ONLINE APPENDIX

Tissue processing

Mouse femurs were immersed in 4% para-formaldehyde for 4h at 4°C; samples were washed three times with PBS, 10 minutes each, and then placed in 0.5M EDTA pH8 for 4 weeks at 4°C. Decalcified bones were then washed three times with PBS, 10 minutes each, embedded in Killik cryostat embedding medium (Bio-Optica, Milan, Italy) and frozen in liquid nitrogen-cooled 2-methylbutane (Sigma-Aldrich). 10 µm thick longitudinal femur sections were obtained with a Leica CM 1950 cryostat (Leica Biosystems S.r.l., Milan, Italy), placed on Superfrost Plus slides (J1800AMNZ, Gerhard Menzel GmbH, Braunschweig, Germany), and stored at -80°C.

Immunohistochemistry and morphometry

Femur sections were air-dried for 20 minutes at RT, then incubated with blocking solution: PBS plus 1% bovine serum albumin (BSA) and 10% normal goat or donkey serum (both from Jackson ImmunoResearch), for 40 minutes at RT. Sections were then incubated with primary antibody at +4C°, diluted in PBS / 1% BSA / 2% normal goat or donkey serum. Sections were incubated with anti-perilipin 1 antibody (1:200 dilution, overnight incubation, Cat. No. 9349 Cell Signaling Technology®) and/or anti-CXCL12 antibody (1:100 dilution, 3 days incubation, gift from Thomas Klein at Boehringer-Ingelheim Pharma), then washed four times with PBS, 5 minutes each, and incubated with secondary antibody for 40 minutes at 37°C, diluted 1:200 in PBS plus 1% BSA. Slides were mounted with some drops of an anti-fade aqueous mounting medium (saturated polyvinyl alcohol solution, dissolved in PBS and added with 30% glycerol). Images were taken with a Leica DM5000B microscope, equipped with a DFC300 FX CCD camera, or with Leica DM6B microscope, equipped with a DFC7000 T CCD camera, or with Cytell (GE Healthcare, Milan, Italy). Images were then processed with Fiji/ImageJ software (1.50, NIH, USA) or with Adobe Photoshop CS2 (9.0.2, Adobe Systems Incorporated, USA). Adipocytes were manually quantified with Fiji/ImageJ, based on perilipin-1 immunostaining: regions of interest (ROIs) corresponding to each adipocyte plasma membrane was manually drawn and added to ROI manger, and then the area was measured by means of the "Measure" command. For images acquired with DM5000B or Cytell, whole femur sections reconstructed by fusion of single images with Adobe Photoshop CS2 (Photomerge function) were considered for these measurements. Whole femur sections were directly reconstructed by LAS X software (Leica), when acquired with DM6B microscope.

Bone marrow fractionation

Femurs and tibias were collected from both hindlimbs and cleaned from muscle and tissue-remnants with sterile PBS. Both ends of femurs and tibias were snipped. Bones were split in two groups: one femur and 2 tibias were processed to isolate bone marrow adipocytes, while the other femur was used to quantify OSM and CXCL12 protein levels. Adipocytes were isolated as previously described (1). Bones were placed in a 0.6-mL microcentrifuge tube that was cut open at the bottom and placed into a 1.5-mL microcentrifuge tube. Bone marrow was spun out by quick centrifuge (from 0 to 10,000 rpm at RT). For adipocytes isolation, red blood

cells were lysed using standard lysing buffer and after centrifugation (3,000 rpm, 5 min, RT), floating adipocytes were collected from the top layer and resuspended in QIAzol® Lysis reagent for RNA isolation. BM pellets isolated from one femur were gently resuspended with 300 uL of PBS and centrifugated to remove cells and debris (5000 rpm, 20 min, RT). OSM and CXCL12 protein levels were quantified in the BM supernatant with ELISA kits (cat. No MSM00 and MCX120, respectively) both from R&D SYSTEMS® (Minneapolis, USA).

Quantification of DPP-4 activity

Snap-frozen femurs were pulverized with liquid nitrogen and resuspended with lysis buffer containing 20mM Tris HCl ph 7.4, 10 mM 2-mercaptoethanol, 100 mM NaCl, 10% glycerol and 1x Complete® EDTA-free protease inhibitor (Roche). After sonication, samples were centrifuged at 13000 g for 30 minutes at 4°C. Supernatant were tested for DPP-4 activity with DPP4 Activity Fluorometric Assay Kit (Catalog #K779-100, BioVision, CA, USA) using an EnSight™ plate reader (Perkin Elmer, USA).

References

1. Scheller EL, Doucette CR, Learman BS, Cawthorn WP, Khandaker S, Schell B, Wu B, Ding SY, Bredella MA, Fazeli PK, Khoury B, Jepsen KJ, Pilch PF, Klibanski A, Rosen CJ, MacDougald OA: Region-specific variation in the properties of skeletal adipocytes reveals regulated and constitutive marrow adipose tissues. Nat Commun 2015;6:7808

Table S1. Primer sequence for qPCR.

Target	Forward	Reverse
m-*Osm*	5'-AGCCCTATATCCGCCTCCAA-3'	5'-GTGTGTCCTCACTGGGGAAG-3’
m-*Cxcl12*	5'-CGGGTCAATGCACACTTGTC-3'	5'-GAGCCAACGTCAAGCATCTG-3'
m-*TNFα*	5'-ATGAGCACAGAAAGCATGA-3'	5'-AGTAGACAGAAGAGCGTGGT-3'
m-*Mrc1*	5'-TTGCACTTTGAGGGAAGCGA-3'	5'-CCTTGCCTGATGCCAGGTTA-3'
m-*IL-1β*	5'-AGCTTCCTTGTGCAAGTGTCT-3'	5'-GACAGCCCAGGTCAAAGGTT-3'
m-*Plin1*	5'-AGCGTGGAGAGTAAGGATGTC-3'	5'-CTTCTGGAAGCACTCACAGG-3'
m-*Abca1*	5'-TCCTTGGGGACAGAATTGCC-3'	5'-TCTGAGAAACACTGTCCTCCTTT-3'
m-*Dpp4*	5’-TCCAAGCAATGTGGTACACGGATGA-3’	5’-TGCTGGAGGAAATGGCTCATGTGG-3’
m-*Cd36*	5’-TCATATTGTGCTTGCAAATCCAA-3’	5’-TGTAGATCGGCTTTACCAAAGATG-3’
m-*Mertk*	5’-GAGGACTGCTTGGATGAACTGTA-3’	5’-AGGTGGGTCGATCCAAGG-3’
m-*Ubc*	5'-GCCCAGTGTTACCACCAAGA-3'	5'-CCCATCACACCCAAGAACA-3'
h-*OSM*	5'-GGGGTACTGCTCACACAGAG-3'	5'-TACGTATATAGGGGTCCAGGAGTC-3'
h-*CXCL12*	5'-ATGCCCATGCCGATTCTT -3'	5'-GCCGGGCTACAATCTGAAGG-3'
h-*ACTβ*	5'-AGAGCTACGAGCTGCCTGAC-3'	5'-GGATGCCACAGGACTCCA-3'

Figure S1 – Stem cell mobilopathy in experimental type 1 and type 2 diabetes. Type 1 diabetes (T1D) was induced in C57Bl6/J mice by streptozotocin (STZ) injection as described in the main manuscript. Type 2 diabetes (T2D) was induced by feeding C57Bl6/J mice with a high fat diet (HFD) for 12 weeks. The diet was composed of 60% calories from fat. At the end of the HFD protocol, mice were obese and insulin resistant (not shown). A) HSPC levels, defined as $Lin^{-}c\text{-}Kit^{+}Sca\text{-}1^{+}$ (LKS) cells, were measured before and after a full-course of G-CSF stimulation in non-diabetic (Ctrl) mice and in T1D and T2D mice (*p<0.05 versus basal; #p<0.05 versus Ctrl). B) Fold-change of HSPC levels (LKS cells) in the same 3 groups of mice. Histograms indicate mean ± SEM with superimposed individual data points.

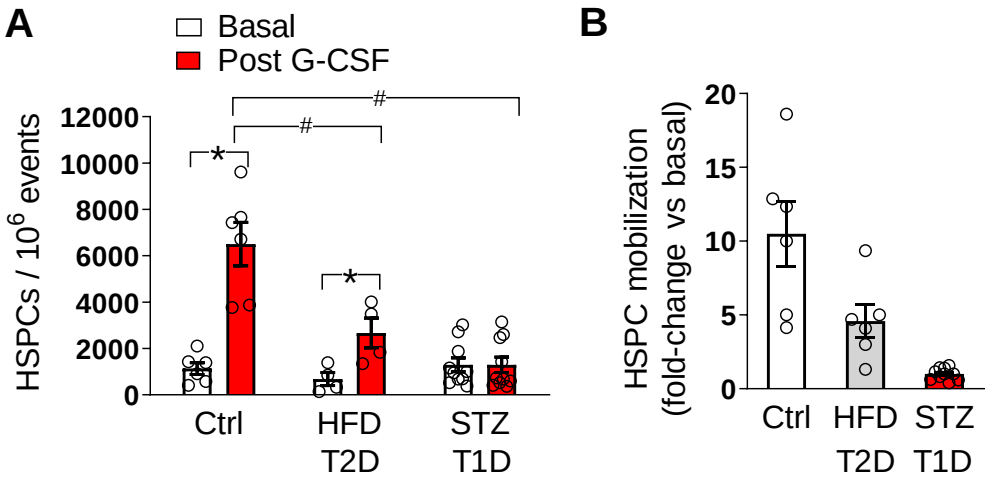

Figure S2 – Gene expression from publicly available dataset. Expression of a series of selected genes was compared between bone marrow (BM) adipocytes and visceral adipocytes in the GEO dataset GSE27017. Individual data points are superimposed histograms showing mean ± SEM (*p<0.05 visceral versus BM adipocytes).

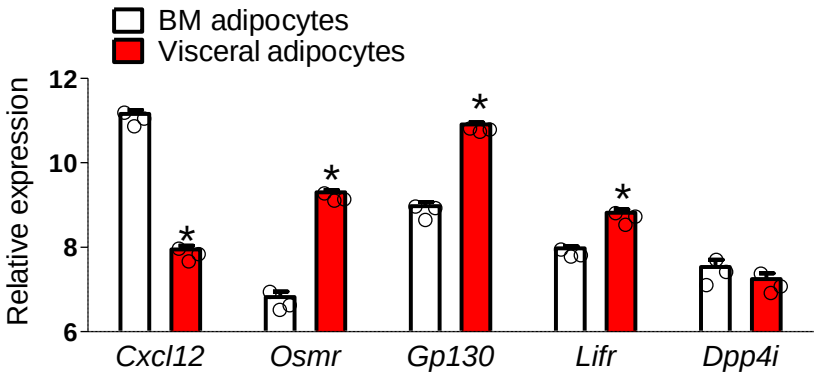

Figure S3 – Adipogenesis in *Osm*$^{-/-}$ mice. This figure complements Figure 3K of the main manuscript. From non-diabetic and streptozotocin-induced diabetic mice, wither wild-type or *Osm*$^{-/-}$, we show low-magnification images of distal femoral epiphyses and part of the diaphysis. Sections are stained with Hoechst (cellularity) and an anti-perilipin antibody (adipocytes).

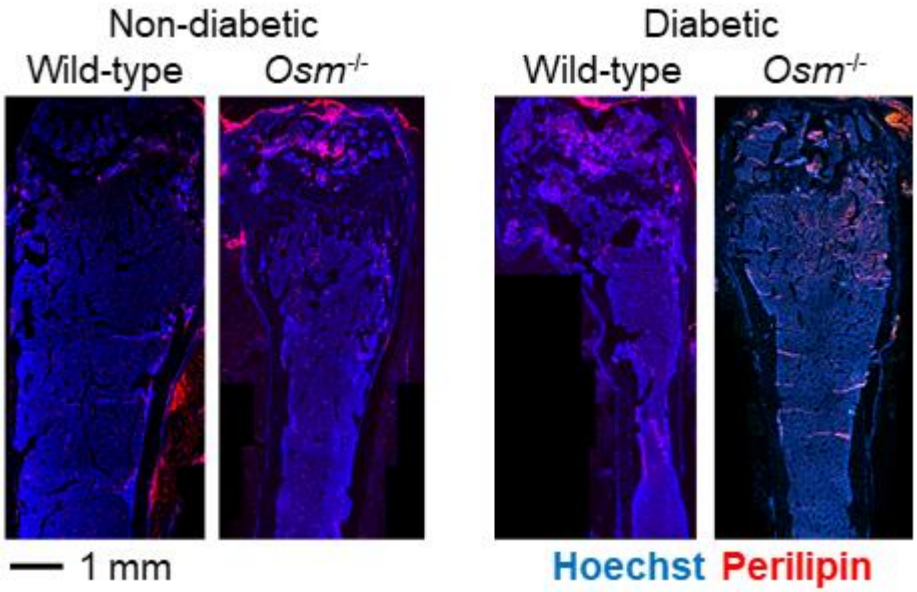

Figure S4. Gene expression of *Cxcl12* in Osm/p66Shc double knock-out (DKO) mice. Expression of *Cxcl12* in BM adipocytes of male DKO mice was compared to that in BM adipocytes of wild type (Wt) and *Osm*$^{-/-}$ male mice. *p<0.05 post-hoc comparison after 1-way ANOVA.