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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	Gen5 software was used to collect absorbance and fluorescence data from the Synergy HT multimode plate reader. Cytosoft 5.3 software was used to collect data from the Guava Flow cytometer (Millipore). BD FACSDiva 8.0.1 was used to collect data from the FACSCanto II (BD Biosciences). Axiovision software version 4.8 was used to acquire fluorescence microscopy data. LSM software ZEN 2011 was used to acquire confocal microscopy data.
Data analysis	FlowJo version 8.5 and 10 were used to analyze flow cytometer data obtained with FACSCanto II (BD Biosciences) and Guava Flow cytometer (Millipore), respectively. QuantStudio Real-Time PCR Software was used to analyze RT-qPCR data. Prism 8 software (GraphPad) was used to generate graphs and to perform statistical analyses. ImageJ version 1.8.0 software was used to analyze confocal microscopy data.

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

Unprocessed Western blot images for ED_Fig3 and Numerical and Statistical data for Fig1, Fig2, Fig3, Fig4, ED_Fig1, ED_Fig2, ED_Fig3, ED_Fig4, ED_Fig5, ED_Fig7, ED_Fig8, ED_Fig9, and Supplementary Fig1 are provided as source data. Additional data supporting the findings of this paper will be made available from the corresponding author upon request.

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 calculations were not performed. All experiments were done with replicates and successfully reproduced 2 or 3 times to demonstrate the magnitude and consistency of measurable differences, as is standard in the field.
Data exclusions	Data were not excluded.
Replication	All experiments were reproduced successfully 2 or 3 times.
Randomization	Samples were not randomized. We only analyzed one variable per experiment.
Blinding	We did not use externally supplied samples. Most experiments from collection to analysis were done by only one Investigator (M.I.C.), therefore blinding was not possible.

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

- Purified mouse anti-human CD365 (HAVCR1) monoclonal antibody, clone 1D12 (BioLegend, Inc., Cat# 353902, Lot# N/A *) was used undiluted at 1 ug/test.
- PE-labeled pur ified mouse anti-human CD365 (HAVCR1) monoclonal antibody, clone 1D12 (ThermoFisher Scientific, Cat# 12-3659-42, Lot# N/A*) was used undiluted at 5 ul/test.
- Purified mouse anti-human CD365 (HAVCR1) monoclonal antibody, clone HAVCR1-1, developed and produced at the Kaplan's Lab was used undiluted at 1 ug/test.
- Biotinylated affinity-purified goat anti-human TIM-1 (HAVCR1) antibody (R&D Systems, Cat# BAF1750, Lot# JVH03) was used undiluted at 1 ug/test.
- Mouse IgG1 isotype control, clone 15H6 (SouthernBiotech, Cat# 0102-01, Lot# N/A*) was used undiluted at 1 ug/test.
- PE-labeled goat anti-mouse IgG polyclonal antibody, human adsorbed (SouthernBiotech. Cat# 1010-09, Lot# K4512-M316Y) was used undiluted at 0.25 ug/test.
- PE-labeled anti-human CD9, clone M-L13 (BD Biosciences, Cat# 555372, Lot# N/A*) was used undiluted at 10 ul/test.
- PE-labeled anti-human CD63, clone H5C6 (BD Biosciences, Cat# 556020, Lot# N/A*) was used undiluted at 10 ul/test.
- PE-labeled anti-human CD81, clone JS-81 (BD Biosciences, Cat# 555676, Lot# N/A*) was used undiluted at 10 ul/test.
- PE-labeled anti-human CD3, clone UCHT1 (BD Biosciences, Cat# 555333, Lot# N/A*) was used undiluted at 10 ul/test.
- Rabbit anti-human 70-kDa heat shock protein (HSP70) polyclonal antibody (System Bioscience, Cat# EXOAB-Hsp70A-1, Lot# N/A*) was used at 1:1,000 dilution.
- Purified peroxidase-labebled goat anti-rabbit IgG antibody (System Bioscience, Cat# EXOAB-HRP, Lot# N/A*) was used at 1:20,000 dilution.
- Mouse anti-human Flotillin-1 (FLOT-1) monoclonal antibody, clone C-2 (Santa Cruz Biotechnology, Inc., Cat# sc-74566, Lot# N/A*) was used undiluted at 0.5 ug/ml.
- Mouse anti-human tumor susceptibility gene 101 protein (TSG101) monoclonal antibody, clone 4A10 (Abcam, Cat# ab83, Lot# N/A*) was used undiluted at 0.5 ug/ml.
- Mouse anti-human golgin subfamily A member 1 (GOLGA1) protein monoclonal antibody, clone CDF4 (ThermoFisher Scientific,

Cat# A-21270, Lot# N/A*) was used undiluted at 0.5 ug/ml.
- Affinity purified peroxidase-labeled goat anti-mouse IgG (H+L) human serum adsorbed (KPL SeraCare, Cat# 5220-0341, Lot# N/A*) was used at 1:20,000 dilution

Lot# N/A*: Because of the lockdown in the US due to the SARS-CoV-2 pandemic, we don't have access to the lot numbers at the present time.

Validation

All commercially available antibodies were validated by the manufacturer, and the data is available in their corresponding websites.
The HAVCR1-1 mAb was raised at the Kaplan's Lab against a HAVCR1-Fc fusion protein. MAb was purified by affinity chromatography in protein G columns. HAVCR1-1 mAb was validated at the Kaplan's lab by reactivity against the IgV domain of HAVCR1, protection of cells against HAV infection, and blocking of binding of apoptotic cells.
Data is provided in the manuscript for the following validations:
- Anti-human HAVCR1 antibodies were validated by comparing flow cytometry results of parental cells vs HAVCR1 KO cells and vector-transfected vs HAVCR1-transfected HAVCR1 KO cells.
- Anti-human CD9, CD63, and CD81 were validated by flow cytometry with Huh7 cells and by comparison of samples with or without exosomes.
- Anti-human FLOT-1, HSP70, TSG101, and GOLGA1 were validated by comparing the Western blot results of samples with or without exosomes produced by Huh7 cells.
- Mouse IgG1 isotype control, clone 15H6 and PE-labeled anti-human CD3, clone UCHT1 were validated by flow cytometry for the lack of cell and exosome surface staining.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Huh7 cells were obtained from the Japanese Collection of Research Bioresources Cell Bank (Cat# JCRB0403) and selected for the stable growth of wild-type HAV
AGMK cells were obtained from the Lab of Dr. Y Moritsugu in Japan.
Human embryonic kidney cells HEK-293 cells (Cat# 11631017, ThermoFisher Scientific)
Vero E6 cells (Cat# CRL-1586, ATCC)

Authentication

Huh7 cells were authenticated by STR profiling. Huh7 HAVCR1 KO and Huh7 NPC1 KO cells were authenticated by nucleotide sequence analysis of the KO genes. Huh7 NPC1 KO were also authenticated by resistance to EBOV cell entry.
AGMK cells were authenticated by staining with anti-HAVCR1 monoclonal antibody 190-4, which stains a HAVCR1 antigenic variant only present in these cells (Kaplan et al., 1996; Feigelstock 1998), and sequencing of monkey HAVCR1 specific allele.
Vero E6 and HEK-293 cells were not authenticated but cells were maintained at low passage from the original stock.

Mycoplasma contamination

Huh7 and AGMK cells were mycoplasma free as assessed by h-Impact Profile II test (IDEXX BioResearch)
The knockout cell lines and HEK293 cells were mycoplasma free as assessed by DAPI staining and fluorescence microscope analysis.
Vero E6 cells were not tested for mycoplasma contamination.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used.

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

Transfected or untransfected cells lines grown in cell culture flasks or plates were trypsinized and resuspended in PBS containing 2% FBS. Approximately 1,000,000 cells were treated with PE-labeled mouse monoclonal antibodies , washed, and fixed in 2% parafolmadehyde or treated with mouse monoclonal antibodies, washed, stained with PE-labled anti-mouse secondary antibodies, washed, and fixed in 2% parafolmadehyde. Staining of the HAVCR1 mucin was performed with biotin-labeled 1750 mAb and Alexa Fluor 488-labeled streptavidin.
Exosomes were absorbed to 2µl of 4 µm Aldehyde/Sulfate Latex Beads, 4% w/v (ThermoFisher Scientific), stained with PE-labled monoclonal antibodies, washed, and analyzed.
For the caspase-3 apoptosis assay, cells were trypsinized, stained with 1µl of cell-permeable caspase-3 inhibitor DEVD-FMK conjugated to sulfo-rhodamine (Red-DEVD-FMK), washed, and analyzed.

Instrument

Guava Flow cytometer (Millipore) and FACSCanto II (BD Biosciences)

Software	Cytosoft 5.3 software was used to collect data from the Guava Flow cytometer (Millipore). BD FACSDiva 8.0.1 was used to collect data from the FACSCanto II (BD Biosciences). FlowJo version 8.5 and 10 were used to analyze flow cytometer data obtained with FACSCanto II (BD Biosciences) and Guava Flow cytometer (Millipore), respectively.
Cell population abundance	The population that we analyzed corresponded to 100% of the cells. We did not perform cell sorting of any other enrichment procedure.
Gating strategy	Cells with a very low FCS/SSC profile that were close to the X- and Y-axis crossing were not included in the analysis. All other cells were included in the analysis using a wide-open gate. Boundaries between positive and negative staining cell populations were determined by using primary antibody isotype controls. Cells stained more than the isotype controls were considered as positive cells. Gating strategy is provided in the the main and exteded data figures next to the corresponding FACS analyses.

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