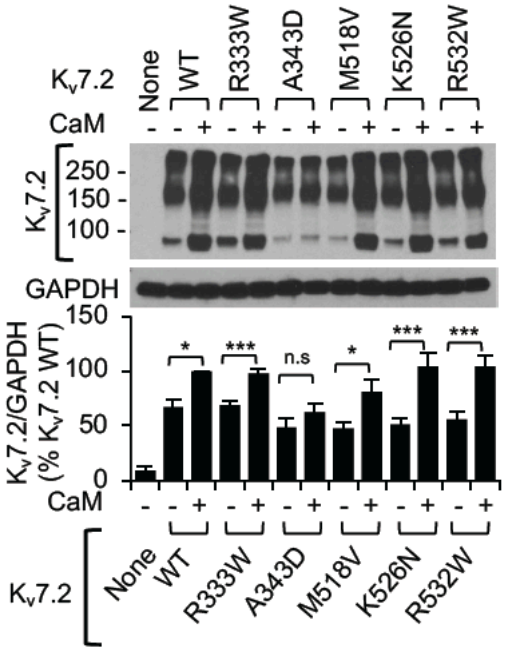
SUPPLEMENTAL INFORMATION

TITLE: Reduced axonal surface expression and phosphoinositide sensitivity in K_v7 channels disrupts their function to inhibit neuronal excitability in *Kcnq2* epileptic encephalopathy.

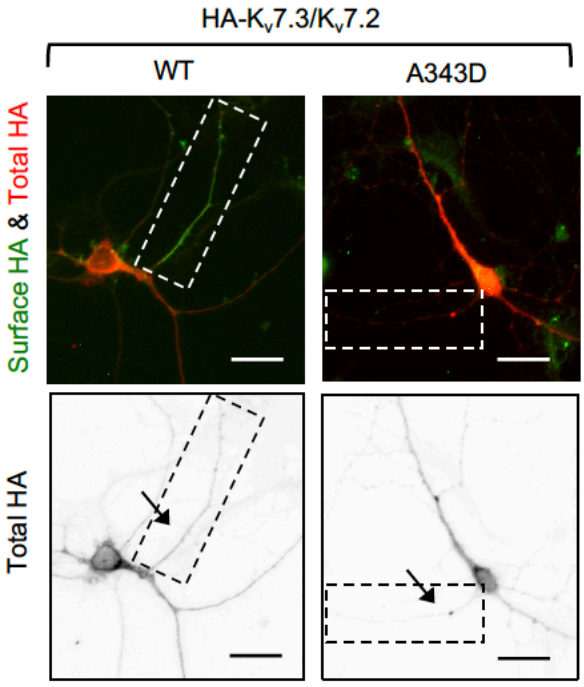
AUTHOR: Eung Chang Kim[1], Jiaren Zhang[1], Weilun Pang[1,*], Shuwei Wang[1,*], Kwan Young Lee[1], John P. Cavaretta[1], Jennifer Walters[1], Erik Procko[2,3], Nien-Pei Tsai[1,3], and Hee Jung Chung[1,3]
[1]Department of Molecular and Integrative Physiology, [2]Department of Biochemistry, [3]Neuroscience Program, and University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, USA.
*These authors contributed equally.

CORRESPONDING AUTHOR: Hee Jung Chung
Department of Molecular and Integrative Physiology,
University of Illinois at Urbana-Champaign,
407 South Goodwin Avenue, 524 Burrill Hall, Urbana, IL 61801, USA.
Telephone number: (217) 244-6839
Email: chunghj@life.illinois.edu; chunghj@illinois.edu

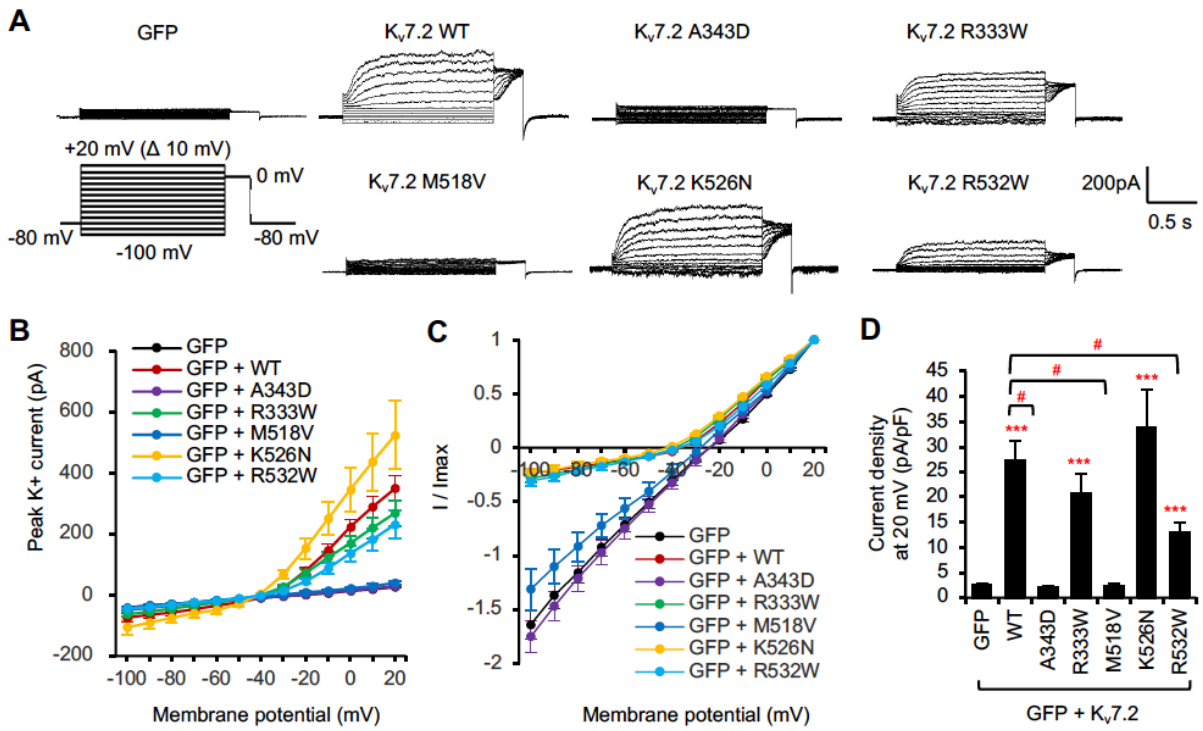
SUPPLEMENTAL FIGURES



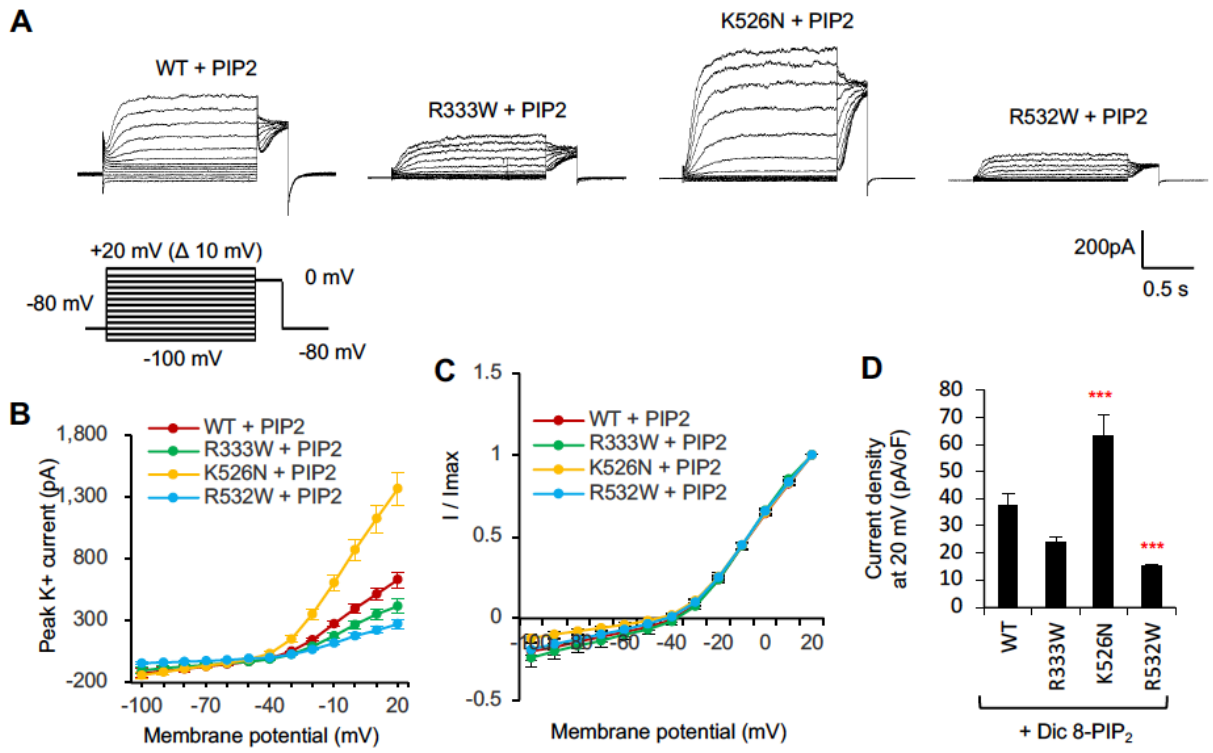
Supplemental Figure 1. Effect of calmodulin on K_v7.2 expression in HEK293T cells. HEK293T cells were transfected with untagged calmodulin (CaM) and wild type K_v7.2 (WT) or K_v7.2 containing epileptic encephalopathy mutations. Immunoblots of cell lysate was performed and quantified (n=4 independent experiments). GAPDH was used as a loading control. Data shown represent the Ave ± SEM (*p<0.05, ***p<0.005). n.s. denotes "not significant".



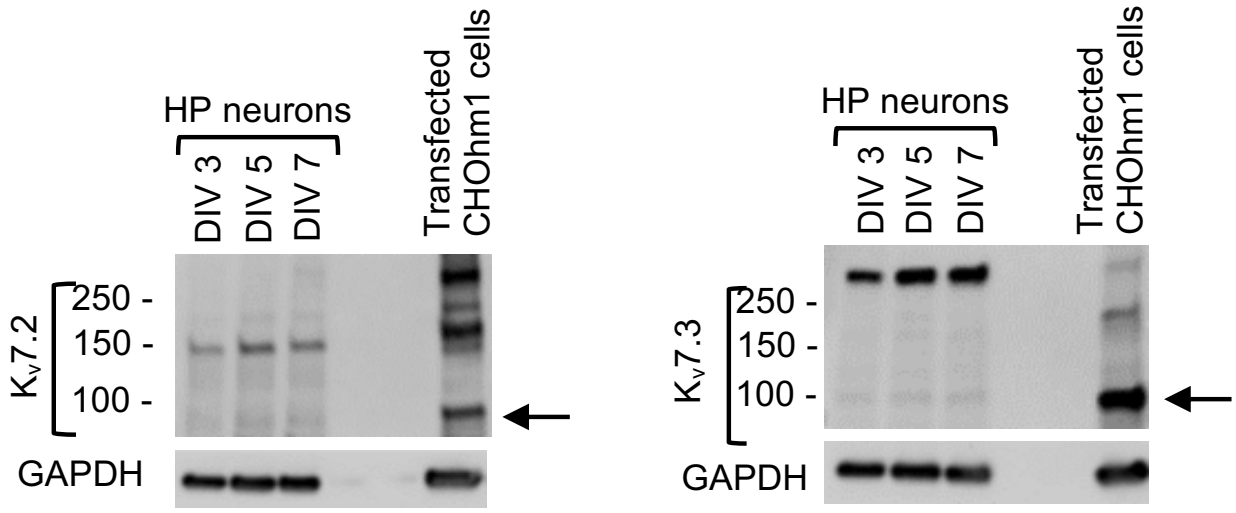
Supplemental Figure 2. The A343D mutation blocks axonal expression of heteromeric K_v7 channels in cultured hippocampal neurons. Immunocytochemistry showing surface and total HA-$K_v7.3$ proteins in hippocampal neurons cotransfected with $K_v7.2$ WT or $K_v7.2$ containing A343D mutation in CaM contact site of helix A. The A343D mutation impairs HA-$K_v7.3$/ $K_v7.2$ channel expression in the axonal surface and intracellularly in the axon.



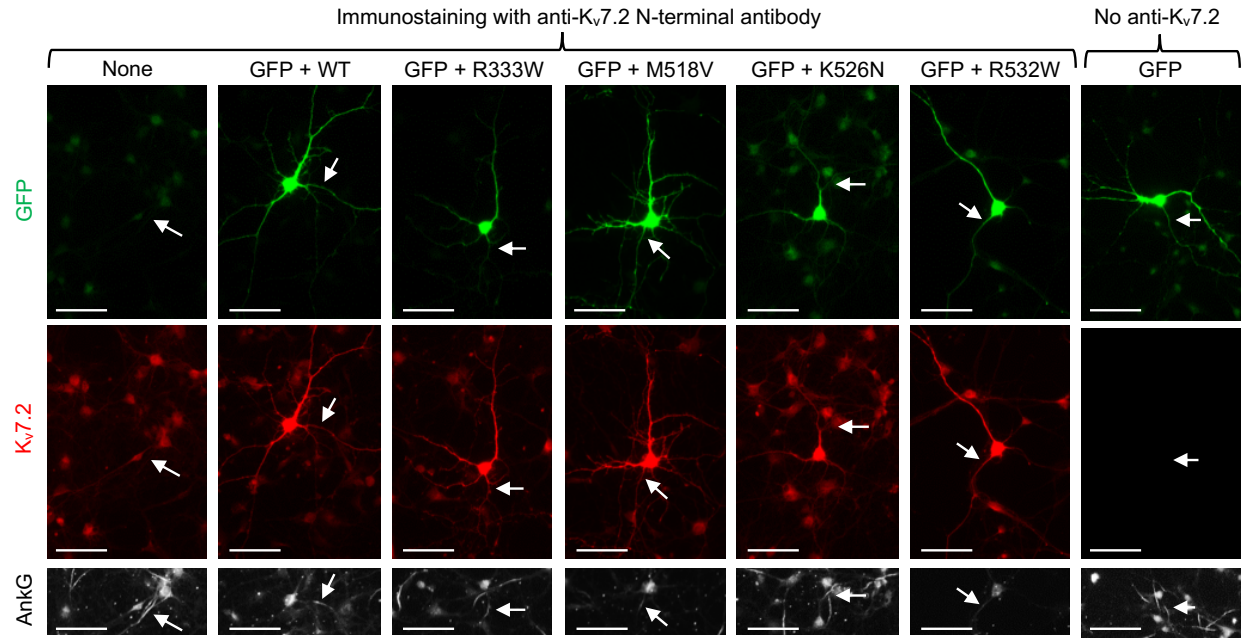
Supplemental Figure 3. Whole cell patch clamp recording of CHO hm1 cells transfected wild-type K_v7.2 or mutant K_v7.2 harboring epileptic encephalopathy mutations. Whole cell voltage clamp recording of macroscopic K^+ currents from CHO hm1 cells transfected with GFP and K_v7.2 WT or K_v7.2 containing epileptic encephalopathy mutations. The cells were held at -80 mV. Currents were evoked by depolarization for 1.5 s from –100 mV to +20 mV in 10 mV increments, followed by a step to 0 mV for 300 ms. The patch pipette solution contained EGTA to sequester free Ca^{2+}. **(A)** Representative traces. **(B)** Average peak currents at all voltage steps. **(C)** Normalized current at all voltage steps. **(D)** Average peak current densities at +20 mV. Leak subtracted current traces and data analysis are shown in Figure 4 of the manuscript.



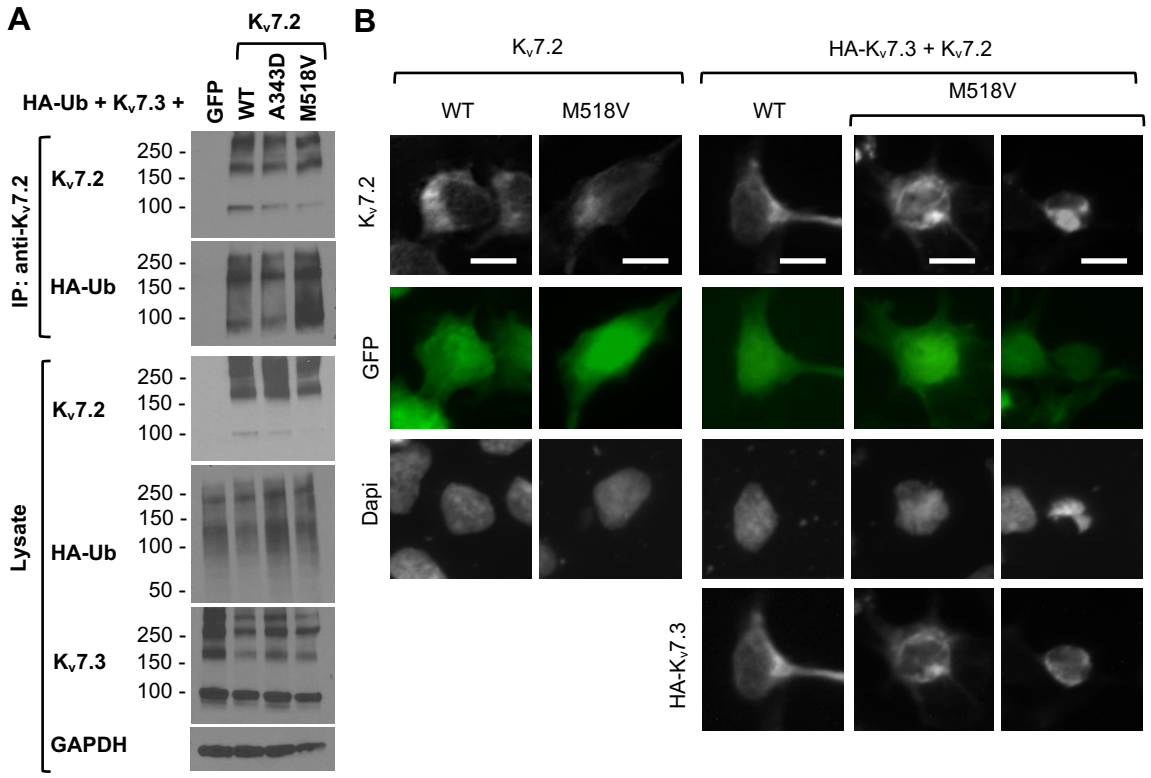
Supplemental Figure 4. PIP2 sensitivity of wild-type $K_v7.2$ channels or mutant $K_v7.2$ channels harboring epileptic encephalopathy mutations. Whole cell voltage clamp recording of macroscopic $K_v7.2$ currents from CHO hm1 cells were repeated in the presence of diC8-PIP_2 (100 μM) in the intracellular solution. The CHO hm1 cells were transfected with GFP and $K_v7.2$ WT or $K_v7.2$ containing epileptic encephalopathy mutations (R333W, K526N, R532W). **(A)** Representative traces. **(B)** Average peak currents at all voltage steps. **(C)** Normalized current at all voltage steps. **(D)** Average peak current densities at +20 mV. Leak subtracted current traces and data analysis are shown in Figure 5 of the manuscript.



Supplemental Figure 5. Immunoblot analysis of endogenous $K_v7.2$ and $K_v7.3$ proteins in cultured hippocampal neurons. Total lysates from rat hippocampal cultured neurons at 3, 5, and 7 days in vitro (DIV) were subjected to immunoblotting with anti-$K_v7.2$ and anti-$K_v7.3$ antibodies (Alomone). Total lysates from CHOhm1 cells transfected with $K_v7.2$ or $K_v7.3$ were used as a positive control. GAPDH was used as a loading control. Arrows point to the monomers of $K_v7.2$ or $K_v7.3$.



Supplemental Figure 6. Expression of wild-type K_v7.2 or mutant K_v7.2 containing epileptic encephalopathy mutation in transfected hippocampal neurons. Dissociated hippocampal cultured neurons were transfected with GFP only or together with K_v7.2 WT or mutant K_v7.2 harboring R333W, M518V, K526N, or R532W mutation. At 24 h post-transfection, untransfected and transfected neurons were immunostained with antibodies against K_v7.2 N-termini and ankyrin-G (AIS marker). Arrows point to the AIS. Fluorescence images were obtained using the same exposure time. Immunostaining with anti-K_v7.2 N-terminal antibody revealed endogenous K_v7.2 expression in untransfected hippocampal neurons whereas immunostaining in the absence of anti-K_v7.2 N-terminal antibody did not detect any K_v7.2 expression (negative control – "No anti-K_v7.2" panels). In transfected neurons, anti-K_v7.2 N-terminal antibody detected both endogenous and transfected K_v7.2 proteins. Scale bars: 50 μm. Quantification of K_v7.2 expression at the AIS is shown in Figure 6E of the manuscript.



Supplemental Figure 7. $K_v7.3$ coexpression prevents degradation of $K_v7.2$-M518V proteins. **(A)** Immunoprecipitation of $K_v7.2$ from HEK293T cells cotransfected with HA-ubiquitin and $K_v7.3$. The $K_v7.2$-M518V proteins are ubiquitinated in the presence of $K_v7.3$. **(B)** Immunocytochemistry of GFP-positive HEK293T cells cotransfected with $K_v7.2$ WT or M518V alone, or together with HA-$K_v7.3$. Scale bars: 10 μm. The M518V mutation decreases $K_v7.2$ expression when expressed alone (left 2 panels). Presence of HA-tagged $K_v7.3$ prevents reduction in expression of $K_v7.2$-M518V, induces aggregation of $K_v7.2$-M518V, and causes cell and nuclear shrinkage (right 3 panels). Quantification of $K_v7.2$ expression and the number of cells that display cell and nuclear shrinkage are shown in Figure 8B-D.