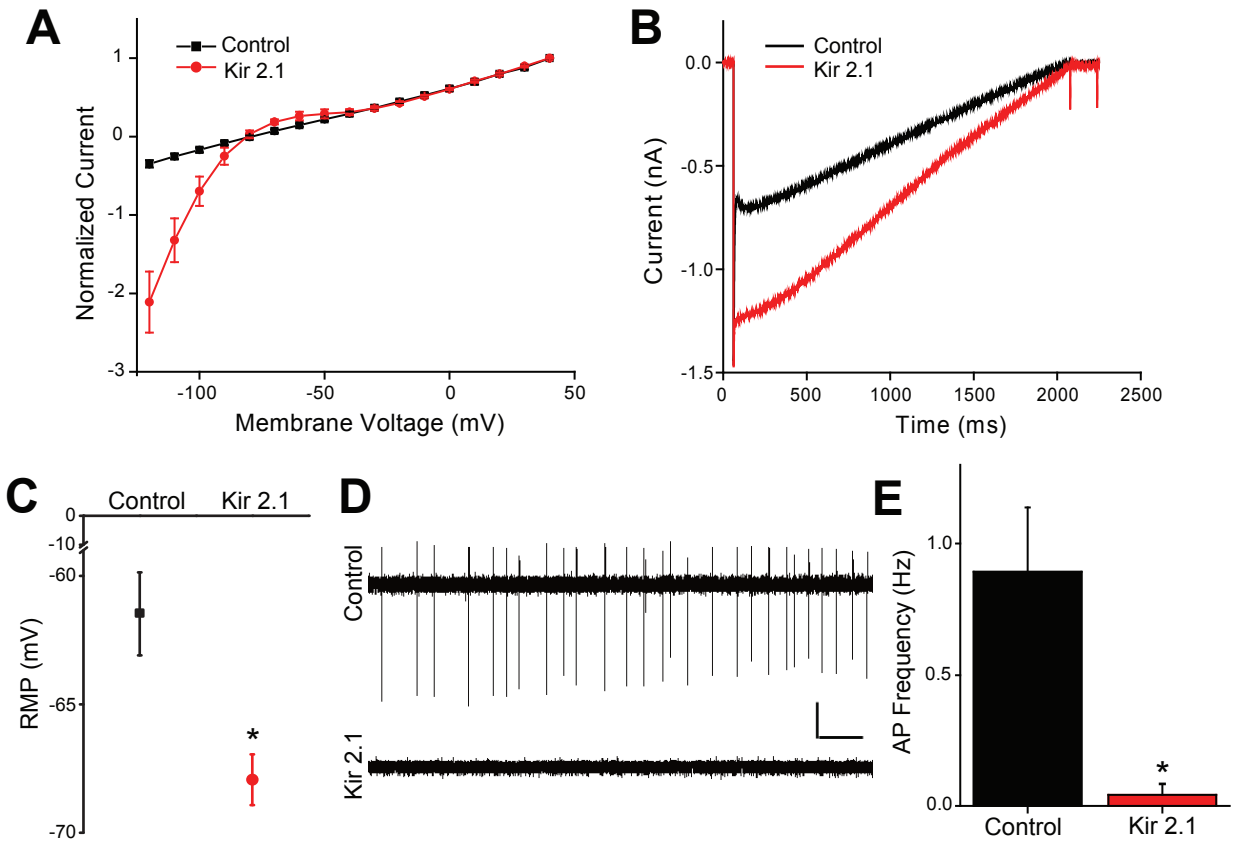

Figure S1. Overexpression of Kir2.1 effectively suppresses intrinsic activity in cultured HEK cells and hippocampal neurons.

A, Current was injected into either HEK cells transfected with Kir2.1 or untransfected (control) HEK cells. Clear inward rectification was seen in transfected HEK cells as compared to untransfeted cells at the more negative current injection. **B,** Example traces of a ramped current injection into a HEK cell transfected with Kir2.1 (red) and an untransfected neighboring HEK cell (black). More inward current is seen in the Kir2.1-expressing HEK cell. **C**, The resting membrane potential (RMP) of cultured hippocampal neurons transfected with Kir2.1 (n = 14) is significantly more hyperpolarized than untransfected control neurons (n = 15; *p = 0.002 by Student's t-test). **D**, Example traces of loose-patch recordings from either untransfected (control) or Kir2.1-transfected hippocampal neurons. There is a distinct lack of action potentials in the Kir2.1-transfected neurons. Scale bars = 10 pA (vertical), 1250 ms (horizontal). **E**, Untransfected control hippocampal neurons (n = 7) have a significantly higher firing rate than Kir2.1-transfected neurons (n = 11) as measured during loose-patch recordings (*p < 0.001 by Student's t-test).

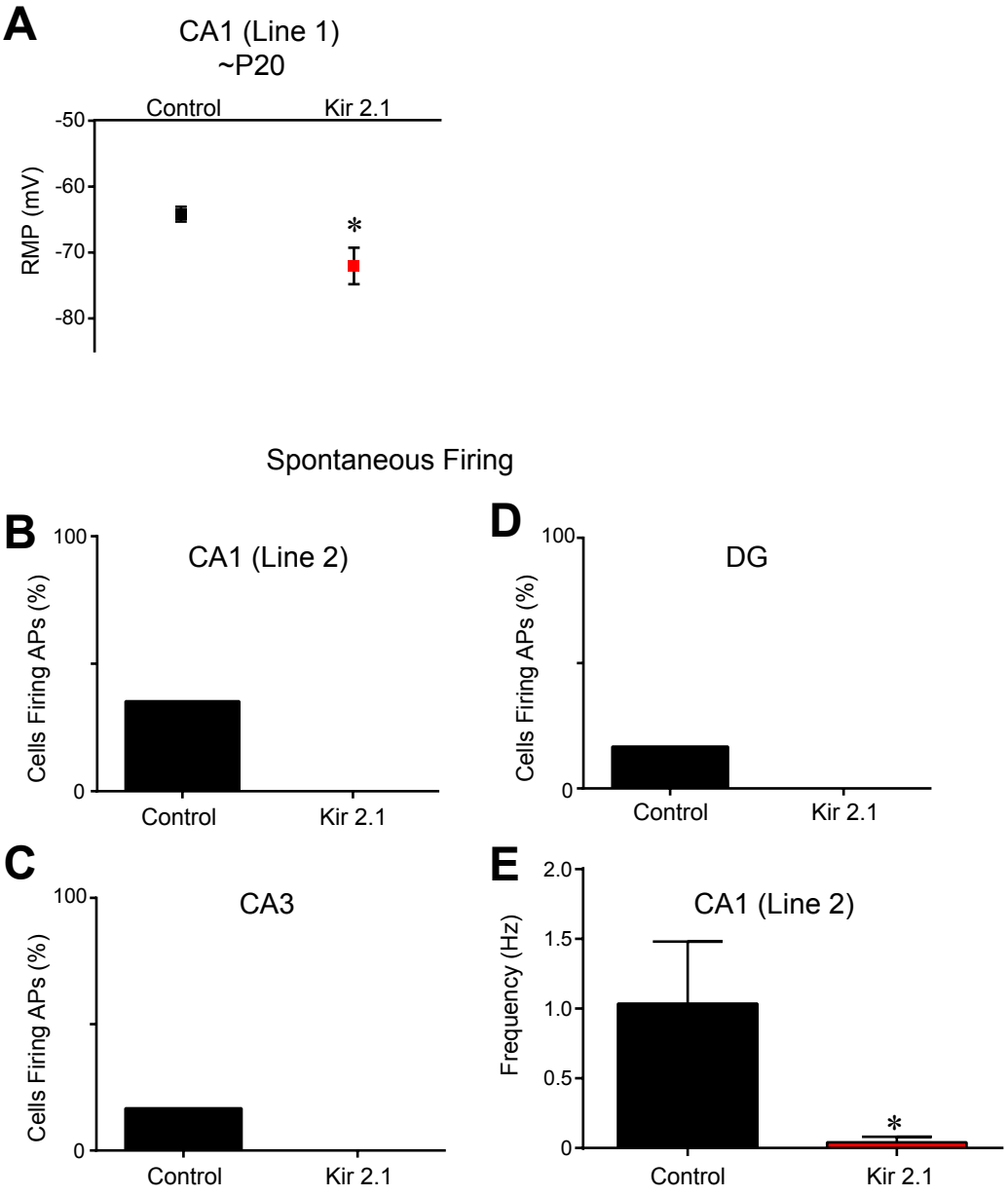

Figure S2. Kir2.1 expression effectively silences hippocampal neurons.
A, Resting membrane potential (RMP) is significantly hyperpolarized in CA1 (n = 17, 17; *p < 0.001 by Student's t-test) in Line-1 mice at P17-P23. **B-D,** The percentage of cells in CA1 (**B**), CA3 (**C**), or DG (**D**) demonstrating spontaneous action potential firing during whole-cell, current clamp recordings where I = 0 at P17-23. No spontaneous firing was seen in any of the hippocampal regions in Kir2.1-expressing neurons; thus, suppression of intrinsic excitability silences the hippocampal neurons. **E**, Loose-patch recordings were conducted in CA1 pyramidal neurons to measure spontaneous action potentials in control or neighboring Kir2.1-expressing neurons at P17-23. Action potentials were significantly less frequent in Kir2.1-expressing neurons (n = 9, 10; *p = 0.031 by Student's t-test).

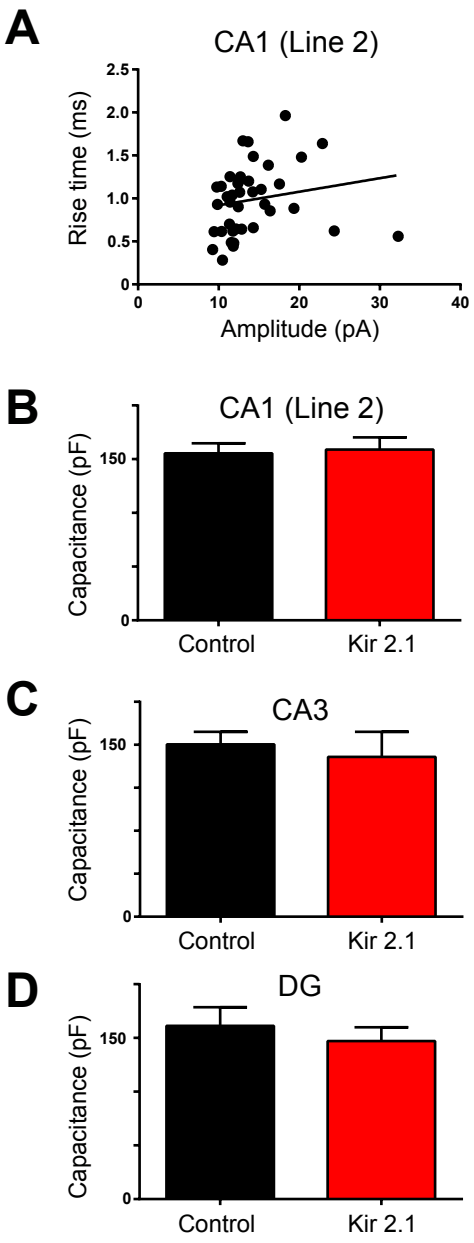

Figure S3. Normal electrophysiological properties of Kir2.1-expressing neurons throughout the hippocampus.

A, There was no correlation between rise time and amplitude of mEPSCs recorded in CA1, suggesting that there are no space clamp issues in Kir2.1 expressing neurons ($r^2 = 0.033$). **B-D,** Capacitance did not differ between control and Kir2.1-expressing neurons in CA1 (**B**, $p = 0.821$ by Student's t-test), CA3 (**C**, $p = 0.625$ by Student's t-test), DG (**D**, $p = 0.563$ by Student's t-test).

Line 1: Used for the analysis of CA1 and CA3 neurons

		Presynaptic Inputs			
Postsynaptic Cell		**Interneuron**	**CA3**	**DGCs**	**EC**
CA1 pyramidal neurons	25.87%	<1%	8.71%	NA	<1% (layer 3)
CA3 pyramidal neurons	8.71%	<1%	8.71%	4.06%	5.27% (layer 2)

Line 2: Used for the analysis of CA1 and DG neurons

		Presynaptic Inputs			
Postsynaptic Cell		**Interneuron**	**CA3**	**DGCs**	**EC**
CA1 pyramidal neurons	21.06%	<1%	3.55%	NA	<1% (layer 3)
Dentate granule cells	23.53%	<1%	NA	NA	9.19% (layer 2)

Supplemental Table 1. Percentage of cells expressing Kir 2.1, as determined by mCherry-immunoreactivity, in Lines 1 and 2. Co-staining with GABAergic markers reveals no apparent overlap between mCherry expression and GABAergic interneurons. Data from the enterhinal cortex reflect percentages in layer 2 for CA3 and DG and layer 3 for CA1.

	Neurogenesis	Peak of neurogenesis	Onset of synaptic defects by Kir2.1-expression	Days from peak of neurogenesis to synaptic defects by Kir2.1	Peak of synaptogenesis
CA1 pyramidal neurons	E12-18[1]	E14-16[1]	P15	30	P7-28[5]
CA3 pyramidal neurons	E12-18[1]	E14-16[1]	N/A	N/A	P3-21[6,7]
Dentate granule cells	E13.5-adulthood[2,3]	P0-P7[4]	P11	8	P1-15[5]

Supplemental Table 2. Relationship between the timing of neuronal development and influence of intrinsic excitability. CA1 and CA3 pyramidal neurons have a similar developmental time course. Dentate granule cells have a different developmental time course than the pyramidal neurons. The synaptic defects by activity suppression in the hippocampus do not appear correlated with the timing of neurogenesis.

[1]Hayashi et al. (2015) Frontiers in Neuroscience; [2]Li and Pleasure (2007) Progress in Brain Research; [3]Nicola et al. (2015) Frontiers in Neuroanatomy; [4]Schlessinger et al. (1975) Journal of Comparative Neurology; [5]Steward et al. (1991) Journal of Comparative Neurology; [6]Marchal and Mulle (2004) Journal of Physiology; [7]Lanore et al. (2012) Journal of Neuroscience.