MicroRNA-dependent regulation of biomechanical genes establishes tissue stiffness homeostasis

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Vertebrate tissues exhibit mechanical homeostasis, showing stable stiffness and tension over time and recovery after changes in mechanical stress. However, the regulatory pathways that mediate these effects are unknown. A comprehensive identification of Argonouate 2-associated microRNAs and mRNAs in endothelial cells identified a network of 122 microRNA families that target 73 mRNAs encoding cytoskeletal, contractile, adhesive and extracellular matrix (CAM) proteins. The level of these microRNAs increased in cells plated on stiff versus soft substrates, consistent with homeostasis, and suppressed targets via microRNA recognition elements within the 3′ untranslated regions (3′UTRs) of CAM mRNAs. Inhibition of DROSHA or Argonaute 2, or disruption of microRNA recognition elements within individual target mRNAs, such as connective tissue growth factor, induced hyper-adhesive, hyper-contractile phenotypes in endothelial and fibroblast cells in vitro, and increased tissue stiffness, contractility and extracellular matrix deposition in the zebrafish fin fold in vivo. Thus, a network of microRNAs buffers CAM expression to mediate tissue mechanical homeostasis.

Cells sense physical forces, including the stiffness of their extracellular matrix (ECM), through mechanosensitive integrins, their associated proteins and actomyosin. These components transduce physical forces into biochemical signals that regulate gene expression and cell function$^{1-11}$. Tissues maintain nearly constant physical properties in the face of growth, injury, ECM turnover and altered external forces (for example, from blood pressure, tissue hydration or body weight)$^{1,4,5}$. These effects imply tissue mechanical homeostasis, in which cells sense mechanical loads, due to both external and internal forces, and adjust their rates of matrix synthesis, degradation and organization to keep tissue properties constant. Cell contractility is critical in this process, as it is a key component of both the stiffness-sensing regulatory pathways and the matrix assembly process that governs resultant matrix properties, including stiffness$^{2,6}$.

Mechanical homeostasis requires that integrin mechanotransduction pathways mediate negative-feedback regulation of the contractile and biosynthetic pathways to maintain optimal tissue stiffness. That is, too soft or low force triggers increased matrix synthesis and contractility, whereas too stiff or high force triggers the opposite. However, in vitro studies have mainly elucidated positive-feedback (or feed forward) circuits, in which rigid substrates or high external forces increase actomyosin contraction, focal adhesions and ECM synthesis$^7$. This type of mechanotransduction signalling characterizes fibrotic tissues, in which sustained contractility and excessive ECM compromise tissue function. Very little is known about negative-feedback pathways that are critical to establish proper stiffness or contractility in normal, healthy tissues.

miRNAs (miRNAs) are processed via the ribonucleases DROSHA/DGCR8 and DICER$^8$ into mature 20–21 nucleotide (nt) RNA that recognize abundant and conserved 7–8 nt miRNA responsive elements (MREs) within mRNAs. MREs reside mainly in the 3′ untranslated regions (3′UTRs) of mRNAs and base pair with the 5′ miRNA mature sequence (SEED region)$^9$. The miRNA–MRE pairs are recognized by the Argonaute 2 (AGO2) protein complex, resulting in mRNA destabilization and/or reduced protein expression$^{10}$. Thus, miRNAs can buffer fluctuations in protein levels caused by changes in transcriptional inputs or extracellular factors.

Although miRNAs participate in regulatory feedback loops that contribute to homeostasis in multiple contexts$^{10,11}$, their role in mechanical homeostasis is currently untested. Here, we describe a miRNA–CAM (cytoskeletal–actin–matrix) mRNA regulatory network that counteracts the effects of the ECM stiffness to promote the mechanical stability of cells and tissues, in both in vitro and in vivo models.

Results

miRNAs preferentially bind to CAM 3′UTRs. To investigate potential roles for miRNAs in mechanical homeostasis, we analysed miRNA–mRNA interactions transcriptome wide using an AGO2-HITS-CLIP (high-throughput sequencing of RNA isolated by cross-linking immunoprecipitation) approach$^{12}$. AGO2-bound miRNAs

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or mRNAs were isolated from two unrelated human endothelial cell types, which are known to respond to mechanical forces, including ECM loads\textsuperscript{14}. We exposed cultured human umbilical artery endothelial cells (HUVECs) and human umbilical vein endothelial cells (HUVECs) to UV light to cross-link protein–RNA complexes. Subsequently, we immunoprecipitated AGO2–RNA complexes, digested unbound RNA (schematic in Fig. 1a) and prepared complementary DNA libraries containing small (∼30 nt AGO2–miRNA) and large RNAs (∼70 nt AGO2–target mRNA) (Supplementary Fig. 1a). To identify conserved AGO2-binding sites, we performed high-throughput sequencing of three libraries for each cell type and selected sequence reads shared in all six samples. We aligned these AGO2-binding sites to human miRNA and genome databases and identified 30–70-nt intervals (peaks) significantly enriched above background (P < 0.05; Supplementary Fig. 1a and Methods). This analysis uncovered 316 AGO2-binding peaks within the 3′ UTRs of 127 human genes. These peaks were preferentially located right after the stop codon or right before the polyadenylation site (Fig. 1b and Supplementary Table 1), consistent with the enrichment of regulatory miRNA-binding sites that destabilize miRNAs\textsuperscript{15}. Importantly, the human AGO2-binding peaks within these 30–70-nt sequences were highly conserved across hundreds of species (Fig. 1b), suggesting functional importance.

Gene Ontology (GO) analysis of AGO2-bound transcripts revealed that 73 of the 127 target mRNAs encode actin- and microtubule-associated proteins, focal adhesion proteins, ECM proteins and functionally related regulatory proteins (Fig. 1c and Supplementary Fig. 1b). We termed this group the CAM genes. The dramatic enrichment of CAM transcripts in the AGO2 complex is not accounted for by their abundance; indeed, the most transcriptionally active genes in cultured endothelial cells pertained to cell division (Supplementary Fig. 1b), which were under-represented in the identified AGO2-binding transcripts. No significant GO terms were associated with the remaining genes identified from the AGO2-HITS-CLIP.

We then searched for specific MRE sequences in AGO2 peaks localized in the 3′ UTRs of the CAM transcripts. We identified 122 miRNA families from AGO2-HITS-CLIP (Supplementary Table 2) that recognize one or more AGO2–CAM MREs (Fig. 1c and Supplementary Table 3). Cytoscape software revealed a highly interconnected network of miRNAs binding to CAM transcripts (Fig. 1c). Altogether, these data reveal pervasive miRNA-mediated post-transcriptional regulation of multiple CAM genes in endothelial cells.

Post-transcriptional regulation of CAM genes is sensitive to matrix stiffness. CAM proteins are highly conserved and play crucial roles in virtually every cell type as determinants of ECM organization and tissue stiffness\textsuperscript{16}. This important function led us to hypothesize that the CAM mRNA–miRNA regulome is mechanosensitive. To test this, we plated endothelial cells on substrates of varying stiffness and used a Sensor-seq strategy\textsuperscript{27} to assess post-transcriptional regulation mediated by 97 selected MREs within 51 different CAM 3′ UTRs (Supplementary Table 4). For this purpose, we created a ‘CAM sensor library’. Each AGO2–3′ UTR peak containing at least one MRE was cloned downstream of an mCherry reporter in a bidirectional lentiviral vector\textsuperscript{18} that co-expressed a green fluorescent protein (GFP) transcript lacking a 3′ UTR (schematic in Fig. 2a). Thus, miRNAs that target the MRE reduce mCherry levels, leading to a decreased mCherry/GFP ratio. Endothelial cells infected with this CAM sensor library at low levels (to avoid multiply infected cells) were seeded for 48 h on substrates with a rigidity of 3 kPa or 30 kPa, which approximate ‘soft’ and ‘rigid’ tissues\textsuperscript{18}, respectively (Supplementary Fig. 2a). miRNA activity on the MRE sensors was compared with the steady-state level of CAM proteins, as well as the expression of CAM RNAs and miRNAs, in the same cellular settings. Thus, proteomics, RNA and miRNA sequencing were assessed in parallel.

To evaluate CAM sensor reporters, endothelial cells were separated by fluorescence-based sorting into bins according to the mCherry/GFP ratio, using an empty sensor as a negative control (not suppressed) and a miR-125 sensor as a positive control (strongly suppressed). Thus, bins were defined as ‘strongly suppressed’, ‘suppressed’, ‘mildly suppressed’ and ‘not suppressed’ relative to these internal standards (Fig. 2b). Wild-type (WT) endothelial cells expressing the CAM sensor library showed a broad distribution between the suppressed and not suppressed bins, on both soft and stiff substrates (Fig. 2b). Importantly, clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR-associated protein 9 (Cas9)-mediated disruption of AGO2 diminished the miRNA levels in endothelial cells (Supplementary Fig. 2b,c) and significantly increased the population of ‘not suppressed’ cells (Supplementary Fig. 2d). Thus, miRNAs are required for post-transcriptional inhibition of CAM sensors.

Sensor vectors from sorted cells were then isolated from each bin and barcoded using PCR primers that recognized each cloned CAM MRE and were compatible with high-throughput sequencing. Combining global miRNA profiling (Supplementary Table 7) and MRE reads from Sensor-seq revealed strong correlations between suppression of CAM sensors and the level of the respective matching miRNAs (Fig. 2c). Notably, both miRNA levels and CAM reporter suppression were present on soft substrate at baseline and elevated in cells on stiff substrates (Fig. 2c). Interestingly, the levels of most CAM mRNA and respective proteins (Supplementary Table 5) were also generally higher in stiff conditions (Fig. 2c). These results suggest transcriptional co-regulation between miRNAs and CAM mRNA targets on stiff substrates. Thus, the CAM MRE–miRNA network has the characteristics of a mechanoregulatory buffer of structural protein-coding genes.

Loss of miRNA biogenesis leads to endothelial cell contractility. To evaluate the function of this miRNA regulatory network, we first examined endothelial cells lacking AGO2 or DROSHA, which have diminished miRNA levels\textsuperscript{20} (Supplementary Fig. 2c). We stained cells to hypothesize that the CAM mRNA–miRNA regulome is mechanosensitive. Genome-wide analyses of the miRNA–CAM mRNA network, we disrupted individual CAM–miRNA interactions. We chose nine of the mechanosensitive CAM MREs (stars in Fig. 2c and Supplementary Table 6) in which the MRE was within 20 nt of a protospacer sequence and thus targetable by a guide RNA (gRNA) and Cas9. Genome-wide analyses of
Fig. 1 | miRNAs regulate CAM genes. a, Left, schematic of AGO2-HITS-CLIP. miRNA–RNA complexes were crosslinked to AGO2 via UV light, and unbound RNAs were removed by RNase treatment. AGO2–RNA complexes were immunoprecipitated, and RNA was labelled with $^{32}$P and isolated. Right, the box plot shows difference in conservation scores across samples scoring using PhastCons (Wilcoxon rank sum test). AGO2 peaks in HUAEC and HUVEC and binned human 3′ UTRs of 100 species. The conservation score is represented as a box plot with minimum, maximum, median and quartiles ($n = 3$ independent replica for HUVEC and HUAEC for a total of 383 for HUAEC, 749 for HUVEC and 125,685 for control individual value).

b, Left, the chart represents positional enrichment of AGO2 peaks within the human 3′ UTR for HUAEC and HUVEC. The lines indicate the nucleotide positional distribution of peak sequences within meta-gene-analysed 3′ UTRs. Right, the box plot shows difference in conservation scores across samples scoring using PhastCons (Wilcoxon rank sum test). AGO2 peaks in HUAEC and HUVEC and binned human 3′ UTRs were compared with binned 3′ UTRs of 100 species. The conservation score is represented as a box plot with minimum, maximum, median and quartiles ($n = 3$ independent replica for HUVEC and HUAEC for a total of 383 for HUAEC, 749 for HUVEC and 125,685 for control individual value).

c, Left, the schematic shows the AGO2 regulome. AGO2–mRNA targets identified via AGO2-HITS-CLIP are highlighted in green. Integrins, TALIN1 and bone morphogenetic protein receptor 1 (BMPR1) proteins (brown) are part of the CAM's GO term but were not detected by AGO2-HITS-CLIP. Arrows point to downstream regulators of CAM proteins targeted by AGO2. CAM proteins and their regulators were identified by database searches (Supplementary Fig. 1a and Supplementary Tables 1–3) and manually curated for accuracy. Right, the chart shows the interactome for 25 of the 73 AGO2-CAM genes in which a complementary MRE (7–8 nt) was identified using TargetScan v.7.0 prediction software and miRNAs were identified from AGO2-HITS-CLIP reads using miRbase (Methods). Colour-coded boxes indicate the number of MREs identified in each of the selected CAM gene 3′ UTRs. The lines indicate interaction between MRE and miRNA family members with similar SEEDs. The miRNA–miRNA network shows high complexity, with numerous miRNAs binding to one or more CAM 3′ UTRs, whereas most CAM genes are targeted by more than one miRNA. ARF2, actin-related protein 2; COL4, collagen IV; HSPG2, heparan sulfate proteoglycan 2; MAPKAPK2, mitogen-activated protein kinase-activated protein kinase-activated protein kinase 2; MYH9, myosin 9; MYL6, myosin light polypeptide 6; NRP1, neuropilin 1; PAI1, plasminogen activator inhibitor 1; RGS5, regulator of G protein signalling 5; TIMP2, metalloproteinase inhibitor 2; TUBA1C, tubulin α-1C chain; TUBB, tubulin β-chain.
**Fig. 2** CAM MREs are actively repressed in stiff substrates. **a**, Schematic for the CAM sensor library. The Sensor-seq lentiviral library (Methods) consisted of (1) mCherry upstream of one of the 97 CAM MRE, and (2) GFP lacking any MRE (control). The numbered MREs indicate different MREs within one CAM 3' UTR; alternatively, one MRE was cloned per CAM 3' UTR (Supplementary Table 4). **b**, Left, mCherry and GFP intensities in HUVECs infected with the lentiviral library at 3 kPa and 30 kPa, a negative control (no MRE) and a positive control (3 perfect MREs for miR-125, abundant in HUVECs\(^{45}\)). The density plots show the relative intensity of cell distribution using contour lines. Each contour line represents 15% probability (higher = lighter grey, lower = darker grey) of containing cells in each bin over total cells (10,000 cells). Sensor-seq library-infected HUVECs at 3 kPa and 30 kPa were sorted into 4 bins, as indicated, based on mCherry/GFP expression relative to controls. Cells in each bin were isolated and genotyped using a specific Illumina primer for sequencing (Methods). Right, the bar graph shows the percentage of mCherry\(^{+}\) cells sorted in each bin from four experiments (mean ± s.e.m.; single experiments are represented by dots). **c**, CIRCOS\(^{\text{TM}}\) graphical representation of CAM miRNA–MRE interactions. Right quadrants: endothelial cell miRNAs with putative SEED matching to CAM MREs sensors displaying differential gene expression between 3 kPa and 30 kPa, divided into 2 groups: expressed at 3 kPa compared to 30 kPa (black line, top right) and expressed at 30 kPa compared to 3 kPa (black line, bottom right). Left quadrants: CAM sensors most suppressed at 30 kPa compared to 3 kPa (black line, bottom left) and vice versa (black line, top left). The colour-coded boxes indicate the categorized bins in **b** at which cells were isolated and genotyped for a specific CAM sensor. The lines indicate a match between individual miRNA (SEED) and CAM MRE in each condition. The colour code indicates the level of complementarity between miRNA SEED and MRE nucleotides. The internal circles show the respective CAM RNAs (red) and proteins (green) log fold change (FC) at 3 kPa compared to 30 kPa and 30 kPa compared to 3 kPa (Supplementary Tables 5 and 6). Asterisks indicate genes that were further validated with MRE targeting guide RNAs in Fig 4. Source data can be found in Supplementary Table 8.
**Fig. 3 | miRNAs limit endothelial cell spreading, YAP signalling and contractility.**

**a**, Representative immunofluorescence images and traction force maps of HUVECs after infection with AGO2 or a non-targeting control pLentiCRISPR virus. Cells on fibronectin-coated 3-kPa PDMS gels were stained for F-ACTIN (phalloidin), focal adhesions (PAXILLIN) and YAP/TAZ (scale bar, 50 µm). Heat maps of traction stress for single cells (scale bar, 20 µm) are also shown. All box plots show the minimum, maximum, median and quartiles. Cell area (based on phalloidin staining) (control: n = 163; AGO2 gRNA: n = 182 cells per group; dots indicate individual cells, representative data from 6 independent experiments, ****P < 0.0001, unpaired, two-sided t-test), the number of PAXILLIN adhesions per cell (control: n = 19 fields of view 49 cells; AGO2 gRNA: n = 20 fields of view 51 cells; dots indicate the average per field of view, representative data from 3 independent experiments, *P = 0.0085, unpaired, two-sided t-test) and nuclear-to-cytoplasmic ratio of YAP/TAZ (control: n = 43 cells; AGO2 gRNA: n = 54 cells; dots represent single-cell measurements, representative data from 2 independent experiments, ****P < 0.0001, unpaired, two-sided t-test). The box plot for total force shows total force per single cell (n = 19 cells per group; dots indicate individual cells, from 2 independent experiments, *P = 0.0119, unpaired, two-sided t-test).

**b**, Box plots show quantification of HUVEC treated with shRNA against DROSHA (Methods) and seeded on fibronectin-coated 3-kPa PDMS gels for the cell spread area (shNeg: n = 156; shDRO#2: n = 138 cells, from 2 independent experiments, ****P < 0.0001, unpaired, two-sided t-test). YAP nuclear localization (shNeg: n = 156; shDRO#2: n = 138 cells, from 2 independent experiments, ****P < 0.0001, unpaired, two-sided t-test) and total force per cell (n = 21 cells per group, from 2 independent experiments, ****P < 0.0001, unpaired, two-sided t-test). The box plot representing the difference in protein expression between HUVEC seeded on 30 kPa versus 3 kPa (x axis) or between HUVECs infected with AGO2 gRNA versus control gRNA (y axis) (n = 3 replicates). Green and red identify CAM proteins with coherent or incoherent differential expression, respectively (Supplementary Table 6). Source data can be found in Supplementary Table 8.
CAM MREs in endothelial cells treated with gRNA–CRISPR–Cas9 revealed that nearly 75% of insertions and deletions were within the desired MRE region (±20 bp) (Fig. 4a and Supplementary Fig. 4a).

To test whether MRE-proximal mutations increased the expression of the cognate proteins, we analysed CAM protein levels via immunofluorescence and western blot. Individual CAM levels increased in the respective MRE mutants compared to control cells (Fig. 4b), consistent with loss of miRNA-mediated suppression23.

Finally, we tested the mechanical properties of each CAM MRE mutant population and found, to varying extents, that cell area, YAP nuclear localization and/or traction stresses were significantly higher than control targeted cells (Fig. 4c).

Whereas multiple genes clearly contributed to each effect, the gene whose MRE mutation gave the most consistent results across multiple assays was CTGF (encoding connective tissue growth factor). CTGF is a matrix protein that modulates the interaction of cells with the ECM24, suggesting that it is a component of a protein-based regulatory network and probably functions via receptor-mediated signalling to control these functions. Blocking CTGF miRNA repression in endothelial cells via a target protector RNA

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**Fig. 4 | Post-transcriptional regulation of individual CAM genes limits endothelial cell spreading, YAP signalling and/or contractility.**

**a.** Experimental strategy to mutate individual MREs in CAM genes 3′ UTRs to block miRNA binding (see Methods and Supplementary Fig. 2b). CDS, coding sequence; Chr. chromosome; pA, poly A; PAM, protospacer adjacent motif. **b.** Representative immunofluorescence images (top; scale bar, 50 µm) and western blot (bottom) of CAM proteins as indicated (CTGF: ~37 kDa, stathmin (STMN1): ~19 kDa, vinculin (VCL): ~116 kDa, RHOB: ~25 kDa, ACTIN: ~47 kDa). HUVECs were infected with lentivirus carrying Cas9 and gRNA targeting specific CAM MREs and no-target gRNA (a) and processed at 7 d post-infection.

**c.** Quantification of cell spreading (n = 99, 75, 91, 94, 88, 87, 79, 54 and 83 from the bottom to the top, representative data from 2 independent experiments), YAP nuclear translocation (n = 91, 81, 87, 93, 79, 88, 67, 54 and 84 cells from the bottom to the top, representative data from 2 independent experiments) and total force per cell (n = 38, 33, 37, 36, 34, 34, 30 and 36 cells from the bottom to the top, from 2 independent experiments) in HUVECs on 3-kPa PDMS gels for 48 h after mutation of the indicated MREs (all box plots indicate the minimum, maximum, median and quartiles, single dots represent single cells, colours represent P values, one-way ANOVA with Fisher’s least significant difference). Source data can be found in Supplementary Table 8.
Fig. 5 | AGO2 activity is required to limit fibroblast contractility in 2D and 3D models. a, Representative immunofluorescence images of HDF after infection with the pLentiCRISPR virus directed at AGO2 or a non-targeting control seeded on fibronectin-coated 3-kPa PDMS gels for 48 h (scale bar, 50 µm). Heat maps of traction stress for single cells are also shown (scale bar, 20 µm). Box plots show the HDF cell area (control: n = 63 cells; AGO2 gRNA: n = 51 cells, representative data from 4 independent experiments, ****P < 0.001, unpaired, two-sided t-test) based on phalloidin staining, the number of PAXILLIN adhesions per cell (n = 19 fields of view 63 cells; AGO2: n = 20 fields of view 51 cells; dots indicate the average per field of view; representative data from 2 independent experiments, ****P < 0.0001, unpaired, two-sided t-test) and the nuclear-to-cytoplasmic ratio of YAP/TAZ (control: n = 58 cells; AGO2 gRNA: n = 34, cells are represented by single dots; representative data from 2 independent experiments, **P = 0.0174, unpaired, two-sided t-test). Single-cell maps of traction stress and quantification of total force per cell (box plots with whiskers indicate the minimum and maximum values with median and quartiles, control: n = 21 cells, AGO2 gRNA: n = 20 cells; *P = 0.0109, unpaired, two-sided t-test). b, Representative 3D matrix constructs with control or Ago2-mutated mouse dermal fibroblasts (scale bar, 1 mm). Bar plots show the average cell number and construct diameter within transverse sections (n = 8, bars indicate the mean ± s.e.m. and dots represent single replicate, **P < 0.01, NS, not significant). c, Transverse sections of control and Ago2-depleted matrix constructs stained for vimentin or pMyosin and DAPI. Source data can be found in Supplementary Table 8.
miRNA–CAM mRNA interactions mediates mechanical homeostasis in cells.

miRNA-dependent regulation limits contractility in 2D and 3D fibroblast models. We next tested the generality of the identified miRNA–CAM gene regulations using fibroblasts as a second model system. Profiling of miRNAs in human dermal fibroblasts (HDFs) seeded on 3- versus 30-kPa substrates showed that miRNAs targeting CAM genes are upregulated on stiff substrates similarly to endothelial cells (Supplementary Fig. 5a and Supplementary Table 7). Depletion of AGO2 in HDFs also increased F-ACTIN and focal adhesion levels, traction forces and YAP localization compared to control cells (Fig. 5a and Supplementary Fig. 5b).

To determine whether the miRNA-mediated network functions at the tissue level, we examined primary mouse dermal fibroblasts in a 3D matrix. Cells suspended in attached fibrin gels contract and replace the fibrin with their own matrix over about 5 d (Fig. 5b), providing a 3D model of cell behaviour. Transduction of freshly isolated cells with a CRISPR–Cas9–gRNA virus targeting Ago2 reduced Ago2 protein levels by ~50–60% (Supplementary Fig. 5c). Ago2-depleted fibroblasts in 3D matrix generated tissue constructs with reduced diameters but no significant change in cell numbers (Fig. 5b). Immunostaining transverse sections of these constructs confirmed the decreased diameter, based on staining with the cytoskeleton protein vimentin (Fig. 5c). Staining for phosphorylated myosin light chain was elevated, consistent with increased contractility (Fig. 5c). These data indicate that reducing miRNA-dependent regulation stimulates fibroblast contraction of the 3D matrix.

miRNA-dependent regulation controls tissue stiffness and wound healing in vivo. We next tested whether miRNAs regulate mechanical homeostasis in vivo using the zebrafish fin-fold regeneration model. The fin fold is a non-vascularized appendage comprising a few layers of epidermis and fibroblast-like cells. Wounding triggers a healing response mediated by a conserved and rapid matrix remodelling- and actomyosin-based process that involves the formation of a provisional matrix, inflammatory cell invasion, cell migration, proliferation and resolution.

To investigate miRNA-dependent regulation of mechanical homeostasis in zebrafish, we first examined embryos that carry a maternal zygotic homozygous mutation in ago2 (mz ago2−/−)28, which showed reduced levels of Ago2 and of miRNAs (Supplementary Fig. 6a). To evaluate miRNA activity in the fin fold, we co-injected an miRNA-sensitive GFP mRNA, containing three perfect miR-24 MREs within the 3′ UTR, with an miRNA-insensitive mCherry control mRNA. As expected, mz ago2 mutants showed elevated levels of GFP, but not of mCherry, when compared to WT embryos, confirming reduced miRNA-mediated suppression (Supplementary Fig. 6b).

We then quantified tissue stiffness using atomic force microscopy (AFM)-based nanoindentation on the central region of the fin fold. The appearance of this tissue was indistinguishable between genotypes (Fig. 6a), ruling out obvious developmental defects. However, the elastic modulus was ~30% higher in mz ago2−/− than in WT embryos, indicating increased mechanical rigidity (Fig. 6a). Importantly, normal tissue stiffness was restored upon injection of in vitro transcribed mRNA encoding human AGO2 (hsAGO2 mRNA), demonstrating that the stiffness of this tissue is dependent on the level of Ago2 (Fig. 6a). Following amputation, mz ago2 mutants exhibited slower repair than WT embryos, which was rescued by hsAGO2 mRNA (Fig. 6a). WT and mz ago2−/− wounds did not display differences in cell cycle progression, detected by proliferating cell nuclear antigen staining, or in apoptosis, detected by TdT-mediated dUTP nick end labelling (TUNEL) assay (Supplementary Fig. 6c,d). These results support that miRNA-dependent suppression restricts tissue stiffness and contributes to tissue healing in vivo.
Fig. 7 | Wound healing in mz ago2 and ctgfa MRE fin-fold mutants. a–c, Top, schematics representing the time course of fin-fold regeneration. Boxes identify the region of interest reported in the images below. Bottom, confocal images of whole-mount fin folds within the boxed region from the schematics at the top, at the indicated stages (scale bars, 120 μm). The white and black dotted lines indicate the edge of the fin fold. The white arrowheads point to staining for the indicated markers. The graphs show box plots with minimum, maximum, median and quartiles. Wild type, gray; mz ago2−/−, red; ctgfa MRE mutant, orange. Intensity profiles for multiple embryos were combined (CTGF: n = 4 embryos per genotype, * in mz ago2−/− 2 h versus ctgfa MRE 2 h = 0.0318 (a); pMyosin: n = 4 embryos per genotype, *** in WT 0 h versus mz ago2−/− 0 h = 0.0004, ** in mz ago2−/− 2 h versus ctgfa MRE 0 h = 0.0069, ** in WT 2 h versus mz ago2−/− 2 h = 0.0014, ** in WT 2 h versus ctgfa MRE 2 h = 0.0041 (b); YAP: n = 6 embryos for each genotype, * in WT uncut versus mz ago2−/− uncut = 0.027, * in ago uncut versus ctgfa MRE uncut = 0.0126, ** in WT 0 h versus mz ago2−/− 0 h = 0.0082, *** in mz ago2−/− 0 h versus ctgfa MRE 0 h = 0.0008, ** in ago 2 h versus ctgf 2 h = 0.0035 (c); single fish are represented by dots, NS, not significant, unpaired, two-sided t-test).

For Yap, the protein nuclear localization was represented. The nucleus/cytosol ratio was obtained using the DAPI channel to generate a binary mask and subtract nuclear YAP-GFP intensity from the total YAP-GFP detected (see Methods and Supplementary Fig. 7b). d, Model for miRNA post-transcriptional regulation of structural protein function in mechanical tissue homeostasis. Increases in matrix stiffness and the resulting cell contractility increase integrin and actomyosin-dependent CAM signalling, which upregulates miRNAs that suppress CAM transcripts, thus restoring normal cell mechanics. PDLIM5, PDZ and LIM domain protein 5; TF, transcription factor; THBS1, thrombospondin 1. Source data can be found in Supplementary Table 8.
miRNA-dependent CAM gene regulation limits tissue contractility during wound healing. Wounding triggers increased contractility and matrix rigidity as a rapid, first response. According to our notion of mechanical homeostasis, these changes should activate negative-feedback mechanisms that restore mechanical equilibrium. Thus, we examined matrix, actomyosin activation and the mechanosensitive translocation of Yap before and after wounding the zebrafish fin fold in the WT embryo versus the mz ago2 mutant. As expected, WT embryos showed increased staining for PMysosin, Ctgfa and fibronectin in the wound area between 0.5 and 2 h post-amputation (h.p.a.) (Fig. 7a,b and Supplementary Fig. 7a). In comparison, mz ago2 wounded fins showed strikingly elevated and persistent PMysosin staining at both 0.5 and 2 h.p.a., and higher levels of Ctgfa and fibronectin at 2 h.p.a. (Fig. 7a,b and Supplementary Fig. 7a). Consistent with the increase in tissue stiffness (Fig. 6a), mz ago2 displayed higher basal Yap nuclear localization than WT embryos that further increased at 0.5 h.p.a. and persisted at 2 h.p.a. (Fig. 7c and Supplementary Fig. 7b). Thus, loss of miRNA-mediated suppression leads to an exaggerated mechanical response and impaired mechanical resolution during wound healing.

To correlate these effects with regulation of individual CAM genes, we generated zebrafish embryos carrying mutations in the two 3’ UTR MREs of the ctgfa gene (Supplementary Fig. 7c,d). These MREs are conserved in the human CTGF 3’ UTR, and their mutation had the largest effect in vitro (Fig. 4c). Accordingly, a GFP sensor miRNA bearing a ctgfa 3’ UTR fragment showed reduced expression in WT relative to mz ago2 embryos, which required the MRE sites (Supplementary Fig. 7c). These results support miRNA-dependent inhibition of ctgfa via the MREs in zebrafish. Embryos with mutated ctgfa MREs showed persistent PMysosin activation compared to WT embryos by 2 h.p.a. (Fig. 7b), consistent with the induction of Ctgfa at 2 h.p.a. in the mz ago2 mutant (Fig. 7a). However, no other differences were detected in the ctgfa MRE mutant embryos (Fig. 7a–c and Supplementary Figs. 6c,d and 7a,b). These results support that post-transcriptional regulation of ctgfa contributes to specific Ago2-mediated mechanical effects within the miRNA–CAM mRNA network.

Discussion
We report that an unbiased analysis of miRNAs and their target genes in endothelial cells, together with functional assays in several biological systems, reveal the existence of a mechanosensitive miRNA-based programme that counteracts cell adhesion, cytoskeletal, contractile and matrix protein expression. This system functions in several cell types, across multiple species, and appears to be conserved throughout vertebrate evolution. Importantly, most of the protein-coding genes for synthesis and assembly of stiff ECM are targeted by miRNAs that are upregulated on stiff substrates. Thus, a ‘buffer’ is generated, in which increased matrix stiffness upregulates both CAM gene transcription and the miRNAs that suppress these transcripts. This miRNA regulome has the molecular and functional characteristics of a homeostatic mechanism, in which changes in cell contraction and matrix are counteracted to maintain normal tissue stiffness (Fig. 7d).

Endothelial cells in vivo are also subject to wall stretch and fluid shear stress from blood pressure and blood flow, which were not examined here but which are likely to interact with cell responses to matrix stiffness. Interestingly, both of these variables are subject to negative-feedback regulation, consistent with the general importance of mechanical homeostasis. Although the current studies dissected the post-transcriptional regulation of CAM proteins in stiffness homeostasis, a deeper understanding of these mechanisms will need to address the interplay between RNA regulation, stiffness, shear stress in endothelial and other cells subject to external forces.

A network-mediated mechanism for stiffness homeostasis, rather than regulation of one or a few CAM genes, would be expected to increase the robustness of the system. Multiple miRNAs can regulate a large cohort of CAM genes via different MREs, whereas different cell types can do so by controlling the expression and processing of tissue-specific mature miRNAs. However, we speculate that these miRNA networks are likely to be subelements within a larger and more robust network of negative and positive circuits, connected by multiple nodes, that mediate tissue homeostasis over the multiple decades of human life. Such nodes could develop within a hierarchy of epigenetic factors in which, for example, the activation of YAP/TAZ and its direct target gene CTGF, may be one of the upstream components.

A role for miRNAs in tissue mechanical homeostasis is supported by the widespread deregulation of miRNAs during lung, renal, cardiac and liver fibrosis, including miRNAs that target ECM proteins. Idiopathic lung fibrosis is also linked to reduced levels of miRNAs that target the ECM, cytoskeletal and transforming growth factor-β pathways genes. All of these studies reported reduced levels of miR-29 species, in contrast to our finding that, in normal cells, miR-29 species are increased on stiff substrates. These results are consistent with the notion that fibrotic disease involves disruption of normal stiffness miRNA-dependent homeostasis.

Cells and tissues with impaired regulation of miRNAs upon AGO2 or DROSHA partial loss of function show defective adaptation to stiffness. The degree of such effects could be related to the corresponding miRNAs levels. Although model organisms that completely lack miRNAs do not develop properly, heterozygous mutants of Ago2 do not manifest obvious phenotypes. Further studies will be necessary to test whether this model has tissue stiffness defects in normal conditions and/or respond abnormally to perturbations, such as wounding, or during ageing.

miRNA-dependent post-transcriptional regulation of structural proteins provides a concrete molecular mechanism that can explain how healthy tissues sustain optimal mechanical properties. Thus, these findings are an important step towards understanding the pathological alterations that result in fibrotic and related diseases. Characterizing the stiffness-dependent RNA metabolism of cytoskeleton and matrix transcripts, their possible regulation under other physical forces and elucidating the complete regulatory network that mediates long-term mechanical robustness are the essential tasks for future studies.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41556-019-0272-y.

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References
1. Cyron, C. J. & Humphrey, J. D. Growth and remodeling of load-bearing biological soft tissues. Meccanica 52, 643–664 (2017).
2. Gilbert, P. M. & Weaver, V. M. Cellular adaptation to biomechanical stress across length scales in tissue homeostasis and disease. Semin. Cell Dev. Biol. 67, 141–152 (2017).
3. Humphrey, J. D., Dufresne, E. R. & Schwartz, M. A. Mechanotransduction and extracellular matrix homeostasis. Nat. Rev. Mol. Cell Biol. 15, 802–812 (2014).
4. Humphrey, J. D. Vascular adaptation and mechanical homeostasis at tissue, cellular, and sub-cellular levels. Cell Biochem. Biophys. 50, 53–78 (2008).
5. Seki, E. & Brenner, D. A. Recent advancement of molecular mechanisms of liver fibrosis. J. Hepatobiliary Pancreat. Sci. 22, 512–518 (2015).
6. Huang, S. & Ingber, D. E. Cell tension, matrix mechanics, and cancer development. Cancer Cell 8, 175–176 (2005).
7. Sun, Z., Guo, S. S. & Fassler, R. Integrin-mediated mechanotransduction. J. Cell Biol. 215, 445–456 (2016).
8. Pasquinielli, A. E. MicroRNAs and their targets: recognition, regulation and an emerging reciprocal relationship. Nat. Rev. Genet. 13, 271–282 (2012).
9. Hafner, M. et al. Transcriptome-wide identification of RNA-binding protein and microRNA target sites by PAR-CLIP. Cell 141, 129–141 (2010).
10. Herranz, H. & Cohen, S. M. MicroRNAs and gene regulatory networks: managing the impact of noise in biological systems. Genes Dev. 24, 1339–1344 (2010).
11. Tsang, J., Zhu, J. & van Oudenaarden, A. MicroRNA-mediated feedback and feedforward loops are recurrent network motifs in mammals. Mol. Cell 26, 753–767 (2007).
12. Kasper, D. M. et al. MicroRNAs establish uniform traits during the architecture of vertebrate embryos. Dev. Cell 40, 552–565.e5 (2017).
13. Chi, S. W., Zang, J. B., Mele, A. & Darnell, R. B. Argonaute HITS-CLIP decodes microRNA–mRNA interaction maps. Nature 460, 479–486 (2009).
14. Byfield, F. J., Reen, R. K., Shentu, T. P., Levitan, I. & Gooch, K. J. Endothelial cell actin and cell stiffness is modulated by substrate stiffness in 2D and 3D. J. Biomech. 42, 1114–1119 (2009).
15. Grimson, A. et al. MicroRNA targeting specificity in mammals: determinants beyond seed pairing. Mol. Cell 27, 91–105 (2007).
16. Saphirstein, R. J. & Morgan, K. G. The contribution of vascular smooth muscle to aortic stiffness across length scales. Microcirculation 21, 201–207 (2014).
17. Mullikandov, G. et al. High-throughput assessment of microRNA activity and function using microRNA sensor and decoy libraries. Nat. Methods 9, 840–846 (2012).
18. Kamata, M., Liang, M., Liu, S., Nagaoka, Y. & Chen, I. S. Live cell monitoring of hiPSC generation and differentiation using differential expression of endogenous microRNAs. PLoS ONE 5, e11834 (2010).
19. Duscher, D. E., Janmey, P. & Wang, Y. L. Tissue cells feel and respond to the stiffness of their substrate. Science 310, 1139–1143 (2005).
20. Kim, Y. K., Kim, B. & Kim, V. N. Re-evaluation of the roles of DROSHA, Export in 5, and DICER in microRNA biogenesis. Proc. Natl Acad. Sci. USA 113, E1881–E1889 (2016).
21. Dupont, S. et al. Role of YAP/TAZ in mechanotransduction. Nature 474, 179–183 (2011).
22. Kumar, A. et al. Talin tension sensor reveals novel features of focal adhesion force transmission and mechanosensitivity. J. Cell Biol. 213, 371–383 (2016).
23. Bassett, A. R. et al. Understanding functional miRNA-target interactions in vivo by site-specific genome engineering. Nat. Commun. 5, 4640 (2014).
24. Shi-Wen, X., Leask, A. & Abraham, D. Regulation and function of connective tissue growth factor/CCN2 in tissue repair, scarring and fibrosis. Cytokine Growth Factor Rev. 19, 133–144 (2008).
25. Kawakami, A., Fukazawa, T. & Takeda, H. Early fin primordia of zebrafish larvae regenerate by a similar growth control mechanism with adult regeneration. Dev. Dyn. 231, 693–699 (2004).
26. Mathew, I. K. et al. Comparative expression profiling reveals an essential role for raldh2 in epimorphic regeneration. J. Biol. Chem. 284, 33642–33653 (2009).
27. Mateus, R. et al. In vivo cell and tissue dynamics underlying zebrafish fin fold regeneration. PLoS ONE 7, e51786 (2012).
28. Cifuentes, D. et al. A novel miRNA processing pathway independent of Dicer regulates Argonaute2 catalytic activity. Science 328, 1694–1698 (2010).
29. Amelio, I. et al. miR-24 triggers epidermal differentiation by controlling actin adhesion and cell migration. J. Cell Biol. 199, 347–363 (2012).
30. Nepochoruk, A. & Keating, M. T. A. Proliferation gradient between proximal and mouse-expressing distal blastema directs zebrafish fin regeneration. Development 129, 2607–2617 (2002).
31. Hasegawa, T. et al. Transient inflammatory response mediated by interleukin-1p is required for proper regeneration in zebrafish fin fold. eLife 6, e22716 (2017).
32. Mateus, R. et al. Control of tissue growth by Yap relies on cell density and F-actin in zebrafish fin regeneration. Development 142, 2752–2763 (2015).
33. Mori, M. et al. Hippo signaling regulates microprocessor and links cell-density-dependent microRNA biogenesis to cancer. Cell 156, 893–906 (2014).
34. Chaulk, S. G., Lattanzi, V. J., Hiemer, S. E., Fahimian, R. P. & Varelas, X. The Hippo pathway effectors TAZ/YAP regulate dicer expression and microRNA biogenesis through Let-7. J. Biol. Chem. 289, 1886–1891 (2014).
35. Davis, B. N., Hilyard, A. C., Lagna, G. & Hata, A. SMAD proteins control DROSHA-mediated microRNA maturation. Nature 454, 56–61 (2008).
36. Felix, M. A. & Wagner, A. Robustness and evolution: concepts, insights and challenges from a developmental model system. Heredity (Edinb.) 100, 132–140 (2008).
37. Mouw, J. K. et al. Tissue mechanics modulate microRNA-dependent PTEN expression to regulate malignant progression. Nat. Med. 20, 360–367 (2014).
38. Liu, G. et al. miR-21 mediates fibrogenic activation of pulmonary fibroblasts and lung fibrosis. J. Exp. Med. 207, 1589–1597 (2010).
39. Cushing, L. et al. miR-29 is a major regulator of genes associated with pulmonary fibrosis. Am. J. Respir. Cell Mol. Biol. 45, 287–294 (2011).
40. Herrera, J. et al. Dicer1 deficiency in the idiopathic pulmonary fibrosis fibroblastic focus promotes fibrosis by suppressing microRNA biogenesis. Am. J. Respir. Crit. Care Med. 198, 486–496 (2018).
41. Parker, M. et al. Fibrotic and proinflammatory microRNAs are activated as a positive feedback loop. J. Clin. Invest. 124, 1622–1635 (2014).
42. Pandit, K. V. & Milosevic, J. MicroRNA regulatory networks in idiopathic pulmonary fibrosis. Biochem. Cell Biol. 93, 129–137 (2015).
43. Wynn, T. A. & Ramalingam, T. R. Mechanisms of fibrosis: therapeutic translation for fibrotic disease. Nat. Med. 18, 1028–1040 (2012).
44. Liu, J. et al. Argonaute2 is the catalytic engine of mammalian RNAi. Science 305, 1437–1441 (2004).
45. McCall, M. N. et al. MicroRNA profiling of diverse endothelial cell types. BMC Med. Genomics 4, 78 (2011).
46. Krzywinski, M. et al. Circos: an information aesthetic for comparative genomics. Genome Res. 19, 1639–1645 (2009).
**Methods**

**Cell culture.** HUVECs and HUAECs were purchased from Cell Applications Inc. (catalogue no. 200-05n and catalogue no. 202-05n). Endothelial cells were cultured on dishes coated with 0.2% w/v gelatin (10 min at room temperature in PBS; Sigma) in endothelial cell growth medium (ECM Bullet Kit, Lonza). For HITs-CLIP assays, cells were used at PS (split 1/3 and 1/5) before UV crosslinking. For other assays, cells were split 1:3 twice per week and used until passage 5. HDFS were purchased from the American Type Culture Collection (ATCC; catalogue no. PCS-201–010, lot no. 65014910) and cultured on 0.2% w/v gelatin-coated dishes in fibroblast growth medium (Fibroblast Growth Kit-Low Serum; PCS-201–041, ATCC). HDFS were split 1:10 twice per week and used until passage 6.

**Primary fibroblasts.** Primary dermal fibroblasts for 3D fibrin gel assays were obtained from 5 to 8-week-old C57BL/6 mice (Envigo). This study is compliant with all relevant ethical regulations regarding animal research. All procedures were in accordance with the UK Home Office Regulation and UK Animals (Scientific Procedures) Act of 1986 for the care and the use of animals. Mice were killed by a schedule 1 procedure by trained personnel. Mouse hair was removed and skin dissected in Hank's buffer supplemented with antibiotic and antimitotic solution (Sigma). Fat and excess connective tissues were removed, the dermis was minced with a scalpel and digested in buffer containing 0.25% trypsin without EDTA (Gibco), collagenase IV (4 mg/ml (Worthington)) and calcium chloride (0.3 mg/ml (Sigma)) for 3 h at 37°C with agitation during the last hour. After mechanical dissociation, cells were passed through a cell strainer (100 µm; Fischer Scientific). Cells were centrifuged at 1,800 r.p.m. for 5 min, resuspended in DMEM supplemented with 10% FBS (Sigma), penicillin (100 U ml⁻¹) and streptomycin (100 µg/ml) (Gibco) and 1% L-glutamine, and seeded in 75-cm² tissue culture flasks. Medium was changed at 3 h and subsequently changed once a day.

**AGO2-HITS-CLIP.** The HITS-CLIP experiment was performed as previously described. Subconfluent endothelial cells in EGM were UV crosslinked two times with 400 mJ/cm² in Stratalinker (model 2400, Stratagene), lysed and treated with DNsase (1,000 Promega RQ1 DNase) and RNase T1 (1:100; Thermo Fisher). Cell lysates and Protein A Dynabeads (Invitrogen) complexed with Ab-panAGO2-A2B (MABE156, Millipore) were incubated at 4°C for 4 h. Beads were subsequently washed and ligated with 3’-F32- radiolabelled linker (RL3; Supplementary Table 6). SDS–PAGE was performed using NuPage 4–12% Bis-Tris Gel (NP0321, Invitrogen), and proteins were transferred onto pure nitrocellulose membrane (BioTrace) using NuPAGE transfer buffer according to the manufacturer’s instructions. High-performance autoradiography film was exposed overnight at ~80°C. The bands corresponding to AGO2–miRNAs (~110 kDa) and AGO2–RNA (~130 kDa) were cut and treated with proteinase K (Roche) to degrade proteins. RNAs were extracted and purified using phenol-chloroform, and AGO2:RNA (~130 kDa) were cut and treated with proteinase K (Roche) and anti-ribo-HRP (1:4,000; Santa Cruz) for 1 h. For blotting, blots were developed with super signal west pico chemiluminescent substrate (Thermo) using a SYNGENE G Box imager.

Single amplicons of ~300 bp were generated using primers equidistant from the putative region of mutation. PCR amplicons were combined and sent to YGCA Sequencing Facility for MiSeq 2x250 analysis. After sequencing, single amplicons were demultiplexed and single reads were used for multiple sequence alignment (msa) against the Wt sequence using the R msa package. The frequency of each mutation was calculated as total reads for each CAM gene mutation divided by the sum of all of the reads aligned to a specific CAM gene, and plot as a bar plot.

**Disruption of the miRNA–MRE interaction with the CTGF gene.** Disruption of the miRNA–MRE interaction with the CTGF gene was performed using a Mscrcl Target Reporter (Qiagen) directed at the MRE within the human CTGF gene. The CTGF target reporter (CTGF_1, T Catalogue no. MTP0079186) or the negative control target reporter (catalogue no. MTP000002) were transfected into HUVECs at 20nm using Lipofectamine.
RNAiMax (Invitrogen) in OPTI-MEM (Gibco) with 4% FBS (Sigma) twice (1 and 3 post-seeding). CTGF expression increases post-transfection were verified by immunoblot as before with a CTGF antibody (1:1000, ab6992, Abcam).

Polydimethylsiloxane and polyacrylamide substrates. Polydimethylsiloxane (PDMS) substrates were cast in the bottom of 10-cm tissue culture dishes or #1.5 cover-glass bottomed 35-mm MatTek dishes (for imaging studies). Soft (3 KPa) gels were made using a 1:1 ratio (by weight) of PDMS components A and B (CY 52–276 A and B, Dow Corning) degassed for 30 min in a vacuum desiccator and cured for 24 h at room temperature. Stiff (30 KPa) gels were made using a 40/1 ratio (by weight) of SYLGARD 184 components B and C (SYLGARD 184, Dow Corning), degassed for 30 min and cured for 3 h at 70 °C. Prior to seeding, gels were washed with PBS, sterilized with UV for 20 min and coated with bovine plasma fibrinogen (10 µg ml⁻¹ in PBS) overnight at 4 °C.

Polyacrylamide gel was prepared using a protocol modified from previously published methods. Briefly, 30-mm glass bottom dishes were activated with glacial acetic acid, 3- (trimethoxysilyl) propyl methacrylate and 96% ethanol solution (1/111/4 ratio, respectively) for 10 min at room temperature. For fibrinectin protein conjugation (1 mg ml⁻¹) on the polyacrylamide gel, acrylic acid N-hydroxysuccinimide ester was partially mixed as a substitute of acrylamide. Each stiffness was prepared with the ratio shown in Supplementary Table 9, which was previously reported[23–25].

RNA, miRNA and Sensor-seq library preparation. Total RNA was extracted from three-cell mixture of U2OS or H1299 cells that shifted to 3 KPa PDMS using TRIzol reagent (Life Technologies) according to the manufacturer’s protocol. For miRNA libraries, total RNA was treated with DNA-free DNase (Ambion) and 500 ng of treated RNA was used to prepare Lexogen QuantSeq 3′ miRNA Seq FWD libraries for Illumina deep–sequencing according to the manufacturer’s protocols. Libraries were amplified with 12 PCR cycles. miRNA libraries were prepared from 1 μg of total RNA using the NEBNext Small RNA Library Kit (NEB) following the gel size selection method in the manufacturer’s protocol and submitted for Illumina sequencing. For the Sensor-seq library, a customized oligonucleotide library was synthesized by Integrated DNA Technologies. The sequence of each individual oligonucleotide was obtained from Piranha analysis (see Supplementary Table 4), extending the genomic coordinate of each peak by 20 nt at the 3′ and 5′ regions. Ninety-seven peaks with at least 1 predicted MRE, representing 51 CAM genes, were selected. In addition, all sensor oligonucleotides contained restriction enzyme sites, Ascl and NheI, allowing for PCR base amplification and cloning. The oligonucleotide library was resuspended in 480 μl of water, diluted 1:100 and PCR amplified using the Phusion HotStart II HF kit and Ascl forward and NheI reverse primers (Supplementary Table 6). PCR amplified libraries were purified using a PCR purification kit (Qiagen) and double digested for 2 h at 37 °C.

Sensor-seq backbone containing a bidirectional promoter for ubiquitous expression of the genes encoding cogFP and mCherry, was kindly provided by J. Lu, Yale University. After sensor backbone digestion with Ascl and NheI, the MRE oligo library was cloned into the 3′UTR of mCherry. Ligation using T4 DNA ligase (Promega) was incubated for 16 h at 16 °C, then transformed into DH5α, and pooled colonies were used to prepare a library Maxi prep (Qiagen).

Lentivirus for the expression of the CAM MRE sensor library were generated as above using Lentiv-X 293T cells. For FACS analysis, additional control lentivirus containing pre-miRNA alone, mCherry MRE sensor with plasmid (CAM empty sensor plasmid, negative control) and miRNA sensor with a synthetic MRE (miR-125 sensor plasmid, positive control) were used.

RNA-seq and miRNA-seq data analysis. Total RNA and miRNA were aligned against the human genome version GRCh38 using the GENCODE 22 transcript annotation, using exact alignment software with the same parameters used for the ENDOXE project (www.endoxygenproject.org).

After alignment, differential gene expression of RNAs or miRNAs between control and treated samples were identified using DESeq or HNSPL metric libraries that were clustered using R. Clustering analysis was performed with the Significance Analysis of Microarrays (SAM) (5000 permutations) followed by one-way ANOVA test and Bonferroni correction. All sample features with a false discovery rate of < 0.05 were considered as significantly regulated.

FACS and Sensor-seq analysis. For FACS experiments, cells were collected and stained with the LIVE/DEAD Fixable Viability Kit (Thermo Fisher Scientific) according to the manufacturer’s protocol. The flow cytometric analysis was performed using a BD FACS Aria III equipped with a laser power of 30 mW and a 488 nm excitation wavelength. The data analysis was performed with the FACSDiva software using the default settings and an exclusion gate of 10,000 events per sample.

To analyze the specificity of the MRE sensors, we performed a sensor-seq assay to quantify the binding of miR-125 to the MREs on the polyacrylamide gel. For this, we used a 3′ biotinylated MRE sensor with restriction enzyme sites at 5′ and 3′ end and a dual-target miR-125 reporter system (Thermo Fisher Scientific). The sensor-seq assay was performed according to the manufacturer’s protocol. The data was analyzed with the RSeqeda R package using the hypergeometric distribution. A maximum of one false positive was allowed for each MRE sensor. The data was clustered using the Ward’s method with the Euclidean distance metric and visualized using the Ggplot2 package.

Computational analysis of the sensor-seq assay was performed using R. The first, the number of reads for each sensor MRE was divided by the total number of reads in the entire experiment and multiplied by 1 million to get the RPM for each sensor. To calculate the frequency of sensor MREs in each bin, the RPM was divided by the total RPM for all four bins of the experiment for that sensor, giving frequency values for each MRE in each bin at each stiffness. MREs that showed a dominant bin (with frequency values for each MRE greater than 0.5) were considered as significantly bound at 3 KPa versus 30 KPa. In Fig. 2c, CAM MREs were then plotted based on the reproducible tendency to be enriched in the same bin at a given stiffness but not the other for three independent experiments. MREs that shifted towards a more suppressed bin on 30 KPa than on 3 KPa were plotted as CAM MRE 30 KPa (bottom left) and 3 KPa (bottom right). MREs that shifted towards a more suppressed bin 3 KPa versus 30 KPa were plotted as CAM MRE 30 KPa (upper right of plot). RNA-seq (red) and proteomics (green) data for each of these proteins were plotted and linked with the miRNA-seq (for miRNAs predicted to bind to these MREs). The regulation of the CAM MREs by miRNAs (stars, Fig. 2c) was further validated by individual MRE mutagenesis followed by functional assays.

Mass spectrometry sample preparation and analysis. Cell pellets were lysed in 50 μl of 25 mM ammonium bicarbonate (Fluka) buffer containing 1.1% SDS (Sigma), 0.3% sodium deoxycholate (Sigma), protease inhibitor cocktail (Sigma) and phosphatase inhibitor cocktail (Merck). Six 1.6 mm steel beads (Next Advance) were added and samples were homogenized with a Bullet Blender (Next Advance) at maximum speed for 2 min. Homogenates were subjected to centrifugation (12,000 rpm for 5 min). Lysates were added to 150 μl 1 digest buffer (1.33 mM CaCl₂, Sigma in 25 mM ammonium bicarbonate) containing immobilized trypsin beads (Perffinity Biosciences) and shaken at 1,400 rpm overnight at 37 °C. The resulting digests were reduced with 5 μl of 500 mM dithiothreitol (Sigma; in 25 mM ammonium bicarbonate) for 10 min shaking at 1,400 rpm at 60 °C and alkylated with 12 μl of 500 mM iodoacetamide (Sigma; in 25 mM ammonium bicarbonate; for 30 min shaking, in the dark, at room temperature). Trypsin beads were removed by centrifugation (10,000 rpm for 10 min). Supernatant were transferred to 1.5 ml LoBind Eppendorf tubes and acidified with 5 μl 10% trifluoroacetic acid (Riedel-de Haën in water) and cleaned by two-phase extraction (3× addition of 200 μl ethyl acetate (Sigma), followed by vortexing and aspiration of the organic layer). Peptides were desalted with POROS R3 beads (Thermo Fisher) using the manufacturer’s protocol and lyophilized. Peptide concentrations were measured by spectrophotometer (Direct Detect 300, Thermo). Peptides were assigned using Mascot (Matrix Science), searching against the SwissProt, TrEMBL mouse databases. The database was modified to search for cysteine acetylation (monoisotopic mass change: 57.021 Da), oxidized methionine (15.995 Da), hydroxylation of asparagine, aspartic acid, proline or lysine (15.995 Da) and phosphorylation of serine, tyrosine or threonine (79.966 Da).

A maximum of two missed cleavages was allowed for each protein. The search was run using the trypsin enzyme with a mass tolerance of 0.1 Da, allowing for the existence of species with a mass difference of 1 Da. The enzyme digestion was set to run with a minimum peptide length of 7 and a maximum of 50 peptides. Peptide identification was filtered via Mascot scores, selecting only those with a Benjamini–Hochberg false discovery rate of <0.05. Raw ion intensities from peptides belonging to proteins with fewer than two unique (by sequence) peptides per protein in the data set were excluded from quantification. Peptides from CAM proteins were only used for quantification if they were unique to the protein (that is not also found in the untreated control or the empty sensor).
is, they did not overlap with other protein sequences). Intensities were logged and normalized by the median logged intensity. Missing values were assumed as missing due to low abundance, as described \ref{10}. Imputation was performed at the peptide-level using a similar to PerkY implementation, where imputed values were imputed randomly from a normal distribution centred on the apparent limit of detection for the experiment. The limit of detection was determined by taking the mean of all minimum logged intensities and downshifting it by 1.66, where $\sigma$ is the standard deviation of the minimum logged peptide intensities. The width of this normal distribution was set to 0.93 as described in ref. \ref{9}. Fold-change differences in protein quantities were calculated by fitting a mixed-effects linear regression model for each protein with Huber weighting of residuals as described in ref. \ref{10} using the fitmix function (MATLAB) with the formula:
$$y_{\text{mix}} = \beta_0 + \beta_1 X + \epsilon,$$
where $\epsilon$ represents the log (intensity) of peptide $p$ belonging to protein $i$, under experimental treatment $t$. $\beta$ represents the effect sizes for the indicated coefficients. Peptide effects were considered as random effects, whereas treatment was considered as a fixed effect. $\beta_i$ denotes the intercept term and $\epsilon$ denotes residual variance. Standard error estimates were adjusted with empirical Bayes variance correction according to ref. \ref{10}. Conditions were compared with two-sided Bayes-adjusted $t$-tests with Benjamini–Hochberg correction for false positives.

Cell immunostaining and quantification. Cells seeded on fibronectin-coated PDMs were fixed with 4% paraformaldehyde (Electron Microscopy Sciences) in PBS. Cells were then permeabilized with 0.1% Triton X-100 in PBS, with 320 mM sucrose and 6 mM MgCl$_2$. Cells were PBS washed 3 times, blocked for 30 min with 1% BSA in PBS, then incubated overnight at 4°C with anti-YAP antibody (1:200; sc-101199, Santa Cruz Biotechnology), anti-RHOB (1:250; 191HCL, Thermo Fisher), anti-VINCULIN (1:200; V9131, Sigma–Aldrich), anti-STMN1 (1:200; ab52630, Abcam), anti-CTGF (1:200, ab6992, Abcam) and anti-PAXILLIN (1:800, RabMAb Y113; ab32084, Abcam) diluted in 1% BSA in PBS. Cells were washed 3 times with PBS and incubated at room temperature for 1 h with secondary antibodies (Alexa-488 anti-rabbit, Alexa-647 anti-mouse, 1:1,000; Thermo Fisher) and Alexa-645-conjugated phallolidin (1:1,000; Molecular Probes). Cells were washed three times with PBS and mounted with 4,6-diamidino-2-phenylindole (DAPI) in Fluoromount-G (SouthernBiotech). Cell areas were quantified using ImageJ by background subtracting, thresholding to generate cell masks and using the analyse particles function. YAP staining was quantified by taking the average nuclear YAP signal (in the area of the DAPI stain), divided by the average cytoplasmic YAP signal (in the area of the non-nuclear cell mask).

Immunostaining matrix constructs. Matrix constructs were rinsed in cold PBS and fixed overnight at 4°C in 4% formaldehyde (Pierce 16% formaldehyde, methanol free) in PBS. Fixation constructs were dehydrated, embedded in paraffin and 5-μm transverse sections cut with a Leica microtome. For immunostaining, we performed a rehydration protocol followed by antigen retrieval for 30 min at 96°C in a citrate buffer (pH 6). Sections were blocked with Odyssey PBS blocking buffer (LI-COR Biosciences) for 1 h and incubated overnight with primary antibodies diluted in blocking buffer: vimentin (1:1000; Cell Signaling) and phospho-myosin light chain (1:1000; Abcam). After extensive rinsing with PBS, sections were incubated with Alexa Fluor-647 anti-rabbit secondary antibody (1:500; Thermostatic) for 1 h at room temperature, thoroughly washed with PBS Tween 0.1% and slides were mounted in Fluoromount-G-DAPI (SouthernBiotech). Slides were imaged with an Olympus slide scanner microscope equipped with a x20 objective.

Zebrafish fin-fold regeneration. This study is compliant with all relevant ethical regulations regarding animal research. Zebrafish 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 (IACUC no. 2017-11473). WT (AB) and $mg\,ag2^−$ mutants were used. To generate the cflg6 MRE mutant, zebrafish AB were injected with 125 ng$\mu$g$\mu$-Cas9 mRNA and 75 ng$\mu$g$\mu$-gRNAs, designed as previously described \ref{13}. The gRNA sequence used to target the conserved MRE within the 3’ UTR human CTGF gene was (CTGF mRNA; Supplementary Table 6). Genomic DNA was isolated from a clutch of 15 injected and uninjected control embryos at 24 h post-fertilization (h.p.f.) using the Qagen DNeasy Blood and Tissue Kit. DNA (250 ng) and the Phoenix HotStart II Kit (Thermo Fisher) used to PCR amplify an approximately 300-bp region surrounding the intended MRE target (MRE Fw Amp, MRE Rev Amp; Supplementary Table 6). The T7 endonuclease I assay was used to detect mutations as described in the manufacturer’s protocol (New England Biolabs). PCR and T7 products were run on 3% agarose gels to verify the occurrence of insertions and deletions in the MRE sequence. The remaining embryos were grown to 48 h.p.f. and used for the fin-fold regeneration experiments (see below).

The zebrafish miR-124 and cflg6 sensor assay and mRNA injection were performed as described \ref{13}. For the fin-fold regeneration assay, we used 14 AB fish, 14 $mg\,ag2^−$ mutant embryos and 15 $mg\,ag2^−$ fish injected at the 1cell stage with 150 pg of mRNA encoding the human FGF10 transcript. At 2 d post-fertilization, the fin fold was cut at the edge of the fin using a 25-G needle. Bright-field images were captured at 0.5, 2, 4, 24, 48 and 72 h.p.f. using a Leica M165 FC stereomicroscope and Leica Application Suite V4 software. The length of the fins over time was measured using Fiji-Image \ref{14} and normalized for the length of the fin before cutting.

Zebrafish immunofluorescence assay. For the fluorescent images: 20 embryos for each genotype (AB, Ago2 mutant (−/−) and Cflg6 5’ UTR mutant) were cut and then at 0.5, 2, 4 and 24 h.p.f. were fixed in 4% paraformaldehyde overnight at 4°C. Embryos were washed 4–5 times with PBS 0.1% Tween, then incubated 2 h in PBS-T (0.1% Tween, 0.1% Triton X-100, 10% normal goat serum, 1% BSA and 0.01% sodium azide in PBS Tween). Zebrafish were stained following the protocol as in ref. \ref{13} using the primary antibody mouse anti-phospho-myosin light chain 2 (1:200; Cell Signaling), mouse anti-proliferating cell nuclear antigen (1:200; Dako), rabbit anti-fibronectin (1:200; Sigma), DAPI (1:1,000; Sigma), rabbit anti-CTGF (1:150; Abcam) and mouse anti-YAP (1:200; Santa Cruz Biotechnology), and the secondary antibody Alexa Fluor-488 anti-mouse (1:250, Thermo Fisher) and Alexa Fluor-596 anti-rabbit (1:250, Thermo Fisher). After staining, images were captured using a Leica Microsystems SP5 confocal microscope using a x40 objective. Max projections were generated and intensity was quantified using Fiji-Image. For each protein staining, the intensity profile of ~4–6 fish was calculated for each section of 80 μm in diameter within the wound and 50 μm from the fin-fold edge. The ratio of nuclear-to-cytosolic YAP was calculated before and during the fin-fold regeneration from confocal images thresholded using the DAPI channel to generate a binary mask for the nuclei. Using ImageJ, the binary mask was used to generate a nuclear and a cytosolic YAP image. Each was ratioed and normalized for the area. For the TUNEL assay, to detect apoptotic cells, embryos were fixed.
in 4% paraformaldehyde overnight and stored in 100% methanol at −20 °C. The TUNEL assay was performed using the ApopTag Red In Situ Apoptosis Detection Kit (Millipore).

AFM. Live zebrafish embryos (48 h.p.f.) were anesthetized using 1× tricaine in egg water and mounted on PDMS gels. The tips of fish tails were probed using a DNP-10 D tip (Bruker; nominal stiffness ~0.06 N m⁻¹) on a Bruker Dimension FastScan AFM immersed in egg water containing 1× tricaine. Probe deflection sensitivity was calibrated by taking indentation curves on glass, and the nominal tip stiffness was calibrated by thermal tuning (assuming a simple harmonic oscillator in water). Force versus deflection curves were collected for a ramp size of 1.5 μm at a rate of 750 nm s⁻¹ for at least 2 locations per fish, with 10–11 fish measured per group. The first 600 nm of the extension curves were fit with NanoScope Analysis Software version 1.5 (Bruker) assuming a Poisson’s ratio of 0.5 and using the Sneddon fit model.⁶⁷

Statistics and reproducibility. All of the statistical analysis were performed using Prism version 7.01 (GraphPad) and R, except for the peak identification, which used Piranha software to measure the significance of read coverage height for each mapped position using the zero-truncated negative binomial model. To confirm changes in cell area, focal adhesion number, YAP localization and traction force generation, t-tests were performed using Prism to assess the change in the mean between WT and AGO2 or MRE CRISPR–Cas9 mutant cells. These data sets contained more than 20 individual measurements for each condition and showed a log-normal distribution. For in vivo analysis of zebrafish WT and Ago2 mutants, changes in fin-fold tissue were analysed using t-tests; fin-fold regeneration was analysed via two-way analysis of variance (ANOVA) and Sidak’s multiple comparisons test for 4–6 individual fish for a data set using Prism. Figure legends indicate the exact number of measurements, the number of independent experiments and the statistical test used for each analysis performed.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
RNA-seq and small RNA-seq data for endothelial cells that support the findings of this study have been deposited in the GEO under accession codes GSE99686 and GSE11021. Small RNA-seq data for HDF have been deposited in the GEO under accession code GSE123008. HTS-CLIP data have been deposited in the Sequence Read Archive (SRA) under accession code PRJNA507245. Proteomics data have been uploaded to the PRIDE depository (PXD011882) and results are summarized in Supplementary Table S. Source data for all figures and supplementary figures have been provided as Supplementary Table 8.

References
47. Dobin, A. et al. STAR: ultrafast universal RNA-seq aligner. Bioinformatics 29, 15–21 (2013).
48. Li, H. et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics 25, 2078–2079 (2009).
49. Kozomara, A. & Griffiths-Jones, S. miRBase: annotating high confidence microRNAs using deep sequencing data. Nucleic Acids Res. 42, D68–D73 (2014).
50. Agarwal, V., Bell, G. W., Nam, J. W. & Bartel, D. P. Predicting effective microRNA target sites in mammalian mRNAs. elife 4, e05005 (2015).
51. Aranguren, X. L. et al. Unraveling a novel transcription factor code determining the human arterial-specific endothelial cell signature. Blood 122, 3982–3992 (2013).
52. Ritchie, M. E. et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res. 43, e47 (2015).
53. Bodenhofer, U., Bonatesta, E., Horejsi-Kainrath, C. & Hochreiter, S. msa: an R package for multiple sequence alignment. Bioinformatics 31, 3997–3999 (2015).
54. Elosegui-Artola, A. et al. Mechanical regulation of a molecular clutch defines force transmission and transduction in response to matrix rigidity. Nat. Cell Biol. 18, 540–548 (2016).
55. Robinson, M. D., McCarthy, D. J. & Smyth, G. K. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26, 139–140 (2010).
56. McCarthy, D. J., Chen, Y. & Smyth, G. K. Differential expression analysis of multifactor RNA-seq experiments with respect to biological variation. Nucleic Acids Res. 40, 4288–4297 (2012).
57. Goeminne, L. J., Gevaert, K. & Clement, L. Peptide-level robust ridge regression improves estimation, sensitivity, and specificity in data-dependent quantitative label-free shotgun proteomics. Mol. Cell. Proteomics 15, 657–668 (2016).
58. Tyanova, S. et al. The Perseus computational platform for comprehensive analysis of (pro)omics data. Nat. Methods 13, 731–740 (2016).
59. Smyth, G. K. Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. Stat. Appl. Genet. Mol. Biol. 3, Article3 (2004).
60. Berginski, M. E. & Gomez, S. M. The Focal Adhesion Analysis Server: a web tool for analyzing focal adhesion dynamics. F1000Res. 2, 68 (2013).
61. Gutierrez, E. & Groisman, A. Measurements of elastic moduli of silicone gel substrates with a microfluidic device. PloS ONE 6, e25534 (2011).
62. Han, S. J., Oak, Y., Groisman, A. & Danuser, G. Traction microscopy to identify force modulation in subresolution adhesions. Nat. Methods 12, 653–656 (2015).
63. Kapacee, Z. et al. Tension is required for fibripositor formation. Matrix Biol. 27, 371–375 (2008).
64. Narayanan, A. et al. In vivo mutagenesis of miRNA gene families using a scalable multiplexed CRISPR/Cas9 nuclease system. Sci. Rep. 6, 32386 (2016).
65. Schindelin, J. et al. Fiji: an open-source platform for biological-image analysis. Nat. Methods 9, 676–682 (2012).
66. Le Guyader, D. et al. Origins and unconventional behavior of neutrophils in developing zebrafish. Blood 111, 132–141 (2008).
67. Sneddon, I. N. The relation between load and penetration in the axisymmetric bousinesq problem for a punch of arbitrary profile. Int. J. Eng. Sci. 3, 47–57 (1965).
68. Uren, P. J. et al. Site identification in high-throughput RNA–protein interaction data. Bioinformatics 28, 3013–3020 (2012).
Reporting Summary

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### 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) |
| Novaalign |
| 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 PRJNAS07245;
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.

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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

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

### Methods

| Involved in the study | n/a |
|-----------------------|-----|
| 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, Santa Cruz, 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, Santa Cruz, 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, Thermo Fisher, 1:1000 IF)
  - Alexa-647 anti-mouse (A-21236, Thermo Fisher, 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, Thermo Fisher, 1:15000 WB)
  - Alexafluor-800 anti-rabbit (A32735, Thermo Fisher, 1:15000 WB)
  - Alexa-647 anti-rabbit (A-21245, Thermo Fisher, 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, 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, Thermo Fisher, 1:250 IF)
  - Alexa Fluor 596 anti-rabbit (A-11037, Thermo Fisher, 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 validated 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 lover 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.