Integrin-dependent Migratory Switches Regulate the Translocation of Toxoplasma-infected Dendritic Cells Across Brain Endothelial Monolayers

Emily C Ross

Stockholm University: Stockholms Universitet

Antonio Barragan (✉ antonio.barragan@su.se)

Stockholms Universitet <https://orcid.org/0000-0001-7746-9964>

Research Article

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1 **Integrin-dependent migratory switches regulate the translocation of *Toxoplasma-***
2 **infected dendritic cells across brain endothelial monolayers**

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4 Emily C. Ross¹ Antonio Barragan^{1,*}

5

6 ¹Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University,
7 Stockholm, Sweden

8 *Correspondence: antonio.barragan@su.se

9

10 **Abstract**

11 Multiple cellular processes, such as immune responses and cancer cell metastasis, crucially
12 depend on interconvertible migration modes. However, knowledge is scarce on how
13 infectious agents impact the processes of cell migration at restrictive biological barriers. In
14 extracellular matrix, dendritic cells (DCs) infected by the obligate intracellular protozoan
15 *Toxoplasma gondii* undergo mesenchymal-to-amoeboid transition (MAT) for rapid
16 integrin-independent migration. Here, in a cellular model of the blood-brain barrier, we
17 report that parasitised DCs shift to integrin-dependent motility and adhesion on polarised
18 endothelium, accompanied by elevated transendothelial migration (TEM). Upon contact
19 with endothelium, parasitised DCs dramatically reduced velocities and adhered under both
20 static and shear stress conditions, thereby obliterating the infection-induced amoeboid
21 motility displayed in collagen matrix. The motility of adherent parasitised DCs on
22 endothelial monolayers was restored by blockade of $\beta 1$ and $\beta 2$ integrins or ICAM-1, which
23 conversely reduced motility on collagen-coated surfaces. Moreover, parasitised DCs
24 exhibited enhanced translocation across highly polarised primary murine brain endothelial
25 cell monolayers. Blockade of $\beta 1$, $\beta 2$ integrins, ICAM-1 and PECAM-1 reduced TEM
26 frequencies. Finally, gene silencing of the pan-integrin-cytoskeleton linker talin (*Tln1*) or
27 of $\beta 1$ integrin (*Itgb1*) in primary DCs resulted in increased motility on endothelium and
28 decreased TEM. Adding to the paradigms of leukocyte diapedesis, the findings provide
29 novel insights in how an intracellular pathogen impacts the migratory plasticity of
30 leukocytes in response to the cellular environment, to promote infection-related
31 dissemination.

32 **Keywords:** leukocyte; blood-brain barrier; Apicomplexa; immune cell; cell migration; cell
33 adhesion molecule

34 Introduction

35 Dendritic cells (DCs) are central to mount immune responses and to combat
36 infections [1]. Their remarkable migratory capacity is in part due to their ability to
37 transition from mesenchymal to amoeboid (MAT) migration modes [2]. This requires
38 major changes in the actin cytoskeleton, and enables high-speed locomotion through
39 extracellular matrix (ECM), when travelling from the periphery to secondary lymphoid
40 organs, or to reach biological barriers, on their way from the blood into tissues [3,4].
41 Amoeboid migration is especially suited for rapid locomotion of leukocytes in cellular
42 networks and tissues [5]. In contrast to integrin-dependent mesenchymal migration on 2-
43 dimensional (D) surfaces, amoeboid migration in 3D confinements occurs independently
44 of adhesion to specific substrates or ECM degradation and relies primarily on the protrusive
45 flow of the actin cytoskeleton [6,7]. Upon reaching biological barriers, however, DCs need
46 to switch their migration mode and slow down to be able to adhere to vascular endothelium.

47 The blood-brain barrier (BBB) regulates traffic of leukocytes and protects the brain
48 from pathogens, toxins, cell damage and inflammation [8]. Specialised endothelial cells,
49 held together by tight junctions (TJs), form a highly restrictive barrier that tightly regulates
50 homeostasis in the central nervous system (CNS) [9]. The cerebral endothelium is in
51 constant crosstalk with astrocytes, microglia, neurons, pericytes and circulating immune
52 cells [10], that all contribute to protect the CNS from external insults.

53 Upon inflammation, recruitment of leukocytes, such as DCs, and their interaction
54 with the vascular endothelium is a critical step in the immune response [11]. Endothelial
55 cells attract and direct leukocytes to enter underlying tissue, by exposing a variety of cell
56 adhesion molecules (CAMs), such as ICAM-1 and PECAM-1, at their surface that capture
57 travelling immune cells in the bloodstream [12]. Leukocytes on the other hand, express
58 various integrins on their surface, which include $\beta 1$ and $\beta 2$ integrins that bind to CAMs on
59 the endothelial cell surface [13]. Talin-1 connects integrins to the cytoskeleton [14].
60 Jointly, these interactions mediate firm adhesion and spreading of leukocytes on the
61 endothelium, which leads to leukocyte crawling and eventually transmigration through the
62 endothelial barrier to reach sites of inflammation [15].

63 Understanding the biology of leukocyte migration can be aided through the study
64 of host-pathogen interactions, owing to the microbial ability to hijack host cell functions.
65 *Toxoplasma gondii* is a model parasite [16] and obligate intracellular pathogen that infects
66 warm-blooded vertebrates and crosses non-permissive barriers to reach immunoprivileged

67 sites, such as the CNS [17]. It is estimated that one third of the global human population
68 encounters *T. gondii* during a life-time [18]. Primary infection is usually subclinical, and
69 once the parasite reaches the CNS, it can chronically persist inside cysts. In
70 immunocompromised individuals or in the developing foetus, reactivation of latent
71 infection or acute disseminated infection, respectively, can be life-threatening and cause
72 encephalitis [19].

73 Mononuclear phagocytes, such as DCs, can act as 'Trojan horses' for *T. gondii*
74 dissemination in mice [20-22]. When actively invaded by *T. gondii*, DCs undergo
75 cytoskeletal changes with redistribution of integrins, consistent with MAT in ECM [23-
76 25]. This migratory activation -termed hypermotility- [26] requires live intracellular
77 parasites [24], does not rely on chemotaxis [27] and is instead mediated by non-canonical
78 GABAergic signaling and MAP kinase activation [22,28-31]. However, it has remained
79 unknown how highly-migratory parasitised DCs interact with polarised endothelium.

80 In the present study, we addressed the interaction of parasitised DCs with polarised
81 primary brain endothelial cell monolayers, and identified a migratory shift associated to
82 transendothelial migration (TEM), with a central role for integrins and CAMs.

83

84 **Results**

85 ***T. gondii*-infected DCs transmigrate across polarised monolayers of primary mouse** 86 **brain endothelial cells (MBECs) and bEnd.3 cells.**

87 To assess if *T. gondii*-infected DCs can cross polarising and TJ forming brain endothelial
88 cell monolayers, primary MBECs from murine brains or bEnd.3 cells were grown to
89 confluency and polarisation in a transwell system (**Fig. 1a, Supplementary Fig. S1**). Upon
90 exposure to *T. gondii*-challenged DCs or unchallenged DCs, MBEC monolayers
91 maintained low permeability to the low molecular weight tracer FITC-dextran (3kDa) (**Fig.**
92 **1b**) and high transendothelial electrical resistance (TEER) (**Fig. 1c**), consistent with
93 characteristics of cerebral endothelium [32]. Importantly, *T. gondii*-challenged DCs
94 transmigrated across polarised MBEC and bEnd.3 monolayers, at significantly higher
95 frequencies compared with unchallenged DCs (**Fig. 1d, g**). In contrast, *T. gondii*-
96 challenged macrophages transmigrated at significantly lower frequencies, with non-
97 significant differences compared with unchallenged macrophages (**Fig. 1e, f, g**). We
98 conclude that *T. gondii*-challenged DCs transmigrate across polarised endothelial
99 monolayers, with undetectable perturbation of cellular barrier integrity.

Absence of hypermotility by *T. gondii*-infected DCs on endothelial cell monolayers

T. gondii-infected DCs are hypermotile in 2D and 3D confinements with collagen [22,29,25]. To determine their migratory behavior on endothelium, we compared the motility of DCs on bEnd.3 endothelial cell monolayers and on collagen-coated surfaces (**Fig. 2a**). In line with previous studies, *T. gondii*-infected DCs exhibited hypermotility on collagen, with elevations in migrated distances and velocity (**Fig. 2b, c**). In stark contrast, we found that *T. gondii*-infected DCs exhibited non-significant differences in velocity and migrated distances on bEnd.3 cell monolayers, compared with unchallenged DCs (**Fig. 2b, c**). The unexpected absence of hypermotility by *T. gondii*-infected DCs on endothelial cell monolayers motivated a further exploration of the cell-cell interactions in play.

CAMs and integrins are implicated in the motility of *T. gondii*-challenged DCs on endothelial cell monolayers

Endothelial CAMs and integrins play central roles in adhesion, crawling and extravasation of leukocytes across endothelium [15]. We previously reported that integrin-blocking monoclonal antibodies (mAbs) non-significantly impacted the migration of parasitised DCs in a 3D collagen matrix [25]. Here, to assess the putative implication of CAMs and integrins on the motility of parasitised DCs on endothelium, analyses were first performed in presence of blocking mAbs. As expected, blockade of $\beta 1$ and $\beta 2$ integrins reduced the motility of parasitised DCs on collagen-coated surfaces (2D), with a non-significant impact by CAM blockade and antibody isotype controls (**Fig. 3a**). In sharp contrast, on bEnd.3 cell monolayers, blockade of ICAM-1 and $\beta 1$ and $\beta 2$ integrins conversely elevated the motility of *T. gondii*-challenged DCs (**Fig. 3b**). Blockade of PECAM-1, $\alpha 4\beta 1$ integrin and isotype controls non-significantly impacted DC motility (**Fig. 3b**). Thus, ICAM-1- and $\beta 1$ - and $\beta 2$ - integrin blockades had opposite effects on DC velocities on collagen and endothelial cell monolayers (**Fig. 3c**). These dramatic and seemingly contraposed motility effects motivated further analyses of parasitised DCs under shear stress.

Adhesion and motility of *T. gondii*-challenged DCs under flow conditions

To assess DC motility under more physiological conditions, unchallenged or *T. gondii*-challenged DCs were tracked under shear stress in flow chambers coated with collagen or bEnd.3 cells (**Fig. 4a, b**). On collagen, the numbers of adhered DCs were reduced upon *T. gondii* challenge (**Fig. 4c**). The mean pathlength of *T. gondii*-challenged DCs (related to

unchallenged) was increased, and their mean velocity was elevated following 0.2 dyn/cm² shear stress conditions (**Fig. 4d, e**). However, non-significant differences were observed between unchallenged and *T. gondii*-challenged DCs at 1 dyn/cm², indicating detachment at higher shear stress (**Fig. 4d, e**). In contrast, on bEnd.3 cell monolayers, adhesion of *T. gondii*-challenged DCs was elevated compared with unchallenged DCs following 0.2 dyn/cm² flow, with non-significant differences after 1 dyn/cm² (**Fig. 4f**). While pathlengths and velocities remained high compared to collagen condition, non-significant differences in pathlength or velocity were observed between unchallenged and *T. gondii*-challenged DCs (**Fig. 4g, h**), in line with observations under static conditions (Fig 2c). Thus, under shear stress, collagen and endothelium had opposite effects on the adhesion of parasitised DCs by reducing and elevating numbers of adhered cells, respectively, related to unchallenged DCs (**Fig. 4i**). Moreover, the pathlengths of adherent parasitised DCs were elevated in collagen but not on endothelium at low shear stress, related to unchallenged DCs (**Fig. 4j**). Altogether, this extends and corroborates results under static conditions (Figs. 2 and 3). Jointly, the data indicate that the *T. gondii*-induced hypermotility of DCs is maintained on collagen under low shear stress but is abolished upon binding of parasitised DCs to the endothelium. Nonetheless, adherent parasitised DCs were motile on endothelial monolayers.

Implication of CAMs and integrins in the transmigration of *T. gondii*-challenged DCs across endothelium

Because CAMs and integrins play a central role in the extravasation of leukocytes across endothelium [12], we assessed the effects of CAM and integrin blockade on the TEM of unchallenged and *T. gondii*-challenged DCs across polarised bEnd.3 cell monolayers. First, we performed a transcriptional profiling for relevant integrins and CAMs (**Supplementary Fig. S2**) and controlled that the treatments non-significantly impacted permeability (**Fig. 5a, b**) and TEER (**Fig. 5c, d**). Interestingly, blockade of ICAM-1, PECAM-1, $\alpha 4\beta 1$, $\beta 1$ and $\beta 2$ integrins significantly reduced the TEM frequency of *T. gondii*-challenged DCs (**Fig. 5e**), but non-significantly impacted the TEM frequency of unchallenged DCs (**Fig. 5f**). Jointly with motility assays (Fig. 3), this indicates that parasitised DCs utilise integrin and CAM interactions to adhere and perform TEM, which is potentiated by *T. gondii* infection.

Gene silencing of talin-1 (*Tln1*) or of $\beta 1$ integrin (*Itgb1*) impacts motility and TEM of *T. gondii*-infected DCs on endothelium

Consistent with paradigms of DC migration [5], we previously showed that amoeboid migration of parasitised DCs in collagen matrix is an integrin-independent process [25]. However, extravasation of DCs across endothelium is integrin-dependent [15] and integrins contribute to the migration of parasitised DCs [29]. To further assess the role of integrins in motility and TEM of *T. gondii*-infected primary DCs, we gene silenced the integrin-linking protein talin (Tln1) and β 1 integrin (ITGB1). In transduced DCs, significant reductions in *Tln1* and *Itgb1* mRNA expression, respectively, were measured, related to control shLuc-transduced DCs (**Fig. 6a, b**). First, to confirm the impact of gene silencing on integrin-mediated motility, transduced cells were challenged with *T. gondii* and allowed to migrate on collagen-coated surfaces (**Supplementary Fig. S3**). Motility and mean velocities of shITGB1 and Tln1-transduced *T. gondii*-infected DCs were significantly reduced on collagen (**Fig. 6c, d**). In contrast, on bEnd.3 cell monolayers, shITGB1- and Tln1-transduced *T. gondii*-infected DCs exhibited increased velocities compared to mock and shLuc-transduced cells (**Fig. 6e, f**). Importantly, the TEM frequency of *T. gondii*-challenged DCs was significantly decreased in shITGB1 and shTln1-transduced cells (**Fig. 6i**) compared to shLuc-transduced cells. We conclude that gene silencing of talin-1 (*Tln1*) or of β 1 integrin (*Itgb1*) inhibits hypermotility and TEM but elevates the motility of *T. gondii*-infected DCs on endothelium.

Discussion

The interconversion between migratory states of leukocytes and the crosstalk between CAMs and integrins are crucial for diapedesis [15]. Here, we explored the interplay between endothelium and *T. gondii*-challenged DCs and found that intracellular parasitisation impacts migration in a cell environment-related fashion, with a pivotal role for integrins.

We report that infection with *T. gondii* induces dramatic migratory changes in primary DCs, which impact on locomotion, adhesion and enhanced transmigration across polarised endothelial cell monolayers. Upon *T. gondii* infection, DCs undergo MAT in extracellular matrix and enhanced amoeboid migration -termed hypermotility- [25,23,29,30], which promotes parasite dissemination *in vivo* [21,27]. Surprisingly, we found that the hypermotility of parasitised DCs in 3D and 2D collagen confinements (**Fig 7a, b**) is lost upon direct contact with endothelium (**Fig. 7c**), and that the mean velocities and migrated distances became comparable between unchallenged and *T. gondii*-infected

201 DCs. The reduction of motility of DCs on endothelium suggests instead, arrest and
202 adhesion, which is regulated by integrin-CAM crosstalk [33,15], and precedes leukocyte
203 TEM (**Fig 7d**).

204 Under shear stress conditions, parasitised DCs elevated or maintained adhesion to
205 endothelium, while adhesion was conversely reduced on collagen. The elevated adhesion
206 on endothelium is in contrast with the MAT-related morphological changes that parasitised
207 DCs present on collagen and in collagen matrix, including round-cell morphology,
208 decreased adhesion and redistribution of integrins [24,25,23]. Moreover, under flow
209 conditions, the pathlengths of parasitised DCs were maintained high with reduced
210 velocities, indicating crawling of DCs [15]. *In vivo*, leukocyte diapedesis mainly occurs at
211 the postcapillary venules of inflamed tissue, at low shear stress levels [12]. Further,
212 exposure to shear stress can increase transcription levels of ICAM-1, PECAM-1 and other
213 endothelial CAMs [11], which interact with leukocyte integrins for diapedesis. This
214 motivated a further investigation of the role of integrins.

215 We demonstrate that integrin signalling plays a pivotal role in the infection-related
216 arrested motility of DCs on endothelium. Interestingly, disruption of the integrin-CAM
217 interaction by blockade of $\beta 1$ and $\beta 2$ integrins, along with ICAM-1, partially restored the
218 hypermotility of infected DCs on endothelium. In contrast, on collagen-coated surfaces,
219 the hypermotility phenotype in *T. gondii*-infected DCs was reduced upon $\beta 1$ and $\beta 2$
220 integrin blockade, and gene silencing of $\beta 1$ integrin or talin. We recently reported a role
221 for $\beta 1$ integrin in a motogenic TIMP1-CD63-ITGB1-FAK signalling axis associated with
222 MAT [29]. Here, we add that $\beta 1$ integrin is equally important for, presumably, amoeboid-
223 to-mesenchymal migratory conversion and adhesion to endothelium. Not in contraposition
224 with the above, amoeboid migration of DCs is integrin-independent in ECM [5], and
225 integrin-blocking antibodies non-significantly impacted the migration of *T. gondii*-infected
226 DCs in a 3D collagen matrix [25]. More recent studies have also shown that talin-deficient
227 T cells, lacking integrin-based force transmission, are unable to adhere to or migrate on 2D
228 surfaces [34]. Similarly, the switch of cancer cells between amoeboid and mesenchymal
229 migration modes appears to be advantageous in tumour invasion, depending on their
230 extracellular environment [35]. Migrating leukocytes have the ability to slow down or
231 speed up migration in seconds, switching from high-speed actin-flow driven motility, to
232 slow adhesion-driven motility mediated by integrins [4]. The difference in motility
233 observed between parasitised DCs on collagen versus endothelium, implies a pivotal role
234 for integrins in each scenario. It also reflects the ability of DCs to switch between different

235 modes of migration depending on the extracellular environment (**Fig. 7**). On the one hand,
236 infection-induced hypermotility in matrix is linked to increased dissemination [27,28,22],
237 but upon reaching barriers such as the BBB, adhesion is necessary for TEM and thus
238 facilitates parasite dissemination across the vasculature.

239 We demonstrate that integrins contribute to the enhanced TEM of *T. gondii*-infected
240 DCs across polarised primary brain endothelial cell monolayers (**Fig. 7d**). Interestingly,
241 transmigration occurred without measurable perturbation of polarisation or disruption of
242 the cell barrier integrity, in line with previous reports [36] and indicative of regulated cell-
243 cell interactions. Indeed, the TEM of infected DCs was reduced upon blockade of adhesion
244 molecules, ICAM-1 and PECAM-1, and $\beta 1$ and $\beta 2$ integrins. Moreover, gene silencing of
245 the integrin-cytoskeleton linker talin in DCs demonstrated a crucial role for integrins.
246 Taken together with the motogenic role of $\beta 1$ integrin [29], here we add a role in adhesion
247 to endothelium and TEM. Thus, while $\beta 2$ integrins contribute, we identify a pivotal role
248 for $\beta 1$ integrins. Jointly, this shows that, potentiated by *T. gondii* infection, integrin-CAM
249 interactions mediated TEM. It remains largely unknown how intracellular pathogens
250 modulate the migration modes of shuttle-leukocytes to enter the CNS, especially initially
251 during infection in the absence of CNS inflammation [37]. However, integrin-CAM
252 interactions are likely to be in play, as shown here for *T. gondii*-infected DCs. A recent
253 report using a fungal infection model in mice showed that $\alpha 4 \beta 1$ integrin (VLA-4) mediated
254 recruitment of infected monocytes to the BBB, which subsequently migrated to the brain
255 parenchyma [38]. In HIV infection, disrupting the interaction between $\alpha L \beta 2$ (LFA-1) on
256 $CD4^+$ T cells and ICAM-1 on DCs by mAbs inhibited viral transmission between T cells
257 and DCs [39,40], and thus similar interactions may be implicated at the BBB. Beyond
258 infection, the pathogenesis of multiple sclerosis implicates a recruitment of DCs and T cells
259 to the CNS [41] and has opened up for mAb-based therapies targeting integrins, for
260 example $\alpha 4 \beta 1$ [42]. Of note, blockade of ICAM-1 in our study, likely has a synergistic
261 effect of blocking both DC and endothelial cell ICAM-1 [43]. Reciprocally, blockade of
262 endothelial integrins may also occur [44].

263 Our data shows that the TEM frequency not only depends on the cellular
264 environment but also is related to cell type. The TEM frequency of *T. gondii*-infected
265 macrophages, derived from the same donor mice, was 10-20-fold inferior to that of DCs.
266 *T. gondii*-challenged monocytic cells have been reported to exhibit hypermotility but
267 reduced transmigration [45,46], contrasting with the elevated transmigration of parasitised
268 DCs and microglia [21,47]. Further, elevated TEM was observed in infected rat peripheral

269 blood mononuclear cells (PBMCs) [48] and, under shear stress conditions on HUVEC
270 monolayers, the motility and TEM frequencies of infected monocytes increased compared
271 to static conditions [49,50]. Altogether, this highlights that the cellular environment likely
272 impacts migratory responses in a cell type-related fashion.

273 In experimental mouse toxoplasmosis, the transportation of *T. gondii* by DCs and
274 other monocytic cells in the circulation has been associated with higher parasitic loads in
275 the CNS [20,21]. DCs migrate into the brain parenchyma in an integrin-dependent fashion
276 during toxoplasmosis [51]. However, it remains unclear if DCs can transport *T. gondii* into
277 the CNS, as extracellular parasites can also invade and transmigrate across endothelium
278 [52,32]. Here, we provide *in vitro* proof-of-concept that transmigration of DCs across
279 highly-polarised primary endothelium is potentiated by infection. In a broader context,
280 leukocytes can be activated to perform diapedesis by chemotactic signalling [53].
281 However, activation does not automatically imply enhanced TEM. For example, upon LPS-
282 stimulation, both reduced and elevated TEM by leukocytes have been reported [54-56].
283 The role of chemokines in homing of leukocytes to the CNS remains partly unelucidated
284 [57]. However, the initial penetration of *T. gondii* to the CNS likely happens in absence of
285 generalized inflammation [58]. We have shown that *T. gondii* infection induces amoeboid
286 migration of DCs and other mononuclear phagocytes by alternative signaling pathways
287 including GABAergic signaling and MAP kinase activation [22,30], which can synergise
288 with chemotaxis *in vitro* [27,24]. Thus, we postulate that the infection-related enhanced
289 TEM shown here *in vitro* could, synergistically with chemotactic and inflammatory cues,
290 facilitate the diapedesis of parasitised DCs to the brain parenchyma. The elucidation of
291 alternative activation pathways that can drive leukocyte TEM will further our knowledge
292 on how leukocytes and other migratory cells regulate their migratory states for vital
293 biological processes.

294

295 **Materials and Methods**

296 **Ethical statement.** The Regional Animal Research Ethical Board, Stockholm, Sweden,
297 approved protocols involving extraction of cells from mice, following proceedings
298 described in EU legislation (Council Directive 2010/63/EU).

299

300 **Parasite culture and cell lines.** The GFP or RFP-expressing ME49 *T. gondii* strains (type
301 II) [59,60] were maintained by serial 48 h passaging in human foreskin fibroblasts (HFFs).
302 HFFs were cultured in Dulbecco's modified Eagle's medium (DMEM, Thermofisher
303 scientific) with 10% heat inactivated fetal bovine serum (FBS, Sigma), gentamicin (20
304 µg/ml, Gibco), L-glutamine (2 mM, Gibco) and HEPES (10 mM, Gibco), referred to as
305 D10. bEnd.3 cells (CRL-2299, American Type Culture Collection) were cultured in D10.
306 All cell cultures and parasites were grown in a humidified atmosphere containing 5% CO₂
307 at 37°C.

308

309 **Primary dendritic cells (DCs) and macrophages.** Murine bone marrow-derived DCs
310 were generated as previously described [27]. Briefly, cells from bone marrow of 6-10 week
311 old male or female C57BL/6NCrl mice (Charles River) were cultivated in RPMI 1640 with
312 10% fetal bovine serum (FBS), gentamicin (20 µg/ml), glutamine (2mM) and HEPES (0.01
313 M), referred to as complete medium (CM), and supplemented with 10 ng/ml recombinant
314 mouse GM-CSF (Peprotech). Loosely adherent cells (DCs) or adherent cells
315 (macrophages) were harvested after 6 or 8 days of maturation.

316

317 **Primary murine brain endothelial cells (MBECs).** Six to 8-week old C57BL/6NCrl
318 mice, housed under specific pathogen-free conditions at Stockholm University, were
319 euthanised and brains were extracted. The brain tissue was freed from the cerebellum,
320 hypothalamus, olfactory bulb and kept in ice-cold PBS. Preparation of cells was performed
321 as described [61], with modifications [32]. The tissue was digested in collagenase IV (1
322 mg/ml, Gibco) for 1 h at 37° C, homogenised and washed with wash buffer (PBS, 0.5%
323 FBS and 2mM EDTA). The tissue suspension was mixed with density gradient medium.
324 Percoll (Easycoll, Biochrom) in PBS was added to the tissue suspension at a final
325 concentration of 30% and centrifuged for 30 min at 500 g. After removal of the myelin on
326 the top layer, the cell pellet was washed with wash buffer. A second digestion was carried
327 out by incubating the cell pellet with collagenase IV for 30 min at 37° C. Positive selection

of CD31-expressing cells was performed by magnetic activated cell sorting (MACS, Myltenyi Biotec) and CD31 MicroBeads (Myltenyi Biotec), according to the manufacturer's instructions. CD31⁺-enriched cell suspensions were finally plated onto transwell filters (8 µm pore size; BD Biosciences) at cell yield from 1 brain per 4 transwell inserts pre-coated with 0.1% gelatin (Gibco), or in 12-well culture plates and monitored for confluence and polarisation. Cells were cultured in EBM-2/EGM-2 medium (Lonza) with 12% FBS, glutamine (200 mM, Gibco) and growth supplements (Bulletkit™, Lonza) minus the vascular endothelial growth factor (mVEGF) supplement. EBM-2/EGM-2 medium was supplemented with puromycin (1 mg/ml, Gibco) for 3 d, after which medium was changed every 2 d in absence of puromycin. The cellular monolayers had high and stable expression of PECAM1/CD31 (>99%), TJ markers ZO-1, occludin and claudin-5, with low expression of astrocyte marker GFAP (<1%), as previously characterised [32].

340

Polarisation parameters: permeability assay and transendothelial electrical resistance (TEER). bEnd.3 cells were cultured to 80% confluence then seeded onto transwells (8 µm pore size; BD Biosciences) and grown for 5 d until they reached polarisation, as defined below. MBECs were seeded directly after isolation and reached a polarisation plateau after ~ 11-12 d, which was maintained beyond 14 d, as defined below. Experiments were performed on d 13-14. For evaluation of cell monolayer permeability following treatments or transmigration, FITC-dextran (3 kDa; Life tech) was added to the upper compartment of the transwell at a concentration of 12.5 µg/ml for 90 min. Medium was collected from the lower compartment, and fluorescence was measured in a fluorometer (EnSpire Multimode Plate Reader, Perkin Elmer) at 485 nm excitation 520 nm emission.

MBECs and bEnd.3 cells were cultured to form polarised monolayers defined by a TEER $\geq 250 \Omega \cdot \text{cm}^2$, as measured using an Ohmmeter (Millipore, Bedford, MA) and correcting measurements with the formula: Unit Area Resistance (TEER) = Resistance (Ω) • Effective Membrane Area (cm^2). TEER was measured before and after transmigration or treatments. Values are shown as percentage (%) of TEER related to TEER prior to treatment or transmigration.

358

Reagents. Blocking antibodies, isotype controls and inhibitor of VLA-4 were used at 1 µg/ml: LEAF™ purified anti-CD29 (clone HMβ1-1, 102209, BioLegend), LEAF™ purified

361 anti-CD31 (clone MEC13.3, 102511, BioLegend), LEAF™ purified anti-CD18 (clone
362 M18/2, 101409, BioLegend), LEAF™ Purified Rat IgG2a, κ Isotype Ctrl (clone RTK2758,
363 400515, BioLegend), Ultra-LEAF™ Purified Armenian Hamster IgG Isotype Ctrl (clone
364 HTK888, 400969, BioLegend), BIO 5192 (R&D systems), anti-CD54 (ICAM-1; clone
365 YN1/1.7.4; eBioscience).

366

367 **Immunostainings.** *T. gondii*-challenged DCs were plated on coverslips coated with bovine
368 collagen I (1 mg/ml, Life Technologies). After fixation (4% PFA, Sigma-Aldrich), cells
369 were permeabilised (0.5% Triton X-100, Sigma-Aldrich) and stained with phalloidin Alexa
370 Fluor 595 (Invitrogen). Micrographs were generated using a 63x objective (DMi8, Leica
371 Microsystems). Primary MBECs were seeded on coverslips pre-coated with 0.1 % gelatin
372 (BioRad). Fixation and permeabilisation steps were carried out as for DCs, followed by
373 blocking (5% FBS in PBS for 2 h). Cells were then incubated with primary antibodies to
374 ZO-1 (Thermofisher) and Occludin (Thermofisher) ON at 4°C at 1:500. Cells were then
375 stained with Alexa Fluor 594-conjugated secondary antibodies (Invitrogen) and DAPI for
376 2 h, mounted and imaged by confocal microscopy (LSM 800, Zeiss).

377

378 **Transmigration assays.** Day 6-8 DCs or macrophages were challenged with freshly
379 egressed tachyzoites (ME49/PTG-GFP or ME49-RFP, MOI 2, 4 h) and then transferred to
380 transwell filters (8 µm pore size; BD Biosciences), with pre-cultured polarised monolayers
381 of bEnd.3 cells in CM or MBECs in EBM-2/EGM-2. After 16 h, transmigrated DCs were
382 put on ice for 1 h and macrophages trypsinised (TrypLE™ Express; Gibco) for 10 min to
383 disassociate adherent cells. Cells were then resuspended and counted in a Bürker chamber.

384

385 **Motility assays.** Motility assays were performed as previously described [24]. Briefly, DCs
386 were cultured with CM ± freshly egressed *T. gondii* tachyzoites (ME49/PTG-GFP or
387 ME49-RFP MOI 3, 4 h) and soluble reagents (as indicated), and then added to 96-well
388 plates pre-cultured with bEnd.3 cells or embedded in bovine collagen I (1 mg/ml, Life
389 Technologies). Live cell imaging was performed for 1 h, 1 frame/ min, at 10x magnification
390 (Z1 Observer with Zen 2 Blue v. 4.0.3, Zeiss). Time-lapse images were consolidated into
391 stacks and motility data was obtained from 30 cells/condition (Manual Tracking, ImageJ)
392 yielding mean velocities (Chemotaxis and migration tool, v. 2.0). Infected cells were
393 defined by GFP⁺ or RFP⁺ cells, as indicated.

394

395 **Flow condition assays.** DCs were cultured as stated in motility assay and then added to
396 fluidic channels (μ -Slide VI^{0.4}; Ibidi) with confluent bEnd.3 cell monolayers or pre-coated
397 with collagen I (1 mg/ml, Life Technologies), and allowed to adhere for 10 min. Phase-
398 contrast and fluorescence images were first captured in static condition. Fluidic shear stress
399 was then applied by flowing CM at 0.2 dyn/cm² through the channels. Live cell imaging
400 was immediately initiated and images acquired every 5 seconds for up to 10 min, at 10x
401 magnification. Fluidic shear stress was then increased to 1 dyn/cm² and live cell imaging
402 immediately initiated. Time-lapse images were consolidated into stacks and motility and
403 path-length data obtained from 20 cells (Manual Tracking, ImageJ) yielding mean
404 velocities and pathlengths (Chemotaxis and migration tool, v .2.0). The percentage of
405 remaining adherent cells was calculated by dividing the number of cells per frame
406 following shear stress, by the number of cells in the same frame in static condition.
407 Representative track plots were created in Python 3.6.9 (<https://www.python.org/>) with
408 pandas 1.1.5 and matplotlib 3.1.3.

409

410 **Lentiviral vector production and *in vitro* transduction.** Self-complementary hairpin
411 DNA oligos targeting *Itgb1* (shITGB1, TRCN0000066645, Genscript) or *Tln1* (shTln1,
412 TRCN0000294832, Genscript) mRNA were on self-inactivating lentiviral vectors
413 (pLL3.7) with eGFP reporter expression (**Table S1**) Transfer plasmid (shRNA targeting
414 ITGB1, Tln1 or Luc) was co-transfected with psPAX2 (12260, Addgene) packaging vector
415 and pCMV-VSVg (8454, Addgene) envelope vector into Lenti-X 293T cells (Clontech)
416 using Lipofectamine 2000 (Invitrogen). The resulting supernatant was harvested 24 h and
417 48 h post-transfection. Supernatants were centrifuged to eliminate cell debris and filtered
418 through 0.45-mm cellulose acetate filters. DCs (3 days post-bone marrow extraction) were
419 transduced by adding lentiviral supernatants in the presence of DEAE dextran (8 μ g/ml;
420 Sigma-Aldrich) to cells for 4 h. After 5-7 days, transduction efficiency was examined for
421 eGFP expression by epifluorescence microscopy (Z1 Observer with Zen 2 Blue v. 4.0.3,
422 Zeiss) followed by expression analysis by qPCR for knockdown of targeted mRNA.

423

424 **Polymerase chain reaction (PCR).** Total RNA was extracted using TRIzol reagent
425 (Sigma-Aldrich). First-strand cDNA was synthesised with Superscript IV Reverse
426 Transcriptase (Invitrogen). Real-time quantitative polymerase chain reaction (qPCR) was
427 performed using SYBR green PCR master mix (Kapa biosystems), forward and reverse

428 primers (200 nM), and cDNA (100 ng) with a QuantStudio™ 5 real-time PCR system
429 (Thermofisher). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a
430 house-keeping gene to generate ΔC_t values; $2^{-\Delta C_t}$ values were used to calculate relative
431 knock-down efficiency. All primers (Invitrogen) were designed using the Get-prime or
432 Primer-BLAST software (**Table S2**).

433

434 **Statistical analysis.** All statistics were performed with Prism software (v. 8, GraphPad).
435 In all statistical tests, values of $P \geq 0.05$ were defined as non-significant and $P < 0.05$ were
436 defined as significant.

437

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442 corresponding author on reasonable request.

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445 **Conflict of interest.** The authors declare that there is no duality of interest associated
446 with this manuscript.

447 **Author's contributions.** E.C.R performed experiments and analysed the data. All
448 authors contributed to the generation of figures and writing of this manuscript.

449

450 **Figure legends**

451 **Figure 1. Transmigration of *T. gondii*-challenged DCs across polarised MBEC and** 452 **bEnd.3 monolayers**

453 **(a)** Top cartoon shows experimental setup in a 2-chamber transwell system and assessment
454 of endothelial cell polarisation and permeability, as indicated in Materials and Methods.
455 (1) Measurement of transcellular electrical resistance (TEER), (2) *T. gondii*-challenged or
456 unchallenged cells were added to upper chamber, (3) The permeability tracer, FITC-
457 dextran (3 kDa), added to upper chamber and measured in lower chamber, (4)
458 Transmigrated cells were collected and counted. Bottom micrograph represents a DC
459 infected with GFP-expressing *T. gondii* (green) and stained for F-actin (red) and nuclei
460 (blue). Scale bar = 10 μ m.

461 **(b)** Permeability of MBEC monolayers to FITC-dextran (3 kDa) following DC
462 transmigration. Data is shown as arbitrary fluorescence units (a.u).

463 **(c)** TEER under same conditions as in (b). For each condition, TEER values ($\Omega \cdot \text{cm}^2$)
464 relative to TEER values at the initiation of the assay (100%) are indicated, as described in
465 Materials and Methods.

466 **(d)** Relative transendothelial migration (TEM) frequency of DCs across MBEC
467 monolayers shown as percentage (%) of DCs added in the upper well, normalised to
468 unchallenged DCs (set to 1.0).

469 **(e)** Permeability of bEnd.3 cell monolayers to FITC-dextran (3 kDa) following
470 transmigration of unchallenged or *T. gondii*-challenged dendritic cells (DCs) or
471 macrophages (M Φ).

472 **(f)** TEER values relative to TEER values at initiation of the assay (100%).

473 **(g)** Relative transendothelial migration (TEM) frequency of DCs or M Φ across bEnd.3
474 monolayers shown as percentage (%) of DCs or M Φ added in the upper well, normalised
475 to unchallenged DCs (set to 1.0).

476 Bar graphs represent the mean $\pm$ s.e.m of 3-4 independent experiments. * $P < 0.05$, ** $P <$
477 0.01, ns: non-significant, by one-way ANOVA with Dunnett's post-hoc test (b, c, e, f),
478 Students' *t*-test (d, g),

479

480 **Figure 2. Motility analyses of *T. gondii*-infected DCs on collagen and endothelium**

481 **(a)** Representative micrographs of unchallenged DCs and GFP-expressing *T. gondii*-
482 challenged DCs on collagen (top row) and bEnd.3 cell monolayers (bottom row). Scale bar
483 = 100 μ m, inset = 10 μ m.

484 **(b)** Representative motility plots of DCs as described and represented in (a).

485 **(c)** Mean velocity of unchallenged and *T. gondii*-infected DCs. Data represent the mean $\pm$
486 s.e.m of 3 independent experiments (n = 3). * $P < 0.05$, ns; non-significant by one-way
487 ANOVA, Sidak's post-hoc test.

488

489 **Figure 3. Motility of *T. gondii*-infected DCs upon blockade of integrins and CAMs**

490 **(a, b)** Mean velocity of unchallenged and *T. gondii*-challenged DCs treated with inhibitors
491 and antibodies as stated, on (a) collagen or (b) bEnd.3 monolayers, respectively.

492 (c) Percentage (%) change in velocity of *T. gondii*-challenged DCs compared to
493 unchallenged DCs for each condition.

494 Data are presented as mean $\pm$ s.e.m from 3 independent experiments (n = 3) * P < 0.05, ** P
495 < 0.01, ns; non-significant by one-way ANOVA, Sidak's post-hoc test.

496

497 **Figure 4. Adhesion and motility of *T. gondii*-challenged DCs under flow conditions**

498 (a, b) Representative cell traces of unchallenged or *T. gondii*-challenged DCs on (a)
499 collagen or (b) bEnd.3 cell monolayers, at indicated shear stress (dyn/cm²). Each color-
500 coded trace shows the euclidian displacement distance of a single cell.

501 (c) Percentage (%) of DCs adhered to collagen, following fluidic shear stress (dyn/cm²),
502 related to total number of DCs in the same frame in static condition as indicated under
503 Materials and Methods.

504 (d, e) Mean pathlength and velocity, respectively, of tracked cells on collagen.

505 (f) Percentage (%) of DCs adhered to bEnd.3 cell monolayers, following fluidic shear stress
506 (dyn/cm²), related to total number of DCs in the same frame in static condition.

507 (g, h) Mean pathlength and velocity, respectively of tracked cells on bEnd.3 cell
508 monolayers.

509 (i, j) Percentage change in adherence and velocity, respectively of *T. gondii*-challenged
510 DCs related to unchallenged DCs, for each condition.

511 Bar graphs show mean $\pm$ s.e.m. from 4 independent experiments (n=4). * P < 0.05, ** P <
512 0.01; ns, non-significant by one-way ANOVA, Sidaks post-hoc test (c, d, e, f, g, h) or
513 Students' *t*-test (i, j).

514

515 **Figure 5. TEM of *T. gondii*-challenged DC upon integrin CAM blockade**

516 (a, b) Permeability of bEnd.3 monolayers to FITC-dextran (3 kDa) after 16 h exposure to
517 *T. gondii*-challenged DCs or unchallenged DCs $\pm$ antibodies or inhibitors, respectively, and
518 as indicated.

519 (c, d) TEER (%) of bEnd.3 monolayers as in (a) and (b), respectively.

520 (e, f) Relative TEM (%) of *T. gondii*-challenged DCs or unchallenged DCs, respectively.
521 Relative TEM frequency of DCs across bEnd.3 monolayers are shown as percentage (%)
522 of DCs added, normalised to unchallenged DC condition for each experimental condition
523 (set to 1.0, light blue bar 'CM').

524 Data are presented as mean $\pm$ s.e.m. from 4 independent experiments (n=4). * P < 0.05, ** P
525 < 0.01, ns; non-significant by one-way ANOVA, Dunnett's post-hoc test (a-f).

526

527 **Figure 6. Impact of gene silencing of talin (*Tln-1*) and β 1 integrin (*Itgb1*) on the**
528 **motility and transmigration of *T. gondii*-challenged DCs**

529 (a) *Tln1* mRNA expression ($2^{-\Delta Ct}$) in DCs transduced with shTln1 or control shLuc
530 lentivirus relative to mock-treated cells (mock set to 1.0).

531 (b) *Itgb1* mRNA expression ($2^{-\Delta Ct}$) in DCs transduced with shITGB1 or control shLuc
532 lentivirus relative to mock-treated cells.

533 (c) Representative motility plots of mock-treated DCs and DCs transduced with lentiviral
534 vectors targeting *Itgb1* mRNA (shITGB1), *Tln1* mRNA (shTln1) or a non-expressed target
535 (shLuc) challenged with *T. gondii*, on collagen.

536 **(d)** Box-and-whisker and scattered dot plots represent median velocities ($\mu\text{m}/\text{min}$) of DCs
537 on collagen, as in (c), with boxes marking 25th to 75th percentile and whiskers marking
538 10th and 90th percentiles of the datasets. Gray circles represent velocities from individual
539 cells.

540 **(e)** Representative motility plots of mock-treated DCs and DCs transduced with lentiviral
541 vectors targeting *Itgb1* mRNA (shITGB1), *Tln1* mRNA (shTln1) or a non-expressed target
542 (shLuc) challenged with *T. gondii*, on bEnd.3 monolayers.

543 **(f)** Box-and-whisker and scattered dot plots represent median velocities ($\mu\text{m}/\text{min}$) of DCs
544 on bEnd.3, as in (e). Gray circles represent velocities from individual cells.

545 **(g, h)** Permeability to FITC-dextran (3 kDa) and TEER (%), respectively, of bEnd.3 cell
546 monolayers following DC transmigration.

547 **(i)** TEM frequency shown as percentage (%) of DCs added in the upper well.

548 Bar graphs show mean $\pm$ s.e.m from 3-4 independent experiments ($n = 3-4$) * $p < 0.05$, ** P
549 < 0.01 , *** $P < 0.001$, **** $P < 0.0001$; ns, non-significant by Students' t -test (a, b), One-
550 way ANOVA, Dunnett's post-hoc test (d, f), repeated measures one-way ANOVA, Sidak's
551 post-hoc test (i).

552

553 **Figure 7. Schematic representation of the *Toxoplasma*-induced migratory switches in**
554 **DCs leading to transendothelial migration**

555 **(a)** *T. gondii* induces rapid integrin-independent amoeboid migration (hypermotility) of
556 DCs in 3D ECM components such as collagen [25].

557 **(b)** Hypermotility of *T. gondii* infected DCs is maintained on 2D collagen, but is integrin
558 dependent, reminiscent of mesenchymal migration. Ab blockade of $\beta 1$ or $\beta 2$ integrin, or
559 gene silencing of Talin (shTln1) or $\beta 1$ integrin (shITGB1) abolishes hypermotility of *T.*
560 *gondii*-infected DCs on a 2D collagen matrix. On endothelium, integrins play a role in
561 leukocyte arrest, crawling and transmigration across endothelium [15].

562 **(c)** In contrast to collagen, on endothelium *T. gondii*-infected DCs switch to mesenchymal
563 migration and exhibit reduced motility. Ab blockade of $\beta 1$ or $\beta 2$ integrins, or gene silencing
564 of Talin (shTln1) or $\beta 1$ integrin (shITGB1) increases *T. gondii*-infected DC motility.

565 **(d)** Finally, Ab blockade of $\beta 1$ or $\beta 2$ integrins, ICAM-1, PECAM-1 or gene silencing of
566 Talin (shTln1) or $\beta 1$ integrin (shITGB1) decreases the transmigration frequency of *T.*
567 *gondii*-infected DCs across endothelium.

568

569

570 **Supplementary material**

571

572 **Supplementary Table S1. Sequences for shRNAs.**

573 **Supplementary Table S2. Sequences for qPCR primers.**

574

575 **Supplementary Figure S1. Immunostainings of endothelial tight junctions**

576 **(a)** Representative micrographs of primary MBECs stained for ZO-1 (red) and DAPI
577 stained nuclei (blue). Scale bar = 50 μ m.

578 **(b)** Representative micrographs of primary MBECs stained for Occludin (red) and DAPI
579 stained nuclei (blue). Scale bar = 50 μ m.

580

581 **Supplementary Figure S2. Transcriptional analyses of bEnd.3 cells and DCs**

582 **(a)** mRNA expression of indicated genes in bEnd.3 cells challenged with *T. gondii*,
583 unchallenged DCs, *T. gondii*-challenged DCs or LPS, relative to CM condition, after 6 h.

584 **(b)** mRNA expression of unchallenged bEnd.3 cells and DCs.

585 Bar graphs show mean $\pm$ s.e.m from 2-3 independent experiments. * $p < 0.05$, ** $P < 0.01$,
586 *** $P < 0.001$, **** $P < 0.0001$; ns, non-significant by two-way ANOVA, Dunnett's post-
587 hoc test.

588

589 **Supplementary Figure S3. Transductions of primary DCs with shRNAs**

590 Transductions were performed as indicated under Materials and Methods. **(a-d)** For each
591 condition, representative micrographs show unchallenged DCs (left) and *T. gondii*-
592 challenged DCs (right), mock-treated or treated with control shRNA (shLuc) or shRNA to
593 indicated target gene. Transduced cells were distinguished by expression of the reporter
594 eGFP (green) and *T. gondii* by expression of RFP (red). Infected cells were defined as
595 green + red cells (indicated by black arrows). Scale bar = 100 μ m.

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Figures

Figure 1

Transmigration of *T. gondii*-challenged DCs across polarised MBEC and bEnd.3 monolayers (a) Top cartoon shows experimental setup in a 2-chamber transwell system and assessment of endothelial cell polarisation and permeability, as indicated in Materials and Methods. (1) Measurement of transcellular

electrical resistance (TEER), (2) *T. gondii*-challenged or unchallenged cells were added to upper chamber, (3) The permeability tracer, FITC-dextran (3 kDa), added to upper chamber and measured in lower chamber, (4) Transmigrated cells were collected and counted. Bottom micrograph represents a DC infected with GFP-expressing *T. gondii* (green) and stained for F-actin (red) and nuclei (blue). Scale bar = 10 μ m. (b) Permeability of MBEC monolayers to FITC-dextran (3 kDa) following DC transmigration. Data is shown as arbitrary fluorescence units (a.u.). (c) TEER under same conditions as in (b). For each condition, TEER values ($\Omega \cdot \text{cm}^2$) relative to TEER values at the initiation of the assay (100%) are indicated, as described in Materials and Methods. (d) Relative transendothelial migration (TEM) frequency of DCs across MBEC monolayers shown as percentage (%) of DCs added in the upper well, normalised to unchallenged DCs (set to 1.0). (e) Permeability of bEnd.3 cell monolayers to FITC-dextran (3 kDa) following transmigration of unchallenged or *T. gondii*-challenged dendritic cells (DCs) or macrophages ($\text{M}\Phi$). (f) TEER values relative to TEER values at initiation of the assay (100%). (g) Relative transendothelial migration (TEM) frequency of DCs or $\text{M}\Phi$ across bEnd.3 monolayers shown as percentage (%) of DCs or $\text{M}\Phi$ added in the upper well, normalised to unchallenged DCs (set to 1.0). Bar graphs represent the mean $\pm$ s.e.m of 3-4 independent experiments. * $P < 0.05$, ** $P < 0.01$, ns: non-significant, by one-way ANOVA with Dunnett's post-hoc test (b, c, e, f), Students' t-test (d, g),

Figure 2

Figure 2

Motility analyses of *T. gondii*-infected DCs on collagen and endothelium (a) Representative micrographs of unchallenged DCs and GFP-expressing *T. gondii*-challenged DCs on collagen (top row) and bEnd.3 cell monolayers (bottom row). Scale bar = 100 μm , inset = 10 μm . (b) Representative motility plots of DCs as described and represented in (a). (c) Mean velocity of unchallenged and *T. gondii*-infected DCs. Data

represent the mean $\pm$ s.e.m of 3 independent experiments (n = 3). *P < 0.05, ns; non-significant by one-way ANOVA, Sidak's post-hoc test.

Figure 3

Motility of *T. gondii*-infected DCs upon blockade of integrins and CAMs (a, b) Mean velocity of unchallenged and *T. gondii*-challenged DCs treated with inhibitors and antibodies as stated, on (a) collagen or (b) bEnd.3 monolayers, respectively. (c) Percentage (%) change in velocity of *T. gondii*-

challenged DCs compared to unchallenged DCs for each condition. Data are presented as mean $\pm$ s.e.m from 3 independent experiments (n = 3) *P < 0.05, **P < 0.01, ns; non-significant by one-way ANOVA, Sidak's post-hoc test.

Figure 4

Figure 4

Adhesion and motility of *T. gondii*-challenged DCs under flow conditions (a, b) Representative cell traces of unchallenged or *T. gondii*-challenged DCs on (a) collagen or (b) bEnd.3 cell monolayers, at indicated

shear stress (dyn/cm²). Each color-coded trace shows the euclidian displacement distance of a single cell. (c) Percentage (%) of DCs adhered to collagen, following fluidic shear stress (dyn/cm²), related to total number of DCs in the same frame in static condition as indicated under Materials and Methods. (d, e) Mean pathlength and velocity, respectively, of tracked cells on collagen. (f) Percentage (%) of DCs adhered to bEnd.3 cell monolayers, following fluidic shear stress (dyn/cm²), related to total number of DCs in the same frame in static condition. (g, h) Mean pathlength and velocity, respectively of tracked cells on bEnd.3 cell monolayers. (i, j) Percentage change in adherence and velocity, respectively of T. gondii-challenged DCs related to unchallenged DCs, for each condition. Bar graphs show mean±s.e.m. from 4 independent experiments (n=4). * P < 0.05, ** P < 0.01; ns, non-significant by one-way ANOVA, Sidaks post-hoc test (c, d, e, f, g, h) or Students' t-test (i, j).

Figure 5

Figure 5

TEM of *T. gondii*-challenged DC upon integrin CAM blockade (a, b) Permeability of bEnd.3 monolayers to FITC-dextran (3 kDa) after 16 h exposure to *T. gondii*-challenged DCs or unchallenged DCs $\pm$ antibodies or inhibitors, respectively, and as indicated. (c, d) TEER (%) of bEnd.3 monolayers as in (a) and (b), respectively. (e, f) Relative TEM (%) of *T. gondii*-challenged DCs or unchallenged DCs, respectively. Relative TEM frequency of DCs across bEnd.3 monolayers are shown as percentage (%) of DCs added,

normalised to unchallenged DC condition for each experimental condition (set to 1.0, light blue bar 'CM'). Data are presented as mean±s.e.m. from 4 independent experiments (n=4). *P < 0.05, **P< 0.01, ns; non-significant by one-way ANOVA, Dunnett's post-hoc test (a-f).

Figure 6

Impact of gene silencing of talin (Tln-1) and $\alpha 1$ integrin (Itgb1) on the motility and transmigration of *T. gondii*-challenged DCs (a) Tln1 mRNA expression ($2^{-\Delta\Delta Ct}$) in DCs transduced with shTln1 or control shLuc

lentivirus relative to mock-treated cells (mock set to 1.0). (b) *Itgb1* mRNA expression ($2^{-\Delta Ct}$) in DCs transduced with shITGB1 or control shLuc lentivirus relative to mock-treated cells. (c) Representative motility plots of mock-treated DCs and DCs transduced with lentiviral vectors targeting *Itgb1* mRNA (shITGB1), *Tln1* mRNA (shTln1) or a non-expressed target (shLuc) challenged with *T. gondii*, on collagen. (d) Box-and-whisker and scattered dot plots represent median velocities ($\mu\text{m}/\text{min}$) of DCs on collagen, as in (c), with boxes marking 25th to 75th percentile and whiskers marking 10th and 90th percentiles of the datasets. Gray circles represent velocities from individual cells. (e) Representative motility plots of mock-treated DCs and DCs transduced with lentiviral vectors targeting *Itgb1* mRNA (shITGB1), *Tln1* mRNA (shTln1) or a non-expressed target (shLuc) challenged with *T. gondii*, on bEnd.3 monolayers. (f) Box-and-whisker and scattered dot plots represent median velocities ($\mu\text{m}/\text{min}$) of DCs on bEnd.3, as in (e). Gray circles represent velocities from individual cells. (g, h) Permeability to FITC-dextran (3 kDa) and TEER (%), respectively, of bEnd.3 cell monolayers following DC transmigration. (i) TEM frequency shown as percentage (%) of DCs added in the upper well. Bar graphs show mean $\pm$ s.e.m from 3-4 independent experiments ($n = 3-4$) * $p < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$; ns, non-significant by Student's *t*-test (a, b), One-way ANOVA, Dunnett's post-hoc test (d, f), repeated measures one-way ANOVA, Sidak's post-hoc test (i).

Figure 7

Figure 7

Schematic representation of the *Toxoplasma*-induced migratory switches in DCs leading to transendothelial migration (a) *T. gondii* induces rapid integrin-independent amoeboid migration (hypermotility) of DCs in 3D ECM components such as collagen [25]. (b) Hypermotility of *T. gondii* infected DCs is maintained on 2D collagen, but is integrin dependent, reminiscent of mesenchymal migration. Ab blockade of β1 or β2 integrin, or gene silencing of Talin (shTln1) or β1 integrin (shITGB1)

abolishes hypermotility of *T. gondii*-infected DCs on a 2D collagen matrix. On endothelium, integrins play a role in leukocyte arrest, crawling and transmigration across endothelium [15]. (c) In contrast to collagen, on endothelium *T. gondii*-infected DCs switch to mesenchymal migration and exhibit reduced motility. Ab blockade of $\beta 1$ or $\beta 2$ integrins, or gene silencing of Talin (shTln1) or $\beta 1$ integrin (shITGB1) increases *T. gondii*-infected DC motility. (d) Finally, Ab blockade of $\beta 1$ or $\beta 2$ integrins, ICAM-1, PECAM-1 or gene silencing of Talin (shTln1) or $\beta 1$ integrin (shITGB1) decreases the transmigration frequency of *T. gondii*-infected DCs across endothelium.

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