**Supplementary Figure 1. Absence of functional expression upon injection of separate N- and C-terminal HERG demi-channels.** Representative currents obtained in 50 mM extracellular K^+ with the protocol shown at the top from uninjected oocytes (*top)* and from cells injected with cRNA encoding the N- (*Demi 1-145 hERG)* and C-terminal (*Demi 546-1159 hERG)* halves of HERG. A current trace from an oocyte expressing Y545 Split HERG is shown for comparison at the *bottom*.
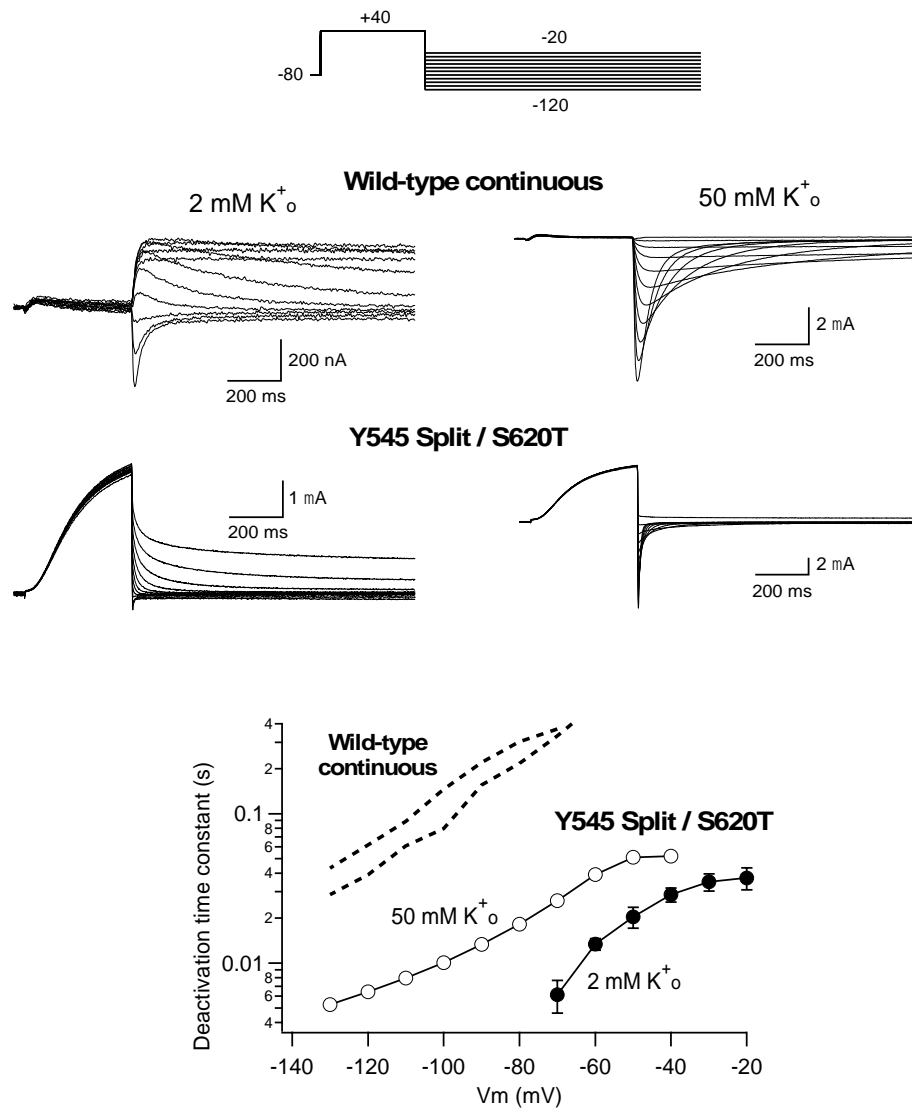
Supplementary Figure 2. Comparison of deactivation properties in wild-type continuous and and Y545 Split HERG channels carrying a S620T point mutation in the pore module that minimizes inactivation. Representative families of current traces obtained with the protocol shown are illustrated at the *top*. Extracellular solutions containing 2 (*left*) and 50 mM (*right*) K^+ were used as indicated. Plots of fast deactivation time constant values at different repolarisation voltages for splits in 2 (*closed circles*, n=4) and 50 mM external K^+ (*open circles*, n=4), and for wild-type non-split channels (*black* and *grey dashed lines*, respectively), are shown at the *bottom*.

Figure 2a: 1. Marker; 2. Continuous (total). 3 Continuous (biotinylated,30μL) 4. Split channel, biotinylated. 5. C Terminus, biotinylated. 6. Control; 7 Marker. The Figure shows only the lanes corresponding to pulled-down, biotinylated samples.
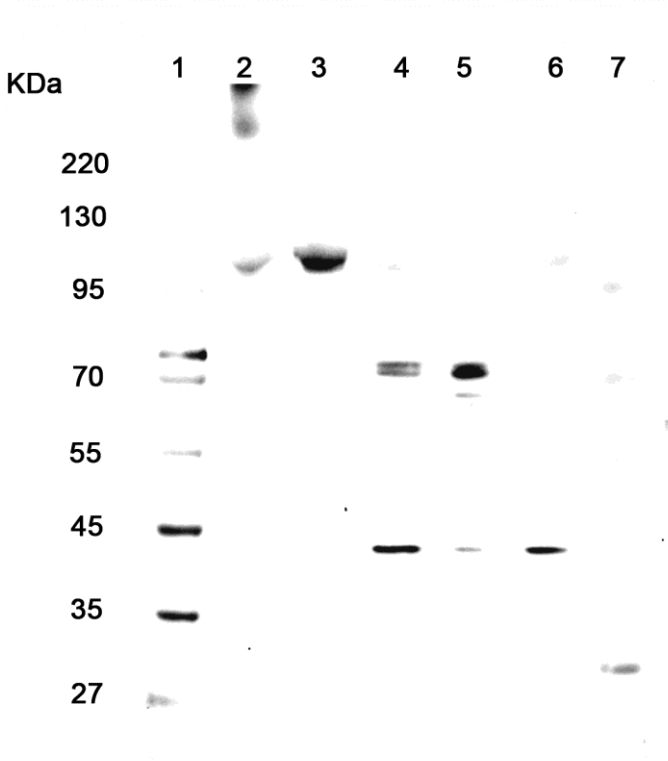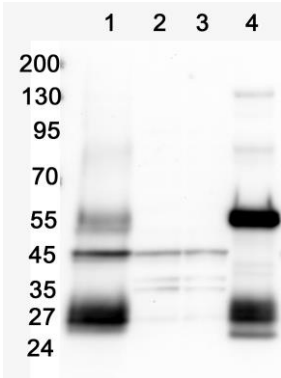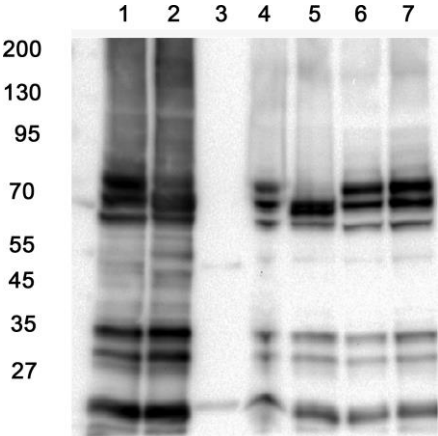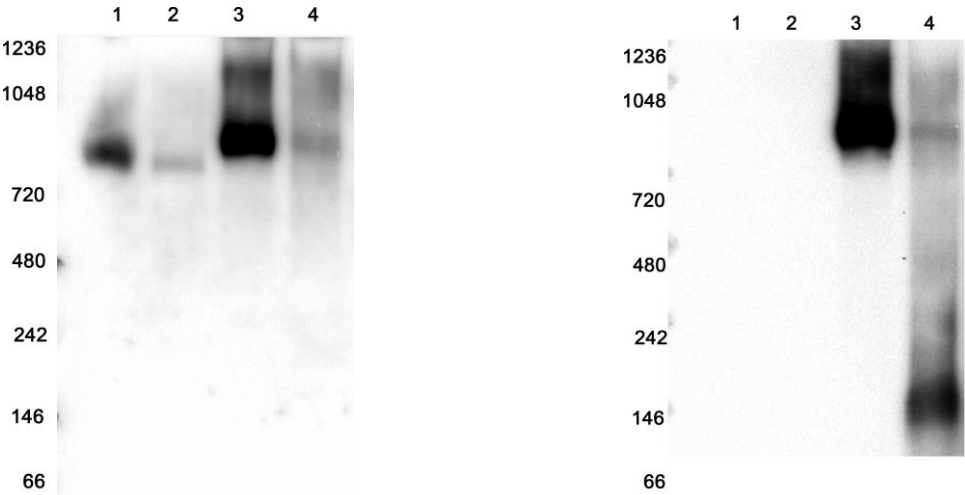

Figure 2b

Figure 2c

In the Figure, lanes 5-7 (eluates from IP, unbound protein) were omitted for clarity.

Figure 2d

In the Figure, lane 1 (continuous $K_V10.1$, 100 ng injection) was omitted for clarity.

Supplementary Figure 3. Images of uncropped blots

Supplementary Table I. Sequences of oligonucleotides

Channel	Purpose	Sequence (5'-3')
$K_V10.1$	Insertion of stop codon, *KpnI* site and start codon	ccactacattgaatattgaggtaccatgggagctgctgtgctggtcc
$K_V10.1$	Insertion of Stop codon, *Eag1* site and start codon	ccactacattgaatattgacggccgatgggagctgctgtgctggtcc
$K_V10.1$	Insertion of stop codon, *BamHI* site and start codon	ccactacattgaatattgaggatccatgggagctgctgtgctggtcc
$K_V10.1$	Deletion of residues from L341 of N-terminal demichannel	cgagtggcccgtaagctgtgagaggtctatttaaaa
$K_V10.1$	Deletion of residues up to A350 of C-terminal demichannel	tggcggccgatggctgtgctggtc
$K_V10.1$	5MycTagging, 1st template and overlapping PCR, forward	ggaccccgagctgctgggaggaaagctatggagcaaaagctc
$K_V10.1$	5MycTagging, 1st and 2nd template PCR, reverse	cggccggaattccgggcccgccggaccccgagctgctgggagg
$K_V10.1$	5MycTagging, 2nd template PCR, forward	cctgcccccagccatggtcatgtcgcccaagctctccatttc
$K_V10.1$	5MycTagging, 3rd template PCR, forward	gaaatggagagcttgggcgacatgaccatggctgggggcagg
$K_V10.1$	5MycTagging, 3rd template and overlapping PCR, reverse	gaatcatcctcaattggctgtttgaaagct
$K_V10.1$	HA tagging, 1st HA forward	caactagttgtacagatcaagcgggatgccctgc
$K_V10.1$	HA tagging, 1st HA reverse	cgcataatctgggacgtcataagggtagctggctccaaaaatgtctct
$K_V10.1$	HA tagging, 2nd HA forward	caactagttgtacagatcaagcgggatgccctgc
$K_V10.1$	HA tagging, 2nd HA forward	atggatccctctagattacgcataatccggaacgtcataagggtatcccgcataatctgggacgtcataagg
$K_V10.1$	Synthetic HA tags 3 and 4, sense	ccggtacgtcataagggtatcccgcataatctggaacgtcataagggtatcccgcataat
$K_V10.1$	Synthetic HA tags 3 and 4, antisense	ccggattatgcgggatacccttatgacgttccagattatgcgggataccttatgacgta
$K_V10.1$	Subcloning 4HA into pSGEM, fragment 1 and overlapping PCR, forward	aaggaggacatcaaggccttaaacgccaaa
$K_V10.1$	Subcloning 4HA into pSGEM, fragment 1reverse	ttttaaatagacctctcacgcataatccgg
$K_V10.1$	Subcloning 4HA into pSGEM, fragment 2, forward	ccggattatgcgtgagaggtctatttaaaa
$K_V10.1$	Subcloning 4HA into pSGEM, fragment 2 and overlapping PCR, reverse	ccctcgaggtcgacggtatcgataagcttg
$K_V10.1$	Mutant G440S	caccagtgtgggcttttcgaacatcgccccatc
$K_V10.2$	Insertion of stop codon, *Eag1* site and start codon	ccattacctagaatattgacggccgatgggagcagcagtcctcgtgctcc
$K_V10.2$	Insertion of stop codon, *HindIII* site and start codon	ccattacctagaatattgaaagcttatgggagcagcagtcctcgtgctcc
$K_V10.1$	Mutant R336Q	tccgtcttgggcaagtggcgcgcaagctggaccac
$K_V11.1$	Mutant G628S	ccagtgtgggcttcagcaacgtctctccc
$K_V11.1$	Synthesis N-terminal demi-channel, forward	ggaagctttcaggatgccggtgcggaggggccacgtc
$K_V11.1$	Synthesis N-terminal demi-channel, reverse	ggggatcctagtactctgagtagcgatccagcttccgc
$K_V11.1$	Synthesis C-terminal demi-channel, forward	ggaagctttcaggatgggcgcggccgtgctgttcttgctcatgtgcacc
$K_V11.1$	Synthesis C-terminal demi-channel, reverse	ggggatcctaactgcccgggtccgagccgtgtctgtgcaggggg