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Gene regulatory mechanisms underlying the intestinal innate immune response

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In the mammalian gastrointestinal tract, distinct types of cells, including epithelial cells and macrophages, collaborate to eliminate ingested pathogens while striving to preserve the commensal microbiota. The underlying innate immune response is driven by significant gene expression changes in each cell, and recent work has provided novel insights into the gene regulatory mechanisms that mediate such transcriptional changes. These mechanisms differ from those underlying the canonical cellular differentiation model in which a sequential deposition of DNA methylation and histone modification marks progressively restricts the chromatin landscape. Instead, inflammatory macrophages and intestinal epithelial cells appear to largely rely on transcription factors that explore an accessible chromatin landscape to generate dynamic stimulus-specific and spatial-specific physiological responses.

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Introduction

The gastrointestinal tract is constantly exposed to microorganisms that can be potentially harmful. Phylogenetically distant species have therefore evolved similar mechanisms to maintain intestinal homeostasis. Indeed, while the adaptive immune system only evolved in vertebrates, the evolutionarily conserved innate immune system in the gastrointestinal tract shares similarities from insects to humans [1]. For example, epithelial cells that line the gut provide a physical barrier between host and commensal or invading bacteria. In addition, they are capable of mounting an innate immune response and produce chemokines and cytokines that signal to phagocytic cells such as macrophages [2,3].

In mammals, intestinal macrophages have a strong phagocytotic capacity against invading bacteria whilst a low competence to release pro-inflammatory mediators, assuring tissue integrity and reducing the undesired elimination of commensal microbes [4]. These responses are orchestrated through the dynamic control of gene expression levels in each of the participating cells, and the molecular mechanisms underlying this control have been intensely studied in recent years. One of the key insights that emerged is that these mechanisms differ from those mediating canonical development and differentiation. In the latter processes, transcription factors (TFs) coordinate the orderly post-translational modification of histones to progressively specify and constrain the responsive chromatin landscape that is inherently linked to the developmental path of the respective cell [5]. While differentiated resident macrophages apparently follow this model [6••,7••], inflammatory macrophages resulting from acute differentiation have a pre-defined open chromatin landscape for nearly all central transcriptional regulators, irrespective of their actual transcription status [8]. A comparable regulatory structure is also observed in intestinal epithelial cells: despite significant differences in gene expression levels between secretory and absorptive cells and their common precursor, they all show similar chromatin accessibility landscapes [9••]. An intriguing hypothesis is that an open chromatin state may enable these cells to react quickly to various stimuli. These observations make the gut an insightful model to study the dynamic properties of gene regulatory networks in normal or infection conditions or in disease contexts.

In this review, we will discuss recent advances in elucidating the gene regulatory mechanisms underlying the innate immune response in mammals. We will first focus on generic or tissue-specific macrophages, after which in a second part we will cover intestinal epithelial cells. We will end with a perspective on outstanding questions in the field and highlight the importance that genetically tractable model organisms such as *Drosophila melanogaster* might have in this domain.

The temporal and spatial properties of macrophage regulatory networks

Gene regulation is controlled by TFs within the context of chromatin, whose fundamental subunit is the nucleosome. Each nucleosome consists of an octamer of two copies of different histones, around which the DNA is wrapped. Post-translational modifications of histones and DNA methylation regulate nucleosome compaction that facilitates or impedes TFs accessibility. For example, while

histone modifications such as H3 lysine 4 mono-methylation, di-methylation and tri-methylation (H3K4me1, H3K4me2 and H3K4me3) and H3 lysine 27 acetylation (H3K27ac) facilitate TF binding and the access of the transcription machinery to DNA, DNA methylation and H3K27me3 are normally associated with reduced DNA binding access and gene repression [5].

Macrophages are phagocytic cells of the mammalian innate immune system that play an important role in tissue homeostasis. In the steady state, they arise from two distinct sources: first, continuously recruited from circulating monocytes in the gut [10], the dermis [11] and the heart [12], and second, from fetal monocytes and yolk sac precursors that colonize the whole embryo between E8.5 and E10.5, becoming self-renewal differentiated tissue-resident macrophages [13,14,15•]. Similar to other systems such as mammalian forebrain, heart and liver [16] as well as cells that arise from hematopoiesis [17•], macrophage development involves substantial reorganization of the chromatin landscape [6••,7••]. This is driven by the hematopoietic-specific TF PU.1, which acts in combination with other TFs such as C/EBPα to establish a macrophage-specific chromatin landscape [7,18••,19,20] (Figure 1).

Tissue-resident macrophages can be found at numerous anatomical locations, presenting considerable phenotypic diversity [21••]. Even after differentiation, they can self-renew in a process mediated by the down-regulation of the TFs MafB and cMaf and the rewiring of the embryonic stem cell self-renewal network [22••]. During mouse embryogenesis, the core macrophage program driven by PU.1 is rapidly diversified by the action of lineage-determining TFs (LDTFs), which integrate specific cues from the microenvironment to orchestrate the deposition of active histone modification marks (Figure 1) [6••,7••,23–26]. Relevant LDTFs involved in this process are first, C/EBPβ in lung and peritoneal cavity macrophages [27], second, nuclear receptor LXRα in splenic marginal zone macrophages [28], third, GATA6 in peritoneal cavity macrophages [24,29], four, PPARγ in alveolar macrophages [23,30], and finally, SPIC in spleen red pulp macrophages [31,32]. The importance of the microenvironment in macrophage differentiation is highlighted by the fact that transferring macrophages from one tissue to another extensively reprograms the enhancer repertoire to a state similar to the one of the residing cell population [6••].

Interestingly, blood monocyte-derived intestinal macrophages also exhibit a high degree of phenotypic diversity [4]. For example, macrophages residing close to the fecal contents activate a robust inflammatory response when the epithelial barrier is damaged. On the other hand, macrophages that are located deeper in the gut wall efficiently eradicate microbes that breach the intestinal epithelial barrier without mounting a potent inflammatory response. This phenotypic difference is orchestrated by interleukin-10 (IL-10), which is secreted locally by T cells, B cells, dendritic cells, and some epithelial cells to limit inflammatory responses [33,34]. The gene regulatory mechanisms controlling this behavior have been recently examined, revealing that the chromatin accessibility landscape of IL-10 knockout intestinal macrophages was similar to that of inflammatory macrophages. This finding suggests that IL-10-deficiency alone is sufficient to poise chromatin for an inflammatory response [35]. Overall, this extensive crosstalk between the microenvironment, LDTFs and SDTFs allows macrophages to control signal-specific transcriptional outputs that are important for their respective tissue of residency [6••,7••].

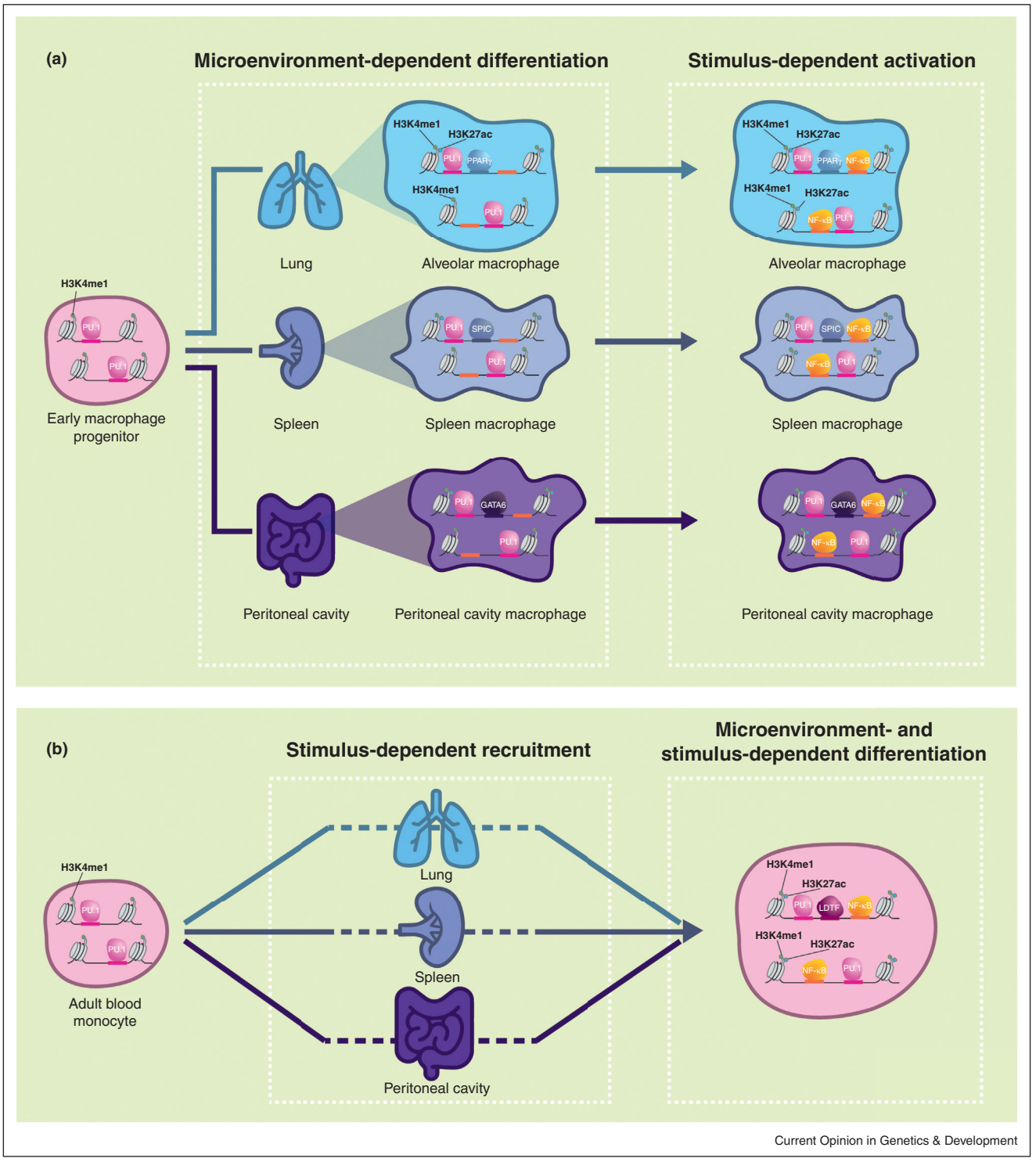
Macrophage regulatory dynamics during inflammation

While the regulatory dynamics of tissue-resident macrophages' response to infection has not yet been addressed, the acute differentiation of blood monocytes in response to microbial products as well as pro-inflammatory or anti-inflammatory cytokines has been well characterized *in vitro* [36•]. These signals activate Signal-Dependent TF (SDTFs) such as NF-κB, STAT factors and nuclear receptors [19,37]. They mainly regulate three classes of regulatory sequences: (i) constitutive (open) enhancers marked by both H3K4me1 and H3K27ac that require no additional modification, (ii) poised enhancers that feature basal H3K4me1 and no H3K27ac levels, and that upon SDTF binding exhibit greater H3K27ac enrichment [18••,38] and (iii) latent or *de novo* enhancers that are devoid of any active marks and acquire both H3K4me1 and H3K27ac upon activation [39••,40]. Latent enhancers constitute a smaller but important fraction of regulated sequences as some retain the H3K4me1 mark upon stimulus removal (i.e. they remain poised), which allows their faster and stronger activation upon re-exposure to identical or heterologous stimuli [39••]. Overall, SDTFs mostly bind to their respective motifs in pre-existing, accessible genomic regulatory sequences (classes i and ii), which might explain why they respond so rapidly to environmental signals [8]. SDTFs then activate directly (class i) or recruit chromatin modifiers to inhibit (class i) or promote (classes ii and iii) the transition of either non-accessible or accessible but inactive (poised) states to fully active enhancers. Thus, by combining stimulus-driven SDTF and environment-driven LDTF activation in an already partially pre-configured chromatin landscape, macrophages induce qualitative and quantitative tissue-specific transcriptional programs [8,39••,40].

Characteristics of developmental and inflammation-responsive regulatory networks in intestinal epithelial cells

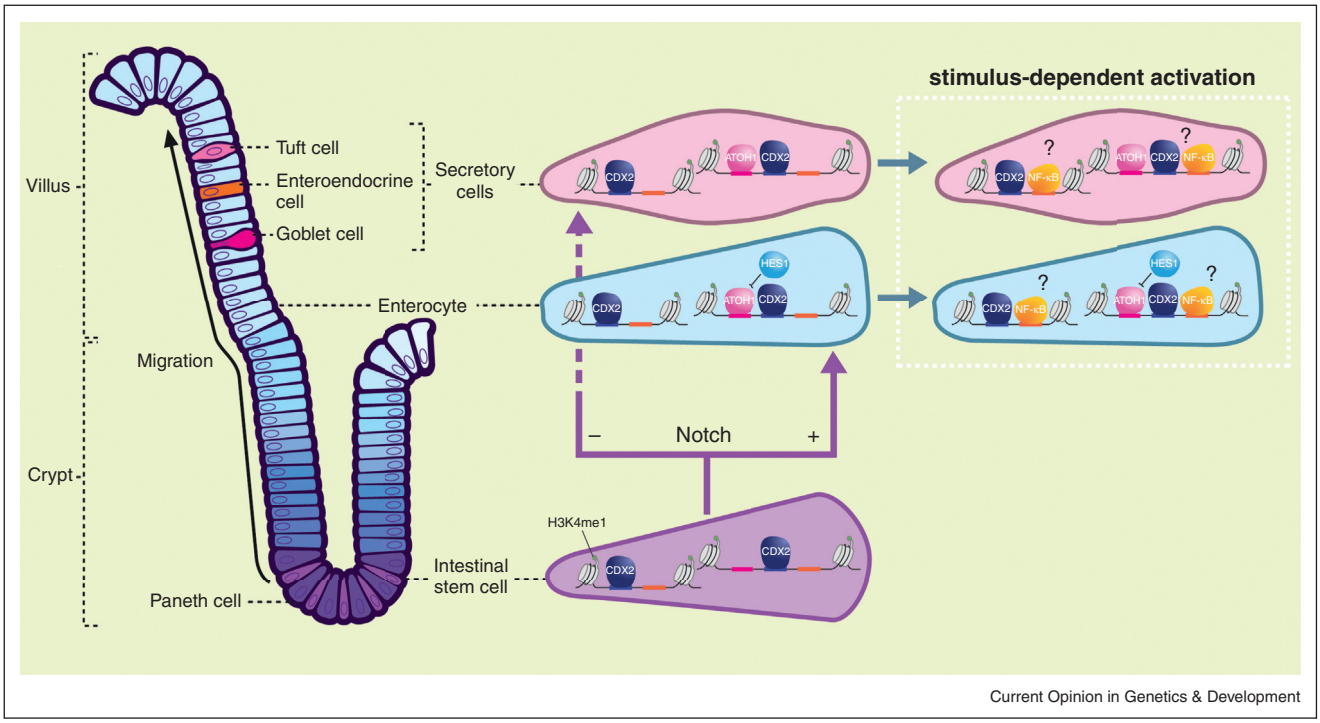
The mammalian intestinal epithelium is an important component in the maintenance of gut homeostasis. Anatomically, it is composed of a single cell layer organized in villi and crypts. The crypt provides a protected niche for

Figure 1



Tissue and environmental signals shape differential gene expression programs in macrophages **(a)** Left — an early macrophage progenitor has accessible chromatin landscape (H3K4me1 marked), also bound by PU.1. Middle — upon local environmental signals, the tissue-specific LDTFs (PPARγ in alveolar macrophages, SPIC in spleen macrophages, and GATA6 in peritoneal cavity macrophages) cooperate with PU.1 at H3K4me1-marked regions and activate the expression of genes with roles in tissue-specific functional pathways. Right — after an environmental challenge, that is, infection or injury, SDTFs such as NF-κB bind and activate open (H3K4me1 and H3K27ac) and poised enhancers (H3K4me1 and no H3K27ac) and *de novo* enhancers (devoid of chromatin modifications, not shown). **(b)** Upon recruitment to different locations in the body, adult blood monocytes differentiate into inflammatory macrophages by integrating the regulatory inputs of both lineage-determining TFs (LDTFs) and signal-dependent TFs (SDTFs) using a globally accessible chromatin landscape.

Figure 2



Intestinal epithelial gene regulation during differentiation **(a)** intestinal stem cells are intercalated with Paneth cells at the crypt base, and continuously differentiate into the various functional cells on the villi. CDX2 is a master regulator of epithelial differentiation and is present at most open chromatin regions in all cell types. Therefore, intestinal stem cells and terminally differentiated enterocytes and secretory cells have similar accessible (permissive) chromatin landscapes (with the H3K4me1 modification) and cell-specific gene expression is achieved by TFs such as ATOH1 in secretory cells, while in enterocytes, *Atoh1* expression is inhibited by HES1. After an environmental challenge, SDTFs such as NF-κB may also make use of the accessible chromatin landscape to control gene expression. Question marks represent hypothesized mechanisms that have not yet been formally assessed.

intestinal stem cells, which migrate upwards and differentiate into enterocyte and secretory (enteroendocrine, goblet, or Paneth cells) lineages, in a process that is dependent on Notch signaling [41]. Absorptive enterocytes constitute around 90% of the intestine, while most of the others are cells from the secretory lineage (Figure 2). Besides absorbing nutrients and providing a physical barrier, they make use of innate immune receptors to sense the luminal microbial composition. This in turn allows them to orchestrate the recruitment of macrophages and dendritic cells, and the migration and differentiation of lymphoid cells at the sub-epithelial connective tissue. Moreover, intestinal epithelial cells also fight the infection directly given their ability to produce antimicrobial proteins and peptides, reactive oxygen species, and several proinflammatory cytokines [42].

There are several key TFs that regulate intestinal epithelial development, maintenance, and proliferation. Among them, CDX2 might be the most important as it is essential for the specification of all intestinal epithelia during mouse endoderm development [43] and required for epithelial cell identity in the adult [44]. CDX2 binds to many regions marked with H3K4me2, a modification associated which enhancers and promoters. In addition, mice that are deficient in *Cdx2* displayed overall reduced H3K4me2 levels, which affected the binding of other partner TFs [45]. But contrary to the canonical development model in which the chromatin landscape of differentiating cells becomes progressively more restricted, reflecting cell identity, the different adult intestinal epithelial cells all have similar accessible chromatin profiles. For example, DNA methylation, which typically acts to repress gene transcription and is widely regarded as essential for normal development, did not change substantially between progenitor stem cells and adult epithelial cells [46–48]. Furthermore, adult intestinal stem cells and their progeny exhibited similar accessible chromatin landscapes, as illustrated by H3K4me2, H3K27ac and DNase I hypersensitivity levels (which all reveal accessible chromatin regions) [9••]. Thus, intestinal epithelial cell development does not seem to require differential chromatin priming, but instead appears to rely on the differential activity of TFs to activate cell-type specific transcriptional programs. For example, while the TF ATOH1 activates the transcription of secretory genes in the secretory lineage, its expression in the absorptive lineage is repressed by HES1 through Notch signaling [41]. Together, these findings support the

notion that differentiation-related genes are already primed for expression in the intestinal stem cell, and that fate choices in the intestinal epithelium are rooted at the gene expression level, as controlled by TFs that operate in a permissive chromatin environment.

One still poorly studied, yet interesting question is how the chromatin landscape responds to microbial colonization and to pathogenic infection. A study of mouse intestinal epithelial cells reared in the presence or absence of microbiota observed that although gene expression programs in these conditions were different, the accessible chromatin landscapes were similar [49••]. These findings suggest that, as in the differentiation process, the transcriptional response of intestinal cells to the microbiota is regulated mainly by the differential binding of SDTFs, independently of changes in chromatin accessibility. One hypothesis is that in cells with such a short lifespan of 3–5 days [50], it may be more effective to have inducible TFs operating in an already accessible, and therefore permissive, chromatin landscape to respond to an insult. Comprehensive experimentation in other cell types that have also a relatively medium to high turnover and are equally capable of mounting an innate response such as lung epithelial cells [51] or skin keratinocytes [52] may allow us to derive a more general picture. The elucidation of how SDTFs respond to microbial agents to control the transcriptional programs underlying the epithelial response to infection promises therefore to be an exciting area for future research.

Conclusions and outlook

In recent years, much progress was made in our understanding of the regulatory networks that mediate the innate immune response in the gastrointestinal tract, leading to the emergence of interesting new questions. For example, will tissue-resident macrophages react as dynamically to environmental stimuli as monocyte-derived macrophages? Will these responses involve epigenomic modifications of the chromatin landscape? It will in this regard be interesting to examine the chromatin dynamics of tissue-resident macrophages during development, where TF activity may be shaped by the local environment to establish the appropriate chromatin landscape that in turn may tune cell identity. Nevertheless, studying tissue-resident macrophages from mammals as well as the intestinal epithelial response in different infection conditions might prove rather difficult, because of the difficulties associated with acquiring reasonable cell numbers for chromatin related studies, and the experimental set-up in general. In addition, when cultured *in vitro*, macrophages lose their tissue-specific chromatin signatures [7••]. Although many of the methodologies utilized in chromatin studies have been considerably improved in terms of efficiency and sensitivity (e.g. [17•]), we think that more genetically tractable model organisms such as *D. melanogaster* will in this regard continue to play an important role in elucidating innate immunity-linked regulatory properties. Indeed, *Drosophila* was fundamental for the discovery of Toll surface receptor's role in pathogen recognition [53••], has contributed to elucidate interactions between gut cells and microbiota [54], and is gaining importance as a useful model to study the etiology of gut-related pathologies [55]. Importantly, the use of *Drosophila* allows assessing the impact of different infection states (i.e. no, mild, or severe infection) on distinct, but constant genetic backgrounds through the use of inbred fly lines [56•]. In addition, it is proving an ideal model to study the molecular and physiological role of the intestinal microbiota in post-embryonic development and homeostasis [57–59]. Finally, the *Drosophila* community has generated an extensive experimental toolkit including TF clone libraries [60] and TF overexpression fly lines [61], *in vivo* transgenic enhancer lines [62,63] as well as genome engineering tools such as CRISPR [64–66], rendering the *Drosophila* gut an ideal model to achieve a level of mechanistic, regulatory understanding that may currently be unattainable for the mammalian gastrointestinal tract.

Conflict of interest statement

None declared.

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