



Figure S1. Pre-mRNA splicing of 4-thioU containing pre-mRNA and crosslinking with non-4-thioU containing pre-mRNA.

(A) *In vitro* splicing of the five different 4-thioU containing *CYH2* pre-mRNA utilized for crosslinking. All the 4-thioU pre-mRNAs are correctly spliced as indicated by the appearance of the first step 5' exon intermediate and the second step mRNA product following 25 min. splicing *in vitro*. The lariat intron-3' exon intermediate and the lariat intron product are not visible by autoradiography as there is a single ^{32}P in the 5' exon. (B) *In vitro* splicing time course using *CYH2* pre-mRNA without 4-thioU. This pre-mRNA is a control for the (-3), (-4) and (-6) 4-thioU containing pre-mRNAs. Zero time point with no UV irradiation (lane 2). Samples were withdrawn at 5 minute intervals from 23°C and UV irradiated (lanes 3-7). (C) *In vitro* splicing time course using *CYH2* pre-mRNA without 4-thioU and a C to U sequence change at position (-5). Lanes as in (B). (D) *In vitro* splicing time course using *CYH2* pre-mRNA without 4-thioU and a G to U sequence change at position (-7). Lanes as in (B).