Pharmacological depletion of microglia and perivascular macrophages prevents vascular cognitive impairment in Ang II-induced hypertension

Danielle Kerkhofs¹,²,⁵, Britt T. van Hagen³,⁶, Irina V. Milanova⁴,⁵, Kimberly J. Schell²,⁵, Helma van Essen⁵, Erwin Wijnands²,⁵, Pieter Goossens²,⁵, W. Matthijs Blankesteijn⁴,⁵, Thomas Unger⁵, Jos Prickaerts³,⁶, Erik A. Biessen²,⁵,⁷, Robert J. van Oostenbrugge¹,⁵,⁶, Sébastien Foulquier⁴,⁵,⁶

Supplementary Figures

Figure S1: Internal diameters of podocalyxin-positive brain capillaries.
Figure S2: FACS analysis from whole brain homogenates.
Figure S3: Microglial morphological analysis.
Figure S4: Microglia activation in the cortex in absence of BBB leakage.
Figure S5: Myelin intensity.
Supplementary Figure S1. Internal diameters of podocalyxin-positive brain capillaries. (A) Representative images of podocalyxin-positive capillaries (green) with the cortex and corpus callosum (scale bar = 50 μm). (B) Internal diameters of cortical capillaries (2-W ANOVA $p_{\text{int}}>0.05$; $p_{\text{PLX6822}}>0.05$; $p_{\text{AngII}}>0.05$). (C) Internal diameters of capillaries in the corpus callosum (2-W ANOVA $p_{\text{int}}>0.05$; $p_{\text{PLX6822}}>0.05$; $p_{\text{AngII}}>0.05$). n=5-6 mice per group.
Supplementary Figure S2. FACS analysis from whole brain homogenates. (A) Representative flow cytometry gating of microglia population (CD45hi, Cx3Cr1hi, CD11bhi). (B) Proportion of microglia as percentage of total cells (2-W ANOVA \( p_{\text{int}} > 0.05; p_{\text{plx5622}} < 0.001; p_{\text{AngII}} > 0.05\); Tukey’s multiple comparison test: *p < 0.05 vs. control). (C) Proportion of MHCIIhi microglia (2-W ANOVA \( p_{\text{int}} > 0.05; p_{\text{plx5622}} > 0.05; p_{\text{AngII}} > 0.05\) (C). n=4-5 per group.
Supplementary Figure S3. Microglial morphological analysis. (A) Representative pictures of Iba1+ cells in cortical areas before (first column), and after automatic analyzes using WIS-NeuroMath software (second column) (scale bar = 50 μm). (B) Microglial cell soma size (2-W ANOVA p_int = 0.027; p_PLX5622 < 0.001; p_{AngII} > 0.05; Sidak’s multiple comparison test: *p < 0.01 vs. control). (C) Microglial ramification lengths (2-W ANOVA p_int > 0.05; p_PLX5622 < 0.001; p_{AngII} = 0.07; Sidak’s multiple comparison test: *p < 0.001 vs. control). n=5-6 per group.
Supplementary Figure S4. Microglia activation in the cortex in absence of BBB leakage. (A) Representative pictures of Cx3Cr1+ cells (green) and CD68+ cells (red) (scale bar = 50 μm). (B) Cx3Cr1+CD68+ densities in cerebral cortex (2-W ANOVA \( p_{\text{int}} > 0.05; p_{\text{plx5622}} < 0.001; p_{\text{AngII}} > 0.05; \) Sidak’s multiple comparison test: * \( p < 0.001 \) vs. Control). n=5-6 per group.
**Supplementary Figure S5. Myelin intensity.** (A) Representative Fire lookup table representation of a stitched coronal brain section stained for Myelin Basic Protein (MBP). (B) Average MBP intensity signal in corpus callosum (2-W ANOVA \( p_{\text{int}} > 0.05; p_{\text{PLX5622}} > 0.05; p_{\text{AngII}} > 0.05 \)). (C) Average MBP intensity signal in Striatum (2-W ANOVA \( p_{\text{int}} > 0.05; p_{\text{PLX5622}} = 0.10; p_{\text{AngII}} > 0.05 \)). n=6 per group.