SUPPLEMENTARY INFORMATION

A novel two-pronged strategy to suppress host protein synthesis by SARS coronavirus Nsp1 protein
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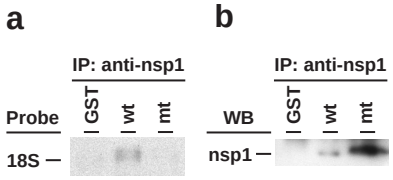
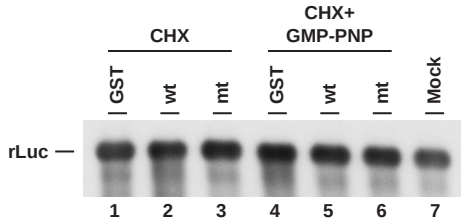
Supplementary Figure 1



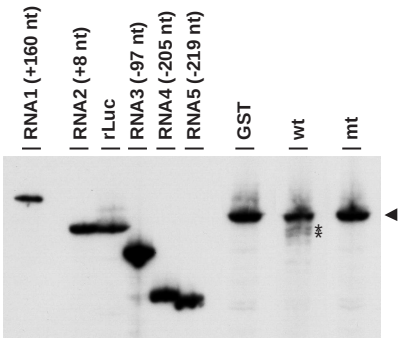
Supplementary Figure 1
Binding of nsp1 to the 40S ribosomal subunits in RRL
After incubation of GST, nsp1 (wt), or nsp1-mt (mt) in RRL at 4^{o}C overnight, the samples were subjected to immunoprecipitation analysis using anti-nsp1 antibody. (a) RNAs were extracted from the immunoprecipitates and subjected to Northern blot analysis using a probe that specifically binds to 18S rRNA. Note the co-immunoprecipitation of 18S rRNA with nsp1, but not with GST or nsp1-mt. (b) To confirm the immunoprecipitation of nsp1 protein by anti-nsp1 antibody, the immunoprecipitates were examined by Western blot analysis using anti-nsp1 antibody. Anti-nsp1 antibody immunoprecipitated both nsp1 and nsp1-mt. The detection of higher levels of nsp1-mt in the immunoprecipitates probably indicated that anti-nsp1 antibody immunoprecipitated nsp1 that bound to the 40S ribosomes less efficiently as compared to nsp1-mt, which did not bind to the 40S ribosomes.

Supplementary Figure 2



Supplementary Figure 2
Northern blot analysis of rLuc RNA
RRL was pre-incubated with GST (lanes 1 and 4), nsp1 (lanes 2 and 5) or nsp1-mt (lanes 3 and 6) for 10 min on ice and then, the samples were incubated with CHX (lanes 1 to 3) or a mixture of CHX and GMP-PNP (lanes 4 to 6) for 5 min at 30^{o}C. Then, rLuc RNA was added to each sample, and the samples were incubated for 10 min at 30^{o}C. RNAs were extracted and subjected to Northern blot analysis using the rLuc probe. Lane 7, rLuc RNA extracted from mock-treated RRL.

Supplementary Figure 3



Supplementary Figure 3
Analysis of nsp1-induced modification of rLuc RNA
3'-end-labeled non-polyadenylated rLuc RNA was incubated with GST, nsp1 (wt), or nsp1-mt (mt) in RRL for 15 min at 30^{o}C in the presence of CHX and GMP-PNP. The RNAs were extracted, resolved on a sequencing gel, and visualized by autoradiography. The untreated 3'-end-labeled non-polyadenylated rLuc RNA, and five RNAs, including RNA1, RNA2, RNA3, RNA4, and RNA5 of known sizes, served as size markers. The size differences (in nucleotides) between each marker RNA and the non-polyadenylated rLuc RNA are shown at the top of the gel. The arrowhead represents the full-length non-polyadenylated rLuc RNA and the asterisks represent modified rLuc RNAs.