nature research

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Last updated by author(s): 9/9/2019

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 Authors & Referees 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	Flow cytometry data were acquired with FACSDiva (BD, v 8.0.1) and with CytExpert (Beckman Coulter, v 2.1.0.92). Luciferase data were acquired with EnVision Manager (PerkinElmer, v 1.14.3049.528). Mass spectrometry data were acquired and analyzed with Byonic (Protein Metrics, v3.5). Octet data were acquired and analyzed with ForteBio (Molecular Devices, v 9.0.0.48). SPR data were acquired with Biacore T200 Control Software (GE Healthcare, v 2.0.2). ELISA data were acquired and analyzed by Softmax Pro (Molecular Devices, v 7). Crystal structure data were acquired and analyzed by HKL2000 (HKL Research, v 719). ITC data were acquired with MicroCal Auto-iTC200 (Malvern Panalytical v 1.21). Tissue imaging data were acquired and analyzed with Living Image (PerkinElmer, v 4.5).
Data analysis	Flow cytometry data were analyzed by FlowJo (BD, v 10.5.3). SPR data were analyzed with Biacore T200 Evaluation Software (GE Healthcare, v 3.1). ITC data were analyzed by MicroCal PEAQ-ITC (Malvern Panalytical, v 1.21). VISTA : PSGL-1 structural modeling was performed with Maestro (Schrodinger, v 10.7.015). PK data were analyzed with Kinetica (ThermoFisher Scientific, v 5.0). Statistics were visualized and generated with Prism (GraphPad, v 8.0.2).

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/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

Data generated or analyzed in this study are included in the manuscript. The VISTA crystal structure described in Figure 2 has been deposited with wwPDB and will be released upon publication.

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	No sample size calculations were performed. Sample sizes were modeled after those from past experience and publications.
Data exclusions	Data were only excluded from assay development and technically failed experiments. In mouse studies, mice that were not enrolled into a treatment group or who reached an endpoint other than study termination or tumor progression (such as morbidity or tumor ulceration) were excluded.
Replication	All technically successful replicate studies reproduced the indicated results. The number of replicates for each study is indicated. Some studies (eg, cynomolgus macaque pharmacokinetics and imaging studies) have only been performed once.
Randomization	In mouse studies, mice were randomized at the start of treatment on the basis of tumor volume.
Blinding	Investigators were not blinded. Data reported for mouse studies (tumor measurements and flow cytometry) are non-subjective.

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
☐	☒ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data

Methods

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

Antibodies

Antibodies used	Commercially sourced antibodies and other flow cytometry reagents: anti-mouse IgG, polyclonal, Alexa Fluor 647, 1:250, Jackson Immunoresearch 115-605-071 lot 131089 anti-mouse CD3, 145-2C11, PE-Cy7, 1:200, Biolegend 100320 lot B268542 anti-mouse CD4, GK1.5, Brilliant Violet 711, 1:400, Biolegend 100447 lot B245638 anti-mouse CD8a, 53-6.7, Brilliant Violet 786, 1:400, Biolegend 100750 lot B273618 anti-mouse CD11b, M1/70, PerCP-Cy5.5, 1:200, eBioscience 45-0112-82 lot 1929457 anti-mouse CD19, 6D5, APC-Cy7, 1:400, Biolegend 115530 lot B253924 anti-mouse CD45, 30-F11, Brilliant Violet 421, 1:500, Biolegend 103133 lot B263588 anti-mouse F4/80, BM8, Brilliant Violet 785, 1:200, Biolegend 123141 (lot unavailable) anti-mouse FoxP3, FJK-16s, FITC, 1:100, eBioscience 11-5773-82 lot 2007700 anti-mouse Gr1, Rb6-8C5, APC, 1:400, BD Biosciences 553129 lot 7121540

anti-mouse LAG-3, eBioC9B7W, PerCP-eFluor 710, 1:200, eBioscience 46-2231-82 lot E15914-105
anti-mouse Ly6C, HK1.4, Brilliant Violet 711, 1:400, Biolegend 128037 lot B247973
anti-mouse MHC-II, M5/114.15.2, APC-Cy7, 1:200, Biolegend 107628 (lot unavailable)
anti-mouse PD-1, J43, APC-eFluor 780, 1:200, eBioscience 47-9985-82 lot 1999029
anti-mouse TIM-3, RMT3-23, PE-Cy7, 1:200, eBioscience 25-5870-82 lot 4342910
anti-mouse VISTA, MIH63, PE, 1:200, Biolegend 150204 (lot unavailable)
anti-human IgG, polyclonal, Alexa Fluor 647, 1:250, Jackson Immunoresearch 709-605-149 lot 129954
anti-human CD3e, SK7, PE, 1:10, Biolegend 344806 lot B246230
anti-human CD4, SK3, Brilliant Violet 650, 1:10, BD Biosciences 563875 lot 7048624
anti-human CD8a, RPA-T8, Brilliant Violet 785, 1:10, Biolegend 301046 lot B221662
anti-human CD14, M5E2, Brilliant Violet 421, 1:10, Biolegend 310830 lot B262218
anti-human CD15, MMA, APC, 1:10, eBioscience 17-0158-42 (lot unavailable)
anti-human CD19, HIB19, APC-R700, 1:10, BD Biosciences 564977 lot 7160751
anti-human CD42b, HIP1, APC, 1:10, Biolegend 303912 (lot unavailable)
anti-human CD56, NCAM 16.2, Brilliant Violet 421, 1:10, BD Biosciences 562752 lot 5295681
anti-human NFkB pS529, K10-895.12.50, unconjugated, 1:100, BD Biosciences 558393 (lot unavailable)
anti-human PSGL-1, KPL1, APC, 1:100, BD Biosciences 562758 lot 7125846
anti-human VISTA, B7H5DS8, APC, 1:10, eBioscience 17-1088-42 (lot unavailable)
anti-human VSIG-3, polyclonal, unconjugated, 1:100, R&D Systems AF4915 (lot unavailable)
CellTrace Violet, ThermoFisher C34557 (lot unavailable)
Live/Dead Fixable Aqua, ThermoFisher L34957 (lot unavailable)
Streptavidin Dextramer, no clone, PE, 32 nM, Immudex DX01-PE (lot unavailable)
human VISTA, no clone, biotinylated, 32-890 nM, Acro Biosystems B75-H82E1 (lot unavailable)
human VISTA-Fc, no clone, unconjugated, 10 ug/mL, R&D Systems 7126-B7-050 (lot unavailable)
mouse VISTA-Fc, no clone, unconjugated, 10 ug/mL, R&D Systems 7005-B7-050 (lot unavailable)

Internally generated antibodies:
anti-human VISTA clones VISTA.4, VISTA.5, VISTA.16, and VISTA.18
anti-mouse VISTA clone VISTA.5

Validation

VISTA.4 validation is depicted in Extended Data Fig 2F-H.
VISTA.5 validation is depicted in Extended Data Fig 2F-H.
VISTA.10 validation is depicted in Extended Data Fig 9A.
VISTA.16 validation is depicted in Extended Data Fig 4D.
VISTA.18 validation is depicted in Figure 2, Extended Data Fig 3D, 4D, 10B, and 10D.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

293T, Raji, Jurkat, MC38, and CHO cell lines were obtained from ATCC and modified as described in the methods. Expi293 cells were obtained from Gibco.

Authentication

No cell line authentication was performed.

Mycoplasma contamination

All cell lines were screened for mycoplasma and found to be negative prior to use. MC38 tumor cells were additionally screened for other microbial contaminants and found to be negative prior to use in mouse studies.

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

Mice used in the study were males and females aged 6-12 weeks. Wildtype (C57BL6), VISTA knockout, and human VISTA knock-in strains were used. Cynomolgus macaques were males 6-24 months of age.

Wild animals

This study did not involve wild animals.

Field-collected samples

This study did not involve field-collected samples.

Ethics oversight

Animal studies were conducted in compliance with all relevant ethical regulations. Animal studies performed at Bristol-Myers Squibb were approved by the Bristol-Myers Squibb Institutional Care and Animal Use Committee. Animal studies performed at Five Prime Therapeutics were approved by the Five Prime Therapeutics Institutional Care and Animal Use Committee. Animal studies performed at Murigenics were approved by the Bristol-Myers Squibb Animal Welfare Risk Assessment Team and by the Murigenics Institutional Care and Animal Use Committee.

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	Human blood donors for experiments were anonymous, and no population information was collected.
Recruitment	Donors were anonymously recruited from Bristol-Myers Squibb employees.
Ethics oversight	Human blood was obtained from a research blood donation program administered by the Bristol-Myers Squibb Occupational Health and Wellness department. The program was operated in compliance with all relevant ethical regulations, and written informed consent was obtained from all donors.

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	Samples were prepared as listed in the methods.
Instrument	Fortessa (BD Biosciences) and Cytoflex (Beckman Coulter) instruments were used to acquire flow cytometry data.
Software	All flow cytometry data were analyzed with FlowJo software (BD Biosciences)
Cell population abundance	*Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.*
Gating strategy	*Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell population, indicating where boundaries between "positive" and "negative" staining cell populations are defined.*

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