Supporting Information

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SI Text

Oligonucleotides Used. The following oligonucleotides were used: wtPRE-top, 5′-AATTCAGAAACGAGACAGAGAGAGAGAGAGAGAGAGAGACGGGGAGAAAAGA-3′; wtPRE-bottom, 5′-AGCTTCTTTTCTCCCCGTCTCTCTCTCT-CTCTCTCTCTGTCTCGTTTCTG-3′; SB41, 5′-GGGAAT-TCCATGGTGAACTCGAACCAG-3′; SB42, 5′-GGGCGGC-CGCTCAATATCTGCGGT-3′; SB43, 5′-GGGAATTCCATG-GCGGAACAAAACGAT-3′; SB44, 5′-GGGCGGCCGC-CTAGTAACGTCTATTGC-3′; SB49, 5′-GGGAATTCAAC-TACGGCAACCAGAATGG-3′; SB50, 5′-GGAAGCTTTT-GTTGCCACCGTTATTGAA-3′; SB60, 5′-AACCAGAAC-CAGAACGGCAACT-3′; SB61, 5′-CCATTCAGCTGATGCT-GCTTTT-3′; SB62, 5′-CCATCAAGAACAAGACCCTGGA-3′; SB63, 5′-CCCTTGTTGAATCCTCCACCA-3′; SB68, 5′-ACTCACGGTCGTTTCAAGGGCA-3′; SB69, 5′-TCATCG-GTGTAGCCCAGGATTC-3′; SB116, 5′-GGTACGCGTA-GAATCGAG-3′; SB117, 5′-GGAAGCTTCAATCGAC-CATCC-3′; SB135, 5′-GGCAATTGGGGGAGATCTATG-GCTTCTAACTTTAC-3′; SB136, 5′-GCTGCCGCCGTA-GATGCCGGAGTTTGC-3′; SB137, 5′-CCACTACTAC-TACTTCTCCCGGGCTCCCCGTCTCTCTCTCTC-3′; SB138, 5′-GAGAGAGAGAGACGGGGAGCCCGGGAGAAGTA-GTAGTAGTGG-3′; SB141, 5′-GGCACCGTGTGCAGCA-CATGAGGATCACCCATGTCTGCTGTCTAGCTGTAG-3′; and SB142, 5′-GGCCCGGGACATGGGTGATCCTCAT-GTTTTCTACAGCTAGACAGCAGACATGG-3′.

Plasmid Construction. pWT-PRE, used to transcribe radiolabeled PRE substrate for in vitro cross-linking studies, was generated by annealing oligonucleotides wtPRE-top and wtPRE-bottom. This duplex harbors restriction sites for EcoR1 and HindIII, and after digesting with these enzymes, the duplex was ligated into the corresponding sites of pGEM3Z (Promega).

pActin-wtpros, used to express the artificial twintron splicing reporter in S2 cells, was constructed by excising the *Drosophila* 5c actin promoter from pActin5C-EGFP (gift from Hervé Agaisse) with EcoRI and BamH1. The ends were blunted with Klenow, and the promoter was ligated into the blunted SpeI site of pMT-wtpros (1). The resulting exon and intron lengths of the splicing reporter were 123 nt for exon 1, 336 nt for the intron, and 360 nt for exon 2. The final 198 nt of exon 2 were derived from pMT vector sequence. Exon and intron lengths are given relative to the U2-type splice sites.

pGEM4Z-RPA, the template used to generate the RPA probe, was constructed by PCR amplifying a sequence from pActin-wtpros by using forward primer SB116 and reverse primer SB117. The amplicon was ligated into the blunted HindIII site of pGEM4Z (Promega).

pGEX6p-3-Hrp38, used to express recombinant, GST-tagged Hrp38, was constructed by PCR amplification of cDNA sequence for *hrp38* by using primers SB41 and SB42, which harbor EcoR1 and NotI sites at the ends. The amplicon was ligated into the EcoR1 and NotI sites of pGEX6p-3 vector (GE/Amersham). pGEX6p-3-Hrp36 was constructed by PCR amplification of the cDNA sequence for *hrp36* by using primers SB43 and SB44, and cloning into the EcoR1/NotI sites of pGEX6p-3 vector. cDNA clones LD383464, for *hrp38*, and LD32727, for *hrp36*, were obtained from the Drosophila Genomics Resource Center (Bloomington, IN).

The template for generating dsRNA corresponding to *hrp38* sequence, pGEM4Z-Hrp38 was constructed by PCR amplifying 400 bp from LD383464 by using primers SB49 and SB50, which harbor EcoR1 and HindIII sites. The amplicon was cloned into the EcoR1 and HindIII sites of pGEM4Z. The template for generating dsRNA corresponding to *hrp36* was constructed by using template GH05625, a cDNA clone obtained from the Drosophila Genomics Resource Center, and primers SB51 and SB52.

1. Scamborova P, Wong A, Steitz JA (2004) An intronic enhancer regulates splicing of the twintron of *Drosophila melanogaster prospero* pre-mRNA by two different spliceosomes. *Mol Cell Biol* 24:1855–1869.

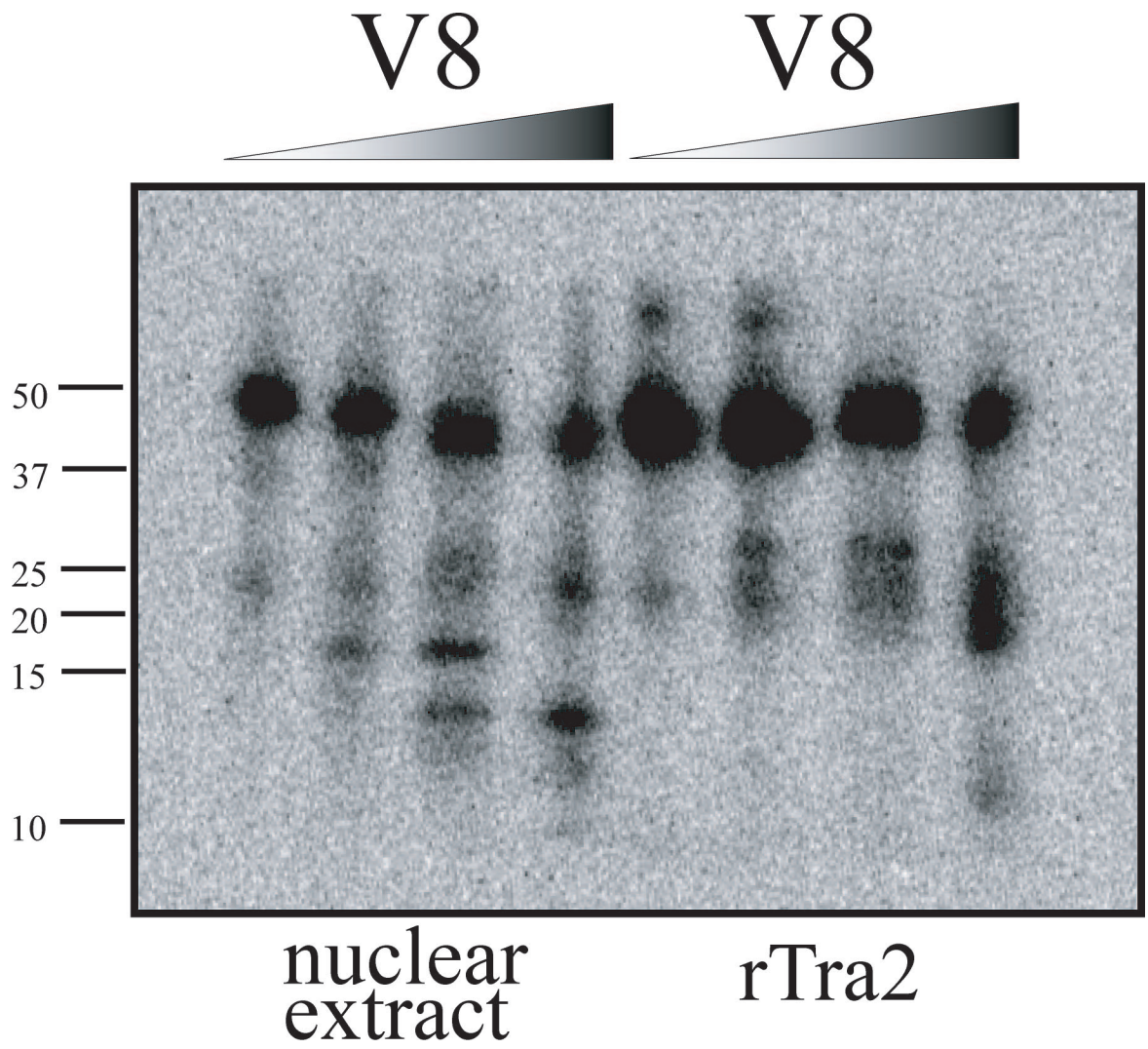

Fig. S1. Partial proteolysis pattern of recombinant Tra2 is distinct from that of the 40-kDa protein in S2 nuclear extract. Radiolabeled PRE substrate was cross-linked by UV irradiation to the 40-kDa protein in S2 nuclear extract or to recombinant Tra2. Partial proteolysis was performed as described.

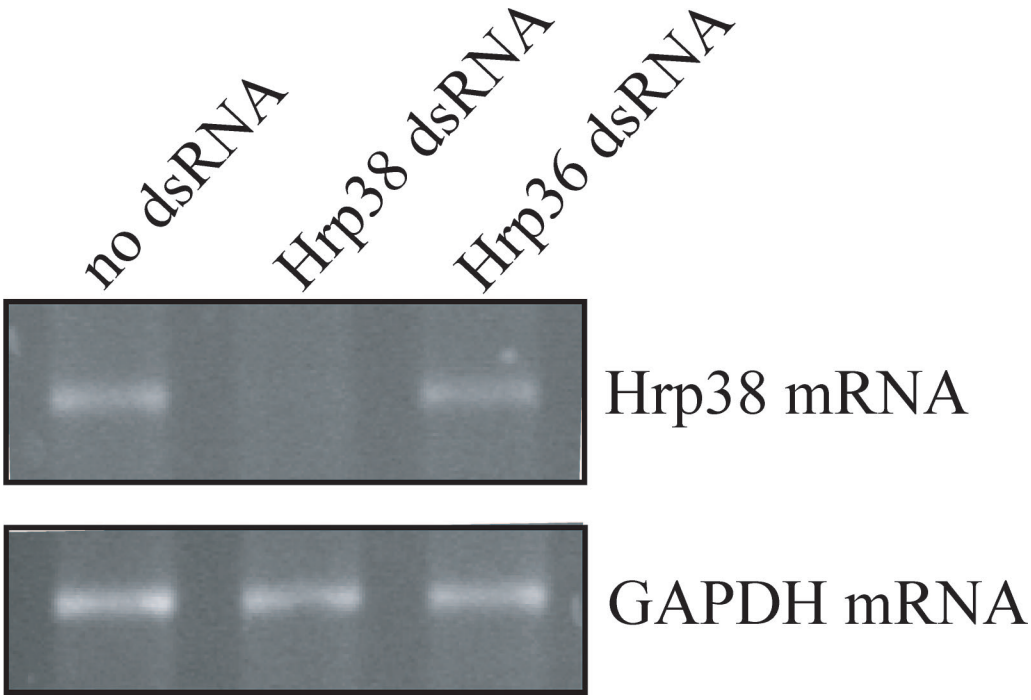

Fig. S2. dsRNA knockdown using anti-Hrp38 dsRNA does not affect mRNA levels of Hrp36. S2 cells were dosed daily with the indicated dsRNA for 5 days before harvesting total RNA. Levels of indicated mRNAs were quantified by RT-PCR. GAPDH mRNA served as a loading control.

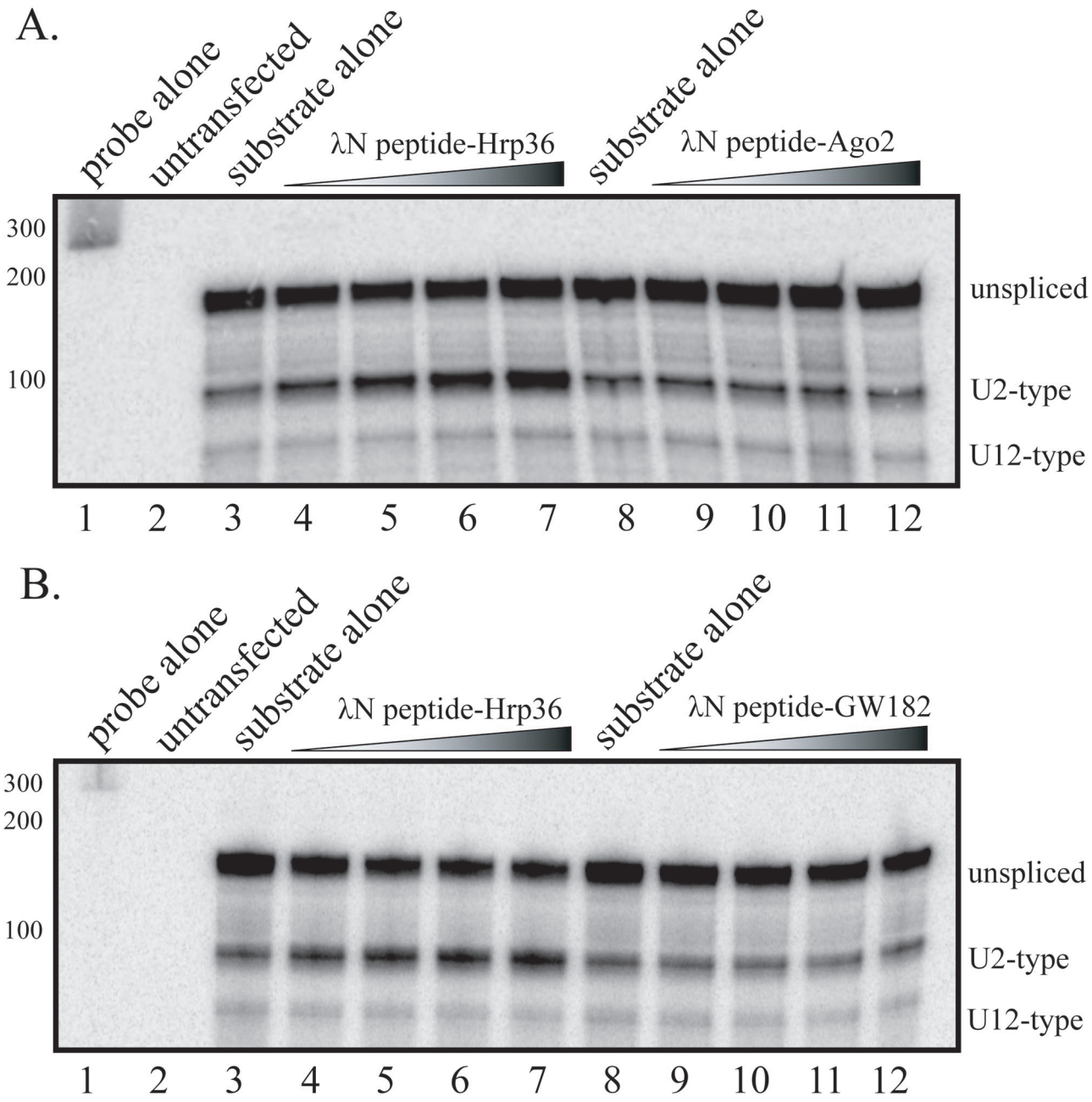

Fig. S3. Tethering of lambda-tagged RNA-binding proteins Ago2 or GW182 does not stimulate U2-type splicing in vivo. Either 0, 1, 5, 10, or 50 ng of lambda peptide-tagged Hrp36 (*A* and *B*, lanes 3–7), lambda-tagged Ago2 (*A*, lanes 9–12), or lambda-tagged GW182 (*B*, lanes 9–12) were cotransfected with 500 ng of twintron substrate containing 3 copies of the bBox hairpin in place of the PRE.

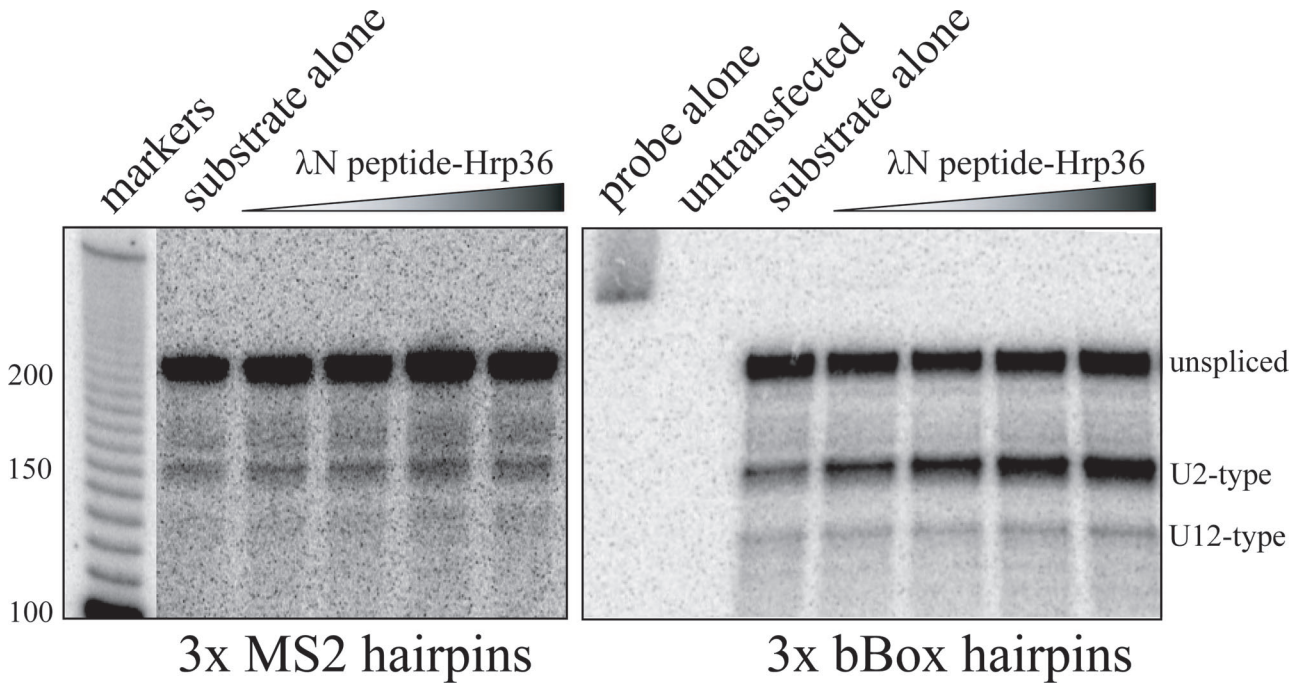

Fig. S4. Lambda peptide-tagged Hrp36 stimulates U2-type splicing of a *prospero* twintron substrate containing bBox hairpins at the PRE site, but not of substrate containing MS2 hairpins. Either 0, 1, 5, 10, or 50 ng of lambda peptide-tagged Hrp36 were titrated into 500 ng of twintron substrate containing either 3 copies of the MS2 hairpin in place of the PRE (*Left*) or 3 copies of the bBox hairpin in place of the PRE (*Right*).