

Reporting Summary

Nature Portfolio 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 Portfolio 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.

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
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☒ | ☐ | 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
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ | 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

Zen Black 2012 (Zeiss) was used for Carl Zeiss 710 LSM confocal laser scanning microscope.
ZEN 2012 v. 8.0.1.273 (ZEISS) software was used for LSM 980 Elyra 7 Airyscan super-resolution microscope.
CellSens dimension software (Olympus) v.4.2 was used for acquiring live-cell imaging videos.
BD FACS Diva software version 8.0.1 (BD Biosciences) for flow cytometry.

Data analysis

Data analysis was done using Fiji v.2.1.0 (NIH). For analysis of particle count and size from confocal micrographs, "analyze particle tool" function of Fiji software was used. For measurement of area, "free hand tool" and "measure tool" functions of Fiji software was used. For measurement of fractional distance, "plot profile tool", "free-hand selection tool" and "clear outside tool" function of Fiji software was used. The threshold was applied using the "MaxEntropy" thresholding option. The percentage area was calculated by selecting the "percentage area" values in the "Analyze Particle" tool. JACoP plugin of Fiji software was used to measure the Pearson correlation coefficient (PCC).
BD FlowJo v.10.0.1. was used to analyze flow-cytometry related experiments.
Statistics and graphing was done using Prism v.8 (GraphPad) and Excel 2013 (Microsoft).
All figures were assembled using Fiji v. 2.1.0 (NIH) and Adobe Illustrator v.24.3.
Figure 10 was created using BioRender. Sharma, M. (2025) <https://BioRender.com/qhvdmo0>
AlphaMissense predictions were done using <https://alphamissense.hegelab.org/>
AlphaFold2 predictions were done using <https://colab.research.google.com/github/sokrypton/ColabFold/blob/main/AlphaFold2.ipynb#scrollTo=kObIAo-xetgx>.
Chimera: 1.17.3 version used to visualize the binding residues between two proteins.

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 Portfolio [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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

All relevant information supporting the findings of this study is presented in the manuscript and supplementary materials. A source file comprising raw data and western blot images that have not been cropped is included in the manuscript.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	n/a
Reporting on race, ethnicity, or other socially relevant groupings	n/a
Population characteristics	n/a
Recruitment	n/a
Ethics oversight	n/a

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

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	The figure legends of the relevant figures list all sample sizes in detail. Sample sizes were determined based on the estimates from preliminary experiments and similar studies (Kumar G. et al., Nature Communications, 1540 (2022), Willett R. et al., Nature Communications, 1580(2017), and Marwaha R. et al., Journal of Cell Biology, 216 (2017)) so that reasonable statistical analysis could be conducted. A minimum of three biological replicates were taken for each experimental set, unless otherwise mentioned.
Data exclusions	Any transfected cell expressing an excessively high amount of proteins that visibly altered the cell morphology or exhibited saturated signal intensities was excluded from the analysis. Also, cells with two nuclei and non uniform morphology were excluded from analysis.
Replication	All the data presented were from biological replicates. Each experiment was repeated multiple times independently, as indicated in the figure legends, and was reproducible. Some of the experiments were replicated in multiple cell lines. All attempts at replication were successful.
Randomization	The randomization of different experimental groups was irrelevant because all of the experiments were conducted on a uniform biological sample, i.e, eukaryotic cell lines obtained from commercial source (ATCC). Furthermore, all the cells were treated or imaged, and they were finally analyzed in the same way.
Blinding	Blinding was not possible because the same investigators were responsible for the experiment preparation and further analysis of the data. Further, the experiments had no preconceptions while analyzing the result.

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 and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Antibodies

Antibodies used

All of the antibodies used in this study are listed in Supplementary Table III, which includes all relevant information about each antibody.

Validation

The validation statements can be found on the supplier's website using the links provided below. The antibodies successfully detected proteins with their expected molecular weights. The specificity of the antibodies against TECPR2, SNX17, FAM21C, EGFR, and SWIP are validated in this manuscript.

1. Rabbit anti-TECPR2 antibody, Sigma-Aldrich was validated in this manuscript for WB. <https://www.sigmaaldrich.com/IN/en/product/sigma/hpa000658>.
2. Mouse anti-Rab5 antibody, BD Biosciences was validated by the supplier for IF and WB. <https://www.bdbiosciences.com/en-us/products/reagents/microscopy-imaging-reagents/immunofluorescence-reagents/purified-mouse-anti-rab5.610724>. It has been previously shown in other publications (PMID: 37423034, 35318705).
3. Mouse anti-MBP-HRP antibody, New England Biosciences was validated by the supplier for WB. <https://www.neb.com/en/products/e8032-anti-mbp-monoclonal-antibody>. It has been previously shown in other publications (PMID: 39218986, 39054315).
4. Rabbit anti-TGN46 antibody, Abcam was validated by the supplier for WB. <https://doc.abcam.com/datasheets/active/ab50595/en-us/tgn46-antibody-ab50595.pdf>
5. Rabbit anti-TECPR2 antibody, Abcam was validated in this manuscript. <https://www.abcam.com/en-us/products/primary-antibodies/tecpr2-antibody-ab121109>.
6. Mouse anti-SNX1 antibody, BD Biosciences was validated by the supplier for IF. <https://www.bdbiosciences.com/en-us/products/reagents/microscopy-imaging-reagents/immunofluorescence-reagents/purified-mouse-anti-snx1.611482>. It has been previously shown in other publications (PMID: 35941155, 30072438).
7. Mouse anti-EEA1 antibody, BD Biosciences was validated by the supplier for IF. <https://www.bdbiosciences.com/en-us/products/reagents/microscopy-imaging-reagents/immunofluorescence-reagents/purified-mouse-anti-eea1.610457>. It has been previously shown in other publications (PMID: 28325809, 25908847, 36282215).
8. Rabbit anti-EEA1 antibody, CST was validated by the supplier for IF and WB. <https://www.cellsignal.com/products/primary-antibodies/eea1-c45b10-rabbit-mab/3288>. It has been previously shown in other publications (PMID: 36282215, 38126335, 38123554).
9. Mouse anti-LAMP1 antibody, BD Biosciences was validated by the supplier for IF and WB. <https://www.fishersci.com/shop/products/antidc107a-clone-h4a3-bd/BDB555798>. It has been previously shown in other publications (PMID: 35314681, 28325809, 25908847, 36282215).
10. Rabbit anti-Rab11 antibody, Abcam was validated by the supplier for IF and WB. <https://www.abcam.com/en-us/products/primary-antibodies/rab11a-antibody-epr7587b-ab128913#application=icc-if>. It has been previously shown in other publications (PMID: 29634932).
11. Rabbit anti-HRS antibody, CST was validated by the supplier for IF. <https://www.cellsignal.cn/products/primary-antibodies/hrs-d7t5n-rabbit-mab/15087>. It has been previously shown in other publications (PMID: 39223601, 3174481).
12. Mouse anti-β-actin antibody, BD Biosciences was validated by the supplier for WB. <https://www.scbt.com/p/beta-actin-antibody-c4>. It has been previously shown in other publications (PMID: 39243763, 37932277).
13. Rabbit anti-APPL1 antibody, CST was validated by the supplier for IF. <https://www.cellsignal.com/products/primary-antibodies/appl1-d83h4-xp-174-rabbit-mab/3858>. It has been previously shown in other publications (PMID: 31253801, 32275659).
14. Rabbit anti-Rabenosyn-5 antibody, Proteintech was validated by the supplier for IF. <https://www.ptglab.co.jp/Products/ZFYVE20-Antibody-22218-1-AP.htm>. It has been previously shown in other publications (PMID: 38509070).
15. Mouse anti-Itgβ1 (12G10) integrin antibody, Abcam was validated by the supplier for IF. <https://www.abcam.com/en-us/products/primary-antibodies/integrin-beta-1-antibody-12g10-ab30394>. It has been previously shown in other publications (PMID: 29712618).
16. Mouse anti-Itgβ1(CD29) integrin antibody, BD Biosciences was validated by the supplier for WB. <https://www.bdbiosciences.com/en-eu/products/reagents/microscopy-imaging-reagents/immunofluorescence-reagents/purified-mouse-anti-cd29.610467>. It has been previously shown in other publications (PMID: 22492727).
17. Rabbit anti-SNX17 antibody, Atlas Antibodies was validated by the supplier for IF and validated in this manuscript for WB. <https://www.atlasantibodies.com/products/primary-antibodies/triple-a-polyclonals/anti-snx17-antibody-hpa043867>. It has been Atlas previously shown in other publications (PMID: 39168982, 37141105).
18. Rabbit anti-Fam21C antibody, Invitrogen was validated in this manuscript for IF and WB. <https://www.thermofisher.com/antibody/product/FAM21C-Antibody-Polyclonal/PA5-35883>.
19. Rabbit anti-WASHC1 antibody, Atlas Antibodies was validated by the supplier for IF and WB. <https://www.sigmaaldrich.com/IN/en/product/sigma/hpa002689?srsltid=AfmBOoqKDAWG6cPnKA-UzxYIWN0vyLWslSPcdnuwAluQ1U8ZuwkGahq>. (PMID: 19922875).
20. Rabbit anti-KIAA1033/SWIP antibody, Atlas Antibodies was validated in this manuscript for WB. <https://www.thermofisher.com/antibody/product/KIAA1033-WASH-complex-subunit-7-Antibody-Polyclonal/A304-919A>.

21. Rabbit anti-Strumpellin antibody, Bethyl Laboratories was validated in this manuscript for IF and WB. <https://www.biomol.com/products/antibodies/primary-antibodies/general/anti-spg8/strumpellin-a304-809a-t>.
22. Rabbit anti-ARPC5/p16 antibody, Abcam was validated by the supplier for IF and WB. <https://www.abcam.com/en-us/products/primary-antibodies/arp5-p16-arc-antibody-ep1551y-ab51243#application=icc-if>. It has been previously shown in other publications (PMID: 21357746, 22447776).
23. Mouse anti-FLAG antibody, Sigma-Aldrich was validated by the supplier for IF. <https://www.sigmaaldrich.com/IN/en/product/sigma/f1804>. It has been previously shown in other publications (PMID: 35314681, 28325809).
24. Rabbit anti-FLAG antibody, Thermo Fisher Scientific was validated by the supplier for WB. <https://www.thermofisher.com/antibody/product/DYKDDDDK-Tag-Antibody-Polyclonal/PA1-984B>. It has been previously shown in other publications (PMID: 36763502, 33966597, 36244455).
25. Mouse anti-GFP antibody, Santa Cruz Biotechnology was validated by the supplier for WB. <https://www.scbt.com/p/gfp-antibody-b-2>. It has been previously shown in other publications (PMID: 28325809, 36282215).
26. Rabbit anti-C16orf62 antibody, Invitrogen was validated by the supplier for WB. <https://www.thermofisher.com/antibody/product/C16orf62-Antibody-Polyclonal/PA5-28553>. It has been previously shown in other publications (PMID: 30959277, 25355947).
27. Mouse anti-SNX27 antibody, Abcam was validated by the supplier for IF and WB. <https://www.abcam.com/en-us/products/primary-antibodies/snx27-antibody-1c6-ab77799>. It has been previously shown in other publications (PMID: 35993307, 35417450).
28. Mouse anti-ATP7A antibody, Santa Cruz Biotechnology was validated by the supplier for WB. <https://www.scbt.com/p/atp7a-antibody-d-9>. It has been previously shown in other publications (PMID: 37248224, 23563491).
29. Mouse anti- α -tubulin antibody, Santa Cruz Biotechnology was validated by the supplier for WB. <https://www.sigmaaldrich.com/IN/en/product/sigma/t9026>. It has been previously shown in other publications (PMID: 3754075, 20534674).
30. Mouse anti-GAPDH antibody, Sigma-Aldrich was validated by the supplier for WB. <https://www.scbt.com/p/gapdh-antibody-h-12>. It has been previously shown in other publications (PMID: 28325809, 35125694).
31. Rabbit anti-TfR antibody, Abcam was validated by the supplier for IF. <https://www.abcam.com/products/primary-antibodies/transferrin-receptor-antibody-ab84036.html>. It has been previously shown in other publications (PMID: 35314681, 36282215).
32. Mouse anti-EGFR antibody, Thermo Fisher Scientific was validated in this manuscript for Flow Cytometry. https://tools.thermofisher.com/content/sfs/manuals/AHR5062_Rev0210.pdf.
33. Rabbit anti-EGFR antibody, CST was validated by the supplier for WB. <https://www.cellsignal.com/products/primary-antibodies/egf-receptor-d38b1-xp-rabbit-mab/4267> (PMID: 40210693).
34. Mouse anti-SV2 antibody, Developmental Studies Hybridoma Bank was validated by the supplier for IF. <https://dshb.biology.uiowa.edu/SV2>. It has been previously shown in other publications (PMID: 31394789, 30699109).
35. Rabbit anti-Rab5 antibody, CST was validated by the supplier for WB. <https://www.cellsignal.com/products/primary-antibodies/rab5-c8b1-rabbit-mab/3547>. It has been previously shown in other publications (PMID: 30627666, 32184389).
36. Mouse anti-Paxillin antibody, BD Biosciences was validated by the supplier for IF. <https://www.bdbiosciences.com/en-us/products/reagents/microscopy-imaging-reagents/immunofluorescence-reagents/purified-mouse-anti-paxillin.610051>. It has been previously shown in other publications (PMID: 34169835, 31816055).
37. Rabbit anti-LAMP1 antibody, Abcam was validated by the supplier for WB and IF. <https://www.abcam.com/products/primary-antibodies/lamp1-antibody-lysosome-marker-ab24170.html>. It has been previously shown in other publications (PMID: 35314681, 28325809, 36282215).
38. Rabbit anti- α 5-integrin antibody, Abcam was validated by the supplier for IF. <https://www.abcam.com/en-us/products/primary-antibodies/integrin-alpha-5-antibody-epr7854-ab150361>. It has been previously shown in other publications (PMID: 28892079).
39. Mouse anti-Rab7 antibody, SCBT was validated by the supplier for IF. <https://www.scbt.com/p/rab-7-antibody-b-3>. It has been previously shown in other publications (PMID: 35314681, 36506095, 28325809, 36282215).
40. Rabbit anti-HA antibody, Sigma-Aldrich was validated by the supplier for IF. <https://www.sigmaaldrich.com/IN/en/product/sigma/h6908>. It has been previously shown in other publications (PMID: 35314681, 36282215).
41. Mouse anti-HA antibody, BioLegend was validated by the supplier for IF. <https://www.biolegend.com/fr-fr/products/purified-anti-ha-11-epitope-tag-antibody-11374?GroupID=GROUP26>. It has been previously shown in other publications (PMID: 35314681, 34103528, 33086052).
42. Alexa-Fluor 488-conjugated goat anti-rabbit IgG, Thermo Fisher Scientific was validated by the supplier. <https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11034>.
43. Alexa-Fluor 568-conjugated goat anti-rabbit IgG, Thermo Fisher Scientific was validated by the supplier. <https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11036>.
44. Alexa-Fluor 488-conjugated goat anti-mouse IgG, Thermo Fisher Scientific was validated by the supplier. <https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11029>.
45. Alexa-Fluor 568-conjugated goat anti-mouse IgG, Thermo Fisher Scientific was validated by the supplier. <https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11031>.
46. Alexa-Fluor 647-conjugated goat anti-mouse IgG, Thermo Fisher Scientific was validated by the supplier. <https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21235>.
47. Alexa-Fluor 488-conjugated α -Bungarotoxin, Thermo Fisher Scientific was validated by the supplier. <https://www.thermofisher.com/order/catalog/product/B13422>. It has been previously shown in other publications (PMID: 31394789, 33456573).
48. Alexa-Fluor 647-conjugated Phalloidin, Thermo Fisher Scientific was validated by the supplier. <https://www.thermofisher.com/order/catalog/product/A22287>.
49. HRP-conjugated goat anti-rabbit IgG, Jackson ImmunoResearch was validated by the supplier. <https://www.jacksonimmuno.com/catalog/products/111-035-144> 62.
50. HRP-conjugated goat anti-mouse IgG, Jackson ImmunoResearch was validated by the supplier. <https://www.jacksonimmuno.com/catalog/products/115-035-166>.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	hTERT-RPE1, HeLa, and HEK293T were purchased from ATCC and cultured following the supplier's instructions.
Authentication	All the cell lines were authenticated by the supplier using STR profiling by the respective repositories. We also assessed the morphology of all the cell lines by microscopy.
Mycoplasma contamination	All cell lines used in this study were regularly screened for the absence of mycoplasma contamination by using the MycoAlert Mycoplasma Detection Kit (LT07-418, Lonza), and all the tests were negative for mycoplasma presence.
Commonly misidentified lines (See ICLAC register)	The study did not use any misidentified cell lines.

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	Zebrafish (wild-type)
Wild animals	Embryos of Zebrafish (wild-type) were used. Embryos were kept in the laboratory as long as they lived after experimentation.
Reporting on sex	Study involved only embryos where both sexes must be involved. Sex determination in Zebrafish is not possible before three months of growth.
Field-collected samples	All samples were made and used in laboratory conditions. No natural collection of any samples involved in this study.
Ethics oversight	No human tissues or samples were used in this study.

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

Plants

Seed stocks	n/a
Novel plant genotypes	n/a
Authentication	n/a

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	To detect the surface level of Itgβ1 in HeLa cells treated with either control siRNA or TECPR2 siRNA, cells were detached from the cell culture dish using a 5 mM EDTA solution prepared in 1X PBS and added to the cells for 15-20 min on ice. The cells were collected by gentle pipetting in a fresh microcentrifuge tube and centrifuged at 400xg for 2 min at 4°C. The cell pellets were washed once with ice-cold flow cytometry buffer (0.2% FBS in 1X PBS), followed by incubation with primary antibody solution prepared in flow cytometry buffer for 1 hr on ice. The cells were washed once to remove unbound primary antibody and allowed to incubate with Alexa Fluor-conjugated secondary antibody solution made in flow cytometry buffer for 30 min on ice. Finally, the cells were washed and resuspended in flow cytometry buffer containing 1% PFA. The amount of surface receptor levels was measured by fluorescence flow cytometry using a BD FACS Aria Fusion Cytometer, and BD FACS Diva software version 8.0.1 (BD Biosciences) were used to acquire the samples. Data analysis was performed using BD FlowJo
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version 10.0.1.

For determining the surface level of EGFR, the above-described methodology was used with some modifications. Briefly, the cells were serum starved by incubating in DMEM media for 30 min at 37°C, followed by fixation on ice for 15 min using 2.5% PFA solution prepared in 1X PBS. The cells were scraped out and resuspended in flow cytometry buffer containing primary antibody against EGFR to label the surface EGFR. The cells were washed once to remove unbound primary antibody and allowed to incubate with Alexa Fluor-conjugated secondary antibody solution made in flow cytometry buffer for 30 min on ice. Finally, the cells were washed and resuspended in flow cytometry buffer and analyzed by flow cytometry.

To determine the surface level of transferrin receptor (TfR) in HeLa cells treated with control or TECPR2 siRNA, the cells were serum starved in DMEM media (with 0.5% BSA) for 30 min at 37°C. The cells were then detached from the cell culture dish by adding 5 mM EDTA solution prepared in 1X PBS to the cells for 15-20 min on ice. The cells were collected by gentle pipetting in a fresh microcentrifuge tube and centrifuged at 400×g for 2 min at 4°C. The cell pellets were washed once with ice-cold flow cytometry buffer (0.2% FBS in 1X PBS), followed by incubation with Alexa 568-Tfn (20µg/ml) prepared in flow cytometry buffer for 1 hr on ice. Finally, the cells were washed twice with ice-cold buffer and resuspended in flow cytometry buffer containing 1% paraformaldehyde (PFA) and analyzed by flow cytometry.

Transferrin recycling assay

To measure transferrin (Tfn) recycling, HeLa cells treated with control and TECPR2 siRNA were serum starved for 30 min at 37°C by addition of DMEM media to culture dishes. The cells were washed once with 1X PBS and pulsed for 5 min at 37°C with 20 µg/mL of Alexa 568-Tfn (Molecular Probes) prepared in DMEM media. After the pulse period, unbound or non-internalized Alexa 568-Tfn was removed by incubating the cells in citric acid buffer, pH 4.5 (0.1 M citric acid anhydrous and 0.1 M tri-sodium citrate dihydrate) for 90 sec at 37°C. After one wash with 1X PBS, for pulse-only samples, cells were collected by trypsinization, pelleted, and resuspended in flow cytometry buffer (0.2% FBS in 1X PBS) for measurement of Alexa 568-Tfn fluorescence intensity by flow cytometry. For the "chase" samples, after the citric acid buffer (pH 4.5) wash step, pre-warmed DMEM media containing 10% FBS was added to the cells for different time periods at 37°C. After the indicated chase period, a second citric acid buffer (pH 4.5) wash was given to cells for 90 sec at 37°C to strip off surface recycled Alexa 568-Tfn at each time point. Cells were collected by trypsinization, pelleted, and resuspended in flow cytometry buffer (0.2% FBS in 1X PBS) for measurement of remaining intracellular Alexa 568-Tfn fluorescence intensity by flow cytometry using a BD FACS Aria Fusion Cytometer, and BD FACS Diva software version 8.0.1 (BD Biosciences) were used to acquire the samples. Data analysis was performed using BD FlowJo version 10.0.1.

Instrument

BD FACS Aria Fusion Cytometer was used in the study

Software

FACS Diva software version 8.0.1 (BD Biosciences).

Cell population abundance

30,000 cells per sample were analyzed.

Gating strategy

Viable cells were gated on a plot of FSC-A versus SSC-A.

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