nature research

Corresponding author(s): Matthew Greenblatt

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study.
For final submission: please carefully check your responses for accuracy; you will not be able to make changes later.

Experimental design

1. Sample size

Describe how sample size was determined.

Generally, sample sizes were calculated on the assumption that a 30% difference in the parameters measured would be considered biologically significant with an estimate of sigma of 10-20% of the expected mean. Alpha and Beta were set to the standard values of .05 and 0.8, respectively.

2. Data exclusions

Describe any data exclusions.

No animals or samples were excluded from analysis.

3. Replication

Describe the measures taken to verify the reproducibility of the experimental findings.

Experiments were repeated 2 times to ensure reproducibility except where otherwise indicated in the figure legends.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

For all experiments utilizing SLIT3 treatment of mice, mice were randomized to SLIT3 or vehicle treatment. Where applicable, mice were randomized to treatment with tamoxifen or vehicle.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

Investigators performing uCT or histomorphometry analysis were blinded to the genotype and treatment group of each sample.

Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ Test values indicating whether an effect is present *Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes noted.*
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars in all relevant figure captions (with explicit mention of central tendency and variation)

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	GraphPad Prism and Microsoft Excel

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.	There are no restrictions

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	SLIT3 (1:500; R&D Systems, AF3629), ROBO1 (1:500; Abcam, ab7279), ROBO2 (1:1000; Abcam, ab75014), YAP (1:1,000; Cell Signaling, 4912), p-YAP (1:1,000; Cell signaling, 4911), AKT (1:1,000; Cell Signaling, 4691), p-AKT (1:1,000; Cell Signaling, 4060), ERK (1:1,000; Cell Signaling, 9102), p-ERK (1:1,000; Cell Signaling, 9101) and beta-actin (1:5,000; sc-47778, Santa Cruz) or Hsp90 (1:1000; sc-13119, Santa Cruz), Purified Rat Anti-Mouse CD16/CD32 (BD Biosciences), APC-conjugated EMCN antibody (ebioscience 50-5851-80), PE-conjugated CD31 (ebioscience 12-0311-81), FITC-conjugated CD45 (Tonbo 35-0451), APC/Cy7-conjugated Ter119 (Biolegend 116223) and PerCP-Cy5.5-conjugated CD146 (BD Biosciences 562231), CD31 (553370, BD Pharmigen, 1:100), CD31 conjugated to Alexa Fluor 488 (FAB3628G, R&D Systems, 1:50), Endomucin (sc-65495, Santa Cruz, 1:100) or Beta Galactosidase antibody (GTX77365, Gene Tex, 1:100), EMCN (sc-65495, Santa Cruz, 1:200) and human Slit3 (ab198726, Abcam 1:50). SLIT3 antibodies were experimentally validated in data provided to reviewers. Other antibodies were validated by the manufacturer.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	Only primary cells were used for this study.
b. Describe the method of cell line authentication used.	No transformed cell lines were used for this study.
c. Report whether the cell lines were tested for mycoplasma contamination.	Yes, all cell lines were routinely tested for mycoplasma contamination.
d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.	No commonly misidentified cell lines were used for this study.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide all relevant details on animals and/or animal-derived materials used in the study.

Shn3-/- (BALB/c), Shn3KI/KI (C57BL/6j), Shn3 floxed allele (C57BL/6j), Slit3-/- (BALB/c), Robo1-/- (ICR) and Robo4-/- (C57BL/6j) mice were all previously reported, with Robo1-/- mice being a generous gift from Dr. Marc Tessier-Lavigne 4,7,8,14,19,40. To generate Slit3 floxed mice, the SLIT3-F08 mouse embryonic stem (ES) cell line in which exon 8 is flanked by loxP sites was obtained from International Mouse Phenotyping Consortium (IMPC). After validation, F08 EC cells were injected into C57BL/6J blastocysts, and the derived chimeras displaying germline transmissions were selected for further breeding. The LacZ and neo cassettes were removed by intercrossing with transgenic mice expressing Flp recombinase. Slit3 floxed mice were backcrossed with C57BL/6j mice for 8 generations.
Transgenic mice expressing Cre recombinase under control of the cdh5 promoter (cdh5-Cre) 41, osterix promoter (osx-Cre) 42 , dmp1 promoter (dmp1-Cre) 43 and Osteocalcin-CreERT mice44 were mated with Shn3 floxed mice or Slit3 floxed mice to obtain various Shn3 or Slit3 conditional KO mouse. For postnatal activation of CreERT, 100mg/kg tamoxifen (Sigma) in corn oil (Sigma) was intraperitoneally injected to 1 month-old mice once a day for five consecutive days. Littermate controls were utilized for all experiments.
All animals were maintained in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were handled according to protocols approved by the Weill Cornell Medical College subcommittee on animal care (IACUC).

For the prophylactic OVX model, 12-week-old female mice (JAX, C57BL/6j) were anesthetized and bilaterally ovariectomized or sham operated. Ovariectomized mice were given twice weekly intravenous injections of 1mg per kg body weight of SLIT3 or vehicle or daily sc injections of 80ug/kg PTH (1-34) for 6 weeks starting 2 weeks after ovariectomy . For the model where SLIT3 was delivered in a therapeutic manner after osteopenia onset, OVX was performed in 12 week old mice, mice were observed for 8 weeks post-OVX to allow for onset of osteopenia, and then mice were treated with SLIT3 (1mg/kg) or vehicle for 6 further weeks.

For fracture model, 5 week old mice of the indicated genotypes underwent fracture as described in the methods section.

For all experiments, group sizes and other cohort details are indicated in figure legends.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

This information is described in the following publication: Han W, He W, Yang W, Li J, Yang Z, Lu X, Qin A, Qian Y. The osteogenic potential of human bone callus. Sci Rep. 2016 Oct 31;6:36330.