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Supplemental Information

Circadian Expression of Migratory Factors Establishes Lineage-Specific Signatures that Guide the Homing of Leukocyte Subsets to Tissues

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Figure S1

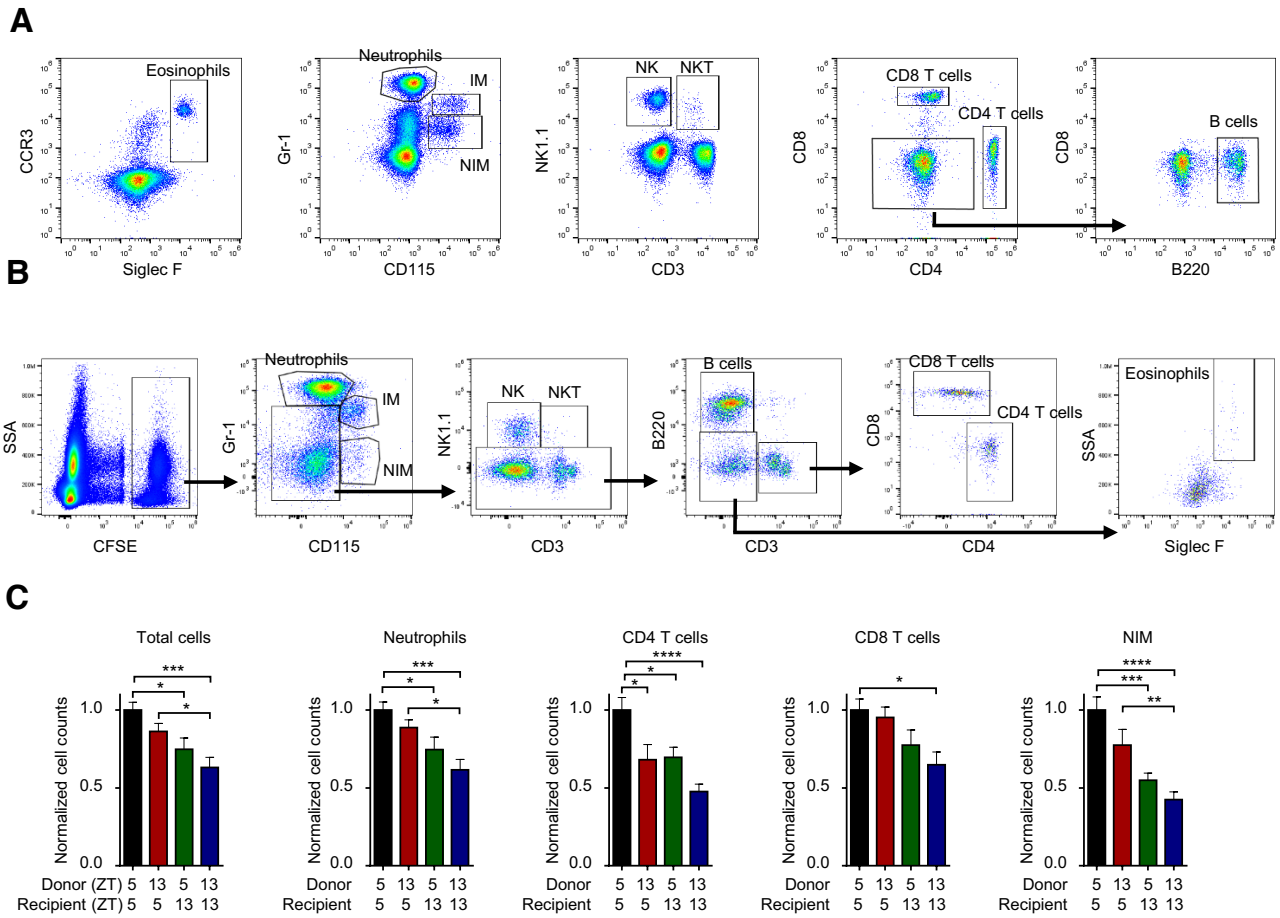

Figure S1 (Related to Figure 1) *Gating strategies and reciprocal homing assays*

(A) Gating strategy of blood leukocyte subsets for Figure 1A. **(B)** Gating strategy for identifying adoptively transferred $CFSE^{+}$ leukocyte subsets in recipient blood. **(C)** Reciprocal 'negative' homing assays with ZT5 and ZT13 donor cells labeled differently and co-injected into ZT5 and ZT13 recipients; n = 21-24 mice, one-way ANOVA followed by Tukey's multiple comparison test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Figure S2

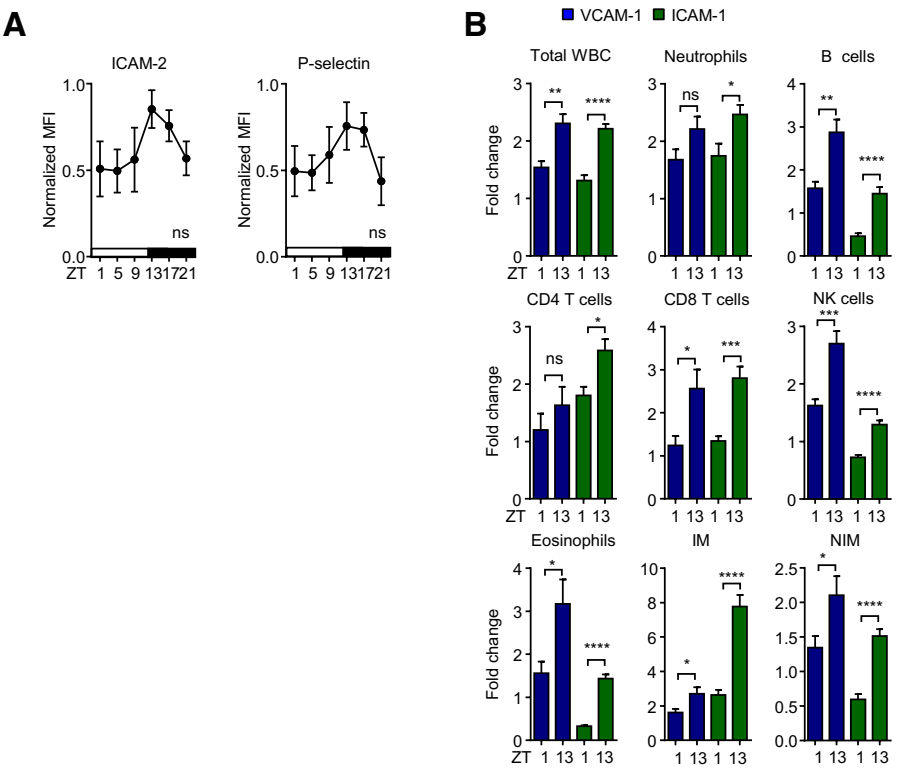

Figure S2 (Related to Figure 2) ***Oscillations in endothelial cell adhesion molecules and associated functions***

(A) Integration of ICAM-2 and P-selectin expression over all organs across the day; n = 3-6 mice with 6 time points measured each, one-way ANOVA. **(B)** Fold change of endogenous leukocyte numbers after treatment with antibodies directed against VCAM-1 or ICAM-1 compared to ZT1 and ZT13 isotype-treated control groups; n = 7-11 mice, unpaired Student's t-test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. ns, not significant.

Figure S3

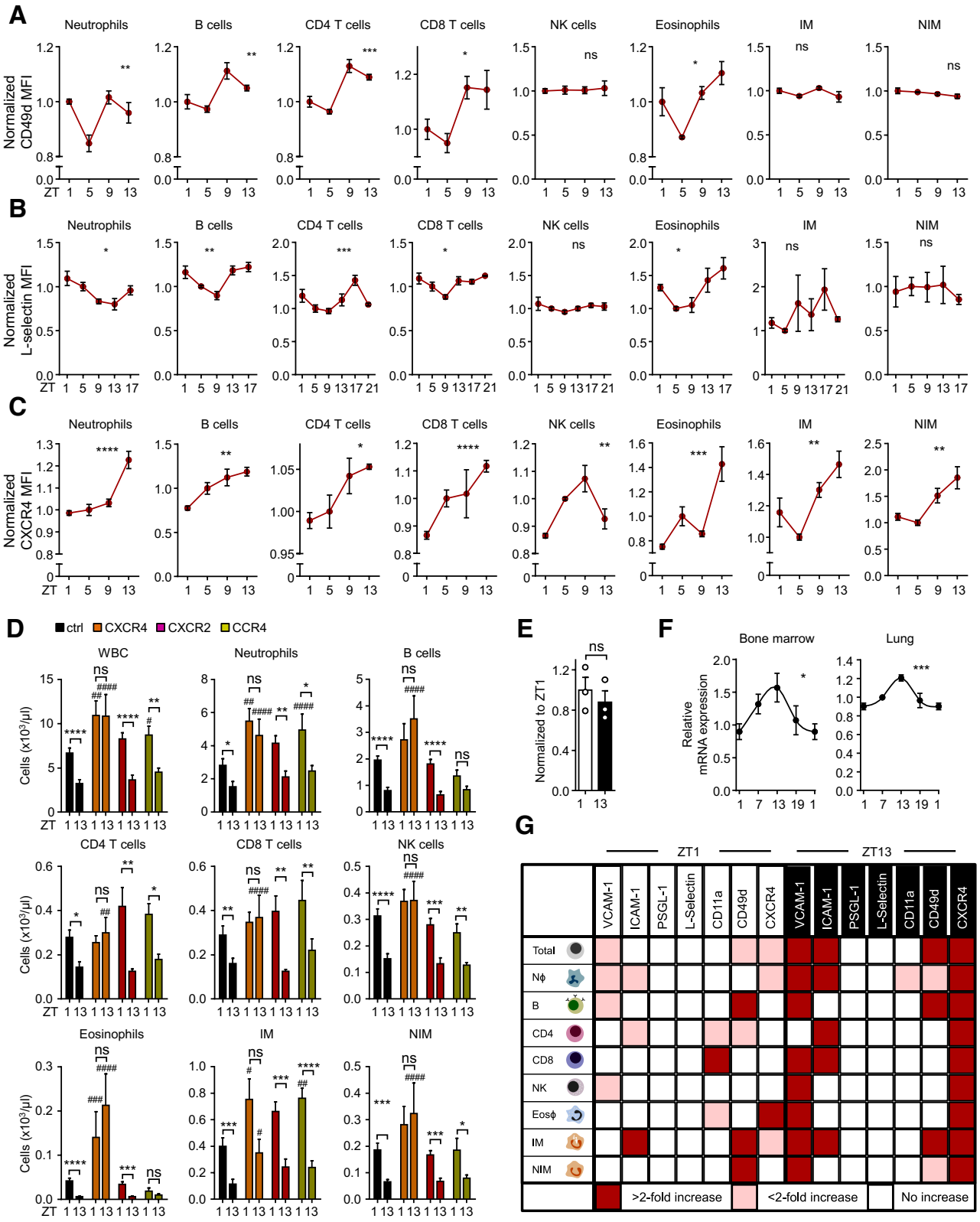
A
Neutrophils
B cells
CD4 T cells
CD8 T cells
NK cells
Eosinophils
IM
NIM
Normalized CD49d MFI
B
Normalized L-selectin MFI
C
Normalized CXCR4 MFI
ZT 1 5 9 13
ZT 1 5 9 13 17
ZT 1 5 9 13 17 21
D
ctrl
CXCR4
CXCR2
CCR4
WBC
Neutrophils
B cells
CD4 T cells
CD8 T cells
NK cells
Eosinophils
IM
NIM
Cells (x10³/μl)
E
Normalized to ZT1
F
Bone marrow
Lung
Relative mRNA expression
G
ZT1
ZT13
VCAM-1
ICAM-1
PSGL-1
L-Selectin
CD11a
CD49d
CXCR4
Total
Nϕ
B
CD4
CD8
NK
Eosϕ
IM
NIM
>2-fold increase
<2-fold increase
No increase

Figure S3 (Related to Figure 3) ***Oscillations in leukocyte pro-migratory factors and associated functions***

(A-C) Expression levels of CD49d **(A)**, L-selectin **(B)** and CXCR4 **(C)** on leukocyte subsets over 24h; n = 3-6 mice with 4-6 time points measured each, one-way ANOVA. **(D)** Endogenous blood leukocyte numbers after treatment with chemokine receptor antagonists; n = 5-12 mice, one-way ANOVA followed by Dunnett comparison to control groups. **(E)** Adoptive transfer of donor cells treated *ex vivo* prior to transfer to recipients with antagonists against CXCR4 at ZT1 and ZT13. Data are normalized to ZT1 levels; n = 3 mice, unpaired Student's t-test. **(F)** *Cxcl12* mRNA levels in bone marrow and lung, ZT1 is double plotted to facilitate viewing; n = 4-5 mice, one-way ANOVA. **(G)** Overview of functional blocking effects on endogenous leukocyte subsets in blood targeting the indicated molecules at ZT1 and ZT13; n = 4-12 mice, one-way ANOVA followed by Dunnett comparison to control groups. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001; $^{\#, \#\#, \#\#\#, \#\#\#\#}$ indicate analogous significance levels to controls. ns, not significant.

Figure S4

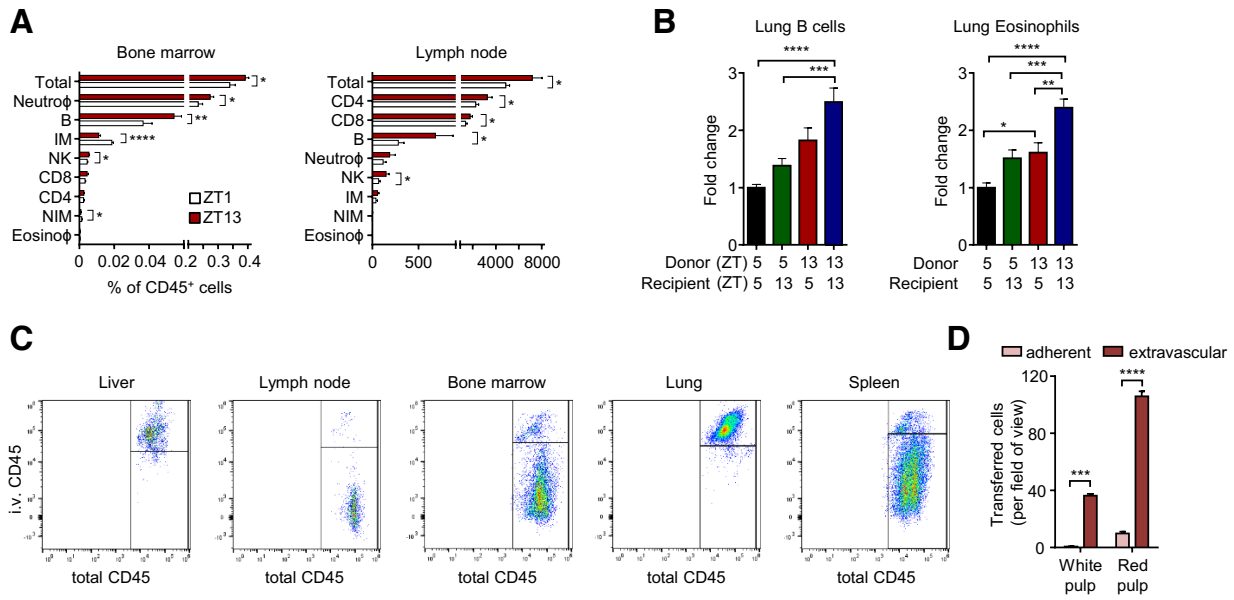

Figure S4 (Related to Figure 4) ***Diurnal homing capacity of leukocyte subsets and localization in specific organs***

(A) Recruitment of donor cells into bone marrow and lymph nodes with ZT1 and ZT13 donor cells and recipients; bone marrow, n = 24-25 mice; lymph node, n = 11-14 mice, unpaired Student's t-test. **(B)** Reciprocal homing assays with ZT5 and ZT13 donor cells labeled differently and co-injected into ZT5 and ZT13 recipients; n = 10 mice, one-way ANOVA followed by Tukey's multiple comparison test. **(C)** Flow cytometry plots of adoptively transferred cells located inside or outside the vasculature in specific organs based on staining with an i.v.-injected anti-CD45 antibody. **(D)** Quantification of numbers and localization of adoptively transferred cells in splenic white pulp and red pulp; n = 2-3 mice, unpaired Student's t-test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Figure S5

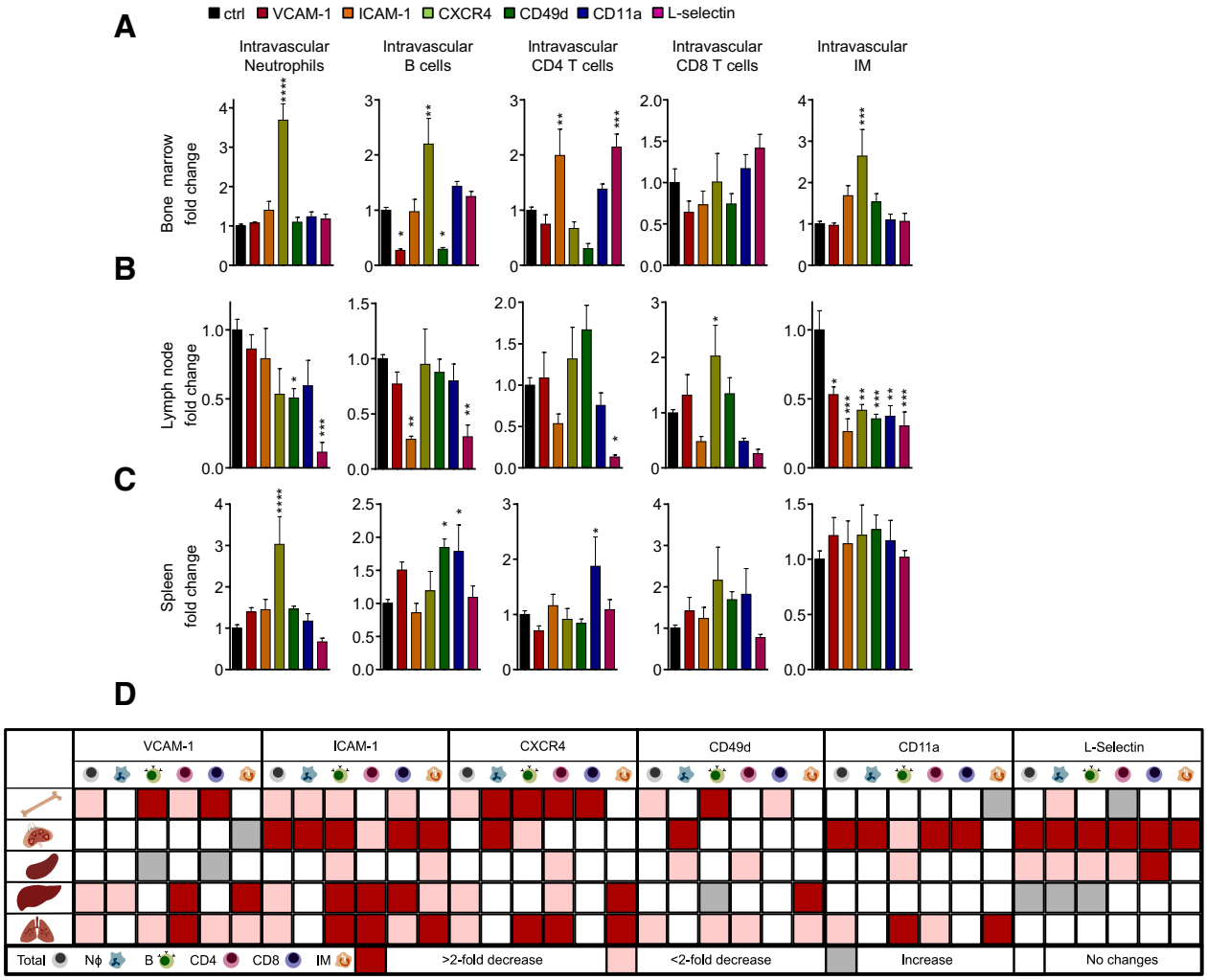

Figure S5 (Related to Figure 5) ***Chronopharmacological targeting of leukocyte homing to tissues***

(A-C) Numbers of intravascular donor cells after adoptive transfer and treatment with antibodies or antagonists directed against the indicated molecules, n = 4-8 mice, one-way ANOVA followed by Dunnett comparison to the control group. **(D)** Overview of functional blocking effects on leukocyte recruitment to organs targeting the indicated molecules, n = 4-8 mice, one-way ANOVA followed by Dunnett comparison to the control group. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Figure S6

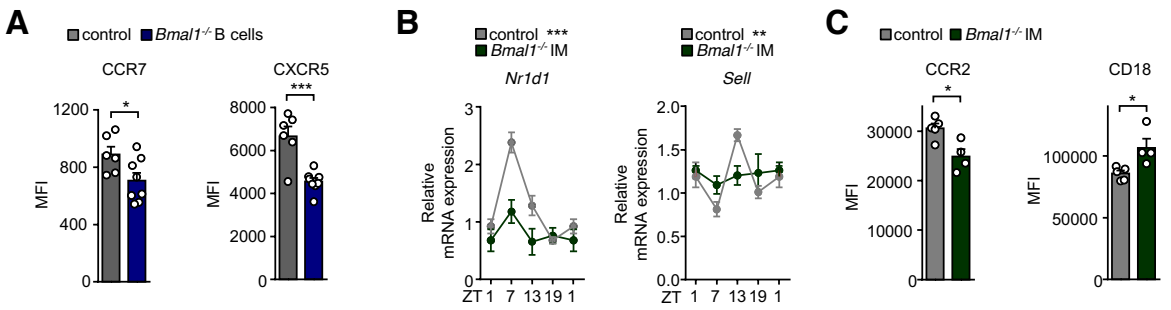

Figure S6 (Related to Figure 6) ***Expression of leukocyte adhesion molecules in lineage-specific Bmal1$^{-/-}$ leukocytes***

(A) Expression levels of CCR7 and CXCR5 at ZT13 in control and *Bmal1*-deficient B cells in blood; n = 6-8 mice, unpaired Student's t-test. **(B)** Q-PCR analyses of *Nr1d1* and *Sell* mRNA levels in isolated control and *Bmal1*-deficient monocytes; n = 3 mice, one-way ANOVA. **(C)** Expression levels of CCR2 and CD18 at ZT13 in control and *Bmal1*-deficient monocytes in blood; n = 4-5 mice, unpaired Student's t-test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Figure S7

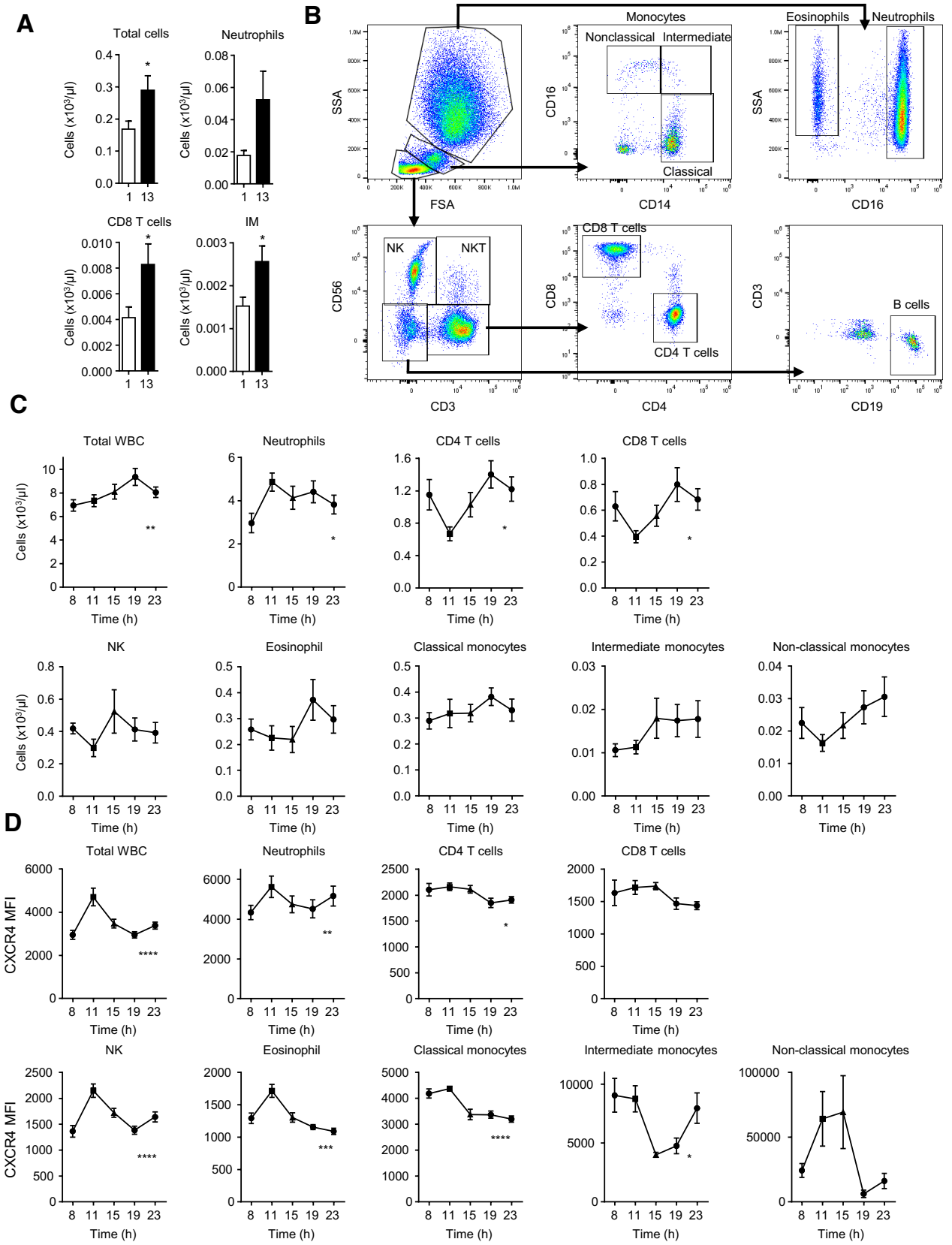
A
Total cells
Neutrophils
CD8 T cells
IM
Cells (x10³/µl)
1 13
B
Monocytes
Nonclassical Intermediate
Classical
Eosinophils
Neutrophils
SSA
FSA
CD16
CD14
NK
NKT
CD56
CD3
CD8 T cells
CD8
CD4
CD4 T cells
B cells
CD19
C
Total WBC
Neutrophils
CD4 T cells
CD8 T cells
NK
Eosinophil
Classical monocytes
Intermediate monocytes
Non-classical monocytes
Time (h)
D
CXCR4 MFI

Figure S7 (Related to Figure 7) ***Oscillations in inflammation and human leukocytes in blood***

(A) Numbers of total leukocytes and subsets in the peritoneal cavity after i.p. LPS challenge at ZT1 and ZT13; n = 12-14 mice, unpaired Student's t-test. **(B)** Gating strategy of human blood leukocyte subsets. **(C)** Numbers of human blood leukocyte subsets over 24h; n = 8 subjects, repeated measures one-way ANOVA. **(D)** Expression of CXCR4 on human leukocytes over 24h; n = 8 subjects, repeated measures one-way ANOVA. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.