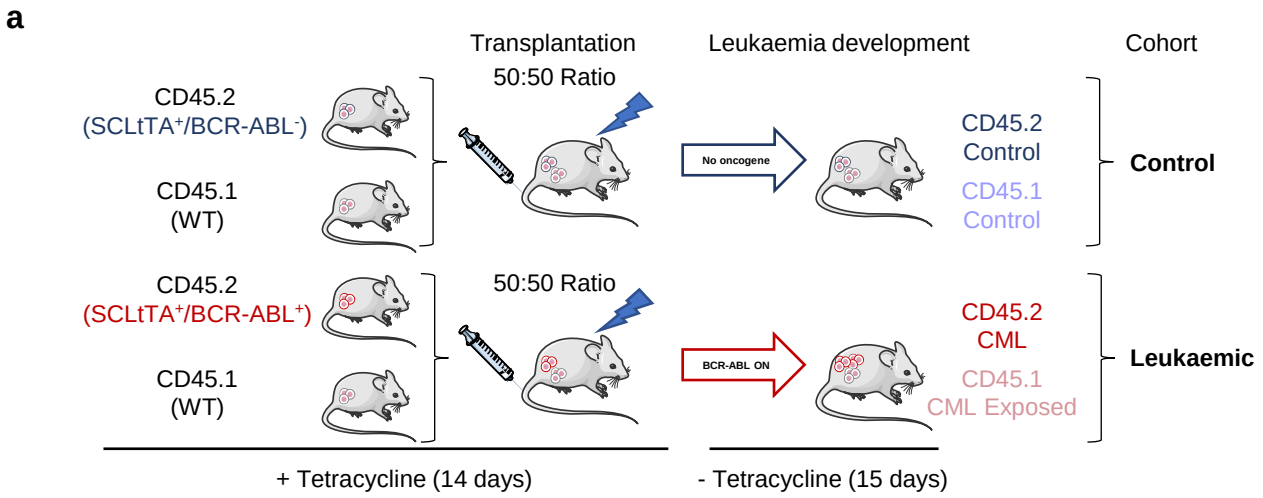
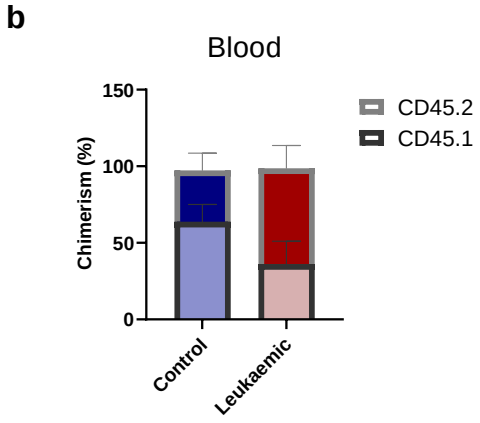
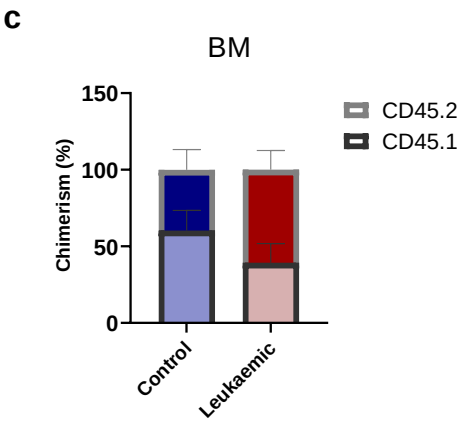
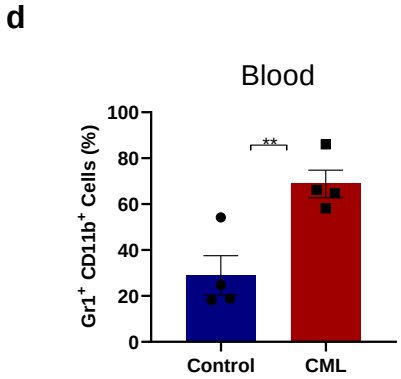
Fig. 1


a
Transplantation
Leukaemia development
Cohort
CD45.2
(SCLtTA+/BCR-ABL-)
CD45.1
(WT)
50:50 Ratio
No oncogene
CD45.2
Control
CD45.1
Control
Control
CD45.2
(SCLtTA+/BCR-ABL+)
CD45.1
(WT)
50:50 Ratio
BCR-ABL ON
CD45.2
CML
CD45.1
CML Exposed
Leukaemic
+ Tetracycline (14 days)
- Tetracycline (15 days)


b
Blood
Chimerism (%)
150
100
50
0
CD45.2
CD45.1
Control
Leukaemic


c
BM
Chimerism (%)
150
100
50
0
CD45.2
CD45.1
Control
Leukaemic


d
Blood
Gr1+ CD11b+ Cells (%)
100
80
60
40
20
0
**
Control
CML

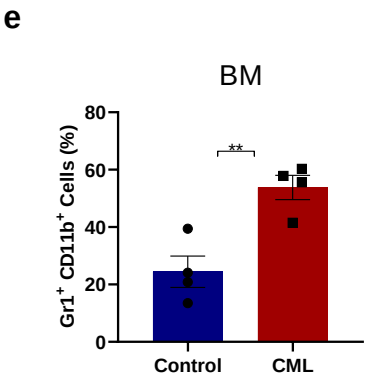

e
BM
Gr1+ CD11b+ Cells (%)
80
60
40
20
0
**
Control
CML

Fig. 1: Establishment of a Chimeric CML Mouse Model

Flow cytometry analysis of blood and bone marrow (BM) of chimeric mice after 15 days off tetracycline. **a:** Schematic outline of experimental design to generate CML chimeric mice. $7.5x10^5$ BM from either CD45.2 $SCLtTA^+/BCR\text{-}ABL^-$ (Control) or $SCLtTA^+/BCR\text{-}ABL^+$ (CML) mice mixed with $7.5x10^5$ CD45.1 BM from wild type (WT) mice was transplanted into WT mice. **b-c:** Chimerism in blood (b) and BM (c) at experimental endpoint. **d-e:** Quantification of myeloproliferation in blood (d) and BM (e). Unpaired Student's t-test statistical analysis, $^{**}p<0.01$. N = 4 mice per experimental arm.

Fig. 2

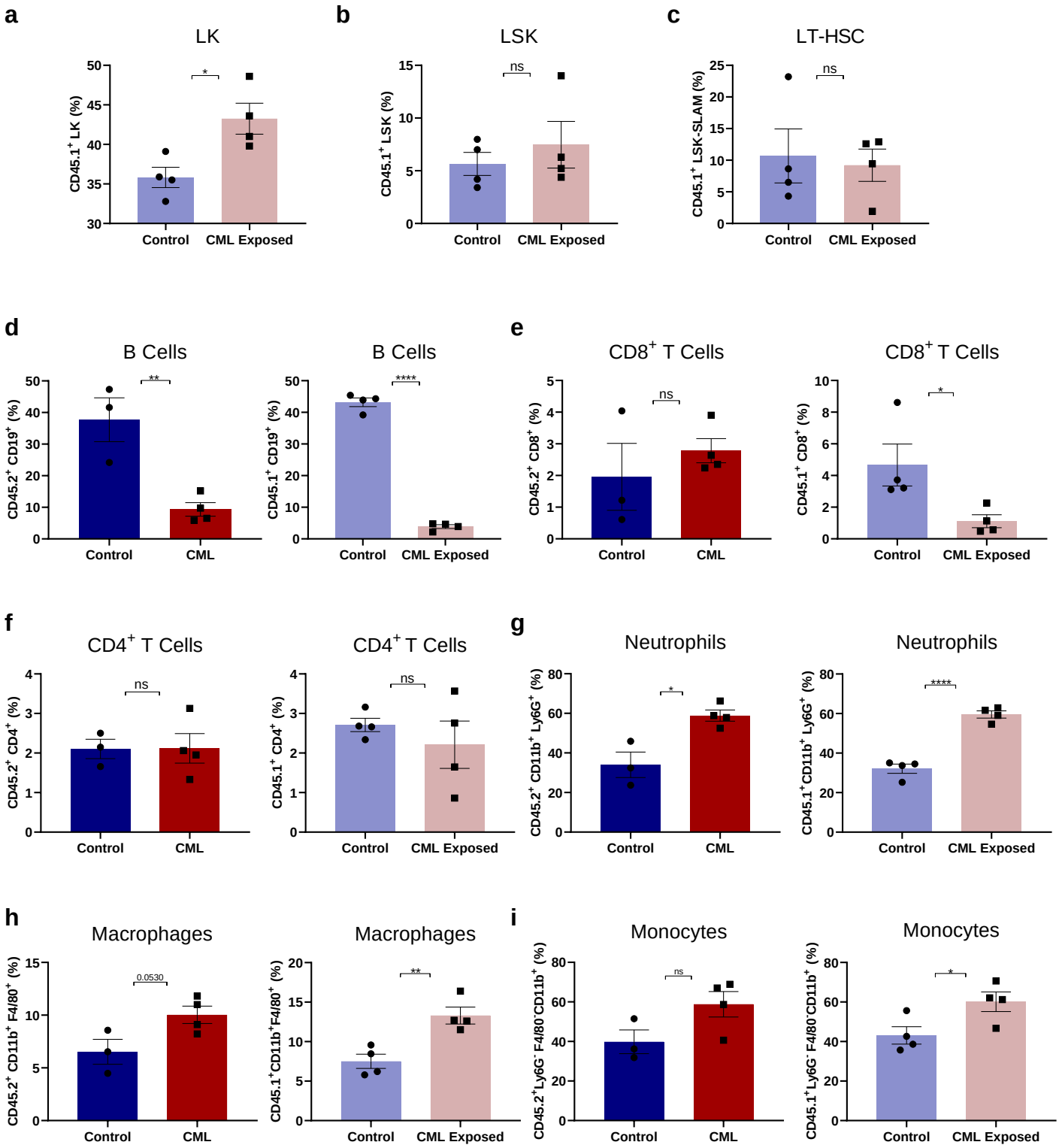

a
LK
CD45.1+ LK (%)
*
Control
CML Exposed
b
LSK
CD45.1+ LSK (%)
ns
Control
CML Exposed
c
LT-HSC
CD45.1+ LSK-SLAM (%)
ns
Control
CML Exposed
d
B Cells
CD45.2+ CD19+ (%)
**
Control
CML
B Cells
CD45.1+ CD19+ (%)
****
Control
CML Exposed
e
CD8+ T Cells
CD45.2+ CD8+ (%)
ns
Control
CML
CD8+ T Cells
CD45.1+ CD8+ (%)
*
Control
CML Exposed
f
CD4+ T Cells
CD45.2+ CD4+ (%)
ns
Control
CML
CD4+ T Cells
CD45.1+ CD4+ (%)
ns
Control
CML Exposed
g
Neutrophils
CD45.2+ CD11b+ Ly6G+ (%)
*
Control
CML
Neutrophils
CD45.1+CD11b+ Ly6G+ (%)
****
Control
CML Exposed
h
Macrophages
CD45.2+ CD11b+ F4/80+ (%)
0.0530
Control
CML
Macrophages
CD45.1+CD11b+F4/80+ (%)
**
Control
CML Exposed
i
Monocytes
CD45.2+Ly6G- F4/80+CD11b+ (%)
ns
Control
CML
Monocytes
CD45.1+Ly6G- F4/80+CD11b+ (%)
*
Control
CML Exposed

Fig. 2: CML Results in an Increase in Ph^- Cells Progenitor Cells and Myeloid Skew at the Expense of Lymphocytes

a-c: Flow cytometry analysis of CD45.1 BM of chimeric mice after 15 days off TET. Quantification of $CD45.1^+$ $Lin^-c\text{-}Kit^+$ (LK; **a**), $Lin^-c\text{-}Kit^+Sca\text{-}1^+$ (LSK; **b**) and $Lin\text{-}c\text{-}Kit^+Sca\text{-}1^+CD150^+CD48^-$ (LT-HSC; **c**) populations (%) of parent population. **d-i:** Flow cytometry analysis of CD45.1 and CD45.2 arms of BM of chimeric mice after 15 days off TET. Percentage of $CD19^+$ B cells (**d**)**,** $CD8^+$ T cells (**e**), $CD4^+$ T cells (**f**), $CD11b^+Ly6G^+$ cells (**g**)**,** $CD11b^+F4/80^+$ cells (**h**), and $CD11b^+Ly6G^-F4/80^-$ cells (**i**). Unpaired Student's t-test statistical analysis, *p<0.05, **p<0.01, ***, ****p<0.0001. N = 3-4 mice per experimental arm.

Fig. 3

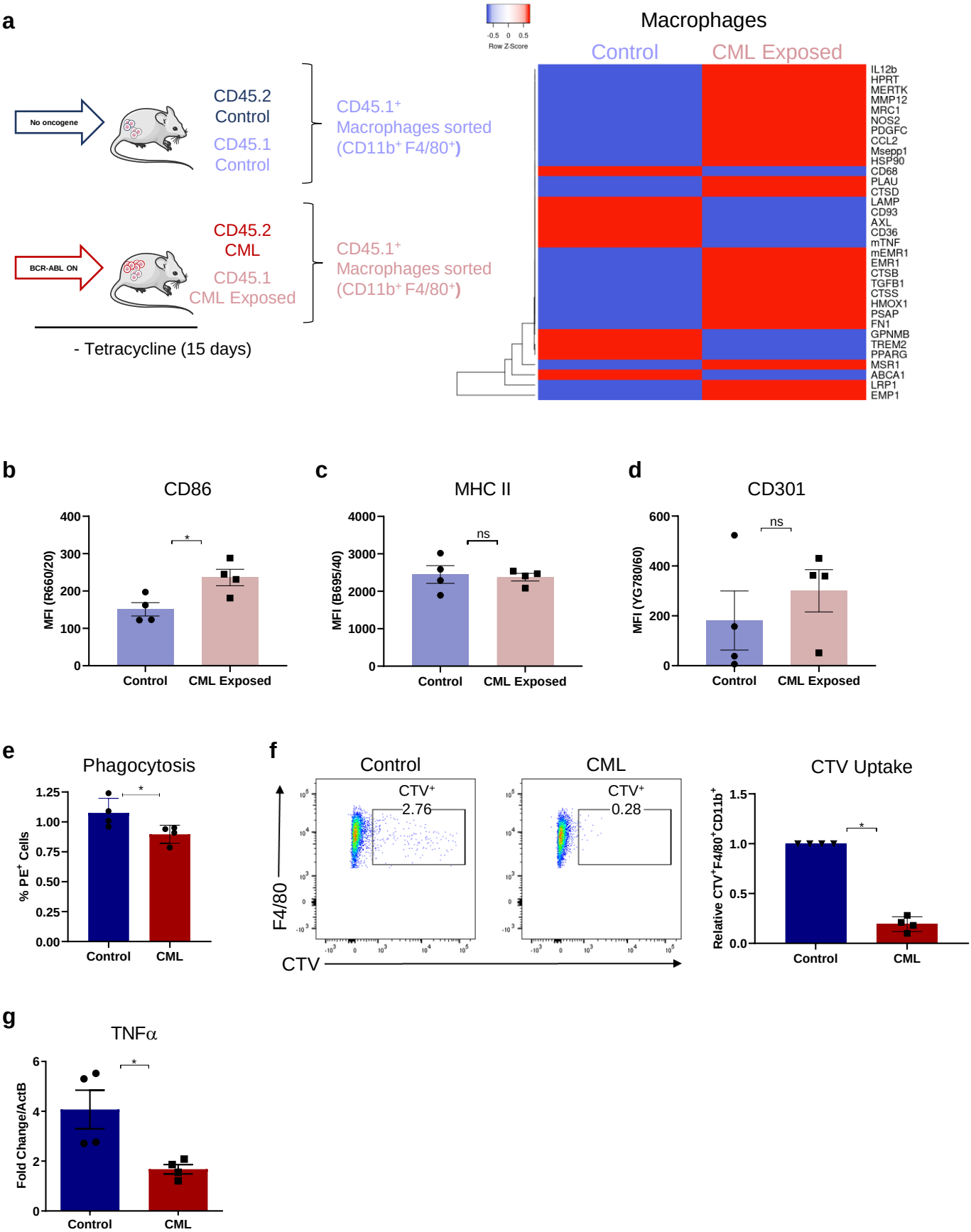
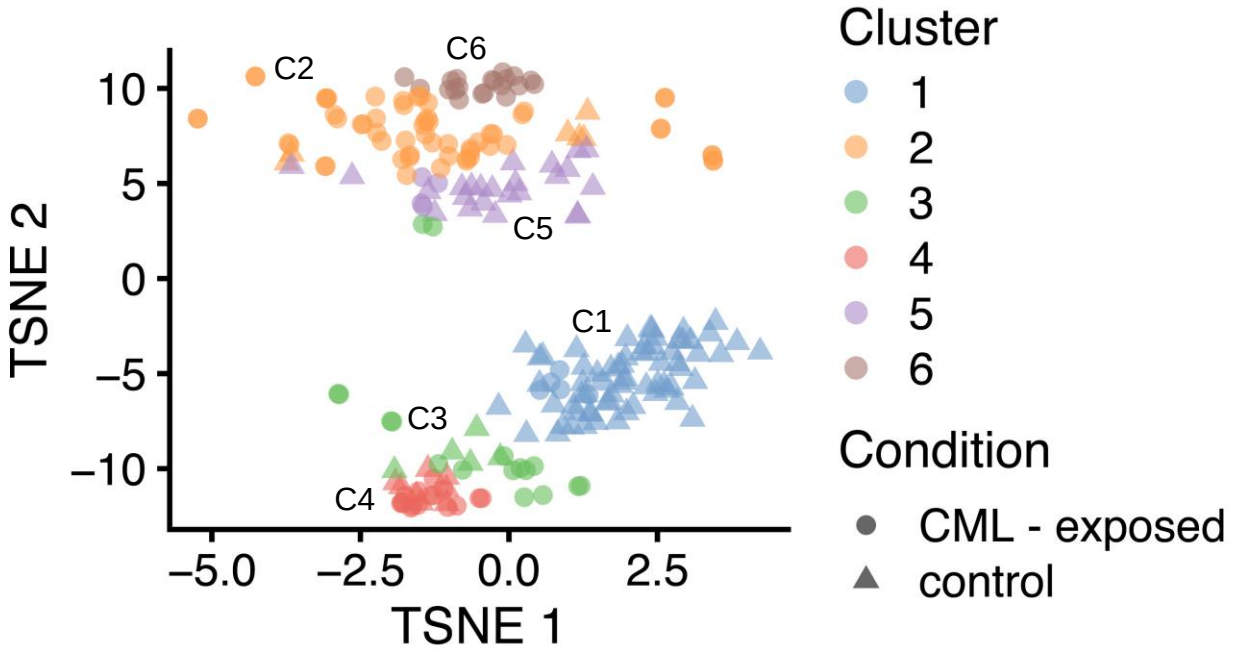

a
No oncogene
CD45.2
Control
CD45.1
Control
CD45.1+
Macrophages sorted
(CD11b+ F4/80+)
BCR-ABL ON
CD45.2
CML
CD45.1
CML Exposed
CD45.1+
Macrophages sorted
(CD11b+ F4/80+)
- Tetracycline (15 days)
Row Z-Score
Macrophages
Control
CML Exposed
IL12b
HPRT
MERTK
MMP12
MRC1
NOS2
PDGFC
CCL2
Msepp1
HSP90
CD68
PLAU
CTSD
LAMP
CD93
AXL
CD36
mTNF
mEMR1
EMR1
CTSB
TGFB1
CTSS
HMOX1
PSAP
FN1
GPNMB
TREM2
PPARG
MSR1
ABCA1
LRP1
EMP1
b
CD86
MFI (R660/20)
Control
CML Exposed
c
MHC II
MFI (B695/40)
ns
Control
CML Exposed
d
CD301
MFI (YG780/60)
ns
Control
CML Exposed
e
Phagocytosis
% PE+ Cells
Control
CML
f
Control
CML
CTV+
2.76
CTV+
0.28
F4/80
CTV
CTV Uptake
Relative CTV+F4/80+CD11b+
Control
CML
g
TNFα
Fold Change/ActB
Control
CML

Fig. 3: Exposure to the CML Bone Marrow Microenvironment Alters Macrophage Gene Expression and Phagocytosis Capacity

a: Schematic outline of experimental design and heatmap of Fluidigm gene expression analysis of $CD11b^{+}F4/80^{+}$ macrophages sorted from CD45.1 (WT/CML exposed) BM (N = 4-7 mice per experimental arm). **b-d:** Surface marker expression of CD86, MHC II and CD301 on control or CML exposed macrophages (N=4 mice per experimental arm). **e:** BMDM phagocytosis of beads after 24hr Control/CML c-Kit^{+} conditioned medium culture (N = 4 mice per experimental arm). **f:** $CTV^{+}CD11b^{+}F4/80^{+}$ flow cytometry analysis following Control/CML CTV^{+} c-Kit^{+} cells co-culture with BMDM following 48hr culture (N=4 mice per experimental arm). **g:** RT-qPCR of TNFα in murine BMDM following conditioned medium treatment (N = 4 mice per experimental arm). Statistical analysis of A-E unpaired Student's t-test, *$p<0.05$.

Fig. 4

a


C2
C6
C5
C1
C3
C4
10
5
0
−5
−10
TSNE 2
−5.0
−2.5
0.0
2.5
TSNE 1
Cluster
1
2
3
4
5
6
Condition
CML - exposed
control

b

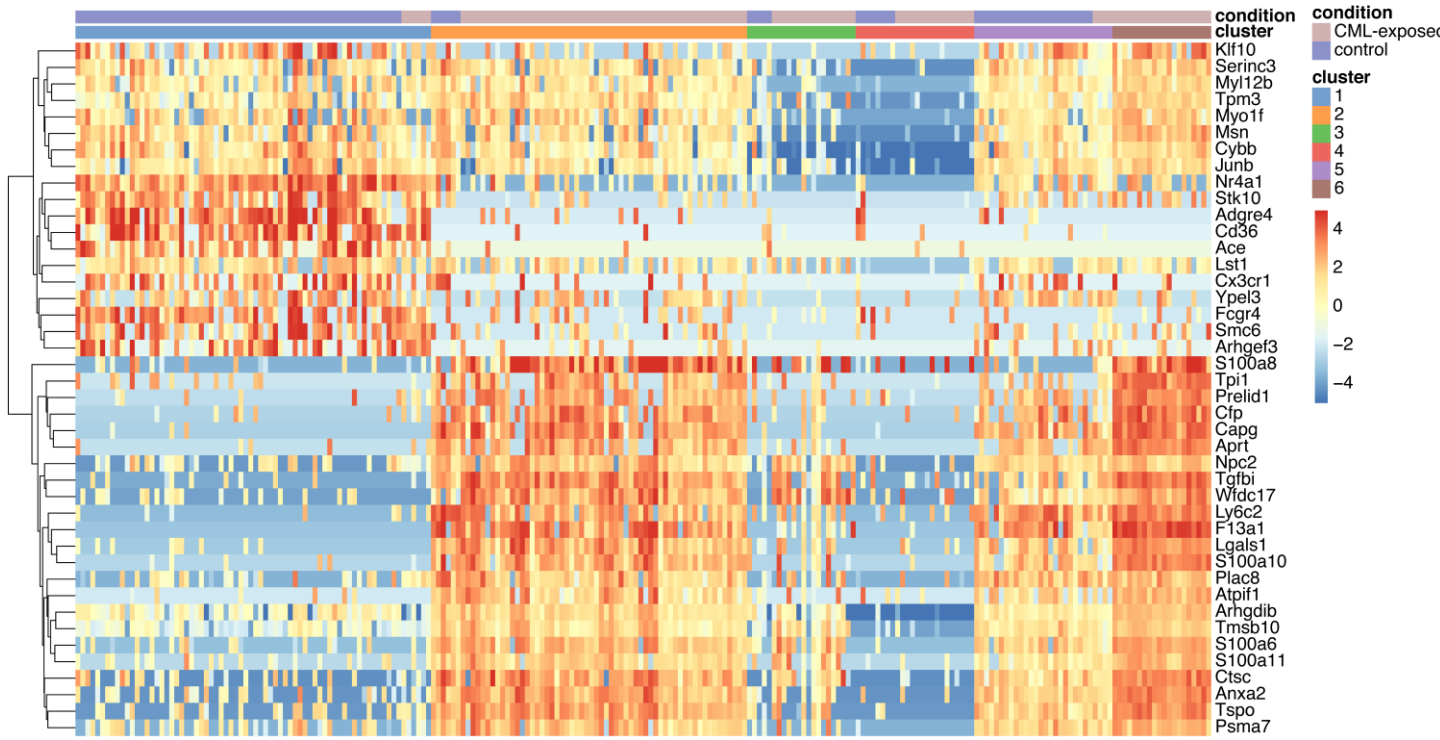
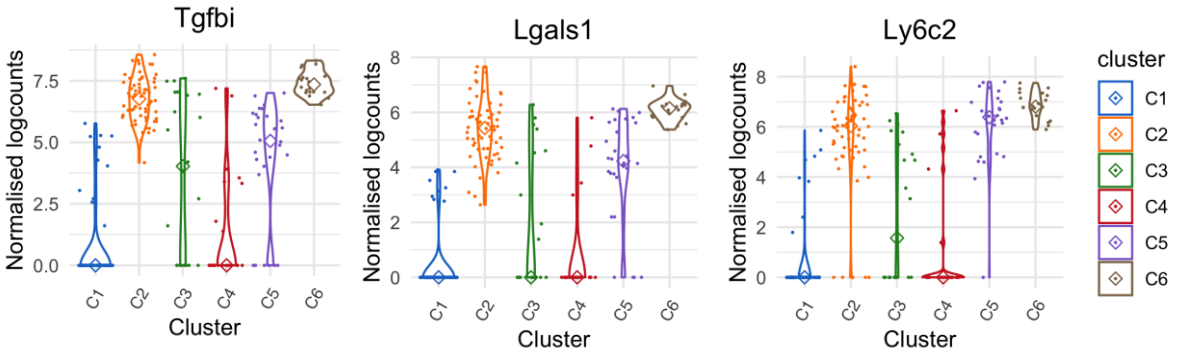

condition
cluster
Klf10
Serinc3
Myl12b
Tpm3
Myo1f
Msn
Cybb
Junb
Nr4a1
Stk10
Adgre4
Cd36
Ace
Lst1
Cx3cr1
Ypel3
Fcgr4
Smc6
Arhgef3
S100a8
Tpi1
Prelid1
Cfp
Capg
Aprt
Npc2
Tgfbi
Wfdc17
Ly6c2
F13a1
Lgals1
S100a10
Plac8
Atpif1
Arhgdib
Tmsb10
S100a6
S100a11
Ctsc
Anxa2
Tspo
Psma7
condition
CML-exposed
control
cluster
1
2
3
4
5
6
4
2
0
−2
−4

Fig. 4 cont.

c


Tgfbi
Lgals1
Ly6c2
Normalised logcounts
Cluster
C1
C2
C3
C4
C5
C6
cluster

d

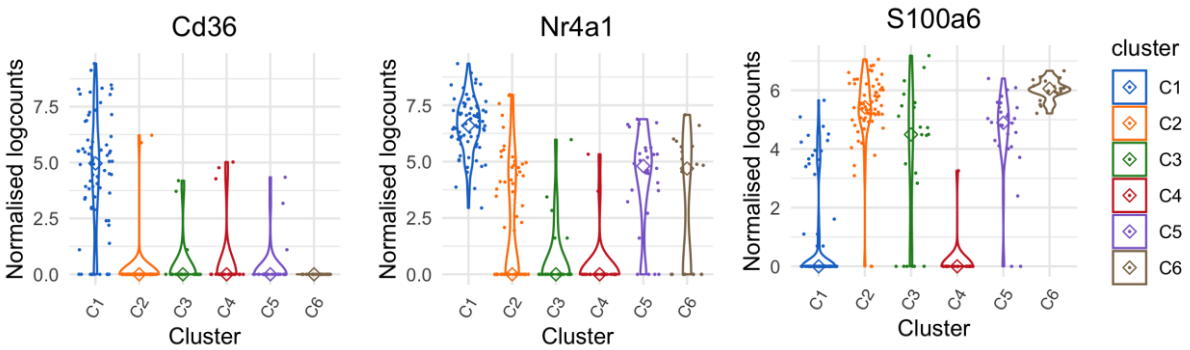
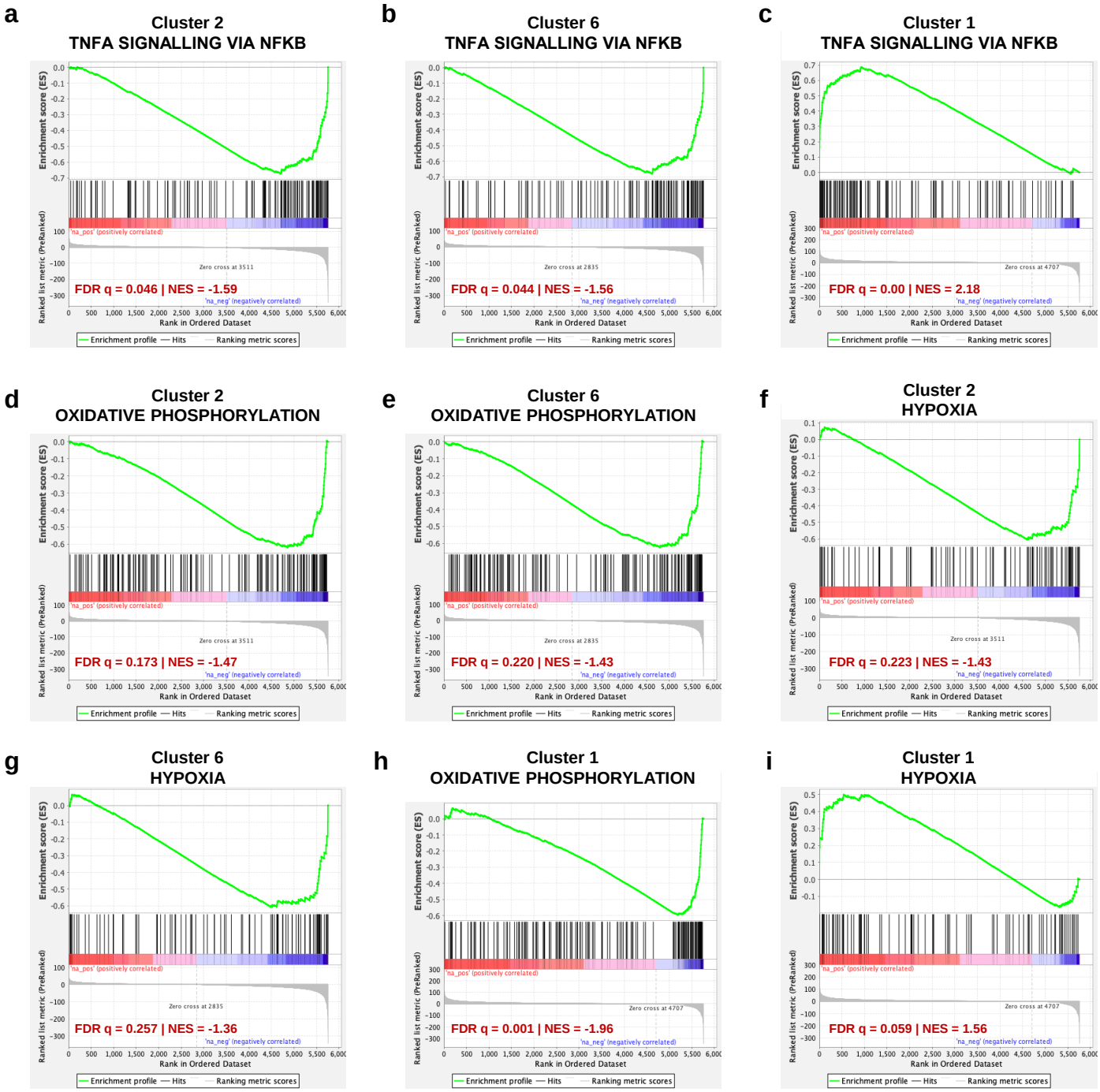

Cd36
Nr4a1
S100a6
Normalised logcounts
Cluster
C1
C2
C3
C4
C5
C6
cluster

Fig. 4: CML Exposed Macrophages Form Unique Subpopulations

a: t-Distributed Stochastic Neighbour Embedding (t-SNE) visualisation of $CD45.1^+$ $CD11b^+F4/80^+$ cells from control and CML BM scRNA seq. **b**: Unsupervised clustering of top 20 marker gene expression heatmap. **c-d**: Violin plots of normalised log counts of *Tgfbi*, *Lgals1*, *Ly6c2* (**c**), *Cd36*, *Nr4a1* and *S100a6* (**d**).

Fig. 5


a
Cluster 2
TNFA SIGNALLING VIA NFKB
FDR q = 0.046 | NES = -1.59
b
Cluster 6
TNFA SIGNALLING VIA NFKB
FDR q = 0.044 | NES = -1.56
c
Cluster 1
TNFA SIGNALLING VIA NFKB
FDR q = 0.00 | NES = 2.18
d
Cluster 2
OXIDATIVE PHOSPHORYLATION
FDR q = 0.173 | NES = -1.47
e
Cluster 6
OXIDATIVE PHOSPHORYLATION
FDR q = 0.220 | NES = -1.43
f
Cluster 2
HYPOXIA
FDR q = 0.223 | NES = -1.43
g
Cluster 6
HYPOXIA
FDR q = 0.257 | NES = -1.36
h
Cluster 1
OXIDATIVE PHOSPHORYLATION
FDR q = 0.001 | NES = -1.96
i
Cluster 1
HYPOXIA
FDR q = 0.059 | NES = 1.56
Enrichment score (ES)
Ranked list metric (PreRanked)
Rank in Ordered Dataset
'na_pos' (positively correlated)
'na_neg' (negatively correlated)
Enrichment profile
Hits
Ranking metric scores

j

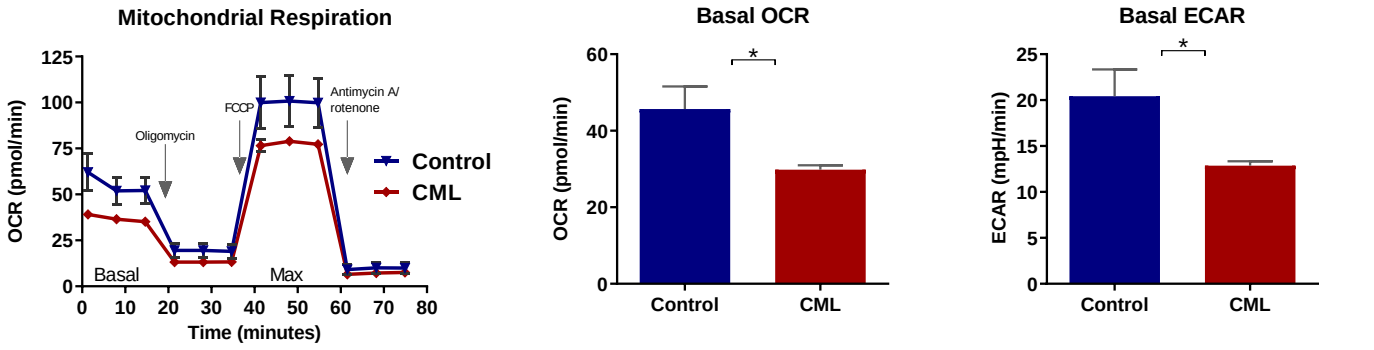

Mitochondrial Respiration
OCR (pmol/min)
Time (minutes)
Oligomycin
FCCP
Antimycin A/
rotenone
Basal
Max
Control
CML
Basal OCR
OCR (pmol/min)
Control
CML
*
Basal ECAR
ECAR (mpH/min)
Control
CML
*

Fig. 5: Subpopulations of CML Exposed Macrophages Down Regulate Inflammatory and Phagocytosis Pathways

a-i: Gene set enrichment analysis (GSEA) of cluster 2 (**a, d, f**), cluster 6 (**b, e, g**) and cluster 1 (**c, h, i**). Normalised enrichment score (NES). False discovery rate (FDR). **j:** Representative oxygen consumption rate (OCR) profile and relative basal OCR and basal ECAR in BMDM following 16h co-culture with control or CML c-Kit^{+} cells (N = 2). Means ± SEM. *P* values were calculated using unpaired Student's *t* test. *p<0.05.

Fig. 6

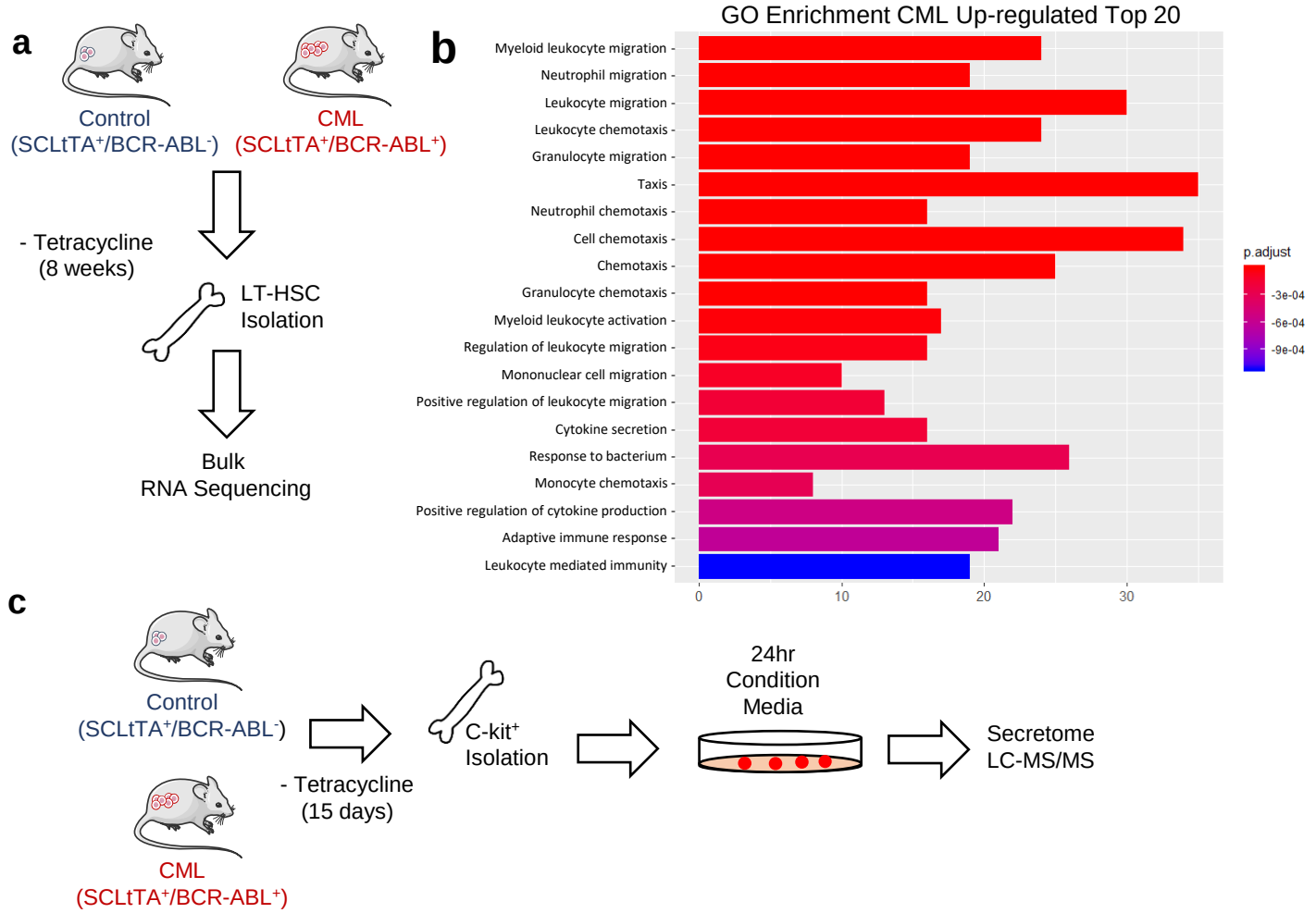

a
Control
(SCLtTA+/BCR-ABL-)
CML
(SCLtTA+/BCR-ABL+)
- Tetracycline
(8 weeks)
LT-HSC
Isolation
Bulk
RNA Sequencing
b
GO Enrichment CML Up-regulated Top 20
Myeloid leukocyte migration
Neutrophil migration
Leukocyte migration
Leukocyte chemotaxis
Granulocyte migration
Taxis
Neutrophil chemotaxis
Cell chemotaxis
Chemotaxis
Granulocyte chemotaxis
Myeloid leukocyte activation
Regulation of leukocyte migration
Mononuclear cell migration
Positive regulation of leukocyte migration
Cytokine secretion
Response to bacterium
Monocyte chemotaxis
Positive regulation of cytokine production
Adaptive immune response
Leukocyte mediated immunity
0
10
20
30
p.adjust
-3e-04
-6e-04
-9e-04
c
Control
(SCLtTA+/BCR-ABL-)
CML
(SCLtTA+/BCR-ABL+)
- Tetracycline
(15 days)
C-kit+
Isolation
24hr
Condition
Media
Secretome
LC-MS/MS
d
CML Secretome
-Log Student's T Test p value
Log2 Fold Change
`q-value`
1.00
0.75
0.50
0.25
0.00
e
CML Differentially Secreted Proteins
FDR<0.05
Log2 FC
HMHA1
KPNA3;KPNA4
NONO
POLR2B
PPP1CB
GNG12
HDLBP
MLEC
ARHGAP25;CDC42BPB
PITPNB
RAP1GDS1
PMM2
CDC37
GUSB
LTF
PEDN2
PLBD1
ELMO1
IL2RG
PCMT1
f
c-Kit+ Secreted LTF
ug/ml
Control
CML
*

Fig. 6: CML c-KIT$^+$ Enriched BM has a Significantly Altered Protein Secretome Compared to WT

A computational and LC-MS proteomic analysis of murine CML cell secretome. **a-b**: Schematic diagram of BM LT-HSC RNA sequencing experimental setup and gene ontogeny (GO) enrichment analysis of significant differentially expressed genes (*FDR <0.05*) in CML LT-HSCs (N = 4 mice per experimental arm). **c**: Schematic diagram of c-kit isolation and conditioned medium generation for secretory proteomics (MS) **d:** Volcano plot representing $\log_2$ fold change between CML and WT mice against $\log_2$ p value for secreted proteins. N = 3 mice per experimental arm). **e:** Log_2 fold change (CML/Control) of significantly changed proteins (FDR <0.05) in CML vs WT. **f:** Lactotransferrin (LTF) ELISA in c-KIT$^+$ conditioned medium (48hr) from SCLtTA$^+$/BCR-ABL$^-$ or SCLtTA$^+$/BCR-ABL$^+$. N = 7-9 samples per experimental arm. Unpaired Student's t-test statistical analysis, *p<0.05, **p<0.01. Secretome data statistical analysis two-sample Student's T test. LT-HSC differential expression calculated by DESeq2 (*fold change < -1 or >1; p<0.05*).

Fig. 7

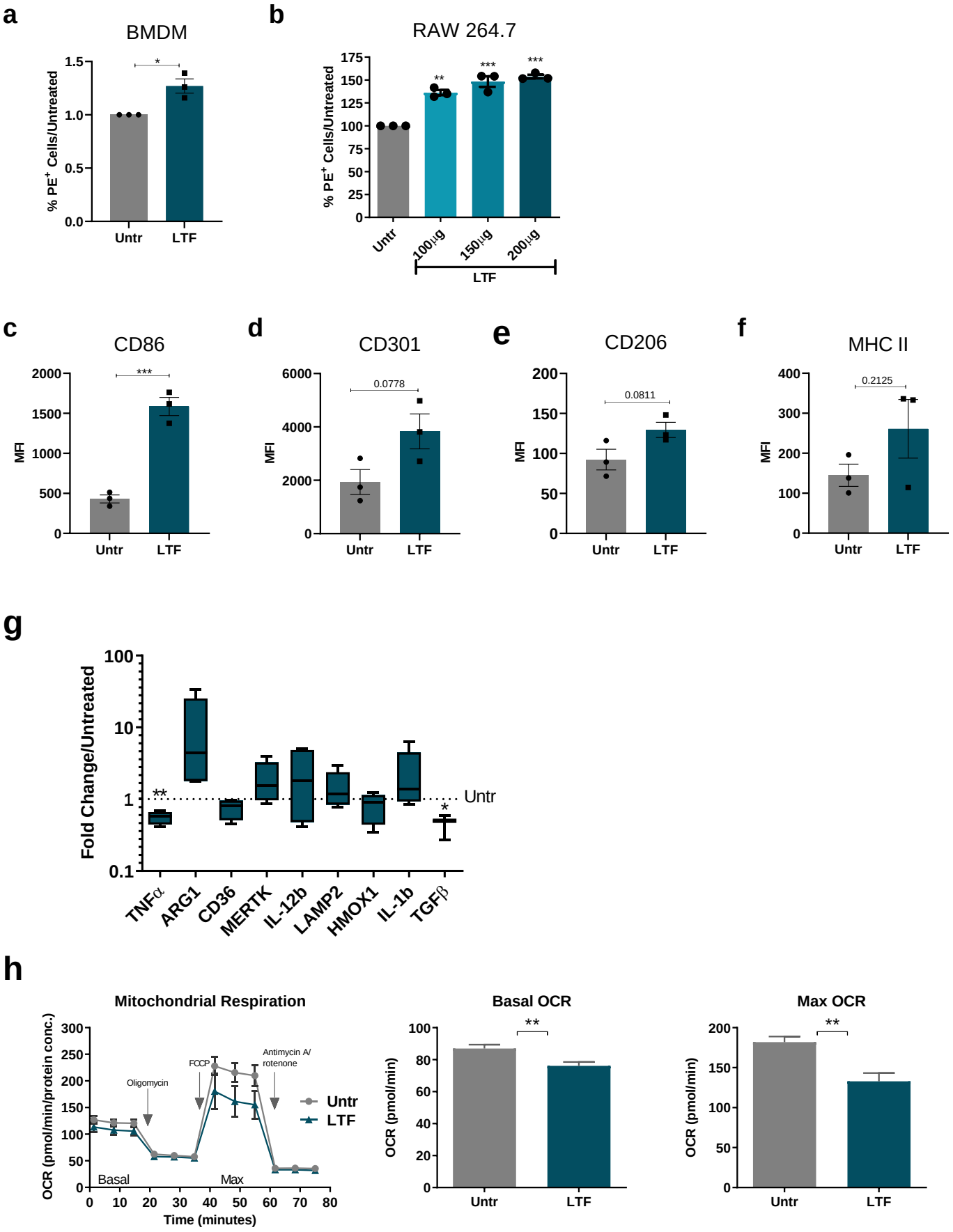

a
BMDM
% PE+ Cells/Untreated
Untr
LTF
b
RAW 264.7
% PE+ Cells/Untreated
Untr
100µg
150µg
200µg
LTF
c
CD86
MFI
d
CD301
0.0778
e
CD206
0.0811
f
MHC II
0.2125
g
Fold Change/Untreated
Untr
TNFα
ARG1
CD36
MERTK
IL-12b
LAMP2
HMOX1
IL-1b
TGFβ
h
Mitochondrial Respiration
OCR (pmol/min/protein conc.)
Oligomycin
FCCP
Antimycin A/ rotenone
Basal
Max
Time (minutes)
Basal OCR
OCR (pmol/min)
Max OCR

Fig. 7: CML c-KIT$^+$ cells Secrete High Levels of Lactotransferrin which Alters Bone Marrow Macrophage Phenotype and Function.

a-b: Murine RAW 264.7 cell line and BMDM were treated with bovine lactotransferrin (LTF) *in vitro* for 24hr (N = 3). Phagocytosis assay of polystyrene beads after treatment with LTF (50-200µg) in BMDM **(a)** and RAW 264.7 cells **(b)**. Cell surface maker expression of CD86 **(c)**, CD301 **(d)**, CD206 **(e)** and MHC II **(f)** on BMDM was measured by flow cytometry. **g:** Log fold change in mRNA expression levels over untreated RAW 264.7 cells following LTF treatment (200µg). **h:** Representative oxygen consumption rate (OCR) profile and relative basal OCR and maximum OCR in BMDM following 24h LTF treatment (N = 2 per experimental arm). Means ± SEM. Statistical analysis of unpaired Student's t-test (**a, c-f, h**), 2-way *ANOVA* (**b, g**), *p<0.05, **p<0.01, *** p<0.001.