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Gene Expression Profiling of Encephalitogenic CD4$^+$ T cells: Identification of Genes Controlling Migration of Effector T cells into the CNS

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von

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Erklärung

Hiermit erkläre ich ehrenwörtlich, dass ich die vorliegende Dissertation selbstständig und ohne unerlaubte Hilfe angefertigt habe. Die Arbeit wird hiermit erstmalig einer Prüfungskommission vorgelegt.

München, den

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Summary

T cells directed against brain antigens are generally held to play a crucial role in the initiation of multiple sclerosis (MS). This was deduced from experimental autoimmune encephalomyelitis (EAE). In this model for MS, T cells reactive for myelin antigens induced a severe paralytic disease upon transfer to healthy syngeneic recipients. Intriguingly, the disease does not start immediately upon transfer of the pathogenic effector T cells. Instead, as earlier studies have shown, the effector T cells attack their target organ only after having migrated in the periphery through secondary lymphoid organs. The aim of the project was to characterize the functional properties of these migrating encephalitogenic T cells during the course of EAE and to identify biological pathways which determine their migratory behaviour and pathogenic potential. To this end, average linkage hierarchical clustering, pathway and gene ontology (GO) analyses of transcriptomes from cultured and ex vivo-isolated myelin basic protein-reactive T cells (T_{MBP} cells) were performed.

At the time of transfer, encephalitogenic T cells in vitro are maximally activated, i.e. they exhibit a prominent upregulation of cell cycle genes such as cyclin A2 (CCNA2) and cyclin B2 (CCNB2) among others. In contrast, T cells isolated from spleen 3 days post transfer, downregulated activation markers such as interleukin 2 receptor (IL2R) and interferon γ (IFNγ), and at the same time upregulated migration specific genes such as CC-chemokine receptor 1 (CCR1), CC-chemokine receptor 2 (CCR2) and CC-chemokine receptor 5 (CCR5). Hierarchical cluster analysis revealed that several transcription regulators known for inhibiting cell cycle progression such as krüppel-like factor 4 (KLF4), B-cell translocation gene 2 (BTG2) and transducer of ERBB2, 1 (TOB1) were clustered together with cell cycle and migration genes. Overexpression of KLF4 in T cells not only inhibited G1/S phase progression of the cell cycle but additionally induced upregulation of CCR2 and CCR5. A novel tetraspan membrane protein called epithelial membrane protein (EMP1), was found to be up regulated in ex vivo-isolated effector T cells. Overexpression of EMP1 in encephalitogenic T cells influenced the migratory behaviour of effector T cells both in vitro and in vivo. EMP1 enhanced T cell motility

within the extracellular matrix milieu in vitro and promoted T cell migration from the connective tissue to lymph nodes in vivo resulting in an accelerated onset of EAE.

In conclusion, gene expression profiling of encephalitogenic T cells revealed interesting genome wide transcriptomic changes and established a correlation between cell cycle progression and cell migration. As a result, in silico analysis put forth several interesting candidate genes that hold promise as potential targets for therapeutic intervention.

1. Introduction

Our organism is constantly exposed to a wide variety of microbial pathogens. The system that comes into play to protect us from these everyday invaders is a complicated and highly precise mechanism called the immune system. The adaptive immune system consists of antigen presenting cells (APCs) and lymphocytes which together coordinate the recognition and elimination of any invading pathogen. In most cases this system is highly effective i.e., the pathogen is eliminated without any lasting damage to host tissues. But there are circumstances, as in the case of autoimmune diseases, when T cells can mount an immune response directed against self-tissues with detrimental consequences. Autoimmune diseases affect approximately 5% of the population in western countries. The exact cause of autoimmune diseases is largely unknown. However, several environmental and genetic factors are known to contribute to the triggering of an autoimmune reaction. Autoimmune disorders are divided into two categories: systemic and localized. Systemic autoimmune disorders, such as rheumatoid arthritis or lupus, affect many organs of the body simultaneously, while localized disorders, such as type I diabetes, Crohn's disease or multiple sclerosis, afflict only one organ.

1.1 Multiple sclerosis

Multiple sclerosis is a chronic inflammatory disease with multiple dispersed lesions, called plaques, within the central nervous system (CNS). These defects result in a diverse spectrum of neurological symptoms such as numbness, impaired vision, loss of balance, paresis, bladder dysfunction and psychological changes. Women are afflicted twice as frequently as men and the disease usually starts between the ages of 20 to 40. One of the salient features of this disease is an inflammatory lesion consisting of T lymphocytes and other mononuclear immune cells.

1.2 Clinical course

In 1996 the United States National Multiple Sclerosis Society standardized four subtype definitions (1). These are termed as the primary-progressive (PP)-, secondary-progressive (SP)-, relapsing-remitting (RR)- and progressive-relapsing (PR)-MS (Figure 1. 1).

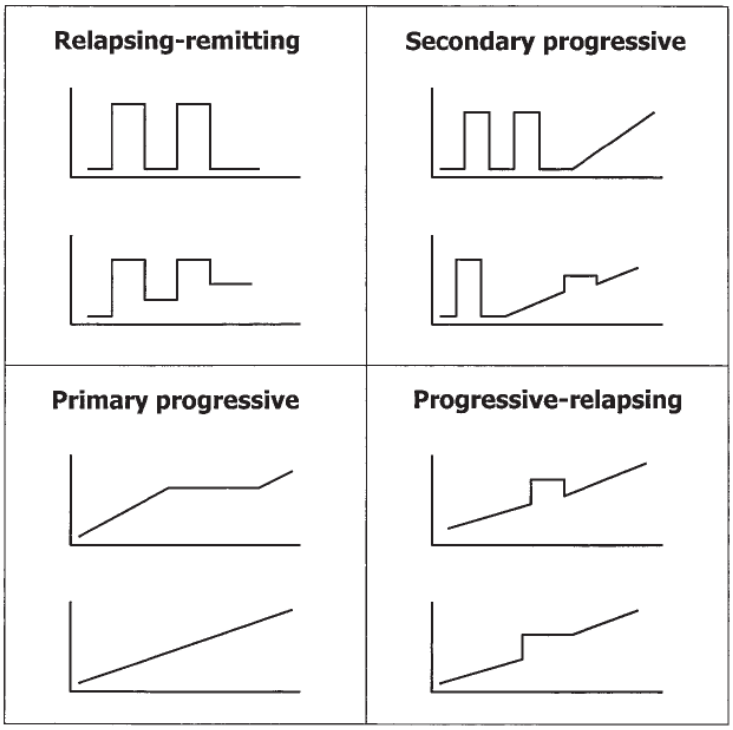

Figure 1. 1 Schematic representation of different clinical forms of MS.
Image adapted from Kieseier et al (2).

1.2.1 Relapsing-remitting MS

Approximately 85% of MS patients present RRMS, which is characterized by disease relapses with full recovery

1.2.2 Secondary progressive MS

SPMS is characterized by disease progression with or without occasional relapses. At least 50% of patients with RRMS will transition into SPMS.

1.2.3 Primary progressive MS

Approximately 10% of MS patients present a disease progression with occasional plateaus from the onset of the disease.

1.2.4 Progressive relapsing MS

This is the least common form presenting a progressive course with acute relapses with or without full recovery.

1.3 Diagnosis

According to the McDonald criteria, magnetic resonance imaging (MRI) evidence of CNS lesions disseminated in time and space is sufficient for a diagnosis of MS even before clinical symptoms have occurred. The McDonald criteria define the Barkhof-Tintore MRI criteria requiring 3 of the following 4 elements: (i) at least one gadolinium-enhancing lesion or 9 T2 hyper intense lesions; (ii) at least one infractentorial lesion; (iii) at least one juxtacortical lesion; and (iv) at least 3 periventricular lesions.

1.4 Pathogenesis

The aetiology of MS is still unclear but recent data point to a combination of genetic and environmental factors. Evidence for the role of genetic factors comes from the MS susceptibility gene localized to the major histocompatibility complex (MHC). The proportion of the total genetic susceptibility explained by the MHC locus is estimated to range between 20% and 50% (3). According to the knowledge obtained from experimental autoimmune encephalomyelitis (EAE) studies, peripherally activated autoreactive $CD4^+$ T cells enter the CNS and recognize auto-antigens, thereby releasing proinflammatory cytokines. Cytokines such as IFNγ and tumour necrosis factor-α (TNF-α) and chemokines recruit additional inflammatory cells and antimyelin antibody forming B cells that amplify tissue injury (4). $CD8^+$ T cells are thought to play a prominent role in MS, because $CD8^+$ T cells were found to be clonally expanded within MS plaques and their numbers correlated with the extent of acute axonal injury. Demyelination is brought about by various contributing factors such as antimyelin antibodies, activated macrophages, microglial cells, complement factors and TNF-α (4,5). Chronic inflammation of the CNS subsequently leads to structural damage including axonal/neuronal degeneration and astrogliosis (6)

1.5 Experimental autoimmune encephalomyelitis

Experimental autoimmune encephalomyelitis is one of the well characterized animal models of human MS. Since its first description in primates (7), EAE has been replicated in a wide range of species including guinea pigs (8), rabbits (9), goats (10), mice (11), rats (12), hamsters (13), dogs (14), sheep (15), marmosets (16) and chickens (17). Studies from animal models have provided strong evidence for brain antigen specific T lymphocytes being the pathogenic mediators of EAE.

1.6 EAE in Lewis rats

1.6.1 Active EAE in Lewis rats

EAE is an acute monophasic paralytic disease in Lewis rats. It can be induced after immunization with either homogenized CNS tissue or isolated myelin basic protein (MBP) emulsified in complete Freund's adjuvant (CFA). About 9-10 days post immunization (p.i.), T cells and a large number of macrophages infiltrate the CNS, forming large perivascular inflammatory lesions (18). Subsequently, paresis and paralysis of the tail and limbs occur owing to oedema and moderate demyelination caused by inflammation in the CNS. In addition to neurological deficits, EAE is also accompanied by a profound loss in body weight (18). The development of clinical and pathological signs of CNS dysfunction in EAE is known to correlate with leukocyte infiltration of the brain (19). Resting lymphocytes commonly do not breach the blood-brain barrier (BBB). However, activated lymphocytes can cross the BBB regardless of their antigen (Ag) specificity, but it is only T cells that recognize their cognate Ag and can recruit other inflammatory cells (20,21).

1.6.2 Adoptive transfer EAE in Lewis rats

Adoptive-transfer EAE (AT-EAE) is an important experimental tool for the investigation of T cell function and regulation in neuroinflammation and autoimmune diseases. AT-EAE in Lewis rat is induced by injection of 5 to 10 million freshly activated T_{MBP} cells. The animals are monitored daily for neurological signs and weight change. Clinical EAE is graded in five scores: 0.5, loss of tail tonus; 1, tail paralysis; 2, gait disturbance; 3, hind limb paralysis; 4, tetraparesis; and 5, death. The disease is highly predictable and is typically monophasic and self-limited (Figure 1. 2). One of the consistent feature of this disease model is that, irrespective of the number of cells injected, clinical and histological changes do not develop in the CNS immediately upon cell transfer but only after a preclinical latency period of at least 3 days (22). Studies using AT-EAE in experimental animals has provided many insights into the behaviour of pathogenic T cells culminating in the design of T cell directed therapies (23).

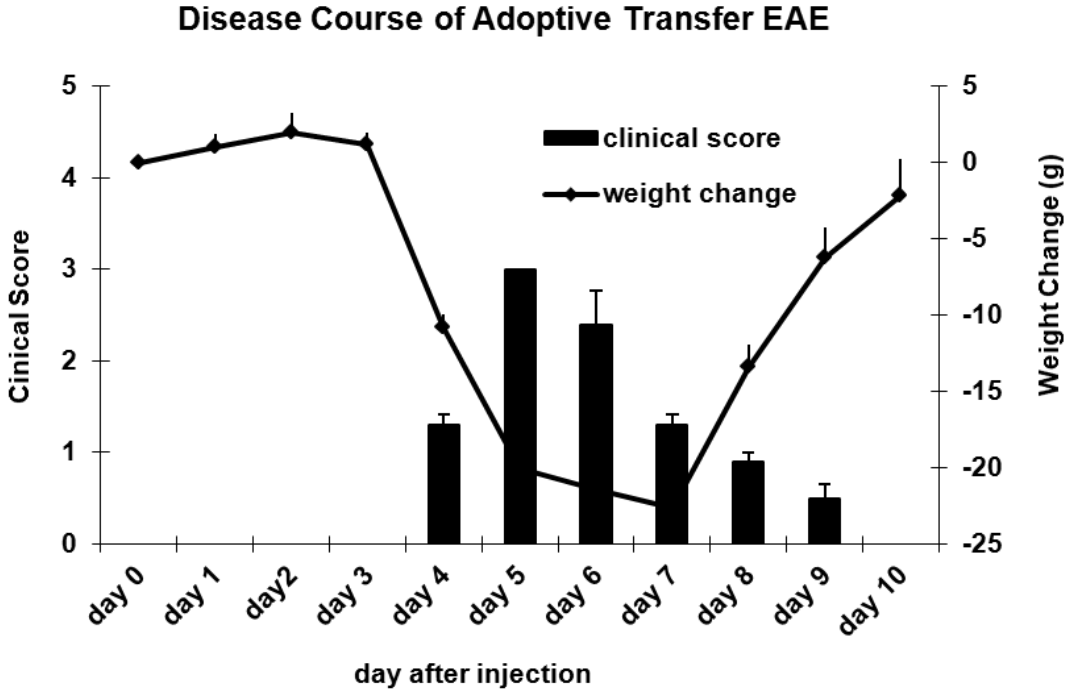

Figure 1. 2 Clinical course of AT-EAE in Lewis rats.
(Image adapted from Odoardi et al. (24).

1.7 T lymphocyte migration to the CNS in AT-EAE

The concept of the BBB was originally introduced by the German bacteriologist, Paul Ehrlich during the late 1800s. He found that intravenous injection of dyes into the bloodstream stained all tissues in most organs except the brain.

The BBB is formed by highly specialized endothelial cells, which inhibit transcellular passage of molecules across the barrier by an extremely low pinocytic activity (39). The endothelial cells of the brain are different in many ways from those found in the peripheral tissues. Brain endothelial cells are joined by tight junctions of high electrical resistance providing an effective barrier against molecules. Only molecules that have a molecular weight less than 500 Daltons can reliably pass through the BBB (40).

The CNS is generally considered as an immunologically privileged site owing to the presence of the BBB, its lack of lymphatic vessels and the absence of classical MHC-positive antigen presenting cells. However, recent evidence points to immunosurveillance of the CNS taking place and that only freshly activated T cells and not resting T cells can enter the CNS. Adoptive transfer experiments using T_{MBP} cells indicate that instead of immediately infiltrating the CNS after activation, they undergo a functional tuning process, which enable them to cross the blood-brain barrier. This preclinical phase normally lasts for 3 to 4 days, during which T_{MBP} cells are distributed in the peripheral lymphoid organs.

The exact molecular and cellular mechanisms guiding activated encephalitogenic T cells into the CNS during this early preclinical phase are not clearly understood. However, according to the traditional view the T cell infiltration into the CNS follows a biphasic course in EAE (20,41). The first phase consists of ‘pioneer T cells’, that infiltrate within a few hours after transfer and encounter their antigen presented by local antigen-presenting cells (42). This first entry of T_{MBP} effector cells supposedly converts the CNS from an immune hostile environment to an immune friendly environment facilitating inflammation. Subsequently, after an interval of 3-4 days, a second wave composed of millions of effector T_{MBP} cells enters the CNS, marking the onset of inflammatory disease. Disease activity reaches a maximum, after which it declines rapidly, resulting in a complete clinical remission within a few days. This clinical

recovery is considered to be due to enhanced apoptosis of inflammatory T cells in the lesion (43).

Recent studies using green fluorescent labelled encephalitogenic MBP-specific T cells ($T_{MBP\text{-}GFP}$) *in vivo* imply that a transcriptomic programming in the periphery is essential for enabling effector T cell migration into the CNS. (44). Importantly, on their way to CNS these $T_{MBP\text{-}GFP}$ cells undergo functional changes in peripheral organs, from maximal activation to migratory state, characterized by downregulation of activation markers, an upregulation of chemokine receptors and an increase of MHC class II molecules on their surface. Eventually, upon arrival to the CNS, migratory $T_{MBP\text{-}GFP}$ cells undergo reactivation as a result of cognate antigen recognition presented by local antigen presenting cells and set the stage for inflammatory leukocyte infiltration. Several pieces of evidence support this hypothesis. Firstly, retransfer of $T_{MBP\text{-}GFP}$ cells from the spleen of an EAE rat 3 days post adoptive transfer to naïve syngeneic rats results in $T_{MBP\text{-}GFP}$ cell infiltration into the CNS within 24 hours (Streyl et al, unpublished). Moreover, surgical connection of blood circulation of an EAE animal (3 days post adoptive transfer of $T_{MBP\text{-}GFP}$ cells) to naïve rat also results in early infiltration of $T_{MBP\text{-}GFP}$ cells. In order to migrate to the CNS $T_{MBP\text{-}GFP}$ cells undergo post activation phenotypic changes in the periphery.

It follows, therefore, that deeper insights into the molecular changes of $T_{MBP\text{-}GFP}$ cells during their migratory path in the periphery might result in the identification of novel targets for interfering with T cell migration to the CNS.

1.8 Targeting T cell migration: Therapeutic strategies for multiple sclerosis

Cell motility is crucial for the effective function of T lymphocyte and immune homeostasis. Priming of lymphocytes for adaptive immune responses requires their migration from the blood stream to lymph nodes to allow their interaction with antigen loaded APCs. Distinct lymphocyte subsets use different combinations of cell migration factors on their surface to orchestrate effector functions and for general immune surveillance. Lymphocyte homing to skin requires the expression of selectin E (SELE) and selectin P (SELP) ligands, CC-chemokine receptor 4 (CCR4) and CC-chemokine receptor 10 (CCR10) (45). Likewise, lymphocyte tropism for the intestines is controlled by expression of the intestine homing receptor α4β7 and CC-chemokine receptor 9 (CCR9) which binds to mucosal addressin cell adhesion molecule 1 (MAdCAM1) and CC-chemokine ligand 25 (CCL25), respectively (46). Up to now, no adhesion molecule combination has been defined that specifically target (effector) T cells to the CNS.

Development of new treatments for MS would require a detailed understanding of the molecular mechanisms of autoreactive T cell migration to the brain. In healthy condition, only a limited number of T lymphocytes are able to cross the BBB and penetrate the CNS. In MS, there is an increased permeability of the BBB leading to an increased transmigration of autoreactive T lymphocytes. After activation, they recruit lymphocytes, macrophages and B cells which induce damage to the CNS. Blocking the migration of T lymphocytes across the BBB has long been considered a promising therapeutic approach to autoimmune diseases of the CNS. The first such agent limiting T cell migration to CNS and representing a breakthrough for the treatment of MS, is a monoclonal antibody (mAb) called natalizumab (Tysabri). Natalizumab is a humanized monoclonal antibody to α4β1 integrin. The integrin heterodimer α4β1 has two binding domains, one to fibronectin and the other to vascular cell adhesion molecule 1 (VCAM1). Studies from both *in vitro* and *in vivo* have established that the interaction between α4b1 integrin and VCAM1 plays an important role in T cell recruitment across the BBB and interference with these molecules significantly reduced adhesion of encephalitogenic T cell blasts to brain endothelium (39) (Figure 1. 3). Moreover, the targeting of α4β1

integrin by natalizumab blocked clinical paralysis in various animal models of MS and has also been approved for treatment of MS.

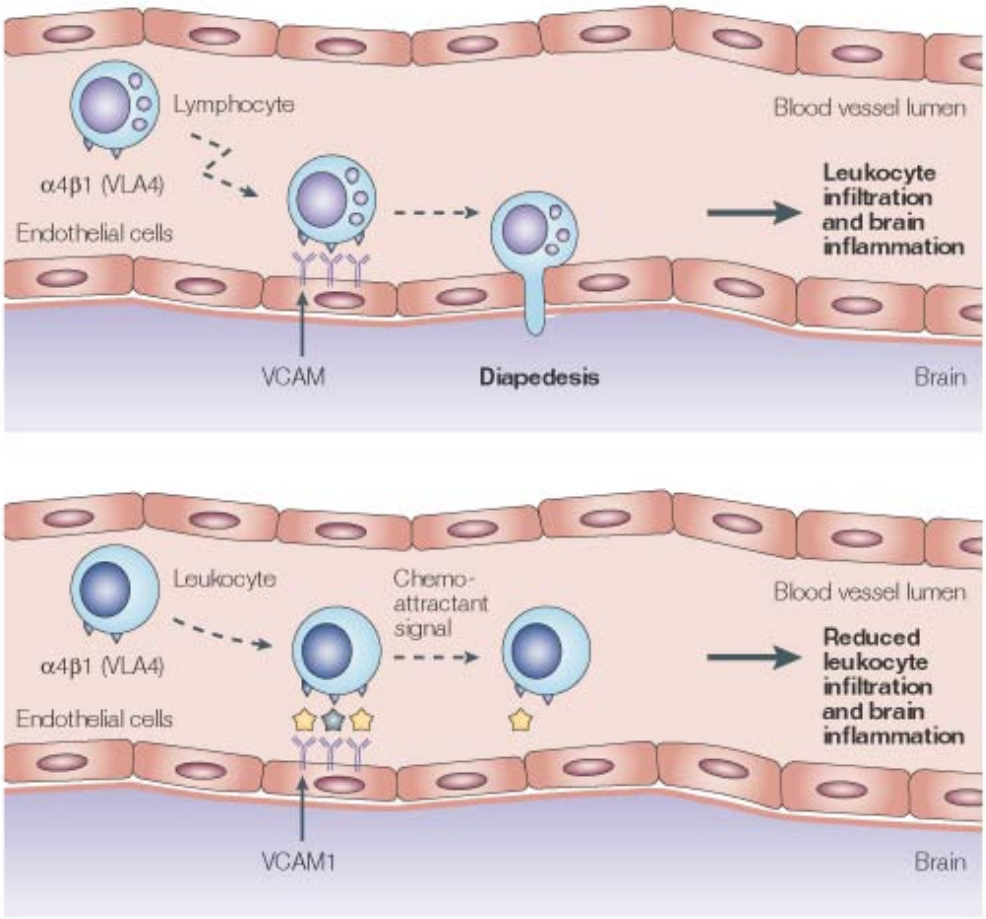

Figure 1. 3 Illustration depicting the mode of action of natalizumab.

Natalizumab is indicated by stars in the lower panel. Image adapted from Steinmann et al (2007) (47)

2 Objectives

Studies using the AT-EAE model for MS have shown that activated encephalitogenic T cells undergo functional changes in the peripheral lymphoid organs such as spleen before migrating to the CNS. There is a phenotypic change from the activated T cell state to migratory T cell state with downregulation of activation markers and upregulation of chemokine receptors. However, still lacking was a detailed understanding of the transcriptomic changes between these different T cell states. The objectives of this thesis were:-

1. To characterize the genome-wide transcriptomic changes in $T_{MBP\text{-}GFP}$ $CD4^+$ T cells during the pre-clinical course of AT-EAE.
2. To identify the molecular link between T cell activation and the T cell migratory state.
3. To identify and test the function of novel membrane molecules implicated in T cell migration to CNS.

3. Materials

3.1 Animals

Lewis rats aged 6-8 weeks old were obtained from the animal facility of Max Planck Institute for Biochemistry (Martinsried, Germany). All animal experiments were performed as per the guidelines of the Bavarian state regulations for animal experimentation.

3.2 Cell lines

Cell Line	**Description**	**Catalogue Number**	**Company**
NIH/3T3	Mouse embryonic fibroblast cell line derived from NIH/Swiss strain	CRL-1658	LGC/ATCC
RBL1	Rat basophilic leukaemia cell line derived from Wistar rat peripheral blood	CRL-1378	LGC/ATCC
293T	Human embryonic kidney cell line transformed with adenovirus E1a and carrying a temperature sensitive T antigen.	CRL-11268	LGC/ATCC
Phoenix™-Eco	293T cell line carrying genes for gag-pol and envelope protein for generation of ecotropic retroviruses.	RVK-10001	Orbigen
GP+E 86 cell line	NIH/3T3 cell line carrying genes for gag-pol and envelope protein for generation of ecotropic retroviruses.	CRL-9642	LGC/ATCC

Table 3. 1 List of mammalian cell lines used.

3.3 Plasmids

Plasmid Name	Use	Company
pMSCVneo	Mammalian ecotropic retroviral expression plasmid DNA	Clonetech
pIRES2-EGFP	Mammalian expression plasmid DNA	Clonetech
pRNAT-H1.4/Retro	Mammalian siRNA retroviral expression plasmid DNA	Genscript
pGEMTEASY	TA cloning plasmid DNA	Promega
pGL3basic	Luciferase reporter plasmid DNA	Promega

Table 3. 2 List of plasmids used.

3.4 Antigens

Myelin basic protein (MBP was prepared from guinea pig brain homogenates as reported (48). S100 calcium binding protein β (S100β) and ovalbumin (OVA) were obtained from Sigma-Aldrich.

3.5 Antibodies

Primary Antibodies (Clone)	Isotype	Specificity	Applications	Company
CD45RC (OX22)	Mouse IgG1κ	Rat	FACS	Serotec
CD4 (W3/25)	Mouse IgG1κ	Rat	FACS	Serotec
αβTCR (R73)	Mouse IgG1κ	Rat	FACS	Serotec
MHC-II (OX6)	Mouse IgG1κ	Rat	FACS	Purified from hybridoma culture supernatant
CD11a	Mouse	Rat	FACS	BD

(WT.1)	IgG1κ			
EMP1	Rabbit IgG	Mouse, Rat, Cow, Dog & Human	WB, IF, IP & ELISA	Santa Cruz biotechnology
KLF4	Rabbit IgG	Human & Rodent	WB, IC, IH	Affinity BioReagents
PAN ACTIN	Rabbit IgG	Human & Rodent	WB, IC	Cell signalling technology

Table 3. 3 List of primary antibodies used.

FACS-Fluorescence Activated Cell Sorting, ELISA-Enzyme Linked Immunosorbent Assay, IC-Immunocytochemistry, IF-Immunofluorescence, IH-Immunohistochem, IP- Immunoprecipitation, WB -Western Blot.

Secondary Antibodies	**Host**	**Conjugation**	**Company**
Anti-mouse	Goat	Allophycocyanin	Invitrogen
Anti-goat	Donkey	Allophycocyanin	Dianova
Anti-rabbit	Goat	Allophycocyanin	Jackson ImmunoResearch

Table 3. 4 List of secondary antibodies used.

3.6 Buffers and Reagents

Cell culture medium and buffers

Dulbecco's modified Eagle's medium (DMEM) for 5 L

DMEM powder (Gibco)	specified amount
Sodium bicarbonate (Merck)	18.5 g
Distilled water	quantum sufficit (q.s.) to 5 L

T-cell medium (TCM) for 1 L

Penicillin/Streptomycin (Gibco) (10000 IU/mL)	10 mL
Sodium pyruvate (100 mM) (Gibco)	10 mL
Non-essential amino acids (100 ×) (Gibco)	10 mL
L-Asparagine (0.36% solution) (Gibco)	10 mL

Glutamine (200 mM) (Gibco)	10 mL
β-mercaptoethanol (Merck)	4 μL
DMEM medium	q.s. to 1000 mL

Re-stimulation medium (RM) for T cells

Fresh autologous rat serum	1 mL
TCM	q.s. to 100 mL

T cell culture medium with growth factor (TCGF)

Supernatant from ConA (Pharmacia Biotech) stimulated mouse splenocytes as a source of IL2 and other growth factors	30 mL
Inactivated horse serum (PAA Lab)	50 mL
TCM	q.s. to 500 mL

Eagle's HEPES solution (EH)

HEPES (Gibco)	125 mL
DMEM medium	q.s. to 5 L

Freezing medium

Inactivated horse serum (PAA Lab)	80 mL
Dimethyl sulfoxide (Sigma)	10 mL
TCM	10 mL

Phosphate buffered saline (PBS)

Sodium chloride (Merck)	137 mM
Potassium chloride (Merck)	2.7 mM
Disodium hydrogen phosphate (Merck)	4.3 mM
Potassium dihydrogen phosphate (Merck)	1.47 mM
Distilled water	800 mL

Adjusted pH to 7.4 and volume made up to 1L using distilled water and sterilized by filtration

Ammonium chloride-potassium (ACK)-erythrocyte lysing buffer

Ammonium chloride (Merck)	0.15 M
Potassium bicarbonate (Merck)	10 mM
Ethylene diamine tetra acetic acid (Merck)	0.1 mM
Distilled water	800 mL

Adjusted pH to 7.4 and volume made up to 1 L using distilled water and sterilized by filtration

Hypoxanthine-Xanthine-Mycophenolic acid (HXM) medium

Hypoxanthine (Sigma)	15 μg/mL
Xanthine (Sigma)	250 μg/mL
Mycophenolic acid (Sigma)	25 μg/mL
Inactivated fetal calf serum (PAA Lab)	50 mL
DMEM medium	q.s. to 500 mL

Reagents for density gradient

Blood lymphocyte gradient

Heparin (5000 U/mL) (Sigma)	100 μL
Optiprep (Nycomed Pharma)	0.63 mL/5 mL blood

CNS lymphocyte gradient

Percol isotonic solution (1.124 g/mL) (Biochem AG)	10.8 mL
Percol underlay solution (1.077 g/mL) (Biochem AG)	10 mL

FACS buffers

FACS buffer for surface staining

Rat serum	3 mL
PBS	q.s. to 100 mL

FACS buffer for surface staining of EMP1	
Bovine serum albumin (BSA) (Sigma)	1 g
Sodium azide (Merck)	1 g
PBS	q.s. to 100 mL
FACS buffer for intracellular staining	
Fixation solution	
Paraformaldehyde (PFA) (Merck)	4 g
PBS	q.s. to 100 mL
Permeabilization solution	
Triton X 100 (Carl Roth)	1 mL
PBS	q.s. to 100 mL
or	
Methanol (Merck)	90% in PBS
Incubation buffer	
PBS	100 mL
BSA (Sigma)	0.5 g
Transfection reagents	
Chloroquine solution 1000×	
Chlroquine diphosphate (Sigma)	100 mM
Distilled water	q.s. to 10 mL
Calcium chloride solution (2 M)	
Calcium chloride (Merck)	29.4 g
Distilled water	q.s. to 100 mL
HEPES-buffered saline (HBS) 2×	
HEPES (Gibco)	50 mM
Sodium chloride (Merck)	280 mM
Disodium hydrogen phosphate (Merck)	1.5 mM

Adjusted pH exactly to 6.95 using Hydrochloric acid (HCl) (Merck) followed by sterilization by filtration through 0.2 μm filter. Small volume aliquot were stored at -20° C

Western Blot buffers

2× Sample Buffer

0.5 M Tris-HCl, pH 6.8	1.25 mL
Glycerol (Carl Roth)	2.5 mL
Sodium dodecyl sulphate (SDS) (Carl Roth) 10% (w/v)	2.0 mL
Bromophenol blue (Sigma) 0.5% (w/v)	0.2 mL
Deionized water	3.5 mL

50 μL of β-mercaptoethanol (Merck) added to 950 μL of sample buffer before use.

SDS-poly acrylamide gel electrophoresis (PAGE) Running Buffer 10×

Tris base (Sigma)	30.3 g
Glycine (Merck)	144.0 g
SDS (Carl Roth)	10.0 g
Deionized water	q.s. to 1 L

SDS-PAGE Transfer Buffer

Tris base (Sigma)	3.03 g
Glycine (Merck)	14.4 g
Methanol (Merck)	200 mL
Deionized water	q.s. to 1 L

TBS-T (Tris-buffered saline with tween) Wash Buffer 10×

Tris-base (Sigma)	31.52 g
Sodium Chloride (Merck)	175.32 g
Tween-20 (Sigma)	10 mL
Deionized water	q.s. to 2 L

4. Methods

4.1 Generation of antigen specific T cells

Antigen specific T cells were generated using the method of Flügel et al, 1999 (49). Lewis rats were immunized by subcutaneous injection of 100 μg of MBP antigen emulsified in CFA (Gibco-BRL) containing 4 mg/mL *Mycobacterium tuberculosis*. Cells from the draining lymph nodes were isolated 10 days after immunization and cultured with a modified limiting dilution method. Lymph node cells (2×10^5 cells/well) were cultured together with MBP (10 μg/mL) in 96-well plates for 3 days and propagated for 5-6 days in IL2 conditioned medium.

4.2 Retroviral transduction of antigen specific T cells

Retroviral packaging cells were generated using GP+E-86 cell lines (50) by adopting the established method of Flügel et al. (49). Phoenix-eco packaging cell lines were transfected with retroviral plasmids pMSCVneo-EMP1-IRES2-EGFP and pMSCVneo-IRES2EGFP (control). Two days after transfection, viral particles containing supernatant were passed through a syringe filter of 0.45 μm and were added to sub-confluent grown GP+E-86 cell lines. Viral transduced GFP positive GP+E-86 cells (GPE-EMP1 and GPE-GFP) were selected in HXM medium for several days and subsequently were FACS sorted several times to obtain 100% GFP positive GP+E-86 packaging cell lines which could produce retroviruses encoding EMP1-IRES2-GFP and GFP respectively. FACS-based viral titration using 3T3NIH cell lines was also performed to determine efficiency of virus production.

Retroviral mediated transduction of primary T lymphocytes was performed by co-cultivation with GP+E-86 packaging cells. The packaging cells were seeded in a 96 well plate (1.4×10^6 cells per plate) and then freshly isolated draining lymph node cells obtained from 10 days post MBP/CFA immunized animals, were added to the wells at a concentration of 2×10^5 cells/well together with MBP (10 μg/mL). Selection with G418 (0.4 mg/mL) was started after 3 days of culture with IL2 conditioned medium and maintained throughout the entire culture period.

For the subsequent re-stimulation, T lymphocytes were incubated with 1×10^6 irradiated (5000 rad) syngeneic thymocytes/well in the presence of MBP (10 μg/mL). T lymphocyte blasts cells were transferred into IL2 conditioned medium and propagated further.

4.3 Recombinant DNA cloning

For construction of a recombinant mammalian retroviral over-expression system, full length cDNA for KLF4 and EMP1 were amplified by polymerase chain reaction (PCR) from *in vitro* activated MBP-specific T cells and were cloned between EcoRI (Fermentas) and SacII (Fermentas) in pMSCVneoIRES2EGFP plasmid DNA. pMSCVneoIRES2EGFP was constructed by sub-cloning IRES2EGFP from pIRES2EGFP into pMSCVneo between HpaI (Fermentas) and EcoRI (Fermentas).

For construction of luciferase assay promoter plasmids, sequences from -1000 to +70 upstream of transcription start site of chemokine receptors viz, CCR2, CCR5 and CXC-chemokine receptor (CXCR3) were amplified by PCR from rat genomic DNA. CCR2 was cloned between XhoI (Fermentas); CCR5 and CXCR3 were cloned between SacI (Fermentas) and XhoI (Fermentas) sites in pGL3 basic vector. Full length KLF4 cDNA for promoter assay was cloned between EcoRI (Fermentas) and SacII (Fermentas) in pMSCVneo vector which lacks EGFP. All the constructs were sequence verified for mutations. Primers sequences used for cDNA and promoter amplification is shown in Table 4. 1.

Gene	**Forward (5'---3')**	**Reverse (5'---3')**
KLF4	5'-TCTGAATTCTTAATGAGGC AGCCACCTGGCGA-3'	5'-TCTCCGCGGTGTGGGTCATG TCCACGATGTGG-3'
EMP1	5'-TCTGAATTCAAGATGTT GGTGCTACTGGCC-3'	5'-GTCCTGAGGAAGAAATAA GCTCGT-3'
CCR2	5'-CTCGAGTCTAATTTGGA GGCAGGATT-3'	5'-CTCGAGGAAATAGAGAA TGAGATGTTGATAGTATG-3'
CCR5	5'-GAGCTCTCAGAAGTGA AGTATCTTGCCA-3'	5'-CTCGAGGGAACGGATGTC TCACCT-3'
CXCR3	5'-CCGTGAACAGAGGAA GTGAA-3'	5'-GTCCATCGGGAGAGAAGA-3'

Table 4. 1 List of primers used for recombinant DNA cloning.

4.4 Proliferation assay for T cells

T cells (5×10^4 cells/well) were co-cultured with irradiated thymocytes (1.2×10^6 cells/mL) in 100 μL RM per well together with antigens viz., MBP, 10 μg/mL or OVA (Sigma), 10 μg/mL or ConA (Pharmacia Biotech) 0.25μg/mL. 48 hrs later, radioactive thymidine [^{3}H]dT (Amerscham Biosciences) (2 Ci/mmol) was added to the culture and radioactive counts were measured after 16-24 hours using a β counter.

4.5 Cytofluorometry FACS

Surface staining

Cells were washed three times in PBS followed by incubation in PBS+3% Rat serum for blocking. Primary antibodies were added in 1:100 dilution in PBS+3% Rat serum for 60 mins on ice followed by three times washing in same buffer. Allophycocyanin-labelled goat anti-mouse was used as secondary antibody.

Intracellular staining

For intracellular staining, cells were fixed in 2% PFA for 10-15 mins then washed in PBS. Permeabilization was carried out using ice cold methanol added slowly to pre-chilled cells, while gently vortexing, to a final concentration of 90% methanol and incubated on ice for 30 mins. Cells were washed in incubation buffer and blocked for 10 mins in incubation buffer at room temperature. Primary antibodies at the appropriate dilutions were added and incubated at room temperature for 30-60 mins. After being washed three times in incubation buffer, secondary antibodies were added and incubated for 30 mins at room temperature before being washed in PBS.

4.6 Quantitative polymerase chain reaction

Total RNA was isolated from cells using Trizol Reagent (Invitrogen) and reverse transcribed to cDNA using Revert-Aid M-MuLV Reverse transcriptase (Fermentas) according the manufacturer's protocol. The cDNA was then analyzed for gene expression by semi-quantitative real time PCR (qPCR) using the GeneAmp 5700 Detection System (Applied Biosystems). β actin gene expression was taken as internal control housekeeping gene. Ready-made Taqman primers and probes were ordered from Applied

Biosystem where applicable. Custom designed primers and Taqman probes were ordered with FAM-TAMRA as fluorophore-quenchers respectively. The list of custom designed Taqman primer probes are listed in Table 4. 2.

Gene	**Forward**	**Reverse**	**FAM-Probe-TAMRA**
β-actin	5´-TACAACCTCC TT GCAGCTCCT-3´	5´-TTGTCGAGAC GAGCGC-3´	5´-CGCCACCAGTTC GCATGGAT-3´
IFNγ	5´-AACAGTAAAG CAAAAAAGGATG CATT-3´	5´-TTCATTGACAG CTTTGTGCTGG-3´	5´-CGCCAAGTTCGAG GTGAACAACCC-3´
IL2	5´-CTCCCCATGA TGCTCACGTT-3´	5´-TCATTTTCCAGG CACTGAAGATG-3´	5´-CAATTCTGTGGC CTGCTTGGG CAA-3´
IL2R	5´-CACAGTCTGT GTACCAGGAGAA CCT-3´	5´-CCACGAAGTG GTAGATTCTCTTG G-3´	5´-CAGGTCACTGCAG GGAGCCCCC-3´
TNFα	5´-TCGAGTGACAA GCCCGTAGC-3´	5´-CTCAGCCACTC CAGCTGCTC-3´	5´-CGTCGTAGCAAAC CACCAAGCAGA-3´
IL4	5´-CGGTGAACTGA GGAAACTCTGTA GA-3´	5´-TCAGTGTTGTG AGCGTGGACTC-3´	5´-CGGTCTGAACTCAC TGAGAAGCTGCACC-3´
IL10	5´-GAAGACCCTC TGGATACAGCTG C-3´	5´-TGCTCCACTGCC TTGCTTTT-3´	5´-CGCTGTCATCGATT TCTCCCCTGTGA-3´
IL17	5´-AGTCCCCGG AGAATTCCA-3´	5´-GAGTACCGCTG CCTTCACTGT-3´	5´-ATGTGCCTGATGC TGTT-3´
CCR2	5´-CACTTAGAC CAGGCCATGCA-3´	5´-TGACAGAGAC TCTTGGAATGACA CACTGCTG-3´	5´-ACTTCTCACCAACA AAGGCATAAAT-3´
CCR5	5´-GTTCTCCTG TGGACCGGGTAT AG-3´	5´-ATTGTCAAACG CTTCTGCAAAC-3´	5´-AGCTTACACGATCA GGATTGACTTGC-3´
CXCR3	5´-AGCAGCCAAG CCATGTACCTT-3´	5´-TAGGGAGATGT GCTGTTTTCCA-3´	5´-AGGTCAGTGAACGT CAAGTGCTAGATGCCT C-3´
EMP1	5´-TCCTCTCGGG ATCCACCAT-3´	5´-TCTACACTCAC CACTACGCCCA-3´	5´-CTGGTGTGCTGGCT GTGCAT-3´
EMP2	5´-CGTCCTGACG GCCATCAT-3´	5´-GACGGAAGCCC CGATCA-3´	5´-CATGTCCTGTCTG TGTGTC-3´
EMP3	5´-GGCCTGCAGT AACGTCAGTGA-3´	5´-GGATGAGAGAC AGCACCATGAG-3´	5´-AACGGCTGGCTG AAG-3´
KLF4	5´-CAGTCGCAA GTCCCCTCTCT-3´	5´-TATCAAGAGC TCATGCCAC-3´	5´-CTCTTTGGCTTGG GCTCCT-3´
RORγ	5’-GACAGGGCC CCACAG AGA-3’	5’-TTTGTGAGGTG TGGG TCTTCTTT-3’	5’-CGAACATCTCGGG AGTTGCTG GCT-3’

T-BET	5'-CCAACAATGTG ACCCAGATGAT-3'	5'-CTGGCTCACC GTCATTCA-3'	5'-TCCTGCAGTCCCTC CATAAGT ACCAGCC-3'
FOXP3	5'-TGGCAAACGG AAGTCTCAA-3'	5'-TCTCATCCAGA GGTGATCTGCTT-3'	5'-AGCCGGGAGAGTT TCTCAAGCACTGC-3'

Table 4. 2 List of Taqman primers and probes used for qPCR.

4.7 EAE induction

AT-EAE in Lewis rats was induced by intraperitoneal (i.p.) or subcutaneous (s.c.) or intravenous (i.v.) injection of 3×10^6 encephalitogenic T lymphocytes. The animals were monitored for weight loss and clinical symptoms. Clinical evaluation was performed by grading clinical scores as follows: 0.5, loss of tail tonus; 1, tail paralysis; 2, gait disturbance; 3, hind-limb paralysis; 4, tetraparesis; and 5, death.

4.8 Memory animal generation

In order to embed $CD4^+$ $T_{MBP\text{-}GFP}$ and $T_{MBP\text{-}EMP1}$ in the immune system of syngeneic neonatal rats, 2.5×10^6 cells in 0.5 mL/animal in EH medium were administered by i.p. injection into new-borns within 48 hrs after birth under hypothermia. After T cell transfer, the new born rats were kept under 30°C humid atmosphere until fully recovered and then returned to their mother. T cells were transferred 4-5 days after re-stimulation with antigen *in vitro*.

4.9 *In vitro* matrigel motility assay

Matrigel (BD Biosciences) is a murine tumour extract, rich in extracellular matrix components such as laminin, collagen IV, heparan sulfate, proteoglycans and entactin. Matrigel (BD Biosciences) was thawed on ice and mixed at a ratio of 1:1 with RM containing 5×10^4-10×10^4 cells. The mixture was incubated in a custom made cylindrical inset in a 35 mm culture dish and imaged after 30 mins incubation at 37° C incubator (Figure 4. 1). Time lapse video microscopy was performed at controlled temperature and CO_2 conditions at 10× objective with a time lapse of 30 secs.

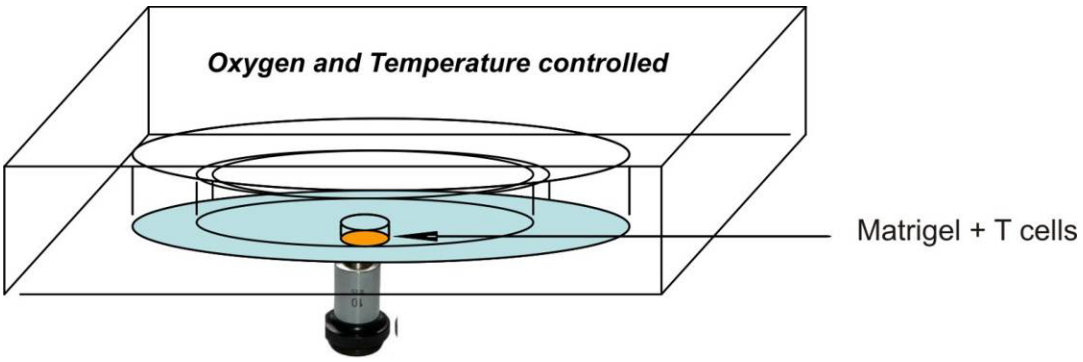

Figure 4. 1 Diagrammatic representation of time lapse video microscopy setup for matrigel motility assay.

The setup consists of a small cylindrical polypropylene tube with an inner radius of 5 mm and height of 7 mm cut out and attached to the centre of 35mm culture dish using 1% agarose. The mixture of matrigel and T cells in a proportion of 1:1 (shown in orange) is then pipetted into the lumen of the cylindrical space and incubated in a closed system with controlled temperature and pH. After 30 mins of incubation, time lapse video microscopy is performed in intervals of 30 seconds over 30 minutes.

4.10 Microarray data analysis

$T_{MBP\text{-}GFP}$ cells were generated as described before in detail (49). Activated T cells (T_{blast}) were obtained 2 days post re-stimulation *in vitro* using thymocytes and MBP antigen followed by FACS sorting. Resting T cells ($T_{resting}$) were obtained 7 days post antigen exposure followed by FACS sorting. AT-EAE was induced as described before by injection of 5 million *in vitro* activated $T_{MBP\text{-}GFP}$ (44). FACS sorting of $T_{MBP\text{-}GFP}$ cells from spleen (T_{spleen}) and $T_{MBP\text{-}GFP}$ cells from CNS (T_{CNS}) was performed, 3.5 days post AT-EAE induction. RNA was isolated and reverted into cDNA followed by cRNA synthesis for microarray analysis. Organs were pooled from several animals to obtain enough starting material. The pooled RNA was divided into three parts for three independent microarray hybridizations and measurements were performed by our collaborators at the Max Planck Institute of Molecular Genetics.

The microarray expression dataset consisted of normalized expression data from three measurements. The dataset essentially pertains to T cells from four different milieus viz, *in vitro* activated (T_{blast}), *in vitro* resting (T_{rest}), *ex vivo* spleen (T_{spleen}) and *ex vivo* spinal cord (T_{CNS}). Comparison analysis was performed to define three T cell state such as $T_{activated}$ (T_{blast} versus $T_{resting}$), $T_{migratory}$ (T_{spleen} versus T_{blast}) and $T_{effector}$ (T_{CNS} versus

T_{blast}). After generating a list of differentially expressed genes in $T_{activated}$, $T_{migratory}$ and $T_{effector}$, all transcripts without annotations were disregarded for further downstream analysis. This resulted in an expression dataset consisting of only 17,694 probesets from a total of 31,256 probesets in the Affymetrix GeneChip® Rat Expression Set 230.

Average linkage hierarchial clustering was performed on the annotated expression dataset using GENESIS (http://genome.tugraz.at/genesisclient/genesisclient_description .shtml). Further, downstream analyses were carried out only on data pertaining to $T_{activated}$ and $T_{migratory}$. For GO based analysis affymetrix probesets were downloaded from GO consortium database (http://www.geneontology.org/). For pathway based analysis, the GenMAPP (Gene Map Annotator and Pathway Profiler) program was used (http://www.genmapp.org/).

4.11 Dual luciferase assay

One day before transfection, 293T cells (4×10^4 cells per well) were plated in a 48 well plate. 24 hours later, cells were transfected with 0.5 μg each of pGL3 reporter constructs (pGL3 basic, pGL3-CCR2, pGL3-CCR5, pGL3-CXCR3), 0.3 μg of pMSCVneo or pMSCVneo-KLF4 vector and 10 ng of pTK-renilla. Two days after transfection, cells were washed once with ice cold PBS and lysed in 65 μL /well of 1× passive lysis buffer. The lysate was clarified by centrifugation and collected in 96 well opaque walled plates. Luciferase activity was measured after an addition of 45μL of LARII™ reagent (Promega) per well. For internal control, Renilla luciferase activity was measured immediately after addition of Stop & Glow™ reagent (Promega) 45 μL per well. Results were normalized to empty vector PMSCVneo.

4.12 Bioinformatics

Extraction of promoter sequences

Chemokine receptor promoter sequences were downloaded using BioMart central portal as follows:- http://www.biomart.org/

Prediction of transcription factor binding site in target DNA sequences

Potential transcription factor binding sites in chemokine receptor promoter sequences CCR1, CCR2, CCR3, CCR4, CCR5 and CXCR3 were predicted using a web based tool called Matinspector™ from Genomatix which can be reached at the following website:- http://www.genomatix.de/online_help/help_matinspector/matinspector_help.html

Multiple sequence alignment of protein sequences

Evolutionarily conserved motifs in EMP1 was identified by a multiple sequence alignment (MSA) of EMP1 protein sequences from different mammalian species such as human, monkey, pig, cattle, rabbit, rat and mouse. PMP22/EMP/MP20 family motifs were illustrated by MSA of EMP1, EMP2, EMP3 and PMP22. The ClustalW2 program used for MSA is available online at the following portal:- http://www.ebi.ac.uk/Tools/clustalw2/index.html

Prediction of transmembrane helices

EMP1 transmembrane helices were determined and illustrated using SOSUI server as reported which is available at the following web portal (51) :- http://bp.nuap.nagoya-u.ac.jp/sosui/sosui_submit.html

5. Results

5.1 Microarray analysis of encephalitogenic T cells

EAE can be induced by adoptive transfer of freshly-activated T_{MBP} cells. In contrast, resting T cells are not pathogenic irrespectively of the number of injected cells. Intriguingly, the T_{MBP} cell blasts do not immediately migrate into their target organ; they rather follow predefined migration paths leading them from peripheral lymph nodes to the spleen and finally into the CNS. Moreover, there is a dramatic downregulation of activation marker and an upregulation of chemokine receptors in the periphery (44). Notably, T_{MBP} cells extracted from the spleen 3.5 days post transfer, readily infiltrate the CNS and induce disease as early as 24 hours post transfer (p.t.) (Streyl et al, PhD dissertation).

In order to investigate the gene expression changes in encephalitogenic T cells $T_{MBP\text{-}GFP}$ cells during the pre-clinical phase of AT-EAE, a genome-wide transcriptional profiling of *in vitro* cultured $T_{MBP\text{-}GFP}$ cells (resting cells: $T_{resting}$ and freshly activated blast cells: T_{blast}) and *ex vivo* -isolated migratory $T_{MBP\text{-}GFP}$ cells (by FACS from spleen (T_{spleen}) and CNS (T_{CNS}) 3.5 days p.t.) (Figure 4. 1) was performed. Microarray analysis was carried out using the affymetrix oligonucelotide microarray (Gene Chip ® Rat Expression Set 230) expression dataset consisting of 30,248 transcripts, including over 28,000 well substantiated rat genes. Microarray was performed by the automation group (Dr. Wilfried Nietfeld, Max Planck Institute of Molecular Genetics, Berlin) and data normalization was executed by the department of computational molecular biology (Prof. Dr. Martin Vingron, Max Planck Institute for Molecular Genetics, Berlin), the resultant expression values being presented as log to the base ‘e’. Thus we started with expression datasets comprising expression values pertaining to four different samples of encephalitogenic T cells termed T_{blast}, $T_{resting}$, T_{spleen} and T_{CNS}. Prior to data analysis three different T cell states or transcriptomes were defined as follows.

1. $T_{activated}$ state: comparison of T_{blast} versus $T_{resting.}$
2. $T_{migratory}$ state: comparison of T_{spleen} versus $T_{blast.}$

3. $T_{effector}$ state: comparison of T_{CNS} versus $T_{blast.}$

Activated T cell state ($T_{activated}$ state) represents the transcriptome of T cell blasts 48 hrs after re-stimulation in vitro whereas migratory T cell state represents the transcriptome of T cell 3.5 days post adoptive transfer in peripheral lymphoid organ, spleen. Effector T cell state on the other hand represents the transcriptome of reactivated T_{MBP} cells within the target organ, CNS.

5.2 Cluster analysis of the microarray expression data

Cluster analysis is the most popular and commonly used method for gene expression analysis. One of the primary goals of clustering is to group together objects such as genes or transcriptomes with similar expression pattern. When genes with similar expression profiles are grouped together they are believed to be co-regulated and functionally related.

	T_{spleen} vs T_{blast}	**T_{CNS} vs T_{blast}**	**T_{blast} vs $T_{resting}$**
Number of upregulated (≥ 2 fold) transcripts	587	514	919
Number of downregulated (≤ 0.5 fold) transcripts	1027	1149	1791
Total number of differentially regulated transcripts	1614	1663	2710
Total number of transcripts	30200	30200	30200

Table 5. 1 A summary of differentially regulated transcripts from microarray dataset.

A summary of the number of differentially regulated transcripts is given in Table 5. 1. A cut off of ≥ 2 fold was chosen for upregulation and ≤ 0.5 fold for downregulation. A comparison between T_{spleen} and T_{blast} state resulted in 1614 differentially regulated transcripts. The number of regulated transcripts in T_{blast} cells (i.e., after activation with specific antigen) compared to $T_{resting}$ cells was 2710 transcripts. This indicates that T_{blast} cells upon migration to spleen experience a dramatic reorganization of their transcriptional program to attain the $T_{migratory}$ state *in vivo.*

Average linkage cluster analysis revealed surprisingly diverse gene expression patterns. Importantly, out of 30248 transcripts only a fraction (~6%) of them showed a differential regulation (Table 5. 1). Moreover, the regulation of genes in $T_{migratory}$ and $T_{effector}$ states is very similar, as can been seen from the heat map illustration of cluster analysis (Figure 5. 3), implying a similar transcriptome between T_{spleen} and T_{CNS}. *In vitro* activated T cells undergo transcriptomic changes that imparts them migratory phenotype facilitating CNS infiltration. Two interesting clustering pattern could be observed from the analysis, namely (i) downregulated in $T_{activated}$ and upregulated in $T_{migratory}$ and $T_{effector}$ and (ii) upregualted in $T_{activated}$ and downregulated in $T_{migratory}$ and $T_{effector}$. Several clustered genes are labelled and depicted in Figure 5. 4. One of the prominent features evident from the cluster analysis is the reciprocal regulation of the same genes in $T_{activated}$ and $T_{migratory}$. Genes that are upregulated in $T_{activated}$ state were downregulated in $T_{migratory}$ state and vice versa (Figure 5. 2, Figure 5. 3 and Figure 5. 4).

As expected, major differences between T_{blast} cells and $T_{resting}$ cells included genes responsible for strong immune activation and proliferation, i.e. genes encoding cytokines for e.g. interleukin 17f (IL17F), CC-ligand 2 (CCL2); cell cycle-associated genes, for e.g. cell division cycle 20 homolog (CDC20), cyclin E1 (CCNE1), cytoskeleton associated protein 5 (CKAP5); factors of the DNA replication machinery, for e.g. deoxyuridine triphosphatase (DUT), minichromosome maintenance complex component 7 (MCM7), replication factor C 3 (RFC3); DNA polymerases, for e.g. minichromosome maintenance complex component 4 (MCM4), minichromosome maintenance complex component (MCM6) and cell metabolism, for e.g. 3-hydroxy-3-methylglutaryl-Coenzyme A reductase (HMGCR), cytochrome P450, family 51 (CYP51), phosphomevalonate kinase (PMVK) (Appendix 1). In striking contrast $T_{migratory}$ state displayed a completely distinct genotype. The genes regulated in T_{spleen} controlled functions such as cell communication for e.g. Rho GTPase activating protein 4 (ARHGAP4), dual specificity phosphatase 5 (DUSP5), dishevelled associated activator of morphogenesis 1 (DAAM1), plasminogen activator, urokinase receptor (PLAUR); cell adhesion for e.g. CD44 antigen (CD44), epithelial membrane protein 1 (EMP1), neuropilin (NRP1), selectin L (SELL); cell migration for e.g.

integrin β1 (ITGβ1), integrin β7 (ITGβ7), (CCR5), vinculin (VCL), and immune response for e.g. CXC-chemokine ligand 2 (CXCL2), GLI pathogenesis-related 1 (GLIPR1), immunoglobulin superfamily, member 6 (IGSF6) (Appendix 2). Overall cluster analysis revealed a strong association of cell cycle genes in a $T_{activated}$ state and cell migration related genes with $T_{migratory}$ state.

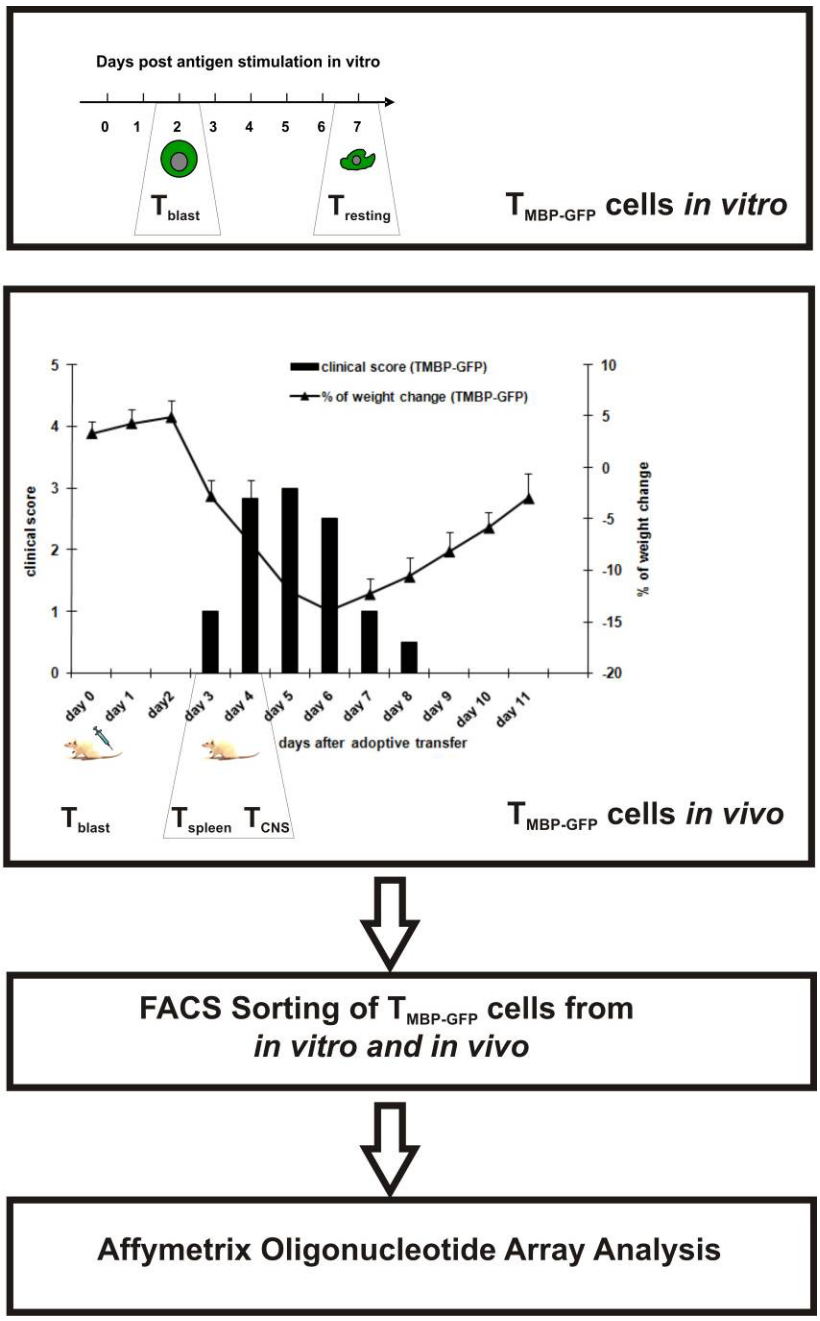

Figure 5. 1 Schematic illustration of green fluorescent $T_{MBP\text{-}GFP}$ cell sorting using FACS from different milieus and subsequent microarray analysis.

$T_{MBP\text{-}GFP}$ were generated as described in detail previously (49). Activated T cells (T_{blast}) were obtained 2 days post re-stimulation *in vitro* using thymocytes and MBP antigen, followed by FACS sorting. Resting T cells ($T_{resting}$) were obtained 7 days post antigen exposure followed by FACS sorting. AT-EAE was induced as described before by injection of 5 million of *in vitro* activated $T_{MBP\text{-}GFP}$ (44). FACS sorting of $T_{MBP\text{-}GFP}$ cells from spleen (T_{spleen}) and $T_{MBP\text{-}GFP}$ cells from CNS (T_{CNS}) was performed, 3.5 days post AT-EAE induction. RNA was isolated and reverted into cDNA followed by cRNA synthesis for microarray analysis.

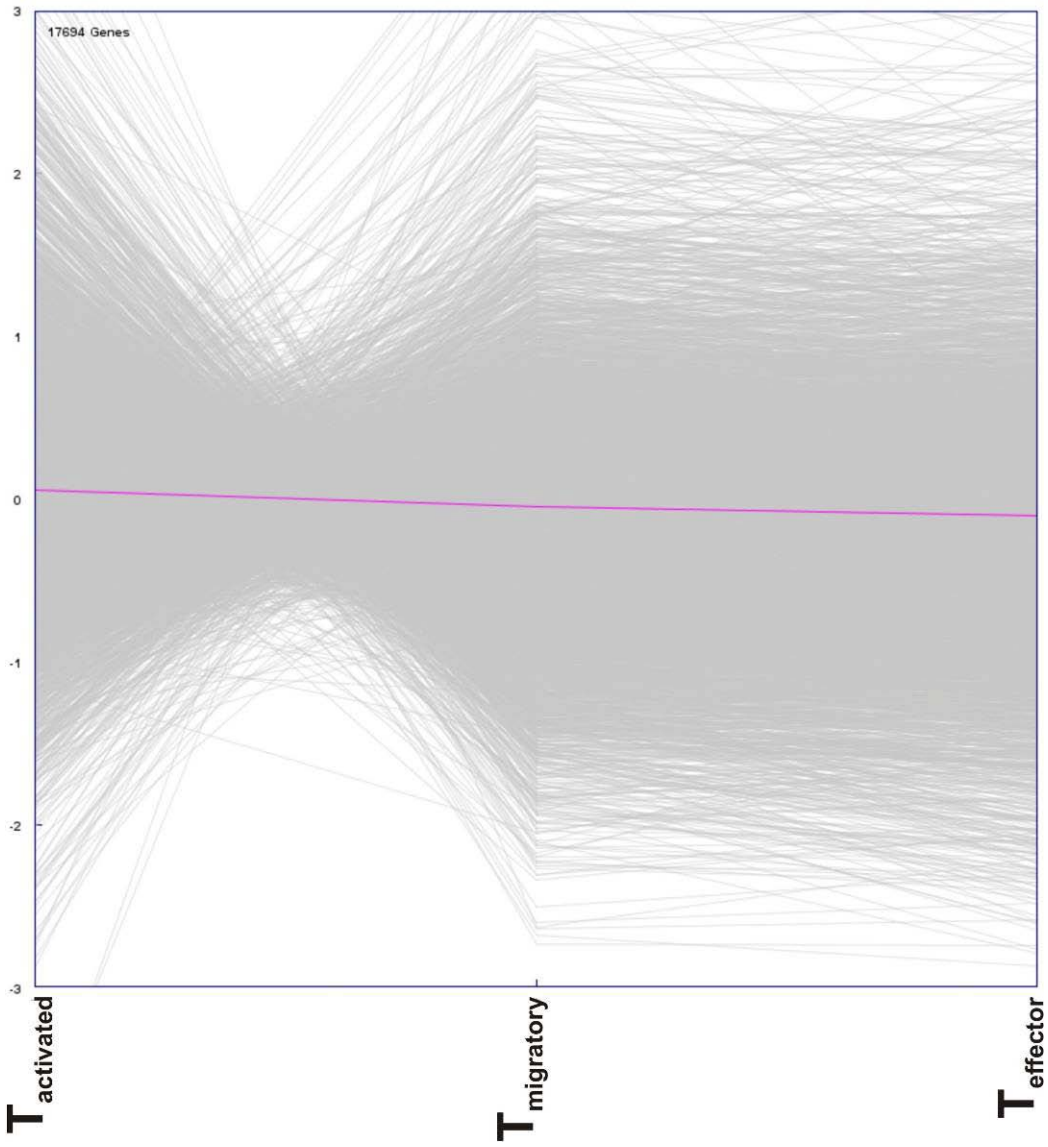

Figure 5. 2 Microarray dataset viewed as expression plot.

Annotated genes (17694 genes) from the microarray dataset were plotted with fold values on ordinate and $T_{MBP\text{-}GFP}$ cell states on abscissa. An imaginary line drawn in magenta indicates zero fold value. The transcriptomes of three T cell states viz, $T_{activated}$, $T_{migratory}$ and $T_{effector}$ were investigated. $T_{activated}$ transcriptome was represented as gene expression fold change values, obtained by dividing gene expression values of T_{blast} by $T_{resting}$. $T_{migratory}$ transcriptome was represented as gene expression fold change values, obtained by dividing gene expression values of T_{spleen} by T_{blast} whereas $T_{effector}$ transcriptome was represented as gene expression fold change values, obtained by dividing gene expression values of T_{CNS} by T_{blast}. Genes that were upregulated in $T_{activated}$ were downregulated in $T_{migratory}$ and $T_{effector}$ and vice versa.

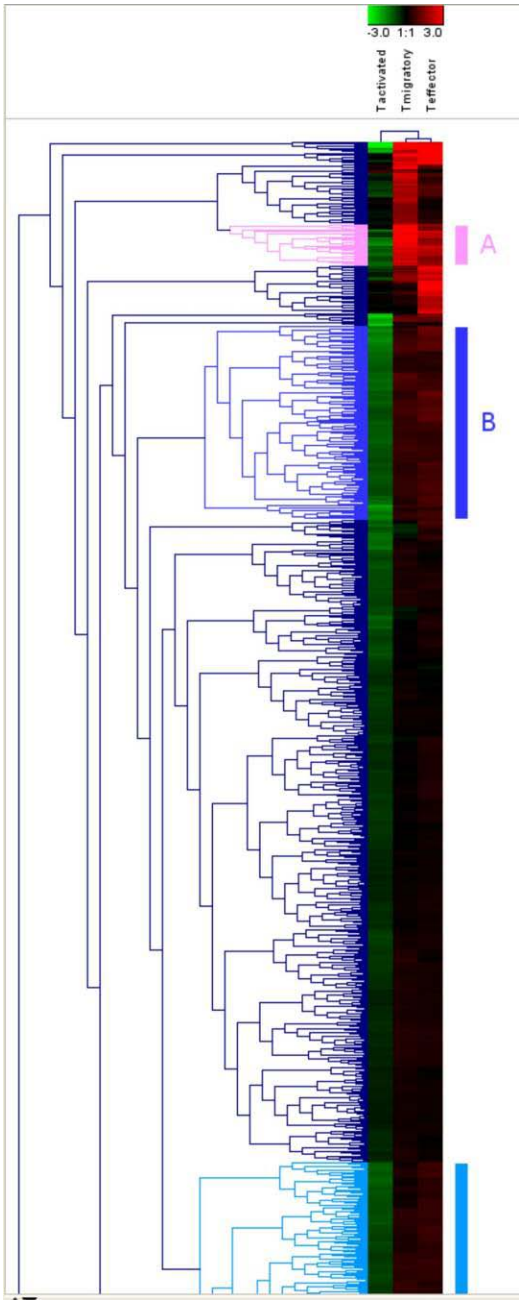

Figure 5. 3 Heat Map representation of cluster analysis of annotated genes.

Average linkage hierarchical clustering of annotated genes is depicted as a heat map with dendrogram. Three T cell states viz., $T_{activated}$, $T_{migratory}$ and $T_{effector}$ were investigated. $T_{activated}$ transcriptome was represented as gene expression fold change values, obtained by dividing gene expression values of T_{blast} by $T_{resting}$. $T_{migratory}$ transcriptome was represented as gene expression fold change values, obtained by dividing gene expression values of T_{spleen} by T_{blast} whereas $T_{effector}$ transcriptome was represented as gene expression fold change values, obtained by dividing gene expression values of T_{CNS} by T_{blast}. Scale for colour code is shown on the top and values are log transformed to the base 2. Coloured bars labelled alphabetically indicate clustered genes.

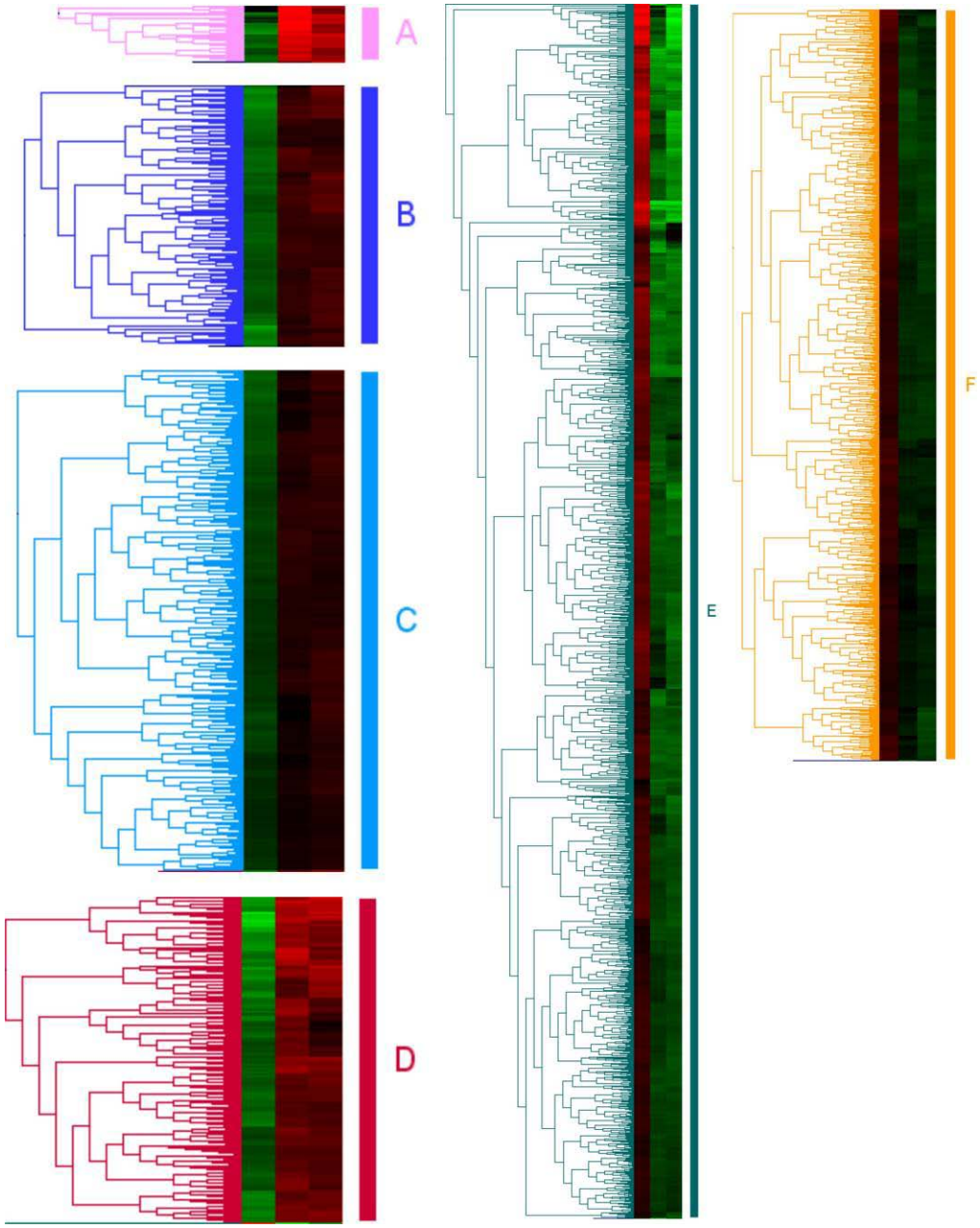

Figure 5. 4 Depiction of clusters A-F.

Annotated genes were analysed via average linkage hierarchical cluster analysis and were grouped into 6 clusters, labelled here A to F. These are depicted as a heat map with a dendrogram separately.

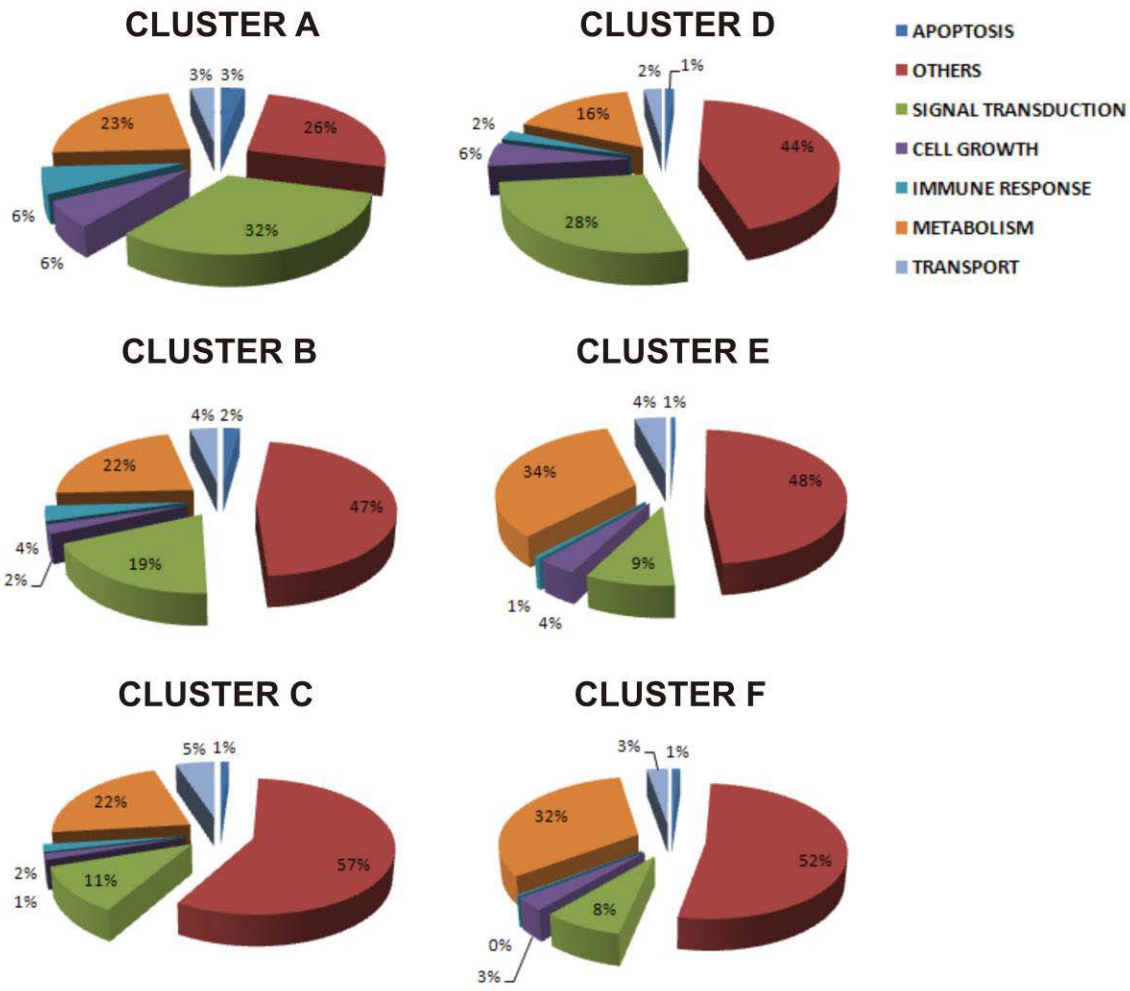

Figure 5. 5 Pie chart analysis of clusters A to F.

Clusters A to F were individually analysed using Gene Ontology annotations. Gene expression changes (either upregulated or downregulated) in cluster A to F were plotted as pie charts.

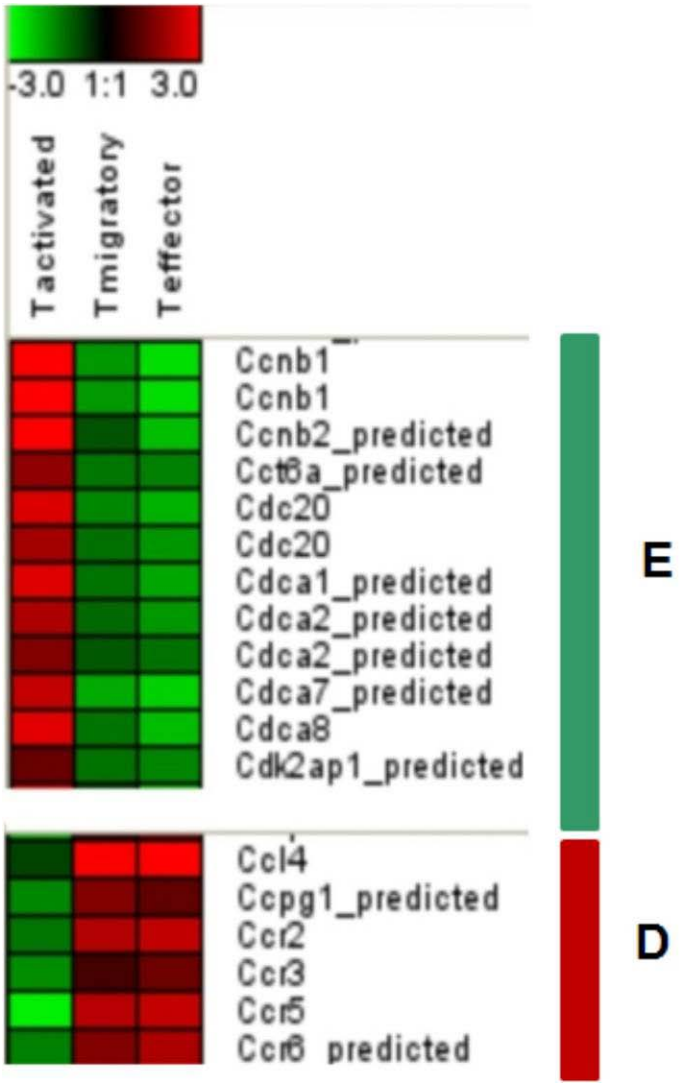

Figure 5. 6 Cell cycle genes and chemokine receptors are clustered.

A part of cluster E depicts clustering of differentially regulated cell cycle genes in $T_{activated}$, $T_{migratory}$ and $T_{effector}$ transcriptomes. A part of cluster D depicting clustering of differentially regulated inflammatory chemokine receptors in $T_{activated}$, $T_{migratory}$ and $T_{effector}$ transcriptomes. Fold values are colour coded and scale is shown on the top.

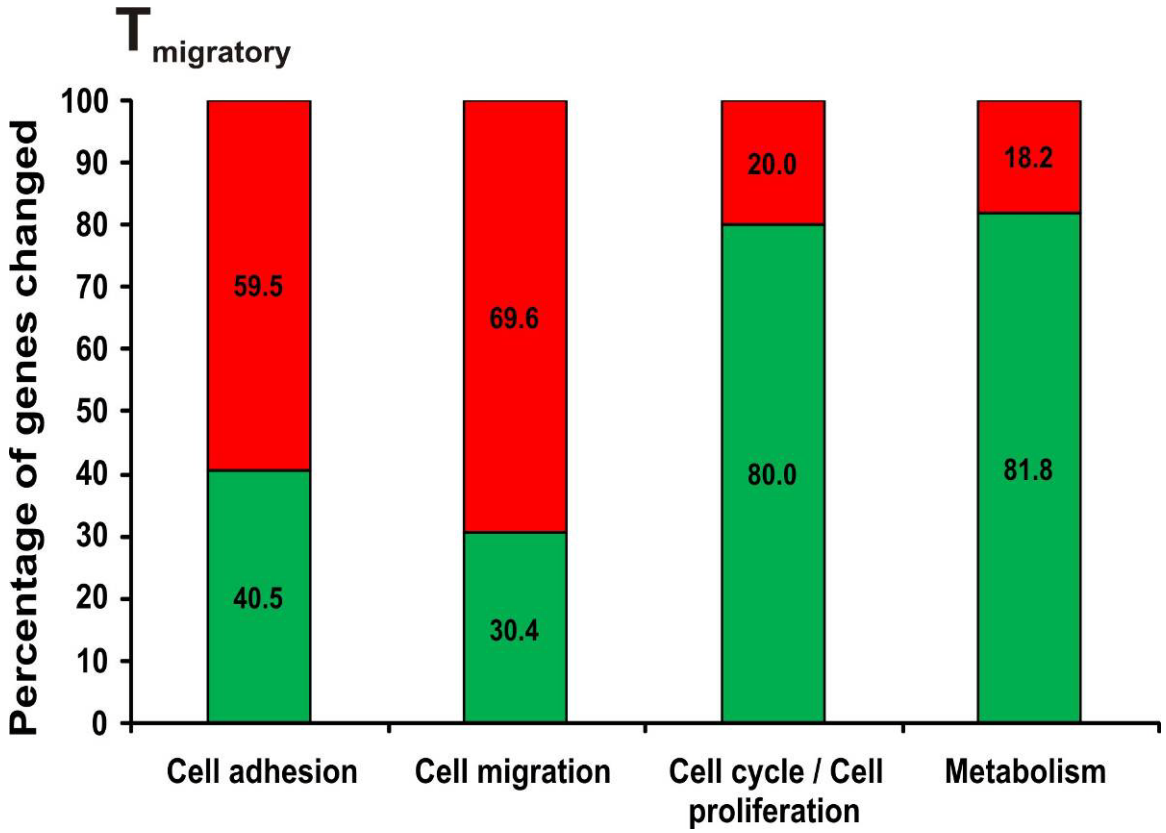

Figure 5. 7 Differential regulation of cell migration and cell cycle processes.

This graph depicts the percentage of annotated genes differentially regulated in four biological processes, viz. cell adhesion, cell migration, cell cycle and metabolism in the $T_{migratory}$ state (T_{spleen}/T_{blast}).

5.3 Gene Ontology based analysis of microarray expression data

GO consortium classifies genes into molecular functions, biological processes and cellular components which is particularly useful for getting an overview of quantitative changes pertaining to each category.

All genes that were differentially regulated were chosen for GO analysis. The clustered genes were assigned the GO annotation and subsequently were plotted in pie charts. In clusters B to F, a major proportion of regulated genes did not belong to any of the biological processes such as apoptosis, signal transduction, cell growth, immune response, metabolism and transport (Figure 5. 5).

Most of the upregulated genes in a $T_{migratory}$, state in GO, belonged either to the plasma membrane (28%) or the gene ontology group of secreted proteins (10%) which was in contrast to $T_{activated}$ cell transcriptome where nuclear (38%) and cytoplasmic (30%) proteins predominated (Table 5. 3). Membrane factors upregulated in a $T_{activated}$ state were involved in auxiliary transport activity for e.g. (solute carrier family 7, member 1, SLC7A1), solute carrier family 16, member 1, SLC16A1, transferrin receptor ,TFRC) (31%), GTPase activity (guanine nucleotide binding protein alpha inhibiting activity polypeptide 1, GNAI, member RAS oncogene family RAB34, RAB34) (12%) or protein binding (phospholipid scramblase 1, PLSCR1) (6%) (Table 5. 2) (Appendix 1). In a $T_{migratory}$ state this group of regulated genes rather belonged to membrane receptor activity (CD44, ITGβ1, killer cell lectin-like receptor subfamily D, member 1, KLRD1, tumour necrosis factor receptor superfamily, member 1B, TNFRSF1B) (35%), calcium ion binding activity (pleckstrin, PLEK, S100 calcium binding protein A4, S100A4, S100 calcium binding protein A9, S100A9) (13%) or fell into the category of undefined molecular functions (GLIPR1, synaptotagmin-like 1, SYTL1) (11%) (Appendix 2). Clearly, membrane molecules in a $T_{migratory}$ state are involved in cell migration machinery.

Biological processes such as cell adhesion, cell migration, cell communication, immune response and signal transduction were the prominently upregulated processes in a $T_{migratory}$ state whereas processes such as metabolism and cell cycle/proliferation formed the majority of the $T_{activated}$ pie chart (Table 5. 4, Table 5. 5).

Cell adhesion molecules such as CD44, ITGβ1 and SELL play a crucial role in T lymphocyte adhesion on the luminal side of blood vessels and were all upregulated in T_{spleen} compared to T_{blast}. In particular, studies involving blocking anti-bodies revealed that both CD44 and ITGβ1 contribute to clinical disease in EAE by inhibiting leukocyte extravasation to the CNS (53). In T_{spleen} compared to T_{blast} in addition to well-known cell adhesion genes, several novel cell adhesion genes were also upregulated, namely, EMP1, CD97 antigen (CD97), and NRP1 (Table 5. 1) (Appendix 2). The majority of cell adhesion genes were downregulated in the $T_{activated}$ state (69% downregulated; 31% upregulated) whereas in the $T_{migratory}$ state 55% of cell adhesion genes that underwent change were upregulated (Table 5. 4, Table 5. 5).

GO biological process that underwent significant change in both $T_{activated}$ and $T_{migratory}$ states were similar such as cell metabolism ($T_{activated}$; 348 genes, $T_{migratory}$; 297 genes), cell communication ($T_{activated}$; 145 genes, $T_{migratory}$; 159 genes), biological processes unknown ($T_{activated}$; 56 genes, $T_{migratory}$; 47 genes) and transport ($T_{activated}$; 53 genes, $T_{migratory}$; 46 genes). However, the number of cell migration genes that underwent changes in the $T_{migratory}$ state was higher than that of the $T_{activated}$ state ($T_{activated}$; 10 genes, $T_{migratory}$; 23 genes) and most of them were upregulated in the $T_{migratory}$ state, implying an apparent migratory genotype acquired by T_{spleen} 3.5 days post adoptive transfer of $T_{MBP\text{-}GFP}$ cells. (Table 5. 4, Table 5. 5).

GO: Molecular Class	Numbers of genes changed	Upregulated in $T_{activated}$ state	Downregulated in $T_{activated}$ state
Centrosome	12	11	1
Cytoplasm	182	114	68
Cytoskeleton	1	0	1
Endoplasmic Reticulum	26	13	13
Extracellular	20	10	10
Golgi apparatus	18	2	16
Kinetochore	1	1	0
Lysosome	10	2	8
Microtubule	3	2	1
Mitochondrion	3	2	1
Nucleus	217	144	73
Perinuclear region	1	0	1
Peroxisome	7	5	2
Plasma membrane	103	28	75
Ribosome	3	3	0

Table 5. 2 GO: Molecular Class based annotations of differentially regulated transcripts in $T_{activated}$ transcriptome.

GO: Molecular Class	Numbers of genes changed	Upregulated in $T_{migratory}$ state	Downregulated in $T_{migratory}$ state
Centrosome	7	1	6
Cytoplasm	84	53	31
Cytoskeleton	1	1	0
Endoplasmic Reticulum	25	7	18
Extracellular	36	20	16
Golgi apparatus	11	8	3
Intermediate filament	1	1	0
Lysosome	5	3	2
Microsome	3	1	2
Mitochondrion	56	4	52
Nucleus	207	49	158
Perinuclear region	1	1	0
Peroxisome	5	0	5
Plasma membrane	87	59	28
Ribosome	2	0	2

Table 5. 3 GO: Molecular Class based annotations of differentially regulated transcripts in $T_{migratory}$ transcriptome.

GO: Biological Process	Numbers of genes changed	Upregulated in $T_{activated}$ state	Downregulated in $T_{activated}$ state
Apoptosis	11	2	0
Process unknown	56	33	23
Cell adhesion	29	9	20
Cell migration	10	4	6
Cell communication	145	56	89
Cell cycle	15	14	1
Cell growth	33	17	16
DNA repair	3	3	0
DNA replication	3	3	0
Energy pathways	1	0	1
Immune response	20	6	14
Metabolism	348	244	104
Protein folding	4	3	1
Signal transduction	13	2	11
Transport	53	28	25

Table 5. 4 GO: Biological Process based annotation of differentially regulated transcripts in $T_{activated}$ transcriptome.

GO: Biological Process	Number of genes changed	Upregulated in $T_{migratory}$ state	Downregulated in $T_{migratory}$ state
Apoptosis	9	5	4
Process unknown	47	15	32
Cell adhesion	37	22	15
Cell migration	23	16	7
Cell communication	159	91	68
Cell cycle	15	3	12
Cell growth	43	19	24
DNA repair	3	0	3
DNA replication	3	0	3
Energy pathways	1	1	0
Immune response	25	20	5
Metabolism	297	54	243
Protein folding	3	0	3
Signal transduction	11	9	2
Transport	46	13	33

Table 5. 5 GO: Biological Process based annotation of differentially regulated transcripts in $T_{migratory}$ transcriptome.

5.4 Pathway based analysis of microarray data

To determine signalling pathways implicated in T cell migration, GenMAPP was used for pathway-based analysis of the expression dataset of T_{blast}, $T_{resting}$ and T_{spleen} cells. The pathways that underwent major changes in activated T_{blast} cells compared to $T_{resting}$ cells ($T_{activated}$) were the cholesterol biosynthetic (Figure 5. 8) and cell cycle pathways (Figure 5. 10). Antigen-specific T cell activation resulted in cell cycle progression by upregulation of the CDC2 kinase which associates with cyclin A (CCNA1) and cyclin B (CCNB). Cyclins required for G1 to S phase transition such as cyclin E1 (CCNE1) and cyclin E2 (CCNE2) and cyclins important for G2 phase to M phase transition such as CCNA2, cyclin B1 (CCNB1) cyclin B2 (CCNB2) and cyclin F (CCNF) were all upregulated. Furthermore, v-myc myelocytomatosis viral oncogene homolog (MYC), a transcription factor driving the synthesis of DNA replication genes was upregulated and at the same time several transcription factors such as krüppel-like factor 2 (KLF2), krüppel-like factor 4 (KLF4), krüppel-like factor 9 (KLF9), B-cell translocation gene 1 (BTG1), and B-cell translocation gene 2 (BTG2) that inhibit the cell cycle progression were downregulated. Of the many signalling pathways involved in the activated phenotype of T cells, the prominent ones were cell division and cell metabolism. In the $T_{activated}$ state, one of the metabolic pathways, the cholesterol biosynthetic pathway, was significantly upregulated (Figure 5. 8). Strikingly, 81% of all the genes involved in cholesterol biosynthesis were upregulated. Thirteen genes out of a total sixteen genes implicated in the cholesterol pathway were upregulated.

At the same time, these pathway specific changes were dramatically opposite in the $T_{migratory}$ state. Both the cell cycle pathway and the cholesterol biosynthetic pathway were downregulated in T_{spleen} cells compared with T_{blast} cells (Figure 5. 9, Figure 5. 11).

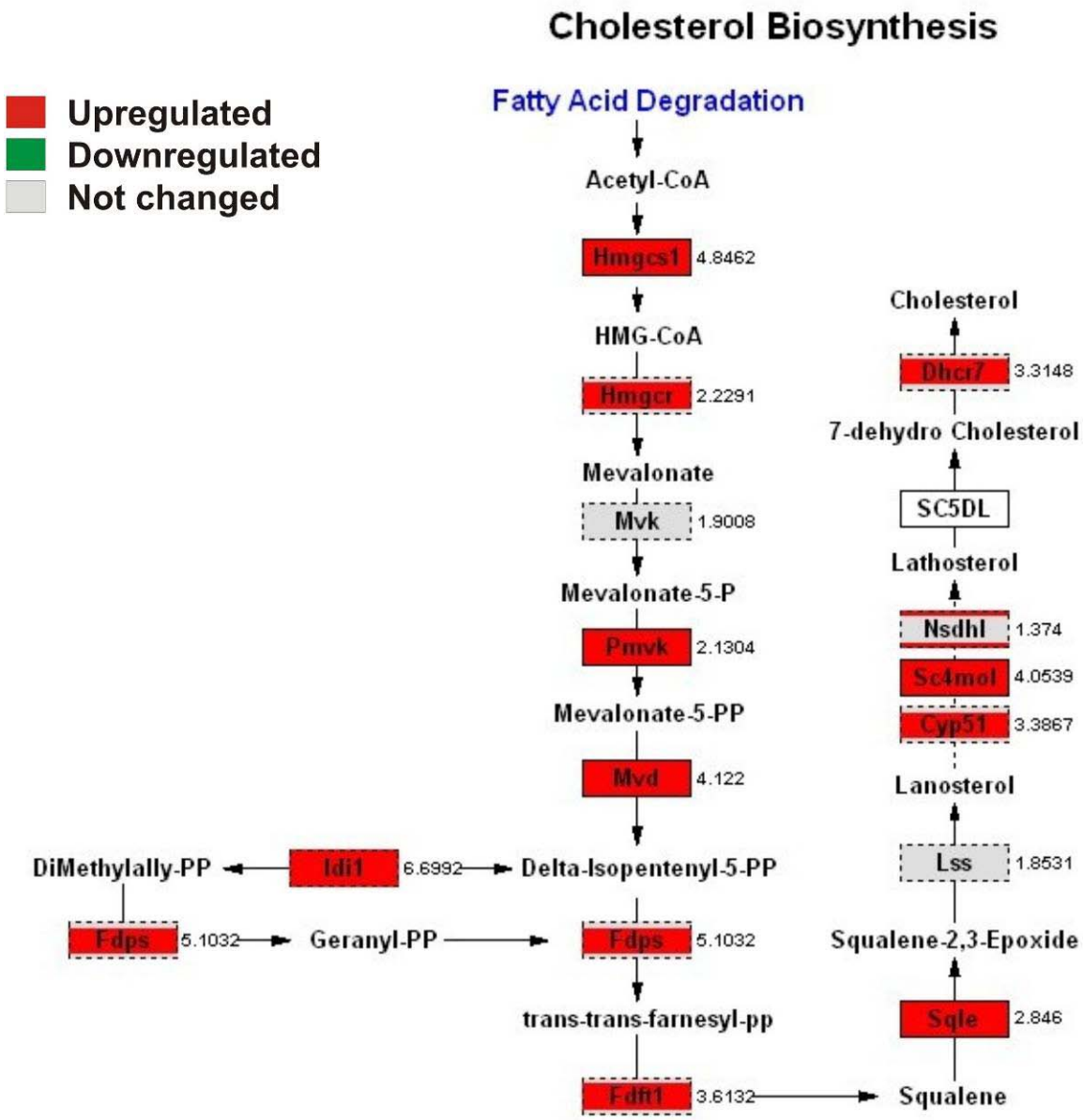

Figure 5. 8 Cholesterol biosynthetic pathway is upregulated in $T_{activated}$ transcriptome.

Overlay of $T_{activated}$ transcriptome dataset on cholesterol biosynthetic pathway using GenMAPP. Fold values of T_{blast} with respect to $T_{resting}$ ($T_{activated}$) is indicated on the right hand side of the gene. Red colour = upregulated; Green colour = downregulated; Grey colour = not changed and No colour = gene not present in microarray.

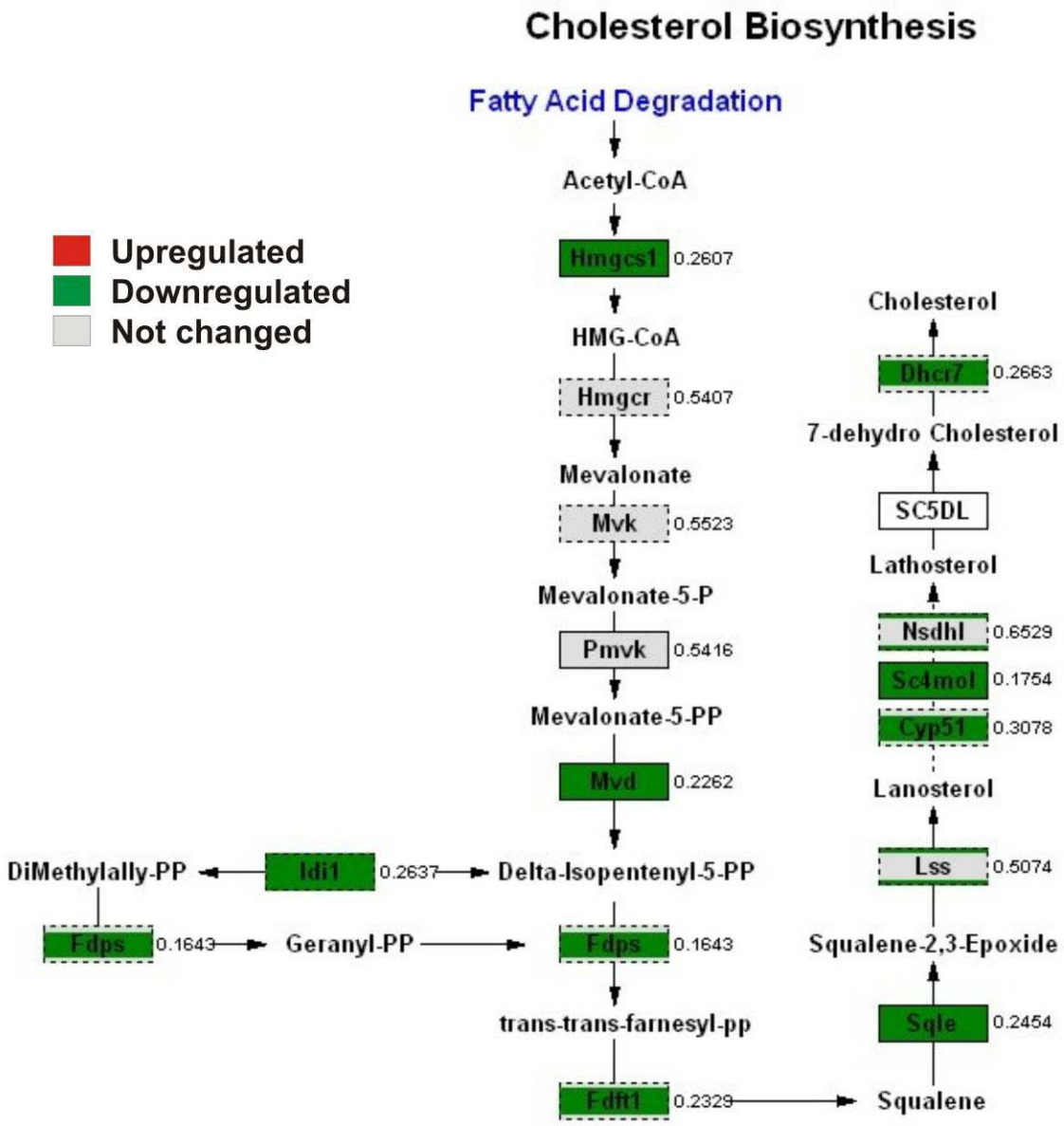

Figure 5. 9 Cholesterol biosynthetic pathway is downregulated in $T_{migratory}$ transcriptome.

Overlay of $T_{migratory}$ transcriptome dataset on cholesterol biosynthetic pathway using GenMAPP. Fold values of T_{spleen} with respect to T_{blast} ($T_{migratory}$) is indicated on the right hand side of the gene. Red colour = upregulated; Green colour = downregulated; Grey colour = not changed and No colour = gene not present in microarray.

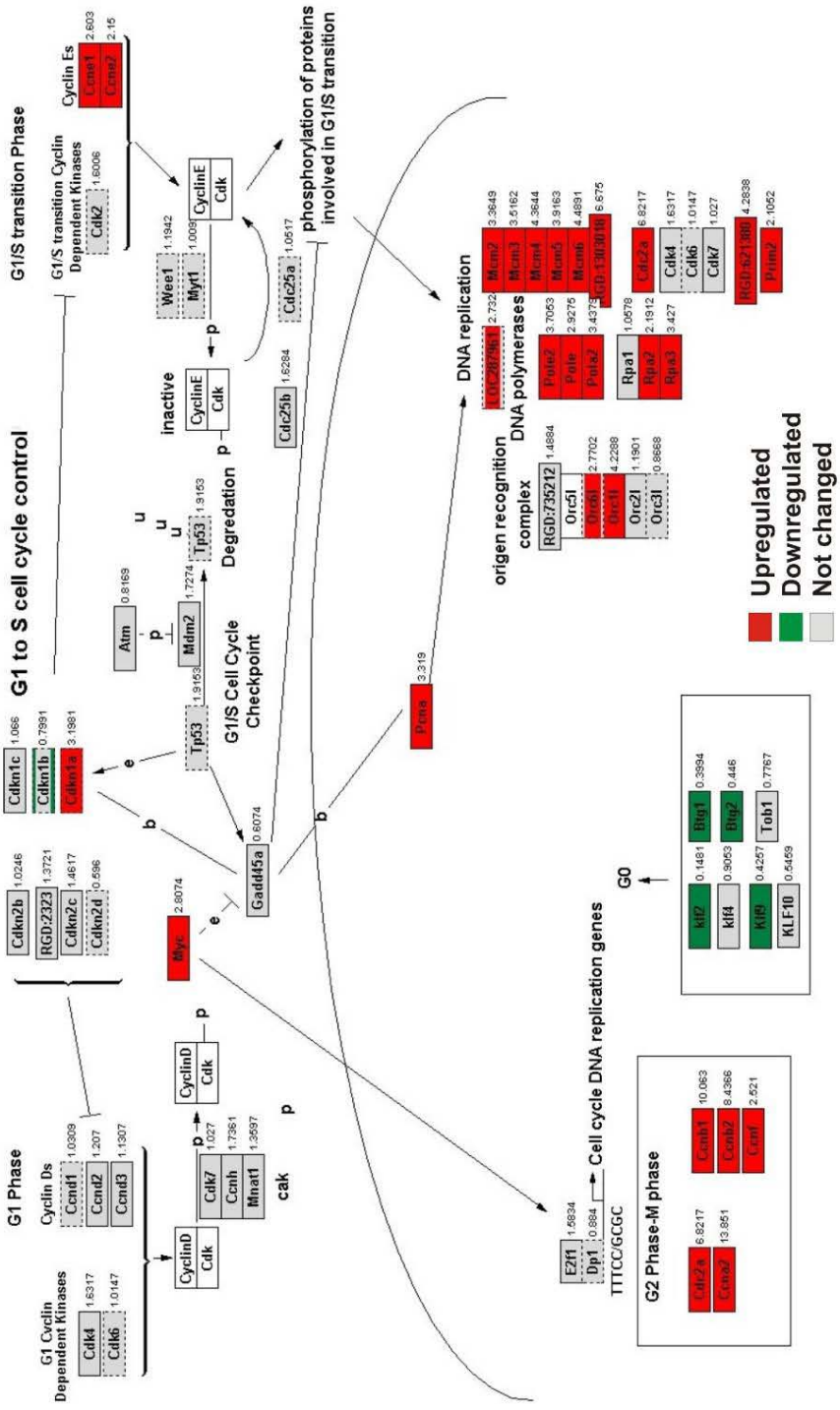

Figure 5. 10 Cell cycle pathway is upregulated in $T_{activated}$ transcriptome.

Overlay of $T_{activated}$ transcriptome dataset on cell cycle pathway using GenMAPP. Fold values of T_{blast} with respect to $T_{resting}$ ($T_{activated}$) are indicated on the right hand side of the gene. Red colour = upregulated; Green colour = downregulated; Grey colour = not changed and No colour = gene not present in microarray.

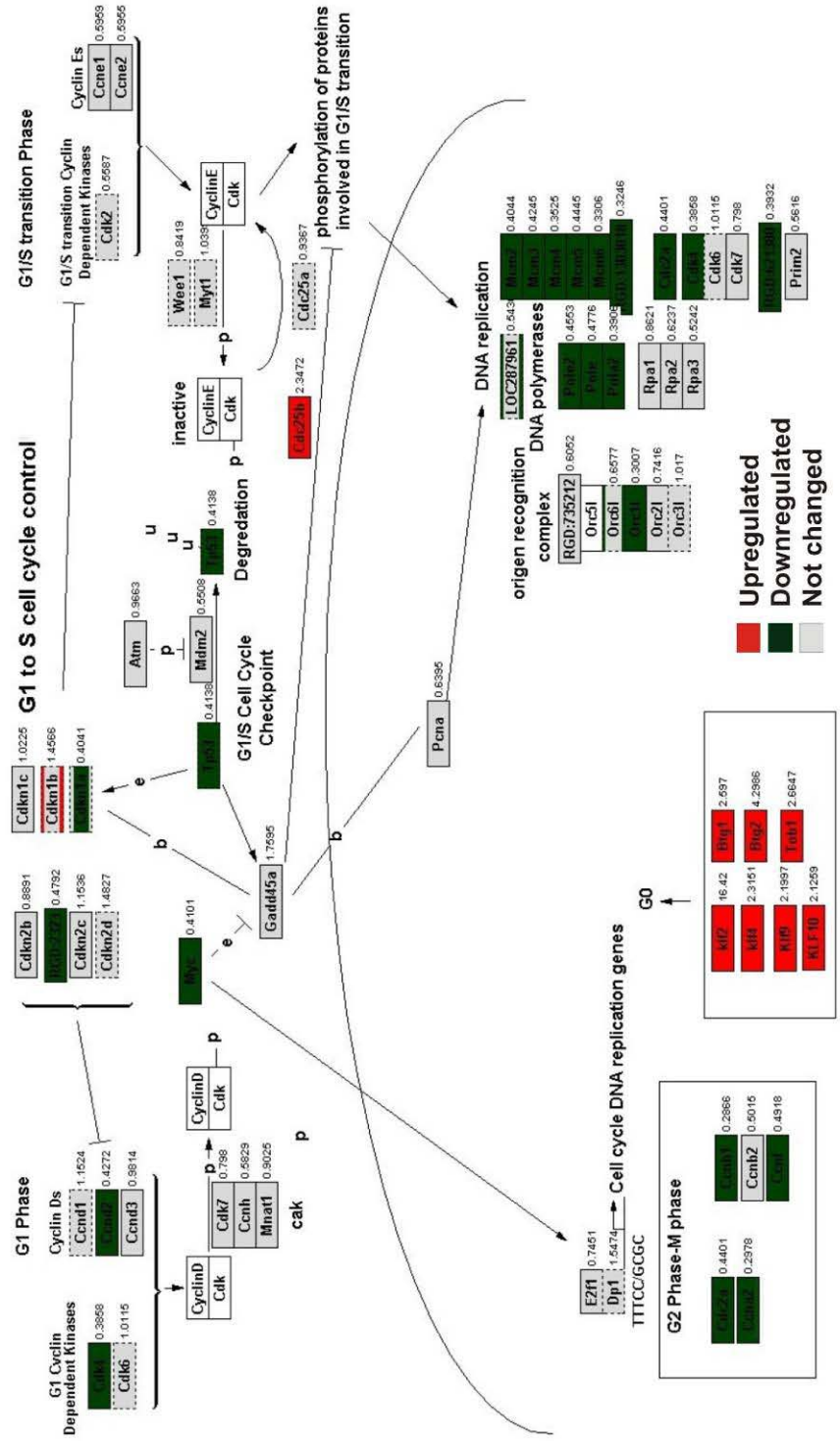

Figure 5. 11 Cell cycle pathway is downregulated in $T_{migratory}$ transcriptome.

Overlay of $T_{migratory}$ transcriptome dataset on cell cycle pathway using GenMAPP. Fold values of T_{spleen} with respect to $T_{resting}$ ($T_{migratory}$) are indicated on the right hand side of the gene. Red colour = upregulated; Green colour = downregulated; Grey colour = not changed and No colour = gene not present in microarray.

	qPCR		Microarray	
GENE	$T_{migratory}$	$T_{effector}$	$T_{migratory}$	$T_{effector}$
Btg1	2.34	1.70	2.60	2.00
Btg2	1.88	1.12	4.30	3.50
Egr1	0.72	7.59	1.79	2.72
Egr2	2.70	45.42	1.61	2.46
Egr3	1.28	11.99	1.69	3.10
Klf4	6.54	20.18	2.32	1.71
Tob1	5.00	13.17	2.66	1.72

Table 5. 6 qPCR and microarray data indicating up regulation of quiescence factors.

This table shows quantitative PCR fold values and microarray fold values for ex-vivo sorted $T_{MBP\text{-}GFP}$ cells from spleen and CNS compared with *in vitro* activated T cell blasts. Values are from an average of three independent measurements.

However, cell quiescence inducing transcription factors such as KLF2, KLF4, KLF9, krüppel-like factor 10 (KLF10), BTG1 and BTG2 crucial for inhibiting cell cycle progression were all upregulated in both the $T_{migratory}$ state and the $T_{effector}$ states This was confirmed by quantitative PCR analyses (Table 5. 6).

A cell migration signalling pathway was generated with the GenMapp program (Figure 5. 11). In this pathway almost all the genes important for the migratory signalling pathway were upregulated in the $T_{migratory}$ state. Genes related to IL2R signalling were decreased. The same held true for genes involved in ERK signalling. However, Tec kinase signalling mediated by T cell-specific non- receptor TXK tyrosine kinases (TXK), which function downstream of integrin receptors and chemokine receptors, was upregulated. The guanine nucleotide Exchange Factors (GEFs) (rho/Rac guanine nucleotide exchange factor 18, ARHGEF18, rho guanine nucleotide exchange factor 3, ARHGEF3) were upregulated. GEFs acting as control switches by stimulating the exchange of GDP for GTP to generate the active form of RhoGTPases are essential for cell migration. Interestingly, the LIM domain containing proteins zyxin (ZYX) and four and a half LIM domains 2 (FHL2) were also upregulated. LIM domain proteins are localized to focal adhesions and nucleus in an

integrin dependent manner to promote cell spreading and migration. Moreover, FHL2 has been reported to inhibit cell proliferation by directly inhibiting mitogen-activated protein kinase 2 (ERK2) (54).

In addition, ZYX, a regulator of actin filament assembly, inhibits mitotic progression by interacting with large tumour suppressor, homolog 1 (LATS1) tumour suppressor (55). Many molecular mediators of cell migration apparently seem to play a role in inhibiting signals for cell proliferation. The well-studied cell cycle progression inhibitor KLF2, expressed in naïve T cells, was recently reported to be involved in controlling cell migration in naïve T cells by directly regulating thymic egress receptor sphingosine-1-phosphate receptor 1 (S1P1) and lymph node homing receptors, SELL and CC-chemokine receptor (CCR7) (56). KLF4, a zinc finger containing transcription factor that inhibits cell proliferation by inhibiting G1/S progression of cell cycle, was upregulated more than 2 fold in T_{spleen} compared to T_{blast}. Moreover, KLF4 expression in $T_{MBP\text{-}GFP}$ cells in different milieus such as spleen, blood and CNS coincide with cell cycle progression as measured by propidium iodide staining, providing functional evidence of cell cycle inhibition in $T_{migratory}$ and $T_{effector}$ state (Figure 5. 13, Figure 5. 15).

These transcriptome analyses reveal a surprisingly reciprocal relationship between cell division and cell migration pathways between the $T_{activation}$ and $T_{migratory}$ states. Hence, the *in-silico* analysis of the microarray data suggests that cell cycle controlling factors play a role in regulating cell migration pathways and vice versa. To consolidate these results transcriptomic data was validated by quantitative PCR analyses and a functional *in vitro* and *in vivo* testing of selected candidate genes was performed.

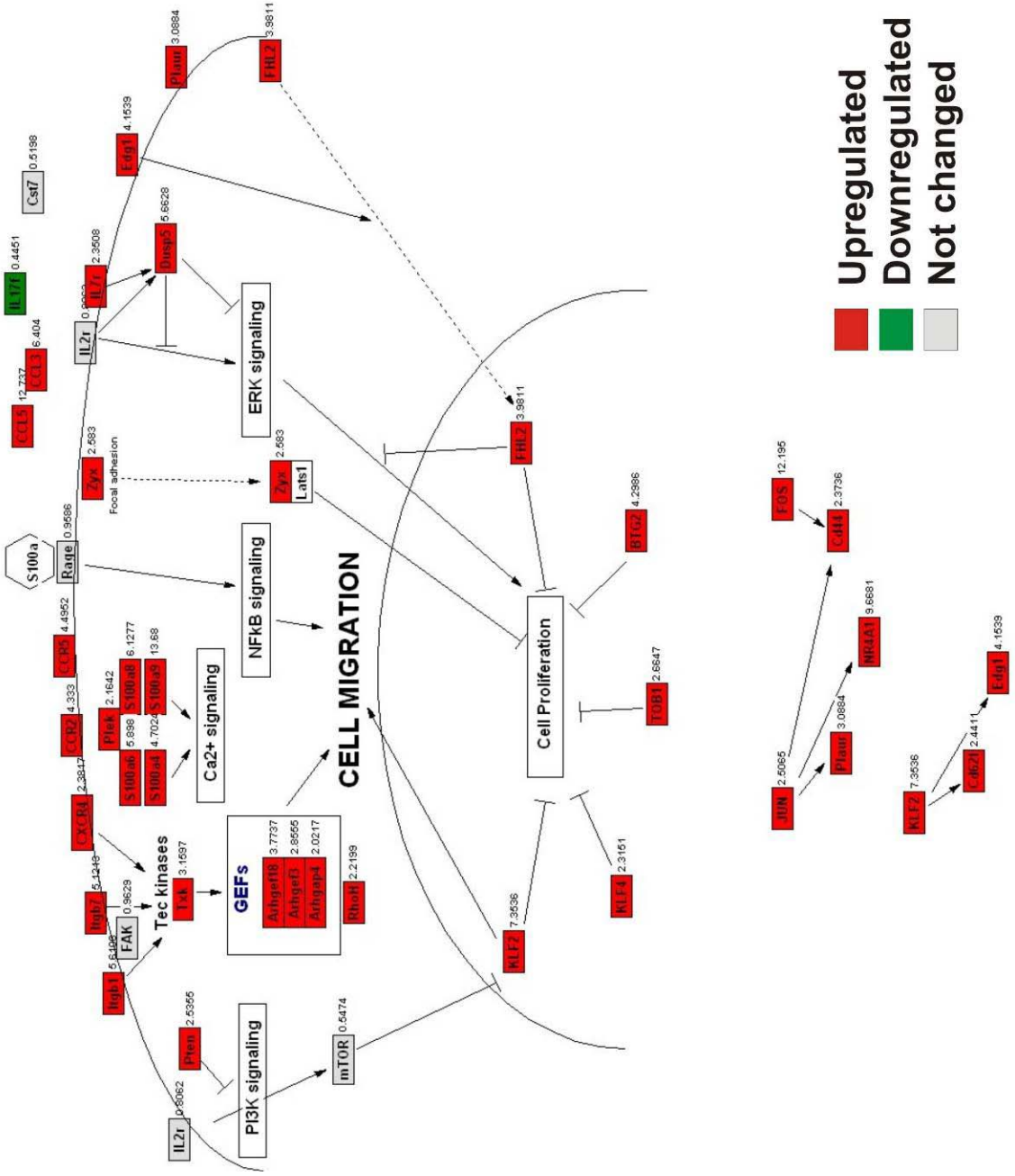

Figure 5. 12 Cell migration pathway is upregulated in $T_{migratory}$ transcriptome.

Overlay of $T_{migratory}$ transcriptome dataset on cell migration pathway generated using GenMAPP. Fold values of T_{spleen} with respect to $T_{resting}$ ($T_{migratory}$) are indicated on the right hand side of the gene. Red colour = upregulated; Green colour = downregulated; Grey colour = not changed and No colour = gene not present in microarray.

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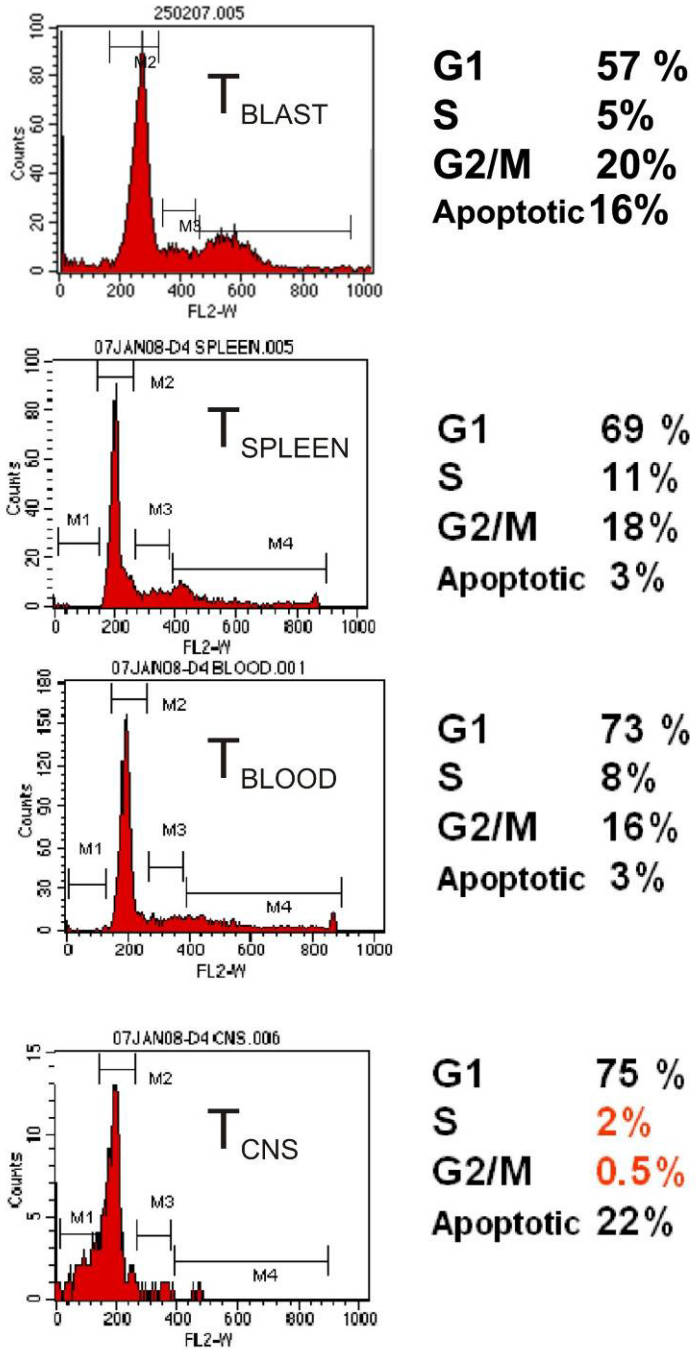

Figure 5. 13 Intracellular propidium iodide staining of DNA for cell cycle analysis by flow cytometry.

In vitro activated T_{blast} cells, and spleen homogenate cells, gradient purified blood leukocytes and gradient purified CNS mononuclear cells from a diseased animal, 3.5 days post AT-EAE induction, were fixed, permeabilized and stained for nuclear DNA using propidium iodide. FACS acquisition was done by gating on GFP positive T cells Percentage of cells in different phases of cell cycle were analysed and shown here. The histograms are representative of three independent experiments.

5.5 Validation of microarray data

Gene name	Gene Description	Primer probe ID	Accession number
ADAM8	A disintegrin and metalloproteinase domain (ADAM) 15 (metargidin)	Rn00571913_m1	BI288110
ADD3	Adducin 3, gamma	Rn00580668_m1	AA894279
ARHGAP 4	Rho GTPase activating protein 4	Rn00595213_m1	BE111827
ASNS	Asparagine synthetase	Rn00565180_m1	U07202
BARD1	BRCA1-associated RING domain protein 1	Rn00575185_m1	NM_022622
BIRC5	Baculoviral IAP repeat-containing 5 (survivin)	Rn00574012_m1	NM_022274
BNIP3	BCL2/adenovirus E1B 19 kDa-interacting protein 3	Rn00821447_g1	NM_053420
BSG	Basigin	Rn00562874_m1	NM_012783
BTG2	B-cell translocation gene 2, anti-proliferative	Custom designed	BI288701
CASP4	Caspase 11	Rn00586960_m1	NM_053736
CCNB1	Cyclin B1	Rn00596848_m1	X64589
CD38	CD38 antigen	Rn00565538_m1	BI289418
CDC25B	Cell division cycle 25B	Rn00592081_m1	NM_133572
CDKN1A	Cyclin-dependent kinase inhibitor 1A	Rn00589996_m1	U24174
DNMT1	DNA (cytosine-5-)-methyltransferase 1	Rn00709664_m1	AI179516
EMP1	Epithelial membrane protein 1	Custom designed	BI275741
FABP5	Fatty acid binding protein 5, epidermal	Rn00821817_g1	U13253
FASLG	Tumour necrosis factor (ligand) superfamily, member 6	Rn00563754_m1	NM_012908
FHL2	Four and a half LIM domains 2	Rn00581565_m1	NM_031677
HDAC3	Histone deacetylase 3	Rn00584926_m1	NM_053448
HMGA1	High mobility group AT-hook 1	Rn00595021_m1	BG378885
HMMR	Hyaluronan mediated motility receptor	Rn00564204_m1	AI171185
IL17F	Interleukin 17F	Custom designed	BI288683
ILF3	Interleukin enhancer binding factor 3	Rn00584682_m1	NM_053412
INSIG1	Insulin induced gene 1	Rn00574380_m1	NM_022392
ITGB1	Integrin beta 1	Rn00566727_m1	NM_017022
JUN	v-jun sarcoma virus 17 oncogene homolog (avian)	Rn00572991_s1	BI288619
KLF4	Krüppel-like factor 4 (gut)	Rn00821506_g1	NM_053713
LGALS1	Lectin, galactose binding, soluble 1	Rn00571505_m1	NM_019904

Gene name	Gene Description	Primer probe ID	Accession number
MIF	Macrophage migration inhibitory factor	Rn00821234_g1	NM_031051
PBEF1	Pre-B-cell colony-enhancing factor	Rn00822046_m1	BM384211
PDCD8	Programmed cell death 8 (apoptosis-inducing factor)	Rn00442540_m1	AF262320
PYCARD	Apoptosis-associated speck-like protein containing a CARD	Rn00597229_g1	BI282953
S100A4	S100 calcium-binding protein A9 (calgranulin B)	Rn00561700_m1	NM_012618
S100A8	S100 calcium-binding protein A9 (calgranulin B)	Rn00587579_g1	NM_012618
S1P1	Sphingosine-1-phosphate receptor 1	Custom designed	BI295971
SLPI	Secretory leukocyte protease inhibitor	Rn00670378_m1	NM_053372
SOD1	Superoxide dismutase 2	Rn00566942_g1	NM_017050
TCP1	T-complex protein 1	Rn00562030_m1	NM_012670
TNFRSF11B	Tumour necrosis factor receptor superfamily, member 11b (osteoprotegerin)	Rn00563499_m1	NM_012870
TNFRSF8	Tumour necrosis factor receptor superfamily, member 8	Rn00569503_m1	NM_019135
TOB1	Transducer of ERBB2, 1	Rn00591220_s1	NM_133317
TP53	Tumour protein p53	Rn00755717_m1	AY009504

Table 5. 7 List of genes selected for microarray data validation by quantitative PCR.

5.5.1 PCR based validation

Quantitative real-time PCR is a commonly used validation tool for confirming gene expression results obtained from microarray analysis. A set of genes was chosen for validation by qPCR, based on their known role in T cell function (Table 5. 7). qPCR was performed on the same source of cDNA that was used for the microarray including *in vitro* activated $T_{MBP\text{-}GFP}$ blast cells and *ex vivo* sorted $T_{MBP\text{-}GFP}$ cells from spleen. Gene expression was normalized with respect to the house keeping gene, β actin as internal control. Comparison of fold values in the $T_{migratory}$ state (T_{spleen} Vs T_{blast}) between microarray and qPCR showed a similar trend in their regulation pattern. However, the magnitude of fold values for corresponding genes differed (Figure 5. 14).

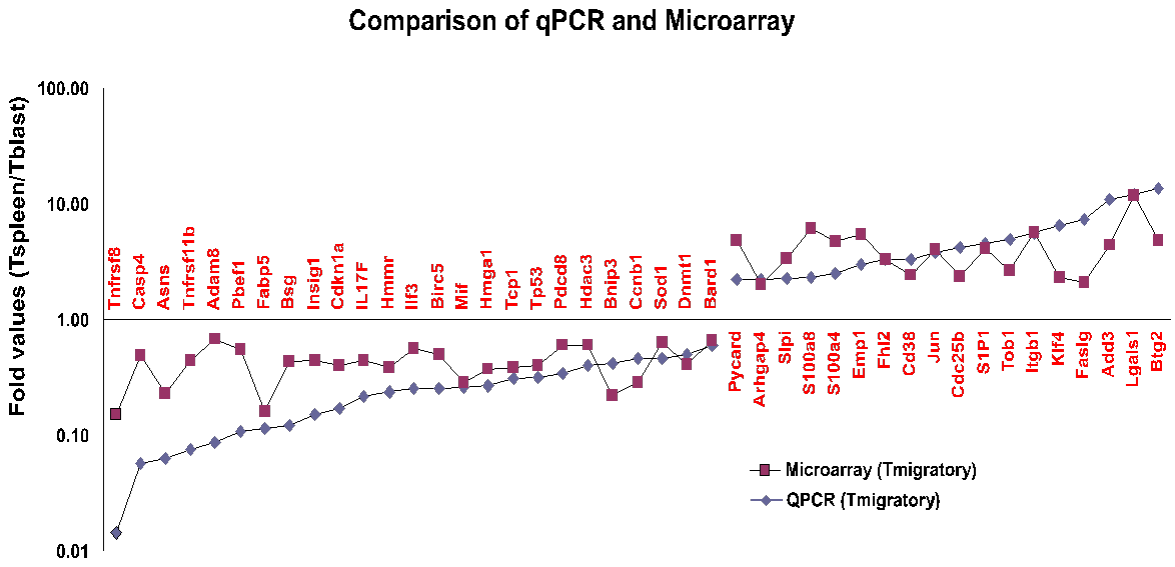

Figure 5. 14 Validation of microarray data by qPCR.

Genes (43 genes) from microarray data were chosen based on their expression in lymphocytes. Gene expression was measured in T_{blast} and T_{spleen} using qPCR. cDNA source was the same as that used for microarray. A comparison of gene expression fold value change in $T_{migratory}$ transcriptome was performed by plotting gene symbols on abscissa and corresponding fold values on ordinate. β actin mRNA expression was used as an internal standard.

5.5.2 Antibody based validation

Earlier studies have already shown via flow cytometry an upregulation of chemokine receptors and a downregulation of activation markers in T_{spleen} on the protein level (44). To consolidate the microarray regulation independently, KLF4 was chosen for antibody-based validation. KLF4 was downregulated in activated T cells (T_{blast}) *in vitro* when compared with *ex vivo* sorted T cells from spleen (T_{spleen}) and CNS (T_{CNS}) (Figure 5. 14).

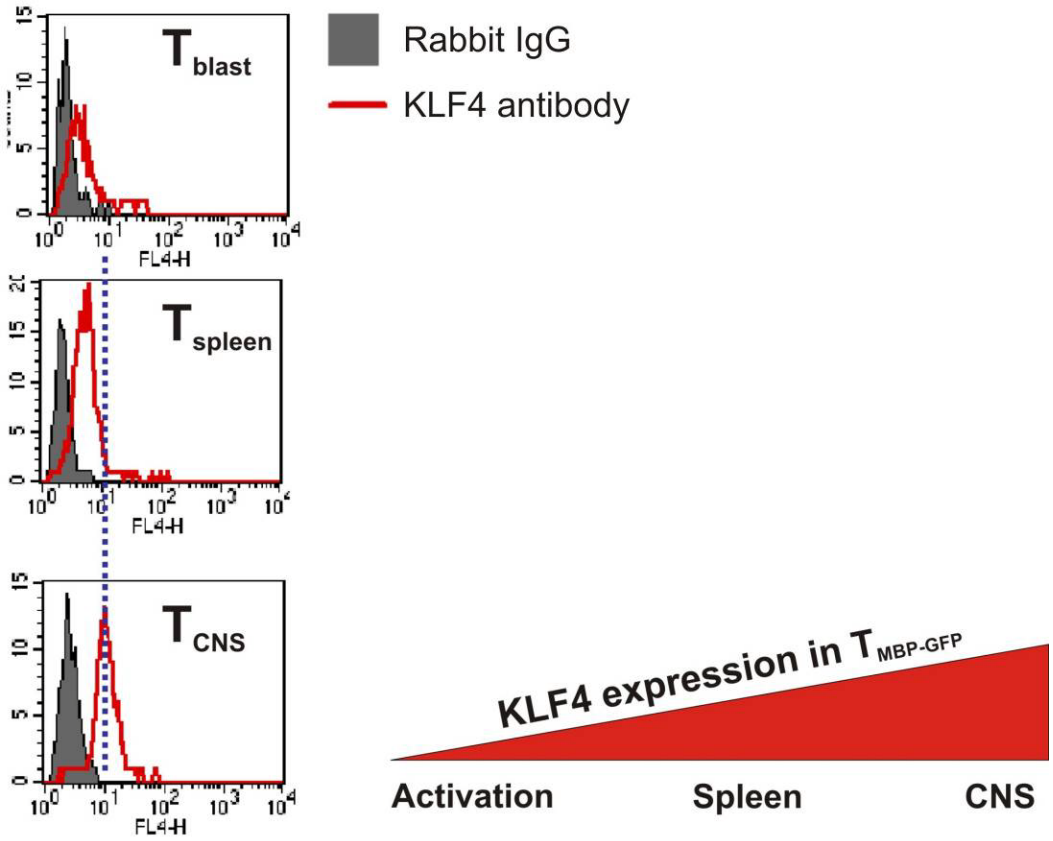

Figure 5. 15 Differential regulation of KLF4 *in vivo.*

Intracellular staining for KLF4 expression by flow cytometry. *In vitro* activated T_{blast} cells, and spleen homogenate cells, gradient purified blood leukocytes and gradient purified CNS mononuclear cells from a diseased animal, 3.5 days post AT-EAE induction, were fixed, permeabilized and stained for KLF4. FACS acquisition was done by gating on GFP positive T cells Overlay of KLF4-specific antibody staining with isotype control staining is shown. (Grey = Isotype control; Red = KLF4 antibody) Shown is a representative histogram of three independent experiments. Illustration depicts increasing amounts of KLF4 expression post activation in spleen and CNS.

5.6 Bioinformatics prediction of KLF4 as a common transcriptional regulator for inflammatory chemokine receptors

The following, describes the results of the study examining the role of KLF4 as potential master regulator that drives the transcriptomic transition from activated T cell state to migratory T cell phenotype. To achieve this aim, Matinspector™ was used, a bioinformatics program that identifies potential transcription factor binding sites (TFBS) in nucleotide sequences (58). The results from this analysis identified KLF4 as a common transcription factor binding to a region within 1000 base pairs upstream of transcription start site (TSS) of CCR1, CCR2, CCR3 and CCR5 but not CXCR3 (Table 5.9). Inflammatory chemokine receptors belonging to the CC-chemokine receptor subfamily showed up in our microarray cluster analysis Figure 5. 6). CCR1, CCR2 and CCR5 were found to be upregulated both at mRNA and protein level in $T_{migratory}$ cells as well as in $T_{effector}$ cells (Table 5. 8). Upregulation of these receptors at protein level has been shown previously (Figure 5. 16) (57). The receptors were also clustered in chromosome 8 of rat genome indicating a potential co-regulatory mechanism (Figure 5. 17).

Gene Symbol	**Microarray fold values**		**qPCR fold values**	
	$T_{activated}$	$T_{migratory}$	$T_{activated}$	$T_{migratory}$
CCR1	1.05	1.60	n.d	n.d
CCR2	0.38	4.33	0.21	4.46
CCR5	0.14	4.50	0.13	1.42

Table 5. 8 Inflammatory chemokine receptors' transcripts are upregulated in $T_{migratory}$ transcriptome.

Comparison of microarray and qPCR data for inflammatory chemokine receptors in the $T_{activated}$ and $T_{migratory}$ states. qPCR and microarray data showing up regulation of CCR1, CCR2 and CCR5. This table shows microarray gene expression fold values and qPCR gene expression fold values of $T_{activated}$ and $T_{migratory}$ transcriptome. cDNA was synthesized from in vitro activated T cells blasts (T_{blast}) and ex-vivo sorted green fluorescent T cells from spleen (T_{spleen}) and CNS (T_{CNS}) β actin mRNA expression was used as an internal standard. Values are from an average of three independent measurements. (n.d not determined)

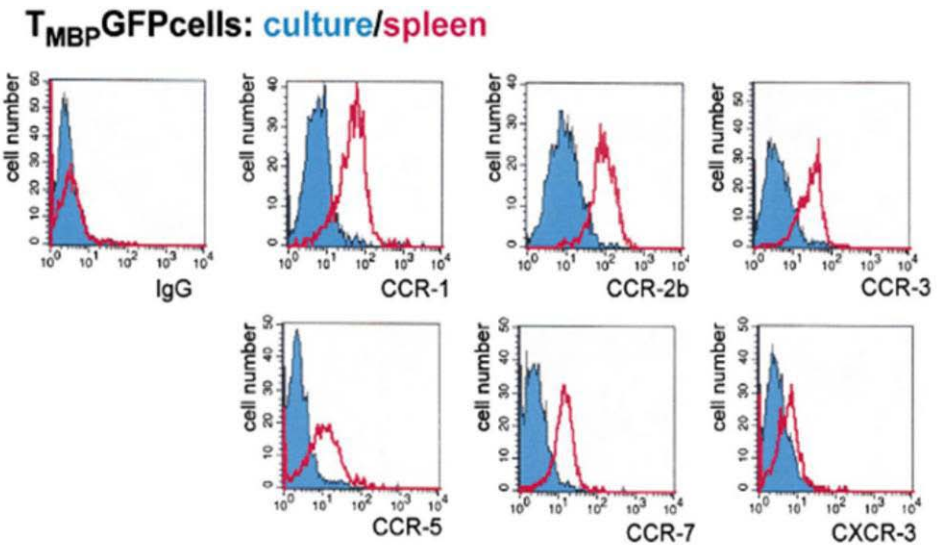

Figure 5. 16 Inflammatory chemokine receptors are upregulated in T_{spleen}.

Intracellular staining for chemokine receptor expression by flow cytometry. Comparsion of chemokine receptor expression between in vitro cultured $T_{MBP\text{-}GFP}$ cells (blue histogram) and T_{spleen} cells (pink histogram) from spleen of AT-EAE rat 84 hrs post adoptive transfer of $T_{MBP\text{-}GFP}$. (Data adapted from Flügel et al (44).

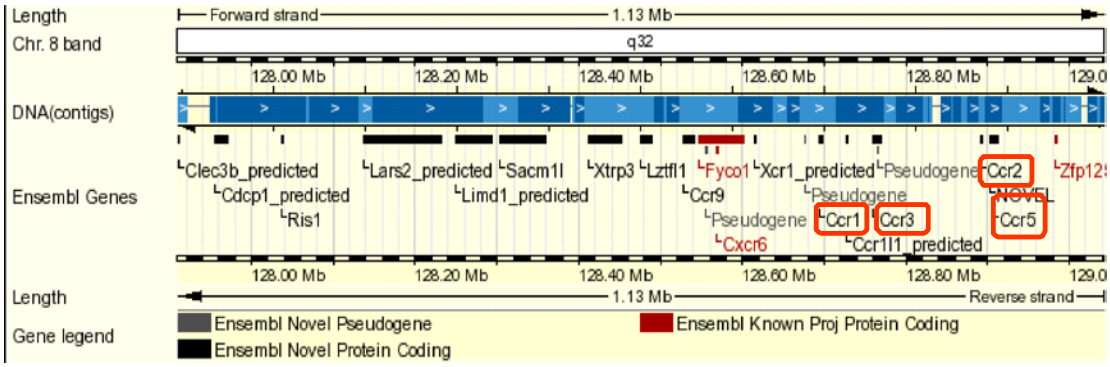

Figure 5. 17 Inflammatory chemokine receptors are clustered in the genome.

Ensemble genome browser image for rat chromosome 8. Inflammatory chemokine receptors CCR1, CCR2, CCR3 and CCR5 are depicted and boxed in red.

Chemokine receptor	Predicted KLF4 binding site	Position (from-to) upstream of TSS
CCR1	aagaagagaAGGG	652 – 664
CCR2	gtcAAAGgcactt	249 – 261
CCR3	cagaaagggAGGG	834 – 846
CCR4	aaaaaaaaaAGGG	571 – 583
CCR5	gaagaccaaAGGG accAAAGggtctc attAAAGgtatta	46 - 58 50 - 62 718 – 730
CXCR3	None	-

Table 5. 9 KLF4 binding motifs are present in inflammatory chemokine receptors.

KLF4 binding motifs upstream of TSS in chemokine receptors, obtained using Matinspector™ is shown. The position of the binding motif from TSS in chemokine receptors is also shown. Base pairs marked in red appear in a position where the transcription factor matrix exhibits a high conservation profile. Base pairs in capital letters denote the core sequences used by Matinspector™.

5.7 Overexpression of KLF4 induces cell cycle arrest and upregulates CCR2 and CCR5

KLF4 is a zinc finger-containing transcription factor which is known to negatively influence the progression of G1/S phase of the cell cycle (59,60). In order to investigate the role of cell cycle inhibition by KLF4 in chemokine receptor regulation, KLF4 was overexpressed in T cells by retroviral-mediated transduction of antigen specific primary T cells. Overexpression was assessed by performing qPCR using standardized qPCR primers for KLF4 (Figure 5. 18). Overexpression of KLF4 full length cDNA in $T_{MBP\text{-}GFP}$ cells resulted in a strong inhibition of G1/S cell cycle progression from 58% to 85% in G1 phase confirming overexpression of functional KLF4 protein and its ascribed function (59) (Figure 5. 19).

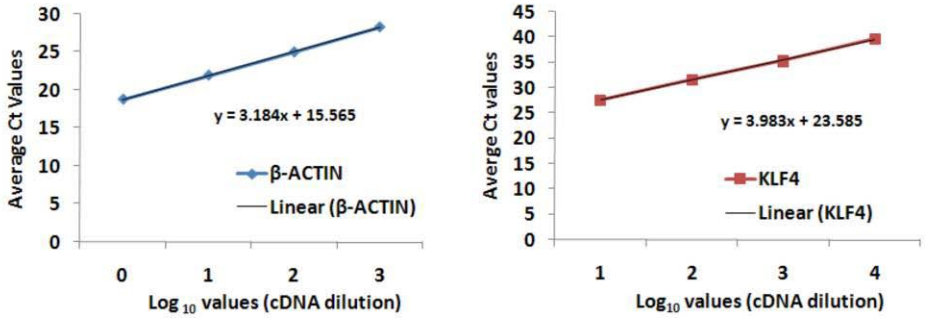

Figure 5. 18 Standardization of KLF4 qPCR primers.

Standard dilution curves were plotted using cDNA prepared from resting T lymphocytes. Average Ct values are plotted on ordinate and cDNA dilutions are plotted on abscissa. A standard curve slope of 3.32 indicates a PCR reaction with 100% efficiency.

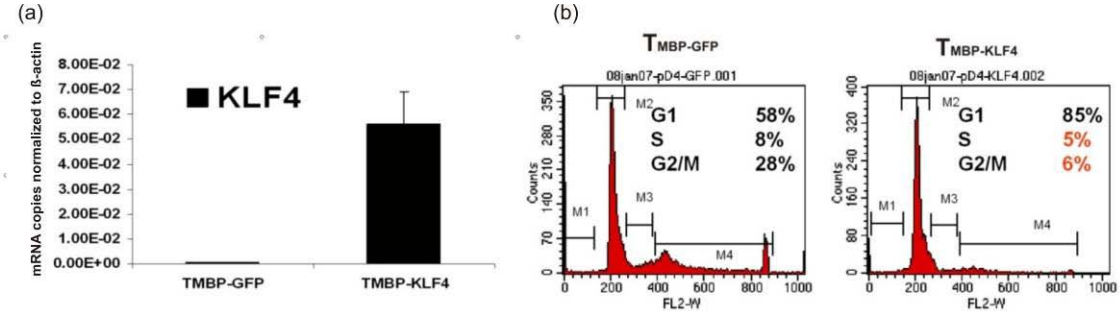

Figure 5. 19 Overexpression of KLF4 inhibits cell cycle progression in MBP-specific T cells.

(a) Quantitative PCR on *in vitro* cultured KLF4-transduced $T_{MBP\text{-}GFP}$ cells compared to control $T_{MBP\text{-}GFP}$ cells four days post retroviral transduction. (b) Propidium iodide staining for cell cycle analysis of $T_{MBP\text{-}GFP}$ and $T_{MBP\text{-}KLF4}$.

Moreover, qPCR performed on RNA isolated from $T_{MBP\text{-}GFP}$ cells overexpressing KLF4 (Figure 5. 19a) indicated upregulation of chemokine receptors CCR2 (~20 fold) and CCR5 (~5 fold), downregulation of, IL2R (~4 fold) but not CXCR3 (Figure 5. 20). Earlier studies reported on upregulation of CCR2 upon IL2 deprivation (61).

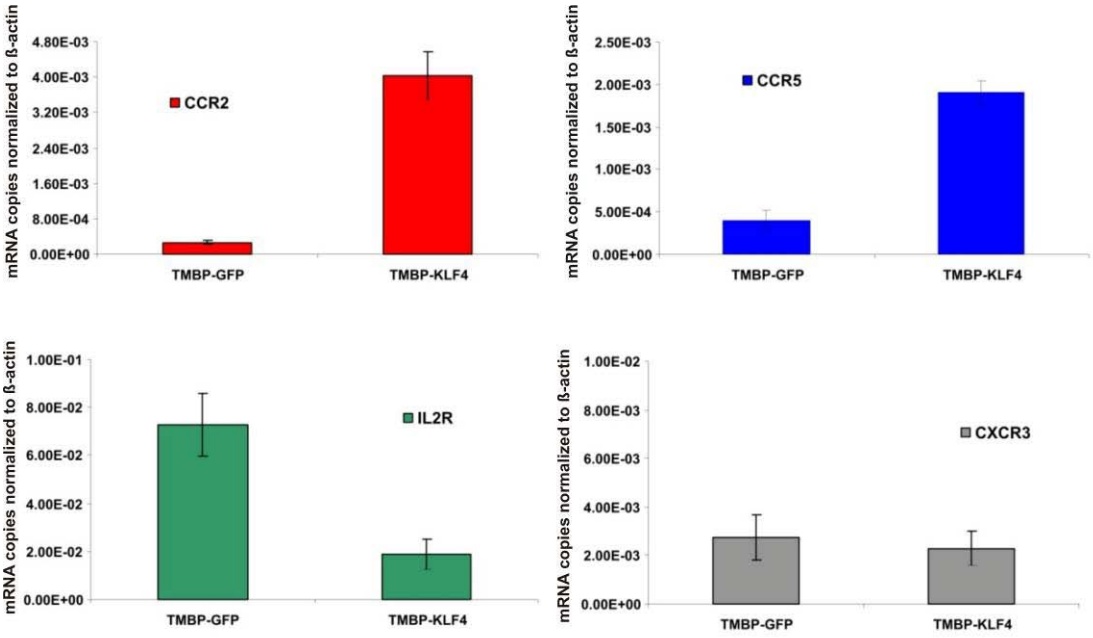

Figure 5. 20 Overexpression of KLF4 upregulates CCR2, CCR5 but not CXCR3 in MBP specific T cells.

Quantitative PCR of KLF4 transduced in vitro cultured $T_{MBP\text{-}GFP}$. $T_{MBP\text{-}GFP}$ cells were retrovirally transduced with full length rat KLF4 cDNA and four days post transduction green fluorescent $CD4^+$ T cells were FACS sorted along with GFP control T cells. Gene expression of chemokine receptors CCR2 (red), CCR5 (blue), IL2R (green) and CXCR3 (grey) was measured using quantitative PCR normalized to β actin as an internal standard. Data shown here is representative of three independent experiments.

Regulation of CCR2 and CCR5 expression by KLF4 was confirmed using different cell lines. Overexpression of KLF4 in OVA-specific T cells, exhibited upregulation of CCR2 and CCR5 both in activated and resting conditions (Table 5. 10). RBL1 (Rat basophilic leukaemia 1) is a histamine releasing cell line commonly used in inflammation, allergy and immunological research. KLF4 overexpression in RBL1 induced at least 2 fold upregulation of CCR2 and CCR5 mRNA compared to control transduced RBL1 cell line (Figure 5. 21).

	$T_{OVA-GFP}$ (ACTIVATED)	$T_{OVA-KLF4}$ (ACTIVATED)	FOLD VALUES ($T_{OVA-KLF4}/T_{OVA-GFP}$)
CCR2	1.15E-03	4.63E-03	4.03
CCR5	1.44E-03	2.98E-03	2.07
	$T_{OVA-GFP}$ (RESTING)	$T_{OVA-KLF4}$ (RESTING)	FOLD VALUES ($T_{OVA-KLF4}/T_{OVA-GFP}$)
CCR2	2.65E-03	5.37E-03	2.03
CCR5	1.32E-03	3.70E-03	2.80

Table 5. 10 Overexpression of KLF4 upregulates CCR2 and CCR5 in ovalbumin-specific T cells.

Quantitative PCR of *in vitro* cultured KLF4-transduced ovalbumin-specific T cells (TOVA-GFP). TOVA-GFP cells were retrovirally transduced with full length rat KLF4 cDNA and green fluorescent CD4+ T cells were FACS-sorted 2 and 6 days post transduction along with GFP control T cells. Gene expression of chemokine receptors CCR2 and CCR5 was measured using quantitative PCR normalized to β actin as an internal standard. Data is representative of three independent experiments.

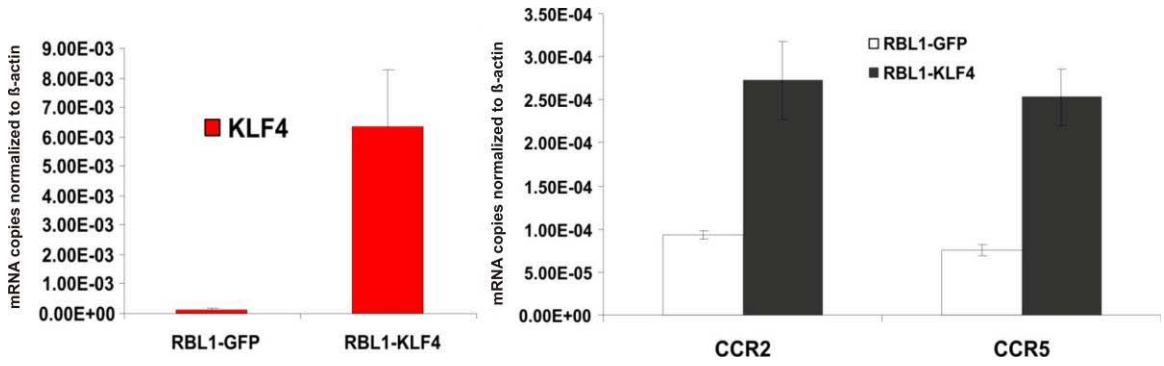

Figure 5. 21 Overexpression of KLF4 upregulates CCR2 and CCR5 in RBL1 cell lines.

Quantitative PCR of *in vitro* cultured KLF4-transduced RBL1 cell line**.** RBL1 cell lines were retrovirally transduced with full length rat KLF4 cDNA and green fluorescent RBL1 cell lines were FACS sorted along with GFP control transduced RBL1 cells. Gene expression levels for KLF4 (red) and chemokine receptors, CCR2 and CCR5 (black) were determined in KLF4- and GFP-transduced RBL1 cells. β actin mRNA expression served as internal standard for normalization. Data obtained were from averages of triplicate measurements.

5.8 Transcriptional activity of KLF4 on CCR2 and CCR5 promoters

In order to further corroborate the role of KLF4 in regulating the expression of inflammatory chemokine receptors, a luciferase assay was performed using reporter constructs containing promoter regions of genes encoding the chemokine receptors CCR2, CCR5 and CXCR3. Promoter regions comprised of 1000 base pairs upstream and 100bp downstream of the transcription start site (TSS). Matinspector™ prediction results indicated a KLF4 binding region in the TSS upstream of CCR2, CCR5 but not CXCR3 (Table 5. 9).

Co-transfection assays with KLF4 and reporter constructs in cultured 293T cells indicated upregulation of both CCR2 and CCR5 promoter activities by at least 2 fold (Figure 5. 22) whereas CXCR3 did not show an increase in promoter activity compared to pGL3 empty reporter transfections. Taken together these results suggest KLF4 inhibits cell cycle progression and regulates migratory molecules such as inflammatory chemokine receptors CCR2 and CCR5. The results also indicate that KLF4 might be one regulator gene which coordinates the transition from activated to migratory state of effector T cells by tuning negatively the proliferative genes and positively, the migratory genes.

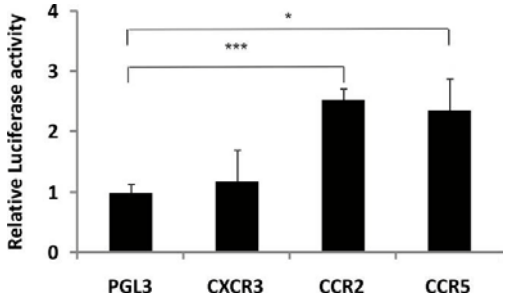

Figure 5. 22 Dual luciferase assay for CCR2, CCR5 and CXCR3 promoter activity.

Luciferase assay was performed after co-transfection of the pMSCVneo vector or of pMSCVneo-KLF4 with PGL3 reporter constructs such as PGL3, PGL3-CXCR3, PGL3-CCR2 and PGL3-CCR5 in 293T cells. Luciferase activity was measured 48 hours after transfection. Luciferase activity is presented relative to that of pMSCVneo vector control after normalization with renilla luciferin. Student's t-Test p value, * denotes $p < 0.5$, ** denotes $p < 0.01$ and *** denotes $p < 0.001$. Date shown is a representative of three independent experiments and measurement was done in triplicates.

5.9 Identification of differentially regulated membrane molecules as potential candidate genes

Genes that are expressed on the T cell surface and upregulated in $T_{migratory}$ state (T_{spleen} vs T_{blast}) can serve as potential candidate genes for therapeutic intervention in AT-EAE models. Retransfer experiments, wherein T_{spleen} cells three days post adoptive transfer were injected into naïve syngeneic rats, clearly demonstrated the early infiltrating capacity of migratory T_{spleen} cells to CNS within 24 hours (Figure 5. 23) (44). Transmembrane molecules that are differentially regulated in T_{spleen} cells, can thus be regarded collectively as imparting the passport to T cells to infiltrate the CNS.

Membrane proteins can be of several types based on their association with their lipid bilayer, such as transmembrane protein, cytosolic membrane protein, external membrane protein and peripheral membrane protein. However, based on their topology, UniProt defines the following classification for transmembrane proteins (62).

- Type I: Protein spanning the membrane once, with its N-terminus on the extracellular side of the membrane and removal of its signal sequence.
- Type II: Protein spanning the membrane once, with its N-terminus on the cytoplasmic side of the membrane. The transmembrane domain is located close to the N-terminus and it functions as an anchor.
- Type III: Protein spanning the membrane once, with its N-terminus on the extracellular side of the membrane and no signal sequence.
- Type IV: Protein spanning the membrane once, with its N-terminus on the cytoplasmic side of the membrane. The transmembrane domain is located close to the C-terminus and it functions as an anchor.
- Multipass: Protein spanning the membrane more than once.
- GPI-anchored: Protein bound to the lipid bilayer of a membrane through a GPI-anchor (glycosylphosphatidylinositol anchor), a complex oligoglycan linked to a phosphatidylinositol group, resulting in the attachment of the C-terminus of the protein to the membrane.

Microarray data analysis revealed several plasma membrane proteins that were differentially regulated in T_{spleen} compared to T_{blast} (Table 5. 11), including ITGB1, S1P1, CCR2 and CC-chemokine receptor 6 (CCR6) (Table 5. 12).

Drug target molecules	Drugs in development	Companies
α4β1	Natalizumab	Biogen-Idec
EDG1 (S1P1)	FTY-720	Novartis
CCR2	MK0812	ChemoCentryx
CCR6	mAb, small molecule	G2 Therapies

Table 5. 11 List of cell membrane receptors which are established drug targets for multiple sclerosis.

Gene symbol	**T_{spleen} vs T_{blast}**	**Molecular function**	**Transmembrane protein type**
BSG	0.4300	Receptor activity	TYPE I
CCR2	4.3330	G-protein coupled receptor activity	MUTI-PASS
CCR5	4.4952	G-protein coupled receptor activity	MUTI-PASS
CCR6	2.9268	G-protein coupled receptor activity	MUTI-PASS
CD200	0.2836	Immunoglobulin receptor activity	TYPE I
CD38	3.0527	Hydrolase activity	TYPE II
CD44	2.4723	Receptor activity	TYPE I
CD69	7.7670	Receptor activity	TYPE II
CD83	1.9859	Molecular function unknown	TYPE I
CD97	2.4615	Receptor activity	MULTI-PASS
CSF1R	2.0229	Transmembrane receptor activity	TYPE I
CXCR4	2.9185	G-protein coupled receptor activity	MUTI-PASS

EDG1	4.1539	G-protein coupled receptor activity	MUTI-PASS
EMP1	5.2180	Cell adhesion molecule activity	MULTI-PASS
FASLG	2.1241	Receptor binding	TYPE II
FXYD5	3.0887	Cell adhesion molecule activity	TYPE I
GABBR1	2.2494	G-protein coupled receptor activity	MUTI-PASS
GLIPR1	3.9142	Molecular function unknown	TYPE I
GPC1	0.4674	Receptor activity	GPI-ANCHORED
GPNMB	2.8633	Molecular function unknown	TYPE I
HCST	2.9049	Receptor signalling complex scaffold activity	TYPE I
IFNGR1	2.5023	Transmembrane receptor activity	TYPE I
IGSF6	2.0765	Antigen binding	TYPE I
ITGB1	5.6106	Receptor activity	TYPE I
ITGB7	5.1213	Receptor activity	TYPE I
JAM3	0.4815	Cell adhesion molecule activity	TYPE I
KDR	0.2806	Transmembrane receptor protein tyrosine kinase activity	TYPE I
KLRD1	8.0694	Receptor activity	TYPE II
LTB	2.1241	Cytokine activity	TYPE II
NRP1	2.4536	Receptor activity	TYPE I
PLAUR	3.0884	Receptor activity	GPI-ANCHORED
RAMP1	2.0671	Transporter activity	TYPE I
RAMP2	2.8365	Transporter activity	TYPE I
SCARB1	0.3199	Receptor activity	TYPE I
SELL	2.4411	Cell adhesion molecule activity	TYPE I
SORL1	2.2598	Transmembrane receptor activity	TYPE I
TFRC	0.2604	Auxiliary transport protein activity	TYPE II

TNFRSF1B	3.1115	Receptor activity	TYPE I
TNFRSF4	0.3819	Receptor activity	TYPE I
TNFRSF8	0.1494	Receptor activity	TYPE I
TYROBP	2.6851	Receptor activity	TYPE I
VAMP3	2.5110	Protein binding	TYPE IV

Table 5. 12 List of cell membrane molecules differentially regulated in T_{spleen} cells compared to T_{blast} cells as indicated by microarray data.

5.10 EMP1 as a novel candidate gene

As a potential effector molecule regulating autoaggressive T cell motility the multipass integral membrane protein, EMP1 or tumour-associated membrane protein (TMP), was tested. EMP1 belongs to the PMP22/EMP/MP20/Claudin superfamily of tetraspan membrane proteins and to a gene family consisting of EMP1, EMP2, EMP3 and peripheral myelin protein 22 (PMP22).

5.11 Bioinformatics based structural features of EMP1

A computer based algorithm called SOSUI, was used to determine the hydropathy plots of EMP1 protein sequence. EMP1 was found to have four transmembrane helices with their amino- and carboxy-terminal tails extending into the cytoplasm (Table 5. 13)-.

Number	N-Terminal	Transmembrane Region	C-Terminal	Type	Length
1	5	LAGLFVVHIATAIMLFVSTIANV	27	PRIMARY	23
2	64	VQAFMILSIIFSIISLVVFVFQL	86	PRIMARY	23
3	96	FLSGSTMLVCWLCILIGVSIYT	117	PRIMARY	22
4	135	YCFILTWICFCFSFIIGILYMVL	157	PRIMARY	23

Table 5. 13 Prediction for transmembrane helix regions in EMP1 amino acid sequence.

Prediction was performed using SOSUI, a WWW-based tool to predict transmembrane helices in a given amino acid sequence (66). Rat EMP1 protein sequence from NCBI (NP_036975.1) was used as input to determine transmembrane regions.

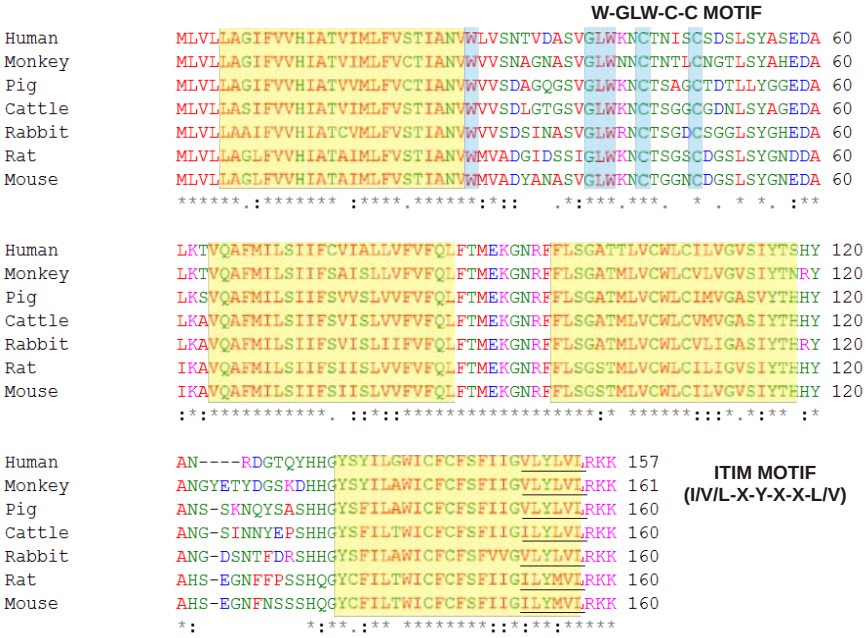

Figure 5. 23 ClustalW multiple sequence alignment of mammalian EMP1 protein sequences.

Transmembrane motifs are highlighted in yellow. Conserved W-GLW-CC motif within the first extracellular loop is highlighted in turquoise blue. Small and hydrophobic including aromatic amino acids (red); Acidic amino acids (blue); Basic amino acids (magenta), hydroxyl, basic, amine and glutamine (green) Predicted Immunoreceptor Tyrosin-based Inhibitory Motif in the C-terminus of EMP1 is underlined. "*" denotes residues in that column are identical in all sequences in the alignment. ":" denotes conserved substitutions. "." denotes semi-conserved substitutions. Predicted Immunoreceptor Tyrosine-based Inhibitory Motif in the C-terminus of EMP1 is underlined.

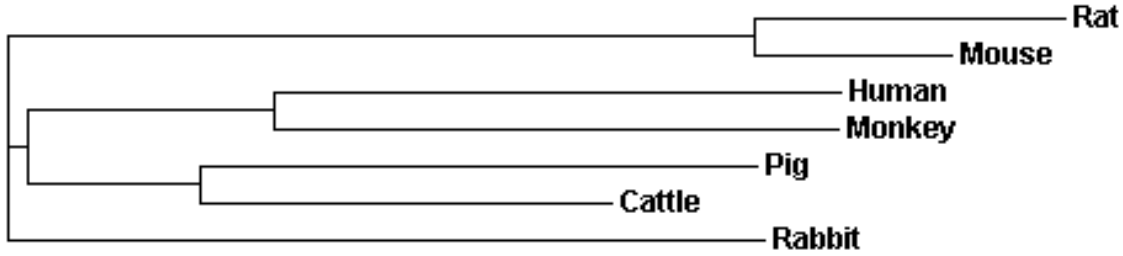

Figure 5. 24 Phylogram tree for mammalian EMP1 are related.

Phylogenetic relationship among mammalian EMP1 protein sequences. Rat and Mouse EMP1 are closely related.

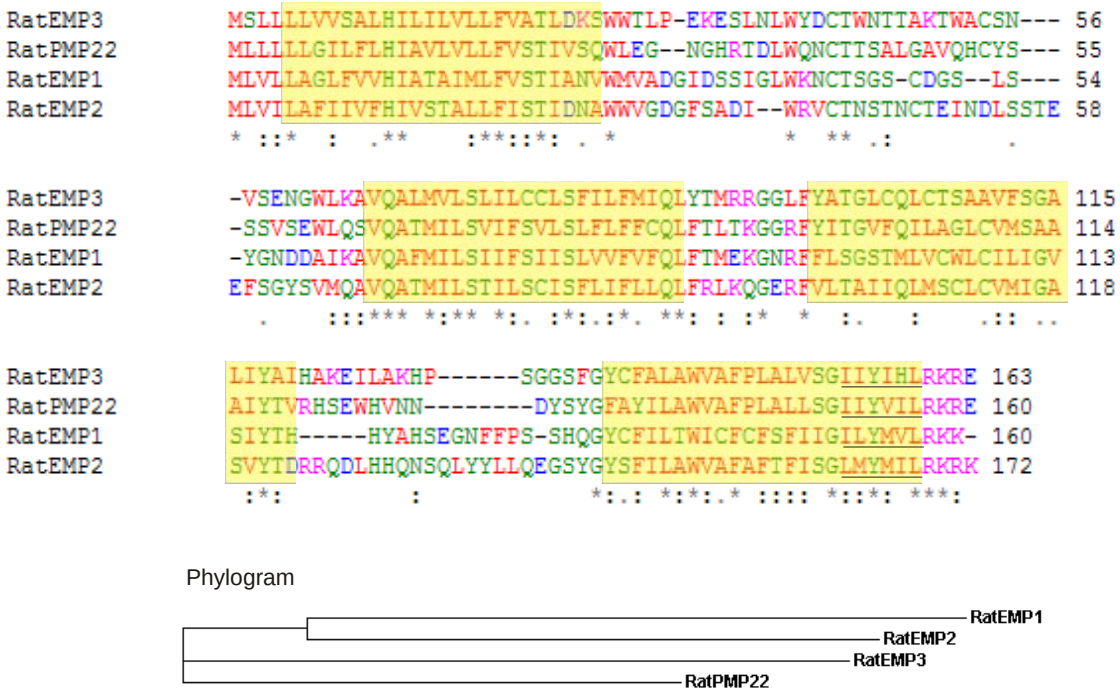

Figure 5. 25 ClustalW multiple sequence alignment of rat EMP1 family amino acid sequences.

Multiple sequence alignment of EMP1, EMP2, EMP3 and PMP22 protein sequences performed using ClustalW algorithm. Transmembrane motifs are highlighted in yellow. Small and hydrophobic including aromatic amino acids (red); Acidic amino acids (blue); Basic amino acids (magenta), hydroxyl, basic, amine and glutamine (green) Predicted Immunoreceptor Tyrosin-based Inhibitory Motif in the C-terminus of EMP1 is underlined. "*" denotes residues in that column are identical in all sequences in the alignment. ":" denotes conserved substitutions. "." denotes semi-conserved substitutions. The phylogeny tree illustrates the close relationship of EMP1 to EMP2.

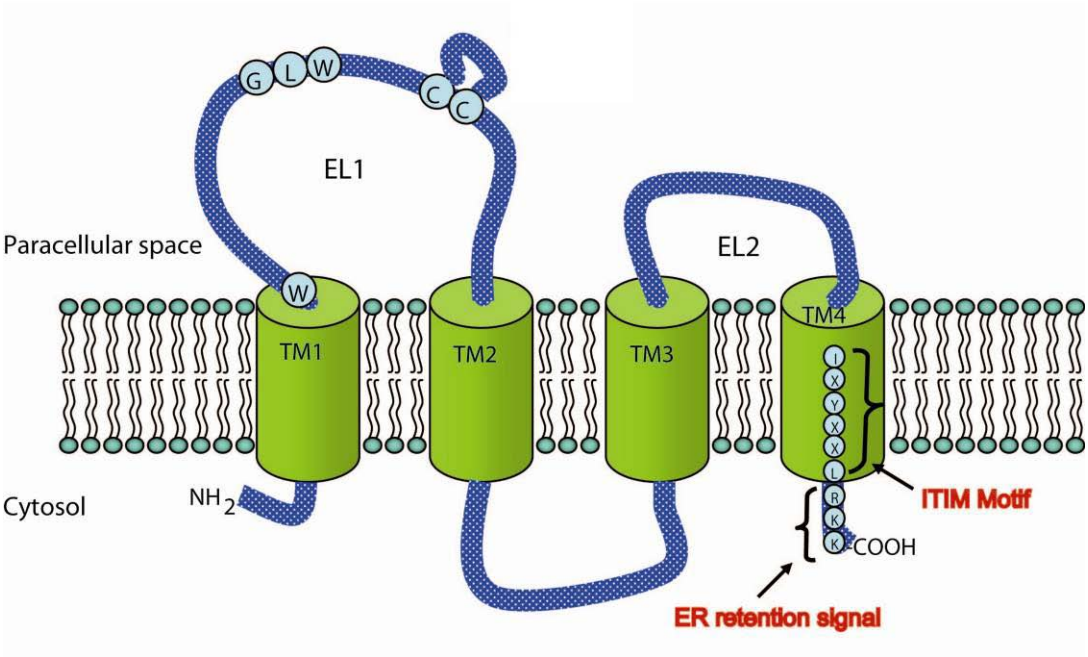

Figure 5. 26 Computer based prediction of EMP1 topology.

This model was generated from sequence analysis results. The image depicts the conserved structural features of the EMP family. EL1 and EL2 denote the extracellular loops 1 and 2. TM1, TM2, TM3 and TM4 are the transmembrane domains.

Mammalian EMP1 harbours a short intracellular cytoplasmic amino-terminal sequence of 4 residues followed by a large extracellular loop (EL1) of 36 residues, a short 9-residue intracellular loop, another extracellular loop (EL2) of about 16 residues and a carboxy-terminal cytoplasmic tail of three positively charged residues (Figure 5. 23). Emp1 is closely related to EMP2 and among different species rat EMP1 closely related to mouse EMP1 (Figure 5. 24, Figure 5. 25)

Moreover, there are structural similarities between EMP1 and tight junction proteins, such as claudins. The topology of EMP1 resembles the tight junction proteins occludin and claudin in the BBB (51). Unlike tetraspanins, the first loop is larger than the second loop. The first extracellular loop of EMP1 contains W-GLW-C-C motif characteristic of the claudin family, indicating a potential functional overlap between EMP1 and claudins (Figure 5. 24). In contrast to claudins and occludin, which are linked to the cytoskeleton via tight junction protein 1 (TJP1) the intracellular tail of EMP1, consisting of only three residues, may lack the ability to bind other cytoplasmic proteins. The short cytosolic C-terminal residues are positively charged

comprising of Arg-Arg-Lys (RKK) (Figure 5. 26). CD3γ also contains RKK motif, in the absence of which the protein is targeted directly from the trans-Golgi network to lysosomes without going through the cell surface and is considered to be an endoplasmic reticulum retention motif (ER) (67). Immunoreceptor tyrosine-based inhibitory motif (ITIM) is defined by the six amino acid consensus sequence (Ile/Val/Leu)-X-Tyr-X-X-(Leu/Val) occurring in the cytosolic domain of many membrane bound receptors (68). Interestingly EMP1 bears such an ITIM motif conserved across species in their predicted fourth transmembrane domain.

5.12 Expression of EMP1 in T cells

Gene expression profiling of T_{spleen} and T_{blast} revealed upregulation of EMP1 in a $T_{migratory}$ state. In order to validate the microarray data for EMP1, qPCR was performed after optimizing primers by a standard dilution curve. (Figure 5. 27). EMP1 and EMP3 are expressed in $T_{MBP\text{-}GFP}$ cells but the expression of EMP2 was too low to be detected in T cells (Figure 5. 28). EMP1 was ~3 fold upregulated in migratory T_{spleen} cells compared to T_{blast} whereas EMP3 was around ~2 fold downregulated in migratory T_{spleen} cells.

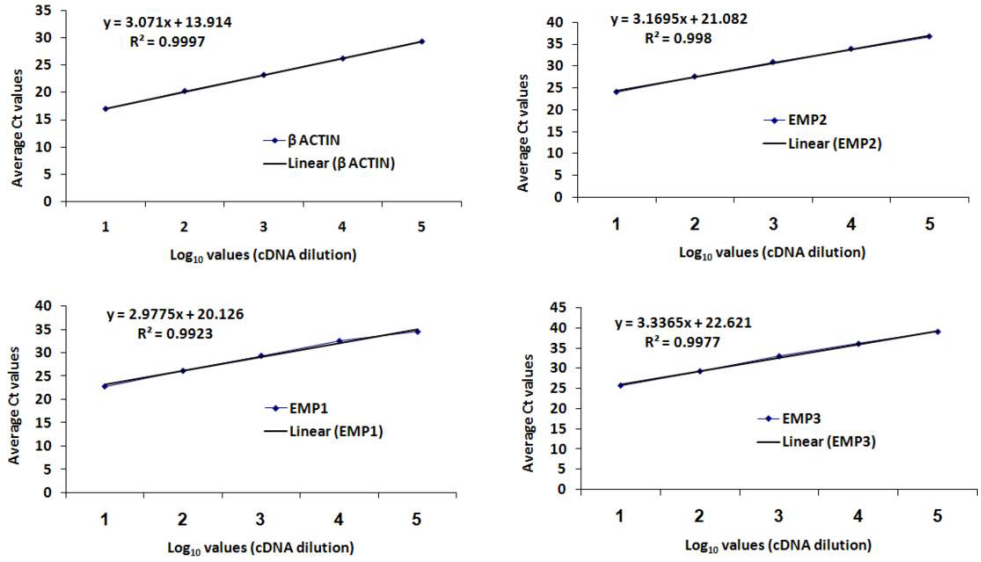

Figure 5. 27 Standardization of EMP1, EMP2 and EMP3 qPCR primers.

Primers were designed to span the exon-exon junction of all the genes. Standard dilution curves were plotted using cDNA which was prepared from total CNS of AT-EAE animal 5 days post transfer of $T_{MBP\text{-}GFP}$ cells. A standard curve slope of 3.32 indicates a PCR reaction with 100% efficiency.

EMP1 protein was detected in activated $T_{MBP\text{-}GFP}$ cells (T_{blast}) by both immunoblot and immunocytochemistry. Intracellular staining for EMP1 in T_{blasts} indicated a granular staining pattern in T cells (Figure 5. 29).

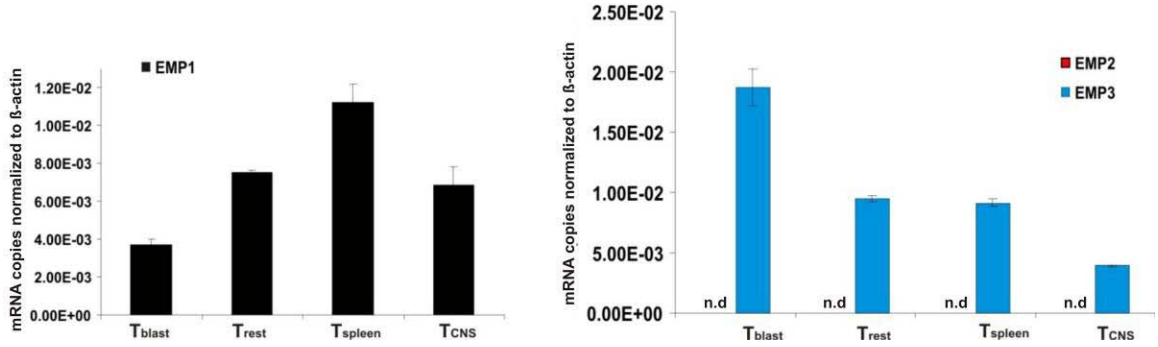

Figure 5. 28 Differential regulation of EMP1 family genes in T cells.

cDNA was prepared from T_{blasts}, $T_{resting}$ and *ex vivo* sorted T_{spleen} and T_{CNS} and qPCR was performed to assess the expression of EMP1, EMP2 and EMP3. EMP2 transcript was not detected indicating absence of EMP2 expression in T cells. Gene expression was normalized using β actin as internal control. Data is representative of three independent experiments.

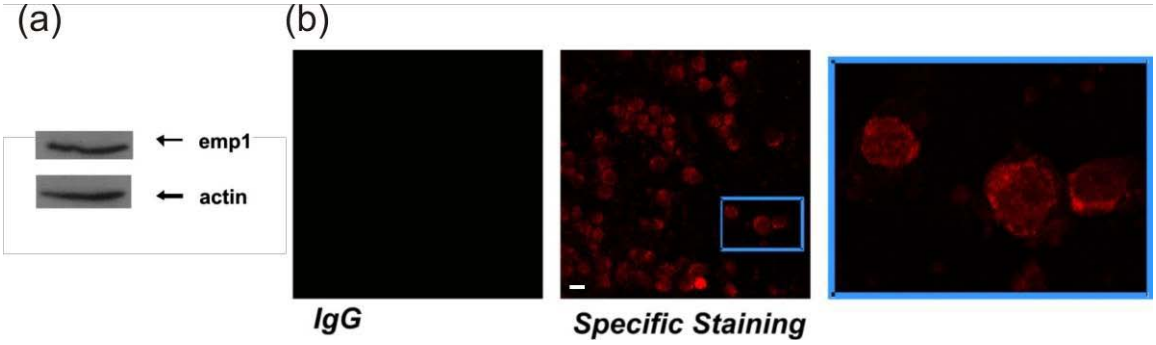

Figure 5. 29 EMP1 protein detection in activated T lymphocytes.

(a) Immunoblot for EMP1 and actin performed on *in vitro* activated $T_{MBP\text{-}GFP}$ cells. Molecular weight of EMP1 is 18 kDa. (b) Immunocytochemistry for EMP1 expression done on *in vitro* activated $T_{MBP\text{-}GFP}$ cells after cytospin. Confocal microscopy was used to examine the intracellular distribution of EMP1 in T cells. 10× magnification; Magnification bar: 10 µm Inset 20 × magnifications.

5.13 Cloning of EMP1 full length cDNA from activated $T_{MBP\text{-}GFP}$ cells

The EMP1 transcript is 2685 base pairs long with a coding region of 483 base pairs (Figure 5. 30). The cDNA was amplified by PCR from *in vitro*-activated $T_{MBP\text{-}GFP}$ cells (Figure 5. 30) and subsequently, cloned into the mammalian retroviral expression vector pMSCVneoIRES2-EGFP (Figure 5. 30). T_{MBP} cells were transduced using GP+E packaging cells producing EMP1-GFP retrovirus particles (50).

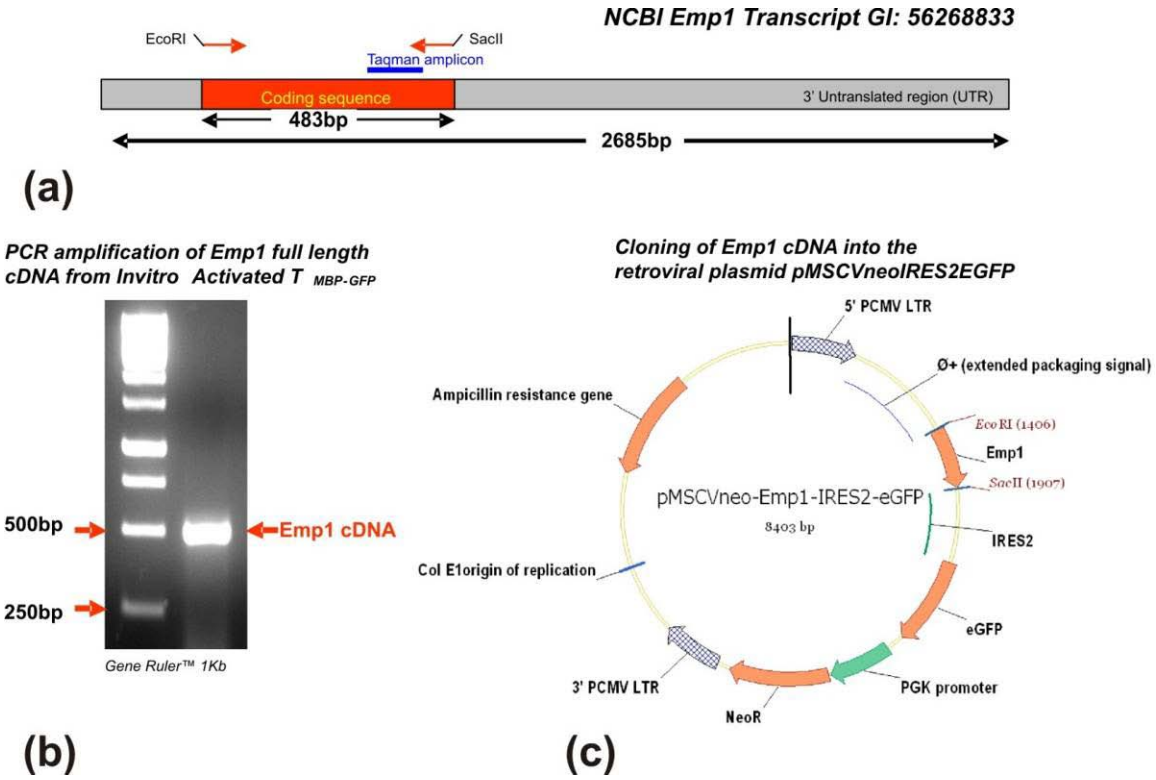

Figure 5. 30 Cloning of rat EMP1 from activated T cells.

(a) Schematic illustration of rat EMP1 mRNA. Full length coding sequence are colour coded in red and untranslated regions are indicated in grey. PCR primers with restriction enzyme for cloning used for amplification of full length cDNA are depicted. (b) EMP1 full length cDNA was amplified by PCR and agarose gel picture of PCR result is shown. (c) Plasmid map of mammalian retroviral expression vector used for GFP and EMP1 transduction in T cells.

5.14 Analysis of gene expression changes in EMP1 overexpressing T cells

In order to characterize the functional properties of $T_{MBP\text{-}EMP1}$ cells, a gene expression study for standard cytokines and transcription factors was performed (Figure 5. 31). Expression of cytokines such as interleukin 4 (IL4), interleukin 10 (IL10), IFNγ and IL17 and transcription factors such as RAR-related orphan receptor gamma (RORγ), forkhead box P3 (FOXP3) and T-box expressed in T cells (TBET) was by qPCR of *in vitro* cultured activated T cells . $T_{MBP\text{-}GFP}$ was always used as a control for comparison. The mRNA expression for cytokines and transcription factor did not change upon EMP1 overexpression in MBP-specific T cells (Figure 5. 32 and Figure 5. 33).

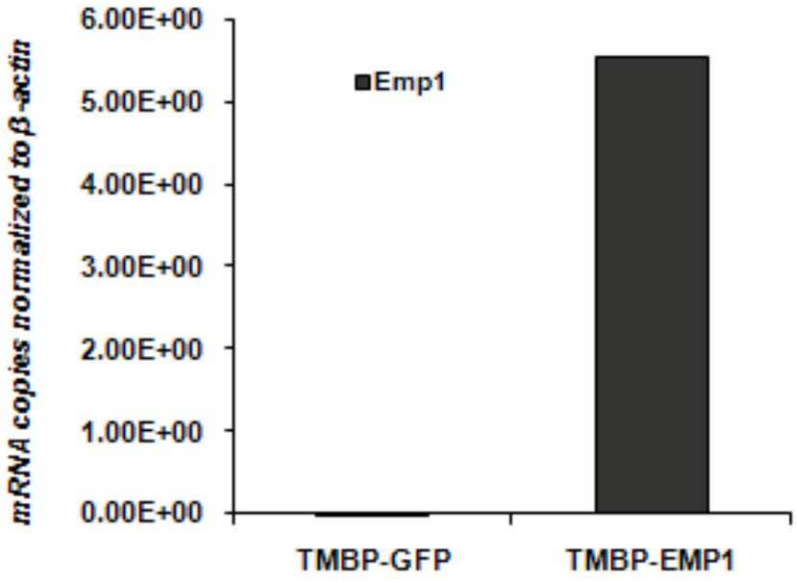

Figure 5. 31 Gene overexpression of EMP1 in activated T cells.

Quantitative PCR was performed on *in vitro* activated EMP1 transduced and GFP transduced control T cell blasts confirming overexpression of EMP1. β actin was used as internal control.

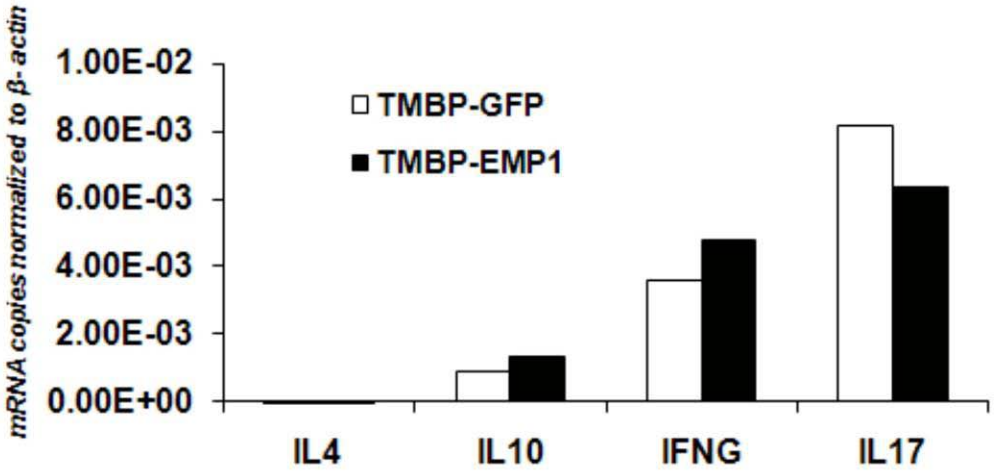

Figure 5. 32 Gene expression of cytokines.

Quantitative PCR performed on *in vitro* cultured activated MBP-specific T cells. $T_{MBP\text{-}EMP1}$, EMP1 overexpressing MBP-specific T cells; $T_{MBP\text{-}GFP}$, GFP control MBP-specific T cells. IL4, Interleukin 4; IL10, Interleukin 10; IFNγ, Interferon Gamma; IL17, Interleukin 17

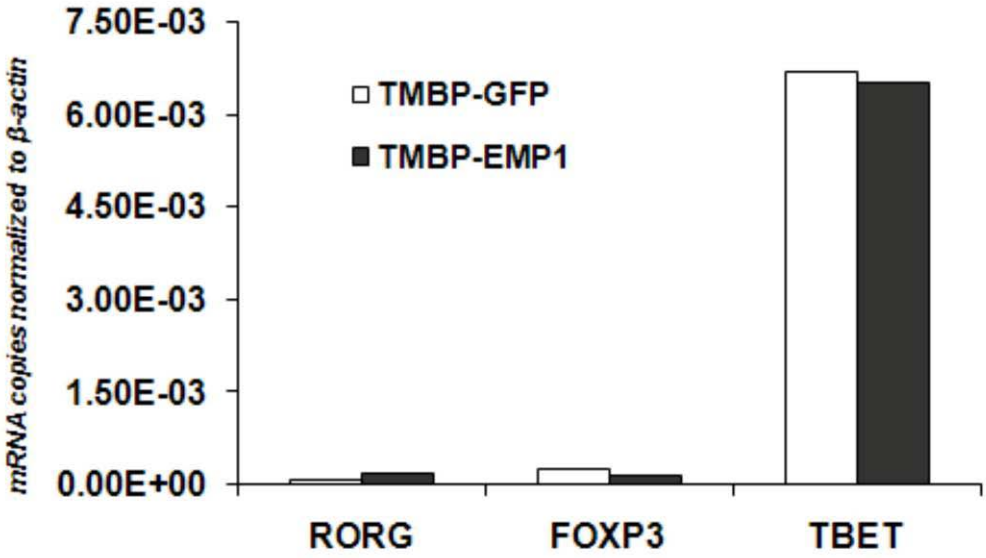

Figure 5. 33 Gene expression of transcription factors.

Quantitative PCR performed on *in vitro* cultured activated MBP-specific T cells. $T_{MBP\text{-}EMP1}$, EMP1 overexpressing MBP-specific T cells; $T_{MBP\text{-}GFP}$, GFP control MBP-specific T cells. RORG, RAR related Orphan Receptor Gamma; FOXP3, Forkhead box P3; TBET, T-Box Expressed in T cells.

5.15 EMP1 overexpressing T cells proliferate normally

It was reported in previous study that overexpression of EMP1 in a cancer cell line inhibits proliferation (69). However, overexpression in T cells did not affect the proliferation ability of T cells *in vitro*. To test the proliferation ability of $T_{MBP\text{-}EMP1}$, a thymidine incorporation assay was performed. Results from proliferation assay also indicated that $T_{MBP\text{-}EMP1}$ are MBP specific (Figure 5. 34).

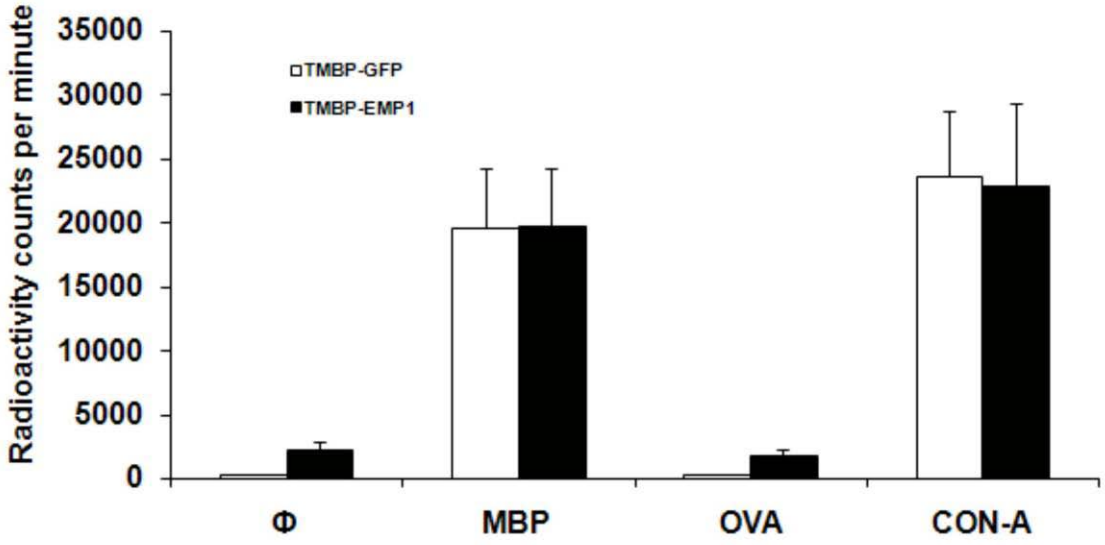

Figure 5. 34 Proliferation assay of *in vitro* cultured T cells.

T cells were stimulated using different antigens viz Φ, no antigen; MBP, Myelin Basic Protein; OVA, Ovalbumin and CON-A, Concanavallin A. 48 hours post antigen exposure thymidine [^{3}H] was added and radioactivity was measured after 16 hours using β counter.

5.16 Overexpression of EMP1 induces enhanced T cell motility in matrigel

In order to characterize the migration ability of EMP1-overexpressing T cells, time lapse video microscopy of T cells in a 3D matrigel was performed. Whereas activated $T_{MBP\text{-}GFP}$ cells showed a largely confined motility pattern within the matrigel, $T_{MBP\text{-}EMP1}$ cell were significantly more agile. Plots of tracks of 100 cells are shown in Figure 5. 35. The majority of $T_{MBP\text{-}EMP1}$ moved with higher speed compared to control T cells with an average velocity (5.6 versus 8.1 µm/min, respectively). Accordingly the cell trajectories of $T_{MBP\text{-}EMP1}$ cells recorded in a 10 min time interval were significantly longer (Figure 4.36).

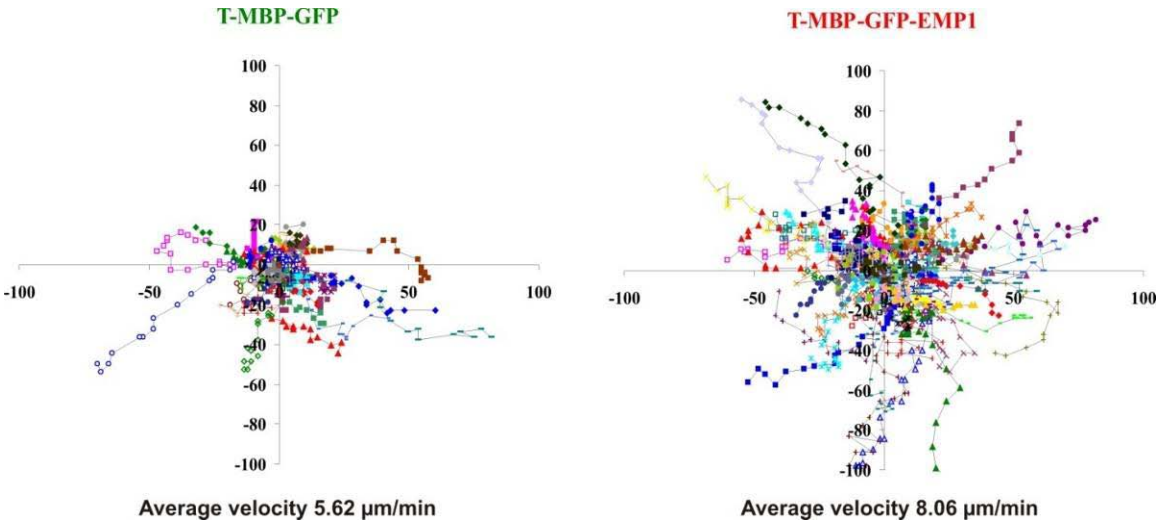

Figure 5. 35 Matrigel T lymphocyte motility assay *in vitro.*

Activated green fluorescent T cells overexpressing EMP1 ($T_{MBP\text{-}GFP\text{-}EMP1}$) were incubated in matrigel and time lapse video microscopy was performed to observe T cell migration in 3D matrigel under controlled oxygen, temperature and pH conditions. The same was done with GFP control T cells ($T_{MBP\text{-}GFP}$). Migratory paths were tracked and motility was analysed using ImageJ software. Individual cell track is colour coded. Both ordinate and abscissa denote distance traveled in µm. Data is representative of three independent experiments.

5.17 EMP1 overexpressing encephalitogenic T cells induce accelerated onset of EAE

Adoptive transfer EAE was induced in Lewis rats by the injection of 3 million activated $T_{MBP\text{-}EMP1}$ or $T_{MBP\text{-}GFP}$ cells. Transfer of T cells was performed using three different routes of administration including i.v., s.c. and i.p. The disease course was observed in these rats for twelve days by monitoring their body weight and clinical score.

$T_{MBP\text{-}EMP1}$ cells induce transfer EAE, which was indistinguishable from the one of $T_{MBP\text{-}GFP}$ cells, when the T cells were applied i.v. (Figure 5. 38). However, when cells were transferred i.p. or s.c., disease started at least 24 h earlier (Figure 5. 36 and Figure 5. 37). This earlier onset included both paralytic symptoms and weight loss. In order to determine the cause of this disease acceleration, the T cell distribution in different organs at day of onset of clinical disease was determined after s.c. and i.v. transfer of $T_{MBP\text{-}EMP1}$ cells. The clinical score in the EMP1 group (s.c. applied $T_{MBP\text{-}EMP1}$ cells) paralleled with the infiltration of $T_{MBP\text{-}EMP1}$ cells into the CNS. At this time point the numbers of control $T_{MBP\text{-}GFP}$ cells within the CNS were significantly lower (Figure 5. 36 and Figure 5. 37). However, there was no significant difference in T cell numbers in the CNS of the EMP1 group and GFP groups of animals that received encephalitogenic T cells after i.v. transfer.

In order to further investigate the early onset of EAE by i.p route of transfer, a time course experiment was performed to count the distribution of $T_{MBP\text{-}GFP}$ cells in different organs at different time points during the course of EAE. The results indicate that $T_{MBP\text{-}EMP1}$ cells appear earlier in the analysed organs, i.e. lung, liver, spleen, paraaortal lymph node and mesenteric lymph nodes (Table 5. 14). Interestingly on day 4 post transfer, the numbers of $T_{MBP\text{-}EMP1}$ cells were similar to $T_{MBP\text{-}GFP}$ in several organs except the parathymic lymph node, meninges and CNS. When injected s.c or intra peritoneally, $T_{MBP\text{-}EMP1}$ cells migrated faster to the draining lymph nodes (Figure 5. 39). This is in concordance with the *in vitro* finding of higher motility in 3D matrigel.

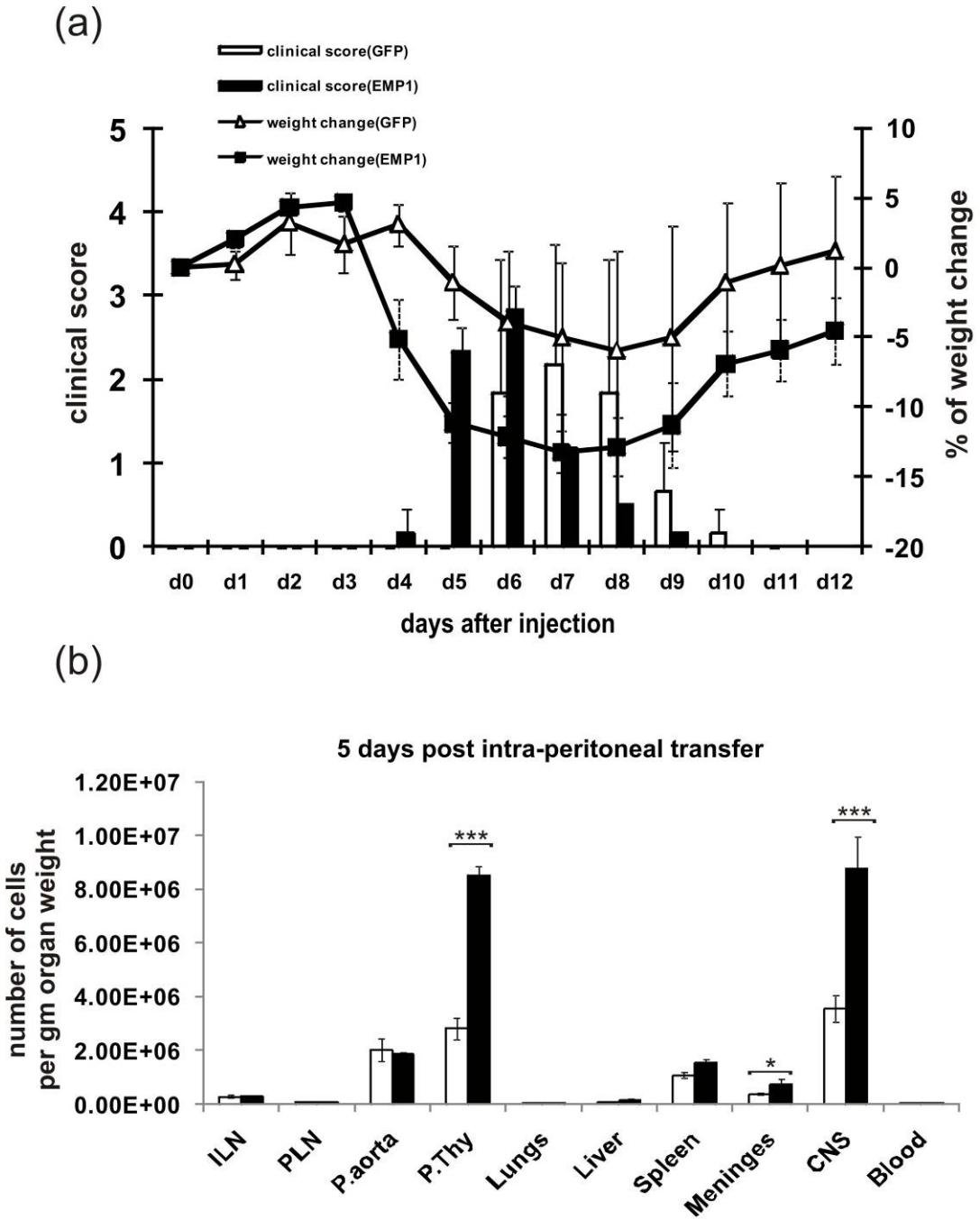

Figure 5. 36 Adoptive transfer EAE induced by intraperitoneal injection and migratory pattern of encephalitogenic T cells *in vivo* 5 days post intra peritoneal injection.

(a) Clinical course of AT-EAE induced by intra-peritoneal (i.p.) transfer. (b) Migration of green fluorescent encephalitogenic T cells to different organs, 4 days post transfer was determined by flow cytometry. Student's t-Test p value, * denotes $p < 0.5$, ** denotes $p < 0.01$ & *** denotes $p < 0.001$.

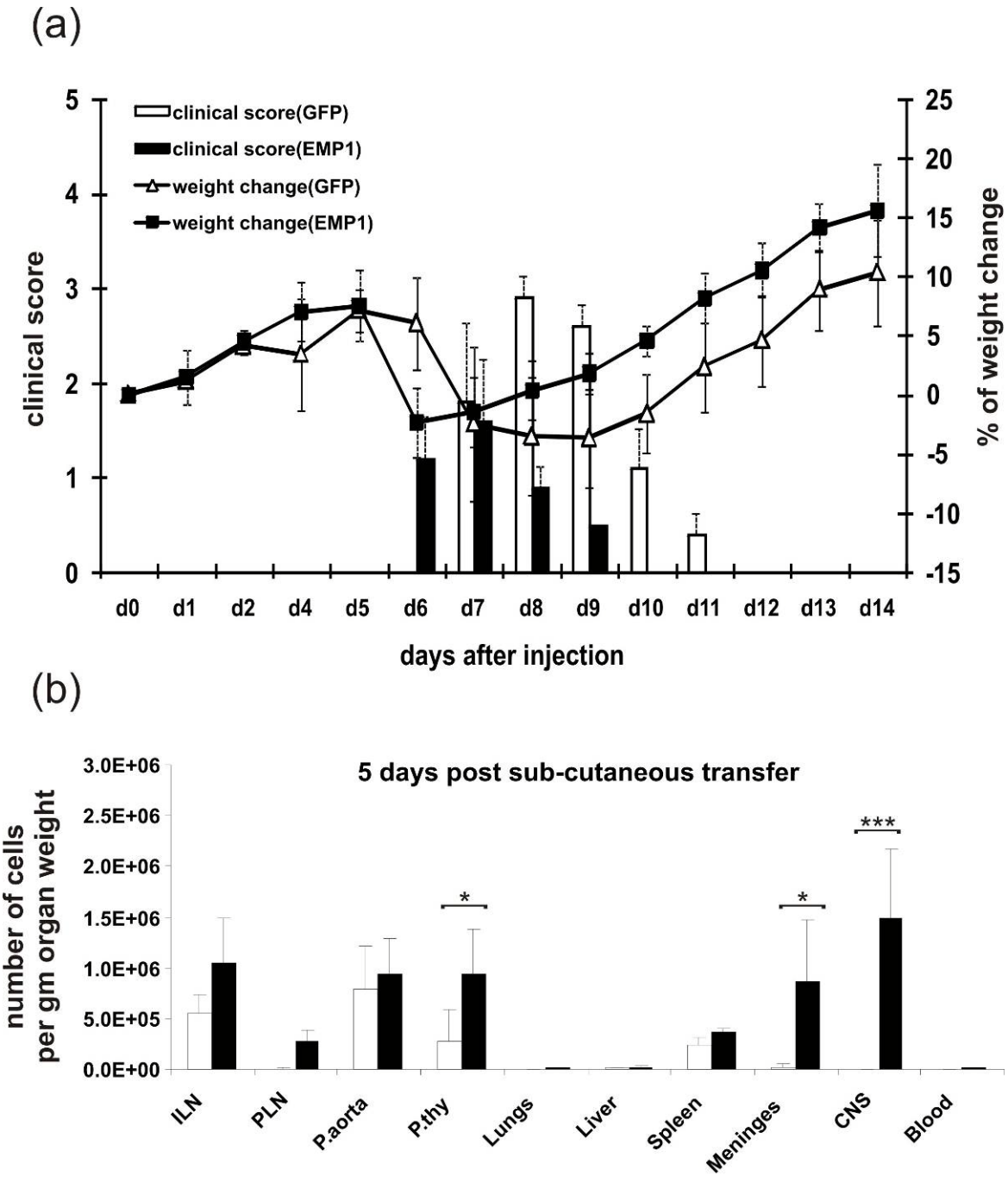

Figure 5. 37 Adoptive transfer EAE induced by sub cutaneous injection and migratory pattern of encephalitogenic T cells *in vivo* 5 days post sub cutaneous injection.

(a) Clinical course of AT-EAE induced by sub-cutaneous (s.c.) transfer. (b) Migration of green fluorescent encephalitogenic T cells to different organs, 4 days post transfer was determined using flow cytometry. Student's t-Test p value, * denotes $p < 0.5$, ** denotes $p < 0.01$ & *** denotes $p < 0.001$.

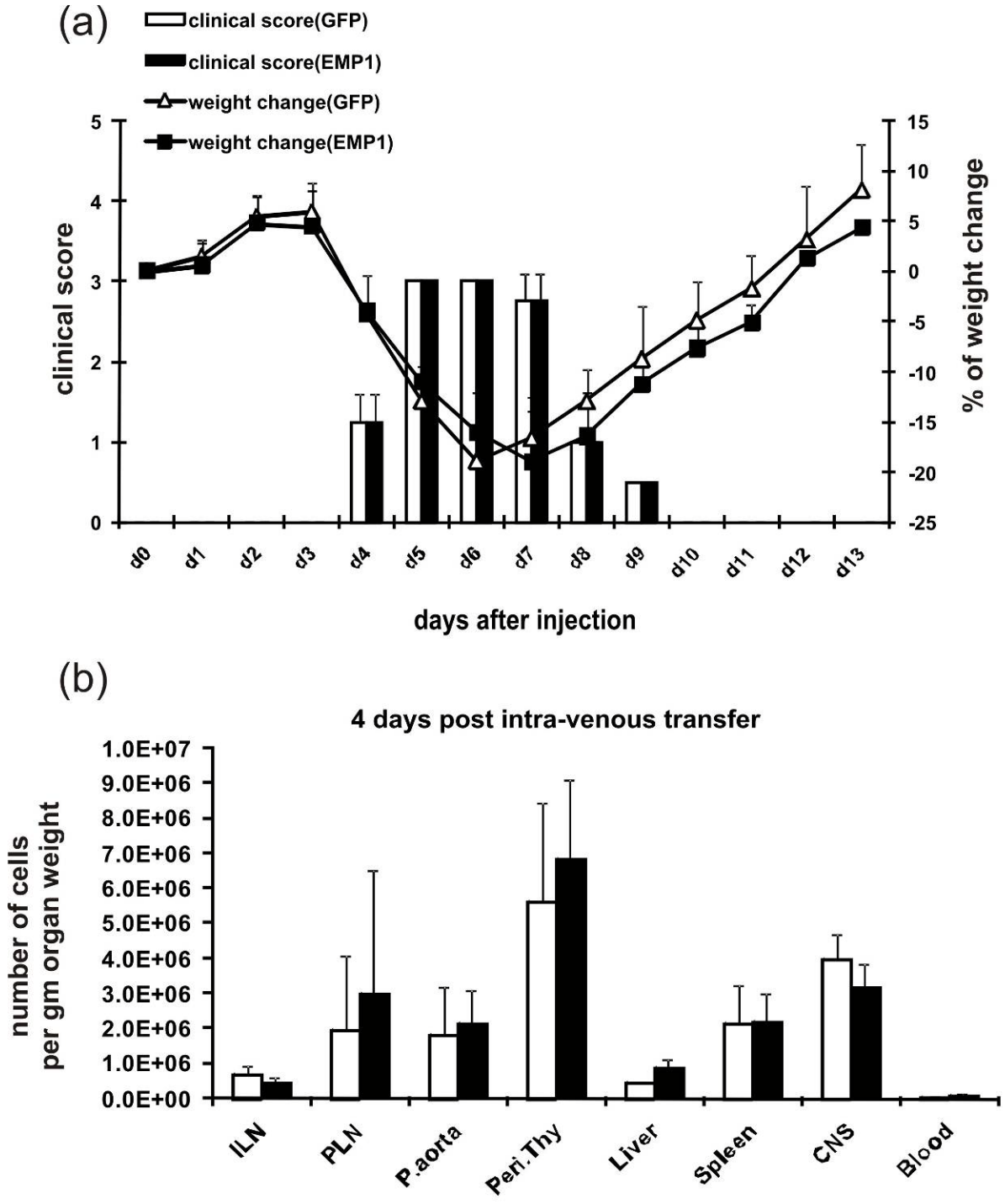

Figure 5. 38 Adoptive transfer EAE induced by intra venous injection and migratory pattern of encephalitogenic T cells 4 days *in vivo* post intra-venous injection.

(a) Clinical course of AT-EAE induced by intra-venous (i.v.) transfer. (b) Migration of green fluorescent encephalitogenic T cells to different organs, 4 days post transfer was determined using flow cytometry. Data is average of three independent experiments.

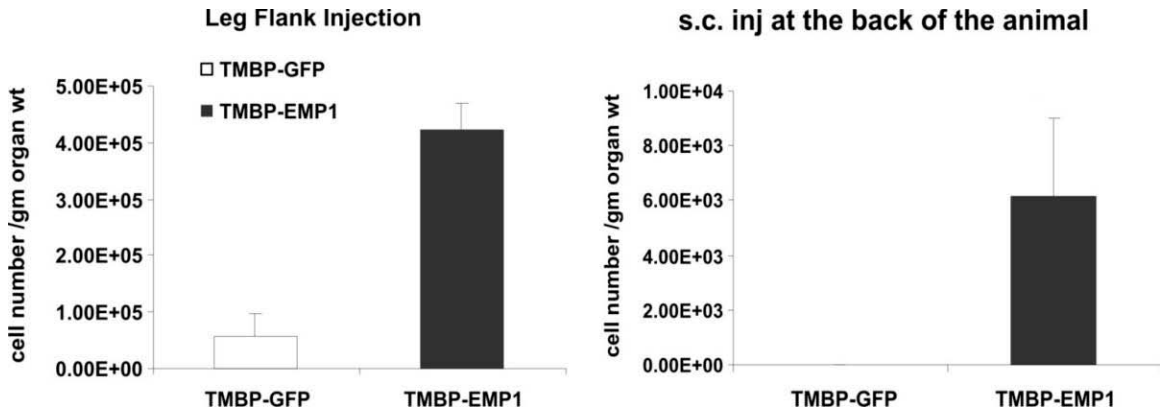

Figure 5. 39 Migration of EMP1 overexpressing T cells to the draining lymph nodes.

10 million green fluorescent EMP1 transduced T cells ($T_{MBP\text{-}EMP1}$) were injected subcutaneously either in leg flank or under the skin of the animal's back in two separate groups. As a control GFP transduced T cells ($T_{MBP\text{-}GFP}$) were injected similarly. 24 hours post sub cutaneous injection, the number of $T_{MBP\text{-}GFP}$ and $T_{MBP\text{-}EMP1}$ reaching the draining lymph nodes were determined by flow cytometry. Data indicate average of three independent experiments.

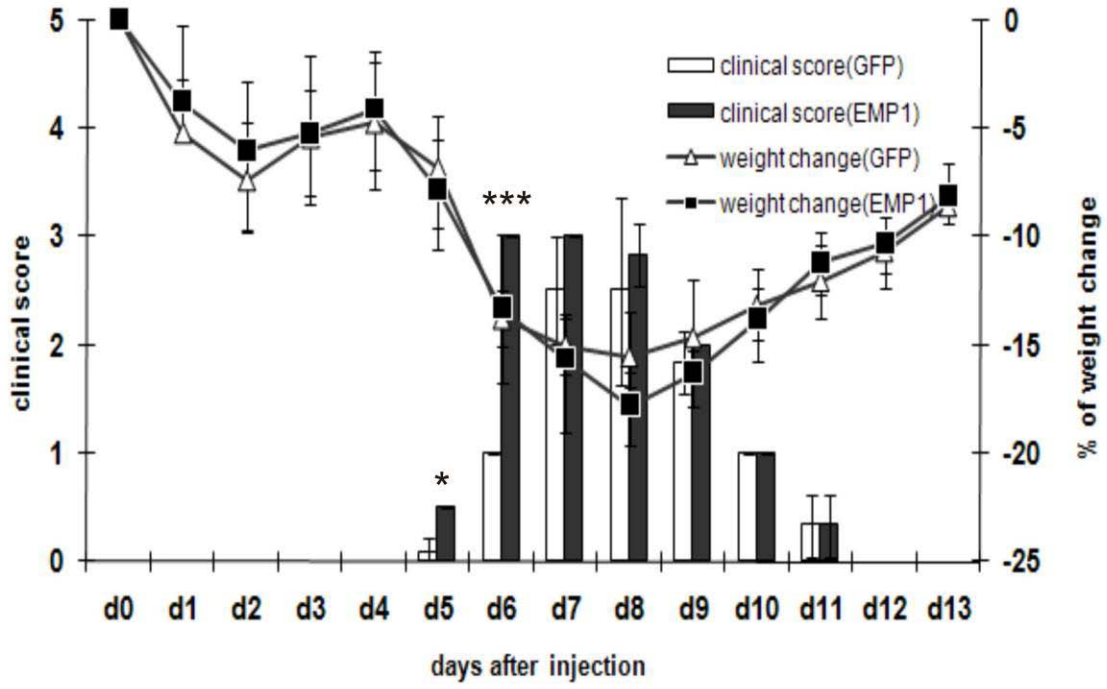

Figure 5. 40 Active EAE induced in memory animals by MBP/CFA immunization.

Memory animals were generated by neonatal transfer of 2.5 million $T_{MBP\text{-}GFP}$ and $T_{MBP\text{-}EMP1}$ cells in two separate groups of animals and EAE was induced after 6-8 weeks. Clinical course was monitored for 13 days post disease induction. Data is average of three animals in a group. Student's t-Test p value, * denotes $p < 0.5$, ** denotes $p < 0.01$ & *** denotes $p < 0.001$.

	Day 1 p.i.p.t		**Day 2 p.i.p.t**		**Day 3 p.i.p.t**	
	$T_{MBP\text{-}GFP}$	$T_{MBP\text{-}EMP1}$	$T_{MBP\text{-}GFP}$	$T_{MBP\text{-}EMP1}$	$T_{MBP\text{-}GFP}$	$T_{MBP\text{-}EMP1}$
ILN	0	0	0	7.16×10^{3}	2.73×10^{5}	2.97×10^{5}
PLN	0	0	0	0	5.69×10^{4}	5.05×10^{4}
P.thy	0	1.10×10^{4}	9.03×10^{4}	4.52×10^{4}	2.81×10^{6}	8.49×10^{6}
Lungs	0	0	5.98×10^{2}	7.62×10^{3}	4.32×10^{4}	2.80×10^{4}
Liver	0	0	9.36×10^{2}	2.37×10^{4}	7.61×10^{4}	1.56×10^{5}
Spleen	0	0	3.78×10^{3}	3.88×10^{5}	1.07×10^{6}	1.52×10^{6}
P.aorta	0	0	0	1.21×10^{6}	2.01×10^{6}	1.85×10^{6}
Mesenteric	0	0	3.11×10^{3}	4.27×10^{5}	1.19×10^{6}	1.30×10^{6}
Meninges	0	0	0	0	3.64×10^{5}	7.28×10^{5}
T.CNS	0	0	0	0	3.68×10^{5}	7.72×10^{5}
Blood	0	0	2.87×10^{2}	1.73×10^{6}	3.90×10^{4}	4.12×10^{4}

Table 5. 14 Encephalitogenic T cells migration to different organs post intra peritoneal transfer (p.i.p.t).

Cell numbers in various organs were counted by flow cytometry day 1,day 2 and day 4 post intraperitoneal transfer of 3 million of $T_{MBP\text{-}GFP}$ (white columns) and $T_{MBP\text{-}EMP1}$ (grey columns). Green fluorescent $T_{MBP\text{-}GFP}$ and $T_{MBP\text{-}EMP1}$ cells were injected in two different groups of animals. ILN, Inguinal lymph Node; PLN, Popliteal lymph node; P.thy, Parathymic lymph node; P.aorta, Para aortal lymph node; T.CNS, Total spinal cord.

Migration to draining lymph nodes by $T_{MBP\text{-}EMP1}$ cells was analysed by two independent experiments. In one experiment, the same animals were injected subcutaneously with both control $T_{MBP\text{-}GFP}$ and $T_{MBP\text{-}EMP1}$ cells but in two different leg flanks. GFP positive cells from the draining lymph nodes were enumerated 24 hours post injection by FACS. In another experiment, two different groups of animals were separately injected subcutaneously with control $T_{MBP\text{-}GFP}$ or $T_{MBP\text{-}EMP1}$ but into the lower back of the animal. GFP positive cells were counted from draining lymph nodes, 24 hours after injection by FACS (Figure 5. 39). EMP1 overexpressing T cells migrated earlier to the draining lymph nodes in both the settings.

Using an autoimmune memory model, migration of genetically modified MBP-specific T cells from periphery to the CNS can be assessed during active EAE. In this model, $T_{MBP\text{-}GFP}$ cells are embedded in the immune system of neonatal rats such that these cells then remain engrafted until adulthood without affecting the health of the hosts. Moreover, $T_{MBP\text{-}GFP}$ cells maintain a memory phenotype with SELL and CD45RC low but high CD44 (70). Active EAE induction in these memory animals by immunization with MBP/CFA induced disease between day 5 and day 6. However, immunization of animals harbouring memory $T_{MBP\text{-}EMP1}$ cells induced an accelerated disease with higher clinical scores on day 5 and day 6 compared to the control group of $T_{MBP\text{-}GFP}$ memory animals (Figure 5. 40). All the animals in EMP1 group became sick on day 5 post immunization with clinical scores of ~0.5 whereas only one animal got a mild score in the GFP group (Figure 5. 40). Moreover, the following day all animals in the EMP1 group developed severe disease with a clinical score of 3 whereas control group exhibited mild clinical scores of 1.

In conclusion, using adoptive transfer and active EAE model in memory animals, the results indicate a pro migratory role of EMP1 in directing $T_{MBP\text{-}GFP}$ cells into circulation subsequently facilitating early entry into the CNS.

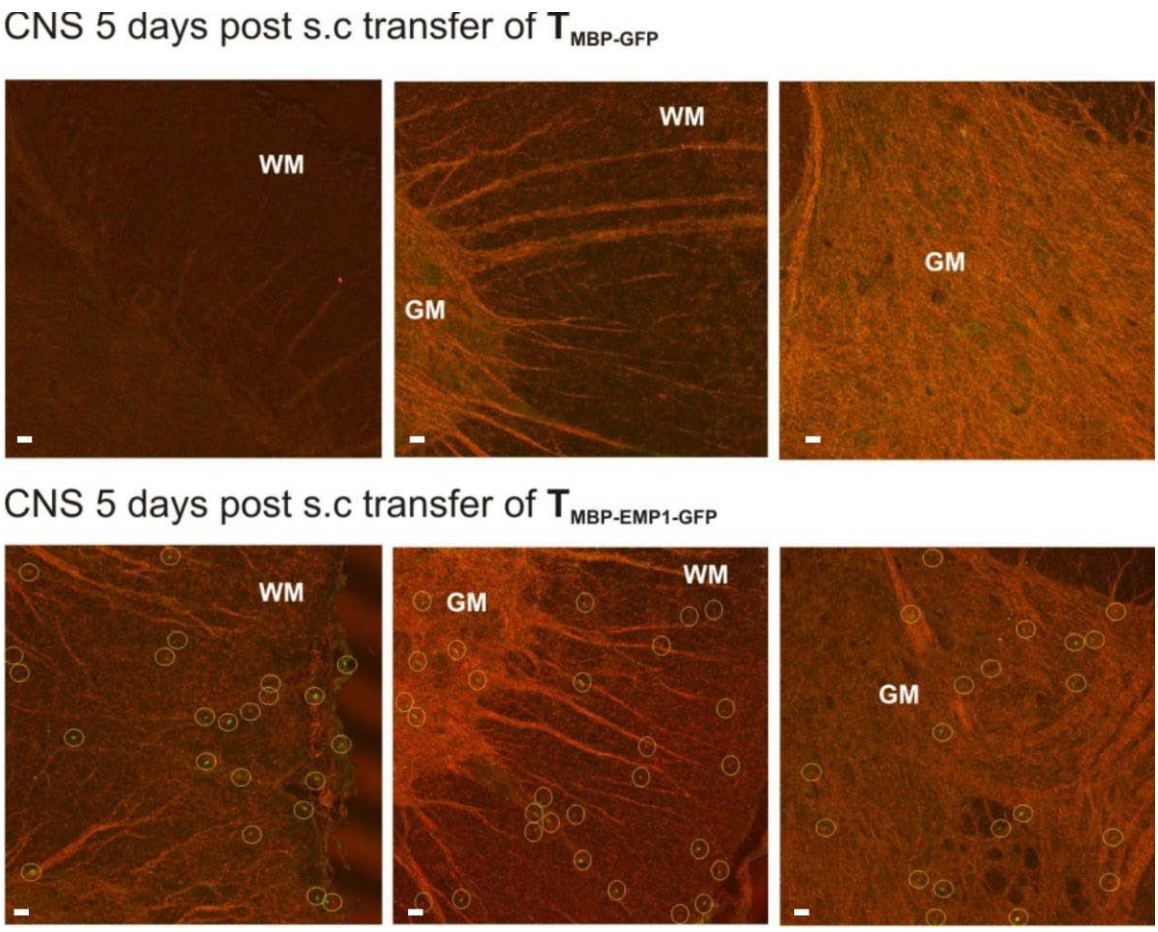

Figure 5. 41 Encephalitogenic T cell infiltration to CNS post AT-EAE induction.

CNS tissue from 5 days s.c. transfer of T_{MBP} cells were fixed in 4% PFA and prepared for microtome sectioning. Slices viewed using 10X objective. Magnification bar: 10 µm. Upper panel images show no GFP positive $T_{MBP-GFP}$ infiltration in CNS tissue of an animal from GFP groups. Lower panel images show many GFP positive $T_{MBP-EMP1}$ cells infiltration into the CNS parenchyma on the onset of disease distributed evenly both in white matter (WM) and grey matter (GM). Data is representative of three independent experiments.

5.18 EMP1 overexpressing T cells infiltrate earlier into the CNS parenchyma

In order to test if the accelerated disease onset correlated with the capacity of $T_{MBP-EMP1}$ cells to invade the CNS parenchyma, morphological analyses using confocal laser scanning microscopy were performed. CNS tissue was prepared from animals of the GFP and EMP1 groups 5 days post s.c. transfer of the T cells. The analyses revealed that $T_{MBP-EMP1}$ cells infiltrated deep into the CNS parenchyma. In contrast at this time point there were no significant $T_{MBP-GFP}$ cell infiltrates detectable (Figure 5. 41). Early migration of $T_{MBP-EMP1}$ cells into CNS following sub-cutaneous transfer paralleled with the T cell specific gene expression in an inflamed CNS (Figure 5. 42). Gene expression was analysed by performing a quantitative PCR on total CNS from two groups of animals (n=3). These data confirm that $T_{MBP-EMP1}$ cells arrive earlier at their target organ, most likely due to enhanced locomotion activity.

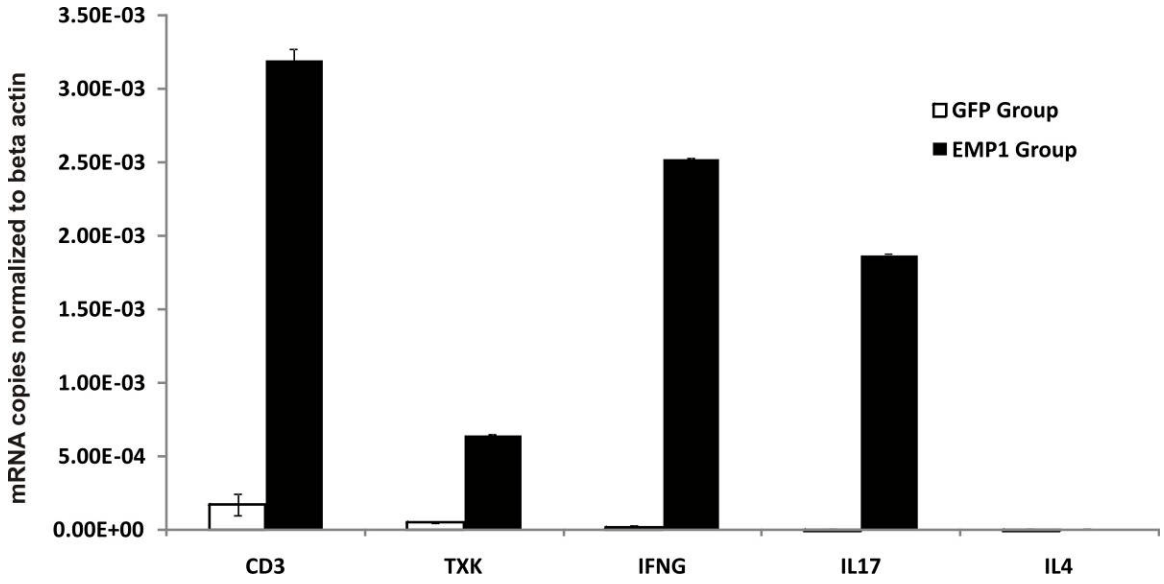

Figure 5. 42 Encephalitogenic T cell migration coincides with expression of T cell specific genes in CNS post AT-EAE.

Quantitative PCR was performed on total CNS of EAE animals 5 days post s.c. injection of $T_{MBP-EMP1}$ (EMP1 Group) and $T_{MBP-GFP}$ (GFP Group) to quantify the expression of CD3, TXK, IFNγ, IL17 and IL4. Data is average of three animals in a group.

6. Discussion

Multiple sclerosis is triggered by infiltration of brain antigen specific T lymphocytes into the CNS. The specific molecular determinants which control the migration of encephalitogenic T cell into the CNS remained unclear. Despite several microarray based gene profiling studies and considerable knowledge of EAE models and human MS, a global survey of gene expression of encephalitogenic T cells in EAE has been lacking. The aim of this PhD thesis was to perform a systematic transcriptomic analysis of encephalitogenic T cells in order to identify novel molecular factors that are crucial for CNS migration in EAE.

Adoptive transfer EAE in Lewis rat is a monophasic disease which follows a highly predictable disease course. Intriguingly, the onset of the disease occurs only after an obligatory latency of at least 3 days, irrespective of the number of autoreactive T cells injected (44). At the time of injection, encephalitogenic T cells are maximally activated and characterized by upregulation of activation markers such as IL2R, IFNγ and OX40 antigen. Before migrating to target organ, they accumulate in the spleen, where aforementioned activation markers are downregulated. Instead, migratory molecules such as CCR1, CCR2, CCR3, CCR5, and CXCR4 are upregulated (44). Once in their target organ, these T cells are reactivated but keep their migratory molecules high. Nevertheless, the molecular and cellular mechanisms which guide migration of encephalitogenic T cells to CNS are largely unknown.

In this study, an oligonucleotide microarray was used to detect differentially regulated genes in encephalitogenic T cells in different milieus such as *in vitro* activated, *in vitro* resting, *ex vivo* spleen and *ex vivo* CNS (Figure 5. 1). Affymetrix Rat 230 Set 2.0, the oligonucleotide array used for analysis consisted of ~31000 transcripts, of which only a small percentage of ~5% underwent differential regulation (Table 5. 1). In this study, it was attempted to identify molecular changes at the transcriptomic level that are necessary to allow activated encephalitogenic T cells to migrate to the CNS and cause inflammation. An average linkage hierarchical cluster analysis of $T_{activated}$, $T_{migratory}$ and $T_{effector}$ transcriptome was performed to identify groups of genes that are co-regulated and to compare different T cell states *in vivo*. Transcriptomic analysis including cluster, gene

ontology and pathway-based analyses, in an unbiased manner, indicated inhibition of cell proliferation or cell cycle progression machinery in T_{spleen}, but induction of cell migration transcriptome (Figure 5. 6). *In vitro*-activated encephalitogenic T cell blasts, when compared to their resting state 7 days post stimulation, exhibited a strong upregulation of cell cycle genes. This cluster of cell cycle regulators included Cyclin E1 and Cyclin E2, proteins required for cell cycle progression from G1 phase to S phase, and Cyclin A2, Cyclin B1, Cyclin B2 and Cyclin F, proteins required for G2 phase to M phase transition. Also within the same upregulated cluster in $T_{activated}$ were genes involved in cell metabolism such as HMGCR, an enzyme involved in cholesterol biosynthesis, and other genes involved in DNA metabolism. At the same time, transcription regulators that inhibit cell cycle progression were downregulated suggesting a tightly controlled transcriptome for cell proliferation and cell metabolism during T cell activation. In contrast, effector T cells in CNS show activation but not cell cycle progression and proliferative response. All the cell cycle inhibiting transcription factors such as BTG2, KLF4 and TOB1 are upregulated and they keep expression of cell migration molecules such as CCR2, CCR5 and EDG1 high. There is a strong upregulation of activation markers such as IL2R and IFNγ.

Around 3 days post transfer of encephalitogenic T cells, a high number of cells accumulate in the spleen with a striking downregulation of activation markers such as IL2R, IFNγ and OX40 antigen (44). Studies extrapolating a direct molecular correlation of T cell activation and T cell migration are lacking. Chchlinska et al has shown that IL2 deprivation inhibits cell cycle progression in lymphoblasts, and other independent studies reported upregulation of chemokine receptors upon IL2 deprivation in lymphocytes (61,71,72). Along the same line, the transcriptomic analysis of T_{spleen} vs T_{blast} also revealed upregulation of a large number of genes important for migratory signalling pathway and downregulation of cell cycle activating genes (Figure 5. 7, Figure 5. 11 and Figure 5. 12). One of the cardinal markers of activation, IL2R, whose stimulation activates ERK signalling subsequently inducing mitosis, was downregulated. At the same time, DUSP5, an inhibitor of ERK signalling was upregulated in the $T_{migratory}$ transcriptome. TXK, T cell specific kinase acting downstream of integrin receptor and chemokine receptor signalling was also upregulated more than 2 fold. Guanine nucleotide

exchange factors, such as ARHGEF18, ARHGEF3, the control switches for RhoGTPases which are essential for cell migration were upregulated. Furthermore, among this upregulated cluster, there were several molecules of the cell locomotion apparatus, such as ZYX and FHL2, and genes involved in cell migration such as CCR2, CCR7 and S1P1 (Figure 5. 12).

Microarray-based studies of gene expression can be extremely informative because the high throughput technology allows the gathering of enormous amounts of information on gene regulation. At the same time, a large dataset needs effective databases and resources for management and analysis. One of the pitfalls of microarray based experiments is the occurrence of false positives and they very often demand validation by more reliable experiments such as real time PCR and antibody based methods to confirm regulation at the protein level. Although the number of regulated transcripts in microarray data reached only ~5% of total available transcripts, this sums up to approximately 1500 genes, which cannot be easily validated by qPCR. Hence, a list of 43 genes with defined functional impact on T cell biology was validated by qPCR. The comparison of the microarray and qPCR data showed a similar regulation pattern, although the magnitude of fold regulation was lower in the microarray (Figure 5. 14).

The comparative transcriptome analysis between highly proliferative activated T cells and migratory T cells demonstrates an inverse correlation between cell cycle and the cell migration pathways. Moreover, effector T cells in CNS display a unique functional state: they upregulate activation molecules but not factors controlling cell cycle progression. Furthermore, they maintain high levels of genes controlling migratory properties. In order to corroborate this finding, an approach was taken which combined bioinformatics with *in vitro* experimental assays such as cell cycle assays, quantitative PCR and luciferase assays. In our transcriptome analysis, very often cell cycle genes and cell migration genes were found to be clustered and among them also were transcription factors known to inhibit cell cycle progression such as KLF4, BTG1, BTG2 and TOB1. All these transcription factors inhibit the cell cycle transition from the G1 phase to the S phase. Among the cell migration molecules that were found to be clustered were CCR1, CCR2, CCR3, CCR5 and CCR6. The upregulation of these inflammatory chemokine receptors in encephalitogenic effector T cells has been published earlier (44).

Furthermore, genes for inflammatory chemokine receptors such as CCR1, CCR2, CCR3 and CCR5 were all clustered in the genome of both rat and mouse (Figure 5. 17).

A bioinformatics approach was employed to identify a common cell cycle inhibiting transcription factor that would additionally be involved in the regulation of inflammatory chemokine receptor expression. KLF4, a zinc finger transcription factor, was identified using the web based transcription factor binding site prediction program Matinspector™ (Table 5. 9) (58). Upon overexpression, KLF4 not only inhibited the cell cycle progression in encephalitogenic T cells but also led to upregulation of CCR2 and CCR5 (Figure 5. 19 and Figure 5. 20). A similar regulatory activity of KLF4 was found in a rat leukaemia cell line (Figure 5. 21). Luciferase promoter assays further corroborated these findings (Figure 5. 22). These data indicate a transcriptional program which simultaneously regulates cell cycle as well as cell migration in effector T cells. Unfortunately, downregulation of the cell cycling proved to be a hindrance for the generation and expansion of encephalitogenic T_{MBP} cells. Therefore, the function of KLF4 in effector T cells could not be tested during AT-EAE. Nevertheless, our *in vitro* results hint to a regulatory role of Krüppel-like factors in $CD4^+$ lymphocyte migration.

Interestingly, Sebzda et al recently showed that KLF2, a different member of the Krüppel-like family, might be involved in regulating the migration of naïve T cells by repressing chemokine receptor gene expression (73). KLF2 is a zinc finger transcription factor that inhibits cell proliferation by inhibiting G1/S phase progression of cell cycle. Several reports over the last few years have established a transcription regulation of migratory receptors by KLF2, for instance that, KLF2 upregulates expression of S1P1 and CCR7 (73,74).

In a summary, a systematic transcriptomic analysis of activated T cells from *in vitro* and migratory encephalitogenic T cells from spleen reveal a link between cell cycle regulators and cell migration control. Moreover, these results provide a molecular explanation for prodromal period of EAE. The current results can be depicted in a model as shown in Figure 4.44

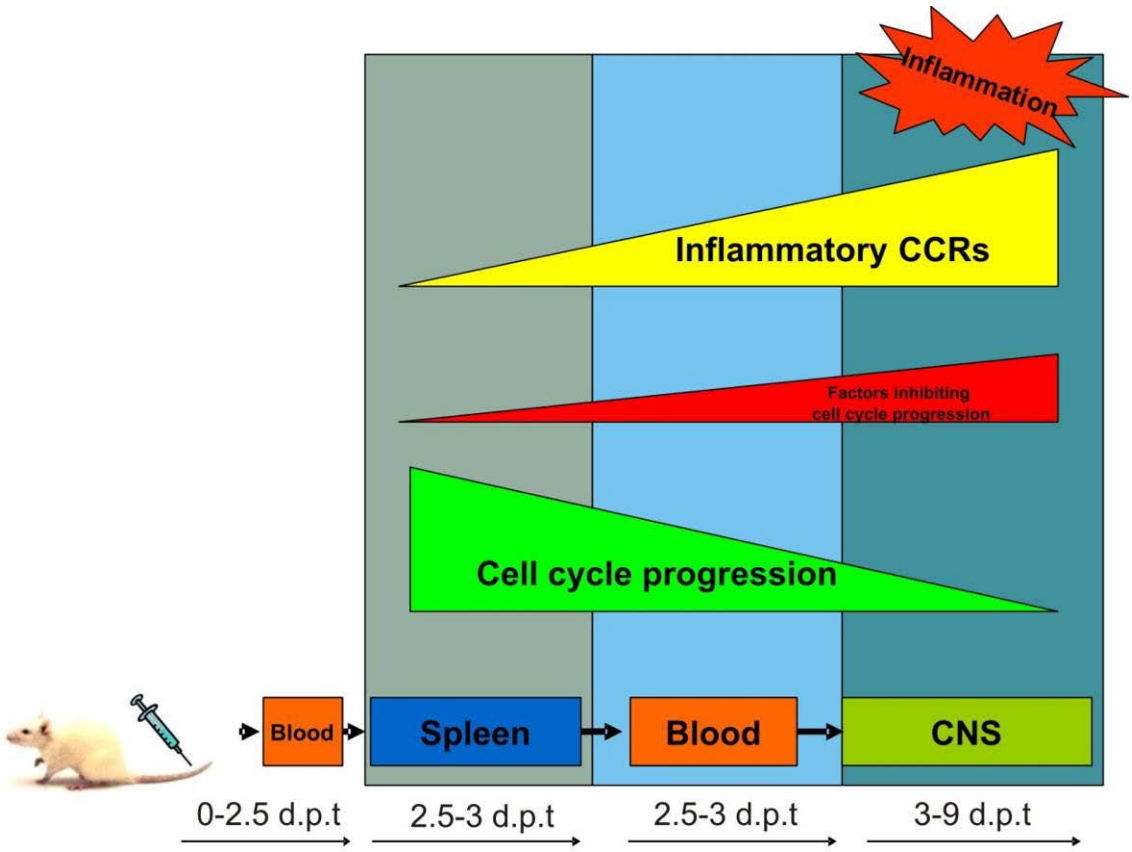

Figure 6. 1 Model illustrating the molecular changes taking place in encephalitogenic T cells from the time of injection until they reach their target organ.

Upregulation is indicated by ascending triangle and downregulation by descending triangle. Time point days post transfer is indicated.

In an attempt to identify and test potential new therapeutic targets on autoaggressive effector T cells, a focus of the transcriptome analysis was put on differentially regulated membrane molecules. Intriguingly, members of regulated genes in migratory T cells included some of the well-known drug targets for multiple sclerosis such as integrin α4β1, S1P1, CCR2 and CCR6.

Here the role of EMP1 was tested in T cell migration during EAE. EMP1 was chosen for the following reasons: i) EMP1 transcript was found upregulated in migratory T_{spleen} cells (Figure 4.29); ii) EMP1 is a 4-transmembrane protein belonging to a novel gene family and was originally isolated as a gene differentially expressed in brain tumours (63). Its role in T cells has not been characterized; iii) Other 4-transmembrane proteins such as CD9, CD81 and CD151 that were first cloned in screens relating to tumorigenesis, were found to promote cell migration by associating with integrins (64,65).

One of the striking features of the members of this family is the shared structural features of both tight junction proteins such as claudins and of tetraspanins such as CD151. Moreover, EMP1 has recently been reported to be a part of the blood brain barrier junction protein. Multiple sequence alignment of EMP1 protein sequences from different species showed certain conserved motifs such as the W-GLW-C-C and ITIM motifs (Figure 5. 23). The W-GLW-C-C motif is a characteristic feature of claudins that occurs in the first extracellular loop is considered to play a role in homophilic and heterophilic interactions. The occurrence of the ITIM motif in EMP1 is surprising, since normally ITIM is a conserved sequence of amino acids found in the cytoplasmic tails of many inhibitory receptors of the immune system. However, there are reports showing a role of ITIM in cell migration and motility (75,76). So far there has been only one report indicating the expression of EMP1 in T lymphocytes (77), however, our RNA and protein data clearly confirm its expression in T cells (Figure 5. 29). Although overexpression of EMP1 in a cancer cell line inhibits cell proliferation (69), there was no such effect on T cell proliferation (Figure 5. 34). Furthermore, encephalitogenic T cells overexpressing EMP1 exhibited similar cytokine production as that of control transduced T cells (Figure 5. 32 and Figure 5. 33). However, EMP1 overexpression induced an increased motility of activated lymphocytes in a 3D matrigel motility assay. Activated T cells are barely motile when incubated with the matrigel but EMP1 T cells exhibited a highly motile behaviour (Figure 5. 35). In order to evaluate this phenotype, EMP1 overexpressing encephalitogenic T cells ($T_{MBP\text{-}EMP1}$), were injected three different routes, i.e. intravenously, intraperitonealy and subcutaneously. It is well conceivable that the latter two milieus confront migrating T cells with distinct structural barriers before the effector T cells can enter their target organ via the blood circulation. Accordingly, the onset of clinical AT-EAE is significantly delayed if the T cells are applied s.c. and i.p. compared to i.v. (Figure 5. 36, Figure 5. 37and Figure 5. 38). Interestingly, EMP1 overexpression in effector T cells clearly accelerated the onset of disease when the T cells were transferred i.p. or s.c., but not after i.v. This could be explained by the early migration of EMP1 T cells from connective tissue to draining lymph nodes (Figure 5. 39) and hence their migration to blood circulation and subsequently early migration to the CNS.

Further investigations using knockdown or knockout approaches could more clearly elucidate the function of EMP1 in T cell function.

7. References

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Abbreviations

ACK	Ammonium Chloride Potassium erythrocyte lysis buffer
APC	Antigen presenting cells
ARHGAP4	Rho GTPase activating protein 4
ARHGEF18	Rho/Rac guanine nucleotide exchange factor 18
ARHGEF3	Rho guanine nucleotide exchange factor
AT-EAE	Adoptive transfer experimental auotimmune encephalomyelitis
BBB	Blood-brain barrier
BSA	Bovine serum albumin
BTG1	B-cell translocation gene 1
BTG2	B-cell translocation gene 2
CCL2	CC-chemokine ligand 2
CCL25	CC-chemokine ligand 25
CCNA1	Cyclin A1
CCNA2	Cyclin A2
CCNB1	Cyclin B1
CCNB2	Cyclin B2
CCNE1	Cyclin E1
CCNF	Cyclin F
CCR1	CC-chemokine receptor 1
CCR10	CC-chemokine receptor 10
CCR2	CC-chemokine receptor 2
CCR4	CC-chemokine receptor 4
CCR5	CC-chemokine receptor 5
CCR9	CC-chemokine receptor 9
CD44	CD44 antigen
CDC20	Cell division cycle 20 homolog
CFA	Complete Freund's Adjuvant
CKAP5	Cytoskeleton associated protein 5
CNS	Central nervous system
CXCL2	CXC-chemokine ligand 2
CXCR3	CXC-chemokine receptor 3
CYP51	Cytochrome P450, family 51
DAAM1	Dishevelled associated activator of morphogenesis 1
DMEM	Dulbecco’s Modified Eagle’s Medium
DUSP5	Dual specificity phosphatase 5
DUT	Deoxyuridine triphosphatase
EAE	Experimental autoimmune encephalomyelitis
EH	Eagle's HEPES medium
EL1	Extracellular loop 1
EL2	Extracellular loop 2
ELISA	Enzyme linked immunosorbent assay
EMP1	Epithelial membrane protein 1
EMP2	Epithelial membrane protein 2

EMP3	Epithelial membrane protein 3
ERK2	Mitogen-activated protein kinase 2
FACS	Fluorescent activated cell sorting
FHL2	Four and a half LIM domains 2
FOXP3	Forkhead box P3
GenMAPP	Gene map annotator and pathway profiler
GLIPR1	GLI pathogenesis-related 1
GM	Grey matter of CNS
GNAI	Guanine nucleotide binding protein alpha inhibiting activity polypeptide 1
GO	Gene Ontology
HBS	HEPES-buffered saline
HCl	Hydrochloric acid
HMGCR	3-hydroxy-3-methylglutaryl-Coenzyme A reductase
HXM	Hypoxanthine xanthine mycophenolic acid medium
i.e.	that is
i.p.	intra peritoneum
i.v.	intra venous
IF	Immunofluorescence
IFNγ	Interferon γ
IGSF6	Immunoglobulin superfamily, member 6
IH	Immunohistochemistry
IL10	Interleukin 10
IL17F	Interleukin 17F
IL2R	Interleukin 2 receptor
IL4	Interleukin 4
IP	Immunoprecipitation
ITGβ1	Integrin β1
ITGβ7	Integrin β7
KLF10	Krüppel-like factor 10
KLF2	Krüppel-like factor 2
KLF4	Krüppel-like factor 4
KLF9	Krüppel-like factor 9
KLRD1	Killer cell lectin-like receptor subfamily D, member 1
LATS1	Large tumour suppressor, homolog 1
mAb	Monoclonal antibody
MAdCAM1	Mucosal addressin cell adhesion molecule 1
MBP	Myelin basic protein
MCM4	Minichromosome maintenance complex component 4
MCM6	Minichromosome maintenance complex component 6
MCM7	Minichromosome maintenance complex component 7
MHC	Major histocompatibility complex
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
MSA	Multiple sequence alignment
MYC	Myelocytomatosis viral oncogene homolog

NRP1	Neuropilin 1
OVA	Ovalbumin
p.i.	post immunization
PAGE	Poly acrylamide gel electrophoresis
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PFA	Paraformaldehyde
PLAUR	Plasminogen activator, urokinase receptor
PLEK	Pleckstrin
PLSCR1	Phospholipid scramblase 1
PMP22	Peripheral myelin protein 22
PMVK	Phosphomevalonate kinase
PPMS	Primary progressive multiple sclerosis
PRMS	Progressive relapsing multiple sclerosis
q.s.	quantum sufficit
qPCR	Quantitative polymerase chain reaction
RAB34	Member RAS oncogene family RAB34
RBL1	Rat basophilic leukemia cell line 1
RM	Restimulation medium
RORγ	RAR-related orphan receptor γ
RRMS	Remitting relapsing multiple sclerosis
s.c.	sub-cutaneous
S100A4	S100 calcium binding protein A4
S100A9	S100 calcium binding protein A9
SDS	Sodium dodecyl sulphate
SELE	Selectin E
SELL	Selectin L
SELP	Selectin P
SLC16A1	Solute carrier family 16, member 1
SLC7A1	Solute carrier family 7, member 1
SPMS	Secondary progressive multiple sclerosis
SYTL1	Synaptotagmin-like 1
TBET	T-box expressed in T cells
TCGF	T cell growth factor
TCM	T cell medium
TFBS	Transcription factor binding sites
TFRC	Transferrin receptor
TJP1	Tight junction protein 1
TMP	Tumour membrane protein 1
TNFRSF1B	Tumour necrosis factor receptor superfamily, member 1B
TNFα	Tumour necrosis factor α
TOB1	Transducer of ERBB2, 1
TSS	Trascription start site
TXK	T cell-specific non- receptor TXK tyrosine kinases
VCAM1	Vascular cell adhesion molecule 1
VCL	Vinculin

WB	Western blot
WM	White matter of the CNS
ZYX	Zyxin

Appendix 1.

List of genes differentially regulated in $T_{activated}$ state.

Gene Symbol	$T_{activated}$	Biological Process
Abca2	0.51	Transport
Abca7	0.54	Transport
Abcg1	0.44	Transport, Lipid transport
Abtb1	0.49	Metabolism, Protein metabolism
Aff4	0.43	Metabolism, nucleotide and nucleic acid metabolism
Agpat3	0.52	Metabolism
Ahnak	0.36	Cell growth, cell differentiation, Muscle contraction
Akap8l	0.38	Metabolism, nucleotide and nucleic acid metabolism
Amigo	0.52	Cell adhesion
Apbb1ip	0.45	Cell communication
Apoe	0.43	Transport
Arhgap4	0.43	Cell communication
Arhgap9	0.49	Cell communication
Arhgef1	0.47	Cell communication
Arhgef18	0.43	Cell communication
Arid4a	0.52	Metabolism, nucleotide and nucleic acid metabolism
Arid5a	0.45	Metabolism, nucleotide and nucleic acid metabolism
Asb2	0.27	Cell communication
Ash1l	0.51	Metabolism, nucleotide and nucleic acid metabolism
Atg16l1	0.54	Transport
Atp2a3	0.51	Transport, Ion transport
Atp2b1	0.55	Transport
B2m	0.44	Immune response
B3galt4	0.54	Metabolism, Protein metabolism
Bcl11b	0.34	Metabolism, nucleotide and nucleic acid metabolism
Bcl6	0.54	Apoptosis
Bcl9l	0.42	Biological process unknown
Bet1l	0.53	Transport
Bin2	0.53	Cell communication
Btg1	0.40	Cell growth and/or maintenance
Btg2	0.46	Metabolism, nucleotide and nucleic acid metabolism
C1qr1	0.42	Cell adhesion
Calca	0.49	Metabolism, Regulation of physiological process
Calcoco1	0.33	Metabolism, nucleotide and nucleic acid metabolism
Card11	0.54	Apoptosis
Cbx7	0.43	Metabolism, nucleotide and nucleic acid metabolism
Ccl3	0.22	Immune response
Ccl5	0.07	Cell communication
Ccnl2	0.49	Metabolism, nucleotide and nucleic acid metabolism

Gene Symbol	$T_{activated}$	Biological Process
Ccnt2	0.54	Metabolism, nucleotide and nucleic acid metabolism
Ccpg1	0.33	Biological process unknown
Ccr2	0.38	Cell communication
Ccr3	0.31	signal transduction,
Ccr5	0.14	Cell communication
Ccr6	0.35	Cell communication
Cd2	0.34	Immune response
Cd37	0.49	Immune response
Cd38	0.50	Metabolism
Cd3d	0.53	Immune response
Cd4	0.47	Immune response
Cd44	0.35	Cell communication
Cd5	0.52	Immune response
Cd53	0.54	Immune response
Cd69	0.37	Immune response
Cd97	0.51	Cell communication
Cdc2l6	0.54	Cell communication
Cdc42ep3	0.39	Cell communication
Cdkn1b	0.44	Cell communication
Centd1	0.50	Cell communication
Cerk	0.38	Cell communication
Chd3	0.42	Metabolism, nucleotide and nucleic acid metabolism
Ches1	0.55	Cell communication
Chsy1	0.46	Metabolism
Churc1	0.50	Metabolism, nucleotide and nucleic acid metabolism
Cish	0.46	Cell communication
Cited2	0.53	Metabolism, nucleotide and nucleic acid metabolism
Ckb	0.33	Metabolism
Cklf	0.51	Immune response, Inflammatory response
Clk1	0.42	Signal transduction
Clk4	0.47	Cell communication
Coq6	0.46	Metabolism
Cox7a2l	0.47	Metabolism
Cpd	0.20	Metabolism, Protein metabolism
Cpe	0.42	Metabolism, Protein metabolism
Cpeb2	0.36	Metabolism, nucleotide and nucleic acid metabolism
Cpt1a	0.51	Transport, Mitochondrial transport
Crebl2	0.51	Metabolism, nucleotide and nucleic acid metabolism
Crot	0.49	Metabolism
Cst3	0.46	Metabolism, Protein metabolism
Ctnnd2	0.54	Cell communication
Ctse	0.34	Metabolism, Protein metabolism
Ctss	0.42	Metabolism, Protein metabolism
Ctsw	0.25	Metabolism, Protein metabolism

Gene Symbol	$T_{activated}$	Biological Process
Cxcr3	0.32	Cell communication
Cyb561d1	0.54	Biological process unknown
Daam1	0.34	Cell communication
Dap	0.48	Apoptosis
Dbp	0.19	Metabolism, nucleotide and nucleic acid metabolism
Ddx17	0.54	Metabolism, nucleotide and nucleic acid metabolism
Dgka	0.39	Cell communication
Dhrs3	0.55	Metabolism
Dnajb9	0.35	Metabolism, Protein metabolism
Dok2	0.36	Signal transduction
Dpp7	0.32	Metabolism, Protein metabolism
Dusp16	0.54	Cell communication
Dusp2	0.28	Cell communication
Dyrk2	0.53	Cell communication
Ech1	0.55	Metabolism
Edg1	0.42	Cell communication
Eef2k	0.48	Metabolism, Protein metabolism
Egr1	0.45	Metabolism, nucleotide and nucleic acid metabolism
Emp1	0.45	Cell growth and/or maintenance
Evl	0.20	Cell growth and/or maintenance
Eya2	0.48	Apoptosis
Ezh1	0.44	Metabolism, nucleotide and nucleic acid metabolism
F2r	0.43	Cell communication
Farp1	0.51	Cell growth and/or maintenance
Fbxl11	0.54	Metabolism, Protein metabolism
Fbxl17	0.45	Metabolism, Protein metabolism
Fbxl3	0.45	Metabolism, Protein metabolism
Fbxo11	0.51	Metabolism, Protein metabolism
Fgl2	0.35	Cell growth and/or maintenance
Flcn	0.54	Biological process unknown
Fli1	0.49	Metabolism, nucleotide and nucleic acid metabolism
Fnbp1	0.53	Biological process unknown
Fos	0.45	Metabolism, nucleotide and nucleic acid metabolism
Foxo1a	0.49	Metabolism, nucleotide and nucleic acid metabolism
Fut8	0.53	Metabolism
Fxyd5	0.52	Transport, Ion transport, Regulation of cellular process
Fyco1	0.53	Cell communication
Fyn	0.46	Cell communication
Gabbr1	0.36	Cell communication
Gbp2	0.48	Cell communication
Gda	0.34	Metabolism
Gdi1	0.40	Cell communication
Ggt1	0.54	Metabolism
Gimap5	0.48	Apoptosis

Gene Symbol	$T_{activated}$	Biological Process
Gimap6	0.54	Signal transduction
Gimap7	0.32	Cell communication
Git2	0.51	Cell communication
Glipr1	0.34	Immune response
Gmfg	0.35	Cell communication
Gns	0.49	Metabolism
Gpsm3	0.44	Cell communication
Grina	0.44	Signal transduction
Gsn	0.19	Cell growth and/or maintenance
Hbp1	0.38	Metabolism, nucleotide and nucleic acid metabolism
Hcst	0.18	Cell communication
Hip1	0.52	Cell growth and/or maintenance
Hpse	0.32	Metabolism
Id2	0.20	Metabolism, nucleotide and nucleic acid metabolism
Ifi44	0.55	Biological process unknown
Il16	0.37	Immune response
Il17re	0.51	Signal transduction
Inpp4a	0.55	Metabolism
Irak2	0.38	Cell communication
Irf1	0.41	Metabolism, nucleotide and nucleic acid metabolism
Irf3	0.45	Metabolism, nucleotide and nucleic acid metabolism
Itgb1	0.38	Cell communication
Itgb2	0.41	Cell communication
Itgb7	0.44	Cell communication
Itm2b	0.53	Cell growth and/or maintenance
Itm2c	0.30	Biological process unknown
Klf10	0.55	cell growth
Klf3	0.51	Metabolism, nucleotide and nucleic acid metabolism
Klf9	0.43	Metabolism, nucleotide and nucleic acid metabolism
Kpna1	0.52	Transport
Lamp1	0.48	Biological process unknown
Laptm5	0.42	Cell communication
Lasp1	0.51	Signal transduction
Lcp2	0.54	Cell communication
Leprot	0.55	Biological process unknown
Lgals3bp	0.21	Immune response
Lgals8	0.43	Signal transduction
Lmbr1l	0.49	Cell communication
Lmbrd1	0.53	Biological process unknown
Lrp10	0.52	Biological process unknown
Ltb	0.28	Immune response, Inflammatory response
Maf	0.25	Regulation of gene expression, epigenetic
Mll	0.52	Metabolism, nucleotide and nucleic acid metabolism
Mll5	0.50	cell growth

Gene Symbol	$T_{activated}$	Biological Process
Mmp13	0.18	Metabolism, Protein metabolism
Mmp9	0.44	Metabolism, Protein metabolism
Mx1	0.29	Cell communication
Myadm	0.41	Biological process unknown
Myd88	0.51	Cell communication
Myo1d	0.55	Cell growth and/or maintenance
Nbr1	0.50	Cell communication
Ncor1	0.48	Metabolism, nucleotide and nucleic acid metabolism
Ndrg4	0.43	Biological process unknown, Cell differentiation
Nedd9	0.53	Cell communication
Nfe2l2	0.52	Metabolism, nucleotide and nucleic acid metabolism
Nr1d1	0.19	Cell communication
Nr1d2	0.54	Metabolism, nucleotide and nucleic acid metabolism
Nr1h2	0.54	Cell communication
Ogt	0.48	Metabolism, Protein metabolism
Olfm1	0.46	Biological process unknown
Optn	0.50	Metabolism, nucleotide and nucleic acid metabolism
Otud5	0.51	Metabolism, Protein metabolism
Pacs1	0.51	Transport
Pbxip1	0.36	Metabolism, nucleotide and nucleic acid metabolism
Pcaf	0.52	Metabolism, nucleotide and nucleic acid metabolism
Pctk2	0.47	Cell communication
Pdcd4	0.48	Apoptosis
Pde3b	0.38	Metabolism
Pde4b	0.31	Signal transduction
Phf1	0.36	Metabolism, nucleotide and nucleic acid metabolism
Picalm	0.52	Transport, Regulation of endocytosis
Pip5k1c	0.45	Cell communication
Pitpnm1	0.35	Metabolism, Lipid metabolism
Pkn1	0.49	Cell communication
Plekha3	0.55	Cell communication
Pnrc1	0.42	Biological process unknown
Pon2	0.54	Metabolism, Lipid metabolism
Ppp1r9b	0.49	Cell communication
Ppt1	0.46	Metabolism
Prei3	0.52	Biological process unknown
Prf1	0.49	Transport
Prkd2	0.52	Cell communication
Prnp	0.41	Metabolism
Psap	0.49	Cell communication
Ptpn18	0.49	Cell communication
Ptprc	0.45	Cell communication
Ptpre	0.47	Cell communication
Pycard	0.33	Apoptosis

Gene Symbol	$T_{activated}$	Biological Process
Rab24	0.53	Cell communication
Rab2b	0.54	Cell communication
Rab7l1	0.44	Cell communication
Rabac1	0.44	Transport
Ramp1	0.25	Cell communication
Ramp3	0.52	Cell communication
Rasa3	0.42	Cell communication
Rasgrp1	0.53	Cell communication
Rbm5	0.46	Metabolism, nucleotide and nucleic acid metabolism
Rgs2	0.39	Cell communication
Rhoh	0.49	Cell communication
Ric8a	0.50	Cell communication
Rnf146	0.51	Metabolism, Protein metabolism
Rnf44	0.47	Metabolism, Protein metabolism
Rps6ka1	0.52	Cell communication
Runx1	0.49	Metabolism, nucleotide and nucleic acid metabolism
S100a4	0.41	Cell growth and/or maintenance
S100a6	0.28	Cell communication
Sdc1	0.40	Cell communication
Sema4a	0.41	Cell communication
Serinc1	0.42	Biological process unknown
Serinc3	0.52	Biological process unknown
Sf3b1	0.39	Metabolism, nucleotide and nucleic acid metabolism
Sfrs5	0.52	Metabolism, nucleotide and nucleic acid metabolism
Sh3bp5	0.53	Signal transduction
Sh3kbp1	0.50	Signal transduction
Sirt2	0.53	Cell communication
Sla	0.51	Cell communication
Slc12a6	0.55	Transport
Slc2a3	0.51	Transport
Slc4a7	0.55	Transport
Slc6a6	0.54	Transport
Slco4a1	0.52	Transport
Smad4	0.50	Metabolism, nucleotide and nucleic acid metabolism
Smpd1	0.47	Signal transduction
Smpdl3a	0.43	Metabolism
Snapap	0.49	Transport
Sos2	0.52	Cell communication
Sqstm1	0.47	Metabolism, Protein metabolism
Ssbp3	0.37	Metabolism, DNA metabolism
Sstr3	0.32	Cell communication
Stat1	0.48	Metabolism, nucleotide and nucleic acid metabolism
Stat3	0.48	Metabolism, nucleotide and nucleic acid metabolism
Stk10	0.44	Cell communication

Gene Symbol	$T_{activated}$	Biological Process
Stk17b	0.43	Cell communication
Stom	0.54	Cell communication
Stxbp3	0.52	Transport
Sv2b	0.34	Transport
Sytl1	0.47	Transport, Vesicle docking
Tacc2	0.42	Cell growth and/or maintenance
Tapbp	0.54	Protein folding
Tcp11l2	0.18	Biological process unknown
Tegt	0.52	Apoptosis
Tgoln2	0.46	Transport
Tle4	0.54	Metabolism, nucleotide and nucleic acid metabolism
Tloc1	0.47	Metabolism, Protein metabolism
Tmem106b	0.51	Biological process unknown
Tmem49	0.38	Apoptosis
Tnfaip8l2	0.54	Biological process unknown
Tnfrsf1b	0.50	Cell communication
Tor1aip1	0.47	Biological process unknown
Tpp1	0.46	Metabolism, Protein metabolism
Tpst2	0.44	Energy pathways
Tsc22d3	0.49	Regulation of gene expression, epigenetic
Txnip	0.18	Cell communication
Ubl3	0.38	Metabolism, Protein metabolism
Wasl	0.52	Cell growth and/or maintenance
Wdr23	0.48	Biological process unknown
Xdh	0.39	Metabolism
Xylt2	0.49	Metabolism
Ypel5	0.35	Biological process unknown
Zap70	0.54	Cell communication
Zbp1	0.31	Metabolism, nucleotide and nucleic acid metabolism
Zfand3	0.41	Metabolism, nucleotide and nucleic acid metabolism
Zfp36	0.43	Metabolism, nucleotide and nucleic acid metabolism
Zfp36l1	0.34	Metabolism, nucleotide and nucleic acid metabolism
Zfp36l2	0.41	Metabolism, nucleotide and nucleic acid metabolism
Znf292	0.44	Transcription
Znrf1	0.49	Metabolism, Protein metabolism
Zyx	0.38	Signal transduction
Aaas	2.84	Cell communication
Aatf	2.32	Apoptosis
Abce1	2.47	Transport
Acaca	2.60	Metabolism
Acat2	4.07	Metabolism
Acly	2.48	Metabolism
Acsl3	2.15	Metabolims, Fatty acid metabolism
Acy1	2.69	Metabolism

Gene Symbol	$T_{activated}$	Biological Process
Adam8	1.97	Metabolism, Protein metabolism
Adk	3.23	Metabolism, nucleotide and nucleic acid metabolism
Adsl	2.08	Metabolism
Ahcy	4.53	Metabolism
Ak2	3.00	Metabolism
Aldh7a1	3.22	Metabolism
Aloxe3	5.09	Metabolism
Anapc5	2.30	Cell communication
Anp32b	2.41	Biological process unknown
Anp32e	2.04	Biological process unknown
Anxa7	2.05	Transport
Ap1s1	1.97	Transport
Apex1	3.60	Metabolism, nucleotide and nucleic acid metabolism
Appbp1	1.97	Cell communication
Asf1a	2.13	Metabolism, Protein metabolism
Asf1b	8.08	Metabolism, Protein metabolism
Ash2l	2.19	Metabolism, nucleotide and nucleic acid metabolism
Asns	3.02	Metabolism, Protein metabolism
Aspm	5.31	Biological process unknown
Atad3a	2.30	Metabolism
Atp5b	2.46	Metabolism
Aurka	4.70	Cell communication
Aurkb	2.67	Cell communication
Banf1	2.41	Biological process unknown
Bcat1	3.86	Metabolism
Bcs1l	2.12	Metabolism
Bex1	2.21	Biological process unknown
Birc5	3.78	Cell communication
Blm	4.45	Metabolism, nucleotide and nucleic acid metabolism
Bop1	2.56	Biological process unknown
Bpnt1	2.34	Cell communication
Bspry	3.39	Biological process unknown
Bub1	9.65	Cell communication
Bub1b	7.18	Cell communication
Bxdc1	2.88	Biological process unknown
Bxdc2	2.33	Biological process unknown
Bysl	2.12	Cell growth and/or maintenance
Bzw2	2.07	Metabolism, Protein metabolism
C1qbp	3.33	Immune response
Cacybp	3.32	Metabolism, Protein metabolism
Cad	3.03	Metabolism
Cand1	2.00	Metabolism, nucleotide and nucleic acid metabolism
Cars	2.96	Metabolism
Casp4	2.35	Apoptosis

Gene Symbol	$T_{activated}$	Biological Process
Cbr1	1.96	Metabolism
Cbx5	2.47	Metabolism, nucleotide and nucleic acid metabolism
Ccdc5	2.54	Cell growth and/or maintenance
Ccl2	1.98	Immune response
Ccna2	13.85	Cell communication
Ccnb1	10.06	Cell communication
Ccnb2	8.44	Cell communication
Ccne1	2.60	Cell communication
Ccne2	2.15	Cell cycle
Ccnf	2.52	Cell communication
Cct2	2.35	Metabolism, Protein metabolism
Cct4	2.17	Metabolism, Protein metabolism
Cct5	2.33	Metabolism, Protein metabolism
Cct7	2.07	Protein folding
Cct8	3.38	Metabolism, Protein metabolism
Cd200	2.20	Immune response
Cd74	2.04	Immune response
Cdc20	6.08	Cell cycle
Cdca2	4.12	Biological process unknown
Cdca4	2.00	Cell division, fate commitment
Cdca7	4.95	Metabolism, nucleotide and nucleic acid metabolism
Cdca8	6.33	Cell communication
Cdkn1a	3.20	Cell cycle
Cenpe	2.18	Metabolism, nucleotide and nucleic acid metabolism
Chchd6	2.63	Biological process unknown
Chek1	5.79	Cell communication
Chtf18	2.41	Metabolism, nucleotide and nucleic acid metabolism
Cit	2.47	Cell communication
Ckap2	5.44	Cell growth and/or maintenance
Ckap5	1.97	Cell cycle, Mitosis
Clic4	2.05	Transport
Clspn	2.32	Cell communication
Cndp2	1.99	Metabolism, Protein metabolism
Cops5	2.17	Metabolism, Protein metabolism
Creld2	2.05	Cell communication
Csda	2.45	Metabolism, nucleotide and nucleic acid metabolism
Cse1l	1.99	Transport
Csrp1	2.07	Cell communication
Ctps	4.16	Metabolism
Ctsz	1.95	Metabolism, Protein metabolism
Cyb5b	2.52	Transport
Cyc1	2.44	Metabolism
Cycs	2.84	Metabolism
Dars	2.22	Metabolism

Gene Symbol	$T_{activated}$	Biological Process
Dazap1	2.29	Metabolism, nucleotide and nucleic acid metabolism
Dbi	3.06	Metabolism
Dcps	1.99	Metabolism
Ddx1	2.43	Metabolism, nucleotide and nucleic acid metabolism
Ddx11	2.69	Metabolism, nucleotide and nucleic acid metabolism
Ddx18	2.00	Transport
Ddx39	2.30	Metabolism, nucleotide and nucleic acid metabolism
Ddx56	2.27	Metabolism, nucleotide and nucleic acid metabolism
Depdc1b	2.93	Biological process unknown
Dhcr24	2.62	Metabolism
Dhcr7	3.31	Metabolism
Dhfr	3.12	Metabolism, nucleotide and nucleic acid metabolism
Dkc1	2.25	Metabolism, nucleotide and nucleic acid metabolism
Dlg7	5.04	Cell cycle
Dnajc9	2.56	Metabolism, Protein metabolism
Dnd1	2.53	Metabolism, nucleotide and nucleic acid metabolism
Dnmt1	3.42	Metabolism, nucleotide and nucleic acid metabolism
Dph5	2.49	Biological process unknown
Drg1	2.43	Cell growth and/or maintenance
Dut	4.72	DNA replication
E2f8	3.04	Cell cycle
Ebna1bp2	3.71	Biological process unknown
Echdc1	2.49	Biological process unknown
Ect2	8.63	Cell communication
Eef1d	2.01	Cell communication
Eftud2	2.10	Metabolism, Protein metabolism
Eif2a	2.68	Metabolism, Protein metabolism
Eif2b3	2.54	Metabolism, Protein metabolism
Eif2s1	2.33	Metabolism, Protein metabolism
Eif2s2	2.49	Metabolism, Protein metabolism
Eif3s6ip	2.55	Metabolism, Protein metabolism
Eif3s7	2.14	Metabolism, Protein metabolism
Eif3s8	2.41	Metabolism, Protein metabolism
Eif3s9	2.42	Metabolism, Protein metabolism
Eif4a1	2.55	Metabolism, Protein metabolism
Eif4ebp1	2.18	Metabolism, Protein metabolism
Eif5a	2.48	Metabolism, Protein metabolism
Elovl6	2.45	Biological process unknown
Emg1	3.42	Metabolism, Protein metabolism
Eprs	2.53	Metabolism, Protein metabolism
Erp29	1.97	Protein folding
Esd	2.53	Metabolism
Espl1	9.86	Metabolism, Protein metabolism
Etf1	2.11	Metabolism, Protein metabolism

Gene Symbol	$T_{activated}$	Biological Process
Ets1	0.55	Metabolism, nucleotide and nucleic acid metabolism
Exo1	4.14	Metabolism, nucleotide and nucleic acid metabolism
Exosc7	2.81	Metabolism, nucleotide and nucleic acid metabolism
Exosc8	2.74	Metabolism, nucleotide and nucleic acid metabolism
Fabp5	27.18	Transport
Fads1	2.10	Metabolism
Fancd2	4.00	Cell communication
Fasn	3.65	Metabolism
Fbl	3.76	Metabolism, nucleotide and nucleic acid metabolism
Fbxo5	2.22	Cell communication
Fdft1	3.61	Metabolism
Fdps	5.10	Metabolism
Fen1	4.01	Metabolism, nucleotide and nucleic acid metabolism
Fkbp11	2.03	Metabolism
Fkbp3	2.64	Metabolism
Fkbp4	3.02	Metabolism
Foxm1	2.24	Metabolism, nucleotide and nucleic acid metabolism
Foxq1	2.01	Metabolism, nucleotide and nucleic acid metabolism
Ftsj3	2.01	Biological process unknown
Fubp1	1.97	Metabolism, nucleotide and nucleic acid metabolism
Fxc1	2.28	Transport
Fxn	2.54	Transport
Fzr1	2.08	Cell communication
Galk1	3.59	Metabolism
Gars	2.91	Metabolism, Protein metabolism
Gart	2.40	Metabolism, nucleotide and nucleic acid metabolism
Gcsh	6.03	Metabolism
Gfer	2.39	Cell communication
Gmps	2.84	Metabolism
Gnai1	2.76	Cell communication
Gnl3	2.60	Cell cycle, Regulation of cell proliferation
Gpd2	2.48	Metabolism
Gpr19	2.32	Cell communication
Gzmb	2.00	Metabolism, Protein metabolism
Hat1	4.03	Metabolism
Hdgf	2.54	Cell communication
Hey1	2.43	Metabolism, nucleotide and nucleic acid metabolism
Hirip3	3.33	Metabolism, nucleotide and nucleic acid metabolism
Hmga1	2.21	Metabolism, nucleotide and nucleic acid metabolism
Hmgcr	2.23	Metabolism
Hmgcs1	4.85	Metabolism
Hmgn1	3.66	Metabolism, nucleotide and nucleic acid metabolism
Hmgn2	3.69	Metabolism, nucleotide and nucleic acid metabolism
Hmgn3	2.39	Metabolism, nucleotide and nucleic acid metabolism

Gene Symbol	$T_{activated}$	Biological Process
Hmmr	4.82	Cell communication
Hnrpa1	3.73	Metabolism, nucleotide and nucleic acid metabolism
Hnrpab	2.22	Metabolism, nucleotide and nucleic acid metabolism
Hnrpm	2.13	Metabolism, nucleotide and nucleic acid metabolism
Hsd17b12	3.31	Metabolism
Hspa14	2.44	Biological process unknown
Hspd1	3.54	Protein folding
Hspe1	4.00	Metabolism, Protein metabolism
Hsph1	2.22	Metabolism, Protein metabolism
Idi1	6.70	Metabolism
Ift74	2.01	Cell growth and/or maintenance
Il17f	5.58	Immune response
Ilf2	3.37	Metabolism, nucleotide and nucleic acid metabolism
Impdh2	2.98	Metabolism
Insig1	2.35	Metabolism, Lipid metabolism
Josd3	2.17	Cell cycle
Kdr	4.01	Cell communication
Kif11	4.72	Cell growth and/or maintenance
Kif15	7.22	Cell communication
Kif22	8.07	Metabolism, nucleotide and nucleic acid metabolism
Kif2c	5.31	Cell growth and/or maintenance
Kpna2	4.76	Cell communication
Kpnb1	2.73	Transport
Lgals3	2.12	Metabolism, nucleotide and nucleic acid metabolism
Lig1	3.86	Metabolism, nucleotide and nucleic acid metabolism
Lmna	2.61	Cell growth and/or maintenance
Lmnb1	3.36	Cell growth and/or maintenance
Lrrc59	2.59	Biological process unknown
Ltv1	2.61	Biological process unknown
Mad2l2	2.10	Cell cycle
Mccc2	2.09	Metabolism
Mcm4	4.36	Metabolism, nucleotide and nucleic acid metabolism
Mcm6	4.49	Metabolism, nucleotide and nucleic acid metabolism
Mcm7	6.68	DNA replication
Mesdc1	2.24	Biological process unknown
Mki67ip	2.09	Metabolism, nucleotide and nucleic acid metabolism
Mrpl12	3.07	Metabolism, nucleotide and nucleic acid metabolism
Mrpl17	1.99	Metabolism, Protein metabolism
Mrpl19	2.01	Metabolism, Protein metabolism
Mrpl23	2.26	Metabolism, Protein metabolism
Mrpl37	2.18	Metabolism, Protein metabolism
Mrpl38	2.51	Metabolism, Protein metabolism
Mrpl47	2.03	Metabolism, Protein metabolism
Mrpl49	1.95	Metabolism, Protein metabolism

Gene Symbol	$T_{activated}$	Biological Process
Mrps15	2.03	Metabolism, Protein metabolism
Mrps18b	2.49	Metabolism, Protein metabolism
Mrps25	2.02	Metabolism, Protein metabolism
Msh2	2.07	Metabolism, nucleotide and nucleic acid metabolism
Mt1a	2.74	Metabolism
Mthfd1	5.31	Metabolism
Mvd	4.12	Metabolism
Mybbp1a	3.12	Metabolism, nucleotide and nucleic acid metabolism
Myc	2.81	Metabolism, nucleotide and nucleic acid metabolism
Myo1g	2.49	Cell growth and/or maintenance
Nap1l1	2.34	Metabolism, nucleotide and nucleic acid metabolism
Nars	2.67	Metabolism, Protein metabolism
Nasp	4.98	Cell cycle
Ncl	2.32	Metabolism, nucleotide and nucleic acid metabolism
Ndufa11	2.11	Metabolism
Nedd4	2.50	Metabolism, Protein metabolism
Net1	4.38	Cell communication
Nfil3	2.01	Metabolism, nucleotide and nucleic acid metabolism
Nkg7	5.34	Biological process unknown
Nme1	2.48	Metabolism
Nme2	4.28	Signal transduction
Nol5a	3.45	Metabolism, nucleotide and nucleic acid metabolism
Nolc1	4.13	Metabolism, nucleotide and nucleic acid metabolism
Npm1	2.23	Metabolism, Protein metabolism
Nrm	1.96	Biological process unknown
Nsdhl	2.20	Metabolism
Nudc	3.48	Cell communication
Nup107	2.34	Transport
Nup155	3.24	Transport
Nup35	2.38	Transport
Nup88	2.13	Transport
Nup93	3.37	Transport
Oat	2.68	Metabolism
Odc1	3.63	Metabolism
Oprs1	2.09	Cell communication
Orc6l	2.77	Metabolism, nucleotide and nucleic acid metabolism
Pa2g4	4.01	Metabolism, nucleotide and nucleic acid metabolism
Pabpc4	3.38	Metabolism, nucleotide and nucleic acid metabolism
Paics	2.78	Metabolism
Pbef1	2.35	Anti-apoptosis
Pcca	2.74	Metabolism
Pcna	3.32	DNA repair
Pebp1	2.00	Cell communication
Pfkp	1.95	Metabolism

Gene Symbol	$T_{activated}$	Biological Process
Pgd	2.02	Metabolism
Phb	3.40	Cell communication
Phb2	2.29	Metabolism, nucleotide and nucleic acid metabolism
Phgdh	5.03	Metabolism
Pi4k2b	2.01	Cell communication
Plk1	5.11	Cell communication
Plscr1	2.93	Cell communication
Pmvk	2.13	Metabolism
Pola1	3.43	Metabolism, nucleotide and nucleic acid metabolism
Pola2	3.44	Metabolism, nucleotide and nucleic acid metabolism
Pold1	4.42	Metabolism, nucleotide and nucleic acid metabolism
Pold2	2.50	Metabolism, nucleotide and nucleic acid metabolism
Pole	2.93	Metabolism, nucleotide and nucleic acid metabolism
Pole3	2.43	Metabolism, nucleotide and nucleic acid metabolism
Polr2f	2.53	Metabolism, nucleotide and nucleic acid metabolism
Ppat	1.98	Metabolism
Ppid	2.55	Metabolism
Ppp1r14b	2.26	Metabolism
Ppp1r7	1.98	Metabolism, nucleotide and nucleic acid metabolism
Ppp3cb	2.18	Cell communication
Prdx1	2.71	Metabolism
Prdx4	2.45	Metabolism
Prim1	4.28	Metabolism, nucleotide and nucleic acid metabolism
Prps1	3.89	Metabolism
Psat1	19.58	Metabolism
Psmb6	2.09	Metabolism, Protein metabolism
Psmb7	2.20	Metabolism, Protein metabolism
Psmc5	2.00	Metabolism, Protein metabolism
Psmc6	2.62	Metabolism, Protein metabolism
Psmd1	2.42	Metabolism, Protein metabolism
Psmd12	2.14	Metabolism, Protein metabolism
Psme3	2.05	Metabolism, Protein metabolism
Psph	2.45	Metabolism
Ptma	2.14	Cell cycle, Cell proliferation
Ptpn5	3.71	Cell communication
Pttg1	5.99	Metabolism, nucleotide and nucleic acid metabolism
Qdpr	2.62	Metabolism
Rab34	2.04	Cell communication
Rae1	1.96	Metabolism, nucleotide and nucleic acid metabolism
Ran	3.02	Cell communication
Rangap1	2.46	Cell communication
Rassf4	2.73	Biological process unknown
Rbbp7	2.22	Metabolism, nucleotide and nucleic acid metabolism
Rbm14	2.72	Metabolism, nucleotide and nucleic acid metabolism

Gene Symbol	$T_{activated}$	Biological Process
Rexo2	2.38	Metabolism, nucleotide and nucleic acid metabolism
Rfc3	3.17	DNA replication
Rhebl1	2.66	Cell communication
Rnf126	2.23	Metabolism, Protein metabolism
Rnps1	2.42	Metabolism, nucleotide and nucleic acid metabolism
Rpa2	2.19	Metabolism, nucleotide and nucleic acid metabolism
Rrm1	4.53	Metabolism, nucleotide and nucleic acid metabolism
Rrm2	16.7	Metabolism, nucleotide and nucleic acid metabolism
Ruvbl1	4.06	Metabolism, nucleotide and nucleic acid metabolism
Ruvbl2	3.35	Metabolism, nucleotide and nucleic acid metabolism
Samm50	2.06	Biological process unknown
Sc4mol	4.05	Metabolims, Fatty acid metabolism
Scarb1	2.87	Cell communication
Scye1	2.06	Immune response
Sfrs2	2.37	Metabolism, nucleotide and nucleic acid metabolism
Shmt1	2.75	Metabolism
Shmt2	4.94	Metabolism
Slc16a1	2.05	Transport
Slc16a3	2.01	Transport
Slc25a4	3.96	Transport
Slc7a1	2.35	Transport
Slc7a5	4.47	Transport
Smagp	2.47	Cell adhesion
Smn1	2.39	Metabolism, nucleotide and nucleic acid metabolism
Sms	2.66	Metabolism
Snrpa	4.95	Metabolism, nucleotide and nucleic acid metabolism
Spag5	4.38	Cell growth and/or maintenance
Sqle	2.85	Metabolism
Srm	3.80	Metabolism
Srpk1	2.42	Metabolism, nucleotide and nucleic acid metabolism
Ssbp1	2.39	Metabolism, nucleotide and nucleic acid metabolism
St13	2.47	Cell communication
Stip1	2.16	Cell communication
Stmn1	7.59	Cell growth and/or maintenance
Stoml2	2.30	Transport
Stra6	2.14	Biological process unknown
Sympk	2.26	Cell growth and/or maintenance
Syncrip	2.43	Metabolism, nucleotide and nucleic acid metabolism
Taf9	2.44	Transcription
Tardbp	2.56	Metabolism, nucleotide and nucleic acid metabolism
Tars	2.81	Metabolism
Tbl3	2.35	Biological process unknown
Tbrg4	2.17	Cell communication
Tcp1	3.69	Metabolism, Protein metabolism

Gene Symbol	$T_{activated}$	Biological Process
Tdp1	2.01	DNA repair
Tfpi	2.18	Metabolism, Protein metabolism
Tfrc	7.14	Transport
Thop1	4.88	Metabolism, Protein metabolism
Timeless	3.38	Metabolism, nucleotide and nucleic acid metabolism
Timm10	2.28	Metabolism, Protein metabolism
Timm13	2.02	Transport
Timm8a	3.52	Metabolism
Tk1	6.64	Metabolism
Tkt	2.61	Metabolism
Tm7sf2	2.79	Metabolism
Tmem147	1.97	Biological process unknown
Tmem97	5.13	Cell cycle, Cell proliferation
Tmpo	2.00	Metabolism, Nuclear organization and biogenesis
Tnfrsf8	6.01	Cell communication
Tnpo1	2.40	Transport
Tomm40	2.28	Transport
Tomm70a	2.12	Transport
Top1mt	1.99	Metabolism, nucleotide and nucleic acid metabolism
Top2a	7.33	Metabolism, nucleotide and nucleic acid metabolism
Trap1	1.99	Metabolism, Protein metabolism
Trnt1	2.08	Metabolism
Troap	2.23	Cell adhesion
Tuba4a	3.18	Cell growth and/or maintenance
Tubb2c	4.95	Cell growth and/or maintenance
Tubb6	2.37	Cell growth and/or maintenance
Tubg1	2.50	Cell growth and/or maintenance
Tusc3	2.30	Cell communication
Txnl1	2.33	Metabolism
Txnl2	2.90	Biological process unknown
Txnrd1	2.13	Metabolism
Tyms	2.71	Metabolism
Ube2v2	2.08	Metabolism, Protein metabolism
Uchl5	3.42	Metabolism, Protein metabolism
Uhrf1	6.35	Metabolism, nucleotide and nucleic acid metabolism
Umps	2.58	Metabolism
Ung	3.04	DNA repair
Usp1	1.96	Metabolism, Protein metabolism
Usp10	1.98	Metabolism, Protein metabolism
Utp14a	1.98	Biological process unknown
Vars2	2.55	Metabolism
Vdac1	3.07	Transport
Vrk1	3.44	Cell communication
Wdr12	2.05	Biological process unknown

Gene Symbol	**$T_{activated}$**	**Biological Process**
Wdr18	2.57	Biological process unknown
Wdr77	2.07	Biological process unknown
Xpo1	4.09	Cell communication
Yars	2.75	Metabolism
Ywhae	2.05	Cell communication

Appendix 2.

List of genes differentially regulated in $T_{migratory}$ state.

Gene Symbol	$T_{migratory}$	Biological Process
Aaas	0.37	Cell communication
Aamp	0.46	Cell adhesion
Aatf	0.49	Apoptosis
Abce1	0.46	Transport
Abcf2	0.43	Immune response
Abhd8	0.48	Biological process unknown
Abi2	0.54	Cell communication
Acaca	0.35	Metabolism
Acat2	0.16	Metabolism
Acly	0.45	Metabolism
Acp6	0.51	Metabolism
Acsl3	0.34	Metabolism, Fatty acid metabolism
Acss2	0.42	Metabolism
Acy1	0.36	Metabolism
Adfp	0.37	Metabolism, Lipid storage
Adk	0.41	Nucleotide and nucleic acid metabolism
Adsl	0.49	Metabolism
Ahctf1	0.53	Nucleotide and nucleic acid metabolism
Ahcy	0.31	Metabolism
Ahcyl1	0.46	Metabolism
Ak3l1	0.20	Metabolism
Akr7a2	0.45	Metabolism
Aldh2	0.55	Metabolism
Aldh7a1	0.21	Metabolism
Aldoa	0.46	Metabolism
Aldoc	0.47	Metabolism
Aloxe3	0.16	Metabolism
Amd1	0.54	Metabolism
Ampd2	0.54	Metabolism
Anapc5	0.49	Cell communication
Anxa7	0.53	Transport
Ap1s1	0.40	Transport
Apex1	0.32	Nucleotide and nucleic acid metabolism
Appbp1	0.45	Cell communication
Arl2	0.50	Cell communication
Armc9	0.53	Biological process unknown
Asf1b	0.34	Metabolism, Protein metabolism
Ash2l	0.47	Nucleotide and nucleic acid metabolism
Asns	0.23	Metabolism, Protein metabolism

Gene Symbol	$T_{migratory}$	Biological Process
Aspm	0.34	Biological process unknown
Atad3a	0.46	Metabolism
Atp5g1	0.52	Metabolism
Aurka	0.40	Cell communication
Bat2	0.53	Biological process unknown
Baz1b	0.50	Regulation of gene expression, epigenetic
Bcat1	0.44	Metabolism
Bckdk	0.38	Metabolism
Bcr	0.49	Cell communication
Bcs1l	0.51	Metabolism
Bex1	0.26	Biological process unknown
Birc5	0.50	Cell communication
Blm	0.40	Nucleotide and nucleic acid metabolism
Bnip3	0.22	Apoptosis
Bola1	0.51	Biological process unknown
Bop1	0.39	Biological process unknown
Bpnt1	0.40	Cell communication
Bsg	0.43	Cell communication
Btbd14b	0.51	Biological process unknown
Bub1	0.31	Cell communication
Bub1b	0.32	Cell communication
Bxdc1	0.43	Biological process unknown
Bxdc2	0.47	Biological process unknown
C1qbp	0.39	Immune response
Cacybp	0.46	Metabolism, Protein metabolism
Cad	0.39	Metabolism
Calca	0.43	Metabolism, Regulation of physiological process
Cand1	0.51	Nucleotide and nucleic acid metabolism
Casp4	0.49	Apoptosis
Cbr1	0.29	Metabolism
Ccdc5	0.44	Cell growth and/or maintenance
Ccna2	0.30	Cell communication
Ccnb1	0.29	Cell communication
Ccnb2	0.50	Cell communication
Ccnd2	0.43	Cell communication
Ccnf	0.49	Cell communication
Ccng1	0.49	Cell cycle, Regulation of cell cycle
Cct3	0.48	Metabolism, Protein metabolism
Cct5	0.44	Metabolism, Protein metabolism
Cct7	0.47	Protein folding
Cct8	0.33	Metabolism, Protein metabolism
Cd200	0.28	Immune response
Cdc20	0.39	Cell cycle
Cdca2	0.48	Biological process unknown

Gene Symbol	$T_{migratory}$	Biological Process
Cdca4	0.55	Cell division, Cell fate commitment
Cdca7	0.25	Nucleotide and nucleic acid metabolism
Cdca8	0.38	Cell communication
Cdk2	0.52	Nucleotide and nucleic acid metabolism
Cdk4	0.39	Cell communication
Cdkn1a	0.55	Cell cycle, Regulation of cell cycle
Cdkn2a	0.48	Cell communication
Cep68	0.53	Biological process unknown
Chchd4	0.55	Biological process unknown
Chchd6	0.49	Biological process unknown
Chek1	0.30	Cell communication
Chtf18	0.53	Nucleotide and nucleic acid metabolism
Ckap2	0.32	Cell growth and/or maintenance
Clpb	0.46	Metabolism
Cog7	0.55	Transport
Cops4	0.53	Metabolism, Protein metabolism
Cpsf2	0.53	Nucleotide and nucleic acid metabolism
Cpsf6	0.52	Nucleotide and nucleic acid metabolism
Cse1l	0.51	Transport
Cst7	0.52	Metabolism, Protein metabolism
Ctps	0.26	Metabolism
Cttn	0.50	Cell growth and/or maintenance
Cyb5b	0.52	Transport
Cyc1	0.52	Metabolism
Cycs	0.46	Metabolism
Cyp2s1	0.50	Metabolism
Dars	0.47	Metabolism
Dazap1	0.53	Nucleotide and nucleic acid metabolism
Dbi	0.47	Metabolism
Ddb1	0.46	Nucleotide and nucleic acid metabolism
Ddx1	0.40	Nucleotide and nucleic acid metabolism
Ddx11	0.50	Nucleotide and nucleic acid metabolism
Ddx18	0.55	Transport
Ddx56	0.48	Nucleotide and nucleic acid metabolism
Dhcr24	0.30	Metabolism
Dhcr7	0.27	Metabolism
Dkc1	0.48	Nucleotide and nucleic acid metabolism
Dlg7	0.39	Cell cycle, Regulation of cell cycle
Dnajc9	0.51	Metabolism, Protein metabolism
Dnd1	0.54	Nucleotide and nucleic acid metabolism
Dnmt1	0.42	Nucleotide and nucleic acid metabolism
Dph5	0.54	Biological process unknown
Drg1	0.39	Cell growth and/or maintenance
Dut	0.51	DNA replication

Gene Symbol	$T_{migratory}$	Biological Process
Dync2li1	0.50	Cell growth and/or maintenance
Ebna1bp2	0.41	Biological process unknown
Echdc1	0.30	Biological process unknown
Ect2	0.28	Cell communication
Eftud2	0.50	Metabolism, Protein metabolism
Egln3	0.42	Metabolism, Protein metabolism
Eif2a	0.54	Metabolism, Protein metabolism
Eif2b3	0.46	Metabolism, Protein metabolism
Eif2s1	0.48	Metabolism, Protein metabolism
Eif2s2	0.55	Metabolism, Protein metabolism
Eif3s6ip	0.40	Metabolism, Protein metabolism
Eif3s8	0.54	Metabolism, Protein metabolism
Eif3s9	0.44	Metabolism, Protein metabolism
Eif4ebp1	0.41	Metabolism, Protein metabolism
Eif4g1	0.47	Metabolism, Protein metabolism
Eif5a	0.46	Metabolism, Protein metabolism
Elovl6	0.47	Biological process unknown
Emg1	0.40	Metabolism, Protein metabolism
Eml2	0.48	Cell growth and/or maintenance
Enah	0.53	Cell growth and/or maintenance
Eno1	0.38	Metabolism
Eno2	0.30	Metabolism
Epor	0.51	Cell communication
Eprs	0.52	Metabolism, Protein metabolism
Ero1l	0.52	Metabolism
Erp29	0.55	Protein folding
Espl1	0.27	Metabolism, Protein metabolism
Exo1	0.37	Nucleotide and nucleic acid metabolism
Exosc5	0.51	Nucleotide and nucleic acid metabolism
Exosc7	0.38	Nucleotide and nucleic acid metabolism
Exosc8	0.41	Nucleotide and nucleic acid metabolism
Fabp5	0.16	Transport
Fads1	0.54	Metabolism
Fads2	0.50	Metabolism
Fancd2	0.40	Cell communication
Fasn	0.34	Metabolism
Fdft1	0.53	Metabolism
Fdps	0.16	Metabolism
Fem1b	0.52	Apoptosis
Fen1	0.42	Nucleotide and nucleic acid metabolism
Fgf13	0.51	Cell communication
Fkbp3	0.45	Metabolism
Fkbp4	0.35	Metabolism
Foxq1	0.48	Nucleotide and nucleic acid metabolism

Gene Symbol	$T_{migratory}$	Biological Process
Frap1	0.55	Cell communication
Fubp1	0.49	Nucleotide and nucleic acid metabolism
Fxc1	0.48	Cell adhesion
Fxn	0.49	Transport
Gadd45gip1	0.52	Cell communication
Galk1	0.20	Metabolism
Gars	0.52	Metabolism, Protein metabolism
Gart	0.37	Nucleotide and nucleic acid metabolism
Gbe1	0.46	Metabolism
Gcat	0.43	Metabolism
Gcsh	0.25	Metabolism
Gemin6	0.55	Nucleotide and nucleic acid metabolism
Gfer	0.41	Cell communication
Gfi1	0.54	Nucleotide and nucleic acid metabolism
Gmps	0.40	Metabolism
Gnai1	0.37	Cell communication
Gnl3	0.42	Cell cycle, Regulation of cell proliferation
Gpc1	0.47	Cell adhesion
Gpiap1	0.41	Transport
Gsta4	0.43	Metabolism
Gsto1	0.45	Metabolism
Gtf2h4	0.52	Nucleotide and nucleic acid metabolism
Gtpbp4	0.48	Cell adhesion
Hat1	0.38	Metabolism
Hdgf	0.49	Cell communication
Hey1	0.43	Nucleotide and nucleic acid metabolism
Higd1a	0.36	Biological process unknown
Hirip3	0.39	Nucleotide and nucleic acid metabolism
Hk2	0.50	Metabolism
Hmbs	0.55	Metabolism
Hmga1	0.37	Nucleotide and nucleic acid metabolism
Hmgcr	0.54	cell migration
Hmgcs1	0.26	Metabolism
Hmgn1	0.45	Nucleotide and nucleic acid metabolism
Hmgn3	0.48	Nucleotide and nucleic acid metabolism
Hmmr	0.38	Cell communication
Hs2st1	0.53	Metabolism
Hsf2	0.35	Metabolism, Protein metabolism
Hspa14	0.42	Biological process unknown
Hspd1	0.42	Protein folding
Hspe1	0.42	Metabolism, Protein metabolism
Hsph1	0.54	Metabolism, Protein metabolism
Idh1	0.45	Metabolism
Idi1	0.26	Metabolism

Gene Symbol	$T_{migratory}$	Biological Process
Ier3	0.42	Anti-apoptosis
Ift74	0.54	Cell growth and/or maintenance
Il17f	0.45	Immune response
Ilf2	0.46	Nucleotide and nucleic acid metabolism
Immt	0.54	Cell growth and/or maintenance
Impdh2	0.40	Metabolism
Insig1	0.44	Metabolism, Lipid metabolism
Jam3	0.48	Cell adhesion
Josd3	0.51	Cell cycle, Regulation of cell cycle
Kdr	0.28	Cell communication
Kif11	0.49	Cell growth and/or maintenance
Kif15	0.47	Cell communication
Kif22	0.36	Nucleotide and nucleic acid metabolism
Kif2c	0.40	Cell growth and/or maintenance
Kifc1	0.44	Cell cycle, Microtubule-based process
Kpna2	0.36	Cell communication
Kpnb1	0.36	Transport
Laptm4b	0.33	Cell cycle, Regulation of cell proliferation
Ldha	0.51	Metabolism
Lig1	0.51	Nucleotide and nucleic acid metabolism
Lmnb1	0.52	Cell growth and/or maintenance
Lrpprc	0.51	Nucleotide and nucleic acid metabolism
Lrrc59	0.45	Biological process unknown
Lss	0.51	Metabolism
Luc7l	0.52	Biological process unknown
Mad2l2	0.52	Cell cycle, Regulation of cell cycle
Mcm4	0.35	Nucleotide and nucleic acid metabolism
Mcm6	0.33	Nucleotide and nucleic acid metabolism
Mcm7	0.32	DNA replication
Mif	0.29	Cell communication
Mina	0.54	Cell growth and/or maintenance
Mki67ip	0.46	Nucleotide and nucleic acid metabolism
Morf4l2	0.55	Nucleotide and nucleic acid metabolism
Mrpl12	0.40	Nucleotide and nucleic acid metabolism
Mrpl19	0.51	Metabolism, Protein metabolism
Mrpl23	0.46	Metabolism, Protein metabolism
Mrpl38	0.43	Metabolism, Protein metabolism
Mrpl47	0.51	Metabolism, Protein metabolism
Mrps18b	0.45	Metabolism, Protein metabolism
Mrps25	0.51	Metabolism, Protein metabolism
Mta1	0.39	Nucleotide and nucleic acid metabolism
Mtf2	0.55	Nucleotide and nucleic acid metabolism
Mthfd1	0.35	Metabolism
Mtss1	0.54	Cell adhesion

Gene Symbol	$T_{migratory}$	Biological Process
Mvd	0.23	Metabolism
Mybbp1a	0.37	Nucleotide and nucleic acid metabolism
Myc	0.41	Nucleotide and nucleic acid metabolism
Nars	0.50	Metabolism, Protein metabolism
Nasp	0.46	Cell cycle, Regulation of cell cycle
Ndufa11	0.46	Metabolism
Nedd4	0.49	Metabolism, Protein metabolism
Net1	0.43	Cell communication
Nif3l1	0.54	Nucleotide and nucleic acid metabolism
Nip7	0.55	Cell growth and/or maintenance
Nme1	0.55	Metabolism
Nme2	0.52	Signal transduction
Nol5a	0.35	Nucleotide and nucleic acid metabolism
Nol9	0.54	Biological process unknown
Nolc1	0.44	Nucleotide and nucleic acid metabolism
Nphp1	0.48	Cell adhesion
Nsdhl	0.47	Metabolism
Nt5dc2	0.50	Biological process unknown
Nts	0.51	Cell communication
Nudc	0.36	Cell communication
Nup107	0.39	Transport
Nup155	0.33	Transport
Nup188	0.53	Biological process unknown
Nup35	0.41	Transport
Nup88	0.52	Transport
Nup93	0.38	Transport
Oat	0.39	Metabolism
Oprs1	0.37	Cell communication
Orc1l	0.30	Nucleotide and nucleic acid metabolism
Orc6l	0.48	Nucleotide and nucleic acid metabolism
P4ha1	0.26	Metabolism
Pa2g4	0.41	Nucleotide and nucleic acid metabolism
Pabpc4	0.42	Nucleotide and nucleic acid metabolism
Paics	0.37	Metabolism
Pbef1	0.42	Anti-apoptosis
Pcca	0.36	Metabolism
Pcgf6	0.49	Nucleotide and nucleic acid metabolism
Pcyt2	0.52	Metabolism
Pdk1	0.24	Metabolism
Pdk3	0.53	Metabolism
Pebp1	0.27	Cell communication
Pfkl	0.28	Metabolism
Pfkm	0.50	Metabolism
Pfkp	0.45	Metabolism

Gene Symbol	$T_{migratory}$	Biological Process
Pgam1	0.54	Metabolism
Pgd	0.34	Metabolism
Pgk1	0.41	Metabolism
Pgm1	0.36	Metabolism
Phb	0.30	Cell communication
Phb2	0.51	Nucleotide and nucleic acid metabolism
Phgdh	0.35	Metabolism
Phlda3	0.54	Cell communication
Pi4k2b	0.45	Cell communication
Pik3r2	0.50	Cell adhesion
Pkig	0.34	Biological process unknown
Plk1	0.35	Cell communication
Plscr1	0.49	Cell communication
Pmvk	0.54	Metabolism
Pola1	0.51	Nucleotide and nucleic acid metabolism
Pola2	0.39	Nucleotide and nucleic acid metabolism
Pold1	0.30	Nucleotide and nucleic acid metabolism
Pold2	0.28	Nucleotide and nucleic acid metabolism
Pole	0.48	Nucleotide and nucleic acid metabolism
Pole3	0.47	Nucleotide and nucleic acid metabolism
Polr2f	0.46	Nucleotide and nucleic acid metabolism
Ppan	0.55	Cell communication
Ppat	0.36	Metabolism
Ppid	0.48	Metabolism
Ppif	0.53	Metabolism, Protein metabolism
Ppp1r14b	0.34	Metabolism
Ppp1r7	0.50	Nucleotide and nucleic acid metabolism
Ppp2r1a	0.44	Cell communication
Prdx1	0.36	Metabolism
Prdx3	0.53	Metabolism
Prim1	0.39	Nucleotide and nucleic acid metabolism
Prps1	0.46	Metabolism
Psat1	0.24	Metabolism
Psmc3	0.53	Metabolism, Protein metabolism
Psmc6	0.54	Metabolism, Protein metabolism
Psmd1	0.51	Metabolism, Protein metabolism
Psmd12	0.48	Metabolism, Protein metabolism
Psmd2	0.49	Metabolism, Protein metabolism
Ptpmt1	0.49	Cell communication
Ptpn5	0.28	Cell communication
Pttg1	0.39	Nucleotide and nucleic acid metabolism
Pus1	0.54	Nucleotide and nucleic acid metabolism
Pycrl	0.54	Metabolism
Qdpr	0.52	Metabolism

Gene Symbol	$T_{migratory}$	Biological Process
Rab26	0.54	Cell communication
Rab34	0.37	Cell communication
Rad23b	0.37	Nucleotide and nucleic acid metabolism
Rad50	0.54	Nucleotide and nucleic acid metabolism
Rae1	0.50	Nucleotide and nucleic acid metabolism
Ran	0.50	Cell communication
Rangap1	0.41	Cell communication
Rassf4	0.49	Biological process unknown
Rbbp7	0.50	Nucleotide and nucleic acid metabolism
Rbm13	0.54	Nucleotide and nucleic acid metabolism
Rbm14	0.55	Nucleotide and nucleic acid metabolism
Rbm17	0.53	Nucleotide and nucleic acid metabolism
Rdbp	0.54	Nucleotide and nucleic acid metabolism
Rfc3	0.53	DNA replication
Rhebl1	0.42	Cell communication
Rnaseh2a	0.54	Nucleotide and nucleic acid metabolism
Rnf126	0.37	Metabolism, Protein metabolism
Rnps1	0.41	Nucleotide and nucleic acid metabolism
Rrm1	0.43	Nucleotide and nucleic acid metabolism
Rrm2	0.39	Nucleotide and nucleic acid metabolism
Rtcd1	0.46	Nucleotide and nucleic acid metabolism
Ruvbl1	0.32	Nucleotide and nucleic acid metabolism
Ruvbl2	0.25	Nucleotide and nucleic acid metabolism
Sap18	0.54	Nucleotide and nucleic acid metabolism
Sc4mol	0.18	Metabolism, Fatty acid metabolism
Scarb1	0.32	Cell adhesion
Scye1	0.54	Immune response
Sf1	0.54	Nucleotide and nucleic acid metabolism
Sf3a2	0.50	Metabolism, Protein metabolism
Sfxn1	0.43	Transport
Shmt1	0.38	Metabolism
Shmt2	0.28	Metabolism
Slc16a1	0.55	Transport
Slc16a3	0.20	Transport
Slc19a1	0.40	Transport
Slc25a1	0.48	Transport
Slc25a4	0.41	Transport
Slc2a1	0.34	Transport
Slc5a3	0.28	Transport
Slc7a5	0.31	Transport
Smagp	0.43	Cell adhesion
Smn1	0.53	Nucleotide and nucleic acid metabolism
Sms	0.46	Metabolism
Snrpa	0.44	Nucleotide and nucleic acid metabolism

Gene Symbol	$T_{migratory}$	Biological Process
Spag5	0.51	Cell growth and/or maintenance
Spr	0.40	Metabolism
Sqle	0.25	Metabolism
Srfbp1	0.53	Metabolism
Srm	0.25	Metabolism
Srpk1	0.42	Nucleotide and nucleic acid metabolism
Ssbp1	0.44	Nucleotide and nucleic acid metabolism
St13	0.31	Cell communication
Stip1	0.43	Cell communication
Stoml2	0.51	Transport
Sympk	0.40	Cell adhesion
Syncrip	0.41	Nucleotide and nucleic acid metabolism
Tardbp	0.51	Nucleotide and nucleic acid metabolism
Tars	0.42	Metabolism
Tbl3	0.45	Biological process unknown
Tcp1	0.38	Metabolism, Protein metabolism
Tdp1	0.48	DNA repair
Tfpi	0.34	Metabolism, Protein metabolism
Tfrc	0.29	Transport
Thop1	0.21	Metabolism, Protein metabolism
Timeless	0.43	Nucleotide and nucleic acid metabolism
Timm10	0.47	Metabolism, Protein metabolism
Timm13	0.52	Transport
Timm8a	0.42	Metabolism
Timm9	0.55	Cell growth and/or maintenance
Tk1	0.43	Metabolism
Tkt	0.39	Metabolism
Tm7sf2	0.34	Metabolism
Tmem55a	0.50	Cell adhesion
Tmem97	0.27	Cell division, proliferation
Tnfrsf11b	0.44	Cell communication
Tnfrsf4	0.38	Cell communication
Tnfrsf8	0.15	Cell communication
Tnpo1	0.45	Transport
Tnpo3	0.54	Cell communication
Tomm40	0.47	Transport
Top1mt	0.51	Nucleotide and nucleic acid metabolism
Top2a	0.53	Nucleotide and nucleic acid metabolism
Tp53	0.41	Nucleotide and nucleic acid metabolism
Tpi1	0.22	Metabolism
Trap1	0.50	Metabolism, Protein metabolism
Trim28	0.45	Nucleotide and nucleic acid metabolism
Trnt1	0.52	Metabolism
Tspan3	0.52	Cell adhesion

Gene Symbol	$T_{migratory}$	Biological Process
Tspan5	0.50	Biological process unknown
Tuba4a	0.42	Cell growth and/or maintenance
Tubb2c	0.38	Cell growth and/or maintenance
Tubb6	0.49	Cell growth and/or maintenance
Tubg1	0.46	Cell growth and/or maintenance
Txnl1	0.53	Metabolism
Txnl2	0.37	Biological process unknown
Txnrd1	0.51	Metabolism
Ubtf	0.55	Nucleotide and nucleic acid metabolism
Uchl5	0.37	Metabolism, Protein metabolism
Uhrf1	0.34	Nucleotide and nucleic acid metabolism
Umps	0.40	Metabolism
Ung	0.34	DNA repair
Usp14	0.51	Metabolism, Protein metabolism
Vars2	0.36	Metabolism
Vdac1	0.42	Transport
Vegfa	0.42	Cell communication
Vegfb	0.51	Cell communication
Vkorc1	0.37	Metabolism, Vitamin metabolism
Vrk1	0.34	Cell communication
Wdr18	0.40	Biological process unknown
Wdr75	0.51	Cell communication
Wdr77	0.50	Biological process unknown
Xpo1	0.45	Cell communication
Xrcc1	0.50	DNA repair
Xylb	0.48	Metabolism
Yars	0.46	Metabolism
2-Mar	2.22	Metabolism, Protein metabolism
9-Sep	2.03	Cell division, proliferation
Abi1	2.07	Cell growth, Regulation of cell growth
Acpl2	3.52	Metabolism
Acss1	2.60	Biological process unknown
Add3	4.42	Cell growth and/or maintenance
Agpat2	2.16	Metabolism, Lipid metabolism
Ahnak	4.45	Cell growth, cell differentiation, Muscle contraction
Aif1	2.61	Cell communication
Ap1s2	2.35	Transport
App	2.06	Cell adhesion
Arhgap4	2.02	Cell communication
Arhgef18	3.77	Cell communication
Arhgef3	2.86	Cell communication
Arid4a	2.33	Nucleotide and nucleic acid metabolism
Atp2a3	3.31	Transport, Ion transport
Atp2b1	2.90	Transport

Gene Symbol	$T_{migratory}$	Biological Process
Axud1	3.11	Apoptosis
Basp1	2.04	Nucleotide and nucleic acid metabolism
Bcl9l	2.46	Biological process unknown
Bin1	2.18	Cell communication
Bin2	2.30	Cell communication
Btg1	2.60	Cell growth and/or maintenance
Btg2	4.92	Nucleotide and nucleic acid metabolism
Ccl3	6.40	Immune response
Ccl4	7.85	Immune response
Ccl5	12.74	Cell communication
Ccpg1	3.56	Biological process unknown
Ccr2	4.33	Cell communication
Ccr5	4.50	Cell communication
Ccr6	2.93	Cell communication
Cd14	2.17	Immune response
Cd2	2.63	Cell adhesion
Cd38	3.05	Metabolism
Cd44	2.47	Cell adhesion
Cd69	7.77	Immune response
Cd83	1.99	Immune response
Cd97	2.46	Cell adhesion
Cdc25b	2.35	Cell communication
Cdkn1b	2.31	Cell communication
Ches1	1.99	Cell communication
Chi3l1	3.43	Cell growth and/or maintenance
Clk1	2.68	Signal transduction
Clk4	2.28	Cell communication
Cotl1	2.38	Cell growth and/or maintenance
Cox7a2l	2.10	Metabolism
Cpeb2	2.13	Nucleotide and nucleic acid metabolism
Csf1r	2.02	Cell communication
Ctse	3.06	Metabolism, Protein metabolism
Ctsh	2.10	Metabolism, Protein metabolism
Ctss	2.19	Metabolism, Protein metabolism
Cxcl10	1.95	Cell migration
Cxcl11	2.93	Cell communication
Cxcl2	2.19	Immune response
Cxcr4	2.92	Cell communication
Cyb561d1	2.18	Biological process unknown
Cybb	2.83	Metabolism
Daam1	4.03	Cell communication
Dap	2.99	Apoptosis
Dgka	2.40	Cell communication
Dnajb9	3.41	Metabolism, Protein metabolism

Gene Symbol	$T_{migratory}$	Biological Process
Dok2	2.73	Signal transduction
Dusp1	4.80	Cell communication
Dusp2	4.43	Cell communication
Dusp5	5.66	Cell communication
Dusp6	2.24	Cell communication
Edg1	4.15	Cell adhesion
Emp1	5.52	Cell growth and/or maintenance
Evl	4.27	Cell adhesion
Faah	2.89	Metabolism
Faslg	2.12	Cell communication
Fcgr2b	3.34	Signal transduction
Fcgr3a	2.13	Immune response
Fgl2	9.87	Cell growth and/or maintenance
Fhl2	3.98	Nucleotide and nucleic acid metabolism
Flna	2.72	Cell growth and/or maintenance
Fn1	1.98	Cell adhesion
Fos	12.19	Nucleotide and nucleic acid metabolism
Foxp1	2.01	Regulation of gene expression, epigenetic
Fxyd5	3.09	Transport, Regulation of cellular process
Fyco1	2.40	Cell communication
Fyn	2.30	Cell adhesion
G0s2	2.11	Cell communication
Gabbr1	2.25	Cell communication
Gas7	2.24	Cell cycle
Gbp2	4.11	Cell communication
Gda	10.63	Metabolism
Gimap4	3.36	Cell communication
Gimap5	1.98	Apoptosis
Gimap7	3.39	Cell communication
Glipr1	3.91	Immune response
Glul	2.54	Metabolism
Gmfg	2.96	Cell communication
Gna15	2.11	Cell communication
Gpnmb	2.86	Biological process unknown
Gpsm3	2.04	Cell communication
Gsn	4.23	Cell adhesion
Hbb	9.01	Transport
Hbp1	2.15	Nucleotide and nucleic acid metabolism
Hck	2.26	Cell communication
Hcst	2.90	Cell communication
Herc6	2.26	Metabolism, Protein metabolism
Hpse	2.23	Metabolism
Hsd11b1	2.10	Metabolism
Ier2	4.65	Nucleotide and nucleic acid metabolism

Gene Symbol	$T_{migratory}$	Biological Process
Ifi44	2.09	Biological process unknown
Ifit2	2.69	Cell growth and/or maintenance
Ifng	3.52	Immune response
Ifngr1	2.50	Immune response
Igsf6	2.08	Immune response
Il1b	6.19	Immune response
Irak2	3.54	Cell communication
Irf7	2.59	Nucleotide and nucleic acid metabolism
Isg20	2.35	Nucleotide and nucleic acid metabolism
Itgb1	5.61	Cell adhesion
Itgb7	5.12	Cell adhesion
Itm2b	2.02	Cell growth and/or maintenance
Jun	4.00	Nucleotide and nucleic acid metabolism
Klf10	2.13	Cell cycle, Regulation of cell growth
Klf3	3.87	Nucleotide and nucleic acid metabolism
Klf4	2.32	Nucleotide and nucleic acid metabolism
Klf6	2.31	Nucleotide and nucleic acid metabolism
Klf9	2.20	Nucleotide and nucleic acid metabolism
Klrd1	8.07	Signal transduction
Klrk1	2.00	Immune response
Kpna1	2.34	Transport
Kras	1.97	Signal transduction
Lamp2	1.99	Cell adhesion
Laptm5	2.16	Cell communication
Lcp2	2.05	Cell communication
Lgals1	11.73	Immune response
Lgals3	2.75	Nucleotide and nucleic acid metabolism
Lgals3bp	5.77	Immune response
Lgals9	3.38	Apoptosis
Litaf	2.08	Nucleotide and nucleic acid metabolism
Lrg1	2.05	Biological process unknown
Ltb	2.12	Immune response, Inflammatory response
Lyz	15.94	Metabolism
Maf	4.77	Regulation of gene expression, epigenetic
Map3k8	2.20	Cell communication
Mbp	2.57	Immune response
Metrnl	2.12	Biological process unknown
Mll5	2.58	cell growth, Regulation of cell growth
Mx1	3.92	Cell communication
Mx2	2.00	Immune response
Myadm	5.25	Biological process unknown
Napsa	2.89	Metabolism, Protein metabolism
Ncald	2.02	Cell communication
Nfkbia	2.63	Nucleotide and nucleic acid metabolism

Gene Symbol	$T_{migratory}$	Biological Process
Nr1d1	3.16	Cell communication
Nr1d2	3.15	Nucleotide and nucleic acid metabolism
Nr4a1	9.67	Cell communication
Nr4a2	3.25	Cell communication
Nr4a3	6.55	Nucleotide and nucleic acid metabolism
Nrp1	2.45	Cell adhesion
Nucb2	2.07	Cell communication
Olfm1	3.03	Biological process unknown
Pbxip1	2.28	Nucleotide and nucleic acid metabolism
Pcsk3	2.17	Cell migration
Pctk2	2.34	Cell communication
Pde4b	4.95	Signal transduction
Pdlim2	2.00	Cell adhesion
Per1	2.18	Nucleotide and nucleic acid metabolism
Phyhd1	2.28	Metabolism
Pip5k2a	2.07	Cell communication
Pitpnm1	1.98	Metabolism, Lipid metabolism
Plaur	3.09	Cell communication
Plek	2.16	Cell communication
Ppapdc2	2.07	Immune response, Innate immune response
Ppp1r9b	1.96	Cell communication
Prf1	2.70	Transport
Psap	2.03	Cell communication
Pten	2.54	Cell communication
Ptpn18	2.49	Cell communication
Pycard	4.92	Apoptosis
Rab7l1	3.33	Cell communication
Ramp1	2.07	Cell communication
Ramp2	2.84	Cell communication
Rasa3	5.55	Cell communication
Rgs1	4.25	Cell communication
Rgs10	2.96	Cell communication
Rgs2	6.78	Cell communication
Rhoh	2.22	Cell communication
Rnf167	2.18	Metabolism, Protein metabolism
Rxra	2.33	Cell communication
S100a10	3.26	Cell communication
S100a11	8.36	Cell communication
S100a4	4.70	Cell growth and/or maintenance
S100a6	5.90	Cell communication
S100a8	6.13	Cell communication
S100a9	13.68	Cell communication
Sell	2.44	Cell adhesion
Sema4b	2.45	Biological process unknown

Gene Symbol	$T_{migratory}$	Biological Process
Sepp1	2.46	Cell adhesion
Serinc1	2.18	Biological process unknown
Sfxn3	2.67	Biological process unknown
Sgk	2.71	Cell communication
Sh3bp5	2.07	Signal transduction
Slc28a2	2.05	Transport
Slc2a3	3.16	Transport
Slc35b2	3.32	Transport
Smpdl3a	2.24	Metabolism
Snf1lk	2.34	Cell communication
Sorl1	2.26	Cell communication
Sqstm1	2.12	Metabolism, Protein metabolism
Ssbp3	2.04	Metabolism, DNA metabolism
Stk17b	2.78	Cell communication
Stk38	2.11	Cell communication
Sv2b	2.73	Transport
Sytl1	2.34	transport, Vesicle docking
Tacc2	3.64	Cell growth and/or maintenance
Tcp11l2	4.69	Biological process unknown
Tgfbi	2.51	Cell adhesion
Tnf	2.48	Cell communication
Tnfrsf1b	3.11	Cell communication
Tob1	2.66	Cell communication
Tpst2	3.23	Energy pathways
Tsc22d3	5.77	Regulation of gene expression, epigenetic
Txk	3.16	Cell communication
Txnip	3.15	Cell communication
Tyrobp	2.69	Cell communication
Ubl3	2.03	Metabolism, Protein metabolism
Vamp3	2.51	Transport
Vcam1	2.42	Cell adhesion
Vcl	1.96	Cell adhesion
Vim	2.36	Cell growth and/or maintenance
Wsb1	2.10	Cell communication
Xdh	5.11	Metabolism
Ypel5	3.11	Biological process unknown
Zbp1	6.44	Nucleotide and nucleic acid metabolism
Zfp36	4.39	Nucleotide and nucleic acid metabolism
Zfp36l1	2.65	Nucleotide and nucleic acid metabolism
Zfp36l2	3.90	Nucleotide and nucleic acid metabolism
Zyx	2.58	Cell adhesion

Appendix 3.

List of genes differentially regulated in $T_{effector}$ state.

Gene Symbol	$T_{effector}$	Biological Process
Aamp	0.40	Cell adhesion
Abcf1	0.39	Protein folding
Abhd4	0.47	Biological process unknown
Abhd8	0.24	Biological process unknown
Ablim2	0.27	Cell growth and/or maintenance
Acin1	0.26	Nucleotide and nucleic acid metabolism
Acp1	0.46	Metabolism
Acrbp	0.54	Biological process unknown
Acsl5	0.51	Metabolism
Actb	0.40	Cell communication
Actn1	0.53	Cell adhesion
Actr2	0.29	Cell communication
Acvr1	0.54	Cell communication
Adarb1	0.54	Nucleotide and nucleic acid metabolism
Adss	0.39	Metabolism
Ak2	0.51	Metabolism
Ak3l1	0.51	Metabolism
Ak7	0.49	Metabolism
Akr1a1	0.36	Metabolism
Akr1d1	0.46	Metabolism
Als2cr4	0.50	Biological process unknown
Amfr	0.51	Protein folding
Angel2	0.48	Biological process unknown
Anxa3	0.38	Cell communication
Ap1s2	0.38	Transport
Ap2m1	0.52	Transport
Apeh	0.48	Metabolism
Aph1a	0.47	Signal transduction
Arcn1	0.48	Transport
Arf4	0.50	Transport
Arid2	0.23	Nucleotide and nucleic acid metabolism
Arid5a	0.50	Nucleotide and nucleic acid metabolism
Arl2bp	0.51	Cell communication
Arsk	0.49	Metabolism
Ascc3l1	0.49	Nucleotide and nucleic acid metabolism
Aspm	0.50	Biological process unknown
Atg3	0.39	Biological process unknown
Atg4b	0.26	Biological process unknown, Proteolysis and peptidolysis
Atp1b3	0.51	Transport

Gene Symbol	$T_{effector}$	Biological Process
Atp5f1	0.40	Metabolism
Atp6v0b	0.44	Transport
Atp6v1f	0.41	Metabolism
Atp6v1g1	0.41	Metabolism
Atrn	0.52	Immune response
Atxn10	0.37	Biological process unknown
Aurkaip1	0.38	Signal transduction
Avp	0.51	Cell communication,Regulation of biological process
Axl	0.53	Cell migration
Bcan	0.54	Cell adhesion
Bin3	0.41	Cell communication
Bloc1s2	0.55	Biological process unknown
Bmp2	0.55	Cell communication
Bmpr2	0.32	Cell communication
Bop1	0.35	Biological process unknown
Brd7	0.23	Cell cycle
Btbd14a	0.53	Biological process unknown
C1qbp	0.44	Immune response
Cacnb1	0.45	Transport
Cacnb3	0.23	Transport
Calr	0.44	Protein folding
Capza2	0.38	Cell adhesion
Capzb	0.25	Cell communication
Cbfb	0.47	Nucleotide and nucleic acid metabolism
Ccnl2	0.47	Nucleotide and nucleic acid metabolism
Ccnt2	0.35	Nucleotide and nucleic acid metabolism
Ccrl2	0.44	Cell communication
Cct5	0.53	Protein folding
Cd44	0.44	Cell adhesion
Cd81	0.41	Metabolism
Cdc16	0.41	Cell communication
Cdc2l5	0.46	Cell communication
Cdc5l	0.44	Cell communication
Cdyl2	0.44	Nucleotide and nucleic acid metabolism
Cetn1	0.52	Cell communication
Churc1	0.35	Nucleotide and nucleic acid metabolism
Clk1	0.30	Signal transduction
Clk4	0.34	Cell communication
Cltc	0.37	Cell communication
Cmklr1	0.52	Cell communication
Cndp1	0.44	Protein folding
Cog6	0.44	Transport
Col12a1	0.44	Cell adhesion
Col15a1	0.37	Cell adhesion

Gene Symbol	$T_{effector}$	Biological Process
Commd3	0.24	Biological process unknown
Commd9	0.44	Biological process unknown
Comp	0.53	Cell adhesion
Cops3	0.45	Cell communication
Cops6	0.48	Cell communication
Cops8	0.39	Signal transduction
Cpne8	0.37	Transport
Crhr1	0.55	Cell communication
Crim1	0.48	Cell communication
Csad	0.40	Metabolism
Cse1l	0.27	Transport
Csrp3	0.51	Cell communication
Ctnnal1	0.50	Apoptosis
Cul3	0.30	Protein folding
Cul4b	0.54	Protein folding
Cuta	0.42	Biological process unknown
Cyb561d2	0.30	Protein folding
Cyp2u1	0.44	Metabolism
Dars	0.52	Metabolism
Dars2	0.50	Biological process unknown
Dclk3	0.55	Cell communication
Dctn3	0.45	Cell communication
Ddb1	0.43	Nucleotide and nucleic acid metabolism
Ddhd1	0.34	Metabolism
Dhrs1	0.49	Metabolism
Dhx15	0.30	Nucleotide and nucleic acid metabolism
Dhx16	0.38	Nucleotide and nucleic acid metabolism
Diap1	0.53	Cell migration
Dicer1	0.49	Regulation of gene expression, epigenetic, Gene silencing
Dmn	0.50	Cell communication
Dnpep	0.33	Protein folding
Dom3z	0.45	Biological process unknown
Dscr6	0.49	Biological process unknown
Dst	0.39	Cell communication
Dtx4	0.29	Signal transduction
Dync1h1	0.49	Metabolism
Dynll2	0.51	Cell communication
Ebp	0.42	Metabolism
Eea1	0.51	Transport
Egr2	0.53	Nucleotide and nucleic acid metabolism
Eif3s3	0.49	Protein folding
Eif3s6ip	0.51	Protein folding
Eif5	0.43	Protein folding
Eps15	0.25	Cell communication

Gene Symbol	$T_{effector}$	Biological Process
Ercc1	0.46	Nucleotide and nucleic acid metabolism
Etv1	0.48	Nucleotide and nucleic acid metabolism
Exoc5	0.42	Transport
Exosc9	0.55	Nucleotide and nucleic acid metabolism
Ezh1	0.33	Nucleotide and nucleic acid metabolism
Fbxl11	0.18	Protein folding
Fbxo7	0.41	Protein folding
Fem1a	0.54	Cell communication
Fgd2	0.41	Cell communication
Fibp	0.34	Cell communication
Fignl1	0.47	Biological process unknown
Fkbp1a	0.44	Cell communication
Folr2	0.48	Cell communication
Foxp4	0.42	Nucleotide and nucleic acid metabolism
Gab1	0.36	Cell communication
Galnt1	0.38	Metabolism
Gba2	0.29	Metabolism
Ghsr	0.54	Cell communication
Gja5	0.49	Transport
Glo1	0.34	Metabolism
Glt8d1	0.50	Biological process unknown
Gnb2	0.38	Cell communication
Gng5	0.50	Cell communication
Gnl3l	0.52	Biological process unknown
Golga7	0.36	Transport
Golph4	0.46	Cell communication
Gorasp1	0.21	Cell communication
Gpr20	0.51	Cell communication
Gpr88	0.26	Cell communication
Gps1	0.43	Cell communication
Gramd3	0.34	Biological process unknown
Grinl1a	0.44	Biological process unknown
Grip2	0.44	Biological process unknown
Gstk1	0.53	Metabolism
Gtpbp3	0.48	Nucleotide and nucleic acid metabolism
H3f3b	0.40	Nucleotide and nucleic acid metabolism
Hapln2	0.24	Cell adhesion
Hbs1l	0.52	Protein folding
Hdac2	0.48	Nucleotide and nucleic acid metabolism
Hdlbp	0.35	Transport
Hk1	0.39	Metabolism
Hnrpk	0.49	Nucleotide and nucleic acid metabolism
Hnrpl	0.48	Nucleotide and nucleic acid metabolism
Homer1	0.21	Cell communication

Gene Symbol	$T_{effector}$	Biological Process
Hoxd3	0.43	Nucleotide and nucleic acid metabolism
Igfals	0.38	Cell adhesion
Il1b	0.52	Immune response
Ilk	0.34	Cell adhesion
Impa1	0.52	Metabolism
Impad1	0.39	Metabolism
Inpp1	0.30	Cell communication
Inpp5e	0.53	Metabolism
Jtb	0.52	Biological process unknown
Kcnh7	0.32	Transport
Kcnk3	0.53	transport, Ion transport
Khdrbs1	0.52	Nucleotide and nucleic acid metabolism
Klc1	0.19	Signal transduction
Klf5	0.54	Nucleotide and nucleic acid metabolism
Klhl7	0.23	Biological process unknown
Lamp1	0.36	Biological process unknown
Lbp	0.44	Immune response
Lemd2	0.35	Biological process unknown
Letm2	0.33	Biological process unknown
Lgals8	0.39	Signal transduction
Lims1	0.51	Cell adhesion
Lrrc23	0.47	Biological process unknown
Magi3	0.46	Cell communication
Mak10	0.54	Cell communication
Man2b1	0.32	Metabolism
Map2k2	0.39	Cell adhesion
Mapk12	0.54	Cell adhesion
Mapk8ip3	0.52	Signal transduction
Mat2a	0.54	Metabolism
Mbtps1	0.44	Protein folding
Mecp2	0.48	Nucleotide and nucleic acid metabolism
Mecr	0.50	Metabolism
Mef2d	0.44	Nucleotide and nucleic acid metabolism
Men1	0.45	Regulation of gene expression, epigenetic
Metap2	0.43	Protein folding
Mkks	0.48	Protein folding
Mkrn2	0.55	Nucleotide and nucleic acid metabolism
Mll	0.52	Nucleotide and nucleic acid metabolism
Mog	0.43	Immune response
Morf4l1	0.29	Regulation of gene expression, epigenetic
Mrpl12	0.39	Nucleotide and nucleic acid metabolism
Mrpl37	0.45	Protein folding
mrpl9	0.40	Protein folding
Mtrr	0.46	Metabolism

Gene Symbol	$T_{effector}$	Biological Process
Mvk	0.46	Metabolism
Mylk2	0.45	Cell adhesion
Myod1	0.41	Nucleotide and nucleic acid metabolism
Myst2	0.50	Nucleotide and nucleic acid metabolism
Nanp	0.43	Metabolism
Napa	0.29	Transport
Narfl	0.24	Biological process unknown
Nck2	0.54	Cell migration
Ncl	0.43	Nucleotide and nucleic acid metabolism
Ncor1	0.31	Nucleotide and nucleic acid metabolism
Nde1	0.47	Cell communication
Ndfip1	0.47	Cell communication
Nedd4l	0.39	Protein folding
Nedd8	0.38	Protein folding
Nfx1	0.46	Regulation of gene expression, epigenetic
Nicn1	0.47	Biological process unknown
Nmt2	0.38	Metabolism
Nmur2	0.54	Cell communication
Nono	0.34	Nucleotide and nucleic acid metabolism
Notch2	0.54	Cell communication
Npc2	0.42	Metabolism
Npm1	0.44	Protein folding
Nrbf2	0.44	Nucleotide and nucleic acid metabolism
Nsdhl	0.51	Metabolism
Nsfl1c	0.41	Cell communication
Ntn1	0.55	Cell communication
Nudt4	0.44	Biological process unknown
Numa1	0.31	Cell communication
Nup155	0.50	Transport
Nup98	0.39	Transport
Ocln	0.54	Cell adhesion
Odf4	0.35	Biological process unknown
Orc6l	0.53	Nucleotide and nucleic acid metabolism
Paics	0.55	Metabolism
Pak1	0.53	Cell adhesion
Pcbp2	0.26	Nucleotide and nucleic acid metabolism
Pcnx	0.37	Biological process unknown
Pdap1	0.35	Cell communication
Pdia3	0.44	Protein folding
Pfkfb2	0.53	Metabolism
Pgam5	0.52	Metabolism
Pigl	0.54	Metabolism
Pitpna	0.44	Transport
Plau	0.46	Protein folding

Gene Symbol	$T_{effector}$	Biological Process
Plekha3	0.33	Cell communication
Plekhc1	0.54	Cell communication
Plxnc1	0.53	Cell adhesion
Poll	0.33	Nucleotide and nucleic acid metabolism
Polr3k	0.21	Nucleotide and nucleic acid metabolism
Ppia	0.43	Protein folding
Ppm1b	0.45	Signal transduction
Ppp2ca	0.24	Cell communication
Ppp5c	0.47	Cell communication
Prl	0.54	Cell communication
Prpf18	0.38	Nucleotide and nucleic acid metabolism
Prpf19	0.51	Nucleotide and nucleic acid metabolism
Psma1	0.38	Protein folding
Psma2	0.32	Protein folding
Psmb1	0.47	Protein folding
Psmb2	0.49	Protein folding
Psmb3	0.47	Protein folding
Psmb4	0.44	Protein folding
Psmb6	0.44	Protein folding
Psmc1	0.45	Protein folding
Psmc4	0.37	Protein folding
Psmd2	0.48	Protein folding
Psmd9	0.49	Protein folding
Ptch1	0.30	Cell communication
Ptpn1	0.50	Cell communication
Pum2	0.39	Protein folding
Rab11a	0.45	Cell communication
Rab15	0.44	Cell communication
Rabac1	0.48	Transport
Rad50	0.48	Nucleotide and nucleic acid metabolism
Rap1b	0.45	Cell adhesion
Rapgef1	0.33	Cell adhesion
Rax	0.43	Nucleotide and nucleic acid metabolism
Rbbp7	0.45	Nucleotide and nucleic acid metabolism
Rbm5	0.48	Nucleotide and nucleic acid metabolism
Rgs1	0.47	Cell communication
Rheb	0.32	Cell communication
Rhoa	0.29	Cell adhesion
Rhobtb2	0.23	Cell communication
Ric8a	0.35	Cell communication
Rnf12	0.43	Regulation of gene expression, epigenetic
Rnf25	0.51	Protein folding
Rnmt	0.40	Nucleotide and nucleic acid metabolism
Rom1	0.42	Cell adhesion

Gene Symbol	$T_{effector}$	Biological Process
Rpl15	0.48	Protein folding
Rpl17	0.48	Protein folding
Rpl21	0.41	Protein folding
Rpl23	0.44	Protein folding
Rpl24	0.52	Protein folding
Rpl28	0.51	Protein folding
Rpl30	0.41	Protein folding
Rpl35a	0.40	Protein folding
Rpl36a	0.39	Protein folding
Rpl37	0.27	Protein folding
Rpl4	0.46	Protein folding
Rpl5	0.31	Protein folding
Rpl7	0.43	Protein folding
Rpn1	0.54	Protein folding
Rpn2	0.46	Protein folding
Rps15a	0.39	Protein folding
Rps21	0.41	Protein folding
Rps5	0.44	Protein folding
Ryr3	0.53	Transport
Scyl1	0.36	Nucleotide and nucleic acid metabolism
Sdcbp	0.41	Cell communication
Serinc3	0.43	Biological process unknown
Sf1	0.50	Nucleotide and nucleic acid metabolism
Sh2d4a	0.47	Cell communication
Skiv2l	0.32	Nucleotide and nucleic acid metabolism
Slc12a3	0.50	Transport
Slc29a1	0.45	Transport
Slc38a6	0.32	Transport
Slc3a2	0.38	Transport
Slc9a3r2	0.28	Cell communication
Smap1l	0.47	Cell communication
Son	0.41	Nucleotide and nucleic acid metabolism
Sparcl1	0.54	Cell communication
Spred1	0.54	Cell communication
Sprn	0.51	Biological process unknown
St13	0.43	Cell communication
Stip1	0.35	Cell communication
Stoml2	0.45	Transport
Sub1	0.37	Nucleotide and nucleic acid metabolism
Sumo1	0.26	Cell cycle
Surf1	0.36	Metabolism
Surf4	0.29	Transport
Tax1bp1	0.33	Cell communication
Tceb1	0.46	Nucleotide and nucleic acid metabolism

Gene Symbol	$T_{effector}$	Biological Process
Tceb2	0.36	Nucleotide and nucleic acid metabolism
Tdrd3	0.51	Nucleotide and nucleic acid metabolism
Thrap4	0.32	Nucleotide and nucleic acid metabolism
Timm13	0.40	Transport
Timm23	0.25	Protein folding
Tm2d2	0.42	Signal transduction
Tmed10	0.49	Transport
Tmed2	0.36	Transport
Tmed5	0.45	Biological process unknown
Tmem39b	0.38	Biological process unknown
Tmem66	0.36	Biological process unknown
Tomm20	0.34	Transport
Tpst2	0.38	Energy pathways
Trpm7	0.53	Transport
Tsc22d1	0.40	Cell growth , Regulation of cell growth
Tsnax	0.37	Nucleotide and nucleic acid metabolism
Tsta3	0.43	Cell adhesion
Tubb4	0.47	Cell communication
Txn2	0.39	Metabolism
Txnrd1	0.29	Metabolism
Uba52	0.42	Protein folding
Ube1c	0.53	Protein folding
Ube2g1	0.41	Protein folding
Ube2n	0.33	Protein folding
Unc50	0.48	Nucleotide and nucleic acid metabolism
Usf1	0.45	Nucleotide and nucleic acid metabolism
Usf2	0.40	Nucleotide and nucleic acid metabolism
Usp19	0.29	Protein folding
Usp48	0.33	Protein folding
Vamp3	0.34	Transport
Vamp5	0.39	Transport
Vapa	0.45	Transport, Vesicle docking
Vps4a	0.30	Cell communication
Vwa1	0.49	Cell adhesion
Wdr44	0.48	Cell migration
Wdr45	0.42	Biological process unknown
Wsb2	0.51	Protein folding
Wwp1	0.51	Protein folding
Ywhab	0.41	Signal transduction
Ywhae	0.35	Cell communication
Zan	0.51	Cell adhesion
Zdhhc13	0.54	Biological process unknown
Zfand3	0.43	Nucleotide and nucleic acid metabolism
Zfp91	0.39	metabolism, RNA metabolism

Gene Symbol	$T_{effector}$	Biological Process
9-Sep	1.98	Cell communication
A2m	2.11	Protein folding
Acadm	2.74	Metabolism
Acads	1.96	Metabolism
Acadvl	2.39	Energy pathways
Accn1	2.01	Transport
Acly	4.16	Metabolism
Aco2	2.09	Metabolism
Acp5	2.37	Metabolism
Acsl1	2.00	Metabolism
Acsl6	2.00	Metabolism
Actn3	2.67	Cell adhesion
Adrm1	1.97	Cell adhesion
Afp	2.06	Transport
Ahcy	2.00	Metabolism
Akap11	2.14	Cell communication
Alad	2.15	Metabolism
Alb	5.03	Transport
Aldh2	2.03	Metabolism
Amacr	2.13	Metabolism
Anapc2	3.93	Cell communication
Ankrd1	3.41	Nucleotide and nucleic acid metabolism
Anxa2	2.05	Siganl transduction, Regulation of enzyme activity
Ap2b1	1.98	Biological process unknown
Ap3d1	3.01	Transport
Apc2	1.96	Cell communication
Apcs	3.08	Protein folding
Api5	4.46	Apoptosis
Apip	6.18	Apoptosis
Apoa1bp	2.53	Biological process unknown
Arf1	10.00	Signal transduction
Arf5	2.10	Cell communication
Arfgap1	2.50	metabolism, Lipid metabolism
Atp1b1	3.58	Transport
Atp2b1	2.00	Transport
Atp5g1	3.49	Metabolism
Atp5g3	2.64	Metabolism
Atp6v0e1	2.37	Transport
Aurkb	2.29	Cell communication
B4galt3	4.12	Metabolism
Bak1	2.07	Apoptosis
Bbs1	3.55	Biological process unknown
Bcap31	3.94	Transport
Becn1	10.45	Cell communication

Gene Symbol	$T_{effector}$	Biological Process
Bgn	3.01	Cell communication
Bmp15	3.00	Cell communication
Btg1	2.21	Cell communication
Btg3	1.96	Cell cycle
Ca2	2.91	Metabolism
Cacna1a	2.24	Transport
Cand1	2.09	Nucleotide and nucleic acid metabolism
Capns1	15.57	Cell adhesion
Ccdc43	2.39	Biological process unknown
Cd36	2.92	Cell adhesion
Cd59	2.76	Immune response
Cd63	1.98	Cell communication
Cdc37	6.61	Protein folding
Cdc42	4.08	Cell adhesion
Cdk5rap3	2.09	Cell cycle
Cdo1	2.17	Metabolism
Ceacam12	2.15	Cell adhesion
Cfi	2.01	Immune response
Chgb	2.04	Cell communication
Cib1	2.58	Cell adhesion
Cited2	2.02	Nucleotide and nucleic acid metabolism
Ckm	2.09	Metabolism
Clns1a	2.27	Transport
Clu	2.21	Immune response
Cnot7	2.10	Nucleotide and nucleic acid metabolism
Copb1	4.15	Transport
Copb2	17.59	Transport
Cox4i1	2.20	Metabolism
Cox6a2	2.72	Metabolism
Cox6c	2.37	Metabolism
Cox7a2	2.02	Metabolism
Crip2	2.09	Nucleotide and nucleic acid metabolism
Cryba4	2.16	Cell communication
Crym	2.81	Cell migration, Osmoregulation
Csn2	2.04	Transport
Ctps2	3.52	Nucleotide and nucleic acid metabolism
Ctsb	2.15	Protein folding
Cubn	2.62	Transport
Cxcr4	8.29	Cell communication
Cyp2e1	3.51	Metabolism
Dad1	3.92	Apoptosis
Dazap2	8.49	Nucleotide and nucleic acid metabolism
Dctn1	2.00	Transport
Decr1	1.99	Metabolism

Gene Symbol	$T_{effector}$	Biological Process
Des	2.54	Cell communication
Dnaja1	1.96	Protein folding
Dnajc8	3.10	Protein folding
Dpep1	3.43	Protein folding
Dtnbp1	3.30	Cell communication
Dusp5	1.98	Cell communication
Eif2b2	2.65	Protein folding
Eif4g2	5.95	Protein folding
Ell2	2.09	Nucleotide and nucleic acid metabolism
Eno1	3.01	Metabolism
Exoc7	2.20	Biological process unknown
F2r	2.15	Cell communication
Fasn	2.01	Metabolism
Fbxw8	3.25	Protein folding
Fdxr	2.64	Metabolism
Fez1	2.10	Biological process unknown
Flot2	3.10	Cell adhesion
Fth1	2.05	Transport
Gabrr1	1.95	Transport
Gap43	3.00	Cell communication
Gata2	2.11	Nucleotide and nucleic acid metabolism
Gatm	2.30	Metabolism
Gdi1	3.33	Cell communication
Gdi2	6.56	Transport
Gfra1	2.05	Signal transduction
Gls	2.36	Metabolism
Glul	2.33	Metabolism
Gnb2l1	2.20	Cell communication
Gng11	2.70	Cell communication
Got1	2.04	Metabolism
Gpx1	2.29	Anti-apoptosis
Gys2	2.26	Metabolism
H1f0	2.31	Nucleotide and nucleic acid metabolism
Hadhb	2.04	Metabolism
Hdgf	2.70	Cell communication
Herpud1	2.17	Biological process unknown
Hmgcs1	4.76	Metabolism
Hspb1	2.33	Protein folding
Ifitm2	5.62	Cell communication
Ifnb1	1.96	Immune response
Ifrd1	1.97	Signal transduction
Igf2r	2.75	Cell communication
Il2rb	2.24	Immune response
Insig1	1.97	Metabolism, Lipid metabolism

Gene Symbol	$T_{effector}$	Biological Process
Itpr3	2.15	Cell communication
Kdr	2.27	Cell communication
Kpna2	2.27	Cell communication
Laptm5	2.72	Cell communication
Ldha	2.27	Metabolism
Lig1	2.44	Nucleotide and nucleic acid metabolism
Lmo4	2.05	Nucleotide and nucleic acid metabolism
Lox	2.21	Metabolism
Lxn	2.31	Metabolism
Lypla1	2.21	Metabolism
Lypla2	9.77	Metabolism
Maf	2.37	Regulation of gene expression, epigenetic
Map2k1	2.05	Cell adhesion
Map3k12	2.19	Cell communication
Mapk14	2.06	Cell communication
Mapt	1.97	Cell communication
Mb	2.03	Transport
Mdk	2.48	Cell communication
Mfge8	4.75	Cell adhesion
Mgp	2.49	Transport
Mif	2.29	Cell communication
Mllt10	2.18	Nucleotide and nucleic acid metabolism
Mlycd	3.54	Metabolism
Mmp10	2.17	Protein folding
Mpg	2.35	DNA repair
mrpl11	3.16	Protein folding
Myl3	2.21	Cell communication
Nat1	2.20	Metabolism
Ncoa2	2.04	Nucleotide and nucleic acid metabolism
Ncstn	3.36	Cell communication
Ndel1	2.20	Cell communication
Nme2	2.01	Signal transduction
Nolc1	2.21	Nucleotide and nucleic acid metabolism
Nppa	2.76	Cell communication
Nppb	3.27	Cell communication
Nudt22	3.08	Biological process unknown
Nup54	2.09	Transport
Orm1	2.13	Immune response
Osbpl2	2.86	Transport, Vesicle-mediated transport
P4hb	2.01	Protein folding
Palm	1.95	Biological process unknown
Pam	2.13	Protein folding
Pcdh21	2.01	Cell adhesion
Pcna	2.61	DNA repair

Gene Symbol	$T_{effector}$	Biological Process
Pcsk3	1.98	Cell migration
Pecr	2.21	Metabolism
Pfn1	2.25	Cell communication
Pga5	1.96	Protein folding
Phb	2.98	Cell communication
Phb2	10.63	Nucleotide and nucleic acid metabolism
Phkg2	2.02	Metabolism
Pim3	2.95	Cell communication
Pitpnb	2.56	Transport
Pitx2	2.05	Cell migration
Ppm2c	2.23	Cell communication
Ppp1cc	3.00	Cell communication
Ppp3r2	2.59	Cell communication
Prap1	2.12	Biological process unknown
Prdm2	6.59	Nucleotide and nucleic acid metabolism
Prdx1	2.10	Metabolism
Prdx2	2.00	Metabolism
Prdx3	2.95	Metabolism
Prdx5	2.28	Metabolism
Prpf8	9.33	Nucleotide and nucleic acid metabolism
Psmb7	2.10	Protein folding
Psme1	1.95	Protein folding
Ptbp1	1.95	Nucleotide and nucleic acid metabolism
Ptdss1	3.04	Metabolism
Ptpns1	2.12	Cell adhesion
Qdpr	2.08	Metabolism
Ralgds	1.95	Cell communication
Ran	2.80	Cell communication
Rara	2.10	Signal transduction
Rcn2	3.32	Cell communication
Rgs2	3.10	Cell communication
Rhoq	2.22	Cell communication
Rpl10	2.52	Protein folding
Rpl10a	2.19	Protein folding
Rpl13a	2.05	Protein folding
Rpl18	6.32	Protein folding
Rpl19	2.73	Protein folding
Rpl27	2.39	Protein folding
Rpl29	5.47	Protein folding
Rpl6	2.55	Protein folding
Rps17	2.05	Protein folding
Rps27	2.09	Nucleotide and nucleic acid metabolism
Rps27a	2.25	Protein folding
Rps3a	2.12	Protein folding

Gene Symbol	$T_{effector}$	Biological Process
Rps6	2.81	Protein folding
Rpsa	2.35	Cell adhesion
Sar1a	6.43	Cell communication
Scamp4	2.01	Transport
Scn10a	2.00	Transport
Sdc1	2.45	Cell adhesion
Sdc4	2.11	Cell adhesion
Sdha	2.37	Metabolism
Selenbp1	2.97	Protein folding
Sepw1	2.57	Metabolism
Shox2	2.39	Nucleotide and nucleic acid metabolism
Slc22a8	2.29	Transport
Slc2a4	2.00	Transport
Slc30a1	2.06	Transport
Slc34a2	2.08	Transport
Slc6a4	2.66	Transport
Slc6a8	2.83	Transport
Slc8a2	4.11	Transport
Snca	2.03	Protein folding
Snx1	1.95	Cell communication
Sod1	2.34	Metabolism
Sparc	2.83	Cell communication
Spp1	2.68	Cell adhesion
Statip1	3.42	Cell communication
Stmn1	3.20	Cell communication
Stx1a	2.84	Cell communication
Suclg1	2.04	Metabolism
Sult4a1	2.16	Metabolism
Syk	2.14	Cell adhesion
Syn3	2.03	transport, Neurotransmitter transport
Synj2bp	2.40	Transport, Mitochondrial transport
Tagln	2.56	Biological process unknown, Muscle development
Tcea1	4.34	Transcription
Tdg	1.97	DNA repair
Thrap3	2.75	Nucleotide and nucleic acid metabolism
Timp1	5.27	Cell communication
Tm9sf4	5.14	Biological process unknown
Tmpo	2.42	Metabolism, Nuclear organization and biogenesis
Tnfrsf1a	2.20	Cell communication
Tomm22	3.61	Transport
Tp53	2.53	Nucleotide and nucleic acid metabolism
Tpi1	2.22	Metabolism
Trmt1	2.77	Nucleotide and nucleic acid metabolism
Tsn	3.02	Nucleotide and nucleic acid metabolism

Gene Symbol	$T_{effector}$	Biological Process
Ttr	3.95	Transport
Tusc3	2.35	Cell communication
Txnrd2	2.46	Cell communication
Ube2d3	13.21	Protein folding
Ube2e2	3.15	Protein folding
Ugdh	2.72	Metabolism
Ung	2.43	DNA repair
Vcp	14.4	Metabolism
Vgf	2.44	Cell communication
Vim	3.75	Cell communication
Vsnl1	1.96	Cell communication
Wdr5	3.85	Cell communication
Ythdf1	2.85	Biological process unknown
Ywhah	2.43	Cell communication

Appendix 4

Description of different T cells used in the dissertation.

$T_{MBP\text{-}GFP}$	Myelin basic protein (MBP) –specific T cells retrovirally transduced with green fluorescent protein (GFP)
$T_{MBP\text{-}KLF4}$	Myelin basic protein (MBP) –specific T cells retrovirally transduced with full length cDNA for Krüppel like factor 4 (KLF4) and green fluorescent protein (GFP)
$T_{MBP\text{-}EMP1}$	Myelin basic protein (MBP) –specific T cells retrovirally transduced with full length cDNA for Epithelial membrane protein 1 (EMP1) and green fluorescent protein (GFP)
$T_{OVA\text{-}GFP}$	Ovalbumin (OVA) –specific T cells retrovirally transduced with green fluorescent protein (GFP)
$T_{OVA\text{-}KLF4}$	Ovalbumin (OVA) –specific T cells retrovirally transduced with full length cDNA for Krüppel like factor 4 (KLF4) and green fluorescent protein (GFP)
T_{blast}	Freshly activated GFP labeled MBP-specific T cells 48 hours post antigen presenting cell (APC) mediated restimulation *in vitro*.
$T_{resting}$	Resting GFP labeled MBP-specific T cells 7 days post APC mediated restimulation *in vitro*.
T_{spleen}	*Ex vivo* FACS sorted GFP labeled MBP-specific T cells from spleen 3.5 days post adoptive transfer of T_{blast} cells.
T_{CNS}	*Ex vivo* FACS sorted GFP labeled MBP-specific T cells from CNS 3.5 days post adoptive transfer of T_{blast} cells.
$T_{activated}$	Activated T cell transcriptome obtained by dividing gene expression values of T_{blast} by $T_{resting}$
$T_{migratory}$	CNS Migratory T cell transcriptome obtained by dividing gene expression values of T_{spleen} by T_{blast}
$T_{effector}$	CNS effector T cell transcriptome obtained by dividing gene expression values of T_{CNS} by T_{blast}

Curriculum Vitae

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Publications

1. Kristina Streyl*, Vijay K. Ulaganathan*, Klaus Heckelsmiller, Ingo Bartholomäus, Francesca Odoardi, Christian Schläger, Naoto Kawakami, Nikolaus Plesnila, Joachim Ellwart, Wolfgang E.F. Klinkert, Hartmut Wekerle, Alexander Flügel (2010). (Auto) antigen-reactive T cells after stimulation assume a post-activatory migratory state before they are enabled to exert (auto)immune effector functions *in vivo* (manuscript in preparation) (* equally contributed)
2. Chiara Cordiglieri, Francesca Odoardi, Bo Zhang, Merle Nebel, Naoto Kawakami, Wolfgang E.F. Klinkert, Vijay Kumar Ulaganathan, Klaus Dornmair, Werner Dammermann, Barry V.L. Potter[#], Andreas H. Guse[#], and Alexander Flügel[#] (2010) NAADP-mediated Ca^{2+} signalling in effector T cells regulates autoimmunity of the nervous system ([#] equal contribution). *Brain*, 133: 1930-43

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