



Supplementary Figure 3 2G-s23 and tFKBP*3R-2G-s23 K_d measurements. **(A)** The 2G-s23 affinity was determined by nitrocellulose filter binding assays, and a representative blot is shown. Triplicate measurements indicate a K_d of $4.6 \pm 0.8 \mu\text{M}$. Binding of 2G to one of the point mutant s23 RNA molecules (G72A) that has a greatly diminished capacity to form a ternary complex was also assayed. At $50 \mu\text{M}$ 2G, binding of G72A is barely detectable, and at $10 \mu\text{M}$ is not detectable, implying that binding of s23 to 2G is specific. **(B)** Representative blot of tFKBP*3R-2G-s23 binding. The K_d for ternary complex formation of $4.3 \pm 0.5 \text{ nM}$ is the average of three independent measurements. Note that no significant binding of s23 to $5 \mu\text{M}$ tFKBP*3R can be seen.