# Supporting Information

**Supporting Information Corrected May 05, 2014**

**Chen et al. 10.1073/pnas.1404545111**

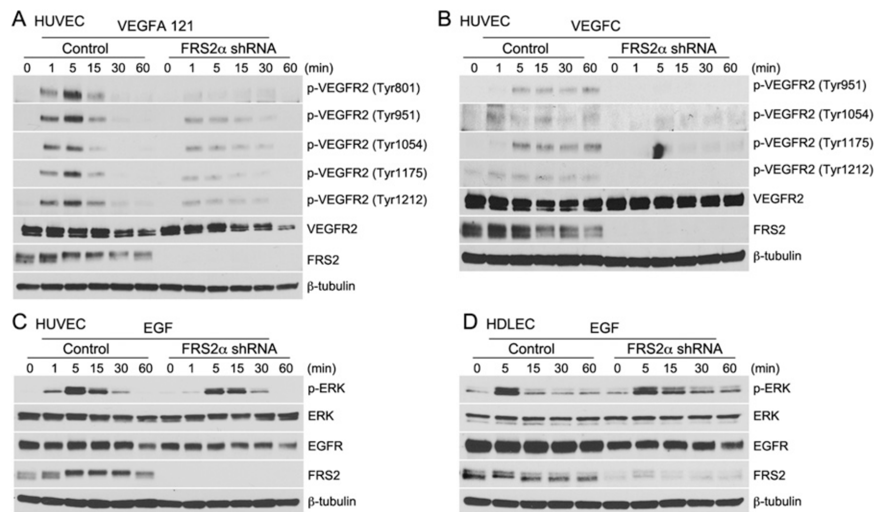


**Fig. S1.** FRS2α knockdown in HUVEC inhibits vascular endothelial growth factor (VEGF) $A_{121}$ and VEGF-C-dependent signaling, but does not impair EGF signaling. (*A* and *B*) Control and FRS2α knockdown HUVEC were serum starved overnight and treated with VEGF-$A_{121}$ (50 ng/mL) or VEGF-C (50 ng/mL) for the indicated times. Cell lysates were blotted with p-VEGFR2, VEGFR2, and FRS2α antibodies. (*C* and *D*) Control and FRS2α knockdown HUVEC and HDLEC were serum starved overnight and treated with EGF (50 ng/mL) for the indicated times. Cell lysates were blotted with p-ERK, ERK, EGFR, and FRS2α antibodies.

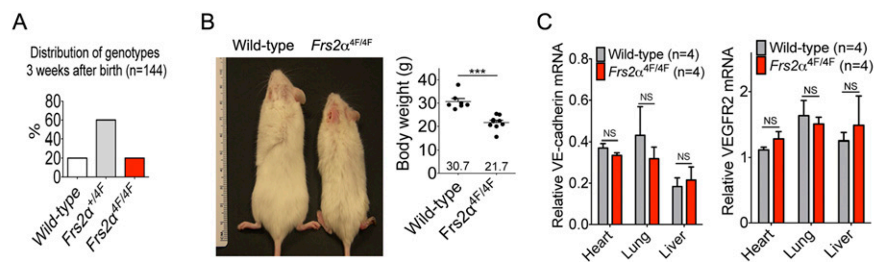


**Fig. S2.** *Frs2*$\alpha^{4F/4F}$ mutant mice have normal baseline vascular density. (*A*) Total genotype distribution from all heterozygous crosses from *Frs2*$\alpha^{+/4F}$ intercrosses: 20% wild-type, 60% *Frs2*$\alpha^{+/4F}$, and 20% *Frs2*$\alpha^{4F/4F}$. (*B*) Body weight analysis of wild-type ($n = 6$, average weight = 30.7 g) and *Frs2*$\alpha^{4F/4F}$ ($n = 8$; average weight = 21.7 g) mice. ***$P < 0.001$. (*C*) Quantitative real-time PCR quantitation of endothelial cell marker gene expression in primary mouse heart, lung, and liver EC isolated from wild-type and *Frs2*$\alpha^{4F/4F}$ mice (NS, not significant compared with control). β-actin was used to normalize the variability in template loading.

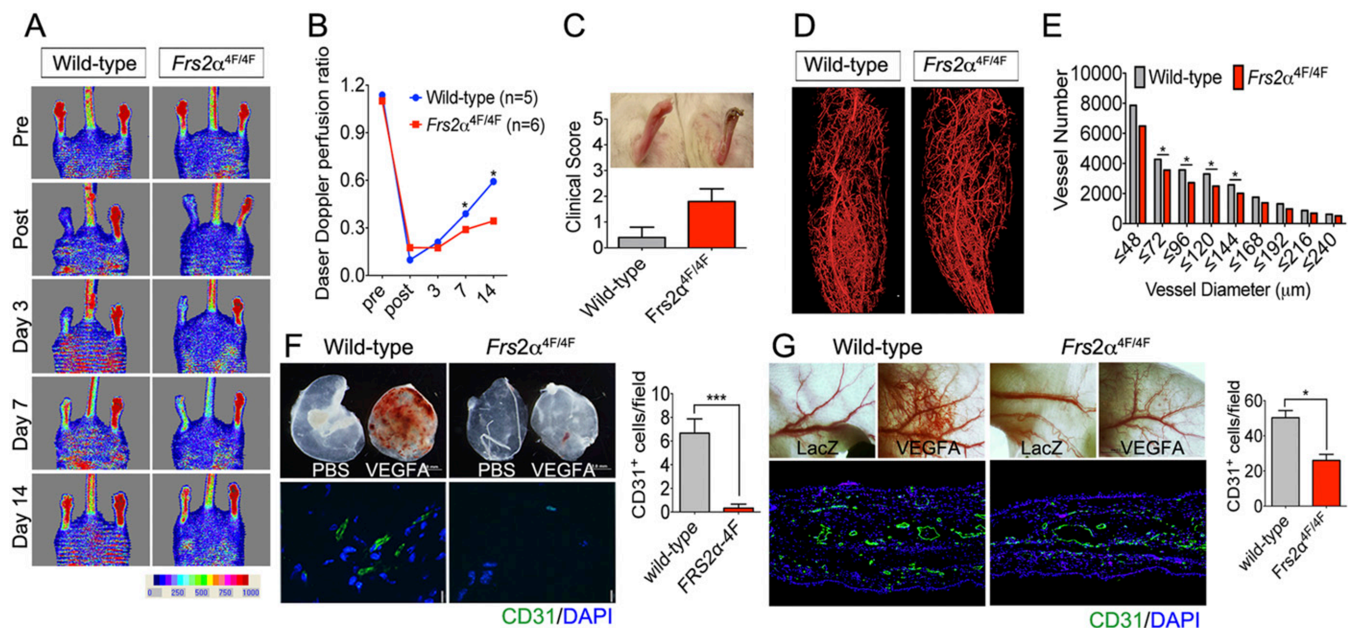


**Fig. S3.** *Frs2α$^{4F/4F}$* mutant mice have arteriogenesis and angiogenesis defects. (*A*) Laser-Doppler images showing blood flow before and after the induction of ischemia to the left hindlimb in wild-type ($n$ = 5) and *Frs2α$^{4F/4F}$* ($n$ = 6) mice. (*B*) Laser-Doppler analysis of blood flow recovery in the left foot, expressed as a ratio of blood flow in left to right foot (L/R). *$P < 0.05$, wild-type vs. *Frs2α$^{4F/4F}$*. (*C*) In *Frs2α$^{4F/4F}$* mice, clinical score indicated a severe phenotype, leading to necrosis of limb. (*D*) Representative reconstructed micro-CT images of ischemic legs (16 μm resolution; $n$ = 3) from wild-type and *Frs2α$^{4F/4F}$* mice. (*E*) Quantitative analysis of micro-CT images in the calf, presented as total number of vascular structures in 250 *z*-axis slices ($n$ = 3 per group). Data are mean ± SEM. (*F*) Matrigel mixed with either PBS or VEGF-$A_{165}$ (50 ng/mL) were placed s.c. in wild-type or *Frs2α$^{4F/4F}$* mice. On day 7, matrigel plugs were sectioned, and the number of vessels was counted (***$P < 0.001$ compared with control) ($n$ = 3 mice per group). (*G*) Wild-type and *Frs2α$^{4F/4F}$* mice were treated with $1 \times 10^9$ pfu of Ad-LacZ or Ad-VEGF-$A_{164}$ virus. VEGF-A–induced angiogenesis was recorded at day 7 by using a stereomicroscope and fluorescent scope. On day 7, mouse ears were sectioned, and the number of vessels was counted (*$P < 0.05$ compared with control) ($n$ = 3 mice per group).