

**Figure S1.** Relative expression of *Atg5* and *Atg7* in BMDMs. (**A, B**) *Atg5* mRNA levels were assessed by real time PCR in CSF1- (**A**) and CSF2- (**B**) derived BMDMs using *Hprt* as a housekeeping control gene to allow for comparison. (**C, D**) *Atg7* mRNA levels were assessed by real time PCR in CSF1- (**C**) and CSF2- (**D**) derived BMDMs using *Hprt* as a housekeeping control gene. (**E, F**) Expression levels of LC3-I and LC3-II in untreated CSF2-derived *atg5*-/-(**E**) and *atg7*-/- (**F**) BMDMs and their respective controls. Positions of LC3-I and LC3-II are indicated.



**Figure S2.** Phagosome maturation of IgG-coated particles is unaffected in autophagy-deficient CSF2-differentiated BMDMs. (**A, B**) CSF2-derived BMDMs were challenged with OpZ and fixed at 30, 60, 90, or 120 min, then stained for LAMP1 and OpZ. The percentage of LAMP1+ OpZ was enumerated in WT and *atg5*-/- (**B**) and WT and *atg7*-/- (**C**) BMDMs. At least 300 OpZ were counted per condition. (**C, D**) CSF2-derived BMDMs were incubated with LysoBrite for 30 min and then challenged with SRBC for 30, 60 or 90 min. The percentage of LysoBrite+ SRBC was enumerated in WT and *atg5*-/-(**C**) and WT and *atg7*-/- (**D**) BMDMs. At least 50 SRBC were counted per condition. (**E, F**) CSF2-derived BMDM were pulsed with DQ-BSA for 1 h, chased for 1 h in regular medium, then challenged with SRBC for 30, 60 or 90 min and imaged live. The percentage of DQ-BSA+ SRBC was enumerated in WT and *atg5*-/-(**E**) and WT and *atg7*-/- (**F**) BMDMs. At least 50 SRBC were counted per condition.



**Figure S3.** Phagosome maturation in *Atg5*-deficient and *Cybb*-deficient BMDMs. (**A, B**) CSF1- (**A**) and CSF2- (**B**) derived BMDMs were challenged with uncoated zymosan and fixed at 30, 60, 90, or 120 min, then stained for LAMP1. The percentage of LAMP1+ zymosan was enumerated in WT and *atg5*-/- BMDMs. At least 300 zymosan particles were counted per condition. The experiment was performed twice (**B**). (**C**) CSF1-derived BMDMs were challenged with IgG-coated zymosan and fixed at 30 or 60 min, then stained for LAMP1 and zymosan. The percentage of LAMP1+ zymosan was enumerated in WT and *cybb*-/- BMDMs. At least 100 OpZ were counted per condition. A p-value of less than 0.05 was considered statistically significant and is denoted by an asterisk (\*).