Secondary Validation **Primary Validation** # of target genes: 28 # of target genes: 20 # of cell lines: 5 # of cell lines: 4 # of siRNA: 3 individual # of siRNA: pooled siRNA plus a pool in triplets in triplets # of time points: 3 # of time points: 3 (1.5, 3, and 4.5 days) (1.5, 3, and 4.5 days) CellTiterGlo (CTG) Assay Apotox (Viability, Caspase) Assay ApoTox (Caspase) Assay n = 12 for each gene: n = 3 for each gene: siRNA2 siRNA3 siRNA pool siRNA1 siRNA pool 00000000000 00000000000 00000000000 00000000000 00000000000 00000000000 00000000000 00000000000 Replicate 1 Replicate 2 Replicate 1 Replicate 2 Replicate 3 Replicate 3

Figure S1. Plate layouts for RNA interference mediated knockdown in HT RNAI primary validation screen and secondary RNAI validation assay. Drawings of 384-well format for primary validation screen and 96-well format for secondary assay. Individual siRNAs and siRNA pooled approach depicted along with replicate plates; CellTiter-Glo assay (Promega) and Apotox-Glo assay (Promega).