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1 **Microtubules tune mechanosensitive cell responses**

2

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16

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21

22 **Abstract**

23

24 Mechanotransduction is a process by which cells sense the mechanical properties of their surrounding
25 environment, and adapt accordingly to perform cellular functions such as adhesion, migration and
26 differentiation. Integrin-mediated focal adhesions are major sites of mechanotransduction, and their
27 connection with the actomyosin network is crucial for mechanosensing as well as the generation and
28 transmission of forces onto the substrate. Despite having emerged as major regulators of cell adhesion
29 and migration, the contribution of microtubules to mechanotransduction still remains elusive. Here, we
30 show that Talin- and actomyosin-dependent mechanosensing of substrate rigidity controls microtubule
31 acetylation (a tubulin post-translational modification) by promoting the recruitment of alpha-tubulin
32 acetyl transferase (α TAT1) to focal adhesions. Microtubule acetylation tunes mechanosensitivity of focal
33 adhesions and YAP translocation, and in turn, promotes GEF-H1-mediated RhoA activation, actomyosin
34 contractility, and traction forces. Our results reveal a fundamental crosstalk between microtubules and
35 actin in mechanotransduction that contributes to mechanosensitive cell adhesion and migration.

36 **Main**

37

38 Cells sense the physical properties of their environment, translate them into biochemical signals and
39 adapt their behaviour accordingly. This process known as mechanotransduction is crucial during
40 development and in the adult, during physiological and pathological conditions such as cell migration,
41 wound healing and cancer^{1,2}. Integrin-mediated focal adhesions (FAs) sense the matrix rigidity, control
42 the generation of actomyosin-dependent forces and the transmission of these traction forces onto the
43 substrate as well as contribute to mechanosensitive cell responses such as migration^{3,4}. In addition to
44 the actin cytoskeleton, microtubules are also key regulators of 2D and 3D cell migration⁵⁻⁸. Several
45 studies have demonstrated the role of the actomyosin cytoskeleton and FAs in mechanotransduction;

46 however, very little is known about microtubules in this context. In this study, we used primary rat
47 astrocytes and primary human umbilical vein endothelial cells (HUVECs) (both types of cells are
48 dependent on efficient microtubule dynamics for migration or invasion⁹⁻¹³), to address the role of
49 microtubules in rigidity sensing and mechanosensitive migration.

50 The crucial factors affecting the functions of the microtubule network are post-translational
51 modifications (PTMs) of tubulin such as acetylation, which occurs at the K40 residue of α -tubulin. The
52 enzyme responsible for microtubule acetylation, α TAT1 (α -tubulin acetyltransferase 1, also called as
53 MEC-17), is present in the lumen of microtubules¹⁴ and is highly specific to α -tubulin K40. On the other
54 hand, the enzymes involved in deacetylation at K40 are histone deacetylase family member 6 (HDAC6)
55 and sirtuin type 2 (Sirt2), both of which target other substrates as well¹⁵. In this study, we used
56 previously characterized siRNAs targeting α TAT1¹³ (set 1: si α TAT1-1 and set 2: si α TAT1-2; [Extended Data](#)
57 [Fig. 1a, 1b and 1c](#)) to decrease acetylation, and Tubacin ([Extended Data Fig. 1a and 1c](#)), a drug which
58 increases microtubule acetylation by inhibiting HDAC6 without modifying the acetylation of other
59 HDAC6 substrates such as histones^{16,17}.

60

61 **Substrate rigidity promotes microtubule acetylation through integrin β_1** 62 **signalling**

63

64 We have previously shown that microtubules come in close proximity to FAs, and that microtubule
65 acetylation promotes FA turnover during migration¹³. Since FAs are major mechanosensitive
66 structures¹⁸, we investigated whether the extracellular matrix rigidity affects microtubule acetylation.
67 Astrocytes were plated sparsely on polyacrylamide hydrogels of different rigidities: 1.26 kPa, 2 kPa, 9
68 kPa and 48 kPa ([Fig. 1a and 1b](#)). Astrocytes on soft substrates exhibited lower levels of acetylated
69 tubulin than cells on stiff substrates ([Fig. 1a and 1b](#)). Tubulin acetylation levels showed a two fold
70 increase with increasing rigidity (1.26 kPa to 48 kPa), a strongly significant change in comparison to the

71 maximal 3.8 fold increase obtained after Tubacin treatment ([Extended Data Fig. 1d](#)). Subsequently, to
72 determine whether microtubule acetylation may be indirectly caused by a mechanism involving
73 differential cell spreading, we plated astrocytes on adhesive micropatterns (area 2500 μm^2) printed on 2
74 kPa and 40 kPa hydrogels ([Extended Data Fig. 1e](#)). Similar to stiff substrates, astrocytes on soft
75 substrates adopted a crossbow shape and identical spread area, and yet, microtubule acetylation was
76 increased on stiff substrates as compared to softer substrates ([Extended Data Fig. 1e](#)). In contrast to
77 tubulin acetylation, tubulin detyrosination (another tubulin PTM), remained unaffected by increased
78 substrate rigidity in astrocytes ([Extended Data Fig. 1f and 1g](#)), and had no effect on astrocyte adhesion
79 and migration¹³, suggesting that rigidity sensing specifically affects microtubule acetylation.

80

81 Integrin-based FAs sense the matrix rigidity and trigger mechanosensitive signalling pathways¹⁹. To
82 determine the role of integrin signalling in controlling microtubule acetylation, we first used a scratch-
83 induced migration assay to trigger integrin activation at the wound edge¹¹. Addition of cyclic RGD (cRGD)
84 peptide, to prevent the binding of integrins to the RGD motif of extracellular matrix proteins, reduced
85 tubulin acetylation ([Extended Data Fig. 2a](#)). Furthermore, integrin activation using MnCl_2 ²⁰ led to an
86 increase in microtubule acetylation as compared to control cells ([Extended Data Fig. 2b](#)). Finally,
87 depletion of β_1 integrin using a siRNA ([Extended Data Fig. 2c](#)) also resulted in a significant decrease in
88 tubulin acetylation as compared to control cells ([Extended Data Fig. 2c and 2d](#)), suggesting that β_1
89 integrins are major players in the control of microtubule acetylation. We then investigated the
90 involvement of two central players in integrin signalling; the proto-oncogene c-Src and the Focal
91 Adhesion Kinase (FAK). In migrating astrocytes, inhibition of Src (using Src kinase inhibitor 1 – referred to
92 as Src kin 1) and FAK (using PF-562271) drastically reduced the levels of acetylated tubulin ([Extended](#)
93 [Data Fig. 2e](#)). In addition, following Src and FAK inhibition, microtubule acetylation induced by cell
94 spreading on 48 kPa substrates was decreased to a level similar to that observed on soft substrates ([Fig.](#)

95 [1C and Extended Data Fig. 2f](#)), indicating that Src and FAK are required for the rigidity-dependent
96 increase in acetylation. Altogether, these results strongly suggest that β_1 integrin-mediated signalling
97 promotes substrate rigidity-induced acetylation of microtubules.

98

99 **α TAT1 interacts with Talin in mechanosensitive manner**

100

101 Microtubule acetylation is mediated by α TAT1; however, the recruitment and/or activation of
102 α TAT1 remains unknown²¹. Therefore, we carried out a quantitative mass spectrometry screen to
103 identify interacting partners of α TAT1 using HEK293 cells. The mass spectrometry data (data available
104 via ProteomeXchange with identifier PXD015871; check methods for details; [Extended Data Fig. 2g](#))
105 revealed interesting potential interactors, amongst which the proteins enriched in the gene ontology for
106 focal adhesions depicted in red in [Extended Data Fig. 2g](#). One of the significant interactors on the mass
107 spectrometry screen was Talin ([Extended Data Fig. 2g](#)), a mechanosensitive partner of integrins¹⁹. We
108 confirmed the interaction of α TAT1 with Talin using pulldown experiments. GST- α TAT1, but not GST
109 alone, associated with endogenous Talin from astrocyte lysates ([Fig. 1d and Extended Data Fig. 2h](#)). The
110 interaction was further confirmed by immunoprecipitation using GFP- α TAT1-transfected astrocytes.
111 Talin co-immunoprecipitated with GFP- α TAT1 but not with GFP alone ([Fig. 1e and Extended Data Fig. 2i](#)).
112 In addition, using TIRF microscopy, we observed that GFP- α TAT1 strongly localised at mCherry-vinculin-
113 positive FAs ([Fig. 1f](#))¹³. We then investigated whether Talin played a role in the recruitment of α TAT1 to
114 FAs and the acetylation of microtubules. Therefore, we used specific siRNAs to reduce Talin expression
115 in primary astrocytes ([Extended Data Fig. 2j](#)). Talin depletion reduced GFP- α TAT1 recruitment to FAs
116 ([Fig. 1f](#)). In addition, Talin-depleted astrocytes plated on 48 kPa substrates exhibited decreased levels of
117 tubulin acetylation as compared to the control cells ([Fig. 1g](#)).

118

119 Since α TAT1 is also present within microtubules (Extended Data Fig. 2k)^{13,14}, we tested whether the
120 recruitment of α TAT1 to FAs was dependent on microtubules. To address this, GFP- α TAT1 and mCherry-
121 vinculin expressing astrocytes were treated with Nocodazole to depolymerize all microtubules.
122 Nocodazole-treated astrocytes displayed larger adhesions and increased stress fibres as observed in
123 other cell types (Extended Data Fig. 2l, Supplementary Video 1)^{22,23}, and increased α TAT1 at FAs
124 (Extended Data Fig. 2l, Supplementary Video 1), suggesting that microtubules are not required to
125 localise α TAT1 at FAs, and that increased actomyosin contractility might facilitate α TAT1 concentration
126 at FAs. Since actomyosin contractility is required for Talin-mediated mechanosensing^{24,25}, we used the
127 ROCK inhibitor, Y-27632 (which strongly reduces actomyosin contractility²⁶) to assess its role in the
128 mechanosensitive acetylation of microtubules. By GST- α TAT1 pulldown as well as TIRF microscopy, we
129 observed that Y-27632 treatment decreased α TAT1 interaction with Talin (Fig. 1h and 1i, Supplementary
130 Video 2) and tubulin acetylation (Fig. 1j and 1k) confirming that actomyosin contractility and
131 mechanosensing at FAs influence microtubule acetylation. Altogether, these results suggest that Talin-
132 and actomyosin-dependent mechanosensing triggers the recruitment of α TAT1 to FAs, and is required
133 for rigidity-dependent microtubule acetylation.

134

135 **Microtubule acetylation influences mechanotransduction at focal adhesions**

136

137 We then tested whether microtubule acetylation may be involved in mechanosensitive cell
138 functions. Astrocytes were sparsely plated on soft (2 kPa) and stiff (48 kPa) polyacrylamide hydrogels
139 and stained for Yes-associated protein (YAP), an actomyosin- and RhoGTPase-dependent transcriptional
140 activator whose nuclear translocation is mechanosensitive²⁷. Like in several cell types²⁸, quantification of
141 nuclear-to-cytoplasmic ratio of YAP indicated that YAP nuclear localisation increased when astrocytes
142 were plated on stiff substrates compared to soft substrates (Fig. 2a and 2b). In α TAT1-depleted

143 astrocytes, the nuclear recruitment of YAP in cells plated on 48 kPa substrates was strongly inhibited
144 and YAP localisation was similar in cells plated on soft and rigid substrates (Fig. 2a). Conversely,
145 increasing microtubule acetylation by Tubacin treatment led to a strong nuclear recruitment of YAP in
146 cells plated on 2 kPa substrates, recapitulating a phenotype of cells on stiff substrates (Fig. 2b). To
147 determine whether the effects of microtubule acetylation on YAP were specific to astrocytes, we
148 performed similar experiments on HUVECs. HUVECs exhibit low level of acetylated tubulin, even when
149 plated on stiff substrates (Extended Data Fig. 3b, 3c). Hence, to test the effects of microtubule
150 acetylation on YAP translocation in HUVECs, cells were treated with Tubacin to increase microtubule
151 acetylation (Extended Data Fig. 3c). Similar to astrocytes, increasing microtubule acetylation in HUVECs
152 plated on 2 kPa substrates resulted in an increased nuclear recruitment of YAP, mimicking the
153 phenotype of cells plated on stiff substrates (Fig. 2c). Altogether, this demonstrates that the
154 manipulation of microtubule acetylation tunes YAP mechanosensitive nuclear-cytoplasmic shuttling
155 (summary cartoon shown in Fig. 2g).

156

157 FAs are crucial mechanosensitive structures which sense and adapt to the matrix rigidity²⁹. Thus, we
158 investigated the effects of substrate rigidity on FAs in astrocytes plated on polyacrylamide hydrogels of
159 different rigidities: 1.26 kPa, 2 kPa, 9 kPa and 48 kPa (Fig. 2d), by immunostaining of the FA-associated
160 protein, Paxillin^{4,30}. Quantification of the density of FAs (Extended Data Fig. 3d) indicates that astrocytes
161 plated on soft substrates display FAs throughout the cell surface, whereas, FAs are predominantly at the
162 periphery of cells adhering to stiffer substrates (Fig. 2d). Thus, changes in substrate rigidity alter FA
163 distribution in astrocytes, allowing us to assess the impact of microtubule acetylation in this
164 phenomenon. Following α TAT1 depletion, FAs were distributed throughout the cell surface
165 independently of the rigidity of the substrate, i.e., α TAT1-depleted cells on stiff substrates showed a
166 distribution of FAs similar as that of control/si α TAT1 cells plated on soft substrates (Fig. 2e, Extended

167 [Data Fig. 3e-3h](#)). To confirm that the changes in FA distribution act through tubulin acetylation, we
168 treated cells plated on different rigidities with Tubacin. Tubacin had no effect on FA distribution in cells
169 plated on 48 kPa substrates ([Fig. 2f, Extended Data Fig. 3e and 3f](#)). In contrast, Tubacin treatment of
170 cells plated on 1.26 kPa substrates mimicked the phenotype (FAs at the cell periphery) observed in
171 control/Tubacin-treated cells plated on stiff matrices ([Fig. 2f, Extended Data Fig. 3e and 3f](#)). Since cell
172 spreading on different substrate rigidities can affect the distribution of FAs, we plated α TAT1-depleted
173 cells on micropatterned hydrogels to observe FAs in cells of similar spread area. In line with our prior
174 results, cells on soft substrates or α TAT1-depleted cells on stiffer substrates displayed FAs throughout
175 the cell spreading area ([Extended Data Fig. 3i](#)). Together with previous findings that microtubule
176 acetylation controls membrane vesicle delivery at FAs and FA dynamics^{13,31}, these results show that the
177 rigidity-dependent microtubule acetylation controls the mechanosensitive distribution of FAs ([Fig. 2g](#)).

178

179 **Microtubule acetylation controls cytoskeletal organization at focal adhesions**

180

181 Next, we investigated the impact of α TAT1 depletion on the FA-associated cytoskeleton. In
182 migrating astrocytes, the actin network comprises of longitudinal stress fibres connected to FAs at the
183 front of the leader cells as well as inter-junctional transverse arcs connecting neighbouring cells at
184 adherens junctions ([Fig. 3a and Extended Data Fig. 4a](#))^{26,32}. In α TAT1-depleted cells, the transverse arcs
185 of actin were dramatically reduced and the longitudinal fibres did not extend to the front of migrating
186 cells ([Fig. 3a, Extended Data Fig. 4a](#)). Associated with these longitudinal fibres, FAs were located further
187 back within the protrusion rather than at the front of leader cells ([Fig. 3a and Extended Data Fig. 4a](#))¹³.
188 Myosin light chain phosphorylation (pMLC) is predominantly seen at the leading edge of migrating
189 control cells; however, in α TAT1-depleted cells, pMLC was barely visible at the leading edge of cells and
190 was only associated with the remaining actin fibres at the cell centre, similar to Myosin IIa distribution

191 (Fig. 3a, 3b and Extended Data Fig. 4a). Moreover, intermediate filaments (visualised using Vimentin),
192 which play a major role in regulating FAs and collective migration of astrocytes³² and normally extend
193 from the perinuclear region to the cell periphery close to FAs^{32,33}, were absent from the front of α TAT1-
194 depleted cells and frequently appeared fragmented (Fig. 3c). We then looked closely at the effect of
195 α TAT1 on the cytoskeletal organisation at FAs by using platinum-replica transmission electron
196 microscopy (EM) on unroofed migrating astrocytes located at the wound edge. As by light microscopy,
197 EM images showed that FAs connected to actin bundles were distributed further within the protrusion
198 in case of α TAT1 cells as compared to a highly organised and parallel set of FAs at the leading edge of
199 control cells (Fig. 3d). From the high magnification views of FAs in the control cells, microtubules were
200 often seen along actin cables reaching FAs (Fig. 3d, marked with white arrows in i.1). Intermediate
201 filaments were also clearly visible, intertwined with the actin filaments at FAs (Fig. 3d, marked with
202 yellow arrows in i. 2 and i. 3). In α TAT1 cells, the actin bundles near FAs were strikingly thinner than
203 those in controls (Fig. 3d). We consistently observed a lack of microtubules and intermediate filaments
204 associated with FAs in α TAT1-depleted cells (Fig. 3d). All these results strongly support a role for α TAT1
205 in the cytoskeletal organisation at FAs in astrocytes. We then tested the effects of microtubule
206 acetylation on the cytoskeletal organisation by treating HUVECs and astrocytes plated on soft substrates
207 with Tubacin to increase microtubule acetylation. In Tubacin-treated cells, we observed prominently
208 larger actin cables, increased Myosin-IIa filaments, together with larger and more peripheral FAs (Fig. 3e
209 and Extended Data Fig. 3b), reminiscent of the phenotype observed in astrocytes plates on stiff
210 substrates (Fig. 3f), confirming that microtubule acetylation regulates actomyosin organization.

211

212

213

214 **Microtubule acetylation increases actomyosin contractility via GEF-H1 and Rho**

215

216 One major impact of mechanosensing is the adaptation of traction forces to the rigidity of the
217 substrate³⁴. We thus investigated whether microtubule acetylation might affect traction forces by
218 plating control or α TAT1-depleted astrocytes on crossbow-shaped micropatterned hydrogels. By
219 traction force microscopy, we observed that α TAT1 depletion resulted in lower traction force production
220 on 40 kPa substrates (Fig. 4a and 4b). In contrast, overexpression of GFP- α TAT1 increased traction
221 energies and forces and also rescued the effect observed on α TAT1 knockdown (si α TAT1 + GFP- α TAT1;
222 Fig. 4a and 4b) when astrocytes were plated on 40 kPa. In addition, Tubacin-treated astrocytes on soft 2
223 kPa hydrogels showed increased traction energies and forces, comparable to control astrocytes plated
224 on 40 kPa (Fig. 4c). We also studied the effects of microtubule acetylation on traction forces in Tubacin-
225 treated HUVECs plated on soft 2 kPa substrates. Similar to astrocytes, increased microtubule acetylation
226 using Tubacin resulted in higher traction energies and forces in HUVECs (Fig. 4d). Thus, the level of
227 microtubule acetylation dictates traction forces exerted on the substrate through FAs. This further
228 illustrates the essential role of the mechanosensitive regulation of microtubules in force transmission.

229 The crucial role of microtubule acetylation in the controlling cytoskeletal organisation and traction
230 forces led us to further investigate the molecular mechanisms involved in this process. We focused on
231 RhoA, a small G protein of the Rho family, well-known for promoting stress fibre formation and
232 actomyosin contractility, via its effector ROCK and MLC phosphorylation. Pulldown of GTP-bound active
233 RhoA using GST-Rhotekin beads showed that α TAT1 depletion reduced RhoA activity (Fig. 4e),
234 suggesting that microtubule acetylation may promote actomyosin contractility by activating RhoA. RhoA
235 activation is mediated by Guanine nucleotide exchange factors (GEFs)³⁵. Amongst these GEFs, GEF-H1
236 (also known as ARHGEF2) is a microtubule-bound RhoGEF which, when released from microtubules
237 triggers the Rho-ROCK signalling cascade and cell contractility^{36,37}. Previously, substrate stiffness was
238 suggested to correlate with GEF-H1 activity and actomyosin contractility³⁸. In addition, GEF-H1 was

239 recently shown to be controlled by the interaction of microtubules with integrin-mediated adhesions³⁹,
240 leading us to investigate whether integrin-mediated microtubule acetylation could affect the association
241 of GEF-H1 with microtubules. In control astrocytes plated on rigid glass coverslips, GEF-H1 localised
242 partially (approximately 42%) on microtubules but also in the cytosol, a localisation that corresponds to
243 the active/released GEF- H1 (Fig. 5a and Extended Data Fig. 5a)⁴⁰. In contrast, in α TAT1-depleted cells,
244 GEF-H1 was predominantly localised on microtubules (approximately 60%; Fig. 5a and Extended Data
245 Fig. 5a). Tubacin treatment of α TAT1-depleted cells led to the release of GEF-H1 into the cytosol,
246 rescuing the effect of α TAT1 depletion and confirming the role of microtubule acetylation in the GEF-H1
247 localisation (Fig. 5a and Extended Data Fig. 5a). In control HUVECs (which exhibit relatively low levels of
248 acetylated tubulin) GEF-H1 was mostly bound to microtubules (approximately 50%; Fig. 5b). Like in
249 astrocytes, the treatment of HUVECs with Tubacin resulted in the release of GEF-H1 into the cytoplasm,
250 with only 38% of GEF-H1 colocalising with microtubules (approximately 38%; Fig. 5b). GEF-H1
251 association with microtubules was confirmed using GFP-immunoprecipitation experiments using HEK
252 cells transfected with GFP-Ctl or GFP-GEF-H1 (Fig. 5c). Like Nocodazole treatment which leads to the
253 depolymerisation of microtubules, Tubacin treatment (which increases microtubule acetylation)
254 abolished the binding of GEF-H1 to microtubules (Fig. 5c). Altogether, these results show that
255 microtubule acetylation promotes the release of GEF-H1 from microtubules into the cytoplasm.

256

257 We then tested whether substrate rigidity and α TAT1 play a role in the association of GEF-H1 with
258 microtubules. Astrocytes plated soft 2 kPa substrates exhibit a higher microtubule-bound portion of
259 GEF-H1 as compared to cells on stiff 48 kPa gels (Fig. 5d and Extended Data Fig. 5b). In α TAT1-depleted
260 cells plated on stiff substrates, GEF-H1 was found predominantly bound to microtubules, mimicking the
261 phenotype observed in cells plated on soft substrates (Fig. 5d and Extended Data Fig. 5b). These results

262 strongly suggest that rigidity-dependent microtubule acetylation contributes to mechanotransduction
263 by enabling RhoA activation and actomyosin contractility.

264

265 **Microtubule acetylation modulates mechanosensitive cell migration by tuning** 266 **mechanotransduction at focal adhesions**

267

268 The involvement of microtubule acetylation in mechanotransduction and in the mechanosensitive
269 regulation of FAs and actomyosin contractility led us to investigate its influence on cell migration, which
270 has often been described as a mechanosensitive cellular response^{2,41}. To this end, we developed a
271 collective migration assay on hydrogels, where microdropping a small amount of a chemical (sodium
272 hydroxide) induced a circular wound in the cell monolayer (Fig. 6A, Supplementary Video 3). Sodium
273 hydroxide treatment did not noticeably alter the hydrogel properties (see Methods). Wild-type
274 astrocytes migrated significantly slower on 2 kPa gels than on 48 kPa gels (Fig. 6B, Supplementary Video
275 4), implying that astrocyte migration speed is affected by substrate rigidity. Most importantly, α TAT1
276 depletion abolished the increase of cell speed observed on stiff 48 kPa gels (Fig. 6C, Supplementary
277 Video 5), so that the migration speed of α TAT1-depleted cells plated on 48 kPa was similar to that of
278 control and α TAT1-depleted cells on 2 kPa substrates. In addition, the effects of α TAT1 depletion in cells
279 plated on 48 kPa gels is in line with our previous results described on the role of α TAT1 during collective
280 migration on glass coverslips¹³. Thus, we demonstrate that microtubule acetylation is required for the
281 mechanosensitive regulation of astrocyte collective migration.

282

283 **Discussion**

284

285 Our results show that microtubules are regulated in response to substrate rigidity sensing and in
286 turn, play a key role in mechanotransduction by participating in mechanosensitive cellular responses,

287 including translocation of the transcriptional coactivator YAP, focal adhesion distribution, actomyosin
288 contractility, generation of traction forces and cell migration (Fig. 6d). Our results point to a mechanism
289 by which Talin-mediated mechanosensing downstream of integrin-mediated signalling (Fig. 6d-i)
290 controls the recruitment of α TAT1 to FAs (Fig. 6d-ii) and induces microtubule acetylation (Fig. 6d-iii). We
291 show that α TAT1 interacts with Talin and that, on rigid substrates, Talin is required for the recruitment
292 of α TAT1 to FAs, and for the acetylation of microtubules (Fig. 6d-ii). Although we do not have any clear
293 evidence of a direct interaction of Talin with α TAT1, the β_1 -integrin- and actomyosin-dependent
294 association of α TAT1 with Talin suggest that tension-induced changes in Talin conformation may be
295 involved in inducing α TAT1 recruitment to FAs and microtubule acetylation.

296

297 How α TAT1 enters the lumen of microtubules still remains unclear although one can speculate that
298 α TAT1 accesses the lumen through microtubule lattice defects or through the open ends²¹. Growing
299 microtubule ends are often seen in close proximity to FAs at the leading edge of migrating cells⁸
300 (Extended Data Fig. 5C). It is very likely that, like other FA-associated proteins, a large proportion of
301 talin-associate α TAT1 rapidly exchange with a cytosolic pool⁴². This lead us to propose a model in which
302 the increased interaction of α TAT1 with talin increases the local concentration of α TAT1 and facilitates
303 its entry in the microtubule lumen through microtubule open ends in the vicinity of adhesions (Fig. 6D-
304 iii; Extended Data Fig. 5C).

305

306 We show that microtubule acetylation reorganises the actomyosin network and promotes traction
307 forces. Therefore, we propose a feedback mechanism involving a crosstalk between microtubules and
308 actin wherein, actomyosin-dependent mechanosensing promotes microtubule acetylation which, in
309 turn, facilitates the release of GEF-H1 from microtubules into the cytosol (Fig. 6D-iv) to increase RhoA
310 activity (Fig. 6D-v), cell contractility (Fig. 6D-vi) and traction forces (Fig. 6D-vii). It was recently shown

311 that uncoupling microtubules from FAs results in a similar release of microtubule-bound GEF-H1 into the
312 cytosol³⁹, which then triggers myosin IIA assembly and increased cell contractility through RhoA.
313 Suppression of RhoA activity in the absence of α TAT1 might be due to the sequestering of GEF-H1 by
314 non-acetylated microtubules. Whether microtubule acetylation directly or indirectly induces the release
315 of GEF-H1 remains unclear. One can speculate that acetylation of Lysine 40 changes the conformation of
316 α -tubulin and modifies the binding site for GEF-H1, thereby, inducing its release from microtubules.
317 Alternatively, acetylation might change the mechanical properties of the microtubule lattice and affect
318 its curvature^{41,42}, which would in turn facilitate the release of GEF-H1 from microtubules. It is also
319 possible that any of the previous mechanisms affect a binding of a partner of GEF-H1, which alters GEF-
320 H1 conformation (via phosphorylation for instance) and promote its release into the cytosol.
321 Interestingly, microtubule acetylation also affects the association of intermediate filaments with actin
322 bundles at FAs. Since intermediate filaments have also been involved in the control of FA dynamics,
323 actomyosin contractility as well as GEF-H1 activity^{32,40}, the microtubule-actin interplay described here
324 may also involve intermediate filaments, whose role in mechanotransduction is still elusive.

325

326 In response to substrate rigidity sensing, cells adapt essential functions such as migration⁴³. In the
327 absence of α TAT1, cells plated on stiff substrates produce less traction forces and migrate slower ([Fig.](#)
328 [6d-viii](#)). The relationship between forces and migration can be considered counter-intuitive for single
329 cell migration where traction forces appear to correlate with migration speed; and contractile forces are
330 required for migration but exaggerated traction forces tend to slow or even stop migration⁴⁴. In fact,
331 measurements of traction forces exerted by astrocytes plated on substrates of various rigidities shows
332 that there is a rigidity optimum (around 50 kPa), at which the forces are maximal ([Extended Data Fig.](#)
333 [5d](#)). When looking at microtubule acetylation, actomyosin contractility, or cell migration, it is clear that
334 all of them increase up to about 48kPa but then reach a plateau or even decrease when cells are plated

335 on glass, indicative of a non-monotonous response. In collective cell migration, the distribution of forces
336 within the migrating monolayer, more than the intensity itself, is crucial for the efficient control of
337 collective cell migration. Also, collective cell migration relies on the transmission of forces between the
338 leaders and followers. Cell-cell junctions not only transmit forces between cells but help maintain the
339 integrity of the monolayer^{26,45}, which improves collective and directed cell migration. Alteration of
340 microtubule acetylation did not induce any detachment of leader cells from followers and did not affect
341 directionality or persistence of migration¹³. Therefore, we propose a model in which, on stiff substrates,
342 increased microtubule acetylation would trigger higher traction forces in leader cells, which would
343 transmit these pulling forces to followers and increase collective migration speed. Microtubule
344 acetylation may also affect cell migration by controlling FA dynamics. We have previously demonstrated
345 that microtubule acetylation promotes the fusion of Rab6-dependent post-Golgi carriers at FAs and
346 increases FA turnover¹³, such that they are continuously renewed at the front of leader cells. Depletion
347 of α TAT1 (as that of Rab6) alters the distribution of FAs in migrating cells¹³. The change in FA distribution
348 between the cell center and the cell periphery suggests that rigidity-induced acetylation of microtubules
349 may promote FA turnover during cell spreading, thereby, excluding them from the cell center. We
350 speculate that the increase in FA turnover together with the increase in cell contractility participate in
351 strengthening the traction forces exerted on FAs concentrated at the leader cell wound edge and
352 improve astrocyte collective migration (Fig. 6d-viii).

353

354 In conclusion, our results depict a crosstalk between the actin and microtubule cytoskeletal
355 networks (Fig. 6d), whereby microtubule acetylation, downstream of rigidity-dependent integrin-
356 mediated signalling, alters actomyosin contractility as well as focal adhesion distribution and dynamics
357 to promote mechanosensitive migration of astrocytes, thus closing a crucial feedback loop governing
358 mechanotransduction at FAs (Fig. 6d).

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Methods

Cell culture

Primary astrocytes were obtained from E17 rat embryos¹². Use of these animals is in compliance with ethical regulations and has been approved from the Prefecture de Police and Direction départementale des services vétérinaires de Paris. Astrocytes were grown in 1g/L glucose DMEM supplemented with 10% FBS (Invitrogen, Carlsbad, CA), 1% penicillin-streptomycin (Gibco) and 1% Amphotericin B (Gibco) at 5% CO₂ and 37°C. Human umbilical vein endothelial cells (HUVECs) were cultured in EGM-2 BulletKit media (Lonza, catalogue no. CC-3162).

Cell nucleofection

Astrocytes were transfected with Lonza glial transfection solution and electroporated with a Nucleofector machine (Lonza). Cells were then plated on appropriate supports previously coated with poly-L-Ornithine (Sigma). Experiments are carried out 3 or 4 days post-transfection and comparable protein silencing was observed. siRNAs were used at 1 nmol and DNA was used at 5 µg. siRNA sequences used were:

Luciferase (control) UAAGGCUAUGAAGAGAUAC; αTAT1 rat (siαTAT1-1): 5'-ACCGACACGUUAAUUUAUGU-3' and 5'-UUCGAAACCGCAGGAACG-3'; αTAT1 rat (siαTAT1-2): 5'-UAAUGGAUGUACUCAUUCA-3' and 5'-UCAUGACUAAUUGUAGAUGA-3'; Talin-1 rat (siTalin1): 5'-GGGACACCAUGGAGUACAGAA-3', 5'-GACCCCCACUCCAGGAUAAU-3', 5'-GCUUGAAGCUGUGGAUAACCU-3', and 5'-GACCUCCACCCCUGAAGAUUU-3'; β₁ integrin (AUUGCCAGAUGGAGUAACA).

Constructs used were: GFP-αTAT1 and GST-αTAT1 (gift from Philippe Chavier, Institut Curie and Guillaume Montagnac, Institut Gustave Roussy) and mCherry-vinculin. siαTAT1-1 and siαTAT1-2 are

385 pools of two siRNAs each. For all experiments, si α TAT1-2 has been used due to better knockdown
386 efficiency as seen in Extended Fig. 1a and previously characterized in¹³. A few major experiments have
387 been carried out with si α TAT1-1 and the results are shown in the respective extended data figures.

388 **Cell treatment**

389 Cells were treated with 5-10 μ M Tubacin (Sigma SMLOO65) or 10 μ M Niltubacin (negative control for
390 Tubacin; Enzo Life Sciences) or DMSO (control) were added cells (3 h treatment for HUVECs; 6 h
391 treatment for astrocytes). Similarly, RGD peptide (Enzo Life Sciences) and RGE Control peptide (Enzo Life
392 Sciences) were added prior to wounding. ROCK inhibitor, Y-27632, and MnCl₂ (1 μ M) were added 6 h
393 after wounding and 2 h before fixation. For TIRF experiments, 10 μ M Nocodazole or Y-27632 were
394 added after 10 min of acquisition. For pull-downs, 10 μ M Nocodazole or Y-27632 were added 1 h before
395 cell lysis.

396 **Preparation of homogenously coated polyacrylamide hydrogels**

397 Protocol to prepare polyacrylamide hydrogels was adapted from^{41,46,47}. Coverslips were plasma cleaned
398 for 3 min and silanised for 10 min using a solution of 1% (v/v) 3-(trimethoxysilyl)propyl methacrylate and
399 1% (v/v) acetic acid in ethanol. Coverslips were then washed twice with absolute ethanol and dried. A
400 solution of polyacrylamide (proportions of acrylamide and bisacrylamide in the solution define the
401 rigidity of the hydrogel) was prepared. 2.5 μ l 10% APS and 0.25 μ l TEMED was added and mixed well
402 with the solution. A 50 μ l drop of the solution was added over each coverslip (20x20 mm) and
403 immediately, an 18x18 mm coverslip was placed gently over the solution. The solution was allowed to
404 polymerise for 1 h at room temperature. HEPES was added over the coverslips to detach the top glass.
405 The polymerised gel was then activated under UV for 5 min using Sulpho-SANPAH and washed with
406 HEPES twice. The hydrogels were then coated with 100 μ g/ml of rat tail collagen I overnight at 4°C. The
407 excess collagen was washed once with PBS and approximately 5 x 10⁴ cells/ml were plated on hydrogels.

408 **Micropatterning**

409 Coverslips were plasma-cleaned for 45 s and incubated with 0.1 mg/ml poly-L-lysine/polyethylene glycol
410 (PLL-PEG) diluted in 10 mM HEPES for 30 min at room temperature. Excess of PLL-PEG was allowed to
411 slide down by gravity, and the coverslips were dried and stored at 4°C overnight before printing.
412 Micropatterns were printed on previously prepared PLL-PEG coverslips for 3 min with specifically
413 designed chrome masks and coated with 50:50 v/v fibronectin/fibrinogen mixture (20 µg/ml each)
414 diluted in fresh NaHCO₃, pH 8.3, 100 mM, for 30 min at room temperature. Micropatterned coverslips
415 were washed thrice in NaHCO₃ and used immediately for transfer on hydrogels. Plated cells
416 (approximately 6 x 10⁴ cells/ml) were allowed to adhere for 16 h before imaging/fixation. Crossbow
417 patterns have an area of ~2500 µm².

418 **Traction force experiments on micropatterned substrates**

419 After printing and coating micropatterned coverslips, gel mixtures were prepared as follows: 40.4 kPa –
420 100 µl 40% acrylamide, 120 µl 2% bisacrylamide, 280 µl water (final concentration of 8% acrylamide and
421 0.48% bisacrylamide in water); 2.61 kPa – 50 µl 40% acrylamide, 25 µl 2% bisacrylamide, 425 µl water
422 (final concentration of 8% acrylamide and 0.048% bisacrylamide in water). 5 µl of fluorescent
423 microbeads (FluoSpheres; Molecular Probes) were incubated with 0.1 mg/ml PLL-PEG on a rotator for 1
424 h at 4°C prior to mixing with the gel mixture. The PLL-PEG coated beads were washed and centrifuged
425 thrice at 1000 rpm for 10 min with 10 mM HEPES. These beads were then mixed with 165 µl of the gel
426 mixture. After adding 1 µl APS and 1 µl TEMED, the solution was added as a 25 µl drop on a silanised
427 20x20 mm coverslip. The protein-coated micropatterned coverslip was then gently placed on the gel
428 mixture. The solution was allowed to polymerize for 25 min at room temperature. After detaching the
429 micropatterned glass from the polymerized gel, 6 x 10⁴ cells/ml were plated on the gels and allowed to
430 adhere and spread for 16 h before TFM experiments. Stacks of single micropatterned cells were
431 acquired before and after trypsin treatment. Acquisitions were performed with a Nikon Eclipse Ti-E
432 epifluorescence inverted microscope, with a 40× 1.49 NA air objective equipped with a pco.edge sCMOS

433 camera and Metamorph software. Cells were maintained at 5% CO₂ and 37°C in normal astrocyte
434 medium during acquisition.

435 **Migration assays**

436 In vitro wound healing assays: cells were plated on appropriate supports (dishes, plates, coverslips or
437 glass-bottom MatTek) and grown to confluence. The cell monolayer was scratched with a p200 pipette
438 tip to induce migration and imaged during 8h.

439 Migration on soft substrates: cells were plated on hydrogels of different rigidities in 6-well glass bottom
440 plates (MatTek) and allowed to grow for 72 h after transfection before creating a chemical wound. 0.05
441 M NaOH was gently dropped on the cells using a microinjector. NaOH is used to instantly kill and detach
442 cells localized in a region of a few hundred microns. The NaOH is added with a microinjector which
443 delivers a fraction of a nanoliter of NaOH (0.05M) on a cell monolayer, which immediately mixes with 2
444 ml of culture medium that is present in the culture dish. The dead cells and debris were gently washed
445 away using PBS. Fresh medium was added to the cells. Cells were placed on the microscope for live
446 imaging. In these conditions, the hydrogel is only very locally and briefly exposed to the chemical. As
447 cells migrate into this space (wounded area of the monolayer), the fibronectin coating and the surface
448 of the hydrogel remains intact with negligible and unnoticeable changes. NaOH treatment did not affect
449 the weight of the gel -indicative of gel swelling-; it did not induce any detectable bead movements –
450 indicative of gel contraction or expansion of the gel-, nor did it affect the traction forces of cells,
451 indicating that cells did not sense any difference in substrate rigidity. Acquisition was started 30 min
452 after wounding. Supplementary Videos were acquired with a Zeiss Axiovert 200M or a Nikon Eclipse Ti-E
453 epifluorescence inverted microscope with cells maintained at 5% CO₂ and 37°C. All images were
454 acquired with dry objective 10X 0.45 NA and an EMCCD camera/pco.edge sCMOS camera and
455 Metamorph software. Images were acquired every 15 min for 24 h. Nuclei of leader cells were manually
456 tracked with Fiji software (Manual Tracking plugin).

457 **Immunostaining**

458 Cells were fixed with cold methanol for 3 min at -20°C, or 4% warm PFA, or 4% PFA + 0.2%
459 glutaraldehyde + 0.25% Triton X-100 for 10 min at 37°C. After fixation with PFA, glutaraldehyde and
460 triton, free aldehyde groups were quenched with a solution of 1 mg/ml sodium borohydride (Sigma-
461 Aldrich) freshly added to cytoskeletal buffer (10 mM MES or MOPS, 150 mM NaCl, 5 mM EGTA, 5 mM
462 MgCl₂, 5 mM glucose, pH 6.1) for 10 min on ice. Cells were permeabilised for 5 min with 0.1% Triton in
463 case of PFA fixation. Coverslips were blocked for 1 h with 5% BSA in PBS. The same solution was used for
464 primary and secondary antibody incubation for 1 h. Nuclei were stained for DAPI and coverslips were
465 mounted with Prolong Gold.

466 Antibody anti-Acetylated Tubulin (1:10000; clone 6-11B-1, mouse monoclonal, T-6793 Sigma Aldrich or
467 1:200; clone 6-11B-1, ab24610, Abcam), anti-Poly-Glu Tubulin (1:1000; AbC0101, ValBiotech), anti- α -
468 Tubulin (1:1000; MCA77G, rat, Biorad), anti-Paxillin (1:500; 610051, mouse monoclonal, lot 5246880;
469 BD; and ab32084, rabbit monoclonal, clone Y133 and lot GR215998-1; Abcam), anti-Talin (1:1000;
470 T3287, mouse monoclonal, clone 8D4, lot 035M4805V; Sigma-Aldrich), anti-GEF-H1 (1:100; ab155785,
471 Abcam), Alexa fluor 647 Phalloidin (1:2000; 176759, lot GR278180-3; Abcam or 1:300; Thermo Fisher
472 Scientific), anti-Vimentin (1:200; V6630, mouse monoclonal lot 10M4831; Sigma-Aldrich), anti-pMLC
473 (1:1000; 3675S, S19, mouse, Cell signalling), anti-Myosin IIA (1:1000; non-muscle, M8064, rabbit
474 polyclonal, Sigma-Aldrich), anti-Vinculin (1:400; V9131, Sigma-Aldrich). Secondary antibodies were Alexa
475 Fluor 488 donkey anti-rabbit (711-545-152), Rhodamine (TRITC) donkey anti-rabbit (711-025-152),
476 Alexa Fluor 647 donkey anti-rabbit (711-695-152), Alexa Fluor 488 donkey anti-mouse (715-545-151),
477 Rhodamine (TRITC) donkey anti-mouse (715-025-151), Alexa Fluor 647 donkey anti-rat (711-605-152),
478 and Alexa Fluor 488 donkey anti-rat (712-545-153); all from Jackson ImmunoResearch. Images were
479 acquired with a Leica DM6000 microscope equipped with 40X 1.25 NA or 63X 1.4 NA objectives and

480 recorded on a CCD camera with a Leica software, or imaged at the basal plane using Yokogawa CSU-W1,
481 Nikon TiE Spinning disk confocal microscope, at 100x magnification.

482 **TIRF microscopy**

483 Acquisitions were performed with a Nikon Eclipse Ti-E epifluorescence inverted microscope, with a 60x
484 1.49 NA oil objective equipped with a pco.edge sCMOS camera with Metamorph software. Cells were
485 maintained at 5% CO₂ and 37°C in normal astrocyte medium. After acquiring a 15 min movie of GFP-
486 αTAT1 and mCherry-vinculin, Nocodazole or Y27 were added and images were acquired every 2 min for
487 1 h.

488 **Transmission electron microscopy**

489 Adherent migrating astrocytes plated on glass coverslips were disrupted by sonication as described
490 previously⁴⁸. Coverslips were unroofed by scanning the coverslip with rapid sonicator pulses in KHMgE
491 buffer (70 mM KCl, 30 mM HEPES, 5 mM MgCl₂, 3 mM EGTA, pH 7.2). Paraformaldehyde
492 2%/glutaraldehyde 2%-fixed cells were further sequentially treated with 0.5% OsO₄, 1% tannic acid and
493 1% uranyl acetate prior to graded ethanol dehydration and Hexamethyldisilazane substitution (HMDS,
494 Sigma-Aldrich). Dried samples were then rotary-shadowed with 2 nm of platinum and 5-8 nm of carbon
495 using an ACE600 high vacuum metal coater (Leica Microsystems). Platinum replicas were floated off the
496 glass by 5% hydrofluoric acid, washed several times by floatation on distilled water, and picked up on
497 200 mesh formvar/carbon-coated EM grids. The grids were mounted in a eucentric side-entry
498 goniometer stage of a transmission electron microscope operated at 80 kV (Philips, model CM120) and
499 images were recorded with a Morada digital camera (Olympus). Images were processed in Adobe
500 Photoshop to adjust brightness and contrast and presented in inverted contrast.

501 **Image analysis**

502 Normalised mean intensity levels of acetylated and detyrosinated tubulin for immunofluorescence
503 images were calculated as shown in equation 1:

504

1

$$\text{Ratio of acetyl or deetyr tubulin level} = \frac{\text{Acetyl/deetyr tubulin intensity}}{\text{Total tubulin intensity}}$$

505 YAP nuclear/cytoplasmic ratio was calculated as shown in equation 2:

506

2

$$\text{YAP nuclear to cytoplasmic ratio} = \frac{\text{Intensity of YAP in the nucleus}}{\text{Intensity of YAP in the cytoplasm}}$$

507 FA density was calculated as shown in equation 3:

508

3

$$\text{FA density} = \frac{\text{Number of FAs in the region}}{\text{Area of the region}}$$

509 Different regions of cells (entire cell, cell periphery-8 μm , 8 μm -16 μm , 16 μm -cell center) were analysed

510 as depicted in Extended Data Fig. 2d.

511 For migration assays, nuclei of cells were manually tracked to determine the speed directionality and

512 persistence of migration using the following formulae:

513 Mean velocity (v), persistence (p) and directionality (d) of cell migration are calculated shown in514 equations 4-6: for a given (x, y) coordinate of leading cell nucleus,

515

4

$$v = \frac{\sum \vartheta}{n}$$

516

5

$$p = \frac{\sqrt{[(x_{24} - x_0)^2 + (y_{24} - y_0)^2]}}{\sum \sqrt{[(x_n - x_{n-1})^2 + (y_n - y_{n-1})^2]}}$$

517

6

$$d = \frac{|(x_0 - x_{24})|}{\sum \sqrt{[(x_n - x_{n-1})^2 + (y_n - y_{n-1})^2]}}$$

518 where n is the number of time points acquired and v is cell velocity.

519 Analysis of TFM on micropatterns was performed with a custom-designed macro in Fiji based on work by
520 ⁴⁹. The topmost planes of beads before and after trypsinisation were selected and aligned using a
521 normalized cross-correlation algorithm (Align Slices in Stack plugin). The displacement field was
522 computed from bead movements using particle image velocimetry (PIV). Parameters for the PIV were
523 three interrogation windows of 128, 64, and 32 pixels with a correlation of 0.60. Traction forces were
524 calculated from the displacement field using Fourier transform traction cytometry and a Young's
525 modulus of 40 kPa or 2 kPa, a regularization factor of 10^{-9} , and a Poisson ratio of 0.5.

526 For the localisation of GEF-H1 on microtubules, the percentage GEF-H1 on microtubules was calculated
527 as shown in equation 7:

528 7

$$\text{Percentage intensity of GEFH1 localisation on MT} = \frac{\text{Intensity of GEFH1 on microtubules}}{\text{Intensity of GEFH1 outside microtubules}} \times 100$$

529 **Immunoprecipitations and pulldown assays**

530 Cell lysates were prepared by scraping cells in 1X lysis buffer (10X buffer recipe: 50 mM Tris pH 7.5,
531 triton 20%, NP40 10%, 2 M NaCl with Complete protease inhibitor tablet (Roche, Indianapolis, IN)) and
532 centrifuged for 30 min at 13,000 rpm 4°C to pellet cell debris. Soluble detergent extracts were incubated
533 with GFP-Trap agarose beads (Chromotek, GTP-20) for immunoprecipitation and with GST or GST-
534 α TAT1 beads for 2 hr at 4°C prior to washing three times with wash buffer (50 mM Tris pH 7.5, 150 mM
535 NaCl, 0.1% triton, 1 mM EDTA, 2.5 mM MgCl₂). The resin was then mixed with Laemmli buffer and used
536 for western blot and mass spectrometry analysis. HEK293 cells were used for mass spectrometry
537 analysis in order to have enough amount of protein from cells expressing α TAT1.

538 **Mass spectrometry**

539 Proteins on beads were washed twice with 100 μ L of 25 mM NH_4HCO_3 and we performed on-beads
540 digestion with 0.2 μ g of trypsin/LysC (Promega) for 1 h in 100 μ L of 25 mM NH_4HCO_3 . Sample were
541 then loaded onto a homemade C18 StageTips for desalting. Peptides were eluted using 40/60
542 MeCN/ H_2O + 0.1% formic acid and vacuum concentrated to dryness. Online chromatography was
543 performed with an RSLCnano system (Ultimate 3000, Thermo Scientific) coupled to an Orbitrap Fusion
544 Tribrid mass spectrometer (Thermo Scientific). Peptides were trapped on a C18 column (75 μ m inner
545 diameter \times 2 cm; nanoViper Acclaim PepMapTM 100, Thermo Scientific) with buffer A (2/98 MeCN/ H_2O
546 in 0.1% formic acid) at a flow rate of 4.0 μ L/min over 4 min. Separation was performed on a 50 cm \times 75
547 μ m C18 column (nanoViper Acclaim PepMapTM RSLC, 2 μ m, 100 \AA , Thermo Scientific) regulated to a
548 temperature of 55 $^\circ$ C with a linear gradient of 5% to 25% buffer B (100% MeCN in 0.1% formic acid) at a
549 flow rate of 300 nL/min over 100 min. Full-scan MS was acquired in the Orbitrap analyzer with a
550 resolution set to 120,000 and ions from each full scan were HCD fragmented and analyzed in the linear
551 ion trap.

552 For identification, the data was searched against the Homo sapiens (UP000005640) SwissProt database
553 using Sequest^{HF} through proteome discoverer (version 2.2). Enzyme specificity was set to trypsin and a
554 maximum of two missed cleavage site were allowed. Oxidized methionine, N-terminal acetylation, and
555 carbamidomethyl cysteine were set as variable modifications. Maximum allowed mass deviation was set
556 to 10 ppm for monoisotopic precursor ions and 0.6 Da for MS/MS peaks. The resulting files were further
557 processed using myProMS⁵⁰ v3.6 (work in progress). FDR calculation used Percolator and was set to 1%
558 at the peptide level for the whole study. The label free quantification was performed by peptide
559 Extracted Ion Chromatograms (XICs) computed with MassChroQ version 2.2⁵¹. For protein
560 quantification, XICs from proteotypic peptides shared between compared conditions (TopN matching)
561 with no missed cleavages were used. Median and scale normalization was applied on the total signal to
562 correct the XICs for each biological replicate. To estimate the significance of the change in protein

563 abundance, a linear model (adjusted on peptides and biological replicates) was performed and p-values
564 were adjusted with a Benjamini–Hochberg FDR procedure with a control threshold set to 0.05. Up-
565 regulated proteins with at least 3 proteotypic peptides (fold change > 1.5 and p-value < 0.05) and the
566 unique proteins identified only in the GFP- α TAT1 were used for gene ontology (GO) enrichment analysis
567 by using GO::TermFinder tools (10.1093/bioinformatics/bth456) through myProMS.

568 The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via
569 the PRIDE^{52,53} partner repository with the dataset identifier PXD015871 (username:
570 reviewer89842@ebi.ac.uk; password: EC4DSdRf; project name: Potential interactors of alpha-tubulin
571 acetyltransferase 1 (α TAT1/MEC17).

572 **Rho activation assay**

573 Rho activation assay was performed using a RhoA Pull-down Activation Assay Biochem Kit from
574 Cytoskeleton Inc (BK036-S). In short, cells were lysed in ice-cold Cell Lysis Buffer plus 1x protease
575 inhibitor cocktail. Cells were centrifuged at 10,000 g at 4°C for 10 min to pellet cell membranes and
576 insoluble material. Part of the supernatant was stored as input for western blot. The remaining
577 supernatant was divided equally into two parts (300-800 μ g protein/tube). 1/15th the volume of Loading
578 Buffer was added to each tube (final conc. 15 mM). Then, 1/100th the volume of GTP γ S was added to
579 one of the tubes and used as positive control (final conc. 0.2 mM). All tubes were incubated at room
580 temperature for 15 min. The reaction was stopped by adding 1/10th the volume of STOP Buffer to all
581 tubes (final conc. 60 mM).

582 Rhotekin-RBD (50 μ g) beads were resuspended and added to the tubes. Tubes were rotated at 4°C for 1
583 h and centrifuged at 5000 g at 4°C for 3 min. Beads were washed with 500 μ l each of Wash Buffer. 10-20
584 μ l of Laemmli sample buffer was added to each tube.

585 **Western blot**

586 Cells lysates were obtained with Laemmli buffer composed of 60 mM Tris-HCl pH6.8, 10% glycerol, 2%
587 SDS and 50 mM DTT with the addition of anti-protease (cOmplete cocktail, Roche 11 873 588 001).
588 Samples were boiled 5 min at 95°C before loading on polyacrylamide gels. Transfer occurred at 100V for
589 1 h on nitrocellulose membranes. Membranes were blotted with TBST (0.2% Tween) and 5% milk and
590 incubated 1 h with the primary antibody and 1 h with HRP-conjugated secondary antibody. Bands were
591 revealed with ECL chemoluminescent substrate (Biorad).

592 Primary antibodies used: Antibody anti-Acetylated Tubulin (1:10000; clone 6-11B-1, mouse monoclonal,
593 T-6793 Sigma Aldrich), anti- α TAT1 (1:400; HPA046816, rabbit, Atlas Antibodies), anti-Poly-Glu Tubulin
594 (1:1000; AbC0101, ValBiotech), anti- α -Tubulin (1:1000; MCA77G, rat, Biorad), anti- β_1 integrin (ab52971,
595 Abcam), anti-Talin (1:1000; T3287, mouse monoclonal, clone 8D4, lot 035M4805V; Sigma-Aldrich), anti-
596 GEF-H1 (1:100; ab155785, Abcam), anti-GAPDH (MAB374, lot 2689153 Millipore). Secondary HRP
597 antibodies were all purchased from Jackson ImmunoResearch. Unmodified western blots of Figures 1, 4
598 and 5, as well as Extended Data Figures 1, 2 and 3 can be found in the corresponding source data files.

599 **Graphs and statistics**

600 All graphs and statistics were done using Graphpad Prism.

601

602 **Data availability**

603

604 The data supporting the findings of this study are available within the manuscript. The mass
605 spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the
606 PRIDE^{52,53} partner repository with the dataset identifier PXD015871 (username:
607 reviewer89842@ebi.ac.uk; password: EC4DSdRf; project name: Potential interactors of alpha-tubulin
608 acetyltransferase 1 (α TAT1/MEC17). Unmodified western blots of Figures 1, 4 and 5, as well as Extended

609 Data Figures 1, 2 and 3 can be found in the corresponding source data files. Other raw data generated
610 during this study are available on reasonable request.

611

612 **Code availability**

613

614 Codes for focal adhesion distribution and traction forces are available from the authors upon request.

615

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763 **Author contributions**

764

765 S.S. designed, performed experiments, analysed and interpreted the results, and wrote the paper; B.V.
766 assisted in the setup of TFM experiments and analysis, helped with data interpretation and discussions;
767 V.R. performed immunoprecipitation and pull down experiments; A. F. carried out a part of the
768 experiments using HUVECs; C. D-P helped with experimental techniques and discussions; B. B optimised
769 the GFP-nanobody and IP conditions used for mass spectrometry sample preparation and set up the
770 chemical wound assay; F. D. carried out the MS experimental work; D. L. supervised the MS experiments
771 and data analysis; S.V. performed EM experiments; A. B. helped with data interpretation and
772 discussions; M.T. provided ideas, helped with data interpretation and discussions; S. E-M. supervised the
773 project, interpreted the results, and wrote the paper.

774 **Competing interests**

775

776 Authors declare no competing interests.

777

778 Main figure legends

779

780 **Figure 1: Integrin-mediated signalling and substrate rigidity regulate microtubule acetylation. a, b, c.**
781 Astrocytes (WT) or treated with **c (i, ii)**. Ctl, Src kin 1 or PF-562271, plated on PAA gels; **(a. i, c. i)** images
782 showing Acetylated tubulin and α -tubulin; **(b. i, b. ii)** western blots showing normalised ratio of
783 acetylated tubulin over GAPDH. **(a)** n = 77 for 1.26 kPa, 88 for 2 kPa, 67 for 9 kPa and 81 for 48 kPa; **(c)** n
784 = 30 for Ctl, 43 for Src kin1, 43 for PF-562271. **d, e.** Astrocyte lysate pulldown (PD; WT cells) and
785 immunoprecipitations (cells transfected with GFP-Ctl or GFP- α TAT1), using (d) GST- α TAT1 and (e) GFP-
786 α TAT1 resin respectively. Westerns showing Talin and Acetylated tubulin. **f.** Images of GFP- α TAT1 and
787 mCherry-vinculin astrocytes transfected along with siCtl or siTalin1. **g.** Astrocytes on 48 kPa PAA gels
788 showing **(g. i)** Acetylated tubulin and α -tubulin; **(g. ii)** ratio of the intensities of Acetylated tubulin over
789 total Tubulin intensity; n = 51 for siCtl and 56 for siTalin1. **h.** Pulldown (PD; Ctl or Y-27632 treated cells)
790 using GST- α TAT1 resin; **(h. i)** Westerns showing Talin and Ponceau staining; **(h. ii)** normalised Talin in
791 DMSO (Ctl) and Y-27632 treated cells. **i.** Images showing GFP- α TAT1 and mCherry-vinculin expressing
792 astrocytes treated with DMSO (Ctl) or Y-27632. **j, k.** Migrating astrocytes, treated with DMSO (Ctl) or
793 ROCK inhibitor Y-27632 for 2 h; **(j)** Western blot showing the levels of Acetylated tubulin and GAPDH in
794 Ctl or Y-27632 treated cells; **(k. i, ii)** ratio of the intensities of Acetylated tubulin over total Tubulin
795 intensity; n = 98 for Ctl, 79 for Y-27632 treated cells respectively. **Scale bar** (a, c, g): 10 μ m, (f, k): 20 μ m.
796 **Number of independent experiments** = 4 (for a, h), 5 (for b, i), 2 (for c), 3 (for d-g, j, k). In box-and-
797 whisker plots, box extends from the 25th to 75th percentile, whiskers show minimum and maximum
798 values, and the line within the box represents the median. **Statistical tests:** One-way ANOVA followed by
799 Tukey's multiple comparison's test (for a, b, g), Paired t-test (for h), Unpaired Student's t-test (two-
800 tailed; for k).

801

802 **Figure 2: Microtubule acetylation tunes cell mechanosensitivity. a, b.** Astrocytes transfected with siCtl
803 and si α TAT1-2, or treated with Niltubacin and Tubacin, or **c.** HUVECs treated with Niltubacin and
804 Tubacin plated on PAA gels of different rigidities; **(a-c. i)** stained for YAP and α -tubulin (not shown) to
805 mark the cell boundary (marked by dotted lines); **(a-c. ii)** ratio of the YAP intensity in the nucleus over
806 cytoplasm; (a) n = 49 for 2 kPa siCtl, 44 for 2 kPa si α TAT1-2, 68 for 48 kPa siCtl, 74 for 48 kPa si α TAT1-2;
807 (b) n = 49 for 2 kPa Niltubacin, 53 for 2 kPa Tubacin, 51 for 48 kPa Niltubacin and 51 for 48 kPa Tubacin;
808 (c) n = 26 for 2 kPa Niltubacin, 25 for 2 kPa Tubacin, 32 for 48 kPa Niltubacin and 26 for 48 kPa Tubacin.
809 **d.** Astrocytes (WT) plated on PAA gels of different rigidities, **(d. i)** stained for α -tubulin and Talin. Images
810 shown correspond to the same cells depicted in Fig. 1A; **(d. ii)** FA density in different regions of the cells;
811 n = 40 for 1.26 kPa, 51 for 2 kPa, 94 for 9 kPa, 77 for 48 kPa. **e, f.** Astrocytes transfected with siCtl and
812 si α TAT1-2, or treated with Niltubacin and Tubacin, plated on PAA gels of different substrate rigidities,
813 and **(e. i, f. i)** stained for Paxillin; **(e. ii, f. ii)** FA density (number of FAs/ μm^2) in the central region (16 μm -
814 cell center) of cells depicted in panels e and f; n = 60 for 1.26 kPa siCtl, 36 for 1.26 kPa si α TAT1-2, 54 for
815 48 kPa siCtl and 47 for 48 kPa si α TAT1-2; n = 59 for 1.26 kPa Niltubacin, 55 for 1.26 kPa Tubacin, 46 for
816 48 kPa Niltubacin and 37 for 48 kPa Tubacin. **g.** Schematic (summarising a-f) showing focal adhesion
817 distribution upon manipulation of microtubule acetylation. **Scale bar** (a-f): 10 μm ; **Number of**
818 **independent experiments** = 3 (for a, b, d-f), 2 (for c); In box-and-whisker plots, box extends from the
819 25th to 75th percentile, whiskers show minimum and maximum values, and the line within the box
820 represents the median; **Statistical tests:** one-way ANOVA followed by Tukey's multiple comparison's
821 test (for a, b, e, f), two-way ANOVA followed by Tukey's multiple comparison's test (for d).

822

823 **Figure 3: Microtubule acetylation reorganises the actomyosin and intermediate filament networks. a,**
824 **b, c.** Astrocytes transfected with siCtl or si α TAT1, and stained for **(a. i)** Actin, pMLC and Paxillin; **(b)**
825 Myosin IIa, Acetylated tubulin and α -tubulin; **(c)** Vimentin and α -tubulin; **(a. ii)** percentage of cells with

826 inter-junctional transverse arcs; n = 199 for siCtl and 159 si α TAT1-2. **d.** Ultrastructural organization of
827 focal adhesion-associated cytoskeleton in siCtl or si α TAT1-2 depleted cells; **d (i)** Platinum replica
828 electron microscopy (PREM) survey view of the cytoplasmic surface of the leading edge in siCtl unroofed
829 cells. Boxed regions correspond to focal adhesions. Extracellular space is pseudo-coloured in purple. (1,
830 2) High magnification views corresponding to the boxed regions in panel (i). White arrows indicate
831 microtubules and yellow arrowheads denote intermediate filaments. (3) Zoom-in region corresponding
832 to the boxed region in marked in region (2). Scale bar: 1 μ m. White arrows indicate microtubules and
833 yellow arrowheads denote intermediate filaments. Scale bar: 2 μ m and 1 μ m. **d (ii, iii)** PREM survey view
834 of the cytoplasmic surface of the leading edge in α TAT1-depleted cells. Extracellular space is pseudo-
835 coloured in purple. Scale bars: 10 μ m, 1 μ m (inset). (4, 5) High magnification views corresponding to the
836 boxed regions in panel ii and iii respectively. Scale bar: 1 μ m. **e.** HUVECs treated with DMSO or Tubacin,
837 stained for Actin, Vinculin and Myosin IIA. **f.** Astrocytes treated with Niltubacin or Tubacin, stained for
838 Actin and Paxillin. **Scale bar** (a-c): 20 μ m, (e, f): 10 μ m. In box-and-whisker plots, box extends from the
839 25th to 75th percentile, whiskers show minimum and maximum values, and the line within the box
840 represents the median. **Number of independent experiments** = 3 (for a-c); **Statistical tests:** Paired
841 Student's t-test (two-tailed; for a).

842

843 **Figure 4: Microtubule acetylation promotes traction force generation and Rho activation. a, b, c, d. (a-**
844 **d. i)** Stress-field maps of astrocytes on crossbow-shaped micropatterned PAA gels of different rigidities,
845 transfected with (a) siCtl and si α TAT1-2, (b) siCtl + GFP- α TAT1 and si α TAT1-2 + GFP- α TAT1, or treated
846 with (c) Niltubacin and Tubacin; or (d) HUVECs treated with Niltubacin and Tubacin; **(a, b, c. ii, d. ii)**
847 corresponding stored energies (in Joules, J; within the range of 0 and 5×10^{-13} J) of cells in the above
848 mentioned conditions; (a) n = 163 for siCtl and 141 for si α TAT1-2, (b) n = 74 for siCtl + GFP- α TAT1, and
849 70 for si α TAT1-2 + GFP- α TAT1, (c) n = 136 for Niltubacin and 141 for Tubacin, (d) n = 83 for Niltubacin

850 and 82 for Tubacin. **e.** GST-Rhotekin pulldowns were performed using siCtl or si α TAT1-transfected
851 astrocytes. Western blots showing Red Ponceau and immunoblotted for RhoA. **Scale bar** (a-d): 10 μ m;
852 **Number of independent experiments** = 3 (for a-e). In box-and-whisker plots, the box extends from the
853 25th to the 75th percentile, the whiskers show the minimum and maximum values, and the line within
854 the box represents the median. **Statistical tests:** one-way ANOVA followed by Tukey's multiple
855 comparison's test (for a, b), unpaired Student's t-test (two-tailed; for c, d).

856

857 **Figure 5: Microtubule acetylation promotes the release of GEF-H1 into the cytosol.** **a.** Migrating
858 astrocytes transfected with siCtl, si α TAT1-2, and si α TAT1-2 followed by treatment with Tubacin, **(a. i)**
859 stained for Acetylated tubulin, α -tubulin and GEF-H1; **(a. ii)** percentage of GEF-H1 colocalised with
860 microtubules; n = 99 for siCtl, 89 for si α TAT1-2, 72 for si α TAT1-2 + Tubacin; **b.** HUVECs treated with
861 DMSO Ctl or Tubacin, **(b. i)** stained for α -tubulin and GEF-H1; **(b. ii)** percentage of GEF-H1 colocalised
862 with microtubules; n = 78 for DMSO Ctl and 84 for Tubacin. **c.** Immunoprecipitations using anti-GFP
863 nanobodies were performed with lysates from HEK293 cells transfected with GFP-Ctl or GFP-GEF-H1,
864 and treated with Nocodazole or Tubacin. Samples were analysed by immunoblotting using α -tubulin,
865 Acetylated tubulin, GEF-H1, GAPDH and GFP antibodies. **d.** Astrocytes transfected with siCtl and
866 si α TAT1-2, and plated on 48 kPa PAA gels, **(d. i)** stained for GEF-H1 and α -tubulin; **(d. ii)** percentage of
867 GEF-H1 colocalised with microtubules; n = 100 for 48 kPa siCtl, 112 for 48 kPa si α TAT1-2, 97 for 2 kPa
868 siCtl, 86 for 2 kPa α TAT1-2 (1-2 regions per cell). **Scale bar** (a): 20 μ m, (b, d): 10 μ m. In box-and-whisker
869 plots, the box extends from the 25th to the 75th percentile, the whiskers show the minimum and
870 maximum values, and the line within the box represents the median. **Number of independent**
871 **experiments** = 3 (for a-d); **Statistical tests:** Ordinary one-way ANOVA followed by Tukey's multiple
872 comparison's test (for a, d); Unpaired Student's t-test followed by Mann-Whitney test (for b).

873 **Figure 6: Microtubule acetylation is required for mechanosensitive migration.** **a, b, c.** Phase contrast
874 images of **(a, b. i)** Astrocytes (WT) or **(c. i)** transfected with siCtl or si α TAT1 migrating in a chemical
875 wound assay on PAA gels of different rigidities for 24 h (pink and orange dotted lines show initial and
876 final wound edge); **(b. ii, c. ii)** migration speed (in $\mu\text{m}/\text{min}$) of cells at the wound edge; $n = 123$ for 2 kPa
877 WT, 117 for 9 kPa WT, 115 for 48 kPa WT, 91 for 2 kPa siCtl, 95 for 2 kPa si α TAT1-2, 93 for 48 kPa siCtl,
878 93 for 48 kPa si α TAT1-2. **Scale bar** (a): 200 μm , (b, c): 100 μm . **Number of independent experiments = 3**
879 (for b, c); **Statistical tests:** one-way ANOVA followed by Tukey's multiple comparison's test. In box-and-
880 whisker plots, the box extends from the 25th to the 75th percentile, the whiskers show the minimum and
881 maximum values, and the line within the box represents the median. **d. Proposed working model:** Cells
882 sensing a soft substrate (d. i), with altered integrin-signalling (d. ii) exhibit less MT acetylation (d. iii). This
883 results in GEF-H1 being bound to MTs (d. iv) and is therefore, unable to activate RhoA (d. v), which
884 renders the cell less contractile (d. vi). In such conditions, cells generate a low amount of traction forces
885 (d. vii) and migrate slower (d. viii). On the other hand, on stiff substrates, integrin- and talin-mediated
886 rigidity sensing (d. i) promotes the tension-dependent recruitment of α TAT1 to FAs (d. ii) and increased
887 microtubule acetylation (d. iii). How α TAT1 enters the lumen of microtubules still remains unclear
888 although one can speculate that the recruitment of α TAT1 at FAs increases the local cytosolic pool of the
889 protein which can then enter microtubules through microtubule lattice defects or through the open
890 ends of microtubules in close proximity to FAs ([Extended Data Fig. 5C](#)). Microtubule acetylation triggers
891 the release of GEF-H1 from MTs (d. iv), which activates the RhoA-ROCK-myosin II pathway (d. v) to
892 promote actomyosin contractility (d. vi), traction forces (d. vii) and cell migration (d. viii). Active GEF-H1,
893 RhoA and ROCK are depicted in bold.

894

895

896 **Extended data figure legends**

897 **Extended Data Figure 1: a, b. (a. i)** Schematic and **(a. ii, b. i)** western blots showing **(a)** acetylation and
898 **(b)** α TAT1 levels using two distinct sets of siRNAs targeting α TAT1 (si α TAT1-1 and si α TAT1-2), and
899 Tubacin (an inhibitor of HDAC6 (deacetylase)); **(a. iii, a. iv)** ratio of the acetylated tubulin or **(b. ii)** α TAT1
900 intensity over GAPDH intensity, normalised to the values observed for the respective controls. **c.**
901 Migrating astrocytes transfected with siCtl, **(c. i)** si α TAT1-1 and **(c. ii)** si α TAT1-2, or treated with Tubacin
902 prior to wounding, showing acetylated tubulin and α -tubulin. **d.** Astrocytes plated on PAA gels of
903 different rigidities and treated with Tubacin; ratio of the intensities of Acetylated tubulin over total
904 Tubulin of each cell; n = 49 for 1.26 kPa WT, 51 for 1.26 kPa Tubacin, 72 for 48 kPa WT, 66 for 48 kPa
905 Tubacin. **e.** siCtl or WT astrocytes plated on crossbow-shaped micropatterned hydrogels of different
906 rigidities, **(e. i)** showing Acetylated tubulin and α -tubulin; **(e. ii)** ratio of the intensities of Acetylated
907 tubulin over total Tubulin of each cell; n = 86 for 2 kPa and 103 for 48 kPa. **f.** Astrocytes plated on PAA
908 gels of different rigidities, **(f. i)** stained with Detyrosinated tubulin and α -tubulin; **(f. ii)** ratio of
909 Detyrosinated tubulin over total tubulin intensities in each cell; n = 39 for 2 kPa, 31 for 9 kPa and 40 for
910 48 kPa. **g. (g. i)** Western blots showing the levels of Detyrosinated tubulin and GAPDH in astrocytes
911 plated on PAA gels of different substrate rigidities; **(g. ii)** ratio of the intensities of Detyrosinated tubulin
912 over GAPDH normalised to the values observed for 2 kPa. **Scale bar** (c, e, f): 10 μ m; **Number of**
913 **independent experiments** = 4 (for a - si α TAT1), 6 (for a - Tubacin, c, g), 3 (for b), 5 (for e), 2 (for f). In
914 box-and-whisker plots, box extends from the 25th to 75th percentile, whiskers show minimum and
915 maximum values, and the line within the box represents the median; **Statistical tests:** one-way ANOVA
916 followed by Tukey's multiple comparison's test or Holm-Šidák's multiple comparisons test (a - si α TAT1,
917 b, d, e, g) or Paired Student's t-test (for a - Tubacin).

918 **Extended Data Figure 2: a, b.** Astrocytes were treated with (a) RGD control or RGD peptides, (b) solvent
919 (Ctl) or MnCl_2 , and **(a. i, b. i)** stained for acetylated tubulin, α -tubulin, paxillin or DAPI; **(a. ii, b. ii)** ratio of
920 the intensities of Acetylated tubulin over total Tubulin; n = 146 for RGD control, 99 for RGD peptide, 116
921 for Ctl and 105 for MnCl_2 . **c, d.** Astrocytes were transfected with siCtl or si β_1 integrin; **(c. i)** Western blots
922 showing levels of β_1 integrin, Acetylated tubulin and GAPDH; **(c. ii, iii)** ratio of intensities of β_1 integrin or
923 Acetylated tubulin over GAPDH, normalised to the levels in siCtl respectively; **(d. i)** stained for
924 Acetylated tubulin, α -tubulin and DAPI; **(d. ii)** ratio of the intensities of Acetylated tubulin over total
925 Tubulin; n = 122 for siCtl and 117 for si β_1 integrin. **e.** Astrocytes were treated with Ctl, Src kin 1 or PF-
926 562271; **(e. i)** stained for Acetylated tubulin, α -tubulin and Paxillin; **(e. ii)** ratio of the intensities of
927 Acetylated tubulin over total Tubulin intensity; n = 78 for Ctl, 70 for Src kin 1, 79 for PF-562271. **f.**
928 Astrocytes were plated on 48 kPa gels and treated with Ctl or Src kin 1. **(f. i)** Western blots showing the
929 levels of Acetylated tubulin and GAPDH; **(f. ii)** ratio of the intensities of Acetylated tubulin over GAPDH
930 normalised to the Ctl. **g.** Volcano plot analysis showing fold changes (GFP- α TAT1/GFP-Ctl) of the
931 quantified proteins with threshold of >3 peptides, minimum absolute fold change of 1.5 (green lines)
932 and maximum adjusted p-value of 0.05 (red line). Enriched protein interactors related to GO:0005925
933 focal adhesion (red boxes; ratio = 1.98 and p = 7.89×10^{-5}). External plots show proteins with peptides
934 identified only in one sample type (left in GFP-Ctl and right in GFP- α TAT1). **h, i.** Normalised Talin
935 interaction in (h) GST-pulldown and (i) GFP-immunoprecipitations. **j.** Western blots **(j. i)** showing the
936 levels of Talin and GAPDH in astrocytes transfected with siCtl and siTalin; **(j. ii)** ratio of the intensities of
937 Talin over GAPDH normalised to the Ctl. **k, l.** Astrocytes showing **(k)** GFP- α TAT1 localisation by
938 epifluorescence, on microtubules, **(l)** by TIRF, at FAs, in cells treated with or without nocodazole. **Scale**
939 **bar** (a, b, d, e, k, l): 10 μm ; **Number of independent experiments** = 3 (for a-d, h-j), 2 (for e), 4 (for f, g), 5
940 (for l). In box-and-whisker plots, the box extends from the 25th to the 75th percentile, whiskers show the

941 minimum and maximum values, and the line within the box represents the median; **Statistical tests:**
942 Student's t-test (for a-j).

943

944 **Extended Data Figure 3:** **a.** HUVECs plated on different substrate rigidities and **(a. i)** stained for
945 Acetylated tubulin (insets shown) and α -Tubulin; **(a. ii)** ratio of the intensities of Acetylated tubulin over
946 total Tubulin of each cell; n = 46 for 2 kPa WT and 70 for 48 kPa. **b.** HUVECs treated with Niltubacin (Ctl)
947 or Tubacin, and stained for Acetylated tubulin (insets shown), α -tubulin and Paxillin. **c.** Western blots
948 showing **(c. i)** Acetylated and α -tubulin levels in HUVECs treated with DMSO (Ctl) and Tubacin; **(c. ii)**
949 ratio of intensities of Acetylated tubulin over α -tubulin. **d.** Schematic representation of the different cell
950 regions used to quantify FA density (Fig. 2D-F). **e, f.** Graphs show FA density (number of FAs/ μm^2) in
951 different regions of astrocytes transfected with siCtl or si α TAT1-2, or treated with Niltubacin or Tubacin,
952 and plated on 1.26 kPa or 48 kPa substrates. **g, h.** Graphs show FA density (number of FAs/ μm^2) in
953 different regions of astrocytes transfected with siCtl or si α TAT1-1, and plated on 1.26 kPa or 48 kPa
954 substrates. **i.** siCtl or si α TAT1-2-transfected astrocytes plated on crossbow-shaped micropatterned
955 polyacrylamide gels of 40 kPa or 2 kPa, **(i. i)** stained with Paxillin and α -tubulin; **(i. ii)** FA density (number
956 of FAs/ μm^2) within 8-16 μm layer of the cell, or **(i. iii)** in different regions of astrocytes transfected with
957 siCtl or si α TAT1-2, and plated on 2 kPa or 40 kPa substrates. **Scale bar** (a, b, i): 10 μm . **Number of**
958 **independent experiments** = 2 (for a), 3 (for b, e-i). In box-and-whisker plots, the box extends from the
959 25th to the 75th percentile, the whiskers show the minimum and maximum values, and the line within
960 the box represents the median. **Statistical tests:** Paired Student's t-test (for c), one-way ANOVA
961 followed by Tukey's multiple comparisons test (for e-i).

962

963 **Extended Data Figure 4: a.** Astrocytes transfected with siCtl or si α TAT1-1, and **(a. i)** stained for Actin,
964 Paxillin and Myosin IIa; **(a. ii)** percentage of siCtl or si α TAT1-transfected astrocytes with transverse
965 interjunctional actin arcs; n = 191 for siCtl and 125 for si α TAT1-1; **Scale bar:** 10 μ m. **Number of**
966 **independent experiments** = 3; **Statistical test:** Paired Student's t-test (two-tailed).

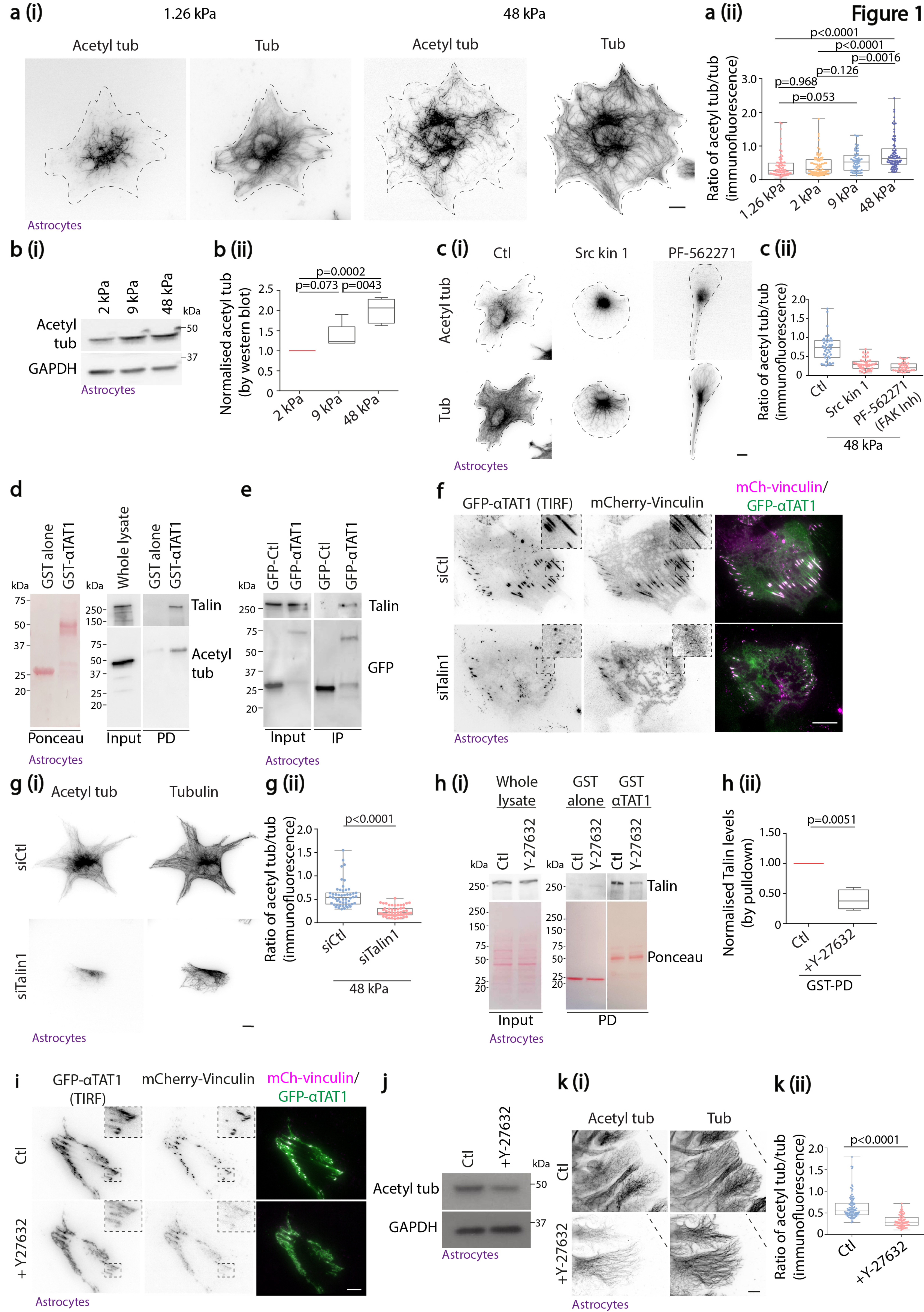
967 **Extended Data Figure 5: a.** Migrating astrocytes transfected with siCtl, si α TAT1-1 and si α TAT1-1
968 treated with Tubacin, **(a. i)** stained for Acetylated tubulin, α -tubulin and GEF-H1; **(a. ii)** percentage of
969 GEF-H1 colocalised with microtubules; n = 119 for siCtl, 131 for si α TAT1-1, 103 for si α TAT1-1 +
970 Tubacin. **b.** Astrocytes transfected with siCtl and si α TAT1-2, and plated on 2 kPa PAA gels, stained for
971 GEF-H1 and α -tubulin. **c.** Ultrastructural organization of a focal adhesion at the leading edge of a
972 control astrocyte. Platinum replica electron microscopy (PREM) high magnification view of a focal
973 adhesion on the cytoplasmic surface of the leading edge in siCtl unroofed astrocytes. Microtubules are
974 both coloured in purple and indicated by white arrowheads. **d.** Traction forces in astrocytes plated on
975 micropatterned polyacrylamide gels of different rigidities; traction forces for cells on different
976 rigidities; Values for 2 kPa and 48 kPa are from experiments shown in Fig. 4a and 4b, pooled along with
977 the values for 50 kPa, 91.8 kPa and 121 kPa; n = 136 for 2 kPa Niltubacin, 163 for 48 kPa siCtl, 31 for 50
978 kPa WT, 33 for 91.8 kPa WT and 30 for 121 kPa WT. In box-and-whisker plots, the box extends from
979 the 25th to the 75th percentile, the whiskers show the minimum and maximum values, and the line
980 within the box represents the median. **Scale bar** (a, b): 10 μ m, (c): 200 nm; **Number of independent**
981 **experiments** = 3 (for a, b, d - 2 kPa Niltubacin and 48 kPa siCtl), 2 (for d - 50 kPa, 91.8 kPa and 121 kPa).
982 **Statistical tests:** One-way ANOVA followed by Tukey's multiple comparison's test (for a).

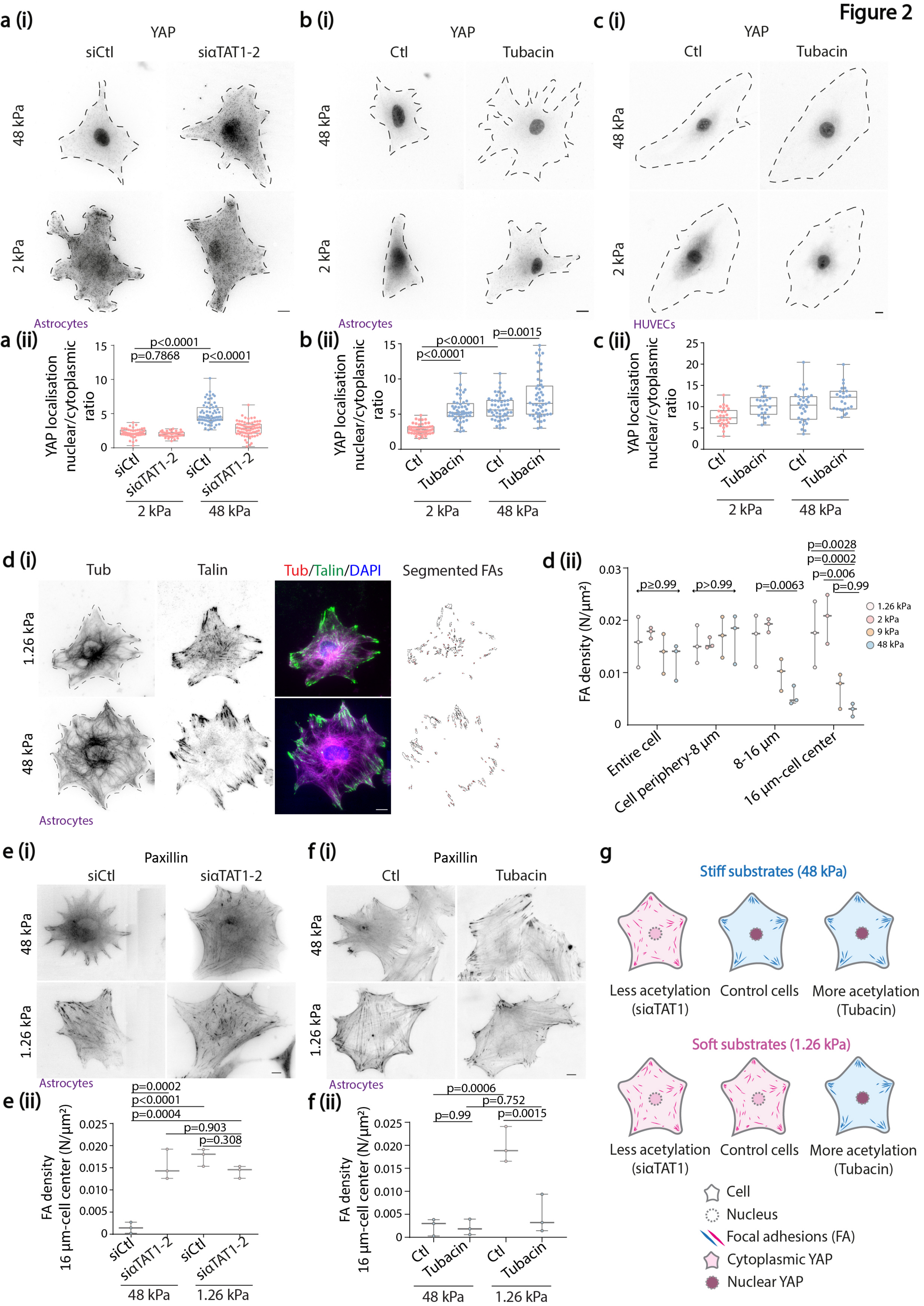
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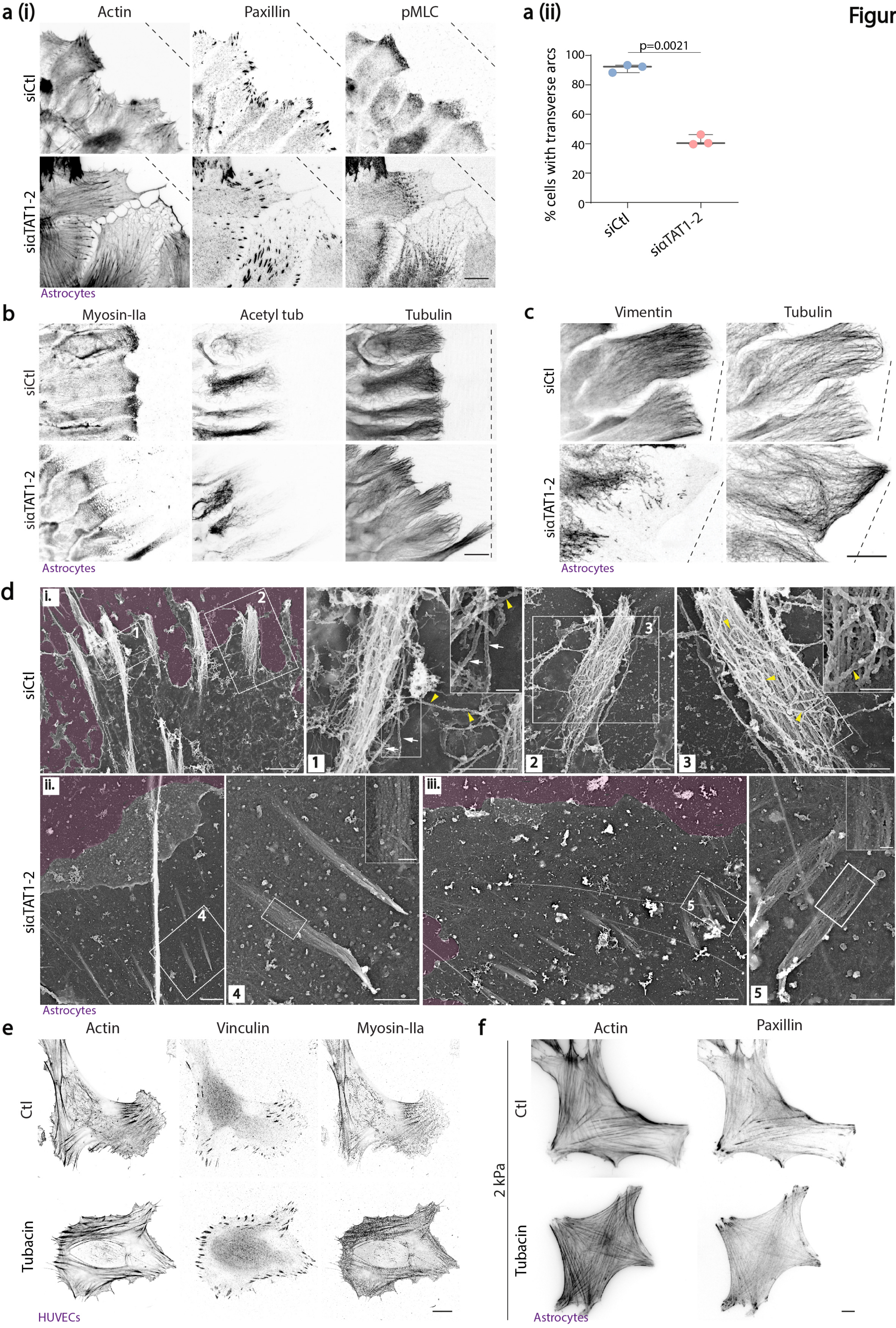
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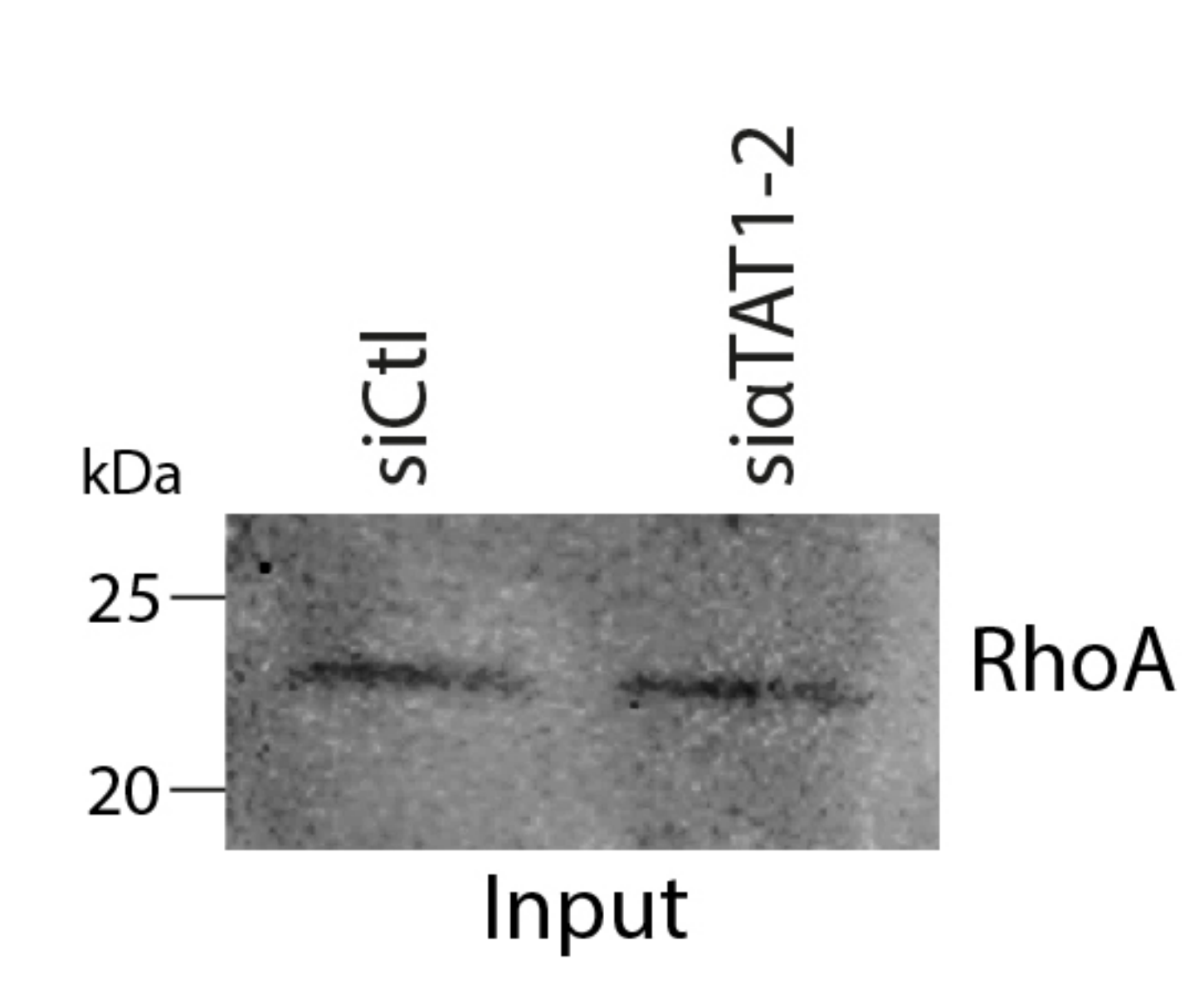
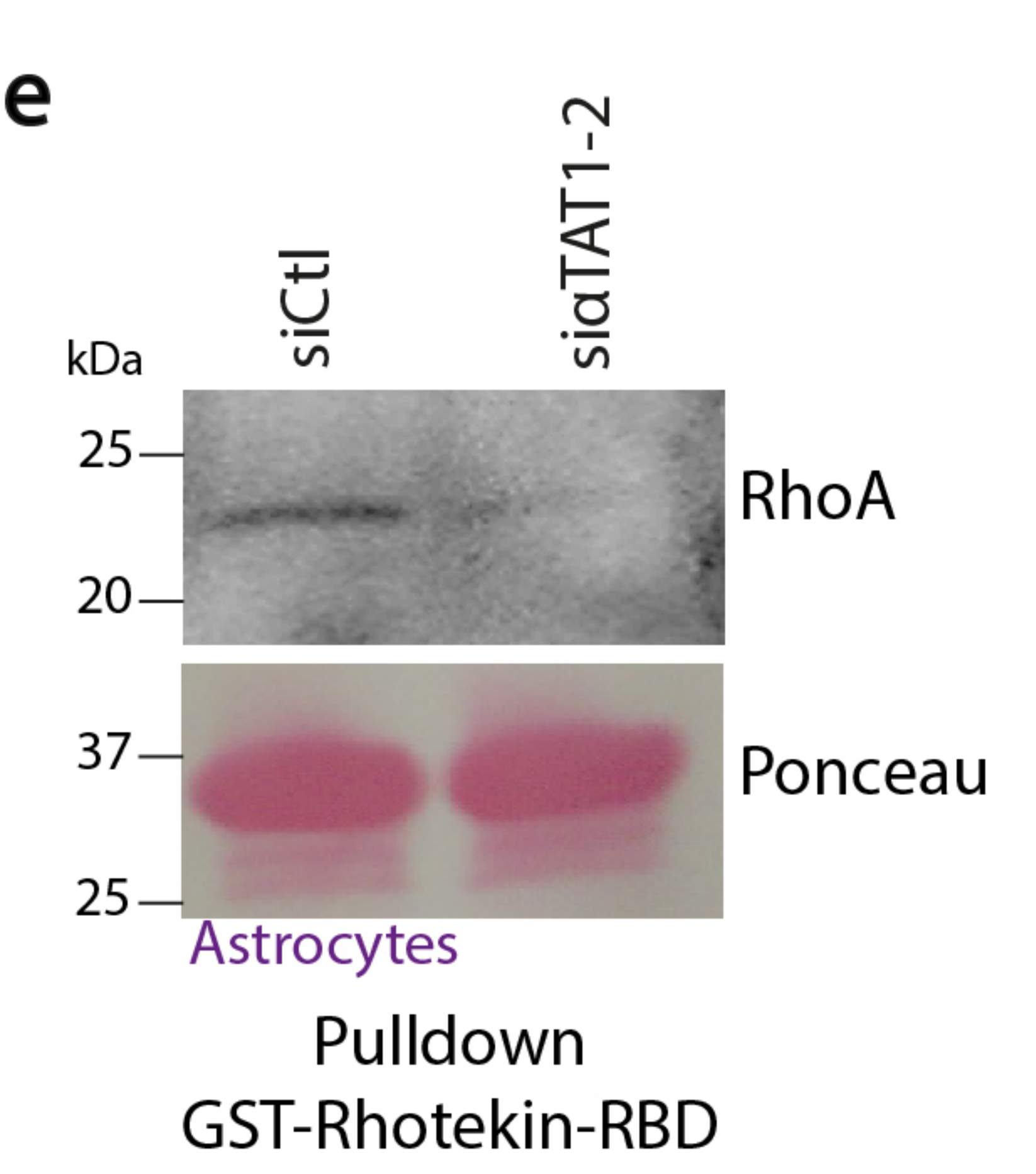
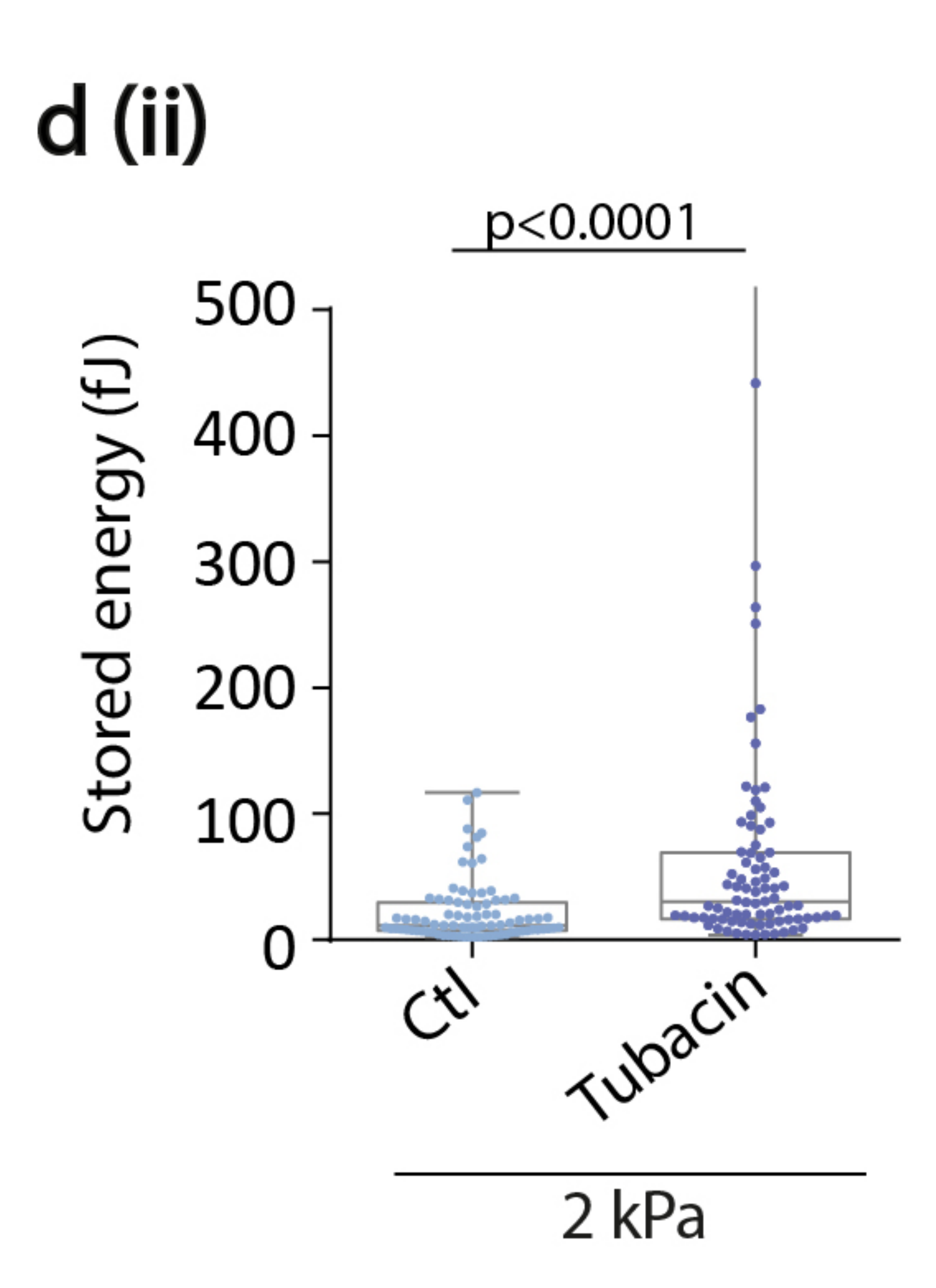
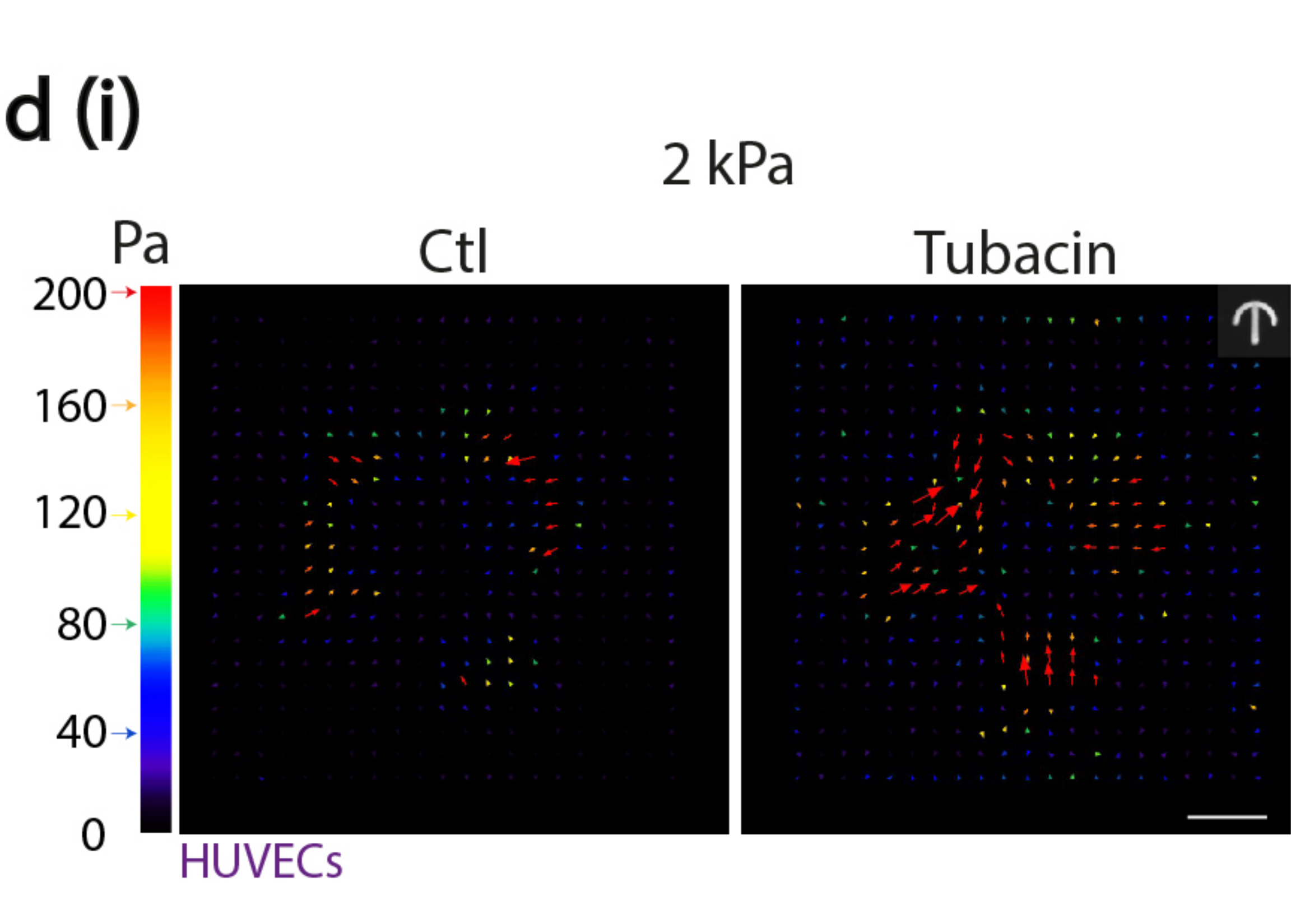
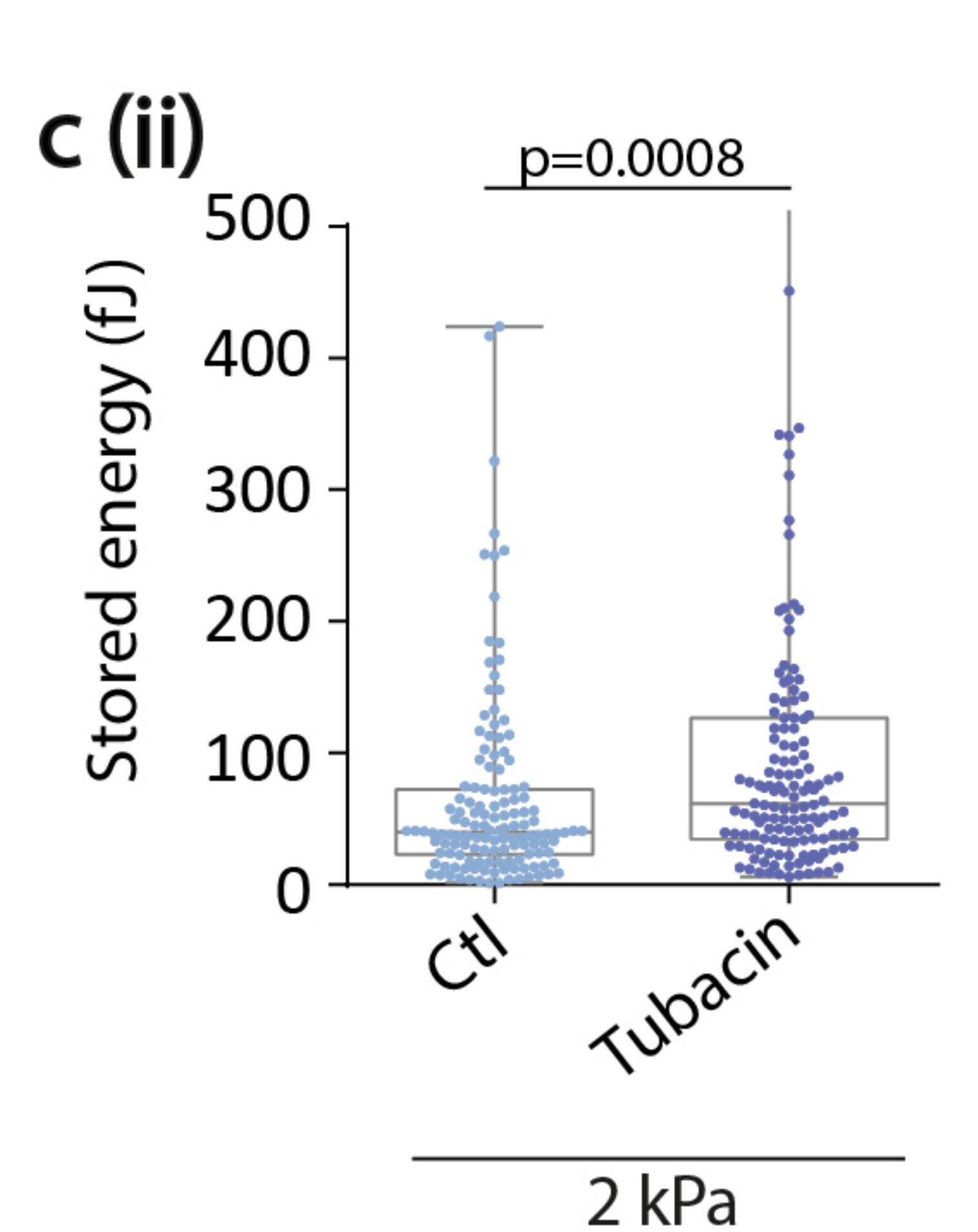
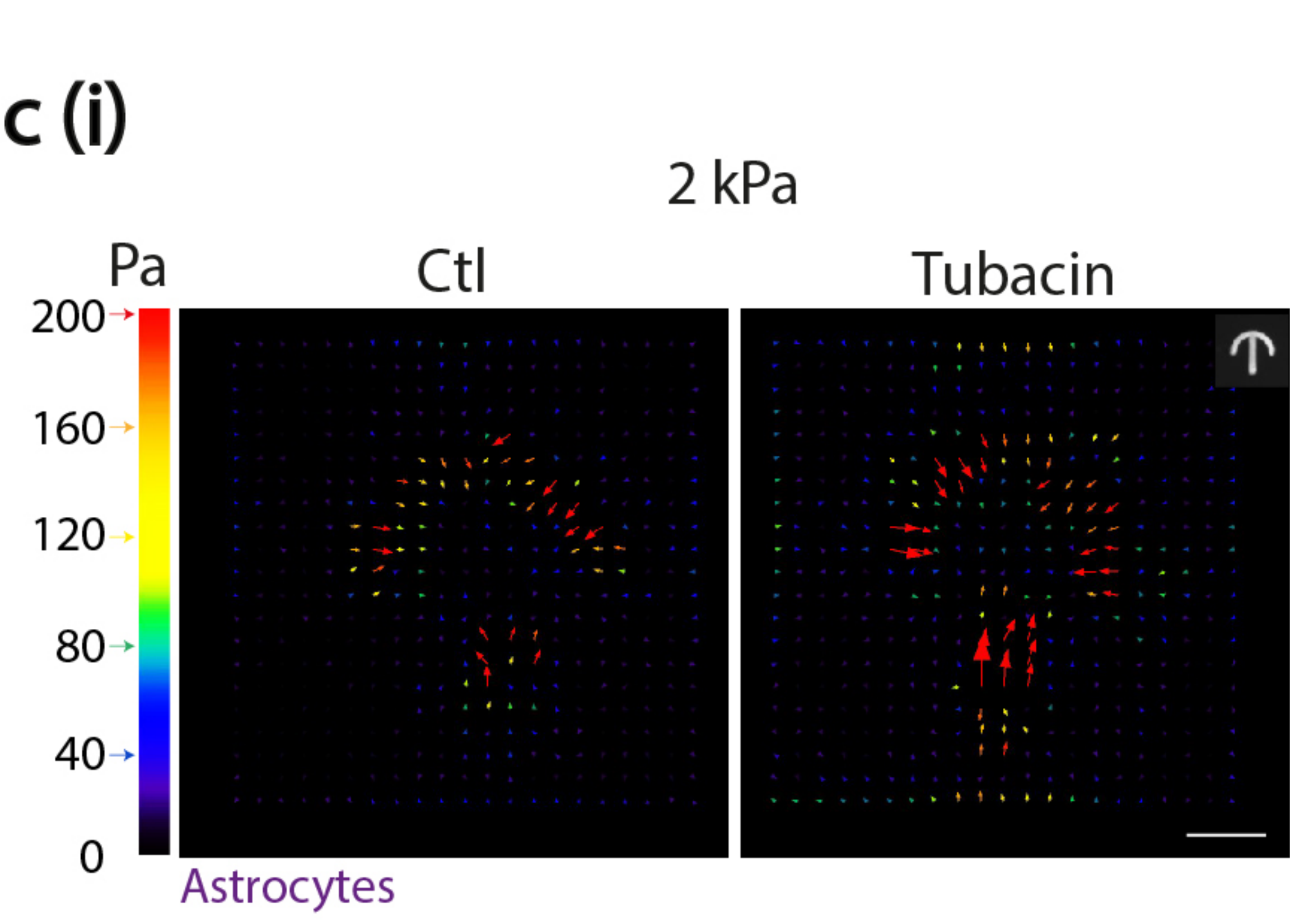
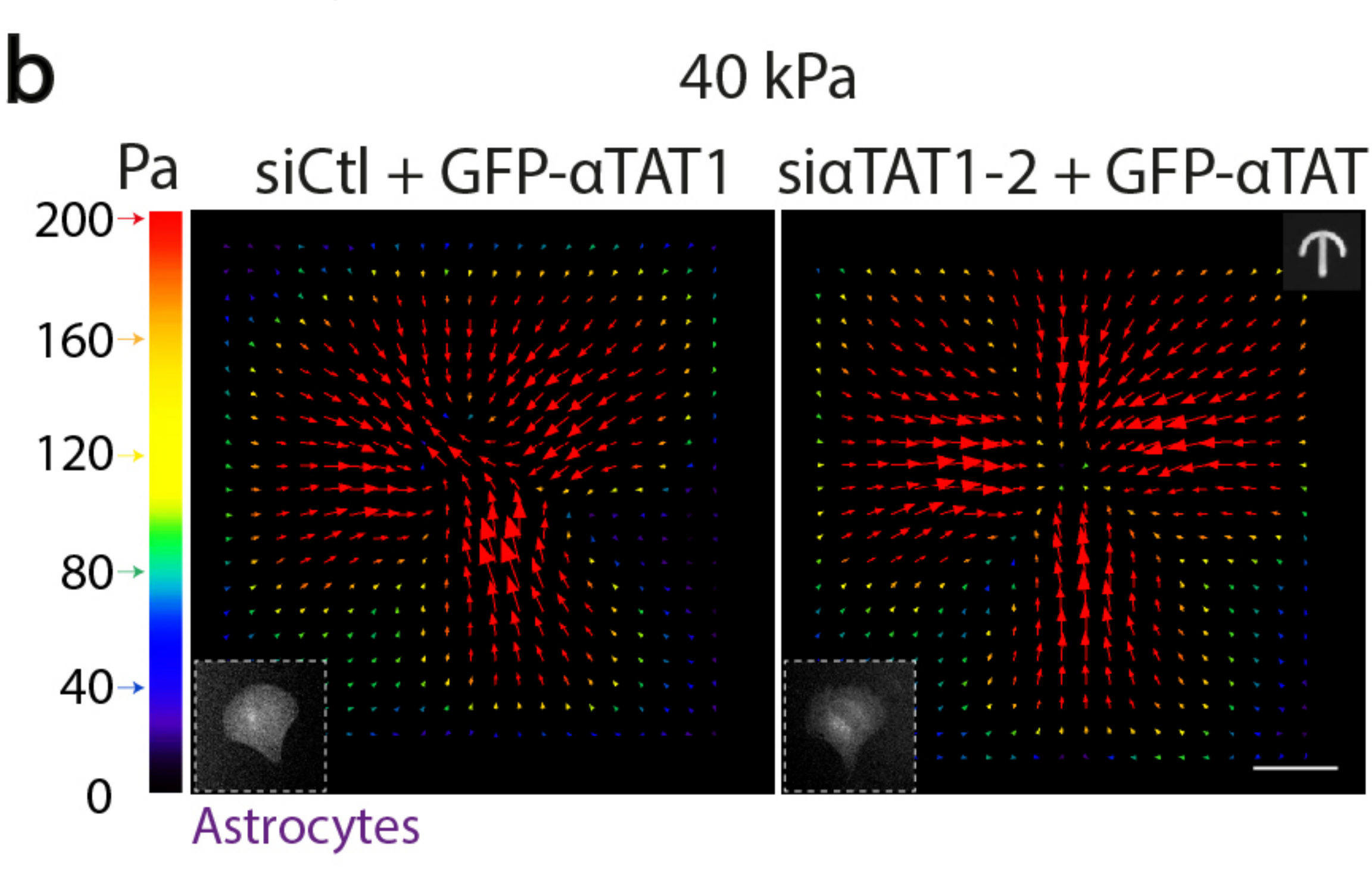
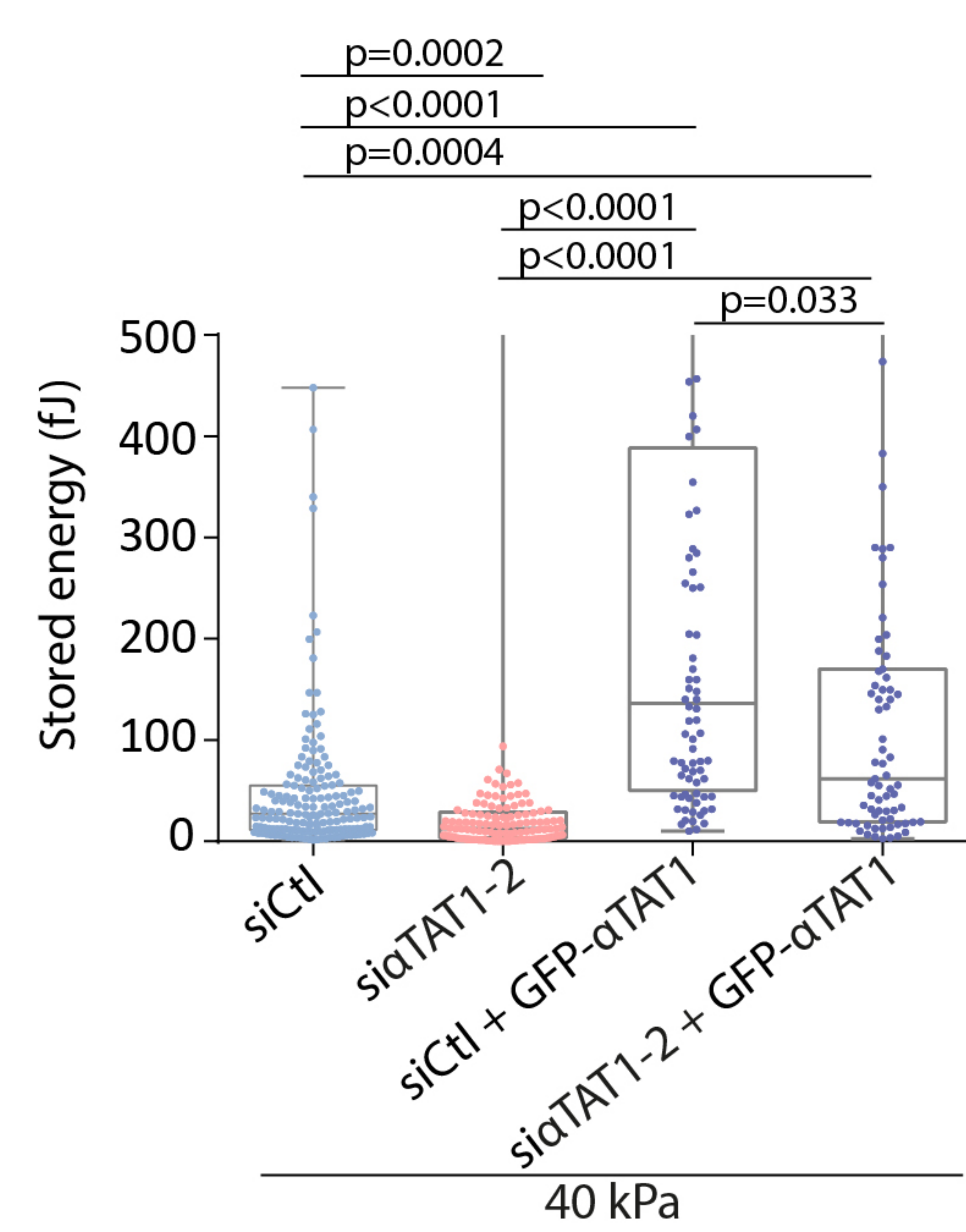
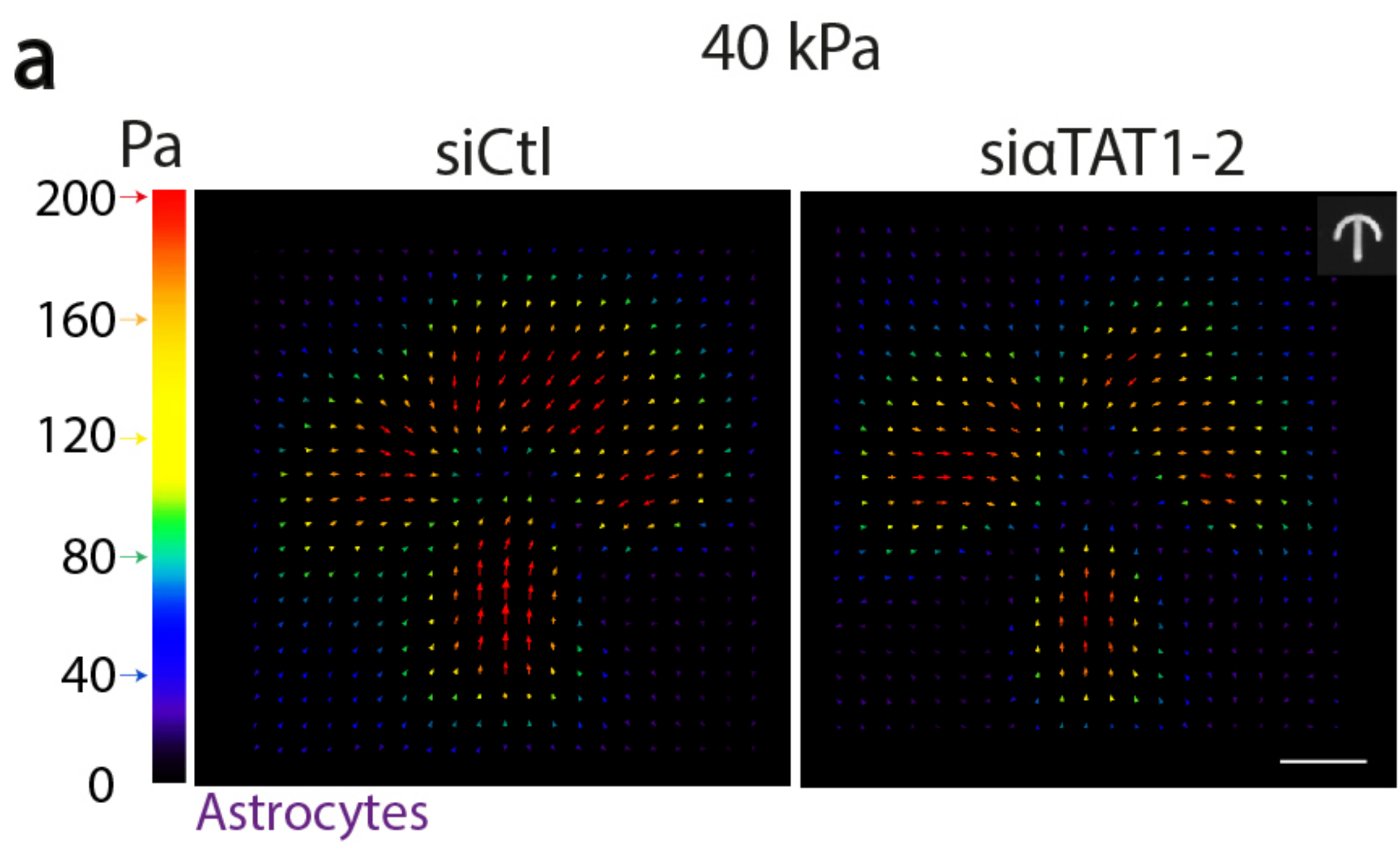
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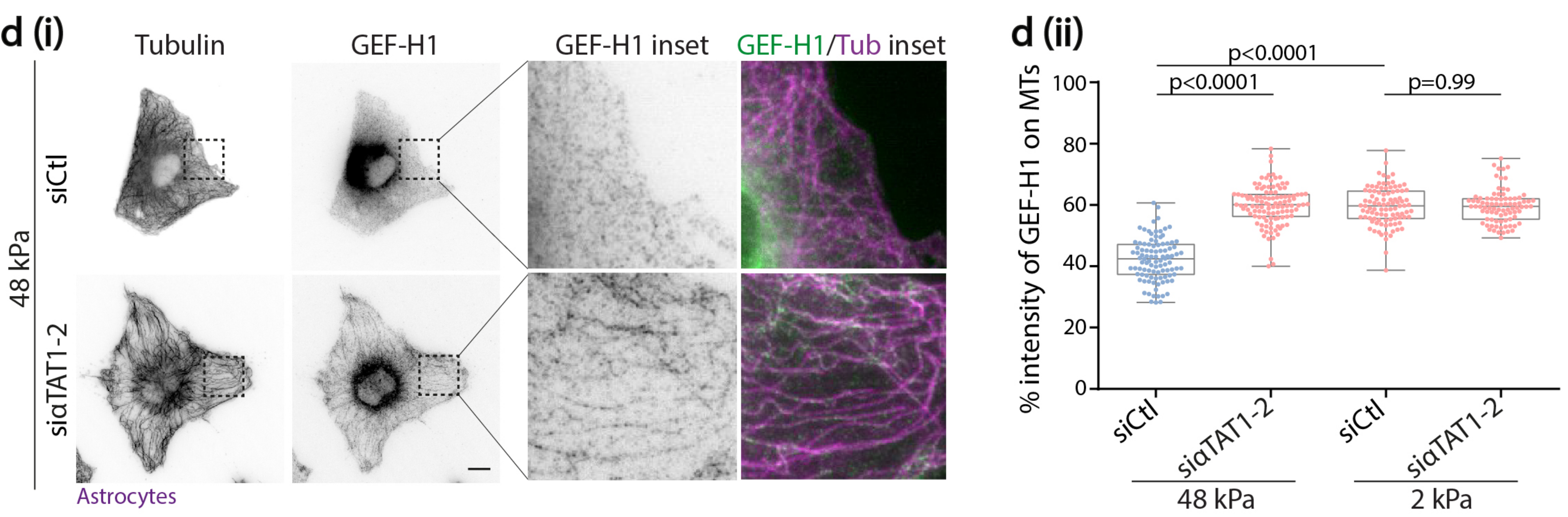
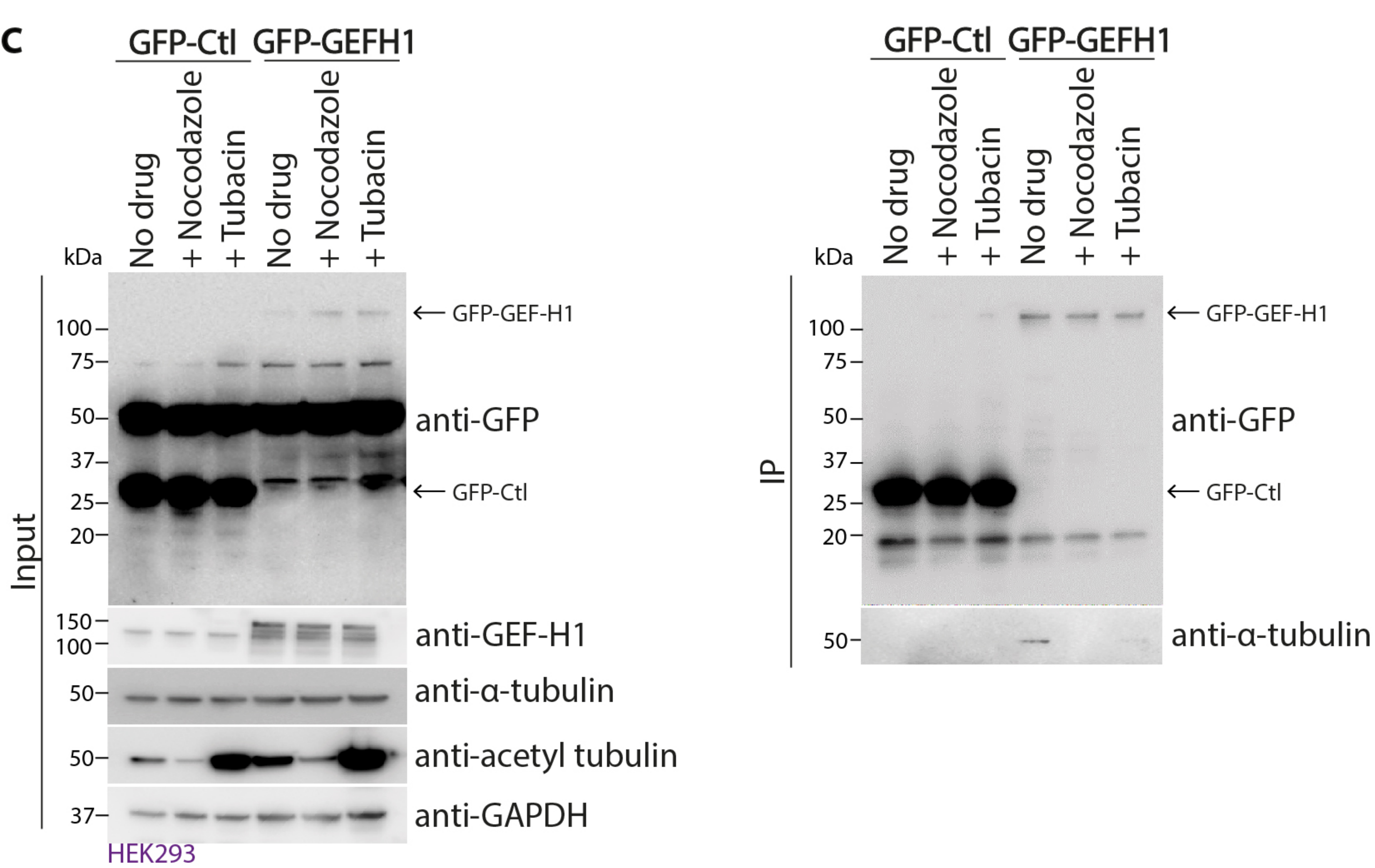
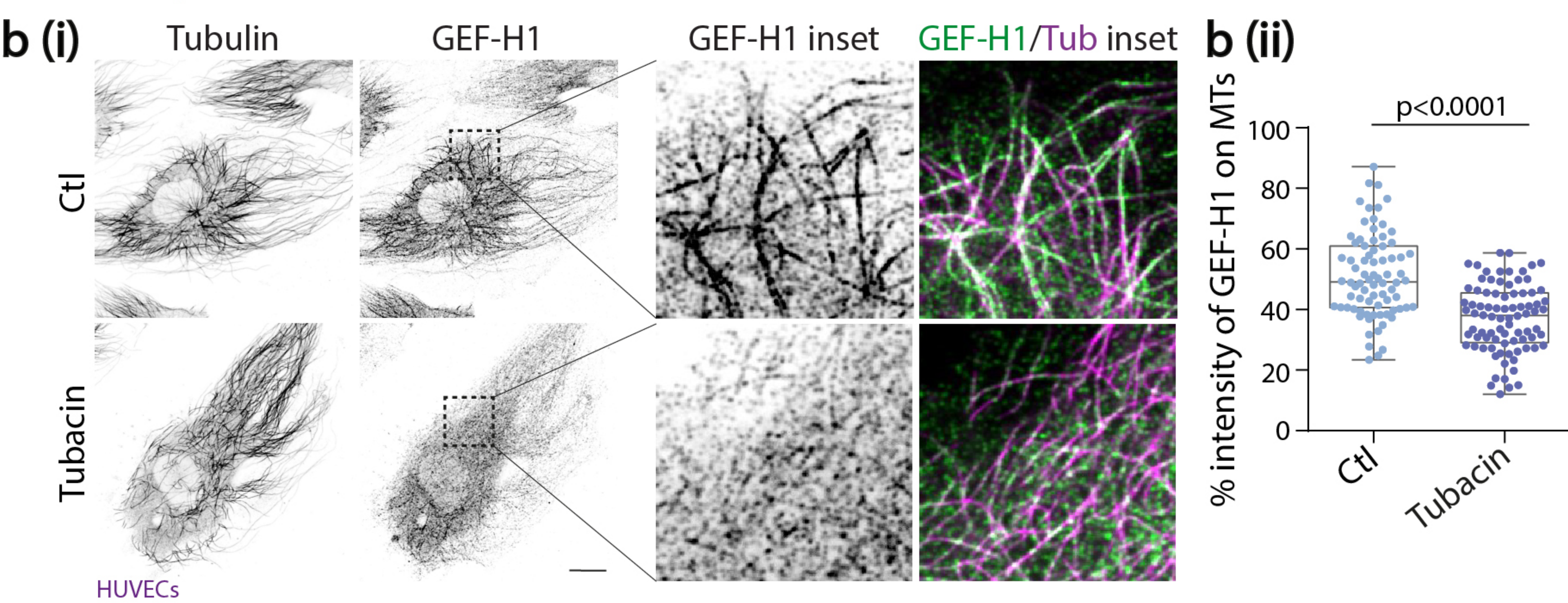
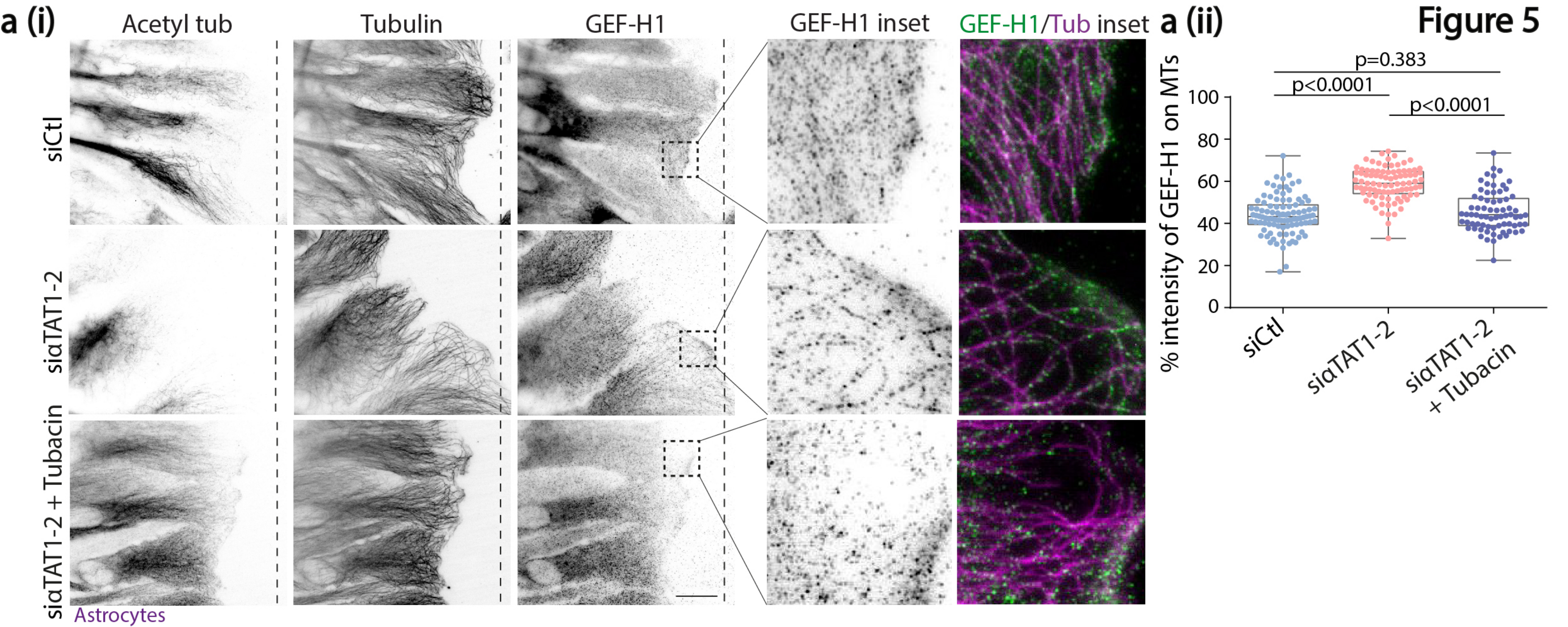
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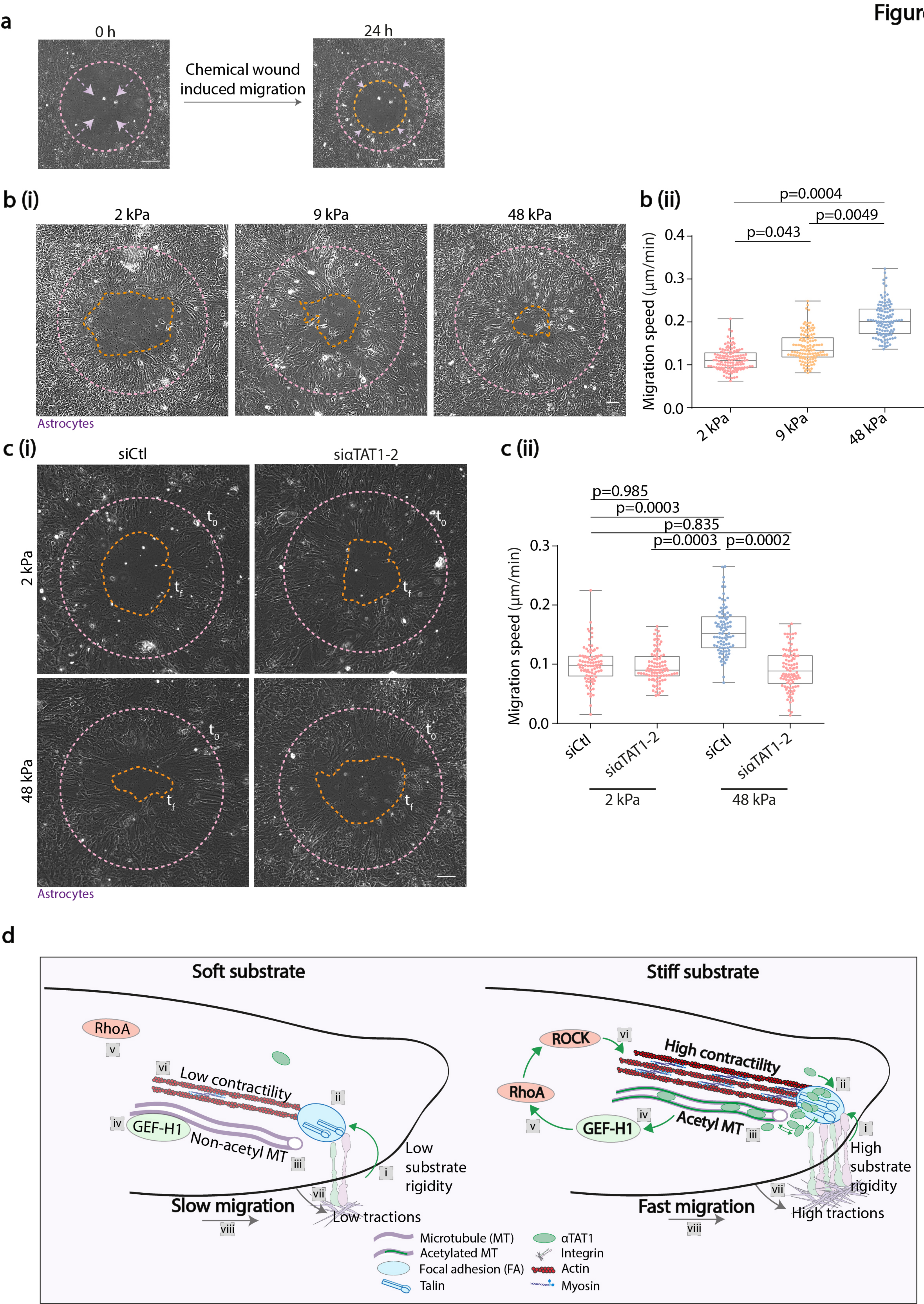


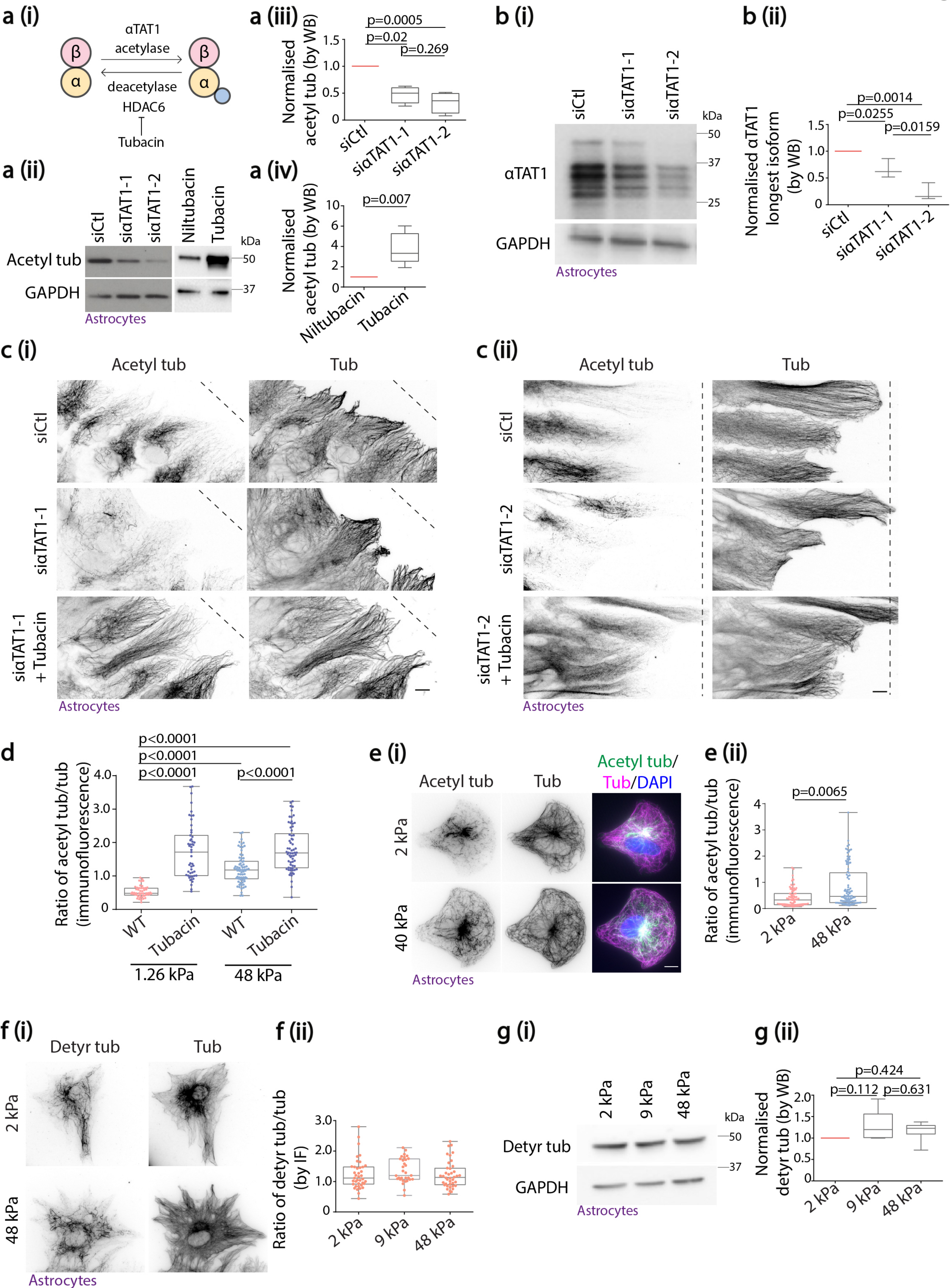


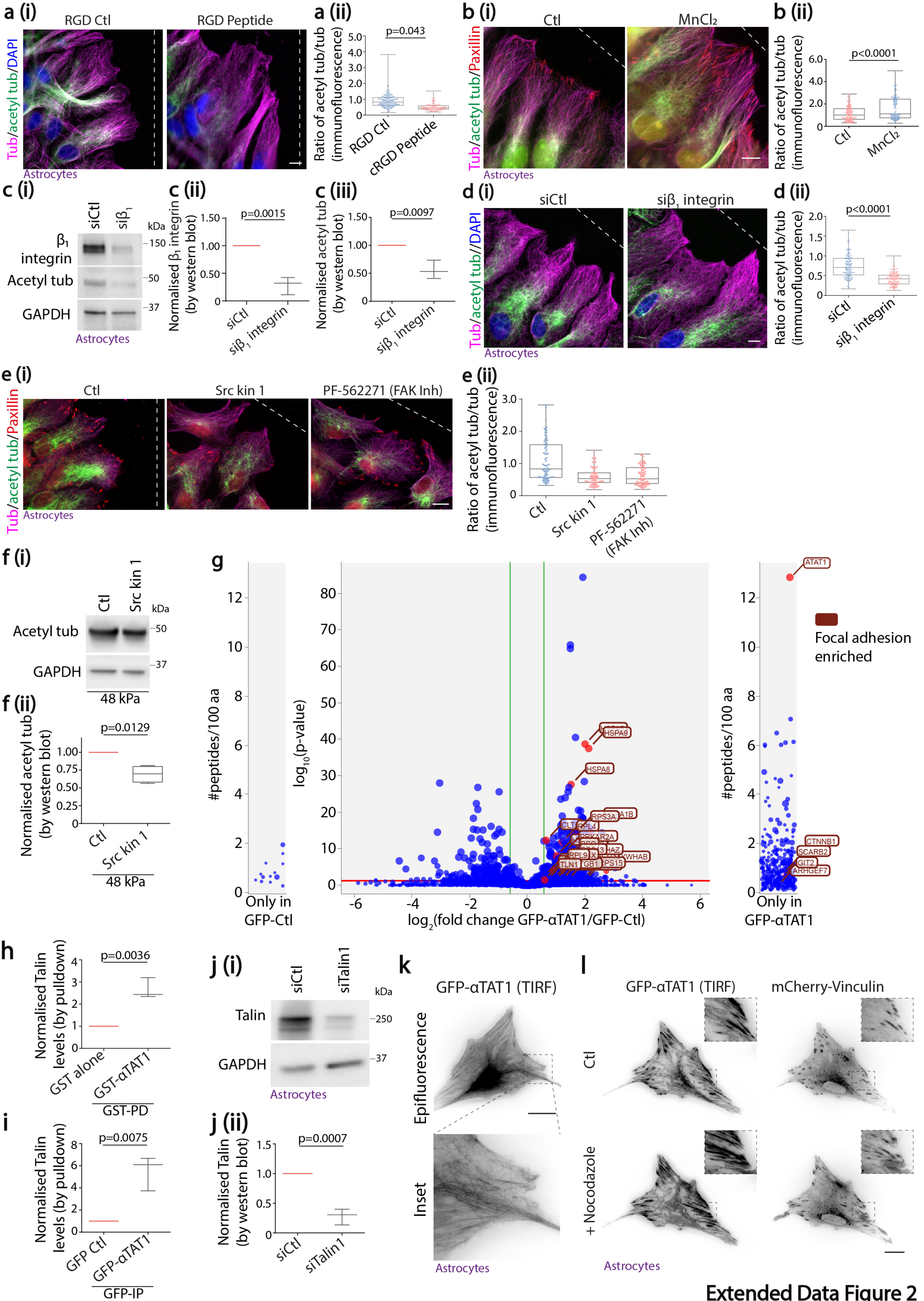




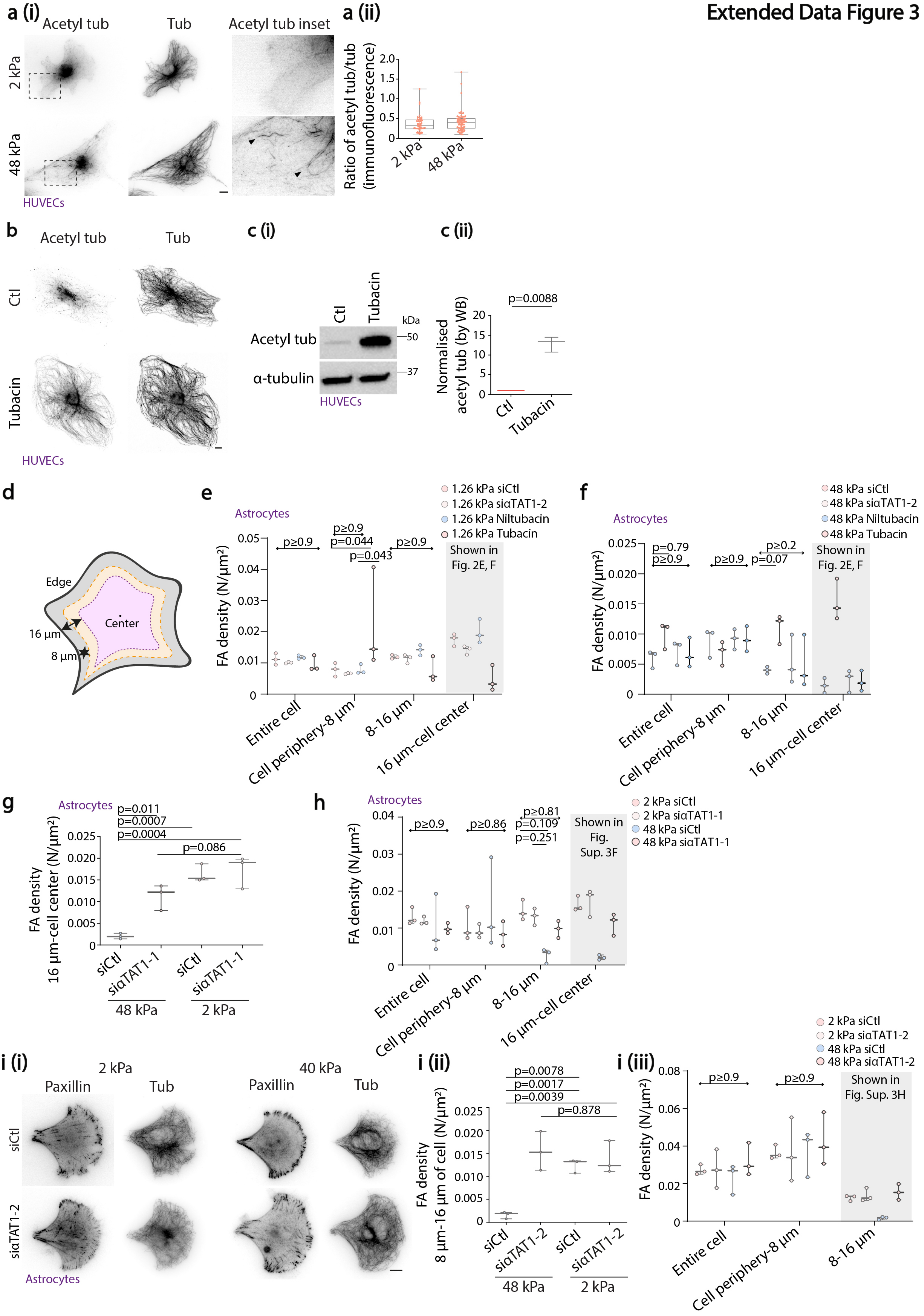


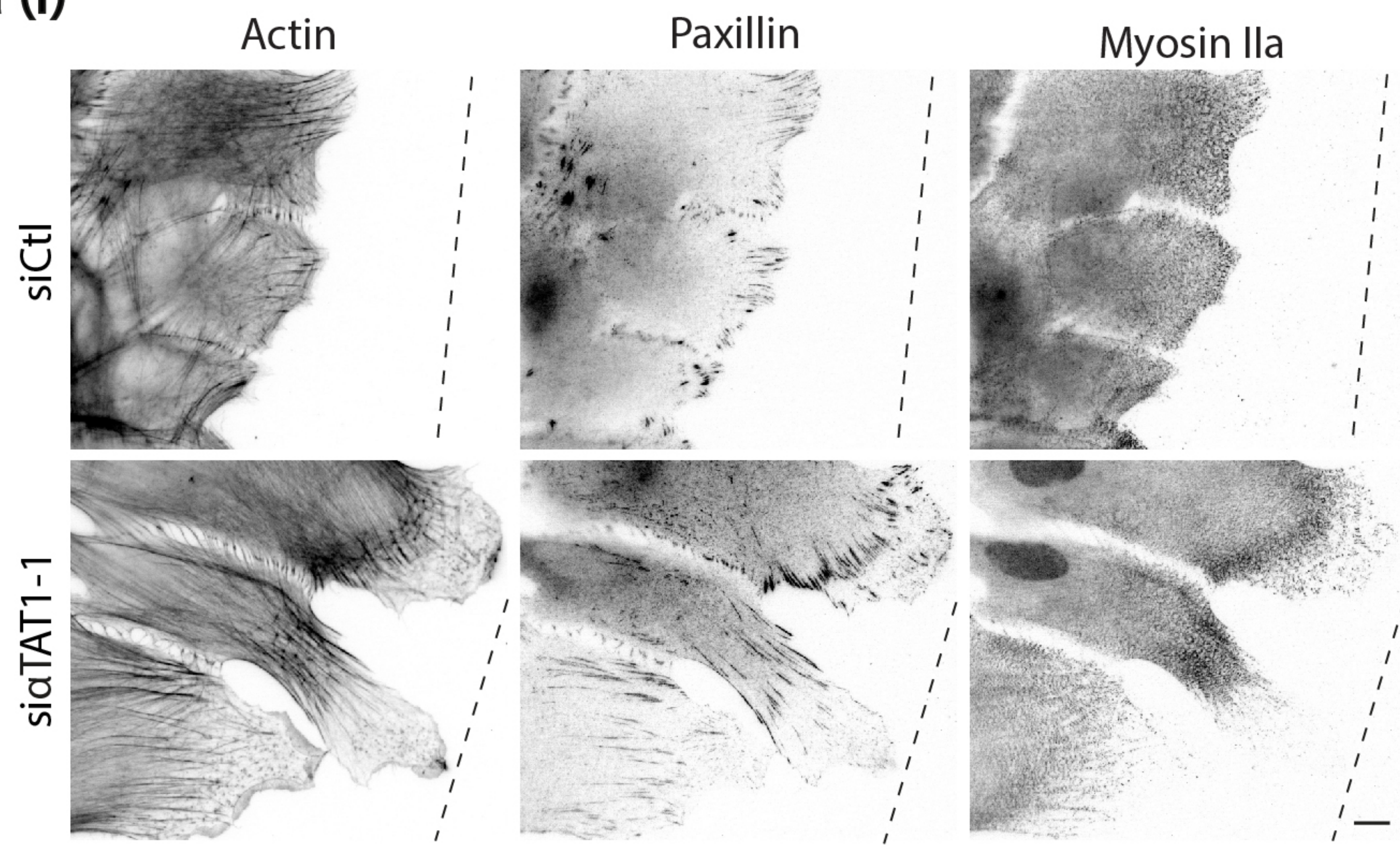






Extended Data Figure 2



a (i)**a (ii)**