Table S1. List of primers used for qRT-PCR

Human gene	
Hepcidin (HAMP)	5'-CTGACCAGTGGCTCTGTTTTC-3'
	5'-GAAGTGGGTGTCTCGCCTC-3'
Zebrafish gene	
RPL	5’-TCTGGAGGACTGTAAGAGGTATGC-3’
	5’-AGACGCACAATCTTGAGAGCAG-3’
zHepcidin	5’-CCTGGCTGCTGTCGTCAT-3’
	5’-TGGTTCTCCTGCAGTTCTTCAC-3’
Mouse gene	
Hepcidin (mHamp-1)	5'-AAGCAGGGCAGACATTGCGAT-3'
	5'-CAGGATGTGGCTCTAGGCTATGT-3'
18S rRNA	5'-CGGCTACCACTCCAAGGAA-3'
	5'-GCTGGAATTACCGCGGCT-3'

Primers used to quantify transcript levels in human HepG2 cells, zebrafish, and mice by quantitative real-time PCR.

Table S2. Treatment of wild-type mice with LDN-193189 for 30 days does not impact circulating erythrocyte, myeloid, lymphocyte, or thrombocyte lineages

	Mean (n=4)	Mean (n=5)
Total WBC	1.9±0.3	2.3±0.2
% Neutrophil	30.8±5.4	24.0±3.1
% Lymphocyte	63.1±7.6	69.7±4.4
% Monocyte	3.8±0.7	4.2±0.9
RBC	9.0±0.2	8.5±0.1
Hb	11.9±0.1	12.5±0.2
Hct	43.8±0.9	42.3±0.3
MCV	48.5±1.0	50.1±0.7
MCHC	27.3±0.3	29.6±0.3
RDW	18.6±0.0	17.7±0.2
Platelet count	830±73	628±86
% Reticulocyte	2.6±0.6	2.9±0.1

Treatment of wild-type mice with LDN-193189 (3 mg/kg i.p. daily) for 30 days did not impact circulating numbers of erythrocyte, myeloid, lymphocyte, or thrombocyte lineages. Data shown are mean±SEM, with no significant differences detected between vehicle or LDN-193189-injected groups.

Figure S1. Kinetics of IL-6–mediated hepcidin induction in human hepatoma (HepG2) cells, and synergy with BMP signaling
(A) RNA was extracted from HepG2 cells incubated with IL-6 (100 ng/ml) for 0 to 120 min. Hepcidin mRNA levels were measured by qRT-PCR (values shown are mean±SEM, UUn=3, *p<0.05 vs. untreated control). Under some conditions (B)U, treatment of HepG2 cells with BMP6 (5 ng/ml) and IL-6 (25 ng/ml) appeared to induce hepcidin mRNA expression in an additive manner as compared to either treatment alone (n=4 per group, one-way ANOVA p<0.01, *p<0.05 vs. untreated control, $^{\#}$p<0.01 vs cells treated with BMP6 alone), whereas, under other condtions (C), treatment of HepG2 cells with BMP6 (10 ng/ml) and IL-6 (50 ng/ml) appeared to induce hepcidin mRNA expression in a synergistic fashion, as compared to either treatment alone (n=4 per group, one-way ANOVA p<0.01, *p<0.05 vs. untreated control, $^{\#}$p<0.05 vs. cells treated with IL-6 or BMP6 alone).

Figure S2. IL-6 expression in cmlc-IL6 transgenic zebrafish embryos
(A) Double-transgenic fish expressing human IL-6 ectopically in the heart (cmlc–IL-6) were fixed at 48 hpf, and in situ hybridization was performed to detect human IL-6 mRNA. Abundant hIL-6 RNA was detected in the hearts of cmlc–IL-6 zebrafish embryos but not in those of WT embryos. (UB) Hepcidin mRNA levels were greater in adult (10 week old) cmlc–IL-6 zebrafish than in WT zebrafish (n=5 zebrafish per group, *p=0.01).

Figure S3. Serum iron levels in mice 6 days after challenge with turpentine
Mice were pretreated with LDN-193189 (3 mg/kg i.p.) or vehicle and then injected intrascapularly with turpentine (5 ml/kg) or saline. LDN-193189 and drug vehicle injections were continued every 12 h for 6 d, at which time serum iron levels were measured. Turpentine injection induced hypoferremia which was evident at 6 d following a single treatment, while concurrent LDN-193189 treatment prevented the turpentine-induced decrease in serum iron levels (n≥5 mice per group, *p<0.05 vs. untreated control, †p<0.05 vs. turpentine-treated).

Figure S4. The number of hematopoetic stem cells are not affected by LDN-193189 injections for 14 and 28 days
Mice were injected with vehicle or LDN-193189 (3 mg/kg i.p) daily for 14 d (A) or 28 d (B). Numbers of BM cells highly enriched for hematopoietic stem and progenitor cells based on the surface phenotype $Lin^{-}CD48^{-}c\text{-}Kit^{+}\ Sca^{+}\ CD150^{+}$ (BM HSC) were measured as a percentage of total mononuclear cells by flow cytometry. No significant impact on the frequency of this BM HSC population were observed as a result of LDN-193189 treatment at 14 or 28 d (Un=5 mice per group).USimilar results were seen based on the analysis of absolute number of HSCs.

Figure S5. Treatment with LDN-193189 for 28 d decreases baseline hepatic hepcidin mRNA levels
Mice were injected with LDN-193189 (3mg/kg i.p.) or vehicle daily for 28 d. Hepatic hepcidin mRNA levels were less in LDN-193189–treated mice than in vehicle-treated animals (n=10, *p<0.05 vs. vehicle-treated mice).

Figure S6. Treatment of mice with LDN-1931819 does not alter numbers of erythroid, myeloid, or granulocytic progentiors, T cells, B cells, or granulocytes
To evaluate the impact of inhibiting BMP signaling on erythropoiesis, we analyzed the abundance (10^3 cells/µl) of several erythroid progenitor cell populations, including the MEPs, the CMPs from which they are derived, as well as GMPs in the BM of mice treated with LDN-193189 (3 mg/kg i.p. daily) or vehicle for 21 d. BMP inhibition did not alter numbers of (A) CMPs, (B) GMPs, or (C) MEPs. Furthermore, LDN-193189 did not alter numbers of $CD3^+$ T cells (D), $B220^+$ B cells, (E) or Mac-1^+ Gr-1^+ granulocytes (F).

Figure S7. BM progenitor cells derived from LDN-193189 treated mice retain normal engraftment capacity following transplantation
Host $CD45.2^+$ C57BL/6 mice were lethally irradiated and injected with a 1:1 mixture of mononuclear bone marrow cells isolated from $CD45.1^+$ mice and from $CD45.2^+$ mice that had received LDN-193189 (3 mg/kg i.p. daily) or vehicle for 14 d. The relative contribution to engraftment from different input populations was assessed by flow cytometry analysis of $CD45.1^+$ and $CD45.2^+$ mononuclear bone marrow cell populations. LDN-193189 treatment of $CD45.2^+$ donors did not influence the functionality of their hematopoietic progenitor cells based upon engraftment efficiency. Results are expressed as percentage of BM cells that were $CD45.2^+$ (U n=5 mice per group).

Figure S1

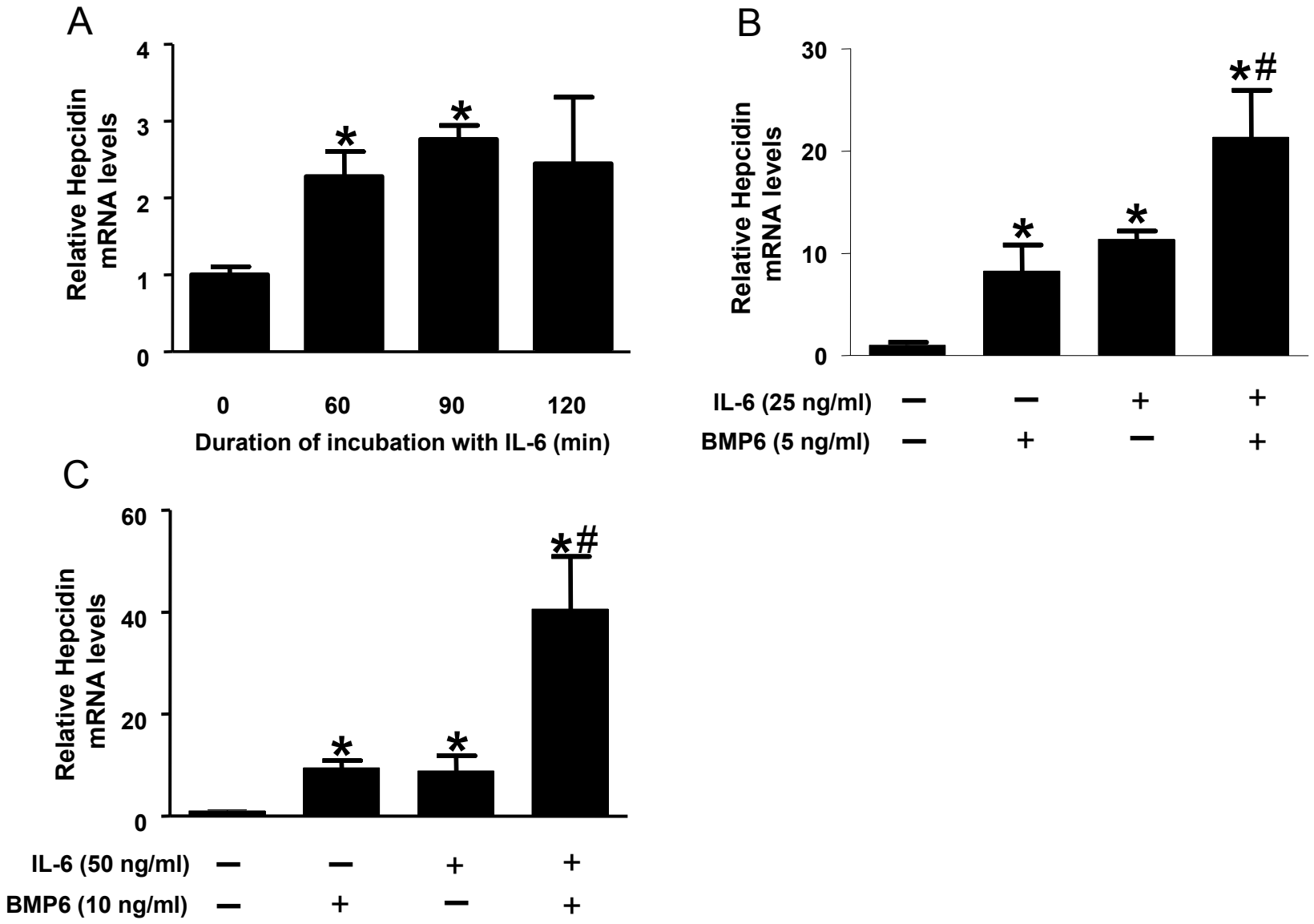
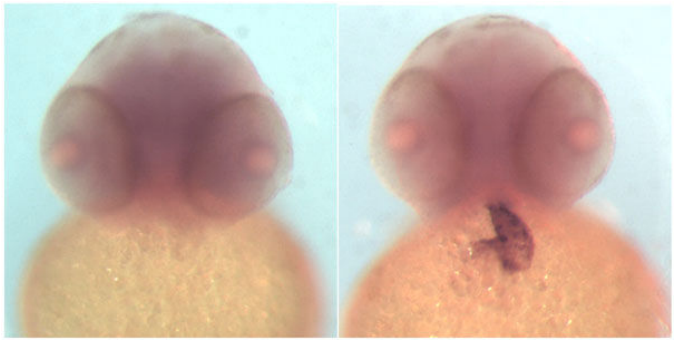

A
Relative Hepcidin mRNA levels
4
3
2
1
0
*
*
0
60
90
120
Duration of incubation with IL-6 (min)
B
Relative Hepcidin mRNA levels
30
20
10
0
*
*
*#
IL-6 (25 ng/ml) — — + +
BMP6 (5 ng/ml) — + — +
C
Relative Hepcidin mRNA levels
60
40
20
0
*
*
*#
IL-6 (50 ng/ml) — — + +
BMP6 (10 ng/ml) — + — +

Figure S2

A


WT
cmlc-IL-6

B

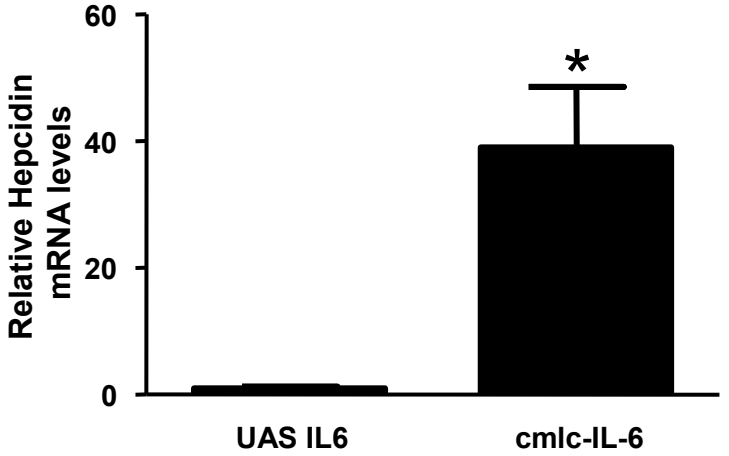

Relative Hepcidin mRNA levels
60
40
20
0
*
UAS IL6
cmlc-IL-6

Figure S3

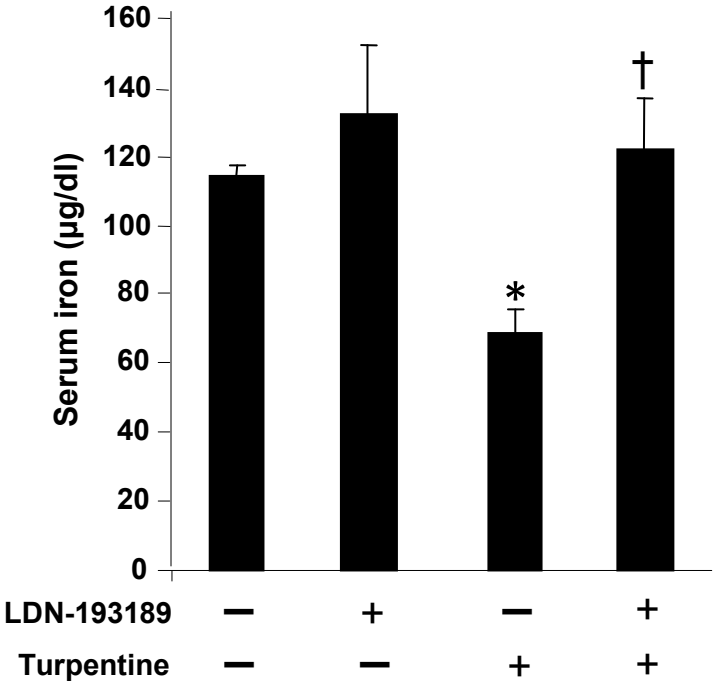

160
140
120
100
80
60
40
20
0
Serum iron (µg/dl)
*
†
LDN-193189
Turpentine
−
+
−
+
−
−
+
+

Figure S4

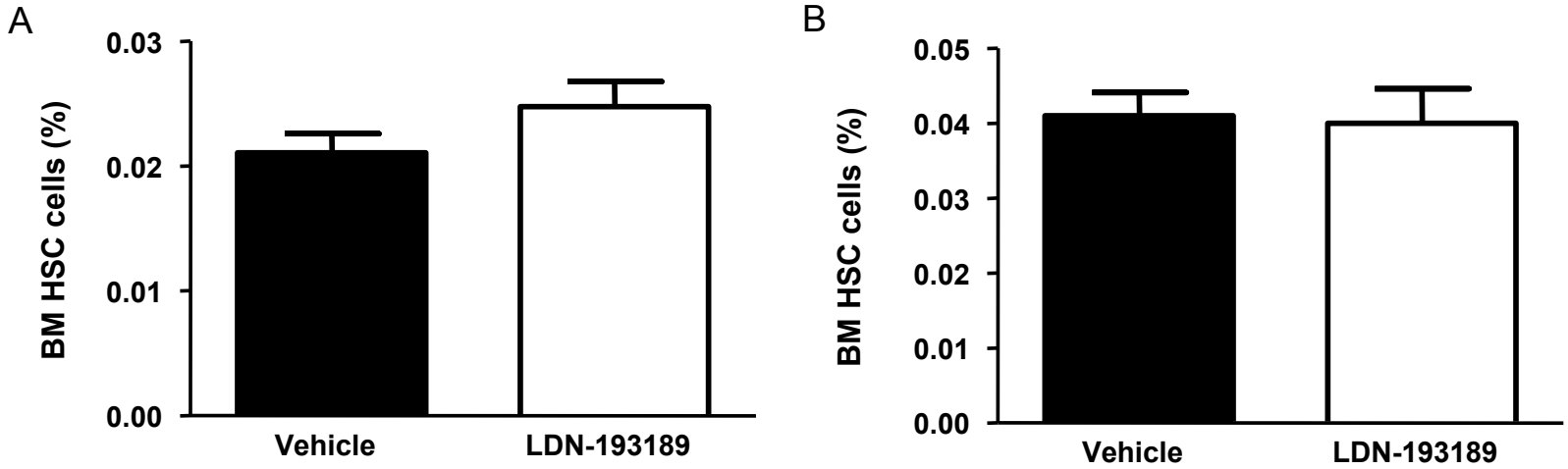

A
BM HSC cells (%)
0.03
0.02
0.01
0.00
Vehicle
LDN-193189
B
BM HSC cells (%)
0.05
0.04
0.03
0.02
0.01
0.00
Vehicle
LDN-193189

Figure S5

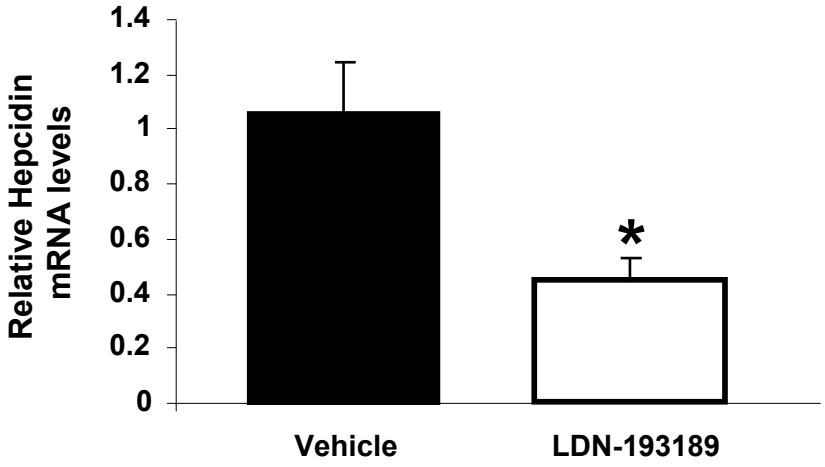

Relative Hepcidin mRNA levels
1.4
1.2
1
0.8
0.6
0.4
0.2
0
*
Vehicle
LDN-193189

Figure S6

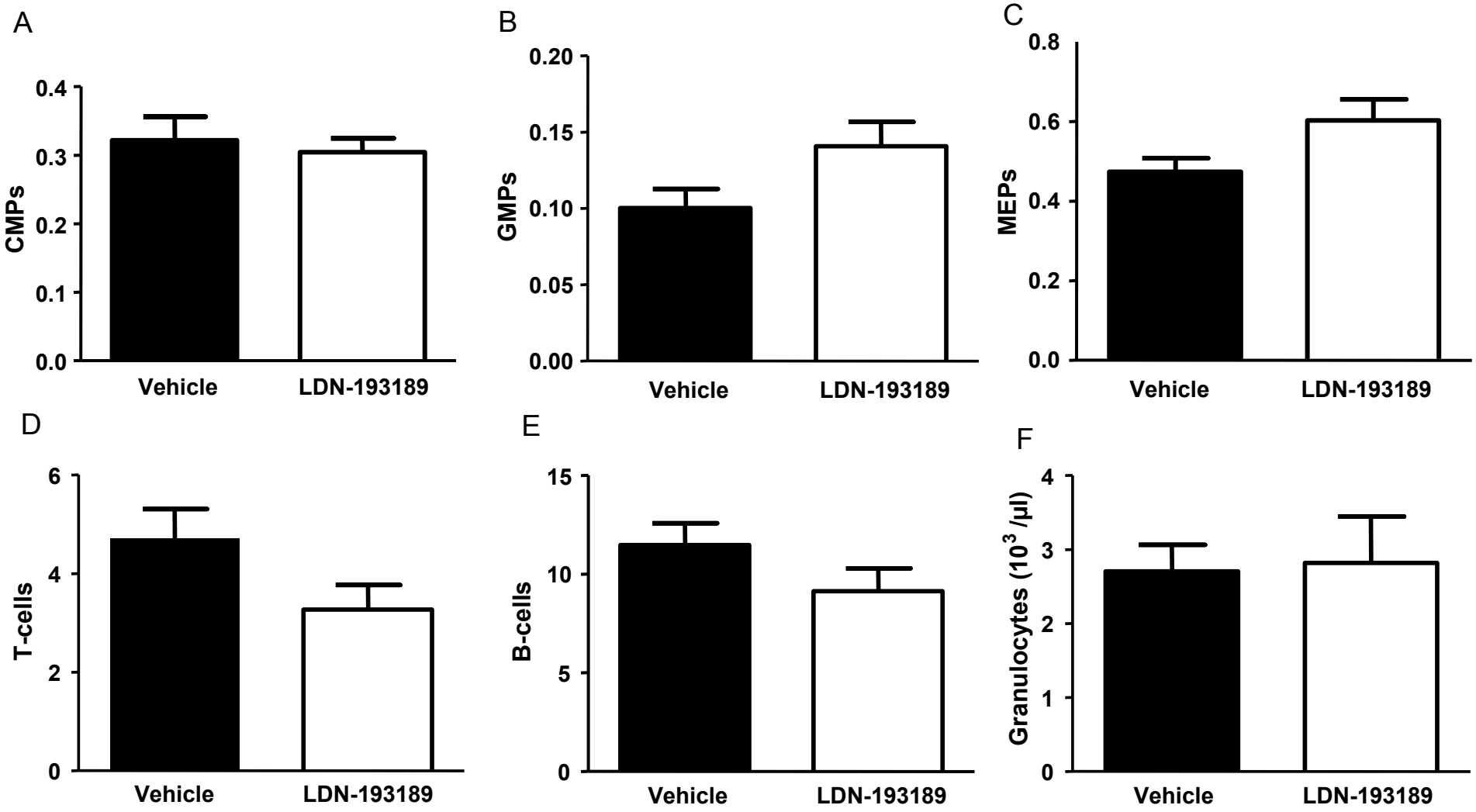

A
CMPs
0.4
0.3
0.2
0.1
0.0
Vehicle
LDN-193189
B
GMPs
0.20
0.15
0.10
0.05
0.00
Vehicle
LDN-193189
C
MEPs
0.8
0.6
0.4
0.2
0.0
Vehicle
LDN-193189
D
T-cells
6
4
2
0
Vehicle
LDN-193189
E
B-cells
15
10
5
0
Vehicle
LDN-193189
F
Granulocytes (10^3 /µl)
4
3
2
1
0
Vehicle
LDN-193189

Figure S7