Supplementary Figure Legends:

Sup. Figure 1: **Cerebral AVMs in PN3 mutant mice**. **A, B.** Gross morphology of control (A) and mutant (B) PN3 mouse heads. Irregular and tortuous blood vessels were evident in the surface of mutant heads. **C, D.** Corrosion casts of blood vessels in the control and mutant brains demonstrate multiple AVM lesions in the mutant brains. The inset shows a magnified view of blood vessels in the hippocampus area. Note that the corrosion casting image is remarkably similar with the images by the latex dye injection shown in Fig 1B,D, and E. **E, F.** H&E staining of coronal sections of brains show dilated vessels in subdural areas (arrows) and hemorrhages in the hippocampus area (arrow with an asterisk). Scale bars indicate 2 mm (A-D), 1 mm (E, F), 0.5 mm (D inset), and 250 μm (F inset).

Sup. Figure 2: Pulmonary AVMs in PN3 mutants. A, B. Gross morphology of PN3 lungs. Abnormally dilated and tortuous vessels (arrows) were visible in the mutant lungs (B). **C-F**. Pulmonary vascular morphologies by the latex dye injection via right heart. The dye visualized arterial branches in the control lungs (C, E), whereas it crossed to venous vessels in the mutants (D, F). Boxes in C and D indicate the areas magnified in E and F, respectively. Nidus-like tangled vessels were indicated by red arrows (F). H, heart; LPa, left pulmonary artery; RPa, right pulmonary artery, Pv, pulmonary vein. **G, H.** H&E staining of lung sections demonstrates abnormal vascular braches with severe dilations and signs of fusions between vessels (asterisks). **I, J.** Staining of lung sections with anti-αSMA antibodies outlines pulmonary vessels. Vascular smooth muscle layers in the mutants (J) are thin, irregular and discontinuous indicated by arrows in the inset (J). Br, bronchus; PA, pulmonary artery; PAVM, pulmonary AVM. **K, L.**

Fluorescence microscopic view of Evans Blue dyes in the control (K) and mutant (L) lungs. Arrows indicate the dye found in the airways. Insets show gross morphology of lungs after Evans Blue dye injection as shown in Fig. 1H and I. Scale bars indicate 1 mm (A-D), 300 μm (E, F), 100 μm (I-L), 50 μm (insets in I, J), and 500 μm (insets in K, L)

Sup. Figure 3: Enlarged heart of TM-treated adult *Alk1* mutants. **A.** Heart morphologies of control (*R26*$^{+/+}$;*Alk1*$^{2loxP/2loxP}$) and mutant (*R26*$^{CreER/+}$;*Alk1*$^{2loxP/2loxP}$) mice 8 days after TM treatment show enlarged heart in the *Alk1* mutants. Scale bar indicates 3 mm. **B.** Heart/body weight ratio is significantly increased in the mutant. * $p < 0.0001$.

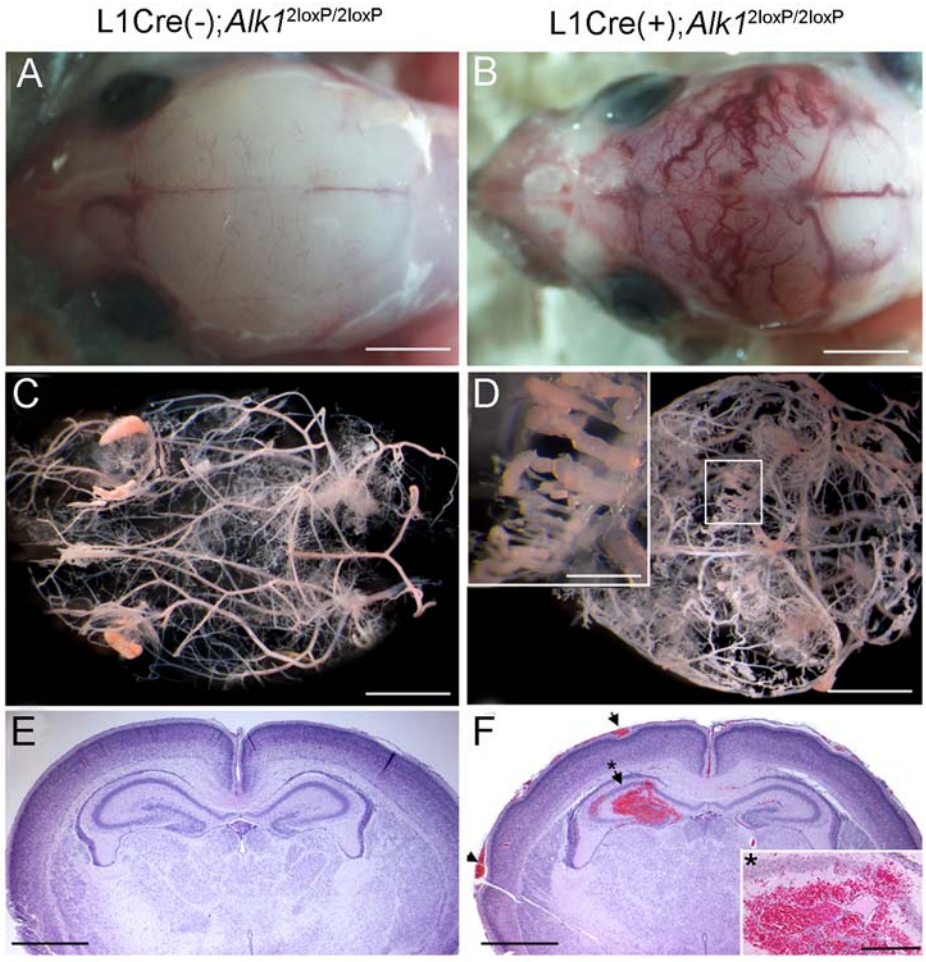
Sup. Figure 4: Hemorrhages and vascular malformations in the lungs and GI tract of TM-treated adult *Alk1* mutants. . **A, B.** Gross morphology of heart and lungs of control and mutant mice 8 days after TM-treatment. Note enlarged mutant heart and lungs and hemorrhagic spots in the mutant lungs (B). **C, D.** Pulmonary arteries are visualized by latex dye injected via right heart. Note enlarged arteries and reduced perfusion to distal microvessels in the mutant lungs. **E, F.** Pulmonary veins (and bronchial arteries) by latex dye injected via left ventricle and left atrium. Photograph of control and mutant lungs was taken side by side. Mutant veins were significantly enlarged and showed roughness along the vessels indicating high permeability. **G, H.** Gross morphology of GI tract. Mutant guts were purplish and displayed signs of hemorrhages. A, appendix. **I-L.** Blood vessels of Peyer's patches in the ilium (I and J) and appendix (K and L) of control (I and K) and mutant (J and L) mice were visualized by the latex dye injection. Dotted circles indicate the represented areas of Peyer's patch. Arrows in J and L

indicate veins containing the latex dye. Scale bars represent 5 mm (A, B, G, H), 500 μm (C-F), and 2 mm (I-L).

Sup. Figure 5: Uterine AVMs in TM-treated adult *Alk1* mutants. Uterine blood vessels of control (*R26*$^{+/+}$;*Alk1*$^{loxP/2loxP}$, A) and mutant (*R26*$^{CreER/+}$;*Alk1*$^{loxP/2loxP}$, B) mice 8 days after TM treatment were visualized by the latex dye injection. Latex dye was confined in the feeding arteries in the controls (A), whereas it was found in both arteries and veins in the mutants (B). Note that latex dye in the venous vessels was appeared as light blue. Scale bars represent 2 mm.

Sup. Figure 6: Overview of the dorsal skin fold chamber and hyperspectral imaging system.

A. A Zeiss microscope connected with digital camera (a), liquid crystal tunable filter (LCTF) for capturing spectral images (b), electron multiplying CCD camera for video recording of blood flow of RBCs labeled with a fluorescence dye (c), connections to a gas anesthetizer (d), fluorescence power lamp (e), and a computer fully equipped with multiple image processing softwares (f). **B.** A mouse installed with the window chamber. **C.** Intravital imaging of blood vessels through the window.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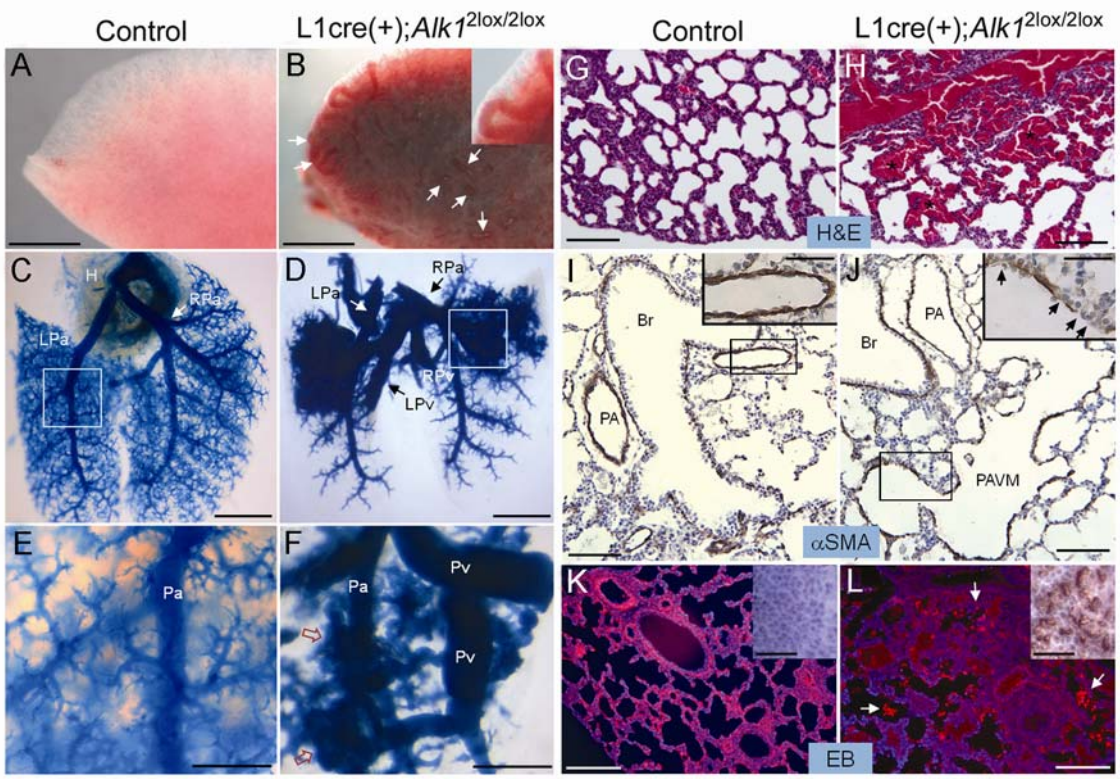
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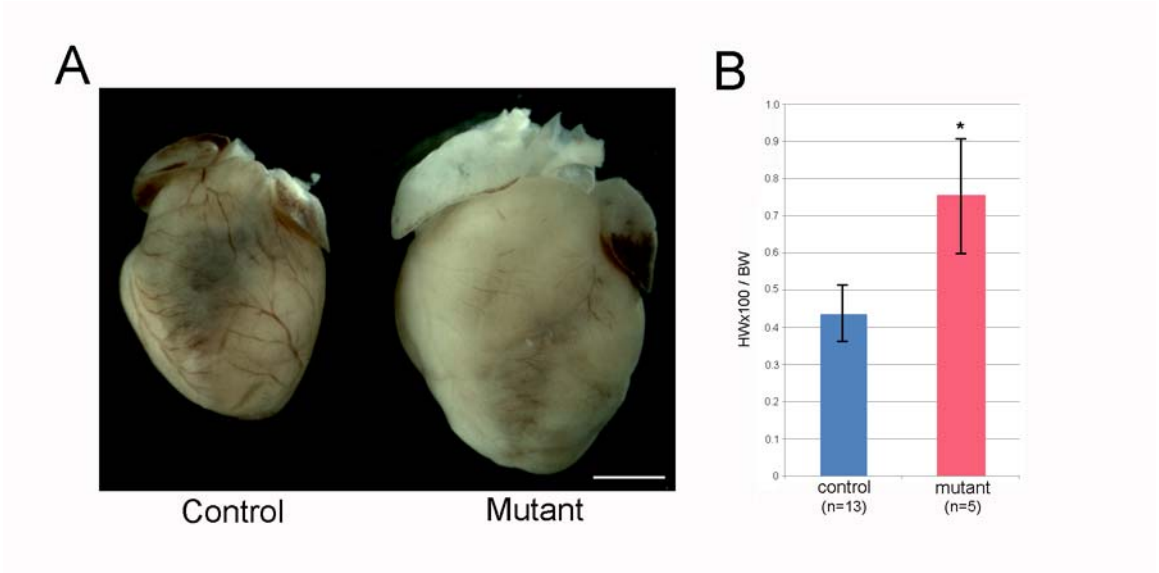
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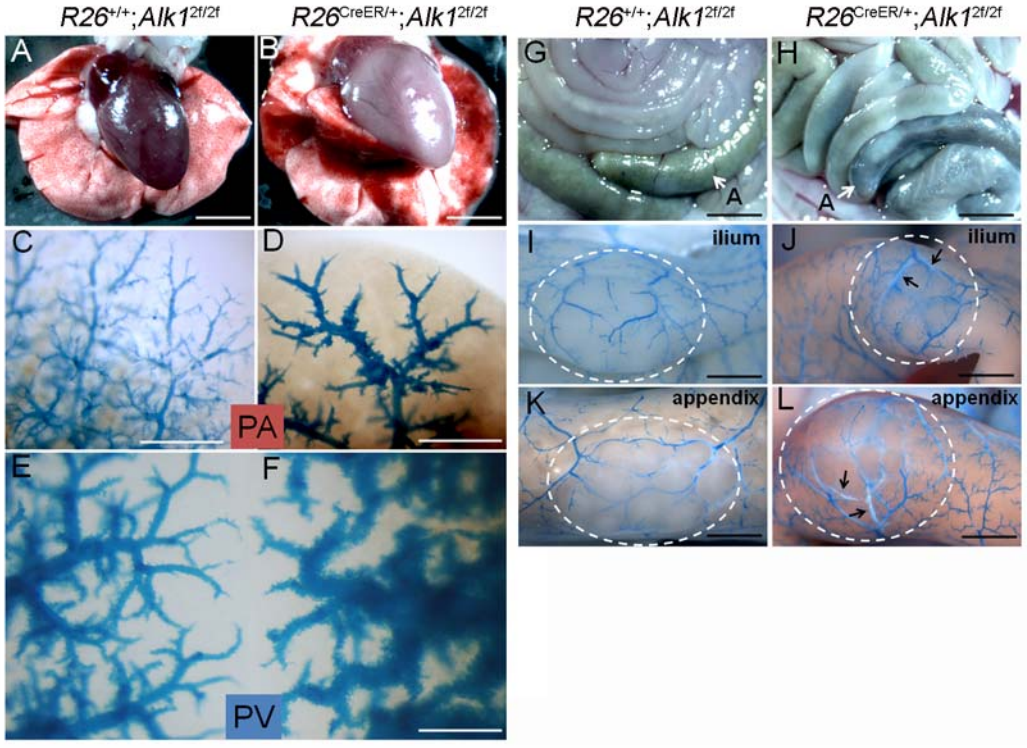
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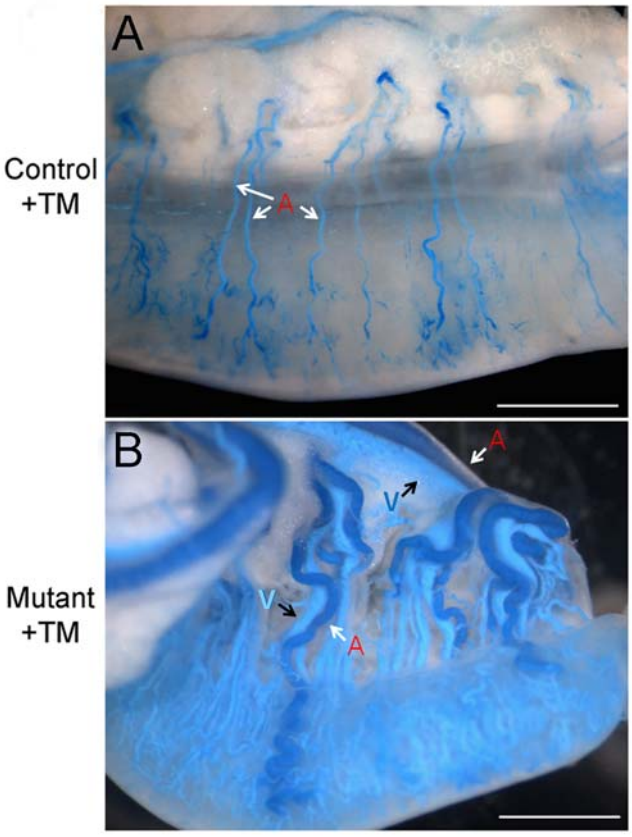
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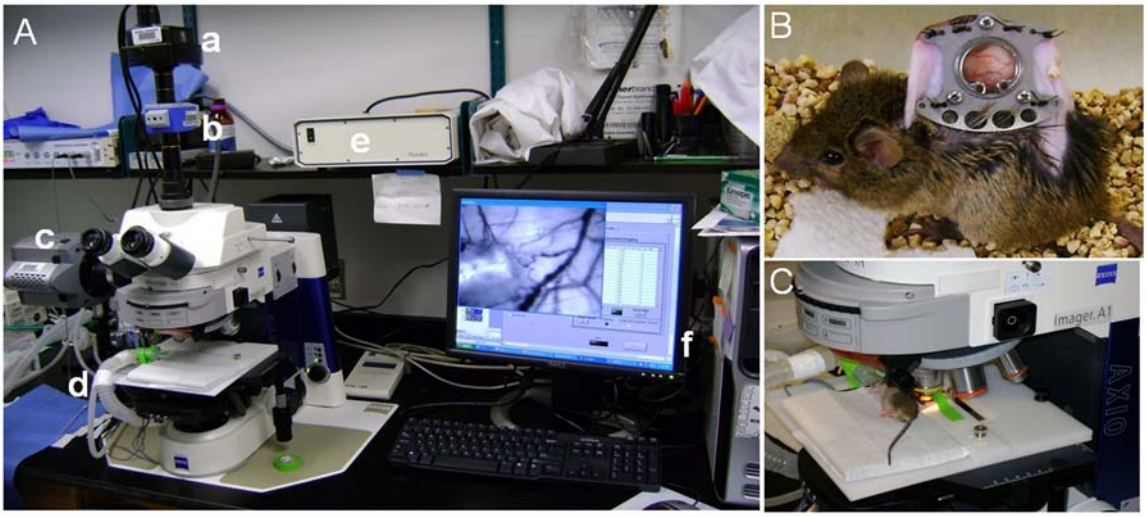
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