



**Supplemental figure S1, related to Figure 1. Exploration of *in vitro* iCLIP data. (A)** U2AF2 binding is significantly enriched at 3' splice sites *in vivo*. Boxplot showing the distribution of U2AF2 *in vivo* iCLIP reads based on their location in different transcript regions. **(B)** U2AF2<sup>RRM12</sup> *in vitro* iCLIP experiments are highly reproducible. Scatter plots of read counts in U2AF2<sup>RRM12</sup> binding sites from independent *in vitro* iCLIP replicate experiments. Pearson correlation coefficients ( $r$ ) and associated  $p$ -values indicated in each panel. **(C)** Binding landscapes correlate well between full-length U2AF2 protein and U2AF2<sup>RRM12</sup>. Scatter plot of read counts from *in vitro* iCLIP experiments with full-length U2AF2 protein and U2AF2<sup>RRM12</sup>. **(D)** GraphProt scores do not significantly differ between *in vivo* and *in vitro* binding sites (two-tailed Mann-Whitney U test,  $p$ -value = 0.7433). Comparison of GraphProt scores for 438,942 genome-wide *in vivo* U2AF2 binding sites (solid black line), the same number of *in vivo* sites that are not bound by U2AF2 (dashed black line), the top 50 *in vivo* U2AF2 binding sites with most crosslink events in the region of the *in vitro* transcripts (blue line), and the top 50 *in vitro* U2AF2<sup>RRM12</sup> binding sites with highest affinity (green line).