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Reporting Summary

Nature Research 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 Research 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	No computer code was used to collect the data
Data analysis	Flow cytometry was performed on Beckman CytoFLEX LX (Version 9). FACS data was collected using (CytExpert, Version 2.3) and was processed using (FlowJo, Version 9). All bulk RNA-seq reads were first trimmed using Trimmomatic (Version 0.36) software with the parameters "ILLUMINACLIP: 654 TruSeq3-PE.fa:2:30:10 LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 655 MINLEN:36" and were further quality-filtered using the FASTX Toolkit (Version 0.0.13, http://hannonlab.cshl.edu/fastx_toolkit/) with the minimum quality score 20 and minimum percent of 80% bases that has a quality score larger than this cutoff value. The high-quality reads were mapped to the GRCh38 genome by HISAT2, a fast and sensitive spliced alignment program for mapping RNA-seq reads, with -dta parameter. PCR duplicate reads were removed using Picard tools (v2.18.2) and only uniquely mapped reads were kept for further analysis. The expression levels of genes were calculated by StringTie (Version v1.3.4d, with -e -B -G parameters) using Release 28 (GRCh38.p12) gene annotations downloaded from GENCODE data portal. To obtain reliable and cross-sample comparable expression abundance estimation for each gene, reads mapped to the reference genome were counted as TPM (Transcripts Per Million reads) based on their genome locations. We adopted "prcomp" and pheatmap function with default parameters for Principal Component Analysis (PCA) and hierarchical clustering, respectively. We used high confident genes characterizing the M1 and M2 states (with the maximum of TPM values among different samples larger than 1) for PCA and hierarchical clustering analysis. The "cellrange count" program, a subcommand included in 10X single-cell gene expression analysis pipeline (https://support.10xgenomics.com/single-cell-gene-expression/), was used to produce gene-cell barcode expression matrix. The single-cell gene expression matrix further was analyzed with Seurat v3.2.1 (https://satijalab.org/seurat/). To guarantee those quality of genes and cells used for downstream analysis, we excluded the genes with expressed cell number smaller than 3 and those cells with expressed genes smaller than 200 or larger than 30000. We also filtered the cells with the expression percentages of mitochondrial genes larger than 0.2. We then adopted 10 Principal Components (PCs) for tSNE and clustering analysis with the cluster resolution of 0.25. The known cell identities (types) of each cell cluster of gene expression data were further predicted by an entropy-based predictor with default parameters (http://scibet.cancer-pku.cn/) (SciBet v1.0). To perform a comprehensive annotation of well-known cell types, we used an atlas database of cell types during human fetal

liver haematopoiesi, and integrate them with 30 major human cell type databases from 42 single cell RNA-seq datasets. To perform Principal Component Analysis (PCA) and hierarchical clustering analysis on bulk RNA-seq data, we have grouped and summed up the normalized expression levels of our single-cell gene expression data to produce the synthetic bulk RNA-seq dataset. We used "prcomp" and pheatmap function with default parameters for PCA and hierarchical clustering, respectively. All the bioinformatic data analyses and resulting visualization were performed in R software (4.0.2)/Bioconductor (v3.13) utilizing custom R scripts.

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 Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

Processed NGS datasets generated in this study have been deposited in our ftp webserver (ftp://47.94.193.106/pub/carmProj). Currently, we are uploading the raw and processed datasets to the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus. Previously published bulk RNA-Seq datasets that were re-analyzed here are available under accession codes GSE55536 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE55536)

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	The sample size was chosen in order to make sure it will be sufficient for statistic analysis. For detecting cytotoxicity of each types of CAR-iMACs in vitro, three or more incubating were performed with FFluc/ tdTomato-expressing U87MG cells. For In vivo anti-tumor assay, five or more animal models were used. No statistical method were used to predetermine the sample size.
Data exclusions	No data were excluded from the analyses.
Replication	For examination of cytotoxicity of CAR-iMACs against antigen-expressing tumor cells, detecting of genes expression, release of cytokines and expression of NF-κB protein, as well as detecting of polarization state of CAR-iMACs in vitro, each type of CAR-iMAC was independently co-cultured with FFluc/ tdTomato-expressing U87MG cell thrice respectively. for detecting of polarization state of CAR-iMACs in vivo, each type of CAR-iMAC was independently utilized to treat three tumor-bearing mice.
Randomization	For in vivo anti-tumor assay, male or female tumor-bearing mice were randomized into different treatment groups.
Blinding	*Describe whether the investigators were blinded to group allocation during data collection and/or analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your 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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used	rabbit anti-human EGFR antibody (CST, #4267. 1:2000), rabbit anti-human EGFRvIII antibody (CST, #64952. 1:2000), and rabbit anti-human β-actin antibody(CST, #4970. 1:10000),rabbit anti-human EGFRvIII (Absin, #abs124275. 1:2000), Alexa Fluor®647 conjugated rabbit antibody against NF-κB/p65 (CST, #D14E12. 1:100), Alexa Fluor® 647 conjugated Donkey anti-rabbit secondary antibody (donkey anti-rabbit IgG. abcom, ab150075. 1:1,000), PE-CD80 antibody (Biolegend, #375410), PE-CD163 antibody (Biolegend, #333605).
Validation	rabbit anti-human EGFR antibody (CST, #4267. 1:2000). This antibody can be used for WB, IHC, IF, IP and Flow Cyt with wide range reactivity according to manufacture's description. There are validation information on the website:https://www.cellsignal.cn/products/primary-antibodies/egf-receptor-d38b1-xp-rabbit-mab/4267?site-search-type=Products&N=4294956287&Ntt=%234267&fromPage=plp&_requestid=657079 rabbit anti-human EGFRvIII antibody (CST, #64952. 1:2000). This antibody can be used IHC and IF. There are validation information on the website: https://www.cellsignal.cn/products/primary-antibodies/egf-receptor-viii-d6t2q-xp-rabbit-mab/64952?site-search-type=Products&N=4294956287&Ntt=%2364952&fromPage=plp&_requestid=659206 rabbit anti-human EGFRvIII antibody (Absin, #abs124275. 1:2000). This antibody can be used for WB, IHC, ELISA, IF, and Flow cyt. There are validation information on the website: https://www.absin.cn/rabbit-anti-egfrv-polyclonal-antibody/abs124275.html rabbit anti-human β-actin antibody (CST, #4970. 1:10000). This antibody can be used for WB, IHC, IF and Flow cyt.There are validation information on the website:https://www.cellsignal.cn/products/primary-antibodies/b-actin-13e5-rabbit-mab/4970?site-search-type=Products&N=4294956287&Ntt=%234970&fromPage=plp&_requestid=660527 Alexa Fluor®647 conjugated rabbit antibodies against NF-κB/p65 (CST, #D14E12. 1:100). This antibody can be used for IF and Flow cyt. There are validation information on the website: https://www.cellsignal.cn/products/antibody-conjugates/nf-kb-p65-d14e12-xp-rabbit-mab-alexa-fluor-647-conjugate/8801?site-search-type=Products&N=4294956287&Ntt=%23d14e12&fromPage=plp Alexa Fluor® 647 conjugated Donkey anti-rabbit secondary antibody (donkey anti-rabbit IgG. abcom, ab150075. 1:1,000) can be used for ICC/IF, ELISA, IHC-P, Flow Cyt, IHC-Fr. There are validation information on the website:https://www.abcam.cn/donkey-rabbit-igg-hl-alexa-fluor-647-ab150075.html PE-CD80 antibody (Biolegend, #375410). This antibody was used for Flow cytometry. The validation information was showed on the website:https://www.biolegend.com/en-us/products/pe-anti-human-cd80-antibody-20589?Clone=W17149D. PE-CD163 antibody (Biolegend, #333605).This antibody was used for Flow cytometry. The validation information was showed on the website:https://www.biolegend.com/en-us/products/pe-anti-human-cd163-antibody-4793?Clone=GHI/61

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	The U87MG cell line was gift from Dr. Chong Liu Lab (Zhejiang University). The iPSCs were reprogrammed from BPMC of volunteer donor via Yamanaka episomal reprogramming plasmids (pCXLE-hSK 1 µg, pCXLE-hUL 1 µg and pCXLE-hOCT3/4 1 µg)
Authentication	For iPSCs, we performed assay to detect expression of stem cell related genes including OCT4 and NANOG. For U87MG cell line, we examined expression of glioblastoma relative genes including EGFR.
Mycoplasma contamination	We regularly perform mycoplasma tests and cells used in this study are negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Four-weeks old male and female B-NDG mice
Wild animals	No wild animal used
Field-collected samples	No field-collected samples were used in this study.
Ethics oversight	All animal experiments and study protocols were approved by the Animal Research Committee guidelines of Zhejiang University.

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	For detecting cytotoxicity of CAR-iMACs against EGFRvIII-expressing U87MG cells, we first incubating EGFP-labled CAR-iMACs with tdTomato-labled the tumor cells, and then collected all the cells by pipetting gently followed with flow cytometry to analyze tdTomato-positive cells. For analysis of polarization state of CAR-iMACs in vivo, we isolated CAR-iMACs from bdominl cvity of tumor-bearing mice using dissociative buffer, and then prepared two equal groups of the cells and stained either of the groups of cells using PE-CD80 antibody and PE-CD163 antibody. Flow cytometry was performed to detect CD80+ and CD163+ CAR-iMACs.
Instrument	Flow cytometry was performed with Beckman CytoFLEX LX
Software	FACS data was collected using CytExpert (Version 2.3), and was processed using FlowJo (Version 9).
Cell population abundance	For detecting cytotoxicity of CAR-iMACs against EGFRvIII-expressing U87MG cells, we sorted 10000 cells, and found that the abundance of tdTomato-positive tumor cells varied along with the different incubating times, just showed in Figure 2 and supplemental Figure 2, determined by tdTomato channel. For analysis of polarization state of CAR-iMACs in vivo, we sorted 10000 EGFP-positive CAR-iMACs for analyzing CD80- and CD163-positive cells. The abundances of the two populations were determined by recognizing PE-CD80 or PE-CD163, and exhibited distinct levels according to different types of CAR-iMACs and treatment times.
Gating strategy	Stringent gating strategies were always applied, leaving a significat gap in between negative and positive populations. Dead cells were excluded in FSC/SSC gating. Single cells were then collected in SSC-A/-H gating. For detecting cytotoxicity, tdTomato channel and EGFP channel were chosen to recognize tumor cells and CAR-iMACs simultaneously from single cells population. For analysis of polarization state of CAR-iMACs, EGFP channel was chosen to detect CAR-iMACs from single cell gating at first. After that, PE-CD80 and PE-CD163 were used for sorting M1 cells and M2 cells from CAR-iMACs.

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