

SUPPLEMENTARY DATA 1

RNA binding properties of the Ty1 LTR-retrotransposon Gag protein

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Figure S1. Raw MST traces for Ty1 Gag-p49 interactions with mTy1 RNA (**A**), non-Psi Ty1 RNA (**B**), 18S rRNA (**C**), and Δ S1a RNA (**D**) at 150mM NaCl.

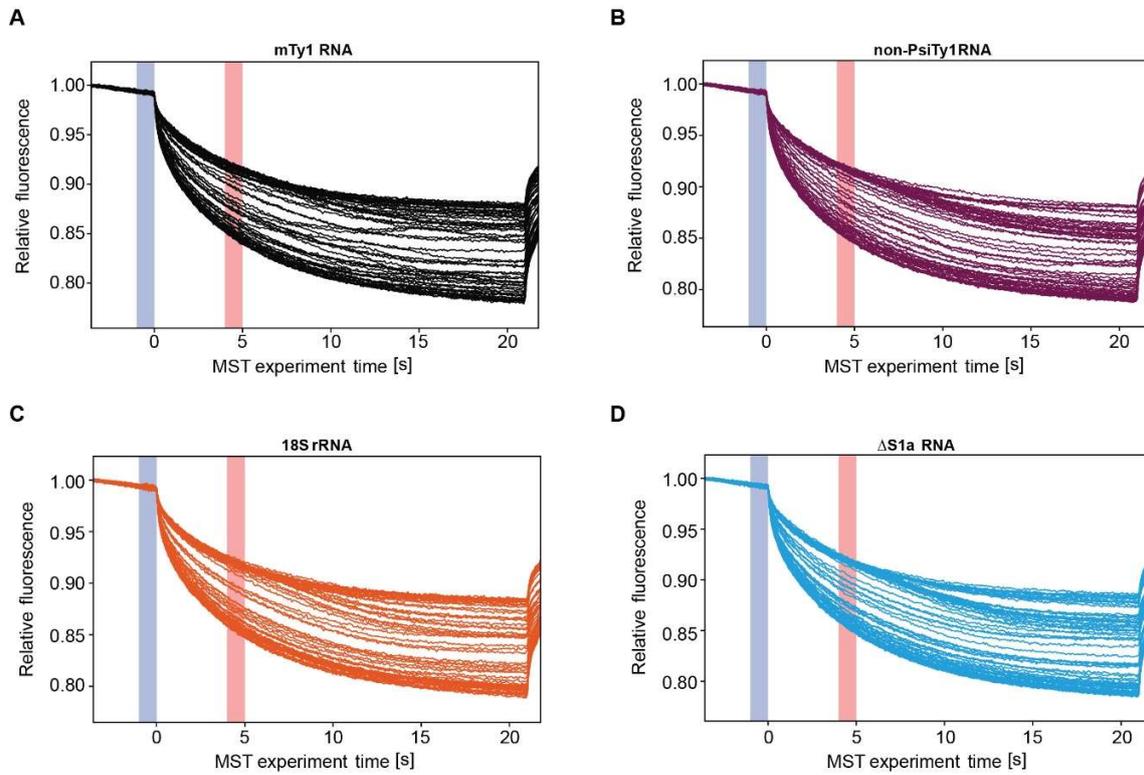


Figure S2. Impact of a large excess of tRNA on Ty1 Gag-RNA binding. Dose–response binding curves of Ty1 Gag-p49 to mTy1 RNA (black) and 18S rRNA (orange) in the presence (dashed lines) or absence (solid lines) of total *E.coli* tRNA in ~133-fold molar excess. Lines represent fits of the data points using the Hill equation. The excess of tRNA resulted in 5.2-fold and 5.7-fold increase in K_D value for mTy1 RNA binding and for 18S rRNA, respectively (K_D 928.7nM and 1445.7nM).

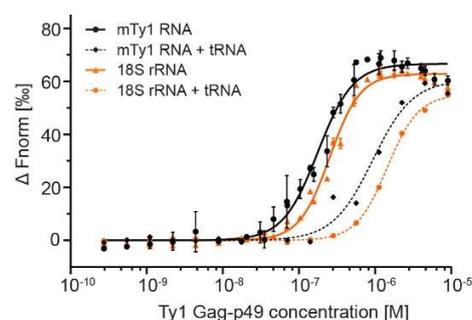


Table S1. Impact of buffer ionic strength on Ty1 Gag-RNA interactions analyzed by EMSA. Binding parameters were obtained based on quantitative analysis of gel images. Mean \pm SD of K_D from at least 3 independent EMSA experiments.

	K_D [nM]	n_H	K_D [nM]	n_H	K_D [nM]	n_H	K_D [nM]	n_H
	150 mM NaCl		300 mM NaCl		500 mM NaCl		800 mM NaCl	
mTy1 RNA-Gag-p49	88.5 \pm 3.6	1.5	136.7 \pm 5.6	2.2	212.4 \pm 27.4	1.5	454.0 \pm 133.5	1.5
18S rRNA-Gag-p49	261.5 \pm 14.2	2.8	551.8 \pm 98	1.9	711.7 \pm 307.9	1.7	634.1 \pm 133.5	2.5
mTy1 RNA-Gag-p45	179 \pm 20.9	1.9	227.9 \pm 20	2	363.1 \pm 56.2	1.9	469.9 \pm 29.1	4.6
18S rRNA-Gag-p45	425 \pm 95.1	1.5	514.7 \pm 56.7	2.5	936 \pm 239.7	2.6	891 \pm 309.8	2.6

Table S2. Impact of RNA competitor on Ty1 Gag-p49 binding to mTy1 RNA and 18S rRNA analyzed by EMSA. Binding parameters for Ty1 Gag-RNA interactions were obtained based on quantitative analysis of gel images. Mean \pm SD of K_D from at least 3 independent EMSA experiments.

Competitor \ Labeled RNA	mTy1 RNA		18S rRNA	
	K_D [nM]	n_H	K_D [nM]	n_H
- competitor	88.5 \pm 3.6	1.5	261.5 \pm 20.7	2.8
mTy1 RNA	390.1 \pm 34.7	2.3	752.7 \pm 217.6	1.9
18S rRNA	191.4 \pm 33.1	1.7	1043 \pm 124	1.3

Figure S3. Comparative analysis of the 3D structure of wild type mTy1 RNA and Δ S1a RNA. Hydroxyl radical (HR) cleavage profiles and difference plot of protein free mTy1 RNA (black) in comparison with protein free Δ S1a RNA (blue). On the difference plot, sites of decreased HR cleavage for Δ S1a RNA are indicated by positive peaks. Regions important for Ty1 retrotransposition are boxed.

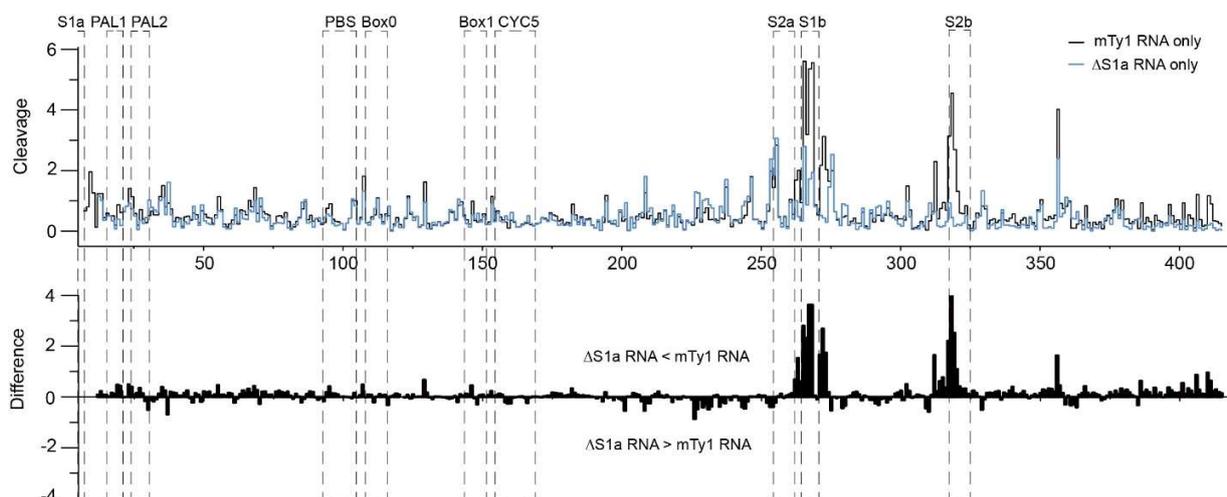


Figure S4. Frequency of nucleotide occurrence within Ty1 Gag-p49 binding sites and their vicinities represented as a logo (<http://weblogo.berkeley.edu/>).



Table S3. Primers used for DNA templates construction.

Primer	Sequence
F-miniRNA	GATTTAGGTGACACTATAGAGGAGAACTTCTAGT
R-miniRNA	ACATTGGTGGTGGTCTGAC
Δ S1a_RNA_PF	GATTTAGGTGACACTATAGAACTTCTAGTATATT
non-Psi Ty1_RNA_PF	TAATACGACTCACTATAGGGTCAAAGACATCCTATCC
non-Psi Ty1_RNA_PR	TTTACTGTAGATTCAGTAAGTTTCTGG
18S_rRNA_PF	GATTTAGGTGACACTATAGTATCTGGTTGATCCT
18S_rRNA_PR	CGCGGCTGCTGGCACCAGAC