

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
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	cisTarget v8 motif collection mc8nr Drosophila reference genome (dmel_r6.19) scRNA-seq dataset for reference mapping: Rust et al., 2020 Nat Commun, DOI: 10.1038/s41467-021-26191-1 All scripts are available from the github repository https://github.com/KatjaRM/Anschoetz-SCS-Hh-and-EGFR-Ras and under https://doi.org/10.5281/zenodo.18611259 . Raw data files of generated scRNA-seq datasets can be accessed under the GEO accession numbers GSE291056 and GSE290241. The reference dataset used for multimodal reference mapping is accessible under GSE136162. Processed Seurat and Monocle3 files can be downloaded from Mendeley Data under https://doi.org/10.17632/dw9fhy3gnv.1 .
Data analysis	R 2022.07 STAR v2.5.1b STARSolo Seurat 5.3.1 Monocle3 1.4.26 SCENIC 1.3.1 miloR BioC 3.22

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](#)

Dataset 1 (duplicates of single pathway activations with four WT controls) is available under the GEO accession number GSE291056. Dataset 2 (HhOE with either EGFRCA or RasV12 in duplicates with two WT controls) is available under GSE290241. Raw data associated with quantifications is available from the Source Data file. The processed single-cell RNA-sequencing datasets are available upon request. Reference mapping was performed using the reference atlas dataset available under GSE136162.

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	No patient data was used in this study , all data were retrieved from female <i>Drosophila melanogaster</i>
Reporting on race, ethnicity, or other socially relevant groupings	No patient data was used in this study
Population characteristics	No patient data was used in this study
Recruitment	No patient data was used in this study
Ethics oversight	Not required

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	Cell numbers for single cell datasets were chosen to be based on previous dataset sizes and designed to cover each cell population of interest. For statistical quantifications we chose a minimum of n = 5. For penetrance quantifications we chose a minimum of 20 from at least three different organisms.
Data exclusions	We excluded low quality cells from our single-cell analysis based on standard parameters and excluded cells with less than 200 detected genes or more than 5% mitochondrial reads. We further excluded genes with less than three reads.
Replication	For in vivo analysis we performed at least three replicates representing at least 15 organisms. For single-cell dataset preparation we performed duplicates from 60 ovarioles each.
Randomization	No randomization was performed.
Blinding	No blinding was performed. Phenotypes studied in this work are easily recognizable, making blinding dispensable.

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

Methods

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

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

Antibodies

Antibodies used

DSHB: mouse anti-Fas3 (7G10, 1:100), mouse anti-Aop (8B12H9, 1:100), mouse anti-LamC (LC28.26, 1:100), mouse anti-Eya (10H6, 1:50), rat anti-ECad (DCAD2, 1:100), mouse anti-Dlg1 (DLG1, 1:100).
 rabbit anti-GFP D5.1 (Cell Signaling #2956, 1:1000), rabbit anti-dpERK (Cell Signaling #4370, 1:100), rat anti-Dpn (abcam ab195173, 1:100), rabbit anti-Bin (gift from M. Frasch, 1:200), rabbit anti-Zfh1 (gift from J. Skeath, 1:500), rabbit anti-Cas (gift from W. Odenwald, 1:1000), mouse anti-GFP JL-8 (Takara Bio 632380, 1:1000), rabbit anti-pH3 (Sigma-Aldrich #06-570, 1:500), rabbit anti-Baz (gift from A. Wodarz, 1:1000).
 Secondary antibodies (Invitrogen): goat anti-mouse 488 (A-11029), goat anti-mouse 568 (A-11031), goat anti-rabbit 488 (A-11034), goat anti-rabbit 568 (A-11036), goat anti-rat 488 (A-11073), goat anti-rat 568 (A-11077), goat anti-rat 647 (A-21247).

Validation

mouse anti-Fas3: DOI 10.1016/j.stem.2007.07.009
 mouse anti-Aop: doi: 10.1016/j.mod.2007.05.002.
 mouse anti-LamC: <https://doi.org/10.1371/journal.pgen.1006790>
 mouse anti-Eya: doi:10.1242/dev.00115
 rat anti-ECad: DOI: 10.1016/j.ydbio.2009.11.027
 mouse anti-Dlg1: DOI: 10.1016/j.ydbio.2009.11.027
 rabbit anti-GFP D5.1: Validation by Western Blot analysis and Immunohistochemistry.
 rabbit anti-dpERK: doi:10.1242/dev.168716
 rat anti-Dpn: Validation by immunohistochemistry. doi: 10.1073/pnas.1602739113.
 rabbit anti-Bin: doi: 10.1101/gad.917101
 rabbit anti-Zfh1: doi: 10.1016/j.stem.2008.05.001
 rabbit anti-Cas: doi: 10.1073/pnas.1300725110.
 mouse anti-GFP JL-8: doi: 10.1369/jhc.2008.951137.
 rabbit anti-pH3: Validated by immunostaining. DOI: 10.15252/embj.201798659
 rabbit anti-Baz: DOI: 10.1038/990128

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

Adult Drosophila melanogaster

Wild animals

No wild animals were used in this study.

Reporting on sex

We used female animals only

Field-collected samples

Not applicable

Ethics oversight

No ethics oversight is required for experimental work using Drosophila melanogaster

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

Plants

Seed stocks

No plants were used in this study.

Novel plant genotypes

No plants were used in this study.

Authentication

No plants were used in this study.