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Supplemental Information

Global Representations of Goal-Directed Behavior in Distinct Cell Types of Mouse Neocortex

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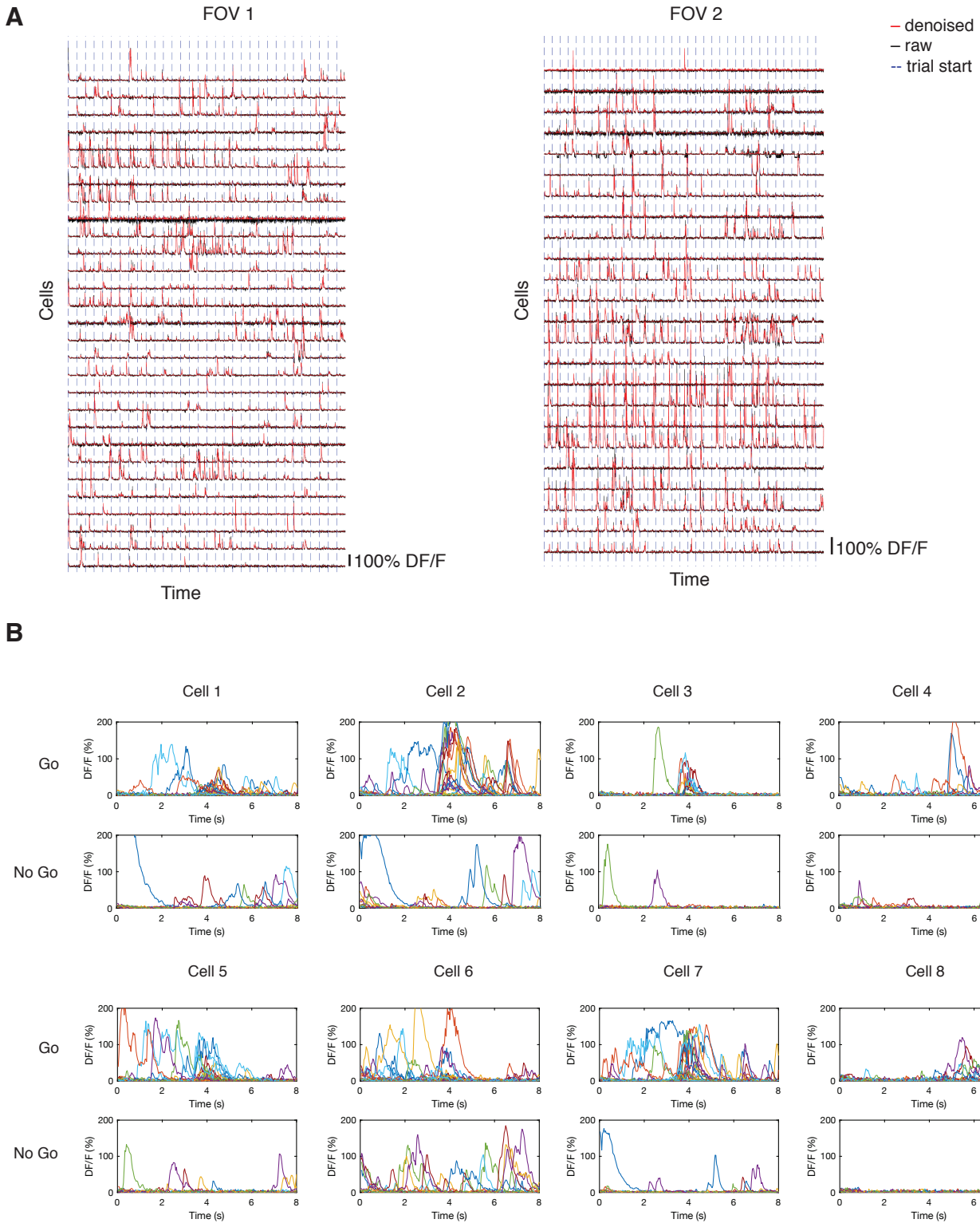

Figure S1. Example single cell two-photon data (related to Figure 1). (A) Representative example traces of all cells from two fields of view in two mice. Trial start times, including both Go and No-Go trials, are indicated with dashed blue lines, raw ΔF/F normalized data as the black trace, and denoised ΔF/F trace as the red trace. (B) Representative denoised ΔF/F normalized traces from eight cells separately overlaid in different colors from single Go and No-Go trials, showing that some cells tend to reliably become activated during a specific point in Go trials.

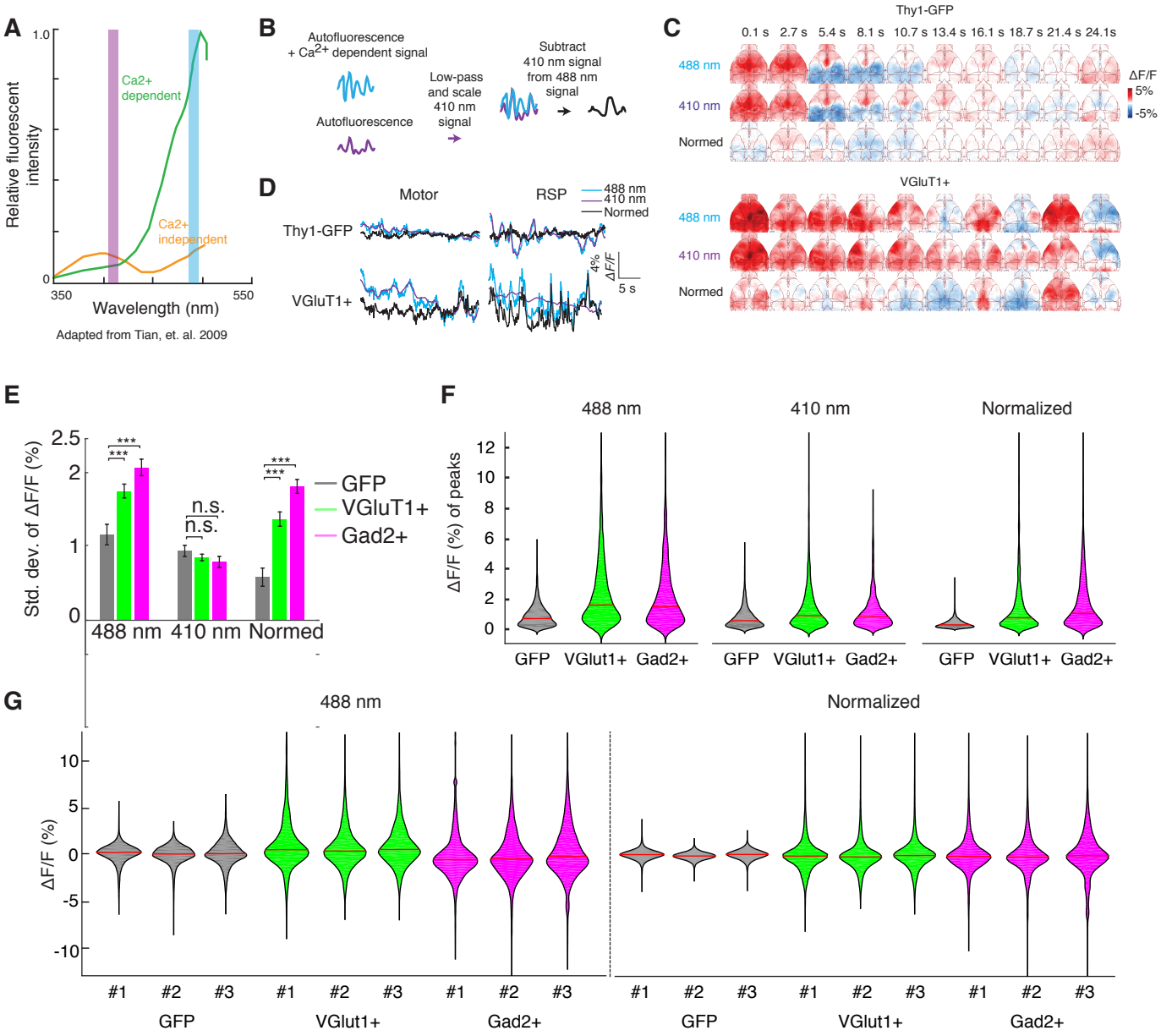

Figure S2. Blood artifact reduction with spectral unmixing (related to Figure 3). (A) Spectra of Ca2+ dependent (green) and Ca2+ independent (orange) GCaMP absorption. The absorption of the two states at 410 nm is nearly identical. (B) Schematic of subtractive normalization procedure, which assumes that autofluorescence and artifacts are additive on to GCaMP fluorescence. (C) Top: Spontaneous fluorescence changes in control Thy1-GFP mouse under 488 nm and 410 nm illumination, and normalized fluorescence. Bottom: Spontaneous fluorescence changes in VGluT1+ triple transgenic mouse, under 488 nm and 410 nm illumination, and normalized fluorescence. (D) Example traces from (C) in motor and visual cortex. (E) Comparison of mean standard deviation across 1 minute trials, in each fluorescence channel and after normalization. N = 3 GFP, N = 3 Gad2, and N = 3 VGluT1+ mice, 10 trials for each mouse. Mean standard deviation for GFP mice is <0.5% ΔF/F, and therefore any normalized signals >1% ΔF/F should be reliable with a 95% confidence interval. In general, normalized signals for GCaMP expressing mice > 2-3% ΔF/F during behavior. (F) Distribution of the value of peaks in the spontaneous time series used in (E), with 488 nm and 410 nm illumination, and normalized. (G) Distribution of fluorescence change (% ΔF/F) values for all mice used in the comparison, before and after normalization.

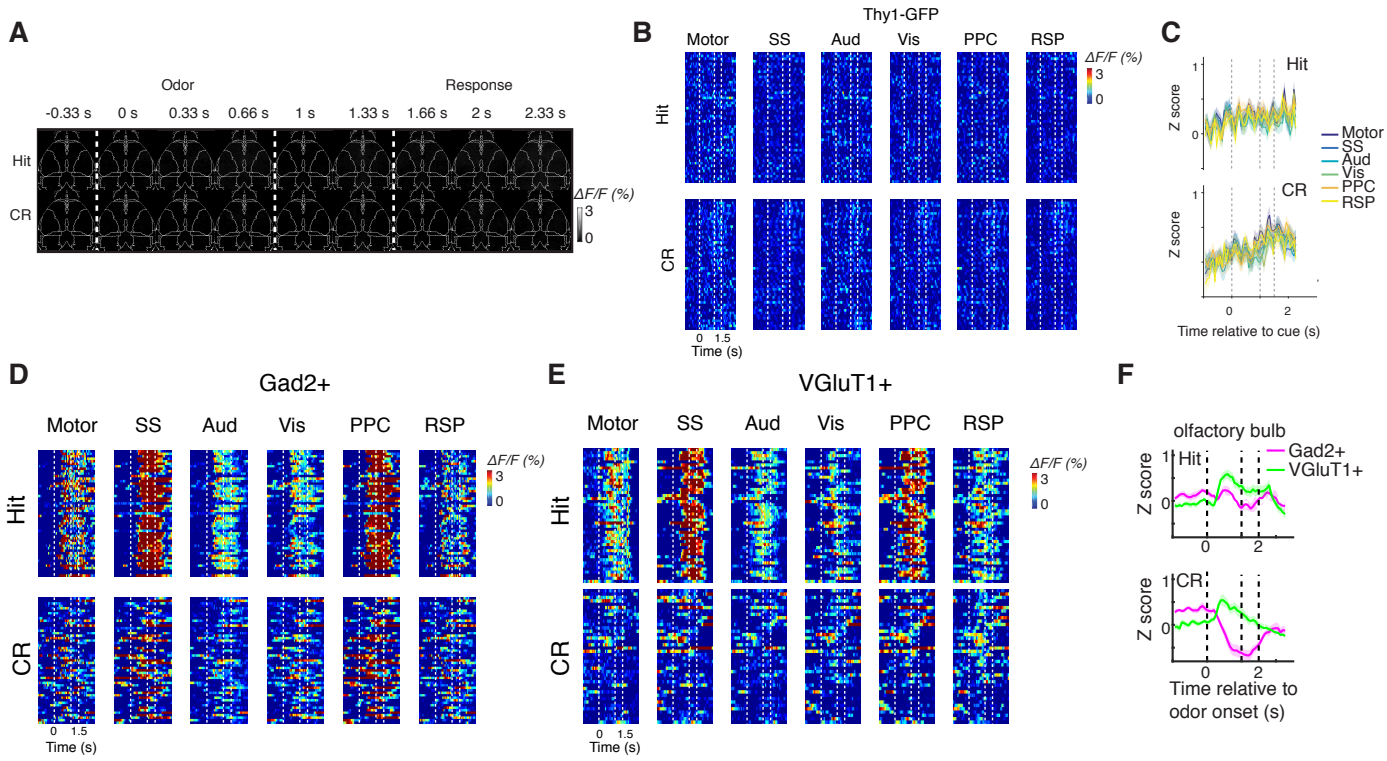

Figure S3. GFP control during learned behavior, consistency of per-trial activity, and olfactory bulb traces (related to Figure 4). (A) Example trial-averaged video sequence in Thy1-GFP mouse performing an olfactory discrimination task. (B) Single trial activity across multiple cortical areas from representative Thy1-GFP in (A). (C) Average traces in six cortical regions across N = 3 Thy1-GFP mice. Mean ± s.e.m. (D) Single-trial data from one Gad2+ mouse across multiple cortical regions over one behavioral session. (E) Single-trial data from one VGluT1+ mouse across multiple cortical regions over one behavioral session. (F) Average traces from olfactory bulb on Hit and Correct Reject (CR), averaged across mice for 9 Gad2+ and 12 VGluT1+ mice. (Means of 62 Hit, 53 CR trials per mouse for VGluT1+ and 57 Hit, 51 CR trials for Gad2+). Error bars = s.e.m.

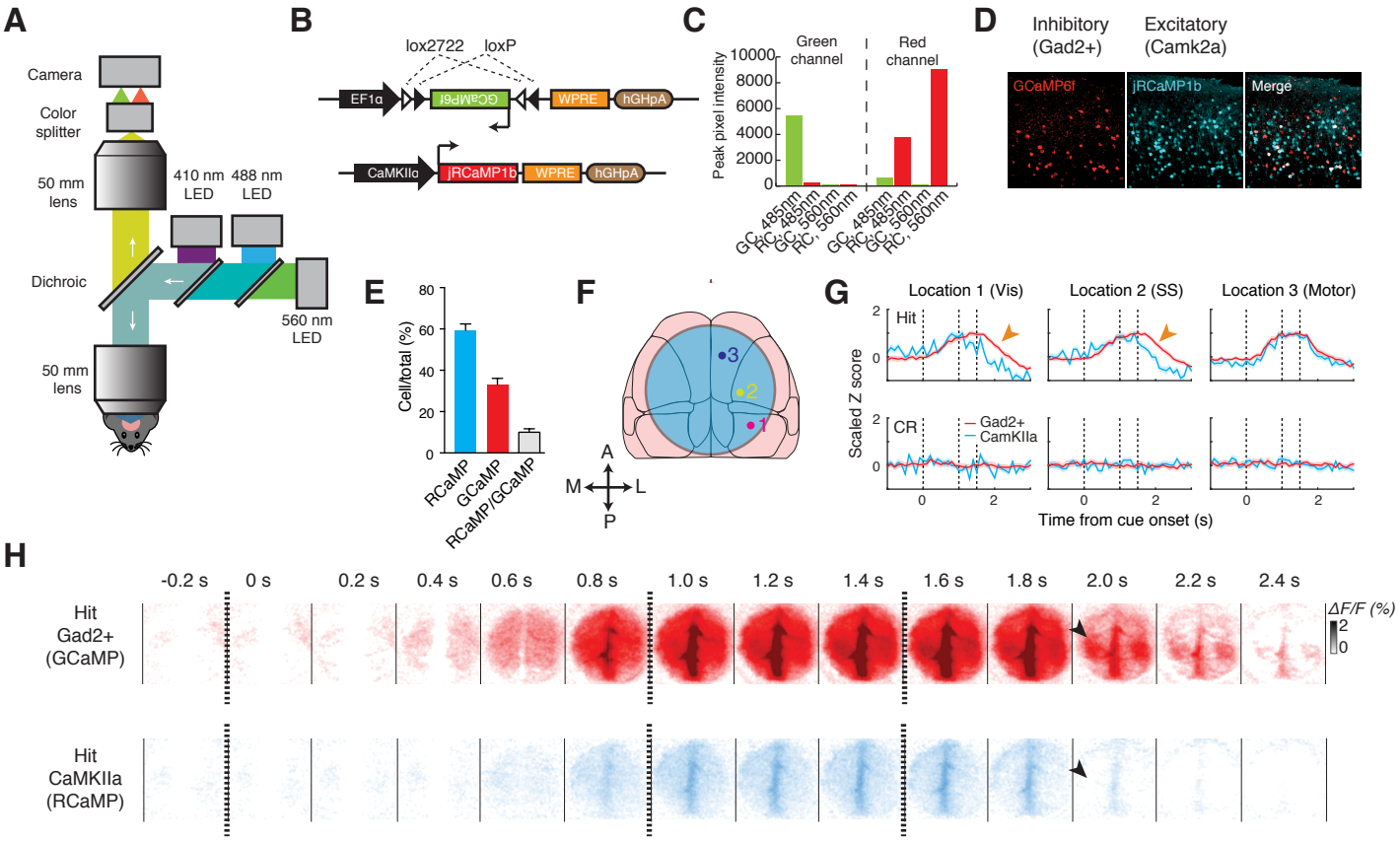

Figure S4. Simultaneous imaging of inhibitory and excitatory dynamics during learned behavior (related to Figure 4). (A) Dual-color macroscope schematic for simultaneous imaging of two populations of neurons. (B) Diagram of viral-genetic strategy for expression of GCaMP6f in all inhibitory neurons and jRCaMP1b in CaMKIIα-expressing (primarily excitatory) neurons. (C) Quantification of RCaMP bleed through into green channel under 485 nm illumination, and lack of GCaMP bleed through into red channel under 560 nm illumination. For this, we measured native fluorescence of fixed brain sections individually expressing either RCaMP or GCaMP in CaMKIIa expressing cortical neurons, using local AAV5 viral injection. (D) Expression of jRCaMP1b and GCaMP6f in cortex. (E) Quantification of fraction of total labeled neurons expressing jRCaMP1b, GCaMP6f, or both. (F) Diagram of 7mm window placement, and locations from which traces were extracted in next panel. (G) Average z-scored traces from three locations on Hit and Correct Reject (CR) trials, simultaneously recorded in Gad2+ and CaMKIIα populations. Mean ± s.e.m. across n = 46 Hit, 48 CR trials in one mouse. (H) Video sequence of average fluorescence across Hit, CR trials in one mouse, simultaneously recorded. Black arrows indicate features that match those seen in Figure 4A.

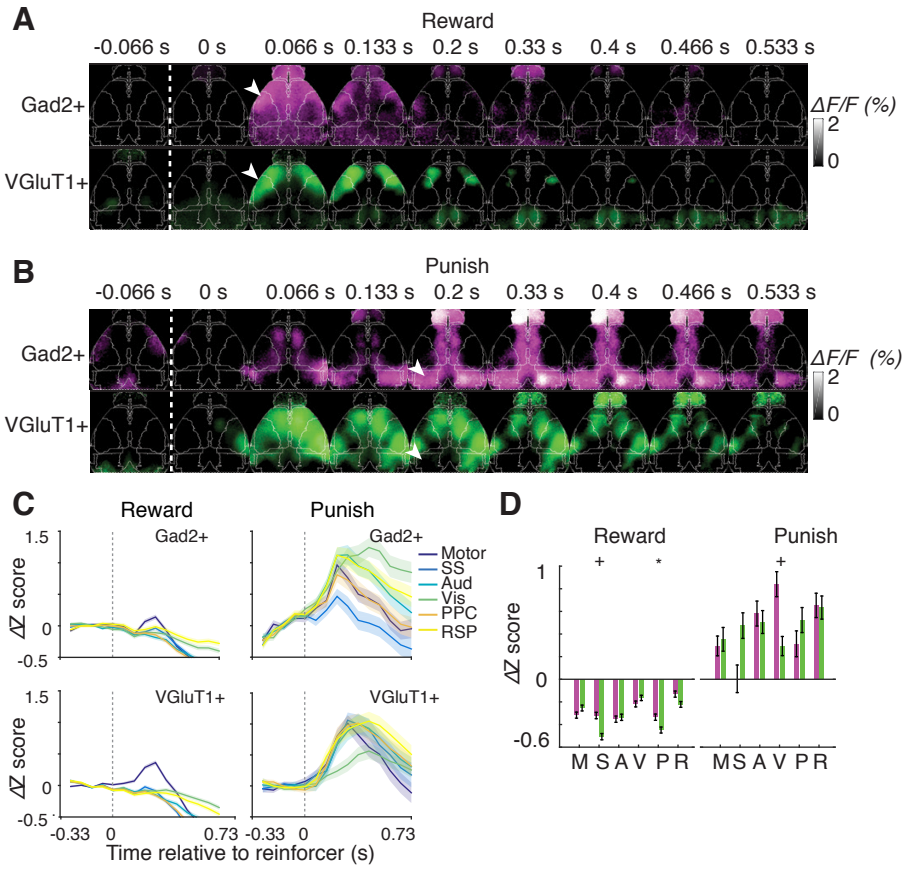

Figure S5. Activity in response to reward or punishment (related to Figure 4). (A-B) Specific increase in neural activity as a function of reward or punishment: Representative video sequence of average change in inhibitory or excitatory fluorescence relative to 0.33 s baseline, in response to reward (A) and punishment (B) (delivered at dotted line). Arrow in (A) indicates secondary activation of somatosensory and motor cortex as a result of reward. Arrow in (B) indicates a difference in persistent activation of visual cortex between Gad2+ and VGluT1+ following punishment. (C) Change in Z-scored fluorescence across six cortical regions, aligned to reward or punishment delivery, in Gad2+ or VGluT1+ mice. n = 362 Hit, n = 182 FA trials across N = 5 Gad2+ mice and n = 419 Hit, n = 144 FA trials N = 5 VGluT1+ mice. (D) Average values from (C) during 0.73 s after reward or punishment across six cortical regions in Gad2+ or VGluT1+ mice. M = motor, S = somatosensory, A = auditory, V = visual, P = PPC, R = RSP. * $P < 0.05$, + $P < 0.005$, Wilcoxon rank-sum test, Bonferroni corrected. All data: mean ± s.e.m.

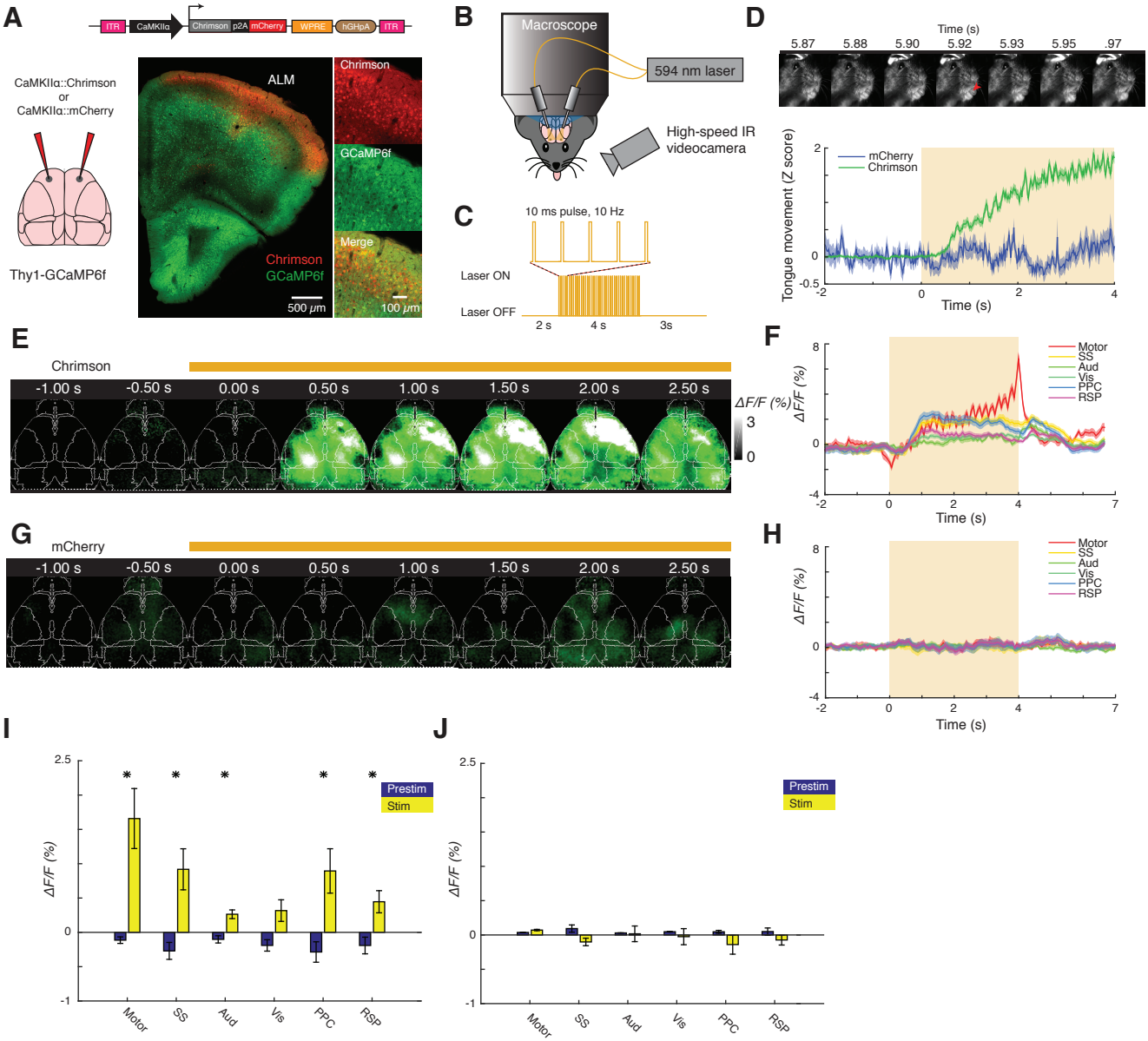

Figure S6. Cortex-wide activation in response to optogenetic ALM stimulation (related to Figure 6). (A) Diagram of CaMKIIα::Chrimson-p2A-mCherry construct, diagram of injection into ALM, and expression of Chrimson-p2A-mCherry in cortex after bilateral injection into ALM of Thy1-GCaMP6f mice. (B) Experiment schematic. Transparent skull-prepared mice were imaged with the macroscope and recorded with a high-speed infrared camera while bilaterally stimulated with fiber-coupled 594nm laser illumination projected onto ALM. (C) Stimulation and imaging parameters (10 ms on, 90 ms off), repeated for 4 s with a 2 s prestimulus period and 3 s poststimulus period. (D) Example video frames demonstrating ALM-stimulation induced licking behavior, and comparison between Chrimson-p2A-mCherry expressing mice and control mice expressing just mCherry. Red arrow indicates tongue extension from mouth. Tongue movement was quantified from video frames. (E, F) Example video sequence and regional traces of average fluorescence across time-locked stimulation trials in a Chrimson expressing mouse. Yellow bar indicates stimulation period. (G, H) Same, but for control mouse expressing just mCherry. (I) Comparison between average, across Chrimson-expressing mice, of fluorescence signal during 2 s preceding stimulation, and the period from 1 to 3 s after beginning of stimulation. * $P < 0.05$, Wilcoxon rank-sum test, Bonferonni corrected, n=5 Chrimson mice. (J) Same comparison but for mCherry-expressing controls, n=2 mCherry mice. All data are presented as mean ± s.e.m.