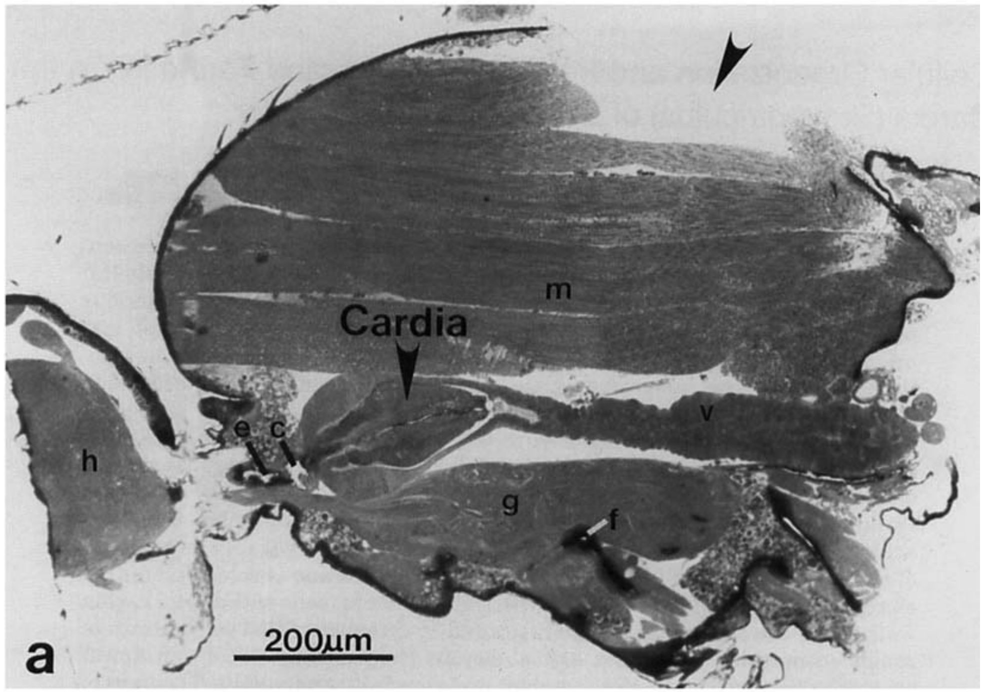
Immunity of Invertebrates, 2014

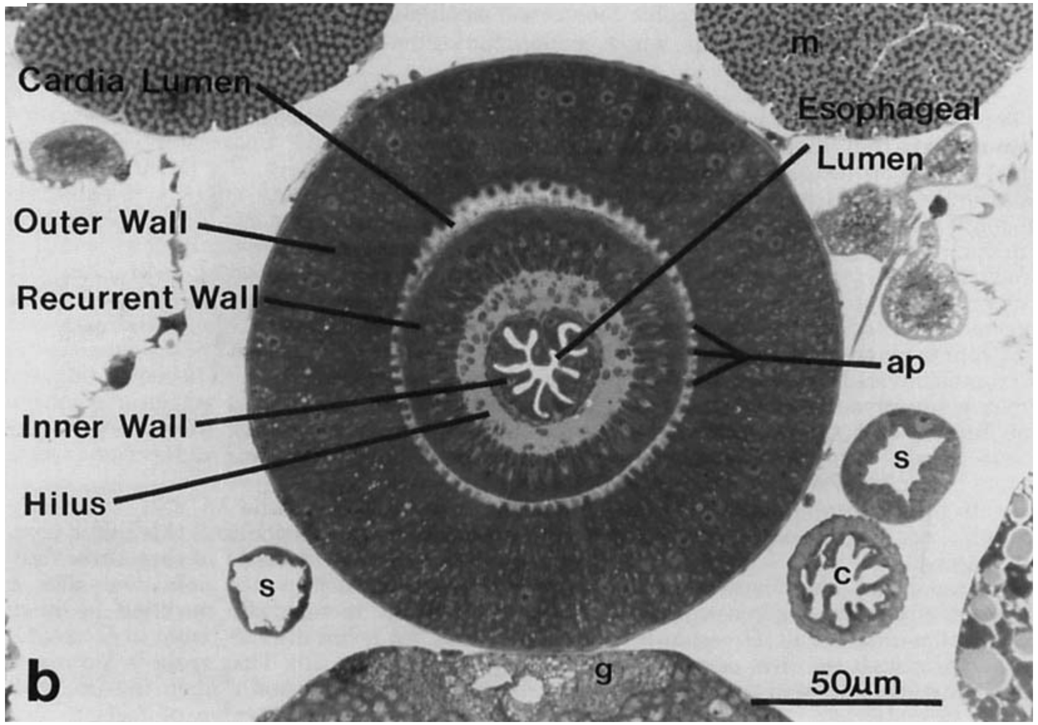
3. Drosophila: the gut battle

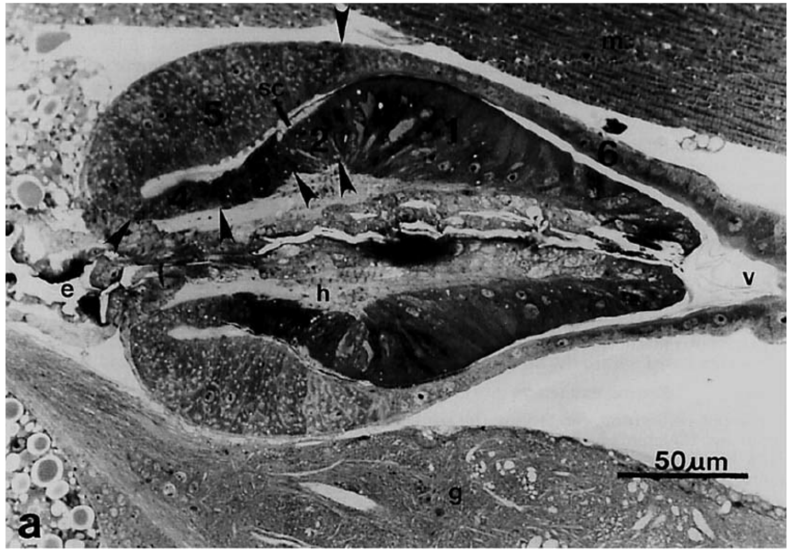
Cellular Organization and Peritrophic Membrane Formation in the Cardia (Proventriculus) of *Drosophila melanogaster*

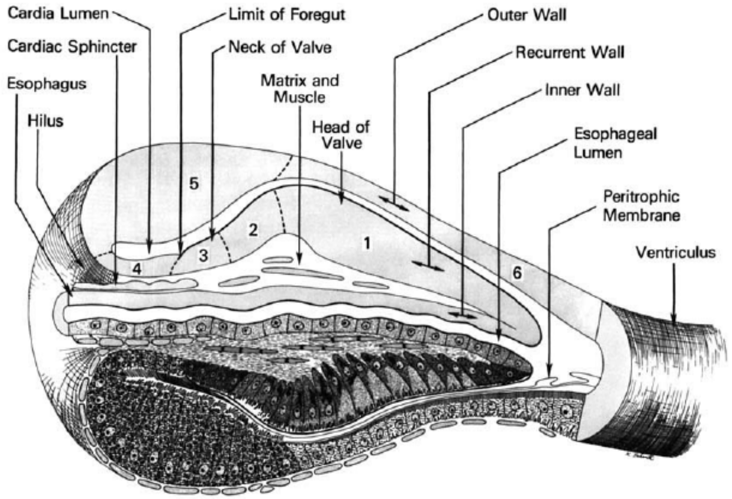
JOURNAL OF MORPHOLOGY 196:253–282 (1988)

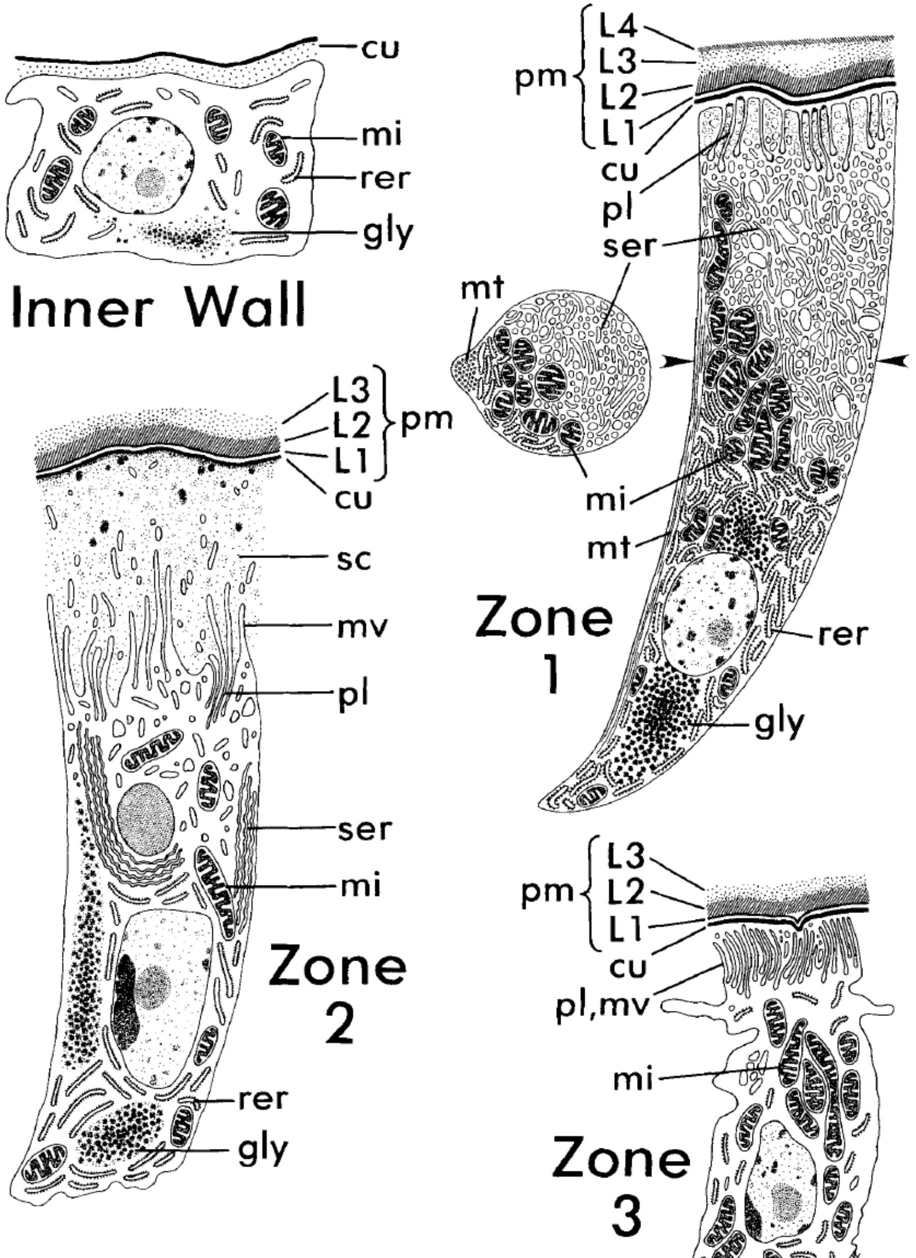
DAVID G. KING



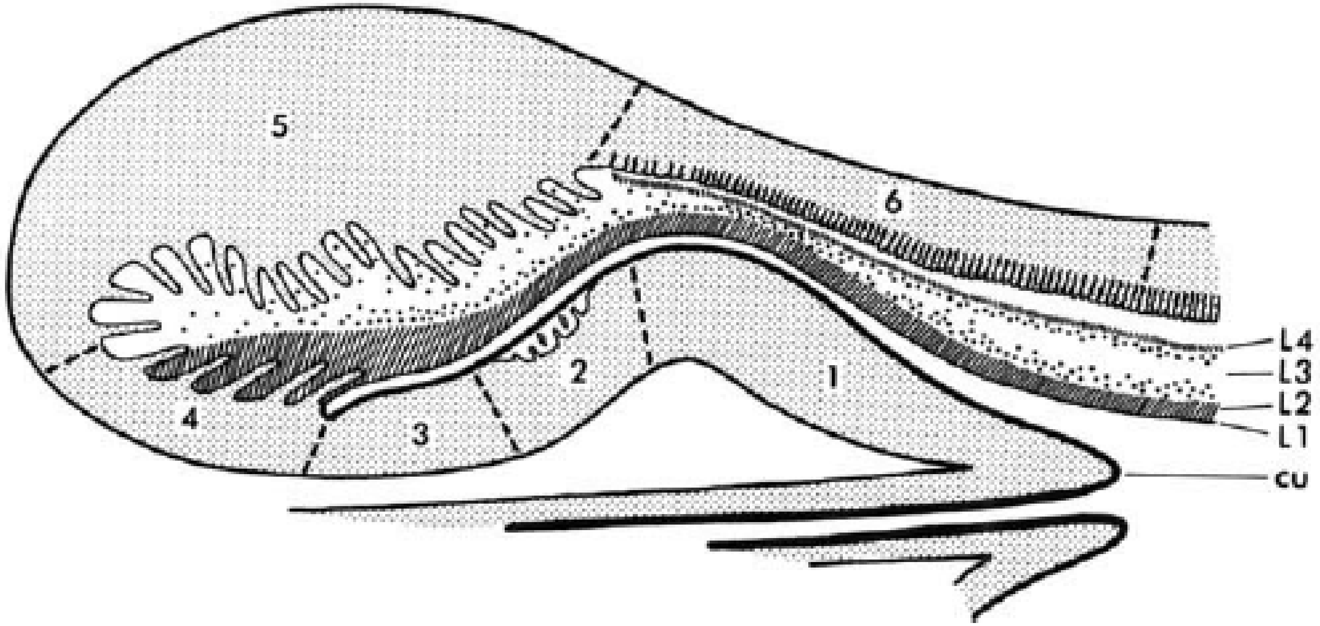


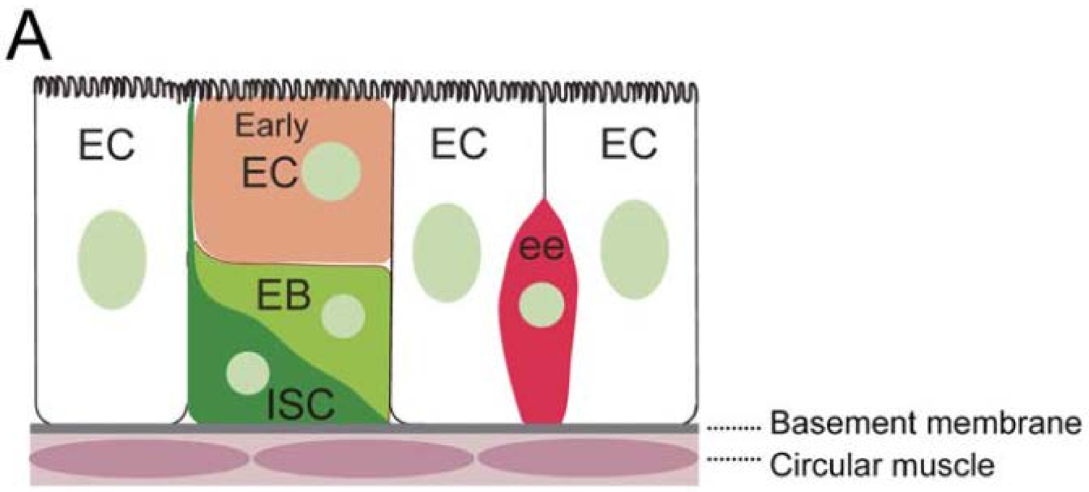


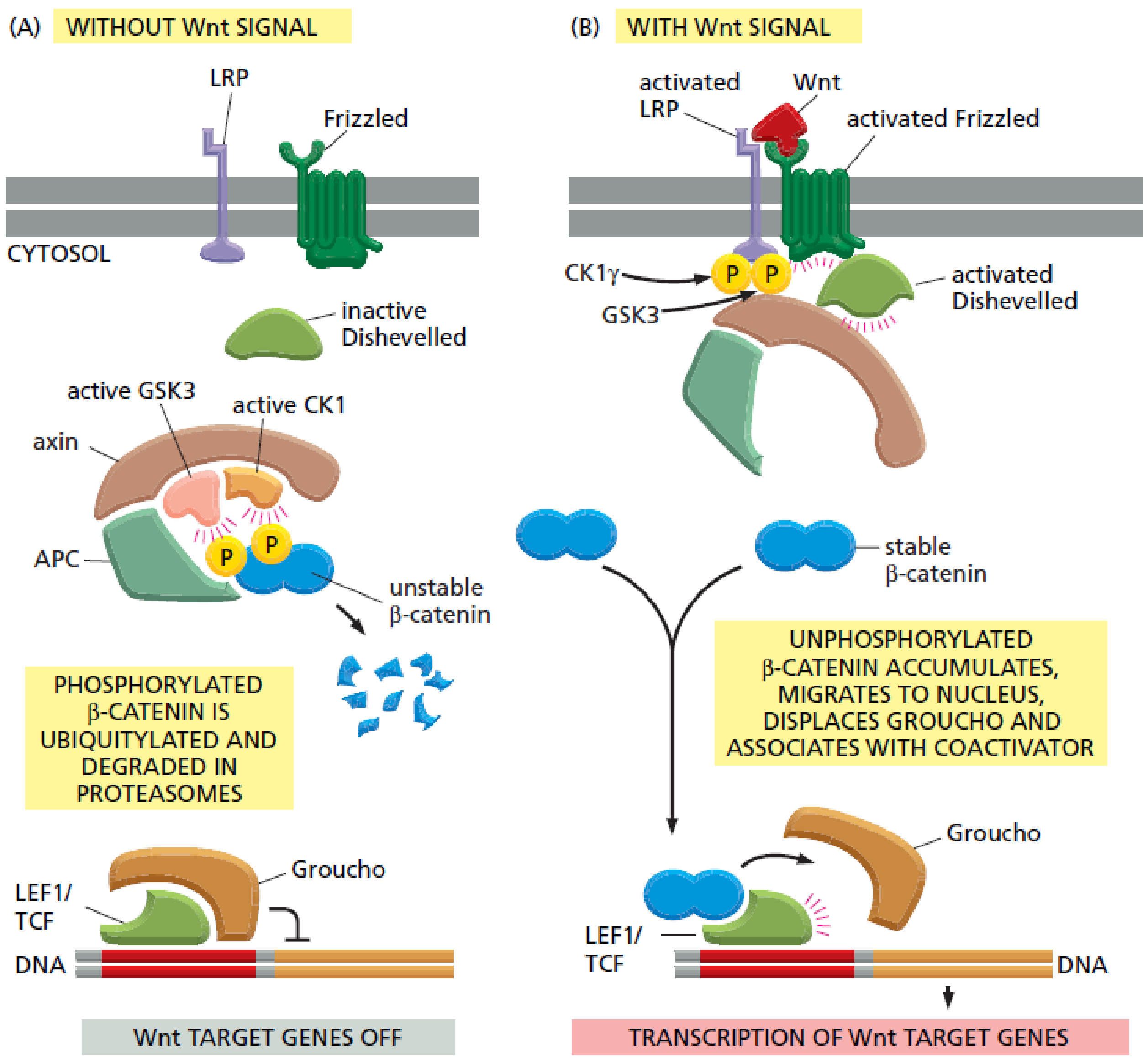




Foregut Cells



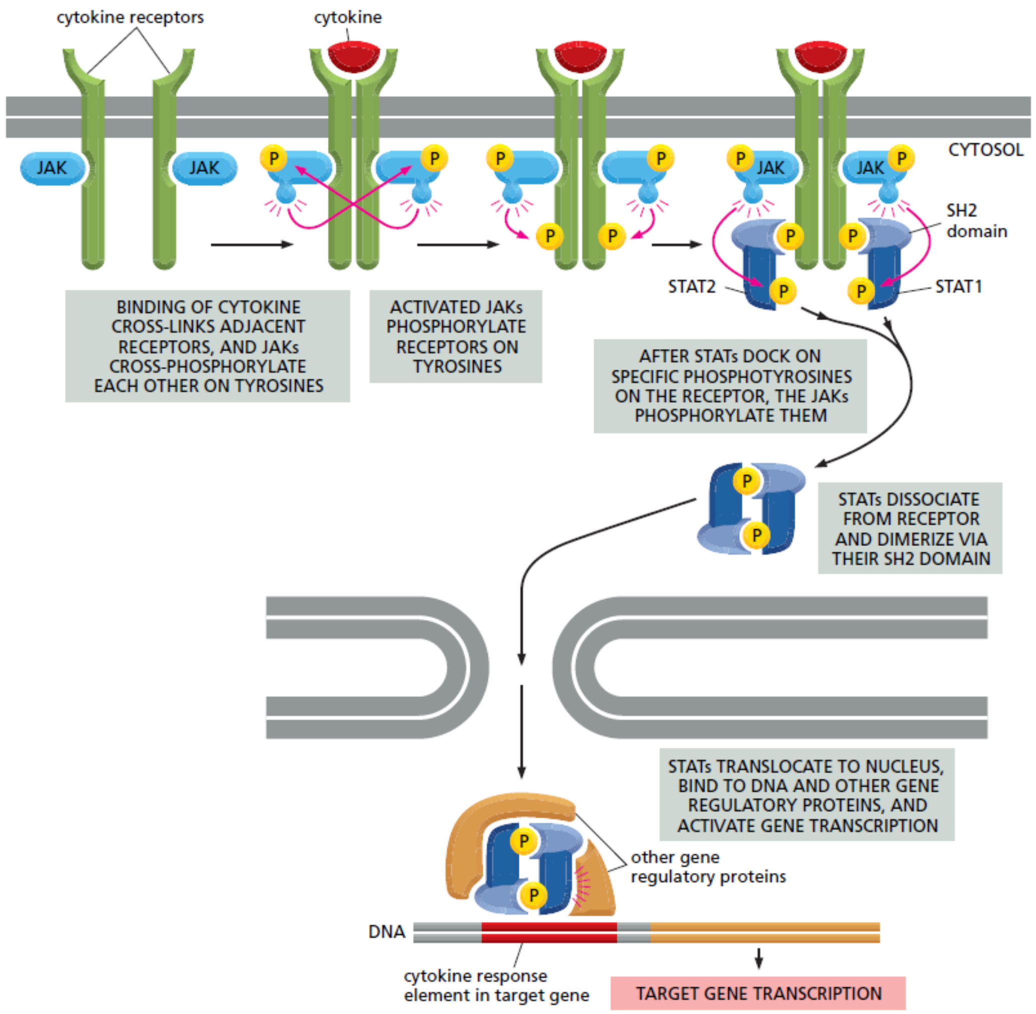

A
EC
Early
EC
EC
EC
EB
ee
ISC
Basement membrane
Circular muscle


(A) WITHOUT Wnt SIGNAL
LRP
Frizzled
CYTOSOL
inactive
Dishevelled
active GSK3
active CK1
axin
APC
P
P
unstable
β-catenin
PHOSPHORYLATED
β-CATENIN IS
UBIQUITYLATED AND
DEGRADED IN
PROTEASOMES
LEF1/
TCF
Groucho
DNA
Wnt TARGET GENES OFF
(B) WITH Wnt SIGNAL
activated
LRP
Wnt
activated Frizzled
CK1γ
GSK3
P
P
activated
Dishevelled
stable
β-catenin
UNPHOSPHORYLATED
β-CATENIN ACCUMULATES,
MIGRATES TO NUCLEUS,
DISPLACES GROUCHO AND
ASSOCIATES WITH COACTIVATOR
Groucho
LEF1/
TCF
DNA
TRANSCRIPTION OF Wnt TARGET GENES

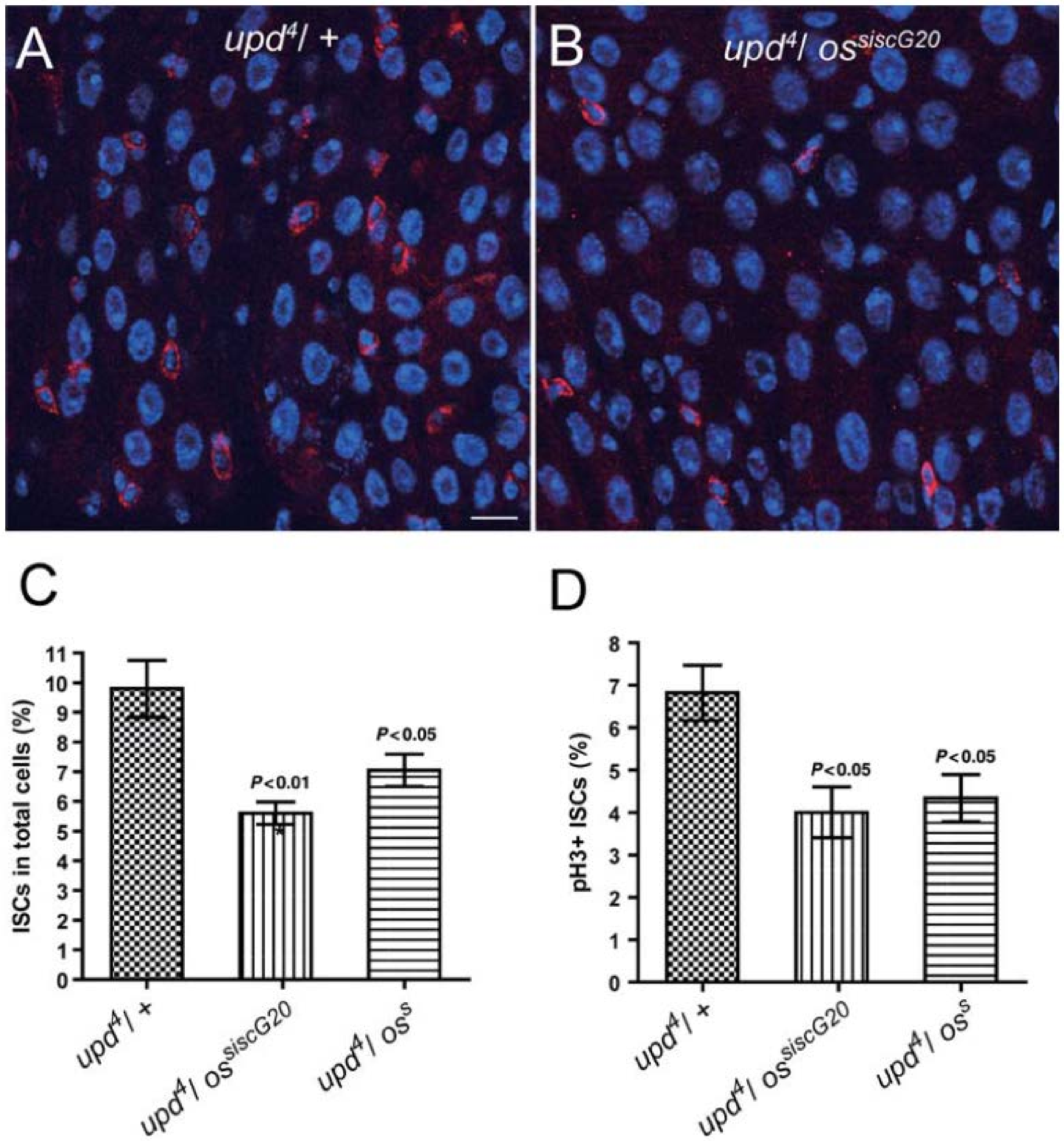
Paracrine Unpaired Signaling through the JAK/STAT Pathway Controls Self-renewal and Lineage Differentiation of *Drosophila* Intestinal Stem Cells

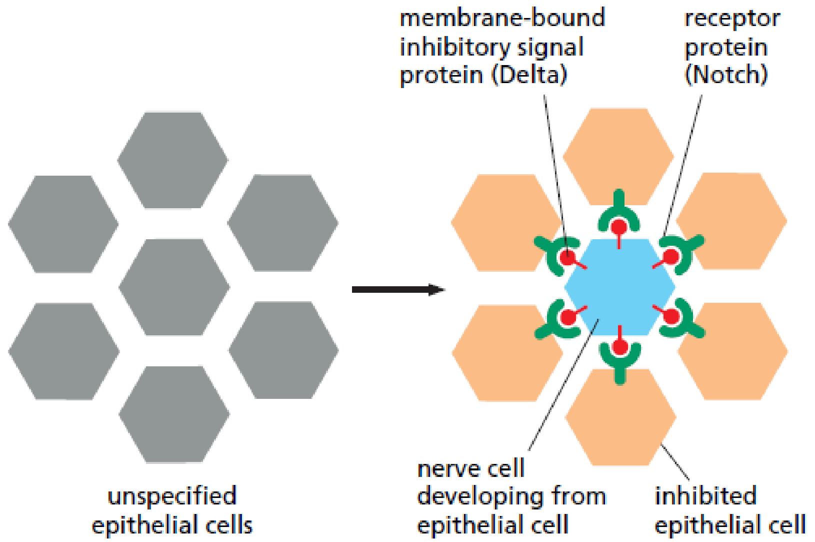
Guonan Lin[1,†], Na Xu[1,2,†], and Rongwen Xi[1,*]

Journal of Molecular Cell Biology (2009), 1–13

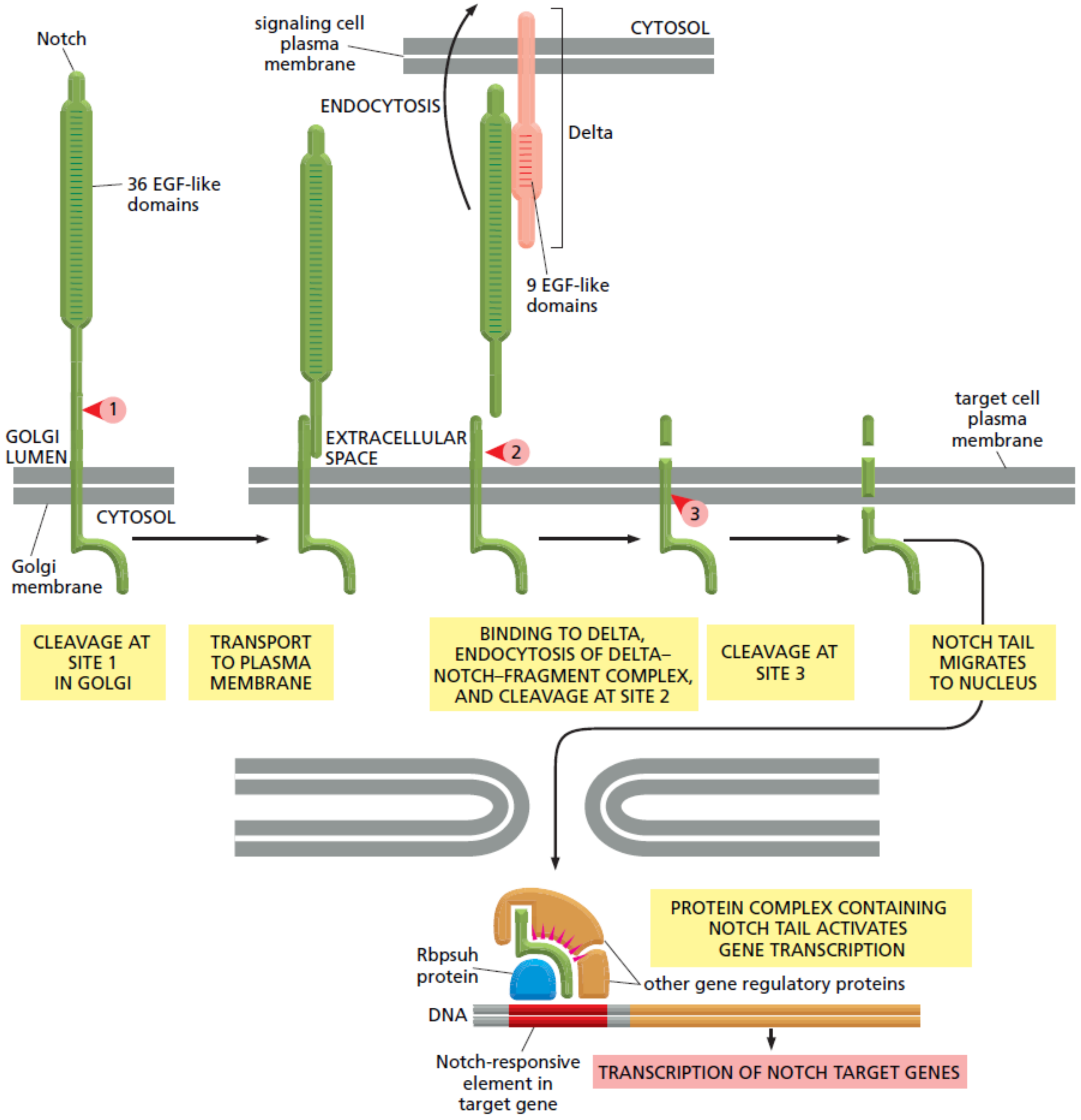


intestines stained with anti-Dl antibody (red) and DAPI (blue)

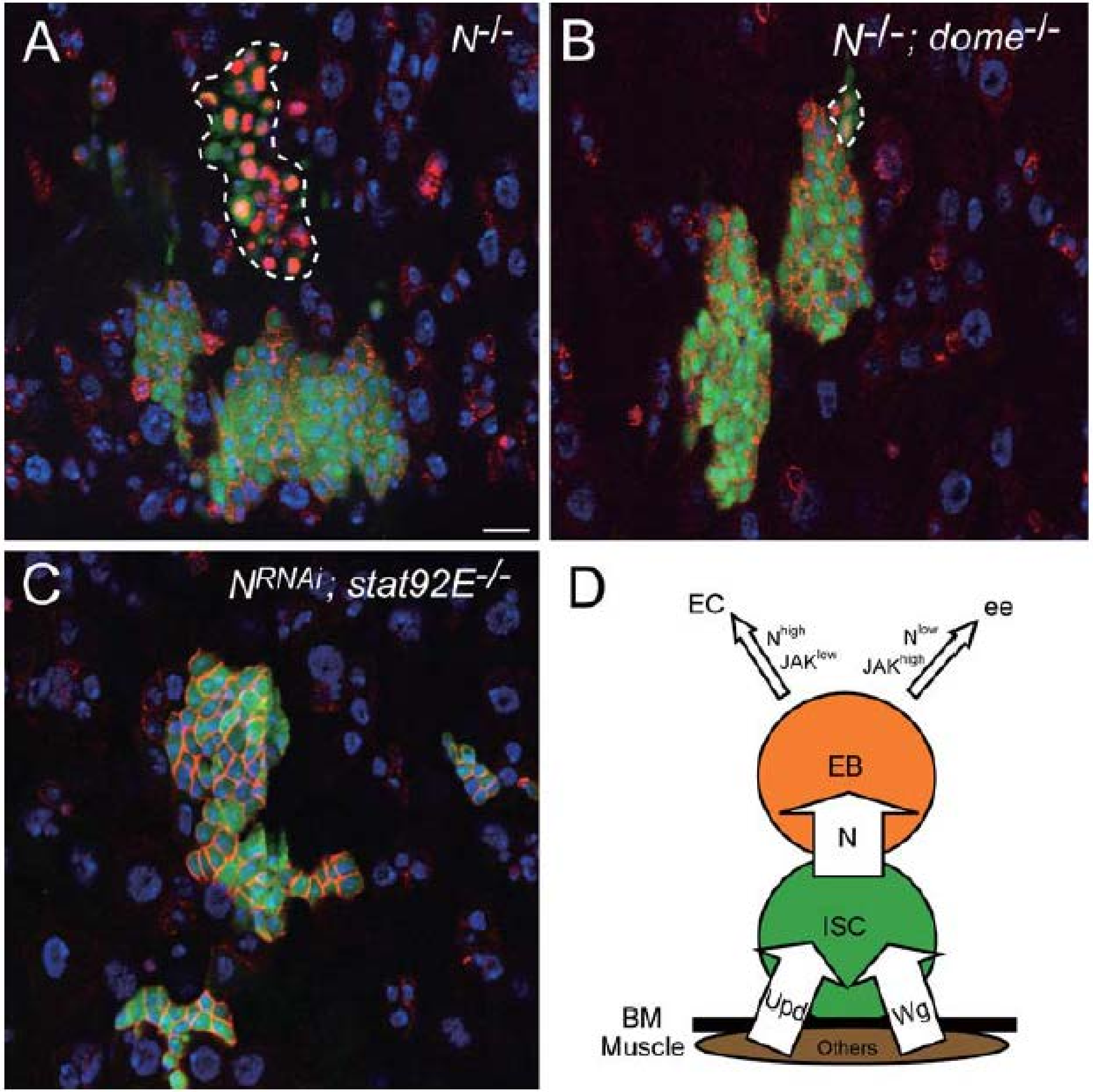




membrane-bound inhibitory signal protein (Delta)
receptor protein (Notch)
unspecified epithelial cells
nerve cell developing from epithelial cell
inhibited epithelial cell



Notch
36 EGF-like domains
signaling cell plasma membrane
CYTOSOL
ENDOCYTOSIS
Delta
9 EGF-like domains
1
2
3
GOLGI LUMEN
CYTOSOL
Golgi membrane
EXTRACELLULAR SPACE
target cell plasma membrane
CLEAVAGE AT SITE 1 IN GOLGI
TRANSPORT TO PLASMA MEMBRANE
BINDING TO DELTA, ENDOCYTOSIS OF DELTA–NOTCH–FRAGMENT COMPLEX, AND CLEAVAGE AT SITE 2
CLEAVAGE AT SITE 3
NOTCH TAIL MIGRATES TO NUCLEUS
PROTEIN COMPLEX CONTAINING NOTCH TAIL ACTIVATES GENE TRANSCRIPTION
Rbpsuh protein
other gene regulatory proteins
DNA
Notch-responsive element in target gene
TRANSCRIPTION OF NOTCH TARGET GENES



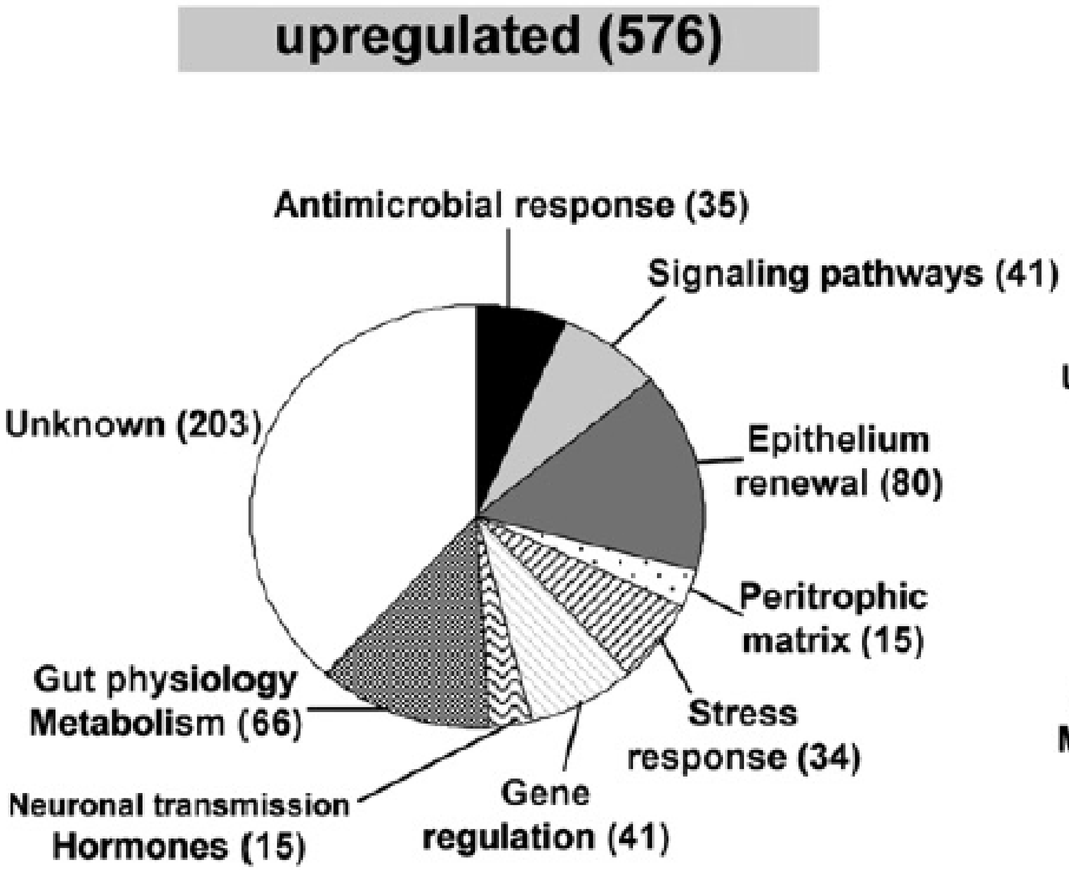
A
$N^{-/-}$
B
$N^{-/-}; dome^{-/-}$
C
$N^{RNAi}; stat92E^{-/-}$
D
EC
N^{high}
JAK^{low}
N^{low}
JAK^{high}
ee
EB
N
ISC
Upd
Wg
BM
Muscle
Others

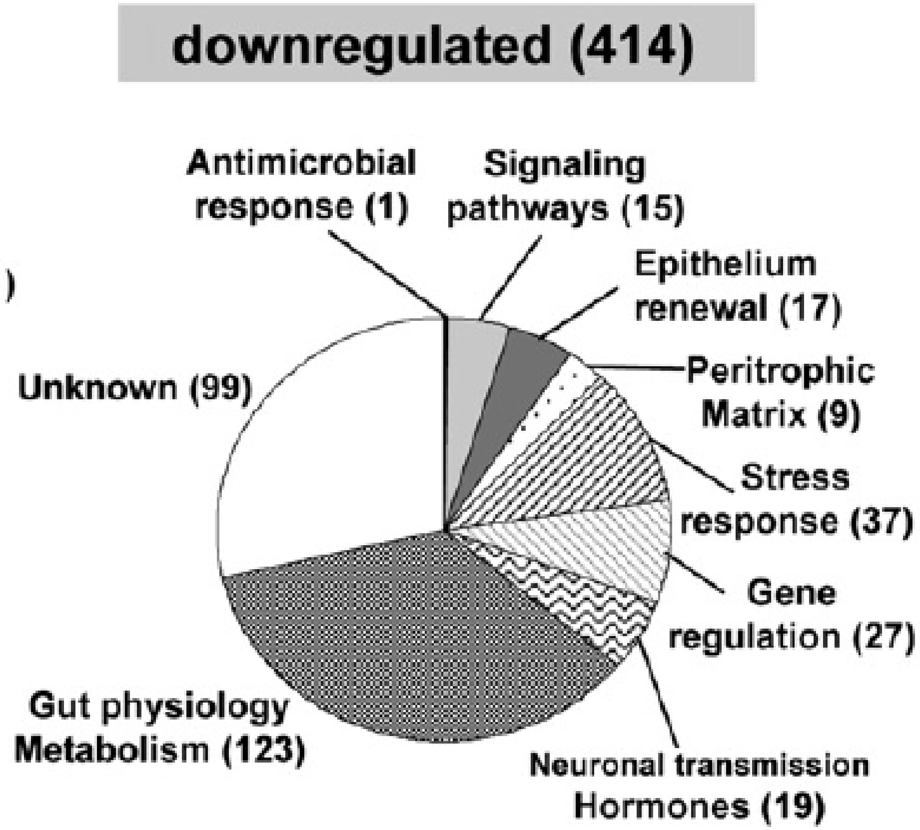
Drosophila Intestinal Response to Bacterial Infection: Activation of Host Defense and Stem Cell Proliferation

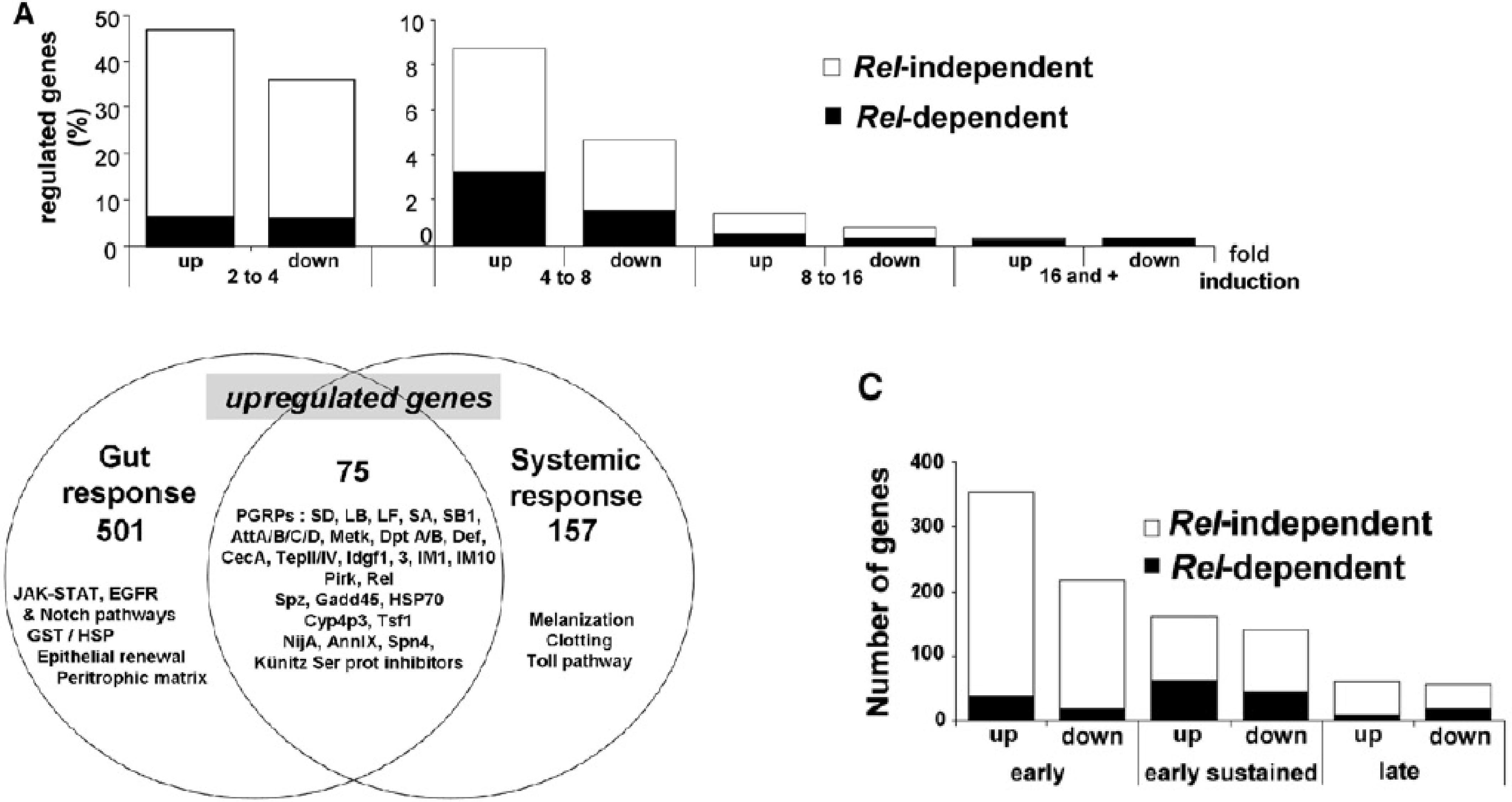
Nicolas Buchon,[1,2] Nichole A. Broderick,[1] Mickael Poidevin,[2] Sylvain Pradervand,[3] and Bruno Lemaitre[1,2,*]

Cell Host & Microbe 5, 200–211, February 19, 2009

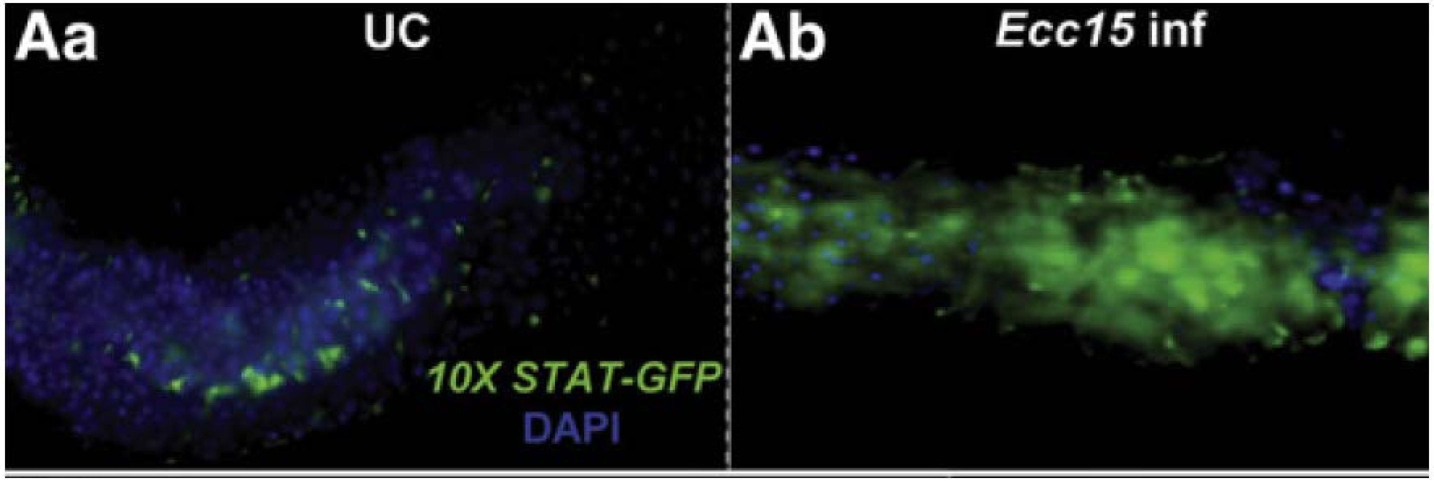
Erwinia carotovora carotovora 15 oral infection



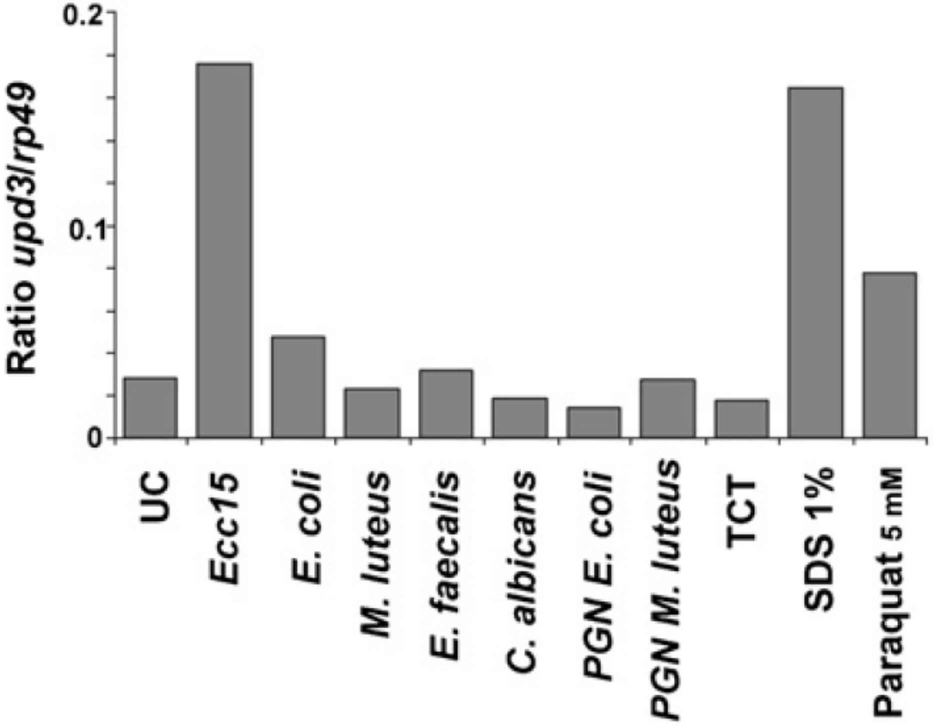



A
regulated genes (%)
Rel-independent
Rel-dependent
up
down
2 to 4
4 to 8
8 to 16
16 and +
fold induction
upregulated genes
Gut response 501
75
Systemic response 157
JAK-STAT, EGFR & Notch pathways
GST / HSP
Epithelial renewal
Peritrophic matrix
PGRPs : SD, LB, LF, SA, SB1,
AttA/B/C/D, Metk, Dpt A/B, Def,
CecA, TepII/IV, Idgf1, 3, IM1, IM10
Pirk, Rel
Spz, Gadd45, HSP70
Cyp4p3, Tsf1
NijA, AnnIX, Spn4,
Künitz Ser prot inhibitors
Melanization
Clotting
Toll pathway
C
Number of genes
early
early sustained
late

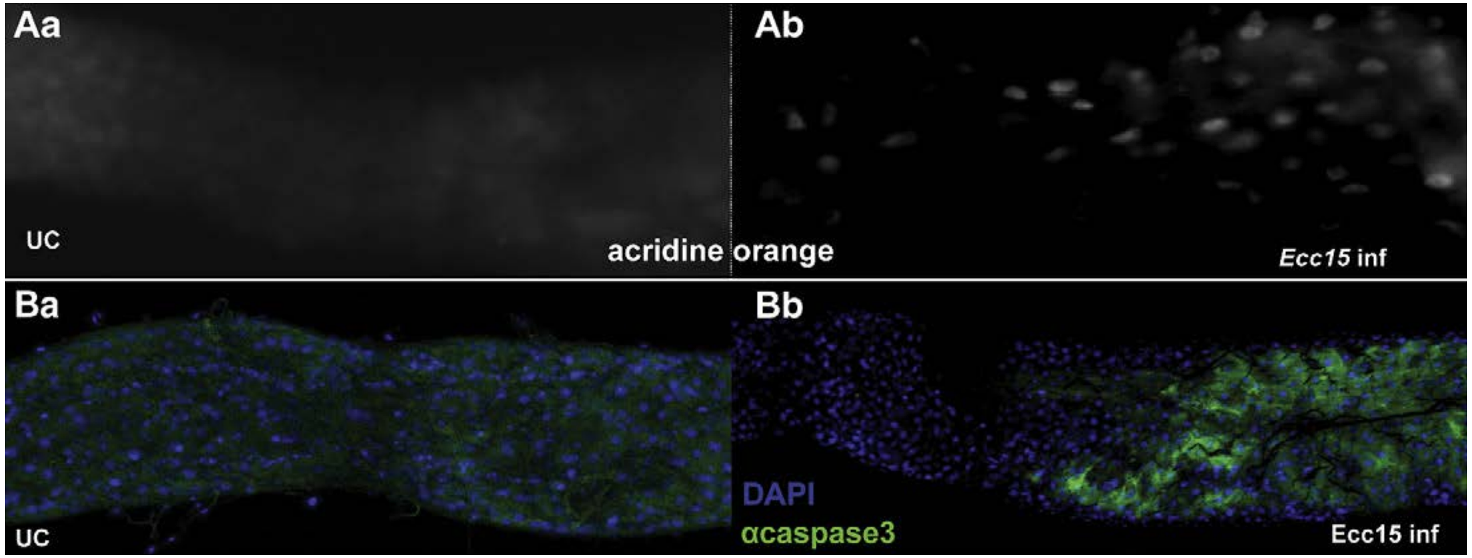
Erwinia carotovora carotovora 15 oral infection

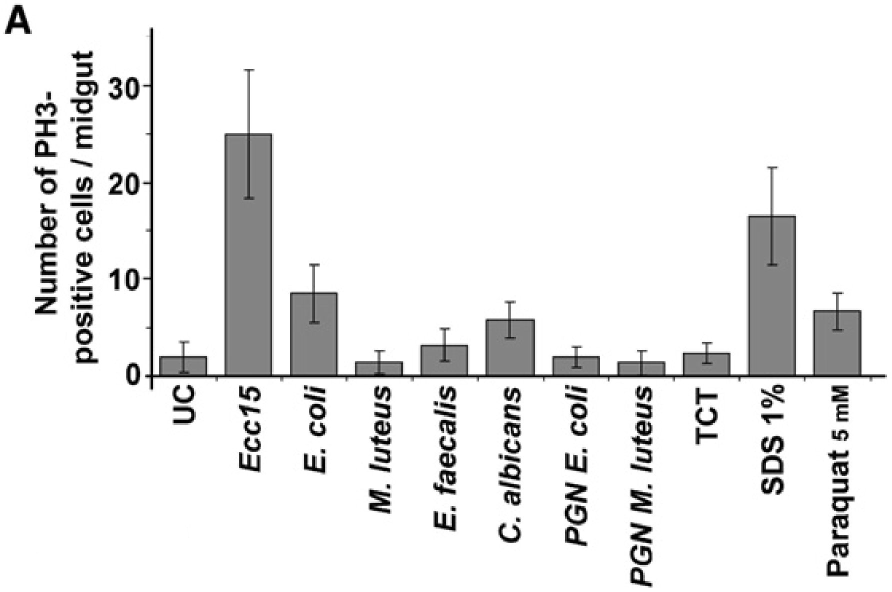


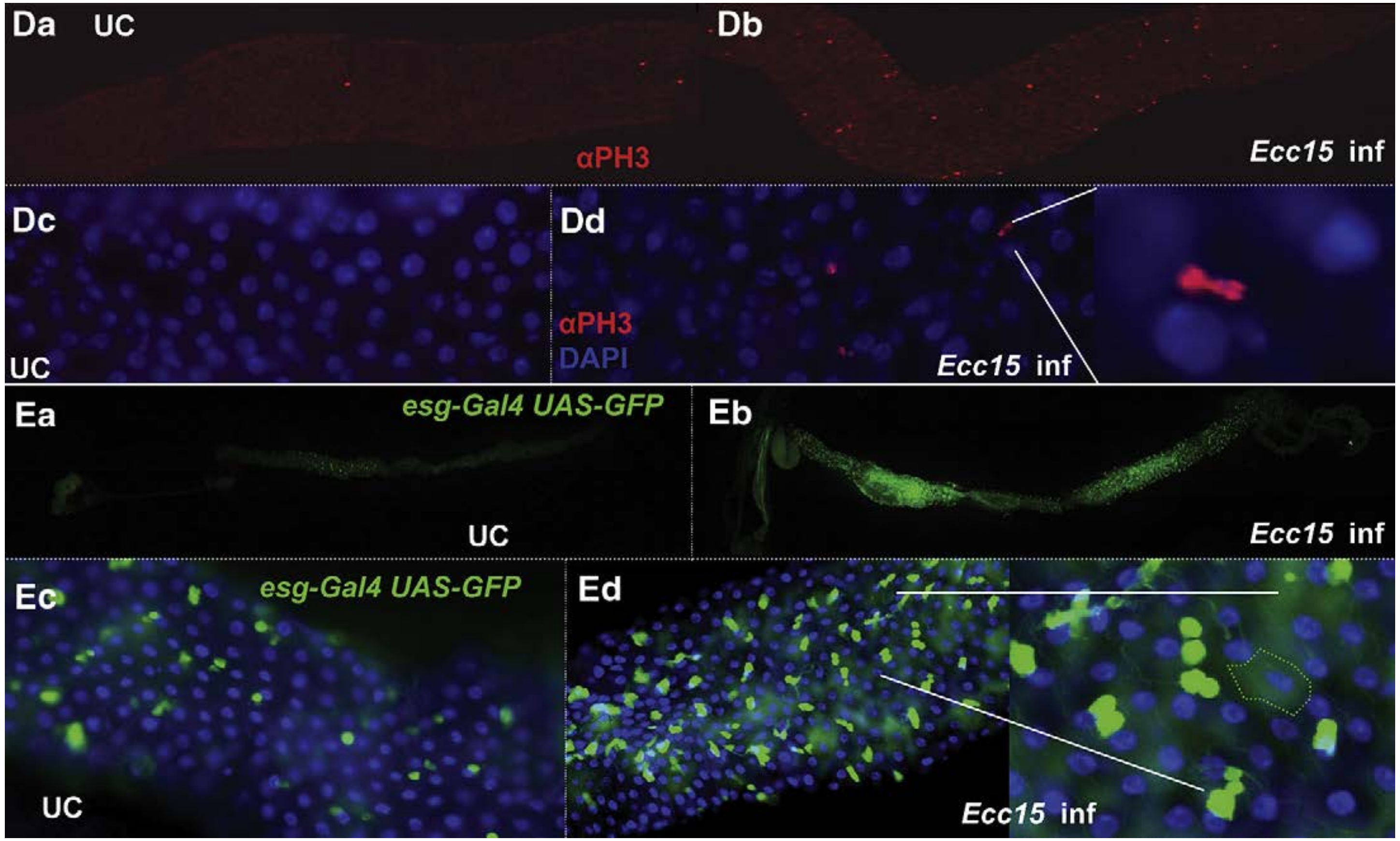
STAT-GFP reporter (green) was expressed in a small population of basal cells in unchallenged flies (a). Ingestion of Ecc15 (16 hr postinfection) induced a strong expression of STAT-GFP along the midgut (b).



RT-qPCR analysis of upd3 induction in gut extracts from unchallenged wild-type flies (UC) or wild-type adult females 16 hr postfeeding with various bacterial strains, polymeric peptidoglycan (PGN), monomeric peptidoglycan (TCT), SDS (0.1%), or paraquat (5 mM).


Aa
Ab
UC
acridine orange
Ecc15 inf
Ba
Bb
DAPI
αcaspase3
UC
Ecc15 inf

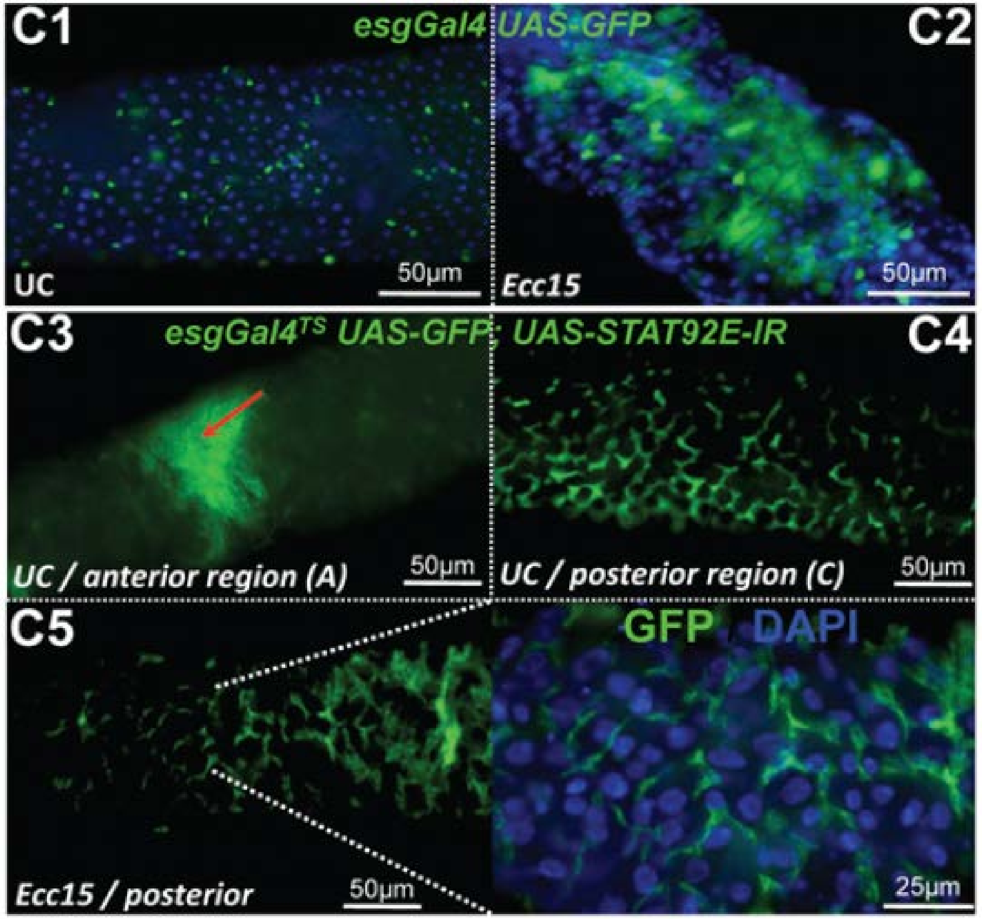

A
Number of PH3-positive cells / midgut
0
10
20
30
UC
Ecc15
E. coli
M. luteus
E. faecalis
C. albicans
PGN E. coli
PGN M. luteus
TCT
SDS 1%
Paraquat 5 mM


Da UC
Db
αPH3
Ecc15 inf
Dc
Dd
αPH3
DAPI
UC
Ecc15 inf
Ea
esg-Gal4 UAS-GFP
Eb
UC
Ecc15 inf
Ec
esg-Gal4 UAS-GFP
Ed
UC
Ecc15 inf

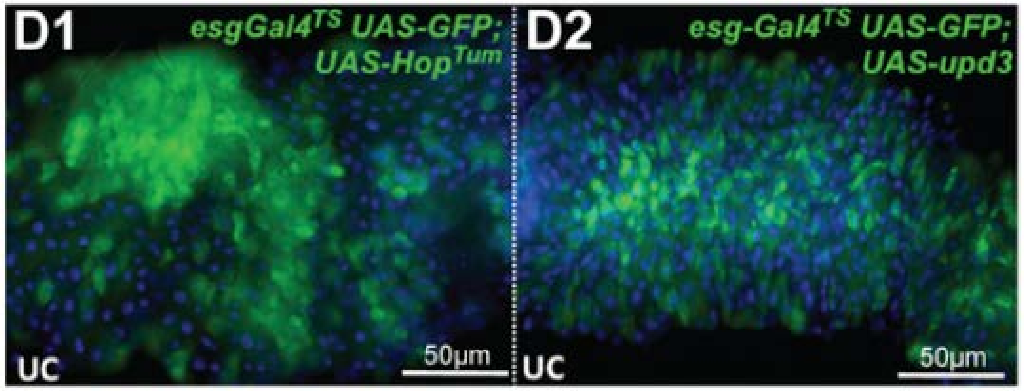
Invasive and indigenous microbiota impact intestinal stem cell activity through multiple pathways in *Drosophila*

Nicolas Buchon,[2] Nichole A. Broderick, Sveta Chakrabarti, and Bruno Lemaitre[1]

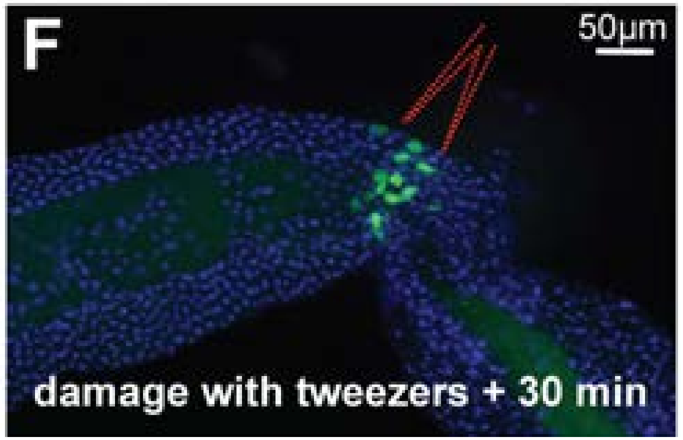
GENES & DEVELOPMENT 23:2333–2344 © 2009



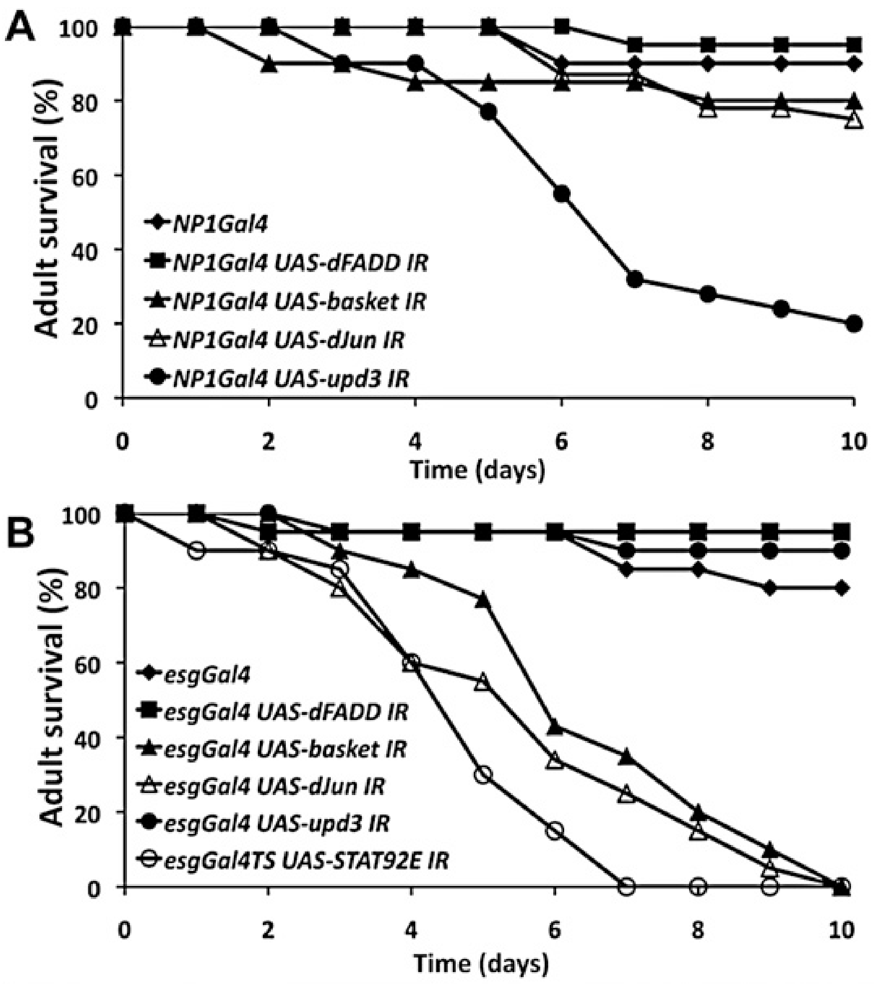
No increase in epithelium renewal was observed in UAS-STAT92E-IR flies upon infection with *Ecc15*, as indicated by the lack of large nuclei cells expressing GFP

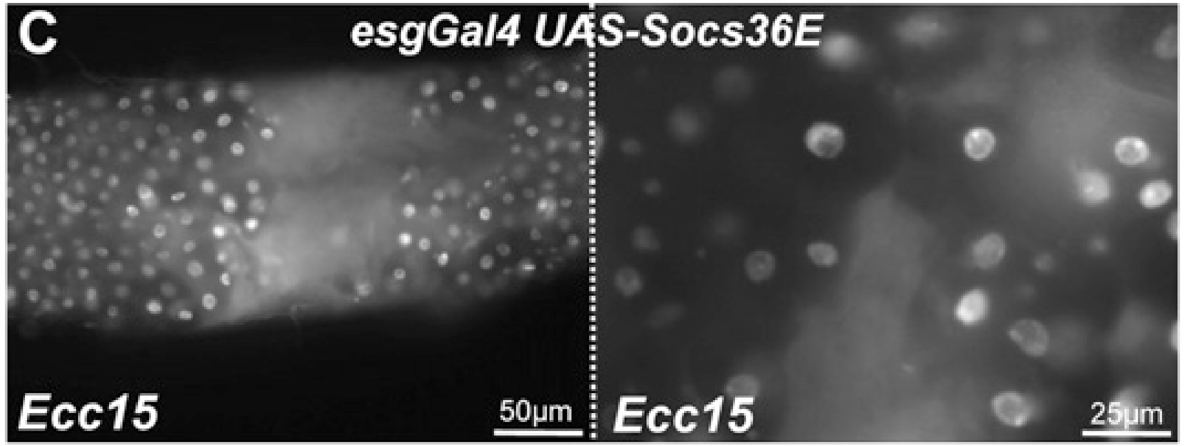


Overexpression of hopTum (D1) or upd3 (D2) in ISCs is sufficient to induce a high level of epithelium renewal in the absence of infection.

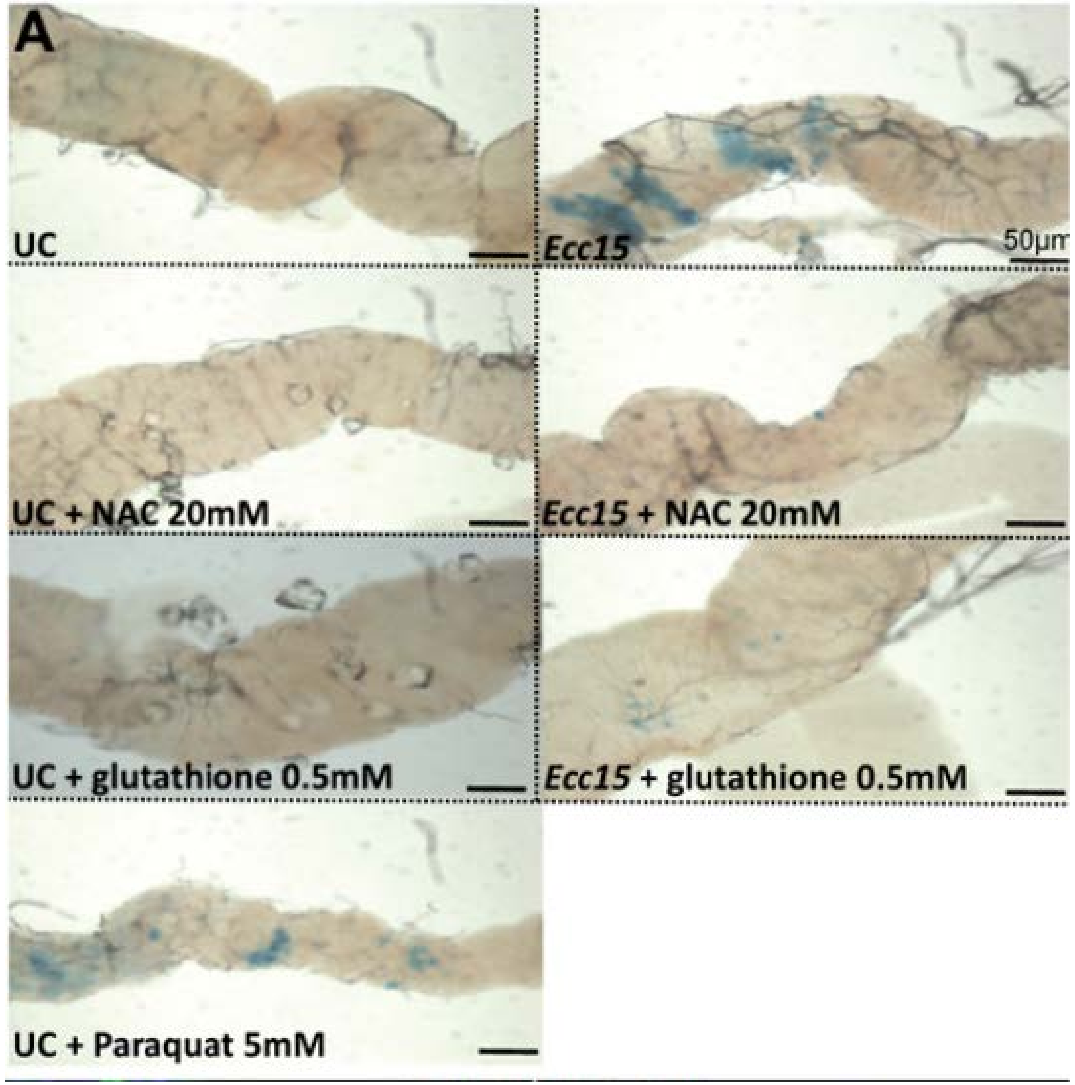


Physical damage to the gut with tweezers induced local expression of the upd3Gal4, UAS-GFP reporter within 30 min following injury.

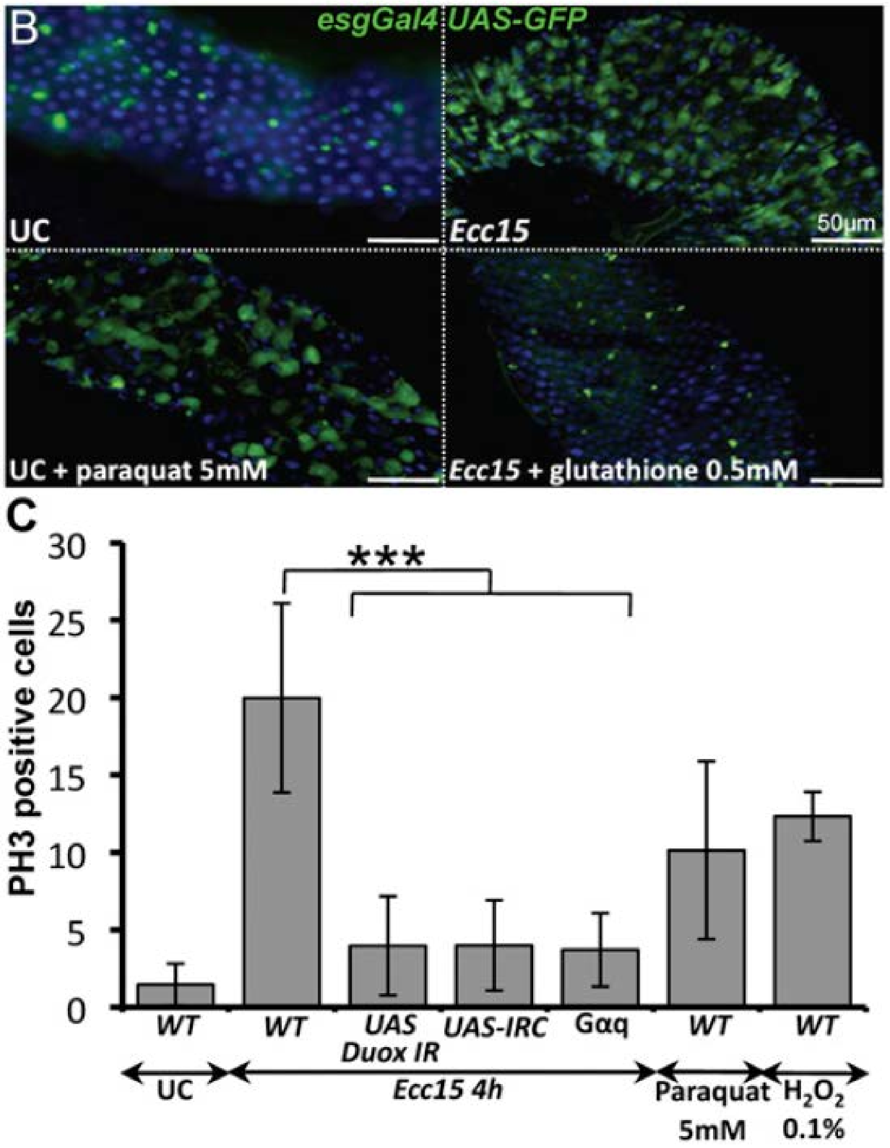




Guts of flies impaired in epithelium renewal displayed altered gut morphology as revealed by the lack of DAPI nuclear staining. Flies were observed 4 d post-infection.



Large lacZ-marked clones containing the tubulin promoter-lacZ fusion due to mitotic recombination are observed in the guts of flies orally infected with Ecc15 or fed with paraquat. No expansion of lacZ clones was detected in the gut of flies fed with both Ecc15 and antioxidants (NAC or gluthathione).

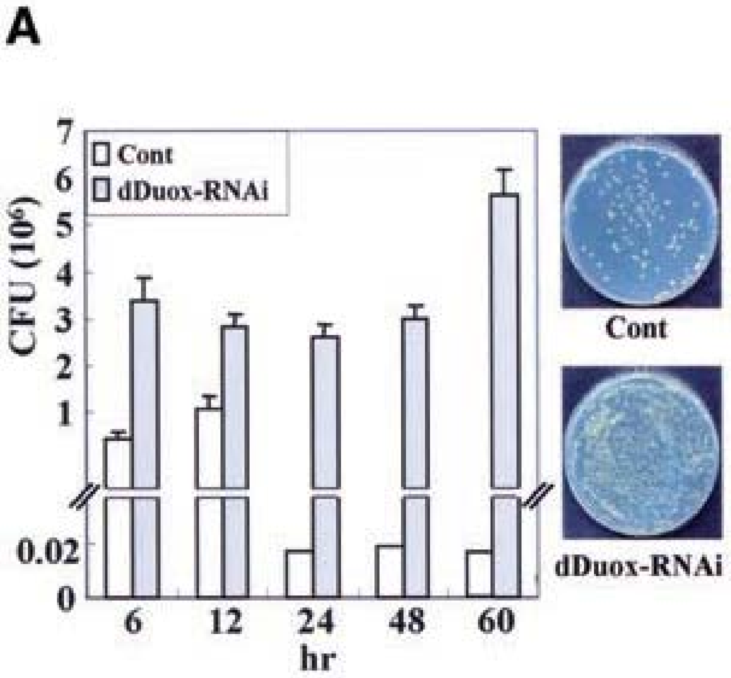


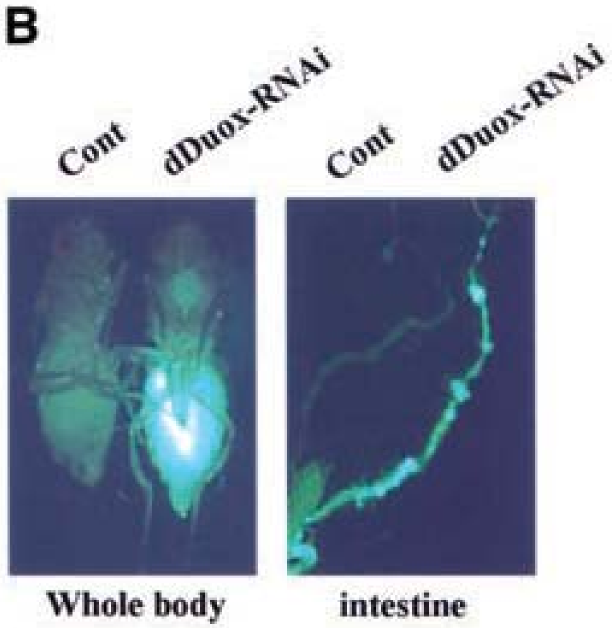
Ingestion of Ecc15 or paraquat induces a marked increase in the number of esg-GFPpositive cells. Coingestion of Ecc15 and glutathione did not induce epithelium renewal.

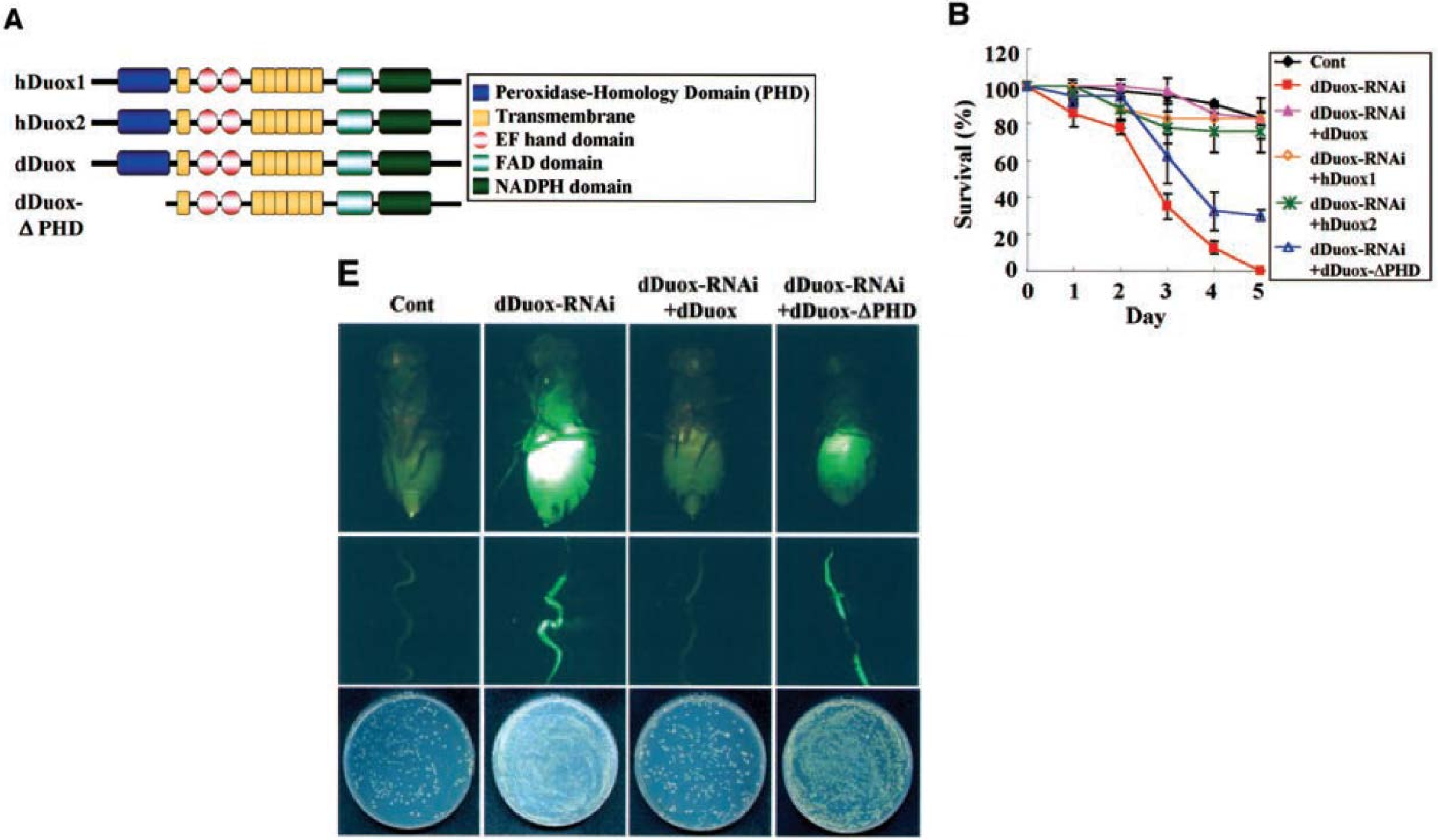
A Direct Role for Dual Oxidase in *Drosophila* Gut Immunity

Eun-Mi Ha,[1] Chun-Taek Oh,[2] Yun Soo Bae,[1] Won-Jae Lee[1,2*]

SCIENCE VOL 310 4 NOVEMBER 2005





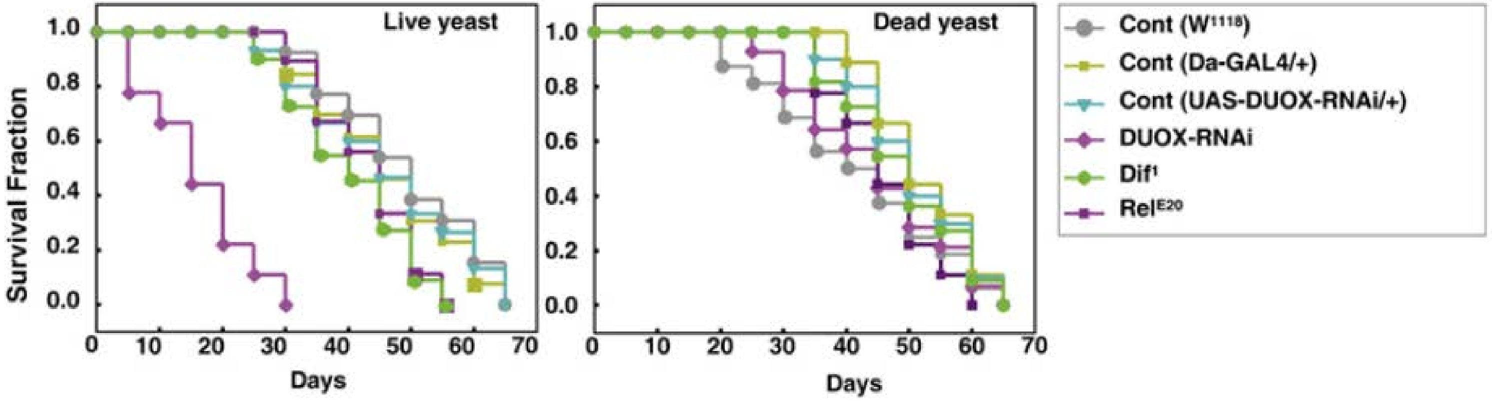

A
hDuox1
hDuox2
dDuox
dDuox-
Δ PHD
Peroxidase-Homology Domain (PHD)
Transmembrane
EF hand domain
FAD domain
NADPH domain
B
Survival (%)
120
100
80
60
40
20
0
0 1 2 3 4 5
Day
Cont
dDuox-RNAi
dDuox-RNAi +dDuox
dDuox-RNAi +hDuox1
dDuox-RNAi +hDuox2
dDuox-RNAi +dDuox-ΔPHD
E
Cont
dDuox-RNAi
dDuox-RNAi +dDuox
dDuox-RNAi +dDuox-ΔPHD

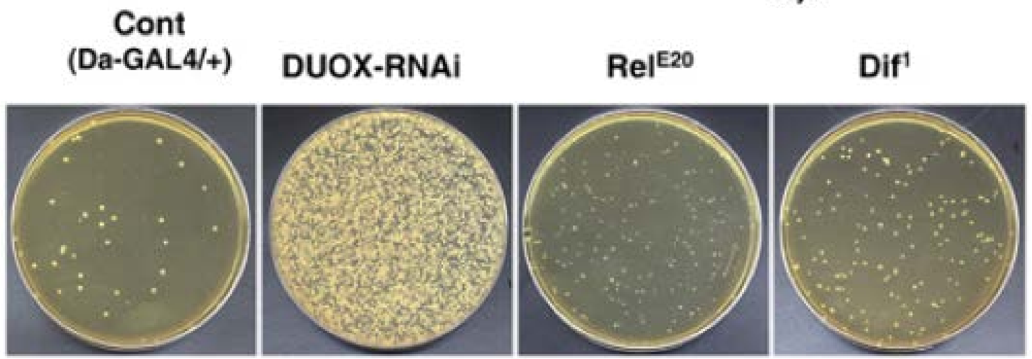
Regulation of DUOX by the Gαq-Phospholipase Cβ-Ca^{2+} Pathway in *Drosophila* Gut Immunity

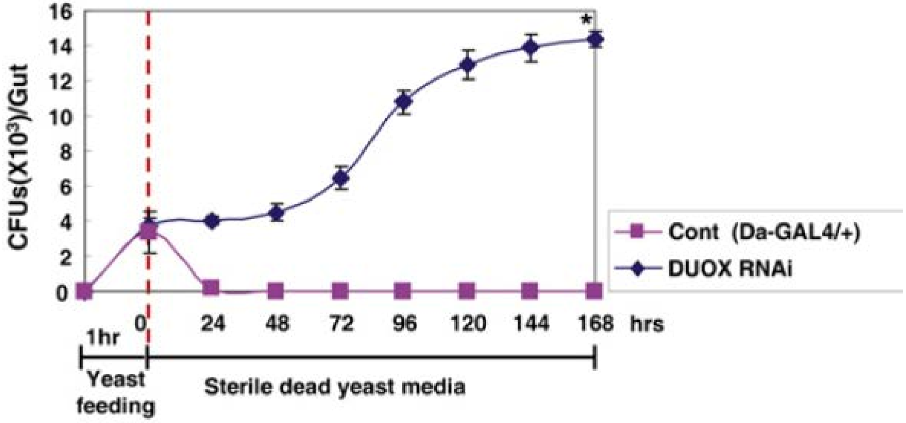
Eun-Mi Ha,[1,2,5] Kyung-Ah Lee,[1,2,5] Seon Hwa Park,[1] Sung-Hee Kim,[1,2] Hyuck-Jin Nam,[1,2] Hyo-Young Lee,[2]
Dongmin Kang,[1] and Won-Jae Lee[1,2,3,4,*]

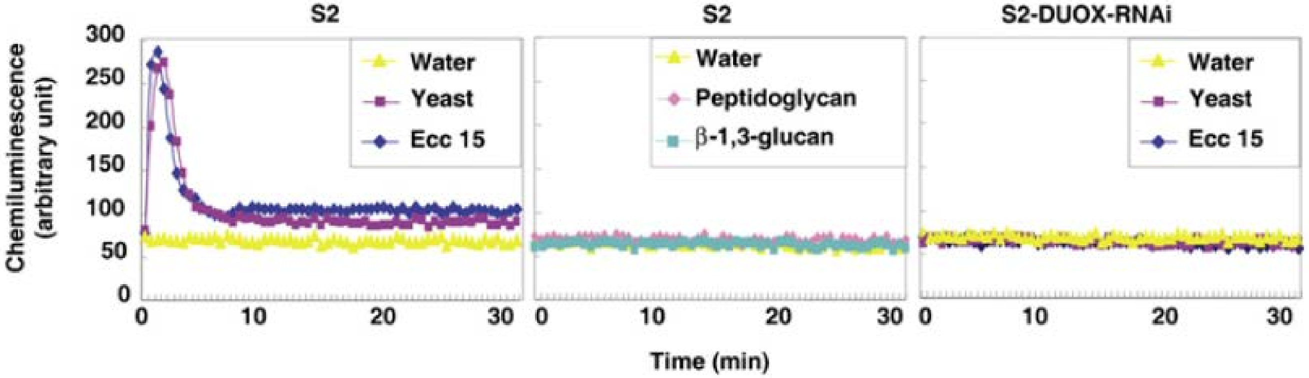
Developmental Cell *16*, 386–397, March 17, 2009

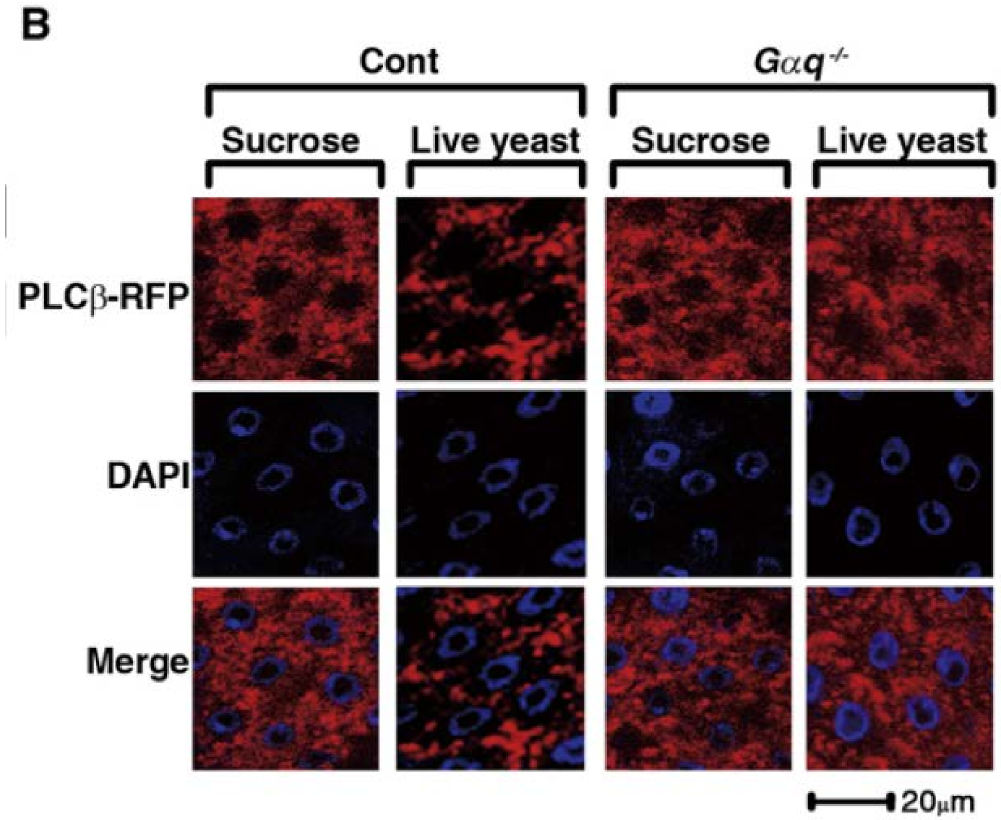
A



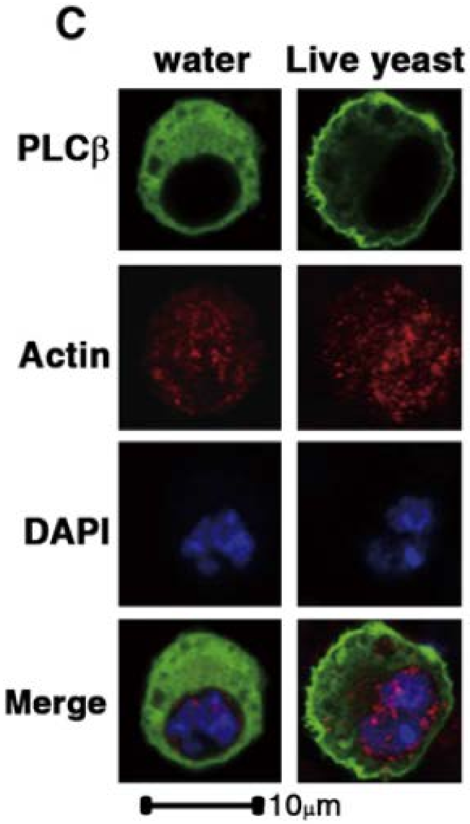




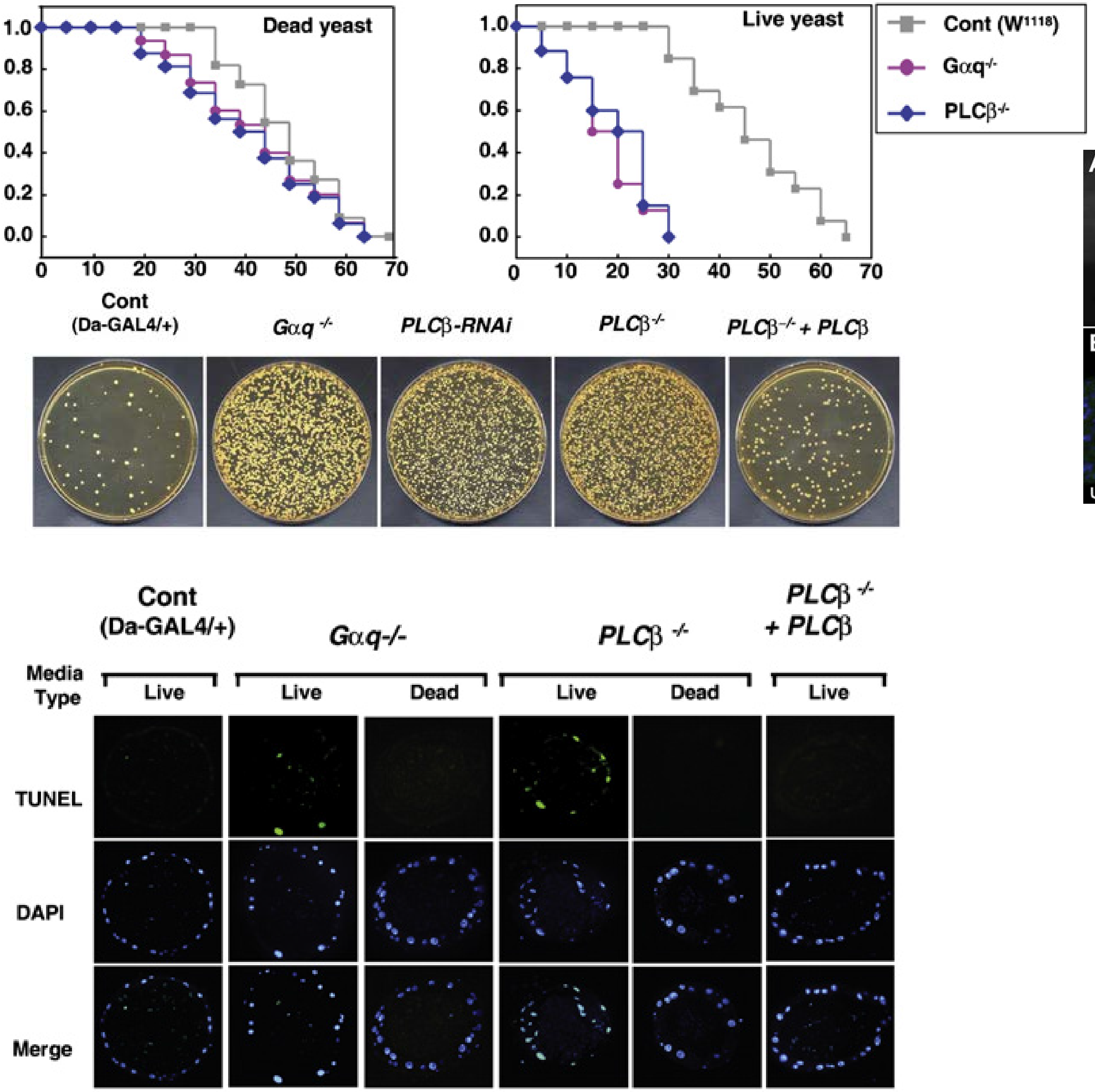


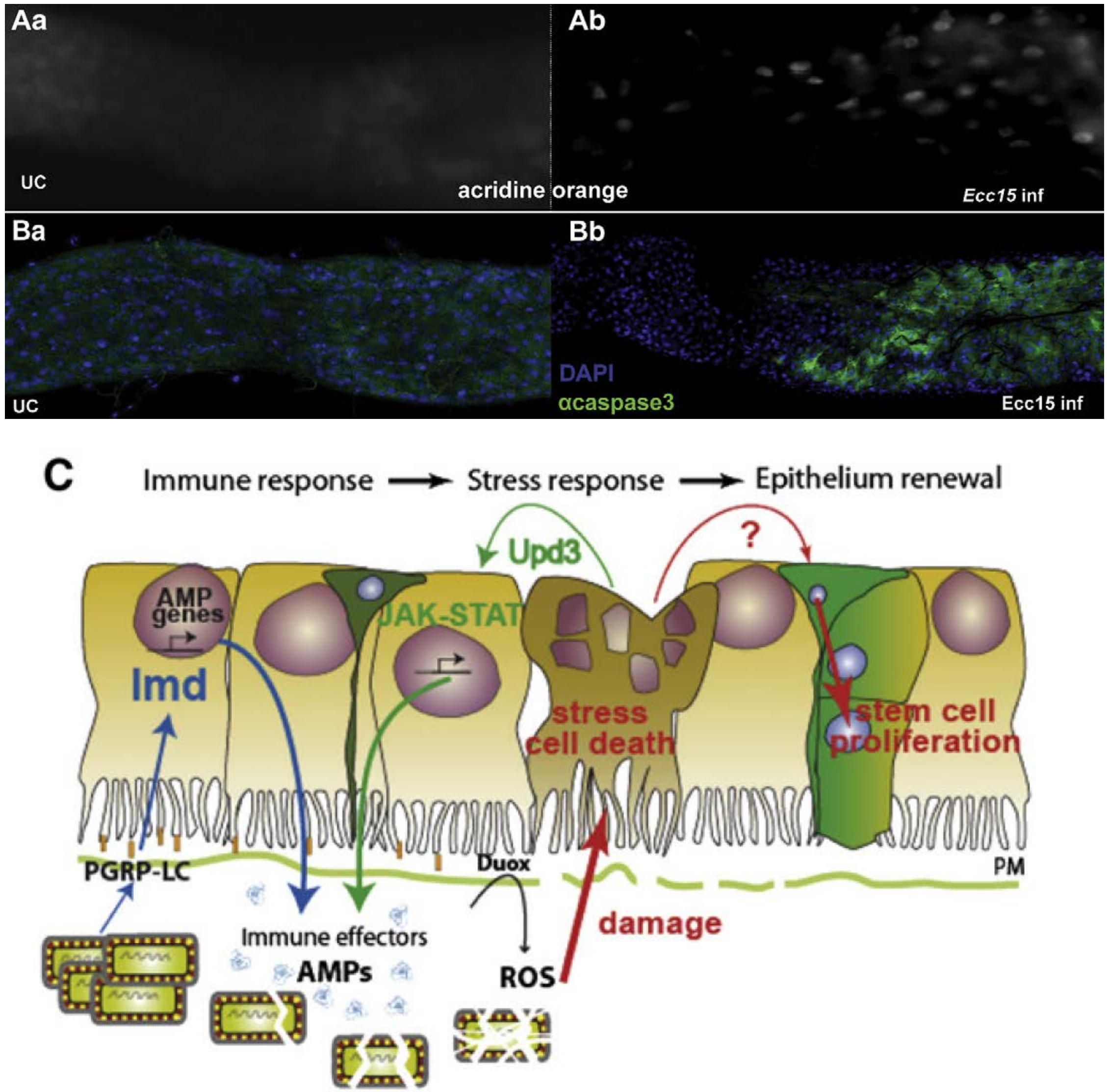


Activation of PLCb subtype-II can be observed in the gut in a Gaq-dependent manner. No PLCb activation was observed in the gut of Gaq mutant flies.



Yeast treatment induces the membrane localization of the activated form of PLCb subtype-II.

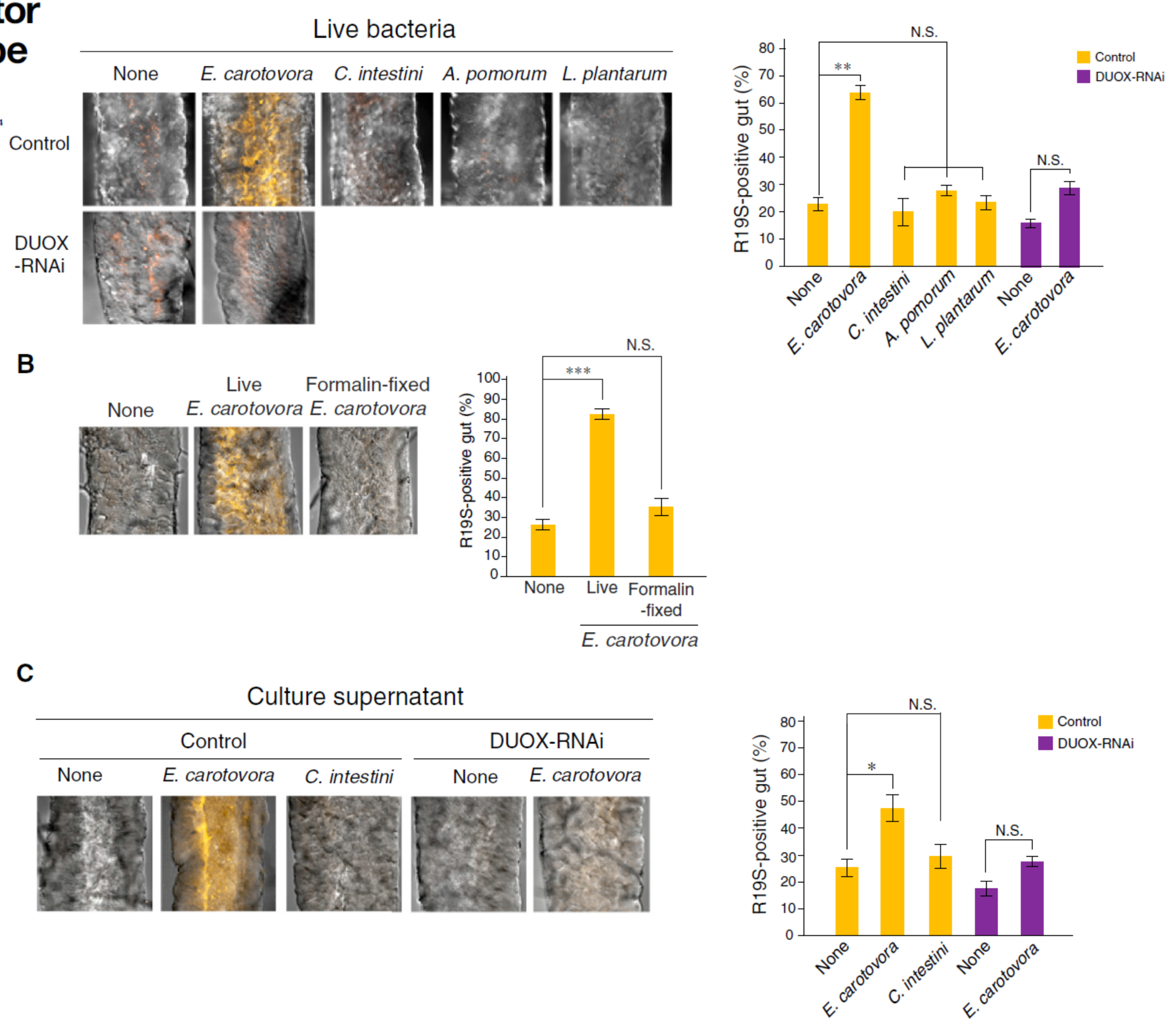

Dead yeast
Live yeast
Cont (W1118)
Gαq-/-
PLCβ-/-
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
50
60
70
Cont
(Da-GAL4/+)
Gαq -/-
PLCβ-RNAi
PLCβ-/-
PLCβ-/- + PLCβ
Cont
(Da-GAL4/+)
Gαq-/-
PLCβ -/-
PLCβ -/-
+ PLCβ
Media
Type
Live
Live
Dead
Live
Dead
Live
TUNEL
DAPI
Merge

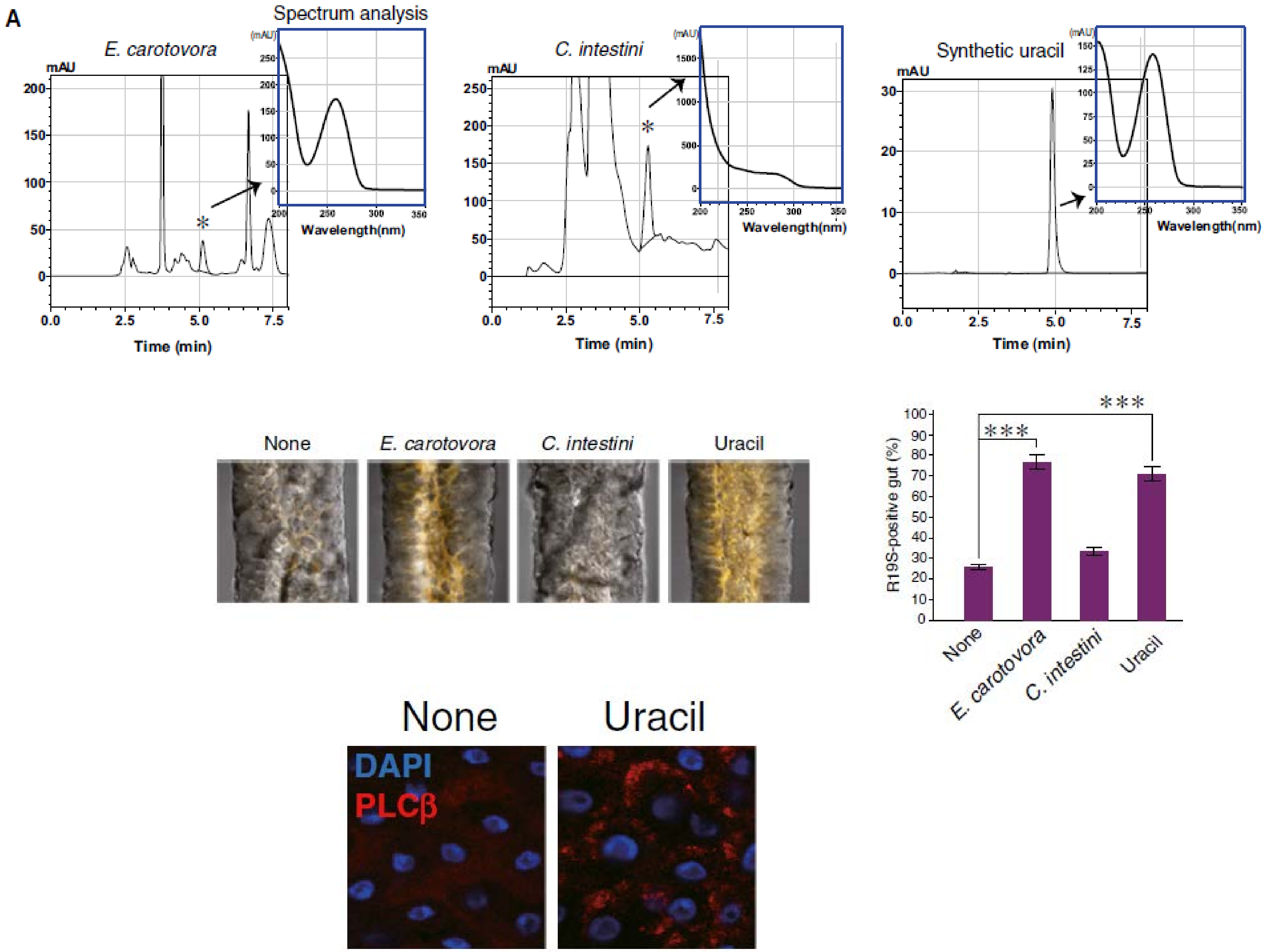

Aa
Ab
UC
acridine orange
Ecc15 inf
Ba
Bb
UC
DAPI
αcaspase3
Ecc15 inf
C
Immune response → Stress response → Epithelium renewal
Upd3
?
AMP
genes
JAK-STAT
Imd
stress
cell death
stem cell
proliferation
PGRP-LC
Duox
PM
Immune effectors
AMPs
ROS
damage

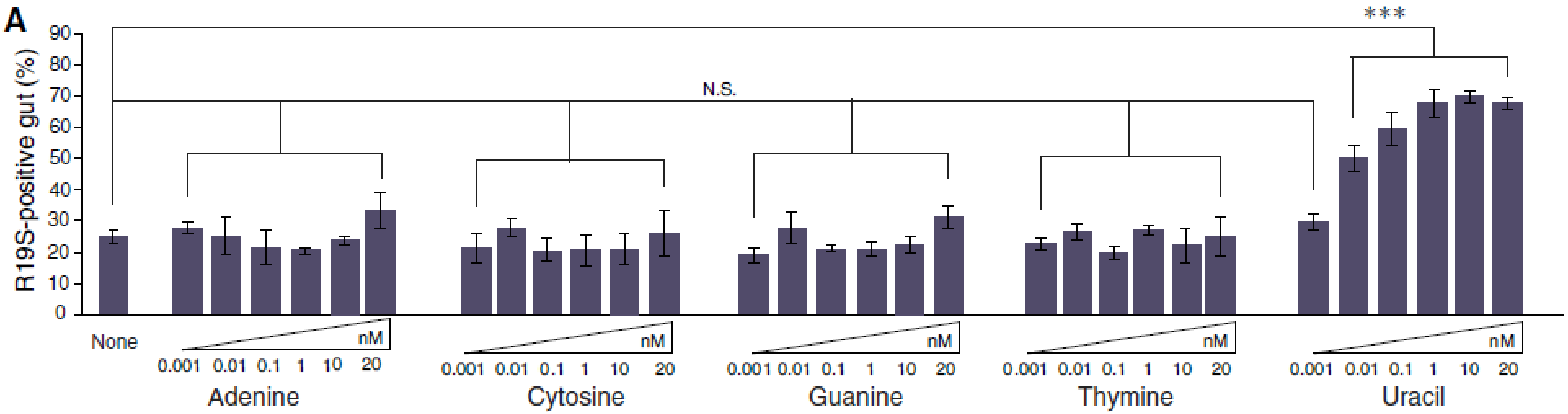
Bacterial-Derived Uracil as a Modulator of Mucosal Immunity and Gut-Microbe Homeostasis in *Drosophila*

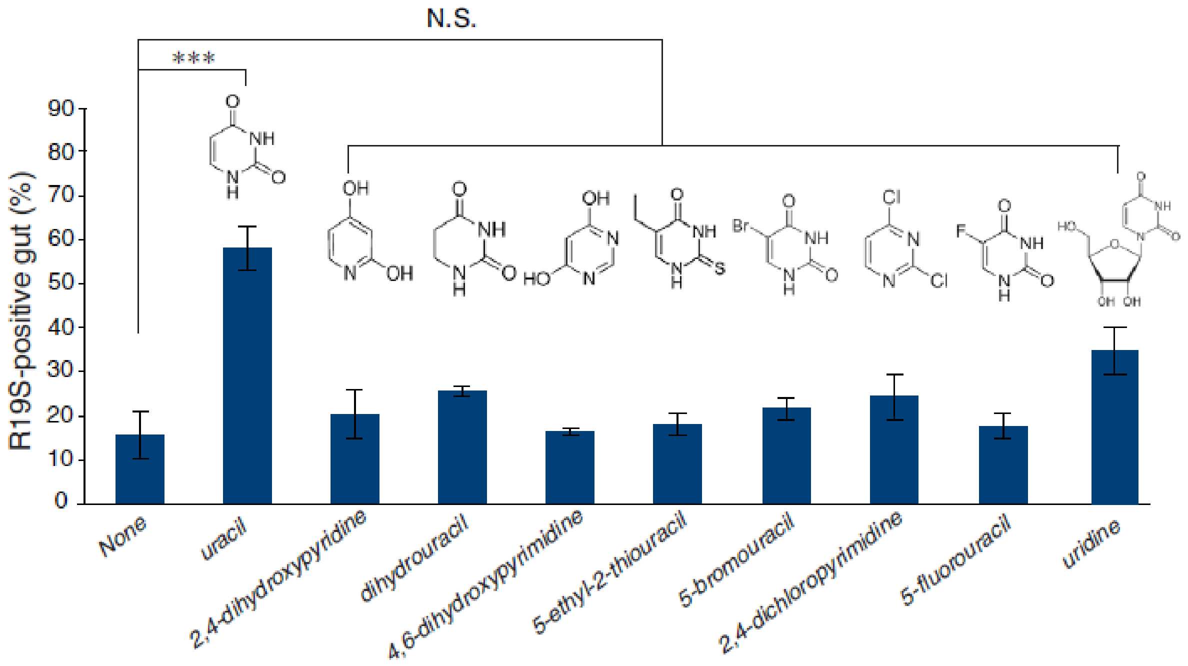
Kyung-Ah Lee,[1,5] Sung-Hee Kim,[1,5] Eun-Kyoung Kim,[1] Eun-Mi Ha,[2] Hyejin You,[1,3] Boram Kim,[1] Min-Ji Kim,[4] Youngjoo Kwon,[3] Ji-Hwan Ryu,[4] and Won-Jae Lee[1,*]

Cell *153*, 797–811, May 9, 2013 ©2013

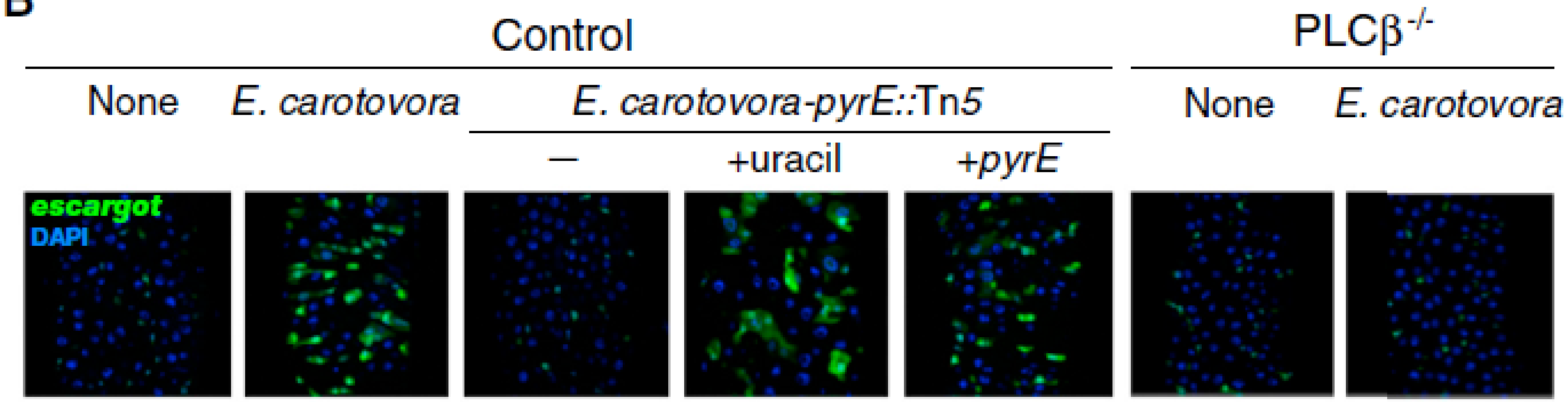



A
Spectrum analysis
E. carotovora
C. intestini
Synthetic uracil
mAU
(mAU)
Wavelength(nm)
Time (min)
None
E. carotovora
C. intestini
Uracil
R19S-positive gut (%)
***
None
Uracil
DAPI
PLCβ

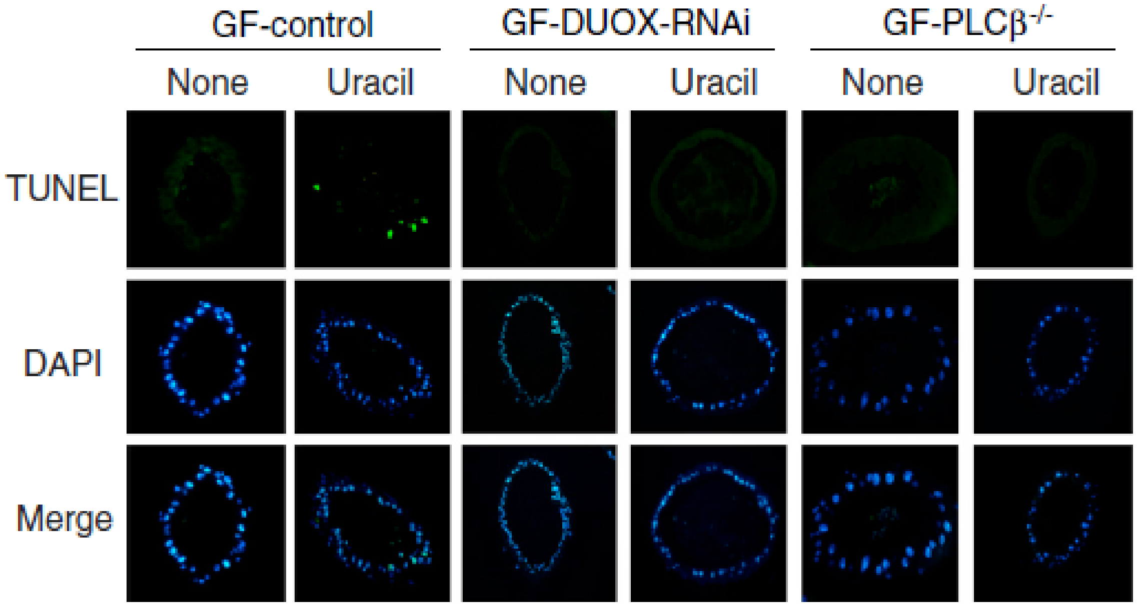

A
R19S-positive gut (%)
90
80
70
60
50
40
30
20
10
0
***
N.S.
None
nM
0.001 0.01 0.1 1 10 20
Adenine
Cytosine
Guanine
Thymine
Uracil


N.S.
***
R19S-positive gut (%)
None
uracil
2,4-dihydroxypyridine
dihydrouracil
4,6-dihydroxypyrimidine
5-ethyl-2-thiouracil
5-bromouracil
2,4-dichloropyrimidine
5-fluorouracil
uridine

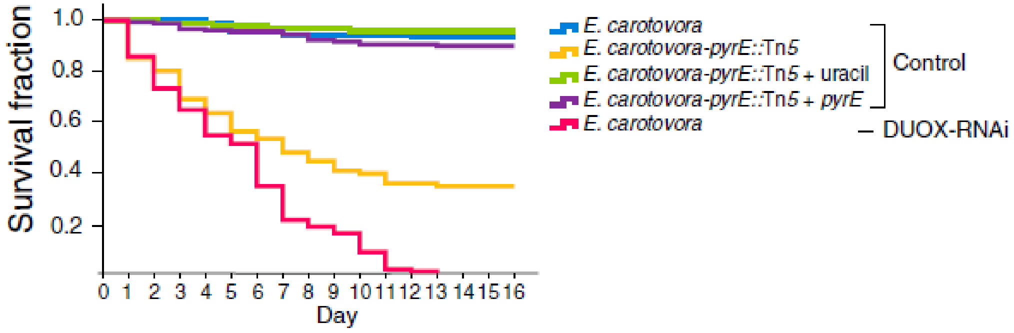
B


Control
PLCβ-/-
None
E. carotovora
E. carotovora-pyrE::Tn5
None
E. carotovora
—
+uracil
+pyrE
escargot
DAPI

C


GF-control
GF-DUOX-RNAi
GF-PLCβ-/-
None
Uracil
None
Uracil
None
Uracil
TUNEL
DAPI
Merge

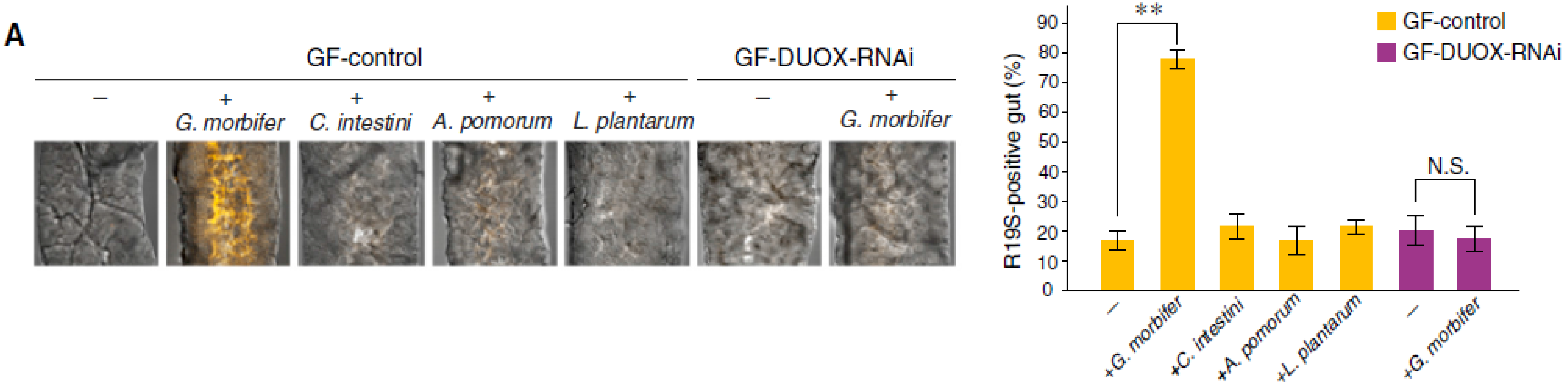
B


Survival fraction
1.0
0.8
0.6
0.4
0.2
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Day
E. carotovora
E. carotovora-pyrE::Tn5
E. carotovora-pyrE::Tn5 + uracil
E. carotovora-pyrE::Tn5 + pyrE
E. carotovora
Control
DUOX-RNAi

One of the fundamental questions is why some bacteria release uracil, whereas others do not?

The result showed that pathogens such as *Vibrio fluvialis*, *Klebsiella pneumoniae*, *Shigella sonnei*, *Pseudomonas aeruginosa*, and *Serratia marcescens* secreted significant amounts of uracil (70–150 ng/10^8 cells).

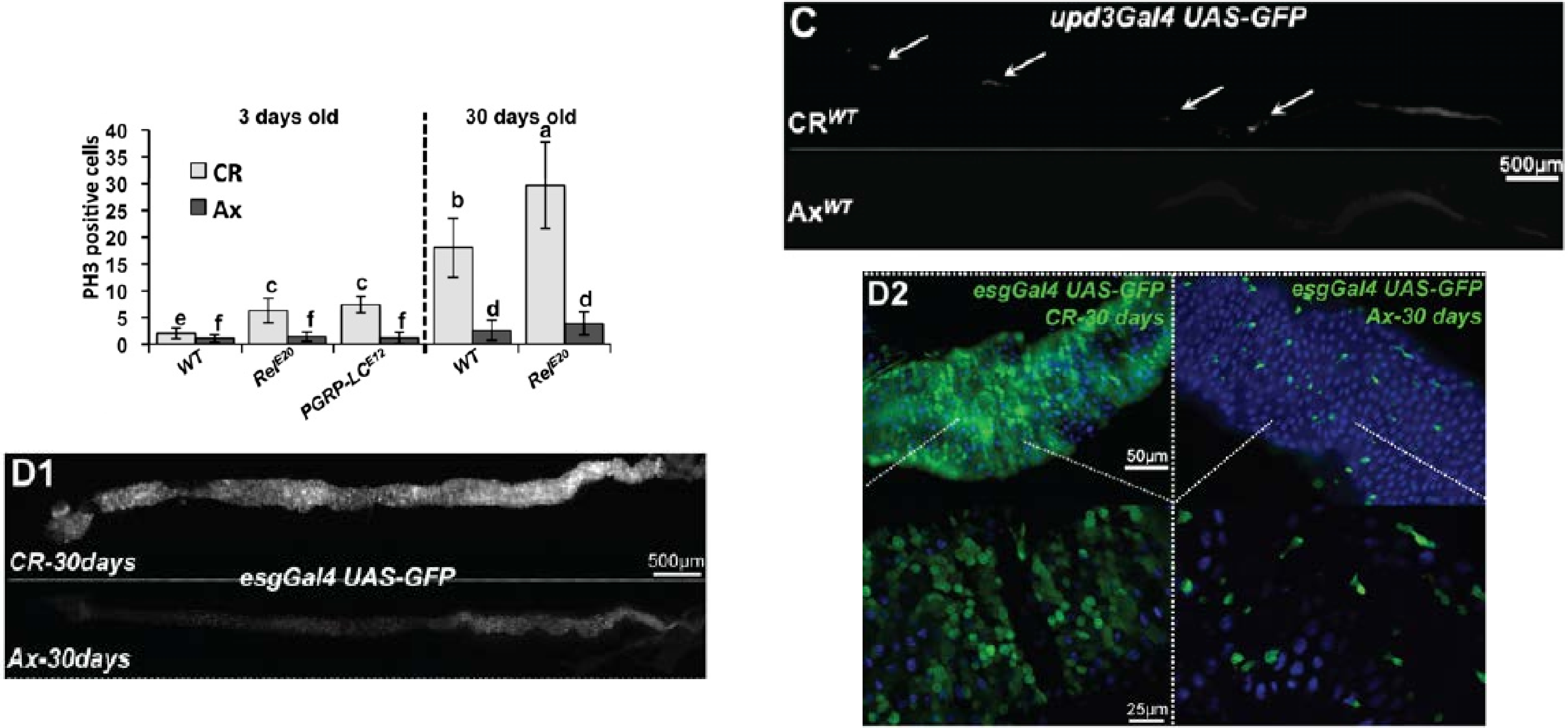
Consistent to the above results, *E. carotovora* secrete high amounts of uracil (200 ng/10^8 cells), whereas *Commensalibacter intestini A911*, *Acetobacter pomorum*, *Lactobacillus plantarum* do not;
but *Gluconobacter morbifer G707* does.



Invasive and indigenous microbiota impact intestinal stem cell activity through multiple pathways in *Drosophila*

Nicolas Buchon,[2] Nichole A. Broderick, Sveta Chakrabarti, and Bruno Lemaitre[1]

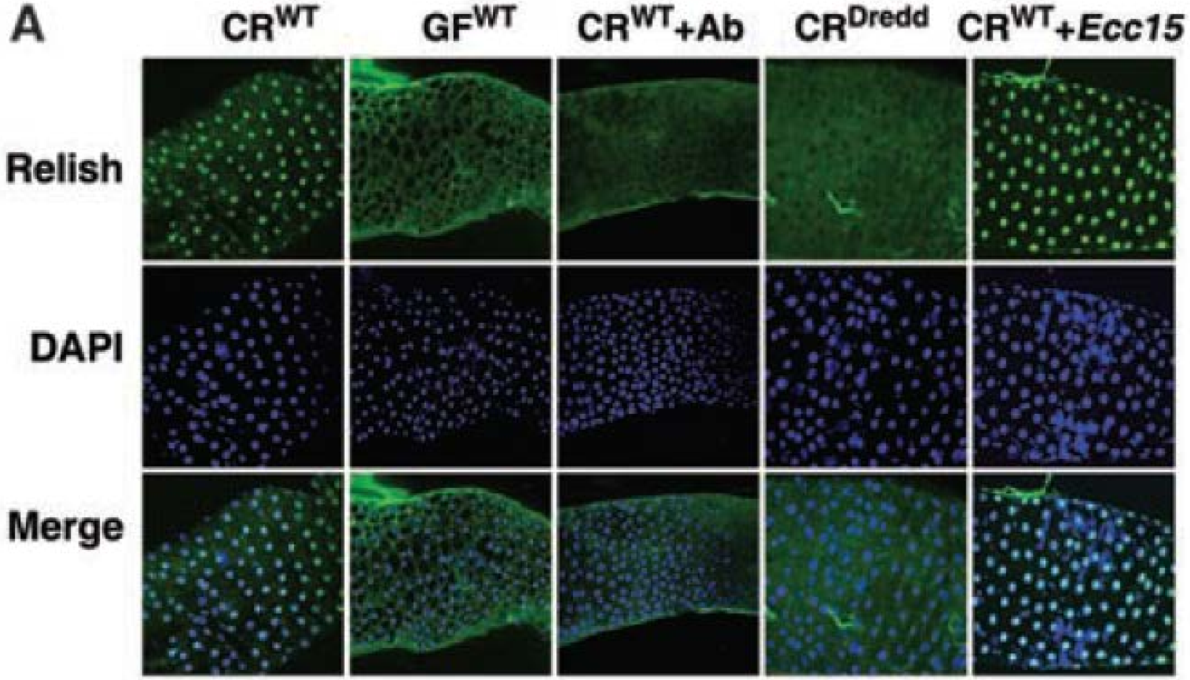
GENES & DEVELOPMENT 23:2333–2344 © 2009

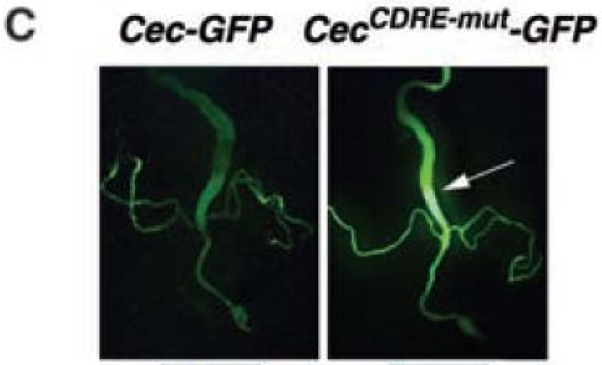


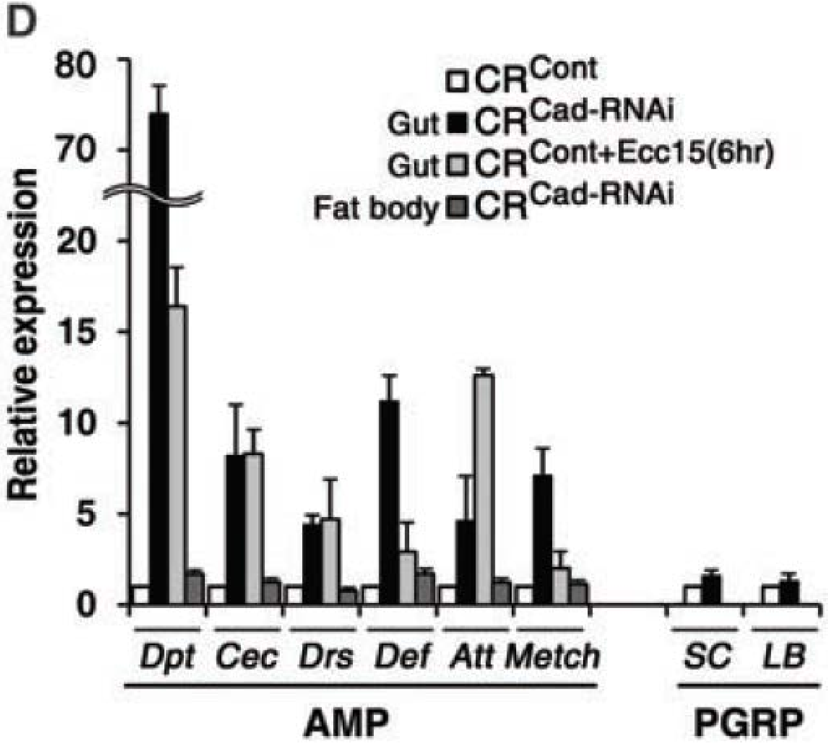
Innate Immune Homeostasis by the Homeobox Gene *Caudal* and Commensal-Gut Mutualism in *Drosophila*

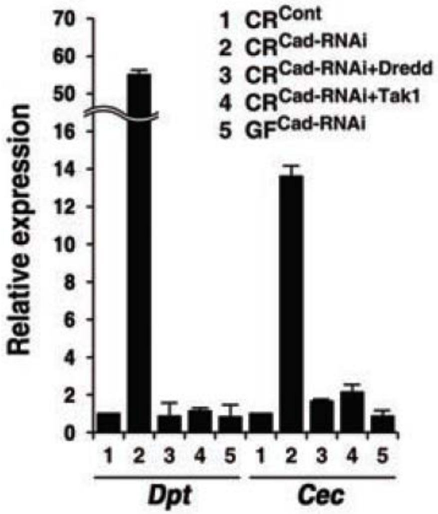
SCIENCE VOL 319 8 FEBRUARY 2008

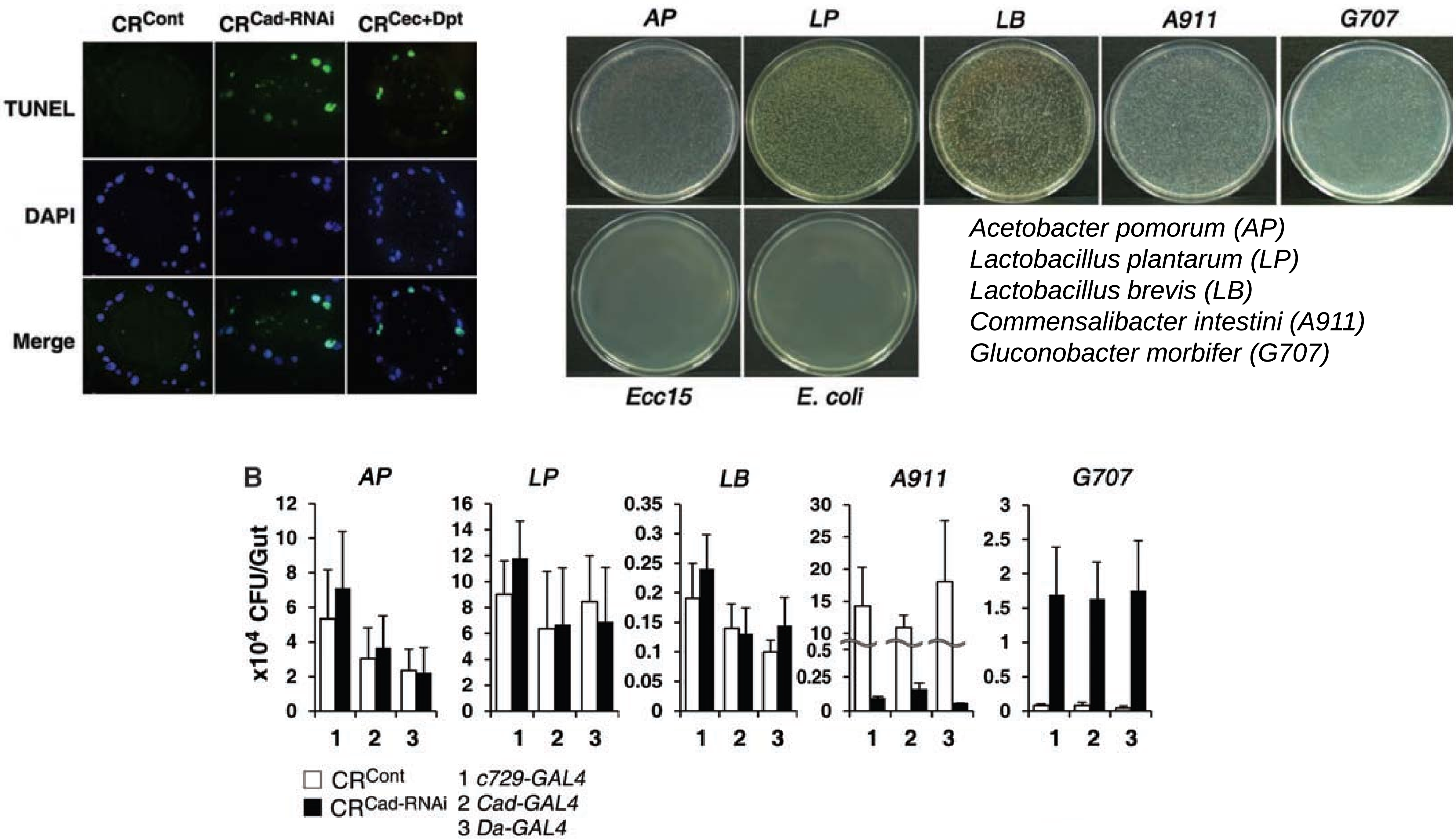
Ji-Hwan Ryu,[1*] Sung-Hee Kim,[1*] Hyo-Young Lee,[1,2] Jin Young Bai,[1] Young-Do Nam,[3] Jin-Woo Bae,[3] Dong Gun Lee,[4] Seung Chul Shin,[1,5] Eun-Mi Ha,[1] Won-Jae Lee[1]†









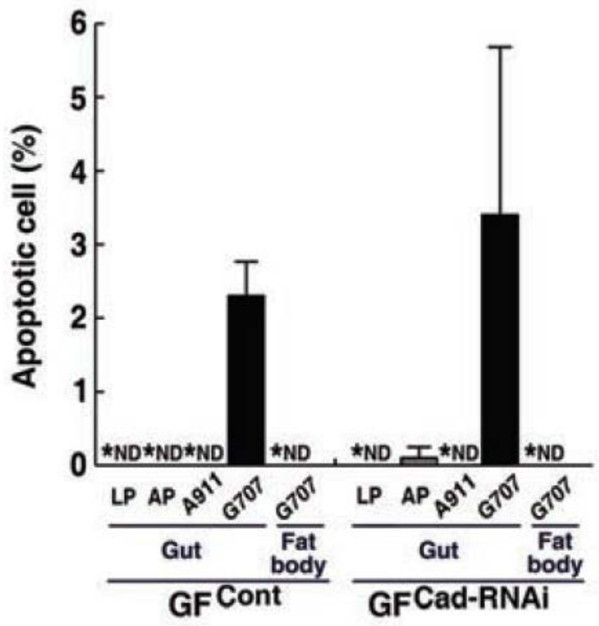


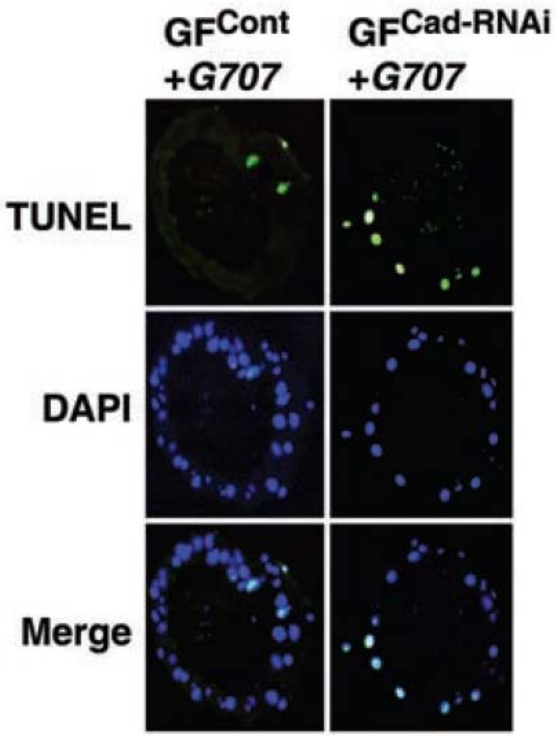
CR^Cont
CR^Cad-RNAi
CR^Cec+Dpt
TUNEL
DAPI
Merge
AP
LP
LB
A911
G707
Ecc15
E. coli
Acetobacter pomorum (AP)
Lactobacillus plantarum (LP)
Lactobacillus brevis (LB)
Commensalibacter intestini (A911)
Gluconobacter morbifer (G707)
B
x10^4 CFU/Gut
1 2 3
CR^Cont
CR^Cad-RNAi
1 c729-GAL4
2 Cad-GAL4
3 Da-GAL4

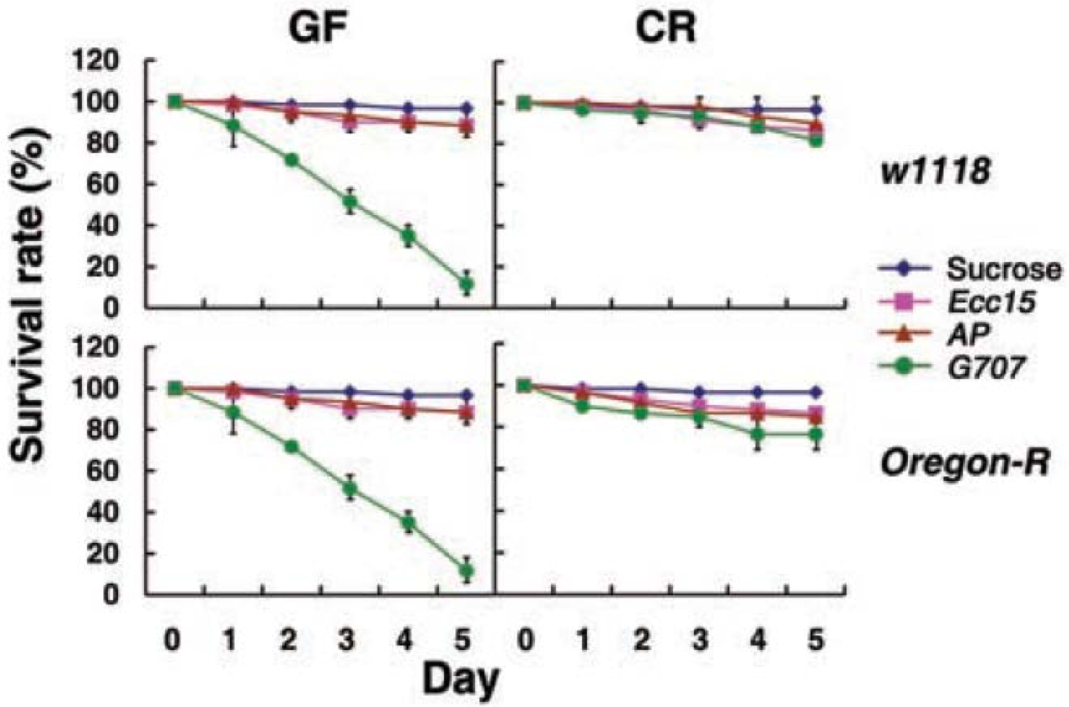
MIC (μg/ml)					
Gram-positive bacteria		Gram-negative bacteria			
LP	*LB*	*AP*	*G707*	*A911*	*E. coli**
10 to 20	10 to 20	2.5	2.5	0.625	1.25

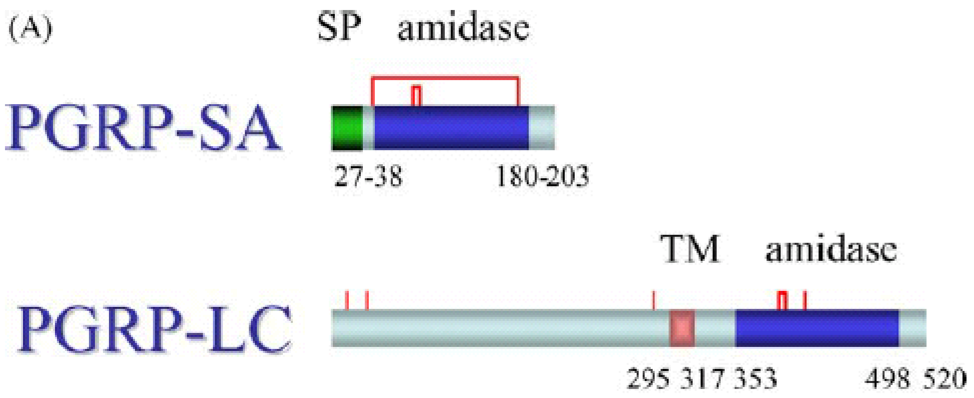
**E. coli* ATCC 25922.

A

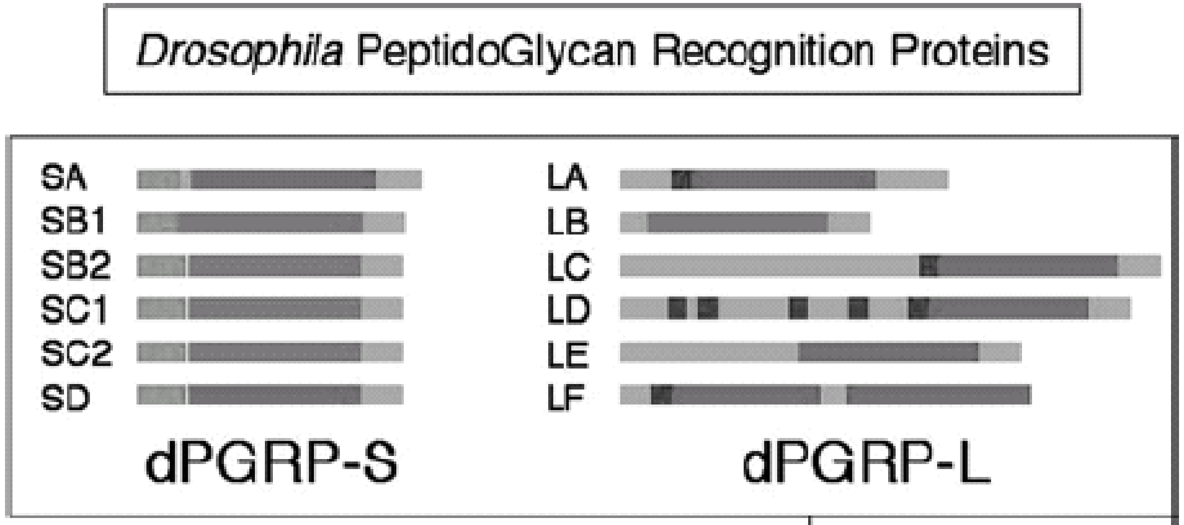




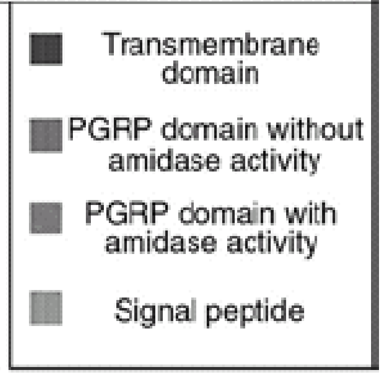




The 160 amino-acid PGRP domain is amidase. The key catalytic residues for amidase activity are not conserved in PGRP-SA and -LC.



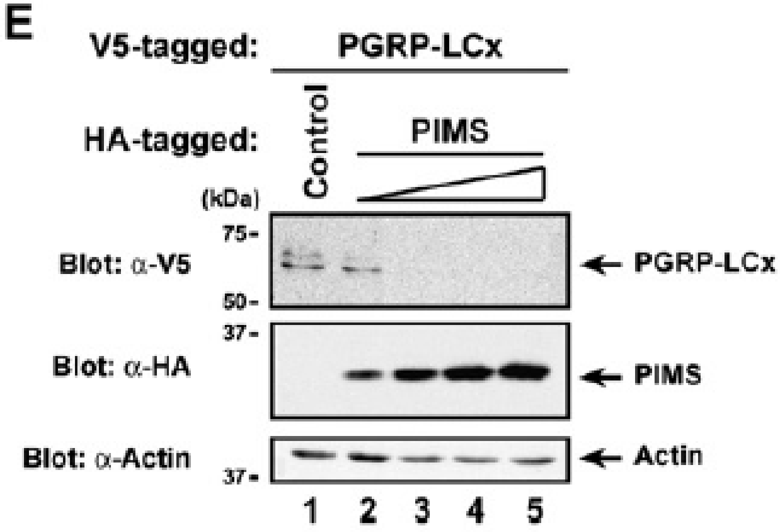
	Long	Short	Int.
Drosophila	6	7	0
Anopheles	4	3	0
Human	1	1	2
Mouse	1	1	2
C. elegans	0	0	0



PIMS Modulates Immune Tolerance by Negatively Regulating *Drosophila* Innate Immune Signaling

Nouara Lhocine,[1,7] Paulo S. Ribeiro,[2,6,7] Nicolas Buchon,[3] Alexander Wepf,[4,5] Rebecca Wilson,[2] Tencho Tenev,[2] Bruno Lemaitre,[1,3] Matthias Gstaiger,[4,5,8] Pascal Meier,[2,8,*] and François Leulier[1,8,*]

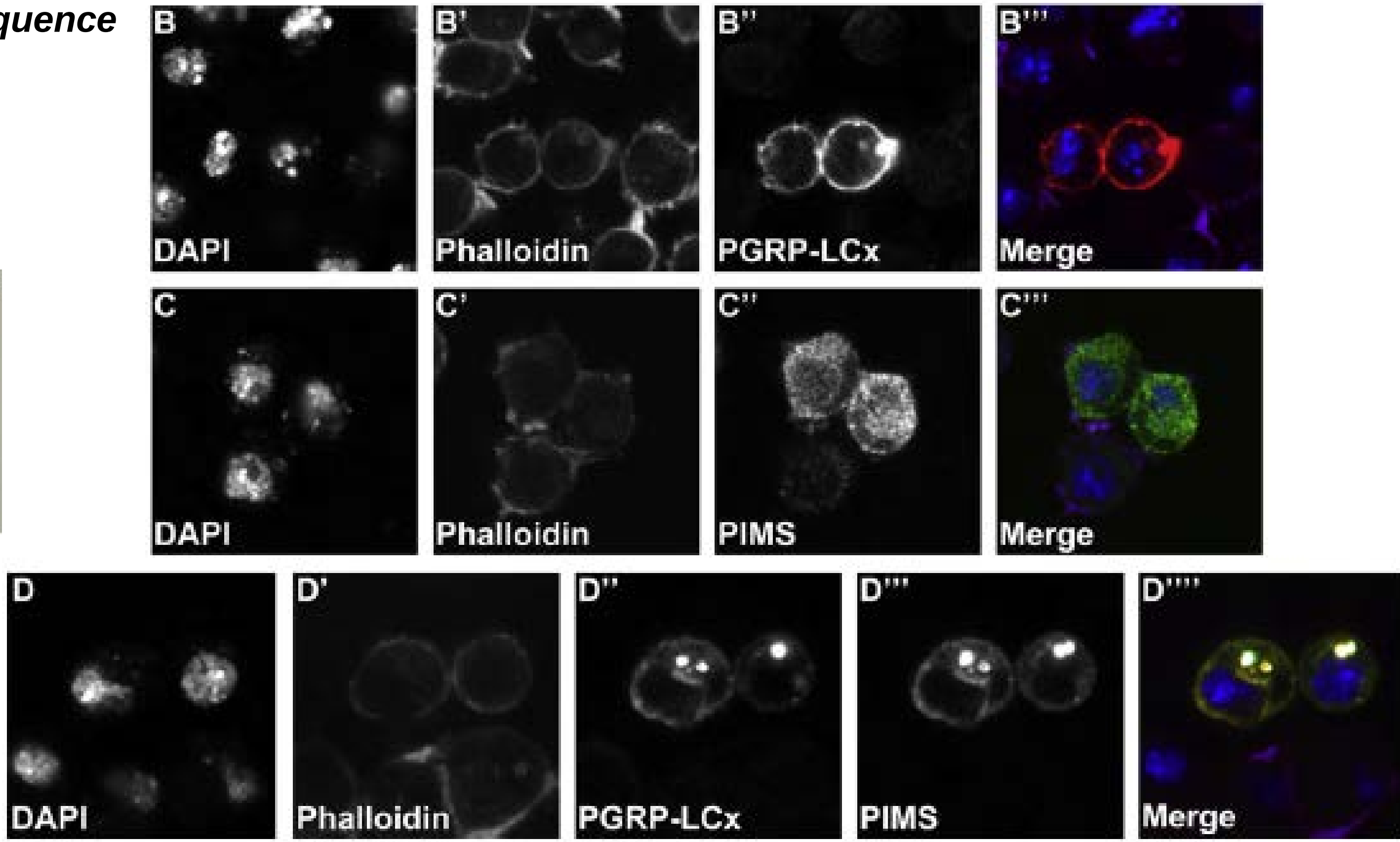
Cell Host & Microbe *4*, 147–158, August 14, 2008

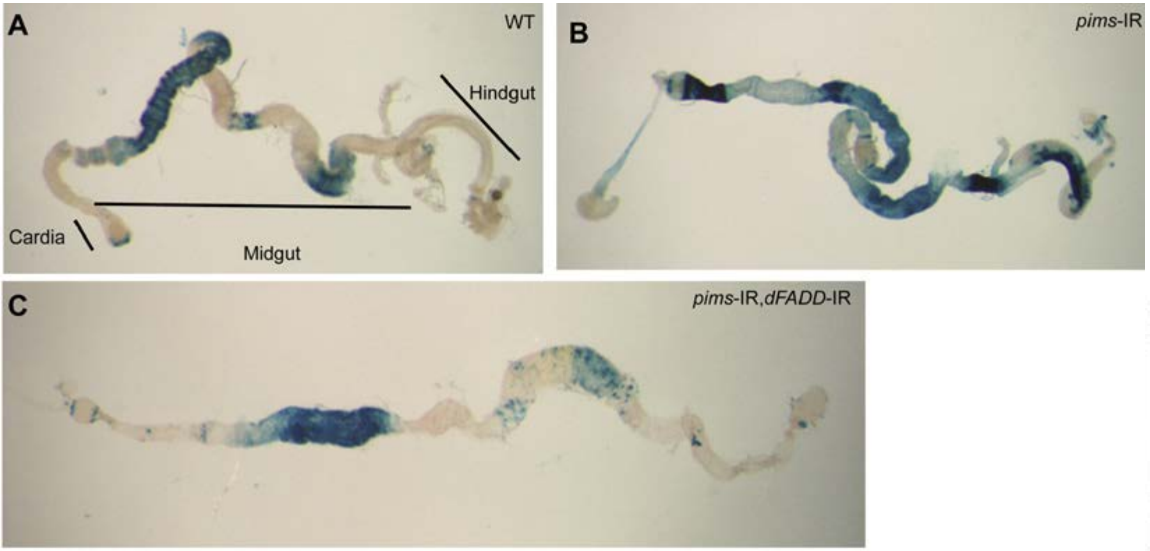


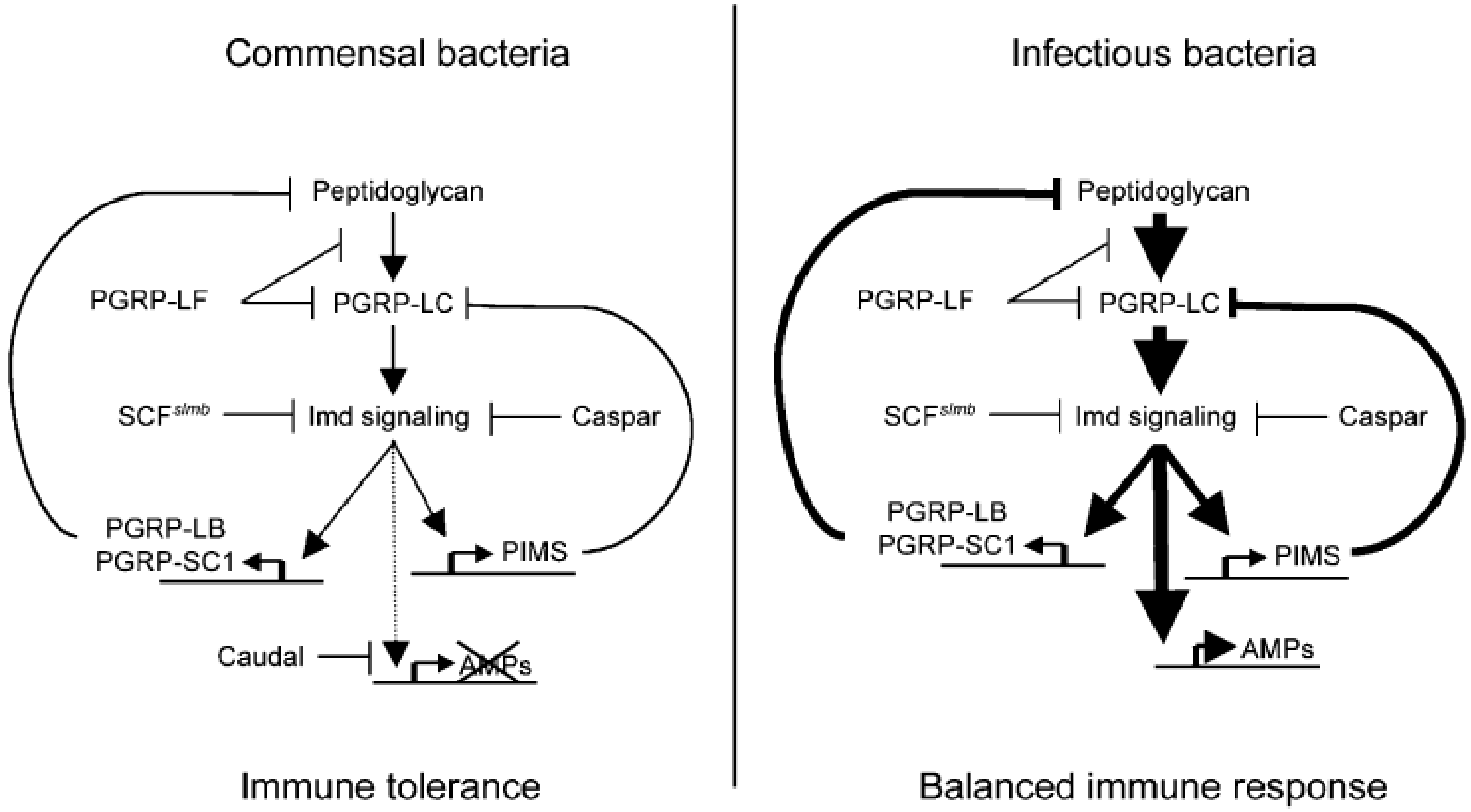
PGRP LC-interacting inhibitor of Imd signaling (PIMS) =
= poor Imd response upon knock-in

Pirk (= PIMS) is a 197-aa protein with no recognizable signal sequence or previously characterized domain structure.

No Pirk homologues have been described from other species






Commensal bacteria
Peptidoglycan
PGRP-LF
PGRP-LC
SCF^{slmb}
Imd signaling
Caspar
PGRP-LB
PGRP-SC1
PIMS
Caudal
AMPs
Immune tolerance
Infectious bacteria
Peptidoglycan
PGRP-LF
PGRP-LC
SCF^{slmb}
Imd signaling
Caspar
PGRP-LB
PGRP-SC1
PIMS
AMPs
Balanced immune response