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**Minhao Wang**

Xuzhou Medical University

**Yiqun Li**

Xuzhou Medical University

**Li Li**

lily9711214@126.com

Xuzhou Medical University

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## Research Article

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# **Ablim3 is upregulated in experimental colitis and its local knockdown attenuates DSS-induced intestinal injury in mice**

Minhao Wang<sup>1,2</sup>, Yiqun Li<sup>1,2</sup>, and Li Li<sup>1,2\*</sup>.

<sup>1</sup> Institute of Digestive Diseases, Xuzhou Medical University, Xuzhou, Jiangsu, China.

<sup>2</sup> Department of Gastroenterology, The Affiliated Hospital of Xuzhou Medical University, Xuzhou, Jiangsu, China

\*Corresponding author: Li Li, Email: lily9711214@126.com;

## **Abstract**

**Background:** Actin cytoskeleton remodeling and epithelial barrier disruption are involved in inflammatory bowel disease (IBD), but the role of actin-binding LIM protein family member 3 (ABLIM3) remains unclear.

**Results:** Integrative bioinformatics analysis identified ABLIM3 as a candidate IBD-related gene. Ablim3 expression was significantly increased in colonic tissues of DSS-treated mice. Local Ablim3 knockdown partially attenuated DSS-induced colitis, as reflected by reduced body weight loss, improved colon shortening, and alleviated histopathological injury. Exploratory proteomic analysis suggested potential associations between Ablim3 and cytoskeleton- or adhesion-related processes.

**Conclusions:** Ablim3 is upregulated in experimental colitis, and its local knockdown partially alleviates DSS-induced intestinal injury, warranting further mechanistic and clinical validation.

## **Keywords**

24 Inflammatory Bowel Disease; ABLIM3; Bioinformatics Analysis; Self-Complementary Adeno-  
25 Associated Virus; Co-IP/MS.

26

## 27 **Background**

28 Inflammatory bowel disease (IBD), mainly including Crohn's disease and ulcerative colitis, is  
29 a chronic relapsing inflammatory disorder of the gastrointestinal tract with a rising global burden,  
30 particularly in newly industrialized regions[1,2]. Although biologics and small-molecule agents  
31 have improved disease management, many patients still experience primary non-response or  
32 secondary loss of response, indicating that additional pathogenic mechanisms and actionable  
33 molecular targets remain to be clarified [3–7].

34 IBD pathogenesis involves complex interactions among genetic susceptibility, microbial  
35 dysbiosis, mucosal immune dysregulation, and epithelial barrier dysfunction[8–12]. Disruption of  
36 the intestinal epithelial barrier is increasingly recognized as a key event in disease initiation and  
37 inflammatory amplification. Barrier integrity depends on the coordinated organization of tight  
38 junctions, adherens junctions, and their dynamic coupling to the cortical actin cytoskeleton.  
39 Therefore, cytoskeletal remodeling may influence junctional stability, epithelial cohesion, cell  
40 extrusion, and mucosal repair, thereby contributing to sustained intestinal inflammation[13,14].

41 Peripheral blood mononuclear cell (PBMC) transcriptomes are widely used to identify  
42 molecular signatures associated with IBD. However, blood-derived transcriptional alterations do not  
43 necessarily reflect local expression patterns or functional effects in the intestinal mucosa [15–17].  
44 Candidate genes identified from peripheral transcriptomic screening therefore require validation in  
45 intestinal tissue and functional assessment in disease-relevant in vivo models.

46 In the present study, we integrated differential expression analysis of the GSE3365 PBMC  
47 dataset with weighted gene co-expression network analysis and machine learning approaches, and

48 identified human ABLIM3 as a candidate IBD-related gene. ABLIM3 belongs to the actin-binding  
49 LIM protein family, whose members contain N-terminal LIM domains and a C-terminal dematin-  
50 like actin-binding domain that may support protein-protein interactions and F-actin-associated  
51 cellular processes[18–21]. Although ABLIM family proteins have been implicated in actin  
52 cytoskeleton organization and junction-associated regulation, direct evidence linking ABLIM3 to  
53 intestinal inflammation remains limited[22].

54 To address this issue, we examined the expression of Ablim3 in colonic tissues from a dextran  
55 sulfate sodium (DSS)-induced acute colitis mouse model, and further evaluated its in vivo function  
56 using self-complementary adeno-associated virus (scAAV)-mediated local shRNA delivery by  
57 enema [23–26]. Because the DSS model is characterized predominantly by epithelial injury with  
58 secondary inflammation, it is particularly suitable for studying barrier disruption and mucosal repair  
59 [27]. Finally, co-immunoprecipitation combined with mass spectrometry (Co-IP/MS) was used to  
60 identify candidate interacting proteins of ABLIM3 and to provide clues for subsequent mechanistic  
61 investigation [28].

62 Overall, this study aimed to determine whether Ablim3 is upregulated in experimental colitis  
63 and whether local Ablim3 knockdown attenuates DSS-induced intestinal injury, thereby providing  
64 preliminary evidence for its involvement in colitis-associated epithelial injury.

65

## 66 **Methods**

### 67 **Public dataset acquisition and bioinformatics analysis**

68 The PBMC transcriptomic dataset GSE3365 was obtained from the Gene Expression Omnibus  
69 database and included 42 healthy controls and 85 patients with inflammatory bowel disease. All  
70 analyses were performed using R software version 4.5.1 with the limma, WGCNA, randomForest,  
71 Boruta, caret, pROC, clusterProfiler, and org.Hs.eg.db packages.

Principal component analysis was used to assess sample distribution. Differential expression analysis was performed using limma, and genes with  $|\log_2 \text{ fold change}| \geq 1$  and  $P < 0.05$  were defined as differentially expressed genes. GO and KEGG enrichment analyses were performed using clusterProfiler. WGCNA was used to identify disease-associated co-expression modules. Random forest and Boruta algorithms were applied for feature selection, followed by model optimization using repeated 10-fold cross-validation. Receiver operating characteristic curve analysis was performed using pROC.

### **Animals and ethics statement**

Specific pathogen-free male C57BL/6J mice aged 6–8 weeks and weighing 20–22 g were obtained from the Experimental Animal Center of Xuzhou Medical University. Mice were maintained under specific pathogen-free conditions with a 12-h light/dark cycle, controlled temperature and humidity, and ad libitum access to standard laboratory chow and water. Animals were allowed to acclimatize for 1 week before the experiments.

Nine mice were included in each experimental group. After acclimatization, mice were randomly allocated to the corresponding experimental groups. The individual mouse was considered the experimental unit.

All animal procedures were approved by the Experimental Animal Ethics Committee of Xuzhou Medical University (approval No. 202508T003) and were performed in accordance with institutional guidelines for the care and use of laboratory animals. Predefined inclusion and exclusion criteria were established before study initiation. Humane endpoints included excessive body weight loss or severe distress, and mice meeting these criteria were euthanized according to animal welfare requirements.

### **Experimental design**

95 The animal experiments consisted of three parts. First, Control and dextran sulfate sodium  
96 (DSS) groups were used to evaluate Ablim3 expression in DSS-induced colitis. Second, AAV-  
97 shNC and AAV-shAblim3 groups were used to verify the efficiency of AAV-mediated Ablim3  
98 knockdown. Third, Sham, DSS, DSS + AAV-shNC, and DSS + AAV-shAblim3 groups were used  
99 to assess the effect of local Ablim3 knockdown on DSS-induced colitis.

100 In the formal intervention experiment, mice in the AAV-treated groups received AAV enema  
101 administration 1 week before DSS exposure, whereas mice in the non-AAV groups received an  
102 equal volume of phosphate-buffered saline by enema to standardize the procedure.

### 103 **AAV vector construction and enema administration**

104 An shRNA targeting mouse Ablim3 and a negative control shRNA were packaged into self-  
105 complementary AAV2/9 vectors by OBiO Technology (Shanghai) Corp., Ltd. The Ablim3-  
106 targeting vector was designated pscAAV-U6-shRNA(Ablim3)-CMV-EGFP-tWPA, and the  
107 negative control vector was designated pscAAV-U6-shRNA(NC2)-CMV-EGFP-tWPA.

108 For local delivery, AAV vectors were diluted in phosphate-buffered saline and administered by  
109 enema at a dose of  $1 \times 10^{11}$  vector genomes per mouse in a total volume of 200  $\mu$ L under  
110 isoflurane anesthesia. One week after AAV administration, colonic transduction was evaluated by  
111 EGFP fluorescence, and Ablim3 protein expression was assessed by Western blotting to verify  
112 knockdown efficiency.

### 113 **DSS-induced acute colitis model and disease assessment**

114 Acute colitis was induced by allowing mice free access to 3% dextran sulfate sodium (DSS;  
115 molecular weight, approximately 40,000 Da; GLP BIO) in drinking water for 7 consecutive days.  
116 Body weight, stool consistency, fecal bleeding, and general condition were recorded daily. Body  
117 weight loss was scored as follows: 0, no weight loss; 1, 1–5%; 2, 5–10%; 3, 10–20%; and 4, >20%.  
118 Stool consistency was scored as 0 for normal, well-formed stools, 2 for loose stools, and 4 for

119 watery diarrhea. Fecal bleeding was scored as 0 for no detectable blood, 2 for positive fecal occult  
120 blood, and 4 for gross rectal bleeding. The disease activity index was calculated as the mean of the  
121 body weight loss, stool consistency, and fecal bleeding scores. At the experimental endpoint, mice  
122 were euthanized under deep anesthesia, and the colons were collected for length measurement,  
123 histological analysis, and molecular assays.

#### 124 **Histological analysis and immunostaining**

125 Colonic tissues were fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned for  
126 histological and immunostaining analyses. Hematoxylin and eosin staining was performed to  
127 evaluate histopathological changes.

128 For immunohistochemistry, sections were subjected to antigen retrieval, endogenous  
129 peroxidase blocking, nonspecific blocking, and incubation with the indicated primary antibodies  
130 overnight at 4°C. Signals were visualized using 3,3'-diaminobenzidine, and nuclei were  
131 counterstained with hematoxylin.

132 For immunofluorescence staining, sections were subjected to antigen retrieval,  
133 permeabilization, blocking, and incubation with the indicated primary antibodies overnight at 4°C.  
134 After incubation with fluorescent secondary antibodies, nuclei were counterstained with DAPI, and  
135 images were acquired using an Olympus fluorescence microscope.

#### 136 **Western blotting**

137 Colonic tissues were lysed in RIPA buffer containing PMSF, and protein concentrations were  
138 determined using a BCA protein assay kit. Equal amounts of protein were separated by SDS-PAGE  
139 and transferred onto PVDF membranes. After blocking, membranes were incubated with the  
140 indicated primary antibodies overnight at 4°C, followed by HRP-conjugated secondary antibodies.  
141 Protein bands were visualized using enhanced chemiluminescence, and band intensities were semi-  
142 quantified using ImageJ software.

143 Detailed information on the primary and secondary antibodies used in this study is provided in  
144 Additional file 1: Table S1.

#### 145 **Co-immunoprecipitation and mass spectrometry**

146 Co-immunoprecipitation combined with liquid chromatography-tandem mass spectrometry  
147 was performed as an exploratory approach to identify potential Ablim3-associated proteins. Briefly,  
148 colonic tissue lysates were incubated with an Ablim3-specific antibody or isotype-matched IgG  
149 control, followed by incubation with Protein A/G magnetic beads. After washing and elution,  
150 immunoprecipitated proteins were submitted to Cosmos Wisdom Mass Spectrometry Data Analysis  
151 Co., Ltd. (Hangzhou, China) for mass spectrometry analysis.

152 Proteins identified in the IP group were compared with those in the IgG control group to  
153 remove nonspecific background signals. IP-specific proteins were retained as the initial candidate  
154 set. Candidate proteins were further filtered using peptide spectrum matches  $\geq 3$ , unique peptides  $\geq$   
155 4, and Sequest HT score  $\geq$  the 75th percentile. These variables were standardized using Z-scores,  
156 and candidate proteins were ranked according to an equally weighted composite score.

157 The complete list of proteins uniquely identified in the ABLIM3 immunoprecipitation group is  
158 provided in Additional file 1: Table S2.

#### 159 **Functional annotation and protein-protein interaction network analysis of candidate Ablim3-** 160 **associated proteins**

161 Candidate Ablim3-associated proteins were subjected to GO and KEGG enrichment analyses  
162 using R, with GO analysis mainly focused on the biological process category. A threshold of  $P <$   
163 0.05 was considered statistically significant.

164 Protein-protein interaction network analysis was performed using STRING database version  
165 12.0, with the species restricted to *Mus musculus*. Network parameters and interaction relationships

166 were exported for downstream visualization and qualitative interpretation of locally connected  
167 protein clusters.

## 168 **Statistical Analysis**

169 Experimental data were analyzed using ImageJ and GraphPad Prism 10.1 and are presented as  
170 mean  $\pm$  standard deviation. Normality and homogeneity of variance were assessed before statistical  
171 analysis. Comparisons between two groups were performed using Student's t-test, whereas  
172 comparisons among multiple groups were performed using one-way ANOVA or nonparametric  
173 tests, as appropriate.

174 For data involving two factors, two-way ANOVA was applied. For repeated measurements  
175 over time, two-way repeated-measures ANOVA was used, with Geisser-Greenhouse correction  
176 applied when the assumption of sphericity was violated. Post hoc multiple comparisons were  
177 performed when appropriate. Exact P values are reported where applicable, except when P values  
178 were less than 0.0001. A P value  $< 0.05$  was considered statistically significant.

## 179 **Results**

### 180 **1. Identification of Ablim3 as a Potential Key Gene by Bioinformatic Screening**

#### 181 **1.1. Identification of Differentially Expressed Genes in the GSE3365 Dataset**

182 The GPL96 annotation file comprised 127 transcriptomic samples, including 42 normal  
183 controls and 85 patients with IBD. To characterize the overall transcriptomic differences between  
184 the two groups, principal component analysis (PCA) was performed first. As shown in Figure 1A,  
185 the normal and IBD samples exhibited partial separation along the PC1 and PC2 axes, although  
186 some overlap remained. This distribution pattern suggested a discernible difference in global gene  
187 expression profiles between the two groups. Differential expression analysis using the limma  
188 package subsequently identified 130 differentially expressed genes (DEGs), including 77  
189 upregulated genes and 53 downregulated genes (Figure 1B). To further illustrate the expression

190 characteristics of these genes across samples, the top 30 DEGs ranked by fold change and statistical  
191 significance were visualized in a heatmap (Figure 1E).

## 192 **1.2. Functional Enrichment Analysis of Differentially Expressed Genes**

193 Enrichment analysis was also performed for the downregulated genes; however, no GO terms  
194 or KEGG pathways met the predefined significance threshold ( $p < 0.01$ ). Therefore, the  
195 downregulated genes were not included in further functional interpretation, and subsequent GO and  
196 KEGG enrichment analyses were focused on the upregulated DEGs only. As shown in Figure 1C-  
197 D, GO analysis indicated that the upregulated genes were mainly enriched in biological processes  
198 and molecular functions related to chemotaxis, leukocyte migration, cytokine activity, and  
199 chemokine receptor binding. KEGG pathway analysis further revealed significant enrichment in  
200 cytokine-cytokine receptor interaction, the chemokine signaling pathway, and inflammation-related  
201 pathways, including the IL-17 and NF-kappa B signaling pathways.

## 202 **1.3. Construction of the Co-expression Network and Identification of the Hub Module**

203 Based on the gene expression profile of GSE3365, weighted gene co-expression network  
204 analysis (WGCNA) was further performed to explore disease-related co-expression patterns in IBD.  
205 Using a soft-thresholding power of 9, genes were clustered into distinct co-expression modules  
206 based on the topological overlap matrix (TOM). To identify the module most closely associated  
207 with ulcerative colitis-related traits, correlations between module eigengenes (MEs) and clinical  
208 phenotypes were subsequently evaluated. A total of 13 modules were identified (Figure 1F-G),  
209 including the dark green, orange, purple, brown, midnight blue, black, dark magenta, light cyan,  
210 tan, dark gray, dark orange, light green, and gray modules. Pearson correlation analysis showed that  
211 the midnight blue module was most strongly correlated with the clinical phenotype ( $r = 0.61$ ,  $p <$   
212  $0.05$ ), suggesting that this module may be closely involved in disease progression.

## 213 **1.4. Screening and Identification of ABLIM3**

214 To identify key features associated with disease classification and evaluate model  
215 performance, a preliminary random forest model was first constructed using the randomForest  
216 package in R. The model comprised 500 decision trees generated under the default parameter  
217 settings. The out-of-bag (OOB) error rate was 5.51%. The OOB-based confusion matrix showed  
218 classification error rates of 11.9% for the Control group and 2.4% for the IBD group, indicating  
219 good preliminary discriminatory performance. Subsequently, Boruta feature selection was  
220 performed to further identify robust disease-related feature genes, and a total of 120 genes were  
221 confirmed as important features (Figure 1H). Their expression distributions in the normal and IBD  
222 groups were visualized using boxplots (Figure 1I). To further assess model stability and  
223 generalizability, repeated 10-fold cross-validation was performed five times for parameter  
224 optimization and final model fitting based on the Boruta-selected feature set. The results showed  
225 that model accuracy varied across different mtry values, with the best classification performance  
226 achieved at mtry = 4; this value was therefore selected for construction of the final model. Based on  
227 the cross-validated results, a receiver operating characteristic (ROC) curve was generated, and an  
228 optimal threshold of 0.236 was selected to construct the confusion matrix for evaluating prediction  
229 performance. The predictive accuracy was significantly higher than the no-information rate (p-value  
230  $[Acc > NIR] < 0.05$ ), and the area under the curve (AUC) reached 0.99, indicating excellent  
231 classification performance (Figure 1J-L).

232 Finally, by intersecting the results of differential expression analysis, WGCNA-based co-  
233 expression analysis, and random forest feature selection, ABLIM3 was identified as a candidate  
234 IBD-related gene, providing a basis for subsequent experimental validation in the DSS-induced  
235 colitis model (Figure 1M).

## 236 **2. Expression Characteristics of Ablim3 in the DSS-Induced Murine Colitis Model**

### 237 **2.1. Establishment and Evaluation of the DSS-Induced Murine Colitis Model**

238 After one week of acclimatization, an acute colitis model was established in mice using DSS  
239 (Figure 2A). Compared with controls, DSS-treated mice showed significantly greater body weight  
240 loss, increased DAI scores, and colon shortening (Figure 2B-E,  $p < 0.01$ ). HE staining revealed  
241 marked mucosal injury, including crypt disruption and inflammatory cell infiltration.  
242 Immunohistochemical analysis further showed reduced Occludin and ZO-1 expression in DSS-  
243 treated colonic tissues (Figure 2F-H,  $p < 0.01$ ), indicating intestinal barrier impairment. These  
244 findings confirmed successful establishment of the DSS-induced acute colitis model.

## 245 **2.2. Expression of Ablim3 Protein in Colonic Tissue**

246 Western blot analysis showed that, compared with the control group, Ablim3 protein  
247 expression was significantly increased in the colonic tissues of DSS-treated mice, and densitometric  
248 analysis confirmed that this difference was statistically significant (Figure 3A-B).  
249 Immunofluorescence staining further demonstrated enhanced Ablim3 expression in the DSS group  
250 (Figure 3C). Semiquantitative analysis of immunofluorescence intensity further confirmed a  
251 significant increase in Ablim3 fluorescence in the DSS group compared with the control group  
252 (Figure 3D).

## 253 **3. Effects of Ablim3 Knockdown on DSS-Induced Colitis**

### 254 **3.1. Establishment of the AAV-Mediated Ablim3 Knockdown Model**

255 Our previous results showed that Ablim3 expression was significantly upregulated in mice  
256 with DSS-induced colitis. To further investigate its role in intestinal inflammation, an in vivo  
257 Ablim3 knockdown model was established by enema-based delivery of AAV-mediated shRNA,  
258 enabling localized gene intervention in the colon (Figure 4A). Representative fluorescence images  
259 of colonic tissues showed clear green fluorescence signals after AAV administration, indicating  
260 successful local transduction of the viral vector in the colon (Figure 4B).

### 261 **3.2. Ablim3 Knockdown Attenuates DSS-Induced Colonic Injury in Mice**

262 After confirming successful local AAV transduction in colonic tissues, a DSS-induced colitis  
263 model was established using four groups: Sham, DSS, DSS + AAV-shNC, and DSS + AAV-  
264 shAblim3 (Figure 5A). Disease severity was comprehensively evaluated based on gross colonic  
265 morphology, colon length, percentage body weight change, and disease activity index (DAI) score.  
266 Compared with the Sham group, DSS-treated mice exhibited marked body weight loss accompanied  
267 by typical manifestations such as diarrhea and bloody stool, indicating successful model  
268 establishment. Further analysis showed that both the DSS group and the DSS + AAV-shNC group  
269 displayed significant body weight loss and marked colon shortening compared with the Sham  
270 group. At the early stage of DSS exposure, no obvious differences in body weight change or DAI  
271 score were observed between the DSS + AAV-shNC group and the DSS + AAV-shAblim3 group.  
272 However, as colitis progressed, mice in the DSS + AAV-shAblim3 group showed a markedly  
273 attenuated decrease in body weight and significantly less colon shortening (Figure 5B-D).  
274 Hematoxylin-eosin (HE) staining revealed pronounced mucosal injury, inflammatory cell  
275 infiltration, and glandular destruction in DSS-treated mice, whereas these histopathological changes  
276 were clearly alleviated in the Ablim3 knockdown group, with better preservation of overall colonic  
277 architecture (Figure 5E). Together, these findings suggest that downregulation of Ablim3 can  
278 alleviate DSS-induced colonic injury to some extent.

279 Western blot analysis with semiquantitative densitometry was further used to assess Ablim3  
280 protein expression in colonic tissues from each group. Under Sham conditions, Ablim3 expression  
281 did not differ significantly between the NO AAV group and the AAV-shNC group, whereas the  
282 AAV-shAblim3 group showed significantly lower Ablim3 protein levels than both control groups.  
283 Under DSS stimulation, Ablim3 protein expression remained similar between the DSS group and  
284 the DSS + AAV-shNC group, but was significantly reduced in the DSS + AAV-shAblim3 group  
285 compared with both groups. These results indicate that AAV-shAblim3 effectively downregulated

286 Ablim3 protein expression in colonic tissues and attenuated the DSS-associated upregulation of  
287 Ablim3 (Figure 5F-G). Combined with the phenotypic and histopathological improvements  
288 observed after knockdown, these findings further support a contributory role of Ablim3 in DSS-  
289 induced colonic injury. Immunohistochemistry and immunofluorescence analyses yielded findings  
290 consistent with the Western blot results, further confirming increased Ablim3 expression in DSS-  
291 injured colonic tissues and its reduction after AAV-shAblim3 intervention. Semiquantitative  
292 analyses supported the statistical significance of these differences (Figure 5H-K).

293 Immunohistochemical staining showed that ZO-1 expression was reduced in the DSS and DSS  
294 + AAV-shNC groups compared with the Sham group, but partially restored in the DSS + AAV-  
295 shAblim3 group. Semiquantitative analysis confirmed that Ablim3 knockdown partially reversed  
296 the DSS-induced decrease in ZO-1 expression, suggesting alleviation of colonic epithelial barrier  
297 injury (Figure 5L-M).

298 Collectively, these results indicate that AAV-mediated Ablim3 knockdown partially alleviated  
299 DSS-induced colonic injury, as reflected by reduced body weight loss, improved colon length, and  
300 attenuated histopathological inflammation, suggesting a functional association between Ablim3 and  
301 colitis progression.

## 302 **4. Preliminary Exploration of the Potential Mechanism of Ablim3**

### 303 **4.1. Screening of Candidate Ablim3-Interacting Proteins Based on Co-IP/MS**

304 To explore potential Ablim3-associated proteins in murine colitis, Co-IP combined with LC-  
305 MS/MS was performed. After removal of nonspecific background proteins detected in the IgG  
306 control, 677 IP-specific proteins were identified (Figure 6A). Further filtering based on PSMs,  
307 unique peptides, and Sequest HT score retained 42 high-confidence candidate proteins. Integrated  
308 ranking using Z-score-standardized metrics identified the top 25 candidates, which were visualized  
309 in a bar plot (Figure 6B). These candidates were mainly associated with cytoskeletal dynamics, cell-

cell adhesion, and inflammation-related processes, providing preliminary clues for the potential involvement of Ablim3 in intestinal inflammation.

#### **4.2. Functional Enrichment Analysis of Candidate Interacting Proteins Based on GO and KEGG**

To investigate the potential functions of the candidate interacting proteins, GO and KEGG enrichment analyses were performed. All 42 candidate proteins were successfully converted for ID mapping, among which 41 proteins had valid annotations in the GO-BP database. GO-BP analysis yielded 10 significantly enriched terms, which were highly concentrated in actin filament-related dynamic regulatory processes, including regulation of actin filament-based processes, regulation of actin polymerization or depolymerization, regulation of actin filament length, actin filament bundle assembly, and regulation of actin cytoskeleton organization (Figure 6C). By contrast, KEGG analysis did not identify any classical signaling pathway reaching the predefined significance threshold.

#### **4.3. PPI Network Analysis of Candidate Interacting Proteins**

The PPI network constructed using the STRING database (version 12.0) showed that the candidate interacting protein network contained 42 nodes and 12 interaction edges, with an average node degree of 0.571 and a local clustering coefficient of 0.214 (Figure 6D). The observed number of interaction edges was significantly higher than the expected number under random conditions (3 edges; PPI enrichment  $p = 9.89 \times 10^{-5}$ ), indicating significant non-random biological connectivity within this protein set. Further interpretation of the network visualization and edge table suggested that, although the overall network was relatively sparse, several locally connected interaction units could still be identified. Among them, Afdn, Lima1, Dbn1, Eps8, and Baiap2l1 formed a core region closely related to cell-cell junctions and actin cytoskeleton regulation; Arhgap17, Atxn2l, G3bp2, Pkp1, and Igf2bp2 formed another local interaction cluster; in addition, independent local

334 interactions were observed among Apobec2, Myl1, Casq2, and Smyd1, as well as between Pnp and  
335 Sh3glb2 (Figure 6E).

336

## 337 **Discussion**

338 In this study, we combined peripheral transcriptomic screening, animal-model validation, and  
339 in vivo gene intervention to evaluate the potential role of Ablim3 in IBD. ABLIM3 was identified  
340 from the GSE3365 dataset through integrative bioinformatics analysis, and Ablim3 was confirmed  
341 to be upregulated in colonic tissues of DSS-treated mice. Local AAV-shRNA-mediated Ablim3  
342 knockdown alleviated body weight loss, colon shortening, and histopathological injury, suggesting  
343 that Ablim3 may be functionally involved in colitic mucosal injury.

344 These findings also support a potential link between Ablim3 and epithelial structural injury in  
345 intestinal inflammation. Mucosal injury in IBD is shaped not only by immune activation but also by  
346 alterations in epithelial barrier integrity and cytoskeletal organization [10,29–32]. As an actin-  
347 binding LIM family member, Ablim3 contains domains compatible with protein-protein interaction  
348 and F-actin binding, and previous work has identified it as a component of adherens-junction-  
349 associated complexes [33–36]. Given the dynamic coupling between junctional complexes and the  
350 cortical actin cytoskeleton, Ablim3 upregulation in DSS colitis and the improvement observed after  
351 knockdown suggest its possible involvement in inflammation-related junction-actin remodeling.

352 The Co-IP/MS results provided preliminary clues supporting this interpretation. Candidate  
353 Ablim3-associated proteins were enriched in processes related to actin filament dynamics,  
354 cytoskeletal remodeling, and cell-cell junction organization. PPI analysis further identified locally  
355 connected proteins involved in adherens-junction organization, membrane-cytoskeleton coupling, or  
356 actin remodeling, such as Afdn, Lima1, Dbn1, Eps8, and Baiap211[37,38]. However, these findings  
357 should be interpreted cautiously, as they were based on exploratory proteomic screening and

358 descriptive network analysis rather than direct validation of specific interactions or signaling  
359 pathways.

360 Several limitations should be acknowledged. First, although partial transcriptional concordance  
361 between peripheral blood and colonic tissue has been reported in IBD, the two compartments are  
362 not equivalent[15,17]: ABLIM3 was identified from PBMC transcriptomic data, whereas its  
363 functional validation was performed in colonic tissue; therefore, the relationship between peripheral  
364 transcriptional alterations and local intestinal pathology requires further clarification. Second, the  
365 current findings are mainly based on a DSS-induced murine colitis model, and direct evidence from  
366 human IBD mucosal samples is still lacking. Future studies using clinical cohorts and diseased  
367 intestinal tissues are needed to confirm the clinical relevance of ABLIM3 in IBD.

368 At the intervention level, although prior studies have supported the feasibility of AAV-  
369 mediated local intestinal gene delivery in experimental colitis[26,39], the present AAV-shRNA  
370 strategy still has limitations. Only a single-dose intervention was used, and knockdown efficiency  
371 was mainly evaluated at the protein level without corresponding mRNA validation. Future studies  
372 should include dose-response assessment, qPCR validation, and additional independent shRNA  
373 sequences to better evaluate knockdown specificity and potential off-target effects[40–42]. In  
374 addition, although enema administration enabled relatively localized delivery, the AAV2/9 vector  
375 and broadly active regulatory elements used here did not confer strict colon specificity. Therefore,  
376 this approach should be interpreted as local intervention rather than tissue-specific targeting, and  
377 future work should incorporate multi-organ tracing, viral genome quantification, and tissue-specific  
378 promoters to improve targeting precision and systemic safety. Finally, Co-IP/MS provided only a  
379 static and exploratory interaction profile, which cannot distinguish direct binding from indirect co-  
380 precipitation. Reciprocal Co-IP, colocalization assays, and domain-level validation are needed to  
381 confirm key interactions and refine the underlying mechanism.

382 In summary, this study suggests that Ablim3 may be functionally involved in DSS-induced  
383 colitic injury, potentially in association with cytoskeletal dynamics and junction-related protein  
384 networks. The protective effect observed after local Ablim3 knockdown supports further  
385 investigation of Ablim3 as a candidate molecule involved in barrier-related injury in IBD.

## 386 **Conclusions**

387 This study provides functional evidence linking ABLIM3/Ablim3 to the progres-sion of  
388 experimental colitis. By integrating peripheral transcriptomic screening with localized in vivo  
389 intervention, we found that Ablim3 expression was upregulated in colonic tissues during DSS-  
390 induced murine colitis. Local AAV-mediated Ablim3 knockdown partially preserved epithelial  
391 barrier integrity, alleviated histopathological injury, and reduced overall disease severity.  
392 Furthermore, Co-IP/MS-based proteomic profiling provided preliminary mechanistic clues  
393 suggesting that Ablim3 may be asso-ciated with cytoskeletal dynamics and intercellular adhesion-  
394 related pathways. Col-lectively, these findings suggest that Ablim3 is not merely a disease-  
395 associated molecule, but may be functionally involved in gut barrier dysfunction, supporting its  
396 further in-vestigation as a potential candidate for barrier-restoring strategies in IBD.

## 397 **List of abbreviations**

|              |                                                                    |
|--------------|--------------------------------------------------------------------|
| AAV          | Adeno-Associated Virus                                             |
| AAV-shAblim3 | Adeno-Associated Virus Carrying Ablim3 Short Hairpin RNA           |
| AAV-shNC     | Adeno-Associated Virus Carrying Negative Control Short Hairpin RNA |
| ABLIM3       | Actin-Binding LIM Protein Family Member 3                          |
| Acc          | Accuracy                                                           |
| AJC          | Apical Junctional Complex                                          |
| AJs          | Adherens Junctions                                                 |
| AUC          | Area Under Curve                                                   |

|       |                                              |
|-------|----------------------------------------------|
| BP    | Biological Process                           |
| DAI   | Disease Activity Index                       |
| DEG   | Differential Expression Gene                 |
| DSS   | Dextran Sulfate Sodium                       |
| GO    | Gene Ontology                                |
| IBD   | Inflammatory Bowel Disease                   |
| IEB   | Intestinal Epithelial Barrier                |
| KEGG  | Kyoto Encyclopedia Of Genes And Genomes      |
| ME    | Module Eigengene                             |
| NIR   | No Information Rate                          |
| OOB   | Out-Of-Bag                                   |
| PBMCs | Peripheral Blood Mononuclear Cells           |
| PCA   | Principal Component Analysis                 |
| ROC   | Receiver Operating Characteristic            |
| scAAV | Self-Complementary Adeno-Associated Virus    |
| TJs   | Tight Junctions                              |
| WB    | Western Blotting                             |
| WGCNA | Weighted Gene Co-Expression Network Analysis |

398

## 399 **Declarations**

## 400 **Availability of data and materials**

401 The dataset analyzed in this study is publicly available in the Gene Expression Omnibus (GEO)  
402 database under accession number GSE3365. The datasets generated and/or analyzed during the  
403 current study are available from the corresponding author on reasonable request.

## 404 **Competing interests**

405 The authors declare that they have no competing interests.

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#### 410 **Authors' contributions**

411 Y.L.: Conceptualization.

412 M.W.: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data  
413 curation, Writing – original draft, Visualization.

414 L.L.: Writing – review & editing, Supervision, Project administration, Funding acquisition. All  
415 authors have read and approved the final manuscript.

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418

#### 419 **Author's information** (optional)

420 Not applicable.

421

#### 422 **Footnotes**

423 Not applicable.

424

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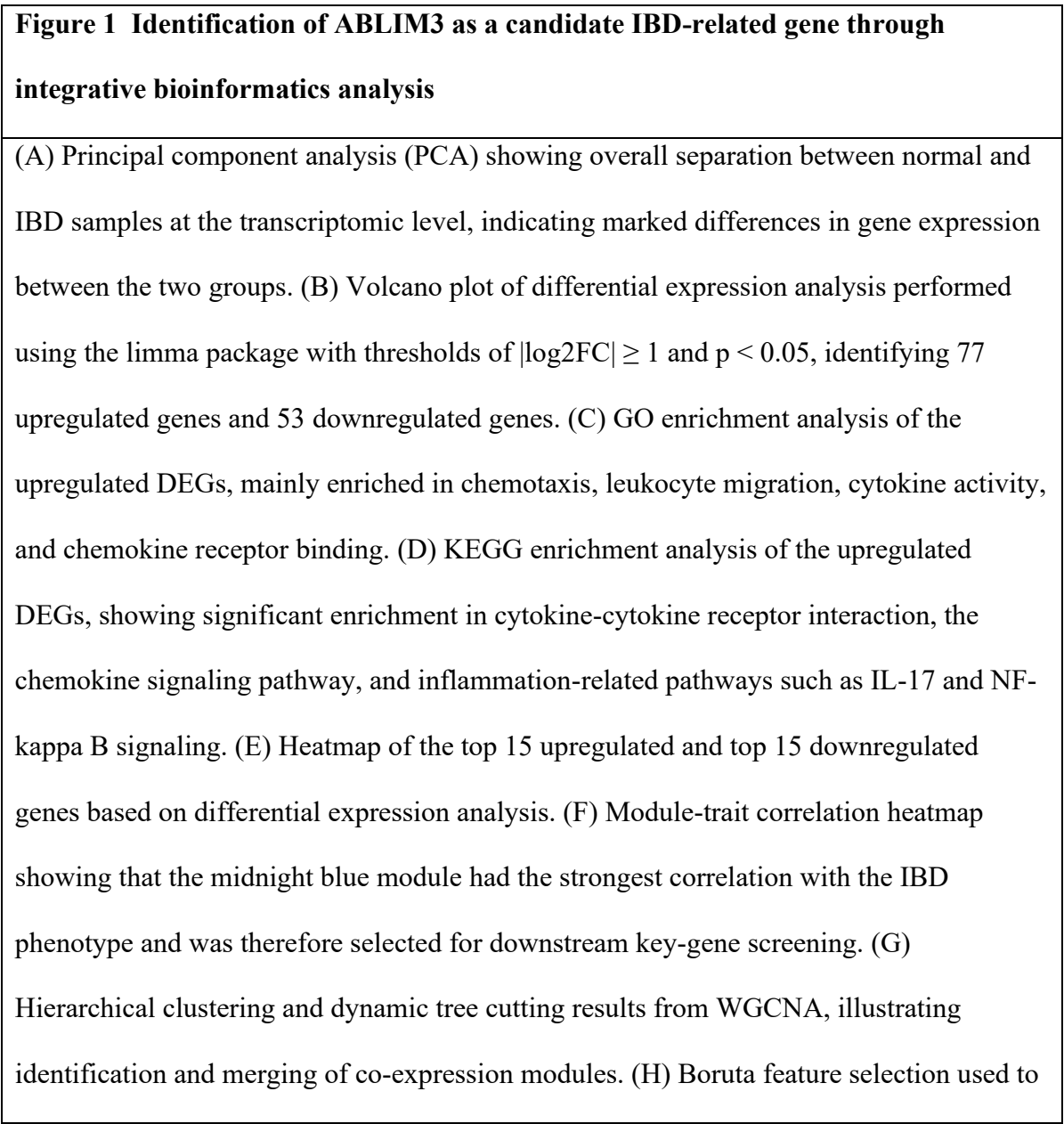
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548

549 **Figure legends**



identify variables closely associated with IBD classification. (I) Boxplot visualization of expression levels for the final key gene in the normal and IBD groups. (J) Model accuracy under different numbers of randomly selected features, used to determine an optimal feature-parameter range. (K) Importance ranking of candidate genes based on the random forest model, showing the contribution of each feature to classification performance. (L) Receiver operating characteristic (ROC) curve assessing diagnostic performance of the model and demonstrating high classification accuracy. (M) Intersection of differential expression analysis (DEGs), WGCNA, and random forest (RF) feature selection, ultimately identifying ABLIM3 as the key gene.

550

## **Figure 2 Establishment and Phenotypic Characteristics of the DSS-Induced Murine Colitis Model**

(A) Schematic workflow for establishment of the DSS-induced murine colitis model. (B) Representative gross images of the colon from each group. (C) Percentage body weight change after DSS treatment, showing progressive weight loss in the DSS group during model induction. (D) Changes in disease activity index (DAI) scores, showing progressive elevation in the DSS group during model induction. (E) Statistical comparison of colon length between the control and DSS groups, showing marked colon shortening in the DSS group. (F) Representative HE staining images and immunohistochemical staining images for the intestinal barrier-related proteins ZO-1 and Occludin in colon tissues from the control and DSS groups. (G) Semiquantitative analysis of Occludin immunohistochemical staining. (H) Semiquantitative analysis of ZO-1 immunohistochemical staining. All data are presented as mean  $\pm$  SD. Compared with the control group, \*\* $p < 0.01$  and \*\*\* $p <$

0.001 indicate statistical significance.

551

**Figure 3 Expression and Distribution of Ablim3 Protein in Colonic Tissue After DSS Treatment**

(A) Representative Western blot images showing Ablim3 protein expression. (B) Densitometric quantification of Ablim3 protein expression, showing significant upregulation in the DSS group compared with the control group. (C) Representative immunofluorescence images of Ablim3 in mouse colonic tissue, showing increased expression in the DSS group. (D) Semiquantitative analysis of Ablim3 immunofluorescence intensity, showing significantly increased fluorescence signal in the DSS group compared with the control group. All data are presented as mean  $\pm$  SD. Compared with the control group, \*\* $p < 0.01$  and \*\*\* $p < 0.001$  indicate statistical significance.

552

**Figure 4 Construction of the AAV-Mediated Ablim3 Knockdown Model and Representative Fluorescence Images of Colonic Transduction**

(A) Schematic diagram of the AAV-mediated Ablim3 knockdown model. (B) Representative fluorescence images showing AAV transduction in colonic tissue in the AAV-shNC and AAV-shAblim3 groups.

553

**Figure 5 Effects of Ablim3 Knockdown on DSS-Induced Colonic Injury and Expression of Related Proteins**

(A) Schematic workflow of Ablim3 knockdown intervention and DSS-induced colitis model establishment (Sham group, DSS group, DSS + AAV-shNC group, and DSS +

AAV-shAblim3 group). (B) Representative gross images of the colon from each group. (C) Statistical analysis of colon length in each group. (D) Percentage body weight change and disease activity index (DAI) score dynamics in each group. (E) Representative HE staining images of colonic tissues from each group. (F) Representative Western blot images of related protein expression in colonic tissues from each group. (G) Semiquantitative densitometric analysis of Ablim3 protein expression in each group. (H) Representative immunohistochemical staining images of Ablim3 in colonic tissues from each group. (I) Semiquantitative analysis of Ablim3 immunohistochemical staining. (J) Representative immunofluorescence images of colonic tissues from each group. (K) Semiquantitative analysis of Ablim3 immunofluorescence intensity. (L) Representative immunohistochemical staining images of ZO-1 in colonic tissues from each group. (M) Semiquantitative analysis of ZO-1 immunohistochemical staining. All data are presented as mean  $\pm$  SD. Colon length was analyzed by one-way ANOVA. The dynamic changes in body weight and DAI scores were analyzed by two-way repeated-measures ANOVA. Semiquantitative analysis of Ablim3 protein gray values was performed by two-way ANOVA, whereas semiquantitative analyses of Ablim3 and ZO-1 immunohistochemistry were performed by one-way ANOVA. Statistical significance among groups is indicated as \* $p < 0.05$ , \*\* $p < 0.01$ , and \*\*\* $p < 0.001$ .

554

**Figure 6 Functional Enrichment and PPI Network Analysis of Candidate Ablim3-Interacting Proteins**

(A) Venn diagram comparing protein identification results between the IgG group and the IP group. (B) Composite confidence scores calculated from PSMs, Unique

Peptides, and Sequest HT Score after Z-score standardization, equal-weight integration, and rescaling to the 0-1 range. The figure shows the top 25 candidate proteins ranked by composite score, where bar height represents overall confidence and the color gradient reflects protein abundance (PSMs). (C) Bubble plot of significantly enriched GO-BP terms for the candidate interacting proteins. (D) Main topological parameters of the candidate interacting protein PPI network, including node number, edge number, expected edge number, average node degree, average local clustering coefficient, and PPI enrichment p value. (E) Candidate interacting protein PPI network constructed using the STRING database (version 12.0), showing relatively concentrated connections among selected protein nodes.

555

## 556 Tables

| Table.STRING PPI network statistics |                       |
|-------------------------------------|-----------------------|
| Parameter                           | Value                 |
| Number of nodes                     | 42                    |
| Number of edges                     | 12                    |
| Expected number of edges            | 3                     |
| Average node degree                 | 0.571                 |
| Avg. local clustering coefficient   | 0.214                 |
| PPI enrichment p-value              | $9.89 \times 10^{-5}$ |

557

## 558 Additional file legends

559 Additional file 1:

560 File format: XLSX;

561 Title: Antibody information and Co-IP/MS data

562 Description: Table S1 provides detailed information on the primary and secondary antibodies  
563 used in this study. Table S2 provides the complete list of proteins uniquely identified in the  
564 ABLIM3 immunoprecipitation group but not detected in the IgG control group.

565 Additional file 2:

566 File format: PDF;

567 Title: Uncropped Western blot images;

568 Description: Uncropped and unprocessed Western blot images corresponding to the Western  
569 blot results presented in the main figures. The relevant lanes and target proteins are indicated.

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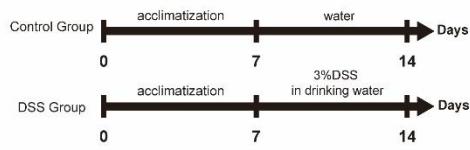
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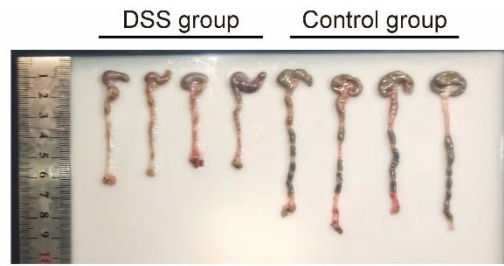
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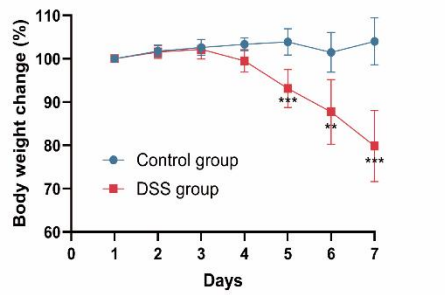
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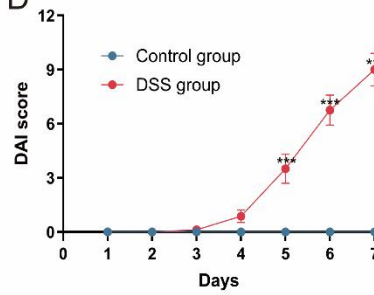
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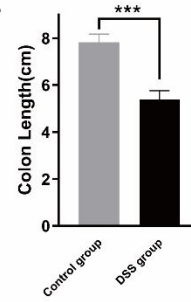
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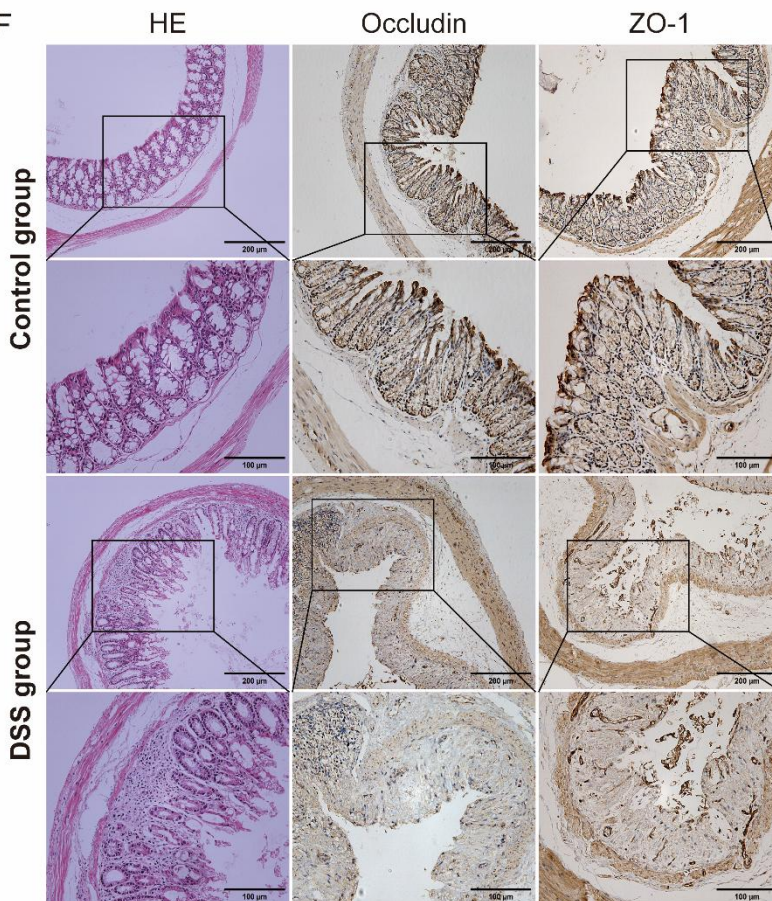
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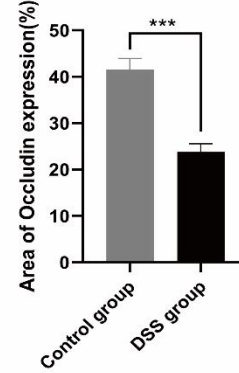
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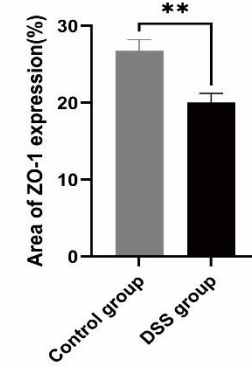
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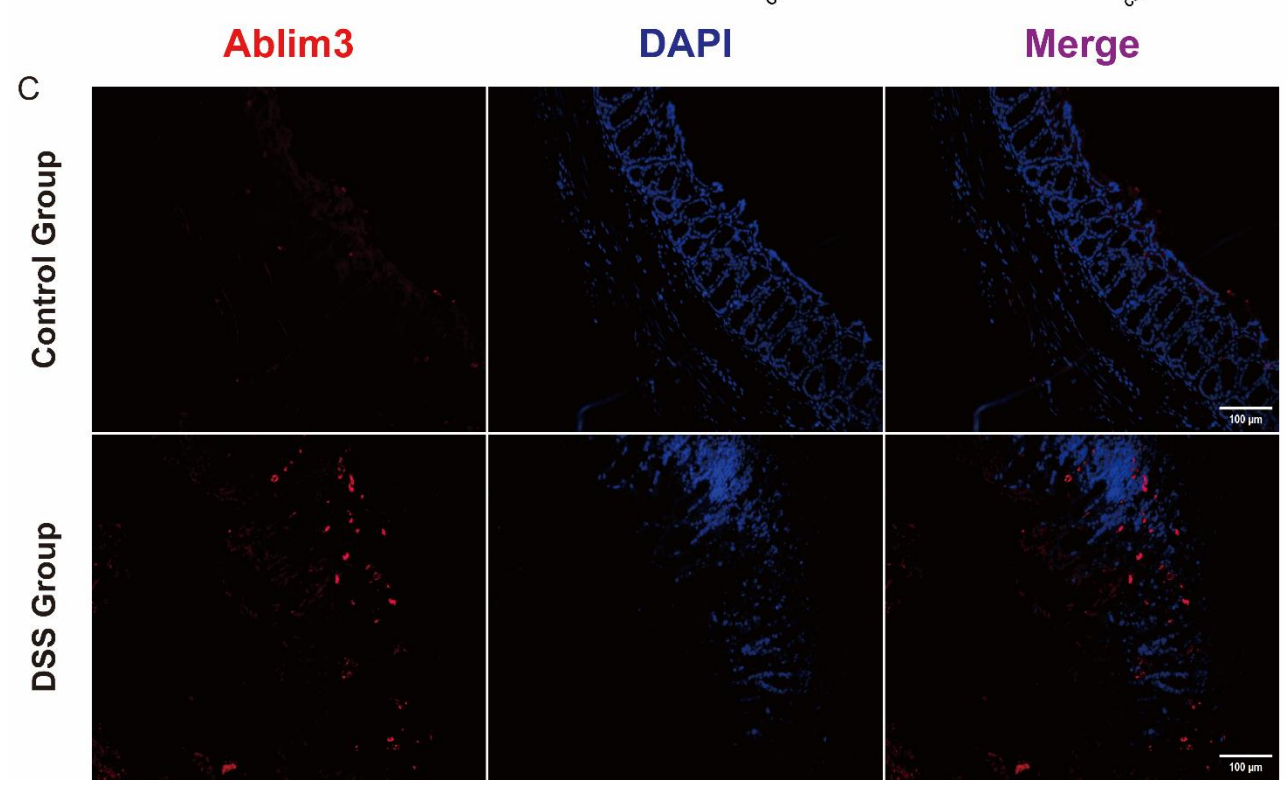
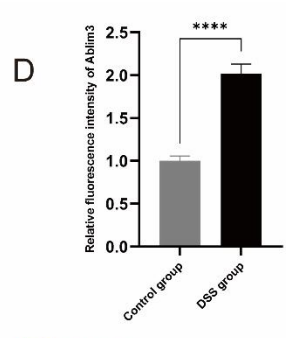
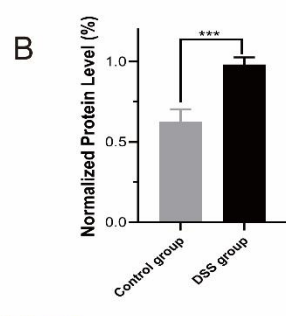
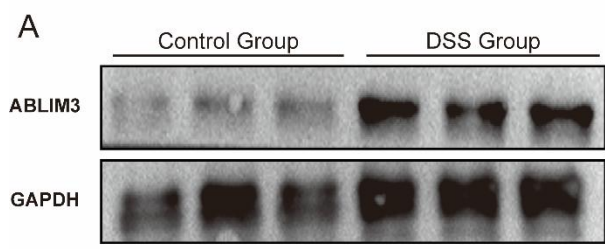
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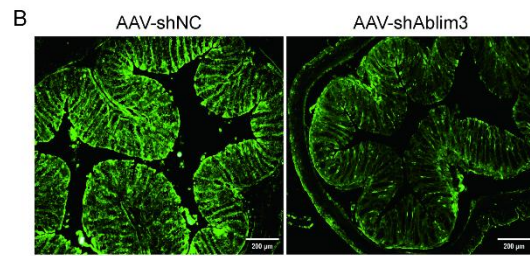
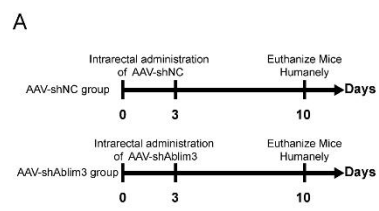
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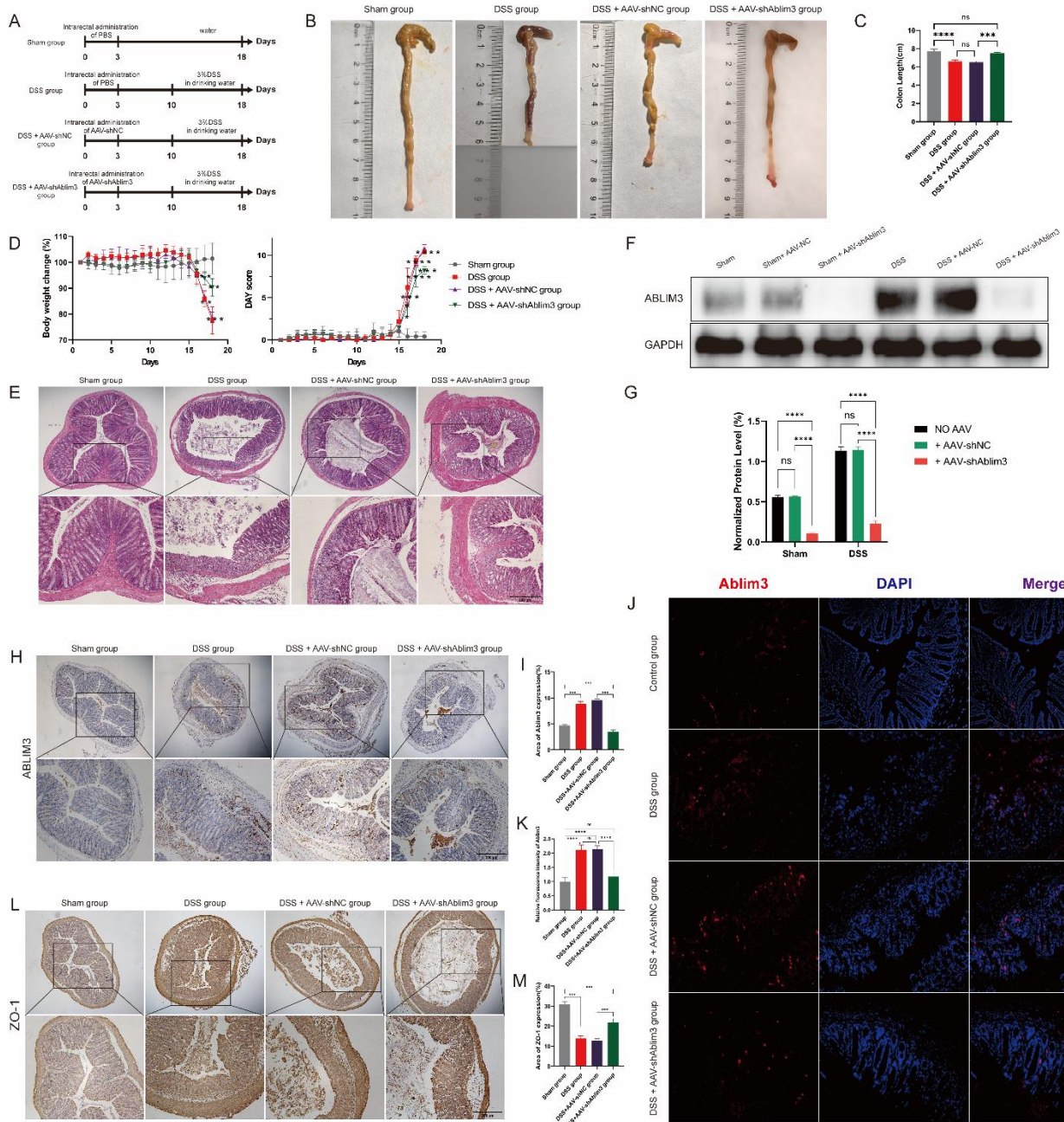
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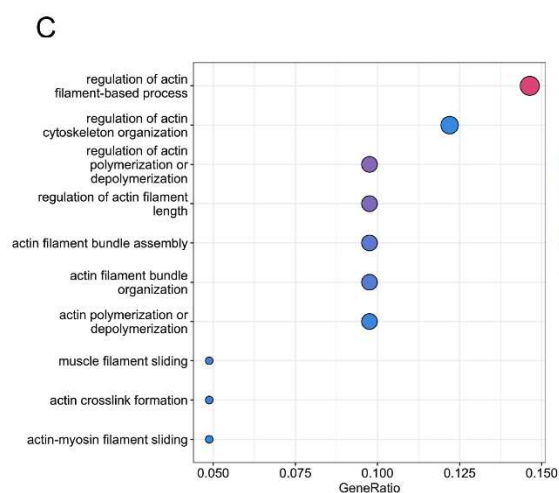
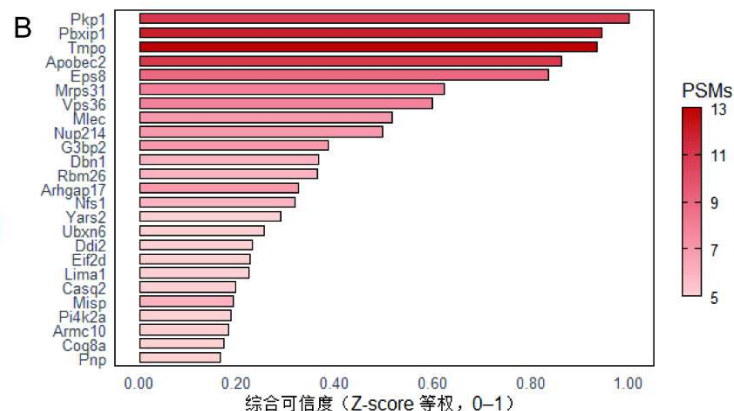
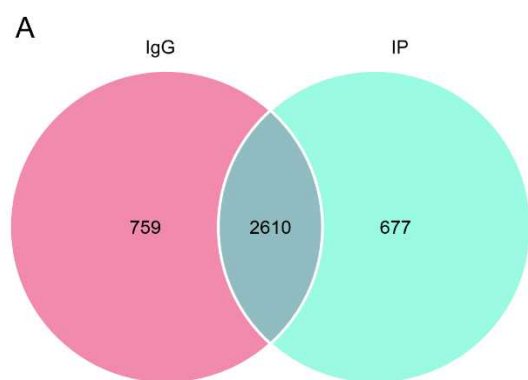
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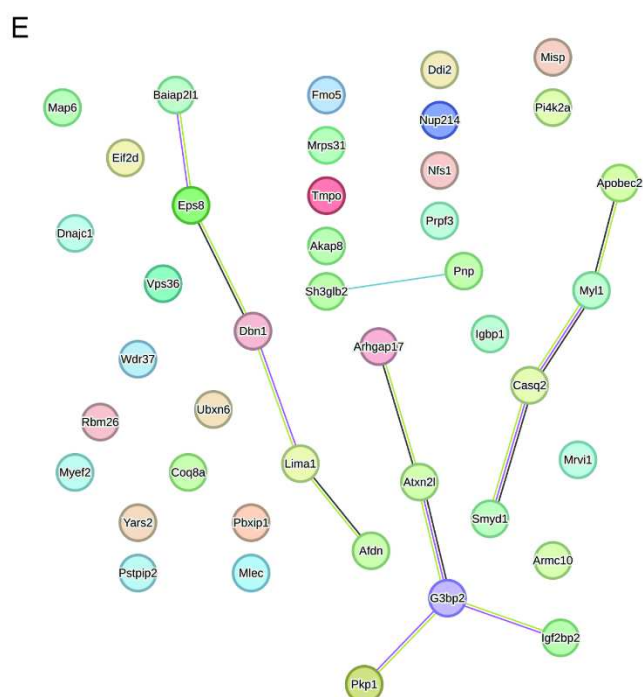
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**D**

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| PPI enrichment p-value              | $9.89 \times 10^{-4}$ |



## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table.xlsx](#)
- [Additionalfile1.xlsx](#)
- [Additionalfile2.pdf](#)