

**iScience, Volume 25**

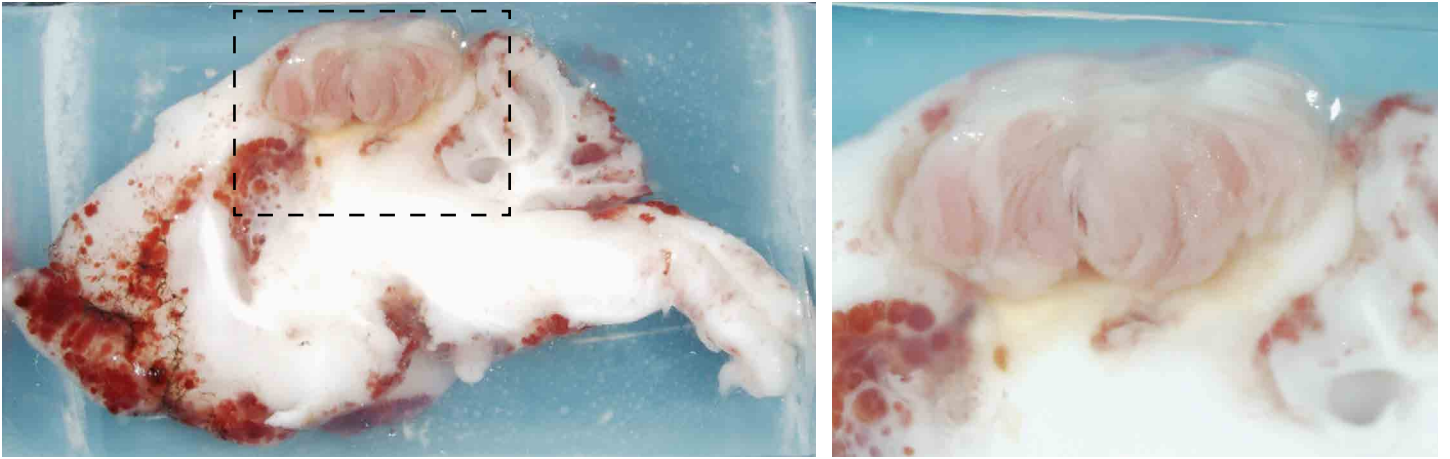
**Supplemental information**

**A murine model of cerebral cavernous  
malformations with acute hemorrhage**

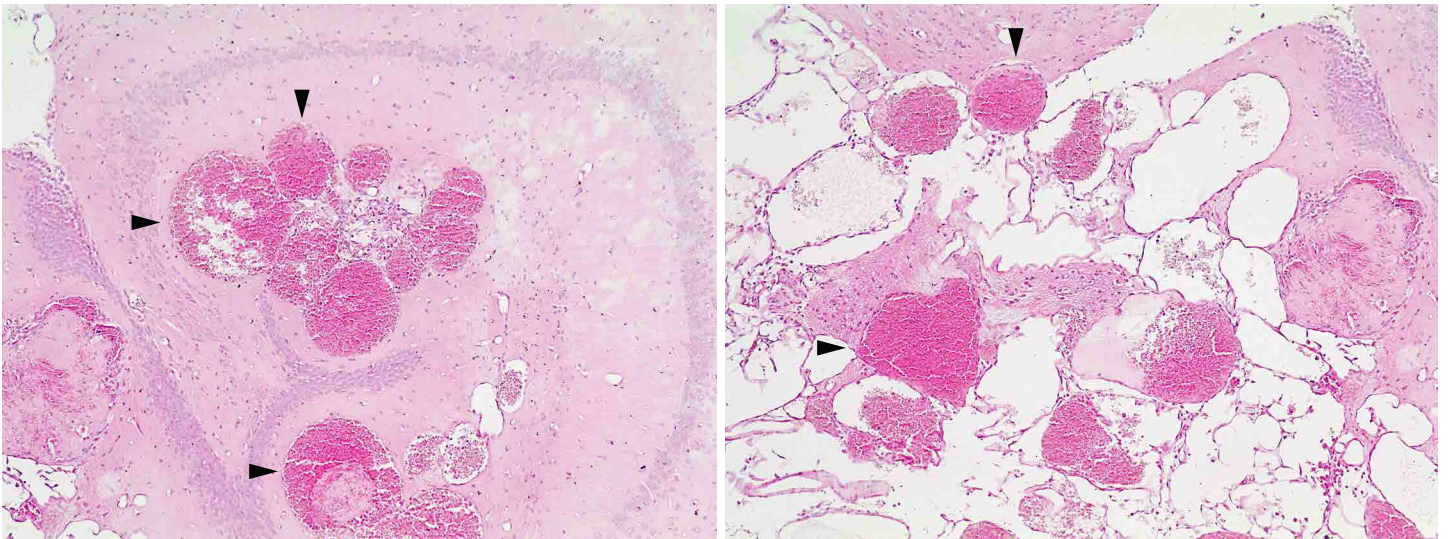
**Claudio Maderna, Federica Pisati, Claudio Tripodo, Elisabetta Dejana, and Matteo Malinverno**

## Supplemental figure 1

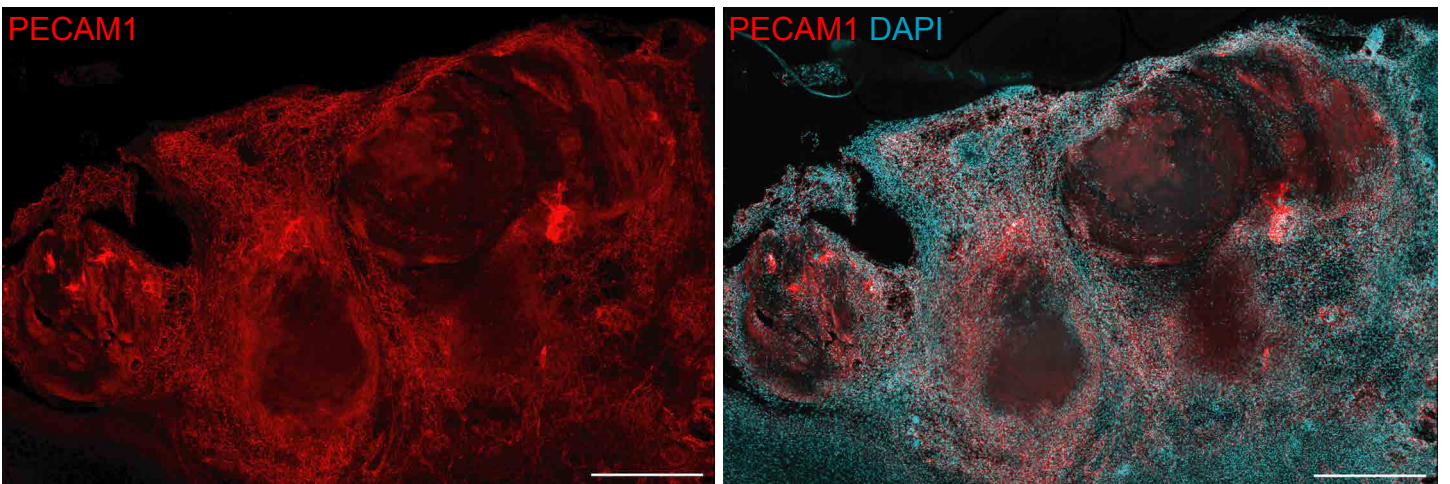
A



B



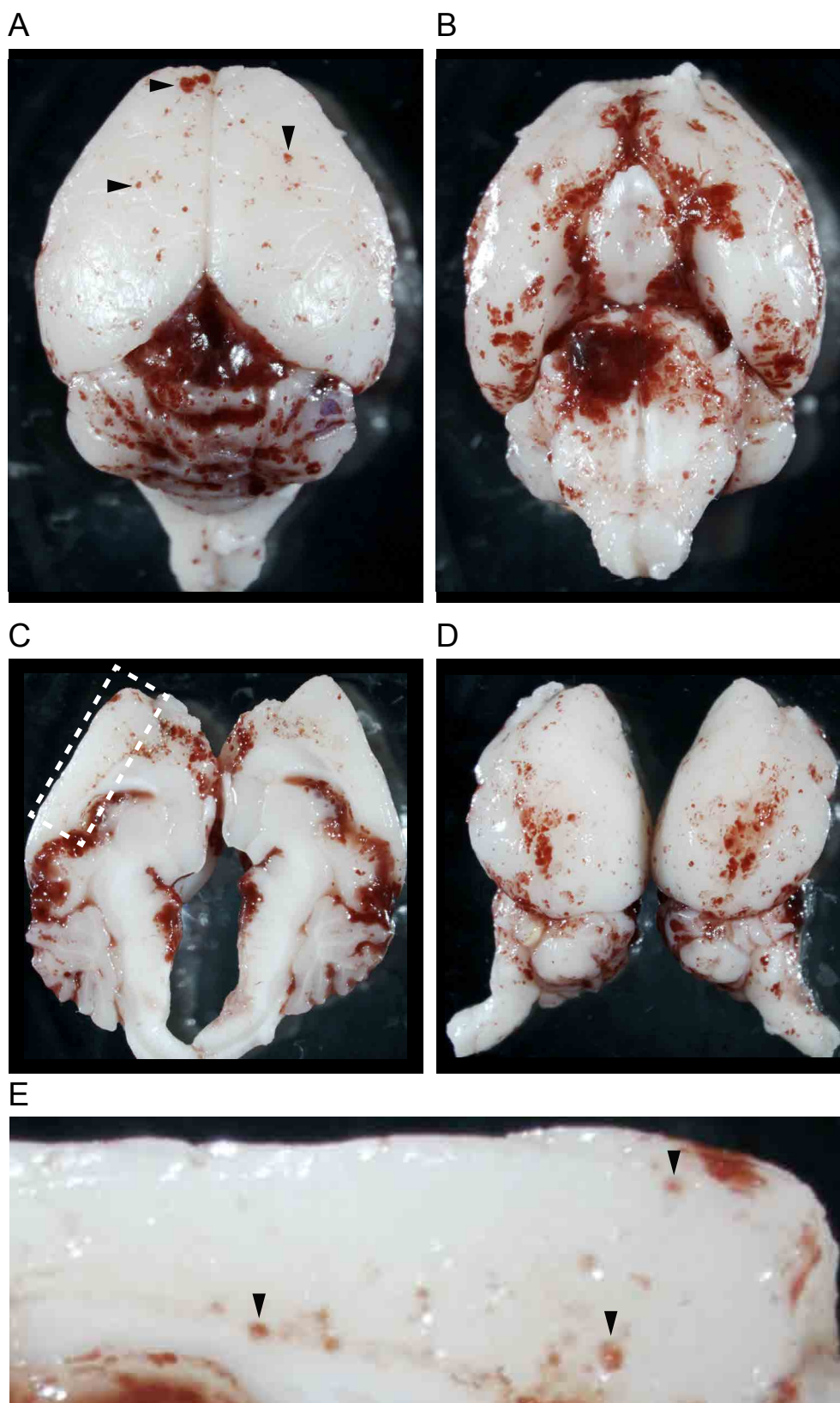
C



**Supplemental figure 1. Giant cavernoma in 6-month-old mouse.** Related to Figure 1.

**A)** Photographs of brain from a 6-month-old *Ccm3*<sup>EPCKO</sup> mouse showing a giant cavernoma in the cortex. **B)** Relative hematoxylin and eosin staining; arrowheads point to multi-lumen blood-filled lesions. **C)** Confocal microscopy image of the cavernoma stained for PECAM1 and DAPI. Scale bars: 500  $\mu$ m.

## Supplemental figure 2

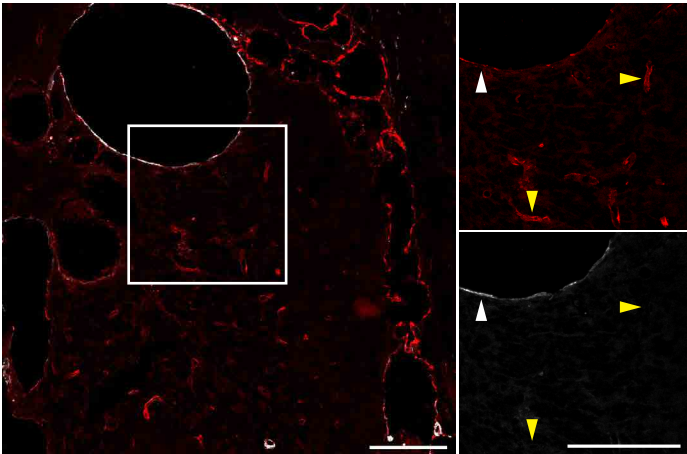
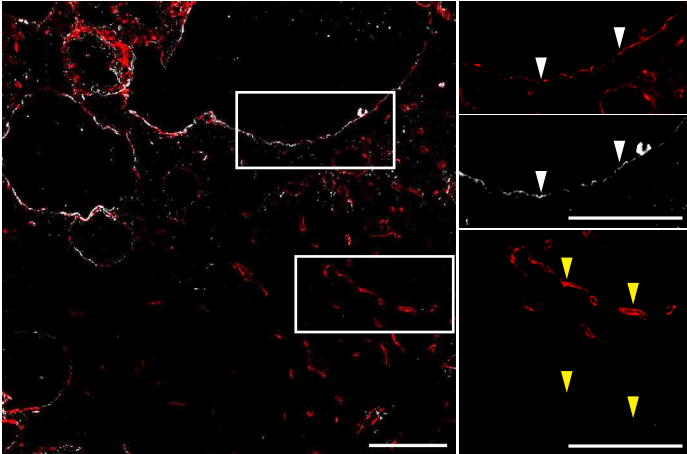
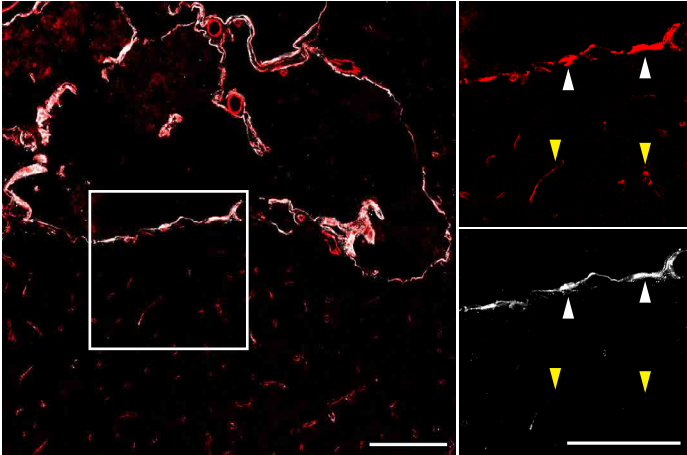
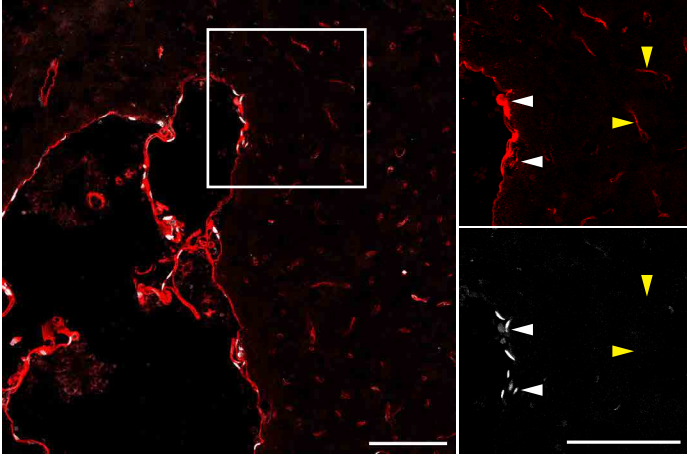


### **Supplemental figure 2. Lesions form throughout the brain. Related to Figure 1.**

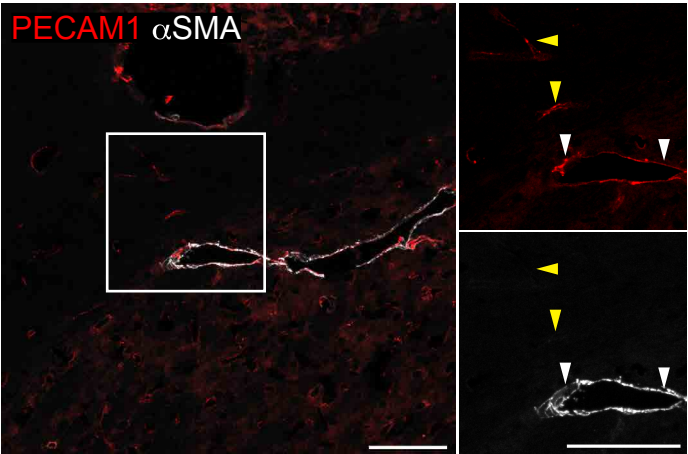
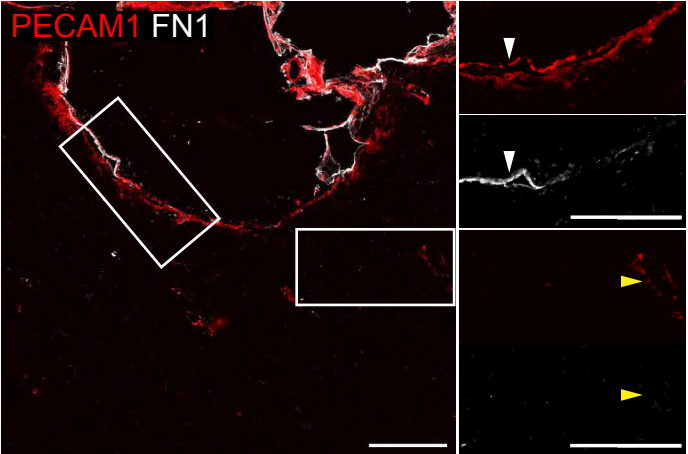
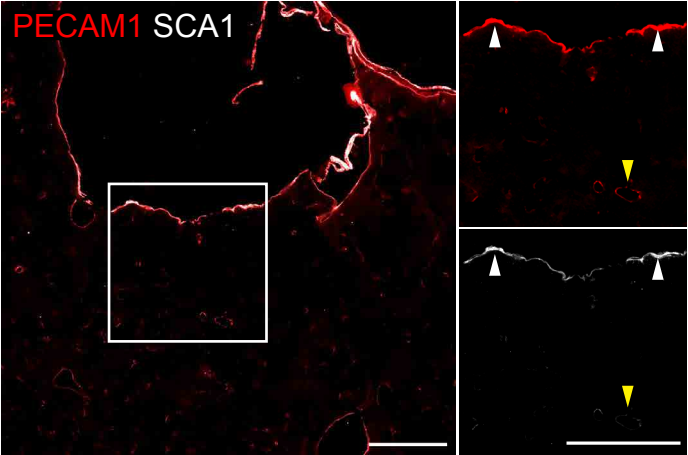
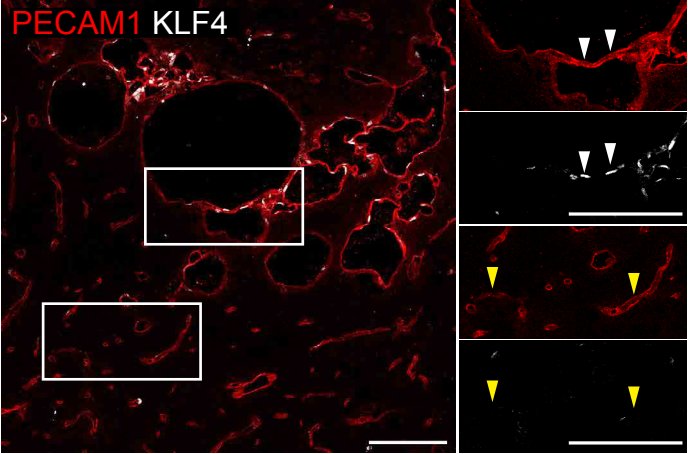
Representative photographs of whole brains from mice analyzed at 90 days. Images show: dorsal **A)** and ventral **B)** views of whole brains; midline **C)** and lateral **D)** sagittal surfaces of hemispheres; **E)** magnification from **C)** showing lesions.

Supplemental figure 3

*Ccm3*<sup>ECKO</sup> - P30



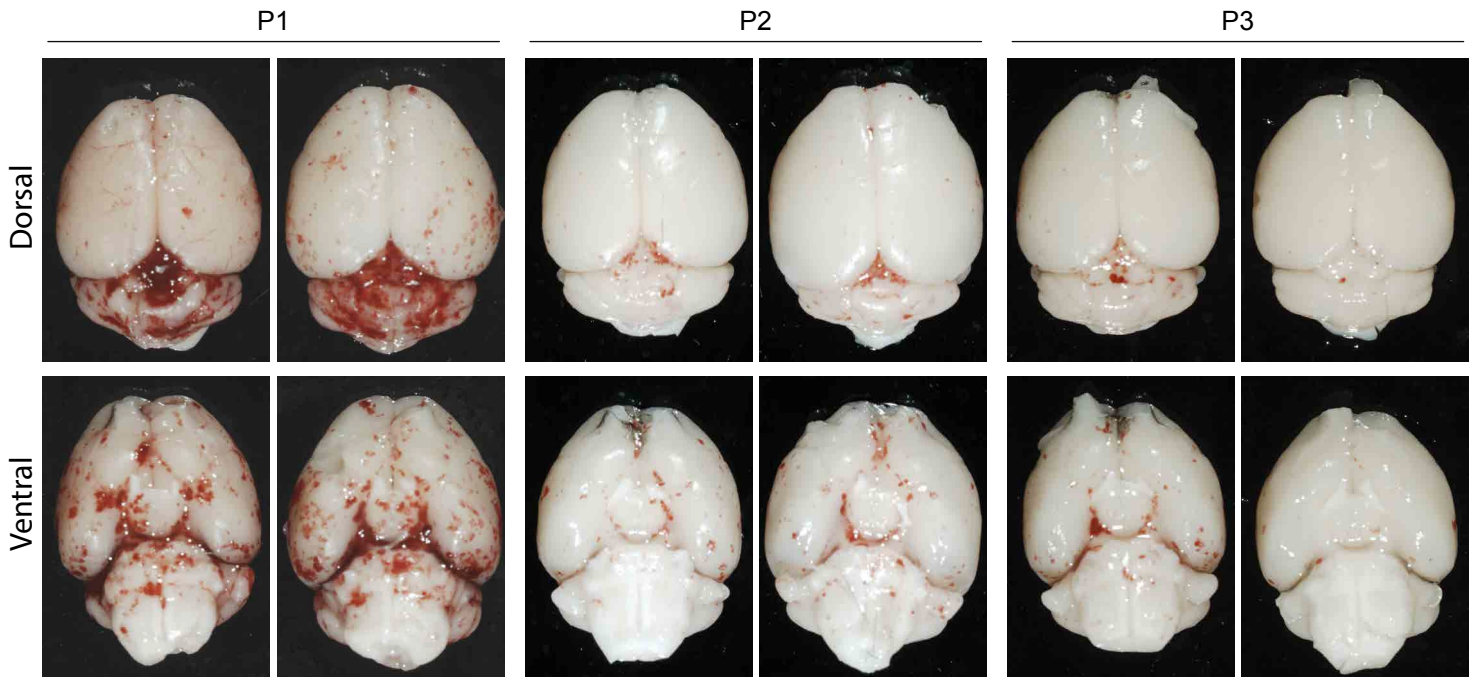
*Ccm3*<sup>EPCKO</sup> - P90



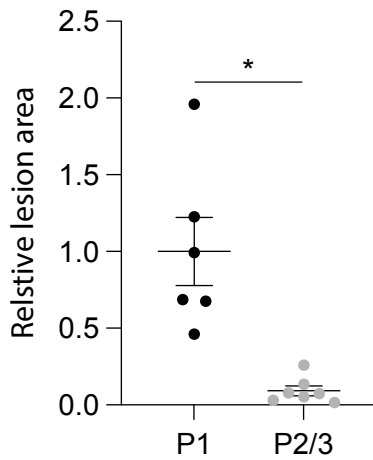
**Supplemental figure 3. ECs lining cavernomas express KLF4 and EndMT genes.** Related to Figure 1. Representative confocal images showing double staining for PECAM1 as endothelial marker (red) and EndMT genes (gray). Images are taken from brains of *Ccm3*<sup>ECKO</sup> mice at P30 and of *Ccm3*<sup>EPCKO</sup> mice at P90. White arrowheads, EC in lesions; yellow arrowheads, EC in normal vessels. Scale bar: 100µm.

# Supplemental figure 4

A



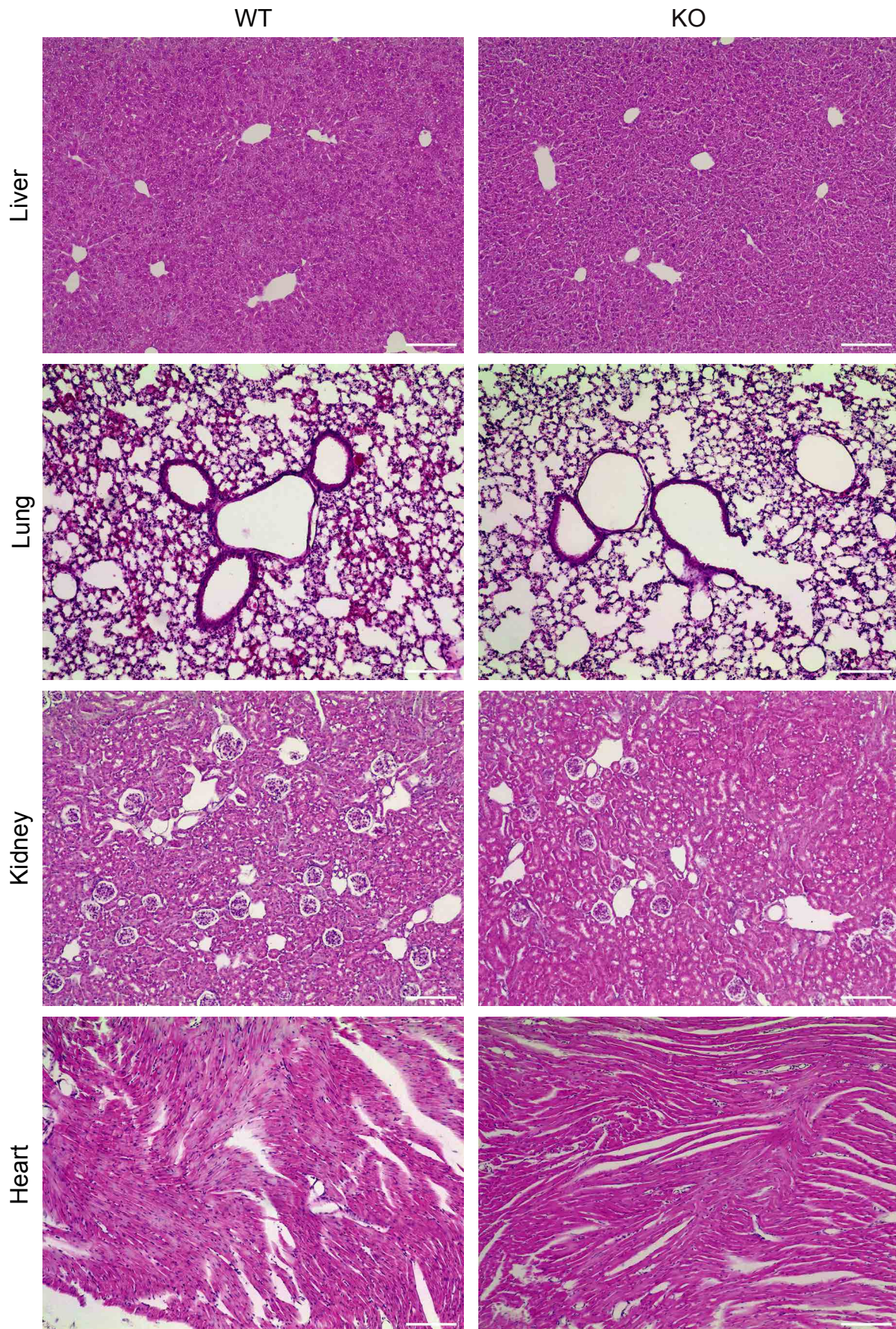
B



**Supplemental figure 4. Delayed tamoxifen administration results in slower disease progression and milder phenotype.** Related to Figure 1.

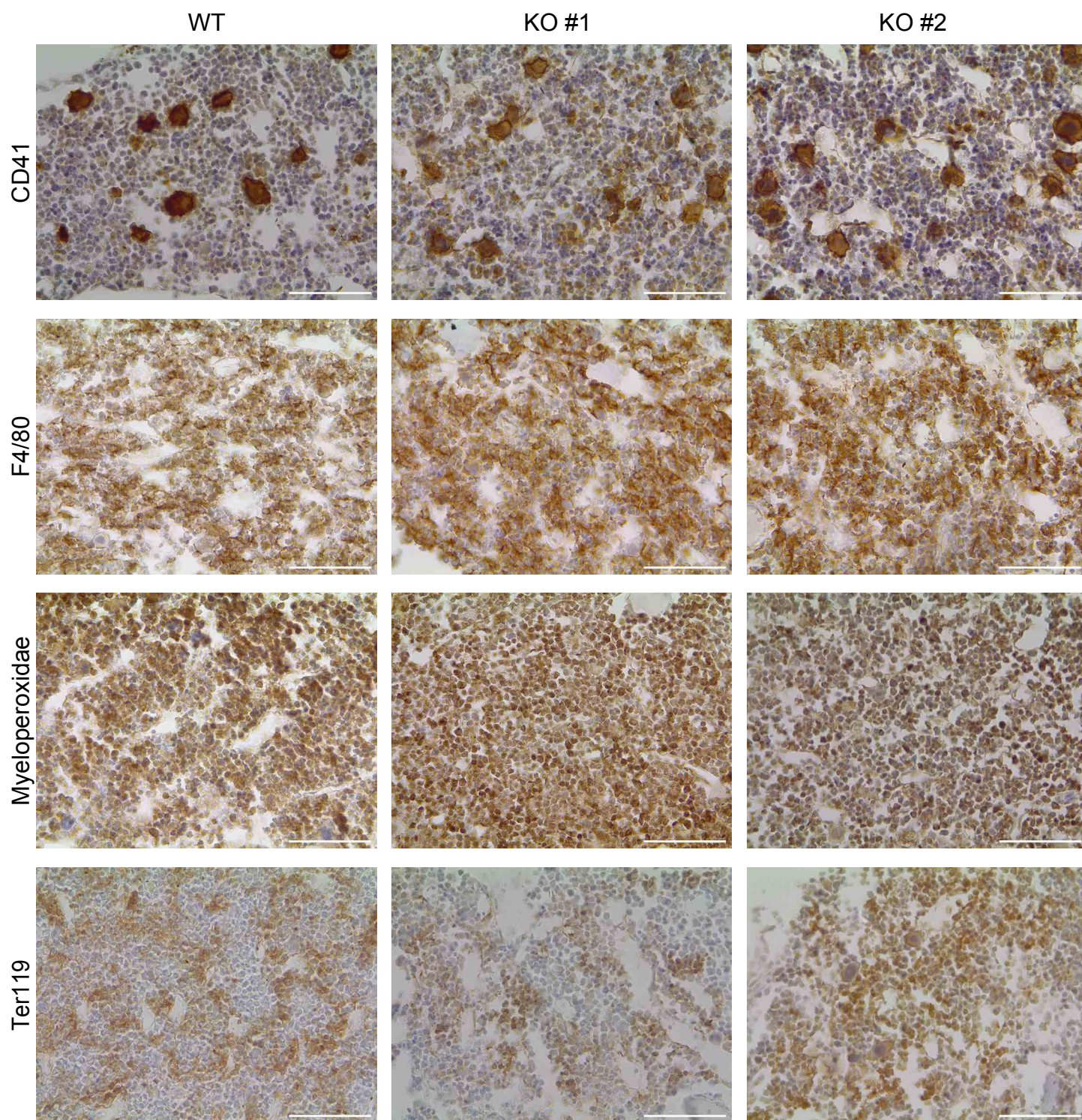
**A)** Representative photographs of whole brains from mice injected with tamoxifen at either P1, P2 or P3 and analyzed at three months of age. **B)** Quantification of total lesioned area normalized on average area of animals injected at P1. Data are means  $\pm$ SE; \* $p < 0.01$  (Student's t-tests).

## Supplemental figure 5



**Supplemental figure 5. Histological analysis of peripheral organs.** Related to figure 4. Representative hematoxylin and eosin staining of liver, lung, kidney, and heart (as indicated) of 3-month-old wild-type (WT) and *Ccm3*<sup>EPCKO</sup> mice. Scale bars: 100  $\mu$ m.

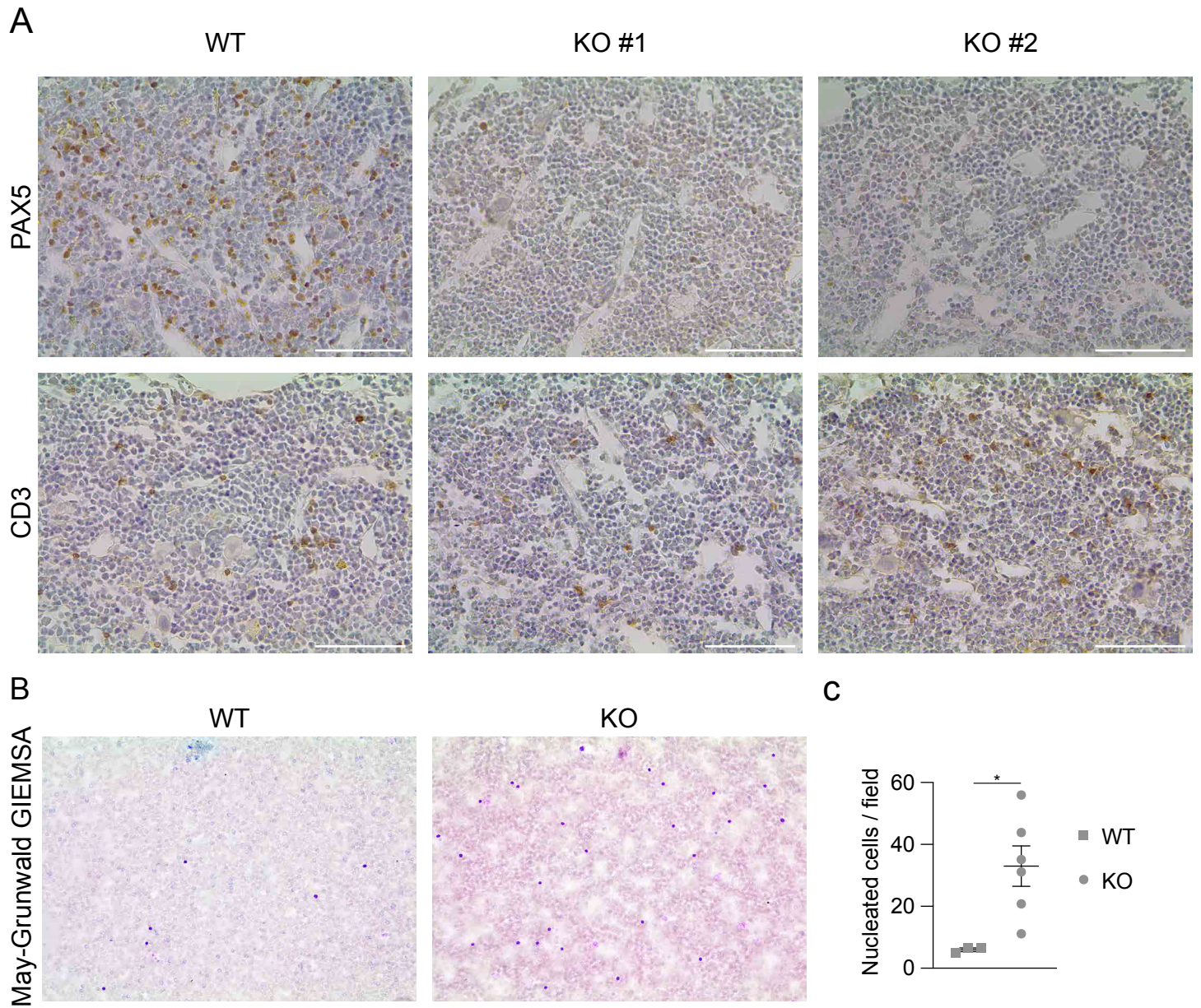
## Supplemental figure 6



**Supplemental figure 6. Bone marrow shows a phenotype similar to the spleen.** Related to Figure 4. Representative images of bone marrow of 3-month-old wild-type (WT) and *Ccm3*<sup>EPCKO</sup> mice stained for CD41 (megakaryocytes), F4/80 (macrophages), myeloperoxidase (myeloid cells), and Ter119 (erythrocytes) (as indicated). A total of four WT and nine knock-out mice were analyzed.

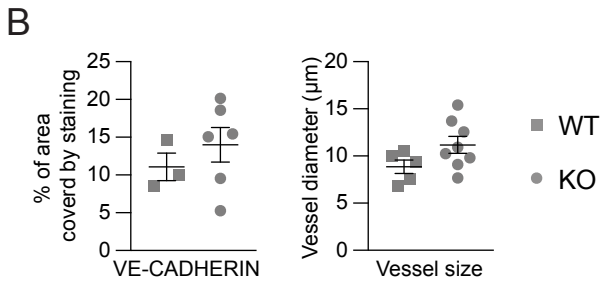
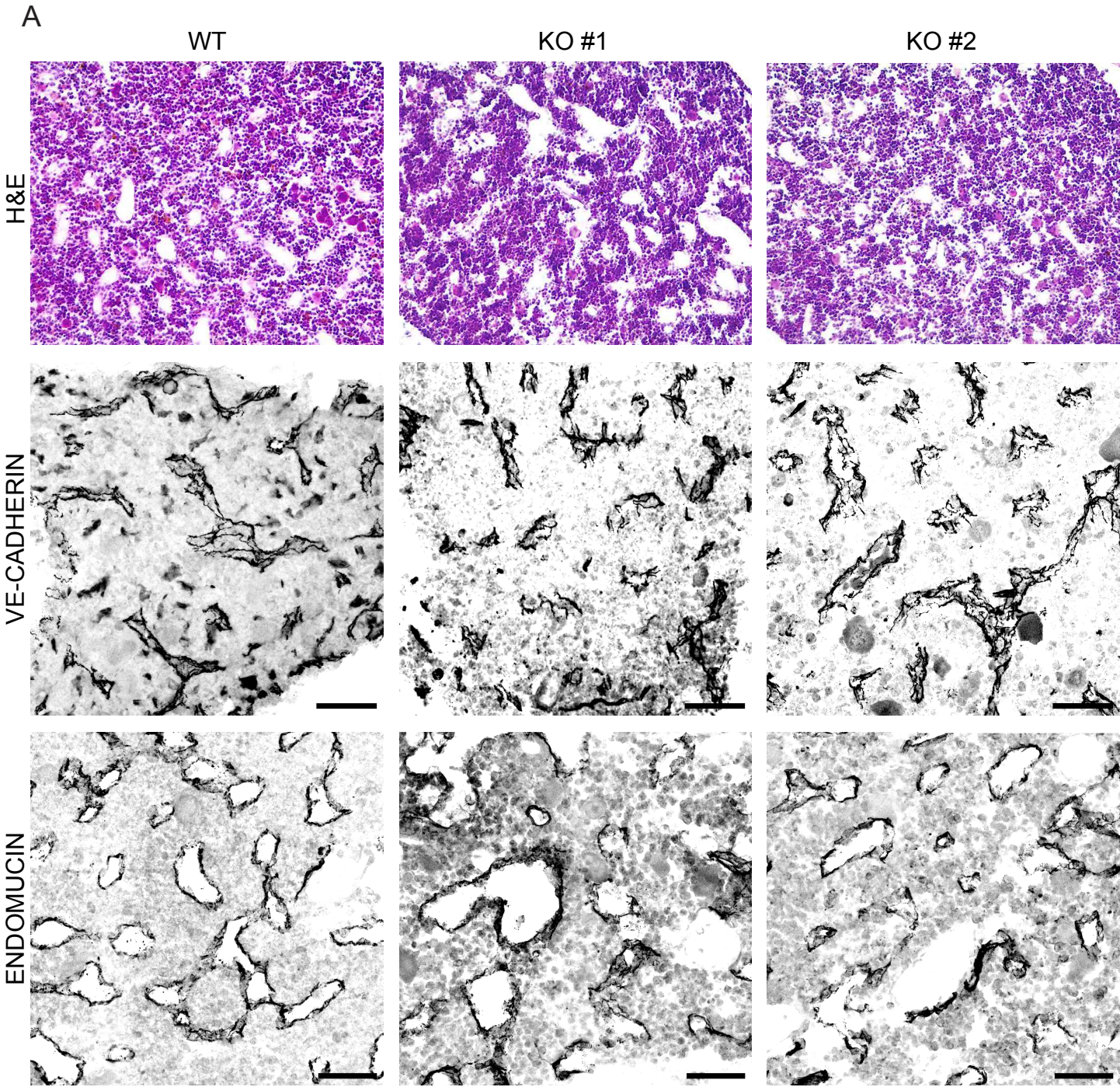


## Supplemental figure 7



**Supplemental figure 7. Bone marrow shows a phenotype similar to the spleen.** Related to Figure 4. **A)** Representative images of bone marrow of a 3-month-old wild-type (WT) and *Ccm3*<sup>EPCKO</sup> mice stained for PAX5 (B-cells) and CD3 (T-cells), as indicated. A total of four WT and nine knock-out mice were analyzed. Scale bars: 50  $\mu$ m. **B)** Representative images of blood smears with May–Grunwald–Giemsa staining. A total of three WT and seven knock-out mice were analyzed. Scale bar: 100  $\mu$ m. (c) Quantification of circulating nucleated cells. Data are means  $\pm$ SE. Each symbol represents an animal; \* $p$  < 0.03 (Student's t-tests).

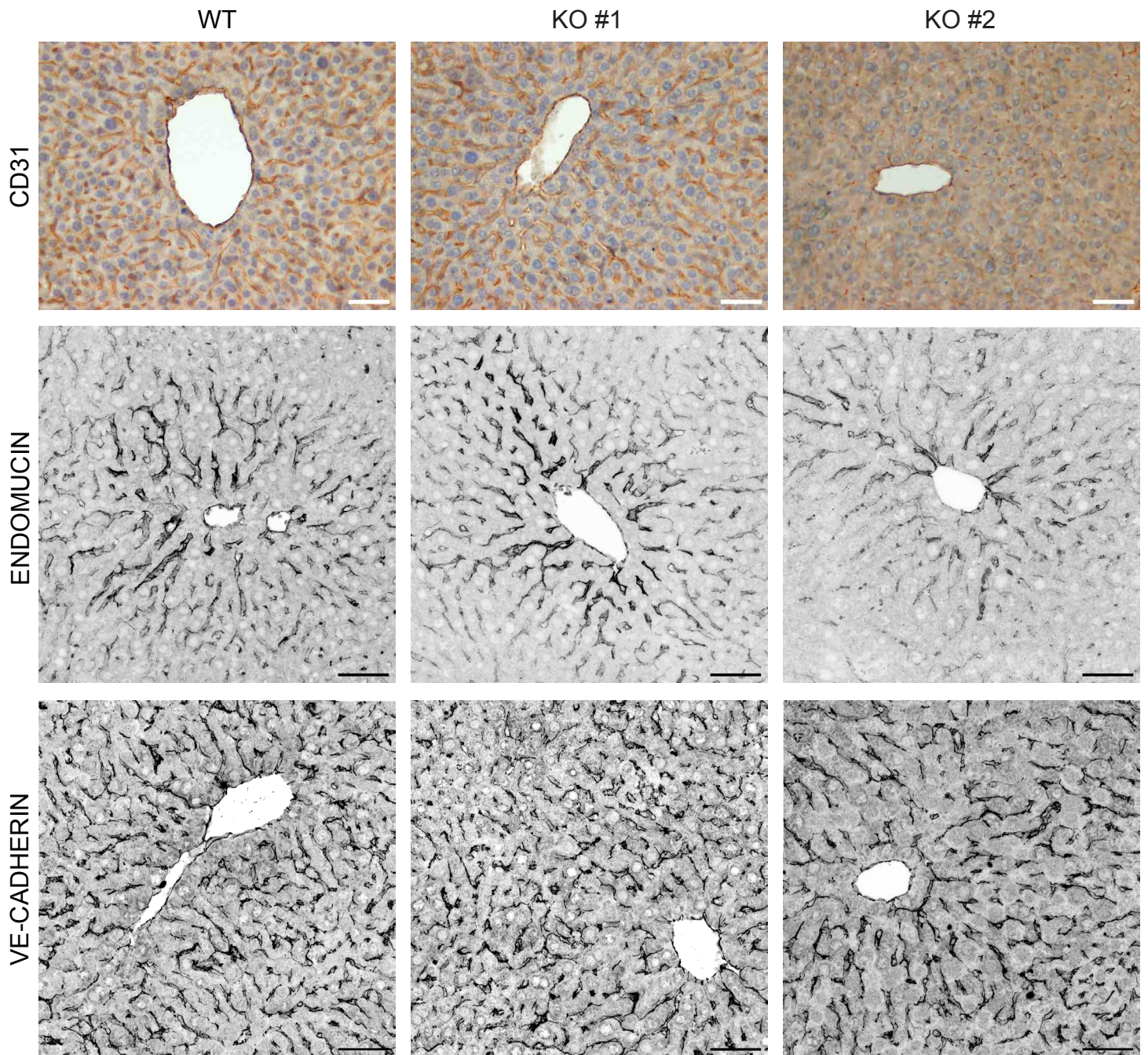
# Supplemental figure 8



**Supplemental figure 8. Bone marrow has a normal vasculature.** Related to Figure 5.

**A)** Representative images of bone marrow of a 3-month-old wild-type (WT) and *Ccm3*<sup>EPCKO</sup> mice stained for hematoxylin and eosin and the endothelial markers VE-cadherin and Endomucin (as indicated). Scale bars: 50  $\mu$ m. **B)** Quantification of vascular density and mean vessel diameter. Data are means  $\pm$ SE. Each symbol represents an animal;  $p > 0.05$  (Student's t-tests).

## Supplemental figure 9



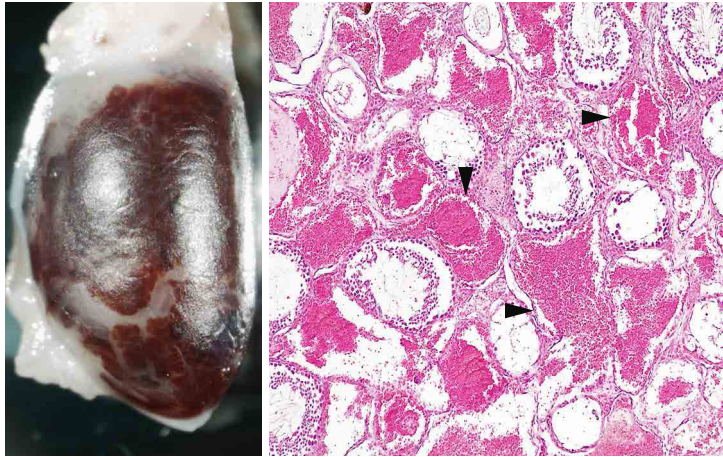
**Supplemental figure 9. The liver has a normal vasculature.** Related to Figure 5.

Representative images of the liver of a 3-month-old wild-type (WT) and *Ccm3*<sup>EPCKO</sup> mouse stained for hematoxylin and eosin and the endothelial markers VE-cadherin and Endomucin (as indicated). Scale bars: 50  $\mu$ m.

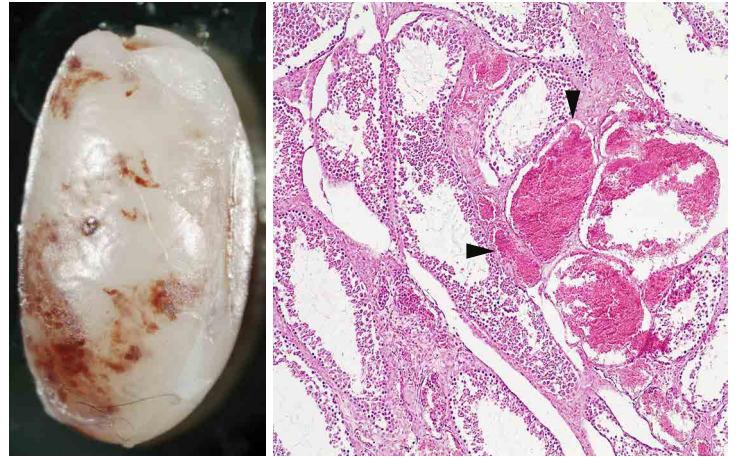
## Supplemental figure 10

A

KO P180 #1

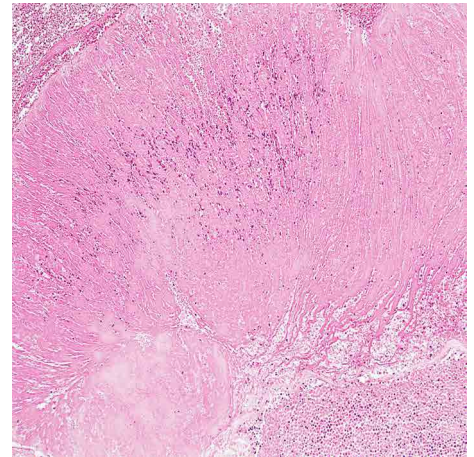
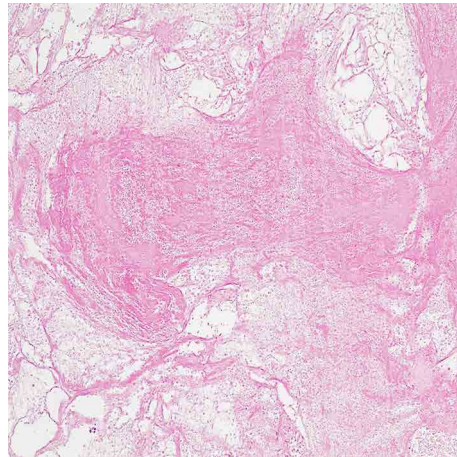
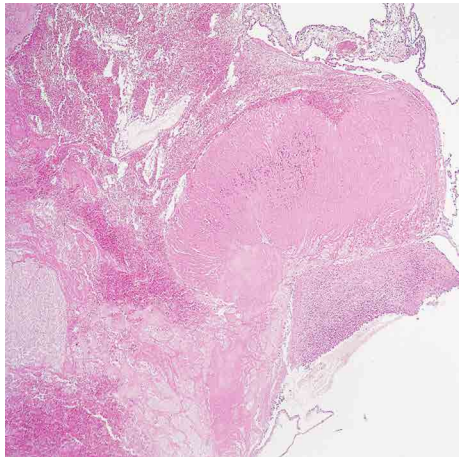
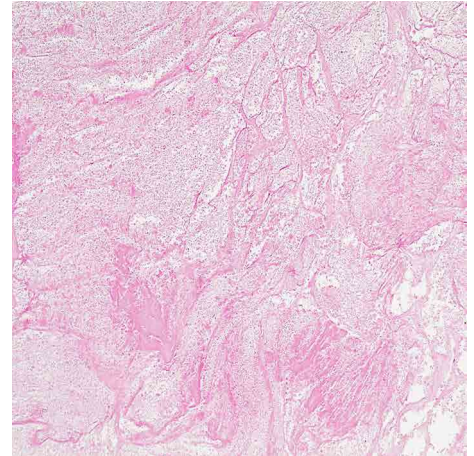


KO P180 #2



B

KO P180 #3



**Supplemental figure 10. Other organs affected in 6-month-old mice. Related to Figure 4.**

**A)** Representative photographs of testis from 6-month-old *Ccm3*<sup>EPCKO</sup> mouse, and relative hematoxylin and eosin staining; arrowhead point to hemorrhage. **B)** Representative photograph of a tumor from the abdominal cavity of a 6-month-old *Ccm3*<sup>EPCKO</sup> mouse, and relative hematoxylin and eosin staining.