



HAL
open science

WHIM Syndrome-linked CXCR4 mutations drive osteoporosis

Adrienne Anginot, Julie Nguyen, Zeina Abou Nader, Vincent Rondeau, Amélie Bonaud, Maria Kalogeraki, Antoine Boutin, Julia Lemos, Valeria Bisio, Joyce Koenen, et al.

► **To cite this version:**

Adrienne Anginot, Julie Nguyen, Zeina Abou Nader, Vincent Rondeau, Amélie Bonaud, et al.. WHIM Syndrome-linked CXCR4 mutations drive osteoporosis. *Nature Communications*, 2023, 14 (1), pp.2058. 10.1038/s41467-023-37791-4 . hal-04077290

HAL Id: hal-04077290

<https://hal.science/hal-04077290>

Submitted on 21 Apr 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

- 1 Using a mouse model harboring a WHIM Syndrome-linked gain-of-function CXCR4 mutation and
- 2 bone marrow samples from WHIM patients, the authors show that proper CXCR4 signaling
- 3 termination is essential for bone tissue homeostasis.

1 **WHIM Syndrome-linked *CXCR4* mutations drive osteoporosis**

2 Adrienne Anginot^{1,2,3,#}, Julie Nguyen^{2,4,#}, Zeina Abou Nader^{1,2,3,#}, Vincent Rondeau^{1,2,3}, Amélie
3 Bonaud^{1,2,3}, Maria Kalogeraki^{1,2,3}, Antoine Boutin⁵, Julia Lemos^{1,2,3}, Valeria Bisio^{1,2,3}, Joyce
4 Koenen^{2,4}, Léa Sakr⁶, Amandine Picart⁶, Amélie Coudert⁶, Sylvain Provot⁶, Nicolas Dulphy^{1,2,3},
5 Michel Aurrand-Lions^{2,7}, Stéphane J.C. Mancini^{2,7}, Gwendal Lazennec^{2,8}, David H.
6 McDermott⁹, Fabien Guidez^{3,10}, Claudine Blin-Wakkach⁵, Philip M. Murphy⁹, Martine Cohen-
7 Solal⁶, Marion Espéli^{1,2,3,£}, Matthieu Rouleau^{5,£}, and Karl Balabanian^{1,2,3,*}

8

9 ¹Université Paris Cité, Institut de Recherche Saint-Louis, INSERM U1160, Paris, France.

10 ²CNRS, GDR3697 "Microenvironment of tumor niches", Micronit, France.

11 ³OPALE Carnot Institute, The Organization for Partnerships in Leukemia, Hôpital Saint-Louis,
12 Paris, France.

13 ⁴Inflammation, Chemokines and Immunopathology, INSERM, Université Paris-Saclay,
14 Clamart, France.

15 ⁵Université Côte d'Azur, CNRS, LP2M, Nice, France.

16 ⁶Université Paris Cité, BIOSCAR Inserm U1132, Department of Rheumatology and Reference
17 Center for Rare Bone Diseases, AP-HP Hospital Lariboisière, Paris, France.

18 ⁷Aix Marseille Univ, CNRS, INSERM, Institut Paoli-Calmettes, CRCM, Marseille, France.

19 ⁸CNRS, SYS2DIAG-ALCEDIAG, Cap Delta, Montpellier, France.

20 ⁹Molecular Signaling Section, Laboratory of Molecular Immunology, National Institute of
21 Allergy and Infectious Diseases, NIH, Bethesda, MD.

22 ¹⁰Université Paris Cité, Institut de Recherche Saint-Louis, INSERM U1131, Paris, France.

23 #AA, JN and ZA-N: these authors contributed equally.

24 £ME and MR: these authors contributed equally.

25 *Correspondence & lead contact: karl.balabanian@inserm.fr.

26

27 **Abbreviations:** BMSC: Bone marrow stromal cell; C-tail: Carboxyl-terminal tail; HSPC:
28 Hematopoietic stem and progenitor cell; MFI: Mean fluorescence intensity; OBL: Osteoblast;
29 OCL: Osteoclast; Ocn: Osteocalcin; OPC: Osteoblastic progenitor; Opn: Osteopontin; Osx:
30 Osterix; SLAM: Signaling lymphocyte activation molecule; SSC: Skeletal stromal/stem cell;
31 WS: Warts, Hypogammaglobulinemia, Infections and Myelokathexis Syndrome.

32

33 **ABSTRACT**

34 WHIM Syndrome is a rare immunodeficiency caused by gain-of-function *CXCR4* mutations.
35 Here we report a decrease in bone mineral density in 25% of WHIM patients and bone defects
36 leading to osteoporosis in a WHIM mouse model. Imbalanced bone tissue is observed in mutant
37 mice combining reduced osteoprogenitor cells and increased osteoclast numbers.
38 Mechanistically, impaired CXCR4 desensitization disrupts cell cycle progression and
39 osteogenic commitment of skeletal stromal/stem cells, while increasing their pro-
40 osteoclastogenic capacities. Impaired osteogenic differentiation is evidenced in primary bone
41 marrow stromal cells from WHIM patients. In mice, chronic treatment with the CXCR4
42 antagonist AMD3100 normalizes *in vitro* osteogenic fate of mutant skeletal stromal/stem cells
43 and reverses *in vivo* the loss of skeletal cells, demonstrating that proper CXCR4 desensitization
44 is required for the osteogenic specification of skeletal stromal/stem cells. Our study provides
45 mechanistic insights into how CXCR4 signaling regulates the osteogenic fate of skeletal cells
46 and the balance between bone formation and resorption.

47

48 **Keywords:** Skeletal stromal/stem cell; Bone marrow; CXCR4 signaling; Osteogenesis; WHIM
49 Syndrome; Osteoporosis.

50

51

52

53 **INTRODUCTION**

54 The bone marrow (BM) is a complex structural and primary immune organ whose development
55 and maintenance depend on multiple cell types including cells of the hematopoietic lineage like
56 hematopoietic stem and progenitor cells (HSPCs), but also vascular cells and numerous skeletal
57 cells encompassing BM stromal cells (BMSCs), skeletal progenitor/precursor cells as well as
58 bone-making osteoblasts (OBLs)^{1,2}. Together these cells compose specialized micro-
59 anatomical structures called “niches” that sustain their survival and differentiation³⁻⁹. For
60 instance, the HSPC niches are thought to be composed of perivascular stromal units associated
61 with sinusoids and arterioles¹⁰⁻¹⁵. Bone and adipose cells are thought to derive from subsets of
62 BMSCs that are located near blood vessels and function as skeletal stromal/stem cells (SSCs)¹⁶⁻
63 ¹⁹. However, the exact localization, composition and crossover of these niches in relation with
64 bone function are not yet established. Bone tissue homeostasis relies on the balance between
65 formation and resorption of bone matrix mediated by effector cells that derive from SSCs and
66 HSPCs respectively. Disequilibrium of this balance can lead to diseases such as osteoporosis
67 or osteopetrosis. In such a landscape, SSCs are key players: not only they give rise to OBLs but
68 they also contribute to perivascular structures important for HSPCs²⁰⁻²⁸. Understanding how
69 SSCs maintain their identity, achieve plasticity and support hematopoiesis in adult BM is thus
70 an important emerging field^{3,6,9,12,29}. Recently, Ambrosi and coll. showed that intrinsic ageing
71 of SSCs skews skeletal and hematopoietic lineage outputs, leading to fragile bones³⁰. Moreover,
72 Jeffery and coll. reported how BM and periosteal SSCs contribute to bone maintenance and
73 repair³¹. However, both extrinsic and intrinsic mechanisms regulating their fate remain
74 incompletely understood.

75 In adult BM, signaling by the G protein-coupled receptor CXCR4 on HSPCs in response
76 to stimulation by the chemokine CXCL12/Stromal cell-derived factor-1, produced by BMSCs
77 constitutes a key pathway through which the stromal niches and HSPCs communicate³²⁻³⁷.

78 Conditional ablation of *Cxcl12* from perivascular stromal cells or OBLs demonstrated that
79 HSCs occupy a perivascular but not an endosteal niche^{21,38}, whereas targeted deletion of *Cxcl12*
80 from BM stromal cells has allowed the identification of specialized niches supporting leukemia
81 stem cell maintenance³⁹. Both *Cxcr4* and *Cxcl12* are broadly expressed by non-hematopoietic
82 tissues and cell types and have multifunctional roles beyond hematopoiesis. Since mice
83 deficient for *Cxcr4* or *Cxcl12* die perinatally, our understanding of the role of the *Cxcl12/Cxcr4*
84 axis in regulating the BM ecosystem is mostly based on relatively selective loss-of-function
85 models^{21,40-44}. Conditional inactivation of *Cxcl12* or *Cxcr4* in paired-related homeobox gene 1
86 (*Prx1*)- or osterix (*Osx*)-expressing cells, *i.e.* respectively multipotent mesenchymal
87 progenitors or osteoprogenitor cells (OPCs) and descendant OBLs, was associated with reduced
88 postnatal bone formation, suggesting a positive regulatory role of this pair in OBL development
89 and/or function^{21,43,44}. Single cell transcriptomics recently suggested heterogeneity within adult
90 *Cxcl12*- or Leptin-receptor (*LepR*)-expressing mesenchymal cells poised to undergo either
91 adipogenic or osteogenic specification^{45,46}. However, it is still unclear whether and how *Cxcr4*
92 signaling regulates intrinsically osteogenic specification of skeletal cells.

93 Here, we addressed this point using as a paradigm the WHIM Syndrome (WS), a rare
94 immunodeficiency caused by viable inherited heterozygous gain-of-function mutations in
95 *CXCR4* affecting homologous desensitization of the receptor, thus resulting in enhanced
96 signaling following CXCL12 stimulation, defective lymphoid differentiation of HSPCs and
97 reduced blood leukocyte numbers⁴⁷⁻⁵⁰. Taking advantage of a mouse strain that harbors the
98 naturally occurring WS-linked heterozygous *CXCR4*^{S338X} mutation (*Cxcr4*^{+/*1013*}, +/*1013*)⁵¹⁻⁵⁴,
99 and of human BM samples from WS donors and clinical data from 19 WS patients, we
100 investigated whether WS mutations affect the SSC landscape with a particular attention given
101 to the bone fraction. WS-linked *CXCR4* mutations were associated with reduced bone mass in
102 mice and humans. In mice, this relied on impaired CXCR4 desensitization that disrupts cell

103 cycle progression and osteogenic commitment of SSCs, while paradoxically increasing the pro-
104 osteoclastogenic capacities of these cells. Impairment in osteogenic potential was also
105 evidenced in BMSCs from WS patients. Thus, proper *CXCR4* desensitization is required for
106 the osteogenic specification of bone SSCs.

107

108 **RESULTS**109 **WS-linked CXCR4 mutations are associated with reduced bone mass in mice and humans**

110 Following CXCL12 stimulation, β -arrestins are recruited to the carboxyl-terminal tail (C-tail)
111 domain of CXCR4, precluding further G-protein activation (*i.e.* desensitization) and leading to
112 receptor internalization⁵⁵. Both processes are dysregulated in WS most often due to autosomal-
113 dominant gain-of-function mutations that result in the distal truncation of the C-tail of CXCR4
114 and a desensitization-resistant, hyperactive receptor⁵⁶. Although the impact of these WS
115 mutations on immune cells is currently being understood^{51-54,57}, nothing is known about their
116 impact on the skeletal landscape. Bone mineral density (BMD) values were measured in 19
117 patients with WS for lumbar spine and femoral neck by total body dual-energy X-ray
118 absorptiometry. BMD T- and Z-scores were found to be low at least in one site in five patients
119 (Table 1). Likewise, this was evidenced in adult (8-12-week-old) *Cxcr4*¹⁰¹³-bearing (*i.e.*
120 heterozygous [+ /1013] and homozygous [1013/1013]) mice, as compared to *Cxcr4*^{+/+} (WT)
121 mice. Analyses of lumbar spine revealed decreased BMD values in mutant mice, in a *Cxcr4*¹⁰¹³
122 allele dose-dependent manner (Fig. 1A). Micro-computed tomography (μ CT) analyses further
123 unraveled a reduction in trabecular and cortical bone in mutant mice (Fig. 1B). In mutant
124 femurs, there was a reduction in the trabecular bone density that followed a *Cxcr4*¹⁰¹³ allele
125 copy number-dependent pattern. This was characterized by a significant decrease in bone
126 volume and trabecular numbers, while the trabecular separation was increased compared to WT
127 mice (Fig. 1C). The cortical bone volume and thickness were also affected (Fig. 1B and 1D).
128 This gene-dependent reduction was observed among both female and male mutant mice.
129 Histomorphometric analyses confirmed decreased bone volume and trabecular numbers in
130 mutant mice as shown by toluidine blue and osteopontin (Opn) staining (Fig. 1E and 1F).
131 Strikingly, staining for Alcian Blue and perilipin that are used for chondrocyte and adipocyte
132 identification respectively, were unaltered in mutant bone (Fig. 1G and 1H). Consistently, the

133 thickness of the growth plate as well as the adipocyte content were similar in mice carrying the
134 *Cxcr4* mutation compared to WT ones (Fig. 1I and 1J), thus suggesting that *Cxcr4* specifically
135 regulates the osteogenic fate in adults. Moreover, adult mutant mice did not exhibit significant
136 changes of body size or weight (Fig. 1K), indicating that the *Cxcr4* mutation likely does not
137 alter the skeletal growth. Overall, these findings revealed an osteopenic skeleton in *Cxcr4*¹⁰¹³-
138 bearing mice and low BMD in 25% of WS patients.

139

140 **Reduction of skeletal stromal cells in *Cxcr4*¹⁰¹³-bearing mice**

141 We then evaluated by flow cytometry the bone composition of WT and mutant mice with a
142 focus on skeletal cells that encompass notably SSCs, OPCs and OBLs⁵⁸. Long bones were
143 flushed and then digested. Total stromal cells in the bone fraction were identified as negative
144 for CD45, Lineage (including Ter119), c-Kit and CD71 expression as previously reported^{12,59}.
145 Endothelial cells were excluded based on CD31 expression. Two distinct CD51⁺ stromal cell
146 subsets were identified based on Sca-1 and PDGFR α : SSCs (Sca-1⁺PDGFR α ⁺) and more
147 differentiated OPCs (Sca-1⁻PDGFR α ^{+/+}) (Fig. 2A). We consider Sca-1⁺PDGFR α ⁺ cells at the
148 top of the hierarchy based on previous works that indicated a highest CFU-F clonogenic
149 potential of these cells as compared to Sca-1⁻PDGFR α ⁺ cells^{12,28,31,60}. We observed a significant
150 decrease of the numbers of OPCs, and to a lesser extent of SSCs, that followed a *Cxcr4*¹⁰¹³
151 allele dose-dependent pattern (Fig. 2B), thus reinforcing that the landscape of the bone stroma
152 is altered in *Cxcr4*¹⁰¹³-bearing mice.

153 We next examined *in vitro* the function of the signaling trio formed by Cxcl12 and its
154 two receptors *Cxcr4* and *Ackr3* in skeletal cells. Membrane expression of *Cxcr4* and *Ackr3* was
155 similar between WT and mutant skeletal cells including SSCs (Fig. S1A and S1B). However,
156 +/1013 and 1013/1013 SSCs displayed both impaired *Cxcr4* internalization following Cxcl12
157 stimulation as well as increased Cxcl12-mediated chemotaxis that was abolished by the specific

158 Cxcr4 antagonist AMD3100 (Fig. S1C and S1D). These dysfunctions likely relied on the
159 enhanced signaling properties of the truncated Cxcr4 receptor as revealed by Erk PhosphoFlow
160 analyses (Fig. S1E). Combined with the apparent preserved capacity of Ackr3 to bind and
161 internalize Cxcl12 *in vitro* (Fig. S1F and S1G), these findings indicated a functional expression
162 of the desensitization-resistant C-tail-truncated Cxcr4¹⁰¹³ receptor on SSCs. Abnormal Cxcr4
163 signaling was not associated with changes in apoptosis of SSCs (Fig. S1H).

164 To determine whether reduction of bone content in *Cxcr4*¹⁰¹³-bearing mice resulted
165 from defects intrinsic to skeletal cells and/or an alteration of the hematopoietic (or another non-
166 stromal) system, we performed reciprocal long (16 weeks)- and short (3 weeks)-term BM
167 reconstitution experiments. First, BM cells from WT CD45.1⁺ mice were transplanted into
168 lethally irradiated 8-wk old CD45.2⁺ WT or mutant (+/1013 and 1013/1013) mice (Fig. 2C).
169 Sixteen weeks later, mutant recipients exhibited CD45.1⁺ chimerism in hematopoietic
170 compartments similar to those of WT recipients (Fig. 2D) but displayed reduced numbers of
171 skeletal cells including SSCs and OPCs (Fig. 2E). Confocal imaging and μ CT analyses
172 confirmed that transplantation of WT BM was not sufficient to rescue the trabecular network
173 in mutant recipients (Fig. 2F-I). This was also evidenced three weeks after WT BM
174 transplantation (Fig. 2G). Although we cannot formally exclude that WT hematopoietic cells
175 may be able to rescue the reduced trabecular bone content in mutant mice earlier in
176 development, these results suggested that skeletal cell-autonomous Cxcr4 regulation
177 contributes to the persistent bone defects in adult *Cxcr4*¹⁰¹³-bearing mice. We then performed
178 reverse chimeras in which irradiated 8-wk old CD45.1⁺ WT mice were reconstituted with WT,
179 +/1013 or 1013/1013 CD45.2⁺ BM (Fig. 3A). Sixteen weeks later, CD45.2⁺ chimerism of LT-
180 HSCs and leukocytes were decreased respectively in BM and blood of CD45.1⁺ WT recipients
181 engrafted with mutant BM, confirming the impaired reconstitution capacity of mutant HSCs
182 (Fig. 3B and ⁴⁹). There were significantly lower numbers of skeletal cells and defective

183 trabecular bone content in *Cxcr4*¹⁰¹³-bearing BM-chimeric mice compared to WT chimeras as
184 early as 3 weeks post-transplantation (Fig. 3C-G), thereby indicating cell-extrinsic Cxcr4-
185 mediated regulation of the skeletal landscape. Likewise, cortical bone content was slightly
186 affected (Fig. 3H and 3I). Altogether, these findings suggest that impaired Cxcr4 desensitization
187 in both skeletal and hematopoietic cells have combinatorial effects on bone landscape
188 dysregulation in adult *Cxcr4*¹⁰¹³-bearing mice.

189

190 **Increased bone resorption and reduced bone formation in *Cxcr4*¹⁰¹³-bearing mice**

191 Bone is maintained by coupled activities of bone-forming OBLs and bone-resorbing osteoclasts
192 (OCLs). Alterations in bone balance can result in pathologic bone loss and osteoporosis. This
193 led us to investigate whether the gain-of-*Cxcr4*-function mutation modulates the OBL/OCL
194 balance. First, we analyzed bone resorption by quantifying OCL numbers in mice using Tartrate
195 Resistant Acid Phosphatase (TRAP) staining⁶¹. We observed increased OCL surface (Oc.S/BS)
196 and number (Oc.N/BV) in mutant mice compared to WT ones (Fig. 4A and 4B). To determine
197 whether the increased bone resorption in mutant mice resulted from OCL-intrinsic defects, we
198 performed *in vitro* OCL differentiation from BM cells in the presence of M-Csf and Rank-L
199 and tested their bone resorption capacity. Similar OCL numbers and bone matrix resorption
200 activities were observed among WT and mutant cultures (Fig. S2A and S2B), suggesting
201 preserved intrinsic capacities of mutant BM myeloid cells to differentiate *in vitro* into functional
202 OCLs. Congruent with this, we observed no changes in expression levels of osteoclastogenic
203 genes in mutant cultures compared to WT ones (Fig. S2C). These findings indicate that the
204 *Cxcr4* mutation does not affect *in vitro* OCL differentiation and function but suggest that
205 osteoclastogenesis and increased bone resorption in mutant mice may be promoted by the BM
206 environment.

207 *Cxcr4*¹⁰¹³-bearing mice exhibited similar bone formation as revealed by osteoid surface
208 (OS/BS) and osteoblast surface (Obl.S/BS) compared to WT mice (Fig. 4C). Dynamic
209 parameters of *in vivo* bone formation were also assessed by quantifying bone surfaces labelled
210 with tetracycline and calcein. Total and double labelled surfaces were lower in mutant than WT
211 mice (Fig. 4D), whilst mineral apposition rate (MAR) were similar in WT and *Cxcr4*¹⁰¹³-
212 bearing mice (Fig. 4E). Overall, bone formation rate (BFR/BS) was significantly reduced in
213 mutant mice (Fig. 4E). This suggests a decrease in bone formation related to a lower number
214 of OPCs and OBLs with maintained activity of individual OBL. In line with preserved intrinsic
215 bone formation capacities of active osteoblastic lineage cells in mutant mice, high-throughput
216 RNA sequencing (RNA-seq) and qPCR analyses of OPC bulk-sorted by flow cytometry from
217 the bone fraction on the basis of the CD51 and Sca-1 markers (Fig. 2A) highlighted a gene
218 signature with preserved mineralized matrix potential in mutant OPCs (Fig. 4F-H and S2D-F).
219 In agreement, sorted OPCs from mutant mice were as efficient as WT ones *in vitro* at producing
220 differentiated OBLs and mineralized nodules after 14- or 21-days culture in osteogenic medium
221 as determined by Alkaline phosphatase (Alp) and Alizarin Red (AR) staining
222 respectively^{24,60,62,63} (Fig. 4I and S2G). This was confirmed by qPCR analyses with no changes
223 in expression of genes encoding osteogenic regulators in mutant cultures (Fig. S2H). These
224 findings suggest reduced osteogenic lineage commitment in *Cxcr4*¹⁰¹³-bearing mice. Given that
225 osteogenic cells support osteoclastogenesis through the production of factors such as Rank-L
226 (*Tnfsf11*)⁶⁴, we questioned our RNAseq data on the related gene expression profile in mutant
227 and WT OPCs. No major changes in expression levels of pro-osteoclastogenic or anti-resorptive
228 genes were revealed in mutant OPCs (Fig. 4J and 4K).

229 To investigate whether increase in OCLs in mutant mice is linked to an altered stromal
230 BM environment, we sought to set up co-culture experiments between *in vitro* expanded
231 osteogenic cells carrying or not the *Cxcr4* mutation and WT OCL precursors, *ie.*, BM CD11b⁺

232 myeloid cells as reported⁶⁵. We showed that mutant osteogenic cells promoted exacerbated
233 OCL differentiation compared to WT cells (Fig. 4L). Soluble factors seem not to be sufficient
234 as the supernatants of such stimulated expanded osteogenic cells (WT or mutant) did not induce
235 OCL differentiation. Additionally, transcriptomic analyses of stimulated osteogenic cells
236 carrying or not the *Cxcr4* mutation did not reveal any major changes in expression levels of
237 master genes regulating osteoclastogenesis (Fig. 4M). These findings suggest a juxtacrine
238 function of osteogenic cells toward OCL differentiation that likely relies on direct interactions
239 between both cell types and involves the Cxcl12/Cxcr4 axis.

240 Taken as a whole, our findings suggest that the hematopoietic contribution to bone loss
241 in *Cxcr4*¹⁰¹³-bearing mice likely involves dysregulation of the OCL compartment regardless of
242 their activity. The overall decrease in bone mass in mutant mice may involve modulation of
243 osteogenic and osteoclastogenic components with decreased bone formation and increased
244 bone resorption. Bone remodeling involves a tight regulation of opposite processes leading to
245 bone resorption and bone formation^{66,67}. The observation that immature and mature osteogenic
246 cells, *i.e.*, OPCs and OBLs, displayed preserved intrinsic functions led us to study the early
247 differentiation process of the osteolineage.

248

249 **Impaired osteogenic specification of *Cxcr4*¹⁰¹³-bearing skeletal stromal cells**

250 We thus investigated the intrinsic characteristics of SSCs carrying the *Cxcr4* mutation.
251 Undifferentiated stem cells are characterized by their slow cell cycle progression in unperturbed
252 conditions^{9,68}. This led us to interrogate by flow cytometry the cycling status of SSCs from the
253 bone fractions of *Cxcr4*¹⁰¹³-bearing mice by performing DAPI/Ki-67 staining. A slight but
254 significant increase in the frequency of cells in the quiescent G0 state (DAPI^{low}Ki-67⁻) was
255 observed among 1013/1013 SSCs but not in the more differentiated osteoblastic pool (Fig. 5A).
256 The turnover of those cells was then studied by performing a 12-day BrdU pulse-chase assay

257 *in vivo* (Fig. 5B). Consistent with previous studies^{28,69}, the fraction of BrdU⁺ cells in WT SSCs
258 reached ~5%, while we observed a *Cxcr4*¹⁰¹³ allele copy number-dependent reduction in BrdU
259 incorporation within mutant SSCs. No changes were observed among mutant OPCs compared
260 to WT ones. Combined to reduced SSC and OPC numbers in mutant bones (Fig. 2B), these
261 findings are suggestive of reduced cycling and osteogenic differentiation capacities of
262 *Cxcr4*¹⁰¹³-bearing SSCs.

263 To gain further mechanistic insights, we investigated the impact of the gain-of-*Cxcr4*-
264 function on the molecular identity of SSCs by performing RNA-seq analyses of bulk-sorted
265 SSCs from the bone fraction of WT and mutant mice. Biological processes related to cell cycle
266 and osteogenic differentiation were significantly modulated in 1013/1013 SSCs as determined
267 by Gene set enrichment analysis (GSEA) (Fig. 5C). The signature for genes related to cell cycle
268 progression and regulation was reduced in 1013/1013 SSCs (Fig. S3A and S3B). Likewise,
269 genes related to osteogenic differentiation appeared to be decreased in mutant SSCs (Fig. 5D
270 and 5E). In contrast, key genes involved in both adipogenesis and chondrogenesis were not
271 differentially expressed in mutant SSCs (Fig. S3C). These results were confirmed by
272 microfluidic-based multiplex gene expression analyses (Fig. 5F and 5G and Fig. S3D), thus
273 suggesting that proper *Cxcr4* signaling is required for regulating osteogenic specification of
274 SSCs. No changes in expression levels of pro-osteoclastogenic genes were detected in mutant
275 SSCs compared to WT ones (Fig. 5H-J). Therefore, these results unravel a *Cxcr4*-mediated
276 transcriptional signature in *Cxcr4*¹⁰¹³-bearing SSCs suggestive of impaired cell cycle
277 progression and defective osteogenic specification.

278 In adult BM, the majority of OBLs derives from OPCs identified by markers such as
279 osterix (*Osx*)^{3,9,17,28,70-72}. They are predominantly found close to the growth plate cartilage along
280 trabecular bone of the primary spongiosa, and along the metaphyseal cortical bone^{70,71,73}. We
281 thus examined whether the gain-of-*Cxcr4*-function mutation alters the number of *Osx*-positive

282 OPCs by immunodetection on bone sections. We found fewer Osx-positive OPCs in mutant
283 bones compared to WT (Fig. 5K and 5L). This decrease was confirmed by flow cytometry in
284 the flushed stromal marrow fraction that encompasses Sca-1-negative and PDGFR α -positive
285 early OPCs with multipotent adipo/osteogenic potential (Fig. 5M)^{28,45,70}. Together, these data
286 suggest that the decrease in early and committed OPCs in mutant mice may arise from a defect
287 in osteogenic specification of BM-residing SSCs.

288

289 **Cxcr4 desensitization intrinsically regulates *in vitro* the osteogenic differentiation of**
290 **skeletal stromal cells**

291 To assess whether Cxcr4 desensitization could regulate SSC fate toward the osteogenic lineage
292 in a cell-intrinsic manner, we first compared *in vitro* the clonogenic capacities of WT and
293 mutant total skeletal cells. There was a significant decrease in the number of colony-forming
294 units-fibroblast (CFU-Fs) in mutant bone cell cultures that followed a *Cxcr4*¹⁰¹³ allele copy
295 number-dependent pattern (Fig. 6A). These results suggested that impaired Cxcr4 signaling
296 might affect *in vitro* overall SSC numbers as well as their proliferation. To test this, we
297 evaluated by flow cytometry cell cycle and proliferation of SSCs expanded *in vitro* using BrdU,
298 Cell Trace Violet (CTV) and DAPI/Ki-67 staining. By day 5 after BrdU pulse, we observed a
299 *Cxcr4*¹⁰¹³ allele dose-dependent reduction in BrdU incorporation within mutant SSCs as
300 compared to WT (Fig. 6B, left panel). Consistently, the fraction of proliferating CTV^{low} cells
301 was reduced among mutant SSCs three days after loading (Fig. 6B, right panel). This altered
302 proliferative capacity of *Cxcr4*¹⁰¹³-bearing SSCs was associated with a slight but significant
303 increase in proportions of SSCs in the quiescent G0 state (DAPI^{low}Ki-67⁻), whereas no changes
304 in apoptosis level were observed (Fig. 6C). This might account for the increased doubling time
305 of mutant SSCs as well as their overall reduced number during the culture (Fig. 6D). Altogether,

306 these findings suggest that *Cxcr4* desensitization is required *in vitro* for appropriate SSC
307 proliferation, expansion and likely maintenance.

308 Next, we investigated *in vitro* the osteogenic potential capacities of *Cxcr4*¹⁰¹³-bearing
309 SSCs^{62,63}. Staining of *in vitro* differentiated osteogenic cells and mineralization capacities by
310 Alp and AR was significantly reduced in cultures from mutant SSCs in an allele dose-dependent
311 manner (Fig. 6E and 6F, upper panels). Real-time PCR analysis revealed decreased expression
312 of genes encoding osteogenic regulators in mutant cultures (Fig. 6G, upper panels). This was
313 more marked for early osteogenic genes downstream the master regulator *Runx2* such as *Osx*
314 and particularly evident in the culture of 1013/1013 SSCs, thus suggesting defects at very early
315 stages in the osteogenic differentiation process. In line with this, higher mRNA expression of
316 *Scal* and *Pdgfra* was observed in mutant cultures three weeks after pro-osteogenic condition
317 initiation (Fig. S4A, left panel). Consistent with the results obtained with Alcian blue and
318 Perilipin staining on bone sections (Fig. 1G and 1H), *Cxcr4*¹⁰¹³-bearing SSCs differentiated into
319 adipocytes or chondrocytes similarly to WT SSCs when cultured *in vitro* with adipogenic or
320 chondrogenic media respectively (Fig. S4B and S4C). Collectively, these data reveal *in vitro* a
321 selective reduction of the osteogenic differentiation capacity of mutant SSCs, and further
322 confirm a pivotal role for *Cxcr4* desensitization in regulating this process at very early stages.

323

324 **Normalization of *Cxcr4* signaling rescues the osteogenic properties of *Cxcr4*¹⁰¹³-bearing** 325 **mouse skeletal cells**

326 We then determined whether targeting *Cxcr4* signaling would counteract the defective
327 osteogenic fate of mutant SSCs. First, we assessed *in vitro* the impact of adding AMD3100
328 every 2 days on the osteogenic capacities of SSCs. AMD3100-mediated inhibition of *Cxcr4*
329 signaling in WT SSCs led to slight changes including decreased numbers of osteogenic cells
330 (Fig. 6E and 6F, lower panels). By contrast, mutant cultures were highly sensitive to AMD3100

331 treatment as shown by the normalization of Alp and AR colorations 14 and 21 days after
332 differentiation respectively (Fig. 6E and 6F) and of the expression of *Scal* and *Pdgfr α* (Fig.
333 S4A). Moreover, AMD3100-mediated reversion of defective osteogenesis within *Cxcr4*¹⁰¹³-
334 bearing SSC cultures was associated with normalized gene expression of osteogenic master
335 regulators (Fig. 6G, lower panels), thus unravelling that Cxcr4 desensitization intrinsically
336 regulates *in vitro* the osteogenic differentiation of SSCs.

337 Then, we assessed the impact of daily intraperitoneal injections for 3 weeks of 5 mg/kg
338 AMD3100 on the bone landscape in adult WT and mutant mice (Fig. 7A). Cxcr4 inhibition
339 decreased slightly the number of WT skeletal cells, and notably OPCs, in the bone fraction (Fig.
340 7B). In line with this, Opn-stained femoral sections revealed minor alterations in the
341 architecture of WT mice trabecular microstructures upon treatment (Fig. 7C). This was
342 extended to lumbar spine that displayed roughly normal BMD values in treated vs untreated
343 WT mice (Fig. 7D). In 1013/1013 mice, chronic AMD3100 treatment reversed the quantitative
344 defect in skeletal cells by normalizing the numbers of SSCs and OPCs (Fig. 7B). This was not
345 evidenced in +/-1013 mice nor associated with a rescue of the trabecular or the cortical network
346 (Fig. 7C and 7E). However, AMD3100 treatment ameliorated slightly but significantly BMD
347 values of lumbar spine in mutant mice (Fig. 7D), suggesting a correcting effect of Cxcr4-
348 dependent signaling dampening on other cell types and mechanisms such as OCLs or LepR-
349 positive BM SSCs as recently reported³¹. Therefore, these data suggest that integrity of Cxcr4
350 signaling is required for maintaining the osteogenic properties of skeletal cells.

351

352 **BM stromal cells from WS patients displayed *in vitro* impaired osteogenic capacities**

353 Finally, we sought to investigate if CXCR4 desensitization was mechanistically involved in
354 regulating *in vitro* the multilineage differentiation capacities of human primary BMSCs that
355 constitute a heterogeneous population containing skeletal progenitors¹⁸. To this end, we

356 analyzed BM samples from two unrelated patients with WS and carrying the heterozygous
357 *CXCR4*^{R334X} mutation. In parallel, we expanded *in vitro* BMSCs from BM aspirates of seven
358 independent healthy donors. All culture-expanded BMSCs were negative for the CD45
359 hematopoietic marker lineage but positive for CD73, CD90 and CD105, a combination of
360 markers that are indicative of stromal/fibroblastic cells (Fig. S5A). Both healthy and WS
361 BMSCs were spindle shaped and fibroblast-like cells and had the ability to form stromal
362 colonies as shown by CFU-F assay (Fig. S5B and S5C). CXCL12 and its two receptors CXCR4
363 and ACKR3 were readily detectable and found at similar levels between cultured healthy and
364 WS BMSCs (Fig. S5D-F). However, real-time PCR analyses revealed decreased expression of
365 genes encoding early and late osteogenic master regulators in WS BMSC cultures compared to
366 healthy controls (Fig. 8A). In line with this, when equal numbers of cells were plated at the start
367 of the assay, WS BMSCs exhibited defective capacities to generate *in vitro* osteogenic progeny
368 in contrast to BMSCs harvested from healthy donors (Fig. 8B). In contrast, WS BMSCs were
369 as efficient as control cells to generate adipocytes in appropriate culture media condition (Fig.
370 8C). Therefore, these findings suggest that *in vitro* osteogenic differentiation of human primary
371 BMSCs requires proper CXCR4 signaling regulation.

372

373 **DISCUSSION**

374 In this study, we investigated the regulatory role of CXCR4 signaling termination in the self-
375 renewal and differentiation capacities of adult BM-residing SSCs. We used a knock-in mouse
376 model expressing a naturally occurring WS-linked heterozygous gain-of-function *Cxcr4*
377 mutation as well as human BM samples and clinical data from healthy and WS donors. We
378 demonstrated a mutated allele dose-dependent effect of the WS-linked *Cxcr4*¹⁰¹³ mutation on
379 trabecular bone microstructures mimicking an osteoporotic-like syndrome, evidenced as well
380 in one quarter of WS patients. This *Cxcr4*-mediated reduction in bone content involved both
381 cell-autonomous and cell-extrinsic defects in SSCs (Fig. 8D). Indeed, we provided
382 unanticipated evidence that *Cxcr4* desensitization is intrinsically required for regulating *in vitro*
383 the quiescence/cycling balance of SSCs and preserving their osteogenic potential, while it was
384 found to be dispensable for their adipogenic and chondrogenic differentiation. Some other BM
385 cellular components contributed to the dysregulation of the bone phenotype. We observed in
386 trabecular area an increase in OCL number. However, the osteoclastogenic differentiation
387 potential of OCL precursors and the resorptive function of differentiated OCLs were not
388 affected *in vitro* by the *Cxcr4*¹⁰¹³ mutation. Therefore, the osteopenia might proceed from a
389 deregulated bone matrix resorption that might not be compensated enough by bone-forming
390 cells, enlightening further the strong entanglement between both actors of bone remodeling.
391 Unravelling the mechanisms leading to such an alteration of the skeletal landscape with a focus
392 on the defective osteogenic capacities of SSCs driving the observed excessive bone defect will
393 require further investigations using specific conditional mouse models. Importantly, defective
394 osteogenic capacities were also evidenced *in vitro* in BMSCs from WS patients. These
395 anomalies establish the C-tail of CXCR4 as an important regulatory domain of the receptor
396 function in BM stromal cell biology in both mice and humans. In light of previous
397 works^{40,41,43,44}, our results also suggest that both increased and decreased *Cxcr4*-mediated

398 signaling negatively impact skeletal stromal elements, thus indicating that fine-tuning of Cxcr4
399 signaling is critical for maintenance and osteogenic specification of adult SSCs. Although the
400 underlying molecular mechanisms remain to be elucidated, Cxcr4 might act as a rheostat
401 regulating the strength and kinetic of signaling pathways involved in osteogenic fate
402 specification of SSCs. Interestingly, a recent work using a new mouse model of WS further
403 illustrated the crucial role of fine-tuned Cxcr4-mediated signaling in mesenchymal
404 stromal/stem cell (MSC) function⁴⁶. Indeed, the lymphopoiesis process was reduced, as
405 observed in our model⁵³, because of a dysregulated transcriptome in MSCs isolated from the
406 flushed marrow fraction and characterized by a switch from an adipogenic to an osteolineage-
407 prone program with limited lymphopoietic activity. This might proceed from reduced
408 expression of IL-7 and excessive signaling through the lymphotoxin beta receptor in MSCs.
409 Whether these changes are observed in the BM of our mouse model and how they would
410 contribute to the bone loss warrant further investigations. Moreover, mice deficient for the gene
411 encoding the transcription factor Ebf3 display an opposite BM phenotype to the one of
412 *Cxcr4*¹⁰¹³-bearing mice, characterized by osteosclerosis with HSC depletion and reduced
413 expression of niche factors⁶⁹. This was related to the uncontrolled ability of *Ebf3*-deficient SSCs
414 to differentiate into OBLs. Further studies are required to address the status of Ebf3 and
415 downstream target genes that act to modulate osteogenic fate of SSCs in *Cxcr4*¹⁰¹³-bearing
416 mice. A potential crosstalk between distinct SSC subsets, either prone to differentiate into
417 osteochondro-lineage cells or perivascular and adipocyte lineage cells, has been reported⁷⁴. This
418 seems to imply ligand-receptor gene pairs such as TGF β , WNT or BMP ligands and their
419 cognate receptors that regulate SSC fate decision. Whether and how the Cxcl12/Cxcr4 signaling
420 axis contributes to these regulatory mechanisms across SSC types remains also to be explored.

421 We reported that loss of Cxcr4 signaling termination impairs overall number, impedes
422 cell cycle progression, and limits osteogenic differentiation of SSCs. Indeed, mutant mice have

423 a global alteration of the bone stromal landscape, including decreased numbers of SSCs and
424 their progeny including early and committed OPCs and impaired architecture of trabecular and
425 cortical bone microstructures that occurred in a mutated allele copy number-dependent manner.
426 Altogether, these findings indicate that the gain-of-*Cxcr4*-function mutation promotes a
427 reduced OBL commitment and differentiation, but does not alter the bone forming activity of
428 individual OBL. Congruent with this, chronic treatment with AMD3100 normalized *in vitro* the
429 osteogenic properties of mutant SSCs. Impaired *Cxcr4* desensitization might alter the balance
430 between quiescence and differentiation of mutant SSCs and reduce the number of osteogenic-
431 endowed precursors. Currently, the prevailing view is that BM *Cxcl12*-expressing stromal cells
432 display slow cell cycle progression and constitute an active source of trabecular and cortical
433 OBLs under physiological conditions, as well as in response to injury^{6,9,28,31,69}. We found a
434 higher proportion of quiescent SSCs in mutant mice that was particularly evident in 1013/1013
435 SSCs, thus suggesting the importance of *Cxcr4* desensitization in controlling SSC proliferation
436 and quiescence and likely their capacity to give rise to osteogenic cells.

437 Loss of bone content in mutant mice was accompanied by a higher number of OCLs
438 within the trabecular bones, possibly reflecting that the *Cxcr4* mutation was intrinsically
439 perturbing the OCL differentiation process. This seems not to be the case since we showed that
440 defective *Cxcr4* desensitization did not increase *in vitro* differentiation of OCLs from mutant
441 BM progenitors, nor their mineral matrix resorbing capacities. BM chimeras leading to a WT
442 hematopoietic development into a mutant bone environment further ruled out the sole
443 involvement of an uncontrolled bone resorption due to defective OCLs with excessive activity.
444 These cells derive from monocytic lineage precursors upon stimulation by RankL and M-
445 *Csf*^{75,76}. In a constant cross interaction between the bone forming and the bone resorbing
446 pathways, these osteoclastic cytokines are produced by mature and immature stromal cell
447 populations within the BM^{9,77,78}. While we did not observe increased *Rank-L*, *M-Csf* or *Opg*

448 (encoding a Rank-L antagonist) gene expression in sorted committed OPCs from mutant bones,
449 we cannot exclude that modification of the bone stroma due to osteogenic defects might in turn
450 modulate the production of osteoclastic factors from the mutant bone environment. Supporting
451 this assumption, co-cultures of WT OCL progenitors with *in vitro* expanded *Cxcr4*¹⁰¹³-bearing
452 bone-derived stromal cells, stimulated to produce osteoclastogenic factors, led to enhanced
453 osteoclastogenesis compared to WT stromal cells. No increase in *Rank-L* or *M-Csf* expression
454 nor decrease in *Opg* level could be detected, suggesting the involvement of other additional
455 mediators. It has recently been shown that intrinsic aging of SSCs resulted in higher proportion
456 of stromal lineages producing pro-inflammatory and pro-resorptive factors, promoting myeloid
457 skewing, and osteoclastic activity³⁰. Whether a similar mechanism occurs in *Cxcr4*¹⁰¹³-bearing
458 mutant mice remains to be characterized. Moreover, BM chimeras leading to a mutant
459 hematopoietic development into a WT bone environment displayed dysregulated bone
460 landscape, thus emphasizing that transplanted hematopoietic cells can participate in bone loss
461 through direct and/or indirect actions on osteoclastogenesis. Despite altered hematopoiesis in
462 such chimeric mice, the myeloid skewing reported elsewhere⁵³ might account for excessive
463 OCL number. As well, the reduced lymphopoiesis observed^{46,53} still led to generation of mature
464 B and T lymphocytes that are present in the BM and hence may act as potential actors in bone
465 erosion as they can produce osteoclastogenic mediators such as Rank-L in non-physiological
466 settings⁷⁹. Whether such action of mutant hematopoietic donor cells recreates a pro-
467 osteoclastogenic environment through a remodeling of the myeloid and lymphoid
468 compartments deserves further investigations. Interestingly, the dysregulated bone landscape
469 in BM chimeras with a mutant hematopoietic compartment in WT recipients was even stronger
470 than that observed in mutant mice at steady-state. This observation raises the intriguing
471 possibility of a pro-osteogenic effect of one or several *Cxcr4*¹⁰¹³-bearing radioresistant cell

472 type(s). To test this possibility, further work will be required using mouse model where the
473 mutation is carried in a cell-specific manner.

474 Osteogenesis is regulated, among different mechanisms, by undifferentiated skeletal
475 cells and more specified osteolineage cells that express factors promoting or preventing their
476 own differentiation into OBLs^{23,59,69,80}. In BM, HSPC niches constitute critical spatio-temporal
477 regulatory units composed of multiple cell populations of hematopoietic and non-hematopoietic
478 origin cross-interacting with each other's in a dynamic setting^{1,3,9,81,82}. This implies that immune
479 and vascular cells among others may influence the osteogenic differentiation process⁸³. Again,
480 in BM chimeras in which *Cxcr4*¹⁰¹³-bearing HSPCs were differentiating into a WT bone
481 environment, we reported a similar bone loss as observed in mutant mice, thus indicating cell-
482 extrinsic *Cxcr4*-mediated regulation of the skeletal landscape. This also suggests that neither
483 the epiphyseal cartilage nor any developmental defect contribute to impaired trabecular bone
484 architecture in adult mutant mice, and further supports the notion that HSPCs, as osteolineage
485 cells do, express regulating osteogenic factors such as BMP-2, BMP-7 and WNT3a, that are
486 particularly involved in SSC osteogenesis specification¹⁷. Whether and how hematopoietic
487 cells, or other BM components such as vascular cells, participate in the defective osteolineage
488 specification of SSCs in *Cxcr4*¹⁰¹³-bearing mice deserves further investigations.

489 Finally, we reported that five out of nineteen patients with WS and carrying distinct
490 autosomal-dominant mutations in *CXCR4* exhibit a decrease in BMD at different anatomical
491 sites. Although this would merit to be extended to a larger cohort before introducing any
492 potential bone-affecting drugs, these data suggest that accelerated osteopenia/osteoporosis and
493 increased risk of fractures may constitute a novel feature of WS. Lack of *CXCR4*
494 desensitization could be mechanistically involved in such anomaly since BMSCs from WS
495 patients carrying a heterozygous *CXCR4* mutation displayed *in vitro* impaired capacities to
496 differentiate into osteogenic, but not adipogenic, cells. Strikingly, we observed that chondro-

497 and adipo-genic differentiation of murine mutant SSCs was normal both *in situ* and *in vitro*.
498 Considering recent studies unraveling human SSCs expressing the CXCL12/CXCR4 axis with
499 osteoblastogenic and, depending on their tissue origin, adipocytic potential^{18,84}, our findings
500 pave the way for exploring the BM of WS patients in search for potential defect(s) in these
501 skeletal populations.

502

503

504 **METHODS**505 **Healthy and WS donors and bone mineral density measurements**

506 Investigations of human BM samples were performed in compliance with Good Clinical
507 Practices and the Declaration of Helsinki. The study was approved by the Ethical Board Ile-de-
508 France X. Recruited WS patients were not compensated and gave their written informed
509 consent for participating to the clinical study that has been approved by NIAID Institutional
510 Review Board (IRB). Cryopreserved BM aspirates from two WS patient (NIH protocol 09-I-
511 0200) were provided through a NIH Material Transfer Agreement. The samples were
512 anonymized. BM samples from seven healthy donors that were matched for age and sex and
513 used as control subjects were isolated from hip replacement surgery samples (Protocol 17-030,
514 n° ID-RCB: 2017-A01019-44). Primary BMSCs from healthy and WS donors were amplified
515 and used at passage 1 to 3. For BMD assessment, data were collected from nineteen WS patients
516 as part of an IRB approved clinical protocol conducted at the NIH (NIAID Protocol #2014-I-
517 0185, IND # 118767). Patients had a baseline bone density scan as part of a drug treatment trial
518 (NCT02231879) comparing 1 year of twice daily filgrastim (Neupogen) versus plerixafor
519 (Mozobil) in a randomized, blinded crossover design. There were 13 women and 6 men with
520 an average age of 30.5 years (range 10-56). The samples were anonymized. Patients had been
521 on filgrastim (Neupogen) for an average of 5.7 years prior to enrolling in the trial (range 0-27).
522 6 of the 19 had not used filgrastim regularly prior to trial enrollment. BMD values expressed as
523 T- or Z-scores were measured by total body dual-energy X-ray absorptiometry with a Lunar
524 iDXA densitometer (GE Healthcare). Five WS patients had abnormal screening bone density
525 by WHO criteria, anonymized at the start of the Phase 3 trial (Table 1), while the other 14
526 patients had normal bone density.

527

528 **Mice and genotyping**

529 All mice were bred in our animal facility under a 12h light/dark cycle, specific pathogen-free
530 conditions (EOPS status) and fed *ad libitum*. For breeding, mice were in conventional cages
531 with filter top. For experimentation, mice were housed in individually ventilated cages. All
532 experiments were performed in accordance with the European Union guide for the care and use
533 of laboratory animals and have been reviewed and approved by institutional review committees
534 (CEEA-26, Animal Care and Use Committee, Villejuif, France and Comité d'Ethique Paris-
535 Nord/N°121, Paris, France). *Cxcr4*^{+/¹⁰¹³} (+/1013) mice were generated by a knock-in strategy⁵¹.
536 Homozygous *Cxcr4*^{1013/1013}(1013/1013) mice were obtained by crossing heterozygous +/1013
537 mice. WT mice were used as controls. Unless specified, all mice were littermates, females and
538 age-matched (8-12 wk-old). Adult Boy/J (CD45.1) (Charles River) mice were used as BM
539 donors. Daily observation was performed to ensure that no animal was left in a state of pain or
540 suffering during experimentation. Euthanasia was performed by increasing gradient of CO₂.

541

542 **Sample isolation in mice**

543 Mouse SSCs were obtained from bones after centrifugation of intact femurs, tibias and hips to
544 flush out the BM cells. Flushed long bones were cut into fine pieces before enzymatic digestion
545 with 2.5 U/mL collagenase type I (Thermofisher) for 45 min at 37°C under agitation. Released
546 cells were filtered and washed with PBS, 2% FBS (Fetal Bovine Serum). Cell numbers were
547 standardized as total counts per two legs. Peripheral blood was collected by cardiac puncture.
548 Freshly isolated cells were either immunophenotyped, incubated at 37°C for 60 min in RPMI
549 20 mM HEPES 0.5% BSA (Euromedex) prior to chemokine receptor internalization studies, or
550 expanded in α MEM medium supplemented with 10% FBS, 1% P/S (penicillin 100 Units/mL,
551 streptomycin 100 Units/mL, Gibco) and 50 μ M β -mercaptoethanol (PAN biotech). For BMD
552 quantification, lumbar spines were fixed overnight in ethanol 70° and analyzed by dual-energy

553 X-ray absorptiometry with an ultrafocus DXA densitometer (Faxitron). Quantifications were
554 made on a ROI of 2 lumbar spines.

555

556 **Flow-cytometric analyses**

557 Mouse and human staining analyses were carried out on an LSRII Fortessa flow cytometer (BD
558 Biosciences) using the antibodies (Abs) described in Table S1. A Live/Dead Fixable Aqua Dead
559 Cell Stain Kit (Biolegend) was used. To assess the compartmentalization of CXCR4 and
560 ACKR3, human BMSCs were incubated with saturating concentrations of non-conjugated
561 mouse anti-human CXCR4 or ACKR3 Abs, washed in PBS, fixed and permeabilized using the
562 BD Cytofix/Cytoperm Fixation/Permeabilization Kit (BD Biosciences). BMSCs were
563 subsequently stained with anti-CXCR4 and -ACKR3 conjugated mAbs, or the corresponding
564 isotype control, at 4°C for 30 min and then analyzed by flow cytometry. FACS Diva software
565 version 7 (BD) were used for collecting data. FLOWJO v10.7 (BD) and GraphPad Prism v8.0e
566 (GraphPad software Inc.) were used for analyzing flow cytometric data.

567

568 ***In vitro* functional assays**

569 Mouse CFU-Fs were performed by plating 1×10^5 bone cells at passage 2-3 from WT and mutant
570 mice. Human CFU-Fs were performed by plating 0.2×10^3 BMSCs into a 25 cm² flask at
571 passage 3 from healthy or WS donors. After 7 or 10 days of culture, colonies were fixed with
572 ethanol 70%, stained with 2% crystal violet (Sigma-Aldrich), and counted with a binocular
573 magnifying glass. For chemotaxis assays, 5×10^4 SSCs were added to the upper chambers of a
574 24-well plate with 8- μ m-pore-size Transwell inserts (EMD Millipore) containing or not 1 nM
575 Cxcl12 (R&D Systems) in the lower chamber. For inhibiting Cxcr4-mediated signaling, 10 μ M
576 AMD3100 (Sigma-Aldrich) was added in the upper and lower chambers. After 24h, membranes
577 were removed and fixed in 4% paraformaldehyde (PFA). The cells that migrated to the lower

578 side of the membrane were stained with 0.1% crystal violet and three fields from each insert
579 were counted under a light microscope. Cxcr4 and Ackr3 internalization assays were performed
580 by incubating total bone cells at 37°C for 45 min with 10 nM Cxcl12. Then the reaction was
581 stopped by adding ice-cold RPMI and quick centrifugation at 4°C. After one wash in acidic
582 glycine buffer at pH= 4.3, levels of Cxcr4 and Ackr3 membrane expression were determined
583 by flow cytometry. Cxcr4 or Ackr3 expression was calculated as follows: (Cxcr4 or Ackr3
584 geometric MFI of treated cells/Cxcr4 or Ackr3 geometric MFI of unstimulated cells) × 100;
585 100% corresponds to receptor expression at the surface of cells incubated in medium alone. For
586 the chemokine scavenging assay, cultured SSCs were harvested by trypsinization and placed in
587 complete medium for 90 min at 37°C and 5% CO₂ to normalize receptor expression. 4x10⁶
588 cells/mL were pre-incubated with 100 μM CCX733, a functional Ackr3 antagonist or vehicle
589 in 1% BSA/PBS for 45 min at room temperature (RT). Then, 2x10⁶ cells/mL were incubated in
590 presence of 5 nM AF647-Cxcl12 (Almac) in 1% BSA/PBS during 45-60 min at 37°C to allow
591 internalization or on ice to inhibit this process. Cells were washed with 1% BSA/PBS and then
592 either treated with an acidic glycine wash buffer pH= 2.7 for 3 min to dissociate cell-surface-
593 bound chemokine, or washed with PBS to estimate internalized plus cell-surface-bound control.
594 AF647 fluorescence (geometric MFI) was determined by flow cytometry. Phosphoflow assays
595 were performed with the PerFix EXPOSE kit (Beckman coulter) on cultured SSCs and an anti-
596 phospho Erk (pT202/pY204) was used. Fold change was calculated as follows: (Phospho-Erk
597 geometric MFI of stimulated cells/Phospho-Erk geometric MFI of unstimulated cells).

598

599 ***In vivo* functional assays**

600 For BM transplantation experiments, 1.5x10⁶ total marrow cells from young CD45.1⁺ WT mice
601 were injected i.v. into lethally irradiated (two rounds of 5.5 Gy separated by 3 h) young CD45.2⁺
602 WT, +/-1013 or 1013/1013 recipient mice. For reverse experiments, 1.5x10⁶ total marrow cells

603 from CD45.2⁺ WT, +/-1013 or 1013/1013 mice were injected into lethally irradiated CD45.1⁺
604 WT recipient mice. Chimerism was analyzed 3 or 16 weeks after transplantation. For Cxcr4
605 blockade experiments, mice were daily injected intraperitoneally with 5 mg/kg AMD3100 or
606 PBS during 3 weeks. BM were harvested 2 h after the last injection and analyzed by flow
607 cytometry and imaging.

608

609 **ELISA**

610 Supernatants of culture-expanded human BMSCs were analyzed using a standardized ELISA
611 for human Cxcl12 (Quantikine; R&D Systems).

612

613 **Bone immunostaining and histomorphometry**

614 Mouse bones were fixed in 4% PFA overnight followed by one-week decalcification in EDTA
615 (0.5 M) at pH 7.4 under agitation. Bones were incubated in PBS with 20% sucrose and 2%
616 polyvinylpyrrolidone (PVP) (Sigma) at 4°C overnight and then embedded in PBS with 20%
617 sucrose, 2% PVP and 8% gelatin (Sigma) before storage at -80°C. Sections of 30 µm-thick
618 were rehydrated in PBS 1X, incubated 20 min at RT in PBS with 0.3% triton X-100, saturated
619 in blocking solution (PBS with 5% BSA) and finally incubated with primary Abs (Table S2).
620 After washing, secondary Abs were incubated for 1h at RT with DAPI for nuclear staining and
621 mounting using Permafluor mounting medium (Thermofisher). Images were acquired using
622 TCS SP8 confocal microscope and processed using Fiji software. For alcian blue and perilipin
623 A staining, fixed and decalcified femur bones were embedded in paraffin, sectioned (7 µm-
624 thick) and deparaffinized with xylene. Staining of cartilage tissues was performed with a 1 %
625 alcian blue solution for 30 min. Images were acquired using a LEICA DM4000B microscope
626 equipped with a DFC425C camera and processed with the Leica Application Suite V3.8
627 software. For perilipin A staining, heat induced epitope retrieval was performed in citrate

628 sodium buffer solution. Sections were saturated for 1h in PBS 1% BSA at RT, washed in PBS
629 0.2% BSA and 0.1% Triton X-100, and incubated with anti-perilipin A Ab in PBS BSA 1%
630 overnight at 4°C. After washing, sections were incubated with TRITC-coupled rabbit anti-
631 guinea pig Ab in PBS 1% BSA for 45 min and counterstained with DAPI. For Osx staining, 16
632 µm frozen sections were permeabilized in TBS-0.3% Triton X-100 for 10 min and blocked in
633 TBS-2.5% BSA-2.5% Donkey Serum for 1h at RT. Sections were incubated with anti-Osx Ab
634 (rabbit, Santa Cruz SC-22536R) in blocking solution overnight at 4°C. After washing with
635 TBS+0.025% Triton X-100, sections were incubated in donkey anti-rabbit secondary Ab
636 dylight 550 (SA5-10039, invitrogen) in blocking solution. After washing, sections were
637 incubated 15 min at RT in DAPI at 0.1µg/mL prior to mounting in GB-Mount (Diagomics).
638 Image acquisitions were done using the ApoTome optical sectioning system (Zeiss) with an
639 inverted microscope (Zeiss Axio Observer Z1). Osx quantification was performed using the
640 ICY software. For human BMSC immunofluorescence studies, cells were plated on coverslips
641 and fixed with 4% PFA in PBS. Fixed cells were permeabilized with Triton X 0.3% for 10 min,
642 blocked with PBS 5% BSA, 5% goat serum and incubated with unlabeled primary CXCL12
643 mAb overnight at 4°C followed by secondary AF633-coupled goat anti-mouse polyclonal Ab
644 (Invitrogen) and the nuclear dye Hoechst 33342. Images were obtained with a Plan-
645 Apochromatic objective using the LSM800 confocal microscope (Carl Zeiss). Sections were
646 acquired as serial z stacks (0.39 µm apart) and were subjected to three-dimensional
647 reconstruction (Zen 2.3 System).

648 Bone histomorphometry was performed in plastic samples, allowing the measurements
649 of bone formation and resorption parameters. Mouse femurs were fixed in ethanol 70°,
650 dehydrated and embedded in methyl methacrylate resin. Five micrometer-thick coronal sections
651 were cut parallel to the long axis of the femur using an SM2500S microtome (Leica, Germany).
652 Sections were deplastified, rehydrated and stained with toluidine blue or with naphthol 3-

653 hydroxy-2-naphthoic acid 4-chloro-2-methylanilide (ASTR phosphate, Sigma, St Louis,
654 France) for detecting mature osteoclasts with TRAP staining. Quantifications were made on a
655 polarizing microscope (Nikon) using a software package (Bonolab) developed for bone
656 histomorphometry (Microvision, France). To allow the measure of dynamic parameters of bone
657 formation, mice were intraperitoneally injected with tetracycline (20 mg/kg) and calcein
658 (10 mg/kg; Sigma) 5 days and 1 day respectively before being killed. Two 12- μ m-thick
659 unstained sections were taken for measurement of the dynamic parameters under UV light. The
660 matrix apposition rate (MAR) was measured using the Microvision image analyzer by a
661 semiautomatic method using tetracycline and calcein double-labeled bone surfaces. The
662 mineralizing surfaces (MS/BS) were measured in the same areas using the objective eyepiece
663 Leitz integrate plate II. When specified, the cortical thickness was measured in paraffin-
664 embedded sections stained with Toluidine Blue. The 2 cortices were measured using
665 histomorphometry software and expressed as mean of both cortices for each sample. All the
666 histomorphometric parameters were recorded in compliance with the recommendation of the
667 American Society for Bone and Mineral Research Histomorphometry Nomenclature
668 Committee. Five to six animals per genotype were analyzed by two different investigators.

669

670 **Bone structure analysis by micro-computed tomography**

671 Femurs were collected for bone microarchitecture analysis after fixation and before
672 decalcification. They were analyzed with high-resolution microcomputed tomography (micro-
673 CT) using a Skyscan 1272 microCT (SkyScan, Kontich, Belgium). Measurements were made
674 on the femurs using the following acquisition parameters: voltage 60kV, pixel size 6 μ m, Filter
675 Alu + 0.5mm. After 3-dimensional images reconstruction with NRecon[®], analyses were
676 performed on the trabecular and cortical region (1.72 mm and 0.43 mm thickness, respectively).
677 Morphometric parameters such as Bone Volume/Tissue volume (BV/TV, %), Trabecular

678 number (Tb.Nb, 1/mm), Trabecular Separation (Tb.Sp, mm), Cortical Bone Volume (Ct.BV,
679 mm³) and Cortical Thickness (Ct.Th, mm) were assessed.

680

681 **Cell culture and differentiation**

682 Mouse osteoblastic differentiation was performed for 3 weeks in α -MEM medium with 10%
683 FBS, 1% P/S, 50 μ M β -mercaptoethanol supplemented with 50 μ g/mL L-ascorbic acid and 10
684 mM glycerophosphate (Sigma) either from SSCs or sorted OPCs. Alkaline phosphatase staining
685 was performed after 14 days of differentiation according to the Alkaline phosphatase Kit
686 (Sigma). At day 21, cultures were fixed with 4% PFA, stained with alizarin red and quantified
687 using the Osteogenesis assay kit (Millipore). When specified, AMD3100 (*versus* vehicle) was
688 added into the osteogenic medium every 2 days at 10 μ M respectively. Chondro- and adipo-
689 genic differentiations of SSCs were performed according to the StemPro-Chondrogenesis or -
690 Adipogenic Differentiation Kits (ThermoFisher) for 2 weeks. After fixation, cells were treated
691 with either Alcian Blue 1% (Sigma) to stain chondrocyte matrix or Oil Red O solution (Sigma)
692 to reveal lipid droplets. For *in vitro* human osteogenic differentiation assays, expanded BMSCs
693 were seeded at 3×10^3 *per* cm² in α -MEM supplemented with 10% FBS and 1% antibiotics.
694 After cell adhesion, medium was replaced by α -MEM supplemented with 10% FBS, 1%
695 antibiotics and 0.1 μ M dexamethasone, 0.05 mM L-ascorbic acid-2-phosphate and 10 mM β -
696 glycerophosphate. Medium was changed every 2 days during 3 weeks. Quantification of
697 mineralization was performed after Alizarin Red S staining⁸⁵. Human adipogenic
698 differentiation assays were performed as described for the murine ones.

699

700 **Osteoclast differentiation and functional analysis**

701 OCLs were differentiated *in vitro*⁸⁶. Briefly, 2.3×10^5 BM cells/cm² were plated in MEM-alpha
702 (ThermoFisher) complemented with 5% serum (Hyclone, GE Healthcare), 1% P/S, 50 μ M 2-

703 mercaptoethanol, 25 ng/ml M-CSf and 30 ng/ml Rank-L (R&D Systems). OCL differentiation
704 (multinucleated TRAP⁺ cells) was quantified at day 5 after TRAP coloration using the
705 leukocyte acid phosphatase kit (Sigma). Matrix dissolution activity was evaluated by seeding a
706 total of 2x10⁴ differentiated OCLs on 96-well osteoassay plates (Corning) in α -MEM
707 containing 10% FBS and 30 ng/ml Rank-L. After 3 days, medium was removed and cells were
708 detached by the addition of water. Resorbed areas were quantified using Fiji/ImageJ software⁸⁷.

709

710 **Co-culture assays between osteogenic cells and osteoclast precursors**

711 Mouse osteogenic cells were isolated from the bone fraction of WT or mutant mice as indicated
712 above. They were expanded in α -MEM medium with 10% FBS, 1% P/S, 50 μ M β -
713 mercaptoethanol for 2 weeks, passaged and plated in 96-well plates (2 x 10⁴ cells/well).
714 Osteoclast precursors were obtained from CD11b⁺-enriched cells (with CD11b-Microbeads,
715 Milteniy Biotec, France) from the flushed marrow fraction of WT mice as previously
716 described⁸⁶. They were added, in co-culture, to WT or mutant expanded osteogenic cells (5 x
717 10⁴ cell/well) and stimulated with 1,25-dihydroxy vitamin D3 (vitD3, 10 nM), prostaglandin
718 E2 (PGE2, 1 μ M) and Dexamethasone (Dex, 50 nM) as previously described⁶⁵ in order to
719 stimulate osteogenic cells to produce RankL/MCSF and to inhibit OPG production^{88,89}. OCL
720 differentiation (multinucleated TRAP⁺ cells) was quantified at day 8 after TRAP coloration as
721 indicated above.

722

723 **Cell cycle, viability, survival and proliferation assays**

724 For flow cytometry-based cell cycle analyses, bone cells were permeabilized, fixed with the
725 FOXP3 permeabilization kit (Foxp3/Transcription Factor Staining Buffer Set; eBioscience) and
726 labelled with a Ki67 Ab and DAPI. For BrdU assays, mice were injected intraperitoneally with
727 180 μ g BrdU (Sigma) and maintained with drinking water containing 800 μ g/ml BrdU and 1%

728 glucose over 12 days. The BrdU labelling was analyzed by flow cytometry using the BrdU-
729 FITC labeling kit (BD Biosciences). For *in vitro* BrdU incorporation, 3 µg/ml of BrdU was
730 added to the culture and after five days the percentage of incorporation was determined as
731 above. Apoptosis was measured using the Annexin V detection kit (BD Biosciences) with DAPI
732 staining. For *in vitro* proliferation assays, SSCs were detached with 0.5% trypsin and loaded at
733 3×10^4 cells/well with cell trace violet (CTV, ThermoFisher) for 15 min at 37°C. CTV dilution
734 was assessed by flow cytometry. To estimate the doubling time values, SSCs were seeded at
735 3×10^3 cells/cm² and counted after 3 days of culture. The doubling time was calculated as
736 follows: (time of culture x log(2))/(log(final number of SSC)-log(initial number of SSC)).

737

738 **Quantitative real time-PCR**

739 For mouse gene expression, total RNA was isolated from cultured or sorted SSCs and OPCs
740 using the RNeasy Plus Mini or Micro Kit (Qiagen) and reverse transcribed with oligo(dT) and
741 SuperScript II Reverse Transcriptase (Invitrogen). Quantitative RT-PCR reactions were
742 performed on a Light Cycler instrument (LC480, Roche Diagnostics) with the LightCycler 480
743 SYBR Green detection kit (Roche Diagnostics) using primers reported in Table S3. For human
744 gene expression, total RNA was isolated from cultured BMSCs using Trizol Reagent
745 (ThermoFisher). Reverse transcription was performed using SuperScriptVilo IV
746 (ThermoFisher). When required, total RNA from WS BMSCs and their related controls were
747 extracted from 0.2×10^3 BMSCs and pre-amplified using CellsDirect One-Step qRT-PCR kit
748 (Invitrogen). PCR reactions were performed using primers reported in Table S3 with Power
749 SYBRGreen (Applied Biosystems) on a 7500 FAST apparatus (Applied Biosystems). Mouse
750 *β-actin* and *36b4* and human *β-ACTIN* and *GAPDH* were used as standards for normalization.
751 Relative quantification was determined by the comparative delta-Ct ($2^{-\Delta CT}$) method (fold

752 changes calculated relative to house-keeping genes) or delta-delta-Ct ($2^{-\Delta\Delta CT}$) method (fold
753 changes calculated by setting the mean values obtained from WT cells as one).

754

755 **Multiplex qPCR**

756 Multiplex qPCR was performed using the microfluidic Biomark system. One hundred SSCs
757 were sorted into PCR tubes containing 5 μ l of reverse transcription/pre-amplification mix
758 containing 2X reaction buffer, SuperScriptIII from the CellsDirect One-Step qRT-PCR kit and
759 0.2X Taqman assay (Life technologies) (Table S4). cDNA pre-amplification was performed
760 during 22 cycles and pre-amplified product was diluted 1:5 in TE buffer before processing with
761 Dynamic Array protocol (Fluidigm). Cells expressing β -actin and control genes (*Runx2*, *Coll α* ,
762 *Alp* and *Ibsp*) and not *Pax5* and/or *Cd3* (negative controls) were considered for analyses.
763 Expression of β -actin was used for normalization. Heatmaps were generated with
764 <http://www.heatmapper.ca> using Z scores.

765

766 **RNA sequencing**

767 Pools of 3 x 10³ SSCs or OPCs were sorted from the bone fraction into RLT buffer (Qiagen)
768 with 1% of β -mercaptoethanol. RNA was isolated using RNeasy Micro Kit. cDNAs were
769 generated from 400 to 1,000 pg of total RNA using Clontech SMART-Seq v4 Ultra Low Input
770 RNA kit for Sequencing (Takara Bio Europe) and amplified with 12 cycles of PCR by Seq-
771 Amp polymerase. For Tn5 transposon tagmentation, 600 pg of pre-amplified cDNAs were used
772 by the Nextera XT DNA Library Preparation Kit (96 samples) (Illumina) followed by library
773 amplification of 12 cycles. Purification was performed with Agencourt AMPure XP and
774 SPRIselect beads (Beckman-Coulter). Sequencing reads were generated, in Paired-End mode,
775 on the GenomEast platform (Illumina). FastQC program was used to evaluate the quality of the
776 raw sequencing data and reads shorter than 50 bp were removed. Reads were aligned to the *Mus*

777 *musculus* genome (mm10 build) using the Star tool⁹⁰. Gene expression quantification was
778 obtained using read counting software Htseq⁹¹. Normalization and differential analysis were
779 carried out with DESeq2 package by applying the Benjamini-Hochberg FDR correction ($p <$
780 0.05; 1.5-fold) for comparison between samples. Heatmaps and volcano plots were obtained
781 using the web server Heatmapper and EnhancedVolcano packages respectively.

782

783 **Statistics**

784 All statistical analyses were conducted using Prism software (GraphPad Software). A Kruskal-
785 Wallis test was used to determine the significance of the difference between means of WT,
786 +/-1013 and 1013/1013 groups. Unless specified, the unpaired two-tailed Student *t* test was used
787 to compare means among two groups.

788

789 **DATA AVAILABILITY**

790 RNA-seq data that support the findings of this study have been deposited in the Gene
791 Expression Omnibus repository with the accession code GSE217422
792 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE217422>). The data that support the
793 findings of this study are available from the corresponding author upon request. Source data
794 are provided with this paper.

795

796 REFERENCES

- 797 1. Wei, Q. & Frenette, P. S. Niches for Hematopoietic Stem Cells and Their Progeny. *Immunity*
798 **48**, 632-648 (2018). <https://doi.org:10.1016/j.immuni.2018.03.024>
- 799 2. Bianco, P. Stem cells and bone: a historical perspective. *Bone* **70**, 2-9 (2015).
800 <https://doi.org:10.1016/j.bone.2014.08.011>
- 801 3. Baryawno, N. *et al.* A Cellular Taxonomy of the Bone Marrow Stroma in Homeostasis and
802 Leukemia. *Cell* **177**, 1915-1932 e1916 (2019).
803 <https://doi.org:10.1016/j.cell.2019.04.040>
- 804 4. Grassinger, J., Haylock, D. N., Williams, B., Olsen, G. H. & Nilsson, S. K. Phenotypically
805 identical hemopoietic stem cells isolated from different regions of bone marrow have
806 different biologic potential. *Blood* **116**, 3185-3196 (2010).
807 <https://doi.org:10.1182/blood-2009-12-260703>
- 808 5. Kiel, M. J. *et al.* SLAM family receptors distinguish hematopoietic stem and progenitor cells
809 and reveal endothelial niches for stem cells. *Cell* **121**, 1109-1121 (2005).
810 <https://doi.org:10.1016/j.cell.2005.05.026>
- 811 6. Nakahara, F. *et al.* Engineering a haematopoietic stem cell niche by revitalizing
812 mesenchymal stromal cells. *Nat Cell Biol* **21**, 560-567 (2019).
813 <https://doi.org:10.1038/s41556-019-0308-3>
- 814 7. Pinho, S. *et al.* Lineage-Biased Hematopoietic Stem Cells Are Regulated by Distinct Niches.
815 *Dev Cell* **44**, 634-641 e634 (2018). <https://doi.org:10.1016/j.devcel.2018.01.016>
- 816 8. Sun, J. *et al.* Clonal dynamics of native haematopoiesis. *Nature* **514**, 322-327 (2014).
817 <https://doi.org:10.1038/nature13824>
- 818 9. Tikhonova, A. N. *et al.* The bone marrow microenvironment at single-cell resolution. *Nature*
819 **569**, 222-228 (2019). <https://doi.org:10.1038/s41586-019-1104-8>

- 820 10. Acar, M. *et al.* Deep imaging of bone marrow shows non-dividing stem cells are mainly
821 perisinusoidal. *Nature* **526**, 126-130 (2015). <https://doi.org:10.1038/nature15250>
- 822 11. Asada, N., Takeishi, S. & Frenette, P. S. Complexity of bone marrow hematopoietic stem
823 cell niche. *Int J Hematol* **106**, 45-54 (2017). [https://doi.org:10.1007/s12185-017-2262-](https://doi.org:10.1007/s12185-017-2262-9)
824 [9](https://doi.org:10.1007/s12185-017-2262-9)
- 825 12. Balzano, M. *et al.* Nidogen-1 Contributes to the Interaction Network Involved in Pro-B Cell
826 Retention in the Peri-sinusoidal Hematopoietic Stem Cell Niche. *Cell Rep* **26**, 3257-
827 3271 e3258 (2019). <https://doi.org:10.1016/j.celrep.2019.02.065>
- 828 13. Comazzetto, S. *et al.* Restricted Hematopoietic Progenitors and Erythropoiesis Require SCF
829 from Leptin Receptor+ Niche Cells in the Bone Marrow. *Cell Stem Cell* **24**, 477-486
830 e476 (2019). <https://doi.org:10.1016/j.stem.2018.11.022>
- 831 14. Ding, L., Saunders, T. L., Enikolopov, G. & Morrison, S. J. Endothelial and perivascular
832 cells maintain haematopoietic stem cells. *Nature* **481**, 457-462 (2012).
833 <https://doi.org:10.1038/nature10783>
- 834 15. Kunisaki, Y. *et al.* Arteriolar niches maintain haematopoietic stem cell quiescence. *Nature*
835 **502**, 637-643 (2013). <https://doi.org:10.1038/nature12612>
- 836 16. Zhong, L. *et al.* Single cell transcriptomics identifies a unique adipose lineage cell
837 population that regulates bone marrow environment. *Elife* **9** (2020).
838 <https://doi.org:10.7554/eLife.54695>
- 839 17. Chan, C. K. *et al.* Identification and specification of the mouse skeletal stem cell. *Cell* **160**,
840 285-298 (2015). <https://doi.org:10.1016/j.cell.2014.12.002>
- 841 18. Chan, C. K. F. *et al.* Identification of the Human Skeletal Stem Cell. *Cell* **175**, 43-56 e21
842 (2018). <https://doi.org:10.1016/j.cell.2018.07.029>

- 843 19. Worthley, D. L. *et al.* Gremlin 1 identifies a skeletal stem cell with bone, cartilage, and
844 reticular stromal potential. *Cell* **160**, 269-284 (2015).
845 <https://doi.org:10.1016/j.cell.2014.11.042>
- 846 20. Calvi, L. M. *et al.* Osteoblastic cells regulate the haematopoietic stem cell niche. *Nature*
847 **425**, 841-846 (2003).
- 848 21. Greenbaum, A. *et al.* CXCL12 in early mesenchymal progenitors is required for
849 haematopoietic stem-cell maintenance. *Nature* **495**, 227-230 (2013).
850 <https://doi.org:10.1038/nature11926>
- 851 22. Jung, Y. *et al.* Hematopoietic stem cells regulate mesenchymal stromal cell induction into
852 osteoblasts thereby participating in the formation of the stem cell niche. *Stem Cells* **26**,
853 2042-2051 (2008). <https://doi.org:10.1634/stemcells.2008-0149>
- 854 23. Mendez-Ferrer, S. *et al.* Mesenchymal and haematopoietic stem cells form a unique bone
855 marrow niche. *Nature* **466**, 829-834 (2010). <https://doi.org:10.1038/nature09262>
- 856 24. Nakamura, Y. *et al.* Isolation and characterization of endosteal niche cell populations that
857 regulate hematopoietic stem cells. *Blood* **116**, 1422-1432 (2010).
858 <https://doi.org:10.1182/blood-2009-08-239194>
- 859 25. Omatsu, Y. *et al.* The essential functions of adipo-osteogenic progenitors as the
860 hematopoietic stem and progenitor cell niche. *Immunity* **33**, 387-399 (2010).
861 <https://doi.org:10.1016/j.immuni.2010.08.017>
- 862 26. Yoshihara, H. *et al.* Thrombopoietin/MPL signaling regulates hematopoietic stem cell
863 quiescence and interaction with the osteoblastic niche. *Cell Stem Cell* **1**, 685-697 (2007).
864 <https://doi.org:10.1016/j.stem.2007.10.020>
- 865 27. Zhang, J. *et al.* Identification of the haematopoietic stem cell niche and control of the niche
866 size. *Nature* **425**, 836-841 (2003).

- 867 28. Zhou, B. O., Yue, R., Murphy, M. M., Peyer, J. G. & Morrison, S. J. Leptin-receptor-
 868 expressing mesenchymal stromal cells represent the main source of bone formed by
 869 adult bone marrow. *Cell Stem Cell* **15**, 154-168 (2014).
 870 <https://doi.org:10.1016/j.stem.2014.06.008>
- 871 29. Wolock, S. L. *et al.* Mapping Distinct Bone Marrow Niche Populations and Their
 872 Differentiation Paths. *Cell Rep* **28**, 302-311 e305 (2019).
 873 <https://doi.org:10.1016/j.celrep.2019.06.031>
- 874 30. Ambrosi, T. H. *et al.* Aged skeletal stem cells generate an inflammatory degenerative niche.
 875 *Nature* **597**, 256-262 (2021). <https://doi.org:10.1038/s41586-021-03795-7>
- 876 31. Jeffery, E. C., Mann, T. L. A., Pool, J. A., Zhao, Z. & Morrison, S. J. Bone marrow and
 877 periosteal skeletal stem/progenitor cells make distinct contributions to bone
 878 maintenance and repair. *Cell Stem Cell* (2022).
 879 <https://doi.org:10.1016/j.stem.2022.10.002>
- 880 32. Ara, T. *et al.* Long-term hematopoietic stem cells require stromal cell-derived factor-1 for
 881 colonizing bone marrow during ontogeny. *Immunity* **19**, 257-267 (2003).
 882 [https://doi.org:10.1016/s1074-7613\(03\)00201-2](https://doi.org:10.1016/s1074-7613(03)00201-2)
- 883 33. Cordeiro Gomes, A. *et al.* Hematopoietic Stem Cell Niches Produce Lineage-Instructive
 884 Signals to Control Multipotent Progenitor Differentiation. *Immunity* **45**, 1219-1231
 885 (2016). <https://doi.org:10.1016/j.immuni.2016.11.004>
- 886 34. Itkin, T. *et al.* Distinct bone marrow blood vessels differentially regulate haematopoiesis.
 887 *Nature* **532**, 323-328 (2016). <https://doi.org:10.1038/nature17624>
- 888 35. Nie, Y., Han, Y. C. & Zou, Y. R. CXCR4 is required for the quiescence of primitive
 889 hematopoietic cells. *J Exp Med* **205**, 777-783 (2008).
 890 <https://doi.org:10.1084/jem.20072513>

- 891 36. Sugiyama, T., Kohara, H., Noda, M. & Nagasawa, T. Maintenance of the hematopoietic
892 stem cell pool by CXCL12-CXCR4 chemokine signaling in bone marrow stromal cell
893 niches. *Immunity* **25**, 977-988 (2006).
- 894 37. Miao, R. *et al.* Competition between hematopoietic stem and progenitor cells controls
895 hematopoietic stem cell compartment size. *Nat Commun* **13**, 4611 (2022).
896 <https://doi.org:10.1038/s41467-022-32228-w>
- 897 38. Ding, L. & Morrison, S. J. Haematopoietic stem cells and early lymphoid progenitors
898 occupy distinct bone marrow niches. *Nature* **495**, 231-235 (2013).
899 <https://doi.org:10.1038/nature11885>
- 900 39. Agarwal, P. *et al.* Mesenchymal Niche-Specific Expression of Cxcl12 Controls Quiescence
901 of Treatment-Resistant Leukemia Stem Cells. *Cell Stem Cell* **24**, 769-784 e766 (2019).
902 <https://doi.org:10.1016/j.stem.2019.02.018>
- 903 40. Hosogane, N. *et al.* Stromal derived factor-1 regulates bone morphogenetic protein 2-
904 induced osteogenic differentiation of primary mesenchymal stem cells. *Int J Biochem*
905 *Cell Biol* **42**, 1132-1141 (2010). <https://doi.org:10.1016/j.biocel.2010.03.020>
- 906 41. Li, G. *et al.* Hypermethylation of microRNA-149 activates SDF-1/CXCR4 to promote
907 osteogenic differentiation of mesenchymal stem cells. *J Cell Physiol* **234**, 23485-23494
908 (2019). <https://doi.org:10.1002/jcp.28917>
- 909 42. Shahnazari, M., Chu, V., Wronski, T. J., Nissenson, R. A. & Halloran, B. P.
910 CXCL12/CXCR4 signaling in the osteoblast regulates the mesenchymal stem cell and
911 osteoclast lineage populations. *FASEB J* **27**, 3505-3513 (2013).
912 <https://doi.org:10.1096/fj.12-225763>
- 913 43. Tzeng, Y. S. *et al.* Imbalanced Osteogenesis and Adipogenesis in Mice Deficient in the
914 Chemokine Cxcl12/Sdf1 in the Bone Mesenchymal Stem/Progenitor Cells. *J Bone*
915 *Miner Res* **33**, 679-690 (2018). <https://doi.org:10.1002/jbmr.3340>

- 916 44. Zhu, W., Liang, G., Huang, Z., Doty, S. B. & Boskey, A. L. Conditional inactivation of the
917 CXCR4 receptor in osteoprecursors reduces postnatal bone formation due to impaired
918 osteoblast development. *J Biol Chem* **286**, 26794-26805 (2011).
919 <https://doi.org:10.1074/jbc.M111.250985>
- 920 45. Matsushita, Y. *et al.* A Wnt-mediated transformation of the bone marrow stromal cell
921 identity orchestrates skeletal regeneration. *Nat Commun* **11**, 332 (2020).
922 <https://doi.org:10.1038/s41467-019-14029-w>
- 923 46. Zehentmeier, S. *et al.* Dysregulated stem cell niches and altered lymphocyte recirculation
924 cause B and T cell lymphopenia in WHIM syndrome. *Sci Immunol* **7**, eabo3170 (2022).
925 <https://doi.org:10.1126/sciimmunol.abo3170>
- 926 47. Dotta, L., Tassone, L. & Badolato, R. Clinical and genetic features of Warts,
927 Hypogammaglobulinemia, Infections and Myelokathexis (WHIM) syndrome. *Curr Mol*
928 *Med* **11**, 317-325 (2011).
- 929 48. Kawai, T. & Malech, H. L. WHIM syndrome: congenital immune deficiency disease. *Curr*
930 *Opin Hematol* **16**, 20-26 (2009). <https://doi.org:10.1097/MOH.0b013e32831ac557>
- 931 49. Murphy, P. M. & Heusinkveld, L. Multisystem multitasking by CXCL12 and its receptors
932 CXCR4 and ACKR3. *Cytokine* **109**, 2-10 (2018).
933 <https://doi.org:10.1016/j.cyto.2017.12.022>
- 934 50. Hernandez, P. A. *et al.* Mutations in the chemokine receptor gene CXCR4 are associated
935 with WHIM syndrome, a combined immunodeficiency disease. *Nat Genet* **34**, 70-74
936 (2003). <https://doi.org:10.1038/ng1149>
- 937 51. Balabanian, K. *et al.* Proper desensitization of CXCR4 is required for lymphocyte
938 development and peripheral compartmentalization in mice. *Blood* **119**, 5722-5730
939 (2012). <https://doi.org:10.1182/blood-2012-01-403378>

- 940 52. Biajoux, V. *et al.* Efficient Plasma Cell Differentiation and Trafficking Require Cxcr4
 941 Desensitization. *Cell Rep* **17**, 193-205 (2016).
 942 <https://doi.org:10.1016/j.celrep.2016.08.068>
- 943 53. Freitas, C. *et al.* Lymphoid differentiation of hematopoietic stem cells requires efficient
 944 Cxcr4 desensitization. *J Exp Med* **214**, 2023-2040 (2017).
 945 <https://doi.org:10.1084/jem.20160806>
- 946 54. Alouche, N. *et al.* Hematologic disorder-associated Cxcr4 gain-of-function mutation leads
 947 to uncontrolled extrafollicular immune response. *Blood* **137**, 3050-3063 (2021).
 948 <https://doi.org:10.1182/blood.2020007450>
- 949 55. Haribabu, B. *et al.* Regulation of human chemokine receptors CXCR4. Role of
 950 phosphorylation in desensitization and internalization. *J Biol Chem* **272**, 28726-28731
 951 (1997). <https://doi.org:10.1074/jbc.272.45.28726>
- 952 56. Balabanian, K. *et al.* WHIM syndromes with different genetic anomalies are accounted for
 953 by impaired CXCR4 desensitization to CXCL12. *Blood* **105**, 2449-2457 (2005).
 954 <https://doi.org:10.1182/blood-2004-06-2289>
- 955 57. Mayol, K., Biajoux, V., Marvel, J., Balabanian, K. & Walzer, T. Sequential desensitization
 956 of CXCR4 and S1P5 controls natural killer cell trafficking. *Blood* **118**, 4863-4871
 957 (2011). <https://doi.org:10.1182/blood-2011-06-362574>
- 958 58. Matsushita, Y., Ono, W. & Ono, N. Flow Cytometry-Based Analysis of the Mouse Bone
 959 Marrow Stromal and Perivascular Compartment. *Methods Mol Biol* **2308**, 83-94 (2021).
 960 https://doi.org:10.1007/978-1-0716-1425-9_7
- 961 59. Mende, N. *et al.* Prospective isolation of nonhematopoietic cells of the niche and their
 962 differential molecular interactions with HSCs. *Blood* **134**, 1214-1226 (2019).
 963 <https://doi.org:10.1182/blood.2019000176>

- 964 60. Morikawa, S. *et al.* Prospective identification, isolation, and systemic transplantation of
965 multipotent mesenchymal stem cells in murine bone marrow. *J Exp Med* **206**, 2483-
966 2496 (2009). <https://doi.org:10.1084/jem.20091046>
- 967 61. Parfitt, A. M. Osteoclast precursors as leukocytes: importance of the area code. *Bone* **23**,
968 491-494 (1998). [https://doi.org:10.1016/s8756-3282\(98\)00140-9](https://doi.org:10.1016/s8756-3282(98)00140-9)
- 969 62. Zhu, H. *et al.* A protocol for isolation and culture of mesenchymal stem cells from mouse
970 compact bone. *Nat Protoc* **5**, 550-560 (2010). <https://doi.org:10.1038/nprot.2009.238>
- 971 63. Abou Nader, Z., Espeli, M., Balabanian, K. & Lemos, J. P. Culture, Expansion and
972 Differentiation of Mouse Bone-Derived Mesenchymal Stromal Cells. *Methods Mol Biol*
973 **2308**, 35-46 (2021). https://doi.org:10.1007/978-1-0716-1425-9_3
- 974 64. Wright, H. L., McCarthy, H. S., Middleton, J. & Marshall, M. J. RANK, RANKL and
975 osteoprotegerin in bone biology and disease. *Curr Rev Musculoskelet Med* **2**, 56-64
976 (2009). <https://doi.org:10.1007/s12178-009-9046-7>
- 977 65. Ciucci, T. *et al.* Bone marrow Th17 TNFalpha cells induce osteoclast differentiation, and
978 link bone destruction to IBD. *Gut* **64**, 1072-1081 (2015). [https://doi.org:10.1136/gutjnl-](https://doi.org:10.1136/gutjnl-2014-306947)
979 [2014-306947](https://doi.org:10.1136/gutjnl-2014-306947)
- 980 66. Feng, X. & McDonald, J. M. Disorders of bone remodeling. *Annu Rev Pathol* **6**, 121-145
981 (2011). <https://doi.org:10.1146/annurev-pathol-011110-130203>
- 982 67. Sims, N. A. & Martin, T. J. Osteoclasts Provide Coupling Signals to Osteoblast Lineage
983 Cells Through Multiple Mechanisms. *Annu Rev Physiol* **82**, 507-529 (2020).
984 <https://doi.org:10.1146/annurev-physiol-021119-034425>
- 985 68. Signer, R. A. & Morrison, S. J. Mechanisms that regulate stem cell aging and life span. *Cell*
986 *Stem Cell* **12**, 152-165 (2013). <https://doi.org:10.1016/j.stem.2013.01.001>

- 987 69. Seike, M., Omatsu, Y., Watanabe, H., Kondoh, G. & Nagasawa, T. Stem cell niche-specific
988 Ebf3 maintains the bone marrow cavity. *Genes Dev* **32**, 359-372 (2018).
989 <https://doi.org:10.1101/gad.311068.117>
- 990 70. Mizoguchi, T. *et al.* Osterix marks distinct waves of primitive and definitive stromal
991 progenitors during bone marrow development. *Dev Cell* **29**, 340-349 (2014).
992 <https://doi.org:10.1016/j.devcel.2014.03.013>
- 993 71. Maes, C. *et al.* Osteoblast precursors, but not mature osteoblasts, move into developing and
994 fractured bones along with invading blood vessels. *Dev Cell* **19**, 329-344 (2010).
995 <https://doi.org:10.1016/j.devcel.2010.07.010>
- 996 72. Shen, B. *et al.* A mechanosensitive peri-arteriolar niche for osteogenesis and lymphopoiesis.
997 *Nature* **591**, 438-444 (2021). <https://doi.org:10.1038/s41586-021-03298-5>
- 998 73. Devignes, C. S. *et al.* HIF signaling in osteoblast-lineage cells promotes systemic breast
999 cancer growth and metastasis in mice. *Proc Natl Acad Sci U S A* **115**, E992-E1001
1000 (2018). <https://doi.org:10.1073/pnas.1718009115>
- 1001 74. Ambrosi, T. H. *et al.* Distinct skeletal stem cell types orchestrate long bone skeletogenesis.
1002 *Elife* **10** (2021). <https://doi.org:10.7554/eLife.66063>
- 1003 75. Cenci, S., Weitzmann, M. N., Gentile, M. A., Aisa, M. C. & Pacifici, R. M-CSF
1004 neutralization and egr-1 deficiency prevent ovariectomy-induced bone loss. *J Clin*
1005 *Invest* **105**, 1279-1287 (2000). <https://doi.org:10.1172/JCI8672>
- 1006 76. Cenci, S. *et al.* Estrogen deficiency induces bone loss by enhancing T-cell production of
1007 TNF-alpha. *J Clin Invest* **106**, 1229-1237 (2000). <https://doi.org:10.1172/JCI11066>
- 1008 77. Cao, J. J. *et al.* Aging increases stromal/osteoblastic cell-induced osteoclastogenesis and
1009 alters the osteoclast precursor pool in the mouse. *J Bone Miner Res* **20**, 1659-1668
1010 (2005). <https://doi.org:10.1359/JBMR.050503>

- 1011 78. Nakashima, T. *et al.* Evidence for osteocyte regulation of bone homeostasis through
1012 RANKL expression. *Nat Med* **17**, 1231-1234 (2011). <https://doi.org/10.1038/nm.2452>
- 1013 79. Wakkach, A., Rouleau, M. & Blin-Wakkach, C. Osteoimmune Interactions in Inflammatory
1014 Bowel Disease: Central Role of Bone Marrow Th17 TNFalpha Cells in
1015 Osteoclastogenesis. *Front Immunol* **6**, 640 (2015).
1016 <https://doi.org/10.3389/fimmu.2015.00640>
- 1017 80. Komori, T. Regulation of osteoblast differentiation by transcription factors. *J Cell Biochem*
1018 **99**, 1233-1239 (2006). <https://doi.org/10.1002/jcb.20958>
- 1019 81. Aurrand-Lions, M. & Mancini, S. J. C. Murine Bone Marrow Niches from Hematopoietic
1020 Stem Cells to B Cells. *Int J Mol Sci* **19** (2018). <https://doi.org/10.3390/ijms19082353>
- 1021 82. Bonaud, A., Lemos, J. P., Espeli, M. & Balabanian, K. Hematopoietic Multipotent
1022 Progenitors and Plasma Cells: Neighbors or Roommates in the Mouse Bone Marrow
1023 Ecosystem? *Front Immunol* **12**, 658535 (2021).
1024 <https://doi.org/10.3389/fimmu.2021.658535>
- 1025 83. Kusumbe, A. P., Ramasamy, S. K. & Adams, R. H. Coupling of angiogenesis and
1026 osteogenesis by a specific vessel subtype in bone. *Nature* **507**, 323-328 (2014).
1027 <https://doi.org/10.1038/nature13145>
- 1028 84. Xu, J. *et al.* Comparison of skeletal and soft tissue pericytes identifies CXCR4(+) bone
1029 forming mural cells in human tissues. *Bone Res* **8**, 22 (2020).
1030 <https://doi.org/10.1038/s41413-020-0097-0>
- 1031 85. Bisio, V., Espeli, M., Balabanian, K. & Anginot, A. Culture, Expansion and Differentiation
1032 of Human Bone Marrow Stromal Cells. *Methods Mol Biol* **2308**, 3-20 (2021).
1033 https://doi.org/10.1007/978-1-0716-1425-9_1

- 1034 86. Ibanez, L. *et al.* Inflammatory Osteoclasts Prime TNF α -Producing CD4(+) T Cells and
1035 Express CX3 CR1. *J Bone Miner Res* **31**, 1899-1908 (2016).
1036 <https://doi.org:10.1002/jbmr.2868>
- 1037 87. Schindelin, J. *et al.* Fiji: an open-source platform for biological-image analysis. *Nature*
1038 *methods* **9**, 676-682 (2012). <https://doi.org:10.1038/nmeth.2019>
- 1039 88. Kaji, H., Sugimoto, T., Kanatani, M., Nishiyama, K. & Chihara, K. Dexamethasone
1040 stimulates osteoclast-like cell formation by directly acting on hemopoietic blast cells
1041 and enhances osteoclast-like cell formation stimulated by parathyroid hormone and
1042 prostaglandin E2. *J Bone Miner Res* **12**, 734-741 (1997).
1043 <https://doi.org:10.1359/jbmr.1997.12.5.734>
- 1044 89. Marino, S., Logan, J. G., Mellis, D. & Capulli, M. Generation and culture of osteoclasts.
1045 *Bonekey Rep* **3**, 570 (2014). <https://doi.org:10.1038/bonekey.2014.65>
- 1046 90. Dobin, A. *et al.* STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* **29**, 15-21
1047 (2013). <https://doi.org:10.1093/bioinformatics/bts635>
- 1048 91. Anders, S., Pyl, P. T. & Huber, W. HTSeq--a Python framework to work with high-
1049 throughput sequencing data. *Bioinformatics* **31**, 166-169 (2015).
1050 <https://doi.org:10.1093/bioinformatics/btu638>
1051
1052

1053 **ACKNOWLEDGMENTS**

1054 We thank ML. Aknin (IPSIT, Facility PLAIMMO, Orsay), F. Mercier-Nomé (IPSIT, Facility
1055 PHIC, Orsay), Drs. V. Parietti-Montcuquet, C. Doliger, S. Duchez and N. Setterblad (Animal
1056 and Flow Cytometry Core Facilities, Institut de Recherche Saint-Louis, Paris), V. Nicolas
1057 (IPSIT, Facility MIPSIT, Orsay), D. Courilleau (IPSIT, Facility CIBLOT, Orsay) and C.
1058 Cordier and J. Megret (Plateau technique de cytométrie, SFR Necker, Paris) for their technical
1059 assistance. We thank the Montpellier Preclinical Platform of the Research Infrastructure
1060 ECELLFRANCE for the microCT analyses as well as the Plateforme d'Irradiation (IRSN,
1061 Fontenay-Aux-Roses, France) for their technical assistance. The study was supported by the
1062 LabEx LERMIT supported by ANR grant ANR-10-LABX-33 under the Program
1063 “Investissements d’Avenir” ANR-11-IDEX-0003-01, an ANR PRC grant (ANR-17-CE14-
1064 0019) to M.A-L., C.B-W. and coordinated by K.B. and by the Association Saint Louis pour la
1065 Recherche sur les Leucémies to KB. J.N. was a PhD fellow from the DIM Cancéropôle and the
1066 FRM. Z.A-N. was a fellowship recipient from the French Ministry and from the FRM
1067 (FDT202204015088). V.R. was supported by the FRM, La Ligue Contre le Cancer and la
1068 Société Française d’Hématologie. A.Bon. was supported by an ANR @RAction grant (ANR-
1069 14-ACHN-0008) and by a JCJC ANR grant (ANR-19-CE15-0019-01) to ME. A.Bou. was
1070 supported by the ANR grant 17-CE14-0019. J.L. was recipient from the People Program (Marie
1071 Curie Actions) of the European Union’s Seventh Framework Program (FP7/2007-2013) under
1072 REA grant agreement n. PCOFUND-GA-2013-609102, through the PRESTIGE Program
1073 coordinated by Campus France, and from an ANR grant (ANR-17-CE14-0019). V.B., N.D. and
1074 K.B. were supported by the INCa agency under the program PRT-K 2017. J.K. was supported
1075 by European Union’s Horizon 2020 MSCA, Program under grant agreement 641833
1076 (ONCORNET). D.H.M and P.M.M. were supported by the Division of Intramural Research of
1077 the National Institute of Allergy and Infectious Diseases, National Institutes of Health.

1078 Graphical abstract and mouse icons were created using the Biorender software
1079 (Biorender.com).

1080

1081 **AUTHOR CONTRIBUTIONS**

1082 A.A., J.N. and Z.A-N. designed and performed experiments, analyzed data and contributed to
1083 manuscript writing; V.R., A.Bon., M.K., A.Bou., J.L., V.B., J.K., L.S., A.P. and A.C. performed
1084 experiments and analyzed data; S.P., N.D., M.A-L., S.J.C.M., G.L., F.G., C.B-W. and M.C-S.
1085 performed experiments, contributed to data analyses and reviewed the manuscript; D.H.M. and
1086 P.M.M provided WS samples and clinical data and reviewed the manuscript; M.E. and M.R.
1087 helped with the study design, performed experiments, contributed to data analyses and reviewed
1088 the manuscript; K.B. conceived, designed and supervised the study, contributed to data
1089 analyses, found funding for the study, and wrote the manuscript.

1090

1091 **COMPETING INTERESTS**

1092 The authors declare no competing interests.

1093

1094 **TABLES**

1095

	Gender	Chronic treatment	Lumbar spine	Femoral neck
P1	Female	No	-3.1 ⁽⁵⁾	0
P2	Female	No	-1.1	-1.8
P3⁽¹⁾	Male	Yes ⁽²⁾	-1.8	-2.3
P4	Female	Yes ⁽³⁾	-2.7	-1.3
P5⁽¹⁾	Male	Yes ⁽⁴⁾	-1.8	-2.2

1096

1097 **Table 1: Abnormal bone mineral density values in WS patients.** Characteristics of each
 1098 patient with low BMD value are shown. Four patients carry the *CXCR4*^{R334X} mutation and one
 1099 displays the *CXCR4*^{S338X} mutation. There were 3 women and 2 men with an average age of 33.2
 1100 years (range 13-52). T-scores for lumbar spine (L1-L4) and femoral neck have been evaluated.
 1101 According to World Health Organization (WHO) criteria, values classify patients as osteopenic
 1102 with a T-score between -1.0 and -2.5 or osteoporotic with a T-score at or below -2.5.

1103

1104

1105

1106

1107

1108 ⁽¹⁾For patients 3 and 5, because of their young age, Z-scores are given with a value at or below
 1109 -2.0 considered as abnormal; ⁽²⁾G-CSF since age of 2; ⁽³⁾G-CSF several years at the time of
 1110 scan; ⁽⁴⁾G-CSF for 6 months at the time of scan; ⁽⁵⁾Values outside the normal range defined by
 1111 WHO are italicized.

1112 **FIGURE LEGENDS**

1113 **Figure 1: WS-linked *Cxcr4* mutations are associated with reduced bone mass in mice. (A)**

1114 The bone mineral density (BMD) of lumbar spine of WT, +/-1013 and 1013/1013 mice was
 1115 measured through Dual-energy x-ray absorptiometry. Results represent means \pm SEM with 5
 1116 mice per group examined over two independent experiments. Statistics were calculated with
 1117 the nonparametric Kruskal–Wallis H test ($^{##}p=0.0042$) and the nonparametric Mann–Whitney
 1118 test, two-sided, +/-1013 $p=0.0556$, 1013/1013 $^{**}p=0.0079$. **(B–D)** 3D representative images of
 1119 trabecular and cortical composites (B) and quantitative μ CT analyses of trabecular (C) and
 1120 cortical (D) parameters of femurs from WT and mutant mice. BV = bone volume; TV =
 1121 trabecular volume; Tb.Nb = trabecular number; Tb.Sp = trabecular separation; Ct.BV = cortical
 1122 bone volume; Ct.Th = cortical thickness. Data (means \pm SEM) are from three independent
 1123 experiments with $n= 15, 15,$ and 7 mice in total for WT, +/-1013 and 1013/1013 groups,
 1124 respectively. Statistics were calculated with the nonparametric Kruskal–Wallis H test
 1125 ($^{###}p=0.0007$, BV/TV; $p=0.0001$, Tb.Nb; $p<0.0001$, Tb.Sp; Ct.BV, $^{##}p=0.0072$; $^{\#}p=0.0241$) and
 1126 the unpaired two-tailed Student’s t test (+/1013 vs WT $^{**}p=0.0556$, 1013/1013 vs WT
 1127 $^{***}p=0.0005$, +/-1013 vs 1013/1013 $^{\$}p=0.032$ for BV/TV; $^{***}p=0.0005$, $^{***}p<0.0001$,
 1128 $^{\$\$}p=0.008$ for Tb.Nb; $^{***}p=0.0038$, $^{***}p<0.0001$, $^{\$ \$ \$}p<0.0001$ for Tb.Sp; $^{**}p=0.0072$,
 1129 $^{**}p=0.0078$ for Ct.BV; $^*p=0.0147$, $^*p=0.02$ for Ct.Th). **(E)** BM sections were stained with
 1130 toluidine blue. Larger images show 2X inserts in trabecular areas. Bars: 200 μ m. Images are
 1131 representative of at least three independent determinations. **(F)** BM sections were
 1132 immunostained for osteopontin (Opn) in association with DAPI. Trabeculae are indicated by
 1133 white arrows. Bars: 250 μ m. Images are representative of five independent determinations. **(G**
 1134 **and H)** BM sections were stained for chondrocyte (alcian blue, G) or adipocyte (perilipin, H)
 1135 markers. Bars: 500 (G) or 20 (H) μ m. Images are representative of at least six independent
 1136 determinations. **(I)** Cartilaginous growth plates were evaluated based on overall growth plate

1137 thickness measured on μ CT scans. Data (means \pm SEM) are from 2 independent experiments
1138 with n= 7, 8, and 7 mice in total for WT, +/1013 and 1013/1013 groups, respectively. **(J)**
1139 Adipocyte counts were evaluated on perilipin-stained BM sections. Six fields of 3 mm² were
1140 analyzed per section. Results (means \pm SEM) are from 4 independent experiments with 13 mice
1141 in total per group. **(K)** Size (left) and weight (right) of WT and mutant mice. Results (means \pm
1142 SEM) are from five independent experiments with n= 6, 9, and 10 mice in total for WT, +/1013
1143 and 1013/1013 groups, respectively. Mice were littermates, females and age-matched (8-12 wk-
1144 old) in A-J and at 8 weeks of age in K. Source data are provided as a Source Data file.

1145

1146 **Figure 2: Reduction of skeletal stromal cells in *Cxcr4*¹⁰¹³-bearing mice.** **(A)** Representative
1147 dot-plots showing the flow cytometric gating strategies used to sort stroma cells (CD45⁻
1148 TER119⁻), differentiated osteoblast progenitor cells (OPCs, CD45⁻TER119⁻CD31⁻Sca-1⁻
1149 CD51⁺PDGFR α ^{+/+}) and SSCs (CD45⁻TER119⁻CD31⁻Sca-1⁺CD51⁺PDGFR α ⁺) in the mouse
1150 bone fraction. **(B)** Absolute numbers of the indicated stroma cell subsets from bone fractions
1151 were determined by flow cytometry. Data (means \pm SEM) are from at least six independent
1152 experiments with n= 31, 31, and 17 mice in total for WT, +/1013 and 1013/1013 groups,
1153 respectively. Statistics were calculated with the nonparametric Kruskal–Wallis H test
1154 (####p<0.0001, OPC) and the unpaired two-tailed Student's t test (1013/1013 vs WT *p=0.0206
1155 for SSC; +/1013 vs WT **p=0.0031, 1013/1013 vs WT ***p<0.0001, +/1013 vs 1013/1013
1156 §p=0.0217 for OPC). **(C)** Schematic diagram for the generation of CD45.1 \rightarrow CD45.2 short (3
1157 wks)- or long (16 wks)-term BM chimeras. **(D)** Proportions of WT donor CD45.1⁺ LSK SLAMF
1158 and leukocytes (Leuko.) recovered from the BM and blood of BM chimeras in CD45.2⁺ WT or
1159 mutant recipients 16 weeks after transplantation. **(E)** Absolute numbers of SSCs and OPCs
1160 determined by flow cytometry in bone fractions of BM chimeras in CD45.2⁺ recipients. Data
1161 (means \pm SEM) in D and E are from three independent experiments with n= 10 (D) or 11 (E),

1162 9, and 5 mice in total for WT, +/-1013 and 1013/1013 recipient groups, respectively. Statistics
1163 were calculated with the nonparametric Kruskal–Wallis H test ($^{##}p=0.0064$) and the
1164 nonparametric Mann–Whitney test, two-sided (+/1013 vs WT $^{**}p=0.0042$, 1013/1013 vs WT
1165 $^{*}p=0.0126$, for SSC; +/-1013 vs WT $^{**}p=0.0031$, 1013/1013 vs WT $^{*}p=0.0398$, for OPC). **(F)**
1166 Sixteen weeks after transplantation, BM sections from WT or mutant CD45.2⁺ recipient mice
1167 were immuno-stained for Opn in association with DAPI (bars: 250 μ m). Trabeculae are
1168 indicated by white arrows. Images are representative of at least three independent
1169 determinations. **(G)** Left: Proportions of WT donor CD45.1⁺ LSK SLAM and leukocytes
1170 recovered from the BM and blood of BM chimeras in CD45.2⁺ WT or mutant recipients 3 weeks
1171 after transplantation. Right: Absolute numbers of SSCs and OPCs. Data (means \pm SEM) are
1172 from three independent experiments with n= 9, 9, and 5 mice in total for WT, +/-1013 and
1173 1013/1013 recipient groups, respectively, except for blood chimerism analysis (5 mice per
1174 group). Statistics were calculated with the nonparametric Kruskal–Wallis H test ($^{#}p=0.0327$)
1175 and the nonparametric Mann–Whitney test, two-sided (1013/1013 vs WT $^{*}p=0.019$, +/-1013 vs
1176 1013/1013 $^{§}p=0.019$ for SSC; 1013/1013 vs WT $^{*}p=0.017$ for OPC). **(H and I)** 3D
1177 representative images of trabecular composites (H) and μ CT analyses of trabecular parameters
1178 (I) of femurs from WT BM-chimeric CD45.2⁺ WT or mutant recipients 4 months after
1179 transplantation. Data (means \pm SEM) are from three independent experiments with n= 8, 5, and
1180 5 mice in total for WT, +/-1013 and 1013/1013 recipient groups, respectively. Statistics were
1181 calculated with the unpaired two-tailed Student’s t test (+/1013 vs WT $^{*}p=0.033$, 1013/1013 vs
1182 WT $^{*}p=0.024$ for BV/TV). WT and mutant mice were littermates, females and age-matched
1183 (8-12 wk-old) and adult Boy/J (CD45.1) WT mice at 8 weeks of age were used as BM donors.
1184 Source data are provided as a Source Data file.
1185

1186 **Figure 3: Cell-extrinsic Cxcr4-mediated regulation of the skeletal landscape. (A)**
 1187 Schematic diagram for the generation of CD45.2→CD45.1 short (3 wks)- or long (16 wks)-
 1188 term BM chimeras. **(B)** Proportions of WT or mutant donor CD45.2⁺ LSK SLAM and
 1189 leukocytes recovered from the BM and blood of BM chimeras in CD45.1⁺ WT recipients 16
 1190 weeks after transplantation. Statistics were calculated with the nonparametric Kruskal–Wallis
 1191 H test (###p=0.0008 for SLAM and <0.0001 for Leukocytes) and the unpaired two-tailed
 1192 Student’s t test (+/1013 vs WT **p=0.0036, 1013/1013 vs WT ***p=0.0004, for SLAM;
 1193 +/1013 vs WT ***p=0.0003, 1013/1013 vs WT ***p<0.0001, for Leukocytes). **(C)** Absolute
 1194 numbers of SSCs and OPCs in bone fractions of BM chimeras in CD45.1⁺ recipients. Statistics
 1195 were calculated with the nonparametric Kruskal–Wallis H test (##p=0.009 for SSC and ###p
 1196 =0.0006) and the unpaired two-tailed Student’s t test (1013/1013 vs WT **p=0.0012, +/1013
 1197 vs 1013/1013 §§p=0.0076, for SSC; +/1013 vs WT *p=0.029, 1013/1013 vs WT ***p<0.0001,
 1198 +/1013 vs 1013/1013 §p=0.022, for OPC). Data (means ± SEM) in B and C are from three
 1199 independent experiments with n= 14 mice in total for WT, +/1013 and 1013/1013 donor groups,
 1200 respectively, except for blood chimerism analysis (n= 11, 9, and 8 mice in total for WT, +/1013
 1201 and 1013/1013 donor groups, respectively). **(D)** Sixteen weeks after transplantation, BM
 1202 sections from WT CD45.1⁺ recipient mice were immunostained for Opn in association with
 1203 DAPI (bars: 250 μm). Trabeculae are indicated by white arrows. Images are representative of
 1204 at least three independent determinations. **(E)** Left: Proportions of WT or mutant donor
 1205 CD45.2⁺ LSK SLAM and leukocytes recovered from the BM and blood of BM chimeras in
 1206 CD45.1⁺ WT recipients 3 weeks after transplantation. Right: Absolute numbers of SSCs and
 1207 OPCs. Data (means ± SEM) are from three independent experiments with n= 6 (SLAM) or 7
 1208 (SSC and OPC), 10, and 8 mice in total for WT, +/1013 and 1013/1013 donor groups,
 1209 respectively, except for blood chimerism analysis (n= 6, 5, and 4 mice in total for WT, +/1013
 1210 and 1013/1013 donor groups, respectively). Statistics were calculated with the nonparametric

1211 Kruskal–Wallis H test (^{##}p=0.0083 for leukocytes; [#]p=0.047 for SSC; [#]p=0.018 for OPC) and
1212 the unpaired two-tailed Student’s t test (+/1013 vs WT *p=0.021, 1013/1013 vs WT
1213 **p=0.0014, for leukocytes; +/1013 vs WT *p=0.046, 1013/1013 vs WT *p=0.029, for SSC;
1214 +/1013 vs WT *p=0.034, 1013/1013 vs WT *p=0.01, for OPC). (F–I) 3D representative images
1215 of trabecular or cortical composites (F and H) and μ CT analyses of trabecular or cortical
1216 parameters (G and I) of femurs from WT or mutant BM-chimeric CD45.1⁺ WT recipients 4
1217 months after transplantation. Ct.BV = cortical bone volume; Ct.Th = cortical thickness. Data
1218 (means \pm SEM) in G and I are from two independent experiments with n= 5, 5, and 4 mice in
1219 total for WT, +/1013 and 1013/1013 donor groups, respectively. Statistics were calculated with
1220 the nonparametric Kruskal–Wallis H test (^{##}p=0.0085 for BV/TV; ^{##}p=0.0069 for Tb.Nb;
1221 ^{###}p=0.0001 for Tb.Sp; [#]p=0.033 for Ct.BV) and the unpaired two-tailed Student’s t test
1222 (1013/1013 vs WT *p=0.028, +/1013 vs 1013/1013 [§]p=0.028 for BV/TV; 1013/1013 vs WT
1223 *p=0.011, +/1013 vs 1013/1013 ^{§§}p=0.0088 for Tb.Nb; +/1013 vs WT *p=0.044, 1013/1013 vs
1224 WT ***p<0.0001, +/1013 vs 1013/1013 ^{§§§}p=0.0006 for Tb.Sp; 1013/1013 vs WT *p=0.014,
1225 +/1013 vs 1013/1013 [§]p=0.016 for Ct.BV). Donor WT and mutant mice and Boy/J (CD45.1)
1226 WT recipient mice were females at 8 weeks of age. Source data are provided as a Source Data
1227 file.

1228

1229 **Figure 4: Increased bone resorption and reduced bone formation in *Cxcr4*¹⁰¹³-bearing**
1230 **mice. (A)** Bone sections were stained for Tartrate Resistant Acid Phosphatase (TRAP) activity
1231 (bars: 100 μ m). OCLs are visualized as brown-stained TRAP-positive cells attached to bone
1232 trabeculae and are indicated by arrows (representative images). **(B)** OCLs were quantified
1233 (Oc.S/BS) and (Oc.N/BV). Results represent means \pm SEM with 6 mice in total per group over
1234 3 independent experiments. Statistics were calculated with the nonparametric Kruskal–Wallis
1235 H test (^{###}p=0.0006) and the unpaired two-tailed Student’s t test (1013/1013 vs WT *p=0.012

1236 for Oc.S/BS; 1013/1013 vs WT * $p=0.049$ for Oc.N/BV). **(C and D)** Dynamic
1237 histomorphometric measures of bone formation. OS/BS = Osteoid number / Bone surface;
1238 Obl.S/BS = Osteoblast surface / Bone surface; MS/BS = Mineralized surface / Bone surface;
1239 Dbl/BS = Double labelled surface / Bone surface. Data (means \pm SEM) are from 3 independent
1240 experiments with $n=6, 6,$ and 5 mice in total for WT, +/1013 and 1013/1013 groups respectively
1241 in C, and $n=6, 6,$ and 5 (for MS/BS) or 6 (for Dbl/BS) mice in total for WT, +/1013 and
1242 1013/1013 groups respectively in D. Statistics were calculated with the nonparametric Kruskal–
1243 Wallis H test ($\#p=0.038$) and the unpaired two-tailed Student’s t test (1013/1013 vs WT
1244 ** $p=0.0097$ for MS/BS; 1013/1013 vs WT * $p=0.035$ for Dbl/BS). **(E)** The mineral apposition
1245 rate (MAR) and bone formation rate (BFR/BS) were determined. Results (means \pm SEM) are
1246 from 3 independent experiments with $n=6, 6,$ and 5 (for MAR) or 6 (for BFR/BS) mice in total
1247 for WT, +/1013 and 1013/1013 groups respectively. Statistics were calculated with the
1248 nonparametric Kruskal–Wallis H test ($\#p=0.039$) and the unpaired two-tailed Student’s t test
1249 (1013/1013 vs WT * $p=0.03,$ +/1013 vs 1013/1013 $\$p=0.044$). **(F)** Volcano plot analysis of
1250 differentially expressed genes obtained by RNA-seq between WT and 1013/1013 OPCs
1251 ($p<0.05;$ $FC\geq 2$) performed on three biological replicates per group with one replicate
1252 representing the pool of 3 mice. Data represent analysis of cpm estimates with a log of fold
1253 change of more than 1.5 fold and $p<0.05$ using enhanced Volcano package. **(G and J)** Heatmap
1254 representing the relative expression levels of selected genes (osteogenic, G and
1255 osteoclastogenic, J) expressed by sorted OPCs. **(H and K)** Normalized counts of osteogenic
1256 (H) and osteoclastogenic (K) genes using the DESeq2 method. Data are represented as floating
1257 bars (min to max and line equal median) of the three biological replicates per group. For
1258 significance testing, DESeq2 uses a Wald test (p values). The Wald test P values from the subset
1259 of genes that pass an independent filtering step, are adjusted for multiple testing using the
1260 procedure of Benjamini and Hochberg (padj values). **(I)** *In vitro* osteoblastic differentiation of

1261 sorted OPCs evaluated at day 21 post-culture by Alizarin Red S coloration. The images are
 1262 representative of 3 independent cultures. The quantification (means \pm SEM) from three
 1263 independent experiments with 6 mice in total per group is shown. **(L)** *In vitro* expanded
 1264 osteogenic cells from bone fractions were cultured with WT CD11b⁺ osteoclast progenitors and
 1265 stimulated with PGE2/Vitamin D3 (VitD3)/Dexamethasone (Dex) for 8 days. OCLs (TRAP-
 1266 positive) were identified (left, representative images, bars: 100 μ m) and quantified (right). Data
 1267 (means \pm SEM) are from 2 independent experiments with 6 mice in total per group. Statistics
 1268 were calculated with the nonparametric Kruskal–Wallis H test ([#]p=0.033) and the unpaired two-
 1269 tailed Student’s t test (+/1013 vs WT *p=0.02 and 1013/1013 vs WT *p=0.014). **(M)** The
 1270 relative expression levels of osteoclastogenic genes were determined by quantitative PCR in
 1271 stimulated osteogenic cells (3 mice per group). Each individual sample was run in triplicate and
 1272 has been standardized for *36B4* expression levels. All mice were littermates, females and age-
 1273 matched (8-12 wk-old). Source data are provided as a Source Data file.

1274

1275 **Figure 5: Impaired osteogenic specification of *Cxcr4*¹⁰¹³-bearing skeletal stromal/stem**
 1276 **cells. (A)** Ki-67 and DAPI co-staining to analyze by flow cytometry the cell cycle status of
 1277 SSCs and OPCs from bone fractions. Bar graphs show the percentage of cells (DAPI^{low}Ki-67⁻)
 1278 in the quiescent G0 phase. Data (means \pm SEM) are from three independent experiments with
 1279 n= 9, 6, and 6 mice in total for WT, +/-1013 and 1013/1013 groups, respectively. Statistics were
 1280 calculated with the nonparametric Kruskal–Wallis H test ([#]p=0.029) and the unpaired two-
 1281 tailed Student’s t test (1013/1013 vs WT **p=0.0091). **(B)** Flow-cytometric detection of BrdU
 1282 staining in SSCs (left). Percentages of BrdU⁺ bone SSCs and OPCs after a 12-day labelling
 1283 period (right). Data (means \pm SEM) are from three independent experiments with six mice in
 1284 total per group. Statistics were calculated with the nonparametric Kruskal–Wallis H test
 1285 (^{##}p=0.0021) and the unpaired two-tailed Student’s t test (+/1013 vs WT *p=0.016, 1013/1013

1286 vs WT ** $p=0.0011$). **(C)** Characterization of some biological processes displaying differential
1287 gene expression signatures in sorted SSCs as defined by GSEA and obtained by RNA-seq on 2
1288 (1013/1013) or 3 (WT and +/1013) biological replicates per group with one replicate
1289 representing the pool of 3 mice. For significance testing, DESeq2 uses a Wald test (p values).
1290 The Wald test P values from the subset of genes that pass an independent filtering step, are
1291 adjusted for multiple testing using the procedure of Benjamini and Hochberg (p_{adj} values). **(D)**
1292 RNA-seq-based heatmap representing the relative expression levels of osteogenic genes. **(E)**
1293 Normalized counts of selected osteogenic genes using the DESeq2 method. Data are
1294 represented as floating bars (min to max and line equal median) of the 2 or 3 biological
1295 replicates per group. For significance testing, DESeq2 uses a Wald test (p values). **(F)** The
1296 heatmap shows the relative expression levels (RQ) normalized for β -actin expression levels in
1297 each sample of selected genes involved in SSC differentiation towards the osteogenic lineage
1298 (6 pools of 100 cells *per* condition) by quantitative PCR. **(G)** RQ of the most regulated genes
1299 involved in differentiation and cell cycle of SSCs. Data (means \pm SEM) are from two
1300 independent experiments with 6 mice in total per group. Statistics were calculated with the
1301 nonparametric Kruskal–Wallis H test ($\#p=0.028$ for *Runx2*; $\#p=0.011$ for *Ccnd3*) and the
1302 unpaired two-tailed Student's t test (1013/1013 vs WT ** $p=0.0063$ for *Runx2*; 1013/1013 vs
1303 WT * $p=0.048$ for *Coll α* ; +/1013 vs WT * $p=0.022$ and 1013/1013 vs WT * $p=0.02$ for *Ccnd3*).
1304 **(H)** RNA-seq-based heatmap representing the relative expression levels of osteoclastogenic
1305 genes expressed by sorted SSCs. **(I)** Normalized counts of selected osteoclastogenic genes
1306 using the DESeq2 method. Data are represented as floating bars (min to max and line equal
1307 median) of the 2 or 3 biological replicates per group. For significance testing, DESeq2 uses a
1308 Wald test (p values). **(J)** Relative expression of osteoclastogenic genes in SSCs by quantitative
1309 PCR. Each individual sample was run in triplicate and has been standardized for β -actin
1310 expression levels and presented as relative expression to WT. Data (means \pm SEM) are from

1311 two independent experiments with 5 mice in total per group. **(K)** Immunofluorescence showing
 1312 in red Osterix (Osx)-positive cells and in blue DAPI-stained nuclei in WT and mutant mice
 1313 femurs (bars: 100 μ m). Dashed lines indicate the limit between the cartilage growth plate (above
 1314 the line) and the bone (below the line). Images are representative of at least 3 independent
 1315 determinations. **(L)** Quantification of Osx⁺ cells per mm² below the growth plate. Data (means
 1316 \pm SEM) are from 5, 5, and 3 independent mice in total for WT, +/1013 and 1013/1013 groups
 1317 respectively. Statistics were calculated with the unpaired two-tailed Student's t test (1013/1013
 1318 vs WT *p=0.0101). **(M)** Absolute numbers of the indicated stroma cell subsets from marrow
 1319 fractions determined by flow cytometry. Data (means \pm SEM) are from four independent
 1320 experiments with n= 9, 10, and 7 mice in total for WT, +/1013 and 1013/1013 groups,
 1321 respectively. Statistics were calculated with the nonparametric Kruskal–Wallis H test
 1322 (###p=0.0004 for stroma; ##p=0.0013 for OPC) and the unpaired two-tailed Student's t test
 1323 (+/1013 vs WT **p=0.0011, 1013/1013 vs WT ***p=0.0002, +/1013 vs 1013/1013 \$p=0.033,
 1324 for stroma; +/1013 vs WT **p=0.0049, 1013/1013 vs WT ***p=0.0003, for OPC). All mice
 1325 were littermates, females and age-matched (8-12 wk-old). Source data are provided as a Source
 1326 Data file.

1327

1328 **Figure 6: Cxcr4 desensitization intrinsically regulates *in vitro* the osteogenic**
 1329 **differentiation of skeletal stromal cells.** **(A)** Number of colonies formed from bone fractions
 1330 in CFU-F assays. Data (means \pm SEM) are from two independent experiments with n= 4, 6,
 1331 and 4 mice in total for WT, +/1013 and 1013/1013 groups respectively. Statistics were
 1332 calculated with the nonparametric Kruskal–Wallis H test (###p=0.002) and the unpaired two-
 1333 tailed Student's t test (+/1013 vs WT *p=0.029, 1013/1013 vs WT ***p=0.0008). **(B)** After *in*
 1334 *vitro* loading with BrdU (5 days) or CTV (3 days), the percentages of BrdU⁺ (left) or CTV^{low}
 1335 (right) cells within WT and mutant bone-derived SSCs were determined by flow cytometry.

1336 Data (means \pm SEM) are from 3 independent experiments with 6 mice in total per group.
1337 Statistics were calculated with the nonparametric Kruskal–Wallis H test ($\#p=0.0231$ and
1338 $\#\#p=0.0047$ for BrdU⁺ and CTV^{low} respectively) and the unpaired two-tailed Student's t test
1339 (+/1013 vs WT $*p=0.031$, 1013/1013 vs WT $**p=0.0023$ for BrdU⁺; +/1013 vs WT $*p=0.015$,
1340 1013/1013 vs WT $**p=0.0054$ for CTV^{low}). (C) Bar graphs show the percentages of cultured
1341 WT or mutant SSCs in the quiescent G0 phase (DAPI^{low}Ki-67⁻, left) or with an apoptotic
1342 phenotype (Annexin V⁺ DAPI⁻, right) as determined by flow cytometry. Data (means \pm SEM)
1343 are from three (right panel) or five (left panel) independent SSC cultures per genotype. Statistics
1344 were calculated using the unpaired two-tailed Student's t test (+/1013 vs WT $*p=0.032$,
1345 1013/1013 vs WT $*p=0.021$). (D) Doubling time (left) and absolute numbers (right) of WT and
1346 mutant SSCs after 3 days of culture. Data (means \pm SEM) are from 8, 6, and 5 independent SSC
1347 cultures for WT, +/1013 and 1013/1013 groups respectively. Statistics were calculated using
1348 the unpaired two-tailed Student's t test (+/1013 vs WT $*p=0.028$, 1013/1013 vs WT $*p=0.033$
1349 for doubling-time; 1013/1013 vs WT $*p=0.048$ for SSC). (E) Alkaline phosphatase (Alp)
1350 staining was performed 14 days after initiation of the culture of WT and mutant SSCs in
1351 osteogenic medium supplemented every two days with 10 μ M AMD3100 or vehicle (PBS)
1352 (bars: 100 μ m). Quantitative analyses (number of Alp⁺ cells) were performed under an inverted
1353 microscope. Data (means \pm SEM) are from 6 independent cultures per genotype. Statistics were
1354 calculated with the nonparametric Kruskal–Wallis H test ($\#\#p=0.0022$) and the unpaired two-
1355 tailed Student's t test (+/1013 vs WT $**p=0.0075$, 1013/1013 vs WT $**p=0.0018$). (F) Alizarin
1356 Red staining was performed 21 days after initiation of the culture. Quantitative analyses (means
1357 \pm SEM) of staining were performed using the osteogenesis assay kit in 12 (vehicle) or 6
1358 (AMD3100) independent cultures per genotype. Statistics were calculated with the
1359 nonparametric Kruskal–Wallis H test ($\#\#\#p=0.0002$) and the unpaired two-tailed Student's t test
1360 (+/1013 vs WT $***p=0.0005$, 1013/1013 vs WT $***p<0.0001$). (G) Expression levels of

1361 osteogenic genes were determined by quantitative PCR in 6 independent WT and mutant SSC
 1362 cultures 14 and 21 days after initiation of the osteogenic culture in the presence or absence of
 1363 AMD3100. Each individual sample was run in triplicate and was standardized for β -actin
 1364 expression levels. Results (means \pm SEM) are expressed as relative expression compared to
 1365 WT samples. Statistics were calculated with the nonparametric Kruskal–Wallis H test
 1366 ($\#p=0.038$ and 0.0205 for Osx days 14 and 21 respectively; $\#p=0.024$ and 0.015 for Alp days 14
 1367 and 21 respectively; $\#\#p=0.0063$ for Opn days 21; $\#p=0.026$ for Ocn days 21) and the unpaired
 1368 two-tailed Student’s t test (1013/1013 vs WT $*p=0.0107$ and 0.013 for Osx days 14 and 21
 1369 respectively; 1013/1013 vs WT $*p=0.035$ and $***p=0.0001$ for Alp days 14 and 21
 1370 respectively; +/1013 vs WT $*p=0.022$ and 1013/1013 vs WT $**p=0.0056$ for Opn days 21;
 1371 1013/1013 vs WT $**p=0.0108$ for Ocn days 21). All mice were littermates, females and age-
 1372 matched (8-12 wk-old). Source data are provided as a Source Data file.

1373

1374 **Figure 7: Normalization of Cxcr4 signaling rescues the osteogenic properties of *Cxcr4*¹⁰¹³-**
 1375 **bearing mouse skeletal cells. (A)** Schematic diagram for daily AMD3100 intra-peritoneal
 1376 (*i.p.*) injection for 21 days in WT and mutant mice. **(B)** Absolute numbers of the indicated
 1377 stroma cell subsets from bone fractions of WT and mutant mice determined by flow cytometry.
 1378 Data (means \pm SEM) are from 2 independent experiments with 6 PBS injected mice and 11
 1379 AMD3100-injected mice in total *per* genotype. Statistics were calculated using the
 1380 nonparametric Mann–Whitney test, two-sided, 1013/1013 vs WT $*p=0.041$, 1013/1013 (AMD)
 1381 vs 1013/1013 (vehicle) $\&p=0.01$ for SSC; 1013/1013 vs WT $**p=0.0022$, WT (AMD) vs WT
 1382 (vehicle) $\&\&p=0.0065$, 1013/1013 (AMD) vs 1013/1013 (vehicle) $\&\&\&p=0.0003$ for OPC. **(C)**
 1383 BM sections from WT and mutant mice treated with vehicle (PBS) or AMD3100 were
 1384 immunostained for Opn in association with DAPI. Bars: 500 μ m. Images are representative of
 1385 3 independent determinations. **(D)** Bone mineral density (BMD) values of lumbar spine from 6

1386 treated mice in total per group are shown. Statistics were calculated using the nonparametric
1387 Mann–Whitney test, two-sided, +/1013 vs WT *p=0.041, 1013/1013 vs WT **p=0.0022,
1388 +/1013 (AMD) vs +/1013 (vehicle) &&p=0.0087, 1013/1013 (AMD) vs 1013/1013 (vehicle)
1389 &p=0.026. **(E)** Cortical thickness was measured in sections stained with Toluidine Blue. Both
1390 cortices were measured and data are presented as a mean of both cortices from 6 mice in total
1391 per group. Statistics were calculated using the nonparametric Mann–Whitney test, two-sided,
1392 1013/1013 vs WT **p=0.026. Data (means \pm SEM) displayed in D and E are from 2
1393 independent experiments. All mice were littermates, females and age-matched (8-12 wk-old).
1394 Source data are provided as a Source Data file.

1395

1396 **Figure 8: BM stromal cells from WS patients displayed *in vitro* impaired osteogenic**
1397 **capacities. (A)** Relative expression levels of osteogenic genes were determined by quantitative
1398 PCR at day 14 in osteogenic-induced cultures of two WS patients-derived BMSCs and 7 healthy
1399 donors-derived BMSCs. Each individual sample was run in triplicate and was standardized for
1400 *36B4* expression levels. Results (means \pm SEM) are expressed as relative expression compared
1401 to healthy samples (set at 1 and representing the mean of the 7 healthy donors) and are from 2
1402 independent experiments. Statistics were calculated using the nonparametric Mann–Whitney
1403 test, two-sided, **p=0.0079 for OSX, RUNX2 and OCN; *p=0.0179 for OPN. **(B)** Alizarin
1404 Red staining was performed 21 days after initiation of the culture of 1.5×10^3 healthy or WS
1405 BMSCs in pro-osteogenic medium (left panel). Representative images for healthy (H) and WS
1406 donors #1 and #2 are shown. Quantitative analyses of staining (means \pm SEM) were performed
1407 using the osteogenesis assay kit in 3 independent cultures with 2 WS and 6 healthy donors (right
1408 panel). Statistics were calculated using the nonparametric Mann–Whitney test, two-sided,
1409 **p=0.0022 for healthy. **(C)** Oil Red O staining was performed 21 days after initiation of
1410 cultures of healthy or WS BMSCs in pro-adipogenic differentiation medium. Bars: 200 μ m. **(D)**

1411 Proposed model: Proper Cxcr4 signaling termination is essential for bone tissue homeostasis.
1412 Absence of Cxcr4 desensitization leads to imbalance in bone remodeling with decreased OBL-
1413 mediated bone formation and increased OCL-mediated bone resorption, leading to severe
1414 trabecular and cortical alterations and a subsequent osteoporotic-like phenotype.
1415 Mechanistically, impaired Cxcr4 desensitization disrupts the osteogenic commitment of SSCs,
1416 while strikingly increasing their pro-osteoclastogenic capacities. Source data are provided as a
1417 Source Data file.

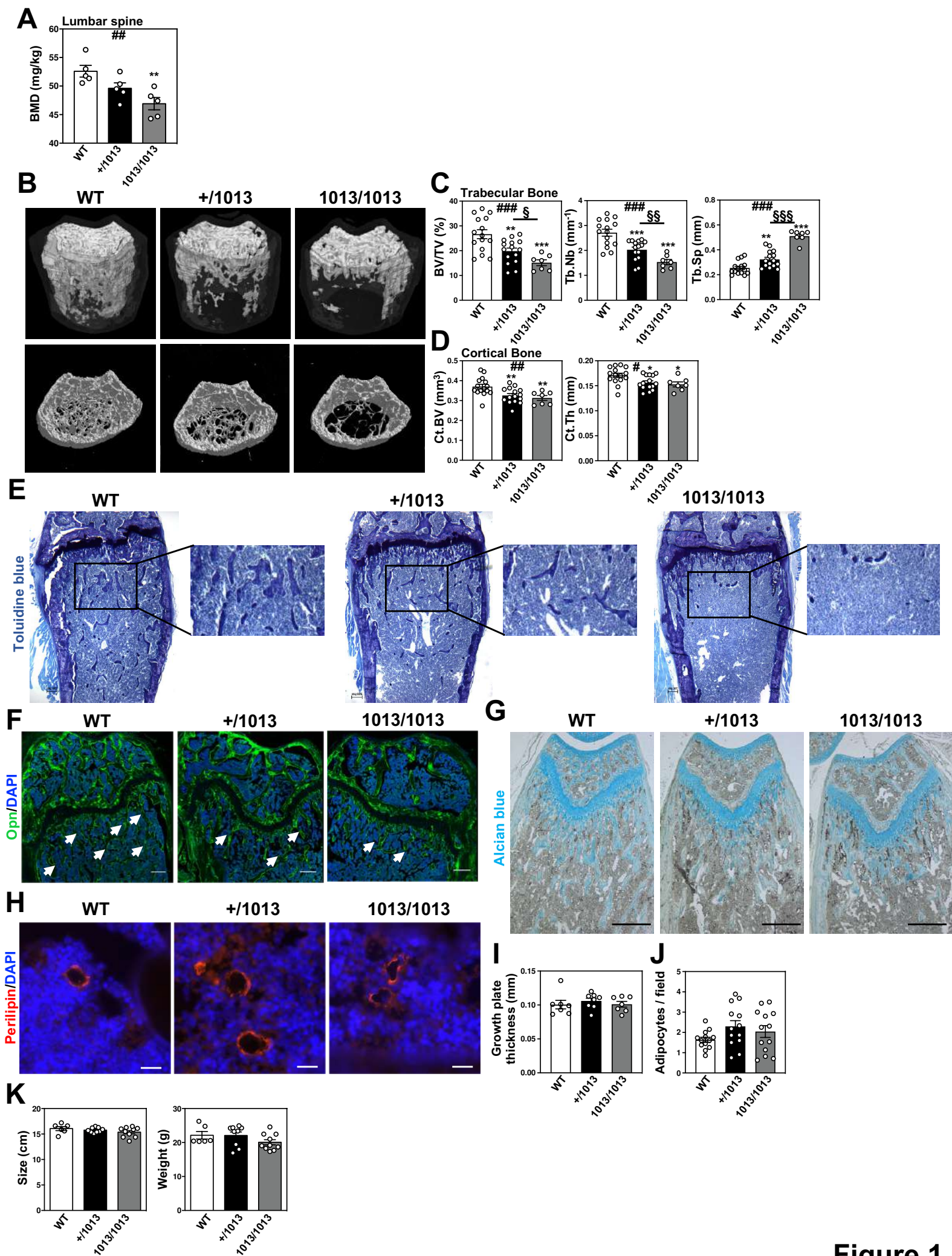


Figure 1

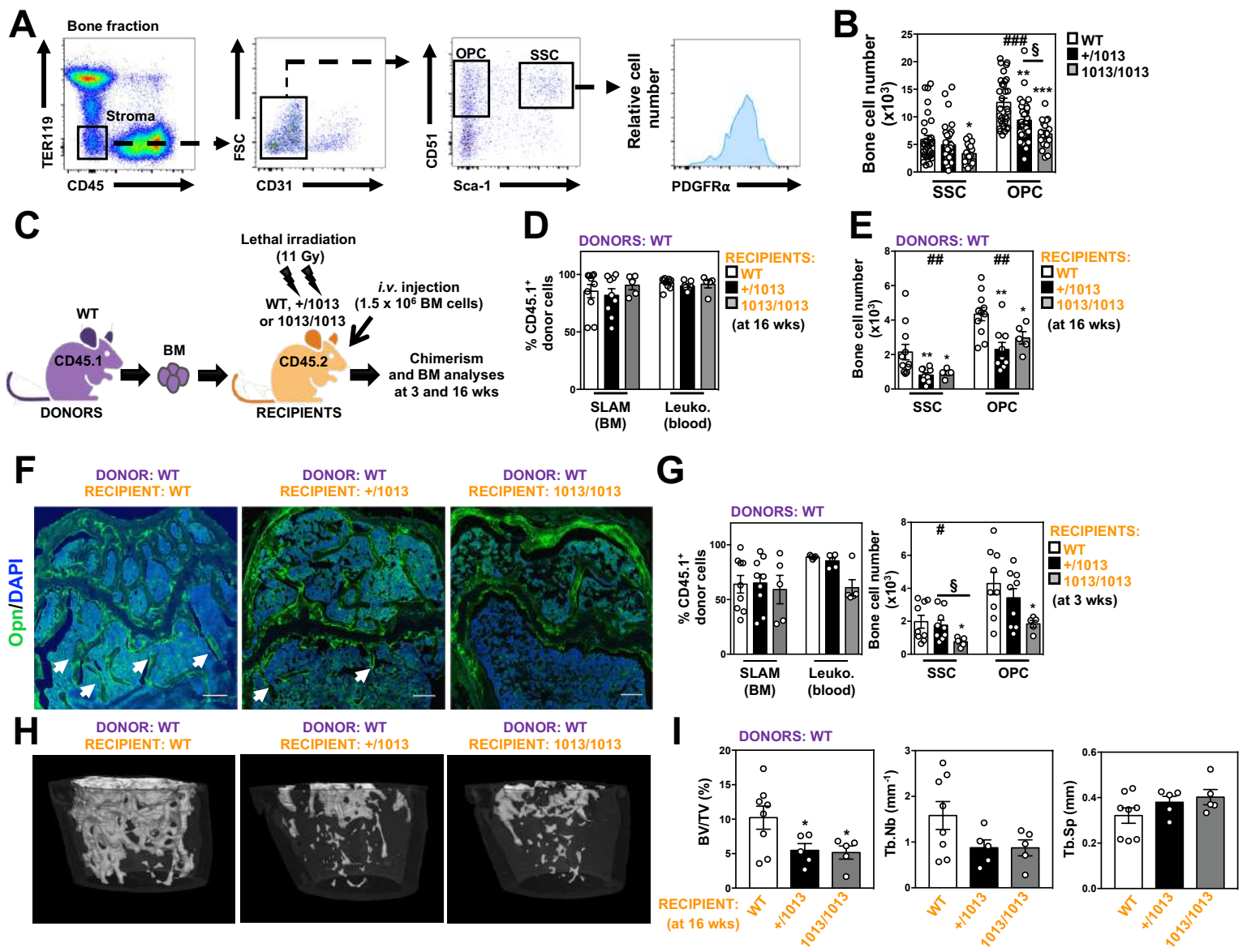


Figure 2

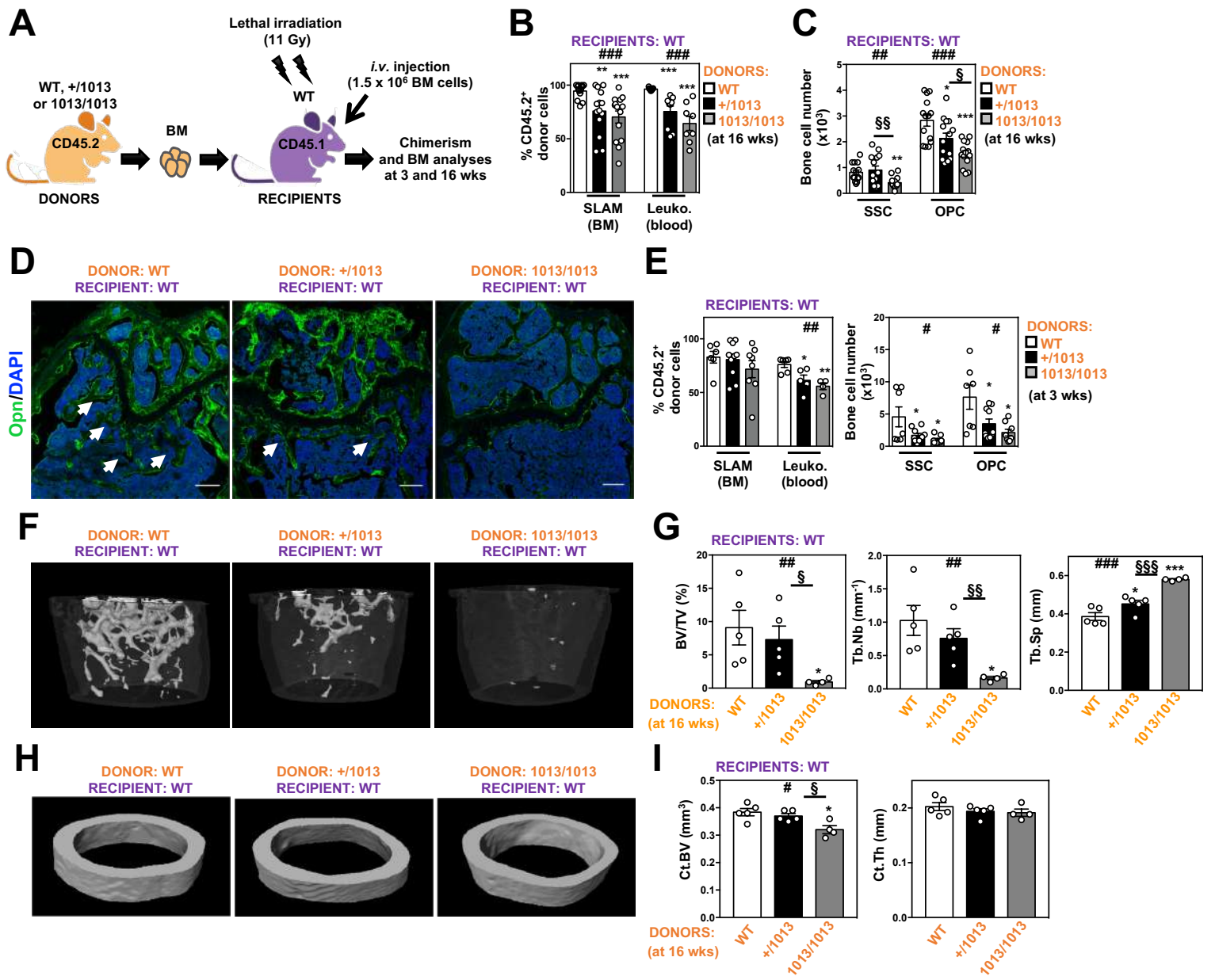


Figure 3

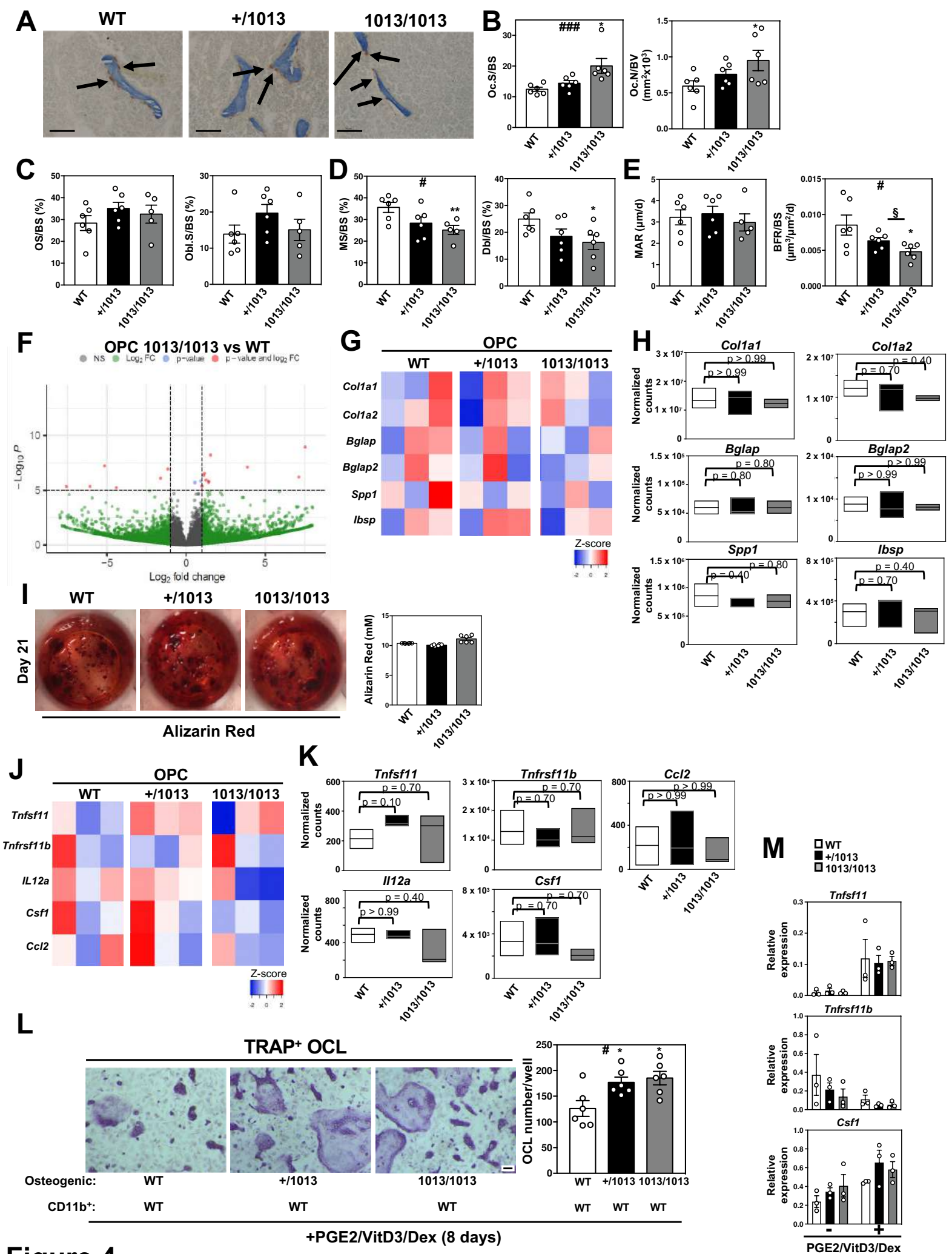


Figure 4

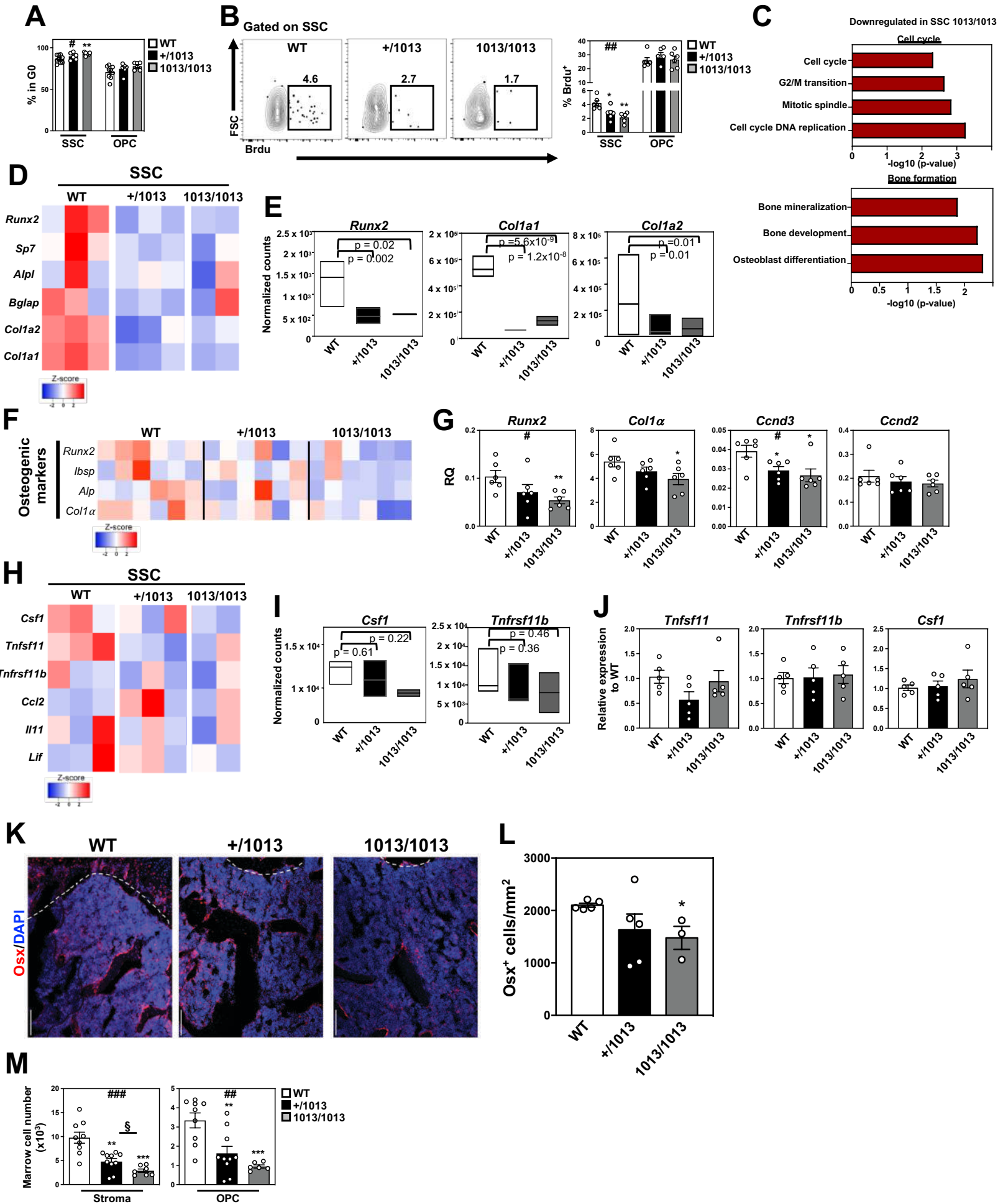


Figure 5

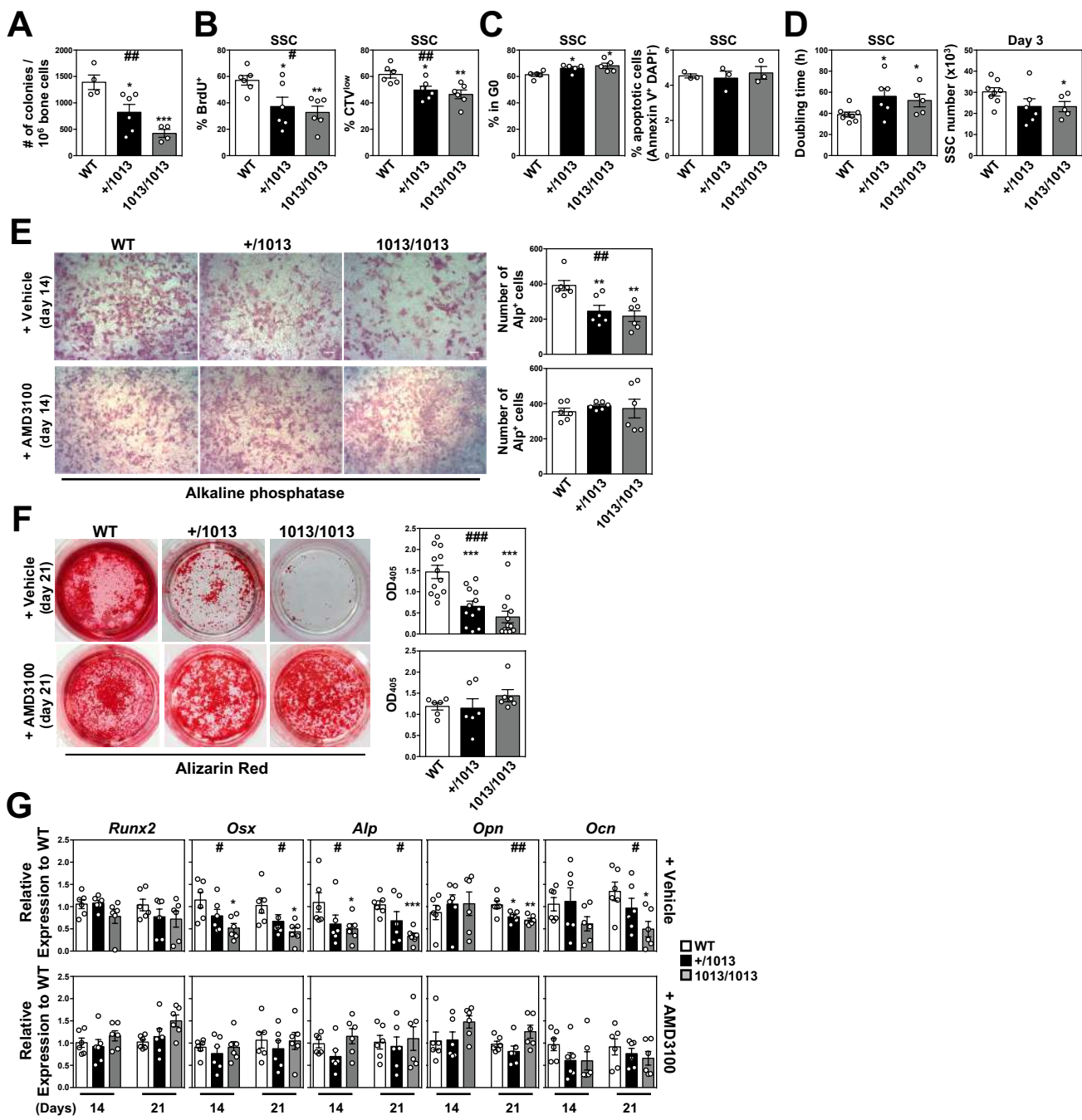


Figure 6

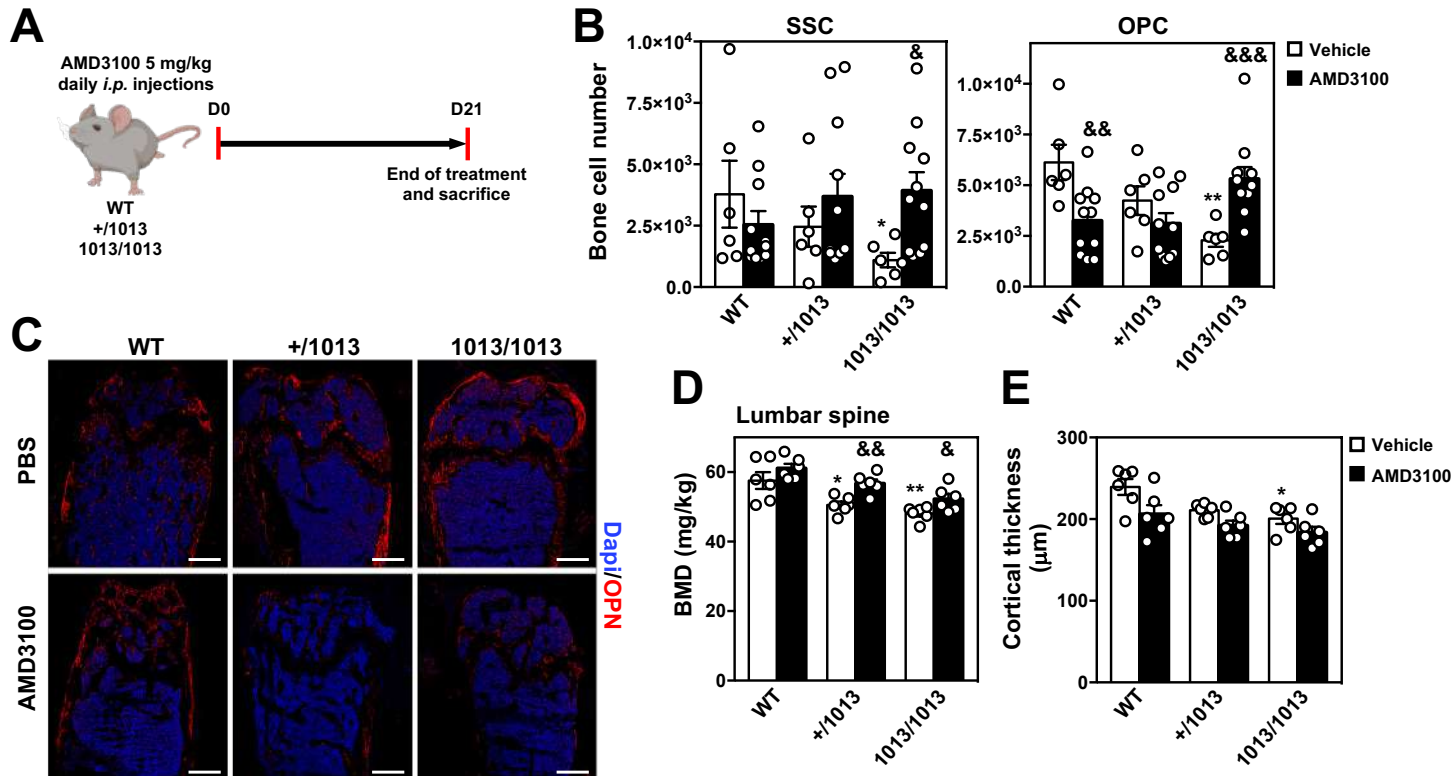


Figure 7

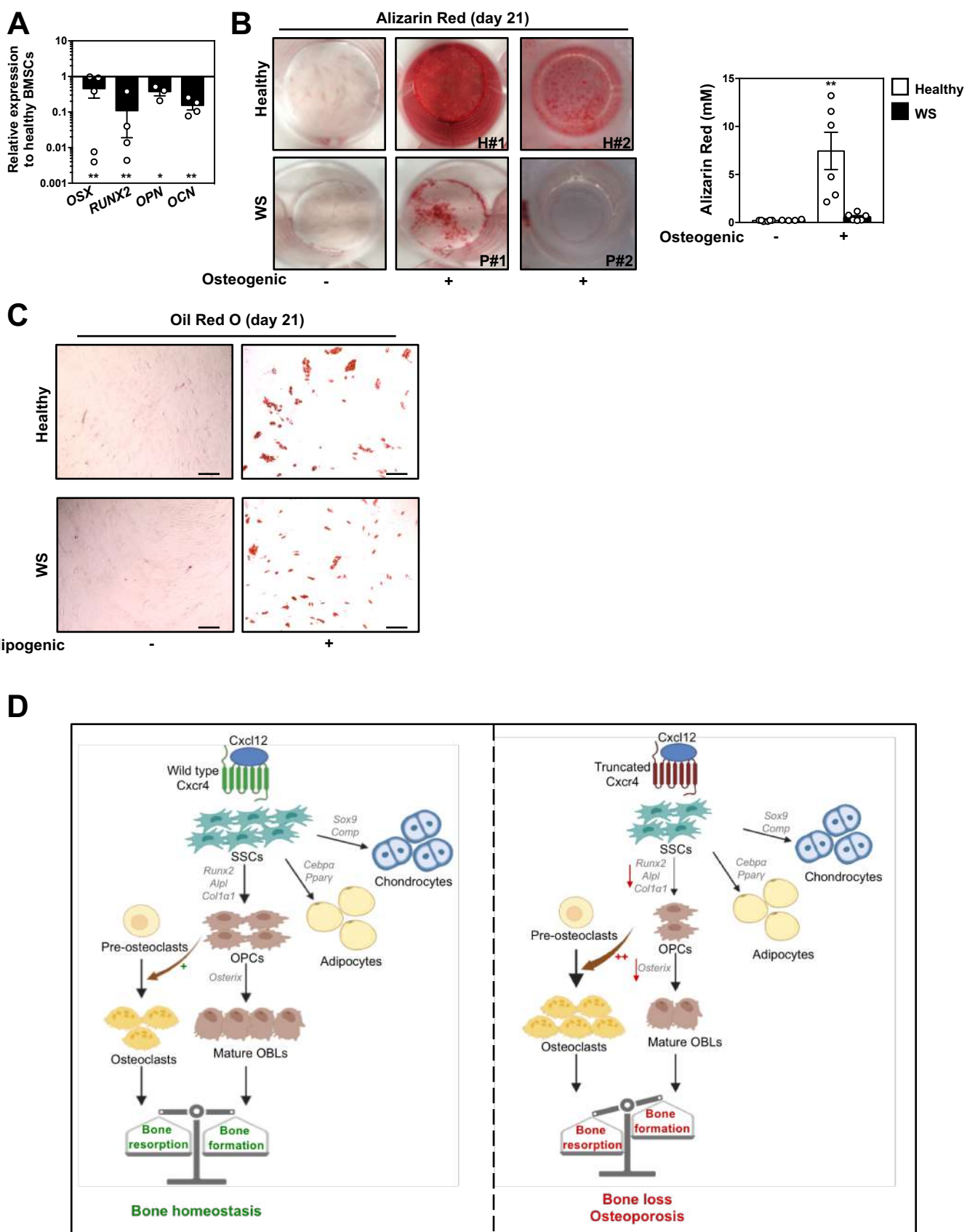


Figure 8