# CRISPR Knockout of the HuR Gene Causes a Xenograft Lethal Phenotype

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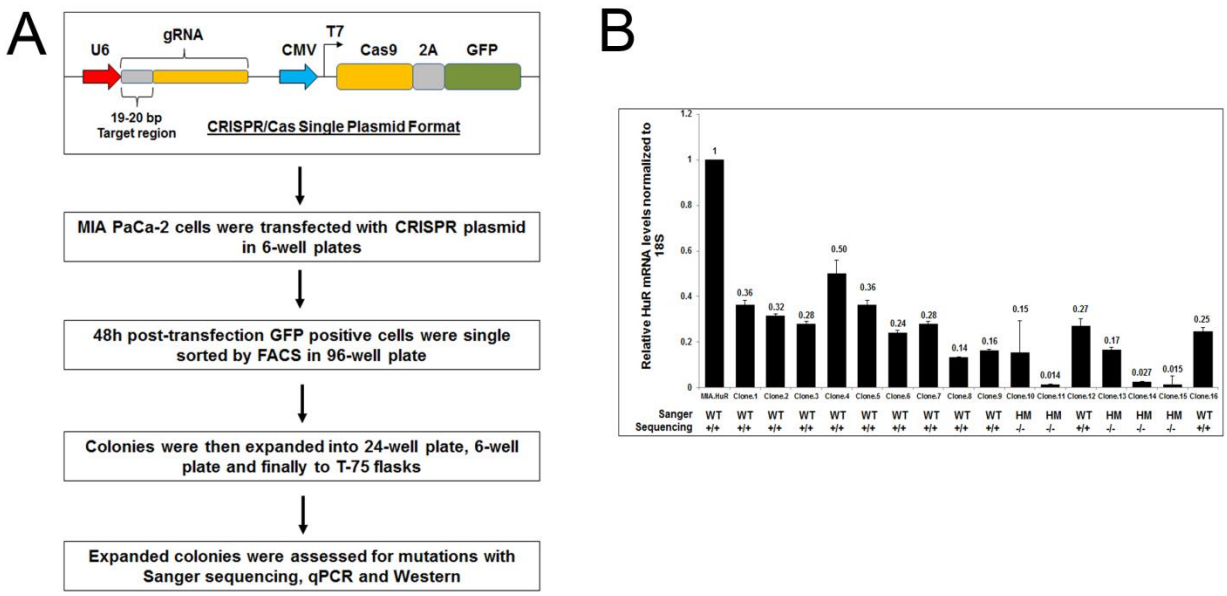
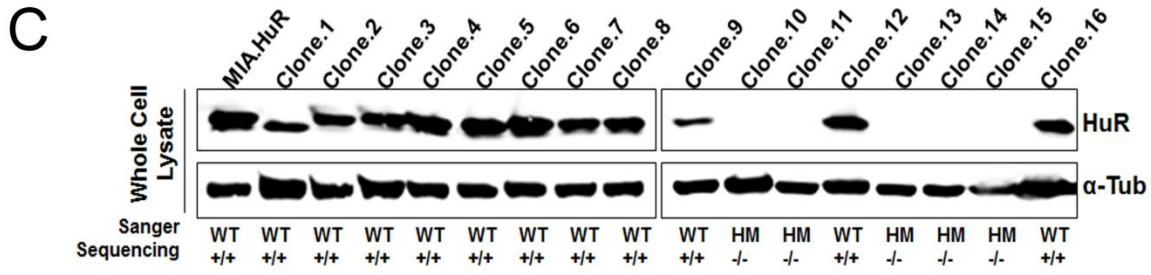
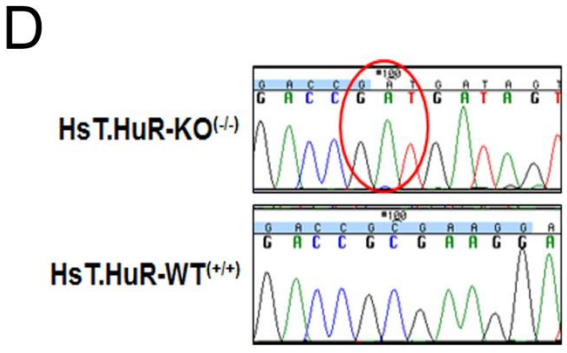
**Figure S1:** ***HuR inhibition was successfully achieved by CRISPR/cas9 system.*** A, Schematic of CRISPR/Cas9 single plasmid and flow chart depicting strategy of the experiment. The U6 promoter is driving the expression of gRNA while CMV promoter is driving the expression of Cas9 which is fused with GFP via 2A peptide linkage at C terminus. GFP positive single cells were sorted by FACS analysis and sorted cell population were expanded and assessed for mutation by sequencing and verified by qPCR and western blot.*CRISPR-cas9 system successfully knockout HuR expression.* B, Relative HuR mRNA expression in all the clones was assessed by qPCR in MIA PaCa-2. C, Relative protein expression of HuR was assessed in all the clones by western blot in MIA PaCA-2. D, Sanger sequencing validation of mutation in Hs 766T CRISPR clones. E, Immunoblot showing complete HuR inhibition after CRISPR knockout in Hs 766T CRISPR clones.


A
U6
gRNA
T7
CMV
Cas9
2A
GFP
19-20 bp
Target region
CRISPR/Cas Single Plasmid Format
MIA PaCa-2 cells were transfected with CRISPR plasmid in 6-well plates
48h post-transfection GFP positive cells were single sorted by FACS in 96-well plate
Colonies were then expanded into 24-well plate, 6-well plate and finally to T-75 flasks
Expanded colonies were assessed for mutations with Sanger sequencing, qPCR and Western
B
Relative HuR mRNA levels normalized to 18S
MIA.HuR Clone.1 Clone.2 Clone.3 Clone.4 Clone.5 Clone.6 Clone.7 Clone.8 Clone.9 Clone.10 Clone.11 Clone.12 Clone.13 Clone.14 Clone.15 Clone.16
Sanger Sequencing
WT +/+ WT +/+ WT +/+ WT +/+ WT +/+ WT +/+ WT +/+ WT +/+ WT +/+ WT +/+ HM -/- HM -/- WT +/+ HM -/- HM -/- HM -/- WT +/+


C
MIA.HuR Clone.1 Clone.2 Clone.3 Clone.4 Clone.5 Clone.6 Clone.7 Clone.8 Clone.9 Clone.10 Clone.11 Clone.12 Clone.13 Clone.14 Clone.15 Clone.16
Whole Cell Lysate
HuR
α-Tub
Sanger Sequencing
WT +/+ WT +/+ WT +/+ WT +/+ WT +/+ WT +/+ WT +/+ WT +/+ WT +/+ WT +/+ HM -/- HM -/- WT +/+ HM -/- HM -/- HM -/- WT +/+


D
HsT.HuR-KO(-/-)
HsT.HuR-WT(+/+)

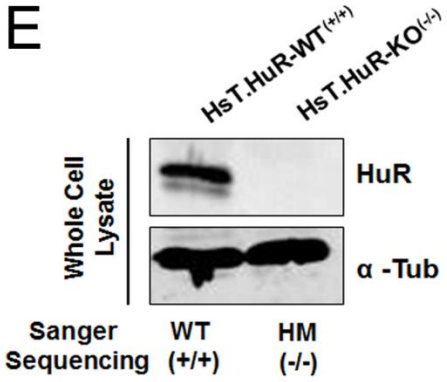

E
HsT.HuR-WT(+/+)
HsT.HuR-KO(-/-)
Whole Cell Lysate
HuR
α -Tub
Sanger Sequencing
WT (+/+)
HM (-/-)

**Figure S1**

**Figure S2:** ***HuR inhibition sensitizes cells to drugs.*** Drug sensitivity (7-day short-term cell survival) on MIA.HuR.WT$^{(+/+)}$, MIA.HuR.KO$^{(+/+)}$ and MIA.HuR.KO$^{(-/-)}$ using A, mitomycin C (MMC) and B, Glucose deprivation (low glucose). C and D, MIA.HuR.WT$^{(+/+)}$ and MIA.HuR.KO$^{(-/-)}$ cells ability to transform from 2D to 3D cultures (i.e., organoids). Error Bars: 95% CI.

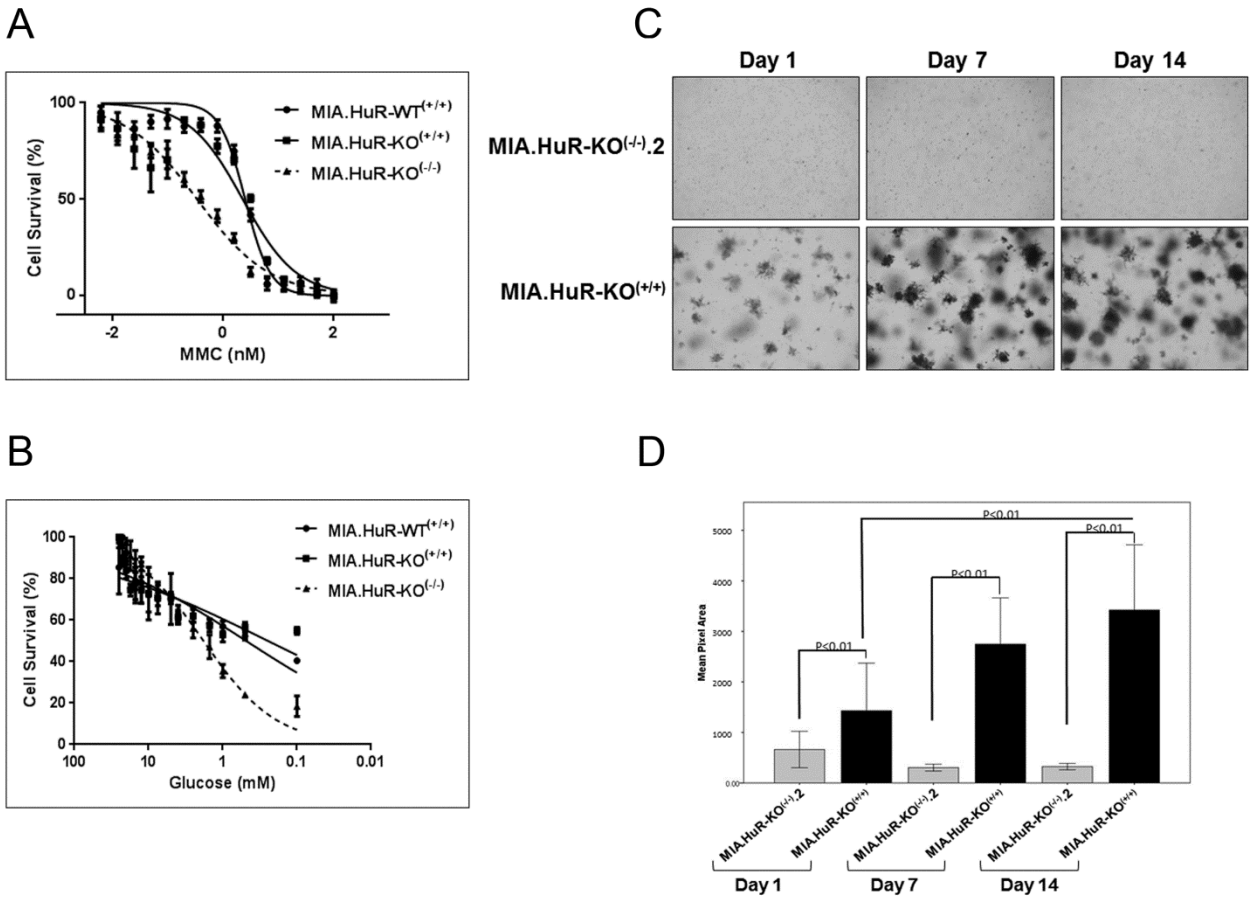



**Figure S2**

**Figure S3:** ***PDA xenografts with HuR inhibition failed to form tumors.*** A, Representative images of subcutaneous tumors on the flanks of nude female mice and their corresponding excised tumors (day 46). B, Tumor growth curves of MIA.HuR.WT$^{(+/+)}$, MIA.HuR.KO$^{(+/+)}$, MIA.HuR.KO$^{(-/-)}$.1 and MIA.HuR.KO$^{(-/-)}$.2 xenografts in nude mice. Data represent means ± SEM of *n* = 5. ns, non-significant,*p<0.001, **p<0.0003.

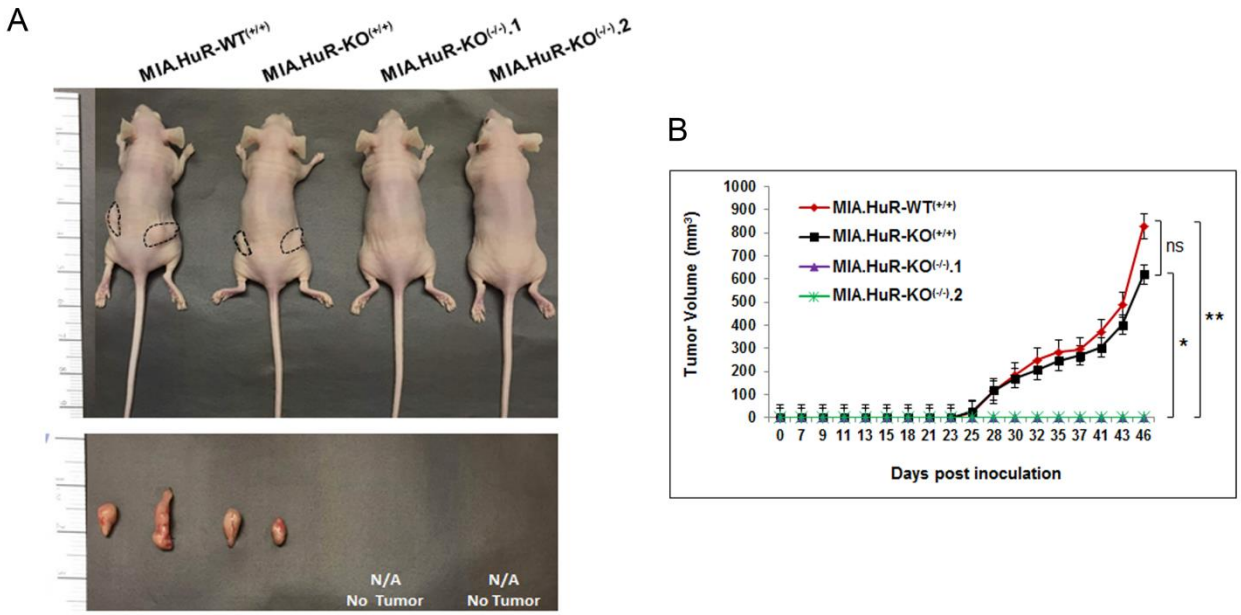



Figure S3

**Table S1:** Lists the three guide RNAs used to generate CRISPR knockout of HuR in MIA PaCa-2 and Hs 766T cell lines.

| Gene Name | RefSeq ID | Exon Position | Nucleotide position in RefSeq | Guide RNA Sequence | Strand |
|---|---|---|---|---|---|
| ELAVL1 (Guide RNA 1) | NM_001419 | 2 | 188 | GCCGAAGACTGCAGG | 5'-N19NGG_sense |
| ELAVL1 (Guide RNA 2) | NM_001419 | 2 | 263 | CCAGGATGAGTTACG | 5'-N19NGG_antisense |
| ELAVL1 (Guide RNA 3) | NM_001419 | 3 | 363 | ACGTGACCGCGAAGG | 5'-N19NGG_sense |

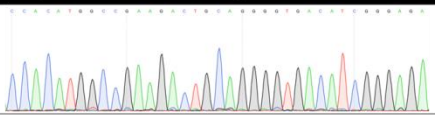
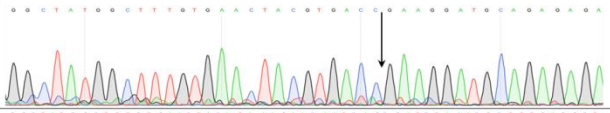
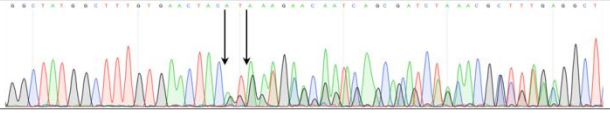
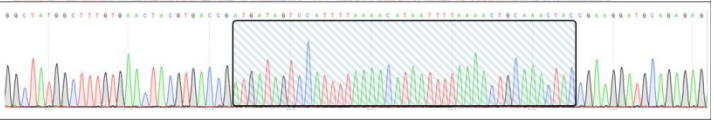
**Table S2:** Lists the primers used to detect the mutation created by all three guide RNAs in CRISPR knockout of HuR MIA PaCa-2 and Hs 766T cell lines.

| Primers | Sequence |
|---|---|
| Guide RNA 1-Forward-1 | TTGGGAACTTATGTCCGATCT |
| Guide RNA 1-Reverse-1 | GCAGTTACTAGTTTTGCCTTCATA |
| Guide RNA 1-Forward-2 | CGATCAAATTCGTTCTCCCG |
| Guide RNA 1-Reverse-2 | GCATAGAAAAGAATCTCTAAGTGC |
| Guide RNA 2-Forward-1 | CACGTGGGACCCAATCAATC |
| Guide RNA 2-Reverse-1 | GTGACATCGGGAGAACGAAT |
| Guide RNA 3-Forward-1 | GAGCTGACGCAACCTGG |
| Guide RNA 3-Reverse-1 | AAGGCCAGGCAGATATAGTG |
| Guide RNA 3-Forward-2 | CACTCATGTAAGGCCTGGGT |
| Guide RNA 3-Reverse-2 | GGAACCTGTGTTCATTGCAGA |
| Guide RNA 3-Forward-3 | TTTACTGTCTGCACCCCCGA |
| Guide RNA 3-Reverse-3 | CCTCACTCGCAGACGGTTG |

**Table S3:** Lists the clones along with their genotypes obtained for all three guide RNAs. All mutations were confirmed by Sanger sequencing. Wild Type: No mutation in both the alleles; and Homozygous Mutant: Both alleles have either same or different mutations.

| | Clone | Genotype |
|---|---|---|
| **Guide RNA 1** | Clone.1 | Wild Type (+/+) |
| | Clone.2 | Wild Type (+/+) |
| | Clone.3 | Wild Type (+/+) |
| | Clone.4 | Wild Type (+/+) |
| **Guide RNA 2** | Clone.5 | Wild Type (+/+) |
| | Clone.6 | Wild Type (+/+) |
| | Clone.7 | Wild Type (+/+) |
| | Clone.8 | Wild Type (+/+) |
| **Guide RNA 3** | Clone.9 | Wild Type (+/+) |
| | Clone.10 | Homozygous Mutant (-/-) |
| | Clone.11 | Homozygous Mutant (-/-) |
| | Clone.12 | Wild Type (+/+) |
| | Clone.13 | Homozygous Mutant (-/-) |
| | Clone.14 | Homozygous Mutant (-/-) |
| | Clone.15 | Homozygous Mutant (-/-) |
| | Clone.16 | Wild Type (+/+) |

**Table S4:** Lists the cell lines produced along with their mutations and chromatograms obtained through Sanger sequencing. WT: Wild Type - No mutation in both the alleles.

| Cell Lines | HGVS Nomenclature | Sequence Chromatogram |
|---|---|---|
| MIA PaCa2: MIA.HuR-KO$^{(+/+)}$ (Clone.4) | NM_001419[WT] |  |
| MIA PaCa2: MIA.HuR-KO$^{(-/-)}$.1 (Clone.11) | NM_001419[c.211delCG];[c.211delCG] |  |
| MIA PaCa2: MIA.HuR-KO$^{(-/-)}$.2 (Clone.14) | NM_001419[c.204delGTGACCGCGA];[c.206delGACCGCGAAGGATGCAG] |  |
| Hs 766T: HsT.HuR-KO$^{(-/-)}$ | NM_001419[c.211_212insATGATAGTCCATTTTAAAACATAATTTTAAAACTGCAAACTAC];[c.211_212insATGATAGTCCATTTTAAAACATAATTTTAAAACTGCAAACTAC] |  |