Supplementary Figure Legends

Figure S1. Temporal gene expression profile for PGRP-LC. Developmental expression pattern of PGRP-LC derived from modENCODE RNA-Seq temporal expression data (Graveley et al., 2011). Time, in days after egg laying, is shown at the top and bottom. The approximate times of 20E and JH titer peaks are shown as yellow and green bars (Dubrovsky, 2004).

Figure S2. Characterization of PGRP-LCx-FLAG cells. Immunoblot (IB) analysis of whole-cell lysates from S2* cells stably transfected with a plasmid encoding metallothionein (MT) promoter expressing C-terminal FLAG-tagged PGRP-LCx (right) or parental S2* cells (left), with or without 3 hour 100 µM CuSO₄ treatment (lower or upper panels, respectively). Samples were also untreated or treated with 20E for 24 hours and/or stimulated with PGN for an additional 10 minutes prior to harvesting for whole cell lysis and immunoblotting. Results are typical of at least three independent experiments.

Figure S3. Characterization of PGRP-LCx-FLAG cells. Immunoprecipitation (IP) – Immunoblot (IB) analysis of whole-cell lysates from PGRP-LCx-FLAG cells and from parental S2* cells with or without 3 hour 100 µM CuSO₄ treatment (lower or upper panels, respectively). Cells with or without exposure to 20E treatment for 24 hours, were stimulated with PGN for an additional 10 minutes or left unstimulated prior to harvesting for whole cell lysis and immunoblotting. PGRP-LCx was first immunoprecipitated (IP) with rabbit anti-FLAG antibody and
then detected by immunoblotting (IB) with mouse anti-FLAG antibody. Results are typical of three independent experiments.

**Figure S4.** Nuclear translocation of Relish is 20E-independent in PGRP-LCx-FLAG cells. Nuclear translocation of Relish protein following PGN stimulation was analyzed by confocal microscopy. S2* cells stably expressing the YFP-Relish (A) & (C) and double-stable cells expressing PGRP-LCx-FLAG and YFP-Relish (B) & (D) were exposed or not to 20E for 24 hours and/or stimulated with PGN for 30 min before imaging. Nuclei are stained with Hoechst 33342. (D) Quantification of Relish nuclear translocation from confocal images; 3 fields were quantified for each cell line and condition and error bars represent standard deviations.

**Figure S5.** 20E treatment of S2* cells induces expression of transcription factors genes. (A) Microarray data (as in Figure 1) was analyzed for twelve 20E inducible transcription factors. The asterisks represent statistical significance (*P<0.05; **P<0.01, ***P<0.001) calculated by unpaired t-test. (B) Expression levels of br-c, Eip78C, Eip93F, Eip74EF, Eip75B, Hr46, srp and pnr transcripts were measured by real-time qRT-PCR from S2* cells treated with 20E for 24 hours or left untreated, as indicated. Normalized gene expression levels are shown for the average of two independent experiments.

**Figure S6.** ERR, Hsf, Hnf4, and luna are not required for IMD signaling. qRT-PCR analysis of Dpt induction in S2* cells treated with RNAi targeting for EcR, ERR, Hsf, Hnf4, luna or mock transfected. Cells with or without exposure to
20E for 24 hours were then stimulated (or not) with PGN for an additional 6 hours, as indicated. Normalized Dpt levels are shown for the average of three biological replicates. Error bars are standard deviation. $P$-values were calculated in comparison to mock RNAi treatment, by one-way ANOVA with Tukey’s Multiple Comparison Test. (***$P<0.001$).

Figure S7. Heterozygous EcR mutant flies display immunodefiency. (A) - (F) Real-time RT-PCR was used to measure the level of Dpt expression in EcR<sup>NP5219</sup>, EcR<sup>A483T</sup> and DTS-3 mutant flies before or 24 hours after infection with <i>E.coli</i>. Normalized Dpt expression is shown. The values represent the mean of three independent experiments and error bars represent standard deviations. All flies in these experiments were reared at 25°C, except for (F) which were reared at the permissive 18°C. $P$ values were calculated by unpaired t-test.

Figure S8. Control male animals from Yp1-Gal4, EcR, PGRP-LC, br-c, Eip78C, Eip93F, Eip74EF, pnr, srp, Eip75B and Hr46 RNAi assays. Real-time RT-PCR was used to analyze the expression of Dpt (A), CecA1 (B) and PGRP-LC (C) in males with the Yp1-Gal4 driver and EcR, br-c, Eip78C, Eip93F, Eip74EF, Eip75B, pnr, or srp hairpin-RNA transgenes before or 24 hours after infection with <i>E. coli</i>. Yp1 does not express in males. Error bars represent standard deviations of three independent experiments. $P$-values were calculated in comparison to Yp1-Gal4 driver alone strain by one-way ANOVA with Tukey’s Multiple Comparison Test, NS for $P>0.05$. (D) Kaplan-Meier plot showing survival of same males genotypes after infection with <i>Erwinia carotovora</i>
carotovora 15 \((n=60)\). The surviving animals were counted every 24 hours. Statistical significance between survival curves was determined by log-rank analysis; all \(P\)-values were non-significant \((P>0.1)\). Data are representative of three independent experiments.

**Figure S9.** \(br-c\), \(Eip93F\), \(Eip78C\), \(Eip74EF\), \(Hr46\), \(pnr\), and \(srp\) knockdown causes immunodeficiency in adult flies. Real-time RT-PCR was used to analyze the expression of \(Dpt\) (A-B) in \(EcR\), \(br-c\), \(Eip78C\), \(Eip93F\), \(Eip74EF\), \(Eip75B\), \(Hr46\), \(pnr\), or \(srp\) RNAi expressing flies before or 24 hours after infection with \(E. coli\). The \(C564\)-Gal4 driver was used to express inverted-repeat transgenes in the fat body and blood cells of both sexes. The \(C564\) strain is presented as a control. Data represent the mean and standard deviation of two biological replicates. **\(P<0.01\), ***\(P<0.001\), were calculated in comparison to \(E. coli\) infected \(C564\)-Gal4 flies, by one-way ANOVA with Tukey’s Multiple Comparison Test.

**Figure S10.** Effect of transcription factors RNAi on growth and survival of *Erwinia carotovora carotovora* 15

Growth and survival of *Erwinia carotovora carotovora* 15 in adult flies depleted of \(EcR\), \(br-c\), \(Eip78C\), \(Eip93F\), \(Eip75B\), \(Eip74EF\), \(Hr46\), \(srp\) or \(pnr\). At 0h, 24h, 48h following septic infection, 20 individual female (A) or male (B) flies were homogenized and serially diluted and plated. For all experiments, the *yolk protein 1 (Yp1)*-GAL4 driver was used to express inverted-repeat RNAs specifically in the adult female fat body, and the *Yp1*-GAL4 strain as well as
males, are presented as controls. CFUs were counted after overnight culture at 37°C. *P<0.05, **P<0.01, ***P<0.001, were calculated by two-way ANOVA with Bonferroni post-test.

**Figure S11. Effect of transcription factors RNAi on growth of *Enterobacter cloacae***

Growth of *Enterobacter cloacae* in adult flies depleted of EcR, br-c, Eip78C or Eip75B at 0h, 24h, 48h following infection. 15 individual female (A) or male (B) flies were homogenized and serially diluted and plated. CFUs were counted after overnight culture at 37°C. The *P*-values were determined by two-way ANOVA with Bonferroni post-test. (*P<0.05, ***P<0.001)

**References**

### Figure S2

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**Notes:**
- CuSO\(_4\)
- 20E
- PGN

**Images:**
- IB: α-FLAG

**Gels:**
- 76
- 52
### Figure S3

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<td>PGN</td>
</tr>
</tbody>
</table>

| + + + +   | CuSO₄               |
| - - + +   | 20E                 |
| - + - +   | PGN                 |

**Images:**
- **S2* Cells:**
  - 76
  - 52

- **PGRP-LCx-FLAG cells:**
  - 76
  - 52

**Immunoprecipitation (IP):** α-FLAG

**Immunoblotting (IB):** α-FLAG
Figure S4

A

(-)20E(-)PGN

(-)20E(+)+PGN

(+)+20E(-)PGN

(+)+20E(+)+PGN

B

(-)20E(-)PGN

(-)20E(+)+PGN

(+)+20E(-)PGN

(+)+20E(+)+PGN

C

S2*-YFP-Relish Cells

%Cells

150

100

50

0

20E

PGN

nuclear translocation

cytoplasmic

D

PGRP-LCx-FLAG;YFP-Relish Cells

%Cells

150

100

50

0

20E

PGN

nuclear translocation

cytoplasmic
Figure S7

- A: 
  - Female flies
  - E. coli infection
  - Dipterin expression
  - Different EcR genotypes
  - Student’s T-test: P = 0.002

- B: 
  - Male flies
  - E. coli infection
  - Dipterin expression
  - Different EcR genotypes
  - Student’s T-test: P = 0.01

- C: 
  - Female flies
  - E. coli infection
  - Dipterin expression
  - Different EcR genotypes
  - Student’s T-test: P = 0.0009

- D: 
  - Male flies
  - E. coli infection
  - Dipterin expression
  - Different EcR genotypes
  - Student’s T-test: P = 0.0068

- E: 
  - Female flies
  - E. coli infection
  - Dipterin expression at 25°C
  - Different EcR genotypes
  - Student’s T-test: P < 0.0001

- F: 
  - Male flies
  - E. coli infection
  - Dipterin expression at 18°C
  - Different EcR genotypes
  - Student’s T-test: NS
Figure S8

A

Diptericin

NS

B

Cecropin A1

NS

C

PGPR-LC

NS

D

Percent survival

P>0.1

days post infection

0 2 4 6 8 10
Figure S9

A

B

Diptericin

Diptericin

no infection

E. coli

no infection

E. coli

** NS

** NS

Legend:
- c564-Gal4
- PGRP-LC RNAi/c564-Gal4
- Eip78C RNAi/c564-Gal4
- Eip93F RNAi/c564-Gal4
- Eip74EF RNAi/c564-Gal4
- pnr RNAi/c564-Gal4
- srp RNAi/c564-Gal4
- Hr46 RNAi/c564-Gal4
- Eip75B RNAi/c564-Gal4

** NS

** NS

*** NS

*** NS

**** NS

**** NS
Figure S10

A

B

**

** NS

*** NS

NS

Yp1-Gal4

EcR RNAi/ Yp1-Gal4

PGRP-LC RNAi/ Yp1-Gal4

br-c RNAi/ Yp1-Gal4

Eip78C RNAi/ Yp1-Gal4

Eip93F RNAi/ Yp1-Gal4

Eip74EF RNAi/ Yp1-Gal4

pnr RNAi/ Yp1-Gal4

srp RNAi/ Yp1-Gal4

Hr46 RNAi/Yp1-Gal4

Eip75B RNAi/ Yp1-Gal4
Figure S11

A

B

Enterobacter cloacae CFUs

Yp1-Gal4

EcR RNAi/ Yp1-Gal4

br-c RNAi/ Yp1-Gal4

Eip78C RNAi/ Yp1-Gal4

Eip75B RNAi/ Yp1-Gal4

0h 24h 48h

0

10^2

10^3

10^4

10^5

10^6

0h 24h 48h

0

10^2

10^3

10^4

10^5

10^6

*** NS

*** NS

* NS

NS

NS