

**Table S1: Association rates for  $E\sigma^{70}$  variants from Figure 4**

$E\sigma^{70}$	6S RNA		P <sub>-35-10</sub> DNA	
	$y_{\max}$	$k_{\text{obs}}^*$ ( $\text{sec}^{-1} \times 10^3$ )	$y_{\max}$	$k_{\text{obs}}^*$ ( $\text{sec}^{-1} \times 10^3$ )
wild type	0.82	30.5	0.77	5.5
R554A	0.53	1.5	0.73	2.9
R562A	0.68	4.0	0.62	2.3
L573A	0.75	5.8	0.42	1.6
E574A	0.86	212.0	0.76	4.3
R584A	0.16	0.3	0.64	1.6
E585A	0.86	210.3	0.64	1.9
R586A	0.46	0.9	0.65	2.9
R588A	0.18	0.2	0.09	0.3

**Table S2: Association rates for  $E\sigma^{70}$  variants from Figure 5**

$E\sigma^{70}$	6S RNA		P <sub>-35-10</sub> DNA	
	$y_{\max}$	$k_{\text{obs}}^*$ ( $\text{sec}^{-1} \times 10^3$ )	$y_{\max}$	$k_{\text{obs}}^*$ ( $\text{sec}^{-1} \times 10^3$ )
wild type	0.82	30.5	0.77	5.5
K593A	0.52	1.0	0.76	6.5
L595A	0.65	4.4	0.57	2.8
R596A	0.44	0.7	0.77	6.4
K597A	0.23	0.5	0.75	6.0
L598A	0.75	10.2	0.73	4.3
R599A	0.65	2.4	0.75	5.9
H600A	0.80	21.4	0.76	6.8

**Table S3: Association rates for  $E\sigma^{70}$  variants from Figure 7**

$E\sigma^{70}$	6S RNA	
	$y_{\max}$	$k_{\text{obs}}^*$ ( $\text{sec}^{-1} \times 10^3$ )
wild type	0.79	6.3
E574A	0.86	90.9
D581G	0.79	12.5
E585A	0.87	116.1
E591A	0.85	67.3
A592K	0.86	111.1

**Legend for Supporting Material:**  $y_{\max}$  and the pseudo first-order rate constants ( $k_{\text{obs}}$ ) were determined from plots of the average fraction of 6S RNA or promoter

DNA (P<sub>-35-10</sub>) bound to E $\sigma$ <sup>70</sup> over time and the equation  $y = y_{\max}(1 - e^{-k_{\text{obs}}t})$  where  $y_{\max}$  is the maximal level of complex formation (see Experimental methods).  $k_{\text{obs}}^*$  is the  $k_{\text{obs}}$  corrected for changes in  $y_{\max}$  for each protein as described by Saecker et al, 2002 and are reported as  $10^3\text{sec}^{-1}$ . Averages were generated from at least 3 independent experiments and standard error associated with these measurements can be seen in Figures 4, 5 and 7. Standard error associated with how well the data fit to the equation was generally < 5% of  $y_{\max}$  or  $k_{\text{obs}}$ , except when values were very low, such as for R588A. Note that RNA binding was done at 18°C for Table S1 and S2 and 9°C for Table S3, DNA binding was at 30°C, and for all binding 2.2 nM E $\sigma$ <sup>70</sup>(active) and 0.5 nM nucleic acid was used. Rate constants can only be compared for binding done under the same conditions; thus it is not possible to directly compare the rate of RNA and DNA binding here.