

**Figure S1. Additional examples of somato-dendritic signals and orientation tuning, related to Figure 1.**

(A) GCaMP6f signals recorded from corresponding somas and dendrites of ten representative neurons.

(B) GCaMP6f signals from a soma and its corresponding dendrite during the presentation of drifting gratings. Gray and light red lines represent individual trials (2 s before the onset and up to 4 s after) while averages are shown in black and red.

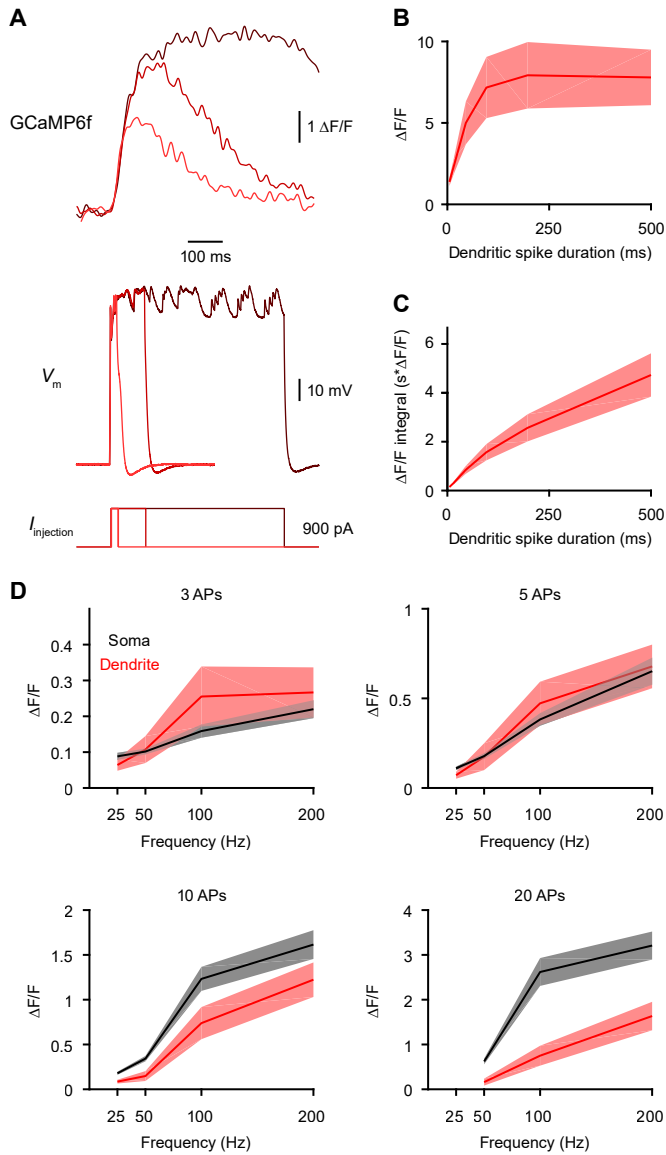
(C) Tuning curve for the soma and dendrite shown in B.

(D) Same as A for another example neuron.

(E) Tuning curve for the soma and dendrite shown in D.

(F) Difference between the preferred orientation of corresponding somas and dendrites for 34 visually-responsive pairs from 3 mice.

(G) Tuning curve for the soma and dendrite of the only pair with different tuning.



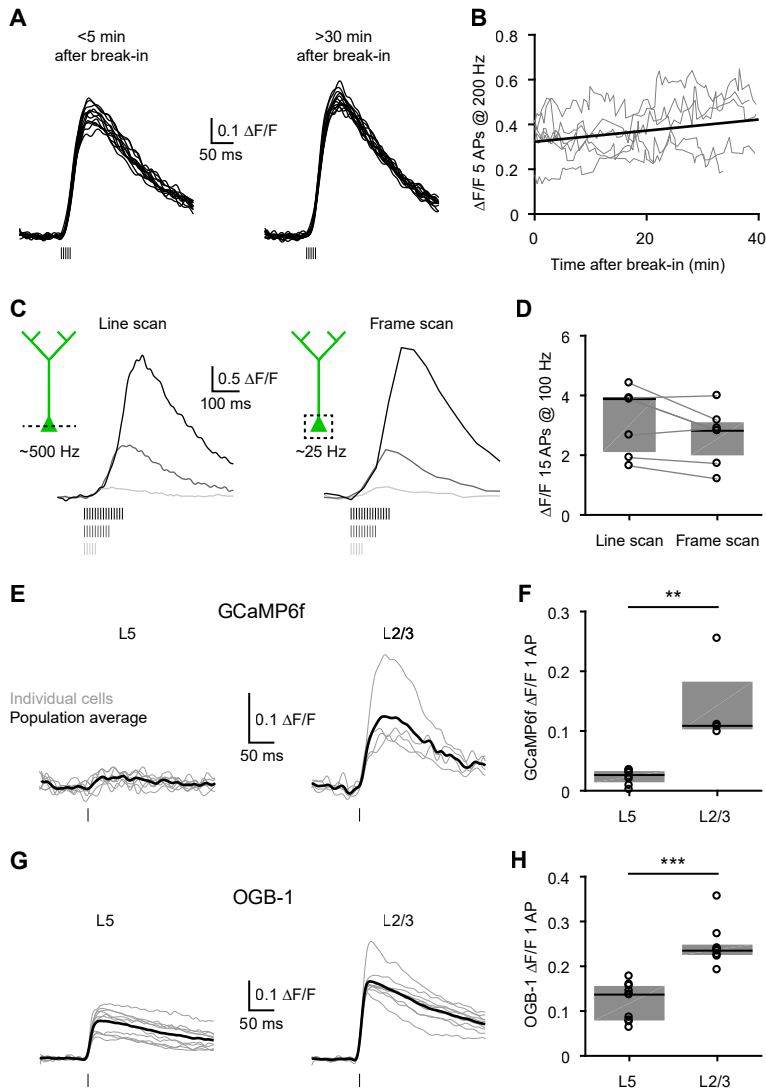
**Figure S2. Additional ex vivo calibration of somatic and dendritic GCaMP6f signals, related to Figure 3.**

(A) Dendritic GCaMP6f signals (top) associated with dendritic spikes (middle) triggered by current injections of different durations (bottom; 20, 100, and 500 ms).

(B) Dendritic GCaMP6f signal peak as a function of dendritic spike duration ( $n=10$  dendrites from 7 mice). Pooled data represent mean  $\pm$  SEM.

(C) Dendritic GCaMP6f signal integral as a function of dendritic spike duration ( $n=10$  dendrites from 7 mice). Pooled data represent mean  $\pm$  SEM.

(D) Somatic (black) and dendritic (red) GCaMP6f peak for 3, 5, 10, or 20 APs as a function of AP frequency ( $n=21$  neurons from 9 mice). Pooled data represent mean  $\pm$  SEM.



**Figure S3. Control experiments for GCaMP6f in slices, related to Figure 3.**

(A) Somatic GCaMP6f signal for 5 APs at 200 Hz within 5 min after break-in and more than 30 minutes after break-in. Tick marks indicate the timing of the APs.

(B) Somatic GCaMP6f signal as a function of time after break-in (n=6 neurons from 1 mouse). Black line represents a linear regression.

(C) Somatic GCaMP6f signal for 5, 10, and 15 APs measured with line scan (left) or frame scan (right). Tick marks indicate the timing of the APs.

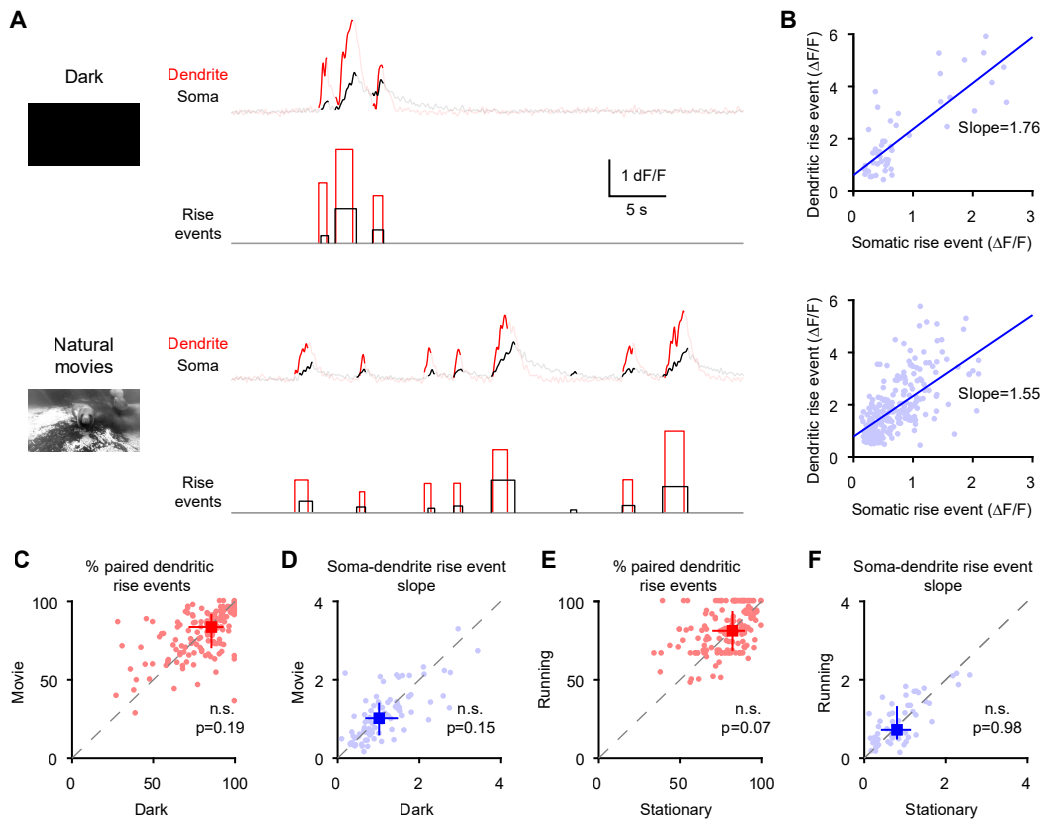
(D) Comparison of somatic GCaMP6f signal for 15 APs measured with line scan or frame scan (p=0.11, Wilcoxon paired test; n=7 from 2 mice). Pooled data represent median and interquartile range.

(E) GCaMP6f signal for single APs in L5 (left) and L2/3 (right) neurons. Gray traces are averages of 3-10 trials for individual cells, while black lines are averages across cells.

(F) Comparison of somatic GCaMP6f signal for single APs in L5 and L2/3 neurons. (\*\*p=0.004, Wilcoxon rank sum test; n=8 from 4 mice & n=4 from 2 mice). Pooled data represent median and interquartile range.

(G) OGB1 signal for single APs in L5 (left) and L2/3 (right) neurons. Gray traces are averages of 3-10 trials for individual cells, while black lines are averages across cells.

(H) Comparison of somatic OGB1 signal for single APs in L5 and L2/3 neurons. (\*\*\*)p<10<sup>-4</sup>, Wilcoxon rank sum test; n=10 from 3 mice & n=9 from 3 mice). Pooled data represent median and interquartile range.



**Figure S4. Visual stimuli and running do not change somato-dendritic GCaMP6f correlation, related to Figure 4.**

(A) GCaMP6f signals from the soma and dendrite with detected rise events shown in solid colors during dark screen (top) or the presentation of natural movies (bottom).

(B) Relationship between the amplitude of paired rise events in the neuron shown in A. The slope was computed as a metric of the relationship between somatic and dendritic rise events during dark screen (Top; slope=1.76,  $n=50$  paired rise events) and the presentation of natural movies (Bottom; slope=1.55,  $n=224$  paired rise events).

(C) Comparison of the percentage of paired dendritic rise events ( $p=0.19$ , Wilcoxon paired test,  $n=151$  pairs from 5 mice) during the dark and natural movie epochs.

(D) Comparison of the soma-dendrite rise event slope ( $p=0.15$ , Wilcoxon paired test,  $n=87$  pairs from 5 mice) during the dark and natural movie epochs.

(E) Comparison of the percentage of paired dendritic rise events ( $p=0.07$ , Wilcoxon paired test,  $n=146$  pairs from 5 mice) during the stationary and running epochs.

(F) Comparison of the soma-dendrite rise event slope ( $p=0.98$ , Wilcoxon paired test,  $n=58$  pairs from 5 mice) during the stationary and running epochs.