Supplemental Information - Supplemental Data

Figure S1. Related to Figure 1: Inhibition generated by photo-activation of NTSR1-Cre expressing layer 6 pyramidal cells is disynaptic in all layers

Inset: Schema illustrates experimental setup of *in vitro* whole cell recordings from NTSR1-ChR2 mice. For each experiment a single whole-cell recording was made in either L2/3, L4, L5 or L6. Current traces: Inhibitory postsynaptic currents (IPSCs) recorded from pyramidal cells voltage clamped at the reversal for excitation (+7mV) in response to the photo-activation of L6 NTSR1-Cre expressing excitatory neurons (blue bar: 1.5s) conditionally expressing ChR2. Bold blue trace: average of IPSCs across cells (L2/3 n=3, L4 n=3, L5 n=5, L6 n=3) with SEM shown in light blue. Black trace: average of IPSCs across cells after perfusion of AMPA receptor and NMDA receptor antagonists (10μM NBQX and 20μM CPP, respectively) to block glutamate transmission abolished IPSCs in all layers (scale bars 100pA/250ms).

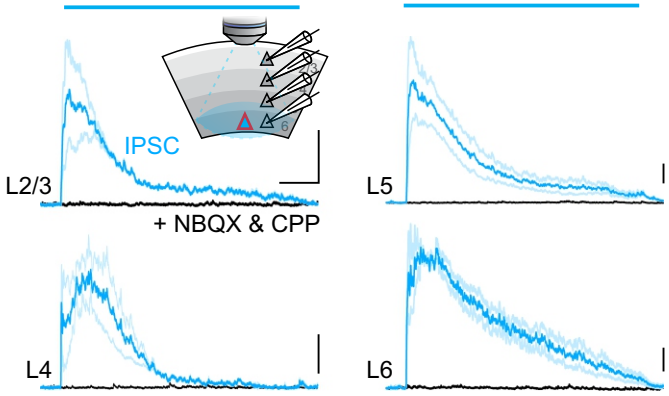

Figure S1.

Figure S2. Related to Figure 4: Pulsed photo-activation of layer 6 pyramidal cells fails to recruit interneurons in superficial layers

Left: Schema shows experimental setup on acute slices of a NTSR1-ChR2 X GAD67-GFP mouse. A single loose-patch recording was made on a GFP-positive inhibitory neuron in either L2/3, L4, L5 or L6 while photoactivating L6CTs. Center: Loose patch recordings from GFP expressing neurons in all layers of a visual cortical slice from NTSR1-ChR2 X GAD67-GFP mouse in response to brief pulses of light (blue triangles, 2ms LED pulses at 2Hz for 2s, 5.3mW; scale bar 50pA/500ms). Note spikes (vertical deflections) triggered by light pulses in deeper layers. Right: Summary graph showing percentage of GFP expressing neurons recruited by photo-activation of L6CTs (same population of neurons as shown in Fig. 4; L1 n=42, L2/3 n=45, L4 n=41, L5 n=54, L6 n=72; 7 mice).

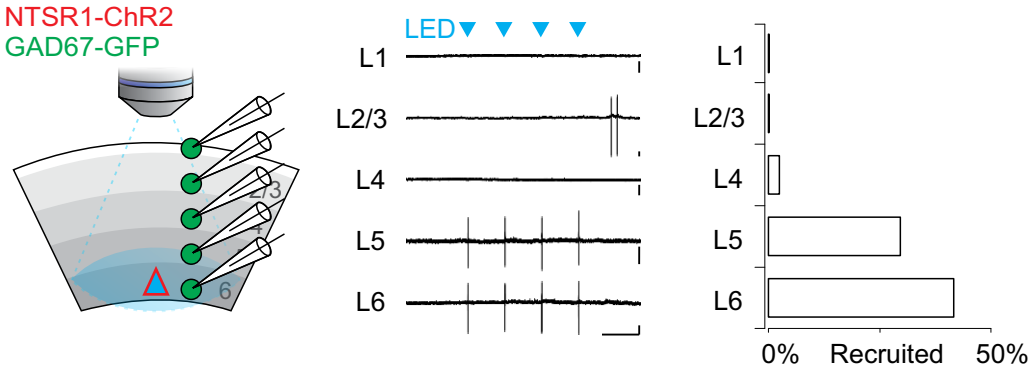
NTSR1-ChR2
GAD67-GFP
2/3
6
LED
L1
L2/3
L4
L5
L6
L1
L2/3
L4
L5
L6
0%
Recruited
50%

Figure S2.

Figure S3. Related to Figure 4: Larger synaptic excitation in inhibitory neurons that fire action potentials in response to photo-activation of layer 6 pyramidal cells

(A) Left: Schematic of experimental setup for i*n vitro* whole cell recordings from GFP expressing cells in NTSR1-ChR2 X GAD67-GFP mouse. A single whole cell recording was made on inhibitory neurons in either L1, L2/3 L4, L5 or L6. Right: Recording from GFP expressing cells voltage clamped at the reversal potential for inhibition. A single LED pulse (2ms) to photo-activate L6 cortico-thalamic neurons (L6CTs) elicited EPSCs in all GFP expressing cells in all layers (L1 n=5, L2/3 n=19, L4 n=5, L5 n=5, L6 n=8; 10 mice; scale bars: L1 10pA/10ms, L2/3 100pA, L4 100pA, L5 500pA, L6 500pA).

(B) Left: Schematic of experimental setup illustrating simultaneous paired recordings of L2/3 and L6 GFP expressing cells. Left graph: Excitatory charges recorded from pairs of cells voltage clamped at the reversal potential for inhibition in response to photo-activation of L6CTs (n=7 pairs; 5 mice; p=0.0029). Inset: example average EPSC elicited in response to photo-activation of L6CTs for the pair labeled by filled circles (L6 red, L2/3 grey, for all insets: blue bar LED photostimulation 1.5s, scale bars 250pA/250ms). Middle graph: Inhibitory charges for the same cell pairs illustrated on the left and recorded at the reversal potential for excitation (p=0.0055). Inset: L6 in blue, L2/3 in grey. Right: Excitatory charge expressed as a percentage of total charge (excitatory + inhibitory) for the same cell pairs illustrated on the left (p=0.0333).

(C) As in (B) but comparing spiking and non-spiking (n.sp.) L6 GFP expressing cells (from left to right p=0.0154, 0.0335 and 0.0396; n=6 pairs 3 mice).

(D) The firing rate of all GFP expressing L6 cells that are recruited in response to photo-activation of L6CTs is plotted against the inhibitory and excitatory charge (p=0.1733 and 0.0008, respectively, n=19 cells 8 mice).

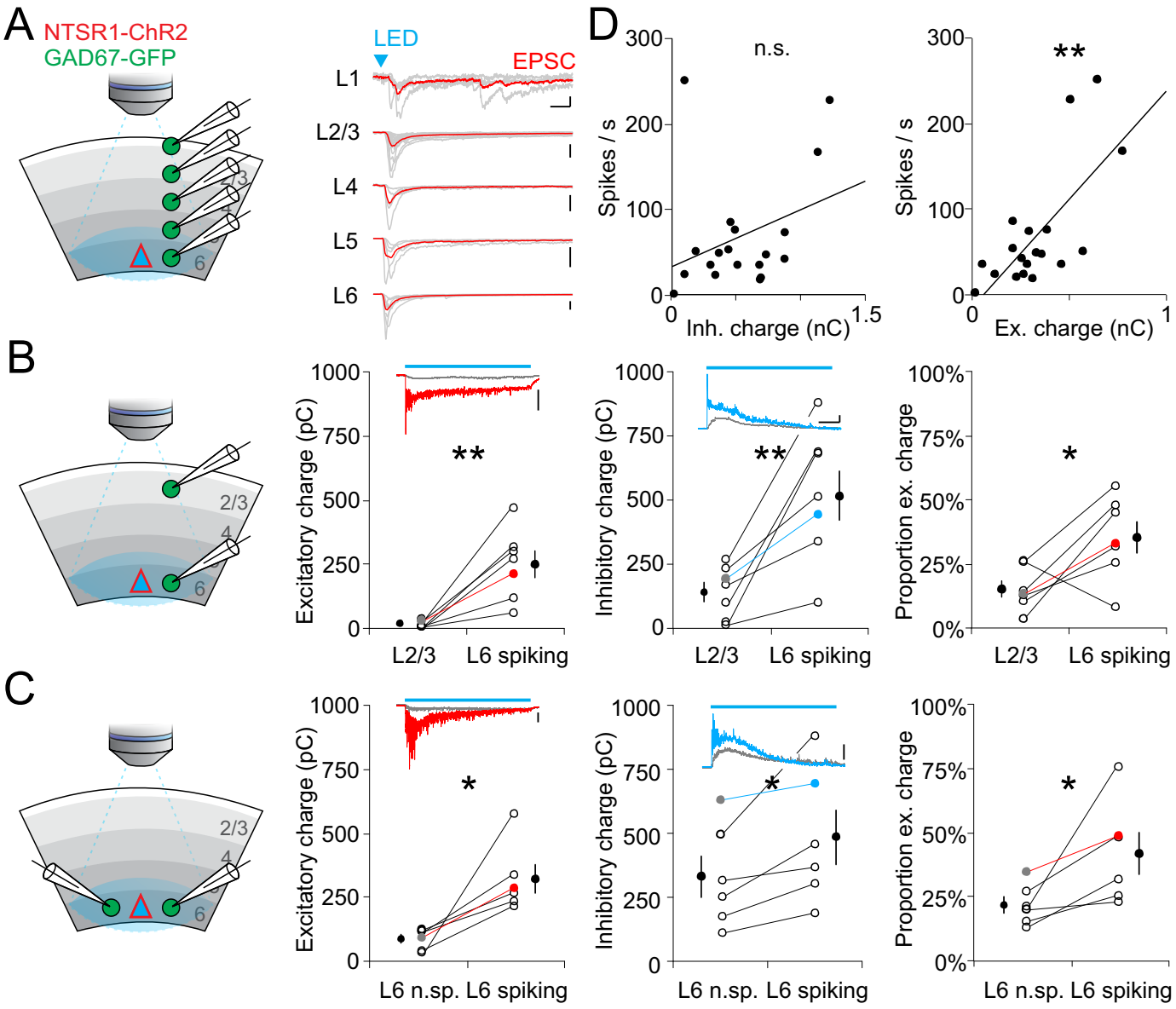
A
NTSR1-ChR2
GAD67-GFP
LED
EPSC
L1
L2/3
L4
L5
L6
2/3
4
6
D
n.s.
**
Spikes / s
Inh. charge (nC)
Ex. charge (nC)
B
Excitatory charge (pC)
Inhibitory charge (pC)
Proportion ex. charge
L2/3
L6 spiking
C
L6 n.sp.
L6 spiking

Figure S3.

Figure S4. Related to Figure 5: Locally projecting FS cells recruited by L6 cortico-thalamic pyramidal cells

(A) Reconstructions of locally projecting FS cells that fired in response photo-activation of L6CTs. Scale bar in black 50μm. Grey tics to right of cells mark layer boundaries. (B) Average heat map of axons (left) and dendrites (middle) of the eleven reconstructed translaminar GFP expressing neurons after normalizing for differences in layer depths. Heat map of axons (left) and dendrites (middle) of biocytin filled, reconstructed neurons after normalizing for differences in layer depths. Right panel shows overlay of axons (red) and dendrites (green). Left: shows the relative density of neurites length for each layer for cells shown above (right bars, n=11) and translaminar FS cells (right bars, from cells shown in Figure 5B, n=11). The relative density is the fraction of total neurite length divided by the fractional layer thickness; the fractional layer thickness is computed as the thickness of a layer divided by the cortical thickness, measured along the radial axis from the pia to the layer 6 white matter border.

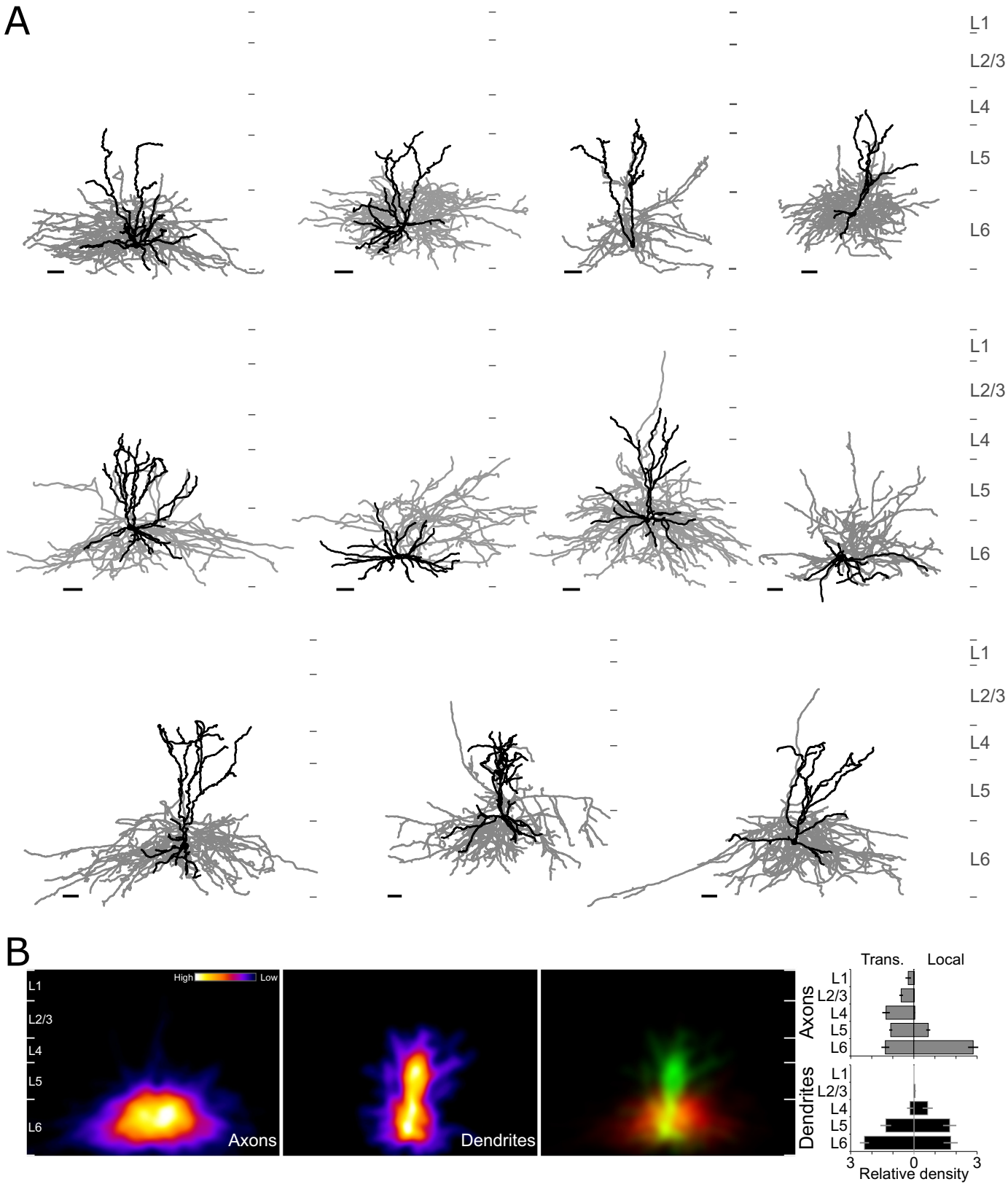
A
L1
L2/3
L4
L5
L6
B
High
Low
Axons
Dendrites
Trans.
Local
Relative density
3
0
3

Figure S4.

Figure S5. Related to Figure 6: Translaminar FS cells lack dendritic spines

Biocytin fill of NTSR1+ pyramidal cell is shown on top with soma on the left and dendrite on the right. Arrowheads indicate spines. Translaminar interneuron (same cell as Figure 5B top middle) soma and dendrite are shown below.

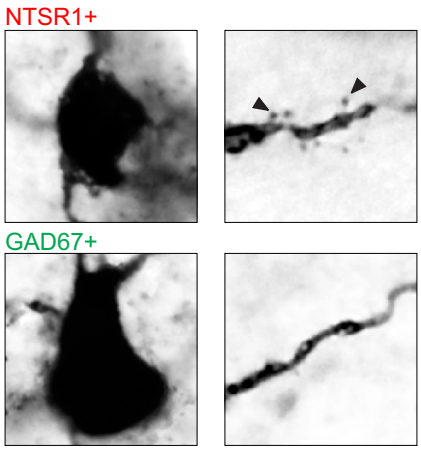

Figure S5.