1 The UPR sensor IRE1 α promotes dendritic cell responses to control *Toxoplasma*
2 *gondii* infection.

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 18 **Key words:** UPR, *Toxoplasma gondii*, dendritic cells, cytokines, antigen presentation.

19
 20 **Abstract**

21 The unfolded protein response (UPR) has emerged as a central regulator of
 22 immune cell responses in several pathologic contexts including infections.
 23 However, how intracellular residing pathogens modulate the UPR in dendritic
 24 cells (DCs) and thereby affect T cell-mediated immunity remains
 25 uncharacterized. Here we demonstrate that infection of DCs by *Toxoplasma*
 26 *gondii* (*T. gondii*) triggers a unique UPR signature hallmarked by a MyD88-
 27 dependent activation of the IRE1 α pathway and an inhibition of the ATF6
 28 pathway. Induction of XBP1s controls pro-inflammatory cytokine secretion in
 29 infected DCs while IRE1 α promotes MHCI antigen presentation of secreted
 30 parasite antigens. In mice, infection leads to a specific activation of the IRE1 α

31 pathway, which was restricted to the cDC1 subset. Mice deficient for IRE1 α and
32 XBP1 in DCs display a severe susceptibility to *T. gondii* and succumb during the
33 acute phase of the infection. This early mortality was correlated with increased
34 parasite burden and a defect in splenic T cell responses. Thus, we identify the
35 IRE1 α /XBP1s branch of the UPR as a key regulator of host defenses upon *T.*
36 *gondii* infection.

37

38 **Introduction**

39 Many chronic infections are caused by intracellular microorganisms, which actively
40 manipulate host responses to escape innate immune defenses. There is growing
41 evidence that activation of appropriate antimicrobial activities of infected immune
42 cells relies both on the detection of microbial compounds by Pattern-Recognition-
43 Receptors (PRRs) and on efficient sensing of variations in cellular homeostasis
44 induced by the intracellular pathogen. An essential stress-sensing pathway is the
45 unfolded protein response (UPR). In metazoans, the UPR is activated by the
46 coordinated action of three endoplasmic reticulum (ER) transmembrane sensors:
47 Inositol-Requiring Enzyme 1 α (IRE1 α), PKR-like ER Kinase (PERK) and Activating
48 Transcription Factors 6 α / β (ATF6 α / β). The downstream transcriptional programs of
49 the UPR are primarily directed towards restoring proteostasis by increasing ER
50 capacity as well as decreasing protein load, through transient translational inhibition
51 and the process of ER-associated peptide degradation (ERAD) (Walter & Ron, 2011).
52 IRE1 α , the most conserved UPR sensor, contains a serine/threonine kinase domain
53 and an endoribonuclease domain. Upon sensing unfolded proteins, IRE1 α
54 oligomerizes, is activated by autophosphorylation, and uses its endoribonuclease
55 activity to excise an intron from the transcription factor XBP1 (X-box Binding
56 Protein). This unconventional splicing reaction of the *Xbp1* mRNA generates the
57 active transcription factor XBP1 spliced (XBP1s) (Yoshida *et al*, 2001). In addition to
58 splicing XBP1, upon prolonged or severe stress, IRE1 α degrades mRNAs in proximity
59 to the ER in a process termed regulated IRE1-dependent decay (RIDD) of mRNA
60 (Hollien *et al*, 2009). XBP1s and ATF6 α up-regulate the expression of genes involved
61 in increasing the protein-folding capacity of the ER (chaperones) as well as ER

62 expansion through phospholipid synthesis (Shoulders *et al*, 2013; Lee *et al*, 2008;
63 Sriburi *et al*, 2007). However, upon irremediable ER stress, the UPR switches to a
64 pro-apoptotic mode by PERK-mediated activation of CHOP expression. During the
65 last decade, a growing number of studies has demonstrated an intricate molecular
66 crosstalk between the immune and UPR pathways, opening new perspectives to
67 treat infections and chronic inflammatory diseases by UPR modulators (Grootjans *et*
68 *al*, 2016; Osorio *et al*, 2018; Reverendo *et al*, 2019). Notably, TLR stimulation of
69 macrophages and dendritic cells (DCs) contributes to XBP1s and CHOP induction that
70 directly activate the transcription of genes encoding pro-inflammatory cytokines,
71 such as IL-6, TNF- α , IFN- β and IL-23 (Cubillos-Ruiz *et al*, 2015; Márquez *et al*, 2017;
72 Martinon *et al*, 2010; Mogilenko *et al*, 2019; Smith, 2018). The IRE1 α /XBP1s arm of
73 the UPR is also a key regulator of antigen presentation in DCs via partially
74 characterized mechanisms involving XBP1s-mediated modulation of lipid synthesis
75 (Cubillos-Ruiz *et al*, 2015) and RIDD-mediated regulation of the antigen presentation
76 machinery (Medel *et al*, 2018; Osorio *et al*, 2014, 2018). Several pathogens interfere
77 with the function of the host ER as part of their life cycle, and can selectively activate
78 or repress distinct arms of the UPR (Celli & Tsolis, 2015; Galluzzi *et al*, 2017; Smith,
79 2018). UPR activation can be beneficial to the host by enabling the establishment of
80 an appropriate innate response directed against the invading pathogen (Celli &
81 Tsolis, 2015; Galluzzi *et al*, 2017; Smith, 2018). However, intracellular pathogens can
82 also selectively modulate UPR pathways to promote their own survival and growth.
83 *Toxoplasma gondii* (*T. gondii*) can be considered as an opportunist parasite as severe
84 symptoms develop in immuno-suppressed patients. However, infection of immuno-
85 competent individuals by type II strains is characterized by bradyzoite-containing
86 cyst development in the brain, leading to life-long chronic infection (Blanchard *et al*,
87 2015). *T. gondii* is a very successful intracellular parasite able to invade any
88 nucleated cells, including immune cells, in which the parasite rapidly replicates
89 within a vacuole. To enable nutrient retrieval from the host and efficient escape
90 from cellular defenses, the parasite secrete numerous effectors that modulate host
91 cell signaling pathways (Hakimi *et al*, 2017). In addition, parasite effectors anchored
92 at the limiting membrane of the vacuole promote recruitment of host organelles,
93 such as mitochondria (Pernas *et al*, 2014, 2018), the Golgi apparatus (Deffieu *et al*,

2019) and the ER (Goldszmid *et al*, 2009; Sinai *et al*, 1997). The impact of *T. gondii* induced ER association with the parasitophorous vacuole (PV) on ER homeostasis has not been investigated. Here we demonstrate that infection of DCs by the parasite induces a specific UPR signature, mainly characterized by a MyD88 dependent activation of the IRE1 α /XBP1s pathway. In addition, the presence of live parasites actively modulates specific branches of the UPR, in contrast to cell stimulated with heat-killed parasites or soluble *T. gondii* antigens. In infected BMDCs, induction of XBP1s leads to enhanced IL-6 and IL-12 secretion while IRE1 α promotes MHCI antigen presentation independently of XBP1s induction. Moreover, activation of the IRE1 α /XBP1s pathway in DC is required to promote the establishment of an efficient T cell response and to control parasite dissemination in infected mice.

Results

T. gondii induces IRE1 α /XBP1s activation in BMDCs

To monitor whether *T. gondii* infection activates the ER stress response, we infected bone marrow derived DCs (BMDCs) with type II Pru parasites for 16h and analyzed IRE1 α , PERK and ATF6 pathway activation by measuring the expression of known down-stream targets. As a positive control, we stimulated cells with tunicamycin (TN), a potent pharmacologic ER-stress inducer. In comparison with TN, *T. gondii* infection led to only partial induction of specific UPR branches (Fig 1A and Appendix Fig S1). Notably, infection did not induce a classic ER-stress signature as illustrated by the lack of induction of the ER chaperones *Hsp90b1* (encoding GRP94) and *Sec61a1* and the slight induction of *Hspa5* (encoding BIP) compared to TN stimulation (Fig 1A, 1B and Appendix Fig S1). On the contrary, the IRE1 α branch was induced as illustrated by the increased expression level of *Ern1* (encoding IRE1 α) and *Xbp1s* and its target genes *Dnajb9* (encoding ERdj4) and *Sec24d* (Fig 1A-B and Appendix Fig S1). RIDD was not activated, as demonstrated by a lack of degradation of the prototypical RIDD target *Bloc1s1* mRNA upon *T. gondii* infection (Fig 1B) (Bright *et al*, 2015, Hollien *et al*, 2009). Western blot analysis of *T. gondii* infected BMDCs revealed an increasing expression in IRE1 α levels with time and the induction of the spliced

version of XBP1, which reached its maximal level at 4h (Fig 1C). Sustained activation of IRE1 α and transient increase in XBP1s expression was confirmed in so-called Flt3/Notch DCs, an alternative system to generate cDC1s *in vitro* (Kirkling *et al*, 2018) (Fig 1D). The PERK branch appeared not activated, neither at mRNA neither at protein level. PERK down-stream effectors ATF4 and CHOP (encoded by *Ddit3*) were not induced, in contrast to TN stimulation (Fig 1A, 1B and Appendix Fig S1). In line with this, phosphorylation of PERK and its target eIF2 α appeared decreased compared to steady state levels, and CHOP levels remained undetectable in infected BMDCs and Flt3/Notch DCs (Fig 1C and 1D). As reported before, splenic DCs also display high level of p-eIF2 α at steady state suggesting that control of protein translation is essential to regulate DC homeostasis (Clavarino *et al*, 2012). GADD34 interacts with the phosphatase protein 1 (PP1) to dephosphorylate eIF2 α to alleviate the protein translation block (Clavarino *et al*, 2012; Novoa *et al*, 2001). *Ppp1r15a* (encoding GADD34) gene expression was strongly increased in *T. gondii* infected BMDCs, consistent with a presumable role of GADD34 in the inhibition of eIF2 α phosphorylation (Fig 1B). Finally, *T. gondii* infection caused a dramatic down-regulation of the *Atf6* gene and its target gene *Herpud1* (Fig 1A and 1B), suggesting that the parasite actively modulates the ATF6 pathway in DCs, as proposed in a previous study (Yamamoto *et al*, 2011). In summary, infection of *T. gondii* leads to a mixed outcome on UPR signaling branches with a down-regulation of the ATF6 branch and an activation of the IRE1/XBP1 branch.

Induction of XBP1s is under the control of TLR signaling

UPR activation and down-stream cytokine responses in DCs and macrophages were demonstrated to be under the control of PRR signaling (Martinon *et al*, 2010; Smith, 2018). Despite the established role of TLR11/12 in parasite detection in infected mice (Andrade *et al*, 2013; Koblanski *et al*, 2013; Hou *et al*, 2011; Yaroviski *et al*, 2005), the innate sensing mechanisms of parasites residing into a intracellular vacuole are not fully elucidated (Poncet *et al*, 2019) and presumably involve distinct signals depending on early parasite attachment to host cells and subsequent partial vacuolar lysis leading to antigen escape into the host cytosol (Fisch *et al*, 2020;

153 Kongsomboonvech *et al*, 2020; Sardhina-Silva *et al*, 2019, Lee *et al*, 2015). Thus, to
154 establish the role of innate sensing in UPR activation, we used BMDCs differentiated
155 from mice lacking the adaptor MyD88 (MyD88KO mice) (Deguine & Barton, 2014).
156 This adaptor is essential to induce signaling down-stream of all TLRs except for TLR3,
157 which relies on the TRIF adaptor.

158 First, we confirmed that *T. gondii* stimulates the secretion of IL-6 and IL-12 over the
159 time-course of infection (Fig EV1A). In addition, and in contrast to the more virulent
160 type I parasites (“RH” strain), which kill their host prematurely and do not establish
161 latent infections in laboratory mice, type II parasites (“Pru” strain) specifically induce
162 the expression and secretion of IL-23 (Fig EV1B) at a late time point of infection (Fig
163 EV1A), suggesting that distinct innate signaling pathways are involved in the
164 detection of these parasite strains. Lack of MyD88 did not impact on parasite
165 invasion as illustrated by a similar rate of infection between WT and MyD88KO
166 BMDCs (Fig 2A), although we did note a slight alteration in parasite growth (Fig 2B).
167 As expected, absence of TLR stimulation led to a drastic loss of pro-inflammatory
168 cytokine production upon infection (Fig 2C), as revealed by a block in IL-6, IL-12 and
169 IL-23 production. Loss of MyD88 strongly impacted the UPR response in *T. gondii*
170 infected BMDCs. The *T. gondii*-mediated increase in *Xbp1s* and *Dnajb9* expression
171 levels was no longer observed and we even noted a down-regulation of their basal
172 expression level upon infection (Fig 2D). This was also observed for *Ddit3* (encoding
173 CHOP) expression levels when comparing infected versus non-infected conditions
174 (Fig 2D). These results therefore indicate that two separate pathways appear to
175 modulate the host UPR response upon infection with *T. gondii*. TLR signaling is
176 required to induce the IRE1 α /XBP1s pathway. In absence of TLR signaling, it
177 becomes obvious that a parasite dependent pathway causes the down-regulation of
178 all UPR branches. Of note, *Ppp1r15a* induction (GADD34) was not dependent on
179 MyD88, suggesting that a distinct MyD88- and PERK-independent signaling pathway
180 induces the expression of this gene during parasite replication (Fig 2D). In line, a
181 previous study demonstrated that GADD34 expression is induced in a IRF3/7-
182 dependent manner after poly:IC stimulation of DCs and controls cytokine production

183 (Dalet *et al*, 2017) (Clavarino *et al*, 2012). Thus, parasite RNA detection following
184 vacuolar lysis could be responsible for GADD34 induction in type II infected DCs.
185 Our results therefore indicate that induction of the IRE1 α /XBP1 signaling branch by
186 *T. gondii* infection relies on TLR dependent triggering, confirming earlier studies
187 (Martinon *et al*, 2010).

188 ***T. gondii* triggers the association of host ER with the parasitophorous vacuole**

189 *T. gondii* actively modulates host cell responses by secreting numerous parasite
190 effectors that hijack host signaling pathways and activate host compartment
191 recruitment at the PV. Notably, host ER (hER) components are recruited in a Sec22b-
192 dependent manner at the PV, a process required to stimulate cross-presentation of
193 soluble secreted OVA antigens by transgenic OVA-expressing parasites (Goldszmid *et*
194 *al*, 2009; Cebrian *et al*, 2011). Whether this process impacts on ER homeostasis has
195 never been investigated, nor whether this is needed for triggering of the UPR. First,
196 we quantified hER recruitment in type II Pru parasite infected BMDCs. Electron
197 microscopy analysis showed that almost all the examined PVs (n=116/119) display
198 hER membranes in close association with the PV delimiting membrane (Fig EV2A).
199 Staining of the hER marker KDEL by immunofluorescence confirmed this association
200 (Appendix Fig Fig S2A). In addition, this process was also detected in infected human
201 fibroblasts, in which KDEL-positive tubular-like structures were also visualized inside
202 the PV space in close proximity to parasites (Appendix Fig Fig S2B). Moreover, we
203 examined hER recruitment in BMDCs incubated with Heat-Killed (HK) parasites that
204 are presumably internalized by phagocytosis or macropinocytosis and do not secrete
205 parasite effectors to modulate host responses. In sharp contrast to live parasite-
206 containing vacuoles, HK-parasite containing vacuoles were never found positive for
207 hER recruitment at their limiting membrane (Fig EV2B). This suggests that hER
208 association with the PV is not specific to immune cells and may be triggered by live
209 parasites to support their survival or replication.

210 **hER recruitment is not required to induce the UPR**

211 In order to determine whether recruitment of the hER to the PV was needed for

induction of the UPR, we monitored activation of the UPR and down-stream cytokine responses upon infection of BMDCs by HK *T. gondii*. Similar to live parasites, we found that *Xbp1s* and its down-stream target *Dnajb9* (coding for ERdj4) were induced in BMDCs incubated with HK parasites both at the mRNA and protein levels (Fig EV3A, EV3B and EV3C). However, in sharp contrast to live parasites, the expression of the UPR-induced ER chaperones *Hspa5* (encoding BIP), *Hsp90b1* (encoding by GRP94) and *Sec61a1* was up-regulated as well as the PERK and ATF6 pathways (Fig EV3A). Indeed, HK parasites internalization induced the expression of the *Atf4* gene and its target genes *Ppp1r15a* (GADD34) and *Ddit3* (CHOP) as well as *Atf6* (Fig EV3A). However, despite increased in *Ddit3* mRNA levels, we still failed to visualize protein expression in HK parasite stimulated BMDCs (Fig EV3B). This result correlates with previous studies showing that a TRIF-dependent pathway triggered upon TLR stimulation attenuates CHOP translation (Woo *et al*, 2009, 2012). In addition, similar to HK parasites, stimulation of BMDCs with an extract of soluble *T. gondii* antigens (STAg) – which presumably only triggers TLRs - also resulted in the activation of the three branches of the UPR (Fig EV3D and EV3E). Therefore, our results on HK parasite and STAg stimulated BMDCs support the hypothesis that triggering of the IRE1 α /XBP1s pathway in infected BMDCs is not dependent on hER recruitment to the PV, but rather relies on TLR dependent signaling.

The presence of replicating parasites modulates the UPR

To further explore a potential modulation of the UPR by replicating parasites, we sorted infected *versus* non-infected bystander BMDCs from a mixed population of infected cells, which included 45% of non-infected cells (Fig 3A) and monitored UPR induction. At 16h post-infection, non-infected bystander BMDCs displayed a significantly higher induction of *Xbp1s* and *Dnajb9* expression compared to infected BMDCs (Fig 3B). These data therefore confirm that parasite invasion/replication is not absolutely required to activate the IRE1 α /XBP1s pathway. Furthermore, they also show that the presence of live parasites correlates with a down-regulation of XBP1s induction triggered by TLR stimulation, in agreement with the observed

repression of *Xbp1s* monitored in infected MyD88 KO cells compared to non-infected conditions (Fig 2D). Of note, the down-regulation of *Xbp1s*, *Dnajb9* and *Ddit3* expression in MyD88 KO infected cells was not observed in cells infected with HK parasites (Fig EV4A), further strengthening the hypothesis that secretion of parasite effectors and/or triggering of other signaling pathways upon invasion by live parasites leads to a dampening of UPR host responses. The inhibitory effect seemed to be specific to *Xbp1s* and its down-stream target genes since *Ern1* (encoding IRE1 α) expression was comparable in infected and bystander cells (Fig 3B), in line with what we monitored on WB (Fig 1C and 1D). Similarly to the modulation of the IRE1 α branch, we also observed an increased expression of the PERK downstream target genes *Atf4* and *Ddit3* in bystander non-infected BMDCs compared to infected BMDCs (Fig 3B). Finally, the down-regulation of the ATF6 target gene *Herpud-1* was no longer observed in bystander infected cells (Fig 3B), suggesting an active down-regulation of the ATF6 branch by parasite derived effector molecules (Fig 3B).

In conclusion, the presence of live parasites induces a down-regulation of the UPR triggered by TLR-mediated sensing of parasite compounds.

The IRE1 α /XBP1s pathway regulates cytokine response in infected BMDCs

The UPR transcription factors XBP1s and CHOP have been previously demonstrated to regulate cytokine response in TLR-stimulated macrophages (Martinon *et al*, 2010; Smith, 2018) and DCs (Márquez *et al*, 2017; Mogilenko *et al*, 2019). Therefore, we investigated whether *T. gondii*-mediated XBP1s activation impacts on cytokine secretion in infected BMDCs.

To establish the function of XBP1s in cytokine production, we used *Xbp1*^{flox/flox}Cre-DC mice lacking XBP1 specifically in the CD11c positive cells (hereafter called XBP1 Δ DC) (Osorio *et al*, 2014; Tavernier *et al*, 2017). Mice with *Xbp1*^{flox/flox} alleles but no expression of Cre (hereafter called XBP1^{fl/fl}) served as controls for XBP1 Δ DC mice (Osorio *et al*, 2014; Tavernier *et al*, 2017). We found that XBP1 Δ DC and control BMDCs display a similar rate of infection (Fig 4A and Fig4B) and parasite replication (Fig 4C). However, deletion of XBP1 in *T. gondii* infected BMDCs correlated with a

271 reduced gene expression and protein secretion of IL-6 and IL-12 but not of IL-23
272 when compared to control cells (Fig 4D and Fig 4E). Of note, the decrease in cytokine
273 production was similar at the mRNA and protein levels strongly supporting that
274 XBP1s regulate cytokine expression at the mRNA level. To investigate whether IRE1 α
275 also contributes to the regulation of cytokine secretion, we used XBP1 Δ DC
276 IRE1 α^{trunc} DC mice. These mice were obtained by crossing XBP1 Δ DC mice with *Ern1*^{fl/fl}
277 mice that bear loxP sites flanking exon 20 and 21 of the *Ern1* gene (encoding IRE1 α)
278 (Tavernier *et al*, 2017). DCs from XBP1 Δ DC IRE1 α^{trunc} DC mice are deficient for XBP1
279 and harbor a low expressed truncated IRE1 α isoform with impaired endonuclease
280 activity. Of note, as previously described (Medel *et al*, 2018), GM-CSF differentiated
281 BMDCs display strongly reduced but not fully abrogated IRE1 α expression (Fig EV5A).
282 We did not measure further decrease in IL-6, IL-12 or IL-23 secretion in XBP1 Δ DC
283 IRE1 α^{trunc} DCs indicating that *T. gondii*-induced XBP1s induction selectively contributes
284 to cytokine production in infected BMDCs upon activation of IRE1 α (Fig 4D and 4E).
285 Importantly, the defect in cytokine production monitored in XBP1 Δ DC BMDCs and
286 XBP1 Δ IRE1 α^{trunc} DC BMDCs was not a result of cell death induced upon infection or
287 impaired differentiation as illustrated by a similar percentage of CD11c⁺/MHCII⁺
288 positive cells (Fig EV5B). Additionally, XBP1 Δ DC BMDCs and XBP1 Δ IRE1 α^{trunc} DC BMDCs
289 displayed similar surface expression levels of the co-stimulatory molecules CD80 and
290 CD86 upon LPS stimulation (Fig EV5C) indicating that deletion of XBP1 and IRE1 α did
291 not impact on BMDCs activation upon immunogenic stimulation. Moreover, similar
292 to BMDCs invaded by live parasites, XBP1 Δ DC BMDCs incubated with HK parasites
293 displayed a drastic inhibition in the gene expression and protein secretion of IL-6 and
294 IL-12 (Fig EV5D and EV5E), further supporting the hypothesis that the IRE1 α /XBP1s
295 pathway is triggered in a TLR-dependent manner to regulate cytokine production. Of
296 note, in contrast to IL-6 and IL-12, IL-23 was less induced by HK parasites, suggesting
297 that the presence of live parasites is required to trigger production of this cytokine
298 (Fig EV6). However, the production of all cytokines induced by HK parasites was fully
299 dependent on TLR/MyD88 signaling (Fig EV4B).

300 Collectively these data demonstrate that upon *T. gondii* infection of BMDCs, IL-6 and
301 IL-12 secretion is partially under the control of IRE1 α /XBP1s signaling activated via

302 MyD88. In contrast, IL-23 production is IRE1 α /XBP1s-independent, requires live
303 parasite invasion, and is only partially dependent on MyD88 signaling.

304 **The IRE1 α /XBP1s pathway regulates cross-presentation of soluble secreted**
305 **parasite antigens**

306 Several studies reported the implication of the IRE1 α branch of the UPR in regulating
307 MHC I antigen presentation in DCs both at steady-state and in activated conditions
308 (Osorio *et al*, 2018). Activation of the IRE1 α /XBP1s axis was found to have beneficial
309 or detrimental outcomes on T cell activation depending on the nature of the
310 stimulation and the antigen. Notably, in the context of tumors, reinforcing XBP1s
311 expression ameliorates cross-presentation and priming of CD8⁺ T cells (Zhang *et al*,
312 2015). Similarly, the inhibition of IRE1 α endoribonuclease activity in cDC1 leads to
313 reduced soluble melanoma antigen cross-presentation to CD8⁺ T cells but has no
314 impact on MHC II antigen presentation (Zhang *et al*, 2015). So far, the implication of
315 the IRE1 α /XBP1s pathway in MHC I antigen presentation has not been addressed in
316 the context of DCs infected by intracellular pathogens. To address this question, we
317 infected BMDCs with type II parasites expressing the soluble model antigen OVA,
318 which is secreted in the vacuolar space of the PV. MHC I presentation of this antigen
319 has been previously demonstrated to depend on hER component recruitment at the
320 PV, notably TAP and the translocon Sec61 (Cebrian *et al*, 2011; Goldszmid *et al*,
321 2009; Gubbels *et al*, 2005). We used LacZ-inducible reporter CD8 T cell hybridomas
322 (B3Z) that specifically respond to H-2K^b-SIINFEKL complexes to assess secreted OVA
323 presentation by control XBP1^{fl/fl} IRE1^{fl/fl}, XBP1 Δ DC and XBP1 Δ DC IRE1 α ^{trunc}DC BMDCs.
324 Since XBP1 Δ DC IRE1 α ^{trunc}DC may display residual IRE1 α activity (Fig EV5A), we
325 additionally treated control cells with 4 μ 8C, a selective and potent inhibitor of the
326 IRE1 α endoribonuclease domain as illustrated by the complete inhibition of *Xbp1s*
327 induction and its target *Dnajb9* upon infection or treatment with tunicamycin
328 (Appendix Fig S3A and Appendix Fig S3B). 4 μ 8C treatment also inhibited IL-6 and IL-
329 12 secretion (but not IL-23 production) induced upon infection (Appendix Fig S3C)
330 similarly to what we observed in XBP1 Δ DC and XBP1 Δ DC IRE1 α ^{trunc}DC BMDCs (Fig
331 4D).

332 We found that inhibition of IRE1 α by 4 μ 8C results in a pronounced decrease in
333 SIINFEKL (SKL8) peptide presentation by infected cells (Fig 4F). A significant inhibition
334 of SKL8 peptide presentation was also observed in XBP1 Δ DC IRE1 α^{trunc} DC BMDCs (Fig
335 4F), although less pronounced, which may reflect the partial deletion of IRE1 α in in
336 these cells. Interestingly, XBP1 Δ DC infected BMDCs were not inhibited in SKL8
337 peptide presentation demonstrating that this process is not dependent on XBP1s
338 induction (Fig 4F). The defect in SKL8 peptide presentation observed in XBP1 Δ DC
339 IRE1 α^{trunc} DC was not due to altered parasite infection (Fig 4G) or to reduced MHCI
340 surface expression (Fig 4H and 4I), also supported by similar exogenous presentation
341 of the SKL8 peptide in all studied conditions (Fig 4H). In conclusion, our data indicate
342 that IRE1 α but not XBP1s contributes to CD8 $^{+}$ T cell priming by *T. gondii* secreted
343 antigens in BMDCs.

344 **The IRE1 α pathway is stimulated in splenic cDC1 during infection**

345 To assess whether the IRE1 α pathway is activated during *T. gondii* infection of mice,
346 we used ERAI reporter mice (Iwawaki *et al*, 2004). These mice express a partial
347 sequence of human XBP1 fused to Venus fluorescent protein (VenusFP) that includes
348 the sites at which IRE1 α splices XBP1 (Iwawaki *et al*, 2004). Thus, detection of the
349 VenusFP signal in cells allows reporting of IRE1 α activation. Our previous findings
350 demonstrated a preferential induction of the IRE1 α arm of the UPR in basal-state
351 splenic cDC1 over cDC2 and pDC (Osorio *et al*, 2014; Tavernier *et al*, 2017). ERAI and
352 control mice were infected with a low dose of Pru parasites and the different
353 subpopulations of splenic DCs were analyzed 6 days post-infection by flow
354 cytometry.

355 In line with our previous findings, a VenusFP signal was detected at steady state in
356 cDC1s (CD26 $^{+}$ /CD11c $^{\text{hi}}$ /MHCI $^{\text{hi}}$ /XCR1 $^{+}$ /CD172a $^{-}$) but not in cDC2s
357 (CD26 $^{+}$ /CD11c $^{\text{hi}}$ /MHCI $^{\text{hi}}$ /XCR1 $^{-}$ /CD172a $^{+}$) (Fig 5A, 5B and 5C). Upon infection, the
358 VenusFP signal greatly increased in the cDC1 subset while it remained unchanged in
359 the cDC2 population (Fig 5A, 5B and 5C) indicating that IRE1 α activation and
360 subsequent XBP1s expression is restricted to cDC1s. We also did not monitor
361 substantial XBP1s induction in splenic neutrophils, monocytes, B and T cells

362 suggesting that the UPR contributes to the regulation of cDC1 functions upon *T.*
363 *gondii* infection (Fig 5D and S4A). To further confirm these results, we sorted splenic
364 cDC1s and cDC2s from 6 days WT infected mice and examined UPR induction by
365 quantifying the mRNA levels of different UPR targets. Consistent with the results
366 obtained in ERAI mice, a strong up-regulation of *Ern1* (IRE1 α) and *Xbp1s* transcripts
367 were monitored only in cDC1s but not in cDC2s. The expression of the PERK target
368 *Ddit3* (CHOP) was not induced in none of the cDC sub-types (Fig 5E and 5F).
369 Importantly, similar to observations made in previous studies (John *et al*, 2009), the
370 percentage of dTomato-positive infected cells was very low in the spleen of ERAI
371 infected mice suggesting that parasite invasion and replication may not be required
372 to trigger the massive activation of the IRE1 α pathway monitored in cDC1s (Fig S4B).
373 This hypothesis correlates with our *in vitro* findings indicating that induction of
374 XBP1s in BMDCs is dependent on innate sensing of particulate antigens and does not
375 require parasite invasion.

376 **IRE1 α /XBP1s pathway activation plays an essential protective role against *T. gondii*** 377 **infection**

378 To determine the contribution of DC-specific activation of IRE1 α in the resistance to
379 *T. gondii* infection, we assessed the survival rate of control XBP1^{fl/fl} IRE1 α ^{fl/fl} mice
380 compared to XBP1 Δ DC IRE1 α ^{trunc}DC mice injected intraperitoneally with Pru
381 parasites. Our data showed that XBP1 Δ DC IRE1 α ^{trunc}DC mice were highly susceptible
382 to infection when compared to control mice and all succumbed between 9 and 12
383 days post infection (Fig 6A). This result indicates that activation of the IRE1 α
384 pathway in DCs plays an essential protective role against *T. gondii* acute infection. To
385 gain insights into the processes leading to XBP1 Δ DC IRE1 α ^{trunc}DC mice early
386 mortality, we isolated splenocytes and cells from the peritoneal exudate (PEC) of
387 infected mice and analyzed parasite load by qPCR. We found a significant increase in
388 parasite burden in both organs in XBP1 Δ DC IRE1 α ^{trunc}DC mice compared to control
389 mice, indicating an altered parasite control (Fig 6B). We did not detect any significant
390 changes in *Il-12* expression in the spleen of infected XBP1 Δ DC IRE1 α ^{trunc}DC mice,
391 presumably due to the contribution of other splenic cell types, notably cDC2s and

monocytes, in the secretion of this cytokine (Fig 6C). However, a reduced *Ifn-g* and *Tnfa* expression was monitored in XBP1ΔDC IRE1α^{trunc}DC mice compared to control mice suggesting a defect in T cell activation that could be caused by a cDC functional alteration in antigen presentation (John *et al*, 2009; Lee *et al*, 2015). Accordingly, while in control mice, CD4 T and CD8 T cell showed increased proliferation upon infection, this expansion was not observed in XBP1ΔDC IRE1α^{trunc}DC mice (Fig 6D and 6E). We also observed a defect in CD8 T cell activation as noted by the decreased number of IFNγ-expressing CD8 T cells but not of IFNγ-expressing CD4 T cells, suggesting an alteration of cross-presentation by cDC1s (Fig 6D and 6E). Finally, we noticed that the number and frequency of splenic cDC1s and cDC2s both increased upon infection of WT mice (Fig 6F). On the contrary, XBP1ΔDC IRE1α^{trunc}DC mice, which exhibit a similar number of the two cDC subsets at steady-state compared to control mice, do not show increased cDC1 number and frequency upon infection (Fig 6F). This defect was not observed for cDC2s, consistent with a specific activation of the IRE1α pathway in cDC1s (Fig 5A and 5B) and a defect in CD8 T cell activation (Fig 6E). These data therefore suggest that the IRE1α pathway may not only regulate cross-presentation to CD8 T cells but also promote the expansion of the cDC1 subset triggered upon infection.

410

411 Discussion

Very few studies addressed the impact of UPR signaling in DCs in response to infection by intracellular pathogens. Our results demonstrate that infection of BMDCs by *T. gondii* specifically activated the IRE1α/XBP1s branch, while the PERK pathway was not activated and the ATF6 branch even repressed. Experiments comparing infected *versus* non-infected bystander BMDCs, or heat-killed *versus* live parasites revealed that triggering of the UPR does not require active invasion with live parasites, but relies on TLR-mediated sensing of parasite compounds (Figure 7A). On the other hand, secretion of parasite effectors and/or triggering of other signaling pathways upon invasion by live parasites lead to a dampening of the UPR, which is especially prominent for the ATF6 and PERK branch (Figure 7B).

422 Manipulation of the UPR has been well documented during viral infections (Smith,
423 2018). For instance, the chikungunya virus shuts-down the PERK pathway to sustain
424 eIF2 α -mediated translation of viral proteins and to avoid CHOP-induced apoptosis of
425 the host cell, but activates the IRE1 α and ATF6 pathways to enhance ER capacity
426 (Rathore *et al*, 2013). Similarly, we found that the presence of live parasites led to a
427 down-regulation of the UPR triggered by TLR stimulation. Along these lines, it has
428 been reported that *TgROP18*, a parasite effector anchored at the limiting membrane
429 of the vacuole phosphorylates ATF6 β , leading to its degradation via the proteasome
430 (Yamamoto *et al*, 2011). Our data indicate that the ATF6 pathway is also repressed at
431 the transcriptional level in infected BMDCs by yet unknown mechanisms. Also ATF4
432 and CHOP, downstream mediators of the PERK pathway, were not induced in
433 infected BMDCs. TLR stimulation has been previously shown to correlate with a TRIF-
434 dependent attenuation of CHOP translation as we also noted in HK or STAg-
435 stimulated parasites (Woo *et al*, 2009, 2012). However, infection with live parasites
436 led to repression of CHOP already at the transcriptional level, which became
437 apparent upon comparison of WT *versus* MyD88 KO cells. This process may allow the
438 infected host cell to avoid CHOP-induced apoptosis.

439 The mechanisms by which infection by live parasites modulates the UPR remain to
440 be elucidated. To escape cellular defenses, *T. gondii* secretes numerous parasite
441 effectors that modulate host cell signaling pathways (Hakimi *et al*, 2017). In addition,
442 parasite effectors anchored at the limiting membrane of the vacuole promote
443 recruitment of host organelles, most likely to stimulate host lipid uptake required to
444 endure the successive divisions of this fast replicating parasite. *T. gondii* stimulates
445 the recruitment of mitochondria (Pernas *et al*, 2014, 2018), fragmented Golgi
446 (Deffieu *et al*, 2019), endosomes (Romano *et al*, 2017) and the hER (Goldszmid *et al*,
447 2009; Sinai *et al*, 1997). It is likely that sensing mechanisms detect changes in
448 organelle structure and functions, which result in the stimulation of specific
449 downstream responses. In support of this hypothesis, the transcriptional response of
450 cells invaded by TgMAF-1-deficient parasites, which are unable to recruit
451 mitochondria at the PV, revealed major changes in cytokine responses (Pernas *et al*,
452 2014). By contrast to mitochondria and Golgi vesicles, the effectors that stimulate
453 hER association with the PVM are not identified. A previous work reported the

454 interaction of *TgGRA3* with calcium modulating ligand (CAMLG), a type II
455 transmembrane protein of the ER, but demonstration of a functional role of *TgGRA3*
456 in this process is lacking (Kim *et al*, 2008). As previously observed by others, we
457 detected hER association with the PV in fibroblasts and BMDCs infected with type II
458 Pru parasites but this process was not required to induce the UPR. However, hER
459 recruitment by the parasite may contribute to the modulation of the UPR pathways
460 induced by TLR-stimulation. Thus, identification of the parasite effectors contributing
461 to hER recruitment at the PV would allow exploring its impact on the regulation of
462 hER functions, the possible modulation of the UPR and down-stream innate immune
463 responses. In general, further characterization of the induced UPR pathways in DCs
464 infected with parasites deficient in the secretion of parasite effectors (Franco *et al*,
465 2016; Naor *et al*, 2018; Panas *et al*, 2019) will also help to identify the mechanisms
466 by which *T. gondii* modulates the UPR.

467

468 Also *in vivo*, infection with live parasites appeared not essential to trigger the UPR in
469 DCs, as non-infected bystander rather than infected DCs showed activation of the
470 IRE1 α branch. Indeed, while activation of IRE1 α in splenic cDC1 was high at day 6
471 post-infection, the level of infected cells at that moment was very low, although
472 parasite DNA was still clearly detected by qPCR. This is consistent with the study of
473 John *et al*. suggesting that cross-presentation by bystander DCs rather than infected
474 DCs is an important route of antigen presentation during toxoplasmosis (John *et al*,
475 2009). Along these lines, it has been demonstrated that during influenza virus
476 infection, efficient cross-presenting cDC1 are non-infected due to their ability to
477 efficiently restrict virus replication by an IFN β -mediated induction of cellular
478 defenses (Helft *et al*, 2012). Thus, also for *T. gondii* infection, detection of infected
479 cells by flow cytometry may not clearly distinguish the cells that have internalized
480 particulate antigens from the ones that have efficiently restricted parasite
481 replication. Activation of the IRE1 α branch was observed specifically in splenic cDC1s
482 but not in cDC2s upon *T. gondii* infection. This result may correlate with the
483 predominant expression of TLR11/12 in cDC1 over cDC2 (Dalod *et al*, 2014; Heng *et al*,
484 2008) and extends earlier findings in steady state showing preferential activation
485 of the IRE1 branch in cDC1s (Osorio *et al*, 2014, Tavernier *et al*, 2017).

486

487 Activation of the IRE1 α pathway upon *T. gondii* infection regulates key DC functions
488 required for optimal host defenses (Figure 7A and 7B). Induction of XBP1s promoted
489 pro-inflammatory cytokine production at the mRNA level in a cytokine specific
490 manner. IL-6 and IL-12p40 appeared XBP1 dependent, while IL-23p19 was not. These
491 data are in line with earlier reports revealing that macrophage stimulation by TLR2
492 and TLR4 agonists activates the IRE1 α pathway via a TRAF6-NOX2 axis (Martinon *et al*,
493 2010). IRE1 α -mediated activation of XBP1s promoted sustained production of
494 inflammatory mediators and XBP1 deficiency resulted in an increased bacterial
495 burden in mice infected with the TLR2-activating intracellular pathogen *Francisella*
496 *tularensis* (Martinon *et al*, 2010). In our hands, absence of MyD88 led to a complete
497 abrogation of *T. gondii* induced XBP1 activation. Still, it remains possible that on top
498 alternative sensing mechanisms are involved. Along these lines, a recent study
499 demonstrated that *T. gondii* infection activates IRE1 α through calcium release from
500 the ER. As described previously (Urrea *et al*, 2018), IRE1 α oligomerization and
501 recruitment of filamin A promoted migration of *T. gondii* infected MEF and BMDCs
502 (Augusto *et al*, 2020). While this study did not investigate activation of additional
503 UPR branches in *T. gondii* infected BMDCs, these findings are consistent with our
504 results showing a sustained activation of IRE1 α in infected BMDCs (Augusto *et al*,
505 2020).

506 Besides stimulating pro-inflammatory cytokine production, we found that IRE1 α , but
507 not XBP1s, was required for optimal MHCI antigen presentation of secreted *T. gondii*
508 antigens to CD8 T cells. We previously demonstrated that IRE1 α -mediated RIDD
509 activity reduces MHCI antigen presentation of dead cell derived antigens by
510 degrading key factors of the cross-presentation machinery (Osorio *et al*. 2014). In
511 contrast to these results, upon infection, we found a role for IRE1 α in promoting
512 MHCI antigen presentation. The mechanisms involved are not yet identified. It is
513 possible that IRE1 α regulates trafficking events impacting on vacuole fate, parasite
514 antigen escape to the cytosol or on antigen processing. IRE1 α induction may thereby
515 facilitate parasite control by stimulating CD8 T cell priming, a process that favors
516 preservation of the host and transmission of the parasites after establishment of a

517 chronic infection. Consistent with this hypothesis, we found that mice lacking XBP1
518 and IRE1 α failed to control the infection and succumbed during the acute phase. This
519 high susceptibility was correlated with a defect in CD4 and CD8 T cell expansion as
520 well as altered CD8 T cell activation in the spleen of infected mice. In line with the
521 altered CD8 T cell proliferation and activation, IRE1 α /XBP1-deficient cells exhibit an
522 altered expansion of cDC1s but not cDC2s upon infection. The defect in CD4 T cell
523 proliferation likely results from a role of cDC1s in assisting CD8⁺ T cell responses
524 through distinct mechanisms, including a process whereby CD4⁺ T cells 'license' cDC1
525 for CD8⁺ T cell priming via CD40 signaling (Smith C *et al*, 2004). While our study
526 revealed that the IRE1 α pathway is essential to protect mice against an acute *T.*
527 *gondii* infection, it is only a first step towards understanding the likely very complex
528 process of IRE1 α -mediated regulation of innate and adaptive immunity against *T.*
529 *gondii*. Future studies will aim to further characterize the mechanisms by which
530 activation of IRE1 α and XBP1s regulates cDC1 functions upon infection, including cell
531 expansion, cytokine production and MHC1 antigen presentation. It will be important
532 to also explore the role of the IRE1 α branch in regulating non-lymphoid tissue cDC
533 responses, notably peripheral migratory CD103⁺-cDC1 after oral ingestion of cysts.
534 For instance, XBP1s-mediated regulation of IL-12 production may be important at
535 early stages of parasite invasion in the intestine to activate IFN γ secretion by NK
536 cells. Thus, our study opens perspectives for the identification of novel strategies to
537 boost immunity against *T. gondii*, notably CD8 T cell responses, by manipulating the
538 UPR and thereby to restrict parasite spreading to the brain and establishment of
539 cerebral chronic infection.

540

541 **Materials & Methods**

542

543 **Mice**

544 Wild-type C57BL/6J (B6) mice were purchased from Janvier (France). C57BL/6
545 XBP1^{fl/fl} (Lee *et al*, 2008), C57BL/6 XBP1 Δ DC (Osorio *et al*, 2014), C57BL/6 XBP1^{fl/fl}
546 IRE1^{fl/fl} (Iwawaki *et al*, 2010), C57BL/6 XBP1 Δ DC IRE1^{trunc}DC (Tavernier *et al*, 2017)
547 and ERAI mice (Iwawaki *et al*, 2004) were provided by S. Janssens (IRC/VIB, Ghent

University). C57BL/6 XBP1^{fl/fl} IRE1^{fl/fl} and C57BL/6 XBP1ΔDC IRE1^{trunc}DC mice were bred at the « Institut Pasteur de Lille » in pathogen-free conditions. Experiments were carried out in accordance with the European regulations (86/609/EEC). All animal procedures were approved by the local Ethical Committee for Animal Experimentation registered by the « Comité National de Réflexion Éthique sur l'Expérimentation Animale » (CEEA-75). All mice were housed and bred at the « Institut Pasteur de Lille » according to the protocole (APAFIS#12804-2017122111233479v2) approved by the French Minister of Higher Education, Research and Innovation.

557

558 **BMDC differentiation and parasite culture**

559 Wild type C57BL/6, C57BL/6 XBP1^{fl/fl}, C57BL/6 XBP1ΔDC, C57BL/6 XBP1^{fl/fl} IRE1^{fl/fl},
560 C57BL/6 XBP1ΔDC IRE1^{trunc}DC and C57BL/6 MyD88^{-/-} (B. Lambrecht, IRC/VIB, Ghent
561 University) mice from 8 to 10 weeks of age were used to obtain bone marrow
562 dendritic cells (BMDCs) from the femur and tibia. BM cells were differentiated for 10
563 days in Petri dish with RPMI (Gibco™ by LifeTechnologies) supplemented with 10%
564 fetal bovin serum (FBS, Gibco™ by LifeTechnologies), 1% penicillin-streptomycin
565 (Gibco™ by LifeTechnologies), 1% sodium pyruvate (Gibco™ by LifeTechnologies),
566 0,2% 2-mercaptoethanol (Gibco™ by LifeTechnologies) and 10% Granulocyte
567 Macrophage-Colony Stimulating Factor. Flt3/Notch-differentiated DC were
568 generated as described in (Kirkling et al., 2018). Flt3 ligand was generated by the
569 protein core facility of VIB Center for Inflammation Research (Ghent, Belgium).

570

571 Tachyzoites of *Toxoplasma gondii* Type I RH Luc GFP (J.Boothroyd; Stanford
572 University of School Medicine), Type II Pru Luc GFP (J.Boothroyd; Stanford University
573 of School Medicine) and Type II Pru Tomato SAG1-Ova (Schaeffer *et al*, 2009)
574 parasites were grown *in vitro* on confluent Human Foreskin Fibroblast (HFF) cells
575 (CCD-1112Sk (ATCC, CRL-2429™)) using Dulbeccos's modified Eagles medium
576 (Gibco™ by LifeTechnologies) supplemented with 10% fetal calf serum (Gibco™ by
577 LifeTechnologies), and 1% penicillin-streptomycin (Gibco™ by LifeTechnologies).
578 Prior to experiments, parasites were extracted from host cells by sequential
579 passages through 17-gauge and 26-gauge needles followed by filtration with a 3-μm

580 polycarbonate membrane filter. Parasite cultures were regularly tested for
581 mycoplasma using MycoAlert™ mycoplasma detection kit (Lonza). If not indicated
582 otherwise, BMDCs were infected at a multiplicity of infection (MOI) of 4 parasites /
583 BMDC. Heat-Killed (HK) parasites were obtained after incubation of live parasites for
584 20min at 56°C. Soluble *T. gondii* Antigens (STAgS) were obtained after lysis of
585 purified parasites by sonication in ice-cold sterile water followed by centrifugation at
586 1400rpm 30min to remove cell debris. The solution was then adjusted to 1X PBS and
587 filtered. Protein concentration was determined using the BCA kit. BMDCs were
588 incubated for 16h with STAgS at a final concentration of 10µg/mL, with tunicamycin
589 (TN) (SIGMA-ALDRICH®) at a final concentration of 1 µg/mL for 16h, with
590 lipopolysaccharide (LPS) (InvivoGen) at a final concentration of 100 ng/mL for 16h
591 and with the IRE1 inhibitor, 4µ8C (SIGMA-ALDRICH®) (Cross *et al*, 2012) at a final
592 concentration of 10µM for 16h. For *in vivo* infection, 500 parasites were injected
593 intra-peritoneally.

594

595 **Immunofluorescence assays (IFA)**

596 Adherent BMDCs on glass coverslips pre-coated with poly-L-lysine were infected
597 with parasites at a MOI of 1 during 16h before fixation with 1% paraformaldehyde
598 for 20min. After quenching with 50mM NH₄Cl for 15min, the cells were
599 permeabilized with 0,1% triton for 3min and incubated with mouse anti-KDEL (10C3
600 Enzo® Life Sciences) antibodies in 0,1% BSA-PBS, followed by DAPI and goat anti-
601 mouse secondary antibodies conjugated to Alexa Fluor 594. Images were acquired
602 using a Zeiss LSM880 confocal microscope.

603

604 **Electron microscopy**

605 BMDCs infected with *T. gondii* parasites at the MOI of 2 were fixed in 4%
606 paraformaldehyde and 2.5% glutaraldehyde in 0.1 M NaCacodylate buffer, pH 7.2
607 and centrifuged at 1500 rpm. Low melting point-agarose was used to keep the cells
608 concentrated for further processing. Cells were fixed for 4 hours at room
609 temperature followed by fixation O/N at 4°C after replacing with fresh fixative. After
610 washing in buffer, they were post-fixed in 1% OsO₄ with 1.5% K₃Fe(CN)₆ in 0.1 M
611 NaCacodylate buffer at room temperature for 1 hour. After washing in ddH₂O, cells

were subsequently dehydrated through a graded ethanol series, including a bulk staining with 1% uranyl acetate at the 50% ethanol step followed by embedding in Spurr's resin. Ultrathin sections of a gold interference color were cut using an ultra-microtome (Leica EM UC7), followed by a post-staining in a Leica EM AC20 for 40 min in uranyl acetate at 20 °C and for 10 min in lead stain at 20°C. Sections were collected on Formvar-coated copper slot grids. Grids were viewed with a JEM 1400plus transmission electron microscope (JEOL, Tokyo, Japan) operating at 80 kV.

619

620 **Flow cytometry**

621 After FcBlock (BD PharmingenTM) incubation, cell suspensions were labeled with a
622 dead cell marker (Aqua Live Dead Molecular Probes[®] by life technologiesTM), anti-
623 CD11c APC (N418, BioLegend[®]), anti-I-A/I-E (MHCII) FITC (M5/114.15.2, BioLegend[®])
624 or anti-CD80 FITC (16-10A1, BioLegend[®]) or anti-CD86 FITC (GL-1, BioLegend[®]) or
625 anti-H2K^b FITC (AF6-88.5.5.3, BioLegend[®]) in PBS. After cells fixation (Fixation buffer
626 BioLegend[®]), samples were analyzed using the Attune NxTTM flow cytometer
627 (ThermoFisher SCIENTIFIC) and analyzed using the FlowJo10 software.

628

629 **Isolation of splenocytes**

630 Spleens were isolated from mice and digested with PBS-2% FBS containing 1mg/mL
631 of collagenase (SIGMA-ALDRICH[®]) and 2µg/mL of DNase I (Roche) for 20min at 37°C
632 followed by lysis of red blood cells (Red Blood Cell Lysing Buffer Hybri-MaxTM,
633 SIGMA-ALDRICH[®]). The cells were then used to perform RTqPCR, cell labeling for
634 flow cytometry or cell sorting.

635

636 **Splenic DC and T cell analysis**

637 After isolation, splenocytes were incubated with FcBlock (BD PharmingenTM). For DC
638 analysis, splenocytes were labeled with a dead cell marker (Zombie VioletTM Fixable
639 Viability, BioLegend[®]), anti-CD45 APC-Cy7 (30-F11, BioLegend[®]), anti-CD3 PE-Cy7
640 (17A2, BioLegend[®]), anti-CD11c APC (N418, BioLegend[®]), anti-I-A/I-E FITC
641 (M5/114.15.2, BioLegend[®]) and anti-CD8α BV605 (53-6.7, BioLegend[®]) antibodies in
642 PBS. CD8α-/+ DCs were isolated by cell sorting using the BD FACSARIATM III with
643 gating on forward scatter and side scatter/singlets/live cells/CD45+ CD3- CD11c+

644 MHCII^{hi} and CD8α⁺ or CD8α⁻. For splenic T cell analysis, splenocytes were labeled
645 with a dead cell marker (Zombie VioletTM Fixable Viability, BioLegend®), anti-CD45
646 APC-Cy7 (30-F11, BioLegend®), anti-CD3 PE-Cy7 (17A2, BioLegend®), anti-CD4 AF700
647 (GK1.5, BioLegend®) and anti-CD8 BV605 (53-6.7, BioLegend®) antibodies. The
648 intracellular labeling of IFN-γ was performed according to the manufacturer's
649 instructions using anti-IFN-γ PE antibodies (XMG1.2, BioLegend®). Samples were
650 analyzed using the Attune NxTTM flow cytometer (ThermoFisher SCIENTIFIC) and the
651 FlowJo10 software.

652

653 **Quantitative real time PCR (RT-qPCR)**

654 Non-infected or infected BMDCs RNA was obtained using the RNeasy® Mini kit
655 (QIAGEN) and treated with DNase I (SIGMA-ALDRICH®). When specified, infected or
656 bystander BMDCs from a mixed population were isolated by cell sorting with gating
657 on forward scatter and side scatter/singlets/live cells/CD11c⁺ MHCII⁺/±Pru Tomato
658 Ova using the BD FACSARIATM III. Total cDNA was generated using the High-Capacity
659 cDNA Reverse Transcription kit (Applied BiosystemsTM by life technologies) according
660 to manufacturer's instructions. RT-qPCR was carried out on a QuantStudio 12K Flex
661 Real-Time PCR system (Applied BiosystemsTM by life technologies) with SYBR®
662 Selected Master Mix (Life technologies) and primers (listed in Appendix Table S1).
663 The expression of individual genes was normalized to the housekeeping gene
664 GAPDH.

665 The RNA of CD8α⁺ DCs was isolated using the RNeasy® Micro kit (QIAGEN) and
666 treated with a DNase I. cDNA was generated using the SuperScript kitTM VILOTM cDNA
667 Synthesis kit (Invitrogen by Thermo Fischer Scientific) and qPCR were performed as
668 previously described.

669

670 **Western blot**

671 Non-infected or infected BMDCs were incubated with lysis buffer (250mM NaCl,
672 20mM Hepes, 1mM EDTA, 1% NP-40, 20mM DTT, phosphatase inhibitor (PhosSTOPTM
673 Roche) and protease inhibitor (PROTEOLOCTM)). After centrifugation, supernatants
674 were complemented with Laemmli 5X and total proteins of 2.10⁵ cells were
675 subjected to electrophoresis in a 6 or 10% polyacrylamide gel. The proteins were

transferred onto a nitrocellulose membrane (AmershamTMProtranTM 0.45μ NC) by a standard western blot procedure. The membrane was blocked with 5% BSA in TBT-T buffer (20mM Tris à pH 7.5, 150mM NaCl and 0.1% Tween 20) and probed with primary antibodies over-night at 4°C with the following antibodies IRE1α (14C10, Cell Signaling TECHNOLOGY[®]), XBP1s (83418, Cell Signaling TECHNOLOGY[®]), PERK (C33E10, Cell Signaling TECHNOLOGY[®]), p-eIF2α (Cell Signaling TECHNOLOGY[®]), CHOP (B-3, Santa Cruz[®]), GAPDH (D16H11, Cell Signaling TECHNOLOGY[®]) and β-tubulin (Cell signaling TECHNOLOGY). Primary antibody incubation was followed by washing using the TBS-T buffer prior to species specific secondary antibodies conjugated to HRP. The membranes were visualized using ECL Western blotting substrate (Pierce) or SuperSignalTM West Femto Maximum Sensitivity Substrate (Thermo ScientificTM) on a ChemiDocTM XRS+ (BIO-RAD).

ELISA

ELISA experiments were conducted on non-infected or infected BMDCs according to the manufacturer's instructions using the IL-6 Mouse Uncoated ELISA kit (Invitrogen by Thermo Fischer Scientific), IL-12/IL-23p40 (Total) Mouse Uncoated ELISA kit (Invitrogen by Thermo Fischer Scientific) and Mouse IL-23 DuoSet[®] ELISA (R&D SYSTEMS[®]). Plates were analyzed on a Spark[®] 10M (TECAN).

Antigen presentation assays

BMDCs were plated into flat-bottom 96-well and infected with serial dilution of tachyzoites. The rate of infected cells (Pru Tomato SAG1-Ova detection) was determined by flow cytometry. After 6h of infection, B3Z (Hosken & Bevan, 1990; Karttunen *et al*, 1992) reporter hybridomas were added. After 16h of incubation with B3Z, presentation of SIINFEKL (SKL8) peptide by H-2K^b was assessed by quantification of β-galactosidase using the chromogenic substrate chlorophenol red-β-D-galactopyranoside (CPRG, Roche). Absorbance was read at 595nm with a reference at 655nm with a Spark[®] 10M (TECAN).

ERAI experiment

707 WT and ERAI mice were infected with 1000 parasites by intra-peritoneal injection.
 708 Splenocytes were isolated 6 days post-infection and labeled with Live/Dead eF506
 709 (eBioscience™), anti-CD45 BV605 (30-F11, BD biosciences), anti-Ly6C PerCP-eF710
 710 (HK1.4, eBioscience™), anti-CD11b BUV395 (M1/70, BD biosciences), anti-CD64
 711 BV711 (X54-5/7.1, BioLegend®), anti-MHCII APC-eF780 (M5/114.15.2, eBioscience™),
 712 anti-CD3e PE-Cy5 (145-2C11, eBioscience™), anti-CD19 PE-Cy5 (eBio1D3(1D3),
 713 eBioscience™), anti-CD11c eF450 (N418, eBioscience™), anti-CD26 APC, anti-XCR1
 714 BV650 (ZET, BioLegend®) and anti-CD172a PE-Cy7 (P84, BioLegend®). The cells were
 715 gated on: Non debris/Singlets/Live/CD45+/Gated out the monocytes (Ly6C^{hi}
 716 CD11b++) and neutrophils (Ly6C^{INT+} CD11b+)/Gated out macrophages (CD64+ MHC-
 717 II+)/Gated out T cells (CD3eCD19+ MHCII-) and B cells (CD3eCD19+ MHCII+) / CD26+
 718 CD11c+ MHCII+ XCR1+ CD172a- for cDC1s and CD26+ CD11c+ MHCII+ XCR1-
 719 CD172a+ for cDC2s.

720

721 **Parasite quantification by qPCR**

722 DNA extractions from splenocytes or PEC were performed with the NucleoSpin
 723 Tissue kit (MACHEREY NAGEL). Individuals qPCR reactions were processed using
 724 TOX9 (5'-AGGAGAGATATCAGGACTGTAG-3') and TOX11 (5'-
 725 GCGTCGTCTCGTCTAGATCG-3') primers (Reischl *et al*, 2003) with SYBR® Selected
 726 Master Mix (Life technologies). qPCR reactions were carried out on a QuantStudio
 727 12K Flex Real-Time PCR system (Applied Biosystems™ by life technologies). Total
 728 parasite number was estimated by using the average Cq for each reaction by
 729 comparison to a standard curve generated with defined numbers of parasites.

730

731 **Statistical analysis**

732 All data were analyzed with Graph Pad Prism 7.0 software (San Diego, California
 733 USA). Statistical analyzes are performed with the unpaired Student's t-test (with
 734 Welch's correction when variances differ significantly), the Tukey's ANOVA one-way
 735 test, the Tukey's ANOVA two-way test and the Log-rank test.

736

737 **Data Availability**

738 No large primary datasets have been generated and deposited.

739

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753

754 **Author contributions**

755 AFP, VB, EH, SC, SR, LH, SMa, VP performed experiments. AFP, VB, EH, NB, SJ and SM
756 conceived and designed the experiments and performed data analysis. NB and JK,
757 contributed reagents, materials, and analysis tools. AFP, VB, NB, SJ and SM wrote the
758 manuscript.

759

760 **Conflict of interests**

761 The authors declare that they have no conflict of interest.

762

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968 **Figure Legends**

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Figure 1. The IRE1α/ XBP1s pathway is activated in BMDCs infected with *T. gondii*.
(A, B) BMDCs were infected with Pru parasites (at a MOI of 4 parasites/BMDC) or treated with tunicamycin (TN) for 16h and the expression of UPR target genes were analyzed by RT-qPCR. The results are expressed as (Log2) Fold change **(A)** or relative mRNA level **(B)** compared to non-infected (NI) conditions. IRE1α/XBP1s pathway: *Ern1* (IRE1α), *Xbp1s*, *Dnajb9* (ERdj4), *Sec24D*, *Bloc1s1* and *Sec61a1*; PERK/ATF4 pathway: *Atf4*, *Ddit3* (CHOP) and *Ppp1r15a* (GADD34); ATF6 pathway: *Atf6* and *Herpud1*; classical ER chaperons: *Hspa5* (BIP) and *Hsp90b1* (GRP94). Results are normalized to the housekeeping gene *Gapdh*. Unpaired Student T-test, ns: p>0.05; **: p<0.01; ***: p<0.001; mean ± S.E.M (n=2-5 independent experiments depending of the studied gene, 3 mice / independent experiment).

(C, D) Immunoblot analysis of IRE1α, PERK, Phospho-PERK (detected by the presence of a second upper band after migration on a 6% acrylamide gel), Phospho-eIF2α, CHOP and XBP1s protein expression in non-infected BMDCs, BMDCs treated with TN and Pru infected BMDCs **(C)** and in non-infected and Pru infected Flt3/Notch-DCs **(D)** for the indicated times. GAPDH and tubulin were used as loading controls. Similar blots are shown in Figure 1C and Figure EV3B-C, but the HK lanes have been removed from Figure 1C.

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Figure 2. UPR and cytokine responses are under the control of MyD88 protein in BMDCs infected with *T. gondii*.

(A) Mean percentage of Pru parasite infection of WT and MyD88KO BMDCs; mean ± S.E.M (n=3 mice, 1 representative experiment from 2 independent experiments is shown). Mann-Whitney U test, ns: p>0.05.

(B) Mean percentage of parasitophorous vacuoles containing 1, 2, 4 or 8 parasites/vacuole (p/v) in WT and MyD88 KO BMDCs after 16h of infection with Pru parasites. Mean values ± S.E.M. n= 3 independent experiments (biological replicates). Unpaired Student T-test, ***p<0.001.

(C) IL-6, IL-12 and IL-23 cytokine levels from WT and MyD88KO BMDCs non-infected (NI) and infected with Pru parasites for 16h. Two-way ANOVA Tukey's multiple

comparisons, ****: $p < 0.0001$; mean $\pm$ S.E.M (n=4-6 mice pooled from 2 independent experiments).

(D) mRNA relative expression level of *Xbp1s*, *Dnajb9* (ERdj4), *Ddit3* (CHOP) and *Ppp1r15a* (GADD34) from WT and MyD88KO BMDCs infected with Pru parasites for 16h compared to non-infected (NI) conditions. Results are normalized to the housekeeping gene *Gapdh*. Two-way ANOVA Tukey's multiple comparisons, ns: $p > 0.05$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$, ****: $p < 0.0001$; mean $\pm$ S.E.M (n=4-5 mice pooled from 2 independent experiments).

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1009 **Figure 3. Replicating parasites modulate the UPR.**

1010 **(A)** BMDCs (CD11c⁺) were incubated with Pru-Tomato parasites for 16h. Infected cells (red gating) and non-infected bystander cells (green gating) were sorted-purified and further analyzed.

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1012 **(B)** The expression of UPR target genes were analyzed by RT-qPCR in infected and non-infected bystander cells compared to non-infected BMDCs (NI). The results are expressed as relative mRNA level compared to non-infected conditions. Results are normalized to the housekeeping gene *Gapdh*. Unpaired Student T-test, ns: $p > 0.05$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; mean $\pm$ S.E.M (n=6 mice pooled from 2 independent experiments).

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1021 **Figure 4. The IRE1 α /XBP1s pathway modulates cytokine responses in infected BMDCs and cross-presentation of soluble secreted parasite antigens.**

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1023 **(A)** Dotplots showing the percentage of CD11c⁺/MHCII^{hi} cells in Pru Tomato SAG1-OVA parasite infected XBP1^{fl/fl}, XBP1 Δ DC, XBP1^{fl/fl}IRE1^{fl/fl} and XBP1 Δ DC IRE1^{trunc}DC BMDCs (upper panels) and of Tomato-positive cells (percentage of infected BMDCs: lower panels).

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1027 **(B)** Mean percentage $\pm$ S.E.M of infection is indicated (n=6 mice pooled from 2 independent experiments). Tukey's ANOVA one-way test, ns: $p > 0.05$.

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1029 **(C)** Mean percentage of parasitophorous vacuoles containing 1, 2, 4 or 8 parasites/vacuole (p/vac) in XBP1^{fl/fl}IRE1^{fl/fl}, XBP1 Δ DC IRE1^{trunc}DC and XBP1 Δ DC BMDCs. Tukey's ANOVA one-way test, ns: $p > 0.05$. Mean values $\pm$ S.E.M. n=3 independent experiments (biological replicates).

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1033 **(D)** IL-6, IL-12 and IL-23 secreted cytokine levels from Pru parasite infected XBP1^{fl/fl}, XBP1 Δ DC, XBP1^{fl/fl}IRE1^{fl/fl} and XBP1 Δ DC IRE1^{trunc}DC BMDCs measured by ELISA. Two-way ANOVA Tukey's multiple comparisons, ns: $p > 0.05$; ***: $p < 0.001$; ****: $p < 0.0001$; mean $\pm$ S.E.M (n=6 mice pooled from 2 independent experiments).

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1037 **(E)** mRNA relative expression level of *Il-6*, *Il-12* and *Il-23* from XBP1^{fl/fl}IRE1^{fl/fl}, XBP1 Δ DC IRE1^{trunc}DC and XBP1 Δ DC BMDCs infected with Pru parasites for 16 h compared to non-infected (NI) conditions. Results are normalized to the housekeeping gene *Gapdh*. Two-way ANOVA Tukey's multiple comparisons, ns: $p > 0.05$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; ****: $p < 0.0001$; mean $\pm$ S.E.M (n=3-5 mice pooled from 2 independent experiments).

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1043 **(F)** H-2K^b-SKL8 MHC class I presentation by XBP1^{fl/fl}IRE1^{fl/fl} treated or not with 4 μ 8C, XBP1 Δ DC IRE1^{trunc}DC and XBP1 Δ DC BMDCs infected with Pru Tomato SAG1-OVA at the indicated ratios, assessed with the B3Z T cell hybridoma. Unpaired Student T-

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test, *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; mean $\pm$ S.E.M (n=3 mice, one representative experiment from 2 independent experiments).

(G) Percentage of Pru Tomato SAG1-OVA infected BMDCs at the indicated ratios of parasites:BMDC. Bar graphs depict mean $\pm$ S.E.M (n=3 mice, one representative experiment from 2 independent experiments). Tukey's ANOVA one-way test, ns ($p > 0.05$) when p values are not identified.

(H) Exogenous MHCI presentation of synthetic SKL8 peptide pulsed on XBP1^{fl/fl}IRE1^{fl/fl} treated or not with 4 μ 8C, XBP1 Δ DC IRE1^{trunc}DC and XBP1 Δ DC BMDCs assessed with the B3Z T cell hybridoma. Bar graphs depict mean $\pm$ S.E.M (n=3 mice, one representative experiment from 2 independent experiments). Unpaired Student T-test, *: $p < 0.05$, ns ($p > 0.05$) when p values are not identified.

(I) Graph depicts the expression (indicated as the Mean Fluorescence Intensity) of H-2K^b by Pru Tomato SAG1-OVA infected XBP1^{fl/fl}IRE1^{fl/fl} treated or not with 4 μ 8C, XBP1 Δ DC IRE1^{trunc}DC and XBP1 Δ DC BMDCs. Bar graphs depict mean $\pm$ S.E.M (n=3 mice, one representative experiment from 2 independent experiments). Tukey's ANOVA one-way test, ns: $p > 0.05$.

Figure 5: The IRE1 α /XBP1s pathway is activated in splenic cDC1 of mice infected with *T. gondii*.

(A, B) Expression of VenusFP by splenic cDC1 (CD26+ CD11c+ MHCII+ XCR1+ CD172a-, upper panels) and cDC2 (CD26+ CD11c+ MHCII+ XCR1- CD172a+, lower panel) from WT **(A)** and ERAI **(B)** mice non-infected (NI) or infected with Pru (6 days post-infection (dpi)) reporting on IRE1 α pathway activation.

(C) Graph depicts the Mean Fluorescence Intensity measured for each condition in ERAI mice (6 dpi). Two-way ANOVA Tukey's multiple comparisons, ns: $p > 0.05$; ***: $p < 0.001$; ****: $p < 0.0001$; mean $\pm$ S.E.M (n=5 mice per genotype and condition, one representative experiment from 2 independent experiments is shown).

(D) Graph depicts the expression (indicated as the Mean Fluorescence Intensity) of VenusFP by splenic monocytes, neutrophils, B and T cells from Pru infected or non-infected (NI) ERAI mice (6 dpi). Bar graphs depict mean $\pm$ S.E.M (n=5 mice per genotype and condition, one representative experiment from 2 independent experiments is shown). Unpaired Student T-test, ns: $p > 0.05$.

(E, F) RT-qPCR analysis of the indicated UPR genes among total RNA prepared from splenic cDC1 **(E)** and cDC2 **(F)** sorted by flow cytometry 6 dpi from wild-type mice, non-infected (NI) or infected with Pru parasites. Results were normalized to the housekeeping gene *Gapdh* and are expressed as relative mRNA level compared to the NI condition. Bar graphs depict mean $\pm$ S.E.M. Unpaired Student T-test, ns: $p > 0.05$; *: $p < 0.05$; **: $p < 0.01$; mean $\pm$ S.E.M (n=3-5 mice pooled from 2 independent experiments).

Figure 6. The IRE1 α pathway protects mice from acute infection and promotes T cell responses.

(A) Survival curves of XBP1^{fl/fl}IRE1^{fl/fl} and XBP1 Δ DC IRE1^{trunc}DC mice infected intraperitoneally with 500 Pru parasites. Log-rank (Mantel-Cox) test, $p = 0.0004$; n=10 mice per genotype.

(B) Number of parasite genomes per spleen and PEC from XBP1^{fl/fl}IRE1^{fl/fl} and XBP1 Δ DC IRE1^{trunc}DC infected intraperitoneally with 500 Pru parasites 6 days post-

infection. Unpaired Student T-test **: $p < 0.01$; mean $\pm$ S.E.M (n=2 independent experiments combined).

(C) mRNA relative expression of *Il-6*, *Il-12*, *Ifn-g* and *Tnf-a* by splenocytes from XBP1^{fl/fl}IRE1^{fl/fl} and XBP1 Δ DC IRE1^{trunc}DC mice infected or not (NI) with 500 Pru parasites for 6 days. Results are normalized to the housekeeping gene *Gapdh*. Two-way ANOVA Tukey's multiple comparisons, ns: $p > 0.05$; *: $p < 0.05$; **: $p < 0.01$; ****: $p < 0.0001$; mean $\pm$ S.E.M (n=2 independent experiments combined).

(D, E) Quantification of the number of total (left histogram) and activated (right histogram) (IFN γ -positive) CD4+ T cells **(D)** and CD8+ T cells **(E)** in the spleen of control mice (XBP1^{fl/fl}IRE1^{fl/fl}) and XBP1 Δ DC IRE1^{trunc}DC infected for 6 days. Two-way ANOVA Tukey's multiple compare, ns: $p > 0.05$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; ****: $p < 0.0001$; mean $\pm$ S.E.M (n=2 independent experiments combined). **(F)** Frequency and number of cDC2 (left) and cDC1 (right) in the spleen of control mice and XBP1 Δ DC IRE1^{trunc}DC infected for 6 days. Two-way ANOVA Tukey's multiple compare, ns: $p > 0.05$; ****: $p < 0.0001$; mean $\pm$ S.E.M (n=2 independent experiments combined).

Figure 7: Modulation of the UPR and down-stream responses in *T. gondii* infected BMDCs.

(A) After incubation of BMDCs with HK parasites or STAg, TLRs are stimulated and induce transcriptional activation of the three pathways of the UPR. The IRE1 α /XBP1s pathway stimulates the secretion of IL-6 and IL-12 by BMDCs. Blue arrows indicate down-regulated pathways and red arrows, up-regulated pathways.

(B) In BMDCs infected with live Pru parasites for 16h, the IRE1 α /XBP1s pathway is activated in a MyD88-dependent manner while the PERK pathway is not induced and the ATF6 pathway is repressed. XBP1s stimulates the secretion of IL-6 and IL-12 and IRE1 α promotes MHC-I presentation of secreted parasite antigens. The secretion of IL-23 is independent of XBP1s and CHOP and only partially depends on MyD88. Our data also indicate that live parasites down-regulate the induction of XBP1s and CHOP induced by TLRs. Blue arrows indicate down-regulated pathways and red arrows, up-regulated pathways.

Expanded View Figure Legends

Figure EV1. Induction of cytokine production from infected BMDCs.

(A) IL-6, IL-12 and IL-23 cytokine levels from non-infected BMDCs (NI) and BMDCs infected with Pru parasites for 6h and 16h measured by ELISA. Unpaired Student T-test, *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; mean $\pm$ S.E.M (n=4-6 mice pooled from 2 independent experiments).

(B) IL-6, IL-12 and IL-23 cytokine levels from non-infected BMDCs (NI) and type I RH or type II Pru infected BMDCs measured by ELISA. ANOVA Tukey's multiple comparisons, ns: $p > 0.05$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$, ****: $p < 0.0001$; mean $\pm$ S.E.M (n=4-6 mice pooled from 2 independent experiments).

Figure EV2. hER is recruited at the PV of infected BMDCs.

(A) Electron microscopy images showing the recruitment of hER at the limiting membrane of vacuole containing live Pru parasites. Right images (scale bars=500nm): zoom of the region indicated by a white frame on the left images (scale bars=1 μ m).

(B) Pru Heat-killed (HK) parasite containing vacuoles do not recruit hER. left image: scale bar=2 μ m; Right image: scale bar=1 μ m.

Figure EV3. The three pathways of the UPR response are activated in BMDCs incubated with Pru HK parasites and STAgS.

(A) BMDCs were incubated with Pru HK parasites for 16h and the expression of UPR target genes were analyzed by RT-qPCR. The results are expressed as relative mRNA level compared to non-infected conditions. Results are normalized to the housekeeping gene *Gapdh*. Unpaired Student T-test, *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; ****: $p<0.0001$; mean $\pm$ S.E.M (n=2-5 independent experiments depending of the studied gene, 3 biological replicates (mice) / experiment).).

(B, C) Immunoblot analysis of IRE1 α , phospho-eIF2 α , CHOP and XBP1s protein expression in non-infected BMDCs, BMDCs treated with tunicamycin (TN), BMDCs incubated with Pru live parasites and BMDCs incubated with Pru HK parasites for the indicated times. GAPDH and tubulin were used as loading controls. Similar blots are shown in Figure 1C and Figure EV3B-C, but the HK lanes have been removed in Figure 1C.

(D) Immunoblot analysis of IRE1 α and XBP1s in non-infected BMDCs (NI), BMDCs infected with Pru parasites for 16h and treated with soluble *T. gondii* antigen extracts (STAgS) for 16h. Tubulin was used as loading control.

(E) BMDCs were incubated with STAgS for 16h and the expression of UPR target genes were analyzed by RT-qPCR. The results are expressed as relative mRNA level compared to non-infected conditions. Results are normalized to the housekeeping gene *gapdh*. Unpaired Student T-test, ns: $p>0.05$; *: $p<0.05$; **: $p<0.01$; mean $\pm$ S.E.M (n=4 mice).

Figure EV4. UPR and cytokine responses are under the control of MyD88 in BMDCs incubated with Heat-Killed parasites

(A) mRNA relative expression level of *Xbp1s*, *Dnajb9* (ERdj4), *Ddit3* (CHOP) and *Ppp1r15a* (GADD34) from WT and MyD88KO BMDCs incubated with Heat-Killed (HK) parasites for 16h compared to non-infected (NI) condition. Results are normalized to the housekeeping gene *Gapdh*. Two-way ANOVA Tukey's multiple comparisons, ns: $p>0.05$; ****: $p<0.0001$; mean $\pm$ S.E.M. (=4-5 mice pooled from 2 independent experiments)

(B) IL-6, IL-12 and IL-23 cytokine levels from WT and MyD88 KO BMDCs non-infected (NI), infected with Pru parasites or incubated with Pru HK parasites for 16 h. Two-way ANOVA Tukey's multiple comparisons, ****: $p<0.0001$; mean $\pm$ S.E.M (n=4-6 mice pooled 2 independent experiments combined).

Figure EV5. IRE1 α /XBP1s depletion does not impact on BMDC differentiation and activation but alters cytokine production by HK parasite-stimulated BMDCs.

1186 **(A)** Immunoblot analysis of IRE1 α and XBP1s protein expression in XBP1^{fl/fl}IRE1^{fl/fl},
1187 XBP1 Δ DC and XBP1 Δ DC IRE1^{trunc}DC BMDCs treated or not with tunicamycin (TN) to
1188 induce ER stress. Please note that there is never 100% excision of the floxed alleles in
1189 GM-CSF BMDCs cultures, as can be noted from the strongly reduced but not fully
1190 abrogated expression of IRE1 α and truncated IRE1 α remaining visible in the
1191 XBP1 Δ DC IRE1^{trunc}DC BMDCs line.

1192 **(B)** Dotplots showing the percentage of live cells (live/dead marker, upper panels)
1193 and of cells expressing CD11c and MHCII molecules (lower panels) in Pru infected
1194 XBP1^{fl/fl}IRE1^{fl/fl}, XBP1 Δ DC IRE1^{trunc}DC and XBP1 Δ DC BMDCs. Graph depicts the mean
1195 percentage of live cells and CD11c+ MHCII^{high} cells in XBP1^{fl/fl}IRE1^{fl/fl}, XBP1 Δ DC
1196 IRE1^{trunc}DC and XBP1 Δ DC BMDCs non-infected (NI), infected with Pru parasites,
1197 incubated with Pru HK parasites or treated with LPS for 16h. Bar graphs depict mean
1198 $\pm$ S.E.M (n=3 mice, one representative experiment from 2 independent
1199 experiments). Tukey's ANOVA one-way test, ns: p>0,05.

1200 **(C)** Staining profiles of LPS treated XBP1^{fl/fl}IRE1^{fl/fl}, XBP1 Δ DC IRE1^{trunc}DC and XBP1 Δ DC
1201 BMDCs indicating the surface expression of CD80 and CD86 molecules. Graph
1202 depicts the quantification (indicated as the Mean Fluorescence Intensity) of CD80
1203 and CD86 expression in LPS treated XBP1^{fl/fl}IRE1^{fl/fl}, XBP1 Δ DC IRE1^{trunc}DC and
1204 XBP1 Δ DC BMDCs. Bar graphs depict mean $\pm$ S.E.M (n=3 mice, one representative
1205 experiment from 2 independent experiments). Tukey's ANOVA one-way test, ns:
1206 p>0,05. **(D)** IL-6, IL-12 and IL-23 cytokine levels from XBP1^{fl/fl}, XBP1 Δ DC,
1207 XBP1^{fl/fl}IRE1^{fl/fl} and XBP1 Δ DC IRE1^{trunc}DC BMDCs infected with Pru parasites or
1208 incubated with Pru Heat-Killed (HK) parasites for 16h compared to non-infected (NI)
1209 BMDCs. Two-way ANOVA Tukey's multiple comparisons test, ns: p>0.05; *: p<0.05;
1210 **: p<0.01; ****: p<0.0001; mean $\pm$ S.E.M (n=3-6 mice pooled from 2 independent
1211 experiments).

1212 **(E)** mRNA relative expression of *Il-6*, *Il-12* and *Il-23* from XBP1^{fl/fl}IRE1^{fl/fl}, XBP1 Δ DC
1213 IRE1^{trunc}DC and XBP1 Δ DC BMDCs infected with Pru parasites or incubated with Pru
1214 Heat-Killed (HK) parasites for 16h compared to non-infected (NI) BMDCs. Results are
1215 normalized to the housekeeping gene *Gapdh*. Two-way ANOVA Tukey's multiple
1216 comparisons test, ns p>0.05; * p<0.05; ** p<0.01; **** p<0.0001; mean $\pm$ S.E.M
1217 (n=3-6 mice pooled from 2 independent experiments).

1218

1219

Figure 1

A **B** **C****BMDCs** **D****Flt3/Notch-cDC1**

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Figure EV1

Figure EV2

A

Live

Live

B

Figure EV3

Figure EV4

Figure EV5

A

B

C

D

E