



Supplemental Fig. S6. In all panels, unless otherwise specified, only introns from transcriptionally active genes where the downstream exon is a fully coding internal exon at least 100 nt long were included. In addition, enough spliced/unspliced reads had to end within the first 100 exonic nucleotides that obtaining a splicing ratio (SR) of 0 or 1 by chance alone was highly unlikely (see the Methods section for details). For genes expressed in late embryos, this threshold was 10 reads for both replicates. For pre-MBT genes, it was 14 for replicate 1 and 9 for replicate 2. For S2P, a threshold of 10 was used for better comparability with S5P data. (A) SR values estimated in two biological replicates of *d*NET-seq/S5P datasets for pre-MBT genes in early embryos (Spearman correlation, $\rho = \sim 0.775$, $P \sim 1.348 \times 10^{-14}$). (B) Distribution of SR values for *d*NET-seq/S5P ($N = 4510$) and *d*NET-seq/S2P ($N = 4373$). (C) SR values estimated in replicate 2 of *d*NET-seq/S5P and *d*NET-seq/S2P from late embryos (Spearman correlation, $\rho = \sim 0.737$, $P < 2.2 \times 10^{-16}$; $N = 3295$). (D-Q) SR values for *d*NET seq/S5P data (replicate 1 and 2) plotted out against several gene architecture parameters. For sample sizes and statistical tests, see Supplementary Table 1. In (D-J, N-Q), the bin ranges have been set so that intron numbers would be as equal possible between bins.