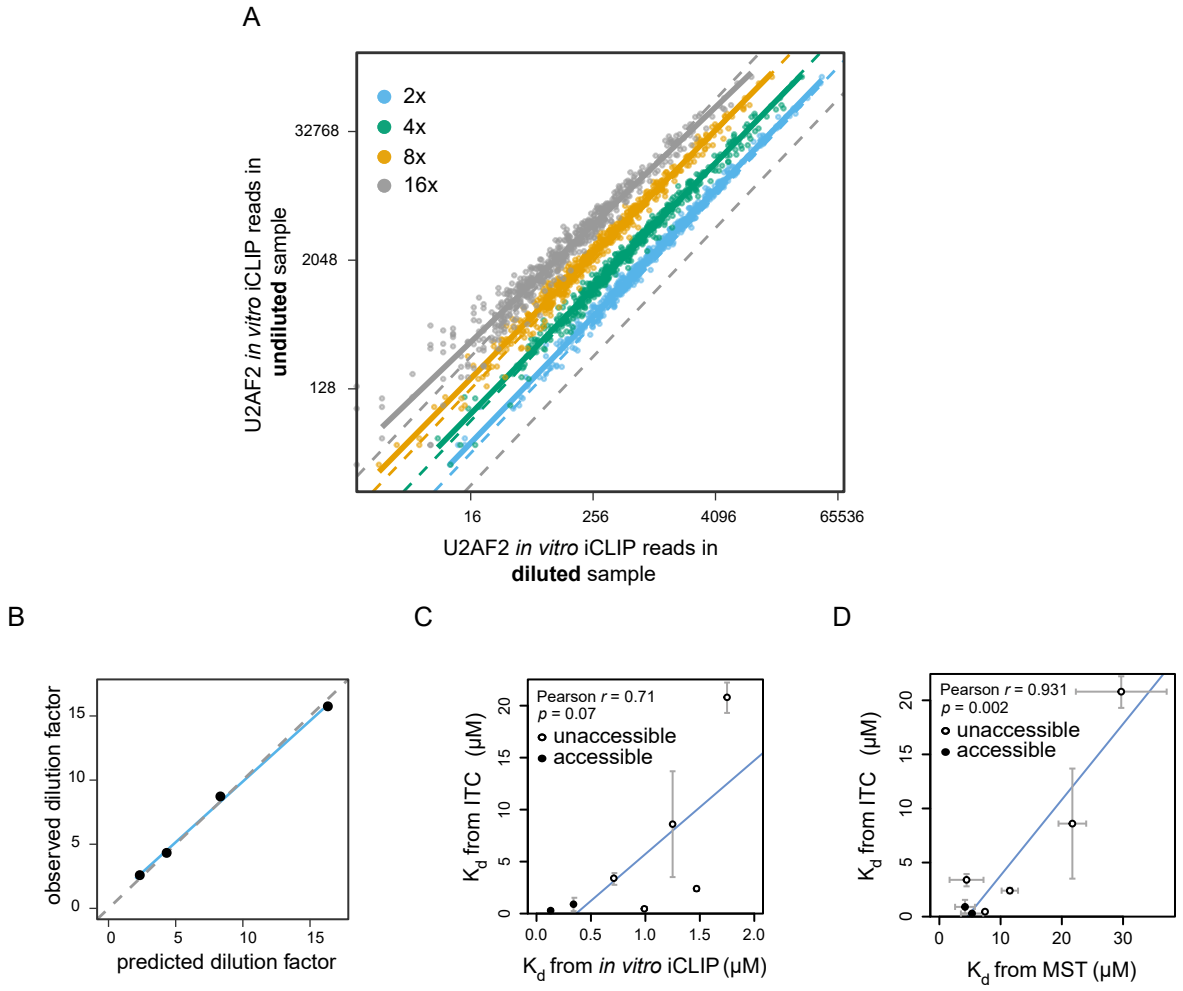

Supplemental figure S2, related to Figure 2. **The experimental measurements and mathematical modeling results are robust. (A)** The *in vitro* iCLIP signal is proportional to the amount of bound RNA. Scatter plot comparing normalized *in vitro* iCLIP read counts from serial dilutions (2x, 4x, 8x, and 16x) to the initial *in vitro* iCLIP reaction mixture (5 μM U2AF2^{RRM12} + 6.75 nM *in vitro* transcripts). Colored solid and dashed lines represent linear regression and expected read distributions for each dilution step, respectively. **(B)** The observed dilution factors precisely reflect the expected values for the four different dilution steps. Linear regression line indicated in blue. **(C)** K_d values from *in vitro* iCLIP are correlated with isothermal titration calorimetry (ITC) measurements for seven selected binding sites (**Supplemental Table S3**). Binding sites are marked as accessible (grey) or unaccessible (white) based on RNA fold predictions (see Methods). Pearson correlation coefficient (*r*) and associated p-value indicated below. **(D)** ITC-derived K_d values correlate well with MST measurements, but differ on absolute terms. ITC-K_d values are on average 2-fold lower than MST-K_d's, and thereby more closely reflect the *in vitro* iCLIP-derived K_d values for the same binding sites.