Supplemental Information

Apelin⁺ Endothelial Niche Cells Control
Hematopoiesis and Mediate Vascular
Regeneration after Myeloablative Injury

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- **Figure S1**, Related to Figure 1
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**Supplemental Tables**

- **Table S1**, Summary of genetic mouse models used in this study
Figure S1. Irradiation induced other changes to bone vasculature, related to Figure 1

(A-B) DAPI labelled (A) and Emcn-immunostained (B) sections from control or lethally irradiated bone (9 Gy; 7 days post irradiation). The overview images are stitched from several individual z-stacked images.

(C) Representative images showing extravasation of intravenously injected Texas red-coupled Dextran (red) in control bone and 7 days post lethal radiation (9 Gy). The overview images are stitched from several individual z-stacked images.

(D) Confocal overview and high-magnification images of diaphyseal vessels at 3 hours, 1 day and 4 days after lethal irradiation (9 Gy). Green: Emcn. The overview images are stitched from several individual z-stacked images.

(E) Validation of overlap between nuclear GFP (nGFP, green) signal and ERG immunostaining (red) in sections from P6 Cdh5-mTnG hearts.

(F) EC labelling in P6 Cdh5-mTnG bone.

(G) Flow cytometry validating Emcn expression in GFP+ cells from Cdh5-mTnG mice. Representative histogram of Emcn expression in GFP- or GFP+ cells and quantification of normalized mean fluorescence intensity (MFI). n=3 in each group. Error bars, mean± s.e.m. P values, two-tailed unpaired Student’s t-Test.

(H) Representative images showing no overlap of nuclear GFP signal (green) in B220+ (red) hematopoietic cells.

(I) Representative images showing no overlap of nuclear GFP signal (Green) in lineage committed (red) hematopoietic cells.

(J) Representative FACS histogram showing clear separation of B220+ cells (or Gr-1+) and GFP+ cells in Cdh5-mTnG mice.

(K) Representative single-plane images showing co-localization of EdU signal and nuclear GFP signal in Ctrl or irradiated (9 Gy 7 days) Cdh5-mTnG mice. About 40mg/kg EdU was injected at 7 consecutive days. Quantification for number and ratio of GFP+EdU+ signals in ctrl or irradiated Cdh5-mTnG mice (Ctrl=30 planes, 10 random planes/mouse; 9 Gy=30 planes, 10 random planes/mouse). Error bars, mean± s.e.m. P values, two-tailed unpaired Student’s t-Test.

(L) Representative images showing expression of active caspase 3 in Ctrl and irradiated (9 Gy 3 hours) Cdh5-mTnG mice. Green, nGFP; red, active caspase 3; blue: Emcn.

(M) Representative histogram of Annexin V staining of Ctrl and irradiated (9 Gy 24 hours) ECs (GFP+) isolated from Cdh5-mTnG mice. Quantification of mean fluorescence intensity (MFI) of Annexin V signal (Ctrl n=6; 9 Gy 3h n=5; 9 Gy 24h n=3). Error bars, mean± s.e.m. P values, two-tailed unpaired Student’s t-Test.

(N) Confocal images of retinal vessels at 7 days after irradiation. Quantification of GFP+ cells per image field (Ctrl n=6 mice; 9 Gy n=5 mice). Error bars, mean± s.e.m. P values, two-tailed unpaired Student’s t-Test.

(O) Confocal images of heart vasculature in left ventricle at 7 days after irradiation. Quantification of GFP+ cells per image field (Ctrl n=6 mice; 9 Gy n=5 mice). Error bars, mean± s.e.m. P values, two-tailed unpaired Student’s t-Test. The overview images are stitched from several individual z-stacked images.

(P and Q) Confocal images of dermal vessels (P) and small intestinal villi (Q) at 7 days after irradiation. Quantification of GFP+ cells per image field (Ctrl n=3 mice; 9 Gy n=3 mice). Error bars, mean± s.e.m. P values, two-tailed unpaired Student’s t-Test. The overview images are stitched from several individual z-stacked images.
Figure S2. Transcriptional changes in irradiated ECs and effect of 5-fluorouracil (5-FU) in bone vasculature, related to Figure 2

(A and B) Projected DAPI (white) and Emcn (green) staining in adult bone vasculature 7 days after 5-FU treatment. The overview images are stitched from several individual z-stacked images.

(C) High magnification confocal images of collagen IV (red) and CD31 (blue) in adult bone vasculature 7 days after 5-FU treatment.

(D) Maximum intensity projections showing morphology of ECs at single cell level in Cdh5-CreER R26-mTmG mice 7 days after 5-FU treatment. Tamoxifen was injected 6 days after 5-FU treatment. Quantification of area, perimeter and shape factor of 73 single ECs from 3 control mice and 67 single ECs from 3 5-FU-treated mice. Error bars, mean± s.e.m. P values, two-tailed unpaired Student’s t-Test.

(E) Representative images and quantification of GFP+ cells in one image field of diaphysis in Cdh5-mT-nG mice 7 days after 5-FU treatment. n=6 in each group. Error bars, mean± s.e.m. P values, two-tailed unpaired Student’s t-Test.

(F) Representative images and quantification of arterial vessels in Bmx-CreER R26-mTmG mice 7 days after 5-FU treatment. GFP(Green), Caveolin-1 (red) and Bcam (blue). Quantification of arterial vessel diameter (Ctrl=75 from 4 mice, 5-FU=63 from 4 mice), mean fluorescence intensity of caveolin-1 (Ctrl=54 from 4 mice, 5-FU=65 from 4 mice) and Bcam (Ctrl=54 from 4 mice, 5-FU=65 from 4 mice). Error bars, mean± s.e.m. P values, two-tailed unpaired Student’s t-Test.

(G) Representative confocal images showing expression of active caspase 3 in Ctrl and 5-FU-treated Cdh5-mTnG mice at 24 hours after treatment. Green, nGFP; red, active caspase 3; blue: Emcn.

(H) Annexin V staining of Ctrl and 5-FU-treated (5-FU 24 hours) ECs (GFP+) from Cdh5-mTnG mice. Quantification of mean fluorescence intensity (MFI) of Annexin V signal (Ctrl n=3; 5-FU 24h n=3). Error bars, mean± s.e.m. P values, two-tailed unpaired Student’s t-Test.

(I) Heatmap showing dynamic change of selected EC marker genes at the indicated times after irradiation.

(J and K) Heatmaps showing selected genes related to DNA damage (J) or senescence (K) after irradiation.
Figure S3. Characterization of DTR<sup>1</sup>H<sub>HC</sub> model, related to Figure 3

(A) Representative images showing no overt co-localization of Emcn+ ECs and GFP signal in Vav1-Cre R26-mTmG BM under steady-state conditions.
(B) Representative images showing co-localization (arrow) of VEGFR2 and GFP signals in Vav1-Cre R26-mTmG liver. The overview images are stitched from several individual z-stacked images.
(C and D) Overview and high-magnification (single plane) images showing Emcn and GFP signals in metaphysis and diaphysis of Vav1-Cre R26-mTmG mice at 7 days after 9 Gy irradiation without transplantation (C) and after transplantation of wild-type Lin- cells (D). Green, GFP; red, Emcn. The overview images are stitched from several individual z-stacked images.
(E) Diphtheria toxin injection in Cre- mice does not influence vessels and hematopoietic cells. Overview and high-magnification image and quantification of bone vessels (Emcn, green) after administration of diphtheria toxin (n=6) or vehicle control (n=5) in Vav1-Cre+/+ R26-DTR mice. The overview images are stitched from several individual z-stacked images. FACS quantification of B220+ B-lymphocytes and CD11b+ Gr-1+ myeloid cells percentage after administration of diphtheria toxin (n=10) or vehicle control (n=5) in Vav1-Cre+/+ R26-DTR mice. Error bars, mean± s.e.m. P values, two-tailed unpaired Student’s t-Test.
(F) Representative FACS plots of Lin- and Lin+ cells 4 days after diphtheria toxin injection in littermate control (Vav1-Cre+/+ R26-DTR) and DTR<sup>1</sup>H<sub>HC</sub> mice.
(G) Projected overview image of DAPI (white) signal 4 days after diphtheria toxin injection in littermate control (Vav1-Cre+/+ R26-DTR) and DTR<sup>1</sup>H<sub>HC</sub> mice. The overview images are stitched from several individual z-stacked images.
(H) Principal component analysis of EC gene expression in DTR<sup>1</sup>H<sub>HC</sub> and littermate control mice. DTA was injected into both DTR<sup>1</sup>H<sub>HC</sub> and control mice, and ECs were isolated 4 days later.
(I) MA plot indicating differentially expressed genes in DTR<sup>1</sup>H<sub>HC</sub> and control ECs 4 days after DTA injection.
(J) Quantification of selected HSC-related niche genes from RNA-seq analysis of DTR<sup>1</sup>H<sub>HC</sub> and control ECs. N=3 per group, error bars, mean± s.e.m. P values, two-tailed unpaired Student’s t-Test.
(K and L) Overview of Emcn-stained bone vasculature 16 days after irradiation and transplantation of 4x10<sup>5</sup> Lin- or Lin+ cells (K) or of 2x10<sup>4</sup> Lin- or LSK cells (L). The overview images are stitched from several individual z-stacked images.
(M) Overview and high-magnification confocal images of Emcn-stained bone vasculature 4 days after irradiation in mice without transplantation (No TP) or after transplantation with 1x10<sup>8</sup> Lin+ cells. The overview images are stitched from several individual z-stacked images. Quantification of Emcn area (No TP n=5; 10<sup>8</sup> Lin+ n=4). Error bars, mean± s.e.m. P values, two-tailed unpaired Student’s t-Test.
(N) Overview images of femur from Cdh5-mTnG recipient (9 Gy 16 days) transplanted with wildtype LSK cells. The overview images are stitched from several individual z-stacked images. Most of Emcn+ BM vessels (green) co-localize with tomato (red) signal of the Cdh5-mTnG reporter.
(O) High-magnification image showing wild-type recipient (9 Gy 16 days) transplanted with LSK cells from Vav1-mTmG donor. Emcn+ vessels (red) show no overt co-localization with GFP signal (green).
Figure S4. Characterization of Apln+ ECs and vascular defects in DTA\textsuperscript{i}A\textsuperscript{pln} in bone, related to Figure 4

(A) Overview and high magnification confocal images of Emcn+ (red) capillaries and GFP+ (green) arteries in Bmx-CreERT\textsuperscript{2} R26-mTmG mice at 7 days after lethal irradiation (9 Gy). The overview images are stitched from several individual z-stacked images.

(B) Representative images of arterial vessels in Bmx-CreER R26-mTmG mice 7 days after irradiation. GFP (green), Caveolin-1 (red) and Bcam (blue).

(C) Overview and high magnification confocal images of Emcn+ (red) capillaries and GFP+ (green) arteries in Bmx-CreERT\textsuperscript{2} R26-mTmG mice at 3 weeks after lethal irradiation and BM transplantation. The overview images are stitched from several individual z-stacked images.

(D) Schematic diagram depicting the isolation of Apln+ ECs from Apln-mTmG whole bone and of Cdh5-mTnG diaphyseal ECs.

(E) Heatmap showing the enrichment of selected endothelial marker genes in Apln+ ECs and diaphyseal (DP) ECs relative to bone marrow cells (BMCs), Lin- cells, LSK cells, and Lepr+ and NG2+ perivascular cells.

(F) Heatmap showing enrichment of specific niche-related genes in diaphyseal ECs relative to Apln+ ECs.

(G) GO biological signaling pathway analysis indicating the top 15 enriched signaling pathways in diaphyseal ECs.

(H) High magnification image showing VE-cadherin immunosignal (red) in Apln+ ECs (arrow) in Apln-mTmG mice.

(I) Apln+ ECs (green) in Apln-mTmG mice express Sca-1 (red, arrows) but are not covered by \(\alpha\)SMA+ vSMCs (blue, arrowheads). The overview images are stitched from several individual z-stacked images.

(J) Heatmap showing expression of Ly6\textsuperscript{a}{\textsuperscript{high}} ECs and Stab2\textsuperscript{high} ECs markers in Apln+ ECs and diaphyseal ECs (Tikhonova et al., 2019).

(K) Heatmap showing expression of “arterial-type” EC markers in Apln+ ECs but not diaphyseal ECs (Baryawno et al., 2019).

(L) Heatmap showing expression pattern of selected anti-inflammatory genes in Apln+ ECs and diaphyseal ECs.

(M) Experimental protocol for DTA-mediated ablation of Apln+ ECs under physiological conditions (top) or in combination with irradiation and transplantation of DTA\textsuperscript{i}A\textsuperscript{pln} recipients.

(N) Confocal overview and high-magnification images of Emcn (green) and ERG (red) stained vessels in metaphysis and diaphysis of adult control and DTA\textsuperscript{i}A\textsuperscript{pln} mice. Arrows indicated EC bulbs near control (Ctrl) growth plate and arrowheads mark local vessel lesions in DTA\textsuperscript{i}A\textsuperscript{pln} samples. Confocal overview image of Emcn (green), extravasation dextran (red) and DAPI (blue) stained BM of adult DTA\textsuperscript{i}A\textsuperscript{pln} and control mice. The overview images are stitched from several individual z-stacked images.

(O) Quantification of vessel buds (ctrl=6, DTA\textsuperscript{i}A\textsuperscript{pln}=6) and ERG+ cells (ctrl=5, DTA\textsuperscript{i}A\textsuperscript{pln}=5) in metaphysis, diameter of diaphyseal vessels (ctrl=6, DTA\textsuperscript{i}A\textsuperscript{pln}=6), dextran leakage area (ctrl=3, DTA\textsuperscript{i}A\textsuperscript{pln}=4). Error bars, mean± s.e.m. P values, two-tailed unpaired Students’ t-Test.
Figure S5. Loss of Apln+ ECs but not of Apln expression causes hematopoietic defects under steady state, related to Figure 5.

(A) Schematic showing protocols for CD45.2/CD45.1 long-term repopulation assay for DTA\textsuperscript{i∆Apln}, Kitl\textsuperscript{i∆Apln} and Vegfr2\textsuperscript{i∆Apln} mice.

(B) Flow cytometric analysis of hematopoietic cell subpopulations in the DTA\textsuperscript{i∆Apln} BM (n=6 in each group). Error bars, mean± s.e.m. P values, two-tailed unpaired Student’s t-Test. BMNC, bone marrow nucleated cells.

(C) Percentage of perivascular cell subpopulations in DTA\textsuperscript{i∆Apln} (n=6) and control (n=7) bone. Error bars, mean± s.e.m. P values, two-tailed unpaired Student’s t-Test.

(D) Blood count analysis of DTA\textsuperscript{i∆Apln} peripheral blood (PB) components (n=6 in each group). Error bars, mean± s.e.m. P values, two-tailed unpaired Student’s t-Test. RBC, red blood cells; WBC, white blood cells; Lym, lymphocytes; GRA, granulocyte.

(E) FACS analysis of DTA\textsuperscript{i∆Apln} peripheral blood (PB) components under steady state (Ctrl=9, DTA\textsuperscript{i∆Apln}=10). Error bars, mean± s.e.m. P values, two-tailed unpaired Student’s t-Test.

(F) Cytokine levels in peripheral blood plasma of DTA\textsuperscript{i∆Apln} mice under steady state. Cytokine level lower than detection limit was excluded from quantification. n=2-8 in each group. Error bars, mean± s.e.m. P values higher than 0.1 are not indicated.

(G and H) FACS analysis of DTA\textsuperscript{i∆Apln} liver (G) and spleen (H) leukocytes under steady-state (Ctrl=7, DTA\textsuperscript{i∆Apln}=6). Error bars, mean± s.e.m. P values, two-tailed unpaired Student’s t-Test.

(I) Blood count analysis of Kitl\textsuperscript{i∆Apln} peripheral blood (PB) components (Ctrl=10; Kitl\textsuperscript{i∆Apln}=9). Error bars, mean± s.e.m. P values, two-tailed unpaired Student’s t-Test. WBC, white blood cells; RBC, red blood cells; GRA, granulocyte.

(J) Blood count analysis of Vegfr2\textsuperscript{i∆Apln} peripheral blood (PB) components (Ctrl=8; Vegfr2\textsuperscript{i∆Apln}=8). Error bars, mean± s.e.m. P values, two-tailed unpaired Student’s t-Test. WBC, white blood cells; RBC, red blood cells; GRA, granulocyte.

(K) Flow cytometric analysis of hematopoietic cell subpopulations in Apln (T/y) and control bone marrow and peripheral blood (PB). Animals in this experiment were not treated with tamoxifen to evaluate the effect of the loss of Apln expression in Apln-CreERT knock-in males (equivalent to full knockout mice) (n=5 per group). Error bars, mean± s.e.m. P values, two-tailed unpaired Student’s t-Test. CLP, common lymphoid progenitors; CMP, common myeloid progenitors; GMP, granulocyte-monocyte progenitors; MEP, Megakaryocyte-erythroid progenitors; RBC, red blood cells; WBC, white blood cells; PLT, platelets; Lym, lymphocytes.

(L) Flow cytometric analysis of hematopoietic reconstitution after loss of Apln expression in Apln-CreERT T/y knockout (n=6) and control males (n=7) without tamoxifen (TMX) administration. Vav1-Cre R26-mTmG hematopoietic cells were used as donors. Error bars, mean± s.e.m. P values, two-tailed unpaired Student’s t-Test.
Figure S6. Role of Apln+ ECs but not Esm1+ ECs in hematopoiesis and transplantation, related to Figure 6.

(A) FACS analysis of DTA\textsuperscript{iApln} peripheral blood (PB) components 3 days after irradiation and transplantation (Ctrl=9, DTA\textsuperscript{iApln} =9). Error bars, mean± s.e.m. P values, two-tailed unpaired Student’s t-Test. 

(B) Cytokine levels in peripheral blood plasma of DTA\textsuperscript{iApln} mice 3 days after irradiation and transplantation. Cytokine level lower than detection limit was excluded from quantification. n=2-8 in each group. Error bars, mean± s.e.m. P values, two-tailed unpaired Student’s t-Test. P values higher than 0.1 are not indicated.

(C) Survival (in days) of DTA\textsuperscript{iApln} mutants (n=5) relative to irradiated (9 Gy) and transplanted control (Apln-CreER+/y, n=11) and DTA\textsuperscript{iApln} mice (n=14).

(D) Genetic lineage tracing of GFP+ cells in Apln-mTmG long bone at 8 days after a single 4-OHT administration with or without irradiation at day 2. Confocal overview images and magnification of diaphysis. The overview images are stitched from several individual z-stacked images. Quantification of GFP+ area relative to Emcn+ area in diaphysis (n=3 in each group). Error bars, mean± s.e.m. P values, two-tailed unpaired Student’s t-Test.

(E) Genetic lineage tracing of GFP+ cells in Apln-mTmG long bone at 7 weeks after a single 4-OHT administration with or without irradiation and transplantation at day 2. Confocal overview images and magnification of diaphysis. The overview images are stitched from several individual z-stacked images. Quantification of GFP+ area relative to Emcn+ area in diaphysis (Ctrl=3; 9 Gy=5). Error bars, mean± s.e.m. P values, two-tailed unpaired Student’s t-Test.

(F) Confocal overview images showing αSMA+ vSMCs and GFP signals in genetic lineage tracing experiments with Apln-mTmG at 7 weeks after irradiation and transplantation. The overview images are stitched from several individual z-stacked images. Dashed box and high magnified images show descendants of Apln+ ECs in association with vSMCs.

(G) Projected confocal image showing Esm1-CreER R26-mTmG mice with genetically labelled Esm1+ BM ECs. Green, GFP(Esm1-CreER); Red, Emcn; Blue, VEGFR2. Quantification of the percentages of Esm1+ ECs and Apln+ ECs in diaphysis (Esm1+ =4; Apln+ =10). Error bars, mean± s.e.m. P values, two-tailed unpaired Student’s t-Test.

(H) Experimental protocol for DTA-mediated ablation of Esm1+ ECs under physiological conditions.

(I) Percentage of BM HSC and LSK cells in control (n=7) and DTA\textsuperscript{iEsm1} mice (n=6) under steady state conditions. Error bars, mean± s.e.m. P values, two-tailed unpaired Student’s t-Test.

(J) Percentage of BM CD45+, B220+ and Gr-1+ cells in control (n=7) and DTA\textsuperscript{iEsm1} mice (n=6) under steady state. Error bars, mean± s.e.m. P values, two-tailed unpaired Student’s t-Test.

(K) Blood counts of BM RBC, WBC and GRA cells in control (n=7) and DTA\textsuperscript{iEsm1} mice (n=6) under steady state. RBC, red blood cells; WBC, white blood cells, GRA, granulocyte. Error bars, mean± s.e.m. P values, two-tailed unpaired Student’s t-Test.

(L) Experimental protocol for DTA-mediated ablation of Esm1+ ECs in combination with irradiation and transplantation of DTA\textsuperscript{iEsm1} recipients.

(M) FACS analysis of CD45+ or CD11b+Gr-1+ cells in PB of control (n=8) and DTA\textsuperscript{iEsm1} mice (n=5) 5 days after irradiation. Error bars, mean± s.e.m. P values, two-tailed unpaired Student’s t-Test.

(N) Representative FACS plot showing gating strategy to isolate Esm1+ ECs (defined as GFP+CD31+CD140b−) from Esm1-mTmG mice.

(O) Heatmap of selective core EC markers genes showing significant enrichment of these genes in Esm1+ ECs relative to BM hematopoietic cells and mesenchymal cells.

(P) Expression levels of core EC marker genes in Apln+ ECs, Esm1+ ECs and total diaphyseal ECs (DP ECs) at transcript level based on RNA-seq data.
Figure S7. VEGFR2 in endothelial and hematopoietic cells have different functions during transplantation, related to Figure 7.

(A) RNA-seq data (fpkm values) of Vegfa in 7 different bone cell groups. n=3 for LSK, Lin-, BMCs, ECs, and irradiated (9 Gy) ECs; n=2 for Lepr+ and NG2+ perivascular cells (Asada et al., 2017). Error bars, mean± s.e.m. P values, two-tailed unpaired Student’s t-Test.

(B) ELISA of cellular VEGF-A in LSK (n=3), Lin- (n=4) and Lin+ (n=4) subpopulations. Error bars, mean± s.e.m. P values, two-tailed unpaired Student’s t-Test.

(C) RT-qPCR analysis of Vegfa levels in whole BM cells 7 days after irradiation or in controls without irradiation (n=5 per group). Error bars, mean± s.e.m. P values, two-tailed unpaired Student’s t-Test.

(D) RNA-seq data (fpkm values) of Kdr/Vegfr2 in 5 different bone cell groups (n=3 each group). Error bars, mean± s.e.m.

(E) VEGFR2 expression at protein level (n=55 each group, from 3 mice) in Apln+ EC and non-Apln+ EC. Error bars, mean± s.e.m.

(F) Schematic diagram depicting the transplantation of VegfaΔHC and littermate control Lin- (3x10^5 ) cells into irradiated wild-type recipients.

(G) Representative image of bone vasculature 2.5 weeks after irradiation and transplantation of 3x10^5 Lin- cells derived from control or VegfaΔHC donor mice. Graphs show Emcn area per field and BMNC number in recipient mice (control=11, VegfaΔHC=10). Quantification of LSK, CD45+, and B220+ cell numbers and LSK percentage (control n=11, VegfaΔHC n=10). Error bars, mean± s.e.m. P values, two-tailed unpaired Student’s t-Test.

(H) Percentage of Lin- cells and percentage of LSK cells in VegfaΔHC (n=10) and control (n=11) BM under steady state conditions.

(I) Schematic diagram depicting the transplantation of Vegfr2ΔHC and control bone marrow cells into wild-type recipients.

(J) Flow cytometric analysis of BMNC number, LSK cell number, B220 cell number and CD11b cell number in recipients transplanted with Vegfr2ΔHC or Ctrl donor cells (n=10 per group). Error bars, mean± s.e.m. P values, two-tailed unpaired Student’s t-Test.

(K) Schematic depiction of the experimental protocol for the transplantation of Lin- (3x10^5 ) cells into Vegfr2ΔEC or Vegfr2ΔApln recipient mice.

(L) Mathematical regression analysis showing the lack of correlation between BMNC number and Emcn+ area in Ctrl (grey, n=11) and Vegfr2ΔApln (turquoise, n=11) samples. R^2, coefficient of determination, range from 0 to 1. R^2=1 means perfect fit of data points and equation.

(M) Quantification of Ki67+ (Ctrl=6; Vegfr2ΔApln=6) and cleaved caspase 3+ (Ctrl=5; Vegfr2ΔApln=5) signal per image field in Ctrl and Vegfr2ΔApln bone sections. Error bars, mean± s.e.m. P values, two-tailed unpaired Student’s t-Test.

(N) Representative high magnification images of Emcn+ bone vasculature in transplanted Vegfr2ΔEC or control recipients. Quantification of Emcn+ area, BMNC number, LSK percentage, B220 cell number, CD11b cell number and Gr-1 cell number in Vegfr2ΔEC (n=8) or control recipients (n=7). Error bars, mean± s.e.m. P values, two-tailed unpaired Student’s t-Test.

(O) Representative overview of Emcn+ bone vasculature in VEGF-A or vehicle control-treated recipient mice. The overview images are stitched from several individual z-stacked images.

(P) Cytokine levels in peripheral blood plasma of irradiated mice 10 days after transplantation together with VEGF-A infusion. Cytokine level lower than detection limit was excluded from quantification. N=4-6 in each group. Error bars, mean± s.e.m. P values, two-tailed unpaired Student’s t-Test. P values higher than 0.1 are not indicated.

(Q) Mathematical regression analysis showing the lack of correlation between BMNC number and Emcn+ area in Ctrl (vehicle, n=14) and VEGF-A-treated bone samples (purple, n=11).

(R) Quantification of Ki67+ (Ctrl=7; VEGF-A=7) and cleaved caspase 3+ (Ctrl=7; VEGF-A=6) signal per image field in Ctrl and VEGF-A-treated bone sections. Error bars, mean± s.e.m. P values, two-tailed unpaired Student’s t-Test.

(S) Quantification of CFU-E, CFU-GEMM number and percentage of cells in tetraploid state (4 nucleus,
| Full Name | Short name | Inducible | Reporter | Purpose |
|-----------|------------|-----------|----------|---------|
| Cdh5 membrane-Tdtomato H2B-EGFP | Cdh5-mTnG | H2B-GFP (nuclear) and mem-Tdtomato | Genetic labeling of ECs for imaging and FACS |
| Rosa26-mT/mG | R26-mTmG | Cre-induced switch from mem-Tomato to mem-GFP | Labeling of Cre-positive cells and their descendants |
| Cdh5(PAC)-CreERT2 | Cdh5-CreERT2 | Yes | - | Labeling of ECs and their descendants |
| Apln-CreERT2 | - | Yes | - | Labeling of Apln+ ECs and their descendants |
| Bmx-CreERT2 | - | Yes | - | Labeling of arterial ECs and their descendants |
| Vav1-Cre | - | No | - | Labeling and knockout of hematopoietic cells |
| VEGFαm2Gne | - | - | Conditional knockout of Vegfa |
| Kdrm1Wag | - | - | Conditional knockout of Vegfr2/Kdr |
| Rosa26-DTR | - | - | Conditional ablation of Cre+ cells, triggered by diphtheria toxin injection |
| Rosa26-DTA<sup>tm1(DTA)Mrc</sup> | - | - | Conditional ablation of Cre+ cells, triggered by tamoxifen injection |
| Cdh5(PAC)-CreERT2 Rosa26-mT/mG | Cdh5-mTmG | mem-GFP after Cre recombination | Tamoxifen injection to label all ECs or cells at single cell level |
| Bmx-CreERT2 Rosa26-mT/mG | Bmx-mTmG | mem-GFP after Cre recombination | Labeling of arterial ECs and their descendants |
| Apln-CreERT2 Rosa26-mT/mG | Apln-mTmG | mem-GFP after Cre recombination | Labeling of Apln+ ECs and their descendants |
| Vav1-Cre Rosa26-mT/mG | Vav1-mTmG | No | mem-GFP after Cre recombination | Labeling of hematopoietic cells for analysis or transplantation (donor) |
| Vav1-Cre Rosa26-DTR | DTR<sup>ΔHC</sup> | No | - | Genetic ablation of hematopoietic cells by diphtheria toxin injection |
| Vav1-Cre Rosa26-DTR Cdh5-mTnG | - | No | H2B-GFP (nuclear) and mem-Tdtomato | Genetic ablation of hematopoietic cells by diphtheria toxin injection and labeling of ECs for imaging and FACS |
| Vav1-Cre VEGFαm2Gne | Vegfa<sup>ΔHC</sup> | No | - | Knockout of Vegfa in hematopoietic cells |
| Vav1-Cre Kdr<sup>tm1Wag</sup> | Vegfr2<sup>ΔHC</sup> | No | - | Knockout of Vegfr2 in hematopoietic cells |
| Apln-CreERT2 Rosa26-DTA | DTA<sup>Δapln</sup> | Yes | - | Inducible ablation of Apln+ ECs, triggered by tamoxifen |
| Mating Scheme | Vegetin Knockout | Lineage Tracing Method | Comments |
|---------------|------------------|------------------------|----------|
| Cdh5(PAC)-CreERT2 Kdr<sup>tm1Wag</sup> | Vegfr2<sup>ΔEC</sup> | Yes | Knockout of Vegfr2 in ECs and their descendants |
| Apln-CreERT2 Rosa26 Kdr<sup>tm1Wag</sup> | Vegfr2<sup>Δapln</sup> | Yes | Knockout of Vegfr2 in Apln+ ECs and their descendants |
| Esm1-CreERT2 | - | Yes | Labeling of Esm1+ ECs and their descendants |
| Esm1-CreER Rosa26-mT/mG | - | Yes | mem-GFP after Cre recombination |
| Esm1-CreERT2 Rosa26-DTA | DTA<sup>ΔEsm1</sup> | Yes | Labeling of Esm1+ ECs and their descendants for lineage tracing |
| Kit<sup>tm2.1Sjm/J</sup> | - | | Genetic ablation of Esm1+ ECs, triggered by tamoxifen |
| Apln-CreERT2 Kit<sup>tm2.1Sjm/J</sup> | Kit<sup>Δapln</sup> | Yes | Conditional knockout of stem cell factor (Kitl) |
| Kit<sup>tm2.1Sjm/J</sup> | - | | Knockout of stem cell factor (Kitl) in Apln+ ECs |