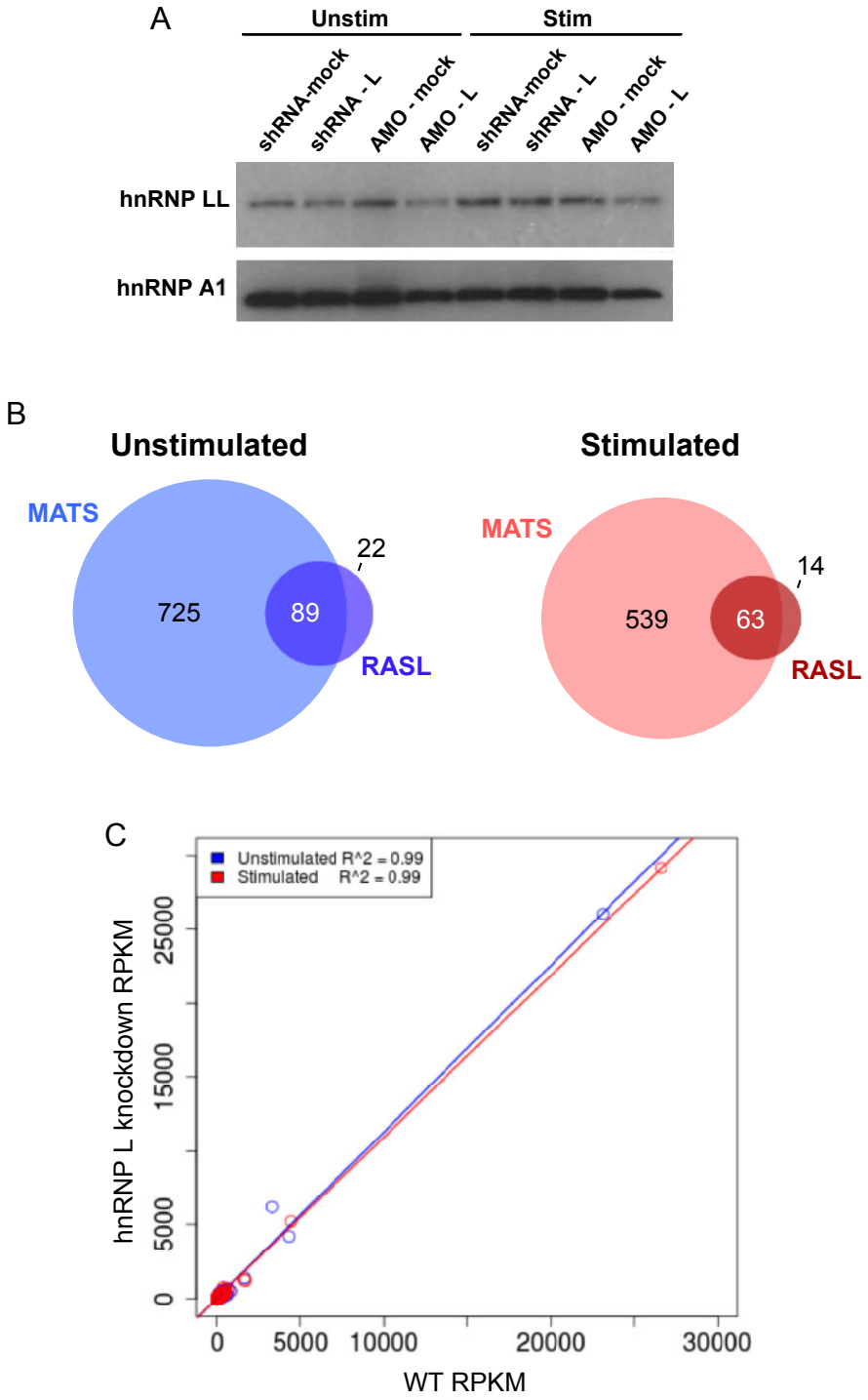
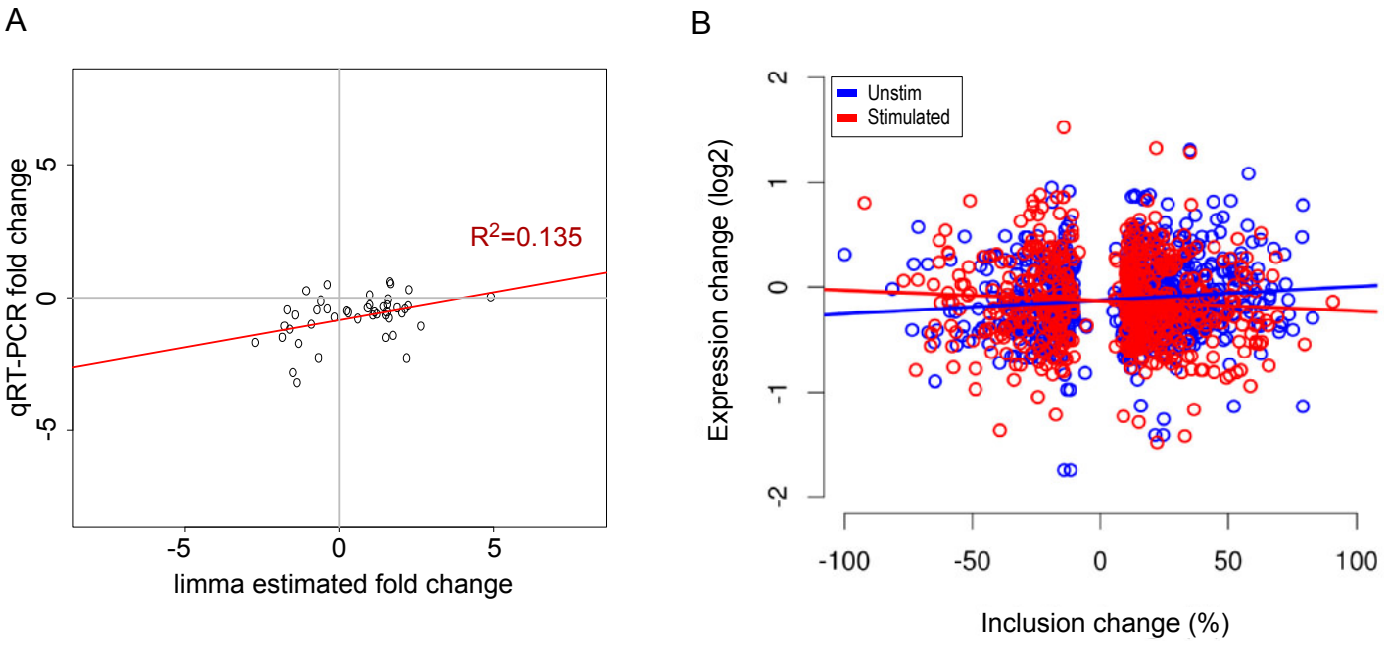
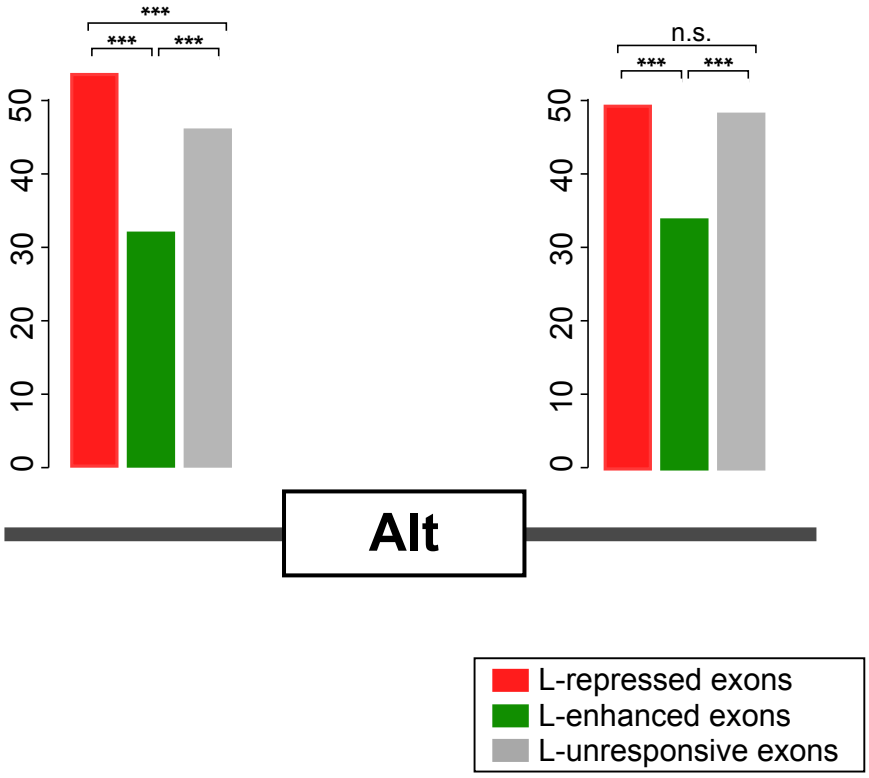


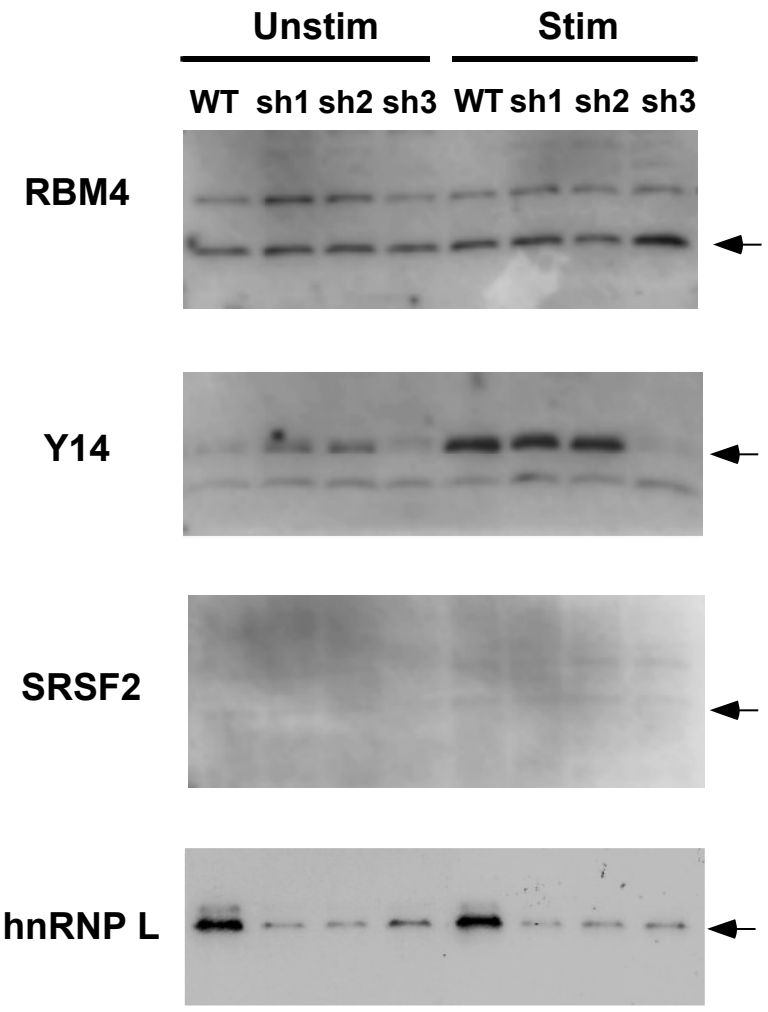
Supplemental Figure 1: Additional analysis of hnRNP L-responsive alternative splicing inferred by RASL-Seq and mRNA-Seq. (A) Western blot of hnRNP LL and hnRNP A1 (loading control) in cells depleted for hnRNP L. (B) Venn diagrams of RASL-Seq vs. RNA-Seq identified hnRNP L responsive exons. Only exons identified as meeting the threshold of significance (ΔPSI>10, p<0.05) are included here. Exons identified only by RASL-Seq typically did not have enough reads in the RNA-Seq analysis for quantification by MATS. (C) Regression analysis comparing RPKM for ~200,000 annotated introns in wildtype or hnRNP L-depleted cells. Slope of best fit line is 1.1 for both unstimulated conditions and stimulated conditions, consistent with hnRNP L knockdown resulting in no more than a 10% shift toward intron retention.



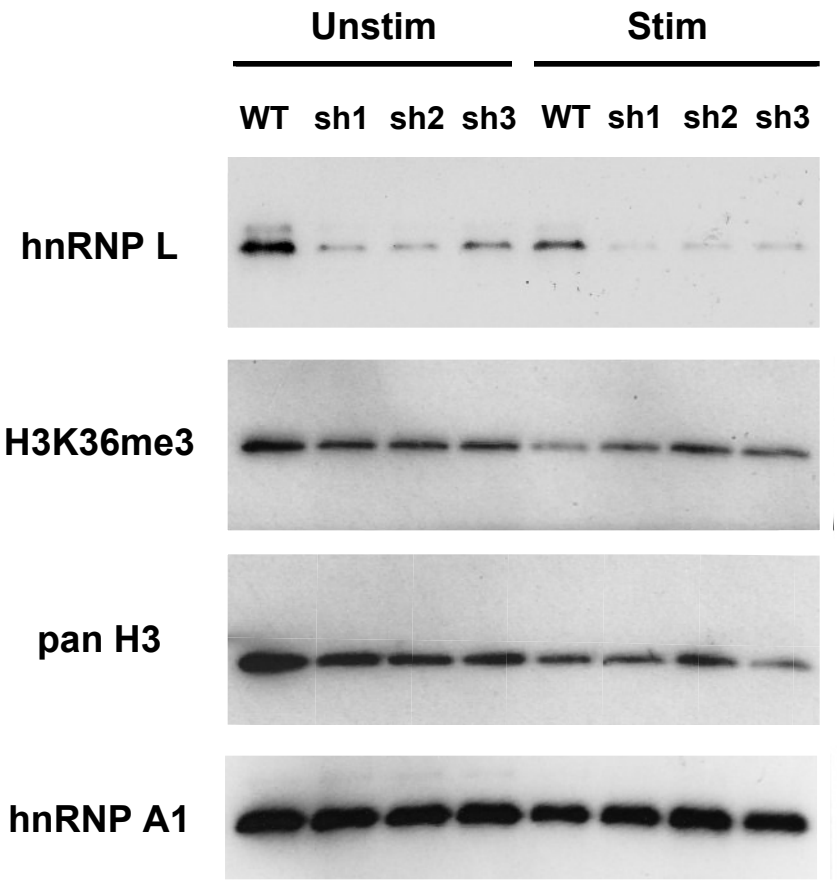
Supplemental Figure 2: hnRNP L does not impact transcript expression level, nor does expression impact hnRNP L-dependent alternative splicing. (A) Regression analysis of expression changes calculated by the limma pipeline versus changes measured by RT-PCR, indicating that the few changes predicted by limma are false. (B) hnRNP L induced changes in splicing (inclusion change %) as a function of expression change induced upon L depletion, demonstrating that hnRNP L-dependent changes in splicing do not correlate with any change in overall expression.



Supplemental Figure 3: hnRNP L does not significantly interact with any sequences within the C1-A-C2 interval around exons it enhances. Percentage of L-repressed, enhanced or unresponsive exons that contain a hnRNP L CLIP signal anywhere in the flanking introns regardless of distance from the alternative exon.



Supplemental Figure 4: hnRNP L depletion does not alter the expression of RNA binding proteins with affinity for GC-rich sequences. Western blots of indicated proteins in lysates from wildtype JSL1 Jurkat cells (WT) or three clones stably transduced with an shRNA against hnRNP L (sh1-3). Size of primary protein species anticipated is indicated by an arrow. Note that while some protein levels are variable, poorly detected or induced upon stimulation, none show a consistent change upon depletion of hnRNP L.



Supplemental Figure 5: hnRNP L depletion does not significantly reduce bulk H3K36 trimethylation in Jurkat cells. Western blots of hnRNP L, H3K36 trimethylation (Cell Signaling), pan histone H3 and hnRNP A1 (loading control) in wildtype JSL1 Jurkat cells (WT) or three clones stably transduced with an shRNA against hnRNP L (sh1-3). Note all proteins are slightly reduced upon stimulation due to underloading of lysates.