

Using BLaER1 as model for *L. major* infection of human macrophages to investigate the role of pyroptosis in parasite spreading

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Abstract

Leishmania is the causative agent of the tropical neglected disease leishmaniasis and infects macrophages as its definitive host cell. In order to sustain and propagate infections, *Leishmania* parasites have to complete cycles of exit and re-infection. Yet, the mechanism driving the parasite spread to other cells remains unclear. Recent studies reported pro-inflammatory monocytes as replicative niche of *L. major* and showed prolonged expression of IL-1 β at the site of infection, indicating an activation of the NLRP3 inflammasome and pointing towards pyroptosis as a possible mechanism of parasite spread. To address the species-specific inflammasome activation of human cells we characterized the BLaER1 macrophages as a model for *L. major* infection. We found that *Leishmania* can infect, activate and develop in BLaER1 macrophages similar as they can do in primary human macrophages. Harnessing the possibilities of this infection model, we first showed that BLaER1 *GSDMD*^{-/-} cells, which carry a deletion of the pore-forming protein gasdermin D, are more resistant to pyroptotic cell death and, concomitantly, display a strongly delayed release of intracellular parasite. Using that knockout in a co-incubation assay in comparison with wild type BLaER1 cells, we demonstrate that impairment of the pyroptosis pathway leads to lower rates of parasite spread to new host cells, thus, implicating pyroptotic cell death as a possible exit mechanism of *L. major* in pro-inflammatory microenvironments.

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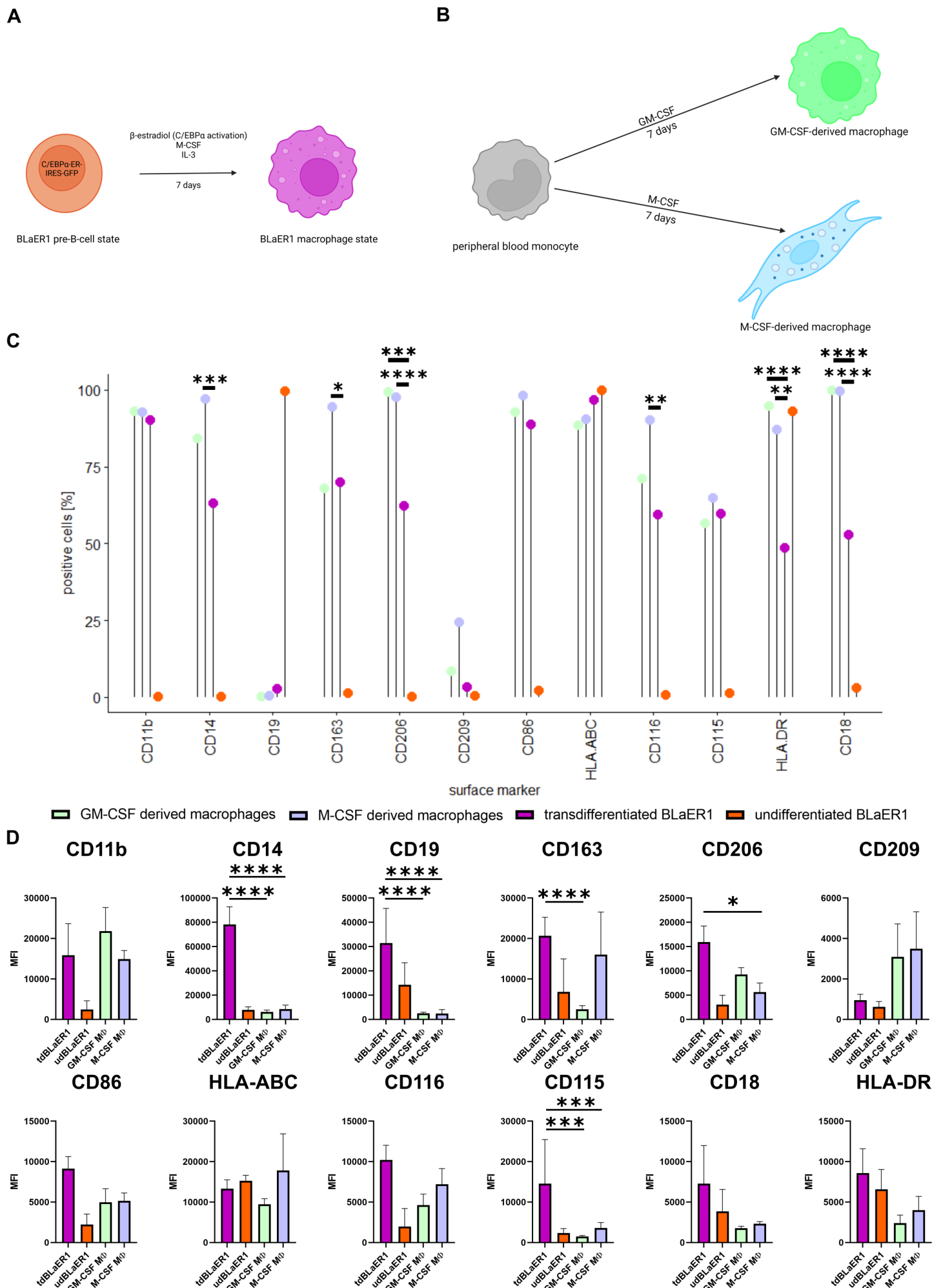


Figure 1: BLaER1 cells show a similar immunophenotype as M-CSF-derived and GM-CSF-derived macrophages.

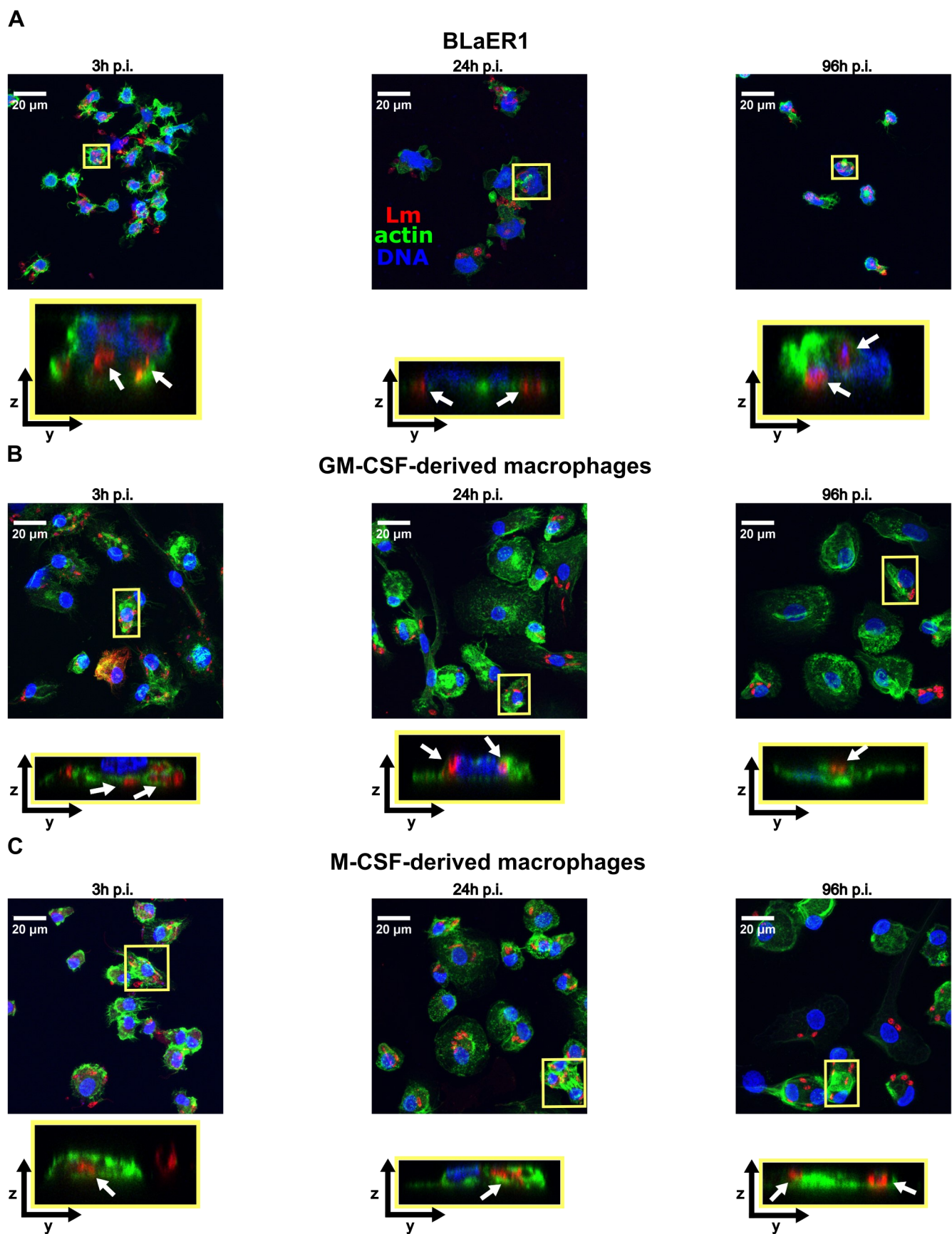


Figure 2: BLaER1 cells are susceptible to infection with *L. major* and able to sustain the infection.

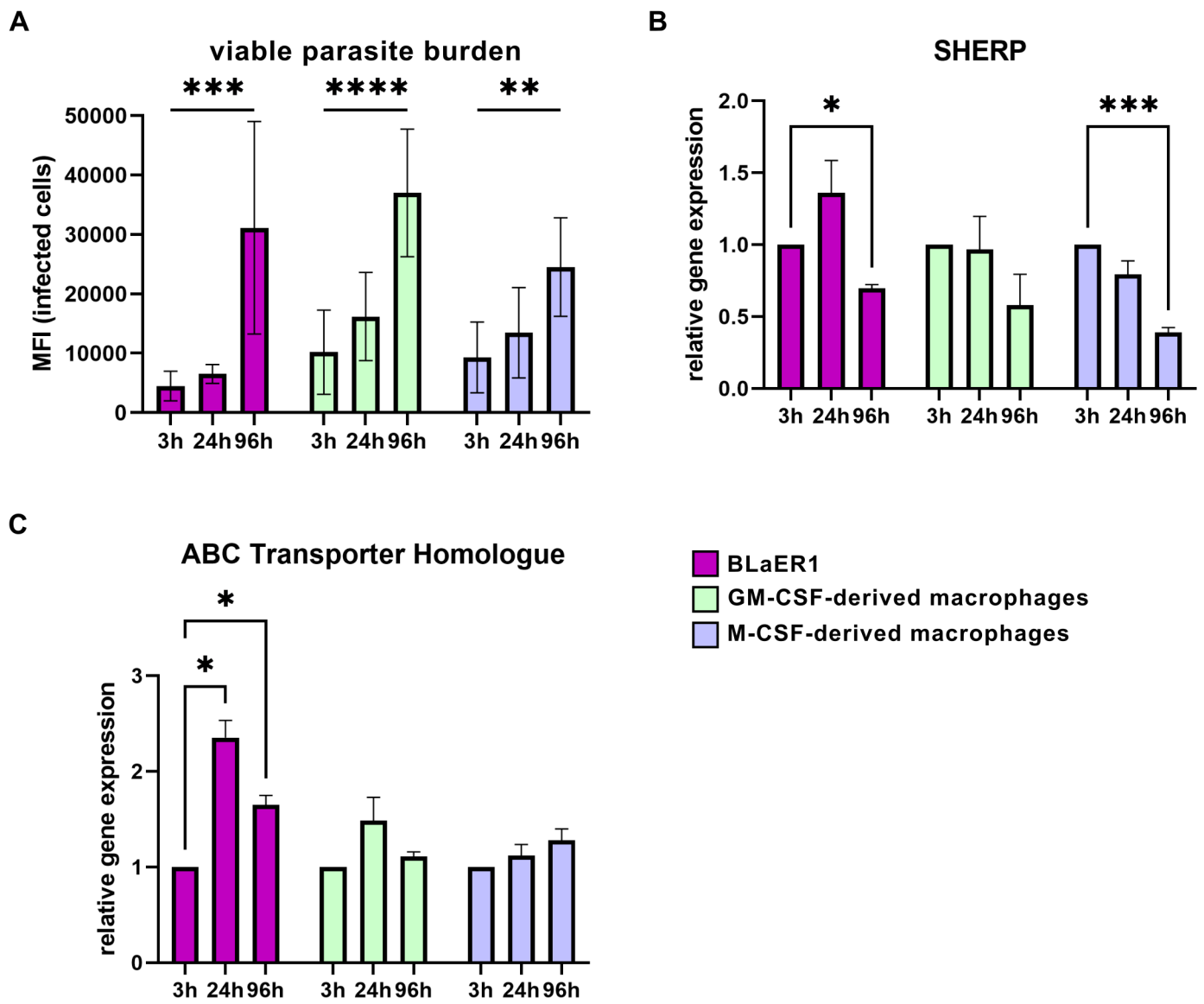


Figure 4: BLaER1 cells support the transformation to the amastigote stage.

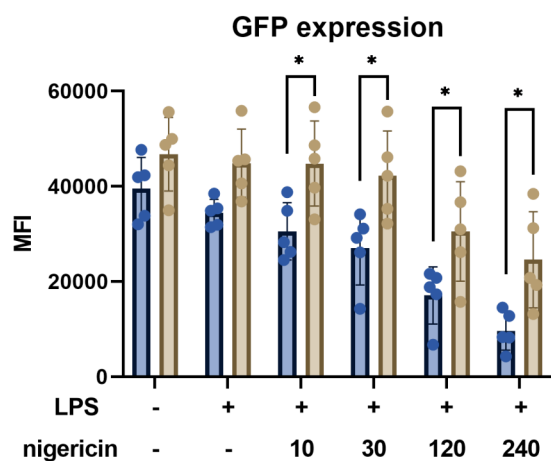
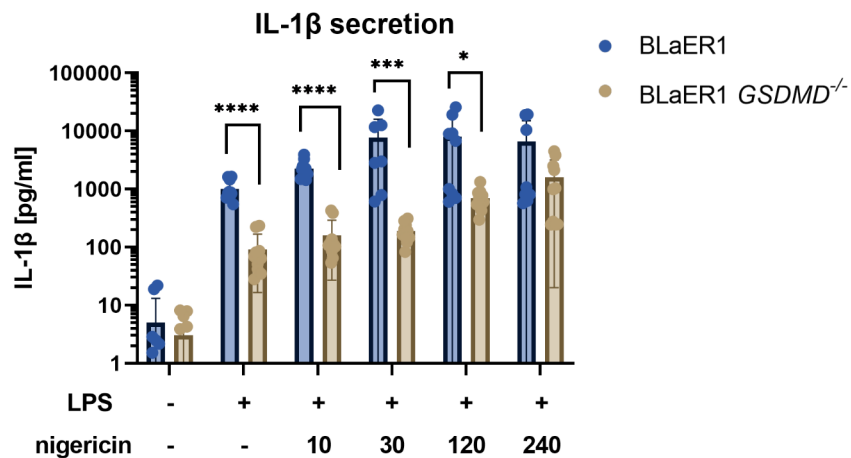
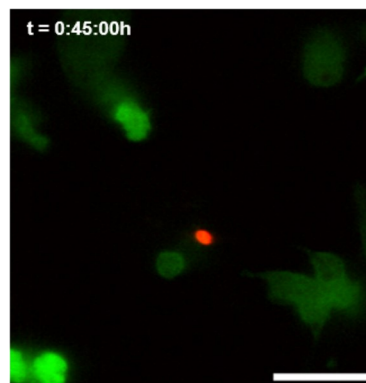
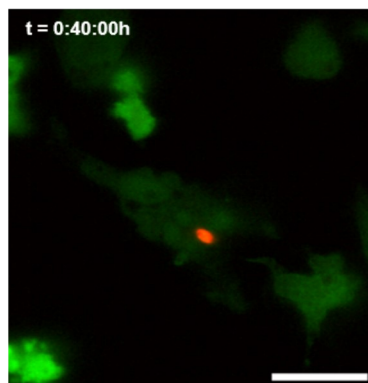
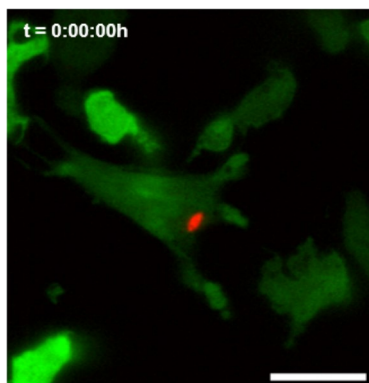
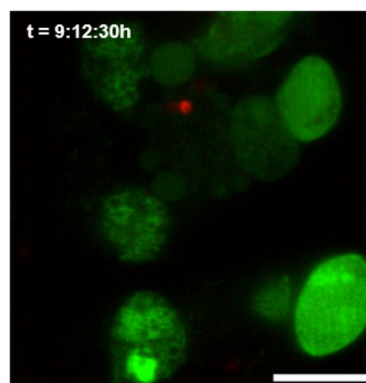
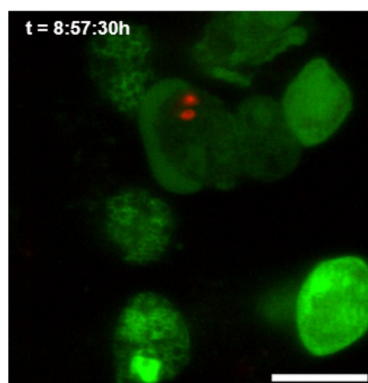
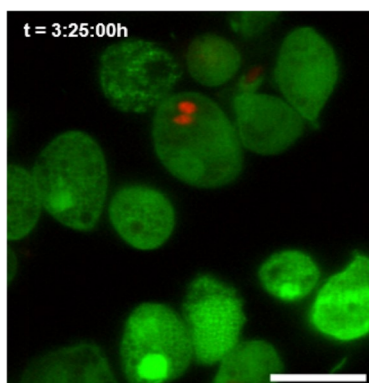
A**B**

Figure 5: BLaER1 *GSDMD*^{-/-} cells are more resistant to pyroptosis than BLaER1 wild type cells

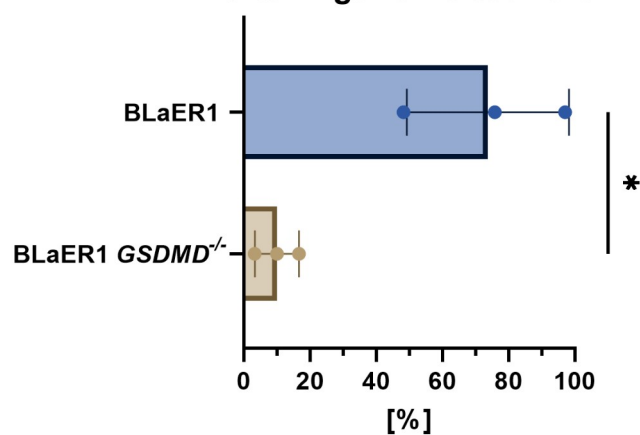
A

BLaER1

BLaER1 *GSDMD*^{-/-}

B

percentage of infected cells
releasing parasites within 8 h
after nigericin treatment



C

time of parasite release
after pyroptosis induction

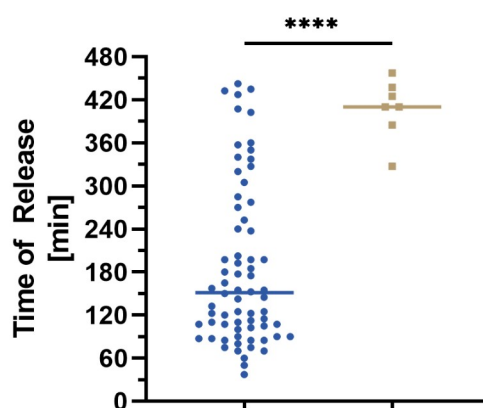


Figure 6: Pyroptosis resistance leads to delayed parasite release from BLaER1 *GSDMD*^{-/-} compared to wild type BLaER1

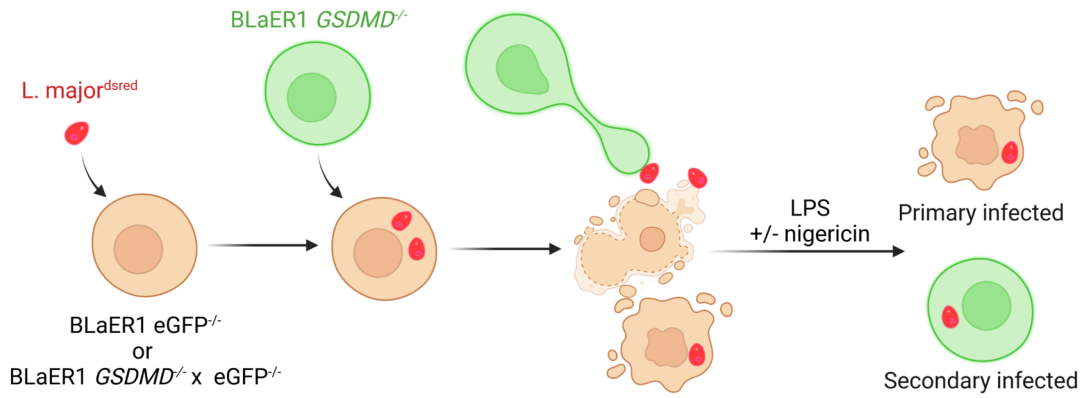
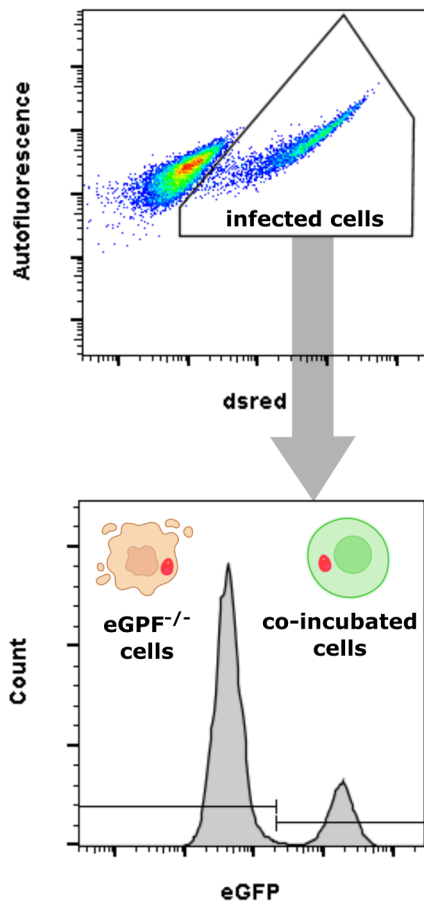
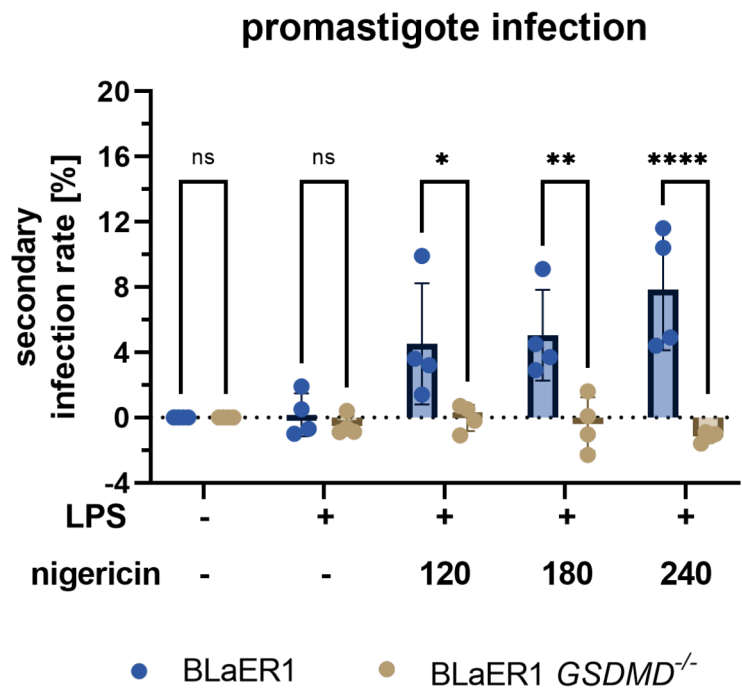
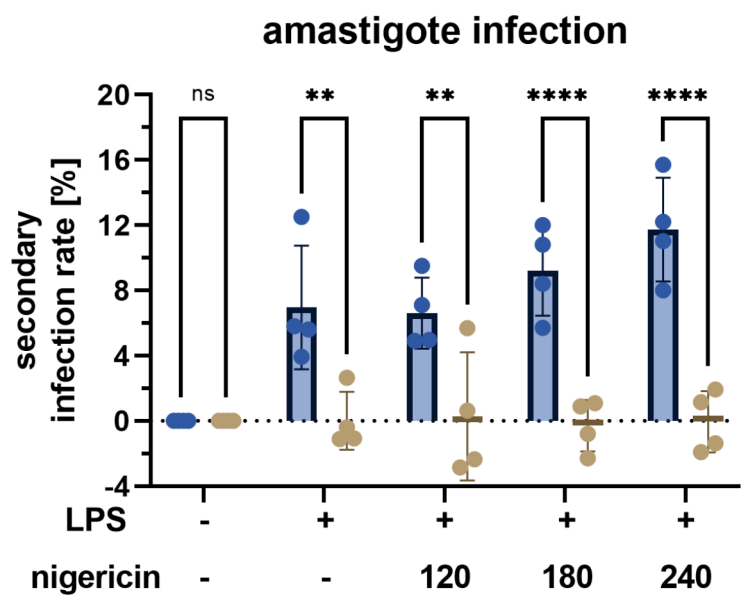
A**B****C****D**

Figure 7: Parasite exit and re-infect new host-cells in pro-inflammatory microenvironment.