Figure legends:

Extended Data Figure 1: Summary of the cross-organ off-target transcriptomic atlas (COOTA).

(a) Top 100 overexpressed genes in AML HSPC (left) and healthy T cells (right) from differential expression analysis. Normalized expression values were logarithmized and scaled to unit variance. **(b)** Overview of 11 scRNA-seq datasets of various healthy tissues used to quantify off-target antigen expression. **(c)** UMAP plots of 11 scRNA-seq datasets with colors highlighting clustering into respective cell types. Cell annotations were provided by the authors of the respective studies. **(d)** Current CAR targets in AML were cross-referenced to filters used for the single cell-based target screening approach. OE HSC-/Prog-like: overexpressed on HSC-/Prog-like cells with log fold change > 2 and FDR-adjusted $p \leq 0.01$, using a t-test with overestimated variance. Red cross: Antigen did not fulfill the respective threshold or criteria. Green check: Thresholds or criteria were passed.

Extended Data Figure 2: CSF1R and CD86 are consistently expressed across multiple patients with differing molecular subtypes.

(a, c) Amount of malignant and normal cells per AML patient of van Galen *et al.* **(a)** or Petti *et al.* **(c)**. **(b, d)** Percentage of malignant and normal cells expressing target genes *CSF1R, CD86* and reference genes *CD123, CD33* for each sequenced AML patient of van Galen *et al.* **(b)** or Petti *et al.* ***(d)*****. (e)** Data from van Galen *et al.*[21] was used as a reference (top left) to map cells from Petti *et al.*[28] (top right) using scANVI[29]. UMAP representation showing the mapped query and reference data together (bottom). **(f)** Computational CAR target antigen identification using the mapped dataset of Petti *et al.* by stepwise evaluation against a set of criteria for an ideal and effective CAR target antigen. The decreasing number of screened AML target genes are shown on the bottom. CSPA: Cell surface protein atlas; HPA: Human protein atlas. **(g)** Volcano plot showing CD86 and CSF1R target genes with their respective FDR-adjusted $\log_{10}$ p-value and $\log_2$ fold changes from differential expression analysis between malignant HSPC-like and healthy HSPC using a t-test with overestimated variance.

Extended Data Figure 3: mCSF1R CART do not persist in immunocompetent mice.

(a) Construct design of mCSF1R or mEpCAM CART or mCherry T cells. **(b)** Representative histograms of mCSF1R or mEpCAM expression on J774A.1 cells. Staining was carried out twice. **(c)** Summary of treatment schedule for *in vivo* toxicity assessment of mCSF1R CART.

(d) Mean weight curves of mice treated with 3 x 10^6 mCSF1R CART or mCherry T cells. n = 10 mice per group. Error bars indicate s.e.m. **(e)** Quantification of tissue-resident CD11b+ cells (left) or mCherry+ T cells of parent population (right, parent population: CD3 and CD8 positive cells) in different organs by flow cytometry. Data are mean ± s.e.m. of n = 10 mice. **(d, e)** Statistical significance was calculated using two-way ANOVA with Šidák multiple comparison correction. **(f)** Scheme of treatment schedule for *in vivo* toxicity assessment of mCSF1R CART. WBI, whole body irradiation. Mice were treated with 3 x 10^6 mCSF1R CART or mCherry T cells per mouse. 6 x 10^6 mEpCAM CART were transferred as a positive control. **(g)** Serum levels of indicated markers one (d1) or seven (d7) days after ACT. Depicted is the fold change of serum levels of the indicated groups from the PBS-treated control group. CRP, C-reactive protein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GLDH, glutamate dehydrogenase; γ-GT, gamma-glutamyl-transferase; LDH, lactate dehydrogenase. n = 3 mice per group. Statistically significant increases in the serum of mEpCAM CART treated mice compared to mCSF1R CART or control-treated mice at day 7 were observed for GLDH ($p < 0.0001$). **(h)** Histopathological analysis of indicated organs after treatment with mEpCAM or mCSF1R CART or mCherry T cells. Representative images of n = 3 mice per group. Signs of organ damage are indicated in the picture: black arrowhead: thickening of alveolar epithel. **(i)** Representative maximum intensity projection of microglia (green) and macrophages (green) in CX_3CR1-GFP mice on days 0 (baseline), 4, 7, 10, 14, 21 and 28 after intravenous injection of 10^7 mCSF1R CART (red). Depth from brain surface: 0-100 µm. Scale bars as depicted: 50 µm and 20 µm. mCSF1R CART: i.c. injection, n = 5 mice; i.v. injection, n = 3 mice; mCherry T cells: i.c./ i.v. injection = 2 mice.

Extended Data Figure 4: Assessment of different hCSF1R CAR constructs *in vitro.*

(a) Construct design of all anti-human constructs used throughout the course of the study. **(b)** Representative flow cytometric images of construct expression on primary human T cells. **(c)** Activation of different hCSF1R CART after incubation with plate-bound hCSF1R protein quantified by flow cytometry. **(d)** T cells expressing different hCSF1R CAR constructs were co-cultured with luc+ target antigen expressing AML tumor cell lines or antigen negative NALM-6 control cells for 48 hours at the indicated E:T ratios. Cell lysis was quantified by BLI. **(e)** Proliferation dye-labeled hCSF1R CART were co-cultured with respective cell lines for 4 or 7 days at a E:T ratio of 0.5:1. Proliferation was subsequently assessed by trace dilution. One representative image of three different donors is shown. **(f)** Bead quantified T cell numbers. **(g)** Secretion of IFNγ by T cells transduced with different hCSF1R CAR constructs after co-culture with AML cell lines. **(h)** Secretion of IFNγ **(left)** or IL-2 **(right)** of hCSF1R,

CD86 or control CART in co-culture with AML cell lines. **(c, d, f - h)** Data are mean ± s.e.m. of three independent donors.

LTR, long terminal repeat; scFv, single chain fragment variable; TM, transmembrane, IC, intracellular, ED, extracellular domain, CTRL-transduced, control-transduced.

Extended Data Figure 5: CSF1R is highly expressed on primary AML blasts.

(a) Representative histograms of CSF1R expression on AML cell lines after freeze and thaw cycles. **(b, c)** Schematic of culture methods used to cultivate primary AML samples throughout the course of the study. **(d)** Expression of CSF1R on primary AML samples after 24 to 48 hours of culture in cytokine rich medium. Left: Percentage positive cells gated to isotype. Each dot represents different primary AML samples. Right: Representative flow cytometric images from three different AML samples. Data are mean ± s.e.m. from six different donors. **(e)** Gating strategy to identify CD34+ CD38± malignant HSPC. **(f, g)** Expression of target antigens **(CSF1R, f; CD86, g)** on malignant HPC (top) and HSC (bottom). **(h)** Expression of CSF1R on PDX-388 sample at indicated time points after thawing. **(i)** IHC staining of human CSF1R in the bone marrow of control-treated PDX-388-bearing mice. Shown are two representative pictures (right, left) in two different magnifications (top 10x, bottom 20x). **(j)** Left: CD33 CART used for i.v. injection into PDX-388-bearing mice **(Fig. 5 k - m)** were co-cultured *ex vivo* with luc+ Mv4-11 tumor cells for 48 hours at indicated E:T ratios. Specific lysis was quantified by BLI. Shown is mean ± s.e.m. of three biological replicates. Experiment was carried out twice. **(k)** Representative flow cytometric image of percentage of CD3 positive T cells (left) and percentage of CAR (c-myc) positive T cells (right) in the blood of PDX-388-bearing mice. **(l, m)** *Ex vivo* CD33 expression on PDX-388 AML blasts in the bone marrow after treatment with CD33 CART or CTRL-transduced T cells (CD19 CART) measured by flow cytometry. Depicted are representative histograms **(l)** or the change of CD33-PE-Cy5 MFI in CD33 CART treated mice compared to CTRL-transduced treated mice **(m)**. **(m)** Data are mean ± s.e.m. from n = 3 mice injected with CD33 CART compared to CTRL-transduced mouse. (**k – m**) n = 3 mice injected with CD33 CART), n = 1 mouse injected with CTRL-transuced T cells.

Extended Data Figure 6: hCSF1R CAR T cells are effective *in vivo*.

(a) Treatment scheme used for PDX-372 model. **(b - d)** BLI images **(b)**, BLI quantification of tumor-burden **(c)** and survival curves **(d)** of PDX-372 tumor-bearing mice injected with 2 x 10^6 hCSF1R, CD33 CART or control-transduced T cells (n = 5 mice per group). **(b)** White cross, censored mice; red cross, mice succumbed to disease. **(e)** IHC staining of human CSF1R in

the bone marrow of control-treated PDX-372-bearing mice. Left: IHC for human CSF1R. Right: Isotype (top) and detection system control (bottom) for CSF1R IHC staining. **(f)** Schematic of treatment scheme used for OCI-AML3 cell line xenograft model. **(g, h)** BLI images **(g)** and survival curves **(h)** of OCI-AML3 tumor-bearing mice injected with 6 x 10^6 hCSF1R CART or control-transduced T cells (n = 3 - 4 mice per group). **(g)** Red cross, mice succumbed to disease. **(a - h)** Statistical significance was calculated using two-way ANOVA with Šidák multiple comparison correction. **(i)** log2 expression of CSF1R and CD86 target antigens or CD123 and CD33 controls in bulk RNA-sequencing dataset of the Leukemia MILE study (n = 615 different patients). HBM, healthy bone marrow. Data was obtained from bloodspot.eu. Dashed line represents the median, dotted line the interquartile ranges. Statistical significance was calculated using ordinary one-way ANOVA with Šidák multiple comparison correction. **(j)** Simple linear regression analysis of *in vitro* lysis of CAR T cells and target antigen density of the indicated AML cell line measured by flow cytometry. r = spearman correlation coefficient, p = p-value. Three independent antigen density measurements were used to perform regression analysis. For Kaplan-Meier-Curves, statistical significance was calculated with log-rank test.

Supplementary Table 1: Summary of patient characteristics of all primary human patient samples used during the study.

(a) Patient characteristics of all primary AML samples. **(b)** Characteristics of PDX samples used for *in vivo* experiments.

References

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