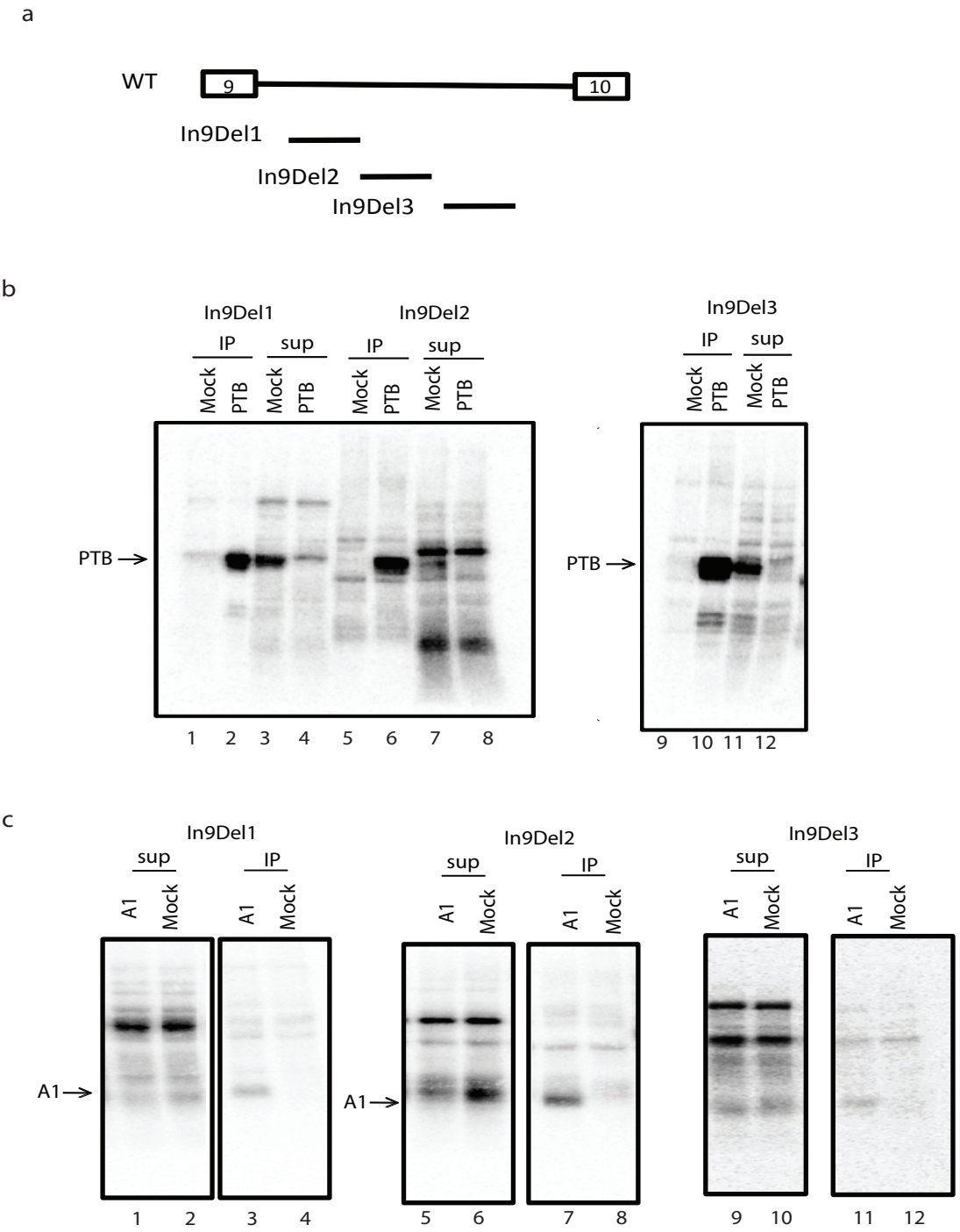
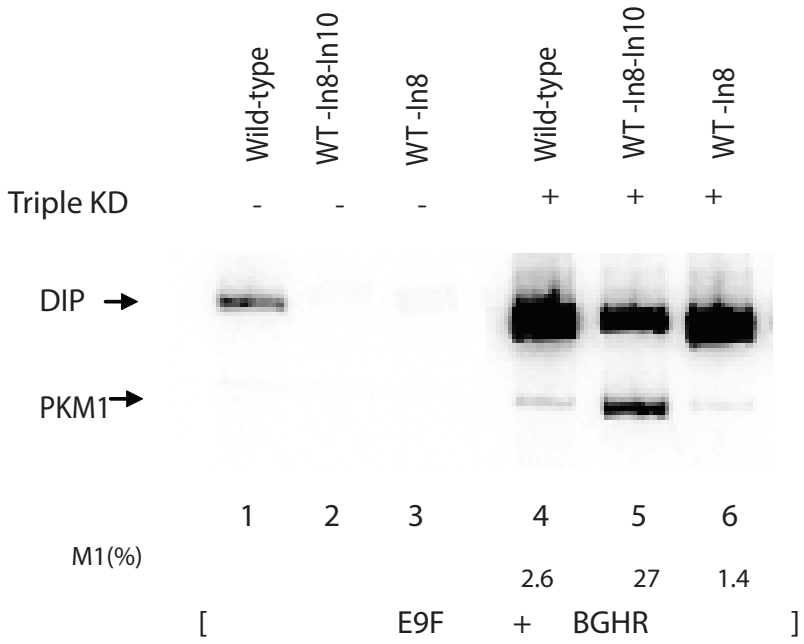
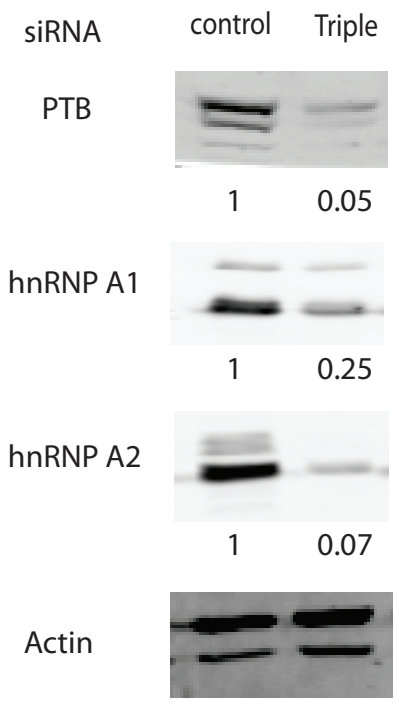


Supplementary Figure 1 UV-crosslinking-Immunoprecipitation of intron 9 truncations Del1, Del2, Del3 in HeLa NE. RNA oligos used are indicated on top. (**a**) Scheme indicates the positions of oligos used in UV-crosslinking experiment. (**b**) UV-crosslinking experiment using RNA oligos indicated in (a) and PTB antibody (BB7). PTB is indicated on the left. (**c**) UV-crosslinking experiment using RNA oligos indicated in (a) and A1 antibody (4B10). A1 is indicated on the left.

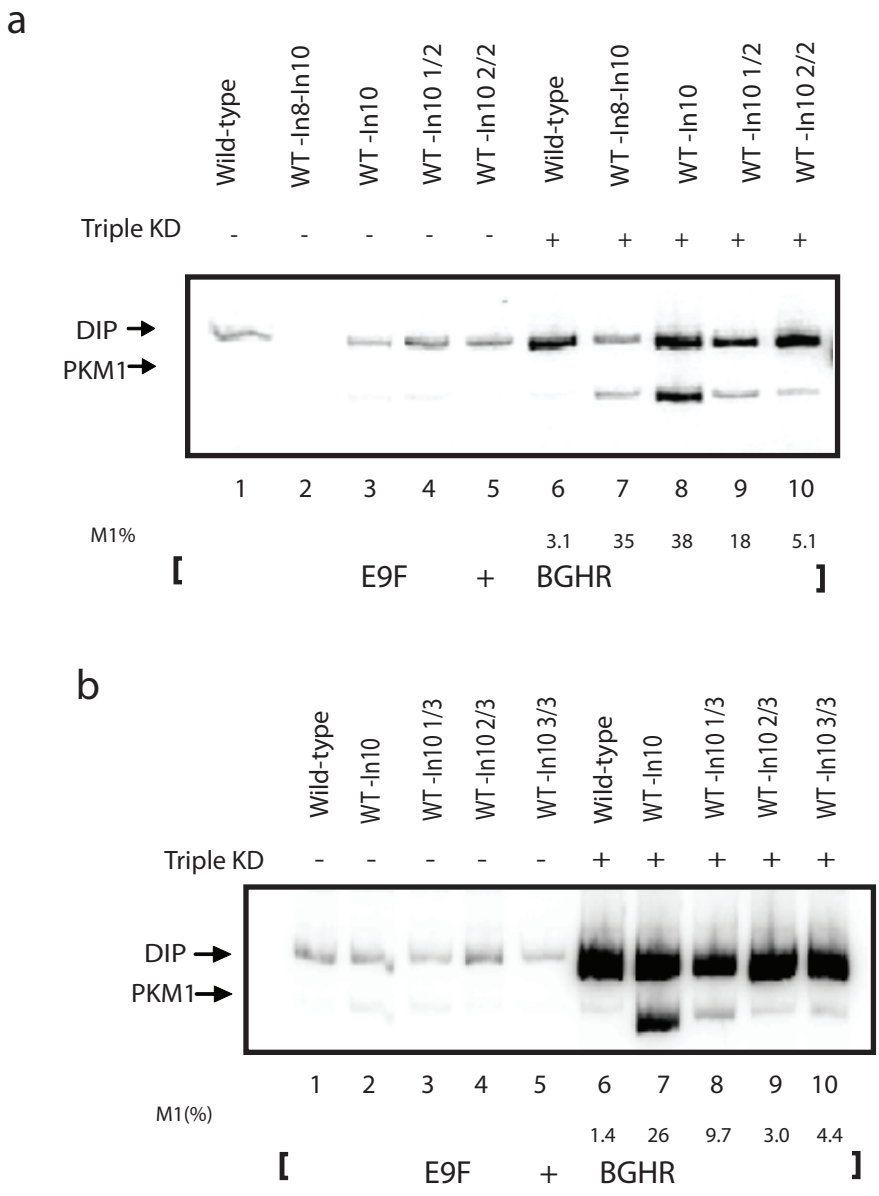


Supplementary Figure-2 (Manley)

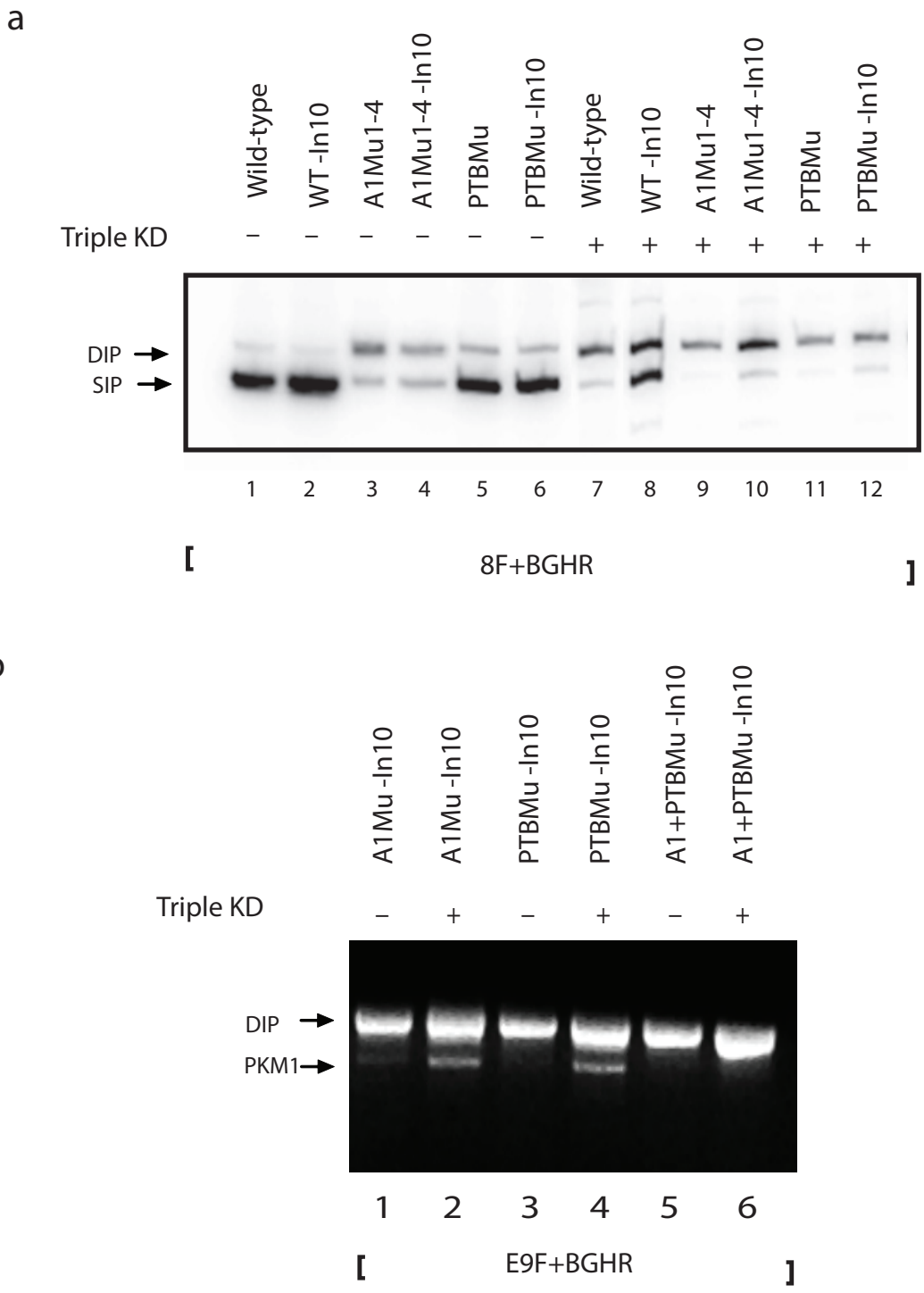
Supplementary Figure 2 Intron 8 does not play a role in reducing double inclusion product. RT-PCR assay was performed with E9-specific primers and total RNA extracted from HeLa cells transfected with wild-type, WT-In8-In10 or WT-In10. Lanes 1-3, HeLa cell treated with control siRNA. Lanes 4-6, HeLa cells treated with PTB, A1 and A2 siRNAs. Splicing products are indicated on the left.



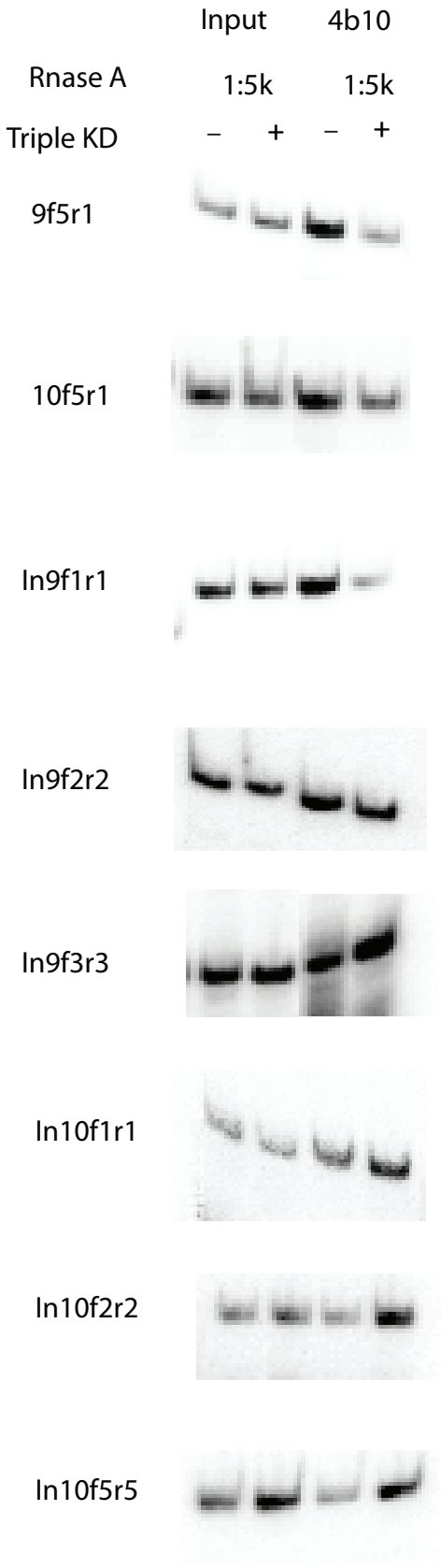
Supplementary Figure 3. Western blotting with antibodies against PTB, hnRNP A1 and hnRNP A2 to estimate siRNA depletion efficiency. Actin was used as loading control. Lane 1, lysate from control siRNA transfected HeLa cells. Lane2, lysate from triple siRNA transfected HeLa cells. Depletion efficiencies of each hnRNP protein are indicated below each panel, with the control siRNA-treated sample set as 1.



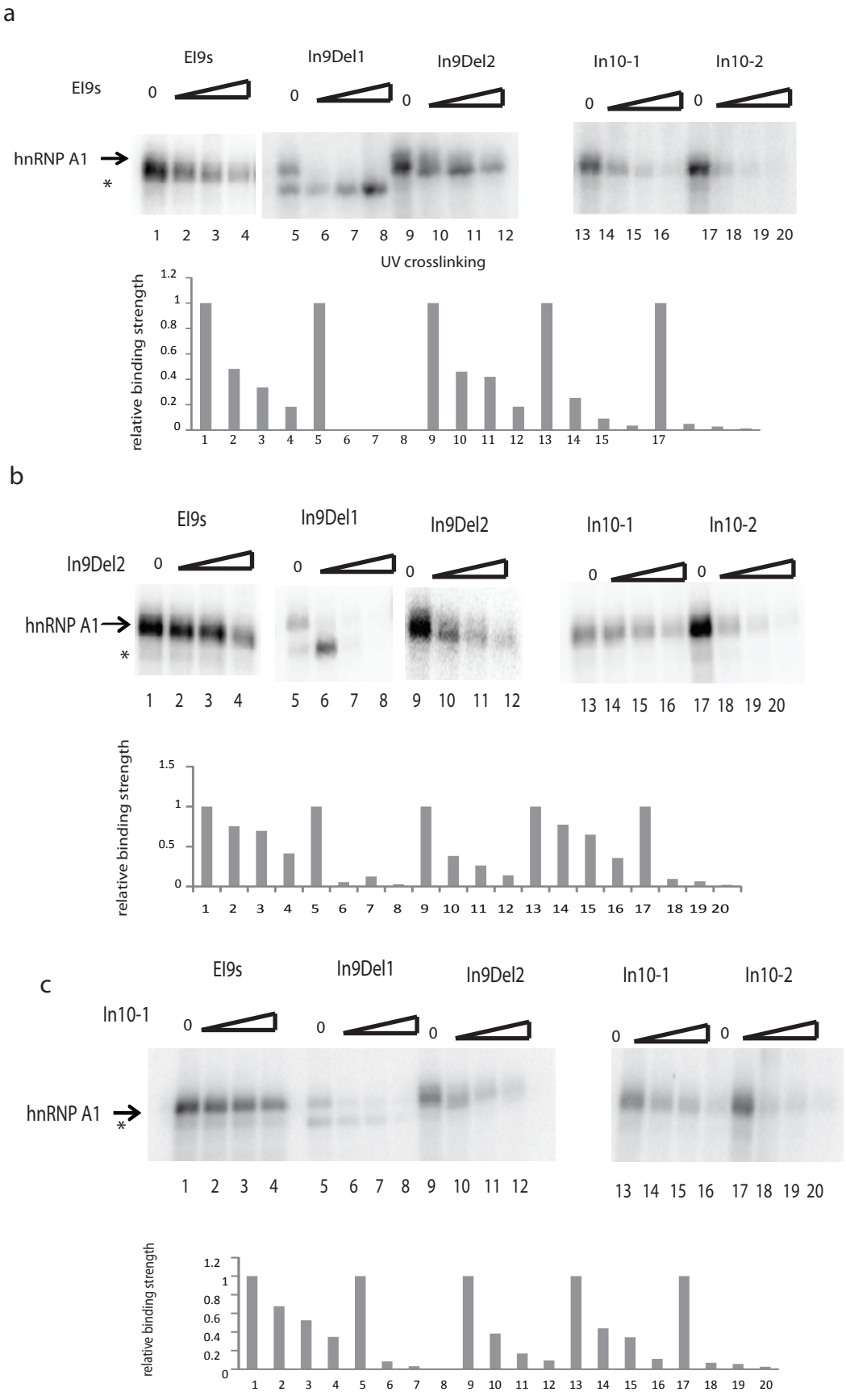
Supplementary Figure 4 Intron 10 sequence prevents double inclusion. (**a**) RT-PCR assays with exon 9-specific primers and total RNA extracted from HeLa cells transfected with wild-type, WT-In8-In10, WT-In10, WT-In10 1/2 or WT-In10 2/2 in either control or A1/A2 and PTB depleted conditions. Splicing products are indicated on the left. M1(%) represents the percentage of M1 in all exon 9 included products. (**b**) RT-PCR assays with exon 9-specific primers and total RNA extracted from HeLa cells transfected with wild-type, WT-In10, WT-In10 1/3, WT-In10 2/3 or WT-In10 3/3 in either control or A1/A2 and PTB depleted conditions. Splicing products are indicated on the left. M1(%) represents the percentage of M1 in all exon 9 included products.



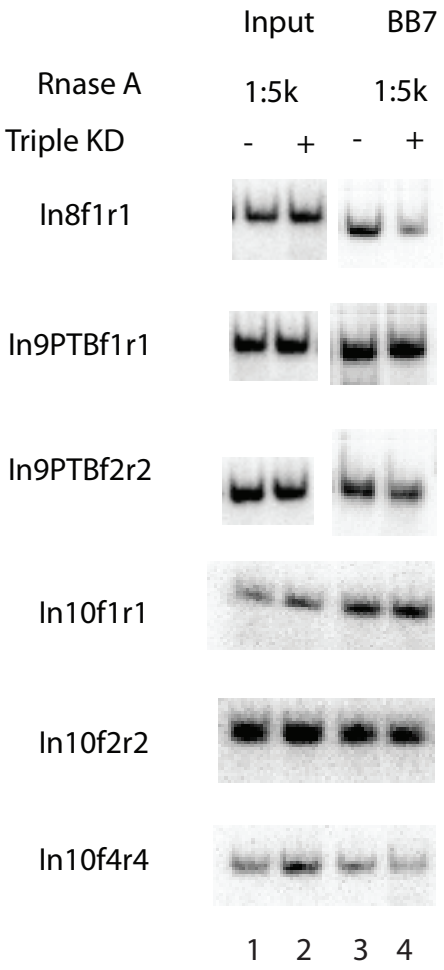
Supplementary Figure 5 Intron 9 sequence prevents double inclusion. (**a**) RT-PCR assays with primers 8F and BGHR and total RNA extracted from HeLa cells transfected with WT, WT-In10, A1Mu1-4, A1Mu1-4-In10, PTBMu and PTBMu-In10 in either control or hnRNP A1/A2 and PTB depleted conditions. Splicing products are indicated on the left. (**b**) RT-PCR assays with exon 9-specific primers and total RNA extracted from HeLa cells transfected with A1Mu1-4-In10, PTBMu-In10 and A1+PTBMu-In10 in either control or A1/A2 and PTB depleted conditions. Splicing products are indicated on the left.



Supplementary Figure 6 CLIP experiments with hnRNP A1 antibody.RT-PCR assays with [α-32P]dCTP and RNAs from CLIP experiments using A1 antibody (4B10) as describe in Figure 6e. Primers used are indicated on the left.



Supplementary Figure 7 hnRNP A1 has higher affinity to EI9s than to intron 9 and intron 10 substrates containing its binding sites. (**a-c**) top, UV crosslinking experiments with [α-32P]UTP-labeled RNA substrates (indicated on top of each gel), purified A1 and increasing amount of cold RNA, indicated on the left of each gel. Bottom, quantification of binding strength. Binding without cold RNA competition was set as "1". *, non-specific band. In10-1 and In10-2 are 200 nt long RNA oligos that contain the amplicons in CLIP assays In10f1r1 and In1f2r2 respectively.



Supplementary Figure 8 CLIP experiments with PTB antibodyRT-PCR assays with [α-32P]dCTP and RNAs from CLIP experiments using PTB antibody (BB7) as describe in Figure 6f. Primers used are indicated on the left.

Supplementary Figure-8 (Manley)