



**Supplemental Fig. S3: Upstream SREs and a second GT at positions -8 and -7 affect the accuracy of TT splice site usage.** HeLa cells were transfected with 1 $\mu$ g of each of the constructs shown in Fig. 4B and 1 $\mu$ g of GH1, which was used as transfection control. 24 hours post transfection, total RNA was isolated, reverse transcribed, and amplified with specific primer pairs (DE: #2648/#2649, S = splice site usage, c1 = c1 usage, ES = exon skipping, BsiWI = -8/-7 GT of BsiWI restriction site, -1 = GT at position -1, -1/+1 = Use of GT at -1 and TT at +1 (see sequencing results); GH1: #1224/#1225). PCR products were separated either on a 10% PAA gel (bottom), or, for higher resolution, on a QIAxcel DNA screening gel cartridge (top).