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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.

## 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<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 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<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 |
| ☐ | ☒ | Clearly defined error bars<br>*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 | Flow cytometry data was collected and performed on a Beckman Coulter CytoFlex S.<br>Western blots were imaged and analyzed using the Li-COR Odyssey Clx. |
| Data analysis | All data was analyzed using Graphpad Prism v7. FlowJo v 10 was used to analyze all flow cytometry experiments. Western blots were analyzed using the Li-COR Odyssey Image Studio Ver 5.2. Genewiz was used to evaluate and confirm colony PCR and sequencing data. Mass spectrometry data was analyzed using Proteome Discoverer 1.4 . |

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

Authors can confirm that all relevant data are included in the paper and/or its supplementary information files

# 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 number of animals used in the experiments in this study is estimated based on a power analysis with the following assumptions: standard deviation will be ~20% of the mean, p-value will be under 0.05 when the null hypothesis is false, the effect size (Cohen's d) is between 1.0-2.0. The minimal number of mice required under these conditions ranges between 6-28 for in vivo experiments. Additionally, we have carefully chosen the sample size listed below based on empirical evidence of what is necessary for interpretation of the data and statistical significance. |
| Data exclusions | No data was excluded from the analysis. |
| Replication | In all experiments reported, each cell culture and animal experiment was attempted at least two times. In all experiments, no attempts at replication failed. |
| Randomization | Groups were established based off genotype and infection status. All other aspects were randomized. |
| Blinding | Investigator blinding was not used or necessary in this study as no quantification of subtle data or phenotypes were required. |

# 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 | All unique materials used are readily available from the authors. |

## Antibodies

| | |
|---|---|
| Antibodies used | Flow: CCR5 (mouse HM-CCR5, 1:200), EpCAM (human, 9C4, 1:500), CD81 (human, 5a6; mouse, Eat-2, 1:200), CD9 (human, HI9a; mouse, MZ3, 1:200), CD63 (human, H5C6; mouse, NVG-2, 1:200), ADAM10 (human, SHM14, 1:200) all purchased from BioLegend. Western Blot: CD9 (Cat# 13174S, Cell Signaling, 1:1000), CD81 (cat# ab109201, Abcam, 1:1000), ADAM10 (Cat# |

| | |
|---|---|
| | AB19026, Millipore, 1:2500), LC3 (Cat# PM026, MBL, 1:1000 ), SQSTM1 (Cat# P0067, Sigma, 1:4000), HLA/a-toxin (Cat# S75531, Sigma, 1:5000), ACTIN (Cat# A5441, Sigma, 1:10,000), STX17 (Cat# NBP1-93968, Novus Biologicals, 1:2500), ARF6 (Cat# 5740S, Cell Signaling, 1:1000), ASS1 (Cat# ab124465, Abcam, 1:1000). |
| Validation | All reported commercial antibody validation can be found on vendor websites. |

## Eukaryotic cell lines

Policy information about cell lines

| | |
|---|---|
| Cell line source(s) | Human lung epithelial purchased from ATCC (A549), and human embryonal kidney cells (293FT, ThermoFisher cat#R70007) were used for lentiviral packaging. |
| Authentication | ATCC Cell Line Authentication, Service Sanger Sequencing |
| Mycoplasma contamination | Confirmed negative for mycoplasm |
| 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 | This study uses mice from the wildtype (WT) and Atg16L1 hypomorph on the C57BL/6 background. Both male and female mice approximately 8-10 weeks of age are used. |
| Wild animals | Study does not involve wild animals. |
| Field-collected samples | Study does not involve field-collected samples. |

## 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

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|---|---|
| Sample preparation | Exosomes were collected from culture supernatants or plasma via high speed centrifugation prior to staining with flow cytomtery antibodies (as described in manuscript). Cells isolated from in vitro assays were removed using 5mM EDTA and washed in PBS containing FBS prior to staining. |
| Instrument | Beckman Coulter CytoFlex S |
| Software | Flow data was collected with BD CytExpert Software and analyzed using FloJo v 10. |
| Cell population abundance | In sorting experiment, purity of samples was determined at the time of sorting by running a sorted sample on the FACSAria. All samples tested were more than 95% pure. |
| Gating strategy | Cells were gated on FCS-A and SSC-A. Doublets were removed by gating FSC-A v FSC-H. Live cells were gated as Zombie-Dye negative. Cells were then gated on for ADAM10 or EpCAM. Exosomes were gated as PKH67+CD81+CD63+CD9+. Exosomes were also stained with ADAM10 unless otherwise mentioned. All gates were set using an antibody isotype control samples. |

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