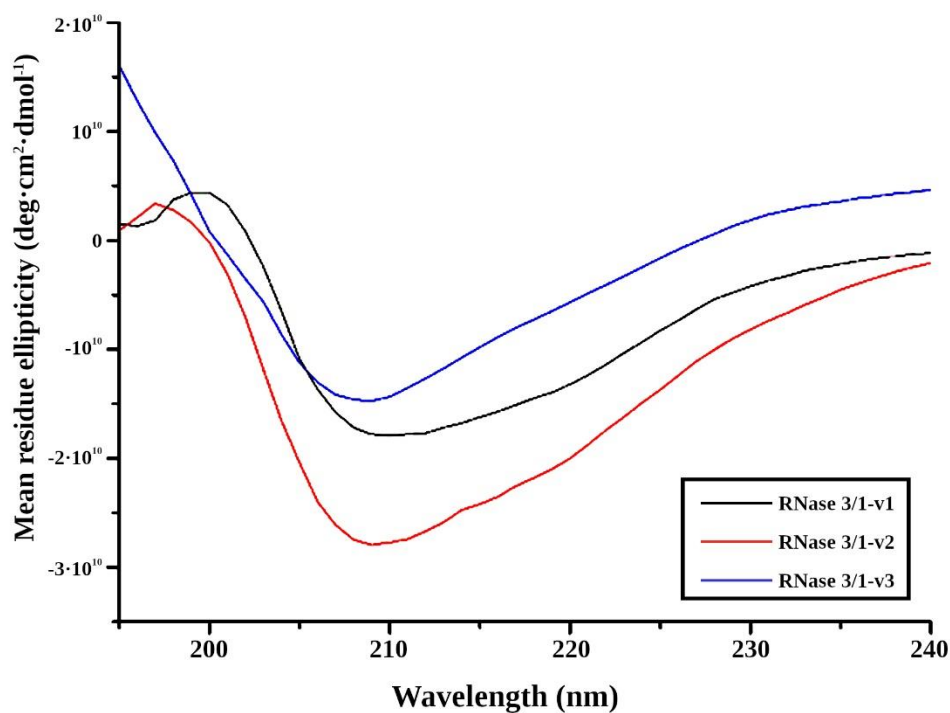
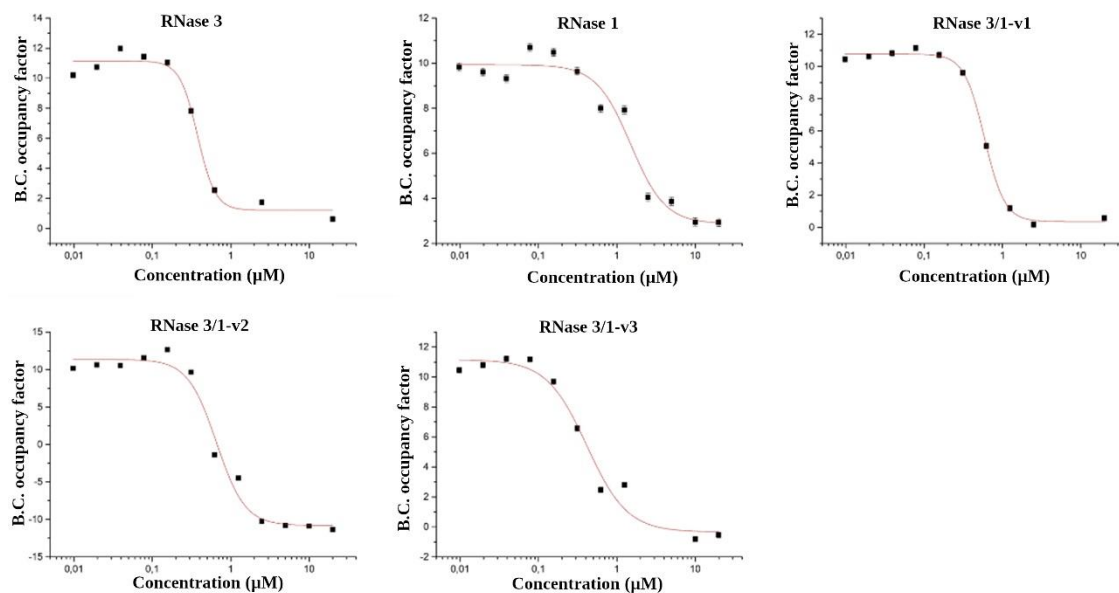


**Figure S1.** Whole trajectory averaged inter-residual distance maps for RNase 3/1 versions. From left to right, RNase 3/1-v1, RNase 3/1-v2 and RNase 3/1-v3 distance maps. Residue numbering, secondary structure labels and active site residues (red arrow) are shown at the bottom of each panel. See also the additional supplementary maps for each variant at high-resolution.

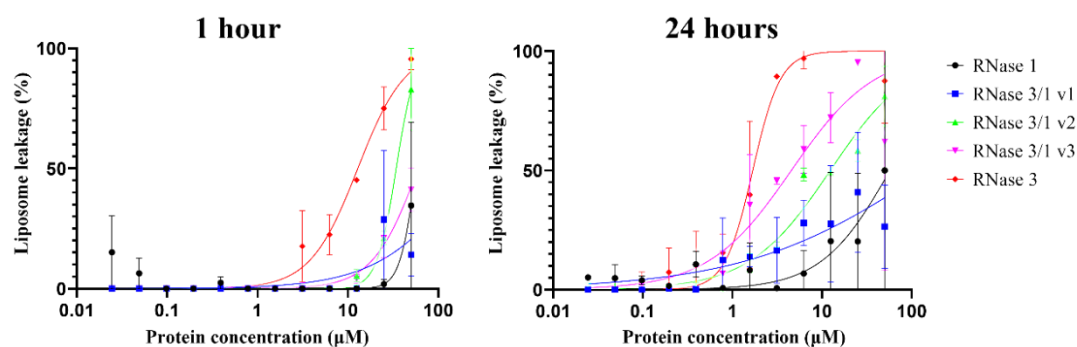


	RNase 3/1-v1	RNase 3/1-v2	RNase 3/1-v3
<i><math>\alpha</math>-helix</i>	18.3	14.1	18.6
<i><math>\beta</math>-sheet</i>	59.4	41.6	62.8
<i>Random coil</i>	16.6	28.7	18.6

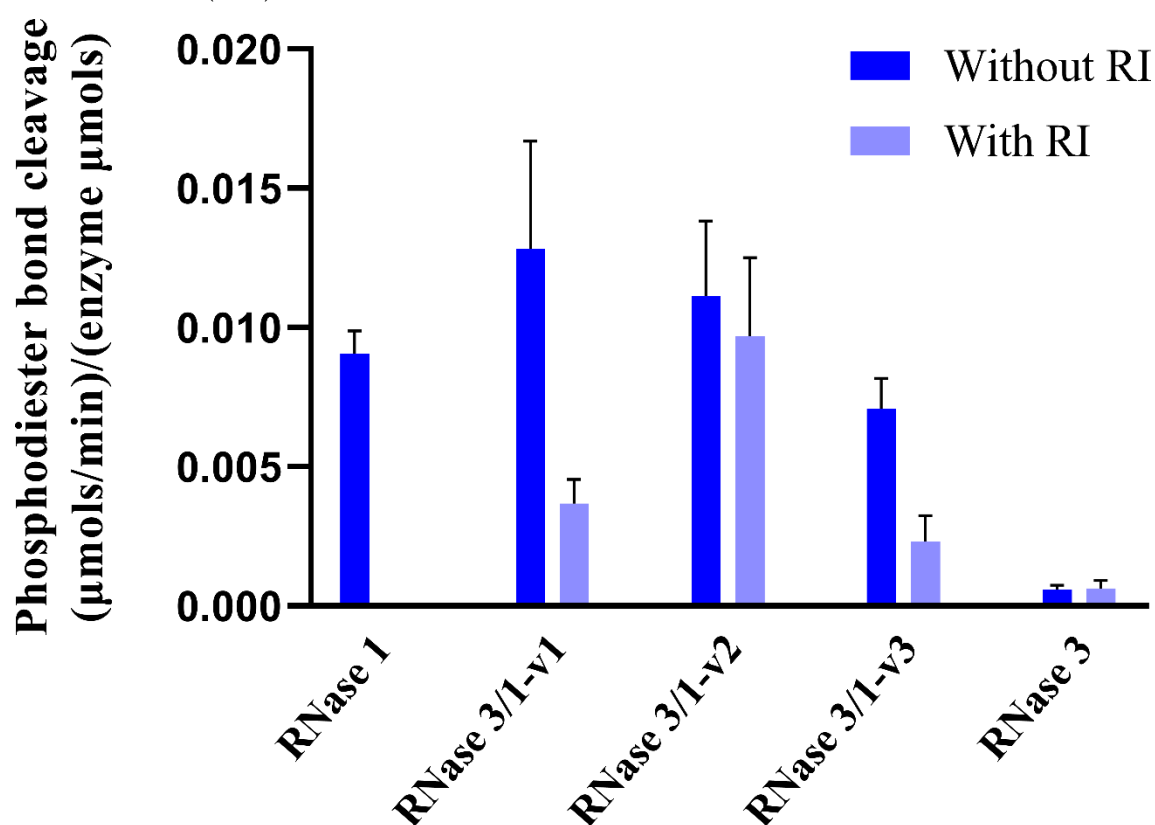
**Figure S2.** Circular dichroism spectra of the three versions of RNase 3/1. The embedded table indicates the estimated % of secondary structures for each construct.



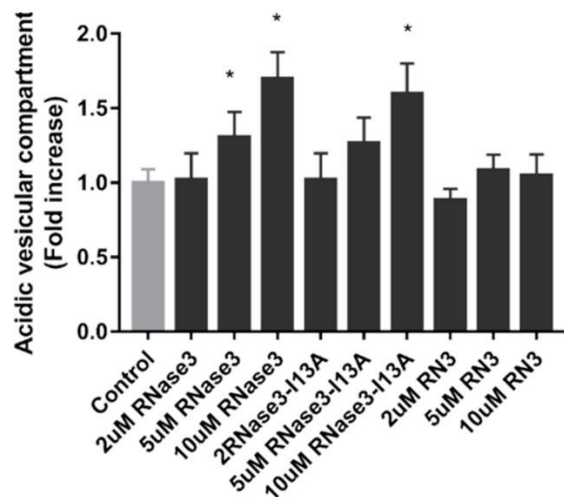
**Figure S3.** LPS binding activity of RNases 1, 3 and the three versions of RNase 3/1 after 1 h of incubation. Dose-response curves were calculated using *OriginPro 8 statistical software*. Results are the average of three replicates.



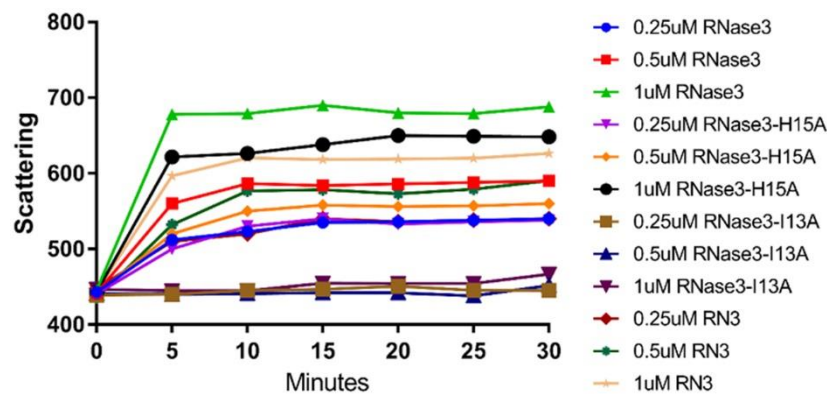
**Figure S4.** Liposome leakage activity of RNases 1, 3 and the three versions of RNase 3/1 after 1 and 24 hours of exposure. Dose-response curves were calculated using *GraphPad Prism 9*. Error bars show the standard error of the mean (SEM).



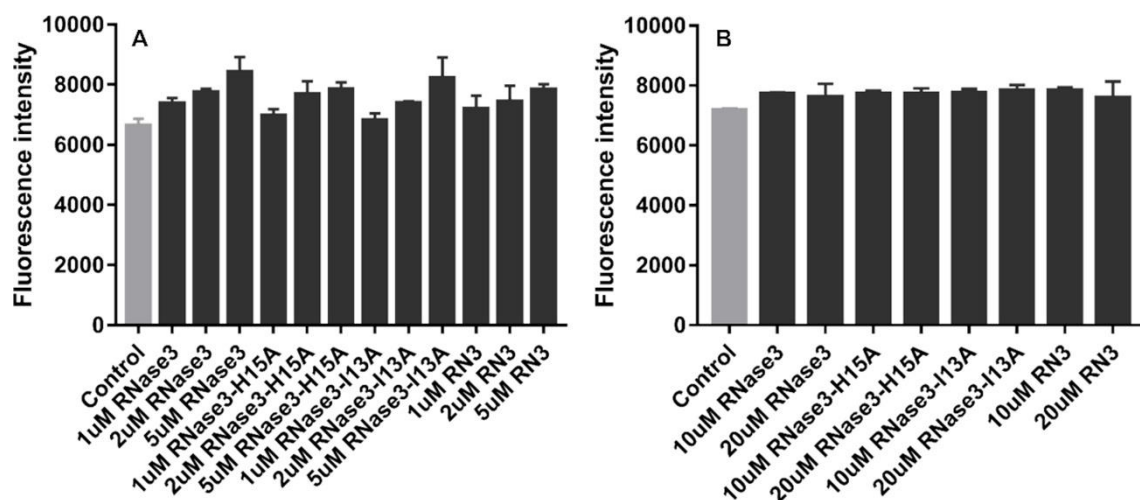
**Figure S5.** Ribonuclease inhibitor (RI) percentage of inhibition was determined by comparing the catalytic activity against CpA with or without presence of the inhibitor. Values represent the activity in  $\mu\text{mols}$  of product per  $\mu\text{mol}$  of enzyme. Results are shown from at least 3 replicates (mean  $\pm$  SEM).



**Figure S6.** Autolysosome formation measured by AO staining. Results are shown from 8 independent experiments (mean  $\pm$  SD).



**Figure S7.** Liposome aggregation by different timing points and/or concentration of protein or peptide. Comparison of kinetic aggregation profile of liposomes by several concentrations of RNase 3 wild-type, mutants and RN3 peptide protein (from 0.25  $\mu$ M to 1  $\mu$ M). Protein or peptide was incubated with 200  $\mu$ M of liposomes at room temperature, the scattering intensity was recorded every 5 minutes, while no protein added is indicated as time 0.



**RNAse1**  $\alpha 1$  TT  $\alpha 2$   $\beta 1$   $\alpha 3$  TT  $\beta 2$   $\beta 3$  TT

RNase1 ...KESRAK**K**TFQCHMDS**TS**SPFSSTSYCNQMRRRNMTQGRCKPVNTFFVHEPLVDVQNVVCFQEKVTCKN...GGQNCYKSNSSMHITDCRLTNGS...  
RNase2 KPPQFTWAQ**K**FETQHINMT...QCCQTNAMQVINNYQRCKNQNTFLITTFANVVNVCGNPMTCPSNKRTRKNCHHSQSQVPLIHCDLITTPSE...  
RNase3 RPPQFTWAQ**K**FAIGHISLN...PFCCTIAMRAINRYRWRCKNQNTFLITTFANVVNVCGNQSIKCPHNRTLKNHRSRFRVPLIHCDLITNPGAQ...  
RNase6 WPKRLTKAH**K**FETQHIOQS...PLQCNRAMSGGINNYT**K**CHCKHNTFLHEDSPQNVAAVCDLLSIYCKN...RRHNCHOSCKPVNMTDCRLTSG...  
RNase7 **K**KPKMTSSSQWFKIQHMVPS...PQACNSAMKNI**K**TKCKDLNTFLHEDPSSVAITCQTFPIACKN...GDKNCHOSHGAVSLTCKLTS...  
RNase8 **K**KPKMTSSSQWFKIQHMVPS...PQACNSAMSI**K**INKYTECKDLNTFLHEDPSSVAITCQTFPIACKN...GDKNCHOSHGAVSLTCKLTS...  
RNase5 ...QDNR**K**YTHFLTQHYDAKPGGRDRDYCESIM**K**RR**K**DT...SECKDINTFIHGKRSIKKAIKENKNGNH...REN**K**IS**K**SS**F**QVITCKLHGGS...  
RNase4 ...QDGM**K**YCF**K**LQHVHEP.ETGGSDRY**K**CNLMQRRKMTLY**K**CKRNTFIHEDIWNIRSI**K**CTIN**K**CKN...GKMNCH**K**GVVKTDCRLTSGS...

**RNAse1** TT  $\beta 4$   $\beta 5$   $\beta 6$

RNase1 **R**Y**P**NCAYRTSPKERHII**V**ACEGS...**P****V****V**VPV**H**T**D**ASVEDST  
RNase2 NI**G**NCRYAQTPANMFY**I**VACDNRDQRRDPPQ**V****P****V**VPVHLDRII...  
RNase3 NI**G**NC**T**YADRPGRRFY**V**VACDNRDPR.DSPRY**P****V****V**VPVHLDTTI...  
RNase6 **K**Y**P**QCRYSAAAQ**K**Y**F**F**I**VACDPPQK.SD.P**P****V**K**L**VPVHLD**S**IL...  
RNase7 **K**HPNCRY**K**KK**F**ONKSYV**V**VACPPQ**K**.KDSQ**F**HLVPVHLDRVL...  
RNase8 **K**Y**P**NCRY**K**KKHLNT**P**Y**I**VACDPPQ**K**.GD.P**G**Y**P**LVPVHLDKV...  
RNase5 **P**WP**P**CCYRATAGFRNVV**V**ACENG...**P**VHLDQ**S**IFRRP  
RNase4 **R**APNCK**Y**RA**T**ASTRRV**V**IACEGN...**P**QVPV**H**FDG...

**Table S1. Prediction of the subcellular localization of human RNases by signal tag**

	Signal	Aminoacid Number/Tag	Localization
RNase 1	<a href="#">Yx{2}[VILFWCM]</a>	91-95/YPNC; 114-118/YVPV	Lysosome
	<a href="#">Kx{3}Q</a>	6-11/KFQRQ	Lysosome
	<a href="#">SPS</a>	17-20/SPS	Nucleus
RNase 2	<a href="#">Yx{2}[VILFWCM]</a>	122-126/YPVV	Lysosome
RNase 3	<a href="#">Yx{2}[VILFWCM]</a>	121-125YPVV	Lysosome
RNase 4	<a href="#">Yx{2}[VILFWCM]</a>	4-8/YQRF; 23-27/YCNL; 93-97/YRAT	Lysosome
	<a href="#">[HK]x{1}K</a>	37-40/HCK	Endoplasmic reticulum
RNase 5	<a href="#">Yx{2}[VILFWCM]</a>	5-9/YTFH	Lysosome
	<a href="#">Kx{3}Q</a>	72-77/KSSFQ	Lysosome
	<a href="#">RRRGL</a>	30-35/RRRGL	Nucleus
RNase 6	<a href="#">Yx{2}[VILFWCM]</a>	87-91/YPQC; 98-102/YKFF; 115-119/YKLV	Lysosome
	<a href="#">[HK]x{1}K</a>	35-38/HCK	Endoplasmic reticulum
RNase 7	<a href="#">Kx{3}Q</a>	66-71/KNCHQ; 93-98/KEKRQ; 110-115/KKDSQ	Lysosome
	<a href="#">[HK]x{1}K</a>	0-3/KPK; 32-35/HTK; 93-96/KEK	Endoplasmic reticulum
RNase 8	<a href="#">Yx{2}[VILFWCM]</a>	87-91/YPNC; 115-119/YPLV	Lysosome
	<a href="#">Kx{3}Q</a>	66-71/KNCHQ	Lysosome
	<a href="#">GYx{2}[VILFWCM]</a>	114-119/GYPLV	Lysosome

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[\[HK\]x{1}K](#)

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0-3/KPK;  
93-96/KEK

Endoplasmic reticulum