Cell Reports, Volume *42*

Supplemental information

Regional and cell-type-specific afferent and efferent projections of the mouse claustrum

Quanxin Wang, Yun Wang, Hsien-Chi Kuo, Peng Xie, Xiuli Kuang, Karla E. Hirokawa, Maitham Naeemi, Shenqin Yao, Matt Mallory, Ben Ouellette, Phil Lesnar, Yaoyao Li, Min Ye, Chao Chen, Wei Xiong, Leila Ahmadinia, Laila El-Hifnawi, Ali Cetin, Staci A. Sorensen, Julie A. Harris, Hongkui Zeng, and Christof Koch

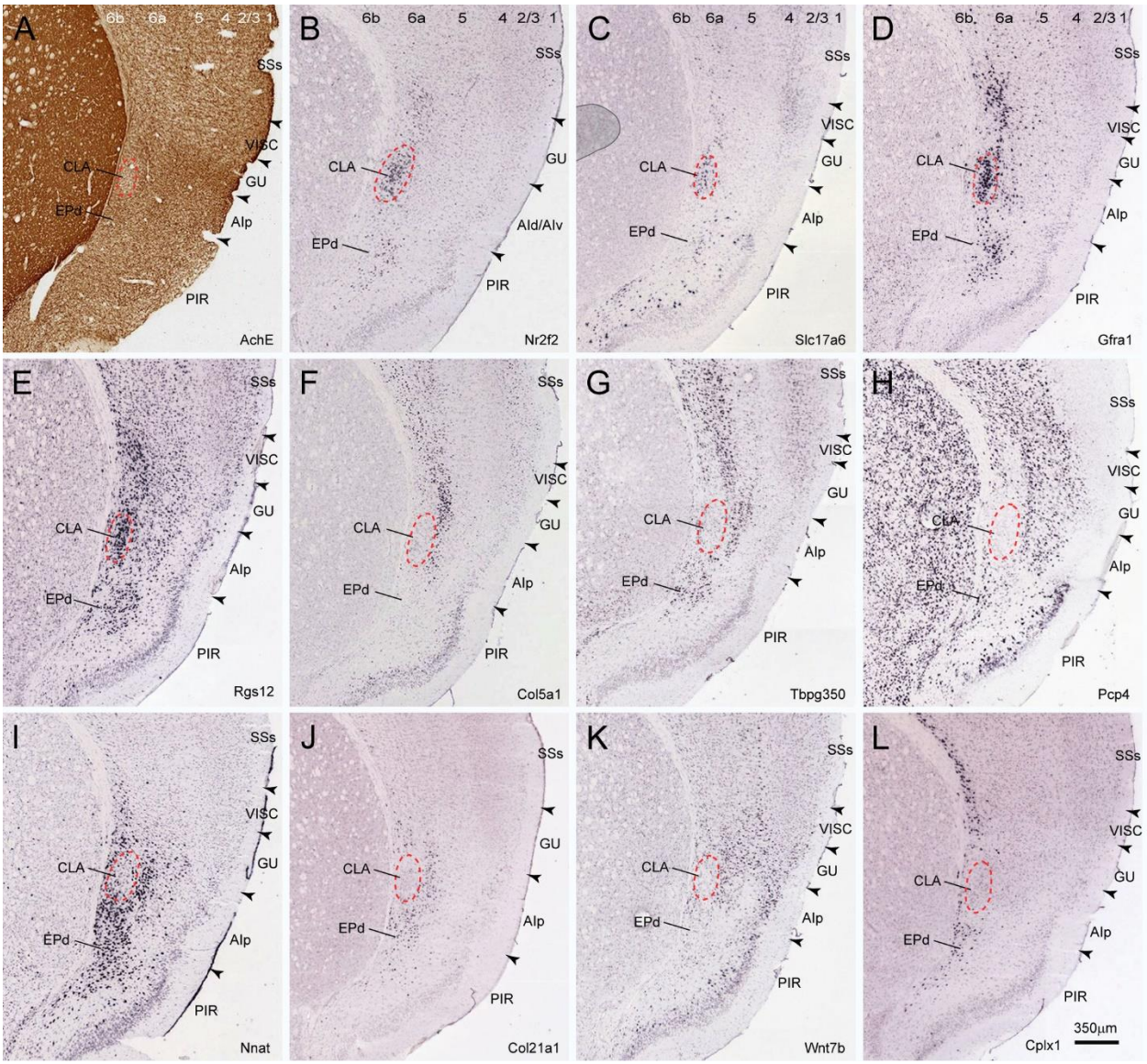

Figure S1. Delineation of the boundary of the mouse CLA. Related to Figure 1

(A) The CLA is an oral shape labeled with weaker AchE-stained fibers than its surroundings. Arrowhead indicates the borders between cortical areas. The dashed line (in red) represents the boundary of CLA (same in A-L). L1 to L6b represent cortical layers (same in B-D).

(B-E) Marker genes show enriched expression in CLA but less expression in AI, GU and EPd.

(F) Marker gene shows enriched expression in L6 of GU, tapering off in L6 of AI but avoids expression in CLA.

(G) Maker gene shows enriched expression in L6 of isocortex, including GU and AI as well as in EPd, but avoids expression in CLA.

(H) Maker gene shows enriched expression in L5 and L6 of isocortex and weak expression in EPd but avoids expression in CLA.

(I) Marker gene expression is stronger in its surroundings than in the CLA itself.

(J) Marker gene expression is in its surroundings but avoids the CLA.

(K) Marker gene shows enriched expression in L6b of iscortex as well as in EPd but avoids CLA.

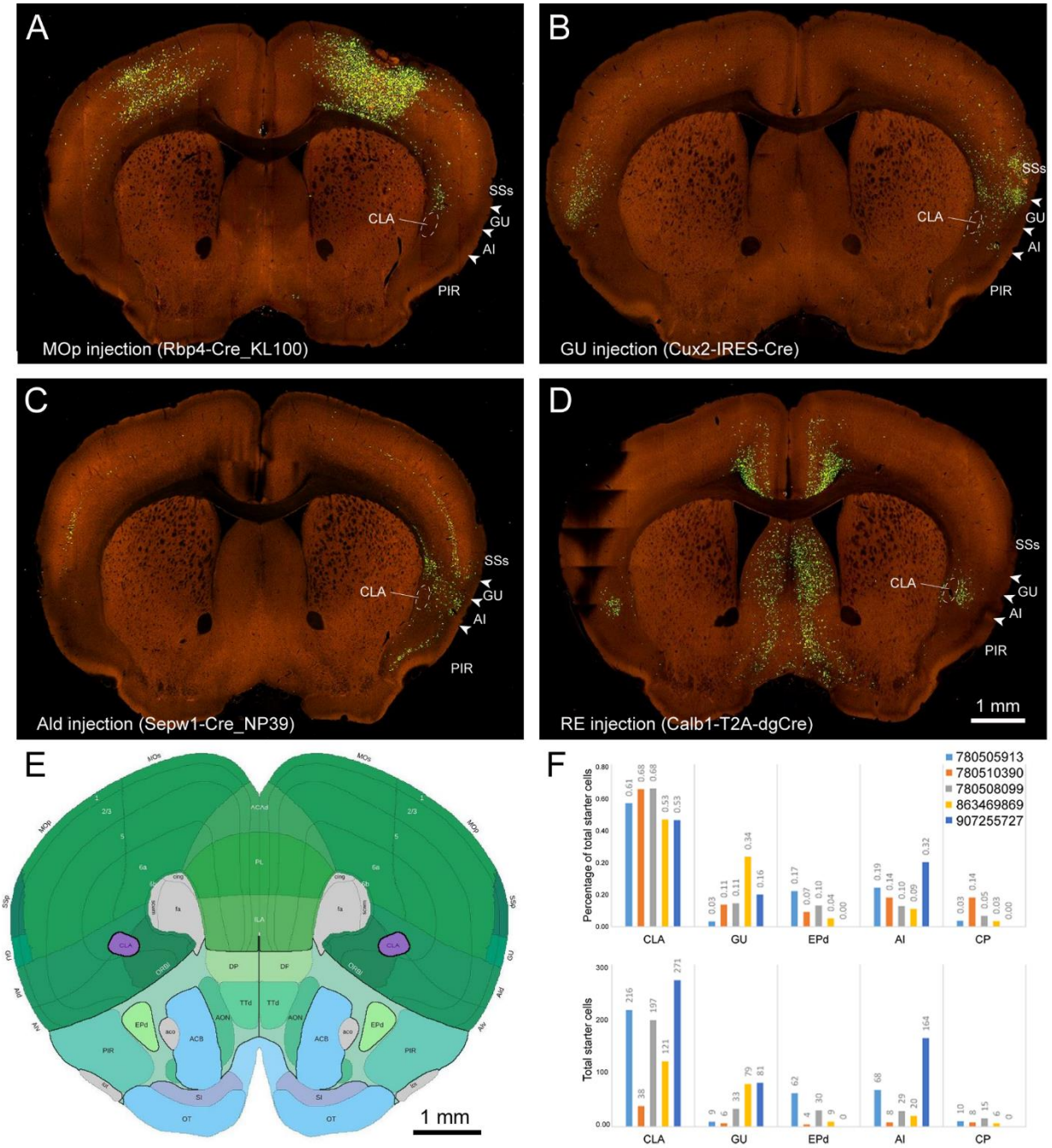

Figure S2. Anterior extent of CLA and its delineation assisted with retrograde rabies tracing data. A-E related to Figure 1. Starter cell distribution F is related to Figure 2.

(A) Retrograde rabies injection into MOp of the transgenic mouse (Rbp4-Cre_KL100) shows retrograde labeling in L6 of GU. The dashed line indicates the CLA border. Arrowhead indicates the border between cortical areas.

(B) Retrograde rabies injection into GU of the transgenic mouse (Cux2-IRES-Cre) shows dense labeling in L6 of GU and spare labeling in deep layers of AI.

(C) Retrograde rabies injection into AId of the transgenic mouse (Sepw1-Cre_NP39) shows dense labeling in L6 of GU and sparse labeling in L2-L6 of AI.

(D) Retrograde rabies injection into RE of the transgenic mouse (Calb1-T2A-dgCre) shows dense labeling in deep layers of AI.
(E) A coronal plate (level 37) in the 2D mouse brain atlas show extension of CLA to the level at the anterior forceps of the corpus callosum. CLA is highlighted in purple.
(F) Bar graphs showing starter cell distribution and numbers in CLA and its adjacent structures (GU, AI, EPd and CP). Each color bar represents one retrograde rabies injection case. X-axis shows structures labeled with starter cells. Y-axis shows the percentage of starter cells in each of these structures. The numbers in the upper right corner are the image series IDs of these experiments.

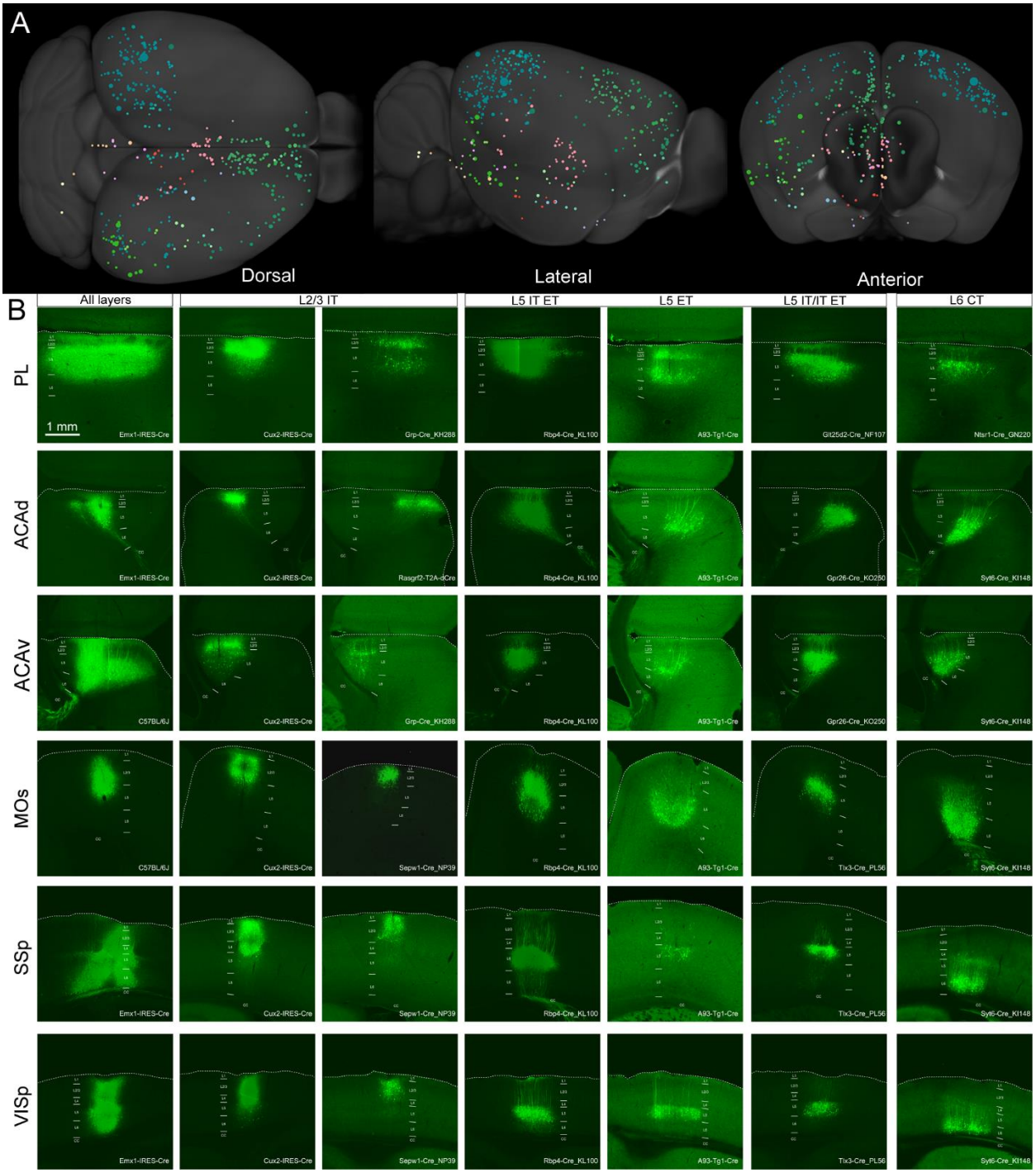

Figure S3. Anterograde AAV injection sites in cortical and subcortical structures. Related to Figure 4.

(A) The anterograde AAV injections into cortical and subcortical structures that send projections to the CLA are shown in dorsal, lateral and anterior views. Colored dots represent injection sites in different major brain divisions.

(B) Examples of injection sites in entire layers, L2/3 IT, L5 IT ET, L5 IT, L5 ET and L6 CT of the six cortical areas PL, ACAd, ACAv, MOs, SSp and VISp.

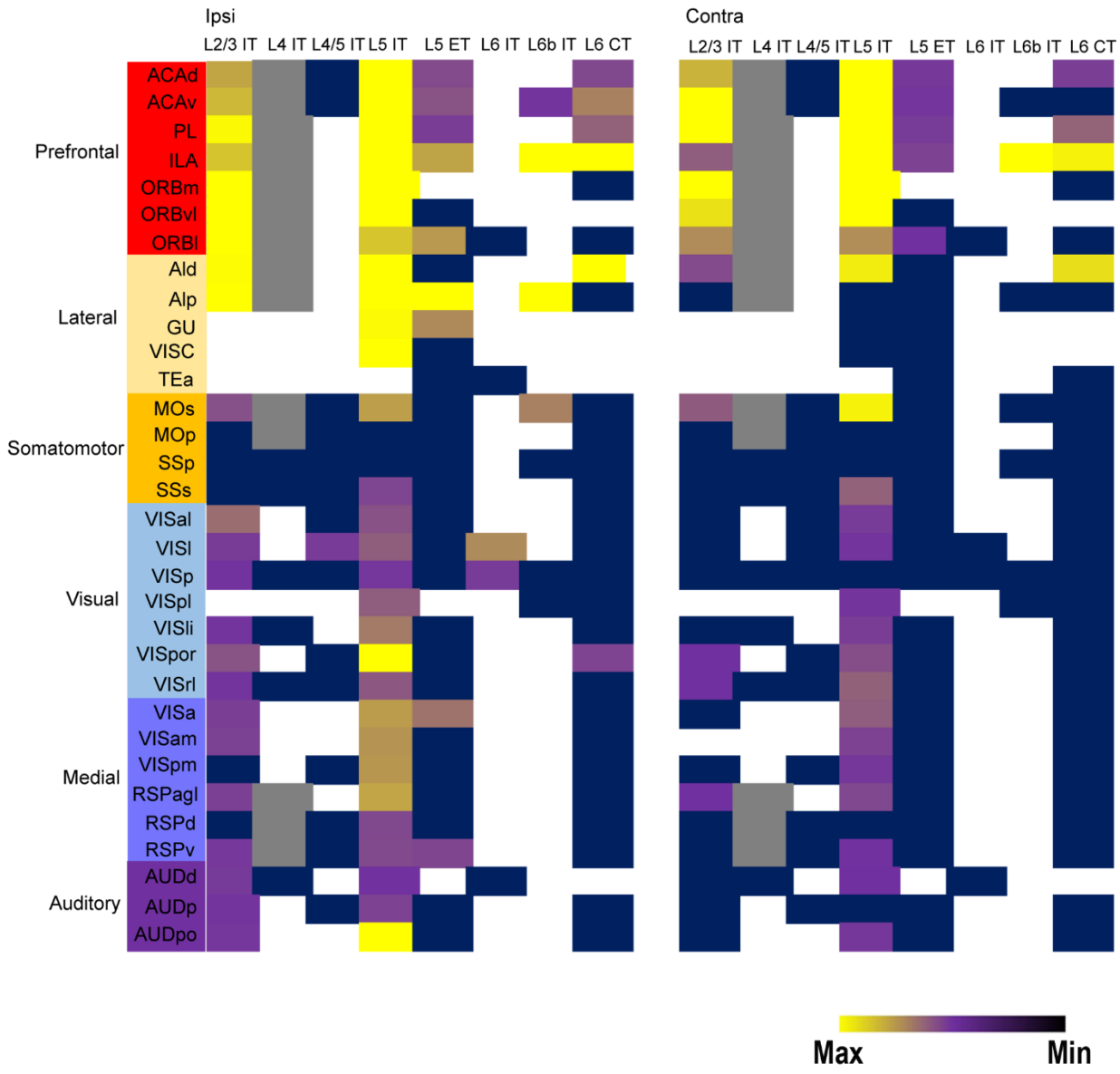

Figure S4. Cortical cell-type-specific inputs to the CLA. Related to Figure 4.
Matrix shows averaged cortical cell-type-specific inputs in CLA. The prefrontal module has more cell types (L2/3 IT, L5 IT, a subset of L5 ET and L6 CT neurons) with denser projections to the CLA than the sensory cortical modules (L2/3 IT, L5 IT and L6 IT neurons) with exception of VISpor and VISa. “Ipsi” and “Contra” stand for ipsilateral and contralateral projections to the injection site.

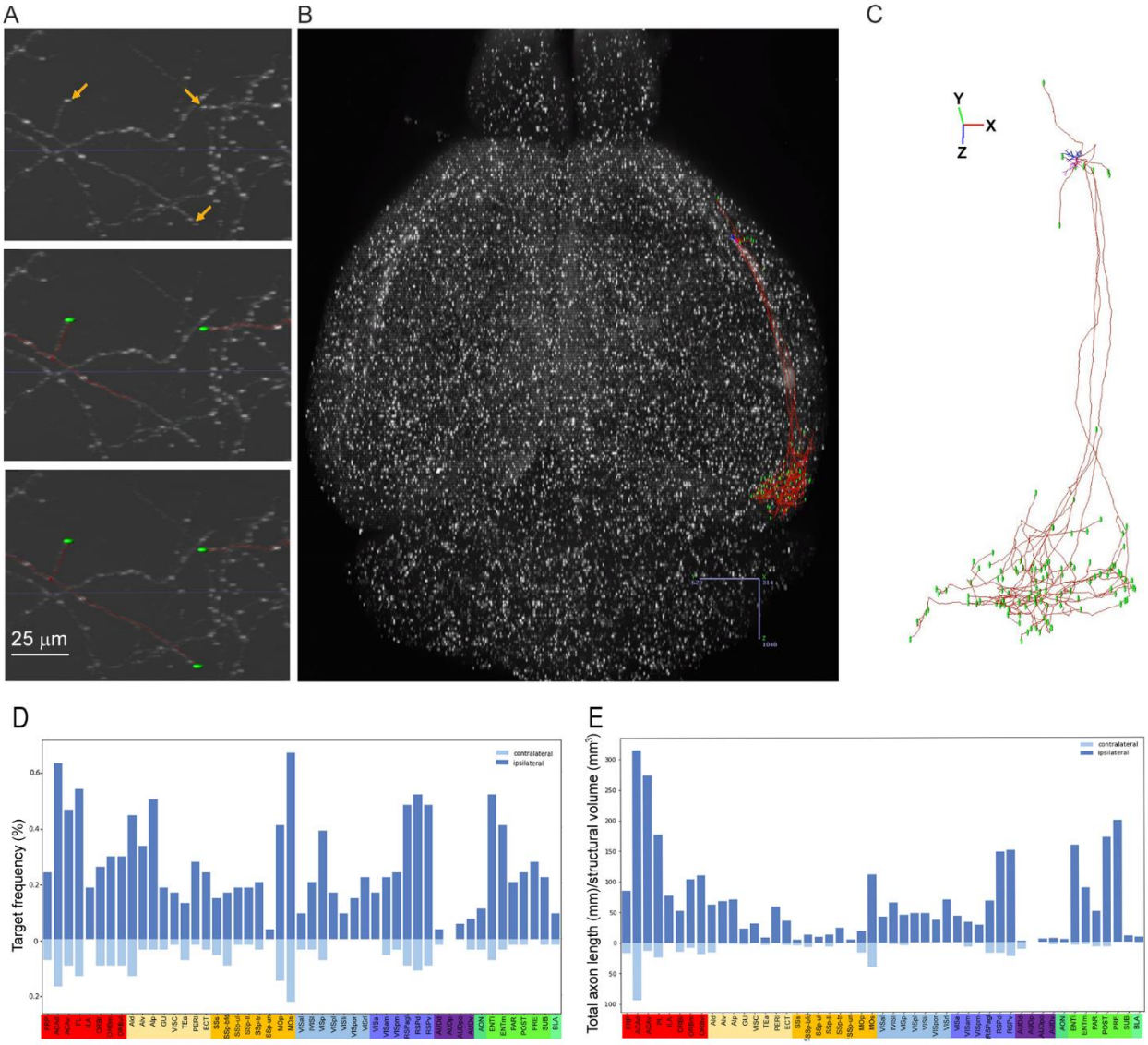

Figure S5. Full morphology, targeting frequency and length of axons per structural volume. Related to Figure 6.

(A) Normal ends of axons with typically enlarged terminal boutons are indicated by orange arrows in the upper panel. Arrows indicate terminal boutons in the upper panel. These boutons are marked at the tracing ends with green markers one by one in the middle and lower panels.

(B) A fully traced CLA neuron (in red) with all its axon terminal boutons marked (in green markers) is shown in the dorsal view of the raw image of a Gnb4-IRES2-Cre brain at low power. White dots are labeled individual neurons on black background.

(C) The completely reconstructed CLA neuron is enlarged from B. Dendrites are presented in blue. Note: markers were removed in the finalizing process.

(D) Bar graph showing percentage of a given structure targeted by 54 principal neurons.

(E) Bar graph shows the ratio of combined axon length (mm) in each target region from all 54 principal neurons divided by the structural volume (mm^3) of each target region.

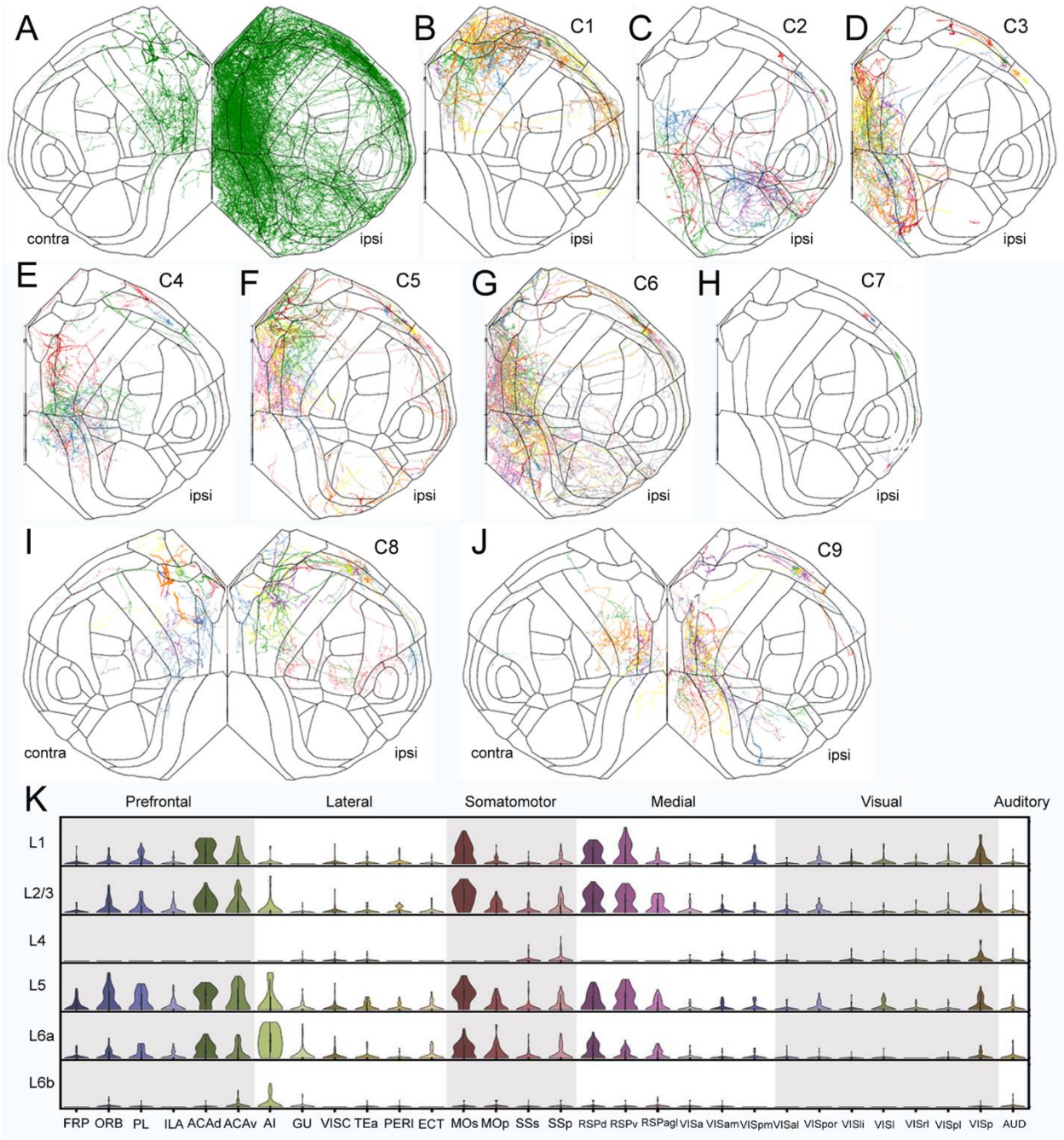

Figure S6. Diverse cell types of CLA principal neurons and their axonal distribution in target cortical areas. Related to Figure 6.

(A) Isocortical flatmap reveals the combined axonal distribution of all reconstructed CLA principal neurons in target cortical areas. “ipsi” and “contra” stands for ipsilateral and contralateral to the soma locations of these principal neurons (same in B-J).

(B-H Ipsilaterally projecting principal neurons classified into 7 clusters are shown in the isocortical flatmaps. Individual neurons are color-coded differently (same in I-J).

(I-J) Bilaterally projecting principal neurons classified into 2 clusters are shown in the isocortical flatmaps.

(K) Violin plot shows the laminar distribution of axonal projections of all principal CLA neurons in each of the isocortical areas. For abbreviations, see Table S1.

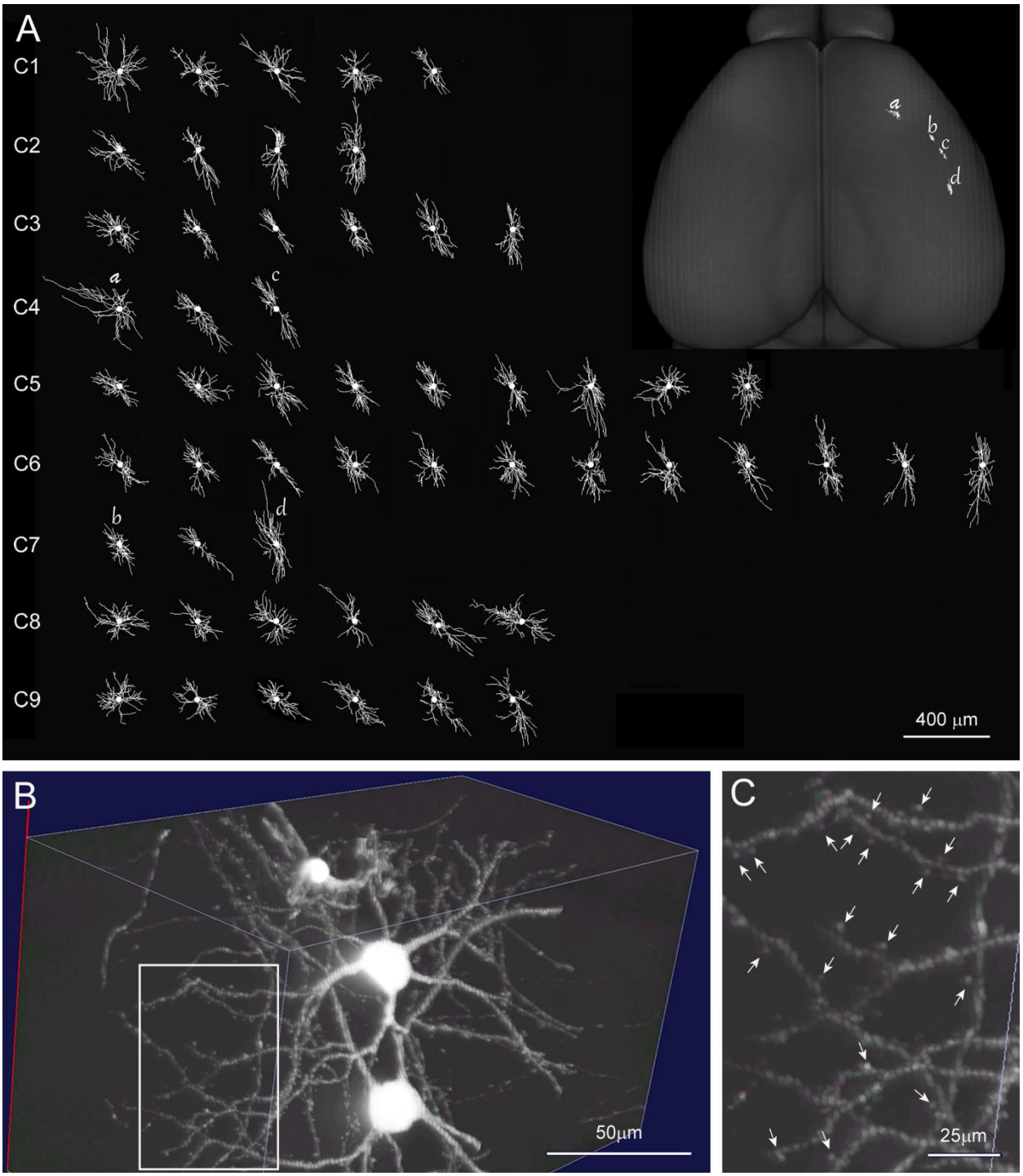

Figure S7. Fully reconstructed dendritic morphology of individual CLA principal neurons. Related to Figure 6.

(A) C1-C9 are CLA principal neuron types classified based on their axon projecting trajectories and target regions. Four examples (a, b, c and d) of the orientations of dendritic arbors within CLA are shown at low magnification in the upper right of the whole brain image, in which a and c, and b and d correspond to a and c, and b and d in C4 and C7, respectively.

(B-C) A representative image shows dendrites of CLA neurons with spines on the dendritic trees (B). C is enlarged from the inset in B. White arrows indicate dendritic spines.