nature research

Corresponding author(s): Naoto Hirano

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study.
For final submission: please carefully check your responses for accuracy; you will not be able to make changes later.

Experimental design

1. Sample size

Describe how sample size was determined.

Samples sizes were estimated based on preliminary experiments. We did not use a statistical method to predetermine sample size.

2. Data exclusions

Describe any data exclusions.

No data were excluded throughout the studies.

3. Replication

Describe the measures taken to verify the reproducibility of the experimental findings.

The in vitro data were reproduced in multiple (at least three) samples. For in vivo studies to see antitumor effects of CAR-T cells, we repeated experiments twice and obtained similar results.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

For in vivo studies, mice were randomly assigned to treatment groups in each experiment.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

The investigators were not blinded to group allocation during data collection or analysis. All of our data analysis both in vitro and in vivo is based on the objectively measurable data: absolute cell counts, frequency, fluorescence intensity, body weight, tumor size, and survival. Blinding does not affect these data values.

Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ Test values indicating whether an effect is present *Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes noted.*
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars in all relevant figure captions (with explicit mention of central tendency and variation)

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

GraphPad Prism 6; FlowJo (version 9.7.6); Image Lab (version 5.2.1); ImageJ (version 1.48); Affymetrix Expression Console (version 1.4.1); Affymetrix Transcriptome Analysis Console (version 3.1.0.5), R (version 3.1.0); Bioconductor (version 2.14); HeatPlus (version 2.10.0); stats (version 3.1.0); ggplot2 (version 2.1.0); GSEA (version 2); Living Image (version 4.3.1).

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.

Artificial antigen presenting cells and CAR constructs will be available upon reasonable request under MTA.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

The following antibodies were used for the flow cytometry analysis: APC-Cy7-anti-CD4 (clone RPA-T4; BioLegend; #300518; lot number B244839), PE-Cy7-anti-CD4 (RPA-T4; BioLegend; #300512; lot number B159354), PE-Cy7-anti-CD8 (clone SFCI21Thy2D3; Beckman Coulter; #6603861; lot number 7617042), PE-anti-CD8 (clone RPA-T8; BioLegend; #301051; lot number 169230), Pacific-Blue-anti-CD8 (clone B9.11; Beckman Coulter; #A82791; lot number 32), PE-anti-CD69 (clone FN50; BioLegend; #310906; lot number B224547), FITC-anti-CD45RA (clone MEM-56; Thermo Fisher Scientific; #MHCD45RA01; lot number 795595F), PE-anti-CD62L (clone DREG-56; BioLegend; #304806; lot number B238376), Pacific Blue-anti-CCR7 (clone G043H7; BioLegend; #353210; lot number B224088), APC-Cy7-anti-CD27 (clone O323; BioLegend; #302815; lot number B213295), APC-anti-CD28 (clone CD28.2; BioLegend; #302911; lot number B208224), PerCP/Cy5.5-anti-CD95 (clone DX2; BD Biosciences; #561655; lot number 4290805), Alexa Fluor 488-anti-CD279 (clone EH12.2H7; BioLegend; #329936; lot number B231939), PE-anti-CD274 (clone 29E.2A3; BioLegend; #329706; lot number B236176), APC/Cy7-anti-CD366 (clone F38-2E2; BioLegend; #345026; lot number B234886), PerCP/Cy5.5-anti-CD223 (clone C9B7W; BioLegend; #125212; lot number B231176), FITC-anti-CD271 (clone ME20.4; BioLegend; #345104; lot number B223717), PerCP/Cy5.5-anti-CD271 (clone ME20.4; BioLegend; #345112; lot number B218745), V450-anti-CD271 (clone C40-1457; BD Biosciences; #562123; lot number 7138758), APC-anti-CD45 (clone HI30; BioLegend; #304012; lot number B190802), FITC-anti-HLA-A2 (clone BB7.2; BioLegend; #343304; lot number B150785), PE-anti-CD19 (clone HIB19, BioLegend; #302208; lot number B231046), biotin-labeled protein L (GenScript; #M00097; lot number 16F001065), streptavidin-PE (Thermo Fisher Scientific; #S866; lot number 1865801), Alexa Fluor 647-anti-phospho-STAT3 (Tyr705) (clone 4/P-Stat3; BD Biosciences; #557815; lot number 7129683), Alexa Fluor 647-anti-phospho-STAT5 (Tyr694) (clone 47/Stat5; BD Biosciences; #612599; lot number 7082834), Alexa Fluor 647-anti-phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (clone 20A, BD Biosciences; #561992; lot number 7088992), anti-phospho-Akt (Thr308) (clone D25E6; Cell Signaling Technology; #13038; lot number 3), Alexa Fluor 647-anti-rabbit IgG (H+L) (Jackson ImmunoResearch; #711-605-152), FITC-anti-IL-2 (clone 5344.111; BD Biosciences; #340448; lot number 7108853), PE-Cy7-anti-IFN-γ (clone 4S.B3; BioLegend; #502528; lot number B182253), and PE-anti-TNF-α (clone MAb11; BioLegend; #502909; lot number B224364). All the antibodies are validated for use in flow cytometry. Data are available on manufacturer's website.
The following antibodies were used for immunoblotting: anti-STAT3 (clone D3Z2G, Cell Signaling Technology; #12640; lot number 4), anti-phospho-STAT3 (Tyr705) (clone D3A7, Cell Signaling Technology; #9145; lot number 31), anti-STAT5 (Cell Signaling Technology; #9363; lot number 11), anti-phospho-STAT5 (Tyr694) (clone D47E7, Cell Signaling Technology; #4322; lot number 7), anti-β-actin (clone C4; Santa Cruz Biotechnology; #sc-477708; lot number C2014), HRP-conjugated anti-mouse IgG (H+L) (Promega, #W4021, lot number 0000262777), and HRP-conjugated anti-rabbit IgG (H+L) (Promega, #W4011, lot number 0000187662). All the antibodies are validated for use in immunoblotting. Data are available on manufacturer's website.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

aAPC/mOKT3 and K562-CD19: derived from the human erythroleukemic cell line K562 directly obtained from American Type Culture Collection (ATCC)
NALM-6: directly obtained from DSMZ (Braunschweig, Germany)
A375 melanoma cell line: directly obtained from ATCC

b. Describe the method of cell line authentication used.

Authentication of cells was carried out by short-tandem repeat (STR) analysis.

c. Report whether the cell lines were tested for mycoplasma contamination.

All cell lines were routinely assessed for the presence of mycoplasma contamination using a PCR-based technology.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

None of the cell lines used are listed in the ICLAC.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide all relevant details on animals and/or animal-derived materials used in the study.

Eight- to twelve-week old male NOD-scid IL2rgnull (NSG) mice bred at the Princess Margaret Cancer Centre animal facility were used.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

Peripheral blood mononuclear cells were obtained from healthy donors at the Princess Margaret Cancer Centre under approval of the Research Ethics Board of the University Health Network, Toronto, Canada. Population characteristcs of individual donors are not available to researchers.
Primary CD19+ B-ALL samples were obtained from newly diagnosed patients at the Princess Margaret Cancer Centre.

nature research

Corresponding author(s): Naoto Hirano

☐ Initial submission ☐ Revised version ☒ Final submission

Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

Data presentation

For all flow cytometry data, confirm that:

☒ 1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ 2. The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ 3. All plots are contour plots with outliers or pseudocolor plots.

☒ 4. A numerical value for number of cells or percentage (with statistics) is provided.

Methodological details

5. Describe the sample preparation.	Peripheral blood mononuclear cells were obtained from healthy donors and T cells were purified as described in Methods. In mouse experiments, peripheral blood was collected from the tail. Red blood cells were lysed before analysis of the peripheral blood and spleen cells. Subcutaneous tumor cells were ground into a single-cell suspension.
6. Identify the instrument used for data collection.	FACSCanto II (BD Biosciences)
7. Describe the software used to collect and analyze the flow cytometry data.	FlowJo software (Tree Star)
8. Describe the abundance of the relevant cell populations within post-sort fractions.	The purity was verified by flow cytometry analysis.
9. Describe the gating strategy used.	The gating strategy for each analysis is provided in Supplementary Figures. After gating on forward scatter (FSC) vs. side scatter (SSC), doublets were excluded by FSC-H vs. FSC-W and SSC-H vs. SSC-W gating. For analysis of spleen cells and subcutaneous tumor cells within the mice, debris was excluded by FSC vs. SSC gating as shown in Supplementary Figures.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information. ☒