Cell Reports, Volume *41*

Supplemental information

Loss of functional System x-c uncouples aberrant postnatal neurogenesis from epileptogenesis in the hippocampus of *Kcna1*-KO mice

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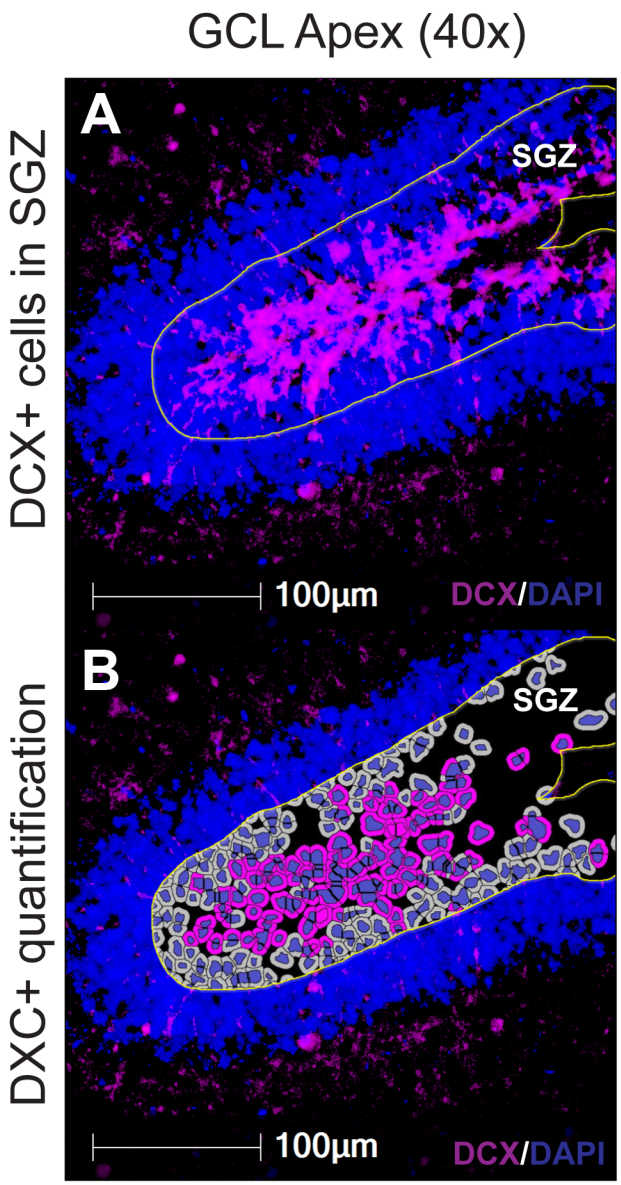
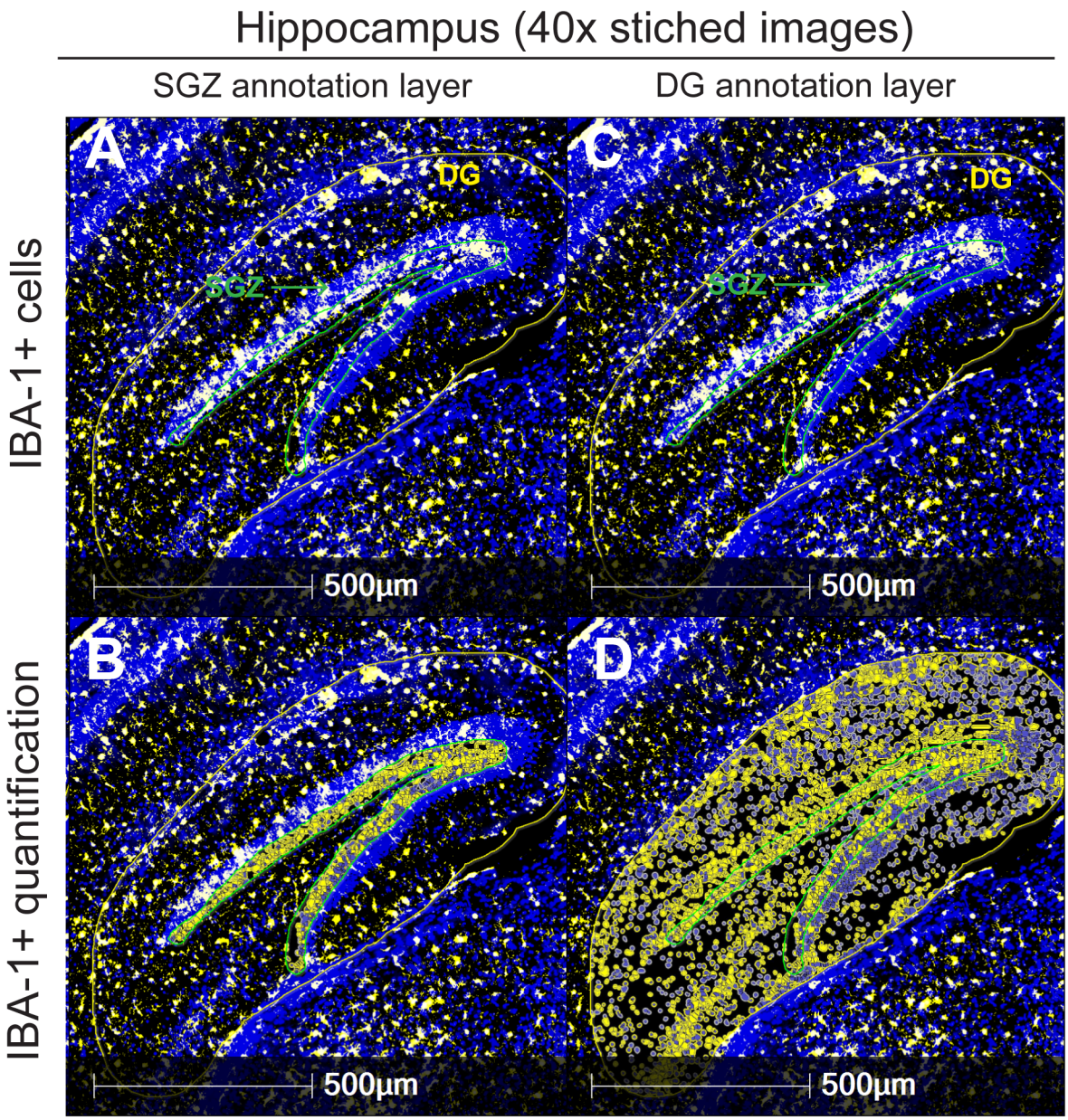
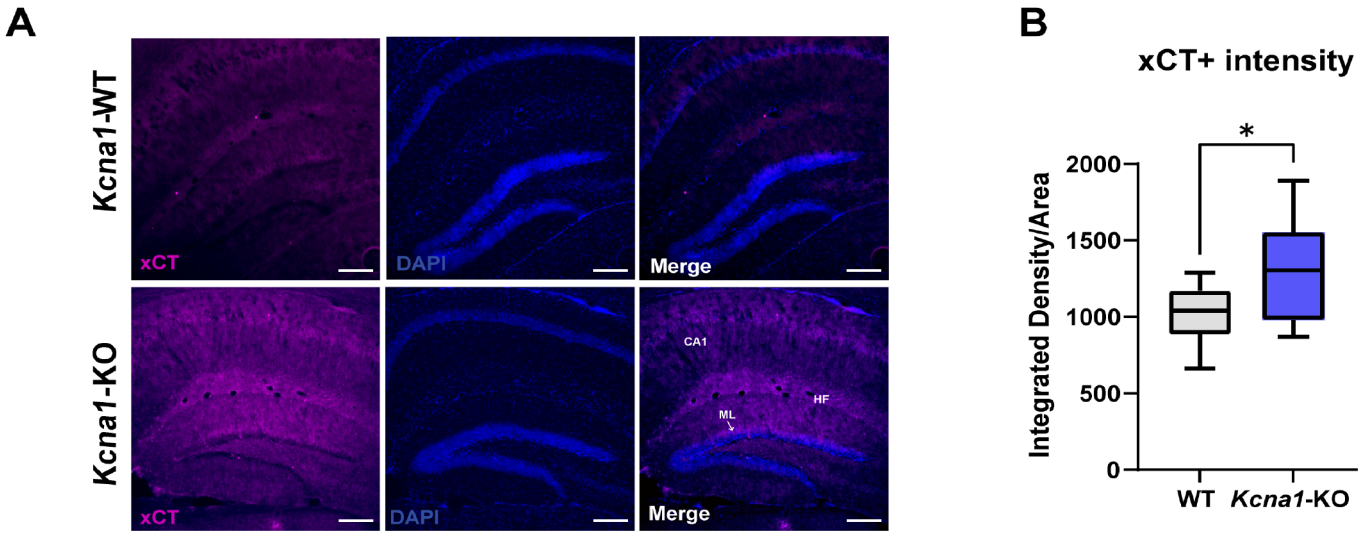


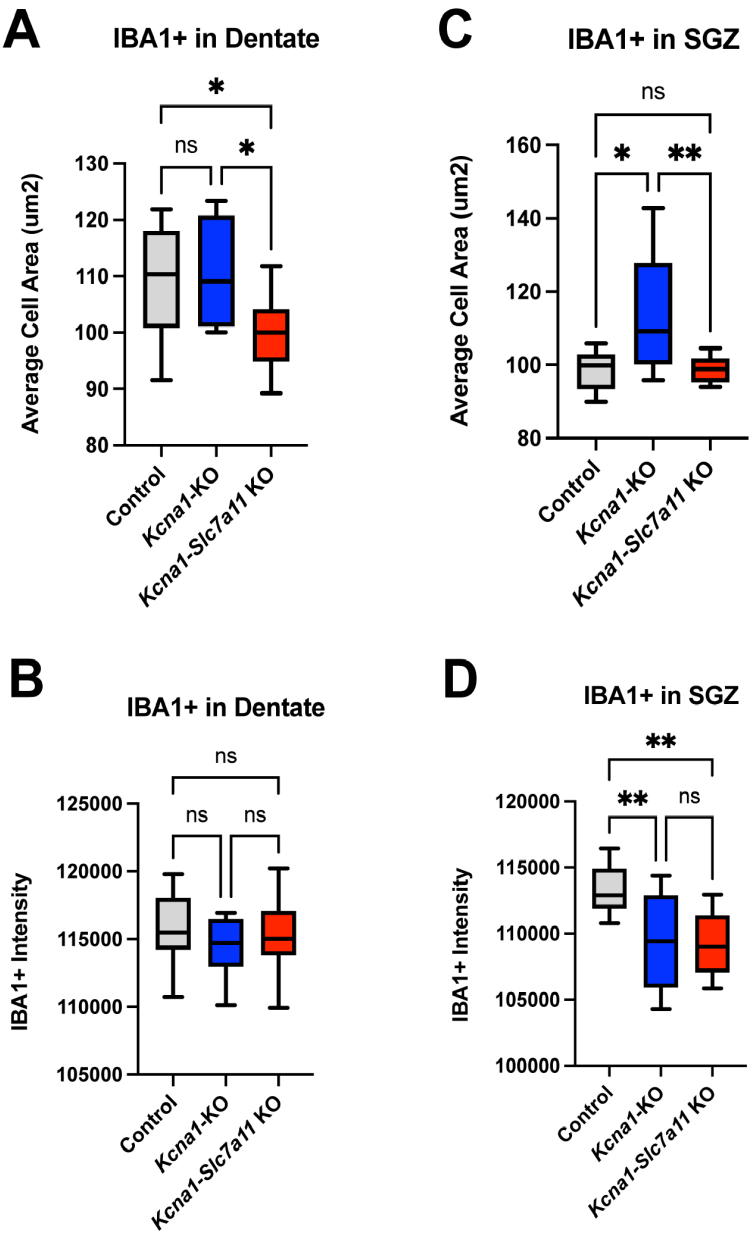
Supplemental Figure 1: Digital pathology using Halo for the quantification of doublecortin-expressing cells at the apex of the granule cell layer in the dentate gyrus. Representative images of higher power (40x) maximum intensity projections from apex of the granule cell layer (GCL) of the dentate gyrus (DG). **A)** Sub-granular zone (SGZ) is defined by positive doublecortin (DCX) stain, allowing demarcation of the SGZ annotation layer. **B)** Identification of DAPI+ nuclei expressing DCX with Halo FISH-IF module. Related to the ***Quantification of Dentate Gyrus Area and Automated Cell Detection*** section of STAR Methods.



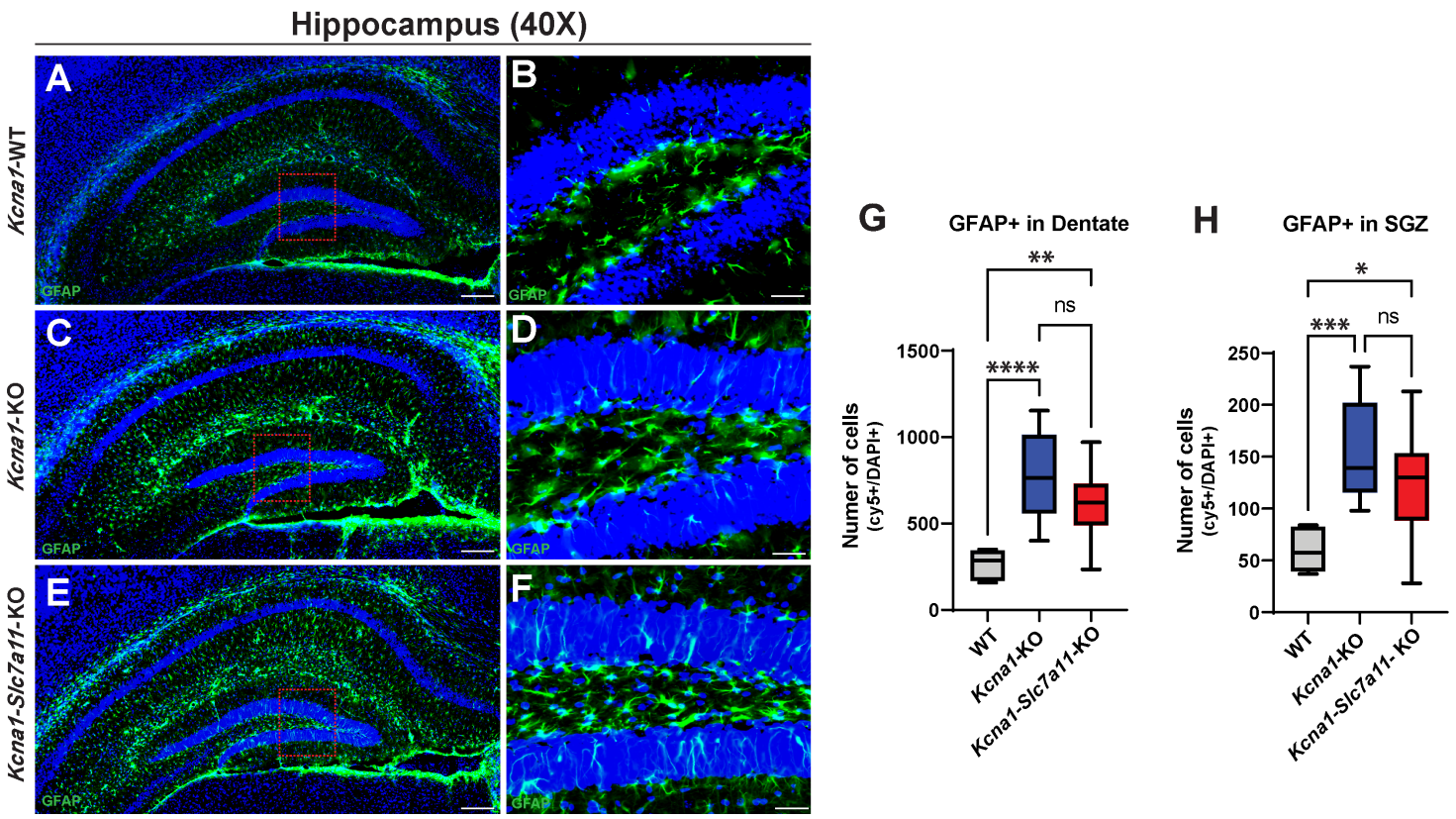
Supplemental Figure 2: Digital pathology using Halo for the quantification of IBA-1-expressing cells in dentate gyrus (DG) and sub-granular zones (SGZ). Representative high power (40x) image tiles stitched to reveal hippocampal structures and microglial (IBA-1+ cell) populations. **A)** The SGZ annotation layer (green) was outlined over the entire GLC. **B)** Representative image showing the identification of DAPI+ nuclei in the SGZ expressing IBA-1 with FISH-IF module. **C)** The DG annotation layer (yellow) was outlined for each stitched image. **D)** Representative image showing the identification of DAPI+ nuclei in the DG expressing IBA-1 with FISH-IF module. We followed the same approach to quantify astrocytosis using glial acidic fibrillary protein (GFAP) as a marker of activated astrocytes (Suppl Fig 5). Related to the ***Quantification of Dentate Gyrus Area and Automated Cell Detection*** section of STAR Methods.



Supplemental Figure 3: The functional unit of Sxc, xCT, is elevated in the Kcna1-KO hippocampus. A) Representative low power (10x) images of hippocampi from littermate *Kcna1*-WT (n=3) and *Kcna1*-KO (n=3) mice immunolabeled with anti-xCT. Staining is intense in inner layer (arrow) and throughout dentate molecular layer (ML), the hippocampal fissure (HF) and pyramidal cell layer (CA1) but does not label granule cells. **B)** Quantification of integrated density per hippocampal area showed increased xCT immunolabeling in the *Kcna1*-KO hippocampus relative to wild-type littermates at P30. Scale bars = 250 µm. Data are represented as mean ± SEM. Related to ***Cells in the Kcna1-KO hippocampus show increased expression of the functional unit of Sxc*** results section.

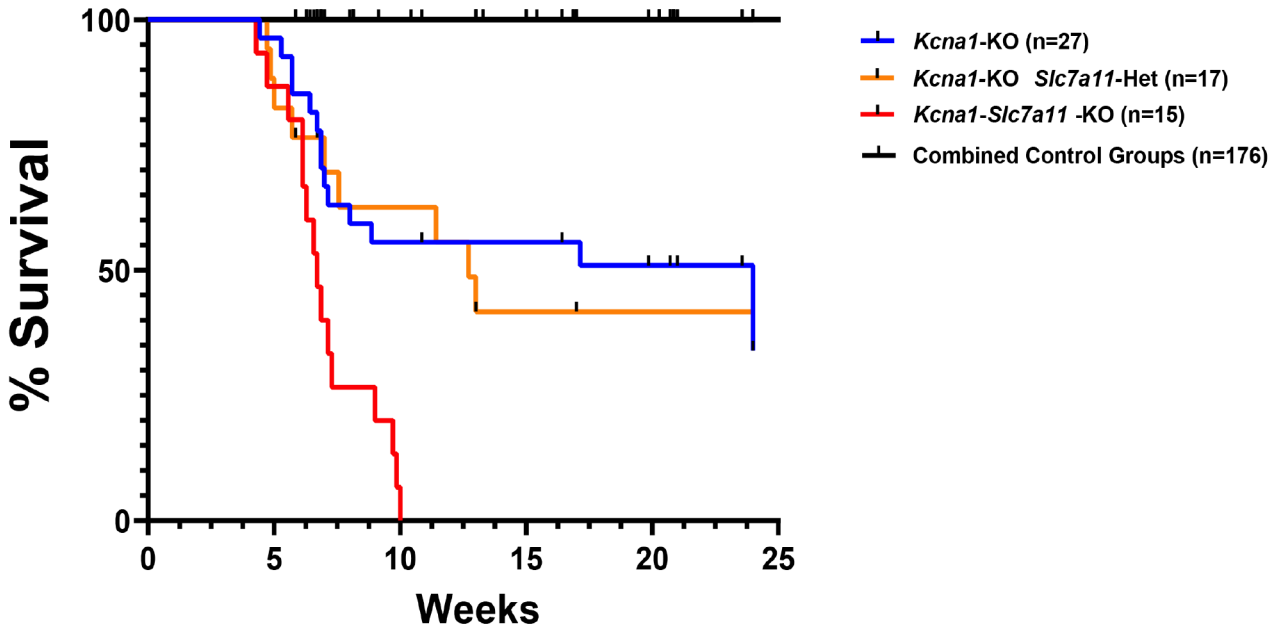


Supplemental Figure 4: Microglial changes during *Kcna1*-KO epileptogenesis are blunted by lack of functional Sxc. A) IBA-1 cells are reduced in area in the *Kcna1-Slc7a11*-KO DG relative to *Kcna1*-KO and wild-type mice. **B)** There is no difference in IBA-1 labeling intensity of microglia in the DG between genotypes. C) In the SGZ IBA-1 positive cells were significantly larger in average area (um2) in the *Kcna1*-KO brain relative to WT and *Kcna1-Slc7a11*-KO. **D)** IBA-1 intensity was also reduced in *Kcna1*-KO and *Kcna1-Slc7a11*-KO. Data were analyzed using HALO Software, Indica Labs and are represented as mean ± SEM. Related to the ***Loss of Sxc in the Kcna1-KO brain decreases microglia density in the dentate gyrus*** results section.



Supplemental Figure 5: Increased Astrogliosis in the Dentate Gyrus and Sub-granular Zone of the *Kcna1*-KO brain is unaffected by developmental absence of functional Sxc. Representative images of high power (40x) stitched images **(A,C,E)** and single field of view **(B,D,F)** of whole hippocampi. **A-B)** *Kcna1*-WT (n=3 mice, 12 stitched images) **C-D)** *Kcna1*-KO (n=3 mice, 12 stitched images), **E-F)** *Kcna1-Slc7a11*-KO (n=3 mice, 12 stitched images) mice immunolabeled with anti-GFAP to mark activated astrocytes. **G)** Quantification of GFAP+ astrocytes in the Dentate Gyrus (DG) and **H)** in the Sub Granular Zone (SGZ) of the granule cell layer (HALO Software, Indica Labs). Scale bars = 250 µm. Data are represented as mean ± SEM. Related to the **Astrogliosis in *Kcna1*-KO hippocampus is unaffected by developmental loss of functional Sxc** results section.

A



B

Summary of pairwise comparisons using Log-Rank test with Bonferroni corrections

	Control	*Kcna1*-KO-*Slc7a11*-Het	*Kcna1*-KO-*Slc7a11*-WT
Kcna1-KO-*Slc7a11*-Het	p<2e-16	n/a	n/a
Kcna1-KO-*Slc7a11*-WT	p<2e-16	0.19068	n/a
Kcna1-Slc7a11-KO	p<2e-16	0.05254	0.00062

Supplemental Figure 6: Genetic loss of functional Sxc results in fully penetrant SUDEP in the *Kcna1*-KO line. **A)** Survival probability plots of *Kcna1*-KO, *Kcna1*-KO-*Slc7a11*-het, *Kcna1-Slc7a11*-KO, and combined control mice population genotypes resulting from crosses including *Slc7a11*-KO, *Slc7a11*-Het, *Kcna1*-Het-*Slc7a11*-WT, *Kcna1*-Het-*Slc7a11*-KO, *Kcna1*-Het-*Slc7a11*-Het, and wild-type ($p<2.0 \times 10\text{-}16$). **B)** Summary of pairwise comparisons using Log-Rank test with Bonferroni corrections. Data were analyzed in R-studio and plotted using Prism v 9.0.0. Related to the **Genetic loss of functional Sxc modifies sudden death risk in *Kcna1*-KO mice** results section.