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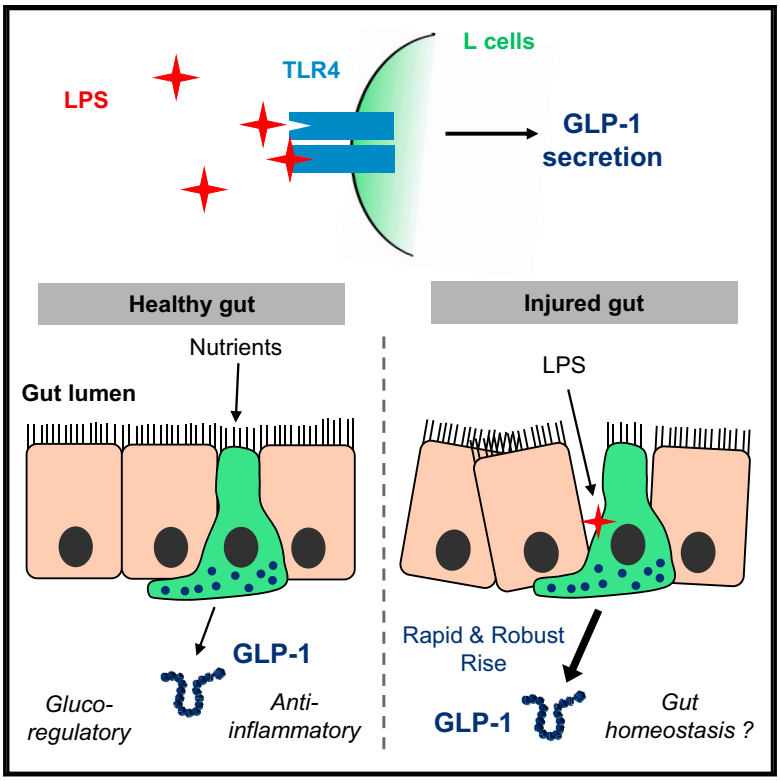
Enteroendocrine L Cells Sense LPS after Gut Barrier Injury to Enhance GLP-1 Secretion

Graphical Abstract



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In Brief

Lebrun et al. demonstrate that enteroendocrine L cells sense lipopolysaccharides (pro-inflammatory bacterial compounds) after gut injury and respond by secreting glucagon-like peptide 1. These findings expand concepts of L cell function to include roles as both a nutrient and pathogen sensor, linking glucagon-like peptide secretion to gut inflammation.

Highlights

- LPSs induce GLP-1 secretion from L cells through a TLR4-dependent mechanism
- Gut ischemic injury is coupled to immediate GLP-1 secretion in mice and humans
- L cells are mucosal sensors of LPS after gut injury
- GLP-1 secretion is closely related to gut inflammation

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Enteroendocrine L Cells Sense LPS after Gut Barrier Injury to Enhance GLP-1 Secretion

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SUMMARY

Glucagon-like peptide 1 (GLP-1) is a hormone released from enteroendocrine L cells. Although first described as a glucoregulatory incretin hormone, GLP-1 also suppresses inflammation and promotes mucosal integrity. Here, we demonstrate that plasma GLP-1 levels are rapidly increased by lipopolysaccharide (LPS) administration in mice via a Toll-like receptor 4 (TLR4)-dependent mechanism. Experimental manipulation of gut barrier integrity after dextran sodium sulfate treatment, or via ischemia/reperfusion experiments in mice, triggered a rapid rise in circulating GLP-1. This phenomenon was detected prior to measurable changes in inflammatory status and plasma cytokine and LPS levels. In human subjects, LPS administration also induced GLP-1 secretion. Furthermore, GLP-1 levels were rapidly increased following the induction of ischemia in the human intestine. These findings expand traditional concepts of enteroendocrine L cell biology to encompass the sensing of inflammatory stimuli and compromised mucosal integrity, linking glucagon-like peptide secretion to gut inflammation.

INTRODUCTION

Glucagon-like peptide 1 (GLP-1) is secreted from enteroendocrine cells (EECs) and was originally described as an incretin hormone, which also exerts other metabolic actions such as reduction in appetite and food intake (Holst, 2007). These actions supported the development of GLP-1 receptor (GLP-1R) agonists for the treatment of type 2 diabetes. GLP-1 acts through a single receptor widely expressed in multiple tissues, suggesting that it could have additional roles beyond glucose lowering. Notably, GLP-1R agonists exert beneficial effects in experimental preclinical models of inflammatory disease (Lee and Jun, 2016). However, the mechanisms through which incretin therapies control inflammation remain incompletely understood (Drucker, 2016).

The gastrointestinal tract, the site of endogenous GLP-1 production, is colonized by billions of bacteria, some of which produce pro-inflammatory lipopolysaccharides (LPSs), which influence gut immunity (Gnauck et al., 2016) and hormone release (Bogunovic et al., 2007). LPSs act mainly through the activation of Toll-like receptor 4 (TLR4) (Poltorak et al., 1998). Under normal conditions, several mechanisms restrict LPSs within the gut lumen; however, in some situations, especially when intestinal barrier function is compromised, LPS molecules enter the blood circulation and trigger systemic inflammation. Intriguingly, LPS or interleukin-6 (IL-6) increased plasma GLP-1 levels in mice, through incompletely understood mechanisms (Ellingsgaard et al., 2011; Kahles et al., 2014; Nguyen et al., 2014).

Here we show that LPSs increase GLP-1 secretion through a TLR4-dependent mechanism. In mice, experimental alteration of the gut barrier led to a rapid rise in plasma GLP-1 levels through mechanisms enabling luminal LPSs to access EECs. Remarkably, LPSs also stimulated GLP-1 secretion in humans, and GLP-1 levels were rapidly increased in humans with acute experimental intestinal ischemia. Hence, gut barrier alteration facilitates LPS exposure, which in turn augments GLP-1 secretion from local L cells. These findings redefine classical nutrient-stimulated concepts of GLP-1 acting as an

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incretin hormone to include a role for GLP-1-producing L cells as sensors of local inflammation and barrier integrity.

RESULTS

LPSs Induce the Expression and Secretion of GLP-1

The actions of LPSs to increase circulating GLP-1 (Nguyen et al., 2014) may be mediated by (1) the enhancement of its secretion and/or (2) the inhibition of its degradation. To distinguish between these mechanisms, we investigated the effects of LPS on GLP-1 metabolism *in vivo*. Mice injected with LPS exhibited marked increases in plasma levels of LPS, measured as 3-hydroxymyristate (3-HM) fatty acids (Figure 1A). Intestinal mRNA transcripts encoding pro- and anti-inflammatory cytokines and TLR4 peaked 3 hr after LPS injection (Figures 1B and 1C), and levels of circulating cytokines increased rapidly (Figure 1D). Ileal expression of proglucagon (*Gcg*, which encodes GLP-1) and prohormone convertase 1/3 (*Pcsk1*) was rapidly and transiently induced (Figures 1E and 1F). As shown in Figures 1G and 1H, both active and total forms of GLP-1 were induced by LPS injection. Due to ethical concerns and in agreement with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines and reduce, replace, and refine (3R) principles, limited analysis at t = 3 and 6 hr revealed that saline administration had no effect compared to LPS injection (Figure 1I). Furthermore, positive correlations between total GLP-1 and LPS were observed (Figure 1J).

We next determined whether intraluminal LPS could stimulate L cell GLP-1 secretion. Both intraluminal glucose and LPS stimulated GLP-1 secretion from mouse ileum explants (Figure 1K). Furthermore, GLP-1 secretion was modestly but rapidly increased after direct LPS stimulation of GLUTag (Figures 1L and 1M) and STC-1 EECs (Figure 1N). *Ex vivo*, quantification of intestinal fatty acid-binding protein (I-FABP), a marker of intestinal cell membrane integrity (Pelsers et al., 2005), did not show any differences with the highest concentration of LPS. *In vitro*, cytotoxicity tests revealed no adverse effects of LPS on the integrity of these two cell lines (data not shown).

LPSs Induce GLP-1 Secretion through a TLR4-Dependent Pathway

Although IL-6 has been proposed as the mediator of LPS-induced GLP-1 secretion (Kahles et al., 2014), injection of LPS in IL-6-deficient (*Il6*$^{-/-}$) mice (Figure S1A) led to an attenuated but detectable increase in GLP-1 secretion (Figure S1B). Furthermore, LPS did not induce GIP secretion (Figure S1C), making indirect GLP-1 secretion through GIP unlikely. We next blocked muscarinic neural transmission with atropine, which resulted in substantially attenuated basal GLP-1 secretion. In contrast, atropine did not abrogate the stimulatory effect of LPS on GLP-1 secretion (Figure S1D).

Although TLR4 has been considered the classical receptor transducing an inflammatory response to LPS, recent data implicate caspases as a non-canonical inflammasome activation pathway that senses LPS (Hagar et al., 2013). After LPS injection, plasma levels of pro- and anti-inflammatory cytokines were robustly increased in wild-type (WT), but not in TLR4-deficient (*Tlr4*$^{-/-}$) mice (Figure 2A); however, the rise in plasma GLP-1 levels was extinguished (Figure 2B), consistent with a critical role for TLR4 in coupling LPS to GLP-1 secretion. As TLR4 is expressed by intestinal EECs (Bogunovic et al., 2007), we next assessed the TLR4 pathway in GLUTag and STC-1 cell lines. GLP-1 secretion was abolished when GLUTag (Figure 2C) or STC-1 cells (Figure 2D) were challenged with LPS in the presence of a TLR4 antagonist, TAK-242 (Figures 2C and 2D). This correlated with an elevation of calcium monitored in single cells in response to LPS in both cell lines (Figures S2A and S2B). Incubation in calcium-free buffer totally blunted this increase, highlighting the importance of extracellular calcium (Figure S2B). Furthermore, LPS-induced calcium fluxes were blocked by a TLR4 antagonist (Figure S2C). Hence, LPSs require TLR4 to induce intracellular calcium flux and GLP-1 secretion *in vitro*.

Luminal LPSs Enhance GLP-1 Secretion after Gut Barrier Injury

LPSs administered intravenously (i.v.) or intraperitoneally (i.p.) induced GLP-1 secretion, whereas no effect was observed when LPSs were given through an oral gavage (Table S1). These findings imply that LPSs may need to reach gut EECs at their basolateral location via the circulation or through luminal exposure in the presence of an altered gut barrier. We next examined animal models in which gut barrier function was compromised. We first studied dextran sodium sulfate (DSS)-induced colitis. As shown in Figure 3A, endotoxemia after an oral administration of LPS in control animals was unchanged over the 6-hr period, yet it increased in DSS-treated mice. Furthermore, plasma GLP-1 levels were increased in DSS-treated mice upon LPS gavage, whereas no increase in GLP-1 was observed in control animals (Figure 3B). Circulating LPS and GLP-1 levels were positively correlated (Figure 3C). Independent of DSS treatment, the oral administration of LPS also led to a marked but transient increase in IL-6 (Figure 3D).

To further assess whether alteration of the gut barrier could facilitate GLP-1 secretion through exposure to endogenous luminal bacterial-derived LPS, we studied mice with mesenteric ischemia/reperfusion (I/R) injury. Gut ultrastructure was highly disorganized after I/R. Short times of I/R were sufficient to damage intestinal villi (Figures S3A and S3B), with further gut injury evident following longer periods of I/R (Figure S3C). Increased GLP-1 levels were observed after a 15-min ischemia/15-min reperfusion period, and they continued to rise after 20-min ischemia/120-min reperfusion (Figure 3E). Levels of *Il6* and *Il10* mRNA transcripts were significantly increased with longer times of reperfusion (Figure 3F). Circulating levels of these two cytokines were detectable after 20 min of ischemia and rose with increased reperfusion time (Figure 3G). We next compared levels of GLP-1 and I-FABP. Only GLP-1 levels rose significantly after a short reperfusion time, suggesting that marked epithelial injury is not required for triggering GLP-1 secretion (Figure 3H), whereas plasma levels of LPS, indirectly quantified as 3-HM, were unchanged during the 2 hr of reperfusion (Figure 3I).

To further investigate the role of endogenous luminal LPS, we used two pharmacological approaches to (1) decrease the quantity of active LPS or (2) decrease signaling through the TLR4 receptor. First, we treated mice with Polymyxin B, an antibiotic that binds to and prevents the activity of LPS. In Polymyxin

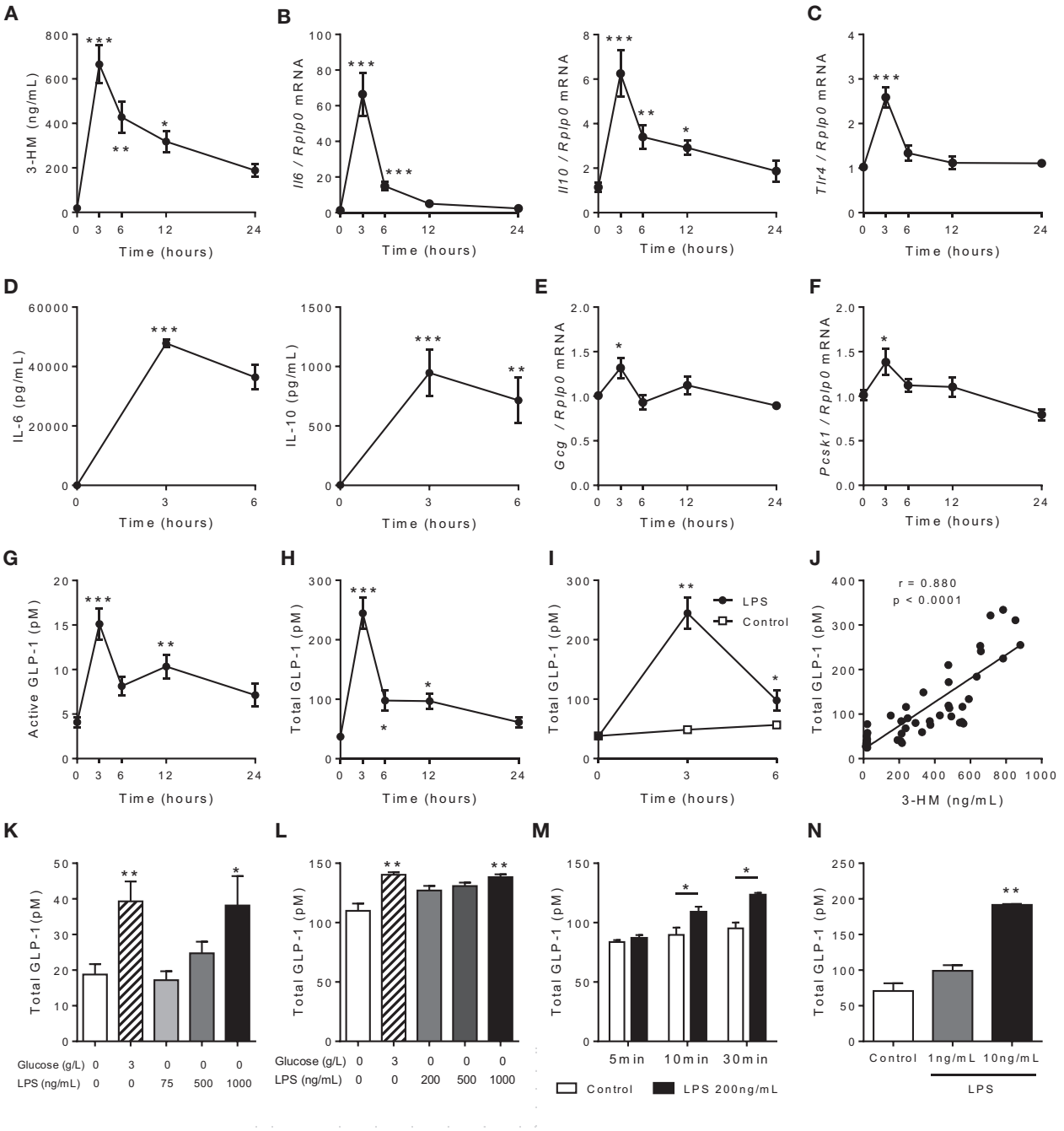


Figure 1. LPSs Enhance GLP-1 Secretion

(A) Plasma 3-HM quantification after LPS i.p. injection in mice (n = 9–10).
(B and C) Ileum cytokines (B) and *Tlr4* (C) mRNA relative expression after LPS i.p. injection in mice (n = 9–10).
(D) Cytokine plasma levels after LPS i.p. injection in mice (n = 8).
(E and F) Mouse ileum *Gcg* (E) and *Pcsk1* (F) mRNA relative expression after LPS i.p. injection (n = 9–10).
(G–I) Plasma active GLP-1 (G) and plasma total GLP-1 (H and I) after LPS i.p. injection in mice (LPS injection, n = 9–10; and saline injection, n = 5).
(J) Correlation between plasma 3-HM and total GLP-1.
(K) GLP-1 secreted by mouse ileum explants challenged by glucose or LPS for 1 hr (n = 11–16).
(L) GLP-1 secreted by GLUTag cells after 90 min of glucose or LPS stimulation (n = 6).
(M) Time course of GLP-1 secretion by GLUTag cells after LPS stimulation (n = 4–5).
(N) GLP-1 secreted by STC-1 cells after 90 min of LPS stimulation (n = 4–5).
All results are expressed as mean ± SEM.

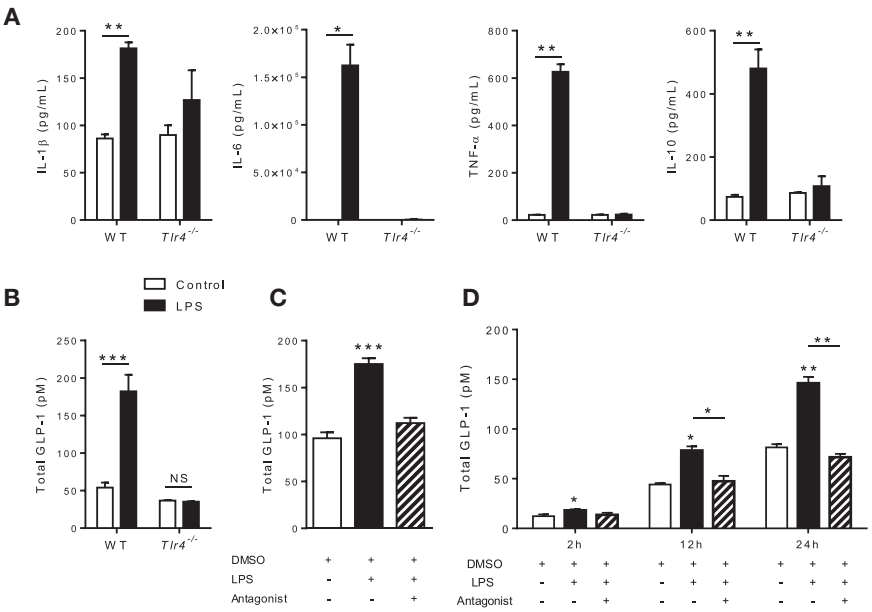


Figure 2. LPSs Induce GLP-1 Secretion through a TLR4-Dependent Mechanism

(A and B) Cytokine (A) and total GLP-1 (B) plasma levels 3 hr after LPS i.p. injection in WT and *Tlr4*$^{-/-}$ mice (n = 10).

(C) GLP-1 secretion by GLUTag cells after LPS stimulation (200 ng/mL) for 90 min ± TLR4 antagonist (n = 4–6).

(D) GLP-1 secretion of STC-1 cells after LPS stimulation (10 ng/mL) ± TLR4 antagonist (n = 4–6). All results are expressed as mean ± SEM. See also Figures S1 and S2.

B-treated mice subjected to I/R, the induction of GLP-1 secretion was blunted (Figures 3J and 3K). The induction of IL-10 was also slightly reduced (Figure 3L). Second, pretreatment of mice with a TLR4 antagonist attenuated the I/R-induced increase in GLP-1 levels (Figure 3M), associated with a lower GLP-1 secretion index (Figure 3N) and reduced levels of IL-10 (Figure 3O). These results suggest that endogenous luminal LPSs induce GLP-1 secretion when gut barrier function is compromised. As intestinal GLP-2 is known to be co-secreted with GLP-1, we tested whether GLP-2 was also increased by LPS or gut injury. Figure S4 shows that LPS injection (Figure S4A) and I/R experiments (Figure S4B) both led to a significant increase in plasma GLP-2 levels.

LPS-Induced GLP-1 Secretion Is Detected in Humans

As shown in Figure 4A, plasma GLP-1 levels were increased 3 hr after LPS, but not saline, injection in human subjects. LPS also induced a transient increase in pro- and anti-inflammatory cytokines (Figure 4B). Finally, we utilized a model of I/R (Grootjans et al., 2010) to evaluate whether I/R injury in the human gut *in vivo* was associated with an increase in GLP-1 secretion. Arteriovenous differences in plasma GLP-1 levels were measured before, after 45 min of ischemia, and after 30 or 120 min of reperfusion. GLP-1 levels were markedly increased after 45 min of ischemia, and they returned to baseline levels after reperfusion of human intestine (Figure 4C). These results demonstrate that even brief periods of human gut injury are associated with a rapid induction of GLP-1 secretion *in vivo*.

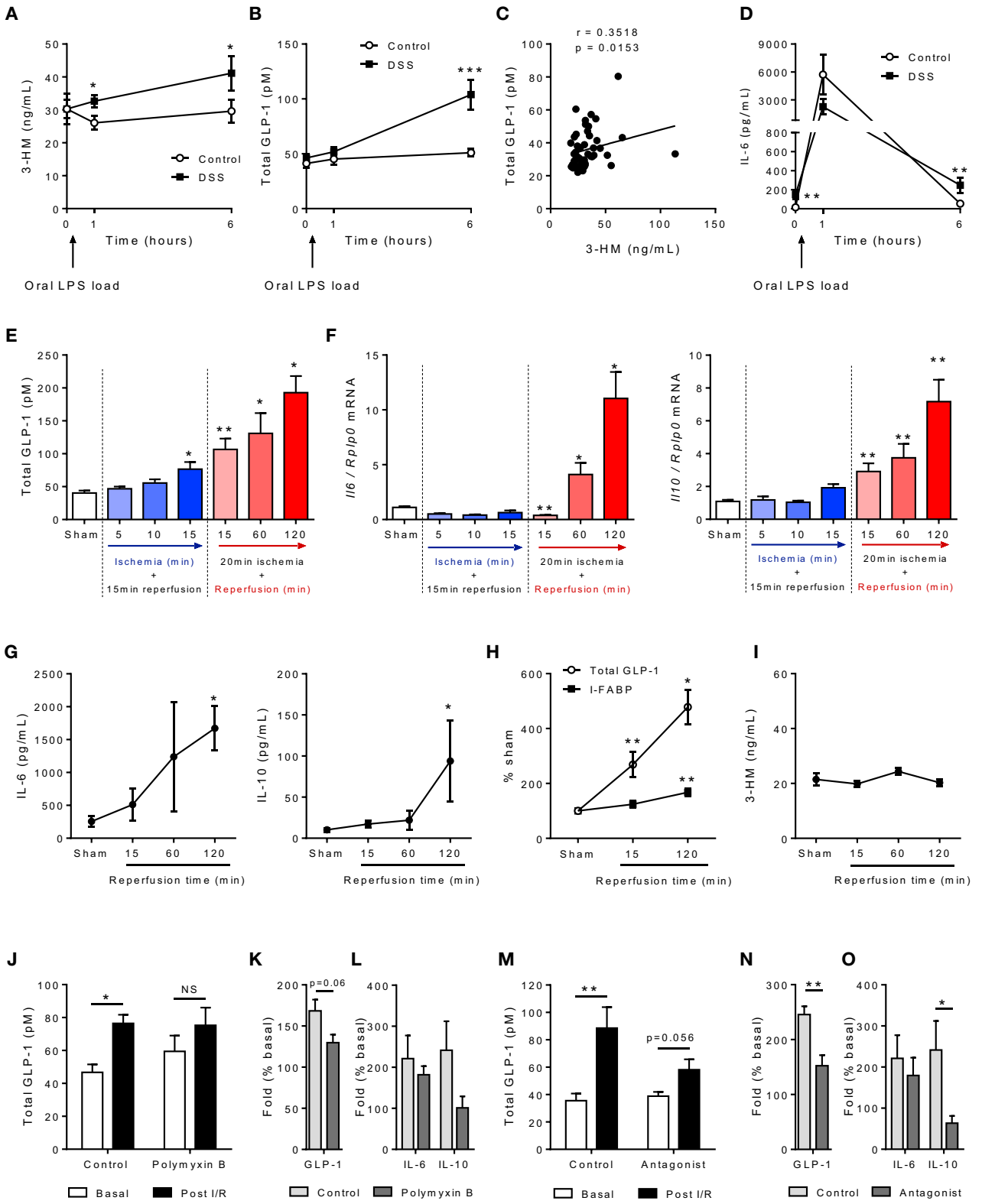
DISCUSSION

We and others have previously shown that LPSs increase plasma levels of GLP-1 in mice (Kahles et al., 2014; Nguyen et al., 2014). Our current study implies that increased secretion is the likely mechanism accounting for the rise in GLP-1 levels following LPS administration. *Tlr4*$^{-/-}$ mice and EECs treated with a TLR4 antagonist established the canonical TLR4 pathway as a key transducer of signals linking LPS to the stimulation of GLP-1 secretion. Moreover, in *Tlr4*$^{-/-}$ mice, plasma cytokines are strongly reduced, consistent with data linking IL-6 and tumor necrosis factor alpha (TNF-α) to the stimulation of GLP-1 secretion (Ellingsgaard et al., 2011; Gagnon et al., 2015).

Under physiological conditions, LPSs do not pass through the gut barrier except in small quantities during nutrient ingestion (Erridge et al., 2007). Indeed, as expected, oral administration of purified LPS molecules did not induce an increase in systemic endotoxemia or GLP-1 secretion. However, when gut barrier function was damaged, such as in ileum explants or in DSS-treated mice, exogenous LPS administered into the lumen led to a significant induction of GLP-1 secretion. In the DSS experiment, the increased levels of IL-6 in both groups of mice (at t = 1 hr) might be explained by the activation of B1 cells of the lamina propria (Murakami et al., 1994) and/or by the rapid sensing of bacterial products by intestinal epithelial cells shown to secrete pro-inflammatory cytokines (Kagnoff and Eckmann, 1997).

In pathophysiological situations exemplified by I/R injury, a very rapid secretion of GLP-1 was observed, findings dependent upon the bioavailability of active LPS. However, for longer time periods of reperfusion, GLP-1 release could also be secondary to cell lysis. Nevertheless, we cannot exclude the possibility that additional molecules present in the gut lumen might simultaneously stimulate GLP-1 secretion during gut injury. Notably, under the defined experimental conditions studied herein, we did not observe changes in circulating LPS or in LPS levels in lymph nodes (data not shown). Nevertheless, LPS stimulated GLP-1 secretion when injected into mice or humans. This suggests that LPS could act through a TLR4 receptor located on the basolateral membranes of EECs, consistent with previous findings (Brighton et al., 2015; Vamadevan et al., 2010) and with our observations linking experimental disruption of gut barrier function with stimulation of GLP-1 secretion.

The LPS-enteroendocrine GLP-1 axis described here is consistent with a putative role for EECs in the innate immune response against pathogens, to maintain mucosal immune



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homeostasis (Bogunovic et al., 2007; Selleri et al., 2008). Moreover, EECs represent key orchestrators of immune responses to pathogens and commensal bacteria (Worthington, 2015). EECs localized within the mucosa without luminal access may be more easily engaged by LPS molecules after mucosal barrier degradation, as occurs upon I/R injury. The increase in permeability associated with gut injury likely facilitates LPS access to and stimulation of enteroendocrine L cells, with resultant GLP-1 secretion. GLP-1 in turn may act as an anti-inflammatory peptide that controls mucosal integrity and attenuates both local and systemic inflammation (Drucker, 2016; Lee and Jun, 2016). Indeed, both Paneth cells (known to produce antimicrobial components) and intestinal intraepithelial lymphocytes have been reported to express a functional GLP-1R (Kedees et al., 2013; Yusta et al., 2015), and loss of the GLP-1R increases the severity of DSS-induced intestinal injury (Yusta et al., 2015).

The physiological relevance of GLP-1 secretion in the control of basal gut barrier homeostasis remains obscure. Nevertheless, liraglutide, a GLP-1 analog, improved gut permeability in rats (Nozu et al., 2017) and induced barrier protective effect by improving Brunner's gland function (Bang-Berthelsen et al., 2016). In addition, GLP-1R agonists promoted intestinal growth (Kissow et al., 2012; Simonsen et al., 2007) through fibroblast growth factor 7 (Koehler et al., 2015), and exogenous GLP-1 protects the gut against oxidative damage (Deniz et al., 2015). Whereas the contribution of endogenously secreted GLP-1 to gut growth appears to be modest (Wismann et al., 2017), endogenous GLP-1 contributes to gut recovery in the pathophysiological context of chemotherapy (Kissow et al., 2013). Thus, the 4.8-fold increase in GLP-1 secretion observed here could also modulate gut homeostasis through local actions to restore gut integrity. L cells also secrete GLP-2, whose role in promoting gut barrier function is well established (Drucker and Yusta, 2014). GLP-2 reduces intestinal permeability through modulation of gut barrier function (Cani et al., 2009; Moran et al., 2012), and it exerts beneficial effects in the rat gastrointestinal tract after intestinal I/R (Zhang et al., 2008). Beyond local gut effects, rising GLP-1 levels during gut ischemia may exert protective effects on heart (Drucker, 2016), kidney (Skov, 2014), lung (Viby et al., 2013), and liver (Wang et al., 2014). Furthermore, central GLP-1 reduces inflammation-induced fever (Rinaman and Comer, 2000). Hence, GLP-1 may act as an early signal to facilitate organ protection following disruption of gut barrier function.

Previous studies have correlated increased levels of circulating GLP-1 with the extent of concomitant critical illness in human subjects (Ingels et al., 2017; Kahles et al., 2014; Lebherz et al., 2017). Our current findings illuminate the importance of GLP-1 in this context by demonstrating that (1) LPSs acutely induce GLP-1 secretion in humans and (2) the injured human gut responds rapidly to ischemic injury with increased GLP-1 secretion. The prompt GLP-1 secretion after gut barrier injury further highlights the role of the enteroendocrine L cell and GLP-1 in the response to injury and inflammation, expanding concepts of L cell function to include roles as both a nutrient and pathogen sensor. Indeed, recent findings have questioned the relative importance of gut versus pancreatic GLP-1 for the control of glucose homeostasis (Chambers et al., 2017). Our current data add to the body of evidence implicating GLP-1 as a component of the mucosal response to external injury, and they suggest that GLP-1 may represent a candidate early biomarker of gut injury in humans. Indeed, in the present I/R experiments, GLP-1 secretion occurs earlier than I-FABP, itself a promising biomarker of gut injury (Khadaroo et al., 2014). Further work will be needed to determine whether GLP-1 levels reflect the severity of gastrointestinal damage in humans with localized or extensive gastrointestinal injury. Collectively, our data position the L cell as a key mucosal sensor of gut injury, which responds to mucosal damage by secretion of the glucagon-like peptides, which in turn could promote restoration of mucosal integrity and attenuation of inflammation.

EXPERIMENTAL PROCEDURES

Animals

All animal procedures were in accordance with institutional guidelines and approved by the University of Burgundy's Ethics Committee on the Use of Laboratory Animals (protocol number 5459). All experiments were performed using male C57BL/6J mice (8–12 weeks old); *Tlr4*$^{-/-}$ mice were provided by Dr. Bernard Ryffel (Hoshino et al., 1999). Blood samples were collected in EDTA-coated tubes. Plasma was separated by centrifugation at 7,200 × *g* for 10 min at 4°C. Blood and plasma samples were stored at −20°C.

Acute LPS Administration

LPSs were i.p. injected at 1 mg/kg. Details are provided in the Supplemental Experimental Procedures.

Gut Injury Models

DSS treatment was adapted from previously described experimental procedures (Kitajima et al., 1999). The oral load of LPS (5 mg/animal) was performed

Figure 3. Gut Injury Induces GLP-1 Secretion in Mice

(A and B) Plasma 3-HM (A) and total GLP-1 (B) levels after LPS gavage in control and DSS-treated mice (n = 8–9).
(C) Plasma 3-HM and total GLP-1 correlation.
(D) IL-6 plasma levels after LPS gavage in control and DSS-treated mice (n = 8).
(E) Total GLP-1 plasma levels after mesenteric I/R (n = 5).
(F) Ileum cytokine mRNA relative expression after mesenteric I/R (n = 5).
(G) Cytokine plasma levels after 20 min of ischemia and 15, 60, or 120 min of reperfusion (n = 4–5).
(H) Total GLP-1 and I-FABP plasma levels after 20 min of ischemia and 15 or 120 min of reperfusion (n = 6).
(I) LPS blood levels after 20 min of ischemia and 15, 60, or 120 min of reperfusion (n = 5–6).
(J) Total GLP-1 plasma levels before and after I/R (20/15 min) in control and Polymyxin B-treated mice (n = 6).
(K and L) Fold induction of GLP-1 (K) and cytokine (L) plasma levels after I/R (20/15 min) in control and Polymyxin B-treated mice (n = 6).
(M) Total GLP-1 plasma levels before and after I/R (20/15 min) in control and TLR4 antagonist-treated mice (n = 6).
(N and O) Fold induction of GLP-1 (N) and cytokine (O) plasma levels after I/R in control and TLR4 antagonist-treated mice (n = 6).
All results are expressed as mean ± SEM. See also Table S1 and Figures S3 and S4.

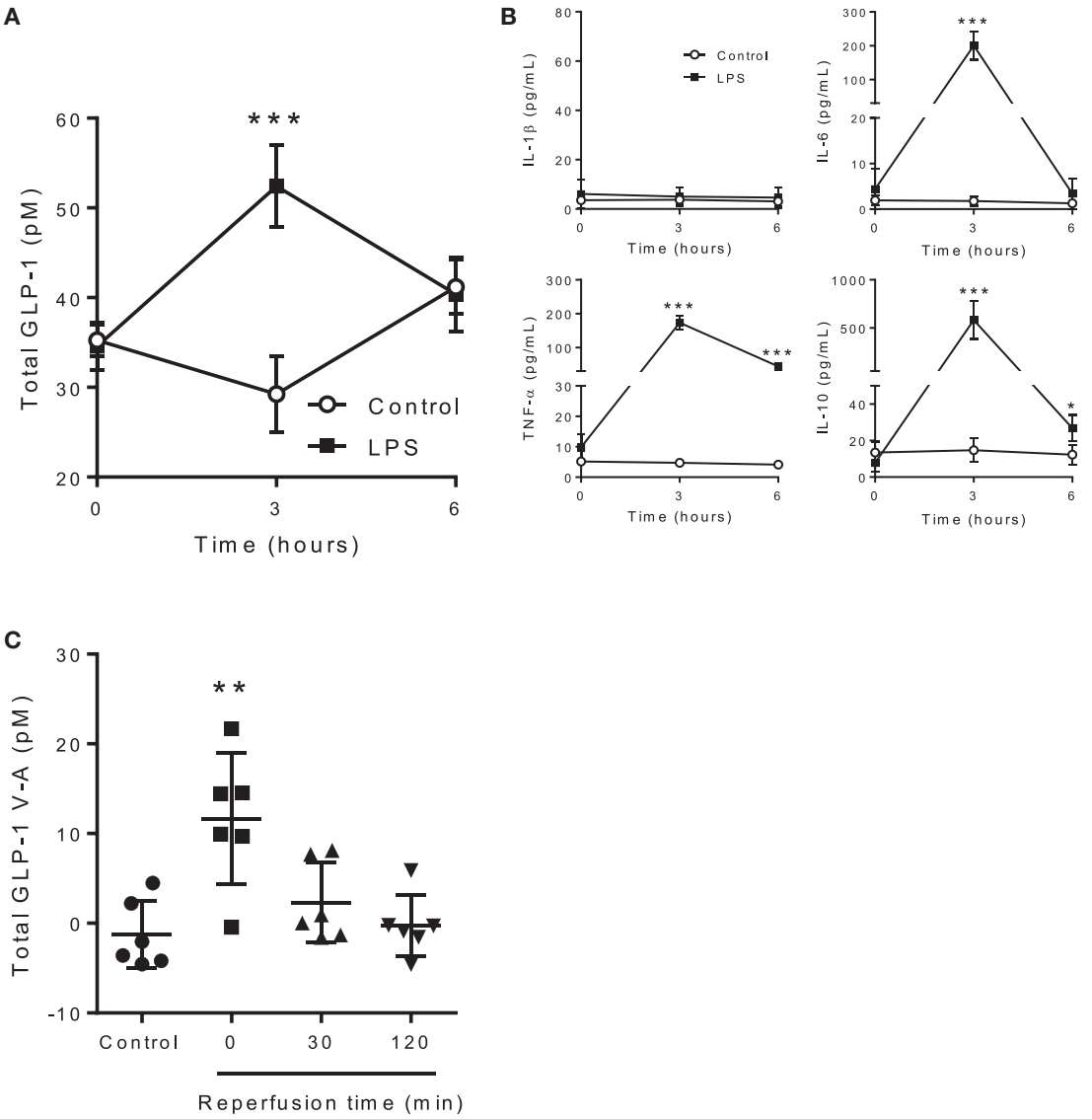

Figure 4. LPS and Intestinal I/R Lead to GLP-1 Secretion in Humans

(A and B) Total GLP-1 (A) and cytokine (B) plasma levels in LPS-injected and control subjects (n = 15).

(C) Total GLP-1 arteriovenous differences (V-A) in patients before and after 45 min of intestinal ischemia and 0, 30, or 120 min reperfusion (n = 6).

All results are expressed as mean ± SEM.

after DSS treatment (2.5% [w/v] for 7 days in drinking water). Intestinal I/R was achieved through an occlusion of the superior mesenteric artery. Polymyxin B (0.2 mg/mL for 14 days in drinking water) and TLR4 antagonist (i.p. injection at 0.2 mg/animal) were administered prior to I/R experiments. Details are provided in the Supplemental Experimental Procedures.

Real-Time qPCR

Details are provided in the Supplemental Experimental Procedures.

Human LPS Injection

Experimental endotoxemia was induced in healthy volunteers (men from 18 to 35 years old) through an i.v. administration of LPS at 2 ng/kg. Experiments were in accordance with the Declaration of Helsinki. After approval from the local ethics committee of the Radboud University Medical Center, volunteers gave written informed consent to participate in the study. Subjects were screened before participation and had a normal physical examination, electrocardiography, and routine laboratory values. Details are provided in the Supplemental Experimental Procedures.

Human Intestinal I/R

The experimental protocol was performed as previously described (Grootjans et al., 2010). Experiments were in accordance with the Declaration of Helsinki. The study was approved by the Medical Ethics Committee of the Maastricht University Medical Center, and written informed consent of all patients (six patients with a median age of 67 years, range of 42–85 years) was obtained. Details are provided in the Supplemental Experimental Procedures.

GLP-1 Secretion Experiments

Ex vivo GLP-1 secretion was studied in a mice model of ileum explants. *In vitro* GLP-1 secretion was investigated using the murine enteroendocrine cell lines

GLUTag and STC-1. Details are provided in the Supplemental Experimental Procedures.

Biochemical Analysis

LPS concentrations were measured through the quantification of 3-HM levels according to the procedure previously described (Pais de Barros et al., 2015). Active/total GLP-1 and I-FABP concentrations were determined by commercially available ELISA Kits (EGLP-35K, Millipore; EZGLP1T-36K, Millipore; E-EL-M0735, Elabscience) in accordance with the manufacturers' protocols. Cytokine plasma levels were measured by Milliplex MAP 5-Plex Kit using mouse cytokine/chemokine magnetic bead panel (MCYTOMAG-70K, Millipore), according to the manufacturer's protocol, and using a LuminexR apparatus (Bio-Plex 200, Bio-Rad).

Statistical Analysis

Data are presented as mean ± SEM. Statistical analyses were performed using Prism (GraphPad). To decide whether to use parametric or non-parametric statistics, the normality of distributions was assessed with the Shapiro-Wilk test (under n = 7, distributions were considered to be non-normal). Statistical significance of differences between two groups was evaluated with the Mann-Withney U test or the Sudent's t test (a statistical correction was applied when variances were different between groups). For more than two groups, Kruskal-Wallis with Dunn's post hoc test or one-way ANOVA with Dunnett's post hoc test was performed. Spearman correlations were calculated between groups. A value of $p < 0.05$ was considered statistically significant (NS, not significant; $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$).

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, and one table and can be found with this article online at https://doi.org/10.1016/j.celrep.2017.10.008.

AUTHOR CONTRIBUTIONS

Conceptualization, Methodology, Validation, and Formal Analysis, L.J.L. and J.G.; Investigation, L.J.L., J.G., P.P., K.L., N.A.K., J.-P.P.d.B., J.P., D.K., M.K., C.H.C.D., I.H.R.H., C.T., V.D., S.M., and T.B.; Resources, N.L.G., D.J.D., P.P., and K.L.; Writing – Original Draft and Visualization, L.J.L. and J.G.; Writing – Review & Editing, L.J.L., K.L., D.K., J.-P.P.d.B., N.L.G., J.P., C.T., T.B., C.H.C.D., M.K., I.H.R.H., N.A.K., S.M., V.D., P.P., D.J.D., L.L., and J.G.; Supervision, J.G.; Funding Acquisition, L.L. and J.G.

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Supplemental Information

Enteroendocrine L Cells Sense LPS after Gut Barrier Injury to Enhance GLP-1 Secretion

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Supplemental Figures

Figure S1

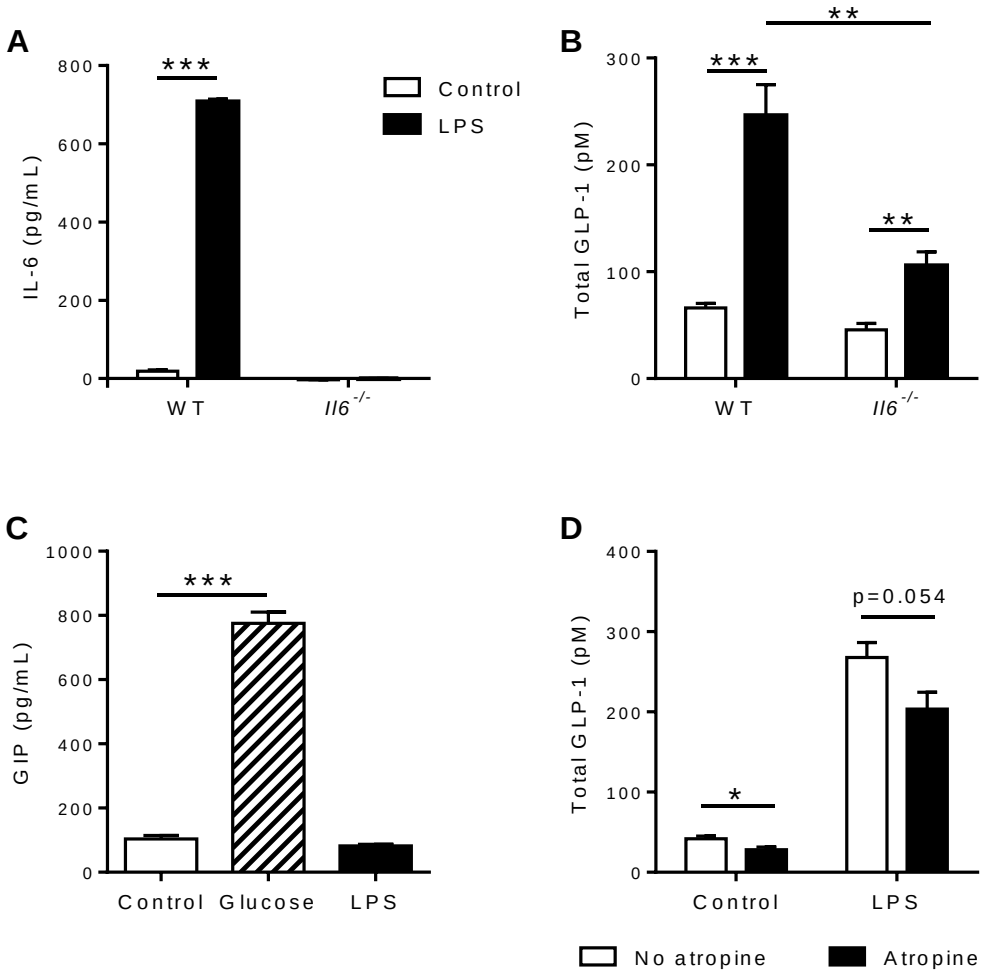

Figure S1 (related to Figure 2). LPS-induced GLP-1 Secretion is not Mediated by Indirect Pathways
(A and B) IL-6 (A) and total GLP-1 (B) plasma levels in WT and *Il6*$^{-/-}$ mice 3 hours after LPS i.p. injection (n=8-10).
(C) GIP plasma levels 15 minutes after glucose gavage or 3 hours after LPS i.p. injection in mice (n=11).
(D) GLP-1 secretion 3 hours after i.p. injection of LPS in mice ± atropine (n=7-8).
All results are expressed as mean ± SEM.

Figure S2

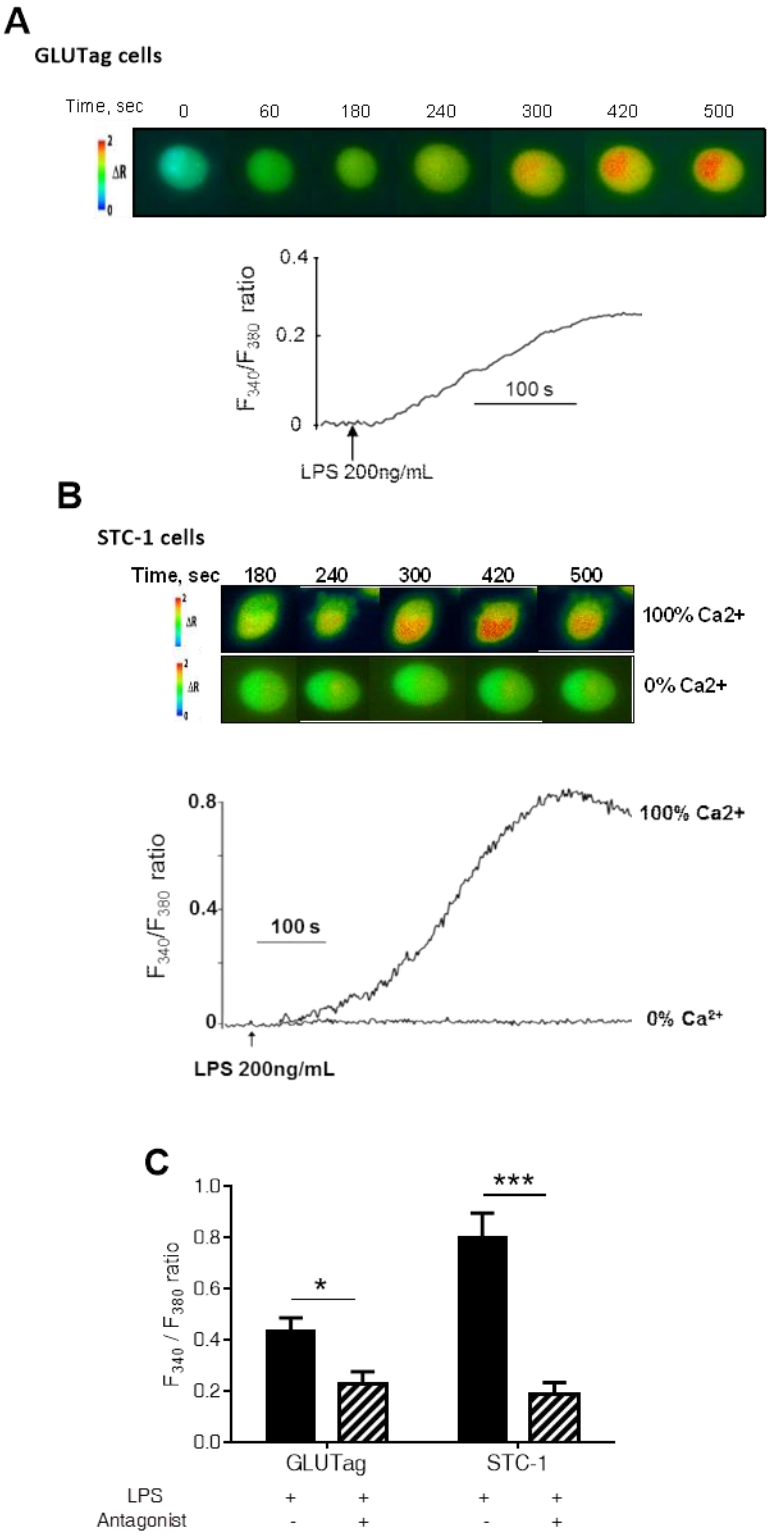

Figure S2 (related to Figure 2). LPS Trigger Calcium Signaling in EECs
(A) Calcium influx in GLUTag cells after LPS stimulation.
(B) Calcium influx in STC-1 cells after LPS stimulation in 0% or 100% calcium buffers.
(C) Calcium influx in GLUTag and STC-1 cells after LPS stimulation ± TLR4 antagonist (n=8). Results are expressed as mean ± SEM.

Figure S3

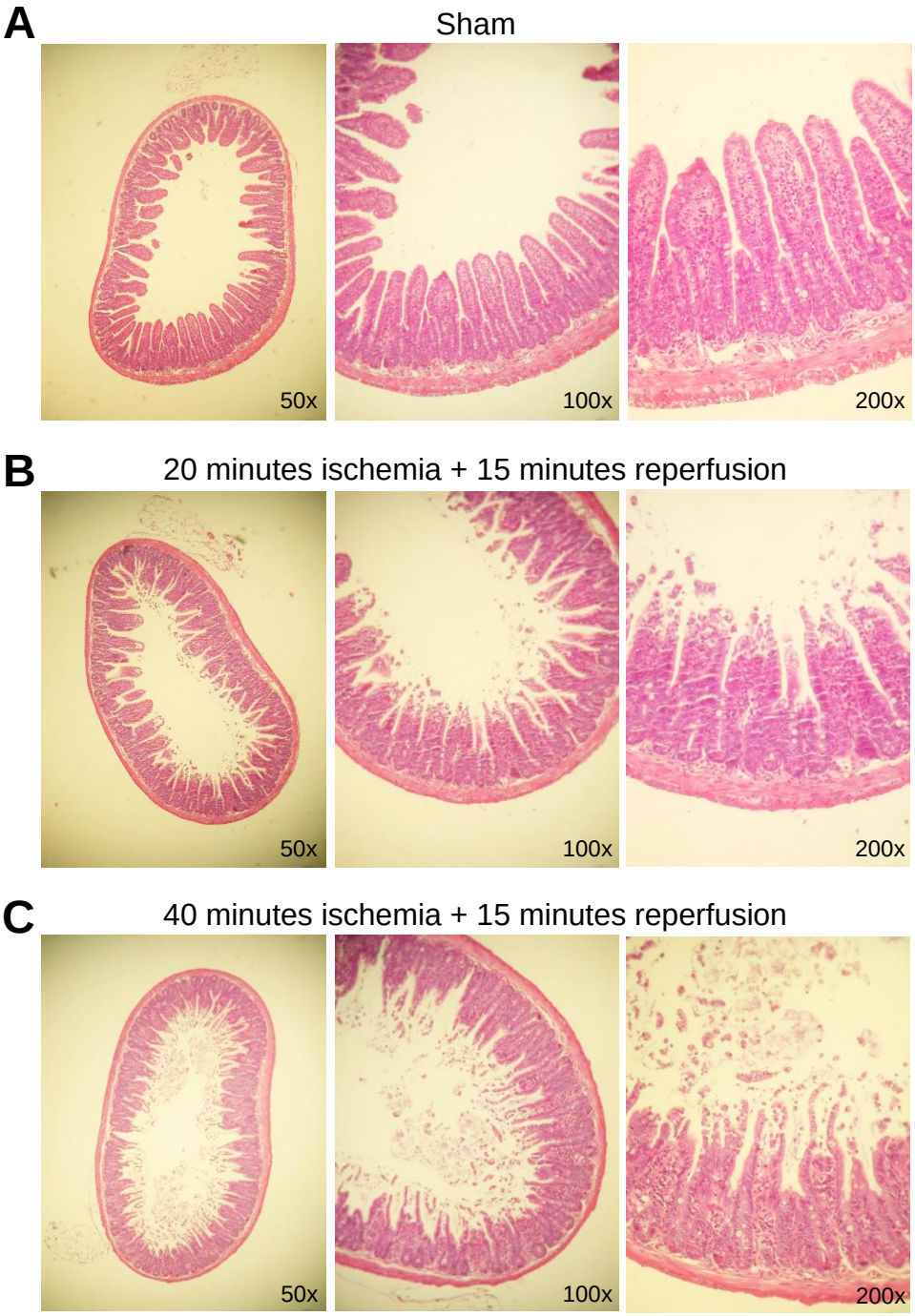

Figure S3 (related to Figure 3). Intestinal Injury after Mesenteric I/R
(A, B and C) Hematoxylin / eosin staining of mouse ileum histological sections from sham group (A), after 20 minutes of ischemia and 15 minutes of reperfusion (B) or after 40 minutes of ischemia and 15 minutes of reperfusion (C).

Figure S4

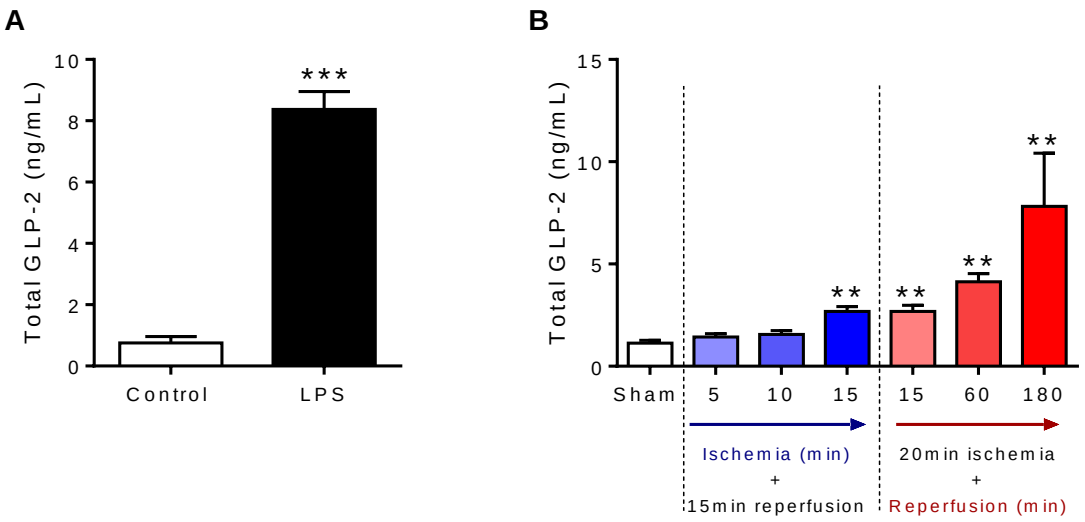

Figure S4 (related to Figure 3). LPS Injection and Gut Injury Induce GLP-2 Secretion in Mice
(A and B) Total GLP-2 plasma levels 3 hours after LPS i.p. injection (A; n=7-8) or intestinal I/R (B; n=5-7). All results are expressed as mean ± SEM.

Supplemental Table

Table S1 (related to Figure 3). Routes of LPS Administration Govern GLP-1 Secretion

Routes of administration	Total GLP-1 (pM)		p values
	Control	LPS	
i.p. injection	10.6 ± 1.7	41.3 ± 3.3	$p < 0.001$
i.v. injection	13.0 ± 3.2	73.7 ± 8.6	$p < 0.001$
Oral gavage	19.8 ± 1.8	21.9 ± 1.9	$p = 0.82$

Total GLP-1 plasma levels in mice 3 hours after i.p LPS injection (n=11), i.v LPS injection (n=7) or oral LPS gavage (n=9-10). All results are expressed as mean ± SEM.

Supplemental Experimental Procedures

Animals

IL-6 deficient (*Il6*$^{-/-}$) mice were from a C57BL6/J background (8-12 weeks old) and were provided by Dr. Victorine Douin.

Drugs administration in mice

LPS from *Escherichia coli* 055:B5 (L2880, Sigma) were solubilized in saline 0.9% when i.p. and i.v. injected at 1mg/kg or in drinking water when administered through an oral load at 5mg/animal. Atropine (4301196, Aguettant) was prepared in saline 0.9% and was i.p. injected at 1mg/kg 10 minutes before and 90 minutes after the injection of LPS. Glucose (G8270, Sigma), prepared in drinking water, was administered at 2mg/kg through an oral load. Polymyxin B Sulfate (P0972, Sigma) was administered at 0.2mg/mL in drinking water for 14 days prior to I/R experiments. TLR4 antagonist (TAK-242, 614316, Millipore) was i.p. injected at 0.2mg/animal 3 hours prior to I/R experiments. Blood and ileum collections were carried out 15 minutes after an oral glucose load as well as before, and 3, 6, 12 and/or 24 hours after LPS administration.

Gut injury models

Mice were given 2.5% (w/v) DSS (molecular weight 40kDa; 42867, Sigma) in drinking water and allowed to drink *ad libitum* for 7 days. The control group received drinking water without DSS. Blood collections were obtained before, 1 and 6 hours after LPS administration (5mg/animal).
For intestinal I/R experiments, mice were separated into sham and I/R groups. Mice were anesthetized with isoflurane inhalation and placed in a supine position on heating pads to maintain body temperature at 37°C. Midline laparotomy was performed and the superior mesenteric artery was isolated. Ischemia was induced by clamping the mesenteric artery for 5, 10, 15, 20 or 40 minutes and was followed by 15, 60 or 120 minutes of reperfusion (removal of the clamp). Gut ischemia was confirmed by intestinal color change and gut reperfusion by the reappearance of pulsation and color. Blood and ileum collections were performed at the specified different time points.

Real-Time Quantitative PCR

Ileum was immediately snap frozen (immersion in liquid nitrogen) after harvest and stored at -80°C until RNA extraction. Total RNA was isolated using RNeasy Mini Kit (74106, Qiagen) according to the manufacturer's instructions. RNA extraction included a DNAse treatment step. RNA was quantified using the NanoDrop 1000 spectrophotometer (Thermo Scientific), and 500ng of RNA from each sample was reverse transcribed using the High-Capacity cDNA Reverse Transcription Kit (Multiscribe® reverse transcriptase, 4368813, Applied Biosystems) according to the manufacturer's instructions. Quantitative PCRs were performed using StepOnePlus (Real-Time PCR System, Applied Biosystems), TaqMan® (4324018, Applied Biosystems) or SYBRGreen® (4367659, Applied Biosystems) technologies. The mRNA level was normalized to levels of *Rplp0* mRNA and the results were expressed as relative expression levels using the $2^{-\Delta\Delta Ct}$ method.

Human LPS injection

Subjects are men from 18 to 35 years old without any medical history and/or special medical treatment. LPS solution (LPS purified from *Escherichia coli*; US Reference Standard Endotoxin *Escherichia coli* O:113, Pharmaceutical Development Section of the National Institutes of Health, Bethestda, USA) was administered i.v. to 15 subjects as a bolus of 2 ng/kg. Control group consists of 15 subjects who received a saline intravenous bolus containing no LPS. Blood samples were drawn using an arterial cannula before as well as 3 and 6 hours after the bolus. Blood tubes were centrifuged within 10 minutes of collection at 2000g for 10 minutes and at 4°C. Plasma was initially stored at -20°C for up to 24 hours before being stored at -80°C (plasma collected on EDTA tubes and lithium heparin).

Human intestinal ischemia/reperfusion

Six patients with a median age of 67 years (range, 42 to 85 years) undergoing pancreaticoduodenectomy for benign or malignant disease were included in this study. Patients with bile duct obstructive disease were stented before surgery. All patients had normal bile flow at the time of the surgical procedure. During pancreaticoduodenectomy, a variable segment of jejunum is routinely resected in continuity with the head of the pancreas and duodenum as part of the surgical procedure. The terminal 6 cm of this jejunal segment was isolated and subjected to 45 minutes of ischemia by placing two atraumatic vascular clamps over the mesentery. Meanwhile, surgery proceeded as planned. After 45 minutes of ischemia, one third (2 cm) of the isolated ischemic jejunum was resected using a linear cutting stapler. Next, clamps were removed to allow reperfusion, as confirmed by regaining of normal pink color and restoration of gut motility. Another segment of the isolated jejunum (2 cm) was resected similarly after 30 minutes of reperfusion. The last part was resected after 120 minutes of reperfusion. Simultaneously, 2 cm of jejunum, which remained untreated during surgery, was resected, serving as internal control tissue. This segment underwent similar surgical handling as the isolated part of jejunum, but was not exposed to I/R. Arterial blood was sampled before ischemia, immediately on reperfusion, and at 30 and 120 minutes after start of reperfusion. Simultaneous with each respective arterial blood sample, blood was drawn from the venule draining the isolated jejunal segment by direct puncture to assess concentration gradients across the isolated jejunal segment. All blood samples were directly transferred to prechilled EDTA vacuum tubes (Becton Dickinson Diagnostics, Aalst, Belgium) and kept on ice. At the end of the procedure all blood samples were centrifuged at 1800g, 4°C for 15 minutes to obtain plasma. Plasma was immediately stored in aliquots at -80°C until analysis.

GLP-1 secretion in a mice model of ileum explants

Mice were anesthetized with isoflurane inhalation and placed in a supine position on heating pads to maintain body temperature at 37°C. Midline laparotomy was performed and the entire small intestine was removed. Only the terminal 3cm of ileum was studied because of its high GLP-1 secretion capacity. Two 1cm pieces were cut from this ileum segment, ligatured at the bottom, filled with either saline 0.9%, glucose 3 g/L or LPS at different concentrations (75, 500 or 1000 ng/mL), closed on the other side and incubated in secretion medium at 37°C for 1 hour. Secretion medium was a glucose-free Krebs-Ringer Bicarbonate Buffer (KRBB; 138mM NaCl, 5.6mM KCl, 2.6mM $CaCl_2$, 1.2mM $MgCl_2$, 4.2mM $NaHCO_3$, 1.2mM NaH_2PO_4 and 10mM hepes) supplemented with 0.5% (w/v) bovine serum albumin (A1595, Sigma) and adjusted to pH 7.4. At the end of the incubation time, medium was collected, centrifuged at 800g for 5 minutes at 4°C to remove any floating particles and frozen at -20°C for subsequent biochemical analysis.

Cell culture and GLP-1 secretion from L cell lines

The murine enteroendocrine cell lines GLUTag and STC-1 (a gift from Dr. Guido Rindi) were cultured in Dulbecco's modified Eagle's medium (DMEM) GlutaMAX™ (21885-025, Gibco) containing 5.6 mM glucose and supplemented with 10% (v/v) fetal bovine serum (10270, Gibco) and 1% (v/v) penicillin-streptomycin (PenStrep, 15140-122, Gibco). Cells were kept in a humidified incubator at 37°C and 5% CO_2. Medium was changed every 3 days and cells were trypsinized and replated when 70%-80% confluence was reached. Both L cell lines have proven to be useful models of GLP-1 secretion. Two days before secretion experiments, cells were plated in 24-well culture plates and allowed to reach 60-80% confluence. On the day of the experiment, cells were washed once with 500µL of secretion medium (GLUTag cells) or culture medium (STC-1 cells). Secretion medium was KRBB, identical to that used for ileum explants. Experiments were performed by incubating the cells with test reagents in 500µL of secretion medium for 5, 10, 30 or 90 minutes (GLUTag) or culture medium for 90 minutes as well as 2, 12 or 24 hours (STC-1) at 37°C and 5% CO_2. Glucose (G8270, Sigma) and LPS (L2880, Sigma) were prepared in saline 0.9%. TLR4 antagonist (TAK-242, 614316, Millipore) was used at 5µM and prepared as 2.8mM stock in dimethyl sulfoxide (DMSO, D8418, Sigma) to adjust the final DMSO concentration to 0.2%. At the end of the incubation time, medium was collected, centrifuged at 800g for 5 minutes at 4°C to remove any floating cells and frozen at -20°C for subsequent biochemical analysis.

Calcium signaling in vitro

STC-1 and GLUTag cells were suspended in fresh Iscove's Modified Dulbecco's Medium IMDM containing 10% fetal bovine serum and seeded ($2x10^5$/well) onto a Willico-Dish wells. The changes in intracellular Ca^{2+} (F_{340}/F_{380}) were monitored using a Nikon microscope (TiU) equipped with EM-CCD (Lucas)

camera for real time recording of 16-bit digital images and an S-fluor 40x oil immersion objective. The planes were taken at *Z* intervals of 0.3 μm, and software (NIS-Elements) was used to analyze the images. The changes in intracellular Ca^{2+} were expressed as ΔRatio, calculated as the difference between the peak F_{340}/F_{380} ratio. The data were summarized from a large number of individual cells (20–40 cells in a single run, with 3–9 identical experiments that included at least three cell preparations). For experiments in Ca^{2+}-free medium, $CaCl_2$ was replaced by EGTA (2mM). Experiments were performed by incubating the cells with test reagents. LPS (L2880, Sigma) and TLR4 antagonist (LPS-RS Ultrapure, tlrl-prslps, Invivogen) were prepared in saline 0.9% at 200ng/mL and 0.5μg/mL respectively.

Light microscopy

The morphologic alterations in the gut were examined by light microscopy (x50, x100 and x200; Axiovert 25, Zeiss). Ileum tissues were promptly taken in sham-operated and I/R groups after 20 or 40 minutes of ischemia and 15 minutes of reperfusion. Gut samples were fixed for 48 hours in 10% neutral buffered formalin at room temperature, dehydrated by graded ethanol and embedded in paraffin for histological analysis. Tissue sections (thickness of 5μm) were deparaffinized with xylene, stained with hematoxylin and eosin.

Biochemical analysis

Total GLP-1, GIP, IL-6 and total GLP-2 concentrations were determined by commercially available ELISA Kits (EZGLP1T-36K, Millipore; EZRMGIP-55K, Millipore; EZMIL6, Millipore and EZGLP2-37K, Millipore) in accordance with manufacturer's protocols.