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Supplementary Materials for

Tuning of fast-spiking interneuron properties by an activity-dependent transcriptional switch

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Materials and Methods

Mice

We generated *Lhx6-Cre;Er81$^{F/F}$;RCE* mice by breeding *Lhx6-Cre* mice (*18*) with mice carrying loxP-flanked *Er81* alleles (*19*) and Rosa26 Reporter CAG-boosted EGFP (*RCE*) mice (*36*). Similarly, we generated *PV-Cre;Er81$^{F/F}$;RCE* mice by breeding *PV-Cre* mice (*20*) with mice carrying *Er81$^{F/F}$* and *RCE* alleles. Controls include mice carrying the corresponding *Cre* alleles. In some experiments, we used *GAD67-GFP* (line G42) mice (*37*) to identify PV+ interneurons, *Er81$^{nLacZ/+}$* mice (*22*) to identify neurons that express or have expressed Er81, *NEX-Cre;RCE* to identify pyramidal cells (*38*), and *Nkx2.1-CreER;RCE* to identify Chandelier cells (*39*). Animal procedures were conducted in accordance with Spanish, United Kingdom and European regulations.

Electrophysiological recordings

P25-60 mice were deeply anaesthetized with isofluorane and perfused with ice-cold oxygenated, modified artificial cerebrospinal fluid (ACSF) containing (in mM): 248 sucrose, 3 KCl, 0.5 $CaCl_2$, 4 $MgCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, and 1 glucose, saturated with 95% O_2 and 5% CO_2. The animals were then decapitated, the brain placed in ice-cold oxygenated modified ACSF and 300 μm coronal slices were cut using a vibratome. Slices were then maintained at room temperature in ACSF containing (in mM): 124 NaCl, 3 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$ and 10 glucose saturated with 95% O_2 and 5% CO_2. For patch clamp recordings in whole-cell configuration, slices were transferred to a chamber and continuously superfused with ACSF at 32°C. We visualized interneurons located in layer II-III of the primary somatosensory cortex with infrared-differential interference optics through a 40x water-immersion objective. For targeting green fluorescent protein (GFP)-expressing neurons, cells were excited at 488 nm. Microelectrodes (6–10 MΩ) were pulled from borosilicate glass (1.5 mm outer diameter x 0.86 inner diameter) using a vertical P10 puller.

For voltage-clamp recordings, we used a cesium-gluconate (CsGlu)-based intracellular solution containing (in mM): 120 Cs-gluconate, 13 CsCl, 1 CaCl2, 10 HEPES, and 10 EGTA (pH 7.2-7.4, 275-285 mOsm). We used the CsGlu solution to measure spontaneous and miniature $GABA_A$ currents at the reversal potential for glutamatergic (+10 mV) events and glutamatergic currents at -70 mV while for current-clamp recordings, we used a potassium-gluconate-based intracellular solution containing (in mM): 140 K-gluconate, 10 HEPES, 2 NaCl, 4 KCl, 4 ATP, and 0.4 GTP. Neurobiotin (2-5 mg/ml) was added for post-recording immunocytochemistry. Interneurons were kept under current-clamp configuration with an Axoclamp 200A amplifier operating in a fast mode. Data were filtered on-line at 2 kHz, and acquired at a 20 kHz sampling rate of using pClamp 6.0.2 software. Series resistance (*R*s) was < 25 MΩ upon break-in and Δ *R*s < 20% during the course of the experiment. To determine the excitability of the PV+ interneurons, we performed 500 ms depolarizing steps of 1 pA (*21*) and used the first spike evoked by the minimum current needed to elicit an action potential applied from -70 mV. We considered a cell as "delayed" when the first elicited spike at threshold potential occurred more than 150 ms after the beginning of the 1 pA depolarizing step. The following parameters were also measured: resting membrane potential (Vrest), membrane resistance, membrane capacitance (Cm), threshold potential for spikes

(Vthreshold, defined as d*V*/dt = 10 mV/ms), rheobase, exponential fit of the slow ramp depolarization that remained just subthreshold during 500 ms current injections, AP amplitude, AP rise and total duration, after-hyperpolarization (AHP) amplitude and duration. We also performed 500 ms depolarizing steps of Δ 25 pA to determine the maximum firing frequency. Data analysis was performed off-line in Clampfit 10.2. For the recording of miniature events, we added TTX (1 μM) and GABAZINE (5 μM) to isolate mEPSCs at -70 mV, and APV (40μM) and NBQX (10 μM) at 0 mV to detect mIPSCs. For the analysis of release properties, stimulus delivery was performed by ISO STIM 01D (NPI) and PSCs induced by a train of stimuli (10Hz and 40Hz, 5 mA, 30 μs in layer V) were recorded in PV+ interneurons under APV (40 μM) and NBQX (10 μM) at + 10 mV for GABAergic synapses. Miniature events were analyzed using Mini Analysis Program.

For in vitro manipulation, Kainate (2.5 μM), KCl (7 mM) and Nifedipine (10 μM) were applied for 2 hours at 32°C, washed for 15 minutes in ACSF and immediately recorded. After recording, cells were fixed in PFA for immunohistochemistry.

Immunohistochemistry and image analysis

Mice were transcardially perfused with 4% paraformaldehyde (PFA) and 60 µm coronal brain sections were cut in a freezing microtome. We used the following primary antibodies: rabbit anti-Er81 (1:1000), mouse anti-PV (1: 2000; Swant), rat anti-somatostatin (1:500; Millipore), pig anti-Vglut1 (1:2000; Millipore), chicken anti-GFP (1:3000, Aves Labs), rabbit anti-Ankyrin-G (1:500; Santa Cruz), mouse anti-Kv1.1 antibodies (1:1000; Neuromab), and mouse anti-βGal (1:1000; Promega). We used the following primary antibodies: donkey anti-rabbit 555, donkey anti-mouse 488, goat anti-rat 633, and goat anti-chicken 488 antibodies (1:200; Molecular Probes), biotin-conjugated anti-guinea pig (1:400; Jackson), horse biotin-conjugated anti-mouse (1:400; Vector), and 647-streptavidine antibodies. The specificity of Er81 antibodies was tested in *Er81* null mice (*22*) (Fig. S12).

Stained sections of control and mutant mice were imaged during the same imaging session using an inverted confocal microscope. Analyses were performed with the same laser power, photomultiplier gain, pinhole and detection filter settings (1024x1024 resolution, 12 bits). Er81 fluorescence signal was quantified using ImageJ by measuring the Er81 immunofluorescence in the nuclear (DAPI+) region of PV+ interneurons, with background subtraction. We binned cells into three groups based on their levels of fluorescence intensity: cells displaying signals lower than 1.3 fold of background levels were considered as non-expressing (-), cells holding signals 1.3 to 1.6 fold higher than the background were considered as expressing (+), and cells with signals higher than 1.6 fold of the background were considered as highly expressing (++). For the quantification of Vglut1+ boutons contacting the soma of PV+ interneurons, we quantified stacks of confocal sections acquired at 0.3 µm step size with a 100x objective and 2x digital zoom magnification, as described before (*40*).

After patch clamp recordings, slices were immediately fixed in 4% PFA at 4°C, rinsed in PB, left for 12h in PB-sucrose 20%, and shock-frozen at -80°C. They were thawed at RT, rinsed in PB and incubated for 30 min in 1% H_2O_2 in PB. Slices were washed with PB and KPBS and then incubated for 3h in ABC complex at a dilution of 1:100 in KPBS with 0.3% triton. We checked the neurochemical content of the recorded

GFP-cells using the following primary antibodies: chicken anti-GFP (1:3000), mouse anti-PV (1:2000) and rabbit anti-Er81 (1:1000-1:5000, kindly provided by T. Jessell and S. Arber). We used the following secondary antibodies: goat anti-chicken 488 (1:400), donkey anti-mouse 488 (1:200) donkey anti-mouse 555 (1:200) or donkey anti-rabbit 555 (1:200). Neurobiotin-ABC complex was revealed by Cy3-, Cy2- or 647-streptavidine (1:200). Slides were finally rinsed in PBS and mounted in Mowiol.

Labeling of chandelier cells

We performed a single tamoxifen injection (1mg/10g mouse) on E17 pregnant *Nkx2.1-CreER;RCE* females to label layer II Chandelier cells, as described before (*39*). Mice were sacrificed at P25 and processed for immunohistochemistry.

Western blots

Mouse somatosensory cortex tissue was homogenized in lysis buffer containing (in mM) 50 Tris pH 7.5, 150 NaCl, 5 EDTA, 0.5 mM prevanadate with protease inhibitor cocktail and 1% Triton X-100. Samples were denatured and run on 10% SDS–PAGE gels. Gels were electrophoretically transferred onto PVDF membranes. Membranes were blocked with 5% BSA in TBST (20 mM Tris-HCl, pH 7.5, 150 mM NaCl and 0.1% Tween20) for 1 h and probed with the following primary antibodies: anti-α-tubulin (1:4000), anti-Kv1.1 external epitope (clone K36/15), or anti-Kv1.1 internal epitope (clone 20/78, 1:1000). Subsequently, blots were treated with horseradish-peroxidase-conjugated secondary antibodies and ECL western blotting detection reagents. Signals were acquired as 16 bit images with a Bio-imager analyzer and quantified with Quantity One 1D Analysis Software.

Fluorescence-Activated Cell Sorting (FACS)

We prepared slices from P60-P65 *PV-Cre;RCE* mice as for electrophysiological recordings and dissected the superficial layers of the somatosensory cortex in ACSF. We then transferred the tissue to a digestion solution (5% trehalose and 10% pronase E in oxygenated ACSF) at 37°C during 30 minutes. We washed the dissociated tissue in ACSF/Trehalose and transferred it to 5 ml triturating solution (optiMEM, glucose, Mg-Kynurenate, AP-V and trehalose) at 4°C, and we triturated it with a glass polish pipette. Subsequently, the cell suspension was centrifuged at 800 rpm for 5 minutes in 4°C. The pellet was resuspended in 200 μl of fresh triturating solution and the cell suspension was filtered through a 40 μm thick mesh filter. The filtered solution was transferred to a snap cap tube, ready for FACS. Immunohistochemistry was performed on a fraction of post-sorted cells (a minimum of 300) to check the purity of the sample (>95%). The remaining cells were collected in 350 μl of lysis buffer prior to storage at -80°C. For quantitative PCR (qPCR), we performed triplicates of each experiment using TagMan Gene Expression Assays.

Single-cell qPCR

Patch clamped neurons were aspirated into a recording pipette by application of gentle negative pressure. The content was ejected into a sterile siliconised tube containing nuclease-free water, DTT 0.1 M, RNase inhibitor (10 U/ μL), random hexanucleotides (3 μg/μl) and placed immediately on dry ice. The mixture was heated at 70ºC for 10',

chilled on ice and then supplemented with 5x first-strand buffer (250 mM Tris HCl, 375 mM KCl, 15 mM $MgCl_2$), mixed deoxynucleotide triphosphates (10 mM) and SuperScript II reverse transcriptase (Invitrogen) during 10' at room temperature. Solution was incubated at 42ºC for 50', at 37ºC for 60' and then at 70ºC for 15' with RNase H (2 U/μl). qPCR was then performed on cDNA with Er81 and GADPH TaqMan Gene Expression Assays.

Chromatin Immunoprecipitation (ChiP) Assay

ChIP assay was performed following the procedures described in the MagnaChiP G tissue kit. Cortical layers (I-IV) were dissected from P60-P65 mice, and DNA-protein complexes were immunoprecipitated with rabbit anti-Er81 (5μg) or rabbit anti-GFP (5μg) antibodies. We designed primers corresponding to conserved (between mouse and human) ETS binding site sequences. Er81 region 1, forward: GGGTGGAGTTTAAGGTGGGAAG; Er81 region 1, reverse: CACATCCTCCTGGCTCTATTGTG); Er81 region 2, forward: CCAGTTCCTCGGAGGTTCAAA; Er81 region 2, reverse: CTTCATCTTCCCTGCTCCGT.

Locomotor training

P60-P65 mice were trained in an accelerating rod (5-50 RPM) following a protocol of 4 trials (5 minutes) every 5 minutes during 7 days. The control group includes mice trained with the same protocol but in a continuous rod at 10 RPM, as previously described (*7*).

In utero retroviral infection

We injected low titer retroviruses in the MGE of E14.5 *Er81*$^{+/+}$ and *Er81*$^{F/F}$ embryos using an ultrasound back-scattered microscope, as described previously (*41*).

In utero electroporation

We performed in utero electroporation in the developing pallium of E14.5 embryos to target superficial cortical pyramidal neurons (layers II-III). In brief, mice were anesthetized with isoflurane (3% during induction, 2.5% during surgery and 1% during suture). A solution containing the expression vector of interest (*Gfp* or *Gfp* and Kir2.1) together with the dye Fast Green (0.3 mg/ml) was injected through the uterine wall into one of the lateral ventricles of the embryos using a 30.5ga needle. Five electrical pulses (amplitude, 50 V; duration, 50 ms; intervals, 100 ms) were delivered through the head of each embryo using a square-wave electroporation generator and tweezer-type circular electrodes (diameter, 1 cm). The uterine horns were then returned into the abdominal cavity, the wall and the skin were sutured, and embryos were allowed to continue their normal development.

Statistics

Values, size of experimental sample and P values for all experiments are summarized in Fig. S13. Statistical analysis was carried out in Origin 7.0 software or SPSS. P values < 0.05 were considered statistically significant. Data are presented as mean and SEM. Principal component analysis (PAC) was performed on R software using

FactoMineR (*42*). In PAC graphs, each circle represents a recorded neuron, the ellipse the 95% confidence, and the square the center of mass. For these analyses, we used different parameters measuring basic membrane properties such as Vrest, Cm, Rm, rheobase, Vthreshold, AP amplitude, AP duration, latency to the first spike, AHP amplitude and AHP duration. Each dimension represents an unbiased clustering of parameters. Statistical differences between groups were examined by non-paired two-tailed Student's *t* test. For rod and Kir2.1 experiments, the Chi-square test was used. To plot data in cumulative probabilities and linear fit, we used Origin 7.0 software.

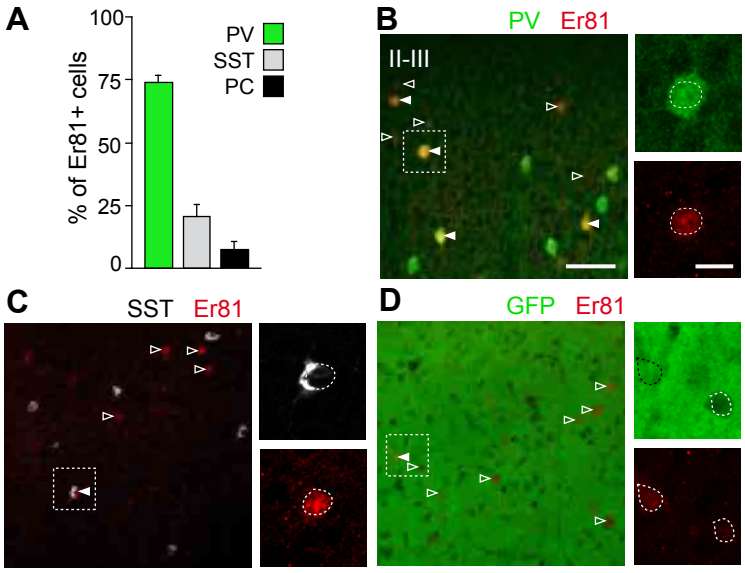

Fig. S1
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Fig. S1. Er81 expression in cortical layer II-III neurons. (**A**) Er81 is primarily expressed by parvalbumin-expressing (PV+) interneurons in layer II-III of the postnatal cortex. A small proportion of Er81 are somatostatin-expressing (SST+) interneurons and pyramidal cells (PC). Representative images of PV+/Er81+ (**B**), SST+/Er81+ (**C**) and PC+/Er81+ (**D**) cells. Pyramidal cells are identified by GFP expression in *NEX-Cre;RCE* mice. Scale bars, 50 μm (**B** to **D**) and 25 μm (insets). Graphs represent mean ± s.e.m. Type or paste caption here. Maintain formatting or style as "SM caption." Create a page break and paste in the figure above the caption.

A

	Er81+	Er81-	*P* values
Vrest (mV)	-70.0 ± 0.6	- 67.5 ± 1.6	0.09
Cm (pF)	31.9 ± 2.8	31.2 ± 3.8	0.88
Rm (MΩ)	135.3± 6.8	124.1 ± 7.3	0.30
Rheobase (pA)	273.0 ± 20.4	186.5 ± 42.2	(*) 0.04
V threshold (mV)	-32.2 ± 0.6	-37.3 ± 2.6	(*) 0.02
AP amplitude (mV)	52.7 ± 1.6	52.2 ± 2.9	0.97
AP rise duration (ms)	0.22 ± 0.01	0.27 ± 0.02	(*) 0.02
AP tot duration (ms)	0.71 ± 0.03	0.90 ± 0.05	(**) 0.002
AHP amplitude (mV)	20.21 ± 0.65	20.50 ± 1.40	0.83
AHP duration (ms)	0.12 ± 0.01	0.15 ± 0.01	0.07
Latency to the 1st spike (ms)	297.0 ± 16.8	147.6 ± 24.5	(*) 0.03
MFF (Hz)	140.9 ± 10.2	151.4 ± 16.0	0.57
Number of cells	22	11	

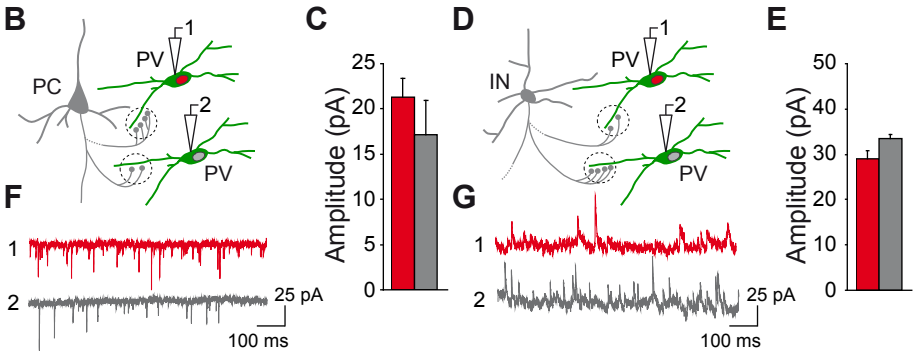

Fig. S2
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Fig. S2. Electrophysiological properties of Er81+ and Er81- PV+ interneurons in cortical layer II-III. (A) Intrinsic electrophysiological properties of Er81+ and Er81- PV+ interneurons in cortical layer II-III at P25. Vrest: resting membrane potential; Cm: membrane capacitance; Rm: membrane resistance; Vthreshold: Threshold potential for spike; AP: Action potential; AHP: After hyperpolarization; MFF: Maximum firing frequency. (**B** and **D**) Schematic of mEPSCs and mIPSCs recordings in PV+ interneurons. PC: pyramidal cell; PV: PV+ interneuron; IN: Interneuron. (**F** and **G**) Representative traces. (**C** and **E**) Measurements of mEPSC (n = 15 and 6 cells, respectively; $P > 0.05$) and mIPSC (n = 7 and 4 cells; $P > 0.05$) amplitudes in PV+ interneurons.

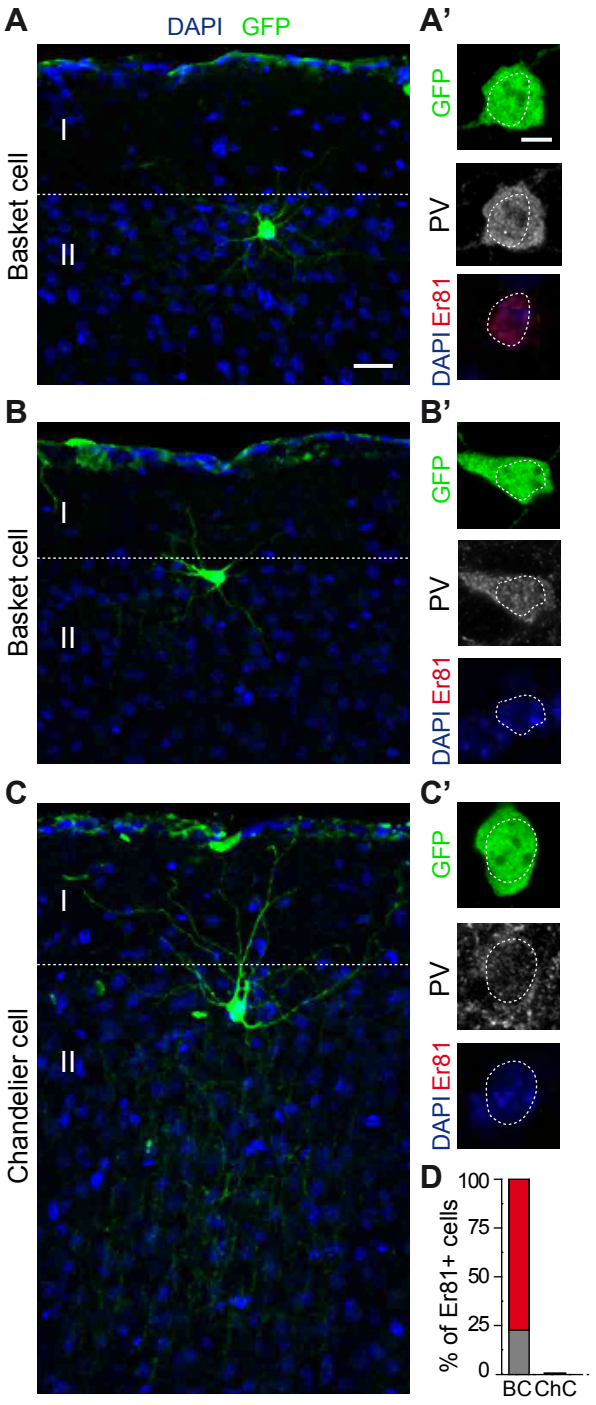

Fig. S3
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Fig. S3. Er81 expression in fast spiking interneurons in cortical layer II-III. (**A** to **B'**) Er81 is expressed in most basket PV+ cells (BC) (A, A'), but not in all **(**B, B'**)**. (**C** and **C'**) Chandeliers cells (CC) do not express Er81. (**D**) Relative proportion of layer II-III basket and chandeliers cells expressing Er81 (*n* = 45, 12 and 32 cells, respectively). Scale bars, 25 μm (**A** to **C)** and 5 μm (**A'** to **C'**). Graphs represent mean ± s.e.m.

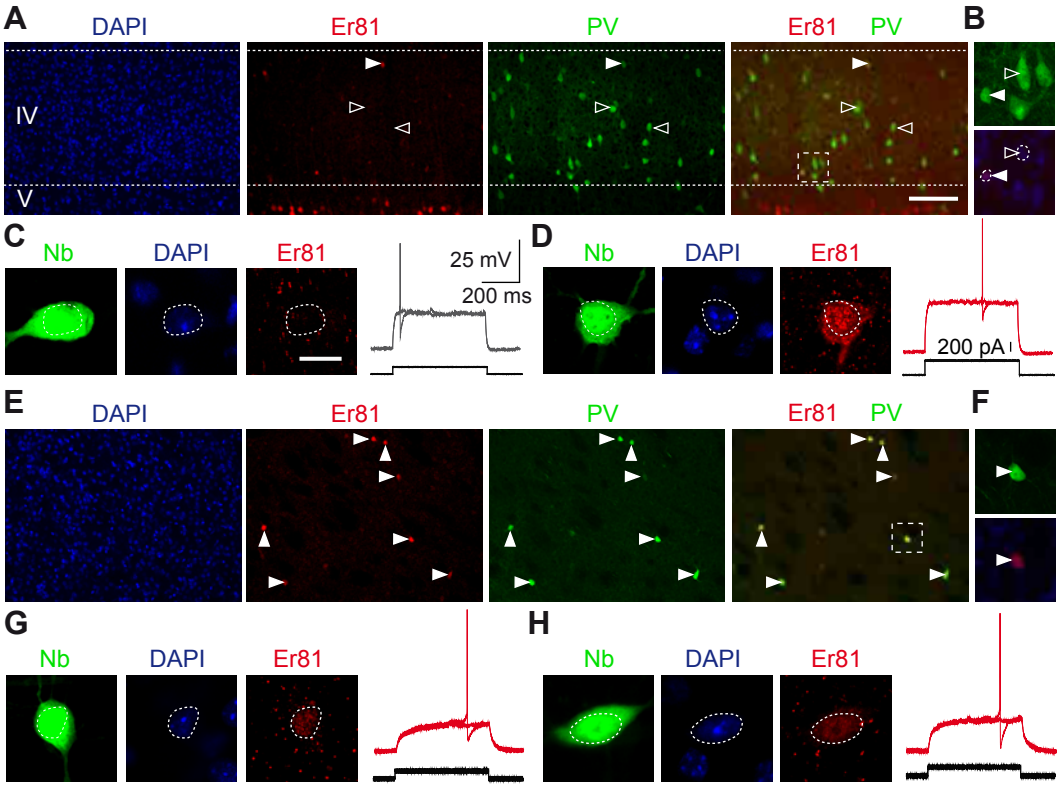

Fig. S4
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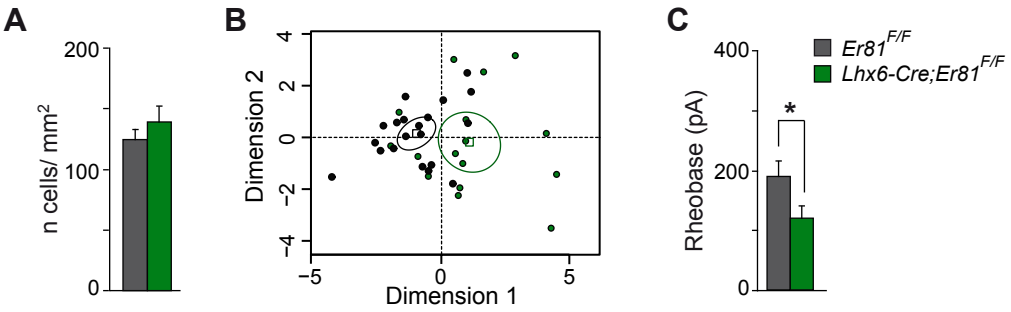
Fig. S4. Er81 expression in cortical layer IV and striatal interneurons. (**A** and **B**) Er81 expression in layer IV PV+ interneurons. (**C** and **D**) Most recorded PV+ interneurons (labeled with neurobiotin, NB) in layer IV exhibit early firing at near threshold potential (**C**), although about a third display latency to the first spike (n = 14 cells). (**E** and **F**) All striatal PV+ interneurons express Er81. (**G** and **H)** All recorded PV+ interneurons in the striatum exhibit delayed firing at near threshold potential (n = 5 cells). Scale bars, 100 μm (**A** and **E)** and 10 μm (**C**, **D**, **G** and **H**).



D

	Lhx6-Cre;Er81$^{+/+}$	*Lhx6-Cre;Er81*$^{F/F}$	***P* values**
Vrest (mV)	-70.1 ± 1.7	-71.3 ± 1.6	0.61
Cm (pF)	31.2 ± 2.3	32.8 ± 3.5	0.69
Rm (MΩ)	134.1± 9.7	165.3 ± 13.3	0.06
Rheobase (pA)	193.5 ± 26.1	122.6 ± 19.1	(*) 0.04
V threshold (mV)	-36.9 ± 1.0	-43.2 ± 2.2	(**) 0.009
AP amplitude (mV)	52.3 ± 2.2	52.2 ± 2.9	0.97
AP rise duration (ms)	0.22 ± 0.01	0.27 ± 0.02	(*) 0.02
AP tot duration (ms)	0.71 ± 0.03	0.90 ± 0.05	(**) 0.002
AHP amplitude (mV)	21.56 ± 0.77	18.56 ± 1.41	(*) 0.04
AHP duration (ms)	0.12 ± 0.01	0.15 ± 0.01	0.07
Latency to the 1st spike (ms)	236.4 ± 28.8	138.8 ± 20.8	(*) 0.01
Exponential fit (mV)	3.8 ± 0.6	2.1 ± 0.6	(*) 0.04
MFF (Hz)	164.9 ± 16.7	142.7 ± 10.9	0.25
Number of cells	20	18	

Fig. S5
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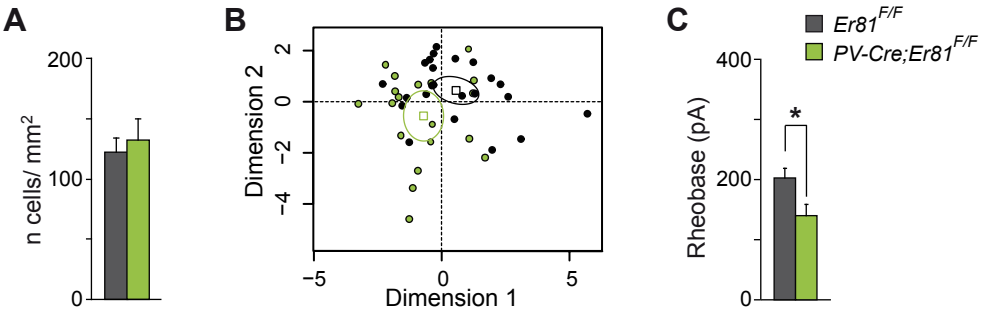
Fig. S5. Embryonic deletion of Er81 from PV+ interneurons impacts their intrinsic properties. (**A**) Density of PV+ interneurons in layer II-III in control and *Lhx6-Cre;Er81* conditional mutants ($n = 3$, $P = 0.3$). (**B**) Individual factor maps from principal component analysis performed on recorded cells segregates PV+ interneurons as distinct clusters in control and *Lhx6-Cre;Er81* conditional mutants. (**C**) Mean rheobase is decreased in *Lhx6-Cre;Er81* conditional mutants compared to controls ($n = 20$ and 18 cells, $P < 0.05$). (**D**) Intrinsic electrophysiological properties of PV+ interneurons in cortical layer II-III of control and *Lhx6-Cre;Er81* conditional mutants. Vrest: resting membrane potential; Cm: membrane capacitance; Rm: membrane resistance; Vthreshold: Threshold potential for spike; AP: Action potential; AHP: After hyperpolarization; MFF: Maximum firing frequency. Graphs represent mean ± s.e.m.



D

	PV-Cre;Er81$^{+/+}$	*PV-Cre;Er81*$^{F/F}$	***P* values**
Vrest (mV)	-72.4 ± 0.9	-69.4 ± 1.3	0.07
Cm (pF)	27.7 ± 1.2	27.8 ± 1.7	0.97
Rm (MΩ)	134.1 ± 7.9	131.4 ± 7.4	0.77
Rheobase (pA)	202.1 ± 16.2	139.4 ± 18.8	(*) 0.02
V threshold (mV)	-37.5 ± 1.2	-42.9 ± 2.0	(*) 0.02
AP amplitude (mV)	48.6 ± 2.2	50.2 ± 2.2	0.62
AP rise duration (ms)	0.28 ± 0.02	0.28 ± 0.02	0.94
AP tot duration (ms)	0.82 ± 0.04	0.87 ± 0.03	0.32
AHP amplitude (mV)	17.08 ± 0.79	17.31 ± 0.94	0.85
AHP duration (ms)	0.16 ± 0.01	0.15 ± 0.01	0.54
Latency to the 1st spike (ms)	220.03 ± 21.38	92.38 ± 13.33	(***) 0.00004
Exponential fit (mV)	4.3 ± 0.4	1.3 ± 0.7	(***) 0.0002
MFF (Hz)	147.7 ± 10.0	182.9 ± 15.0	0.04
Number of cells	29	20	

Fig. S6
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Fig. S6. Postnatal deletion of Er81 from PV+ interneurons impacts their intrinsic properties. (**A**) Density of PV+ interneurons in layer II-III in control and *PV-Cre;Er81* conditional mutants ($n = 3$; $P = 0.54$). (**B**) Individual factor maps from principal component analysis performed on recorded cells segregates PV+ interneurons as distinct clusters in control and *PV-Cre;Er81* conditional mutants. (**C**) Mean rheobase is decreased in *PV-Cre;Er81* conditional mutants compared to controls ($n = 29$ and 20 cells; $P < 0.05$). (**D**) Intrinsic electrophysiological properties of PV+ interneurons in cortical layer II-III of control and *PV-Cre;Er81* conditional mutants. Vrest: resting membrane potential; Cm: membrane capacitance; Rm: membrane resistance; Vthreshold: Threshold potential for spike; AP: Action potential; AHP: After hyperpolarization; MFF: Maximum firing frequency. Graphs represent mean ± s.e.m.

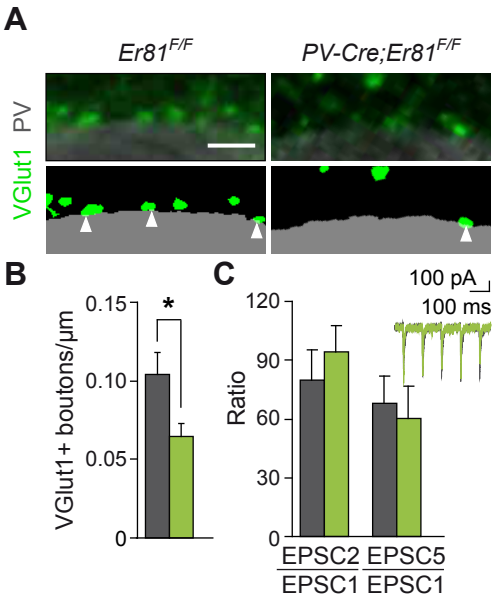

Fig. S7
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Fig. S7. Reorganization of excitatory synapses received by PV+ interneurons in conditional *Er81* mutants. (**A**) Single confocal images and their corresponding software-processed images illustrating VGlut1+ boutons contacting PV+ interneurons in P60 control and *PV-Cre;Er81* conditional mutants. (**B**) Density of VGlut1+ boutons contacting the soma of PV+ cells in control and *PV-Cre;Er81* conditional mutants ($n = 3$; $P < 0.05$). (**C**) Analysis of paired-pulse ratios (PPRs) revealed no significant differences in release probability between control and *Er81* mutant cells ($n = 4$ and 5 cells; sEPSC2/sEPSC1 ratio: $P = 0.5$; sEPSC5/ SEPSC1 ratio: $P = 0.7$). Scale bar, 2 μm (**A**). Graphs represent mean ± s.e.m.

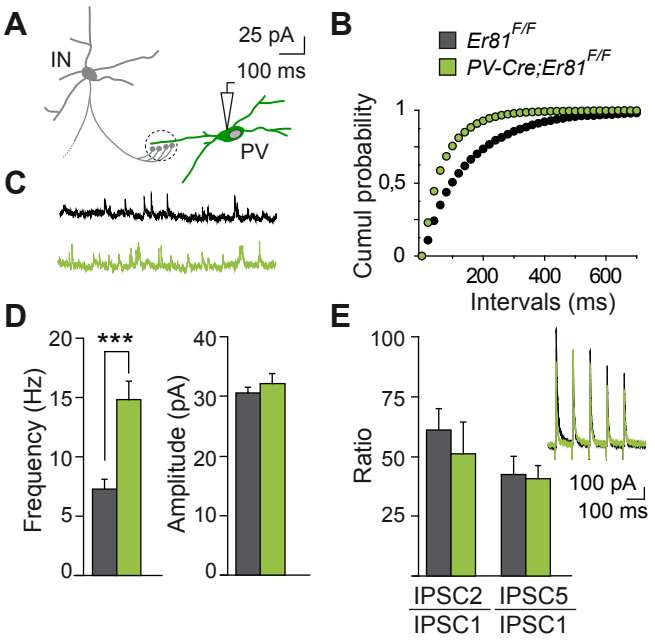

Fig. S8
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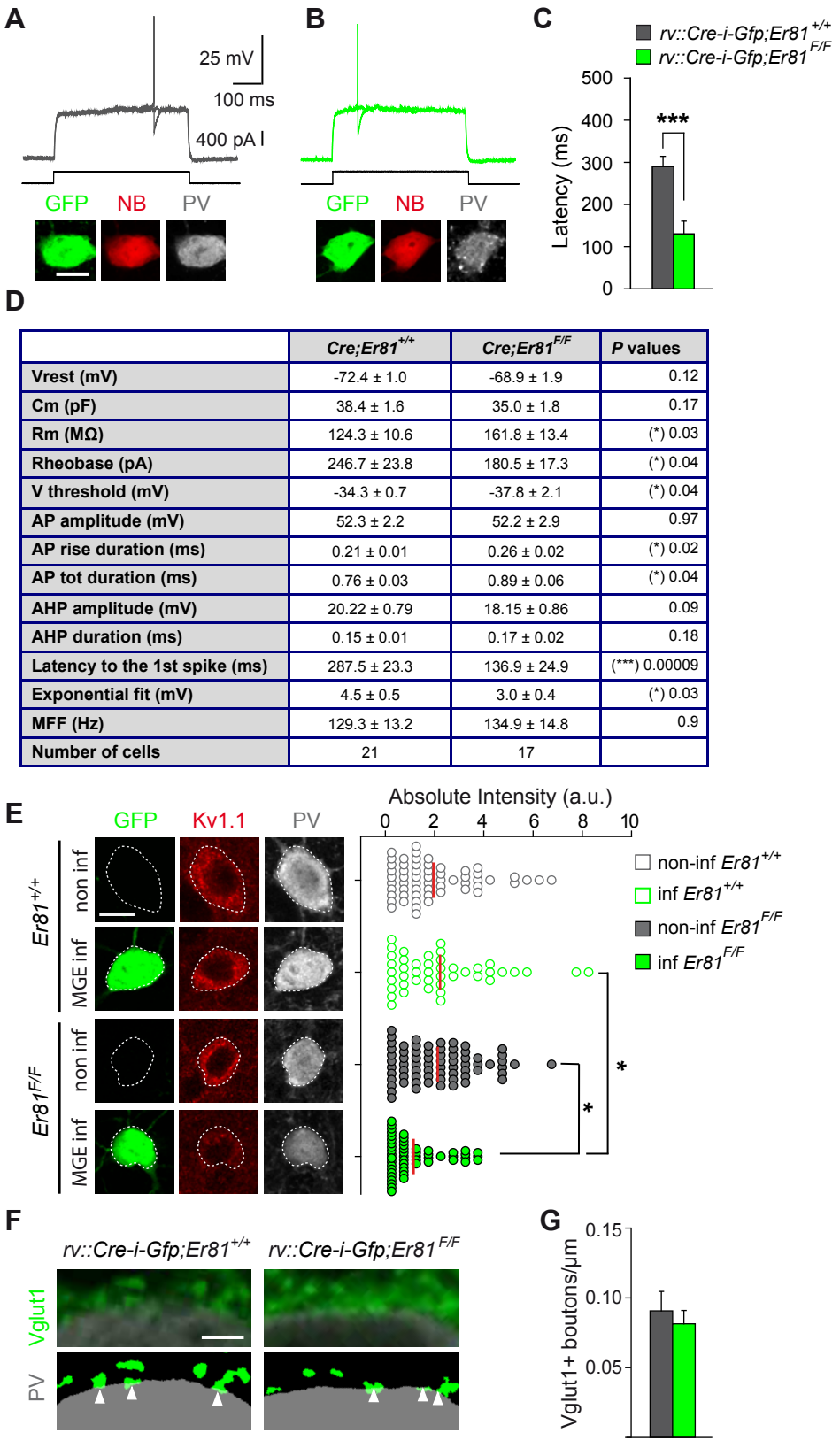
Fig. S8. Reorganization of inhibitory synapses received by PV+ interneurons in conditional *Er81* mutants. (**A**) Schematic of mIPSCs recordings in PV+ interneurons. (**B**) Cumulative probability plot of mIPSCs frequencies. (**C**) Representative traces. (**D**) Mean frequency (n = 11 and 10 cells; $P < 0.001$) and amplitude (n = 11 and 10 cells; P = 0.58) in PV+ cells from control and *PV-Cre;Er81* conditional mutants. (**E**) Analysis of paired-pulse ratios (PPRs) revealed no significant differences in GABA release probability between control and *Er81* mutant cells (n = 6 and 5 cells ; sIPSC2/sIPSC1 ratio: P = 0.5; sIPSC5/ SIPSC1 ratio: P = 0.8). The inset illustrates representative traces of inhibitory currents recorded from control and mutant PV+ interneurons after train stimulation. Graphs represent mean ± s.e.m.



	Cre;Er81$^{+/+}$	*Cre;Er81*$^{F/F}$	*P* values
Vrest (mV)	-72.4 ± 1.0	-68.9 ± 1.9	0.12
Cm (pF)	38.4 ± 1.6	35.0 ± 1.8	0.17
Rm (MΩ)	124.3 ± 10.6	161.8 ± 13.4	(*) 0.03
Rheobase (pA)	246.7 ± 23.8	180.5 ± 17.3	(*) 0.04
V threshold (mV)	-34.3 ± 0.7	-37.8 ± 2.1	(*) 0.04
AP amplitude (mV)	52.3 ± 2.2	52.2 ± 2.9	0.97
AP rise duration (ms)	0.21 ± 0.01	0.26 ± 0.02	(*) 0.02
AP tot duration (ms)	0.76 ± 0.03	0.89 ± 0.06	(*) 0.04
AHP amplitude (mV)	20.22 ± 0.79	18.15 ± 0.86	0.09
AHP duration (ms)	0.15 ± 0.01	0.17 ± 0.02	0.18
Latency to the 1st spike (ms)	287.5 ± 23.3	136.9 ± 24.9	(***) 0.00009
Exponential fit (mV)	4.5 ± 0.5	3.0 ± 0.4	(*) 0.03
MFF (Hz)	129.3 ± 13.2	134.9 ± 14.8	0.9
Number of cells	21	17	

Fig. S9
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Fig. S9. Er81 cell autonomously controls intrinsic properties on PV+ interneurons. (**A** and **B**) Representative traces of recorded PV+ interneurons labeled after MGE infection with Cre-expressing viruses in control (**A**) and $Er81^{F/F}$ (**B**) mice (neurobiotin, NB). In these experiments, very few cells are labeled in each animal through low titer viral infection of their progenitor cells in the embryo. (**C**) Mean latency to the first spike in control and *Er81* mutant PV+ interneurons ($n = 21$ and 17; $P < 0.001$). (**D**) Intrinsic electrophysiological properties of control and Er81 mutant PV+ interneurons in cortical layer II-III. Vrest: resting membrane potential; Cm: membrane capacitance; Rm: membrane resistance; Vthreshold: Threshold potential for spike; AP: Action potential; AHP: After hyperpolarization; MFF: Maximum firing frequency. (**E**) Expression of Kv1.1 in PV+ interneurons labeled after MGE infection with Cre-expressing viruses in control and $Er81^{F/F}$ mice. Adjacent non-labeled cells were also used as controls. The graph illustrates the corresponding distribution of Kv1.1 fluorescence intensities for each group ($n = 44$, 59, 44 and 54; $P < 0.05$ and $P < 0.01$). (**F**) Single confocal images and their corresponding software-processed images illustrating VGlut1+ boutons contacting PV+ interneurons infected with Cre-expressing viruses in control and $Er81^{F/F}$ mice. (**G**) Mean density of VGlut1+ boutons contacting the soma of PV+ interneurons infected with Cre-expressing viruses in control and $Er81^{F/F}$ mice ($n = 3$; $P = 0.6$). Scale bars, 5 μm (**A** and **B)** and 2 μm (**F**). Graphs represent mean ± s.e.m.

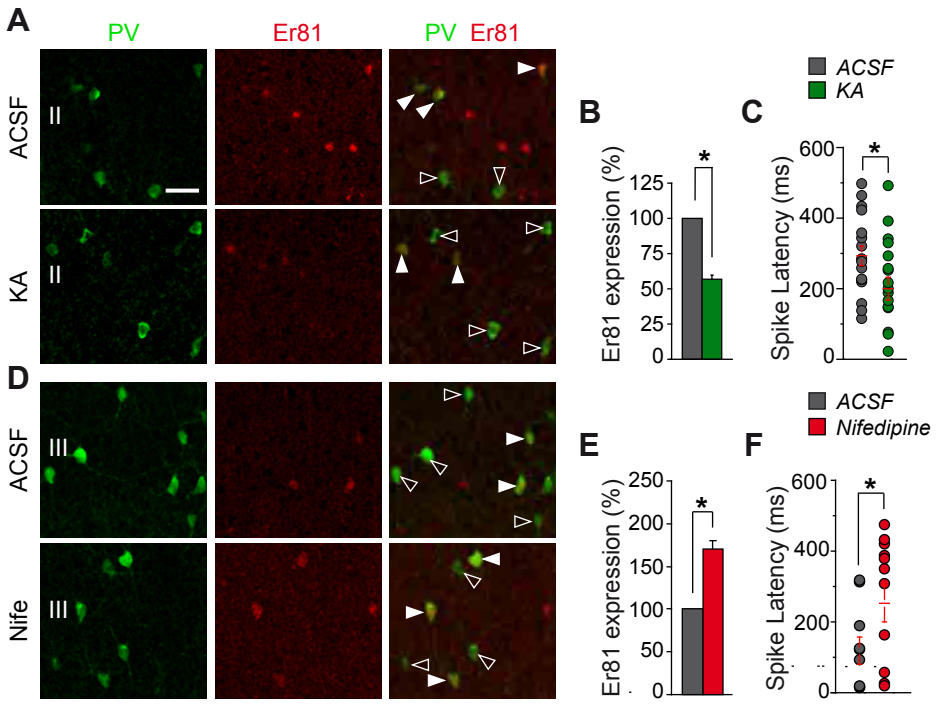

Fig. S10
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Fig. S10. Er81 expression is modified by changes in network activity in vitro. (**A**) Er81 expression in layer II PV+ interneurons in slices treated with artificial cerebrospinal fluid (ACSF) or 2 μM KA. (**B**) Percentage of Er81+/PV+ interneurons in ACSF and KA treated slices (n = 306 and 244 cells; $P < 0.05$). (**C**) Mean spike latency in ACSF and KA treated slices (n = 17 and 18 cells; $P < 0.05$). (**D**) Er81 expression in layer III PV+ interneurons in slices treated with ACSF or 10 μM Nifedipine. **(E**) Percentage of Er81+/PV+ interneurons in ACSF and Nifedipine treated slices (n = 236 and 199 cells; $P < 0.05$). (**F**) Mean spike latency in ACSF and Nifedipine treated slices (n = 13 and 12 cells; $P < 0.05$). Scale bar, 25 μm (**A** and **D**). Graphs represent mean ± s.e.m.

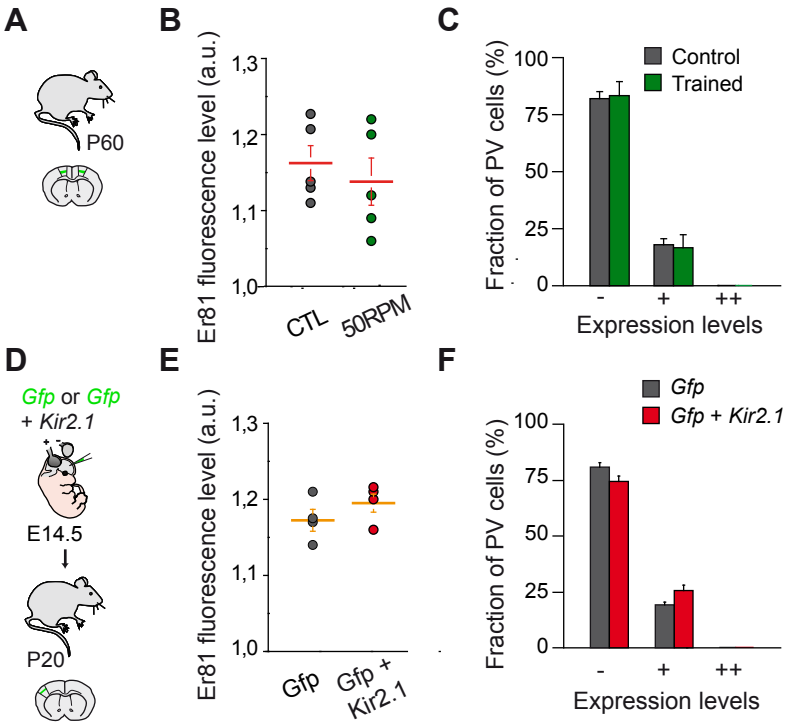

Fig. S11
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Fig. S11. Absence of activity-dependent changes in Er81 expression in layers IV and V. (**A)** Analysis of Er81 expression in layer V of P60 mice after motor training. (**B**) Er81 fluorescence intensity levels in PV+ interneurons from control and trained animals ($n = 5$; $P = 0.54$). (**C**) Distribution of Er81 fluorescence intensities in layer V PV+ interneurons from control and trained mice ($n = 5$; $P = 0.8$). Cells were grouped into three main classes based on their levels of fluorescence intensity for nuclear Er81: < 1.3 fold of background levels (-), 1.3 to 1.6 fold higher than background (+), and > 1.6 fold of background levels (++). (**D**) Experimental design and analysis of Er81 expression in layer IV of P20 mice. (**E**) Mean Er81 fluorescence intensity in PV+ interneurons from control and *Kir2.1*-electroporated mice ($n = 4$; $P = 0.27$). (**F**) Distribution of Er81 fluorescence intensities in layer IV PV+ interneurons from control and *Kir2.1*-electroporated mice ($n = 4$; $P = 0.1$), groups as in (**C**).

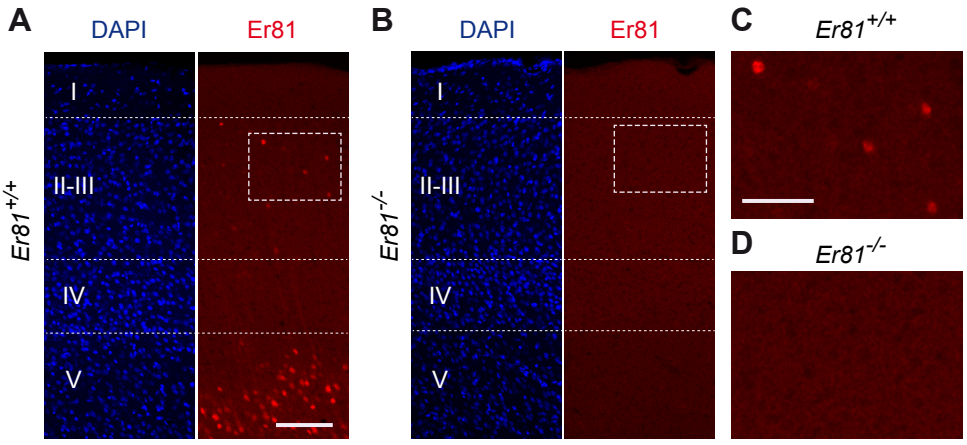

Fig. S12
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Figure S12. Er81 protein expression is absent in *Er81* null mutant mice. (**A** and **C**) Er81 is prominently expressed in layer 5 pyramidal neurons and in scattered cells throughout the entire cortex in wild type mice. (**B** and **D**) Er81+ cells are not the detected in the cortex of *Er81*$^{-/-}$ mice. Scale bars, 100 μm (**A** and **B)** and 50 μm (**C** and **D**).

Fig. S13

FIGURE 1	Measurement	Values	N	Statistical	P value
Fig 1B	PV+/Er81+ cells (all layers) (mean ± SEM)	I: 5.2 ± 5.2 %; II-III: 62.8 ± 2.4 %; IV: 24.7 ± 6.3 % V: 12.5 ± 4.2 %; VI: 11.7 ± 5.2 %	11 mice		
Fig 1B	PV+/Er81+ cells (layer II-III) (mean ± SEM)	II: 74.1 ± 4.4%; layer III: 49.9 ± 5.1%	7 mice		
Fig 1E	Latency to the first spike (mean ± SEM)	Er81+: 297 ± 17 ms; Er81-: 90 ± 13 ms	Er81+: 22 cells; Er81-: 11 cells	Student *t*-test	0.0001 (***)
Fig 1G	AP Threshold (mean ± SEM)	Er81+: -32.2 ± 0.6 mV; Er81- : -37.3 ± 2.6 mV	Er81+: 22 cells; Er81-: 11 cells	Student *t*-test	0.02 (*)
Fig 1L	mEPSCs frequency (mean ± SEM)	Er81+: 22.0 ± 2.1 Hz; Er81-: 12.4 ± 3.7 Hz	Er81+: 15 cells; Er81-: 6 cells	Student *t*-test	0.03 (*)
Fig 1K	mIPSCs frequency (mean ± SEM)	Er81+: 5.5 ± 0.3 pA; Er81-: 10.4 ± 0.9 Hz	Er81+: 7 cells; Er81-: 4 cells	Student *t*-test	0.01 (*)

FIGURE 2	Measurement	Values	N	Statistical	P value
Fig 2B	Latency to the first spike (mean ± SEM)	Control: 236.4 ± 28.8 ms; Mutant: 138.8 ± 20.8 ms	Control: 20 cells; Mutant: 18 cells	Student *t*-test	0.01 (*)
Fig 2B	Threshold potential (mean ± SEM)	Control: -36.9 ± 1.0 mV; Mutant: -43.2 ± 2.2 mV	Control: 20 cells; Mutant: 18 cells	Student *t*-test	0.01 (*)
Fig 2D	Latency to the first spike (mean ± SEM)	Control: 220.0 ± 21.4 ms; Mutant: 92.4 ± 13.3 ms	Control: 29 cells; Mutant: 20 cells	Student *t*-test	0.00004 (***)
Fig 2D	Threshold potential (mean ± SEM)	Control: -37.5 ± 1.0 mV; Mutant: -42.9 ± 2.0 mV	Control: 29 cells; Mutant: 20 cells	Student *t*-test	0.02 (*)
Fig 2E	Kv1.1 protein levels (mean ± SEM)	Control: normalised 100%; Mutant: 65.7 ± 8.2 %	Control: 5 mice; Mutant: 5 mice	Student *t*-test	0.003 (**)
Fig 2F	Kv1.1 mRNA levels (mean ± SEM)	Control: normalised 100%; Mutant: 42.1 ± 9.2 %	Control: 4 mice; Mutant: 4 mice	Student *t*-test	0.008 (**)
Fig 2G	ChiP Site 1 (mean ± SEM)	Er81: 0.36 ± 0.05 AU; GFP: 0.27 ± 0.14 AU	Control: 3 mice; Mutant: 3 mice	Student *t*-test	0.8
Fig 2G	ChiP Site 2 (mean ± SEM)	Er81: 0.56 ± 0.09 AU; GFP: 0.19 ± 0.07 AU	Control: 3 mice; Mutant: 3 mice	Student *t*-test	0.04 (*)

FIGURE 3	Measurement	Values	N	Statistical	P value
Fig 3C	mEPSCs frequency (Lhx6) (mean ± SEM)	Control: 19.0 ± 3.8 Hz; Mutant: 8.7 ± 1.6 Hz	Control: 11 cells; Mutant: 9 cells	Student *t*-test	0.03 (*)
Fig 3C	mEPSCs frequency (PV) (mean ± SEM)	Control: 19.3 ±1.6 Hz; Mutant: 10.5 ± 1.6 Hz	Control: 17 cells; Mutant: 10 cells	Student *t*-test	0.001 (**)
Fig 3E	mEPSCs amplitude (Lhx6) (mean ± SEM)	Control: 20.6 ± 1.3 pA; Mutant: 15.1 ± 1.4 pA	Control: 11 cells; Mutant: 9 cells	Student *t*-test	0.01 (*)
Fig 3E	mEPSCs amplitude (PV) (mean ± SEM)	Control: 15.2 ± 0.7 pA; Mutant: 18.9 ± 2.1 pA	Control: 17 cells; Mutant: 10 cells	Student *t*-test	0.06
Fig 3K	mEPSCs frequency (mean ± SEM)	Control: 17.2 ± 2.7 Hz; Mutant: 19.9 ± 2.2 Hz	Control: 10 cells; Mutant: 11 cells	Student *t*-test	0.45
Fig 3L	mEPSCs amplitude (mean ± SEM)	Control: 20.0 ±1.2 pA; Mutant: 16.3 ± 1.1 pA	Control: 10 cells; Mutant: 11 cells	Student *t*-test	0.03 (*)

FIGURE 4	Measurement	Values	N	Statistical	P value
Fig 4C	βgal+/Er81+ cells (mean ± SEM)	Er81-/βgal-: 10.8 ± 3.6 %; Er81-/βgal+: 24.1 ± 9.1 % Er81+/βgal+: 27.1 ± 7.7 %; Er81+/βgal+: 38.0 ± 8.3 %	3 mice		
Fig 4D	Er81 mRNA levels (mean ± SEM)	Control: 0.21 ± 0.03; Mutant: 0.01 ± 0.00 (a.u)	Control: 11 cells; Mutant: 4 cells	Student *t*-test	0.002 (**)
Fig 4G	Latency to fall (mean ± SEM)	Day 1: 71.9 ± 10.3 ms; Day 7: 224.4 ± 14.2 ms	8 mice	One Way ANOVA	0.001 (**)
Fig 4H	Er81 fluorescence intensity (mean ± SEM)	Control: 2.26 ± 0.08 A.U.; Trained: 1.96 ± 0.07 A.U.	Control: 8 mice; Trained: 8 mice	Student *t*-test	0.01 (*)
Fig 4I	Er81 expression levels (mean ± SEM)	Control (-): 23.2 ± 2.9 %; (+): 43.1 ± 2.6 %; (++): 36.1 ± 3.1 % Trained (-): 27.8 ± 3.4 %; (+): 53.7 ± 4.1 %; (++): 18.8 ± 4.6 %	Control: 8 mice; Trained: 8 mice	Chi-square test	0.0001 (***)
Fig 4K	Er81 fluorescence intensity (mean ± SEM)	Control: 1.67 ± 0.03; Kir2.1: 2.34 ± 0.25	Control: 4 mice; Kir2.1: 4 mice	Student *t*-test	0.03 (*)
Fig 4L	Er81 expression levels (mean ± SEM)	Control (-): 39.3 ± 5.2 %; (+): 58.6 ± 4.7 %; (++): 2.1 ± 1.0 % Kir2.1 (-): 22.8 ± 8.1%; (+): 47.1 ± 10.0 %; (++): 30.1 ± 5.1 %	Control: 4 mice; Kir2.1: 4 mice	Chi-square test	0.0001 (***)

FIGURE S1	Measurement	Values	N	Statistical	P value
Fig S1A	Distribution Er81+ cells (mean ± SEM)	PV+: 74.3 ± 6.6 %; SST+: 20.5 ± 5.0 %; PC: 7.0 ± 3.0 %	PV+: 11 mice; SST+: 4 mice; PC: 3 mice		

FIGURE S2	Measurement	Values	N	Statistical	P value
Fig S2C	mEPSCs amplitude (mean ± SEM)	Er81+: 21.3 ± 1.0 pA Er81-: 17.2 ± 3.0 pA	Er81+: 15 cells; Er81-: 6 cells	Student *t*-test	0.15
Fig S2E	mIPSCs amplitude (mean ± SEM)	Er81+: 29.0 ± 1.9 pA; Er81-: 33.5 ± 0.8 Hz	Er81+: 7 cells; Er81-: 4 cells	Student *t*-test	0.1

FIGURE S3	Measurement	Values	N	Statistical	P value
Fig S3D	Er81 expression (mean ± SEM)	BC: 79.8 ± 12.3 %; CC: 0 ± 0.0 %	BC: 57 cells; CC: 32 cells		

FIGURE S5	Measurement	Values	N	Statistical	P value
Fig S5A	Number of PV+ cells (mean ± SEM)	Control: 124.2 ± 8.3 cells; Mutant: 139.2 ± 12.3 cells	Control: 3 mice; Mutant: 3 mice	Student *t*-test	0.35
Fig S5C	Rheobase (mean ± SEM)	Control: 193.5 ± 26.1 pA; Mutant:122.6 ± 19.1 pA	Control: 20 cells; Mutant: 18 cells	Student *t*-test	0.04 (*)

FIGURE S6	Measurement	Values	N	Statistical	P value
Fig S6A	Number of PV+ cells (mean ± SEM)	Control: 122.1 ± 11.8 cells; Mutant: 132.0 ± 17.9 cells	Control: 3 mice; Mutant: 3 mice	Student *t*-test	0.5
Fig S6C	Rheobase (mean ± SEM)	Control: 202.1 ± 16.2 pA ; Mutant: 139.4 ± 18.8 pA	Control: 29 cells; Mutant: 20 cells	Student *t*-test	0.02 (*)

FIGURE S7	Measurement	Values	N	Statistical	P value
Fig S7B	Density of Vglut1 boutons (mean ± SEM)	Control: 0.104 ± 0.013 boutons; Mutant: 0.064 ± 0.008 boutons	Control: 3 mice; Mutant: 3 mice	Student *t*-test	0.01 (*)
Fig S7C	Paired-pulse ratio EPSC2/EPSC1 (mean ± SEM)	Control: 79.8 ± 15.7 %; Mutant: 94.2 ± 13.5 %	Control: 4 cells; Mutant: 5 cells	Student *t*-test	0.5
Fig S7C	Paired-pulse ratio EPSC5/EPSC1 (mean ± SEM)	Control: 68.0 ± 14.0 %; Mutant: 60.2± 16.7 %	Control: 4 cells; Mutant: 5 cells	Student *t*-test	0.7

FIGURE S8	Measurement	Values	N	Statistical	P value
Fig S8D	mIPSCs frequency (mean ± SEM)	Control: 7.3 ± 0.8; Mutant: 14.8 ± 1.6 Hz	Control: 11 cells; Mutant: 10 cells	Student *t*-test	0.0003 (***)
Fig S8D	mIPSCs amplitude (mean ± SEM)	Control: 30.5 ± 1.4; Mutant: 32.1 ± 2.6 pA	Control: 11 cells; Mutant: 10 cells	Student *t*-test	0.58
Fig S8E	Paired-pulse ratio IPSC2/IPSC1 (mean ± SEM)	Control: 61.0 ± 8.5 %; Mutant: 51.2 ± 12.9 %	Control: 6 cells; Mutant: 5 cells	Student *t*-test	0.5
Fig S8E	Paired-pulse ratio IPSC5/IPSC1 (mean ± SEM)	Control: 42.5 ± 7.1 %; Mutant: 40.8 ± 5.1 %	Control: 6 cells; Mutant: 5 cells	Student *t*-test	0.8

FIGURE S9	Measurement	Values	N	Statistical	P value
Fig S9C	Latency to the first spike (mean ± SEM)	Control: 287.5 ± 23.3; Mutant:136.9 ± 24.9 ms	Control: 21 cells; Mutant: 17 cells	Student *t*-test	9.2.10-5 (***)
Fig S9E	Kv1.1 fluorescence intensity (mean ± SEM)	Infected control: 1.86 ± 0.10 AU; Infected mutant: 1.49 ± 0.07 AU	I-Control: 44 cells; I-Mutant 54 cells	One Way ANOVA	0.03 (*)
		Non-infected mutant: 1.75 ± 0.08 AU; Infected mutant: 1.49 ± 0.07 AU	NI-Mutant 70 cells; I-Mutant 54 cells	One Way ANOVA	0.03 (*)
Fig S9G	Density of Vglut1 boutons (mean ± SEM)	Control: 0.090 ± 0.013 boutons; Mutant: 0.081 ± 0.009 boutons	Control: 3 mice; Mutant: 3 mice	Student *t*-test	0.6

FIGURE S10	Measurement	Values	N	Statistical	P value
Fig S10B	Er81+ cells (mean ± SEM)	ACSF: normalised 100%; KA: 57.1 ± 2.7 %	Control: 306 cells; KA: 244 cells	Student *t*-test	0.04 (*)
Fig S10C	Latency to the first spike (mean ± SEM)	ACSF: 293 ± 28 ms; KA: 200 ± 32 ms	Control: 17 cells; KA: 18 cells	Student *t*-test	0.03 (*)
Fig S10E	Er81+ cells (mean ± SEM)	ACSF: normalised 100%; Nife: 170.3 ± 10.1 %	Control: 236 cells; Nife: 199 cells	Student *t*-test	0.03 (*)
Fig S10F	Latency to the first spike (mean ± SEM)	ACSF: 107 ± 31 ms ; Nife: 252 ± 53 ms	Control: 13 cells; Nife: 12 cells	Student *t*-test	0.02 (*)

FIGURE S11	Measurement	Values	N	Statistical	P value
Fig S11B	Er81 fluorescence intensity (mean ± SEM)	Control: 1.16 ± 0.02 A.U.; Trained: 1.14 ± 0.03 A.U.	Control: 5 mice; Trained: 5 mice	Student *t*-test	0.54
Fig S11C	Er81 expression levels (mean ± SEM)	Control (-): 82.2 ± 2.8 %; (+): 17.9 ± 2.9 %; (++): 0 ± 0 %	Control: 5 mice; Trained: 5 mice	Chi-square test	0.8
		Trained (-): 83.4 ± 6.0 %; (+): 16.6 ± 6.0 %; (++): 0 ± 0 %			
Fig S11E	Er81 fluorescence intensity (mean ± SEM)	Control: 1.17 ± 0.01; Kir2.1: 1.19 ± 0.01	Control: 4 mice; Kir2.1: 4 mice	Student *t*-test	0.27
Fig S11F	Er81 expression levels (mean ± SEM)	Control (-): 80.7 ± 1.7 %; (+): 19.3 ± 1.7 %; (++): 0 ± 0 %	Control: 4 mice; Kir2.1: 4 mice	Chi-square test	0.1
		Kir2.1 (-): 74.5 ± 2.5%; (+): 25.5 ± 2.5 %; (++): 0 ± 0 %			

Fig. S13. Summary of values and statistical analyses.