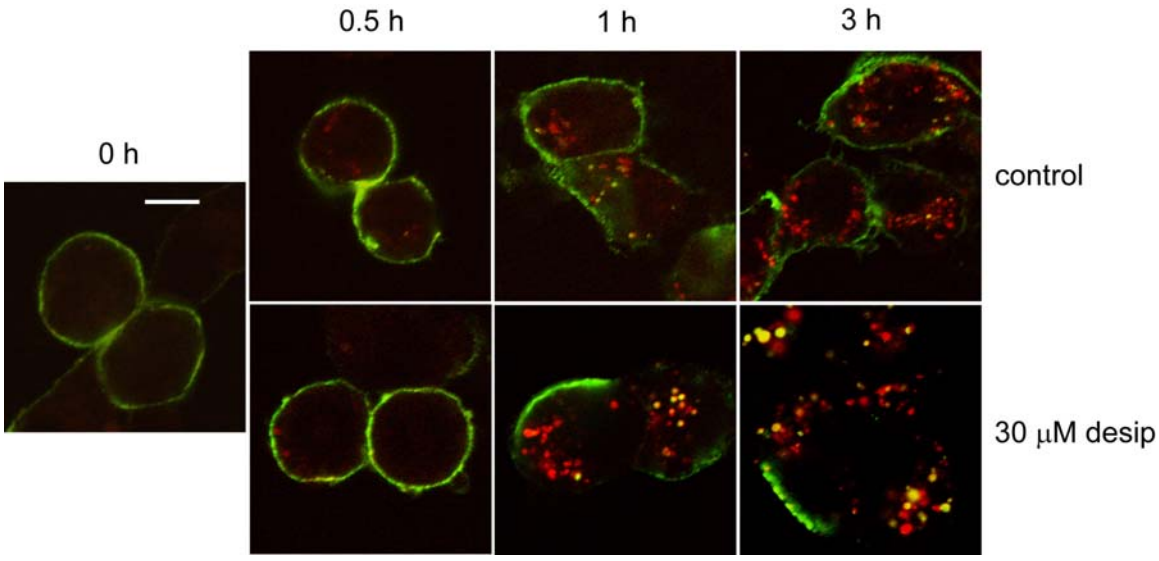
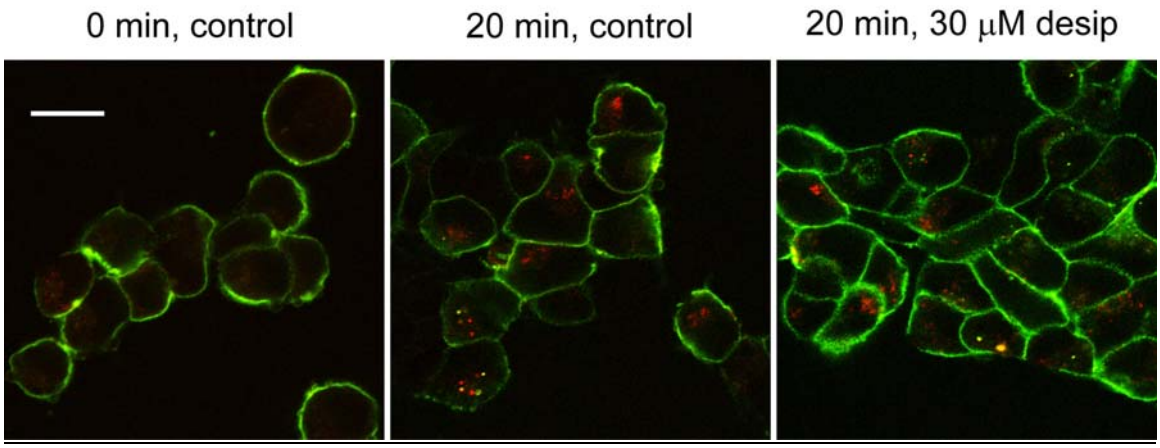
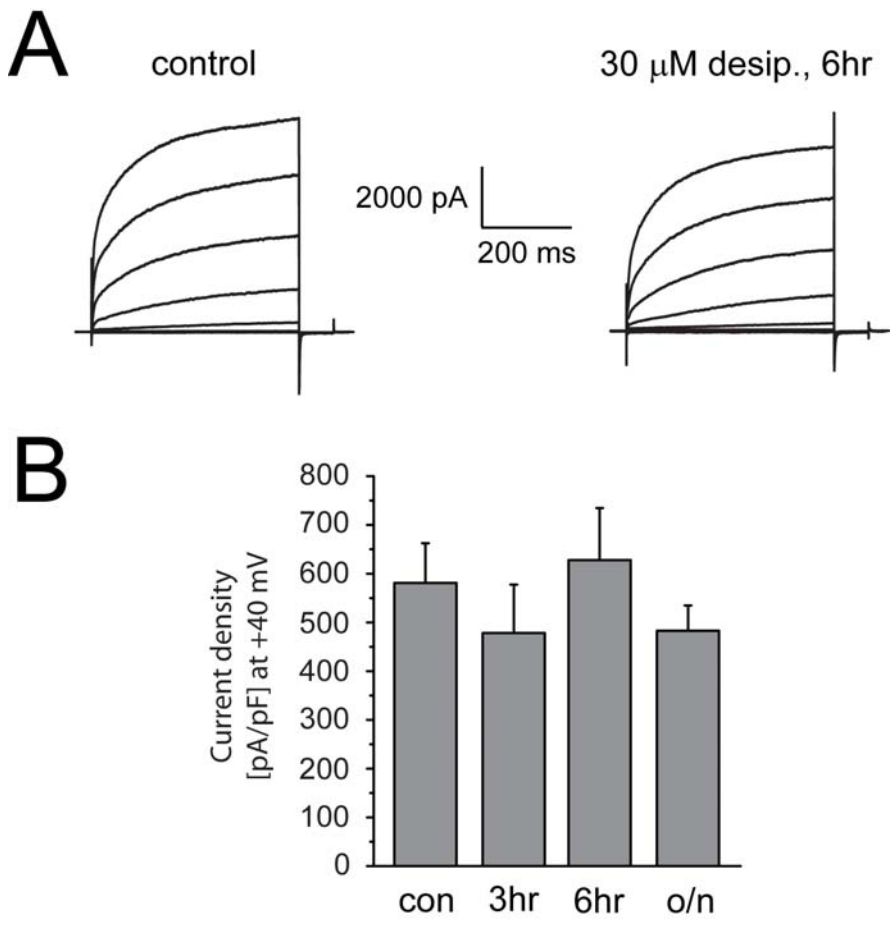


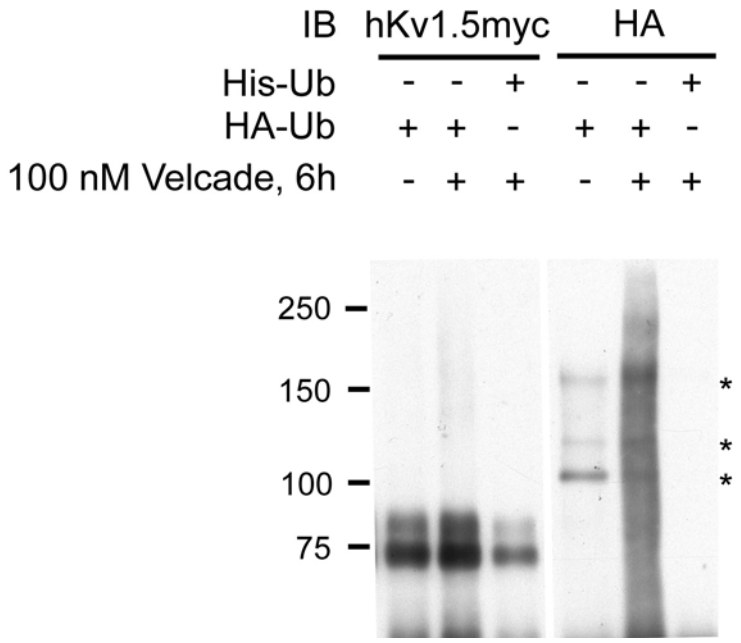
Supplemental Figure 1. Desipramine increases internalization of cell surface hERG channels. Stably transfected hERG-HA_{ex} cells were pre-labeled with anti-HA antibody and incubated for 0.5, 1 and 3h either under control conditions or in the presence of 30 μM desipramine. Following fixation, HEK/hERG-HAex cells were first stained with Alexa Fluor 488-conjugated secondary antibody to label cell surface hERG (green) prior to permeabilization. Next, cells were permeabilized and stained with RedX-conjugated secondary antibody to label internalized hERG (red) in one and the same preparation. Shown are confocal images acquired under control conditions (top row) or following incubation with 30 μM desipramine (bottom row). Note that hERG surface labeling is dramatically reduced in the presence of desipramine. Scale bar: 10 μm.



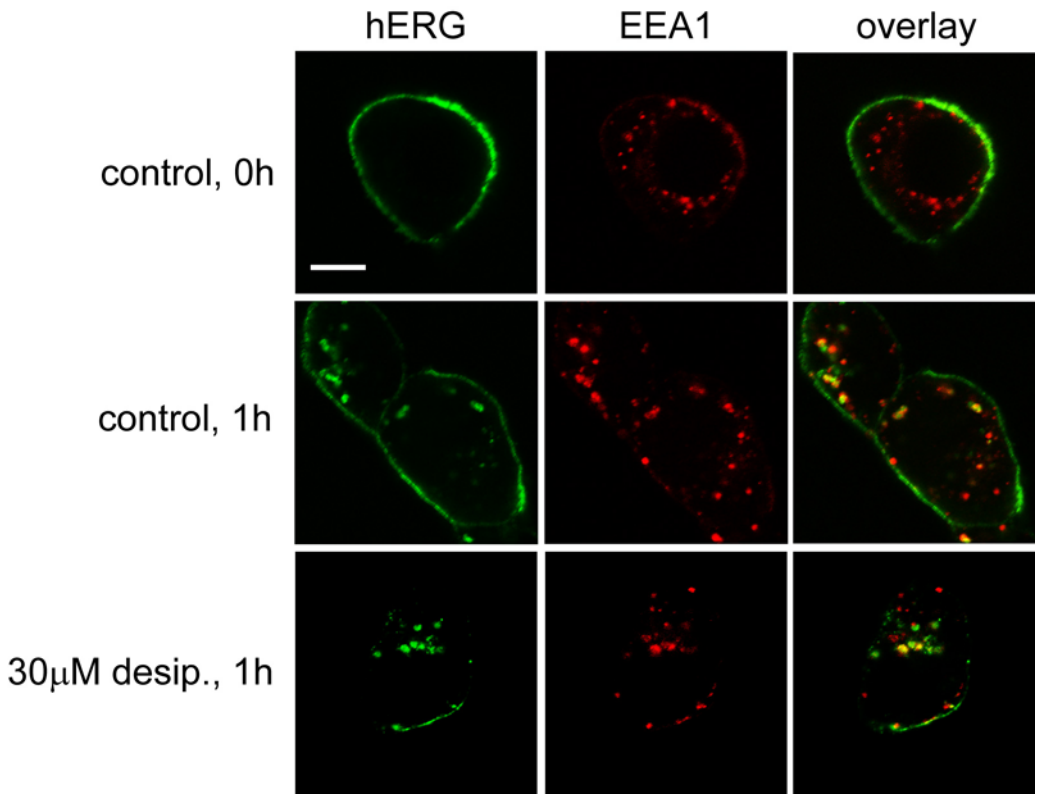
Supplemental Figure 2. Desipramine does not inhibit hKv1.5 internalization. Stably transfected HEK/hKv1.5-HA_{ex} cells were pre-labeled with anti-HA antibody and incubated for 20 min either under control conditions or in the presence of 30 μM desipramine. Following fixation, HEK/hKv1.5 cells were first incubated with Alexa Fluor 488-conjugated secondary antibody to label cell surface hKv1.5 (green) prior to permeabilization. Next, cells were permeabilized and stained with RedX-conjugated secondary antibody to label internalized hKv.15 (red) in one and the same preparation. Shown are confocal images acquired under control conditions, or following incubation with 30 μM desipramine for 20min. Scale bar: 20 μm.



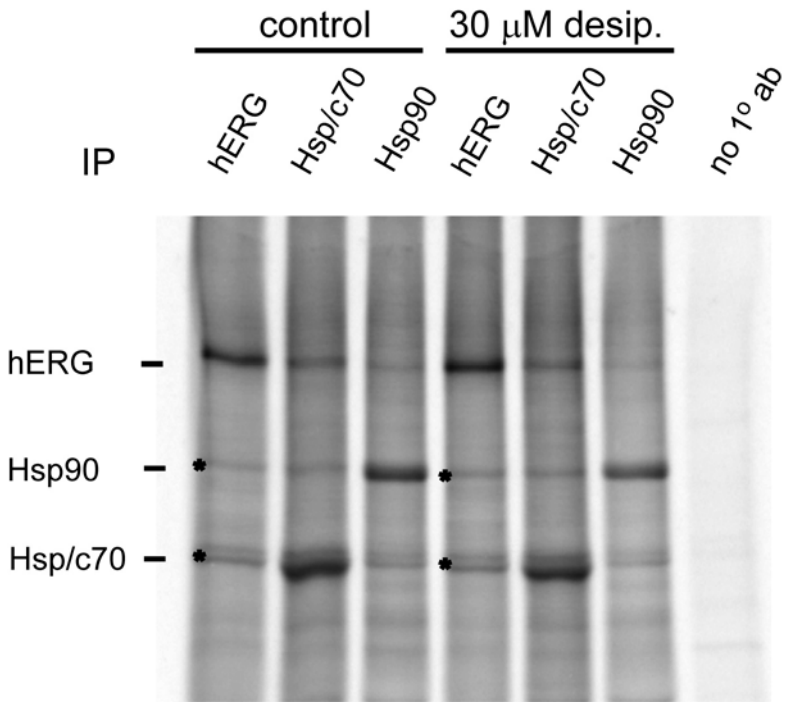
Supplemental Figure 3. Desipramine does not alter bEAG surface expression. A, representative bEAG current families recorded under control conditions or following 6 h exposure to 30 μM desipramine. Currents were elicited using depolarizing voltage steps from -60 to +40 mV. Tail currents were recorded on return to -100 mV. Holding potential was -80 mV. B, quantitative analysis of bEAG current densities measured under control conditions or following incubation with 30 μM desipramine for 3 and 6 h, or overnight. Data are presented as mean ± S.E.



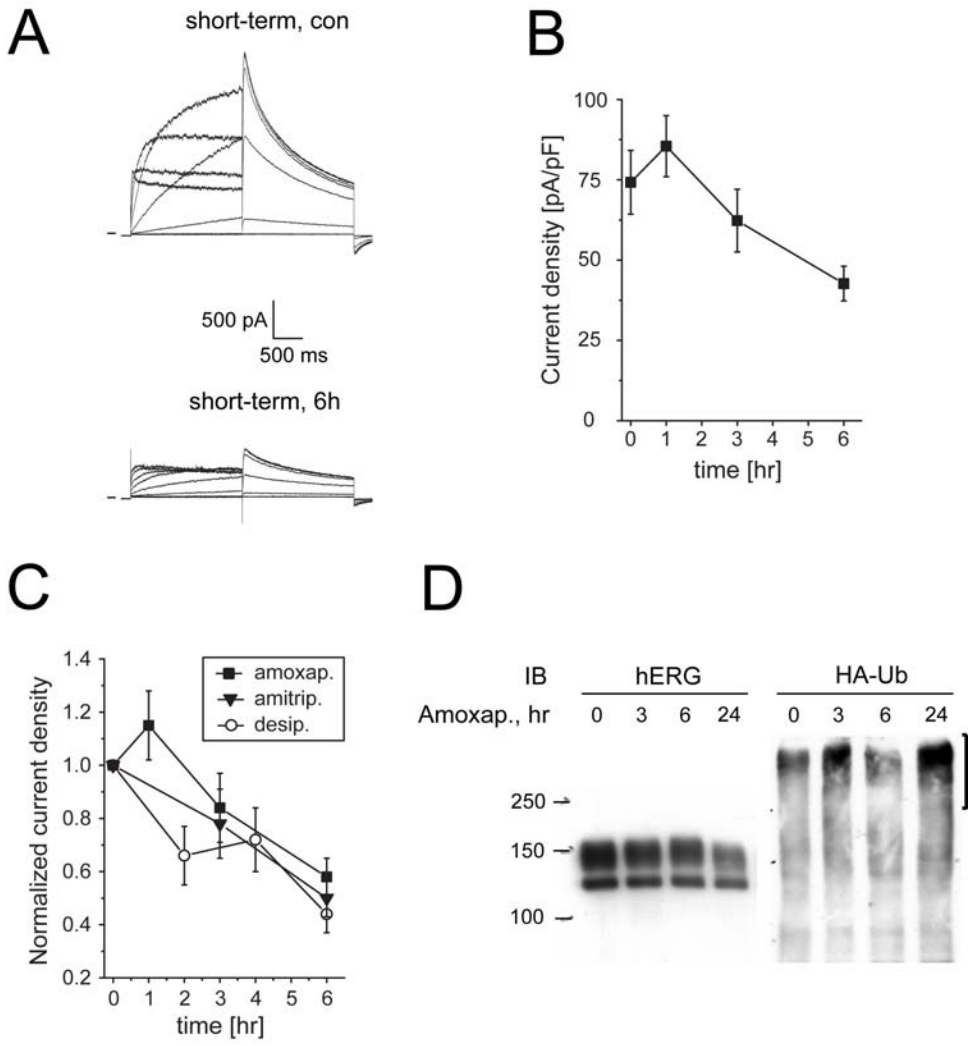
Supplemental Figure 4. Western analysis of HEK cells co-transfected with hKv1.5myc and either HA- or HIS_6-tagged ubiquitin. Shown are immunoprecipitations of hKv1.5myc protein with anti-myc antibody under control conditions or following treatment with 100nM of the proteasomal inhibitor velcade/bortezomib for 6h. Samples were analyzed using anti-HA antibody to identify ubiquitinated forms of hKv1.5myc. Transfection with HIS_8-ubiquitin was used as negative control. Asterisks to the right of panel indicate non-specifically stained protein bands. Note that velcade/bortezominb increases HA-Ub staining.



Supplemental Figure 5. Colocalization of hERG channels with EAA1, a marker for early endosomes. HEK/hERG-HA_{ex} cells were pre-labeled with anti-HA antibody and incubated for 1h either under control conditions or in the presence of 30 μM desipramine. Cells were fixed, permeabilized and double stained with anti-hERG and anti-EEA1 antibody. Shown are confocal images. Scale bar: 10 μm.



Supplemental Figure 6. Desipramine does not interfere with Hsp90 function**.** Shown is an experiment where HEK/hERG cells were radioloabeled, lysed and immunoprecipitated with anti-hERG, anti-Hsp/c70 and anti-Hsp90 antibody either under control conditions or following overnight exposure to 30 μM desipramine. All sample were cross-linked prior to immunoprecipitation with cell-permeable dithiobis(succinimidyl propionate), DSP. Right most lane represents a negative control with no primary antibody added. To the left of the panel the positions of cg hERG, Hsp90 and Hsp/c70 on the autoradiogram are indicated. Note that hERG associates with both Hsp90 and Hsp70 irrespective of desipramine (black dots).



Supplemental Figure 7. Amoxapine increases hERG ubiquitination. A, representative hERG current families recorded under control conditions or following a 6 hr exposure to 30 μM amoxapine. Currents were elicited using depolarizing voltage steps from -60 to +60 mV. Tail currents were recorded on return to -50 mV. Holding potential was -80 mV. B, time-dependent reduction of hERG tail current densities recorded on exposure to 30 μM amoxapine (n=8-9). C, normalized time-dependent changes in tail current levels recorded in the presence of 30 μM desipramine, 30 μM amitriptyline or 30 μM amoxapine. D, Western blot analysis of HEK/hERG cells transiently transfected with HA-tagged ubiquitin and treated for 3 and 6h, or overnight (24h) with 30 μM amoxapine. Shown are immunoprecipitations of hERG under control conditions or

following treatment with 30 μM amoxapine for 3, 6h or overnight (24h). Samples were analyzed using anti-HA antibody to identify putative high molecular weight forms of ubiquitinated hERG. Immunoblotting with anti-HA antibody identifies an increase of ubiquitinated hERG following 3 and 24h exposure to 30 μM amoxapine. Note that 6h exposure to amoxapine did not affect channel ubiquitination in two independent experiments. It is currently not clear why there is a complex time-dependence observed with amoxapine.