CD40 INDUCES MACROPHAGE ANTI-*TOXOPLASMA GONDII* ACTIVITY BY TRIGGERING AUTOPHAGY-DEPENDENT FUSION OF PATHOGEN-CONTAINING VACUOLES AND LYSOSOMES

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Figure S1. A subset of macrophages exhibits vacuole-lysosome fusion in response to CD40 stimulation. Monolayers of mouse resident peritoneal macrophages were incubated with a stimulatory anti-CD40 or control mAbs. Macrophages were infected with *T. gondii*-YFP followed by staining with biotinylated anti-CD40 mAb plus Alexa 643-conjugated streptavidin, and anti-LAMP-1 plus Texas Red-conjugated secondary Abs. Cells were examined by confocal microscopy 8 h post-infection. $CD40^+$ macrophages activated through this receptor show colocalization of LAMP-1 (rings) around *T. gondii*-containing vacuoles (arrows). Bar = 5 μm. Results shown are representative of three independent experiments.

Figure S2. Vacuole-lysosome fusion mediates macrophage anti-microbial activity induced by CD40. Mouse peritoneal macrophages were incubated with control or anti-CD40 mAb or with IFN-γ/LPS followed by infection with *T. gondii*. *A*, *B*, Leupeptin (2 mg/ml) was added 1 h after infection. Percentage of infected macrophages and parasite load were assessed by light microscopy at 1 and 18 h post-challenge. *C- E*, LY294002 (20 nM) was added 1 h after infection with *T. gondii*. *C*, expression of cathepsin D was examined by immunofluorescence 8 h post-infection. *D, E*, percentage of infected

macrophages and parasite load were assessed at 1 and 18 h post-challenge. Results are shown as the mean ± SD and are representative of 3 to 4 independent experiments.

Figure S3. Autophagy induces vacuole-lysosome fusion and anti-microbial activity. *A*, Colocalization of LC3 around PV in RAW 264.7 cells treated with rapamycin. RAW 264.7 cells were transfected with LC3-EGFP followed by infection with *T. gondii*-RFP. Cells were treated with vehicle or rapamycin (1 μM) beginning 2 h after infection. Percentages of vacuoles that colocalized with LC3 were assessed by immunofluorescence 3 h after adding rapamycin. *B*, RAW 264.7 cells transfected with control or Beclin 1 siRNA were infected with *T. gondii* followed by treatment with or without rapamycin. Parasite load was assessed by light microscopy 18 h post-infection. *C*, Mouse resident peritoneal macrophages were infected with *T. gondii* followed by incubation in medium with or without rapamycin and/or 3-MA (10 mM). Parasite load was assessed at 18 h post-infection. Results are shown as the mean ± SD and are representative of 3 independent experiments.

Figure S1

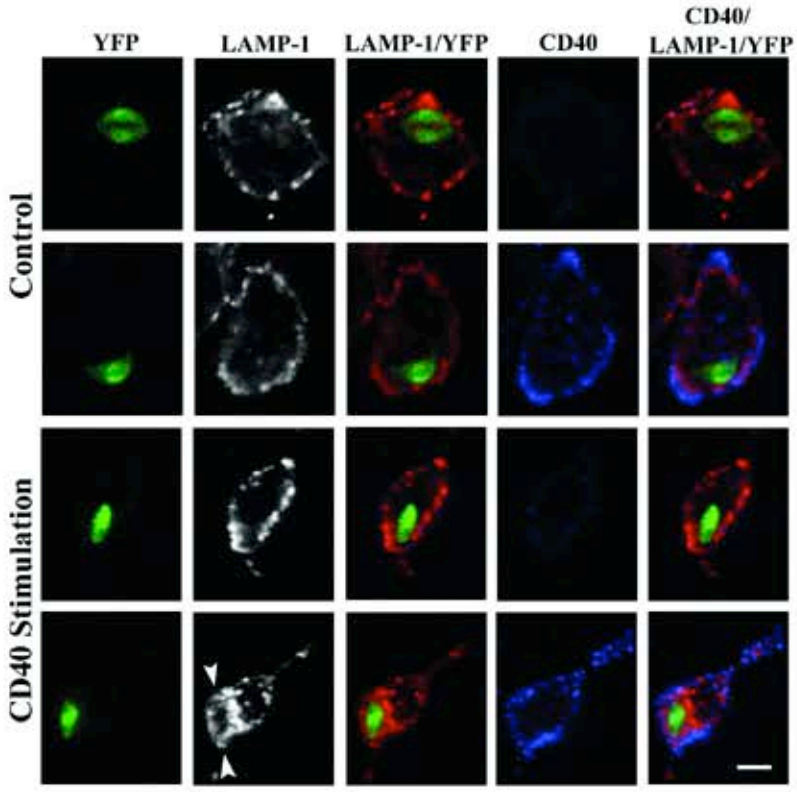

YFP
LAMP-1
LAMP-1/YFP
CD40
CD40/
LAMP-1/YFP
Control
CD40 Stimulation

Figure S2

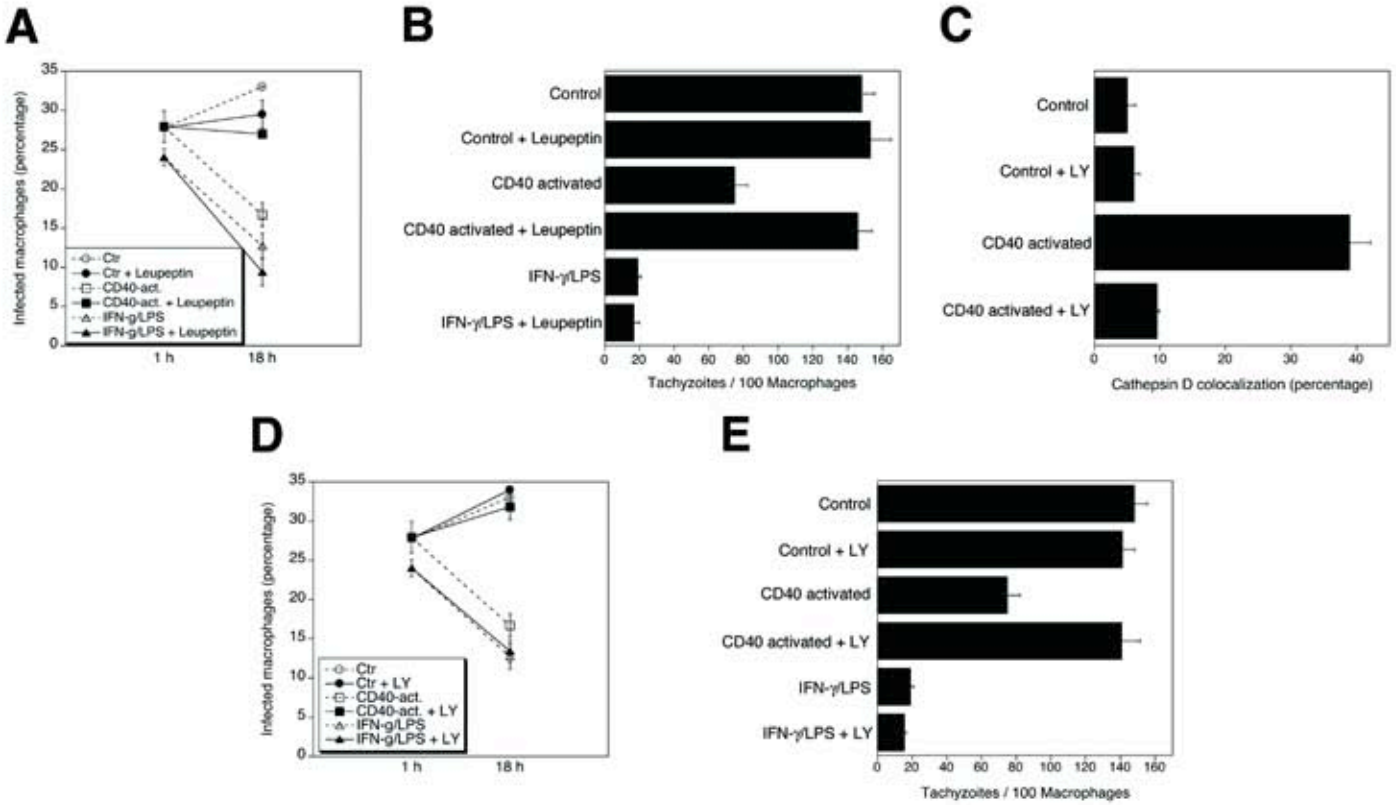
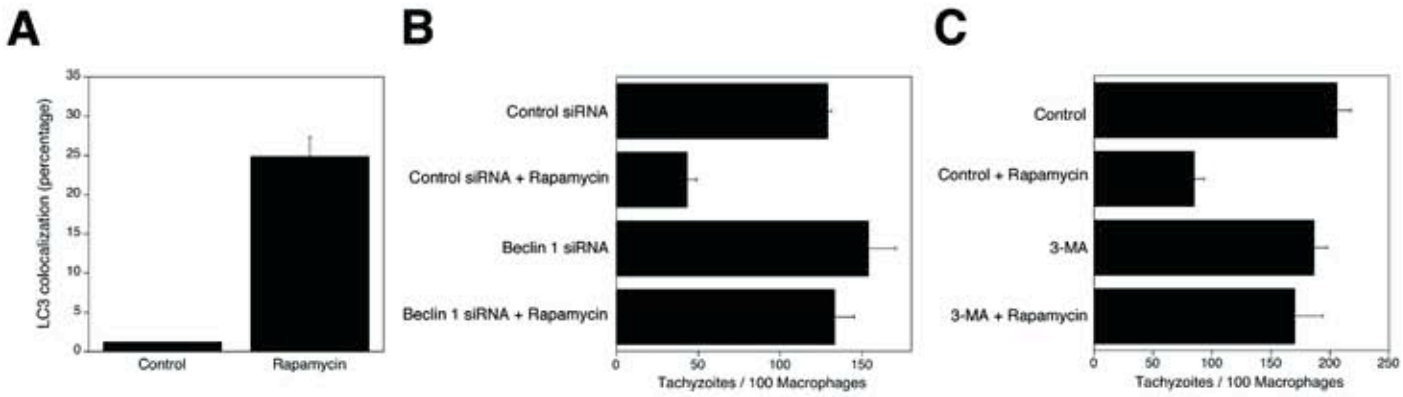

A
Infected macrophages (percentage)
Ctr
Ctr + Leupeptin
CD40-act.
CD40-act. + Leupeptin
IFN-g/LPS
IFN-g/LPS + Leupeptin
1 h
18 h
B
Control
Control + Leupeptin
CD40 activated
CD40 activated + Leupeptin
IFN-γ/LPS
IFN-γ/LPS + Leupeptin
Tachyzoites / 100 Macrophages
C
Control
Control + LY
CD40 activated
CD40 activated + LY
Cathepsin D colocalization (percentage)
D
Infected macrophages (percentage)
Ctr
Ctr + LY
CD40-act.
CD40-act. + LY
IFN-g/LPS
IFN-g/LPS + LY
1 h
18 h
E
Control
Control + LY
CD40 activated
CD40 activated + LY
IFN-γ/LPS
IFN-γ/LPS + LY
Tachyzoites / 100 Macrophages

Figure S3


A
B
C
LC3 colocalization (percentage)
0
5
10
15
20
25
30
35
Control
Rapamycin
Control siRNA
Control siRNA + Rapamycin
Beclin 1 siRNA
Beclin 1 siRNA + Rapamycin
0
50
100
150
Tachyzoites / 100 Macrophages
Control
Control + Rapamycin
3-MA
3-MA + Rapamycin
0
50
100
150
200
250
Tachyzoites / 100 Macrophages