SUPPLEMENTARY APPENDIX

Bone marrow endothelial cell-derived interleukin-4 contributes to thrombocytopenia in acute myeloid leukemia

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Supplementary Figure Legends

Supplementary Figure S1. Decreased platelets and megakaryocytes in AML mice.

a. Schematic diagram of the experimental design to establish the MLL-AF9 leukemia murine model. BM c-kit^{+} cells (CD45.1) were infected with the MSCV-MLL/AF9-PGK-puro retrovirus and then transplanted into lethally irradiated mice (CD45.2). Established CD45.1^{+} MLL-AF9 leukemia cells were serially propagated by transplanting 1 x10^{6} spleen cells from AML mice (day 14) into non-irradiated B6-Ly5.2 recipient mice for subsequent experiments.

b. Platelet counts in peripheral blood at indicated time points of AML. n=5 mice for each time point, three independent experiments.

c. Total number per limb of MKs in bone marrow at indicated time points of AML. n=5 mice for each time point, three independent experiments.

d. Representative flow cytometric plots of megakaryocytes (MKs) from healthy control and AML (Day14) mice bone marrow.

Significant difference: ** $p<0.01$, *** $p<0.001$. Error bars show SEM.

Supplementary Figure S2. Representative flow cytometric plots of HSPCs from healthy control and AML (Day 14) mice bone marrow.

Supplementary Figure S3. Intact survival, cell cycle state and megakaryocytic potential of PreMegE and MkPs from AML bone marrow.

a. Percentage of Annexin V+ apoptotic cells in PreMegE subset at indicated time points of AML. n=5 for each time point, three independent experiments.

b. Percentages of cells in different phase of cell cycle in PreMegE subset at indicated time points of AML. n=5 for each time point, three independent experiments.

c-d. Representative flow cytometric plots (c) and total number (d) of CD150$^{+/high}$ CD41$^{+/high}$ megakaryocytic lineage formed by 1000 PreMegE cells which were isolated from control or AML (Day 14) bone marrow and cultured *in vitro* with

mSCF (50ng/ml), mTPO (50ng/ml), mIL-3 (20ng/ml) and EPO (2U/ml) for 3 days. n=5, three independent experiments.

e-f. Representative flow cytometric plots (e) and percentage of Annexin V+ apoptotic cells (f) in MkP subset at indicated time points of AML. n=5 for each time point, three independent experiments.

g. Percentages of cells in different phases of cell cycle in MkP subset at indicated time points of AML. n=5 for each time point, three independent experiments.

h. Number of megakaryocyte colonies (CFU-MKs) formed from 2000 MkPs isolated from control or AML (Day14) bone marrow. n=4 mice per group, two independent experiments

Significant difference: ** $p<0.01$, *** $p<0.001$. ns, no significant difference. Error bars show SEM.

Supplementary Figure S4. Differential gene expression of MKs from control and AML mice.

a. Volcano plot of differentially expressed genes between control and AML MKs. The X-axis represents the log2 fold change of gene expression levels. The Y-axis represents the –log10 P-value. Significantly upregulated genes are represented as ‘red’ dots and significant downregulated genes are represented as ‘green’ dots.

b. Heatmap of cytokine receptor expression in control and AML MKs. The color scale indicates expression values.

Supplementary Figure S5. TPO protein levels in serum of control and AML mice based on ELISA measurements.

Supplementary Figure S6. Blocked polyploidization of megakaryocytes in AML bone marrow.

a. Proportions in normal hematopoietic cells of MKs with different ploidy in mice bone marrow at indicated time points of AML. Data were normalized to those values of healthy control and presented as the folds of control. n=4-5 for each time point, three independent experiments.

b. Representative flow cytometric plot of MK ploidy distribution in healthy control and AML (Day14) mice bone marrow.
Significant difference: * $p<0.05$, ** $p<0.01$. Error bars show SEM.

Supplementary Figure S7. Inhibition of IL-4 or induction chemotherapy alone could not result in platelet recovery in AML.

a. Schematic diagram of the experimental design: CD45.1+ leukemia cells were injected intravenously into mice on day 1. 10 mg/kg/day of anti-mIL-4 or PBS was administrated intraperitoneally on days 7, 9 and 11. Mice were sacrificed on day 13 for analysis.

b. Counts of platelets, erythrocytes and leukocytes in peripheral blood of mice injected with PBS or anti-mIL-4. n=5-6 mice per group, three independent experiments.

c. Percentage of CD45.1+ leukemia cells engraftment measured via bone marrow treated with PBS or anti-mIL-4. n=5-6 mice per group, three independent experiments.

d. Schematic of the experiment: CD45.1+ leukemia cells were injected intravenously into mice on day 1. 60 mg/kg/day of Arac was administrated intraperitoneally for one week from day 15. PB analysis was performed one day after the last administration of AraC.

e. Percentage of CD45.1+ leukemia cells engraftment measured via PB before (day 15) and after (day 22) chemotherapy. n=5 mice per group, three independent experiments.

f. Survival curves of AML mice treated with Arac or not. n=5 mice per group, three independent experiments.

g. Counts of platelets in peripheral blood of mice injected with AraC or not. n=5 mice per group, three independent experiments.

h. IL-4 protein levels in BM plasma of healthy control, AML control (Day 14) mice and AML mice receiving chemotherapy based on ELISA measurements. n=5 mice per group, three independent experiments.

i. TPO protein levels in serum of AML mice injected with AraC or combined with

anti-mIL-4. n=5 mice per group, three independent experiments.

Significant difference: * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$. ns, no significant difference. Error bars show SEM.

Supplementary Table S1. Cell surface markers for phenotypical analyses of hematopoietic cells and niche cells by flow cytometry

Cell surface markers for flow cytometric analyses

Cell type	Surface markers
LT-HSC	Lin- c-Kit+ Sca1+ CD150+CD48-
MPP2	Lin- c-Kit+ Sca1+ CD150+CD48+
PreMegE	Lin- c-Kit+ Sca1+CD105-CD150+CD41-
CMP	Lin- c-Kit+ Sca1- CD16/32- CD34+
MEP	Lin- c-Kit+ Sca1- CD16/32- CD34-
MkP	Lin- c-Kit+ Sca1- CD150+ CD41+
MK	SSChigh CD41high
EC	CD45- Ter119- CD31+
MSC	CD45- Ter119- CD31- CD51+ Sca1+
OBC	CD45- Ter119- CD31- CD51+ Sca1-

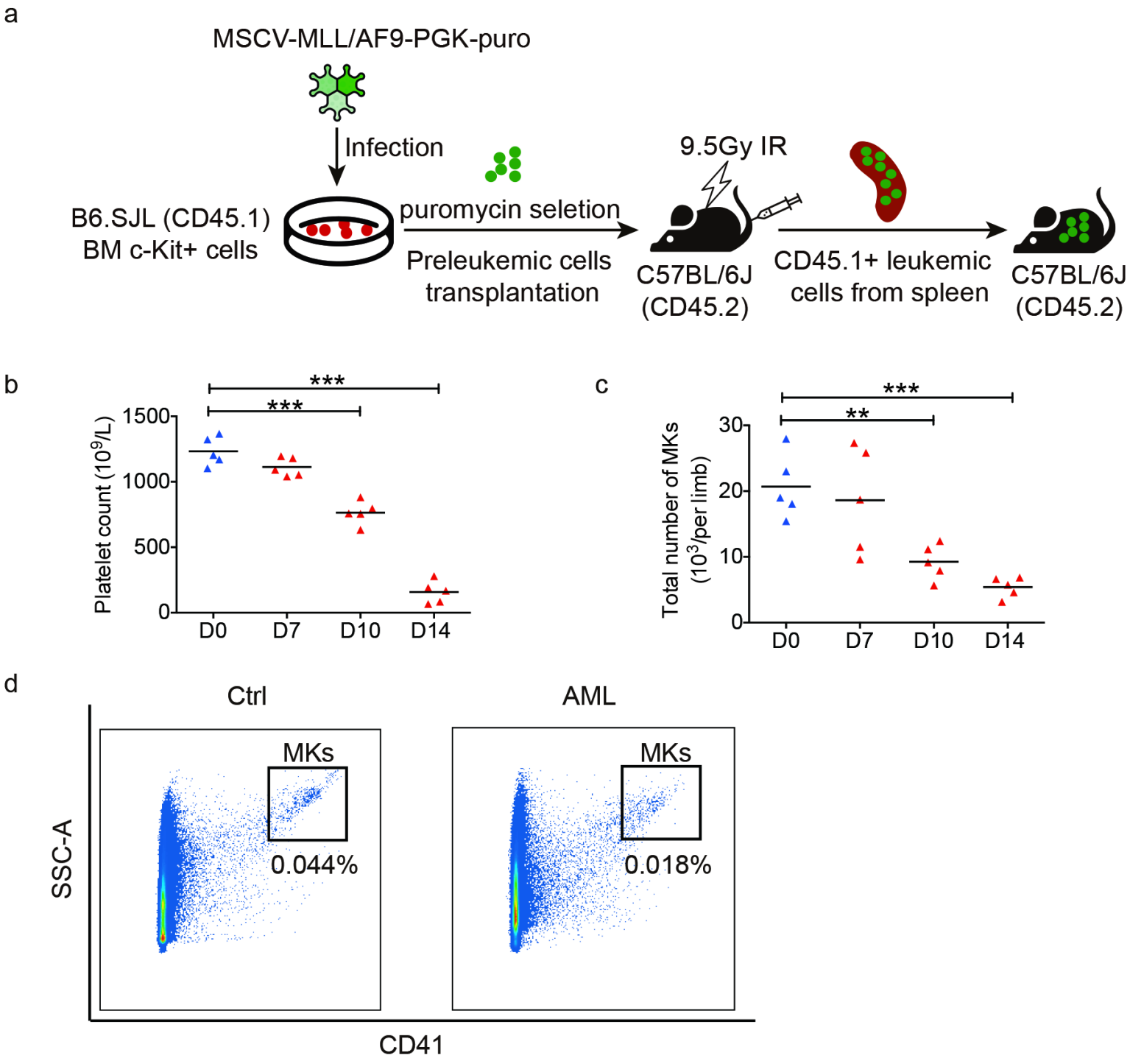
Supplementary Table S2. Antibodies used for flow cytometry

Antibodies used for flow cytometry

Antibody conjugate	Clone	Supplier
B220 PE-CY7	RA3-6B2	eBioscience
B220 Biotin	RA3-6B2	eBioscience
CD3e Biotin	145-2C11	eBioscience
CD3e PE-CY7	145-2C11	eBioscience
CD4 Biotin	RM4-5	eBioscience
CD4 PE-CY7	RM4-5	eBioscience
CD8a Biotin	53-6.7	eBioscience
CD8a PE-CY7	53-6.7	eBioscience
Ter-119 Biotin	Ter119	eBioscience
Ter-119 PE-CY7	Ter119	eBioscience
Ter-119 PE	Ter119	eBioscience
Mac-1 Biotin	M1/70	eBioscience
Mac-1 PE-CY7	M1/70	eBioscience
Mac-1 APC-eFluor 780	M1/70	eBioscience
Gr-1 (Ly-6G) Biotin	RB6-8C5	eBioscience
Gr-1 (Ly-6G) PE-CY7	RB6-8C5	eBioscience
Gr-1 (Ly-6G) APC-eFluor 780	RB6-8C5	eBioscience
Streptavidin APC-eFluor 780	-	eBioscience
Streptavidin BrilliantViolet 421	-	BioLegend
c-Kit (CD117) APC	2B8	eBioscience
CD34 Biotin	RAM34	eBioscience
CD34 FITC	RAM34	eBioscience
CD41 APC	MWReg30	eBioscience
CD41 PerCP-eFluor 710	MWReg30	eBioscience
Flt3 (CD135) PE	A2F10	eBioscience
Sca-1 PE-Cy7	D7	eBioscience
Sca-1 APC-Cy7	D7	BioLegend
CD124 BrilliantViolet 421	mIL-4R-M1	BD Bioscience
CD16/32 PE	93	eBioscience
CD150 PE	TC15-12F12.2	BioLegend

CD45.1 PE-CY7	A20	eBioscience
CD45 FITC	30-F11	eBioscience
CD51 PE	RMV-7	eBioscience
CD31 APC	390	eBioscience
Annexin V FITC	-	BD Bioscience
CD105 biotin	MJ7/18	eBioscience
Anti-Von Willebrand Factor antibody	ab6994	abcam
Ki67 FITC	7B11	eBioscience
Ki67 PE	B56	BD Bioscience
Donkey anti-Rabbit AF488	-	Invitrogen
Stat6 (pY641) Alexa Fluor-647	-	BD Bioscience

Supplementary Figure S1. Decreased platelets and megakaryocytes in AML mice.



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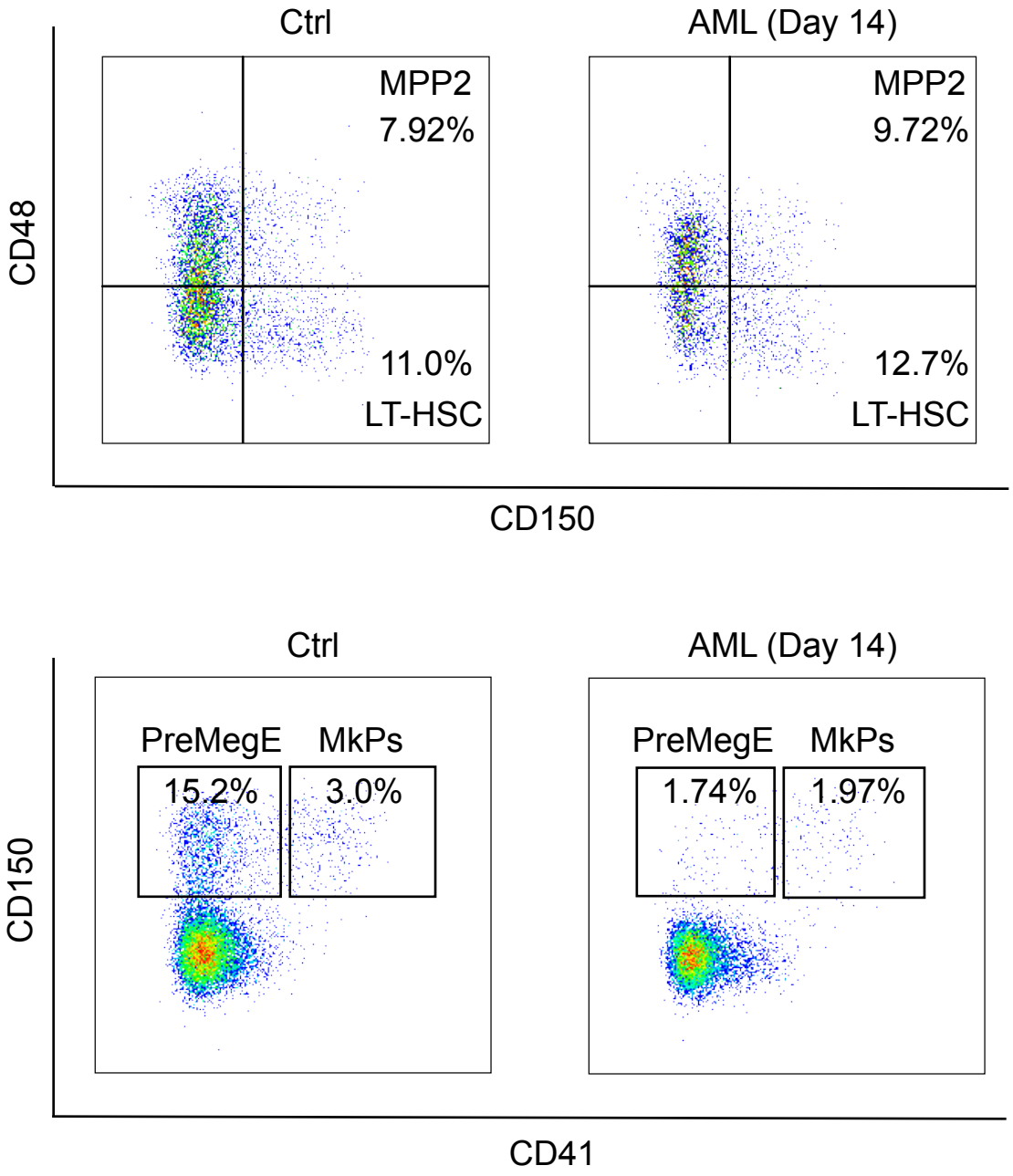

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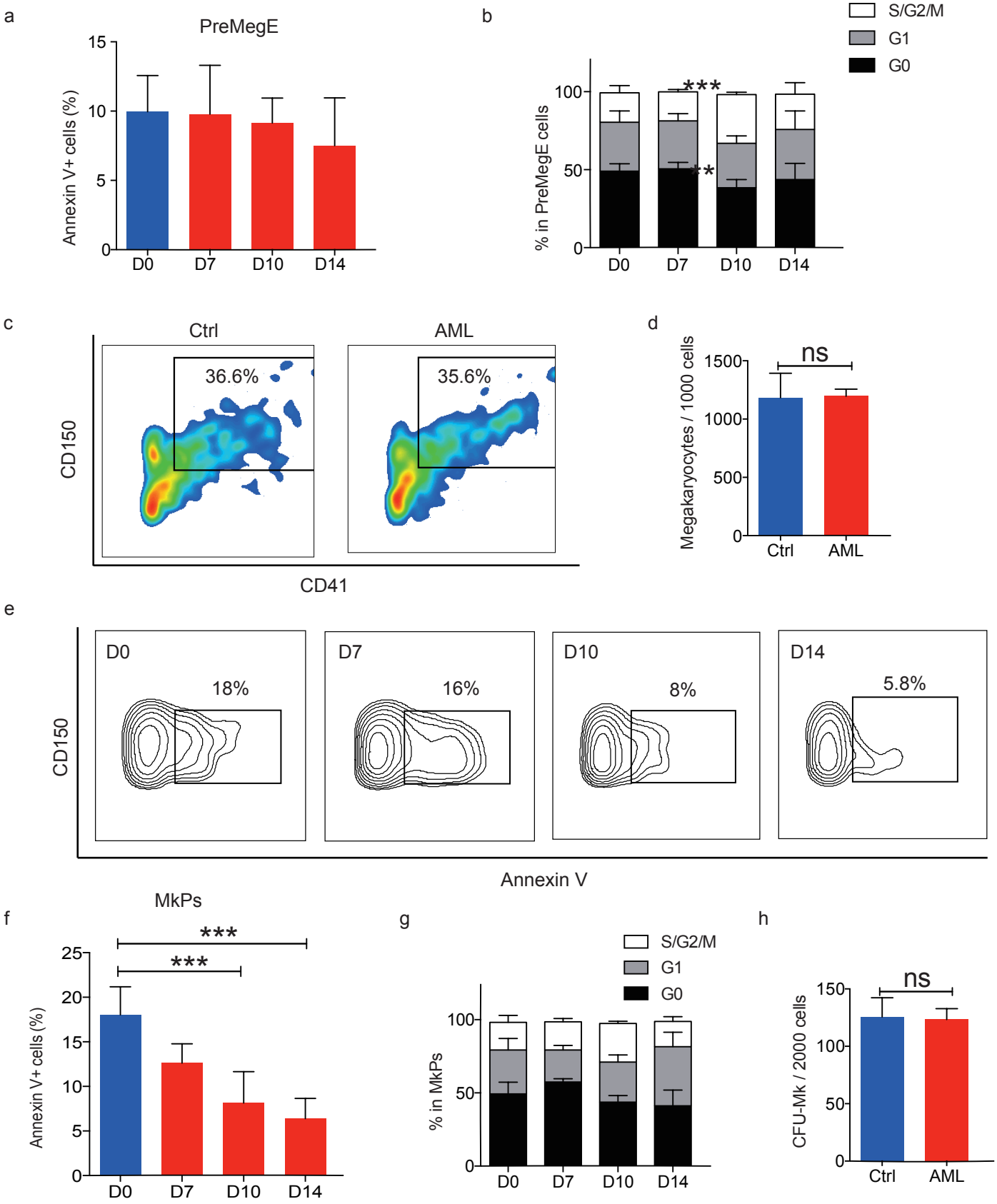

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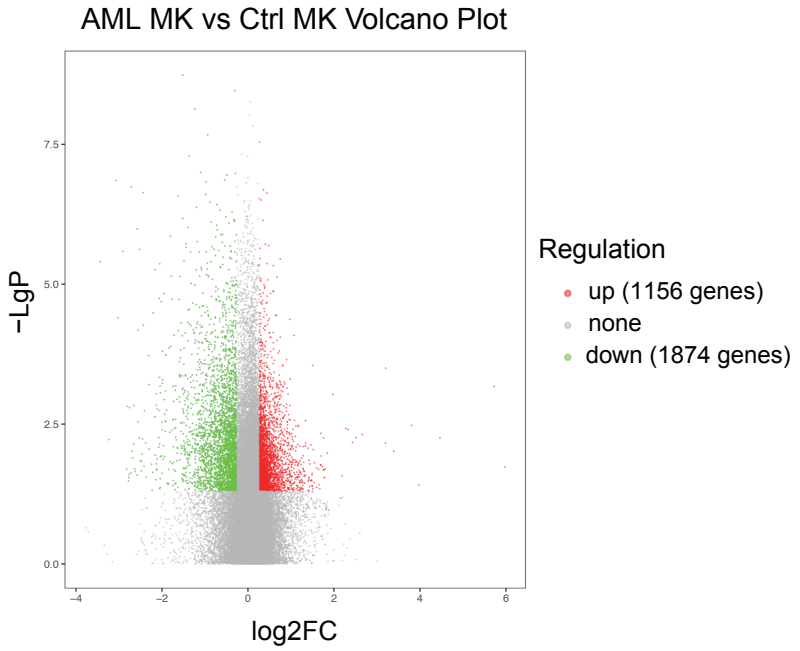
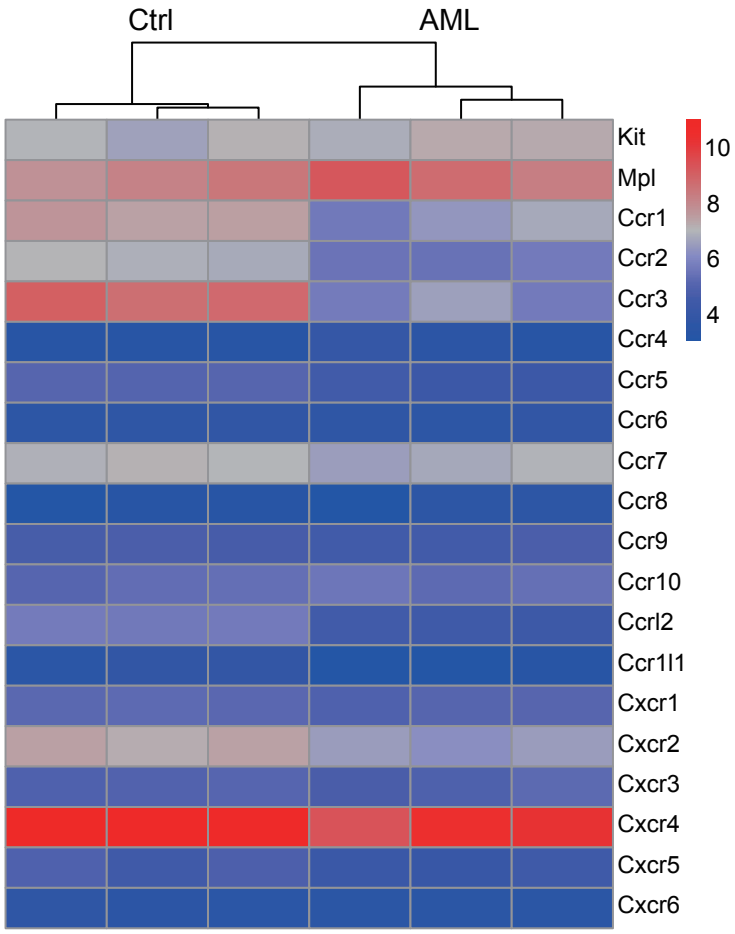
a



b



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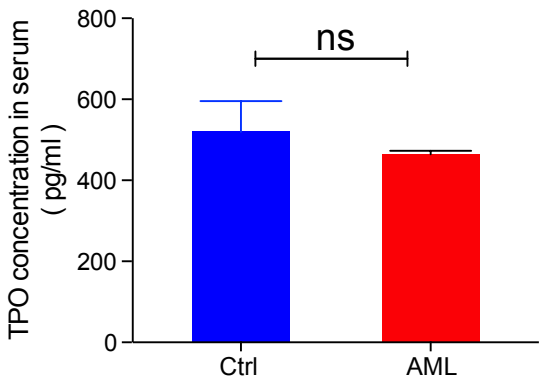

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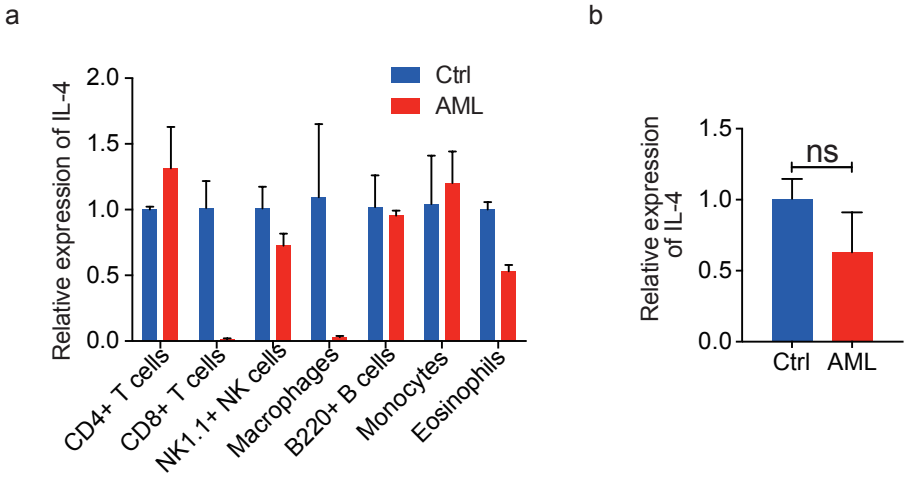

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