High-performance and site-directed *in utero* electroporation with a triple-electrode probe

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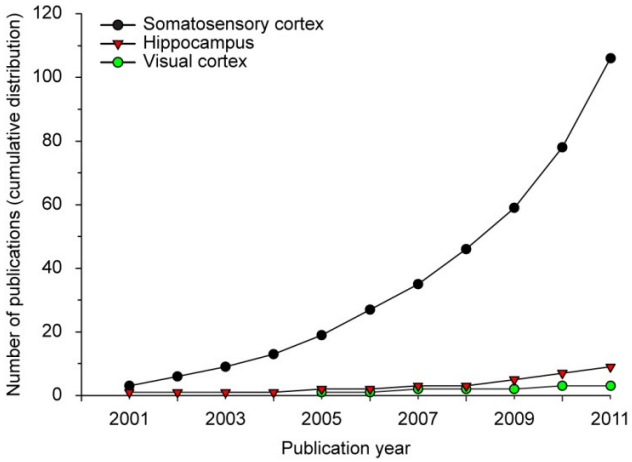
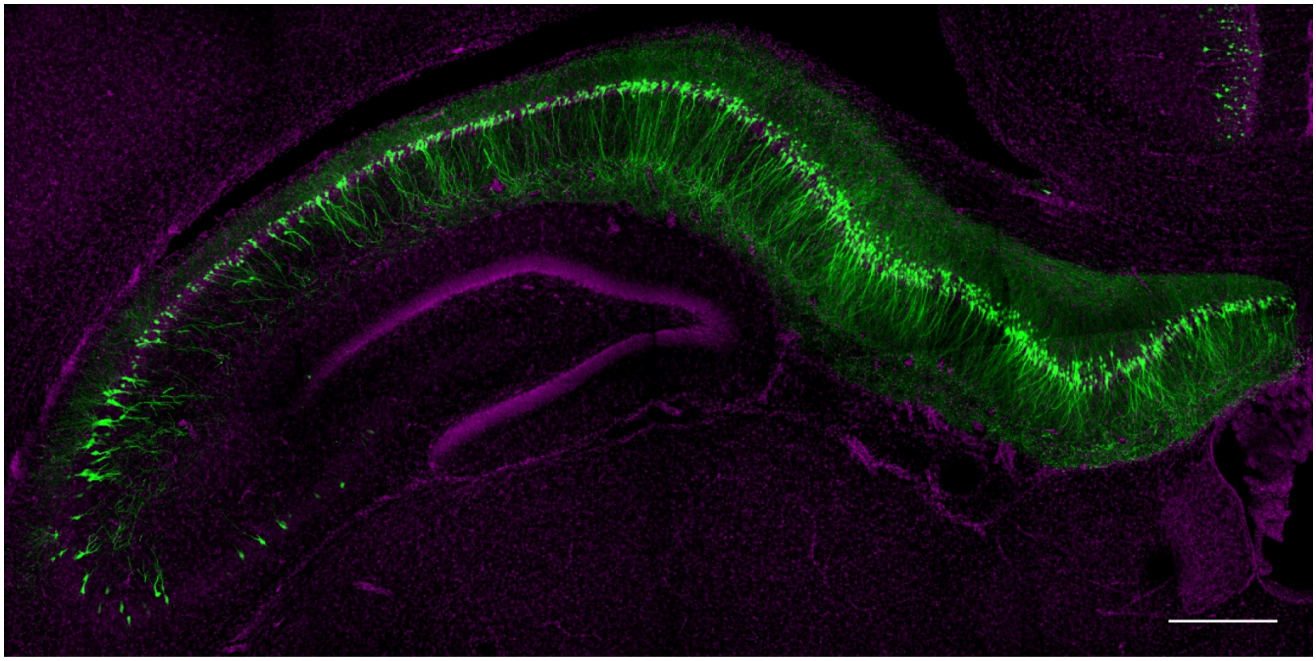
Supplementary Fig. S1: Cumulative distribution of the number of publications/year with description of *in utero* electroporation targeting somatosensory cortex, hippocampus or visual cortex.



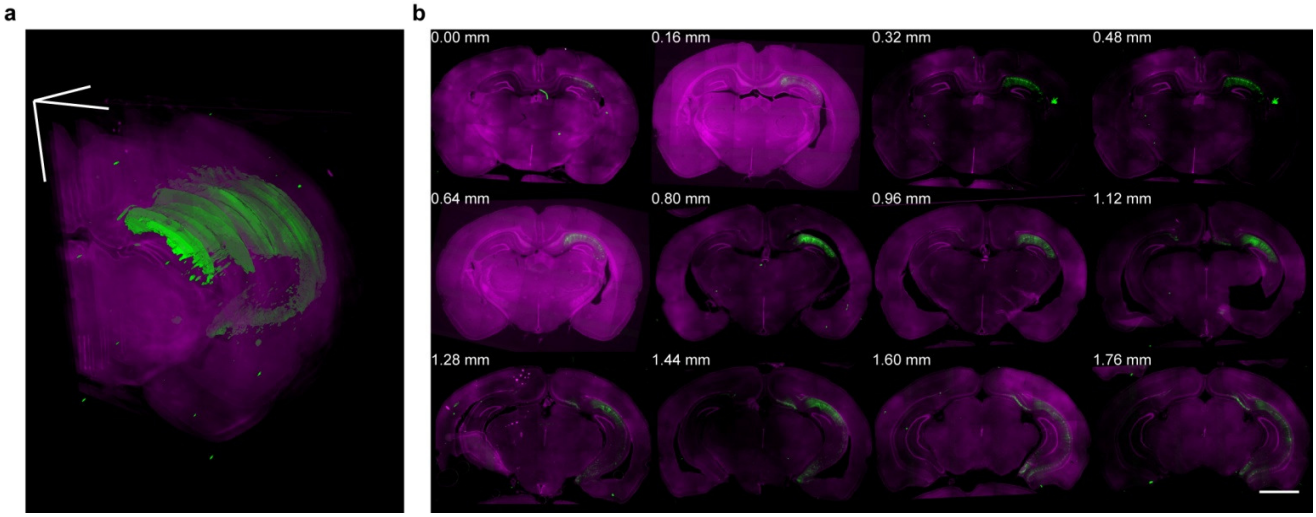
Supplementary Fig. S1: The number of publications on IUE targeting different brain areas indicates technical difficulties in electroporating regions other than the somatosensory cortex. The numbers of publications were obtained upon a pubmed quest with "*in utero* electroporation AND cortex" and subsequent careful screen of the results to separate articles on somatosensory from that on visual cortex, or "*in utero* electroporation AND hippocampus" as key words. Note the exponential increase of the number of publications on the somatosensory cortex in comparison to articles on the hippocampus or visual cortex.

Supplementary Fig. S2: Tiled images of a coronal section of the hippocampus (P21).



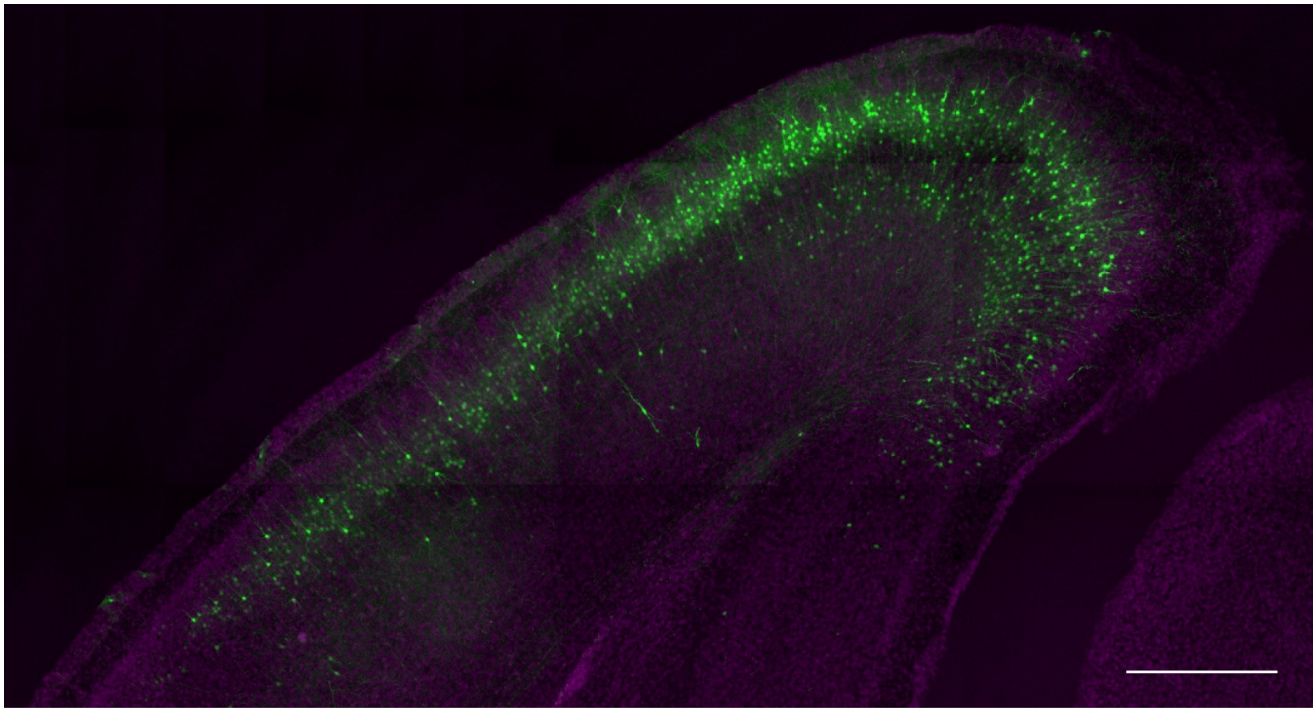
Supplementary Fig. S2: Principal cells transfected in CA1-CA2 regions (green). Sections were counterstained with Hoechst (magenta). Scale bar: 500 µm.

Supplementary Fig. S3: Rostro-caudal coronal series for the hippocampus (P21).



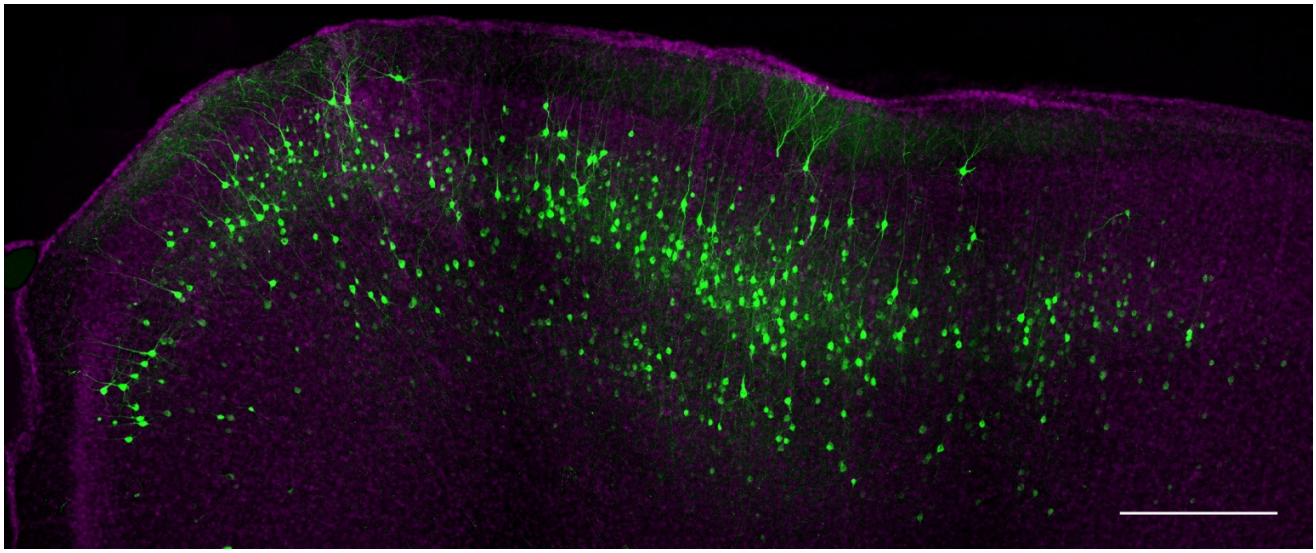
Supplementary Fig. S3: (**a**) 3D rostro-caudal reconstruction of a (P21) brain injected monolaterally and electroporated for targeting the hippocampus. (**b**) Set of the serial coronal slices utilized for reconstruction in a. Sections were counterstained with Hoechst (magenta). Scale bars: 1 mm

Supplementary Fig. S4: Tiled images of a coronal section of the visual cortex (P10).



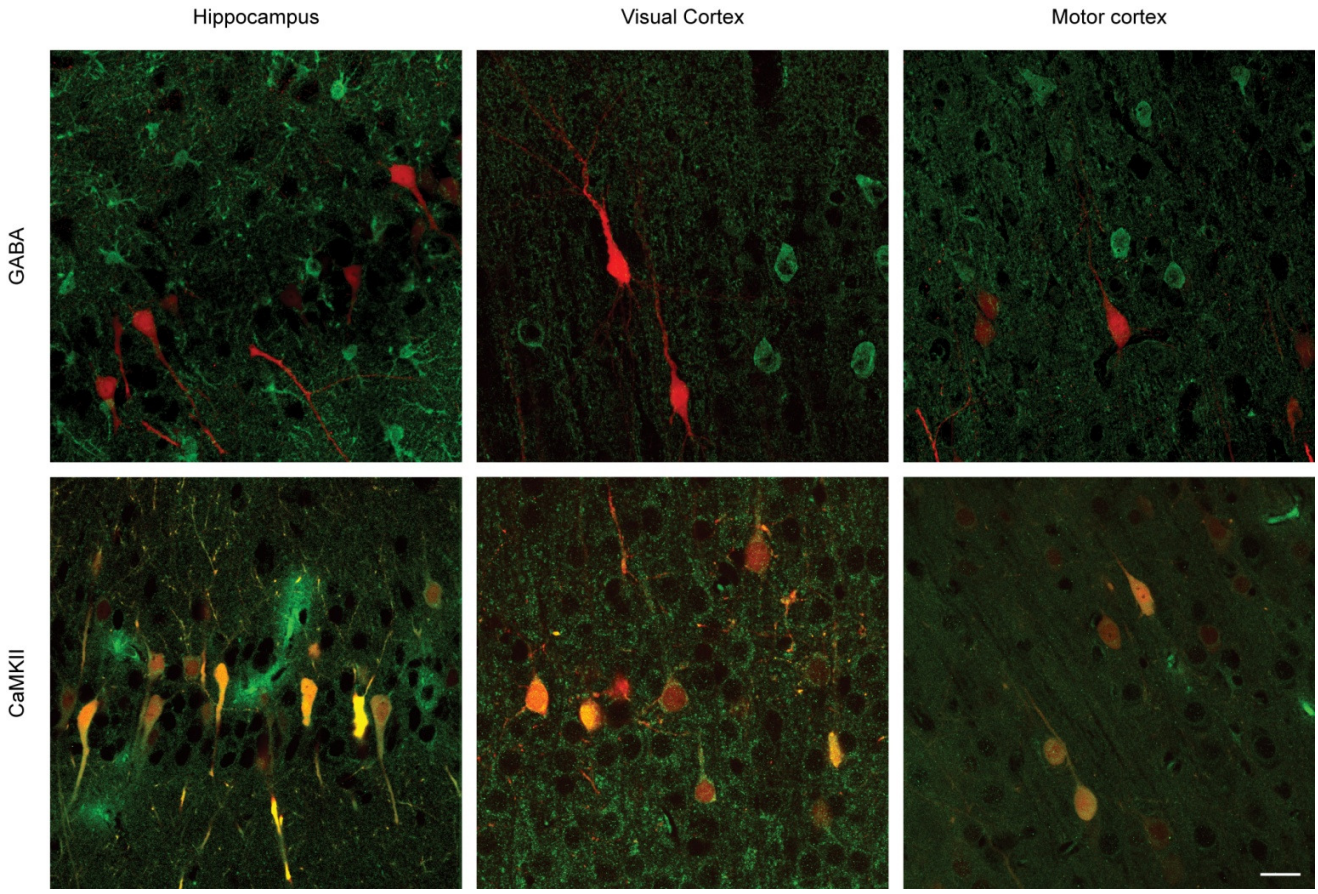
Supplementary Fig. S4: Transfected pyramidal cells in green Sections were counterstained with Hoechst (magenta). Scale bar: 500 µm.

Supplementary Fig. S5: Tiled images of a coronal section of the motor cortex (P20).



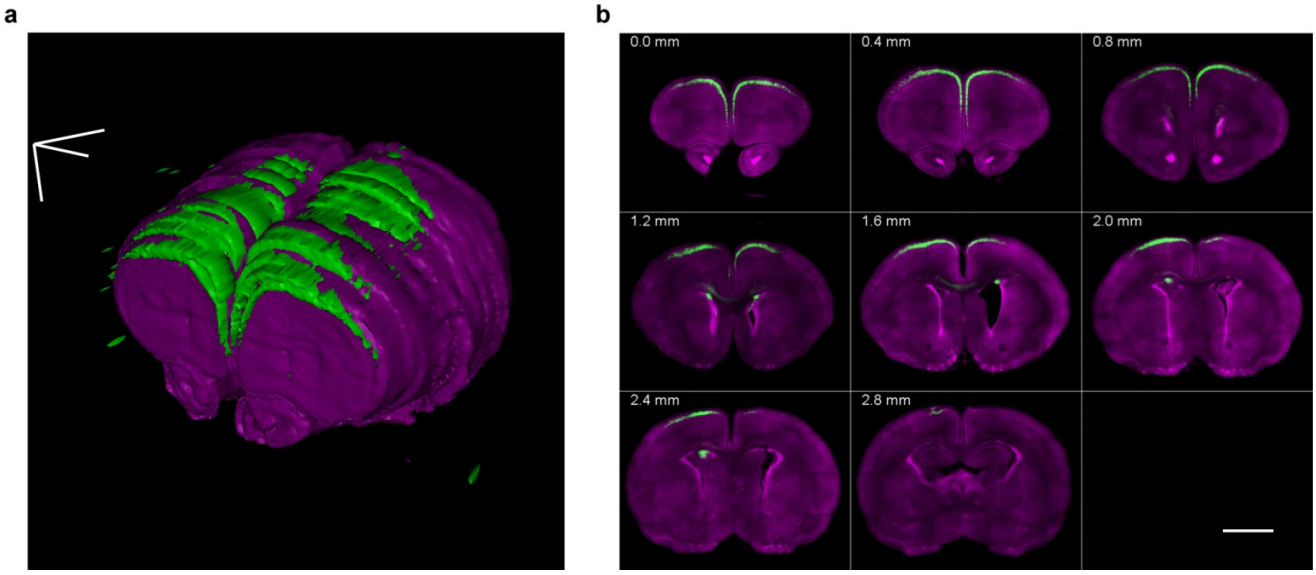
Supplementary Fig. S5: Transfected pyramidal cells in layer II/II (green). Sections were counterstained with Hoechst (magenta). Scale bar: 500 µm.

Supplementary Fig. S6: *In utero* electroporation of hippocampus and visual or motor cortex results in specific transfection of excitatory neurons.



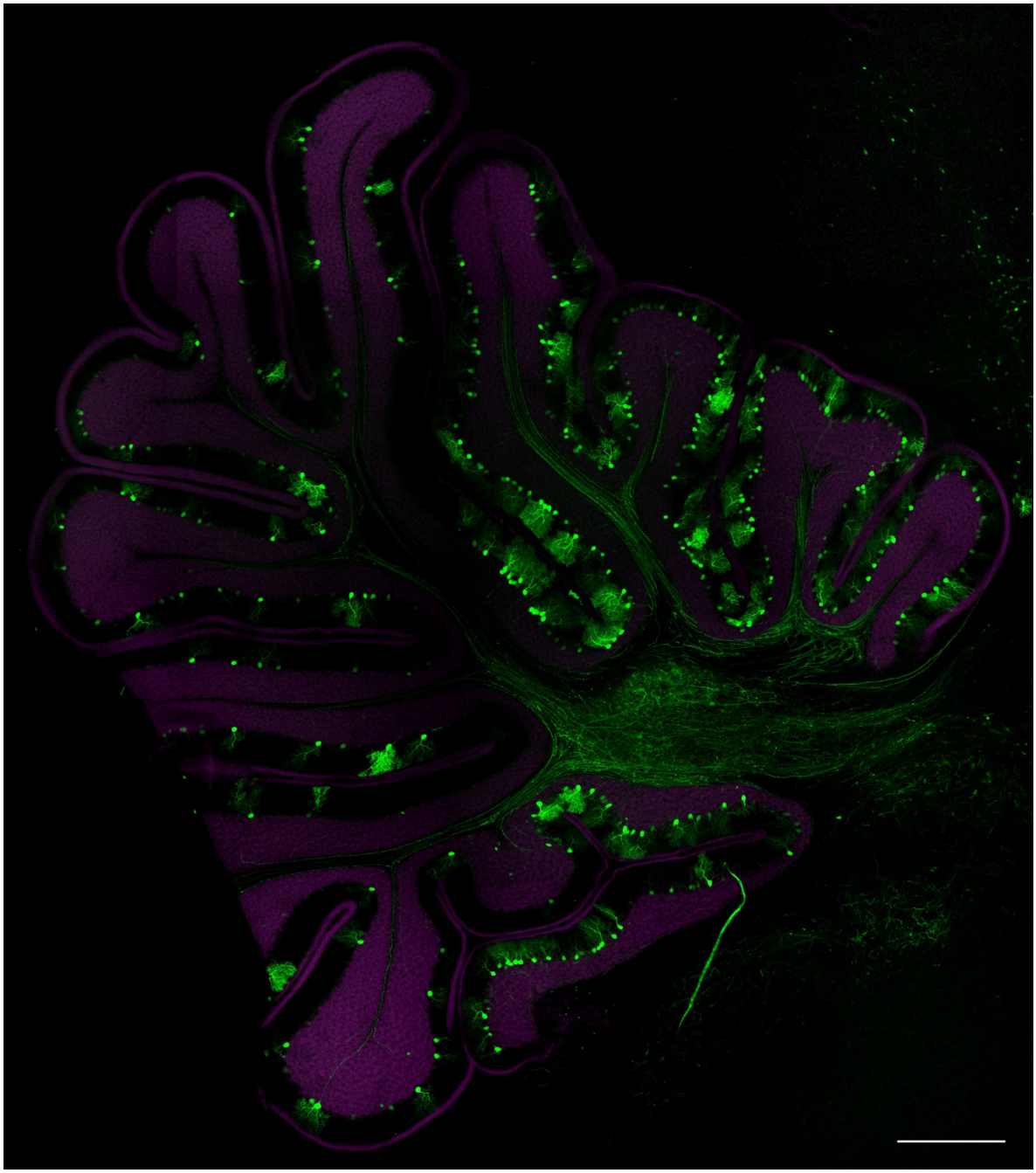
Supplementary Fig. S6: *In utero* electroporation of hippocampus and visual or motor cortex results in specific transfection of excitatory neurons (red, td-Tomato fluorescence). Confocal images of coronal sections (P21) stained for excitatory-cell marker CaMKII and inhibitory-cell marker GABA. Tomato-expressing cells stained positive only for CaMKII. Scale bar: 20 µm

Supplementary Fig. S7: Rostro-caudal coronal series for the motor cortex.



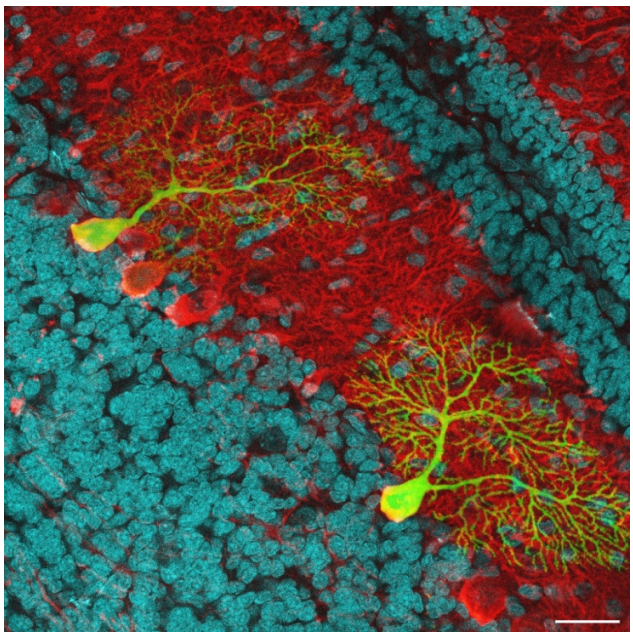
Supplementary Fig. S7: (**a**) 3D rostro-caudal reconstruction of a (P15) brain injected bilaterally and electroporated for targeting the motor cortex. (**b**) Set of the serial coronal slices utilized for reconstruction in a. Sections were counterstained with Hoechst (magenta). Scale bars: 1 mm

Supplementary Fig. S8: Tiled images of a sagittal section of the cerebellum (P14).



Supplementary Fig. S8: Transfected with EGFP Purkinje cells (green). Sections were counterstained with Hoechst (magenta). Scale bar: 500 µm.

Supplementary Fig. S9: *In utero* electroporation of cerebellum at E14.5 results in specific transfection of Purkinje cells.



Supplementary Fig. S9: Confocal images of a coronal section (P14) with Purkinje-cells (green, EGFP fluorescence) stained for their specific marker Calbindin (red, alexa 568) and cell nuclei (cyan, Hoechst). Scale bar: 50 µm

Supplementary Table 1: Electrophysiological functional proprieties of layer II/III visual cortical neurons.

	Membrane Resting Potential (mV)	**Membrane Capacitance (pF)**	**Membrane Resistance (MΩ)**	**sEPSC Frequency (Hz)**
WT (n = 4)	-50.5 ± 0.5	56.74 ± 4.35	704.7 ± 133.3	1.42 ± 0.47
EGFP (n = 5)	-53 ± 3.6	71.22 ± 5.74	621 ± 172.1	1.95 ± 0.5

Electrophysiological functional proprieties of layer II/III visual cortical neurons transfected with EGFP by the three-electrode configuration in comparison to WT cells. Data are expressed as average of all recorded cells ± SEM.