

### Supplemental Figure S8. Sufficiency mutants assayed with DMS-MaPseq in live cells

Related to Figure 6C. For each subfigure: at left, coefficient of determination between DMS signal of this mutant compared to full-length *Insr* 5'UTR across their shared sequence range, considering only signal on As and Cs, calculated in windows (see Methods). Center, mFMI comparison between the predicted structure and full-length. Right, the secondary structure model of the mutant. Indexes in the structures correspond to the mutant UTR, not full-length. **(A)** D1-Solo probed in live cells reveals an intact D1. **(B)** D1D2 cellular models a disrupted D2 that is more paired than D2 in the context of the full-length *Insr* 5'UTR. Base-pairs removed (C9-G13 with G109-C113) are indicated. **(C)** D1D2TL probed in live cells, where the tetraloop rescues the structure of D2. Base-pairs removed for clarity (C9-C12 with G214-G217) are shown. **(D)** D1D2TLTL probed in live cells is intact outside of the TL replacements replacing D3 and D4. **(E)** D1TLTLTL replaces D2, D3, and D4. Cellular probing reveals an intact D1 as well as the small hairpin at the base of D3 (G175-C192). Base-pairs removed for clarity (C9-C14 with G225-G230) are shown.

