Cell Stem Cell, Volume *25*

Supplemental Information

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

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This PDF file includes:

Supplemental Figures and Legends

Supplemental Tables

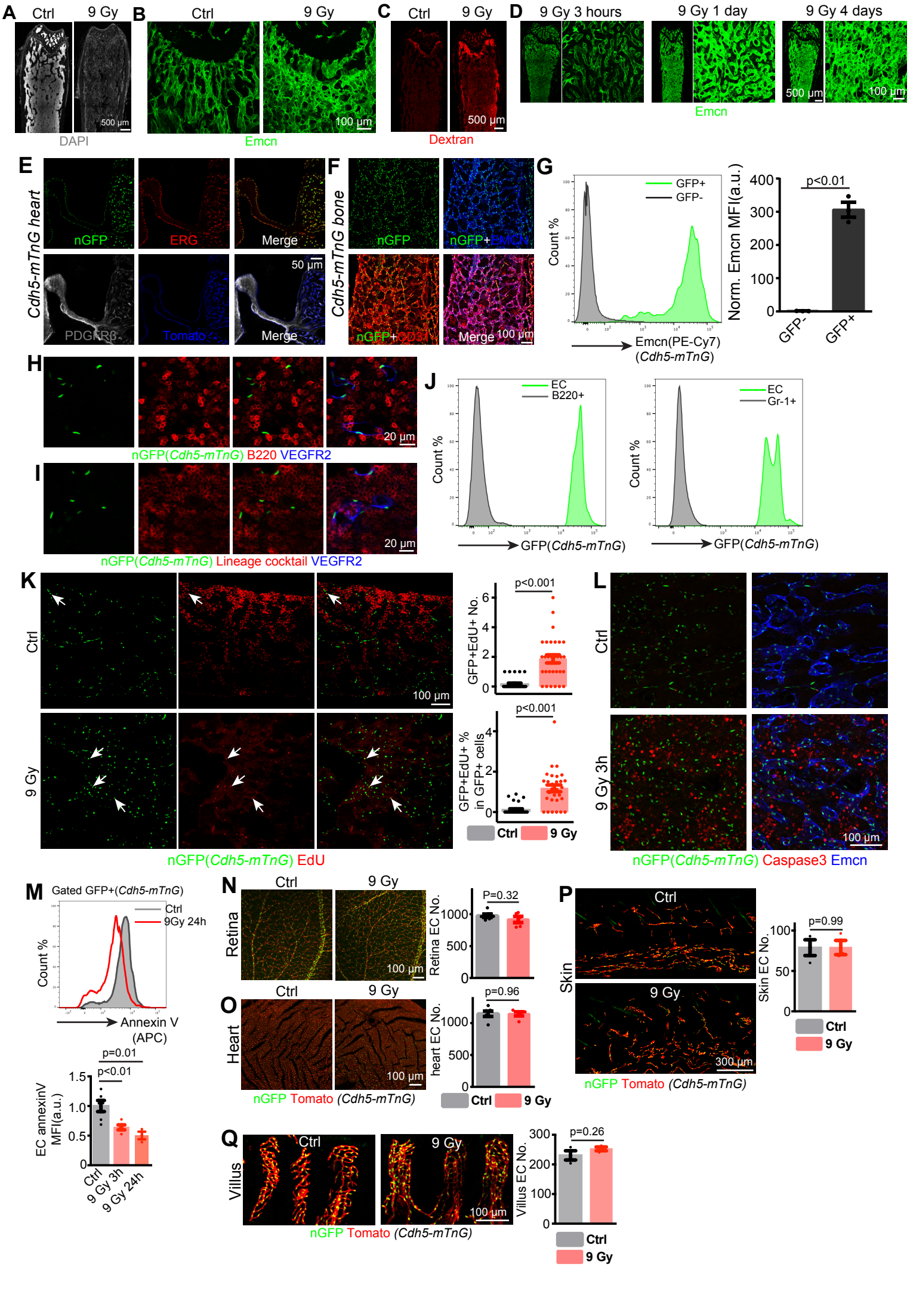
A
Ctrl
9 Gy
500 μm
DAPI
B
Ctrl
9 Gy
100 μm
Emcn
C
Ctrl
9 Gy
500 μm
Dextran
D
9 Gy 3 hours
9 Gy 1 day
9 Gy 4 days
500 μm
100 μm
Emcn
E
Cdh5-mTnG heart
nGFP
ERG
Merge
50 μm
PDGFRβ
Tomato
Merge
F
Cdh5-mTnG bone
nGFP
nGFP+EMCN
nGFP+CD31
Merge
100 μm
G
GFP+
GFP-
Count %
Emcn(PE-Cy7)
(Cdh5-mTnG)
Norm. Emcn MFI(a.u.)
p<0.01
400
300
200
100
0
GFP-
GFP+
H
20 μm
nGFP(Cdh5-mTnG) B220 VEGFR2
I
20 μm
nGFP(Cdh5-mTnG) Lineage cocktail VEGFR2
J
EC
B220+
Count %
GFP(Cdh5-mTnG)
EC
Gr-1+
Count %
GFP(Cdh5-mTnG)
K
Ctrl
9 Gy
100 μm
p<0.001
GFP+EdU+ No.
6
4
2
0
p<0.001
GFP+EdU+ % in GFP+ cells
4
2
0
Ctrl
9 Gy
nGFP(Cdh5-mTnG) EdU
L
Ctrl
9 Gy 3h
100 μm
nGFP(Cdh5-mTnG) Caspase3 Emcn
M
Gated GFP+(Cdh5-mTnG)
Ctrl
9Gy 24h
Count %
Annexin V
(APC)
p=0.01
p<0.01
EC annexinV MFI(a.u.)
1.5
1.0
0.5
0
Ctrl
9 Gy 3h
9 Gy 24h
N
Retina
Ctrl
9 Gy
100 μm
P=0.32
Retina EC No.
1000
500
0
O
Heart
Ctrl
9 Gy
100 μm
p=0.96
heart EC No.
1000
500
0
Ctrl
9 Gy
nGFP Tomato (Cdh5-mTnG)
P
Skin
Ctrl
9 Gy
300 μm
p=0.99
Skin EC No.
100
50
0
Ctrl
9 Gy
nGFP Tomato (Cdh5-mTnG)
Q
Villus
Ctrl
9 Gy
100 μm
p=0.26
Villus EC No.
300
200
100
0
Ctrl
9 Gy
nGFP Tomato (Cdh5-mTnG)

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 irradiation (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; 9Gy 3h n=5; 9Gy 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.

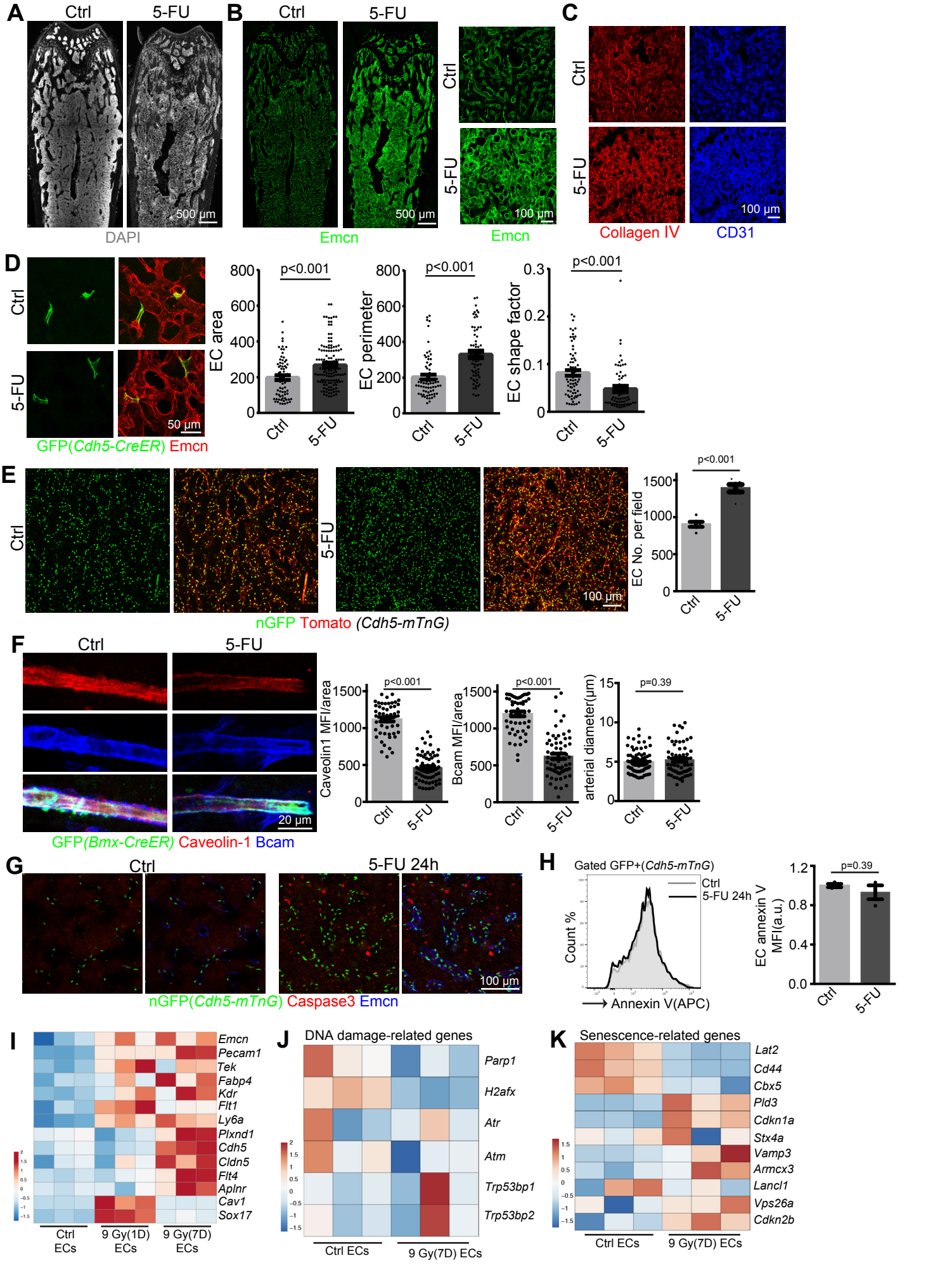
A
Ctrl
5-FU
500 μm
DAPI
B
Ctrl
5-FU
500 μm
Emcn
Ctrl
5-FU
100 μm
Emcn
C
Ctrl
5-FU
100 μm
Collagen IV
CD31
D
Ctrl
5-FU
50 μm
GFP(Cdh5-CreER) Emcn
EC area
p<0.001
EC perimeter
p<0.001
EC shape factor
p<0.001
E
Ctrl
5-FU
100 μm
nGFP Tomato (Cdh5-mTnG)
EC No. per field
p<0.001
F
Ctrl
5-FU
20 μm
GFP(Bmx-CreER) Caveolin-1 Bcam
Caveolin1 MFI/area
p<0.001
Bcam MFI/area
p<0.001
arterial diameter(μm)
p=0.39
G
Ctrl
5-FU 24h
100 μm
nGFP(Cdh5-mTnG) Caspase3 Emcn
H
Gated GFP+(Cdh5-mTnG)
Ctrl
5-FU 24h
Count %
Annexin V(APC)
EC annexin V MFI(a.u.)
p=0.39
I
Emcn
Pecam1
Tek
Fabp4
Kdr
Flt1
Ly6a
Plxnd1
Cdh5
Cldn5
Flt4
Aplnr
Cav1
Sox17
Ctrl ECs
9 Gy(1D) ECs
9 Gy(7D) ECs
J
DNA damage-related genes
Parp1
H2afx
Atr
Atm
Trp53bp1
Trp53bp2
Ctrl ECs
9 Gy(7D) ECs
K
Senescence-related genes
Lat2
Cd44
Cbx5
Pld3
Cdkn1a
Stx4a
Vamp3
Armcx3
Lancl1
Vps26a
Cdkn2b
Ctrl ECs
9 Gy(7D) ECs

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.

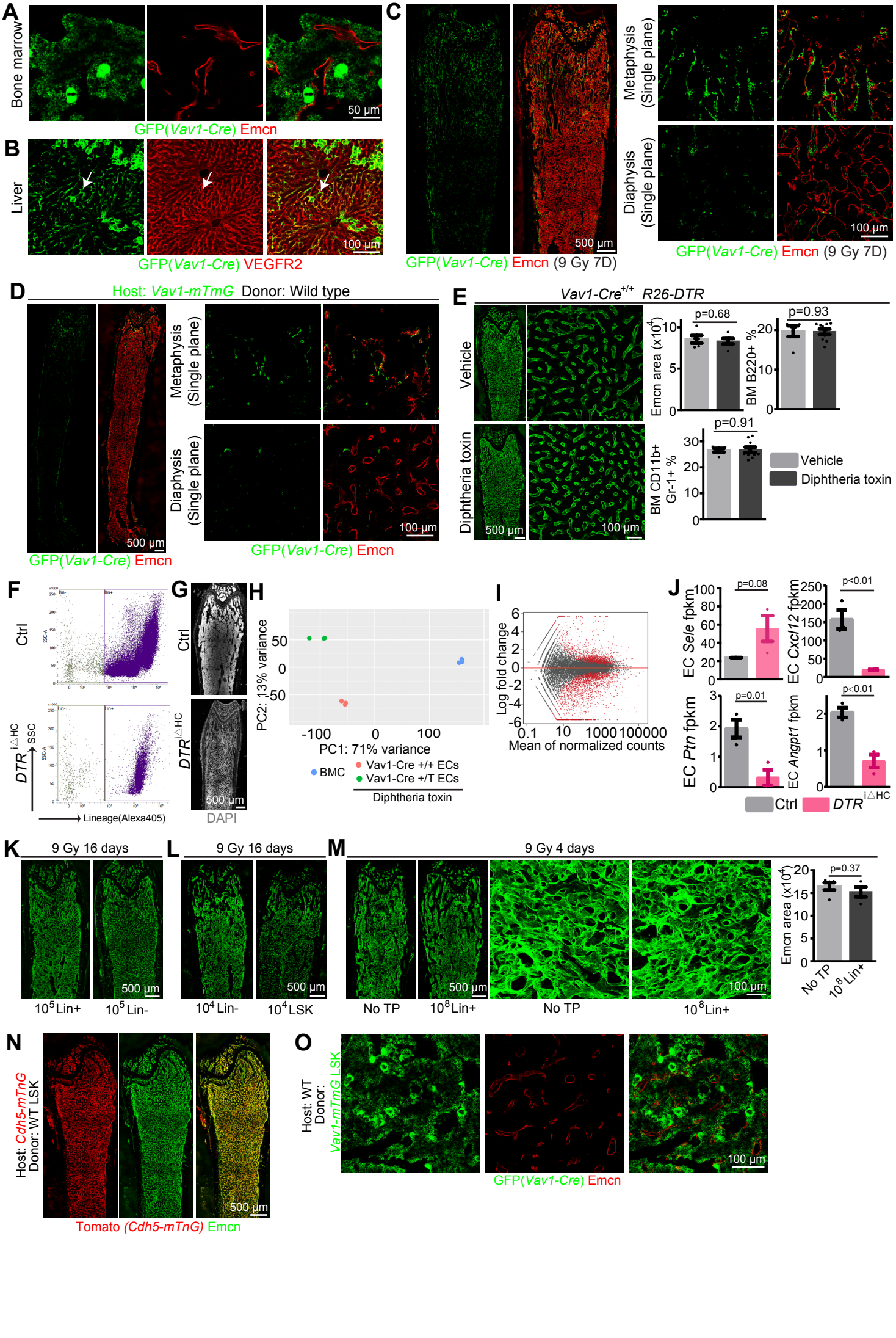
A
Bone marrow
50 μm
GFP(Vav1-Cre) Emcn
B
Liver
100 μm
GFP(Vav1-Cre) VEGFR2
C
500 μm
GFP(Vav1-Cre) Emcn (9 Gy 7D)
Metaphysis (Single plane)
Diaphysis (Single plane)
100 μm
GFP(Vav1-Cre) Emcn (9 Gy 7D)
D
Host: Vav1-mTmG Donor: Wild type
500 μm
GFP(Vav1-Cre) Emcn
Metaphysis (Single plane)
Diaphysis (Single plane)
100 μm
GFP(Vav1-Cre) Emcn
E
Vav1-Cre+/+ R26-DTR
Vehicle
Diphtheria toxin
500 μm
100 μm
Emcn area (x10^4)
p=0.68
BM B220+ %
p=0.93
BM CD11b+ Gr-1+ %
p=0.91
Vehicle
Diphtheria toxin
F
Ctrl
DTR i△HC
SSC
Lineage(Alexa405)
G
Ctrl
DTR i△HC
500 μm
DAPI
H
PC2: 13% variance
PC1: 71% variance
BMC
Vav1-Cre +/+ ECs
Vav1-Cre +/T ECs
Diphtheria toxin
I
Log fold change
Mean of normalized counts
J
EC Sele fpkm
p=0.08
EC Cxcl12 fpkm
p<0.01
EC Ptn fpkm
p=0.01
EC Angpt1 fpkm
p<0.01
Ctrl
DTR i△HC
K
9 Gy 16 days
500 μm
10^5 Lin+
10^5 Lin-
L
9 Gy 16 days
500 μm
10^4 Lin-
10^4 LSK
M
9 Gy 4 days
500 μm
No TP
10^8 Lin+
100 μm
No TP
10^8 Lin+
Emcn area (x10^4)
p=0.37
No TP
10^8 Lin+
N
Host: Cdh5-mTnG
Donor: WT LSK
500 μm
Tomato (Cdh5-mTnG) Emcn
O
Host: WT
Donor: Vav1-mTmG LSK
100 μm
GFP(Vav1-Cre) Emcn

Figure S3. Characterization of DTR$^{i\Delta HC}$ 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$^{i\Delta HC}$ mice.
(G) Projected overview image of DAPI (white) signal 4 days after diphtheria toxin injection in littermate control (Vav1-Cre+/+ R26-DTR) and DTR$^{i\Delta HC}$ mice. The overview images are stitched from several individual z-stacked images.
(H) Principal component analysis of EC gene expression in DTR$^{i\Delta HC}$ and littermate control mice. DTA was injected into both DTR$^{i\Delta HC}$ and control mice, and ECs were isolated 4 days later.
(I) MA plot indicating differentially expressed genes in DTR$^{i\Delta HC}$ and control ECs 4 days after DTA injection.
(J) Quantification of selected HSC-related niche genes from RNA-seq analysis of DTR$^{i\Delta HC}$ 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 $4x10^5$ Lin- or Lin+ cells (K) or of $2x10^4$ 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 $1x10^8$ Lin+ cells. The overview images are stitched from several individual z-stacked images. Quantification of Emcn area (No TP n=5; 10^8 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).

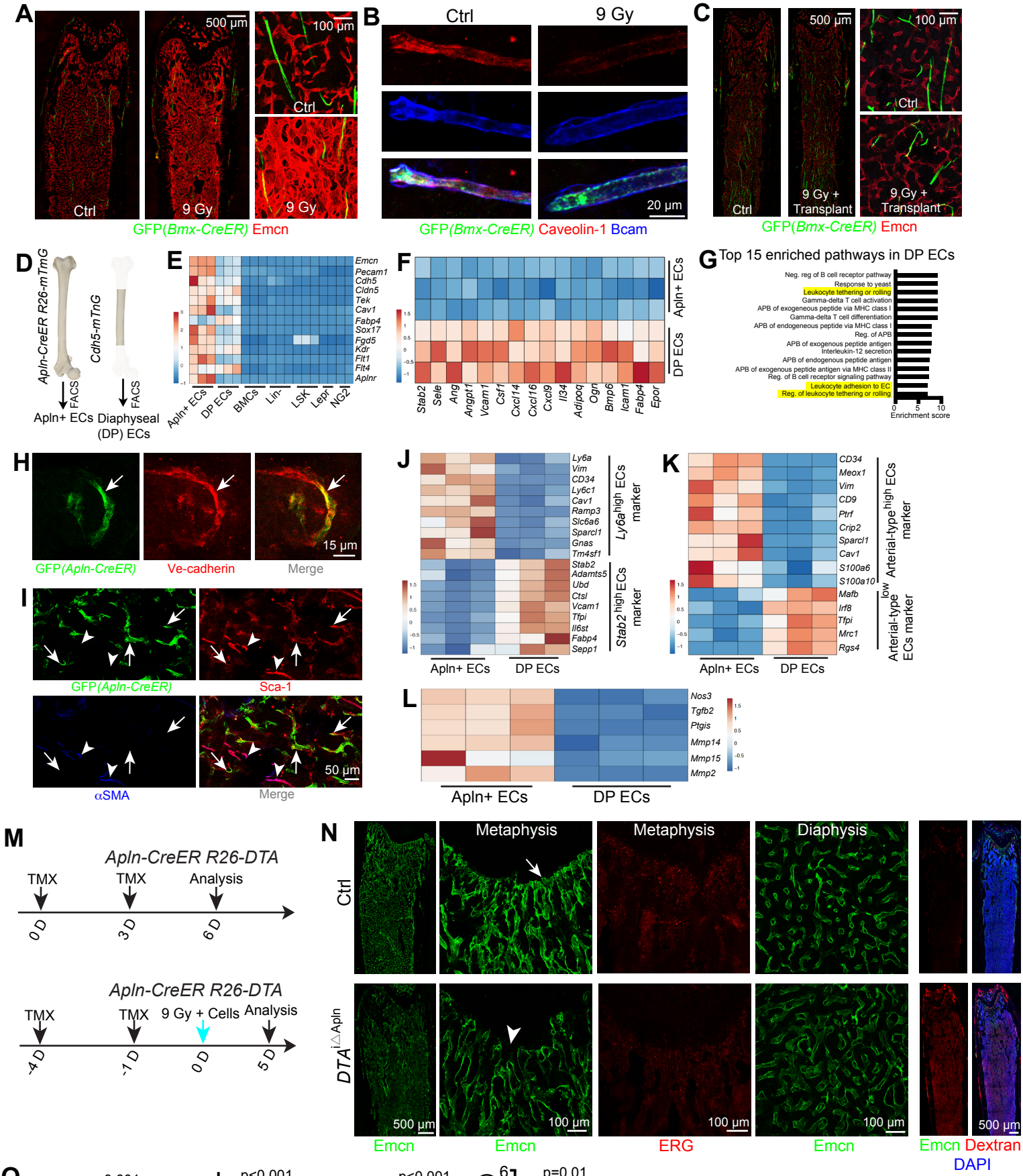
A
500 μm
100 μm
Ctrl
9 Gy
9 Gy
GFP(Bmx-CreER) Emcn
B
Ctrl
9 Gy
20 μm
GFP(Bmx-CreER) Caveolin-1 Bcam
C
500 μm
100 μm
Ctrl
9 Gy + Transplant
9 Gy + Transplant
GFP(Bmx-CreER) Emcn
D
Apln-CreER R26-mTmG
Cdh5-mTmG
FACS
FACS
Apln+ ECs
Diaphyseal (DP) ECs
E
Emcn
Pecam1
Cdh5
Cldn5
Tek
Cav1
Fabp4
Sox17
Fgd5
Kdr
Flt1
Flt4
Aplnr
Apln+ ECs
DP ECs
BMCs
Lin-
LSK
Lepr
NG2
F
Apln+ ECs
DP ECs
Stab2
Sele
Ang
Angpt1
Vcam1
Csf1
Cxcl14
Cxcl16
Cxcl9
Il34
Adipoq
Ogn
Bmp6
Icam1
Fabp4
Epor
G
Top 15 enriched pathways in DP ECs
Neg. reg of B cell receptor pathway
Response to yeast
Leukocyte tethering or rolling
Gamma-delta T cell activation
APB of exogeneous peptide via MHC class I
Gamma-delta T cell differentiation
APB of endogeneous peptide via MHC class I
Reg. of APB
APB of exogenous peptide antigen
Interleukin-12 secretion
APB of endogenous peptide antigen
APB of exogenous peptide antigen via MHC class II
Reg. of B cell receptor signaling pathway
Leukocyte adhesion to EC
Reg. of leukocyte tethering or rolling
0
5
10
Enrichment score
H
15 μm
GFP(Apln-CreER)
Ve-cadherin
Merge
I
GFP(Apln-CreER)
Sca-1
50 μm
αSMA
Merge
J
Ly6a
Vim
CD34
Ly6c1
Cav1
Ramp3
Slc6a6
Sparcl1
Gnas
Tm4sf1
Stab2
Adamts5
Ubd
Ctsl
Vcam1
Tfpi
Il6st
Fabp4
Sepp1
Ly6a high ECs marker
Stab2 high ECs marker
Apln+ ECs
DP ECs
K
CD34
Meox1
Vim
CD9
Ptrf
Crip2
Sparcl1
Cav1
S100a6
S100a10
Mafb
Irf8
Tfpi
Mrc1
Rgs4
Arterial-type high ECs marker
Arterial-type low ECs marker
Apln+ ECs
DP ECs
L
Nos3
Tgfb2
Ptgis
Mmp14
Mmp15
Mmp2
Apln+ ECs
DP ECs
M
Apln-CreER R26-DTA
TMX
TMX
Analysis
0 D
3 D
6 D
Apln-CreER R26-DTA
TMX
TMX
9 Gy + Cells
Analysis
-4 D
-1 D
0 D
5 D
N
Ctrl
DTA iΔApln
Metaphysis
Metaphysis
Diaphysis
500 μm
100 μm
100 μm
100 μm
500 μm
Emcn
Emcn
ERG
Emcn
Emcn Dextran
DAPI
O
MP bulge No./mm
p<0.001
ERG No. in MP
p<0.001
DP Diameter(μm)
p<0.001
Dextran area(x10^6)
p=0.01
Ctrl
DTA iΔApln

Figure S4. Characterization of Apln+ ECs and vascular defects in DTA$^{i\Delta Apln}$ in bone, related to Figure 4

(A) Overview and high magnification confocal images of Emcn+ (red) capillaries and GFP+ (green) arteries in Bmx-CreERT2 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-CreERT2 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 αSMA+ vSMCs (blue, arrowheads). The overview images are stitched from several individual z-stacked images.
(J) Heatmap showing expression of Ly6a^{high} ECs and Stab2^{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$^{i\Delta Apln}$ recipients.
(N) Confocal overview and high-magnification images of Emcn (green) and ERG (red) stained vessels in metaphsis and diaphysis of adult control and DTA$^{i\Delta Apln}$ mice. Arrows indicated EC bulbs near control (Ctrl) growth plate and arrowheads mark local vessel lesions in DTA$^{i\Delta Apln}$ samples. Confocal overview image of Emcn (green), extravasation dextran (red) and DAPI (blue) stained BM of adult DTA$^{i\Delta Apln}$ and control mice. The overview images are stitched from several individual z-stacked images.
(O) Quantification of vessel buds (ctrl=6, DTA$^{i\Delta Apln}$=6) and ERG+ cells (ctrl=5 , DTA$^{i\Delta Apln}$ =5) in metaphysis, diameter of diaphyseal vessels (ctrl=6 , DTA$^{i\Delta Apln}$ =6), dextran leakage area (ctrl=3 , DTA$^{i\Delta Apln}$ =4). Error bars, mean± s.e.m. P values, two-tailed unpaired Students’ t-Test.

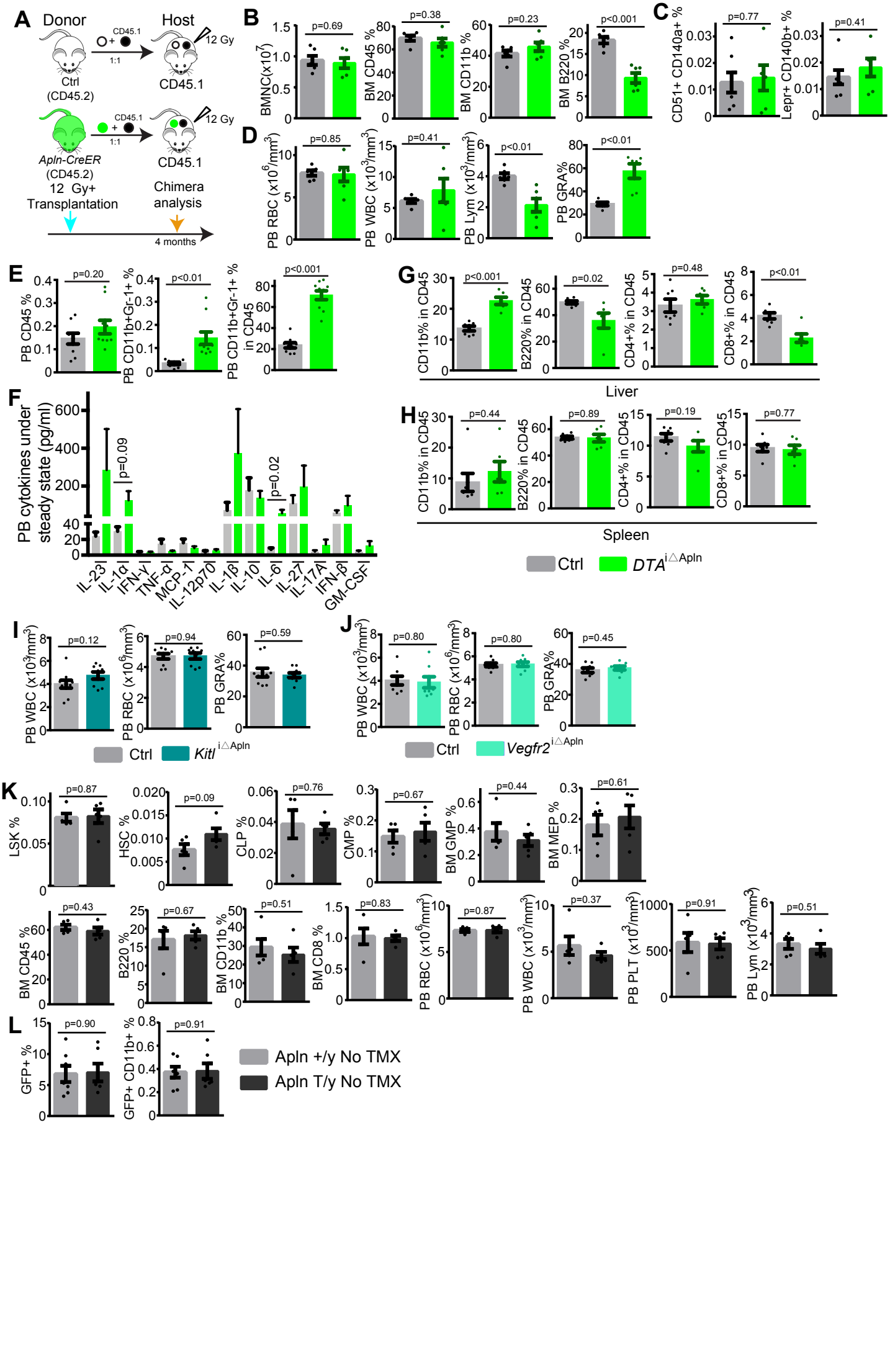
A
Donor
Host
CD45.1
1:1
12 Gy
Ctrl
(CD45.2)
CD45.1
Apln-CreER
(CD45.2)
12 Gy+
Transplantation
Chimera
analysis
4 months
B
BMNC(x10^7)
BM CD45 %
BM CD11b %
BM B220 %
p=0.69
p=0.38
p=0.23
p<0.001
C
CD51+ CD140a+ %
Lepr+ CD140b+ %
p=0.77
p=0.41
D
PB RBC (x10^6/mm^3)
PB WBC (x10^3/mm^3)
PB Lym (x10^3/mm^3)
PB GRA%
p=0.85
p=0.41
p<0.01
p<0.01
E
PB CD45 %
PB CD11b+Gr-1+ %
PB CD11b+Gr-1+ % in CD45
p=0.20
p<0.01
p<0.001
G
CD11b% in CD45
B220% in CD45
CD4+% in CD45
CD8+% in CD45
p<0.001
p=0.02
p=0.48
p<0.01
Liver
F
PB cytokines under steady state (pg/ml)
p=0.09
p=0.02
IL-23
IL-1α
IFN-γ
TNF-α
MCP-1
IL-12p70
IL-1β
IL-10
IL-6
IL-27
IL-17A
IFN-β
GM-CSF
H
CD11b% in CD45
B220% in CD45
CD4+% in CD45
CD8+% in CD45
p=0.44
p=0.89
p=0.19
p=0.77
Spleen
Ctrl
DTA^iΔApln
I
PB WBC (x10^3/mm^3)
PB RBC (x10^6/mm^3)
PB GRA%
p=0.12
p=0.94
p=0.59
Ctrl
Kitl^iΔApln
J
PB WBC (x10^3/mm^3)
PB RBC (x10^6/mm^3)
PB GRA%
p=0.80
p=0.80
p=0.45
Ctrl
Vegfr2^iΔApln
K
LSK %
HSC %
CLP %
CMP %
BM GMP %
BM MEP %
p=0.87
p=0.09
p=0.76
p=0.67
p=0.44
p=0.61
BM CD45 %
B220 %
BM CD11b %
BM CD8 %
PB RBC (x10^6/mm^3)
PB WBC (x10^3/mm^3)
PB PLT (x10^3/mm^3)
PB Lym (x10^3/mm^3)
p=0.43
p=0.67
p=0.51
p=0.83
p=0.87
p=0.37
p=0.91
p=0.51
L
GFP+ %
GFP+ CD11b+ %
p=0.90
p=0.91
Apln +/y No TMX
Apln T/y No TMX

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 $DTA^{i\Delta Apln}$, $Kitl^{i\Delta Apln}$ and $Vegfr2^{i\Delta Apln}$ mice.
(B) Flow cytometric analysis of hematopoietic cell subpopulations in the $DTA^{i\Delta Apln}$ 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 $DTA^{i\Delta Apln}$ (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 $DTA^{i\Delta Apln}$ 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 $DTA^{i\Delta Apln}$ peripheral blood (PB) components under steady state (Ctrl=9, $DTA^{i\Delta Apln}$=10). Error bars, mean± s.e.m. P values, two-tailed unpaired Student's t-Test.
(F) Cytokine levels in peripheral blood plasma of $DTA^{i\Delta Apln}$ 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, two-tailed unpaired Student's t-Test. P values higher than 0.1 are not indicated.
(G and H) FACS analysis of $DTA^{i\Delta Apln}$ liver (G) and spleen (H) leukocytes under steady-state (Ctrl=7, $DTA^{i\Delta Apln}$ =6). Error bars, mean± s.e.m. P values, two-tailed unpaired Student's t-Test.
(I) Blood count analysis of $Kitl^{i\Delta Apln}$ peripheral blood (PB) components (Ctrl=10; $Kitl^{i\Delta Apln}$ =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 $Vegfr2^{i\Delta Apln}$ peripheral blood (PB) components (Ctrl=8; $Vegfr2^{i\Delta Apln}$ =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.

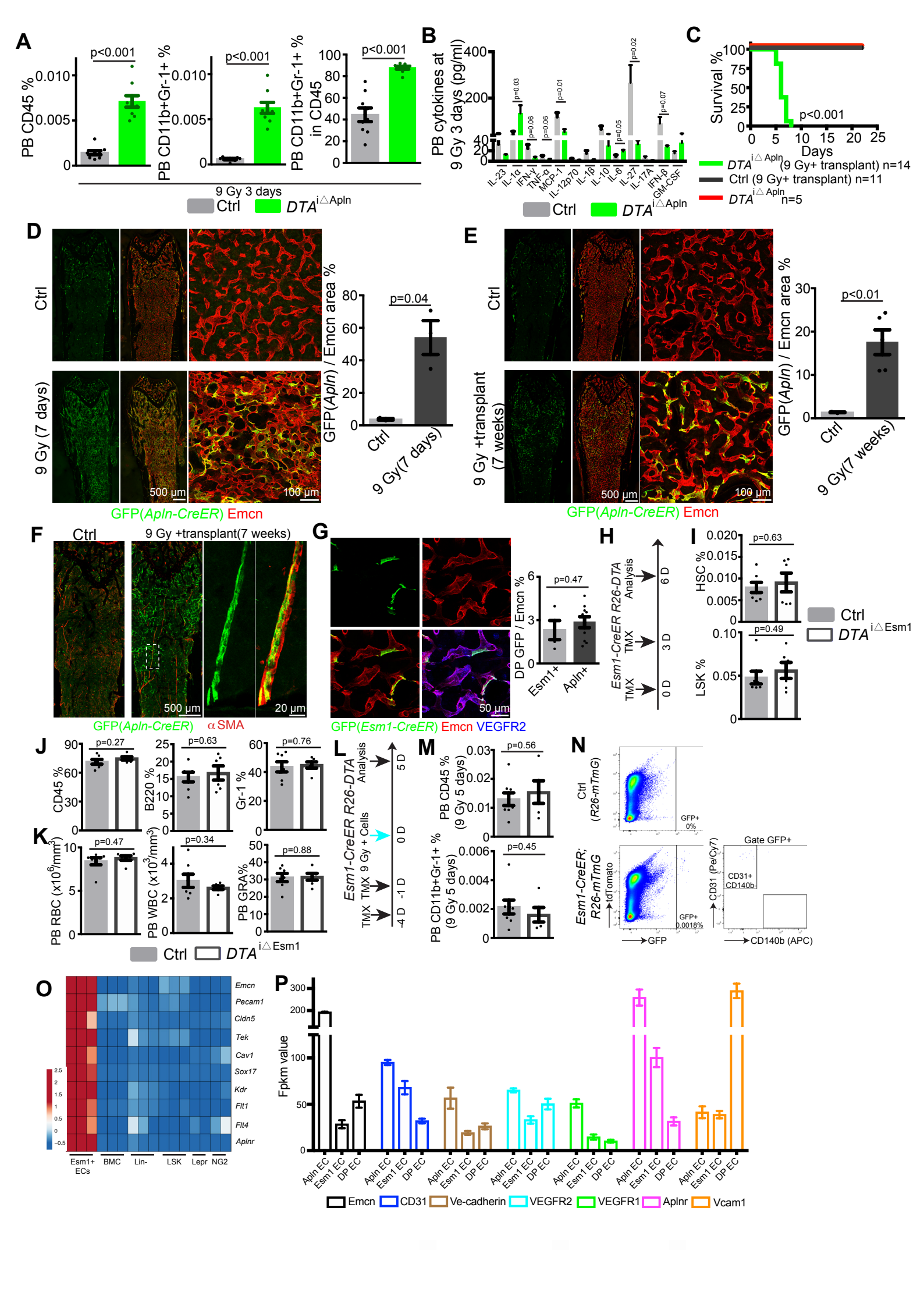
A
PB CD45 %
p<0.001
PB CD11b+Gr-1+ %
p<0.001
PB CD11b+Gr-1+ % in CD45
p<0.001
9 Gy 3 days
Ctrl
DTA iΔApln
B
PB cytokines at 9 Gy 3 days (pg/ml)
IL-23
IL-1α
IFN-γ
TNF-α
MCP-1
IL-12p70
IL-1β
IL-10
IL-6
IL-27
IL-17A
IFN-β
GM-CSF
Ctrl
DTA iΔApln
C
Survival %
p<0.001
Days
DTA iΔApln (9 Gy+ transplant) n=14
Ctrl (9 Gy+ transplant) n=11
DTA iΔApln n=5
D
Ctrl
9 Gy (7 days)
500 μm
100 μm
GFP(Apln) / Emcn area %
p=0.04
Ctrl
9 Gy(7 days)
GFP(Apln-CreER) Emcn
E
Ctrl
9 Gy +transplant (7 weeks)
500 μm
100 μm
GFP(Apln) / Emcn area %
p<0.01
Ctrl
9 Gy(7 weeks)
GFP(Apln-CreER) Emcn
F
Ctrl
9 Gy +transplant(7 weeks)
500 μm
20 μm
GFP(Apln-CreER) αSMA
G
50 μm
DP GFP / Emcn %
p=0.47
Esm1+
Apln+
GFP(Esm1-CreER) Emcn VEGFR2
H
Esm1-CreER R26-DTA
Analysis
TMX
TMX
6 D
3 D
0 D
I
HSC %
p=0.63
LSK %
p=0.49
Ctrl
DTA iΔEsm1
J
CD45 %
p=0.27
B220 %
p=0.63
Gr-1 %
p=0.76
K
PB RBC (x10^6/mm^3)
p=0.47
PB WBC (x10^3/mm^3)
p=0.34
PB GRA%
p=0.88
Ctrl
DTA iΔEsm1
L
Esm1-CreER R26-DTA
Analysis
9 Gy + Cells
TMX
TMX
5 D
0 D
-1 D
-4 D
M
PB CD45 % (9 Gy 5 days)
p=0.56
PB CD11b+Gr-1+ % (9 Gy 5 days)
p=0.45
N
Ctrl (R26-mTmG)
GFP+ 0%
Esm1-CreER; R26-mTmG
tdTomato
GFP+ 0.0018%
GFP
Gate GFP+
CD31+ CD140b-
CD31 (Pe/Cy7)
CD140b (APC)
O
Emcn
Pecam1
Cldn5
Tek
Cav1
Sox17
Kdr
Flt1
Flt4
Aplnr
Esm1+ ECs
BMC
Lin-
LSK
Lepr
NG2
P
Fpkm value
Apln EC
Esm1 EC
DP EC
Emcn
CD31
Ve-cadherin
VEGFR2
VEGFR1
Aplnr
Vcam1

Figure S6. Role of Apln+ ECs but not Esm1+ ECs in hematopoiesis and transplantation, related to Figure 6.

(A) FACS analysis of DTA$^{i\Delta Apln}$ peripheral blood (PB) components 3 days after irradiation and transplantation (Ctrl=9, DTA$^{i\Delta Apln}$ =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$^{i\Delta Apln}$ 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$^{i\Delta Apln}$ mutants (n=5) relative to irradiated (9 Gy) and transplanted control (Apln-CreER+/y, n=11) and DTA $^{i\Delta Apln}$ 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 DTAiΔEsm1 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$^{i\Delta Esm1}$ 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$^{i\Delta Esm1}$ 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 $^{i\Delta Esm1}$ recipients.
(M) FACS analysis of CD45+ or CD11b+Gr-1+ cells in PB of control (n=8) and DTA$^{i\Delta Esm1}$ 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.

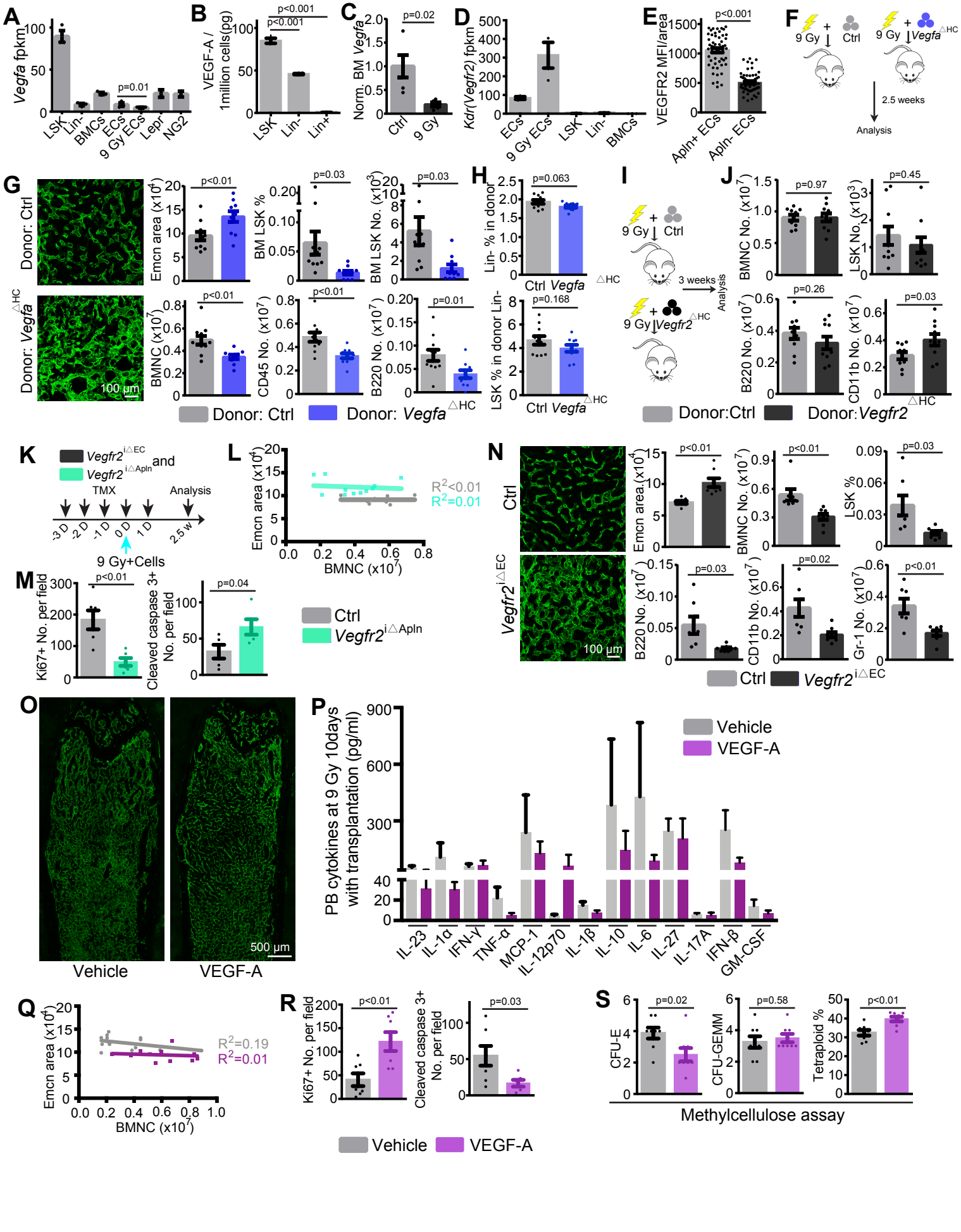
A
Vegfa fpkm
LSK
Lin-
BMCs
ECs
9 Gy ECs
Lepr
NG2
p=0.01
B
VEGF-A / 1million cells(pg)
p<0.001
p<0.001
LSK
Lin-
Lin+
C
Norm. BM Vegfa
p=0.02
Ctrl
9 Gy
D
Kdr(Vegfr2) fpkm
ECs
9 Gy ECs
LSK
Lin-
BMCs
E
VEGFR2 MFI/area
p<0.001
Apln+ ECs
Apln- ECs
F
9 Gy
Ctrl
9 Gy
Vegfa△HC
2.5 weeks
Analysis
G
Donor: Ctrl
Donor: Vegfa△HC
100 μm
Emcn area (x10⁴)
p<0.01
BM LSK %
p=0.03
BM LSK No. (x10³)
p=0.03
BMNC (x10⁷)
p<0.01
CD45 No. (x10⁷)
p<0.01
B220 No. (x10⁷)
p=0.01
△HC
Donor: Ctrl
Donor: Vegfa
H
Lin- % in donor
p=0.063
Ctrl
Vegfa
△HC
LSK % in donor Lin-
p=0.168
Ctrl
Vegfa
△HC
I
9 Gy
Ctrl
3 weeks
Analysis
9 Gy
Vegfr2△HC
J
BMNC No. (x10⁷)
p=0.97
LSK No. (x10³)
p=0.45
B220 No. (x10⁷)
p=0.26
CD11b No. (x10⁷)
p=0.03
△HC
Donor:Ctrl
Donor:Vegfr2
K
Vegfr2 i△EC and Vegfr2 i△Apln
TMX
Analysis
-3 D
-2 D
-1 D
0 D
1 D
2.5 w
9 Gy+Cells
L
Emcn area (x10⁴)
R²<0.01
R²=0.01
BMNC (x10⁷)
Ctrl
Vegfr2 i△Apln
M
Ki67+ No. per field
p<0.01
Cleaved caspase 3+ No. per field
p=0.04
N
Ctrl
Vegfr2 i△EC
100 μm
Emcn area (x10⁴)
p<0.01
BMNC No. (x10⁷)
p<0.01
LSK %
p=0.03
B220 No. (x10⁷)
p=0.03
CD11b No. (x10⁷)
p=0.02
Gr-1 No. (x10⁷)
p<0.01
Ctrl
Vegfr2 i△EC
O
500 μm
Vehicle
VEGF-A
P
PB cytokines at 9 Gy 10days with transplantation (pg/ml)
Vehicle
VEGF-A
IL-23
IL-1α
IFN-γ
TNF-α
MCP-1
IL-12p70
IL-1β
IL-10
IL-6
IL-27
IL-17A
IFN-β
GM-CSF
Q
Emcn area (x10⁴)
R²=0.19
R²=0.01
BMNC (x10⁷)
R
Ki67+ No. per field
p<0.01
Cleaved caspase 3+ No. per field
p=0.03
S
CFU-E
p=0.02
CFU-GEMM
p=0.58
Tetraploid %
p<0.01
Methylcellulose assay
Vehicle
VEGF-A

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 Vegfa$^{\Delta HC}$ 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 Vegfa$^{\Delta HC}$ donor mice. Graphs show Emcn area per field and BMNC number in recipient mice (control=11, Vegfa$^{\Delta HC}$ =10). Quantification of LSK, CD45+, and B220+ cell numbers and LSK percentage (control n=11, Vegfa$^{\Delta HC}$ 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 Vegfa$^{\Delta HC}$ (n=10) and control (n=11) BM under steady state conditions.
(I) Schematic diagram depicting the transplantation of Vegfr2$^{\Delta HC}$ 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 Vegfr2$^{\Delta HC}$ 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 Vegfr2$^{i\Delta EC}$ or Vegfr2$^{i\Delta Apln}$ recipient mice.
(L) Mathematical regression analysis showing the lack of correlation between BMNC number and Emcn+ area in Ctrl (grey, n=11) and Vegfr2$^{i\Delta Apln}$ (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; Vegfr2$^{i\Delta Apln}$ =6) and cleaved caspase 3+ (Ctrl=5; Vegfr2$^{i\Delta Apln}$=5) signal per image field in Ctrl and Vegfr2$^{i\Delta Apln}$ 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 Vegfr2$^{i\Delta EC}$ or control recipients. Quantification of Emcn+ area, BMNC number, LSK percentage, B220 cell number, CD11b cell number and Gr-1 cell number in Vegfr2$^{i\Delta EC}$ (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,

Table S1. Summary of genetic mouse models used in this study.

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
VEGFa^{tm2Gne}	-		-	Conditional knockout of *Vegfa*
Kdr^{tm1Wag}	-		-	Conditional knockout of *Vegfr2/Kdr*
Rosa26-DTR	-		-	Conditional ablation of Cre+ cells, triggered by diphtheria toxin injection
Rosa26-DTA$^{tm1(DTA)Mrc}$	-		-	Conditional ablation of Cre+ cells, triggered by tamoxifen injection
Cdh5(PAC)-CreERT2 Rosa26-mT/mG	*Cdh5-mTmG*	Yes	mem-GFP after Cre recombination	Tamoxifen injection to label all ECs or cells at single cell level
Bmx-CreERT2 Rosa26-mT/mG	*Bmx-mTmG*	Yes	mem-GFP after Cre recombination	Labeling of arterial ECs and their descendants
Apln-CreERT2 Rosa26-mT/mG	*Apln-mTmG*	Yes	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$^{i\Delta HC}$*	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 VEGFa^{tm2Gne}	*Vegfa$^{\Delta HC}$*	No	-	Knockout of *Vegfa* in hematopoietic cells
Vav1-Cre Kdr^{tm1Wag}	*Vegfr2$^{\Delta HC}$*	No	-	Knockout of *Vegfr2* in hematopoietic cells
Apln-CreERT2 Rosa26-DTA	*DTA$^{i\Delta apln}$*	Yes		Inducible ablation of Apln+ ECs, triggered by tamoxifen

Cdh5(PAC)-CreERT2 Kdr^{tm1Wag}	*Vegfr2$^{i\Delta EC}$*	Yes		Knockout of *Vegfr2* in ECs and their descendants
Apln-CreERT2 Rosa26 Kdr^{tm1Wag}	*Vegfr2$^{i\Delta apln}$*	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	Labeling of Esm1+ ECs and their descendants for lineage tracing
Esm1-CreERT2 Rosa26-DTA	*DTA$^{i\triangle Esm1}$*	Yes		Genetic ablation of Esm1+ ECs, triggered by tamoxifen
Kitl$^{tm2.1Sjm/J}$				Conditional knockout of stem cell factor (*Kitl*)
Apln-CreERT2 Kitl$^{tm2.1Sjm/J}$	*Kitl$^{i\Delta apln}$*	Yes		Knockout of stem cell factor (*Kitl*) in Apln+ ECs