A L2/3 labeling
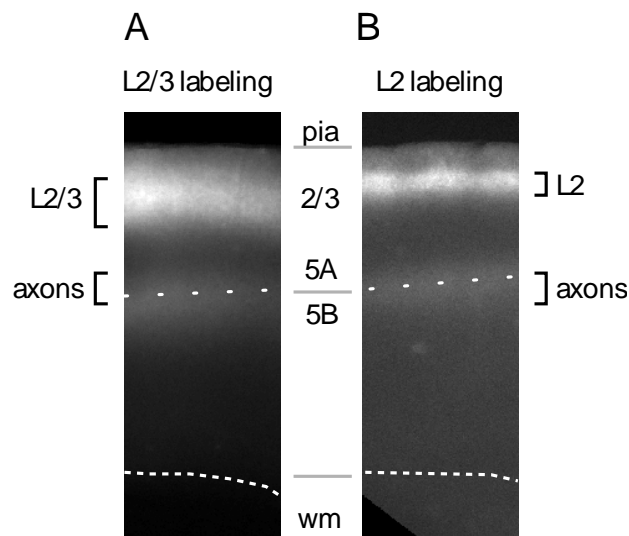
B L2 labeling

Supplementary Fig. 1. Axons of upper layer 2/3 neurons project to the layer 5A/B border. (A) Representative epifluorescence image showing the axonal projection pattern resulting from transfection of both upper and lower layer (L) 2/3 neurons. Neurons were transfected as described in Methods. Upper line (short dashes) marks the L5A/B border (identified in separate bright field image). The descending axonal projection appears as a band at the L5A/B border. The lower (dashed) line marks the border between L6 and white matter (wm). (B) Representative epifluorescence image showing the axonal projection pattern resulting from transfection of just upper L2/3 neurons. Note that the labeling is restricted to upper L2/3 neurons (L2). The descending axonal projection appears as a band straddling the L5A/B border. A similar pattern was observed in slices from 4 additional mice that also exhibited a layer 2-restricted expression pattern.
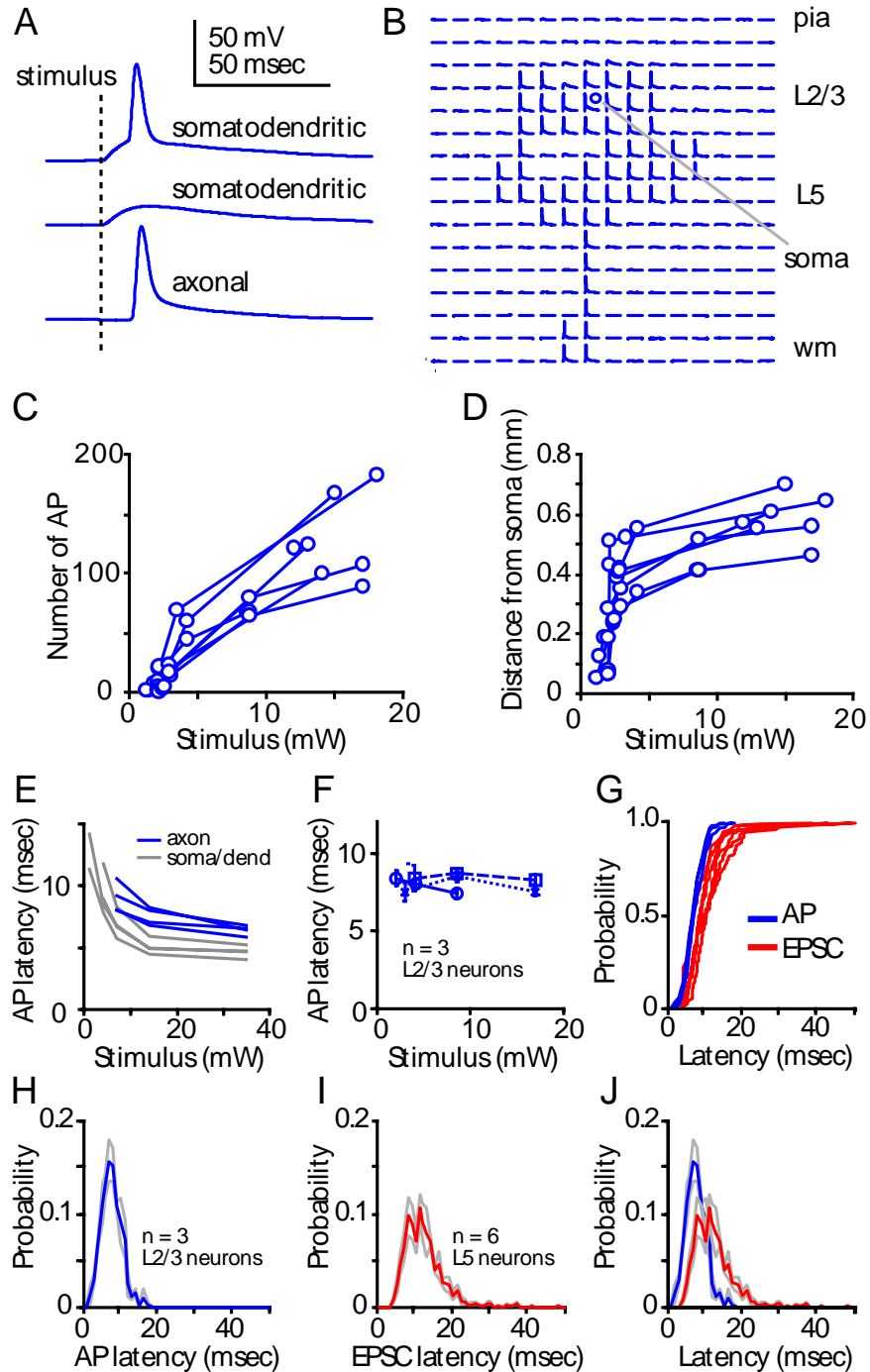
Supplementary Fig. 2. Characterization and calibration of ChR2 photostimulation. (A) Photostimulation-evoked events recorded from a ChR2-expressing layer (L) 2/3 pyramidal neuron. One perisomatic stimulus evoked a slower depolarization and a spike (top) riding on a slow subthreshold depolarization, and another evoked only a subthreshold response (middle). An axonal stimulus evoked an abruptly rising spike (bottom). (B) Map of photoexcitable sites. Soma position indicated by triangles. Excitable sites extend down into white matter. (C) Power dependence of action potentials (AP) per map. Each point is the cell's average for a given power, and lines connect

multiple powers tested for a given cell. (D) Power dependence of the mean distance of spike-evoking sites from the soma. (E) Power dependence of action potential onset latencies, for two somatodendritic sites (gray) and several axonal sites (blue). (F) Power independence of overall mean AP latencies per map. (G) Cumulative probabilities of AP latencies ($n = 3$ L2/3 pyramidal neurons) and synaptic latencies (EPSC; $n = 6$ L5 pyramidal neurons). (H) Mean probability distribution of AP latencies (gray lines: s.e.m.). (I) Mean probability distribution of EPSC latencies. (J) Data from H and I combined.