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Coupling Polar Adhesion with Traction, Spring, and Torque Forces Allows High-Speed Helical Migration of the Protozoan Parasite *Toxoplasma*

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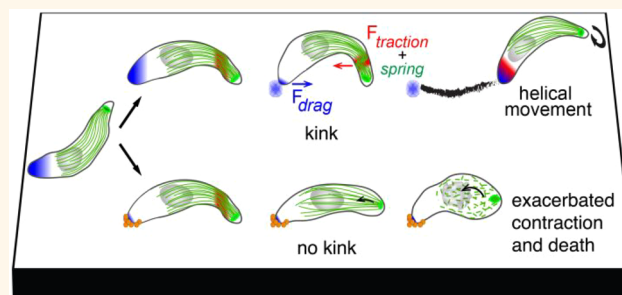
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ABSTRACT: Among the eukaryotic cells that navigate through fully developed metazoan tissues, protozoans from the Apicomplexa phylum have evolved motile developmental stages that move much faster than the fastest crawling cells owing to a peculiar substrate-dependent type of motility, known as gliding. Best-studied models are the *Plasmodium* sporozoite and the *Toxoplasma* tachyzoite polarized cells for which motility is vital to achieve their developmental programs in the metazoan hosts. The gliding machinery is shared between the two parasites and is largely characterized. Localized beneath the cell surface, it includes actin filaments, unconventional myosin motors housed within a multimember glideosome unit, and apically secreted transmembrane adhesins. In contrast, less is known about the force mechanisms powering cell movement. Pioneered biophysical studies on the sporozoite and phenotypic analysis of tachyzoite actin-related mutants have added complexity to the general view that force production for parasite forward movement directly results from the myosin-driven rearward motion of the actin-coupled adhesion sites. Here, we have interrogated how forces and substrate adhesion–de-adhesion cycles operate and coordinate to allow the typical left-handed helical gliding mode of the tachyzoite. By combining quantitative traction force and reflection interference microscopy with micropatterning and expansion microscopy, we unveil at the millisecond and nanometer scales the integration of a critical apical anchoring adhesion with specific traction and spring-like forces. We propose that the acto-myosin motor directs the traction force which allows transient energy storage by the microtubule cytoskeleton and therefore sets the thrust force required for *T. gondii* tachyzoite vital helical gliding capacity.

KEYWORDS: cell migration, cell focal contact, 3D collagen, *Toxoplasma*, traction force microscopy, reflection interference contrast microscopy, expansion microscopy



Across eukaryotic phyla, cell migration is an evolutionary conserved function which appears vital for both free living protozoans and metazoans.¹ In metazoans, cells such as fibroblasts and leukocytes use a wide spectrum of adhesion-dependent motility modes conjointly defined as crawling and primarily driven by the dynamics of the cell actin–myosin cytoskeleton.^{2,3} These cells crawl with the help of dynamic actin-powered membrane protrusions that fold at the leading edge as a flat lamellipodium or as a variety of pseudopodia-like membrane projections. Firmly attached to the substrate upon coordinated assembly of integrin-driven adhesive platforms, the lamellipodium is a hallmark of the mesenchymal cell migration mode in 2D and 3D micro-

environments. These anchoring multimolecular platforms allow the development of actomyosin bundles that tune contractile forces between the front and trailing margins and eventually lead to the release of trailing adhesions, hence allowing cell forward translocation.^{4,5} Likewise, metazoan cells—as well as large free-living protozoans—can display a

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49 more versatile motility mode and cope with heterogeneous 3D
50 microenvironments. In addition to the lamellipodium style,
51 they can adopt diversified pseudopodia mechanisms that,
52 combined with a range of relative cell-matrix adhesion and
53 actomyosin contractility, account for the rapid amoeboid type
54 of motility (~ 10 to $20 \mu\text{m}/\text{min}$ versus less than $1 \mu\text{m}/\text{min}$ for
55 the lamellipodia mode).⁶ Alternatively, fibroblasts can utilize
56 an amoeboid–mesenchymal hybrid type of 3D migration
57 called lobopodial during which a pressure-driven bleb-like
58 membrane protrusion stems at the leading margin from the
59 piston-like forward movement of the nucleus itself driven by
60 the cortical actomyosin contractility.⁷ Biophysical studies have
61 proved instrumental in mapping and measuring the develop-
62 ment of forces in cells, and they brought insights on how cells
63 differentially regulate the adhesion–contractile balance to
64 spatiotemporally control the adhesion assembly deassembly
65 cycle and achieve productive forward movement in different
66 contexts.⁸

67 Intriguingly, unicellular eukaryotic parasites from the ancient
68 phylum of Apicomplexa have evolved an adhesion- and actin-
69 dependent motility mode that differs from the mesenchymal,
70 amoeboid, lobopodial repertoire and enables the microbes to
71 glide within host tissues and across barriers to follow their
72 complex stepwise developmental program. The several
73 thousand members of the Apicomplexa phylum include
74 among the world's most pre-eminent mammal-invasive
75 protozoan parasites. In humans, *Plasmodium* spp. are
76 inoculated in the vascularized dermis extracellular matrix
77 (ECM) by a blood-feeding mosquito which hosts parasites in
78 salivary glands. *Plasmodium* displays a prolonged asexual
79 developmental phase in red blood cells, which results in a
80 range of clinical outcomes known as malaria, impacting human
81 populations in tropical and subtropical regions of the Earth. Its
82 relative *Toxoplasma gondii* is present in a large assortment of
83 endotherm metazoans worldwide. Being ingested with
84 contaminated food and water, *T. gondii* is hosted quasi-silently
85 by possibly up to a third of the human population in tissues
86 such as the brain, retina, and heart and skeletal muscles.
87 However, in case of immune dysfunction, *T. gondii* can re-
88 emerge from these tissues and proliferate, causing life-
89 threatening or debilitating complications.⁹

90 Throughout their life cycle in their respective hosts, both
91 *Plasmodium* and *T. gondii* ensure their perpetuation by
92 undergoing multiple cell differentiation events that give rise
93 to specific developmental stages with sizes of several microns,
94 harboring a crescent shape and endowed with high motility
95 skills.¹⁰ Best studied are the *Plasmodium* slender mature
96 sporozoite inoculated in the host skin and the *T. gondii* bulky
97 highly replicative tachyzoite stages that glide 10 times faster
98 than the amoeboid cells without folding any of the crawling-
99 associated protrusions.^{10–12} Instead, they share a robust
100 longitudinal apico-basal polarity which persists during the
101 generally unidirectional motile activity with the apex leading.¹³
102 Whereas the *Plasmodium* sporozoite moves on a circular path
103 in either counterclockwise (CCW) or clockwise (CW)
104 directions with an irregular stop and go kinetics,¹³ it can also
105 occasionally adopt a random motion or a corkscrew-like
106 pattern *in vivo*.^{14,15} Instead a left-handed helix characterizes the
107 path the mosquito-restricted ookinete *Plasmodium* stage used
108 to move in Matrigel-based 3D matrices.¹⁶ The *Toxoplasma*
109 tachyzoite can similarly glide CCW on a circular path under
110 2D *in vitro* settings but often uses a CCW helical trajectory or a
111 succession of the two mechanisms over the same gliding

sequence.^{11,17} Another intermittent 2D movement termed 112
twirling accounts for the stationary CW rotation executed by 113
the upright-positioned tachyzoite around its posterior end.¹⁸ 114
The corkscrew-like mode of gliding, which is the obligate 115
mechanism in 3D reconstituted or *in vivo* environments was 116
proposed to relate to the 2D helical mode of gliding.¹⁹ The 117
latter typically starts with a CW short motion, follows with a 118
 180° twist; meanwhile, the body flattens on the substrate 119
before twisting up and flipping on the side, which allows 120
starting another helical cycle.^{11,18} 121

To initiate and sustain all types of gliding activity, both the 122
sporozoite and tachyzoite rely on the regulated secretion of 123
adhesive protein oligomers at the cell surface^{20,21} that, once 124
delivered at the apical tip, can coengage with ECM proteins 125
and short cortical actin filaments organized beneath the plasma 126
membrane. Force production driving parasite movement has 127
long been considered to directly translate from the apico-basal 128
translocation of the ECM-bound adhesins coupled to the 129
flowing actin filaments and their enzymatic-mediated release of 130
these adhesins at the trailing edge.^{22,23} It is the Apicomplexa 131
specific, fast, nonprocessive, single-headed motor myosin A 132
(MyoA) that was assigned an essential powering role in pulling 133
backward the apically initiated actin filaments and the captured 134
adhesins.^{24,25} Upstream from MyoA however, the *T. gondii* 135
tachyzoite additionally requires myosin H (MyoH) to propel 136
actin filaments from the parasite apical tip formed by the 137
conoid,²⁶ a retractile appendage missing in *Plasmodium*. Both 138
MyoA and MyoH molecules function within a multisubunit 139
complex identified as glideosome²² that also comprises several 140
regulatory subunits and scaffolding partners.^{27–29} Specifically 141
for MyoA, the glideosome associated proteins (GAPs) anchor 142
the motor machinery to the plasma membrane and the lining 143
internal bilayered membrane complex (IMC) which underside 144
is tethered to a cytoplasmic array of cortical microtubules 145
(cMTs), the number and arrangement of which typify each 146
Apicomplexa species and developmental stage.^{27,30} 147

However, the recent introduction of force microscopy, 148
reflection interference contrast microscopy (RICM) and 149
optical tweezers in conjunction with molecular genetics in 150
the *Plasmodium* field has added more complexity to the 151
prevalent force production model.³¹ A key observation is the 152
discrepancy between the speed of the retrograde flow of 153
optically trapped microbeads on the apical surface of the 154
sporozoite and the speed achieved during typical circular 155
gliding, the former being up to 50% faster than the latter.³¹ In 156
addition, several distinct areas of contact between the moving 157
sporozoite and the substrate were uncovered at the apex, base, 158
and center over the circular path and were shown to display 159
specific on–off dynamics,¹³ whereas the genetic loss of a 160
surface-exposed adhesin reported to function during motility 161
was found to trigger a speed increase in the retrograde flow and 162
a concomitant decrease in force production.³¹ Collectively, 163
these studies bring compelling evidence for the assembly of 164
distinct force-generating adhesive structures which differ- 165
entially control production of the force required for migration. 166

By contrast, how forces and dynamic adhesions coordinate 167
in space and time and integrate with the impressive high-speed 168
helical path that features the *T. gondii* tachyzoite gliding 169
mechanism has been much less investigated. Intriguingly, the 170
rearward capping of nanobeads bound to the tachyzoite surface 171
was shown not to require an intact actomyosin system but 172
rather a polarized secretory–endocytic cycle that would locally 173
drive membrane tension.³² On the other hand, qualitative 174

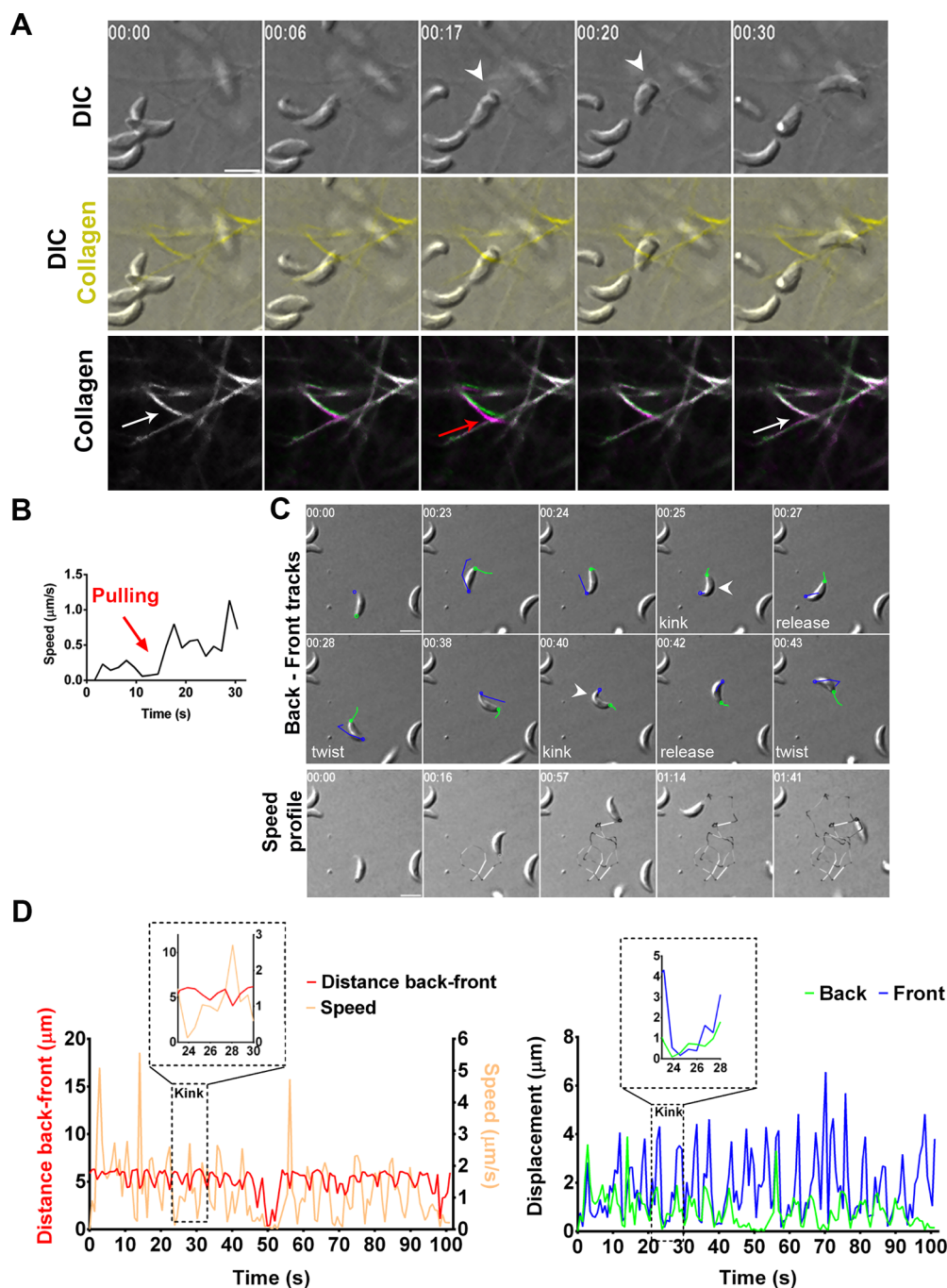


Figure 1. Tachyzoite pulls on the collagen fibers prior to helical acceleration. (A) Successive images from a time lapse of a tachyzoite navigating within a 3D-meshwork of rat collagen1 fibers pre-labeled with the CNA35-GFP collagen chimeric probe. Panels show images of (top) DIC, (middle) DIC-GFP over, giving yellow-colored fibers, (bottom) the fiber initial (t_0 , green) and over time (t_x , magenta) positions when encountered by the moving tachyzoite. The overimposition of the green and magenta signals comes as white (white arrows) when the fiber maintains the t_0 position, whereas the separation between the green (t_0) and magenta (t_x) signals attests two distinct positions overtime. The backward displacement indicates the parasite pulls on the fiber (red arrow), whereas the graph (B) documents that this pulling coincides with the parasite low speed. Next the acceleration step coincides with the merge back of the green and magenta signals that signs for the release of fiber tension post motion. (C) Successive DIC images from a time lapse of a tachyzoite undergoing helical gliding on fibronectin-coated glass. (Top) back (green) and front (blue) tracks over three last frames (t_{x-2} to t_x) are shown, (bottom) speed profile is shown from black to white scaling with increasing speeds. All white arrowheads point to the kink, all scale bars: $5 \mu\text{m}$, time is in minutes/seconds. (D) Graph (left) plots the tachyzoite back–front distance (red line) and the speed based on the back tracking (light orange); note that the value close to zero corresponds to the time the tachyzoite twirls, hence the apex is out of focus and the back-front distance not measurable (right) the xy displacement for both the back (green) and front (blue) sites over the helical sequence. Zoomed of a typical kink step for both graphs shown as inlays.

175 RICM allowed identifying an early tachyzoite contact with the
176 substrate at the cell front and its rearward translocation which

was assumed to directly power the force-producing parasite 177
movement.³³ In this study, we revisited the mechanisms 178

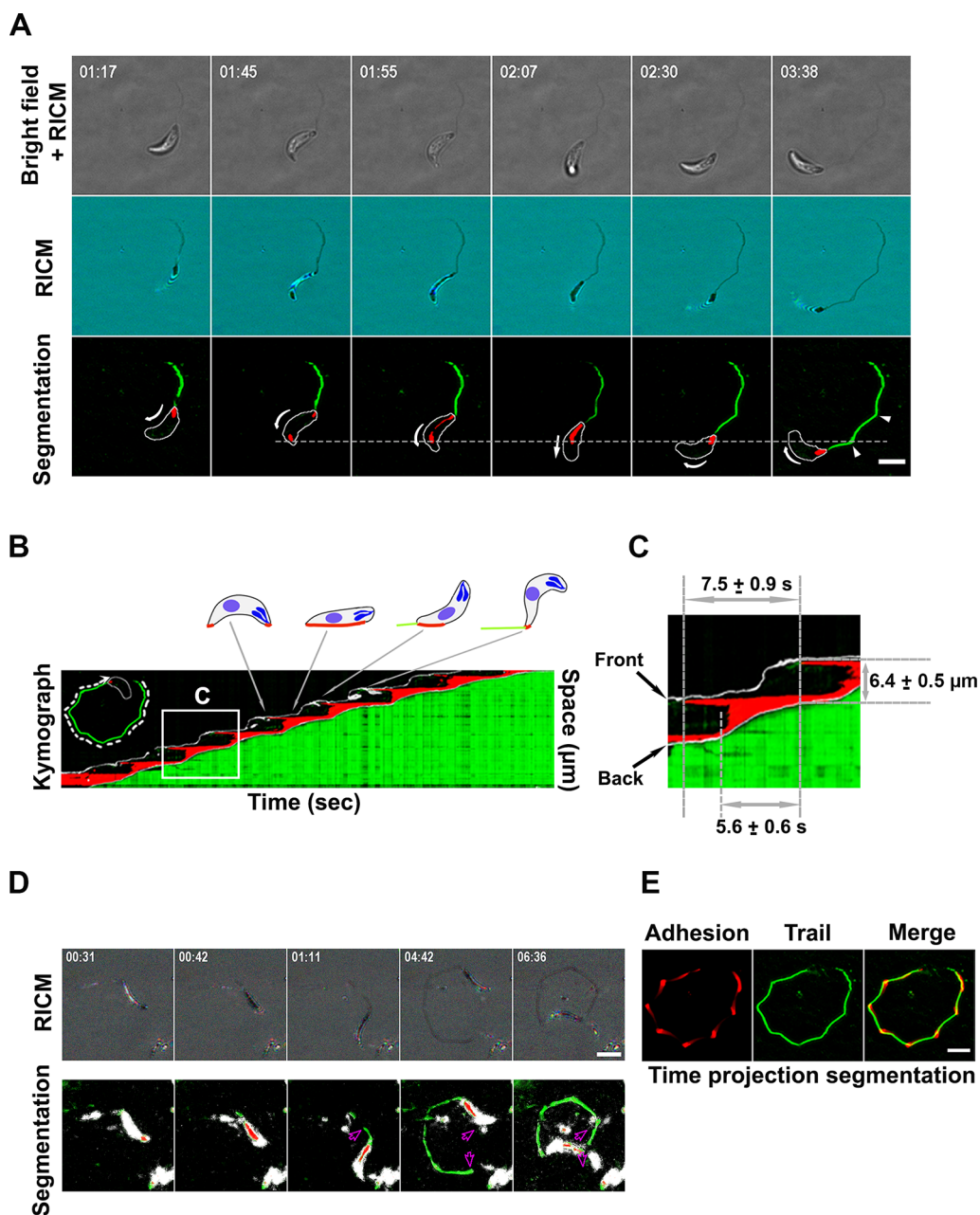


Figure 2. Helical cycle starts with an apical site of adhesion that constraints sliding of both the parasite and the parasite posteriorly shed trail. (A) Successive RICM images from a time lapse of a tachyzoite undergoing helical motion on a glass coverslip. Panels show (top) simultaneous bright-field (BF) and RICM images, (middle) blue and green RICM images superimposed and displayed as RGB, (bottom) segmentation of BF and RICM images showing the parasite outline (white), areas of close contact between the glass and the moving parasite (red), and material shed behind as trail (green). White arrows indicate the direction of the parasite movement. The dotted line marks the position of the initial contact over one helical cycle and delineates the trajectory angular point. (B,C) Kymograph of the whole gliding sequence from (A) and for a single zoomed helical cycle (C) shows the evolution of the close contact area along the trajectory over time. (D) Successive three-color RICM images (shown as RGB) from a time lapse of a tachyzoite undergoing helical motion on a poly-L-lysine-coated glass coverslip (top) and corresponding segmentation with the Weka Fiji plugin (bottom). The pink arrows indicate that over time the tachyzoite pulls on the trail that slides without breaking but follows the polygonal trajectory imposed by the initial anchoring sites. (E) Time projection of the segmented close contact areas over the gliding sequence on which (left) the longest-lived attachment points are brightest, (middle) the trails are green, and (right) adhesion and trails are overimposed. Note that angles in the polygonal path coincide with both the long-lived close contacts and the kinks. All scale bars: 5 μm ; time is in minutes/seconds.

179 underpinning the helical gliding of *T. gondii* by combining
 180 quantitative live imaging with traction force microscopy,
 181 quantitative RICM, micropatterning and expansion micros-
 182 copy. We provided evidence for a spatiotemporal coordination
 183 of an actoMyoA-based traction force produced at a stable
 184 apical adhesion site and a spring-like force which, driven by the

left-handed cMT spiral orientation, directs straightening and
 helical propelling of the *T. gondii* tachyzoite.

RESULTS/DISCUSSION

Tachyzoite Pulls on the Collagen Fibers in a 3D
 Meshwork Prior to Accelerating with a Helical Motion.

Pioneering work on *T. gondii* 3D *in vitro* motility has relied on Matrigel-based matrices under rather elastic and homogeneous conditions.¹⁹ However, *in vivo*, most ECM microenvironments are enriched in a nonlinear, anisotropic, and dynamic fibril-rich meshwork. Because collagens are dominant ECM components that form multiscale fibrils withstanding tensile forces,³⁴ we analyzed how the *T. gondii* tachyzoite moves within a confined collagen fibril-based matrix. Whereas the tachyzoite squeezed its body to pass through the meshwork, it first paused and bent, showing an apical deviation as it next accelerated, possibly with a rotation around its main axis, to escape from the collagen fibrils (Figure 1A,B and Supporting Information Figure S1, Movie 1, and Movie 2). Using a fluorescent probe selectively associated with collagen fibrils,³⁵ we applied a temporal color-code (green and magenta) and identified fibers encountered by the motile tachyzoite that shifted backward before returning to initial position as the parasite propelled forward (Figure 1A, Supporting Information Movie 1). This fiber backward displacement was seen in all cases of moving tachyzoites encountering a fiber ($n = 10$), and qualitatively indicated that the parasite exerted pulling forces on the surrounding fibers.

To get more mechanistic insights on the front-rear spatiotemporal coordination over the helical gliding cycle, we further analyzed the changes in the tachyzoite shape with high spatiotemporal acuity using a 2D setting. We observed a yet undefined apical inward bending immediately post the initial clockwise arc motion and concomitant with the parasite pause (Figure 1C). Indeed, tracking the front and back revealed a shortening of the parasite that coincided with an apical flexure and thus an increased curvature, resembling the observation made within the fibrous 3D matrix, and which we defined as the kink step. Associating with the apical pole arrest we detected the contraction of the cell body and retraction of the posterior pole. Next the apex re-extended and lifted up, a phase defined as the kink release, while the tachyzoite straightened and slid to further twisted in the typical CCW motion (Figure 1C, Supporting Information Movie 3a and Movie 3b, slow motion). Accordingly, not only the instantaneous speed was about null during the kink step and reached maximal value as the latter relaxed back (Figure 1D), resulting in the characteristic periodic speed fluctuations documented under 3D conditions.¹⁹ No such pattern was detected when the parasite performed the 2D-restricted CCW circular trajectory (Supporting Information Figure S1B,C and Movie 4). Of note, high temporal resolution live imaging of *Plasmodium* sporozoites also reveals alternating periods of rapid movement in which they travel up to one body length and periods of slow movement in which they more firmly adhere to the substrate, thereby indicating that distinct adhesion types operate during motility.¹³ Although such kinetics was associated with the sporozoite body stretching upon firm adhesion of its polar and center regions to the substrate and the subsequent thrust in speed,¹³ no peculiar apical deformation that could resemble a kink has been observed to initiate movement. We proposed that the kink deformation and the subsequent body extension might reflect the tension built in the tachyzoite cytoskeleton to achieve the thrust force required for the helical twisting motion.

Apical Focal Adhesion Behaves as a Firm Long-Lived Anchor That Constrains Sliding of the Parasite Body.

Because the apical kink suggests the assembly of a specific contact between the parasite front and the substrate, which

would be appropriate for building up tension in the *T. gondii* cytoskeleton, we first used qRICM to monitor the parasite-substrate distance. In contrast to a recent study,³³ we used high-speed (30 frames/s), multicolor RICM with simultaneous transmitted-light imaging coupled with machine-learning-based image processing to achieve a precise segmentation of close contact regions between the parasite and the substrate at high temporal resolution (33 ms) over thousands of frames (Figure 2 and Supporting Information Movie 5 and Movie 6). In addition to the low-distance patches detected between the parasite basal pole and the substrate even in absence of movement, we found that at the onset of a helical cycle, the membrane of the tachyzoite apex came in contact with the glass surface. Because these RICM-detected signals coexisted with motion, we assumed they accounted for two polar parasite-substrate adhesive areas in agreement with Tosetti and collaborators³³ and similarly to what was found for the *Plasmodium* sporozoite.¹³ Crucially, the apical contact remained static over the full length of the helical cycle (7.5 ± 0.9 s), and the posterior contact progressively expanded to account for $\sim 80\%$ of the tachyzoite length along its major axis (Figure 2A–C, see kymographs, Supporting Information Movie 5). Upon release of the kink, the parasite propelled forward and shifted orientation before it started a new front attachment. For each helical cycle, the kink step could be *a posteriori* inferred through the mix of parasite-derived membrane hydrophobic droplets and surface-exposed proteins/glycoproteins left as a complex trail behind the cell edge onto the substrate, a common feature of Apicomplexa motile stages (Figure 2A,D,E).^{11,36} Quantitative modeling of the RICM signal provides transverse dimensions for these trails of ~ 70 nm (isotropic) or 100 nm (width) and 40–60 nm (height). By comonitoring the parasite helical motion and the trail assembly in real time, we were able to uncover that the tachyzoite not only slid on the adhesive tracks it built itself but could also pull these, an observation that has not been reported for the RICM data collected on *Plasmodium*.¹³ These observations suggest a continuous nature of the shed material that remained close but not firmly fixed on the substrate (Figure 2D and Supporting Information Movie 6), which would agree with membrane components including lipids as previously deduced from the analysis of fixed samples.¹¹ Importantly, the sliding trajectories of the shed material once pulled by the moving tachyzoite appeared constraint by the periodically distributed firm anchors, which could occasionally be stripped off from the substrate (Supporting Information Movie 6). In further support of these periodic stable anchors, the time projection of the trail trajectory postimage segmentation allowed positioning the longest-lived parasite-substrate contacts at each angular point of the trajectory, matching the initial apical anchoring sites (Figure 2E). The relative homogeneity in the trail observed at our RICM resolution cannot inform on a specific distribution or arrangement in proteins and lipids upon specific secretory events or/and due to local physical constraints that would compose the periodic anchors. The microneme-stored MIC2 is seen as the adhesion prototype that bridges the parasite motor system and ECM ligands during motility. Indeed partial or full silencing of the *mic2* expression significantly impairs both the helical and twirling motions.^{37,38} This double default argues more for a MIC2 contribution to the posterior adhesion force,³⁷ hence *mic2* KO parasites show a reduced capacity to bind to host cells and collagen-coated surfaces.³⁸ Consistently, 315

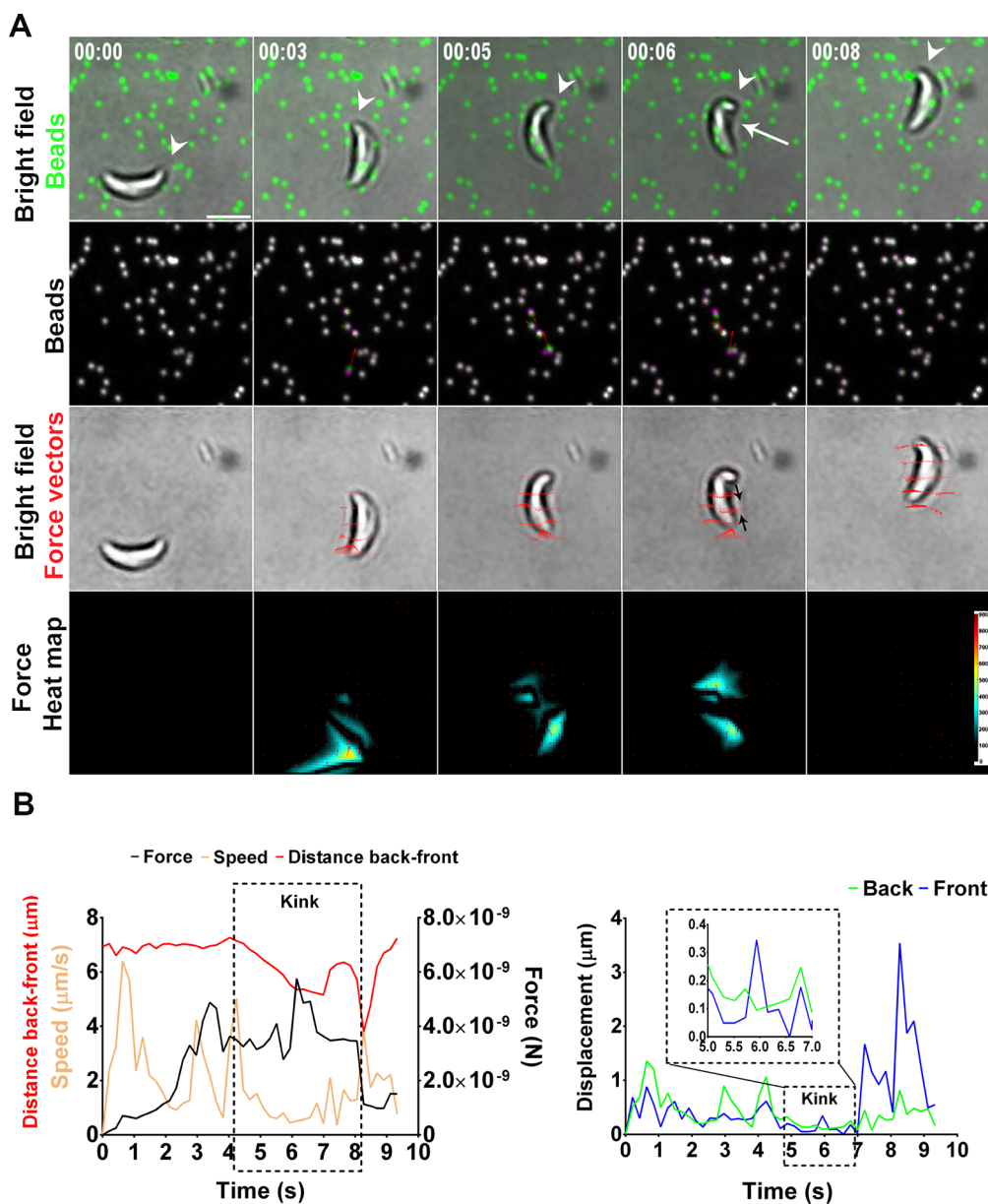


Figure 3. Helical motion includes the development of apical traction at the kink site and basal dragging forces. (A) Traction force microscopy (TFM) images were taken at the time a parasite glides on a 2 kPa PAA gel in which fluorescent TFM beads are embedded underneath the gel surface itself coated with fibronectin (20 $\mu\text{g}/\text{mL}$). Panels show (top) bright-field (BF) and TFM beads composite images; white arrowheads indicate the apex of the tachyzoite, and white arrow marks the kink, (second row) BF with force vectors computed by the MATLAB TFM code. Red arrows indicate the force direction and value; black arrows have been manually added to ease visualization of the two longitudinal forces and their orientation, (bottom) force heat map computed MATLAB TFM code. Scale bar: 5 μm , time is in minutes/seconds; au for force heat map. (B) (Left) Speed (light orange line), the back to front body distance (red line) and the traction force applied by the parasite over time (black line) are shown with a zoomed inlay (black dotted line) at the kink step time, and (right) the back and front displacement (green and blue trajectories, respectively) are shown with a zoomed inlay (black dotted line) at the kink time.

316 when the *T. gondii* tachyzoite expresses only residual levels of
 317 the rhomboid protease (ROM4) in charge of cleaving the
 318 surface-exposed MIC2, MIC2 molecules accumulate at the
 319 surface of the parasite that can no longer glide forward but
 320 instead shows enhanced twirling motility.³⁹ Similarly, when
 321 *Plasmodium berghei* sporozoites express a mutant version of the
 322 MIC2 homologue TRAP that cannot be processed by the
 323 rhomboid protease, the parasite can no longer disengage its
 324 posterior pole from the substrate and move forward.³⁶
 325 Whereas we detected both SAG1 and MIC2 proteins in
 326 patches over successive segments of the helical trails

postfixation and immunolabeling, we did not find MIC2- 327
 specific enrichment at the angular adhesion regions that were 328
 accurately identified using the RICM live sequences as 329
 recognition pattern (Supporting Information Figure S2). 330
 Therefore, it is possible that another unknown adhesin(s), 331
 which would not integrate the retrograde flow, might modulate 332
 force production at the parasite apex in line with the turnover- 333
 specific adhesion sites built by the *Plasmodium* sporozoite 334
 during circular gliding.¹³ In addition, monitoring the apico- 335
 basal flow of optically trapped beads pinned out that specific 336
 adhesions do not integrate the apico-basal capping process, yet 337

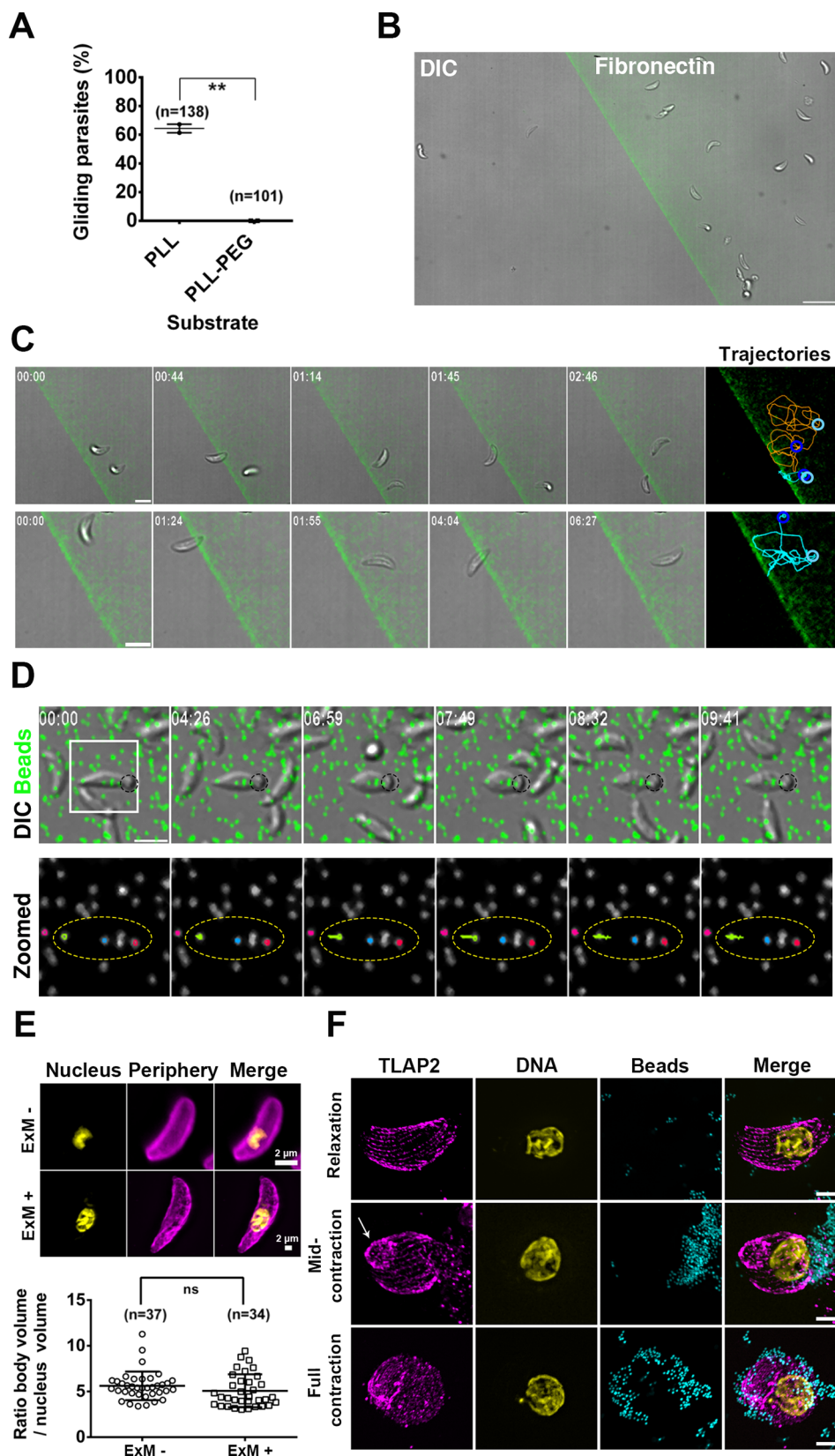


Figure 4. Front adhesion to and back disengagement from the substrate are both required for kink formation and traction force development. (A) Percentage of tachyzoites that moved by at least one body length displacement over a 20 min period post contact with poly-L-lysine (PLL) or poly(ethylene glycol) (PEG) chains grafted on PLL coating, unpaired *t* test, $**p = 0.021$. (B) Large view of the poly-L-lysine PEG–fibronectin composite micropattern shows that tachyzoites are selectively retained in the fibronectin area, which is fluorescently labeled by fibrinogen-Alexa647, scale bar: 15 μm . (C) Images from a sequence of two (top) or one (bottom) representative gliding tachyzoite(s) that fail to pass over the fibronectin–PLL–PEG demarcation line being incapable of interacting apically with PEG. Instead they elicit twirling around their posterior end bound to fibronectin and regain helical motion once apically sensing fibronectin. Blue and

Figure 4. continued

cyan circles in the trajectories window indicate the initial and the final position of the tachyzoite over the several-minute gliding sequence. Trajectories were reconstituted by tracking the tachyzoite base, which unlike the apex stays in focus even during twisting and twirling motions. (D) TFM on a tachyzoite posteriorly immobilized on fibronectin through carboxylated microbeads (black circle). (Top) Selective inward displacement of TFM beads (green) beneath the apex of the immobilized parasite occurs during sustained contraction, whereas the beads relax back to initial position upon parasite relaxation, (bottom) zoomed white square from top, showing the colored trajectories; note the large yellow trajectory that attests significant bead inward displacement, the yellow dotted ellipsoid delineates the tachyzoite. (E) Confocal images showing a representative tachyzoite immunolabeled for the GAP45 protein (pink) that delineates the parasite periphery and PI stained for DNA (yellow) prior ExM (ExM⁻) and after ExM (ExM⁺), maximal projection intensity are presented, compare the scale bars that attests the physical magnification of the ExM specimen. The graph shows the ratio of the body and nucleus volumes after 3D reconstruction prior (ExM⁻) and after ExM (ExM⁺), Kruskal–Wallis test, NS, $p = 0.17$. (F) ExM on tachyzoites posteriorly immobilized on beads (cyan) and immunolabeled for the cortical MT-associated TLAP2 protein (pink) and PI stained for DNA (yellow), (top) no contraction has yet started, (middle) contraction has started, note the apical humpy deformation in the cMT cytoskeleton indicated with a white arrow, (bottom) cMT disruption at a latest time of tachyzoite sustained contraction. Scale bars: 5 μm unless indicated, time is in minutes/seconds. The ExM allowed reaching about a 70 nm xy resolution thanks to a ~ 3 – 5 -fold increase in the biological sample size.

338 they can optimize force production. Accordingly, the trap-like
339 protein (TLP) was proposed to promote firmer adhesion to
340 the substrate by enabling specific actin remodeling underneath
341 the plasma membrane. Such function would explain why TLP
342 causes specific slowdown of the actin-dependent flow.³¹ With
343 these in mind, we next interrogated whether the firm stable
344 apical anchor formed by the moving tachyzoite and localized at
345 the kink could indeed serve as a platform for force transmission
346 by using traction force microscopy (TFM). These assays also
347 allowed testing whether the kink-induced cell shortening could
348 be associated with tension stored in the parasite that would be
349 released to promote the thrust required for productive helical
350 movement.

351 **Traction force Spatiotemporally Associates with the**
352 **Kink and Coincides with Body Dragging.** To design the
353 TFM assay, we first identified a substrate in which the stiffness
354 was sufficient to allow helical gliding while being elastic enough
355 to measure substrate deformations *via* a displacement field
356 analysis performed using fluorescent beads embedded into the
357 polyacrylamide (PAA) hydrogel. Particle image velocity (PIV)
358 combined with single-particle tracking allowed analyzing
359 substrate deformation with a spatial resolution in the range
360 of tenth of nanometers. Tracking in space and time the
361 tachyzoite posterior pole and scoring as motile those that
362 performed at least one body length displacement, we found
363 that stiffer substrates such as glass ($n = 73$) and 40 kPa PAA (n
364 = 55) correlated with optimal gliding skills, whereas lowering
365 stiffness gradually decreased the frequency and duration of the
366 motile behavior. Yet, the tachyzoites retained a short-lasting
367 ability to perform helical motion on the soft 2 kPa PAA
368 substrate ($n = 103$) (Supporting Information Figure S3).
369 Applying TFM⁴⁰ to parasites gliding on 2 kPa fibronectin–
370 PAA substrate, we obtained a traction vector map and
371 identified an early inward-oriented traction force stemming
372 at the cell front and exerted longitudinally in line with the
373 backward displacement of the in-gel beads (*i.e.*, TFM beads)
374 underneath the apical part of the parasite (Figure 3A and
375 Supporting Information Movie 7a and Movie 7b, slow
376 motion). As the force progressively increased, concomitant
377 dragging of the parasite body occurred and accounted for the
378 TFM bead's displacement underneath the posterior pole in the
379 direction of the parasite motion, in line with the previous
380 detection of trails being pulled by the tachyzoite (Supporting
381 Information Movie 6). Relaxation of the TFM beads back to
382 their initial position was recorded immediately after the kink
383 release and the parasite forward acceleration (Figure 3A,B).
384 Pioneering TFM on the *Plasmodium* sporozoite has allowed

385 uncovering two longitudinal forces (applied at each pole during
386 circular gliding, alike what we found for the *T. gondii* tachyzoite
387 undergoing helical gliding. A large force localized at the
388 posterior adhesion site peaked just prior sporozoite de-
389 adhesion, whereas a lower pulling traction force at the apex
390 pointed toward the center. In contrast to what we detected for
391 the tachyzoite, large forces were also found to point
392 orthogonally toward the sporozoite center,¹³ where they
393 promote body stretching and thus favor close contact of the
394 whole cell with the substrate—an obligate requirement for
395 circular gliding—without contributing to force production.⁴¹
396 Therefore, although both the tachyzoite and sporozoite likely
397 use a stalling force at the back, which upon rupture of the
398 adhesive contact contributes to a thrust force, the spatiotem-
399 poral distribution and intensity of the forces during their
400 specific gliding cycle (*i.e.*, helical *versus* circular) likely differ.
401 Accordingly, the overall force during the helical cycle was
402 computed to a few nanoNewtons (~ 1 to 4 nN, $n = 10$) with an
403 increment over the kink step, therefore significantly higher
404 than the 100–200 pN range inferred for cortical forces using
405 laser trap on nongliding *T. gondii* tachyzoites⁴² or gliding
406 *Plasmodium* sporozoites.³¹ By comparison, the nanoNewton
407 range characterize forces applied by both bacteria and
408 mammalian cells on their surroundings.⁴³

409 **Toxoplasma Apical Focal Adhesion Is Essential to**
410 **Switch from Nonproductive Twirling to Productive**
411 **Helical Motion.** To demonstrate the functional contribution
412 of the apical body adhesion to the subsequent helical gliding,
413 we sought interfering with the parasite adhesive step at the
414 apical traction force site. First, we screened for substrates not
415 permissive to parasite adhesion using several chemicals of
416 distinct charge and wettability properties. We found that
417 tachyzoites were unable to stabilize and glide on the densely
418 packed polymeric poly(ethylene glycol) (PEG) brushes grafted
419 on poly-L-lysine (PLL) (Figure 4A) similarly to what was
420 reported for *Plasmodium* sporozoites.⁴⁴ Therefore, composite
421 substrate was photopatterned: the parasites underwent
422 energetic gliding on the fibronectin-coated area when
423 approaching the demarcation line between the fibronectin
424 and PLL–PEG-juxtaposed areas. The tachyzoites stopped
425 when facing PEG, sometimes sliding on it prior to stepping
426 back in a reverse motion while still posteriorly bound to
427 fibronectin (Figure 4B,C and Supporting Information Movie 8
428 and Movie 9). They responded to the exclusive basal
429 attachment by spinning around the main axis in a typical
430 twirling nonproductive motion¹⁸ until the front region was
431 eventually caught again in an interaction with fibronectin,

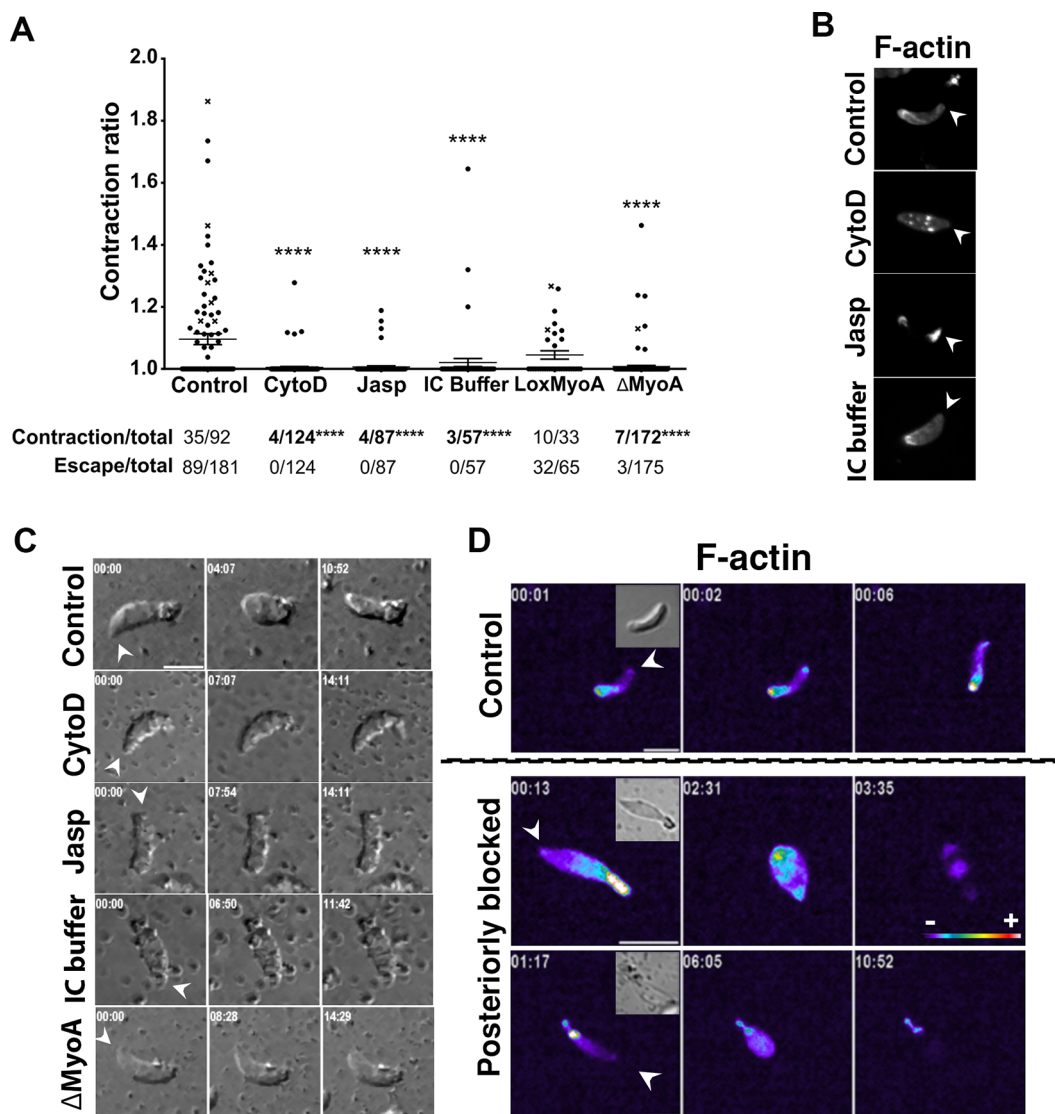


Figure 5. Prolonged and exacerbated tachyzoite contraction relies on intact actin filaments and MyoA motor. (A) Graph shows the contraction ratio after posterior immobilization on beads over a 20 min period for (i) wild-type control tachyzoites expressing the F-actin chromobody-Emerald (Acb-E) and incubated in EC buffer supplemented or not with 1 μ M cytochalasin D or 1 μ M jasplakinolide actin poisons or in IC buffer, and (ii) for tachyzoites before (LoxMyoA) and after (Δ MyoA) Cre-mediated excision of the *myoA* gene. Kruskal–Wallis test, **** $p < 0.001$ Dunn’s multiple comparisons with the control genotype. Table below the graph shows (i) proportions of number of parasites contracted over the total and (ii) proportions of number of parasites that escape over the total. For both, chi square test, **** $p < 0.001$ Fisher’s exact test with the control. (B) Confocal images of tachyzoites showing F-actin prior to record the contractile behavior. Maximal projection intensities are presented. (C) Representative DIC images of tachyzoites posteriorly immobilized to monitor both contractile behavior and bead-capping ability under each setting over a 20 min period (57 > $n > 181$ depending on the actin poison used and 65 > $n > 165$ for *myoA* KO, 3 independent assays). (D) Representative fluorescent images of F-actin dynamics in tachyzoites expressing the Ach-E undergoing helical gliding (top panel) or sustained contraction upon posterior immobilization (middle and bottom panels); DIC images are presented as inlays for each sample. Heat map intensity is presented in arbitrary units.

432 hence restoring the tachyzoite ability to perform helical
 433 trajectory. These data reinforce the view that the tachyzoite
 434 builds an apical stable adhesion site with the substrate that is
 435 compatible with the development of traction force. As we
 436 showed that both adhesion and force spatiotemporally
 437 coincide with the kink-like deformation, we expected then
 438 that the thrust productive force would be at least partly driven
 439 by the relaxation of the kink, which drives the slight lift of the
 440 parasite apex. Of note, this thrust force might also require
 441 some contribution from the parasite basal contact which
 442 loosens when traction operates allowing both dragging and
 443 retraction of the body. Given that tachyzoites must overcome

the resistance of the 3D—nonlinear—meshwork to navigate in
 physiological microenvironments, the acceleration induced
 upon the kink release should provide the right thrust to pass
 through the meshwork of fibers they pulled on. Accordingly,
 we next addressed how the contact and dragging force at the
 cell back identified by, respectively, RICM and TFM
 contribute to the helical gliding mode of the tachyzoite.

**Adhesion–De-adhesion Coupling at Both Poles
 Drives the *Toxoplasma* Apical Kink and Its Release, in
 Turn Promoting the Helical Thrust Force.** We designed
 another TFM assay in which the tachyzoite would be unable to
 disengage its posterior pole from the substrate using submicron

456 sticky beads. As already reported in the context of membrane
457 flow or cortical forces studies,^{32,42} the beads deposited on the
458 tachyzoite surface underwent apico-basal translocation. How-
459 ever, because in our setting the bead's surface engaged covalent
460 interactions not only with the parasite but also with the
461 fibronectin layer in a configuration that forced the former to
462 stay posteriorly immobilized on the latter, the cell forward
463 progression could be hindered (Figure 4D). Such posteriorly
464 constraint tachyzoites never formed the apical kink ($n = 0/92$)
465 and, instead, underwent a directional, minute time scale and
466 repetitive inward-oriented contraction visualized by the whole
467 body rounding (Figure 4D and Supporting Information Movie
468 10). The TFM captured the displacement of in-gel beads
469 suitably positioned underneath the apex of the immobilized
470 parasite consistent with a sustained apical traction force, and
471 therefore a sustained adhesion between the parasite front and
472 the substrate (Figure 4D, zoomed panel, yellow bead and
473 trajectory). The prolonged force application induced pro-
474 nounced shrinkage of the cell body along the longitudinal axis
475 and eventually led to membrane damages to the extent the
476 tachyzoite was likely wounded beyond repair. Meanwhile, we
477 observed that the in-gel beads shifted back to almost the initial
478 position, which we assumed to relate to the sudden relaxation
479 of the cell "ghost" when internal tension fell (Figure 4D and
480 Supporting Information Movie 10). Strong evidence for the
481 rupture of the plasma membrane upon excessive contraction of
482 the YFP-expressing tachyzoite was provided by the acute loss
483 of fluorescence that occurred immediately after maximal cell
484 rounding and concomitantly with the relaxation of the cell
485 (Supporting Information Figure S4). The relaxed tachyzoite
486 cell looked mis-shapen for the rest of the recording time as
487 expected for a cell ghost, therefore attesting that the
488 exacerbated and sustained contraction induced cell death. To
489 next visualize the deleterious effect of the long-lasting
490 contraction on internal cytoskeletal structures at the nanoscale
491 resolution, we applied the expansion microscopy technique
492 (ExM)⁴⁵ and first validated the isotropic expansion of the
493 specimen with the 3D co-reconstruction of the tachyzoite body
494 and nucleus prior and after expansion (Figure 4E). We then
495 analyzed a tachyzoite line engineered to express a fluorescent
496 version of TLAP2, a protein aligned with the 22 cMTs,³⁰ and
497 confirmed that during the prolonged contraction period, the
498 capped beads accumulated posteriorly while the spiral cMTs
499 deformed. We first observed an apical hump in the cMT
500 network (Figure 4F, middle panel), and eventually, the latter
501 fell apart while the capped beads were redistributed over the
502 damaged surface (Figure 4F, bottom panel). Overall, these
503 data support that the kink rose from the interplay between the
504 apical adhesion/base de-adhesion and the forces generated at
505 both poles rather than from the apical traction force only. They
506 also highlight that creating unbalance between the front and
507 back responses can result in a contractile force that imposes a
508 mechanical compression to the cMT network to which it
509 cannot resist. The need for a balanced myosin-A-based
510 contractile activity that stems at the tachyzoite apex and
511 promotes disengagement of the posterior pole can explain why
512 the genetically myosinA-deficient tachyzoites, which retain
513 only residual motility, do not any longer undergo helical
514 gliding.^{37,32}

515 **Tachyzoite Apico-Basal Contraction Requires Intact**
516 **Actin Filaments and Myosin A Motor and Correlates**
517 **Only Partially with the Requirements for Rearward**
518 **Membrane Flow.** Apart from the prolonged contraction and

fatal outcome described earlier, we also observed that the
519 tachyzoites, which were posteriorly immobilized, could either
520 rotate on the beads, slightly contract, and move forward, hence
521 those escaped (Supporting Information Movie 11) or remain
522 inactive, hence those showed no contraction and stayed
523 attached. To verify whether actin dynamics and MyoA motor
524 the function of which are required for motility^{25,46} would also
525 contribute to the apically driven exacerbated contraction, we
526 first defined a quantitative readout for measuring the
527 contraction extend over time. Using the ratio between the
528 resting body length (*i.e.*, no contraction) and the minimal body
529 length (*i.e.*, maximum contraction) as a contraction indicator,
530 we found that nearly half of the wild-type (WT) control
531 parasites were able to escape from the immobilization setting,
532 therefore attesting to the energetically active status of the
533 specimens under study. Approximately 40% of the second half
534 ($n = 35/92$) contracted over a wide range of amplitude,
535 whereas 20% of this contracting population actually showed
536 signs of irreversible damages ($n = 7/35$) (Figure 5A). Blocking
537 actin dynamics with cytochalasin D (0.5 and 1 μM) or
538 jasplakinolide (1 μM) that work through different mecha-
539 nisms^{47,48} almost abolished both the escape (*i.e.*, motile) and
540 contractile behaviors. Of note, the few (less than 5%)
541 tachyzoites that displayed contraction never reached the
542 stage of high and sustained body compression, hence none
543 of them died (Figure 5A). We confirmed the cytochalasin and
544 jasplakinolide potency on tachyzoite actin as parasites
545 expressing the fluorescent chromobody actin-Emerald (Acb-
546 E)⁴⁹ displayed the typical drug-induced redistribution of actin
547 filaments as, respectively, cytoplasmic aggregates and apical
548 acrosome-like.⁵⁰ With the same readout, we also compared the
549 contractile behavior of parasites following or not *MyoA* gene
550 silencing using the well-characterized DiCre Lox *MyoA*
551 strains.²⁴ We observed that loss of *MyoA* (ΔMyoA)
552 significantly altered the contractile capability of tachyzoites
553 immobilized on their base when compared to the *MyoA*-
554 expressing parental line (Lox*MyoA*) ($\Delta\text{MyoA} = 175$, $p <$
555 0.001) (Figure 5A). Finally, we checked if the contractile
556 activity was specific for tachyzoites metabolically ready for
557 initiating motility by measuring the contraction of WT control
558 tachyzoites kept in the Endo buffer which mimics the
559 intracellular buffer (hereafter referred to as IC buffer) and is
560 known to drastically decrease microneme secretion (*i.e.*,
561 adhesin delivery on the tachyzoite surface) and consequently
562 motility.²⁰ Those tachyzoites showed the typical actin posterior
563 network of intracellular tachyzoites⁵¹ and were significantly
564 inefficient at contracting under the posterior microbead setting
565 ($n\text{IC} = 57$, $p < 0.001$) (Figure 5A–C and Supporting
566 Information Movie 12). Finally, we monitored actin filaments
567 in real time in Acb-E-expressing tachyzoites undergoing either
568 helical gliding or forced contraction during the immobilization
569 phase. The F-actin apical focus detected at the onset of the
570 helical cycle remained discrete and rapidly resolved into the
571 large stable basal F-actin pool, as reported^{33,51} and in line with
572 our force mapping assays. In contrast, an increasing F-actin
573 signal was detected within the whole body, in particular, in the
574 cell front upon prolonged contraction of the immobilized and
575 rounding tachyzoites (Figure 5D and Supporting Information
576 Movie 13). Eventually, the F-actin signal vanished, which fits
577 with the loss of fluorescence observed with YFP-expressing
578 tachyzoite artificially immobilized on the substrate through
579 their basal pole (Supporting Information Figure S4), thereby
580 confirming that the membrane had ruptured upon excessive
581

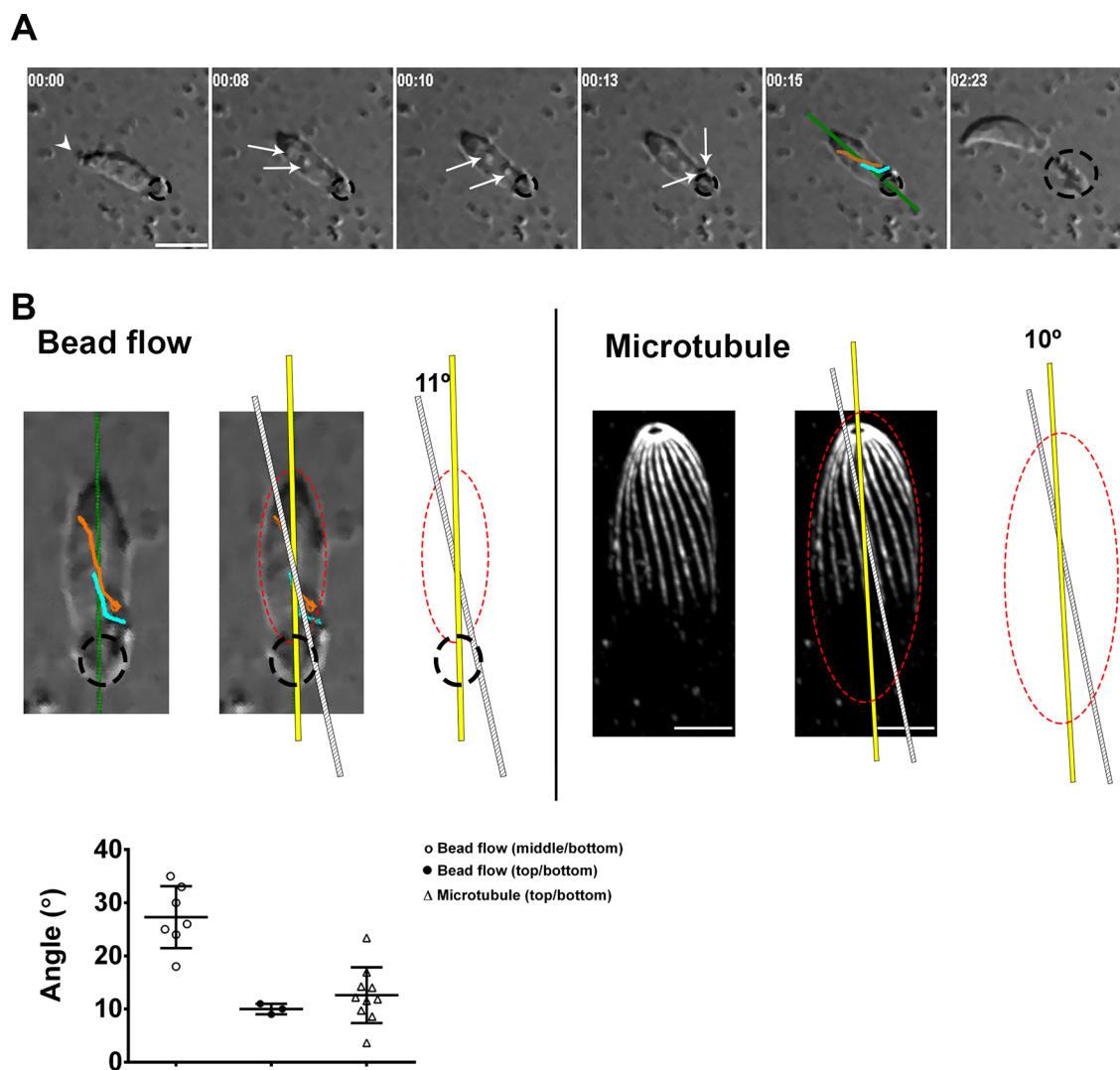


Figure 6. Apico-basal translocation of surface-bound microbeads follows a helical path that approximates the spiral direction of the tachyzoite cMTs. (A) Representative images of apico-basal bead capping monitored in real time for tachyzoites posteriorly immobilized on beads encircled with the dotted black line. The two surface-bound beads are indicated with white arrows, and their trajectory depicted in orange and cyan lines is shown on the fifth panel, before active escape of the tachyzoite from the immobilizing setting. In all panels, white arrowheads mark the tachyzoite apex; all scale bars: 5 μm , time is in minutes/seconds. (B) Zoomed panels show images and schematics for (left) trajectory of two beads over the capping time taken from the sequence shown in A, which deviates from the body main axis (yellow line) of about a 11° angle, while (right) the spiral cMTs visualized on an extracellular tachyzoite treated for U-ExM and stained with antipolyglutamylated tubulin show about a 10° angle orientation. (Bottom) Plot of the different angles calculated for the cMTs; the mean with SD is shown for each group ($n = 10$ tachyzoites, mean of 4 cMTs per tachyzoite) and for the capping bead flow ($n = 9$ tachyzoites). Please note the larger variability shown by the bead angles, which depends on the bead position at the initial time of capture and tracking.

582 contractile activity, leaving a tachyzoite ghost, the apex of
583 which no longer attached to the substrate.

584 As the retrograde membrane flow process has been shown to
585 operate in *T. gondii* tachyzoites in absence of the actomyosin
586 system,³² we analyzed how this process would operate for
587 tachyzoites artificially immobilized on their base. Unlike
588 previously,³² we monitored in real time the bead-capping
589 process and found that the loss of contractility driven by actin
590 poisons and IC conditions did correlate with the inhibition of
591 the rearward bead capping along the parasite surface ($57 > n >$
592 181 depending on the condition) (Figure 5C and Figure 6A
593 and Supporting Information Movie 14), thus recapitulating the
594 phenotype of formin 1(FRM1)-deficient tachyzoites³³ and
595 consistent with the need of apically initiated actin filaments.
596 However, on loss of myosinA,⁴⁶ whereas the tachyzoites
597 showed almost no ability to contract (Figure 5A), they

intriguingly maintained a bead-capping activity almost as 598
efficient as tachyzoites from the parental line ($65 > n > 165$) 599
(Figure 5C and Supporting Information Movie 12), in 600
agreement with a MyoA contribution to force transmission 601
rather than production.³² Furthermore, along with these bead- 602
translocation assays we observed in WT tachyzoites that the 603
bead-capping process could precede productive helical move- 604
ment (Figure 6A and Supporting Information Movie 14), and 605
that the bead apico-basal motion could follow the cMT helical 606
trajectory. To confirm this observation, we applied ExM with a 607
recently refined near-native expansion microscopy (U-ExM) 608
protocol,⁵² and labeled the α,β -tubulin dimer that forms the 609
cMT polymers taking advantage of the polyglutamylation post- 610
translational modification of these isoforms.⁵³ With this level of 611
resolution, we could measure the angle with the longitudinal 612
axis made by the cMTs when they emanate at the apical polar 613

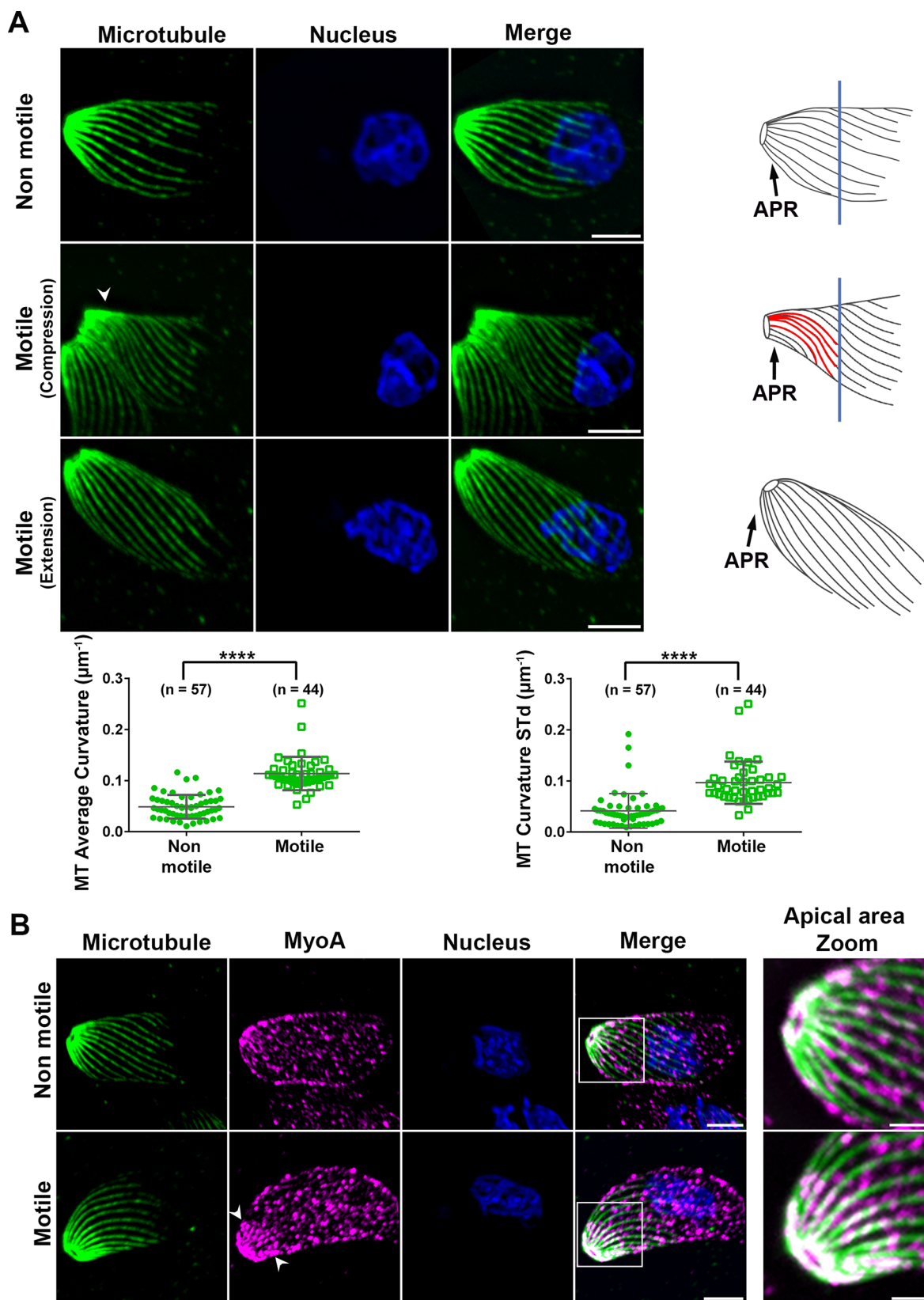


Figure 7. Curvature of cortical microtubules changes during helical gliding suggesting a spring-like mechanism apically driven by myosin-based contractile forces. (A,B) Confocal images representative of extracellular tachyzoites following U-ExM and immunofluorescence co-staining of (A) tachyzoite cMTs using antipolyglutamylated tubulin antibodies (green) and nucleus (blue). Maximal projection intensities of the cMTs from the tachyzoite ventral side are shown for (top) a nonmotile tachyzoite, (middle and bottom) two motile tachyzoites. Note the pronounced torsion and compression (white arrowhead, middle panel) and the extension (white arrowhead, bottom panel) that likely account for the spring like force underlying helical gliding. Right: Schematics of the cMTs for each stage show the highly curved cMTs at the apical region of the motile tachyzoites, drawn in red. (Bottom) Graph on the left shows a significant increase in mean curvature of the cMTs

Figure 7. continued

for the motile tachyzoites in torsion, unpaired *t* test, *** $p < 0.0001$ $n = 44$, when compared to the nonmotile ($n = 57$); graph on the right shows enhanced curvature amplitude over the length of single cMT for motile tachyzoites during the compression step ($n = 47$) when compared to nonmotile ($n = 57$) tachyzoites, unpaired *t* test, *** $p < 0.0001$. (B) Co-staining of the cMTs (green) and MyoA-HA tagged using anti-HA antibodies (purple) and the nucleus (blue). (Top) Nonmotile tachyzoite, (bottom) motile tachyzoite are shown. Note the clear MyoA signal enrichment at the apex of the motile parasite indicated with white arrowheads, which co-aligns with the MT signal. The right frames show zoomed areas delineated in the merge frames with a white square. All scale bars: $5 \mu\text{m}$ except for the B zoomed frames: $2 \mu\text{m}$. The U-ExM protocol gave a 3–5-fold increase of the tachyzoite size and provided a $\sim 70 \text{ nM}$ *xy* resolution. APR: Apical polar ring.

614 ring while approximating the angle made by the microbeads
615 monitored over the capping process (Figure 6B and
616 Supporting Information Movie 14). Although we observed
617 for some beads that the angles were off the range of those
618 made by the well-resolved cMTs, it is worth noting that these
619 gaps mainly correspond to situations where the microbeads
620 were tracked while they had already reached the minor axis
621 plane of the ellipsoid tachyzoite. While traveling toward the
622 posterior pole, the beads approached an area devoid of cMTs
623 and often collided with the substrate before reaching the
624 posterior pole, an observation already reported when
625 monitoring the apico-basal trajectory of $0.6 \mu\text{m}$ beads delivered
626 by laser trap to the surface of resting (*i.e.*, nonmotile)
627 tachyzoites.⁴² Collectively, these data indicate that the
628 actomyosin system mobilized during the rearward bead
629 translocation process has aligned with the longitudinal cMT
630 network, this independently of the apical adhesion and thus
631 before the apical traction force operates.

632 **Curvature of Cortical Microtubules Significantly**
633 **Changes during Helical Gliding, Suggesting a Spring-**
634 **like Mechanism Apically Driven by Myosin-Based**
635 **Contractile Forces.** Triggered upon relaxation of the apical
636 kink, the extension of the tachyzoite body coincided with the
637 thrust force while the parasite rotated with an orientation
638 dictated by the MTs' helical architecture (Figure 1C and
639 Figure 3A and Supporting Information Movie 3a). In
640 metazoans, the general ability of MTs to bear compressive
641 loads imposed by contractile forces translates into a
642 coordinated bending of MTs and to drive a spring-like force
643 mechanism as exemplified with the rhythmically contracting
644 cardiac myocytes.⁵⁴ To interrogate whether the cMTs could
645 transiently store energy upon actomyosin activation and acts as
646 a torsional spring to promote the required thrust for helical
647 propelling, we analyzed the behavior of cMTs during helical
648 motion. Whereas the U-ExM allows nanoscale resolution, it
649 does not permit to affirm that the tachyzoites under
650 observation were actually caught in motion. As under pro-
651 gliding conditions, the tachyzoites displayed a statistically
652 significant elongation of the nucleus as compared to the
653 samples of either extracellular tachyzoites left in IC buffer or
654 tachyzoites which had just invaded host cells ($n > 75$ for each
655 condition, Supporting Information Figure S5A), we used the
656 nucleus sphericity as first criterion. We next selected for
657 tachyzoites associated with helical trails using the surface-
658 exposed glycoprotein SAG1 known as robust trail marker,¹¹
659 which also bore an elongated nucleus ($p < 0.0001$, $n = 12$
660 motile helical and $n = 15$ intracellular) (Supporting
661 Information Figure S5B). To analyze whether individual
662 cMTs could display a specific compression, relaxation and
663 torsion behavior in motile tachyzoites, we applied U-ExM and
664 used both antiacetylated α -tubulin and antipolyglutamylated
665 α, β -tubulin antibodies which have been widely used for labeling
666 *T. gondii* cMTs.⁵³ When quantifying the curvature of the cMTs

667 in nonmotile extracellular (Figure 7A, top panel) or nonmotile
668 intracellular (Supporting Information Figure S5C, top panel)
669 and motile parasites (Figure 7A, middle panel; Supporting
670 Information Figure S5C, bottom panel), we observed for the
671 motile specimens, a highly significant increase in the mean
672 curvature of the MTs as compared to the nonmotile ones
673 (Figure 7A, polyglutamylated tubulin, $p < 0.0001$, $n = 57$
674 nonmotile and $n = 44$ motile parasites) (Supporting
675 Information Figure S5B,C, acetylated tubulin, $p < 0.0001$, n
676 = 24 nonmotile and $n = 22$ motile tachyzoites). Importantly,
677 the curvature amplitude over the length of a single MT was
678 also significantly enhanced in gliding when compared to
679 intracellular parasites as assessed by the standard deviation
680 values of the curvature profile over the full length MTs (Figure
681 7A, polyglutamylated tubulin, $p < 0.0001$, Supporting
682 Information Figure S5B,C, acetylated tubulin, $p < 0.0001$).
683 The decreased interdistance between single cMT nearby the
684 apical ring (APR) from where they emanate together with the
685 observed nonuniform bending over the cMT length strongly
686 argue for a specific compression occurring at the tachyzoite
687 apex during cell movement. In addition, situations where the
688 cMTs straightened at the apical region likely corresponded to
689 the time of the thrust force occurring upon kink release (Figure
690 7A, bottom panel; see also schematics on the right). To further
691 test the hypothesis of cooperation between the actoMyoA-
692 based and the cMT-based cytoskeletons could contribute to
693 force production at the kink, we performed U-ExM imaging of
694 MyoA and cMTs simultaneously on either motile or nonmotile
695 tachyzoites. To this end, we used a tachyzoite line that
696 expresses a chimeric MyoA-HA protein under the endogenous
697 promoter and anti-HA antibodies. A clear co-alignment of the
698 MyoA and the cMTs was detected in the apical region of
699 motile tachyzoites together with a local enrichment in MyoA
700 when compared to nonmotile parasites (Figure 7B). Although
701 it is not possible to ascertain that this situation reflects the kink
702 step, the alignment between the MyoA and the cMTs makes
703 plausible an indirect connection and thus argue for an energy
704 transfer between the two cytoskeletons. In addition, this co-
705 alignment is consistent with the helical path taken by the beads
706 undergoing actin-based rearward capping. In metazoans cells,
707 it was found that the MTs were embedded in a viscoelastic
708 material provided by the surrounding medium and MT-
709 associated proteins—in particular, kinesin motors⁵⁵—that
710 influence the MT ability to buckle and bear energy load.⁵⁴
711 Therefore, it would be worth investigating whether some MT
712 or/and IMC protein partners - including the APR-associated
713 kinesin,⁵⁶ proteins that coat the cMT length and alveolins—
714 positioned at the apical flexure site could behave as pro-elastic
715 elements and thus optimize a cMT driven spring-like reaction.
716 Of note the intrinsic stable left-handed curvature of
717 «resting» cMTs already sign for their ability to withstand
718 large forces within the cytoplasm in agreement with their
719 contribution to shape maintenance.^{30,57} As the activation of

720 the actomyosin system translates into force-powering motility,
721 it is plausible that the significant local increase in cMT
722 curvature of motile tachyzoites accounts for higher compressive
723 loads on the cMTs in response to the increased
724 contractility. Indeed, when the tachyzoite underwent exacerbated
725 actomyosin-driven contraction (Figure 4F) in a failed
726 attempt to disengage its base from the adhesion site, the cMT
727 cytoskeleton overcame its bending capacity and eventually
728 broke up.

729 CONCLUSIONS

730 In this study, by combining surface sensitive biophysical
731 techniques with high resolution-high speed 2D and 3D live
732 imaging and expansion microscopy, we provided insights on
733 how the *T. gondii* parasite couples cycles of adhesion–de-
734 adhesion to the substrate with traction and spring-like force
735 generation to achieve helical gliding, a function which is
736 required for perpetuating *T. gondii* populations in their
737 homeothermic hosts. Owing to its persistent structural polarity
738 over the intracellular/extracellular cycles, the tachyzoite
739 bypasses the canonical symmetry break most eukaryotic cells
740 must undergo to start migrating.⁵⁸ In addition, although
741 lacking the typical actomyosin II motor commonly used to
742 generate traction in eukaryotes, *Toxoplasma* has evolved an
743 Apicomplexa-restricted unconventional myosinA motor that,
744 immobilized within the glideosome unit and between the
745 membrane layers forming the pellicle at the cell surface, works
746 on noncanonical actin filaments to activate motility.²⁹ The
747 pellicle and the subtending twisted 22 microtubules provide
748 the tachyzoite with a peculiar crescent shape and flexibility,
749 which has proposed to act as the primary driver of the helical
750 motion. We now demonstrate that the *T. gondii* tachyzoite
751 must engage its apical region in a specific stable adhesion with
752 the substrate that is compatible with the development of a
753 balanced traction force and drives, in turn, the loosening of the
754 posterior contact point, allowing retraction of the parasite base
755 and dragging force. Therefore, similarly to what was found for
756 the *Plasmodium* motile sporozoite, real time analysis of
757 adhesion dynamics and force mapping supported by recently
758 refined high spatiotemporal resolution imaging approaches and
759 biophysics characterization applied to the *Toxoplasma* motile
760 tachyzoite have challenged the classic model of Apicomplexa
761 gliding motility. In addition to the backward capping of
762 adhesion proteins at the cell surface, both parasite rely on a
763 distinct class of adhesive contact that do not enter the rearward
764 flow but directly contribute to build tension in the parasite
765 cytoskeleton and produce force by strengthening anchors
766 points with a specific on–off dynamics, hence regulating the
767 motile process. Although these studies confirm the need to
768 shift paradigm for a comprehensive mechanistic understanding
769 of Apicomplexa gliding motility, they raised key questions on
770 the nanoscale architecture of the singular adhesive platforms
771 including the adhesin ligand identities on the extracellular side,
772 the actin filament organization on the cytoplasmic side, but
773 also how they would transmit tensional forces from the
774 cytoskeleton to the ECM with the yet to clarify contribution of
775 the MyoA motor, and eventually, it would be worth
776 investigating their putative contribution for mechanotransduction.
777 Providing indication of a spatiotemporally regulated
778 cooperation between the tachyzoite actomyoA-based contrac-
779 tile activity and the helical flexible cMT ability to build tension,
780 this study gives credit to pioneered kinematic analysis.¹⁸ These
781 results also highlight the necessity to delve deeper into the

compressive, torsional, and tensile strengths of *Toxoplasma* 782
tachyzoite and also *Plasmodium* sporozoite cMTs as some 783
differences between the two stages could in part account for 784
their specific motile behavior. 785

METHODS/EXPERIMENTAL

Parasite Strain Maintenance and Preparation for All Motility Live Assays. All media and products used for cell culture were from Gibco-Life Technologies (St Aubin, France) unless specified. *T. gondii* strains were propagated on *Mycoplasma*-free human foreskin fibroblast monolayers (HFFs) and grown in Dulbecco's modified Eagle medium (DMEM) supplemented with glutamax, 10% heat-inactivated fetal calf serum (FCS), penicillin (100 U/mL), and streptomycin (100 µg/mL) at 37 °C and 5% CO₂. The laboratory type 1 RH strain was used as well as the YFP-expressing RH¹⁷ and the mutant deleted for the KU80 protein⁵⁹ (gift from V. Carruthers, Michigan University, USA; see [Molecular Cloning](#) section). The RH Δku80: DiCre/lox MyoA, RH Δku80: DiCre/ΔMyoA,⁶⁰ and the RH cbactinEmFP⁴⁹ expressing strains were given by M. Meissner (MLU Munich University, Germany) and hereafter designated LoxMyoA, ΔMyoA, or Ach-E, respectively. For most motility assays, tachyzoites were collected within a few hours following spontaneous egress from the HFF monolayers, and ~10⁵ cells were centrifuged in HBSS⁺⁺ supplemented with 0.2% FCS (0.2% HBSS). The pellet containing parasites was resuspended in 150 µL of 1% FCS HBSS (1% HBSS) and adjusted at 1.6 mM of CaCl₂ (1% HBSS-Ca²⁺). Typically, when using the 18 mm diameter glass coverslip fitting chamber, 50 µL of the tachyzoite suspension was added to 100 µL of 1% FCS HBSS-Ca²⁺ covering the coverslip. The amounts of FCS, CaCl₂, and pharmacological reagents were adjusted upon need as described in each appropriate section.

Molecular Cloning. A mCherry (mC) tag was fused in frame with the C-terminus of TLAP2 gene ([ToxoDb.org](#), TGGT1_232130) in the ΔKu80 strain using the ligation independent cloning strategy.⁵⁹ A 1.5 kb fragment corresponding to the 3' end of the TLAP2 excluding the stop codon was amplified by PCR with the forward primer 5'-GCGCCCTCCTTCAGTGTTCCTC-3' and reverse primer 5'-TGCACCTGCGGCACCGGCAG-3'. The PCR fragment and vector p-LIC-mCherry-HXG were digested with T4 DNA polymerase and annealed, yielding plasmid pTLAP2mCherry-LIC-DHFR. Freshly egressed Δku80 tachyzoites from a 1/2 T25 flask were transfected with 15 µg of the EcoRV-linearized plasmid. After one cycle without drug, ΔKu80/TLAP2-mC transformants were selected with 2 µg/mL pyrimethamine and single-cell cloned by limiting dilution.

Endogenous tagging of MyoA with HA-tag was achieved using the pLIC-MyoA-HA-HXGPRT vector. To construct this plasmid the coding sequence of MyoA was amplified using primers 5'-TACTTCCAATCCAATTTAGCAAAGCAGCAAATGCCCCA-GCGAAG-3' and 5'-TCCTCCACTTCCAATTTTAGC-GAAGCCGGCTGAACAGTCGCGGG-3' and RH Δku80 genomic DNA as template. The PCR product was cloned to pLIC-mAID-HA-HXGPRT vector using the LIC cloning method as described above.⁶¹ RHku80 OsTir1-(Ty)3 parasites were electroporated with BstBI-linearized plasmid, and recombinant parasites were selected with mycophenolic acid (25 µg/mL) and xanthine (50 µg/mL). Stable transgenic parasites were single-cell cloned by limiting dilution.

Video Microscopy. Time-lapse video microscopy was conducted in ChamSlide chambers (LCI Corp., Seoul, Korea) accommodating 18 mm diameter coverslips or 35 mm MatTek glass bottom dishes (Matek corporation) installed on an Eclipse Ti inverted confocal microscope (Nikon France Instruments, Champigny sur Marne, France) set up with a temperature and CO₂-controlled stage and chamber (LCI Corp., Seoul, Korea). The microscope was equipped with the sCMOS prime camera (Photometrics), a 60× objective, and a CSU X1 spinning disk (Yokogawa, Roper Scientific, Lisses, France). MetaMorph software was used for controlling the microscope (Universal Imaging Corporation, Roper Scientific, Lisses, France). All live microscopy was performed at 37 °C and 5% CO₂.

849 **Image Processing.** For image processing, we combined the use of
850 ImageJ, Icy, MATLAB, ChimeraX, and Amira-Avizo (Thermo-Fisher)
851 software. Details of image processing are included in the appropriate
852 protocol sections. Slow motion videos were created using Adobe
853 Premiere with the optical flow module.

854 **Motility Assays in Collagen1 Fibrous Matrices.** Collagen gels
855 were made using rat tail type I collagen (CellSystems). Nine parts of
856 collagen dissolved in acidic medium were mixed with 1 part of
857 neutralization solution to reach the concentration of 4 mg/mL and
858 promote matrix formation. All solutions were kept in 4 °C during
859 preparation. Then dilution of the gel at a concentration of 2 mg/mL
860 in HBSS⁺⁺ supplemented with 1% FCS was rapidly spread in the
861 middle of a MatTek glass bottom dish and incubated for 30 min at 37
862 °C and 5% CO₂. Labeling of the collagen fibers was achieved with the
863 recombinant CNA35-EGFP produced in *Escherichia coli* (BL21-DE3
864 strain) used at 2 μM final concentration according to the protocol.³⁵
865 A 50 μL volume of the tachyzoite suspension prepared in 1% FCS
866 HBSS-Ca²⁺ was deposited on top of the gel prior to being transferred
867 in the microscopy stage for video microscopy. Once tachyzoites had
868 penetrated within the mesh, images were captured at 1 frame/s. For
869 tracking the fibers during parasite navigation, we used Icy and
870 visualized with green the initial position of the fibers at $t = 0$ s while
871 we duplicated this frame as many times as the number of the time-
872 lapse frames. Then we merged this “time zero” stack with the original
873 time lapse in which the fibers were colored in magenta. The whitish
874 color accounts for the green and magenta overlay and indicates no
875 displacement, whereas the detection of the green and magenta reveals
876 the fiber displacement during the sequence.

877 **Tachyzoite 2D Gliding Assays.** Parasites were prepared as
878 described above, and assays were carried on 18 mm plasma-activated
879 glass coverslips coated with either fibronectin (20–50 μg/mL in
880 phosphate-buffered saline (PBS)) or poly-L-lysine (150–300 kDa
881 PLL, 50 μg/mL in PBS). Image analysis included tracking the
882 tachyzoite back and front parts over the gliding time by using Icy as
883 described.⁶² “Manual tracking” and “Motion profiler” plugins were
884 used to extract the x - y position of the back and front spots over time.
885 Having tracked the x - y positions over time, we used the “Distance
886 profiler” plugin to calculate the distance between the two positions
887 (back and front) over time.

888 **Reflection Interference Contrast Microscopy.** RICM images
889 were acquired on an inverted microscope (IX71, Olympus) on which
890 a white-light source (HPLS 345, Thorlabs) was coupled using a
891 home-built illumination arm incorporating a diaphragm (SM1D12C,
892 Thorlabs) controlling the illumination numerical aperture (NA = 0.46
893 in all experiments shown here) and a triple-band spectral filter (FF01-
894 457/530/628-25, Semrock) as described.⁶³ The usual fluorescence
895 cube in the microscope turret was replaced by polarization optics,
896 allowing separation of the incoming and reflected light (polarizer
897 WP25M-VIS, Thorlabs), polarizing beamsplitter cube (PBS251,
898 Thorlabs), and achromatic imaging flat quarter waveplate (QWP;
899 custom-made, Fichou, France). The sample was imaged with an oil-
900 immersion objective (60XO UPLSApo, Olympus, Japan) onto a
901 sCMOS camera (Orca Flash 4 v2, Hamamatsu) after passing through
902 an autofocus device (CRISP system, ASI imaging, USA) and a home-
903 built image-splitting setup incorporating a variable slit (VA100/M,
904 Thorlabs), achromatic relay lenses (ITL200, Thorlabs), imaging flat
905 dichroic mirrors (FF484-FD01-25X36 and FF560-FD01-25X36,
906 Semrock) gently held through curable silicon paste (Sugru, Form-
907 Form, UK), silver mirrors (PFSQ10-03-P01, Thorlabs) and
908 dichroic filters (blue: Semrock FF01-452/45-25; green: FF01-531/46-
909 25; red: FF01-629/56-25, Semrock). In addition, for bright-field (BF)
910 imaging, a red colored glass filter (FGL610S, Thorlabs) was used to
911 filter the light for the microscope illumination arm that was
912 subsequently focused by a long-distance 0.3 NA condenser (all
913 Olympus, Japan). BF and RICM images were acquired simulta-
914 neously, in the red channel, and thus show a dim RICM image
915 superimposed. Finally, a custom-built thermostated box (Digital Pixel,
916 UK) enclosing most of the microscope was used to control the
917 temperature at the sample. Image processing was done using ImageJ.
918 Blue, green, and red stacks of images were first manually registered.

RICM images were subsequently filtered using the “FFT band-pass
919 filter” plugin (1–100 pixels) to remove high-frequency noise and 920
uneven illumination background. The two (in the case of 921
simultaneous BF/RICM imaging) or three (in the case of only 922
RICM imaging) RICM images were then superimposed and 923
converted into a RGB image. BF images and RGB RICM images 924
were then segmented using the “trainable Weka segmentation” 925
plugin.⁶⁴ For BF images, the classifier was trained to segment the 926
whole parasite, and the outline of the resulting binary image was 927
extracted subsequently. For RICM images, the algorithm was used to 928
separate the “close contact” area defined by the first dark RICM fringe 929
on the parasite surface, the trail (when relevant), the rest of the RICM 930
signal coming from the parasite (when no BF image was present), and 931
the rest of the image. Because the classifier could detect the second 932
dark RICM fringe manual correction of the segmented images was 933
performed afterward to remove incorrect areas classified as “close 934
contact”. A binary image was obtained for the “close contact” area, 935
whereas an 8-bit probability image was extracted for the trail. Analysis 936
of the processed images was also performed using ImageJ. The 937
kymograph was obtained using the plugin “KymoResliceWide”. For 938
measuring the speed of the parasite back end, the corresponding white 939
line on the kymograph was isolated and the positions of the pixels 940
were exported to Origin. After smoothing, the position curve was 941
derived to obtain the velocity of the back end over time. 942

Traction Force Microscopy. TFM setting was adapted from ref 943
65. The 2 kPa PAA gels were made and mixed with TFM beads 944
(FluoSpheres carboxylate-modified microspheres, ThermoFisher 945
Scientific, 0.2 μm dark red fluorescent, λ : 660/680, 2% solid). The 946
gel was top-coated with fibronectin (20 μg/mL, 30 min, 23 °C) 947
diluted in sodium bicarbonate pH 8.3 (100 mM). Typical samples 948
were made from stocks of 0.7 μL beads mixed in 165 μL of gel 949
supplemented with 1 μL of both tetramethylethylenediamine 950
(TEMED) and ammonium persulfate (APS) 10% using 20 μL of 951
bead-gel suspension for an 18 mm diameter silanized glass coverslip. 952
A 50 μL volume of the tachyzoite suspension prepared in 1% HBSS- 953
Ca²⁺ (see above) was deposited on top of the fibronectin-coated PAA 954
gel already filled with 100 μL of 1% HBSS-Ca²⁺ in the appropriate 955
chamber and video recording started upon tachyzoite contact with the 956
substrate. MetaMorph streaming option was used for fast recording 957
(<1 frame/s) in both the DIC and far-red channels. Analysis of the 958
TFM beads displacement for extracting the force values carried out 959
using MATLAB. The TFM bead displacement was assessed using as a 960
reference bead position, the frame with a gel area free of parasite (*i.e.*, 961
relaxed position of the gel). Then using the frames taken over time, 962
the bead's displacement was tracked from their initial relaxed position. 963
To check the tachyzoite gliding behavior on a substrate of different 964
stiffness, gels of different PAA concentration and coated with a layer 965
of fibronectin were made using the same protocol, omitting the bead 966
addition step, and the stiffness was controlled by atomic force 967
microscopy. 968

Micropatterned Devices. Micropatterned PEG-fibronectin 969
composite coverslips were engineered by Alveole using the PRIMO 970
patterning with specific area coated with a mix of fibronectin (100 μg/ 971
mL) and AlexaFluor647-coupled fibrinogen (10 μg/mL) and other 972
coated with PLL-PEG. Fifty microliters of the tachyzoite suspension 973
prepared in 1% HBSS-Ca²⁺ was deposited on top of the fibronectin- 974
coated glass coverslip placed in the chamber and prefilled with 100 μL 975
of buffer prior to video microscopy. 976

Rear-Blocked Parasite Assay. The 0.2 μm diameter beads 977
(FluoSpheres carboxylate-modified microspheres, ThermoFisher 978
Scientific F8807, λ : 660/680, 2% solid) were activated using the 979
microsphere coupling—two-step EDC/Sulfo NHS covalent coupling 980
procedure for Estapor carboxyl-modified dyed microspheres protocol 981
(MerckMillipore). Fifty microliters of the stock-activated bead 982
solution was diluted in 150 μL of MES buffer (50 mM, pH 6.0). 983
Then 30 μL of the bead solution was added to 120 μL of the 984
tachyzoite suspension in 0.15% FCS HBSS⁺⁺ (0.15% HBSS). Fifty 985
microliters of the parasite suspension (from either the RHΔKu80, 986
ΔMyoA, RHΔKu80Acb-E, or YFP tachyzoite lines) was deposited on 987
the (50 μg/mL) fibronectin-coated glass coverslips in the microscopy 988

989 chamber, which was already filled with 200 μL of 0.15% HBSS⁺⁺. In
990 some experiments, parasites were preincubated for 10 min with 0.5 or
991 1 μM of either jasplakinolide or cytochalasin D (Sigma) and video-
992 recorded in the presence of the compounds. For the intracellular
993 mimicking conditions, tachyzoites collected by centrifugation in
994 0.15% HBSS⁺⁺ were rinsed in ENDO buffer referred to here as IC
995 buffer (145 mM KCl; 5 mM NaCl; 1 mM MgCl₂; 15 mM MES; 15
996 mM HEPES; pH 8.3)⁶⁶ before use, and the activated beads were
997 prepared in IC buffer. The mixture prepared as mentioned above was
998 deposited on the microscopy chamber, which was prefilled with 200
999 μL of IC buffer. Images were acquired at 1 s intervals for 20 min. To
1000 describe the contractile potential of tachyzoites under each
1001 experimental condition, we calculated the contraction ratio by
1002 measuring two times the length of the parasite body starting from
1003 the back until its farther apical point but excluding the conoid to avoid
1004 false measurements in case of fluctuating extrusion. The first measure
1005 corresponded to the relax position and the second to the maximal
1006 contraction. Division of the relaxed *versus* the contracted length
1007 provided the contraction ratio. When needed, both DIC and
1008 fluorescence channels were recorded at ~ 1 frame/s.

1009 **Bead Flow Assays.** The assay was performed similarly to the rear
1010 blocked parasite assay. For tracking the beads, visualizing their tracks
1011 and extracting their speed, “Spot detector” and “Track manager”
1012 plugins (Icy) were used.

1013 **Expansion Microscopy.** To analyze gliding tachyzoites, we
1014 collected the parasites in either in prewarmed 1% FCS HBSS-Ca²⁺
1015 or IC buffer, which were centrifuged at low speed to synchronize their
1016 sedimentation and left to glide for 10 min on PLL (50 $\mu\text{g}/\text{mL}$)-coated
1017 plasma-activated glass coverslips (37 $^{\circ}\text{C}$, 5% CO₂). Gliding was
1018 stopped by addition of paraformaldehyde (PFA, 3.2%, 15 min). The
1019 tachyzoite surface and trails were immunolabeled with anti-TgPSAG1
1020 antibodies (mAb, clone TP3), and the chimeric TLAP2 protein was
1021 detected using the rabbit anti-mCherry antibodies (Institut Curie,
1022 TAb-IP Paris, France) directed against the mC tag after cell
1023 permeabilization with TritonX-100 (0.1% in PBS, 5 min). The
1024 samples were next incubated with appropriate secondary highly cross-
1025 adsorbed antibodies (4 $\mu\text{g}/\text{mL}$, 2h). The ExM protocol was adapted
1026 from ref 45. Briefly Acryloyl-X was used at 0.1 mg/mL PBS for 2 h at
1027 23 $^{\circ}\text{C}$ prior to the gelation step, which was performed by placing the
1028 gelation solution (80 μL) on parafilm and the coverslip with the cell
1029 sample face down on top of it. Gelation was allowed to proceed at 37
1030 $^{\circ}\text{C}$ for 1 h followed by the digestion step (0.5% Triton X-100, 8 units/
1031 mL Proteinase K) carried out overnight at 23 $^{\circ}\text{C}$. A 4 mm diameter gel
1032 core sample was collected with a puncher and immersed in 5 mL of
1033 ddH₂O in a 50 mm diameter Petri dish to promote expansion, while
1034 PI staining was concomitantly performed (2.5 μM in PBS). H₂O was
1035 exchanged every 20 min until maximal expansion. The piece of gel
1036 was next gently transferred onto a PLL-coated glass coverslip in the
1037 minimal liquid volume to avoid gel drift. The 0.2 μm Z-image stacks
1038 of parasites in stages were captured using a spinning disk confocal
1039 Eclipse-ti Nikon microscope.

1040 **Ultrastructure Expansion Microscopy.** Gliding assays were
1041 performed with RH Δ Ku80MyoA-HA similarly to that for ExM.
1042 Additionally, for the invasion assay, HFF cells were plated on a poly-L-
1043 lysine-coated glass coverslip to obtain 80% cell confluence on the
1044 following day. Parasites were settled on top of the cells by gentle
1045 centrifugation (2 min, 250g) and left to invade for 1 h (37 $^{\circ}\text{C}$, 5%
1046 CO₂). For U-ExM, we followed the protocol developed by the
1047 Guichard laboratory,⁵² but to optimally preserve both free and
1048 intracellular tachyzoite shapes, we found it better to fix the samples
1049 before expansion in a solution made of 0.7% formaldehyde and 1%
1050 acrylamide in PBS (4–5 h, 37 $^{\circ}\text{C}$). Next, similar to the ExM protocol,
1051 gelation was carried out for 1 h (37 $^{\circ}\text{C}$). Coverslips with gels were
1052 then transferred into ~ 2 mL of the denaturation buffer in a 6-well
1053 plate for 15 min (23 $^{\circ}\text{C}$). Gels were removed from the coverslips with
1054 tweezers and transferred to a 35 mm diameter glass Petri dish filled
1055 fresh denaturation buffer for a 60 min incubation at 95 $^{\circ}\text{C}$. After
1056 denaturation, gels were placed in a 150 mm diameter glass Petri dish
1057 filled with ddH₂O for the first expansion. Water was exchanged at
1058 least twice every 30 min at room temperature, and gels were

incubated overnight in ddH₂O. After limited shrinkage in PBS, several
4 mm diameter pieces of gel were collected and deposited on 24-well
plates. Gels were then incubated for 3 h at 37 $^{\circ}\text{C}$ under shaking in
primary antibodies (antiacetylated α -tubulin, mAb, clone 6-11B-1) or
rabbit α -polyglutamylated (mAb, clone GT335) and rat anti-HA
antibodies (mAb, clone 3F10). Gels were washed in PBS-Tween 0.1%
prior to incubation with appropriate secondary highly cross-adsorbed
antibodies (4 $\mu\text{g}/\text{mL}$) for 150 min at 37 $^{\circ}\text{C}$ and washed again in PBS-
Tween 0.1%. Expansion was achieved by controlled immersion in
ddH₂O. The PI staining, final ddH₂O wash, and gel mounting steps
were all performed as mentioned for ExM.

1070 **ExM and U-ExM Image Processing.** 3D reconstruction was
1071 achieved with the UCSF ChimeraX software from the raw data (*i.e.*,
1072 xyz files) processed with ImageJ to first crop the region of interest and
1073 then to apply the “Iterative Deconvolve 3D” plugin for each channel.
1074 We used the ImageJ “3D Object counter” plugin to check for the
1075 isotropic physical expansion of the specimen by comparing the ratio
1076 of the parasite (SAG1 staining) and the nucleus (PI staining) volumes
1077 in controlled and expanded extracellular tachyzoite samples. The
1078 nucleus sphericity index of motile, nonmotile, and intracellular
1079 tachyzoites was compared by thresholding each image object to create
1080 3D binary images, whereas the “3D Object counter” plugin was next
1081 used to segment Z-stack binary images and retrieve the volume and
1082 surface of the 3D objects (*i.e.*, parasite nucleus and whole body). The
1083 sphericity index was calculated using the formula where V is the
1084 volume and S the surface:

$$\text{sphericity } \Psi = \frac{(36\pi V^2)^{1/3}}{S}$$

The nucleus roundness was obtained by thresholding a Z-Max
projection from the Z-stack images and using the “Particle analysis”
plugin.

1088 For the tubulin curvature analysis in expanded specimens, we used
1089 the “Kappa” plugin in Fiji on the Z-Max projection from Z-stack
1090 images of the tachyzoite ventral cMTs (*i.e.*, facing the substrate),
1091 which allowed tracing each MT shape and retrieving the mean
1092 curvature of each cMT as well as the curvature amplitude over the
1093 cMT length (standard deviation).

1094 **MIC2 and SAG1 Analysis in Helical Trail.** Extracellular
1095 tachyzoites were let to glide for 15 min prior to be fixed with PFA
1096 as described in the ExM section. A double immunolabeling was
1097 performed sequentially using the anti-TgSAG1 protein (clone TP3,
30 min, 23 $^{\circ}\text{C}$) and the anti-TgMIC2 (clone 6D10, overnight, 4 $^{\circ}\text{C}$)
1098 as primary antibodies, each followed by incubation with appropriate
1099 secondary highly cross-adsorbed antibodies (4 $\mu\text{g}/\text{mL}$, 2 h). Images
1100 were acquired on the Axio Imager Z1 Zeiss microscope using the
1101 Zeiss imaging software ZEN before being processed using Fiji
1102 software.

1104 To determine the size and position of the adhesion point formed at
1105 the early step of the helical cycle (*i.e.*, coinciding with the kink) on the
1106 MIC2/SAG1 immunolabeled trails, we used the machine-learning-
1107 based segmentation of the real-time RICM sequence as a template.
1108 The region of interest for the trail trajectory, the angular area, and the
1109 parasite apical adhesion area were defined manually with Fiji: the
1110 distance between the center of adhesion point and the angle point was
1111 measured for six angles over a sequence of successive helical cycles,
1112 which allowed positioning the apical adhesion at about +0.170 μm
1113 downstream the angle point, and the size of the adhesion point was
1114 estimated around 0.5 μm . Applying these values on the MIC2/SAG1
1115 labeled trails, it was possible to accurately identify the adhesion and
1116 angle area over the profiles of fluorescence intensity measured for the
1117 whole trails trajectories that in total defined 40 helical cycles
1118 performed by eight tachyzoites. The fluorescence intensity is given as
1119 a ratio by normalizing on the mean intensity value.

1120 **Quantification and Statistical Analysis.** Data were statistically
1121 analyzed, and plots were made using GraphPad Prism 6.0 software for
1122 Windows (La Jolla, CA, USA). Data are presented as mean \pm
1123 standard error of mean if not indicated otherwise. Figure legends
1124 include the statistical test and resulting comparison when appropriate.

1125 For Figure 4A, Figure 7A, and Figures S3A and S5B,C, an unpaired *t*
 1126 test was used with significance being represented as a *p* value <0.05,
 1127 and the *n* indicated represents the parasite sample size or the number
 1128 of individual microtubules analyzed. For Figure 4E, Figure 5A, and
 1129 Figure S5A, an unpaired Kruskal–Wallis test was performed, and the
 1130 *n* indicates the sample size. For the table in Figure 5A, a chi-square
 1131 test followed by Fisher's exact test was used, with the *n* showing the
 1132 proportions analyzed.

1133 ASSOCIATED CONTENT

1134 **SI** Supporting Information

1135 The Supporting Information is available free of charge at
 1136 <https://pubs.acs.org/doi/10.1021/acsnano.0c01893>.

1137 Helical and circular gliding by *T. gondii* tachyzoites;
 1138 tachyzoite apical adhesion site is not enriched in MIC2
 1139 and SAG1 proteins; frequency and duration of *T. gondii*
 1140 tachyzoite gliding behavior depends on the substrate
 1141 stiffness; prolonged and exacerbated contraction induced
 1142 upon tachyzoite posterior immobilization leads to
 1143 membrane leakage; expansion microscopy allows un-
 1144 covering cMT local selective compression and torsion in
 1145 gliding tachyzoites (PDF)

1146 Movie 1: Parasite moving in 3D collagen fibers1 (AVI)

1147 Movie 2: Parasite moving 3D collagen fibers2 (AVI)

1148 Movie 3a: Parasite helical gliding in 2D (AVI)

1149 Movie 3b: Slow motion but same as movie 3a (AVI)

1150 Movie 4: Parasite circular gliding in 2D (AVI)

1151 Movie 5: RICM analysis of parasite helical gliding (AVI)

1152 Movie 6: RICM-pulling on or breaking the trail (AVI)

1153 Movie 7a: TFM analysis of parasite helical gliding (AVI)

1154 Movie 7b: Slow motion but same as movie 7a (AVI)

1155 Movie 8: Parasite helical gliding on micropattern
 1156 Alveole1 (AVI)

1157 Movie 9: Parasite helical gliding micropattern Alveole2
 1158 (AVI)

1159 Movie 10: Parasite base blocked by microbeads (AVI)

1160 Movie 11: Bead release from the base of a moving
 1161 parasite (AVI)

1162 Movie 12: Parasite contraction in the presence of drugs
 1163 (AVI)

1164 Movie 13: Actin dynamics in gliding or immobilized
 1165 parasites (AVI)

1166 Movie 14: Bead flow and bead release in tachyzoites
 1167 (AVI)

1168 AUTHOR INFORMATION

1169 Corresponding Author

1170 **Isabelle Tardieux** – *Institute for Advanced Biosciences (IAB),*
 1171 *Team Membrane Dynamics of Parasite–Host Cell Interactions,*
 1172 *CNRS UMR5309, INSERM U1209, Université Grenoble*
 1173 *Alpes, 38700 Grenoble, France;* [orcid.org/0000-0002-](https://orcid.org/0000-0002-5677-7463)
 1174 [5677-7463](https://orcid.org/0000-0002-5677-7463); Email: isabelle.tardieux@inserm.fr

1175 Authors

1176 **Georgios Pavlou** – *Institute for Advanced Biosciences (IAB),*
 1177 *Team Membrane Dynamics of Parasite–Host Cell Interactions,*
 1178 *CNRS UMR5309, INSERM U1209, Université Grenoble*
 1179 *Alpes, 38700 Grenoble, France*

1180 **Bastien Touquet** – *Institute for Advanced Biosciences (IAB),*
 1181 *Team Membrane Dynamics of Parasite–Host Cell Interactions,*
 1182 *CNRS UMR5309, INSERM U1209, Université Grenoble*
 1183 *Alpes, 38700 Grenoble, France*

Luis Vigetti – *Institute for Advanced Biosciences (IAB), Team* 1184
Membrane Dynamics of Parasite–Host Cell Interactions, CNRS 1185
UMR5309, INSERM U1209, Université Grenoble Alpes, 1186
38700 Grenoble, France 1187

Patricia Renesto – *Institute for Advanced Biosciences (IAB),* 1188
Team Membrane Dynamics of Parasite–Host Cell Interactions, 1189
CNRS UMR5309, INSERM U1209, Université Grenoble 1190
Alpes, 38700 Grenoble, France; TIMC-IMAG UMR 5525 - 1191
UGA CNRS, 38700 Grenoble, France 1192

Alexandre Bougdour – *Institute for Advanced Biosciences* 1193
(IAB), Team Host–Pathogen Interactions & Immunity to 1194
Infections, CNRS UMR5309, INSERM U1209, Université 1195
Grenoble Alpes, 38700 Grenoble, France 1196

Delphine Debarre – *Laboratoire Interdisciplinaire de Physique,* 1197
UMR CNRS, 5588, Université Grenoble Alpes, Grenoble 1198
38402, France 1199

Martial Balland – *Laboratoire Interdisciplinaire de Physique,* 1200
UMR CNRS, 5588, Université Grenoble Alpes, Grenoble 1201
38402, France 1202

Complete contact information is available at: 1203
<https://pubs.acs.org/10.1021/acsnano.0c01893> 1204

Author Contributions

1205 Conceptualization, I.T., M.B., G.P.; Software, M.B., D.D., G.P.;
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