Supplementary Materials and Methods

Mice, and host cell and parasite cultures

Type I IFN receptor KO mice in the C57BL/6 background (B6.129S2-*Ifnar1*^{tm1Agt}/Mmjax) (83) were purchased from the Mutant Mouse Regional Resource Centers (MMRRC) (Bar Harbor, Maine). All animals were housed and maintained according to the McGill University Animal Care Committee (permit AUC #5380). Hind leg bones from TNF$\alpha^{-/-}$ mice in the C57BL/6 background were used to generate bone marrow-derived pAPCs (TNF$\alpha^{-/-}$ mice were a kind gift from Jörg Fritz (McGill University)). BMMΦ and BMDCs were generated as described above.

Supplementary Results

Ii induction in infected BMMΦ occurs in the absence of endogenous production of TNFα and type I IFNs.

Considering that induction of CD74 expression occurred in cultures without exogenously-added IFNγ, we verified whether endogenous secretion of TNFα or type I IFNs (α and β), occurred in infected cells, which may trigger transcription of class-II genes through different signaling pathways (84, 85). BMMΦ from TNFα and type I IFN receptor (IFN-AR1) KO mice were cultured and infected with *T. gondii*, and CD74 expression within these cells was analyzed by flow cytometry (Fig. S3). Infected KO cells displayed enhanced levels of CD74, arguing that the production of these cytokines is not necessary for the over expression of CD74 within infected cells.

Supplementary Figures and Tables

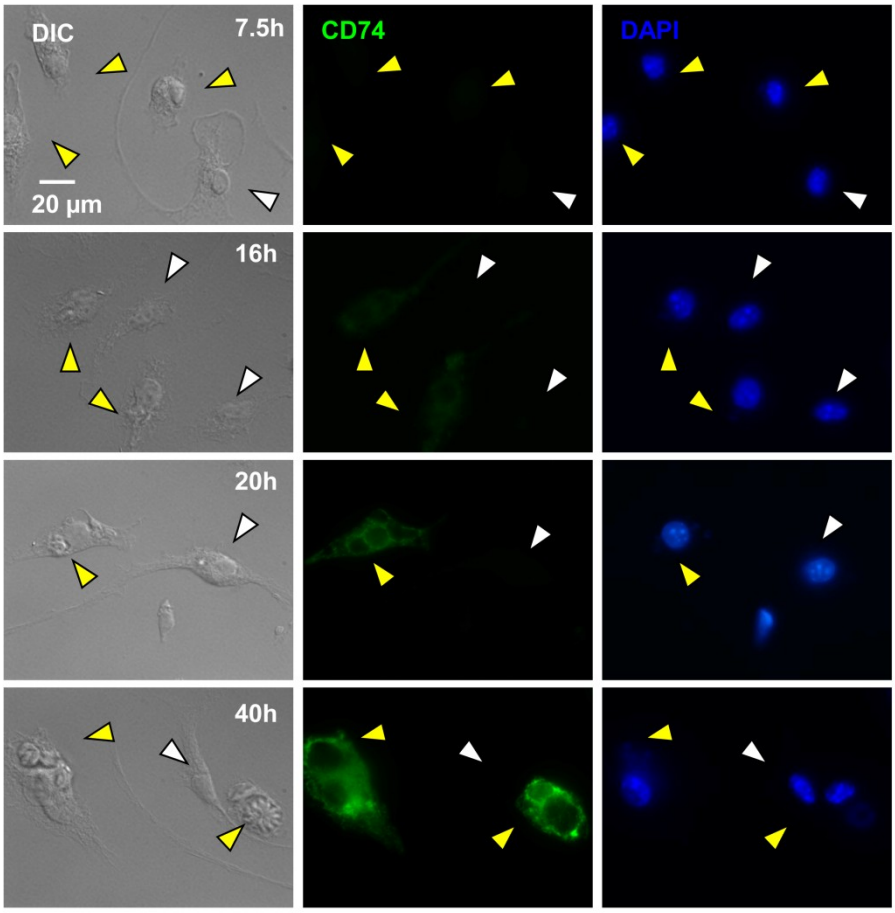

Fig. S1 *T. gondii* up-regulates CD74 protein expression in infected BMMΦ in the absence of IFNγ until egress of the parasite.

BMMΦ cultures were infected with *T. gondii* RH WT tachyzoites at an MOI 2:1, left unstimulated, and fixed with 3.7% PFA in PBS at the indicated times. Cells were stained for CD74 (here shown in green) and DAPI. Samples were visualized by epifluorescence microscopy, and images were deconvolved using AutoQuant X software. CD74 expression levels are shown in uninfected cells (white arrowheads) and infected cells (yellow arrowheads) within same fields. These fields are representative of

entire cultures and of three independent experiments. DIC refers to transmitted light differential interference contrast.

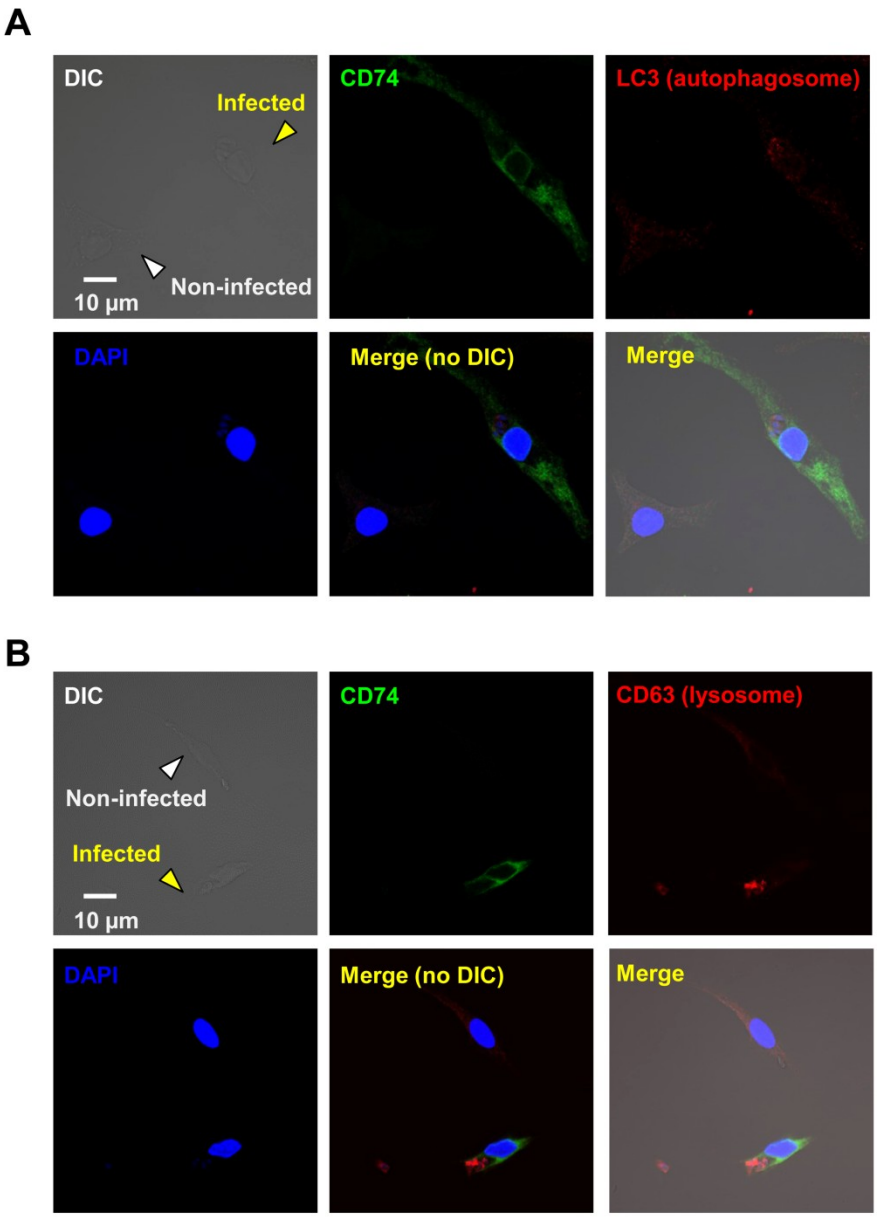

Fig. S2 CD74 does not colocalize with autophagosome or lysosome markers in infected unstimulated BMMΦ.

BMMΦ cultures were infected with *T. gondii* RH WT tachyzoites at an MOI 2:1, and after 4 h, extracellular parasites were rinsed away, fresh medium was added, and cells were fixed with 3.7% PFA in PBS after 20 h. Cells were stained for CD74 and DAPI. Colocalization experiments for CD74 (in green) were carried with different markers (A) for autophagosomes (LC3) and (B) lysosomes (CD63) (all shown in red). CD74 proteins are detected in infected cells (yellow arrowheads), but not uninfected cells (white arrowheads), and do not colocalize with LC3 (R_{coloc} = 0.03, tM = 0.42) nor CD63 (R_{coloc} = 0.05, tM = 0.36). These fields are representative of entire cultures and of two independent experiments. DIC refers to transmitted light differential interference contrast.

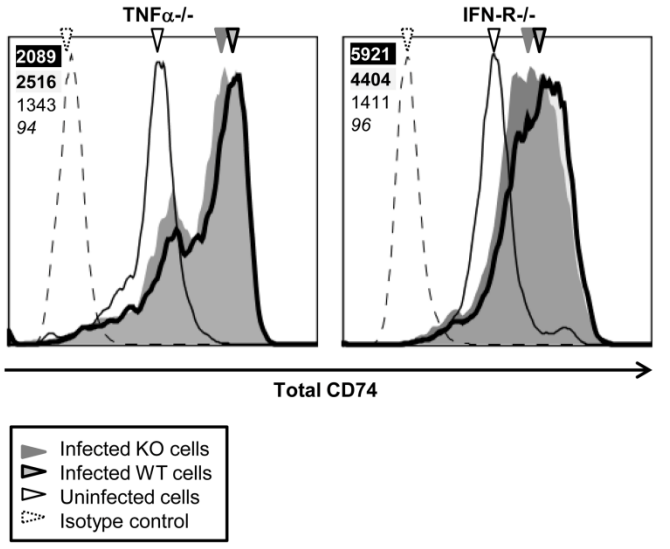

Fig. S3 CD74 induction in infected BMMΦ occurs in the absence of endogenous production of TNFα and type I IFNs.

WT, TNF$\alpha^{-/-}$, and IFN-$R^{-/-}$ BMMΦ cultures were infected with CellTrace Far Red DDAO-stained *T. gondii* RH tachyzoites at an of MOI 3:1, and after 4 h, extracellular

parasites were rinsed away, fresh medium was added, and incubated 20 h. WT uninfected control cultures (thin curves, white arrowheads) and infected cells (bold line shaded curves, gray arrowheads with black contour) are shown as a reference for the KO cells (filled gray curves, gray arrowheads without contour). All cultures were stained for CD74 after permeabilization and analyzed by flow cytometry. Isotype control is indicated by the dashed curve and dashed arrowheads. Mean fluorescence intensity (MFI) values are indicated for each population (i.e. italicized for isotype control, standard font for uninfected control cells, bold font for infected WT cells, and white font in dark box for infected KO cells). Shown here are the results form one representative experiment from two independent experiments.

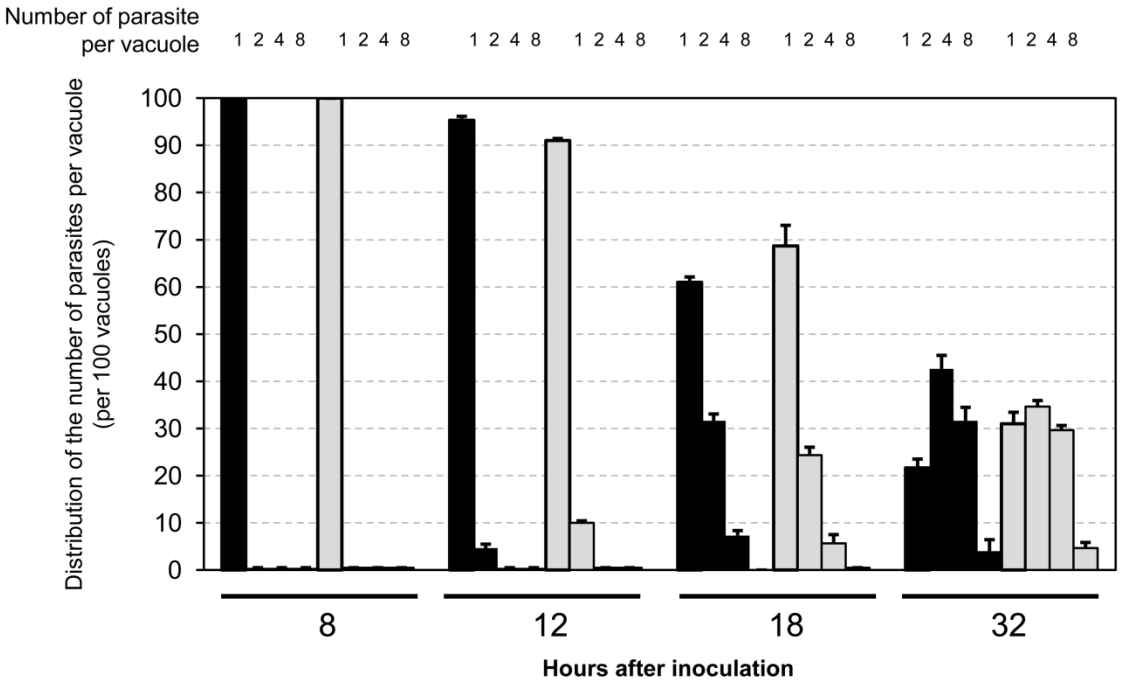

Fig. S4 *In vitro* replication rate of parasites in infected BMMΦ is not affected by the presence or absence of CD74.

WT and CD74$^{-/-}$ BMMΦ cultures were plated on glass coverslips, infected with RH WT parasites at an MOI of 3:1, and fixed with 3.7% PFA at the indicated times after inoculation (8, 12, 18, and 32 h post-inoculation). The number of parasites per vacuole was counted in 100 randomly selected vacuoles for each type point in two different cover slips. Each bar represents the number of vacuoles containing the indicated number of parasites (1, 2, 4, or 8) within WT BMMΦ (black bars) and CD74$^{-/-}$ BMMΦ (grey bars). Shown here are the results form one representative experiment from two independent experiments.

Table S1 PCR primers for mouse genotyping.

	Gene	
Primer type	**Ii (CD74)**	**H2-DM**
WT	5'- AAT CAG CGC TCA CT -3'	5'- CAC ATT CCG GCA CAC -3'
Common	5'- GGC TAG GTC CCA GTG TAG GC -3'	5'- TCT GGA CAC TGG GAT TTG -3'
Mutant	5'- CGC TGA CAG CCG GAA CAC GG -3'	5'- CCT TCT ATC GCC TTC TTG ACG -3'

Table S2 RT-PCR primers for class CD74 genes.

Gene	Sense primer	Anti-sense primer
Ii p31	5'-ACC GAG GCT CCA CCT AAA GAG-3'	5'- TTG ACC CAG TTC CTG CCT G-3'
Ii p41	5'-TTC CTC ACA CCA AGA GCC G -3'	5'- TGT CCA GTG GCT CAC TGC AG -3'
Ii common	5'- GAA CCT GCA ACT GGA GAG CC -3'	5'- GGT TTG GCA GAT TTC GGA AG -3'
H2-Aα	5'- CAT CTT CCC TCC TGT GAT CAA CA -3'	5'- CAC CGT CTG CGA CTG ACT TG -3'
H2-Aβ1	5'- GGA GAC TCC GAA AGG CAT TTC -3'	5'- GCG TCC CGT TGG TGA AGT AG -3'
H2-Eβ1	5'- TGG CAG CTG TGA TCC TGT TG -3'	5'- AAA CCA TGG TCT GGA GTC TCT GA-3'
H2-DMα	5'- GGC GGT GCT CGA AGC A -3'	5'- TGT GCC GGA ATG TGT GGT T -3'
H2-DMβ1/2	5'- CTA TCC AGC GGA TGT GAC CAT -3'	5'- TGG GCT GAG CCG TCT TCT -3'