nature portfolio

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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	Talos L120C, Nova Nano 450, Malvern Zetasizer Nano, UV-2600 spectrophotometer, Beckman Coulter Cytoflex, Beckman Coulter CytoFLEX LX, Olympus IX83-FV3000, ChemiDoc Touch Imaging System, Thermo Scientific Varioskan Flash, SLIDEVIEW VS200 research slide scanner, Illumina NovaSeq 6000 PE150, Bruker 400 spectrometer, AB TripleTOF 5600 plus System, Hitachi Chromaster 5000 system, Roche LightCycler 480 real-time quantitative PCR detecting system.
Data analysis	CytExpert 2.4 and FlowJo 10 were used for flow cytometry data analysis; NMR spectra were processed by Mestre Nova 14; The confocal laser scanning microscopy data were analyzed using Olympus FV31S-SW 2.1 and ImageJ 1.54g; The histological section images were analyzed using Olympus OlyVIA 3.1; 3D rendering was achieved by Imaris 9.7; scRNA sequencing was analyzed using Cell Ranger 7.0.0 and Seurat 4.3.0; GraphPad Prism 8 were used for statistical analysis.

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

> All data supporting the findings of this work are available within the articles or in the Supplementary Information. The scRNA-seq datasets generated for this study have been deposited in the NCBI Gene Expression Omnibus (GEO) under accession number GSE272255. 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	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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 chosen to ensure reproducibility of the experiments in accordance with the replacement, reduction and refinement principles of animal ethics regulation. Sample size are provided in the figure legends for each experiment and reasonable sample sizes were chosen to ensure they are sufficient for statistical comparison between different groups.
Data exclusions	No data was excluded in this study.
Replication	Replication data was not performed for scRNA-seq due to the cost, whereas n = 3 mice or samples were pooled to control for biological variability. The data are represented as mean ± s.d. of a minimum of 3 independent replicates. All experimental findings were reliably reproduced. All experiments were performed as technical or biological replications as appropriate for the experiment design. Details of experimental replicates are given in the figure legends.
Randomization	All samples were randomly assigned to experimental groups.
Blinding	The investigators were blinded during experiment, data acquisition, and analysis with the same parameter to ensure the consistency of measurement and analysis across all groups.

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 anti-PD-1 antibody used in vivo was purchased from Bio X Cell (29F.1A12™, #BP0273, lot 883023J1). The primary antibodies used for immunostaining were against CD45 (30-F11, Biolegend, #103112, lot B353749, 80×), CD11c (N418, Biolegend, #117318, lot B346713, 80×), CD80 (16-10A1, Biolegend, #104706, lot B342942, 80×), CD86 (GL-1, Biolegend, #105028, lot B317511, 80×), MHC II (M5/114.15.2, Biolegend, #107632, lot B360796, 80×), CD3 (17A2, Biolegend, #100228, lot B343124, 80×), CD4 (GK1.5, Biolegend, #100408, lot B334826, 80×), CD8a (53-6.7, BD Biosciences, #750024, lot 2214082, 40×; KT15, Thermo Fisher Scientific, #MA5-16759), CD44 (IM7, BD Biosciences, #560569, lot 1221629, 40×), CD62L (MEL-14, BD Biosciences, #562910, lot 1293954, 40×), intracellular IFN-γ (XMG1.2, Biolegend, #505826, lot B367890, 80×), and CD16/32 (93, Biolegend, #101302, lot B361754, 80×). The primary antibodies used for immunoblotting and immunofluorescence were against Drp1 (EPR19274, Abcam, #ab184247, lot GR3369203-11, 1000×), caspase 9 (C9, Cell Signaling Technology, #9508, lot 7, 1000×), cleaved caspase 9 (D2D4, Cell Signaling Technology, #7237, lot 3, 1000×), caspase 3 (Cell Signaling Technology, #9662, lot 19, 1000×), cleaved caspase 3 (5A1E, Cell Signaling Technology, #9664, lot 22, 1000×), PARP (Cell Signaling Technology, #9542, lot 15, 1000×), Bcl-2 (D17C4, Cell Signaling Technology, #3498, lot 6, 1000×), Bim (Abclonal, #A0295, lot 3100170101, 1000×), Bax (E63, Abcam, #ab32503, 1000×), cytochrome c (6H2.B4, Beyotime, #AC908, lot 091223240402, 500×), TOM20 (ARC5002-01, Abclonal, #A19403, lot 3600001090, 100×), calreticulin (D3E6, Cell Signaling Technology, #12238, lot 5, 500×), HMGB1 (Cell Signaling Technology, #3935, lot 4, 50×), PERK (EPR19876-294, Abcam, #ab229912, 1000×), p-PERK (16F8, Cell Signaling Technology, #3179, lot 21, 300×), eIF2α (D7D3, Cell Signaling Technology, #5324, lot 9, 1000×), p-eIF2α (D9G8, Cell Signaling Technology, #3398, lot 8, 1000×), ATF4 (Abclonal, #A21500, lot 5500038538, 1000×), CHOP (L63F7, Cell Signaling Technology, #2895, lot 15, 1000×), HRI (Proteintech, #20499-1-AP, 1000×), Ubiquitin (ARC50024, Abclonal, #A19686, lot 6100005203, 1000×), Sec61b (Abclonal, #A15788, lot 0161690101, 1000×), 4-HHE/HNE (6F10, Novus Biologicals, #NBP2-59352, 50×), CD11c (D1V9Y, Cell Signaling Technology, #97585, lot 6, 300×), F4/80 (D2S9R, Cell Signaling Technology, #70076, lot 9, 300×), CD86 (E5W6H, Cell Signaling Technology, #19589, lot 5, 300×), CD206 (E6T5J, Cell Signaling Technology, #24595, lot 3, 300×), β actin (8H10D10, Cell Signaling Technology, #3700, lot 21, 1000×), VDAC1/2 (Proteintech, #10866-1-AP, 1000×), NaK ATPase (EP1845Y, Abcam, #ab76020, 100000×), isotype control (E7Q5L, Cell Signaling Technology, #53484, lot 4; DA1E, Cell Signaling Technology, #3900, lot 52). The secondary antibodies used for immunostaining were horse anti-mouse IgG, HRP linked (Cell Signaling Technology, #7076P2, lot 36, 2000×), goat anti-rabbit IgG, HRP linked (Cell Signaling Technology, #7074P2, lot 33, 2000×), donkey anti-rabbit IgG Alexa FluorTM 555 (H+L; Thermo Fisher Scientific, #A31572, 500×), donkey anti-rabbit IgG Alexa FluorTM 488 (H+L; Thermo Fisher Scientific, #A21206, 1000×), goat anti-mouse IgG Alexa FluorTM 488 (H+L; Thermo Fisher Scientific, #A11001, 1000×).
Validation	All antibodies were verified by the supplier and each lot has been quality tested. All the antibodies used are from commercial sources and have been validated by the vendors. Validation data are available on the manufacturer's website. APC anti-mouse CD45 Antibody (103112) has been validated to be used for flow cytometry and mentioned species reactivity with mouse (https://www.biolegend.com/en-us/products/apc-anti-mouse-cd45-antibody-97). PE/Cyanine7 anti-mouse CD11c Antibody (117318) has been validated to be used for flow cytometry and mentioned species reactivity with mouse (https://www.biolegend.com/en-us/products/pe-cyanine7-anti-mouse-cd11c-antibody-3086). FITC anti-mouse CD80 Antibody (104706) has been validated to be used for flow cytometry and mentioned species reactivity with mouse (https://www.biolegend.com/en-us/products/fitc-anti-mouse-cd80-antibody-41). PerCP/Cyanine5.5 anti-mouse CD86 Antibody (105028) has been validated to be used for flow cytometry and mentioned species reactivity with mouse (https://www.biolegend.com/en-us/products/percp-cyanine5-5-anti-mouse-cd86-antibody-4276). Brilliant Violet 421™ anti-mouse I-A/I-E Antibody (107632) has been validated to be used for flow cytometry and mentioned species reactivity with mouse (https://www.biolegend.com/en-us/products/brilliant-violet-421-anti-mouse-i-a-i-e-antibody-7147). Brilliant Violet 421™ anti-mouse CD3 Antibody (100228) has been validated to be used for flow cytometry and mentioned species reactivity with mouse (https://www.biolegend.com/en-us/products/brilliant-violet-421-anti-mouse-cd3-antibody-7326). PE anti-mouse CD4 Antibody (100408) has been validated to be used for flow cytometry and mentioned species reactivity with mouse (https://www.biolegend.com/en-us/products/pe-anti-mouse-cd4-antibody-250). BD OptiBuild™ BUV496 Rat Anti-Mouse CD8a (750024) has been validated to be used for flow cytometry and mentioned species reactivity with mouse (https://www.bdbiosciences.com/zh-cn/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/buv496-rat-anti-mouse-cd8a.750024). BD Pharmingen™ PE-Cy™7 Rat Anti-Mouse CD44 (560569) has been validated to be used for flow cytometry and mentioned species reactivity with mouse (https://www.bdbiosciences.com/zh-cn/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/pe-cy-7-rat-anti-mouse-cd44.560569). BD Horizon™ BV421 Rat Anti-Mouse CD62L (562910) has been validated to be used for flow cytometry and mentioned species reactivity with mouse (https://www.bdbiosciences.com/zh-cn/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv421-rat-anti-mouse-cd62l.562910). PE/Cyanine7 anti-mouse IFN-γ Antibody (505826) has been validated to be used for flow cytometry and mentioned species reactivity with mouse (https://www.biolegend.com/en-us/products/pe-cyanine7-anti-mouse-ifn-gamma-antibody-5865). Purified anti-mouse CD16/32 Antibody (101302) has been validated to be used for flow cytometry and mentioned species reactivity with mouse (https://www.biolegend.com/en-us/products/purified-anti-mouse-cd16-32-antibody-190).

Anti-DRP1 antibody (ab184247) has been validated to be used for western blotting/immunofluorescence and mentioned species reactivity with human/mouse/rat (https://www.abcam.cn/products/primary-antibodies/drp1-antibody-epr19274-ab184247.html).

Caspase-9 (C9) Mouse mAb (9508) has been validated to be used for western blotting and mentioned species reactivity with human/mouse/rat (https://www.cellsignal.cn/products/primary-antibodies/caspase-9-c9-mouse-mab/9508).

Cleaved Caspase-9 (Asp330) (D2D4) Rabbit mAb (7237) has been validated to be used for western blotting and mentioned species reactivity with human (https://www.cellsignal.cn/products/primary-antibodies/cleaved-caspase-9-asp330-d2d4-rabbit-mab/7237).

Caspase-3 Antibody (9662) has been validated to be used for western blotting and mentioned species reactivity with human/mouse/rat (https://www.cellsignal.cn/products/primary-antibodies/caspase-3-antibody/9662).

Cleaved Caspase-3 (Asp175) (5A1E) Rabbit mAb (9664) has been validated to be used for western blotting and mentioned species reactivity with human/mouse/rat (https://www.cellsignal.cn/products/primary-antibodies/cleaved-caspase-3-asp175-5a1e-rabbit-mab/9664).

PARP Antibody (9542) has been validated to be used for western blotting and mentioned species reactivity with human/mouse/rat (https://www.cellsignal.cn/products/primary-antibodies/parp-antibody/9542).

Bcl-2 (D17C4) Rabbit mAb (3498) has been validated to be used for western blotting and mentioned species reactivity with human/mouse (https://www.cellsignal.cn/products/primary-antibodies/bcl-2-d17c4-rabbit-mab/3498).

Bim Rabbit pAb (A0295) has been validated to be used for western blotting and mentioned species reactivity with human (https://abclonal.com.cn/Datasheet/Antibodies/A0295.pdf?v=1719564279).

Anti-Bax antibody (ab32503) has been validated to be used for western blotting and mentioned species reactivity with human/mouse/rat (https://www.abcam.cn/products/primary-antibodies/bax-antibody-e63-ab32503.html).

Cytochrome C antibody (AC908) has been validated to be used for immunofluorescence and mentioned species reactivity with human/mouse/rat (https://www.beyotime.com/product/AC908.htm).

TOM20 Rabbit mAb (A19403) has been validated to be used for immunofluorescence and mentioned species reactivity with human/mouse/rat (https://abclonal.com.cn/catalog/A19403).

Calreticulin (D3E6) XP® Rabbit mAb (12238) has been validated to be used for western blotting/immunofluorescence/flow cytometry and mentioned species reactivity with human/mouse/rat (https://www.cellsignal.cn/products/primary-antibodies/calreticulin-d3e6-xp-rabbit-mab/12238).

HMGB1 Antibody (3935) has been validated to be used for western blotting/immunofluorescence and mentioned species reactivity with human/mouse/rat (https://www.cellsignal.cn/products/primary-antibodies/hmgb1-antibody/3935).

Anti-PERK antibody (ab229912) has been validated to be used for western blotting and mentioned species reactivity with human/mouse/rat (https://www.abcam.cn/products/primary-antibodies/perk-antibody-epr19876-294-ab229912.html).

Phospho-PERK (Thr980) (16F8) Rabbit mAb (3179) has been validated to be used for western blotting and mentioned species reactivity with mouse/rat (https://www.cellsignal.cn/products/primary-antibodies/phospho-perk-thr980-16f8-rabbit-mab/3179).

eIF2α (D7D3) XP® Rabbit mAb (5324) has been validated to be used for western blotting and mentioned species reactivity with human/mouse/rat (https://www.cellsignal.cn/products/primary-antibodies/eif2a-d7d3-xp-rabbit-mab/5324).

Phospho-eIF2α (Ser51) (D9G8) XP® Rabbit mAb (3398) has been validated to be used for western blotting and mentioned species reactivity with human/mouse/rat (https://www.cellsignal.cn/products/primary-antibodies/phospho-eif2a-ser51-d9g8-xp-rabbit-mab/3398).

ATF4 Rabbit pAb (A21500) has been validated to be used for western blotting and mentioned species reactivity with human/mouse/rat (https://abclonal.com.cn/Datasheet/Antibodies/A21500.pdf?v=1718359851).

CHOP (L63F7) Mouse mAb (2895) has been validated to be used for western blotting/immunofluorescence and mentioned species reactivity with human/mouse/rat (https://www.cellsignal.cn/products/primary-antibodies/chop-l63f7-mouse-mab/2895).

EIF2AK1 Polyclonal antibody (20499-1-AP) has been validated to be used for western blotting/immunofluorescence and mentioned species reactivity with human (https://www.ptgcn.com/products/EIF2AK1-Antibody-20499-1-AP.htm).

Ubiquitin Rabbit mAb (A19686) has been validated to be used for western blotting and mentioned species reactivity with human/mouse/rat (https://abclonal.com.cn/catalog/A19686).

SEC61B Rabbit pAb (A15788) has been validated to be used for western blotting and mentioned species reactivity with human/mouse/rat (https://abclonal.com.cn/catalog/A15788).

4-Hydroxy-2-hexenal Antibody (NBP2-59352) has been validated to be used for western blotting/flow cytometry/immunofluorescence and mentioned species reactivity with human/mouse/rat (https://www.novusbio.com/products/4-hydroxy-2-hexenal-antibody-6f10_nbp2-59352).

Anti-CD8 alpha antibody (ab217344) has been validated to be used for western blotting/ immunohistochemistry and mentioned species reactivity with mouse (https://www.abcam.cn/products/primary-antibodies/cd8-alpha-antibody-epr21769-ab217344.html).

CD11c (D1V9Y) Rabbit mAb (97585) has been validated to be used for western blotting/ immunohistochemistry and mentioned species reactivity with mouse (https://www.cellsignal.cn/products/primary-antibodies/cd11c-d1v9y-rabbit-mab/97585).

F4/80 (D2S9R) XP® Rabbit mAb (70076) has been validated to be used for western blotting/ immunohistochemistry and mentioned species reactivity with mouse (https://www.cellsignal.cn/products/primary-antibodies/f4-80-d2s9r-xp-rabbit-mab/70076).

CD86 (E5W6H) Rabbit mAb (19589) has been validated to be used for western blotting/ immunohistochemistry and mentioned species reactivity with mouse (https://www.cellsignal.cn/products/primary-antibodies/cd86-e5w6h-rabbit-mab/19589).

CD206/MRC1 (E6T5J) XP® Rabbit mAb (24595) has been validated to be used for western blotting/ immunohistochemistry and mentioned species reactivity with human/mouse/rat (https://www.cellsignal.cn/products/primary-antibodies/cd206-mrc1-e6t5j-xp-rabbit-mab/24595).

β-Actin (8H10D10) Mouse mAb (3700) has been validated to be used for western blotting/ immunohistochemistry/immunofluorescence and mentioned species reactivity with human/mouse/rat (https://www.cellsignal.cn/products/primary-antibodies/b-actin-8h10d10-mouse-mab/3700).

VDAC1/2 Polyclonal antibody (10866-1-AP) has been validated to be used for western blotting/immunohistochemistry and mentioned species reactivity with human/mouse/rat (https://www.ptgcn.com/products/VDAC1-Antibody-10866-1-AP.htm).

Anti-Sodium Potassium ATPase antibody (ab76020) has been validated to be used for western blotting/immunohistochemistry/immunofluorescence and mentioned species reactivity with human/mouse/rat (https://www.abcam.cn/products/primary-antibodies/sodium-potassium-atpase-antibody-ep1845y-plasma-membrane-loading-control-ab76020.html).

Mouse (E7Q5L) mAb IgG2b Isotype Control (53484) has been validated to be used for flow cytometry/immunohistochemistry/immunofluorescence (https://www.cellsignal.cn/products/primary-antibodies/mouse-e7q5l-mab-igg2b-isotype-control/53484).

Rabbit (DA1E) mAb IgG XP® Isotype Control (3900) has been validated to be used for flow cytometry/immunohistochemistry/

immunofluorescence (https://www.cellsignal.cn/products/primary-antibodies/rabbit-da1e-mab-igg-xp-isotype-control/3900).

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	B16F10 (SCSP-5233), 4T1 (SCSP-5056), CT26 (SCSP-523), AML12 (SCSP-550), HeLa (SCSP-504), and A549 (SCSP-503) were purchased from the cell bank of the Chinese Academy of Sciences (Shanghai, China). MC38 (BTCC-2026) and A2780 (BTCC-1008) were purchased from Bowers Type Culture Collection (Beijing, China). B16F10-OVA was purchased from Crisprbio (Beijing, China). Drug-resistant cell lines HeLa/R, A549/R and A2780/R were established following method of increasing cisplatin concentration. HepLi5 was a gift from Professor Li Lanjuan's team at the State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, First Affiliated Hospital, School of Medicine, Zhejiang University.
Authentication	These cell lines were morphologically confirmed.
Mycoplasma contamination	All cell lines were 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/6 (6~8 weeks) and BALB/c mice (6~8 weeks) were purchased from the Laboratory Animal Center of Hangzhou Medical College (Hangzhou, China). Animals were raised in specific pathogen-free animal experimental center and allowed free access to food and water. All experimental/control animals were co-housed in a habitant under standard conditions (23~26°C, 40%~60% humidity, 12 h light-dark cycle, and 4-6 mice/cage).
Wild animals	No wild animals were used in the study.
Reporting on sex	Female mice were used for mammary carcinoma models because breast cancer is more common in women than men. The sex was not considered in other cancer models because there was no direct correlation between the selected tumor model and sex.
Field-collected samples	No field-collected samples were involved in this study.
Ethics oversight	We performed animal experiments in accordance with the National Institute Guide for the Care and Use of Laboratory Animals. The experimental protocols were approved by the Ethics Committee of the First Affiliated Hospital, Zhejiang University School of Medicine. All studies comply with relevant ethical regulations.

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:

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

[x] 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).

[x] All plots are contour plots with outliers or pseudocolor plots.

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

Methodology

Sample preparation	The sample preparation was described in the Methods.
Instrument	CytoFLEX and CytoFLEX LX were used for flow cytometry data collection.
Software	CytExpert 2.4 and FlowJo 10 were used to collect and analyze flow cytometry data.
Cell population abundance	No sorting was performed by flow cytometry.
Gating strategy	BD™ CompBeads were used for calculation of appropriate compensation. Single cells were first gated with FSC-A and FSC-H. Live cells were determined using Zombie Aqua™ Fixable dye. Total immune cells were identified by CD45 staining. Sebsequent gating was conducted to selected targeted populations.

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