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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	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 all covariates tested
☒	☐	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Beckton Dickinson Facsdiva Software was used for the acquisition of Flow cytometer raw data. Microplate Manager Software V5.2.1 of Benchmark Plus Microplate Spectrophotometer (Bio-Rad) was used for the measure of absorbance for ELISA. qRT-PCR raw data was collected with CFX manager Software using CFX Connect Rea-Time System (Bio-Rad). Bioluminescence imaging data were collected using the Living Image Software V4.7.4 (PerkinElmer).
Data analysis	Flow cytometry data analysis was performed using FlowJo Software V.10 (TreeStar, BD Biosciences). Bioluminescence image analysis was performed using Living Image Software V4.7.4(PerkinElmer). Aperio ImageScope Software V12.3.3 was used for image scan of histology data. Microsoft Excel Professional Pro 2019, Graphpad Prism V5.01 and V8.0 (GraphPad Software, Inc.) and SigmaPlot V8.0 (Systat Software, Inc.) were used for all other data analysis and visualization.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The data that support the findings of this study are available from the corresponding author upon reasonable request. Source data are provided with this paper.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	As anonymous random donors were used for PBMC in this study, we did not collect sex and gender information
Reporting on race, ethnicity, or other socially relevant groupings	As anonymous random donors were used for PBMC in this study, we did not collect race, ethnicity or other socially relevant groupings information.
Population characteristics	As anonymous healthy random donors were recruited, we did not collect population characteristics.
Recruitment	Anonymous healthy random donors were recruited
Ethics oversight	Human PBMCs were isolated from peripheral blood obtained from healthy volunteers according to a protocol approved by the Institutional Review Board of Seoul National University Hospital (IRB No. 1805-153-948).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	In vitro experiments were performed in triplicate or otherwise noted. For mouse studies a sample size of at least 4-5 mice per group was used and proved sufficient to determine statistical significance.
Data exclusions	No data were excluded from the analysis.
Replication	In vitro experiments were performed mainly in triplicate as stated in the methods and figure legends. The replication number is indicated in the legend of corresponding figures and attempts at replication were successful. Most of the in vivo studies were performed independently at least twice, and some data were pooled. Detailed information was provided in the relevant figure legends.
Randomization	For in vitro experiments, all samples and controls were allocated to identical cell-culture wells and treated side-by-side, so randomization was not need. For in vivo experiments, mice were randomly divided into experimental groups after confirmation of successful tumor engraftment by bioluminescence imaging prior to T cell injection.
Blinding	Investigators were not blinded. For animal studies, mice in control and test groups were handled in a consistent manner to prevent study bias, and only quantitative measures (BLI, weight, survival) were used to make conclusions, except GVHD scoring. GVHD scoring were performed objectively by the usage of stringent score sheets and the evaluation by one trained person.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

The following antibodies were used to stain cells for flow cytometry analysis:

Goat anti-Chicken IgY-FITC (IgG Fab Polyclonal, LSBio, #LS-C61573, 1:200 dilution),
AffiniPure Fab Fragment Goat Anti-Rabbit IgG (H+L)-FITC (IgG Fab Polyclonal, Jackson ImmunoResearch, #111-097-003, 1:200 dilution),
anti-cotinine-APC (in house conjugation with Rapid APC Antibody conjugation kit (LNK032APC, Bio-rad Laboratories, 1:500 dilution),
anti-mCD40-FITC (3/23, BD Biosciences, #561845, 1:200 dilution),
Rat IgG2a, κ Isotype Ctr-FITC (R35-95, BD Biosciences, #553929, 1:200 dilution),
anti-mCD86-FITC (GL1, BD Pharmingen, #553691, 1:200 dilution),
anti-mCD40-PE (3/23, BD Biosciences, #553791, 1:500 dilution),
Rat IgG2a, κ Isotype Ctr-PE (R35-95, BD Biosciences, #553930, 1:500 dilution),
anti-mCD4-PE (RM4-4, Biolegend, #116006, 1:1000 dilution),
anti-mCD8a-PerCP/Cy5.5 (53-6.7; Biolegend, #100722, 1:300 dilution),
anti-Thy1.2(CD90.2)-APC (53-2.1, Biolegend, #140312, 1:500 dilution),
anti-Thy1.1(CD90.1)-APC (OX-7, Biolegend, #202526, 1:500 dilution),
anti-mCD19-APC/Cy7 (6D5, Biolegend, #115529, 1:300 dilution),
anti-mCD4-APC/Cy7 (GK1.5, Biolegend, #100414, 1:300 dilution),
anti-mCD3-BV421 (17A2, Biolegend, #100218, 1:500 dilution),
anti-mCD11b-FITC (M1/70, Biolegend, #101205, 1:200 dilution),
anti-mF4/80-PE (BM8, Biolegend, #123109, 1:500 dilution),
anti-mCD11c-PE (N418, Biolegend, #117307, 1:500 dilution),
anti-hCD4-FITC (RPA-T4, Biolegend, #300506, 1:100 dilution),
anti-hCD40-PE (5C3, Biolegend, #334308, 1:100 dilution),
Mouse IgG1, κ Isotype Ctrl-PE (MOPC-21, Biolegend, #400111, 1:100 dilution),
anti-hCD8a-PerCP/Cy5.5 (HIT8a, Biolegend, #300924, 1:100 dilution),
anti-hCD4-PE/Cy7 (RPA-T4, Biolegend, #300512, 1:100 dilution),
anti-human kappa light chain-APC (TB28-2, BD Biosciences, #341108, 1:100 dilution),
anti-hCD4-APC/Cy7 (SK3, Biolegend, #344616, 1:100 dilution),
anti-hCD45-BV421 (HI30, Biolegend, #304032, 1:100 dilution),
anti-hCD4-BV510 (RPA-T4, Biolegend, #300546, 1:100 dilution),
anti-mCD80-BV421 (16-10A1, Biolegend, #104725, 1:500 dilution),
anti-mH-2K^b-APC (AF6-88.5, Biolegend, #116517, 1:500 dilution),
anti-mH-2K^d-APC (SF1-1.1, Biolegend, #116619, 1:500 dilution),
Streptavidin-PE (Biolegend, #554061, 1:1000 dilution),
7-AAD Viability Staining Solution (Biolegend, #420404, 5ul/sample),
TruStain FcX™ PLUS (anti-mCD16/32) (Biolegend, #156604, 2ul/sample)
PKH26 Red Fluorescent Cell Linker Mini Kit for General Cell Membrane Labeling (Sigma-Aldrich, #MINI26, Manufacture's instruction)
123count eBead Counting Beads (Invitrogen, #01-1234-42, 10ul/sample)

Validation

All used antibodies were validated and titrated with appropriate positive and negative controls in our Lab. Antibody validation by the manufacturer is available at each manufacturer's website.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

A20(TIB-208; B cell lymphoma on a Balb/C backgound), Raji(CCL-86; human B cell lymphoma), AU565(CRL-2351; human mammary gland adenocarcinoma), EL4(TIB-39; T cell lymphoma on a B6 background) and PG-13(CRL-3597; retroviral packaging cell line) were purchased from the American Type Culture Collection(ATCC, USA). Daudi(10213; human B cell lymphoma) was from the Korean Cell Line Bank. Phoenix GP and Phoenix Eco cell lines were provided by Garry Nolan(Stanford University, USA).

Authentication

Certificate of Analysis was provided with cell lines by the vendors, and additional authentication was not conducted. Morphology and antigen expressions were confirmed routinely by flow cytometry.

Mycoplasma contamination

All cell lines tested routinely and negative for mycoplasma.

Commonly misidentified lines (See ICLAC register)	No misidentified cell lines were used.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	Balb/C and B6 mice were purchased from Orient Bio, Inc., Korea. Thy1.1 congenic (000406; B6.PL-Thy1a/CyJ), CD40 knockout (002928; B6.129P2-Cd40tm1Kik/J), albino (000058; B6(Cg)-Tyrc-2J/J) on a B6 background, and NSG (005557; NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ) mice were from Jackson Laboratory, USA. CB6F1 mice were from Japan SLC, Inc. All mice were housed in a specific pathogen-free (SPF) animal facility at the Seoul National University College of Medicine and maintained in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC). They were subjected to a 12 h light/dark cycle and maintained at an ambient temperature of 23-27°C and a humidity of 40–60%.
Wild animals	This study did not involve wild animals.
Reporting on sex	Sex- and age-matched 6-8 week old mice were used in the experiment, and the experimental and control groups were raised separately in IVC racks in the same room.
Field-collected samples	This study did not involve samples collected from the field.
Ethics oversight	Animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Seoul National University College of Medicine (SNU-160602-17, SNU-200713-4).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

Confirm that:

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

☒ 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).

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

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

Methodology

Sample preparation	CAR expression on the mouse and human T cells was estimated by surface staining (for 25 min) with Fab Fragment Goat Anti-Rabbit IgG (H+L)-FITC for Cot-CAR or Goat anti-Chicken IgY-FITC for CD40-CAR with lineage markers (CD4/CD8). Dead cells were gated out with 7-AAD staining (for 10 min) and analyzed by flow cytometry with FlowJo software. For FACS-based cytotoxicity assay, target tumor cells were labeled with PKH26 (Sigma-Aldrich) according to manufacturer's instruction. The tumor cells (target; 5x104) and CAR-T cells (effector) were mixed at various effector: target (E:T) ratios (0:1 to 25:1) in 500 µl culture media per reaction and incubated for 6 h with subsequent 7-AAD staining. The number of viable tumor cells (7-AAD-, PKH+) was determined using the cell-counting beads (123count eBeads, ThermoFisher Scientific) via flow cytometry. The percent cytotoxicity was calculated as the following formula: (the number of viable tumor cells in the tube without CAR-T cells – the number of viable tumor cells in the tube with CAR-T cells) / the number of viable tumor cells in the tube without CAR-T cells x 100. For in vitro Cot-saporin-dependent cytotoxicity assay on Cot CAR-T cells, control T cells were generated using the same protocol for Cot CAR-T cell generation except for the retroviral transduction and pre-labeled with PKH26 dye to discriminate from Cot CAR-T cells. Then, the two populations (Cot CAR-T cells (target cells) and the control T cells (bystander non-CAR-T cells)) mixed with 1:1 (50,000 cells each) and incubated with various doses of Cot-saporin for 48 h in human IL-2 (500U/ml)-containing media. At the end of the culture, Cot CAR-T cells were stained with Fab Fragment Goat Anti-Rabbit IgG (H+L)-FITC and dead cell excluded with 7-AAD staining. The number of viable target (7-AAD-, Cot-CAR+) or control (7-AAD-, PKH26+) T cells after incubation were determined using the cell-counting beads via flow cytometry. The percent viability was calculated as the following formula: the number of viable cells in the tube with the drug / the number of viable cells in the tube without the drug x 100.
Instrument	BD FACScanto II or BD FACSLyric flow cytometer, BD FACS ARIA II (for cell sorting)
Software	Beckton Dickinson Facsdiva software was used for Flow cytometry raw data acquisition. FACS data analysis was performed using FlowJo Software V.10
Cell population abundance	In selective Cot CAR-T cell depletion test in allogeneic CAR-T cell transfer model, Cot CAR-T cells generated from Thy1.1 B6 mice were FACS sorted. Purity was at least 90%.
Gating strategy	With FSC-A/SSC-A gating, debris was removed by gating on the main cell population. FSC-H/FSC-A singlet gating was used to

Gating strategy

select a single cell population. In some cases, dead cells were excluded by 7-AAD staining. Multicolor samples were compensated with single color controls.

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