

Supplementary Figures

Autophagic control of *Listeria* through intracellular innate immune recognition in *drosophila*

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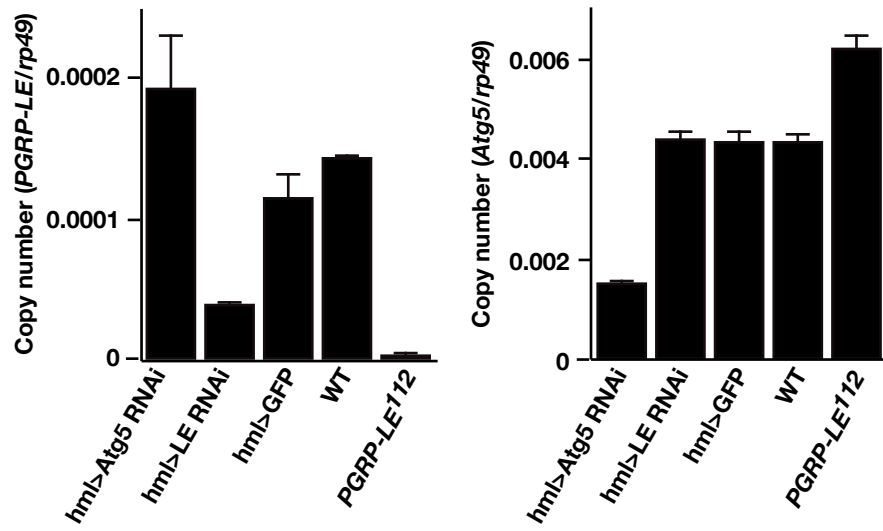


Figure S1

Supplementary Figure 1

RNA interference against PGRP-LE or Atg5 using an hml-GAL4 driver efficiently reduces the expression of the target genes in hemocytes. Quantification of the expression of *PGRP-LE* and *Atg5* in hemocytes from third instar larvae of each genotype by real-time reverse transcription-polymerase chain reaction. *rp49* was used as an internal control. Bars indicate the variance of duplicate measurements. Genotypes: UAS-Atg5IR/+;;*hml-GAL4*/+ (hml>Atg5 RNAi), UAS-PGRP-LE IR/+; *hml-GAL4*/+ (hml>LE RNAi), UAS-GFP/+; *hml-GAL4*/+ (hml>GFP), Oregon R (wild-type), *PGRP-LE*¹¹² (LE¹¹²)

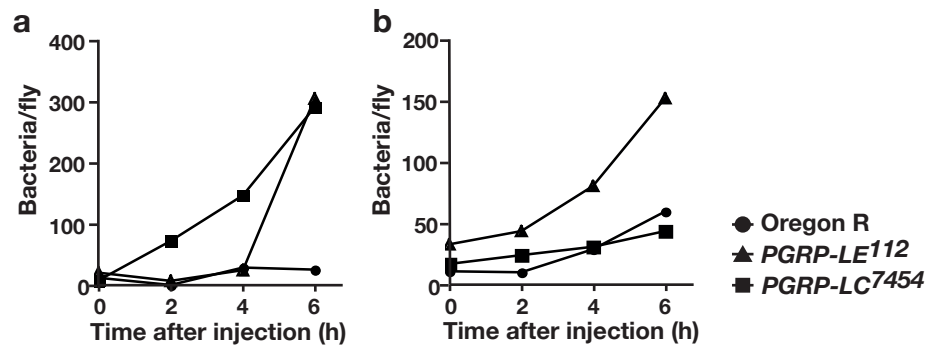


Figure S2

Supplementary Figure 2

Differences in bacterial growth in humoral and cellular fractions of PGRP-LE and PGRP-LC mutant adult flies. After injection of approximately 50 wild-type *L. monocytogenes* per fly, wild-type (Oregon R) flies, *PGRP-LE*¹¹² flies, and *PGRP-LC*⁷⁴⁵⁴ flies were dissected in PBS at the times indicated, the body debris were discarded, and the resultant humoral and cellular fractions were separated by centrifugation. *L. monocytogenes* growth in each fraction was quantified by determining colony-forming units by plate assay. **(a)** *L. monocytogenes* growth in the humoral (hemolymph) fraction. **(b)** *L. monocytogenes* growth in the cellular fraction. Results are representative of two independent experiments.

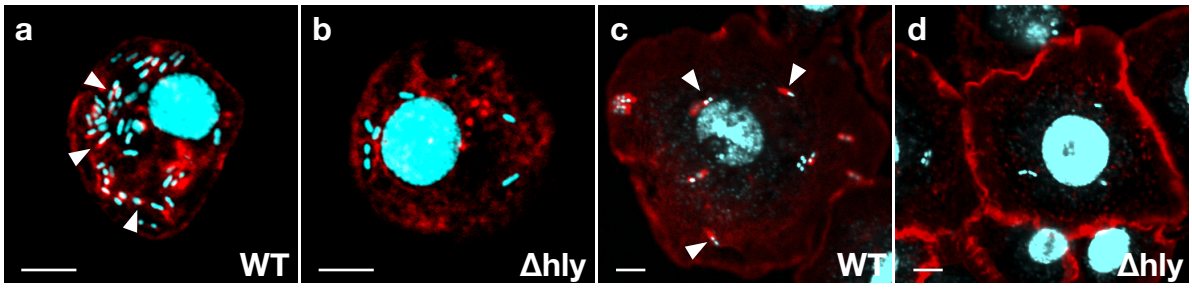


Figure S3

Supplementary Figure 3

Cytoplasmic invasion of wild-type *L. monocytogenes* into drosophila cells. **(a, b)** *Ex vivo*-cultured hemocytes from *PGRP-LE¹¹²* larvae infected with **(a)** wild-type or **(b)** Δhly strain *L. monocytogenes* for 2.5 h (0.5 h incubation with bacteria and additional 2 h incubation in gentamicin-containing medium). **(c, d)** S2 cells were incubated with wild-type **(c)** or Δhly strain **(d)** *L. monocytogenes* for 0.5 h, and additionally incubated in gentamicin-containing medium for 1.5 h. **(a-d)** Fixed cells were stained with rhodamine-labeled phalloidin (red) and DAPI (cyan). Arrowheads indicate some of the actin tails at the poles of bacteria. Scale bars, 5 μm .

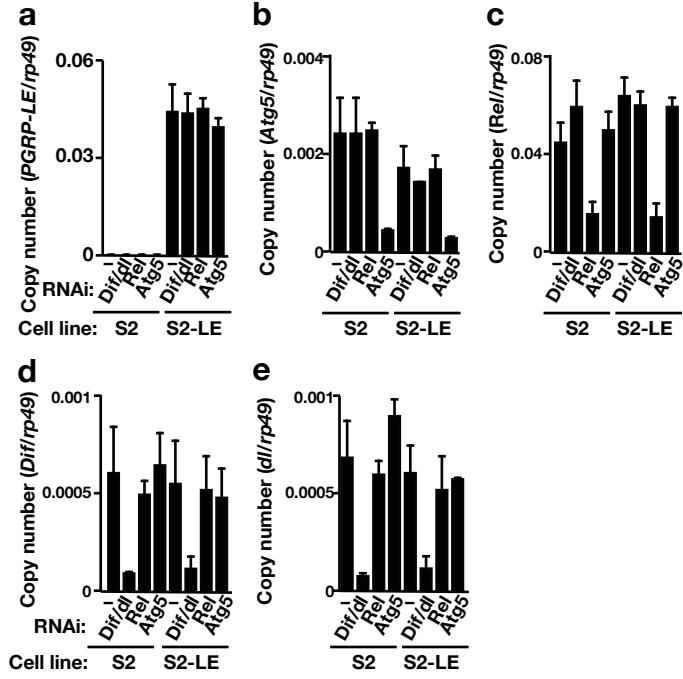


Figure S4

Supplementary Figure 4

Quantification of the expression of *PGRP-LE*, *Atg5*, *Relish*, *Dif*, and *dorsal* in S2 cells and S2 cells expressing PGRP-LE by real-time reverse transcription-polymerase chain reaction. **(a)** *PGRP-LE* **(b)** *Atg5* **(c)** *Relish (Rel)* **(d)** *Dif* **(e)** *dorsal (dl)*. *rp49* was used as an internal control. Bars indicate the variance of duplicate measurements.

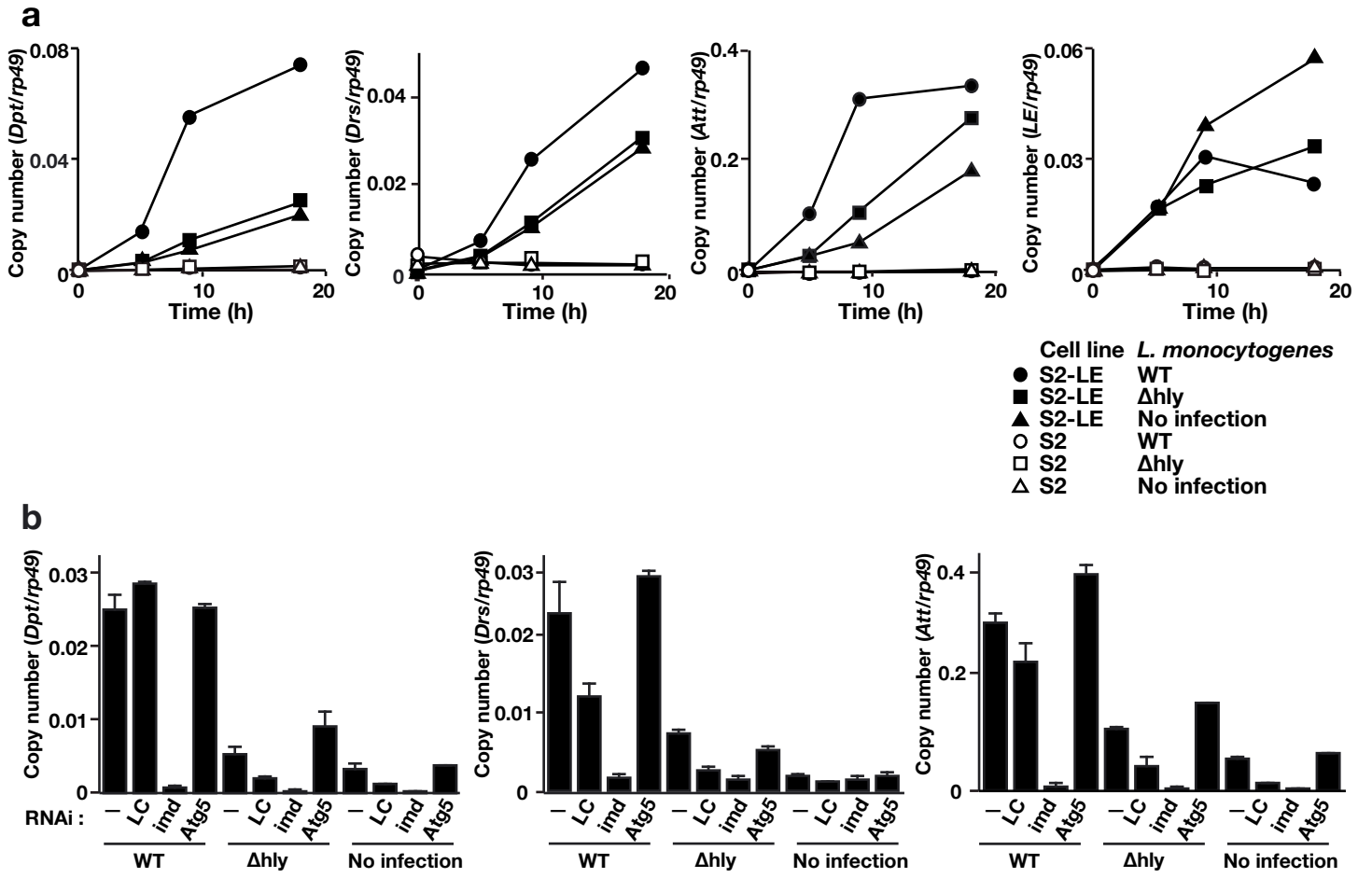


Figure S5

Supplementary Figure 5

AMP induction in response to *L. monocytogenes* infection in S2 cells expressing PGRP-LE is dependent on *imd*, but not on *Atg5*. **(a)** Real-time reverse transcription-polymerase chain reaction of *Diptericin (Dpt)*, *Drosomycin (Drs)*, *Attacin (Att)*, *PGRP-LE (LE)*, and *rp49* (internal control). S2 cells and S2 cell lines stably transfected with a metallothionein-PGRP-LE construct (S2-LE). After 1.5 h infection with wild-type or Δhly *L. monocytogenes*, cells were cultured in CuSO₄- and gentamicin-containing medium, and the RNA was extracted for analysis. **(b)** S2-LE cells were transfected with double-stranded RNA specific for the indicated genes (RNAi, below graph) and then infected with wild-type or Δhly *L. monocytogenes* for 1.5 h. After 9 h incubation in CuSO₄-and gentamicin-containing medium, the RNA was extracted for real-time reverse transcription-polymerase chain reaction analysis. Bars indicate the variance of duplicate measurements.

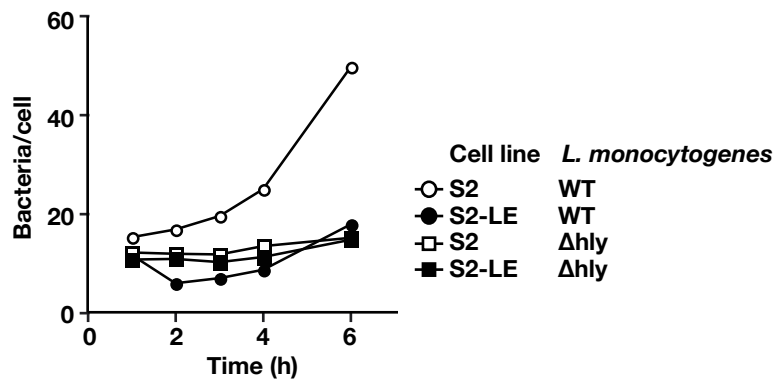


Figure S6

Supplementary Figure 6

PGRP-LE is crucial for the clearance of intracellular *L. monocytogenes*. S2 cells or S2 cells expressing PGRP-LE (S2-LE) were infected with wild-type (WT) or Δhly *L. monocytogenes* (approximately 250 bacteria per cell) for 0.5 h, followed by incubation in CuSO₄- and gentamicin-containing medium for the indicated time. *L. monocytogenes* growth was quantified by determining colony-forming units by plate assay.

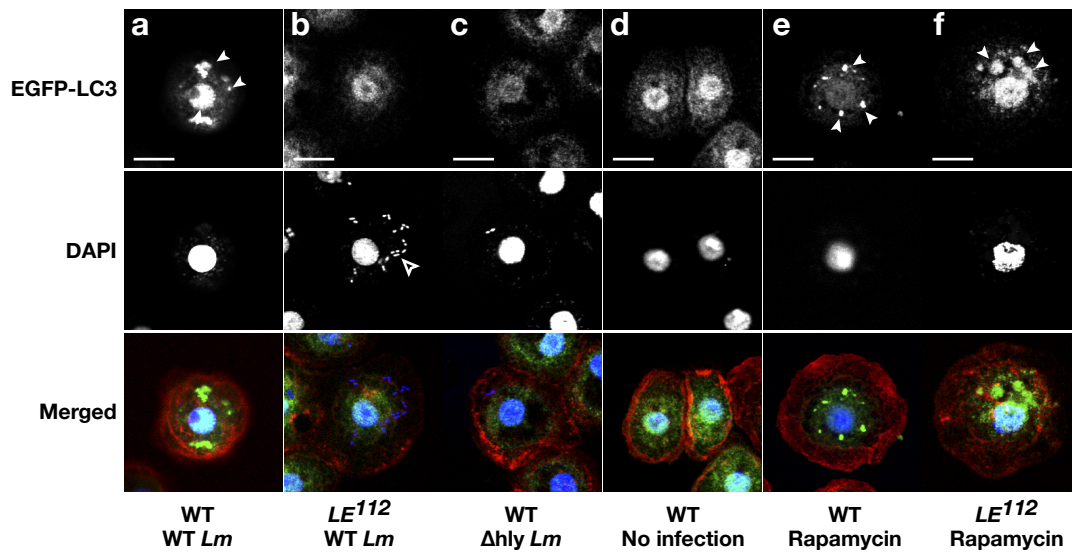


Figure S7

Supplementary Figure 7

PGRP-LE is required for the induction of autophagy in response to *L. monocytogenes* infection in the cytoplasm. *Ex vivo*-cultured hemocytes from EGFP-LC3 expressing wild-type (WT) or *PGRP-LE*¹¹² (*LE*¹¹²) mutant-background third-instar larvae infected with wild-type (WT *Lm*) or Δhly strain (Δhly *Lm*) *L. monocytogenes*. EGFP-LC3 (green or white), rhodamine-labeled phalloidin staining of the actin cytoskeleton (red), and DAPI (blue or white). All images were obtained using a confocal microscope. Filled arrowheads indicate some of the dot-shaped EGFP-LC3 signals. The open arrowhead indicates *Lm* DNA. Bars represent 10 μ m.

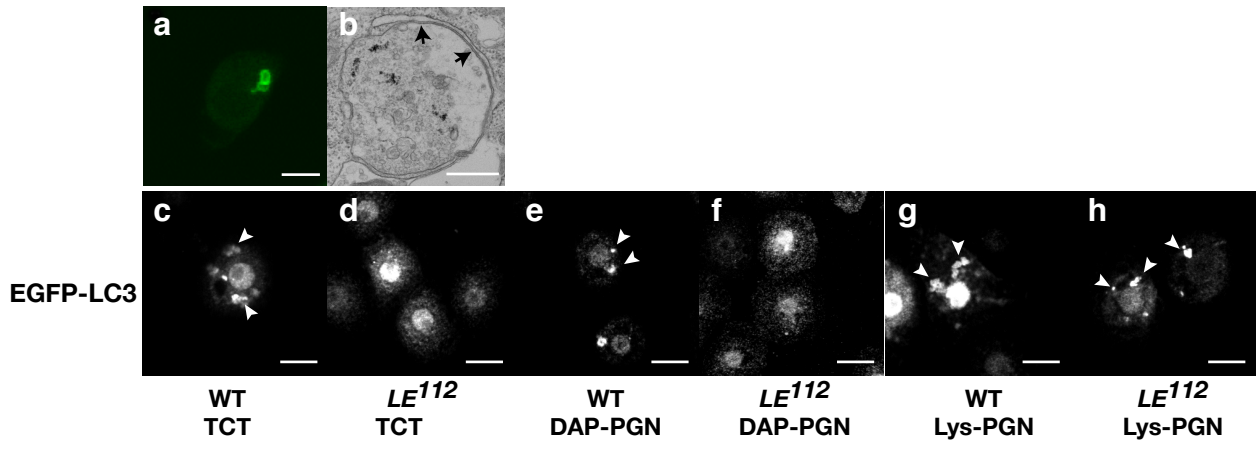


Figure S8

Supplementary Figure 8

PGRP-LE is required for the induction of autophagy in response to TCT or DAP-type peptidoglycans. **(a)** A fluorescence microscopy image of S2 cells expressing PGRP-LE and GFP-LC3 (green) transfected with TCT. **(b)** An electron microscopy image of the fluorescence-positive field in **(a)**. Arrows indicate double-membrane structure. Scale bars represent 5 μm in **(a)** and 500 nm in **(b)**. **(c-h)** *Ex vivo*-cultured hemocytes from GFP-LC3 expressing wild-type **(c, e, g)** or *PGRP-LE*¹¹² **(d, f, h)** mutant-background third-instar larvae treated with 100 nM TCT **(c, d)**, 100 $\mu\text{g/ml}$ highly purified DAP-type peptidoglycans from *L. plantarum* (DAP-PGN) **(e, f)**, or lysine-type peptidoglycans from *S. epidermidis* (Lys-PGN) **(g, h)**. All images were obtained using a confocal microscope. Some of the dot-shaped GFP-LC3 signals are indicated by filled arrowheads. Bars represent 10 μm .