SUPPORTING INFORMATION

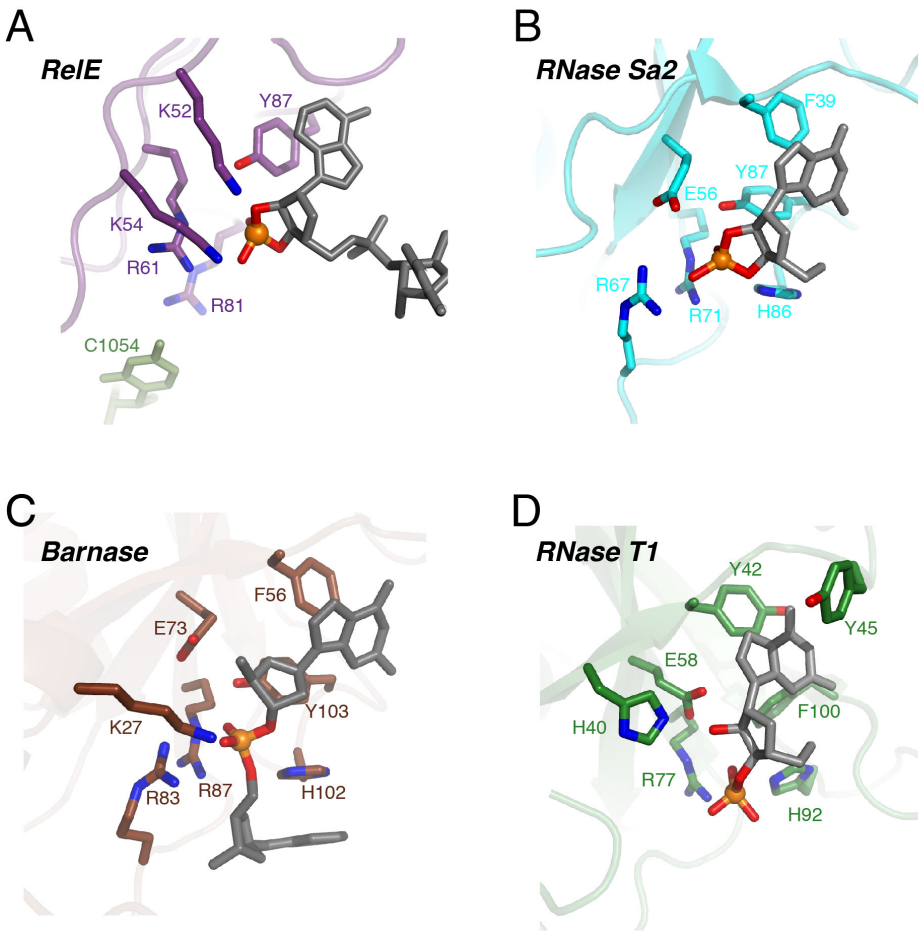

Figure S1. Comparison of RelE active-site structure and composition with other RNases. (A) RelE in the post-cleavage structure with ribosomal-bound mRNA (PDB ID: 3KIU) . (B) RNase Sa2 with guanosine 2′,3′-cylcophosphorothioate (PDB ID: 3D5I) (C) Barnase, with deoxy-guanosine-deoxy-adenosine (PDB ID: 1BRN), and (D) RNase T1 with 3′-guanosine-monophosphate (PDB ID: 1RGA). RNase proteins are shown in cartoon representation, with important active-site residues depicted as sticks. Heteroatoms near the scissile phosphate are colored: nitrogen (blue), hydrogen (red). Bound substrates are shown in dark grey sticks for each RNase, with important heteroatoms colored: oxygen (red), phosphate (orange).

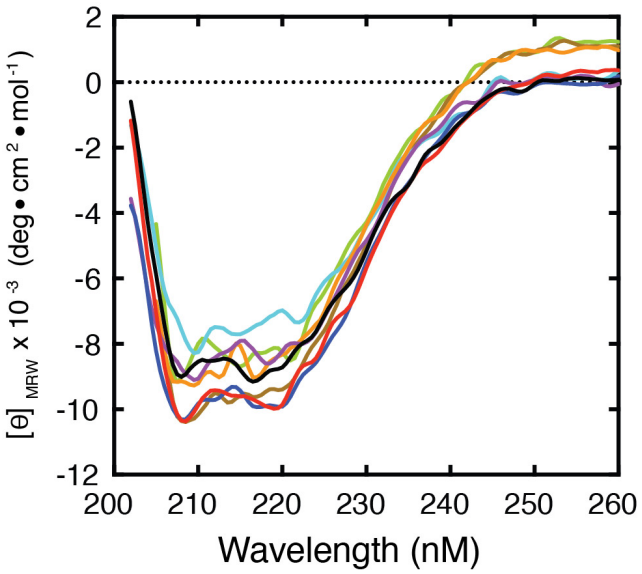

Figure S2. Far-UV circular dichroism analysis of RelE proteins. Wavelength scans collected from 202 - 260 nm at 4 °C for RelE proteins wild-type, black; K52A, light purple; K54A, orange; R61A, red; R81A, light green; Y87F, light blue; Y87A, brown; K52A/Y87F, blue.

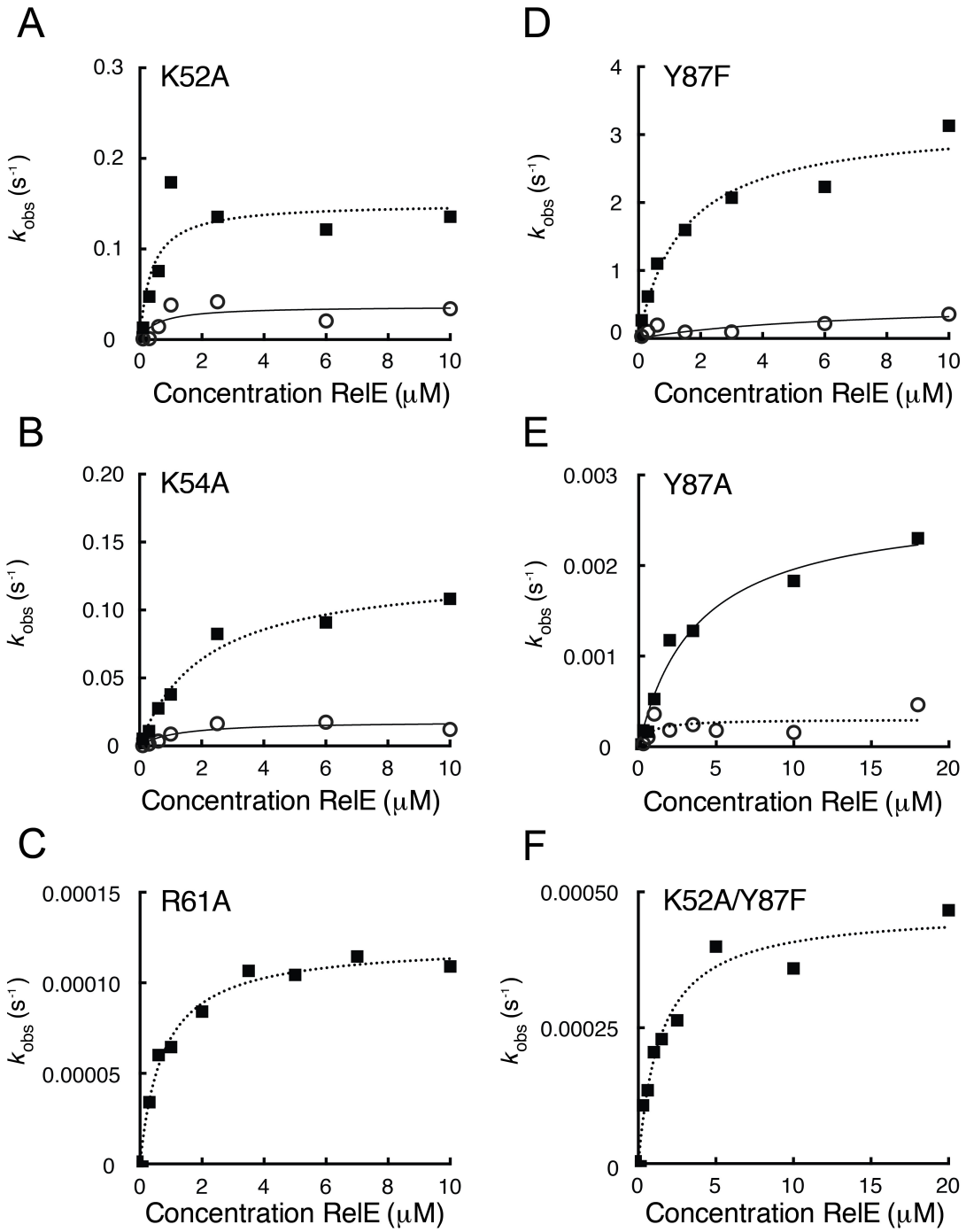

Figure S3. Dependence of mutant single-turnover rates on enzyme concentration. The rate, k_{obs} (s^{-1}), for the fast (■) and slow phases (O) from a single replicate are plotted as a function of RelE concentration (μM) for mutants (A) K52A, (B) K54A, (C) R61A, (D) Y87F, (E) Y87A, (F) K52A/Y87F. The rate constants (k_2) and dissociation constants (K_d) were extracted from hyperbolic fits of each replicate and the mean and SEM for each constant is listed in Table 1 and Table S1.

	$k_{2,\ slow}$ (s^{-1})[a]	Fold Change[b]	$K_{d,\ slow}$ (μM)[a]
Wild type	16 ± 2		1.5 ± 0.3
K52A	0.038 ± 0.011	4.2×10^2	0.63 ± 0.34
K54A	0.027 ± 0.016	5.9×10^2	1.4 ± 0.8
R81A	0.00049 ± 0.00005	3.2×10^4	0.42 ± 0.20
Y87F	0.27 ± 0.04	5.9×10^1	1.0 ± 0.2
Y87A	0.00024 ± 0.00006	6.6×10^4	0.43 ± 0.14

[a] Values are the mean ± SEM from at least three independent determinations.
[b] Values calculated as the change in cleavage rate constants for each mutant relative to the wild-type rate.

Table S1. Single-turnover mRNA cleavage rate constants and dissociation constants for wild-type and mutant RelE proteins. The k_2 and K_d values for the slow phase are reported as the mean ± SEM from at least three independent determinations. Fold changes were calculated as the change in cleavage rate constant for each mutant relative to the corresponding wild-type rate constant.