

Supplemental Figure S4. RT-qPCR of the bicistronic reporters

(A) Schematic of the bicistronic transcript RT-qPCR assay with probes diagrammed. HEX: Hexachlorofluorescein. BHQ1: Black Hole Quencher 1. FAM: 6-Carboxyfluorescein. Insert: assayed UTR placed between the Renilla (REN) and Firefly (FF) open reading frames (ORFs). HEX reports on REN and FAM reports on FF. **(B)** Efficiency plots of the probes under the assay conditions (see Methods). **(C)** Ratios of HEX (assaying the REN ORF) to FAM (assaying the FF ORF) across *Insr*, BG, and HCV controls, as well as 20 *Insr* variants presented in the manuscript. The error bars in (B) and (C) are the standard deviation of technical replicates.

