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Supplemental information

Tissue-resident, extravascular Ly6c^{-} monocytes are critical for inflammation in the synovium

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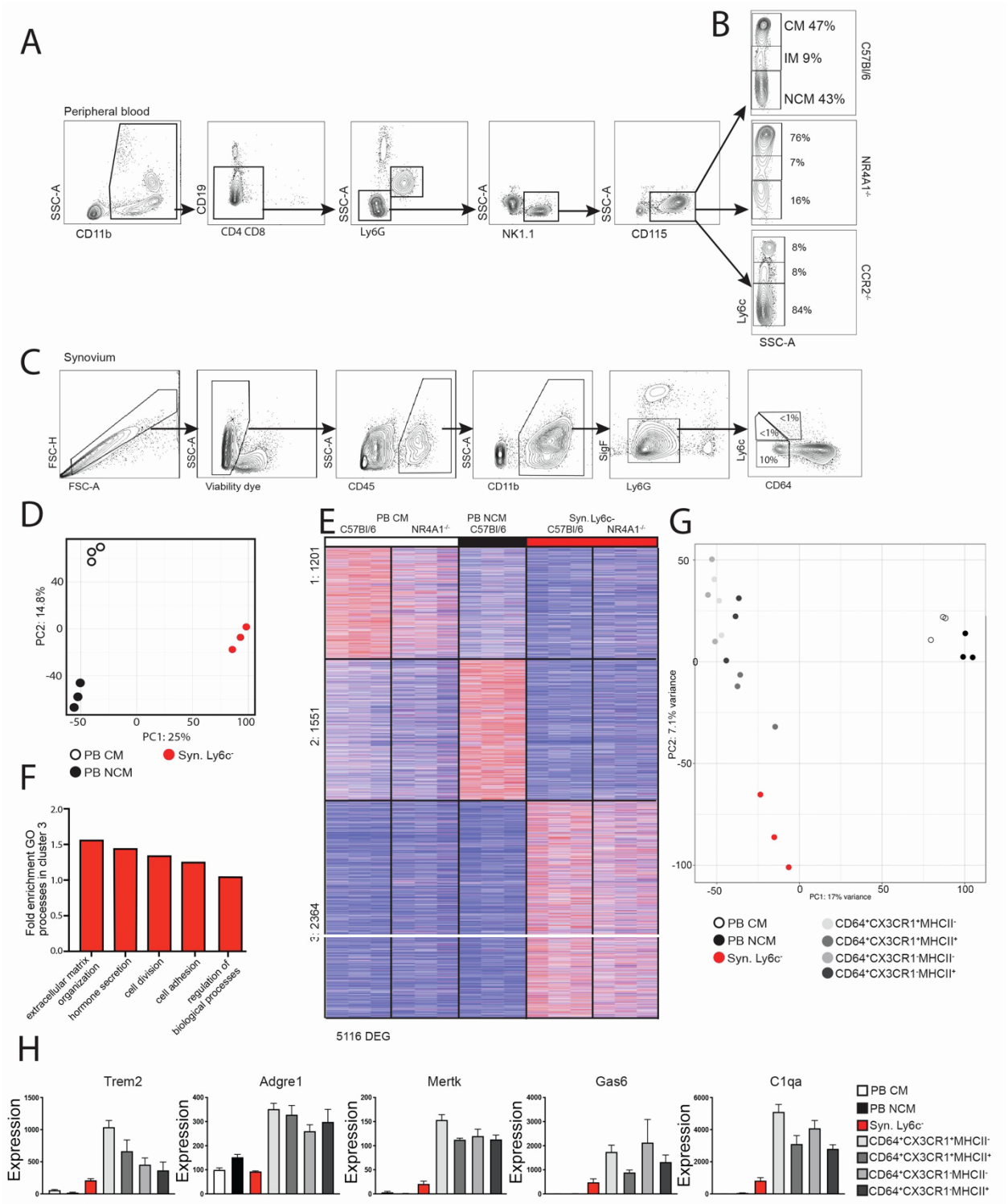
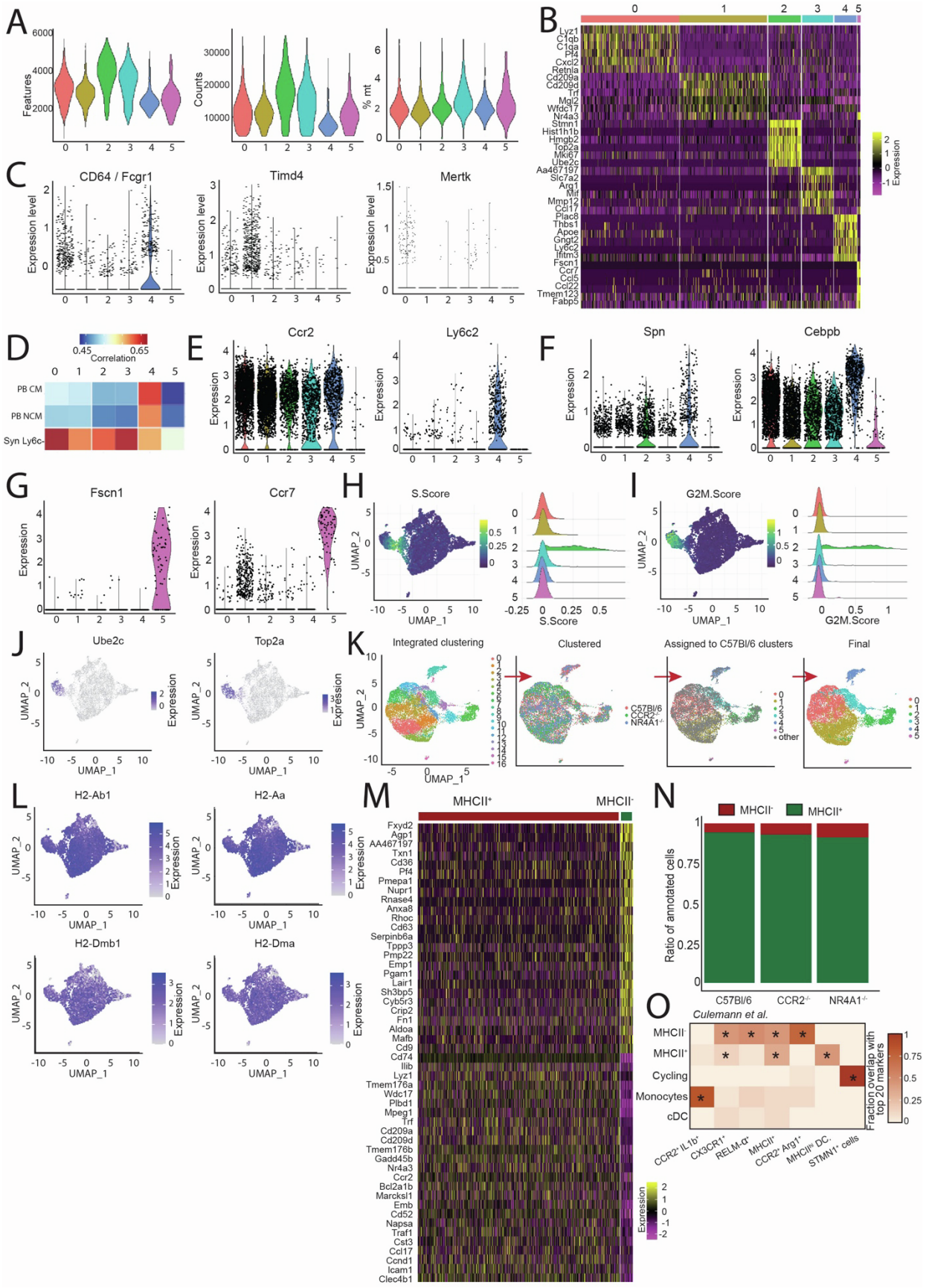
S1: Synovial Ly6c^{-} cells are distinct from circulating NCM. A) Gating strategy for PB monocyte subpopulations in C57Bl/6, B) NR4A1$^{-/-}$ and CCR2$^{-/-}$ mice, and C) Gating strategy for Syn Ly6c^{-} and Ly6c^{+} cells (CD64^{-}) and macrophages (CD64^{+}). D) PCA of 10206 genes expressed by PB CM, PB NCM, and Syn CD64^{-} cells. E) Visualization of clusters from Figure 1L with expression in PB CM and Syn Ly6c^{-} from NR4A1$^{-/-}$ mice. F) Significantly enriched GO processes in cluster 3 from Figure 1L with preferential expression in Syn Ly6c^{-} ($P<0.05$). G) PCA of 9661 genes expressed in PB CM, PB NCM, Syn Ly6c^{-} and Syn CD64^{+}. H) Mean expression of representative macrophage genes from PB CM, PB NCM, Syn Ly6c^{-} and Syn Mac populations (RNA-seq data: n=3, error bars indicate SEM).

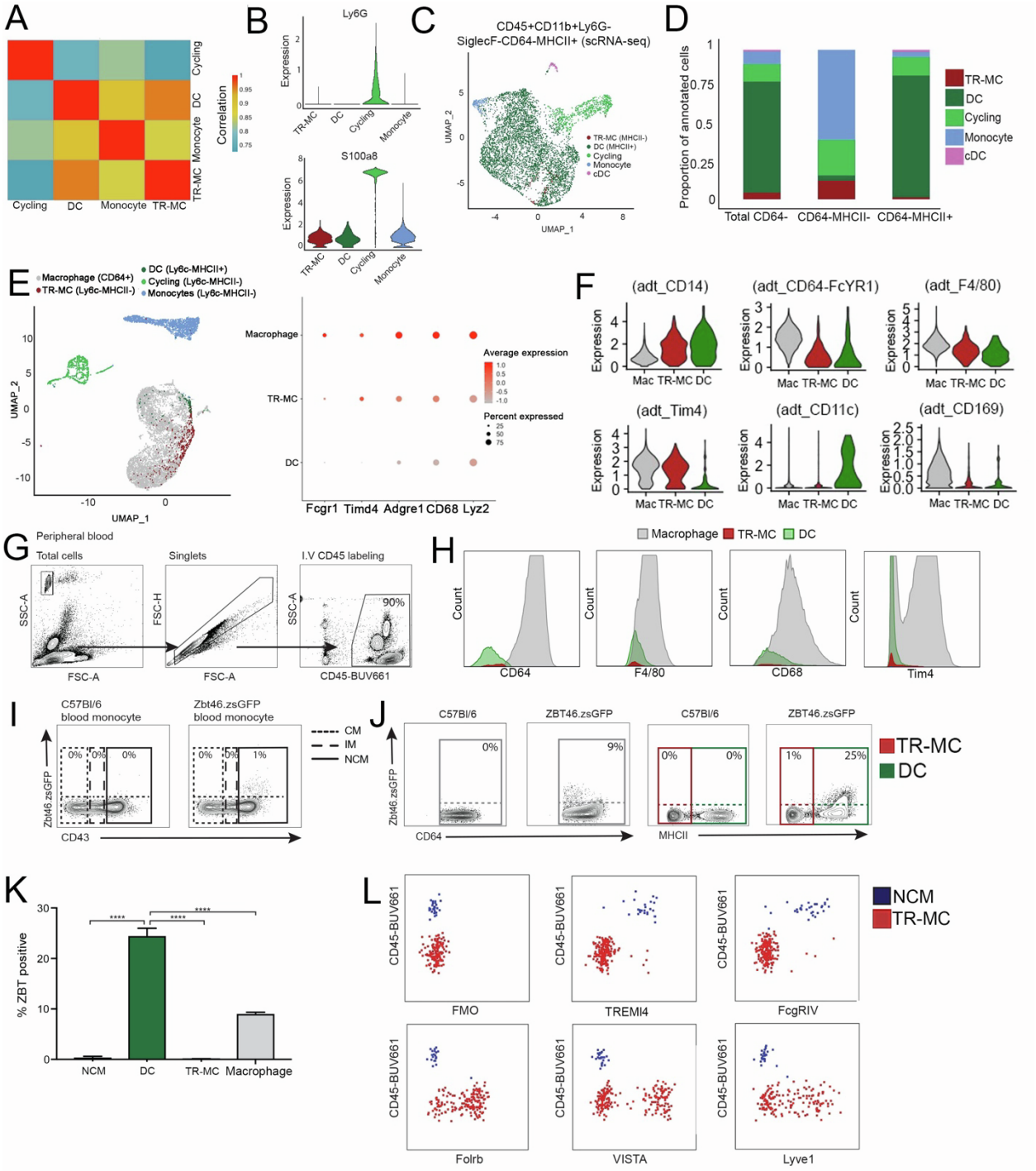


S2: **Synovial myeloid niche is segmented based on MHCII expression.** A) Quality control of $CD45^{+}CD11b^{+}Ly6G^{-}SiglecF^{-}CD64^{-}$ cells by subpopulation from C57Bl/6 mice showing number

of genes, number of UMIs, and percent mitochondrial reads per cell. B) Relative expression of top 5 marker genes (by fold change) across subpopulations 0-5. C) Normalized expression of macrophage-associated genes. D) Pearson's correlation between gene expression in scRNA-seq subpopulations and bulk RNA-seq of PB CM, PB NCM, and Syn Ly6c^{-} cells. E) Normalized expression of CM, F) NCM and G) DC associated genes. H) Normalized expression of S-phase and I) G2-phase module genes. J) Visualization of expression of cell cycle genes. K) Integration of scRNA-seq data from CCR2$^{-/-}$ and NR4A1$^{-/-}$ mice with superimposed C57Bl/6 annotations. L) Expression of MHCII genes. M) Relative expression of top 20 differentially expressed genes between MHCII^{+} and MHCII^{-} cells. N) Ratio of cells annotated either MHCII^{+} or MHCII^{-} in C57Bl/6, CCR2$^{-/-}$ and NR4A1$^{-/-}$ mice. O) Fraction overlap of differentially expressed genes from C57Bl/6 scRNA-seq subpopulations with top 20 markers from (Culemann et al., 2019*)* * indicates significant p-value by hypergeometric test after FWER correction.

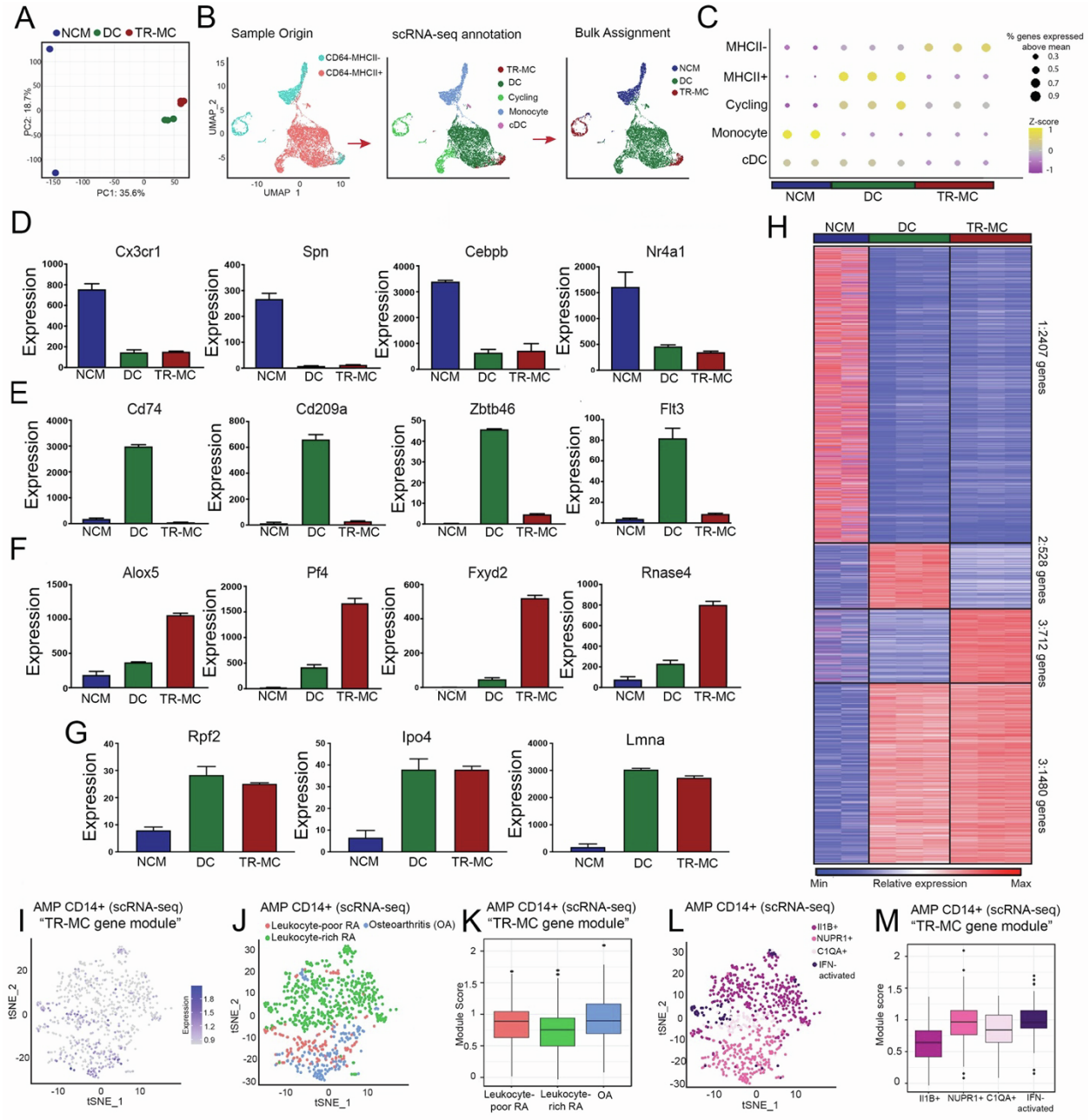

A
Features
Counts
% mt
B
C
CD64 / Fcgr1
Timd4
Mertk
Expression level
D
Correlation
PB CM
PB NCM
Syn Ly6c-
E
Ccr2
Ly6c2
F
Spn
Cebpb
G
Fscn1
Ccr7
H
S.Score
I
G2M.Score
J
Ube2c
Top2a
K
Integrated clustering
Clustered
Assigned to C57Bl/6 clusters
Final
C57Bl/6
CCR2-/-
NR4A1-/-
other
L
H2-Ab1
H2-Aa
H2-Dmb1
H2-Dma
M
MHCII+
MHCII-
N
Ratio of annotated cells
C57Bl/6
CCR2-/-
NR4A1-/-
O
Culemann et al.
MHCII-
MHCII+
Cycling
Monocytes
cDC
Fraction overlap with top 20 markers
CCR2+ IL-1b+
CX3CR1+ RELM-α+
MHCII+
CCR2- Arg1+
MHCIIlo DC.
STMN1+ cells

S3: **Syn Ly6c$^-$ cells are identified by surface marker expression.** A) Pairwise Pearson's correlation between average expression profile of subpopulations in CITE-seq of CD45$^+$CD11b$^+$Ly6G$^-$SiglecF$^-$CD64$^-$MHCII$^-$. B) Expression of neutrophil-associated genes in CD45$^+$CD11b$^+$Ly6G$^-$SiglecF$^-$CD64$^-$MHCII$^-$ CITE-seq data C) Annotation of CD45$^+$CD11b$^+$Ly6G$^-$SiglecF$^-$CD64$^-$MHCII$^+$ CITE-seq based on subpopulations in total Syn Ly6c$^-$ cells (CD45$^+$CD11b$^+$Ly6G$^-$SiglecF$^-$CD64$^-$) (Figure 2H). D) Frequency of cells annotated as TR-MC, DC, cDC, monocytes or cycling cells from CD45$^+$CD11b$^+$Ly6G$^-$SiglecF$^-$CD64$^-$ scRNA-seq (Figure 2H), CD45$^+$CD11b$^+$Ly6G$^-$SiglecF$^-$CD64$^-$MHCII$^-$ CITE-seq (Figure 3A), and CD45$^+$CD11b$^+$Ly6G$^-$SiglecF$^-$CD64$^-$MHCII$^+$ CITE-seq (C above). E) UMAP of merged CD45$^+$CD11b$^+$Ly6G$^-$SiglecF$^-$CD64$^+$CITE-seq with CD45$^+$CD11b$^+$Ly6G$^-$SiglecF$^-$CD64$^-$MHCII$^-$ CITE-seq along with expression of canonical macrophage genes. F) Protein expression (adt) of classical monocyte and macrophage proteins from merged CITE seq samples in E. G) I.V. labeling of PB cells using i.v. antibody. H) Surface expression of CD64, F4/80, CD68 and Tim4 on NCM, TR-MC, macrophage, and DC using flow cytometry. I) GFP in PB monocytes and J) synovial cells from representative zbt46.zsGFP and controls. K) Percent of cells that are ZBT positive (GFP+) in NCM, DC, TR-MC and macrophages. Values are mean of N>4 ±SEM. ***=p<0.005, ****=p<0.001. L) Surface expression of FMO, TREMl4, FcgRIV, Folrb, VISTA, and Lyve1 in NCM (blue) and TR-MC (red) measured by flow cytometry.

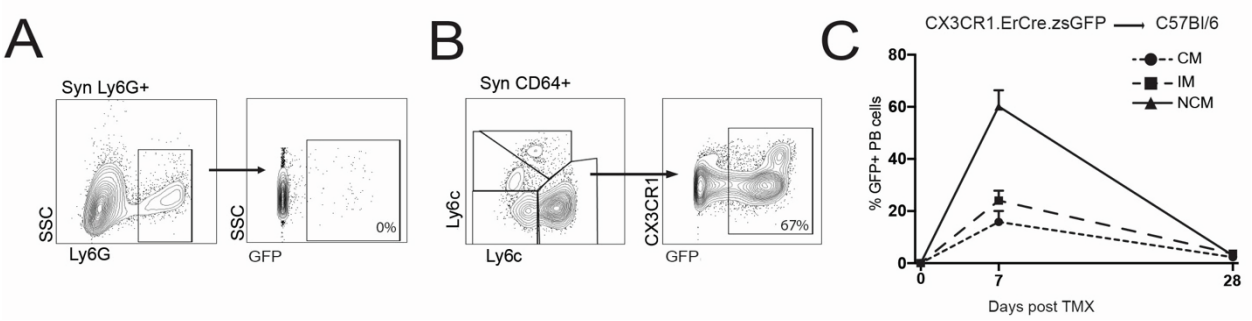


S4: Bulk RNA-seq indicates function in TR-MC A) PCA of 10270 expressed genes from NCM, DC and TR-MC. B) Merged cells from CD45^{+}CD11b^{+}Ly6G^{-}SiglecF^{-}CD64^{-}MHCII^{-} (Figure 3A) and CD45^{+}CD11b^{+}Ly6G^{-}SiglecF^{-}CD64^{-}MHCII^{+} (Figure S3C), annotation by subpopulation, and assignment to NCM, DC or TR-MC populations using bulk transcriptional data. C) Expression of

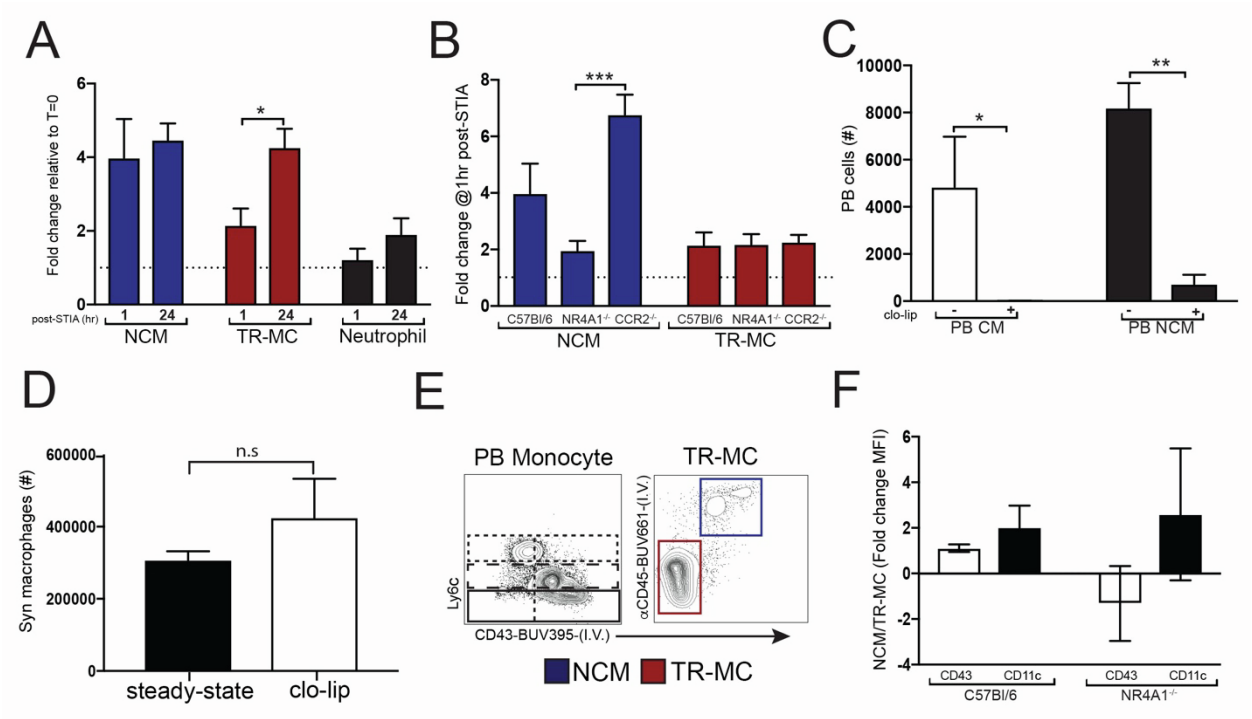
marker gene sets from scRNA-seq on total Ly6c$^-$ subpopulations (Figure 2H) in bulk populations. Color of circle indicates z-score normalized expression while size indicates % of genes in the set expressed in the given sample above their mean. D) Genes with preferential expression in NCM, E) DC, F) TR-MC and G) in DC and TR-MC sorted populations. H) K-means clustering of 5127 differentially expressed genes across sorted NCM, DC and TR-MC. I) MHCII$^-$ gene module expression in scRNA-seq data from published AMP data (Zhang et al., 2019). J-K) Origin of CD14$^+$ cells from Leukocyte-poor RA, Leukocyte-rich RA, and OA patients in AMP data and their MHCII$^-$ module score. L-M) Annotation of CD14$^+$ cells as IL1B$^+$, NUPR1$^+$, C1QA$^+$, or IFN-activated clusters in published AMP data and their MHCII- module score.



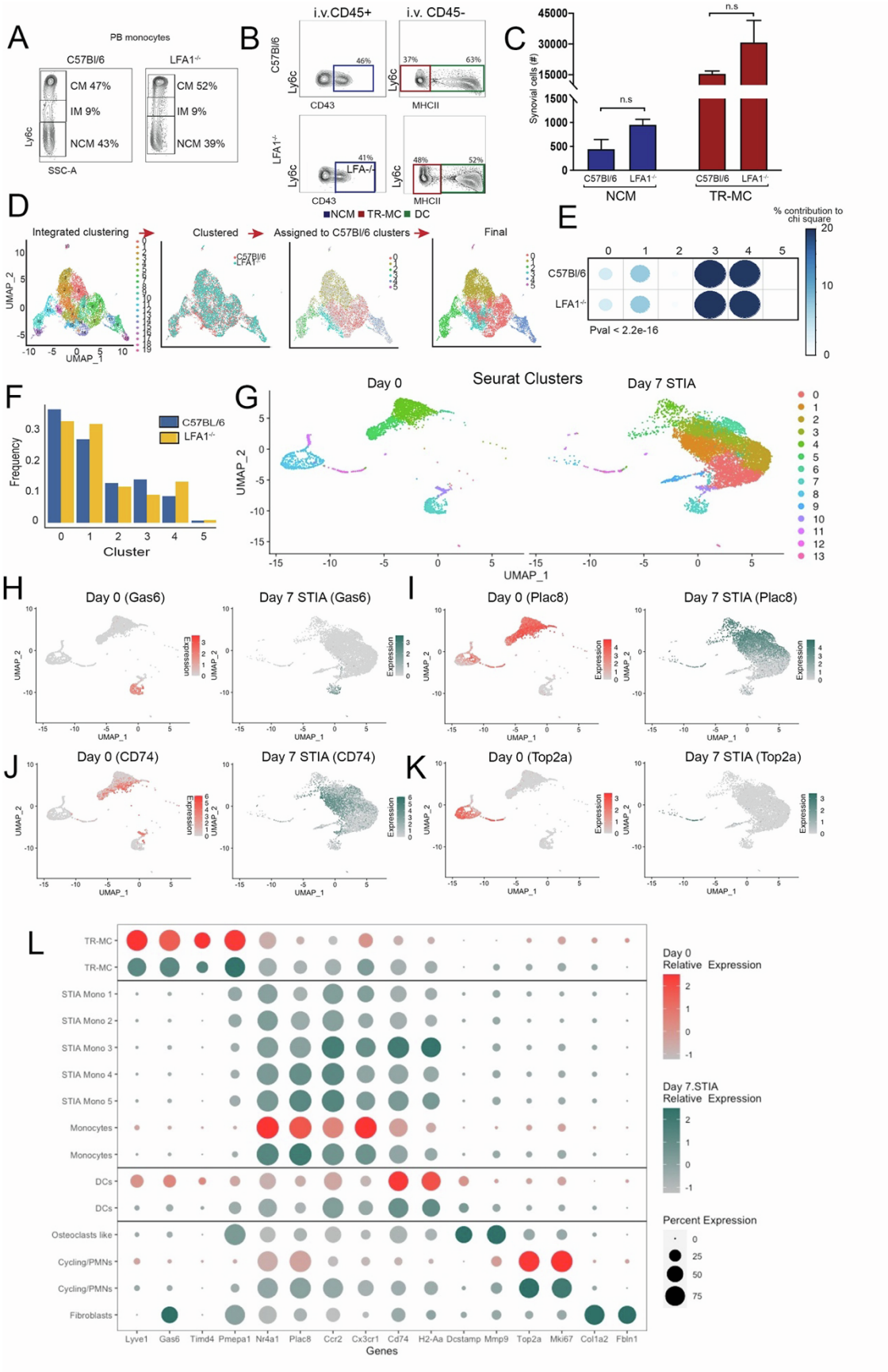
S5: Synovial macrophages have embryonic origins. A) Expression of GFP in synovial Ly6G^{+} and B) Syn CD64^{+} cells in adult CX3CR1$^{Cre.ER}$.zsGFP mice treated with TMX. C) GFP^{+} cells in PB monocytes in CX3CR1$^{Cre.ER}$.zsGFP -> C57Bl/6 chimeric mice 7 and 28-days post TMX. All graphs are mean N>4.



S6: **TR-MC have access to synovial vasculature**. A) Fold-change of NCM, TR-MC and neutrophils 1h and 24h after STIA compared to steady state. B) Fold-change of NCM and TR-MC 1h after STIA in C57Bl/6, NR4A1$^{-/-}$ and CCR2$^{/-}$ mice. C) PB monocytes and D) synovial CD64^{+} macrophages following treatment with clo-lip. E) Expression of Ly6c vs αCD43-BUV395 staining in PB and synovial Syn Ly6c^{-} cells. F) Fold-change MFI CD43 and CD11c of I.V. αCD43-BUV395$^{+/-}$ TR-MC in C57Bl/6 and NR4A1$^{-/-}$ synovial cells. All graphs are mean N>4 +SEM *=p<0.05, **=p<0.01, ***=p<0.005.



S7: **LFA1 is required for TR-MC function**. A) PB monocyte subpopulations in C57Bl/6 and LFA1$^{-/-}$ mice. B, C) NCM (blue) and TR-MC (red) in C57Bl/6 and LFA1$^{-/-}$ mice at steady-state. D) Integration of scRNA-seq data on CD45^{+}CD11b^{+}Ly6G^{-}SiglecF^{-}CD64^{-} cells from LFA1$^{-/-}$ mice with C57Bl/6. E, F) Proportion of cells annotated as each subpopulation in LFA1$^{-/-}$ and C57Bl/6 mice and chi-squared residuals ($p<2.2e-16$). G) Day 0 and Day 7 CITE seq merged. Feature plot analysis of H) Gas6 (TR-MC), I) CD74 (DC), J) Plac8 (monocyte) and K) Top2a (cycling) in Day 0 and Day 7 STIA. L) Expression of genes associated with TR-MC, monocytes, DC, osteoclast-like, cycling/PMN, and fibroblasts across cluster split by Day 0 and Day 7 STIA.


A
PB monocytes
C57Bl/6
LFA1-/-
CM 47%
IM 9%
NCM 43%
CM 52%
IM 9%
NCM 39%
Ly6c
SSC-A
B
i.v.CD45+
i.v. CD45-
C57Bl/6
LFA1-/-
Ly6c
CD43
MHCII
46%
37%
63%
41%
48%
52%
LFA-/-
NCM TR-MC DC
C
Synovial cells (#)
n.s
n.s
C57Bl/6
LFA1-/-
NCM
TR-MC
D
Integrated clustering
Clustered
Assigned to C57Bl/6 clusters
Final
UMAP_1
UMAP_2
E
% contribution to chi square
C57Bl/6
LFA1-/-
Pval < 2.2e-16
F
C57BL/6
LFA1-/-
Frequency
Cluster
G
Day 0
Seurat Clusters
Day 7 STIA
UMAP_1
UMAP_2
H
Day 0 (Gas6)
Day 7 STIA (Gas6)
I
Day 0 (Plac8)
Day 7 STIA (Plac8)
J
Day 0 (CD74)
Day 7 STIA (CD74)
K
Day 0 (Top2a)
Day 7 STIA (Top2a)
Expression
L
TR-MC
STIA Mono 1
STIA Mono 2
STIA Mono 3
STIA Mono 4
STIA Mono 5
Monocytes
DCs
Osteoclasts like
Cycling/PMNs
Fibroblasts
Lyve1 Gas6 Timd4 Pmepa1 Nr4a1 Plac8 Ccr2 Cx3cr1 Cd74 H2-Aa Dcstamp Mmp9 Top2a Mki67 Col1a2 Fbln1
Genes
Day 0 Relative Expression
Day 7.STIA Relative Expression
Percent Expression