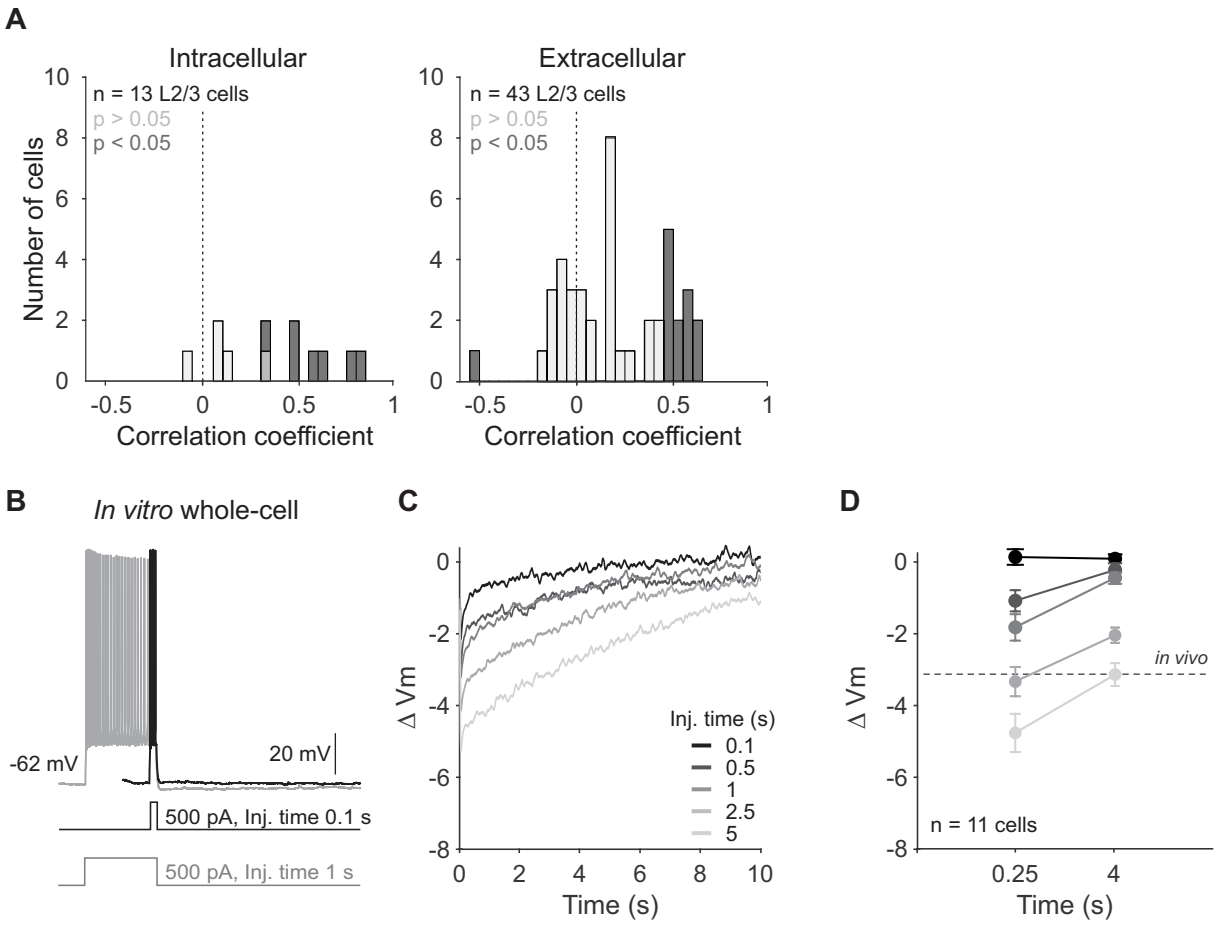

Figure S1. Rapid adaptation does not induce cell-intrinsic fatigue, related to Figure 1. A. Histogram of single trial correlation of spikes elicited in response to baseline and test stimuli for 0.25 ISI condition from intracellular (left; n = 13 cells) and extracellular (right; n = 43 units) L2/3 *in vivo* recordings. Dark gray bars indicate significant correlations. **B.** Current clamp recording in an example L2/3 pyramidal cell in response to current injections of two durations (black = 0.1 s, gray = 1 s). **C.** Change in membrane potential (Vm) following offset of increasing current injection durations in the example cell in **B**. **D.** Average change in membrane potential after current injection offset at recovery times when spike output is suppressed (0.25 s) or recovered (4 s) *in vivo* for increasing current durations. Dashed line is average change in stimulus-evoked membrane potential *in vivo*. Error bar is SEM across cells

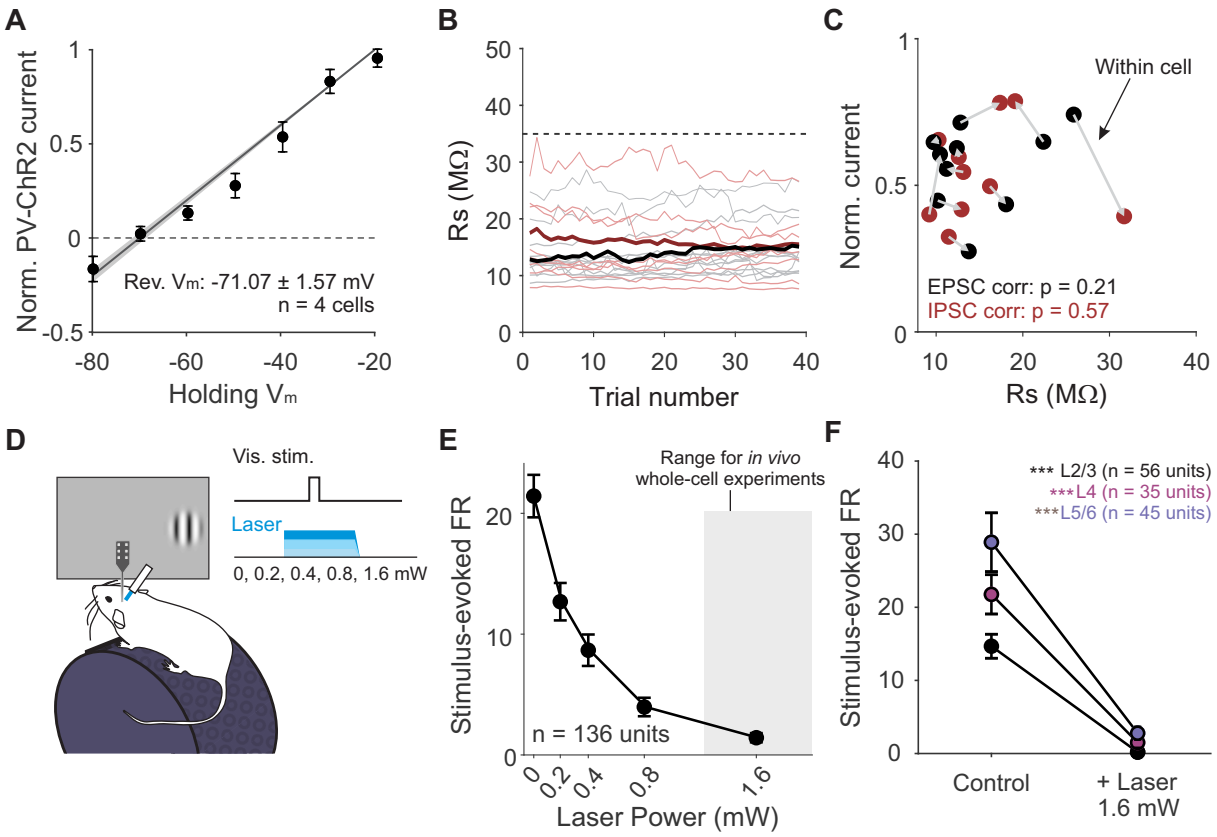

Figure S2. Whole-cell voltage clamp recording of EPSCs and IPSCs *in vivo* and cortical silencing, related to Figure 2. A. Reversal potential of currents evoked with optogenetic activation of parvalbumin-expressing (PV) interneurons expressing Channelrhodopsin-2 (ChR2) to calibrate reversal potential for inhibitory currents *in vivo* (PV-Cre mice injected with AAV2/1.hSyn.ChR2-YFP; n = 4 cells). **B.** Series resistance (Rs) during recording of EPSCs (black) and IPSCs (red). Thick lines are average across cells. Dashed line is cutoff used for series resistance inclusion criteria. **C.** Normalized current (test/baseline; 0.25 s ISI) as a function of series resistance for all recordings. Grey arrows connect currents recorded within the same cell and direction reflects the order of recording. P-value is significance of Pearson correlation. **D.** Schematic of recording setup for measuring effects of PV-ChR2 cortical silencing with extracellular silicone probe and attached optic fiber. Visual stimulus was presented (0.1 s) with photostimulation of PV interneurons at varying laser intensities. **E.** Stimulus-evoked firing rate with increasing laser intensity, averaged across units. Error bar is SEM across units. Grey shaded area indicates range of laser powers used for intracellular recordings. **F.** Suppression of stimulus-evoked response at 1.6 mW laser power, separated by layer. Marker colors indicate layers. Error bar is SEM across units. Asterisks indicate $p < 0.001$ from paired t-test between control and laser on for each layer.

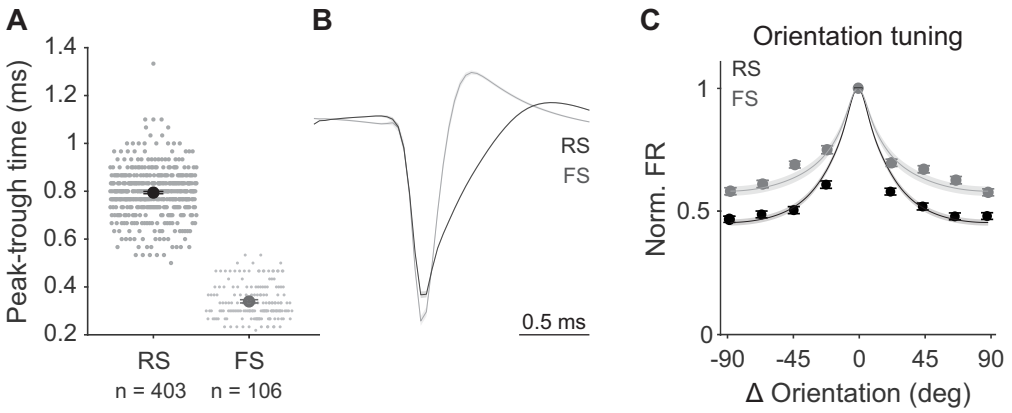

Figure S3. Separation of FS and RS units and comparison of orientation tuning, related to Figures 2 and 3. A. Peak-trough time of spike waveforms from units classified as regular spiking (RS, black) or fast spiking (FS, grey). Each small dot is an individual unit. Large dots are mean and SEM across units. **B.** Average spike waveforms from the units in **A**. Shaded error is SEM across units. **C.** Average orientation tuning curves aligned to preferred orientation for each unit. Points are averaged normalized response across units. Curves are averages of the von Mises fit for individual units.

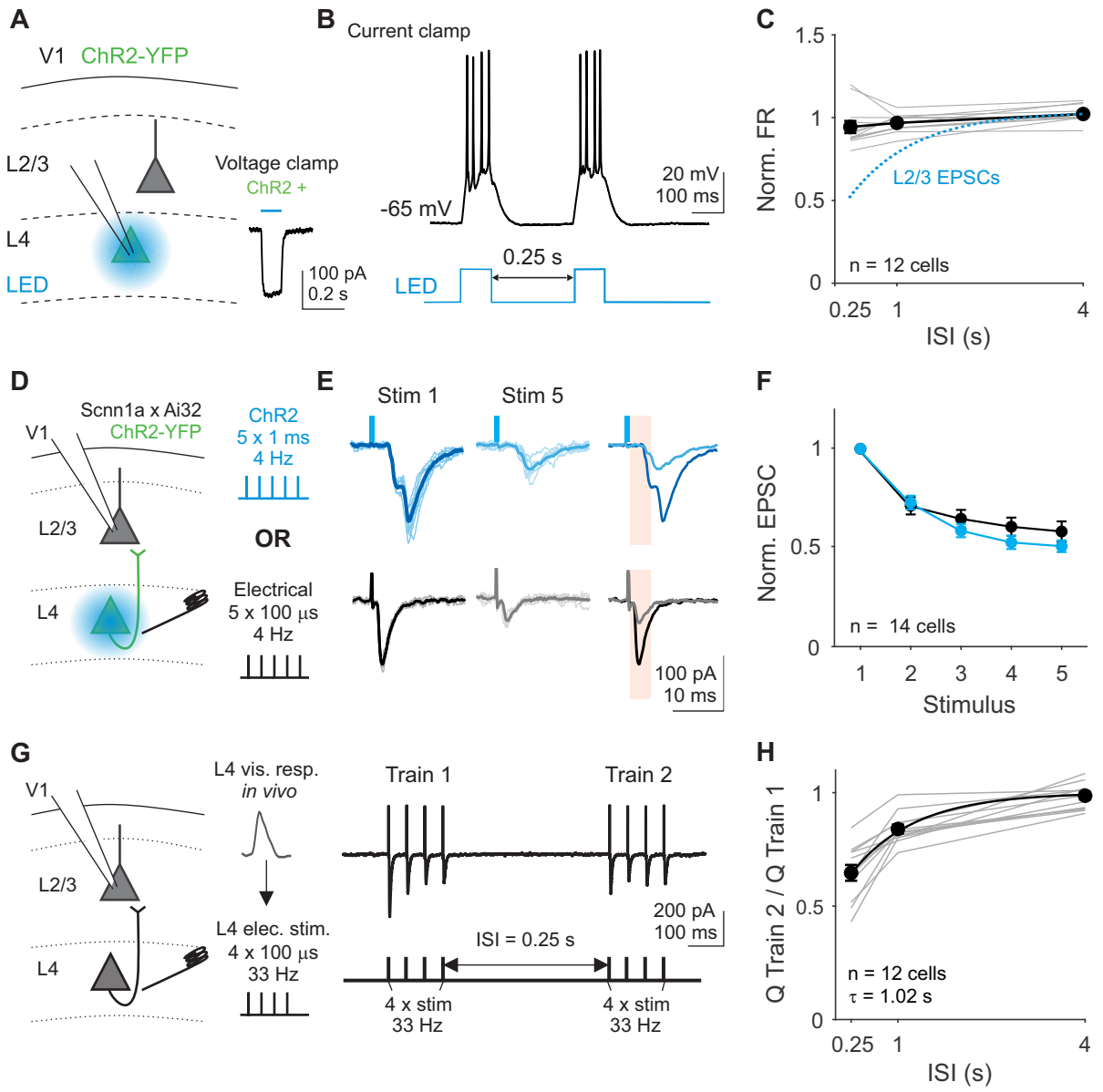

Figure S4. *In vitro* recordings with ChR2 and electrical stimulation, related to Figure 4. A. Schematic of recording from ChR2-expressing L4 neurons during 0.1 s photostimulation. ChR2 expression was first confirmed by the presence of fast-onset, step-like inward currents with LED activation. **B.** Single trial voltage trace from an example cell (black) during LED stimulation (blue) with 0.25 s inter-stimulus interval (ISI). **C.** Normalized firing rate as a function of pulse ISI Grey lines are individual cells, black line is average across cells, error bar is SEM. Fit to normalized L2/3 EPSCs during the same stimulation paradigm (**Figure 4**) is plotted for reference **D.** Schematic of recording EPSCs from a L2/3 pyramidal cell while stimulating L4 neurons optogenetically or electrically on alternating trials. **E.** Average EPSCs from an example cell in response to optogenetic (blue) or electrical (black) stimulation of L4 for the first (dark) and last (light) stimulus in the train. **F.** Average EPSC amplitude normalized to first pulse within stimulation type. Error bar is SEM across cells. Two-way ANOVA, p = 0.11 for effect of stimulation type. **G.** Left, schematic of voltage clamp recording from a L2/3 pyramidal cell while stimulating L4 neurons with a high-frequency (33 Hz) trains. Right, average EPSCs recorded in an example cell in response to a pair of 33 Hz trains at 0.25 s ISI. **H.** Normalized total charge (Q; sum of charge evoked by 4 pulses within a train) as a function of ISI.

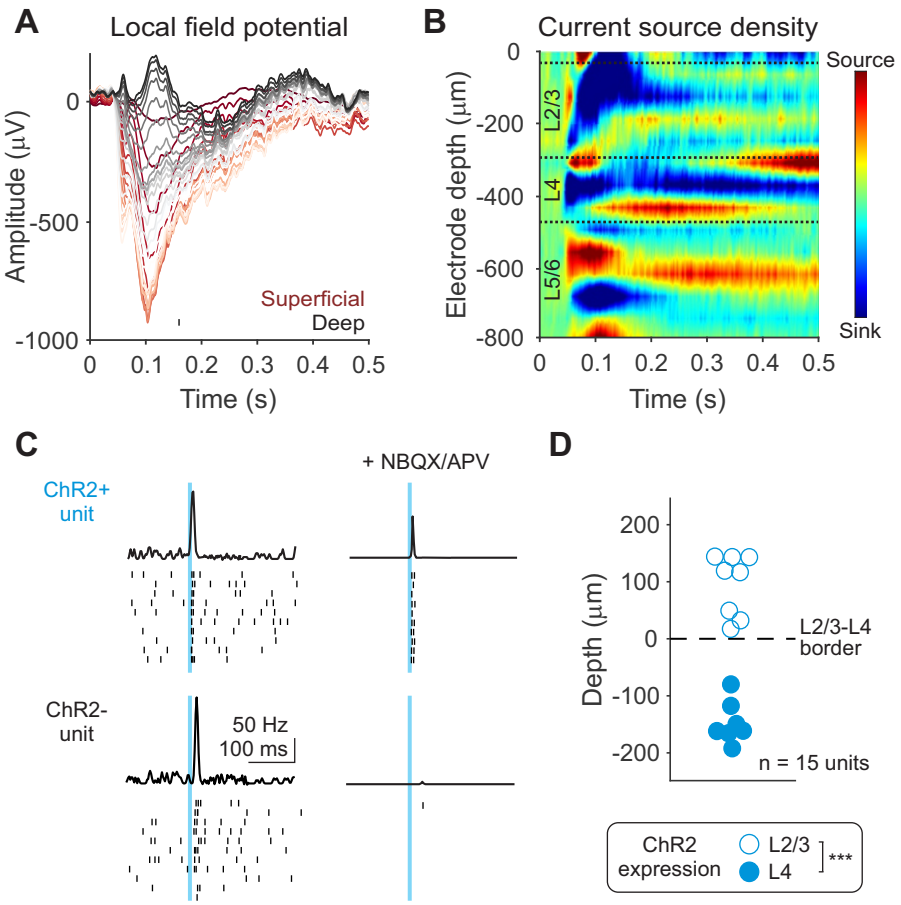

Figure S5. Identification of layer boundaries for classifying units as L2/3 or L4, related to Figures 5, 6 and 8. A. Local field potential (LFP) measured across cortical depths during a drifting grating stimulus from an example recording. Traces are colored according to contact site from superficial (red) to deep (black). **B.** Current source density (CSD) calculated using the LFP in **A**. Dashed lines indicate layer boundaries assigned based on this map. L4 was assigned by identifying an early onset sink and L2/3 was identified as the later onset sink above it. **C.** Example units identified as ChR2+ (top) or ChR2- (bottom). PSTH and spike rasters in response to blue laser pulses (10 ms) before (left) and after (right) pharmacological block of excitatory transmission (**STAR Methods**). **D.** Depth of ChR2-expressing units relative to L2/3-L4 boundary identified using the CSD. Marker fill indicates ChR2 expression layer (unfilled = L2/3, in utero electroporated mice; filled = L4, Scnn1a x Ai32 mice; depth of L2/3 vs L4 expression: $p < 0.001$, un-paired t-test).

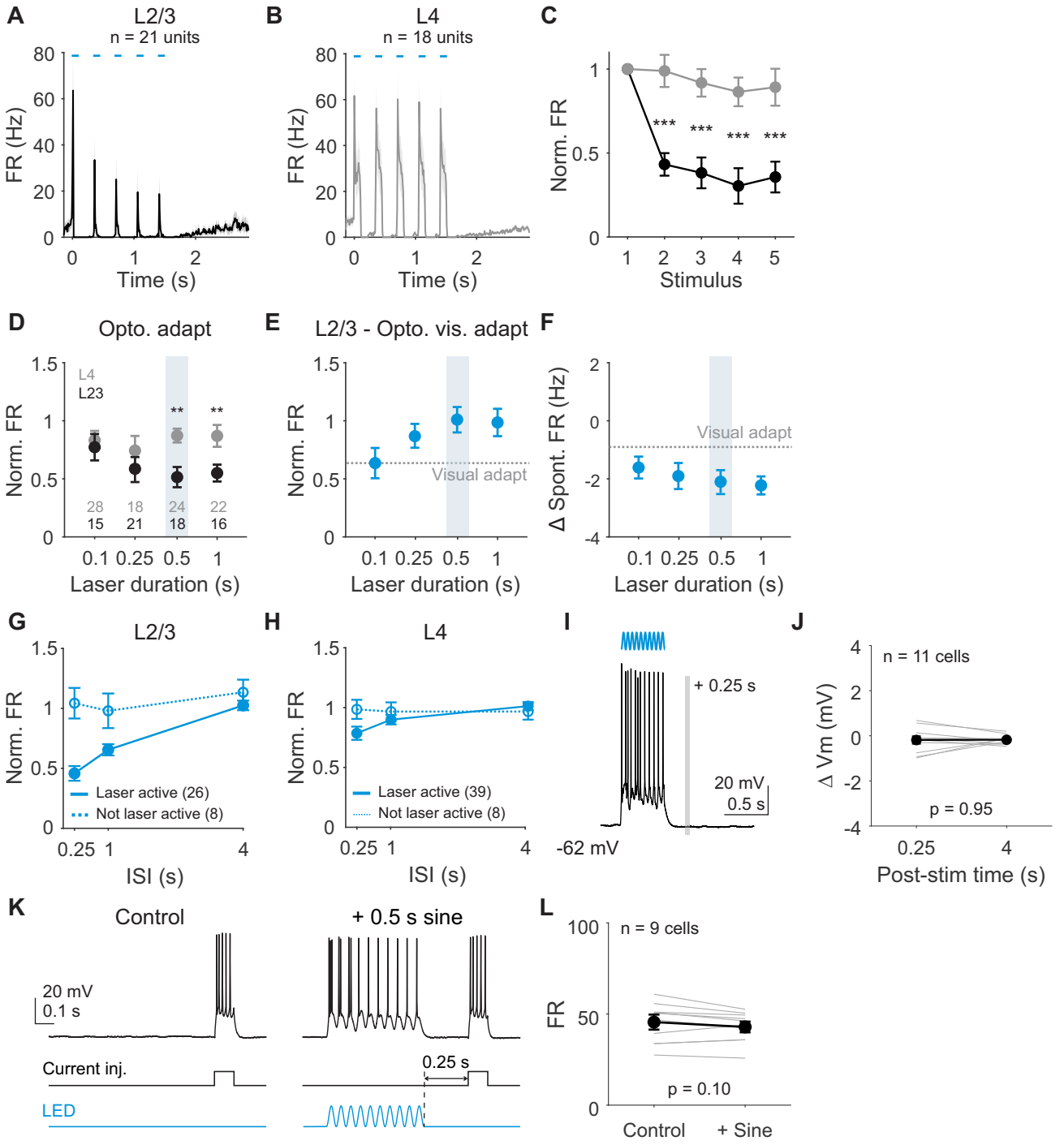

Figure S6. Effects of optogenetic activation of L4 neurons, related to Figure 5. A. Average PSTH of laser active L2/3 units during optogenetic activation of L4 neurons with 5, 0.1 s square pulses of blue light. **B.** Same as **A**, for L4 units. **C.** Normalized peak FR for each pulse to the first pulse in the train for L2/3 (black) and L4 (grey) units. Error bar is SEM across units. L2/3 $p < 0.001$ for stimulus 2-5 vs 1, one-way ANOVA with post hoc Tukey test. **D.** Optogenetic adaptation measured in L2/3 and L4 units across different laser durations (L4 vs L2/3, 0.5 s laser duration: $p = 0.006$; 1 s laser duration: $p = 0.009$, unpaired t-tests). Shaded box is laser duration used for main figure experiments. **E.** Visual adaptation in L2/3 neurons with different durations of optogenetic activation of L4 neurons. Dashed line is visual adaptation without L4 stimulation. **F.** Change in spontaneous FR after different durations of L4 activation. Dashed line is spontaneous FR after visual adaptation. **G.** L2/3 optogenetic adaptation in units divided by units that were significantly modulated by L4 ChR2 stimulation (solid line) or not (dashed line). **H.** Same as **G**, for L4 units. **I.** Voltage trace from a ChR2-expressing L4 neuron *in vitro* with the 0.5 s sinusoidal light stimulation used *in vivo*. **J.** Change in membrane potential at 0.25 and 4 s after light offset. Grey lines are individual cells, black line is mean. Error bar is SEM across cells. **K.** Voltage trace from a ChR2-expressing L4 neuron *in vitro* with current injection in control (left), or 0.25 s after 0.5 s ChR2 stimulation (right). **L.** Firing rate elicited with current injection in control and ChR2 activation trials. Grey lines are individual cells, black line is mean. Error bar is SEM across cells. P value is from paired t-test.

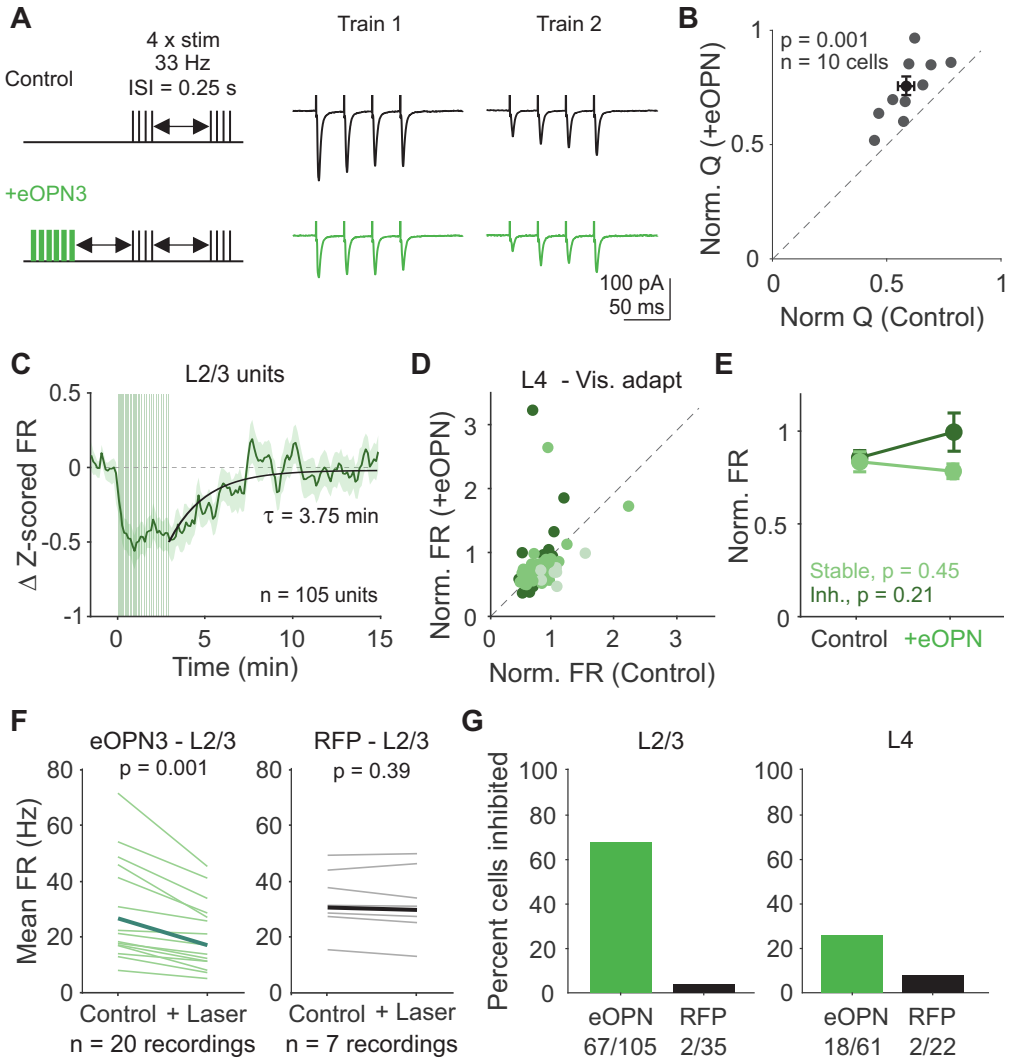

Figure S7. Strength and specificity of the effect of eOPN3 on synaptic transmission and firing rates, related to Figure 8. A. Left: Schematic of *in vitro* L4 electrical stimulation to test the effect of eOPN3 activation with paired high frequency bursts. Following a block of control trials, eOPN3 is activated with green light and the same stimulation is applied. Right: EPSCs from an example neuron during control (top, black) and eOPN3 (bottom, green) trials. **B.** Normalized total charge (Train 2/Train 1) before and after eOPN3 activation. Each grey dot is a cell, black dot is the mean, error bar is SEM ($p < 0.001$, paired t-test). **C.** Average time course of stimulus-evoked, z-scored firing rate aligned to eOPN3 activation for all units recorded in L2/3 *in vivo* (n = 105). Green vertical lines indicate eOPN3 activation trials. Black curve is fit to the recovery from eOPN3 activation. Shaded error is SEM across units. **D.** Comparison of normalized response (test/baseline) in control and eOPN3 activation trials, for all L4 units colored by categorization in **Figure 8E** (dark green = inhibited, medium green = stable, light green = facilitated). **E.** Average normalized response for inhibited (dark green) and stable (light green) units in L4. Error bar is SEM across units. **F.** Average visually-evoked firing rate of L2/3 neurons during control and laser stimulation trials in eOPN3 (left, green) or RFP control (right, black) recordings. Individual lines are average response of all L2/3 neurons in each session, thick line is mean across sessions (eOPN: paired t-test, $p < 0.001$; RFP: paired t-test $p = 0.39$). **G.** Left: Fraction of L2/3 units classified as inhibited from recordings with eOPN3 (green) or RFP control (black) in L4 neurons. Right: Same as left, for L4 units.

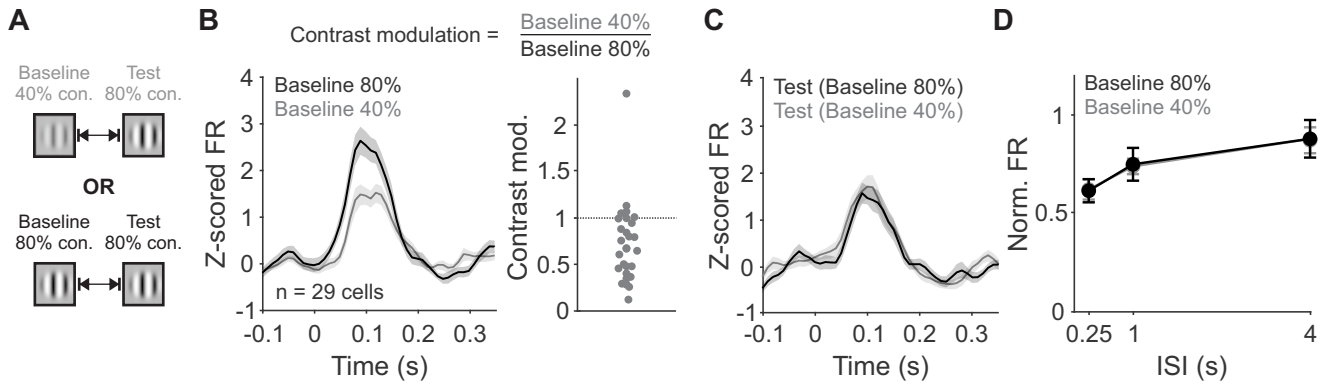

Figure S8. Effect of low contrast baseline stimulus on adaptation in L2/3, related to Figure 8. A. Schematic of visual stimulus. Baseline stimulus was either low (40%) or high (80%) contrast and test stimulus was always high contrast. **B.** Left: Z-scored PSTH of L2/3 units during high contrast (black) or low contrast (gray) baseline visual stimulus presentation. Right: Fractional change in peak firing rate during baseline stimulus for high versus low contrast. **C.** Z-scored PSTH during test visual stimulus presentation with baseline high (black) or low contrast (gray). **D.** Average normalized firing rate (test/baseline) with high or low contrast baseline stimulus. Test responses for both trial types was divided by the baseline response to high contrast (two-way ANOVA, effect of contrast, $p = 0.45$).