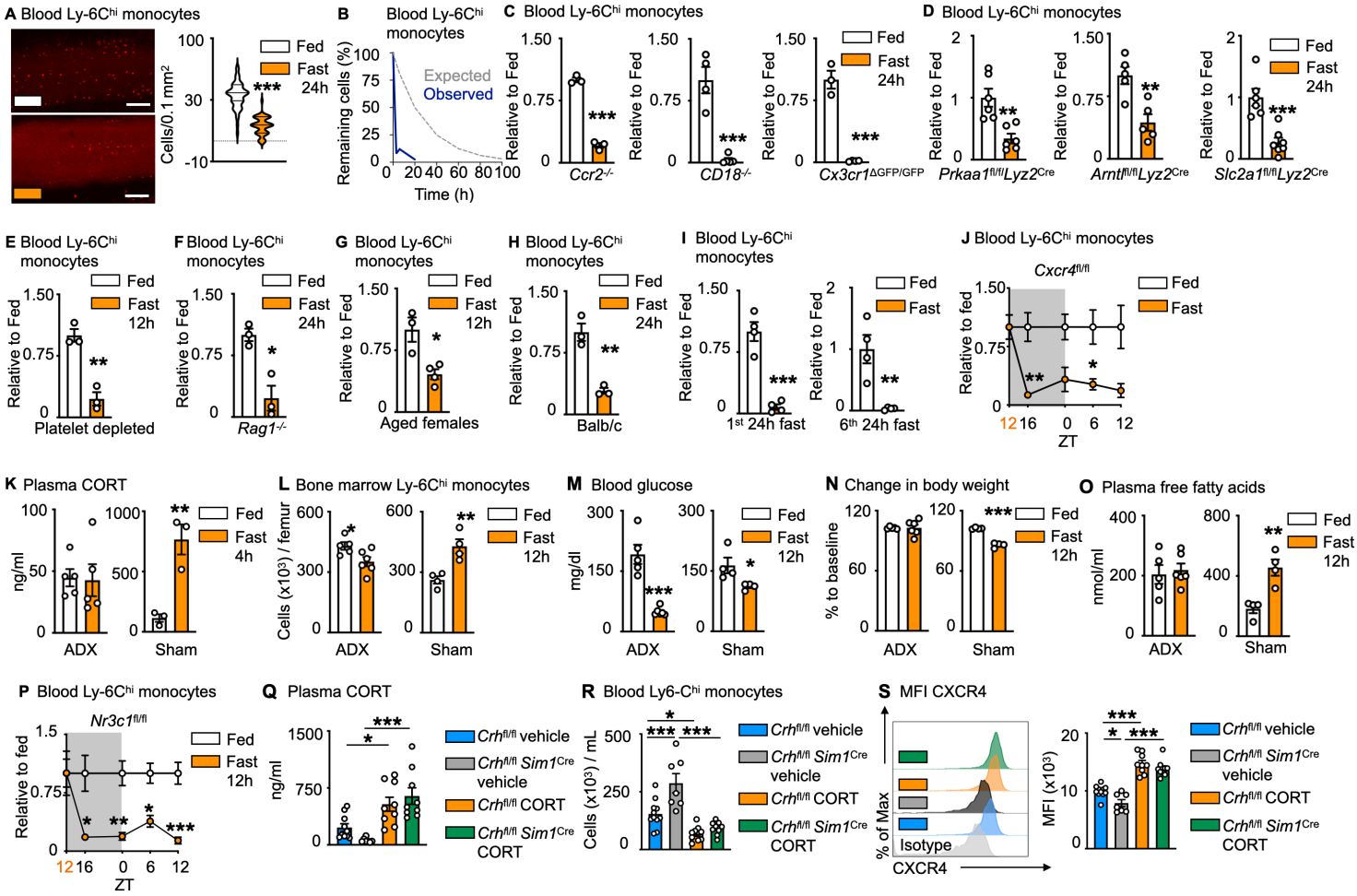

Figure S1: Fasting returns Ly-6C^{hi} monocytes back to the bone marrow and is dependent on corticosterone (related to Figure 2)

A. Intravital microscopy of blood Ly-6C^{hi} monocytes in femoral vessel. Image show representative z-projections of 20x z-stacks collected at 5 μm interval (n=50 images per mouse). Refer to supplemental movie fed and fast. Scale bar: 100 μm. (n=1 per group). Kolmogorov-Smirnov test.

B. Expected: Under the assumptions of a fasting mitigated shutdown of bone marrow Ly-6C^{hi} monocyte egress and a typical half-life of circulating Ly-6C^{hi} monocytes of approximately 20 h, the expected reduction of blood monocytes after a 20h fast, in the absence of any additional mechanisms, would be ~50%. Observed: After a 4h fast, initiated at zeitgeber 12, the reduction of circulating Ly-6C^{hi} monocytes from the blood was ~90%.

C. Blood Ly-6C^{hi} monocytes after fasting normalized to fed control in *Ccr2*$^{-/-}$, *CD18*$^{-/-}$ and *Cx3cr1*$^{\Delta GFP/GFP}$ mice (n=3-5 per group). Unpaired t test.

D. Blood Ly-6C^{hi} monocytes after fasting normalized to fed control in myeloid conditional deletion of *Prkaa1* (AMPK), *Arntl* (Bmal), and *Slc2a1* (GLUT-1) (n=5-7 per group).Unpaired t test.

E. Blood Ly-6C^{hi} monocytes after platelet depletion and fasting normalized to fed control (n=3 per group). Unpaired t test.

F. Blood Ly-6C^{hi} monocytes after fasting normalized to fed control in *Rag1*$^{-/-}$ mice (n=3 per group). Unpaired t test.

G. Blood Ly-6C^{hi} monocytes after fasting normalized to fed control in one year old female mice (n=3-4 per group). Unpaired t test.

H. Blood Ly-6C^{hi} monocytes after fasting normalized to fed control in Balb/c mice (n=3 per group). Unpaired t test.

I. Mice underwent 24h fasting periods every other day and were bled on fasting days. Blood Ly-6C^{hi} monocytes after fasting normalized to fed control after first fasting and after 6th fasting period (n=4 per group). Unpaired t test.

J. Relative circulating Ly-6C^{hi} monocytes in *Cxcr4*$^{fl/fl}$ under fed and fasted conditions normalized to fed mice for each timepoint (n=4-9, timepoints are independent experiments). Start of the fast is indicated by orange zeitgeber (ZT). Two-way ANOVA.

K. Plasma corticosterone (CORT) concentration in mice after bilateral adrenalectomy (ADX) or sham submitted to feeding ad libitum or 4h fasting (n=3-5 per group). Unpaired t test.

L. Bone marrow Ly-6C^{hi} monocytes in mice after bilateral ADX or sham submitted to feeding ad libitum or 12h fasting (n=4-6 per group). Unpaired t test.

M. Blood glucose in mice after bilateral ADX or sham submitted to feeding ad libitum or 12h fasting. (n=4-6 per group). Unpaired t test.

N. Relative change of body weight from pre-fasting measurement in mice after bilateral ADX or sham submitted to feeding ad libitum or 12h fasting (n=4-6 per group). Unpaired t test.

O. Plasma free fatty acid concentration in mice after bilateral ADX or sham submitted to feeding ad libitum or 12h fasting. (n=4-6 per group). Unpaired t test.

P. Relative circulating Ly-6C^{hi} monocytes in *Nr3c1*$^{fl/fl}$ under fed and fasted conditions normalized to fed mice for each timepoint (n=4-11), timepoints are independent experiments). Start of the fast is indicated by orange ZT. Two-way ANOVA.

Q. Plasma CORT in *Crh*$^{fl/fl}$ and *Crh*$^{fl/fl}$ *Sim1*^{Cre} 1h after vehicle or CORT injection i.p. (n=7-11 per group). One-way ANOVA.

R. Blood Ly-6C^{hi} monocytes in *Crh*$^{fl/fl}$ and *Crh*$^{fl/fl}$ *Sim1*^{Cre} 1h after vehicle or CORT injection i.p. (n=7-11 per group). One-way ANOVA.

S. Median Fluorescence Index of CXCR4 on Ly-6C^{hi} monocytes in *Crh*$^{fl/fl}$ and *Crh*$^{fl/fl}$ *Sim1*^{Cre} 1h after vehicle or CORT injection i.p (n=7-11 per group). One-way ANOVA.

Data presented as mean ± SEM, *p < 0.05, **p<0.01, ***p < 0.001. ZT: Zeitgeber, ADX: Adrenalectomy, CORT: Corticosterone, MFI: Median Fluorescence Index.

T.

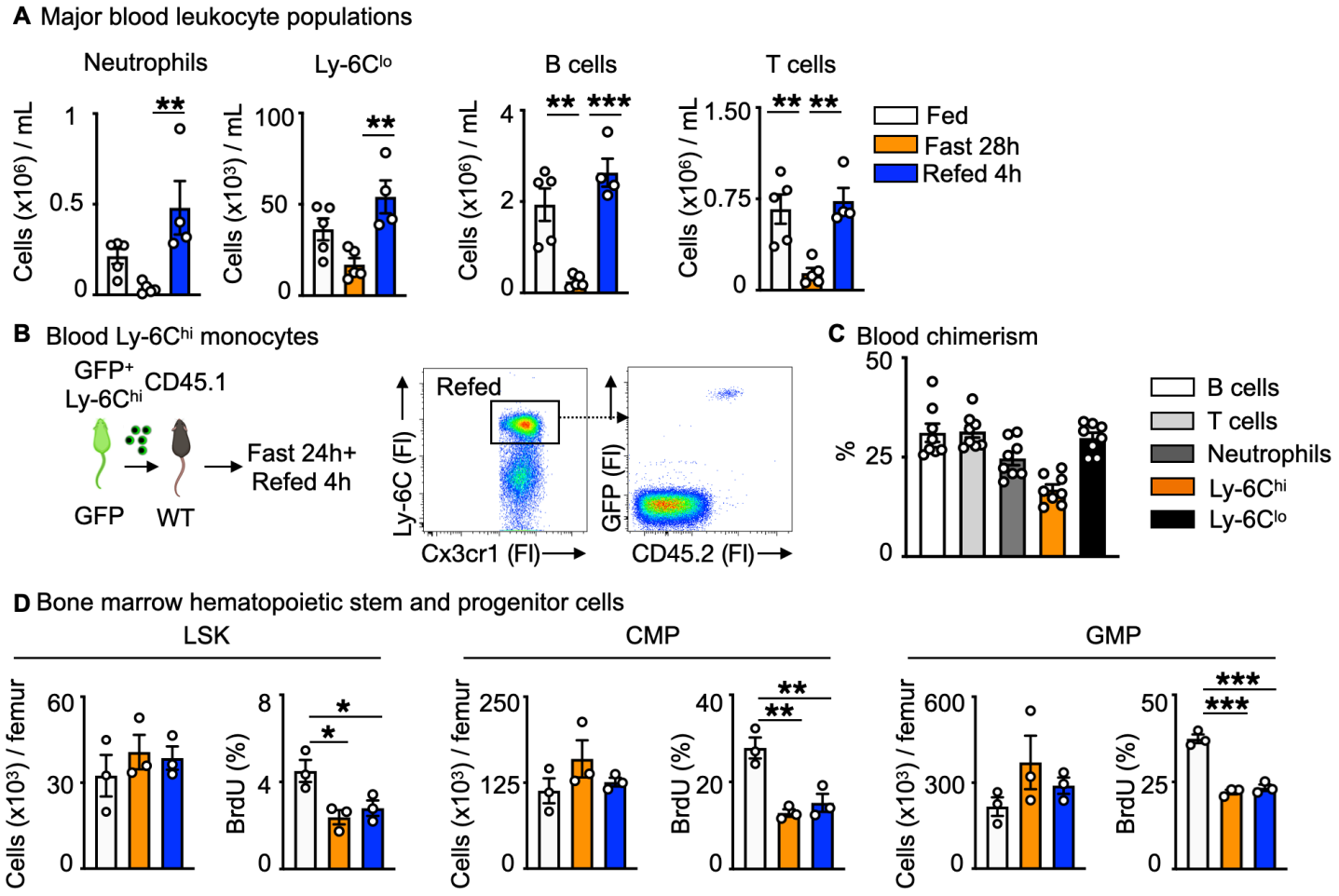

Figure S2: Re-feeding leads to a surge of monocytes and fasting+re-feeding reduces hematopoiesis (related to Figure 3)

A. Major leukocyte populations in the blood after feeding ad libitum, 24h fasting or 24h of fasting and 4h of re-feeding (n=4-5 per group). One-way ANOVA.

B. Adoptive transfer of GFP-positive Ly-6C^{hi} monocytes into CD45.1 mouse, which was then subjected to fasting and re-feeding.

C. Blood chimerism after two weeks of parabiosis between GFP-positive mouse and wild type mouse before separation (n=4 per group).

D. Absolute count and relative BrdU incorporation in bone marrow progenitors after feeding ad libitum, 24h fasting or 24h of fasting and 4h of re-feeding (n=3 per group). One-way ANOVA. Data presented as mean ± SEM, *p < 0.05, **p<0.01, ***p < 0.001. FI: Fluorescence index

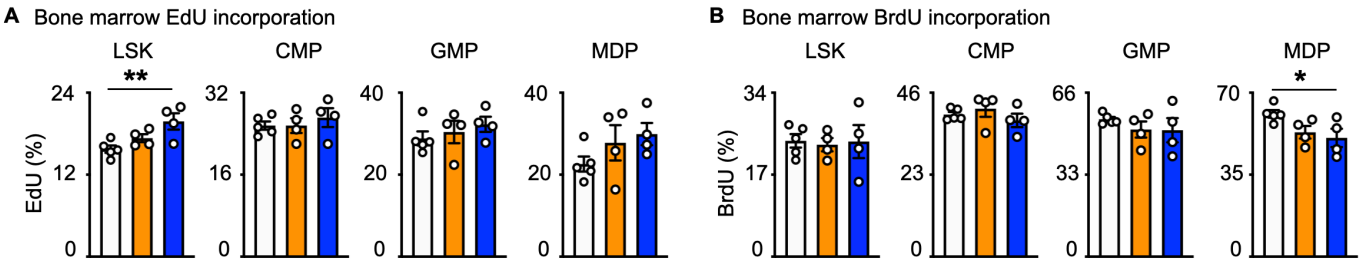

Figure S3: EdU and BrdU is incorporated into progenitors in the bone marrow (related to Figure 4)

A. EdU incorporation in hematopoietic stem and progenitor cells in the bone marrow under feeding ad libitum, 24h fasting or 24 fasting and 4h re-feeding (n=4-5 per group). One-way ANOVA.

B. BrdU incorporation in hematopoietic stem and progenitor cells in the bone marrow under feeding ad libitum, 24h fasting or 24 fasting and 4h re-feeding (n=4-5 per group). One-way ANOVA. Data presented as mean ± SEM, *$p < 0.05$, **$p<0.01$, ***$p < 0.001$.

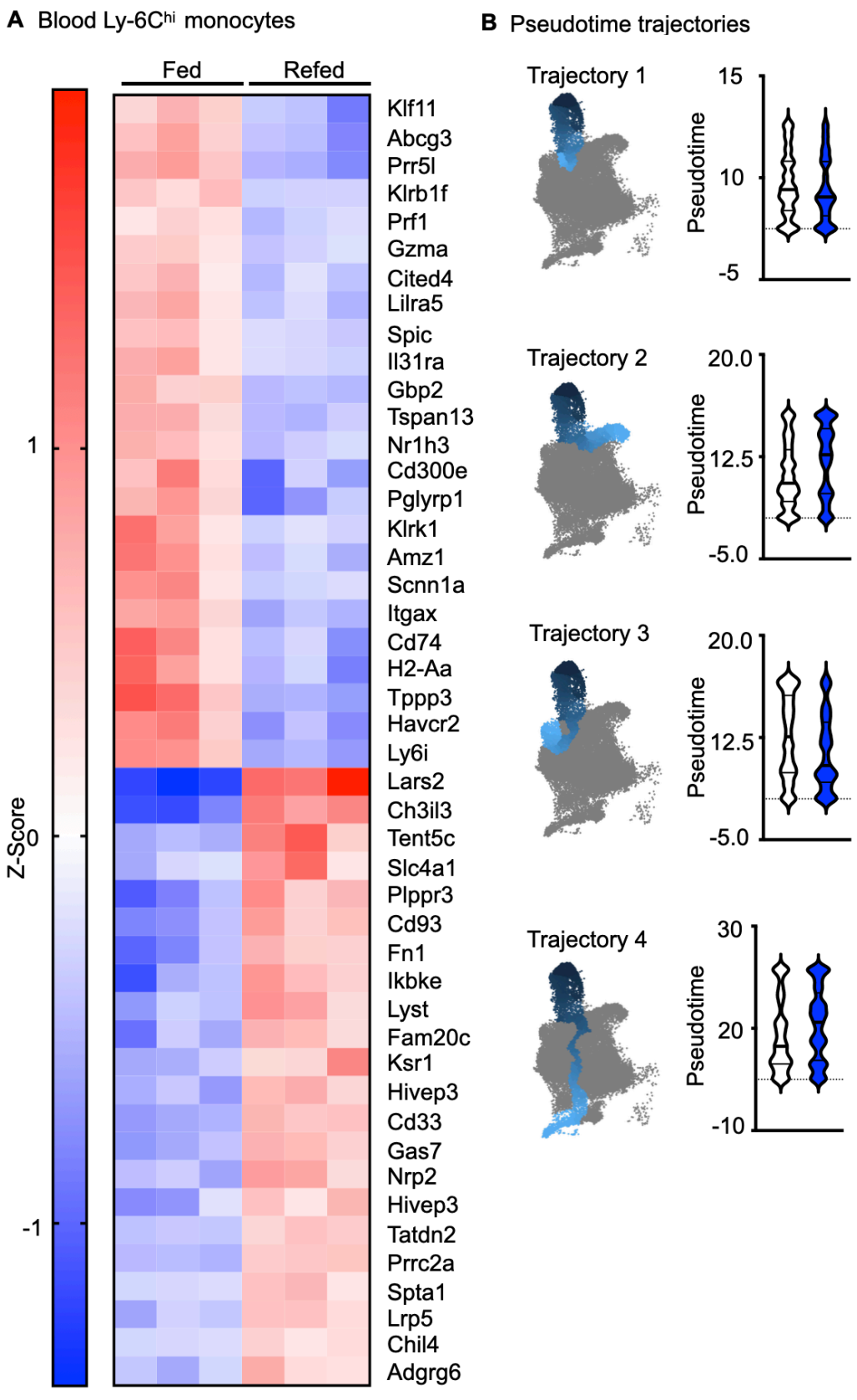

Figure S4: Heatmap and pseudo time trajectories after feeding or fasting+re-feeding of sorted monocytes (related to Figure 5)

A. Heat map of differential expressed genes (FC>1.0, FDR<0.05, p<0.05) in sorted blood Ly-6C^{hi} monocytes after feeding ad libitum and fasting+refeeding (n=3 samples per group, 4 mice pooled per sample).

B. Pseudotime of trajectories 1 to 4 in mice under feeding ad libitum or fasting+re-feeding(n=1 sample per group, 5 mice pooled per sample).

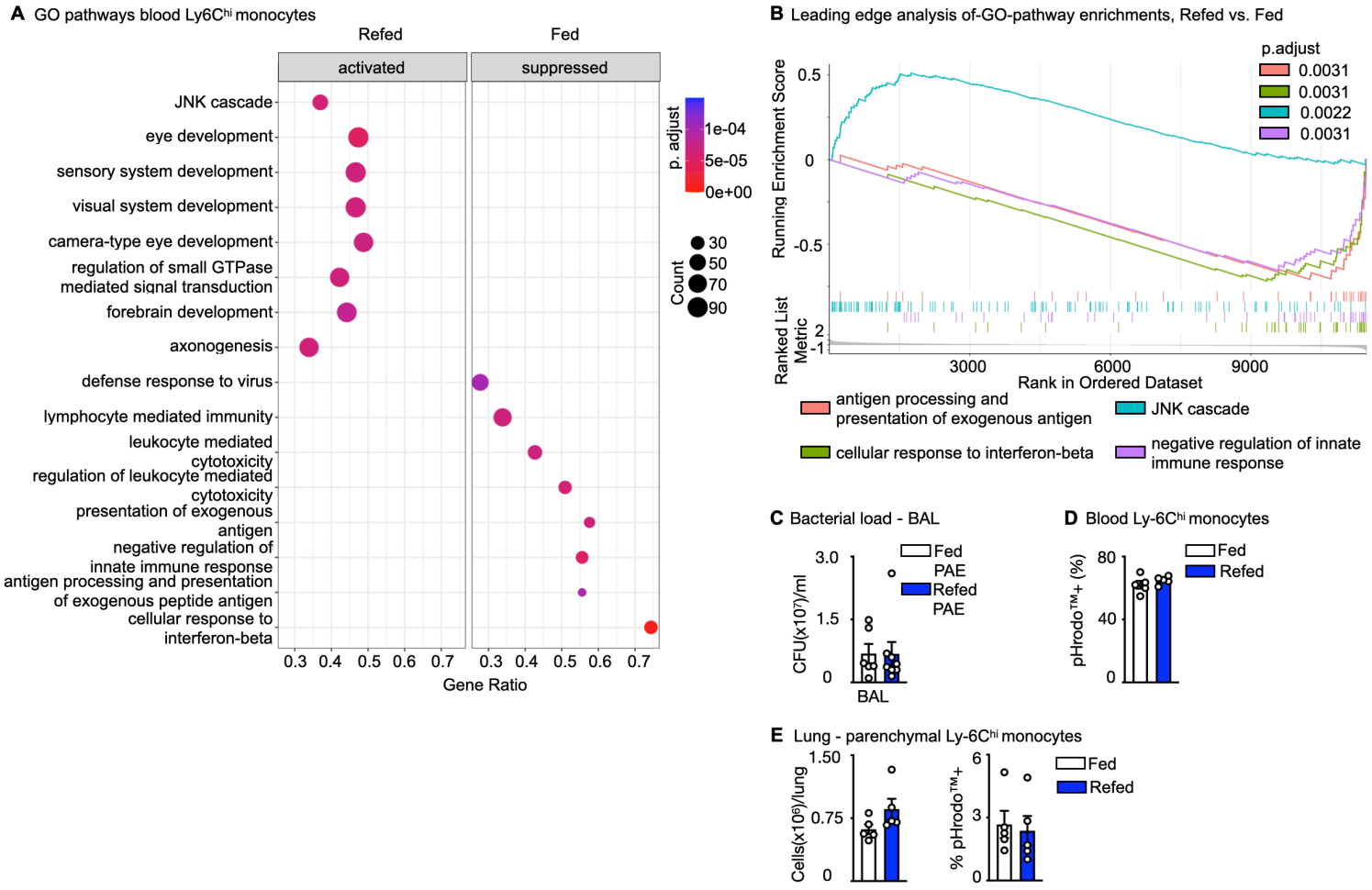

Figure S5: Fasting+re-feeding alters the host repose to infection (related to Figure 6)

A. Gene ontology (GO) pathways. Genes from selected GO-pathways are depicted in Fig. S6. Activated: Enriched in Ly-6C^{hi} monocytes sorted from mice after fasting+re-feeding, suppressed in Ly-6C^{hi} monocytes sorted from mice after feeding ad libitum. Suppressed: Suppressed in Ly-6C^{hi} monocytes sorted from mice after fasting+re-feeding, enriched in Ly-6C^{hi} monocytes sorted from mice after feeding ad libitum. Size of dot shows number of genes belonging to the gene set enriched in the leading edge. X-axis shows gene ratio of enriched genes in the total gene pool of the geneset. Color of the dot shows adjusted p-value.

B. Leading edge analysis of GO-pathway enrichments (GSEA-plot). Ranked list metric: logFC values of genes ordered by logFC; genes enriched in Ly-6C^{hi} monocytes sorted from mice after fasting+re-feeding are on the left, genes enriched in Ly-6C^{hi} monocytes sorted from mice after feeding ad libitum on the right. Colored vertical lines indicate position of genes belonging to the gene set of interest within the ranking. Running enrichment score: Distribution of enrichment score for the gene sets of interest.

C. Bacterial load after infection with Pseduomona aeruginosa (PAE) from bronchoalveolar lavage (BAL) comparing fed and fasted+re-fed mice (n=6-8 per group). Unpaired t test.

D. Whole blood from fed ad libitum and fasted+re-fed mice was incubated with pHrodo™-labelled e.coli particles for 30 min at 37°C and Ly-6C^{hi} monocytes positivity for pHrodo™ assessed by flow cytometry (n=5 per group). Unpaired t test.

E. Fed ad libitum mice and mice after 24h fast followed by 4h of re-feeding were intranasally injected with pHrodo™-labelled PAE particles and sacrificed 4h thereafter. Parenchymal Ly-6C^{hi} monocytes were enumerated and pHrodo™-positivity assessed (n=5 per group). Unpaired t test. Data presented as mean ± SEM, *p < 0.05, **p<0.01, ***p < 0.001. BAL: Broncholaveolar lavage, PAE: Pseudomonas aeruginosa.

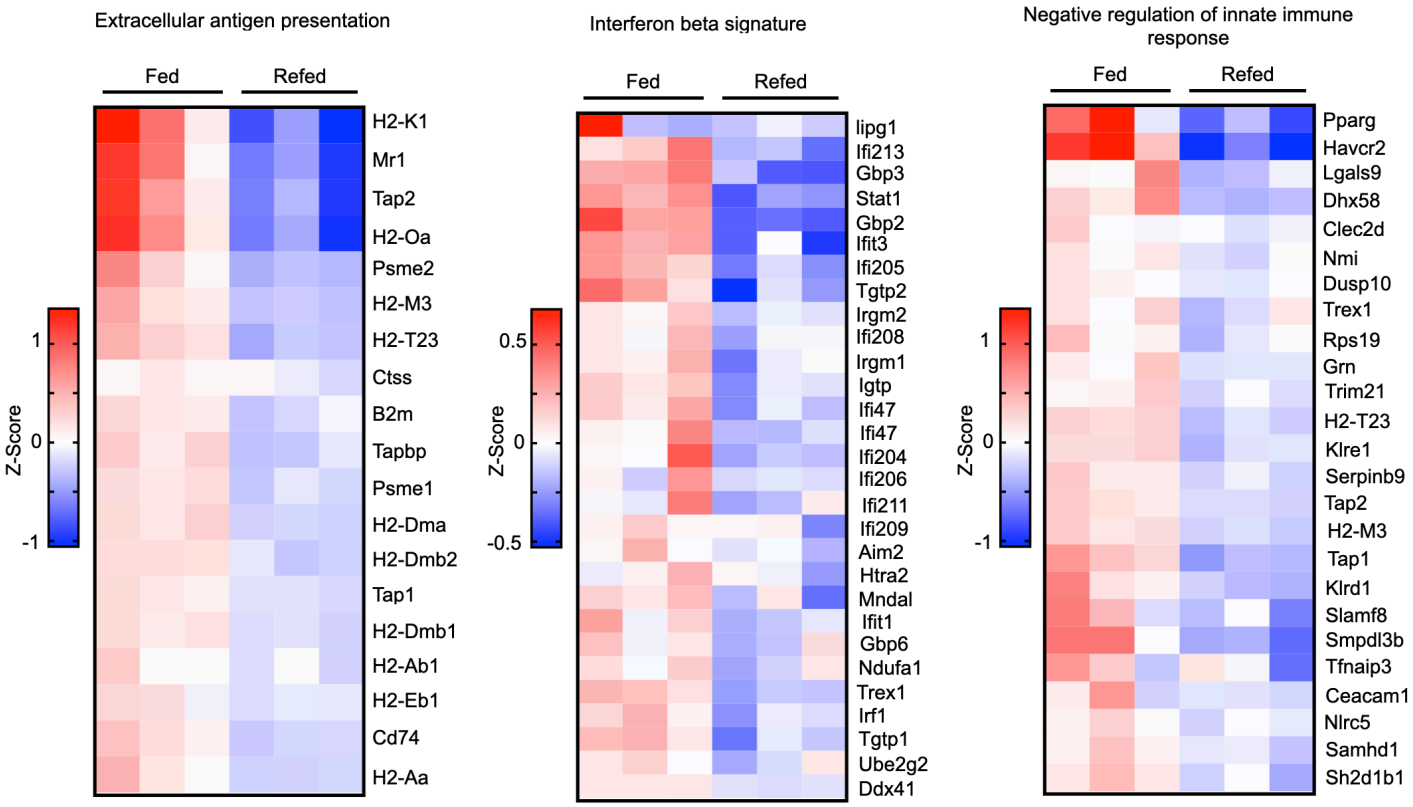

Figure S6: Gene ontology pathways of sorted monocytes after feeding or fasting+re-feeding (related to Figure 6)

Heatmaps of genes of indicated gene ontology (GO) pathway. Variance stabilized, centered and log-transformed counts.

Supplemental Table 1: Gating Strategy for leukocytes (related to Figure 1)

	Monocytes (%)	Neutrophils (%)	B cells (%)	T cells (%)
Panel with B and T cells in Lineage	2.64	5.8	71.2	8.17
Cell defined as	$CD45^{+}LIN^{-}CD11b^{+}Ly\text{-}6G^{-}Cx3cr1^{-}Ly\text{-}6C^{hi}$	$CD45^{+}LIN^{-}CD11b^{+}Cx3cr1^{-}Ly\text{-}6G^{+}$	$CD45^{+}LIN^{+}MHCII^{+}$	$CD45^{+}LIN^{+}MHCII^{-}$
Panel with CD19 and CD3 in separate channels	2.88	8.86	70.8	9.88
Cell defined as	$CD45^{+}CD19^{-}CD3^{-}CD11b^{+}Ly\text{-}6G^{-}Cx3cr1^{-}Ly\text{-}6C^{hi}$	$CD45^{+}CD19^{-}CD3^{-}CD11b^{+}Cx3cr1^{-}Ly\text{-}6G^{+}$	$CD45^{+}CD19^{+}CD3^{-}$	$CD45^{+}CD19^{-}CD3^{+}$

For enumerations in Figure 1, B cells were defined as ($CD45^{+}Lin1^{+}CD11b^{-}MHCII^{+}$) and T cells as ($CD45^{+}Lin1^{+}CD11b^{-}MHCII^{-}$). As a proof of concept, gating was compared to an alternative panel with B cells defined as ($CD45^{+}CD3^{-}CD19^{+}$) and T cells as ($CD45^{+}CD19^{-}CD3^{+}$) to exclude major differences in assignments.