

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

FACSDiva v.9.0 (BD Biosciences): Flow cytometric data acquisition.
NIS-Elements Confocal software (A1, Nikon), and FV10S-SW (FV1000, Olympus) and FV31S-SW (FV3000, Olympus): Confocal microscopy data acquisition.
Metamorph software (IX71, Olympus): Fluorescence microscopy data acquisition.
LivingImage v.2.50.1 (Perkin Elmer) : In vivo Bioluminescence data acquisition
ImageReader LAS-4000 (FUJI): Immunoblotting image collection.
Dionex UltiMate 3000 RS UHPLC system and Q Exactive mass spectrometer: Proteomics data collection.

Data analysis

FlowJo (V10) was used for flow cytometric data analysis.
Image J and IMARIS (V9.8.0) were used for image data analysis.
LivingImage (V2.50.1) were used for In vivo bioluminescence data analysis.
Proteomics data were analyzed by R (V4.2.1), edgeR R package (V3.36.0), Partek Flow Software(V10.0.23), String (V11.0), and Cytoscape (V3.9.1).
Statistical analysis was performed using GraphPad Prism (V9.0)

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 mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD039955. All data are included in the Supplementary Information or available from the authors, as are unique reagents used in this Article. The raw numbers for charts and graphs are available in the Source Data file whenever possible.

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. H-2008-114-1150).

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](https://www.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	All in vitro experiments and in vivo experiments shown are characteristic of at least 3 repeated independent experiments. The replication number is indicated in the legend of corresponding figures and attempts at replication were successful. In vitro and in vivo CAR T-cell experiments were repeated using different blood donors. Most of the in vivo studies were performed independently at least twice. Detailed information was provided in the relevant figure legends.
Randomization	Animals were age matched and randomly assigned to each experimental group. For in vivo bioluminescence experiments, mice were randomly divided into experimental groups after confirmation of successful tumor engraftment by bioluminescence imaging prior to T cell injection.
Blinding	No blinding was used, as data were analyzed by each individual responsible for the experiment.

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	Involvement 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	Involvement in the study
☒	☐ ChIP-seq
☒	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

T cell stimulation:

anti-mouse CD3e (Clone 145-2C11), BD Biosciences, Cat# 553058
 anti-mouse CD28 (Clone 37.51), BD Biosciences, Cat# 553295
 anti-human CD3 (Clone OKT3), BioXcell, Cat# BE0001-2
 anti-human CD28 (Clone 28.2), BD Biosciences, Cat# 556620

Immunoblotting and immunoprecipitation (IP):

anti-Actin, beta (Clone AC-15), Sigma-Aldrich, Cat# 05-829, 1:20000 dilution for immunoblotting
 anti-Tubulin, alpha (Clone DM1A), Millipore, Cat# 05-829, 1:2000 dilution for immunoblotting, 2 ug for IP
 anti-mouse CD99 (Clone EJ2), Dinona, Manually purified, 1:1000 dilution for immunoblotting, 5 ug for IP
 anti-c-Myc (Clone 9E10), Santa Cruz Biotechnology, Cat# sc-40, 1:2000 dilution for immunoblotting, 2 ug for IP
 anti-GFP (Clone 3E6), Thermo Fisher Scientific, Cat# A11120, 2 ug for IP
 anti-IQGAP1 (Clone C-9), Santa Cruz Biotechnology, Cat# sc-376021, 1:1000 dilution for immunoblotting, 2 ug for IP
 anti-Myosin IIA, Cell Signaling Technology, Cat# 3403, 1:1000 dilution for immunoblotting
 anti-Erk1/2 (Clone 3A7), Cell Signaling Technology, Cat# 9107, 1:4000 dilution for immunoblotting
 anti-Zap70 (Clone 99F2), Cell Signaling Technology, Cat# 2705, 1:1000 dilution for immunoblotting
 anti-Phospho-Erk1/2 (Clone E10), Cell Signaling Technology, Cat# 9106, 1:1000 dilution for immunoblotting
 anti-Phospho-Zap70 (Clone 65E4), Cell Signaling Technology, Cat# 2717, 1:1000 dilution for immunoblotting
 Peroxidase AffiniPure Goat Anti-Mouse IgG, light chain specific, Jackson ImmunoResearch Labs, Cat# 115-035-174, 1:2000-1:5000 dilution for immunoblotting
 goat anti-rabbit IgG-HRP, Santa Cruz Biotechnology, Cat# sc-2004, 1:1000-1:2000 dilution for immunoblotting
 normal mouse IgG, Santa Cruz Biotechnology, Cat# sc-2025, 2 ug for IP
 normal rabbit IgG, Santa Cruz Biotechnology, Cat# sc-2027, 2 ug for IP
 normal rat IgG, Santa Cruz Biotechnology, Cat# sc-2026, 5 ug for IP

Cell imaging:

anti-Tubulin, alpha, Abcam, Cat# ab18251, 1:1000 dilution
 anti-Tubulin, alpha (Clone DM1A), Millipore, Cat# 05-829, 1:500 dilution
 anti-NFAT1 (Clone D43B1), Cell Signaling, Cat# 5861, 1:200 dilution
 anti-NFkBp65 (Clone D14E12), Cell Signaling, Cat# 8242, 1:200 dilution
 anti-phosphor-Erk1/2 (D13.14.4E), Cell Signaling, Cat# 4370, 1:200 dilution
 anti-GFP (Clone 3E6), Thermo Fisher Scientific, Cat# A11120, 1:500 dilution
 anti-IQGAP1 (Clone C-9), Santa Cruz Biotechnology, Cat# sc-376021, 1:200 dilution
 anti-Myosin IIA, Cell Signaling Technology, Cat# 3403, 1:200 dilution
 Alexa Fluor 594-conjugated phalloidin, Thermo Fisher Scientific, Cat# A12381, 1:1000 dilution
 Alexa Fluor 647-conjugated phalloidin, Thermo Fisher Scientific, Cat# A22287, 1:1000 dilution
 Alexa Fluor 488 goat anti-mouse IgG (H+L), Thermo Fisher Scientific, Cat# A11001, 1:2000 dilution
 Alexa Fluor 555 goat anti-rabbit IgG (H+L), Thermo Fisher Scientific, Cat# A21430, 1:2000 dilution
 Alexa Fluor 594 goat anti-mouse IgG (H+L), Thermo Fisher Scientific, Cat# A11005, 1:2000 dilution
 Alexa Fluor 647 goat anti-rabbit IgG (H+L), Thermo Fisher Scientific, Cat# A21245, 1:2000 dilution
 FITC anti-mouse TCR beta (Clone H57-597), Thermo Fisher Scientific, Cat# 11-5961-81, 1:500 dilution
 FITC anti-mouse CD11a (LFA-1; Clone 2D7), BD Biosciences, Cat# 553120, 1:500 dilution
 PE anti-mouse ICAM-1 (Clone YN1/1.7.4), Thermo Fisher Scientific, Cat# 12-0541-81, 1:500 dilution
 PE anti-mouse CD99, R and D systems, Cat# FAB3905P, 1:200 dilution

FACS:

PE/Cy7 anti-mouse CD8a (Clone 53-6.7), BD Biosciences, Cat# 552877, 1:2000 dilution
 FITC anti-mouse TCR beta (Clone H57-594), Thermo Fisher Scientific, Cat# 11-5961-81, 1:1000 dilution
 FITC anti-mouse CD11a (LFA-1; Clone 2D7), BD Biosciences, Cat# 553120, 1:1000 dilution
 APC anti-mouse CD25 (Clone PC61.5), Thermo Fisher Scientific, Cat# 17-0251-81, 1:2000 dilution
 APC/Cy7 anti-mouse/human CD44 (Clone IM7), Biolegend, Cat# 103028, 1:2000 dilution
 eFluor450 anti-mouse CD45.1 (Clone A20), Thermo Fisher Scientific, Cat# 48-0453-82, 1:500 dilution

PE anti-mouse CD69 (Clone H1.2F3), Biolegend, Cat# 104508, 1:2000 dilution
 BV605 anti-mouse CD90.2 (Clone 30-H12), Biolegend, Cat# 105343, 1:4000 dilution
 PE anti-mouse CD99, R and D systems, Cat# FAB3905P, 1:500 dilution
 APC anti-mouse CD178 (FasL; Clone MFL3), BioLegend, Cat#106609, 1:500 dilution
 BV421 anti-human CD3 (Clone UCHT1), BioLegend, Cat#300434, 1:500 dilution
 Alexa Fluor 647 mouse anti-human CD99 (Clone DN16), Novus Biologicals, Cat#NB100-64930AF647, 1:1000 dilution
 BV510 anti-human CD8 (Clone SK1), BioLegend, Cat#344732, 1:500 dilution
 APC/Cy7 anti-human CD4 (Clone RPA-T4), BD Biosciences, Cat#557871, 1:500 dilution
 BV605 anti-human CD69 (Clone FN50), BD Biosciences, Cat#562989, 1:250 dilution
 PE anti-human CD25 (Clone M-A251); BioLegend, Cat#985802, 1:1000 dilution
 Biotinylated anti-FMC63 (Clone Y45), Acro Biosystems, Cat#FM3-BY54, 1:50 dilution
 PE/TexasRed Streptavidin, BD Pharmingen, Cat#551487, 1:2000 dilution
 PE Streptavidin, BD Pharmingen, Cat#554061, 1:8000 dilution
 7-AAD Viability Staining Solution, Biolegend, Cat#420404, 5ul/sample
 123count eBead Counting Beads, Invitrogen, Cat#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)

Jurkat (TIB-152), HEK293T (CRL-3216), Raji (CCL86), PG-13 (CRL-3597), B16F10 (CRL-6475) were purchased from the American type Culture Collection (ATCC). Plate-E (RV-101) was purchased from Cell Biolabs. Phoenix GP and Phoenix Eco cell lines were provided by Garry Nolan (Stanford University, USA). The generation of B6 or CD99 knockout H60-CTL, and recombinant CDD99-transduced cells, H60-DC, OVA-DC, B16F10-OVA and Raji-Luc is detailed in Methods.

Authentication

Certificate of Analysis was provided with cell lines by the vendors, and additional authentication was not conducted. B6 or CD99 knockout H60-CTLs, and CD99 knockout H60-CTLs engineered to express recombinant CDD99s were routinely validated via flow cytometry.

Mycoplasma contamination

All cell lines tested negative for mycoplasma contamination.

Commonly misidentified lines
(See [ICLAC](#) register)

No commonly 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

C57BL/6J (wild type, WT; 000664), Thy1.1+ (B6.PL-Thy1a/CyJ; 000406), CD45.1+ (B6.SJL-Ptprca Pepcb/BoyJ; 002014), BALB.B (C.B10-H2b/LiMcdj; 001952), OT1 (C57BL/6-Tg(TcratCrb)1100Mjb/J; 003831), and NSG (NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ; 005557) mouse strains were purchased from The Jackson Laboratory. H60 congenic mouse strain (B6.C-H60C/DCR) is a gift from Dr. Derry Roopenian (The Jackson laboratory), CD99 knockout (B6-Cd99Gt(pU-21T)44Imeg) was obtained from Kumamoto University. J15 TCR transgenic mouse strain was developed in our laboratory.

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%. Mice at the age of 8-12 week old were used for experiments.

Wild animals

This study did not involve wild animals.

Reporting on sex

Only female mice are induced to respond to immunization against H60. Therefore, only H60-specific T cell immunity was tested with female B6 or CD99 knockout mice, and B6 and CD99 knockout H60-CTLs, which were established from splenocytes of the immunized mice, are of female mouse origin. Otherwise, gender was not considered.

Field-collected samples

This study did not involve samples collected from the field.

Ethics oversight

All mice were bred and maintained under specific-pathogen-free (SPF) conditions at the Biomedical Center for Animal Resource Development of Seoul National University College of Medicine. All animal studies were performed with permission from the Institute of Animal Care and Use Committee (IACUC) of Seoul National University College of Medicine (SNU-221207-9-2, SNU-221207-5-1, SNU-221207-4-3, SNU-220704-9).

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

Plants

Seed stocks	Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.
Novel plant genotypes	Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.
Authentication	Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.

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	Cells were washed with 2% FBS in PBS before resuspension, blocked with Fc receptor antibody, and then stained with fluorescent dye-labeled antibodies for cell surface marker detection. Staining was performed in 100 µl 2% FBS in PBS. Cells were incubated for 30 minutes at 4°C, then washed with 2% FBS in PBS prior to analysis by flow cytometry.
Instrument	Flow cytometric data was collected on BD LSR II and Cytek Northern Lights. Sorting was conducted on BD Aria II.
Software	Data collection was performed on FACSDiva V.9.0 (BD Biosciences) and SpectroFlo (Cytek Biosciences) Data analysis was performed on FlowJo V.10 (TreeStar)
Cell population abundance	To sort CD99 knockout CTL lines expressing rWT-CD99, Cyt-Mut-CD99, or TM-Mut-CD99 after transduction with corresponding retrovirus, about 0.1 ~ 0.2 million YFP+ cells were collected. Purity was determined by subsequent flow cytometric analysis after sorted cells were allowed time to proliferate through antigen-re-stimulation. Cells were resorted if relevant purity was below 99.9%. Subsequent verification of purity was accomplished via frequent flow cytometric analysis. Luciferase-GFP-expressing Raji (Raji-luc) cells were sorted based on GFP expression and maintained as described above.
Gating strategy	If a live/dead stain (DAPI) was used, cells were first gated for live cells by capturing the DAPI-negative population. For all flow cytometry experiments in the manuscript, samples were gated for single cells (FSC-A/FSC-H), lymphocyte population (FSC-A/SSC-A), then relevant surface markers (CD8, CD44, CD25, CD69, etc) and/or peptide-MHC tetramers.

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