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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 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 *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	Software built in the microscopes for image acquisition. Numerical simulations were performed on code that was custom written in C++. Source codes are available in Github (https://github.com/zenah12/DppTrafficking-/blob/main/README.md).
Data analysis	Image analysis has been performed using Image j (version 2.0.0-rc-69/1.52p). Spatial gradient analysis performed using Matlab R2019b (9.7.0.1216025). Simulated data and nanobody recovery experimental data was analyzed using Wolfram Mathematica 12.1.1.0. Also used Excel version 16.36 and Prism 9 version 9.3.0.

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

Source data are provided with this paper. Datasets generated during the parameter estimation are available in Github (https://github.com/zenah12/DppTrafficking-/blob/main/README.md).

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	Decay length assay, extracellular fraction assay: Sample sizes were chosen to ensure narrow confidence intervals of fitted variables (with mean/sem > 10) and a high determination coefficient value. Nanobody assay: we chose a sample size for the nanobody experiment that yields sem for each point in the dynamics curve that is substantially smaller than the mean measurement (with mean/sem > 10). FRAP: for the FRAP experiments we progressively increased the sample size until the quality of the fit to a simple FRAP recovery curve described by an effective diffusion and effective degradation no longer improved with additional samples, and similarly the estimates for the effective diffusion and degradation no longer moved when additional samples were incuded (see Extended Data Figure 4c,d and the supplementary information chapter 2.5 for FRAP assay). iFRAP assay: We used a number of samples that yield a mean recovery that was substantially smaller than the sem. Timer assays: We used a number of samples that yield a mean age ratio that was substantially smaller than the sem. The sample size for iFRAP and timer experiments were sufficient to perform the necessary statistical test and the SEM was much smaller than the mean. Note that the sample sizes chosen are equal or above the typical sample sizes used in the field of developmental biology. For the clones experiment in Figure 4a,b, the experiment has been performed in triplicate and a representative image has been chosen for illustration.
Data exclusions	No data exclusion
Replication	For each assay and condition, experiments were replicated independently, in wing discs from different flies at the same developmental stage and of the same genetic background.
Randomization	Flies of the same genetic background were kept separate and each experiment was carried out on discs obtained from randomly chosen fly larvae of the appropriate genetic background and developmental stage. Our study does not explore the impact of different treatments on subjects, nor did it require sampling individuals that belong to different groups from large populations. As such randomization is not strictly relevant to our analysis.
Blinding	Our analysis did not involve quantifying the impact of treatments on different groups, as such blinding was not necessary. Quantification was performed using the same programing scripts for all samples. Furthermore, the quantifications in all experiments were performed at the absence of information regarding the genetic background or developmental stage of samples.

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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used	-Drosophila Ptc (Apa 1) was deposited to the DSHB by Guerrero, I. (DSHB Hybridoma Product Drosophila Ptc (Apa 1)) . -Rabbit-anti-P-Mad (PS-1) (from Tanimoto H, Itoh S, ten Dijke P, Tabata T. Hedgehog creates a gradient of DPP activity in Drosophila wing imaginal discs. Mol Cell. 2000 Jan;5(1):59-71. doi: 10.1016/s1097-2765(00)80403-7. PMID: 10678169.)

-mouse-anti-GFP antibodies (Santa Cruz # sc-9996)
-mouse-anti-GFP antibodies (clones 7.1 and 13.1) (Roche Cat. No. 11 814 460 001)

Validation

-Drosophila Ptc (Apa 1) was deposited to the DSHB by Guerrero, I. (DSHB Hybridoma Product Drosophila Ptc (Apa 1)) : verified in Drosophila embryos in Capdevila J, Pariente F, Sampedro J, Alonso JL, Guerrero I. Subcellular localization of the segment polarity protein patched suggests an interaction with the wingless reception complex in Drosophila embryos. Development. 1994 Apr;120(4):987-98. PMID: 7600973. Additionally verified in Drosophila wing discs in Capdevila J, Estrada MP, Sánchez-Herrero E, Guerrero I. The Drosophila segment polarity gene patched interacts with decapentaplegic in wing development. EMBO J. 1994 Jan 1;13(1):71-82. PMID: 8306973; PMCID: PMC394780.

-Rabbit-anti-P-Mad (PS-1) : this antibody has been verified in Drosophila wing discs to specifically detect phosphorylated MAD as indicator for Dpp signaling activity (see Tanimoto H, Itoh S, ten Dijke P, Tabata T. Hedgehog creates a gradient of DPP activity in Drosophila wing imaginal discs. Mol Cell. 2000 Jan;5(1):59-71. doi: 10.1016/s1097-2765(00)80403-7. PMID: 10678169.)

-mouse-anti-GFP antibodies (Santa Cruz # sc-9996) : cited in 2,892 publications, recommended for western blot and immunoprecipitation. Chosen citations: 1. Hiscox, S., et al. 2002. GPI-anchored GFP signals Ca2+ but is homoge- neously distributed on the cell surface. Biochem. Biophys. Res. Commun. 293: 714-721.
2. Ronkina, N., et al. 2015. Comparative analysis of two gene-targeting approaches challenges the tumor-suppressive role of the protein kinase MK5/PRAK. PLoS ONE 10: e0136138.
3. Kim, S.H., et al. 2016. Tunable regulation of CREB DNA binding activity couples genotoxic stress response and metabolism. Nucleic Acids Res. 44: 9667-9680.
4. Guo, X., et al. 2017. VCP cooperates with UBXD1 to degrade mitochondrial outer membrane protein MCL1 in model of Huntington's disease. Biochim. Biophys. Acta 1863: 552-559.
5. Mahpour, A., et al. 2018. A methyl-sensitive element induces bidirectional transcription in TATA-less CpG island-associated promoters. PLoS ONE 13: e0205608.
6. Sharma, M. and Subramaniam, S. 2019. Rhes travels from cell to cell and transports Huntington disease protein via TNT-like protrusion. J. Cell Biol. 218: 1972-1993.
7. Kwon, Y., et al. 2020. bPix-d promotes tubulin acetylation and neurite outgrowth through a PAK/Stathmin1 signaling pathway. PLoS ONE 15: e0230814.
8. Kim, B., et al. 2021. The trehalose-6-phosphate phosphatase Tps2 regu- lates ATG8 transcription and autophagy in Saccharomyces cerevisiae. Autophagy 17: 1013-1027.

-mouse-anti-GFP antibodies (clones 7.1 and 13.1) (Roche Cat. No. 11 814 460 001) : from supplier's website: "Anti-GFP is tested for functionality and purity relative to a reference standard to confirm the quality of each new reagent preparation.
Purity: Both Anti-GFP mouse monoclonal antibodies (Clones 7.1 and 13.1) are >95% pure as determined by SDS-PAGE and ion-exchange HPLC analyses."
Choisen citations: Nature Communications (2021)
BRCA2 binding through a cryptic repeated motif to HSF2BP oligomers does not impact meiotic recombination
Rania Ghouil Et Al.
Nature Microbiology (2021)
CrvA and CrvB form a curvature-inducing module sufficient to induce cell-shape complexity in Gram-negative bacteria
Nicholas R. Martin Et Al.
Nature Communications (2021)
Pathogen effector recognition-dependent association of NRG1 with EDS1 and SAG101 in TNL receptor immunity
Xinhua Sun Et Al.
Proteasomal degradation of the tumour suppressor FBW7 requires branched ubiquitylation by TRIP12
Omar M. Khan Et Al.
Nature Communications (2021)
STIM-Orai1 signaling regulates fluidity of cytoplasm during membrane blebbing
Kana Aoki Et Al.
Nature Communications (2020)
Molecular variation in a functionally divergent homolog of FCA regulates flowering time in Arabidopsis thaliana
Yunhe Wang Et Al.
Nature Communications (2020)
CHD7 and 53BP1 regulate distinct pathways for the re-ligation of DNA double-strand breaks
Magdalena B. Rother Et Al.
Nature Structural & Molecular Biology (2020)
Atg9 is a lipid scramblase that mediates autophagosomal membrane expansion
Kazuaki Matoba Et Al.
Nature Communications (2020)
PilY1 and minor pilins form a complex priming the type IVa pilus in Myxococcus xanthus
Anke Treuner-Lange Et Al.
Nature Plants (2020)
ARMADILLO REPEAT ONLY proteins confine Rho GTPase signalling to polar growth sites
Ivan Kulich Et Al.
Nature Communications (2020)
TMEM16K is an interorganelle regulator of endosomal sorting
Maja Petkovic Et Al.
Nature Communications (2020)
The netrin receptor UNC-40/DCC assembles a postsynaptic scaffold and sets the synaptic content of GABAA receptors
Xin Zhou Et Al.
Nature Communications (2020)
Pan-active imidazolopiperazine antimalarials target the Plasmodium falciparum intracellular secretory pathway
Gregory M. LaMonte Et Al.
Nature Communications (2020)

Plant Raf-like kinases regulate the mRNA population upstream of ABA-unresponsive SnRK2 kinases under drought stress
Fumiyuki Soma Et Al.
Nature Communications (2020)
UBB pseudogene 4 encodes functional ubiquitin variants
Marie-Line Dubois Et Al.
Nature metabolism (2020)
mTORC1 directly inhibits AMPK to promote cell proliferation under nutrient stress
Naomi Ling Et Al.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Drosophila Melanogaster. All experiments have been performed on wing discs of larvae of ages specified in the figure legends and of random sex (males and females). The genotypes used are specified in Supplementary Tables 1 and 2.
Wild animals	the study did not involve wild animals
Field-collected samples	the study did not involve samples collected from the field
Ethics oversight	no ethical approval or guidance was required

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