Supplemental material

PFKFB3 gene deletion in endothelial cells inhibits intraplaque angiogenesis and lesion formation in a murine model of venous bypass grafting

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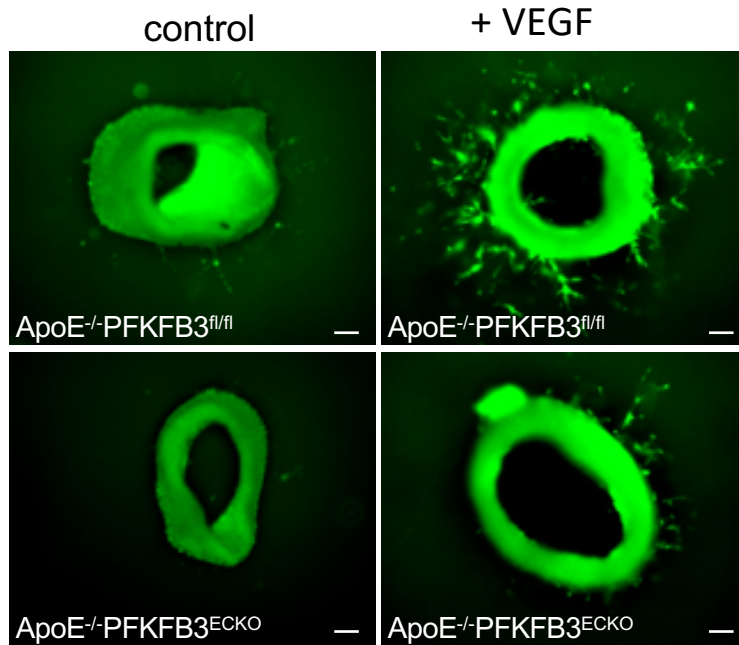[3] Antwerp University Hospital, Laboratory Medicine, Belgium

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Figure S1. PFKFB3 deficiency in endothelial cells inhibits aortic sprouting. (A) Aortic rings from ApoE$^{-/-}$ PFKFB3$^{fl/fl}$ and ApoE$^{-/-}$PFKFB3^{ECKO} mice were embedded in collagen type I and treated with Opti-MEM supplemented with 2.5% FBS (control) in the presence or absence of vascular endothelial growth factor (VEGF, 50 ng/mL). At day 6, rings were fixed and stained to delineate endothelial cells. Images of ring sprouting were obtained and quantified. Scale bar = 500 μm. (B) Sprouts were quantified, n=4 mice. ***P<0.0001 Two-way ANOVA, effect of PFKFB3^{ECKO}: **P<0.005, effect of VEGF: ***P<0.0001.
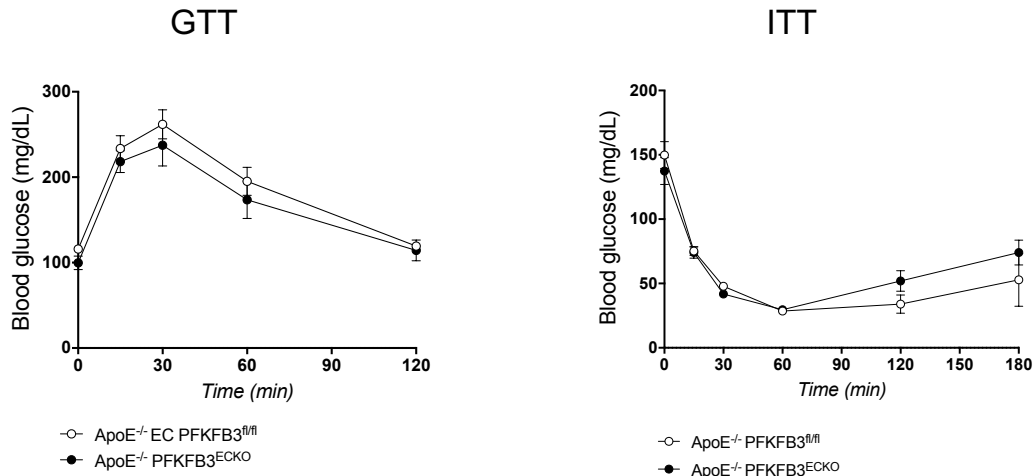
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GTT

ITT

Figure S2. Glucose clearance in ApoE^{-/-}PFKFB3^{fl/fl} and ApoE^{-/-}PFKFB3[ECKO] mice after intraperitoneal injection with glucose or insulin. A glucose tolerance test (GTT) and insulin tolerance test (ITT) was performed after 12 weeks of western diet. Blood glucose was measured at different time points after injection of glucose (1 g/kg, i.p.) or insulin (1 U/kg, i.p.).
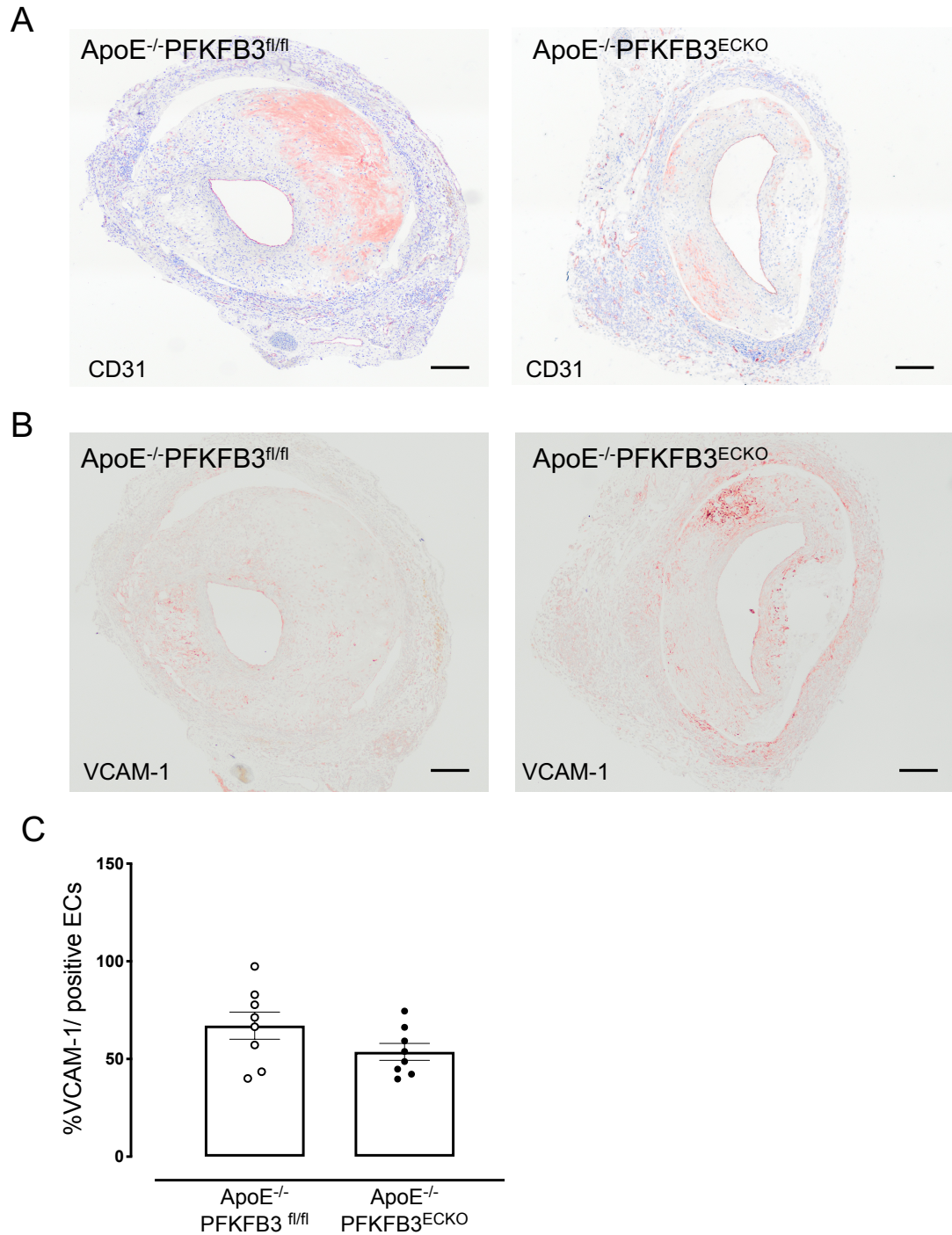
Figure S3. PFKFB3 deficiency in endothelial cells does not influence vascular cell adhesion molecule-1 (VCAM-1) expression in vein graft lesions. (A-B) Immunohistochemical detection of endothelial cells in representative vein graft lesions of ApoE$^{-/-}$PFKFB3$^{fl/fl}$ and ApoE$^{-/-}$PFKFB3^{ECKO} mice using CD31 and VCAM-1 antibody. Scale bar = 200 μm. (C) Quantification of VCAM-1 positive endothelial cells in vein graft lesions of ApoE$^{-/-}$PFKFB3$^{fl/fl}$ and ApoE$^{-/-}$PFKFB3^{ECKO} mice (Independent samples t test, n=8).