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Supplemental Information

Vascular Compartmentalization of Functional Hyperemia from the Synapse to the Pia

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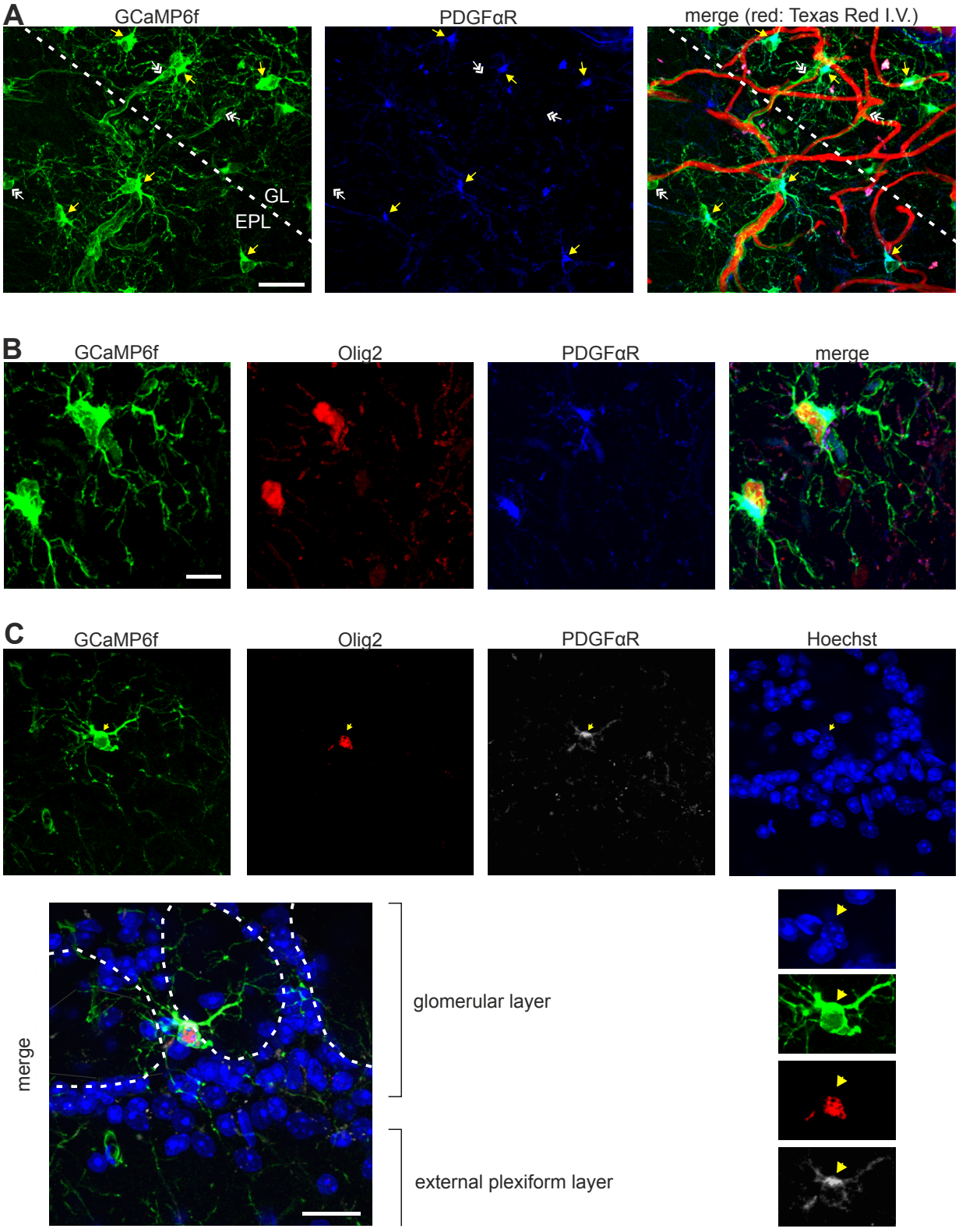

Figure S1. Immunostaining indicates GCaMP6f expression in olfactory bulb OPCs. Related to Figure 1.

(**A**) Single yellow arrows point to OPCs that express GCaMP6f (green) and are positive for

PDGFαR (blue). Double white arrows point to capillary pericytes that express GCaMP6f (green), are negative for PDGFαR (blue), and are found on capillaries (red). Glomerulus layer (GL) and External Plexiform layer (EPL) are separated by dashed white line. Stack size: 18 μm z-projection, scale bar: 25 μm. (**B**) Triple staining shows that GCaMP6f expressing OPCs are positive for both Olig2 and PDGFαR. Stack size: 16 μm z-projection, scale bar: 10 μm. (**C**) Hoechst (blue) indicates location of periglomerular nuclei allowing identification of glomeruli boundaries (dashed white lines encircle individual glomeruli). Olig2 and PDGFαR staining indicate that the GcAMP6f expressing cell is an OPC and it extends processes into more than 1 glomeruli. Yellow arrow indicates typical nuclear expression of Olig2 that does not overlap with surrounding PDGFαR staining. Stack size: 5 μm z-projection, scale bar: 20 μm.

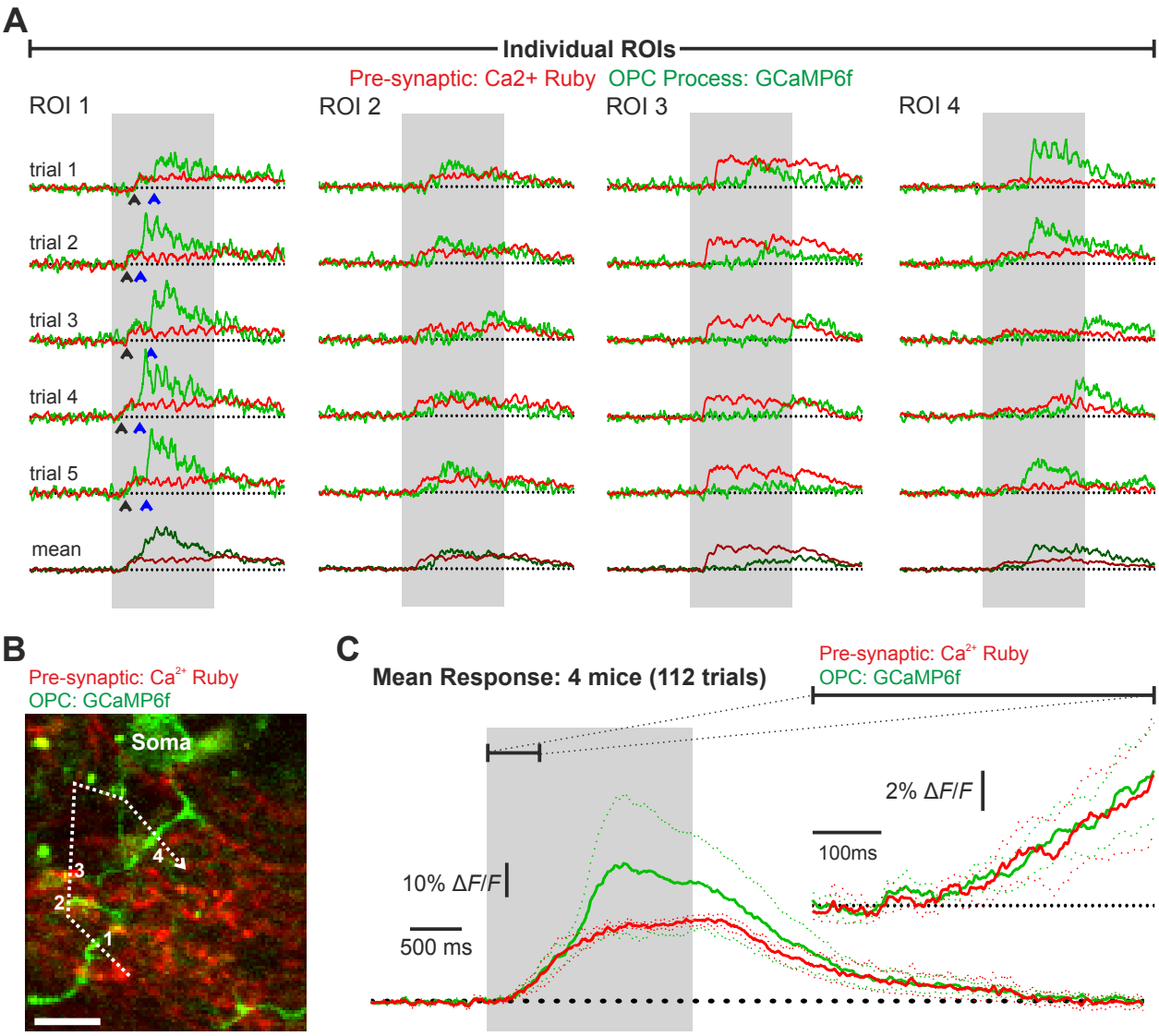

Figure S2. Odor stimulation evokes biphasic Ca^{2+} elevations in glomerular OPC processes. Related to Figure 1.

(A) 5 consecutive trial by trial responses (top) and mean of 5 trials (bottom) showing OSN pre-synaptic Ca^{2+} elevation (red) and adjacent OPC process Ca^{2+} elevation (green) in individual ROIs numbered 1-4 in (**B**). Grey shaded area indicates timing of a 2 second odor application. ROI 1 consistently shows a rapid OPC process signal synchronous with the presynaptic Ca^{2+} elevation (black arrowhead), and a secondary increase which is delayed (blue arrowhead). In ROIs 2-4 the first OPC Ca^{2+} elevation is barely detectable, but the secondary elevation consistently occurs with a varying delay. (**B**) Mean image taken from a time series during an odor application shows presynaptic labeling (red) and OPC-GCaMP6f (green). Dashed line shows path of line scan. Scale bar: 5 µm. (**C**) Mean response of paired pre-synaptic and OPC process Ca^{2+} signals shows that the onset of the pre-synaptic Ca-Ruby signal and the OPC GcAMP6f signal were indistinguishable in response to a natural odor stimulation. Right inset shows expanded traces from between the hash marked line. Data represents mean (solid lines) ± SEM (dashed lines).

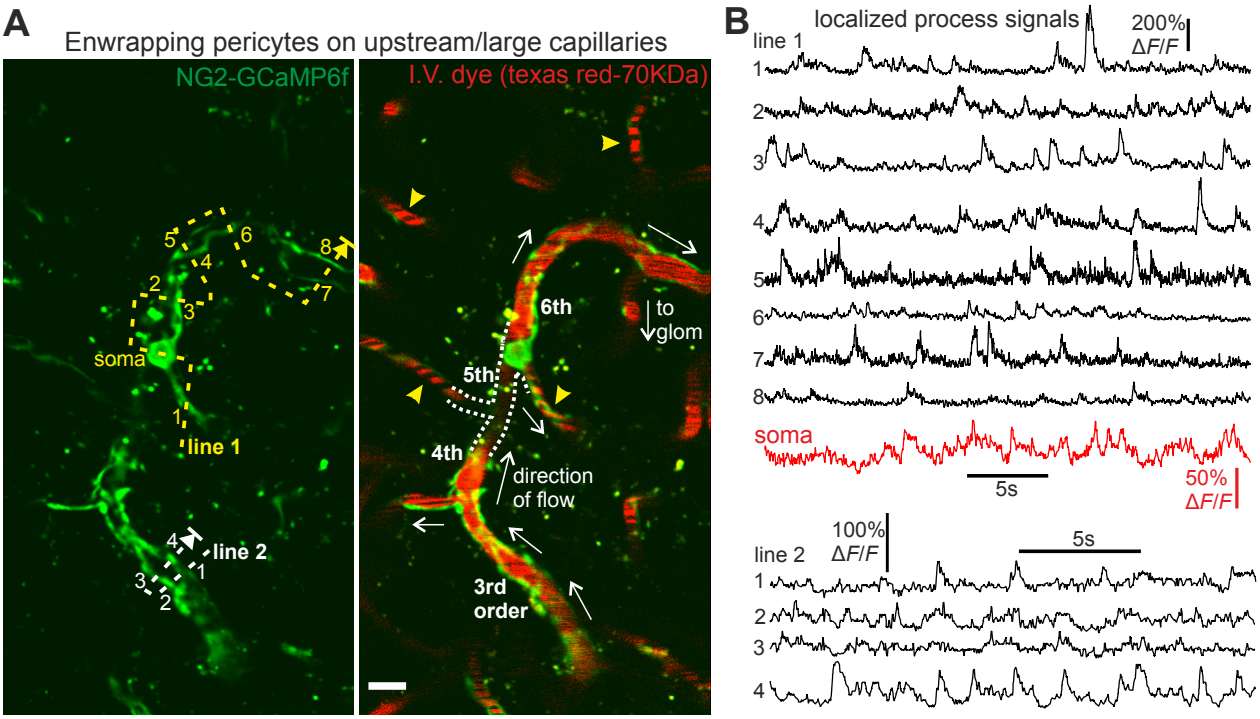

Figure S3. Microdomain Ca^{2+} transients in enwrapping-type pericyte processes. Related to Figure 3 and Movie S3.

(**A**) Single z-plane image shows GCaMP6f fluorescence (green) in enwrapping type pericytes on capillaries located in the external plexiform layer (EPL) and upstream from a glomerulus. These capillaries are larger than downstream glomerulus capillaries that are contacted by thin-strand pericytes as well as surrounding capillaries in the same field of view (yellow arrows, right). Left: Yellow and white dashed arrows lines indicates the path of 2 broken line scans plotted in (B) on the 2 different branches. Right: White dashed line indicates the path of the capillary that dips below the imaged z-plane. White arrows indicate direction of blood flow, which was followed forwards to a glomerulus located above the imaged z plane. (**B**) As in the thin strand pericyte (Figure 3), enwrapping or mesh-type pericytes also show spontaneous Ca^{2+} transients that are spatially localized. Scale bar: 10 µm. Note, different $\Delta F/F$ and time scales were used for the data plots of the 2 line scans and the soma (red).

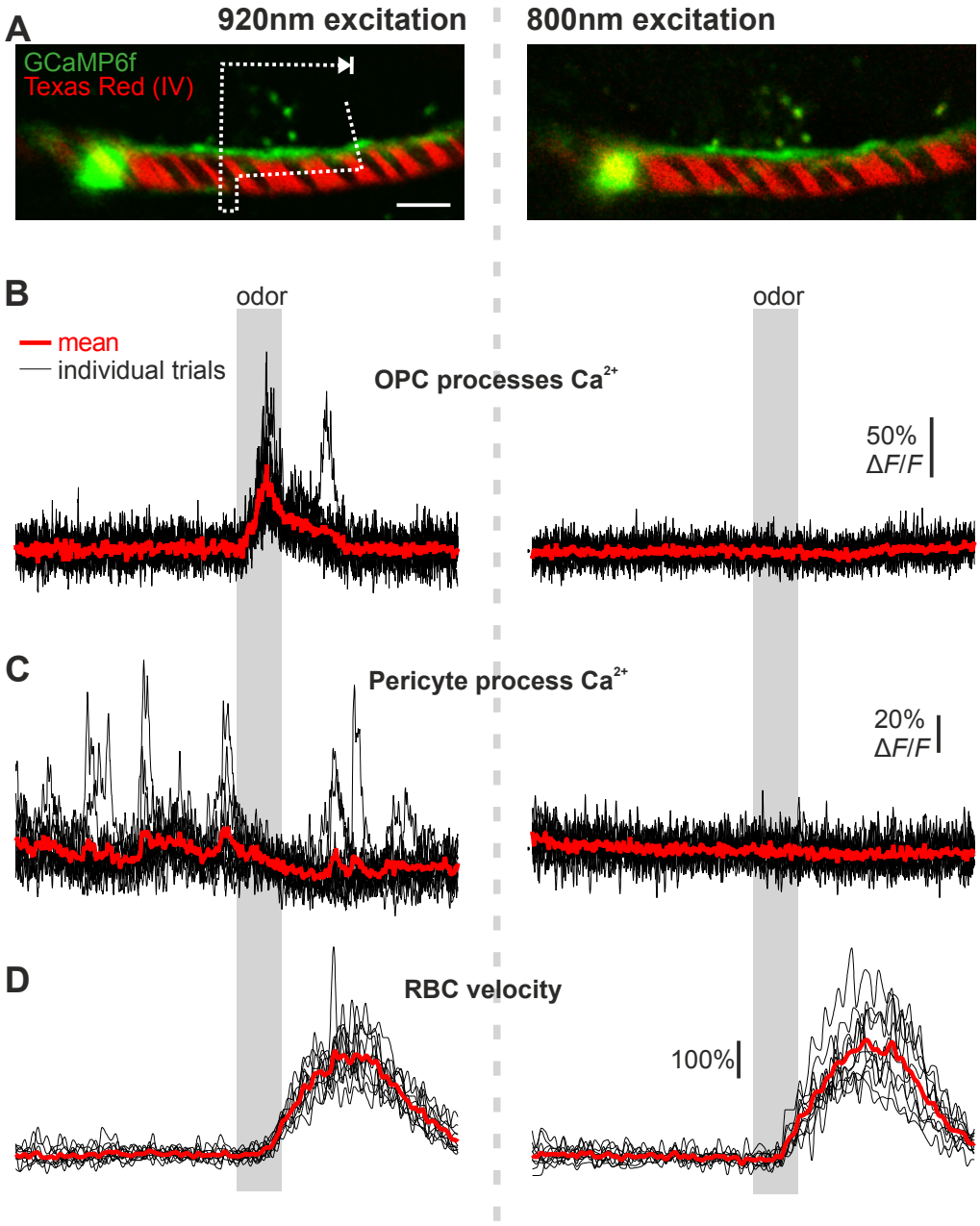

Figure S4. Imaging of GCaMP6f at different wavelengths confirms that activity dependent changes in pericyte Ca^{2+} are real and not due to volume changes. Related to Figures 3 and 4. (**A**) Single z-plane images of GCaMP6f (green) and Texas Red (red) fluorescence excited at 920nm (left) and 800nm (right), shows that the fluorophores can be excited at both wavelengths. Dashed line indicates path of broken line scan quantified in (B-D) to capture OPC process Ca^{2+}, pericyte process Ca^{2+} and RBC velocity simultaneously. Scale bar: 5 µm. (**B**) Imaging with 920nm excitation (left) shows that the glomerulus is activated, as indicated by an increase in OPC process GCaMP6f fluorescence. Imaging at 800nm excitation confirms that GCaMP6f sensitivity is lost at this wavelength as no increase in OPC process Ca^{2+} is observed across multiple trials. (**C**) Synaptic activation decreases frequency of transients and steady state GCaMP6f fluorescence within the pericyte process when imaged at 920nm but not 800nm, indicating that the transients

and decrease in fluorescence are indeed Ca^{2+} dependent. (**D**) RBC velocity increases consistently across multiple trials at both wavelengths. 11 individual trials were performed and are plotted at each 800nm and 920nm. The Ca^{2+} dependency of the GCaMP6f 2-photon excitation spectrum can be found at https://www.janelia.org/lab/harris-lab-apig/research/photophysics/two-photon-fluorescent-probes

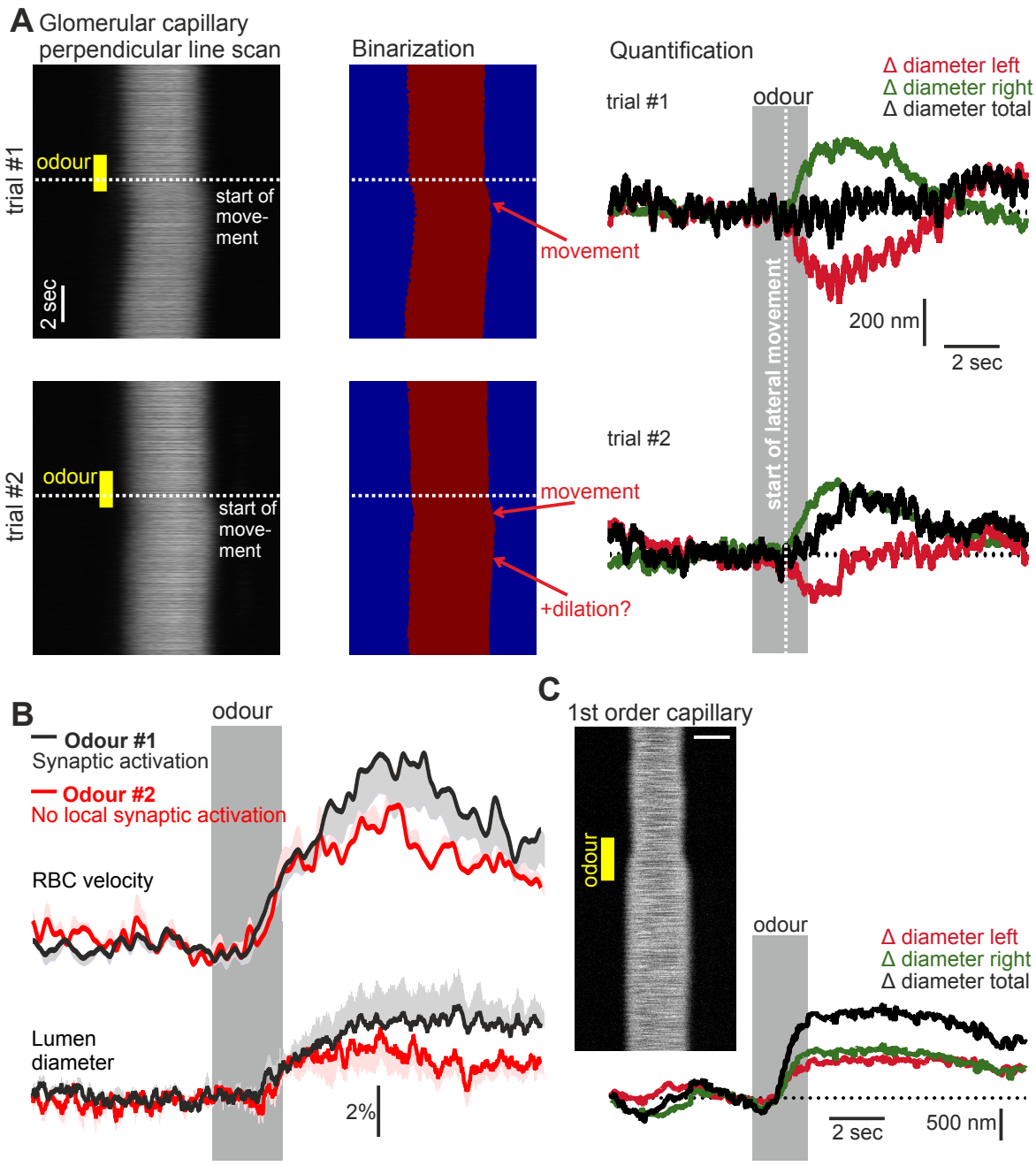

Figure S5. Lumen diameter changes of juxta-synaptic glomerulus capillaries are difficult to interpret and occur in the absence of local synaptic activation and pericyte Ca^{2+} changes. Related to Figures 3 and 4.

(**A**) Two individual trials from the same glomerulus capillary shows that the capillary moves laterally following synaptic activation. In trial #1 (top) the increase in diameter on the right is mostly matched by the decrease in diameter on the left indicating a case where dilation is not detected. In trial #2 (bottom), the increase on the right is initially matched by a decrease on the left, however, the increase on the right continues in the absence of a further decrease on the left and results in the detection of a delayed diameter increase that is unilateral and difficult to interpret. Note, individual RBCs are not visible in the left images because of the compressed time scale. (**B**)

RBC velocity (top, same data from Figure 4C) with quantified lumen diameter changes (bottom), shows that when the lumen diameter is analyzed, the increase in RBC velocity is accompanied by a small increase in diameter. Although these diameter changes were not interpreted, if they were indeed real, the observation that they occurred independently of local synaptic activity or decreases in pericyte Ca^{2+} (red traces, see Figure 4C), suggests that they were passive. n=5 paired experiments from 5 mice. Decreases in diameter were never detected. Data represents mean ± SEM. (**C**) An example of a diameter increase (single trial) at the level of a 1st order capillary, upstream of an activated glomerulus (Example mouse #2, Figure S6), that shows an unambiguous dilation. Scale bar: 5 μm.

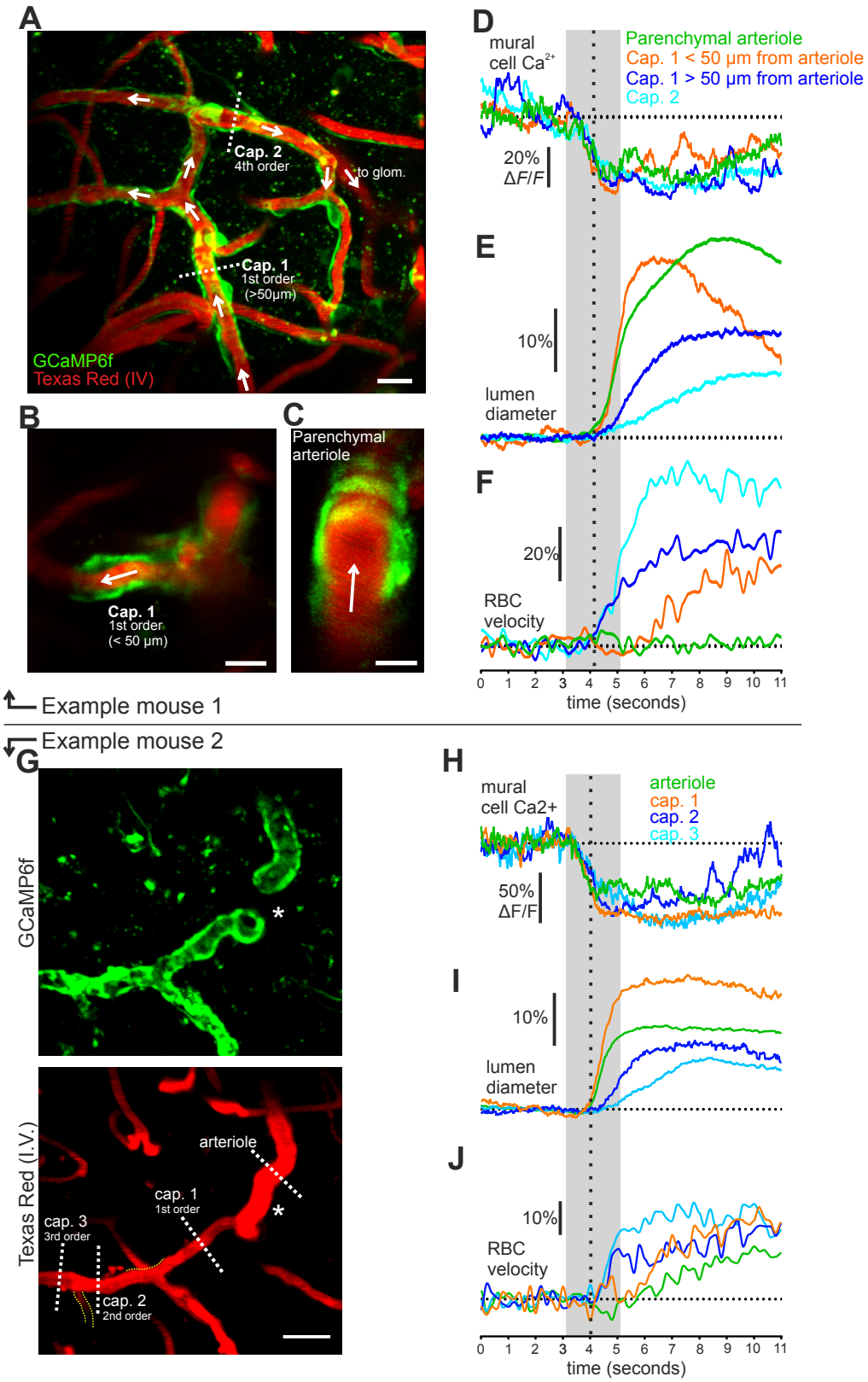

Figure S6. Individual examples of hemodynamics and mural cell Ca^{2+} signaling upstream of an activated glomerulus. Related to Figure 5.

This figure shows data obtained from individual vascular networks. (**A**) 50 µm z-stack max

projection showing GCaMP6f expressing (mesh type) pericytes that are enwrapping capillaries upstream of a glomerulus and > 50 µm downstream of the arteriole. Scale: 20 µm (**B**) single z plane image of the first capillary branch point off of the feeding arteriole. Scale: 10 µm (**C**) 5 µm z-stack max projection showing GCaMP6f expressing SMCs covering the arteriole. Scale: 10 µm. Arrows indicate the direction of RBC flow. (**D**) The Ca^{2+} drop in all mural cells. (**E**) Dilation occurs first and simultaneously in arteriole and the proximal portion of the 1st order capillary and becomes progressively delayed with distance downstream. Note, the speed of the dilation is not dependent on branch order as the portion of the first order capillary furthest from the arteriole dilates more slowly (blue trace). (**F**) The increase in RBC velocity occurs first in the downstream capillaries that dilate more slowly and is delayed in the proximal portion of the capillary. In this example the arteriole RBC velocity remains constant. (**G**) 47 µm z stack max projection showing GCaMP6f expression (top) and Texas Red fluorescence in lumen (bottom) along the arteriole and 1st-3rd order capillaries upstream of an activated glomerulus. Yellow dashed lines show the location of the vessel when it dips out of the focal plane or crosses over a non-connected vessel. Star indicates portion of the arteriole negative for GCaMP6f expression (**H**) Synchronous Ca^{2+} drop in all mural cells. (**I**) Dilation occurs first and simultaneously in arteriole and proximal portion of the capillary and becomes progressively delayed with distance. (**J**) The increase in RBC velocity occurs first in the downstream capillaries that dilate more slowly and is delayed in the proximal portion of the capillary and the arteriole. Dashed white lines indicate the location of the diameter measurements. Dashed vertical lines on traces serve as visual cue to compare timing of events. Grey shaded region represents odor delivery. Traces represent averages of 4-13 trials.

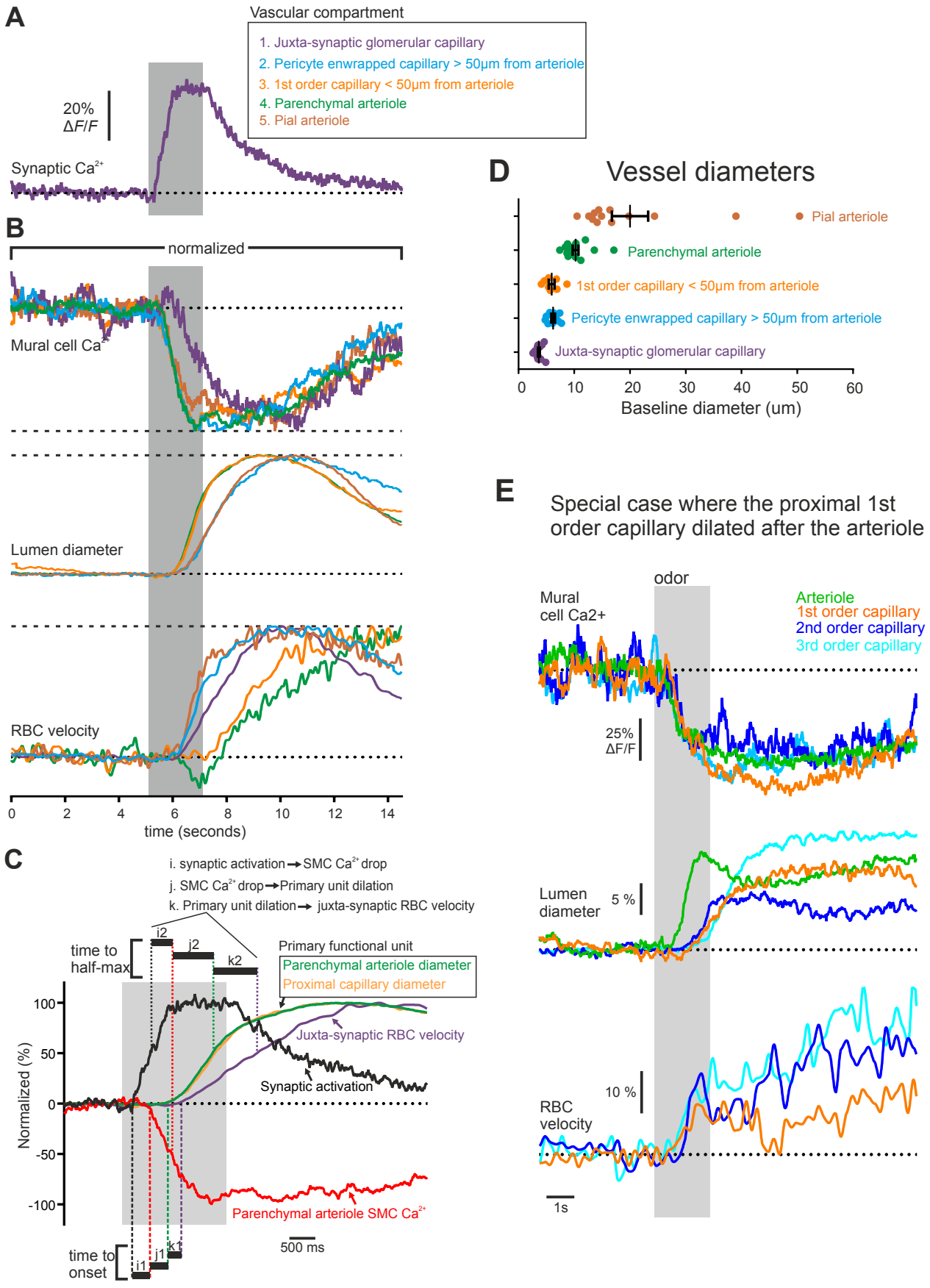
A
Vascular compartment
1. Juxta-synaptic glomerular capillary
2. Pericyte enwrapped capillary > 50µm from arteriole
3. 1st order capillary < 50µm from arteriole
4. Parenchymal arteriole
5. Pial arteriole
20%
ΔF/F
Synaptic Ca2+
B
normalized
Mural cell Ca2+
Lumen diameter
RBC velocity
0 2 4 6 8 10 12 14
time (seconds)
C
i. synaptic activation → SMC Ca2+ drop
j. SMC Ca2+ drop → Primary unit dilation
k. Primary unit dilation → juxta-synaptic RBC velocity
time to half-max
i2
j2
k2
Primary functional unit
Parenchymal arteriole diameter
Proximal capillary diameter
Juxta-synaptic RBC velocity
Synaptic activation
Parenchymal arteriole SMC Ca2+
Normalized (%)
100
50
0
-50
-100
time to onset
i1
j1
k1
500 ms
D
Vessel diameters
Pial arteriole
Parenchymal arteriole
1st order capillary < 50µm from arteriole
Pericyte enwrapped capillary > 50µm from arteriole
Juxta-synaptic glomerular capillary
0 10 20 30 40 50 60
Baseline diameter (um)
E
Special case where the proximal 1st order capillary dilated after the arteriole
odor
Mural cell Ca2+
Arteriole
1st order capillary
2nd order capillary
3rd order capillary
25% ΔF/F
Lumen diameter
5 %
RBC velocity
10 %
1s

Figure S7. Timing of Ca^{2+} signaling and hemodynamics from the synapse to the pia: all measurements combined and one special case. Related to Figure 5.

This supplemental figure serves as an extension to Figure 5. Here the average data from all vessels recorded in this study are combined (**A**) Average timing of the synaptic response (OSN or OPC process Ca^{2+} signal). (**B**) Mean data normalized showing from top to bottom; mural cell Ca^{2+}, lumen diameter and RBC velocity at different vascular compartments. (**C**) Timing of synaptic activation, parenchymal arteriole SMC Ca^{2+} decrease, primary functional unit dilation and resulting RBC velocity increase at the synapse. Black bars and dashed vertical lines indicate the time to half-maximum (top), and the time to onset (bottom). Data in (**A-C**) represents multiple branches from 23 distinct vascular networks in 17 mice. Glomerulus capillary, n= 14; Enwrapped > 50 μm from arteriole, n=10; Enwrapped <50 μm from arteriole, n=8; arteriole, n=18; Pial arteriole, n=13. (Every vascular compartment was not imaged in each vascular network, RBC velocity was only measured in a subset of branches, and in a few cases Ca^{2+} was not measured at all branches due to sparseness of GCaMP6f expression). Data incorporated into (**A-C**) was also presented in Figures 3-5. (**D**) Distribution of baseline diameters for each vessel type. Data represents mean ± SEM. **(E)** Analysis of a case where the proximal 1st order capillary dilated after the arteriole. Top, Arteriole SMC and pericyte (1st to 3rd order capillaries) show "synchronous" Ca^{2+} decreases. Middle, Arteriole dilates before all capillary branch orders. Bottom, RBC velocity increases fast in all capillary branch orders. Vessels were traced backwards from a capillary in an activated glomerulus. Data represents averages of 3-4 trials. Note, Arteriole RBC velocity was not measured as the vessel was never horizontal with the 2D imaging plane.