

## 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

- |                                     |                                     |                                                                                                                                                                                                                                                            |
|-------------------------------------|-------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | The exact sample size ( <i>n</i> ) for each experimental group/condition, given as a discrete number and unit of measurement                                                                                                                               |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly                                                                                                                                    |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | The statistical test(s) used AND whether they are one- or two-sided<br><i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>                                                               |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | A description of all covariates tested                                                                                                                                                                                                                     |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons                                                                                                                                        |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | 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) |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i> ) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted<br><i>Give P values as exact values whenever suitable.</i>                     |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings                                                                                                                                                           |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes                                                                                                                                     |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i> ), 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 | No software was used for data collection                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| Data analysis   | <ul style="list-style-type: none"> <li>Data analysis was performed and graphs were plotted with GraphPad Prism version 8.0 for Windows, GraphPad Software, la Jolla California USA, <a href="http://www.graphpad.com">www.graphpad.com</a></li> <li>Automated analysis of IE positive cells was carried out using ArrayScan XTI instrument using the Target Activation experimental tool in Thermo Scientific™ Store™ Express Image and Database Management Software</li> </ul> |

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

All data associated with this study are present in the paper or in the Supplementary Materials. The source data are provided as a Source Data file.

## 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](https://www.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 samples size was calculated beforehand. For the validation of the nanobody, each experiment is consisting of two or four technical replicates of three to four biological replicates. For the virus experiments, three to six technical replicates of two to four different donors has been used. No outliers were removed from the experiments. Sample sizes were chosen based on previous published research. |
| Data exclusions | No data was excluded.                                                                                                                                                                                                                                                                                                                                                                                              |
| Replication     | For all experiments, replication of the phenotype or observation was successful. For the validation of the nanobody, each experiment is has been performed three times independently. For the virus experiments, two to four independent experiments were performed. Experimental replicates have been stated in the manuscript.                                                                                   |
| Randomization   | During all experiments, samples were randomized.                                                                                                                                                                                                                                                                                                                                                                   |
| Blinding        | Acquisition and analysis of data was performed blind.                                                                                                                                                                                                                                                                                                                                                              |

## 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                                           |
|-------------------------------------|-----------------------------------------------------------------|
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Antibodies                  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Eukaryotic cell lines       |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology                          |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Animals and other organisms            |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Human research participants |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Clinical data                          |

### Methods

| n/a                                 | Involved in the study                           |
|-------------------------------------|-------------------------------------------------|
| <input checked="" type="checkbox"/> | <input type="checkbox"/> ChIP-seq               |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Flow cytometry         |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> MRI-based neuroimaging |

## Antibodies

|                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|-----------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Antibodies used | <ul style="list-style-type: none"> <li>• Mouse anti-Myc-tag antibody. Supplier name: Cell Signaling Technology. Catalog number: 2276S. Clone name: 9B11. Lot number: 24. Dilution: 1:1000</li> <li>• Polyclonal rabbit anti-US28 antibody. Supplier name: Custom generated by Covance. Dilution: 1:1000</li> <li>• Anti-mouse IgG (H+L)-HRP conjugate antibody. Supplier name: Bio-Rad. Catalog number: 170-6516. Lot number: L005680A. Dilution: 1:10000</li> <li>• Anti-rabbit IgG (H+L)-HRP conjugate antibody. Supplier name: Bio-Rad. Catalog number: 170-6515. Lot number: L005679A. Dilution: 1:10000</li> <li>• Alexa Fluor® 488- and Alexa Fluor® 546-conjugated anti-mouse and anti-rabbit antibodies. Supplier name: Thermo Fisher Scientific. Catalog numbers: A11001 and A11010 respectively. Lot numbers: 2090562 and 1813035. Dilution: 1:1000</li> <li>• Mouse-anti-actin antibody. Provider: Sigma-Aldrich. Catalog number: A5316. Clone AC-74. Lot number: 059M4770V. Dilution: 1:2000</li> <li>• Mouse-anti-IE antibody. Provider: Argene. Catalog number: 11-003. Lot number: 1099280. Dilution: 1:1000</li> <li>• Rat-anti-HA antibody. Provider: Roche. Catalog number: 11867423001. Clone 3F10. Lot number: 42155800. Dilution: 1:1000</li> <li>• Anti-Rat-IgG-HRP conjugate antibody. Provider: Thermo Scientific, Pierce. Catalog number: 31470 Lot number: RF236103. Dilution: 1:1000</li> <li>• Mouse-anti-IFI16 antibody. Provider: Santa Cruz. Catalog number: SC-8023 Lot number: G2915. Dilution: 1:500</li> </ul> |
| Validation      | <ul style="list-style-type: none"> <li>• Mouse anti-Myc-tag antibody. Supplier name: Cell Signaling Technology. Catalog number: 2276S. Clone name: 9B11. Lot number: 24</li> </ul> <p>The antibodies were validated for immunoprecipitation and immunofluorescence microscopy by Cell Signaling Technology. We</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |

have validated them by immunofluorescence microscopy on cells transfected with the wild type US28 receptor upon incubation with Myc-tagged nanobodies (Heukers et al, Oncogene, 2018). Cells incubated without Myc-tagged nanobodies served as negative control for specific binding of the anti-Myc-tag antibody (data not shown in manuscript).

- Polyclonal rabbit anti-US28 antibody. Supplier name: Custom generated by Covance.

Method of validation:

The antibodies were previously validated by Bongers et al., J Clin Invest, 2010 and Heukers et al, Oncogene, 2018.

- Anti-mouse IgG (H+L)-HRP conjugate antibody. Supplier name: Bio-Rad.

Catalog number: 170-6516. Lot number: L005680A

Method of validation:

Described by: Knapp et al., Elsevier/North Holland Biomedical Press, 1978

- Anti-rabbit IgG (H+L)-HRP conjugate antibody. Supplier name: Bio-Rad.

Catalog number: 170-6515. Lot number: L005679A

Method of validation:

Described by: Knapp et al., Elsevier/North Holland Biomedical Press, 1978

- Alexa Fluor® 488- and Alexa Fluor® 546-conjugated anti-mouse and anti-rabbit antibodies. Supplier name: Thermo Fisher Scientific. Catalog numbers: A11001 and A11010 respectively. Lot numbers: 2090562 and 1813035

Method of validation:

We have validated them on immunofluorescence microscopy cells US28 overexpressing cells. Non-expressing served as control.

- Mouse-anti-actin antibody. Provider: Sigma-Aldrich. Catalog number: A5316. Clone AC-74. Lot number: 059M4770V

Method of validation:

The antibody was previously validated by others including Kornblum et al, Nature Genetics, 2013.

- Mouse-anti-IE antibody. Provider: Argene. Catalog number: 11-003. Lot number: 1099280

Method of validation:

The antibodies were validated by others including Businger et al, Nature Microbiology, 2019.

- Rat-anti-HA antibody. Provider: Roche. Catalog number: 11867423001. Clone 3F10. Lot number: 42155800

Method of validation:

The antibodies were validated by Roche via western blot and we have validated specificity previously (De Groof et al, Molecular Pharmaceutics, 2019).

- Anti-Rat-IgG-HRP conjugate antibody. Provider: Thermo Scientific, Pierce. Catalog number: 31470 Lot number: RF236103

Method of validation:

The antibodies were validated by Thermo Fischer Scientific via western blot and immunohistochemistry.

- Mouse-anti-IFI16 antibody. Provider: Santa Cruz. Catalog number: SC-8023 Lot number: G2915

The antibodies were validated previously by us in a previous paper Elder et al, mBio, 2019.

## Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)

HEK293T: derived from fetal human kidney. Obtained from ATCC.  
THP-1: derived from human peripheral blood. Obtained from European Collection of Authenticated Cell (ECAC)  
hfff1: derived from human foreskin. Obtained from European Collection of Authenticated Cell (ECAC)

Authentication

none of the cell lines were authenticated

Mycoplasma contamination

All cell lines were mycoplasma negative, as determined via PCR by Microbiome (Amsterdam, the Netherlands), using the R&D Mycoprobe Mycoplasma detection kit and DAPI staining.

Commonly misidentified lines  
(See [ICLAC](#) register)

No commonly misidentified cell lines were used.

## Human research participants

Policy information about [studies involving human research participants](#)

Population characteristics

Monocytes isolated from leukapheresis cones from UK NHS blood and transplant are anonymised so population characteristics were unknown. For PBMC isolation from arm bleeds, this was carried out by venous puncture of healthy donors who were chosen solely on the basis of HCMV seropositive status with no other Tier 1 reportable items according to the Biospecimen Reporting for Improved Study Quality guidelines.

Recruitment

Leukapheresis cones were obtained from the UK NHS Blood and Transplant from consenting donors. These were anonymised so population characteristics were unknown. We know of no bias of selection. PBMC from arm bleeds were performed on healthy donors chosen solely on the basis of HCMV seropositive status.

Ethics oversight

Human samples were obtained under ethical approval from Cambridgeshire 2 Research Ethics Committee (REC reference 97/092) conducted in accordance with the Declaration of Helsinki.

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