

1 **Interleukin 27 is a potential rescue therapy for acute severe colitis via interleukin-10 dependent, T**
2 **cell independent attenuation of colonic mucosal innate immune responses**

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28

29 Abstract

30 Background; IBD patients with acute severe colitis face systemic anti-TNF biologic rescue therapy or
31 colectomy, if treatment with intravenous steroids fail. Interleukin (IL)-27 is a cytokine with an
32 immunosuppressive role in adaptive immune responses. However, the IL-27 receptor complex is
33 also expressed on innate immune cells, and there is evidence that IL-27 can impact the function
34 of innate cell subsets, although this particular functionality *in vivo* is not understood. Our aim was
35 to define the efficacy of IL-27 in acute severe colitis and characterize novel IL-27 driven mechanisms of
36 immunosuppression in the colonic mucosa.

37 Methods; We assessed oral delivery of *Lactococcus lactis* expressing an IL-27 hyperkine on the innate
38 immune response *in vivo* in a genetically intact, non-infective acute murine colitis model induced by
39 intra-rectal instillation of 2,4,6-Trinitrobenzenesulfonic acid in SJL/J mice.

40 Results; IL-27 attenuates acute severe colitis through reduction of colonic mucosal neutrophil infiltrate
41 associated with a decreased CXC chemokine gradient. This suppression was T cell-independent and IL-
42 10-dependent, initially featuring enhanced mucosal IL-10. IL-27 was associated with a reduction in
43 colonic pro-inflammatory cytokines and induced a multifocal strong positive nuclear expression of
44 phosphorylated STAT-1 in mucosal epithelial cells.

45 Conclusion; We have defined novel mechanisms of IL-27 immunosuppression towards colonic innate
46 immune responses *in vivo*. Mucosal delivery of IL-27 has translational potential as a novel therapeutic for
47 IBD and is a future mucosal directed rescue therapy in acute severe inflammatory bowel disease.

48

49 Keywords; interleukin-27, cytokine, IBD, colitis

50 Introduction

51 Inflammatory bowel disease is a progressive relapsing remitting inflammation of the
52 gastrointestinal tract ¹ and is increasing in incidence globally ². Biologic therapy has revolutionized
53 patient management ^{3, 4}. Despite this and the use of other immunosuppressant drugs, there remains a
54 significant cohort of patients with refractory or relapsing disease, or those that cannot tolerate available
55 treatments. The systemic nature of current immunosuppressant therapies is associated with an appreciable
56 risk of significant adverse events and carries a financial burden. Novel, safer, and more affordable
57 treatment strategies are required for maintenance therapies.

58 Acute severe colitis requires hospital admission for intravenous steroid therapy and close
59 monitoring, due to potential life threatening complications such as perforation and toxic dilatation ⁵⁻⁷. Up
60 to 15% of patients present with acute severe disease. Clinical and laboratory severity markers predict the
61 need for emergent colectomy and 85% of patients with >8 stools/day or 3-8 stools/day and raised CRP
62 >45mg/L on day 3 need urgent consideration of escalated medical therapy with rescue infliximab anti-
63 TNF biologic therapy or intravenous ciclosporin, or surgery. If medical therapy fails by day 7 or life
64 threatening complications occur, emergency colectomy with ileostomy is required as a lifesaving
65 treatment. This clinical need for life saving and life changing surgery unfolds rapidly and can be
66 associated with post-operative psychosocial morbidity, especially in adolescent and young adult
67 patients ⁸⁻¹⁰. There is a need for novel rescue therapies for the treatment of acute severe colitis.

68 We previously demonstrated that oral delivery of *Lactococcus lactis* (*L. lactis*) expressing an IL-
69 27 hyperkine (LL-IL-27), composed of both EBI3 and p28 subunits, a linker molecule and secretory
70 peptide, is immunosuppressive in murine chronic enterocolitis induced by CD4⁺CD45Rb^{hi} T cell
71 transfer ¹¹. In this T cell driven colitis model, representing adaptive immune responses, LL-IL-27 led to
72 significant histological improvement and survival advantage, through induction of IL-10 derived from
73 intra-epithelial mucosal T cells. We also described an immunosuppressive action on acute DSS colitis ¹¹,

74 although the mechanism was not explored. We then hypothesized that IL-27 may exert an
75 immunosuppressive role in innate immune responses.

76 IL-27, a heterodimeric cytokine composed of EBI3 and p28 subunits, is secreted from
77 antigen presenting cells and signals via a heterodimeric receptor complex composed of the
78 widely expressed Gp130, and the specific IL-27R α ¹²⁻¹⁴. IL-27 was initially considered pro-
79 inflammatory through promotion of Th1 responses ¹⁵. There is now appreciation that IL-27 has a
80 wide functional repertoire, including profound anti-inflammatory effects through promotion of
81 IL-10 secreting Tr1 regulatory T cells and inhibition of Th2 and Th17 responses ¹²⁻¹⁴.
82 Knowledge of IL-27 functionality has largely come from T cell biology, and its role in the
83 adaptive immune response is well described ¹²⁻¹⁴. However, the IL-27 receptor complex is also
84 expressed on other immune cell types, including granulocytes ¹⁶ and macrophages ¹⁷, indicating
85 that the functional capabilities of IL27 may be even wider and in particular IL27 may play a role
86 in mediating innate immunity. Indeed, IL-27 can impact the function of innate cell subsets,
87 including macrophages ¹⁸, neutrophils ^{16,19}, and dendritic cells ²⁰⁻²². It has been shown that IL-27
88 influences innate responses to bacterial challenge in the context of systemic sepsis in genetic
89 manipulation models such as IL-27R α knock-out murine strains. ^{23,24}. To date, there is no report
90 on the immunosuppressive mechanism of IL27 in acute inflammation of the colon driven by
91 innate cells in a genetically intact, non-infective environment.

92 Here, by demonstrating an immunosuppressive effect of IL-27 on the innate, rather than adaptive,
93 immune response *in vivo* in a genetically intact, non-infective acute murine colitis model induced by
94 intra-rectal instillation of 2,4,6-Trinitrobenzenesulfonic acid (TNBS), we offer novel mechanistic insights
95 into the biology of IL-27 *in vivo*. Our data highlights the immunosuppressive role of IL-27 in innate
96 immune responses offering translational potential as a novel rescue therapy in acute severe colitis.

98 Materials and Methods

99 **Study approval** - Animal experiments were conducted under approved protocols by the NCI Animal
100 Care and Use Committee, in keeping with federal regulations governing care and use of animals in
101 biomedical research. Frederick National Laboratory is accredited by AAALAC International and follows
102 the Public Health Service Policy for the Care and Use of Laboratory Animals. Animal care was provided
103 in accordance with the procedures outlined in the “Guide for Care and Use of Laboratory Animals”
104 (National Research Council; 2011; National Academies Press; Washington, D.C.).

105 **Animals** – Experiments were performed on 6-10 week old male SJL/J, B6 Rag^{-/-} (01BJ2 - B6.129S7-
106 Rag1), and B6 IL-10^{-/-} (01IL6 - IL-10 GFP/C) mice, maintained within the NCI-Frederick animal facility.
107 Mice were fed normal chow, had free access to water and exposure to a 12 hourly light/dark cycle.

108 **Acute colitis induction** –100 µl TNBS (Sigma, ~1M in H₂O, stored at -20°C, 92823) in 45% ethanol
109 vehicle was administered intra-rectally to isoflurane anaesthetized mice with a 3cm flexible catheter. The
110 administered dose was optimized within our facility and dependent on the sensitivity of the mouse strain;
111 2mg for SJL/J, 4mg for B6/Rag^{-/-} and 6mg for B6/IL-10^{-/-}. The mice were held vertically for > 30secs post
112 instillation to ensure retention. Mice were given supportive care (wet food/ice chips/heat pad) throughout
113 the protocol. Prior to colitis induction, mice were fasted to solid food overnight and given access to 5%
114 sucrose water.

115 **Bacterial handling and administration** – *Lactococcus lactis* (*L. lactis*) expressing IL-27 (LL-IL-27) and
116 *L. lactis* empty vector control (LL-C) were prepared as described previously¹¹. Briefly, *L. lactis* strain
117 MG1363 was used for bacterial preparations. A bacterial research bank was prepared and IL-27 secretion
118 confirmed by IL-27p28 ELISA (R&D systems, M2728) before storage in 50% glycerol at -80°C. For oral
119 administration, stock bacteria were cultured 1:1000 in Difco M17 broth (BD Biosciences,218561)
120 supplemented with 0.5% glucose and 5mg/ml erythromycin (Sigma, E5389) (GM17E) at 30°C for 16
121 hours. The bacteria were harvested by centrifugation and resuspended in buffered M9 salt media. Each

122 mouse was administered 100 µl of this bacterial suspension by oral gavage at 24 hour intervals within the
123 treatment protocol, commencing on the day of colitis induction following recovery from anesthesia.

124 **Clinical assessment of colitis activity** – Disease activity index was assessed daily as previously
125 reported ¹¹. This is a composite score (maximum 12) of three parameters: weight loss from baseline ($\leq 1\%$
126 0, $1 < \leq 5\%$ 1, $5 < \leq 10\%$ 2, $10 < \leq 20\%$ 3, $> 20\%$ 4), consistency of stool (normal pellets 0, soft-semi formed 2,
127 diarrhea 4) and presence of fecal blood (none 0, occult blood positive 2, overt blood 4). Macroscopic
128 colitis score was assessed by colon weight (mg) and length (cm) immediately after harvest. Histological
129 colitis score (maximum 14) was reported by a veterinary pathologist (MRA) based on multiple
130 parameters: severity of inflammatory cell infiltrate (none 0, mild 1, moderate 2, severe 3), goblet cell
131 depletion (none 0, mild 1, moderate 2, severe 3), crypt hyperplasia (none 0, mild 1, moderate 2, severe 3),
132 degree of ulceration of epithelium, (none 0, erosion 1, mild 2, moderate 3, severe 4 ulceration), presence
133 (1) or absence (0) of granulomas.

134 **Measurement of systemic inflammatory response** – Blood was collected on day 2 post TNBS
135 instillation in a serum separator tube, centrifuged and the supernatant stored at -80°C for analysis. Serum
136 C-reactive protein (CRP) was measured using ELISA kit (Life Diagnostics Inc, 2210-1) as per
137 instructions.

138 **Gene expression** – RNA was extracted from cells or snap frozen distal colon tissue with the RNeasy mini
139 kit (Qiagen, 74104) as per instructions. Quality and yield were assessed by Nanodrop spectrophotometry.
140 cDNA was synthesized using the QuantiTect reverse transcription kit (Qiagen, 205310) with inclusive
141 genomic DNA wipeout buffer. Further details of gene expression assays are available in the
142 supplementary materials.

143 **Total protein extraction and protein expression assays**– Total protein was extracted by mechanical
144 homogenization from distal colon tissue in 1X RIPA buffer (Cell signaling 10X, 9806) containing 1:1000
145 protease inhibitors at 4°C . Following 20 min incubation on ice, tissue homogenates were centrifuged at

146 14,000rpm for 10 minutes and the supernatant stored at -80°C for analysis. Total protein concentration
147 was established using the Pierce BCA protein assay kit (23225) as compared to albumin standard. Further
148 details of protein expression ELISA assays are available in the supplementary materials.

149 **Colonic lamina propria cell isolation** – Single cell suspension from freshly harvested distal colon tissue
150 was extracted with a lamina propria dissociation kit (Miltenyi Biotec, 130-097-410) on the gentleMACS
151 dissociator as per instructions, incorporating chemical and physical dissociation.

152 **Flow cytometry & cell sorting** - Nonspecific staining of cells was blocked by 20 minute incubation with
153 anti-mouse CD16/CD32 Fc block (1:100 dilution) (BD Pharmingen, 553142). Macrophages and
154 neutrophils were identified by cell surface expression of F4/80 and Ly6G using anti-mouse APC-F4/80
155 (eBiosciences, 17-4801-82, clone BM8) and anti-mouse Pacific Blue-Ly6G (Biolegend, 127611) on 4%
156 paraformaldehyde fixed cells using the BD FACSCanto II flow cytometer. Single stain positive and
157 unstained negative cell controls were included. F4/80⁺ cells were collected using a FACS Aria II (BD
158 Biosciences) cell sorter. Data was analyzed using FlowJo software (Tree Star, Inc, Ashland, OR).

159 **Assessment of mucosal inflammatory cell phenotype, proliferation index and phosphorylated**
160 **STAT-1 response**– Immunohistochemistry was performed on 10% NBF fixed, paraffin embedded tissue
161 sections of distal colon. Further details of immunohistochemistry protocols are available in the
162 supplementary materials and methods.

163 **Macrophage culture protocols** – Details of bone marrow derived and thioglycollate peritoneal
164 macrophage culture protocols are available in the supplementary materials and methods.

165 **In vitro cell stimulation assay** – Cells were stimulated with *E. coli* 0111:B4 derived LPS (Sigma,
166 L4130) from 10mg/ml stock, or 5mM Adenosine 5'-triphosphate disodium salt hydrate (ATP) from
167 90mM stock (Sigma, A22383-5G), stored at -20°C. Recombinant mouse IL-27 (NSO expressed, R&D
168 systems, 2799-010/CF) was added to stimulation assay media as appropriate at 100ng/ml. Culture
169 supernatant was collected and stored at -80°C for analysis.

170 **Statistics** - SigmaPlot 11.0 software was used for statistical analysis. Survival curves were analyzed
171 according to the Kaplan-Meier estimator on GraphPad 6. Statistical differences were determined by
172 ANOVA and non-parametric Rank Sum tests, with p values of ≤ 0.05 considered to represent a
173 statistically significant difference between groups. If groups displayed normal variance, Student's t-test
174 was performed (as indicated in the figure legend text). Data is presented as mean +/- SEM.

175

176

177 Results178 **IL-27 attenuates acute severe colitis in vivo**

179 Intra-rectal instillation of TNBS in SJL/J mice resulted in an acute severe left-sided colitis, with a
180 rapid increase in disease activity index (DAI) within 24 hours. Mice treated with TNBS were included if
181 their DAI was greater than 4 on day 1, reflecting appropriate response to TNBS. Mucosal delivery of LL-
182 IL-27 attenuated TNBS colitis with a decreased DAI and significantly less weight loss from baseline
183 starting weight, compared to *L.lactis* control (LL-C) (Figure 1a & 1b). LL-IL-27 was associated with a
184 significantly lower colon weight and increased colon length (Figure 1c), resulting in a reduced colon
185 weight:length ratio (p=0.005).

186 Oral administration of LL-IL-27 is presumed to act locally since there was no evidence of
187 systemic absorption, as determined by IL-27 p28 ELISA (Figure 1d). Although mucosally-delivered IL-
188 27 was not measurable systemically, we observed a decrease in serum C-reactive protein (CRP) in LL-IL-
189 27 treated mice (Figure 1e). This can be attributed to indirect effects on liver production of CRP, possibly
190 downstream of inflammatory cytokines such as IL-6, as will be discussed.

191 LL-IL-27 resulted in a significant reduction in total histology colitis score compared to LL-C
192 (Figure 2a & d), although this was surprisingly modest compared to clinical improvement. Assessment of
193 the independent histological parameters of the composite score revealed significant protection against
194 mucosal ulceration in those receiving LL-IL-27 (Figure 2b). We hypothesized that IL-27 may protect the
195 epithelial barrier, shielding the innate immune system from contact with intestinal microbiota. IL-27 was
196 previously shown to increase colonic epithelial cell proliferation, and to promote epithelial wound
197 restitution in vitro ²⁵. To explore this in vivo, we assessed epithelial cell proliferation by Ki67
198 immunohistochemistry. Eliciting colitis increased epithelial proliferation by 50% but there was no effect
199 on proliferation index associated with LL-IL-27 (Figure 2c).

200 Our *in vivo* data shows that oral IL-27 attenuates acute severe colitis when administered shortly
201 after the time of colonic insult. An important question is whether pre-exposure to IL-27 could impact
202 disease induction as a prophylactic effect. Mice were pre-treated with LL-IL-27 for 2 weeks prior to intra-
203 rectal administration of TNBS. This did not affect acute colitis induction (Figure 3), suggesting that an
204 active inflammatory microenvironment is required for IL-27 to suppress inflammation in the colonic
205 mucosa.

206

207 **IL-27 reduces colonic neutrophil infiltrate associated with decreased CXC chemokine expression**

208 Previous studies have shown that lack of IL-27 signaling increased neutrophil influx to the site of
209 acute bacterial infection in the peritoneal cavity ²⁶ and lung ²⁴. This raised the question of whether
210 mucosal IL-27 might suppress neutrophil infiltration. To explore this as a potential mechanism, neutrophil
211 and macrophage infiltration was assessed by myeloperoxidase (MPO) and F4/80 immunohistochemistry,
212 respectively. Induction of acute TNBS colitis led to a precipitous increase in mucosal neutrophils
213 ($p=0.001$) and a modest increase in macrophages ($p<0.05$). LL-IL-27 led to a significant reduction in
214 MPO⁺ neutrophil infiltration into the colonic mucosa (Figure 4a & Supplementary Figure 1a). F4/80⁺
215 macrophage infiltrate did not alter with LL-IL-27 treatment (Figure 4b & Supplementary Figure 1b).

216 Neutrophils move to areas of acute inflammation via chemokine gradients, therefore expression
217 of several candidate chemokines in the distal colon was assessed. On day 2 post-TNBS instillation,
218 CXCL1 and CXCL2 but not CXCL5 protein was significantly reduced in colitic mice treated with LL-IL-
219 27 compared to LL-C (Figure 4c). CXCL2, a potent neutrophil chemokine, was taken forward in further
220 investigation and at the end of the treatment protocol (day 4 or time of death), the significant suppression
221 persisted (Figure 4d).

222

223 Oral IL-27 reduces CXCL2 secretion in ex-vivo colon derived F4/80⁺macrophage

224 Macrophages are major producers of chemokines that induce a neutrophil influx ²⁷. The cellular
225 source of CXCL2 in our acute TNBS colitis model was assessed by selecting F4/80⁺ cells from distal
226 colon lamina propria cell suspension, on day 2 post-TNBS instillation, cultured for 24 hours to assess
227 CXCL2 response. These colitis derived F4/80⁺ macrophages secreted CXCL2, and this was significantly
228 reduced in cells exposed to LL-IL-27 in vivo (Figure 4e).

229

230 IL-27 does not directly suppress macrophage CXCL2 expression ex vivo

231 Having shown that IL-27 suppressed chemokine expression by colonic macrophages in vivo, we
232 next evaluated whether this was a direct effect by examining IL-27 responses of different macrophage
233 preparations.

234 Bone marrow derived macrophage (BMDM) expressed IL-27R α (Figure 5a) demonstrating their
235 capability to respond to IL-27. LPS stimulation provoked robust CXCL2 secretion. However, addition of
236 recombinant IL-27 (rIL-27) for the 5 hour duration of LPS stimulation did not suppress this response
237 (Figure 5b). To investigate whether the macrophage chemokine response was suppressed by priming with
238 IL-27 prior to LPS challenge, BMDM were cultured in media containing rIL-27 for 48 hours prior to LPS
239 stimulation. Again, exposure to LPS led to CXCL2 secretion and this was not reduced by rIL-27 pre-
240 exposure (Figure 5c).

241 To evaluate a different macrophage population, thioglycollate-induced peritoneal cells were
242 examined, perhaps better representing the acute inflammatory macrophages in this *in vivo* colitis model.
243 At day 4 post-thioglycollate injection, the majority of cells were F4/80⁺ macrophages, representing
244 F4/80^{intermediate} inflammatory and F4/80^{high} resident macrophages, co-existing with Ly6G⁺ neutrophils
245 (Figure 5d). Adherent cells were used as a macrophage enriched population, and expressed IL-27R α . As

246 in BMDM, LPS induced robust CXCL2 expression and this was not altered by presence of rIL-27 (Figure
247 5e).

248 Overall, LPS induced CXCL2 protein expression in macrophages was not inhibited by rIL-27 in
249 two independent *in vitro* cell culture models, suggesting this is not the direct mechanism for CXCL2
250 suppression *in vivo*.

251 However, it remained possible that macrophage responses would be site specific to the colon. To
252 explore this, F4/80⁺ cells isolated from murine colon lamina propria were LPS stimulated for 24 hours,
253 with or without rIL-27. As seen in the other macrophage culture models, LPS induced a striking increase
254 in CXCL2 compared to unstimulated cells (Figure 5f). Once again, there was no significant IL-27
255 suppression of CXCL2 production in response to LPS. This supports the hypothesis that mucosal delivery
256 of IL-27 does not directly interfere with the ability of colonic lamina propria macrophages to secrete
257 CXCL2 after an inflammatory stimulus.

258

259 **IL-27 suppression of acute severe colitis is T cell-independent**

260 Since IL-27 inhibits several T cell subsets, we assessed whether T cell mediated
261 immunosuppression could be central to IL-27 effect in acute colitis.

262 TNBS colitis was evoked in Rag1-deficient (Rag1^{-/-}) mice that lack mature T and B cells, and
263 responses to LL-C or LL-IL-27 assessed. There was reduced disease severity evoked in the C57BL/6
264 strain of mice due to relative resistance to TNBS (compared to SJL/J strain)²⁸. LL-IL-27 led to a
265 significantly improved DAI (Figure 6a). LL-IL-27 treated mice lost significantly less weight initially,
266 though the difference between the groups narrowed during the protocol with a strong trend for
267 improvement in the LL-IL-27 treated group (Figure 6b). CXCL2 protein in distal colon homogenates was
268 significantly inhibited by LL-IL-27 (Figure 6c), and there was a significant reduction in histological

269 colitis score (Figure 6d). Taken together, these data show that IL-27 mediated inhibition of acute colitis
270 persists in Rag1-deficient mice, implying the mechanism is T cell-independent.

271

272 **IL-27 immunosuppression in acute severe colitis is IL-10-dependent**

273 It is known that IL-27 can induce the immunosuppressive cytokine, IL-10^{29, 30} from Tr1 T
274 regulatory cells³¹, and other cell types, such as macrophages¹⁷. Our previous data revealed that LL-IL-27
275 evoked a significant colonic IL-10 response, from CD4⁺CD8 α intra-epithelial T cells, and overall clinical
276 improvement mediated by IL-27 was dependent on production of this cytokine in a chronic enterocolitis
277 model¹¹. To evaluate whether IL-10 played a major role in the immunosuppressive function of IL-27 in
278 the context of acute innate cell driven colitis, we employed an IL-10 deficient (C57BL/6 IL-10^{-/-}) mouse
279 model in our experimental colitis protocol. There was no significant difference in DAI (Figure 6e) or
280 weight loss (Figure 6f) between mice treated with LL-C versus LL-IL-27. There was no reduction in
281 CXCL2 (Figure 6g) nor total histology score (Figure 6h) associated with LL-IL-27. This suggests IL-27
282 mediated immunosuppression in acute colitis is IL-10-dependent and CXCL2 reduction is downstream of
283 this IL-10 dependency.

284 Next, IL-10 protein expression was assessed in distal colon homogenate on either day 2 (Figure
285 6i) or day 4/time of death post-TNBS instillation (Figure 6j). On day 2, distal colonic IL-10 was increased
286 by 25% in mice exposed to IL-27. Later in the treatment protocol there was no significant difference in
287 mucosal IL-10 detected between colitic mice who received LL-C or LL-IL-27. This suggests that early in
288 disease pathogenesis, *L.lactis* delivered IL-27 hastens the immunoregulatory IL-10 response, increasing
289 IL-10 in the colonic mucosal microenvironment, impacting clinical disease course and outcome.

290

291 **IL-27 induces phosphorylated STAT-1 in mucosal epithelial cells**

292 To identify the cell type directly impacted by IL-27 treatment, immunohistochemistry was
293 employed to identify STAT-1 phosphorylation in response to orally administered LL-IL-27. Multifocal
294 mucosal epithelial cells and scattered inflammatory cells within the lamina propria and submucosa
295 showed strong positive nuclear labeling for phosphorylated STAT 1 (Figure 7a). This suggests efficacy of
296 LL-IL-27 is a consequence of both immune and non-immune cell mediated effects. In keeping with this,
297 IL-27 was previously shown to promote epithelial wound restitution *in vitro*²⁵. As expected, gavage of
298 LL-IL-27 in healthy non colitic mice did not result in STAT-1 phosphorylation response, again
299 demonstrating that an inflammatory microenvironment is required for LL-IL-27 immunosuppression.

300

301 **IL-27 significantly reduces pro-inflammatory cytokine profile *in vivo***

302 Gene expression of pro-inflammatory cytokines were assessed in distal colon homogenate,
303 namely *Il6*, *Il1 β* , *Tnf*, *Il13*, *Il12*, *Ifng*, *Il17a*, and *Il22*, along with the regulatory T cell associated
304 transcription factor *Foxp3*. The majority of these genes were expressed at low abundance or undetectable
305 and not investigated further. *Il6*, *Il1 β* , and *Tnf* were significantly increased in all TNBS treated mice
306 compared to ethanol controls (Figure 7b). *Il6* expression was significantly lower in mice treated with LL-
307 IL-27 compared to LL-C. No differential expression of *Il1 β* or *Tnf* was seen with LL-IL-27 treatment.

308 In contrast, distal colon protein expression of IL6, IL1 β and TNF were significantly reduced in
309 colitic mice receiving LL-IL-27 treatment (Figure 7c), highlighting a discrepancy between gene and
310 protein expression for the latter two cytokines. This suggests potential influences on translational or post-
311 translational regulation. Thus we hypothesized that IL-27 may inhibit the inflammasome as a mechanism
312 of immunosuppression. However, there was no impact on expression of distal colon *il-18* or
313 inflammasome components (*Nlrp3*, *Caspase-1* and *Asc*), nor LPS induced IL1 β secretion from *in vitro*
314 macrophage preparations (Supplementary Figure 2), suggesting IL-27 does not directly influence acute
315 colonic inflammasome activation *in vivo*.

317 Discussion

318 Our data show a novel immunosuppressive activity of the cytokine IL-27 in acute severe colitis, directed
319 toward gut mucosal innate immune responses. Mucosal delivery of IL-27 reduced clinical parameters of
320 colonic disease activity, repressed systemic inflammatory response, and improved colitis histology score.
321 LL-IL-27 improved mucosal ulceration. This is important because mucosal healing is defined by the
322 absence of ulcerated lesions in the gut mucosa during endoscopy, and this represents a primary endpoint
323 in assessing effectiveness of IBD therapy.

324 The mechanism of IL-27 directed immunosuppression was multifactorial. This data represents
325 first in field evidence of IL-27 evoked reduction in colonic mucosal neutrophil infiltrate, associated with a
326 decreased CXC chemokine gradient. This was T cell independent and IL-10 dependent, with early
327 enhanced mucosal IL-10. IL-27 also significantly attenuated pro-inflammatory cytokine protein
328 expression. Although there was no evidence of direct impact on epithelial cell proliferation, IL-27
329 provoked an epithelial phosphorylated STAT-1 response. For the first time, we have revealed that IL-27
330 attenuates acute immune responses in the colon in vivo in a non-infective, genetically intact host.

331 IL-27 evoked reduction in myeloid cell infiltrate has been reported in different body sites,
332 identified through genetic manipulation of IL-27 receptor signaling. Atherosclerotic prone *Ldlr*^{-/-} mice
333 had attenuated disease with hematopoietic cell IL-27R α deficiency, mediated partly via reduced plaque
334 chemokine gradient and infiltration of myeloid cells³². *Ebi3*^{-/-} mice demonstrated reduced granulocyte
335 flux to acute peritonitis²⁶. When IL-27 receptor signaling was interrupted by genetic deficiency or
336 antibody blocking, mice with systemic sepsis secondary to cecal puncture ligation and exposed to a
337 secondary pneumonic bacterial insult, exhibited enhanced neutrophil infiltrate to the lungs. This was
338 associated with increased chemokine expression including CXCL2 and pro-inflammatory mediators such
339 as IL-6, TNF, and IL1 β ²⁴. This IL-27 mediated blunting of granulocyte response was deleterious overall
340 and associated with inability to resolve the secondary bacterial infection.

341 Neutrophils are necessary for efficient resolution of acute microbial attack through a variety of
342 mechanisms³³. The suppression of neutrophil influx by IL-27 complicates IL-27 therapy for application
343 to human disease. However, LL-IL-27 represents a localized rather than systemic therapy, delivered
344 orally to act directly on the inflamed colonic mucosa, with no evidence of systemic IL-27 absorption as
345 reported here and in our previous publication¹¹. The balance of beneficial to deleterious IL-27 inhibition
346 of innate immunity appears site specific. Thus, IL-27 is beneficial in the circumstance of acute non-
347 infective colitis, by limiting granulocyte directed tissue damage, release of pro-inflammatory mediators
348 and activation of downstream adaptive immune responses.

349 CXCL2 is a potent neutrophil chemokine, secreted mainly by monocytes and macrophages, in
350 response to LPS stimulation²⁷. There is existing evidence that IL-27 can modulate the functional capacity
351 of macrophages and other innate cells. It is unclear whether IL-27 effects on innate cells are pro-
352 inflammatory or anti-inflammatory and it appears that the outcome is dependent on stimulus, cell type and
353 surrounding or experimental microenvironment¹⁴, akin to its pleiotropic effect on T cell biology. For
354 example, IL-27 can enhance pro-inflammatory cytokine and chemokine expression from blood derived
355 human monocytes cultured and stimulated *in vitro*^{34,35}. In the gut, host or recipient IL-27R knockout
356 mice, with or without genetic silencing of TCR revealed a role of IL-27-induced, APC-derived, cytokine
357 mediated Th17 responses in T cell driven colitis³⁶. Conversely, lung macrophages from IL-27R α
358 knockout mice exert enhanced pro-inflammatory cytokine and chemokine secretion, along with increased
359 anti-microbial killing capacity²⁴ exemplifying a suppressive activity of IL-27 on innate immune cells.
360 Iyer¹⁷ revealed macrophage IL-10 secretion in response to LPS or type I interferons is dependent on
361 endogenous IL-27 signaling. Therefore, it was reasonable to speculate that our orally delivered IL-27 may
362 suppress the proinflammatory function of the colonic macrophage population. Indeed, our data suggest
363 that colonic macrophages are a potent source of CXCL2 during acute colitis and this was blunted by *in*
364 *vivo* exposure to IL-27. We did not find evidence to support a direct immunosuppressive effect of IL-27
365 on macrophage chemokine response, despite utilizing three independent *ex vivo* macrophage culture
366 models including colonic macrophages.

367 We then assessed the involvement of T cells in the IL-27-mediated immunosuppression in this
368 acute colitis model. IL-27 inhibits Th2 and Th17 responses ^{13,14}, although this was not likely to be
369 prominent in our acute model given the rapid onset of pathology. T cells present in the normal colonic
370 mucosa are part of the ‘physiological immunity’ that evokes tolerance to luminal antigens and appropriate
371 response to pathogenic insult. IL-27 is known to promote T regulatory cell subsets ^{31,37}. However in our
372 model, IL-27 immunosuppression of innate cell driven colonic pathology was T cell independent.

373 We acknowledge that the direct cell target of orally delivered IL-27 in acute colitis remains
374 elusive. We predict that IL-27 in acute severe colitis acts on epithelial cells and inflammatory cells of
375 unknown phenotype. There are several immune cell types that may, independently or in conjunction, act
376 in response to exogenous IL-27, such as neutrophils, dendritic cells or innate lymphoid cells and although
377 outside the scope of this current study, warrants further investigation to reveal novel biological functions
378 of IL-27. Neutrophils can secrete chemokines and attract an increasing neutrophil influx ³⁸. IL-27 can
379 inhibit neutrophil ROS production ^{16, 19}, reduce their expression of the surface integrin Mac-1 ^{16, 19} and
380 provoke short lived IL1 β secretion either independently ¹⁶ or in response to bacterial challenge ^{19, 24}. IL-27
381 receptor deficiency led to enhanced neutrophil intracellular bacterial killing ²⁴. Neither neutrophil survival
382 nor apoptosis is known to be affected by IL-27 ¹⁶. Another potential cell candidate is dendritic cells
383 (DCs). Human immature DCs exposed to IL-27 displayed reduced antigen presenting capacity ²². IL-27
384 provokes DC derived IL-10, and suppresses downstream Th1 and Th17 response ^{20, 21}. This mechanism
385 impacts disease outcome in CNS autoimmune pathology, with vaccination of IL-27 primed DCs
386 attenuating murine EAE ²¹. Another potential cell candidate is colonic epithelial cells, as this cell
387 compartment can secrete chemokines ^{39, 40} and our results indicate that these cells strongly express
388 phosphorylated STAT1 in response to mucosally delivered LL-IL-27 in vivo.

389 This data confirms the potential for oral *L. lactis* IL-27 to be a potential rescue medical therapy in
390 acute severe colitis. *Lactococcus lactis* is a non-pathogenic, non-colonizing and non-transmissible food
391 grade bacterium. The use of this bacteria as a mucosal delivery system for therapeutic proteins and

392 vaccines has been reviewed elsewhere ⁴¹⁻⁴³. We showed previously that orally delivered IL-27 attenuates
393 chronic T cell derived colonic inflammation ¹¹ and now present novel evidence to show efficacy towards
394 acute innate cell derived colonic pathology, highlighting translational impact as a new or adjunct medical
395 treatment strategy in acute severe colitis.

396

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402

403 Author contributions

404 MHM designed and executed experiments, analyzed data, and wrote the manuscript. LS provided *L. lactis*
405 strains. WQL designed the IL-27 construct and assisted with experiments. MRA provided pathology
406 reporting. All other authors assisted with design, execution and analysis of experiments. Specific input
407 with macrophage in vitro culture and flow cytometry (WAB & LCD), preventative assessment (CS), ex
408 vivo colonic cell isolation, pathology, and digital photography (CA) is noted. SKD designed experiments,
409 wrote the manuscript and provided overall direction. All authors edited and agreed on the final
410 manuscript.

411

412 Abbreviations; IL-27, interleukin 27; LL-IL-27, *Lactococcus lactis* expressing IL-27; LL-C, *Lactococcus*
413 *lactis* empty vector control; DAI, Disease activity index;

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- 517

518 Figure Legends

519 **Figure 1. Oral delivery of IL-27 suppresses acute severe colitis.** (a) LL-IL-27 evokes a decreased
520 disease activity index (DAI) compared to LL-C; 5.8 vs. 8.2 on day 1, 5.3 vs. 8.9 on day 2 and 4.1 vs. 8.2,
521 on day 3, maximum 12, * $p=0.001$. (b) LL-IL-27 led to significantly less weight loss than TNBS+LL-C or
522 TNBS alone, $p<0.01$ and $p<0.05$ on days 2 and 3, respectively. Data in (a) and (b) combined from 3
523 separate experiments. $n=14, 21, 25, 25$ for ethanol, TNBS, TNBS+LL-C and TNBS+LL-IL-27 groups,
524 respectively. (c) Macroscopic colitis score represented by colon weight (mg) and length (cm). Colitis
525 renders the colon shorter and heavier. Both parameters improved with LL-IL-27 vs. LL-C; colon length,
526 $p=0.001$ and colon weight, $p=0.017$. Data combined from 2 separate experiments. $n=7, 16, 18, 19$ for
527 ethanol, TNBS, TNBS+LL-C and TNBS+LL-IL-27, respectively. (d) SJL/J colitic mice (2mg TNBS)
528 receiving daily gavage of LL-IL27 for 4 days ($n=10$) showed serum IL-27 of 2.9pg/ml, indistinguishable
529 from non-colitic untreated mice ($n=8, 4.1$ pg/ml), $p=0.469$ (t-test). (e) IL-27 provoked an improved
530 systemic inflammatory response measured by serum CRP (day 2); LL-C vs. LL-IL-27, $p=0.019$ (t-test).
531 Data from one experiment. $n=5, 3, 10, 9, 10$ for untreated, ethanol, TNBS, TNBS+LL-C and TNBS+LL-
532 IL-27, respectively. All data (a-e) presented as mean+SEM.

533 **Figure 2. Oral delivery of IL-27 leads to improved histological colitis score and protection against**
534 **ulceration in acute severe colitis.** (a) Cumulative histological colitis score based on 5 parameters
535 (degree of inflammatory cell infiltrate/ mucosal ulceration/ crypt hyperplasia/ goblet cell depletion,
536 presence of granuloma) with a maximum of 14. TNBS+LL-C vs. TNBS+ LL-IL-27, $p=0.035$. (b) Degree
537 of distal colonic mucosa ulceration score, graded on a scale of 0-4. 0=none, 1=erosion, 2=mild ulceration,
538 3=moderate ulceration, 4=severe ulceration. TNBS+LL-C vs. TNBS+ LL-IL-27, $p=0.008$. Data generated
539 from randomly selected animals from 2 experiments. $n=5,5,6,7$ for ethanol, TNBS, TNBS+LL-C and
540 TNBS+LL-IL27, respectively. Mean + SEM. (c) Epithelial proliferation index was assessed by Ki67
541 immunohistochemistry on paraffin embedded distal colon sections. $n=5$ /group from 2 experiments. Index
542 derived from number of Ki67 positive cells/total number epithelial cells/ HPF, expressed as percentage.

543 Mean + SEM % from 5 HPF per sample used in analysis. Colitis evoked a significant increase in
544 proliferation index across all groups compared to ethanol control; $p=0.009$, 0.001 & 0.002 for TNBS
545 alone, +LL-C and +LL-IL-27, respectively. There was no significant difference between TNBS alone vs.
546 +LL-C vs. +LL-IL-27 ($p=0.243$), t-test. **(d)** Representative distal colon photomicrographs of each
547 experimental group. There is full-thickness necrosis of the mucosa and marked submucosal inflammation
548 and edema in both the TNBS alone and TNBS+LL-C treated mice. LL-IL-27 treatment significantly
549 reduced mucosal necrosis and decreased inflammatory infiltrates. Slides were optically scanned with an
550 Aperio AT2 digital slide scanner. Hematoxylin and eosin staining.

551 **Figure 3. An active inflammatory microenvironment is required for oral IL-27 to suppress**
552 **inflammation in the colonic mucosa.** Pre-exposure to LL-IL-27 (10 oral gavages over 2 week period)
553 prior to intra-rectal instillation of 2mg TNBS (SJL/J 6-8 week old male mice) did not impact acute colitis
554 induction as measured by **(a)** disease activity index, **(b)** weight loss from starting weight, or **(c)**
555 macroscopic colitis score (colon length and weight). Data presented combined from 2 separate
556 experiments, $n=10$ /group, as mean + SEM; ANOVA on Ranks & Rank Sum test for pairwise
557 comparisons.

558

559 **Figure 4. Oral IL-27 reduces colonic neutrophil infiltrate associated with decreased CXC**
560 **chemokine expression.** Distal colon inflammatory cell phenotype assessed by immunohistochemistry
561 using **(a)** myeloperoxidase (MPO) and **(b)** F4/80 positivity to identify neutrophils and macrophages,
562 respectively. Data generated from randomly selected animals from 2 experiments. $n=5,5,6,6,8$ for
563 untreated, ethanol, TNBS, TNBS+LL-C and TNBS+LL-IL-27, respectively. Mean + SEM. **(a)** Treatment
564 with IL-27 led to a significant reduction in MPO⁺ neutrophil infiltration into the colonic mucosa
565 ($p=0.002$, t-test). **(b)** F4/80⁺ macrophage infiltrate did not alter with LL-IL-27 treatment ($p=0.250$, t-test).
566 **(c)** On day 2 post TNBS instillation, CXCL1 and CXCL2 but not CXCL5 protein (measured by ELISA

567 and normalized to mg total protein concentration) were significantly reduced in distal colon homogenate
568 in colitic mice treated with LL-IL-27 compared to LL-C ($p=0.026$, $p=0.038$ & $p=0.427$ (t-test),
569 respectively). Data represents mean + SEM, generated from 2 separate experiments, $n=10$ /group. **(d)**
570 This IL-27 associated reduction in CXCL2 expression persisted as measured later in the experimental
571 protocol (day 4 or time of death), $p=0.001$ and $p=0.003$, compared to TNBS plus LL-C and TNBS alone,
572 respectively. Mean + SEM, generated from one experiment, $n=3, 3, 8, 8, 11$ for untreated, ethanol, TNBS,
573 TNBS+LL-C and TNBS+LL-IL27, respectively. **(e)** Ex-vivo derived colitis associated lamina propria
574 F4/80⁺ macrophage cells secreted CXCL2 measured by ELISA after 24 hours culture at 37°C (10^5
575 cells/200 μ l), and this was significantly reduced in cells exposed in vivo to LL-IL-27, $p=0.001$ (t-test).
576 Data generated from 5 pooled distal colon samples per group.

577

578 **Figure 5. IL-27 does not directly suppress macrophage CXCL2 expression.** To investigate the
579 mechanism of IL-27 mediated immunosuppression, chemokine response in bone marrow derived
580 macrophage (BMDM) **(a-c)**, thioglycollate induced peritoneal macrophage (TPM) **(d & e)** and colonic
581 lamina propria F4/80⁺ macrophage **(f)** were assessed. **(a)** >90% of M-CSF cultured bone marrow derived
582 cells expressed F4/80 and IL-27R α . **(b)** BMDM were stimulated for 5 hours with various concentrations
583 of LPS as labelled +/- rIL27 (100ng/ml) and CXCL2 measured in supernatant by ELISA. Combined data
584 from several experiments, generated from >3 wells/experimental condition. LPS resulted in a robust
585 CXCL2 response but there was no differential response in association with IL-27, t-test. **(c)** Exposure for
586 >48 hours prior to LPS stimulation did not impact CXCL2 response, t-test. **(d)** Peritoneal cell phenotype
587 day 4 post IP thioglycollate injection. Cells expressed IL-27R α . **(e)** Adherent cells were stimulated with
588 50ng/ml of LPS +/- 100ng/ml rIL27, for 5 or 72 hours. Again, LPS resulted in a robust CXCL2 response
589 but there was no differential response in association with IL-27. **(f)** This was also seen in ex vivo colonic
590 lamina propria F4/80⁺ cells stimulated for 24 hours with 50ng/ml of LPS +/- 100ng/ml rIL27, t-test.

591 Results in C, E & F presented from one experiment, with the same pattern of expression seen in repeat
592 experiment. All data presented as mean + SEM.

593

594 **Figure 6. IL-27 immunosuppression in acute TNBS colitis is T cell-independent and IL-10-**
595 **dependent, evoking early IL-10 secretion in the colonic mucosa. (a-d)** 4mg TNBS instilled intra-
596 rectally into Rag^{-/-} mice. Data combined from 2 separate experiments. n=10/group. There was a
597 significant difference in **(a)** DAI in mice treated with LL-IL-27 compared to LL-C on day 1 and 2,
598 p=0.013 & p=0.040 respectively, t-test, and **(b)** degree of weight loss from baseline on day 1 (p=0.019),
599 then a trend just under significance on days 2&3 (p=0.06), t-test. **(c)** Distal colon homogenate CXCL2
600 protein was significantly reduced with LL-IL-27, p=0.028, **(d)** as was distal colon histology score,
601 p=0.028, t-test. **(e-h)** 6mg TNBS instilled intra-rectally into IL-10^{-/-} mice. Data combined from 2 separate
602 experiments. n=8/group. **(e)** DAI, p=0.237, 0.903, 0.681, respectively on day 1, 2 & 3, LL-IL-27 vs. LL-
603 C, t-test, **(f)** weight loss from baseline, p=0.727, 0.751, 0.716, respectively on day 1, 2 and 3, LL-IL-27
604 vs. LL-C, t-test. **(g)** CXCL2 protein in distal colon homogenate, p=0.959 and **(h)** distal colon histology
605 score, p=0.608, t-test, were not significantly different in mice treated with LL-IL-27 compared to LL-C.
606 **(i)** On day 2 post TNBS instillation in SJL/J mice, there was a significant increase in distal colon IL-10
607 protein associated with IL-27, p=0.01, t-test. **(j)** Later in the treatment protocol, at day 4 or time of death,
608 this difference in IL-10 protein expression was not apparent, p=0.653. At both time points, n=10/group,
609 combined data from 2 experiments.

610

611 **Figure 7. IL-27 is associated with phosphorylated STAT-1 in colonic epithelial cells and**
612 **significantly reduces pro-inflammatory cytokine profile in vivo. (a)** Immunohistochemistry for
613 phosphorylated STAT-1 (Tyr701). Multifocal mucosal epithelial cells and scattered inflammatory cells
614 within the lamina propria and submucosa show strong positive nuclear labeling for phosphorylated

615 STAT-1 in response to oral LL-IL-27. Occasional cells show both strong nuclear and variable
616 cytoplasmic labeling. There is no phosphorylated STAT-1 immunopositivity in colitic mice who received
617 no treatment or treatment with LL-C. Diaminobenzidine chromogen and hematoxylin counterstain. This
618 pattern of expression was seen in all mice per group (n=3). **(b)** Cytokine gene expression profile in distal
619 colon homogenate was assessed by Taqman RT-PCR assays (Applied Biosystems) and analyzed with
620 ddCt method of relative quantification; normalization to endogenous GAPDH control and level of
621 expression compared to ethanol control group. Data, combined from 2 experiments, presented as mean +
622 SEM. *Il6* (p=0.003), *Il1b* (p=0.001) and *Tnf* (p=<0.01) were significantly increased in TNBS treated mice.
623 Expression of *Il6* was significantly reduced with LL-IL-27 compared to LL-C (p=0.036), whereas no such
624 effect was seen for *Il1b* and *Tnf*. n=7, 10-13, 10-15, 10-15, for ethanol, TNBS alone, TNBS+LL-C and
625 TNBS+LL-IL-27, respectively. **(c)** Cytokine protein expression in distal colon homogenate measured by
626 ELISA and normalized to mg total protein. Combined data presented from 2 experiments as mean +
627 SEM. n=5, 8-10, 7-10, 10, for ethanol, TNBS alone, TNBS+LL-C and TNBS+LL-IL-27, respectively.
628 Protein expression of IL-6 (p=0.002), IL-1 β (p=0.003) and TNF (p=0.031) were significantly reduced in
629 the distal colon of colitic mice receiving LL-IL-27 treatment compared to LL-C.

630

631

Figure 1

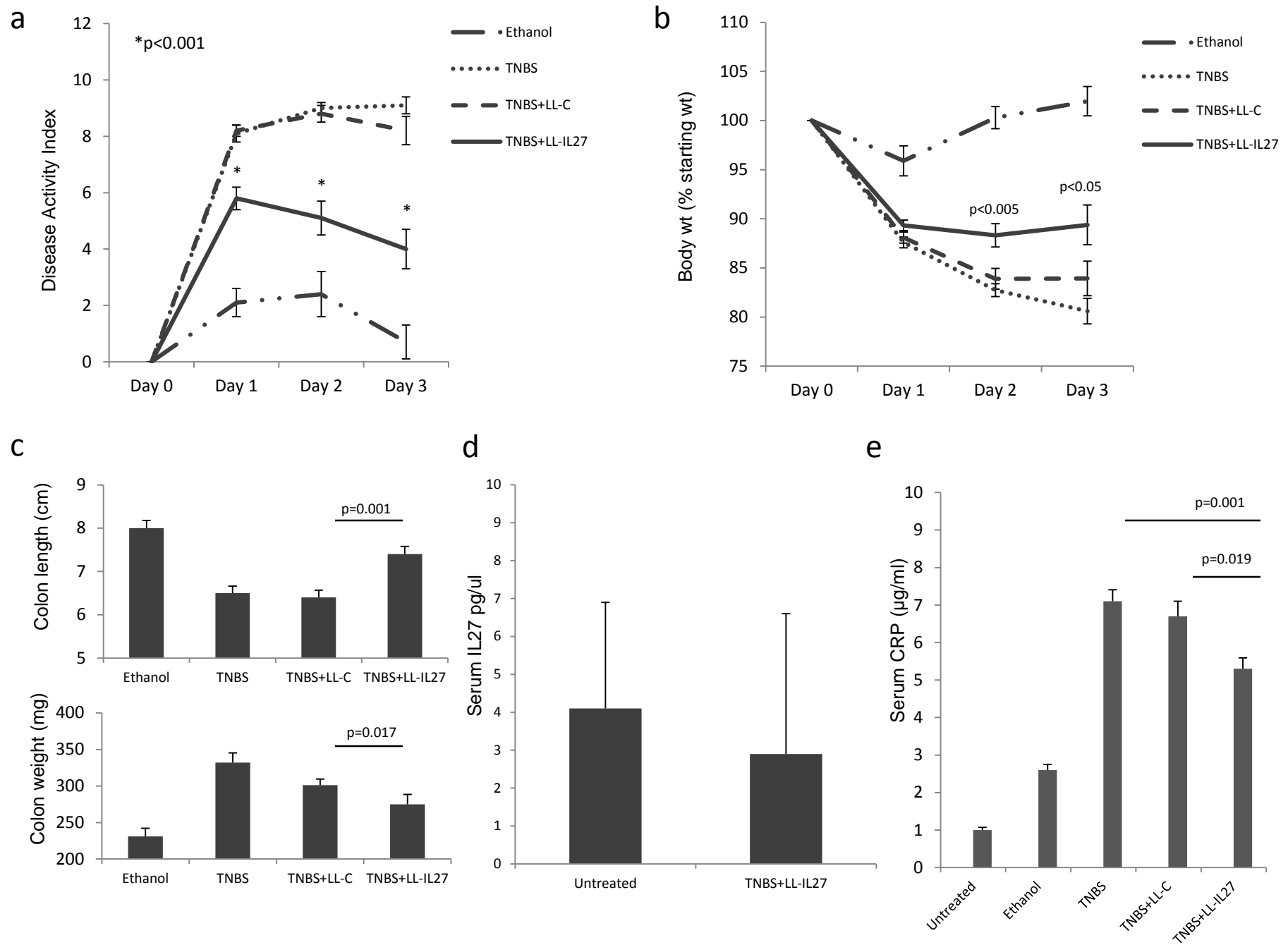


Figure 2

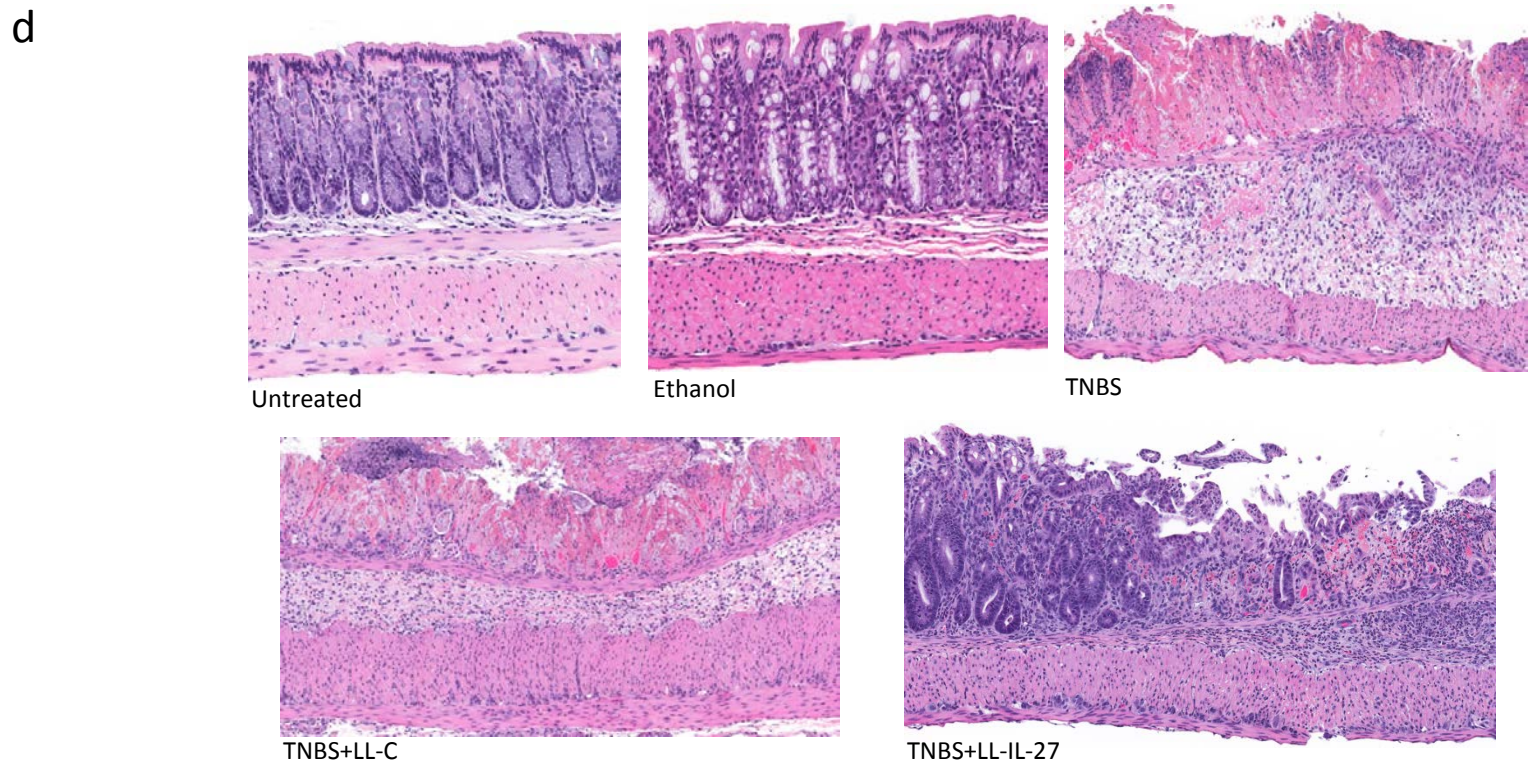
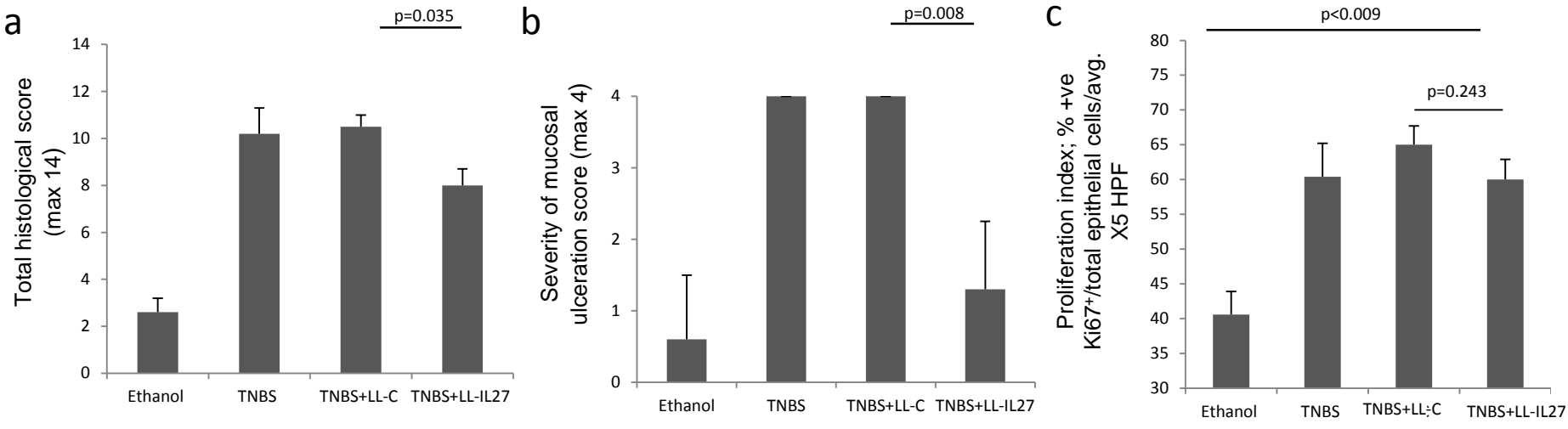


Figure 3

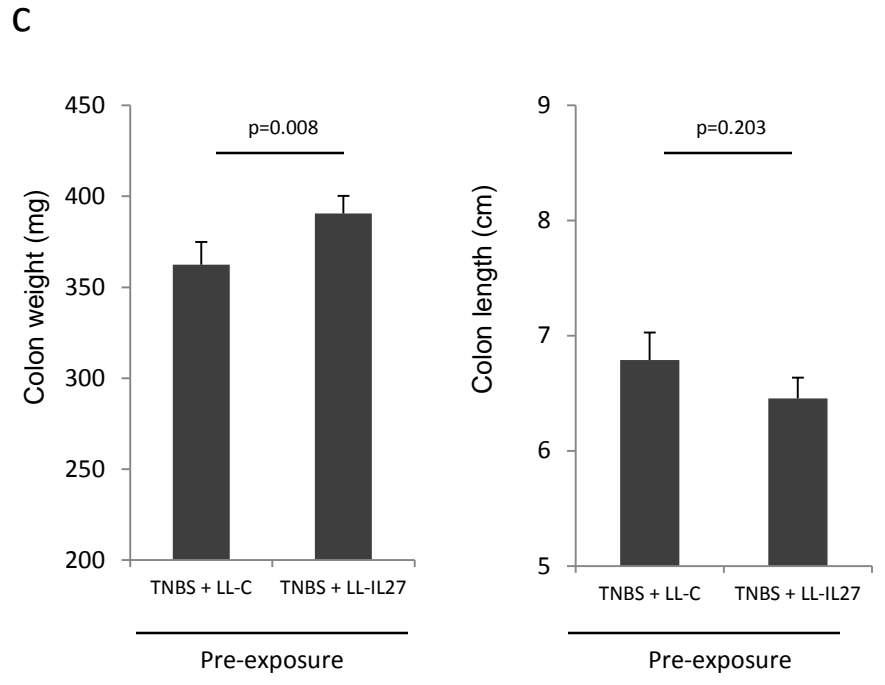
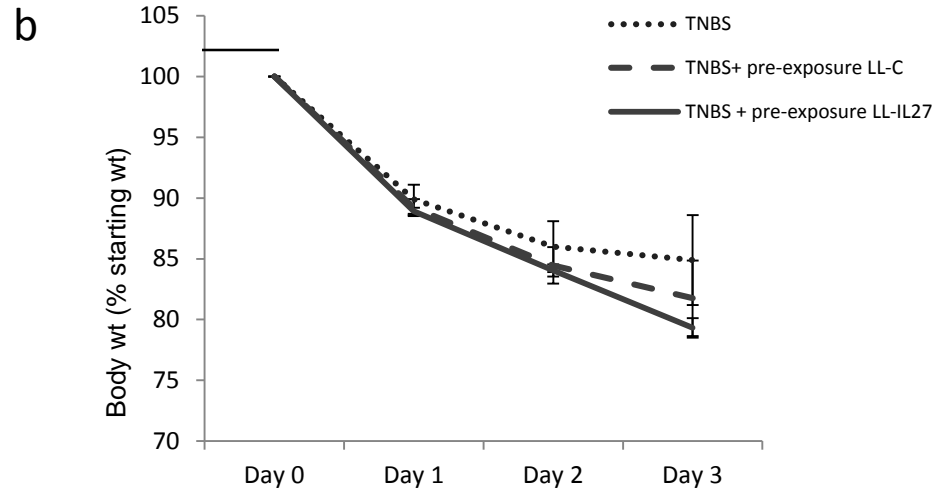
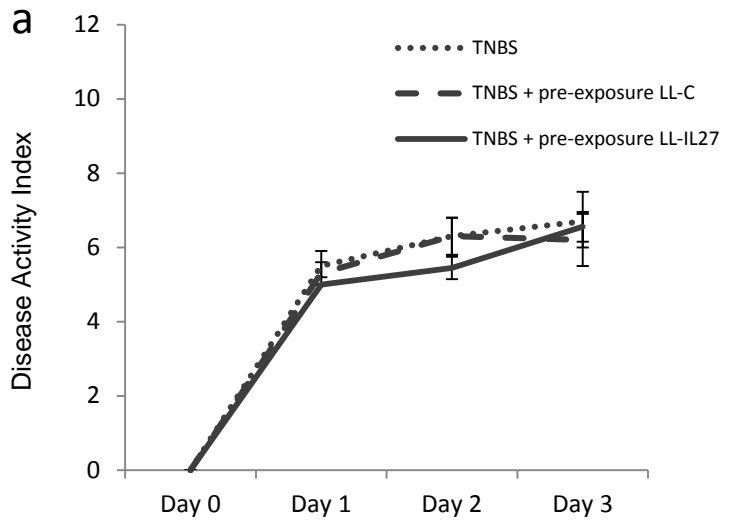


Figure 4

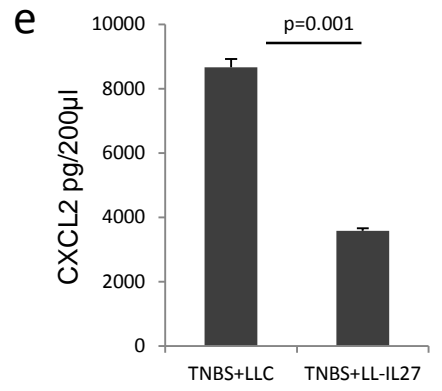
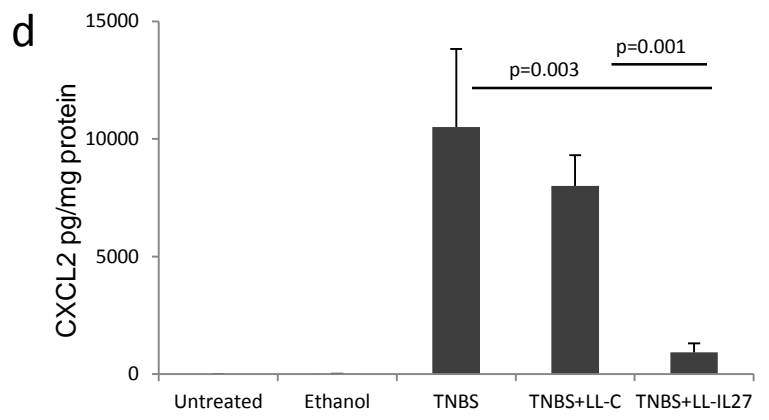
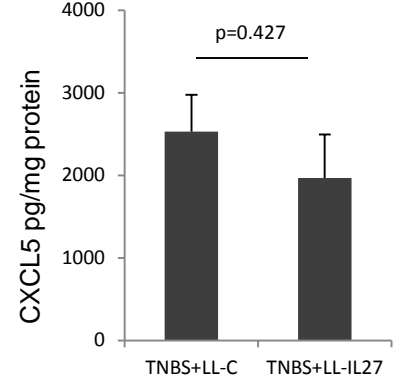
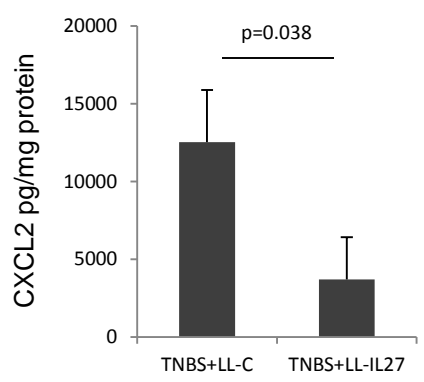
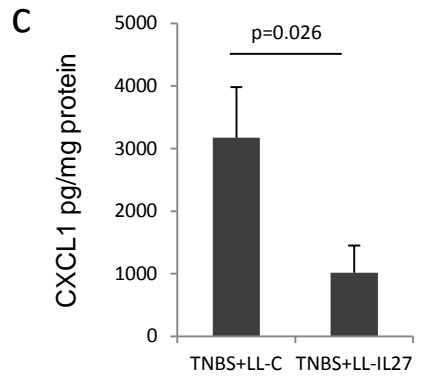
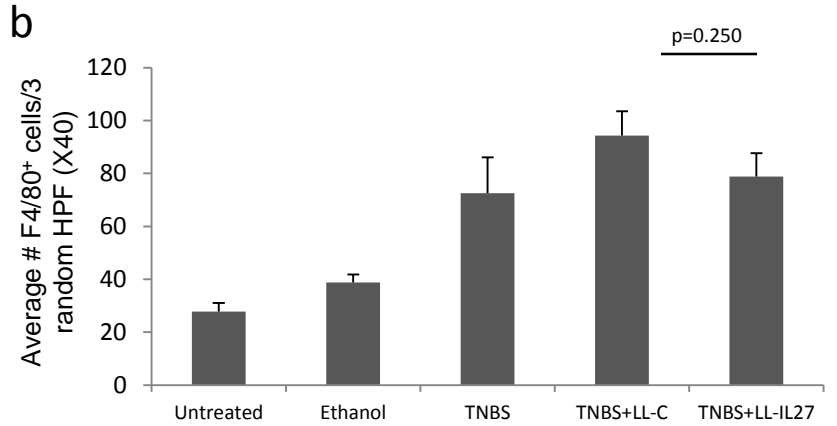
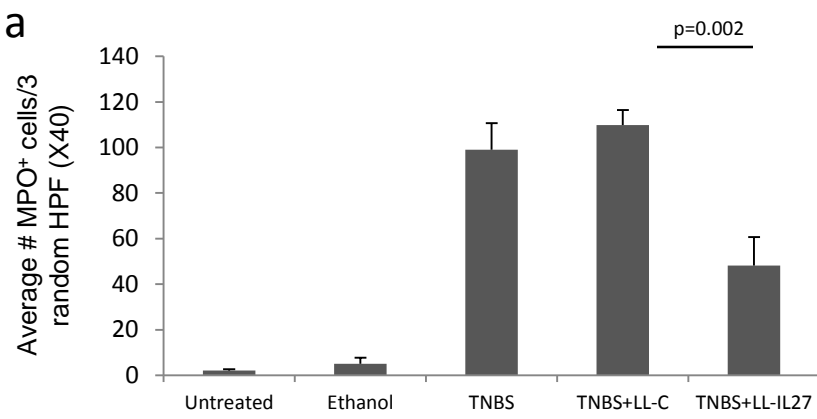


Figure 5

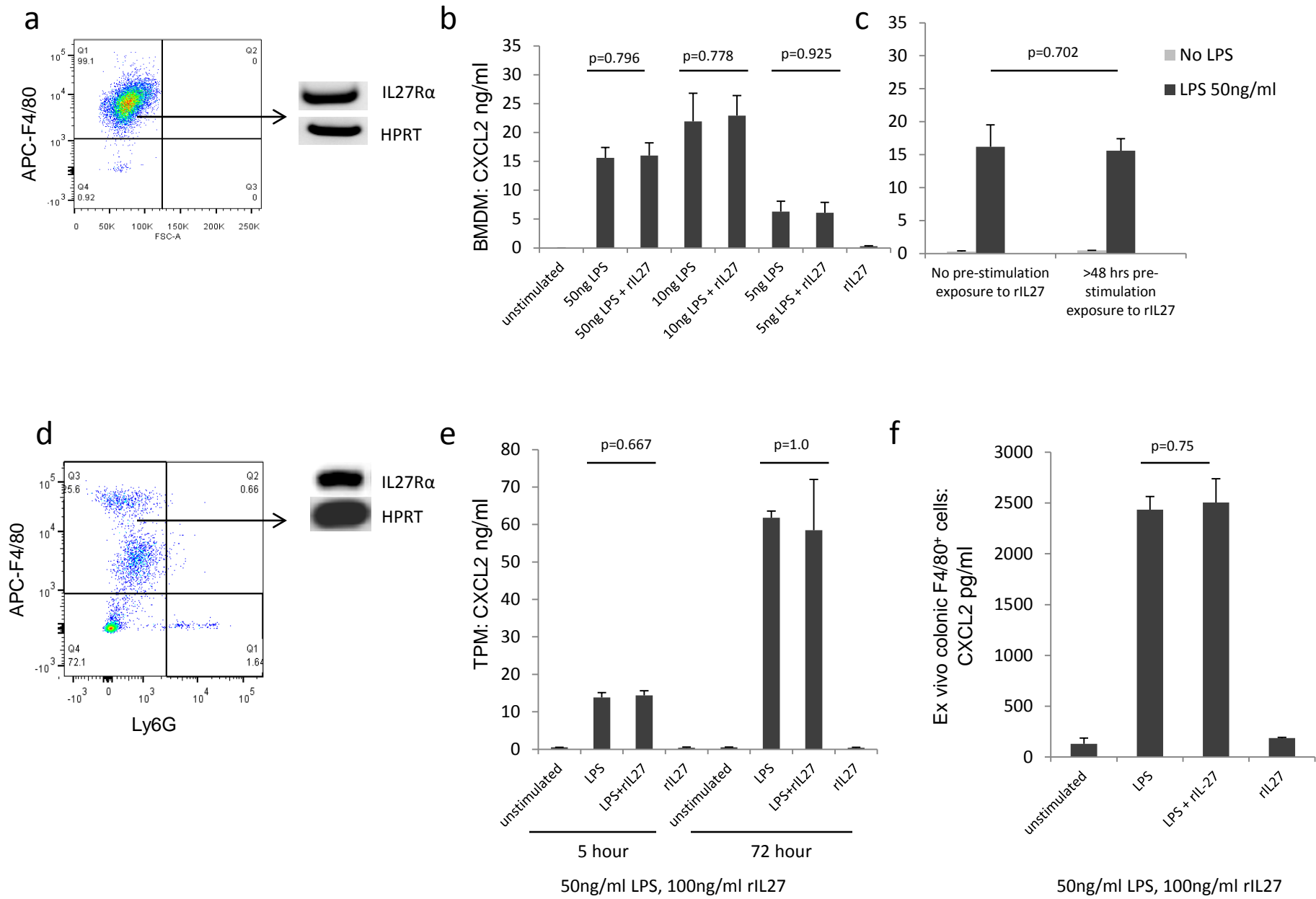
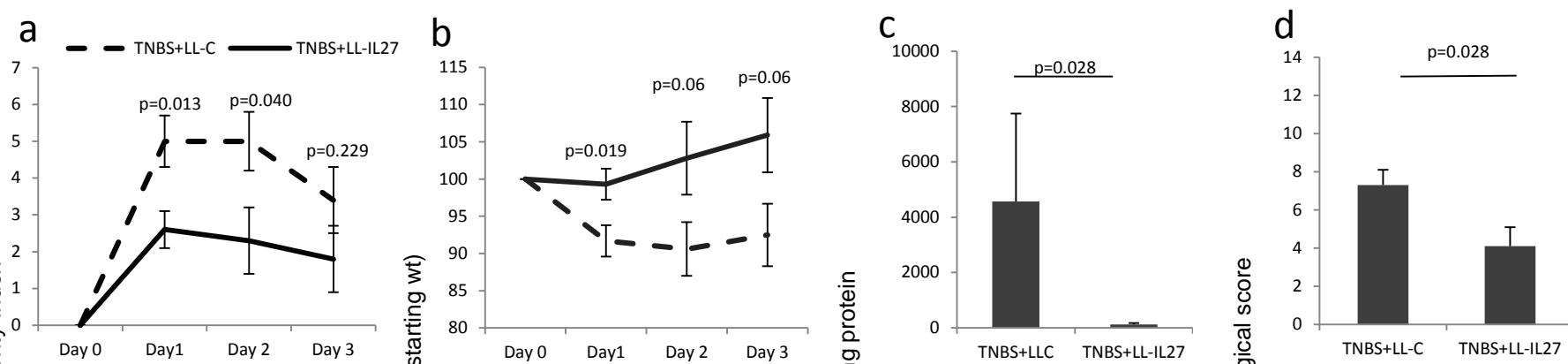


Figure 6

Rag^{-/-} mice



IL10^{-/-} mice

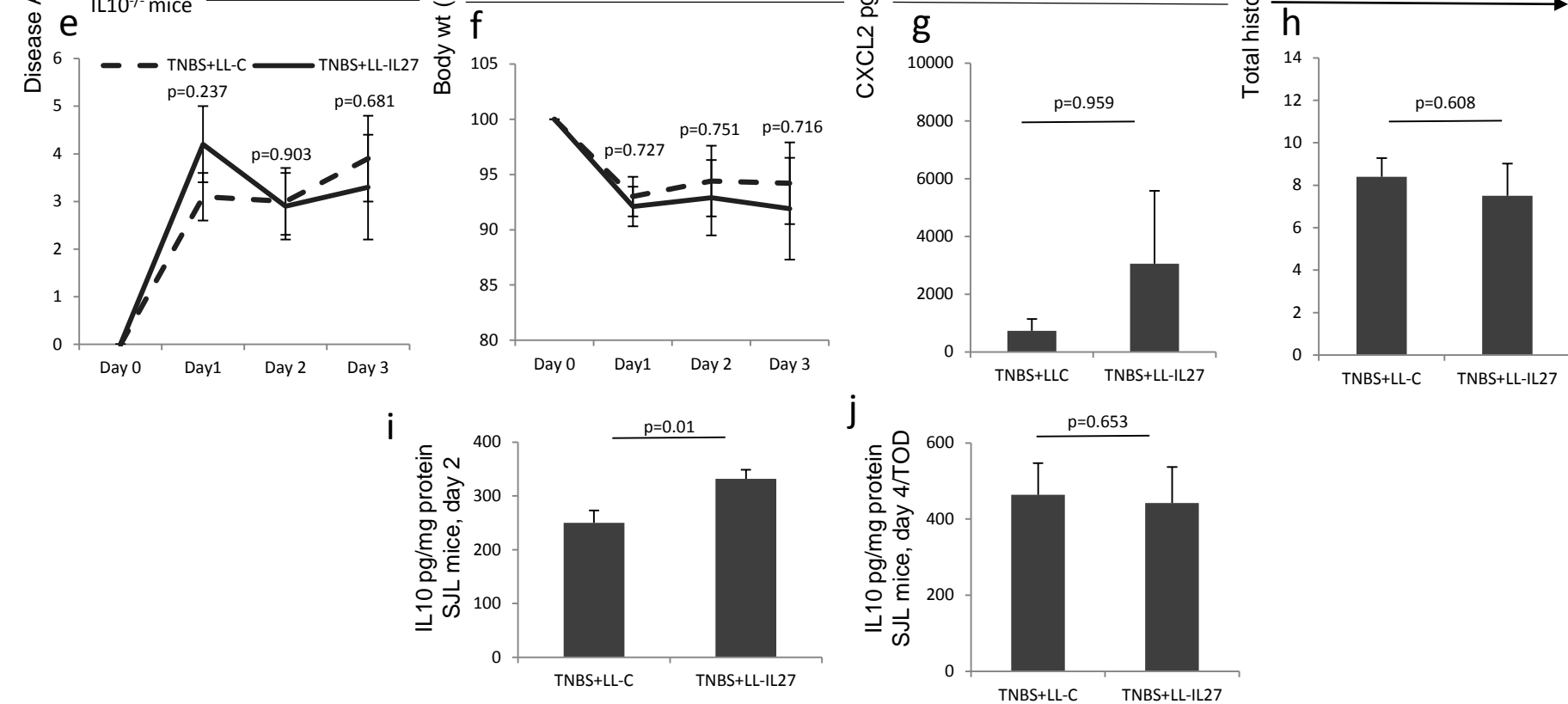
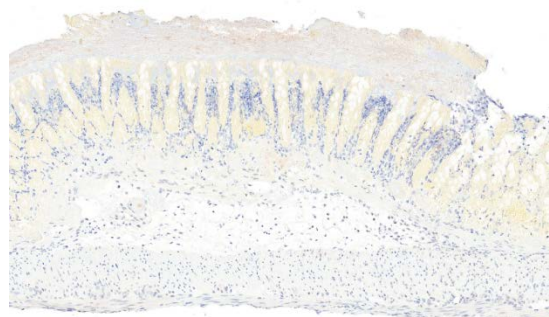
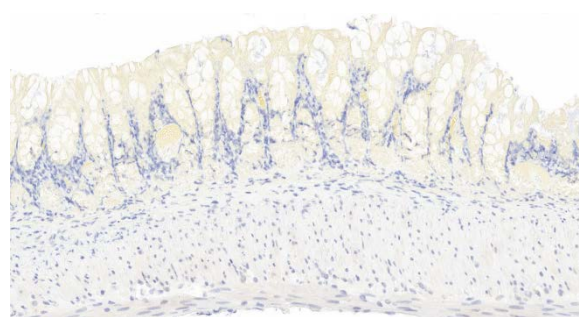


Figure 7

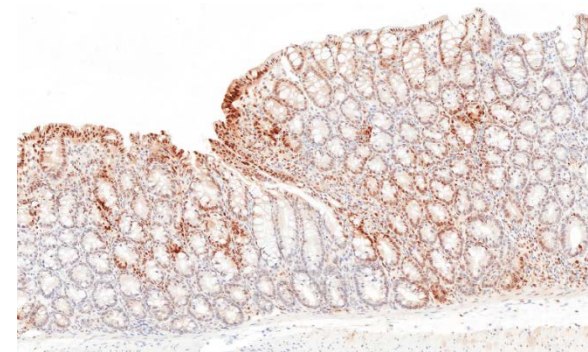
a



TNBS

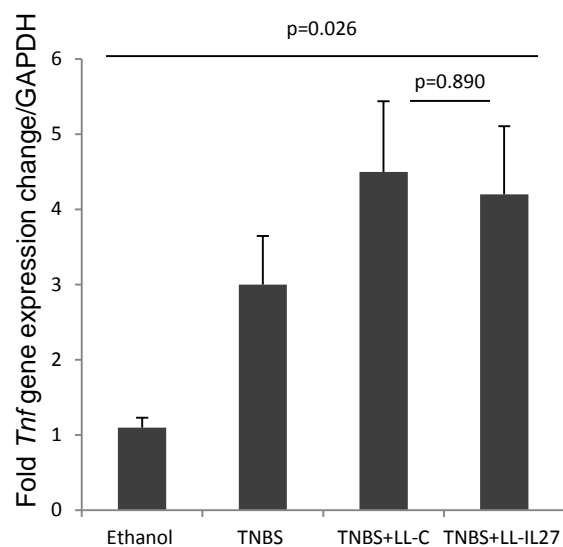
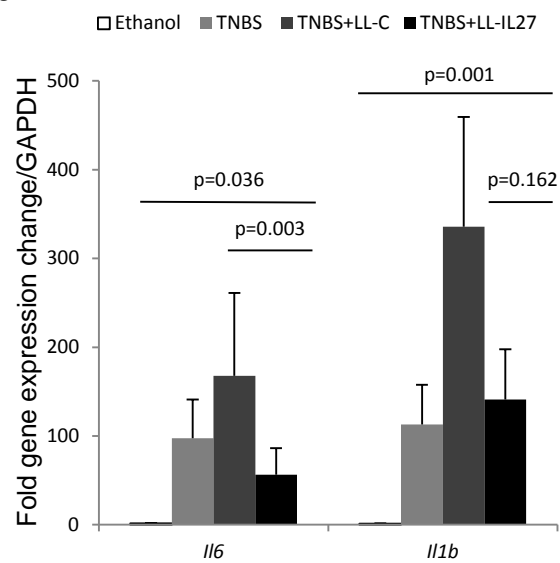


TNBS+LL-C

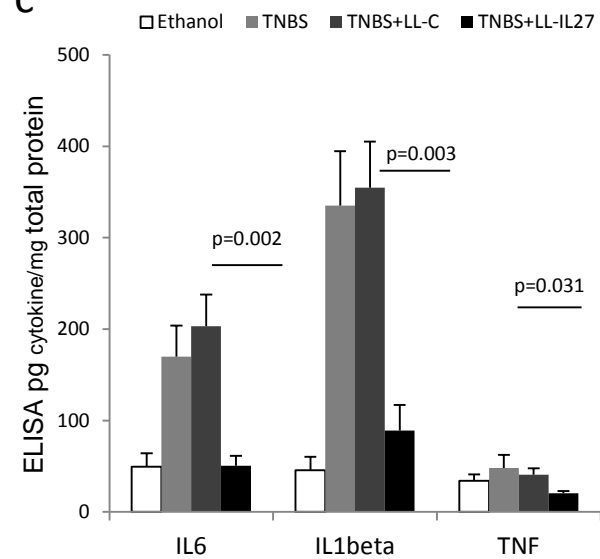


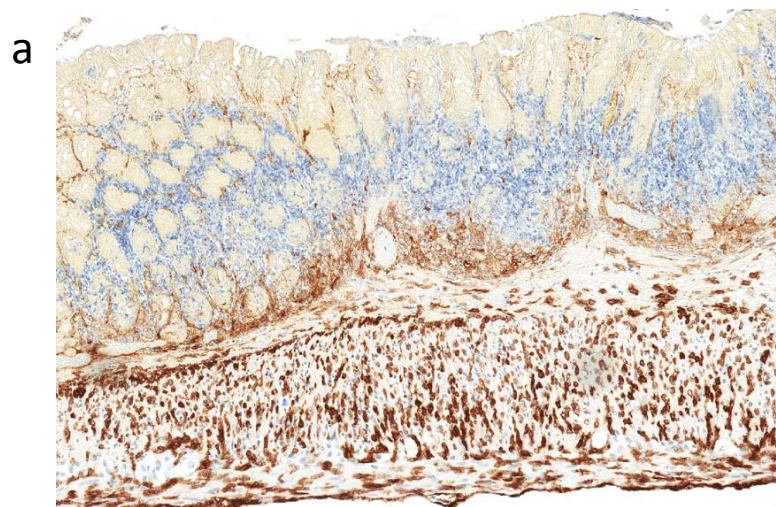
TNBS+LL-IL27

b

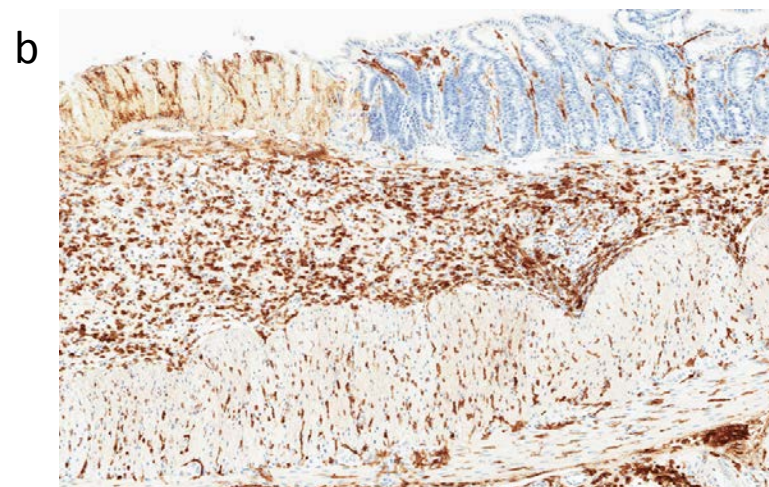


c

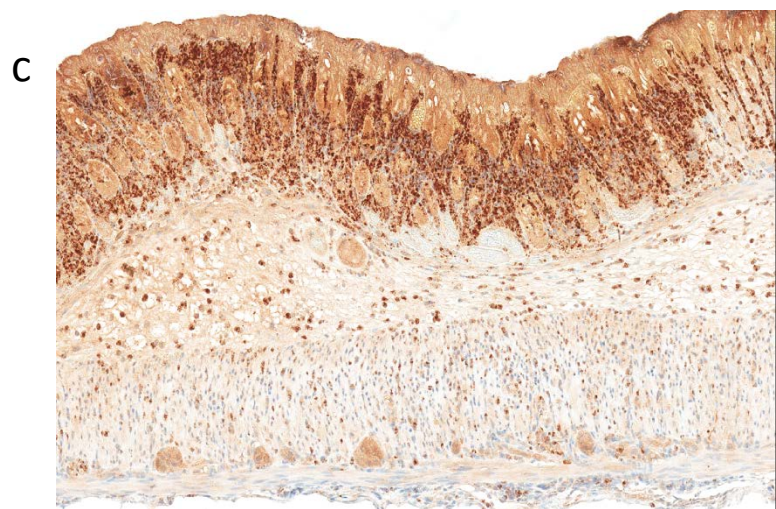




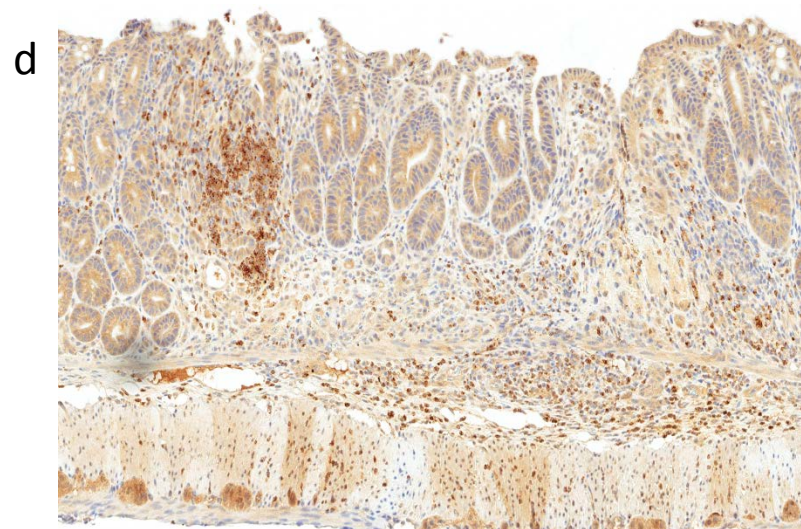
F4/80 TNBS+LL-C



F4/80 TNBS+LL-IL-27

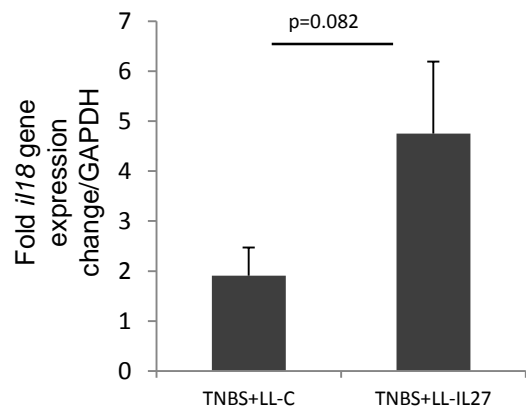


MPO TNBS+LL-C

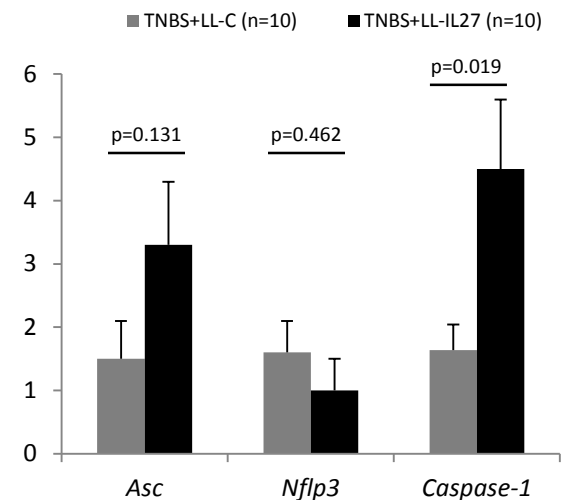


MPO TNBS+LL-IL-27

a

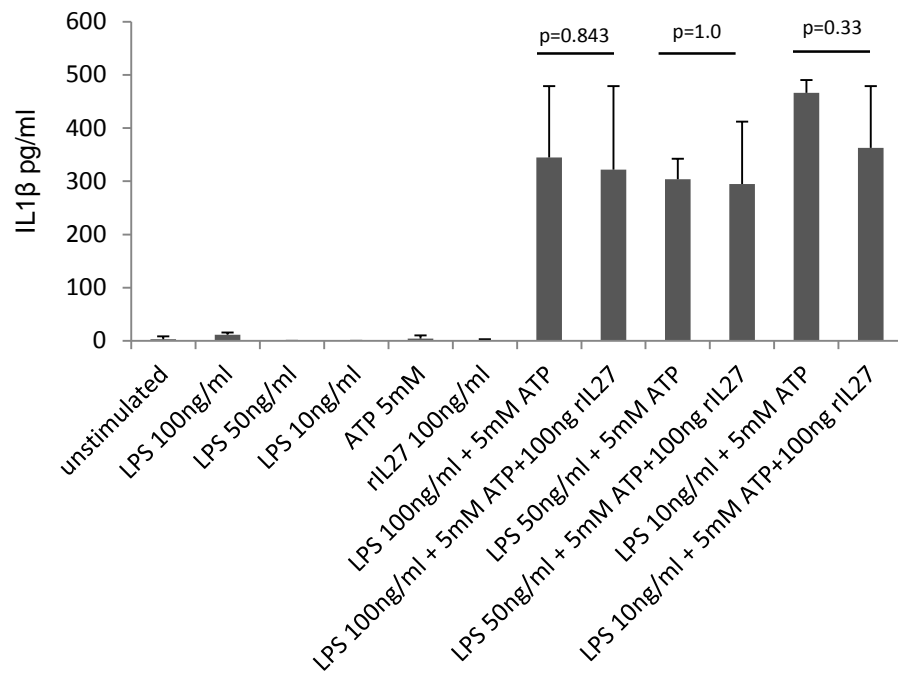


b



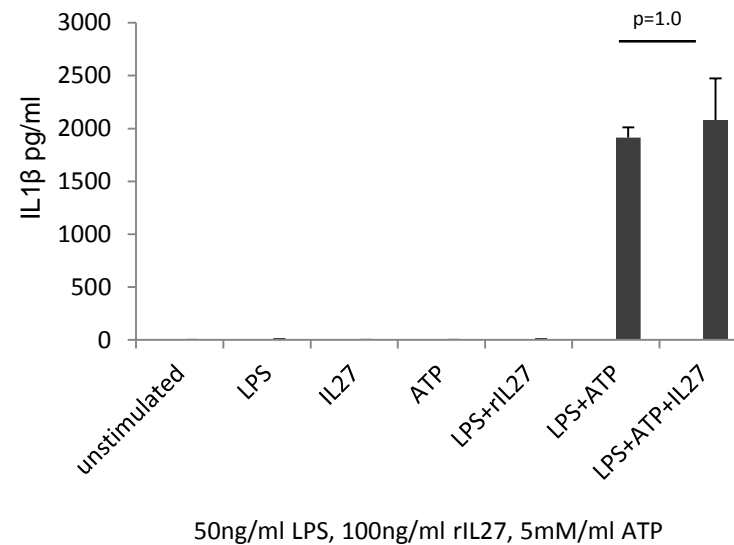
c

Bone marrow derived macrophage



d

Elicited peritoneal macrophage



Supplementary Figure Legends_McLean et al_ Interleukin 27 is a potential rescue therapy for acute severe colitis via interleukin-10 dependent, T cell independent attenuation of colonic mucosal innate immune responses

Supplementary Figure 1 show representative distal colon photomicrographs of F4/80⁺ and MPO⁺ macrophages and neutrophils, respectively in each experimental group as labeled. This was determined by immunohistochemistry with positive staining shown as brown colour on counterstained formalin fixed paraffin embedded tissue sections. TNBS precipitated an increase in macrophages and neutrophils. Colitic mice treated with LL-IL27 had a significantly reduced colonic mucosal neutrophil infiltrate compared to those treated with LL-C (p=0.002, t-test). Slides were optically scanned with an Aperio AT2 digital slide scanner.

Supplementary Figure 2. IL-27 does not directly influence acute colonic inflammasome activation *in vivo*. There was no evidence of IL-27 mediated suppression of the NLRP3 inflammasome (*il18*, *Asc*, *Nflp3*, *Caspase-1*) in distal colon homogenate, assessed by Taqman RT-PCR gene expression assays (Applied Biosystems) and analyzed with ddCt method of relative quantification; normalization to endogenous GAPDH control and level of expression compared to ethanol control group (**a & b**). n=10/group, t-test. Bone marrow derived macrophage (BMDM) (**c**) and thioglycollate-induced peritoneal macrophage (TPM) (**d**) were stimulated for 5 hours or 72 hours with various concentrations of LPS as labeled, +/- 5mM ATP, +/- rIL27 (100ng/ml). IL-1 β was measured in supernatant by ELISA. (**c**) Combined data from several experiments, generated from >2 wells/experimental condition. (**d**) Data presented from one experiment, with the same pattern of expression seen in repeat experiment. LPS plus ATP resulted in a robust IL-1 β response but there was no differential response in association with IL-27 in either macrophage population.

Mean + SEM. BMDM 100ng LPS dose data passed normality test and analyzed by t-test, all others Rank Sum test.