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Albertomaria Moro, Tristan P. Driscoll, William Armero, Liana C. Boraas ...+13 more authors

Institutions: Yale University, Wellcome Trust Centre for Cell-Matrix Research, Université libre de Bruxelles, University of Manchester

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1 **microRNA-dependent regulation of biomechanical genes establishes tissue**
2 **stiffness homeostasis**

3

4 Albertomaria Moro^{1#}, Tristan Discroll^{1#}, William Armero¹, Liana C. Boraas¹, Dionna M.
5 Kasper¹, Nicolas Baeyens², Charlene Jouy², Venkatesh Mallikarjun^{2,3}, Joe Swift^{2,3}, Sang
6 Joon Ahn¹, Donghoon Lee^{4,5}, Jing Zhang^{4,5}, Mengting Gu^{4,5}, Mark Gerstein^{4,5,6}, Martin
7 Schwartz^{1,2,9*} and Stefania Nicoli^{1,7,8*}

8

9

10

11 ¹Yale Cardiovascular Research Center, Department of Internal Medicine, Section
12 of Cardiology, Yale University School of Medicine, New Haven, CT 06511, USA

13 ² Wellcome Trust Centre for Cell-Matrix Research, ³ Division of Cell Matrix Biology and
14 Regenerative Medicine, School of Biological Sciences, Faculty of Biology, Medicine and
15 Health, Manchester Academic Health Science Centre, University of Manchester,
16 Manchester M13 9PL, UK

17 ⁴ Program in Computational Biology and Bioinformatics, ⁵ Department of
18 Molecular Biophysics and Biochemistry, ⁶ Department of Computer Science, Yale
19 University, New Haven, CT 06520, USA

20 ⁷ Department of Genetics, ⁸ Department of Pharmacology, Yale University School of
21 Medicine, New Haven, CT 06510, USA

22 ⁹Departments of Cell Biology and Biomedical Engineering, Yale University

23

24

25

26

27 # These authors contributed equally.

28 *Correspondence: martin.schwartz@yale.edu and stefania.nicoli@yale.edu.

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32 **Summary.** The mechanical properties of tissues, which are determined primarily by their
33 extracellular matrix (ECM), are largely stable over time despite continual turnover of
34 ECM constituents^{1,2}. These observations imply active homeostasis, where cells sense
35 and adjust rates of matrix synthesis, assembly and degradation to keep matrix and
36 tissue properties within the optimal range. However, the regulatory pathways that
37 mediate this process are essentially unknown³. Genome-wide analyses of endothelial
38 cells revealed abundant microRNA-mediated regulation of cytoskeletal, adhesive and
39 extracellular matrix (CAM) mRNAs. High-throughput assays showed co-transcriptional
40 regulation of microRNA and CAM genes on stiff substrates, which buffers CAM
41 expression. Disruption of global or individual microRNA-dependent suppression of CAM
42 genes induced hyper-adhesive, hyper-contractile phenotypes in multiple systems *in vitro*,
43 and increased tissue stiffness in the zebrafish fin-fold during homeostasis and
44 regeneration *in vivo*. Thus, a network of microRNAs and CAM mRNAs mediate tissue
45 mechanical homeostasis.

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49 Cells sense physical forces, including the stiffness of their ECM, through
50 mechanosensitive integrins, their associated proteins, and actomyosin. These factors
51 transduce physical forces into biochemical signals that regulate gene expression and
52 cell function^{2,3}. Tissues maintain nearly constant physical properties in the face of
53 growth, injury, ECM turnover, and altered external forces (e.g. from blood pressure,
54 tissue hydration or body weight)^{1,4,5}. These effects imply tissue mechanical homeostasis,
55 in which cells sense mechanical loads, due to both external and internal forces, and
56 adjust their rates of matrix synthesis, degradation and organization to keep tissue
57 properties constant. Cell contractility is critical in this process, as it is a key component of
58 both the stiffness-sensing regulatory pathways and of the matrix assembly process that
59 governs resultant matrix properties, including stiffness^{2,6}.

60 Mechanical homeostasis requires that integrin mechanotransduction pathways
61 mediate negative feedback regulation of the contractile and biosynthetic pathways to
62 maintain optimal tissue stiffness. That is, too soft/low force triggers increased matrix
63 synthesis and contractility, while too stiff/high force triggers the opposite. However, *in*
64 *vitro* studies have mainly elucidated positive feedback (or feed forward) circuits, where
65 rigid substrates or high external forces increase actin myosin contraction, focal

66 adhesions and ECM synthesis⁷. This type of mechanotransduction signaling often
67 characterizes fibrotic tissues, where sustained contractility and excessive ECM
68 compromise tissue function. Very little is known about negative feedback pathways that
69 are therefore critical to establish proper stiffness/contractility in normal, healthy tissues.

70 MicroRNAs (miRNAs) regulate gene expression at the post-transcriptional level⁸⁻
71 ¹⁰, and often function to reduce fluctuations in protein levels caused by changes in
72 transcriptional inputs or extracellular factors. miRNAs therefore participate in regulatory
73 feedback loops that contribute to homeostasis in multiple contexts¹¹⁻¹³. Studies of
74 miRNA regulation of biological processes often focus on a single or a few miRNA-target
75 gene interactions¹⁴⁻¹⁸. However, miRNAs appear to function within larger networks that
76 are likely critical for cellular functions^{11,19}.

77 miRNAs regulate target mRNAs via homologous base pairing. After transcription,
78 miRNAs are processed via the ribonucleases DROSHA/DRG8 and DICER²⁰ into mature
79 20-21 nucleotide (nt) hairpins that recognize abundant and conserved 7-8 nt miRNA
80 responsive elements (MREs) within mRNAs. MREs reside mainly in the 3' untranslated
81 regions (3'UTR) of mRNAs and base-pair with the 5' miRNA mature sequence (SEED
82 region)²¹. The miRNA-MRE pairs are recognized by the Argonaute2 (AGO2) protein
83 complex, resulting in mRNA destabilization and/or reduced protein expression²⁰.

84 To investigate a potential role for miRNAs in mechanical homeostasis, we
85 analyzed miRNA-mRNA interactions transcriptome-wide using an AGO2- HITS-CLIP
86 approach²². AGO2-bound miRNAs/mRNAs were isolated from two unrelated human
87 endothelial cells (EC) lines, which are known to respond to mechanical forces, including
88 ECM loads^{3,23}. We exposed cultured human umbilical artery ECs (HUAECs) and human
89 venous umbilical ECs (HUVECs) to UV light to cross-link protein-RNA complexes.
90 Subsequently, we immunoprecipitated AGO2-RNA complexes, digested unbound RNA
91 (schematic in Fig. 1a), and prepared cDNA libraries containing small (~30 nt AGO2-
92 miRNA) and large RNAs (~70 nt AGO2-target mRNA) (Fig. 1b). To identify conserved
93 AGO2 binding sites, we performed high throughput sequencing of three libraries for each
94 cell type and selected sequence reads shared in all six samples. We aligned these
95 AGO2 binding sites to human miRNA and genome databases, and identified 30-70 nt
96 interval (peaks) significantly enriched above background (p -value < 0.05, Fig. 1c and
97 methods). This analysis uncovered 316 AGO2-binding peaks within the 3'UTRs of 127
98 human genes. These peaks were preferentially located right after the stop codon or right
99 before the polyadenylation site (Fig. 1d and e, Supplementary Table 1), consistent with

100 the enrichment of regulatory miRNA binding sites that destabilize mRNAs²⁴. Importantly,
101 the human AGO2-binding peaks within these 30-70 nt sequences showed high
102 conservation across hundreds of species (Fig. 1e), suggesting functional significance.

103 Gene ontology (GO) analysis of AGO2-bound transcripts revealed that 73 of the
104 127 target mRNAs encode actin- and microtubule-associated proteins, focal adhesion
105 proteins, ECM proteins and functionally related regulatory proteins (Fig. 1f,
106 Supplementary Fig. 1a). We termed this group the cytoskeleton-adhesion-matrix (CAM)
107 genes. The dramatic enrichment of CAM transcripts in the AGO2 complex is not
108 accounted for by their abundance; indeed, the most transcriptionally active genes in
109 cultured ECs pertained to cell division (Supplementary Fig. 1b), which were under-
110 represented in the identified AGO2-binding transcripts. No significant GO terms were
111 associated with the remaining genes identified from the AGO2-HITS-CLIP.

112 We then searched for specific MRE sequences in AGO2-peaks localized in the
113 3'UTRs of the CAM transcripts. We identified 122 miRNA families from AGO2-HITS-
114 CLIP (Supplementary Table 2) that recognize one or more AGO2-CAM MREs (Fig. 1g,
115 Supplementary Table 3). Cytoscape software revealed a highly interconnected network
116 of miRNAs binding to CAM transcripts (Fig. 1g). Altogether, these data reveal pervasive
117 miRNA-mediated post-transcriptional regulation of multiple CAM genes in ECs.

118 CAM proteins are highly conserved and play crucial roles in virtually every cell
119 type, as determinants of ECM organization and tissue stiffness²⁵. This important
120 function led us to hypothesize that the CAM mRNA-miRNA regulome is
121 mechanosensitive. To test this, we plated ECs on substrates of varying stiffness and
122 used a Sensor-Seq strategy²⁶ to assess post-transcriptional regulation mediated by 97
123 selected MREs within 51 different CAM 3'UTRs (Supplementary Table 4). For this
124 purpose, we created an "MRE-sensor library". Each AGO2-3'UTR-peak containing at
125 least one MRE was cloned downstream of an mCherry reporter in a bidirectional
126 lentiviral vector²⁷ that co-expressed a GFP transcript lacking a 3'UTR (schematic in Fig.
127 2a). miRNAs that target the MRE thus reduce mCherry levels and decrease the
128 mCherry/GFP ratio. ECs infected with this sensor library at low levels (to avoid multiply
129 infected cells) were seeded for 48 hours on substrates with rigidity of 3 kPa
130 (kilopascal) or 30 kPa, which approximate "soft" and "rigid" tissues²⁸, respectively (Fig.
131 2a, Supplementary Fig. 2a). miRNA activity on the MRE sensors was compared with the
132 steady state level of CAM proteins, CAM RNAs and miRNAs expression in the same

133 cellular settings. Thus, proteomics, RNA and miRNA sequencing were assessed in
134 parallel (Fig. 2a).

135 To assess CAM-MREs sensor reporters, ECs were separated by fluorescence-
136 based sorting into bins according to the mCherry/GFP ratio, using an empty-Sensor as a
137 negative control (not suppressed) and a miR-125-Sensor as a positive control (strongly
138 suppressed). Thus, bins were defined as strongly suppressed, suppressed, mildly
139 suppressed, and not suppressed relative to these internal standards (Fig. 2b). Wild-type
140 ECs infected with our CAM-MREs Sensor library showed a broad distribution between
141 the suppressed and not suppressed bins, on both soft and stiff substrates (Fig. 2b).
142 Importantly, CRISPR/Cas9-mediated disruption of AGO2 diminished the miRNA levels in
143 CAM-MREs Sensor ECs (Supplementary Fig. 2b and c), and significantly increased the
144 population of 'not suppressed' cells (Supplementary Fig. 2d). Thus, miRNAs are required
145 for post-transcriptional inhibition of CAM-MRE Sensors.

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

157 To evaluate the function of this miRNA regulatory network, we first examined
158 ECs lacking AGO2 or DROSHA, which have diminished miRNA levels (Supplementary
159 Fig. 2 b and c and ²⁹). We used immunofluorescence to detect F-actin, the focal
160 adhesion marker paxillin, and the mechanosensitive transcription factor YAP³⁰, and also
161 assayed for traction stresses using elastic substrates with embedded beads³¹. Relative
162 to control cells, AGO2 mutant cells showed increased actin stress fibers, focal
163 adhesions, Yap nuclear localization and traction stress on both 3 or 30 kPa substrates
164 (Fig. 3a), as well as on polyacrylamide substrates over a wider range of stiffnesses
165 (Supplementary Fig. 3a and b). Consistent with these observations,
166 immunofluorescence analysis revealed that cell spreading and YAP nuclear activation

167 were inversely correlated with AGO2 levels (Supplementary Fig. 3c). Furthermore,
168 proteomic analysis of AGO2 mutant cells showed increased levels of several CAM
169 proteins, reminiscent of the increased CAM levels in ECs plated on 30 kPa versus 3kPa
170 substrates (Fig. 3b and Supplementary table 5). Importantly, human dermal fibroblasts
171 deficient in AGO2 protein had similar hyper-adhesive and contractile behaviors, as did
172 ECs lacking DROSHA (Supplementary Fig. 3d and e). Together these data suggest that
173 the loss of miRNA-mediated suppression of mRNAs increases CAM protein levels and
174 enhances cell contractility and adhesion in different cell types.

175 To further validate the function of the miRNA-CAM mRNA network, we disrupted
176 individual CAM-miRNA interactions. We chose nine of the mechanosensitive CAM
177 MREs (stars in Fig. 2c, and Supplementary table 6) in which the MRE was within 20 nt of
178 a protospacer sequence (PAM) and thus targetable by a guideRNA (gRNA) and Cas9.
179 Genome-wide analyses of CAM MREs in ECs treated with gRNA/CRISPR/Cas9
180 revealed insertions and deletions within the desired MRE region (Fig. 3c and
181 Supplementary Figs. 4 and 5). We found that mutation of individual CAM MREs de-
182 repressed CAM protein levels, as predicted for impaired miRNA-mediated suppression³²,
183 and increased, to varying extents, cell area, YAP nuclear localization and/or traction
184 stresses (Fig. 3d, Supplementary Fig. 6a).

185 While multiple genes clearly contributed to each effect, the gene whose MRE
186 mutation gave the most consistent effects across multiple assays was Connective
187 Tissue Growth Factor (*CTGF*). *CTGF* is a matrix protein that modulates the interaction of
188 cells with the ECM³³, suggesting that it is a component of a protein-based regulatory
189 network and likely functions via receptor-mediated signaling to control these functions.
190 Blocking *CTGF* miRNA-repression in ECs via a target protector RNA oligonucleotide or
191 MRE mutation had similar effects (Fig. 3d, Supplementary Fig. 6 a and b), providing
192 independent support. Notably, no single MRE mutation reproduced the strong phenotype
193 observed after AGO2 downregulation, suggesting that a network of miRNA-CAM mRNA
194 interactions mediates mechanical homeostasis in cells.

195 As a first approach to determine if the miRNA-mediated network functions at the
196 tissue level, we examined primary mouse dermal fibroblasts grown in a 3-dimensional
197 (3D) matrix. Cells suspended in attached fibrin gels contract and replace the fibrin with
198 their own matrix over about 5 days (Fig. 4a), providing a 3D model of cell behavior.
199 Transduction of these cells at passage 0 with a CRISPR/Cas9/gRNA virus targeting
200 *Ago2* reduced *Ago2* protein levels by ~50-60% (Supplementary Fig. 7a). *Ago2*-depleted

201 fibroblasts grown in 3D matrix generated tissue constructs with reduced diameters but
202 no significant change in cell numbers (Fig. 4b). Immunostaining transverse sections of
203 these constructs confirmed the decreased diameter, based on staining with the
204 cytoskeleton protein Vimentin (Fig. 4c). We observed elevated staining for
205 phosphorylated myosin light chain, demonstrating that reduced Ago2 levels led to
206 increased myosin activation and contractility³⁴ (Fig. 4c). These data suggest that
207 reducing miRNA-dependent regulation stimulates contractility in fibroblasts in a 3D
208 setting.

209 We next tested whether miRNAs regulate mechanical homeostasis *in vivo* using
210 the zebrafish fin fold regeneration model³⁵. The fin fold is a non-vascularized appendage
211 comprised of a few layers of epidermis and fibroblast-like cells³⁶. Wounding triggers a
212 healing response mediated by a conserved and rapid matrix remodeling- and
213 actomyosin-based process that involves formation of a provisional matrix, inflammatory
214 cell invasion, cell migration, proliferation and resolution³⁷.

215 To investigate miRNA-dependent regulation of mechanical homeostasis in
216 zebrafish, we first examined embryos that carry a maternal zygotic homozygous
217 mutation in *ago2* (*mz ago2 -/-*)³⁸, which show reduced levels of Ago2 and of miRNAs
218 (Supplementary Fig. 7b). To evaluate miRNA activity in the fin fold of *mz ago2 -/-*
219 embryos, we co-injected an miRNA-sensitive GFP mRNA, containing three perfect miR-
220 24 MREs within the 3'UTR³⁹, with an miRNA-insensitive mCherry control mRNA. As
221 expected, *mz ago2* mutants showed elevated levels of GFP, but not mCherry, when
222 compared to wild-type (WT) embryos, confirming reduced miRNA-mediated suppression
223 (Supplementary Fig. 7c). We then quantified tissue stiffness using atomic force
224 microscopy (AFM)-based nanoindentation on the central region of the fin fold. The
225 appearance of this tissue was indistinguishable between genotypes (Fig. 4d), ruling out
226 obvious developmental defects. However, the elastic modulus was ~30% higher in *mz*
227 *ago2 -/-* than WT embryos, indicating increased mechanical rigidity (Fig. 4d).

228 Importantly, normal tissue stiffness was restored upon injection of *in vitro* transcribed
229 mRNA encoding human AGO2 (*hsAGO2 mRNA*), demonstrating that the stiffness of this
230 tissue is dependent upon the level of Ago2 (Fig.4d). Following amputation, *mz ago2*
231 mutants exhibited slower repair than WT embryos and *mz ago2 -/-* embryos expressing
232 *hsAGO2 mRNA* (Fig. 4e). WT and *mz ago2 -/-* wounds did not display differences in cell
233 cycle progression, detected by Proliferating Cell Nuclear Antigen (PCNA) staining⁴⁰, or in
234 apoptosis, detected by TUNEL assay (Supplementary Figs. 8 a and b and 9a). These

235 results support that miRNA-dependent suppression is required mainly to restrain tissue
236 stiffness and contributes to tissue healing *in vivo*.

237 Wounding triggers increased contractility and matrix rigidity as a rapid, first
238 response. According to our notion of mechanical homeostasis, these changes should
239 activate negative feedback mechanisms that restore mechanical equilibrium³. We
240 therefore examined matrix, actomyosin activation and the mechanosensitive
241 translocation of Yap³⁰ before and after wounding the zebrafish fin fold in WT vs. *mz ago2*
242 mutant. As expected³⁷, WT embryos showed increased staining for pMyosin, Ctgfa and
243 Fibronectin^{34,37} in the wound area between 0.5 and 2 hours post amputation (hpa), (Fig.
244 5a and b, Supplementary Figs. 8 c and 9a). In comparison, *mz ago2* *-/-* wounded fins
245 showed strikingly elevated and persistent pMyosin staining at both 0.5 and 2 hpa, and
246 higher Ctgfa and Fibronectin at 2 hpa (Fig. 5a and b, Supplementary Figs.8c and 9a).
247 Consistent with the increase in tissue stiffness (Fig.4d), *mz ago2* *-/-* showed higher basal
248 Yap nuclear localization compared to WT embryos that further increased at 0.5 hpa and
249 persisted at 2 hpa after wounding (Fig.5c and Supplementary Fig. 9b). Thus, loss of
250 miRNA-mediated suppression leads to an exaggerated mechanical response and
251 impaired mechanical resolution during wound healing.

252 To correlate these effects to regulation of individual CAM genes, we generated
253 zebrafish embryos carrying mutations in the two 3'UTR MREs of the *ctgfa* gene
254 (Supplementary Fig. 8 d and e). These MREs are conserved in the human *CTGF* 3'
255 UTR, and their mutation had the largest effect *in vitro* (Fig. 3d, Supplementary Figs. 4
256 and 6). Accordingly, a GFP sensor mRNA bearing a *ctga* 3'UTR fragment showed
257 reduced expression in WT relative to *mz ago2* *-/-* embryos (Supplementary Fig. 8 d),
258 which required the MRE sites (Supplementary Fig. 8 d). These results support miRNA-
259 dependent inhibition of *ctgfa* via the MREs in zebrafish. We found that zebrafish with
260 mutated *ctgfa* MREs showed persistent p-myosin activation compared to WT by 2 hpa
261 (Fig. 5b), consistent with the induction of Ctgfa at 2 hpa in the *mz ago2* mutant (Fig. 5a).
262 However, no other differences were detected in the *ctgfa* MRE mutant embryos (Fig. 5a-
263 c, and Supplementary Figs.8 a-c and 9). These results support that post-transcriptional
264 regulation of *ctgfa* contributes to specific Ago2-mediated mechanical effects within the
265 miRNA-CAM mRNA network.

266

267 **Discussion**

268 We report that an unbiased analysis of miRNAs and their target genes in
269 endothelial cells, together with functional assays in several biological systems, reveal the
270 existence of a mechanosensitive miRNA-based program that counteracts cell adhesion,
271 cytoskeletal, contractile and matrix protein expression. This system is highly conserved,
272 functioning in several cell types, across multiple species, and with evidence of high
273 evolutionary conservation. Importantly, most of the protein-coding genes for synthesis
274 and assembly of stiff ECM are targeted by miRNAs on stiff substrates. Thus, a “buffer” is
275 generated, in which increased matrix stiffness not only upregulates cytoskeleton-
276 adhesion-matrix gene transcription, but also upregulates miRNA-mediated suppression
277 of cytoskeleton-adhesion-matrix transcripts. This miRNA-regulome has the molecular
278 and functional characteristics of a homeostatic mechanism in which changes in cell
279 contraction and matrix are counteracted to maintain normal tissue stiffness (Fig. 5c).

280 A network-mediated mechanism for stiffness homeostasis, rather than regulation
281 of one or a few CAM genes, would be expected to increase the robustness of the
282 system. Multiple miRNAs can regulate a large cohort of CAM genes via different MREs,
283 while different cell types can do so by controlling expression and processing of tissue
284 specific mature miRNAs⁴¹⁻⁴³. We speculate, however, that these miRNA networks are
285 likely to be sub-elements within a larger and more robust network of negative and
286 positive circuits, connected by multiple nodes, that mediate tissue homeostasis over the
287 multiple decades of human life⁴⁴. Such nodes could develop within a hierarchy of
288 epigenetic factors in which, for example, the activation of YAP/TAZ and its direct target
289 gene *CTGF*, may be one of the upstream components.

290 A role for miRNAs in tissue mechanical homeostasis is supported by the
291 widespread de-regulation of miRNAs in lung, renal, cardiac and liver fibrosis, including
292 miRNAs that target ECM proteins⁴⁵⁻⁴⁷. Idiopathic lung fibrosis is also linked to reduced
293 levels of miRNAs that target ECM, cytoskeletal and TGF β pathways genes⁴⁸⁻⁵⁰. All of
294 these studies reported reduced levels of miR-29 species, in contrast to our finding that in
295 normal cells, miR-29 species are increased on stiff substrates. These results are
296 consistent with the notion that fibrotic disease involves disruption of normal stiffness
297 miRNA-dependent homeostasis⁵¹.

298 miRNA-dependent post-transcriptional regulation of structural proteins provides
299 a concrete molecular mechanism that can explain how healthy tissues sustain optimal
300 mechanical properties. These findings are therefore an important step toward
301 understanding the initial pathological alterations resulting in fibrotic and related diseases.

302 Characterizing the stiffness-dependent RNA metabolism of cytoskeleton and matrix
303 transcripts, their possible regulation under other physical forces, and elucidating the
304 complete regulatory network that mediates long-term mechanical robustness are the
305 essential tasks for future studies.

306

307

308 **Methods**

309 Cell Culture

310 Human umbilical vein endothelial cells (HUVECs) and human umbilical artery
311 endothelial cells (HUAECs) were purchased from Cell Applications Inc. (Cat # 200-05n
312 and Cat #202-05n). Endothelial cells were cultured on tissue culture dishes coated with
313 0.2% w/v gelatin (10 min at room temperature in PBS, Sigma) in endothelial cell growth
314 medium (EGM Bullet Kit, LONZA). For HITS-CLIP assays, cells were used at P3 (split
315 1:3 and 1:5) before UV crosslinking. For other assays, cells were split 1:3 twice per week
316 and used until passage 5. Human dermal fibroblasts (HDFs) from normal donors were
317 purchased from ATCC (Cat #PCS-201-010, Lot# 63014910) and cultured on 0.2% w/v
318 gelatin-coated dishes in fibroblast growth medium (Fibroblast Growth Kit-Low Serum,
319 ATCC, PCS-201-041). HDFs were split 1:10 twice per week and used until passage 6.

320

321 Primary fibroblasts

322 Primary dermal fibroblasts for 3D fibrin gel assays were obtained from 5 to 8
323 week old C57BL/6 mice (Envigo, UK). All procedures were in accordance with UK Home
324 Office regulation and UK animals (Scientific Procedures) Act of 1986 for the care and the
325 use of animals. Mice were sacrificed by a schedule 1 procedure by trained personnel.
326 Mouse hair was removed with a hair clipper and skin dissected in Hank's buffer
327 supplemented with antibiotic and antimycotic solution (Sigma). Fat and excess
328 connective tissues were removed, the dermis was minced with a scalpel and digested in
329 buffer containing 0.25% trypsin without EDTA (Gibco), collagenase IV (4mg/mL
330 (Worthington) and calcium chloride (0.3mg/mL, Sigma) for 3 hours at 37°C with frequent
331 agitation during the last hour. After mechanical dissociation, cells were passed through a
332 cell strainer (100 µm, Fischer Scientific) to remove debris and hairs. Cells were
333 centrifuged at 1800 rpm for 5 minutes, resuspended in DMEM supplemented with 10%
334 Fetal Bovine Serum (Sigma), Penicillin (100U/mL), Streptomycin (100 ug/mL) (Gibco)
335 and 1% L-glutamine, and seeded in 75cm² tissue culture flasks. Medium was changed at
336 3 hours and subsequently changed once a day.

337

338 AGO2-HITS-CLIP

339 HITS-CLIP experiment was performed as previously described²². Three
340 independent replicas were analyzed for each cell line (HUVEC and HUAEC). For each
341 replica, five 10 cm dishes of sub-confluent endothelial cells (ECs) in EGM Bullet Kit

342 supplemented media (LONZA) were UV crosslinked two times with 400mJ/cm² in
343 Stratalinker (model 2400, Stratagene), lysed and treated with DNase (1:1000 Promega
344 RQ1 DNase) and RNase T1 (1:100, Thermo Fisher). Cell lysates and Protein A
345 Dynabeads (Invitrogen) complexed with Ab-panAGO-2A8 (MABE56 Millipore) were
346 incubated at 4°C for 4 hours. Beads were subsequently washed and ligated with 3' -P32
347 radiolabeled linker (RL3: 5'-PGUGUCAGUCACUCCAGCGG-3'). SDS-PAGE was
348 performed using NuPage 4-12% Bis-Tris Gel (NP0321 Invitrogen), and proteins were
349 transferred onto Pure Nitrocellulose membrane (BioTrace) using NuPAGE transfer buffer
350 according to manufacturer's instructions. High performance autoradiography film was
351 exposed overnight at -80°C. The bands corresponding to AGO2-miRNAs (~110 kDa),
352 and AGO2: RNA (~130 kDa) were cut and treated with proteinase K (Roche) to degrade
353 proteins. RNAs were extracted and purified via phenol-chloroform, then a 5'-linker
354 oligonucleotide (RL5: 5'-AGGGAGGACGAUGCGG-3') was ligated to the ends. cDNA
355 libraries were generated using DNA oligos complementary to RL3 and SuperScriptIII
356 reverse transcriptase (Invitrogen) according to manufacturer's instructions. Products
357 were then PCR amplified using specific primers (DP5: AGGGAGGACGATGCG, DP3:
358 GCCGCTGGAAGTGAAGTACTGACAC) and purified via agarose PAGE 1% using a Gel
359 extraction kit (Qiagen). A second round of PCR was performed, using custom Illumina
360 Hi-Seq primers with three different barcodes to multiplex the libraries (DSFP5 5' to 3'
361 sequence: AATGATACGGCGACCACCGACTATGG, DSFP3-Index1:
362 CAAGCAGAAGACGGCTATCGAGATTGGTCAAGTGTGACTGGAGTTCACCGCTGGAAGT
363 GACTGACAC, DSFP3 5' to 3' sequence -Index4:
364 CAAGCAGAAGACGGCTATCGAGATCGTGATGTGACTGGAGTTCACCGCTGGAAGT
365 GACTGACAC
366 DSFP3 5' to 3' sequence -Index8
367 CAAGCAGAAGACGGCTATCGAGATTCAAGTGTGACTGGAGTTCACCGCTGGAAGT
368 GACTGACAC). Products were PAGE purified using the Gel extraction kit, and libraries
369 were analyzed by the YCGA Sequencing facility using a customized Illumina primer
370 (SSP1 5' to 3' sequence: CTATGGATACTTAGTCAGGGAGGACGATGCGG).

371

372 Data Analysis

373 Human AGO2 peaks were called using Piranha peak caller (version 1.2.1)⁵².
374 Prior to aligning sequencing reads, the raw data were analyzed for quality steps to
375 reduce artifacts: adapters were removed from raw reads, filtered according to quality

376 scores and exact sequence duplicates were collapsed. Remaining reads were aligned
377 using STAR RNA-seq aligner (version 2.4.1a)⁵³ using UCSC hg19 reference human
378 genome. A minimum of 10 bases matched was enforced, only unique reads were used,
379 and a maximum of 3 mismatches were allowed. Replicates were merged using Samtools
380 (version 1.2)⁵⁴ and the aligned reads were analyzed with Piranha using a bin size of 30
381 bp. All identified peaks with p-value less than 0.05 were mapped to Gencode version 22
382 annotation.

383 Conservation between artery and vein samples was calculated for each identified
384 peak using PhastCons 100 conservation scores. Using Piranha peaks with a 30bp bin
385 setting, a Wilcoxon rank sum test was performed to compare the difference in
386 distribution of conservation score across samples.

387 For microRNA identification, reads were aligned using Novoalign (Novocraft,
388 <http://www.novocraft.com/products/novoalign/>) against human microRNA sequences
389 from miRBase (release 21)⁵⁵. The miRNA expression levels were quantified as the
390 number of reads mapped to individual miRNA genome sequence and normalized to the
391 total number of mapped reads in miRbase per million (RPM). Endothelial microRNAs
392 identified in AGO2-HITS-CLIP were divided into families based on 8mer SEED regions.
393 Using TargetScan software⁵⁶, these microRNA SEED families were associated to the
394 AGO2-HITS-CLIP peaks based on the putative MRE.

395 To test expression of CAM vs. other genes in cultured ECs, we examined
396 previously published microarray data performed in freshly isolated versus cultured
397 HUVEC and HUAEC (GEO ID: GSE43475)⁵⁷. Standard microarray analysis for
398 differential gene expression (DEG) was performed using Bioconductor library of
399 biostatistical packages (<http://www.bioconductor.org/>) and specific packages simplyaffy
400 (<http://bioinformatics.picr.man.ac.uk/simpleaffy/>) and limma⁵⁸.

401

402 CRISPR/Cas9 strategy to generate mutant primary cells

403 To mutate AGO2 in HUVECs and HDFs, a pLentiCRISPR vector containing an
404 AGO2 or a non-targeting guide RNA (control, which doesn't target known mouse or
405 human genome sequences) was used (AGO2(Fw): 5'-
406 CACCGGGGCGGCTCCCGAGTACA-3', AGO2(Rv):5'-
407 CACCGGCGTTACACGATGCACTTTC-3') (NT(Fw): 5'-GCGAGGTATTCGGCTCCGCG,
408 NT(Rv): 5'-CGCGGAGCCGAATACCTCGC-3'). Lentiviruses were generated by
409 transfecting Lenti-X 293T cells (Clontech) (10cm dishes) with packaging vectors (2.5µg

410 VSV; 5µg pxPAX2, Addgene) and the pLentiCRISPR DNA vector (7.5ug) using
411 lipofectamine 2000 (Invitrogen). Virus containing supernatant was collected 36 and 60
412 hours post transfection. In Supplementary Fig. 2b shows a schematic of approach. Cells
413 were infected with pLentiCRISPR virus containing AGO2 gRNAs or non-targeting
414 gRNAs in the presence of polybrene (8 µg/ml). To generate cells with mutant MREs,
415 similar vectors were generated to target the selected MRE sequences identified in the
416 AGO2 HITC-CLIP experiment and confirmed via Sensor-seq. The complete lists of
417 genes and gRNAs are reported in Supplementary Table 6. Cells were cultured for 7-10
418 days (up to a maximum of passage 5) prior to seeding on gels for immunostaining or
419 traction force microscopy. Reduced AGO2 expression was confirmed by Western blot at
420 7 and 10 days post-infection. Cells were lysed in RIPA buffer with protease and
421 phosphatase inhibitor cocktail (Roche) on ice. Samples were loaded onto 8% or 4-12%
422 SDS-PAGE gels and run 2h at 130 V. Transfer was performed using Tris-
423 Glycine/Methanol buffer on Immun-Blot PVDF membrane (Biorad). Membranes were
424 blocked with 10% milk for 2 hours and incubated with rabbit anti-AGO2 (Cell Signaling)
425 and mouse anti-βActin (Santa Cruz) in 2% milk overnight at 4°C. After washing,
426 membranes were incubated with secondary antibody anti-mouse-HRP and anti-rabbit-
427 HRP (Santa Cruz) for 1h. For blots of other proteins following MRE mutation, target
428 protector or knockdown, membranes were blocked with 5% w/v BSA in PBS 0.1%
429 Tween for 1 hour and incubated with primary antibody for RhoB (1:200, sc-8048, Santa
430 Cruz), CTGF (1:1000, ab6992, Abcam), Vinculin (1:2500, V9131, Sigma-Aldrich),
431 STMN1 (1:10000, ab52630, Abcam), DROSHA (1:5000, ab183732, Abcam), or GAPDH
432 (1:4000, 2118, Cell Signaling) overnight at 4°C. After washing, membranes were
433 incubated with secondary antibody anti-rabbit-HRP or anti-mouse-HRP (1:4000, 7076P2
434 and 7074S, Cell Signaling) for 1 hour at room temp in 5% BSA TBS-T. After washing,
435 blots were developed with super signal west pico chemiluminescent substrate (Thermo)
436 using a SYNGENE G-Box imager.

437 For single CAM MRE mutations, T7 Endonuclease I assay⁵⁹ was first used to
438 verify the occurrence of indels in the MRE sequence as described in manufacturer's
439 protocol (New England BioLabs)(data not shown). Then, single amplicons of ~300 bp
440 were generated using primes equidistant from the putative region of mutation. PCR
441 amplicons were combined and sent to Yale Sequencing Facility for MiSeq 2x250
442 analysis. After Illumina sequencing, single amplicons were demultiplexed and single
443 reads were used for msa (Multiple sequence alignment) against the wild-type sequence

444 using R msa package⁶⁰. The frequency of each mutation was calculated as total reads
445 for each CAM gene mutation divided the sum of all the reads aligned to specific CAM
446 gene, and plot as bar plot.

447 To mutate *Ago2* in mouse fibroblast, pLentiCRISPRV2 (Addgene) vectors
448 containing *Ago2* or non-targeting guide RNAs (control, as above) were used. P0
449 fibroblasts at 80% confluence were infected with lentivirus containing either non-
450 targeting or *Ago2* guide RNA in the presence of 4mg/mL polybrene for 16 hours. Culture
451 medium was changed and cells were incubated for 72 hours. Infected cells were
452 selected in medium with 0.5 ug/mL puromycin for 48 hours (this concentration efficiently
453 kills all control cells) and then cultured for another 96 hours before use in matrix
454 constructs. Reduced *Ago2* expression in mouse fibroblasts was confirmed by Western
455 blot of protein extracts from the matrix constructs at 5 days. Matrix constructs were
456 washed with cold PBS and immediately immersed in liquid nitrogen. Frozen constructs
457 were then homogenized with metallic beads in a Bullet Blender (Strom 24, Next
458 Advance) in protein extraction buffer (1.1% Sodium dodecyl sulphate, 0.3% sodium
459 deoxycholate, 25mM dithiothreitol, in 25mM ammonium bicarbonate with Complete anti-
460 protease and anti-phosphatase, Roche). Protein samples were loaded in a 4-12% Nu-
461 Page pre-casted gel (ThermoScientific) for electrophoresis (200V, 50 minutes). Transfer
462 was performed using Tris-Glycine/Methanol buffer on nitrocellulose membrane (Biorad).
463 The membrane was then blocked for 1 hour with Odyssey PBS blocking buffer (LiCor
464 biosciences) and incubated overnight with 2 primary antibodies against *Ago2* (Cell
465 Signaling) and beta-actin (Abcam) at 1/5000. After extensive rinsing in PBS-Tween, the
466 membrane was incubated with 2 secondary antibodies: AlexaFluor 680 anti-mouse to
467 detect α -actin and AlexaFluor 800 anti-rabbit to detect α -*Ago2* (both from
468 ThermoScientific, 1/15000). The membrane was scanned with an Odyssey CLX NIR
469 scanner (Licor biosciences) and fluorescence intensity of the bands quantified with the
470 Image Studio software (Licor biosciences).

471

472 shRNA knockdown of DROSHA and miRNA Target Protector for CTGF

473 Knockdown of DROSHA was performed using Dharmacon shRNA
474 SMARTvectors (GE Healthcare). Lentivirus was prepared in Lenti-X 293T cells as
475 before using a non-targeting negative control shRNA (5'-CCTAAGGTTAAGTCGCCCT-
476 3') and two shRNAs directed at DROSHA (shDRO#1, 5'-ACCAATGCCTTGTCCTAAT-
477 3') (shDRO#2, 5'-GCAAAGGCATGATTGTTAC-3'). Experiments were performed with

478 shDRO#2 since it achieved ~95% knockdown at 5 days post infection. DROSHA
479 knockdown was verified by immunoblot as before with DROSHA antibody (Abcam,
480 ab183732, 1:5000 in 5% BSA).

481 Disruption of the miRNA-MRE interaction with the CTGF gene was performed
482 using a miScript Target Protector (Qiagen) directed at the MRE within the human CTGF
483 gene. The CTGF target protector (CTGF_1_TP, Cat#MTP0079186, 5'-
484 AACTAGAAAGGTGCAAACATGTAACCTTTTG-3') or the Negative control target protector
485 (Cat#MTP0000002) were transfected into P2 HUVECs at 20nM using Lipofectamine
486 RNAiMax (Invitrogen) in OPTI-MEM (Gibco) with 4% FBS (Sigma). CTGF increases
487 post transfection were verified by immunoblot as before with a CTGF antibody (Abcam,
488 ab6992, 1:1000 in 5% BSA).

489

490 PDMS and Polyacrylamide Substrates

491 Polydimethylsiloxane (PDMS) substrates were cast in the bottom of 10cm tissue
492 culture dishes or #1.5 cover-glass bottomed 35mm Mattek dishes (for imaging studies).
493 Soft (3kPa) gels were made using a 1:1 ratio (by weight) of PDMS component A and B
494 (CY 52-276 A and B, Dow Corning), degassed for 30 minutes in a vacuum desiccator,
495 and cured for 24 hours at room temperature. Stiff (30kPa) gels were made using a 40:1
496 ratio (by weight) of Sylgard 184 components B and C (SYLGARD 184, Dow Corning),
497 degassed for 30 minutes and cured for 3 hours at 70°C. Prior to seeding, gels were
498 washed with PBS, sterilized with UV for 20 minutes, and coated with bovine plasma
499 fibronectin (10µg/ml in PBS) overnight at 4°C.

500 Polyacrylamide gels were prepared using a protocol modified from previously
501 published methods in ref⁶¹. Briefly, 30-mm glass bottom dishes were activated with
502 glacial acetic acid, 3-(trimethoxysilyl) propyl methacrylate, and 96% ethanol solution
503 (1:1:14 ratio, respectively) for 10 minutes in room temperature. For fibronectin protein
504 conjugation (1mg/ml) on the polyacrylamide gel, acrylic acid N-hydroxysuccinimide ester
505 was partially mixed as a substitute of acrylamide. Each stiffness was prepared with the
506 ratio in the table below which was previously reported by ref⁶¹.

Stiffness (Pa)	40% aa (ul)	2% bis-aa (ul)	60% NHS acrylate (ul)	Citric buffer (pH 4) (ul)	APS (ul)	TEMED (ul)
490	45	7.5	33	394	20	1
1551	56.3	10	42	372	20	1
5083	84	11	62	323	20	1
13380	84.4	25	63	308	20	1
17765	85	40	63	292	20	1
30027	135	37.5	100	208	20	1

507

508

509 RNA, miRNA and Sensor-seq library preparation

510 Total RNA was extracted from three replicates of cultured HUVEC cells seeded
511 on 3kpa or 30kpa PDMS using TRIzol reagent (Life Technologies) according to the
512 manufacturer's protocol. For mRNA libraries, total RNA was treated with DNA-free
513 DNase (Ambion) and 500 ng of treated RNA was used to prepare Lexogen QuantSeq 3'
514 mRNA-Seq FWD libraries for Illumina deep-sequencing according to the manufacturer's
515 protocols using the i7 barcode indices. Libraries were amplified with 12 PCR cycles.
516 miRNA libraries were prepared from 1µg of total RNA using the NEBNext® Small RNA
517 Library Kit (NEB) following the gel size selection method in manufacturer's protocol and
518 submitted for Illumina sequencing. For Sensor-Seq library, a customized oligonucleotide
519 library was synthesized by Integrated DNA Technologies (IDT). The sequence of each
520 individual oligonucleotide was obtained from the piranha analysis (see Supplementary
521 Table 4) extending the genomic coordinate of each peak by 20 nucleotides at the 3 and
522 5 prime region. 97 peaks with at least 1 predicted MRE, representative of 51 CAM genes
523 were selected. In addition, all the sensor oligonucleotides contained restriction enzyme
524 sites, *Ascl* and *NheI*, to allow for PCR base amplification and cloning. The library of
525 oligonucleotides was resuspended in 480 µl of water, diluted 1:100 and PCR amplified
526 as follows: in a 50 µl reaction, 10 µl 5X Phusion HotStart II HF Buffer, 1 µl 10 mM dNTP,
527 2.5 µl 10 mM *Ascl* forward (5' - GGCCATCTGGCGGCC) and *NheI* reverse primers
528 (5'- GGCCGATAAGCTAGC), 1 µl of diluted library, 1 µl DMSO, 0.5 µl Phusion HotStart
529 II Polymerase. Cycling parameters were: 98°C for 2 min, 20 cycles of 98°C for 20 sec,
530 63°C for 20 sec, 72°C for 20 sec and 72°C for 2 min. PCR-amplified libraries were
531 purified using a PCR purification kit (Qiagen) and double digested for 2 h at 37°C.
532 Sensor-seq backbone²⁷ containing a bidirectional promoter for UbC upstream of copGFP
533 and mCherry genes, was kindly provided by Dr. Jun Lu, Yale University. After sensor
534 backbone digestion with *Ascl* and *NheI*, the MRE oligo library was cloned into the 3'UTR
535 of mCherry. Ligation was performed in 20 µl reactions containing 50 ng vector, 3 ng
536 insert, 2 µl 10x T4 Ligation Buffer, 2500 U T4 DNA Ligase (Promega), incubated 16 h at
537 16°C. After transformation into DH5α, colonies were collected, and a pool of plasmids
538 was prepared using a Maxi prep kit (Qiagen).

539 Lentiviruses for expression of the CAM-MRE Sensor library were generated by
540 transfecting Lenti-X 293T cells (10cm dishes) with packaging vectors (2.5µg VSV; 5µg

541 pxPAX2) and the DNA vector library (7.5ug) with lipofectamine 2000 (Invitrogen). For
542 FACS analysis additional control lentiviruses expressing GFP alone, mCherry alone,
543 miRNA sensor lacking a MRE (Empty-Sensor plasmid, negative control), and miRNA
544 sensor with a synthetic MRE for miR125 (miR125-Sensor plasmid, positive control) were
545 used.

546

547 RNA and miRNA seq data analysis

548 Data analysis for RNA and miRNA seq was performed using STAR alignment
549 software and R software environment for statistical computing (<http://www.r-project.org/>).
550 Total RNA and microRNA were aligned against the human genome version GRCh38
551 using the GENCODE 22 transcript annotation, using STAR alignment software with
552 same parameters used for the ENDOCE project (www.encodeproject.org)
553 After alignment, differential gene expression (DEG) of RNAs or miRNAs between ECs
554 seeded on 30 and 3 kPa substrates was computed using the negative binomial
555 distributions via edgeR using standard parameter Genes^{62,63}. The levels (log 2 Fold
556 Change) of CAM RNAs and SEED matching miRNAs to CAM-MRE sensors, were
557 combined and represented in the CIRCOS plot.

558

559 FACS and Sensor-Seq Analysis

560 For FACS experiments, cells were infected with low levels of the library or control
561 lentivirus in the absence of polybrene to avoid multiply infected cells (10-20% of cells
562 infected). After 48 h, cells were trypsinized and seeded onto soft (3kPa) or stiff (30kPa)
563 fibronectin-coated PDMS dishes at low density (150k cells per 10cm dish) for 2 days.
564 After 2 days on PDMS gels, cells were washed once with PBS, trypsinized, centrifuged
565 for 5 min at 300xg, and re-suspended in PBS at 500k cells per ml immediately before
566 FACS analysis. Infected cells were sorted on a BD FACSAria II and analyzed with
567 FACSDiva 7. Four sorting gates were set based on the 2 control plasmids (Empty-
568 Sensor and miR125-Sensor). The upper limit bin (Not Suppressed) was designed to
569 contain 90% or more events/cells infected with the Empty-Sensor and less than 0.5% of
570 events for the miR125-Sensor. Conversely, the lower two bins (Strongly Suppressed and
571 Suppressed) were designed to contain 90% of events coming from cells infected with
572 miR125-Sensor, in a ratio close to 3:2 (~60% of events in Strongly Suppressed bin and
573 ~40% of events in Suppressed). The 3rd bin (Mildly Suppressed) was set between the
574 Not Suppressed and the Suppressed bins. For clarity, the contour plot represents the

575 total percentage of event in each single bins, grouped in “island” of 15% probability were
576 shown for the Empty-Sensor, miR125-Sensor, Sensor-Library at 3 kPa and Sensor-
577 Library at 30 kPa.

578 After sorting of cells into each bin, genomic DNA was isolated using the DNeasy
579 Blood & Tissue Kit (Qiagen) and the MREs were PCR amplified using specific forward
580 primers to barcode each bin:

581 strongly suppressed 5'- AGCACTCGAGCTGTACAAGTAGTG- 3',

582 suppressed 5'-TCGGAACGAGCTGTACAAGTAGTG- 3',

583 mildly suppressed 5'- CCAGTACGAGCTGTACAAGTAGTG- 3' and

584 not suppressed 5'- GACATCCGAGCTGTACAAGTAGTG – 3', and the reverse primer

585 5'- TGTAATCCAGAGGTTGATTATCG. The PCR protocol used was: in 50 μ l reaction,

586 10 μ l 5X Phusion HotStart II HF Buffer, 1 μ l 10 mM dNTP, 1 μ l 10 mM 5'and 3'primers,

587 100 ng genomic DNA, 0.5 μ l Phusion HotStart II Polymerase. Cycling parameters were:

588 98°C for 2 min, 35 cycles of 98°C for 20 sec, 59°C for 20 sec, 72°C for 20 sec and 72°C

589 for 5 min. Library were purified on a 1% agarose gel using gel extraction kit (Qiagen).

590 The primers used contain barcodes for multiplexing (underline sequences) and were

591 designed to hybridize with Illumina sequencing.

592 Computational analysis of Sensor-seq was performed using R software

593 environment for statistical computing, using customized pipeline. First, the number of

594 reads for each sensor-MRE were normalized by dividing by the total number of reads in

595 the entire experiment. This was then multiplied by one million to get the Reads Per

596 Million (RPM) for each sensor. To calculate the frequency of sensor-MREs in each bin,

597 the RPM was divided by the total RPM for all 4 bins of the experiment for that

598 sensor. This gave frequency values for each MRE in each bin at each stiffness. MREs

599 that showed a dominant bin (with frequency values above 0.375, i.e. non-random) were

600 compared at 3 vs. 30 kPa. In Figure 2c CAM MREs were then plotted based on the

601 reproducible tendency to be enriched in the same bin at a given stiffness but not the

602 other for 3 independent experiments. MREs that shifted towards a more suppressed bin

603 on 30kPa compared to 3kPa were plotted as CAM-MRE 30kPa (bottom left of plot).

604 MREs that that shifted towards a more suppressed bin on 3kPa compared to 30kPa

605 were plotted as CAM-MRE3kPa (upper right of plot). RNA-seq (red) and Proteomics

606 (green) data for each of these proteins was also plotted and linked with the miRNA-seq

607 (for miRNAs predicted to bind to these particular MREs). The regulation of CAM-MREs

608 by miRNAs (stars in the Figure 2c) was further validated by individual MRE mutagenesis
609 followed by functional assays.

610

611 Mass spectrometry sample preparation and analysis

612 Cells were lysed in 25 mM ammonium bicarbonate (AB) buffer containing 1.1 %
613 sodium dodecyl sulfate (SDS, sigma), 0.3 % sodium deoxycholate (Sigma), protease
614 inhibitor cocktail (Sigma), phosphatase inhibitor cocktails (Merck). Six 1.6 mm steel
615 beads (Next Advance Inc.) were added to the tube and samples were homogenized with
616 a Bullet Blender (Next Advance Inc.) at maximum speed for 2 minutes. Resulting
617 homogenates were cleared by centrifugation (12 °C, 10000 rpm, 5 minutes).

618 Immobilized-trypsin beads (Perfinity Biosciences) were suspended in 150 µL of digest
619 buffer (1.33 mM CaCl₂, Sigma, in 25 mM AB) and 50 µL of cell lysate and shaken
620 overnight at 37 °C in a Thermocycler at 1400 rpm. The resulting digest was then
621 reduced by the addition of 4 µL of 500 mM dithiothreitol (DTT, Sigma, in 25 mM AB; 10
622 min. shaking at 1400 rpm at 60 °C) and alkylated by the addition of 12 µL 500 mM
623 iodoacetamide (Sigma, in 25 mM AB; 30 min. shaking in the dark at room temperature).
624 Immobilized trypsin beads were removed by centrifugation at 10,000 rpm for 10 min.
625 Supernatant containing reduced, alkylated peptides were transferred to 1.5 mL 'LoBind'
626 Eppendorf tubes and acidified by addition of 5 µL 10 % trifluoroacetic acid (Riedel-de
627 Haën) in water, and cleaned by two-phase extraction (3 x addition of 200 µL ethyl
628 acetate, Sigma, followed by vortexing and aspiration of the organic layer). Peptides were
629 desalted, in accordance with the manufacturer's protocol, using POROS R3 beads
630 (Thermo Fisher) in accordance with the manufacturer's protocol and lyophilized. Peptide
631 concentrations (measured by Direct Detect spectrophotometer, Millipore) in injection
632 buffer (5 % HPLC grade acetonitrile, Fisher Scientific, 0.1% trifluoroacetic acid in
633 deionized water) were adjusted to 200 ng µL⁻¹ prior to MS analysis.

634 Digested samples were analysed by LC-MS/MS using an UltiMate® 3000 Rapid
635 Separation LC (RSLC, Dionex Corporation, Sunnyvale, CA) coupled to a Q Exactive HF
636 (Thermo Fisher Scientific, Waltham, MA) mass spectrometer. Peptide mixtures were
637 separated using a multistep gradient from 95% A (0.1% FA in water) and 5% B (0.1% FA
638 in acetonitrile) to 7% B at 1 min, 18% B at 58 min, 27% B in 72 min and 60% B at 74 min
639 at 300 nL min⁻¹, using a 75 mm x 250 µm i.d. 1.7 µM CSH C18, analytical column
640 (Waters). Peptides were selected for fragmentation automatically by data dependent
641 analysis.

642

643 Mass spectrometry data processing and protein quantification

644 Spectra from multiple samples were automatically aligned using Progenesis Q1
645 (Nonlinear Dynamics) with manual placement of vectors where necessary. Peak-picking
646 sensitivity was set to 4/5 and all other parameters were left as defaults. Only peptides
647 with charge between +1 to +4, with 2 or more isotopes were taken for further analysis.
648 Filtered peptides were identified using Mascot (Matrix Science UK), by searching against
649 the SwissProt and TREMBL mouse databases. The peptide database was modified to
650 search for alkylated cysteine residues (monoisotopic mass change, 57.021 Da), oxidized
651 methionine (15.995 Da), hydroxylation of asparagine, aspartic acid, proline or lysine
652 (15.995 Da) and phosphorylation of serine, tyrosine or threonine (79.966 Da). A
653 maximum of 2 missed cleavages was allowed. Peptide detection intensities were
654 exported from Progenesis Q1 as Excel (Microsoft) spread sheets for further processing.
655 Peptide identifications were filtered via Mascot scores so that only those with a
656 Benjamini-Hochberg FDR < 0.05 remained. Raw ion intensities from peptides belonging
657 to proteins with fewer than 2 unique (by sequence) peptides per protein in the dataset
658 were excluded from quantification. Remaining intensities were logged and normalized by
659 the median logged peptide intensity. Missing values were assumed as missing due to
660 low abundance, an assumption others have shown is justified⁶⁴. Imputation was
661 performed at the peptide level following normalization using a method similar to that
662 employed by Perseus⁶⁵ whereby missing values were imputed randomly from a normal
663 distribution centered on the apparent limit of detection for this experiment. The limit of
664 detection in this instance was determined by taking the mean of all minimum logged
665 peptide intensities and down-shifting it by 1.6σ , where σ is the standard deviation of
666 minimum logged peptide intensities. The width of this normal distribution was set to 0.3σ
667 as described in⁶⁵. Fold-change differences in the quantity of proteins detected in different
668 time-points were calculated by fitting a mixed-effects linear regression model for each
669 protein with Huber weighting of residuals as described in⁶⁴ using the fitglm Matlab (The
670 MathWorks, USA) function with the formula:

$$y_{ipt} = \beta_0 + X_p\beta_p + X_t\beta_t + \varepsilon_{ipt}$$

671 Where y_{ipt} represents the \log_2 (intensity) of peptide p belonging to protein i , under
672 experimental treatment t . β s represent effect sizes for the indicated coefficients. *Peptide*
673 effects were considered as random effects whereas *treatment* was considered as a fixed

674 effect. β_0 denotes the intercept term and ε_{pt} denotes residual variance. Standard error
675 estimates were adjusted with Empirical Bayes variance correction according to ⁶⁶.
676 Conditions were compared with Student's t-tests with Benjamini-Hochberg correction for
677 false positives.

678

679 Cells Immunostaining and Quantification

680 Cells seeded on fibronectin-coated PDMS were fixed with 4% paraformaldehyde
681 (Electron Microscopy Sciences) in PBS. Cells were then washed and permeabilized with
682 0.05% Triton X-100 in PBS supplemented with 320 mM sucrose and 6 mM MgCl₂. Cells
683 were washed 3 times with PBS and blocked for 30 minutes with 1% BSA in PBS. Cells
684 were incubated overnight at 4°C with anti-YAP antibody (1:200, no sc-101199, Santa
685 Cruz Biotechnology), anti-RhoB (1:250, 19HCLC, Thermo Fisher), anti-Vinculin (1:200,
686 V9131, Sigma-Aldrich), anti-STMN1 (1:200, ab52630, Abcam), anti-CTGF (1:200,
687 ab6992, Abcam) and anti-paxillin (1:800, RabMAb Y113, ab32084, Abcam) diluted in 1%
688 BSA in PBS. Cells were washed 3 times with PBS and incubated at room temperature
689 for 1 hour with secondary antibodies (alexa-488 anti-rabbit, alexa-647 anti-mouse,
690 1:1000, Molecular Probes) and alexa-565 conjugated phalloidin (1:1000, molecular
691 probes). Cells were washed again 3 times with PBS and mounted with DAPI in
692 Fluoromout-G (SouthernBiotech). Cell areas were quantified using ImageJ by
693 background subtracting, thresholding to generate cell masks, and using the analyze
694 particles function (n=51-150 cells / group, experiment replicated 3 times for each cell
695 type). YAP staining was quantified by taking the average nuclear YAP signal (in the
696 area of the DAPI stain), divided by the average cytoplasmic YAP signal (in the area of
697 the non-nuclear cell mask). Focal adhesions were analyzed using the focal adhesion
698 analysis server ⁶⁷ with the minimum adhesion size set to 0.5 μm^2 and the default settings
699 for only static properties.

700

701 Traction Force Microscopy

702 PDMS TFM substrates were fabricated as described ⁶⁸. Briefly, cover-glass
703 bottom dishes were spin-coated to obtain a ~40 μm thick layer of polydimethylsiloxane
704 (PDMS; Sylgard 184 by Dow Corning mixed at various B/C ratios, 67:1 3kPa, 40:1
705 30kPa) and cured at 70°C for 3 hours. Gels were then treated with 3-aminopropyl
706 trimethoxysilane for 5 min and incubated for 10 min at room temperature with 40nm
707 Alexa Fluor 647 beads (Molecular Probes) suspended in a 100 $\mu\text{g}/\text{ml}$ solution of 1-Ethyl-

708 3-(3-dimethylaminopropyl) carbodiimide (Sigma) in water to covalently link the beads to
709 the gel surface. Elastic moduli for each batch was measured using a microfluidic device
710 as described⁶⁸ and is reported as the Young's modulus (E).

711 TFM gels were coated with fibronectin (10µg/ml) in PBS overnight at 4°C and
712 washed 3 times with PBS. HUVECs and HDFs were seeded on the gels in EGM or low
713 serum fibroblast growth medium, respectively, 24h before analysis at low density (~3000
714 cells per cm²). Cells and florescent beads were imaged on a spinning disk confocal
715 microscope (UltraVIEW VoX, Perkins Elmer) attached to a Nikon A-1 microscope
716 equipped with a temperature and CO₂ controlled incubation chamber and 60x 1.4NA
717 lens. Florescent images of Alexa Fluor 647 beads and DIC images of cells were
718 acquired before and after cell lysis with 0.05% SDS. Images were drift corrected and
719 bead displacements were quantified using a previously developed open source traction
720 force microscopy software in MATLAB 2015a⁶⁹. Force fields and traction stresses were
721 calculated using FTTC force reconstruction with the regularization parameter set to
722 0.007. Total force per cell was calculated as the average stress under the cell multiplied
723 by the area.

724

725 3D matrix constructs

726 A method to generate three-dimensional cell-derived uniaxial matrix constructs
727 (3D matrix constructs) based on the "tendon construct" developed by Karl Kadler's group
728 was used⁷⁰. Six well plates were coated with a 2 mm layer of SYLGARD 184 and
729 incubated overnight at 65°C to induce polymerization. After cooling, the (hydrophobic)
730 SYLGARD layer was incubated 15 minutes with Pluronic® F-127. Custom-made
731 rectangular Teflon molds (15x10x2mm) were sterilized with Virkon (10 minutes) then
732 Ethanol 70% (15 minutes). Inside the molds, two 8mm segments of size 0 silk sutures
733 were pinned to the PDMS using insect pins exactly 10mm apart. Inside each mold, we
734 added 12 µL of thrombin stock solution (200U/mL, Sigma). Primary fibroblasts were
735 detached with 0.05% trypsin, centrifuged at 1800 rpm and counted. For each matrix
736 construct, 2x10⁵ cells were resuspended in 300 µL of DMEM containing 8mg/mL
737 fibrinogen (Sigma) and 0.2 mM of L-ascorbate-2- phosphate. Cell suspensions were
738 injected inside the molds and placed at 37°C for 15 min in incubator for polymerization.
739 After polymerization, the Teflon mold was removed and one additional insect pin added
740 to maintain the suture thread. Matrix constructs were cultured with DMEM/F12
741 supplemented with 10% fetal bovine serum (Sigma), Penicillin (100U/mL), Streptomycin

742 (100 ug/mL, Gibco), 1% L-glutamine and 0.2mM of L-ascorbate-2- phosphate. The 3D
743 matrix constructs were cultured for 5 days and the culture medium changed every other
744 day. After 5 days, photographs of the constructs were taken with a Nikon reflex camera
745 equipped with a 50mm macro-objective at a focal distance of 1:1. Constructs diameter
746 was obtained by averaging the diameter at 3 different locations (each extremity and the
747 middle).

748

749 Immunostaining matrix constructs

750 Matrix constructs were rinsed in cold PBS and fixed overnight at 4°C in 4%
751 formaldehyde (Pierce 16% formaldehyde, methanol free) in PBS. Fixation constructs
752 were dehydrated, embedded in paraffin and 5 µm transverse sections cut with a Leica
753 microtome. For immunostaining, we performed a rehydration protocol followed by
754 antigen retrieval for 30 minutes at 96°C in a citrate buffer (pH 6). Sections were blocked
755 with Odyssey PBS blocking buffer (LiCor biosciences) for 1 hour and incubated
756 overnight with primary antibodies diluted in blocking buffer: vimentin (1/400, Cell
757 Signaling), phospho-myosin light chain (1/400, Abcam). After extensive rinsing in PBS
758 Tween 0.1%, slides were incubated with AlexaFluor 647 anti-rabbit secondary antibody
759 (1/500, ThermoScientific) for 1 hour at room temperature, thoroughly washed with PBS
760 Tween 0.1%, and slides mounted in FluoromountG-DAPI (Southern Biotech). Slides
761 were imaged with an Olympus slide scanner microscope equipped with a 20x objective.

762

763 Zebrafish Fin Fold Regeneration

764 Zebrafish were raised and maintained at 28.5°C using standard methods and
765 according to protocols approved by Yale University Institutional Animal Care and Use
766 Committee (# 2015-11473). Wild-type (AB) and *mz ago2* ³⁸ mutants were used. To
767 generate the *ctgfa* MRE mutant, zebrafish AB were injected with 125 ng/µl *Cas9* mRNA
768 and 75 ng/µl gRNAs, designed as previously described⁵⁹. The gRNA sequence used to
769 target the conserved MRE within the 3'UTR human *CTGF* gene was (5'-
770 GGTGAAAACATGTAACATTT-3'). Genomic DNA was isolated from a clutch of 15
771 injected and uninjected control embryos at 24 hpf using the Qiagen DNeasy Blood and
772 Tissue Kit. Genomic DNA (250 ng) and the Phusion HotStart II Kit (ThermoFisher) used
773 to PCR amplify an approximately 300 bp region surrounding the intended MRE target
774 (Fwd: 5'-TTGGGAAAGAGCCAGTATCC-3', Rev: 5'-TGGTGCCATTATTGTGTGGT-3').
775 T7 Endonuclease I assay was used to detect mutations as described in the

776 manufacturer's protocol (New England BioLabs). PCR and T7 products were run on 3%
777 agarose gels to verify the occurrence of indels in the MRE sequence. The remaining
778 embryos were grown to 48 hpf and used for the fin fold regeneration experiments (see
779 below).

780 The zebrafish miR-124 and ctgfa sensor assay and mRNA injection were
781 performed as described⁵⁹. For the fin fold regeneration assay, we use 14 AB fish, 14 mz
782 ago2 mutant *-/-* embryos and 15 mz ago2 *-/-* fish injected at the one cell stage with 200
783 pg of in vitro transcribed mRNA encoding the human AGO2 protein. At 2 days post
784 fertilization (dpf), the fin fold was cut at the edge of the fin using a 25G needle. Bright
785 filed images were captured at 0.5, 2, 4, 24, 48 and 72 hours post amputation (hpa) using
786 a Leica M165 FC stereomicroscope and Leica Application Suite V4 software. The length
787 of the fins over time was measured using FIJI-ImageJ^{70,71} and normalized for the length
788 of the fin before cutting. Analysis and graph were generated using Graphpad Prism7
789 statistical software.

790

791 Zebrafish immunofluorescence assay

792 For the fluorescent images: 20 embryos for each genotype (AB, Ago2 mutant (*-/-*)
793 and ctgfa 3' UTR mutant) were cut and then, at 0.5, 2, 4, 24 hpa were fixed in PFA 4%
794 overnight at 4°C. Embryos were washed 4-5 times with PBS 0.1% Tween, then incubated
795 2 hours in blocking solution (0.8% Triton-X, 10% normal goat serum, 1% BSA, 0.01%
796 sodium azide in PBS Tween). Zebrafish were stained following the protocol as in¹³ using
797 the primary antibody mouse anti-Phospho-Myosin Light Chain 2 (1:200; Cell Signaling),
798 mouse anti-Proliferating Cell Nuclear Antigen (1:200; PCNA, Dako), rabbit anti-
799 Fibronectin (1:200; Cell Signaling), DAPI (1:1000; Sigma), rabbit anti-Connective Tissue
800 Growth Factor A (1:150; Abcam), and mouse anti-YAP (1:200; Santa Cruz
801 Biotechnology) and the secondary antibody Alexa Fluor 488 anti-mouse (1:250,
802 ThermoFisher) and Alexa Fluor 596 anti-rabbit (1:250, ThermoFisher). After staining,
803 images were captured using a Leica Microsystems SP5 confocal microscope using a
804 40X objective. Max projections were generated with the Leica application suite or Perkin
805 Elmer Volocity software. Intensity was quantified using FIJI-Image. For each protein
806 staining, a line profile of 80 µm in diameter within the wound was calculated for the first
807 50 µm from the fin fold edge. The intensity profile of 4 to 6 fish was calculated using R
808 statistical software. To determine the ratio of Nuclear and Cytosolic YAP in before and
809 during the fin fold regeneration confocal images were split by channel and a threshold

810 was used on the DAPI channel to generate a binary mask for the nuclei. Using the
811 Image Calculator function of ImageJ, the binary mask was subtracted from the YAP
812 channel to generate the cytosolic YAP image. After inverting the binary mask and
813 subtracting it from the YAP channel, the nuclear YAP image was generated. Each were
814 measured and normalized to the area. For TUNEL assay to detect apoptotic cells
815 embryos were fixed in 4% PFA overnight and stored in 100% methanol at -20 °C. The
816 TUNEL assay was performed using the ApopTag Red *In situ* Apoptosis Detection kit
817 (Millipore).

818

819 Atomic Force Microscopy

820 Live zebrafish embryos (48 hpf) were anesthetized using 1x tricaine in egg water
821 and mounted on PDMS gels. The tips of the fish tails were probed using a DNP-10 D tip
822 (Bruker, nominal stiffness ~0.06 N/m) on a Bruker Dimension FastScan AFM immersed
823 in egg water containing 1x tricaine. Probe deflection sensitivity was calibrated by taking
824 indentation curves on glass and the nominal tip stiffness was calibrated by thermal
825 tuning (assuming a simple harmonic oscillator in water). Force vs. deflection curves
826 were collected for a ramp size of 1.5 μ m at a rate of 750 nm/s for at least 2 locations per
827 fish, with 10-11 fish measured per group (n=27-49 total measurements per group). The
828 first 600nm of the extension curves were fit with NanoScope Analysis Software version
829 1.5 (Bruker) assuming a Poisson's ratio of 0.5 and using the Sneddon fit model⁷³.

830

831 Statistical Analyses.

832 All the of statistical analysis were performed using Prism version 7.01
833 (GraphPad) and R software environment for statistical computing, except for the peak
834 identification, which used piranha software⁵² to measure the significance of read
835 coverage height for each mapped position using the zero-truncated negative binomial
836 model (ZTNB). To confirm changes in cell area, focal adhesion number, YAP localization
837 and traction force generation, t-tests were performed using Prism to assess the change
838 in the mean between Wild-type and AGO2 or MRE CRISPR/Cas9 mutant cells. These
839 data sets contained more than 30 individual measurements for each condition and
840 showed a log-normal distribution. For the *in vivo* analysis of zebrafish wild-type and
841 Ago2 mutants, changes in fin fold tissue were analyzed using t-tests; fin fold
842 regeneration was analyzed via 2 way ANOVA and Sidak's multiple comparisons test

843 using Prism. The distribution of fluorophore intensity within 20 μm from the edge of the
844 wound was calculated using Prism 4th order smoothing with 2 neighbors.

845

846 Data Availability

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

848 HITS-CLIP: GEO: GSE99686; RNA-seq and sRNA-seq for HUVEC cells at 3 kPa and 30
849 kPa: GEO: GSE110211; Proteomics results are reported as excel file

850 Supplementary_table2

851

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864

865 **Author contributions.**

866 SN and MS conceived the project. AM and TD performed experiments, analyzed data
867 and AM developed the computational data analysis. WA performed and analyzed
868 zebrafish fin fold regeneration experiments. LB performed and analyzed the experiments
869 in Supplementary Fig 2b and c, Supplementary figure 3b-d and 6. SJA performed cell
870 culture experiments in Supplementary Fig.3a. NB and CJ performed the 3D cell-culture
871 experiments and analyzed the data. DL, MG, JZ, and MG develop the sequence data
872 processing, analysis (mapping, peak calling) of the AGO2-HITS-CLIP experiment. VM
873 and JS performed mass spectrometry proteomic analysis. DMK made the miRNA and
874 RNA-sequencing libraries. AM, TD, MS and SN designed the experiments. MS and SN
875 wrote the manuscript. All the authors edited the manuscript.

876

877 **Competing Financial Interests.** The authors have no competing financial, professional
878 or personal interests.

879

880

881

882 **Figures legend**

883

884 **Figure 1. miRNA-cytoskeleton-adhesion-matrix (CAM) interactions in endothelial**
885 **cells.**

886 **(a)** Schematic of AGO2-HITS-CLIP approach. miRNA-RNA complexes were cross-linked
887 to AGO2 (green) via 254nm-UV light. RNase treatment removed unbound RNA. P32-
888 radiolabeling allowed isolation of radioactive RNA from AGO2-immunoprecipitated
889 complexes (purple). **(b)** SDS-PAGE of RNA-AGO2 or control IPs with nonspecific IgG
890 were transferred to nitrocellulose and exposed to X-Ray film to reveal P32-RNAs bound
891 to AGO2 (dotted square). P32 RNAs were isolated (dotted line) from nitrocellulose
892 membranes and cDNAs generated with specific primers containing an Illumina barcode
893 for high throughput sequencing (Methods). AGO2-mRNA complexes appear as ~200 nt
894 bands (70 nt AGO-mRNA+ 120 nt Illumina primer), while AGO2-miRNAs are ~150 nt (30
895 nt miRNA+120 nt Illumina primer). DNA MW markers are indicated in the left lane of the
896 gel. **(c)** Computational pipeline to identify reads from miRNAs and mRNAs in AGO2 IPs.
897 Three replicates for each cell type (HUAEC and HUVEC) were processed as described
898 in **a** and **b**. 68 million (M) total reads were analyzed. Upon removal of Illumina adapters
899 and duplicates from PCR-based library preparation, reads were mapped to the human
900 genome (UCSC hg19), resulting in 1M unique reads for each cell type. Reads were
901 mapped to miRBase to identify miRNAs and processed using Piranha software to
902 identify significant AGO2-binding sites (peaks) (methods). **(d)** Integrative Genomics
903 Viewer (IGV) display of HITS-CLIP reads. Reads accumulated within 30 to 70 nt
904 intervals (AGO2 peak) within the 3'UTR region of the representative genes. Both
905 HUAEC and HUVEC share the most significant peaks (green) while reads mapping
906 outside the 3'UTR gene region (gray) accumulated below backgrounds (supplementary
907 table). **(e)** Top chart represents positional enrichment of AGO2 peaks within the human
908 3'UTR for HUAEC (light gray) and HUVEC (black). Lines indicate the nt positional
909 distribution of peak sequences within meta-gene analyzed 3'UTRs (methods). We
910 observed a positional bias for AGO2 peaks at the 5' and 3' ends of 3'UTRs, which are
911 typically associated with increased miRNA functionality. Bottom chart shows difference
912 in conservation scores across samples scoring using PhastCons (Wilcoxon Rank Sum
913 Test). AGO2 peaks in HUAEC and HUVEC and binned human 3'UTRs were compared
914 with binned 3'UTRs of 100 species. Conservation score is represented as a boxplot.
915 AGO2 peak sequences showed a higher conservation score compared to randomly

916 binned human 3'UTRs. **(f)** The cytoskeleton-adhesion-matrix (CAM) AGO2-regulome.
917 AGO2-mRNA targets identified in **a-c** highlighted in green. Integrins, Talin1 and BMPR1
918 proteins (boxed in brown) are part of CAM's GO term but were not detected in AGO2-
919 HITS-CLIP complexes. Arrows point to downstream regulators of CAM proteins targeted
920 by AGO2. CAM proteins and their regulators were identified by database searches
921 (Supplementary Fig. 1a) and manually curated for accuracy. **(g)** Interactome showing 25
922 of 73 AGO2-CAM genes in which a complementary MRE (7-8 nt) was identified using
923 Target Scan v.7.0 prediction software and miRNAs were identified from AGO2-HITS-
924 CLIP reads using miRbase (methods). Color-coded boxes indicate the number of MREs
925 identified in each of the selected CAM-gene 3'UTRs. Interactions indicate interaction
926 between MRE and miRNA family members with similar SEEDs. The mRNA-miRNA
927 network shows high complexity in which numerous miRNAs bind one or more CAM-
928 3'UTRs, while most CAM genes are targeted by more than one miRNA.

929

930 **Figure 2. AGO2-CAM-MREs are actively repressed.**

931 **(a)** Schematic for Sensor-Seq assay of AGO2-CAM-MREs and miRNA activity. The
932 Sensor-Seq lentiviral library (see Methods) consisted of the mCherry sequence with a 3'
933 noncoding sequence containing 97 CAM-MREs, plus GFP lacking any MREs as an
934 internal control. Numbered MREs indicate different MRE positions in each clone from
935 one CAM 3'UTR, alternatively one MRE was cloned per CAM 3'UTR (Supplementary
936 Table 4). Titration of virus infection was performed to allow single vector copy integration
937 and expression of physiological amounts of each GFP/mCherry transcript per cell.
938 HUVECs were seeded on 3 and 30 kPa PDMS substrates for 48h and analyzed as
939 described. **(b)** Intensity of mCherry and GFP in HUVECs infected with the lentiviral
940 Sensor-Seq library; a negative control vector with no MRE, and a positive control vector
941 with 3 perfect MREs for miR-125, which is abundant in HUVECs⁷⁴, were included.
942 Density plots, show relative intensity of cells distribution using contour lines. Each
943 contour line represents 15% of probability (from higher- lighter grey- to lower-darker
944 grey-) to contain cells in each bin over total cells (10,000 cells). Based on mCherry/GFP
945 expression in controls, Sensor-seq library infected HUVECs at 3 kPa and 30 kPa were
946 sorted into 4 bins as indicated. Cells in each bin were isolated and genotyped using
947 specific Illumina primer for sequencing (Methods). The bar graph shows the percentage
948 of cells from 4 experiments **(c)** CIRCOS⁷⁵ graphical representation of CAM miRNA-MRE
949 interactions. The right half of the plot shows the ECs miRNA with putative SEED

950 matching to CAM MREs sensors differential gene expression (DEG) between 3 kPa and
951 30 kPa, divided in two groups: expressed at 3 kPa compared to 30 kPa (black line, top
952 right) and expressed at 30 kPa compared to 3 kPa (black line, bottom right). The left half
953 of the graph shows CAM-MRE Sensors most suppressed at 30 kPa compared to 3 kPa
954 (black line, bottom left) and vice versa (black line, top left). Color-coded boxes indicate
955 the categorized bins in **(b)** at which cells were isolated and genotyped for a specific
956 CAM-Sensor MRE. Lines indicate match between individual miRNA (SEED) and CAM
957 MRE in each condition. Color code indicates the level of complementarity between
958 miRNA SEED and MRE nucleotides. The internal circles show the respective CAM-
959 RNAs (red) and proteins (green) log₂ fold change at 3 kPa compared to 30 kPa (top
960 right) and 30 kPa compared to 3 kPa (bottom right).

961

962 **Figure 3. miRNA interactions limit cell spreading, YAP signaling and contractility.**

963 Representative immunofluorescence images and traction force maps of HUVECs **(a)**
964 after infection with AGO2 or non-targeting control pLentiCRISPR virus. Cells on
965 fibronectin-coated 3kPa PDMS gels were stained for F-Actin (phalloidin), focal
966 adhesions (paxillin), and YAP/TAZ. Quantification shows cell area (based on phalloidin
967 staining) (n=281-396 cells/group), number of paxillin adhesions per cell (n=49-51
968 cells/group), and nuclear to cytoplasmic ratio of YAP/TAZ (n= 43-54 cells / group).
969 Single cell maps of average traction stress and quantification of total force per cell (n=19
970 cells / group, bars indicate standard error, * p<0.05, **** p<0.0001,). **(b)** Scatter plot
971 representing difference in proteins expression between HUVEC seeded on 30 vs. 3 kPa
972 (x axis) or between HUVECs infected with AGO2 gRNA vs. control gRNA (y axis). Green
973 and red identify CAM proteins with coherent or incoherent differential expression,
974 respectively (Supplementary Table 6). **(c)** Experimental strategy to mutate individual
975 MREs in CAM gene 3UTR's to block miRNA binding (See methods and Supplementary
976 Figs 4 and 5). **(d)** Quantification of cell spreading (n=68-99 cells/group), YAP nuclear
977 translocation (n=68-99 cells/group) and traction stress (n=13-21 cells/group) in HUVECs
978 on 3 kPa PDMS gels for 48h after mutation of the indicated MREs (bars indicate
979 standard error).

980

981 **Figure 4. AGO2 activity limits tissue contractility and stiffness.**

982 **(a)** Representative 3D matrix constructs with control or Ago2-mutated mouse dermal
983 fibroblasts. **(b)** quantification of cell number and construct diameter within transverse

984 sections (n=8, bars indicated standard error, ** p<0.01, ns= non significant). **(c)**
985 Transverse sections of control and Ago2 depleted matrix constructs stained for Vimentin
986 or pMyosin. **(d)** Bright field images of fin fold tissues in the indicated genotypes. Elastic
987 modulus of 48 hpf zebrafish fin fold surfaces of Wild-Type, *mz ago2*^{-/-} and *mz ago2*^{-/-}
988 rescued with 200 pg of *in vitro* transcribed human Wild-Type AGO2 mRNA (*hs AGO2*
989 *mRNA*). Embryos were harvested and adhered to a soft surface of PDMS in egg water.
990 Elastic moduli were measured and AFM using NanoScope Analysis 1.5 software to fit
991 force-deflection curves using the Sneadon model. At least two fin fold regions within
992 each of 10-11 embryos were tested for each genotype (n=27-49 measurements per
993 region; bars indicate standard deviation, *p<0.05). **(e)** Bright field images of zebrafish fin
994 folds at the indicated stages and genotypes (head is to the left). Dotted black line
995 outlines the edge of the fin fold. Fin fold regeneration was assessed from the distance
996 between the wound edge and the embryo body. One cell stage Wild-Type, *mz ago2*^{-/-},
997 and *mz ago2*^{-/-} embryos rescued with 200 pg of *hs AGO2 mRNA*. Values were
998 normalized for the size of the fin-fold prior to injury (n=14, bars = standard deviation,
999 *p<0.05, ** p<0.01; ***p<0.001; ****p<0.0001).

1000

1001 **Figure 5. Wound healing in *mz ago2* and *ctgfa* MRE fin fold mutants.**

1002 **(a-c)** Top, schematics representing the time course of fin fold regeneration. Boxes
1003 identify the region of interest reported in the images below. Bottom confocal images of
1004 whole mount fin folds within the boxed region from the cartoons at the top, at the
1005 indicated stages. White and black dotted lines indicate the edge of the fin fold. White
1006 arrows point to staining for the indicated markers. Graphs show box plot of the
1007 respective measurements (methods and Supplementary Fig. 9a). Intensity profiles for
1008 multiple embryos were combined (n= 6 embryos for each genotype, *p<0.05, n.s.= non
1009 significant). For Yap the protein nuclear localization was represented. The
1010 nucleus/cytosol ration was obtained using the DAPI channel to generate a binary mask
1011 and subtract nuclear YAP-GFP intensity from the total YAP-GFP detected (methods and
1012 Supplementary Figure 9b). **(d)** The cartoon represents a model for miRNA-post-
1013 transcriptional regulation of structural proteins function in mechanical tissue
1014 homeostasis. Increases in matrix stiffness and the resulting cell contractility increase
1015 integrin and actomyosin-dependent CAM signaling, which upregulates miRNAs that
1016 suppress CAM transcripts, thus restoring normal tissue mechanics.

1017

1018 **Supplementary Figures and Table.**

1019

1020 **Supplementary Figure 1. AGO2-peaks are positioned preferentially on**
1021 **cytoskeleton-adhesion-matrix (CAM) genes.** (a) Bar graph of the Log(FDR) for the
1022 significant enriched Gene Ontology terms resulting from the HITS-CLIP assay and
1023 identified by DAVID software. (b) Bar graph of the Log(FDR) for the significant enriched
1024 Gene Ontology terms resulting from the microarray analysis of endothelial cells in culture
1025 versus freshly isolated.

1026

1027 **Supplementary Figure 2. Validation of CRISPR/Cas9 targeting of AGO2 in**
1028 **HUVECs.** (a) Quantification of elastic modulus of PDMS gels by compression testing on
1029 an instron 5848 (a, mean \pm SEM, n=2 gels per condition). Gels were compressed with a
1030 cylindrical indenter to 10% strain at 0.1%/s and allowed to stress-relax for 150 seconds,
1031 modulus was measured at equilibrium. Quantification of Fibronectin staining intensity on
1032 the two PDMS gels with representative images showing uniform Fibronectin deposition
1033 on the gel surface (mean \pm SEM, n=14 fields of view per stiffness). (b) Schematics
1034 showing the experimental approach to generate AGO2 CRISPR/Cas9 mutant cells in
1035 primary human and mouse cells. (c) Representative Western blot of three independent
1036 replicates showing reduced AGO2 levels in HUVECs with a lentivirus co-expressing
1037 Cas9 and AGO2 gRNAs, or a no-target gRNA (control, has no homology to any known
1038 mammalian gene). The bar graphs show quantification of the proteins normalized to
1039 GAPDH (mean \pm SEM; n = 3). qRT-PCR analysis of endothelial specific miRNAs in
1040 HUVECs infected with Cas9 and gRNA targeting AGO2 or DROSHA and no-target
1041 gRNA (control) at 7 days post infection (dpi). Results are shown normalized to controls
1042 (mean \pm SEM; n = 3). (c) Bar plot representing distribution obtained upon FACS analysis
1043 of 10,000 wild-type or AGO2 gRNA mutant HUVECs infected with CAM MRE Sensors
1044 and treated as indicated (mean \pm SEM; n = 3, * p<0.05)., mCherry/GFP ratio of CAM
1045 MRE Sensors increase in AGO2 mutant ECs, as the cell distribution is shifted
1046 significantly toward the Empty-Sensor control bin in both 3 and 30 kPa stiffness
1047 conditions.

1048

1049 **Supplementary Figure 3. Phenotype in cells with CRISPR/Cas9 targeting of AGO2**
1050 **and DROSHA.** (a) Cell spread area and nuclear/cytoplasmic YAP ratio using
1051 fibronectin coated polyacrylamide gels over a wider range of stiffness display

1052 results consistent with results from PDMS gel experiments (Fig. 3a) (mean \pm
1053 SEM, n=32-79 cells / group). **(b)** Bar plot representing HUVEC and HDF cell area for
1054 cells infected with AGO2 gRNA and non-targeting control seeded on fibronectin coated
1055 30kPa PDMS gels (mean \pm SEM, **** p<0.0001, HUVEC n=239-393, HDF n=201-
1056 273). **(c)** Left: Representative immunofluorescence images of HUVECs after infection
1057 with pLentiCRISPR virus directed at AGO2 or a non-targeting control seeded on
1058 fibronectin coated 3kPa PDMS gels. Right: Quantification of AGO2 expression in ~100
1059 cells. Intensity of the staining in each cell is normalized for the average intensity of all
1060 AGO2 cells and transformed in %. Cell area based on F-ACTIN staining or nuclear to
1061 cytoplasmic ratio of YAP/TAZ are plotted as individual value per cell. Highlighted in red
1062 cells infected with gRNA targeting AGO2, in black with no-target gRNA and in blue with
1063 gRNA targeting AGO2 expressing ~50% expression of AGO2 compared to control cells.
1064 **(d)** Top, representative Western blot of three independent replicates showing reduced
1065 levels of AGO2 in human dermal fibroblasts (HDFs) infected with Cas9 and AGO2
1066 gRNAs or a no-target gRNA (control) at 7 days post infection (dpi). Bar plot indicates
1067 Western blot quantification of the AGO2 protein normalized for GAPDH at 7 dpi (mean \pm
1068 SEM; n = 3). Bottom, quantifications of HDFs after infection with pLentiCRISPR virus
1069 directed at AGO2 or a non-targeting control seeded on fibronectin coated 3kPa PDMS
1070 gels. Bar plot shows HDF cell area (n=229-309 cells/group) based on phalloidin staining,
1071 number of paxillin adhesions per cell (n=34-58 cells/group), and nuclear to cytoplasmic
1072 ratio of YAP/TAZ (n=34-58 cells/group). (mean \pm SEM, *p<0.05,
1073 **p<0.01, ****p<0.0001). Single cell maps of average traction stress and quantification
1074 of total force per cell (mean +/- SEM, n=20-21 cells per group, * p<0.05). **(e)** Western
1075 blot for DROSHA after knockdown with shRNA delivered via lentivirus (5 days post
1076 infection, pSMART Dharmacon) with quantification on 3kPa PDMS gels of cell spread
1077 area, YAP nuclear localization (mean +/- SEM, n=138-156 cells / group, **** p<0.0001),
1078 and total force per cell (mean +/- SEM, n=21 cells / group, *** p<0.001).

1079

1080 **Supplementary Figure 4 and 5. Sequences of CAM MREs resulting from**
1081 **CRISPR/Cas9 mutagenesis.** MiSeq 2x250 analysis of PCR amplicons derived from
1082 genomic DNA of HUVEC mutated with the indicated CAM MRE gRNAs. MREs are
1083 highlighted with red boxes, PAMs are highlighted with black boxes. Bar blot represent
1084 the % wild-type and mutated CAM 3UTR sequences. Only mutations with a frequency >

1085 of 2% are represented. MSA= Multiple Sequence Alignment; nt= nucleotide. Numbers
1086 indicate the mutations as nt inserted (+) or deleted (Δ).

1087

1088 **Supplementary Figure 6. Effects of CAM MRE mutations in HUVECs. (a)**

1089 Representative Western blot and immunofluorescence images showing the upregulation
1090 of the target CAM protein in human endothelial cells (HUVECs) infected with Cas9 and
1091 gRNA targeting specific CAM MREs and no-target gRNA (control) at 7 days post
1092 infection (dpi). Bar plot indicates Western blot quantification of the CAM protein
1093 normalized to β -ACTIN at 7 dpi. **(b)** Bar plots representing the quantification of
1094 mechanical parameters as in Fig. 3a of HUVECs transfected with miRTP (miRNA target
1095 protector) directed at CTGF 3UTR for 4 days. miRTP is a single-stranded, modified RNA
1096 oligonucleotide that blocks a miRNA interaction with an individual MRE. Negative control
1097 target protector (QIAGEN) has no homology to any known mammalian gene (mean \pm
1098 SEM, Area and YAP: n=99-101 cells / group, TFM: n=13-15 cells/ group). *p<0.05,
1099 **p<0.01, ****p<0.0001.

1100

1101 **Supplementary Figure 7. Reduced levels of Ago2 in Mouse Dermal Primary**

1102 **fibroblasts, and in *mz ago2* $-/-$ zebrafish embryos. (a)** Representative blot showing
1103 reduced Ago2 levels in mouse skin fibroblast for 3D culture assay infected with gRNAs
1104 targeting Ago2 and no-target gRNA (control, has no homology to any known mammalian
1105 gene). Blots were obtained with Odyssey system from Licor to quantify near-IR
1106 fluorescence emitted by the secondary antibodies. Bar plots indicate densitometry
1107 measurements the AGO2 protein normalized for β -Actin at 7 dpi (mean \pm SEM, n=2). **(b)**
1108 Top, Confocal lateral view of whole mount zebrafish embryos at 48 hours post
1109 fertilization (hpf). Wild-type and *ago2* maternal zygotic homozygous mutant (*mz ago2* $-/-$)
1110 were stained with the Ab-panAGO2-2A8 and secondary only as a control of the total
1111 staining background. Bar plots indicate average of Ago2 fluorescence intensity
1112 normalized for the DAPI staining for each genotype (mean \pm SEM, n=10 embryos /
1113 group). Bottom, qPCR representing miRNAs level in wild-type vs *mz ago2* $-/-$ at 72 hpf
1114 (mean \pm SEM, n=3). **(c)** Right panel, schematics representing the experimental
1115 procedure to test defective miRNA-mRNA interaction in the *ago2* $-/-$ fin fold model. *In*
1116 *vitro* transcribed RNAs encoding for mCherry control and GFP coding sequence
1117 upstream of three perfect MREs for miR-24, a miRNA expressed in epidermis³⁰. 25
1118 picograms (pg) of each mRNA was co-injected into wild-type and *ago2* $-/-$ zebrafish

1119 embryos at the one cell stage post fertilization. Left panel, confocal whole mount image
1120 of 48 hpf embryos expressing GFP and mCherry (head is to the left). GFP and mCherry
1121 pixel intensities were quantified and the GFP/mCherry ratio was plotted for each
1122 genotype (mean \pm SEM, n~5 embryo / group). *p<0.05, **p<0.001

1123

1124 **Supplementary Figure 8. Fin fold regeneration model in zebrafish wild-type and**

1125 **mutant embryos . (a-c)** Top, Confocal lateral view of whole mount zebrafish embryos
1126 treated as indicated. White dotted line shows the edge of the wound. Bottom,
1127 quantification of each whole mount stained embryos as indicated. Pixel matrix of
1128 intensity was reconstructed for 50 μ m within the wound edge to the fish body. Pixel
1129 mean \pm SEM was calculated for 4-6 fish within each group. *p<0.05, n.s.= non-significant

1130 **(d)** Alignment of human and zebrafish *ctgfa* 3'UTR sequence containing the conserved
1131 MREs. This 3' UTR sequence was used to generate a wild-type and mutated miRNA
1132 sensor vector (as above). An in vitro transcribed mRNAs encoding for a GFP reporter
1133 was under the post-transcriptional regulation of *ctgfa* 3UTR (wild-type), a mutated
1134 version lacking the MREa sequence and co-injected with an mCherry mRNA with no
1135 3UTR regulation as negative control. 75 picograms (pg) of each mRNA was co-injected
1136 into wild-type and *mz ago2*^{-/-} zebrafish embryos at the one cell stage post fertilization.
1137 GFP and mCherry pixel intensities were quantified and the GFP/mCherry ratio was
1138 plotted for each genotype (mean \pm SEM, n~10 embryo / group *p<0.05). **(e)** Sequence
1139 alignment of the *ctgfa* 3UTR of zebrafish wild-type and *ctgfa* MRE embryos injected with
1140 Cas9 and the gRNAs which PAM region was highlighted in black. Mutations are
1141 represented as nucleotide (nt) inserted (+) or deleted (Δ) and were cloned from individual
1142 embryos 24 hours post injection. Red boxes represent the MREs targeted.
1143 Representative agarose gel for T7 Endonuclease I assay for Wild-Type and *ctgfa* MRE
1144 mutant zebrafish. hpa= hour post amputation. ns= non significant.

1145

1146 **Supplementary Figure 9. Spatial profiles of CAM proteins and YAP**

1147 **Nuclear/Cytosol quantification. (a)** Fluorescent intensity profiles for the indicated
1148 proteins and treatments depicting the protein quantification per zebrafish fin fold
1149 represented in Fig.5 a-c and Supplementary Fig. 7 a-c). Pixel matrix of intensity was
1150 reconstructed for 50 μ m within the wound edge to the fish body. Pixel mean was
1151 calculated and normalized for the maximum value to obtain pixel intensity profile. The
1152 intensity profile of 4-6 fish was combined and the smooth distribution of intensity was

1153 calculated. Solid lines show the mean for each genotype, while the grey ribbons show
1154 standard error. **(b)** Representative confocal lateral images of YAP immunostaining in the
1155 uncut fin fold. A DAPI binary mask was used to generate the Nuclear YAP (Fig. 5 c) and
1156 Cytosol YAP images.

1157

1158

1159 **Supplementary Table 1. AGO2-peaks localization.**

1160 The table shows the chromosome location and strand, gene name and Ensembl Gene
1161 ID and the peak p-value calculated with the Piranha (Methods). The last two columns
1162 show the number of genes associated with AGO2 and the number of peaks identified
1163 from the AGO2-HITS-CLIP.

1164

1165 **Supplementary Table 2. AGO2-miRNAs are clustered in 155 families.**

1166 The table lists the miRNAs associate with AGO2 complex in endothelial cells (HUVEC
1167 and HUAEC). For each miRNA, the mature sequence and the miRbase ID are shown.
1168 MiRNAs are grouped in families based on the 7-mer SEED region (column Family and
1169 SEED).

1170

1171 **Supplementary Table 3. miRNA-CAM-MRE interaction network.**

1172 The table represents the interaction network between the selected CAM-MRE and the
1173 endothelial miRNA within the AGO2 complex. The network was generated using
1174 TargetScan v. 7.0 (method) using CAM peaks listed in Table 1 and miRNA family listed
1175 in Table 2. Site_type, UTR_start and UTR_end columns represent the seed interaction
1176 type base (6mer < 7mer-1a < 7mer-m8 < 8mer-1a) and the localization of the interaction
1177 site within the Sensor_MRE_sequence (Table 4).

1178

1179 **Supplementary Table 4. CAM-MRE sequences.**

1180 The table lists the selected CAM sequence used for the Sensor-seq assay (method) and
1181 reported in Fig. 2. Canonical Gene name, MRE identity, extended MRE sequence, and
1182 length of the sequences cloned into the Sensor-Seq vector (method) are reported.

1183

1184 **Supplementary Table 5. Proteomics results.**

1185 The table lists the proteomics analysis performed on HUVEC cells seed on soft (3 kPa)
1186 or stiff (30 kPa) PDMS gel, or infected with AGO2 gRNA or not-target gRNA. The fold
1187 change values were used to generate the scatter plot in Figure 3b.

1188

1189 **Supplementary Table 6. CAM-MRE gRNA.**

1190 The table lists the selected CAM-MRE sequence, and the gRNA used for destabilizing
1191 the interaction with the miRNA (method).

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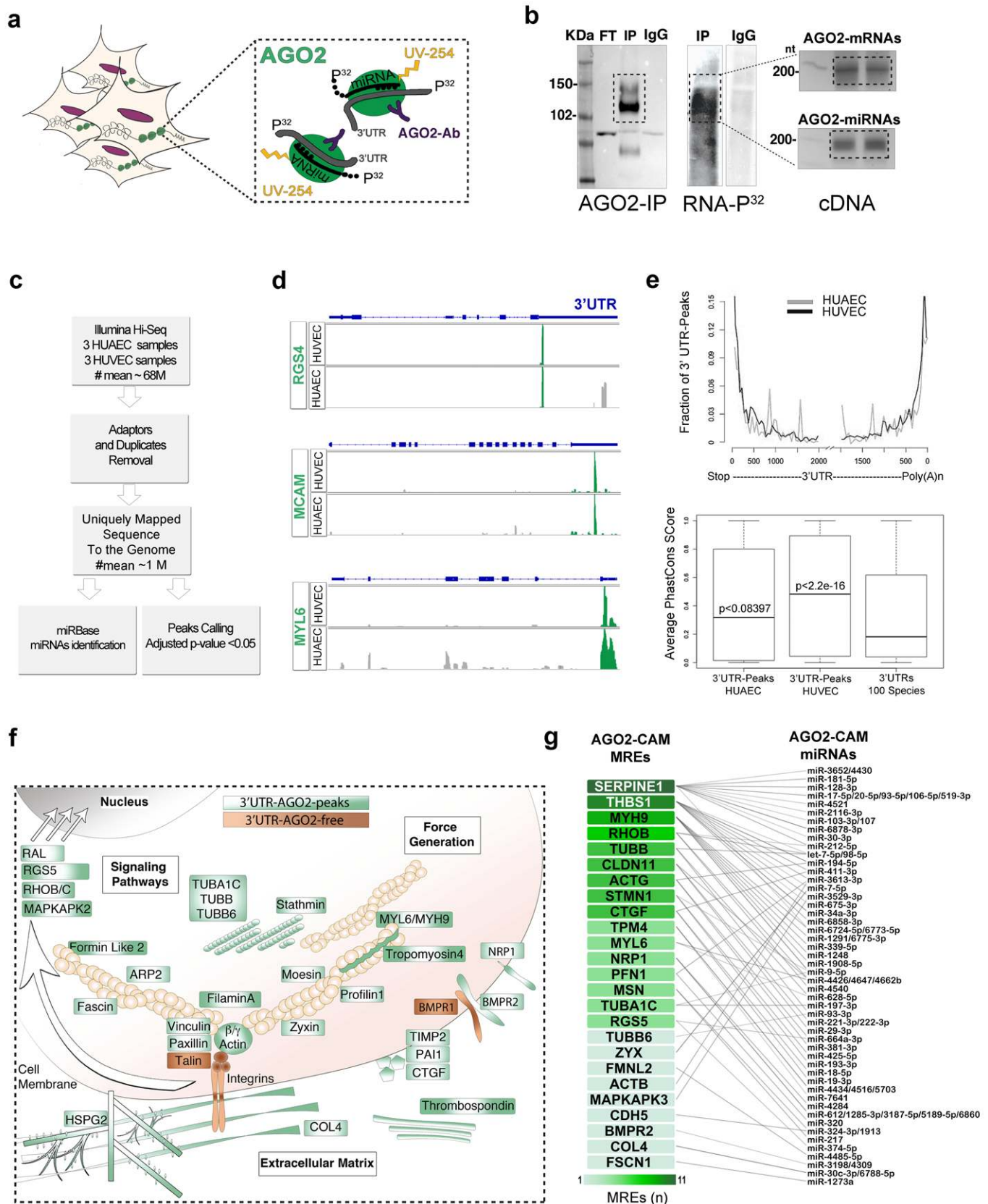
1195 **References**

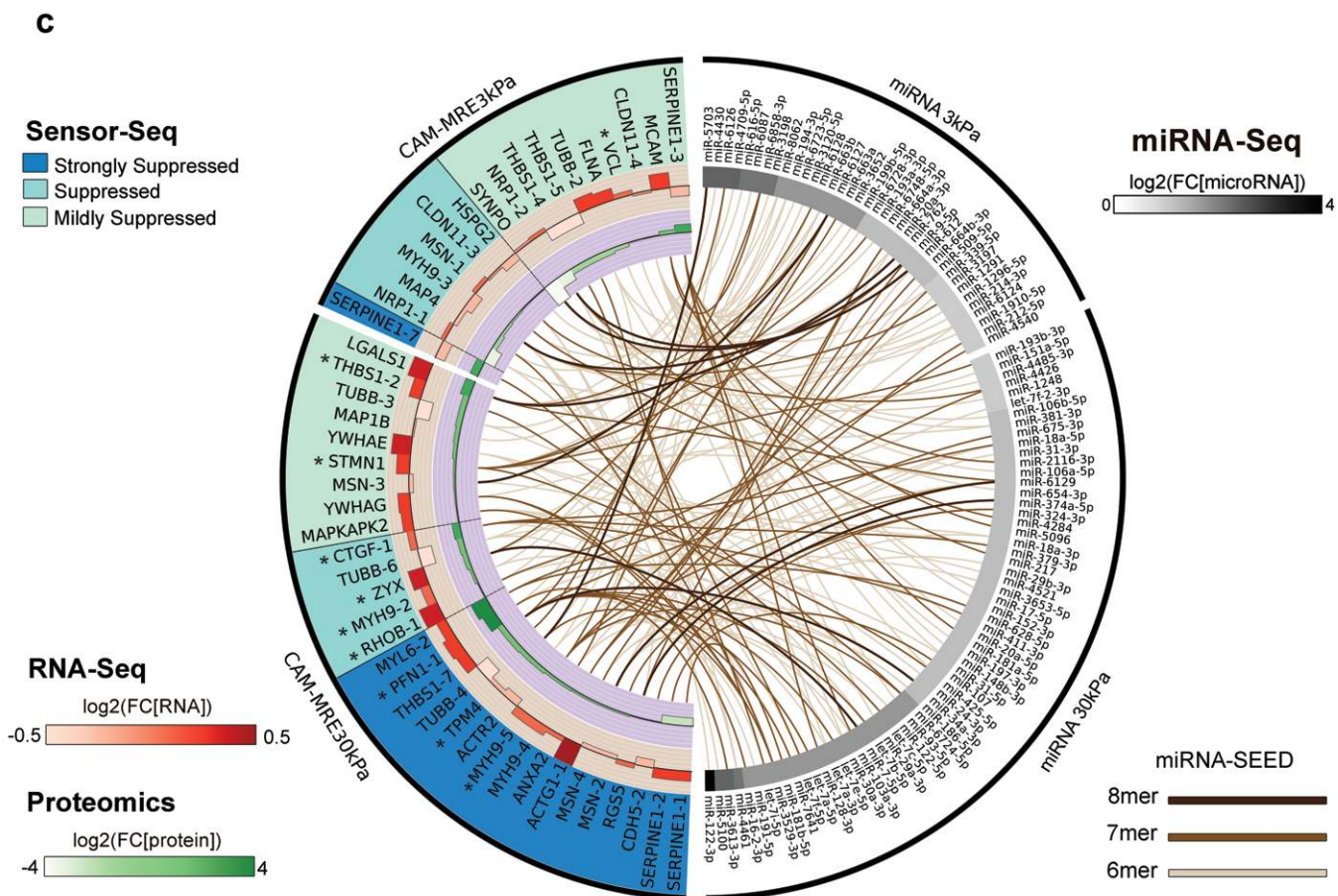
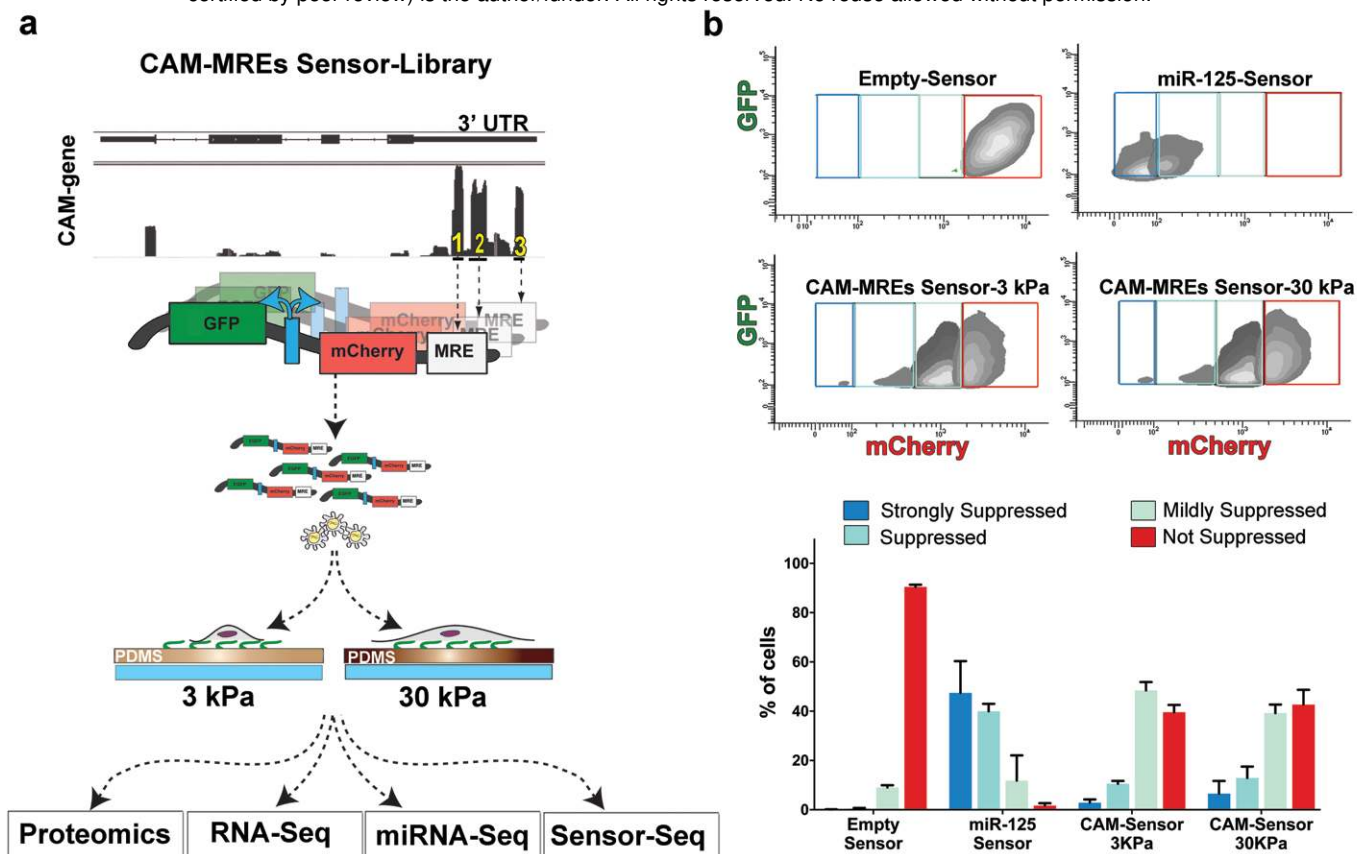
- 1196 1. Cyron, C.J. & Humphrey, J.D. Growth and Remodeling of Load-Bearing
1197 Biological Soft Tissues. *Meccanica* **52**, 645-664 (2017).
- 1198 2. Gilbert, P.M. & Weaver, V.M. Cellular adaptation to biomechanical stress
1199 across length scales in tissue homeostasis and disease. *Semin Cell Dev Biol* **67**,
1200 141-152 (2017).
- 1201 3. Humphrey, J.D., Dufresne, E.R. & Schwartz, M.A. Mechanotransduction and
1202 extracellular matrix homeostasis. *Nat Rev Mol Cell Biol* **15**, 802-12 (2014).
- 1203 4. Humphrey, J.D. Vascular adaptation and mechanical homeostasis at tissue,
1204 cellular, and sub-cellular levels. *Cell Biochem Biophys* **50**, 53-78 (2008).
- 1205 5. Seki, E. & Brenner, D.A. Recent advancement of molecular mechanisms of
1206 liver fibrosis. *J Hepatobiliary Pancreat Sci* **22**, 512-8 (2015).
- 1207 6. Huang, S. & Ingber, D.E. Cell tension, matrix mechanics, and cancer
1208 development. *Cancer Cell* **8**, 175-6 (2005).
- 1209 7. Sun, Z., Guo, S.S. & Fassler, R. Integrin-mediated mechanotransduction. *J Cell*
1210 *Biol* **215**, 445-456 (2016).
- 1211 8. Bartel, D.P. MicroRNAs: target recognition and regulatory functions. *Cell* **136**,
1212 215-33 (2009).
- 1213 9. Ebert, M.S. & Sharp, P.A. Roles for microRNAs in conferring robustness to
1214 biological processes. *Cell* **149**, 515-24 (2012).
- 1215 10. Guo, H., Ingolia, N.T., Weissman, J.S. & Bartel, D.P. Mammalian microRNAs
1216 predominantly act to decrease target mRNA levels. *Nature* **466**, 835-40
1217 (2010).
- 1218 11. Herranz, H. & Cohen, S.M. MicroRNAs and gene regulatory networks:
1219 managing the impact of noise in biological systems. *Genes Dev* **24**, 1339-44
1220 (2010).
- 1221 12. Tsang, J., Zhu, J. & van Oudenaarden, A. MicroRNA-mediated feedback and
1222 feedforward loops are recurrent network motifs in mammals. *Mol Cell* **26**,
1223 753-67 (2007).
- 1224 13. Kasper, D.M. *et al.* MicroRNAs Establish Uniform Traits during the
1225 Architecture of Vertebrate Embryos. *Dev Cell* **40**, 552-565 e5 (2017).
- 1226 14. Olson, E.N. MicroRNAs as therapeutic targets and biomarkers of
1227 cardiovascular disease. *Sci Transl Med* **6**, 239ps3 (2014).
- 1228 15. Nicoli, S., Knyphausen, C.P., Zhu, L.J., Lakshmanan, A. & Lawson, N.D. miR-221
1229 is required for endothelial tip cell behaviors during vascular development.
1230 *Dev Cell* **22**, 418-29 (2012).
- 1231 16. Nicoli, S. *et al.* MicroRNA-mediated integration of haemodynamics and Vegf
1232 signalling during angiogenesis. *Nature* **464**, 1196-200 (2010).
- 1233 17. Ristori, E. *et al.* A Dicer-miR-107 Interaction Regulates Biogenesis of Specific
1234 miRNAs Crucial for Neurogenesis. *Dev Cell* **32**, 546-60 (2015).
- 1235 18. Bracken, C.P., Scott, H.S. & Goodall, G.J. A network-biology perspective of
1236 microRNA function and dysfunction in cancer. *Nat Rev Genet* **17**, 719-732
1237 (2016).
- 1238 19. Pelaez, N. & Carthew, R.W. Biological robustness and the role of microRNAs: a
1239 network perspective. *Curr Top Dev Biol* **99**, 237-55 (2012).

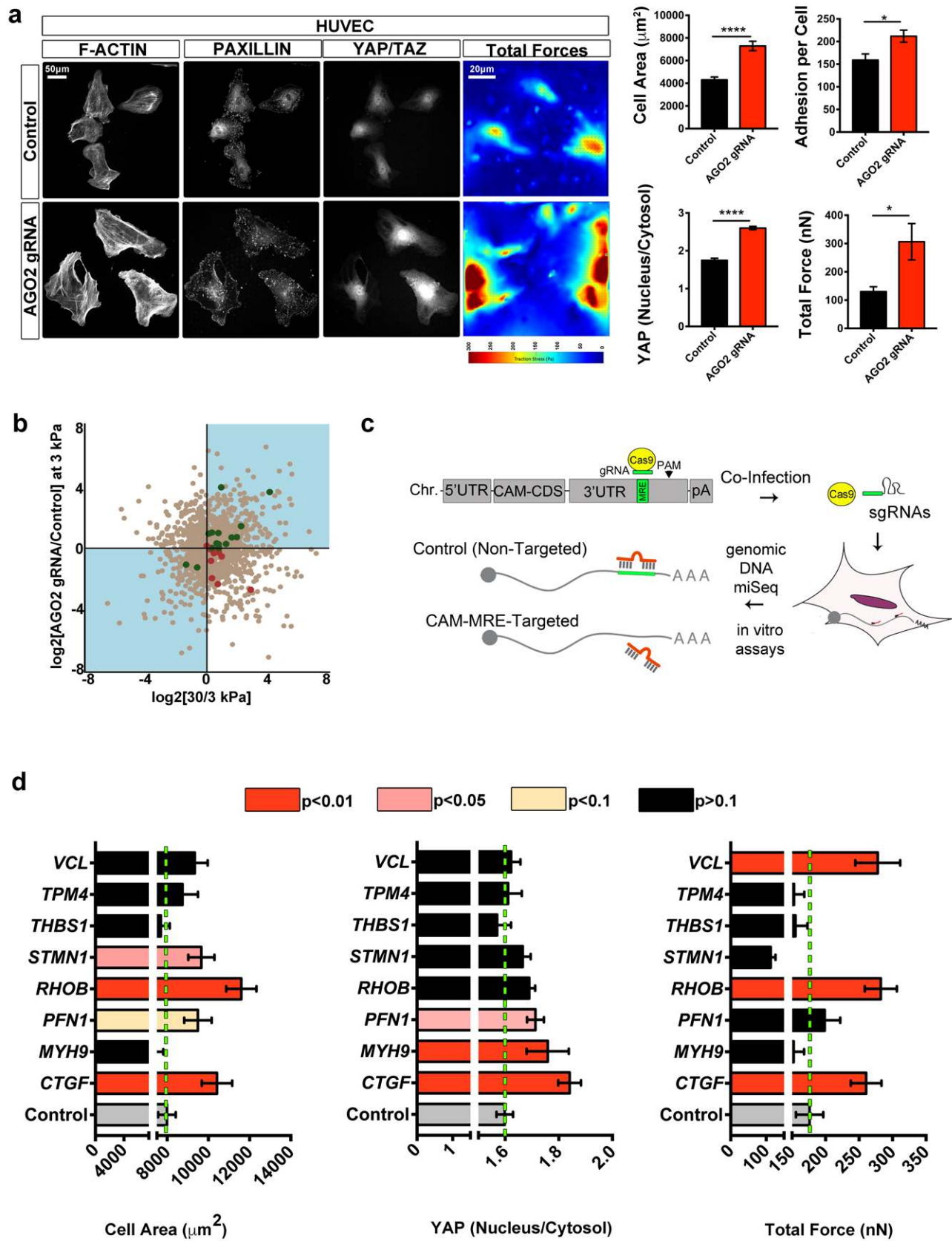
- 1240 20. Pasquinelli, A.E. MicroRNAs and their targets: recognition, regulation and an
1241 emerging reciprocal relationship. *Nat Rev Genet* **13**, 271-82 (2012).
- 1242 21. Hafner, M. *et al.* Transcriptome-wide identification of RNA-binding protein
1243 and microRNA target sites by PAR-CLIP. *Cell* **141**, 129-41 (2010).
- 1244 22. Chi, S.W., Zang, J.B., Mele, A. & Darnell, R.B. Argonaute HITS-CLIP decodes
1245 microRNA-mRNA interaction maps. *Nature* **460**, 479-86 (2009).
- 1246 23. Byfield, F.J., Reen, R.K., Shentu, T.P., Levitan, I. & Gooch, K.J. Endothelial actin
1247 and cell stiffness is modulated by substrate stiffness in 2D and 3D. *J Biomech*
1248 **42**, 1114-9 (2009).
- 1249 24. Grimson, A. *et al.* MicroRNA targeting specificity in mammals: determinants
1250 beyond seed pairing. *Mol Cell* **27**, 91-105 (2007).
- 1251 25. Saphirstein, R.J. & Morgan, K.G. The contribution of vascular smooth muscle
1252 to aortic stiffness across length scales. *Microcirculation* **21**, 201-7 (2014).
- 1253 26. Mullokandov, G. *et al.* High-throughput assessment of microRNA activity and
1254 function using microRNA sensor and decoy libraries. *Nat Methods* **9**, 840-6
1255 (2012).
- 1256 27. Kamata, M., Liang, M., Liu, S., Nagaoka, Y. & Chen, I.S. Live cell monitoring of
1257 hiPSC generation and differentiation using differential expression of
1258 endogenous microRNAs. *PLoS One* **5**, e11834 (2010).
- 1259 28. Discher, D.E., Janmey, P. & Wang, Y.L. Tissue cells feel and respond to the
1260 stiffness of their substrate. *Science* **310**, 1139-43 (2005).
- 1261 29. Kim, Y.K., Kim, B. & Kim, V.N. Re-evaluation of the roles of DROSHA, Export in
1262 5, and DICER in microRNA biogenesis. *Proc Natl Acad Sci U S A* **113**, E1881-9
1263 (2016).
- 1264 30. Dupont, S. *et al.* Role of YAP/TAZ in mechanotransduction. *Nature* **474**, 179-
1265 83 (2011).
- 1266 31. Kumar, A. *et al.* Talin tension sensor reveals novel features of focal adhesion
1267 force transmission and mechanosensitivity. *J Cell Biol* **213**, 371-83 (2016).
- 1268 32. Bassett, A.R. *et al.* Understanding functional miRNA-target interactions in
1269 vivo by site-specific genome engineering. *Nat Commun* **5**, 4640 (2014).
- 1270 33. Shi-Wen, X., Leask, A. & Abraham, D. Regulation and function of connective
1271 tissue growth factor/CCN2 in tissue repair, scarring and fibrosis. *Cytokine*
1272 *Growth Factor Rev* **19**, 133-44 (2008).
- 1273 34. Mateus, R. *et al.* Control of tissue growth by Yap relies on cell density and F-
1274 actin in zebrafish fin regeneration. *Development* **142**, 2752-63 (2015).
- 1275 35. Kawakami, A., Fukazawa, T. & Takeda, H. Early fin primordia of zebrafish
1276 larvae regenerate by a similar growth control mechanism with adult
1277 regeneration. *Dev Dyn* **231**, 693-9 (2004).
- 1278 36. Mathew, L.K. *et al.* Comparative expression profiling reveals an essential role
1279 for raldh2 in epimorphic regeneration. *J Biol Chem* **284**, 33642-53 (2009).
- 1280 37. Mateus, R. *et al.* In vivo cell and tissue dynamics underlying zebrafish fin fold
1281 regeneration. *PLoS One* **7**, e51766 (2012).
- 1282 38. Cifuentes, D. *et al.* A novel miRNA processing pathway independent of Dicer
1283 requires Argonaute2 catalytic activity. *Science* **328**, 1694-8 (2010).
- 1284 39. Amelio, I. *et al.* miR-24 triggers epidermal differentiation by controlling actin
1285 adhesion and cell migration. *J Cell Biol* **199**, 347-63 (2012).

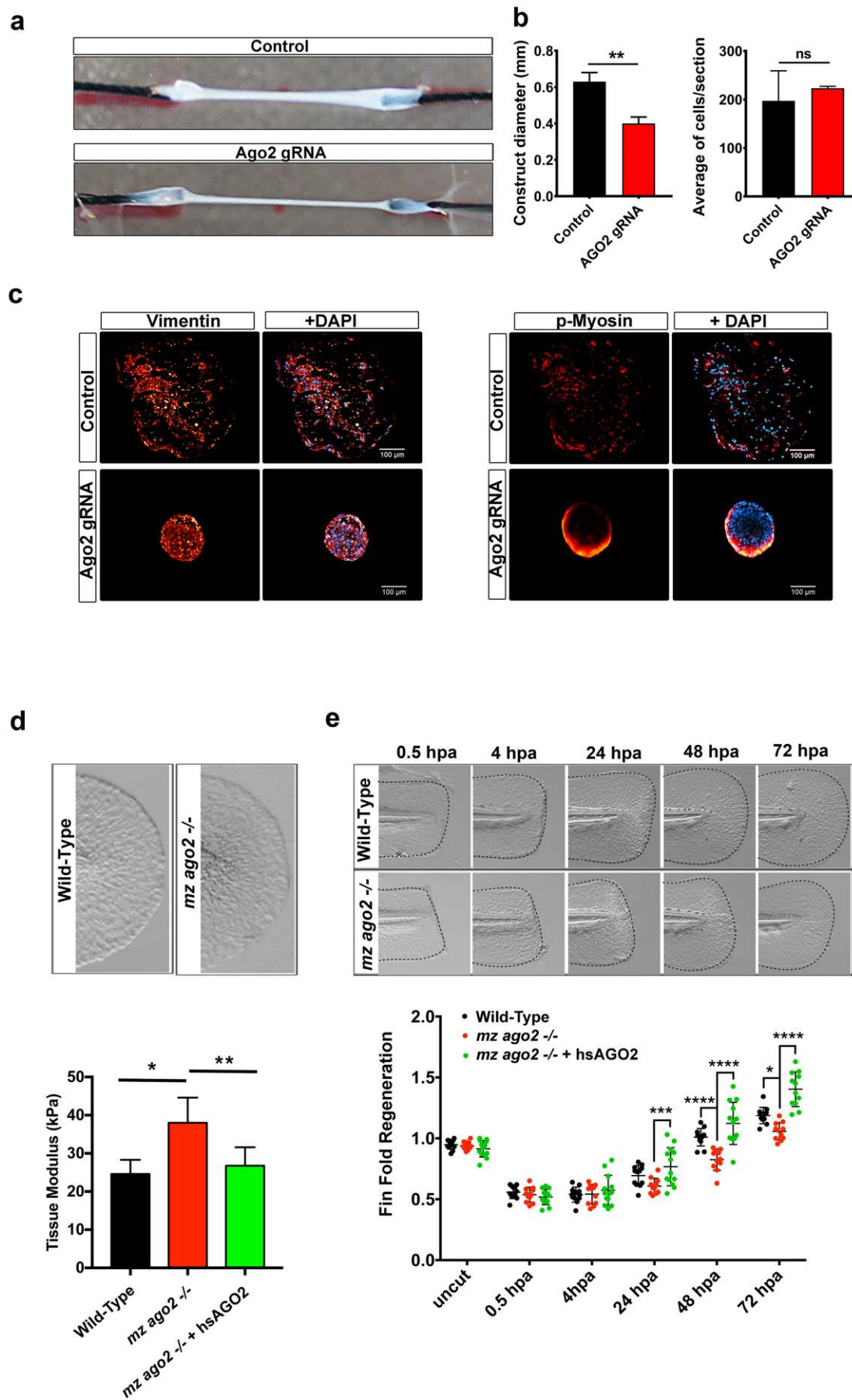
- 1286 40. Nechiporuk, A. & Keating, M.T. A proliferation gradient between proximal
1287 and msxb-expressing distal blastema directs zebrafish fin regeneration.
1288 *Development* **129**, 2607-17 (2002).
- 1289 41. Mori, M. *et al.* Hippo signaling regulates microprocessor and links cell-
1290 density-dependent miRNA biogenesis to cancer. *Cell* **156**, 893-906 (2014).
- 1291 42. Chaulk, S.G., Lattanzi, V.J., Hiemer, S.E., Fahlman, R.P. & Varelas, X. The Hippo
1292 pathway effectors TAZ/YAP regulate dicer expression and microRNA
1293 biogenesis through Let-7. *J Biol Chem* **289**, 1886-91 (2014).
- 1294 43. Davis, B.N., Hilyard, A.C., Lagna, G. & Hata, A. SMAD proteins control DROSHA-
1295 mediated microRNA maturation. *Nature* **454**, 56-61 (2008).
- 1296 44. Felix, M.A. & Wagner, A. Robustness and evolution: concepts, insights and
1297 challenges from a developmental model system. *Heredity (Edinb)* **100**, 132-
1298 40 (2008).
- 1299 45. Mouw, J.K. *et al.* Tissue mechanics modulate microRNA-dependent PTEN
1300 expression to regulate malignant progression. *Nat Med* **20**, 360-7 (2014).
- 1301 46. Liu, G. *et al.* miR-21 mediates fibrogenic activation of pulmonary fibroblasts
1302 and lung fibrosis. *J Exp Med* **207**, 1589-97 (2010).
- 1303 47. Cushing, L. *et al.* miR-29 is a major regulator of genes associated with
1304 pulmonary fibrosis. *Am J Respir Cell Mol Biol* **45**, 287-94 (2011).
- 1305 48. Herrera, J. *et al.* Dicer1 Deficiency in the IPF Fibroblastic Focus Promotes
1306 Fibrosis by Suppressing MicroRNA Biogenesis. *Am J Respir Crit Care Med*
1307 (2018).
- 1308 49. Parker, M.W. *et al.* Fibrotic extracellular matrix activates a profibrotic
1309 positive feedback loop. *J Clin Invest* **124**, 1622-35 (2014).
- 1310 50. Pandit, K.V. & Milosevic, J. MicroRNA regulatory networks in idiopathic
1311 pulmonary fibrosis. *Biochem Cell Biol* **93**, 129-37 (2015).
- 1312 51. Wynn, T.A. & Ramalingam, T.R. Mechanisms of fibrosis: therapeutic
1313 translation for fibrotic disease. *Nat Med* **18**, 1028-1040 (2012).
- 1314 52. Uren, P.J. *et al.* Site identification in high-throughput RNA-protein interaction
1315 data. *Bioinformatics* **28**, 3013-20 (2012).
- 1316 53. Dobin, A. *et al.* STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* **29**,
1317 15-21 (2013).
- 1318 54. Li, H. *et al.* The Sequence Alignment/Map format and SAMtools.
1319 *Bioinformatics* **25**, 2078-9 (2009).
- 1320 55. Kozomara, A. & Griffiths-Jones, S. miRBase: annotating high confidence
1321 microRNAs using deep sequencing data. *Nucleic Acids Res* **42**, D68-73 (2014).
- 1322 56. Agarwal, V., Bell, G.W., Nam, J.W. & Bartel, D.P. Predicting effective microRNA
1323 target sites in mammalian mRNAs. *Elife* **4**(2015).
- 1324 57. Aranguren, X.L. *et al.* Unraveling a novel transcription factor code
1325 determining the human arterial-specific endothelial cell signature. *Blood*
1326 **122**, 3982-92 (2013).
- 1327 58. Ritchie, M.E. *et al.* limma powers differential expression analyses for RNA-
1328 sequencing and microarray studies. *Nucleic Acids Res* **43**, e47 (2015).
- 1329 59. Narayanan, A. *et al.* In vivo mutagenesis of miRNA gene families using a
1330 scalable multiplexed CRISPR/Cas9 nuclease system. *Sci Rep* **6**, 32386 (2016).

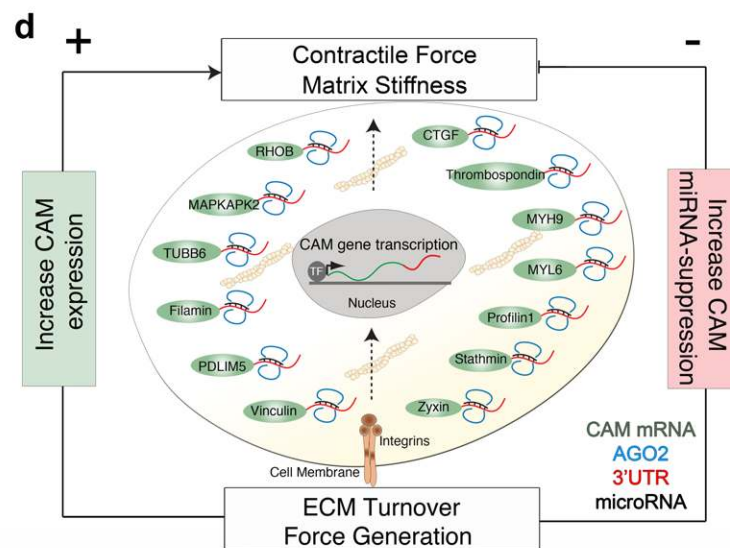
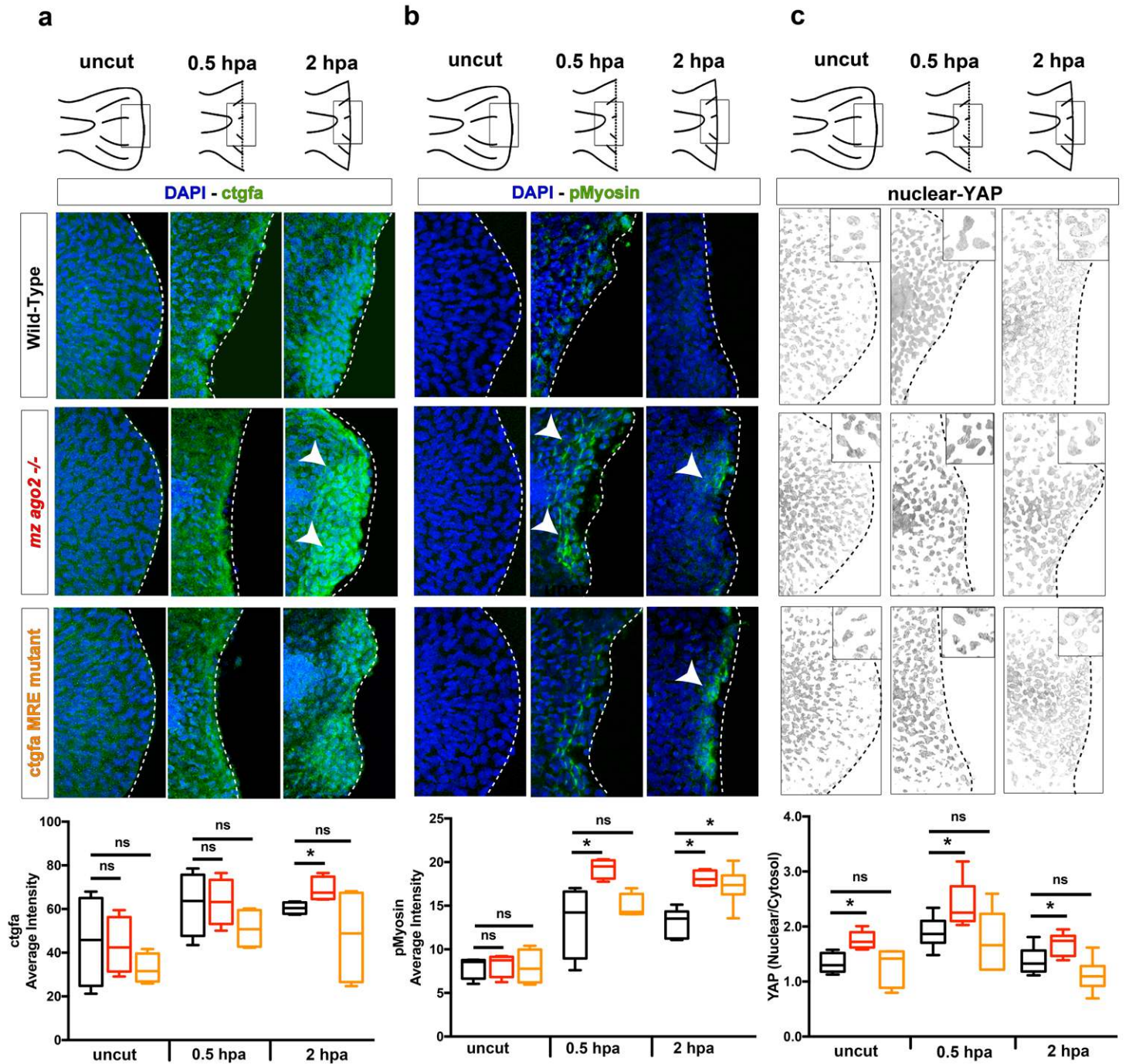
- 1331 60. Bodenhofer, U., Bonatesta, E., Horejs-Kainrath, C. & Hochreiter, S. msa: an R
1332 package for multiple sequence alignment. *Bioinformatics* **31**, 3997-9 (2015).
- 1333 61. Elosegui-Artola, A. *et al.* Mechanical regulation of a molecular clutch defines
1334 force transmission and transduction in response to matrix rigidity. *Nat Cell*
1335 *Biol* **18**, 540-8 (2016).
- 1336 62. Robinson, M.D., McCarthy, D.J. & Smyth, G.K. edgeR: a Bioconductor package
1337 for differential expression analysis of digital gene expression data.
1338 *Bioinformatics* **26**, 139-40 (2010).
- 1339 63. McCarthy, D.J., Chen, Y. & Smyth, G.K. Differential expression analysis of
1340 multifactor RNA-Seq experiments with respect to biological variation. *Nucleic*
1341 *Acids Res* **40**, 4288-97 (2012).
- 1342 64. Goeminne, L.J., Gevaert, K. & Clement, L. Peptide-level Robust Ridge
1343 Regression Improves Estimation, Sensitivity, and Specificity in Data-
1344 dependent Quantitative Label-free Shotgun Proteomics. *Mol Cell Proteomics*
1345 **15**, 657-68 (2016).
- 1346 65. Tyanova, S. *et al.* The Perseus computational platform for comprehensive
1347 analysis of (prote)omics data. *Nat Methods* **13**, 731-40 (2016).
- 1348 66. Smyth, G.K. Linear models and empirical bayes methods for assessing
1349 differential expression in microarray experiments. *Stat Appl Genet Mol Biol* **3**,
1350 Article3 (2004).
- 1351 67. Berginski, M.E. & Gomez, S.M. The Focal Adhesion Analysis Server: a web tool
1352 for analyzing focal adhesion dynamics. *F1000Res* **2**, 68 (2013).
- 1353 68. Gutierrez, E. & Groisman, A. Measurements of elastic moduli of silicone gel
1354 substrates with a microfluidic device. *PLoS One* **6**, e25534 (2011).
- 1355 69. Han, S.J., Oak, Y., Groisman, A. & Danuser, G. Traction microscopy to identify
1356 force modulation in subresolution adhesions. *Nat Methods* **12**, 653-6 (2015).
- 1357 70. Kapacee, Z. *et al.* Tension is required for fibripositor formation. *Matrix Biol*
1358 **27**, 371-5 (2008).
- 1359 71. Schindelin, J. *et al.* Fiji: an open-source platform for biological-image analysis.
1360 *Nat Methods* **9**, 676-82 (2012).
- 1361 72. Le Guyader, D. *et al.* Origins and unconventional behavior of neutrophils in
1362 developing zebrafish. *Blood* **111**, 132-41 (2008).
- 1363 73. Sneddon, I.N. The relation between load and penetration in the axisymmetric
1364 boussinesq problem for a punch of arbitrary profile. *Int. J. Engng Sci.* **3**, 47-57
1365 (1965).
- 1366 74. McCall, M.N. *et al.* MicroRNA profiling of diverse endothelial cell types. *BMC*
1367 *Med Genomics* **4**, 78 (2011).
- 1368 75. Krzywinski, M. *et al.* Circos: an information aesthetic for comparative
1369 genomics. *Genome Res* **19**, 1639-45 (2009).
- 1370

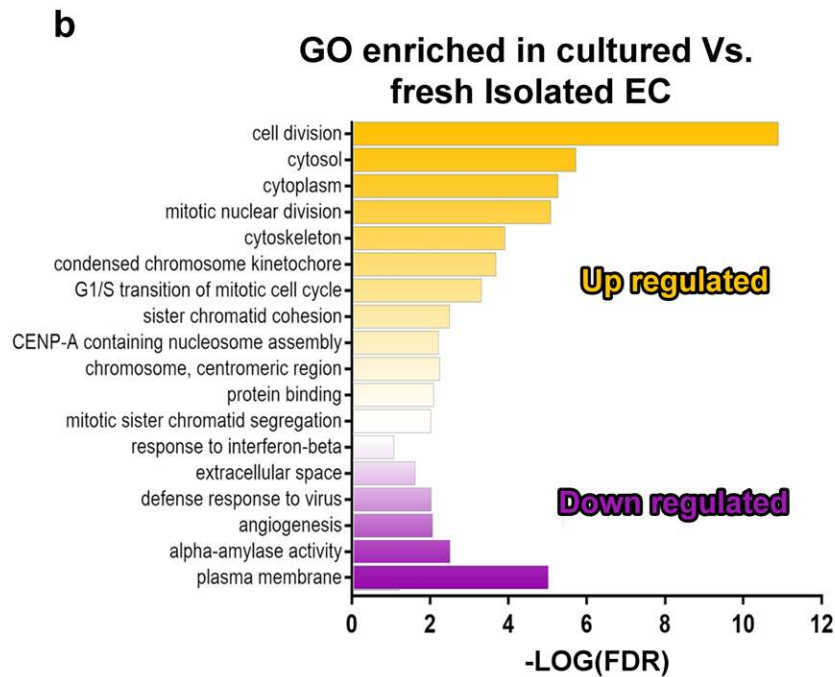
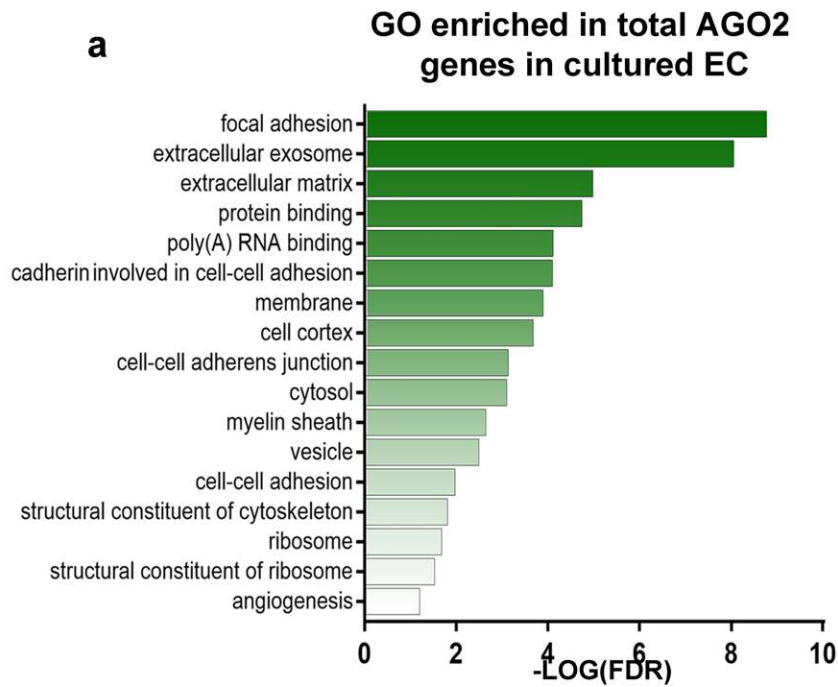


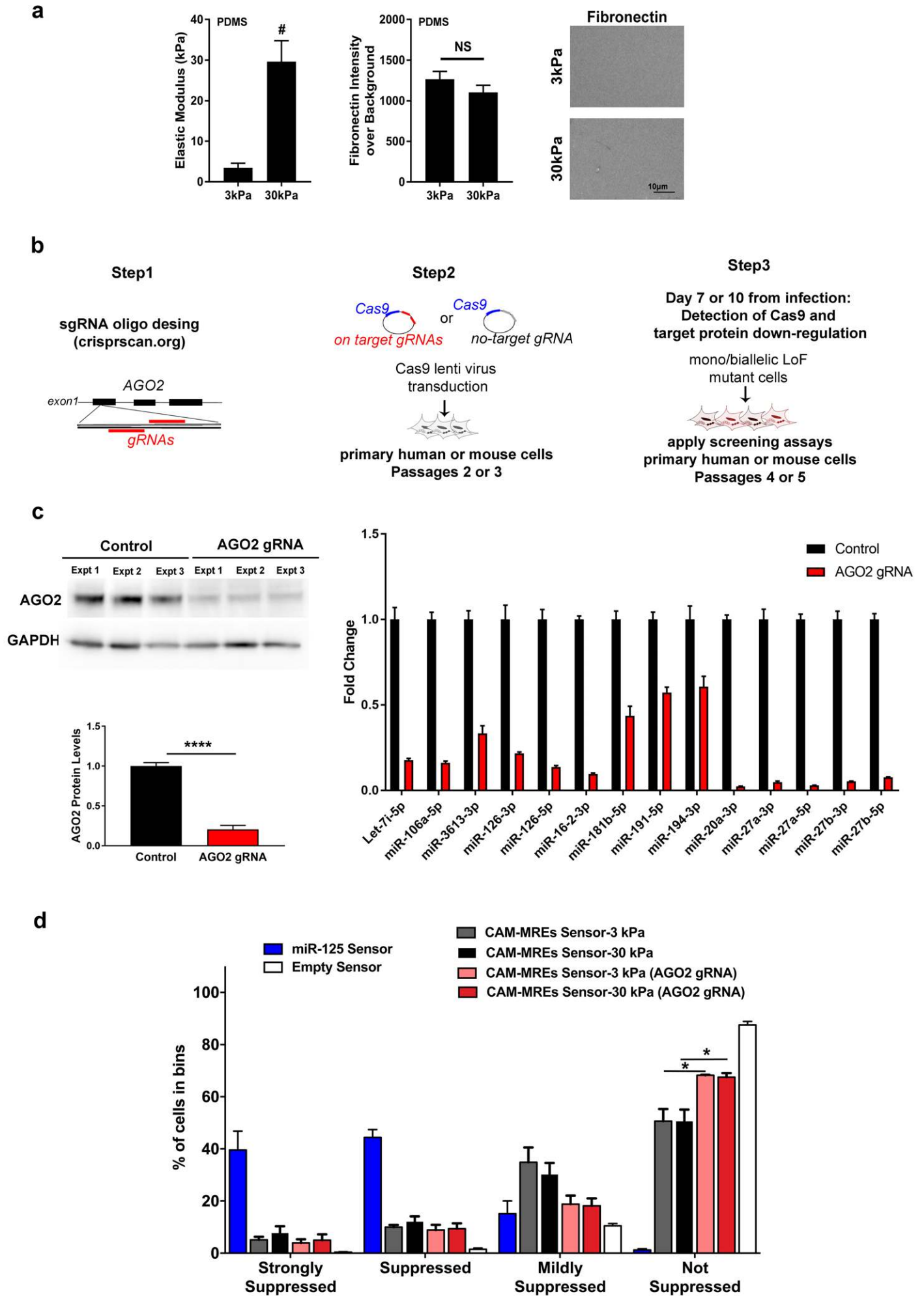


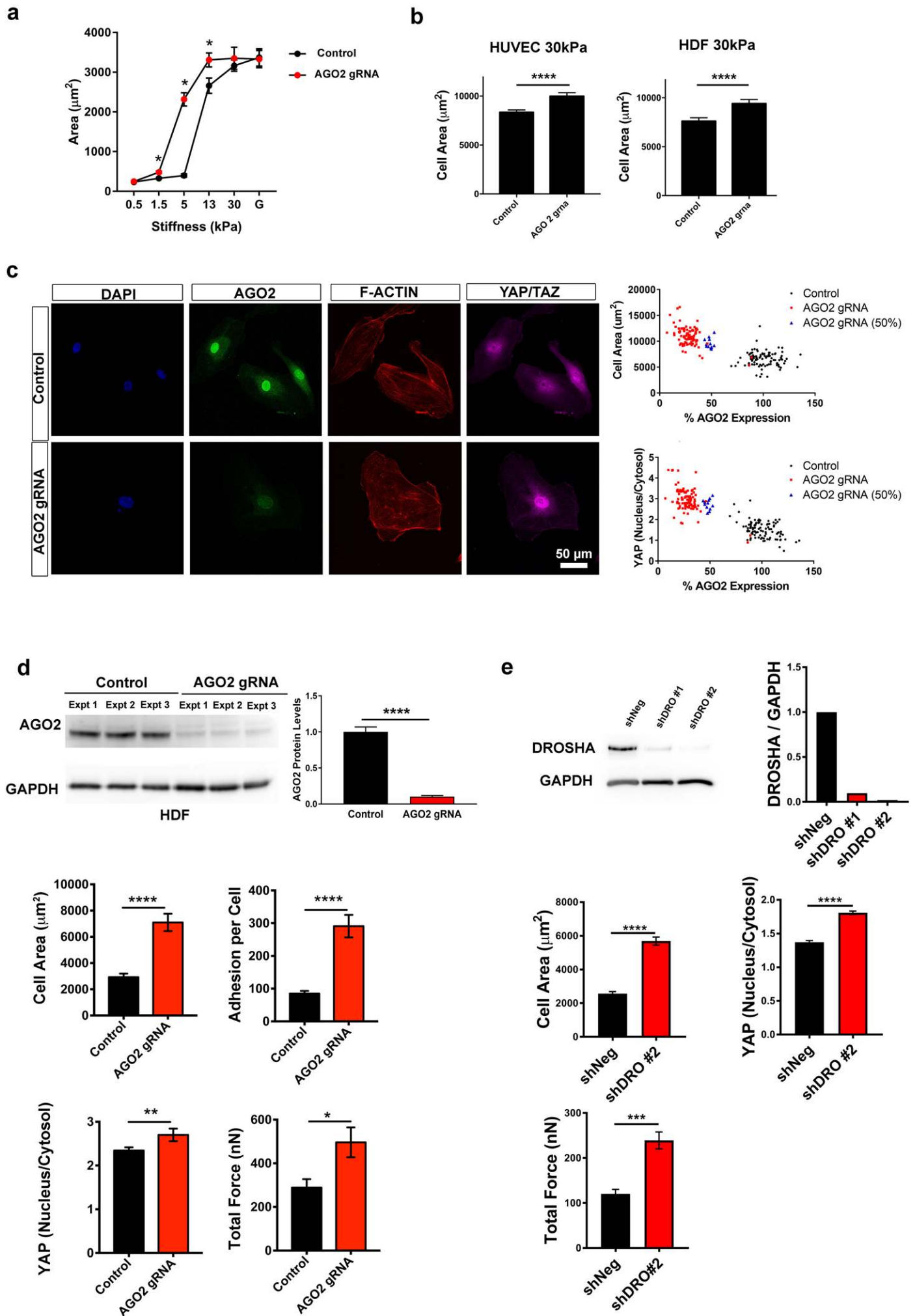


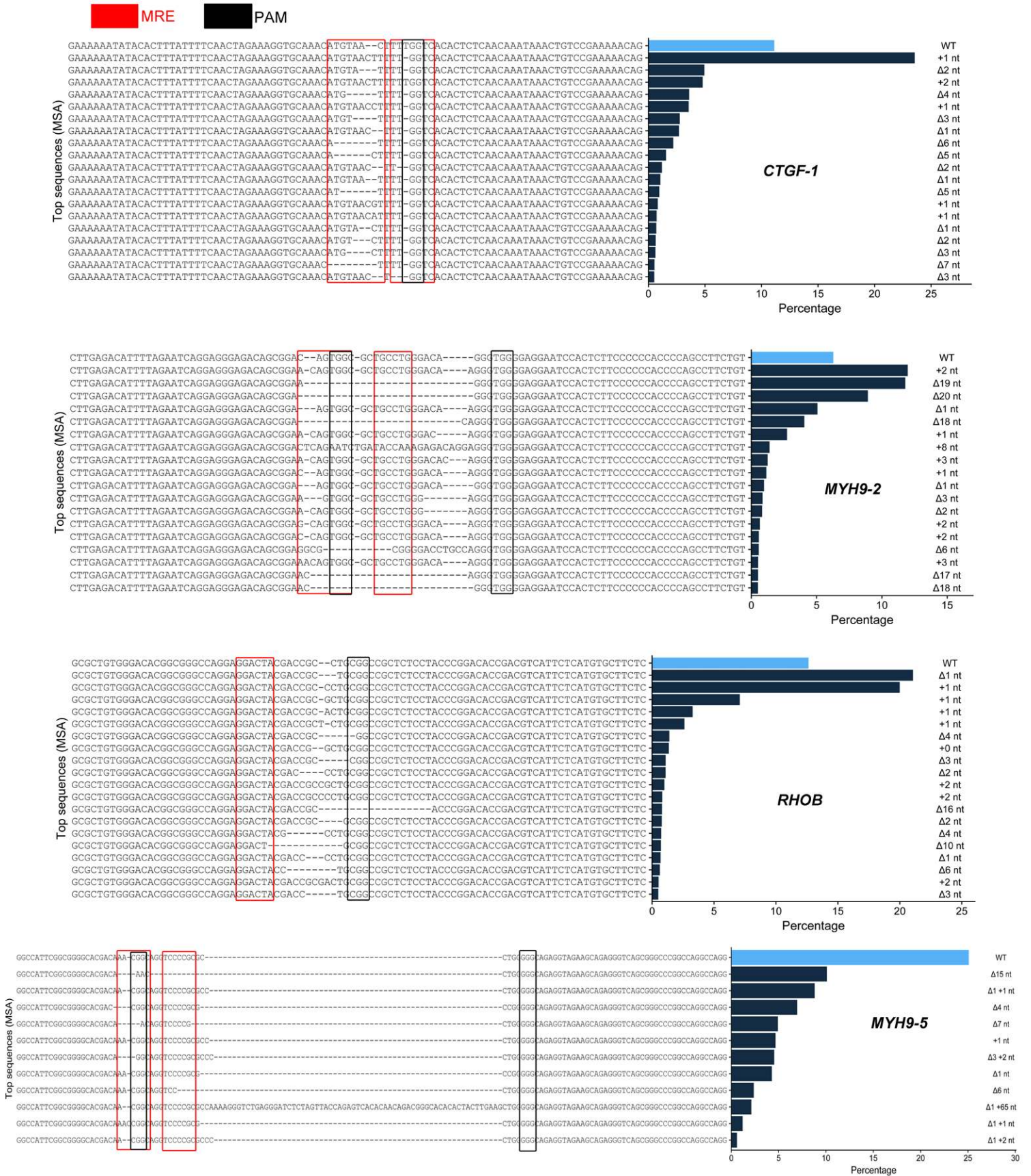


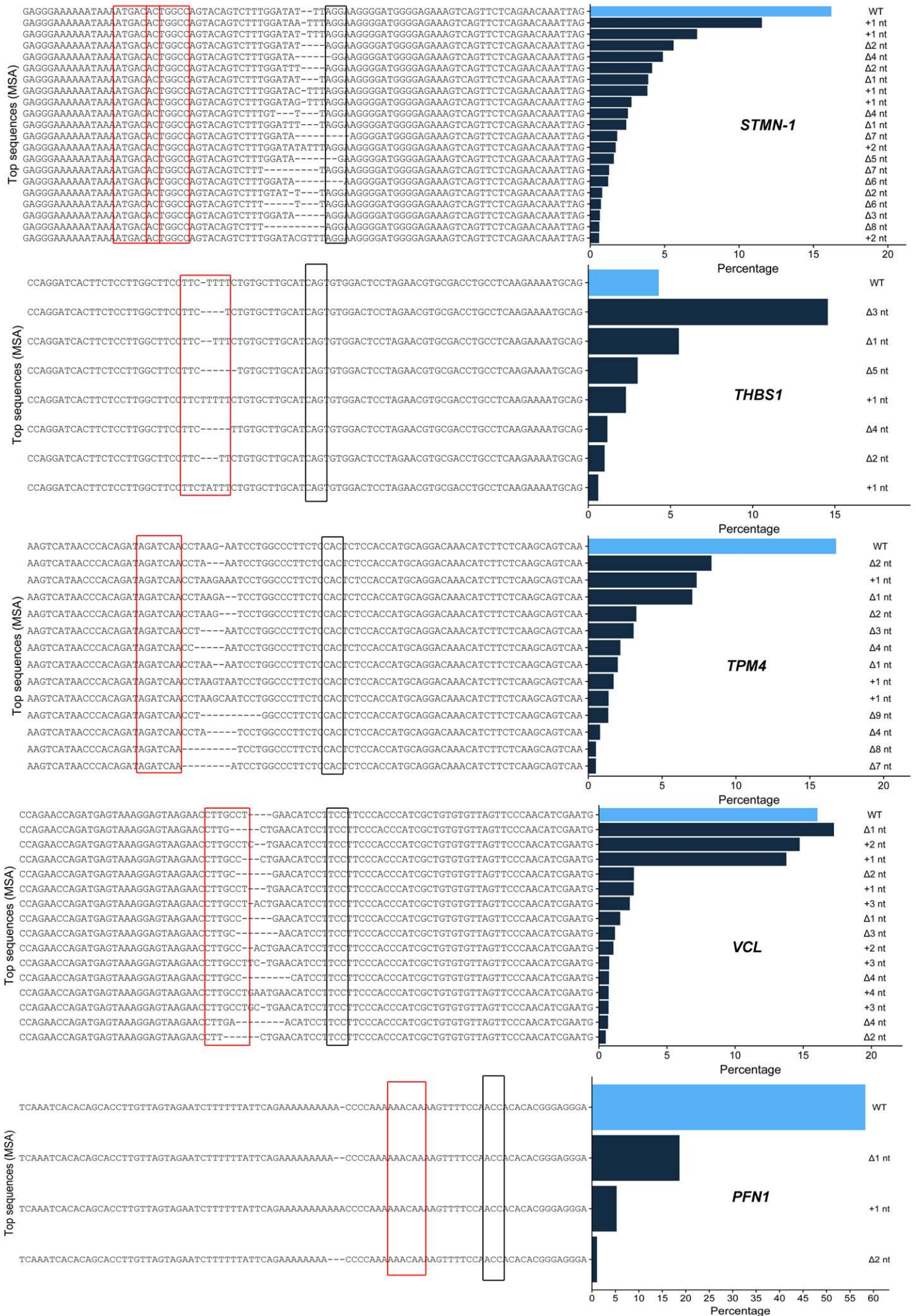


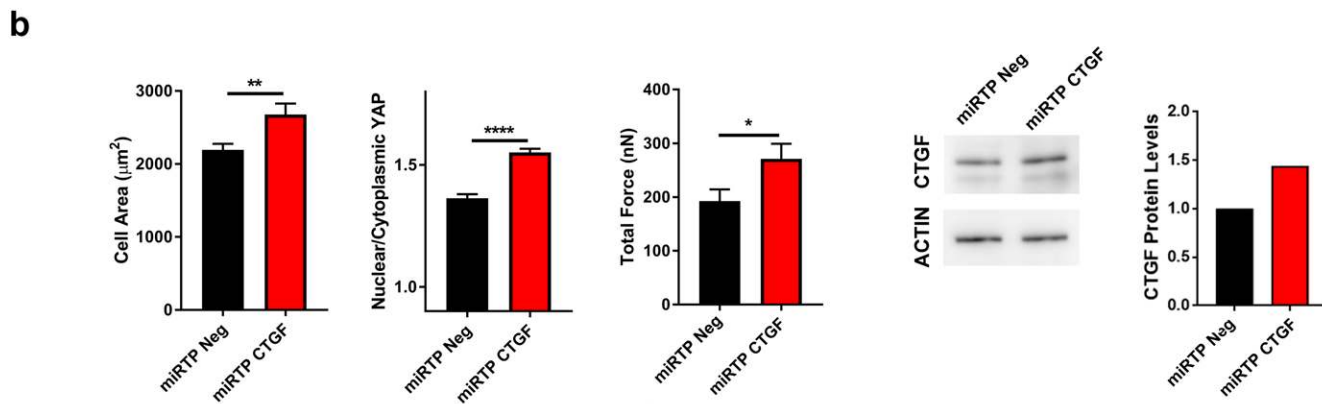
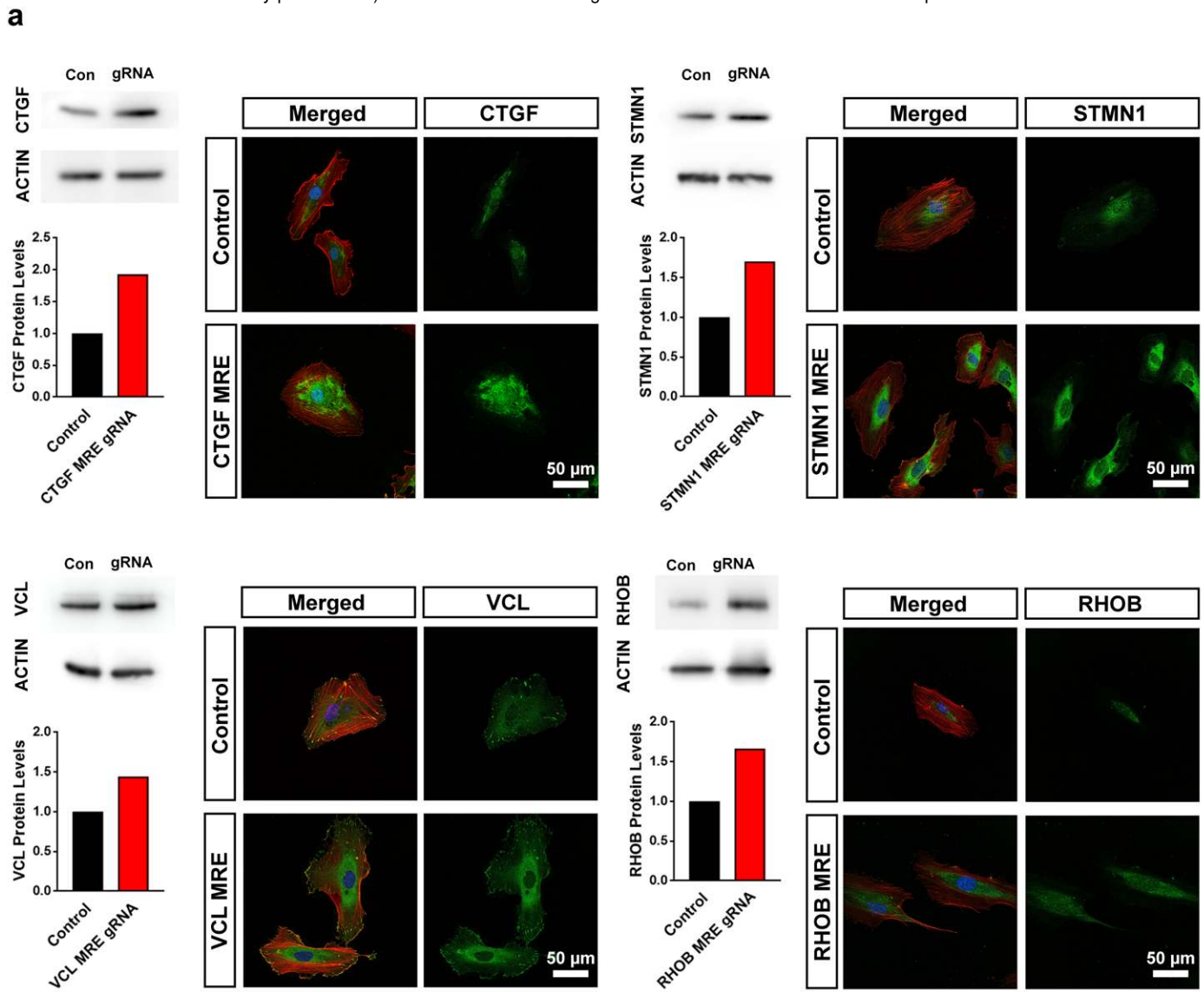












Mouse Dermal Primary Fibroblasts

