nature research

Corresponding author(s): Yong Tao, Di Yu, Wei Wei

Last updated by author(s): Apr 11, 2021

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	The images of immunohistochemistry and Opal multicolor immunofluorescence staining were visualized by using an automatic multispectral imaging system (Vectra II, PerkinElmer). Images of rEXS-cL-aV with/without immune-antibody-linked Au nanoparticles were obtained using TEM (JEM-1400, JEOL). The size distribution and zeta potential of rEXS-cL-aV were determined using nanoparticle tracking analysis (Particle Metrix). The images of simple western were obtained using a ProteinSimple Wes capillary western blot analyser (PS-MK15, ProteinSimple). Fluorescent images were collected using CLSM (UltraVIEW VoX, PerkinElmer) and STED (SP8, Leica) with associated software. Images of nanodrug distribution were collected using an in vivo imaging system (530 FX Pro, Kodak) with associated software. Two-photon microscopy imaging and 3D reconstruction were performed with IMARIS 9.0 software (Bitplane). Flow-cytometry data were obtained using Flow Jo 7.6.1 (Tree Star, Inc., USA). RNA-seq analysis was performed by using the R package DEGseq(R-3.6.2). OCT angiography images of the CNV monkeys model were acquired using the Ultra-Widefield SS-OCTA software (SVisionResearch, Inc., China).
Data analysis	Statistical analyses were carried out via GraphPad Prism 8.0.1.

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

The main data supporting the results in this study are available within the paper and its Supplementary Information. Source data for the figures are available as Supplementary Information and also at figshare: https://figshare.com/articles/dataset/Source_Data_xlsx/14401208. The RNA-sequencing data are available at the NCBI BioProject via the identifier PRJNA721185.

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	Power analysis of the animal experiments indicated that the chosen sample sizes per group are sufficient. We also referred to relevant literature to determine sample sizes. For the in vitro and in vivo experiments, we followed standards of good scientific practice. We used at least 3 biological replicates or 3 animals per group, to calculate means and standard deviations and to perform statistical analyses.
Data exclusions	In the analysis of areas of laser-induced choroidal neovascularization, only burns that produced a bubble without haemorrhage were used for analysis. As a quality control, genes or pathways were filtered out from the RNA-seq analysis if they were not in top 100 gene clusters or top 50 KEGG pathways.
Replication	For all murine and non-human primate experiments, we report pooled results from multiple experiments or the data shown correspond to one representative experiment of at least 3 experiments.
Randomization	For the in vitro experiments, samples were randomly allocated into experimental groups. For the in vivo studies, animals were randomly grouped.
Blinding	For image-based data analysis, at least two blinded graders performed the evaluation.

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	Neutralization: anti-mouse VEGF (AF-493-NA; R&D Systems) bevacizumab (HY-P9906; MedChemExpress) Histology: anti-mouse MMP2 (sc-13595; Santa Cruz; 1:200 dilution)

anti-mouse MMP9 (sc-393859; Santa Cruz; 1:200 dilution)
anti-mouse CD105 (ab221675; Abcam; 1:100 dilution)
anti-mouse F4/80 (ab90247; Abcam; 1:100 dilution)

Simple Western:
anti-mouse CD47 (ab175388; Abcam; 1:50 dilution)
anti-mouse ALIX (ab186429; Abcam; 1:50 dilution)
anti-mouse TSG101 (ab125011; Abcam; 1:50 dilution)
anti-mouse CTLA-4 (ab237712; Abcam; 1:50 dilution)
anti-mouse β-tubulin(ab179513; Abcam; 1:50 dilution)
anti-mouse CD9 (ab92726; Abcam; 1:50 dilution)
anti-mouse CCR6 (ab273580; Abcam; 1:50 dilution)
anti-mouse IL-10 (ab189392; Abcam; 1:50 dilution)
anti-mouse CD4 (ab183685; Abcam; 1:50 dilution)
anti-mouse CD25 (ab264557; Abcam; 1:50 dilution)
anti-mouse FOXP3 (ab215206; Abcam; 1:50 dilution)

Flow cytometry:
APC Rat Anti-Mouse CD11b (561039; BD; 1:75 dilution)
PE Rat Anti-Mouse CD86 (561963; BD; 1:75 dilution)
APC Rat Anti-Mouse CD4 (561091; BD; 1:75 dilution)
FITC Rat anti-Mouse CD25 (558689; BD; 1:75 dilution)
PE Rat anti-Mouse FOXP3 (61-5773-82; eBioscience; 1:75 dilution)

Validation

We relied on publications and on validation sources cited by manufacturer. Links for each antibody are given below:

anti-mouse VEGF (AF-493-NA; R&D Systems)
https://www.rndsystems.com/cn/products/mouse-vegf-164-antibody_af-493-na
bevacizumab (HY-P9906; MedChemExpress)
https://www.medchemexpress.cn/Bevacizumab.html
anti-mouse MMP2 (sc-13595; Santa Cruz; 1:200 dilution)
https://www.scbt.com/zh/p/mmp-2-antibody-8b4?requestFrom=search
anti-mouse MMP9 (sc-393859; Santa Cruz; 1:200 dilution)
https://www.scbt.com/zh/p/mmp-9-antibody-e-11?requestFrom=search
anti-mouse CD105 (ab221675; Abcam; 1:100 dilution)
https://www.abcam.cn/cd105-antibody-epr21846-ab221675.html
anti-mouse F4/80 (ab90247; Abcam; 1:100 dilution)
https://www.abcam.cn/f480-antibody-f480-macrophage-marker-ab90247.html
anti-mouse CD47 (ab175388; Abcam; 1:50 dilution)
https://www.abcam.cn/cd47-antibody-ab175388.html
anti-mouse CD9 (ab92726; Abcam; 1:50 dilution)
https://www.abcam.cn/cd9-antibody-epr2949-ab92726.html
anti-mouse ALIX (ab186429; Abcam; 1:50 dilution)
https://www.abcam.cn/alix-antibody-epr15314-n-terminal-ab186429.html
anti-mouse TSG101 (ab125011; Abcam; 1:50 dilution)
https://www.abcam.cn/tsg101-antibody-epr7130b-ab125011.html
anti-mouse CTLA-4 (ab237712; Abcam; 1:50 dilution)
https://www.abcam.cn/ctla4-antibody-cal49-ab237712.html
anti-mouse β-tubulin(ab179513; Abcam; 1:50 dilution)
https://www.abcam.cn/beta-tubulin-antibody-epr16774-ab179513.html
anti-mouse CCR6 (ab273580; Abcam; 1:50 dilution)
https://www.abcam.cn/ccr6-antibody-18b9e6-ab273580.html
anti-mouse IL-10 (ab189392; Abcam; 1:50 dilution)
https://www.abcam.cn/il-10-antibody-jes5-2a5-ab189392.html
anti-mouse CD4 (ab183685; Abcam; 1:50 dilution)
https://www.abcam.cn/cd4-antibody-epr19514-ab183685.html
anti-mouse CD25 (ab264557; Abcam; 1:50 dilution)
https://www.abcam.cn/il-2-receptor-alpha-antibody-epr22816-65-ab264557.html
anti-mouse FOXP3 (ab215206; Abcam; 1:50 dilution)
https://www.abcam.cn/foxp3-antibody-epr22102-37-ab215206.html
PE Rat Anti-Mouse CD86 (561963; BD; 1:75 dilution)
https://www.bdbiosciences.com/ds/pm/tds/561963.pdf
APC Rat Anti-Mouse CD4 (561091; BD; 1:75 dilution)
https://www.bdbiosciences.com/cn/applications/research/t-cell-immunology/th-1-cells/surface-markers/mouse/apc-rat-anti-mouse-cd4-rm4-5-also-known-as-rm45/p/561091
FITC Rat anti-Mouse CD25 (558689; BD; 1:75 dilution)
https://www.bdbiosciences.com/ds/pm/tds/558689.pdf
PE Rat anti-Mouse FOXP3 (61-5773-82; eBioscience; 1:75 dilution)
https://www.thermofisher.com/cn/zh/antibody/product/FOXP3-Antibody-Monoclonal/61-5773-82

Animals and other organisms

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

Laboratory animals

C57BL/6 mice (6–8 weeks, female, weight of 20 ± 1 g) were purchased from Charles River (Beijing, China). The mice were housed in

Laboratory animals	an environmentally controlled room (23 °C, with 55 ± 5% humidity and under a 12 h–12 h light–dark cycle). Cynomolgus monkeys (3–3.5 years, males, weight of 3.5 ± 0.5 kg) were purchased from the Animal Center of Military Medical Sciences and were also housed in the Center. The monkeys were housed in individual cages, which were 0.95 m in height, 0.9 m in depth and 0.8 m in width, and had a constant ambient temperature (23 ± 3 °C) and humidity (55% ± 5%).
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	The cynomolgus monkey experiments were approved and performed in accordance with the institutional guidelines of the Academy of Military Medical Sciences Institutional Animal Care and Use Committee (Number: IACUC-DWZX-2019-010). The mouse experiments were approved by the Institutional Animal Care and Use Committee at the Institute of Process Engineering, Chinese Academy of Sciences (Number: IPEAECA2018156).

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

Human research participants

Policy information about studies involving human research participants

Population characteristics	The patient population covered retinal neovascularization (RNV) and choroidal neovascularization (CNV).
Recruitment	Aqueous samples were obtained from the patients diagnosed with RNV or CNV in Beijing Chaoyang Hospital (Beijing, China).
Ethics oversight	Collection and measurement of aqueous humor samples was approved by the Ethics Committee of Beijing Chaoyang Hospital (Number: 2018-S-125).

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	CECs and primary macrophages were collected from either upper or lower chambers of the transwell system, filtered into single cell suspensions, and stained using the antibodies described above.
Instrument	CytoFLEX LX flow cytometer (Beckman Coulter)
Software	Cyt-Expert software
Cell population abundance	3×10^4 CECs and 1×10^5 primary macrophages were sorted from 3 transwell chambers for flow cytometry.
Gating strategy	Cells were gated on the starting cell population in a FSC/SSC plot, followed by a FCA/FCH plot to gate single cells. For cell-apoptosis experiments, CECs were then gated on Annexin and PI double-positive cells. For primary-macrophage-phenotype identification, primary macrophages were gated on CD86-positive cells.

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