

Figure S1. Determination of Tex:25nt ssRNA binding stoichiometry using fluorescence polarization. Fluorescein labeled RNA was held at a constant concentration 20-fold above the  $K_d$ . Tex protein was titrated in at increasing concentration. An inflection point representing the molar ratio where the binding sites have become saturated occurs at equimolar concentrations of Tex and 25nt ssRNA indicating a 1:1 binding stoichiometry. The assay was performed in 15mM Tris 7.5, 100mM NaCl, 5% glycerol and measured using a Tecan fluorimeter.

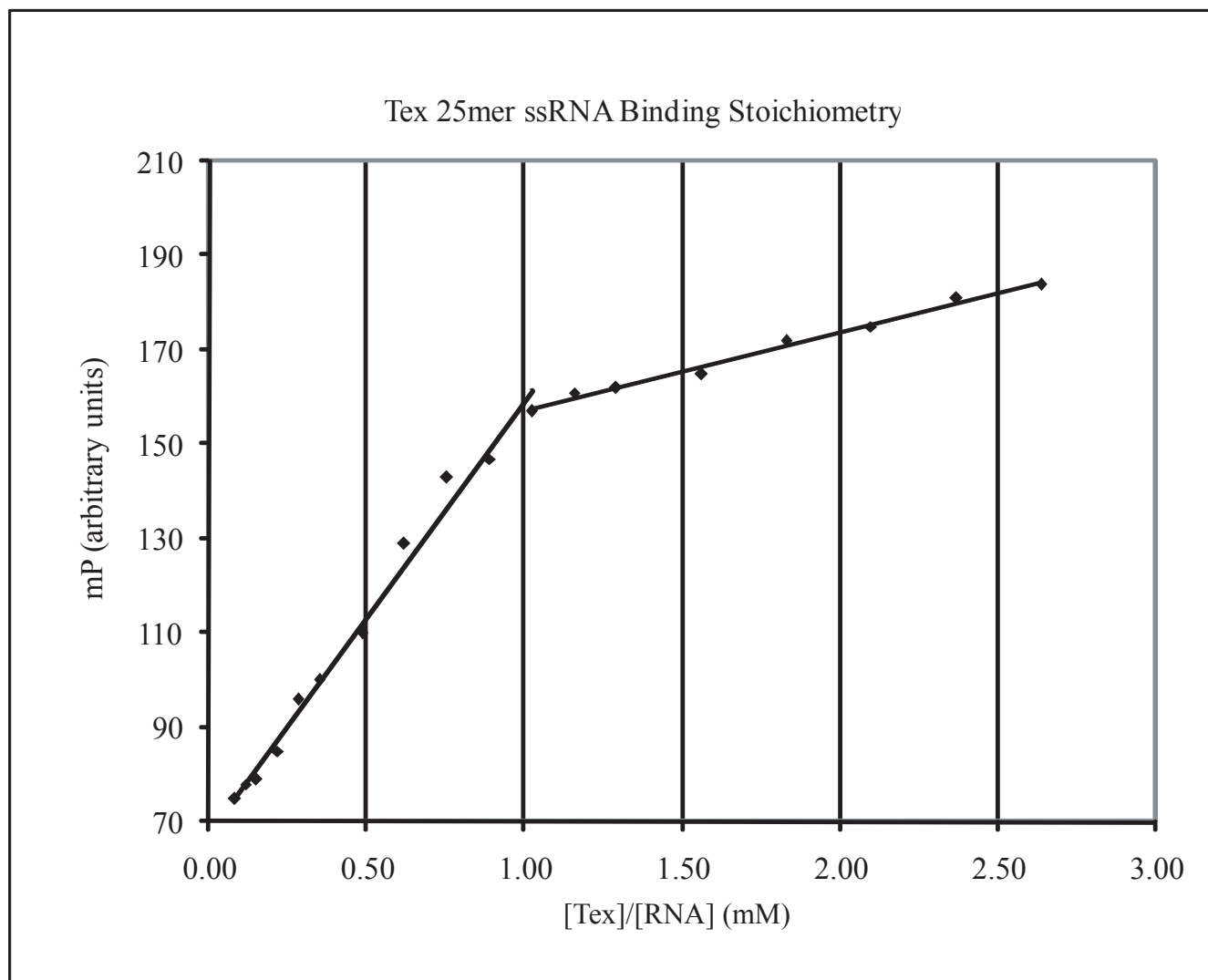
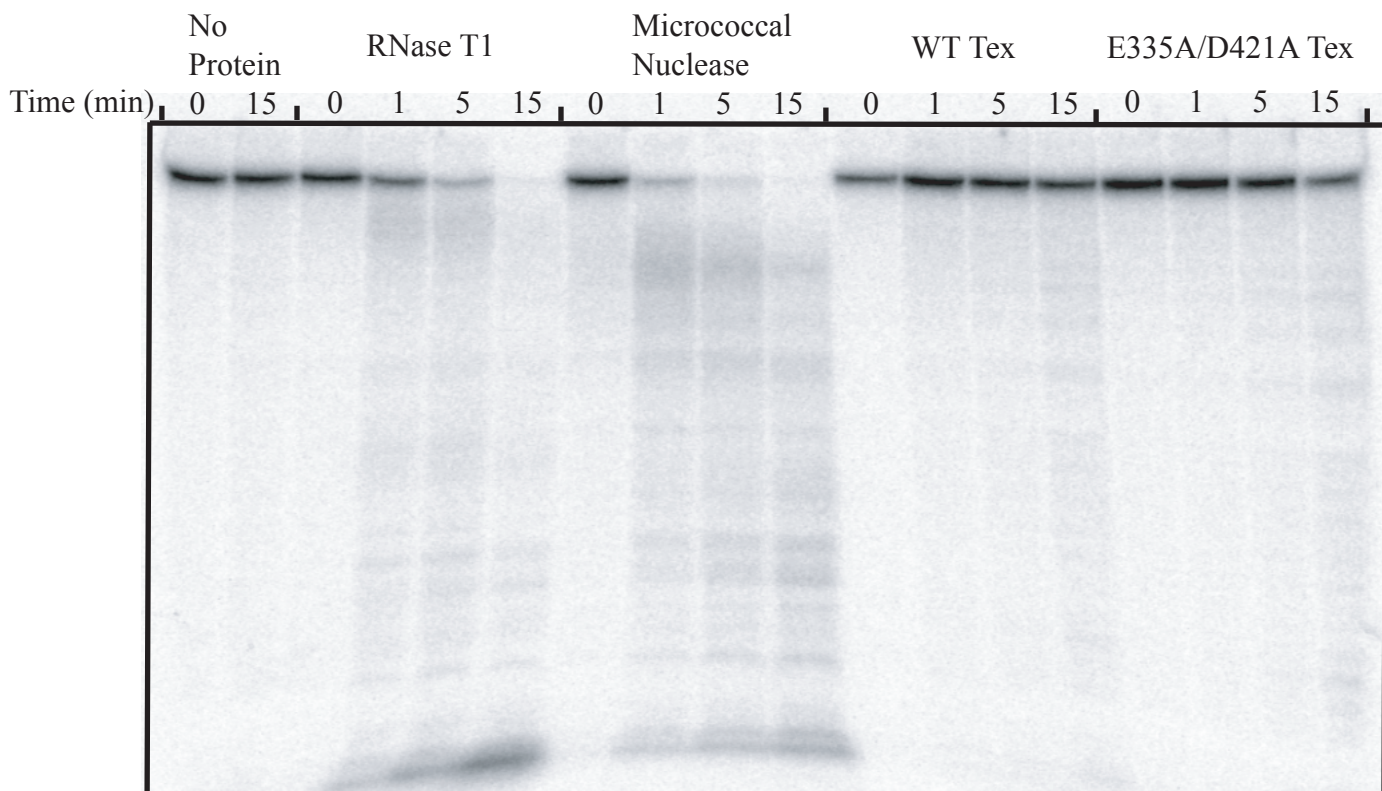
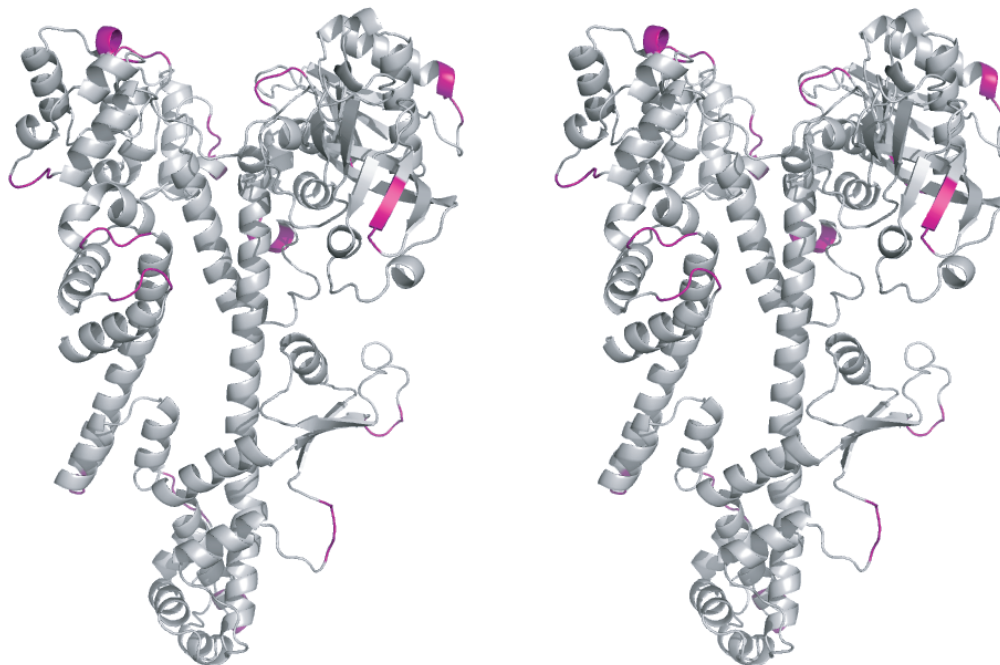


Figure S2. Tex does not appear to have nuclease activity. WT Tex was assayed in a time course experiment in parallel with RNase T1, Micrococcal Nuclease, and the Tex YqqF domain active site mutant E335A/D421A. In the assay, the Tex proteins were at a molar concentration 100x that of Micrococcal Nuclease. Substrate used in this assay is a 5' radio-labeled 300 nt single-stranded RNA and the assay was performed in 15mM Tris 7.5, 100mM NaCl, 5% glycerol, 3mM MgCl<sub>2</sub>, 1mM CaCl<sub>2</sub> at 37 C. Reactions were quenched by the addition of an equal volume of phenol/chloroform pH 6.6 and 25mM EDTA. Samples were spun down and the aqueous phase extracted and mixed with 2x formamide loading dye. Samples were run on a 12% acrylamide (19:1)/7M Urea denaturing gel in TBE buffer and the gel was scanned using a TYPHOON imaging system (GE Healthsciences).

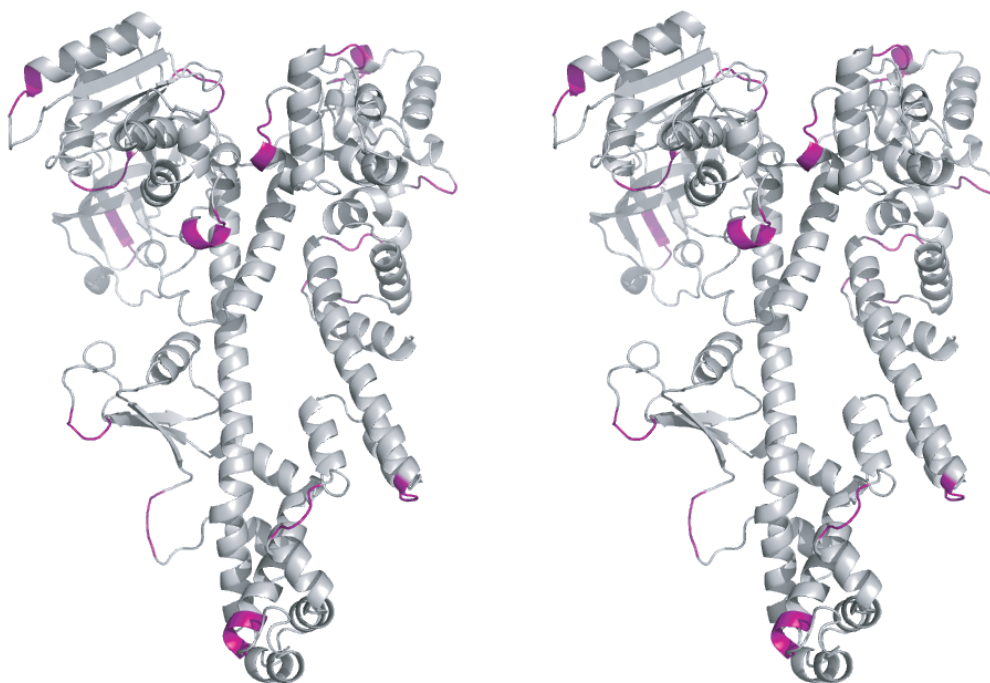


**Figure S3.** Predicted sites of sequence insertion for Spt6, with respect to the Tex structure. Front (A) and back (B) stereo views of the Tex structure. Highlighted regions (purple) indicate sites where additional sequence exists in Spt6 when aligned with Tex sequences. All highlighted regions lie on the surface of the Tex structure and appear to be able to accommodate additional sequence without disrupting the core structural scaffold.

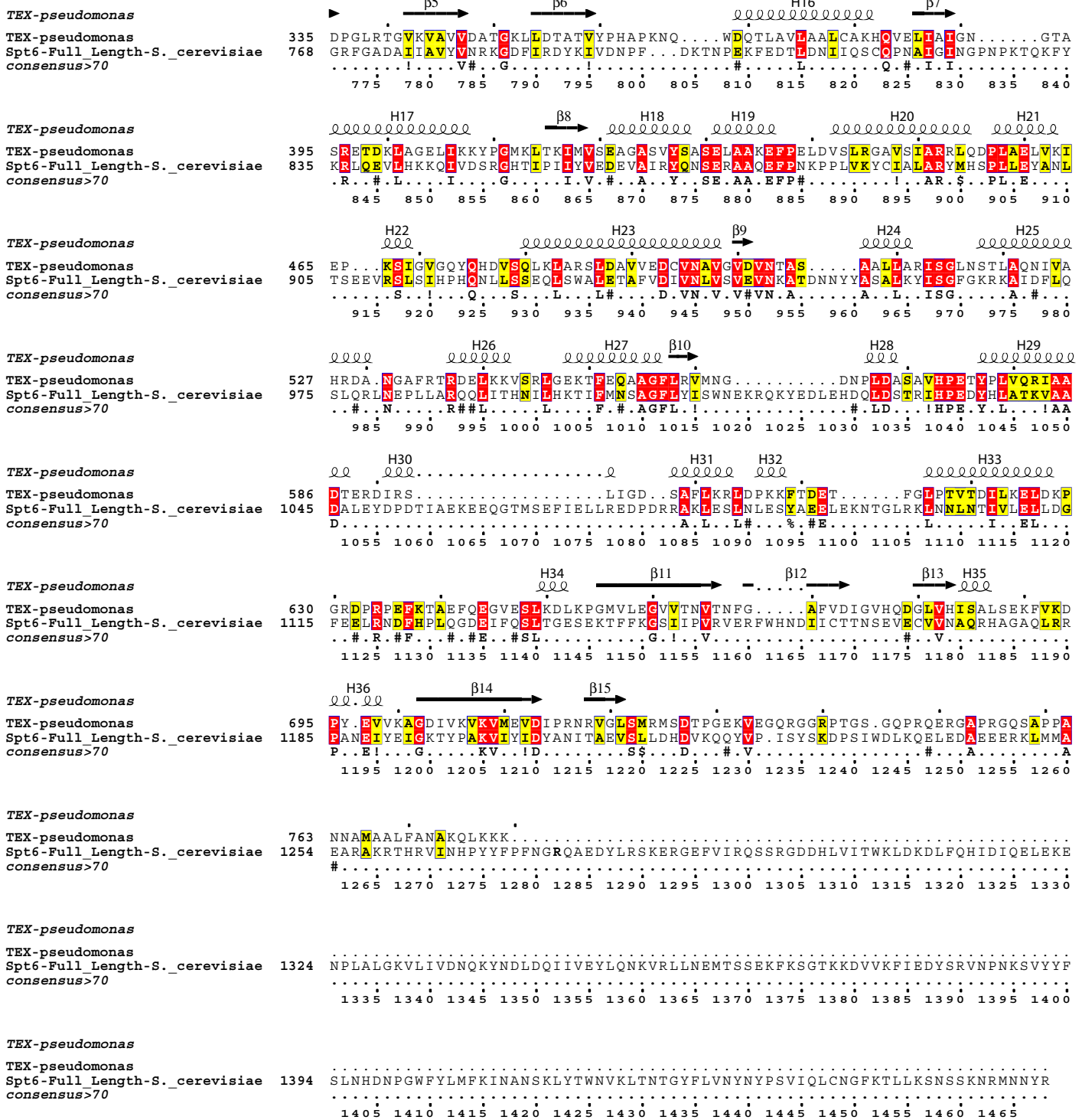
A



B







## References

- Higgins D., Thompson J., Gibson T., Thompson J.D., Higgins D.G., Gibson T.J. (1994). CLUSTAL W: improving the sensitivity of progressively multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**, 4673-4680.
- Gouet, P., Courcelle, E., Stuart, D.I. & Metoz, F. (1999). ESPript: multiple sequence alignments in PostScript. *Bioinformatics* **15**, 305-308.