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<br>*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<br>*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 | N/A |
| Data analysis | Biacore 8K Control Software(version 3.0.12.15655)<br>Biacore Insight Evaluation(version 3.0.12.15655)<br>SoftMax Pro (version 7.0)<br>GraphPad Prism software (version 10)<br>SnapGene (6.1.1)<br>ChimeraX (1.6.1)<br>CryoSPARC (v3.3.1)<br>Coot (0.9.4.1)<br>CCP-EM (1.5.0) |

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

*Provide your data availability statement here.*

## 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 determined based on common standards for animal, cell biology and biochemistry experiments, with three biological replicates. The number of animals used for each experiment was estimated based on previous and pilot studies in vivo assays. Normally, 6-8 mice for each group were analyzed to ensure the statistical significance. We performed more than three independent biological replicates for most of in vitro experiments. |
| Data exclusions | No data were excluded. |
| Replication | All experiments in this study had a minimum of three biological replicates. |
| Randomization | Mice were randomly grouped for different samples. |
| Blinding | N/A |

# 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

Antibodies used in HDX, negative staining and cryo-electron microscopy analysis:
Anti CD73 antibody: HB0038, in-house
Anti CD73 antibody: HB0039, in-house

Antibodies used in animal experiments:
Anti CD73 antibody: HB0038, in-house
Anti CD73 antibody: HB0039, in-house
Anti CD73 antibody: HB0045, in-house
Anti CD73 antibody (AstraZeneca): Oleclumab, in-house
Anti 4-1BB antibody: HB0027, in-house
Anti-PD-L1/VEGF bispecific antibody: HB0025, in house
IgG control: in-house

Antibodies used in SPR binding assay:
Anti CD73 antibody: HB0038, in-house
Anti CD73 antibody: HB0039, in-house

Antibodies used in CD4+ or CD8+ T cell proliferation:
Anti CD73 antibody: HB0038, in-house
Anti CD73 antibody: HB0039, in-house
Anti CD73 antibody: HB0045, in-house
Anti CD73 antibody (AstraZeneca): Oleclumab, in-house
Ultra-LEAF™ Purified anti-human CD3 Antibody: Cat NO: 317326, 1 μg/mL, Bilegend
CD28 Monoclonal antibody, Cat NO: 14-0289-82, 1 μg/mL, Thermo Fisher Scientific
FITC anti-human CD4 Antibody, Cat NO: 317408, 1:100, Biolegend
APC anti-human CD4 Antibody, Cat NO: 317416, 1:200, Biolegend
APC/Cyanine7 anti-human CD4 Antibody, Cat NO: 317418, 1:100, Biolegend
Brilliant Violet 510™ anti-human CD8 Antibody, Cat NO: 344732, 1:100, Biolegend
APC anti-human CD8 Antibody, Cat NO: 344722, 1:200, Biolegend
Propidium iodide, Cat NO: P4170, 2 μg/mL, Sigma

Antibodies used in intro CD73 binding, enzymatic inhibition activity:
Anti CD73 antibody: HB0038, in-house
Anti CD73 antibody: HB0039, in-house
Anti CD73 antibody: HB0045, in-house
Anti CD73 antibody (AstraZeneca): Oleclumab, in-house
Anti CD73 antibody (Corvus): Mupadolimab , in-house
Anti CD73 antibody (Akeso): AK119, in-house
Anti CD73 antibody: HB0038 Fab, in-house
Anti CD73 antibody: HB0039 Fab, in-house
IgG control: in-house
PE Goat anti-Human IgG, Fcγ: # 109-115-098, 1:200, Jackson

Antibodies used in antibody-mediated internalizaiton :
Anti CD73 antibody: HB0038, in-house
Anti CD73 antibody: HB0039, in-house
Anti CD73 antibody: HB0045, in-house
IgG control: in-house

Antibodies used in flow cytometric analysis of CD73 expression in CD4+ cell and CD8+ cell:
CD73 Monoclonal Antibody (AD2), PE, Cat NO: 12-0739-42, eBioscience™, Invitrogen
Mouse IgG1 kappa Isotype Control (P3.6.2.8.1), PE, eBioscience™, Invitrogen

| | |
|---|---|
| | |
| Validation | HB0038, HB0039, HB0045, HB0027, and HB0025 were validated in this study or our previous work (doi.org/10.3389/fimmu.2021.778978) by a number of approaches such as SPR binding assay and structural analyses. Oleclumab, Mupadolimab, and AK119 targeting CD73 were developed by AstraZeneca, Corvus, and Akeso, respectively. The rest of antibodies were validated by manufacturers. |

## Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

| | |
|---|---|
| Cell line source(s) | B-hCD73 MC38: CD73 humanized MC38 cell line B-hCD73 MC38 was prepared by Biocytogen Pharmaceutical (Beijing) Co.<br>MDA-MB-231: MDA-MB-231 cells were purchased from Cell Bank, Chinese Academy of Science, catalog number: TCHu227<br>BxPC-3: BxPC-3 cells were purchased from Nanjing Cobioer Biosciences Co. LTD. catalog number: CBP60542 A375: A375 cells were purchased from Cell Bank, Chinese Academy of Science, catalog number:TCHu155 HepG2: HepG2 cells were purchased from the American Type Culture Collection (ATCC). CHO-K1-CD73-3F4: CHO-K1 cell line stably expressing full-length CD73 (CHO-K1-CD73-3F4) was made in-house. CHO-K1 was purchased from ATCC.<br>Human PBMC: Human PBMC were purchased from Milestone (Shanghai) Biological Science & Technology Co. Ltd. (Reference number: PB010C) |
| Authentication | All these cell lines were authenticated by corresponding manufacturers. |
| Mycoplasma contamination | We routinely conducted mycoplasma contamination checks on cell lines utilized in this study, and the results consistently confirmed their negativity. |
| Commonly misidentified lines (See ICLAC register) | N/A |

## 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 | 1. The B-hCD73/hCD39(v3) humanized C57BL/6 mice (CD73 and CD39 humanized C57BL/6) used in Supplemental Figure 1B-D; female, 7-8 weeks.<br>2. The immunodeficient Prkdcscid Il2rgnull (NPG) mice used in Figure 5B-D, and Supplemental Figure 8A-E; female, 7-8 weeks.<br>3. The immunodeficient NOD-Prkdcem26Cd52Il2rgem26Cd22/NjuCrl (NCG) mice used in Figure 6A-D female, 9-10 weeks.<br>4. The immunodeficient NOD-Prkdcscid Il2rgem1/Cyagen (C-NKG) mice used in Figure 6E, F, female, 5-8 weeks.<br>5. The immunodeficient Prkdcscid Il2rgnull (NPG) mice used in Supplemental Figure 8I-K; male, 5-8 weeks. |
| Wild animals | N/A |
| Reporting on sex | Female mice were used in all in vivo animal efficacy experiments. Compared with female mice, male mice are more aggressive and prone to fight. This has a negative impact on the animal's living conditions, and might also lead to tumor rupture in the later stages. This difference in survival status will indirectly affect the body weight and tumor volume of the mice. Therefore, we chose to use female mice for our in vivo animal efficacy experiments.<br>Male mice were used in pharmacokinetics studies, this avoids the effects of changes in hormone levels on pharmacokinetics. |
| Field-collected samples | Our study did not involve field-collected samples. |
| Ethics oversight | Experiments complied with the relevant laws and institutional guidelines, as overseen by the Animal Studies Committee of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). |

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

## Plants

| | |
|---|---|
| Seed stocks | N/A |
| Novel plant genotypes | N/A |
| Authentication | N/A |