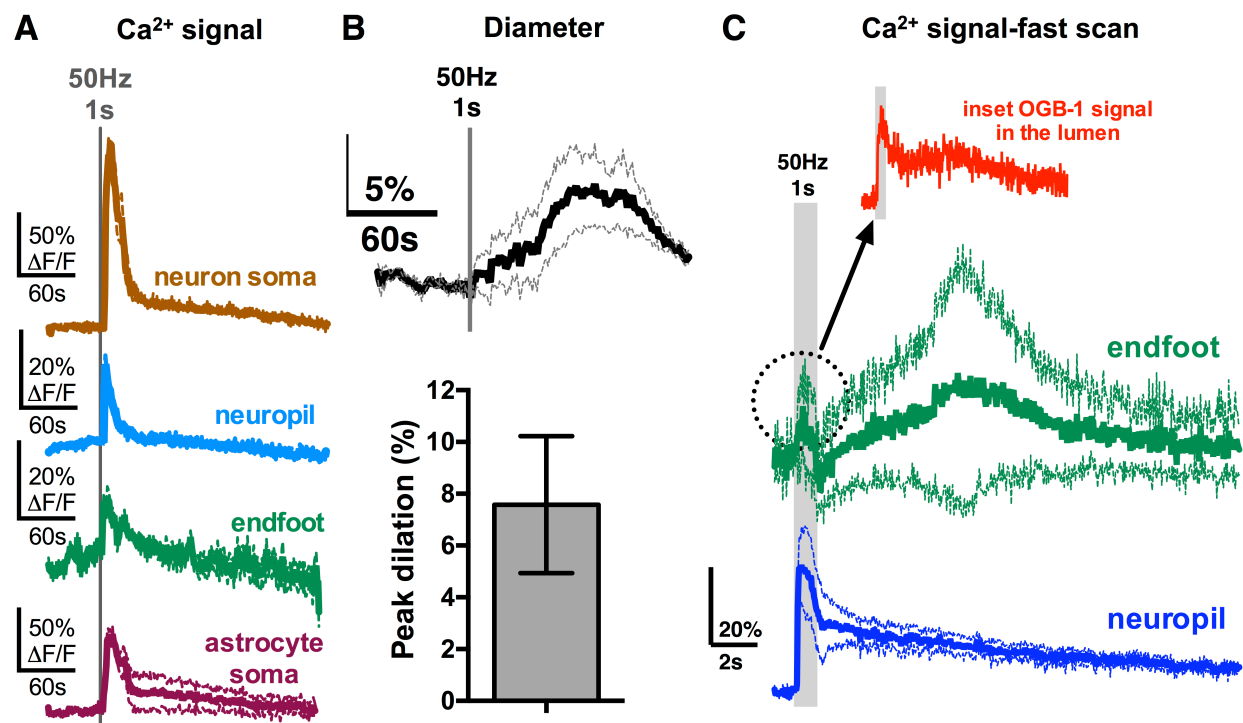


Supplementary Video. **Vasodilation in response to afferent stimulation without endfoot Ca^{2+} transients.** Low intensity (1.5V, 50Hz), short (1s) afferent stimulation with neuropil and neuronal Ca^{2+} transients but no astrocyte somata or endfoot Ca^{2+} signals preceding vasodilation. A small, localized endfoot transient can be seen but it occurs 40s after dilation onset. Vascular lumen (green); Rhod-2/AM Ca^{2+} indicator (white). Video speed is 50x of real-time events. The movie refreshes at peak dilation and repeats three times.

Supplementary Figure. High intensity afferent neuronal stimulation in the presence of the kinetically fast, high-affinity Ca^{2+} indicator OGB-1/AM ($K_d \approx 200\text{nM}$) reveals the same threshold effect for astrocytic Ca^{2+} signals as other synthetic and genetic Ca^{2+} indicators tested. **A:** Higher voltages (supra-threshold) of brief (1s), high frequency (50Hz) electrical stimulation evoked Ca^{2+} transients in all neuronal elements and in astrocyte somata and endfeet. **B:** Averaged stimulus-evoked arteriolar dilation to supra-threshold voltages, and the summary of peak dilations. **C:** Ca^{2+} signals from endfeet and surrounding neuropil (n=4) during supra-threshold stimulation, imaged at 31.25Hz (32msec frame rate) detected a fast neuropil signal starting <32msec after the onset of stimulation (blue trace). The same stimulation evoked a rapid (32-64msec latency) and a delayed (2.9s latency) Ca^{2+} transient in the endfeet (green trace). The rapid Ca^{2+} signal was only detected in 1 of 4 cases, where a Ca^{2+} signal in the center of the arteriole lumen could also be detected (inset red trace), indicating leakage of signal from out-of-plane structures.



Supplementary Figure