



Figure S2: Acute depletion of DR5 via siRNA knockdown substantially inhibits ER stress-induced apoptosis.

HCT116 Cas9 parental cells were transfected with siRNA against DR5 or a non-targeting control (Nt) for 48 hours prior to treatment with 100 nM Tg for 24 hours. Cells were then stained with Annexin V-AlexaFluor647 and SytoxBlue Dead cell stain to quantify the population of live (Q4), early apoptotic (Q3), and late apoptotic (Q2) through flow cytometry (A-C). Lysates of Cas9 parental cells treated with siRNA against DR5 were analyzed for PARP cleavage via Western blotting, where one representative replicate is shown, (D) and caspase-3/7 activity through GLO assay (F, mean \pm standard deviation for $n = 3$ biological replicates). The ratio of cleaved to uncleaved PARP was quantified using ImageJ to measure the intensity of the bands at 130 and 100 kDa in the anti-PARP blot (E, mean \pm standard deviation for $n = 3$ biological replicates).