**Figure S1.**

Death receptor surface protein expression and TRAIL responsiveness in PDA cell lines. A, PDA cell lines were immunostained with Ab against DR4 and DR5 (black line) and analyzed by flow cytometry to determine the MFI (indicated in top right panel of each histogram) for each cell line. An isotype matched control Ab (grey line) was used to determine non-specific binding. B, TRAIL responsiveness was determined for each PDA cell line by treating cells with 2-fold serial dilutions of sTRAIL for 7 days and measuring cell viability with PicoGreen staining. Cell lines are arranged from most sensitive (top) to most resistant (bottom) based upon their determined IC50 values indicated in each panel. (n=3, P < 0.05). C, Comparative regression analysis comparing flow cytometric examination of DR5 surface protein levels, expressed as MFI, to TRAIL responsiveness, expressed as IC50 values, for each PDA cell line. R2 = 0.298.

**Figure S2.**

Cytoplasmic HuR expression levels in PDA cell liens. Representative immunoblots of cytoplasmic fractions of cell lysates from PDA cells, probed with Ab against HuR and α-tubulin, used as a loading control. (n=3, P < 0.01).

**Figure S3.**

Quantification of total mRNA used for RNP-IP samples. Comparison of the deltaCT of each input mRNA used for RT-qPCR for the RNP-IP analysis in Fig. 3C, normalized to *18S* rRNA. The mRNA samples analyzed were taken prior to IP to determine the transfection efficiency of each sample.

**Figure S4.**

HuR regulates TRAIL sensitivity in PDA cell lines. A and C, RT-qPCR determination of the amount of HuR mRNA in total mRNA samples isolated from 3 PDA cell lines transfected with control siRNA or HuR-specific siRNA, expressed as a percentage of HuR mRNA in control siRNA-treated samples. Validations correspond to experiments in Supplemental Fig. 4B for MiaPaCa-2 and BxPC3 cells and Fig 4A for MiaPaCa-2 and Panc-1 cells. B, TRAIL responsiveness of PDA cell lines pre-treated with control (siCtrl) or HuR siRNA (siHuR) and measured for cell viability on day 5. MiaPaCa2, *P* = 0.004 and BxPc3, *P* < 0.05 (n = 3). Corresponding TRAIL IC50 values in Supplemental Table 2. D, to validate mRNA knockdown for Fig. 4E, quantification of HuR, DR4 or DR5 mRNA in total mRNA samples isolated from 2 PDA cell lines transfected with a combination of either control siRNA, HuR-specific siRNA, DR4-specific siRNA or DR5-specific siRNA, as indicated. RNA amounts were determined by RT-qPCR and normalized to the amount of HuR, DR4 or DR5 mRNA in respective control siRNA-treated samples.

**Figure S5.**

HuR overexpression induces TRAIL resistance. A, Immunoblot of MiaPaCa-2 cells stably transfected with a GFP-tagged HuR expression construct (GFP-HuR) or GFP-only empty vector (GFP) and probed with an Ab against GFP. B, immunoblot of MiaPaCa-2 cell GFP-HuR or GFP stable transfects probed with an Ab against HuR. α-tubulin was used as a loading control. C, TRAIL responsiveness of MiaPaCa-2 GFP-HuR or GFP only (GFP) stable transfectants measured for cell viability on day 5, *P* < 0.05. Corresponding TRAIL IC50 values in Supplemental Table 3.

**Figure S6.**

The drug MS-444 inhibits cytoplasmic HuR accumulation. Confocal immunofluorescence photomicrographs (40x) of BxPc-3 cells pre-treated with MS-444 (15 mM) or vehicle only (DMSO), then treated for 2 h with sTRAIL (120 ng/mL) or for 4 h with Gemcitabine (1mM) and stained with anti-HuR Ab (green) and Hoechst 33342 nucleic acid stain (blue).

**Table S1.**

TRAIL responsiveness in PDA cell lines treated with control, HuR, DR4 or DR5-specific siRNA. Comparison of TRAIL IC50 values calculated from survival curves in Fig. 4E.

**Table S2.**

TRAIL responsiveness in PDA cell lines treated with control or HuR-specific siRNA. Comparison of TRAIL IC50 values calculated from survival curves in Fig. S5A.

**Table S3.**

TRAIL responsiveness in MiaPaCa-2 PDA cells transfected with GFP or GFP-HuR. Comparison of TRAIL IC50 values calculated from survival curves in Fig. S5D.

**Table S4.**

TRAIL responsiveness in MiaPaCa-2 PDA cells transfected with HuR and/or DR4 expression constructs. Comparison of TRAIL IC50 values calculated from survival curves in Fig. 4G.

**Table S5.**

TRAIL responsiveness in PDA cell lines treated with the drug, MS-444. Comparison of TRAIL IC50 values calculated from survival curves in Fig. 6E.