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Supplemental information

SNARE protein SNAP25 regulates the chloride-transporter KCC2 in neurons

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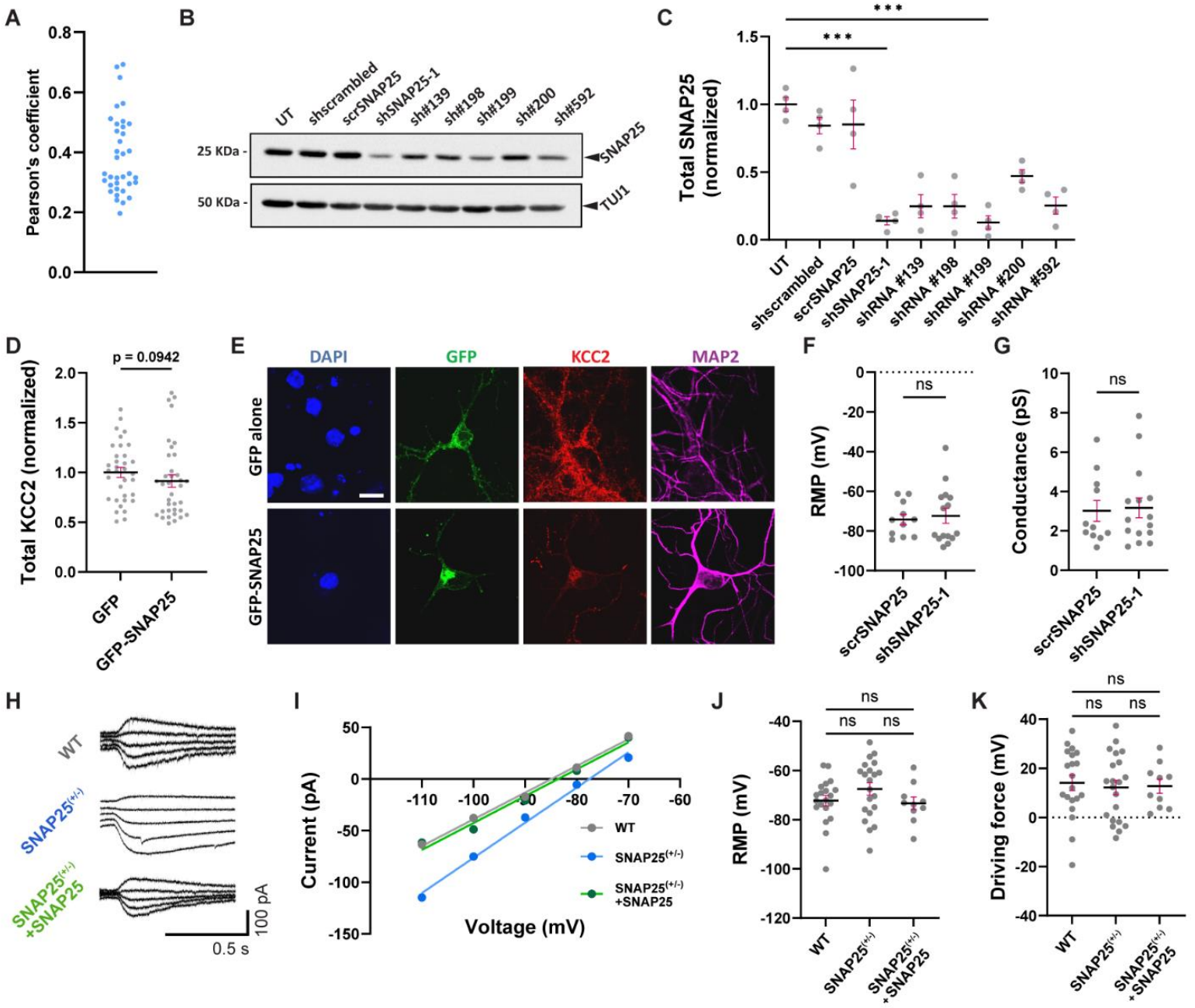

Figure S1. SNAP25 overexpression did not change total endogenous KCC2 level in neurons. Related to Figure 1 and Figure 2. A) Pearson's correlation coefficient for colocalization of SNAP25 and KCC2 in mouse cortical neurons. Each data point represents a single neuron (n=36), and the graph contains data from four independent cultures. **B)** Immunoblot showing total endogenous SNAP25 in neuro-2a cells transfected with scrambled shRNAs (shscrambled, scrSNAP25) or shRNAs targeting SNAP25 (shSNAP25-1, sh#139, sh#198, sh#199, sh#200, sh#592). The blot was probed with the antibodies indicated on the right. **C)** Summary graph (n=4) corresponding to (B), normalized to un-transfected cells (UT). Statistical significance was determined using Brown-Forsythe and Welch ANOVA tests followed by Dunnett's T3 multiple comparisons test. Asterisks denote significance from UT, and only statistical significance for shSNAP25-1 and sh#199 (shSNAP25-2) are depicted in the figure. **D)** Summary graph of total endogenous KCC2 fluorescence (normalized to volume) from primary mouse cortical neurons transfected with GFP alone (n = 34) or GFP-SNAP25 (n = 36). **E)** Representative images (maximum intensity projection) from neurons corresponding to (D), immunostained with anti-KCC2 (red) and anti-MAP2 (magenta) antibodies. Green fluorescence (GFP) represents transfected neurons. Scale bar, 10 µm. Statistical significance was determined using Mann-Whitney test. **F)** Summary graph showing resting membrane potential and **G)** conductance of neurons transduced with scrSNAP25 (n=11) or shSNAP25-1 (n=15). n values represent individual cells obtained from three independent cultures. Statistical significance for (F) and (G) was determined using an unpaired t-test with Welch's correction. **H)** Sample current traces, **I)** representative current-voltage plot, **J)** resting membrane potential, and **K)** Cl^- driving force for WT neurons (n=20), $SNAP25^{(+/-)}$ neurons (n=21) and $SNAP25^{(+/-)}$ neurons with exogenous SNAP25 transduction (n=10). n values indicate cells obtained from cultures of at least five individual pups. All graphs represent mean ± SEM. ns - not significant, ***p<0.001.

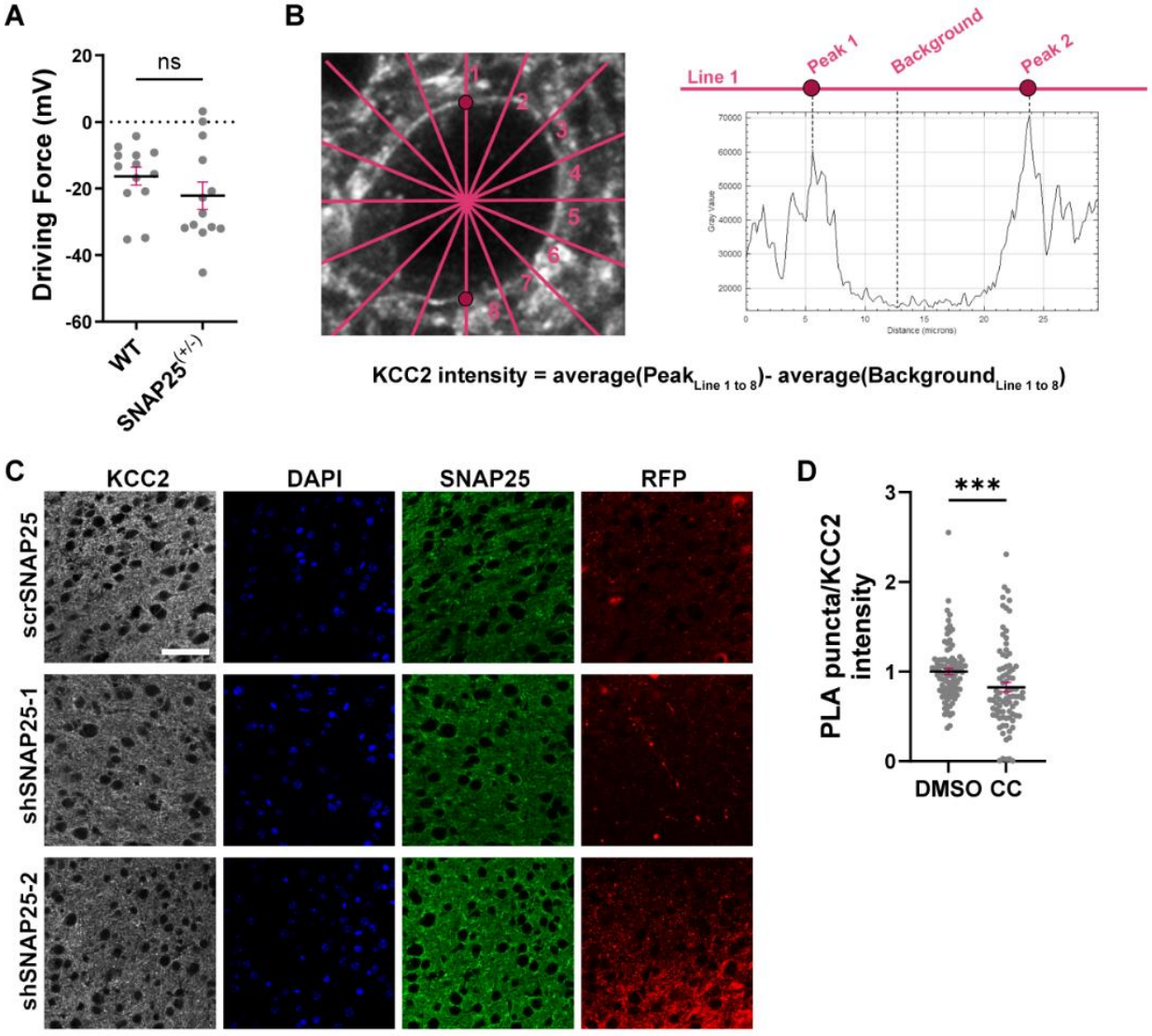

Figure S2. SNAP25 knockdown reduced KCC2 in mice. Related to Figure 3 and Figure 4. A) Driving force for Cl^- from $SNAP25^{(+/-)}$ mice (n=13) and WT littermates (n=18). n values represent individual cells obtained from at least four animals. Statistical significance was determined using an unpaired t-test with Welch's correction. **B)** Schematic figure showing the method used to quantify KCC2 in brain slices **C)** Representative images (maximum intensity projection) at a lower magnification from C57BL/6 mice cortex transduced with scrambled shRNA (scrSNAP25) or SNAP25 shRNA (shSNAP25-1, shSNAP25-2), and immunostained for KCC2 (gray) and SNAP25 (green). Scale bar, 50 µm. **D)** Summary graph of PLA puncta intensity (KCC2-SNAP25 interaction) in neuro-2a cells transfected with KCC2 and treated with DMSO (n=92) or chelerythrine chloride (CC, n=84). n values indicate individual cells from three independent coverslips. Statistical significance was determined using Mann-Whitney test. ns- not significant, ***p<0.001.

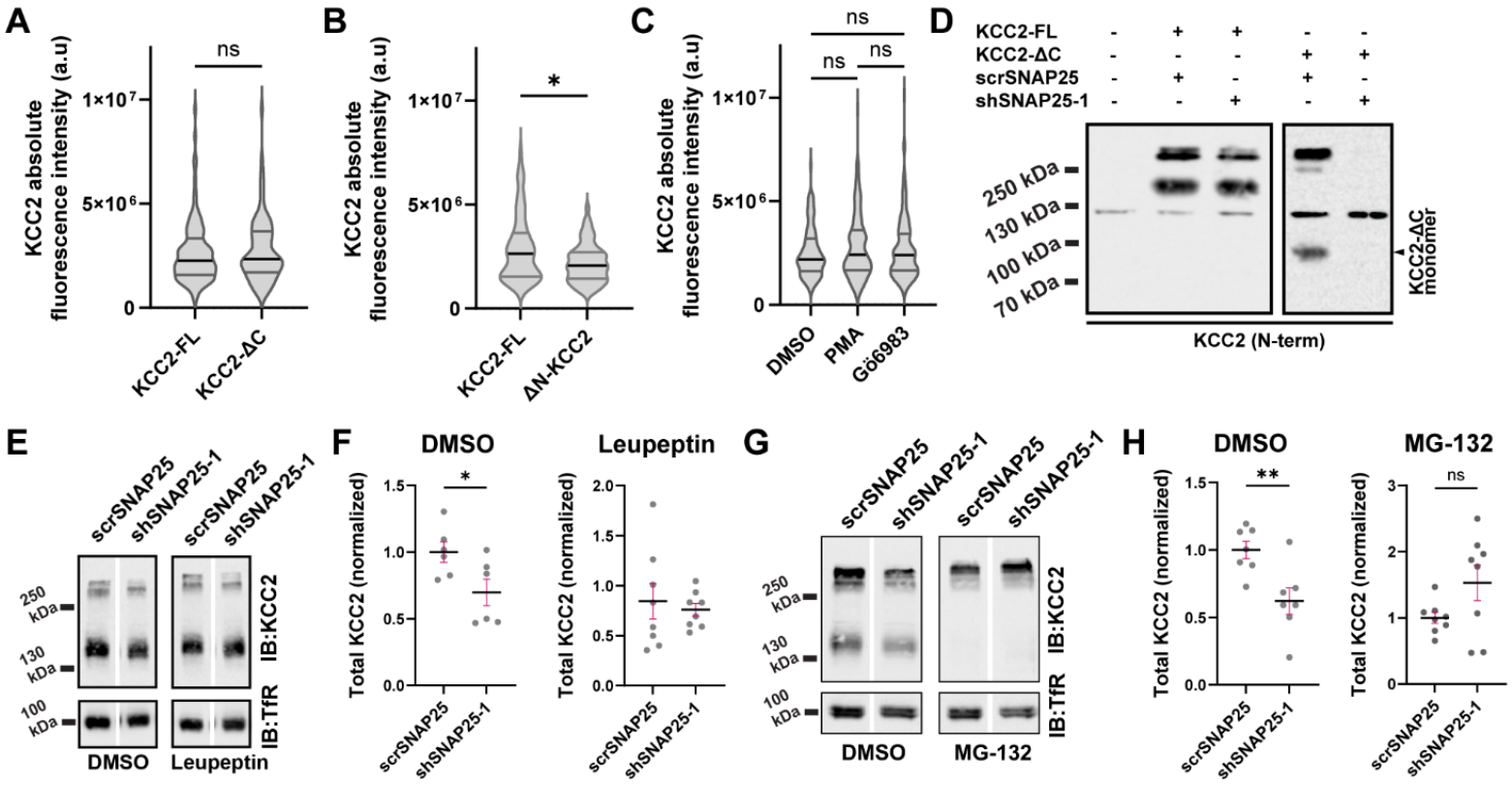

Figure S3. SNAP25 knockdown reduces KCC2 through lysosomal-proteasomal degradation. Related to Figure 5. A) Summary plot showing absolute KCC2 fluorescence intensity in neuro-2a cells (selected for quantification in Figure 5 C) transfected with KCC2-FL (n=99) or KCC2-ΔC (n=80) cDNA. n values indicate individual cells from four independent coverslips. Statistical significance was determined using Mann-Whitney test. **B)** Summary plot showing absolute KCC2 fluorescence intensity in neuro-2a cells (selected for quantification in Figure 5 E) transfected with KCC2-FL (n=69) or ΔN-KCC2 (n=63) cDNA. n values indicate individual cells from three independent coverslips. Statistical significance was determined using Mann-Whitney test. **C)** Summary plot showing absolute KCC2 fluorescence intensity in neuro-2a cells (selected for quantification in Figure 4 J) transfected with KCC2 cDNA and treated with DMSO (n=106), PMA (n=141) or Go6983 (n=134). n values indicate individual cells from four independent coverslips. Statistical significance was determined using Kruskal-Wallis test and Dunn's multiple comparisons test. **D)** Representative immunoblot showing KCC2-FL and KCC2-ΔC in neuro-2a cells co-transfected with scrambled (scrSNAP25) or SNAP25 shRNA (shSNAP25-1), and probed with N-terminus directed anti-KCC2 antibody. **E)** Immunoblot and **G)** summary graph showing total KCC2 abundance in neuro-2a cells transfected with KCC2 and scrSNAP25 or shSNAP25-1, and treated with DMSO (scrSNAP25, n=6; shSNAP25-1, n=6) or Leupeptin (scrSNAP25, n=8; shSNAP25-1, n=8). Statistical significance was determined using unpaired t-test. **H)** Immunoblot and **I)** summary graph showing total KCC2 abundance in neuro-2a cells transfected with KCC2 and scrSNAP25 or shSNAP25-1, and treated with DMSO (scrSNAP25, n=7; shSNAP25-1, n=7) or MG-132 (scrSNAP25, n=8; shSNAP25-1, n=8). Statistical significance was determined using unpaired t-test. The violin plots show median with quartiles and all other graphs represent mean ± SEM. ns - not significant, *p<0.05, **p<0.01.

Table S1. Complete details and dilutions of all antibodies used in this study are as follows. Related to STAR Methods.

Antibody	Catalog #	Manufacturer	Dilution (or amount) and corresponding figures		
			Immunoblot (IB)	Immunofluorescence (IF)/ Proximity ligation assay (PLA)	Immunoprecipitation (IP)
KCC2 (rabbit)	07-432	Sigma-Aldrich	1:2000 (Figure 1 A, C, E; 3 B; 4 A, F; 5 G, H; 6 A, B, C, D)	1:1000 (Figure 1 B; 2 A) 1:400 (Figure 3 J) 1:500 (Figure 4 D, I, L, N; 5 D)	N/A
KCC2 (mouse)	75-013	NeuroMab	N/A	N/A	5µg/1000µg total protein (Figure 1 A)
KCC2 (rabbit)	ab97502	Abcam	1:2000 (Figure 5 F)	1:500 (Figure 5 B)	N/A
SNAP25 (mouse)	111011	Synaptic Systems	N/A	1:1000 (Figure 1 B; 2 A) 1:500 (Figure 4 I, L, N; 5 B, D)	N/A
SNAP25 (rabbit)	ab5666	Abcam	1:2000 (Figure 1 A, C, E; 3 B)	N/A	N/A
b-Actin (rabbit)	4967S	Cell Signaling Technology	1:2000 (Figure 1 C)	N/A	N/A
TfR (mouse)	13-6800	Invitrogen	1:3000-5000 (Figure 1 E; 3 B; 4 A, F; 5 F, G, H; 6 A, B, C, D)	N/A	N/A
MAP2 (chicken)	AB5543	EMD Millipore	N/A	1:2000 (Figure 1 B)	N/A
TUJ1 (mouse)	801202	BioLegend	1:5000 (Figure 3 B; 4 A, F; 5 F, G)	N/A	N/A
FLAG (mouse)	F1804	Sigma-Aldrich	N/A	1:500 (Figure 4 D)	N/A
Purified Mouse IgG	43-637-0010	Antibodies Inc	N/A	N/A	5µg/1000µg total protein (Figure 1 A)