

Supplemental Information

A living enzymatic adenosine switch couples metabolic rescue to IL-12-driven antitumor immunity

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Supplementary materials**Supplementary Table 1. Cell names, media and source of cell lines**

Cell line name	Species	Disease model	Cell culture media	Supplements	Purchased from
NOMO-1	Human	Acute myeloid leukemia	RPMI	10% FBS; 1% P/S	ATCC
OCI-AML3	Human	Acute myeloid leukemia	RPMI	10% FBS; 1% P/S	ATCC
AsPC1	Human	Pancreatic adenocarcinoma	RPMI	10% FBS; 1% P/S	ATCC
B16-F10	Mouse	Melanoma	DMEM	10% FBS; 1% P/S	ATCC
WEHI-3B	Mouse	Myelomonocytic leukemia	RPMI	10% FBS; 1% P/S	Cytion
4T1	Mouse	Mammary carcinoma	RPMI	10% FBS; 1% P/S	ATCC
HEK-Blue-GFP™ IL-12 Cells	Human	Human embryonic kidney cells	DMEM	10% HI-FBS; HEK-Blue Selection; Normocin; 1% P/S	InvivoGen

Supplementary Table 2. Antibodies and Live/Dead dye

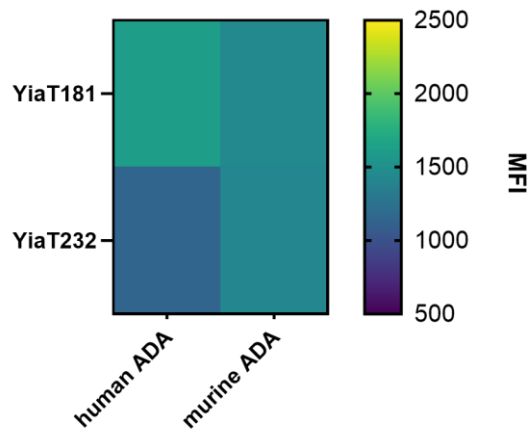
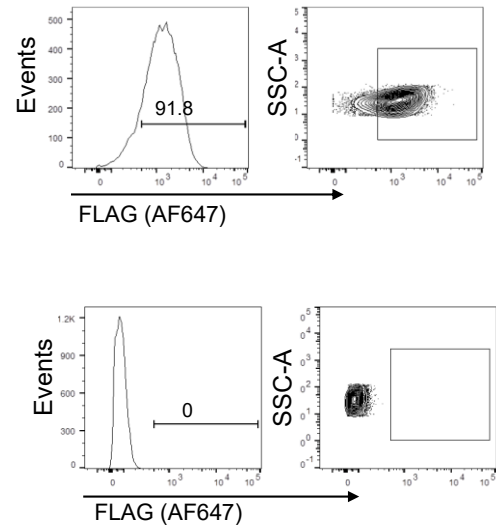
Target	Species	Fluorochrome	Clone	Supplier
MitoTracker FM	—	FITC	—	MedChemExpress
anti- <i>E. coli</i>	<i>E. coli</i>	FITC	Polyclonal	Abcam
A2aR	Human	PE	—	R&D Systems/Bio-Techne
A2bR	Human	AF700	—	R&D Systems/Bio-Techne
CD107a	Human	BV650	H4A3	BioLegend
CD16	Human	BV605	3G8	BioLegend
CD16	Human	PerCP/Cy5.5	3G8	BioLegend
CD16	Human	APC-Cy7	3G8	BioLegend
CD203a	Human	APC	—	BioLegend
CD25	Human	Pacific Blue	BC96	BioLegend
CD26	Human	PerCP/Cy5.5	BA5b	BioLegend
CD3	Human	APC-CY7	HIT3a	BioLegend
CD3	Human	BV510	HIT3a	BioLegend
CD3	Human	Spark UV395	SK7	BioLegend
CD3	Human	PerCP/Cy5.5	HIT3a	BioLegend
CD38	Human	AF700	HIT2	BioLegend
CD39	Human	PE/Dazzle 594	A1	BioLegend
CD4	Human	PE/Cy7	RPA-T4	BioLegend
CD4	Human	AF700	RPA-T4	BioLegend
CD45	Human	PerCP/Cy5.5	HI30	BioLegend
CD45	Human	FITC	2D1	BioLegend
CD45	Human	APC/Cy7	2D1	BioLegend
CD56	Human	BV570	HCD56	BioLegend
CD69	Human	APC/Cy7	FN50	BioLegend
CD69	Human	BV650	FN50	BioLegend
CD69	Human	Pacific Blue	FN50	BioLegend
CD73	Human	PE/Cy7	AD2	BioLegend
CD8	Human	APC/Cy7	SK1	BioLegend
CD94	Human	APC/Cy7	DX22	BioLegend
CTLA-4	Human	APC	BNI3	BioLegend
DNAM-1	Human	BV785	11A8	BioLegend
FoxP3	Human	R718	236A/E7	BD Biosciences
HA.11	—	APC	16B12	BioLegend
IFN- γ	Human	PE/Cy7	—	BD Pharmingen
Ki-67	Human	BV711	ki-67	BioLegend
KIR2DL2/3 (CD158b)	Human	PE/Cy7	HP-MA4	BioLegend
KIR2DL2/3 (CD158b)	Human	APC	HP-MA4	BioLegend
NKG2D	Human	BV785	1D11	BioLegend
NKp30	Human	PE/Dazzle 594	P30-15	BioLegend
NKp30	Human	BV711	P30-15	BioLegend
NKp46	Human	AF700	9E2	BioLegend
PD-1	Human	APC	A17188A	BioLegend
PD-1	Human	PE	A17188A	BioLegend
TIM-3	Human	BV650	A18087E	BioLegend
TNF- α	Human	FITC	MAb11	BD Pharmingen
Live/Dead	Human/Mouse	Zombie NIR	—	BioLegend
DYKDDDDK	Human/Mouse	AF647	L5	BioLegend
B220	Mouse	PE/Dazzle 594	RA3-6B2	BioLegend

CD11b	Mouse	FITC	M1/70	BioLegend
CD11b	Mouse	BV711	M1/70	BioLegend
CD11c	Mouse	PerCP/Cy5.5	N418	BioLegend
CD19	Mouse	PE	6D5	BioLegend
CD25	Mouse	PerCP/Cy5.5	3C7	BioLegend
CD3	Mouse	Alexa Fluor 700	17A2	BioLegend
CD4	Mouse	PE-Cy7	GK1.5	BioLegend
CD4	Mouse	APC/Cy7	RM4-5	BioLegend
CD4	Mouse	RB705	GK1.5	BioLegend
CD44	Mouse	Spark Red 718	IM7	BioLegend
CD44	Mouse	R718	IM7	BioLegend
CD45	Mouse	APC/Cy7	30-F11	BioLegend
CD45	Mouse	PE	30-F11	BioLegend
CD49b	Mouse	APC	HMA2	BioLegend
CD62L	Mouse	Spark PLUS UV395	W18021D	BioLegend
CD69	Mouse	Pacific Blue	H1.2F3	BioLegend
CD8a	Mouse	BV750	S18018E	BioLegend
F4/80	Mouse	APC	BM8	BioLegend
FoxP3	Mouse	BV421	MF-14	BioLegend
Ly6C	Mouse	BV785	HK1.4	BioLegend
Ly6G	Mouse	BUV395	1A8	BioLegend
NKp46	Mouse	PE/Cy7	29A1.4	BioLegend
PD-1	Mouse	PE	RMP1-30	BioLegend
TIM-3	Mouse	BV785	RMT3-23	BioLegend
XCR1	Mouse	Pacific Blue	ZET	BioLegend
Immunohistochemistry				
E. coli serotype O/K	Escherichia coli	Unconjugated	Polyclonal	Thermo Fisher Scientific (Invitrogen)
DYKDDDDK	species-independent	Unconjugated	D6W5B	Cell Signaling Technology
Immunofluorescence				
<i>E. coli</i>	Escherichia coli	FITC	Polyclonal	Abcam
Nucleus	species-independent	DAPI		Biotium

Supplementary Table 3. Apparent Michaelis–Menten kinetic parameters of bacterial surface-displayed adenosine deaminase (ADA) constructs

Construct / control	V _{max}	V _{max} CI lower	V _{max} CI upper	K _m	K _m CI lower	K _m CI upper	R ²	Sum of squares	Sy.x	n
Y181-hADA	19.0	15.0	23.2	0.6	NE	2.8	0.2	517.8	6.1	4.0
Y181-mADA	41.7	34.8	49.0	6.5	1.7	18.0	0.7	816.9	7.6	4.0
Y232-hADA	23.3	19.4	27.7	1.5	0.4	6.4	0.5	410.1	5.4	4.0
Y232-mADA	33.7	28.3	39.7	3.4	0.9	11.7	0.7	493.5	5.9	4.0
Y181-mADA-hIL12	36.4	30.0	44.9	2.3	0.5	12.4	0.6	932.7	8.2	4.0
Y181-mADA-mIL12	39.6	33.6	46.2	3.7	1.2	10.5	0.7	766.5	7.4	4.0
Y181-hIL12-mADA	35.1	30.6	39.9	3.1	1.3	7.2	0.8	476.7	5.8	4.0
Y181-mIL12-mADA	34.5	28.5	41.5	1.9	0.5	8.5	0.5	897.5	8.0	4.0
YiaT181 ctr	10.1	8.5	11.8	0.1	NE	0.6	0.0	98.2	2.7	4.0
YiaT232 ctr	9.0	7.5	10.6	0.1	NE	0.7	0.0	89.3	2.5	4.0
Soluble ADA (0.5 U)	40.2	34.8	45.7	1.1	0.4	3.0	0.6	817.9	7.6	4.0

Abbreviations: ADA, adenosine deaminase; hADA, human ADA; mADA, murine ADA; IL-12, interleukin-12; V_{max}, maximum reaction velocity (U/L); K_m, Michaelis constant (μM); CI, confidence interval; R², coefficient of determination; Sy.x, residual standard deviation (U/L); NE, not estimable; n, independent biological replicates.

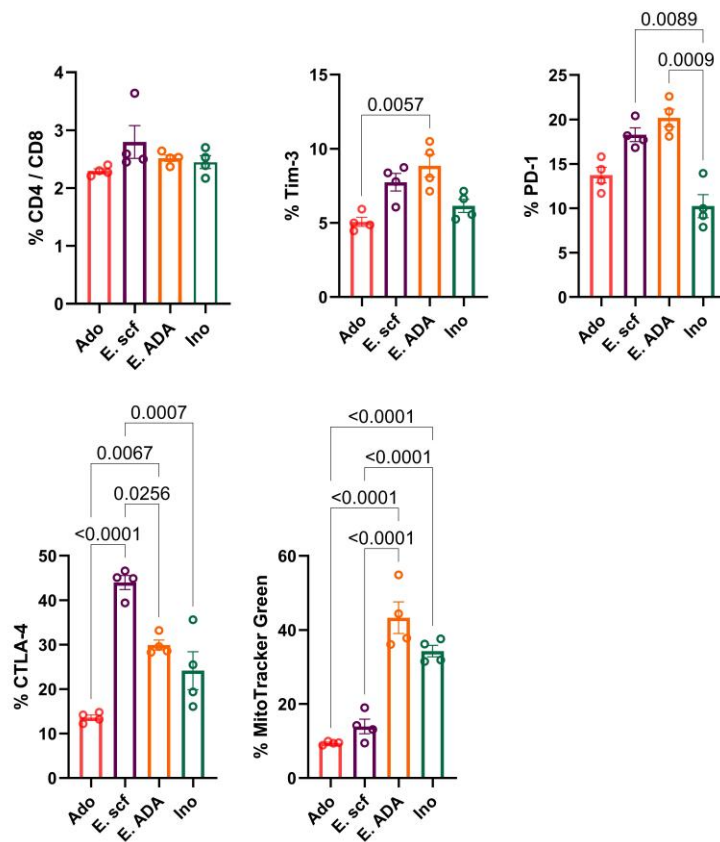
Supplementary Figure 1**a****b**

Supplementary Fig. 1. Non-pathogenic *E. coli* surface display ADA. **a**, Median fluorescence intensity (MFI) of FLAG staining for human adenosine deaminase (hADA) and murine ADA (mADA) displayed using YiaT181 or YiaT232 scaffold constructs. **b**, Representative flow-cytometric histograms and dot plots of FLAG staining in YiaT181-ADA-displaying bacteria (top) and FLAG-negative control bacteria (bottom). FLAG fluorescence histograms (left) and corresponding FLAG versus side-scatter area (SSC-A) plots (right) show the gates used to identify FLAG-positive bacteria; numbers indicate the percentage of FLAG-positive events.

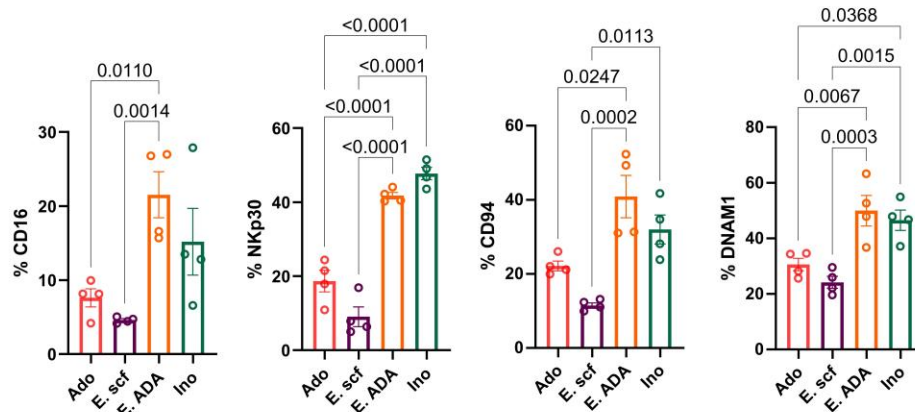
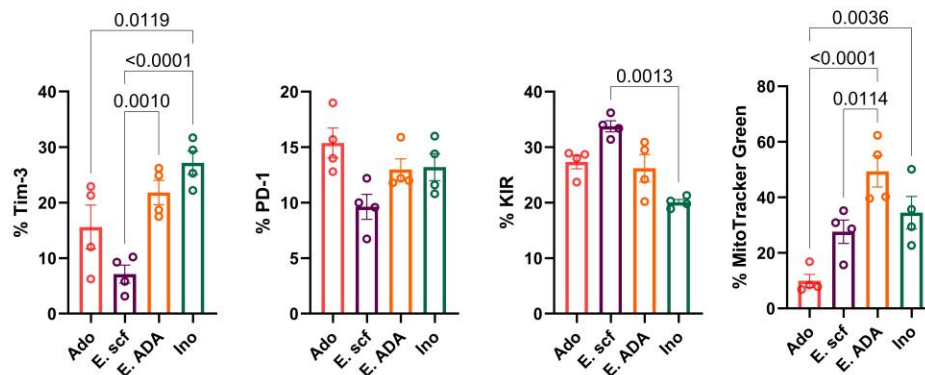
Supplementary Figure 2

a

b



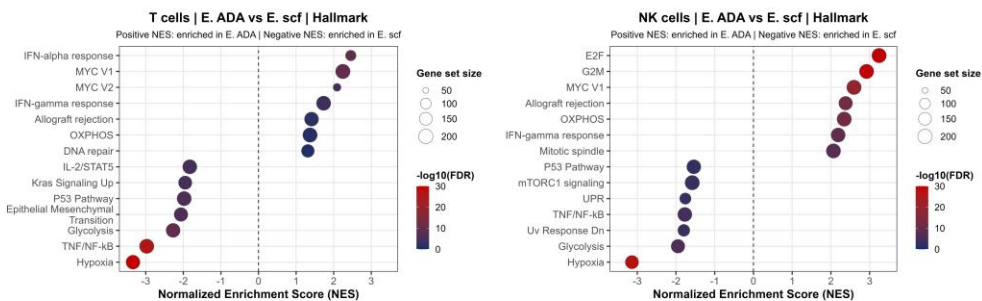
Supplementary Fig. 2. ADA modulates T-cell immune-regulatory phenotype under adenosine-mediated stress. **a**, CD4 to CD8 ratio, and **b**, expression of the immune-regulatory receptors TIM-3, PD-1, CTLA-4 and MitoTracker Green on CD3⁺ T cells following the same treatments. Mean±s.e.m. of n=4 independent donors. Individual points represent biologically independent donors. One-way ANOVA with Tukey's post-hoc test.

Supplementary Figure 3**a****b**

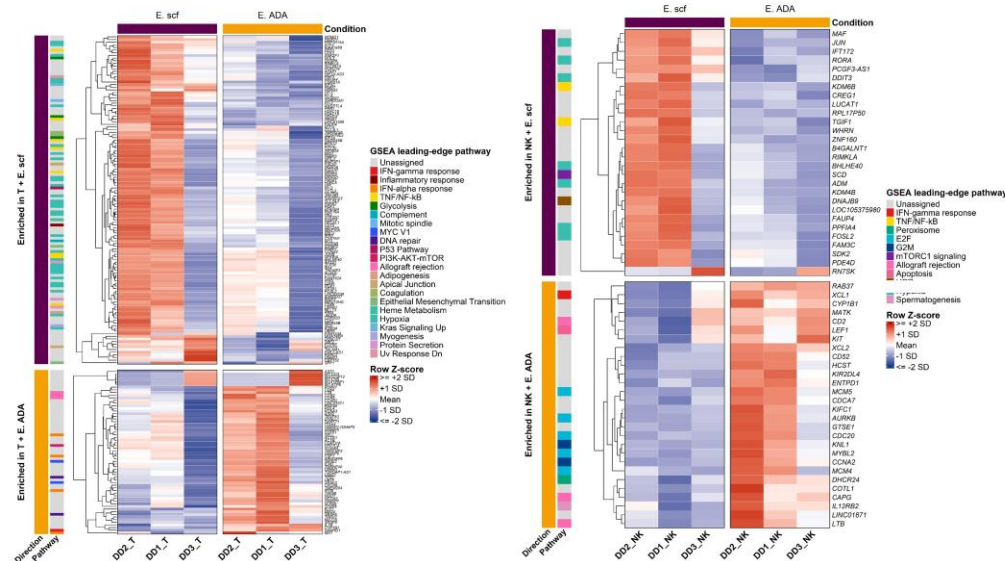
Supplementary Fig. 3. ADA modulates NK-cell immune-regulatory phenotype under adenosine-mediated stress. a, Expression of the NK-cell phenotypic and activation-associated markers CD16, NKp30, CD94 and DNAM-1 following exposure to ADO alone or treatment with scaffold-control (E. scf) or ADA-displaying (E. ADA) bacteria. **b**, Expression of the immune-regulatory receptors TIM-3, PD-1 and KIR on NK cells following the same treatments. Mean $\pm$ s.e.m. of n=4 biological replicates and independent donors. Individual points represent biologically independent donors. **(a-b)** One-way ANOVA with Tukey's post-hoc test.

Supplementary Figure 4

a



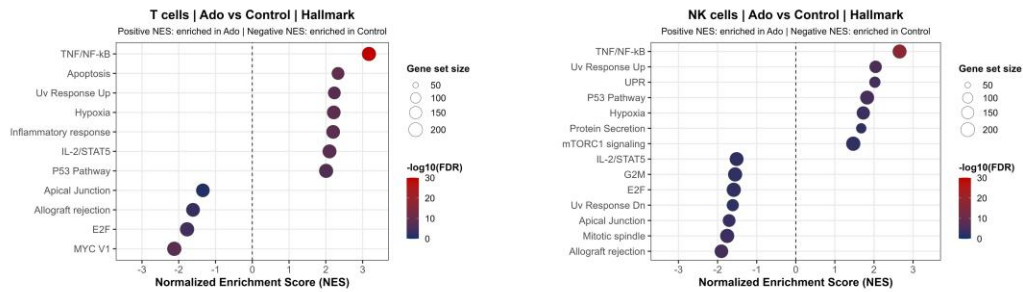
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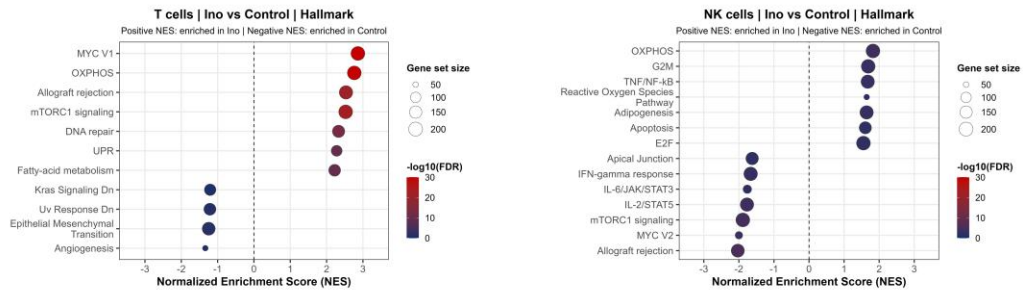
Supplementary Fig. 4. ADA induces distinct transcriptional programs in human T and NK cells under adenosine-mediated suppression. **a**, Gene set enrichment analysis (GSEA) of MSigDB Hallmark pathways in primary human T cells (left) and NK cells (right) ($n=3$ donors) exposed to E. ADA compared with E. scf. Positive normalized enrichment scores (NES) indicate enrichment in E. ADA and negative NES enrichment in E. scf. Dot size indicates gene-set size and color indicate $-\log_{10}$ false-discovery rate (FDR). **b**, Pathway-independent heatmaps of differentially expressed genes in T cells (left) and NK cells (right) ($n=3$ donors) exposed to E. ADA versus E. scf. Differentially expressed genes were identified independently of pathway membership, separated according to the direction of differential expression and hierarchically clustered within each direction. Columns represent individual donors and colors indicate gene-wise z-scores of variance-stabilized expressions, truncated at ± 2 . Side annotations indicate subsequent membership in selected Hallmark GSEA leading-edge gene sets; genes not represented in the displayed leading edges are labelled unassigned. GSEA pathway membership did not influence gene selection or clustering. Differential expressions were analyzed using DESeq2 with donors included in the model.

Supplementary Figure 5

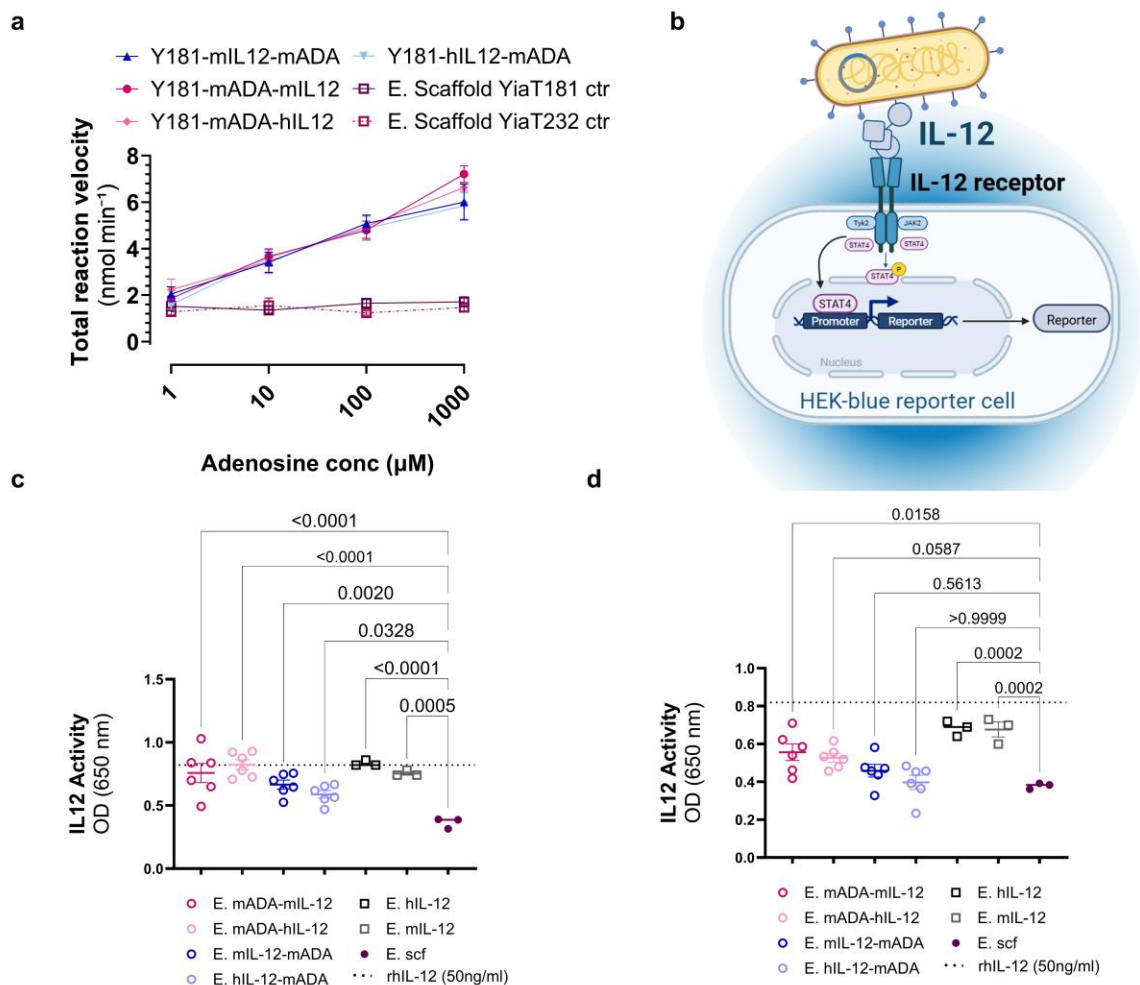
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b



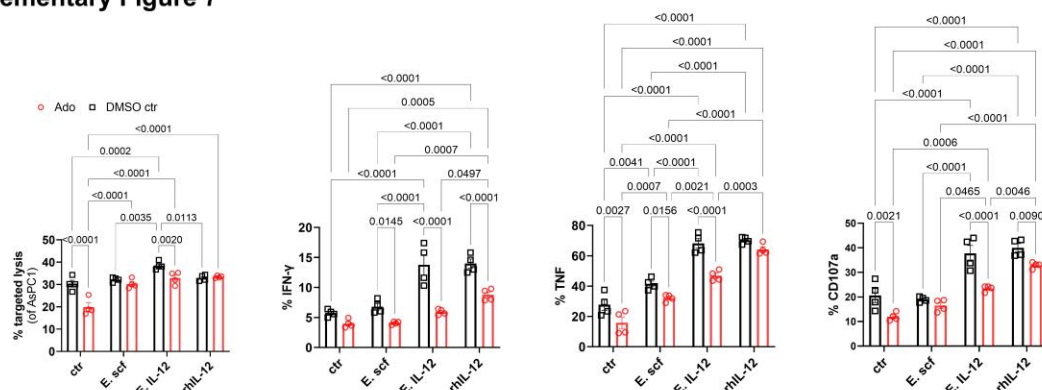
Supplementary Fig. 5. ADO and INO induce distinct transcriptional programs in human T- and NK cells. **a**, Gene set enrichment analysis (GSEA) of MSigDB Hallmark gene sets in primary human T cells (left) and NK cells (right) from n=3 biologically independent donors following exposure to 1 mM adenosine (ADO) or untreated control. **b**, Gene set enrichment analysis of MSigDB Hallmark gene sets in primary human T cells (left) and NK cells (right) from n=3 biologically independent donors following exposure to 1 mM inosine (INO) or untreated control. In **a,b**, positive normalized enrichment scores (NES) indicate enrichment following ADO or INO exposure, respectively, whereas negative NES indicate enrichment in untreated controls. Each dot represents a gene set; dot size indicates gene-set size and color indicate $-\log_{10}$ false-discovery rate (FDR). FDR, false-discovery rate; GSEA, gene set enrichment analysis; NES, normalized enrichment score.

Supplementary Figure 6**Supplementary Fig. 6. Functional characterization of dual ADA–IL-12 bacterial constructs.**

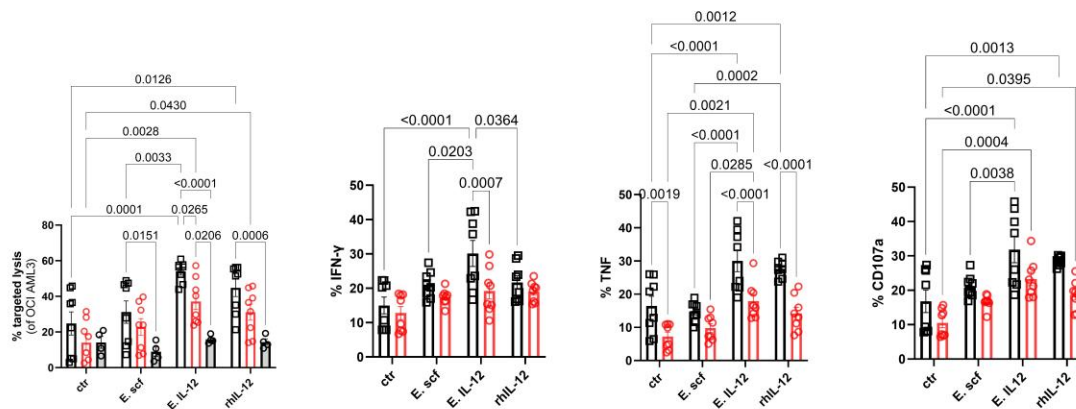
a, ADA-dependent reaction velocity across increasing adenosine concentrations for four dual ADA–IL-12 construct combinations displaying mADA tethered to either mouse or human IL-12 in both fusion orientations, compared with the corresponding YiaT181 and YiaT232 scaffold controls. Data represents four replicates from two independent experiments. **b**, Schematic of the HEK-Blue IL-12 reporter assay used to assess IL-12 bioactivity. **c,d**, HEK-Blue IL-12 reporter activity following exposure to the indicated dual ADA–IL-12 constructs, bacteria displaying IL-12 alone, scaffold control and recombinant IL-12, tested at bacteria-to-reporter-cell ratios of 1:100 (**c**) and 1:10 (**d**). Reporter activity was quantified by absorbance at 655 nm. Mean±s.e.m. of $n=4$ (**a**), $n=6$ (E. ADA-IL-12 and E. IL-12-ADA constructs), $n=3$ (E. IL-12 and E. scf) (**c,d**) biological replicates from two independent experiments. V_{max} and K_m estimates with 95% confidence intervals are provided in Supplementary Table S3 (**a**). Two-way ANOVA with Tukey's multiple-comparisons test (**a,c,d**).

Supplementary Figure 7

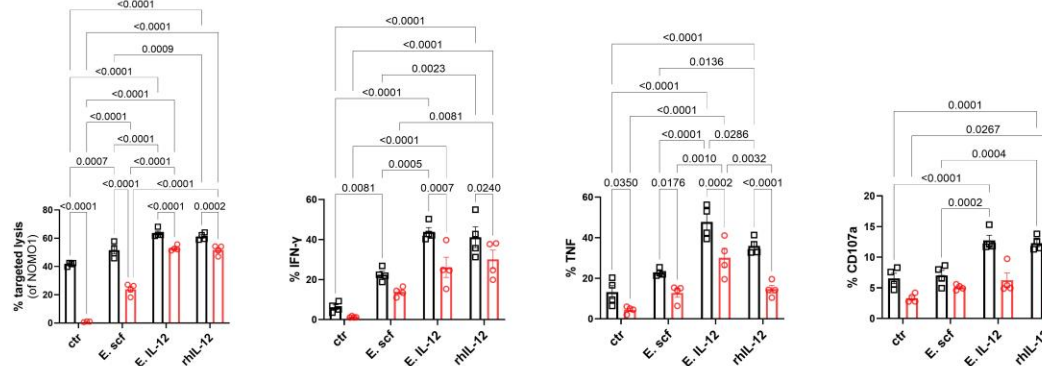
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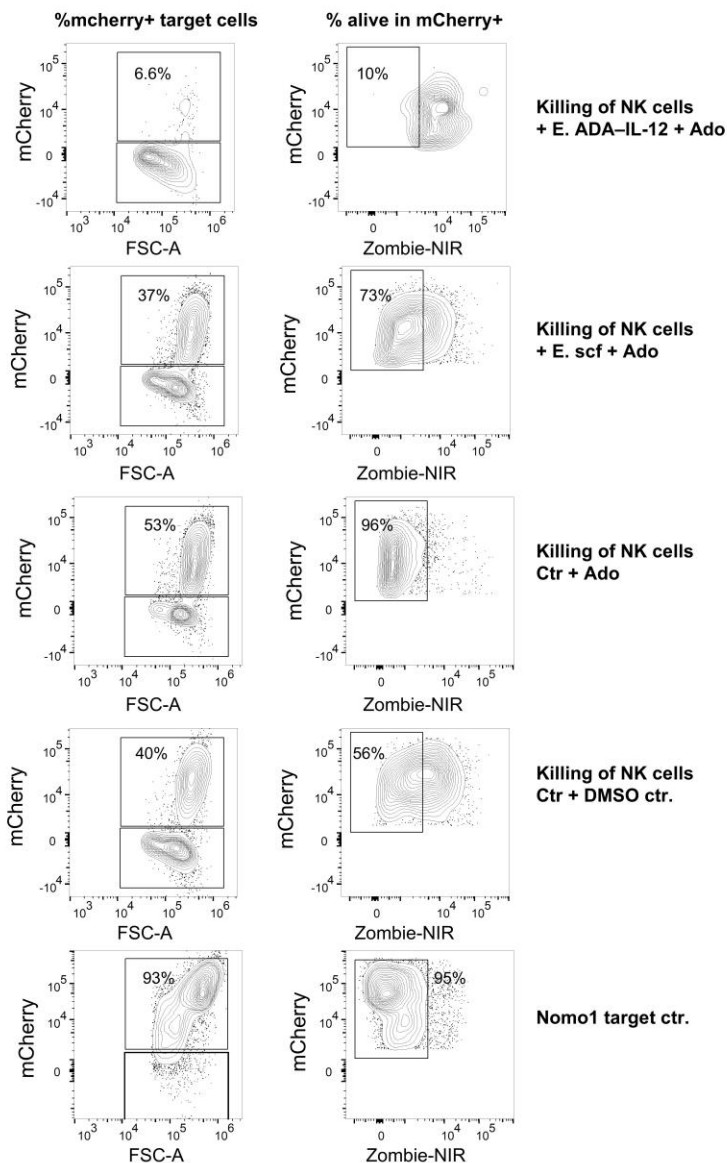


c



Supplementary Fig. 7. IL-12 enhances NK- and CAR-T-cell effector function under adenosine stress. **a–c**, Effector-cell cytotoxicity and corresponding TNF, IFN- γ and CD107a responses following exposure to scaffold-control bacteria (E. scf), IL-12-displaying bacteria (E. IL-12) or recombinant human IL-12 (rhIL-12), in the presence or absence of adenosine (ADO). **a**, NK-cell cytotoxicity against AsPC1 pancreatic cancer cells after 24–36 hours ($n=4$ biologically independent donor-derived NK-cell samples). **b**, CD70 CAR-T-cell cytotoxicity against OCI-AML3 acute myeloid leukemia cells after 16 hours ($n=8$ biologically independent donor-derived CAR-T-cell samples); untransduced T cells (UTD) served as CAR-negative controls for cytotoxicity. **c**, NK-cell cytotoxicity against NOMO-1 acute myeloid leukemia cells after 16 hours ($n=4$ biologically independent donor-derived NK-cell samples). All cytotoxicity assays were performed at an

effector-to-target ratio of 1:1. Individual points represent biologically independent donors. Mean $\pm$ s.e.m. Two-way ANOVA with Tukey's multiple-comparisons test.

Supplementary Figure 8

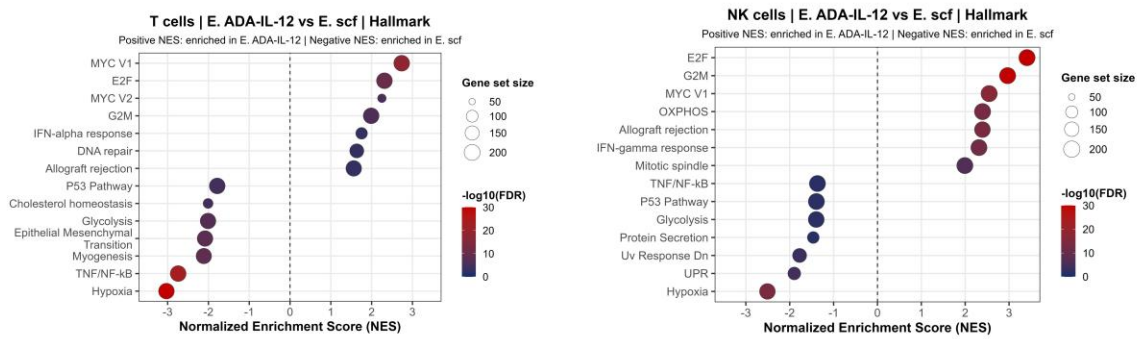
Supplementary Fig. 8. Representative flow-cytometric analysis of NK-cell-mediated cytotoxicity, shown for Nomo1. Representative flow-cytometry plots showing identification of mCherry⁺ Nomo1 target cells by gating on mCherry⁺ cells, followed by assessment of target-cell viability. Cytotoxicity was assessed 14 to 16 hours after coculture with activated NK cells (E:T, 1:1) in the presence of E. ADA-IL-12 or scaffold control (E. scf) with adenosine (ADO, 10 mM), or without bacteria under ADO or DMSO control conditions. Values indicate the percentage of parent cells within each gate. NK-cell-mediated cytotoxicity was calculated as the percentage of viable mCherry⁺ target cells normalized to the Nomo1 target-only control.

Supplementary Fig. 9. ADA–IL-12 modulates T- and NK-cell activation and regulatory phenotypes under adenosine stress. a–d, Flow-cytometric phenotyping of T cells (**a,b**) and NK cells (**c,d**) following exposure to scaffold-control bacteria (*E. scf*), IL-12-displaying bacteria (*E. IL-12*), ADA-displaying bacteria (*E. ADA*) or dual ADA–IL-12-displaying bacteria (*E. ADA–IL-12*), in the presence or absence of adenosine (ADO). **a**, T-cell CD4/CD8 ratio and expression of CD25, CD69 and NKG2D. **b**, T-cell expression of Ki-67, TIM-3, PD-1 and CTLA-4. **c**, NK-cell expression of NKp30, CD25, CD69 and NKG2D. **d**, NK-cell expression of Ki-67, TIM-3, PD-1 and KIR. n=4 biologically independent donor-derived T-cell samples (**a,b**) and n=4 biologically independent donor-derived NK-cell samples (**c,d**). Individual points represent biologically independent donors. Mean±s.e.m. Statistical significance was assessed by two-way ANOVA with Tukey's multiple-comparisons test.

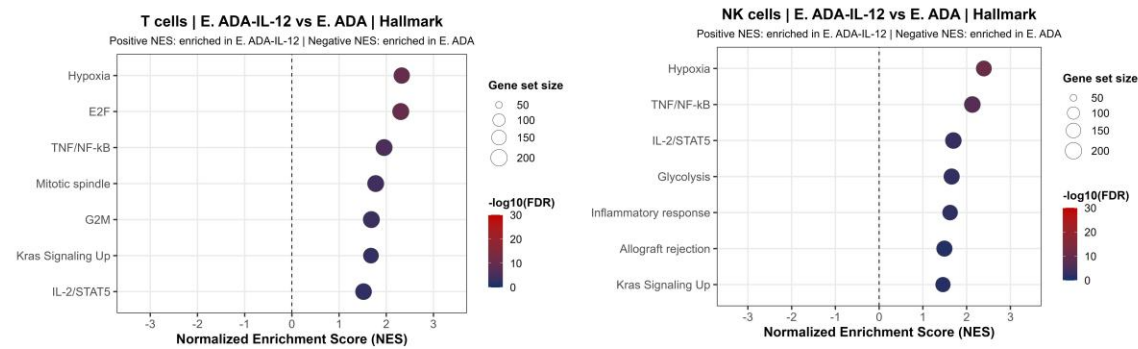
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Supplementary Figure 10

a

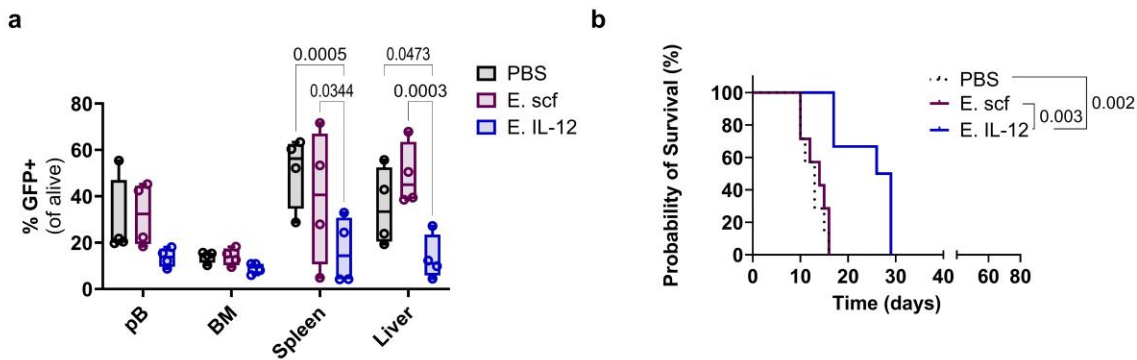


b

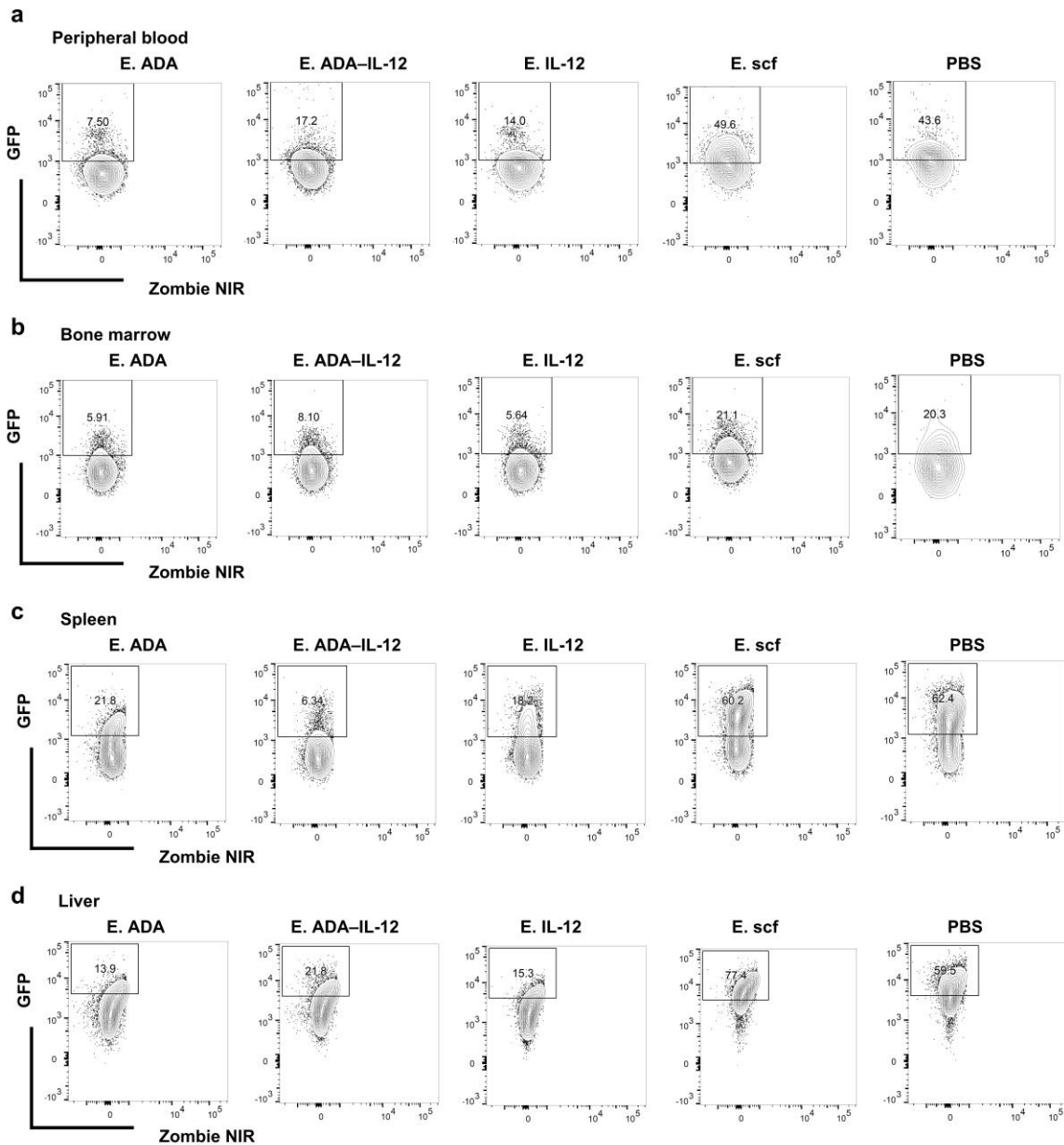


Supplementary Fig. 10. ADA–IL-12 induces distinct proliferative, metabolic and immune-response programs in NK and T cells. Hallmark gene-set enrichment analysis (GSEA) of activated primary human T cells (left) and NK cells (right) from n=3 independent donors comparing **a**, E. ADA–IL-12 with scaffold control (E. scf) and **b**, E. ADA–IL-12 with E. ADA. Positive and negative normalized enrichment scores (NES) indicate enrichment in E. ADA–IL-12 and the respective comparator, respectively. Circle size represents gene-set size, and color indicates $-\log_{10}(\text{FDR})$. GSEA was performed using Hallmark gene sets. FDR, false discovery rate; NES, normalized enrichment score.

Supplementary Figure 11

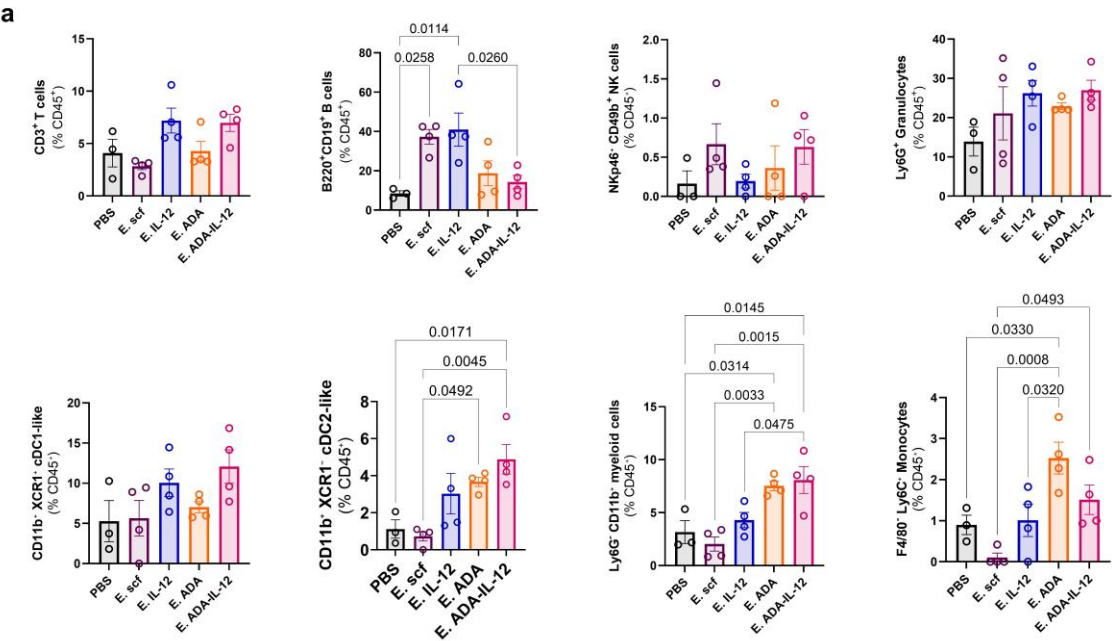


Supplementary Fig. 11. IL-12-displaying bacteria reduce leukemia burden and prolong survival in systemic WEHI-3B leukemia. a, Flow-cytometric quantification of GFP⁺ WEHI-3B cells among viable cells in peripheral blood (PB), bone marrow (BM), spleen and liver following treatment with PBS, scaffold-control bacteria (E. scf) or IL-12-displaying bacteria (E. IL-12). n=4 mice per group in E. scf and E. IL-12 and n=3 PBS treated mice were analyzed. **b**, Kaplan-Meier survival of WEHI-3B-bearing mice following treatment with PBS, E. scf or E. IL-12. **a**, Mean±s.e.m.; One-way ANOVA with Tukey's multiple comparison test; individual mice with box plots indicating the median and interquartile range; **b**, Log-rank (Mantel-Cox) test.

Supplementary Figure 12

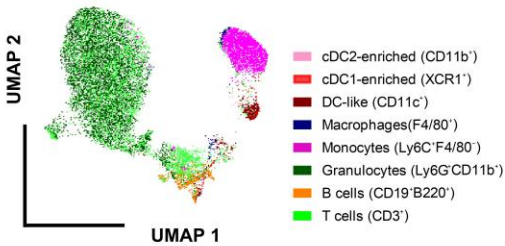
Supplementary Fig. 12. Representative dot plots of live GFP⁺ target cells. a–d, Representative flow-cytometry dot plots showing GFP-positive bacteria and viability staining in peripheral blood (PB; **a**), bone marrow (BM; **b**), spleen (**c**) and liver (**d**) following treatment with E. ADA, E. ADA-IL-12, E. IL-12, scaffold-control bacteria (E. scf) or PBS. Percentages of positive gated cells among parent cells are indicated.

Supplementary Figure 13

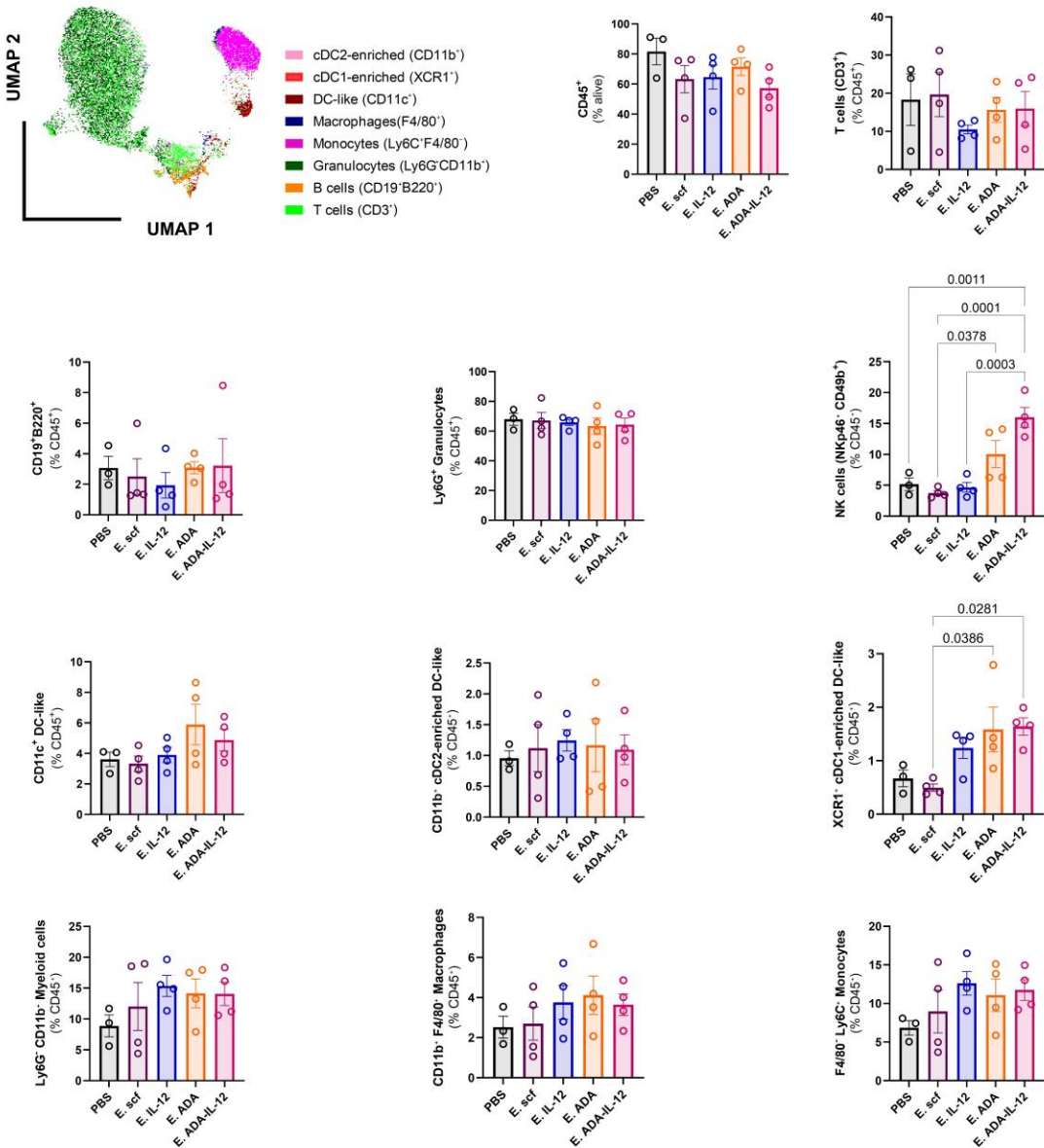


Supplementary Figure 13

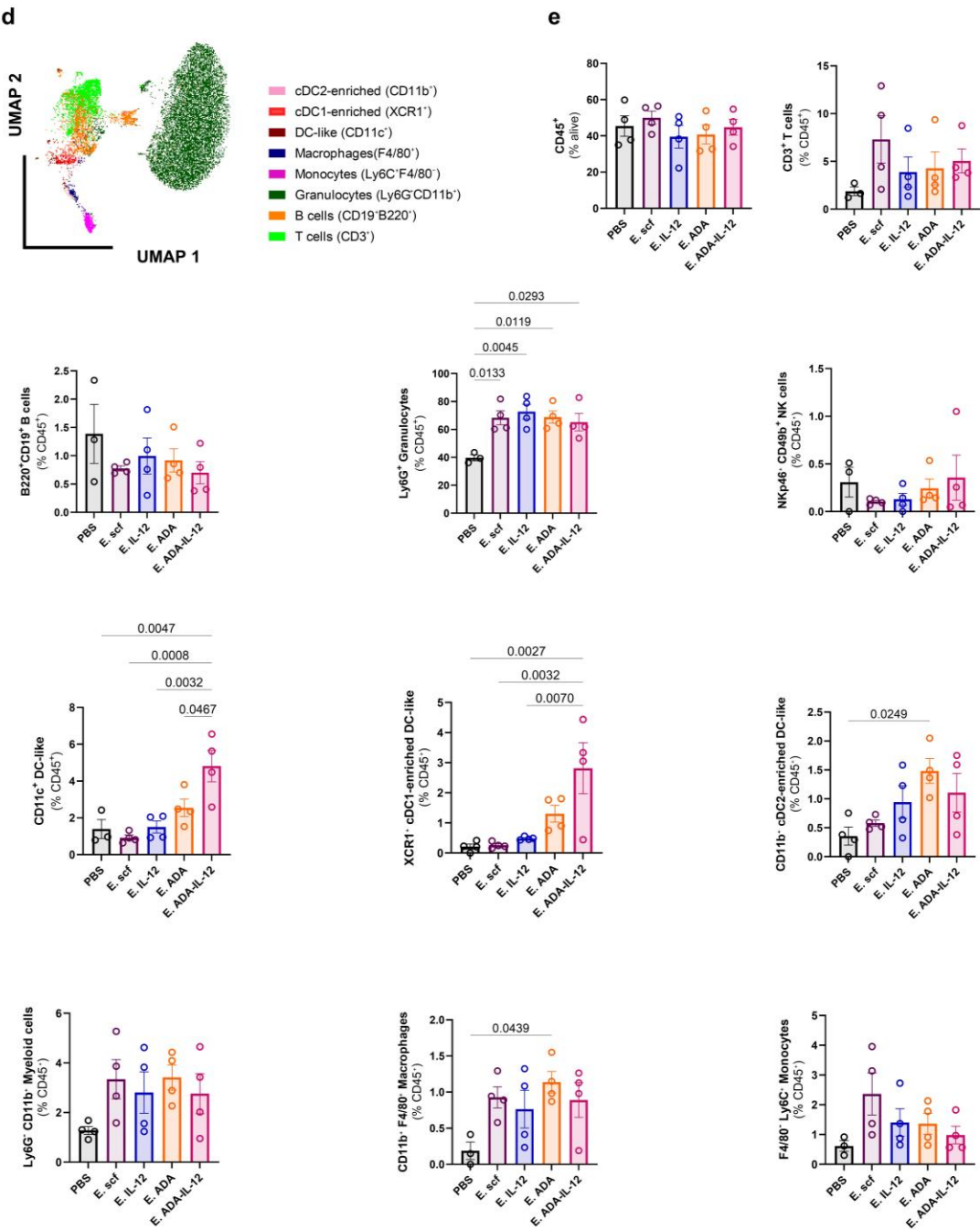
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c

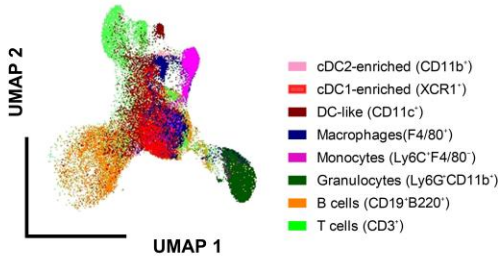


Supplemental Figure 13

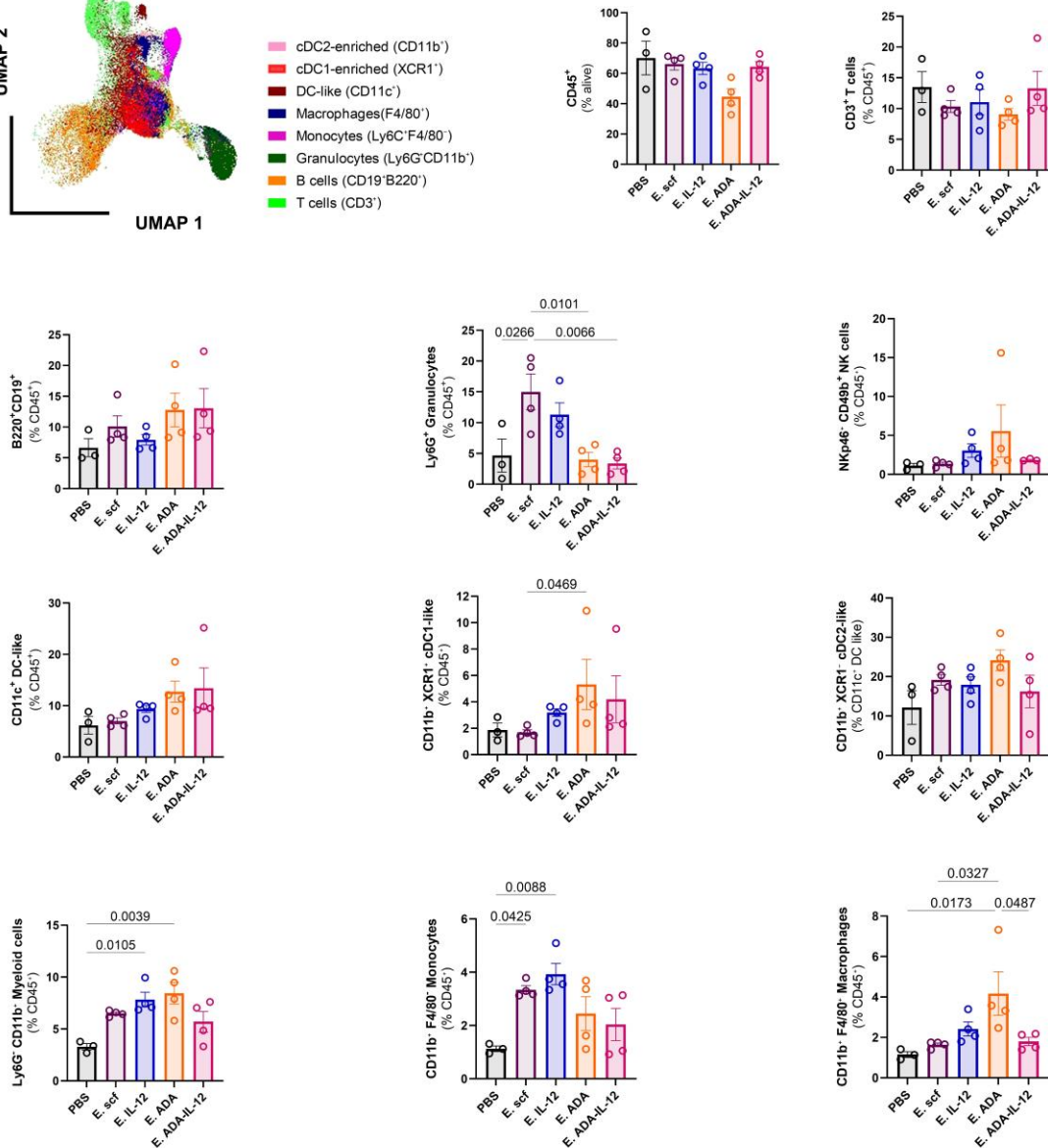


Supplementary Figure 13

f



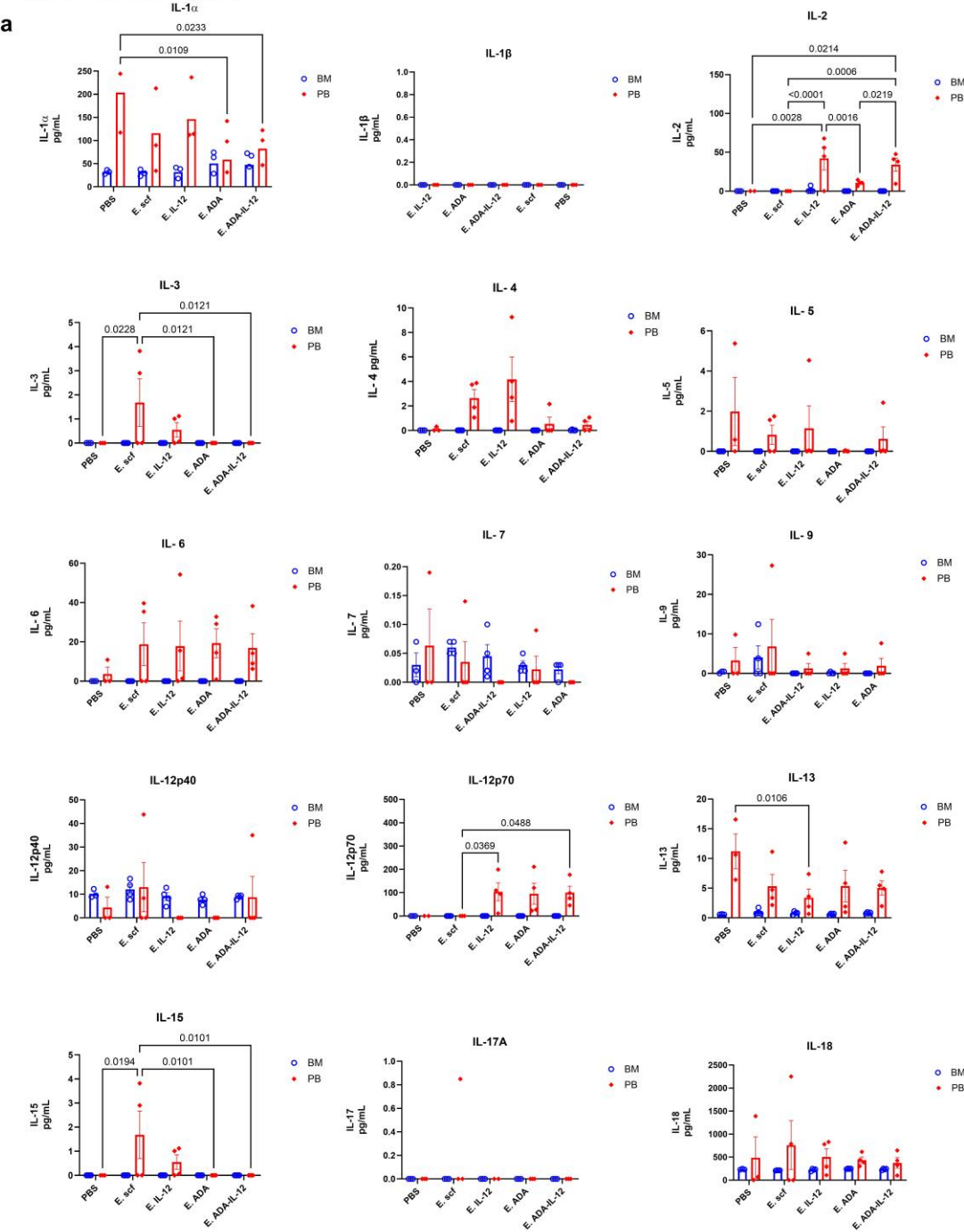
g



Supplementary Fig. 13. Tissue-specific immune remodeling following systemic bacterial therapy in WEHI-3B leukemia. a–g, Flow-cytometric immune profiling of liver (a), peripheral blood (b,c), bone marrow (d,e) and spleen (f,g) collected on day 14 from WEHI-3B-bearing mice treated with PBS (n=3), scaffold-control bacteria (E. scf; n=4), IL-12-displaying bacteria (E. IL-12; n=4), ADA-displaying bacteria (E. ADA; n=4) or dual ADA–IL-12-displaying bacteria (E. ADA–IL-12; n=4). **b,d,f,** UMAP visualization of immune-cell populations identified as T cells (CD3⁺), B cells (CD19⁺CD20⁺), granulocytes (Ly6G⁺CD11b⁺), DC-like cells (CD11c⁺), comprising cDC2-enriched (CD11b⁺) and cDC1-enriched (XCR1⁺) populations, and macrophages (F4/80⁺) and monocytes (Ly6C⁺F4/80⁺). **a,c,e,g,** Quantification of CD45⁺ leukocytes and the indicated lymphoid and myeloid populations and subsets within the corresponding tissues, including CD3⁺ T cells, CD4⁺

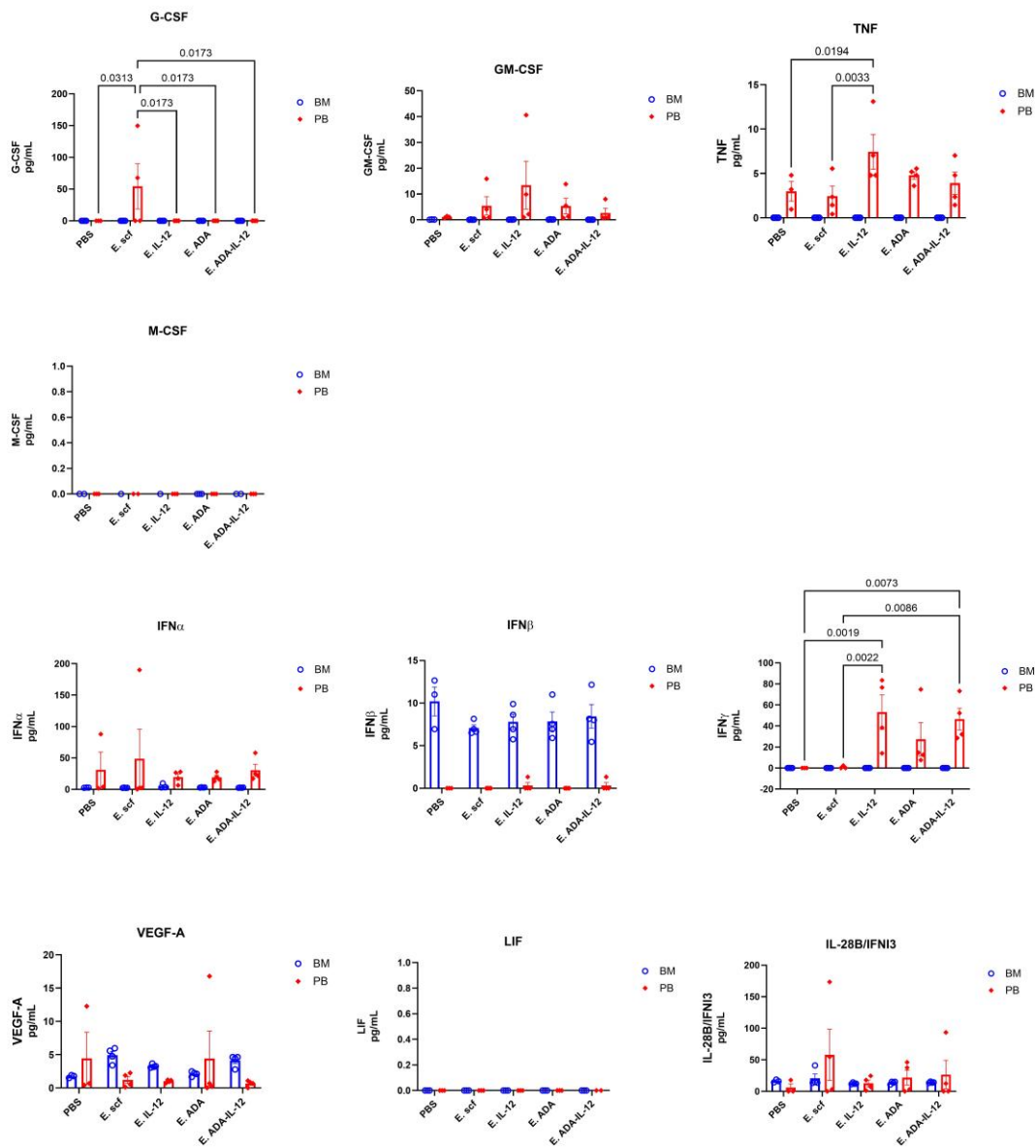
and CD8⁺ T-cell subsets, CD11c⁺ DC-like cells, XCR1⁺ cDC1-enriched and CD11b⁺ cDC2-enriched populations, Ly6G⁻CD11b⁺ myeloid cells, Ly6C⁺F4/80⁺ monocytes, Ly6G⁺CD11b⁺ granulocytes and F4/80⁺ macrophages. Individual points represent independent biological replicates from individual mice. Mean±s.e.m.; One-way ANOVA with Tukey's multiple comparison test.

Supplementary Figure 14



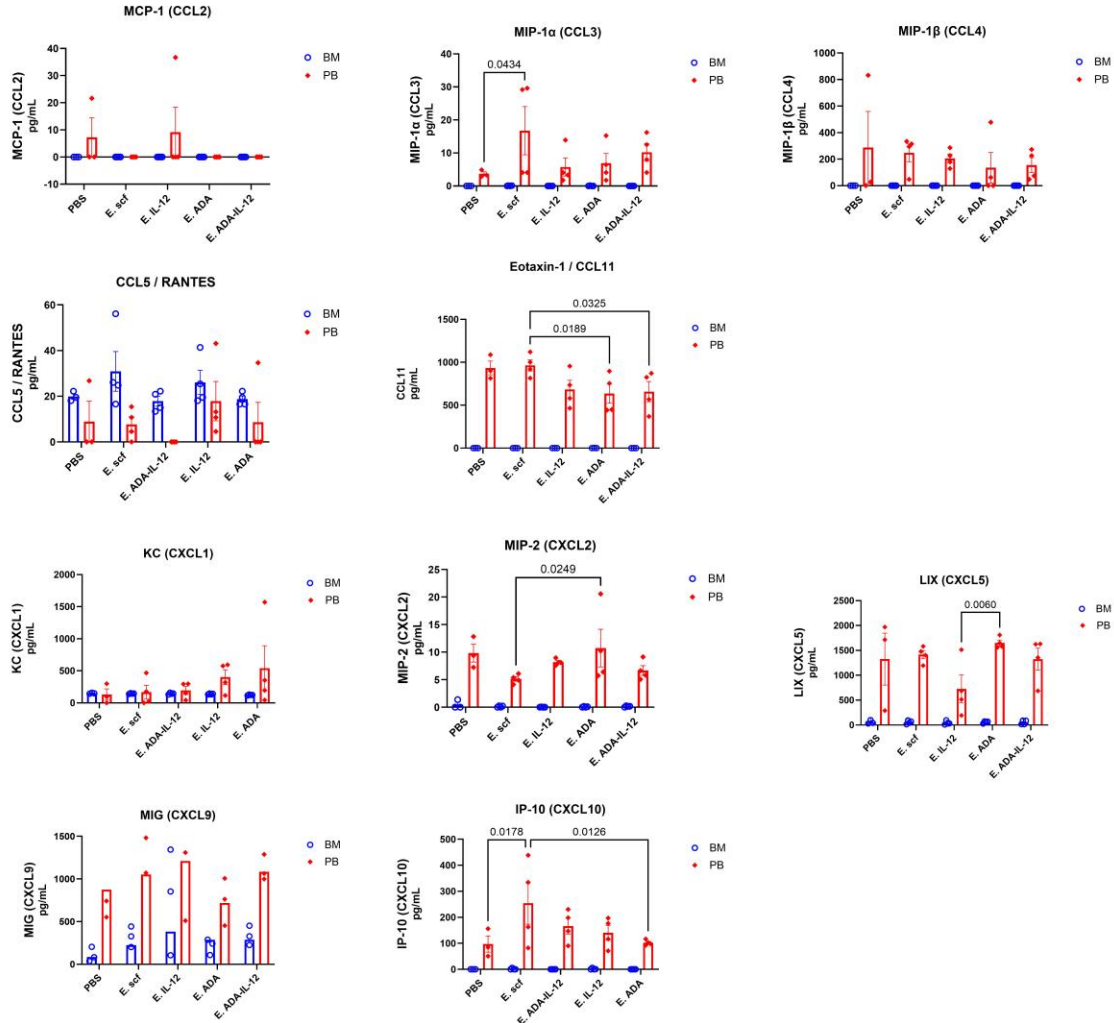
Supplementary Figure 14

b



Supplementary Figure 14

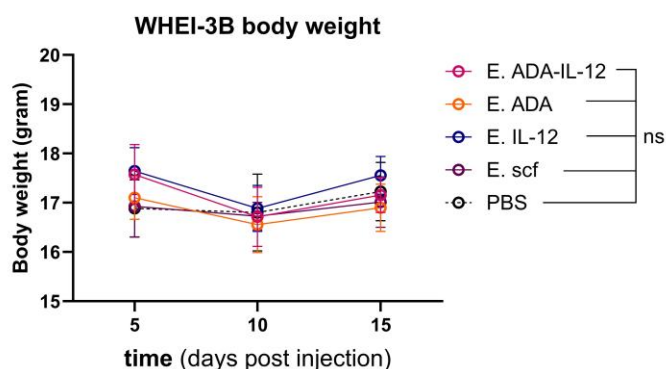
c



Supplementary Fig. 14. Systemic cytokine and chemokine profiles following bacterial treatment in WEHI-3B-bearing mice. Plasma cytokine, chemokine and growth-factor concentrations in peripheral blood (PB) and bone marrow (BM) of WEHI-3B-bearing mice on day 14 after tumor-cell injection, 4 days after the final bacterial administration, following treatment with PBS, scaffold-control bacteria (E. scf), IL-12-displaying bacteria (E. IL-12), ADA-displaying bacteria (E. ADA) or dual ADA-IL-12-displaying bacteria (E. ADA-IL-12). **a**, Interleukin profiles comprising IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-12p40, IL-12p70, IL-13, IL-15, IL-17A and IL-18. **b**, Colony-stimulating factors, interferons and additional cytokines and growth factors comprising G-CSF, GM-CSF, TNF, M-CSF, IFN-α, IFN-β, IFN-γ, VEGF-A, LIF and IL-28B (IFN-λ3). **c**, Chemokine profiles comprising MCP-1 (CCL2), MIP-1α (CCL3), MIP-1β (CCL4), CCL5 (RANTES), eotaxin-1 (CCL11), KC (CXCL1), MIP-2 (CXCL2), LIX (CXCL5), MIG (CXCL9) and IP-10 (CXCL10). Analytes were quantified by multiplex cytokine analysis. n=3 (PBS) and n=4 mice per group (E. scf, E. IL-12, E. ADA, E. ADA-IL-12). Individual points represent individual

mice. Mean $\pm$ s.e.m.; Two-way ANOVA with Tukey's multiple comparison test.

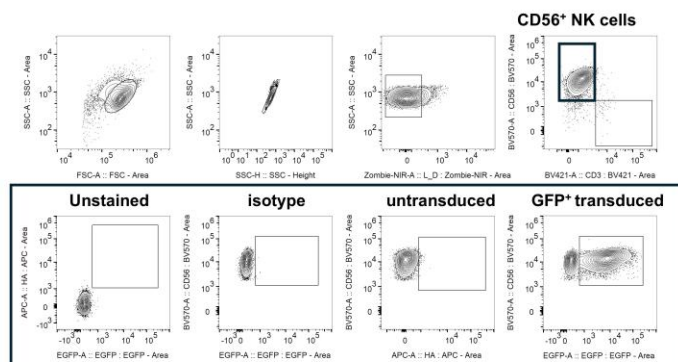
Supplementary Figure 15



Supplementary Fig. 15. Body-weight in WEHI-3B-bearing mice.

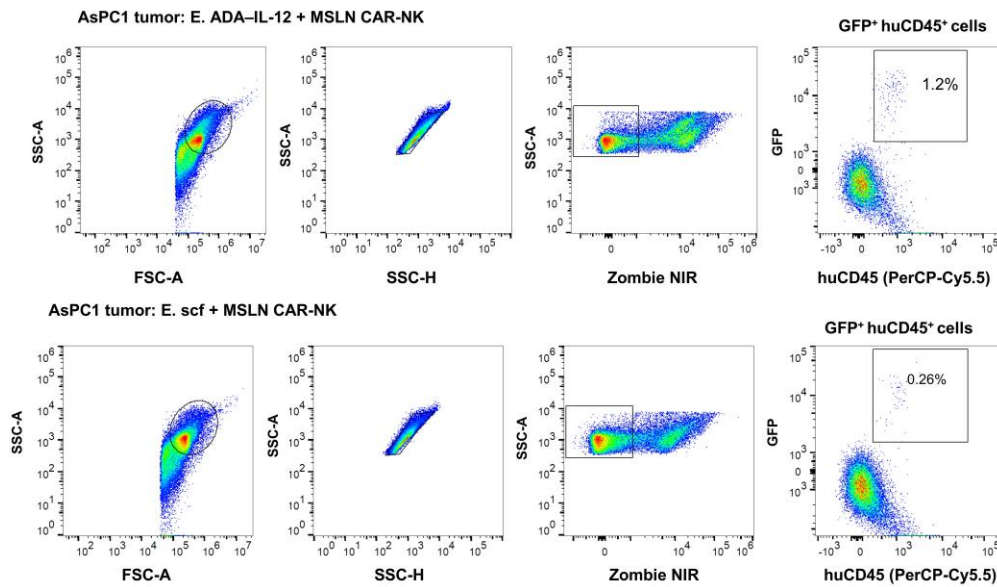
Body weight in mice bearing WEHI-3B leukemia following treatment with PBS $n=7$, scaffold-control bacteria (E. scf, $n=7$), IL-12-displaying bacteria (E. IL-12, $n=7$), ADA-displaying bacteria (E. ADA, $n=7$) or dual ADA-IL-12-displaying bacteria (E. ADA-IL-12, $n=7$). Mean $\pm$ s.e.m. Two-way ANOVA with Tukey's multiple comparison test.

Supplementary Figure 16



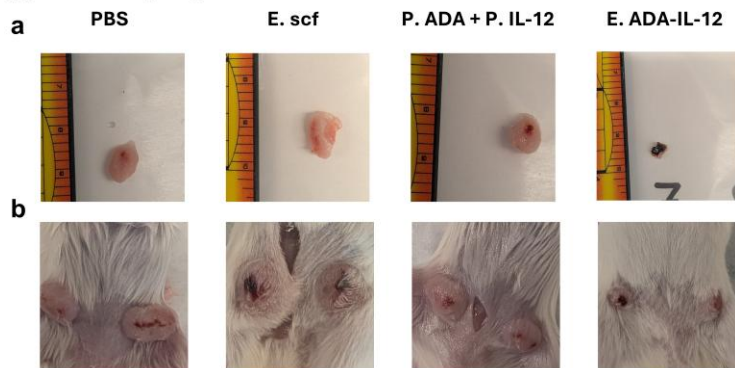
Supplementary Fig. 16. Flow-cytometric assessment of MSLN CAR-NK-cell transduction.

Representative flow-cytometric dot plots for MSLN CAR-NK cells used in Extended Data Fig. 8. NK cells were identified as CD56⁺ cells and CAR-transduced cells as the EGFP⁺ subpopulation. Unstained, isotype and untransduced NK-cell controls were used to define the EGFP⁺ transduced NK cells as CAR transduced population.

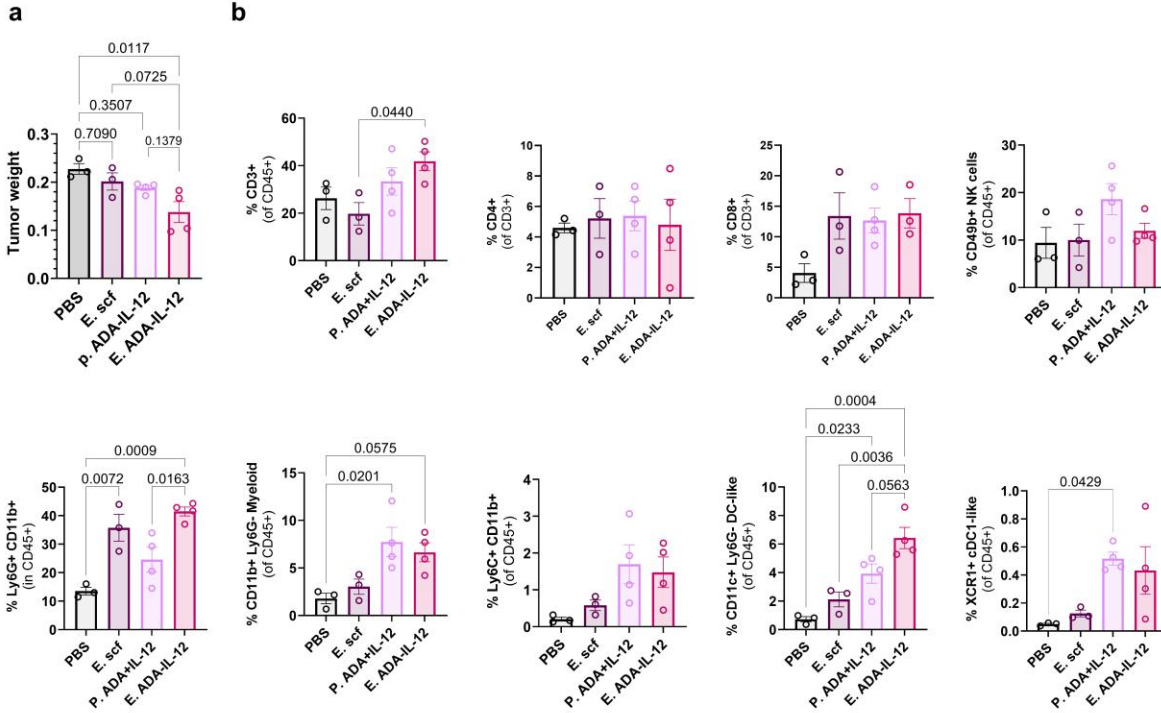
Supplementary Figure 17

Supplementary Fig. 17. Flow-cytometric gating of MSLN CAR-NK cells in AsPC1 tumors. Representative flow-cytometric gating dot plots for detection of human MSLN CAR-NK cells in AsPC1 tumors from mice receiving MSLN CAR-NK cells in combination with E. ADA-IL-12 (top) or E. scf (bottom). Cells were sequentially gated based on forward- and side-scatter characteristics, singlets and viability, followed by identification of GFP⁺huCD45⁺ MSLN CAR-NK cells. Numbers indicate the percentage of GFP⁺hCD45⁺ cells within the parent population.

Supplementary Figure 18



Supplementary Fig. 18. Tumor regression following E. ADA-IL-12 treatment in an orthotopic 4T1 breast cancer model.
a, Representative images of excised tumors collected on day 14 from mice across the indicated treatment groups. **b**, Representative images of mice showing the tumor site on day 14 across the indicated treatment groups.

Supplementary Figure 19

Supplementary Fig. 19: E. ADA-IL-12 remodels the immune environment of orthotopic 4T1 tumors. **a**, Tumor weight and **b**, Flow-cytometric profiling of tumor-infiltrating immune cells on day 14 in mice bearing orthotopic 4T1 mammary tumors following treatment with PBS (n=3), scaffold-control bacteria (E. scf, n=3), recombinant ADA plus recombinant IL-12 (P. ADA+IL-12, n=4) or ADA-IL-12 displaying bacteria (E. ADA-IL-12, n=4). Frequencies of CD3⁺, CD4⁺ and CD8⁺ T cells, CD49b⁺ NK cells and CD11b⁺Ly6G⁺ granulocyte-enriched, CD11b⁺Ly6G⁻ myeloid cells, Ly6C⁺CD11b⁺ monocyte-enriched cells, CD11c⁺Ly6G⁻ DC-like cells and XCR1⁺CD11c⁺MHC-II⁺ DC-like cells among the indicated parent populations. Individual points represent individual mice. Mean±s.e.m. One-way ANOVA with Tukey's multiple comparison test.