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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	All code is available at GitHub: https://github.com/yezhengSTAT/CAR-T_Histone. All software and platforms used are listed in Supplementary Table 13.
Data analysis	All code is available at GitHub: https://github.com/yezhengSTAT/CAR-T_Histone. All software and platforms used are listed in Supplementary Table 13.

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

Source data are provided as a Source Data file that was used to generate the corresponding figures. All raw data and workflow are available as an open-source

resource. The raw sequencing data and the processed normalized bedGraph files generated in this study have been deposited in the NCBI GEO database under the accession code GSE254807 for the CUT&RUN data and GSE254808 for the matching RNA-seq data. Both are available for the public without any restrictions.

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	Sex-information is recorded in Table S10. Gender information was not recorded and not relevant to the findings of this study.
Reporting on race, ethnicity, or other socially relevant groupings	Race, ethnicity and other socially relevant grouping information was not recorded and was not relevant to the analysis and findings of this study.
Population characteristics	Clinical outcome data following CAR-T cell therapy was ascertained from a de-identified patient database where only patient numbers were recorded. All other population characteristics was not recorded and was not relevant to the analysis and findings of this study.
Recruitment	Healthy donors were recruited from Bloodworks Northwest as apheresis donors for research (https://bloodworksbio.org/research-products/leukopaks/). Patients for NCT01865617 were recruited from the Fred Hutchinson Cancer Center/ Univeristy of Washington clinical trial. All trial recruitment is detailed as previously in Turtle et al. J Clin Invest 2016;126(6):2123-38.
Ethics oversight	All ethics oversight was by the Fred Hutchinson Cancer Center Institutional Review Board and the University of Sydney Human Research Ethics Committee.

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	Sample size was determined by known variation in CUT&RUN and RNA-seq of healthy donor T cells.
Data exclusions	All healthy donor data was included except biological replicates that failed QC assessments. All CM-enriched patient data was included that had matched RNA-seq performed previously.
Replication	All samples were run as technical duplicates. Biological replicates n=6 HD, 3 T cell subsets/HD; Fig. S3 and CUT&RUN data n=4 HD, 3 T cell subsets/HD and n=15 for patient CM-enriched CAR-T cells
Randomization	Randomization was not relevant to this study.
Blinding	Blinding was not relevant to this study.

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	All antibodies, suppliers and catalogue numbers are listed in Table S15.
Validation	All antibodies are commercially available and have been verified by Western blotting or flow cytometry described on the manufacturer's specification sheets. All antibodies used in this study are confirmed to recognize the human protein as stated on manufacturer's website.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Human TM-LCL and parental K562 cell lines and K62 transduced with CD19 used for generation and testing of CAR-T cells were a gift from the Riddell Laboratory at the Fred Hutchinson Cancer Center.
Authentication	TM-LCL is a widely used feeder cell line for T cell immunotherapy (see Terakura et al. Blood 2012; 119(1):72-82). K562 cells and CD19-K562 cells have been widely used in previous CAR-T cell studies (see Hirayama et al. Blood Adv. 7 (11): 2479–2493)
Mycoplasma contamination	All cell lines were tested negative for mycoplasma contamination. All primary T cells were tested negative for mycoplasma.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified lines were used in this study.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	Clinical trial is registered with clinicaltrials.gov under accession number NCT01865617
Study protocol	Study protocol overview is available on https://clinicaltrials.gov/study/NCT01865617
Data collection	Data collection was at time of apheresis and CAR-T cell manufacture.
Outcomes	Primary outcomes were determined as detailed in the study protocol.

Plants

Seed stocks	Not applicable
Novel plant genotypes	Not applicable
Authentication	Not applicable

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 into a flow cytometry (PBS + 1% foetal calf serum) and stained as per manufacturer protocol.
Instrument	Cells were acquired on a BD FACSymphony A5 Special Order Research Product or Aurora Cytek.
Software	Data was collected with FACSDiva (BD) or Aurora Cytek and analyzed by FlowJo Version 10.0 (BD)
Cell population abundance	Purity was confirmed at time of sort for fractions on the BD FACSAria software with all sort purities > 95%.
Gating strategy	Gating strategy was based on fluorescence minus one for each individual sample.

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