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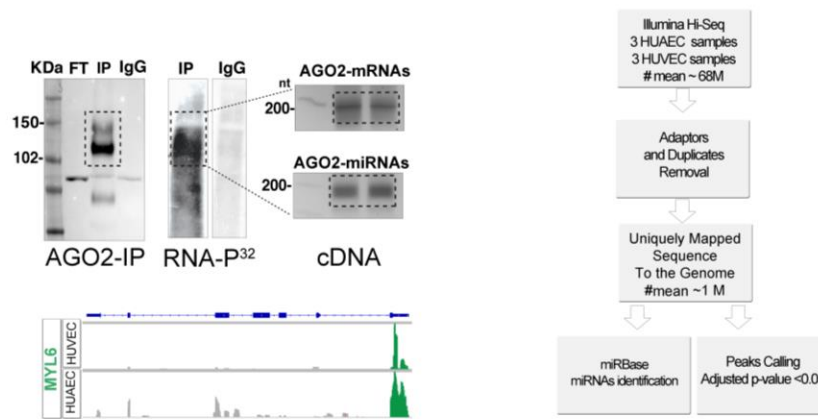
MicroRNA-dependent regulation of biomechanical genes establishes tissue stiffness homeostasis

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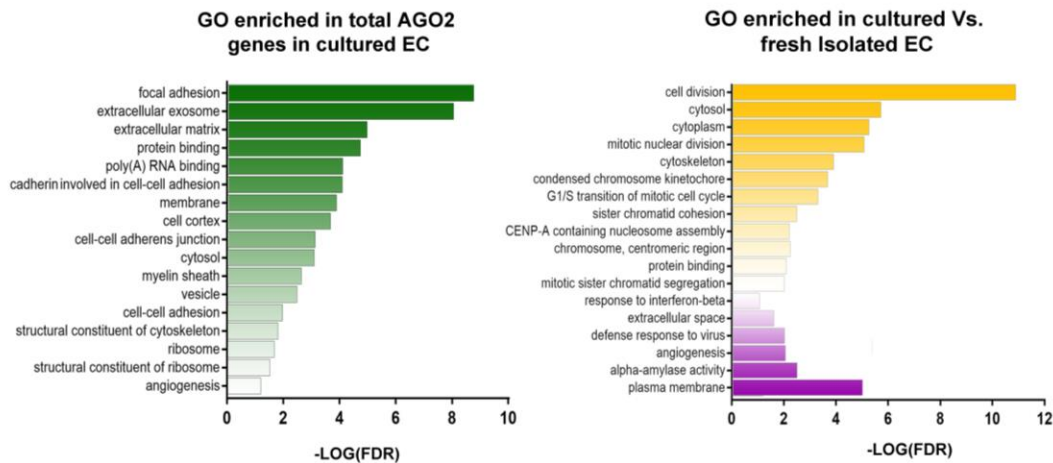
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a



b



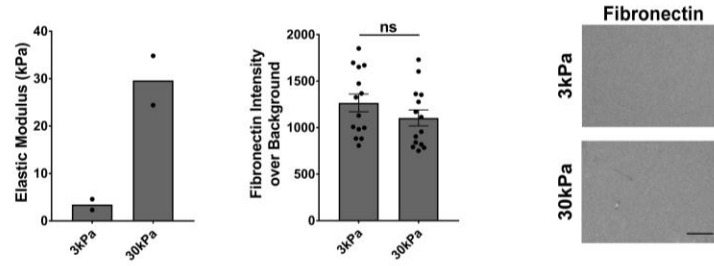
Supplementary Figure 1

AGO2-HITS-CLIP.

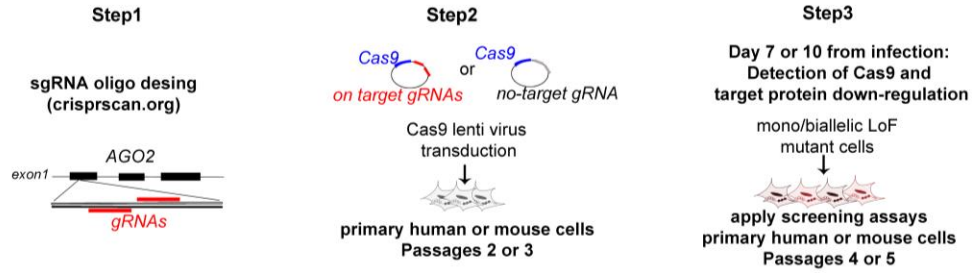
(a) Top left, SDS-PAGE of RNA-AGO2 or control IPs with nonspecific IgG were transferred to nitrocellulose and exposed to X-Ray film to reveal P32-RNAs bound to AGO2 (dotted square). P32 RNAs were isolated (dotted line) from nitrocellulose membranes and cDNAs generated with specific primers containing an Illumina barcode for high throughput sequencing (Methods). AGO2-mRNA complexes appear as ~200 nt bands (70 nt AGO-mRNA+ 120 nt Illumina primer), while AGO2-miRNAs are ~150 nt (30 nt miRNA+120 nt Illumina primer). DNA MW markers are indicated in the left lane of the gel. Bottom left, Integrative Genomics Viewer display of HITS-CLIP reads. Right, computational pipeline to identify reads from miRNAs and mRNAs in AGO2 IPs. Three replicates for each cell type (HUAEC and HUVEC) were processed as described in Fig.1a. 68 million

(M) total reads were analyzed. Upon removal of Illumina adapters and duplicates from PCR-based library preparation, reads were mapped to the human genome (UCSC hg19), resulting in 1M unique reads for each cell type. Reads were mapped to miRBase to identify miRNAs and processed using Piranha software to identify significant AGO2-binding sites (peaks) (methods). **(b)** Left, Bar graph of the Log(FDR) for the significant enriched Gene Ontology terms resulting from the HITS-CLIP assay and identified by DAVID software. Right, Bar graph of the Log(FDR) for the significant enriched Gene Ontology terms resulting from the microarray analysis of endothelial cells in culture versus freshly isolated.

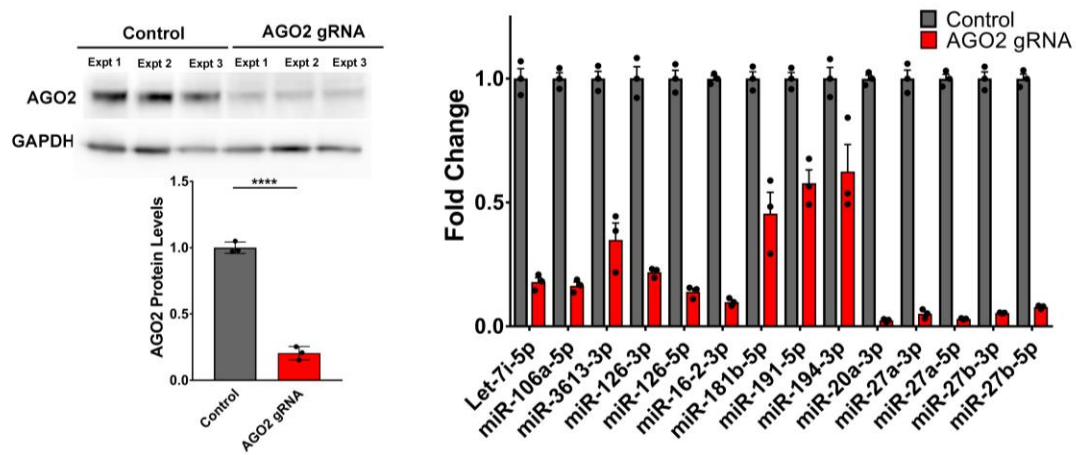
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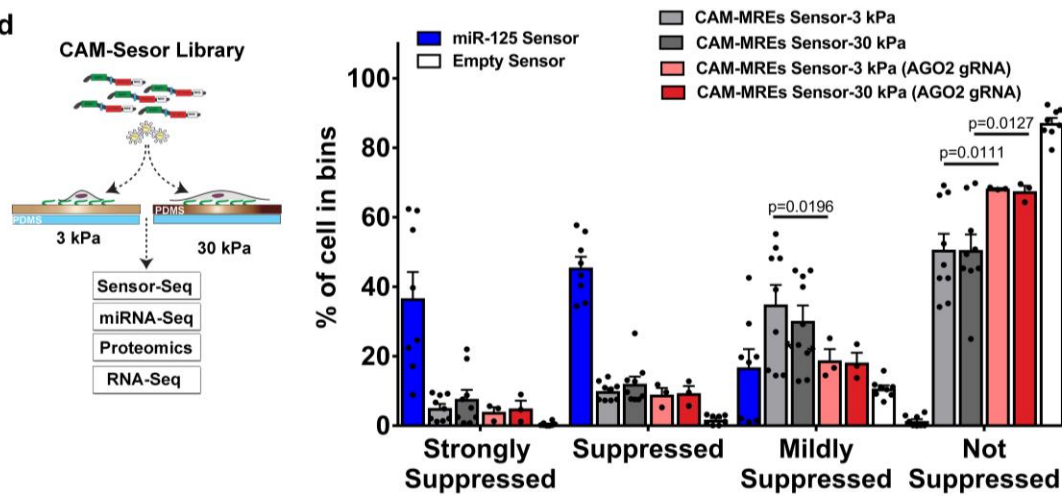
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c



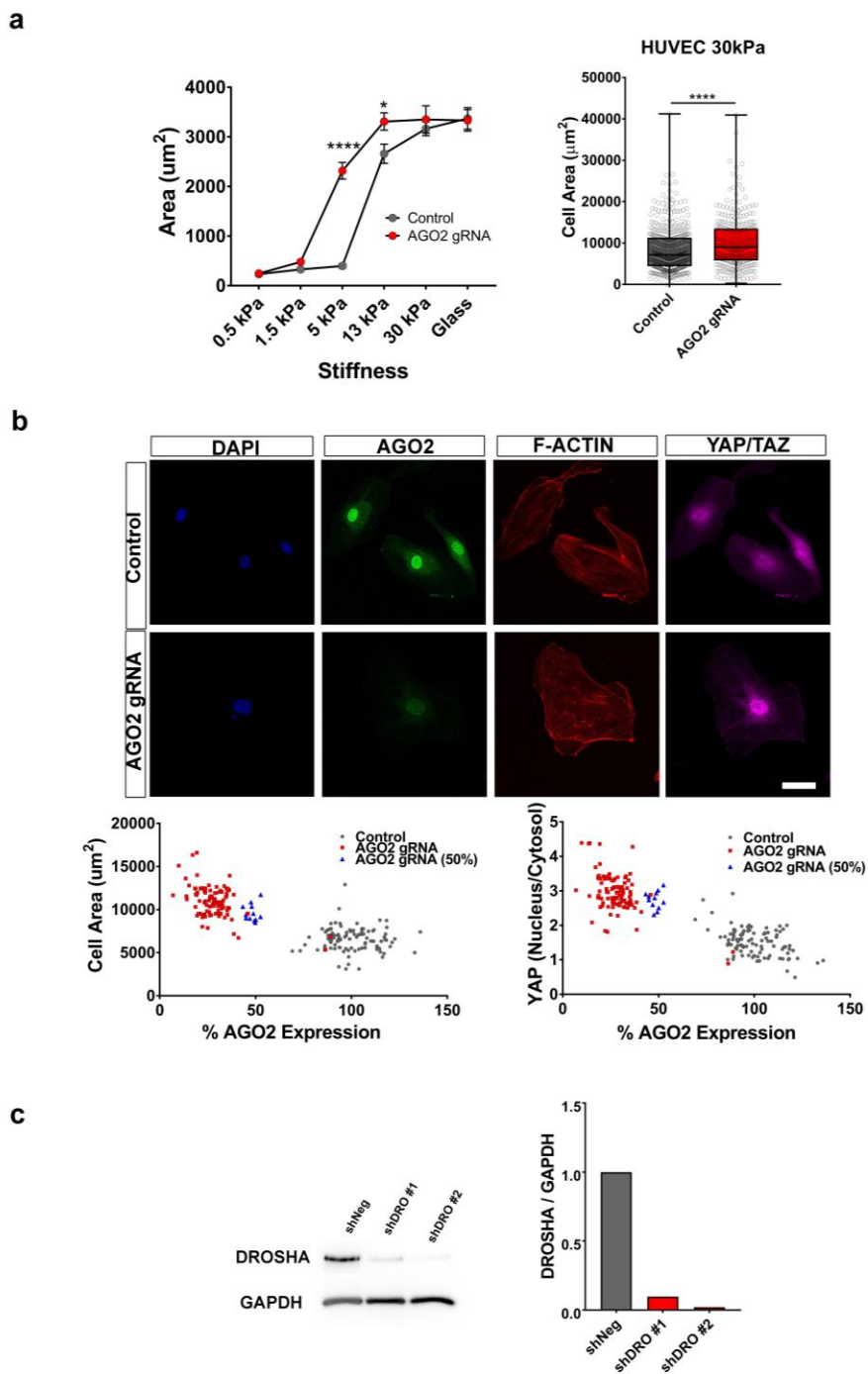
d



Supplementary Figure 2

Validation of AGO2 CRISPR/Cas9 targeting in HUVECs.

(a) Quantification of elastic modulus of PDMS gels by compression testing on an instron 5848 (bar = mean, n=2 independent gels per condition plotted as dots). Gels were compressed with a cylindrical indenter to 10% strain at 0.1%/s and allowed to stress-relax for 150 seconds, modulus was measured at equilibrium. Quantification of Fibronectin staining intensity on the two PDMS gels with representative images showing uniform Fibronectin deposition on the gel surface (mean $\pm$ SEM, n=14 fields of view per stiffness, ns = not significant, p=0.2167, unpaired two-sided t-test, scale bar = 10 μ m). (b) Schematics showing the experimental approach to generate AGO2 CRISPR/Cas9 mutant cells in primary human and mouse cells. (c) Left, Representative western blot of three independent replicates showing reduced AGO2 (~92kDa) levels in HUVECs with a lentivirus co-expressing Cas9 and AGO2 gRNAs, or a no-target gRNA (control, has no homology to any known mammalian gene). The bar graphs show quantification of the proteins normalized to GAPDH (mean $\pm$ SEM; n = 3 biologically independent experiments, **** p<0.0001, unpaired two-sided t-test). Right, qRT-PCR analysis of endothelial specific miRNAs in HUVECs infected with Cas9 and gRNA targeting AGO2 or no-target gRNA (control) at 7 days post infection (dpi). Results are shown normalized by one control for each microRNA (mean $\pm$ SEM; n = 3 biologically independent experiments). (d) Left, Schematics of CAM Sensor-Seq strategy. Sensor-seq lentivirus library was titrated to allow single vector copy integration and expression of physiological amounts of each GFP/mCherry transcript per cell. HUVECs were seeded on 3 and 30 kPa PDMS substrates for 48 h and analyzed as described. Right, bar plot representing distribution obtained upon FACS analysis of 10,000 wild-type or AGO2 gRNA mutant HUVECs infected with CAM MRE Sensors and treated as indicated (mean $\pm$ SEM; n=9 biologically independent experiments for control 3kPa, control 30kPa; n=8 biologically independent experiments miR125 and empty sensor; n=3 biologically independent experiments for AGO 3kPa and AGO 30kPa, p values indicated on plot, two-way ANOVA with Fisher's LSD). mCherry/GFP ratio of CAM MRE Sensors increase in AGO2 mutant ECs, as the cell distribution is shifted significantly toward the Empty-Sensor control bin in both 3 and 30 kPa stiffness conditions. Source data can be found in Supplemental Table 8. Unprocessed blots for 2c can be found in Supplementary Figure 8.

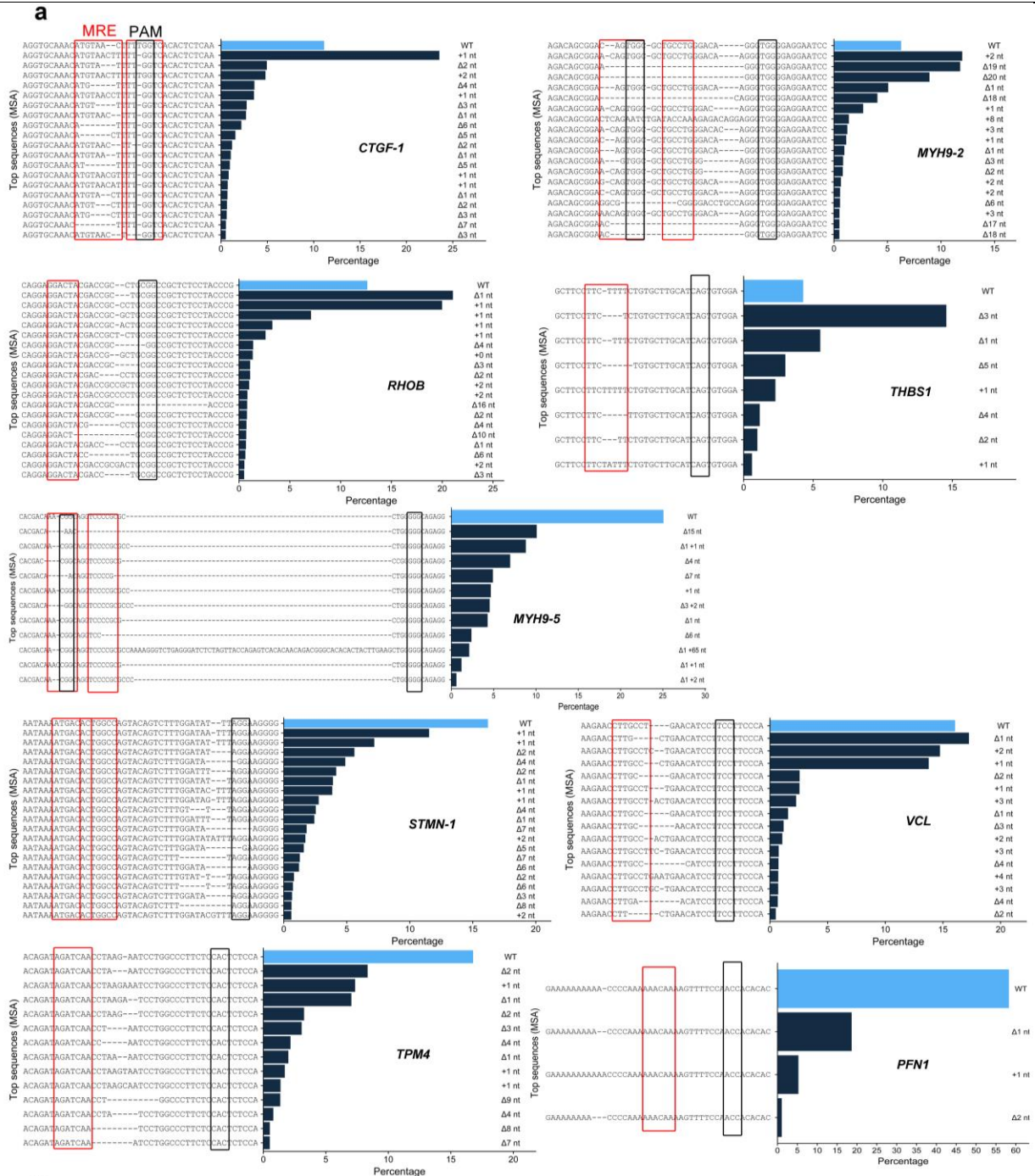


Supplementary Figure 3

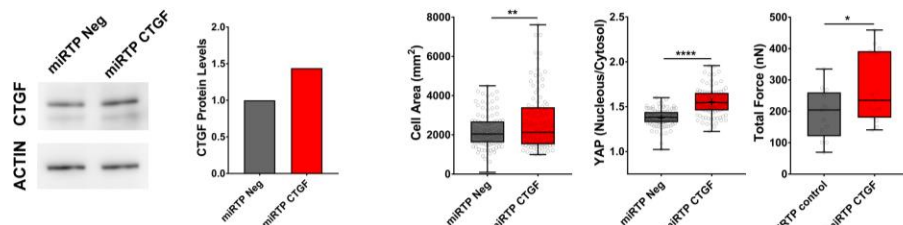
Validation of AGO2 or DROSHA loss of function in HUVECs.

(a) Left, Cell spread area using fibronectin coated polyacrylamide gels over a wider range of stiffness display results consistent with results from PDMS gel experiments (mean $\pm$ SEM, NT n=43, 52, 78, 79, 89, 59 cells from soft to stiff, AGO2 n=52, 52, 73, 63, 72, 54 cells from soft to stiff, * $p=0.0250$, **** $p<0.0001$, two-way

ANOVA with Sidak's multiple comparisons test). Right: Box plot representing HUVEC cell area for cells infected with AGO2 gRNA and non-targeting control seeded on fibronectin coated 30 kPa PDMS gels (box plots indicate minimum, maximum, median and quartiles, dots show single cell values, Control n=440, AGO2 n=289, **** p<0.0001, unpaired two-sided t-test). **(b)** Top, representative immunofluorescence images of HUVECs after infection with pLentiCRISPR virus directed at AGO2 or a non-targeting control seeded on fibronectin coated 3 kPa PDMS gels (scale bar = 50µm). Bottom: Quantification of AGO2 expression in n=100 Control and n=112 AGO2 cells. Intensity of the staining in each cell is normalized for the average intensity of all AGO2 cells and transformed in %. Cell area based on F-ACTIN staining or nuclear to cytoplasmic ratio of YAP/TAZ are plotted as individual value per cell. Highlighted in red cells infected with gRNA targeting AGO2, in black with no-target gRNA and in blue with gRNA targeting AGO2 expressing ~50% expression of AGO2 (~92kDa) compared to control cells. **(c)** Western blot and quantification of DROSHA (~150kDa) after knockdown with two distinct shRNAs delivered via lentivirus (5 days post infection, pSMART Dharmacon), normalized to GAPDH. Source data can be found in Supplemental Table 8. Unprocessed blots for 3c can be found in Supplementary Figure 8.



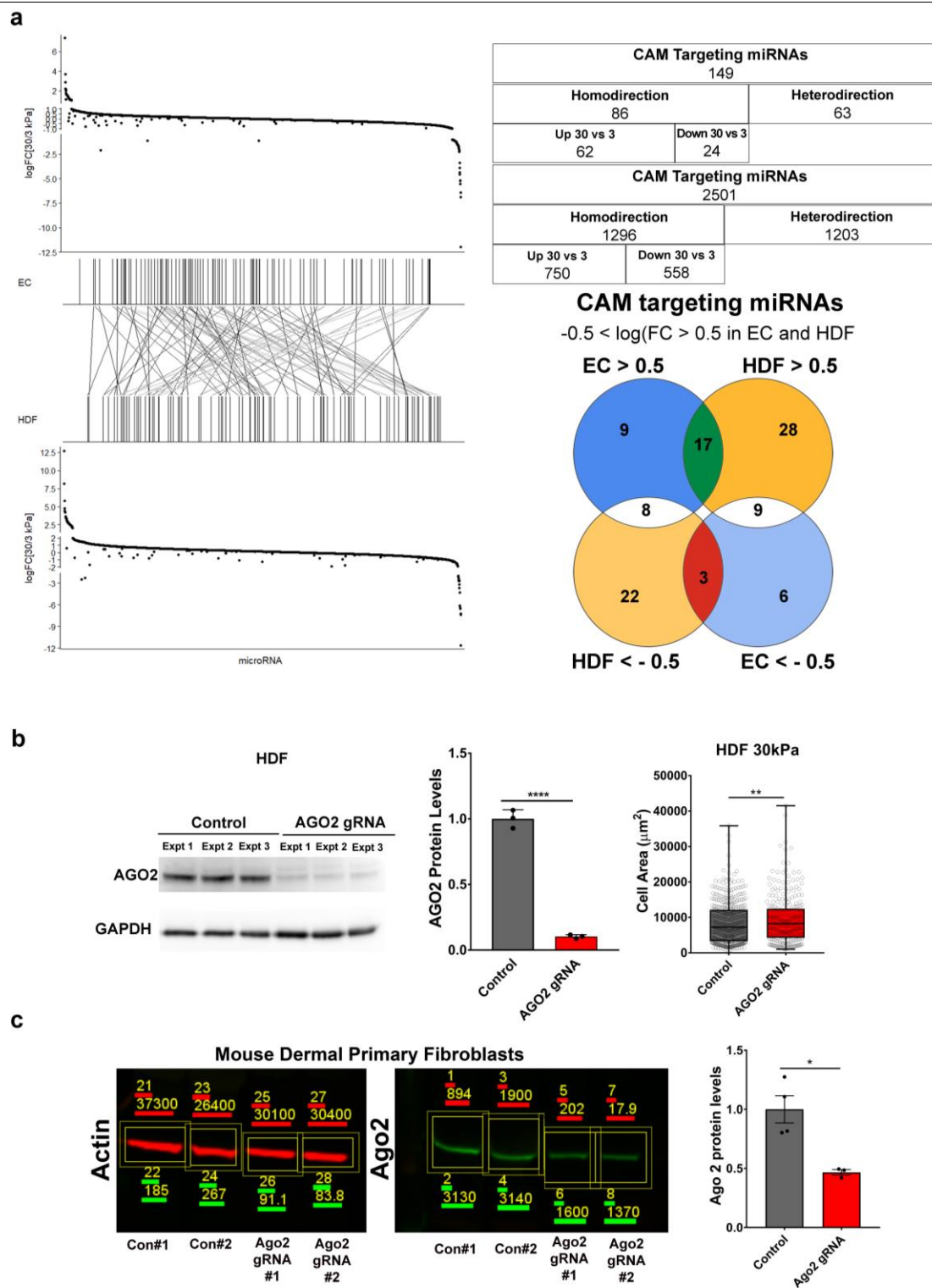
b



Supplementary Figure 4

Mutagenesis and targeting of CAM MREs.

(a) MiSeq 2x250 analysis of PCR amplicons derived from genomic DNA of HUVEC mutated with the indicated CAM MRE gRNAs. MREs are highlighted with red boxes, PAMs are highlighted with black boxes. Bar blot represent the % wild-type and mutated CAM 3'UTR sequences. Only mutations with a frequency > of 2% are represented. MSA= Multiple Sequence Alignment; nt= nucleotide. Numbers indicate the mutations as nt inserted (+) or deleted (Δ). **(b)** Left, Western blot of the CTGF protein (~37 kDa) normalized to β -ACTIN at 4 days post transfection. Right, box plots representing the quantification of mechanical parameters as in Fig. 4c of HUVECs transfected with miRTP (miRNA target protector) directed at CTGF 3'UTR for 4 days. miRTP is a single-stranded, modified RNA oligonucleotide that blocks a miRNA interaction with an individual MRE. Negative control target protector (QIAGEN) has no homology to any known mammalian gene. Box plots indicate minimum, maximum, median and quartiles. Cell spread area (miRTP Neg n=109 cells, miRTP CTGF n=103 cells, **p<0.004, unpaired two-sided t-test), YAP nuclear/cytosol ratio (miRTP Neg n=98 cells, miRTP CTGF n=101 cells, **** p<0.0001, unpaired two-sided t-test), and total force per cell (miRTP Neg n=13 cells, miRTP CTGF n=15 cells, * p=0.0398, unpaired two-sided t-test). Source data can be found in Supplemental Table 8. Unprocessed blots for 4b can be found in Supplementary Figure 8.

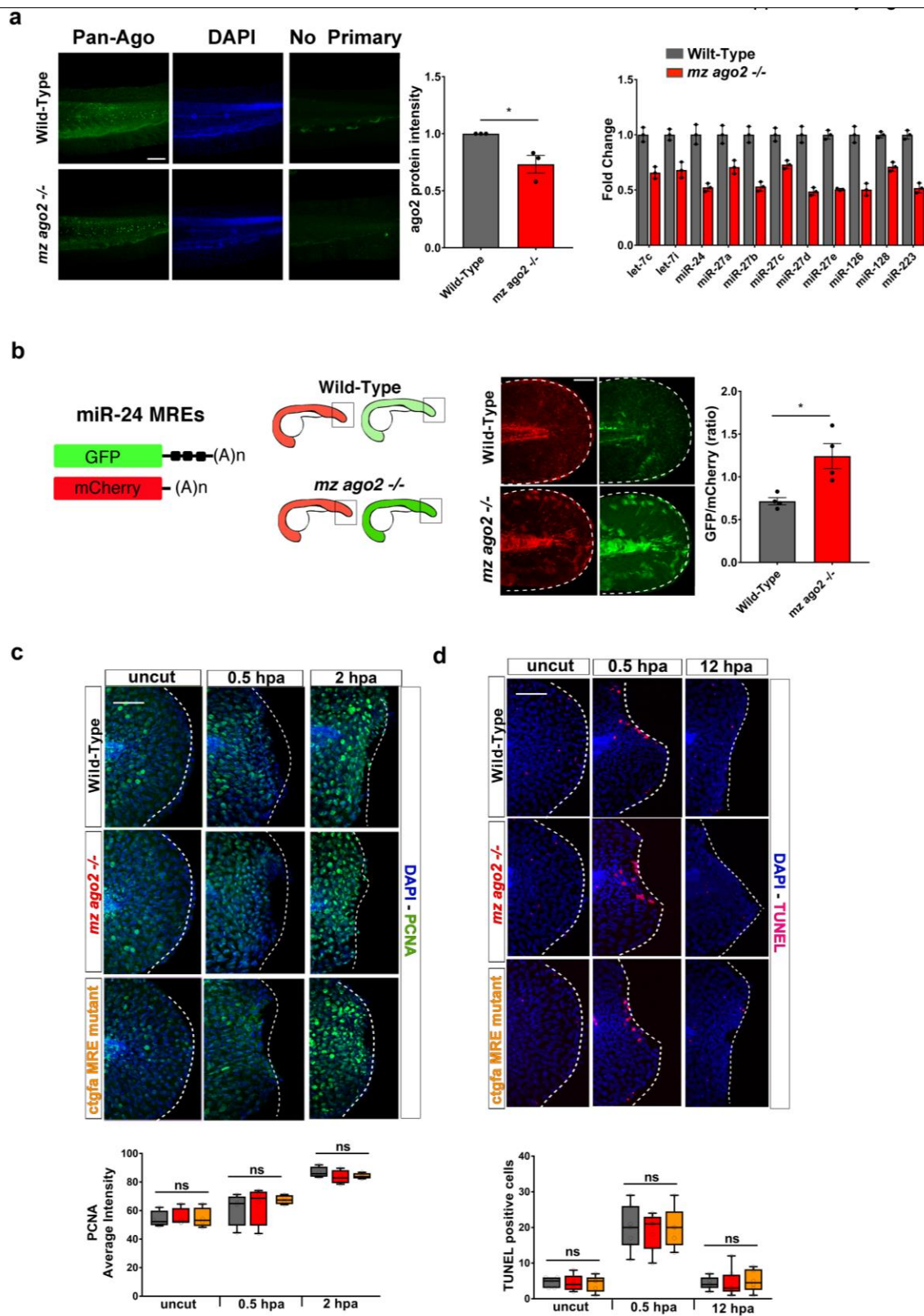


Supplementary Figure 5

Analysis of miRNAs and AGO2 loss of function in fibroblast.

(a) DEG for miRNAs at 30 kPa vs. 3 kPa (left) and table with the results (right top). Scatterplots show miRNA

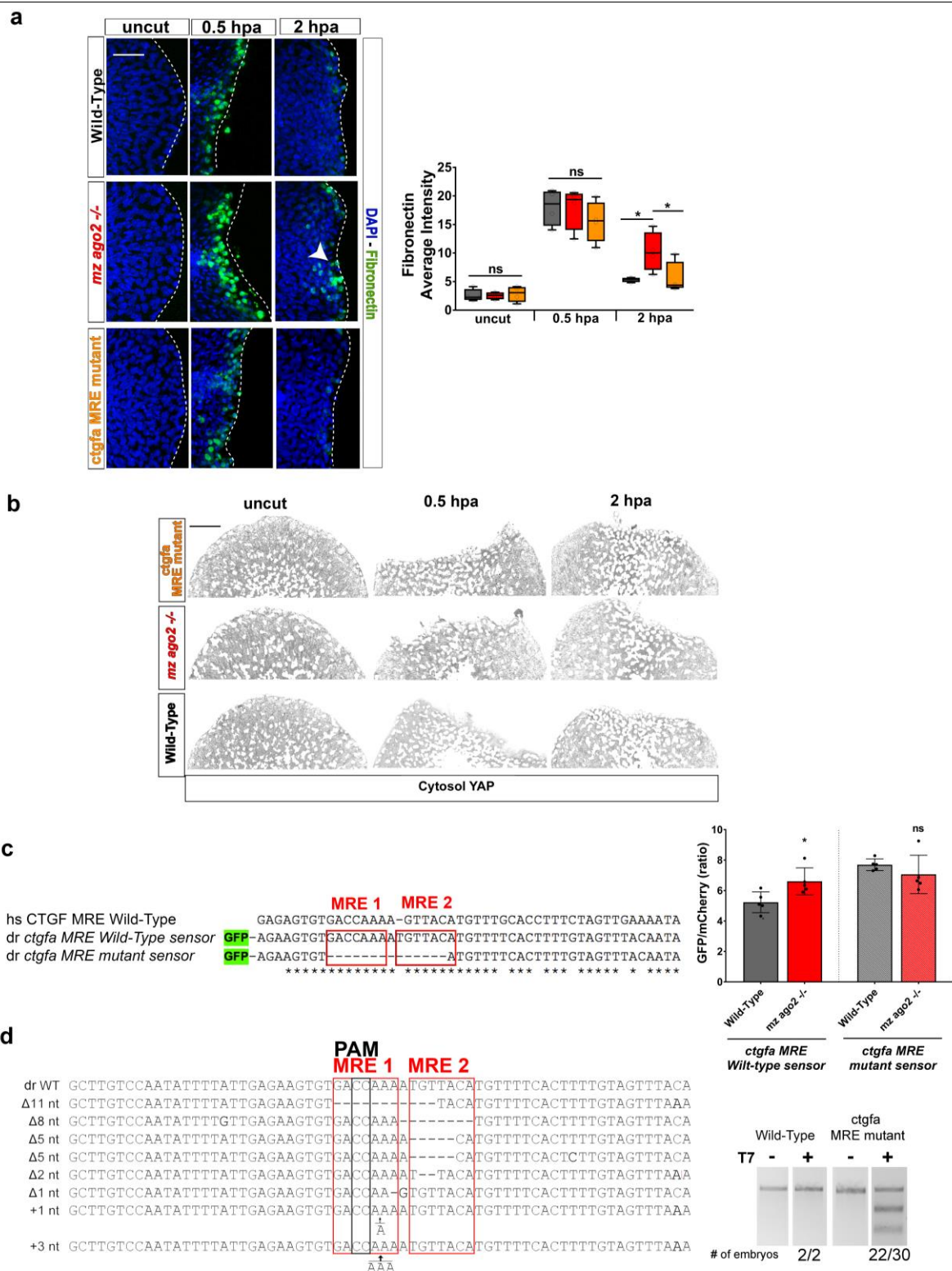
in HUVEC and HDF. miRNAs are plotted following a decreasing DEG in both graphs. The rug plot shows the localization of the miRNA targeting CAM-MRE (CAM targeting miRNAs). Each miRNA in the EC rug plot is connected with the same miRNA in HDF plot. miRNAs with similar behavior in both conditions (overexpression or down-regulation at 30 vs 3 kPa in EC and HDF) are 58%, compared to 51% of total miRNAs with same behavior (p-Value=0.1946). Within the miRNA group with similar behavior, 72% are up regulated on 30 vs. 3 kPa (against a total trend of 56% [Z-score pValue = 0.006432]). Bottom right, Venn diagram representing the overlap between CAM targeting miRNAs with a cut off of $|\log_2(FC)| > 0.5$ in EC (Blue circle) and HDF (Yellow circle). With a proportion of 31% upregulated CAM targeting miRNAs [17/54 in green sector] against a proportion of 10% downregulated [3/31 in red sector] the Z score proportion test reveals a significant enrichment of common upregulated miRNAs (two tail Z-score test pValue = 0.04385). **(b)** Left, western blot of 3 experiments showing AGO2 in HDFs infected with Cas9 and AGO2 gRNAs or a non-targeting gRNA (control) at 7 dpi with quantification of AGO2 normalized to GAPDH (n=3 experiments, **** p<0.0001, unpaired two-sided t-test). Right, box plot representing HDF cell area for cells infected with AGO2 gRNA and non-targeting control seeded on fibronectin-coated 30kPa PDMS gels (box plot indicates minimum, maximum, median and quartiles, dots show single cell analysis, Control n=311 cells AGO2 n=264 cells, **p=0.0009, unpaired two-sided t-test). **(c)** Representative blot of Ago2 levels in mouse skin fibroblast for 3D culture infected with gRNAs targeting Ago2 and no-target gRNA. Blots were obtained with Odyssey system from Licor to quantify near-IR fluorescence emitted by the secondary antibodies. Bar plots indicate densitometry measurements of Ago2 protein normalized for β -Actin at 7 dpi (mean $\pm$ SEM, Control n=4, AGO2 n=3, *p=0.0261). Source data can be found in Supplemental Table 8. Unprocessed blots for 5b can be found in Supplementary Figure 8.



Supplementary Figure 6

Reduced levels of Ago2 in *mz ago2* ^{-/-} zebrafish model.

(a) Left, Confocal lateral view of whole mount zebrafish embryos at 48 hpf. Wild-Type and *ago2* maternal zygotic homozygous mutant (*mz ago2*^{-/-}) were stained with the Ab-panAGO-2A8 and secondary only as a control of the total staining background (scale bar = 100µm). Bar plots indicate average of Ago2 fluorescence intensity normalized for the DAPI staining for each genotype (mean ± SEM, n=3 replica with 10 embryos / group, * p=0.0261, unpaired two-sided t-test). Right, RT-qPCR representing miRNAs level in wild-type vs *mz ago2*^{-/-} at 72 hpf (mean ± SEM, n=3 embryos per group, value normalized for one wild-type embryo). (b) Left panel, schematics representing the experimental procedure to test defective miRNA-mRNA interaction in the *mz ago2*^{-/-} fin fold model. *In vitro* transcribed RNAs encoding for mCherry control and GFP coding sequence upstream of three perfect MREs for miR-24, a miRNA expressed in epidermis²⁹. 25 picograms (pg) of each mRNA was co-injected into wild-type and *ago2*^{-/-} zebrafish embryos at the one cell stage post fertilization. Right panel, confocal whole mount image of 48 hpf embryos expressing GFP and mCherry (head is to the left, scale bar = 60µm). GFP and mCherry pixel intensities were quantified and the GFP/mCherry ratio was plotted for each genotype (mean ± SEM, n=4 embryos / group, *p=0.0137, unpaired two-sided t-test). (c) and (d) Top, Confocal lateral view of whole mount zebrafish embryos treated as indicated after zebrafish fin fold assay (scale bar = 120µm). White dotted line shows the edge of the wound. Bottom, box plot with minimum, maximum, median and quartiles representing the quantification of each whole mount stained embryos as indicated. Average intensity was calculated for 4 individual fish for PCNA and 4 to 6 fish for TUNEL assay. (Single dots represent single embryos, n.s.= non-significant). Source data can be found in Supplemental Table 8.

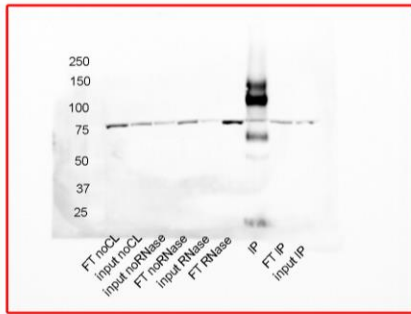


Supplementary Figure 7

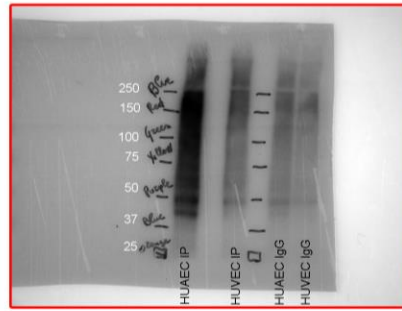
Fin fold regeneration model in zebrafish wild-type and mutant embryos.

(a) Left, Confocal lateral view of whole mount zebrafish embryos treated as indicated (scale bar = 120 μ m). White dotted line shows the edge of the wound. Right, quantification of each whole mount stained embryos as indicated. Pixel matrix of intensity was reconstructed for 50 μ m within the wound edge to the fish body. Average intensity was calculated for 4 fish within each group and show as box plot with minimum, maximum, median and quartiles (Single dots represent single embryos, * in wt 2h vs. ago 2h = 0.0127; * in ago 2h vs. ctgfa 2h = 0.0174; n.s.= non-significant; unpaired two-sided t-test) **(b)** Representative confocal lateral images of YAP immunostaining in the uncut fin fold. A DAPI binary mask was used to generate the Nuclear YAP (Fig. 7c) and Cytosol YAP images. **(c)** Alignment of human and zebrafish *ctgfa* 3'UTR sequence containing the conserved MREs. This 3' UTR sequence was used to generate a wild-type and mutated miRNA sensor vector (as above). An in vitro transcribed mRNAs encoding for a GFP reporter was under the post-transcriptional regulation of *ctgfa* 3'UTR (wild-type), a mutated version lacking the MREa sequence and co-injected with an mCherry mRNA with no 3UTR regulation as negative control. 75 picograms (pg) of each mRNA was co-injected into Wild-Type and *mz ago2*^{-/-} zebrafish embryos at the one cell stage post fertilization. GFP and mCherry pixel intensities were quantified and the GFP/mCherry ratio was plotted for each genotype (mean $\pm$ SEM, n=5 embryo / group, *p=0.0253, unpaired two-sided t-test). **(e)** Sequence alignment of the *ctgfa* 3UTR of zebrafish wild-type and *ctgfa* MRE mutant embryos injected with Cas9 and the gRNAs which PAM region was highlighted in black. Mutations are represented as nucleotide (nt) inserted (+) or deleted (Δ) and were cloned from individual embryos 24 hours post injection. Red boxes represent the MREs targeted. Representative agarose gel for T7 Endonuclease I assay for Wild-Type and *ctgfa* MRE mutant zebrafish. Source data can be found in Supplemental Table 8. Unprocessed blots for 7b and d can be found in Supplementary Figure 8.

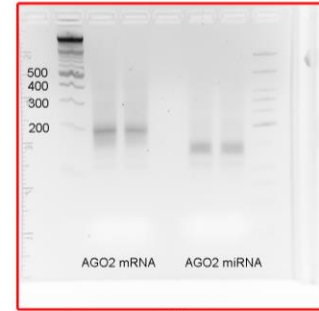
Figure 1_b



Western Blot AGO2-IP

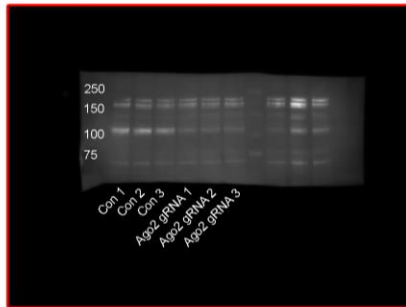


Autoreadiography RNA-P32

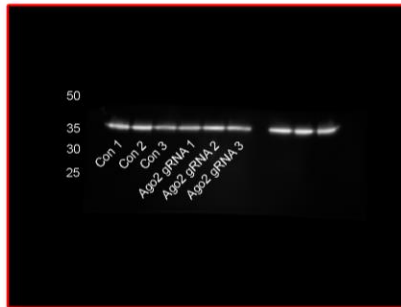


cDNA

Supplementary Figure 2_c

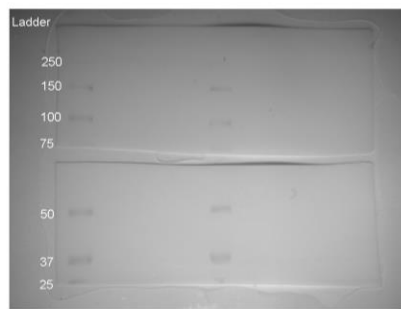
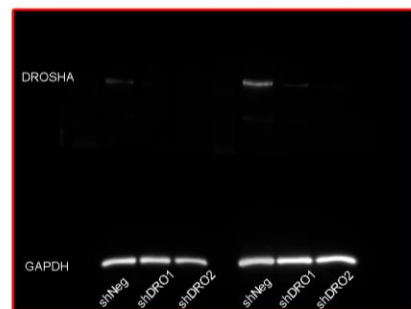


Western Blot AGO2 HUVECs

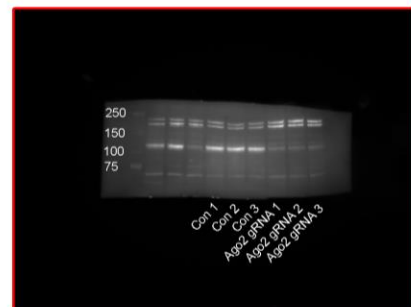


Western Blot GAPDH HUVECs

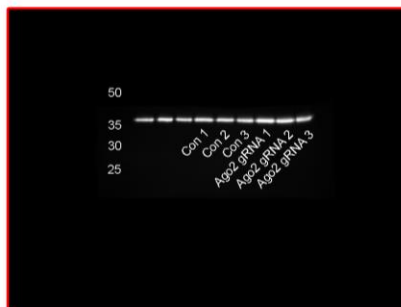
Supplementary Figure 3_c



Supplementary Figure 4_b



Western Blot AGO2 HDFs

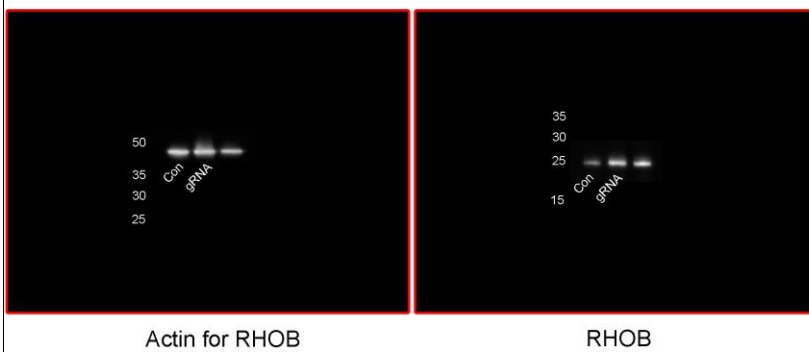
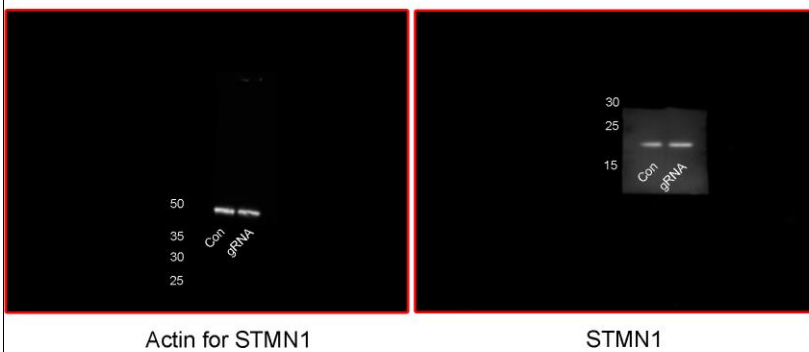
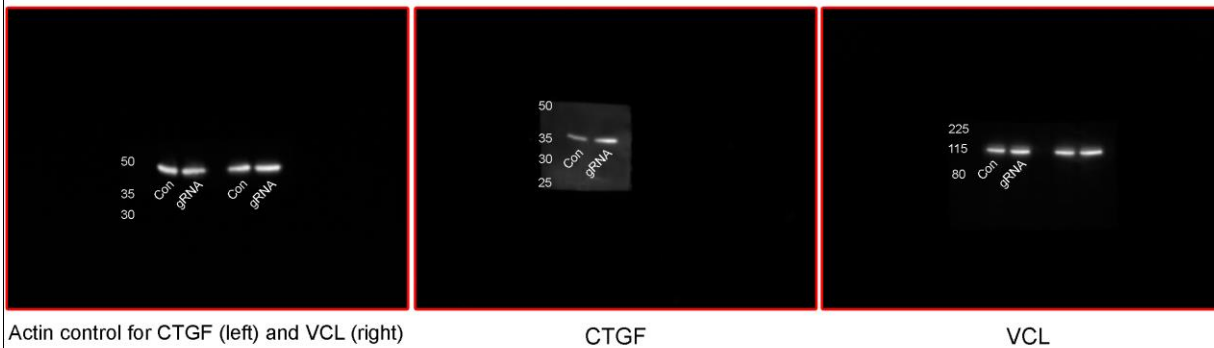


Western Blot GAPDH HDFs

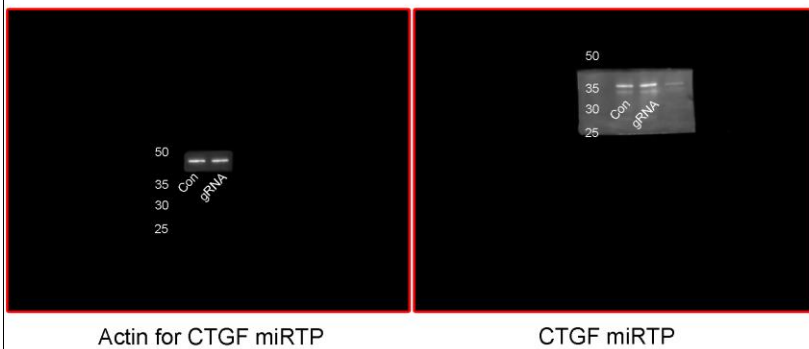
Supplementary Figure 8: Unprocessed scans of blots and gels

This figure contains the unprocessed scans of blots and gels shown in the figures. Each blot or gel is labeled with the lanes, antibody used, and the corresponding figure.

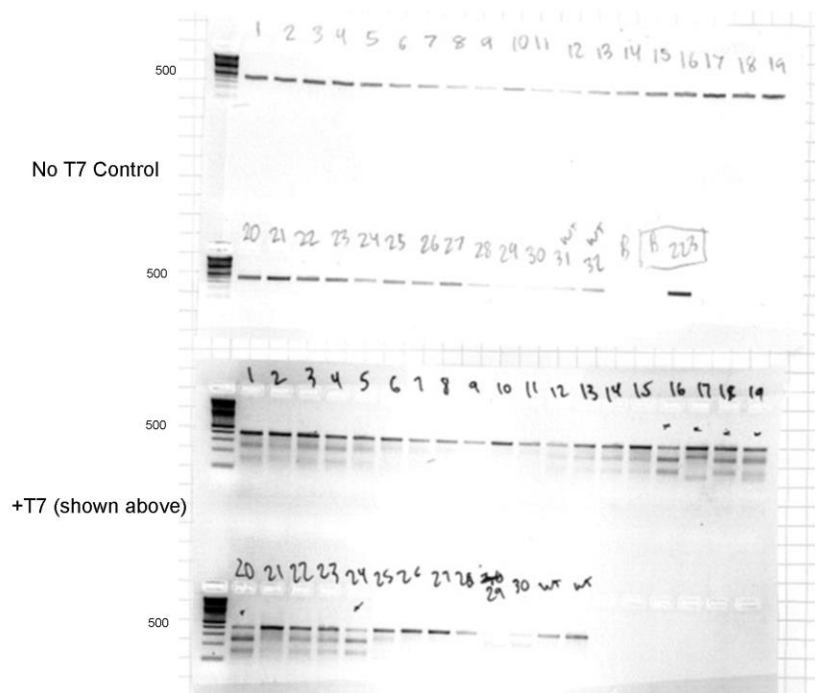
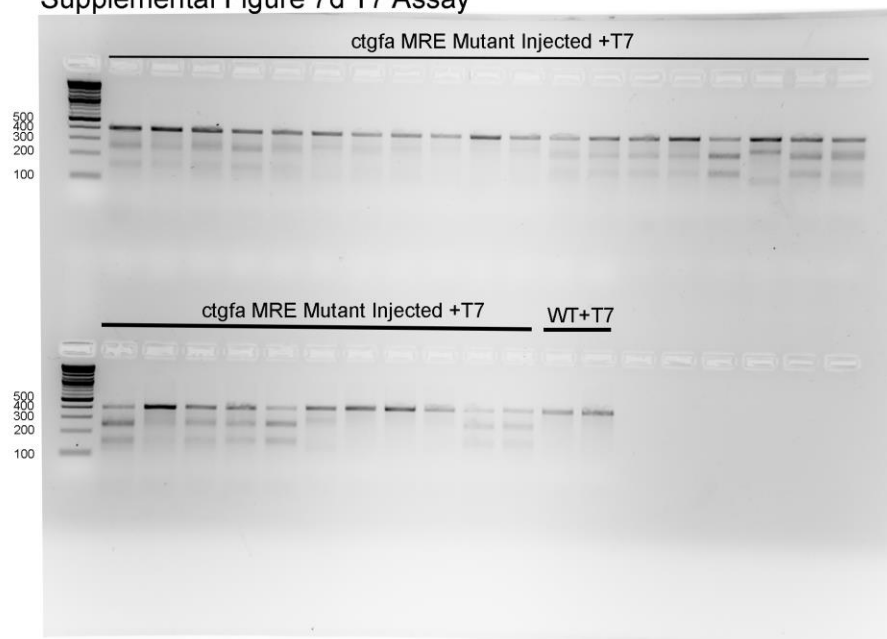
Figure 4b



Supplementary Figure 4_b



Supplemental Figure 7d T7 Assay



Supplementary Figure 8 Continued

Supplementary Table 1. AGO2-peaks localization.

The table shows the chromosome location and strand, gene name and Ensembl Gene ID and the peak p-value calculated with the Piranha (Methods). The last two columns show the number of genes associated with AGO2 and the number of peaks identified from the AGO2-HITS-CLIP.

Supplementary Table 2. AGO2-miRNAs are clustered in 155 families.

The table lists the miRNAs associate with AGO2 complex in endothelial cells (HUVEC and HUAEC). For each miRNA, the mature sequence and the miRbase ID are shown. MiRNAs are grouped in families based on the 7-mer SEED region (column Family and SEED).

Supplementary Table 3. miRNA-CAM-MRE interaction network.

The table represents the interaction network between the selected CAM-MRE and the endothelial miRNA within the AGO2 complex. The network was generated using TargetScan v. 7.0 (method) using CAM peaks listed in Table 1 and miRNA family listed in Table 2. Site_type, UTR_start and UTR_end columns represent the seed interaction type base (6mer < 7mer-1a < 7mer-m8 < 8mer-1a) and the localization of the interaction site within the Sensor_MRE_sequence (Table 4).

Supplementary Table 4. CAM-MRE sequences.

The table lists the selected CAM sequence used for the Sensor-seq assay (method) and reported in Fig. 2. Canonical Gene name, MRE identity, extended MRE sequence, and length of the sequences cloned into the Sensor-Seq vector (method) are reported.

Supplementary Table 5. Proteomics results.

Table listing fold-changes to proteins in HUVEC cells seeded on soft (3 kPa) vs. stiff (30 kPa) gels and in cells infected with AGO2 gRNA vs. non-target gRNA. Columns indicate the gene names, the number of peptides that were used for quantification, log2 fold-changes (FC) and Benjamini-Hochberg false-discovery corrected p-values (FDR; two-sided Empirical Bayes adjusted t-tests). Note that fold-changes to CAM proteins were calculated using only peptides unique to that protein (i.e. no overlaps with other proteins). Fold-change values were used to generate the scatter plot in Figure 3b.

Supplementary Table 6. List of oligos. The table list all the oligos used for gRNA preparation, cloning, library preparation and all the shRNA used (methods). Oligos are grouped base on the assay they were used, and ID and sequence are reported.

Supplementary Table 7. miRNA differentially expressed between EC and HDF. This table list the miRNAs differentially expressed in HUVEC and HDF seed on soft (3 kPa) or stiff (30 kPa) PDMS gel. DEG and pValue were calculate as exact test for the negative binomial distribution using edgeR.

Supplementary Table 8. Compiled figure source data.

This excel sheet contains all the source data used to generate the plots in the figures. Each figure and supplemental figure is separated as its own sheet in the file with each panel of the figure labeled and boxed in bold. Independent replicates for experiments where representative data are shown are included below the main data.

Supplementary Table 9. Polyacrylamide gels of varying stiffness.

Volumes of acrylamide (aa) and bis-acrylamide (bis-aa) used to generate poly-acrylamide gels of varying stiffness. NHS-acrylate incorporation allows for covalent linkage of extracellular matrix (fibronectin) to the gels. Gels were polymerized using the volumes of 10% ammonium persulfate and TEMED listed.