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
☐ | 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 -
For data analysis, the study involved the use of various computational tools and software. The raw data on RTK interactomes were accessed through MassIVE with dataset identifier D613.2, and the protein-protein interaction information on PSIQUIC (http://www.ebi.ac.uk/Tools/webservices/psiquic/view/main.xhtml) and sequence information in Uniprot (https://www.uniprot.org/) can be accessed through the respective website of the database.

For image analysis, the study utilized Fiji (Schindelin, J. et al. Fiji: An open-source platform for biological-image analysis. Nature Methods 9, 676–682 (2012), versions 1.50 and 1.51) was used. For quantification of Western blot analyses, Image Studio Lite, version 5.2 (LI-COR Biosciences, NE, USA) was used. For live-imaging analysis, Incucyte ZOOM 2018b (Sartorius, Germany) software was used. For mass spectrometry data analysis, FlAnLFQ (Milliken, R. J., Solntsev, S. K., Shortreed, M. R. & Smith, L. M. Ultrafast Peptide Label-Free Quantification with FlAnLFQ. J. Proteome Res. 17, 386–391 (2018), version 1.1.2) and Metamorphes v. 0.0.304 (Solntsev, S. K., Shortreed, M. R., Frey, B. L. & Smith, L. M. Enhanced Global Posttranslational Modification Discovery with MetaMorphes. J. Proteome Res. 17, 1844–1851 (2018)) were used. For standard normalization, differential expression, complex analysis and statistical analysis standard functions in Matlab 2016a (Mathworks, MA, USA) and R versions 3.0.3-3.6.2 (R Core Team. R Core Team (2017): R: A language and environment for statistical computing. R Found. Stat. Comput. Vienna, Austria. URL http://www.R-project.org/) was used. For a reference copy of the document with all sections, see 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

The raw MS proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE65 and Panorama Public67 partner repositories with the data set identifiers PXD017783, PXD026546, PXD026617, and PXD026617. The raw data from the glycan screen are provided as a Supplementary Data 8. The raw data on the molecular dynamics simulations have been supplied to the corresponding author upon reasonable request. The additional source data was not deposited to a public repository since the public repositories do not support the deposition of over 1 TB of data of various data file types. The subcellular localization data in Compartments database were accessed through their website (Janos X. Binder, Sune Pletscher-Frankild, Kalliopi Tsafou, Christian Stolte, Seán I. O’Donoghue, Reinhard Schneider, Lars Juhl Jensen, COMPARTMENTS: unification and visualization of protein subcellular localization evidence, Database, Volume 2014, 2014, 8-10, accessed 19.4.2021). The protein-protein interaction information on PSIQUIC database was accessed through a R vignette (Shannon P (2016). PSIQUIC: Proteomics Standard Initiative Common Query Interface. R package version 1.28.0.). The protein-protein interaction information on the STRING database were accessed through their website (Szklarczyk et al. Nucleic acids research 47 D1 (2018): D607-D613.2, "string-db.org/", v.11.0). The sequence information of human receptor tyrosine kinases was accessed through the Uniprot website (The UniProt Consortium UniProt: the universal protein knowledgebase. Nucleic Acids Res. 46: 2699 (2018), https://www.uniprot.org/, release-2018-03) and the protein structure information was accessed through the Protein Databank website (H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, P.E. Bourne. (2000) The Protein Data Bank Nucleic Acids Research, 28: 235-242, pdb.org, accessed 5.12.2017).

**Field-specific reporting**

Please select the one below that is the best fit for your research. If you are not sure, please 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**

The study was conducted with cell lines, which is why the traditional sense of sample size does not apply. For quantitative studies, the amount of analyzed samples was determined by how many replicates were needed to gain a significant P-value (under 0.05) for observed differences.
Data exclusions
Data were excluded from analyses due to technical concerns. In live-imaging experiments, cell plate wells with a significantly different confluence in the beginning of the experiments were excluded. Western blot bands with smeared wells or unfortunate air bubbles or very low signal against the background were excluded. In immunofluorescence imaging experiments, cells that had highly irregular morphology against other cells in the slide were excluded. Experiments with failed positive and/or negative controls were excluded.

Replication
The findings were replicated in different cell lines to ensure reproducibility. All experiments were replicated at least twice in the exact same setting to ensure reproducibility. Different approaches to show the same phenomenon were applied to ensure reproducibility. All attempts at replication that were not successful were due to technical concerns.

Randomization
Randomization of samples to group allocation is not relevant to the study. The experimental model used was cell lines. By using cell lines the background of each treatment is identical.

Blinding
Blinding to group allocation during collection or analysis was not feasible since most experiments were conducted independently by one investigator and the sample order needed to be recorded for accurate interpretation of results (western analyses, immunofluorescence analyses). The experiments conducted with more than one investigator were blinded by not divulging the details of the samples to the second investigator running the analyses (mass spectrometry experiments).

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**

Mouse monoclonal anti-β-actin (AC-74), Sigma, Catalog no: A5441, Lot no: 026M4780V, 1:2000 dilution in western analyses; Goat polyclonal anti-Actin (I-19), Santa Cruz, Catalog no: sc-1616, Lot no: (Antibody not available anymore), 1:1000 dilution in western analyses; Mouse monoclonal anti-β1-integrin (4B7R), Santa Cruz, Catalog no: sc-9970, Lot no: j129, 1:100 dilution in immunofluorescence analyses; Mouse monoclonal anti-β1-integrin (K-20), Santa Cruz, Catalog no: sc-18887, Lot no: #G1713, 1:100 dilution in immunofluorescence analyses; Rabbit monoclonal anti-β1-integrin (EP1041Y), Abcam, Catalog no: ab52971, Lot no: 927161, 1:1000 dilution in western analyses; Rat monoclonal anti-β1-integrin (Mab 13), BD Pharmingen, Catalog no: 552828, Lot no: 8054, 1ug per 1mg of protein in immunoprecipitation; Rabbit monoclonal anti-β4-integrin (D8P6C), Cell Signaling, Catalog no: #14803, Lot no: 1, 1:1000 dilution in western analyses; Rabbit polyclonal anti-β-Tubulin (H-235), Santa Cruz, Catalog no: sc-9104, Lot no: (Antibody not available anymore), 1:1000 dilution in western analyses; Mouse monoclonal anti-β-Tubulin (SAP.4G5), Sigma, Catalog no: T7816, Lot no: 025M4776V, 1:2000 dilution in western analyses; Rabbit polyclonal EGF Receptor Antibody, Cell Signaling, Catalog no: #2232, Lot no: 16, 1:1000 dilution in western analyses; Rabbit monoclonal Phospho-EGF Receptor (Tyr1068) (D7A5), Cell Signaling, Catalog no: #3777, Lot no: 10, 1:500 dilution in western analyses; Rabbit polyclonal ErbB2 Antibody (C-18) Santa Cruz, Catalog no: sc-284, Lot no: #H2411, 1:1000 dilution in western analyses; Rabbit monoclonal Phospho-HER2/ErbB2 (Tyr1248) Antibody, Cell Signaling, Catalog no: #2247, Lot no: 9, 1:500 dilution in western analyses; Rabbit monoclonal anti-ErbB4 (E200), Abcam, Catalog no: ab32375, Lot no: GR3869848-2, 1:100 and 1:100 dilution in western and immunofluorescence analyses respectively; Mouse monoclonal anti-ErbB4 (HFR-1), Abcam, Catalog no: ab19391, Lot no: GR155999330-8, 1:50 dilution in immunofluorescence analyses; Rabbit monoclonal anti-Phospho-HER4/ErbB4 (Tyr1284) (21A9), Cell Signaling, Catalog no: #4757, Lot no: 6, 1:500 dilution in western analyses; Rabbit polyclonal anti-GFP, Abcam, Catalog no: ab6556, Lot no: GR3216972-1, 2ug per 1 mg of protein for western analyses.
immunoprecipitation, 1:1000 dilution in western analyses;
Mouse monoclonal anti-HA-Tag (6E2), Cell Signaling, Catalog no: #2367, Lot no: 5, 1:500 dilution in western analyses;
Mouse monoclonal anti-HA (HA-7), Sigma, Catalog no: H3663, Lot no: 092M4827V, 1:200 dilution in immunofluorescence analyses;
Rabbit monoclonal anti-JAK2 (D2E12), Cell Signaling, Catalog no: #3330, Lot no: 8, 1:1000 dilution in western analyses;
Rabbit monoclonal anti-Lamin B1 (D4Q4Z), Cell Signaling, Catalog no: #12586, Lot no: 2, 1:1000 dilution in western analyses;
Goat polyclonal anti-Lamin B (M-20), Santa Cruz, Catalog no: sc-6217, Lot no: J1712, 1:1000 dilution in western analyses;
Rabbit monoclonal p44/42 MAPK (Erk1/2), Cell Signaling, Catalog no: #9102, Lot no: 26, 1:2000 dilution in western analyses;
Rabbit polyclonal P-p44/42 MAPK (T202/Y204), Cell Signaling, Catalog no: #9101, Lot no: 29-30, 1:1000 dilution in western analyses;
Rabbit polyclonal anti-β1-integrin (4B7R), Validated for immunofluorescence at manufacturer's website for human, -actin (AC-74), Validated for Western analysis at manufacturer's website for human and mouse, in Fig. 8B the antibody produced a clear band of correct size in green monkey cells in Western analysis;
Mouse polyclonal anti-Actin (I-19), Validated for Western analysis at manufacturer's website for human and mouse;
Mouse monoclonal anti-β-actin (AC-74), Validated for Western analysis at manufacturer's website for human and mouse;
Mouse monoclonal anti-β1-integrin (k-20), Validated for immunofluorescence at manufacturer’s website for human, immunofluorescence staining pattern similar to the one in manufacturer’s datasheet for green monkey cells
Rabbit monoclonal anti-β4-integrin (D8P6C), Validated for Western analysis at manufacturer’s website for human, In Fig. 4D the antibody produced a clear band of correct size in green monkey cells
Rabbit polyclonal anti-β-Tubulin (H-235), Validated for Western analysis at manufacturer’s website for human and mouse
Mouse monoclonal anti-β-Tubulin (SAP 4G5), Validated for Western analysis at manufacturer’s website for human and mouse, In Fig. 4A,C and S7C the antibody produced a clear band of correct size in green monkey cells
Rabbit polyclonal EGF Receptor Antibody, Validated for Western analysis at manufacturer’s website for human and mouse, In Fig. 5A produced a clear band of correct size in mouse cells
Mouse monoclonal anti-β2- and β3-integrin (9E10), Validated for Western analysis at manufacturer’s website for human and mouse
Rabbit polyclonal anti-β3- and β5-integrin (4H8), Validated for Western analysis at manufacturer’s datasheet for green monkey cells
Mouse monoclonal anti-ErbB4 (HFR-1), Validated for immunoprecipitation and Western analysis at manufacturer’s website for mouse and human
Rabbit monoclonal anti- Phospho-HER4/ErbB4 (Tyr1284) (21A9), Validated for Western analysis at manufacturer’s website for human; In Fig. 5A antibody shows reactivity against phosphorylated murine ErbB4 only after ErbB4 ligand treatment
Rabbit polyclonal anti-HER2/neu, Validated for immunoprecipitation, immunofluorescence and Western analysis at manufacturer’s website for transfected human cells, validated to recognize all variants of Aequorea victoria GFP such as S65T-GFP, RS-GFP, YFP, CFP, RFP and EGFP.
Mouse monoclonal anti-HA-Tag (6E2), Validated for Western analysis at manufacturer’s website for mammalian cells transfected with the HA epitope tag only
Mouse monoclonal anti-HA (HA-7), Validated for Western analysis at manufacturer’s website for mammalian cells transfected with the HA epitope tag only
Rabbit polyclonal anti-JAK2 (D2E12), Validated for Western analysis at manufacturer’s website for human, in Fig. 1E,F the reactivity of the antibody is reduced in cells treated with JAK2 siRNA
Rabbit monoclonal anti-Lamin B1 (D4A4Q), Validated for Western analysis at manufacturer’s website for human and mouse, in Fig. 4D, S6A the antibody produced a clear band of correct size in green-monkey cells in Western analysis
Rabbit monoclonal anti-Lamin B (M-20), Validated for Western analysis at manufacturer’s website for human and mouse
Rabbit polyclonal anti-Myc-Tag, Validated for immunofluorescence at manufacturer’s website for transfected cells
Rabbit monoclonal p44/42 MAPK (Ert1/2), Validated for Western analysis at manufacturer’s website for mouse and human, , In Fig. 5A produced a clear band of correct size in mouse cells
Rabbit polyclonal p-44/42 MAPK (T202/Y204), Validated for Western analysis at manufacturer’s website for mouse and human, In Fig. 5A produced a clear band of correct size in mouse cells
Rabbit monoclonal anti-PDGFRα antibody, Validated for Western analysis at manufacturer’s website for mouse and human, In Fig. 5A produced a clear band of correct size in mouse cells
Rabbit polyclonal anti-PDGFRα antibody, Validated for Western analysis at manufacturer’s website for mouse and human, in Fig. 3D the antibody produced a clear band of correct size that was enriched by immunoprecipitation with a STAT5b antibody
Mouse monoclonal anti-PDGFRα antibody, Validated for Western analysis at manufacturer’s website for mouse and human, In Fig. 5A produced a clear band of correct size in mouse cells
Rabbit monoclonal anti-PDGFRα antibody, Validated for Western analysis at manufacturer’s website for mouse and human, In Fig. 5A produced a clear band of correct size in mouse cells
Mouse monoclonal anti- POL II (8WG16), Validated for Western analysis at manufacturer’s datasheet for human and mouse
Mouse monoclonal anti-Sodium Potassium ATPase (EP1845Y), Validated for Western analysis and immunofluorescence at manufacturer’s website for human and mouse
Mouse monoclonal anti-Stat5a (224H6) (W), Cell Signaling, #9139; Validated for western analysis at manufacturer’s sheet for human. In Fig. 4C produced a band of correct size in green monkey cells that was recognized also with a pSTAT5a antibody
Rabbit monoclonal anti-Phospho-Stat5a (yr705) (D5A7) XP*,Cell Signaling, #9135; Validated for western analysis at manufacturer’s sheet for human. In Fig. 4C produced a band of correct size in green monkey cells that was recognized also with a pSTAT5a antibody
Rabbit monoclonal anti-Phospho-Stat5b (yr694) (14H2), Validated for Western analysis at manufacturer’s website for human and mouse. In Fig. 3D the antibody recognized a clear band of correct size that was enriched by immunoprecipitation with a STAT5b antibody
Rabbit monoclonal anti-Phospho-STAT5 (yr694), Validated for Western analysis at manufacturer’s website for human and mouse, In Fig. 3D, 4A, 4C, S8C and S10A-B the antibody recognized a clear band of correct size that was enriched by immunoprecipitation with STAT5a and STAT5b antibodies and recognized by other STAT5 antibodies in Western analysis in green monkey cell samples
Rabbit monoclonal anti-STAT5a (L-20), Validated for Western analysis, immunoprecipitation and immunofluorescence at manufacturer’s datasheet for human and mouse
Mouse monoclonal anti-STAT5a (C-6), Validated for Western analysis at manufacturer’s website for human and mouse, was validated in house for immunoprecipitation in green monkey samples (Fig. S10A-C) by detecting enrichment of a band that was detected with other STAT5a and pSTAT5a antibodies in Western analysis
Rabbit polyclonal anti-STAT5a (Ab 8700), Validated for Western analysis at manufacturer’s website for human, was validated in house for immunoprecipitation in green monkey samples (Fig. S8C) by detecting enrichment of a band that was detected with other STAT5a and pSTAT5a antibodies in Western analysis
Rabbit monoclonal anti-STAT5a Prestige Antibodies, Validated for Western analysis at manufacturer’s website for human, was validated in house for immunoprecipitation in and green monkey samples (Fig. 4A) by detecting enrichment of a band that was detected with other STAT5a and pSTAT5a antibodies in Western analysis (more data available upon request)
Rabbit polyclonal anti-STAT5 (C-17) (recognizes STAT5b), Validated for Western analysis, immunoprecipitation and immunofluorescence at manufacturer’s datasheet for human and mouse, was validated in house with Western analysis of cells treated with STAT5a- and STAT5b-specific siRNAs to selectively recognize STAT5b (data available upon request), in Fig. 2B,4C,
Eukaryotic cell lines
Policy information about cell lines

Cell line source(s)
- MCF7, European Collection of Cell Cultures
- HC11, a kind gift from Dr. Lars-Arne Haldosén, Karolinska Institutet, Huddinge, Sweden, original commercial source: ATCC
- COS-7, a kind gift from Dr. Lea Sistonen, Turku Bioscience Centre, University of Turku, Turku, Finland, original commercial source: ATCC
- MDA-MB-468, a kind gift from Dr. Johanna Ivaska, Turku Bioscience Centre, University of Turku, Turku, Finland, original commercial source: ATCC
- Phoenix Ampho HEK293 cells, ATCC

Authentication
None of the cell lines used were authenticated

Mycoplasma contamination
Mycoplasma contamination was sporadically tested. Cells that tested positive for mycoplasma contamination were disposed.

Commonly misidentified lines
No commonly misidentified cell lines were used