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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	Long-read sequencing data were acquired using the MinKNOW software platform (version 6.2.8). Base calling was subsequently performed with Dorado (version 0.7.4) to generate FASTQ files for downstream analysis.
Data analysis	Off-target data were analyzed using UMI-tools (v1.1.6), SAMtools (v1.18), minimap2 (v.2.28-r1209) and python 3.10.8. Long-read data consensus clustering were analyzed using MMseqs2(version f2498a2acda2d8b24fc7cc3b2243c9ded3c7272b). Custom scripts are available at https://github.com/BaeLab/PrimeAssemblyAnalysis .

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

Sequencing datasets generated in this study have been made publicly available through the NCBI Sequence Read Archive under BioProject accession PRJNA1506632. The analysis code used in this study is publicly available on GitHub at https://github.com/BaeLab/PrimeAssemblyAnalysis.

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	*Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used. Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.*
Reporting on race, ethnicity, or other socially relevant groupings	*Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status). Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.) Please provide details about how you controlled for confounding variables in your analyses.*
Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

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	No statistical methods were used to predetermine sample size for experiments. Sample sizes were chosen based on previous experience with similar genome editing studies and are consistent with those generally employed in the field.
Data exclusions	No data were excluded from the analyses. Representative images and quantifications are shown for clarity, and all replicates yielded consistent results.
Replication	All experiments using cultured cell lines were independently replicated at least twice (biological replicates), and key findings were confirmed in three or more independent experiments.
Randomization	Samples were not randomized because experimental conditions and treatments were predetermined by study design and plasmid constructs.
Blinding	Investigators were not blinded to group allocation during experiments or outcome assessment, as the assays involved objective quantification by dPCR, next-generation sequencing, and flow cytometry analyses.

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:
APC-conjugated anti-human TCRα/β antibody (BioLegend, cat#: 306718, clone IP26, lot#: B417584, 1 µl per sample)
APC/Cyanine7-conjugated anti-human CD4 antibody (BioLegend, cat#: 344616, clone SK3, lot#: B474303, 1 µl per sample),
PE/Cyanine7-conjugated anti-human CD8 antibody (BioLegend, cat#: 344712, clone SK1, lot#: B463703, 0.1 µl per sample),
PE-conjugated anti-FMC63 antibody (ACROBiosystems, cat#: FM3-PY54A2, clone Y45, 1 µl per sample). The lot number for the anti-FMC63 antibody was not recorded.
7-AAD Viability Staining Solution (BioLegend. cat. no. 420404, lot#: B495080, 5 µl per sample).

Validation

All antibodies used in this study were validated by the manufacturers for flow cytometry applications in human cells, as stated on the manufacturers' websites. No additional validation was performed.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

HEK293T, HEK293, HeLa, k562 from ATCC.
Huh7, Hepa-1c1c7 from Korean Cell Line Bank(KCLB).
Human peripheral blood mononuclear cells (PBMCs) from (StemCell Technologies, 70025)

Authentication

No cell lines were authenticated

Mycoplasma contamination

Cells were not tested for mycoplasma contamination

Commonly misidentified lines
(See ICLAC register)

None

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 mice (Mus musculus), 4–8 weeks old, were purchased from Orient Bio. Female NSG mice (Mus musculus; NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ; stock no. 005557) , 6–8 weeks old, were obtained from The Jackson Laboratory, USA. C57BL/6J mice were maintained under specific-pathogen-free conditions at 22 ± 2 °C and 50–60% relative humidity under a 12-h light–dark cycle, with ad libitum access to food and water. NSG mice were housed in a specific-pathogen-free animal facility at Seoul National University College of Medicine at 23–27 °C and 40–60% relative humidity under a 12-h light–dark cycle, with ad libitum access to food and water.

Wild animals

This study did not involve wild animals.

Reporting on sex

Only female C57BL/6J and NSG mice were used in this study. Female C57BL/6J mice were used to minimize variability associated with sex-dependent physiological differences and to maintain consistency across hydrodynamic tail-vein injection experiments. For the NSG mouse experiments, age- and sex-matched animals were assigned to the experimental and control groups and housed separately in individually ventilated cage racks in the same room. Sex-based analyses were not performed.

Field-collected samples

This study did not involve field-collected samples.

Ethics oversight	Experiments involving C57BL/6J mice were approved by the Sungkyunkwan University Institutional Animal Care and Use Committee (SKKUIACUC2025-06-64-1). Experiments involving NSG mice were approved by the Institutional Animal Care and Use Committee of Seoul National University College of Medicine (SNU-250213-4-1). All animal experiments were conducted in accordance with the relevant institutional guidelines and approved protocols.

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, mosiacism, 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	At 72 h post-transfection, cells were harvested using 0.05% trypsin–EDTA (Welgene, LS015), centrifuged at 455 × g for 3 min, and resuspended in 200 µL of flow cytometry buffer (DPBS supplemented with 2% FBS). For primary human T-cell experiments, cells were collected at the indicated time points after electroporation, washed, and resuspended in flow cytometry buffer before antibody staining.Instrument
Instrument	Fluorescence was measured using BD FACSymphony A5 and BD LSRFortessa X-20 instruments (BD Biosciences)
Software	Flow cytometry data were acquired using BD FACSDiva™ Software and analyzed using FlowJo v10 (Tree Star).
Cell population abundance	Reporter-positive (GFP^+) cells were quantified as a percentage of total viable singlets. Representative plots are shown in the figures, and mean ± s.d. values from independent replicates are summarized in the corresponding bar graphs. For human T-cell experiments, viable singlets were identified by exclusion of DAPI-positive events and sequential FSC/SSC-based doublet discrimination. TCRαβ-positive and TCRαβ-negative populations were defined based on APC fluorescence, and CAR-positive cells were quantified based on PE fluorescence within each population. Representative gating plots are shown in the figures, and mean ± s.d. values from independent biological replicates are summarized in the corresponding bar graphs.
Gating strategy	For cell line experiments, cells were first gated on forward and side scatter (FSC-A vs SSC-A) to exclude debris.Singlet cells were subsequently isolated using two sequential gating steps: first on FSC-H vs FSC-W and then on SSC-H vs SSC-W.Live cells were identified based on scatter characteristics, and GFP^+ populations were defined relative to unstained and donor-only negative controls. For human T cell experiment, Cells were first gated based on forward and side scatter parameters to exclude debris. Live cells were identified by exclusion of DAPI-positive events. Doublets were excluded by sequential gating on FSC-H versus FSC-W and SSC-H versus SSC-W to obtain single-cell populations. Single cells were then gated based on TCRαβ expression, and CAR expression was assessed by PE fluorescence within TCRαβ-positive and TCRαβ-negative populations. Gates were defined relative to unstained and donor-only negative controls.

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