Expanded View Figures

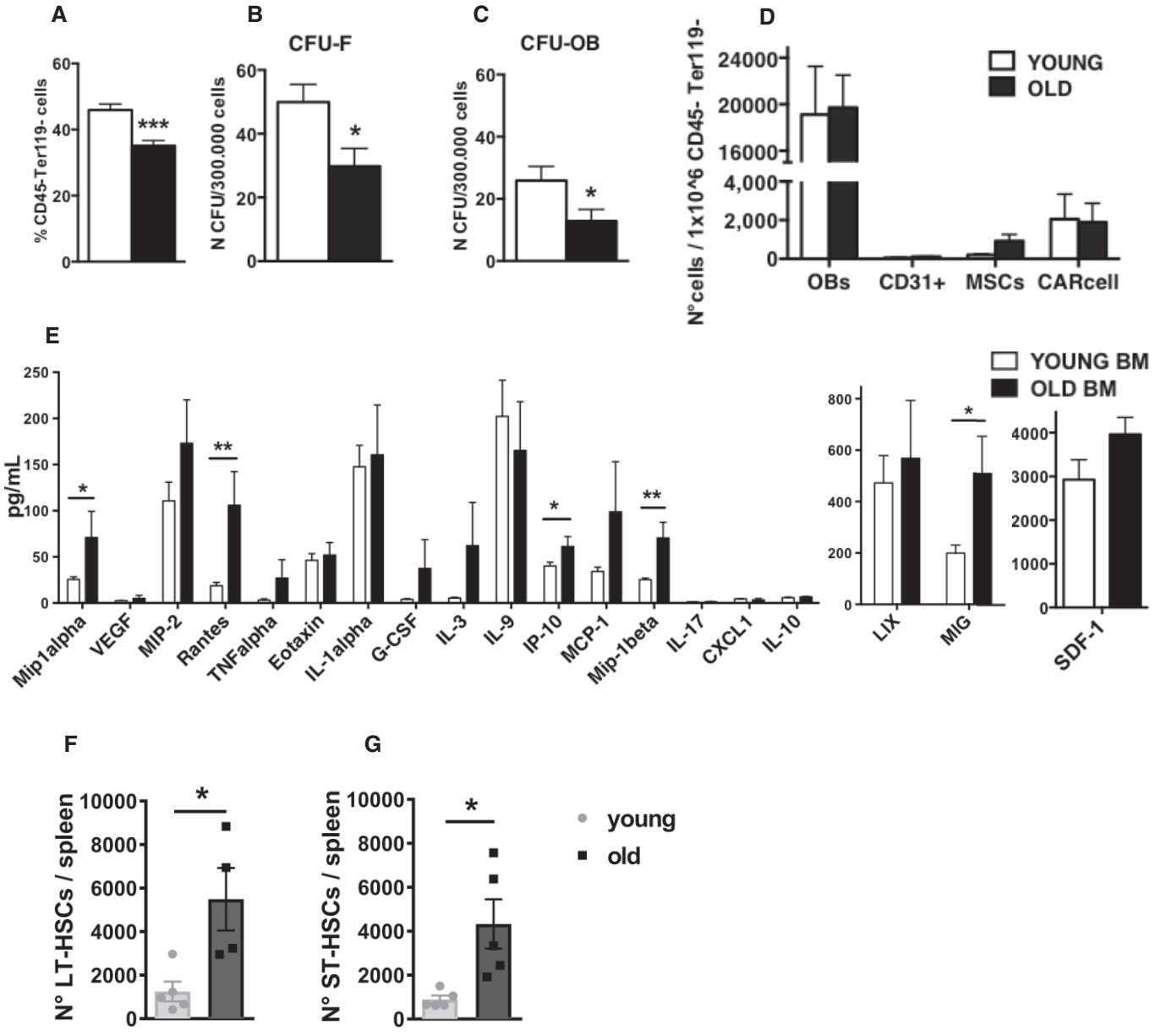

Figure EV1. BM niche microenvironment changes upon aging with respect to decreased cell frequency, decreased CFU-F/OB ability and altered cytokines secretion.

A Relative frequency of $CD45^{-}$ $Ter119^{-}$ 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, $CD31^{+}$ 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 + 1 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 + 1 s.e.m. *$P < 0.05$.

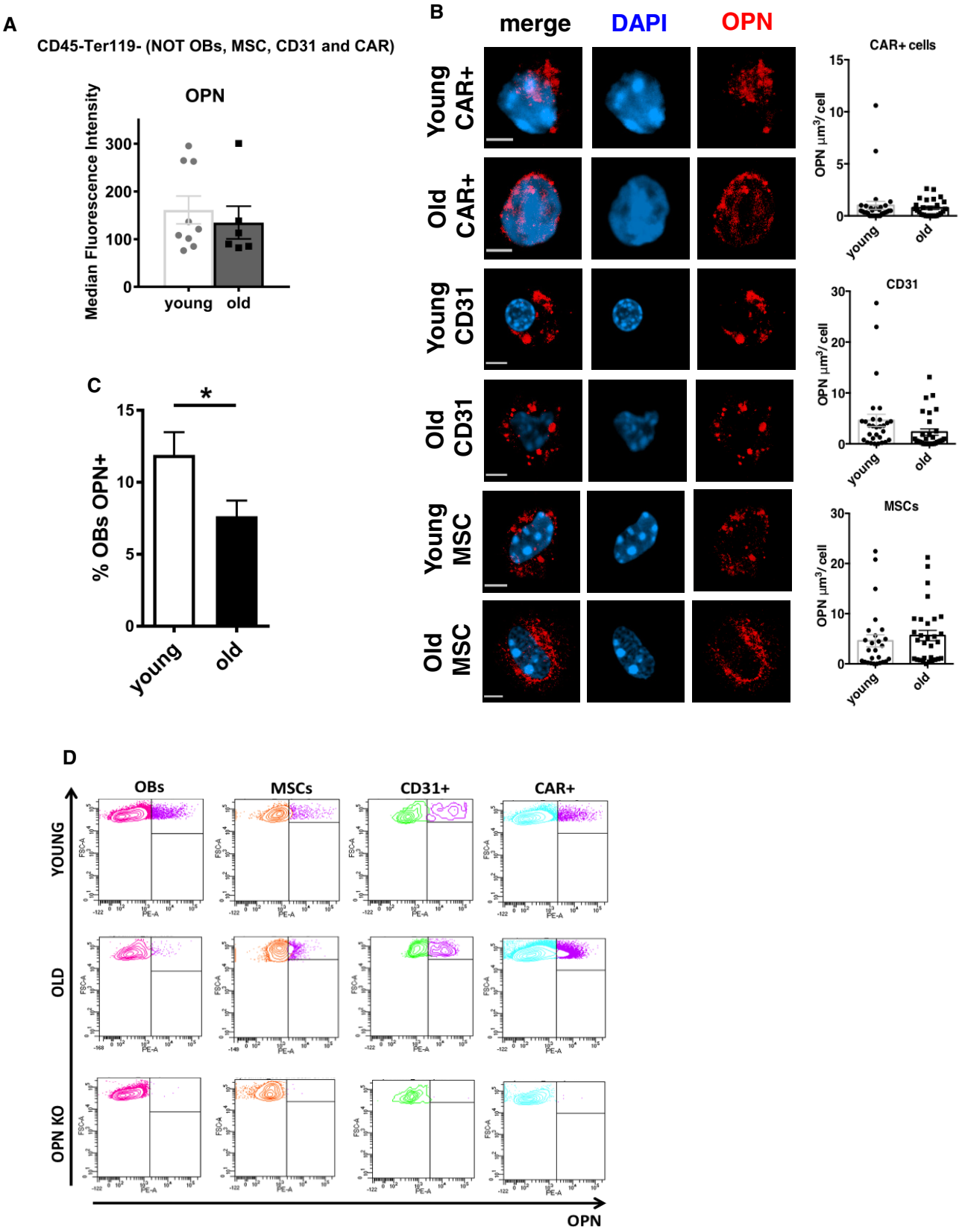
A
CD45-Ter119- (NOT OBs, MSC, CD31 and CAR)
OPN
Median Fluorescence Intensity
300
200
100
0
young
old
B
merge
DAPI
OPN
Young CAR+
Old CAR+
Young CD31
Old CD31
Young MSC
Old MSC
CAR+ cells
CD31
MSCs
OPN µm³/cell
young
old
C
*
% OBs OPN+
15
10
5
0
young
old
D
OBs
MSCs
CD31+
CAR+
YOUNG
OLD
OPN KO
OPN

Figure EV2.

Figure EV3. $OPN^{-/-}$ 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 $Ly5.1^+$ Annexin V negative (A) and $BrdU^+$ (B) co-cultured onto young, young OPN KO and old endosteal-enriched stroma population (n = 6).

C Relative frequency of osteoblasts, $CD31^+$ 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 AcH4k16 (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 μm. 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. *$P < 0.05$, **$P < 0.01$.

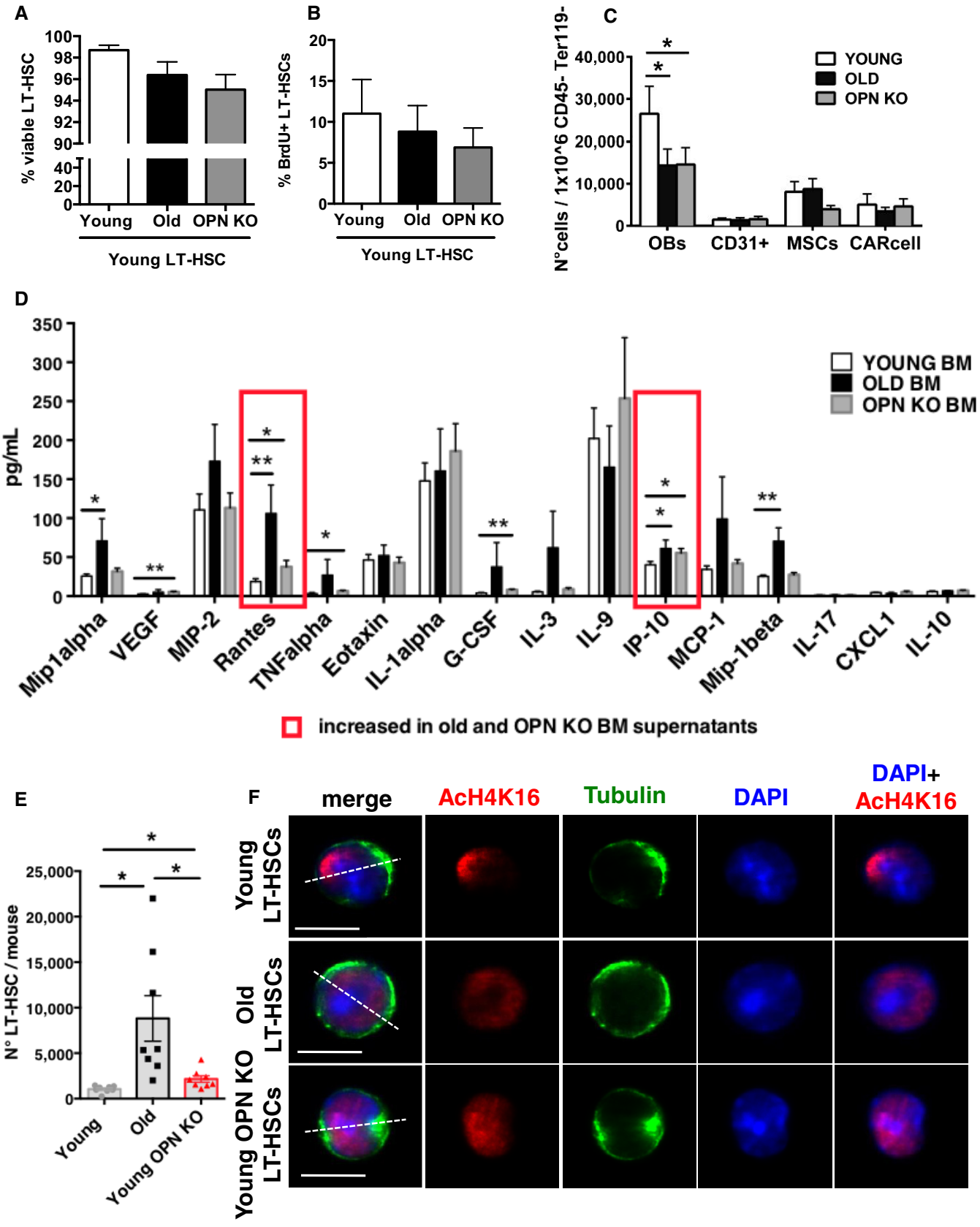
A
% viable LT-HSC
Young
Old
OPN KO
Young LT-HSC
B
% BrdU+ LT-HSCs
Young
Old
OPN KO
Young LT-HSC
C
N°cells / 1x10^6 CD45- Ter119-
YOUNG
OLD
OPN KO
OBs
CD31+
MSCs
CARcell
D
pg/mL
YOUNG BM
OLD BM
OPN KO BM
Mip1alpha
VEGF
MIP-2
Rantes
TNFalpha
Eotaxin
IL-1alpha
G-CSF
IL-3
IL-9
IP-10
MCP-1
Mip-1beta
IL-17
CXCL1
IL-10
increased in old and OPN KO BM supernatants
E
N° LT-HSC / mouse
Young
Old
Young OPN KO
F
merge
AcH4K16
Tubulin
DAPI
DAPI+ AcH4K16
Young LT-HSCs
Old LT-HSCs
Young OPN KO LT-HSCs

Figure EV3.

Figure EV4. Increased HSCs number in OPN$^{-/-}$ 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; ~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$^-$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) (Ly5.2$^+$) 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) (Ly5.2$^+$) 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) (Ly5.2$^+$) 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$^+$ 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. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

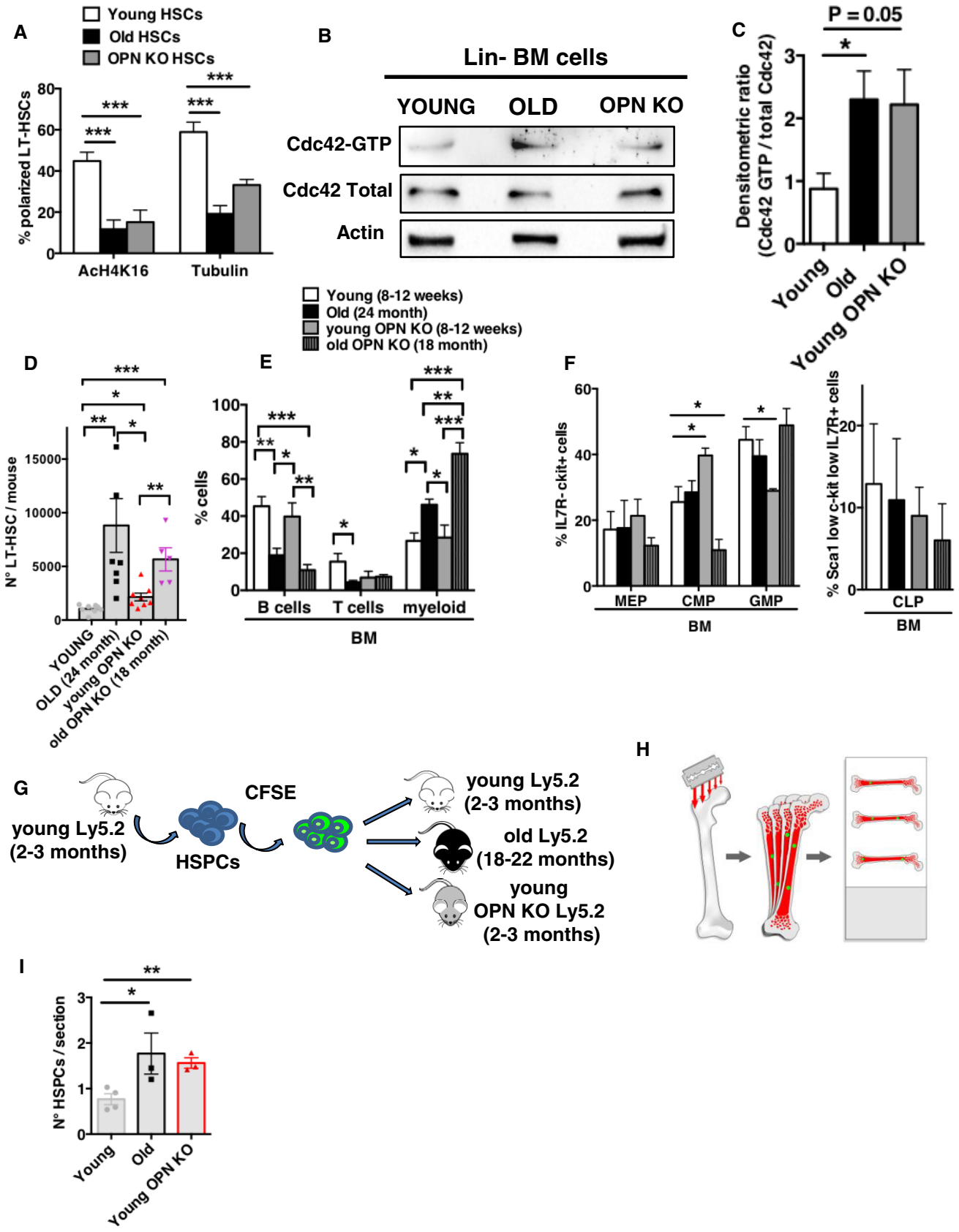
A
Young HSCs
Old HSCs
OPN KO HSCs
% polarized LT-HSCs
AcH4K16
Tubulin
B
Lin- BM cells
YOUNG
OLD
OPN KO
Cdc42-GTP
Cdc42 Total
Actin
C
P = 0.05
Densitometric ratio (Cdc42 GTP / total Cdc42)
Young
Old
Young OPN KO
Young (8-12 weeks)
Old (24 month)
young OPN KO (8-12 weeks)
old OPN KO (18 month)
D
N° LT-HSC / mouse
YOUNG
OLD (24 month)
young OPN KO
old OPN KO (18 month)
E
% cells
B cells
T cells
myeloid
BM
F
% IL7R- ckit+ cells
MEP
CMP
GMP
BM
% Sca1 low c-kit low IL7R+ cells
CLP
BM
G
young Ly5.2
(2-3 months)
HSPCs
CFSE
young Ly5.2
(2-3 months)
old Ly5.2
(18-22 months)
young
OPN KO Ly5.2
(2-3 months)
H
I
N° HSPCs / section
Young
Old
Young OPN KO

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 + 1 s.e.m. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

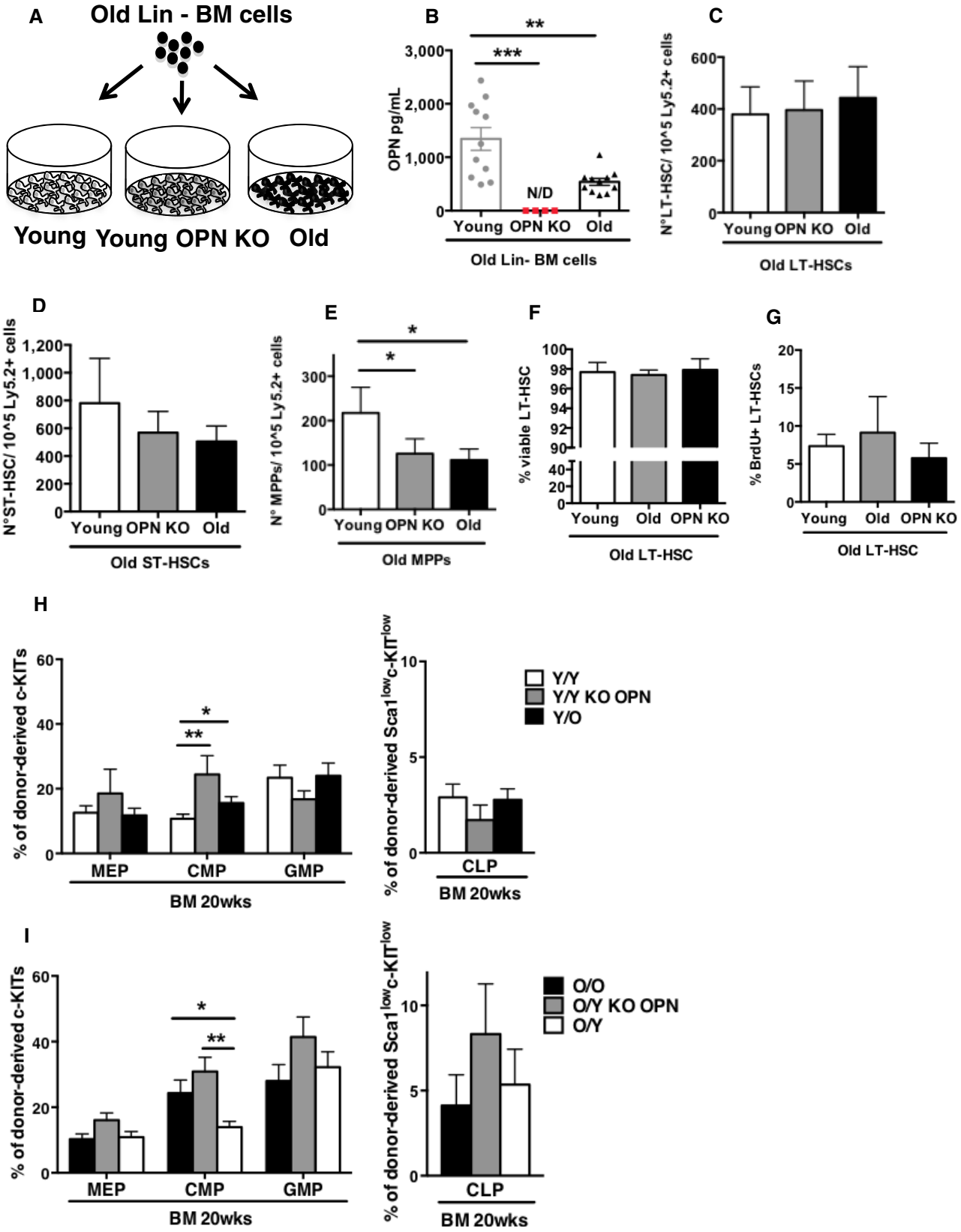

Figure EV5.

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 + 1 s.e.m. $*P < 0.05$, $**P < 0.01$, $**P < 0.001$.

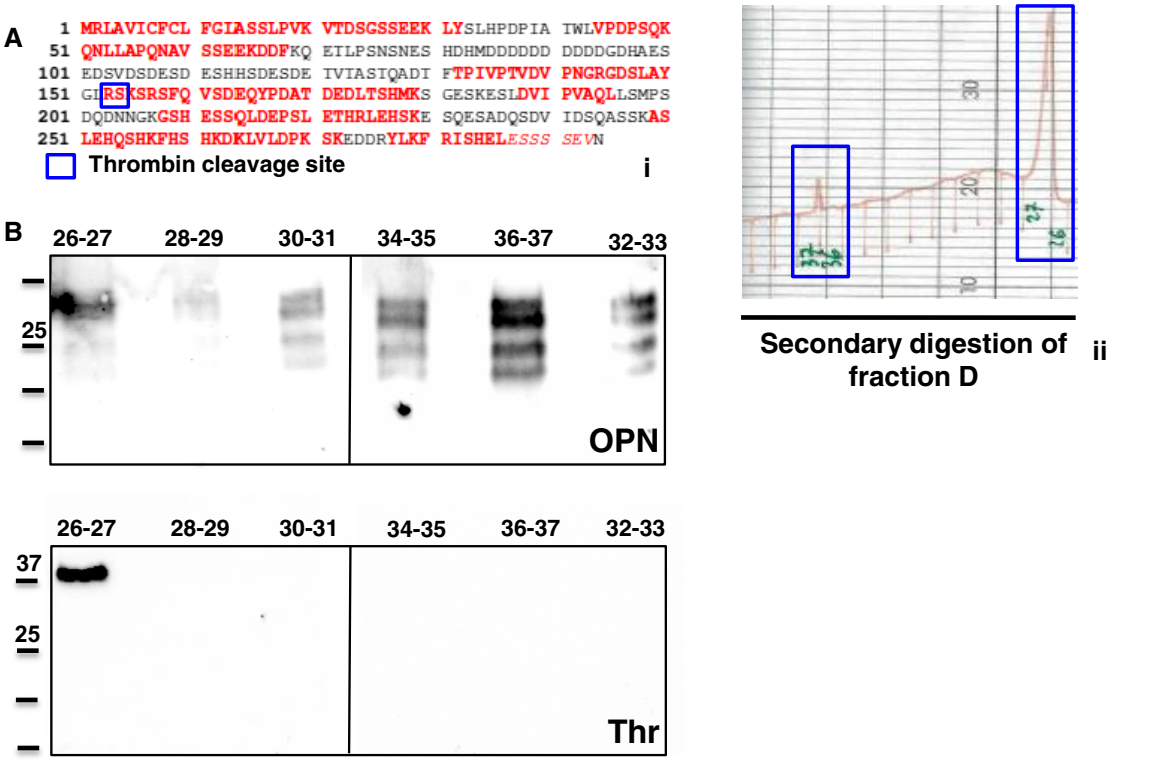
A
1 MRLAVICFCL FGIASSLPVK VTDSGSSEEK LYSLHPDPIA TWLVPDPSQK
51 QNLLAPQNAV SSEEKDDFKQ EILPSNSNES HDHMDDDDDD DDDDGDHAES
101 EDSVDSDESD ESHHSDESDE IVIASIQADI FTPIVPTVDV PNGRGDSLAY
151 GIRSKSRSFQ VSDEQYPDAT DEDLTSHMKS GESKESLDVI PVAQLLSMPS
201 DQDNNGKGSH ESSQLDEPSL ETHRLEHSKE SQESADQSDV IDSQASSKAS
251 LEHQSHKFHS HKDKLVLDPK SKEDDRYLKF RISHELESSS SEVN
Thrombin cleavage site
i
B
26-27
28-29
30-31
34-35
36-37
32-33
25
OPN
37
25
Thr
Secondary digestion of fraction D
ii
C
AcH4K16
Tubulin
% polarized LT-HSCs
young
old
old full-lenght
old D
old 26-27
old 28-29
old 30-31
old 32-33
old 34-35
old 36-37
D
% polarized LT-HSCs
young
old
old D
old + α4β1
old + α9β1
old + αVβ3
old + α4β1,α9β1, αVβ3

Figure EV6.