Supplemental Fig. 1. iCLIP analysis of HNRNPA1 and SRSF1 from control HEK 293 cells and HNRNPA1 over-expression cells. (A) Top left panel, autoradiograph from HNRNPA1 IP from control cells. Top left panel, autoradiograph from HNRNPA1 IP from HNRNPA1 over-expression cells. Middle left panel, annotation of HNRNPA1 peaks identified by CLIPper and consensus motifs identified by HOMER from control cells. Middle right panel, annotation of HNRNPA1 peaks identified by CLIPper and consensus motifs identified by HOMER from HNRNPA1 over-expression cells. (B) Top left panel, autoradiograph from SRSF1 IP from control cells. Top left panel, autoradiograph from SRSF1 IP from HNRNPA1 over-expression cells. Middle left panel, annotation of SRSF1 peaks identified by CLIPper and consensus motifs identified by HOMER from control cells. Middle right panel, annotation of SRSF1 peaks identified by CLIPper and consensus motifs identified by HOMER from HNRNPA1 over-expression cells.
Supplemental Fig. 2. Distribution of changes to U2AF2 AND SRSF1 crosslinking peaks within specific genomic features. (A) Comparison of the density (y-axis) of crosslinking peaks with a given log₂ (fold-change) between HNRNPA1 over-expression vs. control (x-axis) across multiple genomic features from two U2AF2 iCLIP experiments. (B) Comparison of the density (y-axis) of crosslinking peaks with a given log₂ (fold-change) between HNRNPA1 over-expression vs. control (x-axis) across multiple genomic features from SRSF1 iCLIP experiment. (C) Stacked Bar graph showing the proportion of experiment of SRSF1 iCLIP crosslinking peaks showing No Change (top), Increased crosslinking peaks (Increased Binding) or decreased crosslinking peaks (Decreased Binding) associated with found within Alu elements, 3' splice site regions, both Alu and 3' splice site regions (both), or other regions outside of these (other). Peaks included in this plot have a log₂ fold-change greater than +/- 2 and a $-\log_{10}$ (adjusted p-value) less than 0.05.
Supplemental Fig. 3. Distribution of HNRNPA1, SRSF1 and U2AF2 peaks relative to splice sites. The frequency of peaks occurring at different positions relative to splice sites is shown for each protein under control and HNRNPA1 over-expression conditions.
Supplemental Fig. 4. Distribution of SRSF1 and HNRNPA1 crosslinking sites relative to 3' splice sites in control and HNRNPA1 over-expressing HEK293T cells. (A, B) Normalized crosslinking distribution for HNRNPA1 and SRSF1 (right and left panels, respectively) in wild-type (blue line) and HNRNPA1 overexpression cell lines (red line) with 95% confidence interval (gray area). Data is divided between constitutive (A) and skipped (B) exons. (C) Nature log fold change distribution of SRSF1 within 200bp intron regions near 3' splice sites of cassette exons. Blue bars correspond to annotated alternative splicing events with no evidence of HNRNPA1 crosslinking in either condition and pink represents annotated events with detectable HNRNPA1 crosslinking.
Supplemental Fig. 5. Examples of HNRNPA1-dependent modulation of U2AF2 crosslinking. UCSC genome browser examples of two genes SRSF6 (A) and PIEZO1 (B) and iCLIP read coverage data for U2AF2 under control and HNRNPA1 overexpression. Alternative splicing changes can be observed in figure 3 (SRSF6) and supplemental figures 13 and 16 (PIEZO1 and SRSF6, respectively).
Supplemental Fig. 6. HNRNPA1 over-expression correlates with global redistribution of U2AF2 signal to Alu RNA elements. (A) Distribution of aggregated U2AF2, HNRNPA1, and SRSF1 iCLIP peaks on Alu subtype AluSc under control and HNRNPA1 over-expression conditions. (B, D) Aggregated crosslinking sites on Alu elements and nearby regions for (B) HNRNPA1 and (D) SRSF1. Blue represents wild-type binding of the given RNA binding protein and red represents HNRNPA1 over-expression of the $\log_{10}$ number of iCLIP read counts across all anti-sense (left graph) and sense (right graph) Alu elements (C, E) Scatter plot of all human cassette exons measuring the proportion of (C) HNRNPA1 and (E) SRSF1 iCLIP crosslinks found within Alu elements relative to the total number of crosslinks observed throughout the alternative event.
**Supplemental Fig. 7. HNRNPC protein levels and sub-cellular localization are not affected by HNRNPA1 over-expression.** (A) Nuclear extracts were subjected to SDS-PAGE and transferred to nitrocellulose blotting paper. Samples were interrogated with antibodies for α-T7 peptide tag (with which overexpressed HNRNPA1 has been tagged), α-HNRNPA1 (4B10; Santa Cruz Biotechnology), α-SRSF1 (96; Santa Cruz Biotechnology), α-U2AF2 (MC3; Santa Cruz Biotechnology), α-HNRNPC1/C2 (Santa Cruz Biotechnology, 4F4) and α-EWSR1 (C-9; Santa Cruz Biotechnology) as positive control. (B) Immunostaining of control HEK293T cells or HNRNPA1 over-expression cells with α-HNRNPC1/C2, α-HNRNPA1 and anti-U2AF2.
Supplemental Fig. 8. Trans- vs. Cis-Alu competition model of U2AF2 redistribution. Model representing two potential modes by which U2AF2 may associate with Alu RNA: trans- competition suggests U2AF2 binds to Alu elements on other RNAs, while a cis-competition suggest U2AF2 binds to Alu elements within the same RNAs that a particular exon is associated
Supplemental Fig. 9. U2AF2 Peaks in proximal and distal introns are enriched in positions overlapping Alu elements. Comparison of observed (Actual) Alu-element overlap of increasing distal intronic peaks to the Alu-element overlap by peaks randomly shuffled (Randomized) within the same population of distal intronic regions. Random shuffling was performed 1000 times to create a null distribution for each definition of distal intronic regions (> 200, 500, or 2000 bp from the nearest exon boundary).
Supplemental Fig. 10. HNRNPA1-dependent modulation of U2AF2 crosslinking and alternative splicing of ACIN1. (A) Genome browser snap shot of iCLIP read coverage surrounding alternative exon. The 3’ splice site is indicated by a red box. (B) Sashimi plot showing read coverage, junction read counts and estimated percent spliced in values (PSI) from aggregated RNA seq data obtained from control (red) or HNRNPA1 over-expression HEK293T cells (gold).
Supplemental Fig. 11. HNRNPA1-dependent modulation of U2AF2 crosslinking and alternative splicing of CALM2. (A) Genome browser snap shot of iCLIP read coverage surrounding alternative exon. The 3' splice site is indicated by a red box. (B) Sashimi plot showing read coverage, junction read counts and estimated percent spliced in values (PSI) from aggregated RNA seq data obtained from control (red) or HNRNPA1 over-expression HEK293T cells (gold).
Supplemental Fig. 12. HNRNPA1-dependent modulation of U2AF2 crosslinking and alternative splicing of COG4. (A) Genome browser snap shot of iCLIP read coverage surrounding alternative exon. The 3’ splice site is indicated by a red box. (B) Sashimi plot showing read coverage, junction read counts and estimated percent spliced in values (PSI) from aggregated RNA seq data obtained from control (red) or HNRNPA1 over-expression HEK293T cells (gold).
Supplemental Fig. 13. HNRNPA1-dependent modulation of U2AF2 crosslinking and alternative splicing of HNRNPL. (A) Genome browser snap shot of iCLIP read coverage surrounding alternative exon. The 3’ splice site is indicated by a red box. (B) Sashimi plot showing read coverage, junction read counts and estimated percent spliced in values (PSI) from aggregated RNA seq data obtained from control (red) or HNRNPA1 over-expression HEK293T cells (gold).
Supplemental Fig. 14. HNRNPA1-dependent modulation of U2AF2 crosslinking and alternative splicing of PIEZO1. (A) Genome browser snap shot of iCLIP read coverage surrounding alternative exon. The 3’ splice site is indicated by a red box. (B) Sashimi plot showing read coverage, junction read counts and estimated percent spliced in values (PSI) from aggregated RNA seq data obtained from control (red) or HNRNPA1 over-expression HEK293T cells (gold).
Supplemental Fig. 15. HNRNPA1-dependent modulation of U2AF2 crosslinking and alternative splicing of SON. (A) Genome browser snap shot of iCLIP read coverage surrounding alternative exon. The 3' splice site is indicated by a red box. (B) Sashimi plot showing read coverage, junction read counts and estimated percent spliced in values (PSI) from aggregated RNA seq data obtained from control (red) or HNRNPA1 over-expression HEK293T cells (gold).
Supplemental Fig. 16. HNRNPA1-dependent modulation of U2AF2 crosslinking and alternative splicing of SREK1. (A) Genome browser snap shot of iCLIP read coverage surrounding alternative exon. The 3' splice site is indicated by a red box. (B) Sashimi plot showing read coverage, junction read counts and estimated percent spliced in values (PSI) from aggregated RNA seq data obtained from control (red) or HNRNPA1 over-expression HEK293T cells (gold).
Supplemental Fig. 17. HNRNPA1-dependent modulation of U2AF2 crosslinking and alternative splicing of SRSF6. (A) Genome browser snap shot of iCLIP read coverage surrounding alternative exon. The 3’ splice site is indicated by a red box. (B) Sashimi plot showing read coverage, junction read counts and estimated percent spliced in values (PSI) from aggregated RNA seq data obtained from control (red) or HNRNPA1 over-expression HEK293T cells (gold).
Supplemental Fig. 18. HNRNPA1-dependent modulation of U2AF2 crosslinking and alternative splicing of SRSF7. (A) Genome browser snap shot of iCLIP read coverage surrounding alternative exon. The 3’ splice site is indicated by a red box. (B) Sashimi plot showing read coverage, junction read counts and estimated percent spliced in values (PSI) from aggregated RNA seq data obtained from control (red) or HNRNPA1 over-expression HEK293T cells (gold).
Supplemental Fig. 19. HNRNPA1-dependent modulation of U2AF2 crosslinking and alternative splicing of SRSF11. (A) Genome browser snap shot of iCLIP read coverage surrounding alternative exon. The 3’ splice site is indicated by a red box. (B) Sashimi plot showing read coverage, junction read counts and estimated percent spliced in values (PSI) from aggregated RNA seq data obtained from control (red) or HNRNPA1 over-expression HEK293T cells (gold).
Supplemental Fig. 20. HNRNPA1-dependent modulation of U2AF2 crosslinking and alternative splicing of TRA2A. (A) Genome browser snap shot of iCLIP read coverage surrounding alternative exon. The 3' splice site is indicated by a red box. (B) Sashimi plot showing read coverage, junction read counts and estimated percent spliced in values (PSI) from aggregated RNA seq data obtained from control (red) or HNRNPA1 over-expression HEK293T cells (gold).