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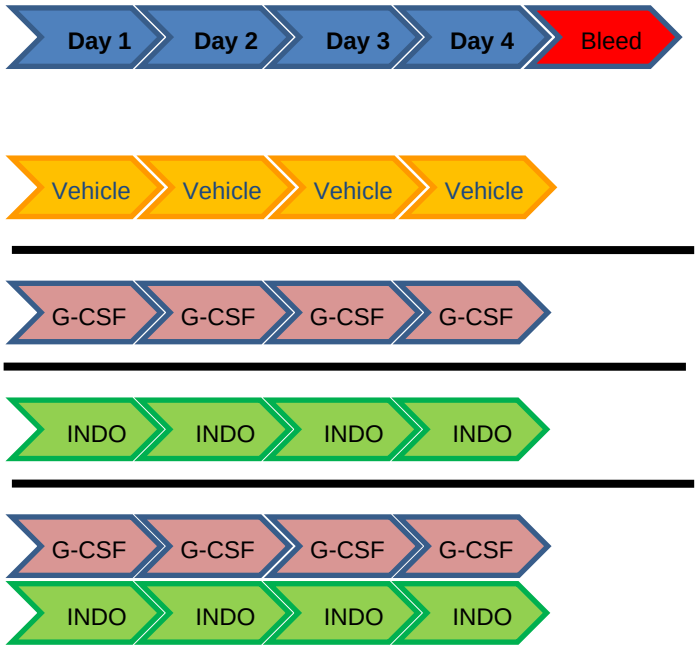

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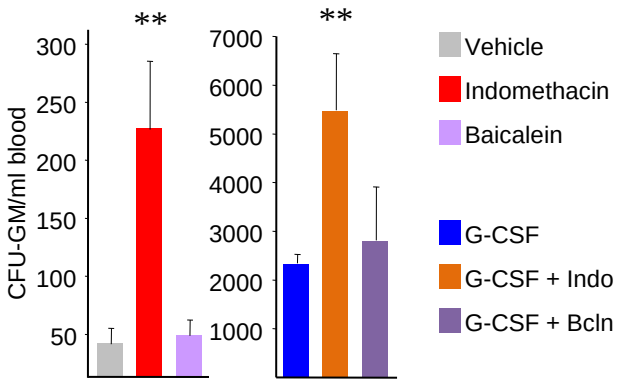
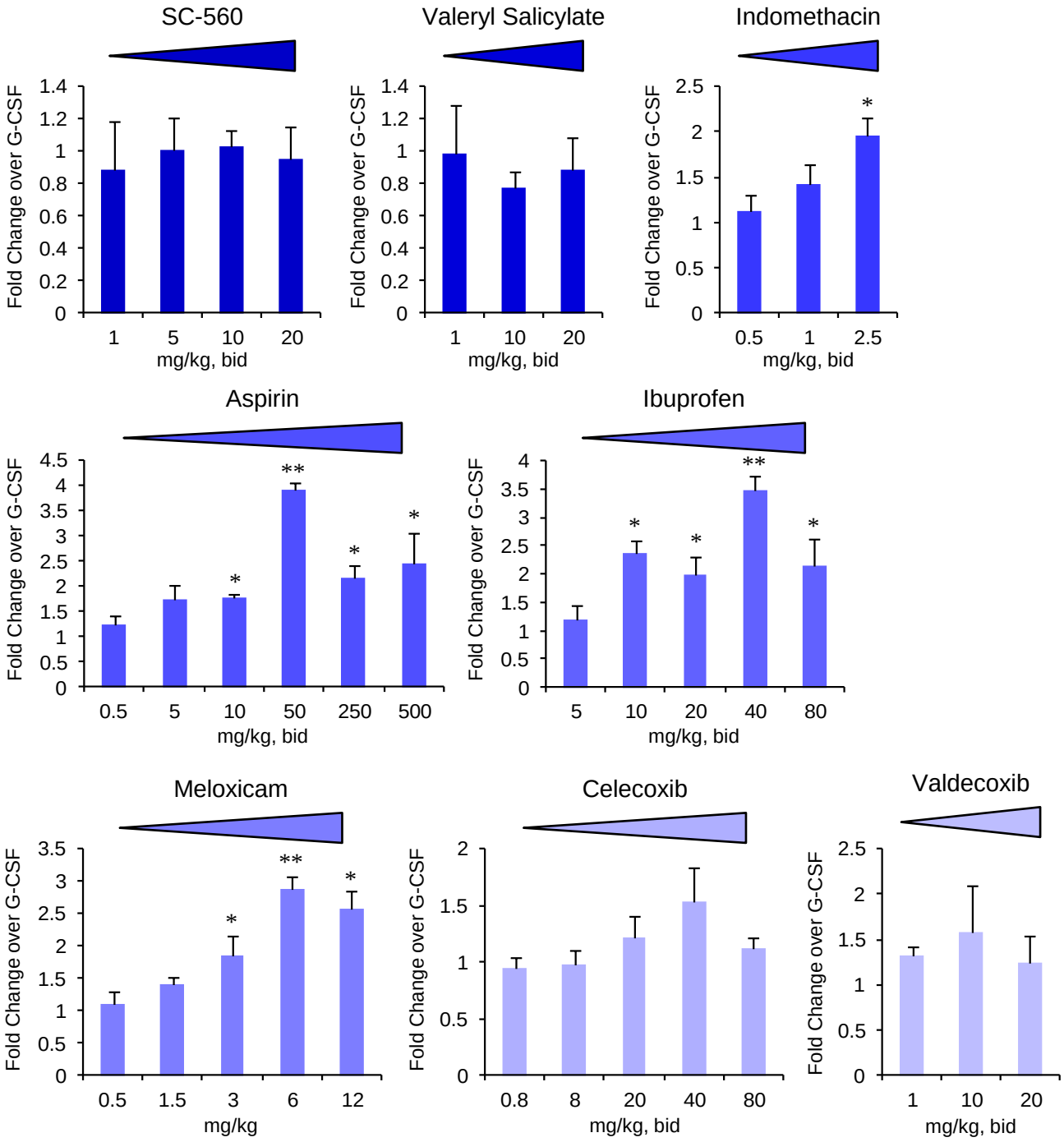


c

	WBC	PMN	LYMPH	MONO	RBC	PLT
Ctrl	7.04±0.59	1.24±0.18	5.40±0.39	0.37±0.06	8.88±0.20	1076±30
Indo	6.63±0.67	1.51±0.18	4.34±0.51	0.62±0.09*	8.04±0.17*	1110±75

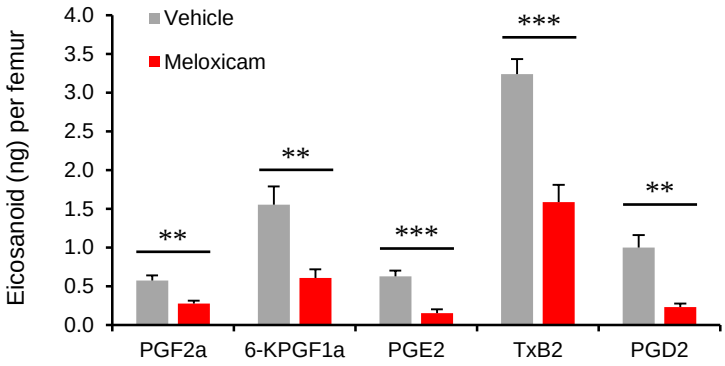
Supplementary Figure 1. Mobilization regimens and CBC after indomethacin treatment. **a,** Schematic of the dosing regimen used to evaluate the mobilization of CFU-GM using G-CSF, Indomethacin, or the combination of G-CSF plus Indomethacin. **b,** Mobilization of hematopoietic progenitors by Indomethacin, G-CSF, or combination treatment (n=5 mice per group, each assayed individually). **c,** Complete blood counts in control or NSAID treated mice. *P<0.05; unpaired two-tailed t-test.



Supplementary Figure 2. Enhancement in HPC niche egress requires inhibition of both COX-1 and COX-2.

Mice were mobilized by a 4 day regimen of G-CSF (50 µg/kg, twice daily) with co-administration of NSAIDs ranging in COX1 and COX2 selectivity. Data are expressed as fold change over G-CSF alone, n=4 or 5 mice per group, each assayed individually. *P<0.05, **P<0.01; one-way ANOVA with Bonferroni post-hoc analysis. All error bars represent mean ± s.e.m.

a



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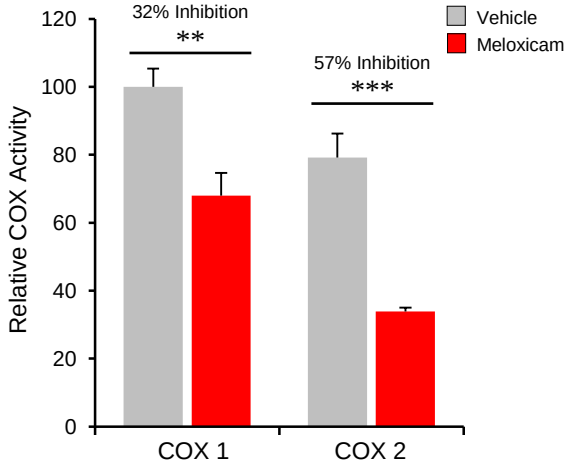
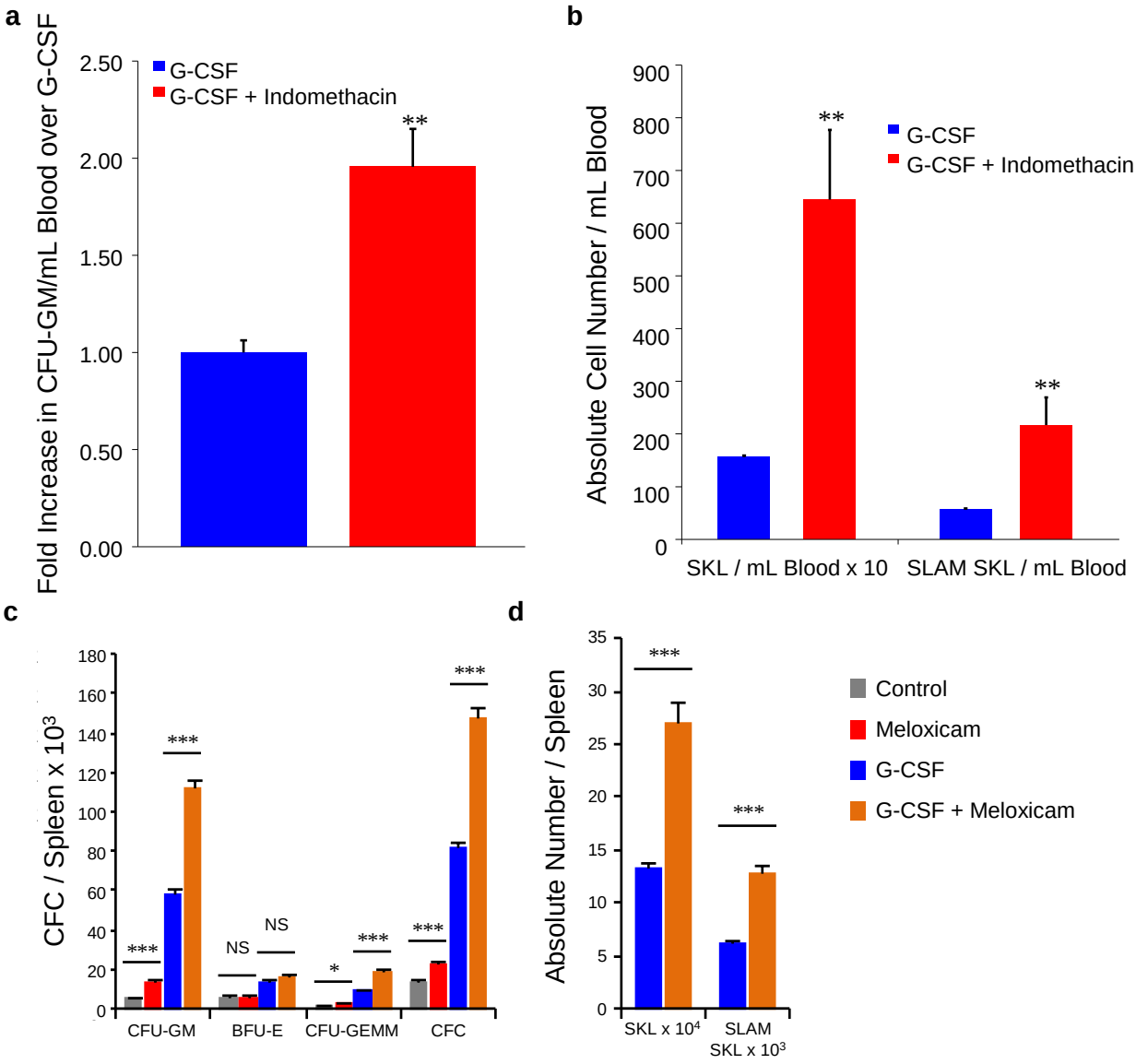


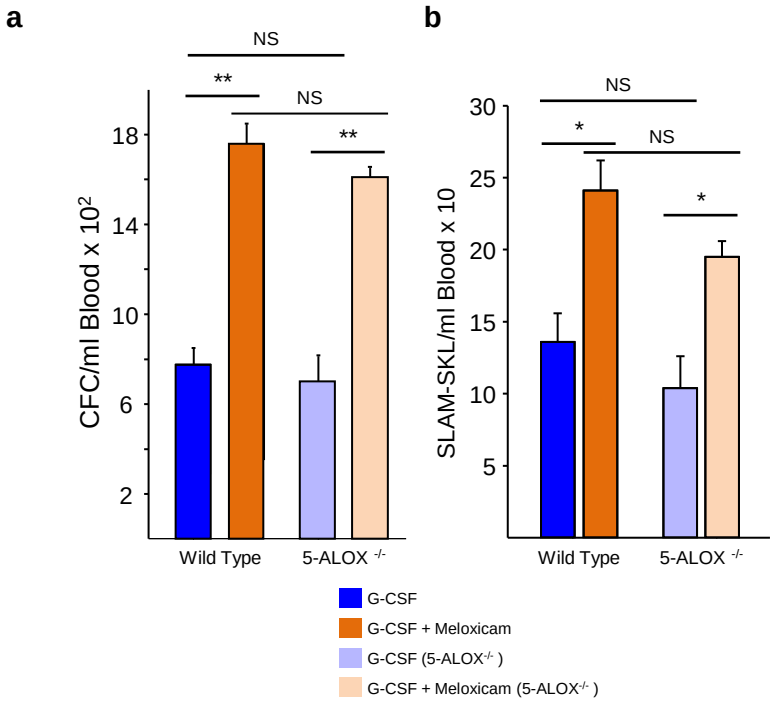
Supplementary Figure 3. Meloxicam inhibits both COX-1 and COX-2 in the bone marrow of mice.

a, Analysis of COX-1 and COX-2 derived eicosanoids in the bone marrow of mice after treatment with meloxicam or vehicle control. **b,** Functional assessment of COX-1 and COX-2 activity from bone marrow cells after treatment with meloxicam or vehicle control. n=5 mice per group, each assayed individually. *P<0.05, **P<0.01, ***P<0.001; unpaired two-tailed t-test. All error bars represent mean ± s.e.m.

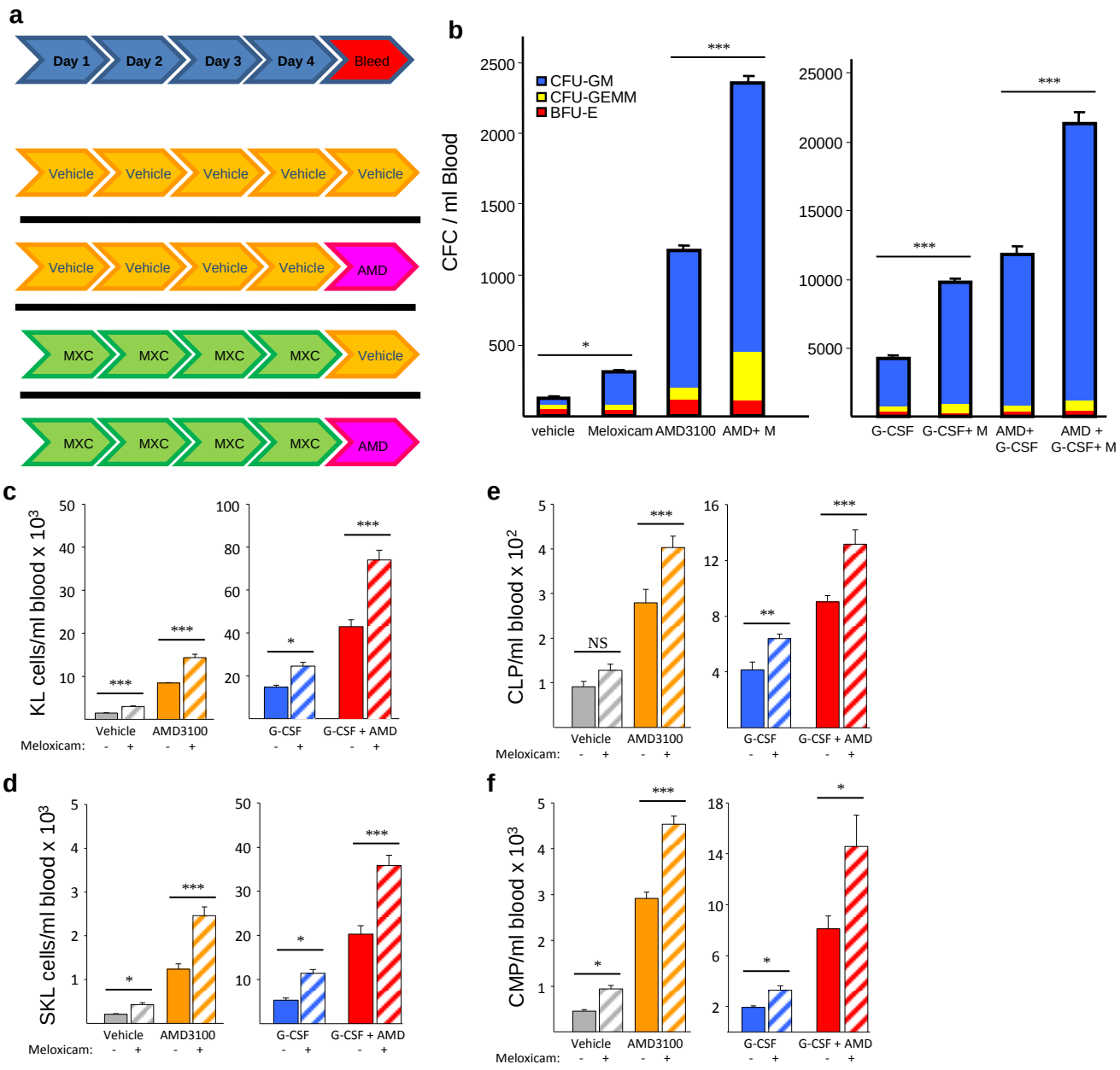


Supplementary Figure 4. Indomethacin and meloxicam increase niche egress of both functionally and phenotypically defined HPC and HSC.

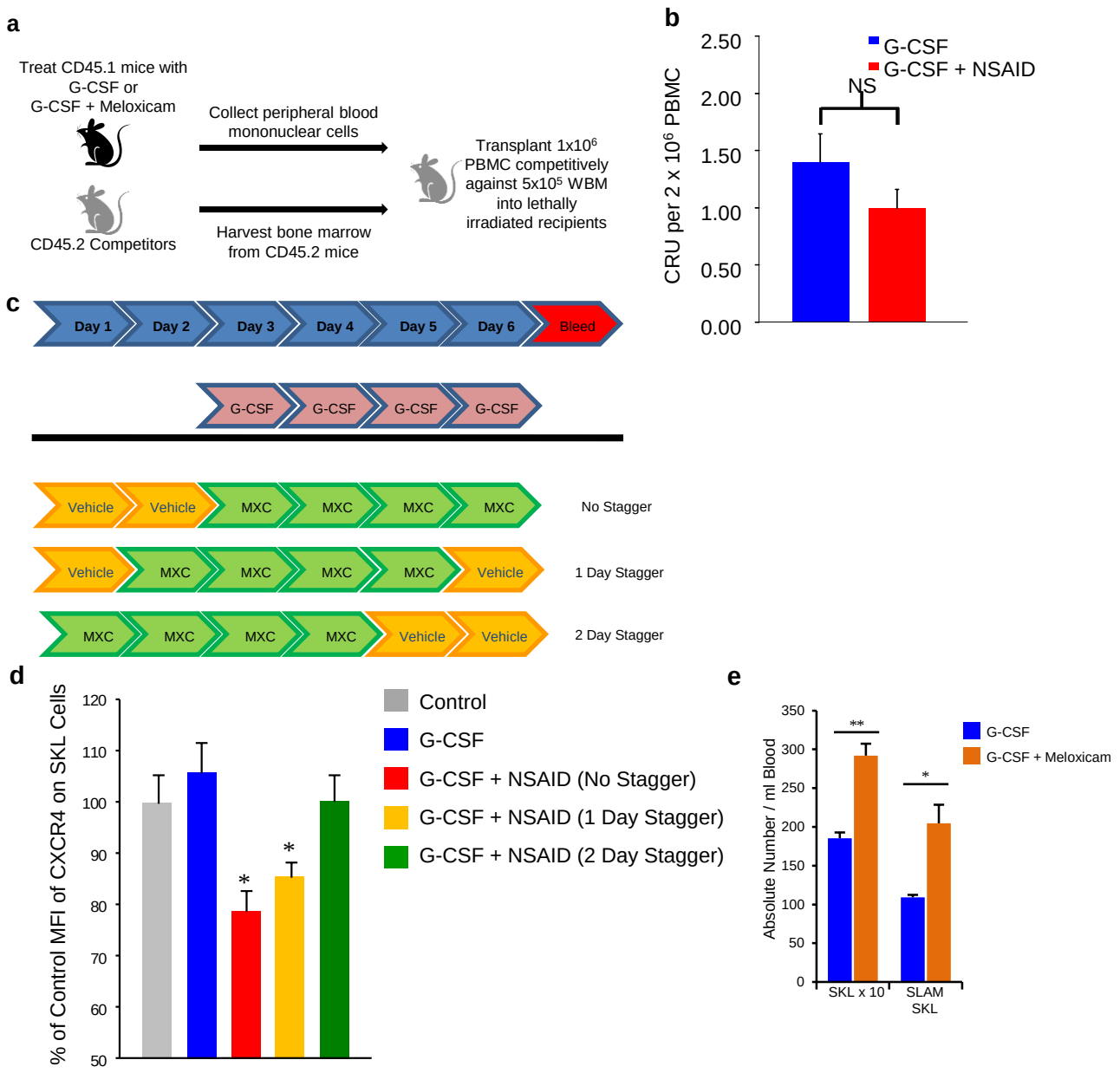
a, Mobilization of CFU-GM with G-CSF, or the combination of G-CSF and Indomethacin. Data are expressed as mean ± s.e.m. from 3 experiments, n=12 mice total per group, each assayed individually. **b,** Flow cytometric analysis of phenotypically defined HSC in peripheral blood of mice treated with G-CSF or the combination of G-CSF and Indomethacin. (n=5 mice per group, each assayed individually) **c,** Mobilization of CFC with meloxicam, G-CSF or the combination of G-CSF + Meloxicam. **(**n=4-5 mice per group, per experiment; 3 separate experiments). **d,** Mobilization of phenotypically defined HSC in peripheral blood of mice treated with G-CSF or the combination of G-CSF and Meloxicam. (n=4-5 mice per group, per experiment; 3 separate experiments). Data are expressed as mean ± s.e.m *P<0.05, **P<0.01, ***P<0.001 unpaired two tailed t-test.



Supplementary Figure 5. Enhanced HSC and HPC mobilization in 5-ALOX knockout mice. **a,** Mobilization of CFC and **b,** SLAM SKL cells with G-CSF or G-CSF + meloxicam in wild type or 5-ALOX knockout mice. n=4 mice per group, each assayed individually. *P<0.05, **P<0.01; one-way ANOVA with Bonferroni post-hoc analysis. All error bars represent mean ± s.e.m.

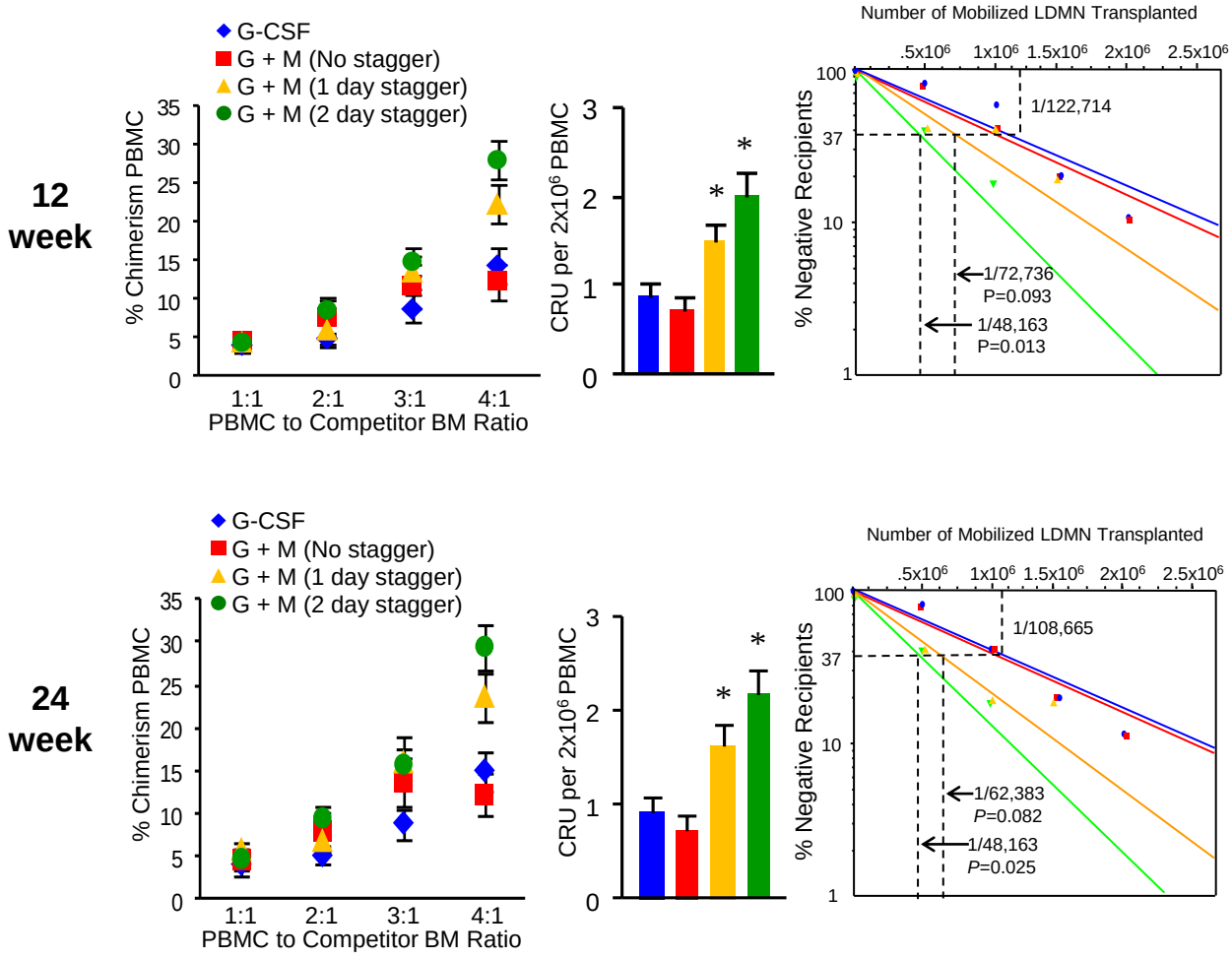


Supplementary Figure 6. Enhanced HSC and HPC mobilization with AMD3100.
a, Schematic of the dosing regimens used to evaluate NSAID enhancement of AMD3100 mobilization. Mice were treated with meloxicam, AMD3100, AMD3100 + meloxicam, G-CSF, G-CSF + meloxicam, AMD3100 + G-CSF, or AMD3100 + G-CSF + meloxicam and peripheral blood **b,** colony forming cells, **c,** c-kit^{+} lineage^{-} (KL) cells, **d,** Sca-1^{+}c-kit^{+}lineage^{-} (SKL) cells, **e,** c-kit^{low} Sca-1^{low} IL-7Rα^{+} Flk2^{hi} lineage^{-}, common lymphoid progenitors (CLP), and **f,** c-kit^{+} Sca-1^{-} CD34^{+} FcγR^{low} lineage^{-}, common myeloid progenitors (CMP), were determined (n=5 mice per group, each assayed individually). *P<0.05, **P<0.01, ***P<0.001, one-way ANOVA, Tukey-Kramer post-hoc analysis. All error bars represent mean ± s.e.m.



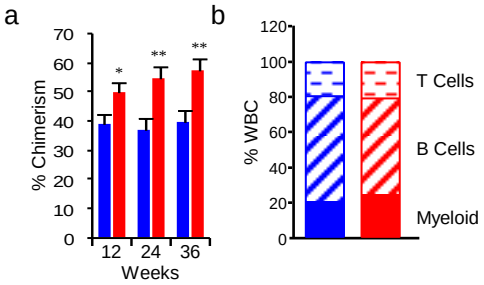
Supplementary Figure 7. Staggered dosing regimens restore peripheral HSC/HPC CXCR4 receptor expression.

a, BoyJ mice were treated with G-CSF with or without NSAID for 4 days. On day 5, LDMC from peripheral blood were acquired and transplanted at a 2:1 ratio with C57Bl/6J WBM competitors into lethally irradiated C57Bl/6J recipient mice. **b,** Competitive repopulating units were calculated 3 months post-transplant (mean ± s.e.m.). Data represent 2 pooled experiments, n=5 mice per group, per experiment, each assayed individually. **c,** Schematic of staggered dosing regimens of G-CSF with Meloxicam (MXC). A 4-day regimen of Meloxicam was either co-administered with G-CSF for 4 days (no stagger) or was staggered 1 or 2 days to allow for restoration of endogenous PGE_2 biosynthesis. **d,** Mice were bled and peripheral blood mononuclear cells (PBMC) were stained for SKL and the CXCR4 receptor and evaluated by FACS. Data represent the percent of control mean fluorescence intensity (MFI) for CXCR4 expression on SKL cells, expressed as mean ± s.e.m., n=5 mice per group, each assayed individually. **e,** Assessment of SKL and SLAM SKL cells in the blood after G-CSF or G-CSF + meloxicam (2-day stagger) (n=4 mice per group, each assayed individually). *P<0.05, **P<0.01; unpaired two-tailed t-test.

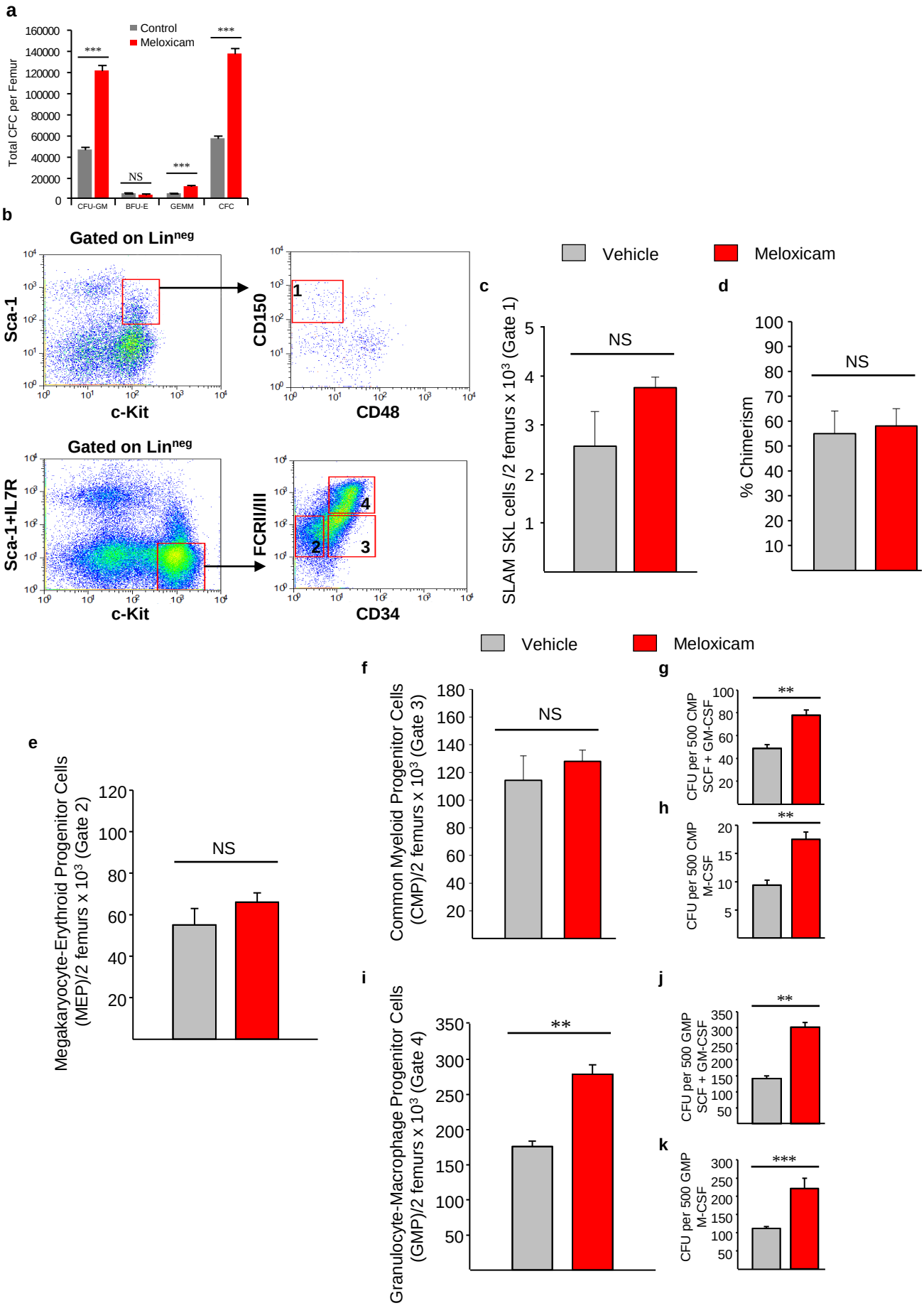


Supplementary Figure 8. Enhanced long-term competitive repopulation with NSAID mobilized grafts.

Chimerism, competitive repopulating units (CRU), and long-term repopulating HSC frequency (Poisson distribution) 12 and 24 weeks after limiting dilution competitive transplants of PBMC from mice mobilized with G-CSF and combination regimens, n=8 mice per group, each assayed individually. *P<0.05; unpaired two-tailed t-test.



Supplementary Figure 9. Enhanced secondary repopulation with NSAID mobilized grafts
Bone marrow from mice originally transplanted with PBMC mobilized by G-CSF or G-CSF + Meloxicam (see Figure 1f) was secondarily transplanted and **a**, chimerism and **b,** multilineage reconstitution were determined (n=10 mice per group, each assayed individually).

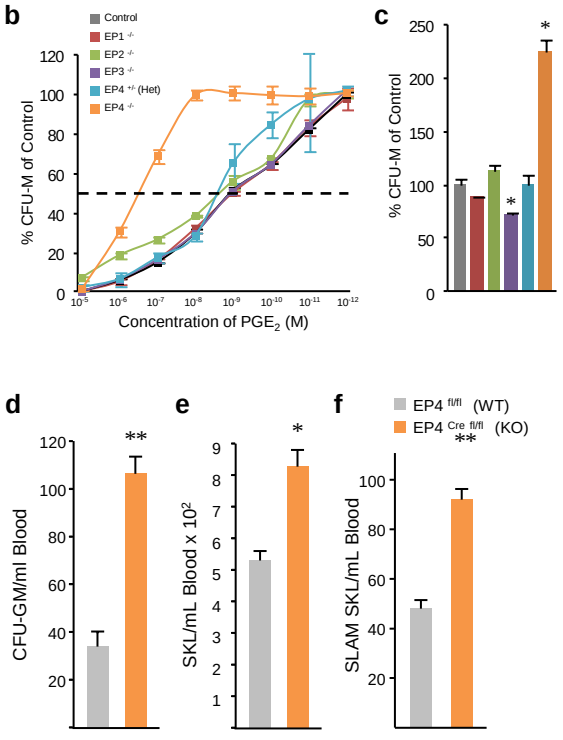

a
Control
Meloxicam
Total CFC per Femur
160000
140000
120000
100000
80000
60000
40000
20000
0
***
***
NS
***
CFU-GM
BFU-E
GEMM
CFC
b
Gated on Lin^neg
Sca-1
c-Kit
CD150
CD48
1
Gated on Lin^neg
Sca-1+IL7R
c-Kit
FCRII/III
CD34
2
3
4
c
Vehicle
Meloxicam
SLAM SKL cells /2 femurs x 10^3 (Gate 1)
NS
d
% Chimerism
NS
e
Megakaryocyte-Erythroid Progenitor Cells (MEP)/2 femurs x 10^3 (Gate 2)
NS
f
Common Myeloid Progenitor Cells (CMP)/2 femurs x 10^3 (Gate 3)
NS
g
CFU per 500 CMP SCF + GM-CSF
**
h
CFU per 500 CMP M-CSF
**
i
Granulocyte-Macrophage Progenitor Cells (GMP)/2 femurs x 10^3 (Gate 4)
**
j
CFU per 500 GMP SCF + GM-CSF
**
k
CFU per 500 GMP M-CSF
***

Supplementary Figure 10. Enhanced bone marrow myeloid progenitors with no alteration of HSCs in NSAID treated mice.

a, Expansion of bone marrow HPC after meloxicam treatment (n=4 mice/group/experiment; two separate experiments.) **b,** Representative flow cytometry gating of SLAM SKL cells (gate 1), megakaryocyte erythroid progenitor (MEP) cells (gate 2), common myeloid progenitor (CMP) cells (gate 3) and granulocyte macrophage progenitor (GMP) cells. **c,** SLAM SKL cell number between vehicle and meloxicam treated mice (n=4 mice per group, each assayed individually). **d,** Chimerism 16 weeks post-transplant after a competitive 1:1 transplant of vehicle or staggered meloxicam (2 day stagger) treated mice (n=5 mice per group, each assayed individually. **e,** MEP number in vehicle or meloxicam treated mice. (n=4 mice per group, each assayed individually). **f,** CMP number in vehicle or NSAID treated mice (n=4 mice per group, each assayed individually). CMPs were sorted and plated in **g,** GM-CSF 10 ng/ml) + SCF (50 ng/ml) or **h,** M-CSF (2 ng/ml)**,** and CFU per 500 cells determined (n=10 plates per treatment and condition group). **i,** GMP number in vehicle or NSAID treated mice (n=4 mice per group, each assayed individually). GMPs were sorted and plated in **j,** GM-CSF + SCF or **j,** M-CSF**,** and CFU per 500 cells determined (n=10 plates per treatment and condition group). *P<0.05, **P<0.01, ***P<0.001; unpaired two-tailed t-test. All error bars represent mean ± s.e.m.

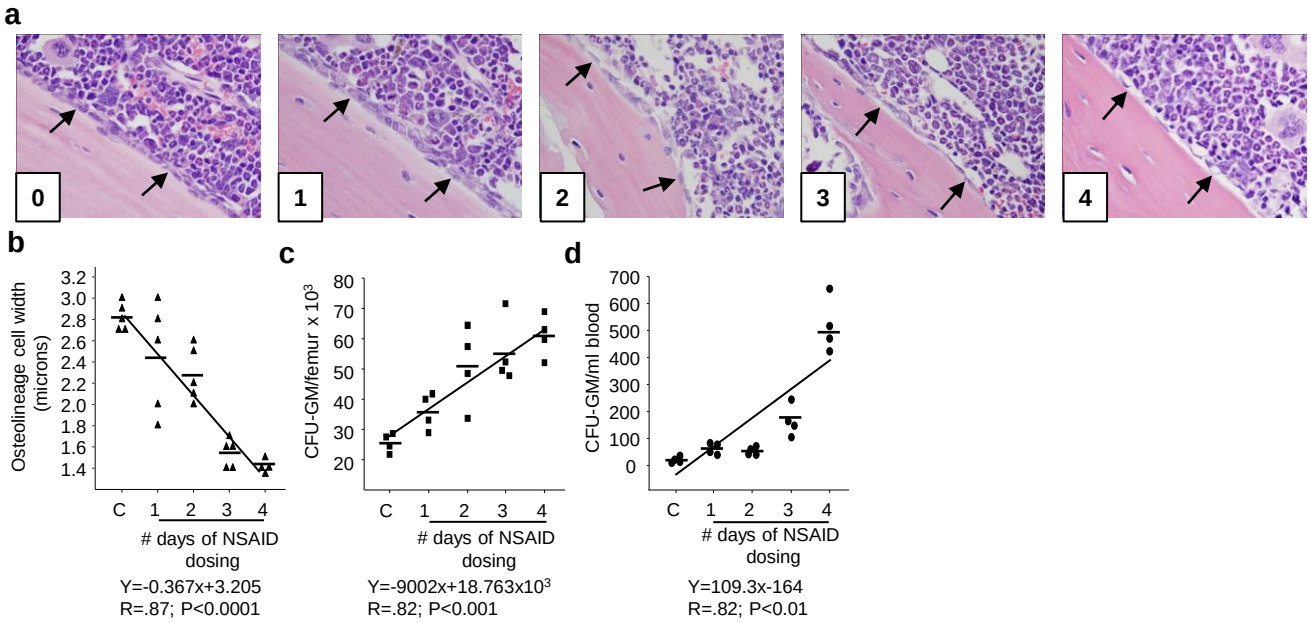
a

Inhibition of CFU-M			
Compound	Agonist Specificity	IC_{50} (nM)	Ratio
PGE_2	EP1, EP2, EP3, EP4	2.37±0.3	1
$dmPGE_2$	EP2, EP3, EP4	3.1±0.9	1.3
17-phenyl trinor PGE_2	EP1, EP3	1392±383	587
Butaprost	EP2	89,100±2145	37,595
Sulprostone	EP3, EP1	6990±349	2949
11-deoxy PGE_2	EP2, EP3, EP4	11.5±5	4.8
L-902,688	EP4	2.9±0.7	1.2
PGE_2		2.4±0.3	1
+ 5μM AH23848	EP4 antagonist	10.6±0.2	4.5
+ 10μM AH23848	"	14.6±0.6	6.2
+ 30μM AH23848	"	36.8±4.5	15.5
+ 60μM AH23848	"	92.1±14.6	38.9
Schild Plot Kb 1.69x10^{-6} M: R^2= 0.95 A/A'=-5.57: = Competitive Antagonist			



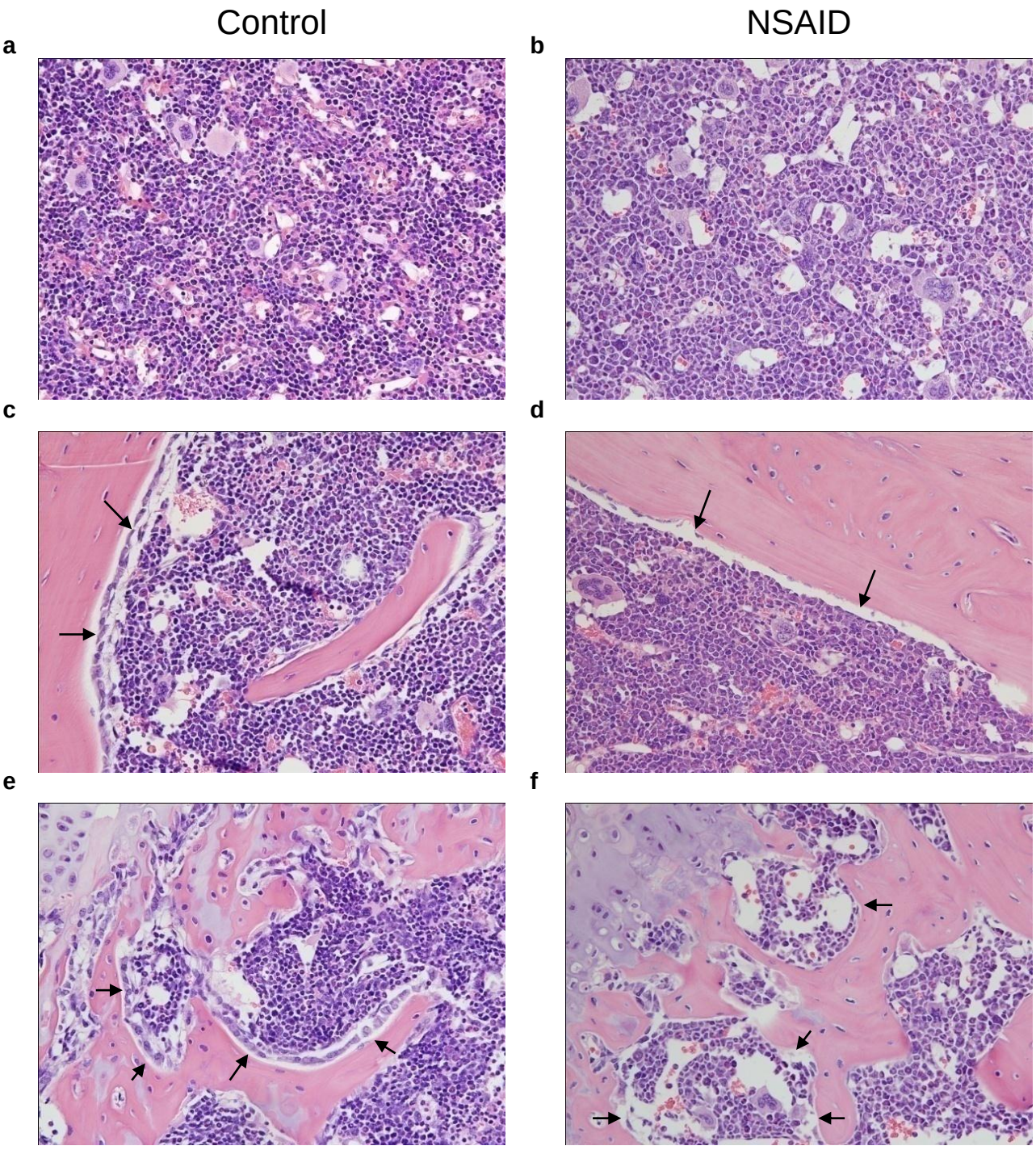
Supplementary Figure 11. Prostaglandin E_2 EP4 receptor antagonism or knockout expands bone marrow progenitors and enhances progenitor and stem cell mobilization

a, Inhibition of CFU-M formation in the presence of PGE_2 agonists and antagonists. **b,** Inhibition of CFU-M formation by PGE_2 from bone marrow of wild type, $EP1^{-/-}$, $EP2^{-/-}$, $EP3^{-/-}$, $EP4^{+/-}$, or $EP4^{-/-}$ bone marrow cells. **c,** Increased CFU-M in bone marrow of $EP4^{-/-}$ mice. **d,** Conditional deletion of EP4 results in enhanced CFU-GM, **e,** SKL cells and **f,** SLAM SKL cells in the peripheral blood (n=3 mice per group, each assayed individually). *P<0.05, **P<0.01; unpaired two-tailed t-test. All error bars represent mean ± s.e.m.



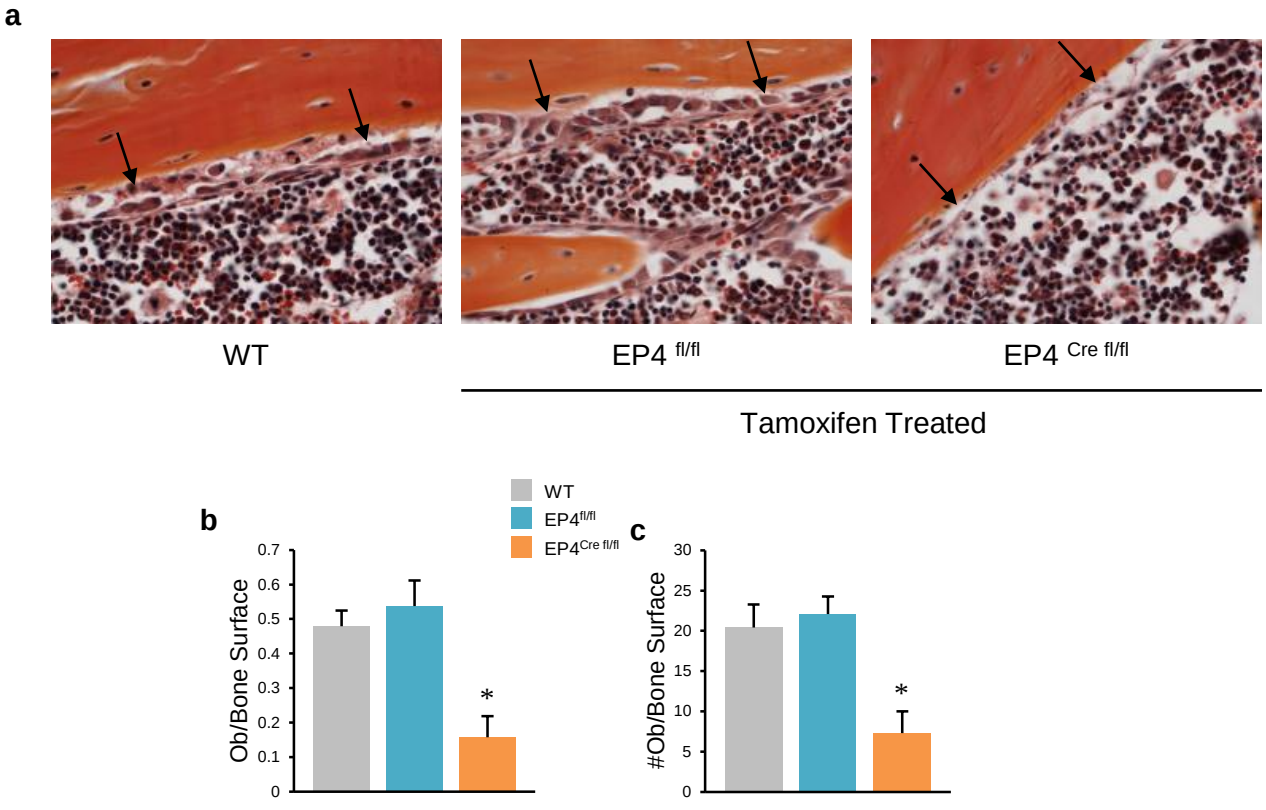
Supplementary Figure 12. Progressive attenuation of endosteal lining osteolineage cells after NSAID treatment.

a, Attenuated endosteal lining osteolineage cells after 0-4 days of NSAID treatment. Original magnification 400X. **b,** Osteolineage cell width after 0-4 days of NSAID treatment (50 measured osteolineage cells per mouse femur, n=4-5 mice per group, each assayed individually). **c,** Expansion of bone marrow hematopoietic progenitors, and **d,** increased progenitor mobilization after 0-4 days.



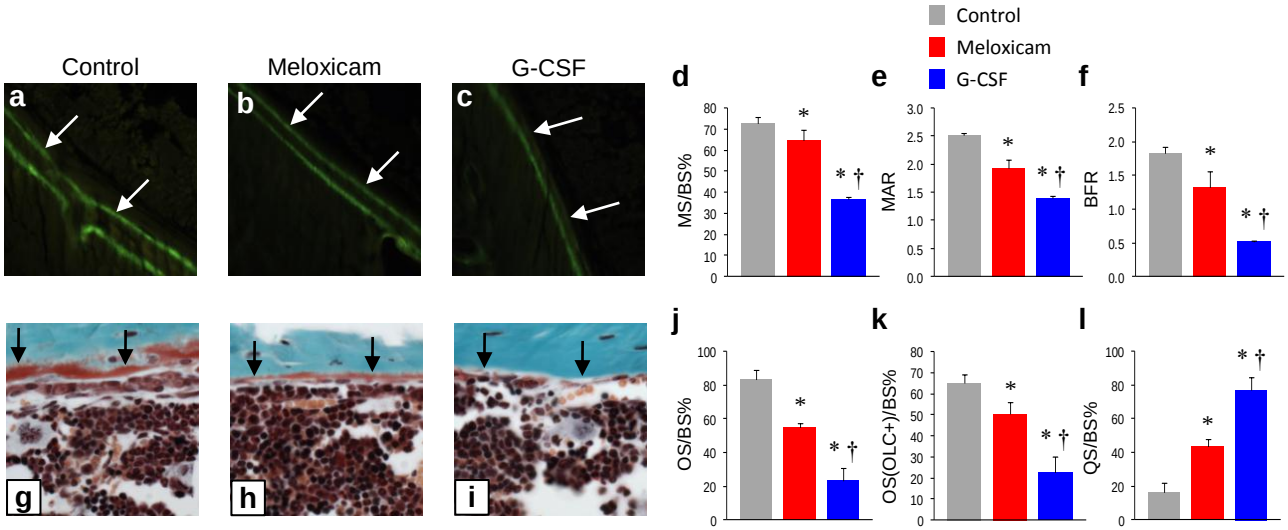
Supplementary Figure 13. Enhanced myelopoiesis and attenuated osteolineage cells after NSAID treatment.

Mice were treated with a 4 day regimen of vehicle or NSAIDs, femurs were acquired, sectioned and H&E stained. Shown is the marrow from **a,** control and **b,** NSAID treated mice, showing increased myelopoiesis and reduced erythropoiesis after treatment. **c,** Control mice maintained normal osteolineage cell lining of bone (arrows) in the diaphyseal region of the femur, while **d,** NSAID treated mice showed a less distinct layer of osteolineage cells, with marked flattening. **e,** Control mice also had normal osteolineage lining within the epiphyseal region of the femur, while **f,** NSAID treated mice showed a less distinct layer of osteolineage cells, with marked flattening. 400X magnification.

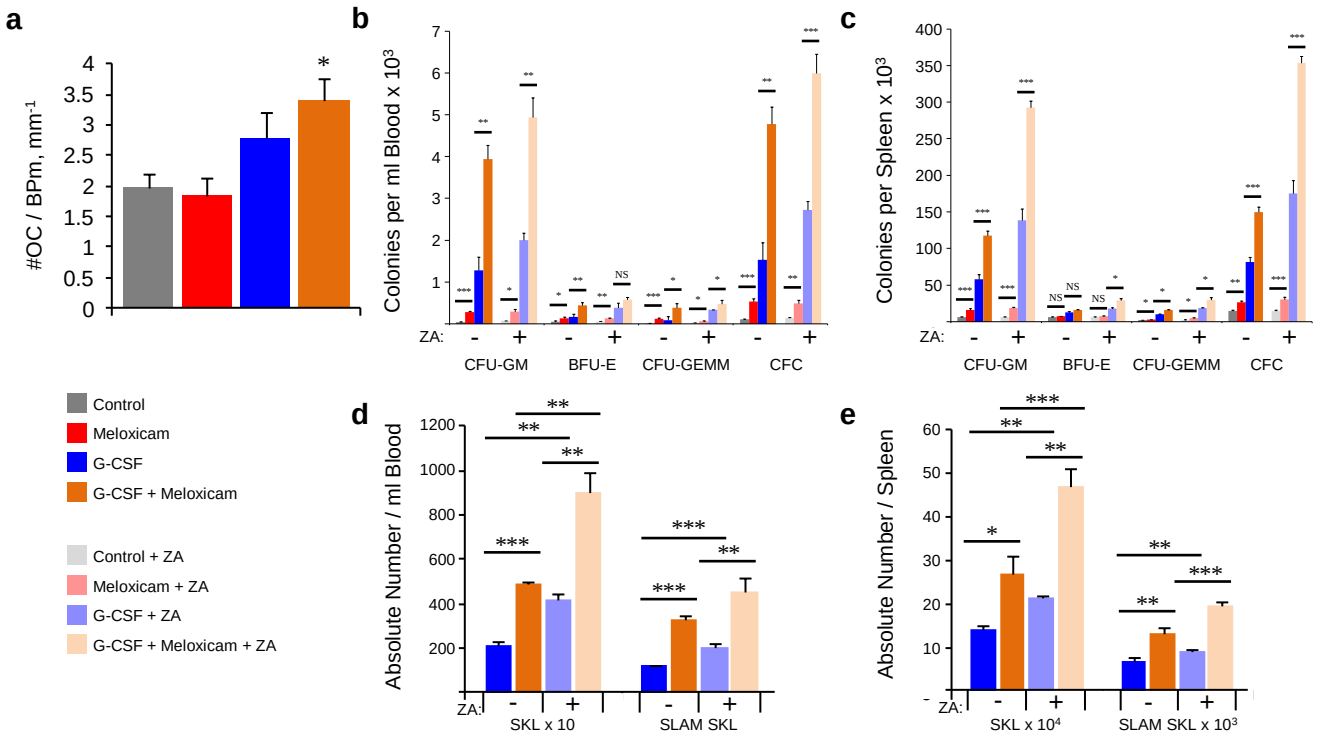


Supplementary Figure 14: Endosteal lining osteolineage cells in EP4 conditional knockout mice.

a, Shown are endosteal lining regions from a wild type mouse compared to an EP4 $^{fl/fl}$ or EP4 $^{Cre\ fl/fl}$ (knockout) 14 days after tamoxifen treatment. **b,** Conditional deletion of EP4 resulted in reduced proportion of osteolineage lining bone surfaces and **c,** osteolineage number. (n=3 mice per group, each assayed individually). *P<0.05; unpaired two-tailed t-test. All error bars represent mean ± s.e.m.

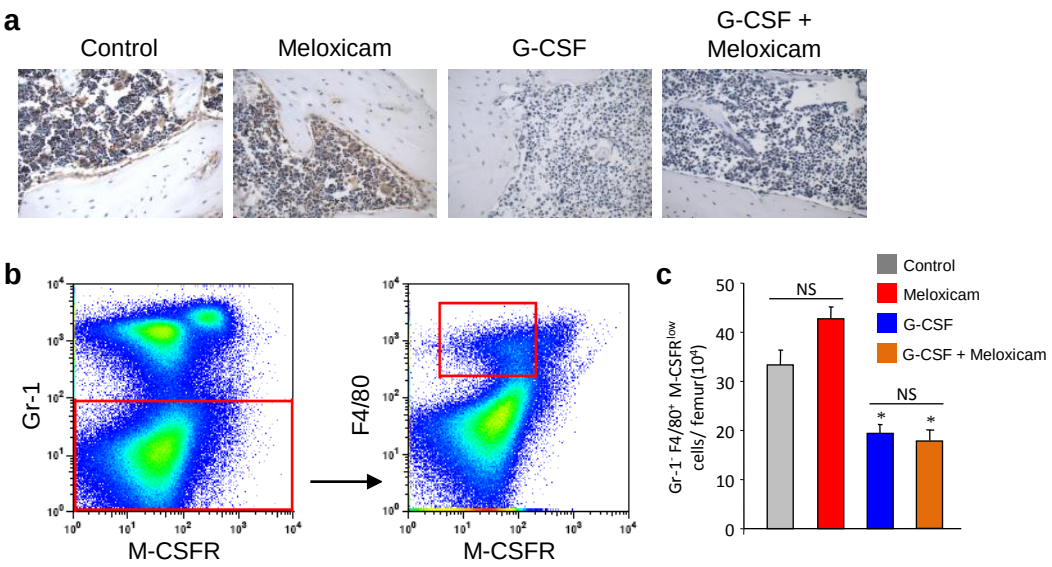


Supplementary Figure 15. Reduced osteolineage function in NSAID treated bones. Calcein uptake during dynamic bone formation assay in **a,** control, **b,** meloxicam, or **c,** G-CSF treated mice, respectively, demonstrates reduced **d,** mineralized surfaces, **e**, mineral apposition rate (MAR) and **f,** bone formation rate (BFR) after treatment with meloxicam or G-CSF (n=5 mice per group). **g, h, i,** Modified Goldner's trichrome staining for osteoid bone in control, meloxicam or G-CSF treated mice, respectively, demonstrates reduced **j,** osteoid surface (OS), **k**, osteoid/osteolineage cell (OLC) positive surfaces, and **l,** increased quiescent (QS) bone surfaces after treatment with Meloxicam or G-CSF (n=5 mice per group, each assayed individually). *P<0.05, unpaired two-tailed t-test. †P<0.05 compared to Meloxicam. All error bars represent mean ± s.e.m.



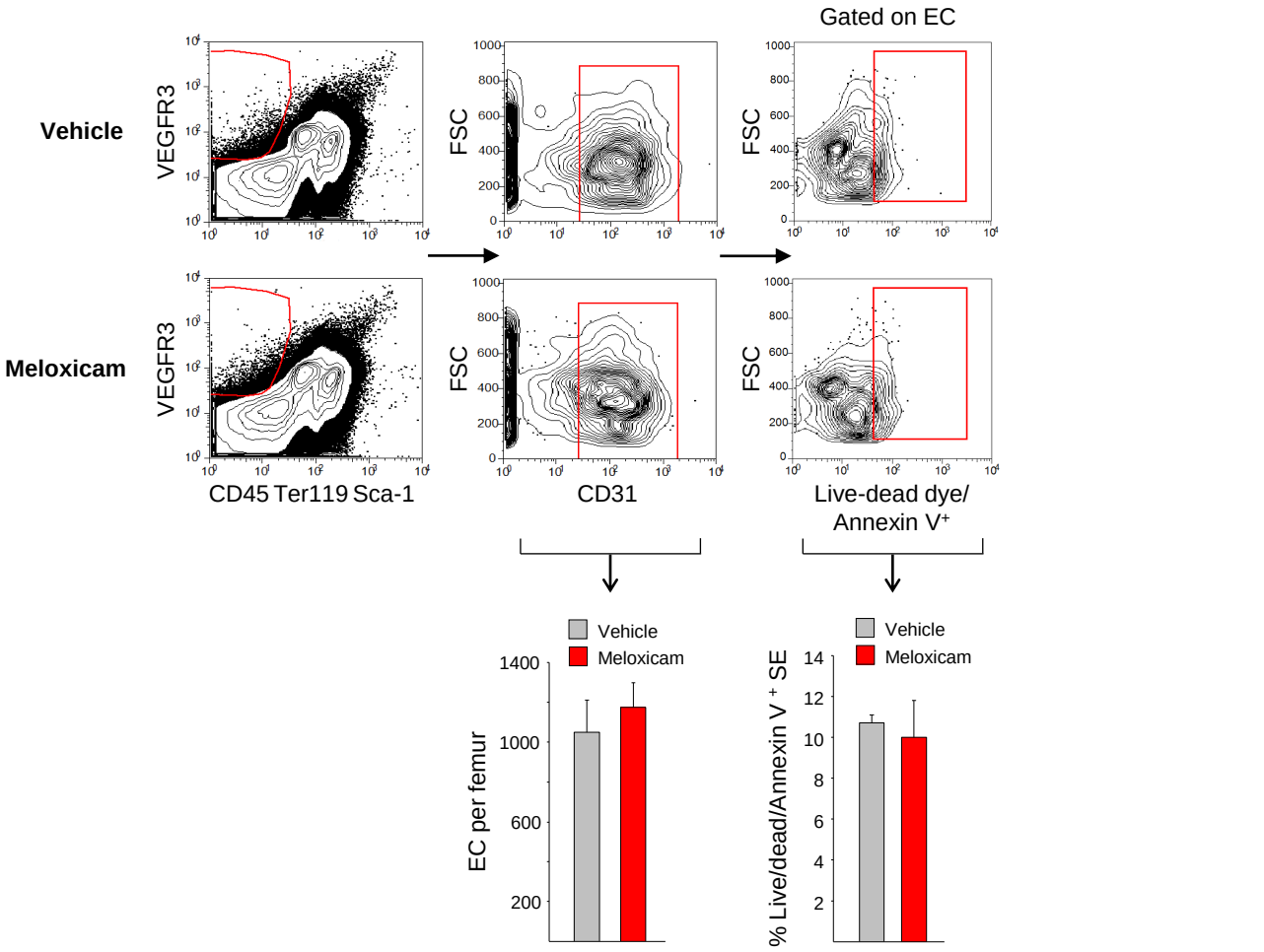
Supplementary Figure 16. Osteoclast function does not alter meloxicam enhancement of mobilization.

a, Osteoclast (OC) number after treatment with vehicle, meloxicam, G-CSF or G-CSF + meloxicam (n=5 mice per group, each assayed individually). **b,** Assessment of HPC in the peripheral blood and **c,** spleen, comparing mobilization strategies with or without zoledronic acid treatment. **d,** Assessment of SKL and SLAM SKL cells in the peripheral blood and **e,** spleen, comparing mobilization strategies with or without zoledronic acid treatment (n=5 mice per group, assayed individually.)). *P<0.05, **P<0.01, ***P<0.001; unpaired two-tailed t-test. All error bars represent mean ± s.e.m.

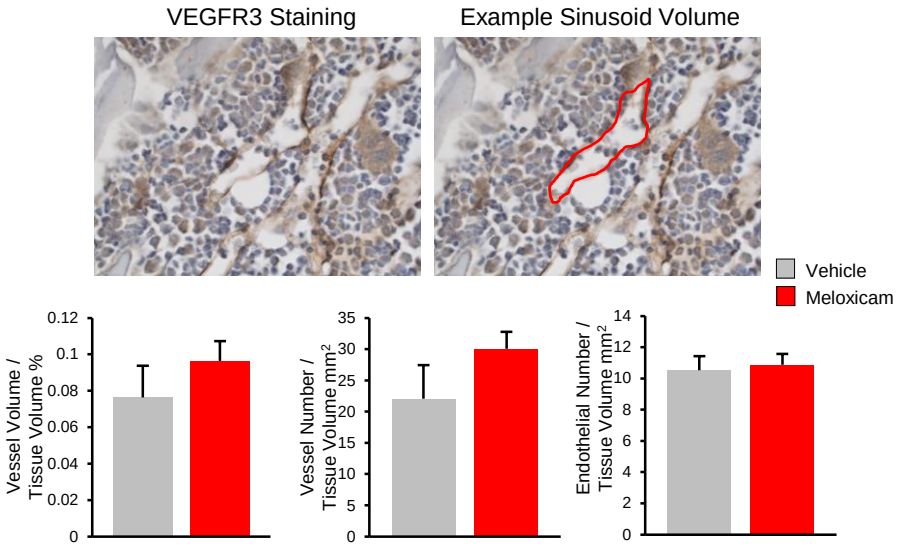


Supplementary Figure 17. No alteration in bone marrow-resident macrophages in NSAID treated mice.

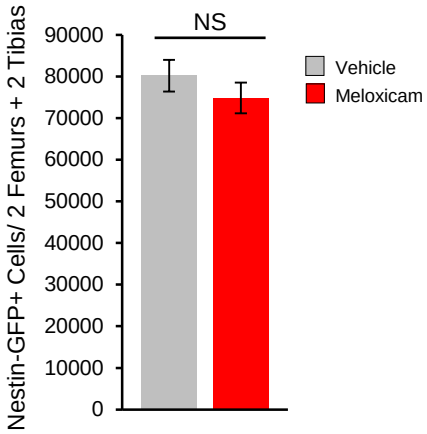
a, Immunohistochemical staining for resident F4/80^{+} bone marrow macrophages. Original magnification 200X. **b,** Representative flow gating of bone marrow macrophages. **c,** Reduced macrophages after G-CSF, but not meloxicam treatment as determined by FACS (n=5 mice per group, each assayed individually). *P<0.05; unpaired two-tailed t-test. All error bars represent mean ± s.e.m.



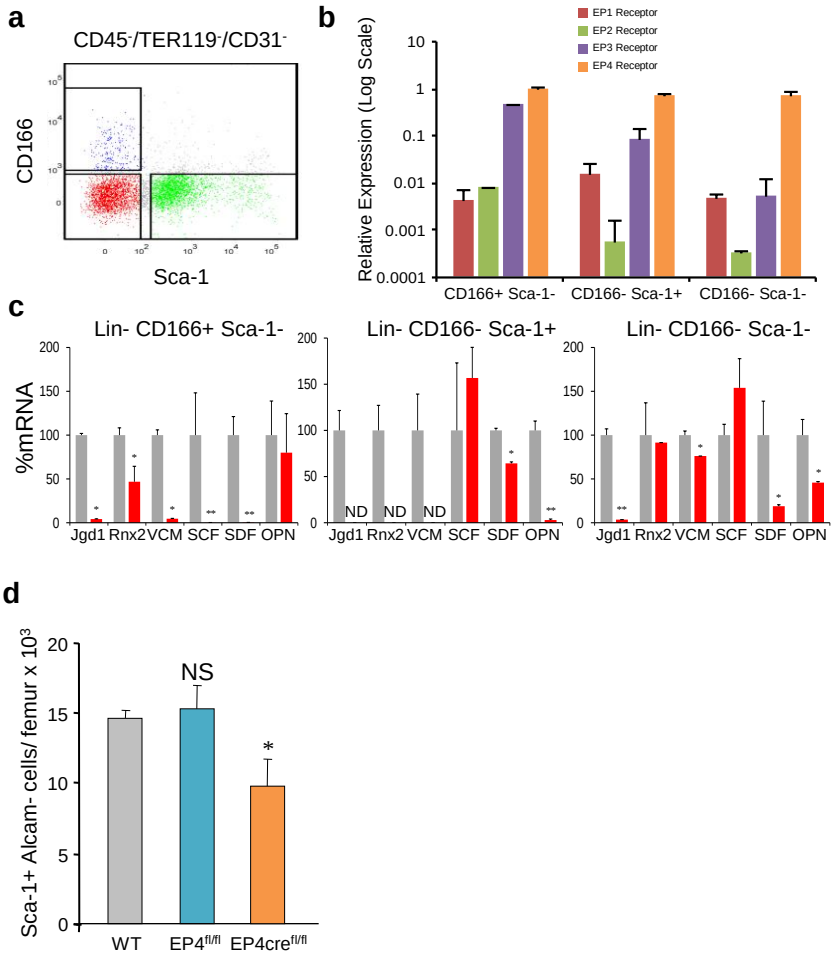
Supplementary Figure 18. No alterations in sinusoidal endothelial cell number or apoptosis in NSAID treated bone marrow.
Shown are representative flow cytometry plots of vehicle or NSAID treated mice for analysis of bone marrow sinusoidal endothelial cell number and apoptosis (n=5 mice/group, assayed individually). All error bars represent mean ± s.e.m.



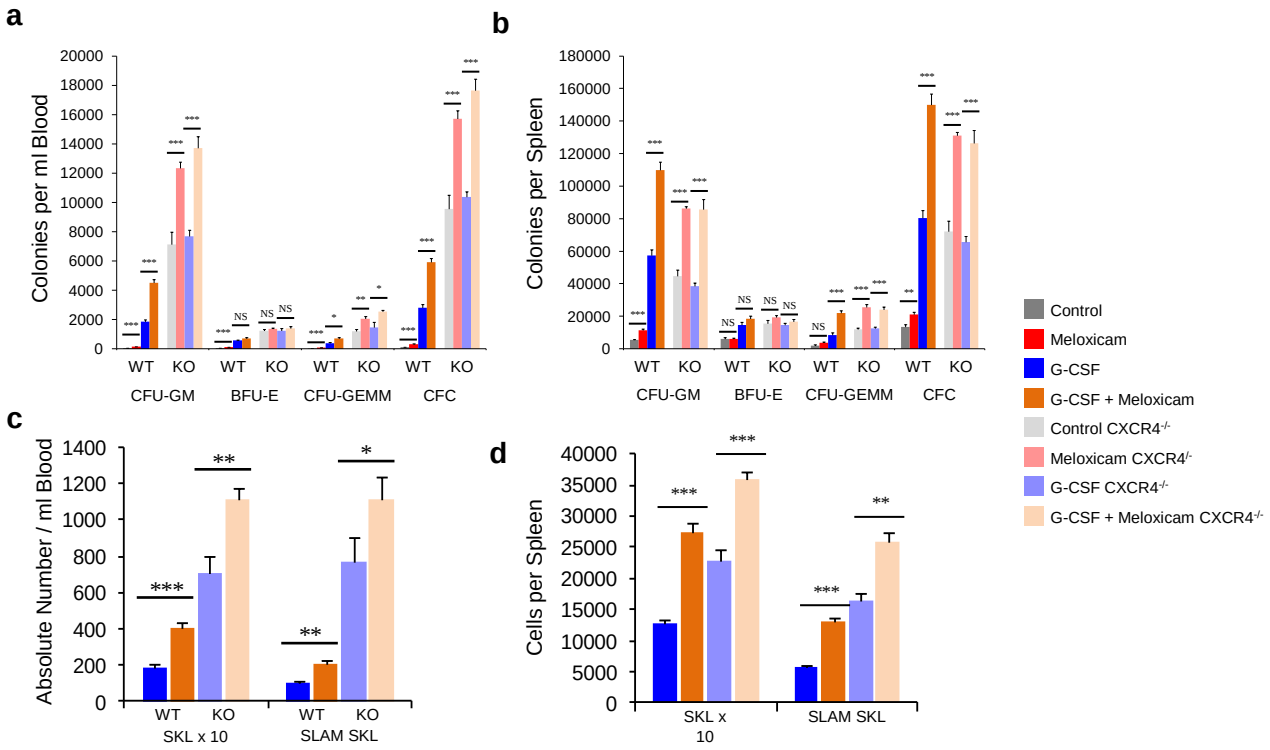
Supplementary Figure 19. No alterations in sinusoid vessel number, volume or endothelial cell number in NSAID treated bone marrow.
Shown is a representative IHC analysis of VEGFR3 stained sinusoids, showing an example sinusoid volume (red outline), with analysis of vessel volume, vessel number and endothelial cell number between meloxicam and vehicle treated mice. (n=3,4 mice per group, each assayed individually). All error bars represent mean ± s.e.m.



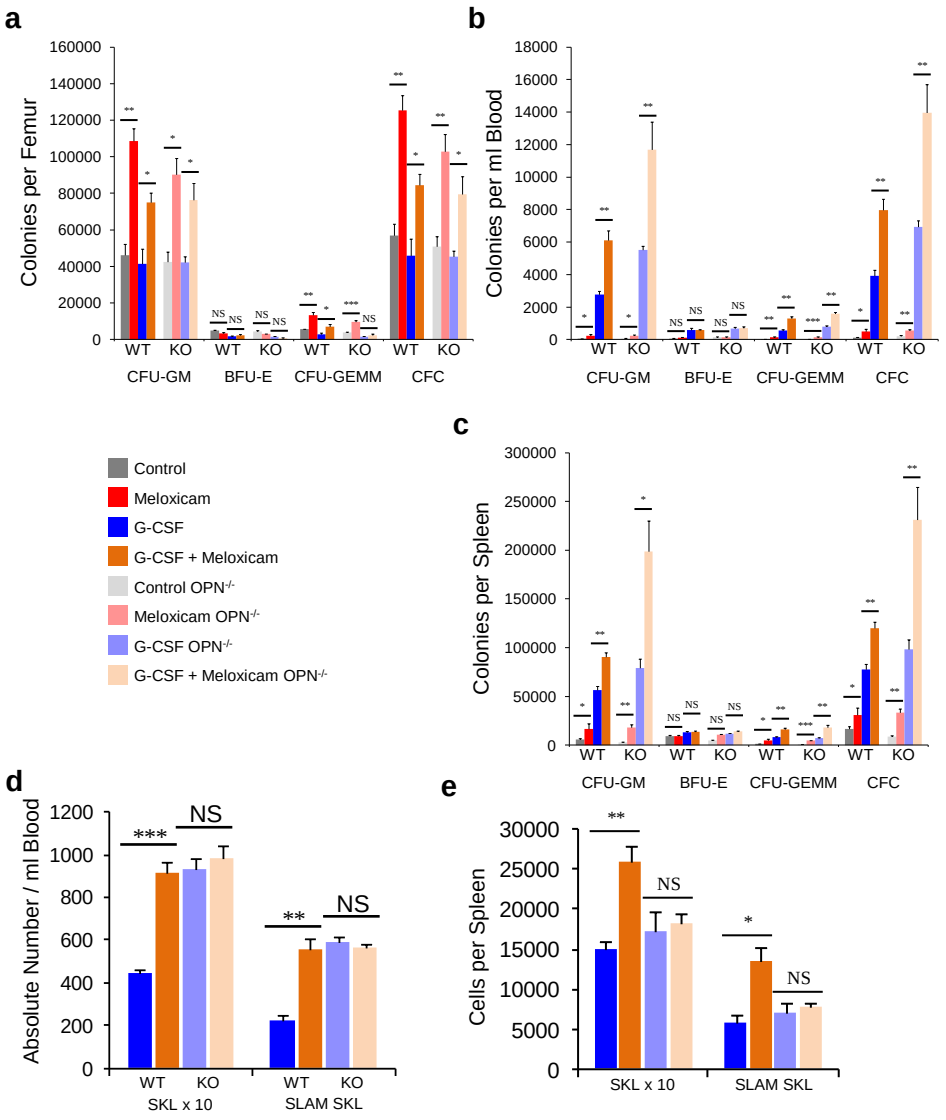
Supplementary Figure 20. No alteration in Nestin+ cell number in NSAID treated bone marrow. Nestin-GFP mice were treated with vehicle or meloxicam for 4 days and GFP^+ cells assessed by flow cytometry (n=3 mice per group, each assayed individually). All error bars represent mean ± s.e.m.



Supplementary Figure 21. NSAIDS reduce osteolineage cell production of hematopoietic supportive molecules and mesenchymal progenitor cells are reduced in EP4 knockout mice. **a,** Representative gating strategy for osteolineage cell populations. "Lineage (Lin)" refers to CD45, CD31 and TER119. **b,** Relative EP receptor expression on osteolineage cells (n=5 mice per group, each assayed individually). **c,** Expression and regulation of HSC maintenance genes by osteolineage cells; Jagged-1 *(Jgd1)*, Runx-2 *(Rnx2)*, VCAM-1 *(VCM)*, stem cell factor/kit ligand *(SCF)*, stromal derived factor-1 *(SDF)*, and osteopontin *(OPN)*. n=4 independent groups per condition. d, Analysis of CD45/TER119/CD31^{-} Sca-1^{+} Alcam^{-} cells in WT, or tamoxifen treated EP4$^{fl/fl}$ and EP4$^{Cre\ fl/fl}$ (knockout mice) (n=4 mice per group, each assayed individually). *P<0.05; unpaired two-tailed t-test. All error bars represent mean ± s.e.m.



Supplementary Figure 22. HPC and candidate HSC mobilization in CXCR4 and knockout mice. CFU-GM, BFU-E, and CFU-GEMM mobilization in CXCR4 knockout mice or Cre(-) littermate controls in **a,** peripheral blood or **b,** spleen, and HPC and HSC mobilization to **c,** peripheral blood or **d**, spleen (n=5 mice per group, each assayed individually). *P<0.05, **P<0.01, ***P<0.001; unpaired two-tailed t-test. All error bars represent mean ± s.e.m.



Supplementary Figure 23. HPC and candidate HSC mobilization in OPN knockout mice. **a,** Bone marrow **b**, peripheral blood and **c**, spleen CFU-GM, BFU-E, and CFU-GEMM in wild type or OPN knockout mice before and after treatment and HSC mobilization to **d**, peripheral blood and **e**, spleen in OPN knockout mice after treatment (n=4 mice per group, each assayed individually). *P<0.05, **P<0.01, ***P<0.001; unpaired two-tailed t-test. All error bars represent mean ± s.e.m.