

SATB2-SELENBP1 program supports colonic epithelial regeneration during altered methionine availability

Yao Liu

yao.liu@njgluy.com

Nanjing Drum Tower Hospital, the Affiliated Hospital of Nanjing University Medical School
<https://orcid.org/0000-0001-5572-723X>

Lanqing Wu

Nanjing Drum Tower Hospital, the Affiliated Hospital of Nanjing University Medical School

Yongyu Chen

Medical College of Soochow University

Juan Chen

Nanjing Drum Tower Hospital, the Affiliated Hospital of Nanjing University Medical School

Haoran Zhuang

Nanjing Drum Tower Hospital, the Affiliated Hospital of Nanjing University Medical School

Lei Lei

Nanjing Drum Tower Hospital, the Affiliated Hospital of Nanjing University Medical School

Xiaoli Li

Medical College of Soochow University

Danni Chen

Nanjing Drum Tower Hospital, the Affiliated Hospital of Nanjing University Medical School

Yu Chen

Nanjing Drum Tower Hospital, the Affiliated Hospital of Nanjing University Medical School

Wen Tang

the Second Affiliated Hospital of Soochow University

Qi Sun

Nanjing Drum Tower Hospital, Medical School of Nanjing University

Yanmiao Dai

Kunshan Hospital of Chinese Medicine

Jianming Li

The First Affiliated Hospital, Jiangxi Medical College, Nanchang University <https://orcid.org/0000-0002-2862-9922>

Article

Keywords: SATB2, SELENBP1, epithelial regeneration, colonic organoid, methionine

Posted Date: October 28th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-8820025/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: There is no conflict of interest

SATB2-SELENBP1 program supports colonic epithelial regeneration during altered methionine availability

Lanqing Wu^{1,2*}, Yongyu Chen^{2*}, Juan Chen^{1,2*}, Haoran Zhuang^{1,3}, Lei Lei¹, Xiaoli Li², Danni Chen^{1,3}, Yu Chen¹, Wen Tang⁴, Qi Sun¹, Yanmiao Dai^{5†}, Jianming Li^{2†}, Yao Liu^{1,2,3†}

¹ Department of Pathology, Nanjing Drum Tower Hospital, Affiliated Hospital of Medical School, Nanjing University, Nanjing 210008, Jiangsu, China

² Department of Pathology and Pathophysiology, Medical College of Soochow University, Suzhou 215123, Jiangsu, China

³ Department of Pathology, Nanjing Drum Tower Hospital, Affiliated Hospital of Nanjing Medical University, Nanjing 210008, Jiangsu, China

⁴ Department of Gastroenterology, the Second Affiliated Hospital of Soochow University, Suzhou 215004, Jiangsu, China

⁵ Department of Gastroenterology, Traditional Chinese Medicine Hospital of Kunshan, Kunshan 215300, China

*These authors contributed equally to this work.

†Correspondence to:

Yao Liu, M.D., Ph.D., Doctor, Associate Professor, Department of Pathology, Nanjing Drum Tower Hospital, the Affiliated Hospital of Nanjing University Medical School, Nanjing 210008, Jiangsu, China. (Tel: +86-25-83304616; E-mail: yao.liu@njglyy.com)

27 Jianming Li, M.D., Ph.D., Professor, Department of Pathology and Pathophysiology,
28 Medical College of Soochow University, Suzhou 215123, Jiangsu, China. (Tel:
29 +86-512-65880129; E-mail: jianmingli@suda.edu.cn)

30

31 Yanmiao Dai, M.D., Doctor, Department of Gastroenterology, Traditional Chinese
32 Medicine Hospital of Kunshan, Kunshan 215300, Jiangsu, China. (Tel:
33 +86-512-55031807; E-mail: 85_dym@163.com)

ABSTRACT

Colonic epithelial regeneration requires transcriptional programs that remain functional during injury, but the nutrient-sensitive regulators of this process are incompletely understood. Here, we identify SELENBP1 as a direct transcriptional target of the colonic epithelial regulator SATB2. Intestinal epithelial deletion of *Selenbp1* exacerbated irradiation-induced mucosal injury and impaired organoid survival and budding, supporting a role for SELENBP1 in epithelial regeneration. Methionine restriction was associated with preservation of SELENBP1-positive epithelial cells during recovery and partially improved survival and budding of *Selenbp1*-deficient organoids. Methionine-restricted conditions increased nuclear SELENBP1 abundance and its association with SATB2, together with SATB2-associated SUMO2/3 signals. SELENBP1 deficiency was accompanied by reduced SUMO2/3 pathway components and lower SATB2 protein abundance. These observations support that SELENBP1 may regulate SATB2 SUMOylation. In human inflammatory bowel disease and chronic radiation enteritis samples, reduced SELENBP1 expression tracked with histological injury and poorer organoid budding. These findings identify a SATB2–SELENBP1 epithelial regeneration program and establish methionine availability as a context in which this program is altered.

KEY WORDS

SATB2; SELENBP1; epithelial regeneration; colonic organoid; methionine

INTRODUCTION

Colorectal mucosal injury is a common pathological feature of inflammatory diseases including inflammatory bowel disease (IBD) and radiation-induced enteritis. In IBD, persistent epithelial damage and barrier dysfunction drive chronic inflammation and increase the risk of colorectal cancer¹. Radiation enteritis is a frequent complication of abdominal radiotherapy and remains difficult to treat, leading to substantial morbidity². Effective mucosal repair requires coordinated epithelial regeneration, immune modulation, and extracellular matrix remodeling, yet the epithelial-intrinsic mechanisms governing regenerative competence remain incompletely understood^{3,4}. Although current therapies primarily target immune activation, accumulating evidence indicates that defective epithelial regeneration often underlies persistent tissue injury^{5,6}.

The colonic epithelium comprises absorptive and secretory populations that together maintain the mucus barrier and luminal environment¹. Disruption of epithelial differentiation and mucus-barrier maintenance is a prominent feature of ulcerative colitis and radiation enteritis^{7–10}. However, the epithelial programs that coordinate mucosal regeneration after injury remain incompletely defined.

SELENBP1 is a methanethiol oxidase involved in detoxifying methionine-derived metabolites, including methanethiol¹¹. Its expression is reduced in colonic biopsies from patients with ulcerative colitis¹², but its contribution to epithelial recovery after injury is unknown. Methionine metabolism is relevant to this question because methionine is required for epithelial proliferation, whereas microbial and host-derived sulfur metabolites can accumulate during inflammation and epithelial injury^{13–15}. Complete methionine deprivation impairs intestinal stem-cell proliferation, while dietary methionine supplementation exacerbates radiation-induced gastrointestinal

injury^{16,17}. These observations indicate that the effect of methionine availability depends on context and dose.

SATB2 is a transcriptional regulator required for colonic epithelial identity. SATB2 loss produces a small-intestinal-like shift in the colon and predisposes to inflammation^{18,19}. SATB2 also regulates the enteroendocrine hormone PYY and the anion exchanger SLC26A3, which contribute to epithelial homeostasis and repair^{18,20}. We therefore asked whether SATB2 controls a broader epithelial program that includes SELENBP1 and whether this program is altered by methionine availability.

Here, we identify SELENBP1 as a direct transcriptional target of SATB2 and show that epithelial Selenbp1 deficiency compromises tissue recovery and organoid regeneration after injury. We further investigate how methionine availability influences SELENBP1-associated epithelial regeneration and examine the relationship between SELENBP1, SATB2, and the SUMO pathway. Finally, we assess the clinical relevance of this epithelial program in human inflammatory bowel disease and radiation enteritis.

RESULTS

SATB2 maintains a colonic epithelial program that includes SELENBP1

We previously reported that SATB2 regulates colonic epithelial ion transport through direct activation of SLC26A3 and that its loss predisposes to colonic mucosal inflammation¹⁸. Consistent with the small-intestinal-like phenotype reported after SATB2 deletion¹⁹, our transcriptomic analysis identified broad downregulation of colonic epithelial genes, including Selenbp1¹⁸. We therefore asked whether SELENBP1 is part of a SATB2-dependent epithelial program relevant to repair.

Immunofluorescence co-staining for SELENBP1 and TFF3 showed that, in control mice, SELENBP1 was detected predominantly in colonocytes and in a subset of upper-crypt TFF3-positive cells. SELENBP1 signal was markedly diminished in colonic tissue of *Satb2^{ff};Vil^{cre}* mice (*Fig. 1A*), and quantification showed fewer SELENBP1-positive epithelial cells per crypt (*Fig. 1B*). TFF3-positive cells remained detectable in SATB2-deficient colonic tissue (*Fig. 1A and 1C*). These data support loss of a SELENBP1-expressing colonic epithelial population after SATB2 deletion, without assigning SELENBP1 to a discrete goblet-cell lineage.

To test whether the relationship extended to human tissue, we analyzed adjacent non-tumor colorectal tissue from the TCGA cohort. Among colonic epithelial genes altered in *Satb2*-deficient mice, SELENBP1 showed the strongest positive correlation with SATB2 ($r = 0.69$; *Fig. 1D-E*). SELENBP1 was also co-expressed with SLC26A3 ($r = 0.70$; *Fig. 1D*), a direct SATB2 target¹⁸. Together, these observations identify SELENBP1 as a component of a SATB2-associated colonic epithelial program and motivate direct tests of transcriptional regulation.

SATB2 directly activates SELENBP1 transcription.

Selenbp1 expression was substantially higher in the colon than in the small intestine of C57BL/6 mice (*Fig. 2A*). In HCT116 and RKO cells, which retained detectable basal SELENBP1 expression, SATB2 overexpression increased SELENBP1 transcripts (*Fig. 2B-C*). Published SATB2 ChIP-seq data (GSE167287)¹⁹ showed a promoter-proximal SATB2-binding peak at SELENBP1 (*Supplementary Fig. 1*). Luciferase assays showed increased SELENBP1 promoter activity after SATB2 expression in 293T, HCT116, and RKO cells (*Fig. 2D*), and ChIP-qPCR showed enrichment of the SELENBP1 promoter with anti-SATB2 relative to vector and IgG controls (*Fig. 2E*). These complementary data support direct transcriptional activation of SELENBP1 by SATB2.

Epithelial SELENBP1 loss impairs regeneration after colonic injury

To examine the functional role of SELENBP1, we subjected intestinal epithelial-specific *Selenbp1* knockout mice (*Selenbp1*^{f/f}; *Vil*^{creER}) to irradiation-induced injury. Compared with controls, *Selenbp1* knockout mice had a higher colon weight-to-length ratio (*Supplementary Fig. 2A-B*). Histology showed epithelial damage and inflammatory infiltration at baseline, with persistent injury at 5 days post-irradiation (dpi) in knockout mice while control tissue showed recovery (*Fig. 3A-B*). Ki67 staining and Wnt target-gene expression were lower in *Selenbp1*-deficient tissue (*Fig. 3C; Supplementary Fig. 3A-B*). In parallel, irradiated crypt-derived organoids from knockout mice showed lower survival, smaller size, fewer buds, and reduced Wnt target-gene expression than control organoids (*Fig. 3D-E; Supplementary Fig. 3C*). Together, these findings identify epithelial SELENBP1 as a contributor to mucosal recovery after injury.

SELENBP1 loss is associated with a pro-inflammatory epithelial–macrophage signature

Before irradiation, colonic tissue from epithelial-specific *Selenbp1* knockout mice had higher mRNA levels of *Tnf- α* , *Il1b*, and *Il6* and higher expression of Cd64, Cd86, and iNos (*Fig. 4A-B*). In SW480 and DLD-1 cells, SELENBP1 knockdown increased TNF- α mRNA and reduced IL-10 mRNA (*Fig. 4C-D*). Together, these epithelial-cell data indicate that SELENBP1 deficiency is accompanied by a pro-inflammatory transcriptional shift.

Conditioned medium from *Selenbp1*-deficient colonic organoids altered marker expression in primary bone marrow-derived macrophages (BMDMs), increasing selected M1-associated transcripts and reducing selected M2-associated transcripts (*Fig. 4E-F*). THP-1 Transwell co-cultures gave a similar direction of change at the

mRNA and protein levels (*Supplementary Fig. 4A,B*). Collectively, these findings show that epithelial SELENBP1 deficiency alters macrophage-associated gene expression in vitro.

Methionine availability modulates SELENBP1-associated epithelial regeneration

Given the methanethiol oxidase activity of SELENBP1, we examined whether methionine availability was associated with epithelial recovery. Before irradiation, methionine-restricted (MR) mice had thinner colonic mucosa than methionine-supplemented (MS) mice (*Supplementary Fig. 5A-B*), while the number of SELENBP1-positive cells per crypt did not show a clear difference (*Fig. 5A-B*). After irradiation, MS mice showed mucus extrusion and incomplete mucosal recovery, whereas MR mice retained more SELENBP1-positive epithelial cells at 5 dpi (*Fig. 5C-D; Supplementary Fig. 5C-D*). RNA-seq identified diet-associated transcriptomic differences, including inflammatory-response pathways (*Supplementary Fig. 6*). In cell culture, methionine supplementation reduced SELENBP1 mRNA and increased TNF- α mRNA (*Supplementary Fig. 7*). Together, these findings show that methionine availability is associated with epithelial responses during mucosal recovery. In irradiated organoids, methionine-restricted medium improved survival and partially restored budding in *Selenbp1*-deficient cultures (*Fig. 5E-F*). The differential effects on organoid survival and budding suggest that methionine availability modulates multiple components of epithelial recovery.

SELENBP1 deficiency is accompanied by reduced SUMO-pathway components and lower SATB2 protein abundance

SUMOylation is implicated in stress responses and epithelial repair. During colonic injury, SUMO2/3, but not SUMO1, showed a visually distinct temporal pattern in epithelial tissue (*Supplementary Fig. 8*). SATB2 has been reported to undergo SUMOylation in embryonic stem cells²¹. Because SELENBP1 and SATB2 are

co-expressed in colonic epithelium and SELENBP1 was detected in both cytoplasmic and nuclear fractions (*Fig. 6A*), we examined whether SELENBP1 loss was accompanied by changes in SUMO-pathway components.

Colonic mucosa from *Selenbp1^{fl/f};Vil^{creER}* mice showed reduced Sumo3 expression at baseline and lower Sumo2 and Sumo3 expression during recovery (*Fig. 6B*). In epithelial cell lines, SELENBP1 knockdown was accompanied by lower SUMO2/3, ZNF451, PIAS1, and SATB2 protein levels, while SATB2 mRNA changed little (*Fig. 6C-D*). In colonic tissue, immunoblotting detected a higher-molecular-weight SATB2-reactive band in the presence of N-ethylmaleimide that was compatible with SUMO-modified SATB2 (*Fig. 6E*). In addition, SATB2-positive epithelial cells from knockout mice had lower SUMO2/3 signal at 5 dpi (*Fig. 6F*). These observations are consistent with a role for SELENBP1 in regulating SUMO-pathway components and SATB2 protein abundance.

Methionine-restricted conditions increase nuclear SELENBP1–SATB2 association and SATB2-associated SUMO2/3 signals

Given that methionine availability influenced epithelial recovery and altered SATB2 staining in the irradiated colon (*Supplementary Fig. 9*), we next examined whether methionine-restricted conditions were associated with changes in SATB2-related SUMO2/3 signals. In MR mice, immunofluorescence and flow cytometry showed more SATB2/SUMO2/3 co-localization and a higher proportion of SATB2-positive/SUMO2/3-positive epithelial cells than in MS mice (*Fig. 7A-B*). In DLD-1 cells, methionine-restricted medium increased nuclear SELENBP1 abundance (*Fig. 7C*). SATB2 immunoprecipitates from methionine-restricted cells showed greater SELENBP1, SUMO2/3, ZNF451, and PIAS1 signal than those from normal or MS conditions (*Fig. 7D*). In cell lines, SELENBP1 knockdown was accompanied by lower SUMO2/3, ZNF451, PIAS1, and SATB2 signals, and MR medium was

associated with partial restoration of these signals (*Fig. 7E*). Together, these data link methionine-restricted conditions to nuclear SELENBP1 accumulation, enhanced SELENBP1–SATB2 association, and altered SUMO-pathway readouts.

Reduced SELENBP1 expression tracks with chronic colonic injury in human samples

SELENBP1 expression was lower in radiation-induced enteritis than in normal colonic mucosa, and co-staining with MUC2 was reduced (*Supplementary Fig. 10*). In a previously established cohort of organoids derived from ascending-colon biopsies of 19 patients with Crohn’s disease in clinical remission²⁰, SELENBP1 mRNA expression positively correlated with organoid budding rate after passaging and 10 days of culture (*Fig. 8A-B*). In 26 ulcerative-colitis biopsy samples, SATB2/SUMO2/3 and SELENBP1/SATB2 staining decreased with increasing Nancy Histology Index score (*Fig. 8C-D; Supplementary Fig. 11*). These clinical observations are correlative and consistent with reduced expression of the SATB2–SELENBP1 program in chronic mucosal injury.

DISCUSSION

Here, we identify SELENBP1 as a direct transcriptional target of SATB2 and link this relationship to colonic epithelial regeneration after injury. Epithelial Selenbp1 deficiency was associated with persistent mucosal injury, reduced Ki67 staining, impaired Wnt-associated regenerative responses, and defective organoid survival and budding after irradiation. Methionine-restricted conditions were associated with improved epithelial recovery, increased nuclear SELENBP1 abundance, and enhanced SELENBP1–SATB2 association. Together, these findings define a SATB2–SELENBP1 epithelial program that is altered by methionine availability during mucosal repair.

Our previous work showed that SATB2 preserves colonic epithelial homeostasis through direct regulation of epithelial genes, including the chloride exchanger SLC26A3, thereby limiting intestinal inflammation and colitis-associated tumorigenesis¹⁸. The present study extends this SATB2-dependent program by identifying SELENBP1 as a direct transcriptional target. Promoter reporter assays, ChIP-qPCR, and published SATB2 ChIP-seq data support direct SATB2 binding and transcriptional activation of SELENBP1. In turn, epithelial Selenbp1 loss impaired mucosal recovery in vivo and regenerative capacity in colonic organoids. SELENBP1 deficiency was also accompanied by pro-inflammatory epithelial transcriptional changes and altered macrophage-associated gene expression in vitro. These observations support a role for SELENBP1 in linking epithelial regenerative state with the local inflammatory environment.

SUMOylation is a reversible post-translational modification that regulates protein localization, stability, and activity in response to cellular stress. The SUMO conjugation cascade involves E1-activating, E2-conjugating, and E3-ligating enzymes and primarily modifies lysine residues on target proteins²². SUMOylation has been implicated in epithelial stress responses and inflammatory regulation²³, while ZNF451-dependent SUMOylation of SATB2 has been described during embryonic stem-cell differentiation²¹. In the present study, SELENBP1 deficiency was accompanied by reduced SUMO2/3, ZNF451, PIAS1, and SATB2 protein abundance. In addition, a higher-molecular-weight SATB2-reactive band detected in the presence of N-ethylmaleimide was compatible with SUMO-modified SATB2, and methionine-restricted conditions increased SATB2-associated SUMO2/3 signals. Because SELENBP1 lacks a canonical SUMO E3-ligase domain²⁴, these findings do not support direct catalytic SUMOylation by SELENBP1. Rather, they suggest that SELENBP1 may influence the availability, recruitment, or activity of SUMO-pathway components associated with SATB2. Defining the relevant E3 ligase,

SATB2 acceptor site, and functional consequence of this modification will be important for testing this model.

Methionine availability provided a metabolic context for the SATB2–SELENBP1 program. Epithelial proliferation and differentiation are central to mucosal recovery following radiation-induced intestinal injury²⁵. Amino acids are increasingly recognized as regulators of epithelial renewal and stem-cell function²⁶. Consistent with the context-dependent effects of methionine metabolism, complete methionine deprivation can impair intestinal stem-cell proliferation, whereas methionine supplementation can exacerbate radiation-induced gastrointestinal injury^{16,17}. In our model, moderate methionine restriction was associated with improved epithelial morphology during recovery and greater retention of SELENBP1-positive epithelial cells after irradiation. In organoids, methionine-restricted medium improved survival and partially restored budding in Selenbp1-deficient cultures. The distinct effects on survival and budding suggest that methionine availability regulates multiple components of epithelial recovery. Methionine-restricted conditions also increased nuclear SELENBP1 abundance and its association with SATB2, linking nutrient availability to this epithelial program.

The clinical observations extend these findings to chronic intestinal injury. SELENBP1 expression was reduced in radiation enteritis, and lower SELENBP1 expression in patient-derived colonic organoids was associated with reduced budding capacity. In ulcerative-colitis biopsies, SATB2/SUMO2/3 and SELENBP1/SATB2 staining decreased with increasing histological activity. These findings are consistent with reduced activity of the SATB2–SELENBP1 epithelial program in chronic mucosal injury. Although these human data are associative, they support the relevance of this program across experimental and clinical settings.

Several limitations should be considered. The study does not identify the molecular mechanism linking SELENBP1 to SATB2-associated SUMO2/3 signals, the relevant SUMO E3 ligase, or the modified SATB2 lysine residue. The regenerative defect associated with Selenbp1 loss was not resolved into its relative contributions from proliferation, survival, stem-cell dynamics, apoptosis, and barrier function. In addition, the epithelial mediator responsible for altered macrophage-associated gene expression remains to be identified. Finally, the contribution of methionine-derived metabolites, residual microbiota, and systemic consequences of dietary methionine manipulation requires further investigation.

In summary, SATB2 directly activates SELENBP1, and epithelial SELENBP1 supports colonic mucosal regeneration after injury. Methionine availability is associated with altered SELENBP1 abundance, SATB2 interaction, and SUMO-pathway readouts. These findings establish a framework for understanding how nutrient context may shape epithelial regenerative competence while identifying mechanistic questions for future study.

METHODS

Animal Models

Selenbp1^{f/f} mice were generated using CRISPR/Cas9-mediated gene editing to insert LoxP sites flanking exons 2–7 (Jiangsu GemPharmatech Co., Ltd.). Genomic DNA was extracted from toe clips using dBiozol reagent (Bioflux, Cat. No. BSC16M1). Genotyping was performed by PCR using primers listed in *Supplementary Table 1*. All mice were housed under specific pathogen-free conditions (12-h light/dark cycle, ad libitum access to food and water) at the Laboratory Animal Center of Soochow University. All animal experiments were approved by the Committee on Animal Research of Soochow University. Intestinal epithelial-specific Selenbp1 knockout mice were generated by crossing Selenbp1^{f/f} mice with Villin-CreER mice.

Constitutive deletion of *Selenbp1* in the intestinal epithelium (*Selenbp1^{f/f};Vil^{cre}*) resulted in postnatal lethality; therefore, all subsequent experiments were performed using inducible *Selenbp1^{f/f};Vil^{creER}* mice. To induce Cre-mediated recombination, 6-week-old *Selenbp1^{f/f};Vil^{creER}* mice and littermate controls were administered tamoxifen (Sigma-Aldrich) dissolved in corn oil by intraperitoneal injection at 75 mg/kg body weight once daily for 5 consecutive days. Experiments were initiated 2 weeks after the final injection. *Satb2* knockout mice (*Satb2^{f/f};Vil^{cre}*) were used as previously described^{18, 20}. All mice used in this study were male and 6–8 weeks of age at the start of experiments unless otherwise stated. Mice were randomly assigned to experimental or control groups using a random number table, with sample sizes ($n = 3–5$ per group) determined based on preliminary data and power analysis. Investigators were blinded to group allocation and outcome assessment when feasible. Radiation-induced colonic injury was performed as our previous study²⁰. In brief, mice received a single dose of 12 Gy abdominal X-ray irradiation using an X-RAD 320 biological irradiator (Precision X-Ray), with shielding of non-abdominal regions. For flow cytometry analysis, colonic epithelial cells were isolated from control and experimental mice as previously described²⁰.

Dietary Intervention

C57BL/6 mice (4-6 weeks old, male) were randomly assigned to two dietary groups: methionine-restricted (MR) diet and methionine-supplemented (MS) diet. Mice were housed in a controlled environment with a 12-hour light/dark cycle. The methionine-restricted diet (Research Diets, Cat. No. A11051301B) contained 0.12% methionine, while the methionine-supplemented diet was standard chow (containing approximately 0.86% methionine) supplemented with 0.5% L-methionine in drinking water. To minimize microbial contributions to methionine metabolism, all mice received an antibiotic cocktail (vancomycin 0.5 g/L, neomycin 1 g/L, ampicillin 1 g/L, and metronidazole 1 g/L) in drinking water for 2 weeks before and throughout the

362 dietary intervention. Mice were maintained on their respective diets for 8 weeks.
363 Following the dietary intervention, RNA was extracted from mouse colonic tissues
364 and subjected to RNA-seq as described in our previous study²⁰. The mRNA
365 sequencing was performed by the Nanjing YiKe Population Health Research Institute
366 Co., Ltd., and the raw data have been deposited in the OMIX database (NGDC;
367 <https://ngdc.cncb.ac.cn/omix/>; accession no. OMIX015132).

369 **Clinical Samples**

370 For human organoid analyses, ascending-colon biopsy specimens were obtained from
371 19 patients with Crohn's disease at the Second Affiliated Hospital of Soochow
372 University between November 2023 and January 2024. All patients had
373 pathologically confirmed Crohn's disease and were in clinical remission at biopsy
374 collection. The Harvey–Bradshaw Index and Simple Endoscopic Score for Crohn's
375 Disease recorded at initial diagnosis and follow-up have been reported previously²⁰.

376
377 Formalin-fixed, paraffin-embedded (FFPE) colonic tissue specimens were obtained
378 from 26 patients with ulcerative colitis (UC) at the Department of Pathology, Nanjing
379 Drum Tower Hospital. Histological inflammation was graded according to the Nancy
380 Histology Index (NHI). Clinical and pathological information, including age, sex,
381 NHI, and biopsy location, was summarized in *Supplementary Table 2*. For
382 immunofluorescence analysis of UC FFPE specimens, rectal biopsies were
383 preferentially selected when available. If rectal tissue was not obtained, biopsies from
384 the sigmoid colon were used instead. FFPE surgical specimens of radiation enteritis
385 were obtained from the Department of Pathology at the Sixth Affiliated Hospital of
386 Sun Yat-Sen University, the same as our previous study²⁰. All samples were collected
387 with informed consent, and the study was approved by the institutional ethics
388 committee.

Colonic Organoid Culture

Mouse and human colonic organoids were established from isolated colonic crypts as previously described²⁰. Mouse colonic organoids were derived from tamoxifen-treated *Selenbp1^{f/f};Vil^{creER}* mice and age-matched *Selenbp1^{f/f}* controls (6-week-old). Human colonic organoids were generated from endoscopic biopsy specimens from the Second Affiliated Hospital of Soochow University, as described previously²⁰. Organoids were passaged once after crypt isolation and allowed to recover before use in experiments. For irradiation experiments, passage 1 organoids were used. Organoids were pre-cultured for 12–24 hours in either standard medium (containing methionine) or methionine-deprived medium. After this period, organoids were exposed to 2 Gy irradiation, and subsequently monitored for survival and budding. Budding was defined as a distinct crypt-like protrusion extending from the organoid body. Organoids were cultured and irradiated at the same time point to ensure relatively uniform sizes across groups. Because organoids are distributed three-dimensionally within Matrigel, at least 10 fields per group were imaged for quantification. All organoids within each field were scored by two independent observers blinded to genotype.

Cell Culture

Human colorectal cancer cell lines SW480, DLD-1, HCT116 and RKO were maintained in our laboratory and authenticated annually by short tandem repeat (STR) profiling. Mycoplasma testing was performed routinely. SW480 and DLD-1 cells were maintained in DMEM/F12 supplemented with 10% FBS and 1% penicillin–streptomycin at 37°C in 5% CO₂. HCT116 and RKO cells were maintained in RPMI-1640 with 10% FBS and 1% penicillin–streptomycin under the same conditions. SELENBP1 knockdown was performed using siRNA transfection with Lipofectamine RNAiMAX (Thermo Fisher) for 24 h, followed by metabolic treatment. SATB2 overexpression was performed by transfection of a pcDNA3.1-FLAG-SATB2

expression vector using Lipofectamine 3000 (Thermo Fisher), and cells were harvested 48 h post-transfection.

Methionine-restricted (MR) and methionine-supplemented (MS) media

MR medium was prepared using methionine-free RPMI-1640 (Sigma-Aldrich, Cat. No. R7513) with 10% FBS, 1% penicillin–streptomycin, 1 mM sodium pyruvate, 1 mM non-essential amino acids, and 2 mM L-glutamine. MS medium was generated by adding L-methionine to MR medium to 0.5 mM final concentration. Cells were cultured in MR or MS medium for 12–24 h prior to assays.

Co-culture

Twenty-four hours after siRNA transfection, epithelial cells were seeded in the lower chamber of a 24-well transwell (0.4 μ m pores) to form a monolayer. THP-1 monocytes were differentiated into M0 macrophages with PMA (50 ng/mL, 24 h) and recovery for 24 h, then seeded in the upper chamber. Co-cultures were maintained for 48 h, after which macrophages were collected for RT-qPCR and Western blot analyses of M1/M2 markers.

Nuclear–cytoplasmic fractionation, Western blot, and immunoprecipitation were performed as described²⁰ (antibodies in *Supplementary Table 3*). RT-qPCR and ChIP assays were performed as described²⁰ (primers in *Supplementary Table 4*).

Luciferase Reporter Assay

The human SELENBP1 promoter region was synthesized (covering nucleotides –1000 to +150 relative to the transcription start site) and cloned into the pGL4 vector (Promega) upstream of the firefly luciferase gene. HEK293T, HCT116 and RKO cells were co-transfected with the promoter reporter construct, a Renilla luciferase control plasmid (pRL-TK), and either a pcDNA3.1-SATB2 expression vector or empty vector

using Lipofectamine 3000. Cells were harvested 48 h post-transfection, and dual-luciferase activities were measured using the Dual-Luciferase Reporter Assay System (Promega). Firefly luciferase activity was normalized to Renilla luciferase activity for each sample. All transfections were performed in three independent experiments.

TCGA Data Download and Analysis

RNA-seq data from adjacent non-tumor colorectal tissue in The Cancer Genome Atlas (TCGA) cohort were obtained from the Genomic Data Commons portal. Gene-level expression data were used for correlation analysis between SATB2 and colonic epithelial genes of interest. Pearson correlation coefficients were calculated in R and visualized with the ggplot2 and pheatmap packages.

Statistical Analysis

All in vitro experiments were performed as three independent biological replicates, each with three technical replicates unless otherwise stated. Data are presented as mean $\pm$ SEM unless otherwise stated. Statistical analysis was performed using GraphPad Prism (version 10.0) or R (version 4.3.1). Normality of data distribution was assessed using the Shapiro-Wilk test. Homogeneity of variances was assessed using Levene's test. For comparisons between two groups, unpaired two-tailed Student's t-test (for parametric data) or Mann-Whitney U test (for non-parametric data) was used. For comparisons among three or more groups, one-way ANOVA was applied. A p-value < 0.05 was considered statistically significant.

Data Availability

RNA-seq data generated for the dietary-intervention cohort are available in the OMIX database (NGDC; accession no. OMIX015132). Other data supporting the findings of

this study are available within the Article and its Supplementary Information files or from the corresponding author upon reasonable request.

Conflict of Interest

The authors disclose no conflicts.

Author Contributions

L. Wu, Y. Chen, and J. Chen contributed equally to the experimental work. J. Chen and H. Zhuang conducted additional experiments. J. Chen also drafted the initial manuscript. L. Lei assisted with experimental procedures and data interpretation. X. Li contributed to the mouse studies. D. Chen supported histological analyses. Yu Chen, W. Tang, and Q. Sun acquired and scored clinical samples. Y. Dai contributed to data interpretation and funding acquisition. J. Li contributed to the overall project design. Y. Liu oversaw project design, manuscript writing, and funding acquisition.

Acknowledgments

We sincerely thank Associate Professor Lina Sun for her support, especially at a time when our students were graduating and the corresponding author was leaving Soochow University. This work was supported by the National Natural Science Foundation of China (81972259, 82273394), Suzhou Applied Basic Research (Healthcare) Science and Technology Innovation Project (No. SYW2025076), the Clinical Trials Fund from Nanjing Drum Tower Hospital and the Affiliated Hospital of Nanjing University Medical School (2024-LCYJ-PY-01), the Nanjing Municipal Health Science and Technology Development Collaborative Project (CGB25035), and the 2025 Zijing Talent Program – High-Level Innovative Talent Project.

499 **REFERENCE**

- 500 1. Peterson LW, Artis D. Intestinal epithelial cells: regulators of barrier function
501 and immune homeostasis. *Nat Rev Immunol* **14**, 141-153 (2014).
- 502 2. Tabaja L, Sidani SM. Management of Radiation Proctitis. *Dig Dis Sci* **63**,
503 2180-2188 (2018).
- 504 3. Brazil JC, Quiros M, Nusrat A, Parkos CA. Innate immune cell-epithelial
505 crosstalk during wound repair. *J Clin Invest* **129**, 2983-2993 (2019).
- 506 4. Vilardi A, Przyborski S, Mobbs C, Rufini A, Tufarelli C. Current
507 understanding of the interplay between extracellular matrix remodelling and
508 gut permeability in health and disease. *Cell Death Discov* **10**, 258 (2024).
- 509 5. Rath E, Haller D. Intestinal epithelial cell metabolism at the interface of
510 microbial dysbiosis and tissue injury. *Mucosal Immunol* **15**, 595-604 (2022).
- 511 6. Eri RD, *et al.* An intestinal epithelial defect conferring ER stress results in
512 inflammation involving both innate and adaptive immunity. *Mucosal Immunol*
513 **4**, 354-364 (2011).
- 514 7. Nystrom EEL, *et al.* An intercrypt subpopulation of goblet cells is essential for
515 colonic mucus barrier function. *Science* **372**, eabb1590 (2021).
- 516 8. Leupin N, Curschmann J, Kranzbuhler H, Maurer CA, Laissie JA,
517 Mazzucchelli L. Acute radiation colitis in patients treated with short-term
518 preoperative radiotherapy for rectal cancer. *Am J Surg Pathol* **26**, 498-504
519 (2002).
- 520 9. Sunderhauf A, *et al.* Loss of Mucosal p32/gC1qR/HABP1 Triggers Energy
521 Deficiency and Impairs Goblet Cell Differentiation in Ulcerative Colitis. *Cell*
522 *Mol Gastroenterol Hepatol* **12**, 229-250 (2021).
- 523 10. Singh V, *et al.* Chronic Inflammation in Ulcerative Colitis Causes Long-Term
524 Changes in Goblet Cell Function. *Cell Mol Gastroenterol Hepatol* **13**,
525 219-232 (2022).
- 526 11. Pol A, *et al.* Mutations in SELENBP1, encoding a novel human methanethiol
527 oxidase, cause extraoral halitosis. *Nat Genet* **50**, 120-129 (2018).
- 528 12. Schniers A, Goll R, Pasing Y, Sorbye SW, Florholmen J, Hansen T.
529 Ulcerative colitis: functional analysis of the in-depth proteome. *Clin*
530 *Proteomics* **16**, 4 (2019).
- 531 13. Sanderson SM, Gao X, Dai Z, Locasale JW. Methionine metabolism in health
532 and cancer: a nexus of diet and precision medicine. *Nat Rev Cancer* **19**,
533 625-637 (2019).
- 534 14. Walker A, Schmitt-Kopplin P. The role of fecal sulfur metabolome in
535 inflammatory bowel diseases. *Int J Med Microbiol* **311**, 151513 (2021).
- 536 15. Furne J, Springfield J, Koenig T, DeMaster E, Levitt MD. Oxidation of
537 hydrogen sulfide and methanethiol to thiosulfate by rat tissues: a specialized
538 function of the colonic mucosa. *Biochem Pharmacol* **62**, 255-259 (2001).

16. Albrecht LV, Bui MH, De Robertis EM. Canonical Wnt is inhibited by targeting one-carbon metabolism through methotrexate or methionine deprivation. *Proc Natl Acad Sci U S A* **116**, 2987-2995 (2019).
17. Miousse IR, *et al.* Methionine dietary supplementation potentiates ionizing radiation-induced gastrointestinal syndrome. *Am J Physiol Gastrointest Liver Physiol* **318**, G439-G450 (2020).
18. Ni H, *et al.* SATB2 Defect Promotes Colitis and Colitis-associated Colorectal Cancer by Impairing Cl-/HCO₃- Exchange and Homeostasis of Gut Microbiota. *J Crohns Colitis* **15**, 2088-2102 (2021).
19. Gu W, *et al.* SATB2 preserves colon stem cell identity and mediates ileum-colon conversion via enhancer remodeling. *Cell Stem Cell* **29**, 101-115.e10 (2022).
20. Liu Y, *et al.* The protective role of PYY in intestinal mucosal defects induced by SATB2 deficiency in inflammatory bowel disease. *Cell Death Discov* **11**, 227 (2025).
21. Antonio Urrutia G, *et al.* ZFP451-mediated SUMOylation of SATB2 drives embryonic stem cell differentiation. *Genes Dev* **35**, 1142-1160 (2021).
22. Vertegaal ACO. Signalling mechanisms and cellular functions of SUMO. *Nat Rev Mol Cell Biol* **23**, 715-731 (2022).
23. Karhausen J, Ulloa L, Yang W. SUMOylation Connects Cell Stress Responses and Inflammatory Control: Lessons From the Gut as a Model Organ. *Front Immunol* **12**, 646633 (2021).
24. Yunus AA, Lima CD. Structure of the Siz/PIAS SUMO E3 ligase Siz1 and determinants required for SUMO modification of PCNA. *Mol Cell* **35**, 669-682 (2009).
25. Jang H, *et al.* Metformin Protects the Intestinal Barrier by Activating Goblet Cell Maturation and Epithelial Proliferation in Radiation-Induced Enteropathy. *Int J Mol Sci* **23**, 5929 (2022).
26. Chi F, *et al.* Dietary cysteine enhances intestinal stemness via CD8(+) T cell-derived IL-22. *Nature* **647**, 706-715 (2025).

Figure Legend

Figure 1. SATB2-associated colonic epithelial program includes SELENBP1.

(A) Immunofluorescence staining for SELENBP1 and TFF3 in colorectal tissues from *Satb2^{f/f};Vilcre* and control mice. SELENBP1 is detected predominantly in colonocytes and in a subset of TFF3-positive cells. Scale bar: 50 μ m. (B) Quantification of SELENBP1-positive epithelial cells per crypt. Data are mean $\pm$ SEM (n = 3 mice per group; at least five fields per mouse; Mann–Whitney test, $P < 0.05$). (C) Quantification of TFF3-positive cells per crypt. Data are mean $\pm$ SEM (n = 3 mice per group; at least five fields per mouse). (D) Heatmap of Pearson correlations between SATB2 and colonic epithelial genes altered in *Satb2*-deficient mice, based on adjacent non-tumor TCGA colorectal tissue. Blue indicates positive correlation and red indicates negative correlation (range, -1 to 1). (E) Scatter plot of SATB2 and SELENBP1 expression in the same cohort; each dot is one sample; Pearson correlation analysis.

Figure 2. SATB2 transcriptionally upregulates SELENBP1 through direct promoter binding.

(A) RT-qPCR analysis of *Selenbp1* mRNA levels in jejunum, ileum, proximal colon, distal colon, and rectum of C57BL/6 mice. Data are shown as mean $\pm$ SEM. (n = 3). (B-C) SATB2 overexpression in HCT116 and RKO cells (B) and corresponding SELENBP1 mRNA expression measured by RT-qPCR (C). Baseline expression in control cells is shown. Data are shown as mean $\pm$ SEM. (n = 3 independent experiments, $*P < 0.05$). (D) Luciferase reporter assays demonstrating that SATB2 activates the SELENBP1 promoter in 293T, HCT116, and RKO cells. Data are shown as mean $\pm$ SEM. (n = 3 independent experiments, $*P < 0.05$). (E) ChIP-qPCR analysis of SATB2 binding at the *SELENBP1* promoter in SATB2-overexpressing versus

control HCT116 and RKO cells. IgG was used as a negative control. Data are shown as mean $\pm$ SEM. (n = 3 independent experiments, * P < 0.05).

Figure 3. Epithelial SELENBP1 loss impairs regeneration after colonic injury.

(A) Representative H&E staining of colonic tissue from control Selenbp1^{f/f} and epithelial-specific Selenbp1-deficient (Selenbp1^{f/f}; Vil^{creER}) mice before irradiation (0 dpi) and at 3 and 5 dpi. Scale bar, 50 μ m.

(B) Distribution of histological crypt-damage, inflammation-depth, and overall-inflammation scores at 3 and 5 dpi. n = 3 mice per group; Mann–Whitney U test for per-mouse scores.

(C) Immunofluorescence co-staining of Ki67 and SELENBP1 in colonic tissue before irradiation and at 5 dpi. Representative images from n = 3 mice per genotype are shown. Scale bar, 50 μ m.

(D) Representative bright-field images of colonic organoids derived from control and Selenbp1-deficient mice after 2 Gy irradiation, monitored over 10 days. Scale bar, 100 μ m.

(E) Quantification of organoid viability and buds per organoid over time. Data are mean $\pm$ SEM from 3 mice per group. Two-way ANOVA with an appropriate multiple-comparisons test; * P < 0.05, ** P < 0.01, *** P < 0.001.

Figure 4. SELENBP1 deficiency is associated with inflammatory epithelial and macrophage marker expression.

(A) RT-qPCR analysis of Tnf- α , Il1b, and Il6 in colonic tissue from Selenbp1^{f/f}; Vil^{creER} and control Selenbp1^{f/f} mice at 0 dpi. Data are mean $\pm$ SEM (n = 3 mice per group; Mann–Whitney test, P < 0.05). (B) RT-qPCR analysis of Cd64, Cd86, and iNos in the same tissues. Data are mean $\pm$ SEM (n = 3 mice per group; Mann–Whitney test, P < 0.05). (C,D) SELENBP1 knockdown in DLD-1 and SW480 cells and corresponding TNF- α and IL-10 mRNA expression. Data represent three

independent experiments, mean $\pm$ SEM (Mann–Whitney test, $P < 0.05$). (E,F) BMDMs were cultured with conditioned medium from colonic organoids of Selenbp1f/f;VilcreER or control Selenbp1f/f mice, followed by RT-qPCR analysis of selected M1-associated (E) and M2-associated (F) markers. Data are mean $\pm$ SEM ($n = 3$ independent experiments; unpaired t-test, $P < 0.05$, $P < 0.01$, or $P < 0.001$).

Figure 5. Methionine availability modulates SELENBP1-associated epithelial regeneration.

(A,B) Immunofluorescence co-staining of MUC2 and SELENBP1 in colonic mucosa before irradiation, with quantification of SELENBP1-positive cells per crypt. Data are mean $\pm$ SEM (20 fields per mouse; $n = 3$ mice per group; Mann–Whitney test). (C,D) H&E staining and MUC2/SELENBP1 immunofluorescence at 5 dpi, with quantification of SELENBP1-positive cells per crypt. Data are mean $\pm$ SEM (scale bar, 50 μm ; 20 fields per mouse; $n = 3$ mice per group; Mann–Whitney test, $P < 0.05$). (E,F) Crypt-derived organoids from Selenbp1f/f;VilcreER and control Selenbp1f/f mice were pre-cultured for 12–24 h in standard or methionine-depleted medium and exposed to 2 Gy irradiation. Morphology is shown over 2–10 days (E), with survival and budding quantified in F. Data are mean $\pm$ SEM (scale bar, 100 μm ; two-way ANOVA, $P < 0.05$).

Figure 6. SELENBP1 deficiency is accompanied by altered SUMO-pathway components and SATB2-associated SUMO2/3 signals.

(A) Nuclear–cytoplasmic fractionation and immunoblotting of SELENBP1 in DLD-1 cells. (B) RT-qPCR analysis of Sumo2 and Sumo3 expression in colonic mucosa from Selenbp1f/f;VilcreER and control mice before irradiation and during recovery. Data are mean $\pm$ SEM (t test; $P < 0.05$; ns, not significant). (C) Immunoblot analysis of SELENBP1, SUMO2/3, ZNF451, PIAS1, and SATB2 after SELENBP1 knockdown in SW480 and DLD-1 cells. Representative blots from three independent experiments

are shown. (D) RT-qPCR analysis of SATB2 mRNA in the same cells. Data are mean $\pm$ SEM (t test; $P < 0.05$; ns, not significant). (E) Immunoblot of colonic tissue from C57BL/6 mice with or without N-ethylmaleimide (NEM), showing a higher-molecular-weight SATB2-reactive band compatible with SUMO-modified SATB2. (F) SATB2/SUMO2/3 co-staining in colonic tissue at 5 dpi; representative images from $n = 3$ mice per genotype are shown.

Figure 7. Methionine-restricted conditions increase nuclear SELENBP1–SATB2 association and SATB2-associated SUMO2/3 signals.

(A) SATB2/SUMO2/3 co-staining in colonic mucosa from methionine-restricted (MR) and methionine-supplemented (MS) mice. (B) Flow-cytometric quantification of SATB2-positive and SATB2-positive/SUMO2/3-positive colonic epithelial cells. Data are mean $\pm$ SEM ($n = 3$ per group; t test, $P < 0.05$; ns, not significant). (C) Nuclear–cytoplasmic fractionation of DLD-1 cells after 12–24 h in standard or methionine-restricted medium. (D) SATB2 immunoprecipitation from DLD-1 cells cultured in standard, MS, or MR medium, followed by immunoblotting for SUMO2/3, ZNF451, PIAS1, and SELENBP1. (E) Immunoblot analysis of SELENBP1, SUMO2/3, ZNF451, PIAS1, and SATB2 after SELENBP1 knockdown and 12–24 h culture in standard or methionine-restricted medium. Representative blots from three independent experiments are shown.

Figure 8. Reduced SELENBP1 expression tracks with chronic colonic injury.

(A,B) Colonic organoids derived from ascending-colon biopsies of 19 patients with Crohn’s disease in clinical remission. This previously established patient-derived organoid cohort was reported in our earlier study²⁰; the present analysis examines the association between SELENBP1 mRNA expression and organoid budding rate. Each dot represents one patient-derived organoid culture; Pearson correlation analysis was used. Representative organoid morphology at low and high SELENBP1 expression is

682 shown in B. (C,D) SATB2/SUMO2/3 and SELENBP1/SATB2 co-staining in
683 ulcerative-colitis biopsies across Nancy Histology Index categories. Representative
684 images are shown; quantitative analyses of 26 samples are provided in *Supplementary*
685 *Fig. 11*.
686

Figure 1

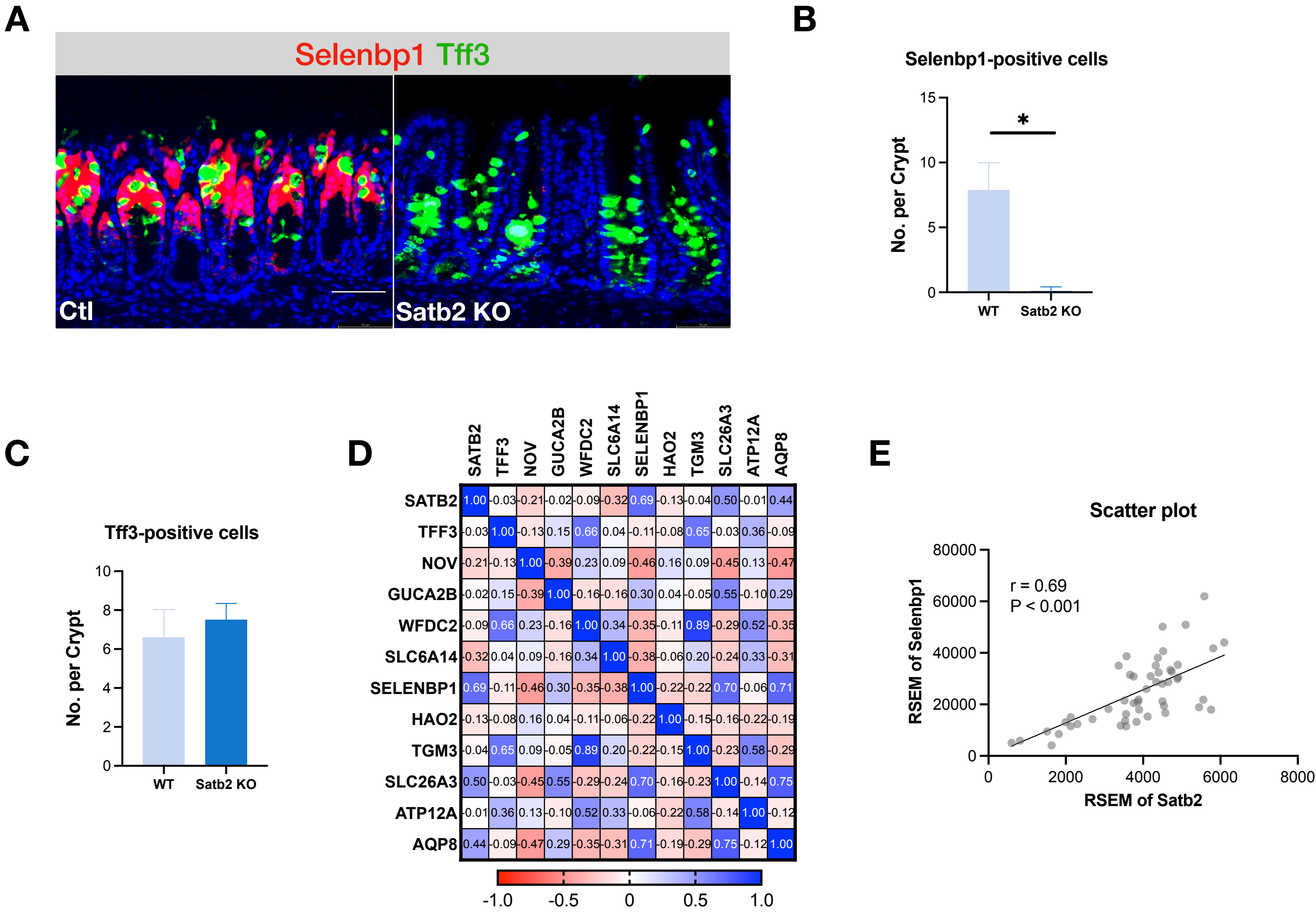


Figure 2

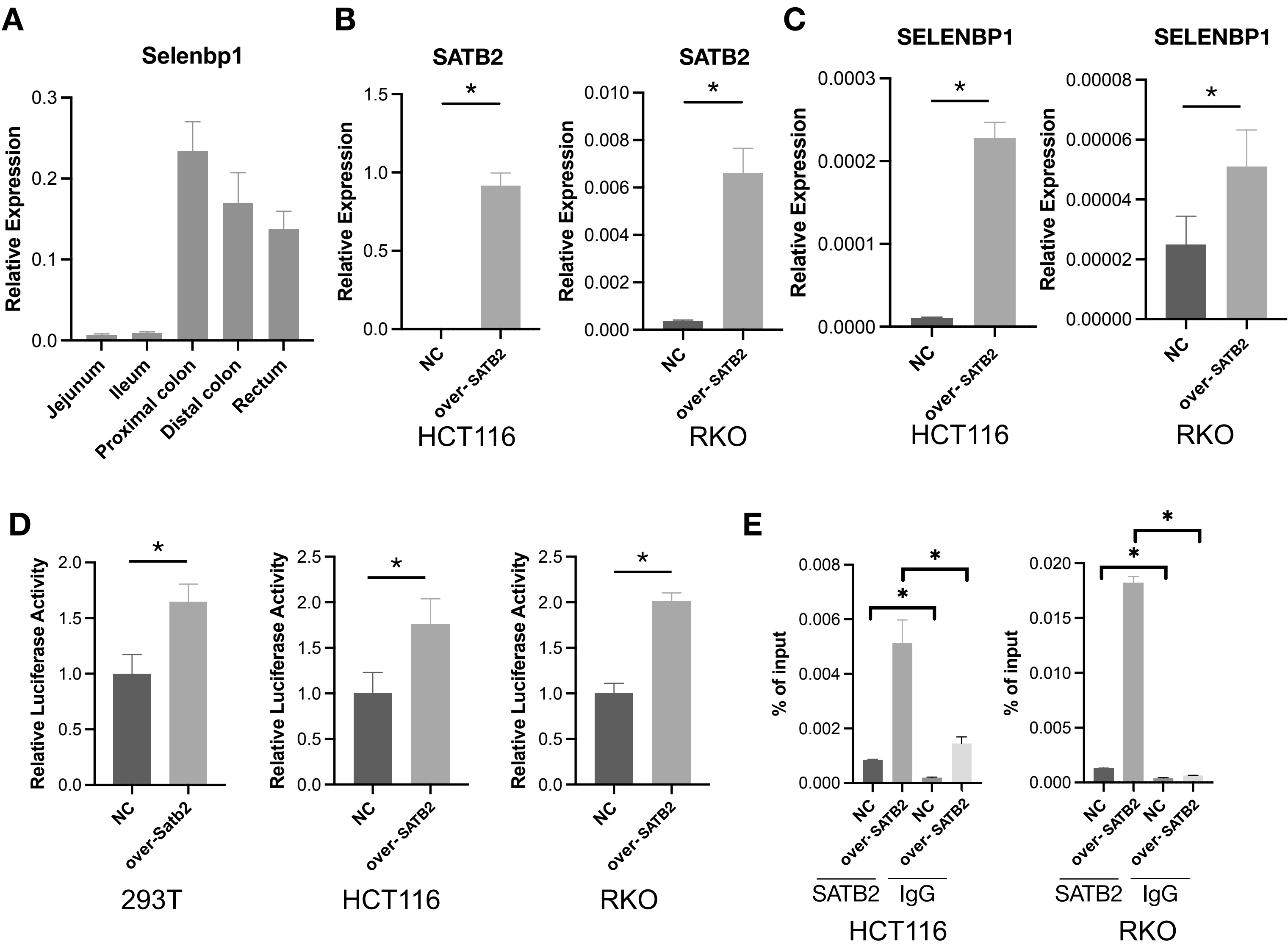


Figure 3

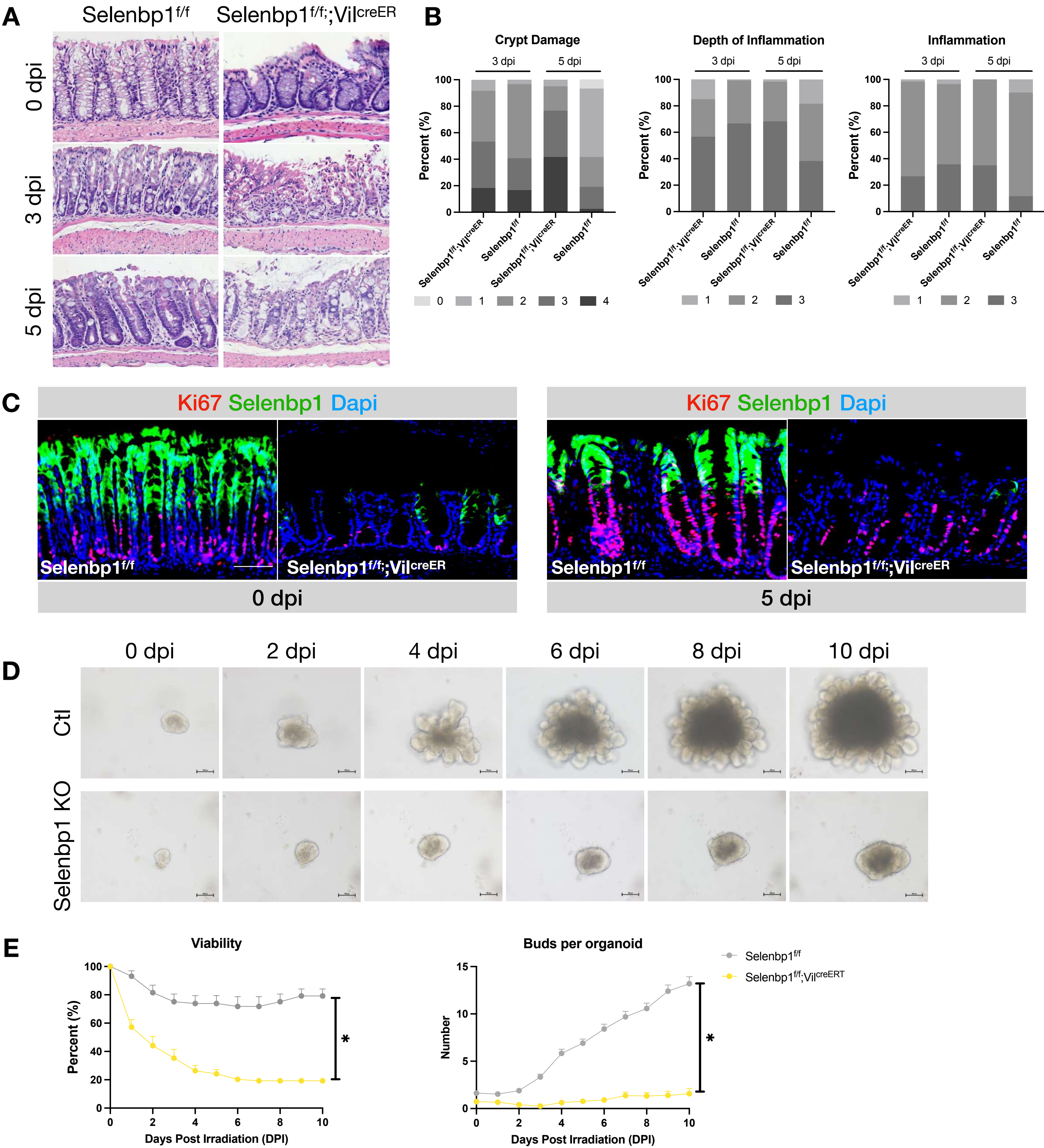


Figure 4

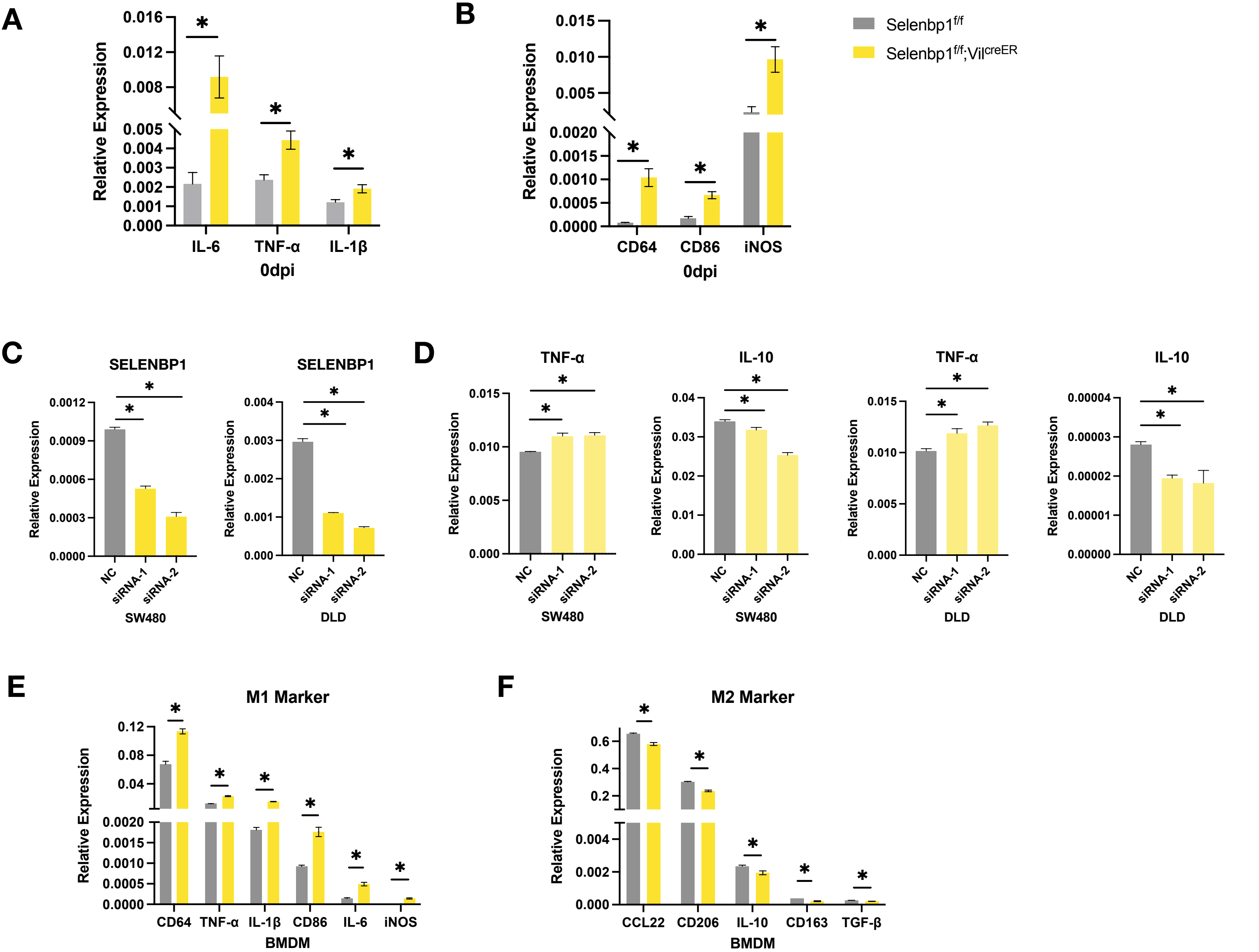


Figure 5

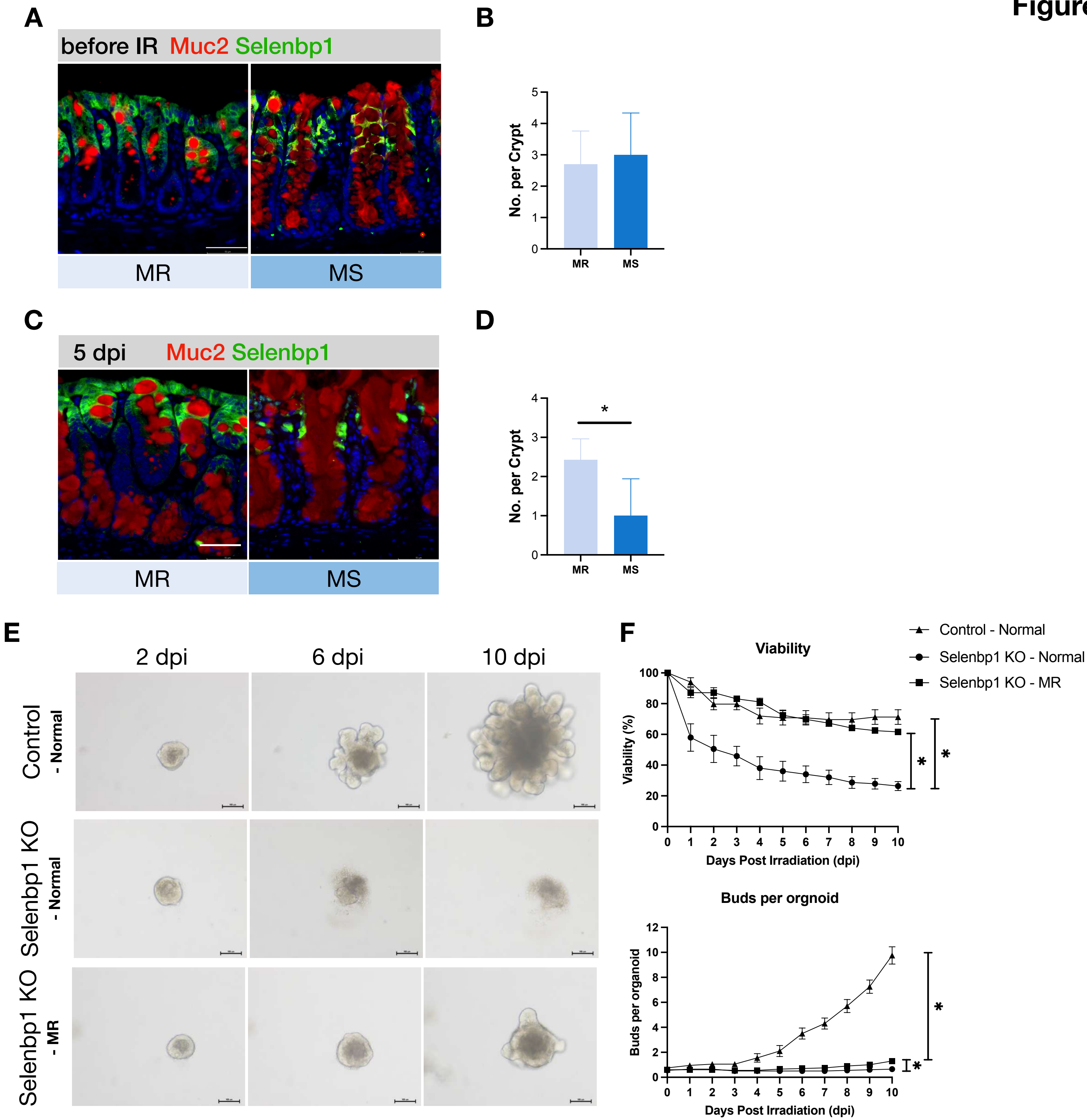


Figure 6

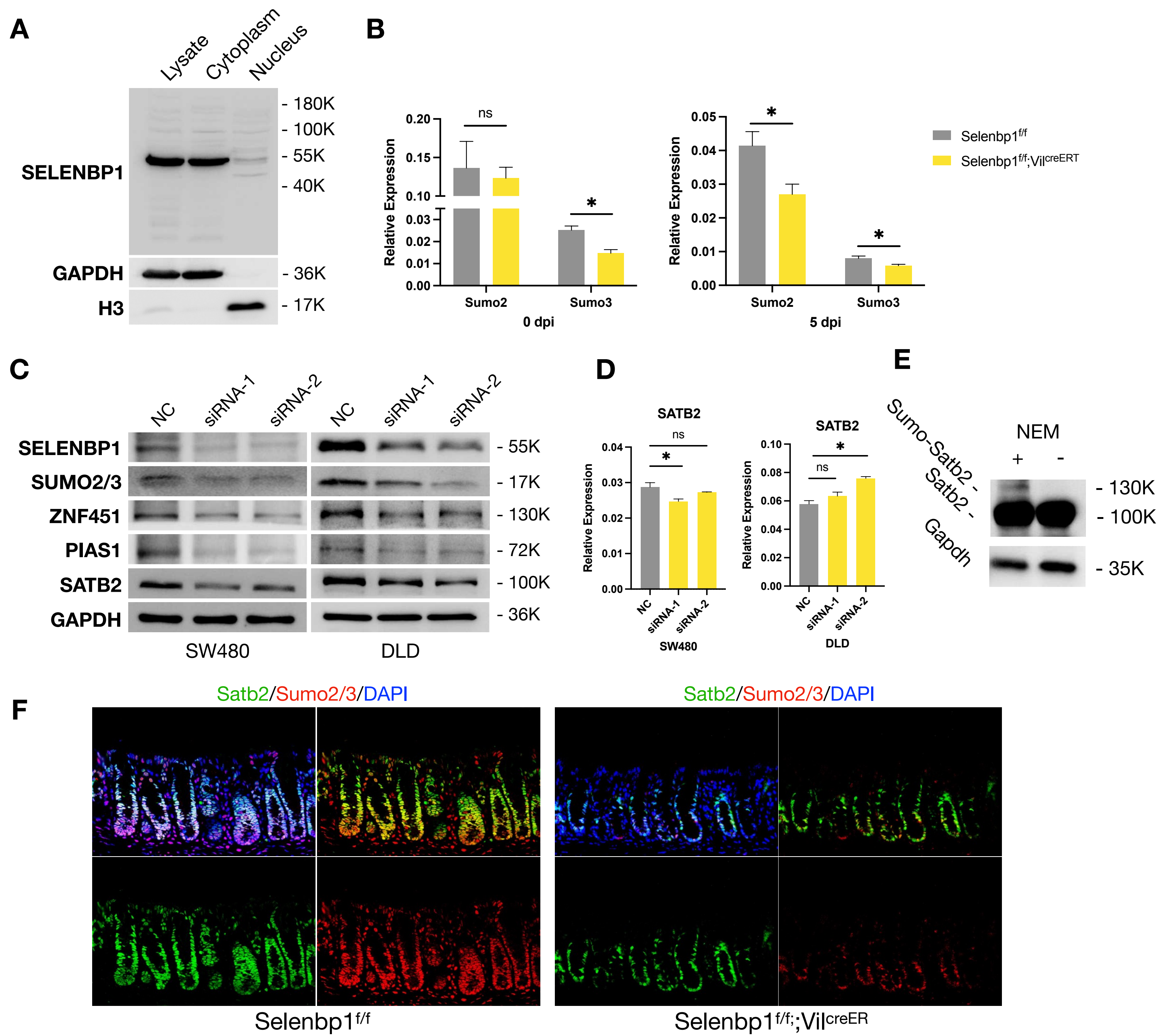


Figure 7

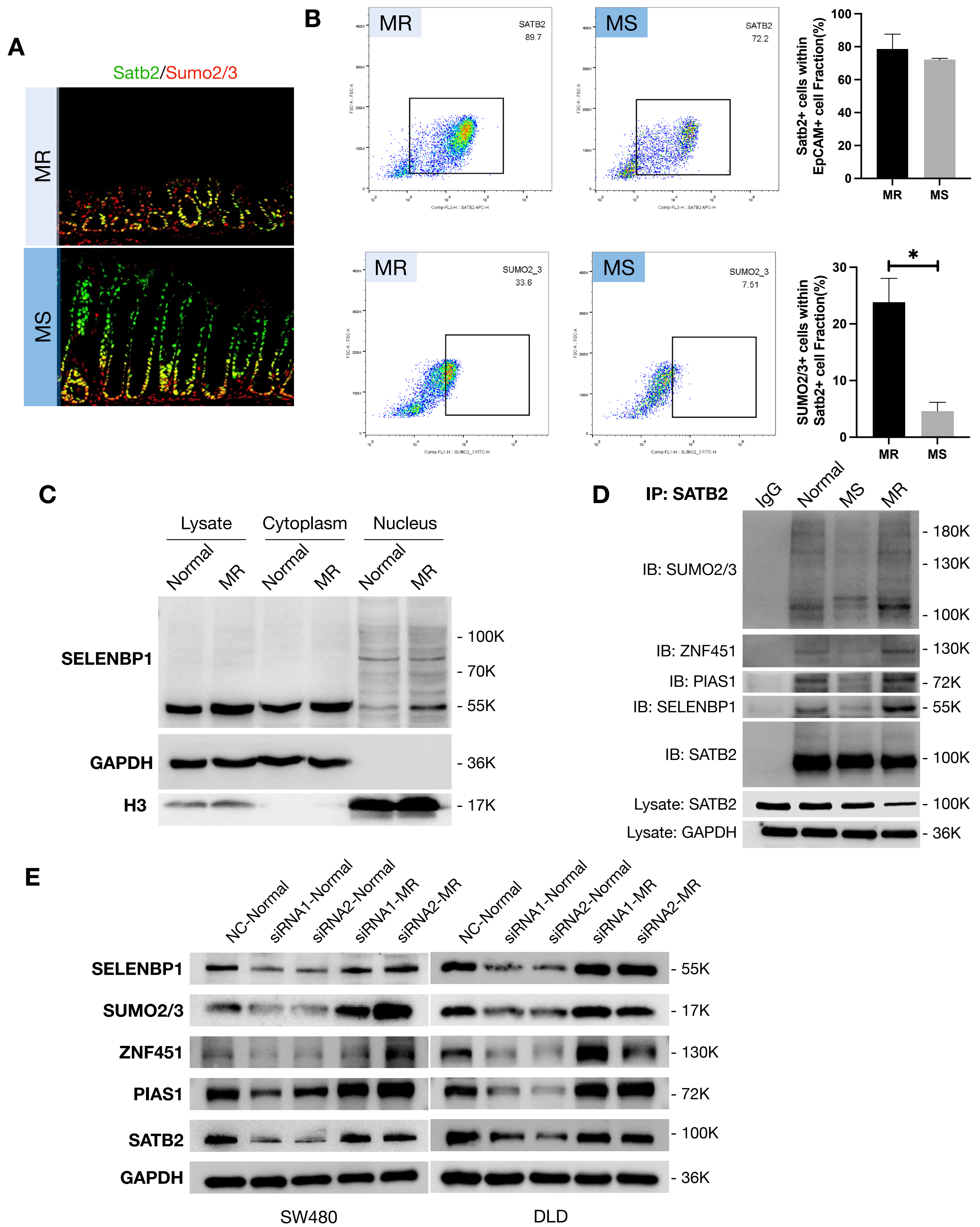
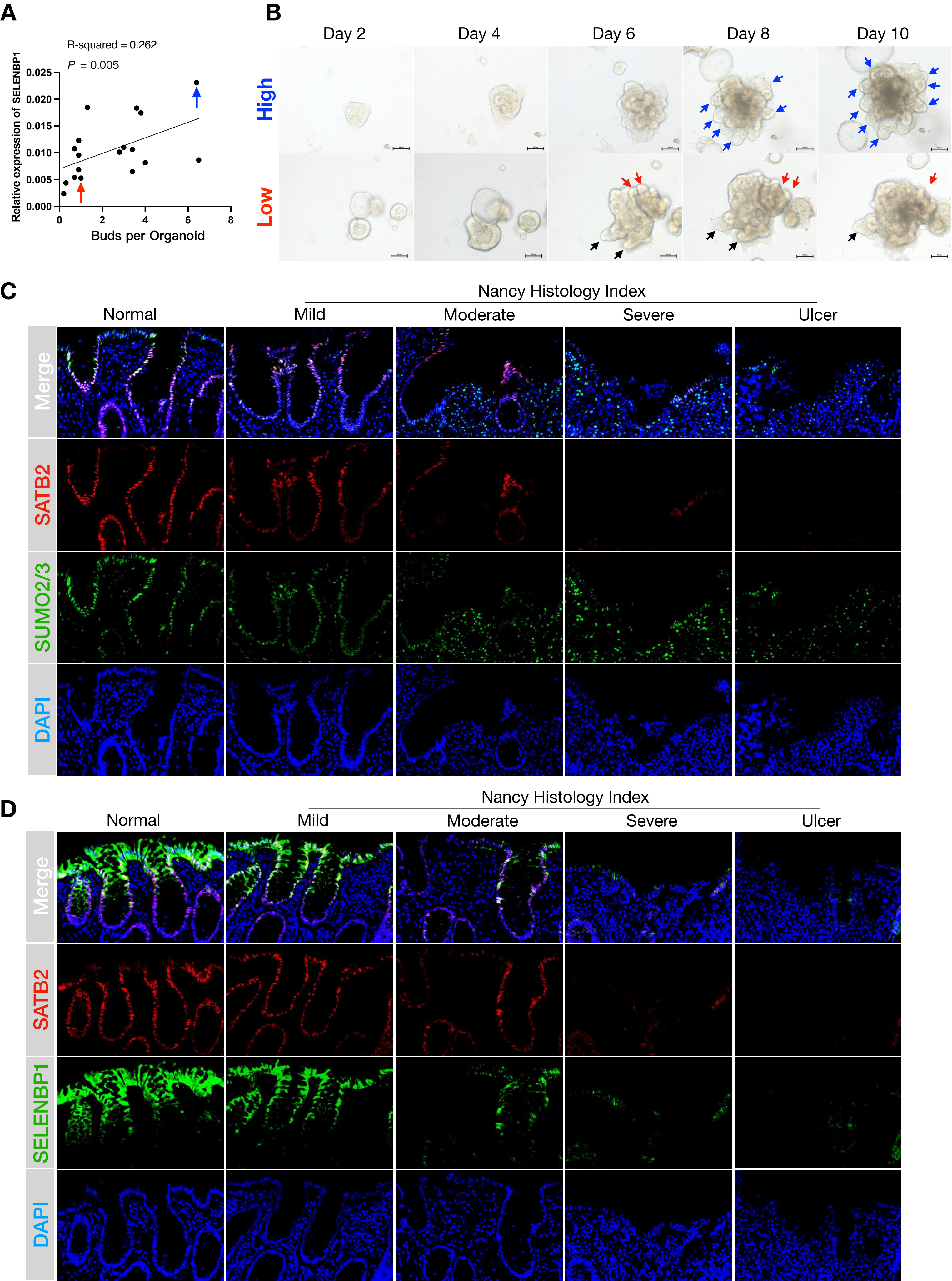


Figure 8



Supplementary Files

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

- [SupTables.doc](#)
- [SupFigs.pdf](#)
- [SupFigLegend.docx](#)