

Supplemental Figure S1. DMS-MaPseq analysis pipeline and quality control

(A) The typical DMS-MaPseq analysis pipeline with an additional barcode splitting step added upstream to support the sequencing of many DMS-MaPseq fragments at once. **(B-E)** DMS-MaPseq data probing the HCV IRES in cells and *in vitro*. At left, representative scatterplots of mutation rates between two replicates in cells and *in vitro*, respectively. Red: As. Blue: Cs. Black: Gs and Us. At right, frequency histogram counting the number of mutations per 150x150 paired end read. **(F-I)** In the same format, DMS-MaPseq data for the EMCV IRES probed in tandem in cells and *in vitro*. **(J-M)** DMS-MaPseq data for *Insr* probed as depicted in Figure 1. These data were used for secondary structure predictions.

