1 **MyD88 Mediates Colitis- and RANKL-induced Microfold Cell Differentiation**

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21 **Abbreviations**

22 CCL9, C-C motif ligand 9; DSS, dextran sulfate sodium; FAE, follicle-associated
 23 epithelium; GP2, glycoprotein 2; Lgr5, leucine-rich repeat-containing G-protein
 24 coupled receptor 5; M cells, microfold cells; MyD88, myeloid differentiation factor
 25 88; NALT, nasopharynx-associated lymphoid tissue; RANK, receptor activator of
 26 NF- κ B; RANKL, receptor activator of the Nuclear Factor- κ B ligand; TNF- α , tumor
 27 necrosis factor- α ; TRAF6, tumor necrosis factor receptor-associated factor 6;

28

29 **Abstract**

30 Intestinal microfold (M) cells are critical for sampling antigen in the gut and
 31 initiating the intestinal mucosal immune response. In this study, we found that the
 32 differentiation efficiency of M cells was closely related to the colitis severity. The
 33 expression levels of M cells differentiation-related genes were synchronized with the
 34 kinetics of pro-inflammatory cytokines expression originated from dextran sulfate
 35 sodium (DSS) induction and *Salmonella* infection. Compared with wild-type (WT)
 36 mice, *MyD88*^{-/-} mice exhibited significantly lower expression levels of M cells
 37 differentiation-related genes. However, DSS could induce colitis in *MyD88*^{-/-} mice but
 38 failed to promote M cells differentiation. Furthermore, the receptor activator of the
 39 Nuclear Factor-κB ligand (RANKL) induced M cells differentiation in murine
 40 intestinal organoids prepared from both WT and *MyD88*^{-/-} mice. However, less M
 41 cells differentiation were found in *MyD88*^{-/-} mice as compared with WT mice. Hence,
 42 we concluded that myeloid differentiation factor 88 (MyD88) is an essential molecule
 43 for colitis- and RANKL-related M cells differentiation.

44 **Keywords:** Colonic M cells, MyD88, Colitis, DSS

45 Introduction

46 M cells are specialized epithelial cells located mainly at follicle-associated
 47 epithelium (FAE) [1] and villous epithelium [2] of the small intestines. These cells are
 48 also found at the epithelium of colonic mucosa [3] and nasopharynx-associated
 49 lymphoid tissue (NALT) [4]. Although M cells are not antigen-presenting cells (APC),
 50 they play essential roles in mucosal immune surveillance [5]. M cells possess unique
 51 morphological features, such as irregular brush border, pocket structure, reduced
 52 glycocalyx, and microvilli [6]. They are conventionally known for capturing luminal
 53 antigens and delivering antigens particles to underlying immune cells. Many intestinal
 54 pathogens, such as murine Norovirus [7, 8], Reovirus [8], *Salmonella typhimurium* (*S.*
 55 *Typhimurium*) [9], and *Candida albicans* (*C. albicans*)[10] utilize M cells as the entry
 56 for invasions.

57 M cells are differentiated from leucine-rich repeat-containing G-protein coupled
 58 receptor 5⁺ (Lgr5⁺) stem cells [11]. In this differentiation process, the receptor
 59 activator of the Nuclear Factor-κB ligand (RANKL) is indispensable. Enterocytes
 60 expressing receptor activator of NF-κB (RANK) are stimulated by RANKL, then
 61 differentiated into M cells [12]. Spi-B, a transcription factor of the Est family, also
 62 mediates this process [11, 13]. Some gut microbiota impact M cells differentiation as
 63 well. For example, bacterial flagellin recovers the age-related decline of FAE M cells
 64 in mice [14]. The number of M cells in specific pathogen-free (SPF) mice increases
 65 after 7-day feeding in a typical animal house environment. *Salmonella enterica*
 66 serovar Typhimurium type III could also increase the number of M cells[15].

67 Additionally, tumor necrosis factor- α (TNF- α), an inflammatory cytokine, induces M
68 cells differentiation in the colon [3]. TNF- α is induced by MyD88 or tumor necrosis
69 factor receptor-associated factor 6 (TRAF6) through stimulating the production of
70 NF- κ B [16, 17]. As the downstream molecule of RANKL-RANK signaling,
71 TRAF6-mediated Nuclear Factor- κ B (NF- κ B) signaling pathway is responsible for
72 developing M cells [18]. Therefore, we hypothesized that MyD88 might participate in
73 colonic M cells differentiation.

74 In this study, we show that DSS and *Salmonella choleraesuis* (*S. choleraesuis*)
75 cause severe colitis in mice. M cells differentiation is attenuated when MyD88 is
76 knocked out. Meanwhile, DSS-induced colitis promotes glycoprotein 2⁺ (GP2⁺) M
77 cells production in WT mice but not in *MyD88*^{-/-} mice. Furthermore, RANKL
78 upregulates M cell-specific gene expression on intestinal organoids partly dependent
79 on MyD88. Therefore, we concluded that MyD88 mediates colitis- and
80 RANKL-induced colitis-related colonic M cells differentiation.

81 **Methods and Materials**

82 **Animals and related materials**

83 *MyD88^{-/-}* (B6.129P2(SJL)-*MyD88^{tm1.1Defr}*/J, Stock No: 009088) mice were
 84 originally purchased from The Jackson Laboratory (Anthony L. DeFranco, University
 85 of California, San Francisco). All mice used in this study were 6-8 weeks old and
 86 were bred in the SPF facility of Lanzhou Veterinary Research Institute (LVRI).
 87 Dextran sodium sulfate (DSS) was purchased from HWRK Chemical Company. *S.*
 88 *choleraesuis* (Strain NO. CVCC79102) was purchased from the China Institute of
 89 Veterinary Drug Control. SPF WT (wild type) C57BL/6 mice were purchased from
 90 the Experimental Animal Center of LVRI. All experimental procedures and animal
 91 care protocols were carried out by following Care and Use of Laboratory Animals of
 92 LVRI, Chinese Academy of Agricultural Sciences, China.

93 **Inflammatory models**

94 For the DSS-induced inflammatory model, DSS 3% (M.W 40000) was added to the
 95 drinking water for the free intake for seven days. DSS-free drinking water was used as
 96 a control for the mice in the control group. For the *S. choleraesuis*-induced
 97 inflammatory model, *S. choleraesuis* were collected by centrifugation and
 98 re-suspended in PBS buffer. The mice in the infected group were gavaged with 10⁴
 99 CFU of *S. choleraesuis* or with PBS as control. All mice were kept in the same
 100 environment, weighed daily and sacrificed at day 8.

101 **Histological analysis**

102 After euthanasia, mice colons were fixed in the FinePix tissue-fix solution

103 (RightTech, China) for 24h, dehydrated according to the standard protocol, and then
104 embedded in paraffin. Then tissue blocks were sectioned and deparaffinized in xylene,
105 stained with Haematoxylin and Eosin (H&E). Finally, double-blinded histological
106 analysis was performed to evaluate the colitis level as described [19].

107 **Western Blot analysis**

108 Total proteins were prepared from tissue samples of the colon using RIPA lysis
109 buffer (Beyotime, China) with protease inhibitor. A total of 20 µg of protein were
110 loaded in each well for SDS-PAGE, transferred, and immunoblotted overnight at 4°C
111 with the primary antibody against GP2 (1:1000, MBL D278-3, Japan) and GAPDH
112 (1:5000, Proteintech Group, U.S.). Goat anti-Rat IgG (OriGene Technologies, U.S.)
113 and anti-Rab IgG secondary rabbit antibody (OriGene Technologies, U.S.) were
114 diluted at 1:5000 for incubation with the membrane. Finally, the immunoblots were
115 developed with chemiluminescence detection reagents (Advansta, U.S.) [20, 21].

116 **Murine intestinal organoids culture**

117 Mice were sacrificed to harvest the small intestines. After being washed by PBS
118 buffer, the intestines were cut into pieces and dissociated with 2 mM EDTA buffer in
119 15 mL centrifuge tubes for 20 min at 4°C. The samples were centrifuged at 300 ×g for
120 5 min to remove the EDTA buffer, repeatedly pipetted with 5 mL PBS buffer. The
121 crypts were then collected through a 70 µm strainer (BD, U.S.), centrifuged at 300 ×g
122 for 5 min, and cultured in the 24-well tissue culture plate (Corning, U.S.) with
123 Matrigel Matrix (Corning, U.S.) and Murine Intestinal Organoids Growth Medium
124 (STEMCELL, Canada). For the RANKL stimulation assay, organoids were stimulated

125 with 100 ng/mL recombinant human RANKL (CST, U.S.) for one day (PBS buffer
126 was used as control). The RANKL and mock control reagents were added daily.

127 **RNA extraction and quantitative real-time PCR**

128 After the mice were sacrificed, their colon was washed by cold PBS then harvested.
129 The total RNA was extracted from the colon samples using RNAiso reagent (TaKaRa,
130 Japan) and used for cDNA preparation using Honor II 1st Strand cDNA Synthesis
131 SuperMix (Novogene, China) with hexamer random primers. To evaluate the
132 inflammation responses and M cells differentiation within mouse colon, the relative
133 expression levels of TNF- α , IL-1 β , IL-6, GP2, Spi-B, RANK, TNF- α -induced protein
134 2 (Tnfaip2), and C-C motif ligand 9 (CCL9) were determined by RT-qPCR using
135 ChamQ SYBR qPCR Master Mix (Vazyme, China). The primers used in this study are
136 listed in Table 1.

137 **Statistical analysis**

138 The data were presented as the means $\pm$ SEM. The significance between groups
139 was analyzed by one-way analysis of variance or Student's *t*-test with GraphPad Prism
140 7 software and *P* values.

141 **Results**

142 **DSS induces colitis and GP2⁺ M cells differentiation**

143 To investigate the relationship between colonic M cells differentiation and colitis, a
 144 model of DSS-induced colitis was used. After oral administration of DSS for 7 days,
 145 the DSS-treated mice lost weight compared with the control group (Figure 1A). The
 146 spleen weight increased in DSS-treated mice, but their colon weight and length were
 147 reduced (Figure 1B). The mRNA levels of pro-inflammatory cytokine TNF- α , IL-1 β ,
 148 and IL-6 in the colon were quantified by RT-qPCR. DSS-treated mice had
 149 significant-high mRNA levels of TNF- α , IL-1 β , and IL-6 (Figure 1C). Histological
 150 changes, including inflammatory cell infiltration in the basal layer and crypt, edema
 151 and reduced mucus, were observed in the colon of DSS-treated mice (Figure 1D). To
 152 assess the differentiation of colonic M cells, the mRNA level of M cells
 153 differentiation-related genes, including GP2, Spi-B, RANK, Tnfaip2, and CCL9, were
 154 measured by RT-qPCR. The results showed that the mRNA levels of GP2, Tnfaip2,
 155 and CCL9 from the colon of the DSS-treated group were significantly upregulated
 156 compared to - control - (Figure 1E). The immunoblotting results showed that DSS
 157 treatment promoted the expression of GP2 (Figure 1F). These results indicated that
 158 oral administration with DSS successfully induced colitis in mice. In addition,
 159 RT-qPCR suggested that DSS treatment induced GP2⁺ M-cell differentiation.

160 ***S. choleraesuis* infection induces colitis and GP2⁺ M cells differentiation**

161 To further verify that colitis is related to colonic M cells differentiation, mice were
 162 administrated with *S. choleraesuis* orally. Mice in the *S. choleraesuis*-treated group

suffered weight loss compared to the controls (Figure 2A). Typical symptoms, such as the increase in spleen weight, and a reduction of colon weight and length, were observed in the *S. choleraesuis*-treated group (Figure 2B). Concomitantly, bacterial colonized the liver and spleen (Figure 2C). The mRNA levels of TNF- α , IL-1 β , and IL-6 in the colon were detected by RT-qPCR. The results illustrated that the *S. choleraesuis* infection significantly upregulated the mRNA levels of TNF- α and IL-6 in mouse colons (Figure 2D). Pathological changes, including inflammatory cell infiltration in the basal layer and crypt, fibrinous exudation and reduced mucus, indicated that the *S. choleraesuis* induced colitis (Figure 2F).

To evaluate the M cells differentiation, the mRNA levels of GP2, Spi-B, RANK, Tnfaip2, and CCL9 were measured by RT-qPCR. The result showed that the mRNA levels of GP2, Tnfaip2, and CCL9 in the colon from the *S. choleraesuis*-infected group were significantly higher than those in the mock-control group (Figure 2E). Western-blot analysis confirmed that *S. choleraesuis* infection induced the GP2 expression (Figure 2G). Taken together, these data demonstrate that *S. choleraesuis* infection induces colitis and GP2⁺ M cells differentiation

MyD88 is a critical factor for colonic M cells differentiation

We speculated that MyD88 might play a role in the differentiation of murine colonic M cells. To test this hypothesis, we compared WT and *MyD88*^{-/-} mice. During 7-day housing in the same environment, the bodyweight of both *MyD88*^{-/-} and WT mice remained stable (Figure 3A). On day 7, the spleen weight of *MyD88*^{-/-} mice were lower than that of WT mice, but their colon weight and length were not significantly

different (Figure 3B). Histological analysis of the colons did not reveal differences between *MyD88*^{-/-} and WT mice (Figure 3C). To investigate whether *MyD88*^{-/-} affects the differentiation of colonic M cells, mRNA level of M cells differentiation-related genes was detected by RT-qPCR while the GP2 protein expression was measured by western-blot. The results showed that the *MyD88*^{-/-} mice had significantly lower mRNA levels of GP2 and Spi-B, as well as GP2 protein levels compared to WT mice (Figure 3D, 3E). These results indicated that the lack of MyD88 restrained the differentiation of colonic M cells and suggested that the MyD88 is a critical factor during the colonic M cells differentiation.

MyD88 is involved in colitis induced M cells differentiation

To explore whether MyD88 participates in colitis-induced M cells differentiation, *MyD88*^{-/-} mice were given DSS orally for 7days. Compared to the control group, weight loss was observed in the DSS-treated mice (Figure 4A). Moreover, the DSS treatment group was associated with more severe clinical symptoms, characterized by increased spleen weight, decreased colon weight, and colon length (Figure 4B). Pathological changes, including inflammatory cell infiltration, exfoliation of epithelial cells, crypt branching and decreased mucus (Figure 4C) and the higher mRNA levels of TNF- α , IL-1 β , and IL-6 in the colon of the DSS-treated group (Figure 4D), indicated that DSS still induced colitis in *MyD88*^{-/-} mice. However, during this severe inflammatory response, M cells differentiation-related markers decreased (GP2, Spi-B and Rank) or remained unchanged (Figure 4E). These results indicate that MyD88 was involved in colitis-induced M cells differentiation.

207 **MyD88 is involved in RANKL-induced M cells differentiation**

208 We hypothesize that MyD88 was also involved in RANKL-induced M cells
 209 differentiation. To test this hypothesis, murine intestinal organoids were prepared for
 210 further study (Figure 5A). Expression of key M cell markers was evaluated by
 211 RT-qPCR in the organoids generated from WT and *MyD88*^{-/-} mice. The results showed
 212 that M cells differentiation markers were down-regulated in *MyD88*^{-/-} mice (Figure
 213 5B). This result supported that MyD88 was indispensable for M cells differentiation
 214 in our previous results. Then the organoids from WT mice and *MyD88*^{-/-} mice were
 215 treated with RANKL 24 hours before analysis. The results illustrated that RANKL
 216 could induce M cells differentiation in both WT mice and *MyD88*^{-/-} mice (Figure 5C),
 217 characterized as increased expression levels of GP2, Spi-B, RANK, Tnfaip2, and
 218 CCL9. However, the expression levels of M cell markers in *MyD88*^{-/-} mice were much
 219 lower than that in WT mice (Figure 5C). Taken together, these results indicate that
 220 MyD88 is also involved in M cells differentiation induced by RANKL.

221

222 Discussion

223 M cell is a potential route for promoting drug and oral vaccine delivery. M
224 cell-dependent antigen transport was reported to have an essential function in
225 alleviating colitis [22]. However, the knowledge about colonic M cells differentiation
226 is limited. The research on the differentiation of colonic M cells will help to better
227 understand the role of M cells in maintaining intestinal homeostasis.

228 RANKL induces M cells differentiation. We found that GP2, used as a mature M
229 cell marker [23], is upregulated by 2-day RANKL stimulation [24]. Our study further
230 demonstrates that RANKL induces M cells differentiation in both WT and *MyD88*^{-/-}
231 mice organoids, even though *MyD88*^{-/-} mice exhibit lower expression levels of M cells
232 differentiation-related genes comparing to that in WT mice organoids.

233 Another study showed that gut microbiota could also regulate the differentiation of
234 M cells [14]. As reported, *S. Typhimurium* induces the differentiation of FAE
235 enterocytes into M cells based on a type III effector protein SopB-dependent pathway
236 [15]. In our study, we found that *S. choleraesuis*-induced colitis also promotes M cells
237 differentiation. Combined with the results that DSS-induced colitis promotes M cells
238 differentiation, we speculated that inflammation induces M cells differentiation.
239 Moreover, we found that MyD88 plays a crucial role in both colitis- and
240 RANKL-induced M cells differentiation. MyD88 is an essential adaptor molecule in
241 the toll-like receptors signaling pathway, which is related to bacterial receptors [25]
242 and intestinal microorganism homeostasis [26]. In the intestines, MyD88 also
243 participates in regulating gut microbial ecology and maintaining homeostasis [27, 28].

244 Here, we found that the differentiation of M cells is attenuated in both *MyD88*^{-/-}
 245 intestinal organoids and colon of *MyD88*^{-/-} mice as compared with its WT control.
 246 These data suggested potential crosstalk between MyD88-mediated signaling and M
 247 cells differentiation.

248 MyD88 has been proved to play a crucial role in regulating RANKL signaling.
 249 MyD88 mediates RANKL transcription introduced by LPS and IL-1 α [29]. In bone
 250 marrow stromal cells, MyD88 is also required for up-regulating RANKL transcription
 251 and down-regulating osteoprotegerin (OPG) mRNA by the TLR and IL-IR signaling
 252 [30]. As a soluble inhibitor of RANKL, OPG mediates the self-regulation of M cells
 253 differentiation in FAE [31]. These studies imply the relationship between the MyD88
 254 mediated pathway and RANKL signaling. Hence, the deletion of MyD88 may
 255 promote the transcription of OPG and potentially inhibit M cells differentiation.

256 In summary, the differentiation of M cells may be regulated by a complicated
 257 signaling pathway related to immune response and gut microbiota. Here, we highlight
 258 crosstalk between inflammation response and M cells differentiation and demonstrate
 259 that MyD88 was a critical molecular for colitis- and RANKL-induced M cells
 260 differentiation. Unfortunately, due to the unsuccessful immunostaining of colonic M
 261 cells with 2 antibodies against GP2, we failed to carry out a quantitative analysis
 262 regarding the differentiation of colonic M cells in tissue. The detailed mechanism that
 263 regulates colonic M cells differentiation still needs further study.

264

265 **Compliance with Ethical Statements**

266 **Conflict of interest**

267 The authors declare no competing interests.

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272 **Ethics approval**

273 The care and use of animals were approved by the Institutional Animal Care and
274 Use Committee of LVRI, Chinese Academy of Agricultural Sciences in compliance
275 with the NIH guidelines for the care and use of laboratory animals.

276 **Author contributions**

277 YL, SY, XH, NY, and GL conceived the study. YL, SY, and GL designed the
278 experiments. YL, SY, XH, CW, JZ performed the experiments. ZJ provided *MyD88*^{-/-}
279 mice. YL and SY analyzed data. YL, SY, LW and GL prepared the draft. GL edited the
280 manuscript.

281

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359

360 **Figure legends**

361 **Figure. 1 DSS induces colitis and GP2⁺ M cells differentiation**

362 WT mice in the DSS-treated group (n=3) and control group (n=3) were allowed to
 363 intake DSS-contained or normal water for 7 days freely. Their body weights were
 364 monitored daily (A). The mice were sacrificed on day 8, and their spleen weight,
 365 colon weight, and colon length were measured for assessing pathological changes
 366 caused by DSS treatment (B). Total RNA from colon samples was used to detect the
 367 mRNA levels of TNF- α , IL-1 β , IL-6 by RT-qPCR (C). Partial colon samples were
 368 used for histological analysis to evaluate the colitis (D). Total RNA from colon
 369 samples was used to detect the mRNA levels of GP2, Spi-B, RANK, Tnfaip2, and
 370 CCL9 by RT-qPCR (E). The data were calculated using the $2^{-\Delta\Delta Ct}$ method. *, $P<0.05$;
 371 **, $P<0.01$; ***, $P<0.001$. The GP2 expression levels in total protein from colon
 372 samples were evaluated by Western blot (F).

373 **Figure. 2 *S. choleraesuis* infection induces colitis and GP2⁺ M cells differentiation**

374 WT mice were gavaged with *S. choleraesuis*-PBS buffer mix (*S.*
 375 *choleraesuis*-infected group, n=3) or normal PBS buffer (control group, n=3). Their
 376 body weight was monitored daily (A). The mice were sacrificed on day 8, and the
 377 spleen weight, colon weight, and colon length were evaluated to assess the
 378 pathological changes caused by *S. choleraesuis* infection (B). The bacterial loads in
 379 the liver, spleen were analyzed (C). Total RNA from colon samples was used to detect
 380 the mRNA level of TNF- α , IL-1 β , IL-6 by RT-qPCR (D). Total RNA from colon
 381 samples was used to detect the mRNA levels of GP2, Spi-B, RANK, Tnfaip2, and

382 CCL9 by RT-qPCR (E). Tissue samples from the colon were used for histological
383 analysis for assessing colitis (F). The data were calculated using the $2^{-\Delta\Delta Ct}$ method. *,
384 $P<0.05$; **, $P<0.01$; ***, $P<0.001$. The GP2 expression levels in total protein from
385 colon samples were evaluated by Western-blot (G).

386 **Figure. 3 MyD88 is a critical factor for colonic M cells differentiation**

387 The body weight of WT and *MyD88*^{-/-} mice (n=3) were monitored for 8 days (A).
388 After sacrificing, their spleen weight, colon weight, and colon length were measured
389 (B). Partial colon samples were fixed for histological analysis (C). Total RNA from
390 colon samples was used to detect the mRNA levels of GP2 and Spi-B by RT-qPCR
391 (D). The GP2 expression levels in complete protein from colon samples were
392 evaluated by Western-blot (E).

393 **Figure. 4 MyD88 is involved in colitis induced M cells differentiation**

394 *MyD88*^{-/-} mice in the DSS-treated group (n=3) and control group (n=3) were allowed
395 to intake DSS-contained or normal water for 7 days freely. The body weight of mice
396 was monitored daily (A). The mice were sacrificed on day 8, and their spleen weight,
397 colon weight, and colon length were measured to assess the pathological changes
398 caused by DSS treatment (B). Total RNA from colon samples was used to detect the
399 mRNA levels of TNF- α , IL-1 β , IL-6 by RT-qPCR (C). Tissue samples from the colon
400 were used for histological analysis to evaluate their colitis (D). Total RNA from colon
401 samples was used to detect the mRNA levels of GP2, Spi-B, RANK, Tnfaip2, and
402 CCL9 by RT-qPCR (E). The data were calculated using the $2^{-\Delta\Delta Ct}$ method. *, $P<0.05$;
403 **, $P<0.01$; ***, $P<0.001$. The GP2 expression levels in total protein from colon

404 samples were evaluated by Western blot (F).

405 **Figure. 5 MyD88 is involved in RANKL-induced M cells differentiation**

406 Murine intestinal organoids were established (A). Images were developed under a
 407 ZEISS Vert A1 microscope. Scale bar = 200 μ m. Both WT and *MyD88*^{-/-} organoids
 408 were stimulated with human recombinant RANKL for one day (PBS was used as
 409 control) and then were collected for RNA isolation. The mRNA levels of
 410 representative M-cell related markers, GP2, Spi-B, RANK, Tnfaip2, and CCL9, in
 411 both WT and *MyD88*^{-/-} organoids, were detected by RT-qPCR (B). The fold change of
 412 these markers was compared after RANKL-treatment between WT and *MyD88*^{-/-}
 413 group (C). The data were calculated using the $2^{-\Delta\Delta Ct}$ method. *, $P < 0.05$; **, $P < 0.01$;
 414 ***, $P < 0.001$.

415 Table 1. Primers used in this study

Target gene	Primer/probe	Sequence (5' – 3')
GAPDH	Forward	GCCTTCCGTGTTCTCTACCC
	Reverse	TGCCTGCTTCACCACTTC
Spi-B	Forward	GGGGGCCTTGACTCTA
	Reverse	CTCTGGGGGGTACACC
GP2	Forward	CCTGCGTTCTGACACTG
	Reverse	GCCGTGCAGGTTATCA
RANK	Forward	ATGCGAACCAGGAAAGT
	Reverse	TGCCTGCATCACAGACT
Tnfaip2	Forward	GTGCAGAACCTCTACCCCAATG
	Reverse	TGGAGAATGTCGATGGCCA
CCL9	Forward	GCCCAGATCACACATGCAAC
	Reverse	AGGACAGGCAGCAATCTGAA
TNF- α	Forward	TCAGTTCCATGGCCCAGAC
	Reverse	GTTGTCTTTGAGATCCATGCCATT
IL-1 β	Forward	CCCTGAACTCAACTGTGAAATAGCA
	Reverse	CCCAAGTCAAGGGCTTGGA
IL-6	Forward	TAGTCCTTCCTACCCCAATTTCC
	Reverse	TTGGTCCTTAGCCACTCCTTCC

Fig.1 DSS induced colitis and GP2⁺ M cells differentiation

Fig.2 *S. choleraesuis* infection induced colitis and GP2⁺ M cells differentiation

Fig.3 MyD88 is a critical factor for colonic M cells differentiation

Fig.4 MyD88 is involved in colitis induced M cells differentiation

Fig.5 MyD88 is involved in RANKL-induced M cells differentiation

A

B

C