Alternative Splicing and Polyadenylation Contribute to the Generation of hERG1 C-Terminal Isoforms

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SUPPLEMENTAL FIGURES:

-130
CACAAATACACACCCCCACAAAACTAAAATCAAAGTTTCACTACATAACACTGGGCCTTACTGCATGTGGT
TCATTCTAGCATTTCTGTTCTGTGCTGTGCTAAGCTATACTACTGTATGTTCTTTC**AGTAAA**AAAAAAAAA
AAAAAAAAAAAAATGCTGGTTTTGATTCACTACTGTGTTCTGATCTTTGGTTTGAAGAACATTGCTTATAA
GGGTGCAGTGATTGGCTAAGAGGGTGTTTGGGACCTGGGGTTTGGGGTAGTCCTGCTGGCTGGGTCTCATT
TGCTGGGAGACCCTGGGCTCC
+172

Figure S1. Sequence surrounding hERG1 intron 9 poly(A) signal. The hERG1 intron 9 poly(A) signal and flanking sequences (-130/+172 bp) are shown. The noncanonical poly(A) signal (AGTAAA) is shown in bold. Putative downstream U/GU rich elements are underlined.

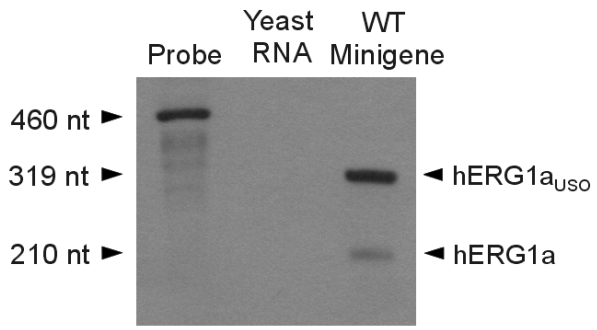

Figure S2. RPA analysis of hERG1 mRNA alternative processing. Analysis of the wild-type minigenes by RPA with a probe containing hERG1a$_{USO}$ sequences from exon 9 to intron 9. This probe generated a protected fragment of 319 nt for hERG1a$_{USO}$ transcript and a 210 nt fragment for hERG1a transcript. The length of the probe was 460 nt and contained sequences from the pCRII vector at both ends.

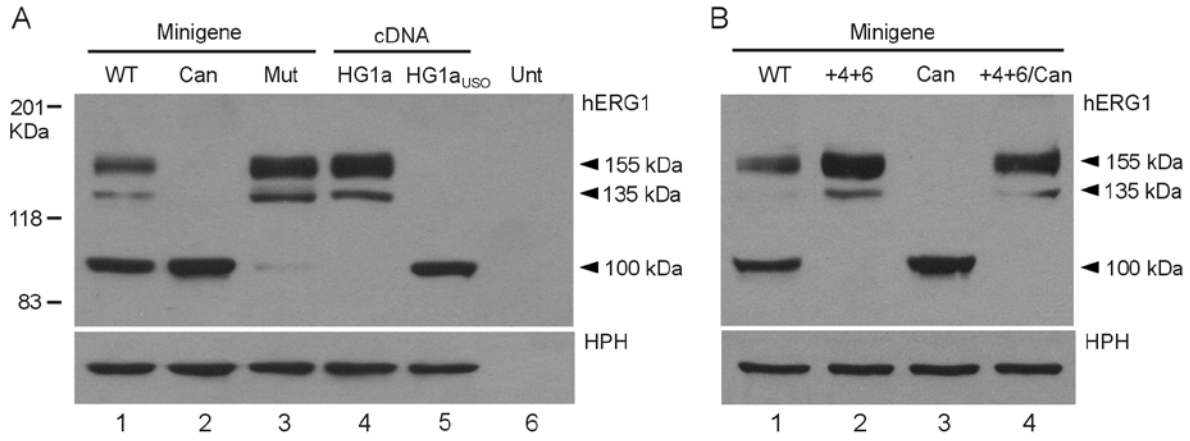

Figure S3. Immunoblot analysis of hERG1a and hERG1a$_{USO}$ proteins. A, Flp-In HEK293 cells were stably transfected with minigenes carrying the wild-type (WT), canonical (Can) and mutant (Mut) intron 9 poly(A) signals, and hERG1a (HG1a) and hERG1a$_{USO}$ (HG1a$_{USO}$) cDNAs. Untransfected control (Unt). Cell lysates were subjected to SDS-PAGE and immunoblotted with an anti-hERG1 antibody against the N-terminus of hERG1 protein. The expression level of hygromycin B phosphotransferase (HPH) encoded by hygromycin B resistant gene in pcDNA5 vector served as a loading control. Results shown are representative of four independent experiments. B, Flp-In HEK293 cells were stably transfected with minigenes carrying the wild-type (WT), 5' splice site mutation +4U>A/+6G>U (+4+6), canonical (Can) intron 9 poly(A) signal and +4U>A/+6G>U plus canonical poly(A) signal mutation (+4+6/Can). Results shown are representative of three independent experiments.