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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 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<br>*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<br>*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 | Raw spectral data were processed using Proteome Discoverer 2.1.0.81 (Thermo). |
| Data analysis | MS2 spectra were identified using the Sequest HT node, searching against the Human Uniprot database (downloaded: 5/11/2017) with the zebrafish Fzd9b sequence appended. False discovery rate (FDR) estimation was performed using a reverse decoy database. Search parameters were as follows. Mass tolerances were set to 50 ppm and 0.6 Da for MS1 and MS2 scans, respectively. Full trypsin digestion was specified with a maximum of two missed cleavages per peptide. Modifications included static 10-plex TMT tags on peptide n-termini and lysine, static carbamidomethylation of cysteine and variable oxidation of methionine. Data were filtered to a 1% FDR at both the peptide and protein level.<br><br>The intensities of TMT reporter ions were extracted from the MS3 scans for quantitative analysis. Prior to quantitation, spectra were filtered to have an average signal to noise of 10 across all labels and an isolation interference less than 25%. Data were normalized in a two-step process by normalizing each protein the pooled bridge channel value and then normalizing to the median of each reporter ion channel and the entire dataset.<br><br>Immunoblot intensities from pulldowns were quantified by densitometry using ImageJ 1.46, normalized to input controls.<br><br>GraphPad Prism 7.04 was used for statistical analyses.<br><br>Gene ontology of the significant proteins was performed using the database for annotation, visualization and integrated discovery (DAVID version 6.8) server. |

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/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

Mass spectrometry data have been deposited in ProteomeXchange with the primary accession code PXD010649 through MassIVE (MSV000082677). Source data for main and supplementary figures have been provided as Supplementary Table 3.
Previously published sequencing data that were re-analysed here are available from the European Nucleotide Archive under the accession number PRJEB4197 https://www.ebi.ac.uk/ena.
All other data supporting the findings of this study are available from the corresponding author on reasonable request.
Summary data is seen in Extended data Figs. 6e, 6f, 7d, 7e.

# 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 | Based on preliminary studies, we determined that examining 5-10 fish and n=3-6 for luciferase assays are sufficient for at least 95% statistical power. |
| Data exclusions | Samples were only excluded as outliers in the case of obvious error in instrument detection, or transfection failure. The exclusion criteria was pre-established as a zero-read on the instrument, or transfection efficiency lower than 20%, as measured by GFP+cells/total cells. Zebrafish cell counts were only excluded in case of staining failure. |
| Replication | To ensure reproducibility, all experiments were conducted with multiple samples, and reproduced successfully with a similar trend at least once. Replicates of luciferase assays that did not have sufficient transfection efficiency (more than 20%) did not signal. See figure legends for specific details. |
| Randomization | Zebrafish offspring were pooled from several clutches and separated into control or experimental groups at random for injection experiments. In the case of genetic experiments, randomization was not possible, but blinding (see below) was carried out. |
| Blinding | For zebrafish genetic experiments, heterozygous animals were crossed, and clutchmates were genotyped after experimental analysis (blinded). For MO injected fish, blinding was not performed. In luciferase reporter experiments, data was collected and analyzed blindly and labeled post analysis. |

# 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 | Fzd9b (1:1000, in house, rabbit polyclonal), Wnt9a (1:1000, in house, rabbit polyclonal), V5 (1:5000, genetex, mouse monoclonal |

clone GT1071, cat#GTX628529 lot 41288), phospho-Y (1:1000, BioLegend, mouse monoclonal Clone PY20, cat#309301 lot B232939), LRP6 (1:2000, cell signaling, rabbit monoclonal C5C7, cat#2560S lot 11), B-actin (1:20,000, mouse monoclonal C47E12, Sigma, cat#A2228-100UL lot 058M4808), EGFR (1:1000, abcam, rabbit monoclonal EP38Y, cat#ab52894), HBEGF (500ng/mL, R&D systems, cat#AF-259-SP lot PX101812A)

Validation

in house antibodies were validated against transfected HEK293T cell lysate.

manufacturer validations:
v5: V5-tagged-NR0B1-transfected 293T western blot
LRP6: Western blot analysis of total cell lysates from HepG2, HeLa and Rat2 cells using LRP6 (C5C7) Rabbit mAb.
pTyr, actin: Each lot is quality tested in Western blot.
EGFR: western blot using A431 (human squamous carcinoma) lysate which naturally overexpresses the EGFR protein.
HBEGF: Measured by its ability to neutralize HB-EGF-induced proliferation in the Balb/3T3 mouse embryonic fibroblast cell line.

## Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

WiCell (H9), ATCC (Chinese Hamster Ovary, HEK293T)

Authentication

None of the cell lines used were authenticated.

Mycoplasma contamination

All cell lines tested negative for mycoplasma contamination, with the exception of 293T TOP:FLASH reporter cells, which tested positive. This was controlled for by using the parental cells as the control group with matched levels of transfected DNA.

Commonly misidentified lines
(See ICLAC register)

HEK293 cells are known to have potential cross-contamination with HeLa cells. This was irrelevant to our study, as these cells were used only as a reporter of Wnt activity, where any cell line would be used.

## Animals and other organisms

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

Laboratory animals

Zebrafish (Danio Rerio) were used; wild-type strain AB*, Tg(kdrl:Cherry-CAAX)y171, Tg(fli1a:eGFP)zf544, Tg(cdh5:Gal4)mu101, Tg(UAS:CA-β-catenin)sd47Tg Tg(gata2b:KalTA4sd32; UAS:Lifeact:eGFPmu271), wnt9ad28/d28,sd49, Tg(UAS:Cre)t32240Tg Tg(bactin2:loxP-BFP-loxP-DsRed)sd27.

Adult fish were mated between 3-15 months of age. Offspring were generated with a male x female cross; the sex of zebrafish embryos is not readily detectable, but a 50:50 distribution is assumed. Offspring ages are noted in each figure.

Wild animals

This study did not involve wild animals.

Field-collected samples

The study did not involve samples collected from the field.

Ethics oversight

Zebrafish were maintained and propagated according to University of California and local Institutional Animal Care and Use Committee (IACUC) policies.

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

## 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

cells were harvested with Accutase, pelleted, resuspended in PBS with 1% BSA and 1mM EDTA, filtered through an 80um filter and sorted using a BD Fortessa flow cytometer.

Instrument

BD Fortessa LSR II

Software

Flow Jo (X.0.7) and FACS Diva (6)

| | |
|---|---|
| Cell population abundance | a minimum of 10,000 cells per sample were analyzed. |
| Gating strategy | Gates were set to unstained cells with no primary antibody present. All gates were compared to control samples within the same experiment, using the same gates. Gating strategy is found in extended data figures 3g-i. |

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