

## Supplemental material, biochemical experiments

Table S1

**Table S1: Data collection, structure solution and refinement statistics**

	Csl4-exosome + RNA	Csl4-exosome Y70A <sup>Rrp42</sup> + RNA
<b>DATA COLLECTION</b>		
space group	P4 <sub>3</sub> 22	P4 <sub>3</sub> 22
cell dimensions	a = b = 138.2 Å; c = 262.0 Å $\alpha = \beta = \gamma = 90^\circ$	a = b = 137.7 Å; c = 261.8 Å $\alpha = \beta = \gamma = 90^\circ$
beamline	ID-14-2, ESRF	PX I, SLS
wavelength	0.933 Å	1.006 Å
resolution	2.4 Å	3 Å
R <sub>sym</sub>	5.6 % (41.3 %)	6.7 % (31.5 %)
I/ $\sigma$ I	18.9 (3.82)	23.7 (6.10)
completeness	94.5 % (86.4%)	98.9 % (96.9 %)
# of unique reflect.	94,417	50,904
redundancy	4.90	7.22
<b>REFINEMENT</b>		
resolution	85 – 2.4 Å	20 – 3 Å
# of reflect.	99,465	51,207
R <sub>work</sub> /R <sub>free</sub>	19.4 % / 24.8 %	18.8 % / 26.6 %
ligand	6mer RNA (CCCCUC)	6mer RNA (CCCCUC)
water molecules	901	18
rmsd: bond lengths bond angles	0.006 Å 1.019°	0.007 Å 1.036°

Values in parentheses are for highest-resolution shell. R<sub>free</sub> is calculated for a randomly chosen 5% of reflections.

**Table S2: Oligonucleotides used in this study**

<b>oligonucleotide</b>	<b>sequence (5' → 3' direction)</b>
<b><i>DNA oligonucleotides used for cloning</i></b>	
afRrp41 for NdeI	AAAAAA CATATG TCGGAATTCAATGAAAAACCAGAA
afRrp41 rev Hind III	AAAAAA AAGCTT TCAGGCATCTTCACCACCCTCTG
afRrp42 for Nco I	AAAAAA CCATGG GCCCTGAAGACATCCTTGTGGACATT
afRrp42 rev Not I	AAAA GCGGCCGC TTAAATTCCTTAAATTTCTCCCTCAG
afRrp4 for Nco I	AAAAAA CCATGG GCAGGAAGATAGTACTGCCAGGAGAT
afRrp4_cHis rev Not I	AAAAAA GCGGCCGC TTGAATTCCGACATCTGCCTTCCT
afCsl4 for Nde I	AAAAAA CATATG AGATTCGTAATGCCGGGAGAT
afCsl4 rev Hind III	AAAAAA AAGCTT CTACCACTCTCCCTTGCCGTAAT
<b><i>DNA oligonucleotides used for mutagenesis</i></b>	
afRrp41_R65E_for	GAAGTGCATCCAGAACACCTTCAGGAT
afRrp41_R65E_rev	ATACCTGAAGGTGTTCTGGATGCACTTC
afRrp41_D180G_for	ATGAAAGAGGAGGGCAATTTTGGTGAG
afRrp41_D180G_rev	CTCACCAAAATTGCCCTCCTCTTTCAT
afRrp42_Y70A_for	CCGGGCGAGCCC GCT CCGGACACCC
afRrp42_Y70A_rev	GGGTGTCCGG AGC GGGCTCGCCCGG
afRrp41_K51E_for	CCTTGAAATGGGGAAGAAC GAA GTAATTGCAGCGGTTTTTC
afRrp41_K51E_rev	GAAAACCGCTGCAATTAC TTC GTTCT TCCCCATTTC AAGG
afRrp41_K37C_for	GCAAGCGTTTTA TGT AGGGCTGACGGG
afRrp41_K37C_rev	CCCGTCAGCCCT ACA TAAAACGCTTGC
afRrp42_D143C_for	ATCCACGCTCTT TGT GATGACGGAAAC
afRrp42_D143C_rev	GTTTCCGTCATC ACA AAGAGCGTGGAT
<b><i>RNA oligonucleotide used for crystallization</i></b>	
6-mer	CCCCUC

**Table S3: RNA sequences identified from co-purification**

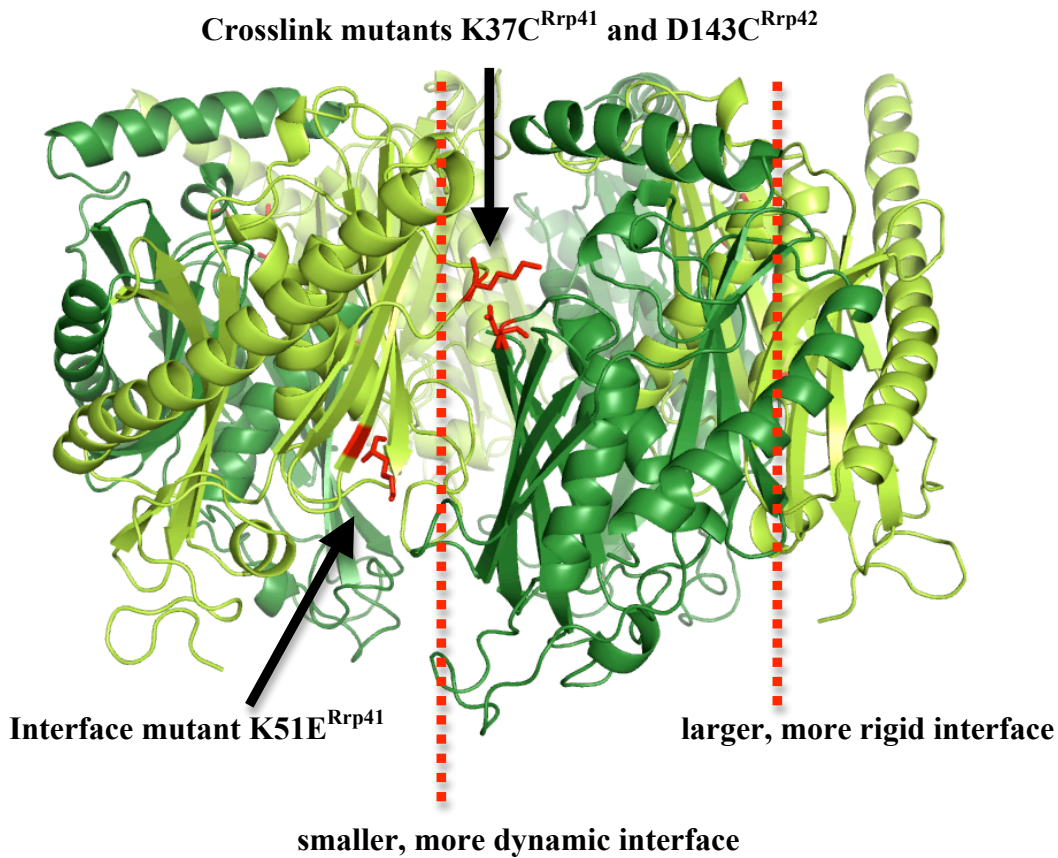
clone #	sequence
1	CAGTAGTAGGTT
2	CTAGCATAACCCCTTGGGGCC
3	CTTGTACACTCTGGACCTTGGCTGTCACTCACTTGATTAGGGTG
4	ATATATACAGCC
5	CTAGCATAACCC
6	ATTAGCCAAAAAA
7	CTATCTGCC
8	CTTACGACCAGG
9	CATGGAGCCGG
10	ATATGGGG
11	AATTTGCCTGGC
12	CGGAATTGG
13	CTAGCATAACCC
14	CTTTTGTATAATGGGTCAG
15	CTTGTACACTCTGGACCTTGGCTGTCACTCACTTGATTAGGGTG
16	CTGAAATGGCTCA
17	CTTTTGTATAATGGGTCAGAA
18	TTTGTAGTGGT

The obtained sequences from the isolated cDNA clones are shown without their polyA-tails.



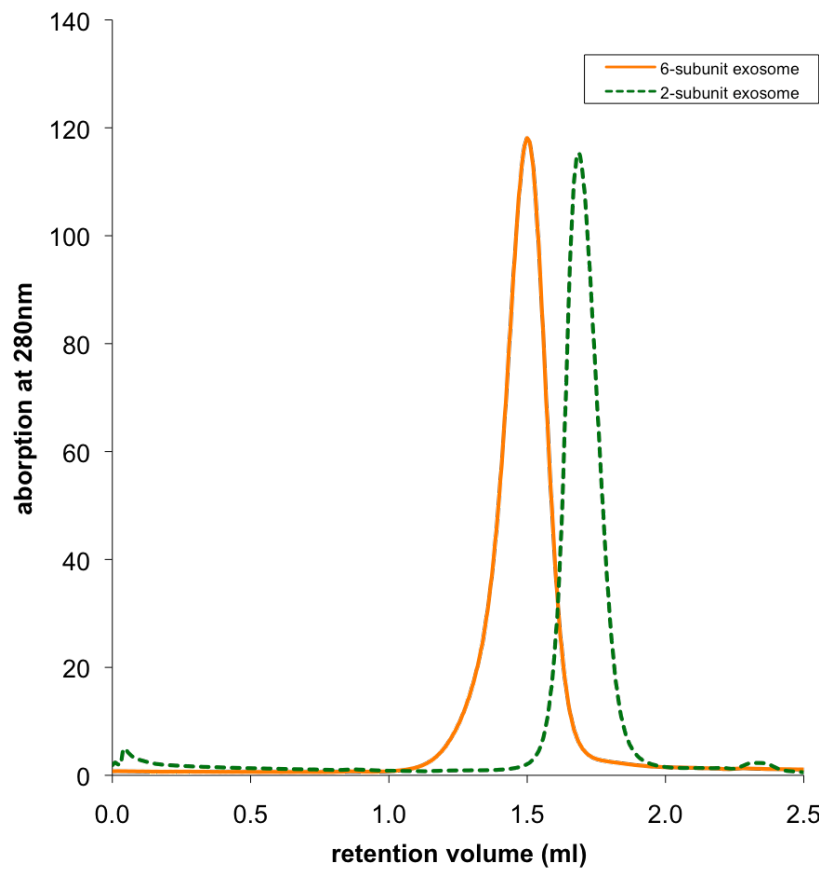
**Figure S1:** Denaturing PA gel of the RNA substrate, that was purified with the exosome from *E. coli* cells (left). On the right the bands of an RNase assay are shown that were used for a calibration curve to estimate the size of the RNA on the left.

**Figure S2**



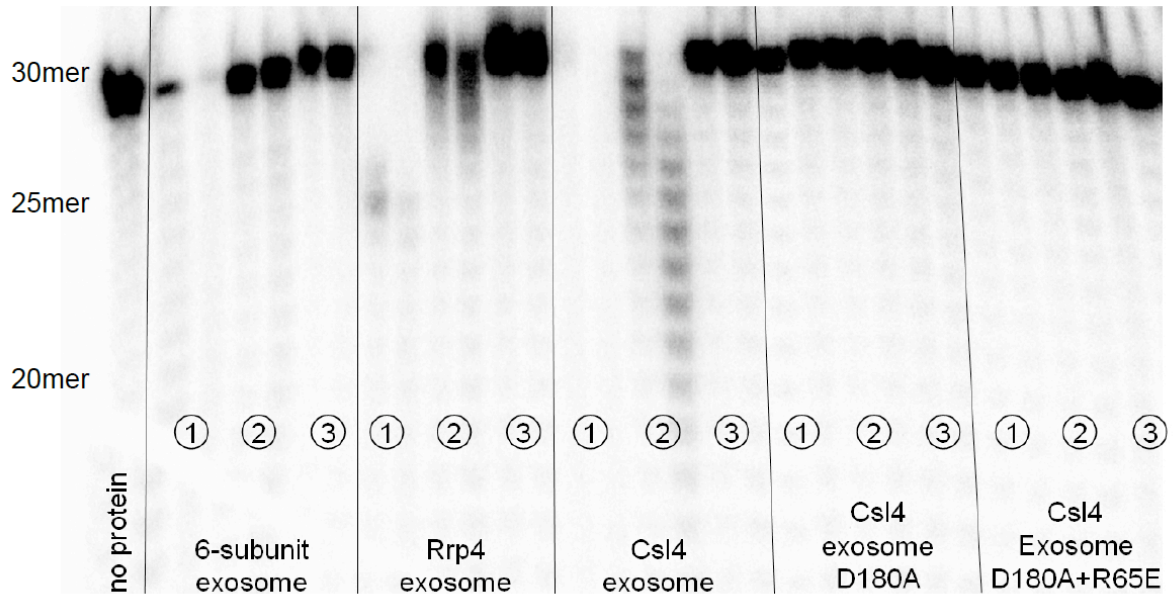
**Figure S2: Location of the interface mutation  $K51E^{Rrp41}$  and the crosslink mutations  $K37C^{Rrp41}$  and  $D143C^{Rrp42}$  within the core exosome ring.** Rrp41 is shown in light green, Rrp42 is shown in dark green. Dashed lines indicate the two different interfaces that are present in the ring of the processing chamber. The interface mutation induces purely dimeric exosomes (only one copy of each Rrp41 and Rrp42). The crosslinked exosome consists of three covalently linked Rrp41:Rrp42 dimers and is more rigid than the wild-type.

**Figure S3**



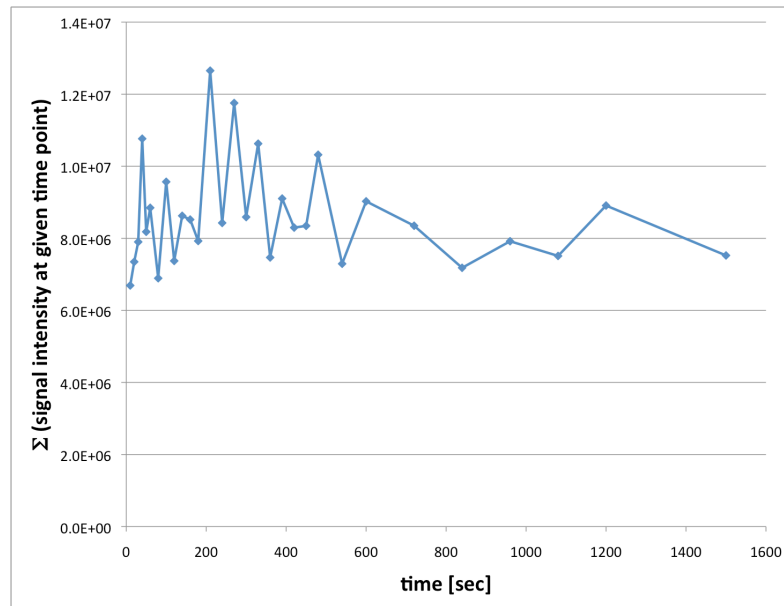
**Figure S3:** Dimeric (Rrp41:Rrp42)<sub>1</sub> exosomes (green) can be easily distinguished and separated from hexameric (Rrp41:Rrp42)<sub>3</sub> exosomes (orange) using size exclusion chromatography.

**Figure S4**



**Figure S4: Denaturing PA gel for the analysis of RNase activity of different exosome complexes.** Radioactively labeled RNA was visualized. Every complex was incubated with 10 mM MgCl<sub>2</sub> and 10 mM phosphate (1); only with 10 mM MgCl<sub>2</sub> (2) and with none of both (3). Samples were taken after 2 min and 6 min. For clearness only the upper part of the gel is shown. The final product of degradation is a three-mer of RNA (not visible on this gel, see Figure 1).

**Figure S5**



**Figure S5: Integrated intensities of gel bands are linear with time.** Plotting the sum of the intensities of all integrated bands for a given time point shows linearity of the signal. (Data of the Csl4 wild-type exosome is used as an example)