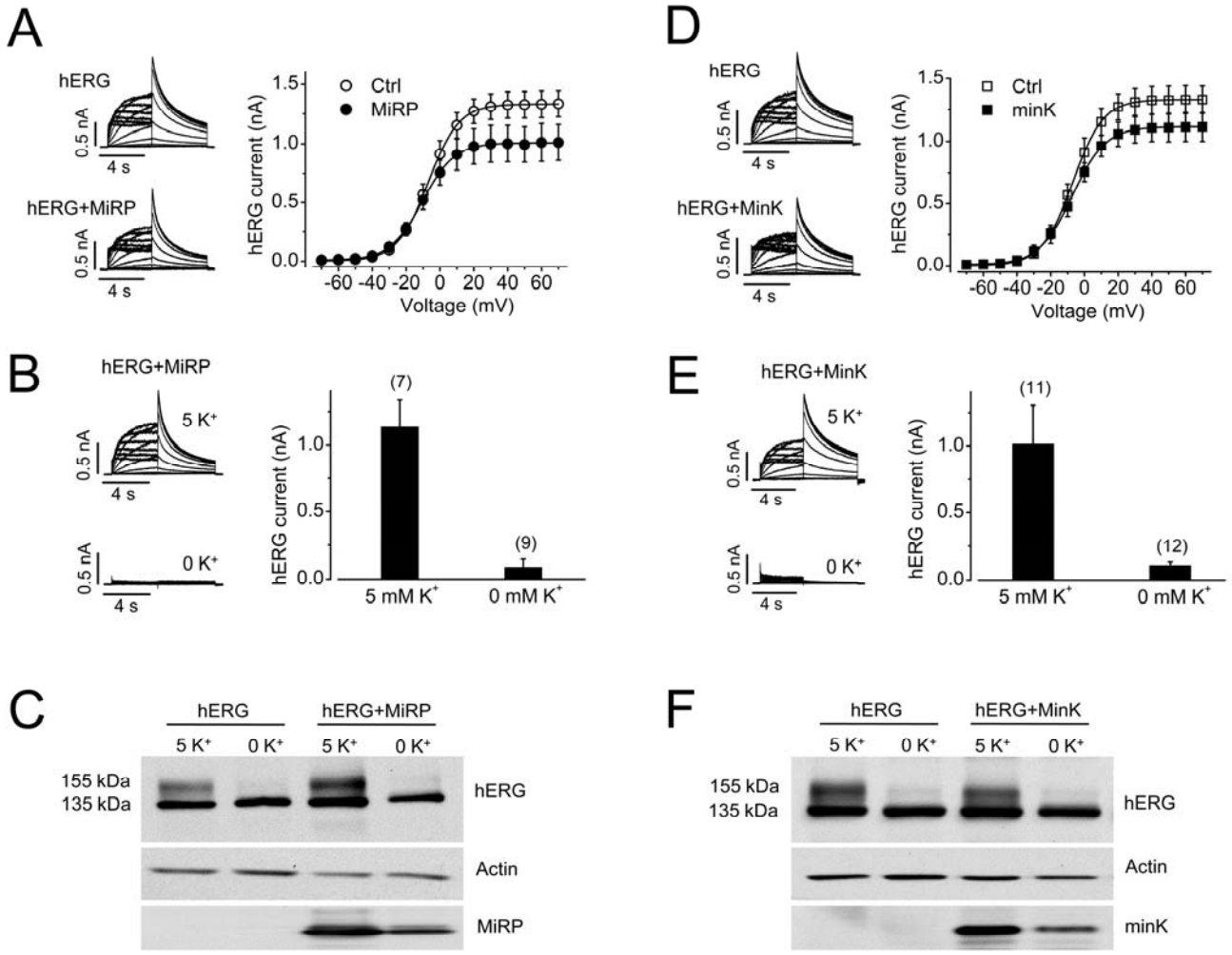
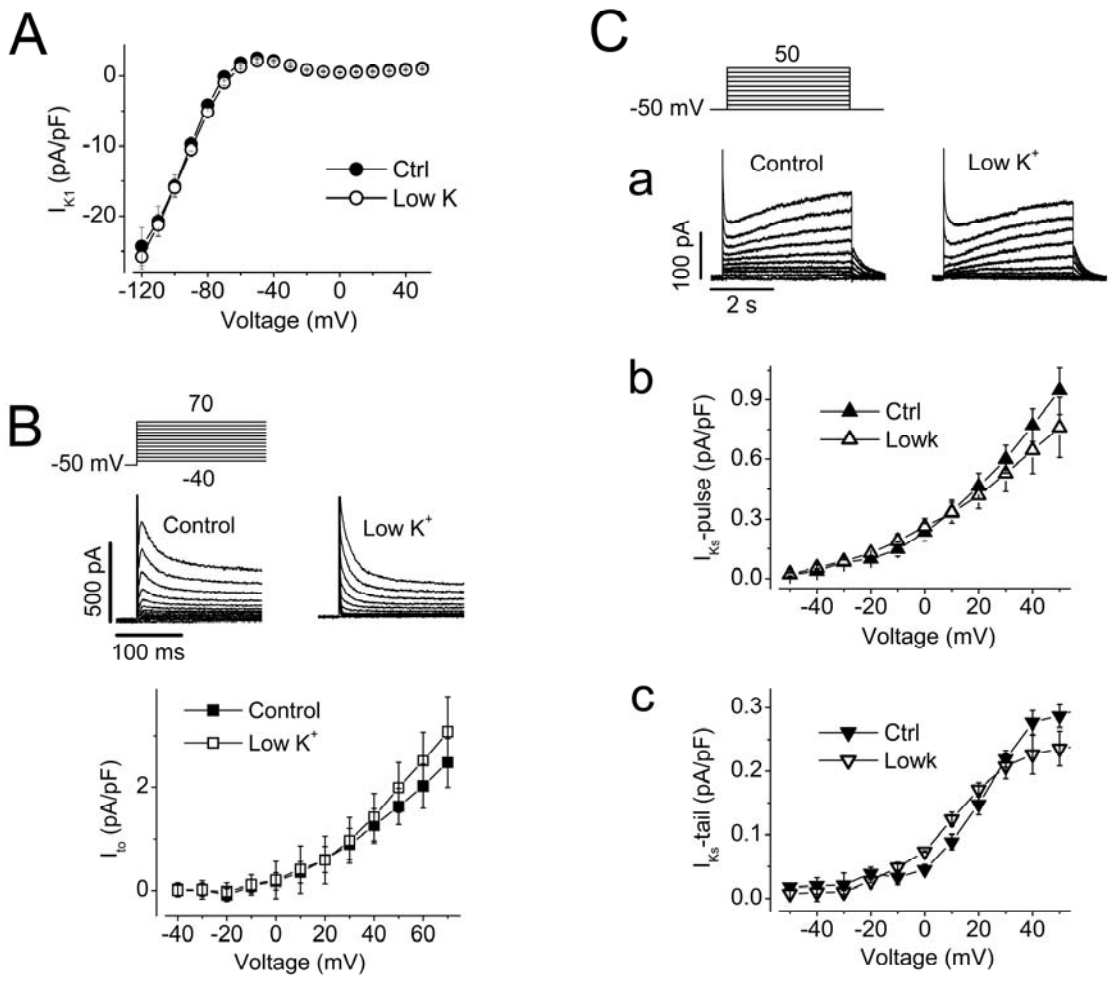
Supplemental Figure 1



Supplemental Figure 1. Co-expressing MiRP or minK does not affect 0 K^+-induced reduction of I_{hERG} and the 155-kDa band of hERG protein. (A) Activation curve of the hERG current with or without MiRP co-expression. The voltages for half maximal activation ($V_{1/2}$) and the slope factor (k) of hERG channels were −10.5±1.7 mV and 9.4±1.6 mV in hERG-HEK cells co-expressing MiRP (n=12), and they were −7.1±0.7 mV and 9.1±0.7 mV in hERG-HEK cells without MiRP co-expression (n=15). The $V_{1/2}$ and k were not different between two groups ($P > 0.05$). However, the amplitude of the hERG tail current in hERG-HEK cells with MiRP co-expression was slightly smaller than that without MiRP co-expression ($P < 0.05$, n=12 or 15). **(B)** hERG currents in hERG-HEK cells with MiRP co-expression under 5 or 0 mM K^+ MEM culture for 12 h. The averaged I_{hERG} was 1.14 ± 0.2 nA (n=7) under 5 mM K^+ MEM culture and was 0.09 ± 0.06 nA (n=9, P <0.001)

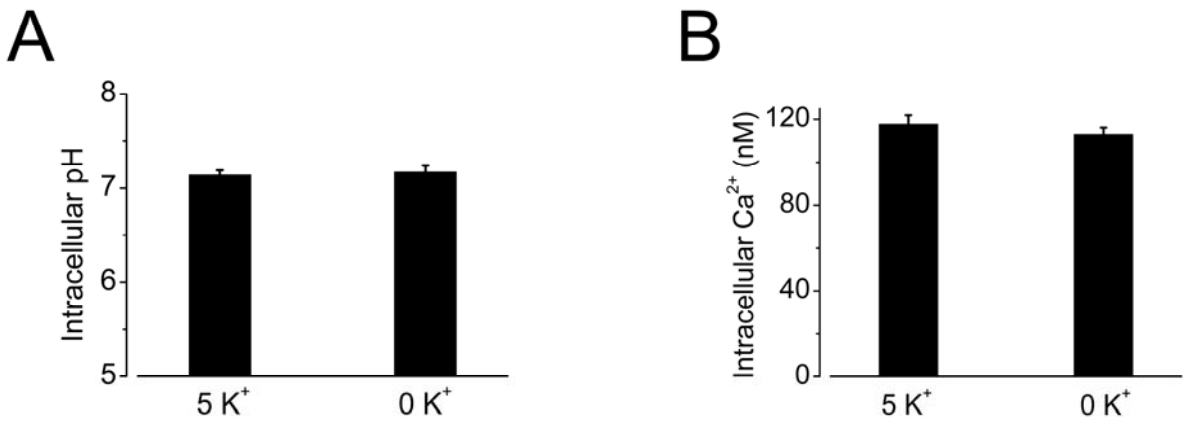
under 0 mM K^+ MEM culture. **(C)** hERG protein expression levels in hERG-HEK cells without or with MiRP co-expression under 5 or 0 mM K^+ culture for 12 h. Expression of MiRP in hERG-HEK cells was evident by the presence of the 25-kDa MiRP band in the MiRP-transfected hERG-HEK cells. Exposure of cells to 0 K^+ also reduced the MiRP expression levels (n = 5). **(D)** Activation curve of the hERG currents with or without minK co-expression. The $V_{1/2}$ and k of hERG channels with minK co-expression were −7.3±1.7 mV and 9.8±1.5 mV (n=9), and they were −7.1±0.7 mV and 9.1±0.7 mV in hERG-HEK cells without minK co-expression (n=15). The $V_{1/2}$ and k as well as tail current amplitude of hERG currents were not different between two groups ($P > 0.05$). **(E)** hERG currents of hERG-HEK cells with minK co-expression under 5 or 0 mM K^+ MEM culture for 12 h. The averaged I_{hERG} was 1.11 ± 0.3 nA (n=11) under 5 mM K^+ MEM culture and was 0.11 ± 0.02 nA under 0 K^+ MEM culture (n=12, $P < 0.001$). **(F)** hERG expression levels in hERG-HEK cells without or with minK co-expression under 5 or 0 mM K^+ culture for 12 h. Expression of minK in hERG-HEK cells was evident by the presence of the 25-kDa minK band in minK-transfected hERG-HEK cells. Exposure of cells to 0 K^+ reduced the minK expression levels as well (n = 6).

Supplemental Figure 2



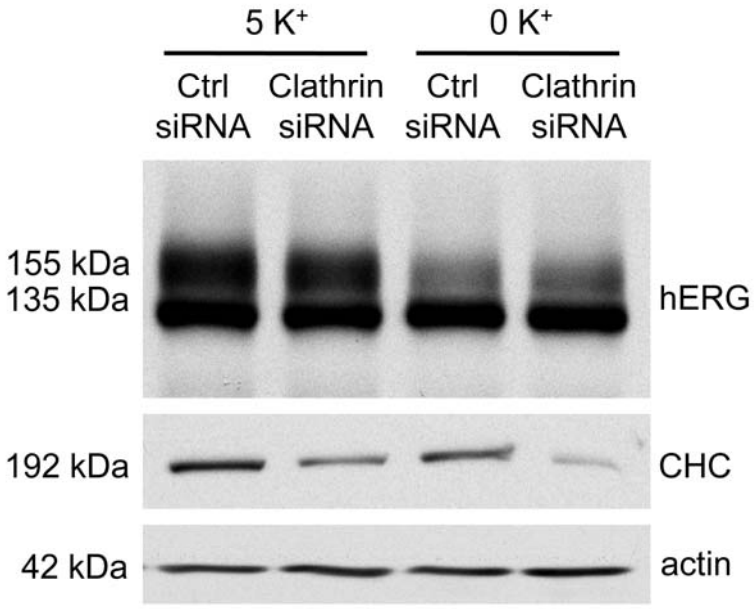
Supplemental Figure 2. Effects of hypokalemia on I_{K1}, I_{to}, and I_{Ks} recorded from isolated rabbit ventricular myocytes. After 4 weeks of feeding with either control or low K^+ diet, ventricular myocytes were isolated. Various currents were compared between control and low K^+ diet groups. **(A)** Current-voltage relationships of I_{K1} in control (n=12) and hypokalemic rabbits (n=11). **(B)** Representative I_{to} traces and the summarized peak currents of I_{to} at various depolarizing voltages. I_{to} was not significantly different between control (n=8) and low K^+ diet rabbits (n=8). **(C)** Representative I_{Ks} currents (**a**), summarized I_{Ks} pulse currents during the end of 4-s depolarizing steps (**b**) and I_{Ks} tail currents upon repolarizing step to -50 mV (**c**). Although I_{Ks} was slightly decreased in ventricular myocytes of low K^+ diet rabbits, this change did not reach statistical significance compared to that from control rabbits (n=7 in control, n=12 in low K^+ diet group). n represents the number of ventricular myocytes tested from at least three rabbits in each group.

Supplemental Figure 3



Supplemental Figure 3. Exposure of hERG-HEK cells to 0 mM K^+ MEM for 4 h did not alter either intracellular pH or intracellular Ca^{2+} concentration. (A) Intracellular pH of hERG-HEK cells cultured in 5 or 0 mM K^+ MEM for 4 h. There was no difference in intracellular pH between the two groups (7.14 ± 0.05 for 5 mM K^+ and 7.17 ± 0.07 for 0 K^+, n=2 independent flow cytometry measurements). **(B)** Intracellular Ca^{2+} concentrations of hERG-HEK cells cultured in 5 or 0 mM K^+ MEM for 4 h. There was no difference in intracellular Ca^{2+} concentrations between the two groups (117.7 ± 4.2 nM for 5 mM K^+, n=78 cells, and 113.0 ± 3.1 nM for 0 K^+, n=64 cells in two independent treatments).

Supplemental Figure 4



Supplemental Figure 4. Clathrin knockdown did not alter 0 K^+-induced reduction of the 155-kDa hERG expression. Knockdown of clathrin did not significantly affect hERG expression levels in either 5 or 0 mM K^+ culture (n=8). Clathrin siRNA (200 nM) or scrambled (control) siRNA (200 nM, Santa Cruz) were transfected in stable hERG-expressing HEK 293 cells. Thirty six hours after transfection, the cell culture media were changed to either 5 or 0 mM K^+ MEM, and the whole cell lysates were extracted 6 h after the cells were exposed to 5 or 0 mM K^+ MEM. Knockdown of clathrin by its siRNA transfection was indicated by the reduced expression levels of the clathrin heavy chain (CHC).

Supplemental Methods

Intracellular pH (pHi) measurement: For each experiment the fluorescent intensity of pHi from $2.5x10^4$ cells were measured using Cytomic FC500 flow cytometer (Beckman Coulter) with a pH sensitive dye SNARF-1 (Invitrogen). Details of this method have been described previously by Wieder *et al.* (Wieder ED, Hang H, and Fox MH. 1993. Measurement of intracellular pH using flow cytometry with carboxy-SNARF-1. *Cytometry* 14:916-921). Briefly, cells cultured in 5 mM K^+ or 0 K^+ MEM were loaded with 3 M SNARF-1-AM for 30 min at 37 °C. Cells were then washed with 5 or 0 mM K^+ solution and placed in 37 °C for 15 min to permit de-esterification of SNARF-1-AM. After loading, the cells were trypsinized and re-suspended in 5 mM K^+ or 0 K^+ solution for flow cytometry analysis. SNARF-1 was excited with argon ion laser at 514 nm and its emission was recorded 575 nm and 675 nm. The emission ratio was used for generation of pH standard curve and intracellular pH calculation. SNARF-1 calibration curve was performed by incubating cells in a high K^+ solution which contained (in mM) 130 KCl, 20 NaCl, 5 dextrose, 1 $CaCl_2$, 1 KH_2PO_4, 0.5 $MgSO_4$, and 10 HEPES with different pH in the presence of 5 M nigericin (K^+/H^+ ionophore, Sigma) to equilibrate the intracellular with extracellular pH.

Intracellular calcium measurement: Intracellular calcium (Ca^{2+}_i) levels in hERG-HEK cells were measured using an InCyt dual-wavelength imaging system (Intracellular Imaging, Cincinnati, OH) with a calcium indicator Fura-2 (Invitrogen). Briefly, cells were grown overnight on glass bottom FluoroDish (World Precision Instrument). After incubation of cells in either 5 mM K^+ or 0 K^+ MEM for 6 h, cells were loaded with 3 M Fura-2-AM for 30 min at 37°C under either 5 mM K^+ or 0 K^+ MEM. Cells were then washed with 5 mM K^+ or 0 K^+ MEM and placed in 37°C for 15 min to permit de-esterification of Fura-2-AM. Intracellular calcium was measured by exciting the cells with 340 and 380 nm wave length and recording the emission at 505 nm. The system was calibrated with a known calcium standard prior to calcium measurements.

Supplemental Table 1: Compositions of solutions (in mM) for recording various ionic currents and action potentials from rabbit ventricular myocytes. The pH of the bath solutions was 7.4, and the pH of the pipette solutions was 7.2, adjusted using appropriate hydroxide salts or HCl.

I_{Ba}	Bath:	140 TEACl, 5.4 $BaCl_2$, 1 $MgCl_2$, 10 glucose, 10 HEPES
	Pipette:	135 CsCl, 10 EGTA, 1 $MgCl_2$, 5 MgATP, 10 HEPES
I_{Kr-K}	Bath:	5 KCl, 130 NaCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 glucose,10 HEPES
	Pipette:	135 KCl, 10 EGTA, 1 $MgCl_2$, 5 MgATP, 10 HEPES
I_{Kr-Cs}	Bath:	135 CsCl, 1 $MgCl_2$, 10 glucose, 10 HEPES, 10 μM Nifedipine
	Pipette:	135 CsCl, 10 EGTA, 1 $MgCl_2$, 5 MgATP, 10 HEPES
I_{K1}	Bath:	5.4 KCl, 130 NMG, 1 $MgCl_2$, 2 $CaCl_2$, 10 glucose, 10 HEPES, 1 M Nifedipine
	Pipette:	135 KCl, 10 EGTA, 1 $MgCl_2$, 5 MgATP, 10 HEPES
I_{to}, I_{Ks}	Bath:	140 NMG, 1 $MgCl_2$, 10 glucose, 10 HEPES, 5 M E4031
	Pipette:	135 KCl, 10 EGTA, 1 $MgCl_2$, 5 MgATP, 10 HEPES
AP	Bath:	5 KCl, 130 NaCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 glucose, 10 HEPES
	Pipette:	135 KCl, 10 EGTA, 1 $MgCl_2$, 5 MgATP, 10 HEPES

AP: Action potential; NMG: N-methyl-D-glucamine.