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Supplemental information

The brain-specific kinase LMTK3 regulates neuronal excitability by decreasing KCC2-dependent neuronal Cl^- extrusion

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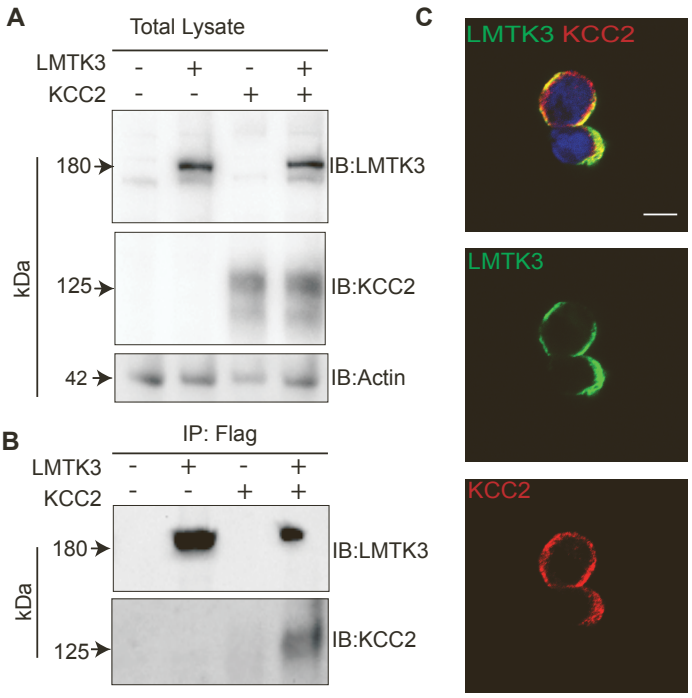
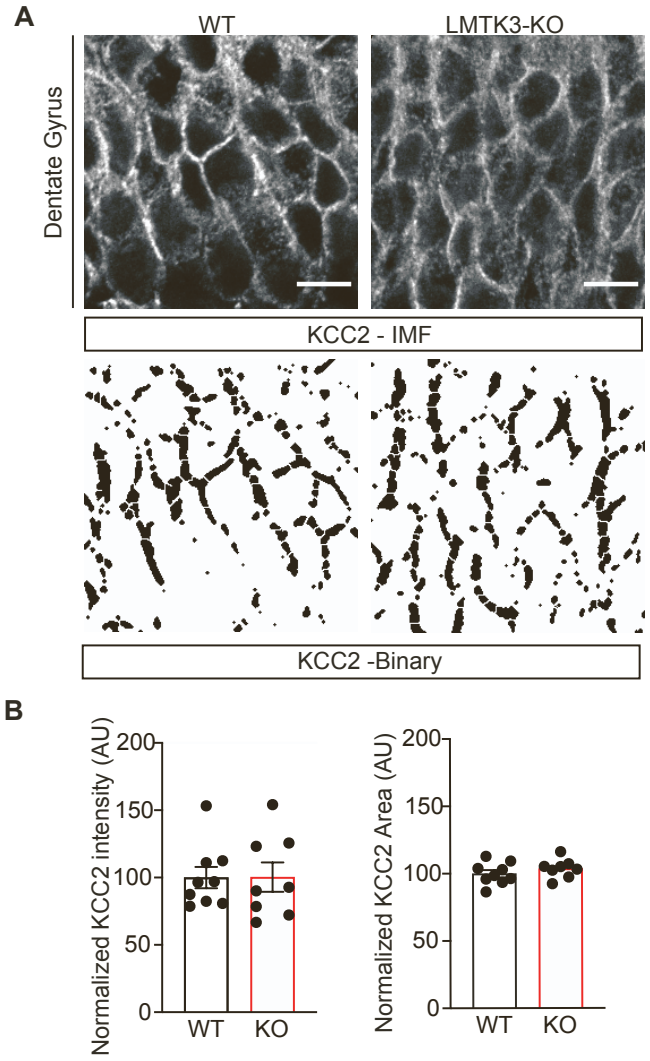
Figure S1. LMTK3 and KCC2 co-purify when expressed in HEK-293 cells, Related to Figure 1.



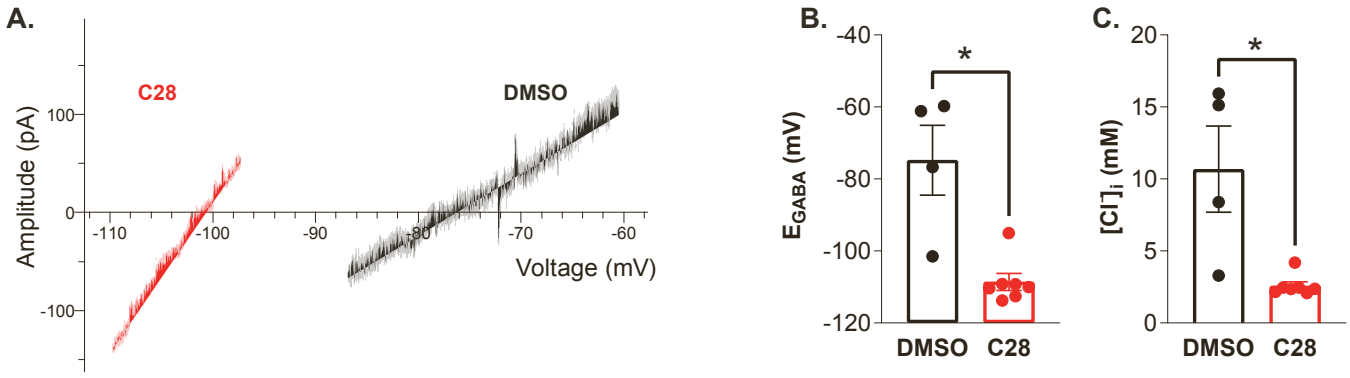
A.HEK cell lysates were subjected resolved on SDS-PAGE and immunoblotted for LMTK3 and KCC2 (input). **B.** LMTK3 was isolated from the lysates by immunoprecipitation (IP), resolved on SDS-PAGE and immunoblotted for LMTK3 and KCC2, confirming their co-immunoprecipitation. **C.** Confocal images of HEK cells expressing LMTK3 and KCC2, stained with DAPI and immuno-stained using an anti-flag and and an anti-KCC2 antibody. Scale Bar = 10 μm.

Figure S2. Examining the subcellular distribution of KCC2 in the dentate gyrus of WT and LMTK3-KO mice, related to Figure 3.



A. In the upper panel representative confocal images are show of dentate gyrus of the stratum dentate gyrus granule cell layer from WT and LMTK3-KO mice stained with KCC2 antibody, scale bar = 10 μm. The raw images were analyzed via ImageJ and this data was used to create background subtracted binary distribution of KCC2 expression that are shown in the lower panels. **B.** Image J was used to compare the intensity and area of KCC2 puncta between genotypes, n=12 images from 3 mice/genotype.

Figure S3. Examining the effects of C28 on E_{GABA} in cultured neurons using gramicidin-perforated patch clamp recording, related to figure 5.



A. Representative I-V traces are shown for the polarity of currents induced by rapid application of muscimol in 18-22 Div neurons as measured using perforated patch-clamp recording following 1h exposure to V or 10 μM C28. The recordings were used to determine E_{GABA} and [Cl^-] which were then compared between treatments as shown in **B** and **C** respectively. *= p<0.01, t-test, n=4-6 cells, in all panels data represent mean ± SEM.

Table S1 Protiens recoverey with LMTK3 as measured using LC-MS/MS

Table S2 Proteins recovered with KCC2 as measured using LC-MS/MS