Zhao et al., http://www.jcb.org/cgi/content/full/jcb.200912094/DC1

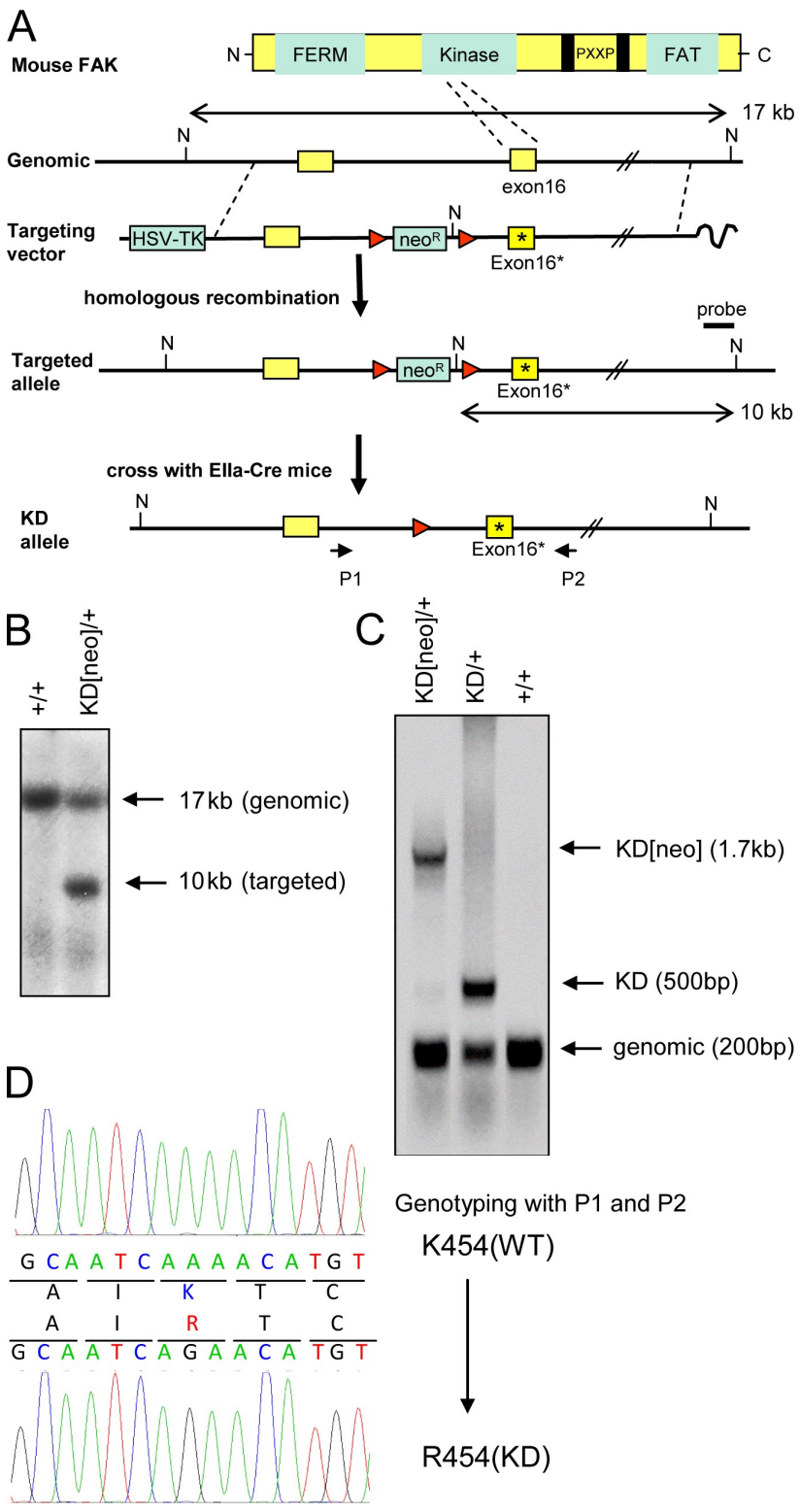

Figure S1. **Generation of the KD FAK knockin allele.** (A) Schematic of mouse FAK protein, targeting vector, and the genomic and targeted allele in FAK loci. Large closed triangles represent loxP sites. The K454 to R mutation (i.e., KD) in the knockin allele is in exon 16 (marked by asterisks). Horizontal lines with arrows on both sides indicate the expected sizes of DNA bands in Southern blotting analysis of the genomic or targeted alleles as detected by the probe (thick horizontal bar) just 3′ end downstream from the targeting vector. Crosses of the mice with targeted allele to Ella-Cre mice result in the deletion of the floxed neomycin cassette to create the FAK KD knockin allele. The relevant restriction sites (NheI [N]) and primers (P1 and P2) for PCR genotyping are indicated. (B) Southern blotting analysis of the tail DNA from representative mice after digestion with NheI. (C) PCR genotyping of the tail DNA from representative mice, using primers P1 and P2 as indicated in A. (D) The PCR fragments corresponding to the wild-type (WT) and KD FAK that were amplified in C were sequenced to confirm the mutation of the codon for K454 to R.

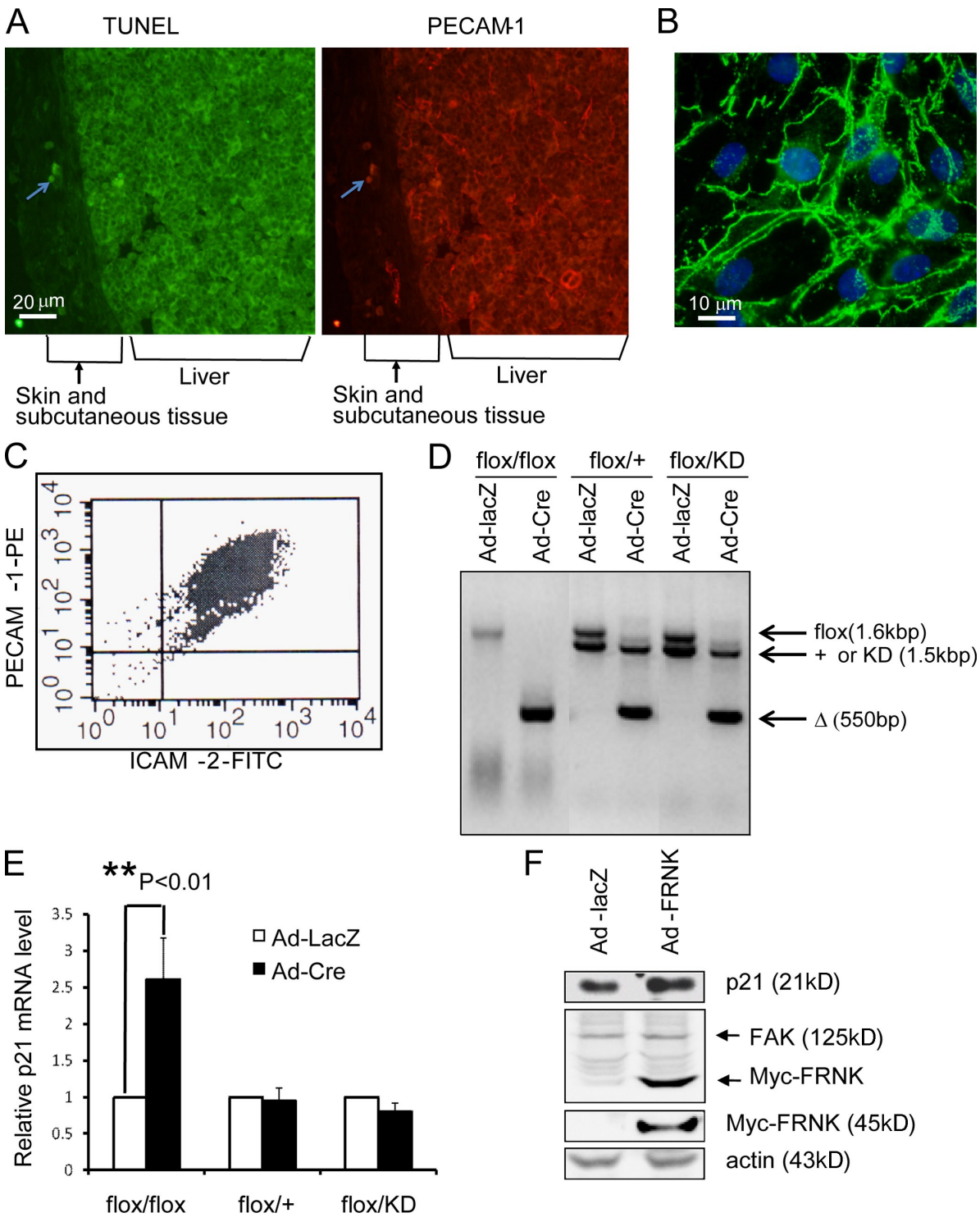

Figure S2. **Analysis of embryonic liver sections in vivo and preparation and analysis of primary ECs in vitro.** (A) Liver sections of CFKO embryos at E13.5 were subjected to TUNEL analysis (left) followed by immunofluorescent staining using anti–PECAM-1. Note the high nonspecific background signal in the liver. Arrows mark possible apoptotic EC in the skin area. (B and C) Primary ECs were isolated as described previously (Shen et al., 2005). They were analyzed by staining with VE-cadherin (green) and Hoechst (blue; B) or flow cytometry using PECAM-1–phycoerythrin (PE) and ICAM-2–FITC (C) to verify their EC natures. (D and E) Primary ECs from $FAK^{flox/flox}$, $FAK^{+/flox}$, and $FAK^{flox/KD}$ mice were infected with Ad-Cre to delete the floxed FAK allele or Ad-lacZ as a control, as indicated. Genomic DNA was then analyzed by PCR. The expected positions of flox, +, and deleted alleles are marked on the right (D). Total RNAs were extracted for analysis of p21 mRNA levels by quantitative RT-PCR. The mean ± SEM of relative values of three experiments (normalized to lacZ-infected cells) is shown (E). (F) Primary ECs from control mice were infected with Ad-lacZ or Ad-FRNK, as indicated. Lysates from the infected cells were analyzed by Western blotting using antibodies against p21 (top), FAK (detecting both FAK and the Myc-tagged FRNK as marked by arrows on the right), Myc (for Myc-FRNK), or actin, as indicated.

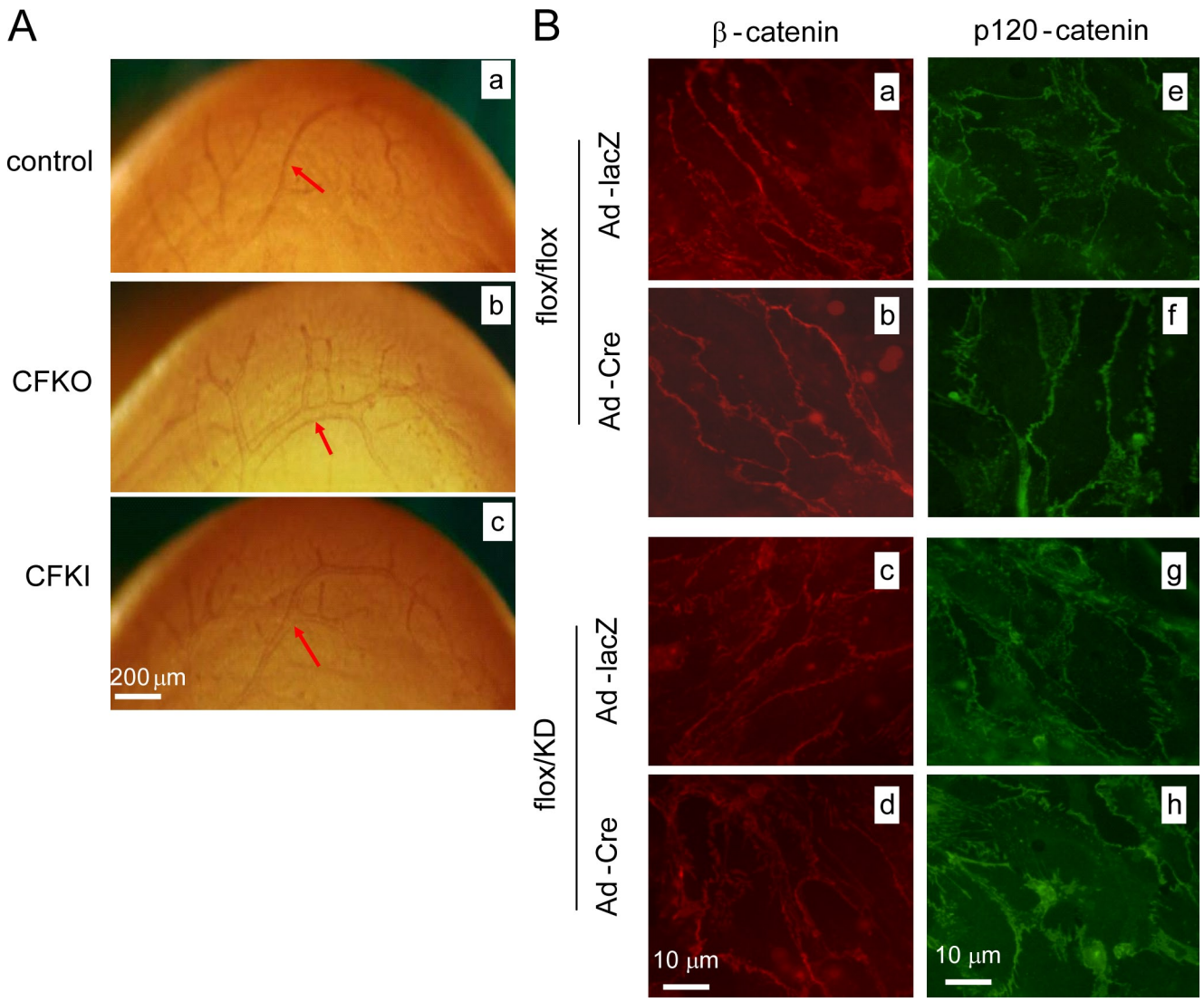

Figure S3. **Whole-mount analysis of embryos and immunofluorescent staining of isolated primary ECs.** (A) Superficial vasculatures in control, CFKO, and CFKI embryos at E12.5 were visualized by staining of whole mount with anti–PECAM-1. Note the apparently different size of the vessels in similar areas of the embryos (arrows). (B) Primary ECs from *FAK*$^{flox/flox}$ and *FAK*$^{flox/KD}$ mice were infected with Ad-Cre to delete the floxed FAK allele or Ad-lacZ as a control, as indicated. The cells were then subjected to immunofluorescent staining using anti–β-catenin (a–d) or anti–p120-catenin (e–h).

Reference

Shen, T.L., A.Y. Park, A. Alcaraz, X. Peng, I. Jang, P. Koni, R.A. Flavell, H. Gu, and J.L. Guan. 2005. Conditional knockout of focal adhesion kinase in endothelial cells reveals its role in angiogenesis and vascular development in late embryogenesis. *J. Cell Biol.* 169:941–952. doi:10.1083/jcb.200411155