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Supplemental Information

Immune Receptor Signaling and the Mushroom Body Mediate Post-ingestion Pathogen Avoidance

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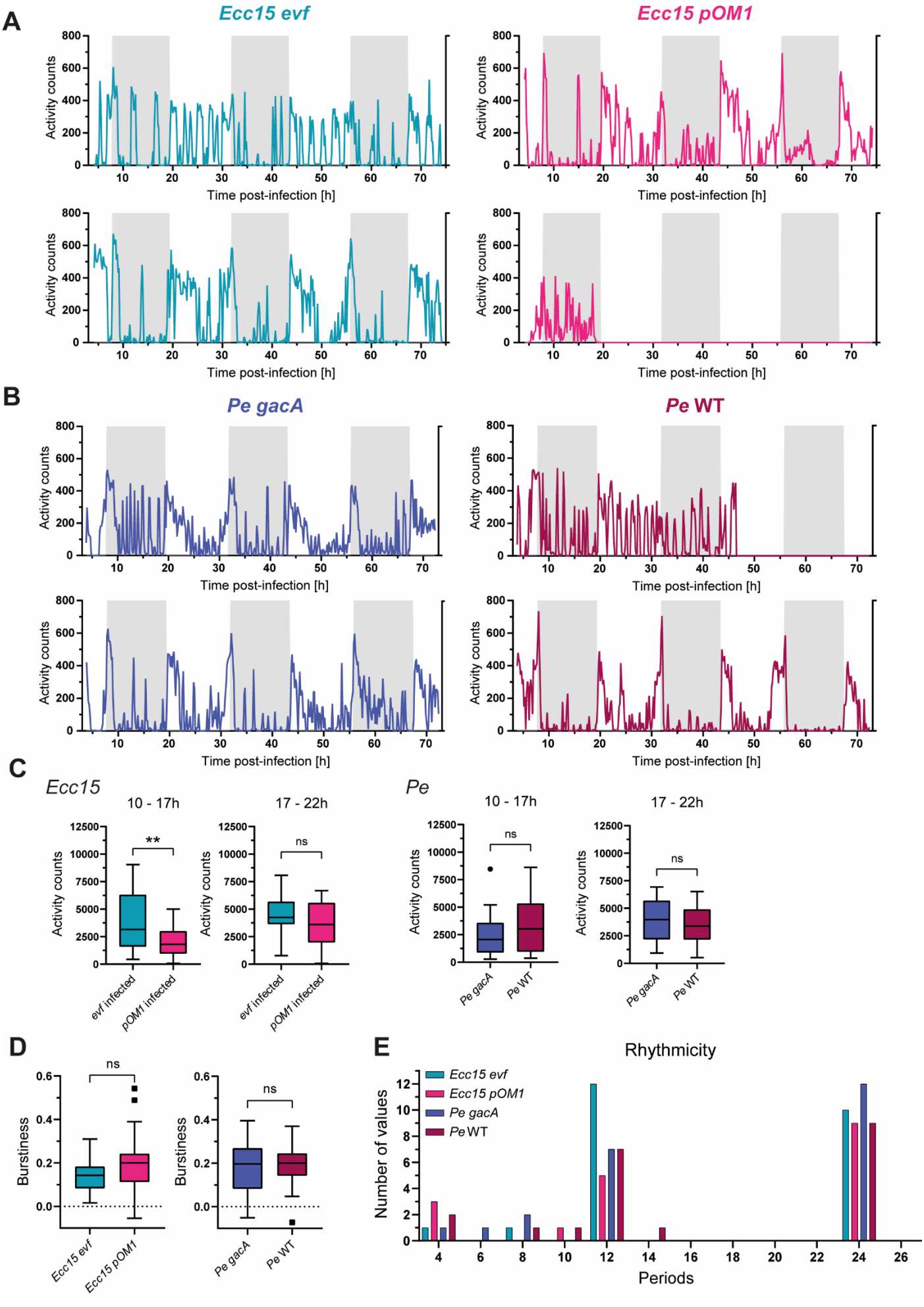
A
Ecc15 evf
Ecc15 pOM1
Activity counts
Time post-infection [h]
B
Pe gacA
Pe WT
C
Ecc15
Pe
10 - 17h
17 - 22h
**
ns
evf infected
pOM1 infected
Pe gacA
Pe WT
D
Burstiness
Ecc15 evf
Ecc15 pOM1
E
Rhythmicity
Number of values
Periods

Figure S1: Analysis of effect of pathogen ingestion on fly activity. Related to Figure 1.

(A) and **(B)** Activity counts of exemplary single flies after feeding with *Ecc15 evf* or *Ecc15 pOM1* as well as *Pe gacA* or *Pe* WT. **(C)** Total activity counts in the *Drosophila* activity monitor for two 5h-long periods after *Ecc15* or *Pe* feeding. *Ecc15 evf/pOM1*: n=24/21 (10-17h) and n=24/20 (17-22h); *Pe*: all n = 23. p-values calculated via unpaired t-test with Welch's correction. **(D)** Burstiness of locomotor activity patterns after bacteria feeding, n=24 (*Ecc15*), n=23 (*Pe*), p-values calculated via unpaired t-test with Welch's correction. **(E)** Circadian rhythms of infected flies. Chi-square test of independence showed no significant differences for periodicity of flies fed with different bacterial strains (p=0.8676).

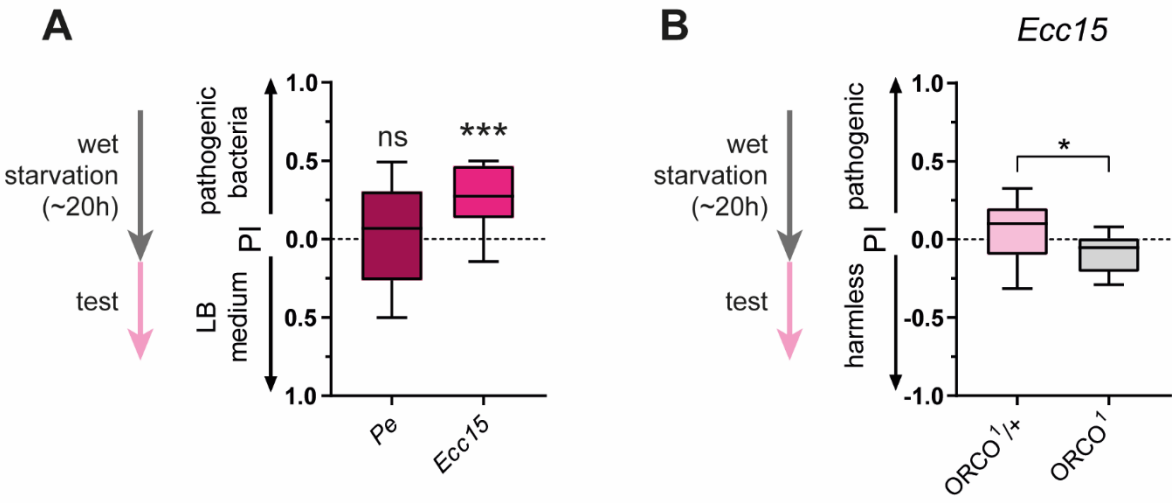

Figure S2: Olfactory choices of control and mutant flies in olfactory arena assay. Related to Figure 2.

(A) Preferences of starved, naïve OrR flies for the olfactory choice between pathogenic *Pe* WT or *Ecc15 pOM1* and LB medium, respectively. n=21 (*Pe*), n=16 (*Ecc15*). p-values calculated via one-sample t-test comparing to 0. **(B)** Preferences of heterozygous $ORCO^{1}$/+ and anosmic $ORCO^{1}$ null mutant flies for the olfactory choice between harmless *Ecc15 evf* and pathogenic *Ecc15 pOM1*. n=16, p-value calculated via unpaired t-test with Welch's correction.

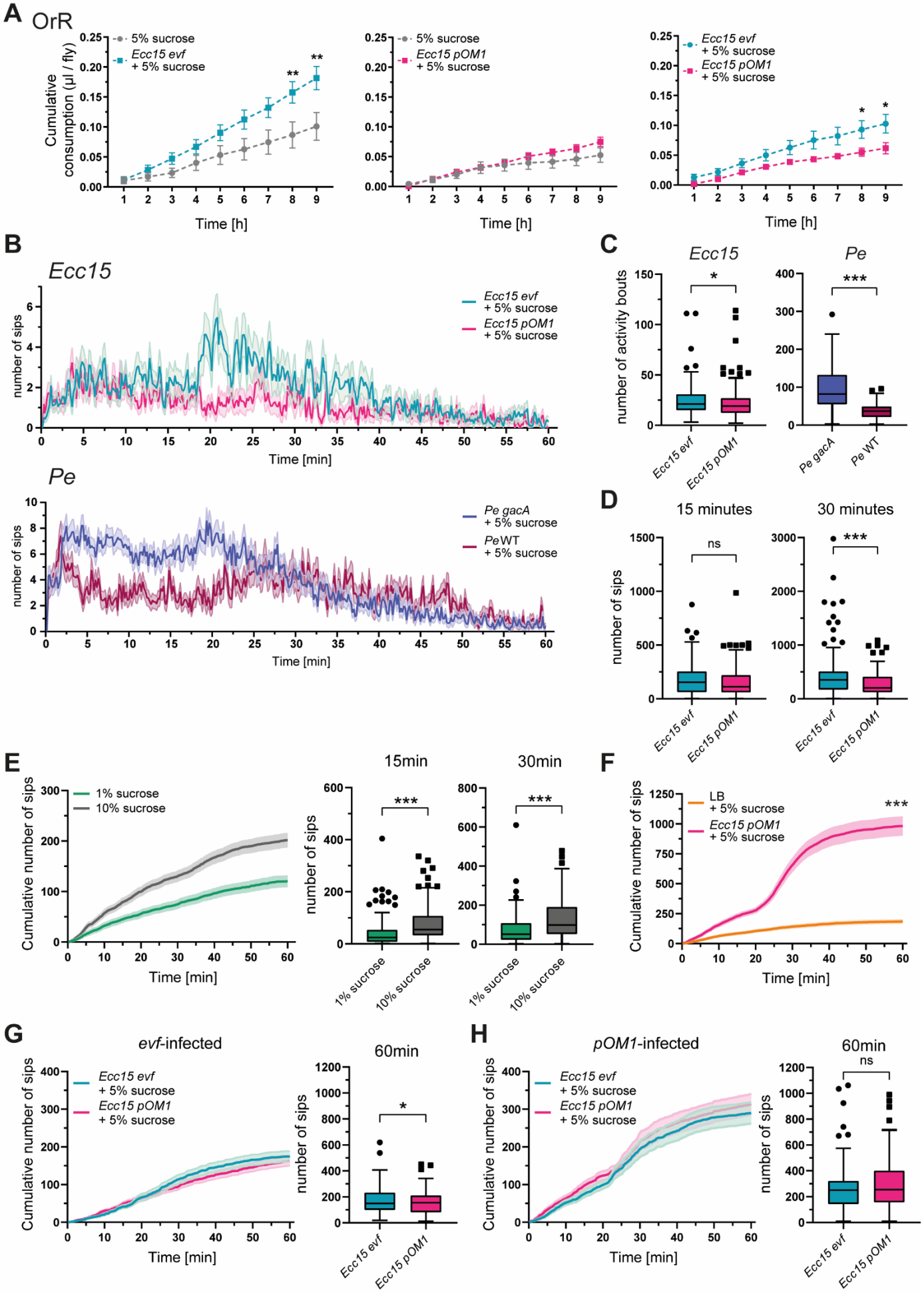
A
OrR
Cumulative consumption (µl / fly)
5% sucrose
Ecc15 evf + 5% sucrose
Ecc15 pOM1 + 5% sucrose
Time [h]
B
Ecc15
Pe
number of sips
Time [min]
Pe gacA + 5% sucrose
PeWT + 5% sucrose
C
number of activity bouts
Ecc15 evf
Ecc15 pOM1
Pe gacA
Pe WT
D
15 minutes
30 minutes
ns
E
1% sucrose
10% sucrose
Cumulative number of sips
15min
30min
F
LB + 5% sucrose
G
evf-infected
60min
H
pOM1-infected

Figure S3: Feeding induced changes in behavior to pathogenetic bacteria. Related to Figure 3.
(A) *Ecc15* feeding of wild-type OrR flies in the CAFE. Cumulative consumption in µl/fly for the feeding choices sucrose vs. harmless *Ecc15 evf* (n=19), sucrose vs. pathogenic *Ecc15 pOM1* (n=20) and *Ecc15 evf* vs. *Ecc15 pOM1* (n=20). p-values calculated via repeated-measures two-way ANOVA followed by Bonferroni's post hoc test for multiple comparisons. **(B-D)** Additional parameters for the bacterial feeding preferences of wild-type CS flies in the flyPAD shown in Figure 3F-I, n=132 (*Ecc15*), n=137 (*Pe*). **(B)** Non-cumulative number of sips for the feeding choice between harmless and pathogenic *Ecc15* or *Pe*, respectively. **(C)** Number of activity bouts, p-value calculated via the Wilcoxon matched-pairs signed rank test. **(D)** Number of sips 15 and 30 minutes after feeding onset for the feeding choice between harmless *Ecc15 evf* and pathogenic *Ecc15 pOM1*. **(E)** Cumulative feeding of wild-type CS flies and number of sips 15 and 30 minutes after feeding onset for the choice between 1% and 10% sucrose in the flyPAD, n=126. **(F)** Cumulative feeding of wild-type CS flies for the choice between pathogenic *Ecc15 pOM1* and LB medium in the flyPAD, n=133. **(G), (H)** Feeding preferences of wild-type CS flies for the choice between harmless *Ecc15 evf* and pathogenic *Ecc15 pOM1* in the flyPAD following overnight feeding on *Ecc15 evf* (G) or *Ecc15 pOM1* (H). Cumulative feeding and total number of sips after 60 minutes, n=65. **(D-H)** p-values calculated by comparing feeding ratios to 1 via the Wilcoxon signed rank test.

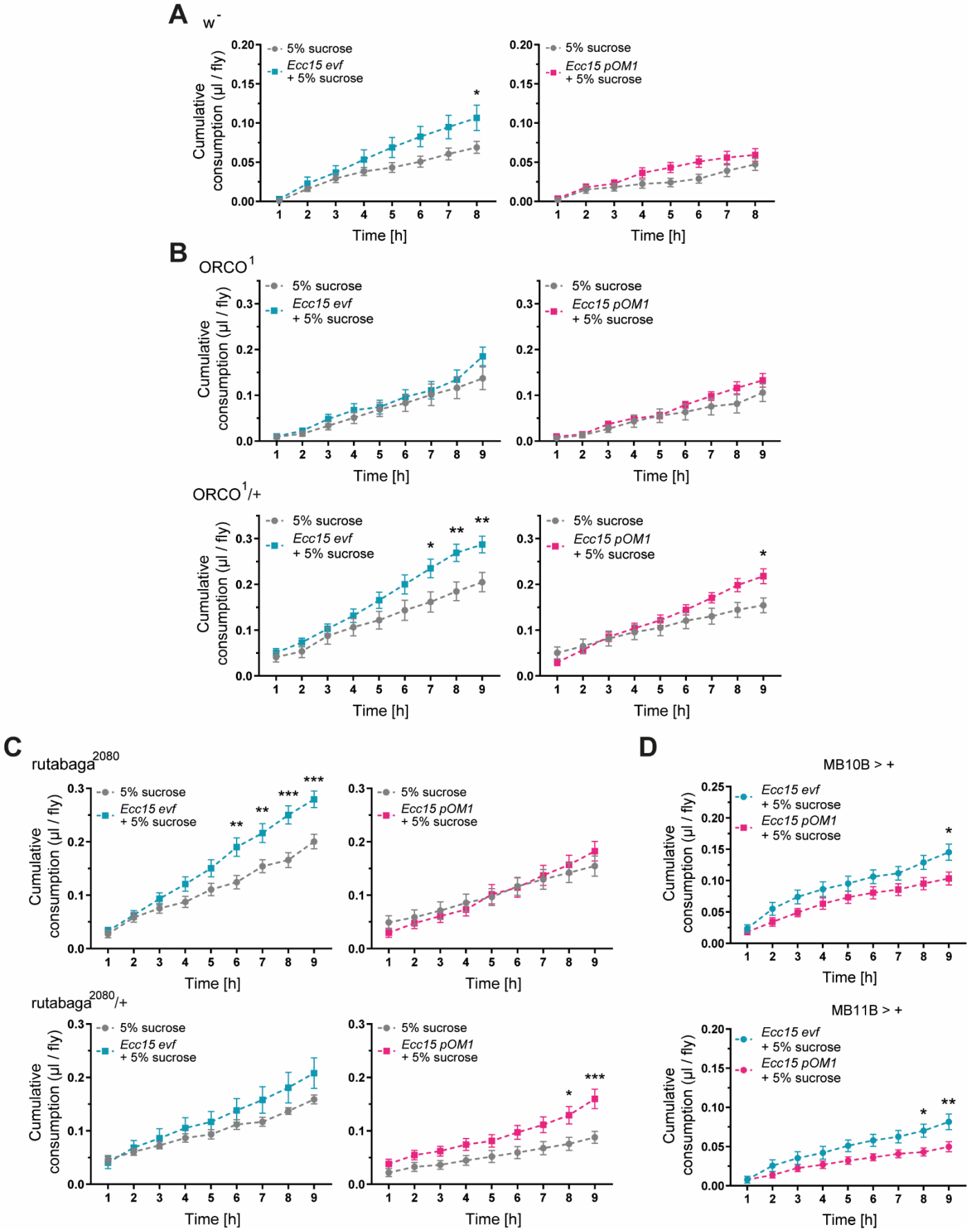
A
w-
5% sucrose
Ecc15 evf
+ 5% sucrose
Ecc15 pOM1
+ 5% sucrose
Cumulative consumption (µl / fly)
Time [h]
B
ORCO1
ORCO1/+
C
rutabaga2080
rutabaga2080/+
D
MB10B > +
MB11B > +

Figure S4: The mushroom body is involved in adaptive post-ingestion behavior to pathogenic bacteria. Related to Figure 4.

(A) Feeding preferences of control w^- flies for the choice between sucrose and harmless *Ecc15 evf* (n=22) as well as between sucrose and pathogenic *Ecc15 pOM1* (n=23), (experiment at 30°C). **(B)** Feeding preferences of anosmic $ORCO^1$ and heterozygous $ORCO^1$/+ control flies for the choices sucrose vs. *Ecc15 evf* and sucrose vs. *Ecc15 pOM1* (all n=17). **(C)** Preferences of $rutabaga^{2080}$ learning mutant flies (n=17) and of heterozygous $rutabaga^{2080}$/+ control flies (n=16/17) for the feeding choice between sucrose and harmless *Ecc15 evf* and between sucrose and pathogenic *Ecc15 pOM1*. **(D)** GAL4-driver controls for MB10B and MB11B Split-GAL4 lines. Feeding preferences of MB10B > + (n=21) and MB11B > + (n=19) flies for the choice between harmless *Ecc15 evf* and pathogenic *Ecc15 pOM1*. **(A-D)** cumulative consumption in µl/fly in the CAFE, p-values calculated via repeated-measures two-way ANOVA followed by Bonferroni's post hoc test for multiple comparisons.

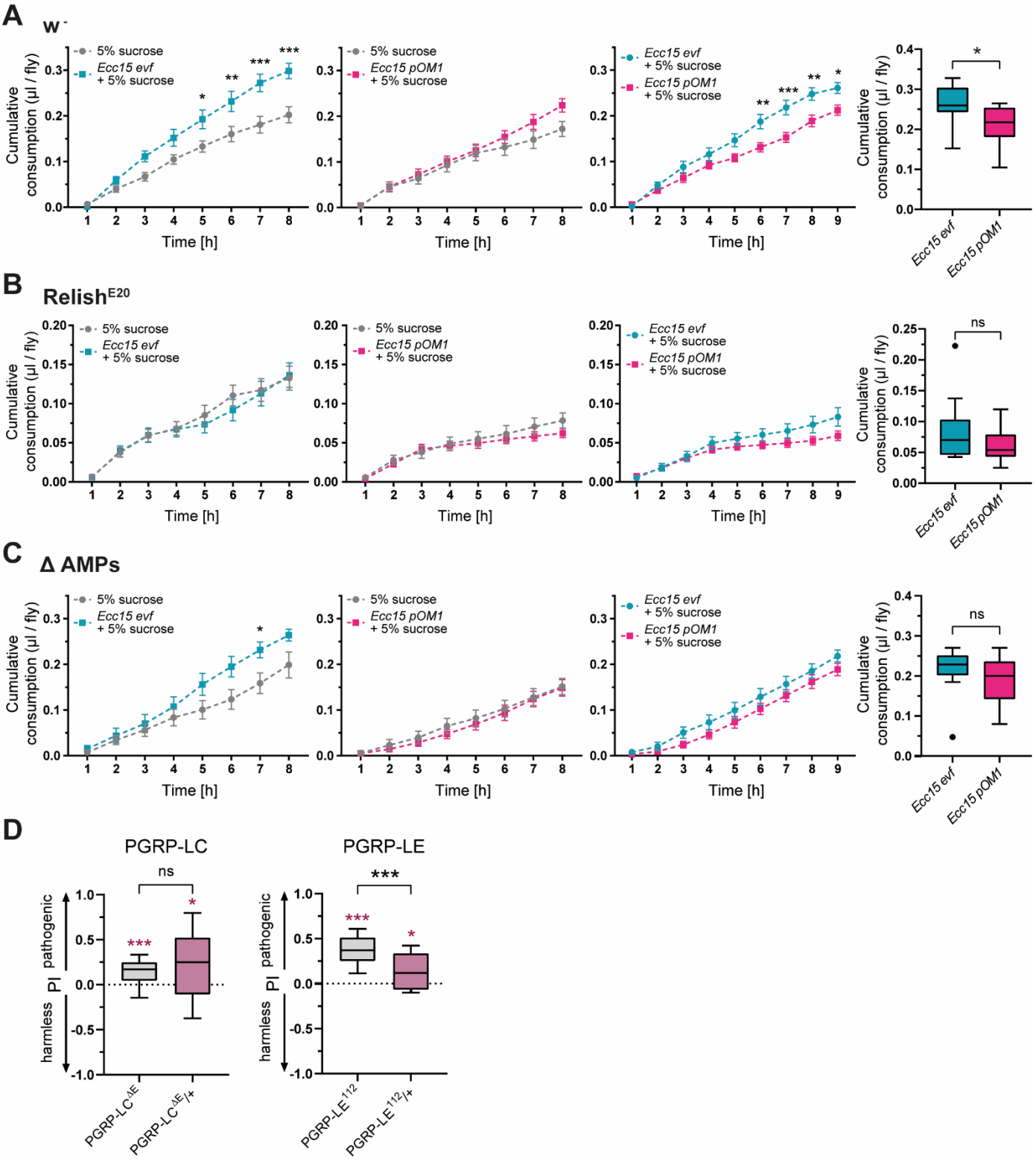
A
w-
5% sucrose
Ecc15 evf + 5% sucrose
Ecc15 pOM1 + 5% sucrose
Cumulative consumption (µl / fly)
Time [h]
Ecc15 evf
Ecc15 pOM1
B
Relish^E20
C
Δ AMPs
ns
D
PGRP-LC
PGRP-LE
pathogenic
harmless
PI
PGRP-LC^ΔE
PGRP-LC^ΔE/+
PGRP-LE^112
PGRP-LE^112/+

Figure S5. Analysis of immune signalling components in feeding assay. Related to Figure 5.
(A) Feeding preferences of isogenized control w^- flies for the choices sucrose vs. harmless *Ecc15 evf* (n=15), sucrose vs. pathogenic *Ecc15 pOM1* (n=14) and *Ecc15 evf* vs. *Ecc15 pOM1* (n=16). **(B)** Feeding preferences of flies lacking the NF-κB transcription factor Relish for the choices sucrose vs. *Ecc15 evf*, sucrose vs. *Ecc15 pOM1* and *Ecc15 evf* vs. *Ecc15 pOM1* (all n=16). **(C)** Feeding preferences of flies deficient for all AMPs except for cecropins for the choice between sucrose and *Ecc15 evf*, sucrose and *Ecc15 pOM1*, and between *Ecc15 evf* and *Ecc15 pOM1* (all n=16). **(D)** Preferences of starved, naïve PGRP-LC$^{\Delta E}$ and PGRP-LE^{112} mutant flies as well as of corresponding heterozygous controls for the odor of pathogenic *Ecc15 pOM1* over the odor of harmless *Ecc15 evf* in the 4-field arena (all n=16). p-values calculated via one-sample t-test comparing to 0 are indicated in red; p-values for group comparisons calculated via unpaired t-test with Welch's correction are indicated in black. **(A-C)** cumulative consumption in µl/fly in the CAFE and box plots of total consumption at the end of the experiment (9h). p-values calculated via repeated-measures two-way ANOVA followed by Bonferroni's post hoc test for multiple comparisons.

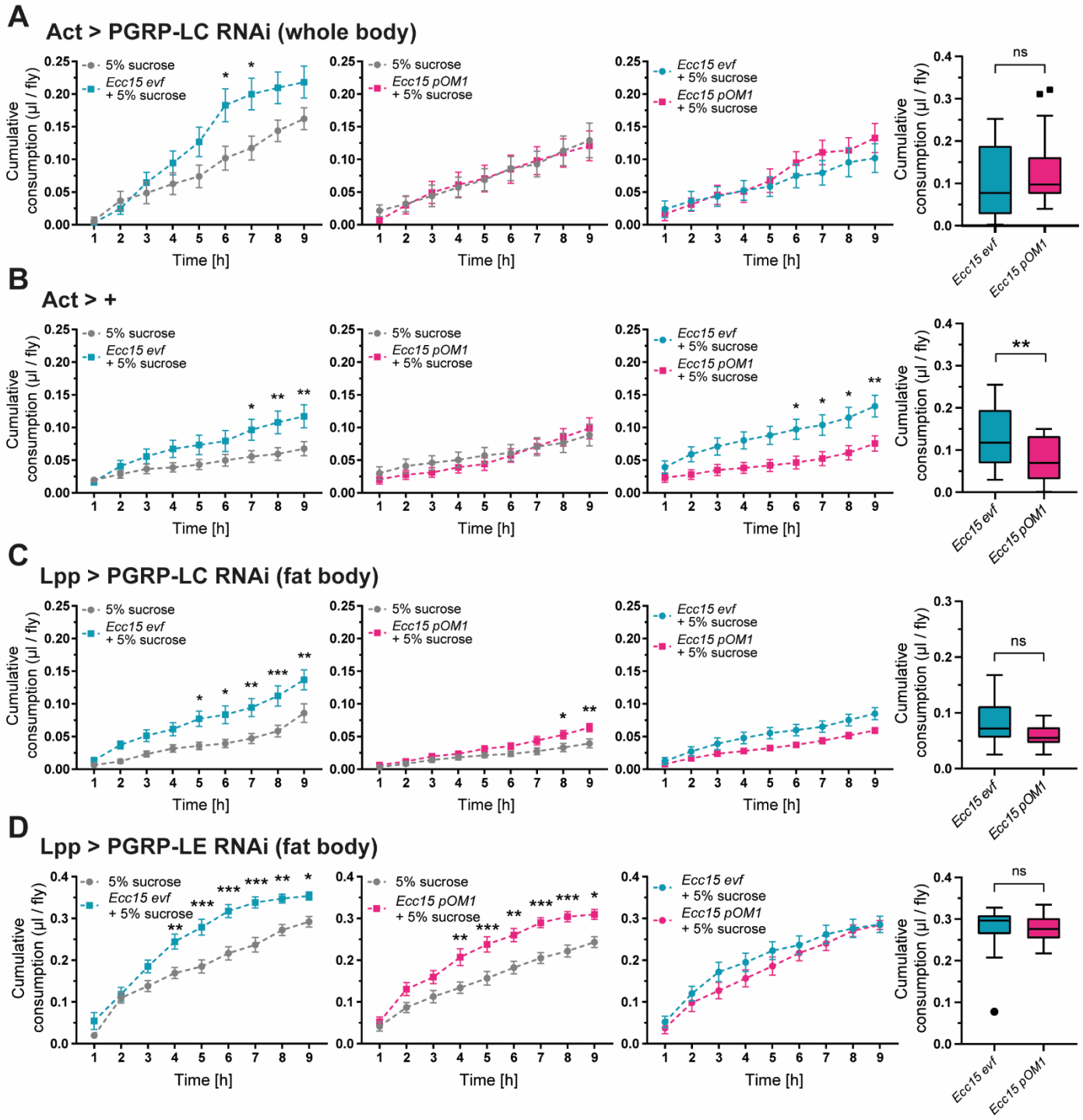
A
Act > PGRP-LC RNAi (whole body)
B
Act > +
C
Lpp > PGRP-LC RNAi (fat body)
D
Lpp > PGRP-LE RNAi (fat body)
5% sucrose
Ecc15 evf + 5% sucrose
Ecc15 pOM1 + 5% sucrose
Cumulative consumption (µl / fly)
Time [h]
ns
Ecc15 evf
Ecc15 pOM1

Figure S6: Imd signaling components are involved in pathogen feeding choice. Related to Figure 6. **(A)** Feeding preferences of Act > PGRP-LC^{RNAi} flies that similarly to PGRP-LC$^{\Delta E}$ mutant flies lack PGRP-LC in the whole body for the choice between sucrose and harmless *Ecc15 evf*, sucrose and pathogenic *Ecc15 pOM1* or between harmless and pathogenic *Ecc15* (all n=16). **(B)** Feeding preferences of Act > + control flies for the choices sucrose vs. *Ecc15 evf* (n=19), sucrose vs. *Ecc15 pOM1* (n=20) and *Ecc15 evf* vs. *Ecc15 pOM1* (n=20). **(C)** Feeding preferences upon downregulation of PGRP-LC specifically in the fat body using Lpp > PGRP-LC^{RNAi} flies for the choices sucrose vs. *Ecc15 evf* (n=20), sucrose vs. *Ecc15 pOM1* (n=19) and *Ecc15 evf* vs. *Ecc15 pOM1* (n=20). **(D)** Preferences of Lpp > PGRP-LE^{RNAi} flies that lack PGRP-LE specifically in the fat body for the feeding choices between sucrose and *Ecc15 evf* (n=14), sucrose and *Ecc15 pOM1* (n=17) and between *Ecc15 evf* and *Ecc15 pOM1* (n=16). **(A-D)** cumulative consumption in µl/fly in the CAFE and box plots of total consumption at the end of the experiment (9h). p-values calculated via repeated-measures two-way ANOVA followed by Bonferroni's post hoc test for multiple comparisons.

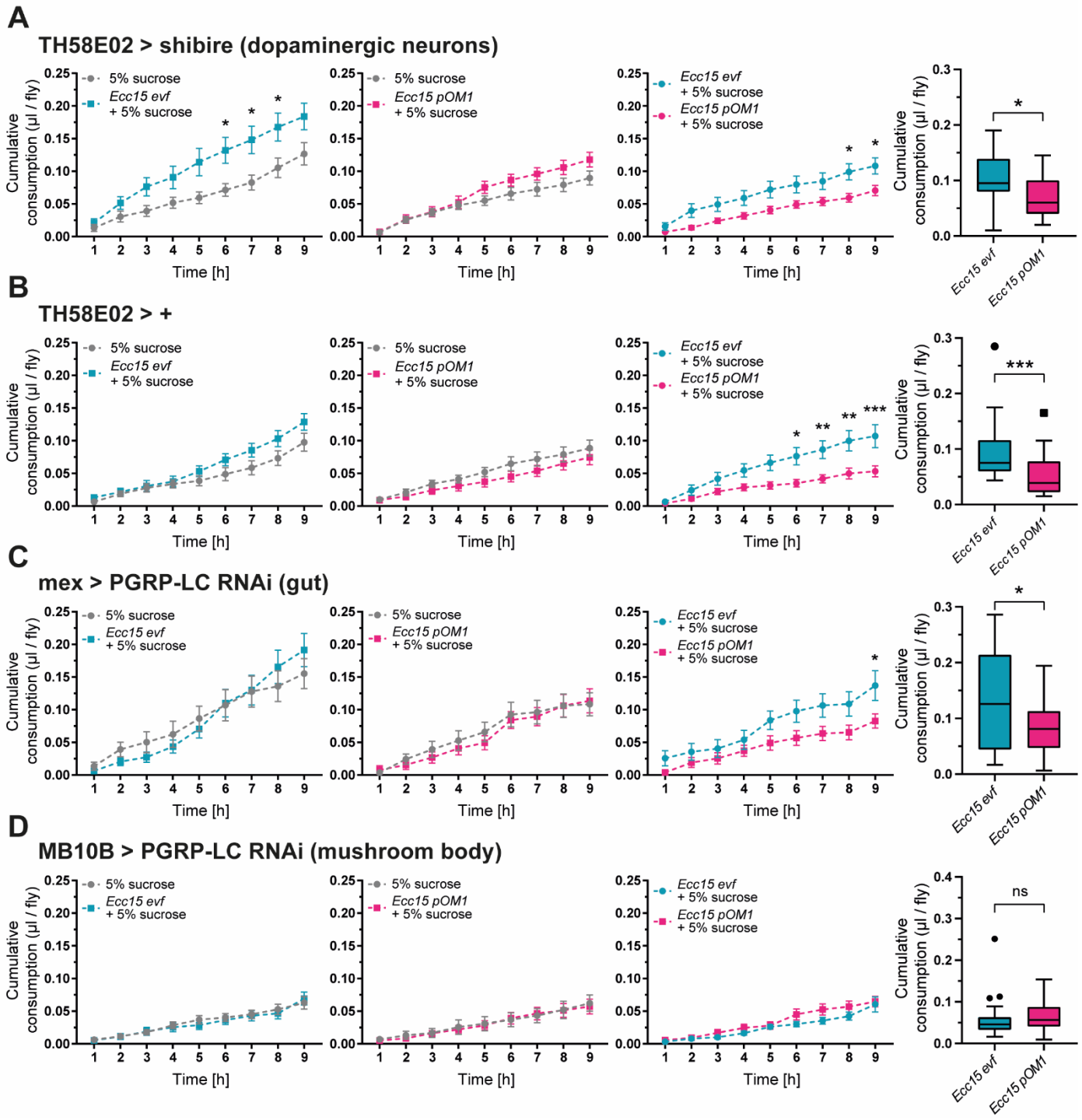
A
TH58E02 > shibire (dopaminergic neurons)
B
TH58E02 > +
C
mex > PGRP-LC RNAi (gut)
D
MB10B > PGRP-LC RNAi (mushroom body)
5% sucrose
Ecc15 evf
+ 5% sucrose
Ecc15 pOM1
+ 5% sucrose
Cumulative consumption (µl / fly)
Time [h]
ns

Figure S7: Dopamine is not essential for pathogenic bacteria feeding suppression. Related to Figure 7.

(A) Preferences for the feeding choice between sucrose and harmless *Ecc15 evf* (n=21), sucrose and pathogenic *Ecc15 pOM1* (n=21) and between *Ecc15 evf* and *Ecc15 pOM1* (n=23) upon inactivation of broad clusters of dopaminergic neurons using TH58E02 > shibire^{ts1} flies. **(B)** Feeding preferences of TH58E02 > + control flies for the choices sucrose vs. *Ecc15 evf* (n=22), sucrose vs. *Ecc15 pOM1* (n=21) and *Ecc15 evf* vs. *Ecc15 pOM1* (n=21). **(C)** Feeding preferences of mex > PGRP-LC^{RNAi} flies that lack PGRP-LC specifically in midgut enterocytes for the choices between sucrose and harmless *Ecc15 evf*, sucrose and pathogenic *Ecc15 pOM1* and between *Ecc15 evf* and *Ecc15 pOM1* (all n=16). **(D)** Feeding preferences of MB10B > PGRP-LC^{RNAi} flies that are deficient for PGRP-LC in all KCs of the MB for the choices sucrose vs. *Ecc15 evf* (n=18), sucrose vs. *Ecc15 pOM1* (n=16) and *Ecc15 evf* vs. *Ecc15 pOM1* (n=20). However, note the overall low consumption. **(A-D**) cumulative consumption in µl/fly in the CAFE and box plots of total consumption at the end of the experiment (9h). p-values calculated via repeated-measures two-way ANOVA followed by Bonferroni's post hoc test for multiple comparisons.