



FIG S3 IRE1 KD prevents sustained ROS production by macrophages. (A) Total cellular ROS production in live cells as assessed by flow cytometry using CM-H2DCFDA. The percentage of ROS⁺ cells were determined by gating against stained mock infected cells. Representative histograms are shown (left) with the mean percentage of ROS⁺ cells from n≥3 independent experiments +/- SD. Mean fluorescent intensity (right) for the indicated conditions was calculated as geometric mean from n≥3 independent

experiments +/- SD. **(B)** Live-cell fluorescence images of ROS association with MRSA-containing phagosomes. RAW264.7 macrophage cell lines expressing NT-control or IRE1 shRNA were infected with MRSA-mCherry (MOI 20) and stained with ROS fluorescence indicator, CM-H2DCFDA (Green), at 8h pi. The percent of cells with ROS⁺ phagosome were determined by counting cells with at least one enriched ROS area localized with MRSA from at least 100 ROS⁺ infected cells. Representative images are shown with the mean percent of cells with ROS⁺ phagosome from n≥3 independent experiments +/- SD. **(C)** Percent of ROS⁺ cells was quantified by flow cytometry from NT-control, or XBP1 KD macrophages at 8h pi. The percentage of ROS⁺ cells are measured by gating against a stained mock cells. **(D)** Mean fluorescent intensity (MFI) was measured from NT-control, or XBP1 KD macrophages. The cells were either left untreated (mock) or infected with MRSA for 8h. MFI was analyzed as geometric mean using FlowJo software. **(C-D)** Graphs are presented as mean of n≥3 independent experiments +/- SD. *p < 0.05.