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Gelsolin regulates intestinal stem cell regeneration and Th17 cellular function

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Abstract

Intestinal stem cells (ISCs) are responsible for intestinal homeostasis and are important for the regeneration of damaged intestine. We established an ionizing radiation (IR)-induced intestinal injury model and observed that Gelsolin KO mice had increased radiosensitivity. The deletion of Gelsolin aggravated intestinal damage and reduced the number of ISCs after lethal IR. The intestinal organoid experiments showed that Gelsolin deletion inhibited ISCs function after IR. Notably, RNA sequencing and RT-PCR results showed IL-17 signaling pathway was down-regulated and Th17 cells differentiation was inhibited in Gelsolin KO mice. Moreover, recombinant IL-17 A ameliorated IR-induced intestinal injury and promoted ISCs regeneration. To figure out the role of Gelsolin in Th17 cells differentiation, flow cytometry was used and we found that Gelsolin targets Th17 cells functionality *via* the p-STAT3/RORγt axis. By establishing the co-culture system, we proved that Th17 cells promoted self-renewal and budding abilities in Gelsolin-deficient organoids. Finally, we found that Gelsolin was protective against DSS-induced colitis and that this protective effect was not specific or limited to the IR induced intestinal injury model. Based on these results, we proved Gelsolin maintained the regeneration of ISCs by sustaining Th17 cells functions *via* the p-STAT3/RORγt axis.

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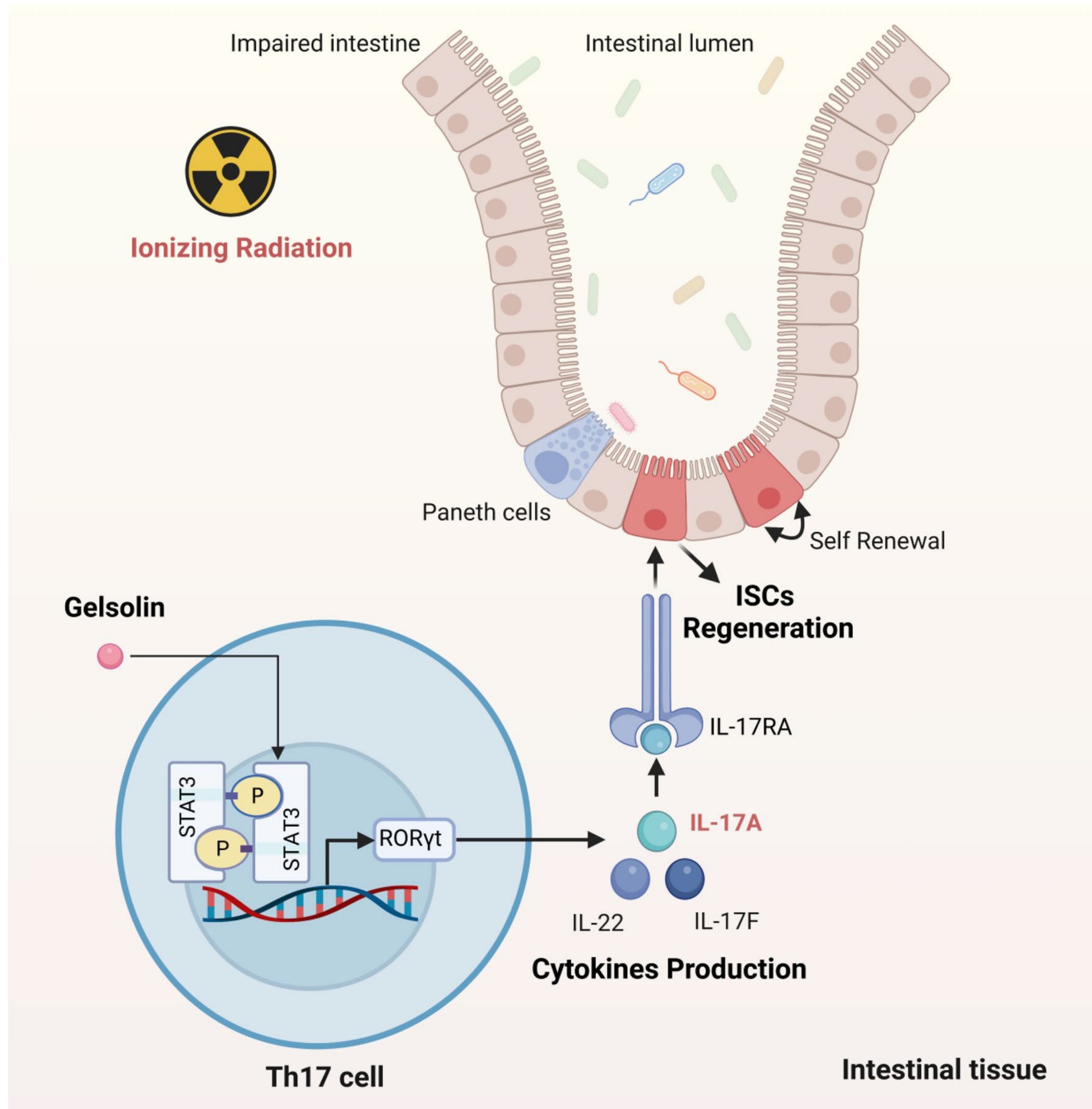
Graphical abstract



Keywords IR induced intestinal injury, Intestinal stem cells (ISCs), Gelsolin (Gelsolin), IL-17 signaling pathway, T helper cells 17 (Th17 cells)

Introduction

The intestinal epithelium maintains the intestine to achieve the functions such as absorption, secretion, and barrier function [1]. The intestine is renewed approximately every 5 days and this renewal is controlled by ISCs [2, 3]. As the fundamental source of intestinal regeneration, ISCs are essential for intestinal regeneration after injury [4, 5]. ISCs, marked by several markers such as Lgr5 and OLFM4, are located at the base of the crypts and interspersed with Paneth cells [6]. ISC repairs damaged intestinal epithelial rapidly and efficiently when infected with pathogens, as well as damage caused by IR [5]. This reaction can occur during pathogen infection and IR exposure [7].

Under physiological conditions, ISCs interact with lymphocytes, myeloid cells, epithelial cells, and nerve cells to synergistically regulate intestinal homeostasis [8, 9]. In combination with scRNA-seq, knockout mice, and intestinal organoids technology, Wu et al. identified MRISCs, a critical component of ISCs niche, specifically depends on MAP3K2 to regulate WNT signaling for the intestinal epithelium regeneration, providing proofs for intestinal

disease treatment [10]. By using scRNA-seq, Biton et al. identified the function of MHC class II (MHCII) in Lgr5+ ISCs, and proved that the stimulation of cytokines could regulate Lgr5+ ISCs functions in diametrically opposing ways: pro-inflammatory cytokines facilitated differentiation, while regulatory cytokines and cells inhibited it [11].

As a multifunctional protein, Gelsolin (Gelsolin) can regulate the function of actin by cleavage, capping, and nucleation [12]. Gelsolin plays critical roles in biological processes such as cell fate, inflammatory response, and tumor immunity [13]. Giampazolias et al. reported that sGelsolin could inhibit DNGR-1 binding to F-actin and repress DNGR-1-dependent cross-presentation of dead cell-associated antigens, suggesting that Gelsolin could be a novel target for tumor immunotherapy [13]. Asare-Werehene et al. found that Gelsolin could inhibit CD8+ T cell function and prompt glutathione mediated cisplatin resistance in ovarian cancer [14]. Recent research has confirmed the function of Gelsolin in intestinal inflammation [15]. Roy et al. constructed Villin-1 and Gelsolin double KO mice and found that Villin-1 and Gelsolin could affect intestinal functions and intestinal inflammatory response by regulating Actin [12].

In this work, we found that Gelsolin played a critical role in IR-induced intestinal injury in vivo and *in vitro.* Gelsolin could promote the regeneration of ISCs *via* the IL-17 signaling pathway. By establishing the co-culture system of intestinal organoids and Th17 cells, we found that Gelsolin may maintain the regeneration of ISCs by sustaining Th17 cells functions via the p-STAT3/RORγt axis.

Materials and methods

Additional materials and methods can be found in online supplemental materials.

Animals and treatment

C57BL/6 mice, male, 6–8 weeks old, were purchased from China Academy of Science (Shanghai, China). Gelsolin KO mice, male, 6–8 weeks old, were purchased from Viewsolid (Beijing, China). IL-17 A-EGFP mice were provided by Cyagen (Jiangsu, China). The mice were treated with Recombinant IL-17 A (50 μg/kg for 5 days before IR, dissolved in normal saline), Anti-mouse IL-17 A-InVivo (10 mg/kg, 24 and 2 h before IR), or PBS (200 μL/mice, 24 and 2 h before IR) *via* peritoneal injection. To establish acute colitis mice model, WT and Gelsolin KO mice were administered 2.5% (w/v) DSS (36000–50000 Da, dissolved in sterile water) in daily drinking for 10 days.

Irradiation

^{60}Co (Naval Medical University, China) was used to irradiate the mice and the intestinal organoids at room temperature. The mice were irradiated at 8.0 and 9.5 Gy to establish the Total Body Irradiation (TBI) model. In order to establish the Abdominal Irradiation (ABI) model, mice hind limbs were shielded with lead plate to avoid hematopoietic cell death before 18.0 Gy IR exposure.

RNA sequencing and functional enrichment analysis

At 24 h after 9.5 Gy IR, intestines from WT and Gelsolin KO mice were isolated for RNA-Seq (3:3) analysis. Total RNA from the intestines was extracted using Trizol (Invitrogen, USA), and its purity was assessed using NanoVue (GE, USA) to ensure A260:A280 > 1.8 and A260:A230 > 2.0. Subsequently, sequencing was carried out using the Illumina HiSeq 2500 system at Oebiotech, Shanghai, China. The raw data underwent filtration to generate high-quality clean data, which was then utilized for all subsequent analyses. Finally, the differentially expressed genes (DEGs) obtained were employed for generating heat maps and conducting functional enrichment analyses.

Intestinal organoid culture

The small intestine was extracted from the mice and rinsed with cold PBS. Then the small intestine was cut into 2 mm fragments and subsequently transferred to PBS solution containing 15 mM EDTA. Following an incubation period of 2 h at 4 ℃, the fragments were shaken and centrifuged with cold PBS 3 times (300 rpm/min, 5 min). Then isolated crypts were suspended in Matrigel and placed into 24-well plates (250 crypts/50 μL per well). Subsequently, 500 μL organoid culture medium was added into each well. Th17 cells were cultured with intestinal organoids at a ratio of 20:1 in Matrigel and 50:1 in the culture medium for co-cultured experiments (Fig. 1A). The intestinal organoids were irradiated 6 Gy at 24 h after inoculation, then were observed microscopically at day 7 post-irradiation. The relative surface area and budding rate of intestinal organoids were then analyzed using Image J (Bethesda, MD, USA).

Antibody staining and flow cytometry analysis

CD4+ T cells and Th17 cells were isolated following the method described previously. Then cells were added with various antibodies and stained for 20 min at 4℃ in the dark for flow cytometry analysis. The antibodies were used as follows: FITC anti-Hu/Mo Phospho-STAT3, FITC anti-Hu/Mo RORγt, PE anti-mouse IL-17 A, and FITC anti-mouse CD4. The mean fluorescence intensity (MFI) of IL-17 A, RORγt, and p-STAT3 were analyzed by flow cytometry (Beckman Cytoflex).

Results

Gelsolin is essential for basal radio-resistance in vivo

As a key intracellular regulator of actin filament dynamics, all cells product cytoplasmic Gelsolin [13]. Gelsolin

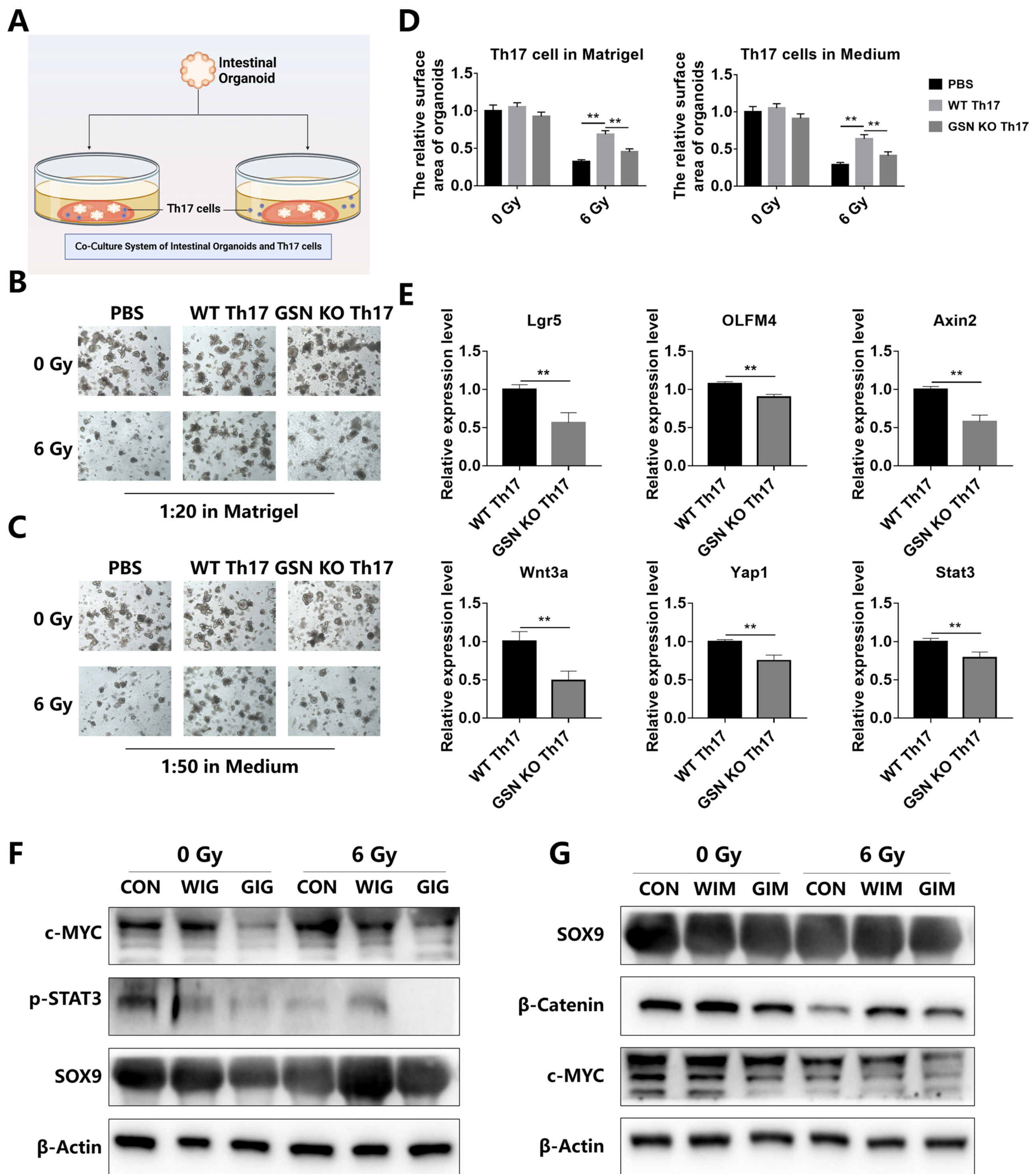


Fig. 1 The decreased formation efficiency of intestinal organoids from Gelsolin KO mice were rescued by Th17 cells co-culture. **(A)** The diagram of co-cultured experiments. **(B)** Th17 cells were co-cultured with organoids derived from WT mice and Gelsolin mice at a ratio of 20:1 in Matrigel and then exposed to 6 Gy IR. **(C)** Th17 cells were co-cultured with organoids derived from WT mice and Gelsolin mice at a ratio of 50:1 in culture medium and then were exposed to 6 Gy IR. **(D)** The relative surface area of intestinal organoids was analyzed and measured using Image J. **(E)** The RT-PCR of Lgr5, OLFM4, AXIN2, WNT3A, YAP1 and STAT3 using RNA extracted from the organoids. **F/G.** The expression levels of key ISCs regulators were detected by using WB. WIG: WT Th17 cells in Matrigel; GIG: Gelsolin KO Th17 cells in Matrigel; WIM: WT Th17 cells in in the culture medium; GIM: Gelsolin KO Th17 cells in the culture medium

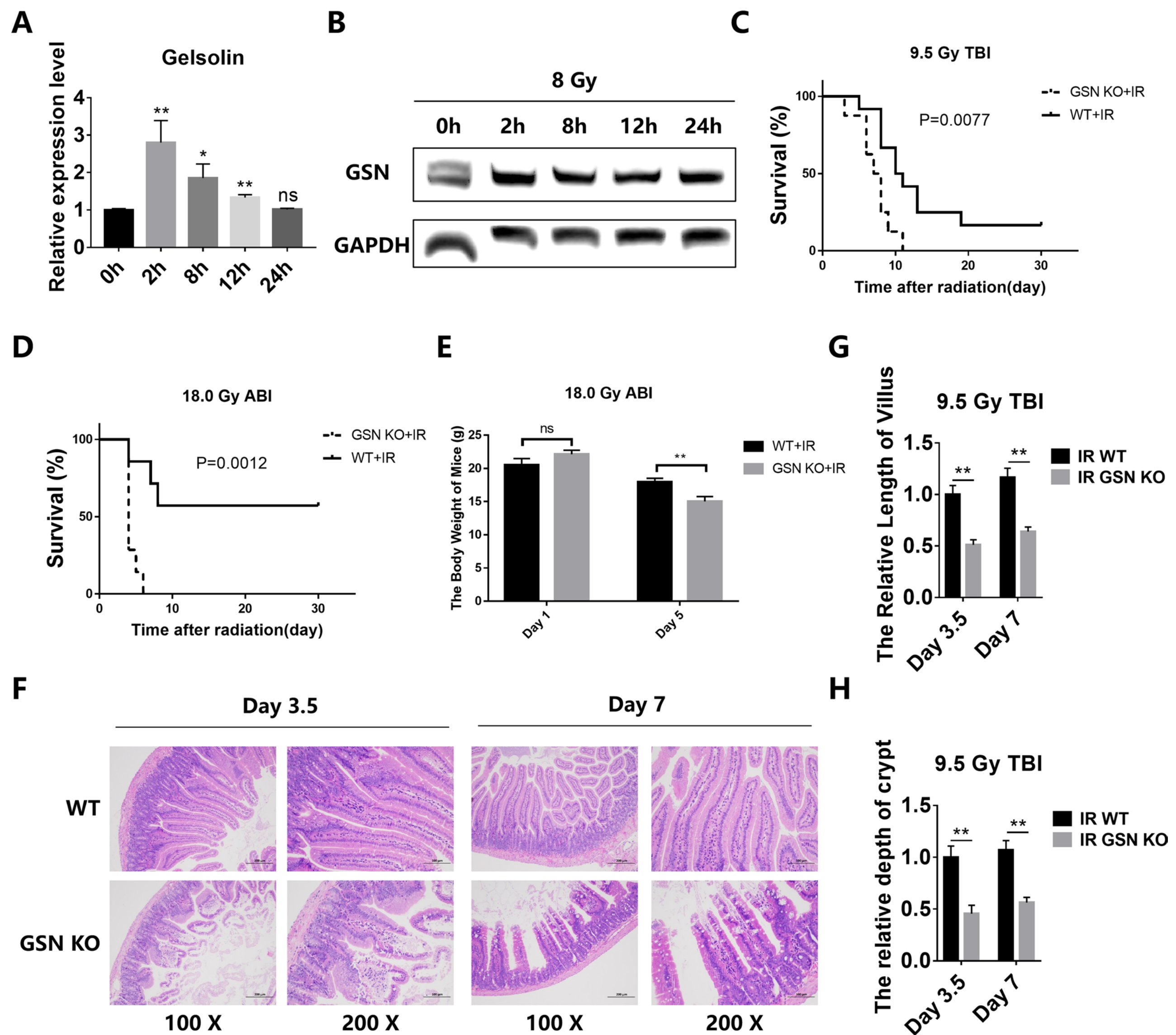


Fig. 2 Gelsolin is essential for basal radio-resistance in vivo. **(A)** WT mice were exposed to irradiation to establish TBI model. Total RNA was extracted from the intestine of irradiated WT mice at different time points after irradiation for PCR experiments. The expression level of Gelsolin was detected by using RT-PCR. **(B)** WT mice were exposed to irradiation to establish TBI model. Total protein was extracted from the intestine of irradiated WT mice at different time points after irradiation. The expression level of Gelsolin(GSN) was detected by using WB after IR. **C/D.** Percent survival of WT and Gelsolin KO mice after 9.5 Gy TBI and 18.0 Gy ABI. **E.** The body weight of mice after 18.0 Gy ABI. **F.** Representative images of HE staining intestinal sections at Day 3.5 and Day 7 after 9.5 Gy TBI. **G.** The relative villus length. **H.** The relative crypts depth

was induced by IR (Fig. 2A/B), which was consistent with our previous study [16]. To discuss the function of Gelsolin in basal radio-resistance in vivo, WT mice and Gelsolin KO mice received 9.5 Gy total body irradiation (TBI). The survival rates of Gelsolin KO mice decreased after 9.5 Gy TBI (Fig. 2C). Moreover, 100% Gelsolin KO mice died within 6 days, while 60% of WT mice survived after 18.0 Gy abdominal body irradiation (ABI) (Fig. 2D). In addition, Gelsolin KO mice had significant body weight loss after 18.0 Gy ABI (Fig. 2E). The intestinal structure of Gelsolin KO mice was severely damaged after IR, as demonstrated by HE results of mice intestines 3.5days after IR(Fig. 2F). The relative villi length (Fig. 2G) and the relative depth of crypts (Fig. 2H) in Gelsolin KO mice were much worse than that in the WT mice. Taken together, these results revealed that the radiosensitivity of Gelsolin KO mice was significantly increased and Gelsolin is essential for basal radio-resistance in vivo.

The intestine of Gelsolin KO mice was susceptible to IR exposure

Microcolony formation assay is used to evaluate ISCs survival [17]. Ki67 immunohistochemistry was conducted to visualize the microcolony formation assay.

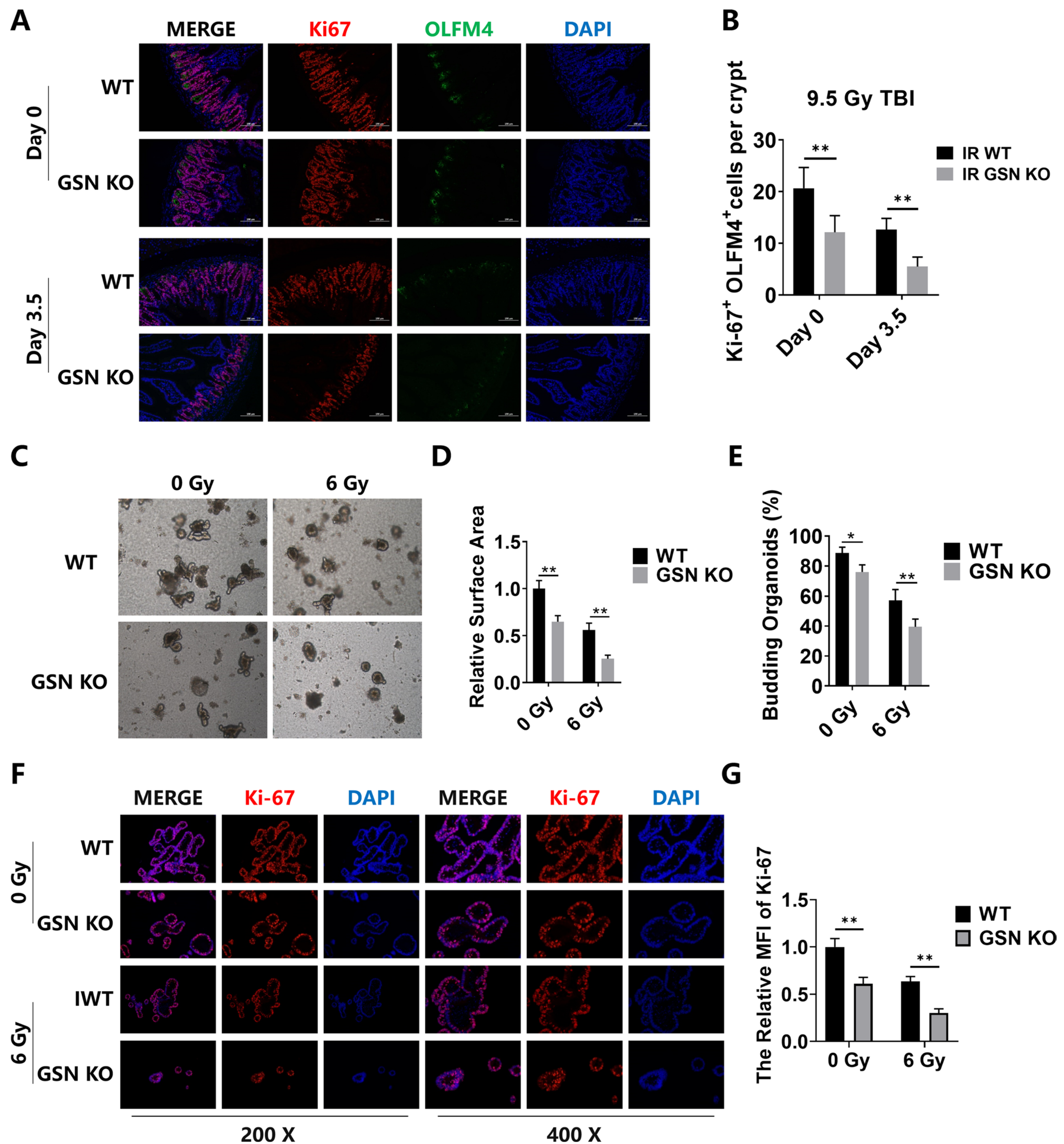


Fig. 3 The intestine of Gelsolin KO mice was susceptible to IR exposure.**(A)** Representative images of Ki-67 staining intestinal sections at Day 1 and Day 3.5 after 9.5 Gy TBI. **(B)** The Ki-67^{+} cells per crypts. **(C)** Intestinal crypts of WT and Gelsolin KO mice were extracted for organoid culture and then exposed to 6.0 Gy IR. Intestinal organoids were evaluated at day 7 after IR. Images of organoids were collected 4 days after irradiation exposure. **(D)** The relative surface area of organoids was analyzed and measured using Image J. **(E)** The rate of budding organoids. **(F)** Representative images of Ki-67 immunofluorescence organoids. **(G)** The MFI of Ki-67

The results revealed Gelsolin KO mice had fewer Ki-67+ cells in crypts after IR, which meant that Gelsolin deficiency could impair ISCs regeneration (Fig. 3A/B). Intestinal organoids have an integrated intestinal epithelial structure and all types of cells [18, 19]. Subsequently, intestinal crypts of WT and Gelsolin KO mice were isolated for organoid culture, and Gelsolin deficiency significantly impaired intestinal organoids ability (Fig. 3C). The relative surface area of single intestinal organoids in the Gelsolin KO mice decreased (Fig. 3D). The budding

rate also decreased in the Gelsolin KO group (Fig. 3E). Next, Ki-67 immunofluorescence was conducted and the results showed that the MFI of Ki-67 was decreased in Gelsolin KO intestinal organoids at 7 days after 6.0 Gy IR (Fig. 3F/G). Above all, we proved that Gelsolin protects against intestinal injury and improve the regeneration of irradiated ISCs.

Gelsolin mediated ISCs regeneration utilizing Th17 cells differentiation and IL-17 signaling pathway

We conducted RNA-Seq to explore the mechanism by which Gelsolin promotes ISCs regeneration. There were 772 DEGs screened out (290 upregulated genes and 482 downregulated genes) (Supplementary Table S1). KEGG enrichment analysis revealed that DEGs were enriched in Th17 cells differentiation and IL-17 signaling pathway, etc. (Supplementary Table S2). Next, we screened out 34 DEGs, which played critical roles in the process of IL-17 signaling pathway, Th17 cell differentiation, Antimicrobial Peptides, Increased barrier Integrity, and ISCs regulations to conduct secondary analysis (Fig. 4A, Supplementary Table S3). Compared with WT mice, the levels of IL-17 A, IL-22, MMP13, MMP3, Reg3γ, and Reg3β were down-regulated in Gelsolin KO mice (Fig. 4B).

Interleukin (IL)-17 A, predominantly produced by Th17 cells, has vital roles in multicellular immunity [20]. Previous reports have proposed a protective role for IL-17 against IR-induced injury [21]. Firstly, we proved that IL-17 A was inhibited after IR exposure (Figure S1A). Then we explored the influence of IR on Th17 cells differentiation by using RT-PCR. As expected, Th17 cells differentiation regulators, including STAT3, RORC, RORA, IL1R2, BATF, AHR, IL-23R, and IRF4, were down-regulated in mice after 9.5 Gy TBI (Figure S1B). Next, recombinant IL-17 A and Anti-mouse IL-17 A-InVivo were used to assess whether IL-17 could provide a radioprotective function. Recombinant IL-17 A protected mice from IR induced death (Figure S2A), and reduced intestinal damage (Figure S2C/D), while Anti-mouse IL-17 A-InVivo aggravated IR-induced mice death (Figure S2B) and intestinal damage (Figure S2E/F). These results proved that recombinant IL-17 A and IL-17 signaling pathway could ameliorate irradiation-induced intestinal injury in vivo and in vitro.

IL-17 A ameliorated IR-induced intestinal injury in Gelsolin KO mice

RNA-seq results implied IL-17 signaling pathway was significantly down-regulated in the intestine of Gelsolin KO mice. Subsequently, we discussed the function of IL-17 signaling pathway in Gelsolin-mediated radioresistance. Recombinant IL-17 A was used to sensitize IL-17 signaling pathway. By establishing TBI mice model, we observed recombinant IL-17 A improved intestinal integrity of Gelsolin KO mice (Fig. 4C). The relative villi length (Fig. 4D) and crypts depth (Fig. 4E) in the Gelsolin KO + IL-17 A group were higher than that in the control group. Ki67 immunofluorescence on these intestinal sections was used to evaluate microcolony formation assay. The results showed that the relative fluorescence intensity of Ki-67 was increased in Gelsolin KO mice crypts after recombinant IL-17 A treatment (Fig. 4F/G). These results proved that recombinant IL-17 A ameliorated IR-induced intestinal injury in Gelsolin KO mice.

IL-17 A promoted the ISCs regeneration in Gelsolin KO mice after IR

The Lgr5+ FISH was used to determine whether the IL-17 signaling pathway acted directly on ISCs. As expected, IR reduced Lgr5+ ISCs in the Gelsolin KO mice compared with WT mice, while recombinant IL-17 A increased Lgr5+ ISCs (Fig. 5A/B). Furthermore, OLFM4 immunofluorescences result showed that recombinant IL-17 A increases the number of OLFM4+ ISCs after IR exposure (Fig. 5C/D). Next, we evaluated the expression of Lgr5, OLFM4, and ASCL2 by using RT-PCR. The expression of these key genes was significantly down-regulated in Gelsolin KO mice after IR, and IL-17 A stimulation led to the induction of these genes in Gelsolin KO + IL-17 A groups, which was consistent with the results of the previous immunofluorescence (Fig. 5E). These data proved that IL-17 A promoted the ISCs regeneration in Gelsolin KO mice. Moreover, recombinant IL-17 A promoted organoid formation in the Gelsolin KO + IL-17 A group compared with control group (Fig. 5F). The relative surface area and budding rate of intestinal organoids were increased in the Gelsolin KO + IL-17 A group (Fig. 5G/H). Taken together, recombinant IL-17 A could promote ISCs regeneration in vivo and in vitro and IL-17 signaling pathway may play an indispensable role in Gelsolin-mediated ISCs regeneration.

Gelsolin sustained Th17 cell functionality via p-STAT3/RORγt axis

RNA-Seq revealed the Th17 cell differentiation pathway were enriched (Fig. 4). Next we discussed Th17 cells function in Gelsolin-mediated radioresistance. Th17 cells exist in various configurations, ranging from homeostatic to pathogenic [22]. Firstly, we investigated whether Gelsolin affected systemic Th17 cells responses. We found the proportions of Th17 cells (CD4+ IL-17 A+) in PBCs and SPCs were decreased in Gelsolin KO mice after IR (Fig. 6A). To understand the mechanism underneath Th17 cells differentiation in Gelsolin deficient background, the level of key Th17 cells regulators were evaluated and the expression of STAT3, RORC, RORA, and IL-6 were significantly downregulated in Gelsolin KO mice after 9.5 Gy IR (Fig. 6B). Indeed, we found STAT3

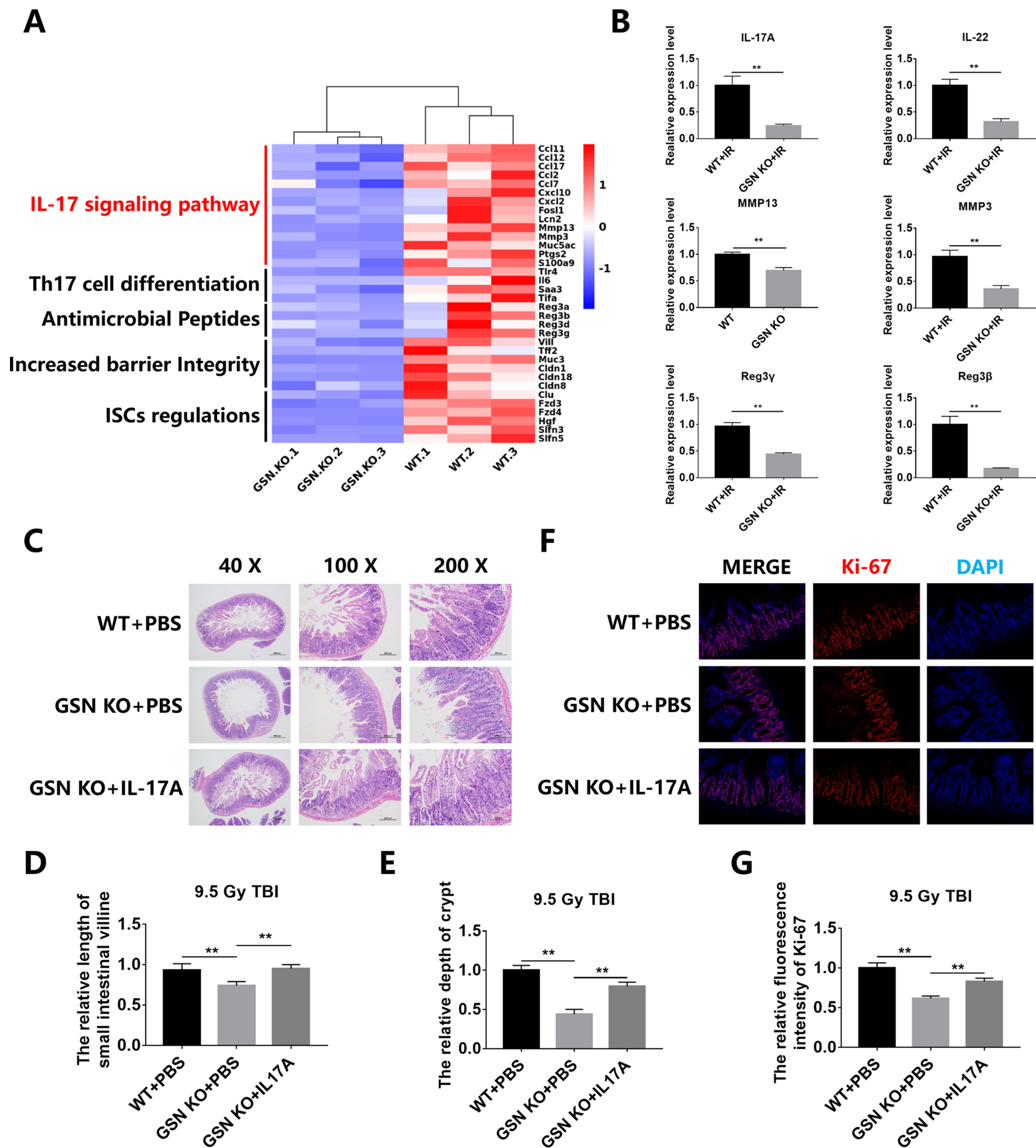


Fig. 4 IL-17 A ameliorated IR-induced intestinal injury in Gelsolin KO mice. **(A)** Heatmap of downstream target genes (Supplementary Table 2) in the intestine was drawn based on RNA-seq results. **(B)** Total RNA was extracted from WT mice and Gelsolin KO mice. Then the RT-PCR of IL-17 A, IL-22, MMP13, MMP3, Reg3γ, Reg3β was conducted. **(C)** The mice were treated with Recombinant IL-17 A (50 μg/kg, 5 days before IR, dissolved in NS), Anti-mouse IL-17 A-InVivo (10 mg/kg, 24 and 2 h before IR), or PBS (200 μL/mice, 24 and 2 h before IR) *via* peritoneal injection.Representative images of HE staining intestinal sections at Day 3.5 after 9.5 Gy TBI. **(D)** The relative villus length. **(E)** The relative crypts depth. **(F)** Representative images of Ki-67 immunofluorescence intestinal sections at Day 3.5 after 9.5 Gy TBI. **(G)** The relative fluorescence intensity of Ki-67

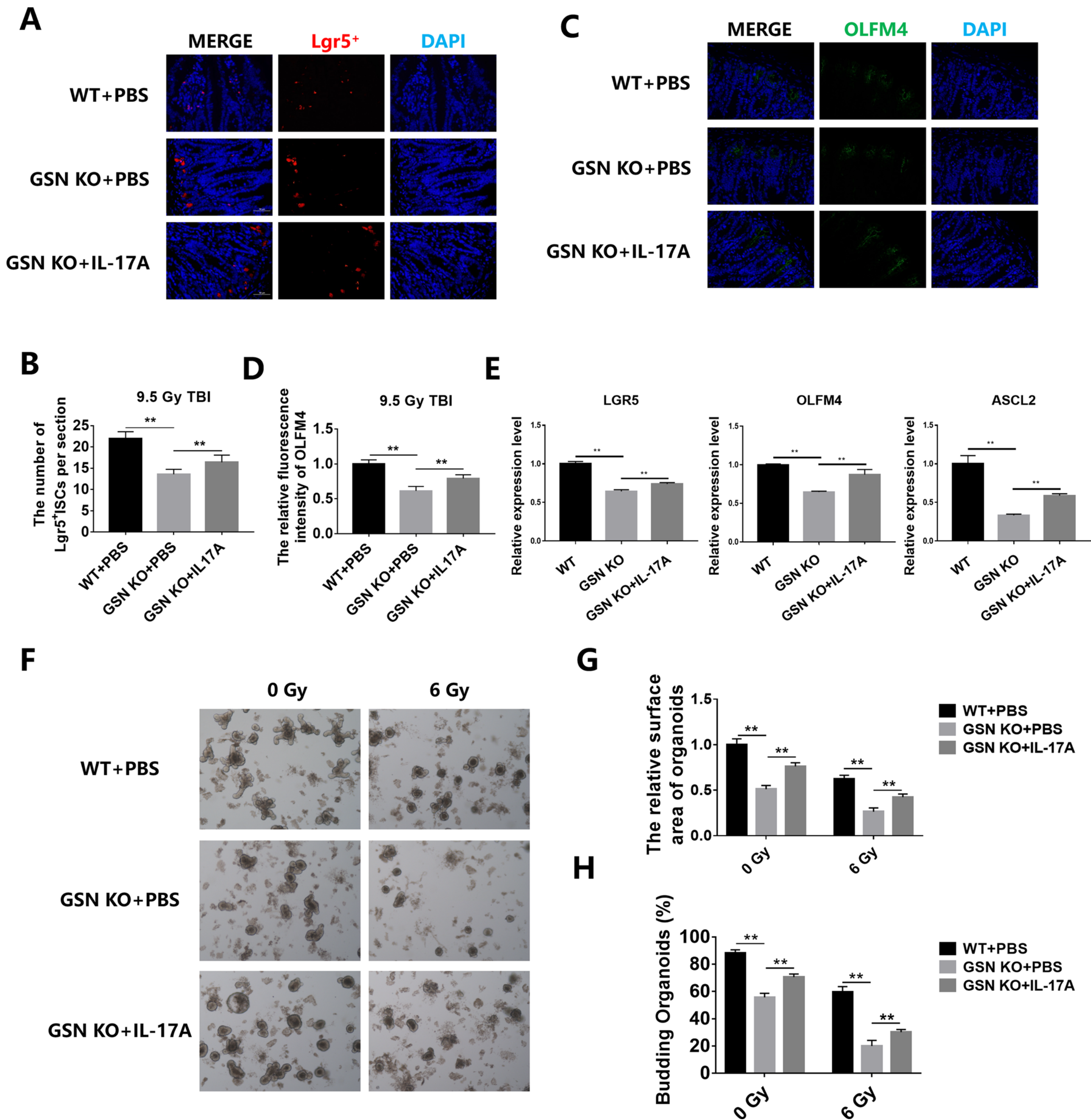


Fig. 5 IL-17 A promoted the ISCs regeneration in Gelsolin KO mice after IR. The mice were treated with Recombinant IL-17 A (50 μg/kg for 5 days before IR, dissolved in NS), Anti-mouse IL-17 A-InVivo (10 mg/kg, 24 and 2 h before IR), or PBS (200 μL/mice, 24 and 2 h before IR) *via* peritoneal injection.**A.** Representative images of $Lgr5^+$ FISH intestinal sections at Day 3.5 after 9.5 Gy TBI. **B.** The number of $Lgr5^+$ cells per crypt. **C.** Representative images of OLFM4 immunofluorescences intestinal sections at Day 3.5 after 9.5 Gy TBI. **D.** The relative fluorescence intensity of OLFM4 per section. **E.** RNAobtained from the mice intestines was used to perform the RT-PCR of Lgr5, OLFM4, ASCL2. **F.** Intestinal organoid derived from WT mice or Gelsolin KO mice were treated with IL-17 A (10 ng/ml) or PBS before 6.0 Gy IR. **G.** The relative surface area of intestinal organoids was analyzed and measured using Image J. **H.** The budding rate of intestinal organoids

phosphorylation was markedly inhibited in the absence of Gelsolin in the intestine after IR (Fig. 6C). Furthermore, we found that the expression of p-STAT3 and RORγt in PBCs, SPCs, and LPLs was significantly downregulated in Gelsolin KO mice (Fig. 6D). These data proved that Gelsolin deletion inhibited Th17 cells differentiation *via* the p-STAT3/RORγt signaling pathway in vivo.

To explore the function of Gelsolin in Th17 cells differentiation in vitro, we isolated naive CD4+ T cells and activated PKC by using PMA, ionomycin, IL-23

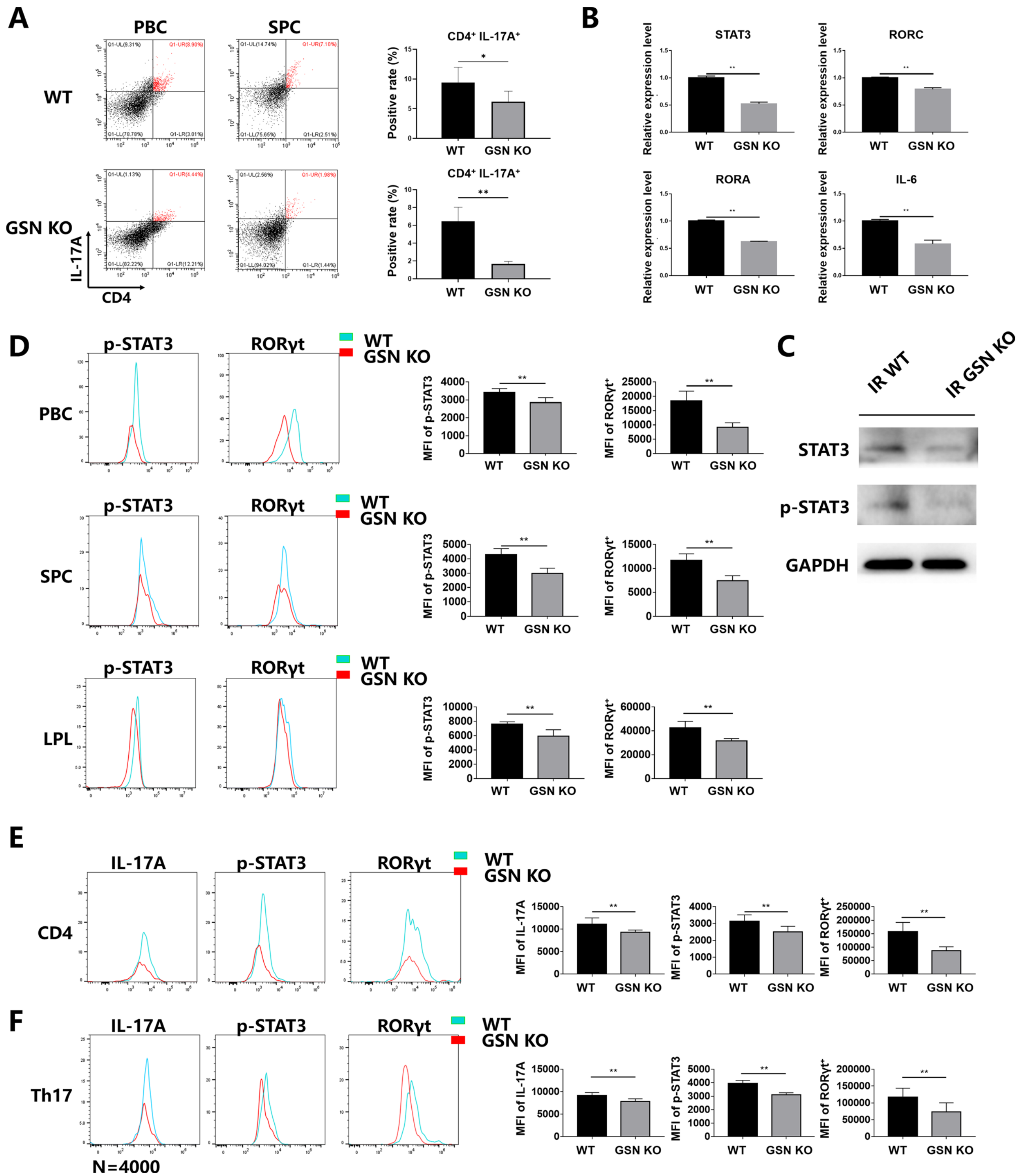


Fig. 6 Gelsolin targeted Th17 cell functionality via the STAT3/RORγt signaling pathway. **(A)** $CD4^+$ T cells in PBCs and SPCs were isolated from the indicated mice according to the manufacturer's instructions and induced into Th17 cells (See Supplementary Material and methods for details). **(B)** Total RNA was extracted from WT mice and Gelsolin KO mice after 9.5 Gy IR. Then the RT-PCR of STAT3, RORC, RORA, and IL-6 was performed. **(C)** The levels of STAT3 and p-STAT3 were detected in the intestine after IR. **(D)** CD4 + T cells were isolated from mice according to the manufacturer's instructionsthe expression of p-STAT3 and RORγt in $CD4^+$ PBCs, SPCs, and LPLs cells were detected. **(E)** The intracellular level of IL-17 A, p-STAT3, and RORγt in $CD4^+$ T cells. **(F)** The intracellular level of IL-17 A, p-STAT3, and RORγt in Th17 cells

and BFA. The expression of IL-17 A, p-STAT3 and RORγt was markedly decreased by using flow cytometry (Fig. 6E). Moreover, naive CD4+ T cells were cultured under Th17 cell-polarizing conditions (IL-6+TGF-β1+IL-1β+IL-23). Flow cytometry analysis revealed that Gelsolin deletion markedly inhibited the differentiation of Th17 cells by intracellular staining of IL-17 A. Moreover, Gelsolin deletion significantly reduced the levels of Th17 cell transcription factors, including p-STAT3 and RORγt (Fig. 6F). Taken together, these observations indicated that deletion of Gelsolin in CD4+ T cells impaired Th17 cells differentiation and Gelsolin may target Th17 cell functionality *via* p-STAT3/RORγt axis.

The decreased formation efficiency of intestinal organoids from Gelsolin KO mice were rescued by Th17 cells co-culture

A co-cultured system was established to assess the specificity of Th17 cells responses to intestinal organoids (Fig. 1A). The Th17 cells were cultured with intestinal organoids at a ratio of 20:1 in Matrigel and 50:1 in culture medium before IR. The results showed that the ability of organoid formation was improved when intestinal crypts were co-cultured with Th17 cells from WT mice at a ratio of 1:20 in Matrigel and 1:50 in culture medium after 6 Gy IR, which reminded us that Th17 cells could modulate ISCs renewal and differentiation. However, the function of intestinal organoids which were co-cultured with Th17 cells from Gelsolin KO mice was weakened (Fig. 1B/C). The relative surface area budding rate of intestinal organoids were attenuated after co-culturing with Gelsolin KO Th17 cells compared with WT groups (Fig. 1D). Moreover, the effects of Th17 cells on the ISCs were explored, and the RT-PCR results suggested Th17 cells modulated the key genes (Lgr5, OLFM4, AXIN2, WNT3A, YAP1, and STAT3) of ISCs regeneration, which in turn affected organoid formation (Fig. 1E). Next, we assessed the expression of key genes including c-Myc, SOX9, and β-Catenin by using WB. Consistently, ISCs key regulators were increased in intestinal organoids when co-cultured with WT Th17 cells while they had a significant reduction after co-culturing with Gelsolin KO Th17 cells (Fig. 1F/G). Taken together, we confirmed that Th17 cells impacted ISCs regeneration and the ablation of Gelsolin in Th17 cells decreased the function of co-cultured intestinal organoids.

Gelsolin protected mice from DSS induced colitis

To figure out whether the protection of Gelsolin was specific to IR induced intestinal damage, we evaluated the protection of Gelsolin by establishing DSS-induced colitis models. Throughout DSS-induced colitis, we found that Gelsolin KO mice had a more severe DSS-colitis phenotype. The survival results showed that 100% of Gelsolin KO mice died within 14 days, while 20% of WT mice survived beyond 30 days (Fig. 7A). In addition, Gelsolin KO mice had significant body weight loss after DSS treatment after 10 Days of DSS administration (Fig. 7B). To observe the function of Gelsolin on DSS-induced colitis, we observed the gross changes in mice colons, which showed Gelsolin KO mice had severe bleeding and edema in the colons compared with WT mice (Fig. 7C). Consistently, Gelsolin KO mice had higher clinic scores (Fig. 7D) and shorter colons (Fig. 7E). Moreover, HE results showed that Gelsolin deficiency led to more severe disruption of the mucosal epithelium in response to DSS treatment (Fig. 7F). The relative villi length in Gelsolin KO mice was much worse than that in the WT mice (Fig. 7G). In addition, TUNEL immunofluorescence result showed that Gelsolin deficiency promoted the apoptosis of colons after DSS treatment (Fig. 7F/G). These results indicated that Gelsolin revealed protection against DSS-induced colitis and also indicated that the protection of Gelsolin was not limited to radiation induced enteritis.

Discussion

As actin-regulating proteins, Gelsolin could remove pathological actin filaments which were released from necrotic cells after tissue injury [13]. Gelsolin is an actin-binding protein that potentially plays key roles in the process of cell motility and division [23]. Gelsolin participates in immunologic function and its roles with different cells of the immune system, Gelsolin is a potential candidate for various therapeutic applications [23]. Cui et al. demonstrated that Gelsolin could alleviate neonatal inflammatory responses after rhinovirus infection [24]. Gelsolin could bind to the β-amyloid in Alzheimer's disease and remove Aβ from brain [25].

As a key gene that regulates critical pathways such as coagulation, immunity, inflammation, and tumor development, the key role of Gelsolin in ISCs regeneration after IR is not fully understood. Although there are reports of the radioprotective effects of recombinant Gelsolin protein both in vitro and in vivo, and the genetic blockade or inhibition of Gelsolin at the cellular level can significantly enhance the radiosensitivity of tumor cells, there is limited knowledge about the key role and mechanisms of Gelsolin in regulating radiation-induced intestinal injury. Here we used Gelsolin KO mice for the first time internationally to show the essential role of Gelsolin in radiation protection and resistance in vivo. In this work, we found that the expression of Gelsolin was induced by IR, and the survival rates of Gelsolin KO mice were decreased after IR. Moreover, the intestine of Gelsolin KO mice was susceptible to IR exposure. It meant that Gelsolin played key roles in radio-resistance. ISCs are susceptible to chemicals, pathogens, or IR [26, 27]. The Ki-67 results

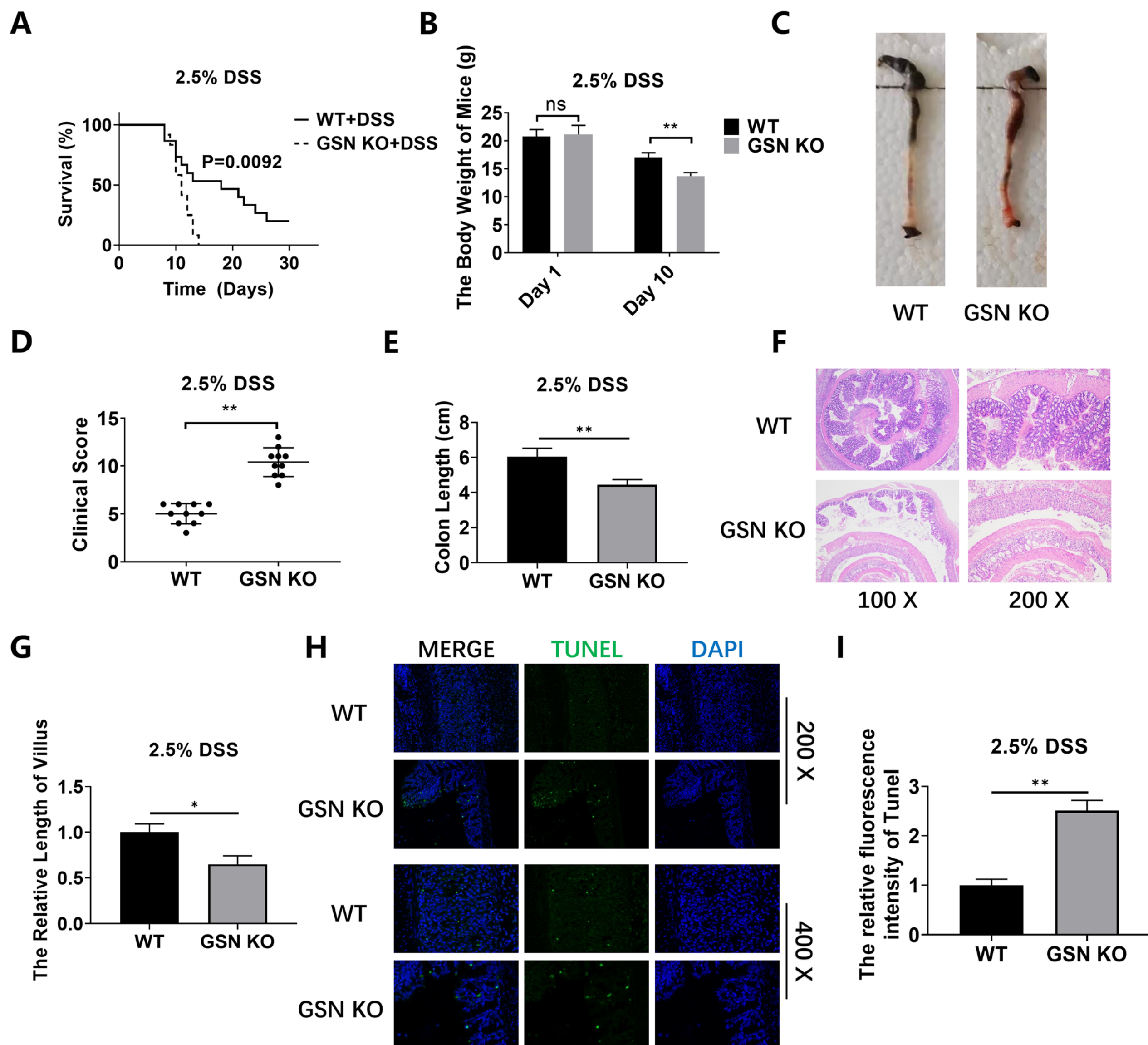


Fig. 7 Gelsolin protected mice from DSS induced colitis. WT and Gelsolin KO mice were administered 2.5% (w/v) DSS (36000–50000 Da, dissolved in sterile water) in daily drinking for 10 days to establish acute colitis mice model. **(A)** Percent survival of WT and Gelsolin KO mice after DSS administration was recorded. **(B)** The body weight of WT and Gelsolin KO mice. **(C)** Gross pathological view of mouse colon after 10 Days DSS administration. **(D)** The clinical score of mice after 10 Days of DSS administration. **(E)** The length of colons of WT and Gelsolin KO mice after 10 Days DSS administration. **(F)** Representative images of HE-stained colon of WT and Gelsolin KO mice after 10 Days DSS administration. **(G)** The relative villus length. **(H)** Representative TUNEL immunofluorescence staining colon sections. **(I)** The relative fluorescence intensity of TUNEL per section

revealed that Gelsolin deficiency could attenuate ISCs regeneration. Using Lgr5+ FISH and intestinal organoids technology, we found that Gelsolin deficiency resulted in a significant decrease in ISC numbers in vivo and *in vitro.*This finding suggests that Gelsolin plays a crucial role in enhancing the function of irradiated ISCs. Further, RNA-Seq reminded us that the IL-17 signaling pathway and Th17 cells differentiation was downregulated.

Next, the function of IL-17 signaling pathway were explored in IR-induced intestinal injury. IL-17 A could regulate intestinal epithelial barrier, intestinal microbiota, and IgA transcytosis [20, 28]. Enamorado et al. revealed that injury promoted the expression of IL-17RA in dorsal root ganglion sensory neurons and IL-17/IL-17RA axis promoted peripheral sensory neuron regeneration [29]. Lin et al. found that IL-17 A could promote Lgr5+ ISCs to express Atoh1, which promote ISCs differentiate to Atoh1+ cells. IL-17 A also protect mice from DSS induced intestinal damage [20].

Using IL-17 A-EGFP mice, we found IL-17 A expression was significantly down-regulated after IR. Through animal and intestinal organoids experiments, we proved that recombinant IL-17 A ameliorated gut injury induced by IR both in vivo and in vitro while anti-IL-17 A promoted IR-induced mice death, which suggested IL-17 A protected mice from IR-induced injury. Further, we discussed the role of IL-17 in the regeneration of ISCs mediated by Gelsolin. Activation of the IL-17 signaling pathway significantly up-regulated Lgr5+ ISCs and OLFM4+ ISCs in Gelsolin KO mice. Moreover, the results of the intestinal organoids also suggested that the impaired function observed in Gelsolin-deficient organoids was restored following IL-17 A treatment. Thus, we proved that Gelsolin may promote the ISCs regeneration via IL-17 signaling pathway.

RNA-Seq results revealed the Th17 cell differentiation pathway was enriched. Th17 cells exists in various configurations, ranging from homeostatic to pathogenic [22, 30]. Th17 cells induce inflammation response as well as play roles in host defense against bacteria [31, 32]. IL-6 and transforming growth factor-β (TGF-β) are key regulators to of Th17 cells differentiation [33]. IL-6 could activate the STAT3 transcription factor [33]. As a master transcription factor, RORγt is indispensable to drive Th17 cells differentiation [34]. The up-regulation of RORγt requires IL-6, IL-21 and IL-23[[35].

We investigated whether Gelsolin affected systemic Th17 cell responses in vivo. Th17 cells proportions in ABCs and PCs were decreased in Gelsolin KO mice after IR. Consistently, the expression of STAT3, RORC, RORA, and IL-6 were significantly down-regulated in Gelsolin KO mice after IR. Flow cytometry showed the level of p-STAT3 and RORγt in ABCs, PCs, and LPLs was significantly down-regulated in Gelsolin KO mice. These results proved that the deletion of Gelsolin inhibited Th17 cells differentiation *via* p-STAT3/RORγt signaling pathway in vivo. Next, naive CD4+ T cells were stimulated with PMA, ionomycin, and IL-23. And the level of IL-17 A, p-STAT3, and RORγt were markedly decreased in naive Gelsolin KO CD4+ T cells. Moreover, by using flow cytometry, we found that Gelsolin deletion markedly inhibited the levels of IL-17 A, p-STAT3, and RORγt in Th17 cells under Th17 cell-polarizing conditions. These observations indicate that deletion of Gelsolin in CD4+ T cells impaired Th17 cells differentiation in vivo and in vitro and Gelsolin may target Th17 cell functionality *via* p-STAT3/RORγt signaling pathway.

Both epithelial and non-epithelial cells can regulate the differentiation of ISCs [11, 36]. Studies have shown that intestinal innate immune cells play key roles in ISCs functions [37]. By using scRNA-seq, Moshe Biton et al. revealed crosstalk between ISCs and T cells on intestinal remodeling [11]. Here, we used the co-culture system of intestinal organoids and Th17 cells to explore interaction between Lgr5+ ISCs and the Th17 cells and their role on ISCs regeneration after IR. To our surprise, Th17 cells could modulate ISCs renewal and differentiation. However, the function of intestinal organoid co-culture with Th17 cells from Gelsolin KO mice was weakened. Further, ISC key regulators were increased when intestinal organoids were co-cultured with WT Th17 cells while they had a significant reduction after co-cultured with Gelsolin KO Th17 cells compared with Th17 cells from WT mice. These results proved Th17 cells impacted ISCs regeneration and the ablation of Gelsolin in Th17 cells decreased the function of co-cultured intestinal organoids. In addition, we also found that Gelsolin revealed protection against DSS-induced colitis and also indicated that the protection of Gelsolin was not limited to radiation induced enteritis.

Conclusion

By using Gelsolin KO mice, we comprehensively characterized and functionally dissected the effect of Gelsolin in intestinal homeostasis. Gelsolin could promote ISCs regeneration *via* the IL-17 signaling pathway. By establishing the co-culture system, we found that Th17 subpopulations played an indispensable role in intestinal homeostasis and Gelsolin sustained Th17 cell function *via* the p-STAT3/RORγt axis. The Th17 cells-ISCs axis may remind us to integrate epithelial and immune cells to avoid continuous inflammation. The interaction between Th17 cells and ISCs may provide a critical mechanism that immune cells regulate ISCs regeneration to restore intestinal homeostasis.

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4: Table 1 - The list of 772 differentially expressed genes.

Supplementary Material 5: Table 2 - The list of KEGG pathway top 20 (Total) enrichment terms.

Supplementary Material 6: Table 3 - The list of downstream target genes in Gelsolin KO mice.

Author contributions

Du J and Gao F designed the research. Du J, Fang L, and Wang Y conducted the animal experiments. Feng Z, Zhao J, Fang D, Huang D, and Zhai X conducted the intestinal organoids experiments. Du J, Gao F, and Liu C analyzed the data. Min R, Gao F, and Liu C supported fund assistance.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
All animal experiments conformed to the National Institute of Health Guide for the Care and Use of Laboratory Animals' (NIH Publication No. 85 – 23, National Academy Press, Washington, DC, revised 1996), with the approval of the Laboratory Animal Center of the Naval Medical University, Shanghai.

Consent for publication
Written informed consent for publication was obtained from all participants.

Competing interests
The authors declare no competing interests.

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