Autocrine and paracrine unpaired signaling regulate intestinal stem cell maintenance and division

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Summary

The Janus kinase (JAK) signal transducer and activator of transcription (STAT) pathway is involved in the regulation of intestinal stem cell (ISC) activity to ensure a continuous renewal of the adult Drosophila midgut. Three ligands, Unpaired 1, Unpaired 2 and Unpaired 3 (Upd1, Upd2 and Upd3, respectively) are known to activate the JAK/STAT pathway in Drosophila. Using newly generated upd mutants and cell-specific RNAi, we showed that Upd1 is required throughout the fly life to maintain basal turnover of the midgut epithelium by controlling ISC maintenance in an autocrine manner. A role of Upd2 and Upd3 in basal conditions is discernible only in old gut, where they contribute to increased ISC abnormal division. Finally, upon an acute stress such as oral bacterial infection, we showed that Upd3 is released from enterocytes and has an additive effect with Upd2 to promote rapid epithelial regeneration. Taken together, our results show that Upd ligands are required to maintain the midgut homeostasis under both normal and pathological states.

Key words: Intestinal stem cells, JAK/STAT, Proliferation, Upd, Infection

Introduction

Recent studies on Drosophila intestinal stem cells (ISCs) have provided new insights into the regulation of stem cell activity as well as their role in maintaining tissue homeostasis. The Drosophila midgut contains multi-potent ISCs that undergo self-renewal throughout adult life and give rise to transient progenitors, the enteroblasts, that differentiate gradually into either absorptive enterocytes (ECs) or secretory entero-endocrine cells (Micchelli and Perrimon, 2006; Ohlstein and Spradling, 2006). The turnover of differentiated cells is continuous; the entire midgut being renewed within ~7–10 days (Micchelli and Perrimon, 2006). In addition to their function in basal gut maintenance, ISCs are also critical to repair intestinal damage. In Drosophila, ingestion of cytotoxic compounds or exposure to enteric pathogens cause the loss of gut cells, which promotes ISC proliferation and differentiation to repair the epithelium damage (Amcheslavsky et al., 2009; Buchon et al., 2009a; Chatterjee and Ip, 2009; Jiang et al., 2009).

The Janus kinase/signal transducer and activator of transcription (JAK/STAT) and the epidermal growth factor receptor (EGFR) pathways both regulate ISC-mediated epithelium renewal. It has been shown that gut damage induces the production of secreted ligands of Unpaired and EGF families, which activate respectively the JAK/STAT and EGFR pathways in progenitors to promote their division and differentiation, thereby establishing homeostatic regulatory loops (Buchon et al., 2009a; Buchon et al., 2010; Cronin et al., 2009; Jiang et al., 2009; Jiang et al., 2011). While a wide array of cytokines regulate the JAK/STAT pathway in mammals, only three ligands of the Unpaired family (Upd1, Upd2 and Upd3) are known to activate Domeless, the unique receptor of the JAK-STAT pathway in Drosophila (Agaisse et al., 2003; Harrison et al., 1998). The function of Unpaired ligands in the adult midgut has been previously investigated using either a upd3 RNAi transgene or a Drosophila line carrying upd1 hypomorphic alleles (Buchon et al., 2009a; Lin et al., 2010). However, no clear picture has emerged concerning their expression pattern in the midgut and their individual roles in gut homeostasis. Here, we used both RNAi approach and new Drosophila lines deleted for upd2 and/or upd3 to unravel the function of all the three Upds in the renewal of midgut epithelium.

Results and Discussion

upd genes are differentially expressed in the adult midgut

Previous study has shown that oral infection with the pathogenic bacterium Erwinia carotovora carotovora 15 (Ecc15) damages the gut epithelium and induces upd3 expression in enterocytes, resulting in the proliferation of ISCs and gut repair (Buchon et al., 2009a). We extended this study by quantifying the expression levels of all upds in 5-day-old flies (referred to as unchallenged) or flies collected 4 hours and 16 hours after infection with Ecc15. Fig. 1A shows that all the upd ligands were upregulated in the midgut upon Ecc15 infection, upd3 being the most induced. To determine the sources of Upds in the midgut, we used transcriptional reporters, in situ hybridization and in vivo RNAi approaches. Both upd1 reporters (upd1-Gal4 >UAS-GFP and upd1-lacZ) and in situ hybridization experiments showed that upd1 is expressed in small-nucleated cells corresponding to progenitors (ISCs and enteroblasts) (Fig. 1B and supplementary...
material Fig. S1A,B). Following Ecc15 oral infection, the number of cells expressing upd1-GFP slightly increased compared to the unchallenged condition. In contrast, upd3-GFP reporter (upd3-Gal4 > UAS-GFP) is weakly expressed in large nucleated enterocytes under basal condition (Fig. 1B), but it is strongly induced in most enterocytes of Ecc15 infected flies. To confirm these results, we knocked-down each Upd ligand in either progenitor cells or enterocytes using the thermosensitive Gal4 drivers Escargot-Gal4a and MyoIA-Gal4a, respectively. We then quantified by RT-qPCR the expression levels of individual upd transcripts in unchallenged and Ecc15-infected guts. We observed a decrease in upd1 and upd2 expression when these genes were silenced in progenitors while both upd2 and upd3 expression was decreased when downregulated in enterocytes (Fig. 1C-E). Importantly, the knock-down of each upd in visceral muscles (How-Gal4a > UAS-RNAi) did not affect their expression (supplementary material Fig. S1C). Taken together, our data suggest that upd1 is expressed only in progenitor cells in contrast with a previous report indicating that Upd1 is expressed autonomously ISC proliferation (Lin et al., 2010). As upd2 expression was altered when silenced with both progenitor and enterocyte drivers, our results indicate that upd2 could be produced in both progenitors and enterocytes or in young differentiating enterocytes. Finally, our study confirmed that upd3 expression is induced in enterocytes upon infection. To track the localization of Upd3, we generated a transgenic line expressing the upd3 coding sequence tagged with GFP under the control of the upd3 promoter. Although Upd3GFP was not detected in the gut of unchallenged flies, a punctate staining was observed in the basal side of the midgut epithelium after Ecc15 infection (supplementary material Fig. S2). This is consistent with the notion that Upd3 is released from enterocytes upon cellular damage to non-autonomously activate the ISCs scattered basally in the midgut epithelium.

Upds impact JAK/STAT transcriptional activity in the midgut

The three upd genes are clustered within a 70 kb genomic region in the proximal part of the X chromosome. It has been reported that a null mutation in upd1 induces embryonic lethality similar to mutations affecting the JAK kinase (hopscotch) and the STAT transcription factor (Harrison et al., 1998). To determine the function of the other Upd ligands in the midgut, we generated Drosophila lines deleted for either upd2 or upd3 that we named upd2- and upd3- respectively (supplementary material Fig. S3). Although separated by only 32 kb, we were able to establish a upd2- and upd3- double mutant line (upd2,3-) by meiotic recombination (supplementary material Fig. S4). upd2-, upd3- and upd2,3- were viable, fertile and did not show any major developmental defect, revealing that Upd1 is the only Unpaired required for viability. RT-qPCR experiments on gut extracts showed that upd3 was strongly induced in most enterocytes of Ecc15 infected guts, whereas upd3-GFP is strongly induced in enterocytes upon Ecc15 infection. Arrows indicate progenitor cells. (C–E) upd genes were knocked down with RNAi (IR) either in progenitors or in enterocytes of the gut using the thermosensitive Gal4 drivers Escargot-Gal4a and MyoIA-Gal4a, respectively. upd expression was then quantified by RT-qPCR. Mean ± s.e.m. of three experiments are shown. *P<0.05, **P<0.001; Student’s t-test.

Fig. 1. upd genes are differentially expressed in the adult Drosophila midgut.

(A) RT-qPCR experiments show that upd genes are induced in the gut of wild-type flies orally infected with Ecc15, compared with unchallenged (UC).

(B) Immunostaining with an anti-GFP reveals that upd1-GFP reporter is expressed in small-nucleated cells in both unchallenged and Ecc15-infected guts, whereas upd3-GFP is strongly induced in enterocytes upon Ecc15 infection. Arrows indicate progenitor cells. (C–E) upd genes were knocked down with RNAi (IR) either in progenitors or in enterocytes of the gut using the thermosensitive Gal4 drivers Escargot-Gal4a and MyoIA-Gal4a, respectively. upd expression was then quantified by RT-qPCR. Mean ± s.e.m. of three experiments are shown. *P<0.05, **P<0.001; Student’s t-test.

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enterocytes (Fig. 2D). In contrast to wild-type and upd2
flies, no expansion of STAT-GFP signals was observed upon
Ecc15 infection in the midgut of upd3
flies with reduced
expression (esgts
; Fig. 2D).

In addition to its role in epithelium renewal, the JAK-STAT
pathway contributes to antimicrobial defense in the gut since it
regulates the expression of the antimicrobial peptide
drosomycin-3 (dro3) (Buchon et al., 2009b). RT-qPCR analysis showed that
both Upd2 and Upd3 contribute to dro3 expression in the midgut
of Ecc15 infected flies (Fig. 2E); Upd3 having stronger effect. To
further characterize the immune role of JAK-STAT in the gut, we
established a reporter transgenic fly line containing a fusion
between the GFP gene and 657 bp of the dro3 regulatory
sequence upstream the start codon (dro3-GFP). Immunostaining
with an anti-GFP antibody revealed almost no expression of
dro3-GFP in the midgut of unchallenged flies (Fig. 2F).

Interestingly, dro3-GFP was highly induced after Ecc15
infection in enterocytes of the anterior midgut (Fig. 2F, and
data not shown). Consistent with the RT-qPCR results, Ecc15-
induced dro3-GFP expression was strongly reduced in upd3
and to a lesser extent in upd2
(Fig. 2F). Altogether, we provide
evidence that Upd3 is the main cytokine controlling the immune
function of JAK-STAT in the gut.

Upd1 but not Upd2 or Upd3 is required for ISC maintenance
To explore the contribution of each Upd in basal midgut turnover,
we first used a flip-out lineage system, referred to as esg
(Jiang et al., 2009). This technique enables the conditional knock
down of individual ups in ISCs and their subsequent lineage,
which allows tracking of both ISC proliferation and the
differentiation of their newborn progeny. We found that
epithelium turnover was blocked in flies with reduced expression
of upd1, as shown by the lack of esg
clonal expansion at 8 days
following the depletion (Fig. 3A). In contrast, using the esg
system, the knock-down of upd2 or upd3 did not affect ISC
proliferation and differentiation, as illustrated by the appearance of
newly synthesized GFP-positive enterocytes along the gut (Fig. 3A).
Importantly, no escargot positive cells were detectable
when upd1 was silenced for 3 weeks in the midgut whereas
inactivating upd2 or upd3 did not disrupt ISC activity (Fig. 3A).
This highlights a specific role of Upd1 in the maintenance of ISC
activity along the midgut in unchallenged condition. We further
investigated the contribution of each Upd in basal midgut renewal
by counting the number of dividing ISCs using an anti-phospho-
histone H3 (anti-PH3) antibody. In agreement with the results
above, upd1 knockdown in ISCs strongly inhibited ISC division, as
evidenced by the absence of any PH3 positive cells compared to
wild type (Fig. 3B). Moreover, we found a low number of PH3-positive cells in midguts of upd2\textsuperscript{D}, upd3\textsuperscript{D} and upd2,3\textsuperscript{D} 5-day-old flies as in wild type (Fig. 3C).

In old flies, a deterioration of the intestinal epithelium is reflected by an aberrant enterocyte differentiation and excessive ISC proliferation. This deregulation of gut homeostasis correlated with increased signaling activities of JNK, p38b and PDGF pathways (Biteau et al., 2008; Choi et al., 2008; Park et al., 2009). We tested whether Upd ligands are part of the aberrant ISC division in aged flies. As previously reported, we observed a 10-fold higher number of PH3 positive cells in the midguts of 40-day-old flies in comparison to 5-day-old flies (Fig. 3B,C). In contrast, the number of PH3 positive cells was reduced in upd2\textsuperscript{D} and upd3\textsuperscript{D} compared to wild-type old flies (Fig. 3C) and nearly abolished in old flies lacking upd1 in progenitors (esg\textsuperscript{ts} > upd1-IR, Fig. 3B). Taken together, our data highlight the requirement of Upd1 in maintaining basal gut homeostasis throughout life and reveal that Upd2 and Upd3 are involved in the increased ISC proliferation during aging.

Upd2 and Upd3 regulate the gut renewal upon bacterial infection

We finally explored the relative role of Upds in infection-induced midgut renewal. While increased ISC proliferation was observed in wild-type flies infected orally with Ecc15 (Fig. 4A), the upd2\textsuperscript{D} and upd3\textsuperscript{D} mutations significantly decreased the number of mitotic ISCs detected by anti-PH3 staining (Fig. 4A). Furthermore, both Upd2 and Upd3 contributed in an additive manner to increase the level of ISC activity as the double mutant upd2,3\textsuperscript{D} displayed a lower proliferative response than each single mutant. Consistent with the role of Upd1 in ISC maintenance shown above, epithelium renewal

Fig. 3. Upd1 regulates intestinal stem cell maintenance. (A) Knockdown of each upd gene with the esg\textsuperscript{ts} system shows that Upd1 but not Upd2 or Upd3 is required for the gut turnover and maintenance of ISCs in basal condition. Progenitor cells and their progeny are marked with an anti-GFP staining. Guts were collected 8 days or 21 days after flipase induction at 29°C. (B,C) Quantification of PH3-positive cells per midgut of unchallenged (UC) young flies shows that esg\textsuperscript{ts} > upd1-IR flies have a lower mitotic index compared to WT controls and upd2\textsuperscript{D}/ upd3\textsuperscript{D} single and double mutants. However, all Upd ligands promote ISC division in 30-day-old flies. Mean ± s.e.m. of three repeats are shown. **P < 0.01, ***P < 0.001; Student’s t-test.

Fig. 4. The three Upds promote epithelium renewal upon Ecc15 oral infection. (A,B) Quantification of PH3-positive cells per midgut of flies dissected 8 hours after Ecc15 ingestion reveals that upd2 and upd3 promote ISC division in an additive manner (A), whereas upd1 is absolutely required for ISC division (B). Mean ± s.e.m. of three repeats are shown. **P < 0.01; Student’s t-test. (C) Survival analysis of females orally infected with Ecc15 at 29°C reveal a lower survival rate of esg\textsuperscript{ts} > upd1-IR, upd3\textsuperscript{D} and upd2,3\textsuperscript{D} flies compared with wild type. Results are pooled data from four independent experiments, n=20 flies per genotype.
was blocked in infected guts with upd1 knocked down (esg<sup> GFP</sup> >upd1-IR, Fig. 4B). Hence, these experiments demonstrate that the three Upds participate in midgut regeneration, with a particular requirement for Upd1 in basal epithelium renewal.

The rapid regeneration of the midgut epithelium is considered as an essential component of the <i>Drosophila</i> defense against <i>Ecc15</i> infection, as reduced JAK-STAT signaling in progenitors resulted in lower ISC proliferation and flies succumbed to <i>Ecc15</i> few days following infection (Buchon et al., 2009a). To assess the role of each Upd in the resistance to <i>Ecc15</i>, we monitored the survival rate of flies lacking Upds after <i>Ecc15</i> ingestion. Fig. 4C shows that upd3<sup>−</sup> and to a lesser extent esg<sup> GFP</sup> >upd1-IR flies succumbed within 10–15 days post-infection whereas upd3<sup>−</sup> only showed a mild susceptibility. Once more, upd2,3<sup>−</sup> displayed higher susceptibility than single mutant flies (Fig. 4C). These data indicate that all Upds contribute to host survival upon oral bacterial infection.

**Concluding remarks**

Using newly generated fly stocks deleted for <i>upd2</i> and/or <i>upd3</i> in addition to an RNAi approach, we investigated the role of three JAK/STAT ligands in the regeneration of an adult homeostatic tissue, the <i>Drosophila</i> midgut. Our study showed that Upd1 is produced by ISCs to control the basal activity of ISCs ensuring gut epithelium turnover. In absence of <i>upd1</i> expression, ISCs failed to divide and were undetectable after 3 weeks, highlighting an important role of Upd1 in maintaining ISCs. In addition, we demonstrated that Upd3 is released from enterocytes, and is required to stimulate <i>Drosophila</i> ISC division in response to infection. Moreover, we show that Upd2 and Upd3 exhibit an additive effect in inducing ISC proliferation in infected guts. A role of Upd2 and Upd3 in absence of infection is however discernible in old flies, where they are involved in the increased ISC abnormal division probably as a response to the senescence or to the age related disruption of enterocytes. Collectively, our data draw a model in which Upd1, in agreement with its developmental function, ensure the maintenance of ISCs while Upd2 and Upd3 induce the replenishment of enterocytes upon damage, functioning as global stress sensors in the gut epithelium. Thus, the three ligands allow ISCs to integrate signals from different cell populations and fine-tune their activity depending on gut integrity and environment. However, we still do not fully understand how three distinct Upds can interact with the same receptor and mediate different ISC behaviors. It has been suggested that Upd1 strongly interacts with the extracellular matrix, whereas Upd2 is more diffusible (Hombria et al., 2005). Therefore, the different biophysical properties of Upds and/or their varying capacities to activate JAK/STAT signaling in terms of amplitude and duration could explain how different cytokine signaling are integrated together (Wright et al., 2011). In agreement, our findings suggest that Upd1 has a low range of action to maintain in a cell-autonomous manner basal ISC activity while Upd3 diffuses from its enterocytes source to regulate ISCs, which are scattered basally in the midgut. As different cell types express Upds, it would be interesting to unravel their differential transcriptional regulation and to better characterize the crosstalk between the Upd/JAK/STAT cascade and other signaling pathways in maintaining midgut homeostasis. Finally, our study revealed an immune role of Upd2 and Upd3 in the midgut upon an oral bacterial infection. We showed that Upd3 and to a lesser extent Upd2 are involved in the transcriptional regulation of the antifungal peptide Dro3. Moreover, using a newly generated dro3-GFP reporter, we found that the regulation of dro3 by JAK-STAT is confined to the anterior compartment of the gut. How the Upd/JAK/STAT signaling pathway displays distinct immune and homeostatic activities in the adult midgut remains to be elucidated.

**Materials and Methods**

**RT-qPCR**

Total RNA was extracted from 15 guts (including the crop, the cardia and the midgut) with Trizol reagent (Invitrogen) and dissolved in 10 μl of RNase-free water. cDNA was then synthesized from 1 μg total RNA using SuperScript II enzyme (Invitrogen) and qPCR was carried out using a LightCycler 2.0 and the SYBR Green I kit (Roche). Relative expression data were normalized to <i>Rpl32</i>.

**Immunostaining and in situ hybridization**

<i>Drosophila</i> guts were fixed for 30 minutes in PBS 0.1 Tween 4% paraformaldehyde. Blocking (1 h), washing (10 min each) and 4°C overnight incubation with primary and secondary antibodies were performed in PBS 0.1 Tween 1% BSA. Antibodies used were 1:1000 mouse anti-GFP (Roche), 1:300 mouse anti-β-galactosidase (Promega), 1:1000 rabbit anti-PH3 (Millipore), 1:500 Alexa-488 anti-mouse and 1:500 Alexa-594 anti-rabbit (Invitrogen). 1:15,000 DAPI (Sigma) was used to stain DNA. Samples were scanned with an Axioplan imager (Zeiss). Digoxigenin-labeled <i>upd1</i> RNA probe were used for in situ hybridization as described by (Tsai and Sun, 2004). In situ hybridization experiments were performed as previously described (Osman et al., 2009 and see supplementary material Fig. S1 legend).

**Infection, survival experiments and fly stocks**

<i>Erwinia carotovora carotovora</i> 15 (<i>Ecc15</i>) bacterium was grown in LB medium at 29°C overnight. For oral infection, and after starvation 3 h at 29°C, flies were flung onto fly medium covered with filter disks soaked in a 1:1 mix of bacterial pellets at O<sub>D</sub>.<sub>600</sub> 200 and 2.5% sucrose. Flies were then left to feed on this mix 4 h and 16 h for RT-qPCR or immunostaining experiments, and 24 h for survival analysis before being flung onto a fresh fly medium. For fly stocks and crosses, see supplementary material Table S1.

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**References**


Unpaired cytokines regulate *Drosophila* midgut homeostasis
**Fig. S1. upd1 is expressed in the midgut progenitor cells but not in the visceral muscles.** (A) Immuno-staining with an anti-β-galactosidase antibody reveals that upd1-lacZ reporter is expressed in small-nucleated progenitor cells in unchallenged guts. (B) An *in situ* hybridization experiment confirms that *upd1* is expressed in progenitor cells but not in visceral muscles. Arrows indicate two progenitor cells expressing *upd1* mRNA. (C) Each *upd* gene was knocked down by RNAi (IR) in muscles surrounding the midgut using the thermosensitive Gal4 drivers *How-Gal4* ts. *upd* expression was then quantified by RT-qPCR in unchallenged (UC) flies or flies collected 4 hrs and 16 hrs after *Ecc15* oral infection. Data are representative of at least 3 independent experiments, Mean ± SEM are shown. Methods related to S1B: DNA template for the *upd* probe was derived from *upd1* coding region by PCR (primers designed were 5'-GAATTCATGGCTCGTCCGCTCCT-3' and 5'-TCTAGATCACGTGCGCTGCACGCG-3') as described in Tsai and Sun, 2004. For *in situ* hybridization experiments, guts were fixed for 30 min in 4% formaldehyde 5 mM EGTA diluted in PBS (fixation solution), rinsed twice with PBS, rinsed 5 times in methanol, washed 5 times in ethanol, washed 1 hour in 1:1 Xylenethanol, rinsed 5 times in methanol, post-fixed 30 min in fixation solution. After several washes in PBS-0.1% Tween 20 (PBST), gut were incubated with proteinase K and treated for 5 min in glycine solution (2 mg/ml). The reaction was stopped by performing several washes with cold PBST. Guts were then incubated with fixation solution overnight at 4°C and washed with PBST. Guts were pre-incubated for 2 h at 60°C in Hybridization Buffer (HB: 50% formamide, 2X SSC, 1 mg/ml Torula RNA, 0.05mg/ml Heparin, 2% Roche blocking reagent, 0.1% CHAPS, 5mM EDTA, 0.1% Tween 20). After incubation overnight at 60°C with *upd1* anti-sense DIG-labeled RNA probe diluted in HB, guts were washed with HB and PBST solutions, blocked with PBST-1% BSA and incubated overnight at 4°C with sheep anti-DIG primary antibody conjugated to alkaline phosphatase (1:2000; Roche). After several washes with PBST, the *in situ* hybridization signal was revealed with NBT/BCIP kit (Promega).
**Fig. S2. Induction and diffusion of the Upd3 protein upon Ecc15 infection.** The upd3-Upd3GFP is an Upd3GFP fusion under the control of the upd3 promoter. Immunostaining with an anti-GFP revealed puncta at the basal side of the gut of flies collected 4 hrs and 16 hrs after Ecc15 infection.

**Fig. S3. Generation of upd2 and upd3 null mutants.** Schematic representation showing the upd2 and upd3 locus as well as the P elements used to induce the genomic deletions. P{XP}03621 is inserted upstream of upd2 and PBac{WH}f04435 locates downstream of upd2. Both contain FRT and mini-white+ gene. upd2Δ is a deletion of 4.7 kb that was generated by FLP/FRT recombination between P{XP}03621 and PBac{WH}f04435 by screening for white eye phenotype. We confirmed that the coding region of upd2 was deleted in the upd2Δ mutant by PCR on genomic DNA. The upd3Δ mutant was generated by imprecise excision of P{XP}d00871, which is inserted in the third intron of the upd3 gene. PCR and sequencing experiments showed that 13,464 bps upstream of the p{XP} d00871 insertion site were deleted in upd3Δ, removing the first three exons of upd3.
Fig. S4. Schematics for generation of the upd2,3Δ double mutant. (A) The transposon insertion sites of P{XP}03621, PBac{WH}f04435 and P{XP}00871 in the upd2 and upd3 loci. (B,C) To generate upd2,3Δ, the upd2 gene was deleted in the upd3Δ mutant background by FLP-FRT mediated deletion. Firstly, meiotic recombinants of upd3Δ and P{XP}03621 were generated. upd3Δ showed small eye phenotype. Recombinant were screened on the [upd3Δ] small eye phenotype and [white+] P{XP}03621. Two recombinants were obtained from 9010 flies (B). Then the upd2,3Δ were generated by FLP–FRT based recombination between P{XP}03621, upd3Δ and PBac{WH}f04435 (C). An hybrid transposon between P{XP} and PBac{WH} is generated if the FLP-FRT mediated recombination occurs. The upd2,3Δ candidates were analysed by PCR on genomic DNA using upd2, upd3-gene specific and transposon-specific primers. RpL32 was used as an internal control (D). P{XP}03621, PBac{WH}f04435, P{XP}d00871 were from the Harvard Exelixis stock center.
Fig. S5. *upd2Δ* and *upd3Δ* mutants display no effect on *upd1* expression. RT-qPCR experiments on gut RNA extracts show that *upd2Δ* and *upd3Δ* single and double deletions do not affect the expression of *upd1* in unchallenged (UC) flies or flies collected 4 hrs and 16 hrs after *Ecc15* oral infection. Data are representative of at least 3 independent experiments, Mean ± SEM are shown.

Table S1. *Drosophila* stocks. Unless specified, all the experiments were done on 5-7 day old adult females, raised on a standard medium (maize flour, dead yeast, agar and fruit juice). *Cantons (Cans)* and *w1118* flies were used as wild-type controls. The F1 progeny expressing the UAS-RNAi constructs under the control of the different Gal4 drivers were maintained at 18°C until 3-4 days post hatching and then transferred to 29°C for maximum activity of the UAS/GAL4 system. To establish the *upd3-Upd3GFP* and *dro3-GFP* reporter lines, we amplified by PCR 1080 bp and 657 bp upstream of start codon of *udp3* and *dro3* genes respectively. Amplicons were merged then with the eGFP sequence by PCR overlap. These fragments were cloned into pDONR221 then into pCasper-DESTSV40 plasmid using the Gateway technology (Invitrogen™),