SUPPLEMENTAL MATERIAL

Muto et al., http://www.jem.org/cgi/content/full/jem.20101854/DC1

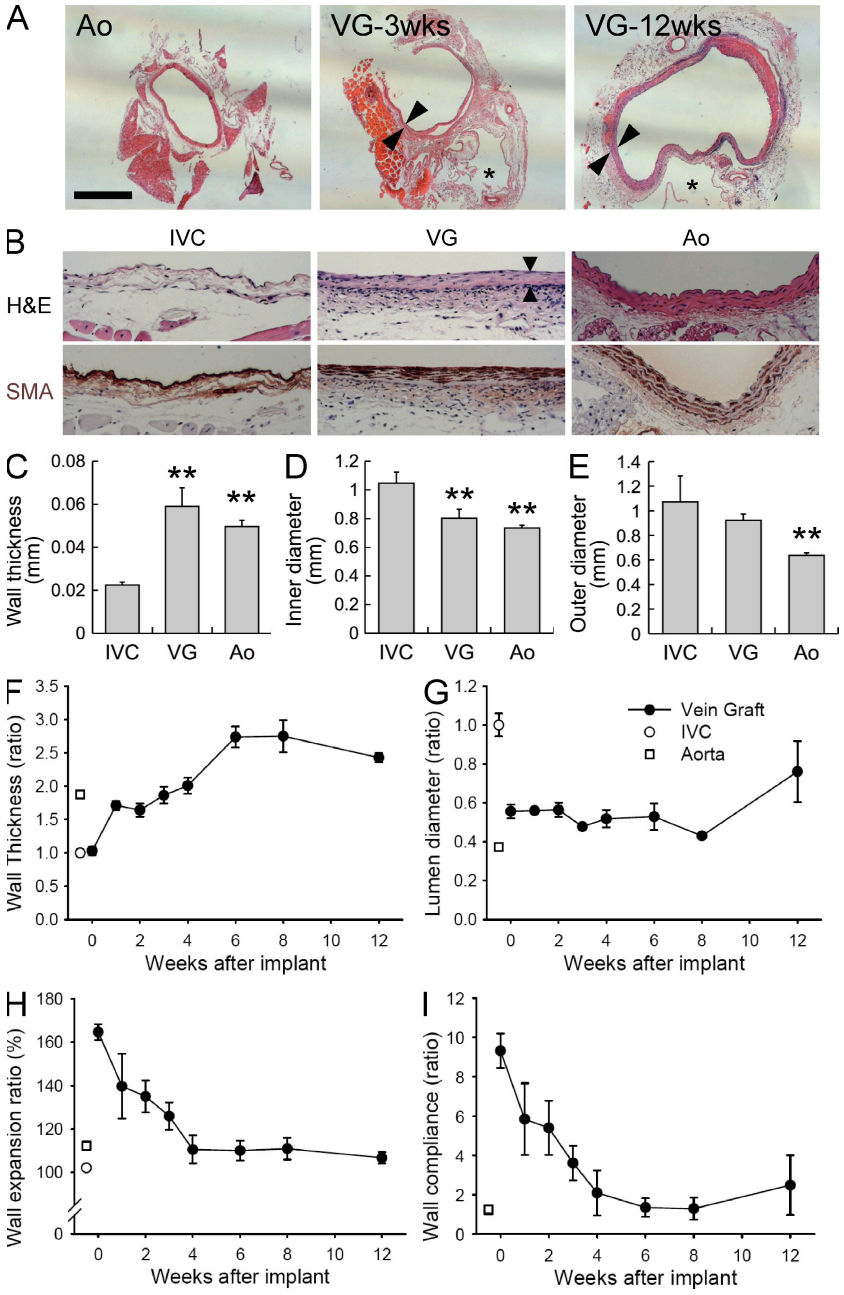


**Figure S1. Morphological and physiological changes during vein graft adaptation in the mouse model.** (A and B) Histology of vein graft adaptation, H&E stained. *n* = 6. (A) Low power magnification of aorta (Ao), vein graft (VG) excised after 3 wk, and vein graft excised after 12 wk. *, inferior vena cava (IVC). Arrowheads show thickened VG wall. (B) High-power magnification of inferior vena cava, vein graft, and Ao, all at 3 wk. Arrowheads show thickened vein graft wall. (C–E) Vein, vein graft, and aorta morphology at 3 wk, showing vessel wall thickness (C), inner diameter (D), and outer diameter (E). **, $P < 0.01$. Error bars denote SEM. (F–I) Serial measurements of vessels using ultrasound, showing vessel wall thickening (F), lumen diameter (G), relative expansion ratio (H), and relative compliance ratio (I). Six mice were followed; one mouse died at 11 wk as a result of spontaneous graft rupture. Error bars denote SEM.

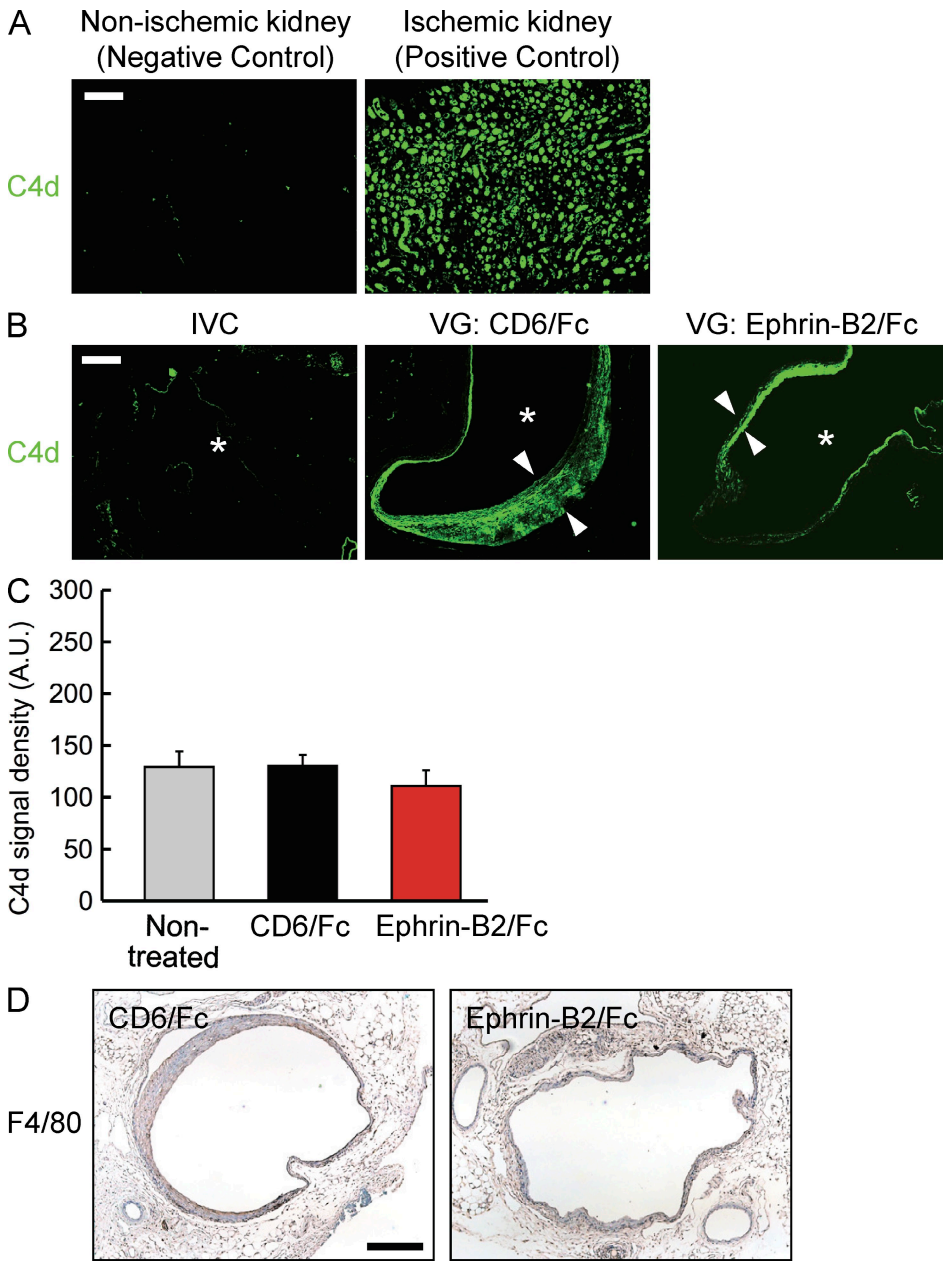


**Figure S2. Lack of excessive complement activation or macrophage accumulation in vein grafts derived from Ephrin-B2/Fc–treated mice.** (A) Expression of C4d in ischemic kidney tissue. *n* = 5–7. Bar, 50 µm. (B) Expression of C4d in veins or vein grafts treated with control CD6/Fc or Ephrin-B2/Fc. *n* = 5–7. Arrowheads show vein graft wall. *, lumen. Bar, 50 µm. (C) Densitometry analysis of C4d signal in untreated vein grafts or vein grafts treated with control CD6/Fc or Ephrin-B2/Fc. *n* = 5–7. Error bars denote SEM. (D) Expression of F4/80 in vein grafts treated with control CD6/Fc or Ephrin-B2/Fc. *n* = 5–7. Bar, 100 µm.

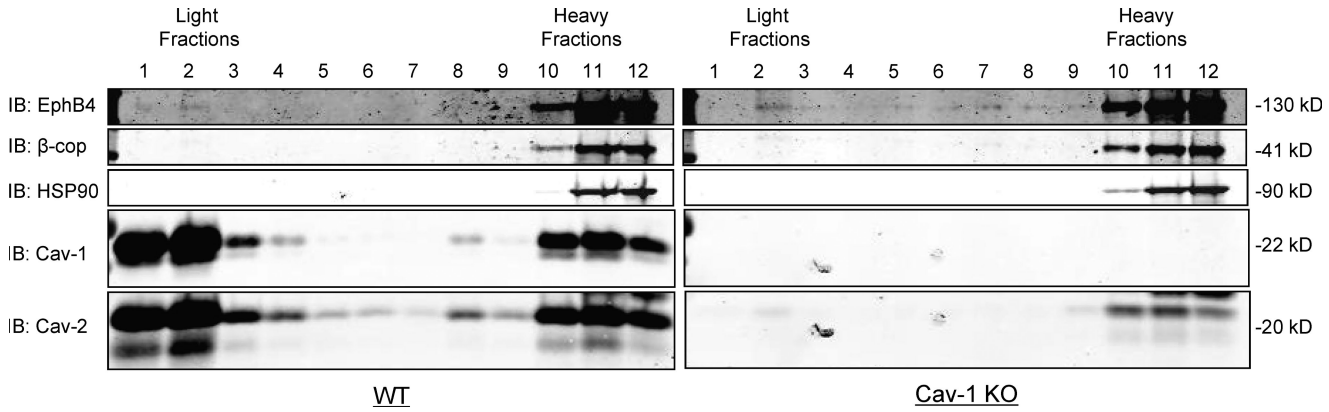


**Figure S3. Cav-1 is colocalized with Eph-B4 in vivo.** Sucrose gradient analysis of lungs isolated from WT or Cav-1 KO mice. *n* = 3.

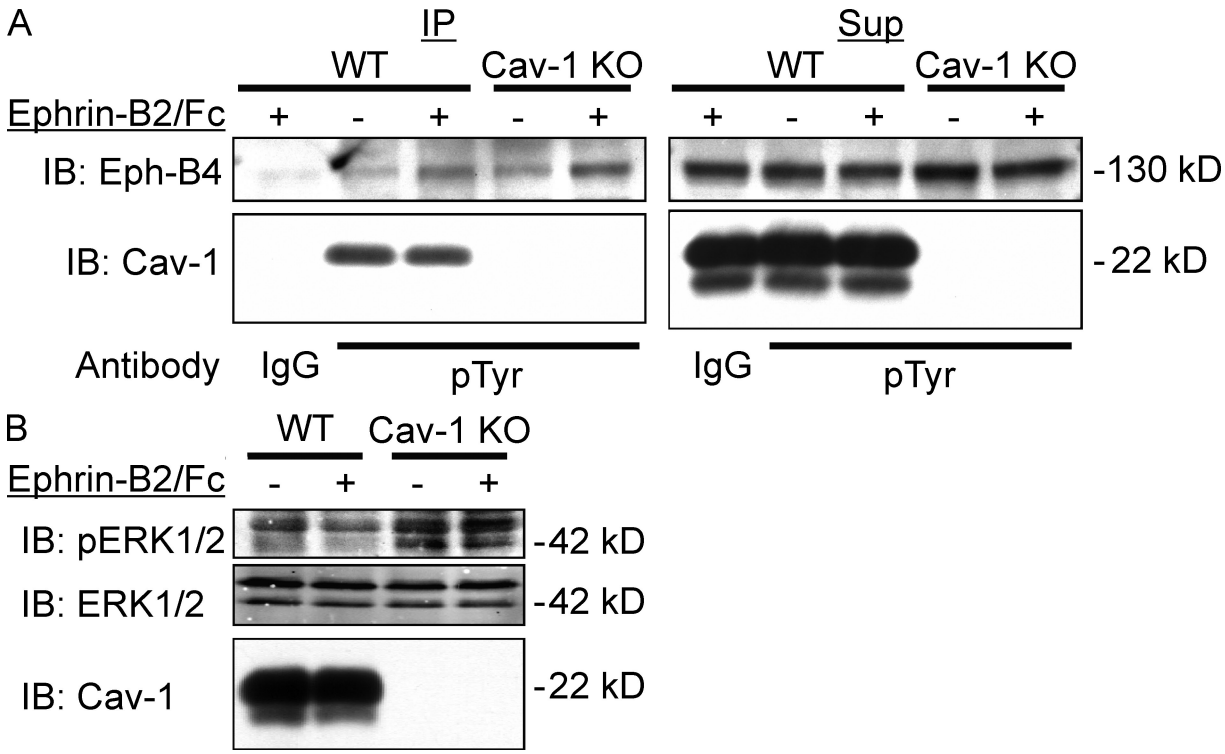


**Figure S4. Cav-1 is not critical for Eph-B4 tyrosine phosphorylation but is important for Eph-B4 downstream signaling.** (A) Eph-B4 tyrosine phosphorylation in response to Ephrin-B2/Fc, in both WT and Cav-1 KO–derived EC. *n* = 3. (B) ERK1/2 dephosphorylation in response to Ephrin-B2/Fc, in both WT and Cav-1 KO–derived EC. *n* = 3.

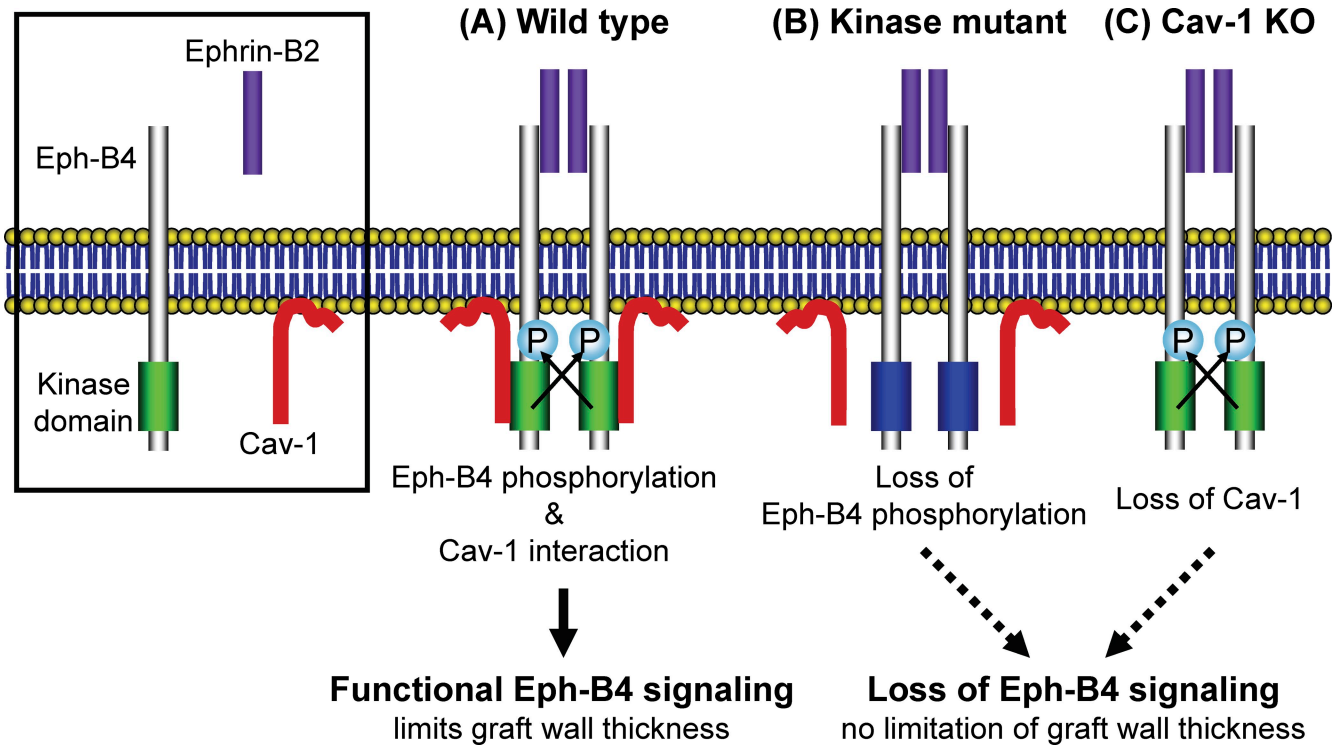


**Figure S5. Schematic model of Cav-1 mediation of Eph-B4 downstream signaling during vein graft adaptation.** (A) Ephrin-B2 ligand stimulates Eph-B4 phosphorylation and association with Cav-1, ultimately resulting in functional signal transduction and Eph-B4–dependent limitation of venous wall thickness. (B) In the mutant Eph-B4 receptor there is no tyrosine phosphorylation. (C) In the Cav-1-KO mouse, there is no association of Cav-1 with phosphorylated Eph-B4. In both B and C, the lack of functional Eph-B4 signaling prevents limitation of venous wall thickness, with consequent development of thick walls (Figs. 4 and 9), during vein graft adaptation.