**Supporting Information**

**Figure S1**

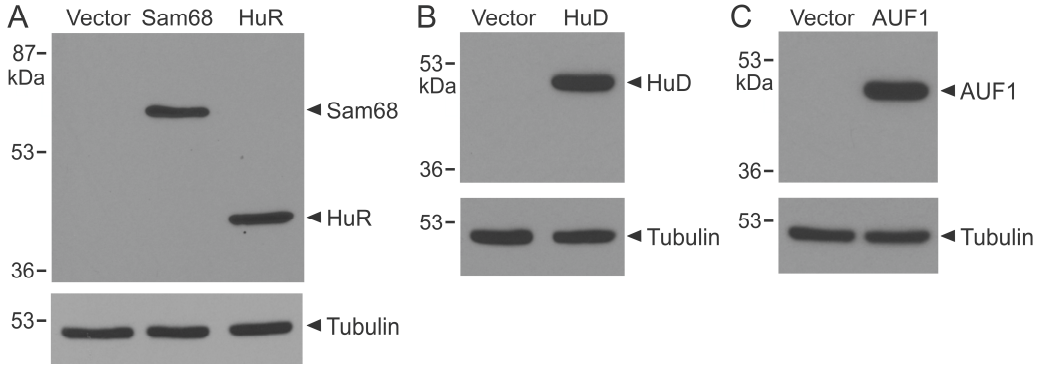


**Figure S1. Immunoblot analysis of HuR, HuD, Sam68 and AUF1 expression following transfection.** A: Xpress epitope tagged HuR and Sam68 were detected by anti-Xpress antibody. B: Myc epitope tagged HuD was detected by anti-Myc antibody. C: Flag epitope tagged AUF1 was detected by anti-Flag M2 antibody**.**

**Figure S2**

-130
CACAAAUACACACCCCCACAAAACUAAAAUCAAAGUUUCACUACAUAACACUGGGCCUUACUGCAUGUGGU

UCAUUCUAGCAUUUCUGUUCUGUGCUGUGCUAAGCUAUACUACUGUAUGUUCUUUC**AGUAAA**AAAAAAAAA
Upstream Oligo
AAAAAAAAAAAAAUGCUGGUUUUGAUUCACUACUGUGUUCUGAUCUUUGGUUUGAAGAACAUUGCUUAUAA
Downstream Oligo
GGGUGCAGUGAUUGGCUAAGAGGGUGUUUGGGACCUGGGGUUUGGGGUAGUCCUGCUGGCUGGGUCUCAUU

UGCUGGGAGACCCUGGGCUCC
+172

**Figure S2. Sequence surrounding KCNH2 intron 9 poly(A) signal.** The *KCNH2* intron 9 poly(A) signal and flanking sequences (-130/+172 bp) are included in the tandem poly(A) signal construct used in Fig. 2. The noncanonical poly(A) signal (AGUAAA) is shown in bold. The upstream sequence element (USE) and downstream sequence element (DSE) RNA oligos used in Fig. 3 are underlined.

**Figure S3**

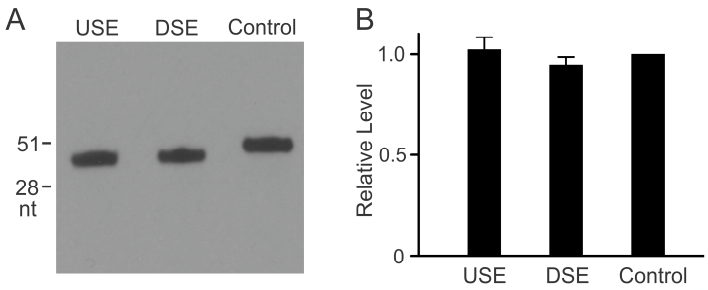


**Figure S3. Biotinylation and purification of RNA oligos.** A: Denaturing polyacrylamide gel electrophoresis of biotinylated RNA oilgos corresponding to *KCNH2* intron 9 USE, DSE and the control HuR binding sequence, a 51 nt sequence from the 3'-UTR of androgen receptor mRNA. B: RNA signals were quantified and plotted as the relative level compared to the control oligo.

**Figure S4**

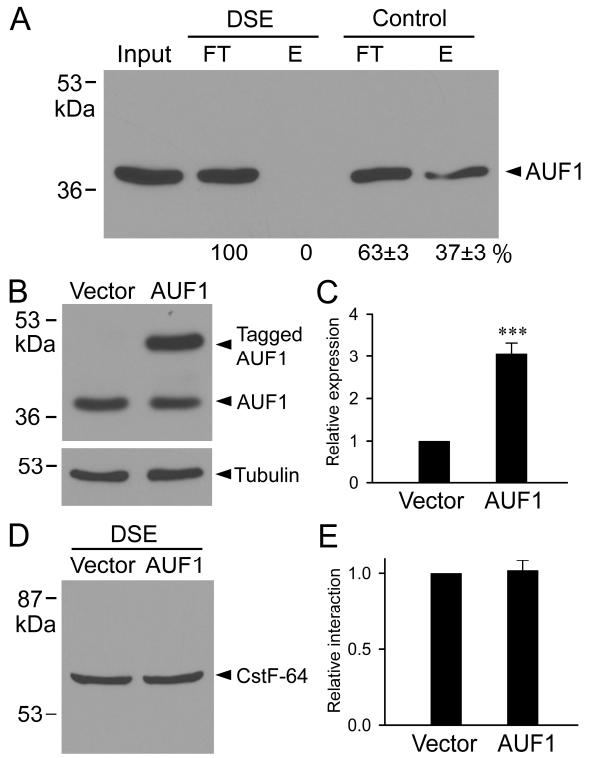


**Figure S4. RNA binding protein AUF1 does not interact with intron 9 poly(A) signal downstream sequence.** A: Cell lysate from HEK293 cells were incubated with biotinylated and streptavidin bead-bound RNA oligos corresponding to DSE or the control sequence from the 3'-UTR of androgen receptor mRNA. The RNA-bound protein fractions (E) and unbound fractions (FT) were analyzed by immunoblotting with anti-AUF1 antibody. Signals were quantified and expressed as percentage of total (FT+E) protein (n=4). B: Immunoblot analysis of total nuclear extracts from vector or AUF1 transfected cells. C: Histogram showing the significant increase in AUF1 expression following transfection compared to vector-transfected control

(***$P$ <0.001, n=4). Protein bands were quantified, normalized to the tubulin and plotted as relative expression of the vector control. D: Total nuclear extracts from HEK 293 cells co-transfection with vector or AUF1 were incubated with biotinylated RNA oligo corresponding to DSE. Immunoblot analysis of RNA-bound protein fractions probed with anti-CstF-64 antibody. E: Histogram showing no significant changes in the interaction between CstF-64 and DSE following AUF1 transfection compared to vector-transfected control ($P$>0.05, n=4). Protein bands were quantified and plotted as the relative to interaction in the vector-transfection conditions.

**Figure S5**

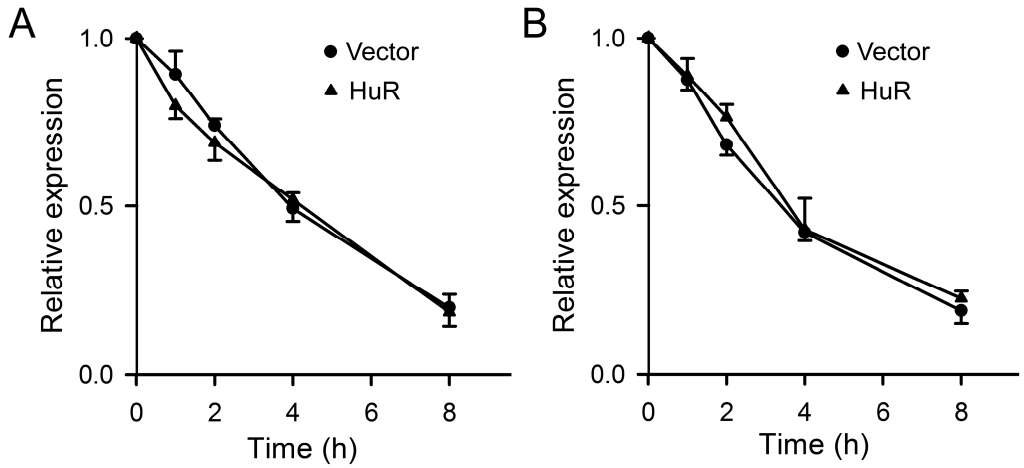


**Figure S5. Effect of HuR on RNA stability of KCNH2 isoforms.** Flp-In HEK293 cells stably expressing *KCNH2* short gene were transiently transfected with HuR-pcDNA3.1.His construct. After 2 days, the cells were labeled by 5-ethynyl uridine (EU) for 2 hours and then chased in normal growth medium for 0, 1, 2, 4 and 8 hours. The EU-labeled RNAs were biotinylated by Click reactions and then captured by streptavidin magnetic beads. The captured RNAs were analyzed by real-time PCR using specific primers for Kv11.1a and Kv11.1a-USO and normalized to 18s ribosomal RNA. The data are presented as ratios of Kv11.1 transcripts at each time point to time 0. A: The half-lives of Kv11.1a transcripts in vector and HuR transfected cells were 4.17±0.48 and 4.08±0.49 hours, respectively ($P$>0.05, n=4). B: The half-lives of Kv11.1a-USO transcripts in vector and HuR transfected cells were 3.41±0.16 and 3.85±0.51 hours, respectively ($P$>0.05, n=4).

**Supplemental Methods:**

**mRNA stability assay.** Click-iT Nascent RNA Capture Kit (Life Technology, Carlsbad, CA) and real time PCR were used to study stability of Kv11.1 isoform mRNA transcripts. Flp-In HEK293 cells stably expressing *KCNH2* short gene were transiently transfected with HuR-pcDNA3.1.His construct. After 2 days, the cells were labeled by 0.4 mM of 5-ethynyl uridine (EU) for 2 hours. The cells were then chased in normal growth medium (DMEM with 10% fetal bovine serum) for 0, 1, 2, 4 and 8 hours and harvested for total RNA isolation with TRIzol reagent. Five µg of total RNA was used for biotinylation by Click reactions, and the biotin-labeled RNAs were captured by streptavidin magnetic beads. After washing, the RNA captured on the beads was immediately used as a template for cDNA synthesis with High Capacity cDNA Reverse Transcription Kits (Thermo Fisher Scientific, Waltham, MA). Real time PCR was performed as described previously (11). The sequences of the primers used for Kv11.1a and Kv11.1a-USO are: Kv11.1a forward (5'-CTTTGGGGAGCCTCTGAACC-3'), Kv11.1a reverse (5'-GGACCAGAAGTG

GTCGGAGAA-3'), Kv11.1a-USO forward (5'-CAGTCACTGGGGCTGTGGA-3'), and Kv11.1a-USO reverse (5'-GAGAATGTGGGAACCCCAGAG-3'). The primers for 18s ribosomal RNA are: forward (5'-TGTGATGCCCTTAGATGTCC-3') and reverse (5'-TTATGACCCGCACTTACTCG-3'). The serial dilutions of a stock of RT reaction were used for generating standard curves. After normalized to the amount of 18s ribosomal RNA, the ratios of Kv11.1 transcripts at each time point to the time 0 were calculated, and the half-lives of the Kv11.1a and Kv11.1a-USO transcripts were estimated.