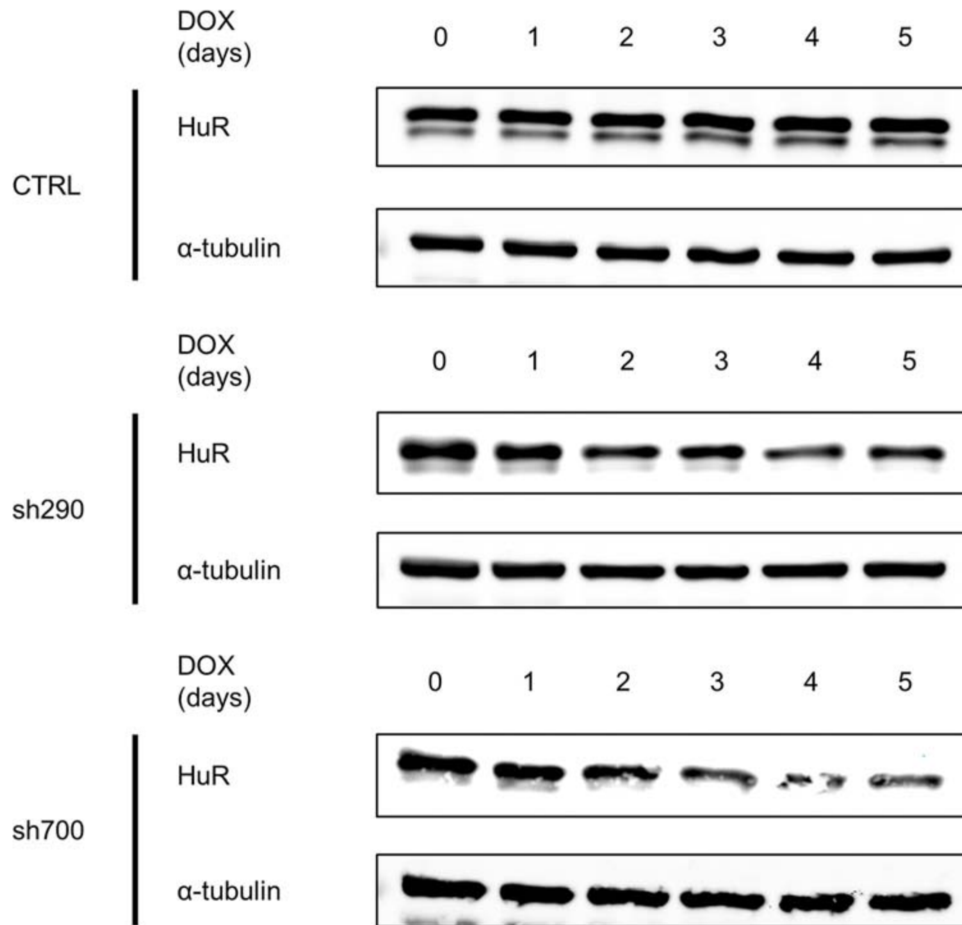
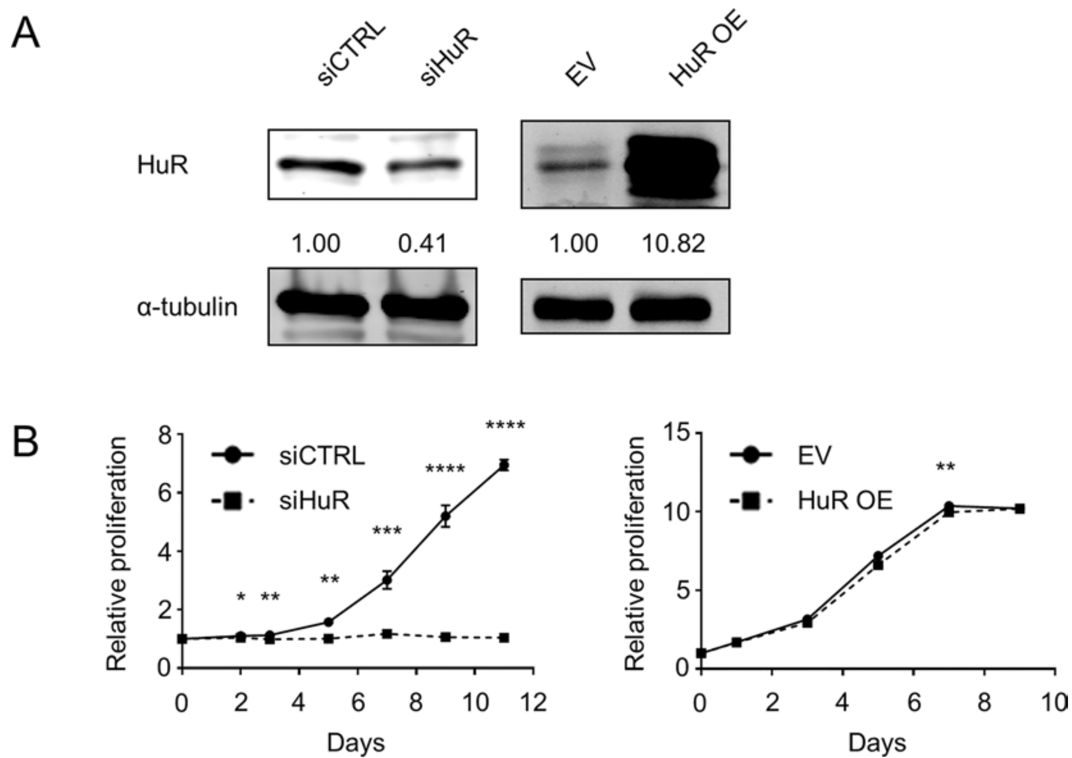
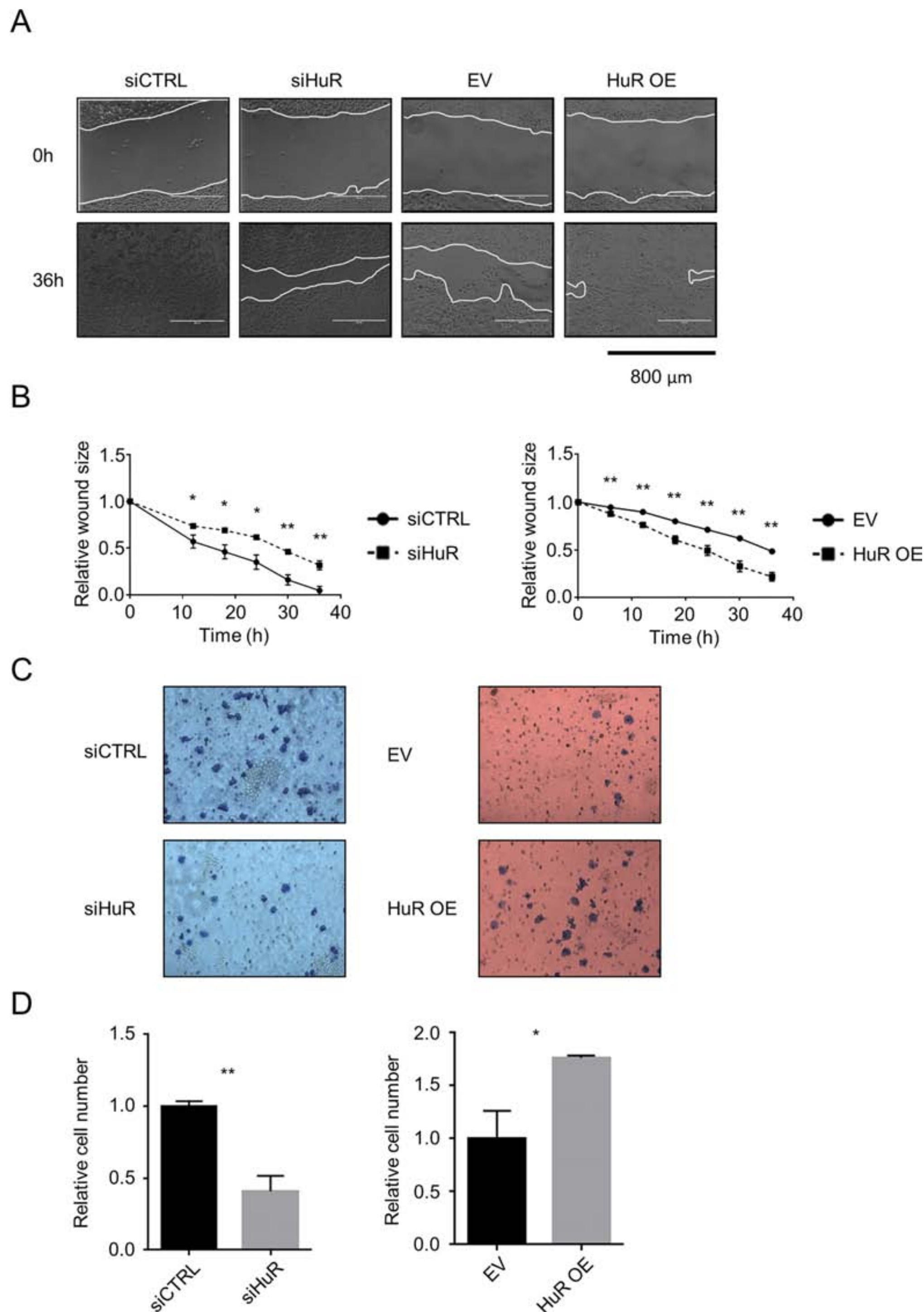
## SUPPLEMENTARY FIGURES AND TABLES



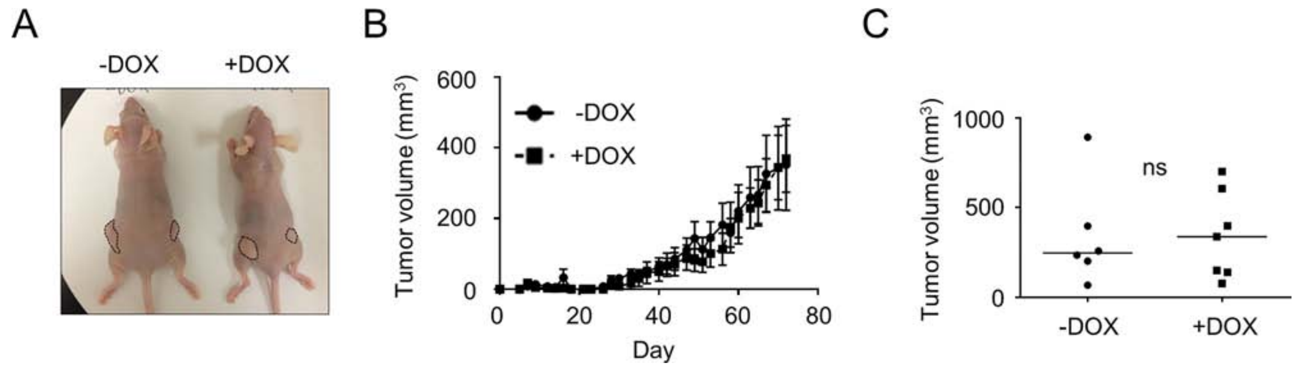

**Supplementary Figure S1: Time course of DOX-induced HuR silencing.** Western blot of HuR protein expression in Mia.CTRL, Mia.sh290, and Mia.sh700 cells treated with 2 μg/ml DOX for the indicated time points. Alpha-tubulin was used as normalization control.



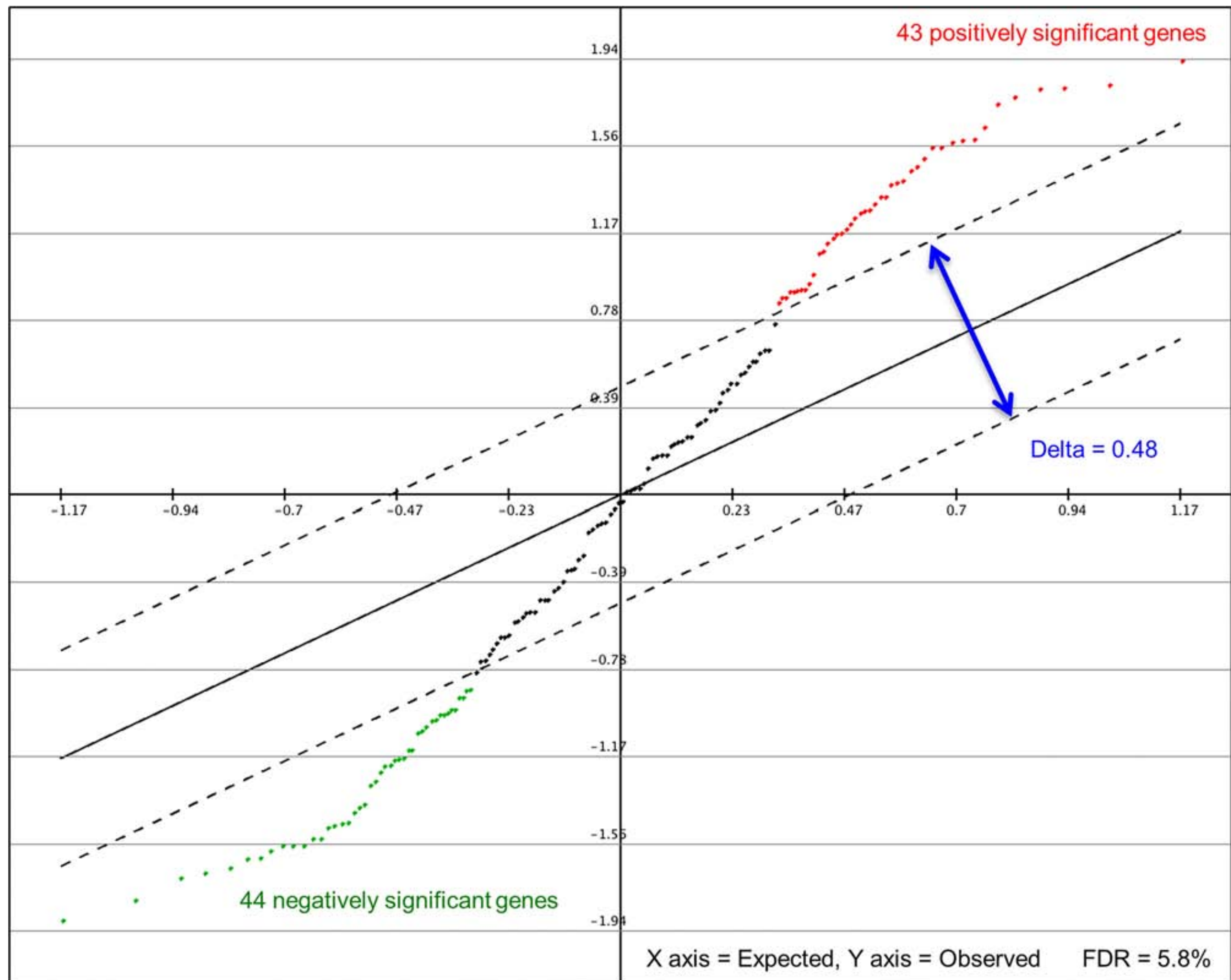

**Supplementary Figure S2: Short-term proliferation of transiently transfected PL5 cells. A.** Representative western blot of HuR protein expression in PL5 cells transiently transfected with control siRNA (siCTRL), HuR siRNA (siHuR), empty vector (EV), or HuR overexpression plasmid (HuR OE) for 3 days. Alpha-tubulin was used as normalization control. **B.** Relative proliferation of transiently transfected PL5 cells, as determined by measurement of dsDNA content by PicoGreen staining. Each data point represents the mean of 5 independent experiments ± SEM. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; **** = $p < 0.0001$.



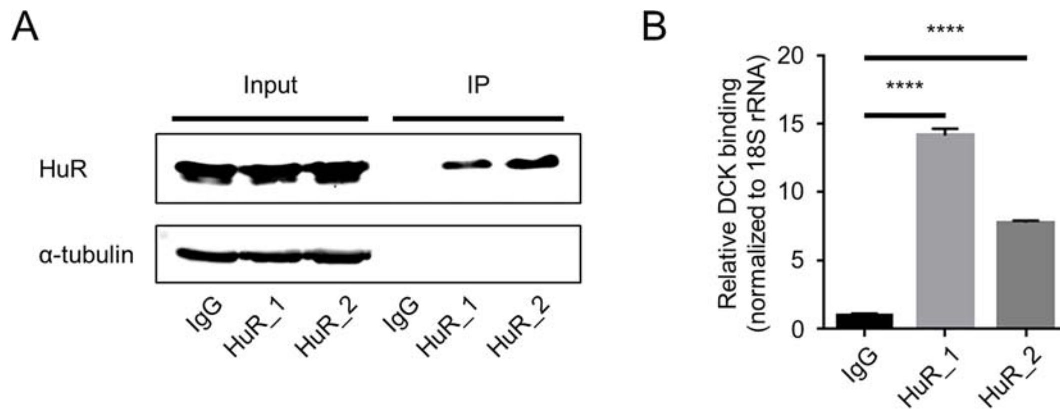

**Supplementary Figure S3: Effects of transient HuR silencing and overexpression on PL5 migration and invasion.** **A.** Representative images of *in vitro* scratch assays performed with PL5 cells transiently transfected with control siRNA (siCTRL), HuR siRNA (siHuR), empty vector (EV), or HuR overexpression plasmid (HuR OE) for 3 days. Images were taken at 0 h and 36 h post-scratch. Leading edges were outlined in white for ease of visualization. **B.** Quantification of the rate of scratch closure, as measured by change in wound size relative to the 0 h time point. Each data point represents the mean of 3 independent experiments ± SEM. **C.** Representative images of Matrigel invasion assays performed with PL5 cells transiently transfected with siCTRL, siHuR, EV, or HuR OE for 3 days. Cells that invaded through the Matrigel and onto the basal surface of transwell inserts were stained with Differential Quik and photographed at 20X magnification. **D.** Quantification of Matrigel invasion assays. Values were normalized to the number of cells in the control groups. Each bar represents the mean of 3 independent experiments ± SEM. * = $p < 0.05$; ** = $p < 0.01$.




**Supplementary Figure S4: Tumor growth of Mia.CTRL xenografts. A.** Representative images of subcutaneous Mia.CTRL tumors on the flanks of nude female mice, at the termination of the experiment (day 72). **B.** Tumor growth curves of Mia.CTRL xenografts. Mice were fed normal diet or 200 mg/kg DOX diet starting on day 0 (date of xenograft injection). Each data point represents the mean ± SEM (*n* = 6 for –DOX group, and *n* = 7 for +DOX group). **C.** Plot of all tumor volumes on the final day of the Mia.CTRL xenograft experiment (day 72). Horizontal bars represent the median tumor volumes. *ns* = non-significant.




**Supplementary Figure S5: Significance analysis of microarrays (SAM).** SAM of mRNA transcripts differentially regulated in MIA PaCa-2 cells transfected for 72 hours with HuR siRNA, compared to cells transfected with control siRNA. FDR = false discovery rate.




**Supplementary Figure S6: mRNP-IP validation.** **A.** Western blot validation of mRNP-IP experiment. **B.** qPCR validation of mRNP-IP experiment. The relative expression of *DCK* mRNA, a previously identified HuR binding target, in the mRNP-IP RNA samples is shown. 18S rRNA was used as normalization control. IP = immunoprecipitate. **** = $p < 0.0001$.

**Supplementary Table S1. Complete gene list of NanoString Technologies nCounter® GX Human Cancer Reference Kit.**

**Supplementary Table S2. HuR-bound transcripts identified by NanoString nCounter® analyses.**

| Gene | Fold enrichment |
|---|---|
| SFPQ | 1,289.24 |
| BIRC5 | 1,273.03 |
| TP53 | 1,068.58 |
| CTNNB1 | 788.09 |
| CD44 | 758.39 |
| PRKAR1A | 606.66 |
| MYC | 285.82 |
| PCTK1 | 238.12 |
| ITGB1 | 194.52 |
| DEK | 176.90 |
| CASP2 | 173.47 |
| TOP1 | 169.16 |
| TFDP1 | 164.53 |
| ETV6 | 147.55 |
| MTA1 | 137.25 |
| TUBB | 134.86 |
| YES1 | 128.01 |
| PTEN | 122.45 |
| PGK1 | 120.63 |
| GAPDH | 106.52 |
| HIF1A | 103.17 |
| PTPN11 | 87.13 |
| CSK | 80.97 |
| KRAS | 77.88 |
| PDGFA | 73.38 |
| HPRT1 | 72.85 |
| NQO1 | 70.36 |
| YY1 | 68.76 |
| HSP90AB1 | 67.34 |
| CCND1 | 66.65 |
| STAT3 | 60.78 |
| RB1 | 59.83 |
| S100A4 | 44.72 |
| NRAS | 34.04 |
| NF1 | 31.62 |

Full list of transcripts that were enriched in MIA PaCa-2 HuR mRNP-IP samples, compared to control IgG mRNP-IP samples. Fold enrichment = average of the transcript expression in HuR mRNP-IP samples vs. average of the transcript expression in IgG mRNP-IP samples.

**Supplementary Table S3. Biological processes enriched in transcripts regulated by HuR.** Lists of biological process gene ontology (GO) terms significantly enriched in the HuR-regulated transcripts, as analyzed by DAVID. Positive = GO terms significantly enriched in the list of transcripts upregulated upon HuR silencing (see Table 1, *left*). Negative = GO terms significantly enriched in the list of transcripts downregulated upon HuR silencing (see Table 1, *right*). Binders = GO terms significantly enriched in the list of transcripts that are direct HuR targets (see Table S2). Count = number of genes associated with the GO term. Fold enrichment = fold enrichment of the GO term in the associated genes, compared to the background. FDR = false discovery rate.