



**Supplemental Figure S3: PCR validation of alternative splicing.**

(A) PCR of select splicing events, using primers spanning cassette exons or retained introns. The right-hand side of each gel indicates the splice variants and their predicted sizes, including for cassette exons that are not observed in any of the samples (e.g., polycomb group ring finger 1, *PCGF1*). Splicing events were selected to include a range of possible outcomes, such as where alternative splicing was only observed in a subset of snRNA-knockdown samples (e.g., chromodomain helicase DNA binding protein 8, *CHD8*, and EMAP like 3, *EML3*); where it was the degree of differential splicing differed (e.g., ubiquitin conjugating enzyme E2 F, *UBE2F*, and pumilio

RNA binding family member, *PUM2*); or where multiple alternatively spliced exons or introns were observed within the same transcript (e.g., interferon lambda 1, *IFNL1*, and endoplasmic reticulum metalloproteinase 1, *ERMP1*)

(B) Dosage-dependent differential splicing following U2 knockdown with increasing concentration of antisense oligo (ASO), from 0 to 250 pmole.

(C) Time-dependent differential splicing following U2 knockdown, at 0 to 48 hours following 200 pmole ASO treatment.