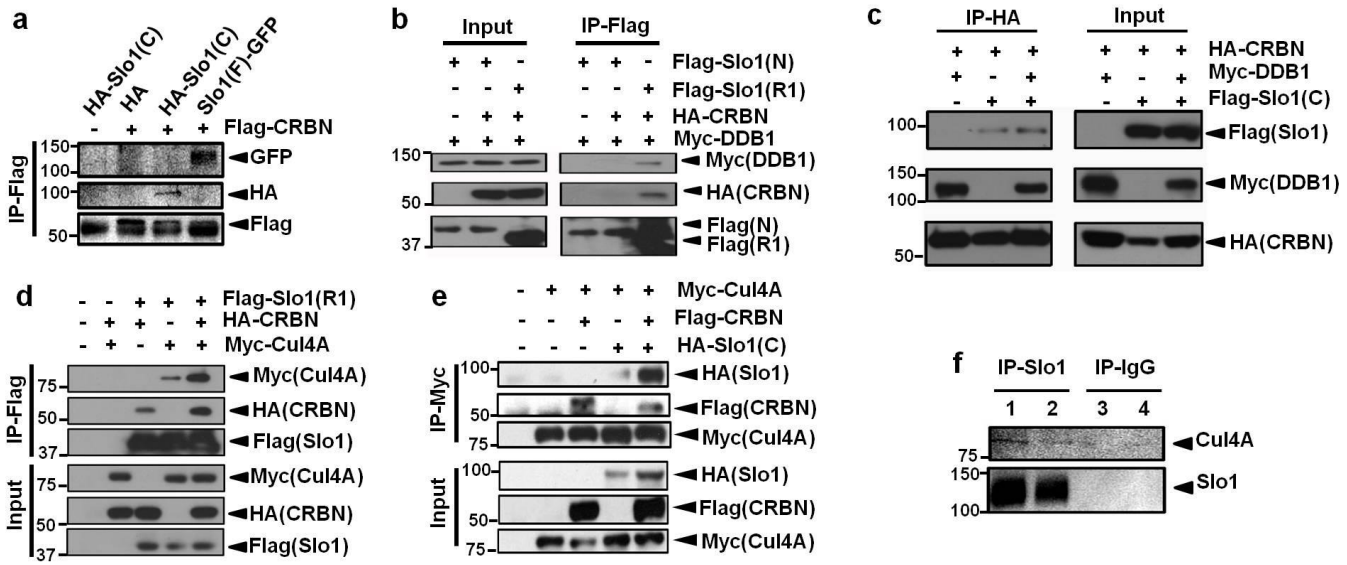
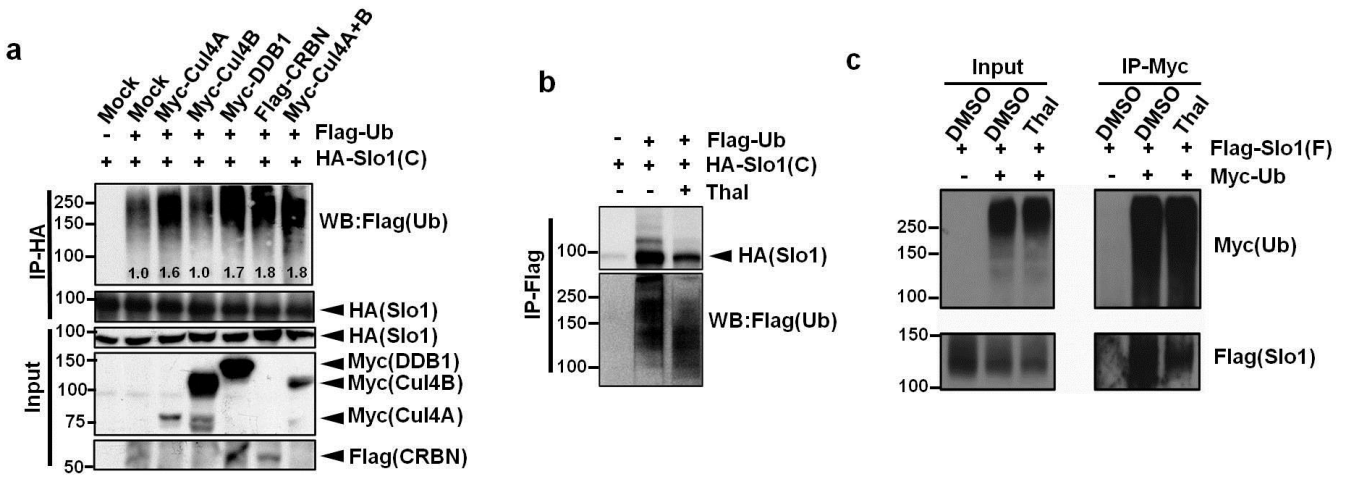
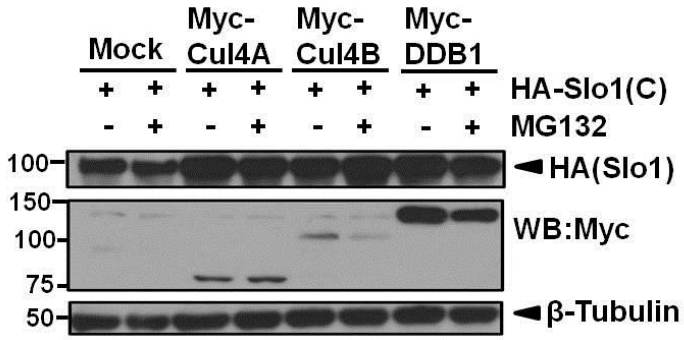
SUPPLEMENTARY INFORMATION



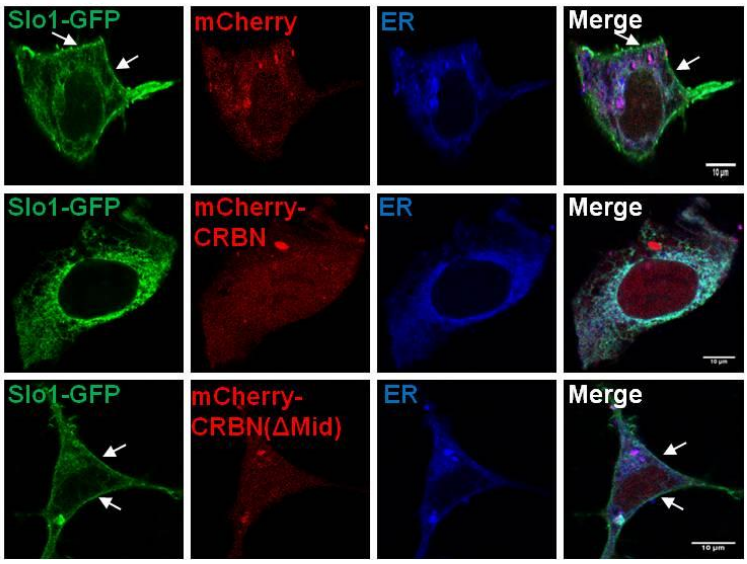
Supplementary Figure 1. Formation of a complex between Slo1 and CRL4A^{CRBN} E3 ligase. (a) HEK 293T cells were transfected with indicated expression vectors and the whole-cell extracts were subjected to co-immunoprecipitation (co-IP) assays. Slo1(F), full length of Slo1. Slo1(C), intracellular domain of Slo1. **(b)** Flag-Slo1(N) or Flag-Slo1(R1), as a control, were transfected into HEK 293T cells together with CRBN and DDB1 expression constructs. Protein extracts were immunoprecipitated using Flag M2 beads. Slo1(N), membrane-spanning N terminus of Slo1. Slo1(R1), RCK1 domain of Slo1. **(c)** HEK 293T cells were transfected with indicated expression vectors and the whole-cell extracts were subjected to co-immunoprecipitation (co-IP) assays. **(d)** HEK 293T cells were co-transfeted with vectors encoding Flag-Slo1(R1), HA-CRBN and Myc-Cul4A. Protein extracts were immunoprecipitated using Flag M2 beads. **(e)** HEK 293T cells were co-transfected with vectors encoding Flag-Slo1(C), HA-CRBN and Myc-Cul4A. Protein extracts were immunoprecipitated using anti-Myc antibody. **(f)** Total tissue lysates extracted from 4 wild type mice cerebrum were immunoprecipitated by BK channel antibody and analysed with indicated antibodies. Non-specific mouse IgG was used as control.



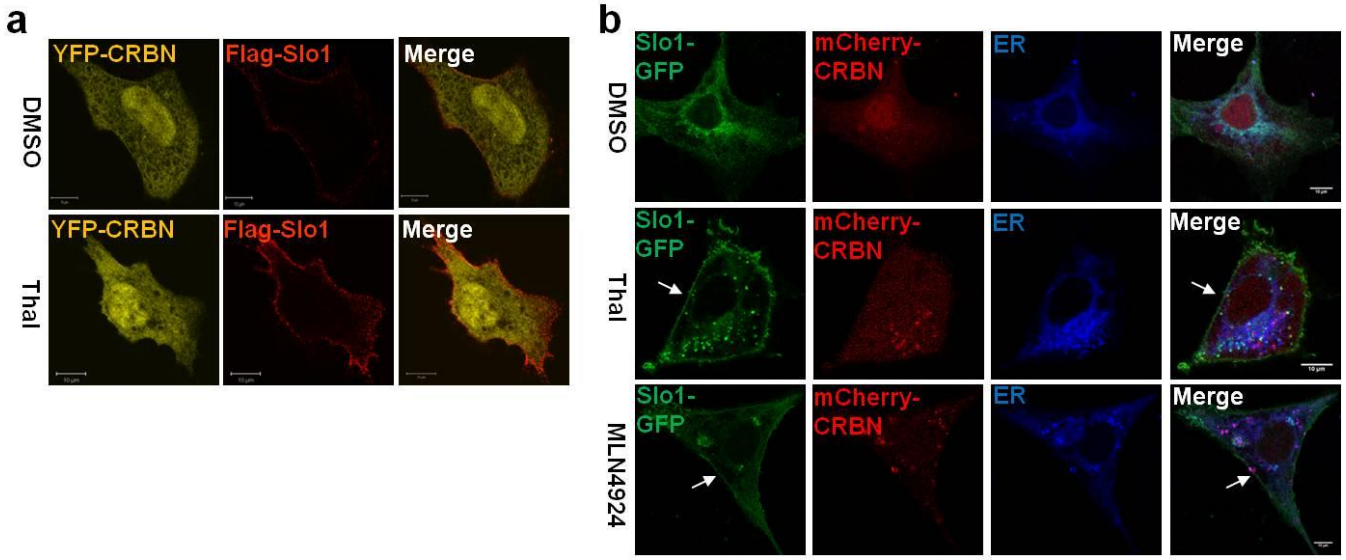
Supplementary Figure 2. Slo1 is ubiquitinated by CRL4A^{CRBN} E3 ligase in HEK 293T cells. (a) HEK 293T cells were transfected with indicated expression vectors and subjected to Slo1 ubiquitination (upper) and protein expression (lower) assays. Numerical band intensity was measured by NIH Image J and indicated on the figure. **(b,c)** Effects on BK channel ubiquitination by treating HEK 293T cells with thalidomide (50 μM) for 6 hrs before harvest. Cell lysates were immunoprecipitated and analysed by Western blot. Ub, ubiquitin.



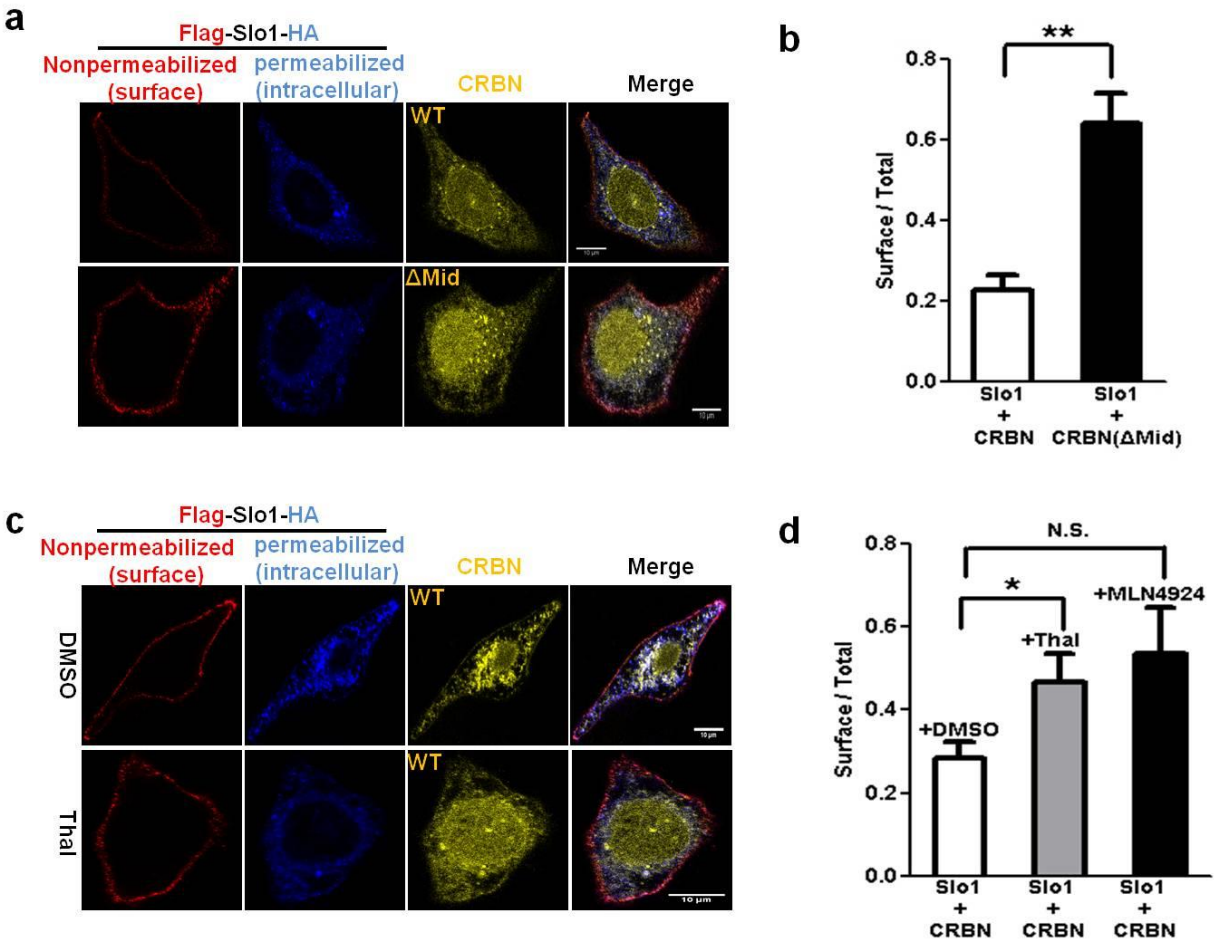
Supplementary Figure 3. B**K channels are not targeted for proteasome-dependant degradation by CRL4A^{CRBN} E3 ligase.** Effects on protein levels of Slo1 by treating cells with MG132 (20 μM) for 6 hrs before harvest when co-transfected with either Cul4A, Cul4B or DDB1 in HEK 293T cells.



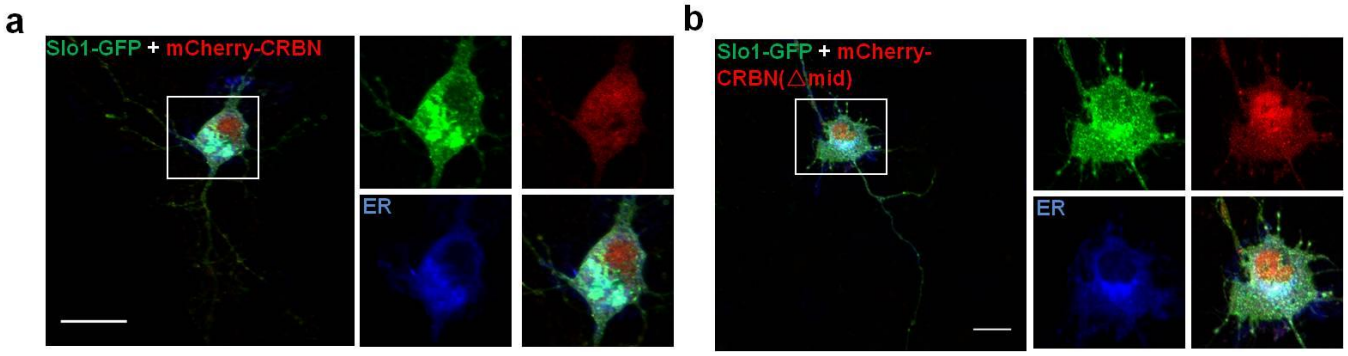
Supplementary Figure 4. CRL4A^{CRBN} E3 ligase inhibits the trafficking and surface expression of BK channels in HEK 293 cells. Confocal fluorescent imaging of HEK 293 cells transfected with expression vectors for Slo1-GFP and mCherry-CRBN or mCherry-CRBN(ΔMid). ER-Tracker^{TM} was used to stain ER. White arrows indicate the distribution of GFP fusion BK channels on cell membranes. Scale bar, 10μm.



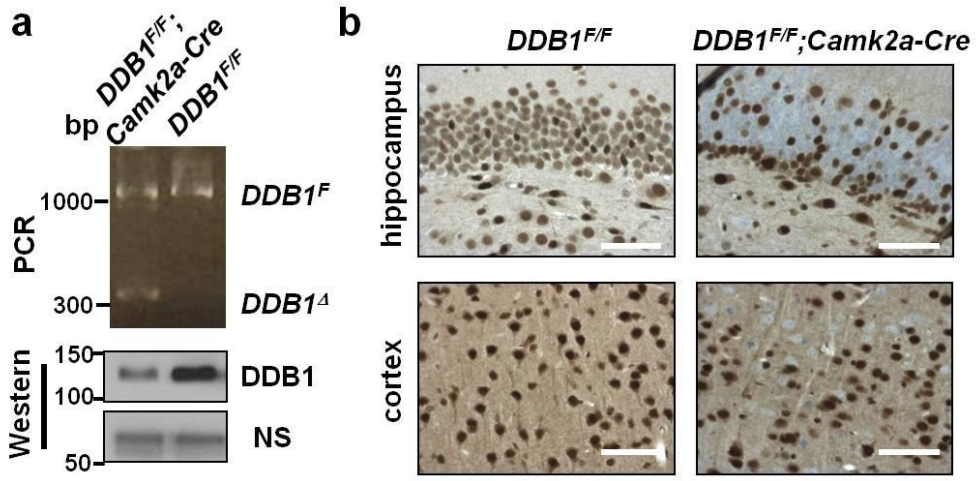
Supplementary Figure 5. Thalidomide and MLN4924 inhibit the effect of CRL4A^{CRBN} E3 ligase on the distribution of BK channels. (a) Immunofluorescence of HeLa cells under nonpermeabilized conditions, which were transfected with YFP-CRBN and Flag-Slo1 and treated with 50 μM thalidomide (Thal) for 6 hrs before harvest. Scale bar, 10μm. **(b)** Confocal fluorescent imaging of HEK 293 cells transfected with Slo1-GFP and mCherry-CRBN and treated with 50 μM thalidomide or 0.1 μM MLN4924 for 6 hrs. White arrows indicate the distribution of GFP fusion BK channels on cell membranes. Scale bar, 10μm.



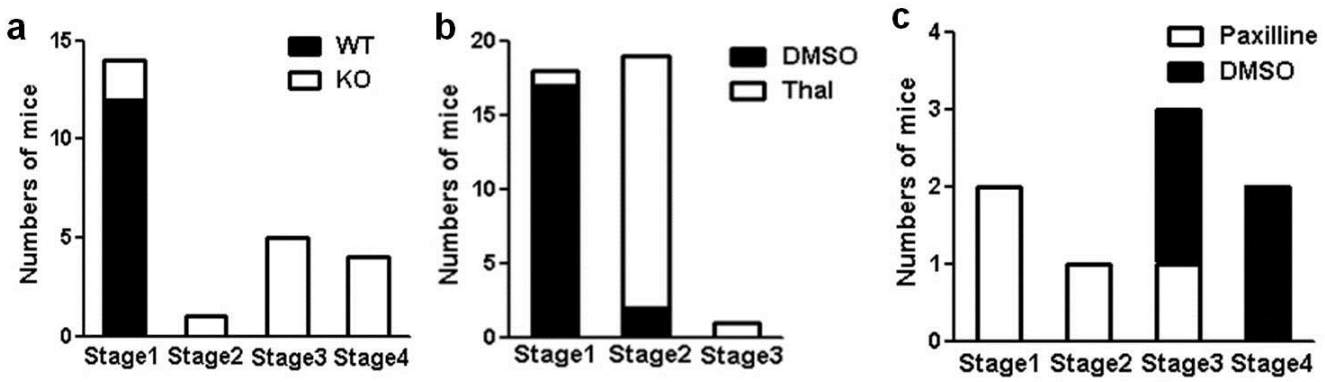
Supplementary Figure 6. The inhibition of CRL4A^{CRBN} E3 ligase activity enhanced the surface expression of BK channels. (a) Confocal imaging of HeLa cells transfected with Flag-Slo1-HA and CRBN or CRBN(ΔMid). The surface expressed BK channels and total expressed BK channels were labeled by Flag antibody and HA antibody under nonpermeabilized and permeabilized conditions separately. Scale bar, 20μm. **(b)** Quantification the ratio of surface expressed BK channels to total expressed channels in HeLa cells by densitometry with the presence of CRBN(WT or ΔMid). n=9. **(c)** Confocual imaging of HeLa cells transfected with Flag-Slo1-HA and YFP-CRBN and treated with thalidomide (50 μM) 6 hrs before harvest. DMSO was used as control. Scale bar, 10μm. **(d)** Quantification the ratio of surface expressed BK channels to total expressed channels in HeLa cells. n=4-6. Results in **(b)** and **(d)** are shown as means and s.e.m. *p<0.05, N.S., not significant. determined by the Student's t-test.



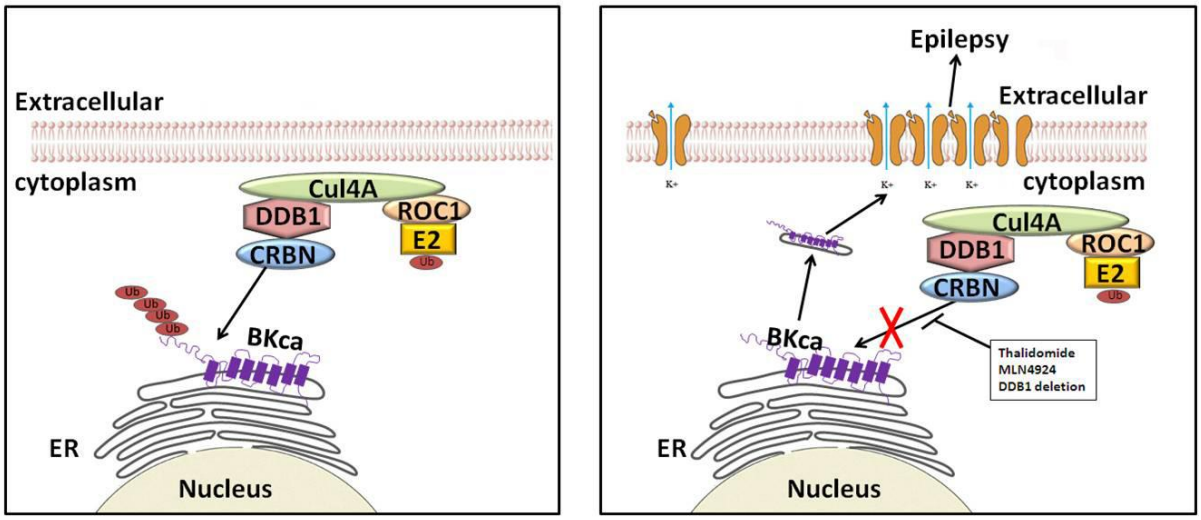
Supplementary Figure 7. CRL4A^{CRBN} E3 ligase inhibit trafficking of BK channels to neuronal surface. (a,b) Confocal images of rat primary hippocampal neurons transfected with expression vectors for Slo1-GFP and mCherry-CRBN or mCherry-CRBN(ΔMid). Cell treatment and Images acquirement were done as in Figure 2a. Scale bar, 10μm.



Supplementary Figure 8. ***DDB1*** **is conditionally knocked out in hippocampal and cortex neurons of** ***DDB1*** $^{F/F}$***; Camk2a-Cre*** **mice. (a)** The genotypes were detected by PCR and the expression levels of DDB1 were assessed using Western blot. **(b)** Immunohistochemistry staining of DDB1 in mouse hippocampus and cortex. Scale bar, 50μm.



Supplementary Figure 9. The function of CRL4A^{CRBN} E3 ligase blocked *in vivo* lead to more serious epilepsy in PTZ-induced seizure mice model. (a) Quantitative distribution of mice at different seizure stages of epilepsy between *DDB1$^{F/F}$* and *DDB1$^{F/F}$; Camk2α-Cre* mice after PTZ treatment. **(b,c)** Quantitative distribution of mice at different seizure stages of epilepsy between **(b)** DMSO and thalidomide injected mice, or **(c)** DMSO and paxilline injected mice after PTZ treatment.



Supplementary Figure 10. Schematic model for the molecular mechanism of epilepsy prevented by $CRL4A^{CRBN}$ through ubiquitinating BK channels. Normally, Slo1 is ubiquitinated by $CRL4A^{CRBN}$ and retained in the ER. When the activity of $CRL4A^{CRBN}$ E3 ligase activity is compromised by chemical inhibitors or genetic alteration, the de-ubiquitinated BK channel will move from the ER to the plasma membrane, leading to enhanced channel activity and possibly the onset of epilepsy.

Fig. 1b

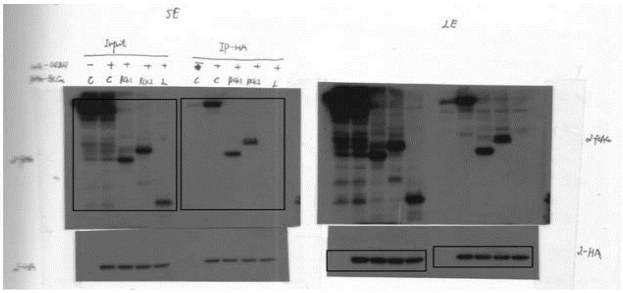


Fig. 1d

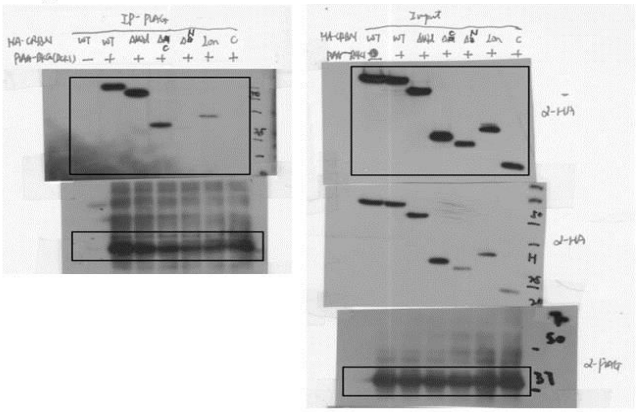


Fig. 2b

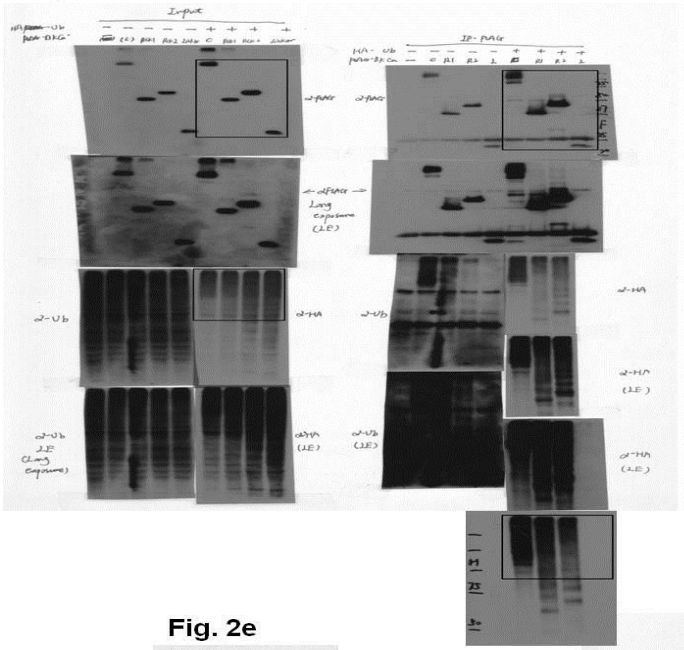


Fig. 2d

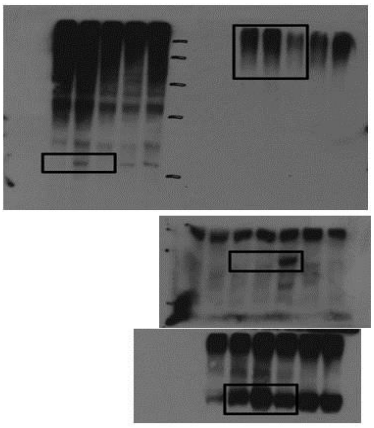


Fig. 2e

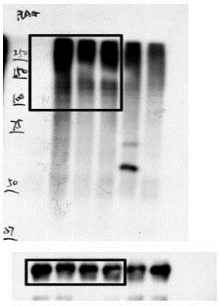
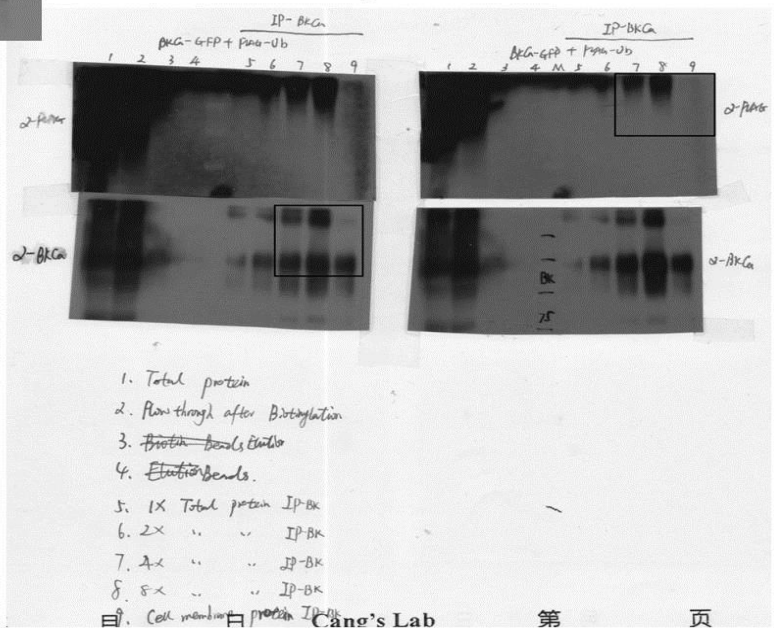


Fig. 3f



Supplementary Figure 11. Western blot scanned films. Boxes highlight lanes used in figures.