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 $CuSO_4$ 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 $CuSO_4$ 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^{NP5219}, EcR^{A483T} and *DTS-3* mutant flies before or 24 hours after infection with *E.coli.* 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 *E. coli*. 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 *Erwinia carotovora*

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

Dubrovsky, E.B. (2004). Hormonal cross talk in insect development. Trends Endocrinol Metab *16*, 6-11.
Graveley, B.R., Brooks, A.N., Carlson, J.W., Duff, M.O., Landolin, J.M., Yang, L., Artieri, C.G., van Baren, M.J., Boley, N., Booth, B.W., *et al*. (2011). The developmental transcriptome of Drosophila melanogaster. Nature *471*, 473-479.

Figure S1

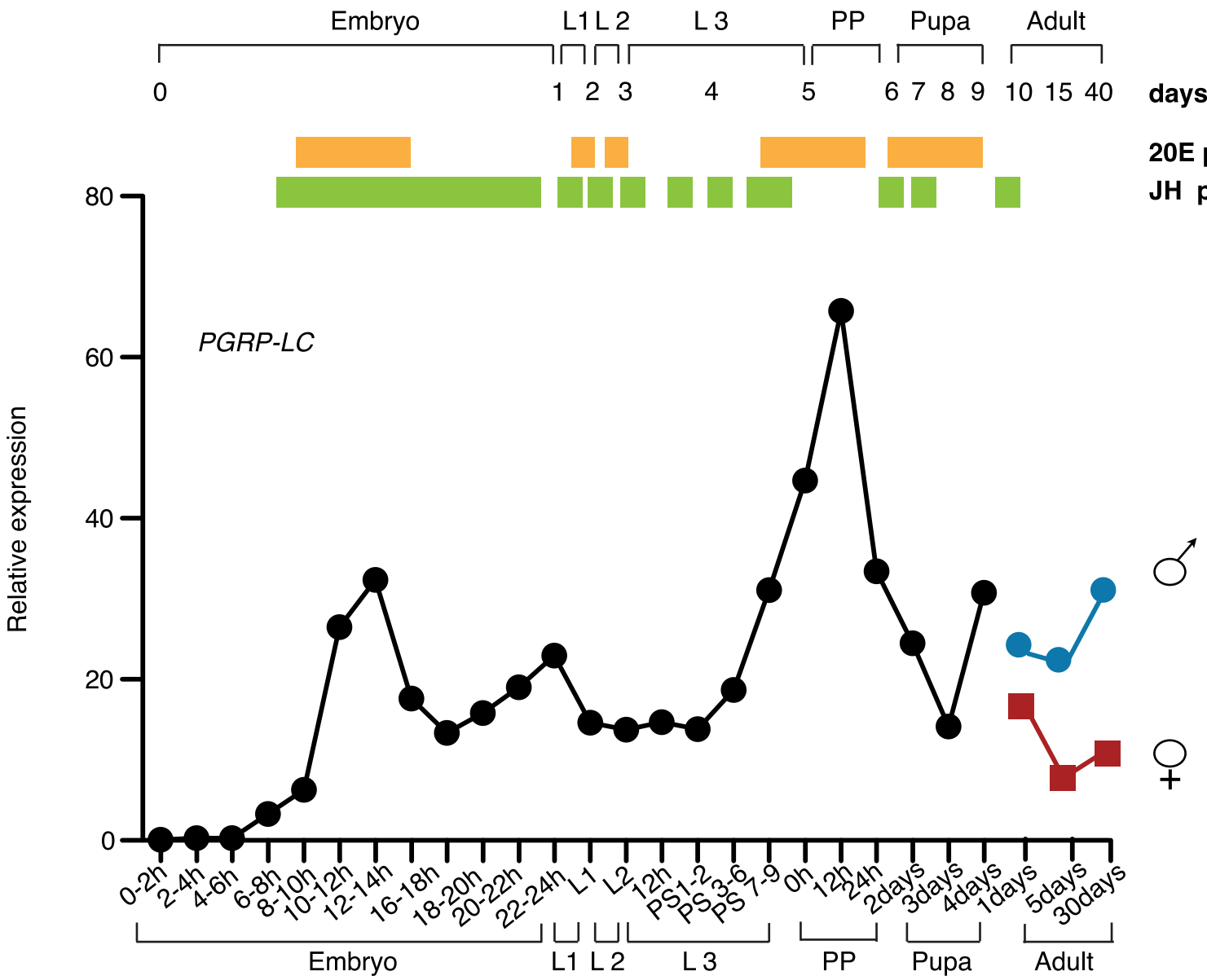

Embryo
L1
L2
L3
PP
Pupa
Adult
0
1
2
3
4
5
6
7
8
9
10
15
40
days
20E peaks
JH peaks
PGRP-LC
Relative expression
80
60
40
20
0
0-2h
2-4h
4-6h
6-8h
8-10h
10-12h
12-14h
16-18h
18-20h
20-22h
22-24h
L1
L2
12h
PS1-2
PS3-6
PS 7-9
0h
12h
24h
2days
3days
4days
1days
5days
30days
Embryo
L1
L2
L3
PP
Pupa
Adult
♂
♀

Figure S2

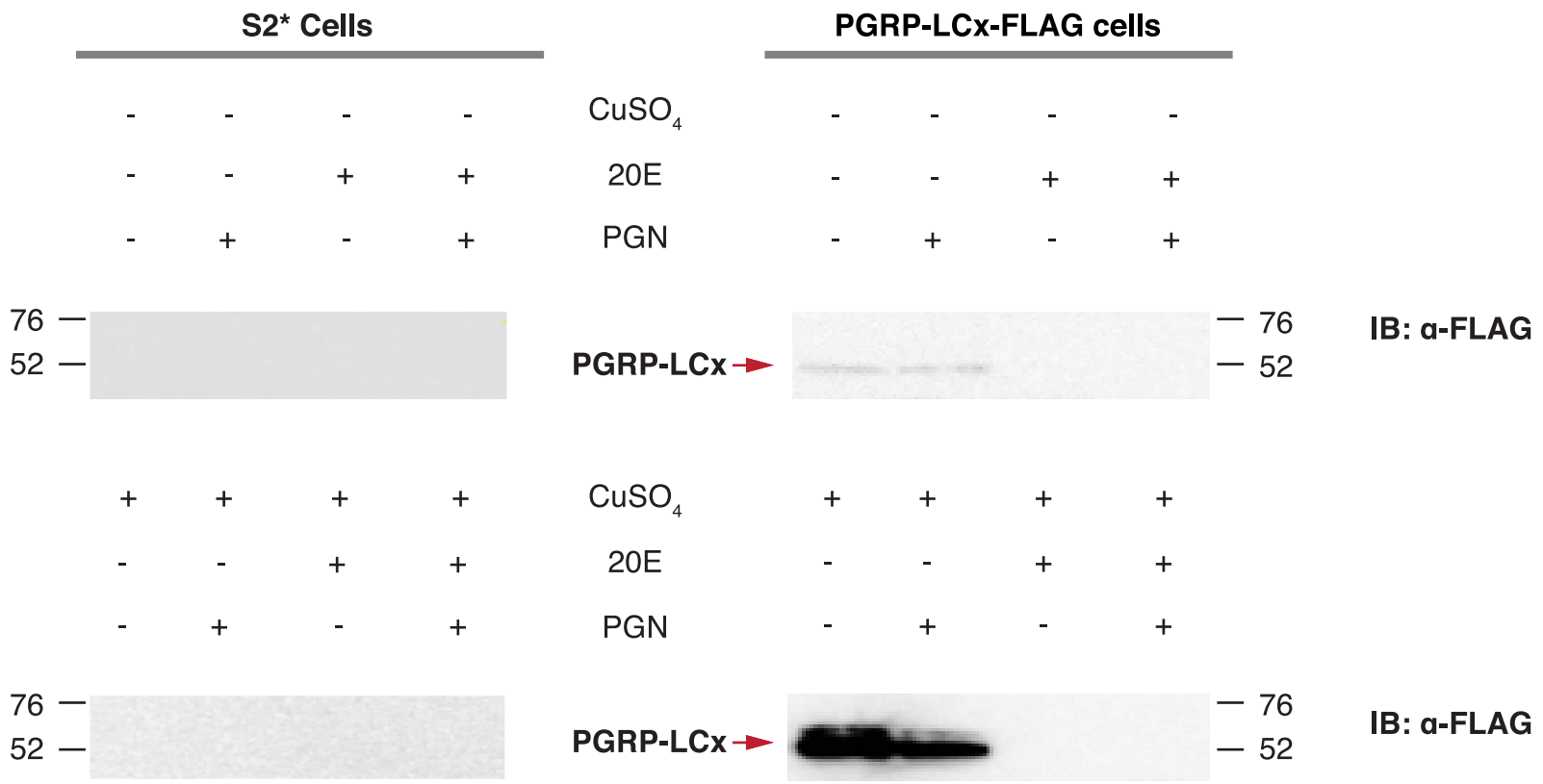

S2* Cells
PGRP-LCx-FLAG cells
- - - - CuSO4 - - - -
- - + + 20E - - + +
- + - + PGN - + - +
76
52
PGRP-LCx
76
52
IB: α-FLAG
+ + + + CuSO4 + + + +
- - + + 20E - - + +
- + - + PGN - + - +
76
52
PGRP-LCx
76
52
IB: α-FLAG

Figure S3

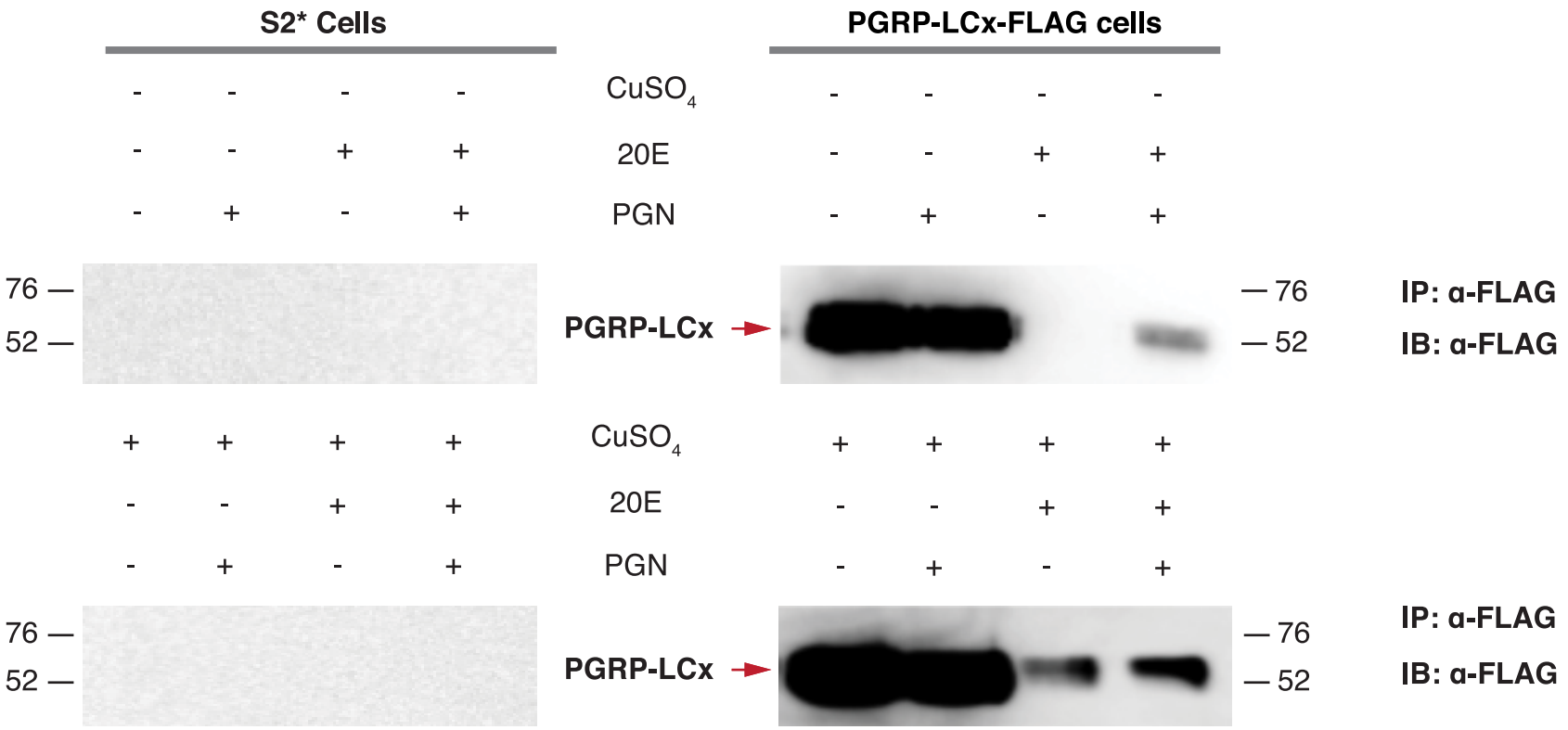

S2* Cells
PGRP-LCx-FLAG cells
CuSO4
20E
PGN
- - - -
- - + +
- + - +
+ + + +
76 —
52 —
PGRP-LCx
— 76
— 52
IP: α-FLAG
IB: α-FLAG

Figure S4

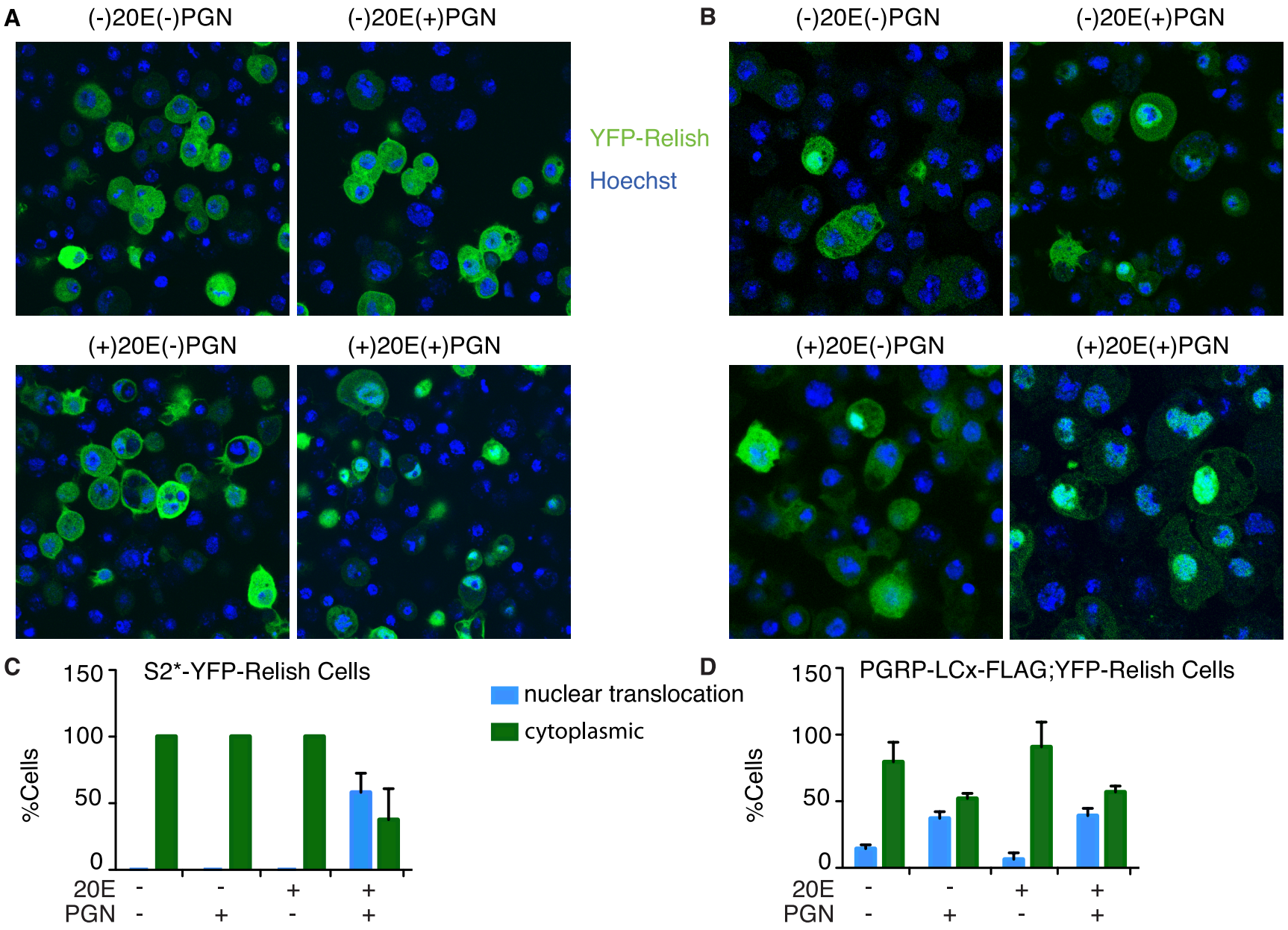

A
(-)20E(-)PGN
(-)20E(+)PGN
(+)20E(-)PGN
(+)20E(+)PGN
YFP-Relish
Hoechst
B
(-)20E(-)PGN
(-)20E(+)PGN
(+)20E(-)PGN
(+)20E(+)PGN
C
S2*-YFP-Relish Cells
nuclear translocation
cytoplasmic
%Cells
150
100
50
0
20E - - + +
PGN - + - +
D
PGRP-LCx-FLAG;YFP-Relish Cells
%Cells
150
100
50
0
20E - - + +
PGN - + - +

Figure S5

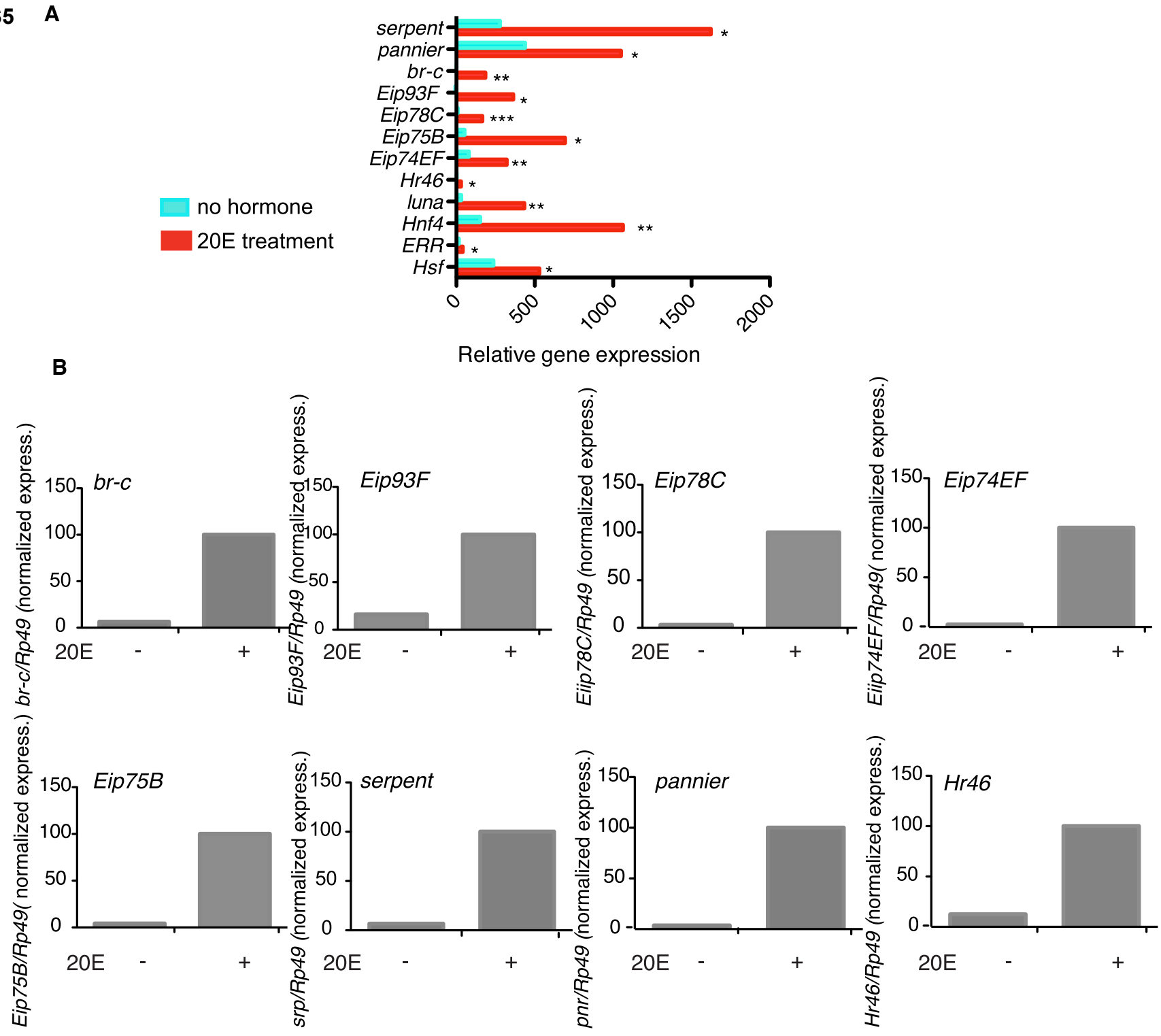

A
serpent
pannier
br-c
Eip93F
Eip78C
Eip75B
Eip74EF
Hr46
luna
Hnf4
ERR
Hsf
no hormone
20E treatment
0
500
1000
1500
2000
Relative gene expression
B
br-c
Eip93F
Eip78C
Eip74EF
Eip75B
serpent
pannier
Hr46
br-c/Rp49 (normalized express.)
Eip93F/Rp49 (normalized express.)
Eiip78C/Rp49 (normalized express.)
Eiip74EF/Rp49(normalized express.)
Eip75B/Rp49(normalized express.)
srp/Rp49 (normalized express.)
pnr/Rp49 (normalized express.)
Hr46/Rp49 (normalized express.)
150
100
50
0
20E
-
+

Figure S6

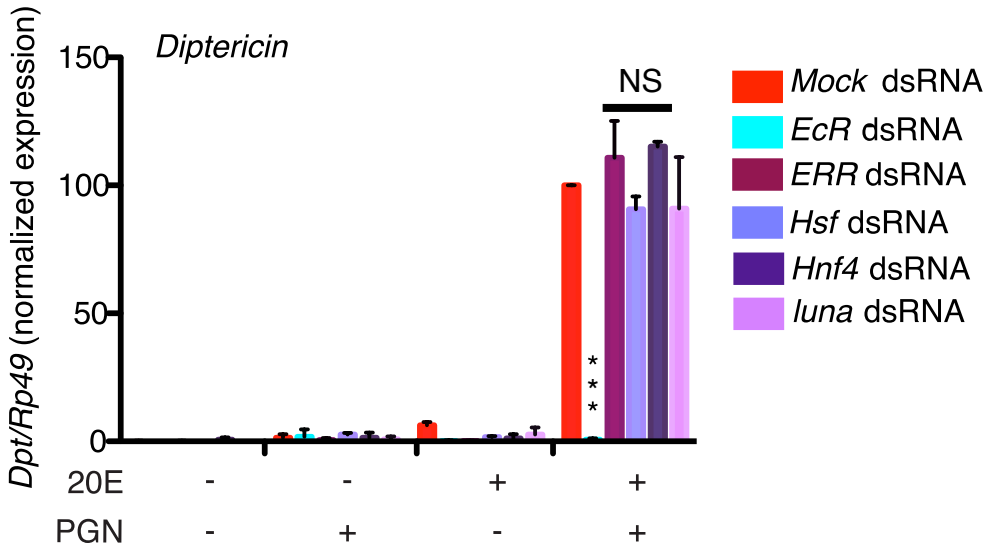

Diptericin
Dpt/Rp49 (normalized expression)
150
100
50
0
NS
***
20E - - + +
PGN - + - +
Mock dsRNA
EcR dsRNA
ERR dsRNA
Hsf dsRNA
Hnf4 dsRNA
luna dsRNA

Figure S7

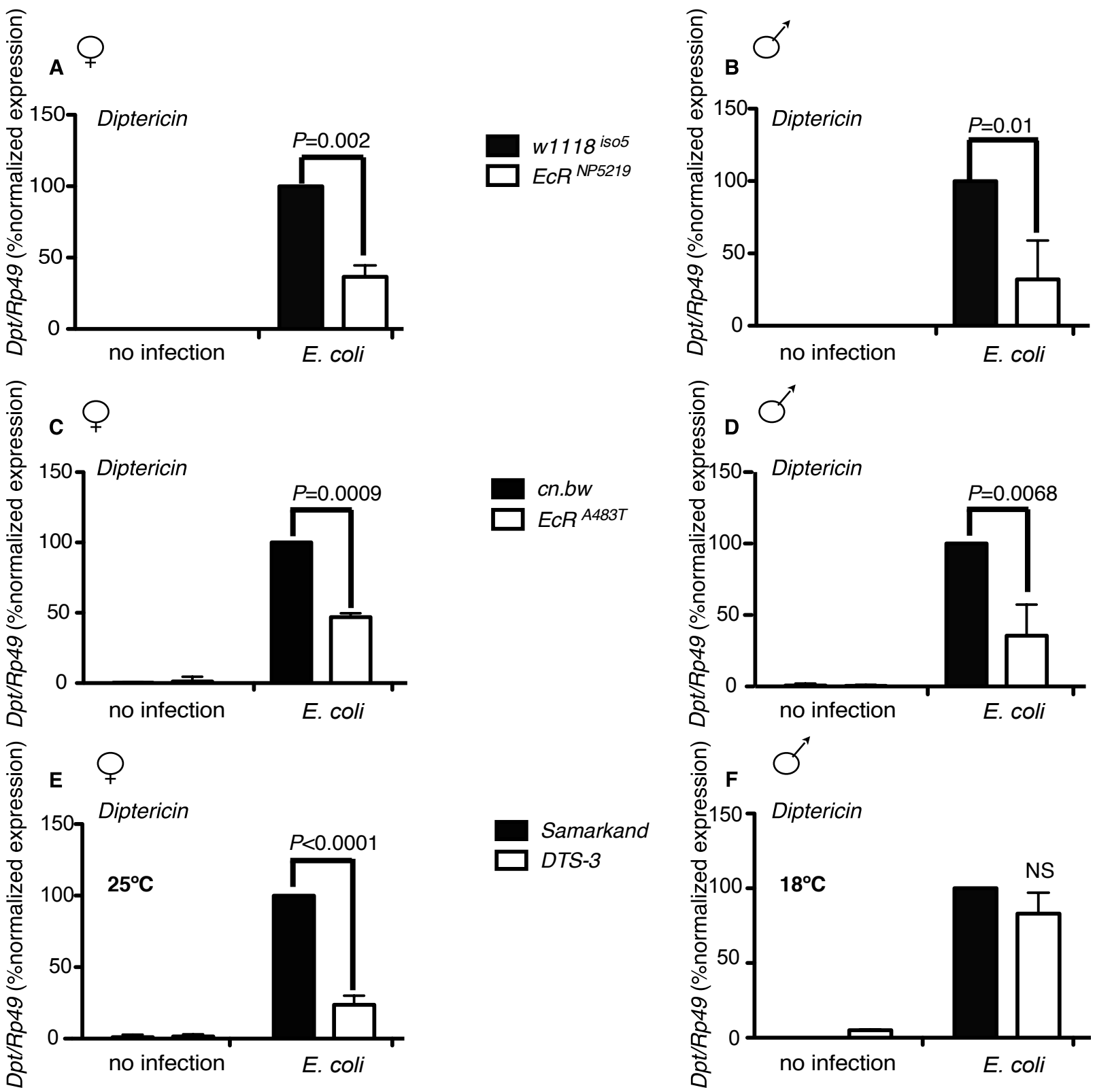

A
B
C
D
E
F
Diptericin
Dpt/Rp49 (%normalized expression)
150
100
50
0
P=0.002
P=0.01
P=0.0009
P=0.0068
P<0.0001
NS
25°C
18°C
no infection
E. coli
w1118 iso5
EcR NP5219
cn.bw
EcR A483T
Samarkand
DTS-3

Figure S8

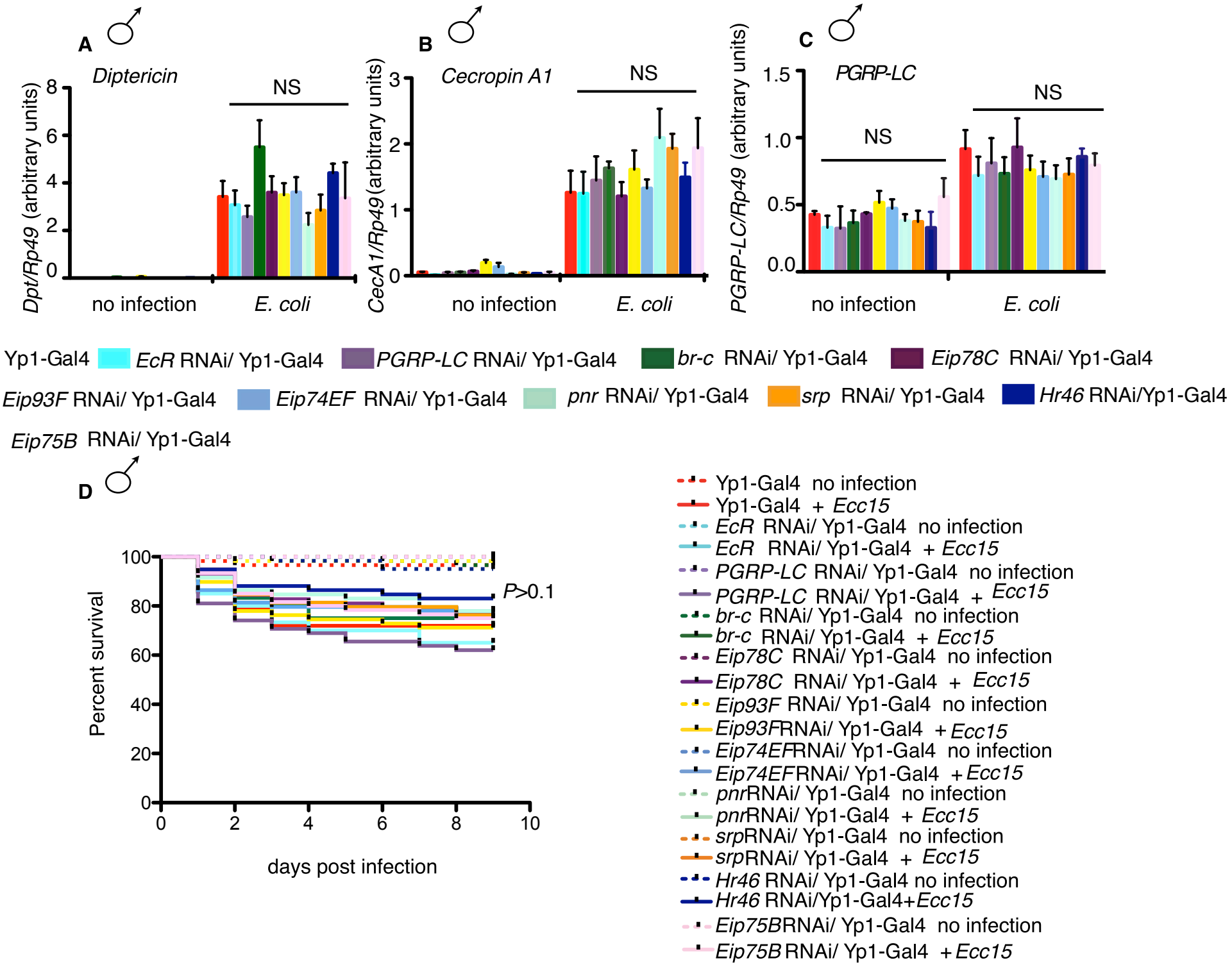

A
Diptericin
NS
Dpt/Rp49 (arbitrary units)
no infection
E. coli
B
Cecropin A1
NS
CecA1/Rp49 (arbitrary units)
no infection
E. coli
C
PGRP-LC
NS
NS
PGRP-LC/Rp49 (arbitrary units)
no infection
E. coli
Yp1-Gal4
EcR RNAi/ Yp1-Gal4
PGRP-LC RNAi/ Yp1-Gal4
br-c RNAi/ Yp1-Gal4
Eip78C RNAi/ Yp1-Gal4
Eip93F RNAi/ Yp1-Gal4
Eip74EF RNAi/ Yp1-Gal4
pnr RNAi/ Yp1-Gal4
srp RNAi/ Yp1-Gal4
Hr46 RNAi/Yp1-Gal4
Eip75B RNAi/ Yp1-Gal4
D
Percent survival
P>0.1
days post infection
Yp1-Gal4 no infection
Yp1-Gal4 + Ecc15
EcR RNAi/ Yp1-Gal4 no infection
EcR RNAi/ Yp1-Gal4 + Ecc15
PGRP-LC RNAi/ Yp1-Gal4 no infection
PGRP-LC RNAi/ Yp1-Gal4 + Ecc15
br-c RNAi/ Yp1-Gal4 no infection
br-c RNAi/ Yp1-Gal4 + Ecc15
Eip78C RNAi/ Yp1-Gal4 no infection
Eip78C RNAi/ Yp1-Gal4 + Ecc15
Eip93F RNAi/ Yp1-Gal4 no infection
Eip93F RNAi/ Yp1-Gal4 + Ecc15
Eip74EF RNAi/ Yp1-Gal4 no infection
Eip74EF RNAi/ Yp1-Gal4 + Ecc15
pnr RNAi/ Yp1-Gal4 no infection
pnr RNAi/ Yp1-Gal4 + Ecc15
srp RNAi/ Yp1-Gal4 no infection
srp RNAi/ Yp1-Gal4 + Ecc15
Hr46 RNAi/ Yp1-Gal4 no infection
Hr46 RNAi/Yp1-Gal4+Ecc15
Eip75B RNAi/ Yp1-Gal4 no infection
Eip75B RNAi/ Yp1-Gal4 + Ecc15

Figure S9

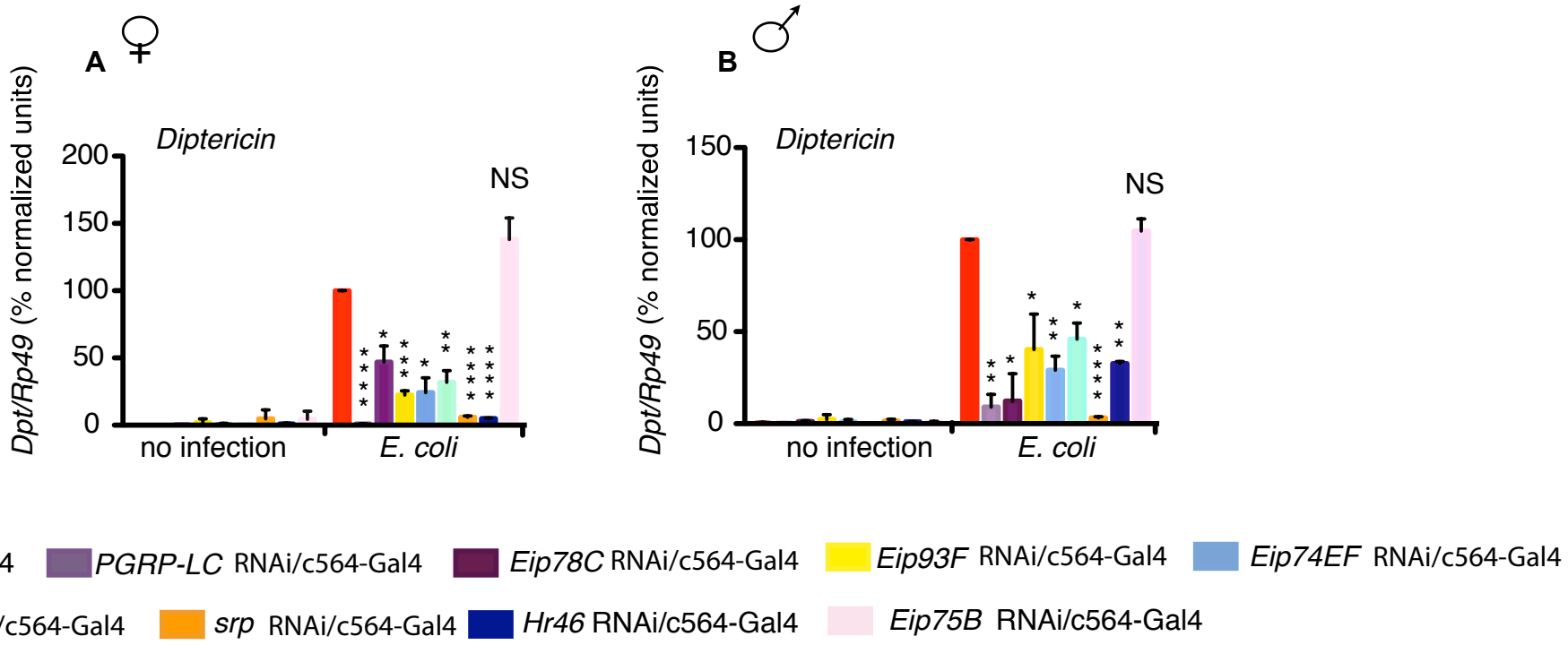

A
♀
Diptericin
Dpt/Rp49 (% normalized units)
200
150
100
50
0
NS
no infection
E. coli
B
♂
Diptericin
Dpt/Rp49 (% normalized units)
150
100
50
0
NS
no infection
E. coli
c564-Gal4
PGRP-LC RNAi/c564-Gal4
Eip78C RNAi/c564-Gal4
Eip93F RNAi/c564-Gal4
Eip74EF RNAi/c564-Gal4
pnr RNAi/c564-Gal4
srp RNAi/c564-Gal4
Hr46 RNAi/c564-Gal4
Eip75B RNAi/c564-Gal4

Figure S10

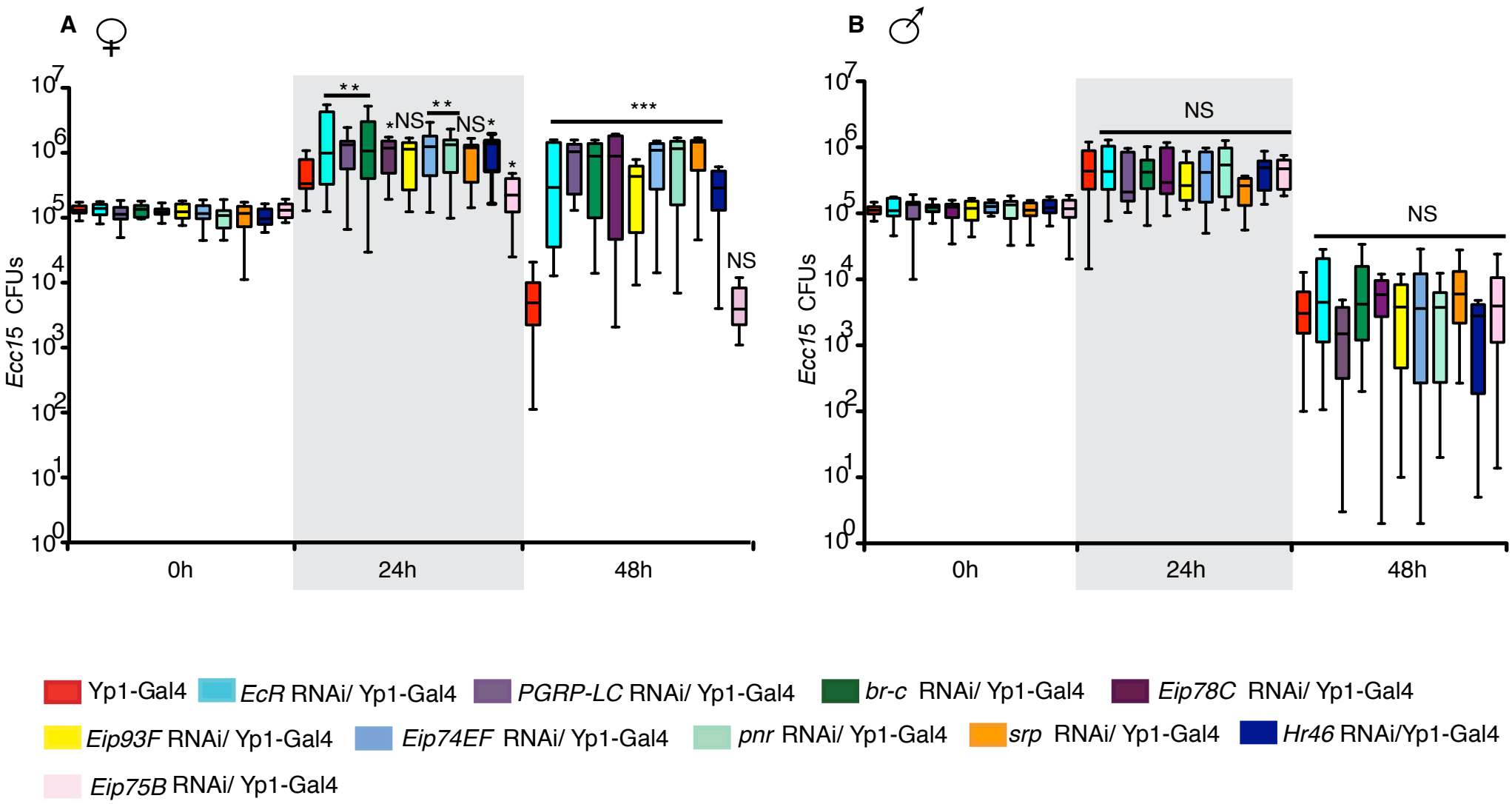
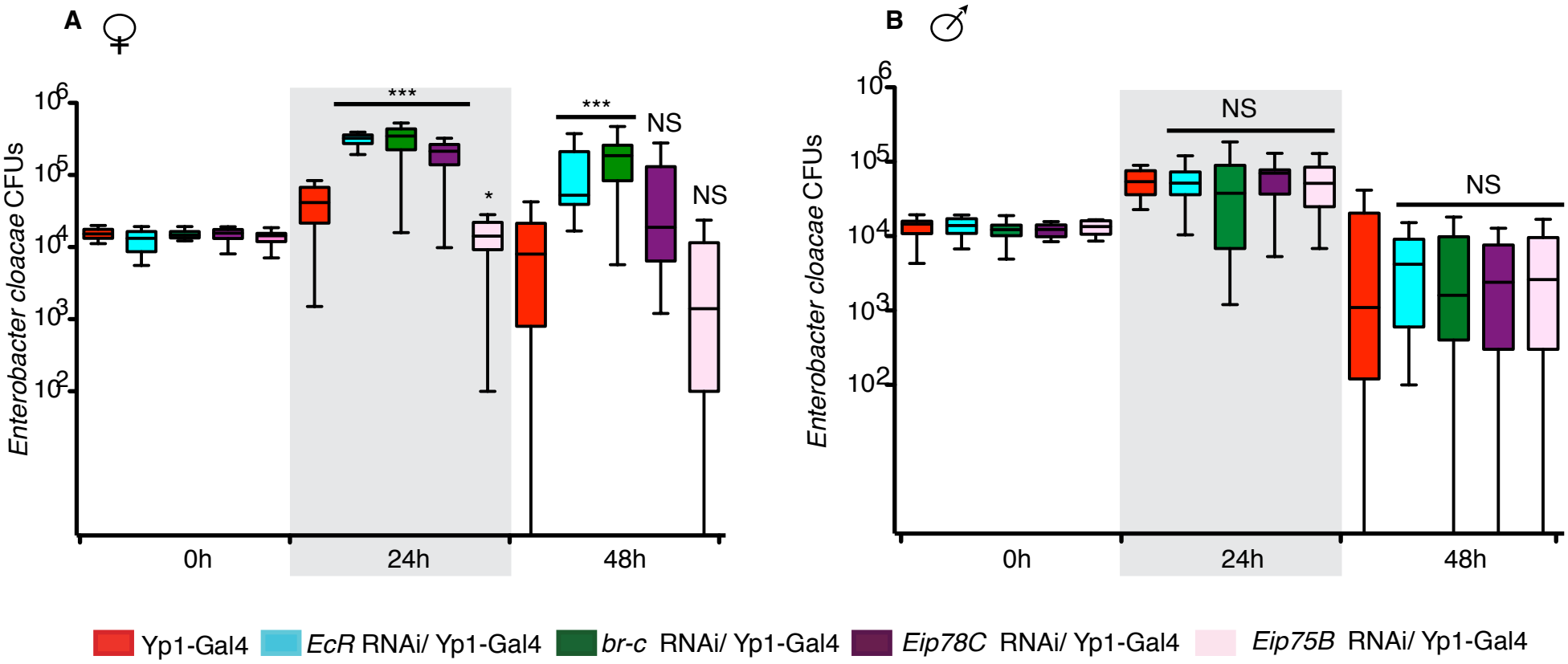

A
B
Ecc15 CFUs
0h
24h
48h
**
*NS
**
NS*
*
***
NS
NS
NS
Yp1-Gal4
EcR RNAi/ Yp1-Gal4
PGRP-LC RNAi/ Yp1-Gal4
br-c RNAi/ Yp1-Gal4
Eip78C RNAi/ Yp1-Gal4
Eip93F RNAi/ Yp1-Gal4
Eip74EF RNAi/ Yp1-Gal4
pnr RNAi/ Yp1-Gal4
srp RNAi/ Yp1-Gal4
Hr46 RNAi/Yp1-Gal4
Eip75B RNAi/ Yp1-Gal4

Figure S11


A
♀
B
♂
Enterobacter cloacae CFUs
10^6
10^5
10^4
10^3
10^2
***
***
*
NS
NS
NS
NS
0h
24h
48h
Yp1-Gal4
EcR RNAi/ Yp1-Gal4
br-c RNAi/ Yp1-Gal4
Eip78C RNAi/ Yp1-Gal4
Eip75B RNAi/ Yp1-Gal4