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 our Editorial Policies 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.

- 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.

Software and code

Policy information about availability of computer code

Data collection
Confocal images were collected using a Laser Scanning Microscope (SP8, Leica) with LAS-X (Leica) software. RNA-seq libraries were sequenced on a NextSeq500 (Illumina) as 76 bp single reads.

Data analysis
Image processing and analysis were performed using LAS-X (Leica) and Fiji software. The statistics analysis was performed using Excel (Microsoft) and BellCurve for Excel (Social Survey Research Information) software.

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 and 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

RNA-seq read data are available in the DDBJ Sequenced Read Archive under the accession numbers DRAxxxxx (under registration). All the other data generated or analyzed during this study are included in this manuscript and its supplementary information files. Raw data as well as all other data that support the findings of this study are available from the corresponding author upon reasonable 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.

- [x] 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 statistical methods were used to predetermine sample size. Sample sizes were chosen based on prior knowledge in the respective experiments and their intrinsic variability as performed in previous studies.
- Data exclusions: No data were excluded in analyses.
- Replication: All attempts at replication were successful. All of data in this manuscript was obtained with more than three biological replicates and/or more than two independent experiments. These obtained data was combined for statistical testing and presentation in figures.
- Randomization: Samples and organisms were allocated to the respective group based on their genotype or treatment. These are randomly chosen in each group, then processed and analyzed in parallel. Image acquisition was performed with randomly selected cells/fields.
- Blinding: Blinding was not relevant to most of our study as the readout of experiments was based on quantitative measurements and not based on subjective assessments. In some analysis with possible subjective view such as evaluation of vesicle formation, two investigators confirmed the results.

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.

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Antibodies

Antibodies used

For immunofluorescence
- anti-discs large monoclonal antibody (4F3) (Developmental Studies Hybridoma Bank)
- anti-cleaved caspase3 monoclonal antibody (Asp175) (Cell Signaling Technology, 9661)
- Alexa Fluor 546 conjugated goat anti-mouse IgG (Life Technology, A11003)
- anti-Rabbit IgG (H+L) Cross-Absorbed Secondary Antibody, Alexa Fluor 568 (Life Technology, A11011)

For western blotting
- anti-Arf6 (3A-1) (Santa Cruz, sc-7971)
- anti-Glyceraldehyde-3-Phosphate Dehydrogenase Polyclonal (Trevigen, 2275-PC-100)
- Anti-DYKDDDDK tag, Monoclonal Antibody (1E6) (Wako, 014-22383)
- Peroxidase-AffiniPure Donkey Anti-Rabbit IgG (H+L) (Jackson ImmunoResearch, 711-035-1522)
- Anti-Mouse IgG (H+L)-HRP Conjugate (Biorad, 170-6516)

Validation

All antibodies are commercially available and have been validated for immunofluorescence and/or western blotting by the manufacturers.
- anti-discs large (4F3), https://dshb.biology.uiowa.edu/4F3-anti-discs-large
Eukaryotic cell lines

<table>
<thead>
<tr>
<th>Policy information about cell lines</th>
<th>MDCKI and MDCKII cells were originally from ATCC, Eph4 cells were provided by Dr. E. Reichmann (University Children's Hospital Zurich, Switzerland).</th>
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<tbody>
<tr>
<td>Authentication</td>
<td>All cell lines were authenticated via ATCC or the providers.</td>
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<tr>
<td>Mycoplasma contamination</td>
<td>Cell lines were not tested for mycoplasma contamination but no indication of contamination was observed.</td>
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<td>Commonly misidentified lines</td>
<td>Such cell lines were not used in this study.</td>
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Animals and other organisms

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<th>Policy information about studies involving animals</th>
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<td>Laboratory animals</td>
<td>Six-week-old female C57BL/6N mice (SLC) were used for intestinal organoid preparation.</td>
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<td>Wild animals</td>
<td>No wild animals were used in this study.</td>
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<tr>
<td>Field-collected samples</td>
<td>No field-collected samples were used in this study.</td>
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<td>Ethics oversight</td>
<td>All experiments using animals were performed under the ethical guidelines of Kyoto Sangyo University.</td>
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Note that full information on the approval of the study protocol must also be provided in the manuscript.