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Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	Surface plasmon resonance was collected using the Biacore T100 control software version 2.0.4. Yeast binding data were collected with BD Accuri C6 software version 1.0.264.21. Size exclusion chromatography data were collected using UNICORN version 7.1. For surface marker expression experimentes, data were collected using FACSDiva version 8.0.2. Screen of additional cancer cell lines for biased marker expression were collected on a Beckman Coulter Cytoflex instrument with Cytoflex software version 2.0. Next-generation sequencing of A549 IFNG treated cells to compare changes in the human transcriptome of over 20,000 genes was performed on an Ion S5 XL instrument with a 550 chip (Thermo Fisher). Transcript mapping and relative abundance was performed using Torrent suite version 5.10.0. For mass spec, a Q-Exactive Orbitrap mass spectrometer (Thermo Fisher) equipped with an Easy nano-LC HPLC system with a C18 EasySpray PepMap RSLC C18 column (50 µm x 15 cm, Thermo Fisher Scientific) was used. For on-cell receptor dimerization experiments, images were acquired using the OLYMPUS cellSens Dimensions 1.18 software.
Data analysis	Microsoft Excel (version 16) and Prism7 (version 7.0d) for Mac OsX were used for analyzing plotting the data from the binding, functional signaling, marker and gene expression, and cytokine secretion experiments. FlowJo version 10.4.2 was used for surface marker expression experiments. Sequence alignment was generated in Geneious version 6. SPR data were analyzed using the Biacore T100 evaluation software versino 2.0.4. qPCR data was analyzed using the RQ Thermo cloud app on Jul 19, 2018. Heatmap and principle component analysis of gene expression data was performed using Matlab version R2018b (Mathworks).

Mass spec raw data files were analyzed using Proteome Discoverer v2.1 (Thermo Fisher Scientific) with Byonic v (Protein Metrics) as a module for automated identification of (glyco)peptides. Extracted ion chromatograms (EICs) of all identified (glyco)peptides were generated using Xcalibur v (Thermo Fisher Scientific).

For NGS data collection, gene mapping, and analysis, Ion Torrent Suite version 5.10.0 (Thermo Fisher) was used. As part of the software, gene target regions were mapped by referencing the hg19 genome build.

Software for crystallography includes: XDS June 1, 2017 (6E3K), Jan 16, 2018 (6E3L); Aimless 0.5.3.2 (6E3K), 0.7.1 (6E3L); Phaser 2.8.0 (6E3K), 2.8.1 (6E3L); Buster 2.10.3 (6E3K); Phenix 1.13_2998 (6E3K, 6E3L); Coot 0.8.9 (6E3K, 6E3L); Molprobity 4.4 (6E3L).

For on-cell receptor dimerization studies, data was analyzed using published software as described in Wilmes et al. J Cell Biol (2015), Ho et al. Cell (2017), Kirchhofer et al. Nat Struct Mol Biol (2010), Serge et al. Nat Methods (2008), and Appelhans et al. JoVE (2018).

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 upon request. 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

Structure factors and coordinates have been deposited in the Protein Data Bank with identification numbers PDB: 6E3K and 6E3L. Diffraction images have been deposited in the SBGrid Data Bank (http://dx.doi.org/591, 592). Next-generation sequencing data files from the human transcriptome study were deposited to the NCBI Gene Expression Omnibus (GEO) data repository with accession number GSE122672. Other data and materials are available upon request from the corresponding author.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	The variability of the effect size induced by the ligand was determined by performing three individual biological replicates. Experimental outliers were identified based on the controls, the experimental unit was in this case repeated, leading to sample sizes from (3-n).
Data exclusions	Experiments were excluded if the controls indicated experimental problems e.g. negative data for the positive controls. Data points were excluded when there was a technical mistake in the ligand preparation or during the procedure of the experiment. Data exclusion was not pre-established.
Replication	The ligands have been tested in at least three individual biological replicates including preparation of the dilutions. All attempts of experimental replication were successful.
Randomization	Plating of cells used in assays produces randomization. For cytokine secretion and surface marker expression, PBMCs were obtained from multiple donors at random.
Blinding	Blinding was not used for these studies. Engineering of the IFNg variants and pSTAT1 was performed in a single lab at Stanford. This information alone would not have predicted effects on surface expression of PD-L1 and MHC I which were performed by an independent laboratory.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☐	☒ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☒	☐ Human research participants

Methods

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

Unique biological materials

Policy information about availability of materials

Obtaining unique materials: Materials for the signaling, functional, yeast-display, and structural experiments are not limited.

Antibodies

Antibodies used: For detection of Myc-tag on yeast, the anti-Myc mAb conjugated with Alexa 647 purchased from Cell signaling: Clone 9B11, Lot 18, and Cat# 2233S, 1/100. The anti-pSTAT1 (Y701) antibody conjugate with Alexa 488 was purchased from Cell signaling: Clone 5806, Lot 14, Cat# 9174S, 1/100.

PDL1, MHC I marker experiments on A549, monocytes, macrophages, DCs, MeWo, HT-29, Hap1, Hep G2, HeLa, and Panc-1 cells: anti-HLA-ABC V450, clone G46-2.6, BD, Cat# 561346, Lot# 8092888, 1/50; anti-HLA-DR FITC, clone L243, Biolegend, Cat# 307604, Lot#B223614, 1/50; anti-CD80 BV650/alexa647, clone 2D10, Biolegend, Cat# 305227, Lot# B253507, 1/50; anti-CD86 BV605, clone IT2.2, Biolegend, Cat# 305430, Lot# B241340, 1/50 ; anti-PD-L1 PE/Dazzle 594, clone 29E.2A3, Biolegend, Cat# 329732, Lot# B246318, 1/100; anti-CD40 PE-Cy7, clone 5C3, Biolegend, Cat# 334322, Lot# B237825, 1/50; anti-CD56 FITC, clone 5.1H11, Biolegend, Cat# 362546, Lot# B261824, 1/50; anti-CD19 PE-Cy5, clone J3-119, Beckman Coulter, Cat# IM2643U, Lot# 39, 1/10; anti-CD3 Alexa700, clone UCHT1, Biolegend, Cat# 300424, Lot# B172725, 1/50; anti-CD11c PE-Cy7, clone B-ly6, BD, Cat# 561356, Lot# 6132737, 1/50; anti-HLA-DR v500, clone G46-6, BD, Cat# 561224, Lot# 6007692, 1/20; anti-CD14 APC-H7, clone 560180, BD, Cat# 560180, Lot# 6057707, 1/50; anti-CD304 PE, clone AD5-17F6, MACs Miltenyi Biotech, Cat# 130-090-533, Lot# 5120327140, 1/10.

Validation: Negative control samples were run during every experiment. The anti-myc antibody (Cell Signaling) is specific for the Myc epitope at either N or C-termini and validated for FAC applications as used in this manuscript. anti-pSTAT1 (Y701) antibody (Cell Signaling) has been validated for use in FAC applications and is reactive to both human and mouse phosphorylated STAT1 proteins. Validation for the pSTAT1 data is supported in the dose-response curves in which protein is varied but anti p-STAT1 ab is held constant including having an untreated control. For the anti-HLA-ABC anti-body (BD), the anti-body is validated for use with Human, Rhesus, Cynomolgus, and Baboon by FAC. Validation data by manufacture includes staining of whole blood. For the anti-HLA-DR FITC (Biolegend) is reactive against Human, African Green, Baboon, Chimpanzee, Cynomolgus, Canine, Marmoset, Rhesus, Squirrel Monkey, and Tamarin for FAC applications and validation shown on L243 cells by manufacturer. For the anti-CD80 antibody (Biolegend), it is reactive against Human and Rhesus in use for FAC applications and manufacturer validated on Raji cell lines. For the anti-CD86 antibody (Biolegend), the Ab is cross-reactive for human and other primates. Manufacture validation data provided are whole blood monocytes treated with Ab for FAC applications. The anti-PDL1 antibody (Biolegend) is cross-reactive against Human, African Green, Baboon, Cynomolgus, and Rhesus. It has been validated for use by FAC using human peripheral blood lymphocytes by the manufacturer. For the anti-CD40 ab (Biolegend), the manufacturer states cross-reactivity for human and other primates for use in FAC applications with validation data using blood lymphocytes. The anti-PDL1 anti-body is cross-reactive against human and other primate cells for use by FAC. Manufacturer validation data includes staining of human peripheral blood lymphocytes. For the anti-CD56 antibody (Biolegend), this ab is validated for FAC using Human cells and validation data by the manufacturer includes staining of peripheral blood lymphocytes. For the anti-CD19 ab (Beckman), the ab is validated for use by FAC on human biological samples. For the anti-CD3 ab (Biolegend), the anti-body is validated for FAC applications in human and chimpanzee samples. Manufacturer validation includes human peripheral blood lymphocytes stained with the anti-body and a negative control. The anti-CD11c antibody (BD) is reactive against human samples and validated for use in FAC applications. The manufacturer provides supporting validation data of stained dendritic cells, and other cell types expressing CD11c. The anti-HLA-DR v500 ab (BD) has been validated to be cross-reactive with Human, Rhesus, Cynomolgus, and Baboon in FAC applications. For the anti-CD14 ab (BD), the ab has been validated for Human samples in FAC applications and manufacturer validation includes staining of Human peripheral blood monocytes. For the anti-CD304 ab (Miltenyi), the manufacture validation includes data of human PBMCs stained with CD304 ab.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s): Hap1s are from the Koen Verhoef lab at the NKI-AVL. Validation method was whole-genome sequencing. A549 cells are from ATCC (CCL-185) and were validated by ATCC via Sanger Sequencing. EBY100 yeast cells were provided by Prof. Dane Wittrup (MIT), genotyping was performed by sequencing. HeLa (ATCC CCL-2) was validated by the ATCC Cell Line Authentication Service.

Hi5 cells were from Invitrogen (BTI-TN-5B1-4); no authentication/validation information is provided by the vendor.

The following cell lines from ATCC do not provide authentication/validation information: HEK293 GnTI- cells are from ATCC (CRL-3022), SF9 cells were from ATCC (CTL-1711), MeWo (ATCC HTB-65), Hep G2 (ATCC HB-8065), HT-29 (ATCC HTB-38), and Panc-1 (ATCC CRL-1469).

Authentication	Cell line authentication was guaranteed by the sources. Hap1s were validated by whole-genome sequencing. A549 cells were validated by Sanger Sequencing, EBY100 yeast cells by genotyping and sequencing, HeLa cells were validated by the ATCC Cell Line Authentication Service. Invitrogen does not indicate authentication methods for Hi5 cells. ATCC does not provide authentication/validation information for: HEK293 GnTI- cells are from ATCC (CRL-3022), SF9 cells were from ATCC (CTL-1711), MeWo (ATCC HTB-65), Hep G2 (ATCC HB-8065), HT-29 (ATCC HTB-38), and Panc-1 (ATCC CRL-1469).
Mycoplasma contamination	All eukaryotic cell lines used for signaling and functional assays were tested negative for mycoplasma on a regular basis, before and during tissue culture. Cell lines used for protein production were not tested for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No ILAC listed cell lines that are commonly misidentified were used in this study.

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 yeast, EBY100 yeast cells were washed and incubated with proteins or antibodies diluted in PBSA. For pSTAT1, Hap1s were fixed with PFA, incubated with methanol, and washed with PBS. For surface marker studies, all cells eg A549, DCs, etc were maintained in PBS for FACs analysis.
Instrument	A BD Accuri C6 instrument was used for yeast and signaling experiments. For determination of surface markers on immune cells, a BD FACsAria II was used for sorting dendritic cells and a BD LSR was used for analysis of surface markers.
Software	The BD Accuri C6 software was used for collection and analysis of yeast and signaling experiments. For surface marker expression studies, FACSDiva was used for data collection and FlowJo was used for analyses.
Cell population abundance	To ensure complete coverage of the library after each round of selection, the total number of yeast were calculated by the number of events by FACS. 10x the number of yeast were then carried grown for the next round of selection. Dendritic cell purity post sort was assessed by flow cytometry and found to be 94% pure.
Gating strategy	For binding titrations and pSTAT1 on the analyzer, in fluorescent channels boundaries were set such that the positive gates included less than 0.3% of the samples described above. PD-L1 and MHC I expression on A549 and DCs: A549 and dendritic cells were first gated based on FSC-A and SSC-A to exclude debris. Doublets were excluded by size by gating based on FSC-A vs FSC-H. Dead cells were excluded by staining with LIVE/DEAD™ Fixable Aqua Dead Cell Stain Kit. Positive and negative antibody staining boundaries were determined based on fluorescence minus one controls. Dendritic cells were selected as HLA-DR and CD11c positive; CD3, CD56, CD19, CD14 and CD304 negative.

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