SUPPLEMENTAL MATERIAL

Jorgensen et al., http://www.jem.org/cgi/content/full/jem.20151613/DC1

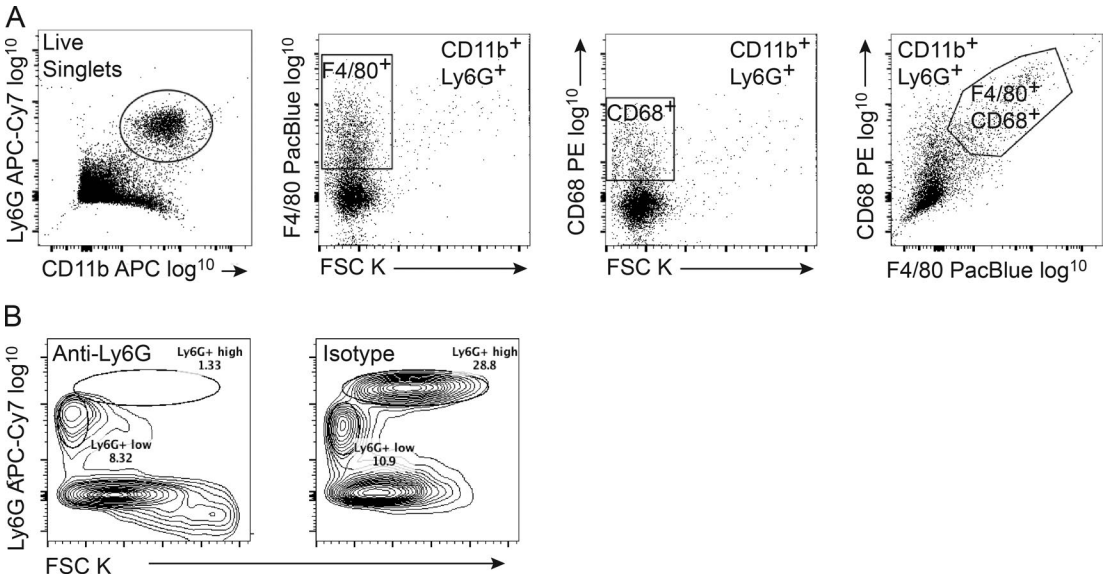
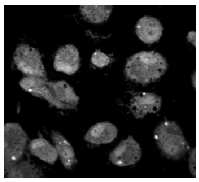
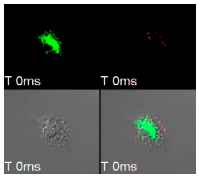


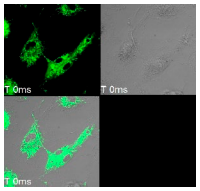
Figure S1. **Spleens from mice infected IP with control or FliC^{ind} GFP–*S.* Typhimurium for 24 h and treated with doxycycline for 3.5 h were harvested and prepared for flow cytometry.** (Related to Figs. 5, 6, and 7.) (A) Neutrophils with intracellular macrophage markers and GFP–*S.* Typhimurium (CD45$^+$ CD11b$^+$ Ly6G^{+high} CD68$^+$ F4/80$^+$ GFP$^+$) were identified by flow cytometry. (B) WT mice (six animals per group) were injected with 500 µg isotype or anti-Ly6G antibodies and infected at 24 h after antibody treatment. Depletion of CD11b$^+$ Ly6G$^{+ high}$ neutrophils was confirmed by flow cytometry.



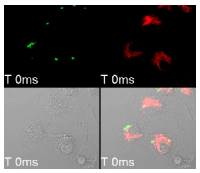
Video 1. **Soluble GFP protein is released during pyroptosis (related to Fig. 1).** 10^6 GFP-BMMs (white pseudocolor) were treated with 3 µg/ml FlaTox in the presence of PI (red) and imaged by live cell confocal microscopy 2 h.



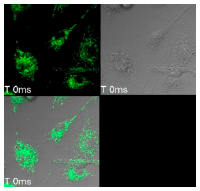
Video 2. **Bacteria are trapped in the pyroptotic cell corpse (related to Fig. 2).** 10^6 WT BMMs were labeled with ER tracker green and infected with SPI1-induced mCherry–*S.* Typhimurium MOI 25 for 30 min, washed, and treated with 15 µg/ml gentamicin 30 min and imaged in the absence of antibiotics by live cell confocal microscopy for 2 h.



Video 3. **Macrophages phagocytose pyroptotic cell debris (related to Fig. 2).** 10^6 WT BMMs were labeled with MitoTracker Green and treated with 3 µg/ml FlaTox. Cells were imaged by live cell confocal microscopy for 2 h.



Video 4. **Macrophages phagocytose the entire pyroptotic cell corpse and trapped bacteria (related to Fig. 4).** 10^6 WT BMMs were labeled with Dextran Alexa555 and infected with SPI1-induced GFP–*S.* Typhimurium MOI 25 30 min, washed and treated with 15 µg/ml gentamicin for 30 min, and imaged in the absence of antibiotics by live cell confocal microscopy for 2 h.



Video 5. **Multiple macrophages phagocytose a piece of the pyroptotic cell corpse each (related to Fig. 4).** 10^6 WT BMMs were labeled with MitoTracker Green and treated with 3 µg/ml FlaTox. Cells were imaged by live cell confocal microscopy for 2 h.

Table S1. **Bacterial strains and growth conditions**

Name of strain	Designation	Culture condition in vitro		Source
		Inflammasome activation	No inflammasome activation	
S. Typhimurium, WT	ATCC 14028s	Overnight culture 37°C, then 1:40 dilution 3 h 37°C, MOI 25, 2 h	Overnight culture 37°C MOI 25, 2 h	S. Miller, University of Washington, Seattle, WA
S. typhimurium flgB (used as WT)	14028s *flgB*::Tn *10*	NA		K. Hughes, University of Utah, Salt Lake City, UT
S. typhimurium 1 ST-FliC^{ind}	14028s *flgB*::Tn *10* pEM087	NA		Miao et al., 2010
C. rodentium	DBS100 ATTC 51459	NA	Overnight culture 37°C MOI 25, 2 h	B. Vallance, The University of British Colombia, Vancouver, Canada
L. monocytogenes, WT	10403S	NA	Overnight culture 30°C MOI 20, 2 h	M. Bevan, University of Washington, Seattle, WA
L. monocytogenes GFP	10403s *DinlAB InlAmB*, pGFP, cmR	NA	Overnight culture 30°C MOI 20, 2 h	Pentecost et al., 2010
B. thailandensis GFP	E264 pBBR2-eGFP	NA	Overnight culture 37°C MOI 25, 2 h	S. Miller, University of Washington, Seattle, WA
F. novicida, WT	U112	NA	Overnight culture 30°C MOI 25, 2 h	S. Miller, University of Washington, Seattle, WA

Table S2. **Plasmids and growth conditions**

Plasmid	Resistance	Notes	Reference
pEM087	Amp, Tet	pWSK29 expressing *fliC fliS* from *tetA* promoter	Miao et al., 2010
pWSK129	Kan, Tet	Low copy vector	Wang and Kushner, 1991
pWSK29	Amp, Tet	Low copy vector	Wang and Kushner, 1991
mCherry	mCherry::amp	Constitutive mCherry expression	Drecktrah et al., 2008
GFP	GFP::kan	Constitutive GFP expression	Valdivia and Falkow, 1997

REFERENCES

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