

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](#).

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
- ☐ ☒ An indication of 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 statistics 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
- ☐ ☒ Clearly defined error bars
State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on [statistics for biologists](#) may be useful.

Software and code

Policy information about [availability of computer code](#)

Data collection

Volocity (PerkinElmer) v6.3.1
BD FACSDiva 7
Bruker NanoScope v1.5 (AFM)

Data analysis

ImageJ v1.6.0 (masking cells)
Matlab v R2015a (TFM)
TFM software (open source, available from the Danuser lab website: <https://www.utsouthwestern.edu/labs/danuser/software/>)
Graphpad Prism v 7.01 (statistical analysis)
Bruker NanoScope Analysis v1.5 (AFM)
R and Rstudio v 3.3.3 and v 1.0.143 (statistical analysis)
Samtools (version 1.2)
Gencode (version 22)
Novoalign
miRBase (release 21)
TargetScan
Bioconductor Library with simplyaffy and limma packages
Progenesis QI (Nonlinear Dynamics)
Mascot (Matrix Science UK) with Swiss Prot and TrEMBL mouse databases

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 upon request. 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

The accession number for all the sequence reads reported in this paper are:

HITS-CLIP in Sequence Read Archive (SRA) under accession code PRJNA507245;

RNA-seq and sRNA-seq for HUVEC cells at 3 kPa and 30 kPa in the Gene Expression Omnibus (GEO) under accession codes GSE99686 and GSE11021;

sRNA-seq for HDF cells at 3 kPa and 30 kPa in the Gene Expression Omnibus (GEO) under accession codes GSE123008;

Proteomics results are reported as excel file Supplementary_table2.

All data is available with no restrictions. Source data plotted in figures can be found in supplemental table 8.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf](https://www.nature.com/authors/policies/ReportingSummary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

For fixed cell culture based assays, minimum sample size was chosen based on previously acquired data sets using power analysis so that a 10% change (in area, YAP, FA#) could be detected with 95% confidence. For live cell TFM experiments (n=15), sample size was chosen to allow for detection of a 30% change in total force with 95% confidence. For AFM measurements of stiffness, minimum sample size (n=25) was chosen based on previous tissue stiffness measurements (bovine meniscus) to allow for detection of a 20% difference in stiffness with 95% confidence. For Mass spec and HITS-CLIP, sample size was chosen to be 3 based on previously published data. For MRE-Sensor Seq, cell number collected for each bin was chosen so that enough material could be collected for illumina sequencing.

Data exclusions

No data was excluded

Replication

Once the protocols were established, all attempts of replication were successfully reproduced at least once and in most cases more than once (stated in methods and figure legends).

Randomization

Each cell culture experiment was performed by starting with a single population of cells that was randomly split after trypsinization into each experimental group. Fixed cell images were acquired by taking many (20-30) random fields of view. Fish experiments were performed on fish of each genotype, and for rescue experiments some fish from the AGO background were randomly chosen to be injected with human AGO for rescue.

Blinding

Authors were not blinded.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants

Methods

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

Antibodies

Antibodies used

Antibodies Used in Cultured Cells
 YAP (sc-10119, Santa Cruz, 1:200 IF)
 panAGO-2A8 antibody (MABE56, Millipore, 1:100 IP)
 AGO2 (2897, Cell Signaling, 1:200 IF, 1:2000 WB)
 Paxillin (ab32084, Abcam, 1:800 IF)
 RhoB (sc-8048, SantaCruz, 1:250 IF, 1:200 WB)
 CTGF (ab6992, Abcam, 1:200 IF, 1:1000 WB)
 Vinculin (V9131, Sigma-Aldrich, 1:200 IF, 1:2500 WB),
 STMN1 (ab52630, Abcam, 1:200 IF, 1:10000 WB)
 DROSHA (ab183732, Abcam, 1:5000 WB)
 GAPDH (2118, Cell Signaling, 1:4000 WB)
 Beta-Actin (sc-47778, SantaCruz, 1:2000 WB)
 anti-rabbit-HRP (7076P2, Cell Signaling, 1:4000 WB)
 anti-mouse-HRP (7074S, Cell Signaling, 1:4000 WB)
 Alexa-488 anti-rabbit (A-11008, ThermoFisher, 1:1000 IF)
 Alexa-647 anti-mouse (A-21236, ThermoFisher, 1:1000 IF)

Antibodies Used in 3D Constructs
 Ago2 (2897, Cell Signaling, 1:2000 WB)
 beta-actin (ab8227, Abcam, 1:5000 WB)
 Vimentin (2897, Cell Signaling, 1:400 IF)
 phospho-Myosin light chain (ab2480, Abcam, 1:400 IF)
 Alexafluor-680 anti-mouse (A-21058, ThermoFisher, 1:15000 WB)
 Alexafluor-800 anti-rabbit (A32735, ThermoFisher, 1:15000 WB)
 Alexa-647 anti-rabbit (A-21245, ThermoFisher, 1:500)

Antibodies Used in Zebrafish
 mouse anti-Phospho-Myosin Light Chain 2 (3671; Cell Signaling, 1:200 IF)
 mouse anti-Proliferating Cell Nuclear Antigen PCNA (clone PC10, M0879, Dako, 1:200 IF)
 rabbit anti-Fibronectin (F3648, Sigma, 1:200 IF)
 rabbit anti-Connective Tissue Growth Factor A (ab6992, Abcam, 1:150 IF)
 mouse anti-YAP (sc-10119, Santa Cruz Biotechnology, 1:200 IF)
 Alexa Fluor 488 anti-mouse (A-11034, ThermoFisher, 1:250 IF)
 Alexa Fluor 596 anti-rabbit (A-11037, ThermoFisher, 1:250 IF)

Validation

YAP antibody was purchased from Santa Cruz (sc-10119) and previously validated for IHC (Dupont, Nature 2011). Paxillin antibody [Y113] was purchased from abcam (ab32084) and has been extensively used and verified in the focal adhesion literature. panAGO-2A8 antibody was purchased from Millipore and validate per HITS-CLIP (Moore, Nature Protocols, 2014), more information are available from antibodypedia: <https://antibodypedia.com/gene/27626/AGO2/antibody/554013/MABE56>. AGO2 antibody was purchased from Cell Signaling(2897) and has been extensively used and verified: <https://antibodypedia.com/gene/27626/AGO2/antibody/106152/2897>. Vimentin antibody was purchased from Cell Signaling (3877) and has been extensively used and verified

The RhoB, CTGF, Vinculin, STMN1, DROSHA, GAPDH, beta-Actin, Vimentin and Myosin light chain antibodies were verified by appropriate band size with western blot.

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)

293tx cells were purchased from Clontech
 Human Dermal Fibroblasts were purchased from ATCC (PCS-201-010, Lot#63014910)
 Human Umbilical Vein Endothelial Cells were purchased from Cell Applications Inc (200-05n, Lot#3051)
 Human Umbilical Arteries Endothelial Cells were purchased from Cell Applications Inc (202-05n)

Authentication

Human Dermal Fibroblasts were isolated from human neonatal foreskin tissue samples based on attachment and outgrowth on tissue culture plastic.

HUVECs were verified by Cell applications Inc to express VEGFR2, stain for EC-specific Dil-Ac-LDL and form vessel-like structures when cultured with HDF in the presence of VEGF.
HUAECs were verified by Cell applications Inc to express Factor VIII-related antigen and for EC-specific Dil-Ac-LDL and from the ability to attach and spread on tissue culture ware surface, and proliferate in Endothelial Cell Growth Medium.

Mycoplasma contamination

Yes, cells were tested and found to not have mycoplasma contamination.

Commonly misidentified lines
(See [ICLAC](#) register)

No commonly misidentified cell lines were used in this study.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals

Wild-type (AB) Danio rario animals and ago2 mutant Danio rerio animals (Cifuentes, Science 2010) were raised and maintained at 28.5°C using standard methods and according to protocols approved by Yale University Institutional Animal Care and Use Committee (# 2015-11473)

Wild animals

No wild animals were used in this study.

Field-collected samples

No field collected samples were used in this study.

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 trypsinized from surfaces, washed and resuspended in PBS without Calcium or Magnesium.

Instrument

BD FACSAria II

Software

BD FACSDIVA 7

Cell population abundance

To minimize the possibility of cells expressing multiple MRE-Sensors, cells were infected to achieve 10-20% of the population as GFP/RFP positive.

Gating strategy

FCS/SSC gate was set for single cells to exclude cell debris and doublets. GFP and RFP positive gates and compensation settings were determined using cells infected with only GFP or only RFP. Four sorting gates were set based on the 2 control plasmids (Empty-Sensor and miR125-Sensor). The upper limit bin (Not Suppressed) was designed to contain 90% or more events/cells infected with the Empty-Sensor and less than 0.5% of events for the miR125-Sensor. Vice versa, the lower bins (Strongly Suppressed and Suppressed) were designed to contain 90% of events coming from cells infected with miR125-Sensor, in a ratio close to 3:2 (~60% of events in Strongly Suppressed bin and ~40% of events in Suppressed). The 3th bin (Mildly Suppressed) represent the conjunction between the Not Suppressed and the Suppressed bins and derive for subtraction between the two bins. For clarity, the contour plot represents the total percentage of event in each single bins, grouped in "island" of 15% probability were shown for the Empty-Sensor, miR125-Sensor, Sensor-Library at 3 kPa and Sensor-Library at 30 kPa.

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