Figure EV1. BM niche microenvironment changes upon aging with respect to decreased cell frequency, decreased CFU-F/OB ability and altered cytokines secretion.

A. Relative frequency of CD45<sup>-</sup> Ter<sub>119</sub><sup>-</sup> cells (endosteal-enriched stroma population) in the cell fraction close to the endosteum in young and aged mice (n = 9).

B, C. Frequency of CFU-F (B) and CFU-OB (C) among 300,000 young or old cells isolated from the endosteal bone region (n = 4).

D. Relative frequency of osteoblasts OBs, CD31<sup>+</sup> endothelial cells, MSCs and CAR cells in BM central stroma population of young and old mice (n = 5).

E. Concentration of cytokines in the BM supernatant of young and old mice (n = 3).

F, G. Numbers of LT-HSCs (F) and ST-HSCs (G) in spleen of young and old mice (n = 4–5 per group).

Data information: A paired Student’s t-test was used to determine the significance of the difference between means of the two groups. Shown are mean values ± s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001.
Figure EV2. OPN staining in stroma OBs, CD31+ endothelial cells, CAR+ cells and MSCs.

A Relative frequency of left over endosteal-enriched stroma population OPN-positive nonosteoblasts, CD31+ endothelial cells, MSCs and CAR cells of young and old mice (n = 4).

B Representative immunofluorescence three-dimensional images of DAPI (blue) and OPN (red) localization in young and old CAR+ cells (scale bar 1.40 μm), CD31+ endothelial (scale bar 1.80 μm) and MSCs (scale bar 1.90 μm) with relative OPN signal volume quantification. Representative of two experiments with ~15–20 cells scored per sample in each experimental repetition.

C Relative frequency of OPN-positive osteoblasts in endosteal-enriched stroma population of young and old mice (n = 4).

D Representative plot showing specificity of the anti-OPN antibody in young, old and OPN KO endosteal-enriched stroma population.

Data information: A paired Student's t-test was used to determine the significance of the difference between means of the two groups. Shown are mean values ± s.e.m. *P < 0.05.
Figure EV2.
Figure EV3. OPN\textsuperscript{−/−} mice, like old mice, show decreased OB number, increase in inflammatory cytokines and increase in LT-HSCs with associated apolarity.

A, B Frequency of young LT-HSCs Ly\textsuperscript{5.1}\textsuperscript{+} Annexin V negative (A) and BrdU\textsuperscript{+} (B) co-cultured onto young, young OPN KO and old endosteal-enriched stroma population (n = 6).

C Relative frequency of osteoblasts, CD\textsuperscript{31}\textsuperscript{+} endothelial cells, MSCs and CAR cells in endosteal-enriched stroma population of young, old and young OPN KO mice (n = 6–7).

D Concentration of cytokines in the BM supernatant of young, old and young OPN KO mice (n = 3).

E Number of LT-HSCs per mouse sorted from young, old and young OPN KO mice (n = 8).

F Representative distribution of AcH\textsuperscript{4k16} (red) and tubulin (green) in LT-HSCs sorted from young, old and young OPN knockout mice (Ly5.2 background). Nuclei are stained with DAPI (blue). Scale bar, 5 \textmu m. Merged pictures on a dark background are shown.

Data information: Two-way ANOVA statistic test was used to compare means among the three groups. Shown are mean values + 1 s.e.m. *P < 0.05, **P < 0.01.
Figure EV3.
Figure EV4. Increased HSCs number in OPN<sup>−/−</sup> mice present with augmented Cdc42 activity and premature lineage skewing.

A Frequency of LT-HSCs polarized for Ach4K16 and tubulin sorted from young, old and young OPN KO mice. n = 6; 3–40 cells scored per sample in each experimental repetition.

B Cdc42 activity in young, old and young OPN KO lineage-depleted bone marrow cells (Lin<sup>−</sup> BM) was determined by pull-down/Western blot assay. Active Cdc42 (Cdc42-GTP) was normalized with respect total Cdc42 and actin.

C Ratio of the densitometric score of the Cdc42-GTP form and the total Cdc42 expression from panel (B), n = 5.

D Number of LT-HSCs per mouse sorted from young, old (24 months), young OPN KO mice and old OPN KO (18 months) (Ly<sup>5.2</sup>) mice (n = 5–8).

E Frequency of B cells, T cells, and myeloid cells in BM in young, old (24 months), young OPN KO and old OPN KO (18 months) (Ly<sup>5.2</sup>) mice (n = 5).

F Frequency of MEPs, CMPs, GMPs and CLPs progenitors in BM cells in young, old (24 months), young OPN KO and old OPN KO (18 months) (Ly<sup>5.2</sup>) mice (n = 5).

G Schematic representation of the experimental setup: the recipient mice were analyzed after 15 h from the cell injection.

H Cartoon demonstrating processing of the bones for analysis.

I Ratio of CFSE<sup>+</sup> HSPCs per section (3–4 biological repeats/group). Average HSPC numbers scored/mouse in young n = 39, old n = 63 and OPN KO = 70 in three biological repeats.

Data information: Two-way ANOVA statistic test was used to compare means among the three groups. Shown are mean values ± 1 s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001.
Figure EV4.
Figure EV5. A young stroma microenvironment supports the increase in old MPPs in vitro and the decrease in old CMPs in vivo.

A Schematic representation of the experimental setup.
B Concentration of OPN in the co-culture supernatant of old BM lineage negative onto young, young OPN KO and old endosteal-enriched stroma population (n = 4).
C–E Number of old LT-HSCs (C), ST-HSCs (D) and MPPs (E) Ly5.1+ onto young, young OPN KO and old endosteal-enriched stroma population (n = 4).
F, G Frequency of old LT-HSCs Ly5.2+ Annexin V negative (F) and BrdU+ (G) co-cultured onto young, young OPN KO and old endosteal-enriched stroma population (n = 4).
H, I Frequency of young (H) and old (I) (Ly5.1+) MEPs, CMPs, GMPs and CLPs progenitors in BM cells in young, old and young OPN KO recipients (Ly5.2+) mice after 20 weeks upon transplantation. Data are based on six experimental repeats with five recipient mice per group (e.g., n = 25–30 per group).

Data information: Two-way ANOVA statistic test was used to compare means among the three different groups. Shown are mean values ± s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001.
Figure EV5.
Figure EV6. OPN fragments 20–25 kDa re-polarize old LT-HSCs.

A OPN fraction D (20–25 kDa) sequence after enzymatic digestion. In blue, the thrombin cleavage site is shown. (ii) Chromatogram of the second digestion of fraction D with 8 M urea.

B Western blot analysis of all the fractions obtained from the digestion of fraction D. Antibodies anti-OPN and anti-thrombin were used.

C All the subfractions were tested on LT-HSCs. Percentage of LT-HSCs polarized for AcH4K16 and tubulin are shown for all the experimental groups. n = 3. ~40 cells scored per sample in each experimental repetition.

D Percentage of LT-HSCs polarized for AcH4K16 and tubulin in the experimental groups listed. n = 3; ~30 cells scored per sample in each experimental repetition. The percentage of polarized cells is plotted over the total number of cells scored.

Data information: Two-way ANOVA statistic test was used to compare means among the different groups. Shown are mean values ± s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001.
Figure EV6.