Figure S1

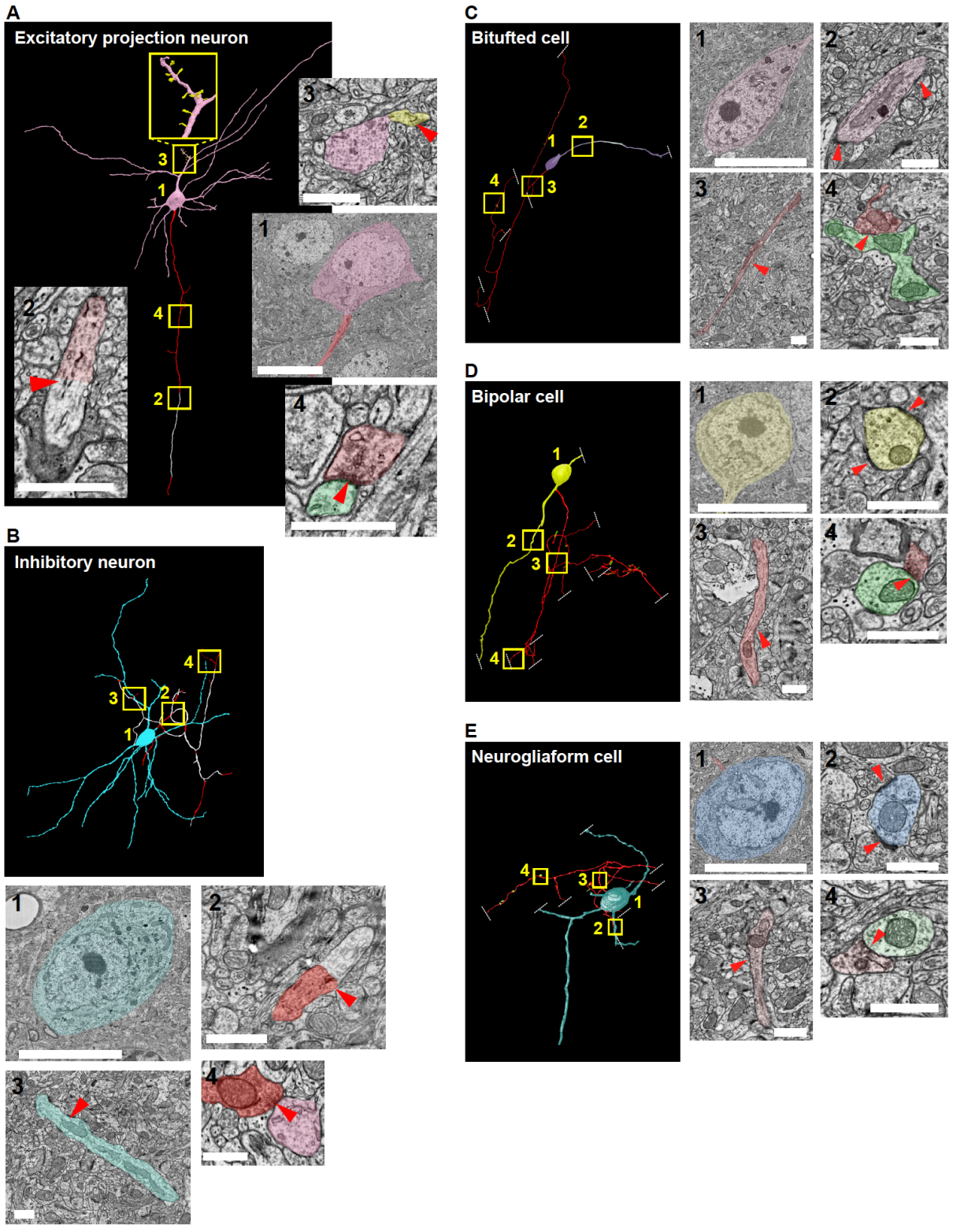

A
Excitatory projection neuron
B
Inhibitory neuron
C
Bitufted cell
D
Bipolar cell
E
Neurogliaform cell

Figure S1. Morphological characterization of an excitatory neuron and myelinated and non-myelinated interneurons in V1. Related to Figure 1.

(A) Representative 3D rendering of a cortical pyramidal cell reconstructed from a high-resolution EM dataset of V1 cortex (Bock et al., 2011); soma and dendrites shown in pink, spines in yellow, non-myelinated axon in red, and myelin in white. EM images are representative single-section images of the pyramidal-shaped cell body (**1**), the descending myelinated axon (**2**), spiny dendrites (**3**), and an example of an asymmetrical excitatory synapse (**4**) (red arrowheads). (**B**) 3D reconstruction and EM images of a neuron with morphological features of a cortical interneuron, with soma and dendrites labeled in blue, non-myelinated axon in red, and myelin in white. EM images are representative single-section images of the ovoid shape cell body (**1**), ascending myelinating axon (**2**), aspiny dendrites receiving multiple shaft synapses (red arrowhead), (**3**) and a symmetrical axo-dendritic synapse onto a pyramidal neuron (pink) (**4**) (Red arrowheads). Scale bars, 10μm for cell bodies, 1μm for axons and synapses. 3D reconstructions of three interneurons with morphological and synaptic connectivity profiles of a cortical bitufted cell (**C**), a bipolar cell (**D**), and a neurogliaform cell (**E**). Dashed white lines indicate the end of EM tracing due to the axon or dendrites leaving the V1 EM volume, or where the quality of the EM data precluded further tracing. Right, EM images are representative single sections of the cell bodies (**1**), dendrites (**2**), axons (**3**), and symmetrical synapses (red arrowhead) (**4**). Scale bars, 10μm for panels 1 and 1μm for panels 2-4.

Figure S2

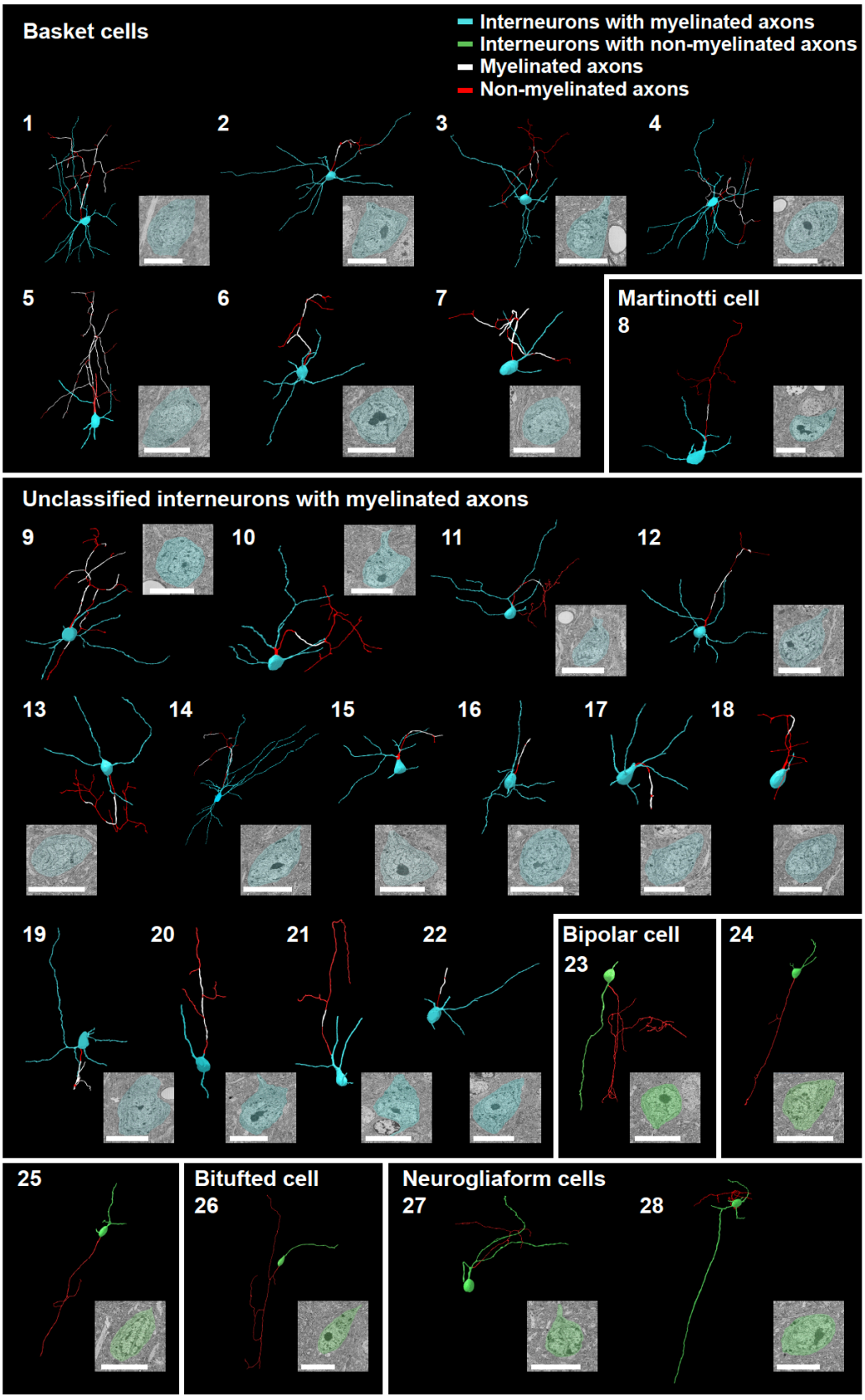

Basket cells
Interneurons with myelinated axons
Interneurons with non-myelinated axons
Myelinated axons
Non-myelinated axons
1
2
3
4
5
6
7
Martinotti cell
8
Unclassified interneurons with myelinated axons
9
10
11
12
13
14
15
16
17
18
19
20
21
22
Bipolar cell
23
24
25
Bitufted cell
26
Neurogliaform cells
27
28

Figure S2. Identification of cortical interneuron subtypes in layer II/III of the V1 cortex. Related to Figure 1.

3D reconstructions of 28 neurons with morphological and synaptic profiles of cortical interneurons traced from the high-resolution EM dataset of V1 cortex. Non-myelinated axons are shown in red, myelin in white, cell bodies and dendrites of interneurons with myelinated axons in blue, and cell bodies and dendrites of interneurons with non-myelinated axons in green. EM images of the cell body for each of the interneurons are displayed next to the 3D renderings. Scale bar 10μm. 12 of the interneurons were classified according to distinctive morphological and synaptic connectivity profiles (Petilla Interneuron Nomenclature Group et al., 2008) as basket cells (**1-7**), Martinotti cell (**8**), bipolar (**23**), bitufted (**26**), and neurogliaform cells (**27-28**) (see **Table S1**).

Figure S3

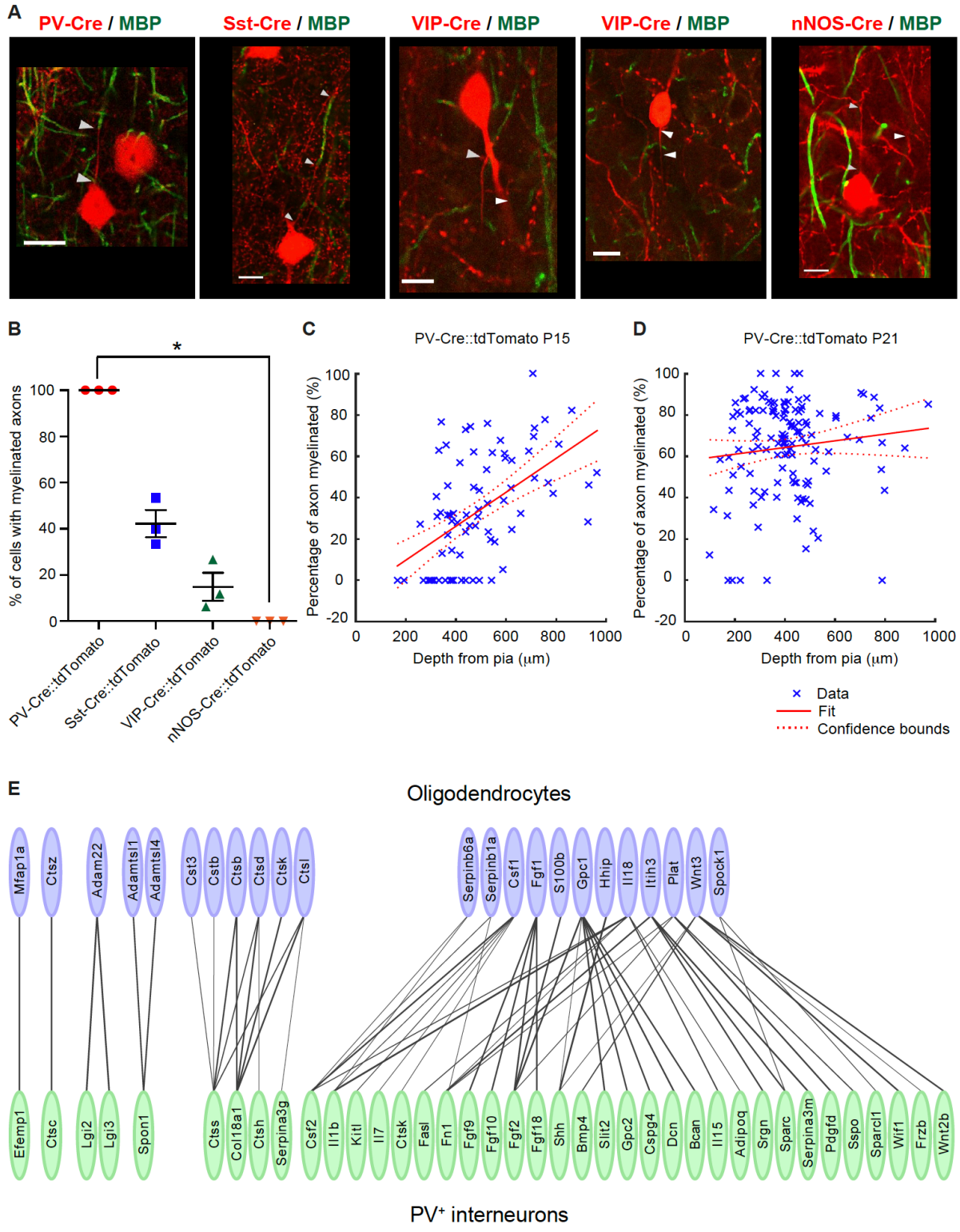

A
PV-Cre / MBP
Sst-Cre / MBP
VIP-Cre / MBP
VIP-Cre / MBP
nNOS-Cre / MBP
B
% of cells with myelinated axons
PV-Cre::tdTomato
Sst-Cre::tdTomato
VIP-Cre::tdTomato
nNOS-Cre::tdTomato
C
PV-Cre::tdTomato P15
Percentage of axon myelinated (%)
Depth from pia (μm)
D
PV-Cre::tdTomato P21
Data
Fit
Confidence bounds
E
Oligodendrocytes
Mfap1a
Ctsz
Adam22
Adamtsl1
Adamtsl4
Cst3
Cstb
Ctsb
Ctsd
Ctsk
Ctsl
Serpinb6a
Serpinb1a
Csf1
Fgf1
S100b
Gpc1
Hhip
Il18
Itih3
Plat
Wnt3
Spock1
Efemp1
Ctsc
Lgi2
Lgi3
Spon1
Ctss
Col18a1
Ctsh
Serpina3g
Csf2
Il1b
Kitl
Il7
Ctsk
Fasl
Fn1
Fgf9
Fgf10
Fgf2
Fgf18
Shh
Bmp4
Slit2
Gpc2
Cspg4
Dcn
Bcan
Il15
Adipoq
Srgn
Sparc
Serpina3m
Pdgfd
Sspo
Sparcl1
Wif1
Frzb
Wnt2b
PV⁺ interneurons

Figure S3. Axonal myelination profiles of specific interneuron populations across cortical layers and candidate genes involved in PV^+ interneuron and oligodendrocyte interaction. Related to Figure 2.

(A) Representative confocal images of neurons from the V1 of PV^+ (PV::tdTomato), Sst^+ (Sst::tdTomato), VIP^+ (Sst::tdTomato) and $nNOS^+$ (nNOS::tdTomato) animals at P60, labeled with anti-MBP (green) (**See Video S1**). White arrowheads indicate the axons and ends of myelin sheaths where visible. **(B)** Pooled data showing the percentage of PV^+, Sst^+/PV^-, VIP^+ and $nNOS^+$ neurons with myelinated axons at P60 (n = 3 animals each). Data are presented as mean ± s.e.m. $*P < 0.05$ (Fisher's exact test). (**C**-**D**) Scatter plots of the percentage of axon length that is myelinated for all axons with myelin (excluding the initial axon segment, defined as the axon between the cell body and the edge of the first myelin sheath) from individually traced PV^+ (PV::tdTomato) cells located in the lower through upper layers of the V1 at P15 (**C**) and P21 (**D**). Linear regression fit, solid red line; 95% confidence intervals, red dotted lines (P15 n = 5 and $P = 3.2262e-07$; P21 n = 3 and $P = 0.19443$, by Spearman rank-order correlation). **(E)** A network interaction map of the PV^+ and oligodendrocyte partner genes constructed using Cytoscape (Shannon et al., 2003). The color intensity of the line connecting two nodes indicates the general strength of the interaction between that protein pair as computed by StringDB.

Figure S4

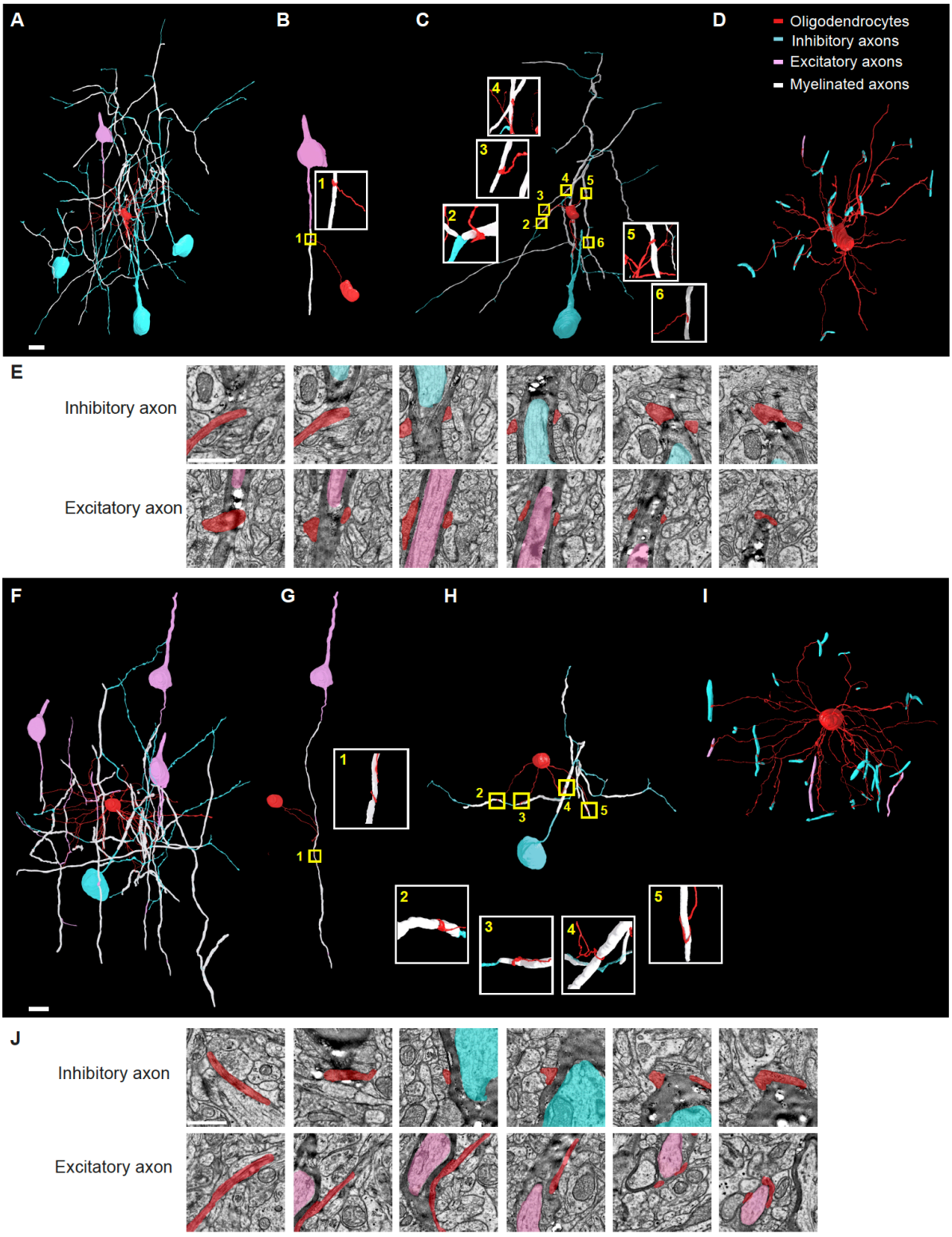
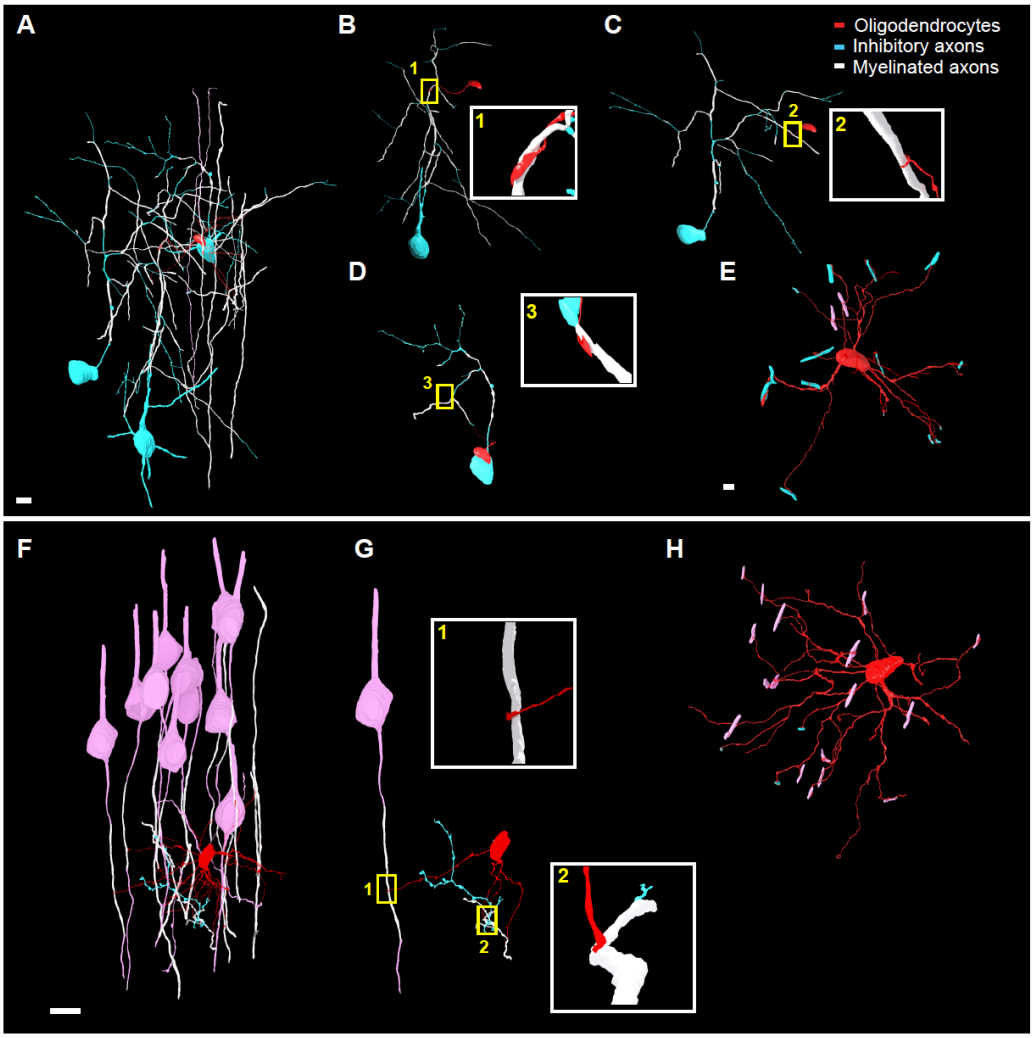

A
B
C
D
Oligodendrocytes
Inhibitory axons
Excitatory axons
Myelinated axons
E
Inhibitory axon
Excitatory axon
F
G
H
I
J
Inhibitory axon
Excitatory axon

Figure S4. Additional examples of individual cortical oligodendrocytes predominantly myelinating the axons of inhibitory and some excitatory neurons. Related to Figure 4 and Figure 5.

(A, F) 3D renderings of oligodendrocytes **6** and **5** (**Figure 5A**) myelinating multiple inhibitory and excitatory axons. Scale bar 1μm. (**B, G**) 3D reconstruction of processes extending from oligodendrocyte 6 and 5 myelinating the axon of a pyramidal neuron. **(C, H)** Reconstructions of oligodendrocytes 6 and 5 repeatedly myelinating the axon of a single inhibitory neuron. (**D, I**) 3D reconstructions of the oligodendrocytes with all of the inhibitory myelinated axons labeled in blue and excitatory axons shown in pink. Scale bar 10μm. **(E, J)** Representative EM images of two branches (red) originating from oligodendrocytes 6 and 5 myelinating the axon of a single inhibitory neuron (blue) and an axon of a single excitatory neuron (pink). (**See Video S2**).

Figure S5


A
B
C
D
E
F
G
H
Oligodendrocytes
Inhibitory axons
Myelinated axons
1
2
3

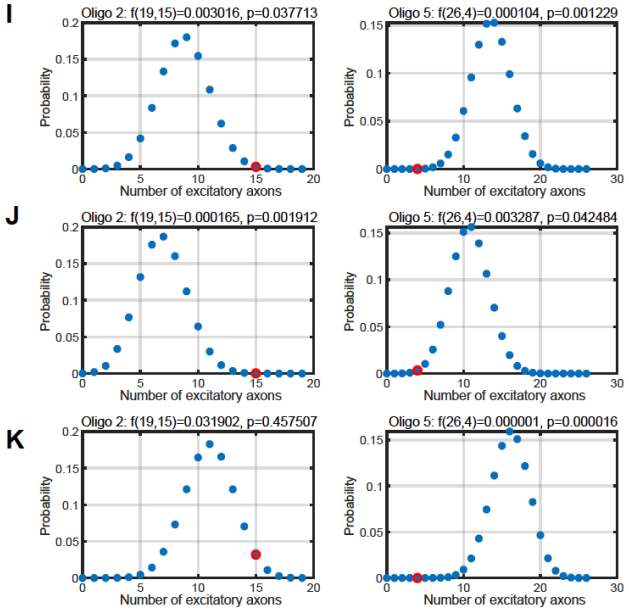

I
J
K
Oligo 2: f(19,15)=0.003016, p=0.037713
Oligo 5: f(26,4)=0.000104, p=0.001229
Oligo 2: f(19,15)=0.000165, p=0.001912
Oligo 5: f(26,4)=0.003287, p=0.042484
Oligo 2: f(19,15)=0.031902, p=0.457507
Oligo 5: f(26,4)=0.000001, p=0.000016
Probability
Number of excitatory axons

Figure S5. Additional examples of oligodendrocytes predominantly myelinating either inhibitory or excitatory axons. Related to Figure 5.

(**A**) 3D reconstructions of a single oligodendrocyte (Oligodendrocyte **3**, **Figure 5A**) myelinating the axons of multiple inhibitory neurons. The oligodendrocyte and all branches are labeled in red, cell somata, dendrites and non-myelinated axons in blue, and myelinated axons in white. (**B-D**) Example 3D reconstructions of individual oligodendrocyte processes (red) myelinating the axons of these interneurons. (**E**) 3D reconstructions of oligodendrocyte 3 showing branches myelinating inhibitory axons (blue) and excitatory axons (pink). Scale bar 10μm. (**F**) 3D reconstruction of a single oligodendrocyte from layer II/III predominately myelinating the axons of excitatory neurons (Oligodendrocyte **1**, **Figure 5A**). The oligodendrocyte is labeled in red; myelinated axons in white; inhibitory non-myelinated axons in blue; and excitatory non-myelinated axons in pink. (**G**) Yellow boxed regions correspond to the oligodendrocyte processes ensheathing the axons of inhibitory and excitatory axons enlarged at right. Image to the right shows the oligodendrocyte with only the axons of the single excitatory and inhibitory neurons shown in the enlargements, for clarity. (**H**) 3D reconstruction of the oligodendrocyte 1, showing the total number of branches myelinating excitatory axons (pink) and inhibitory axons (blue). Scale bar 10μm. (**I-K**) Binomial distribution plots for oligodendrocyte 2 and 5, comparing the actual number of myelinated excitatory axons (red circles) with the number expected from the average ratio of locally available, classifiable myelinated axons in this dataset (**I,** reproduced from **Figure 5D** for ease of comparison), or assuming extreme scenarios wherein the 20% of unidentifiable axons are exclusively inhibitory (**J**) or excitatory (**K**).

Supplementary Tables and Legends

Table S1. Location of interneurons and oligodendrocytes traced in V1 EM dataset. Related to Figure 1 and Figure 4.

Location of the cell bodies and synapses of 28 individually traced inhibitory neurons in the V1 EM dataset (Sheet1; **Figure 1A**). The location of the inhibitory axo-somatic synapses used to classify basket cells are highlighted in red. Location of the 10 oligodendrocytes and each of the axons myelinated in the V1 EM dataset (Sheet2; **Figure 4B**). [**separate Excel file**]

PV-Cre::tdTomato

Age	Total number of cells traced	Number of cells with myelinated axons	Number of cells w/o myelinated axons	Percentage of cells with myelinated axons (%)
P15	78	58	20	74
P21	131	126	5	96
P30	111	111	0	100
P60	156	156	0	100

P values for indicated comparison	
P15 vs P21	7.79E-06
P15 vs P30	4.04E-09
P15 vs P60	4.59E-11
P21 vs P30	*ns*
P21 vs P60	0.019
P30 vs P60	*ns*

Sst-Cre::tdTomato/PV$^-$

Age	Total number of cells traced	Number of cells with myelinated axons	Number of cells w/o myelinated axons	Percentage of cells with myelinated axons (%)
P15	52	0	52	0
P21	52	10	42	19
P30	36	21	15	58
P60	75	39	36	52

P values for indicated comparison	
P15 vs P21	0.0012
P15 vs P30	5.59E-11
P15 vs P60	5.07E-12
P21 vs P30	2.43E-04
P21 vs P60	2.00E-04
P30 vs P60	*ns*

PV-Cre::tdTomato vs. Sst::tdTomato PV$^-$

P values for indicated comparison	
P15 vs P15	3.74E-15
P21 vs P21	1.54E-19
P30 vs P30	2.62E-06
P60 vs P60	9.81E-19

Table S2. Percent myelination of PV^+ and Sst^+/PV^- interneurons. Related to Figure 2.

Statistical analysis examining the effect of age (P15-P60) on the total number of PV^+ and Sst^+/PV^- interneurons with myelinated axons **(Figure 2C)**.