Protease-activated receptor 2 contributes to Toxoplasma gondii-mediated gut inflammation

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Protease-Activated Receptor 2 contributes to *Toxoplasma gondii*–mediated gut inflammation

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Abstract

Toxoplasma gondii is a widespread intracellular parasite, which naturally enters the organism via the oral route and cross the intestinal barrier to disseminate. In addition to neuronal and ocular pathologies, this pathogen also causes gut inflammation in a number of animals. This infection-triggered inflammation has been extensively studied in the C57BL/6 mice, highlighting the importance of the immune cells and their mediators in the development of gut pathology. However, despite their importance in inflammation, the role of Protease-Activated Receptors (PAR) was never reported in the context of *T.gondii*-mediated small intestine inflammation. Using genetically modified mice, we show that PAR_2 plays a pathogenic role in the development of gut inflammatory lesions. We find that PAR_2 controls the innate inflammatory mediators IL-6, KC/CXCL1, PGE_2 as well as neutrophil infiltration in *T. gondii*-triggered gut damage. These results bring new knowledge on the mechanisms operating in the gut in response to *T. gondii* infection.

Keywords: *Toxoplasma gondii*, gut infection, inflammation, Protease-Activated Receptor, ileitis

Introduction

The mucosal barrier constitutes the first line of exchange and defense between our body and the surrounding environment. This natural obstacle has to be crossed in order for a number of harmful enteric pathogens such as *Salmonella*, but also parasites like *Toxoplasma gondii (T. gondii)*, to penetrate the host. *T. gondii* belongs to the group of Apicomplexa protozoan parasites. *T. gondii* infection is widespread in humans with dramatic consequences in immuno-compromised patients and in fetus of primo-infected pregnant women. Recent growing evidences suggest that *T. gondii* infection might also be a risk factor for developing neuropsychiatric disorders (1).

Apart from neurological and ocular pathologies, another level of interest linked to *T. gondii* invasion in the gut is the observation that a number of mammals and primates display small intestine inflammation upon *T. gondii* infection (2). This pinpoints an acute effect of *T. gondii* infection on gut pathology in animal kingdom. This *T. gondii*-driven small intestine inflammation is observed in the susceptible C57BL/6 (B6) mice. As referred in the literature (3), ingestion of *T. gondii* cysts in B6 mice results in ileitis associated with a massive necrosis causing death of the animals within 10 days.

Previous studies have established a major role for immune cells in the development of intestinal inflammation in this model, including CD4+ T cells (3), Th17 (4), intra-epithelial lymphocytes (5), Natural Killer (NK) cells (6) and innate lymphoid cells (7). Furthermore, the collapse of regulatory T cells together with the subversion of their suppressive capacity is a major event in *T. gondii*-induced intestinal

pathology (8). $Gr1^+$ inflammatory monocytes play a protective role but not neutrophils that rather contribute to the intestinal damage (9, 10). In addition, the intestinal microflora is believed to play a critical role in the development of gut lesions (11). Regarding the molecular mechanisms, adaptive and innate immunity components have been shown to positively or negatively impact the development of inflammation in this model. Pathogenic functions have been attributed to IFN-γ, TNFα, iNOS (3, 12) and TLR9 (13), while IL-10, TGF-β and NOD2 appear protective (14-16). However, the role of proteases and their signaling pathways have never been investigated in this pathology.

Proteolytic homeostasis plays a prominent role at mucosal surfaces (17), particularly in the context of intestinal inflammation (18), (19). As a matter of fact, proteases, historically considered as simple digestive enzymes, are now well recognized for their capacity to turn on and off cellular signaling pathways (17). That concept is well illustrated by the Protease-Activated Receptor (PAR) family (20). PARs are G protein-coupled receptors (GPCRs), which are present at the surface of a large number of cell types. These receptors are activated through proteolytic cleavage of their extracellular N-terminal domain, thereby creating a new N-terminal fragment that serves as a tethered ligand able to bind to the second extracellular loop of the cleaved receptor. This results in signal transduction generally leading to the initiation of a host inflammatory response. PARs are indeed involved in a number of inflammatory or infectious conditions of the oral mucosa and digestive tract (20, 21), but their specific role during *T. gondii*-triggered gut pathology has never been addressed.

Using genetically modified mice, our study demonstrates that PAR_2 has a pro-inflammatory role during *T. gondii*-triggered small intestine pathology. Moreover, our results indicate that PAR_2 is involved in the up-regulation of pro-inflammatory mediators like IL6 and KC/CXCL1, as well as in the local production of bioactive lipids such as PGE_2. This sheds new light on the pathways involved in *T. gondii*-induced intestinal inflammation.

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Materials and Methods

Mouse model and *in vivo* infection

$PAR_2^{-/-}$ animals on C57BL/6 (B6) background (22) were compared to either purchased C57BL/6 wild-type mice from Janvier Laboratory or $PAR_2^{+/+}$ littermates. Parasite cysts were maintained in CBA/J mice through serial passage of cysts every 8 weeks. Seven week old $PAR_2^{+/+}$ and $PAR_2^{-/-}$ mice were orally infected with 30 cysts of 76K *T. gondii* strain as described (23). Mice were euthanized at day 7 post-infection. Experimental data results from at least 2 independent experiments using a minimum of n=7 animals per group. For the kinetics experiments using WT B6 animals **(Fig. 1)**, a minimum of n=4 animals per group was used. Animal studies were carried out under the control of the French National Veterinary Services and in accordance with the European regulations (EEC directive 86/609 dated 24 November 1986). The protocol was approved by the local Ethics Committee CEEA122 (Approval CE n°2015-02).

Macroscopic and microscopic scoring of inflammation

The macroscopic score was based on visual evaluation of small intestinal damage, using criteria adapted from Motta *et al.* (18). Strictures and presence of blood in the feces could not be used since those two parameters were never observed in the present model. The following criteria were: tissue swelling (0= no swelling, 1= minor, 2= prominent, 3= important, 4= major), change in luminal content color (0 = no change, 1= change) and presence of pus (0= no, 1= minor, 2= intermediate, 3= important) (maximum score: 8). Small intestine tissues were fixed in 4% formaldehyde for 24 hours and dehydrated in EtOH 70% before being embedded in paraffin. Three-micron sections were cut, mounted on slides and stained for hematoxylin & eosin (HE). Histology analysis was used to assess microscopic scores, which combines defects in mucosal architecture (0-3), cellular infiltration (0-3), crypt morphology (normal = 0, abnormal = 1), diminution of Goblet cell

number (no diminution = 0, diminution =1), diminution of Paneth cell (no diminution = 0, diminution =1) and muscle thickening (0-3) (Maximum scores: 12).

QPCR and RT-qPCR analysis

RNA was extracted from small intestine tissue using Trizol Reagent (Invitrogen). Reverse transcription was performed from 5 µg RNA using the Maxima First Strand cDNA Synthesis Kit (Thermo Scientific). QPCR was performed using SYBR Green master mix on LightCycler 480 apparatus (Roche). The graphs represent means of ΔCt between *Hprt* and target mRNA. *Gapdh* was also used as a second house-keeping gene to confirm our results (data not shown). Parasite genomic DNA was extracted from mesenteric lymph nodes (MLN) using blood and tissues kit (Qiagen). Parasite burden in MLN was quantified by qPCR of parasite gDNA. The target gene that was chosen was described earlier and the primers allow the amplification of a 162 bp amplicon (24). The absolute number of parasites per mg of tissue was calculated using a standard curve realized with genomic DNA extracted from a known number of *T. gondii* parasites. All primer sequences and GenBank accession numbers are indicated on **Table S1**.

Immunofluorescence

Mouse small intestine tissues were prepared as described above in the paragraph "Macroscopic and microscopic scoring of inflammation". After removal of paraffin at 60°C, slides were boiled 20 minutes in a citrate buffer pH6. After rehydration, slides were incubated in phosphate-buffered saline (PBS) containing 4% goat serum. Slides were then incubated overnight in the same buffer containing primary antibodies, i.e. rat anti-Ly6G (Biolegend, clone 1A8) or rat IgG2a isotype antibodies (Biolegend). After three washes in PBS, slides were incubated with goat anti-rat Alexa 555 secondary antibodies (Invitrogen), and in a solution of Dapi (500 ng/mL)

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before mounting with Prolong (Invitrogen). Tissues were observed using a Zeiss 710 confocal microscope with a 20x objective.

Quantification of Poly Unsaturated Fatty Acid metabolites

Analysis was performed by LC-MS/MS as described (25).

Statistical analysis

All statistical analysis were performed on Graphpad Prism 6 software. Results are expressed as mean ± SD. Groups were compared using non-parametric tests, i.e. Kruskal-Wallis, Dunn's multiple comparison tests. For neutrophil infiltration in infected animals, Mann-Whitney test was applied. For survival curves, a Mantel-Cox test was used. Unless otherwise stated, experimental data results from at least 2 independent experiments using a minimum of n=7 animals per group.

Results

PAR_2 mRNA level is increased in inflamed murine small intestine following *Toxoplasma gondii* infection

Inflammation was induced by oral infection of B6 mice by gavage with 30 cysts of the 76K *T. gondii* strain. An initial time-course experiment performed at days 3, 5 and 7 post-infection showed a gradual increase in parasite burden quantified by RT-qPCR of SAG1 in small intestine tissues and qPCR on genomic DNA from mesenteric lymph nodes (**Fig. 1A,B**). This correlated with the progressive setting of small intestine inflammation, as illustrated by increased macroscopic and microscopic scores of inflammation **(Fig. 1C,D)**. Significant increased levels of IFNγ and TNFα were observed at day 7 post-infection **(Fig. 1E,F)**. Since sustained PAR_2 activation promotes a regulatory feedback loop that augments the level of its own mRNA (26),

we investigated the impact of *T. gondii* infection on PAR_2 transcript level in the small intestine of infected B6 mice. Our results showed that PAR_2 mRNA was significantly increased at day 7 post-infection, suggesting a possible involvement of PAR_2 in *T. gondii*-driven inflammation **(Fig. 1G).**

PAR_2 deficiency reduces gut inflammation induced by *Toxoplasma gondii*

To evaluate the role of PAR_2 in the *T. gondii* gut inflammation model, we next infected B6 mice invalidated or not for PAR_2 expression. No difference was observed in weight loss between infected WT and KO animals during the week following infection (**Fig. 2A**). However, higher survival rate was observed in PAR_2 deficient infected mice, compared to WT infected mice (**Fig 2B**). Mice were euthanized at day 7 post-infection to assess gut inflammation. As illustrated in **Fig. 3**, in infected animals, the macroscopic and microscopic inflammatory scores were significantly decreased in the absence of PAR_2 7-days post infection. This protection was not due to a better control of parasite growth in the gut of PAR_2 deficient animals since measurement of *T. gondii* load in the small intestine by RT-qPCR of SAG1 showed similar parasite burden between infected WT and $PAR_2^{-/-}$ animals (**Fig. 4A**). Similar parasite burden was also found in gut draining mesenteric lymph nodes (**Fig.4B**). Therefore, our results suggest that the development of small intestine inflammation following oral gavage of mice with *T. gondii* cysts involves active PAR_2 signaling.

PAR_2 is involved in the up-regulation of IL6, KC/CXCL1 cytokines and production of PGE2 and 8-isoPGA2 during *Toxoplasma gondii* – induced gut pathology

To decipher which mediators could be regulated by PAR_2 during the development of *T. gondii*-mediated gut lesions, we quantified the gene expression level of several inflammatory mediators in the gut tissues of infected and non-infected WT and knockout animals. Gene

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expression of IL-6, KC/CXCL1, CCL2/MCP-1, CCL3/MIP-1A, CCL4/MIP-1B, CCL5/RANTES, NOS2, CXCL2/MIP2, IFNγ, TNFα, IL-10 and TGF-β was measured by RT-qPCR analysis **(Fig. 5)**. This showed that the amount of IL-6 mRNA level was significantly reduced in infected PAR_2-deficient mice compared to WT animals (**Fig. 5A**). Moreover, the up-regulation of KC/CXCL1 level observed upon *T. gondii* infection in WT mice was lost in the absence of PAR_2 (**Fig. 5B**). In contrast, CCL2/MCP-1, CXCL10/IP10, CCL3/MIP-1α, CCL4/MIP-1β, CXCL2/MIP2, TNFα, IFNγ and IL-10 were up-regulated similarly between infected WT and $PAR_2^{-/-}$ mice (**Fig. 5C-J)**. Surprisingly, CCL5/RANTES expression was down-regulated by *T. gondii* infection in WT animals but up-regulated in PAR_2 KO animals (**Fig. 5K**). A similar tendency was found for iNOS expression (**Fig. 5L**). Finally, no difference in TGF-β expression was detected among the 4 groups (**Fig. 5M**). These results indicate that the up-regulation of some innate pro-inflammatory cytokines, such as IL-6 and KC/CXCL1, depends at least partially on the presence of PAR_2 in this infectious model.

PolyUnsaturated Fatty Acids (PUFA) constitute a source of bioactive lipids playing major roles in innate immunity during infection and inflammation (27). We therefore quantified PUFA metabolites present in the gut during *T.gondii* infection in WT or PAR_2 - deficient mice (**Fig. 6**). Results illustrated in **Fig. 6A,B** revealed that in wild-type mice, the amount of PGE_2 and 8-isoPGA2 were both elevated in the small intestine of *T. gondii*-infected mice in comparison with non-infected animals. The absence of PAR_2 completely abolished these effects. In contrast, infection had no impact on the level of other bio-active lipids such as 6KPGF1a, 15d-PGJ2, TxB2 and LTB4 (**Fig. 6C-F**).

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PAR_2 is involved in the infiltration of neutrophils in the small intestine tissue during *Toxoplasma gondii* infection

Since KC/CXCL1 was reduced in PAR_2-deficient mice compared to WT, and given the major neutrophil chemo-attracting role of KC/CXCL1, we evaluated neutrophil infiltration within the small intestine at day 7 post-infection. For this purpose, immunostaining was performed in tissue cross sections using anti-Ly6G antibodies (**Fig. 7A**). Total number of neutrophils present on the whole tissue section was reported for WT and PAR_2 infected animals, as shown in **Fig. 7B**. Neutrophil infiltration was lower in PAR_2 KO animals compared to WT. Moreover, in WT tissues, a significant proportion of neutrophils was localized at the top of the villi or in the lumen and was associated with epithelial layer erosion, while the majority of neutrophils counted in PAR_2 KO tissues was detected in the middle or bottom part of the villi.

Altogether, our data showed the implication of PAR_2 in the inflammatory phenotype developed in the small intestine infected with *T. gondii*. More specifically, innate immunity mediators IL6, KC/CXCL1 as well as PGE_2 and 8-isoPGA_2 are under the control of PAR_2 during *T.gondii*-triggered gut pathology. Moreover, our results suggest that PAR_2-dependent mechanisms rely on the recruitment of neutrophils in the small intestine, thereby proposing the implication of this immune cell population in the inflammatory response to *T. gondii*.

Discussion

The present study sheds light on the role played by PAR_2 in the establishment of gut mucosal immune response associated with *Toxoplasma gondii* pathogenic infection. We here show an alleviated gut inflammatory phenotype in PAR_2 KO mice infected by *T. gondii*,

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associated with a better survival rate. In the absence of parasite load difference between WT and PAR_2 KO infected mice, our results suggest that the increased survival rate observed in PAR_2 KO animals likely results from a lower gut inflammation level compared to WT. However, there was no impact on body weight loss. Although body weight loss is a gross parameter to follow gut inflammation, it does not always correlate perfectly. In particular, PAR_2-deficient mice submitted to DSS-colitis protocol do not loose less weight than WT mice despite of a significantly alleviated phenotype as described in Hyun *et al*.(28).

We showed that among a panel of inflammatory chemokines, cytokines, and lipid mediators, only a few were specifically regulated by PAR_2 during *T.gondii* infection. We found that expression of IL-6 and KC/CXCL1 cytokines as well as PGE_2 and 8-isoPGA_2 lipid production were lower in infected PAR_2-deficient animals, compared to WT. In line with the control of KC/CXCL1 by PAR_2 and in agreement with the strong neutrophil chemo-attracting activity of this chemokine, we found that neutrophil infiltration of the tissue in infected animals was significantly reduced in the absence of PAR_2. Our results suggest that the mechanisms behind the pathogenic role of PAR_2 rely on the control by PAR_2 of a specific subset of innate immunity mediators. Our results propose that PAR_2-dependent neutrophil infiltration would be due to KC/CXCL1 chemokine up-regulation and that this could constitute a major pathway in the establishment of intestinal lesions during *T. gondii* infection of the gut.

Our manuscript supports the idea of parallel PAR_2 -dependent and independent mechanisms to control inflammatory mediators and inflammation level in *T. gondii*-mediated gut inflammation model. The fact that inflammation in the gut was not fully abolished in the absence of PAR_2 suggests that a part of this inflammation is regulated by PAR_2-independent mechanisms that are still in place during infection. This is likely reflected by the fact that the levels of IFNγ

and TNFα, which are described as important mediators in this inflammation model, are up-regulated similarly in both groups of infected animals, regardless of PAR_2 presence. Interestingly, a lack of correlation between the level of these two major cytokines and the level of gut inflammation has been reported in the literature. This is exemplified in the paper of Guiton *et al*. (4) where the same *T. gondii*-mediated ileitis model was used. The authors showed a decrease of gut inflammation in the absence of IL-17 receptor but this was not correlated with decreased levels of IFNγ and TNFα, which were similarly induced in WT and IL17RA KO infected mice. In addition, akin to our situation, there was a similar parasite burden in the small intestine of both animals. Even more surprising, Bonfa *et al*. (2014) showed an inverse correlated phenotype where *T.gondii*-infected CCR5 knockout mice displayed increased gut inflammatory lesions but decreased levels of mRNA levels IFNγ and TNFα (and many more cytokines) in the ileum (29). These studies including ours, underline the complexity of mechanisms controlling gut inflammation during *T. gondii* infection and indicate that pathways, unrelated to IFNγ and TNFα, operate during infection and control the intensity of inflammatory gut lesions.

Several non-exclusive sources of proteases are potentially able to activate PAR_2 during *T. gondii*–triggered gut pathology (17). Firstly, proteases with known PAR_2-modulating activities are brought by pancreatic secretion (trypsin), or produced *in situ* by intestinal epithelial cells and/or inflammatory cells (kallikreins, cathepsin S, neutrophil elastase, cathepsin G and proteinase 3)(30-32). Although host cells are considered as the main sources of PAR_2-activating proteases, a number of studies show that microbial proteases also participate to the modulation of PAR activity (33, 34) and it is logical to speculate that serine proteases from the microbiota as well as *T.gondii* itself may also cleave the receptor. Although the number of *T. gondii* parasites is low at the initial invasion step, the parasite burden in the small intestine tissue is increasing

substantially during inflammation progression as a consequence of intra-vacuolar replication in infected cells and neutrophil-mediated spreading throughout the small intestinal lumen (35). *T. gondii* contains intra-vesicular pools of proteases that are secreted during the invasion process (36) and which remain at the vicinity of membrane receptors during invasion. It is thus conceivable that some of these parasitic proteases from the parasite-secreted microneme vesicles, may directly activate PAR_2 and thereby participate to the switch of the intestine towards a pro-inflammatory phenotype. Among those potential candidate proteases are MPP2, MPP3, TgSUB1, TgROM1 and Rhomboid-like protease 5. Future work will be needed to identify the precise nature of these PAR_2-activating proteases and their relevance in the inflammatory response.

Our work demonstrates for the first time the contribution of PAR_2 in gut inflammatory responses mediated by *T. gondii* infection. Our study favors a model in which PAR_2 controls the level of inflammation and neutrophil infiltration by regulating the level of KC/CXCL1 in the small intestine tissue during *T. gondii* infection. These results highlight the importance of proteolytic homeostasis in the control of the host response to gut infection. Our findings may be relevant for other gut protozoan parasites such as *Cryptosporidium,* which also belong to Apicomplexa, targets the small intestine and is the causal agent of severe diarrheal pathology worldwide.

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Figure Legends

Figure 1. Kinetics of parasite load, inflammatory parameters and PAR_2 expression in wild-type B6 mice at days 3, 5 and 7 post-infection. A) Parasite burden quantified in the small intestine of B6 mice by RT-qPCR of SAG1 at days 3, 5 and 7 post-infection. Murine HPRT was used as a house keeping gene for normalization. **B**) Absolute parasite burden quantified in mesenteric lymph nodes by qPCR of *T. gondii* genomic DNA at days 3, 5 and 7 post-infection. **C)** and **D)** Macroscopic and microscopic scores of inflammation in the small intestine during *T. gondii* infection. **E-F)** Quantitative RT-PCR showing the relative expression of IFNγ **(E)** and TNFα **(F)** in the small intestine tissue. Panels A-F are representative of one experiment using n=4 infected mice for each time point and n=3 to 12 mice for the non infected group. **G)** RT-qPCR of small intestine tissue showing increased expression level of PAR_2 mRNA (relative to HPRT) at day 7 post-infection. This figure represents two experiments. NI: Non Infected ; *T. gondii: T. gondii* - infected animals. Statistics were done to compare infected groups with the non infected control group. Only significant differences to the non infected group are indicated. p-value *< 0,05 ; **<0,01.

Figure 2. Body weight loss and survival curves of WT and PAR_2 KO mice infected with *Toxoplasma gondii*. A) The body weight of non infected or *T. gondii*-infected animals was measured during the week following infection. The graph shows the variation of weight compared to day 0 (corresponding to 100%). WT and PAR_2 KO infected animals showed no difference in weight loss (Kruskal-Wallis test). **B)** Survival curves of *T. gondii*-infected WT and PAR_2 deficient mice showing a better survival in KO animals compared to WT (statistical

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analysis using Mantel-Cox test). This figure represents one experiment. p-value *< 0,05 ; n.s : not significant.

Figure 3. Inflammatory scores in the small intestine of WT and PAR_2 KO animals infected with *Toxoplasma gondii* . A) Macroscopic scores of inflammation in the small intestine of animals harvested 7 days post-infection. **B)** H&E staining of the small intestine of animals harvested 7 days post-infection. Scale bar: 200 µm. Microscopic scores of inflammation were based on H&E picture examination. **C-D)** Magnification of H&E pictures. **(C)** Top of the villi showing erosion of the epithelium layer with decreased in the number of Goblet cells (arrow) and strong immune cell infiltration (numerous small nuclei) in WT infected animals. $PAR_2^{-/-}$ infected animals showed less severe erosion, and some goblet cells were still visible. **(D)** Bottom of the crypt showing the presence of Paneth cells (red vesicles - containing cells) in non infected WT and PAR_2 KO mice. Upon infection, strong reduction of Paneth cell was observed in WT animals whereas some Paneth cell were still present in PAR_2 deficient animals Scale bar: 50 µm, objective 40X. n = 3 independent experiments. NI: Non Infected *; T. gondii* : *T. gondii* - infected animals.

Figure 4. Parasite burden in WT and PAR_2 KO infected mice. A) Parasite burden quantification in WT and $PAR_2^{-/-}$ small intestine tissues at day 7 post-infection. RT-qPCR analysis showed similar mRNA expression level of SAG1 (major surface antigen of tachyzoite) relative to murine HPRT. No expression of SAG1 was detectable in non infected animals. **B)** Parasite burden quantified in mesenteric lymph nodes by QPCR at day 7 post-infection, showing similar load between WT and PAR_2 mice.

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Figure 5. RT-qPCR quantification of inflammatory mediators in the small intestine of WT and PAR_2 KO animals infected with *Toxoplasma gondii*. Quantitative RT-PCR at day 7 post-infection showing the relative expression of IL6 **(A)**, KC/CXCL1 **(B)**, CCL2 **(C)**, CXCL10 **(D)**, CCL3 **(E)**, CCL4 **(F)**, CXCL2 **(G)**, TNFα **(H)**, IFNγ **(I)**, IL10 **(J)**, CCL5 **(K)**, iNOS **(L)** and TGF-b **(M)** in WT or $PAR_2^{-/-}$ murine small intestine tissue infected or not with *T. gondii*. The graph represents 2 experiments for IL6, KC/CXCL1, IFNγ and TNFα analysis, and 1 experiment for the other mediators. NI: Non Infected *; T. gondii* : *T. gondii* - infected animals. p-value *< 0,05 ; **<0,01 ; ***<0,001 ; ****<0,0001 ; n.s : not significant.

Figure 6. Quantification of lipid metabolites in the small intestine of WT and PAR_2 KO animals infected with *Toxoplasma gondii*. Quantification of lipid metabolites in WT or $PAR_2^{-/-}$ small intestine tissues at day 7 post-infection : PGE2 **(A),** 8-isoPGA2 **(B)**, 6KPGF1a **(C)**, 15d-PGJ2 **(D)**, TxB2 **(E)** and LTB4 **(F)**. NI: Non Infected ; *T. gondii : T. gondii* - infected animals. The graph results from 2 pooled experiments. p-value *< 0,05 ; **<0,01 ; ***<0,001; n.s : not significant.

Figure 7. Neutrophil infiltration in the small intestine of infected WT and PAR_2 KO animals at day 7 post-infection. (A) Immunostaining was performed on tissue cross sections using anti-Ly6G antibodies. Pictures show the presence of neutrophils (Ly6G staining in red) associated with areas of tissue erosions in the small intestine from WT infected animals. Tissues from PAR_2 KO showed few Ly6G positive cells, which were restricted to the lamina propria compartment. Scale bar: 100 µm. DAPI: blue staining. **B)** Quantification of neutrophils present on tissue cross sections in WT and $PAR_2^{-/-}$ infected animals. The total number of Ly6G positive

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cells per cross section of WT or $PAR_2^{-/-}$ was reported and showed diminished neutrophil infiltration in PAR_2 KO animals compared to WT. This figure represents one experiment. p-value *< 0,05.

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Figure 1 *Bonnart et al.*

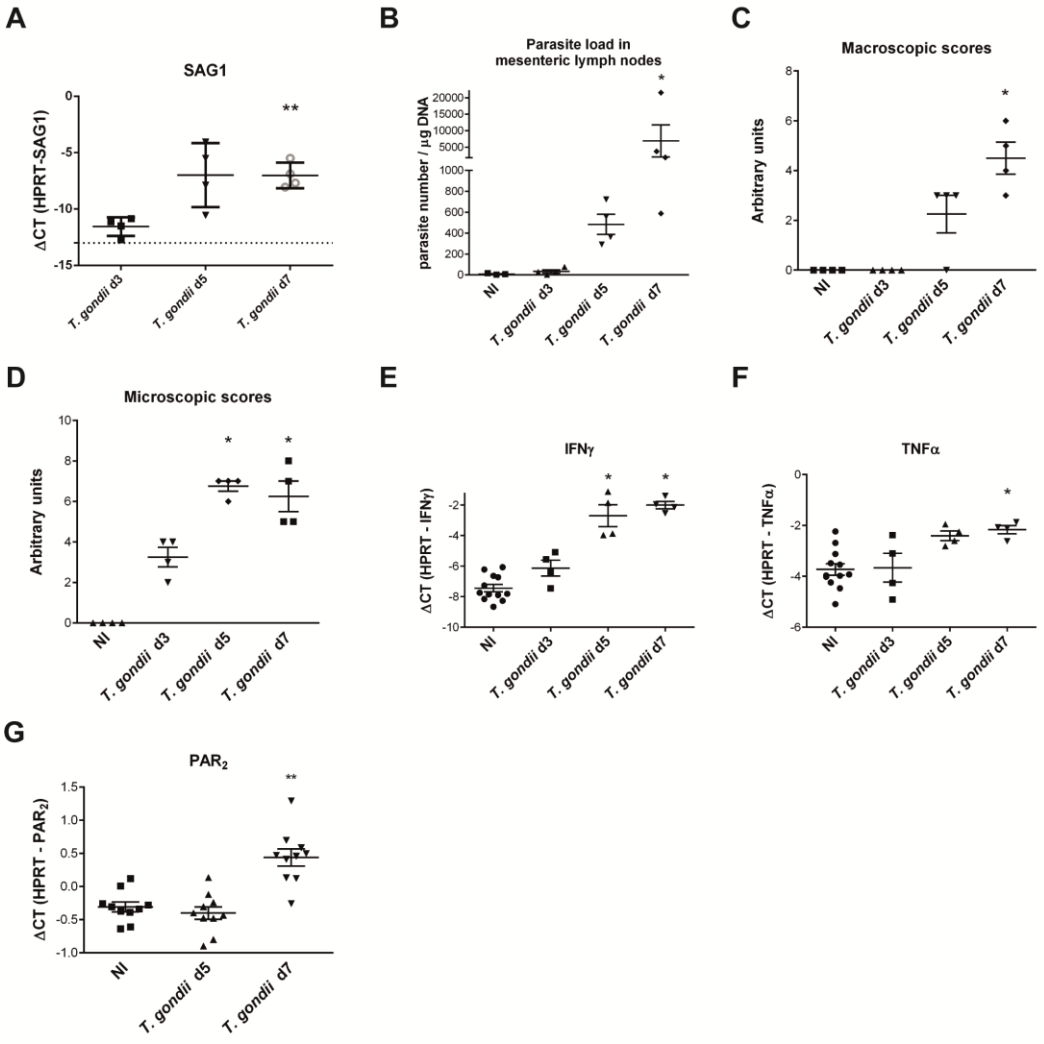

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Figure 2 *Bonnart et al.*

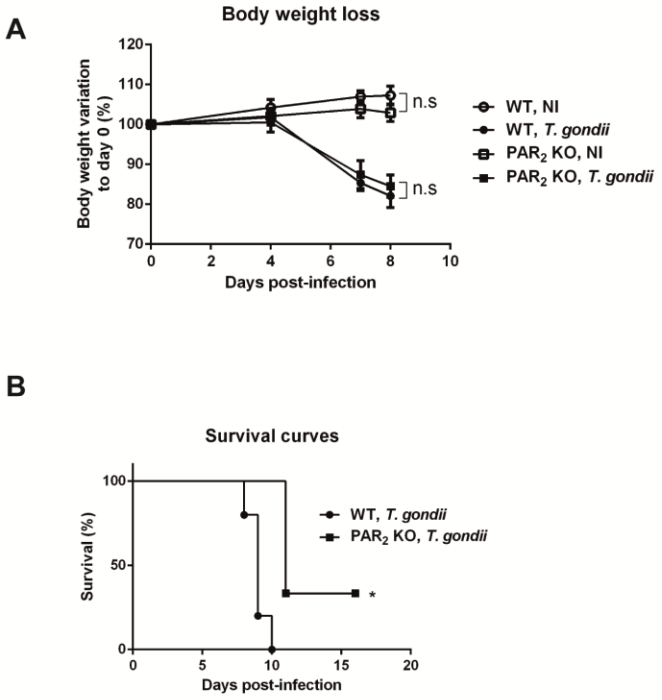

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Figure 3 (A-B) *Bonnart et al.*

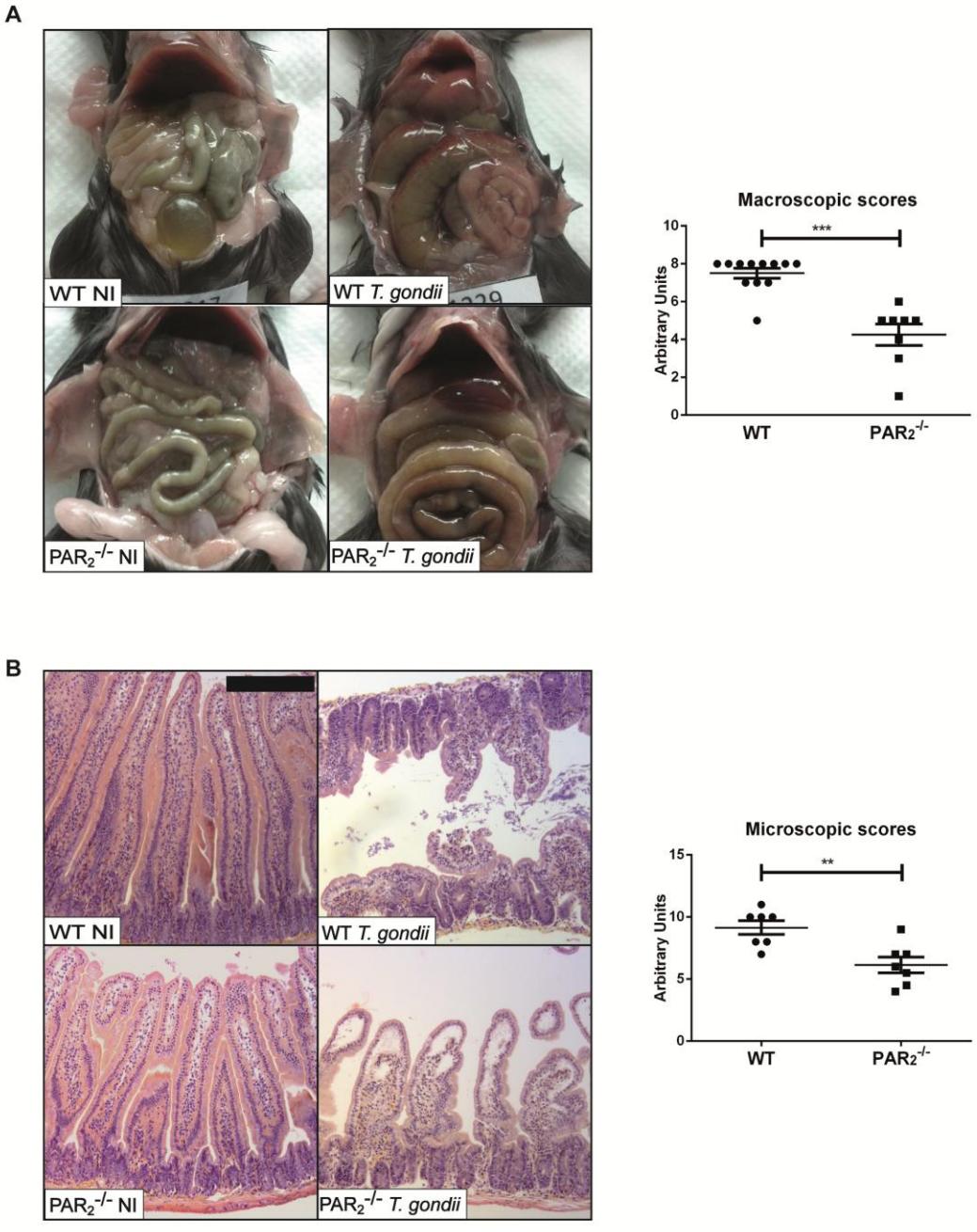

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Figure 3 (C-D) *Bonnart et al.*

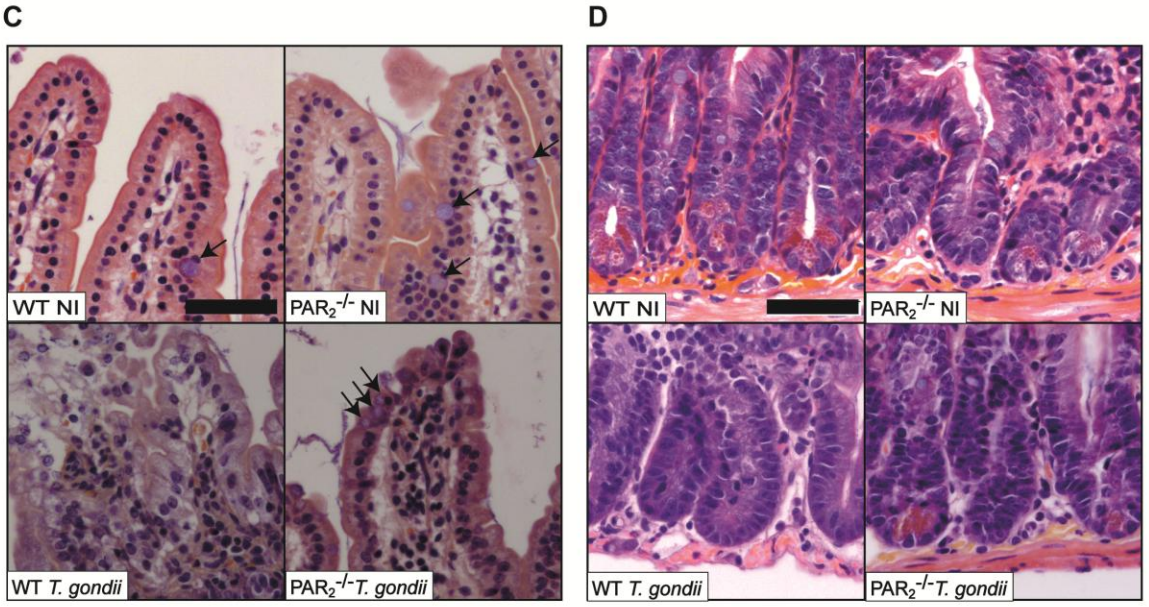

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Figure 4 *Bonnart et al.*

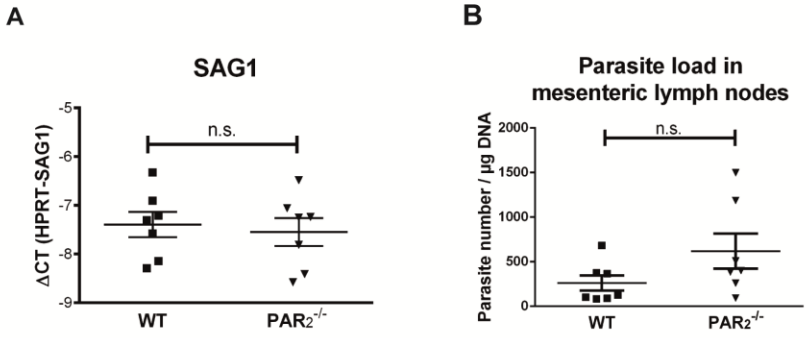

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Figure 5 *Bonnart et al.*

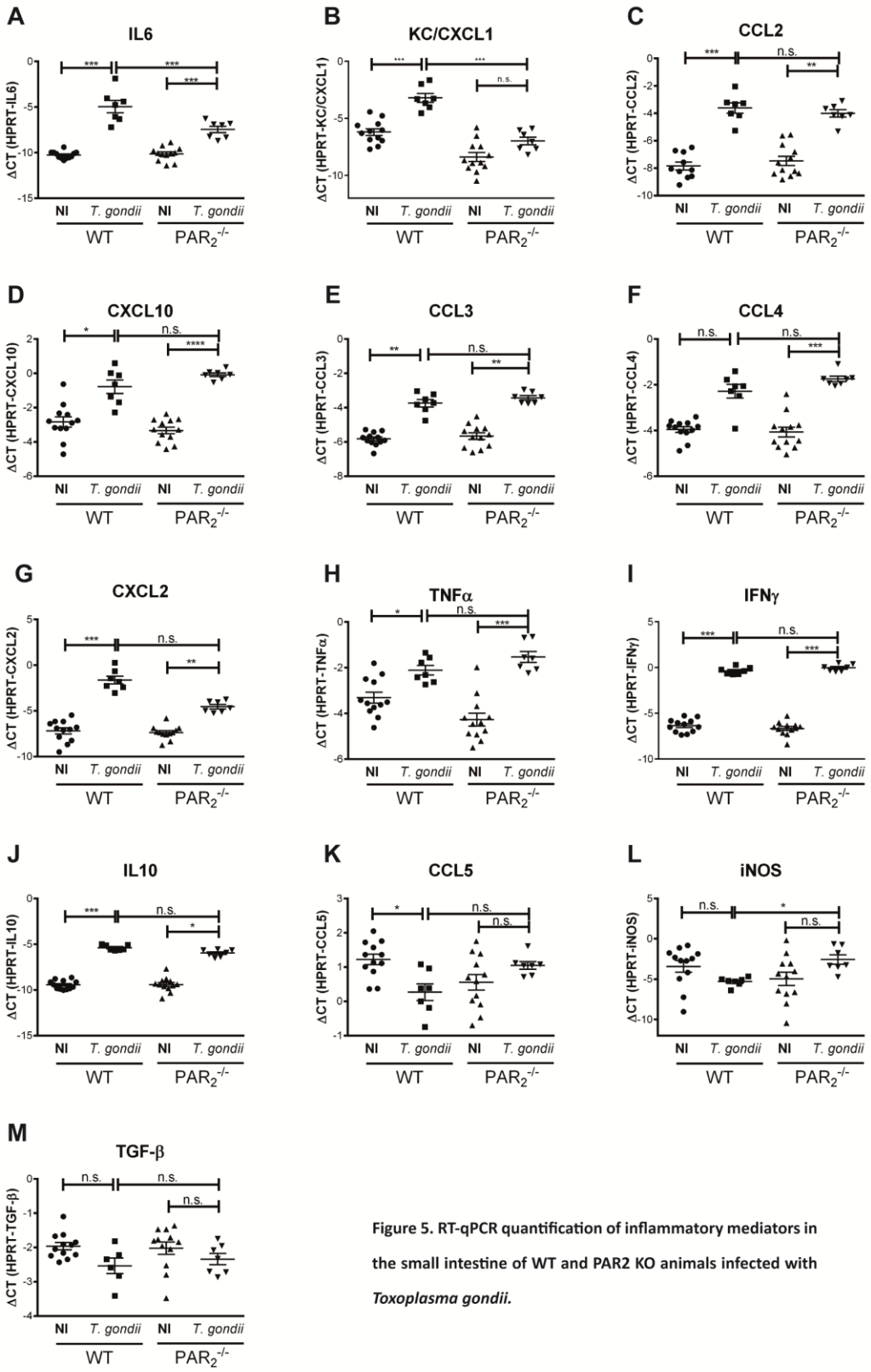

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Figure 6 *Bonnart et al.*

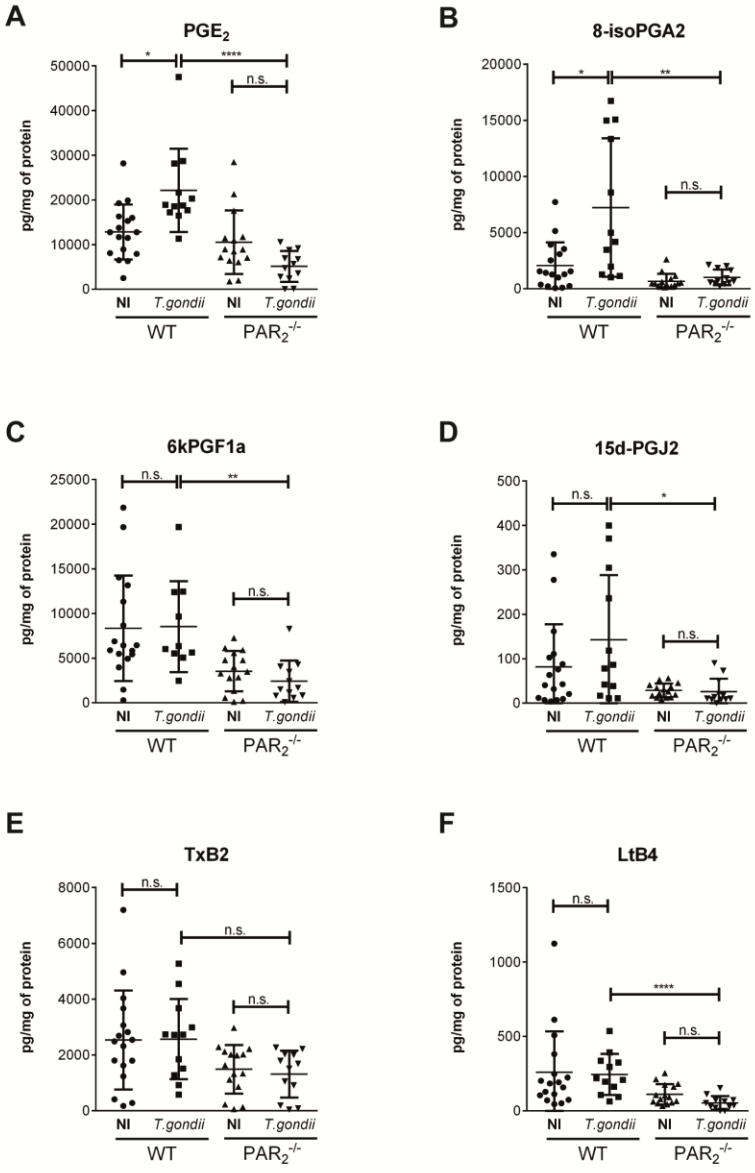

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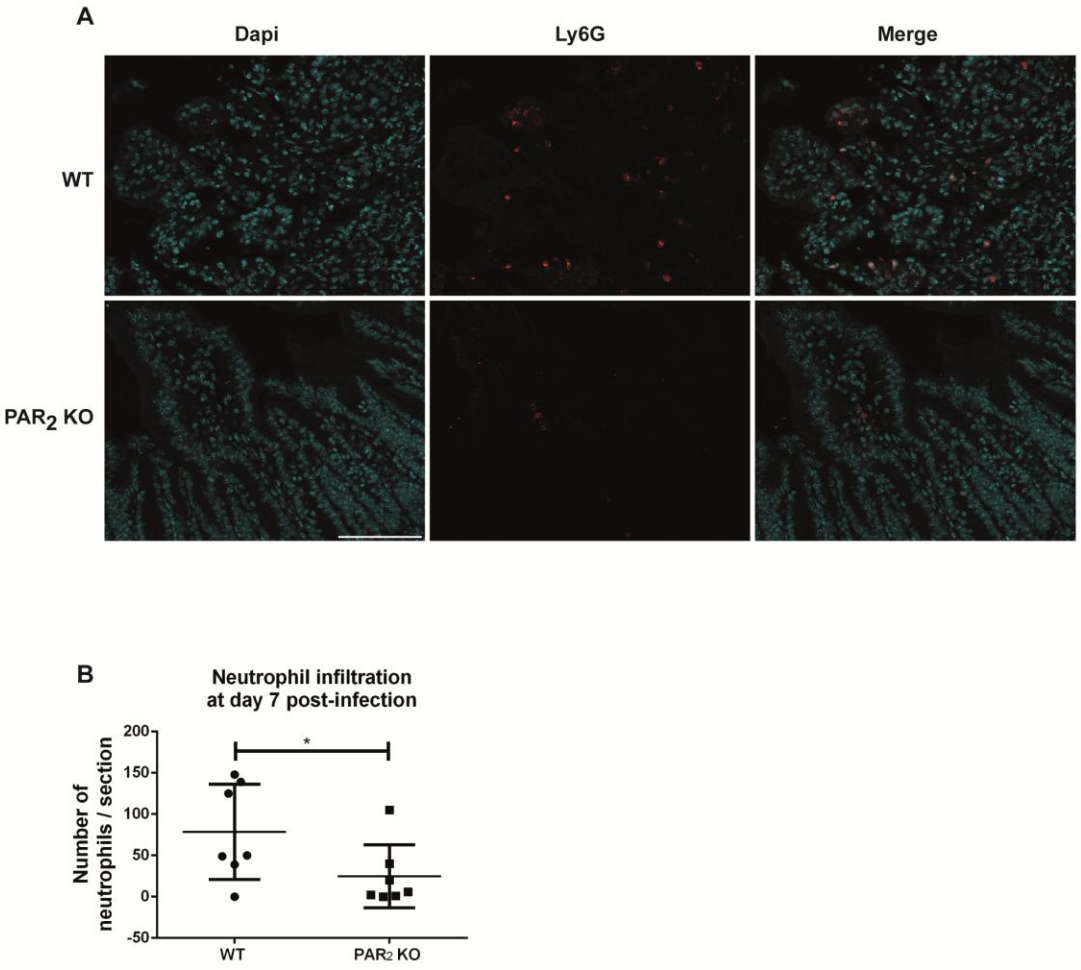
Figure 7 *Bonnart et al.*



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