Supplementary note on numerical simulations of cerebral blood flow changes induced by capillary occlusions for: "Neutrophil adhesion in brain capillaries reduces cortical blood flow and impairs memory function in Alzheimer's disease mouse models," by J.C. Hernandez, et al.

In previous work, we studied how the occlusion of a single cortical capillary influenced blood flow in downstream vessels[20] and found strong reductions in blood flow (10% of baseline value 1 branch downstream; 25% at 2 branches; 50% at 3 and 4 branches), suggesting that even the small fraction of occluded capillaries we observed in APP/PS1 mice could cause a significant decrease in overall brain blood flow. To test this idea, we simulated blood flow in anatomically accurate blood vessel networks from mice and humans and examined how flow changed when we occluded a random selection of capillaries.

Validation of simulations by comparison to in vivo measurements in mouse: As described in the Supplementary Methods, our simulations resulted in calculated values for flow (Supplementary Fig. 15d), pressure (Supplementary Fig. 15e), and hematocrit (Supplementary Fig. 15f) in each vessel segment in the volume. We validated the simulation by comparing *in vivo* measurements of blood flow at different levels in the microvascular hierarchy acquired by 2PEF from the top 300 μm of mouse cortex (data from Santisakultarm, *et al.*[53]) with the simulation predictions. The simulation results are highly dependent on the boundary conditions imposed on capillaries at the lateral edges of the simulation volume. The calculated velocity distribution using pseudo-periodic boundary conditions in capillaries up to 300 μm in depth and using the vessel diameter corrections described in the Supplementary Methods matches the experimental distribution well (Supplementary Fig. 15g). For comparison, the velocity distribution calculated using diameters from the raw datasets (without correction for the difference in vessel size between *in vivo* and post mortem measurements) and that calculated using a no-flow boundary condition both led to an order of magnitude underestimation of capillary flow speeds (Supplementary Fig. 15g). Our new pseudo-periodic boundary condition, together with the correction of vessel diameters, led to a velocity distribution that approaches the distribution of experimental velocities. The experimental distribution has a sharper peak, which might be due to experimental bias associated with

the limited number of vessels in which these measurements have been performed (147 *in vivo* measurements vs. 3,400 capillaries in the simulations). The simulated speeds in penetrating arterioles and ascending venules as a function of their diameters also closely matched experimental results from Santisakultarm, et al.[53] and from Taylor, et al.[61] (Supplementary Fig. 15h).

Numerical simulation of cerebral blood flow reductions caused by capillary occlusions: The effect of occlusions in capillaries was investigated by randomly selecting a given proportion of capillaries and reducing their flow by imposing a 100-fold reduction in diameter (pressure change: Supplementary Fig. 15i, flow change: Fig. 6a). To quantify the effects of the occlusions, we calculated the normalized cortical perfusion as the summed flow in the penetrating arterioles feeding the region, normalized by the value calculated with no capillary occlusions (Fig. 6c). While the magnitude of this summed flow is highly dependent on the boundary conditions, the decrease in flow due to capillary occlusions was much less sensitive to the choice of boundary conditions (Supplementary Fig. 15j). For the mouse network shown in Fig. 6a with pseudo-periodic boundary conditions and diameter correction, we found a linear decrease in the normalized perfusion with a slope S=-2.3±0.2 %baseline perfusion/% capillaries stalled (mean±SD) (Fig. 6c). This linear behavior was very robust to variations in the parameters chosen for the computations, with slopes equal to -2.2±0.1 (-2.1±0.2) with no-flow boundary conditions and diameter correction (no diameter correction). In order to evaluate the influence of boundary conditions with regard to the size of the simulated volume, 300 µm-thick sub-volumes of the mouse anatomical datasets were randomly extracted. The decrease in blood flow with increasing numbers of stalled capillaries was slightly larger when 300 µm-thick sub-volumes of the datasets were used (-2.6±0.4 and -2.9±0.5 with the pseudo-periodic boundary condition and the no-flow boundary condition, respectively), as compared to the full ~1 mm-thick volume. In Fig. 6c, only computations on the maximum simulation volume with the corrected diameters and pseudo-periodic boundary conditions are presented.

The simulations in the human network (Fig. 6b) using pseudo-periodic boundary conditions yielded a slope of S=-2.3±0.6, very similar to the mouse results. This linear decrease was also observed in synthetic periodic networks of order three (i.e. three edges per node; S=-2.9, Fig. 6c).

Limitations and methodological considerations: The human dataset used in the simulations was only 300 μm thick, raising concerns about the influence of boundary conditions. The broad agreement between simulation results in mouse datasets with 1-mm and 300-μm thickness reduces this concern. The simulations predicted a similar CBF increase across mouse and human vascular networks when stalls were reduced, suggesting that the blood flow improvements we observed in APP/PS1 mice may be achievable in humans.

The simulations predicted a smaller impact of capillary stalling on CBF than we observed experimentally. One possible explanation is that the simulations used vascular networks from wt mice, while AD mouse models have different vascular densities and topologies[71] that may influence the sensitivity of CBF to capillary stalls, although the vascular density differences between APP/PS1 and wt mice have been reported to be relatively minor[31,32]. In addition, increased leukocyte adhesion in APP/PS1 mice may lead not only to complete stalls, but also to slowed flow in some capillaries when a leukocyte is present in the segment, which is not captured in the simulations.

Supplementary Note-only References:

71 Brown, W. R. & Thore, C. R. Review: cerebral microvascular pathology in ageing and neurodegeneration. *Neuropathol Appl Neurobiol* **37**, 56-74, doi:10.1111/j.1365-2990.2010.01139.x (2011).