nature medicine

SUPPLEMENTARY INFORMATION

https://doi.org/10.1038/s41591-018-0036-4

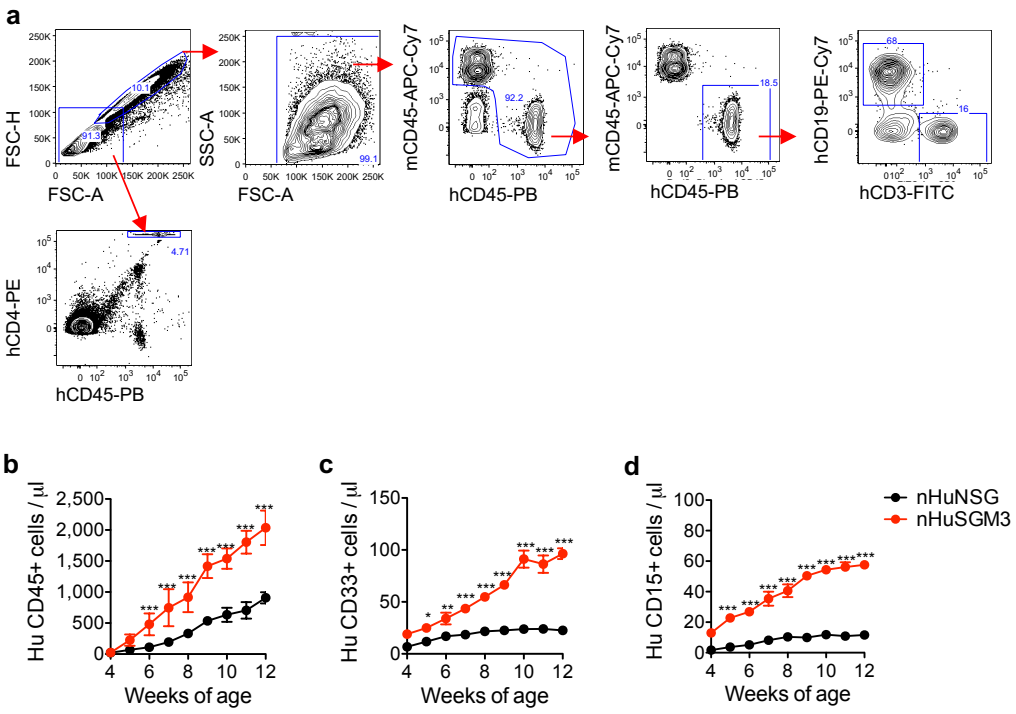
In the format provided by the authors and unedited.

Monocyte-derived IL-1 and IL-6 are differentially required for cytokine-release syndrome and neurotoxicity due to CAR T cells

Margherita Norelli[1,2], Barbara Camisa[1], Giulia Barbiera[3], Laura Falcone[1], Ayurzana Purevdorj[1], Marco Genua[3], Francesca Sanvito[4], Maurilio Ponzoni[4], Claudio Doglioni[4], Patrizia Cristofori[5], Catia Traversari[6], Claudio Bordignon[2,6], Fabio Ciceri[2,7], Renato Ostuni[3], Chiara Bonini[2,8], Monica Casucci[1] and Attilio Bondanza[1,2]*

[1]Innovative Immunotherapies Unit, San Raffaele Hospital Scientific Institute, Milano, Italy. [2]Vita-Salute San Raffaele University, Milano, Italy. [3]Genomics of the Innate Immune System Unit, San Raffaele-Telethon Institute for Gene Therapy (SR-Tiget), Milano, Italy. [4]Pathology Unit, San Raffaele Hospital Scientific Institute, Milano, Italy. [5]San-Raffaele-Telethon Institute for Gene Therapy (SR-Tiget), Milano, Italy. [6]Molmed Spa, Milano, Italy. [7]Hematology and Bone Marrow Transplantation Unit, San Raffaele Hospital Scientific Institute, Milano, Italy. [8]Experimental Hematology Unit, San Raffaele Hospital Scientific Institute, Milano, Italy. *e-mail: attilio.bondanza@gmail.com

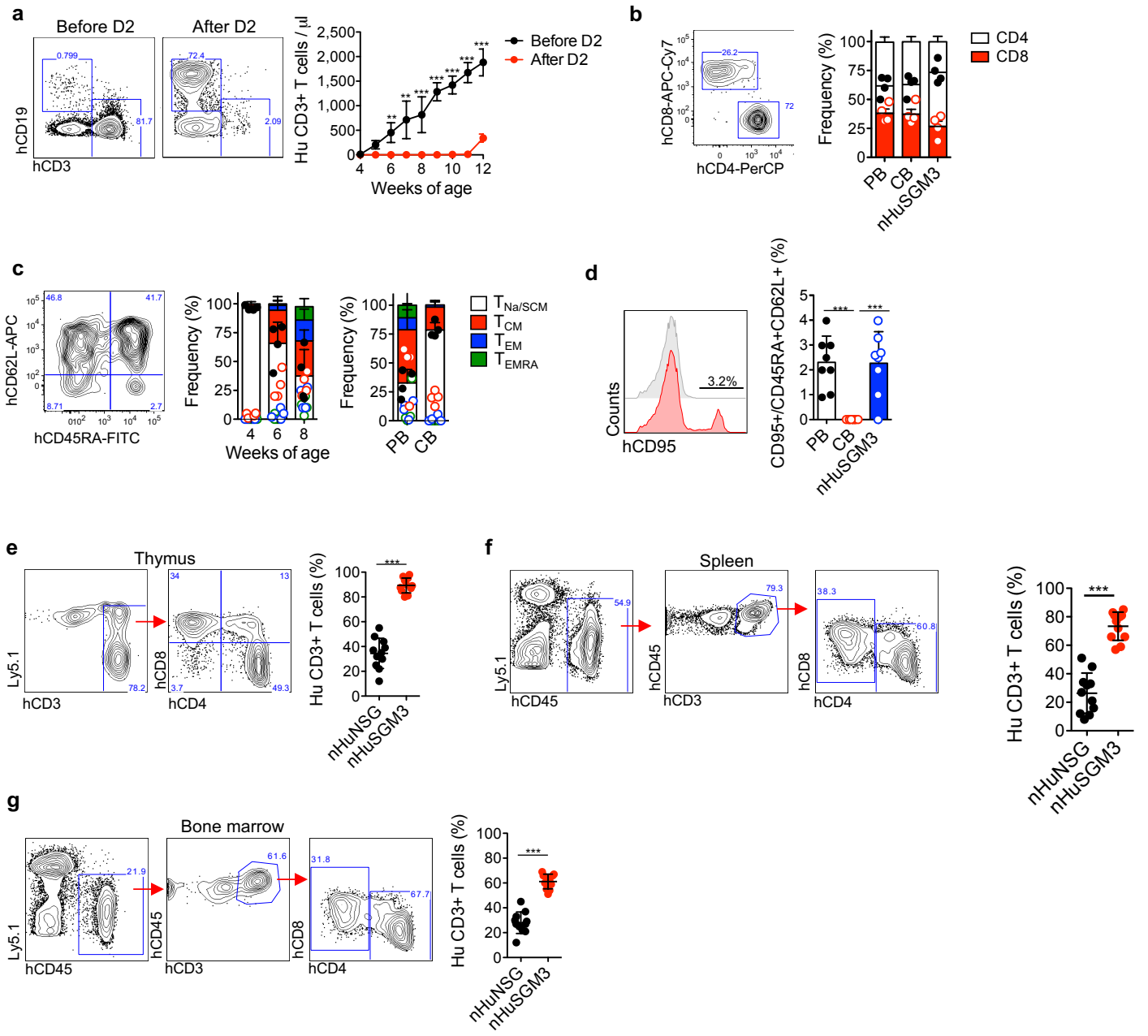
Supplementary Fig. 1



Human lympho-hematopoietic reconstitution in HuSGM3 mice. 10^5 human hematopoietic stem/progenitor cells (HSPCs) were injected intra-liver into newborn NSG mice (nHuNSG) or newborn NSG mice triple transgenic for human stem cell factor, GM-CSF and IL-3 (nHuSGM3). (**a**) Peripheral blood of HSPC-humanized mice was incubated with fluorochrome-conjugated monoclonal antibodies, lysed with ACK and run through a FACS Canto II apparatus. Serial gating strategy for cells (upper plots) and counting fluoro-spheres (lower plots). (**b-d**) Mean Hu $CD45^+$, $CD33^+$ or $CD15^+$ cell counts ± SD (n=15 from 3 independent experiments each group). ***, P<0.001 by a two-way ANOVA test.

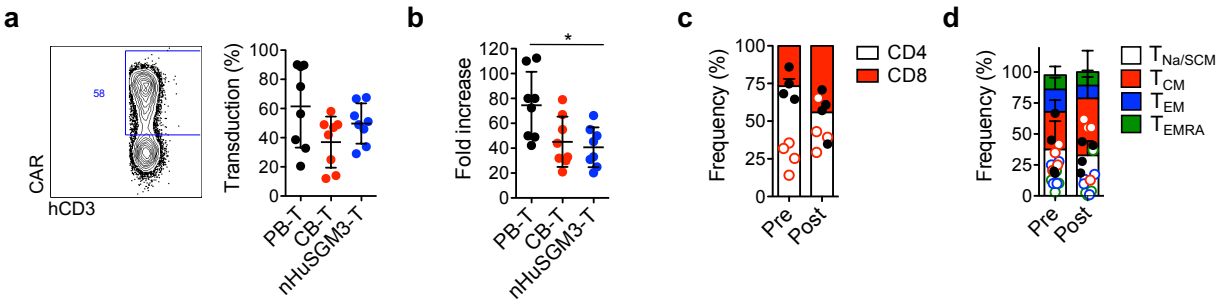
*

Supplementary Fig. 2



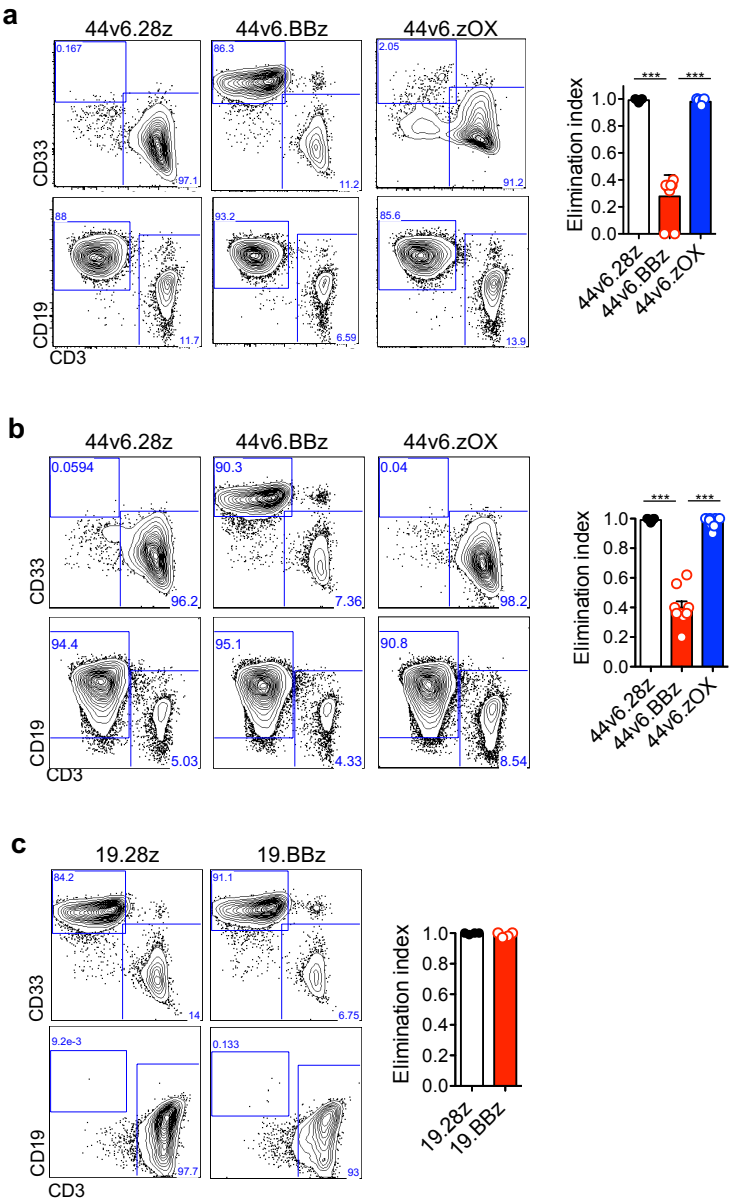
Human T cell repopulation of lymphoid organs in nHuSGM3 mice. Newborn HSPC-humanized NSG (nHuNSG) or newborn SGM3 mice (nHuSGM3) were analyzed for T cell reconstitution in peripheral blood and lymphoid tissues. (**a**) Representative plot (5-weeks old) and mean human CD3$^+$ counts ± SD in nHuSGM3 mice transplanted before or after post-natal day 2 (n=5 each group). **, P<0.01; ***, P<0.001 by a two-way ANOVA test. (**b**) Representative plot and mean CD4/CD8 frequencies ± SD in human peripheral blood (PB, n=4), cord blood (CB, n=4) or 8-weeks old nHuSGM3 mice (n=4). (**c**) Representative plot and mean frequencies of CD45RA$^+$/CD62L$^+$ naïve/stem cell memory ($T_{Na}/_{SCM}$), CD45RA-/CD62L+ central memory (T_{CM}), CD45RA$^-$/CD62L$^-$ effector memory (T_{EM}) and CD45RA+/CD62L- effector memory RA (T_{EM}RA) cells ± SD in 4, 6 or 8 weeks-old nHuSGM3 mice, human PB or CB (n=4 each group). (**d**) Representative plot (grey, isotype control; red, specific antibody) and mean CD95$^+$ percentages over CD8$^+$/CD45RA$^+$/CD62L$^+$ T cells ± SD in human PB, CB or 4 weeks-old nHuSGM3 mice (n=8 each group). ***, P<0.001 by a one-way ANOVA test. (**e-g**) Representative gating strategy and mean human CD3$^+$ percentages ± SD in the thymus, spleen or bone marrow of 8 weeks-old nHuNSG or nHuSGM3 mice (n=11 each group). ***, P<0.001 by a Mann-Whitney test.

Supplementary Fig. 3



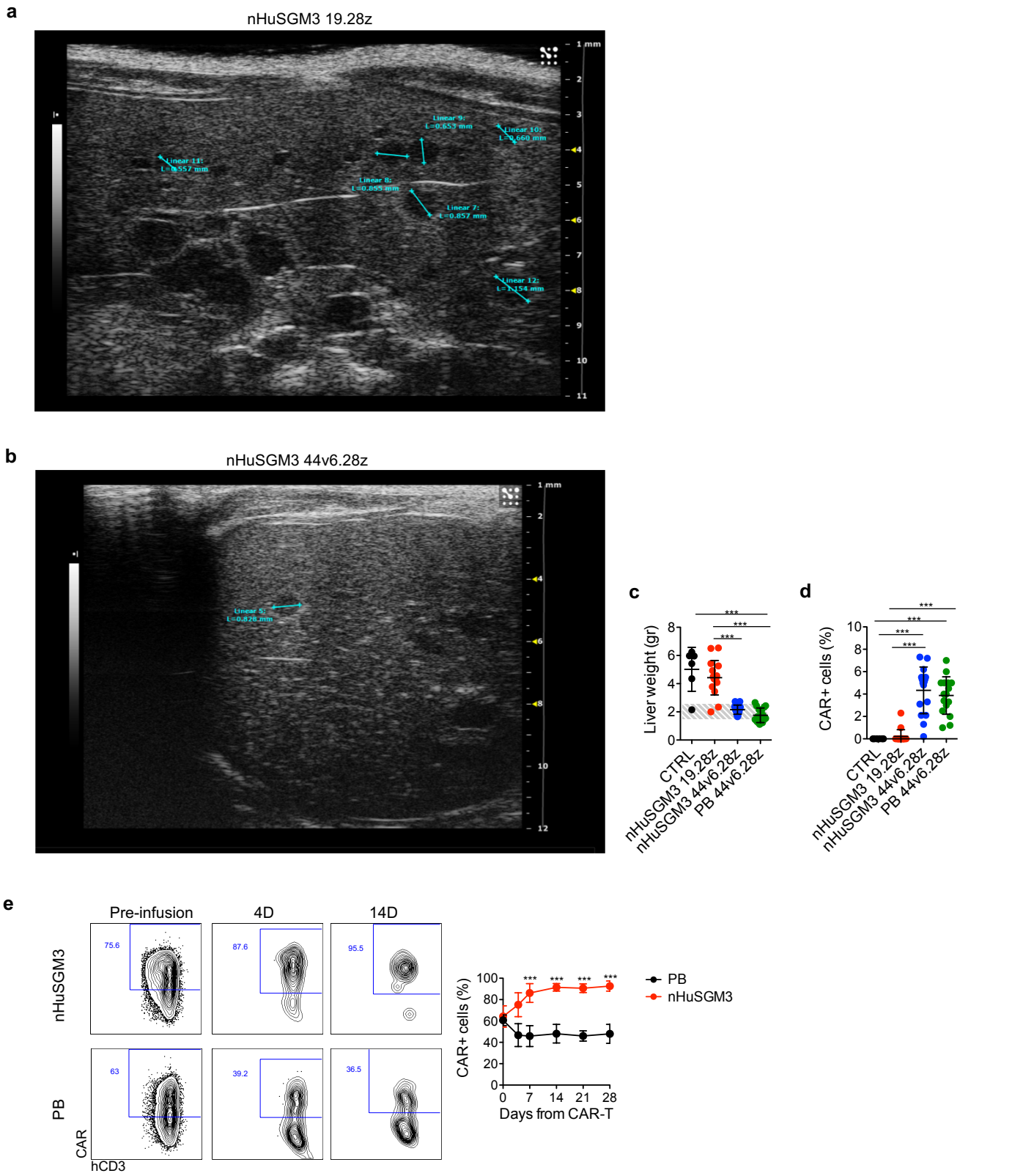
CAR transduction of T cells from nHuSGM3 mice. T cells from human peripheral blood (PB), cord-blood (CB) or from the spleen of newborn HSPC-humanized SGM3 mice (nHuSGM3) were activated with anti-CD3/CD28 beads, transduced with a CD44v6.28z CAR and cultured with IL-7/IL-15. (**a**) Representative plot of CAR positivity and transduction percentages ± SD after 7 days (n=8 each group). (**b**) Fold increases over initial numbers ± SD after 15 days (n=8 each group). *, P<0.05 by a one-way ANOVA test with Bonferroni correction. (**c-d**) Mean human CD4/CD8 frequencies ± SD and mean human CD45RA$^+$/CD62L$^+$ naïve/stem cell memory ($T_{Na}/_{SCM}$), CD45RA$^-$/CD62L$^+$ central memory (T_{CM}), CD45RA-/CD62L- effector memory (T_{EM}) and CD45RA$^+$/CD62L$^-$ effector memory RA (T_{EM}RA) frequencies ± SD in T cells from nHuSGM3 mice before beads activation (Pre, n=4) or after 15 days (Post, n=4).

Supplementary Fig. 4



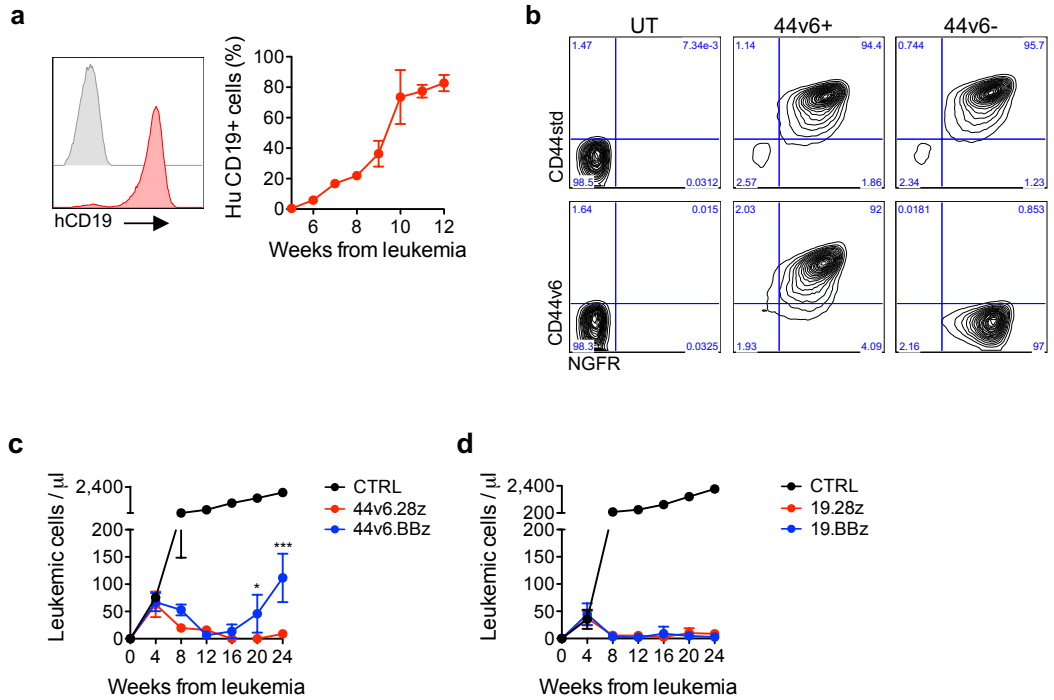
***In vitro* functionality of CAR-T cells from nHuSGM3 mice.** T cells from newborn HSPC-humanized SGM3 mice (nHuSGM3) or from human peripheral blood (PB) were activated with CD3/CD28-beads, CAR transduced and cultured with IL-7/IL-15. After 15 days, CAR-T cells were co-cultured at a 1:10 effector-to-target ratio with either $CD33^{+}CD44v6^{+}$ THP-1 leukemic cells (upper plots) or $CD19^{+}CD44v6^{-}$ BV173 leukemic cells (lower plots). (**a**-**b**) Representative 4-days co-culture plots and mean THP1 leukemia elimination indexes ± SD (see Methods) by either (**a**) nHuSGM3 or (**b**) PB T cells transduced with a CD44v6.28z, a CD44v6.BBz or a CD44v6.zOX CAR (n=9 independent experiments each group). ***, $P<0.001$ by a one-way ANOVA test. (**c**) Representative 4-days co-culture plots and mean BV173 leukemia elimination indexes ± SD by nHuSGM3 T cells transduced with either a CD19.28z or a CD19.BBz CAR (n=4 independent experiments each group).

Supplementary Fig. 5



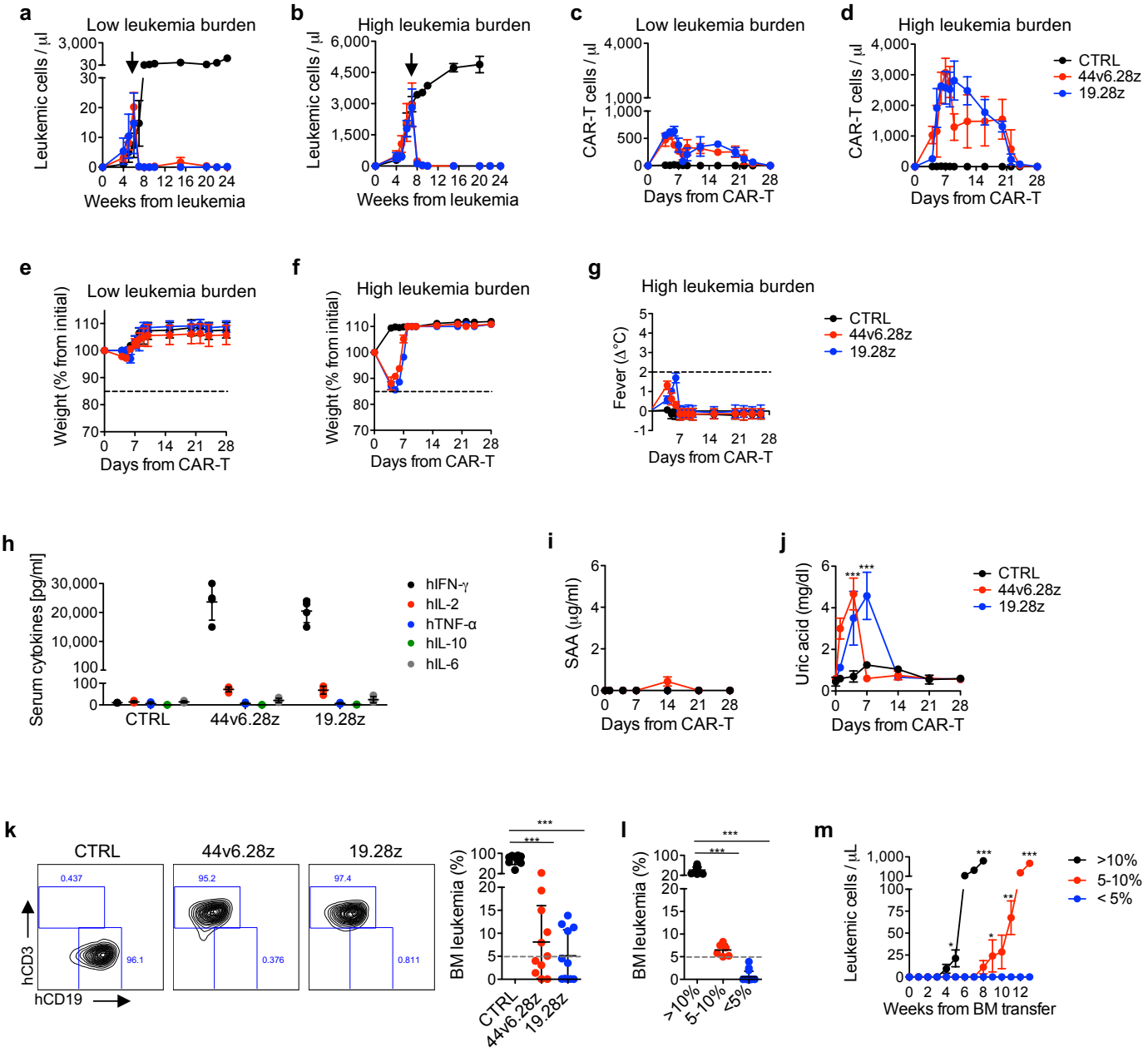
***In vivo* antileukemia effects by 28z CAR-T cells from nHuSGM3 mice.** $2x10^6$ $CD19^-CD44v6^+$ THP-1 leukemic cells were infused i.v. in 6-8 weeks-old NSG mice. After 1 week, leukemic NSG mice received $5x10^6$ T cells derived from newborn HSPC-humanized SGM3 mice (nHuSGM3), and either un-transduced as control (CTRL) or transduced with a CD19.28z (nHuSGM3 19.28z) or a CD44v6.28z CAR (nHuSGM3 44v6.28z). A group of leukemic NSG mice received T cells from human peripheral blood transduced with a CD44v6.28z CAR (PB 44v6.28z). (**a-b**) Representative NSG liver ultrasound images. Light arrows: liver sarcomas. (**c-d**) Mean liver weights ± SD and mean percentages of intra-liver CAR^+ T cells ± SD (CTRL, n=6 from 2 independent experiments; nHuSGM3 19.28z, n=16 from 3 independent experiments; nHuSGM3 44v6.28z, n=16 from 3 independent experiments; PB 44v6.28z, n=16 from 3 independent experiments). Dashed area: range of mouse normal liver weight. ***, P<0.001 by a one-way ANOVA test with Bonferroni correction. (**e**) Representative plots and mean percentages of CAR^+ cells over circulating human T cells ± SD from either 44v6.28z PB or nHuSGM3 mice. ***, P<0.001 by a two-way ANOVA test with Bonferroni correction.

Supplementary Fig. 6



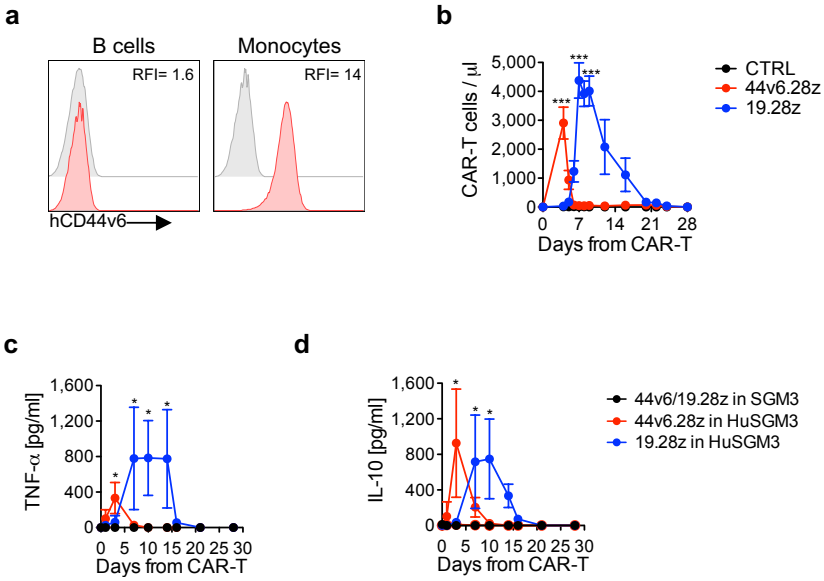
In vivo* antileukemia effects by BBz CAR-T cells from nHuSGM3 mice.** 5x10^6 CD19$^+$ ALL-CM leukemic cells were infused i.v. in 6-8 weeks-old NSG mice. (**a**) Representative plot (grey, isotype control; red, specific antibody) and mean circulating CD19$^+$ leukemic cell percentages ± SD (n=8 from 2 independent experiments). (**b**) Representative plots of un-transduced ALL-CM cells (UT, left plots) or ALL-CM cells transduced with NGFR-marked CD44 constructs containing (CD44v6+, middle plots) or not (CD44v6-, right plots) the CD44v6 isoform. (**c-d**) 5x10^6 CD19$^+$CD44v6$^+$ ALL-CM leukemic cells were infused in 6-8 weeks-old SGM3 mice. After 5 weeks, leukemic SGM3 mice received 2x10^6 T cells from newborn HSPC-humanized SGM3 mice (nHuSGM3), either un-transduced as control (CTRL), or transduced with either a CD44v6.28z (44v6.28z) or a CD44v6.BBz (44v6.BBz) CAR, or with either a CD19.28z (19.28z) or a CD19.BBz CAR (19.BBz)). Mean leukemic cell counts ± SD (n=6 each group from two independent experiments)., P<0.001 by a two-way ANOVA with Bonferroni correction.

Supplementary Fig. 7



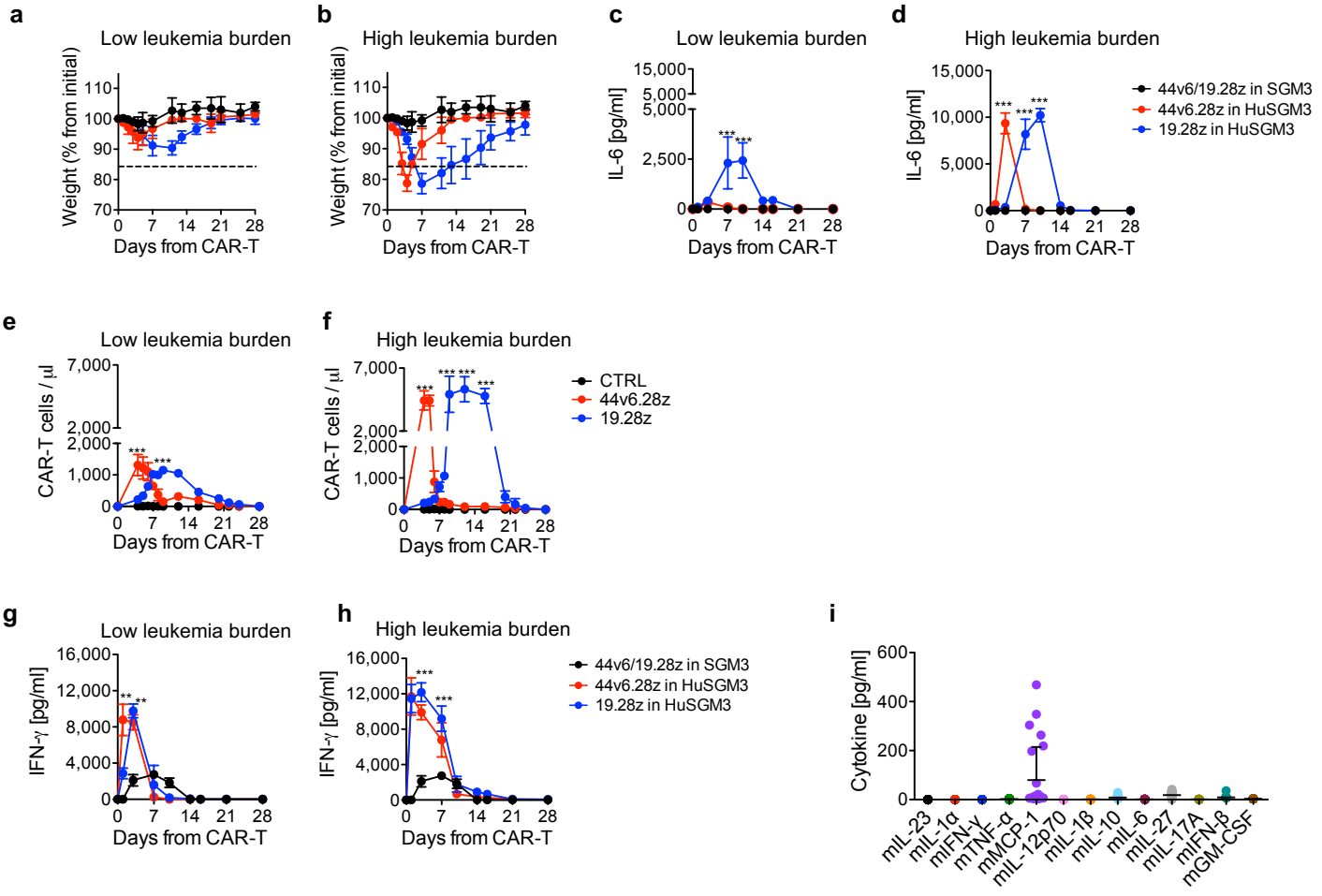
Tumor lysis syndrome by CAR-T cells from nHUSGM3 mice. 5x10^6 CD19$^+$CD44v6$^+$ ALL-CM leukemic cells were infused in 6-8 weeks-old SGM3 mice. After 5 (low leukemia burden) or 7 weeks (high leukemia burden), leukemic SGM3 mice received 2x10^6 T cells from newborn HSPC-humanized SGM3 mice (nHuSGM3), either un-transduced as control (CTRL, n=6 from 2 independent experiments), or transduced with a CD44v6.28z (44v6.28z, n=12 from 2 independent experiments) or a CD19.28z CAR (19.28z, n=12 from 2 independent experiments). (**a-b**) Mean leukemic cell counts ± SD. Arrow indicated CAR-T cell infusion. (**c-d**) Mean CAR-T cell counts ± SD. (**e-f**) Mean body weight variation percentages ± SD. Dashed lines: threshold for severe weight loss (>15%). (**g**) Mean body temperature variations ± SD. Dashed line: threshold for high fever (ΔT>2°C). (**h**) Mean human IFN-γ, IL-2, TNF-α, IL-10 or IL-6 serum concentrations ± SD 7 days after CAR-T cell infusion in high leukemia burden mice. (**i-j**) Mean mouse SAA and uric acid serum concentrations ± SD in high leukemia burden mice. ***, P<0.001 by a two-way ANOVA test with Bonferroni correction. (**k**) Representative plots and mean bone marrow (BM) CD19$^+$ leukemic cell percentages ± SD 24 weeks after CAR-T cell infusion. ***, P<0.001 by a one-way ANOVA test. (**l**) Mean BM CD19$^+$ leukemic cell percentages ± SD categorized as follows: >10%, n=10 from 2 independent experiments; 5-10%, n=11 from 2 independent experiments; <5%, n=10 from 2 independent experiments. (**m**) Mean leukemic cell counts ± SD after CD3-depleted BM cell infusion in secondary 6-8 weeks-old SGM3 recipients according to previous category. *, P<0.05; ***, P<0.001 by a two-way ANOVA test with Bonferroni correction.

Supplementary Fig. 8



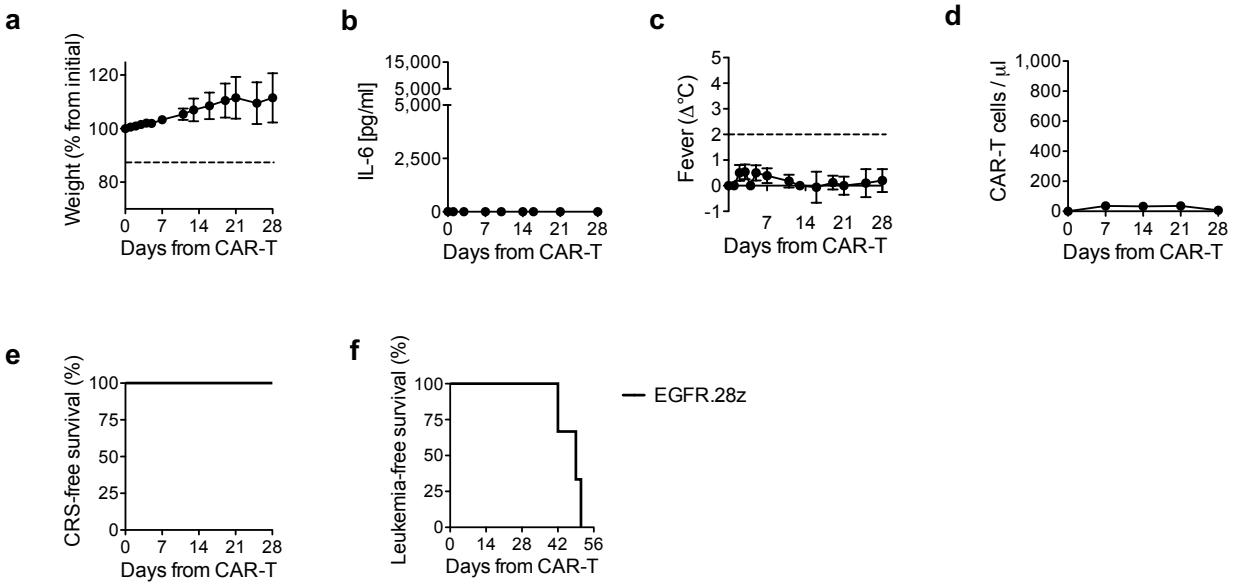
CAR-T cell expansion in HuSGM3 mice. 10^5 HSPCs were infused i.v. in 6-8 weeks-old SGM3 mice (HuSGM3). After 4 weeks, HuSGM3 mice received $2x10^6$ T cells from newborn HuSGM3 mice, either un-transduced as control (CTRL), or transduced with a CD44v6.28z (44v6.28z) or a CD19.28z CAR (19.28z). n=15 from 3 independent experiments each group. (**a**) Representative CD44v6 expression plots on circulating B cells and monocytes (grey, isotype control; red, specific antibody) before CAR-T cell infusion. (**b**) Mean CAR-T cell counts ± SD. (**c-d**) Besides 44v6.28z in HuSGM3 and 19.28z in HuSGM3, a group of 6-8 weeks-old SGM3 mice received CAR-T cells without prior HSC humanization (44v6.28z/19.28z in SGM3, n=18 from 3 independent experiments). Mean serum TNF-α and IL-10 concentration ± SD. *, P<0.05; ***, P<0.001 by a two-way ANOVA test with Bonferroni correction.

Supplementary Fig. 9



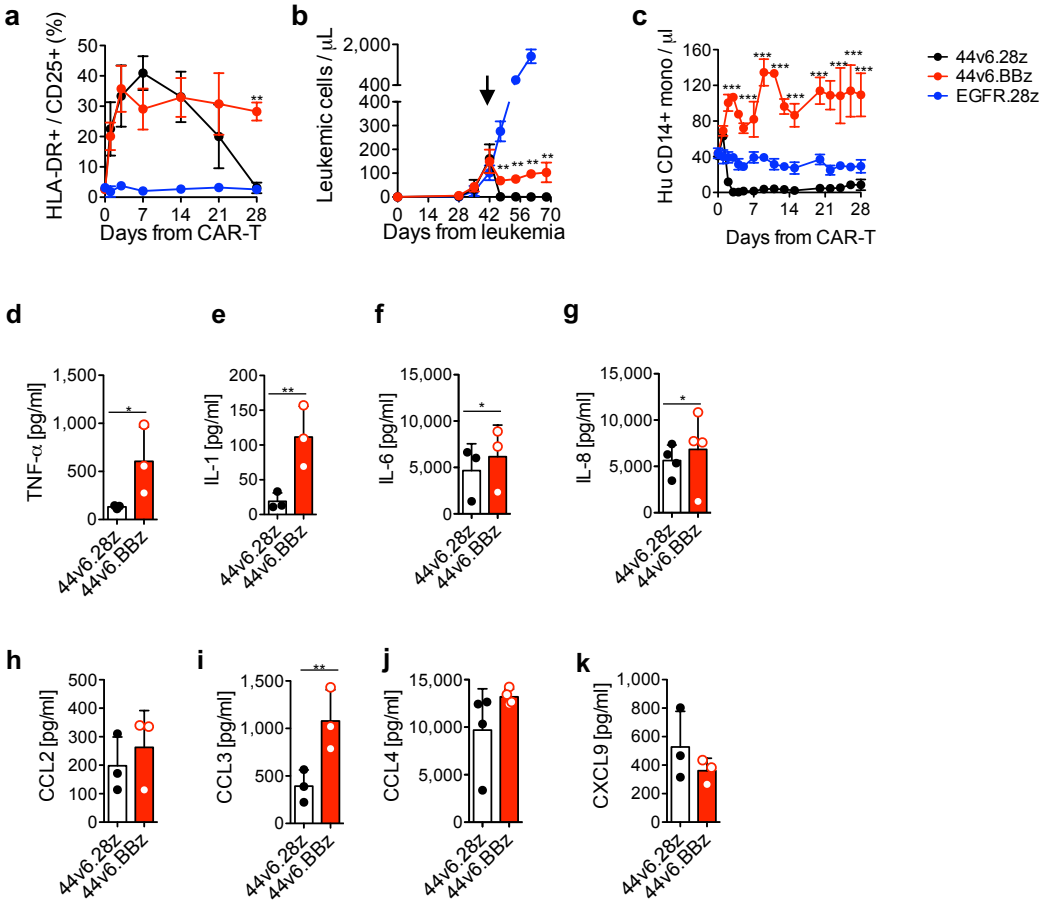
Correlation between leukemia burden, CAR-T cell expansion and cytokine release in HuSGM3 mice. 10^5 HSPCs and $5x10^6$ $CD19^+CD44v6^+$ ALL-CM leukemic cells were co-infused i.v. in 6-8 weeks-old SGM3 mice (HuSGM3). After 5 (low leukemia burden) or 7 weeks (high leukemic burden), HuSGM3 mice received $2x10^6$ T cells from newborn HuSGM3 mice, either un-transduced as control (CTRL), or transduced with a CD44v6.28z (44v6.28z in HuSGM3) or a CD19.28z CAR (19.28z in HuSGM3). A group of 6-8 weeks-old SGM3 mice received CAR-T cells without prior HSC humanization (44v6/19.28z in SGM3). n=15 from 3 independent experiments each group. (**a**-**b**) Mean body weight variation percentages ± SD. (**c**-**d**) Mean human IL-6 serum concentrations ± SD. ***, P<0.001 by a two-way ANOVA test with Bonferroni correction. (**e**-**f**) Mean CAR-T cell counts ± SD. ***, P<0.001 by a two-way ANOVA test with Bonferroni correction. (**g**-**h**) Mean human IFN-γ serum concentrations ± SD. ***, P<0.001 by a two-way ANOVA test with Bonferroni correction. (**i**) Mean mouse cytokine serum concentrations ± SD 7 days after CAR-T cell infusion.

Supplementary Fig. 10



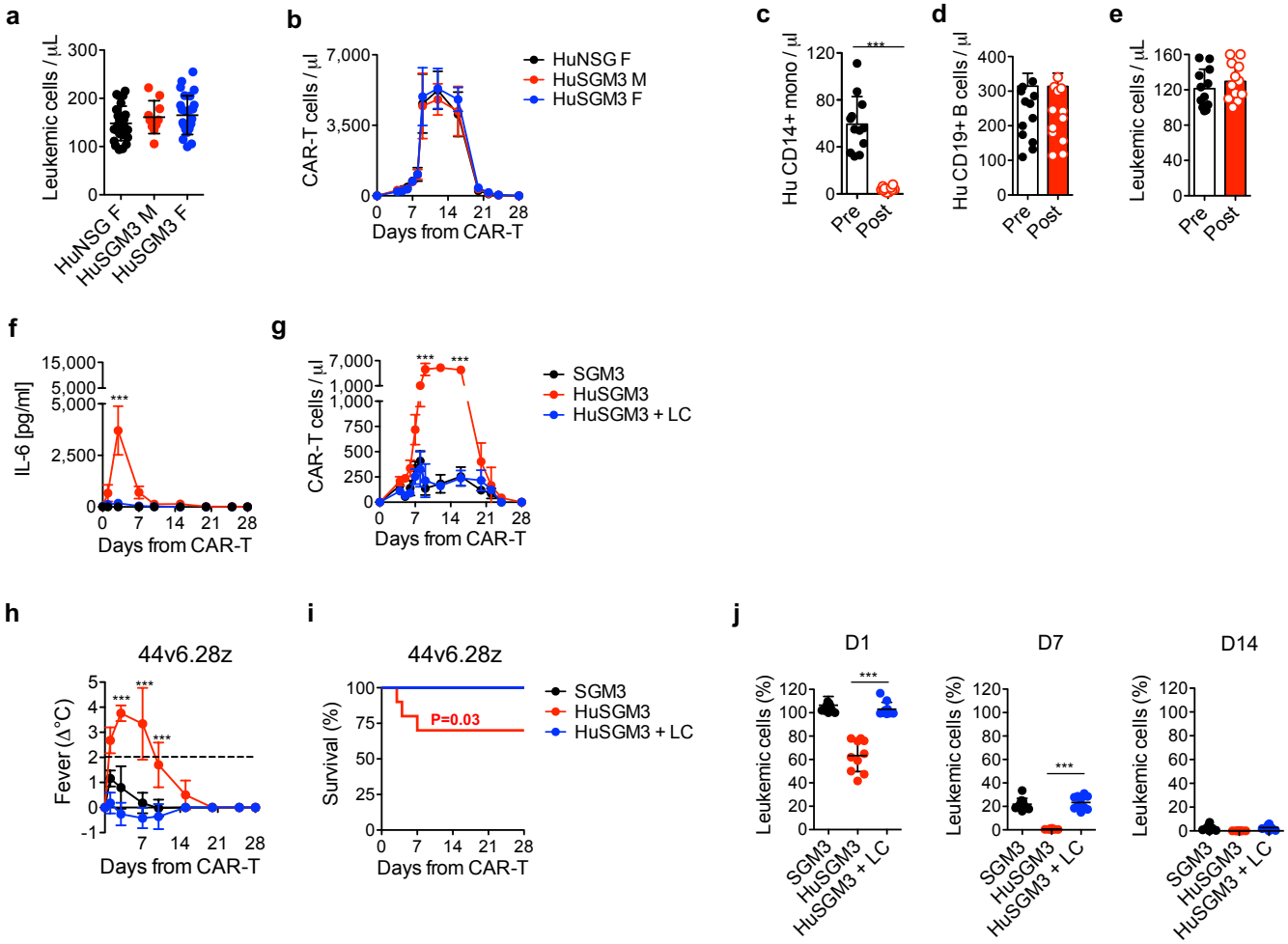
Lack of CRS by irrelevant CAR-T cells in leukemic HuSGM3 mice. 10^5 HSPCs and $5x10^6$ $CD19^+CD44v6^+$ ALL-CM leukemic cells were co-infused i.v. in 6-8 weeks-old SGM3 mice (HuSGM3). After 7 weeks, leukemic HuSGM3 mice received $2x10^6$ newborn HuSGM3 mice T cells transduced with an irrelevant EGFR.28z CAR (EGFR.28z, n=10 from 2 independent experiments). (**a**) Mean body weight variation percentages ± SD. (**b**) Mean human IL-6 serum concentration ± SD. (**c**) Mean body temperature variations ± SD. (**d**) Mean CAR-T cell counts ± SD. (**e**) CRS-free survival (see Methods). (**f**) leukemia-free survival (see Methods).

Supplementary Fig. 11



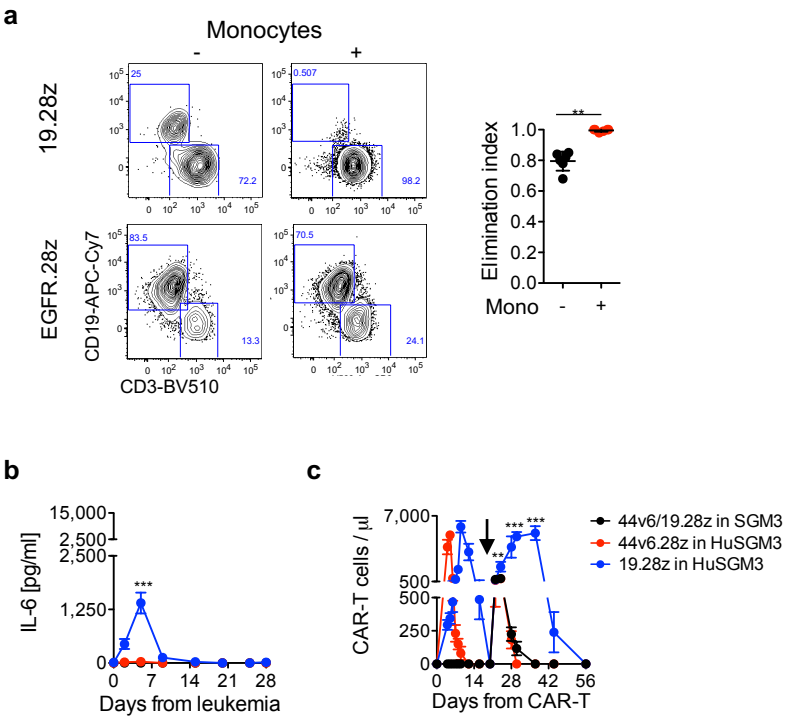
44v6.BBz CAR-T cell-induced monocytosis in leukemic HuSGM3 mice. 10^5 HSPCs and $5x10^6$ $CD19^+CD44v6^+$ ALL-CM leukemic cells were co-infused i.v. in 6-8 weeks-old SGM3 mice (HuSGM3). After 7 weeks, leukemic HuSGM3 mice received $2x10^6$ T cells from newborn HuSGM3 transduced with either a CD44v6.28z CAR (44v6.28z), a CD44v6.BBz CAR (44v6.BBz), or a EGFR.28z CAR (EGFR.28z), n=10 from 2 independent experiments each group. (**a**) Mean $HLA\text{-}DR^+CD25^+$ CAR-T cell percentages ± SD. (**b**) Mean leukemic cell counts ± SD. Arrow: CAR-T cell infusion. (**c**) Mean $CD14^+$ monocyte counts ± SD. **, P<0.01; ***, P<0.001 by a two-way ANOVA test with Bonferroni correction. (**d**-**k**) Mean human TNF-α (n=3 each group), IL-1 (n=3 each group), IL-6 (n=3 each group), IL-8 (n=4 each group), CCL2 (n=3 each group), CCL3 (n=3 each group), CCL4 (n=4 each group) and CXCL9 (n=3 each group) serum concentrations ± SD 7 days after CAR-T cell infusion. *, P<0.05; **, P<0.01 by a Mann-Whitney test with Bonferroni correction.

Supplementary Fig. 12



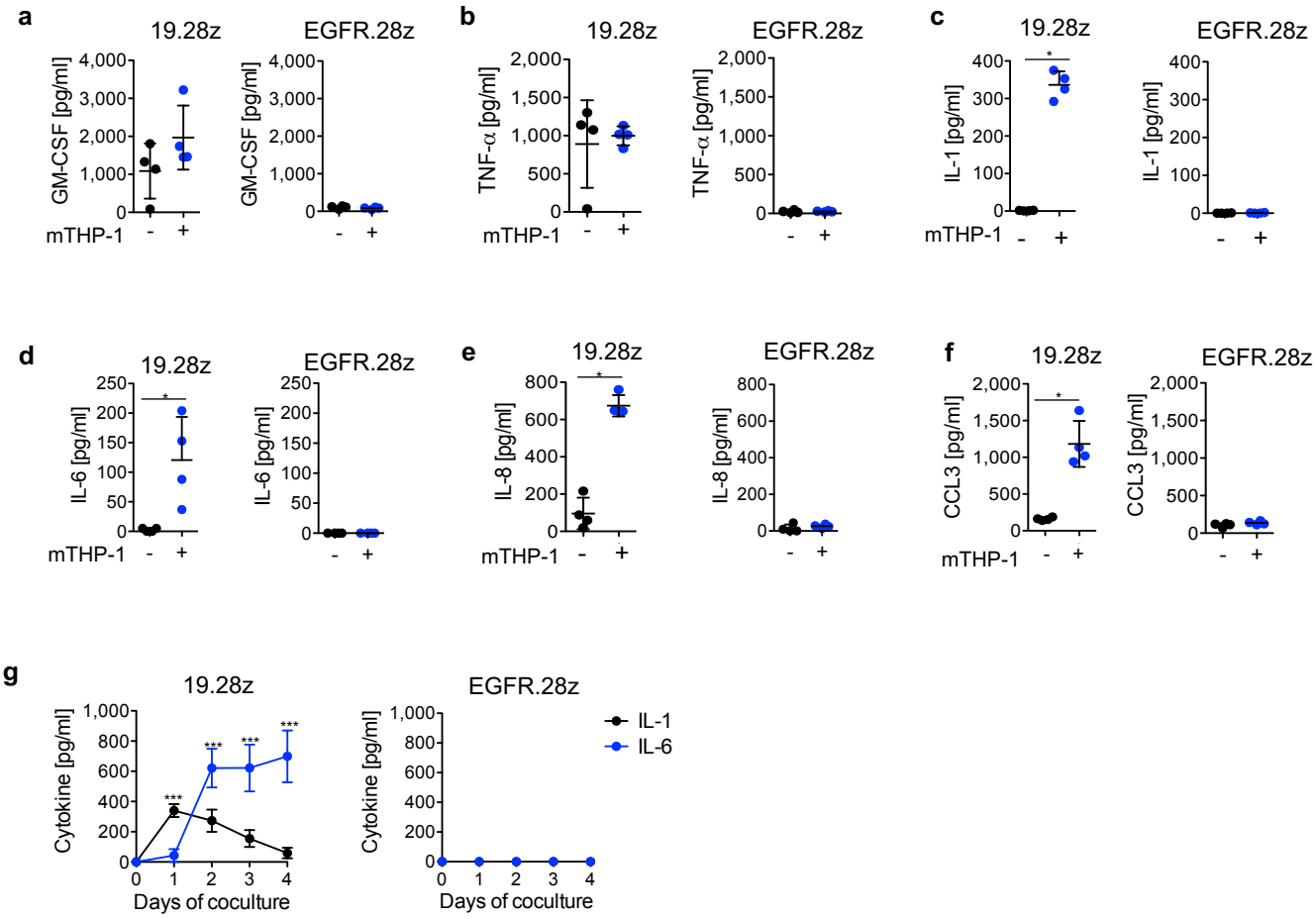
Lack of CAR-T cell expansion in monocyte-depleted leukemic HuSGM3 mice. 10^5 HSPCs and $5x10^6$ $CD19^+CD44v6^+$ ALL-CM leukemic cells were co-infused i.v. in 6-8 weeks-old female NSG (HuNSG F, n=26 from 3 independent experiments), male SGM3 (HuSGM3 M, n=10 from 2 independent experiments) or female SGM3 (HuSGM3 F, n=26 from 3 independent experiments,). After 5 weeks, leukemic HSPC-humanized mice received $2x10^6$ newborn HuSGM3 T cells transduced with a CD19.28z CAR. (**a**) Mean leukemic cell counts ± SD after 5 weeks from leukemia. (**b**) Mean CAR-T cell counts ± SD. (**c-e**) Leukemic non-HSC humanized and HuSGM3 mice were treated with lyposomal clodronate (+LC) prior to receiving HuSGM3 T cells transduced with either a CD19.28z (n=10 from 2 independent experiments) or a CD44v6.28z CAR (n=10 from 2 independent experiments). Mean Hu $CD14^+$ monocyte, $CD19^+$ B cell and leukemic cell counts ± SD one day before (Pre) or three days after (Post) LC administration. ***, P<0.001 from a one-way ANOVA test with Bonferroni correction. (**f**) Mean human IL-6 serum concentrations ± SD. (**g**) Mean CAR-T cell counts ± SD. ***, P<0.001 by a two-way ANOVA test with Bonferroni correction. (**h**) Mean body temperature variations ± SD. Dashed lines: threshold for high fever (ΔT>2°C). ***, P<0.001 by a two-way ANOVA test with Bonferroni correction. (**i**) Kaplan-Meyer survival curve with exact P value from a Mantel-cox two-sided log-rank test. (**j**) Mean leukemic cell percentages at day 1, 7 and 14 from CD44v6.28z CAR-T cell infusion. ***, P<0.001 from a one-way ANOVA test with Bonferroni correction.

Supplementary Fig. 13



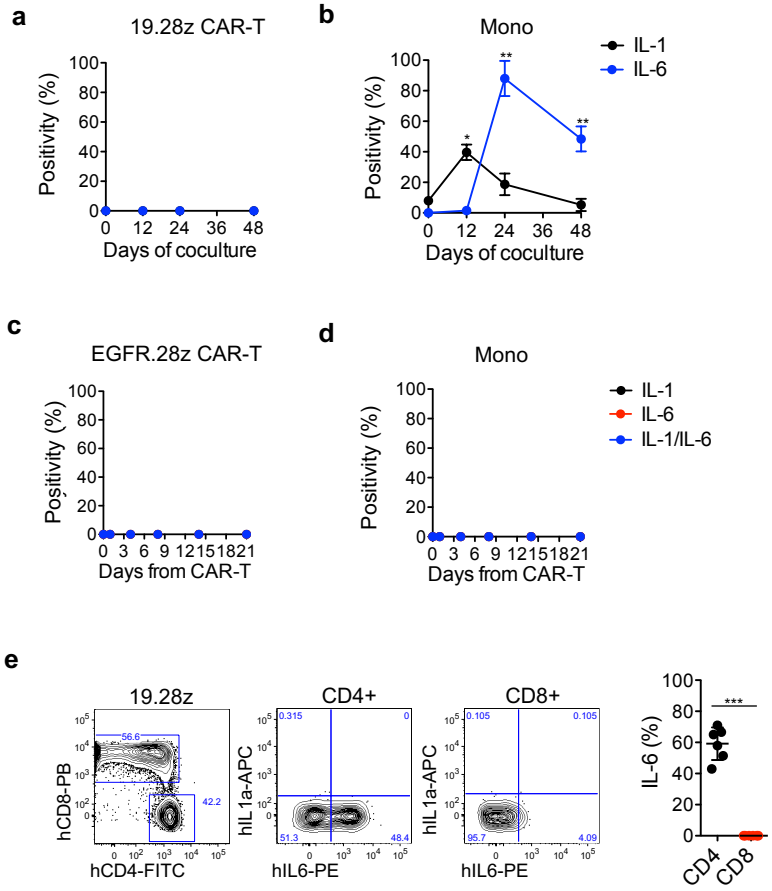
Contribution of monocyte-like cells to *in vitro* leukemia killing and *in vivo* IL-6 release by CAR-T cells. Human peripheral blood T cells were activated with CD3/CD28-beads, transduced with either a CD19.28z or an irrelevant EGFR.28z and cultured with IL-7/IL-15. After 15 days, CAR-T cells were co-cultured at a 1:10 effector-to-target ratio with $CD19^+$ BV173 leukemic cells with or without autologous monocytes (see Methods, n=6 independent experiments each). (**a**) Representative 4-days co-culture plots and mean BV173 leukemia elimination indexes ± SD. **, P<0.01 by a Mann-Whitney test with Bonferroni correction. (**b-c**) 10^5 HSPCs were infused i.v. in 6-8 weeks-old SGM3 mice (HuSGM3). After 4 weeks, HuSGM3 mice received $2x10^6$ newborn HuSGM3 T cells transduced with either a CD44v6.28z (44v6.z8z in HuSGM3) or a CD19.28z CAR (19.28z). A group of 6-8 weeks-old SGM3 mice received CAR-T cells without prior HSC humanization (44v6/19.28z in SGM3). n=15 from 3 independent experiments each group. After additional 3 weeks, mice were challenged with $5x10^6$ $CD19^+CD44v6^+$ ALL-CM leukemic cells (arrow). Mean human IL-6 serum concentrations ± SD. Mean CAR-T cell counts ± SD. **, P<0.01; ***, P<0.001 by a two-way ANOVA test with Bonferroni correction.

Supplementary Fig. 14



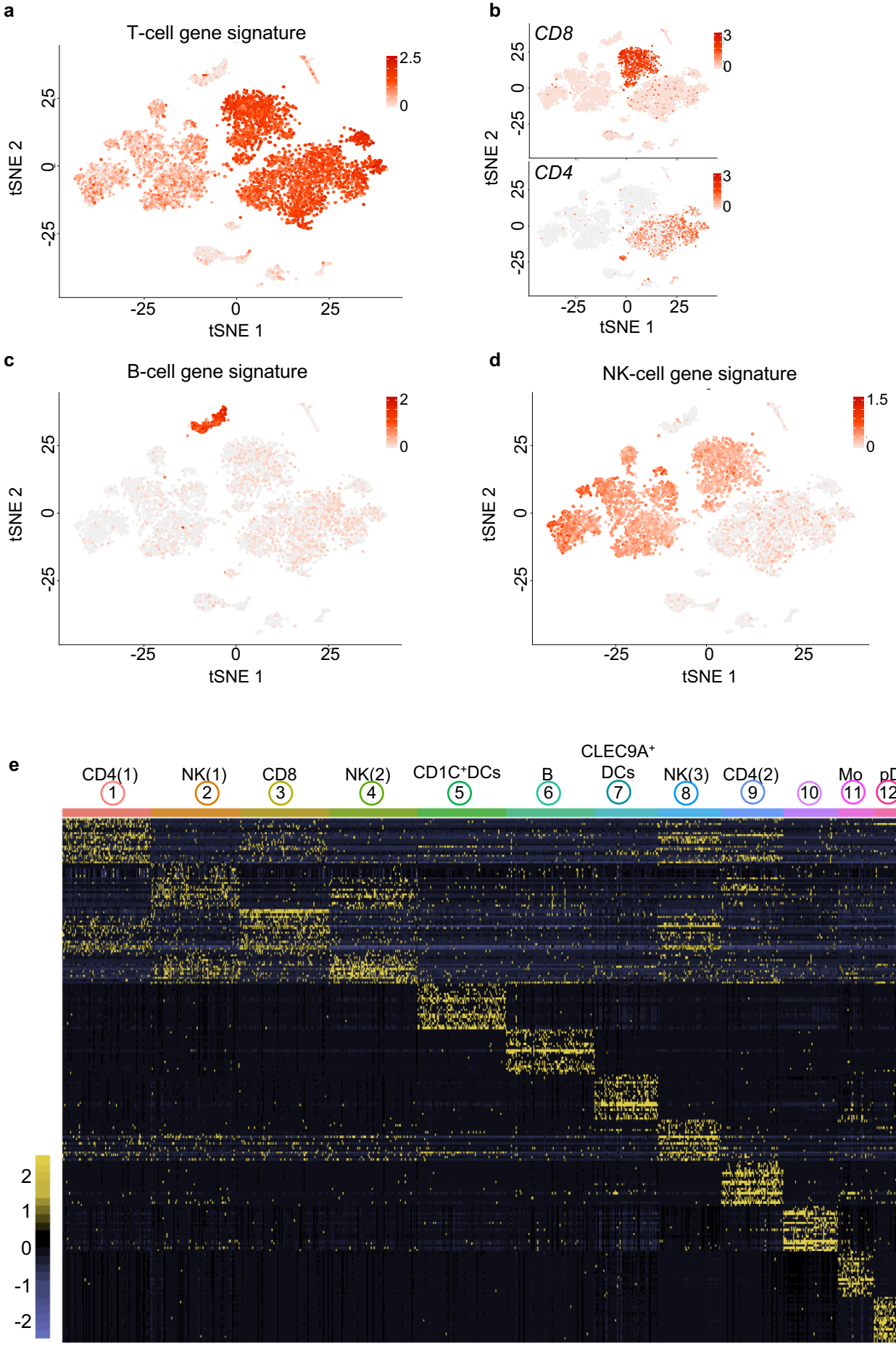
***In vitro* IL-1/IL-6 induction in monocyte-like cells by CAR-T cells.** Human peripheral blood T cells were activated with CD3/CD28-beads, transduced with either a CD19.28z or an irrelevant EGFR CAR and cultured with IL-7/IL-15. After 15 days, CAR-T cells were co-cultured at a 1:10 effector-to-target ratio with CD19^{+} BV173 leukemic cells. 48 hrs co-culture supernatants were added to THP1 monocyte-like cells (see Methods). (**a-f**) Mean human GM-CSF, TNF-α, IL-1, IL-6, IL-8 and CCL3 concentrations in 24 hrs THP1 monocyte-like supernatants ± SD (n=4 independent experiments). *, P<0.05 by a two-tailed Student's t test. (**g**) Mean human IL-1 and IL-6 concentrations ± SD in THP1 monocyte-like cell supernatants. ***, P<0.001 by a two-way ANOVA test.

Supplementary Fig. 15

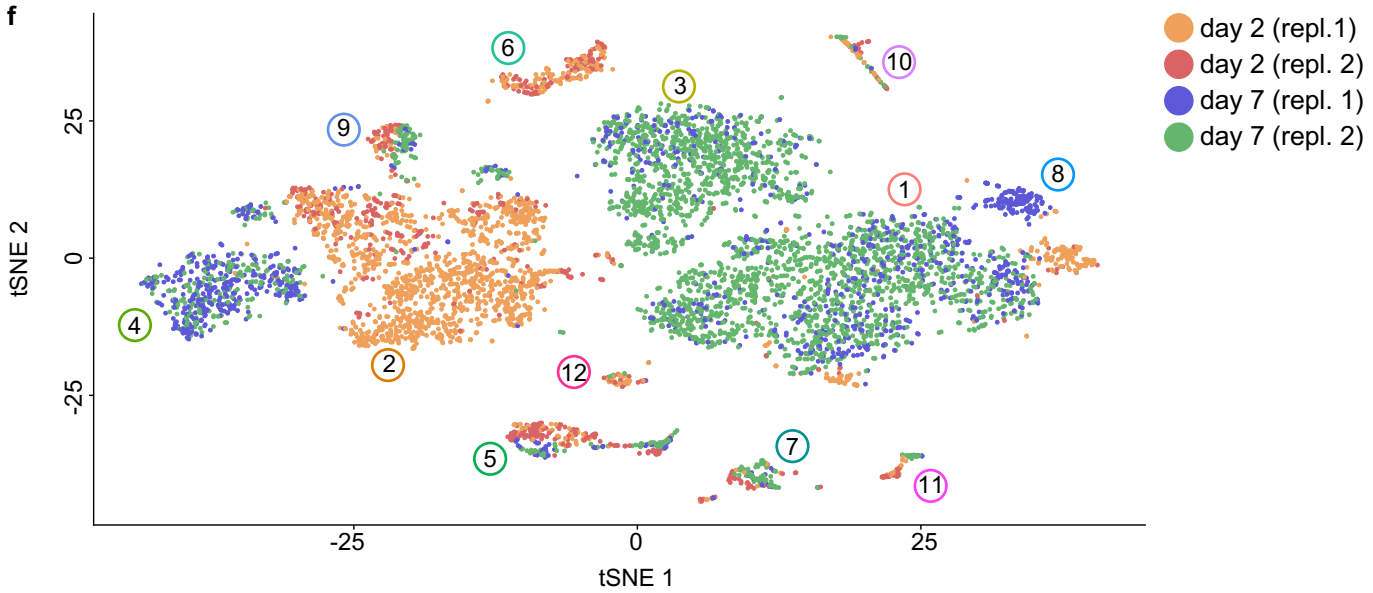


***In vivo* induction of IL-1/IL-6 release in primary monocytes by CAR-T cells.** Human peripheral blood T cells were activated with CD3/CD28-beads, transduced with either a CD19.28z or an irrelevant EGFR.28z CAR and cultured with IL-7/IL-15. After 15 days, CAR-T cells were co-cultured at a 1:10 effector-to-target ratio with CD19$^+$ BV173 leukemic cells, with or without primary autologous monocytes. (**a-b**) Mean intracellular IL-1/IL-6 positivity ± SD (n=3 independent experiments). *, P<0.05; **, P<0.01 by a two-way ANOVA test. (**c-e**) 10^5 HSPCs and $5x10^6$ CD19$^+$CD44v6$^+$ ALL-CM leukemic cells were co-infused i.v. in 6-8 weeks-old SGM3 mice (HuSGM3). After 7 weeks, leukemic HuSGM3 mice received $2x10^6$ T cells from newborn HuSGM3 mice transduced with either a CD19.28z CAR or irrelevant EGFR.28z CAR. (**c-d**) Mean intracellular IL-1/IL-6 positivity ± SD (n=3 independent experiments). (**e**) Representative plots and mean human IL-1/IL-6 positivity ± SD in CD4$^+$ or CD8$^+$ CD19.28z CAR-T cells 7 days after infusion (n=6). ***, P<0.001 by a two-tailed Student's t test.

Supplementary Fig. 16

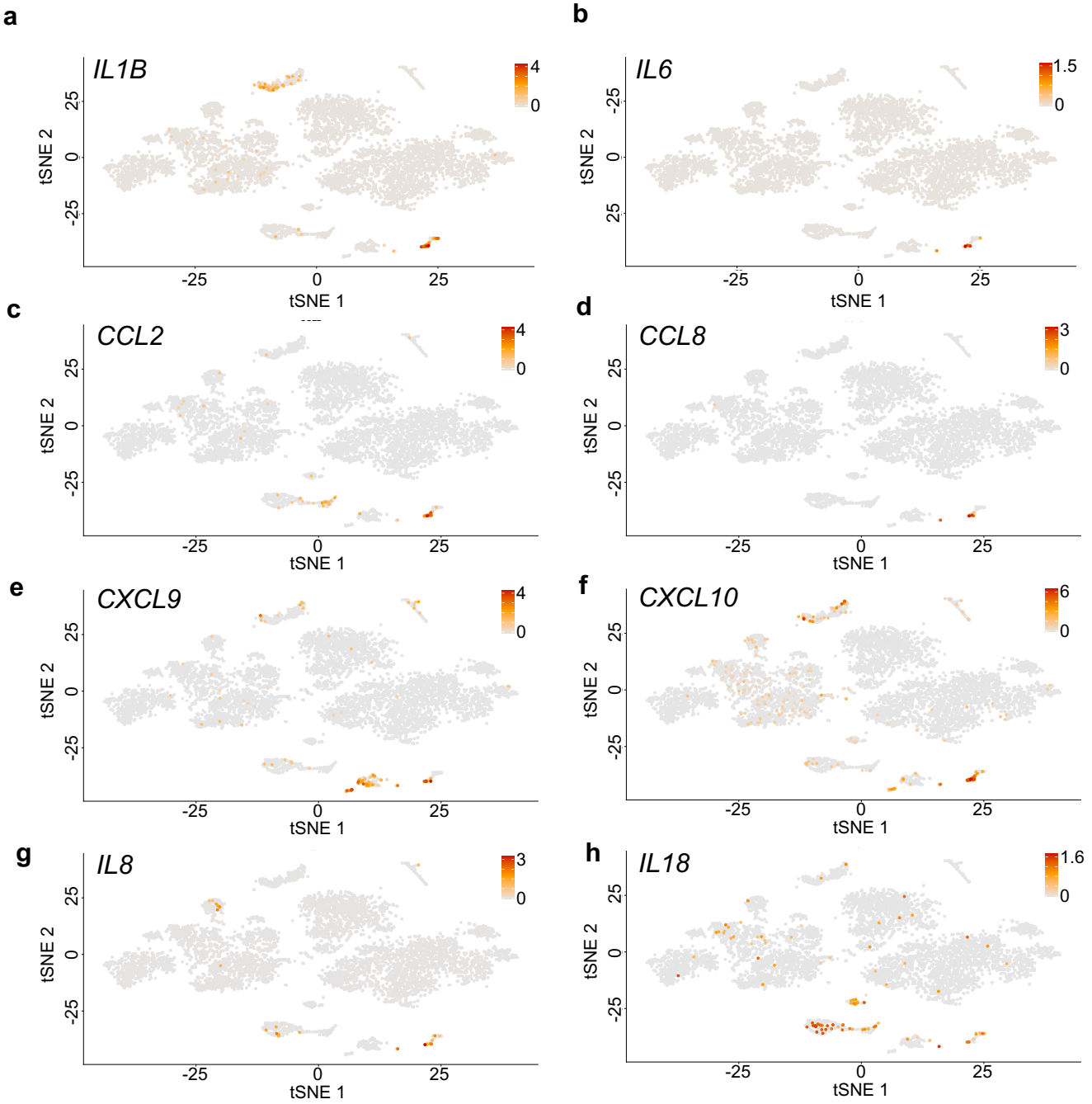


Supplementary Fig. 16



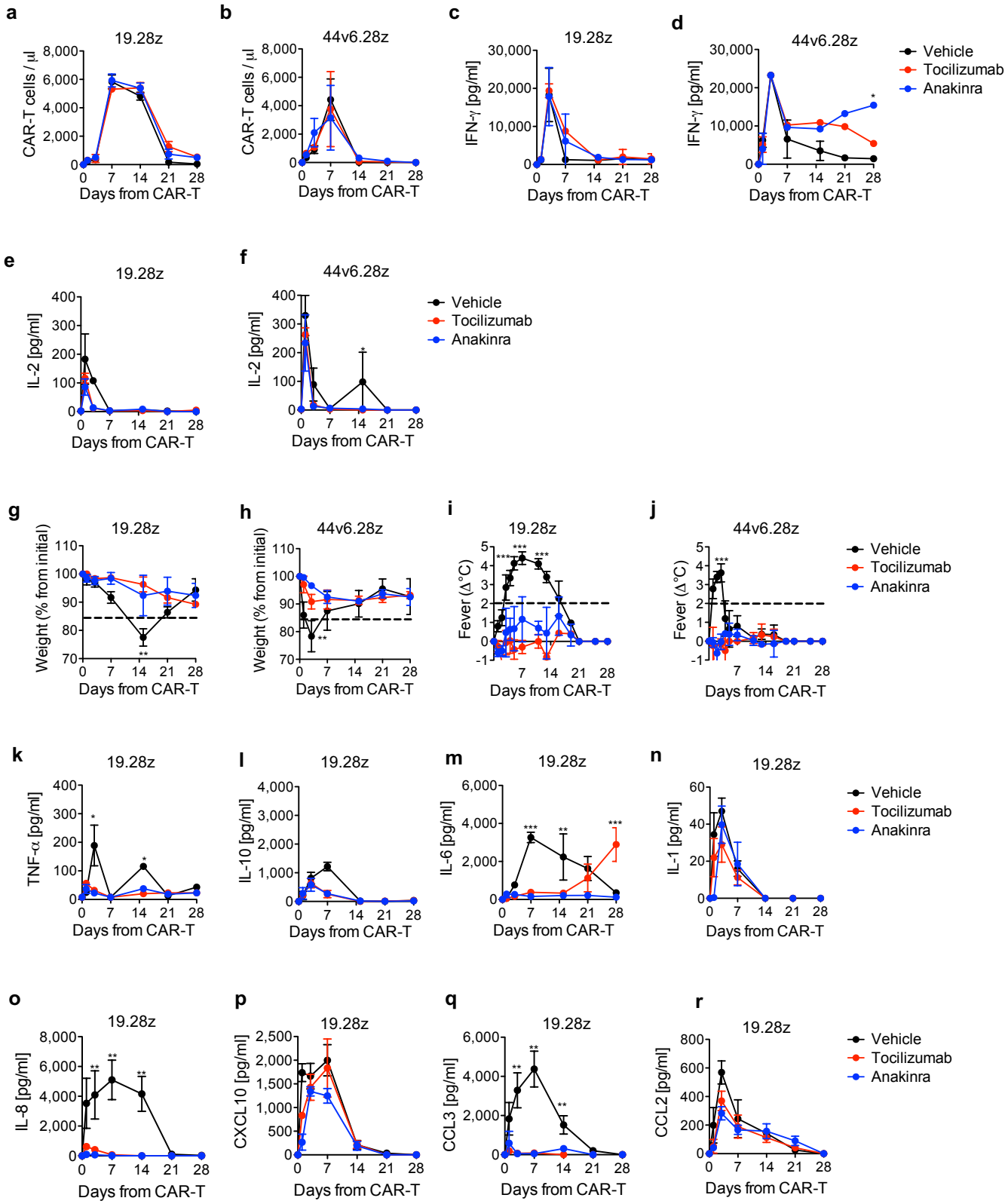
scRNA-Seq identification of human lymphoid and myeloid cell populations in leukemic HuSGM3 during CRS. 10^5 HSPCs and $5x10^6$ $CD19^+CD44v6^+$ ALL-CM leukemic cells were co-infused i.v. in 6-8 weeks-old SGM3 mice (HuSGM3). After 7 weeks, leukemic HuSGM3 mice received $2x10^6$ T cells from newborn HuSGM3 mice transduced with a CD19.28z CAR. At day 2 and day 7 from fever onset, human $CD45^+$ cells were isolated from the spleen and analyzed by Single-cell RNA Sequencing (scRNA-Seq). ScRNA-Seq tSNE plots of data from (**a**) T cell (CD3D, CD3E, CD3G, CD27, CD28), (**b**) CD8/CD4, (**c**) B cell (CD19, MS4A1, CD79A, CD79B, BLNK) and (**d**) NK cell signatures (FCGR3A, FCGR3B, NCAM1, KLRB1, KLRC1, KLRD1, KLRF1, KLRK1). Color scale: log transformed TPM across genes within each signature. (**e**) Heat map of log transformed TPM for top 20 discriminative genes within each cluster (cl.) and selected representative genes for each cl. (circled numbers, 200 cells per cl.). (**f**) scRNA-Seq tSNE plots of data from mice at day 2 and day 7 from fever onset. Each colored dot are referred to a single experimental sample and replicate.

Supplementary Figure 17



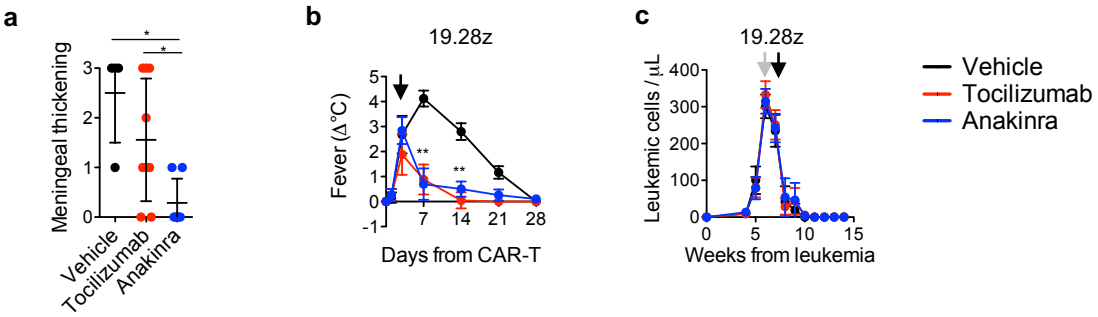
Myeloid-specific expression of genes encoding for inflammatory cytokines and chemokine in leukemic HuSGM3 mice during CRS. For experimental design description, see legend of **Supplementary Figure 16**. (**a-h**) tSNE plots showing single-cell expression levels of the indicated genes. Color scale reflects gene expression in log(TPM+1).

Supplementary Fig. 18



CAR-T cell expansion upon tocilizumab/anakinra prophylaxis in leukemic HuSGM3 mice. 10^5 HSPCs and $5x10^6$ CD19$^+$CD44v6$^+$ ALL-CM leukemic cells were co-infused i.v. in 6-8 weeks-old SGM3 mice. After 7 weeks, leukemic HSPC-humanized SGM3 mice (HuSGM3) received $2x10^6$ T cells from newborn HuSGM3 mice transduced with either a CD44v6 CAR.28z (44v6.28z) or a CD19.28z CAR (19.28z), n=50 from 3 independent experiments each group. Immediately prior to CAR-T cell infusion, mice were administered either vehicle (n=14 per group), tocilizumab (n=18 per group) or anakinra (n=18 per group). (**a-b**) Mean CAR-T cell counts ± SD. (**c-f**) Mean human IFN-γ and IL-2 concentrations ± SD. *, P<0.05 from a two-way ANOVA test with Bonferroni. (**g-h**) Mean body weight variation percentages ± SD. Dashed lines: threshold for severe weight loss (>15%). (**i-j**) Mean body temperature variations ± SD. Dashed lines: threshold for high fever (ΔT>2°C). **, P<0.01; ***, P<0.001 by a two-way ANOVA test with Bonferroni correction. (**k-r**) Mean human TNF-α, IL-10, IL-6, IL-1, IL-8, CXCL10, CCL3 and CCL2 serum concentrations ± SD. *, P<0.05; **P<0.01; ***,P<0.001 by a two-way ANOVA test with Bonferroni correction.

Supplementary Fig. 19



Prevention of CAR-T cell-mediated neuropathology by anakinra administration. 10^5 HSPCs and $5x10^6$ $CD19^+CD44v6^+$ ALL-CM leukemic cells were co-infused i.v. in 6-8 weeks-old SGM3 mice. After 7 weeks, leukemic HSPC-humanized SGM3 mice (HuSGM3) received $2x10^6$ T cells from newborn HuSGM3 mice transduced with a CD19.28z CAR. (**a**) 30-days meningeal thickening score ± SD (see Methods) in leukemic HuSGM3 mice administered vehicle (n=4), tocilizumab (n=9) or anakinra (n=7) prophylaxis. *, P<0.05 by a Mann-Whitney test. (**b-c**) Leukemic HuSGM3 mice receiving CD19.28z CAR-T cells from nHuSGM3 were administered vehicle (n=10), tocilizumab (n=10) or anakinra (n=10) at fever onset (black arrow). **, P<0.01 by a two-way ANOVA test. Mean body temperature variations ± SD. Mean leukemic cell counts ± SD. Light arrow: CAR-T cell infusion; arrow: drug administration.