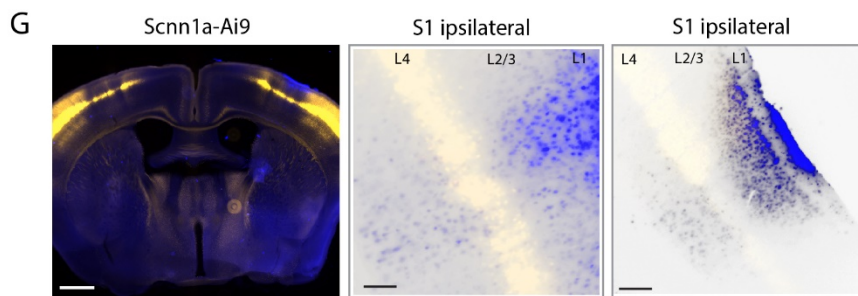
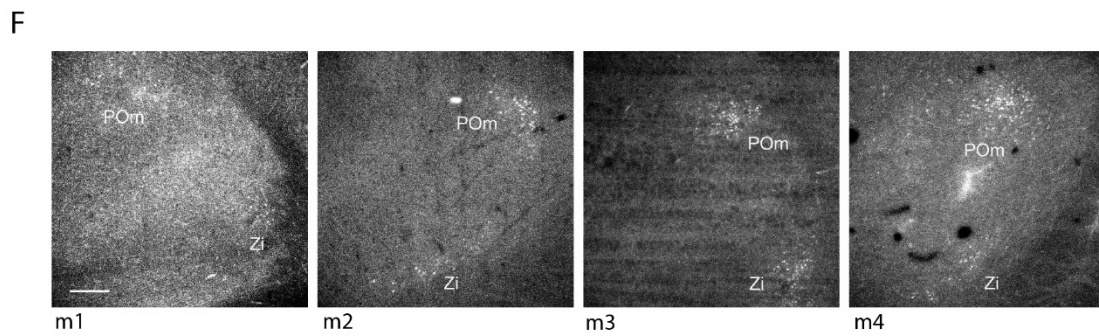
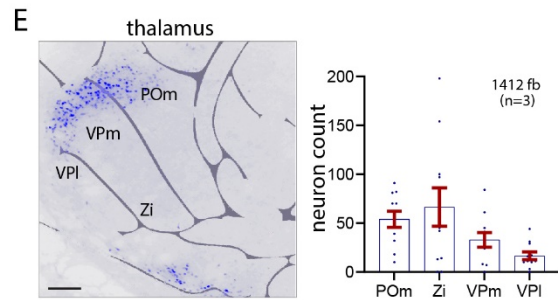
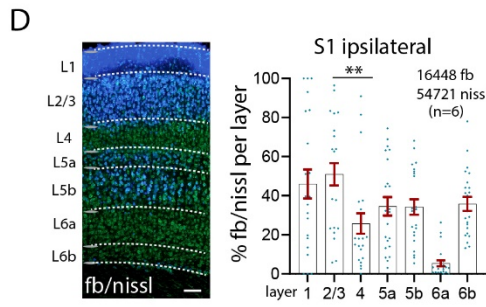
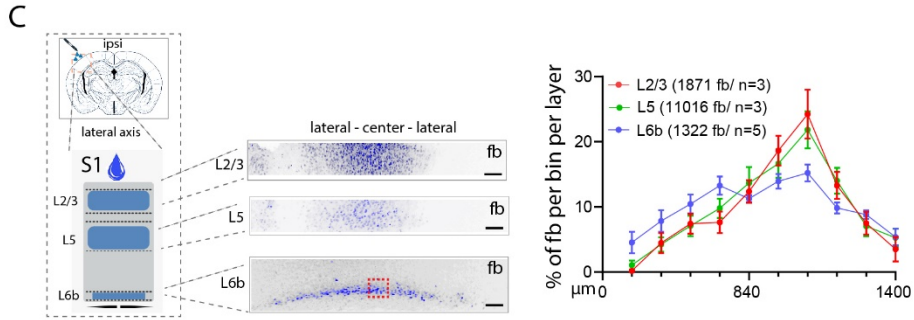
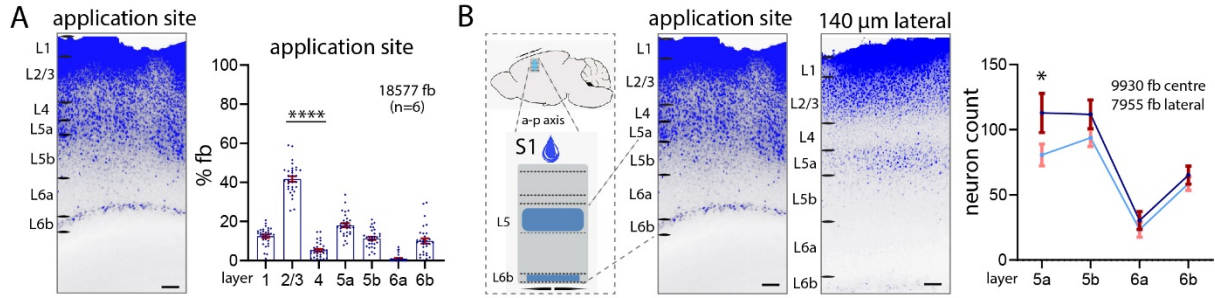
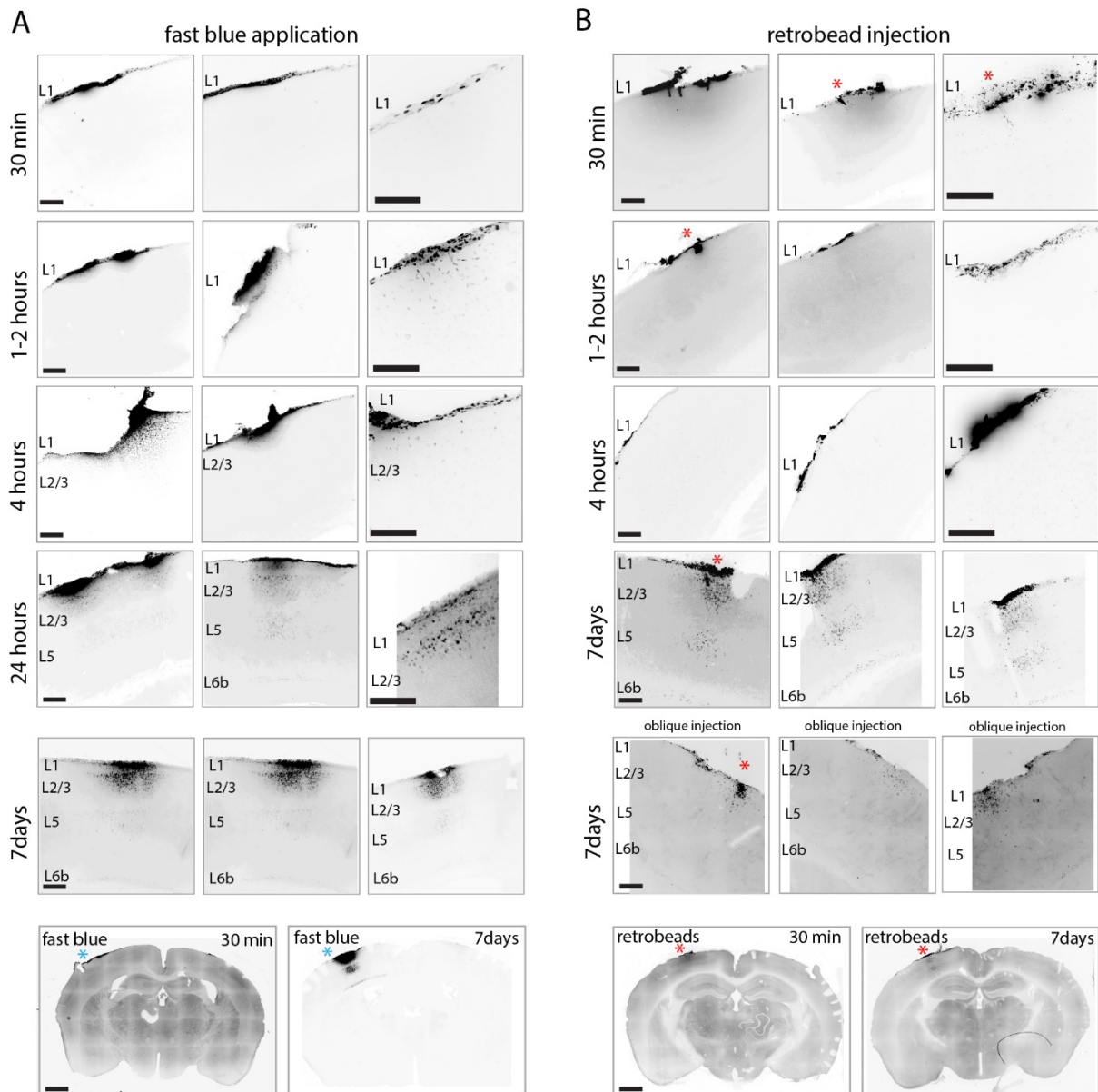


Supplementary Figures and Tables

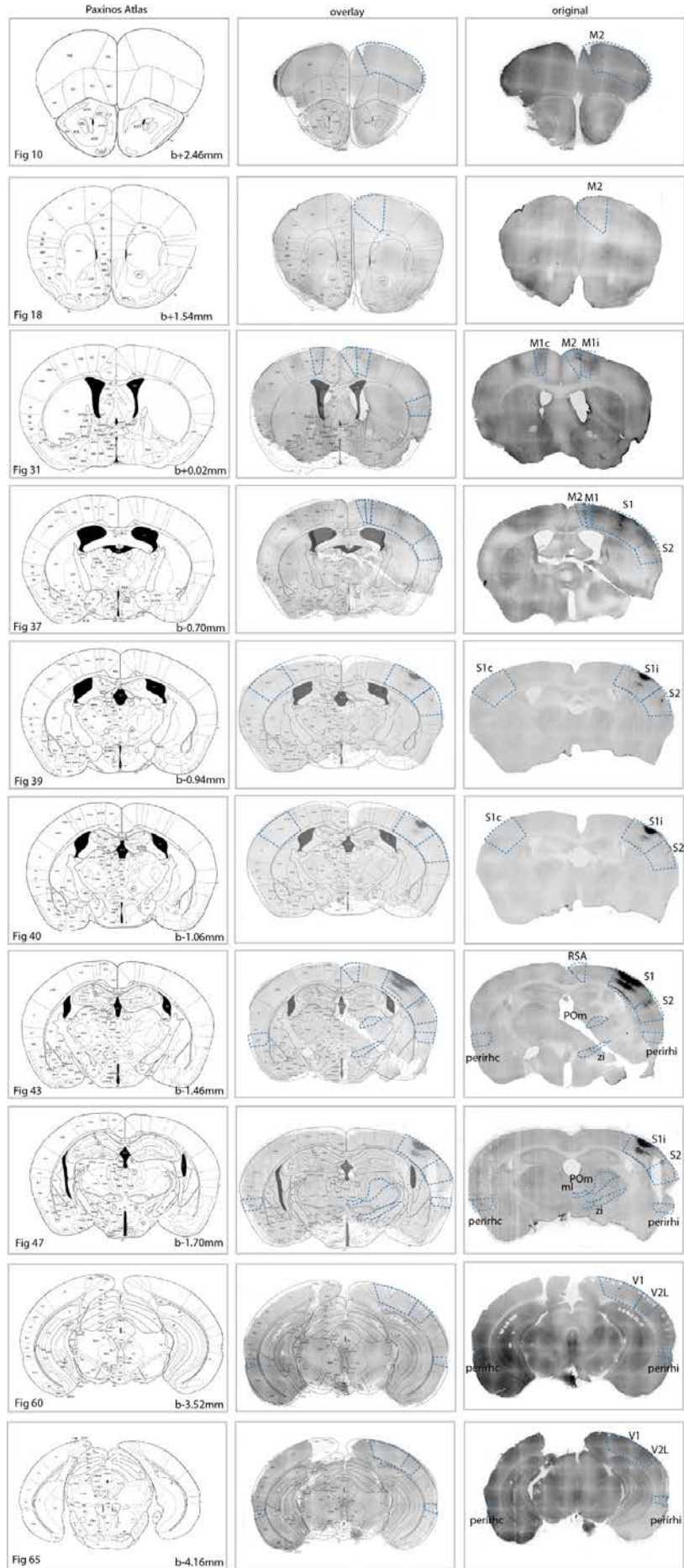


Supplementary Figure 1. Local input to S1 L1. (A) Uptake of fb at the centre of the application site in S1 cortex. (B) Centre of application site and 140 microns lateral to the application site. Fewer fb neurons were labelled in L5 and L6 (7955 fb, 31 brain sections) at 140 microns lateral to the application site, compared to the number labeled at the application site (9930 fb, 31 brain sections, one-way ANOVA * $p < 0.05$). (C) Comparison of fb label in L6b, L5 and L2/3 at +/- ~700 microns from the center of the application site. (D) Laminar profile --percentage of fb labeled neurons in each layer, binned at 100 microns. (E) Brains with fb label in VPI and VPm. Even though, these brains were excluded from analysis, total numbers of neurons labeled in each thalamic area were counted. (F) Example images showing fb label in POM and Zi in four brains. (G) Example images of fb label in Scnn1a-Cre-Ai9 brain sections showing fb label is not taken up by cells in L4. In the panels with binned data, each dot represents a count or a percentage from a brain section. Number of mice is indicated in brackets. Analysis details are presented in **Supplementary Table 1A**. Scale bars in **A, B, D**, 100 μm , in **C** and **G** (*right*) 50 μm , in **E, F**, and **G** 500 μm .

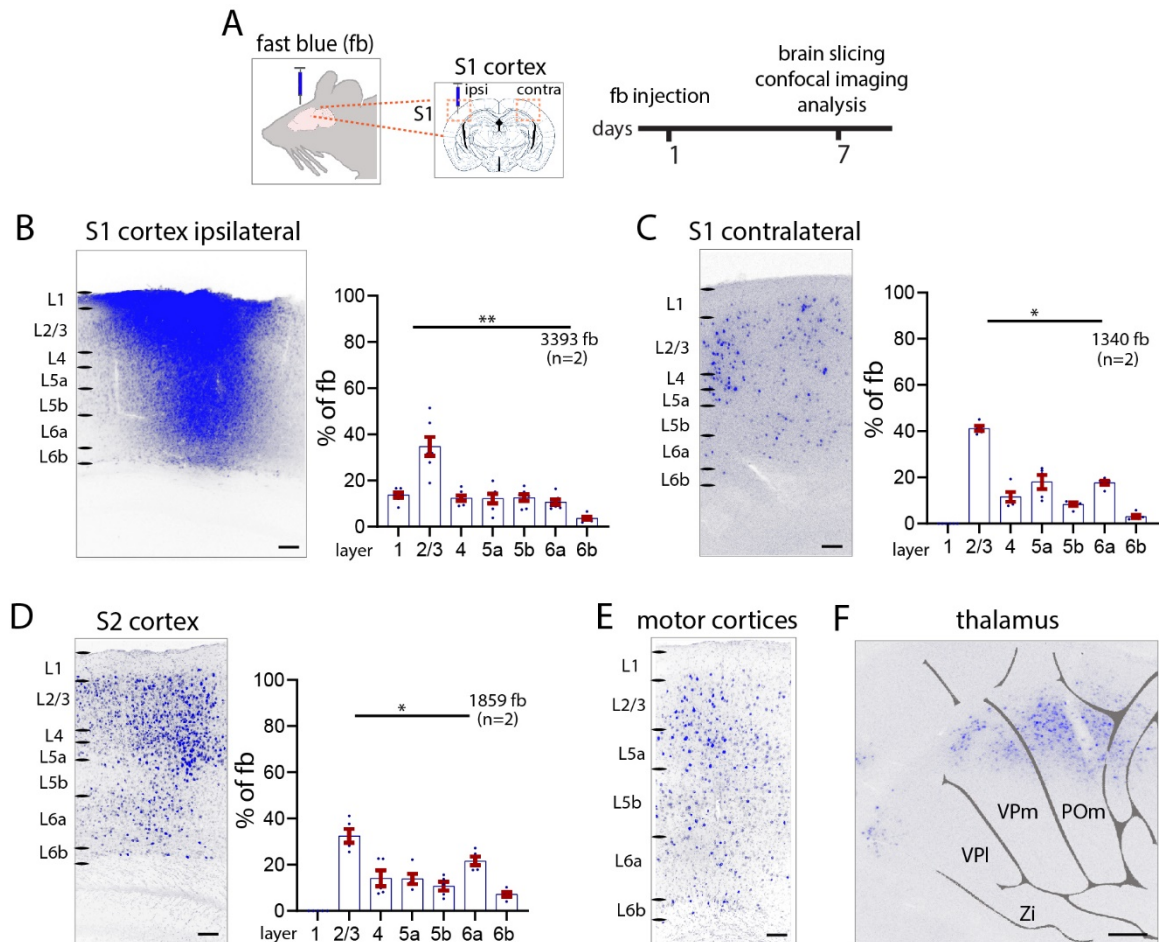


Supplementary Figure 2. Application of fast blue and injection of retrobeads for a time series analysis. (A) Measuring fb uptake at the application site ½ hour, 1-2 hours, 4 hours, 24 hours and 7 days after application of fb on the cortical surface. Uptake of fast blue was evident in layers 2/3 at ~ 4 hours. Blue asterisks mark the application site. (B) Measuring uptake of retrobeads ½ hour, 1-2 hours, ~ 4 hours, and 7 days after injection of retrobeads with an oblique injection). Labeled neurons were evident in L2/3 at ~ 4 hours after injection. Red asterisks

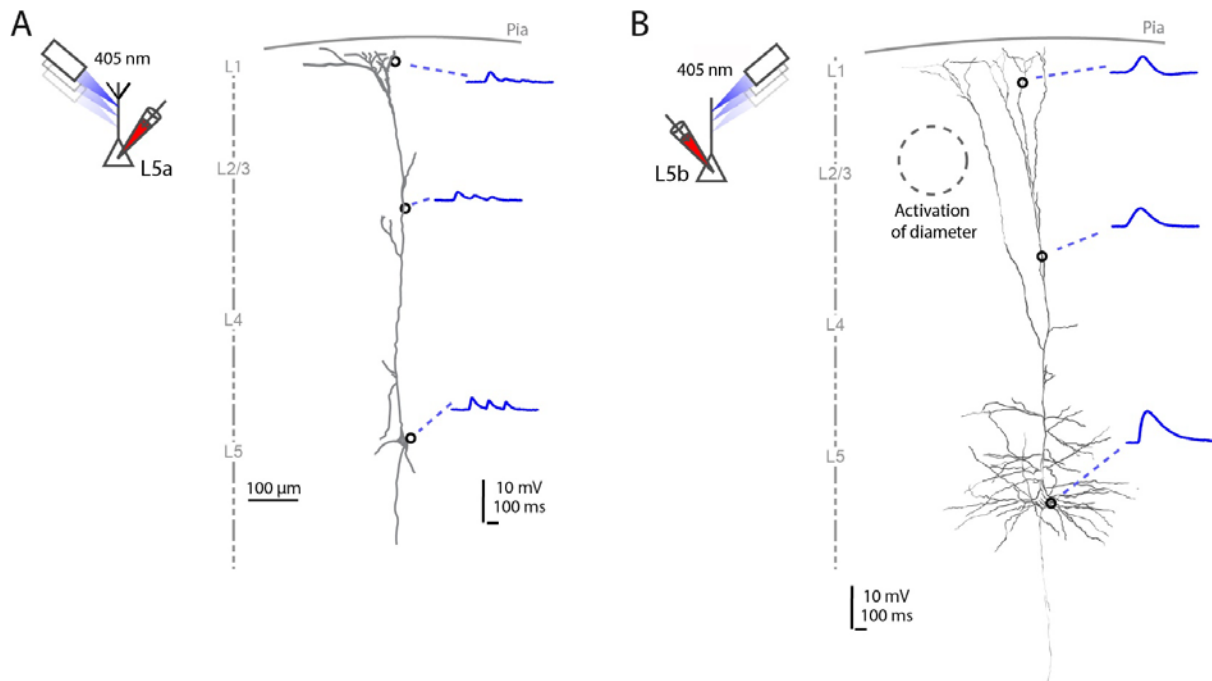
mark the injection site. Scale bars in **A, B**, 100 μm , in higher magnification images 50 μm , in overview images 500 μm .



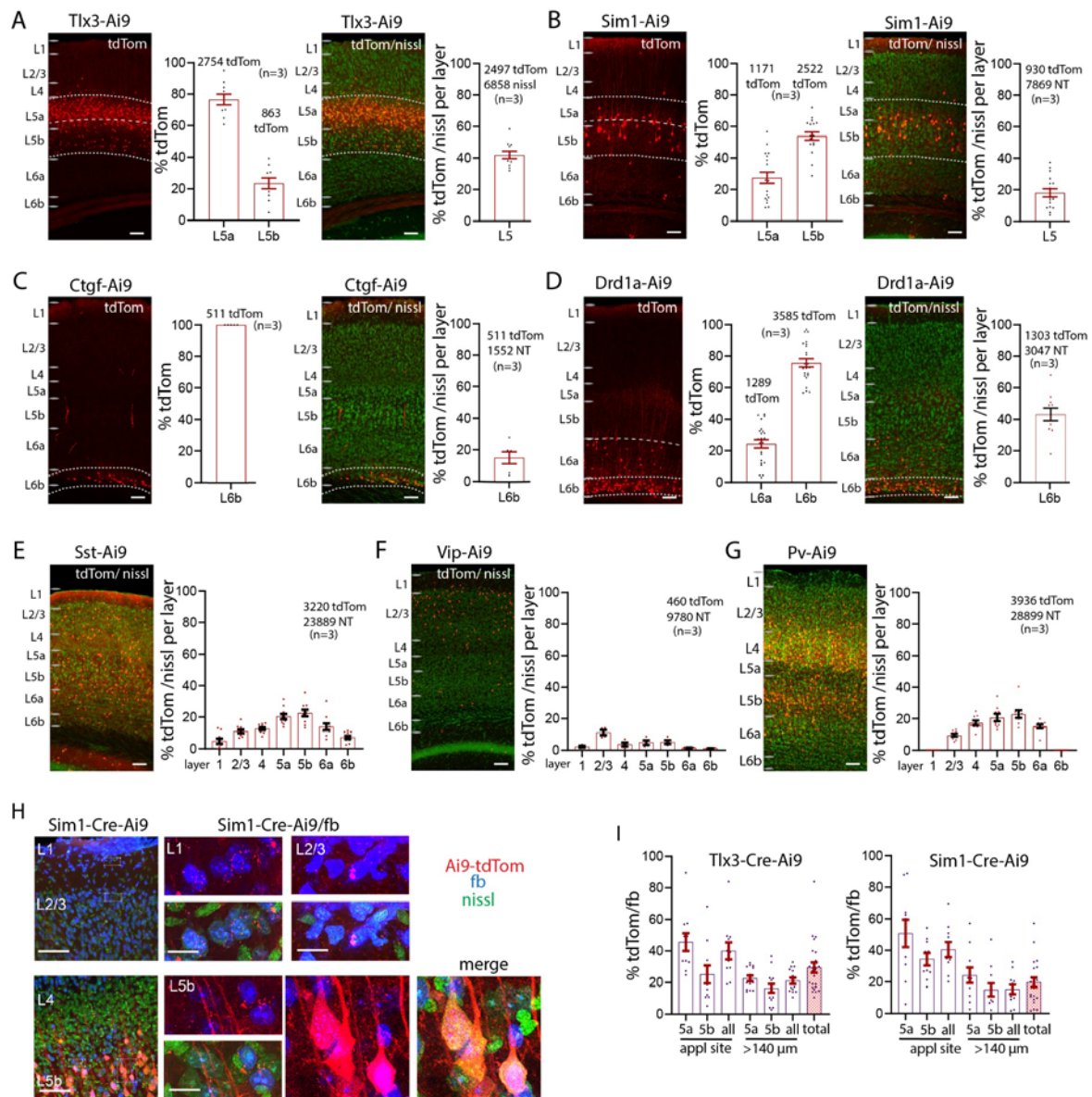
Supplementary Figure 3. Brain sections labeled with fast blue aligned to a the Paxinos reference atlas. Images in anterior-posterior direction showing fb label in distinct brain areas. Areas with fb label are marked with blue outlines and indicated as motor cortices M1, M2, somatosensory cortices S1, S2, (i, ipsi, c, contra), visual cortices V1, V2L, perirhinal cortices, perirhi (ipsi), perirhc (contra), POm thalamus, Zi zona incerta.



Supplementary Figure 4. Fast blue injection in S1 cortex. (A) Schematic for injection of fb into S1 cortex. (B) When fb was injected into cortex, fb labelled neurons were distributed in a different pattern than when applied on cortex L1. Fast blue labelled neurons were found in all cortical layers, primarily in L2/3. (C) Example images and quantification of fb labelling in contralateral S1, and (D) in ipsilateral S2 cortex. (E) Example images of motor cortex show fb labelled neurons in all layers. (F) Example images of fb uptake in POm, VPm, and VPI thalamus. Each dot in the graphs represents one brain section. Total number of neurons counted and mice used (in brackets) are shown in each panel. Data from two mice. One-way ANOVA, Bonferroni post-hoc test, ** $p < 0.01$. Analysis details are presented in **Supplementary Table 1C**. Scale bars in **B-E**, 100 μm , in **F** 500 μm .

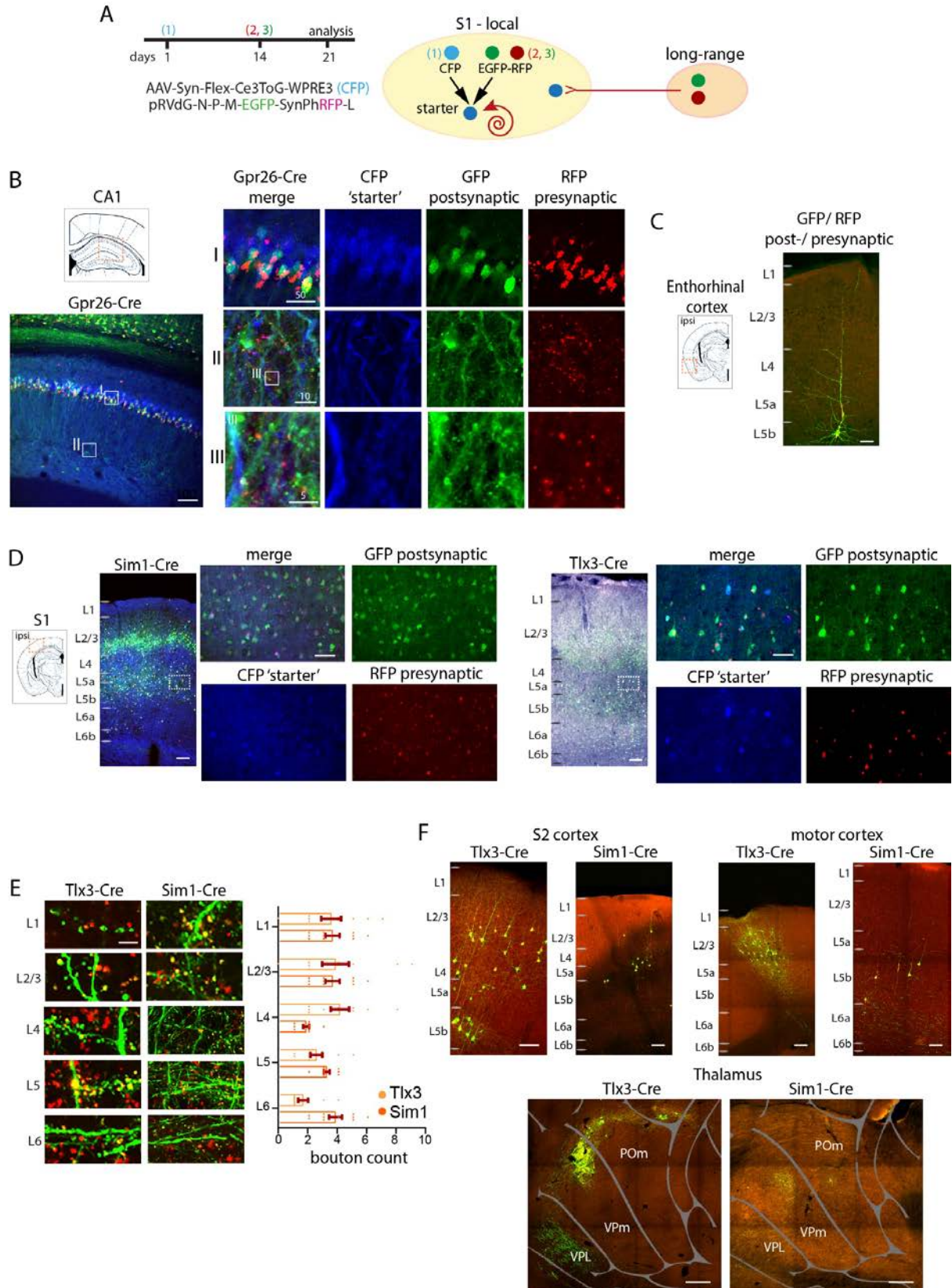


Supplementary Figure 5. Glutamate uncaging in L5a and L5b neurons revealed EPSPs evoked by stimulation on the dendrites. Experimental setup to test input along the L5a (A) and L5b (B) reconstructed neurons used in glutamate uncaging. EPSP traces after glutamate uncaging was performed at the soma, at the apical dendrites, and at the apical tufts in L1 (n=5 neurons, three mice per genotype).

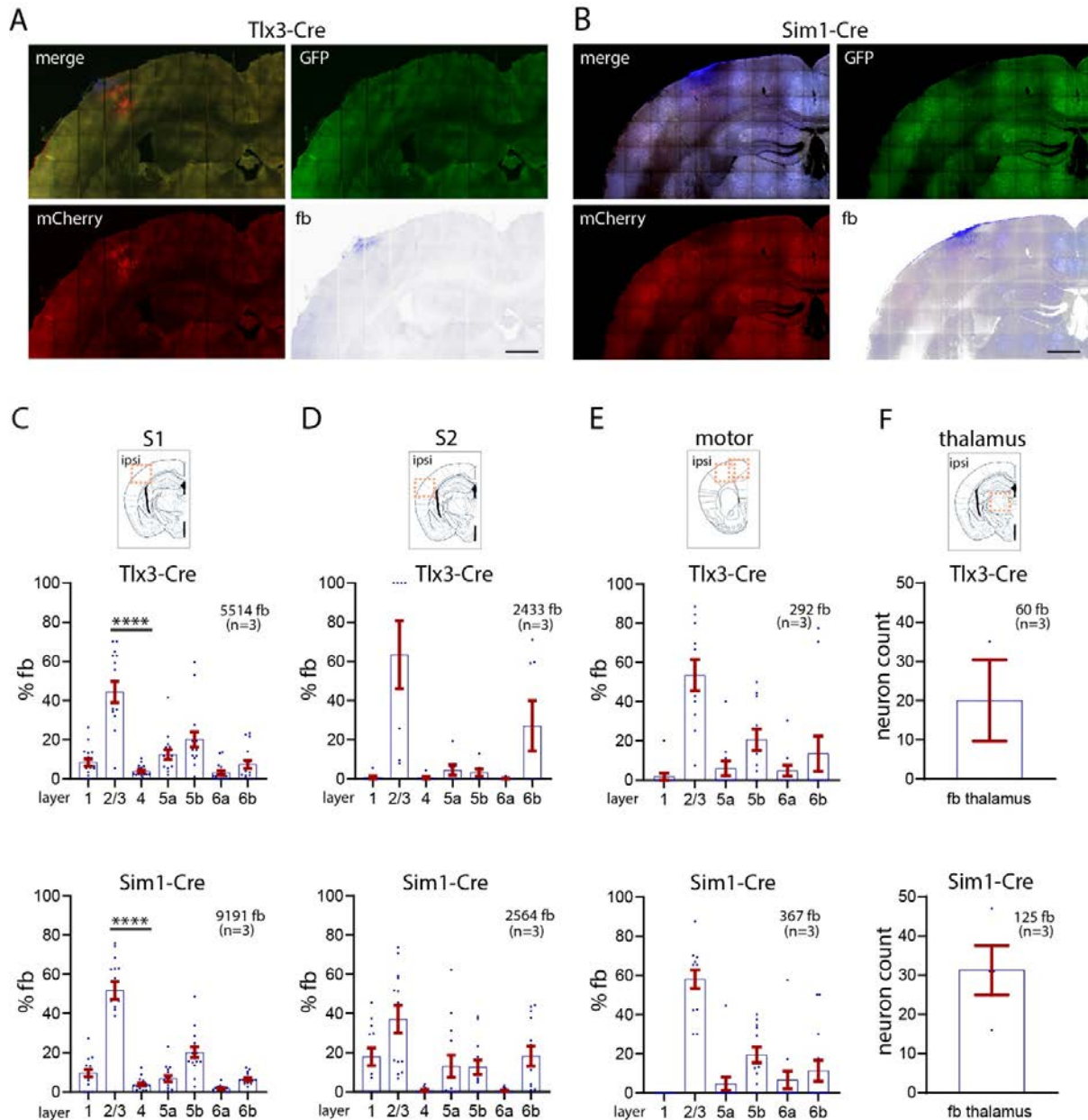


Supplementary Figure 6. Laminar profile of L5, L6b, and inhibitory cells. (A) Images of sections showing IT Tlx3-Cre/ tdTom positive neurons (*left*) and adjacent nissl stained sections (*right*) in L5. Seventy-six percent of tdTom positive cells in this line were in L5a. Forty-two percent of all L5 cells were tdTom positive. **(B)** Sections showing PT Sim1-Cre/ tdTom positive (*left*) and nissl stained (*right*) neurons and quantification. In this line, 18% of L5 neurons were tdTom positive. **(C)** Sections showing proportion of L6b neurons labelled neurons in Ctgf-Cre line, Ctgf-Cre line / tdTom positive (*left*) and nissl (*right*). All Ctgf neurons

were located in L6b, but these neurons only constituted 15% of all L6b neurons. **(D)** In the *Drd1a*-Cre line 76% of tdTom positive cells are located in L6b, the other remaining in L6a. Forty-two percent of all L6b neurons in L6b were *Drd1a* positive. **(E)** Laminar pattern of *Sst*/tdTom neurons (*left*). Quantification of laminar pattern of *Sst* positive neurons in each layer (*right*). **(F)** Laminar pattern of *Vip*/tdTom neurons. Quantification of laminar pattern shows percentages of *Vip* neurons in each layer. **(G)** Laminar pattern of *Pv*/tdTom labelled neurons. Quantification of laminar pattern shows percentages of *Pv* neurons in each layer. **(H)** High magnification images of fb label in *Sim1*-Cre-tdTom neurons show that adjacent L5 pyramidal neurons with dendrites going toward L1 have variable uptake of fb in dendrites. Some show no uptake of fb. **(I)** Quantification of fb uptake in *Tlx3*-Cre-tdTom and in *Sim1*-Cre-tdTom in L5a, L5b, and total L5, at the centre and >140 microns distant from the application site. In the panels with binned data, each dot represents a count or a percentage from a brain section. Total number of neurons counted and mice (in brackets) used are shown in each panel. Data from three mice each genotype. Analysis details in **Supplementary Tables 2A, 2B, 2E**. Fast blue pseudo colored in cyan. Scale bars 100 μm .

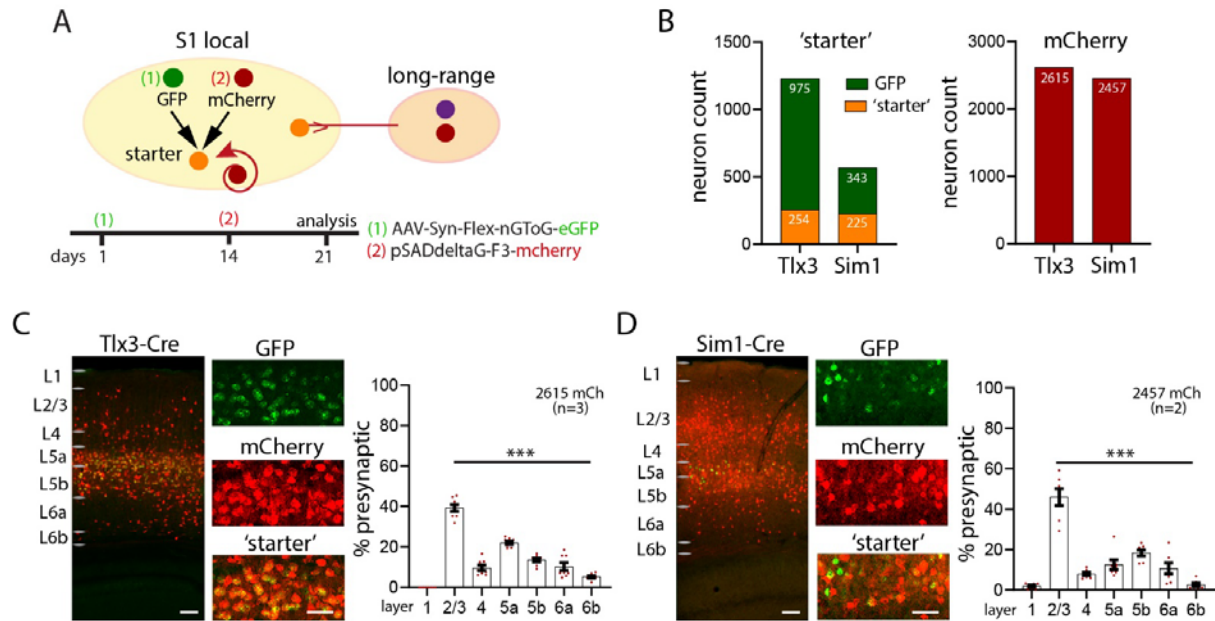


Supplementary Figure 7. Measuring synaptic input using the rabies approach in L5 and POM transgenic lines. (A) Schematic showing injection scheme for rabies virus tracing in Gpr26-Cre, Tlx3-Cre and Sim1-Cre mice. (B) Example images of CA1 hippocampus in Gpr26-Cre mice, with CFP positive starter neurons, GFP positive postsynaptic neurons, RFP positive presynaptic boutons and merged images. (C) Example image of entorhinal cortex confirming long-range projections to CA1, suggesting that rabies approach can work at the tuft dendrites. (D) Example images of Tlx3-Cre and Sim1-Cre brains of S1 cortex with CFP positive starter neurons, GFP positive postsynaptic neurons, RFP positive presynaptic boutons and merged images. (E) Quantification of boutons (yellow) in Tlx3-Cre and Sim1-Cre brains across the layers (Tlx3, n=369 synapses, Sim1, n=158 synapses, counting from 10 boxes in each layer with size of 20 μm x 10 μm , 1 dot represents 1 box). (F) Example images in S2, motor cortex and thalamus showing presynaptic neurons. Three brains per genotype, one-way ANOVA, Bonferroni post-hoc test, ***p<0.001. Data shown as mean \pm S.E.M. Data from three mice each genotype. Scale bars in **B-D, F**, 100 μm , in zoom-ins as indicated or 50 μm , in **E** 5 μm , in **F** thalamus 500 μm .



Supplementary Figure 8. S1 injection sites in Tlx3-Cre and Sim1-Cre brains. (A, B) Example images of injection sites of rabies virus in Tlx3-Cre and Sim1-Cre brains. **(C-F)** Quantification of fb labelled neurons in Tlx3-Cre and Sim1-Cre brains of rabies tracing. **(C)** In S1 cortex ipsilateral, **(D)** S2 cortex ipsilateral, **(E)** Motor cortices ipsilateral, **(F)** Thalamus (neuron counts). Data shown as mean \pm S.E.M. Each dot in the graphs represents one brain section. Total number of neurons counted and mice used (in brackets) are shown in each panel. Data from three mice each genotype. Statistical analysis with one-way ANOVA, Bonferroni

post-hoc test, ****p<0.0001. Analysis details in **Supplementary Tables 3A, 3B**. Scale bars in **A, B**, 800 μm .



Supplementary Figure 9. Rabies virus without fb in Tlx3-Cre and Sim1-Cre brains. (A) Schematic showing timeline for synaptic rabies virus tracing without fb application. **(B)** In Tlx3-Cre, the presynaptic input (2615 mCherry) was derived from 254 starter neurons (975 GFP neurons). In Sim1-Cre, the presynaptic input (2457 mCherry) was derived from 225 starter neurons (343 GFP neurons). **(C, D)** Example images of Tlx3-Cre brains and Sim1-Cre brains in S1 cortex at the injection sites. Higher magnification shows starter, GFP, and mCherry neurons. Quantification of presynaptic neurons in Tlx3-Cre and Sim1-Cre brains in graphs. Each dot represents one brain section. Total number of neurons counted and mice used (in brackets) are shown in each panel. One-way ANOVA, Bonferroni post-hoc test, *** $p < 0.001$. Data shown as mean \pm S.E.M. Analysis details in **Supplementary Table 3C**. Scale bars in **C, D** 100 μ m, in zoom-ins 50 μ m.

Supplementary Tables

Supplementary Table 1A: Input to L1 with fast blue application and retrobead injection.

% fb, cortices (number of neurons, average per brain)	L1	L2/3	L4	L5a	L5b	L6a	L6b	Mice/ brain sections
fb S1 ipsi without centre sections (9868 fb)	12.7±1.3	39.8±1.9 ****	4.8±0.9	17.9±1.4	10.5±1.2	1.4±0.5	12.8±1.9	6/13 (Figure 1B)
fb S1 ipsi all sections (18577 fb, 3096±459)	12.4±0.8	41.7±1.4 ****	5.3±0.7	18.1±1.0	11.4±0.8	1.0±0.3	10.0±1.3	6/31 (Suppl Figure 1A)
fb/nissl S1 ipsi, 30-micron bin (1071 fb, 3664 nissl)	42.7±15.7	45.2±11.9 **	4.2±0.8	32.9±8.8	20.4±6.3	0.4±0.3	31.9±5.5	3/8 (Figure 1C)
fb/nissl S1 ipsi (16448 fb, 54721 nissl)	46.1±7.4	51.0±5.8 ****	25.8±5.1	34.6±4.7	34.3±4.0	5.4±1.4	35.9±3.5	6/34 (Suppl Figure 1D)
retrobeads (5391 retrobeads, 2490±374)	15.3±2.0	44.9±2.4 ****	0.1±0.09	11.6±1.0	11.3±0.9	0.3±0.3	16.4±2.1	3/30 (Figure 1D)

Values are percentage mean ± standard error. Data from mice as indicated in right column (**Figure 1, Supplementary Figure 1**). Statistical analysis one-way ANOVA, ****p<0.0001. P-values indicate significance level for comparison between L2/3 and other layers. Comparison between layers with fast blue application and retrobead injections; L6a** (Kruskal-Wallis test, p<0.0001).

Supplementary Table 1B: Input from other cortical areas to L1 S1 cortex.

% fb, cortices (number of neurons, average per brain)	L1	L2/3	L4	L5a	L5b	L6a	L6b
fb S1 contra (1883 fb, 366±55)	0±0	61.0±2.9 ****	1.7±0.5	24.9±2.3	12.0±1.7	0.3±0.2	0.03±0.03
fb/nissl S1 contra (1221 fb, 102, 4239 nissl)	0±0	9.4±8.0 ****	0±0	4.0±1.5	2.9±1.2	0±0	0±0
fb M2 ipsi (1311 fb)	0.1±0.1	36.6±5.2	-	48.4±5.7	9.9±2.0	4.6±1.1	0.4±0.4
fb/nissl M2 ipsi (1311 fb, 13327 nissl)	0.2±0.1	14.2±3.1	-	34.7±6.0 ****	6.6±1.5	2.3±0.8	0.1±0.1
fb M1 ipsi (1343 fb)	0±0	50.2±6.2	-	31.1±7.1	9.4±2.1	2.9±1.3	6.5±5.4
fb/nissl M1 ipsi (1343 fb, 12285 nissl)	0±0	19.4±3.0	-	20.8±5.4	5.4±1.3	1.9±0.9	3.6±2.9
fb S2 ipsi (1663 fb)	0±0	41.8±6.2	8.8±1.2	12.9±1.6	30.4±10.7	10.1±5.3	56.0±1.7
fb/nissl S2 ipsi (1663 fb, 11763 nissl)	0±0	42.3±14.0	15.9±4.1 ****	14.0±1.1	8.6±2.0	10.7±2.1	20.1±2.2
fb visual ipsi (1056 fb)	1.6±1.6	50.7±2.4	5.7±14.8	14.9±2.5	9.7±3.9	2.0±1.4	15.4±7.4
fb/nissl visual ipsi (1056 fb, 5544 nissl)	6.1±6.1	41.5±14.2	18.5±15.0 ****	23.3±10.2	14.4±4.5	4.1±3.5	11.1±2.4
fb perih ipsi (51 fb)	0±0	1.6±1.6	0±0	90.3±5.6	8.1±4.9	0±0	0±0
fb perih contra (146 fb)	0±0	7.2±4.2	0±0	70.5±7.4	22.4±8.6	0±0	0±0

Values are percentage mean ± standard error, data from four mice (**Figure 2**). Statistical analysis one-way ANOVA, ****p<0.0001. P-values indicate significance level for comparison between L2/3 (or L5a, M2) and other layers.

Supplementary Table 1C: Fast blue injection into S1 cortex.

% fb, cortices/ layers, (number of neurons)	L1	L2/3	L4	L5a	L5b	L6a	L6b	Mice/ brain sections
fb injection S1 ipsi (3393 fb)	13.8±1.1	34.8±4.1 **	12.5±1.2	12.2±2.0	12.6±1.4	10.7±1.2	3.7±0.6	2/5 (Suppl Figure 4B)
fb injection S1 contra (1340 fb)	0±0	41.3±1.1 *	11.6±2.1	18.0±3.0	17.7±1.0	3.0±0.7	0±0	2/5 (Suppl Figure 4C)
fb injection S2 ipsi (1859 fb)	0±0	32.5±3.0 *	14.2±3.5	13.8±2.2	10.7±1.9	21.7±1.9	7.2±1.0	2/5 (Suppl Figure 4D)

Values are percentage mean \pm standard error, data from two mice (**Supplementary Figure 4**). Statistical analysis one-way ANOVA, ****p<0.0001. P-values indicate significance level for comparison between L2/3 and other layers. Comparison between layers with fast blue application, retrobead injections into L1, and fast blue injection into cortex; L4****, L6a**** (Kruskal-Wallis test, p<0.0001).

Supplementary Table 2A: tdTom expression in excitatory Ai9-Cre lines.

% fb, cortices/ layers, (number of neurons)	L5a	L5b	L6b	Mice/ brain sections
Tlx3-Ai9, S1 ipsi (3617 tdTom)	75.6±2.4	23.8±2.3	-	3/16 (Suppl Figure 6A)
Tlx3-Ai9, S1 ipsi, nissl (2497 tdTom, 6858 nissl)	42.1±2.4	-	-	3/11 (Suppl Figure 6A)
Sim1-Ai9, S1 ipsi (3693 tdTom)	27.5±3.6	54.0±2.5	-	3/18 (Suppl Figure 6B)
Sim1-Ai9, S1 ipsi, nissl (930 tdTom, 7869 nissl)	-	18.1±2.6	-	3/16 (Suppl Figure 6B)
Ctgf-Ai9, S1 ipsi, nissl (1552 tdTom, 511 nissl)	-	-	14.9±3.8	3/6 (Suppl Figure 6C)
Drd1a-Ai9, S1 ipsi (4874 tdTom)	-	-	75.7±2.6	4/23 (Suppl Figure 6D)
Drd1a-Ai9, S1 ipsi, nissl (1303 tdTom, 3047 nissl)	-	-	42.8±4.0	3/11 (Suppl Figure 6D)

Values are percentage mean ± standard error, data from three mice of each genotype and as indicated (**Supplementary Figure 6**).

Supplementary Table 2B: tdTom expression in inhibitory Ai9-Cre lines.

Cortices / layers, Tlx3 (%)	L 1	L 2/3	L4	L5a	L5b	L6a	L6b	Mice/ brain sections
Sst-Ai9, S1 ipsi, nissl (3220 tdTom, 23889 nissl)	4.4±1.6	11.0±1.1	12.7±0.8	20.3±1.7	22.5±1.7	13.9±2.2	7.3±1.0	3/10 (Suppl Figure 6E)
Vip-Ai9, S1 ipsi, nissl (460 tdTom, 9780 nissl)	2.3±0.5	11.3±1.6	3.6±1.2	4.9±1.4	5.2±1.2	1.5±0.2	1.2±0.2	4/10 (Suppl Figure 6F)
PV-Ai9, S1 ipsi, nissl (3936 tdTom, 28899 nissl)	0±0	9.6±0.9	17.3±1.5	20.7±2.3	22.9±2.4	15.1±1.3	0±0	3/9 (Suppl Figure 6G)

Values are percentage mean ± standard error, data from three mice of each genotype and as indicated (**Supplementary Figure 6**).

Supplementary Table 2C: Input from classes of L5 and L6b cells to L1.

% fb, cortices/ layers, (number of neurons)	L5a	L5b	L6b	Mice/ brain sections
Tlx3-Ai9 S1 ipsi, tdTom/fb (7706 tdTom, 2361 tdTom+fb)	29.4±3.2 ****	-	-	3/25 (Figure 4A)
Sim1-Ai9, S1 ipsi, tdTom/fb (1832 tdTom, 382 tdTom+fb)	-	19.7±3.0	-	3/22 (Figure 4B)
Ctgf-Ai9, S1 ipsi, fb (578 tdTom, 288 tdTom+fb)	-	-	51.5±9.9	3/12 (Figure 4C)
Drd1a-Ai9, S1 ipsi, fb (3447 tdTom, 510 tdTom+fb)	-	-	12.7±7.0	3/18 (Figure 4D)
Tlx3-Ai9, S1 contra, tdTom/fb (2100 tdTom, 111 tdTom+fb)	7.7±1.4	-	-	3/18 (Figure 4H)
Tlx3-Ai9, S2 ipsi, tdTom/fb (439 tdTom, 49 tdTom+fb)	11.1±1.1	-	-	3/3 (Figure 4H)
Tlx3-Ai9, motor ipsi, tdTom/fb (1249 tdTom, 141 tdTom+fb)	10.7±1.7	-	-	3/15 (Figure 4H)
Sim1-Ai9, S1 contra, tdTom/fb (4522 tdTom, 73 tdTom+fb)	-	1.3±0.5	-	3/18 (Figure 4I)
Sim1-Ai9, S2 ipsi, tdTom/fb (412 tdTom, 19 tdTom+fb)	-	8.2±1.5	-	3/13 (Figure 4I)
Sim1-Ai9, motor ipsi, tdTom/fb (1083 tdTom, 42 tdTom+fb)	-	10.6±2.1	-	3/12 (Figure 4I)

Values are percentage mean ± standard error, data are from three mice of each genotype (**Figure 4**).

Supplementary Table 2D: Input from inhibitory Sst, Vip, and Pv cells to L1.

% fb, cortices/ layers, (number of neurons)	L 1	L 2/3	L4	L5a	L5b	L6a	L6b	Mice/ brain sections
Sst-Ai9, S1 ipsi, tdTom/fb (3819 tdTom, 377 tdTom+fb)	28.3±7.2	19.7±2.3	12.1±2.2	11.6±1.5	8.9±1.1	1.5±0.6	3.0±1.0	3/25 (Figure 4E)
Vip-Ai9, S1 ipsi, tdTom/fb (777 tdTom, 93 tdTom+fb)	4.0±2.5	81.2±4.9 ****	2.0±1.2	5.3±2.4	5.9±2.7	0±0	1.6±1.6	3/7 (Figure 4F)
Pv-Ai9, S1 ipsi, tdTom/fb (3942 tdTom, 318 tdTom+fb)	0±0	14.2±2.7	0±0	15.9±3.3	19.7±3.5	0±0	0±0	3/15 (Figure 4G)

Values are percentage mean ± standard error, data are from three mice of each genotype (**Figure 4**).

Supplementary Table 2E: Fast blue application - Tlx3-Cre-Ai9 and Sim1-Cre-Ai9 in L5.

% fb, cortices/ layers, (number of neurons)	L5a	L5b	L5	Brain sections
Tlx3-Ai9, S1 tdTom/fb (5746 tdTom, 1575 tdTom +fb)	45.7±6.6 (c), 22.6±1.8 (l)	34.3±8.7 (c), 15.0±4.2 (l)	40.0±5.4 (c), 21.0±2.0 (l)	25 (Suppl Figure 6)
Sim1-Ai9, S1 tdTom/fb (4038 tdTom, 1209 tdTom +fb)	50.7±8.7 (c), 24.2±4.9 (l)	25.2±5.7 (c), 16.1±2.8 (l)	40.4±4.8 (c), 15.2±3.0 (l)	22 (Suppl Figure 6)

Values are percentage mean ± standard error, data are from three mice of each genotype; c=centre, l=lateral (**Supplementary Figure 6**).

Supplementary Table 3A: Rabies virus and fast blue application in Tlx3-Cre mice.

Cortices / layers, Tlx3 (%)	L 1	L 2/3	L4	L5a	L5b	L6a	L6b	Brain sections
mCherry S1 (6138 total, 4728 local)	3.6±1.4	37.8±2.8 ****	8.9±0.9	23.7±1.8	16.8±1.8	7.9±1.6	1.2±1.1	15 (Figure 5C)
fb S1 (9596 total, 5514 local)	9.7±1.9	47.1±4.8 ****	3.3±0.8	14.6±1.0	13.4±3.5	1.4±0.6	10.4±2.9	10 (Suppl Figure 8C)
fb-mCherry S1 (449 total, 391 local)	4.78±1.4	26.8±3.78 ****	9.43±1.9	28.1±3.1	30.9±3.17	0±0	0±0	15 (Figure 5C)
mCherry S2 (761)	1.79±3.65	46.2±3.38 ***	2.80±1.31	25.1±4.57	16.8±4.16	5.22±2.53	2.13±1.37	9 (Figure 6A)
fb S2 (2433)	0.8±0.8	63.3±17.4	0.6±0.6	4.5±2.7	3.3±1.9	0.2±0.2	27.1±12.9	9 (Suppl Figure 8D)
mCherry motor (229)	0±0	49.9±4.99 ***	-	12.3±3.56	23.7±4.94	12.0±6.20	2.17±2.17	12 (Figure 6B)
fb motor (292)	1.8±1.8	53.5±26.4	-	5.9±3.7	20.5±5.5	4.8±2.7	13.5±9.0	11 (Suppl Figure 8E)

Values are percentage mean ± standard error, data are from three mice (**Figures 5, 6, Supplementary Figure 8**). Statistical analysis one-way ANOVA, ****p<0.0001. P-values indicate significance level for comparison between L2/3 and other layers.

Supplementary Table 3B: Rabies virus and fast blue application in Sim1-Cre mice.

Cortices / layers, Sim1 (%)	L 1	L 2/3	L4	L5a	L5b	L6a	L6b	Brain sections
mCherry S1 (6495 total, 3869 local)	1.5±0.2	38.6±1.8 ****	6.6±1.0	8.5±0.7	36.7±1.33 ****	6.94±1.1	1.18±0.18	15 (Figure 5D)
fb S1 (13131 total, 9191 local)	13.7±3.9	43.0±4.8 ****	1.2±0.4	26.0±4.7	3.8±1.3	0.6±0.3	11.6±2.8	12 (Suppl Figure 8C)
fb-mCherry S1 (823 total, 754 local)	3.9±0.9	38.1±5.6 ****	2.4±1.1	7.6±1.9	46.6±5.5 ****	0.3±0.3	0.3±0.2	15 (Figure 5D)
mCherry S2 (1095)	0.1±0.1	58.6±5.8 ***	5.4±2.01	13.1±3.0	20.6±4.0	1.9±1.2	0.1±0.13	14 (Figure 6D)
fb S2 (2564)	17.9±4.5	37.1±7.0	0.6±0.4	13.0±5.6	12.5±3.6	0.6±0.3	18.2±5.10	14 (Suppl Figure 8D)
fb-mCherry S2 (31)	0±0	19.0±7.7	0±0	66.2±14.5	14.8±8.4	0±0	0±0	6 (Figure 6D)
mCherry motor (542)	0±0	48.8±6.1 ****	-	7.1±3.3	39.2±5.7	3.9±1.9	1.0±1.0	17 (Figure 6E)
fb motor (367)	0±0	58.0±4.7	-	4.7±3.4	19.4±3.9	6.5±4.5	11.4±5.4	13 (Suppl Figure 8E)
fb-mCherry motor (13)	0±0	30.7±11.9	-	1.8±1.8	3.2±2.2	0±0	0±0	9 (Figure 6E)

Values are percentage mean ± standard error, data are from three mice (**Figures 5, 6, Supplementary Figure 8**). Statistical analysis one-way ANOVA, ****p<0.0001. P-values indicate significance level for comparison between L2/3, L5b and other layers.

Supplementary Table 3C: Rabies virus in Tlx3-Cre and Sim1-Cre brains.

mCherry, layers S1 (%)	L 1	L 2/3	L4	L5a	L5b	L6a	L6b	Brain sections
Tlx3-Cre (2615 mCherry)	0±0	39.3±1.7 ***	9.6±1.4	22.1±0.8	13.5±0.9	10.3±1.9	5.2±0.5	10
Sim1-Cre (2457 mCherry)	1.9 ±0.5	45.9±4.1 ***	7.9±0.8	12.5±2.4	18.4±1.5	10.7±2.7	2.5±0.8	9

Values are percentage mean ± standard error, data are from three Tlx3-Cre, and two Sim1-Cre mice (**Supplementary Figure 9**). Statistical analysis one-way ANOVA,

***p<0.001. P-values indicate significance level for comparison between L2/3 and other layers.

Supplementary Table 3D: Rabies virus and fast blue application in Tlx3-Cre and Sim1-Cre mice.

% (average per brain)	Local	Long-range	S1c	M1i	M2i	M1c	S2i	Perirhc	V1i, V2Li	Thal	other
Tlx3 mCherry	80.3±5.3	19.7±5.3	0±0	0.8±0.3	2.5±2.5	0±0	12.0±2.4	0.01±0.01	2.3±1.2	1.7±0.9	0.4±0.4
Tlx3 mCherry+fb	89.1±7.7	10.9±7.7	0±0	0.7±0.4	0±0	0±0	8.3±8.3	0±0	0.5±0.5	1.3±0.7	0±0
Sim1 mCherry	62.8±5.5	37.2±5.5	0.1±0.1	10.3±5.0	0.6±0.4	1.9±1.9	13.8±5.1	0.4±0.4	9.5±2.9	0.8±0.04	0±0
Sim1 mCherry+fb	93.1±2.0	6.9±2.0	0±0	0.7±0.7	0.2±0.2	0±0	3.8±0.1	0±0	1.5±1.5	0.1±0.1	0±0

Values are percentage mean ± standard error, data are from three mice each genotype (**Figure 6**).

Supplementary Table 4: Fast blue application and retrobead injection on S1.

% (number of neurons, average per brain)	Local	Long-range	S1c	M1i	M2i	M1c	S2i	Perirh-i	Perirh-c	V1i	V2Li	Thal	other
fb (32943, 11956±2469)	68.0±5.6	31.8±5.6	0.7±0.5	7.7±4.8	5.2±3.2	0.2±0.2	2.9±1.9	0.2±0.2	0.5±0.5	1.6±1.1	1.6±1.4	8.7±2.6	0.9±0.1
retrobead (15810, 3487±868)	64.7±5.3	35.3±5.3	0.9±0.5	9.2±1.6	3.6±1.6	0.9±0.5	4.1±1.0	2.8±0.9	0.9±0.1	4.2±2.7	0.7±0.4	6.1±2.0	1.8±1.6

Values are percentage mean \pm standard error, data are from four mice (fb), three mice (retrobeads) (**Figure 7**). i=ipsi, c=contra.