## SUPPLEMENTARY INFORMATION

# Ty1 retrovirus-like element Gag contains overlapping restriction factor and nucleic acid chaperone functions

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**Table S1. Primers used for construction of expression plasmids.**

| Primer name | Sequence[a] |
|---|---|
| A-outGAL1F | ATAATACCGCGCCACATAGC |
| D-PR1R | CATTGATAGTCAATAGCACTAGACC |
| B-GCG1-R | AAAGAATTTTCGCGATATCCGTATAATCAACG |
| C-GCG1-F | GGATATCGCGAAAATTCTTTCCAAAAGTATTG |
| B-GCG2-R | TATCAGATTGCGCTTTTTCAATACTTTTGG |
| C-GCG2-F | TGAAAAAGCGCAATCTGATACCCAAGAGGC |
| B-C1040-R | GAATTTTGCATGATATCCGTATAATCAACGGATAGGATG |
| C-C1040-F | GATTATACGGATATCATG**C**AAAATTCTTTCCAAAAGTATTG |
| 294EcoRIF | CATGTTTC***GAATTC***ATGGAATCCCAACAATTATCTCAAC |
| 810EcoRIF | CATGTTTC***GAATTC***ATGCCAATGTTAACCTCACCTAATG |
| 1038EcoRIF | CATGTTTC***GAATTC***ATGAAAATTCTTTCCAAAAGTATTG |
| 1068EcoRIF | CATGTTC***GAATTC***ATGCAATCTGATACCCAAGAGGCAA |
| AUG1GCG2EcoRIF | TC***GAATTC***ATGAAAATTCTTTCCAAAAGTATTGAAAAAGCG CAATCTGATACCCAAGAGG |
| GCG1AUG2EcoRIF | CATGTTTC***GAATTC***GCGAAAATTCTTTCCAAAAGTATTG |
| GCG1GCG2EcoRIF | TC***GAATTC***GCGAAAATTCTTTCCAAAAGTATTGAAAAAGCG CAATCTGATACCCAAGAGG |
| C1040EcoRIF | CATGTTTC***GAATTC***ATG**C**AAAATTCTTTCCAAAAGTATTG |
| 1355XhoIR | CATGTTTC***CTCGAG***TTATCTCGATCCCTGTTGTTCTT |
| 1496XhoIR | CATGTTTC***CTCGAG***TTAGTGAGCCCTGGCTGTTTTCG |
| 1613XhoIR | CATGTTTC***CTCGAG***TCAGTAAGTTTCTGGCCTAAGATG |
| 810NdeIF | CATGTTTC***CATATG***CCAATGTTAACCTCACCTAATG |
| 1038NdeIF | CATGTTTC***CATATG***AAAATTCTTTCCAAAAGTATTG |
| 1068NdeIF | CATGTTC***CATATG***CAATCTGATACCCAAGAGGCAA |
| 810XbaIF | CTAG***TCTAGA***CATGCCAATGTTAACCTCACCTAATG |
| 1496HindIIIR | CCC***AAGCTT***TTAGTGAGCCCTGGCTGTTTTCG |

[a]Restriction enzymes cleavage sites are annotated in bold and italic, start codons are underlined.

**Table S2. Primers used for reverse transcription and construction of templates for *in vitro* transcription.**

| Primer name | Sequence[a] |
|---|---|
| F-AUG1AUG2 | **TAATACGACTCACTATA**GGGTCAAAGACATCCTATCC |
| F-GCG1AUG2 | **TAATACGACTCACTATA**GGGTCAAAGACATCCTATCCGTT GATTATACGGATATCGCGAAAATTCTTTCCAAAAGTATTG AAAAAATGCAATCTGATACCCAAGAGGCAAACGACA |
| F-AUG1GCG2 | **TAATACGACTCACTATA**GGGTCAAAGACATCCTATCCGTT GATTATACGGATATCATGAAAATTCTTTCCAAAAGTATTGA AAAAGCGCAATCTGA TACCCAAGAGGCAAACGACA |
| R-p22 | TTTACTGTAGATTCAGTAAGTTTCTGG |
| F-miniRNA | **GATTTAGGTGACACTATA**GAGGAGAACTTCTAGT |
| R-miniRNA | ACATTGGTGGTGGTCTGAC |
| PR3 | TCAGGTGATGGAGTGCTCAG |

[a]Promoters of SP6 and T7 polymerases are annotated in bold, start codons are underlined.

**Table S3. Calculated dissociation constants for CTR, AUG1p18 and AUG2p18.**

| **NaCl [mM]** | **CTR** | **AUG1p18** | **AUG2p18** |
|---|---|---|---|
| 10 | 5.57 ± 0.4 nM | 5.19 ± 0.17 nM | 5.98 ± 0.11 nM |
| 50 | 7.18 ± 0.54 nM | 8.44 ± 0.31 nM | 6.49 ± 0.19 nM |
| 100 | 7.91 ± 0.62 nM | 6.98 ± 0.7 nM | 15.84 ± 0.83 nM |
| 150 | 12.23 ± 1.13 nM | 26.78 ± 2.3 nM | 61.41 ± 4.03 nM |
| 200 | 21.67 ± 2.04 nM | 56.07 ± 4.87 nM | 133.96 ± 7.74 nM |
| 250 | 46.31 ± 10.2 nM | 72.3 ± 6.51 nM | 652.85 ± 61.38 nM |
| 500 | n.d. | n.d. | n.d. |

**Figure S1. Ty1 protein levels following induction of pGTy1*his3-AI* and pYES2-*GAG* derivatives.** A shorter exposure of the immunoblot in Figure 2C is presented to show differences in Gag protein levels.

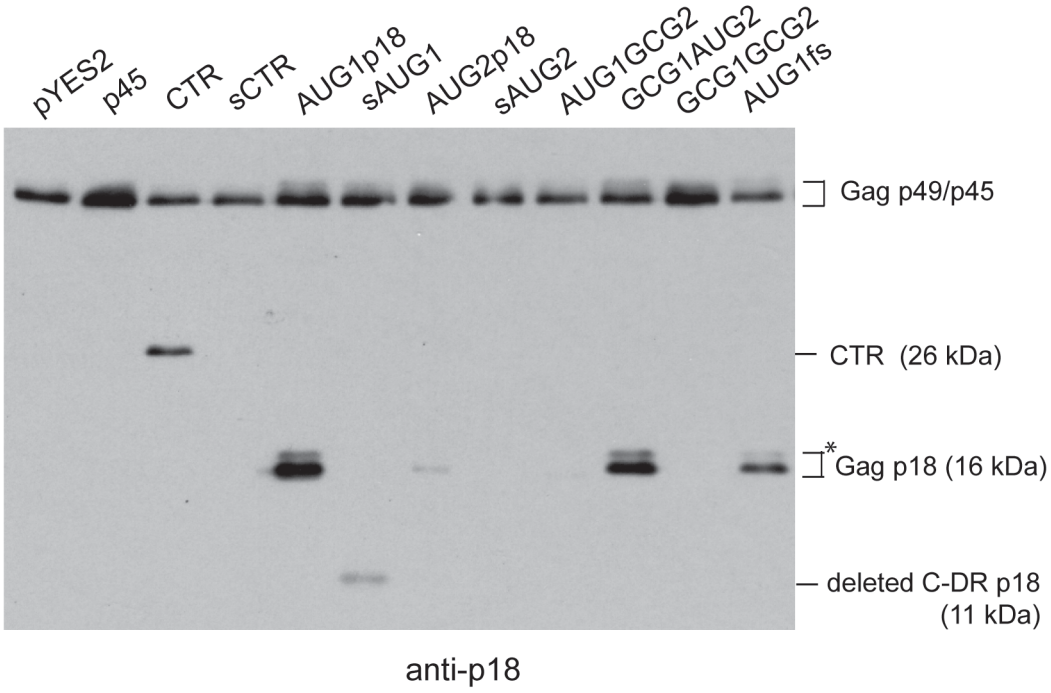



**Figure S2. Ty1 transcripts expressed from pYES2-*GAG* derivatives.** Total RNA from a Ty1-less strain induced for expression of pGTy1*his3-AI* and pYES2-*GAG* derivatives was subjected to Northern blot analysis with a Ty1 $^{32}$P-labeled strand-specific riboprobe (nts 238 – 1702) that spans the *GAG* region (Ty1 RNA). Ty1*his3-AI* mRNA served as a loading control. Ty1/Ty1*his3-AI* RNA ratios were determined by phosphorimage analysis.

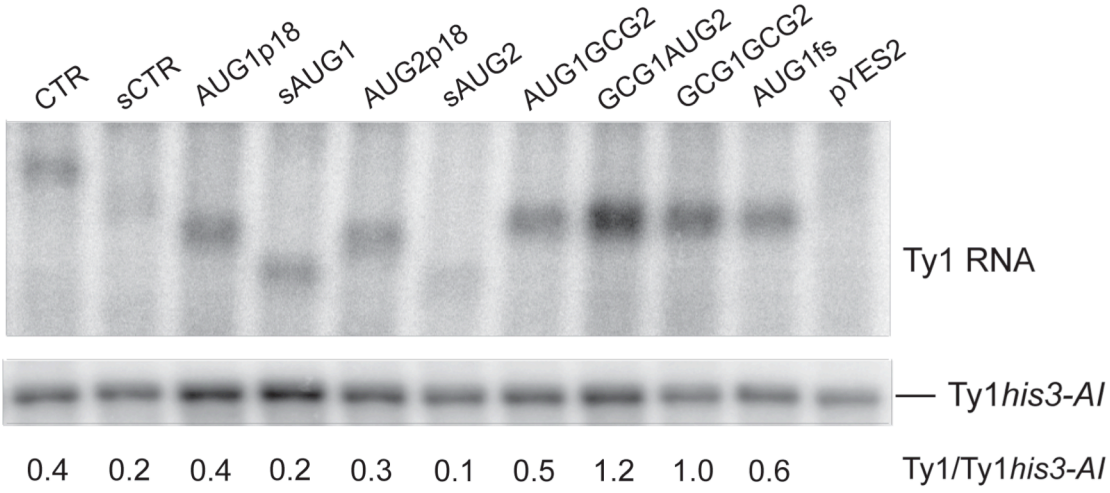
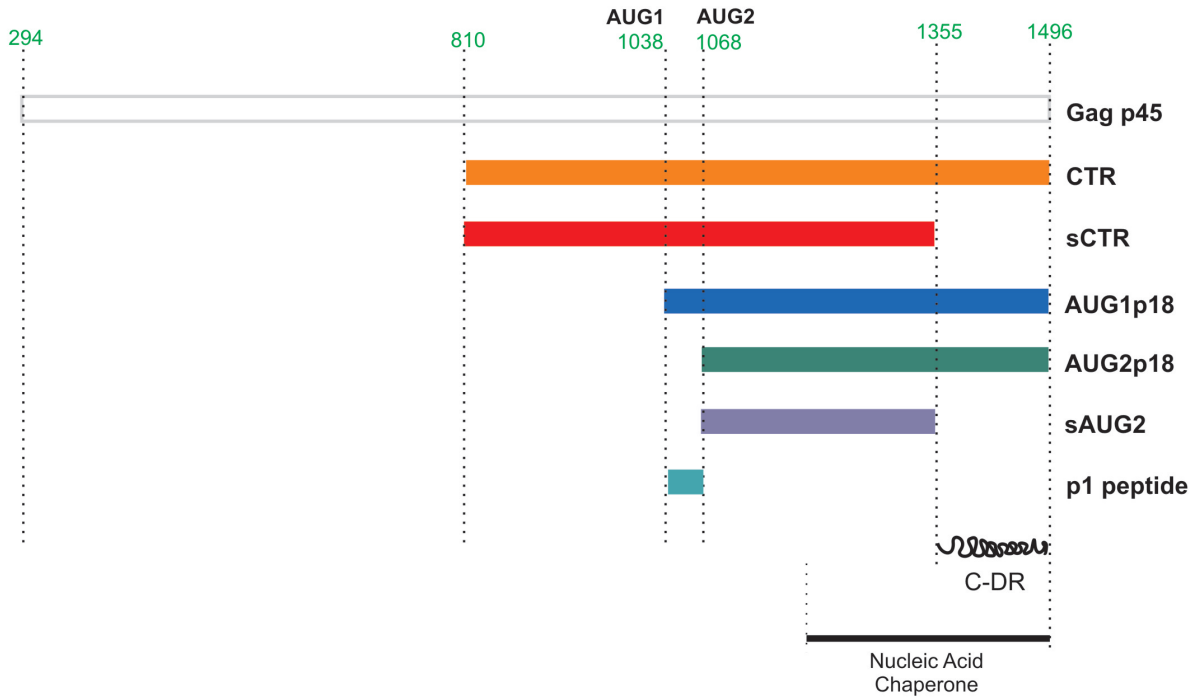


**Figure S3. The Ty1 Gag derived proteins. (A)** Schematic representation of Gag-derived proteins used in *in vitro* assays. Gag p45 is shown as a reference. **(B)** SDS-PAGE of recombinant proteins used in this study. His-tagged Gag proteins were purified by Talon affinity resin from *E. coli* strain BL21.

**A**




**B**

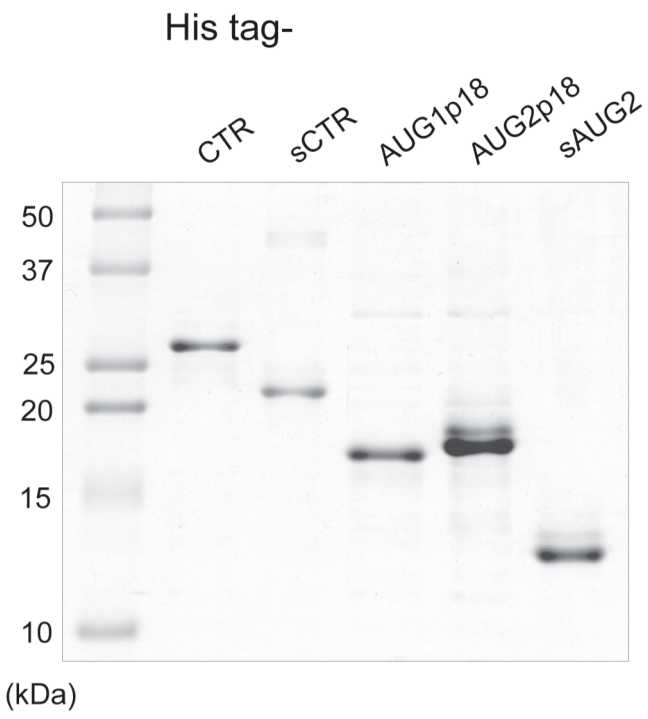


**Figure S4. TAR(-) DNA / TAR(+) DNA annealing activity of CTR and AUG2p18 proteins.** The assays were performed as a function of protein concentration (0; 0.1; 0.15; 0.2; 0.3 μM) that corresponds to 1:8; 1:5; 1:4; 1:2.6 protein to nt ratios. The graphs represent averaged data from three independent annealing experiments for each protein. The error bars represent standard deviations.

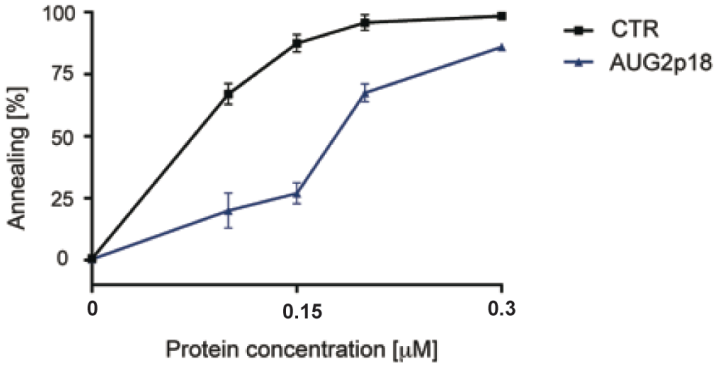



**Figure S5. Determination of RNA binding properties of sCTR, sAUG2 and p1 peptide.** Representative raw autoradiograms from filter binding experiments showing RNA binding properties of sCTR, sAUG2 and p1 peptide at concentrations ranging from 10 to 10000 nM. For comparison, autoradiogram for AUG1p18 protein (concentration from 1 to 1250 nM) is included.

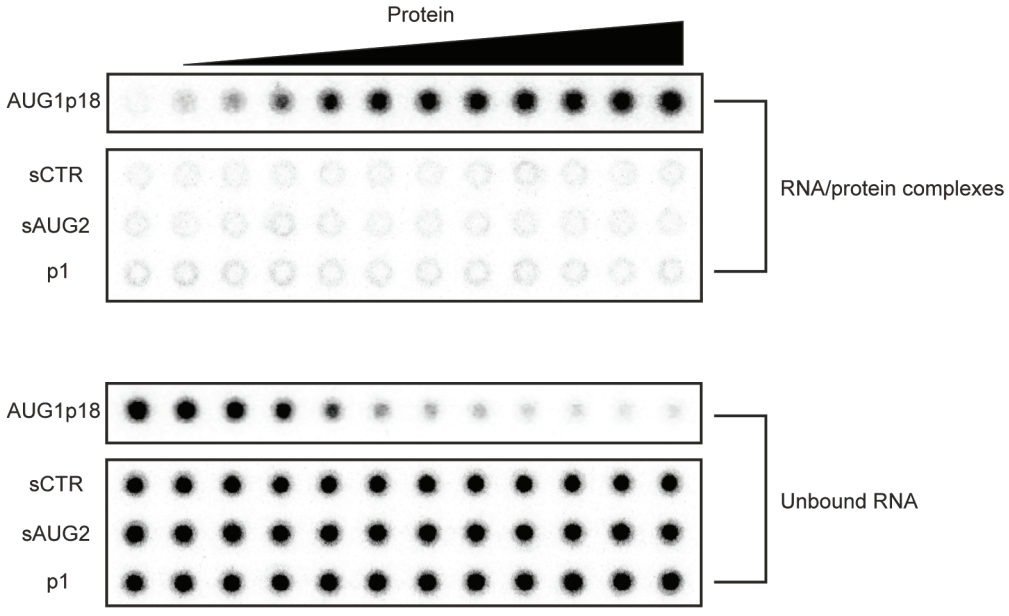


**Figure S6. Influence of p18 proteins on the CTR mediated annealing of $tRNA_i^{Met}$ to the Ty1 mini RNA.** The graph represents the averaged percent of annealed strands in the presence of CTR (1.5 μM) and increasing concentrations of AUG2p18 or AUG1p18. The error bars represent standard deviations.

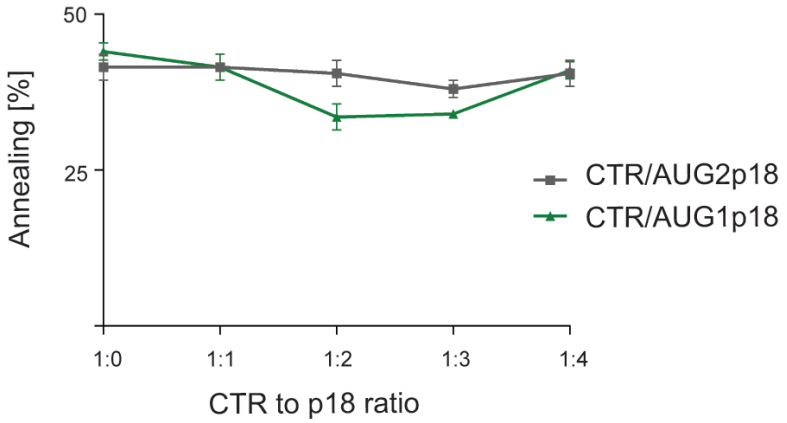



**Figure S7. Expression of GST, GST-CTR, and p18.** Total protein extracts from a Ty1-less strain induced for expression of GST alone, GST-CTR alone, GST and p18, GST-CTR and p18, and p18 alone were immunoblotted with GST or p18 antisera. The putative p18 degradation product (*) appeared only when co-expressed with GST or GST-CTR.