

SUPPLEMENTS

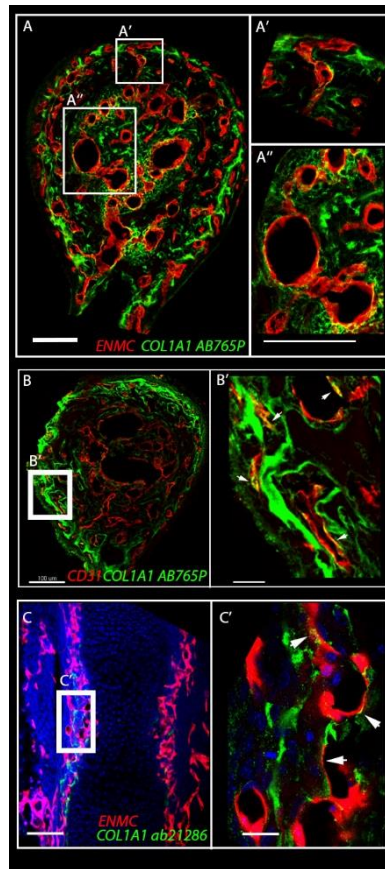


Figure S1. Collagen type I coats bone vasculature. (A) Confocal images of cross-sections of humeri from mouse embryo at E15.5 immunostained for collagen I (green) using Anti-Collagen I antibody (AB765P; EMD Millipore) and for blood vessels (red) using anti-EMCN antibody. A' and A'' show magnifications of the boxed areas. High concentration of collagen I is seen placed on ECs. (B) Confocal images of cross-sections of humeri from mouse embryo at E16.5 immunostained for collagen I (green) using Anti-Collagen I antibody (AB765P; EMD Millipore) and for blood vessels (red) using anti-CD31 antibody. (B') Magnification of the boxed area. Arrows indicate blood vessels coated with collagen I. (C) Confocal images of longitudinal section of humeri from mouse embryo at E14.5 immunostained for collagen I (green) using Anti-Collagen I antibody (ab21286; Abcam) and for blood vessels (red) using anti-EMCN antibody. (C') Magnification of the boxed area in A. Arrows indicates collagen I deposition on ECs. Scale bars: 100 μm (A, A', A'', B and C) and 20 μm (B' and C').

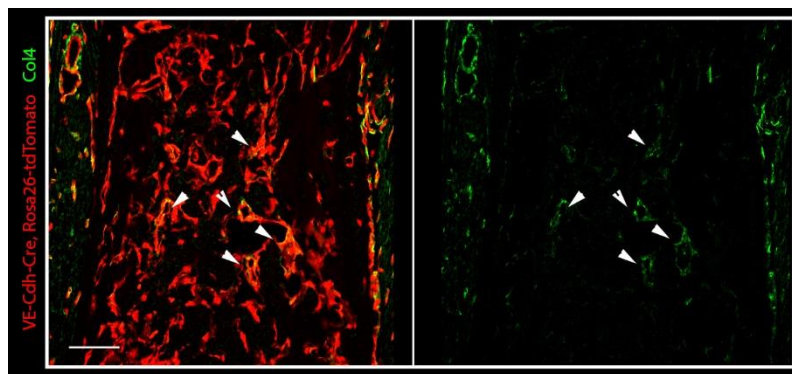


Figure S2. Most of the blood vessels in the bone lack basement membrane. Sagittal section of humerus from *VECad-Cre*, *tdTomato* (red) mouse embryo at E16.5 immunostained for BM marker collagen type IV (green). Only a few blood vessels in the bone center display collagen IV staining (arrows). Scale bar: 100 μm .

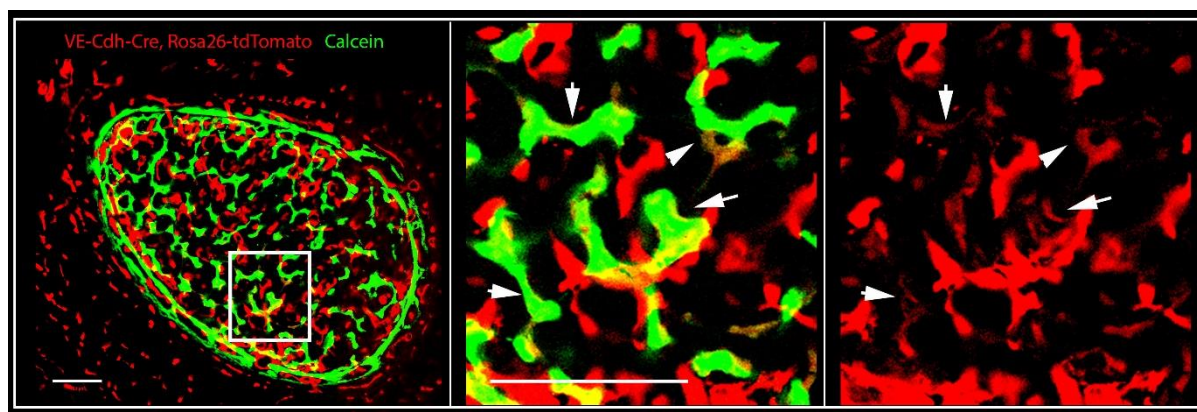
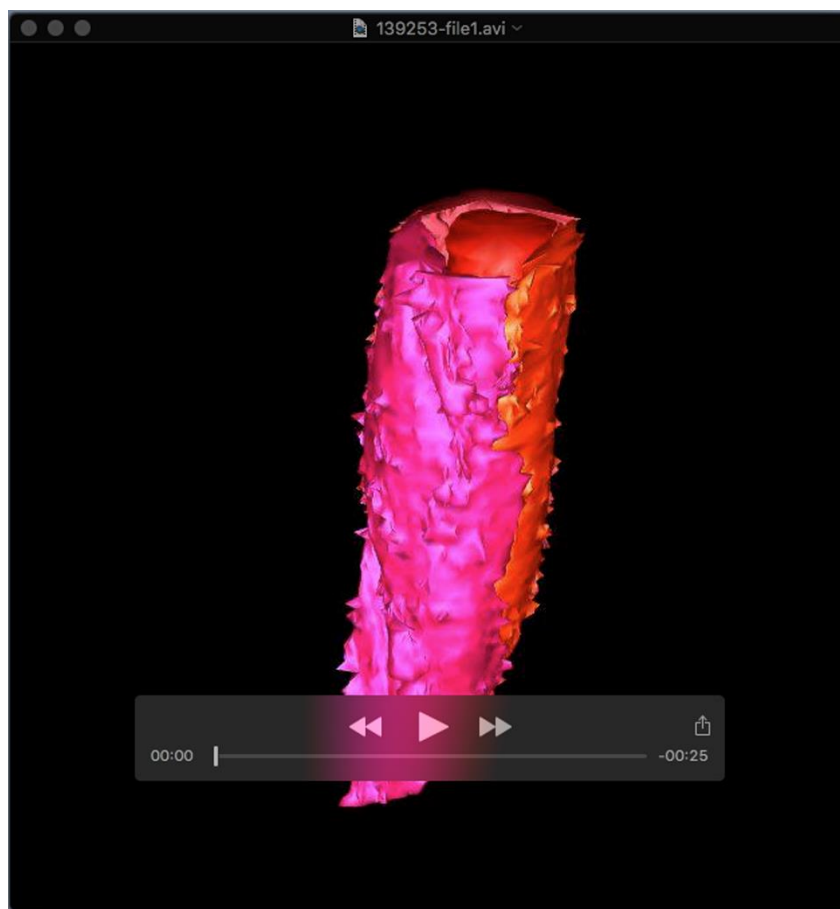


Figure S3. Low expression of EC marker in newly mineralized areas. Cross-section of humerus from *VECad-Cre*, *tdTomato* (red) mouse embryo at E17.5 injected with calcein (green). Right: Magnifications of the box area on the left. Arrows indicate low expression of *VECad* by ECs in areas of newly deposited mineral, indicated by weak calcein signal. Scale bars: 100 μ m.



Movie 1. Serial block-face scanning electron microscopy shows a blood vessel coated with collagen I and associated with osteoblast. 3D reconstruction using 321 images generated by SBF-SEM analysis of humerus from an E16.5 mouse embryo. A blood vessel consisting of three ECs and carrying a red blood cell (different shades of red) is covered with collagen (green) and associated with osteoblast (blue).