

1 **Elasticity of dense actin networks produces nanonewton protrusive forces**

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16 **Abstract**

17 Actin filaments assemble into force-generating systems involved in diverse cellular functions,
18 including cell motility, adhesion, contractility and division. It remains unclear how networks of actin
19 filaments, which individually generate piconewton forces, can produce forces reaching tens of
20 nanonewtons. Here we use *in situ* cryo-electron tomography to unveil how the nanoscale
21 architecture of macrophage podosomes enables basal membrane protrusion. We show that the
22 sum of the actin polymerization forces at the membrane is not sufficient to explain podosome
23 protrusive forces. Quantitative analysis of podosome organization demonstrates that the core is
24 composed of a dense network of bent actin filaments storing elastic energy. Theoretical modelling
25 of the network as a spring-loaded elastic material reveals that it exerts forces of up to tens of
26 nanonewtons, similar to those evaluated experimentally. Thus, taking into account not only the
27 interface with the membrane but also the bulk of the network, is crucial to understand force
28 generation by actin machineries. Our integrative approach sheds light on the elastic behavior of
29 dense actin networks and opens new avenues to understand force production inside cells.

30

31 **Introduction**

32 Actin, one of the most abundant proteins in eukaryotic cells, organize into force-generating
33 filamentous networks, which play pivotal roles in cell motility, adhesion, endocytosis and vesicular
34 traffic ^{1,2}. Thermodynamics showed that actin polymerization generates mechanical forces by the
35 addition of new monomers at the end of a fluctuating filament ^{3,4}. Typical stall forces of a
36 polymerizing actin filament were estimated in the 1-10 pN range ^{3,5,6}, in agreement with
37 experimental values of 1.5 pN found by optical trap measurements ⁷. Thus, polymerization of actin
38 filaments against a membrane is capable of extruding thin membrane tubes or forming plasma
39 membrane invaginations in mammalian cells in a force range of a few tens of pN ⁸⁻¹⁰.

40 The forces exerted by actin filaments can also reach a much higher regime, in the
41 nanonewton (nN) range ^{11,12}. At the leading edge of motile cells, branched actin networks in
42 lamellipodial protrusions produce local forces of ~1 nN ^{13,14}. During yeast endocytosis, the actin
43 machinery generates similar forces to overcome the turgor pressure pushing the invagination
44 outwards ¹⁵. At the basal membrane of myeloid cells, podosomes probe the stiffness of the
45 extracellular environment through the generation of forces reaching tens of nN ¹⁶. Unlike the lower
46 force regime, the mechanisms by which meshworks of actin filaments produce nN forces remain
47 unknown.

48 If the force generated by an actin network corresponds to the sum of the polymerization

49 forces generated by single filaments pushing against a load, then hundreds to thousands of
50 filaments would need to continuously grow against the surface to reach the nN range.
51 Alternatively, filaments could push on the membrane with shallow angles ^{17,18}. It was also
52 proposed that the actin network could store elastic energy, and thus exert a restoring force. In the
53 low force regime, bending of endocytic filaments observed in animal cells has been proposed to
54 store elastic energy for pit internalization ¹⁹. In the large force regime, a theoretical model with
55 non-deformable filaments showed that a large force would cause elastic energy to be stored in
56 cross-linkers deformation ²⁰. However, none of these hypotheses have been explored in native
57 actin machineries generating forces of several nN, due to the difficulty to combine direct
58 observation of the actin network architecture and the knowledge of the exerted force.

59 To date, cryo-electron tomography (cryo-ET) is the only technique that resolves single
60 actin filaments inside unperturbed cellular environments ²¹⁻²³. Here we use cryo-ET to unveil the
61 three-dimensional (3D) architecture of human macrophage podosomes and elucidate their force
62 generation mechanism. These submicrometric structures are composed of a protrusive core of
63 actin filaments surrounded by an adhesion ring. The balance of forces requires the protrusion
64 force applied by the core on the substrate to be counteracted by a force of equal magnitude.
65 Protrusion force microscopy (PFM) revealed that this balance of forces takes place locally through
66 traction at the adhesion ring ^{16,24,25}, which has been proposed to be transmitted by radial actin
67 cables connecting the core to the ring ²⁵. Here we visualize these radial filaments and
68 quantitatively analyze the 3D organization of the core and ring networks. We show that the
69 protrusive forces generated by podosomes cannot be explained by the sum of the actin
70 polymerization forces at the core membrane but by the storage of elastic energy into the dense
71 network of bent actin filaments. These results explain how cellular actin networks can act as a
72 spring-loaded elastic material to exert forces of up to tens of nanonewtons.

73

74 **Results**

75 **Cryo-ET allows quantitative analysis of filament organization in podosomes**

76 Owing to their size and location at the basal cell membrane, native podosomes are amenable to
77 cryo-ET exploration using cryo-focused ion beam (cryo-FIB) milling sample preparation. We
78 prepared thin vitrified sections (so-called wedges) containing podosomes using shallow incidence
79 angles of the ion beam and subjected them to cryo-ET (**Figure 1A and Movie S1**). Segmentation
80 of the tomograms revealed that podosomes are made of a core of oblique filaments surrounded
81 by radial filaments (**Figure 1B-C and Movie S1**), as predicted previously ²⁵. Other cytoskeletal

82 elements, cellular organelles, ribosomes and glycogen granules are excluded from the core and
83 radial actin networks, gathering either at the periphery or on top of podosomes (**Figure 1A-B and**
84 **Movie S1**).

85 Quantitative analysis of filament organization highlighted the specificity of the actin core
86 in terms of filament length, density and orientation with respect to the basal membrane (**Methods**
87 **and Figures S1-S3**). All of these parameters exhibit a sharp transition as a function of the radial
88 distance from the core center (**Figure 1D-F**). Density is higher inside the core by a factor of 2 to 3
89 (**Figure 1D**) and filaments display a mean orientation of $50 \pm 21^\circ$ relative to the plasma membrane,
90 as compared to the flatter $23 \pm 21^\circ$ outside the core (**Figure 1E and Figure S4A-B**). Core filaments
91 are shorter than the surrounding radial filaments, with mean lengths of 119 ± 52 nm and 181 ± 134
92 nm, respectively (**Figure 1F and Figure S4C-D**). Further analysis of the transition curves for the
93 filament orientation provided a mean core radius of 203 ± 38 nm for a total of ten podosomes
94 (**Figure 1E, inset**). This agrees with the values obtained from the fits of the other parameters
95 (**Figure S5**).

96 Since the milling procedure removed the top of the podosomes, we also imaged
97 podosomes exposed by cell unroofing prior to cryo-fixation to get a complete picture of podosome
98 organization (**Figure S6 and Movie S2**). In addition to the core and radial filaments observed
99 previously, we detected horizontal filaments on top of the core as well as in between neighboring
100 cores. These filaments, which may have been lifted up from the plasma membrane during
101 podosome growth, could also participate in the generation of the traction forces that
102 counterbalance the protrusion forces generated by podosomes²⁵.

103

104 **The sum of the actin polymerization forces at the core membrane is below 1 nN**

105 To evaluate the forces generated by actin polymerization on the plasma membrane beneath the
106 podosome core, where protrusion occurs^{16,24,25}, we identified filament segments in the close
107 vicinity of and protruding against the plasma membrane (**Figure 2A and Movie S3**). We found an
108 average of 45 ± 29 filaments per podosome, with a mean orientation of $61 \pm 6^\circ$ relative to the
109 plasma membrane (**Figure 2B**). This rules out the first two hypotheses, namely that 1) hundreds
110 to thousands of filaments are growing at the same time onto the plasma membrane, or that 2)
111 filaments are pushing with shallow angles. In addition, using the upper limit of 10 pN for the stall
112 force of a single filament growing perpendicularly to the membrane^{3,5,6,26}, taking into account
113 filament orientation¹⁷, and considering that all these filaments are polymerizing concomitantly,
114 we evaluated a maximal polymerization force of 615 ± 396 pN per podosome (ten podosomes

115 evaluated; **Methods and Figure 2C-D**). This is one order of magnitude lower than the experimental
116 values ¹⁶. We therefore concluded that the total force produced by polymerization of actin
117 filaments against the plasma membrane is not sufficient to generate the protrusive forces exerted
118 by podosomes.

119

120 **The actin core stores elastic energy**

121 Next, we tested the third hypothesis, that is, the storage of elastic energy in the network. Visual
122 inspection of the networks indicated that podosome filaments are bent (**Figure 3A and Movie S4**).
123 The projection of each filament on the vertical plane passing through its two ends highlighted the
124 variation of their local curvature along the filament length (**Methods and Figure 3B-C**). Quantitative
125 analysis of filament curvature revealed an average compressive strain of $4.2 \pm 0.4\%$ for all
126 filaments, independently of their localization within the podosome (**Figure 3D and Figure S7**). We
127 therefore evaluated the elastic energy using the theory of linear elasticity: actin filaments can be
128 modeled as semi-flexible polymers with the following elastic energy at the single filament level ²⁷:

$$129 \quad u_{elastic} = \frac{\kappa}{2} \int \left\| \frac{\partial t(s)}{\partial s} \right\|^2 ds \quad (\text{Eq. 1})$$

130 where $\kappa \sim 4 \cdot 10^{-26}$ N.m² is the bending modulus ²⁸, $t(s)$ is the tangent vector as a function of the
131 arc-length coordinate s , and the integrand represents the square of the local curvature along the
132 filament (**Methods and Figure S8A**). We evaluated the elastic energy per unit volume as the sum
133 of the energies over all filaments in a given interval at a radial distance r from the core (**Figure**
134 **S8B**). The density of elastic energy stored inside the core is much larger than that outside the
135 core, by factors ranging from 3 to 10 (**Figure 3E**). This is consistent with the larger actin density
136 by factors of 2 to 6 measured inside the core (**Figure 1D**). Therefore, this specific architecture
137 allows the system to store elastic energy inside the podosome core, ranging from $\sim 10^4 k_B T$ to
138 $\sim 5 \cdot 10^4 k_B T$. This corresponds to $40 k_B T$ per filament on average, which is much larger than the
139 scale of thermal fluctuations.

140

141 **The actin core generates an elastic force in the nN range**

142 To test whether the stored elastic energy can account for podosome protrusion forces, we next
143 evaluated the elastic force generated through the compression of the actin network. Assuming
144 that the podosome core behaves as a homogeneous elastic material, the force exerted by the
145 core is such that its work for a small deformation of amplitude δh_{core} is equal to $U_{elastic}^{core}$ (the total
146 elastic energy stored in the podosome core), with $\delta h_{core} = \epsilon_{core} \times h_{core}$ (ϵ_{core} is the average

147 filament compressive strain in the core and h_{core} is the core height; **Methods**). We thus find:

$$148 \quad F_{elastic}^{core} \approx \frac{U_{elastic}^{core}}{\epsilon_{core} \times h_{core}} \quad (\text{Eq. 2})$$

149 Using **Eq. 2**, we found an average elastic force of 9.7 ± 4.2 nN, close to the mean value of 10.4 ± 3.8
150 nN reported from PFM measurements ¹⁶. The elastic force varies significantly between
151 podosomes, with values ranging from 4.1 to 14.4 nN (**Figure 4A**). This can be explained by the
152 disparity in the core size in our tomograms. Consistently with this, the force per unit area of the
153 core exhibits less variation with an average value of $P = 71.1 \pm 12.9$ kPa (**Methods**). This is in
154 agreement, within the experimental margin of error, with the pressure estimated by PFM ¹⁶
155 (**Methods and Figure 4B**) and in the same order of magnitude as that produced during endocytosis
156 in yeast ¹⁸. Note that the elastic energy stored by the network, and thus the elastic force, are
157 underestimated here since we do not take into account the elastic energy stored by crosslinkers
158 ²⁰.

159

160 **Mechanical properties of the podosome**

161 Knowing the elastic force, and thus the pressure P , allowed us to estimate the Young's modulus of
162 the core: $Y = P / \epsilon_{core} = 1.7$ MPa. While this value is several orders of magnitude larger than
163 reported values for reconstituted and cellular actin networks ^{29,30}, it is compatible with the very
164 high actin density in the core. Actin itself has a Young's modulus $Y_a = 2.3$ GPa ³¹. The elastic
165 modulus that can be reached by an actin network can be estimated as $Y = Y_a \phi^2$, with ϕ the
166 volume fraction occupied by actin filaments ³², yielding an upper limit of 12 MPa in podosomes.
167 Thus, the Young's modulus we find for the podosome core is well within expected values and
168 helps understand how a compressive strain of less than 5% translates into forces in the
169 nanonewton range.

170 The protrusive force exerted by the core on the substrate is balanced by an opposing force
171 transmitted by the radial filaments to the adhesion ring, which prevents the core from relaxing
172 towards the cell interior. We can estimate the surface tension, σ , of the 2D meshwork of radial
173 filaments that is required to balance the force $F_{elastic}^{core}$, knowing the mean radius of the core,
174 r_{core} , and the mean angle of the radial filaments, θ_{radial} , with respect to the membrane plane
175 (**Figure 4C and Figure S4B**):

$$176 \quad \sigma = \frac{F_{elastic}^{core}}{2\pi r_{core} \sin \theta_{radial}} \sim 20 \text{ mN/m} \quad (\text{Eq. 3})$$

177 While this tension is one order of magnitude larger than the cortical tension of rounding mitotic

178 cells³³, it is the same order of magnitude as the tension inferred for some adhering cells, from 6
179 mN/m³⁴ to 100 mN/m³⁵.

180

181 Discussion

182 In summary, our results showed that mechanical energy accumulates inside the podosome core
183 through bending of the dense network of actin filaments (Figure 1 and Figure 3). To relax, the
184 elastic energy produces an elastic force, which is balanced by radial tension between the actin
185 core and the adhesion ring. Indeed, we found that the elastic force computed from the bending
186 energy matches the protrusive force measured experimentally (Figure 4). We revealed that the
187 load borne by actin filaments in contact with the membrane is greater than the stall force, making
188 their growth thermodynamically unfavorable (Figure 2). In addition, the need to synchronize their
189 growth against the membrane would drastically slow down the polymerization process³⁶. Thus,
190 how the network assembles under force remains to be understood.

191 One possibility is that actin filaments do not grow directly against the load at the
192 membrane, which would instead be borne by the existing dense and compressed network. They
193 would rather grow bent in the bulk of the podosome core in a dense environment, thus increasing
194 the elastic energy stored by the network, which can then relax by pushing against the membrane.
195 An estimation of the energy released by actin polymerization in the core, by summing over all
196 actin monomers¹⁷, yields $1 - 3 \cdot 10^5 k_B T$, which is one order of magnitude larger than the elastic
197 energy $U_{elastic}^{core}$. While some of the polymerization energy will be dissipated as heat, the
198 polymerization reaction can still significantly contribute to the loading of the network.

199 Another possibility is that the filaments grow under a much smaller force, and that the
200 network is then loaded by the active tension of the actomyosin cables driven by myosin II. This
201 would be consistent with the decrease in pushing force when myosin II is inhibited²⁴. In this
202 scenario, the energy source would be mostly ATP consumed by myosin II rather than the
203 polymerization energy. We expect that a combination of experiments and simulation will be able
204 to clarify the assembly of such a spring-loaded network.

205 In conclusion, to understand the forces applied by actin assemblies, it is not sufficient to
206 consider only the local actin-membrane interactions; rather, the whole system must be taken into
207 account. Through a combination of quantitative analysis and modelling of the native 3D
208 architecture of macrophage podosomes, we showed that elastic energy is stored in actin networks
209 *in vivo*, allowing forces of the order of ten nN to be produced. These results highlight the
210 possibilities opened by exploring the architecture of actin networks *in situ* at the molecular scale.

211 Given the rapid progress of cryo-ET, we expect that, building on our biophysical approach, it will
212 soon be possible to address other degrees of freedom for elastic energy storage, such as
213 crosslinker deformation and filament twisting, and to shed light on other modes of force generation
214 by cellular machineries.

215 **Methods**

216 **Differentiation and culture of primary monocyte-derived macrophages**

217 Human monocytes were isolated from the blood of healthy donors as described previously ³⁷.
218 Cells were resuspended in cold phosphate buffered saline (PBS) supplemented with 2 mM EDTA,
219 0.5% heat-inactivated Fetal Calf Serum (FCS) at pH 7.4 and magnetically sorted with magnetic
220 microbeads coated with antibodies directed against CD14 (Miltenyi Biotec). Monocytes were then
221 seeded on glass coverslips at 1.5×10^6 cells/well in six-well plates in RPMI 1640 (Invitrogen)
222 without FCS. After 2h at 37°C in a humidified 5% CO₂ atmosphere, the medium was replaced by
223 RPMI containing 10% FCS and 20 ng/mL of Macrophage Colony-Stimulating Factor (M-CSF)
224 (Peprotech). For experiments, cells were harvested at day 7 using trypsin-EDTA (Fisher
225 Scientific) and centrifugation (320g, 10 min).

226

227 **Cell vitrification**

228 Gold EM grids with R1/4 holey SiO₂ film (Quantifoil) were glow-discharged in a EasiGlow (Pelco)
229 glow discharge system. After grid sterilization under UV light, the cell suspension containing
230 fiducials was seeded onto the grids and incubated for 2 h at 37°C to let the cells adhere to the
231 grids, resulting in 3 to 4 cells per grid square. For cell vitrification, grids were loaded into the
232 thermostatic chamber of a Leica EM-GP automatic plunge freezer, set at 20°C and 95% humidity.
233 Excess solution was blotted away for 10 s with a Whatman filter paper n°1, and the grids were
234 immediately flash frozen in liquid ethane cooled at -185°C.

235

236 **Unroofing**

237 When indicated, macrophages plated on grids were unroofed prior to vitrification. Cells were
238 unroofed using distilled water containing cOmplete™ protease inhibitors (Roche) and 10 µg/mL
239 phalloidin (Sigma-Aldrich P2141) for 30 s.

240

241 **Cryo-FIB milling**

242 Plunge-frozen EM grids were clipped into Autogrid frames modified for wedge milling under
243 shallow angles ³⁸. Autogrids were mounted into a custom-built FIB-shuttle and transferred using
244 a cryo-transfer system (PP3000T, Quorum) to the cryo-stage of a dual-beam Quanta 3D FIB/SEM
245 (Thermo Fisher Scientific) operated at liquid nitrogen temperature ³⁹. The support film close to the

246 cells of interest was sputtered away with high beam currents of 0.5-1.0 nA to provide a reference
247 in Z direction for wedge milling. Cells were first milled roughly at very shallow angles (typically 2-
248 5° of the incident ion beam) with beam currents of 300-500 pA. Advancement of the milling was
249 monitored by SEM at 5 kV and 5.92 pA. Closer to the cell surface, beam currents of 50-100 pA
250 were used for fine milling. Once all the wedges were prepared on the grid, a final polishing step
251 at 30-50 pA was performed to limit surface contamination.

252

253 **Cryo-ET and tomogram reconstruction**

254 Wedges were loaded vertically to the tilt axis in a Titan Krios transmission electron microscope
255 (Thermo Fisher Scientific) equipped with a 300 kV field-emission gun, Volta phase plates (VPPs)
256 (Danev et al., 2014), a post-column energy filter (Gatan, Pleasanton, CA, USA) and a 4k x 4k K2
257 Summit direct electron detector (Gatan) operated with SerialEM. The VPPs were aligned and
258 used as described previously ⁴⁰. Low-magnification images were recorded at 2250x. High-
259 magnification tilt-series were recorded at 33,000x (calibrated pixel size 0.421 nm) with a target
260 defocus for phase-plate imaging of 0 μm. Bi-directional tilt series were acquired typically from -
261 30° to +60° and -32° to -60° with a tilt increment of 2° and a total dose between 150 and 200 e-
262 /Å². Frames were aligned with in-house software (K2Align) based on procedures developed by Li
263 et al. ⁴¹. Tilt series were aligned using the gold beads deposited on the surface of the support film
264 as fiducial markers. 3D reconstructions with final pixel sizes of 1.684 nm were obtained by
265 weighted-back projection using the IMOD software ⁴².

266

267 **Automated filament segmentation**

268 The 4 x binned tomograms (pixel size of 1.684 nm) were subjected to nonlocal-means filtering
269 using the Amira software ⁴³ provided by Thermo Fisher Scientific. Actin filaments were traced
270 using an automated segmentation algorithm based on a generic filament as a template ⁴⁴, with a
271 diameter of 8 nm and a length of 42 nm. To reduce background noise, short filamentous structures
272 with lengths below 60 nm (or 50 nm for the unroofed podosomes) were filtered out.

273 The coordinates of the segmented filaments were exported from Amira and used as input for data
274 analysis in MATLAB (The MathWorks) and in python (<https://gitlab.com/jhervy/podosome-demo>).
275 The coordinates of the filaments were resampled every 3 nm to give the same weight to every
276 point along a filament ²¹.

277

278 **Radial distance analysis**

279 All the parameters (filament length, density and orientation, compressive strain and density of
280 elastic energy) were evaluated as detailed in **Figures S1-S3, S7 and S8**, respectively. The plots
281 as a function of the radial distance were computed by considering all the values in a given interval
282 $[r, r + \delta]$ at a radial distance r from the core center. A list of 21 linearly-spaced values starting
283 from 0 to 500 nm was used for the binning of the radial distance, which corresponds to an interval
284 value of $\delta=25$ nm (**Figure 1D-F, Figure 3D-E, Figure S5A**).

285

286 **Estimation of the core radius**

287 All the parameters except the compressive strain were fitted as a function of the radial distance r
288 from the core center using the following equation:

$$289 \quad m(r) = \frac{1}{2} \left[(a + b) + (b - a) \tanh \left(\frac{r - r_0}{r_s} \right) \right] \quad (\text{Eq. 4})$$

290 $m(r)$ is either a decreasing function (for the filament density, filament orientation and the density
291 of elastic energy) or an increasing function (for the filament length) with two saturation values set
292 by the parameters a and b (**Figure 1D-F and Figure 3E**). The parameter r_0 corresponds to the
293 radial distance for which the slope of the tangent line is maximum; the parameter r_s defines the
294 transition range between the two saturating values (**Figure S5A**). We used the parameter r_0 from
295 the fit of the orientation as a measure of the core radius (**Figure S5B**).

296

297 **Polymerization force**

298 To estimate the polymerization force generated at the core membrane, the protrusive filaments,
299 *i.e.* those in the immediate vicinity of the plasma membrane, were considered. Specifically, all the
300 filament portions up to 10 nm away from the membrane were taken into account (**Figure 2A**). A
301 single filament growing perpendicularly to the membrane generates a force given by ⁴:

$$302 \quad f_a = \frac{k_B T}{\delta} \ln \left(\frac{C}{C_c} \right) \quad (\text{Eq. 5})$$

303 where $k_B T = 4.11 \cdot 10^{-21}$ J, $\delta = 2.75$ nm is the displacement induced by the addition of one actin
304 monomer, C is the concentration of actin monomers in solution and C_c is the critical concentration.
305 Using the values $C = 150 \mu\text{M}$ as estimated in ⁴⁵ and $C_c = 0.06 \mu\text{M}$ for the critical concentration at
306 the plus end as measured *in vitro* ^{46,47}, we found an upper limit of 11 pN for the stall force. Thus,
307 the factor $\ln(C/C_c)$ was set to 7 leading to a stall force of 10 pN for a single filament growing
308 perpendicularly to the membrane. This value was increased by a factor $1/\sin(\theta)$ for a filament
309 having an orientation θ relative to the membrane ¹⁷ (**Figure 2C**). Therefore, the total

310 polymerization force was computed as follows:

$$311 \quad F_{polym}^{core} = f_a \sum_{i=1}^{n_p} \frac{1}{\sin(\theta_i)} \quad (\text{Eq. 6})$$

312 where n_p is the number of protrusive filaments and θ_i is the average orientation for the protrusive
313 filament i (Figure 2D).

314 Local curvature

315 The local curvature was evaluated between two consecutive points using their respective tangent
316 vector t as follows:

$$317 \quad \gamma_{i,i+1} = \frac{1}{a} \arccos(t_i \cdot t_{i+1}) \quad (\text{Eq. 7})$$

318 where a is their relative distance set to 3 nm in our segmentation procedure.

319 Local elastic energy

320 The local energy is proportional to the square of the local curvature and can be discretized as
321 follows:

$$322 \quad u_{i,i+1}^{elastic} = \frac{\kappa}{2} (\gamma_{i,i+1})^2 \quad (\text{Eq. 8})$$

323 where κ is the bending modulus²⁸.

324

325 Total elastic energy

326 The total elastic energy inside the core was computed by summing the local energy over all the
327 filament points that are within the radial distance domain $r \in [0, r_{core}]$, where r_{core} is the core
328 radius (Figure S8).

329 Elastic force

330 The elastic force pushing perpendicularly to the membrane is the derivative of the core elastic
331 energy with respect to the height h_{core} of the core:

$$332 \quad F_{elastic}^{core} = \frac{dU_{elastic}^{core}}{dh_{core}} \approx \frac{\delta U_{elastic}^{core}}{\delta h_{core}} \quad (\text{Eq. 9})$$

333 with δ indicating small changes. Indeed, in the core, the average compressive strain of the
334 filaments is $\epsilon_{core} \sim 0.04$ (Figure 3D) and therefore considered to be small. Assuming all elastic
335 energy to be released in the resting state, we have $\delta U_{elastic}^{core} = U_{elastic}^{core}$ and $\delta h_{core} = \epsilon_{core} \times h_{core}$.

336 Therefore, we find:

$$337 \quad F_{elastic}^{core} \approx \frac{U_{elastic}^{core}}{\epsilon_{core} \times h_{core}} \quad (\text{Eq. 2})$$

338 Note that this is an order of magnitude estimate: since the actin network is assembled under
339 pressure, it is likely that its resting state has a non-zero elastic energy, in which case the force is
340 overestimated. The precise architecture of the network could also yield a prefactor in the relation
341 between δh_{core} and ϵ_{core} . Lastly, the elastic energy could be underestimated: part of it might be
342 stored in crosslinker elasticity and in tension of the non-bent segments of actin filaments.

343

344 **Pressure induced in the podosome core**

345 Assuming a circular shape of radius r_{core} for the core, the pressure was computed as:

$$346 \quad P = \frac{F_{elastic}^{core}}{\pi r_{core}^2} \quad (\text{Eq. 10})$$

347 In the rest of this section, the values used to compute the pressure from the cryo-ET data and the
348 PFM force measurements reported in ¹⁶ are detailed.

349

350 **a) From the tomograms**

351 The pressure induced by the compression of the actin filaments (labelled as “Elastic”, **Figure 4A**)
352 and by the polymerization at the membrane (labelled as “Polymerization”) were evaluated using
353 the values for r_{core} estimated from the fits of the orientation data (**Figure 1E, inset**).

354

355 **b) From PFM force measurements**

356 The pressure P_{PFM} was computed using the force value $F = 10.4 \pm 3.8$ nN reported in ¹⁶ and
357 $r_{core} = 203.1 \pm 37.8$ nm estimated from the average of 10 podosomes (black dashed line in **Figure**
358 **1E, inset**). The error bar for this value was computed using the propagation of uncertainty method
359 as follows:

$$360 \quad \Delta P_{PFM} = \frac{2F_{elastic}^{core}}{\pi r_{core}^3} \Delta r_{core} + \frac{1}{\pi r_{core}^2} \Delta F_{elastic}^{core} \quad (\text{Eq. 11})$$

361 We found $P_{PFM} = 80.0 \pm 59.0$ kPa, with the margin of error ΔP_{PFM} represented as a rectangular
362 blue area filled in **Figure 4B**.

363

364 **Resource availability**

365 All data and code used in the analysis are available upon request to the corresponding authors.

366

367 References

- 368 1 Pollard, T. D. & Cooper, J. A. Actin, a central player in cell shape and movement. *Science*
369 **326**, 1208-1212, doi:10.1126/science.1175862 (2009).
- 370 2 Harris, A. R., Jreij, P. & Fletcher, D. A. Mechanotransduction by the Actin Cytoskeleton:
371 Converting Mechanical Stimuli into Biochemical Signals. *Annual Review of Biophysics*,
372 *Vol 47* **47**, 617-631, doi:10.1146/annurev-biophys-070816-033547 (2018).
- 373 3 Mogilner, A. & Oster, G. Cell motility driven by actin polymerization. *Biophysical Journal*
374 **71**, 3030-3045 (1996).
- 375 4 Peskin, C. S., Odell, G. M. & Oster, G. F. Cellular motions and thermal fluctuations: the
376 Brownian ratchet. *Biophysical Journal* **65**, 316-324, doi:10.1016/S0006-3495(93)81035-
377 X (1993).
- 378 5 Hill, T. L. & Kirschner, M. W. Subunit treadmill of microtubules or actin in the presence
379 of cellular barriers: possible conversion of chemical free energy into mechanical work.
380 *Proceedings of the National Academy of Sciences of the United States of America* **79**,
381 490-494, doi:10.1073/pnas.79.2.490 (1982).
- 382 6 Hill, T. L. & Kirschner, M. W. Bioenergetics and kinetics of microtubule and actin filament
383 assembly-disassembly. *Int Rev Cytol* **78**, 1-125 (1982).
- 384 7 Footer, M. J., Kerssemakers, J. W., Theriot, J. A. & Dogterom, M. Direct measurement of
385 force generation by actin filament polymerization using an optical trap. *Proceedings of the*
386 *National Academy of Sciences of the U.S.A.* **104**, 2181-2186,
387 doi:10.1073/pnas.0607052104 (2007).
- 388 8 Derenyi, I., Julicher, F. & Prost, J. Formation and interaction of membrane tubes. *Physical*
389 *review letters* **88**, 238101, doi:10.1103/PhysRevLett.88.238101 (2002).
- 390 9 Atilgan, E., Wirtz, D. & Sun, S. X. Mechanics and dynamics of actin-driven thin membrane
391 protrusions. *Biophysical journal* **90**, 65-76, doi:10.1529/biophysj.105.071480 (2006).
- 392 10 Ding, B. *et al.* Recording the dynamic endocytosis of single gold nanoparticles by AFM-
393 based force tracing. *Nanoscale* **7**, 7545-7549, doi:10.1039/c5nr01020a (2015).
- 394 11 Giardini, P. A., Fletcher, D. A. & Theriot, J. A. Compression forces generated by actin
395 comet tails on lipid vesicles. *Proceedings of the National Academy of Sciences of the*
396 *United States of America* **100**, 6493-6498, doi:10.1073/pnas.1031670100 (2003).
- 397 12 Parekh, S. H., Chaudhuri, O., Theriot, J. A. & Fletcher, D. A. Loading history determines
398 the velocity of actin-network growth. *Nature cell biology* **7**, 1219-1223,
399 doi:10.1038/ncb1336 (2005).
- 400 13 Mueller, J. *et al.* Load Adaptation of Lamellipodial Actin Networks. *Cell* **171**, 188-200 e116,
401 doi:10.1016/j.cell.2017.07.051 (2017).
- 402 14 Prass, M., Jacobson, K., Mogilner, A. & Radmacher, M. Direct measurement of the
403 lamellipodial protrusive force in a migrating cell. *The Journal of cell biology* **174**, 767-772,
404 doi:10.1083/jcb.200601159 (2006).
- 405 15 Basu, R., Munteanu, E. L. & Chang, F. Role of turgor pressure in endocytosis in fission
406 yeast. *Molecular biology of the cell* **25**, 679-687, doi:10.1091/mbc.E13-10-0618 (2014).
- 407 16 Proag, A. *et al.* Working together: spatial synchrony in the force and actin dynamics of
408 podosome first neighbors. *ACS nano* **9**, 3800-3813, doi:10.1021/nn506745r (2015).
- 409 17 Dmitrieff, S. & Nedelec, F. Amplification of actin polymerization forces. *The Journal of cell*
410 *biology* **212**, 763-766, doi:10.1083/jcb.201512019 (2016).

- 411 18 Mund, M. *et al.* Systematic Nanoscale Analysis of Endocytosis Links Efficient Vesicle
412 Formation to Patterned Actin Nucleation. *Cell* **174**, 884-896 e817,
413 doi:10.1016/j.cell.2018.06.032 (2018).
- 414 19 Akamatsu, M. *et al.* Principles of self-organization and load adaptation by the actin
415 cytoskeleton during clathrin-mediated endocytosis. *eLife* **9**, doi:10.7554/eLife.49840
416 (2020).
- 417 20 Ma, R. & Berro, J. Structural organization and energy storage in crosslinked actin
418 assemblies. *PLoS computational biology* **14**, e1006150,
419 doi:10.1371/journal.pcbi.1006150 (2018).
- 420 21 Jasnin, M. *et al.* Three-dimensional architecture of actin filaments in *Listeria*
421 *monocytogenes* comet tails. *Proceedings of the National Academy of Sciences of the*
422 *United States of America* **110**, 20521-20526, doi:10.1073/pnas.1320155110 (2013).
- 423 22 Jasnin, M. *et al.* The Architecture of Traveling Actin Waves Revealed by Cryo-Electron
424 Tomography. *Structure* **27**, 1211-1223 e1215, doi:10.1016/j.str.2019.05.009 (2019).
- 425 23 Jasnin, M., Ecke, M., Baumeister, W. & Gerisch, G. Actin Organization in Cells
426 Responding to a Perforated Surface, Revealed by Live Imaging and Cryo-Electron
427 Tomography. *Structure* **24**, 1031-1043, doi:10.1016/j.str.2016.05.004 (2016).
- 428 24 Labernadie, A. *et al.* Protrusion force microscopy reveals oscillatory force generation and
429 mechanosensing activity of human macrophage podosomes. *Nat. Commun.* **5**, 5343,
430 doi:10.1038/ncomms6343 (2014).
- 431 25 Bouissou, A. *et al.* Podosome Force Generation Machinery: A Local Balance between
432 Protrusion at the Core and Traction at the Ring. *ACS nano* **11**, 4028-4040,
433 doi:10.1021/acsnano.7b00622 (2017).
- 434 26 Hill, T. L. Microfilament or microtubule assembly or disassembly against a force.
435 *Proceedings of the National Academy of Sciences of the United States of America* **78**,
436 5613-5617, doi:10.1073/pnas.78.9.5613 (1981).
- 437 27 Broedersz, C. P. & MacKintosh, F. C. Modeling semiflexible polymer networks. *Rev Mod*
438 *Phys* **86**, 995-1036, doi:10.1103/RevModPhys.86.995 (2014).
- 439 28 De La Cruz, E. M. & Gardel, M. L. Actin Mechanics and Fragmentation. *Journal of*
440 *Biological Chemistry* **290**, 17137-17144, doi:10.1074/jbc.R115.636472 (2015).
- 441 29 Planade, J. *et al.* Mechanical stiffness of reconstituted actin patches correlates tightly with
442 endocytosis efficiency. *PLoS biology* **17**, e3000500, doi:10.1371/journal.pbio.3000500
443 (2019).
- 444 30 Bieling, P. *et al.* Force Feedback Controls Motor Activity and Mechanical Properties of
445 Self-Assembling Branched Actin Networks. *Cell* **164**, 115-127,
446 doi:10.1016/j.cell.2015.11.057 (2016).
- 447 31 Gittes, F., Mickey, B., Nettleton, J. & Howard, J. Flexural rigidity of microtubules and actin
448 filaments measured from thermal fluctuations in shape. *The Journal of cell biology* **120**,
449 923-934, doi:10.1083/jcb.120.4.923 (1993).
- 450 32 Satcher, R. L., Jr. & Dewey, C. F., Jr. Theoretical estimates of mechanical properties of
451 the endothelial cell cytoskeleton. *Biophysical journal* **71**, 109-118, doi:10.1016/S0006-
452 3495(96)79206-8 (1996).
- 453 33 Fischer-Friedrich, E., Hyman, A. A., Julicher, F., Muller, D. J. & Helenius, J. Quantification
454 of surface tension and internal pressure generated by single mitotic cells. *Scientific reports*
455 **4**, doi:Artn 621310.1038/Srep06213 (2014).
- 456 34 Paul, R., Heil, P., Spatz, J. P. & Schwarz, U. S. Propagation of mechanical stress through
457 the actin cytoskeleton toward focal adhesions: Model and experiment. *Biophysical journal*

- 458 **94**, 1470-1482, doi:10.1529/biophysj.107.108688 (2008).
- 459 35 Manificier, I. *et al.* The consequence of substrates of large-scale rigidity on actin network
460 tension in adherent cells. *Comput Method Biomec* **22**, 1073-1082,
461 doi:10.1080/10255842.2019.1629428 (2019).
- 462 36 Tsekouras, K., Lacoste, D., Mallick, K. & Joanny, J. F. Condensation of actin filaments
463 pushing against a barrier. *New journal of physics* **13**, doi:Artn 10303210.1088/1367-
464 2630/13/10/103032 (2011).
- 465 37 Van Goethem, E., Poincloux, R., Gauffre, F., Maridonneau-Parini, I. & Le Cabec, V. Matrix
466 Architecture Dictates Three-Dimensional Migration Modes of Human Macrophages:
467 Differential Involvement of Proteases and Podosome-Like Structures. *Journal of*
468 *immunology* **184**, 1049-1061, doi:10.4049/jimmunol.0902223 (2010).
- 469 38 Rigort, A. *et al.* Micromachining tools and correlative approaches for cellular cryo-electron
470 tomography. *J. Struct. Biol.* **172**, 169-179, doi:10.1016/j.jsb.2010.02.011 (2010).
- 471 39 Rigort, A. *et al.* Focused ion beam micromachining of eukaryotic cells for cryoelectron
472 tomography. *Proceedings of the National Academy of Sciences of the United States of*
473 *America* **109**, 4449-4454, doi:10.1073/pnas.1201333109 (2012).
- 474 40 Fukuda, Y., Laugks, U., Lucic, V., Baumeister, W. & Danev, R. Electron cryotomography
475 of vitrified cells with a Volta phase plate. *Journal of structural biology* **190**, 143-154,
476 doi:10.1016/j.jsb.2015.03.004 (2015).
- 477 41 Li, X. *et al.* Electron counting and beam-induced motion correction enable near-atomic-
478 resolution single-particle cryo-EM. *Nature methods* **10**, 584-590, doi:10.1038/nmeth.2472
479 (2013).
- 480 42 Kremer, J. R., Mastrorade, D. N. & McIntosh, J. R. Computer visualization of three-
481 dimensional image data using IMOD. *Journal of structural biology* **116**, 71-76,
482 doi:10.1006/jsbi.1996.0013 (1996).
- 483 43 Stalling, D., Westerhoff, M., Hege H-C Amira: A highly interactive system for visual data
484 analysis. *Elsevier, Butterworth-Heinemann*, 749-767 (2005).
- 485 44 Rigort, A. *et al.* Automated segmentation of electron tomograms for a quantitative
486 description of actin filament networks. *J. Struct. Biol.* **177**, 135-144,
487 doi:10.1016/j.jsb.2011.08.012 (2012).
- 488 45 Koestler, S. A. *et al.* F- and G-actin concentrations in lamellipodia of moving cells. *PLoS*
489 *one* **4**, e4810, doi:10.1371/journal.pone.0004810 (2009).
- 490 46 Pollard, T. D., Blanchoin, L. & Mullins, R. D. Molecular mechanisms controlling actin
491 filament dynamics in nonmuscle cells. *Annu Rev Biophys Biomol Struct* **29**, 545-576,
492 doi:10.1146/annurev.biophys.29.1.545 (2000).
- 493 47 Le Clainche, C. & Carlier, M. F. Regulation of actin assembly associated with protrusion
494 and adhesion in cell migration. *Physiol Rev* **88**, 489-513, doi:10.1152/physrev.00021.2007
495 (2008).

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505

506 **Contributions**

507 MJ, RP designed the project. AB, RP prepared the cells. SB vitrified the cells. MJ performed cryo-
508 FIB milling, cryo-ET, tomogram reconstruction and segmentation. MJ, JH, AP, RP designed and
509 performed the experimental analysis. RV, SD designed the theoretical analysis. JH, SD performed
510 the theoretical analysis. MJ, SD, RP supervised the project. MJ, IMP, WB, SD, RP obtained
511 funding. MJ, JH, SD, RP wrote the manuscript with input from the others.

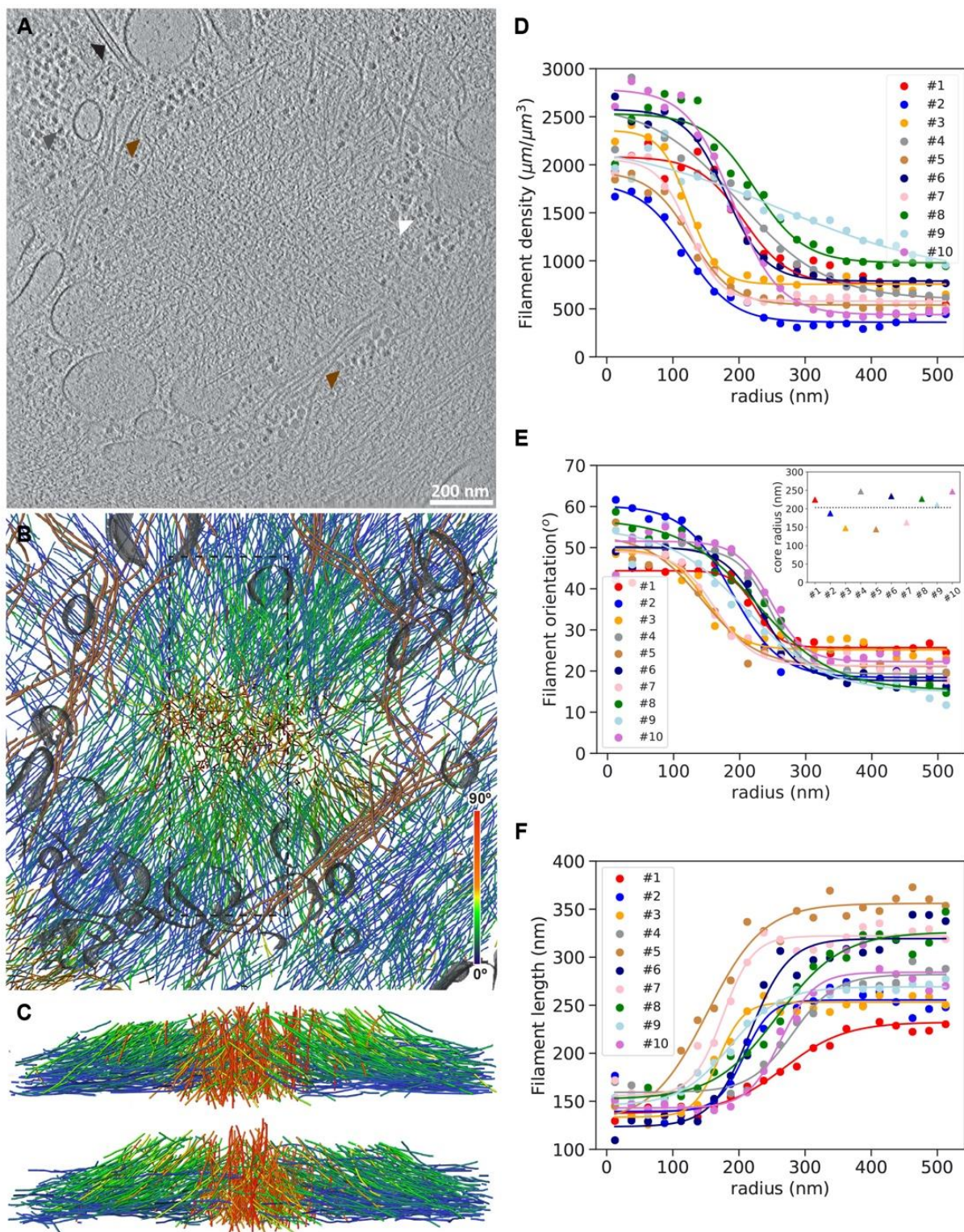
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513 **Ethics declaration**

514 The authors declare no competing interests.

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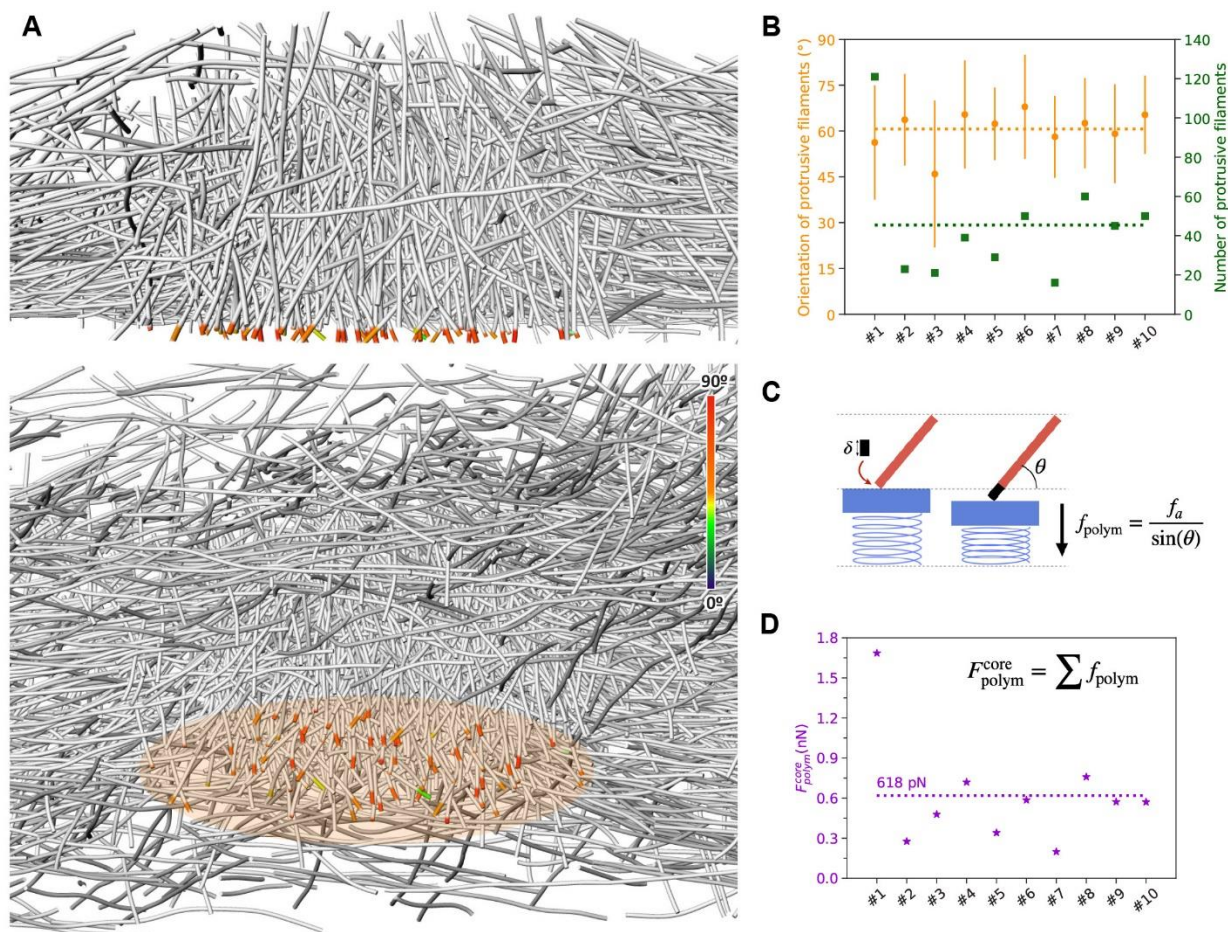


517

518 **Fig. 1: Cryo-electron tomography allows quantitative analysis of actin filament**
519 **organization in native podosomes.**

520 **A:** Slice from a tomographic volume acquired in a frozen-hydrated human macrophage revealing
521 the podosome environment. Colored arrows point to ribosomes (grey), glycogen granules (white),

522 a microtubule (black), and intermediate filaments (brown). See also **Movie S1**.
523 **B**: Orthographic view of the corresponding 3D segmentation of the actin filaments showing their
524 relative orientation with respect to the basal membrane, intermediate filaments (brown), and
525 organelle membranes (grey).
526 **C**: Perspective views of the actin filaments in the volume indicated by a dotted rectangle in (B)
527 shown from the left (top) and right (bottom) sides.
528 **D-F**: Average filament density (**D**), orientation (**E**) and length (**F**) as a function of the radial
529 distance from the core center for ten tomograms. Inset in (**E**): Corresponding values estimated
530 for the core radius (**Methods**). The podosome shown in (**A-C**) corresponds to #9.



531

532 **Fig. 2. The sum of the actin polymerization forces at the core membrane is below 1 nN.**

533 **A:** (Top) Perspective view of a cross-section through podosome #8 displaying in color the part of
 534 the core filaments pushing against the plasma membrane. (Bottom) Orthographic view of the
 535 same podosome from a different angle. The color map corresponds to the filament orientation
 536 with respect to the basal membrane. See also [Movie S3](#).

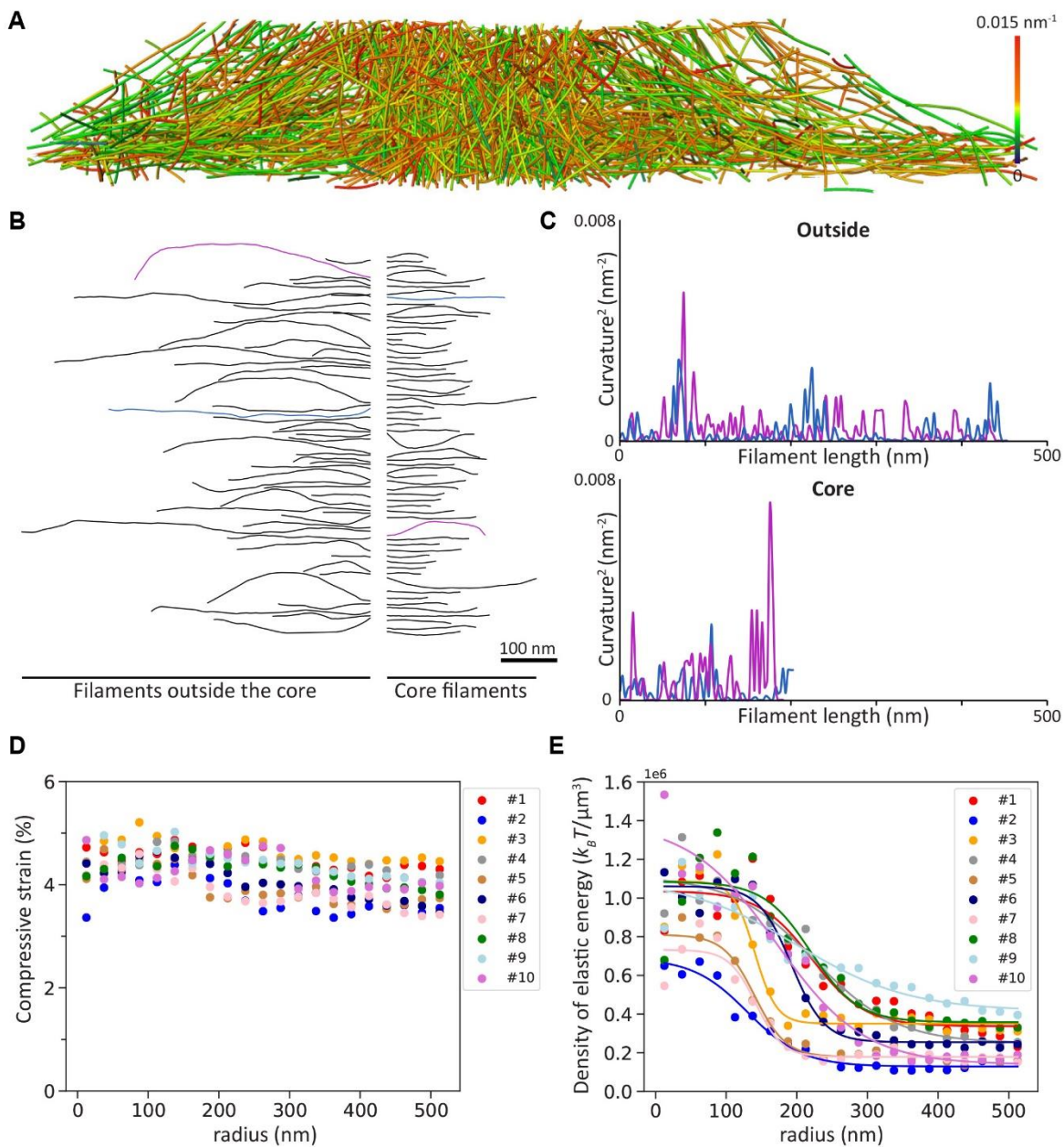
537 **B:** Mean orientation (orange) and number (green) of polymerizing filaments at the core
 538 membrane.

539 **C:** Scheme representing the polymerization force generated by the addition of a new monomer
 540 at the growing end of a filament with an inclination θ relative to the membrane.

541 **D:** Estimated polymerization force generated at the core membrane.

542

543



544

545 **Fig. 3. Podosome filaments are compressed and store high elastic energy inside the**
 546 **core.**

547 **A:** Perspective view of a cross-section through podosome #1 displaying the mean curvature of
 548 the filaments. See also [Movie S4](#).

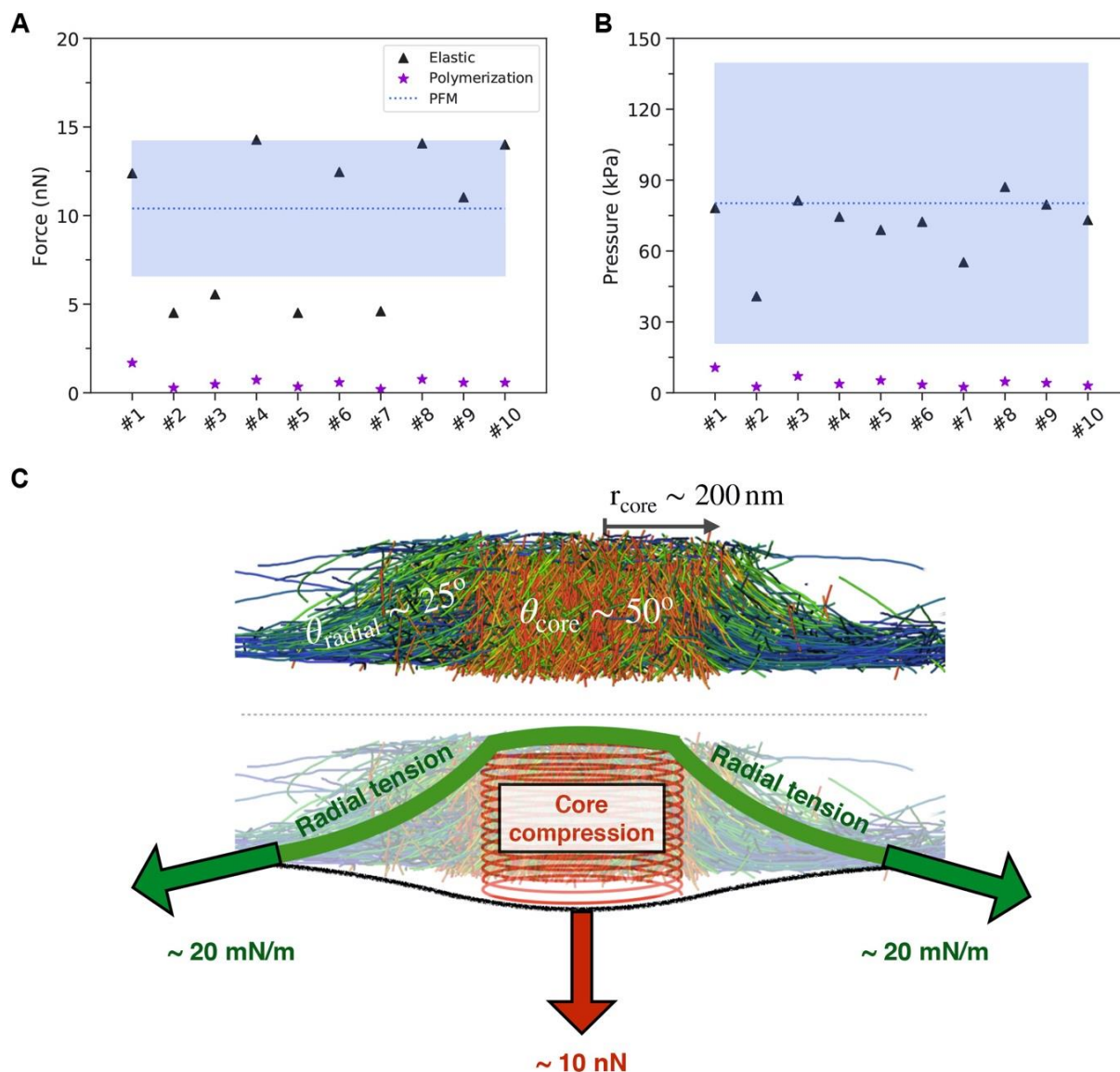
549 **B:** Gallery of a random selection of actin filaments from podosome #1. Filaments from the core
 550 (right) and outside the core (left) are shown.

551 **C:** Square of the local curvature along the filament length for the blue and purple filaments from
 552 the galleries of filaments outside (top) and inside (bottom) the core shown in (B).

553 **D:** Average compressive strain as a function of the radial distance from the core center for ten
 554 tomograms.

555 **E:** Density of elastic energy as a function of the radial distance from the core center for ten
 556 tomograms.

557



558

559

Fig. 4. The actin core generates elastic forces in the nanonewton range.

560 **A:** Comparison of the elastic force (“Elastic”) generated by the core through the compression of
 561 the actin network (black triangle) with the estimated polymerization force (“Polymerization”)
 562 generated at the core membrane (purple stars). The blue dashed line corresponds to the mean
 563 force derived from PFM measurements (“PFM”) ¹⁶ and the rectangular area filled in blue
 564 represents its standard deviation.

565 **B:** Same comparison plot as in (A) for the estimated pressure assuming a perfect circular shape
 566 for the podosome core (Methods).

567 **C:** Summary scheme showing the podosome organization revealed by cryo-ET, the elastic force
 568 resulting from core compression and the radial surface tension counterbalancing it.

569

570

571 **Supplemental information**

572

573 **Movie S1. Native podosome architecture revealed by *in situ* cryo-ET.**

574 This movie shows the tomogram of the podosome presented in [Figure 1A-C](#) followed by its
575 segmentation. Actin filaments are colored as a function of their orientation with respect to the
576 basal membrane. Intermediate filaments are in brown and membranes in grey. Successive
577 rotation, sectioning and zoom in the segmented volume allow the visualization of the dense
578 organization of the actin filaments of the podosome core.

579

580 **Movie S2. Architecture of unroofed podosomes by cryo-ET.**

581 This movie shows the tomogram of unroofed podosomes presented in [Figure S6](#) followed by its
582 segmentation. Actin filaments are colored as a function of their orientation with respect to the
583 basal membrane. Successive rotations, sectioning and zoom in the segmented volume reveal the
584 nanoscale actin organization of neighboring podosomes.

585

586 **Movie S3. Visualization of the actin filaments in the vicinity of the plasma membrane
587 beneath a podosome core.**

588 This movie shows the actin segmentation of the podosome presented in [Figure 2A](#). Core filament
589 segments in the vicinity of the basal membrane are colored as a function of their orientation. A
590 series of rotations, zoomed and unzoomed sections through the segmented volume allow to
591 visualize the number and orientation of the protrusive filaments at the core.

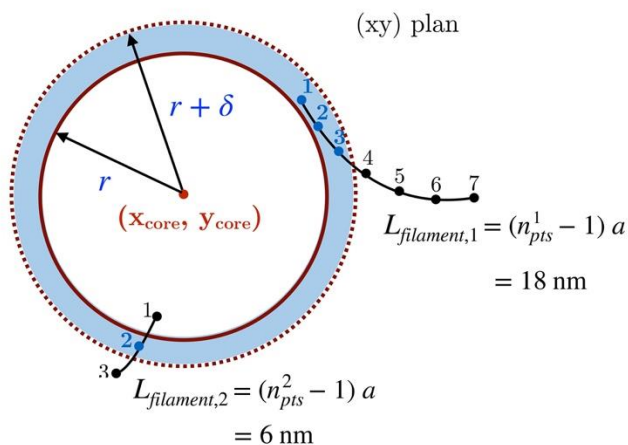
592

593 **Movie S4. Podosome actin filaments are bent.**

594 This movie shows the actin segmentation of the podosome presented in [Figure 3A](#). Actin filaments
595 are colored as a function of their mean curvature. Successive zoomed sections through the
596 segmented volume allow to visualize the curvature of the filaments in a native podosome.

Average filament length: $\langle L \rangle_{[r, r+\delta]}$

A



filament 1:

	x	y	z	r
1				
2				
3				
4				
5				
6				
7				

filament 2:

	x	y	z	r
1				
2				
3				

B

$$\langle L \rangle_{[r, r+\delta]} = \frac{1}{n_{pts,in}^{tot}} \sum_{i=1, \in [r, r+\delta]}^{n_{fil}} n_{pts,in}^i L_{filament,i}$$

$$= \frac{1}{4} \left[\underbrace{L_{filament,1} + L_{filament,1} + L_{filament,1}}_{\text{counted 3 times}} + \underbrace{L_{filament,2}}_{\text{counted 1 time}} \right] = 15 \text{ nm}$$

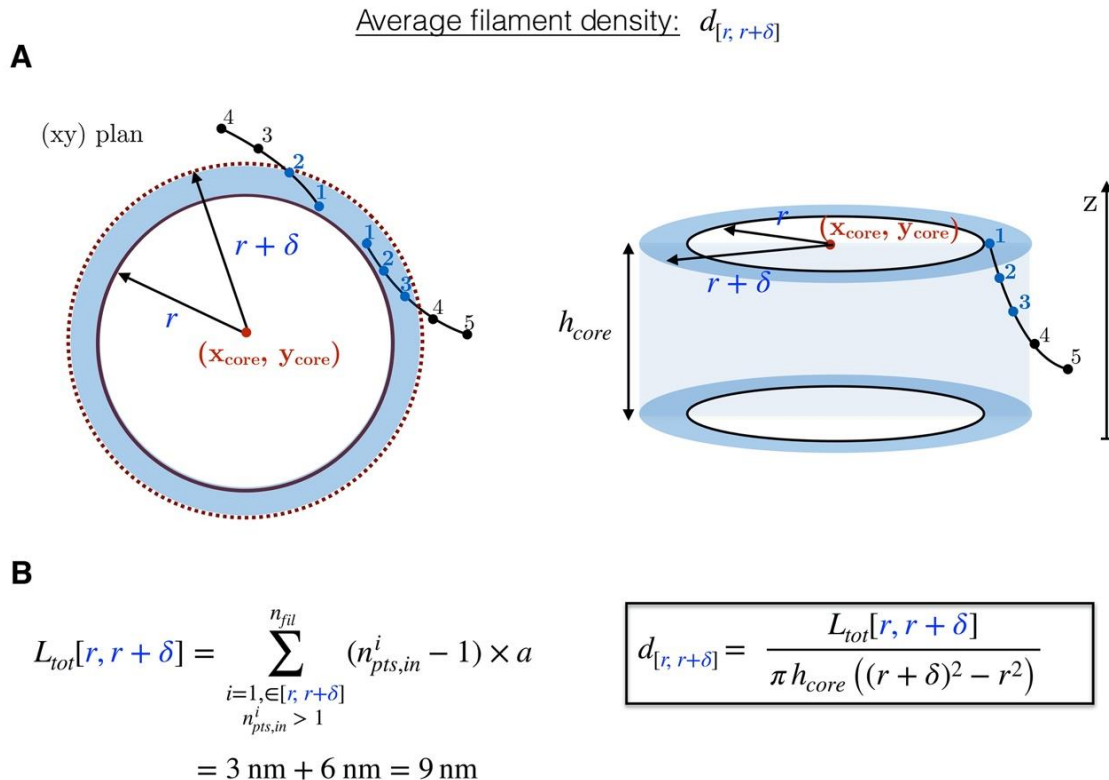
597

598 **Figure S1. Illustration of the computation of the average filament length as a function of**
 599 **the radial distance.**

600 **A:** For each distance bin $[r, r+\delta]$, the points inside the bins (shown in blue) are identified for each
 601 actin filament (left). Each point is represented both in cartesian and radial coordinates (right).

602 **B:** The average length in the bin is the mean of the filament lengths weighted by their number of
 603 points in the bin.
 604

605



606

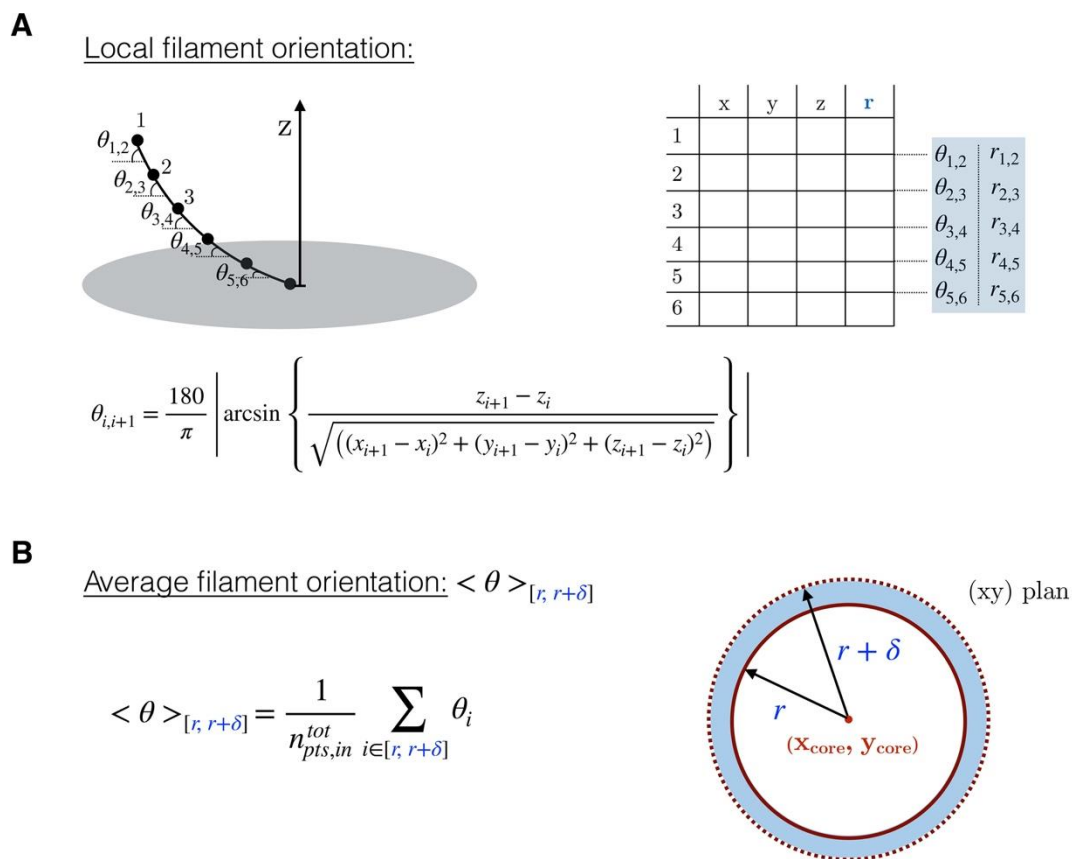
607 **Figure S2. Illustration of the computation of the average filament density as a function of**
 608 **the radial distance.**

609 **A:** For each bin $[r, r+\delta]$, the points inside the bins (shown in blue) are identified for each actin
 610 filament.

611 **B:** The total actin length is calculated in each bin and divided by the bin volume to compute the
 612 density.

613

614



615

616 **Figure S3. Illustration of the computation of the average filament orientation as a function**
 617 **of the radial distance.**

618 **A:** To each segment between two filament points is associated an angle with respect to the
 619 membrane plane.

620 **B:** The average filament orientation in a bin is the mean of the orientations of all segments inside
 621 the bin.

622

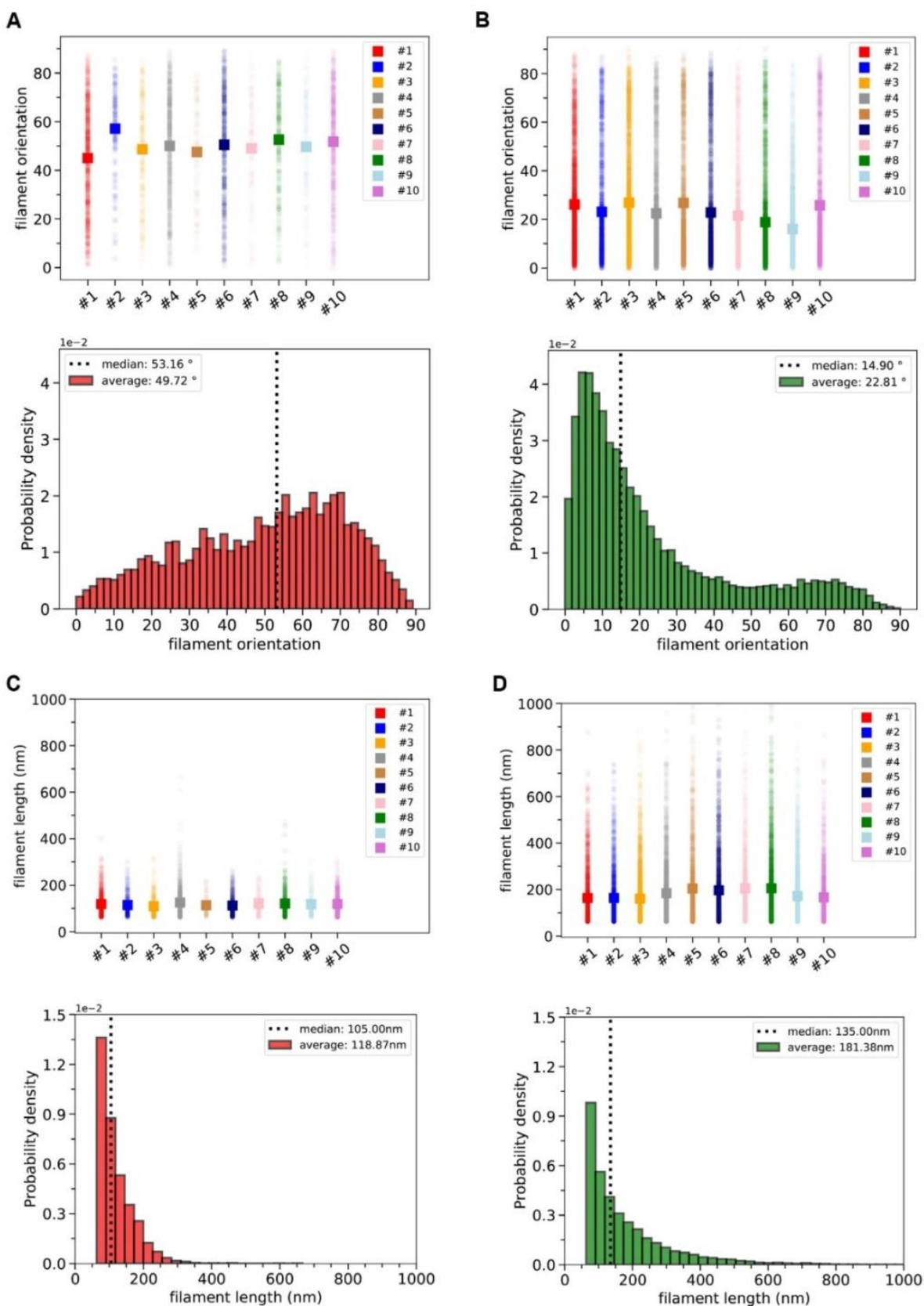
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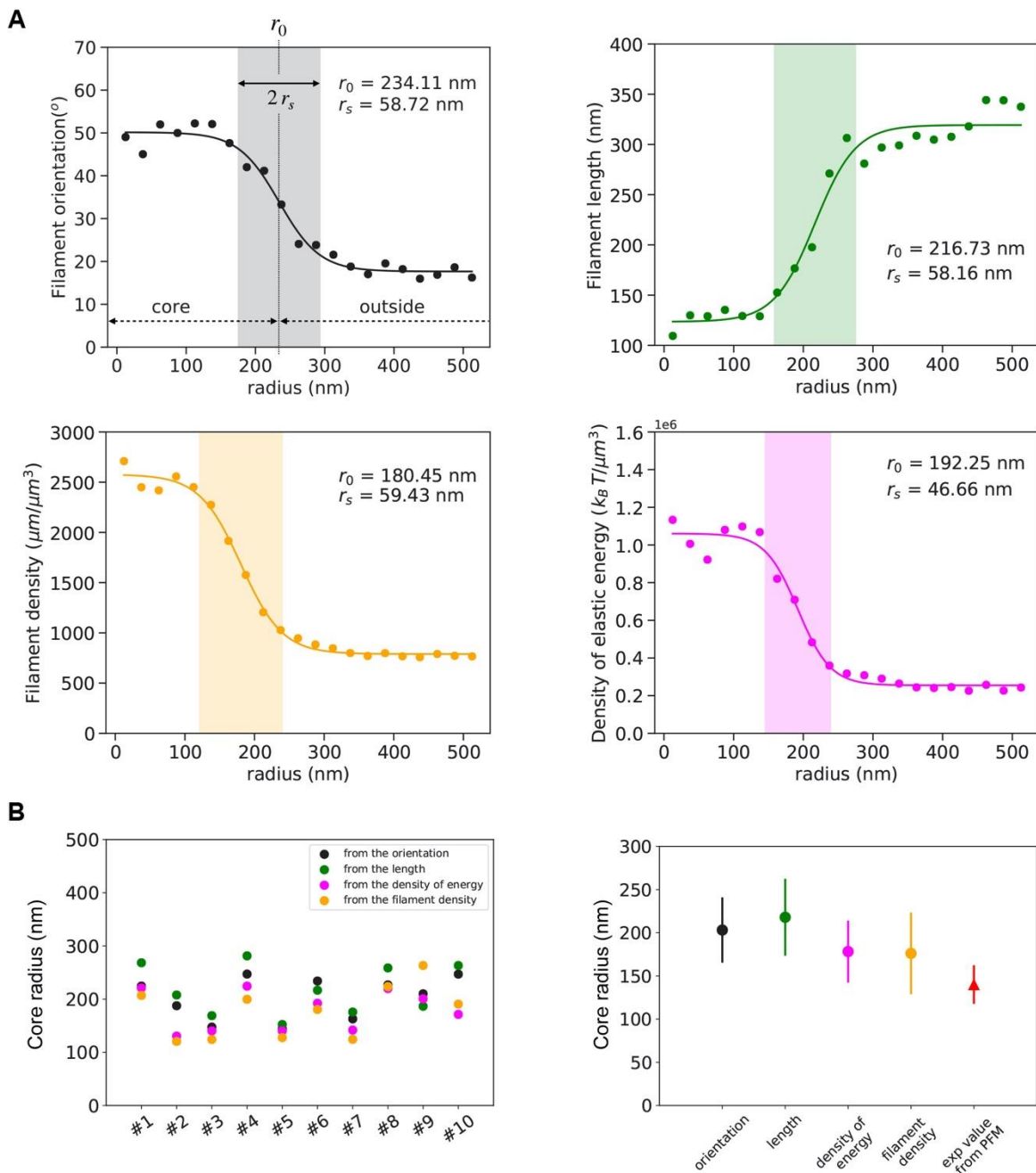
628

629 **Figure S4. Orientation and length distributions of the innermost and outermost filaments,**
630 **respectively, in ten podosomes.**

631 **A:** (Top) Mean orientation of the innermost filaments within the radial distance range $r \in [r_0 - r_s, r_0]$.
632 r_0 and r_s are the inflection point and transition range parameters, respectively, and were obtained
633 from the fit of the orientation as a function of r for each tomogram. (Bottom) Corresponding
634 distribution obtained by merging all the data from the top panel into a single dataset. The vertical
635 dashed line indicates the median value of the dataset.

636 **B:** Same distributions for the outermost filaments, *i.e.* within the radial distance range $r \in$
637 $[r_0 + r_s, r_{\max}]$ where r_{\max} is the highest radial distance between a point filament and the core center.

638 **C-D:** Same plots as in **A-B** for the filament length.



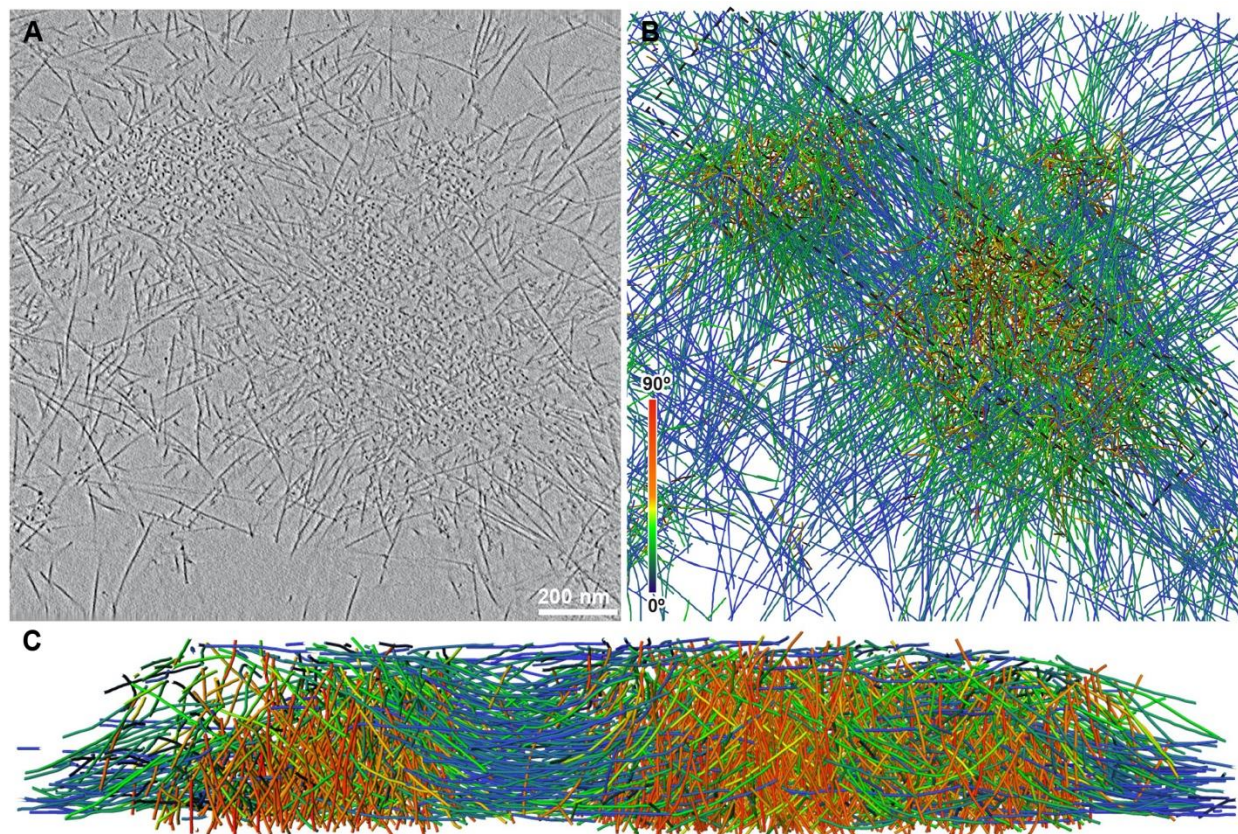
639

640 **Figure S5. Estimation of the core radius from the distribution of each parameter as a**
 641 **function of the distance to the core center.**

642 **A:** Filament orientation, length, actin density and density of energy for podosome #6 with their
 643 respective fits are shown. The parameters r_0 and r_s and the transition (colored) zone are indicated.

644 **B:** (Left) Comparison of the core radius values obtained from the fits of the different parameters
 645 as a function of the radial distance for the ten tomograms. (Right) Average radius of the podosome
 646 core estimated from the quantitative analysis of the cryo-ET data and compared to the reported
 647 value from PFM (mean \pm s.d.).

648



649
650

651 **Figure S6. Unroofed podosome architecture revealed by cryo-ET.**

652 **A:** Slice from a tomographic volume acquired in a plunge-frozen, unroofed human macrophage
653 revealing neighboring podosomes.

654 **B:** Orthographic view of the corresponding 3D segmentation of the actin filaments showing their
655 relative orientation with respect to the basal membrane.


656 **C:** Perspective view of the volume indicated by a dotted rectangle in (D) and shown from the left
657 side.

658 See also [Movie S2](#).

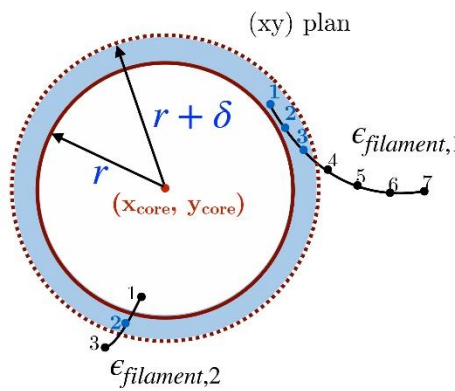
659

Compressive strain: ϵ

$$\epsilon = 1 - \frac{c}{L}$$


$\epsilon = 0$	$\epsilon \rightarrow 1$
	

Average compressive strain : $\langle \epsilon \rangle_{[r,r+\delta]}$



$$\langle \epsilon \rangle_{[r,r+\delta]} = \frac{1}{n_{pts,in}^{tot}} \sum_{i=1, \in [r,r+\delta]}^{n_{fil}} n_{pts,in}^i \epsilon_{filament,i}$$

$$= \frac{1}{4} \left[3 \times \epsilon_{filament,1} + 1 \times \epsilon_{filament,2} \right]$$

660

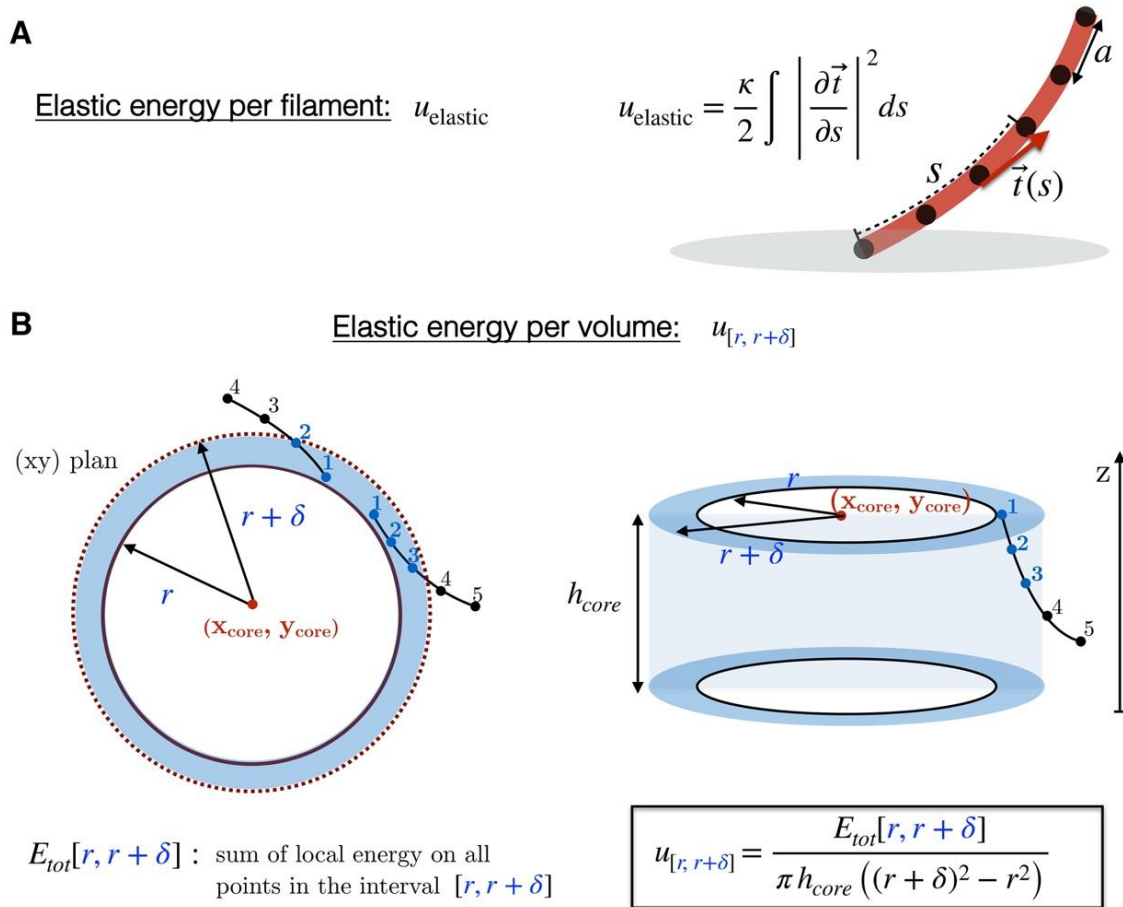
661 **Figure S7. Illustration of the computation of the average compressive strain as a function**
 662 **of the radial distance.**

663 **A:** The compressive strain is computed as 1 minus the ratio between the end-to-end distance, c ,
 664 and the filament length, L . Thus, a straight filament has a zero strain.

665 **B:** The average compressive strain in a bin is computed as the mean of the compressive strains
 666 weighted by the number of filament points in the bin.

667

668



669

670 **Figure S8. Illustration of the computation of the elastic energy per volume as a function of**
 671 **the radial distance.**

672 **A:** Definition of the elastic energy of a filament as the integral of the squared curvature over the
 673 filament length.

674 **B:** The density of elastic energy as a function of the radial distance is computed as the sum of
 675 the elastic energy of all the filament points in the bin divided by the volume.
 676
 677