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# Targeting skeletal endothelium to ameliorate bone loss

Ren Xu<sup>1</sup>, Alisha Yallowitz<sup>1</sup>, An Qin<sup>2</sup>, Zhu hao Wu<sup>3</sup>, Dong Yeon Shin<sup>1</sup>, Jung-Min Kim<sup>4</sup>, Shawon Debnath<sup>1</sup>, Gang Ji<sup>5,6</sup>, Mathias P. Bostrom<sup>5,7</sup>, Xu Yang<sup>5</sup>, Chao Zhang<sup>8</sup>, Han Dong<sup>9,10</sup>, Pouneh Kermani<sup>11</sup>, Sarfaraz Lalani<sup>1</sup>, Na Li<sup>1</sup>, Yifang Liu<sup>1</sup>, Michael G. Poulos<sup>11</sup>, Amanda Wach<sup>12</sup>, Yi Zhang<sup>1</sup>, Kazuki Inoue<sup>13,14</sup>, Annarita Di Lorenzo<sup>1</sup>, Baohong Zhao<sup>13,14</sup>, Jason M. Butler<sup>11</sup>, Jae-Hyuck Shim<sup>14</sup>, Laurie H. Glimcher<sup>9,10\*</sup> and Matthew B. Greenblatt<sup>1\*</sup>

<sup>1</sup>Department of Pathology and Laboratory Medicine, Cornell University, New York, NY, USA. <sup>2</sup>Department of Orthopaedics, Shanghai Key Laboratory of Orthopaedic Implant, Shanghai Ninth People's Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China. <sup>3</sup>Laboratory of Brain Development and Repair, The Rockefeller University, New York, NY, USA. <sup>4</sup>Division of Rheumatology, Department of Medicine, University of Massachusetts Medical School, Worcester, MA, USA. <sup>5</sup>Research Division, Hospital for Special Surgery, New York, NY, USA. <sup>6</sup>Department of Joint Surgery, The Third Hospital of Hebei Medical University, Shijiazhuang, China. <sup>7</sup>Division of Adult Reconstruction and Joint Replacement, Department of Orthopaedic Surgery, Hospital for Special Surgery, New York, NY, USA. <sup>8</sup>Institute for Computational Biomedicine, Cornell University, New York, NY, USA. <sup>9</sup>Department of Cancer Immunology and Virology, Dana-Farber Cancer Institute and Harvard University Medical School, Boston, MA, USA. <sup>10</sup>Department of Medicine, Harvard Medical School and Brigham and Women's Hospital, Boston, MA, USA. <sup>11</sup>Division of Regenerative Medicine, Department of Medicine, Ansbary Stem Cell Institute, Cornell University, New York, NY, USA. <sup>12</sup>Department of Biomechanics, Hospital for Special Surgery, New York, NY, USA. <sup>13</sup>Arthritis and Tissue Degeneration Program and David Z. Rosensweig Genomics Research Center, Hospital for Special Surgery, New York, NY, USA. <sup>14</sup>Department of Medicine, Weill Cornell Medical College, Cornell University, New York, NY, USA. \*e-mail: [laurie\\_glimcher@dfci.harvard.edu](mailto:laurie_glimcher@dfci.harvard.edu); [mag3003@med.cornell.edu](mailto:mag3003@med.cornell.edu)

## Supplementary Figure legends

Supplementary Figure 1: *Shn3*<sup>-/-</sup> mice have higher levels of CD31<sup>hi</sup>EMCN<sup>hi</sup> endothelium.

**(a)** Representative confocal images ( $n = 3$  total images per group) of heart, brain, lung and kidney from 2 week old mice stained for EMCN (red) and DAPI (blue). Bars, 100 $\mu$ m. **(b)** Representative confocal images and relative quantification of CD31-positive (upper) and EMCN-positive (lower) vessel area in the femurs of neonatal mice.  $n = 3$  per group. Growth plate is marked. **(c)** Schematic representation of the strategy used for flow cytometry for analysis of CD31<sup>hi</sup>EMCN<sup>hi</sup> endothelial cells. Values represent mean  $\pm$  s.e.m.; \* $P < 0.05$  and \*\* $P < 0.01$  by an unpaired two-tailed Student's  $t$ -test in all panels.

Supplementary Figure 2: Ablation of *Shn3* in osteoblasts enhances osteogenesis and angiogenesis in vivo.

**(a)**  $\mu$ CT analysis of trabecular bone volume/total volume (BV/TV) (left) and cortical (right) bone thickness in OSX-Cre ( $n = 3$ ), *Shn3*<sup>ff</sup> ( $n = 3$ ) and *Shn3*<sup>osx</sup> male mice ( $n = 4$ ) at 8-weeks of age. **(b)** Representative confocal images ( $n = 3$  total images per group) of DAPI (left) and direct eGFP/tdTomato (mT mG) fluorescence (right) in metatarsals from OSX-Cre; R26 mT/mG male mice at 7-days of age. Red: membrane-targeted tdTomato; Green: membrane-targeted eGFP; Blue: DAPI. Endosteal surface (ES), Cortical bone (CB) and Periosteal surface (PS). Scale bars, 50 $\mu$ m. **(c)** Representative  $\mu$ CT images (left) and relative cortical bone analysis (right) in *Shn3*<sup>ff</sup> and *Shn3*<sup>dmp1</sup> mice at 8-weeks of age (male,  $n = 5$ ). Scale bars, 500  $\mu$ m. **(d)** Histomorphometric quantification of mineral apposition rate (MAR) and bone formation rate/bone surface (BFR/BS) at the endosteal and periosteal bone surfaces of the L3 vertebrae in *Shn3*<sup>ff</sup> and *Shn3*<sup>dmp1</sup>



mice (male,  $n = 5$ ) at 8-weeks of age. **(e)** Histomorphometry of osteoblast surface/bone surface (Ob.S/BS) and the number of osteoclasts/bone perimeter (No. Oc./B. Pm) at the endosteal bone surfaces of the L3 vertebrae in *Shn3<sup>ff</sup>* and *Shn3<sup>dmp1</sup>* mice (male,  $n = 5$ ) at 8-weeks of age. **(f,g)** Representative confocal images ( $n = 3$  total images per group) of femur sections from 2 week-old male mice stained for CD31 (red) with DAPI (blue) **(f)** or EMCN (red) with DAPI (blue) **(g)**. Growth plate and cortical bone are marked. Scale bars, 300 $\mu$ m. **(h)** Quantification of CD31-positive (upper,  $n = 3$ ) and EMCN-positive (lower,  $n = 4$ ) vessels in the BM cavity in *Shn3<sup>ff</sup>* and *Shn3<sup>dmp1</sup>* male mice at 2-weeks of age. **(i)** Real-time PCR analysis of *Shn3* expression in BM derived endothelial cells sorted from *Shn3<sup>ff</sup>* and *Shn3<sup>cdh5</sup>* male mice at 2-weeks of age ( $n = 4$ ). **(j,k)** Representative confocal images ( $n = 4$  total images per group) from 2 week-old *Shn3<sup>ff</sup>* and *Shn3<sup>cdh5</sup>* male mice stained for CD31 (red) with DAPI (blue) **(j)** or EMCN (red) with DAPI (blue) **(k)**. Growth plates and cortical bone are marked. Scale bars, 300 $\mu$ m. **(l)** Quantification of CD31-positive and EMCN-positive vessels in the BM cavity in *Shn3<sup>ff</sup>* and *Shn3<sup>cdh5</sup>* male mice at 2-weeks of age. ( $n = 4$ ). **(m)** Representative confocal images ( $n = 3$  total images per group) of CD31 (green) and EMCN (red) immunostained sections from the femur sections of 2 week-old *Shn3<sup>ff</sup>* and *Shn3<sup>cdh5</sup>* male mice. Growth plate is marked. **(n)** Representative  $\mu$ CT images of trabecular bone in the distal femur (left) and relative BV/TV analysis (right) in *Shn3<sup>ff</sup>* ( $n = 7$ ) and *Shn3<sup>cdh5</sup>* ( $n = 8$ ) male mice at 8-weeks of age. **(o)** Quantitative  $\mu$ CT analysis of cortical bone thickness in *Shn3<sup>ff</sup>* (oil = 7; tamoxifen = 7) and *Shn3<sup>ocn-ert2</sup>* mice (oil = 6; tamoxifen = 8) at 12-weeks after tamoxifen injection or corn oil injection at 4-weeks of age. Values represent mean  $\pm$  s.e.m; \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$  by a two-way ANOVA followed by Sidak's

test (panel d) and unpaired two-tailed Student's *t*-test or by one-way ANOVA followed by a Dunnett's test in all other panels.

### Supplementary Figure 3: *Slit3* is expressed in osteoblasts

**(a)** Real-time PCR analysis of *Slit3* expression in mouse brain, lung, kidney, heart, liver, spleen, bone marrow (BM), bones (femur and tibia after BM flush), primary osteoblasts (POBs) and osteoclasts ( $n = 4$ ). **(b)** Real-time PCR analysis of *Slit3* expression during sorted human CD14<sup>+</sup> cell differentiation with macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor kappa-B ligand (RANKL) ( $n = 4$ ). Human brain cDNA (Clontech) is used as a positive control. **(c)** Representative confocal images ( $n = 2$  total images per group) of femur sections from 2 week-old *Slit3*<sup>+/-</sup> or *Robo1*<sup>+/-</sup> male mice with a lacZ knock-in and the control mice stained for beta-galactosidase (green), EMCN (red) and DAPI (blue). Growth plate is marked. Scale bars, 100μm (top); 50μm (bottom). **(d)** Real-time PCR analysis of *Alpl*, *Ibsp* and *Slit3* in hMSCs, respectively ( $n = 4$ ). **(e)** mRNA (left) and protein (right) levels of *Slit3* in murine POBs transduced with a control construct expressing Cre or a construct expressing SHN3 and subsequently cultured under osteogenic conditions.  $n = 3$  per group. **(f)** ELISA for platelet-derived growth factor-BB (PDGF-BB) in osteoblast CM ( $n = 4$ ) and serum ( $n = 3$ ) from *Shn3*<sup>-/-</sup> mice and the littermate controls. **(g)** Real-time PCR analysis of *Slit3* in POBs treated with the indicated doses of trametinib (TTNB).  $n = 6$  per group. Values represent mean  $\pm$  s.e.m.; \*\* $P < 0.01$  and \*\*\* $P < 0.001$  by an unpaired two-tailed Student's *t*-test or by one-way ANOVA followed by a Dunnett's test in all panels. All immunoblots are cropped from the original.

#### Supplementary Figure 4: SLITs have proangiogenic activity

**(a)** Representative images (left) and relative quantification (right) from a wound healing assay of BM-derived endothelial progenitors outgrowth cells (EPOCs) after SLIT3 stimulation.  $n = 3$  per group. Scale bars, 200 $\mu$ m. **(b)** Alamar blue based proliferation assay in EPOCs with SLIT3 treatment.  $n = 5$  per group. **(c,d)** Representative images (**c**, left and **d**, left) and relative quantification of tube branch numbers (**c**, right and **d**, right) of a Matrigel tube formation assay in EPOCs after SLIT1 or SLIT2 treatment.  $n = 5$  per group. **(e)** Effects of SLIT3 treatment on YAP, AKT and ERK phosphorylation in mouse EPOCs after serum starvation as determined by immunoblotting. **(f)** Representative flow cytometry plots (left) and relative frequency of CD31<sup>hi</sup>EMCN<sup>hi</sup> endothelial cells (right) in cultures of EPOCs after SLIT3 stimulation, FGF2 as a positive control (Control = 6, SLIT3 = 6, FGF2 = 3). **(g)** Representative flow cytometry plots (left) and relative frequency of CD31<sup>hi</sup>EMCN<sup>hi</sup> endothelial cells (right) in the Ter119<sup>-</sup>, CD45<sup>-</sup> cell fraction isolated from the femurs of 8 week-old B6 male mice (JAX) after twice weekly IV injection of SLIT3 (1mg/kg) or vehicle initiated 21 days prior to sacrifice. (Vehicle = 5; SLIT3 = 4). **(h)** Representative flow cytometry dot plots (left), and relative frequency of CD31<sup>hi</sup>EMCN<sup>hi</sup> endothelial cells (right) in femurs of 8 week-old B6 male mice (JAX) after twice weekly IV injection of SLIT2 (1mg/kg) or vehicle initiated 21 days prior to sacrifice.  $n = 4$  per group. Values represent mean  $\pm$  s.e.m.; \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$  by an unpaired two-tailed Student's  $t$ -test or by one-way ANOVA followed by a Dunnett's test in all panels. All immunoblots are cropped from the original.

Supplementary Figure 5: SLIT3 does not directly regulate osteoblast differentiation or activity

**(a)** Body weight of 1month (left) and 3 month (right) old *Slit3*<sup>-/-</sup> male mice and littermate controls (1month:  $n = 4$ ; 3month: *Slit3*<sup>+/+</sup> = 6, *Slit3*<sup>-/-</sup> = 5). **(b)** Histomorphometry of the trabecular No. Oc./B. Pm of the L3 vertebrae in *Slit3*<sup>-/-</sup> mice and littermate controls mice at 6-weeks of age ( $n = 7$ ). **(c)** ELISA for type I collagen carboxy-terminal telopeptide (CTX) levels in serum samples collected from *Slit3*<sup>-/-</sup> male mice ( $n = 8$ ) and littermate controls ( $n = 6$ ) at 6 weeks of age. **(d)** Real-time PCR analysis of *Sp7* ( $n = 3$ ), *Runx2* ( $n = 3$ ), *Ibsp* ( $n = 4$ ) and *Bglap* ( $n = 4$ ) expression in mouse POBs derived from neonatal *Slit3*<sup>-/-</sup> mice and littermate controls after 6 days of culture under osteoblast differentiation conditions. **(e,f)** Alkaline phosphatase (ALP) activity **(e)**, representative images of Alizarin red staining **(f, left)** and relative mineralization assay **(f, right)** in mouse POBs derived from neonatal *Slit3*<sup>-/-</sup> and littermate control mice ( $n = 6$ ) after 6 days of culture under osteoblast differentiation conditions. **(g,h)** ALP activity assay in hMSCs treated with different doses of recombinant SLIT3 **(g)** or Robo1-Fc **(h)** after 6 days of osteogenic culture.  $n = 5$  per group. Values represent mean  $\pm$  s.e.m.; \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$  by an unpaired two-tailed Student's *t*-test or by one-way ANOVA followed by a Dunnett's test in all panels.

Supplementary Figure 6: Ablation of *Slit3* in osteoblasts but not in endothelial cells or neurons impairs osteogenesis and angiogenesis in vivo.

(a) Protein levels of SLIT3 in POBs collected from *Slit3<sup>ff</sup>* mice subsequently transduced with *Cre* expressing or control lentivirus. (b,c) Representative  $\mu$ CT images of the trabecular bone in the distal femur (b) and relative quantitative analysis of BV/TV and cortical bone thickness (c) in *Slit3<sup>ff</sup>* ( $n = 4$ ) and *Slit3<sup>osx</sup>* male mice ( $n = 6$ ) at 8-weeks of age. Scale bars, 1mm. (d) Representative  $\mu$ CT images of the trabecular bone in the distal femur (left) and relative BV/TV analysis (right) in *Slit3<sup>ff</sup>* ( $n = 6$ ) and *Slit3<sup>dmp1</sup>* ( $n = 7$ ) male mice at 8-weeks of age. Scale bars, 1mm. (e) Representative flow cytometry plots (left) and relative frequency of CD31<sup>hi</sup>Emcn<sup>hi</sup> endothelial cells (right) within the Ter119<sup>-</sup>, CD45<sup>-</sup> cell fraction isolated from the femur of 2-week-old *Slit3<sup>ff</sup>* and *Slit3<sup>dmp1</sup>* mice ( $n = 4$  per group). (f) Representative  $\mu$ CT images of the trabecular bone in the distal femur (b) and relative BV/TV analysis in *Slit3<sup>ff</sup>* ( $n = 6$ ) and *Slit3<sup>cdh5</sup>* ( $n = 5$ ) male mice at 8-weeks of age. Scale bars, 1mm. (g) Representative  $\mu$ CT images of the trabecular bone in the distal femur (left) and relative BV/TV analysis (right) in *Slit3<sup>ff</sup>* ( $n = 5$ ) and *Slit3<sup>syn1</sup>* ( $n = 6$ ) male mice at 8-weeks of age. Scale bars, 1mm. Values represent mean  $\pm$  s.e.m.;  $P$  value,  $*P < 0.05$  and  $**P < 0.01$  by an unpaired two-tailed Student's  $t$ -test in all panels.

Supplementary Figure 7: The interaction between *Slit3* and *Shn3* null alleles is intrinsic to osteoblasts

(a,b) Representative  $\mu$ CT images of the trabecular bone in the distal femur metaphysis (a) and relative BV/TV analysis of 8 week-old *OSX cre* ( $n = 5$ ), *OSX cre-Slit3<sup>f/+</sup>* ( $n = 6$ ), *Osx cre-Shn3<sup>f/+</sup>* ( $n = 5$ ) and *OSX cre-Slit3<sup>f/+</sup>Shn3<sup>f/+</sup>* ( $n = 5$ ) male mice. (b). Scale bars,

1mm. Values represent mean  $\pm$  s.e.m.; \*\*\* $P < 0.001$  by a one-way ANOVA followed by a Dunnett's test in all panels.

### Supplementary Figure 8: ROBO1 is a key receptor of SLIT3 signals regulating skeletal angiogenesis and bone mass accrual

**(a)** Heatmap of SLIT/ROBO expression from three sets per genotype of independently derived CD31<sup>hi</sup>EMCN<sup>hi</sup> endothelial cells sorted from femur of *Shn3<sup>fl/fl</sup>* and *Shn3<sup>dmp1</sup>* male mice at 2-weeks of age. **(b,c)** Real-time PCR analysis of *Robo1-4* expression in murine osteoblasts **(b)** and osteoclasts **(c)**, using a murine total brain mRNA preparation as a positive control, (**n** = 4). **(d)** Representative  $\mu$ CT images of the trabecular bone in the distal femur metaphysis (left) and relative BV/TV analysis (right) in *Robo1<sup>-/-</sup>* (**n** = 4) and *Robo1<sup>+/+</sup>* (**n** = 8) female mice at 6-weeks of age. Scale bars, 1mm. **(e)** Representative  $\mu$ CT images of the trabecular bone in the distal femur metaphysis (left) and relative BV/TV analysis (right) in *Robo4<sup>-/-</sup>* and *Robo4<sup>+/+</sup>* mice at 6-weeks of age (female, **n**=6). Scale bars, 1mm. **(f)** Representative flow cytometry dot plots (left) and relative frequency of CD31<sup>hi</sup>EMCN<sup>hi</sup> endothelial cells (right) from femur in *Robo1<sup>-/-</sup>* (**n** = 3) and *Robo1<sup>+/+</sup>* (**n** = 5) male mice at 2-weeks of age. **(g)** Heatmap of SLIT/ROBO expression from two sets per genotype of independently derived CD31<sup>hi</sup>EMCN<sup>hi</sup> endothelial cells sorted from *Robo1<sup>+/+</sup>* and *Robo1<sup>-/-</sup>* male mice at 2-weeks of age. **(h,i)** mRNA and protein levels of *Robo1* **(h)** and *Robo2* **(i)** in mouse EPOCs expressing a control vector or *Robo1* shRNA. Brain is included as a positive control for *Robo2* expression. Values represent mean  $\pm$  s.e.m.; \* $P < 0.05$  and \*\* $P < 0.01$  by an unpaired two-tailed Student's *t*-test in all panels. All immunoblots are cropped from the original.

Supplementary Figure 9: The hippo pathway is a key mediator of SLIT3/ROBO1-induced tube formation.

(a) Representative images from EPOCs transduced with a *Robo1* or control shRNA, treated with SLIT3 or FGF2 and subjected to a Matrigel tube formation assay (left), including quantification of tube branch numbers (right).  $n = 5$  per group. Scale bars, 200 $\mu$ m. (b) EPOCs were transduced with a *Robo1* or control shRNA, and immunoblotting was conducted with the indicated antibodies after SLIT3 treatment. (c) Protein levels of YAP were determined by immunoblotting of mouse EPOCs expressing a YAP or control shRNA. (d) EPOCs were transduced with a *Robo1* or control GFP shRNA, treated with SLIT3 or FGF2 and placed in a Matrigel tube formation assay (left) with quantification of tube branch numbers (right).  $n = 5$  per group. Scale bars, 200 $\mu$ m. Values represent mean  $\pm$  s.e.m.; \* $P < 0.05$  and \*\*\* $P < 0.001$  by a one-way ANOVA followed by a Dunnett's test in all panels. All immunoblots are cropped from the original.

Supplementary Figure 10: SLIT3 enhances bone-fracture healing

(a) Representative images ( $n = 3$  total images) of IHC for SLIT3 in human fracture callus. Scale bars, 50 $\mu$ m. Osteoblasts and are indicated by arrows. (b) H&E (top) and CD31 IHC (bottom) performed on serial sections from human fracture callus tissue. Scale bars, 100 $\mu$ m.  $n = 2$  per group. Micro vessels in BM are indicated. (c,d) Quantification of EMCN positive vessel volume/total volume (Relative vessel volume (%)) in the callus area as determined by histomorphometry (c) and bio-mechanical testing of femurs 21 days after fracture (d) in male *Shn3<sup>+/+</sup> Slit3<sup>+/+</sup>*, *Shn3<sup>+/+</sup> Slit3<sup>-/-</sup>*, *Shn3<sup>-/-</sup>*

*Slit3*<sup>+/+</sup> and *Shn3*<sup>-/-</sup> *Slit3*<sup>-/-</sup> mice. *n* = 4 per group. **(e)** Fracture was performed and mice were treated with IV injection of SLIT3 or vehicle. 21 days post-fracture callus tissue was harvested and immunostained for CD31 (green) and EMCN (red). Scale bars, 100µm, *n*=3 per group. Values represent mean ± s.e.m.; \*\**P* < 0.01 and \*\*\**P* < 0.001 by one-way ANOVA followed by a Dunnett's test in all panels.

Supplementary Figure 11: Systemic or local administration of SLIT3 does not cause abnormalities in the amount or morphology of non-skeletal vasculature

**(a)** Representative confocal images of retinal flat-mounts of 8 week old mice stained with VE-cadherin (green) from mice 21 days post-fracture with IV injection of SLIT3 or Vehicle. Scale bars, 500µm. *n* = 3 per group. **(b)** Representative confocal images of heart, kidney and lung sections stained for EMCN (red) and DAPI (blue) from mice 21 days post-fracture with IV injection of SLIT3 or Vehicle. Scale bars, 100µm. *n* = 3 per group. **(c)** Representative images of the whole brain sections stained for neurofilament-M (NF-M, green) and Nissl (red) from mice 21 days post-fracture with IV injection of SLIT3 or Vehicle. Scale bars, 500µm, *n* = 2 per group. **(d)** Representative images of the whole brain sections stained for EMCN from mice 21 days post-fracture with IV injection of SLIT3 or Vehicle. Scale bars, 500µm (top), 50 µm (bottom). *n* = 2 per group. **(e)** Representative µCT images of mouse femurs (*n* = 4) harvested 21 days post-fracture with insertion of a gelatin sponge soaked with SLIT3 or vehicle. A no sponge control is included. Scale bar = 1 mm. **(f)** Representative confocal images of retinal flat-mounts of 8 week old mice stained with ve-cadherin (green). Retinas were harvested from mice 21 days post-fracture with implantation of a gelatin sponge soaked with SLIT3 or vehicle at the time of fracture. Scale bars, 500µm, *n* = 3 per group. **(g)** Representative confocal



images of heart, kidney and lung sections stained for EMCN (red) and DAPI (blue) of mouse femurs 21 days post-fracture with insertion of a gelatin sponge soaked with SLIT3 or vehicle at the time of fracture. Scale bars, 300 $\mu$ m. *n* = 3 per group. **(h)** Representative images of the whole brain sections stained for neurofilament-M (NF-M, green) and Nissl (red). Brains were harvested from mice 21 days post-fracture after implantation of a gelatin sponge soaked with SLIT3 or vehicle. Scale bars, 500 $\mu$ m. *n* = 2 per group.

**Supplementary Figure 12: SLIT3 treatment counteracts the bone loss and impaired skeletal endothelium in the mouse OVX model**

**(a)** Representative images (*n* = 4 total images per group) of the uterus from sham-operated (Sham) and ovariectomized (OVX) mice. Scale bar, 500 $\mu$ m. **(b)** Uterus weight in Sham and OVX mice. *n* = 4 per group. **(c)**  $\mu$ CT analysis of cortical bone thickness, trabecular number (Tb. N), trabecular bone space (Tb. Sp) and trabecular thickness (Tb. Th) of distal femoral metaphyseal regions (Sham = 9, OVX+Vehicle = 10, OVX+Slit3 = 9, OVX+PTH = 8). **(d-f)** Representative confocal images of CD31 (green) and EMCN (red) dual immunostained bone sections **(d)**, *n* = 3) and quantification of histomorphometric parameters **(e, f)**, of the L3 vertebrae from mice after sham operation, OVX with vehicle, OVX with SLIT3 injection and OVX with PTH injection. Periosteal and endosteal BFR/BS (Sham = 10, OVX+Vehicle = 9, OVX+Slit3 = 10, OVX+PTH = 7) and No. Oc./B. Pm (Sham = 10, OVX+Vehicle = 10, OVX+Slit3 = 9, OVX+PTH = 8) and Ob.S/BS (Sham = 10, OVX+Vehicle = 9, OVX+Slit3 = 10, OVX+PTH = 8) in the endosteum. **(g,h)** Representative  $\mu$ CT images of the trabecular

bone in the distal femur (**g**) and relative quantitative analysis of BV/TV (**h**, left) and cortical bone thickness (**h**,right) in mice after sham operation, OVX, or OVX with SLIT3 injection ( $n = 8$ ). Scale bar, 1mm. (**l,j**) Representative flow cytometry dot plots (**i**) and relative frequency of CD31<sup>hi</sup>EMCN<sup>hi</sup> endothelial cells (**j**) of the femur in mice (Sham = 4, OVX+Vehicle = 3, OVX+Slit3 = 4). Values represent mean  $\pm$  s.e.m.; \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.01$  by an unpaired two-tailed Student's  $t$ -test or by one-way ANOVA followed by a Dunnett's test in all panels.

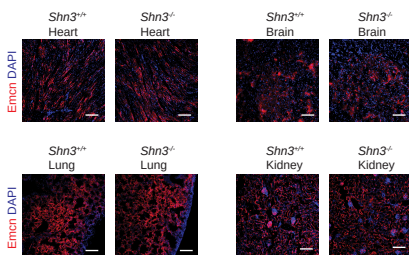
#### Supplementary Figure 13: Uncropped Western blots

The region corresponding to the cropped images is indicated with a dotted line.

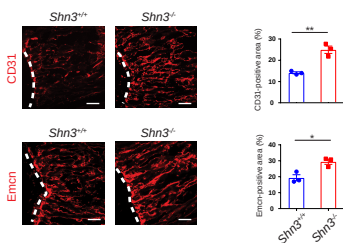
#### Supplementary Table 1: Real-time PCR primer sequences

# Supplementary figure 1

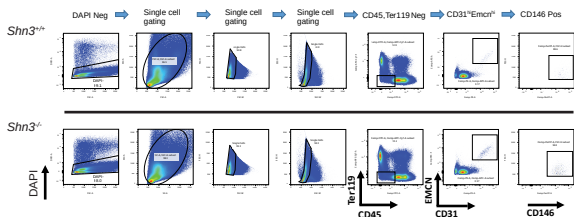
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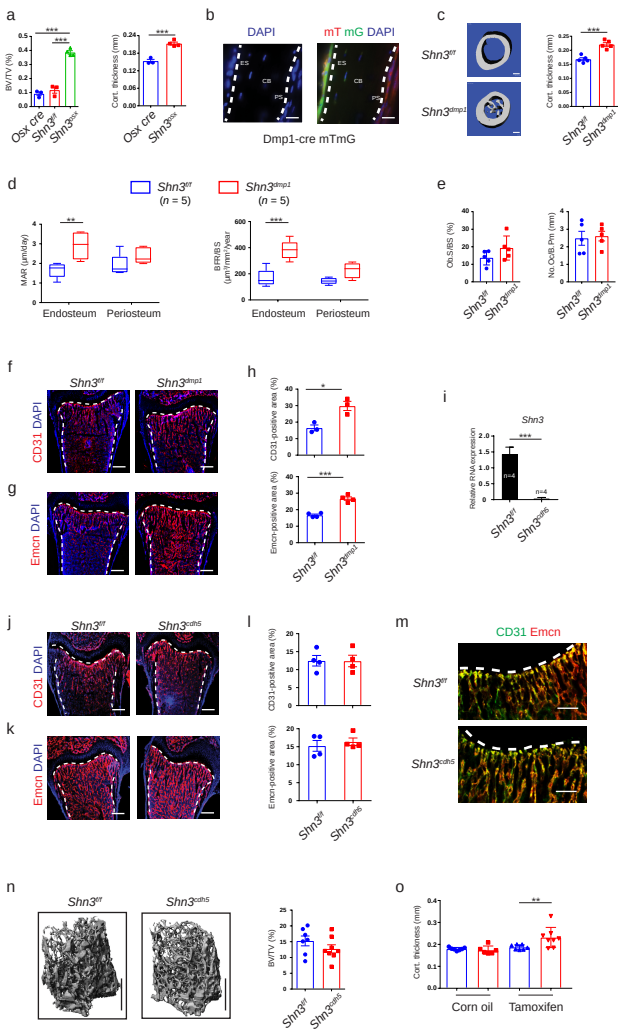
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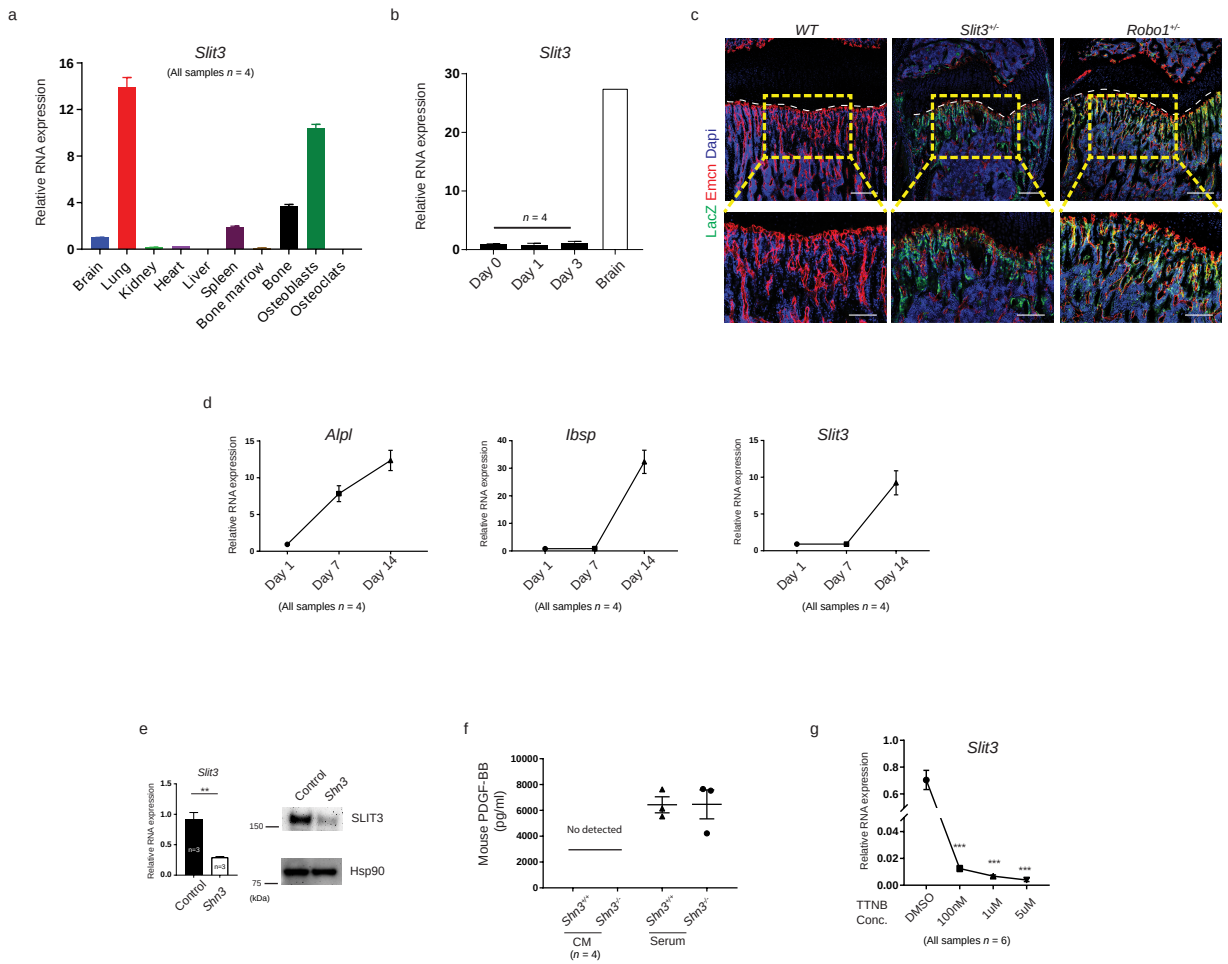
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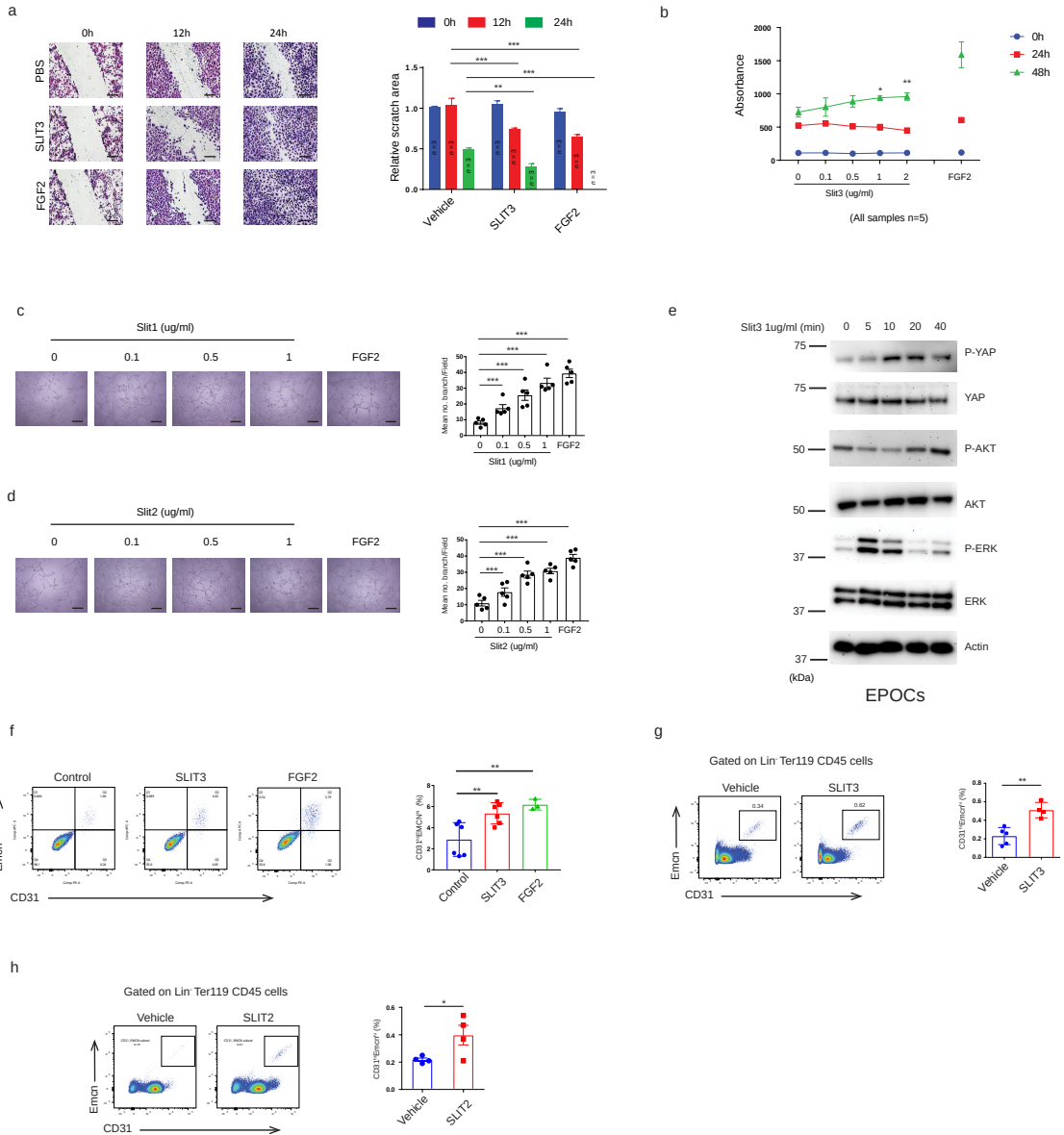
Supplementary figure 2



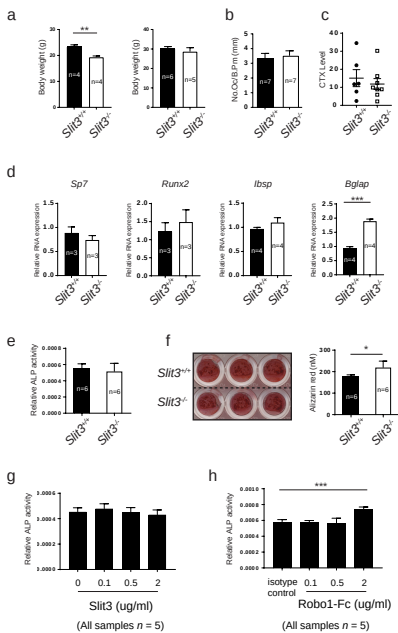
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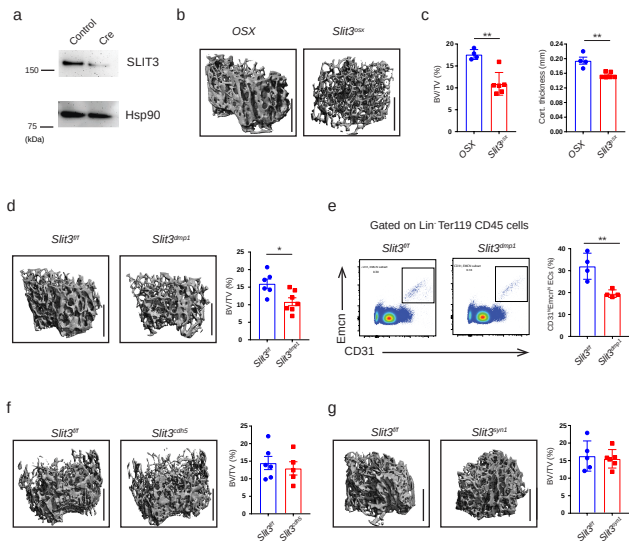
Supplementary figure 4



# Supplementary figure 5



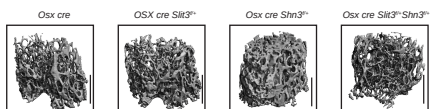
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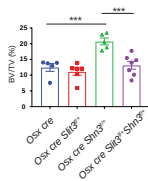


## Supplementary figure 7

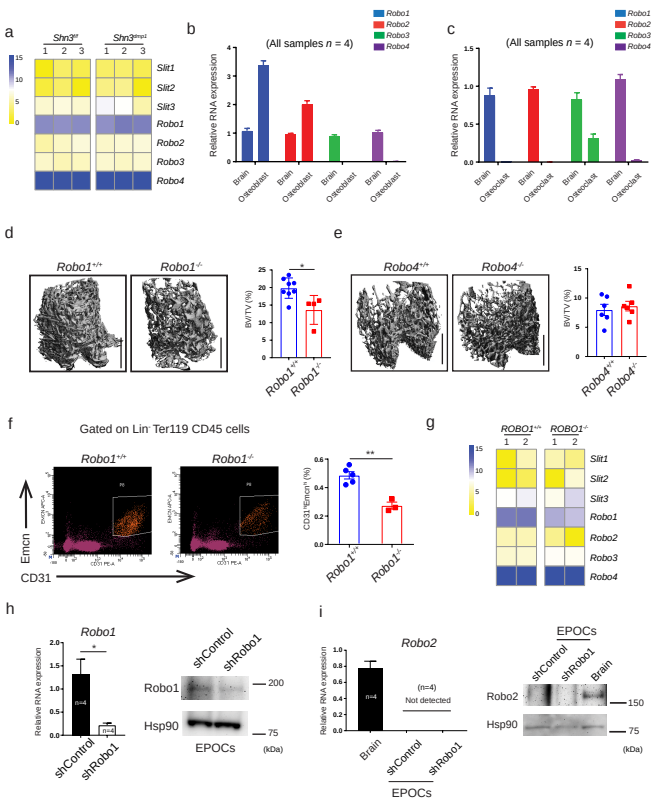
a



b

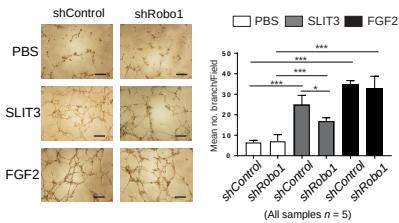


# Supplementary figure 8

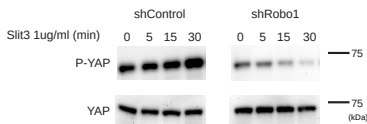


# Supplementary figure 9

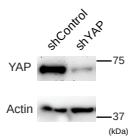
a



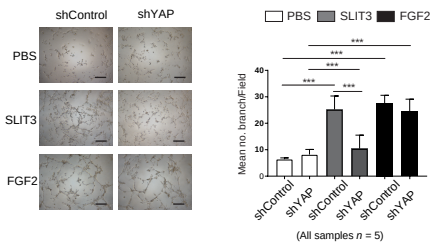
b



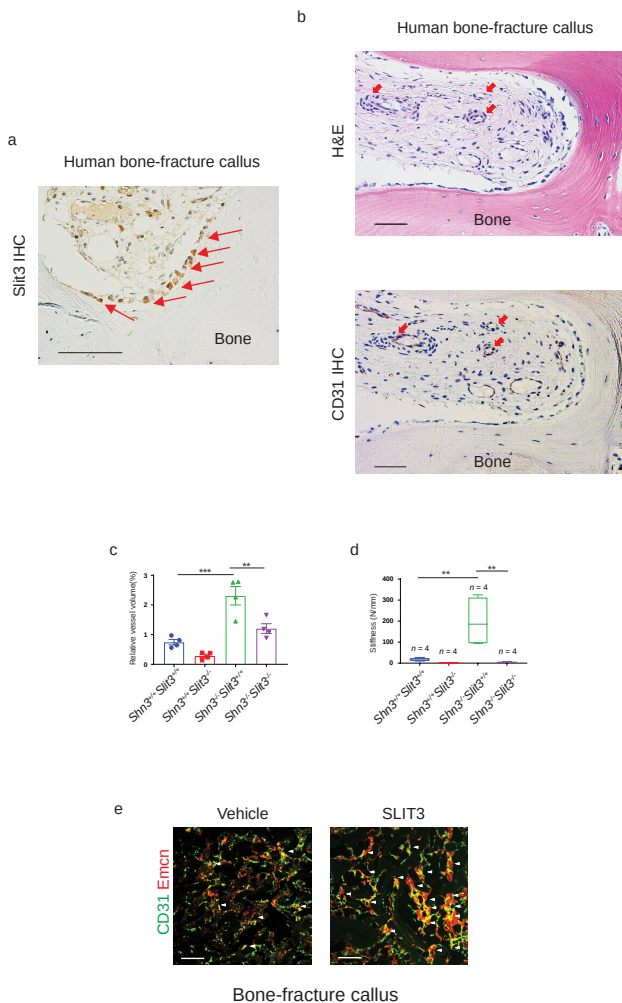
c



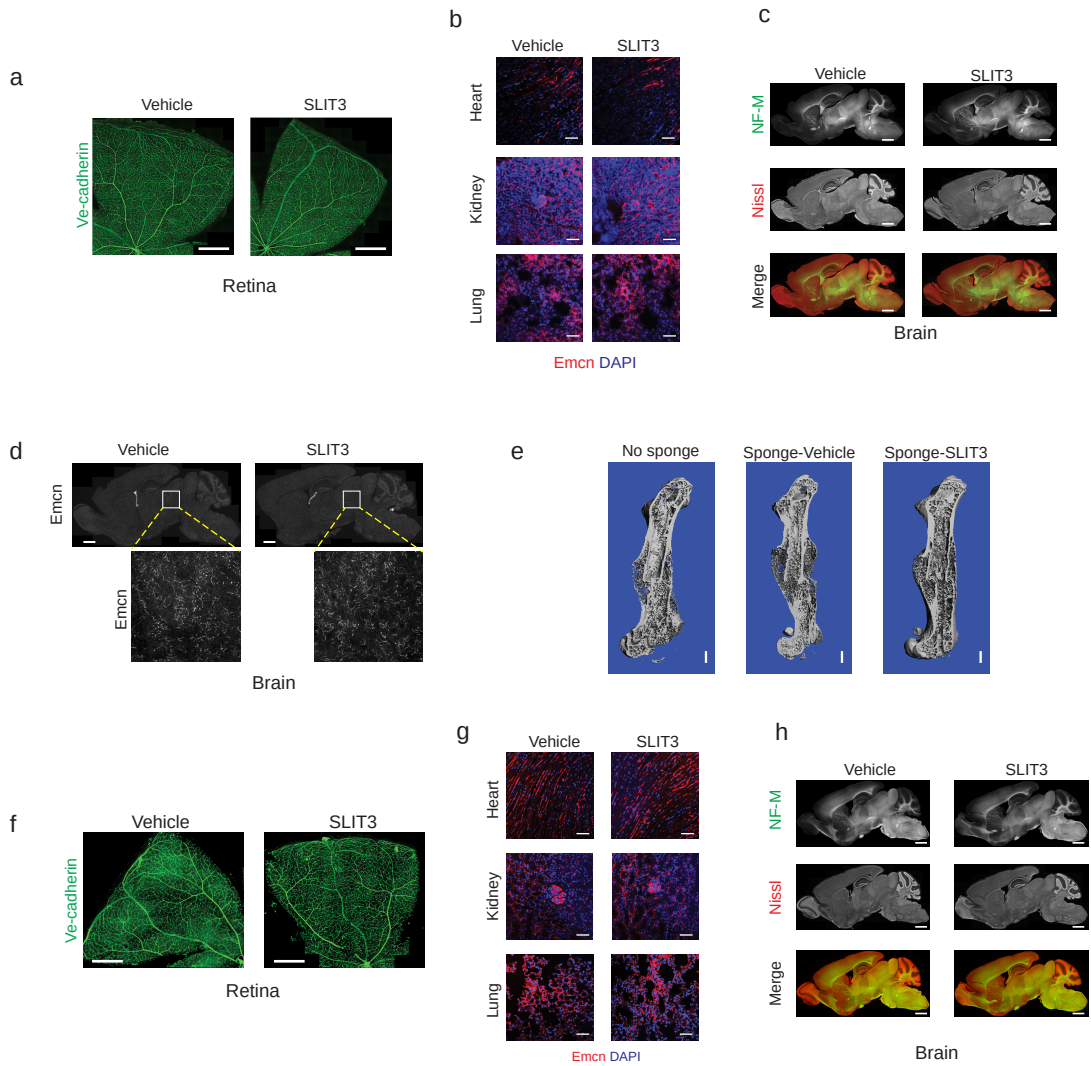
d



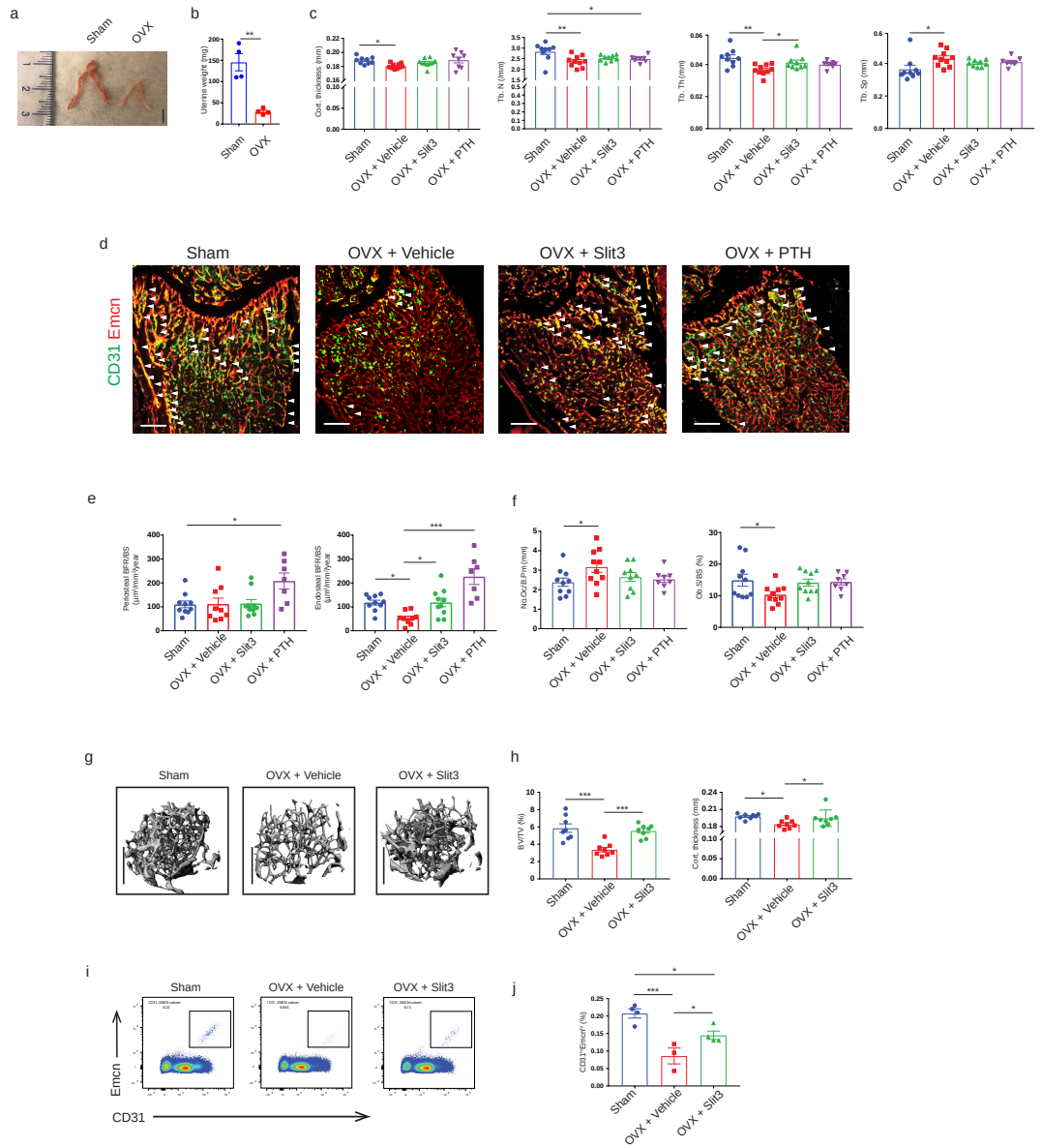
Supplementary figure 10



Supplementary figure 11



Supplementary figure 12



## Supplementary figure 13

Figure 3h

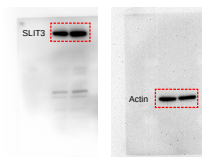
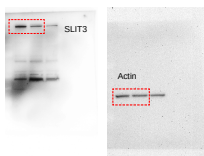
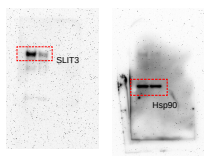


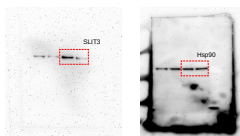
Figure 3i



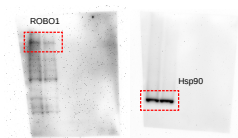
Supplementary Figure 3e



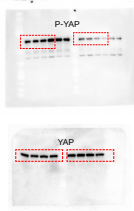
Supplementary Figure 6a



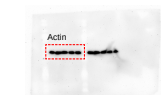
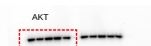
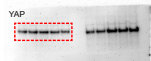
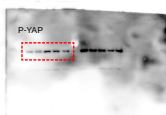
Supplementary Figure 8h



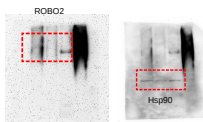
Supplementary Figure 9b



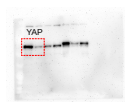
Supplementary Figure 4e



Supplementary Figure 8i



Supplementary Figure 9c



**Supplementary Table 1.**

| Gene        | Forward                   | Reverse                  |
|-------------|---------------------------|--------------------------|
| Human HPRT  | CATTATGCTGAGGATTTGGAAAGG  | CTTGAGCACACAGAGGGCTACA   |
| Human SLIT3 | CCAGATGACTGTGAGGACAACG    | CACAGTGGTCAATCACCTCGTC   |
| Human ALPL  | TAACATCAGGGACATTGACG      | TGCTTGTATCTCGGTTTGAA     |
| Human IBSP  | CAACAGCACAGAGGCAGAAA      | TTGTGGTGGGGTTGTAGGTT     |
| Mouse HPRT  | CTGGTGAAAAGGACCTCTCGAAG   | CAAGATATCGTTGAAACGTGGA   |
| Mouse Runx2 | TACAAACCATACCCAGTCCCTGTTT | AGTGCTCTAACCACAGTCCATGCA |
| Mouse IBSP  | CAGGGAGGCAGTGACTCTTC      | AGTGTGGAAAGTGTGGCGTT     |
| Mouse SP7   | ATGGCGTCCTCTCTGCTTGA      | GAAGGGTGGGTAGTCATTTG     |
| Mouse BGLAP | GCAGCACAGGTCCTAAATAG      | GGGCAATAAGGTAGTGAACAG    |
| Mouse SLIT3 | TCCAGTGTTCTGAAGGCTCCT     | TGGCAATGCCAGGCTCCTTGTA   |
| Mouse ROBO1 | GGCAGCCTGTTGGATTATCCTC    | GTTGGCGTGAAGGTAAAGGACG   |
| Mouse ROBO2 | CCACCATCCAAACCTCAGGTCA    | TCTGCCAGCTATTGCTCACCGA   |
| Mouse ROBO3 | CTACAGAAGGGAGCACAGTC      | ATGGCTCAGGAAGCTTGGTC     |
| Mouse ROBO4 | GTCATTGCCAGTAGTGCTGTCC    | AATGGCGTCCTCGCTGGTGTAT   |