Cell-specific Imd-NFκB responses enable simultaneous antibacterial immunity and intestinal epithelial cell shedding upon bacterial infection

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SUMMARY

Intestinal infection triggers potent immune responses to combat pathogens and concomitantly drives epithelial renewal to maintain barrier integrity. Current models propose that epithelial renewal is primarily driven by damage caused by reactive oxygen species (ROS). Here we found that in Drosophila, the Imd-NFκB pathway controlled enterocyte (EC) shedding upon infection, via a mechanism independent of ROS-associated apoptosis. Mechanistically, the Imd pathway synergized with JNK signaling to induce epithelial cell shedding specifically in the context of bacterial infection, requiring also the reduced expression of the transcription factor GATAe. Furthermore, cell-specific NFκB responses enabled simultaneous production of antimicrobial peptides (AMP) and epithelial shedding in different EC populations. Thus, the Imd-NFκB pathway is central to the intestinal antibacterial response by mediating both AMP production and the maintenance of barrier integrity. Considering the similarities between Drosophila Imd signaling and mammalian TNFR pathway, our findings suggest the existence of an evolutionarily conserved genetic program in immunity-induced epithelial shedding.

KEYWORDS

Imd-NFκB signaling, innate immunity, enterocyte shedding, enteric infection, enhancer, JNK, GATAe, Drosophila
INTRODUCTION

Epithelial tissues such as the skin and the epithelial linings of the digestive tract form dynamic barriers between the body and the external environment. They perform diverse physiological functions while fending off constant challenge from a variety of factors including microorganisms. Damaged epithelial cells are shed from the epithelium and rapidly replenished to maintain tissue integrity through the action of stem cells. Stem cell proliferation and differentiation are tightly adjusted to compensate for the number of cells lost, so as to maintain an internal steady state known as tissue homeostasis (Blanpain and Fuchs, 2014). Recent studies have uncovered the complex mechanisms underlying stem cell activation and maintenance of tissue homeostasis, notably through feed-back signals sent from stressed epithelial cells to stem cells to promote their proliferation (Barker, 2014; Guo et al., 2016; Jiang and Edgar, 2012). While research has mostly focused on stem cells, epithelial cell shedding constitutes an integral part of epithelial turnover (Patterson and Watson, 2017; Vereecke et al., 2011). Aberrant epithelial cell shedding can lead to unsealed breaches and underlies inflammatory bowel diseases. However, the genetic program that epithelial cells use to sense damage and delaminate into the gut lumen is not well understood.

Research in Drosophila has provided insights into the maintenance of intestinal homeostasis and epithelial immunity (Buchon et al., 2013a; Guo et al., 2016). In the adult Drosophila midgut, intestinal stem cells (ISCs) differentiate into either polyploid absorptive enterocytes (ECs) or diploid secretory enteroendocrine cells (EEs). Differentiating ISC daughter cells called enteroblasts (EBs) are precursors of ECs, and ISCs and EBs are collectively referred to as midgut progenitors. In Drosophila, enteric infection rapidly leads to EC death through shedding into the gut
lumen (Buchon et al., 2010). Infection-induced EC death in *Drosophila* is so far largely attributed to the production of reactive oxygen species (ROS) by the *Drosophila* NADPH Dual Oxidase (Duox). While ROS neutralize invading microbes, they are also believed to damage ECs leading to their elimination (Buchon et al., 2009a; Lee et al., 2013).

In addition to triggering ROS production, enteric infection also activates the immune deficiency (Imd) pathway in the *Drosophila* gut (Buchon et al., 2013a). This pathway regulates the transcription of genes encoding antimicrobial peptides (AMPs) in the gut during ingestion of pathogenic bacteria or in response to beneficial gut microbiota. Imd signaling is triggered by the recognition of diaminopimelic acid (DAP)-type peptidoglycan, a component of the cell wall of Gram-negative bacteria and *Bacillus* species. Peptidoglycan sensing is mediated through the surface-bound pattern recognition receptor PGRP-LC or the cytosolic receptor PGRP-LE. Activation of these receptors initiates a complex signaling cascade, involving the adaptor protein Imd, the caspase 8-like protease Dredd, the E3 ubiquitin ligase Diap2, the MAPKK kinase dTAK1 and the IKK complex and eventually leads to the activation and cleavage of the NFκB-like transcription factor Relish (Rel) (Kleino and Silverman, 2014). The N-terminal part of Relish then translocates into the nucleus to induce the transcription of genes coding AMPs (*e.g.* *Diptericin* (*Dpt*)) and negative regulators of the pathway including *pirk* and the amidase *PGRP-LB*.

Transcriptomic analyses have revealed that the Imd pathway regulates not only the production of AMPs in the gut, but also genes not associated with immune functions (Broderick et al., 2014; Buchon et al., 2009b; Erkosar et al., 2014), suggesting that this pathway executes non-immune programs (reviewed in Zhai et al., 2017b). Indeed, Imd signaling is implicated in apoptosis (Georgel et al., 2001), and
some of the Imd components have been implicated in cell death in non-immune contexts, such as eliminating unfit cells during cell competition (Meyer et al., 2014) and neuronal cell death (Petersen et al., 2012). In the adult Drosophila midgut, increased Imd activity upon infection (Buchon et al., 2009b; Jiang et al., 2009) or upon loss of negative regulators (Mistry et al., 2017; Paredes et al., 2011; Ryu et al., 2008) is also associated with cell death. Yet, the molecular mechanisms linking elevated Imd immune signaling to cell death in the intestinal epithelium are not known.

Here we found that the Imd pathway controlled EC shedding upon bacterial infection. By analyzing the cis-regulatory sequence of the unpaired 2 (upd2) gene, we identified an enhancer sequence that was turned on specifically in damaged ECs upon bacterial infection. This sequence harbored an NFκB motif and could be used as a marker to visualize shedding ECs. Using this reporter, we found that the Imd pathway was not only involved in the antibacterial immune response but also contributed to EC shedding upon enteric infection. EC shedding upon bacterial infection required both the Imd and JNK pathways and was negatively regulated by the GATAe transcription factor. Thus, the Imd pathway enables a dual response to infection via both promoting the production of AMPs and through regulating epithelial cell shedding to ensure appropriate epithelial turnover and the maintenance of barrier integrity during infection.

RESULTS

An Infection-Inducible Enhancer of upd2 Marks Delaminating ECs
Enteric infection with the Gram-negative bacterium *Erwinia carotovora* 15 (*Ecc15*) increases the rate of intestinal epithelial renewal in *Drosophila*, a process that involves the shedding of ECs and the production of new epithelial cells by resident stem cells (Buchon et al., 2010). Intestinal homeostasis is maintained by a feedback loop in which damaged ECs promote stem cells to divide and differentiate through the release of secreted factors. Notably, secretion of the Upd2 and Upd3 non-cell-autonomously activate the JAK-STAT pathway to stimulate stem cells by binding to the cell surface receptor Domeless (Dome), a homolog of JAK receptors, in the neighboring ISC and EBs (Buchon et al., 2009b; Jiang et al., 2009) (Fig 1A). We hypothesized that cis-regulatory elements of these *Upd* genes harbor a combination of binding sites for transcription factors activated upon damage.

To better understand the damage-sensing program involved in EC shedding, we systematically surveyed the regulatory sequences over a 6 kilobase pair (kb) region upstream of the *upd2* coding sequences (Fig 1B). Transgenic reporter lines were generated to study the enhancer activities of these fragments *in vivo* under both basal (i.e. unchallenged) conditions and oral infection with *Ecc15*. Two DNA fragments (*upd2_A* and *upd2_B*) showed enhancer activity in midgut progenitors, while the *upd2_D* sequences drove reporter expression in a subset of EEs in the middle midgut, under both conditions. We also identified a 1kb region, *upd2_C*, which conferred inducible reporter expression. The *upd2_C* reporter gene showed almost no expression under basal conditions but was strongly induced in ECs after oral infection with *Ecc15* (Fig S1A). A 498bp sub-region of *upd2_C*, the *CB* fragment, recapitulated the expression profile of *upd2_C*. Further dissection of the *CB* enhancer allowed us to identify a 204bp fragment that we named *CBM* (*CB* minimal enhancer), which completely recapitulated the enhancer activity of the *CB* fragment (Fig S1B).
In contrast, a GFP reporter driven by three other sub-fragments of upd2_CB (CB_S1-3) did not show any expression in both conditions. Examination of the CBM sequences revealed the presence of conserved binding sites for the JNK transcription factors API (TGANTCA), GATA factor (GATAR) and homeobox protein (TTATT or TAATT) (Fig 1B and S1C). The CBM fragment also harbored an NFkB motif (GGGRNNYYYY), which is usually found in the regulatory DNAs of immune responsive genes.

The midgut of 4-10-day-old adult flies carrying one copy of CB transgenic reporter was formed by a mono-layered epithelium with few dying cells occasionally found in the gut lumen (Fig 1C). Oral infection of flies with Ecc15 caused massive EC shedding starting from 4 hours post infection (Fig S1D). Dying ECs present in the gut lumen became easily detectable at 8-12 hours post infection (Fig 1D). The EC identity of the delaminating cells was confirmed by their large nuclear size, apical localization, expression of EC maker Myo1A>GFP and absence of the progenitor marker esg::GFP (Fig 1D-E and S1E). Nearly all the ECs at the early stages of detaching from the epithelium expressed the CB-mCherry reporter (Fig 1E), indicating that the CB enhancer is specifically activated in shedding ECs. However, ECs that had been shed into the gut lumen did not appear to consistently maintain CB-mCherry expression. The dynamic reporter expression in shedding and/or shed ECs was further supported by quantitative measurements of CB reporter levels in ECs according to their basal to luminal positions (Fig S1F). Analyses of nuclear morphology revealed that ECs detached from the epithelium without any sign of apoptosis, but underwent cell death at a later step of shedding in the lumen (orange arrows, Fig 1E and S1E). Shed ECs displayed classic apoptotic features, including the ring or necklace-shaped chromatin condensation (white arrows, Fig 1E and S1E)
followed by nuclear collapse and disassembly (yellow arrows, Fig 1E and S1E) (Tone et al., 2007). EC shedding as revealed by the induction of the CB>CD8GFP reporter was more prominent in the posterior midgut (R4bc according to Buchon et al., 2013b) and was associated with a strong contraction of the visceral muscle (Fig S1G). Thus, upd2_CB provides a valid marker of shedding ECs as well as a tool to uncover the genetic program leading to EC shedding. In the next sections of the paper, we used the CB reporter to visualize EC shedding. However, it is important to note that upd2 itself is not required for the EC shedding process. In fact, the JAK-STAT receptor and therefore the pathway activity is restricted to progenitors, excluding a role of this pathway in ECs.

**The upd2_CB Enhancer is Specifically Activated by Gram-negative Bacteria**

Oral infection with Ecc15 activates the Imd pathway (Fig 2A) as well as the production of ROS through the activity of the NADPH oxidase Duox (Dual oxidase). A series of experiments demonstrated that the CB element was not activated by ROS but by determinants associated with Gram-negative bacteria. Oral infection with the uracil-deficient Ecc15 strain, which does not activate Duox (Lee et al., 2013), still activated the CB>CD8GFP reporter to the same extent as a wild-type strain of Ecc15 (Fig 2B-C). In addition, RNAi of Duox in ECs did not attenuate Ecc15 infection-induced Imd activation, CB-mCherry reporter expression or EC shedding (Fig S2A-B). Moreover, the CB element was also induced by a derivative of the Gram-negative bacterium Pseudomonas entomophila, P. entomophila gacA, which is completely avirulent but retains the capacity to trigger Imd signaling (Liehl et al., 2006) (Fig S2C). In contrast, ingestion of either a Gram-positive bacterium, Micrococcus luteus for 12 hours (Neyen et al., 2014) or 10% dextran sulfate sodium (10% DSS), a corrosive agent that damages the intestine (Amcheslavsky et al., 2009), for 36 hours...
did not activate the reporter (Fig 2D-E). Oral infection with the Gram-negative *Serratia marcescens* Db11 strain, which causes thinning of the gut epithelium without inducing EC death or Imd activity (Lee et al., 2016), failed to induce CB reporter expression (Fig S2D). Collectively, these data suggest that CB reporter expression in delaminating ECs is specifically induced by determinants associated with Gram-negative bacteria and activation of the Imd pathway.

The CB Reporter is Regulated by the NFκB-like Transcription Factor Relish

We next investigated whether the Imd pathway regulates the CB reporter. This pathway can be activated by oral bacterial infection or by over-expressing *PGRP-LC* in ECs. RNAi depletion of Relish or Dredd in ECs of *Ecc15*-infected flies reduced the expression of the antibacterial gene *Dpt*, a read-out of the Imd pathway, as expected. Loss of Relish or Dredd also abolished the expression of the CB-mCherry reporter. In contrast, the activation of the widely used *upd3* reporter, *upd3.1-lacZ* (Jiang et al., 2011), which is known to respond to epithelial damage, was not affected (Fig 2F and S2E-F). Activation of the Imd pathway by over-expressing *PGRP-LCx* in ECs was sufficient to activate both *Dpt* and the CB-mCherry reporter, and their expression required the Imd pathway components Relish, Imd, Dredd or TAK1 (Fig 2G and S2G-I). Thus, induction of the CB-mCherry reporter upon bacterial infection requires the Imd pathway.

We then tested whether the CB enhancer is directly regulated by the NFκB-like factor Relish. To this aim, we expressed two transcriptionally active forms of Relish, *Rel-VP16* and *RelD*, in ECs and then examined the activation of CB-mCherry. *Rel-VP16*, a strong activator of Imd signaling, is a fusion of the VP16 activation domain to the N-terminal of the full-length Relish protein, while *RelD*, a weak activator of Imd signaling, is the N-terminal DNA binding domain of Relish without
the inhibitory ankyrin region (DiAngelo et al., 2009). Expressing RelD mildly activated CB-mCherry as well as Dpt, pirk and PGRP-LB, while expressing Rel-VP16 for three days led to a 130-fold induction of the CB-mCherry reporter, a level significantly higher than that achieved by over-expressing PGRP-LCx (Fig 2H-K). Of note, although Rel-VP16 greatly activated the CB-mCherry reporter and the negative regulators pirk and PGRP-LB, it did not increase the expression of Dpt (Fig 2K). This is in line with the notion that AMP gene expression requires not only the Imd pathway but also additional cell type-specific transcriptional factors (Zhai et al., 2017b).

Consistent with a direct regulation of the CB element by Relish, a mutated version of the CB reporter, CB.mtNFkB-mCherry, in which the NFkB site was abolished, was not activated upon Ecc15 infection, despite the presence of many delaminating cells in the gut lumen (Fig 2L-N). Moreover, CB.mtNFkB-mCherry was not induced by over-expressing Rel-VP16 or RelD in ECs (Fig 2O). This indicates that the CB fragment of the upd2 gene is a target of Relish downstream of the Imd pathway.

**Distinct Expression Pattern of Dpt and CB Reporters along the Gut**

Having shown that the CB-mCherry reporter was a target of Imd signaling, we next tested the range of cell types along the gut that are responsive to Imd activation. For this, we applied mosaic analysis with the esgF/O system (Jiang et al., 2009) to generate GFP-labeled clones of cells that contained both progenitors and their differentiated progenies that over-expressed PGRP-LCx or Rel-VP16 (Fig 3A). Only ECs within the GFP-positive clones expressed the CB-mCherry reporter, indicating a cell-autonomous activation by the Imd pathway restricted to ECs (Fig 3B and S3A-C). Similarly, the Imd-responsive Dpt reporters (Dpt-lacZ or Dpt-mCherry) were also
exclusively induced in ECs (Fig 3C and S3D). These results suggest that ECs are the primary Imd-responsive cell type in the *Drosophila* midgut.

The adult midgut is a compartmentalized organ showing differences in morphology, stem cell activity, metabolic, and digestive function along its length (Buchon et al., 2013b; Marianes and Spradling, 2013). We found that the CB and Dpt reporters were expressed in a non-overlapping and nearly complementary manner (Fig 3D, 3F and S3E-F). While strong CB reporter (CB>CD8GFP or CB-mCherry) expression was found at regions R2bc, R3 and R4bc (midgut regions according to Buchon et al., 2013b), the Dpt reporter gene was induced at regions R0, R1, R2a, BR2-R3, BR3-R4 and R4a, that did not show EC shedding upon Ecc15 infection (Fig 3E). Dpt expression was mostly observed in gut domains with limited radius and reduced lumen size, notably the two constrictions BR2-R3 and BR3-R4 that surround the copper cell region in the middle midgut. We speculated that production of AMPs in these bottlenecks can maximize the effectiveness of AMPs in neutralizing invading bacteria. On the other hand, strong CB reporter expression coincided with regions showing higher epithelial renewal rate except R3 (Marianes and Spradling, 2013). We conclude that AMP production and EC shedding as revealed by the Dpt and CB reporters, two different gut responses to infection, take place in distinct gut regions.

**Bacterial Infection-induced EC Shedding Requires the Imd Pathway**

The observation that the CB reporter, a marker of shedding ECs, was regulated by the Imd pathway prompted us to investigate whether the Imd pathway is required for EC shedding. Midguts from Rel*E20* or Dredd*Bl18* flies did not exhibit delaminating ECs upon infection with *Ecc15* (Fig 4A-D, G). Expressing a full-length Relish in the ECs of Rel*E20* flies restored the ability of ECs to delaminate upon infection (Fig S2J-K). Previous studies have shown that the Imd pathway is activated in the midgut by
the intracellular receptor PGRP-LE but not PGRP-LC (Bosco-Drayon et al., 2012; Neyen et al., 2012). Consistent with a role of the Imd pathway in EC shedding, PGRP-LE but not PGRP-LC was found to be necessary for infection-induced EC shedding (Fig 4E-G). We conclude that beyond its well-established role in AMP production, the Imd pathway is also required for EC shedding upon infection.

We then investigated whether Imd activation is sufficient to trigger EC shedding. Consistent with this, over-expressing PGRP-LCx or Relish-VP16 using the EC-specific driver, Myo1AT5, induced massive EC shedding into the gut lumen (Fig 4H-I and S2G-H). We next generated mosaic clones using the esgF/O system, to study whether EC shedding is cell-autonomously activated by Imd signaling. Confocal sections revealed that many CB-mCherry expressing ECs that over-expressed PGRP-LCx were extruding apically into the gut lumen. This phenotype was cell-autonomous as neither their wild-type neighbors nor GFP-marked wild-type ECs displayed the same migratory behavior (Fig 4J-K). Furthermore, activation of the effector caspase, Caspase 3, was observed in the midgut of wild-type but not Relish mutant flies upon infection (Fig 4L-N), suggesting that EC detachment precedes activation of the apoptotic machinery. Collectively, this indicates that the Imd pathway controls EC shedding into the gut lumen upon bacterial infection. As such, the Imd pathway represents a bona fide cell elimination pathway.

**ISC Proliferation upon Bacterial Infection does not Require the Imd Pathway**

It is generally assumed that ISC proliferation is coupled to EC elimination through feedback mechanisms, notably through the production of secreted factors (e.g. Upd2 and Upd3) activating stem cells during regeneration (Buchon et al., 2009b; Jiang et al., 2009; Liang et al., 2017). According to this notion, Relish flies should exhibit reduced ISC proliferation upon infection, as ECs did not delaminate in this
mutant. However, we did not detect any difference in the mitotic index at 10 hours post infection between the w1118 control and RelE20 mutant flies (Fig 5A), in agreement with a previous study (Buchon et al., 2009b). Consistent with this, qPCR showed that upd2 and upd3 were induced to the same level in the midgut of Relish and wild-type flies upon infection (Fig 5B). The induction of upd2 in Relish flies was at first sight surprising, considering our data showing that the CB enhancer of upd2 was activated in a Relish-dependent manner in ECs. We reasoned that since upd2 is also expressed in midgut progenitors (Zhai et al., 2015), changes of upd2 expression in ECs could have been masked when assayed in whole midgut extracts. Further qPCR measurements using FACS-sorted ECs confirmed that upd2 but not upd3 was less induced in ECs of RelE20 flies upon infection (Fig 5C). We conclude that Relish is required for EC shedding and upd2 expression in ECs upon infection, but that Relish does not affect ISC proliferation. Thus, the processes of EC delamination and ISC proliferation can be regulated independently.

Relish is Specifically Required for Bacterial Infection-induced EC Death

We then examined whether Relish is required for other forms of EC death that are not linked to an infection. EC death can be triggered by expressing i) the proapoptotic gene reaper (rpr), or ii) a constitutively active form of the JNK kinase hemipterous (hepCA) (Jiang et al., 2009). These manipulations significantly increased the expression of CB-mCherry and upd3.1-lacZ reporters, and upd2 and upd3 endogenous genes, but did not prominently induce the expression of Dpt (Fig S4A). This indicates that very strong JNK activation can overcome the requirement of the Imd pathway to induce CB reporter expression. Expressing reaper rapidly induced massive EC apoptosis and resulted in a much-shortened midgut. However, this phenotype was not blocked in RelE20 flies (Fig S4B). Similarly, over-expressing either
reaper or hep<sup>CA</sup> led to the same level of EC delamination, ISC proliferation, and expression of upd2, upd3, keren (encoding one of the EGFR ligands) and the JNK target gene puckered (<i>puc</i>), in wild-type and Relish mutant flies (Fig 5D and S4C). In sharp contrast, increased ISC proliferation caused by over-expressing PGRP-LCx was completely dependent on Relish (Fig 5D). ISC tumors induced by the loss of Notch also cause EC shedding (Patel et al., 2015). Despite the fact that CB-mCherry was induced in the detaching ECs (Fig S4D), Notch tumor-induced EC death and proliferation of the tumor cells was not inhibited in Rel<sup>E20</sup> flies (Fig 5E and S4E).

Taken together, these data show that Relish is exclusively required for EC death triggered by bacterial infection but not by other abiotic stresses. The observations that CB-mCherry was strongly induced by JNK activation via expressing hep<sup>CA</sup> (Fig S4A), and that ISC tumors that lead to JNK activation in surrounding ECs (Patel et al., 2015) also induced CB-mCherry therein, suggest that the CB enhancer likely also receives transcriptional input from the JNK pathway, under conditions where the Imd pathway is not activated.

**JNK signaling Cooperates with the Imd Pathway to Induce EC shedding during Infection**

JNK signaling has been widely implicated in apoptosis and tissue remodeling (Pastor-Pareja et al., 2004; Uhlirrova and Bohmann, 2006), as well as EC stress and renewal of the gut epithelium (Biteau et al., 2008; Zhou et al., 2017). The presence of an AP1 site in the CB element prompted us to analyze the contribution of JNK activity to the Imd-dependent EC shedding in the context of bacterial infection.

qPCR indicated that the JNK activity reporter gene puckered (<i>puc</i>) was induced about 2-fold during the course of Ecc15 infection (Fig 6A), albeit to a much lower level than that obtained by over-expressing hep<sup>CA</sup> in ECs, which induced <i>puc</i>
expression by 40-fold (compare Fig 6A to S4C). Using a *puckered* reporter line
(*puc*\textsuperscript{E69}-Gal4/UAS-GFP) as a readout for JNK activity (Pastor-Pareja et al., 2004), we
found that *CB-mCherry* was induced upon infection in a subset of ECs with high
levels of JNK activity (Fig 6B). Inhibiting JNK signaling by expressing a dominant
negative form of the JNK Basket (*bsk\textsuperscript{DN}*) in ECs significantly reduced the expression
of *CB-mCherry* but not *upd3.1-lacZ* reporter (Fig 6C-D), and suppressed EC
shedding upon *Ecc15* infection (Fig 6D-E). However, decreased *CB-mCherry*
to JNK inhibition was not accompanied by a significant drop in *Dpt*
levels (Fig 6C). This indicates that JNK signaling is specifically required in EC
shedding but not in AMP production, in contrast to Imd signaling which controls both
processes. Furthermore, preventing apoptosis by expressing the baculovirus P35
protein had no effect on the induction of *CB-mCherry*, *upd3.1-lacZ* and *Dpt* (Fig 6C),
and on EC shedding upon infection (Fig 6E). Thus, the classic apoptotic pathway is
not essential to prime EC shedding upon infection. This suggests that ECs are most
likely extruded alive but undergo apoptosis at a later step in the gut lumen (Fig 1E
and S1E). Of note, caspase-independent cell shedding also occurs in *C. elegans*
embryos and mammalian intestinal epithelium (Coopersmith et al., 1999; Denning et
al., 2012).

To assess the role of the JNK pathway in regulating the *CB* element, we
generated a transgenic reporter containing the *CBM* element with a mutated AP1 site.
While the *CBM.mtAP1-GFP* reporter displayed a basal-level expression similar to its
wild-type counterpart, it was not activated upon *Ecc15* infection (Fig 6F-G). This
confirms that the *CBM* enhancer is directly targeted by the JNK transcription factors
through the AP1 site. Strong JNK activation by expressing *hep\textsuperscript{CA}* in ECs for 15 hours
induced *CB-mCherry* more than 30-fold but *CB.mtNF\kappaB-mCherry* only 6-fold (Fig
6H and S5A-B), indicating that the NFκB binding site can modulate the magnitude of
induction in the CB element by JNK. This supports the notion that the JNK pathway
cooperates with Imd signaling to regulate CB reporter expression and EC shedding.
This cooperation is, however, not required for the expression of antibacterial genes in
the fly intestine.

The Imd pathway bifurcates downstream of TAK1 to activate both JNK
signaling and IKK kinase, the latter leading to the activation of Relish (Silverman et
al., 2003). However, we found that activation of the JNK pathway in infected ECs
was not a direct consequence of Imd activation. First, induction of puc and two other
JNK targets coding for matrix metalloproteinases (Mmp1 and Mmp2) (Uhlirova and
Bohmann, 2006) was not blocked in RelE20 guts during infection (Fig S5C). Second,
although TAK1 was absolutely necessary for activation of the Imd pathway, it proved
dispensable for induction of JNK signaling in the midgut upon oral infection (Fig
S5D). Third, JNK activation upon infection was not reduced in PGRP-LC, PGRP-LE
or imd mutants (Fig S5E). Fourth, flip-out clones over-expressing PGRP-LCx showed
puc-lacZ expression, but this induction was not cell-autonomous and thus not a direct
consequence of PGRP-LCx expression (Fig S5F). Fifth, increased ISC proliferation
induced by over-expressing PGRP-LCx in ECs was completely Relish-dependent (Fig
S5D), arguing against a role of the TAK1-JNK branch in this process. Although it is
unclear at this stage how JNK signaling is activated by enteric infection, Imd and JNK
signaling encompass two independent infection-induced pathways whose cooperation
is required for EC shedding.

Relish-dependent inhibition of GATAe is Required for EC Shedding

Next, we explored the molecular processes downstream of Imd signaling that
promote EC shedding. An attractive hypothesis is that Relish might regulate a factor
that promotes cell detachment. The GATA transcription factor GATAe has been implicated in the maintenance of midgut compartmentalization (Buchon et al., 2013b). Depleting GATAe in ECs led to massive EC death via apical extrusion (Fig 7A). GATAe-deficient ECs also showed dramatic induction of CB-mCherry and upd3.1-lacZ reporters (Fig 7A and S7C). The crucial role of GATAe in EC survival prompted us to examine whether GATAe is repressed by the Imd pathway upon infection, a process that would permit ECs to delaminate. To test this hypothesis, we first analyzed the kinetics of GATAe transcription in the midgut following Ecc15 infection by qPCR (Fig 7B). A 40% reduction of GATAe was observed at 4 hours post infection, a time point when the mitotic response is just about to begin. During the regeneration phase (8 hours post-infection and onwards), the level of GATAe transcripts was gradually restored and further reached a slightly higher level than that in unchallenged guts. In contrast to wild-type control, GATAe expression was not significantly decreased in Relish mutant flies observed at 6 hours post infection although GATAe levels in this group showed greater variation (Fig 7C). To better visualize the cellular expression of GATAe in the midgut, we examined, using reporter genes, over 6kb regulatory sequences upstream of GATAe (Fig S6A-E). Expression of the GATAe reporter was indeed decreased in ECs at 4 hours post infection with Ecc15, notably in regions where massive EC shedding usually took place (Fig 7D and S6C). In contrast, the high level of GATAe expression in midgut progenitors was not affected during infection (Fig S6C-E). An increase of progenitor numbers during the regeneration phase could explain the increase of GATAe transcription at later time points (Fig 7B). Although the repression of GATAe by Relish in ECs is likely indirect since no NFκB site was found in the regulatory DNA of GATAe responsible for its
expression in ECs, our data show that infection-triggered Imd signaling decreases
GATAe expression in ECs, a process that promotes EC shedding.

To reinforce the notion that GATAe functions downstream of Imd signaling, we analyzed whether increased expression of GATAe could block EC shedding upon Imd activation. Indeed, EC shedding induced upon Ecc15 infection was suppressed in flies over-expressing GATAe (Fig 7E-F). Similarly to Relish mutant, such flies were also more susceptible to oral infection (Fig S7A-B). Furthermore, Ecc15 infection and over-expressing PGRP-LCx-induced CB-mCherry activation was significantly inhibited upon GATAe over-expression (Fig S7C-E), consistent with a reduction in EC shedding. Conversely, although depleting GATAe in ECs was associated with low-level activation of Imd signaling under basal conditions as indicated by the levels of pirk, PGRP-LB and Dpt expression (Fig S7C), inhibiting the Imd pathway did not suppress EC shedding induced by the loss of GATAe (Fig S7F). This is consistent with a role of GATAe downstream of Imd signaling. It is likely that the primary cause for EC shedding upon GATAe depletion was excessive JNK activation rather than Imd activity (Fig S7G), but this requires further investigation. Collectively, our data are consistent with a model in which Imd promotes EC shedding upon infection by decreasing GATAe expression, which in turn further amplifies JNK activity over a threshold required for EC shedding.

DISCUSSION

Intestinal infection in Drosophila triggers the production of ROS and AMPs to combat pathogens and concomitantly drives increased epithelial renewal to repair the collateral damage. Current models propose that epithelial damage is primarily caused
by ROS produced by Duox, while confining the role of the Imd pathway to the
induction of antimicrobial peptides (Buchon et al., 2013a). Here we found that the
Imd pathway controlled the shedding of intestinal epithelial cells upon infection,
challenging the notion that ROS-associated apoptosis is central to EC shedding. Interestingly, the Imd pathway synergized with JNK signaling to induce epithelial cell
shedding specifically in the context of bacterial infection and not in other scenarios of
EC damage. Furthermore, Imd signaling contributed to cell shedding by decreasing
the expression of GATAe, a GATA factor critical for EC morphological identity in
Drosophila (Buchon et al., 2013b). Future studies will be necessary to define the
mechanisms by which the Imd pathway regulates GATAe and how GATAe is linked to
epithelial shedding. An intriguing hypothesis is that GATAe is required to maintain
epithelial cell polarity, whose disruption can lead to JNK activation and cell extrusion
(Ohsawa et al., 2018).

In a tissue replenished by the activity of stem cells, such as the intestine,
promoting EC shedding likely acts an effective way to dump damaged ECs especially
upon infection. This raises the question whether EC shedding per se is an integral part
of the host intestinal defense. The susceptibility of Relish mutant flies to oral infection
is usually explained by the defective AMP production (Liehl et al., 2006). Our
findings raised an alternative hypothesis, namely that it could be simultaneously due
to defects in epithelial turnover. Supporting this notion, over-expression of GATAe in
ECs inhibited EC shedding and significantly compromised fly survival during
infection. Thus, EC shedding may represent an additional layer of the Imd-dependent
gut response to pathogenic bacteria, working to enhance host tolerance to infection
(Soares et al., 2017) alongside with its well-known function in antibacterial immunity.
Indeed, epithelial cell shedding has also been implicated in mammalian mucosal
immunity, where it is associated with the expulsion of infected epithelial cells, thereby reducing the chance of bacterial colonization (Sellin et al., 2015; Sellin et al., 2014).

Using CB and AMP reporters, our study uncovered that EC shedding and antibacterial immunity, two Imd-dependent processes, could be simultaneously induced because the two responses were spatially separated. Such cell-specific NFκB responses to infection should well coordinate different host defense strategies, namely resistance and tolerance, for optimal host survival. It is likely that specific transcription factors together with the NFκB factor Relish can shape distinct outputs of Imd activation. In the case of EC shedding, it appears that JNK activity provides the second signal that intersects with Imd activation leading to cell elimination. Consistently, implication of JNK signaling in EC elimination has previously been described in other contexts in the fly gut (Patel et al., 2015; Zhai et al., 2015). Moreover, using both immunity (Dpt) and EC shedding (CB) reporters, we also showed that ECs were the primary Imd-responsive cell type in the Drosophila midgut. Restricting Imd activation to ECs likely serves to protect midgut progenitors from damage.

Our study together with others points to an ancestral link between epithelial immunity and cell shedding. The Drosophila Imd pathway mirrors aspects of tumor necrosis factor receptor (TNFR) signaling in mammals (Leulier et al., 2002). Both pathways share many components and signaling steps, notably the ubiquitination and caspase-dependent cleavage of the adaptors Imd and RIP1 respectively, and the involvement of TAK1 kinase and IKK complex for NFκB activation. Of note, epithelium-intrinsic TNFR1 signaling is also necessary and sufficient to trigger intestinal epithelial cell shedding (Marchiando et al., 2011; Piguet et al., 1998;
Thus, the dual functions of the Imd and the TNFR pathways in immunity and epithelial cell shedding extend from flies to mammals. In mammals, additional immune pathways such as Nod-like receptor (NLR) signaling have also been implicated in the shedding of infected intestinal epithelial cells from the mucosa (Knodler et al., 2010; Rauch et al., 2017; Sellin et al., 2014). In contrast, neither fly Toll nor mammalian Toll-like receptor (TLR) signaling has an epithelium-intrinsic role in promoting epithelial shedding (Abreu, 2010; Buchon et al., 2014). However, specific mechanisms that each pathway adopts to regulate shedding may have been diversified during evolution. As shown here, Imd-induced shedding relied entirely on a transcriptional response controlled by the NFκB transcription factor Relish, while NLR-dependent shedding acts via caspase-centered inflammasome activation (Sellin et al., 2015). In contrast, the transcriptional response downstream of NFκB factors partially contributes to the shedding process induced by TNFR1 activation (Williams et al., 2013). Additionally, TNFR1 signaling appears essential for homeostatic enterocyte turnover in mice (Matsuoka and Tsujimoto, 2015), while the role of Imd signaling in shedding was restricted to the context of bacterial infection. Collectively, our findings suggest an evolutionarily conserved genetic program of immunity-induced epithelial cell shedding. In future, comparative studies on the mechanisms of epithelial shedding in diverse organisms should serve to better understand the evolution and diversification of epithelial immunity.

**AUTHOR CONTRIBUTIONS**

Z.Z. designed the research; Z.Z. and J-P.B. performed experiments; Z.Z. interpreted the data; Z.Z. and B.L. discussed the project and wrote the paper.
ACKNOWLEDGEMENTS

We thank Drs. Claudine Neyen, Zheng Guo and Mark Hanson for comments on the manuscript; Won-Jae Lee, Julien Royet, Sara Cherry, Luis Teixeira, Leanne Jones, Mirka Uhlírova, BDSC, DGRC, VDRC for fly stocks; DSHB for antibodies; the FCCF and BIOP platforms at EPFL for technical help. This project was supported by the SNSF grant 3100A0-12079/1 (to B.L.) and the Hunan Natural Science grant 2018JJ1015 (to Z.Z.). Z.Z. was also supported by a Marie-Curie IEF fellowship (gutENCODE).

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES


Bosco-Drayon, V., Poidevin, M., Boneca, I.G., Narbonne-Reveau, K., Royet, J., and Charroux, B. (2012). Peptidoglycan sensing by the receptor PGRP-LE in the
Drosophila gut induces immune responses to infectious bacteria and tolerance to microbiota. Cell Host Microbe 12, 153-165.


FIGURE LEGENDS
Figure 1. Identification of an infection-inducible enhancer fragment of upd2

(A) Working model of JAK-STAT signaling in Drosophila midgut. Note that shedding enterocytes (ECs) release Upd2 and Upd3 ligands, which bind to the JAK-STAT receptor Domeless (Dome) expressed only in the progenitors (intestinal stem cell (ISC) and enteroblast (EB)).

(B) Cis-regulatory elements of upd2 tested for enhancer activity. Fragments shown in red are activated in ECs upon Ecc15 oral infection.

(C-D) Sagittal view (Sag.) of midgut epithelium from unchallenged (UN, C) and infected (Ecc15, 10-11 hours post infection (hpi), D) flies. Myo1A>nlsGFP labels ECs and esg-GFP labels ISC and EBs, respectively. DAPI stains nuclei. Some shedding cells are indicated with arrows.

(E) Sagittal view of midgut epithelium from Ecc15-infected flies carrying both Myo1A>nlsGFP (EC marker) and CB-mCherry reporter. A shedding EC (orange arrow), a shed EC with ring or necklace-shaped chromatin condensation (white arrow) and two shed ECs showing nuclear collapse and disassembly (yellow arrows) are indicated.

Scale bars 50μm. See also Figure S1.

Figure 2. The CB enhancer is regulated by the Imd pathway

(A) Schematic representation of the Imd pathway.

(B-E) CB-Gal4/UAS-CD8GFP (CB>CD8GFP) expression in the midgut of flies upon different challenges. Bottom panel shows sagittal view highlighting shed cells in the gut lumen.

(F-G) Dpt, mCherry (CB-mCherry) and lacZ (upd3.1-lacZ) expression in midguts of unchallenged and Ecc15-infected flies (F) or midguts over-expressing PGRP-LCx in ECs (G). RNAi was performed for 3 days (F) and 6 days (G), respectively, using Myo1ATS as driver. Means and SEMs (n=3).

(H-J) Immunofluorescence showing the activation of CB-mCherry upon over-expression of Rel-VP16 and RelD for 3 days at 29°C.

(K) Expression of various genes upon EC-specific over-expression of Rel-VP16, RelD or PGRP-LCx using Myo1ATS for 4 days. Means and SEMs (n>4).

(L-M) mCherry reporter expression in the midgut of CB-mCherry and CB.mtNFkB-mCherry flies following Ecc15 infection (10hpi).
qPCR quantification of mCherry levels in the midgut of flies with the indicated genotype under basal conditions (UN) and upon infection (Ecc15, 10-11hpi). Means and SEMs (n=3).

(Differential activation of CB-mCherry and CB.mtNFxB-mCherry upon EC-specific expression of Rel-VP16 or RelD for 4 days. pirk expression was monitored to reveal Imd activity. Means and SEMs (n=3).

***p < 0.001, **p < 0.01, *p < 0.05, ns: p > 0.05; One-way ANOVA. Scale bars 50μm. See also Figure S2.

Figure 3. Different expression pattern of Dpt and CB reporters along the gut

(A) Schematic representation of the esgF/O system.

(B-C) Frontal view of midgut epithelium from flies over-expressing PGRP-LCx using esgF/O for 4-7 days. White arrows indicate progenitors; yellow arrows indicate newly generated ECs.

(D-F) Expression of CB and Dpt reporters (CB-mCherry / Dpt-lacZ) in the midgut of Ecc15-infected flies (D), sagittal view of regions R1 and R4a (E) and quantification of reporter intensity profile of the midgut shown in D (F).

Scale bars 50μm except D 500μm. See also Figure S3.

Figure 4. EC shedding upon infection requires Imd signaling

(A-F) Sagittal view of midgut of Ecc15-infected wild-type control (A and C) and Imd pathway deficient flies (10-12hpi). All these flies carry CB>CD8GFP reporter. F-Actin is in red.

(G) Quantification of shed cells present in the gut lumen of Ecc15-infected control and Imd pathway deficient flies. ***p < 0.001, ns: p > 0.05; One-way ANOVA.

(H-I) Sagittal view of the midgut epithelium from control (H) and Relish-VP16-overexpressing flies (I) using Myo1A\textsuperscript{TS} for 4 days.

(J-K) Midgut epithelium from control (J) and flies over-expressing PGRP-LCx (K) for 7 days using esgF/O. Cells extruding apically into the gut lumen are indicated by arrows.

(L-N) Immunostaining detecting activated Caspase 3 in Ecc15-infected flies with indicated genotype (10-12hpi) (L-M) and quantification of Caspase 3 signal intensity (N). ***p < 0.001; Student’s t test.

Scale bars 50μm.
Figure 5. *Relish* is specifically required for EC shedding upon infection

(A) Midgut mitotic index (PH3 count) of 7 day-old *w*118 (wild type) and *Rel*E20 isogenic flies infected with *Ecc15* (10hpi).

(B-C) Expression of *upd2*, *upd3* and *Dpt* in wild-type (*Rel*E20/+ and *Rel*E20 flies under unchallenged conditions (UN) or upon infection (*Ecc15*, 4hpi). B: whole midguts; C: FACS-sorted ECs. Means and SEMs (n=4; ***p < 0.001, **p < 0.01, ns: p > 0.05; One-way ANOVA).

(D) Midgut mitotic index of flies with indicated genotype. *Myo1A*TS was used as the Gal4 driver. The time window of transgene expression is indicated.

(E) Midgut mitotic index of flies bearing ISC tumors via depletion of *Notch* in progenitor cells, both in wild-type and *Relish* mutant background. Dots indicate wild-type control; triangles indicate *Relish* mutant background. Means and SEMs in A, D and E (**p < 0.001, **p < 0.01, ns: p > 0.05; Student’s *t* test). See also Figure S4.

Figure 6. The JNK pathway cooperates with Imd signaling to induce EC shedding

(A) Kinetics of *puc* expression in midgut of wild-type flies upon *Ecc15* oral infection. Means and SEMs (n=4).

(B) Concurrent detection of JNK activity (*puc*E69>GW*P*) and *CB-mCherry* expression in flies infected with *Ecc15* (10hpi).

(C) Expression of *Dpt*, *CB-mCherry* and *upd3.1-lacZ* in midgut with EC-specific over-expression of indicated genes for 3-4 days, under both unchallenged conditions (UN) and *Ecc15* infection (11-12hpi). Means and SEMs (n=4).

(D) Representative midgut of indicated flies infected with *Ecc15* (12hpi) showing reduced EC shedding and *CB-mCherry* expression upon JNK inhibition by expressing *bsk*DN in ECs using *Myo1A*TS.

(E) Quantification of shed cells present in the gut lumen of *Ecc15*-infected wild-type control flies and flies with inhibition of JNK signaling (>bsk*DN*) or apoptosis (>p35) in ECs using *Myo1A*TS.

(F-G) GFP expression (F) in the midgut of *CBM-GFP* and *CBM.mtAP1-GFP* flies following *Ecc15* infection (10hpi), and qPCR quantification of GFP expression (G) under basal conditions (UN) and upon infection (10hpi). Means and SEMs (n=3).
(H) mCherry expression (from the CB-mCherry or CB.mtNFκB-mCherry reporter) in midguts over-expressing hep<sup>CA</sup> in ECs for 15 hours as determined by qPCR. puc is used as a readout of JNK activity. Means and SEMs (n=3).

***p < 0.001, ns: p > 0.05; One-way ANOVA. Scale bars 500μm for B, 50μm for D, F and the closeup image in B. See also Figure S5.

Figure 7. Repression of GATAe by Relish is required for EC shedding

(A) Sagittal view of the midgut epithelium of wild-type control and flies with EC-specific depletion of GATAe for 3 days.

(B) Kinetics of GATAe expression in midgut collected after Ecc15 infection as determined by qPCR. Means and SEMs (n=4; *p < 0.05; One-way ANOVA).

(C) GATAe expression level in the midgut of Rel<sup>E20</sup>/+ (wild type) or Rel<sup>E20</sup> flies either unchallenged or infected (6hpi). Means and SEMs (*p < 0.05, ns: p > 0.05; One-way ANOVA). Each dot represents one independent measurement.

(D) Expression of the GFP reporter driven by a GATAe-Gal4 in unchallenged and Ecc15-infected midguts. Arrows indicate examples of ECs lacking GFP expression.

Prospero (in red) marks EEs.

(E-F) Sagittal view of the midgut epithelium (E) and quantification of shed cells in the gut lumen (F) from Ecc15-infected wild-type flies and flies with EC-specific over-expression of GATAe for 3 days. Observations were made at 12hpi. Means and SEMs (**p < 0.001; Student’s t test).

Scale bars 50μm. See also Figures S6-7.

STAR★METHODS

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Bruno Lemaitre (bruno.lemaitre@epfl.ch).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Drosophila stocks and their use in this study

Driver lines used in the study were Myo1A-Gal4, tub-Gal80<sup>FS</sup>, UAS-GFP (referred as Myo1A<sup>TS</sup>) (Jiang et al., 2009); esg-Gal4, tub-Gal80<sup>FS</sup>, UAS-GFP (referred as esg<sup>TS</sup>) (Micchelli and Perrimon, 2006); esg-Gal4, tub-Gal80<sup>FS</sup>, UAS-GFP, UAS-Flp, Act>CD2>Gal4 (referred as esgF/O) (Jiang et al., 2009). UAS-Rel-IR (KK), UAS-imd-IR (KK), UAS-Dredd-IR (KK), UAS-dTAK1-IR (KK), UAS-Notch-IR (KK), UAS-GATAe-IR (v10420, GD), GATAe-Gal4<sup>TS</sup> (construct ID: 242357-242360) were obtained
from Vennia *Drosophila* Resource Center (VDRC). The following UAS lines were used, *UAS-PGRP-LC* (BDSC30917), *UAS-PGRP-LC* (BDSC30918 and 30919), *UAS-PGRP*-LE (BDSC33054), *UAS-Rel* (BDSC9459), *UAS-Rel-VP16* (BDSC36547), *UAS-RelD* (gift from Sara Cherry), *UAS-RelF88* (BDSC55778), *UAS-Rel49* (BDSC55779), *UAS-Doxo-IR* (gift from Won-Jae Lee), *UAS-hskE20* (BDSC 6409), *UAS-hskE20* (gift from Mirka Uhlírova, on 3rd chromosome), *UAS-p35* (BDSC5072), *UAS-rpr* (BDSC5824), *UAS-hspCA* (BDSC9306), *UAS-GATAe* (Zhai et al., 2017a), *UAS-GATAe-IR* (BDSC34907), *UAS-mCD8::GFP* (BDSC32185 and 32186). Reporter lines were used were *esg::GFP* (gift from Leanne Jones), *upd3.1-lacZ* (Jiang et al., 2011), *Dpt-lacZ* (BDSC30918 and 55707), *Dpt-mCherry* (gift from Julien Royet), *pucE20-lacZ* (DGRC109029), *puc-Gal4* > *UAS-GFP* (Pastor-Pareja et al., 2004), and *upd2* reporters generated in this study. Null mutants for the Imd pathway were used were *Rel*E20*, *Dredd*, *PGRP-LC*E20*, *PGRP-LE*E20*, *TAK1*E20* and *imd*. Isogenic w¹¹¹ and *Rel*E20* lines were kindly provided by Luis Teixeira.

Female flies were used in all experiments. Fly strains were kept on a standard medium (maize flour, yeast, agar and fruit juice) at room temperature, unless otherwise indicated. The age and rearing of flies used were noted within the text, figures, legends, and STAR Methods. In most cases, the driver lines (*Myo1A*E5, esgE5 or esgF0) were crossed to the w¹¹¹ strain, and the progenies were used as control for over-expression experiments.

**METHOD DETAILS**

**Generation of transgenic reporter lines**

*pbPGUw-eGFP/mCherry* gateway reporter vectors were constructed by replacing the *Gal4* coding sequences and yeast terminator in the *pbPGUw* vector (Pfeiffer et al., 2008) with *eGFP* or *mCherry* coding sequences as *KpnI*-HindIII fragments (Zongzhao Zhai and Ingrid Lohmann, unpublished). To generate reporter constructs, primers shown below were used to amplify the regulatory regions of *upd2*.

The PCR products were first cloned into *pENTR-D-TOPO* (ThermoFisher Scientific) vector, and then swapped into *pbPGUw*, *pbPGUw-eGFP* or *pbPGUw-mCherry* destination vector. Site-specific integration was performed to insert the transgenic reporters at predefined genomic locations. The transgene insertion sites were indicated in Figure S1A. Putative transcription factor binding sites were mutated via overlapping PCR whereby point mutations were introduced through PCR primers. The following motifs, NFkB (GTGAATTCCTC→GTTCCGTGTC) and AP1 (TGAATCA→CCAAATGG),
were mutated in the way indicated above. Transgenic reporters controlled by the mutated CB or CBM fragment were inserted in the attP2 site, and reporter expression level was compared to the wild-type CB or CBM reporter at the same attP2 site. All the constructs were verified by sequencing.

Oral infection of adult flies

Bacterial strains Erwinia carotovora carotovora15 (Ecc15), Ecc15 ΔPyrE (gift from Won-Jae Lee), Micrococcus luteus, Pseudomonas entomophila gau,A, Serratia marcescens Db11 were grown in LB medium at 29°C with shaking overnight, and harvested by centrifugation at 3000g at 4°C for 30 minutes. The pellet was then re-suspended in the residual LB. 4-7 day-old mated female flies (15-20 per vial) were first dry-starved in an empty tube for 2 hours, and then transferred into a classical food vial containing a filter paper that totally covers the food and was soaked with a solution consisting of 140μL 2.5% sucrase and bacteria at final OD_{600} = 100-200, except for Serratia marcescens Db11 at final OD_{600} = 50. Unchallenged control flies were fed with 140μL 2.5% sucrase. Infected flies were kept at 29°C until dissection.

Conditional expression of UAS-linked transgenes

The TARGET system was used in combination with the indicated Gal4 drivers to conditionally express UAS-linked transgenes (McGuire et al., 2004). Flies were grown at 18-22°C to limit Gal4 activity.

After being maintained 3-4 days at 18-22°C, newly hatched adult flies with the appropriate genotypes were shifted to 29°C, a temperature inactivating the temperature-sensitive Gal80’s ability to suppress Gal4 and in turn allowing for the expression of UAS-linked transgenes in cell-type and/or tissue-specific manner, and dissected after indicated time of transgene activation.

Mosaic analysis was done using the esg/F0 system (esg-Gal4, tab-Gal80Δ5, UAS-GFP; UAS-Flp, Act>CD2>Gal4) (Jiang et al., 2009). Combining the TARGET system, this tool allows activating UAS-Flp recombinase in progenitor cells with esgΔ5 by temperature shift. Flp in turn excises the CD2 cassette from Act>CD2>Gal4 (> indicates the FRT site recognized by the Flp) and converts it to a ubiquitous Act-Gal4 driver that is inherited in the stem cell progenies. ECs were identified by their large nuclei size, round cell shape and relatively weak GFP signal compared to progenitor cells. UAS-linked transgenes were only expressed in cells indicated by the presence of GFP.

Immunohistochemistry

Flies were transferred overnight into a classical fly food vial containing a filter paper soaked with a solution consisting of 5% sucrase to clean the digestive tract. Then, intestines of adult females were dissected in phosphate-buffered saline (PBS), and fixed for at least one hour at room temperature in 4% paraformaldehyde (PFA) in PBS. Flies infected with bacteria were directly dissected for staining. They were subsequently rinsed in PBS+0.1% Triton X-100 (PBT), permeabilized and blocked in 2% BSA 1% NGS PBT for one hour, and incubated with primary antibodies in 2% BSA 1% NGS PBT overnight at 4°C. After one hour of washing, secondary antibodies, DAPI and phalloidin when necessary were applied at room temperature for two hours.

Primary antibodies used are: mouse anti-Pros (1:100), rabbit anti-pH3 (1:1000), rabbit anti-Cleaved Casp3 (1:100), Chicken anti-GFP (1:1000), mouse anti-βGal (1:1000), and Rat anti-mCherry (1:500). Alexa488-, Alexa555- or Alexa647-conjugated secondary antibodies (ThermoFisher Scientific) were used at a final concentration of 1:1000. Nuclei were counterstained by DAPI (1:10’000). Filamentous actin (F-actin) was visualized by phalloidin (1:100) staining.

Image acquisition and processing

All the images were taken on a Zeiss LSM 700 confocal microscope by using a 20x objective. Images were processed using Fiji-Image J and Adobe Photoshop software. Shown in figures are maximal intensity projections of all the confocal z stacks. Sagittal view (indicated by “Sag.” in Figures) was shown to highlight the drying cells present in the gut lumen, and other pictures were frontal view.

Enterocyte sorting through FACS

w; Myo1A<sup>T7</sup>; Rel<sup>20</sup> virgin females were crossed to either isogenic w<sup>1118</sup> or isogenic Rel<sup>20</sup> at 25°C. Closed progenies (control: w; Myo1A<sup>T7</sup>; Rel<sup>20</sup>/+; Relish mutant: w; Myo1A<sup>T7</sup>; Rel<sup>20</sup>/Rel<sup>20</sup>) were maintained at 25°C for 4-6 days, and then shifted to 29°C for at least 24hours to activate Myo1A>GFP that labels ECs prior to an Ecc15 infection. Oral infection with Ecc15 was performed as described above. Around 30 flies for each biological replicate were dissected in ice-cold 1xPBS made with DEPC-treated water. Four biological replicates were performed. Cell dissociation and FACS sorting were performed as described (Dutta et al., 2013). ECs were directly sorted into lysis buffer, and total
RNA was isolated using RNeasy mini kit (Qiagen). Around 10ng total RNA was used for cDNA synthesis and subsequent qPCR.

**qRT-PCR analysis of gene expression**

Total RNA was extracted from dissected midguts (15-20 guts per sample) using Trizol. cDNA was synthesized using the PrimeScript RT reagent Kit (TaKaRa). 0.5μg total RNA was used for reverse transcription with oligo dT, and the 1st strand cDNA was diluted 10-20 times with water and further used in real time PCR. Real time PCR was performed in at least duplicate for each sample using SYBR Green (Roche) on a LightCycler 480 System (Roche). Expression values were calculated using the ΔΔCt method and relative expression was normalized to Rp49. The expression in control sample was further normalized to 1. Primer sequences used for qPCR are available upon request.

**Lifespan analysis**

Genetic crosses were set up at 18-20°C to avoid developmental effects using the TARGET system, and progenies were collected and mated for 3-4 days at 18-20°C. Then, female flies (20-30 per vial) were shifted to 29°C to induce transgene expression. Isogenic w^{1118} and Rel^{E20} flies were grown at 25°C. Flies were infected in triplicates with Ecc15 at OD<sub>600</sub> 100-200 as described above and kept at 29°C. New Ecc15 were added every two days, and dead flies were counted daily.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

For all quantifications, n represents the number of biological replicate, and error bar represents SEM. Each independent test was performed typically with 12-15 midguts, unless otherwise noted. Statistical significance was determined using either the unpaired t test or one-way ANOVA with Tukey post hoc tests where multiple comparisons were necessary, in GraphPad Prism Software, and expressed as P values. (*) denotes p < 0.05, (**) denotes p < 0.01, (***) denotes p < 0.001, and (ns) denotes values whose difference was not significant.

Results of mRNA expression obtained with qPCR are shown as mean ± SEM of at least 3 independent biological samples (Figures 2F-G, 2K, 2N-O, 5B-C, 6A, 6C, 6G-H and 7B-C; S2A, S2I, S4A, S4C, S5C-E, S7C, S7E and S7G). Quantification of Casp3 signal intensity in regions of interest (ROI) (Figure 4N) and analysis of line profiles of relative expression level of Dpt and CB reporters (Figures 3F and S3F) were directly done with Fiji software. Midgut mitotic index was calculated by manually counting PH3-positive progenitor cells along the length of the midgut, and the results (Figures 5A and 5E) are representative of three independent analyses. Fiji macros for automated counting of nuclei in ROI (Figures 4G, 4N, 5A, 5D-E, S2B) or for simultaneously measuring the intensity of CB reporter expression and the distance of respective cell nuclei to the tissue border in sagittal confocal sections (Figure S1F), were kindly developed by Dr. Romain Guiet at the BioImaging & Optics Platform (BIOP) in EPFL. The image window of ROI was set to 320 μm x 320 μm when preforming confocal scanning. The macros are available upon request. Survival data were pooled and analyzed in Prism software using the log-rank test (Figures S7A-B). In Figures 4G, 4N, 5A, 5D-E, 6E, 7F and S2B, one dot or one triangle represents one gut.
### Key Resource Table

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Oligonucleotides

- Primer for cloning of upd2_1.9kb, forward: caccACAGTGAGTATGGATCGGTT (Microsynth)
- Primer for cloning of upd2_1.9kb, reverse: GATCACTAGCAGCACCTGCC (Microsynth)
- Primer for cloning of upd2_A, forward: caccCTAGCTGTCACCGCCCTC (Microsynth)
- Primer for cloning of upd2_A, reverse: GATTGGAATTGTGTGTCGC (Microsynth)
- Primer for cloning of upd2_B, forward: caccCATACCTGCCCCACG TAAAG (Microsynth)
- Primer for cloning of upd2_B, reverse: GAGGGGCGTGGCACAGCTAG (Microsynth)
- Primer for cloning of upd2_C, forward: caccTAGCGCCAGGTGCTAAGCTG (Microsynth)
- Primer for cloning of upd2_C, reverse: TGGAAAACTTTACCGTGGGC (Microsynth)
- Primer for cloning of upd2_D, forward: caccTCATAGGCTTTAAAGTGATG (Microsynth)
- Primer for cloning of upd2_D, reverse: GCACATCCAATTAACCCAATC (Microsynth)
- Primer for cloning of upd2_CA, forward: caccTAGCGCCAGGTGCTAAGCTG (Microsynth)
- Primer for cloning of upd2_CA, reverse: AGGATGCCACCATACATAGGC (Microsynth)
- Primer for cloning of upd2_CB, forward: caccGCATAGTATGGTGCCACTCTT (Microsynth)
- Primer for cloning of upd2_CB, reverse: TGAAAAACTTTACCGTGGGC (Microsynth)
- Primer for cloning of upd2_BM, forward: caccGCTAGCCAGTCCGATTACCA (Microsynth)
- Primer for cloning of upd2_BM, reverse: GACATTGCGAGGGTGGCAC (Microsynth)
- Primer for cloning of upd2_BM-S1, forward: caccGCATAGTATGGTGCCACTCTT (Microsynth)
- Primer for cloning of upd2_BM-S1, reverse: ATATCGCTTCATGGATATAC (Microsynth)
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Figure 3

Gal80<sup>ts</sup> suppresses Gal4, no expression of UAS-X/GFP.

1. at 18°C

2. shifted to 29°C ISC/EBs express UAS-X/GFP

3. after a few days at 29°C ISC/EBs and new EC/EEs express UAS-X/GFP
Figure S1. Identification of an infection-inducible enhancer fragment of upd2. Related to Figure 1.

(A) Summary of the reporter expression pattern in the midgut controlled by various cis-regulatory elements of upd2. Reporter types (Gal4, mCherry or GFP), transgene insertion sites in the Drosophila genome, and expression pattern of respective reporters both under basal conditions and upon Ecc15 infection are listed. (B) Overlap of CBM-GFP and CB-mCherry reporters upon Ecc15 infection (10hpi). (C) The sequences of the 204bp CBM enhancer with putative transcription factor binding sites. Conservation by phastCons scores was obtained from the UCSC genome browser. (D) Frontal view of the posterior midgut of wild-type flies at different time points post Ecc15 infection (UN, 4 and 8hpi). Yellow arrows indicate ECs showing nuclear condensation. (E) Sagittal view of midgut epithelium from Ecc15-infected flies carrying Myo1A>nlsGFP (EC marker). Shedding ECs (orange arrows), shed ECs with ring or necklace-shaped chromatin condensation (white arrows) and shed ECs showing nuclear collapse and disassembly (yellow arrows) are indicated. (F) Quantitative measurements of CB-mCherry reporter levels and the distance of respective cell nuclei to the basal tissue border in Ecc15-infected gut. mCherry signal intensity below 25 is treated as background. Trend line and SEM are shown. n=13 guts. (G) Posterior midgut from Ecc15-infected CB>CD8GFP flies (10hpi). F-Actin is in red. Scale bars 50µm.
Figure S2. CB enhancer activity coincides with the levels of Imd signaling. Related to Figure 2.

(A) Expression of mCherry (CB-mCherry) and pirk (an Imd readout) as measured by qPCR in the midgut of flies RNAi of Duox in ECs using Myo1A^TS for 3 days. Results are presented as fold change of infected guts over unchallenged control. (B) Quantification of shed cells present in the gut lumen of Ecc15-infected control flies and flies depleting Duox using Myo1A^TS for 3 days. The image window was set to 320 µm x 320 µm. One dot represents one gut. (C-D) Induction of the CB-Gal4/UAS-CD8GFP reporter gene in the posterior midgut of flies challenged with P. entomophila gacA (C) or S. marcescens Db11 (D), at 10hpi. (E-F) Representative images of the posterior midgut of wild-type flies (upper panel, E) and flies with EC-specific depletion of Relish by RNAi (bottom panel, F) upon oral infection with Ecc15 (12hpi). Note that the expression of CB-mCherry but not upd3.1-lacZ was affected by Relish depletion. (G-H) Sagittal view of the anterior midgut epithelium from wild-type flies (G) and PGRP-LCx-overexpressing flies (H) using Myo1A^TS (4 days at 29°C). (I) Expression of Dpt and mCherry (CB-mCherry) as measured by qPCR in the midgut of flies over-expressing various genes using Myo1A^TS for 2 days. (J-K) Expression of CB-mcherry in the posterior midgut of an Ecc15-infected (12hpi) Relish mutant fly (J) and a Relish mutant fly expressing a full-length form of Relish in ECs (K) with Myo1AT^TS. Note that both EC shedding and CB-mCherry (in red) expression were restored when Relish was rescued. F-actin (in green) is shown for the upper panel and ECs (Myo1A>GFP, in green) for the bottom panel. Means and SEMs (n=3; ***p < 0.001, **p < 0.01, ns: p > 0.05; One-way ANOVA). Scale bars 50µm.
ECs are the only Imd-responsive cell type in the fly midgut. Related to Figure 3.

(A-D) Frontal view of midgut epithelium from wild-type flies and flies over-expressing various Imd pathway components (PGRP-LCx, PGRP-LE and Rel-VP16) using esgF/O for 4-7 days. White arrows indicate progenitors; yellow arrows indicate newly generated ECs. Only GFP-marked cells over-express UAS-linked genes. ECs can be identified based on their large nuclei size and round cell shape. Expressing PGRP-LCx, Rel-VP16 and PGRP-LE activated both CB-mCherry and Dpt-mCherry only in ECs in a cell-autonomous manner. No expression of these reporters was found in the GFP-marked progenitors suggesting that these reporter genes are only induced in ECs. Scale bars 50µm. (E-F) Expression of CB and Dpt reporters (CB>CD8GFP / Dpt-lacZ) in the midgut of Ecc15-infected flies (E) and quantification of respective reporter intensity profile along the length of the midgut (F). The two reporters were expressed in ECs of different gut regions. Arrow in E indicates non-specific staining of β-Gal due to massive EC shedding in this region. Scale bar in E 500µm.
Figure S4. Relish is not required for other forms of EC death. Related to Figure 5.

(A) Expression of various reporter genes or endogenous genes in the midgut of flies with EC-specific expression of reaper (rpr) or hep$^{CA}$ for 15 hours as monitored by qPCR. (B) Over-expressing the pro-apoptotic gene reaper in ECs induced cell death and midgut shortening in both wild-type and Relish mutant flies. reaper was over-expressed for 36 hours at 29°C. (C) Expression of upds and Keren but not Dpt were induced upon expression of reaper or hep$^{CA}$ in ECs. The induction of upds and Keren was not blocked in RelE20 flies. (D) CB-mCherry reporter activation in ECs at the vicinity of Notch-deficient ISC tumors. Notch was silenced by RNAi in the midgut progenitors using esg$^{TS}$ for 4 days. Progenitors are marked with esg$>$GFP (green). Yellow arrows indicate shedding ECs; white arrows indicate progenitor cells. (E) Representative images of posterior midgut bearing ISC tumors in wild-type (upper panel) or Relish mutant background (bottom panel). Arrows indicate remaining ECs that had not been eliminated by the ISC tumors. Means and SEMs (n=3). Scale bars 50µm.
Figure S5. JNK activation upon infection is independent of the Imd pathway. Related to Figure 6.

(A-B) Representative images showing activation of CB-mCherry (A) and CB.mtNFκB-mCherry (B) reporters in the midgut upon EC-specific over-expression of hepCa for 15 hours. (C) Expression of JNK target genes (puc, Mmp1 and Mmp2) in control (Ref20/+ and Ref20) mutants under basal conditions (UN) and upon infection (Ecc15, 6hpi). (D) Expression of the JNK target gene puc and the Imd-target gene pirk in wild-type control (yw) and yw, dTAK1 mutant under basal conditions (UN) and upon infection (Ecc15, 8hpi). (E) Expression of puc as measured by qPCR in the midgut of flies with indicated genotype. Results are presented as fold change of infected guts over unchallenged control. (F) Midgut with GFP-labeled clones over-expressing PGRP-LCx via esgF/O for 5 days. puc-lacZ expression was detected by immunostaining. The white arrow indicates a GFP+ EC that does not express puc-lacZ reporter, while the yellow arrow indicates a GFP-negative EC that expresses puc-lacZ. Means and SEMs (n>3; ***p < 0.001, ns: p > 0.05; One-way ANOVA). Scale bars 50µm.
Figure S6. Analysis of the enhancer activity of GATAe cis-regulatory elements. Related to Figure 7.

(A) Schematic representation of the GATAe cis-regulatory sequence analyzed in the present study (lines 357-360). (B-E) Expression pattern of various GATAe-Gal4/UAS-CD8GFP reporters in the posterior midgut of flies either unchallenged (UN) or orally infected with Ecc15 (4hpi). The line 357 marks ECs; line 358 marks both ECs and midgut progenitors; both lines 359 and 360 mark midgut progenitors and a subset of EEs. The absence of GFP signal in some ECs in the region where massive EC shedding will take place is indicated with arrows in C. Pros staining shown in red marks EEs. Scale bars 50µm.
Figure S7. GATAe is essential for EC survival. Related to Figure 7.

(A) Survival analysis of isogenic wild-type and RelE20 female flies upon infection with Ecc15. (B) Survival of wild-type flies and flies over-expressing GATAe in ECs upon Ecc15 infection. ***p < 0.0001, **p < 0.001; Log-rank test. (C) qPCR analysis of the expression of the indicated reportors or genes in the midgut of unchallenged or infected (Ecc15, 16hpi) flies with EC-specific depletion (>GATAe-IR) or over-expression (>GATAe) of GATAe for 2-3 days. Means and SEMs (n=4; **p < 0.01, ns: p > 0.05; One-way ANOVA). (D) Immunofluorescence showing the activation of CB-mCherry reporter upon over-expression of PGRP-LCx alone or in combination with GATAe with Myo1A15S for 4 days. (E) qPCR quantification of mCherry, pirk and Dpt expression in the midgut of indicated flies. Means and SEMs (n=3; ***p < 0.001, **p < 0.01, *p < 0.05; One-way ANOVA). (F) Sagittal view of midguts from indicated flies shifted to 29°C for 3-4 days. EC shedding induced upon RNAi of GATAe in ECs was not suppressed in RelE20 flies. (G) qPCR quantification of gene expression (puc, Dpt and pirk) in the midgut of wild-type flies and flies with a specific depletion of GATAe in ECs. Means and SEMs (n=3; ***p < 0.001, **p < 0.01; Student’s t test). Scale bars 50µm.