Telomere dysfunction instigates inflammation in inflammatory bowel disease

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Inflammatory bowel disease (IBD) is a chronic inflammatory condition driven by diverse genetic and nongenetic programs that converge to disrupt immune homeostasis in the intestine. We have reported that, in murine intestinal epithelium with telomere dysfunction, DNA damage-induced activation of ataxia-telangiectasia mutated (ATM) results in ATM-mediated phosphorylation and activation of the YAP1 transcriptional coactivator, which in turn upregulates pro-IL-18, a pivotal immune regulator in IBD pathogenesis. Moreover, individuals with germline defects in telomere maintenance genes experience increased occurrence of intestinal inflammation and show activation of the ATM/YAP1/pro-IL-18 pathway in the intestinal epithelium. Here, we sought to determine the relevance of the ATM/YAP1/pro-IL-18 pathway as a potential driver of IBD, particularly older-onset IBD. Analysis of intestinal biopsy specimens and organoids from older-onset IBD patients documented the presence of telomere dysfunction and activation of the ATM/YAP1/precursor of interleukin 18 (pro-IL-18) pathway in the intestinal epithelium. Employing intestinal organoids from healthy individuals, we demonstrated that experimental induction of telomere dysfunction activates this inflammatory pathway. In organoid models from ulcerative colitis and Crohn's disease patients, pharmacological interventions of telomerase reactivation, suppression of DNA damage signaling, or YAP1 inhibition reduced pro-IL-18 production. Together, these findings support a model wherein telomere dysfunction in the intestinal epithelium can initiate the inflammatory process in IBD, pointing to therapeutic interventions for this disease.

inflammatory bowel disease | telomere dysfunction | pro-IL-18 | Yap1 | DNA damage

Inflammatory bowel disease (IBD), classified mainly as Crohn's disease (CD) and ulcerative colitis (UC), has limited therapeutic options (1). While IBD targets primarily a young adult population (2, 3), 10 to 15% of IBD diagnoses manifest in individuals over age 50 and is termed elderly or older-onset IBD (4, 5). Moreover, young-onset IBD is characterized by recurrent episodes of progressively decreasing intervals and increasing severity (6). IBD has been linked to dysregulation of many biological processes driven by diverse etiological factors (7). These biological processes include 1) epithelial damage (8, 9); 2) immune system dysfunction with inflammation (10); 3) germline variants in genes governing cellular response to bacteria (*NOD2* and *TLR4*) (11, 12), autophagy (*ATG16L1* and *IRGM*) (13), and epithelial barrier function (*CDH1* and *LAMB1*) (13–15); and 4) altered gut microbiome and/or other environmental factors (15–17). Notably, none of these factors appears singularly capable of driving the disease, suggesting cooperative interactions (18–20).

Significance

Inflammatory bowel disease (IBD) is a chronic debilitating condition with limited treatment options. Recently, we found that telomerase reactivation can ameliorate intestinal inflammation in a mouse model of IBD through the regulation of the YAP1/pro-IL-18 axis. This study aims to validate the therapeutic potential of this axis genetically and with the use of small molecule activators and inhibitors in the intestinal epithelium of IBD patients.

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More recently, we reported that telomere dysfunction can drive an inflammatory process (21). Specifically, intestinal epithelium of mice engineered to experience telomere dysfunction showed an IBD-like inflammatory condition with up-regulation of precursor of interleukin 18 (pro-IL-18) (21). As pro-IL-18 is a major driver of IBD (22, 23), we audited and confirmed that individuals with germline variants associated with impaired telomere maintenance show activation of this pathway (21). Such experimental findings inform clinical observations where germline mutations in *TERC* (RNA component) or *TERT* (telomere enzyme subunit) are associated with increased risk of fibrotic and inflammatory diseases as well as cancer (24–26). On the pathological level, individuals with germline mutations in telomerase components show progressive villous atrophy, enterocolitis, and intraepithelial lymphocytosis (27).

A potential link between telomeres and IBD builds on prior work documenting shorter telomeres in UC intestinal epithelium, which may drive genomic instability and fuel increased cancer incidence in these patients (28). However, in this work, telomere erosion was considered to be a consequence of the inflammatory process, which increases epithelial damage and turnover as well as increases local reactive oxygen species (ROS) levels. In active IBD, high local ROS is generated by intestinal epithelial cells, neutrophils, and macrophages as a defense mechanism against invading pathogens (29, 30). In addition to telomere shortening associated with cell division, ROS-induced oxidation of telomere guanines leads to the formation of 8-oxoguanine, which impairs shelterin binding, leading to the disruption of telomere length maintenance and loss of capping function (31, 32). Thus, the shorter telomeres in IBD intestinal epithelia have been considered a consequence, rather than an instigator, of the inflammatory process (28, 33).

The generation and characterization of mice null for *TERC* or *TERT* have revealed that telomere shortening and loss of function cause age-associated degenerative pathology, including inflammation (34–36). With respect to ROS and telomeres, it is worth noting that telomere dysfunction activates an ATM- and ATR-dependent DNA damage response (37, 38), which up-regulates p53, which in turn represses peroxisome proliferator activating receptor gamma coactivator 1-alpha gene product (PGC1α) and peroxisome proliferator activating receptor gamma coactivator 1-beta gene product (PGC1β), the major regulators of mitochondrial biogenesis and function and of many genes involved in oxidative defense (39). Thus, as ROS levels increase, a feed-forward loop is established with increased p53-mediated repression of PGC with diminishing mitochondrial function, oxidative defense, and increasing telomere damage (39, 40). The importance of mitochondrial ROS in IBD is further evidenced by amelioration of disease in mouse models of experimental colitis upon treatment with mitochondrial ROS neutralizer mitoQ (41) as well as by findings of suppression of several mitochondrial genes in the mucosa of UC and CD patients (42–44).

Together, these studies point to an intimate link between telomere biology and inflammatory pathways of relevance to IBD. In particular, our previous work in telomere dysfunctional mice revealed that telomere dysfunction (and the DNA damage signal induced by short telomeres) activates ATM/cABL-driven phosphorylation and activation of the transcriptional coactivator YAP1 at the Y357 residue. Activated YAP1 up-regulates many IBD-relevant genes encoding the cytokine pro-IL-18 and inflammasome components, Nod-like receptor-containing proteins, NLRP1, NLRP3, NLRP6, and NLRC4 (21). Gut microbiome-mediated inflammasome activation of caspase-1 results in caspase-1–directed cleavage of pro-IL-18 into its mature form. Mature IL18 leads to the recruitment of T cells, which secrete IFNγ, further fueling local tissue inflammation (21). Thus, in the current study, we sought to document the role of telomeres in the regulation of pro-IL-18 in biopsies and organoids from older-onset IBD patients. Consistent with a role for telomere dysfunction as a disease driver, we demonstrate that the induction of telomere dysfunction can drive this inflammatory axis in healthy epithelial organoids, and, conversely, that telomerase activation can quell this pathway in IBD organoids. Together, these findings support a role for telomere dysfunction in IBD pathogenesis and provide therapeutic strategies for the treatment of this intractable disease.

Results

Telomere Shortening in Inflammatory Bowel Disease Correlates with Disease Severity. Telomere shortening has been documented in the intestinal epithelium of IBD patients (33), albeit without reported age stratification. Given that IBD affects both young and older adults, we undertook a systematic analysis of IBD patients who had a first diagnosis at age 50 or older and compared these data with age-matched healthy controls (Fig. 1 *A–C* and *SI Appendix*, Tables S1 and S2). Telomere length determinations via telomeric fluorescence in situ hybridization (FISH) assay documented a significant decrease in telomere reserves in the crypt epithelial cells of older-onset IBD patients (both ulcerative colitis and Crohn's disease) compared with age-matched healthy controls (Fig. 1 *A–C* and *SI Appendix*, Fig. S1 *A* and *B*). In patient biopsies, Kendall's rank analysis showed a significant negative correlation of disease severity score and telomere reserves (P = 5.3e-05) (Fig. 1*C*). Together, these data suggest a potential role for telomere dysfunction in the development of older-onset IBD.

DNA Damage in the IBD Patient Epithelium Activates YAP1-Mediated Inflammation. DNA damage signaling, ROS accumulation, and telomere attrition have been observed in both CD and UC (33, 45, 46). To understand the signaling networks activated by telomere damage in the human intestine and its relationship to human IBD, we intersected the well-established human 92 IBD gene signature of UC and CD colons (47) with the differentially expressed genes of the intestinal epithelium of telomere intact (G0, LSL-mTERT) heterozygotes versus telomere dysfunctional (G4, LSL-m TERT homozygotes) mice obtained from our previous publication (21). Strikingly, 59 of 92 genes were shared cross-species and included chemokines and their receptors (*CXCL1*, *CXCL2*, *CXCL5*, *CXCL9*, *CXCL10*, and *CCL20*), cytokines and receptors (*IFNγ* and *TNF*), bacterial sensing genes (*LCN2*, *LTF*, and *LYZ2*), intracellular signaling markers (GATA3 and STAT1), adhesion molecules (*ICAM1* and *VCAM1*), and genes associated with mucosal barrier (*MUC1*), tissue remodeling (*COL1A2* and *COL6A3*) and the complement system (*C3*). Ingenuity pathway analysis of these common murine and human genes showed expected connections to immune pathways such as T and B cell signaling, activation of IRF by cytosolic pattern recognition receptors, interferon signaling, and Th1- and Th2-mediated signaling, among others. Notably, this overlap also included the "role of BRCA1 in DNA damage response" pathway, reinforcing the notion that DNA damage signaling plays an active role in this disease in humans (Fig. 2*A*).

The findings of telomere shortening in the intestinal biopsies of IBD patients prompted analysis of telomere-associated DNA damage in the epithelium of these patients. Increased γH2AX signal was present in IBD epithelium compared with age-matched controls (Fig. 2 *B* and *C*) and this signal colocalized with telomere repeat sequences, consistent with extensive telomere dysfunction-induced foci (TIF) formation in the IBD epithelial biopsies (*SI Appendix*, Fig. S2*A*). Contemporaneously, we documented activation of the YAP1/IL18 axis in the IBD patient epithelium, consistent with our previous findings (21) of increased nuclear YAP1 and pro-IL-18 expression in the intestinal epithelium of telomere dysfunctional mice and of individuals with germline defects in telomere maintenance genes (Fig. 2 *D–G*).

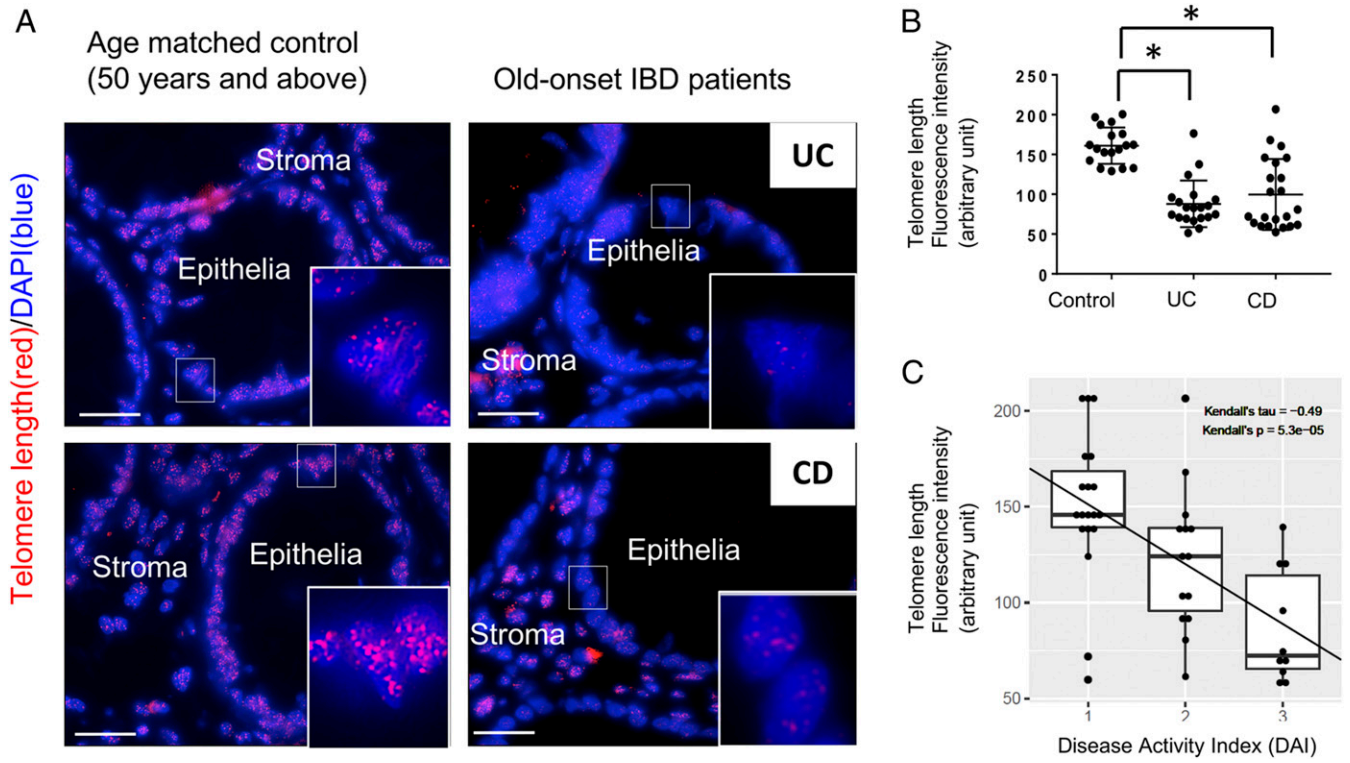

Fig. 1. Telomere shortening in inflammatory bowel disease patients correlates with disease severity. (*A*) FISH for telomeres in tissue from healthy controls or IBD patient biopsies (control, $n = 17$; UC, $n = 20$; CD, $n = 23$). Fluorescence was quantified as detailed in *Materials and Methods* indicating telomere length. (Scale bars, 10 μm.) *Insets* show magnification of the epithelial cells. (*B*) Quantification of telomere FISH from healthy and IBD biopsies (control, $n = 17$; UC, $n = 20$; CD, $n = 23$). (*C*) Kendall's correlation test was performed indicating significant association between telomere length and DAI (disease activity index). (Kendall's tau = –0.49, Kendall's $P = 5.3e\text{-}05$). *Statistically significant, $P < 0.05$ by unpaired Student's *t* test. *n*, number of mice or patient biopsies used in the study. Data are represented as mean ± SEM. Experiments were conducted at least two independent times. Also refer to *SI Appendix*, Table S1.

Induction of Telomere Dysfunction Mediates Immune Response in the Intestinal Epithelium. Our prior findings using a conditional inducible telomerase model (LSL-mTert; Lgr5-CreERT2), established that telomere dysfunction in the epithelium can initiate tissue inflammation, which can be reversed by telomerase reactivation in the epithelium (21). To determine whether telomere dysfunction and its activation of YAP1-pro-IL-18 in intestinal epithelia could be an early event in promoting inflammation in human IBD, telomere dysfunction was acutely induced in normal jejunal (J2) and duodenal (D109) epithelial organoids by expressing a dominant-negative mutant form of TRF2 (iTRF2ΔBΔM) (*SI Appendix*, Fig. S3*A*) known to induce telomeric fusions, TIFs, and cellular senescence (48). In human epithelial intestinal organoids, induction of iTRF2ΔBΔM expression led to γH2AX foci and pATM-mediated DNA damage signaling activation, activation of YAP1 (Y357 phosphorylation), and up-regulation of pro-IL-18 expression (Fig. 3*A* and *SI Appendix*, Fig. S3*B*). Colocalization of dysfunctional telomeres with DNA damage signals confirmed increased TIFs and no changes in telomere length as quantified by Southern blotting in the iTRF2ΔBΔM-expressing organoids (Fig. 3 *B* and *C* and *SI Appendix*, Fig. S3 *C* and *D*). Finally, gene set enrichment analysis (GSEA) of RNA-sequencing (RNA-seq) profiles from two independent iTRF2ΔBΔM-expressing organoids (J2 and D109) highlighted prominence of inflammatory pathways including the topmost pathways of "interferon alpha and interferon gamma," "TNFα signaling via NF-κB," and "inflammatory response" (Fig. 3 *D* and *E* and *SI Appendix*, Fig. S3*E*). Together, these results show that acute telomere dysfunction can rapidly up-regulate inflammatory pathways, further suggesting the possibility of dysfunctional telomeres as an instigator of intestinal inflammation.

Genetic and Pharmacologic Activation of Telomerase or Inhibition of YAP1 Suppresses Immune Activation Pathways in Human CD and UC Organoids. To further investigate the relevance of the ATM/YAP1/pro-IL-18 pathway in maintaining the proinflammatory state of the IBD epithelium, we established colon organoids derived from tissue biopsies from UC or CD patients aged 50 y or older. First, consistent with prior studies (33), extensive reduction in telomere signal was detected in the IBD organoids compared with the normal age-matched human colon organoids (*SI Appendix*, Fig. S4 *A* and *B*). The reduction in median telomeric length in UC was greater than CD, mirroring the telomere length measurements in the IBD patient biopsies (Fig. 1 *A* and *B* and *SI Appendix*, Fig. S4 *A* and *B*). Second, a CD duodenal organoid model with documented short telomeres and activation of the γH2AX/ATM/YAP1/pro-IL-18 pathway (Fig. 4 *A* and *B* and *SI Appendix*, Fig. S4*C*) showed that a 2-wk induction of TERT decreased DNA damage activation (pATM and pYAP1) and inflammatory (pro-IL-18) signaling (Fig. 4*C*). Correspondingly, TERT induction resulted in decreased TIFs consistent with reduced DNA damage signaling from telomere ends (Fig. 4 *A* and *B*). Finally, transcriptomic analysis revealed that TERT activation was associated with a significant reduction in inflammatory pathways (interferon alpha and interferon gamma) and oxidative phosphorylation and increases in cell proliferation (MYC, and E2F, and KRAS) and DNA repair pathways (*SI Appendix*, Fig. S4*D*).

To explore the therapeutic potential of our findings, older-onset human CD and UC organoids exhibiting high levels of DNA damage and the induction of the ATM/YAP1/IL18 pathway compared with the age-matched controls were treated with a well-established TERT activator (TA-65) and a known suppressor of DNA damage (nicotinamide adenosine dinucleotide [NAD]) (49),

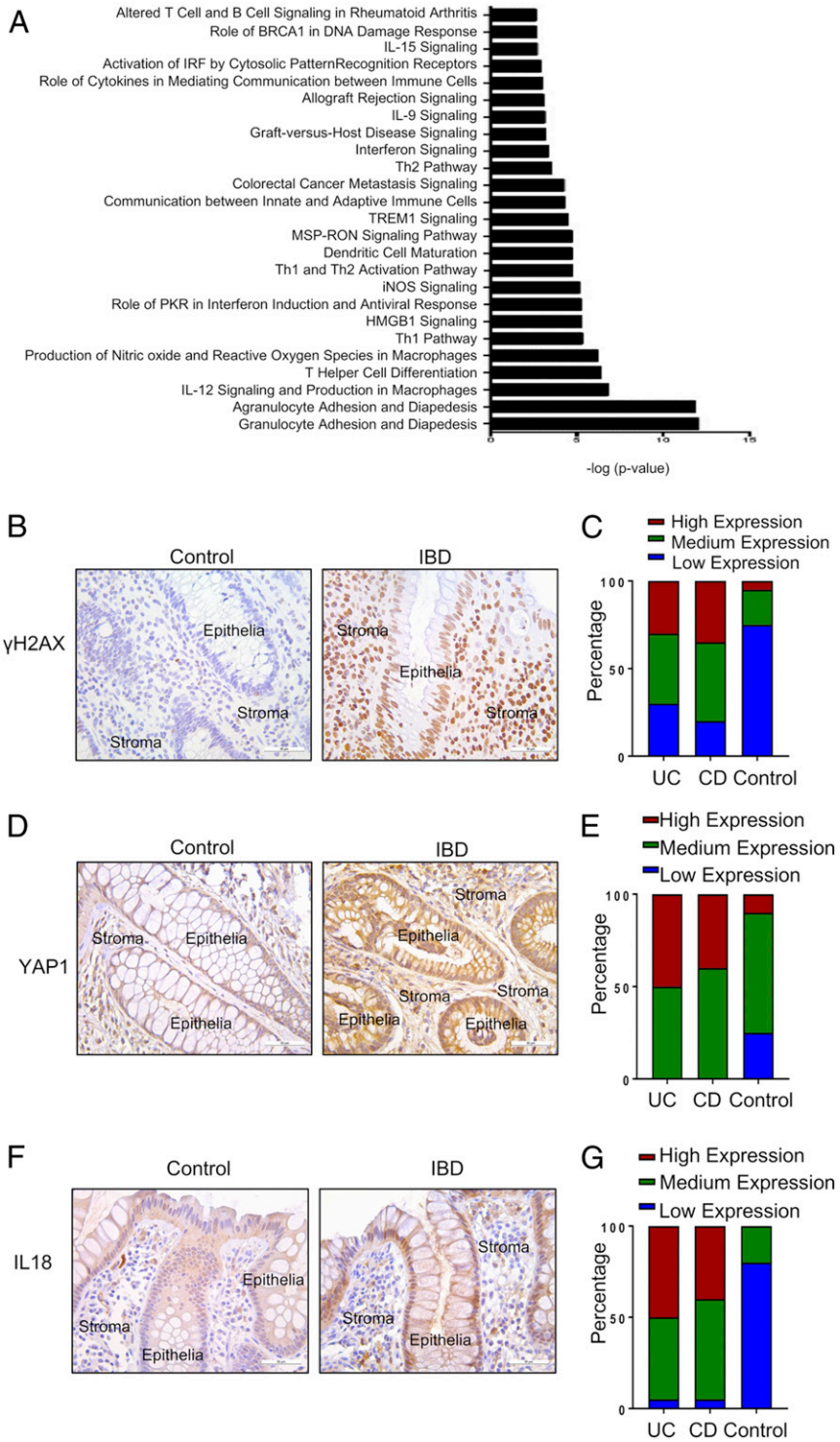

Fig. 2. DNA damage in the IBD patient epithelium activates YAP1-mediated inflammation. (*A*) Differentially expressed genes from human IBD RNA-seq and mouse RNA-seq (G0 vs. G4) overlapped to identify similarities in the genes and pathways that are deregulated in IBD patients. Top 25 pathways are shown. (*B*) Representative images of γH2AX immunohistochemistry in colonic biopsies from either healthy (control) or IBD patients showing representative images from each group. (Scale bars, 100 μm.) (*C*) Histogram representing quantification of staining intensity for γH2AX of colon biopsies from either healthy or IBD patients (control, $n = 20$; CD, $n = 20$; UC, $n = 20$). control vs. UC, $P = 0.0001$ and control vs. CD, $P = 4.088e\text{-}06$. (*D*) Immunohistochemistry on the colonic epithelium from healthy (control) and IBD patient biopsies for YAP1 showing representative images from each group (control, $n = 20$; UC, $n = 20$; CD, $n = 20$). (Scale bars, 30 μm.) (*E*) Histogram depicting the quantification of staining intensity for YAP1 in the colonic epithelium of healthy (control) and IBD patients. Control vs. UC, $P = 0.0031$ and control vs. CD, $P = 0.0001$. (*F*) IL-18 immunohistochemistry in colonic biopsies from either healthy (control, $n = 20$) or IBD patients ($n = 40$) showing representative images from each group. (Scale bars, 100 μm.) (*G*) Histogram shows the quantification of staining intensity for IL-18 from the colon biopsies of both healthy and patients with IBD. Control vs. UC, $P = 2.097e\text{-}05$ and control vs. CD, $P = 2.933e\text{-}05$. **n* represents number of patient biopsies used in the study. Each experiment was conducted at least two times.

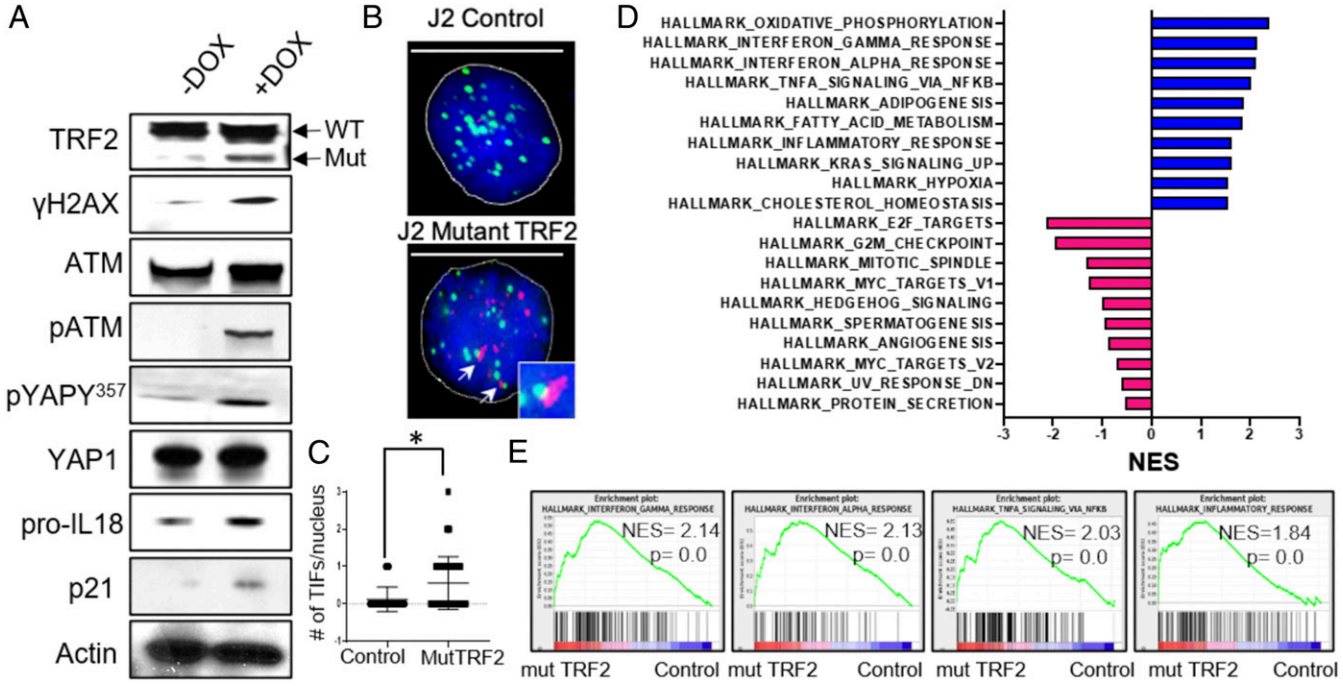

Fig. 3. Induction of telomere dysfunction mediates immune responses in human intestinal epithelium. (*A*) Western blotting performed with the indicated antibodies on protein lysates from organoids transfected with the DOX-inducible mutant TRF2 (iTRF2ΔBΔM) plasmid and treated with or without doxycycline for 7 d. (*B*) Micrographs of TIF assessment of the cultured organoids treated with or without doxycycline for 7 d. (*C*) Measurement of TIFs per nucleus counted in 50 cells of the organoids treated with or without doxycycline for 7 d. (Scale bar, 10 μm.) (*D*) RNA-seq analysis identifying differentially regulated pathways in the mutant organoids compared with the controls. Top 10 up-regulated (red) and down-regulated (blue) pathways are depicted. (*E*) GSEA graphs for the indicated pathways up-regulated in organoids expressing mutant TRF2 (TRF2ΔBΔM). *Statistically significant, $P < 0.05$ by unpaired Student's *t* test. Data are represented as mean ± SEM. Experiments were conducted at least two independent times.

which indirectly up-regulates sirtuins (Sirt1) and has been shown to be a substrate of sirtuins as well (50). Validation of Sirt1 protein up-regulation as well as up-regulation of its activity indicated increased expression of Sirt1 on treatment with NAD as well as enhanced deacetylation of p53 at K382, reflecting up-regulation of Sirt1 activity in both the UC- and CD-treated organoid lines (*SI Appendix*, Fig. S4*E*) (51). As expected, increased TERT levels were present in the TA-65–treated organoids, while no change in TERT levels was detected in the NAD-treated organoids (Fig. 4 *E–H*). A 10-d treatment period with either TA-65 or NAD resulted in reduced DNA damage signaling (γH2AX) and activation of the ATM/YAP1/pro-IL-18 axis (Fig. 4 *E–H*). Furthermore, reduced telomere damage signal was documented in the CD and UC organoids treated with either TA-65 or NAD compared with untreated controls (*SI Appendix*, Fig. S4 *F–I*). Our previous work demonstrated that YAP1 inhibition ameliorates intestinal inflammation in the telomere dysfunctional mouse model (21). Correspondingly, short-term treatment of human IBD organoids with the YAP1 inhibitor, verteporfin, produced a reduction in both total and activated YAP1 and pro-IL-18 levels (Fig. 4 *I–L*). Together, these data support a role for telomere dysfunction as a primary instigator of inflammation in human IBD via DNA damage-mediated activation of the ATM/YAP1/pro-IL-18 axis.

Discussion

In this study, analysis of human IBD tissues and derivative epithelial organoids established that telomere dysfunction activates ATM/Yap1-mediated up-regulation of immune network genes, including pro-IL-18. While prior work has established that inflammation and associated increases in ROS can drive telomere damage (52–54), our study establishes that telomere erosion/dysfunction is not merely a consequence of chronic inflammation but can serve as a key instigator of the inflammatory process in IBD. We demonstrate that pharmacological activation of TERT or inhibition of YAP1 blocks the production of pro-IL-18 in human UC and CD organoids, thereby providing a potential therapeutic strategy for this obdurate disease.

Our findings in human IBD build on our recent observations (21) that humans with germline telomere maintenance defects and mice with telomere dysfunction show activation of the ATM/YAP1/pro-IL-18 axis and associated intestinal inflammation, and that this inflammatory process can be quelled upon telomerase reactivation and restoration of telomere function. Of additional relevance to IBD where the gut microbiome plays a key cooperating role, these prior studies also demonstrated that microbiome-induced inflammasome activation results in caspase-1 activation, which cleaves pro-IL-18 into its mature form, resulting in IFNγ secretion by resident T cells to initiate an inflammatory cascade and colossal local tissue damage. Together, this body of work implicates telomere dysfunction as a driver of intestinal inflammation and identifies tractable targets for therapeutic intervention.

Germline alterations can directly alter function in immune cells, which can initiate and perpetuate intestinal inflammation in human IBD (13, 55–57). This study expands relevant cell types to include the intestinal epithelia as a prime source of key inflammatory factors known to drive disease pathogenesis. This concept aligns well with recent work identifying the intestinal epithelium as a source of mature IL18, which initiates downstream signaling through its binding to its receptor IL18R on the epithelial cells and exacerbates colitis in a murine model (58). In addition, chromatin immunoprecipitation (ChIP)-seq data of murine intestinal epithelium with activation of the telomere dysfunction/ATM/YAP1 pathway established a direct role for YAP1 in the transcriptional up-regulation of key inflammatory molecules, including pro-IL-18 (21). Together with our findings in the intestinal tissues of patients with IBD (Fig. 2 *D–G*), this mounting evidence

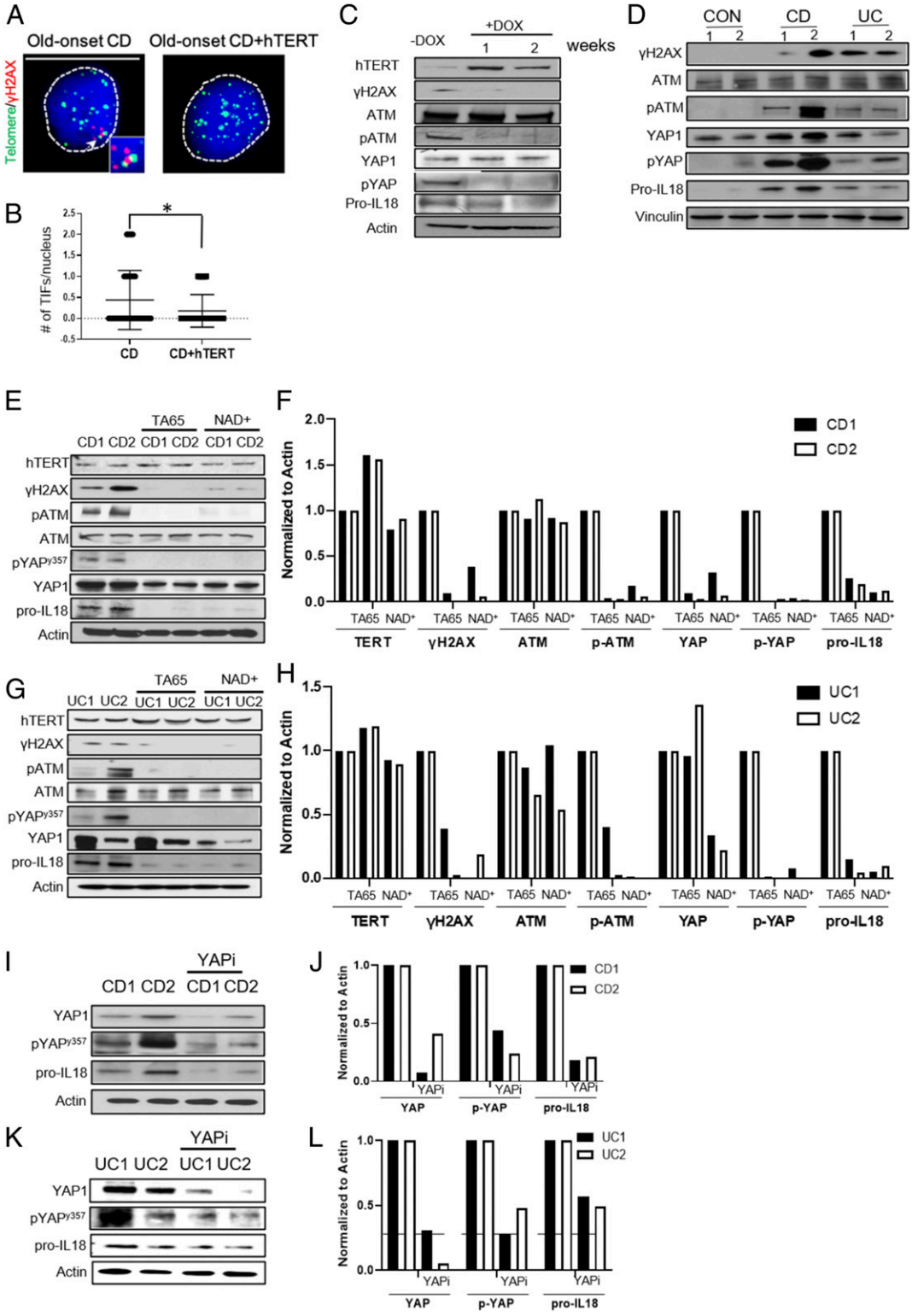

Fig. 4. Genetic and pharmacologic activation of telomerase or inhibition of YAP1 suppresses immune activation pathways in IBD epithelium. (*A*) Micrograph of TIFs in CD duodenal organoids with or without overexpression of telomerase. (Scale bar, 10 μm.) (*B*) Quantification of TIFs in duodenal organoids from CD patient with or without overexpression of telomerase. Fifty cells from each group were quantified for TIFs. (*C*) Western blotting of lysates from the CD organoid line transduced with an inducible hTERT overexpression plasmid treated with or without (–DOX) doxycycline for 1 or 2 wk. (*D*) Western blotting of lysates from control ($n = 2$), CD ($n = 2$), and UC ($n = 2$) organoids with the indicated antibodies, indicating up-regulation of DNA damage and the ATM/YAP1/IL18 axis. (*E* and *F*) Western blotting for the indicated antibodies of lysates from older-onset CD organoids treated with or without TA-65 or NAD for 10 d (*E*) and quantification (*F*). ($n = 2$). (*G* and *H*) Western blotting for the indicated antibodies of lysates from older-onset UC organoids treated with or without TA-65 or NAD for 10 d (*G*) and quantification (*H*). ($n = 2$). (*I* and *J*) Western blotting with the indicated antibodies of lysates from YAP inhibitor treated or untreated CD organoids (*I*) and quantification (*J*). ($n = 2$). (*K* and *L*) Western blotting with the indicated antibodies of lysates from YAP inhibitor treated or untreated UC organoids (*K*) and quantification (*L*). ($n = 2$). *n* represents number of organoid lines used in the study. Each experiment was conducted at least two times.

strongly implicates telomere dysfunction-induced activation of YAP1 in the intestinal epithelium as a key mediator of disease pathogenesis in IBD.

The pathogenic relevance of telomere dysfunction or genotoxic stress as an instigating factor in IBD is further reinforced by transcriptomic overlap of our telomere-deficient model and IBD tissues, which share the "BRCA-mediated DNA damage pathway" as a prominent signature. Correspondingly, analysis of biopsies from patients with UC and CD show elevated levels of DNA damage signaling compared with normal tissue controls (45, 59). A connection between development of IBD and genotoxic stress is also implied by the presence of single nucleotide polymorphisms (SNPs) in antioxidant gene loci such as *SOD2*(rs4880) and *GPX1*(rs1050450), which show reduced function in IBD patients (45). This link to oxidative defense genes is particularly relevant as oxidative stress can shorten and damage telomeres, which could potentially set in motion a feed forward loop of the ATM → Yap1 → pro-IL-18 inflammatory cascade, thus instigating or perpetuating IBD pathology along with the gut microbiome. The circumstantial link to DNA damage signaling, coupled with our experimental data, supports the hypothesis that telomere dysfunction-mediated YAP activation and induction of the inflammatory network could be a major pathway driving older-onset IBD. It is also tempting to speculate that telomere biology may also be relevant to young-onset IBD following an initial inflammatory insult; the associated increased ROS levels could damage telomeres and further drive inflammation as well as predispose individuals to disease recurrence. On the basis of this hypothesis, we suggest that further investigation into whether telomere shortening exists in the epithelium of young-onset IBD patients with recurrent disease is warranted. This model parallels the disease etiology of both Bloom and Werner syndrome patients, where genetic mutation of *WRN* or *BLM* does not initiate premature aging in mice but rather requires additional events mediated through telomere shortening (60–62). The early age of onset of the inflammatory conditions in patients with mutations in *ACD*, *TINF2*, and *TERT* are similar to the phenotypes found in very early onset (VEO)-IBD patients, who exhibit similar symptoms at a very early age (27).

Transcriptomic analyses of the telomere dysfunctional human organoids overexpressing mutant TRF2 revealed abundant immune-associated pathways as well as altered metabolism pathways, which have also been linked to inflammation (63). The prominence of altered metabolism would be consistent with our previous work establishing that telomere dysfunction activates p53-mediated suppression of PGC-1α/β, which impairs mitochondrial biogenesis and oxidative defense resulting in increased ROS levels (39). With respect to inflammation, the increased ROS associated with telomere dysfunction gains added significance in the context of this study as ROS can further provoke inflammation through the activation of NLRP3 (63) as well as NF-κB, which can up-regulate TNFα, a key inflammatory cytokine. Furthermore, ROS can also induce DNA damage particularly in the G-rich telomere sequences, which can lead to DNA double-strand breakage-induced inflammation (30). Finally, ROS has been shown to activate ATM, which can phosphorylate and activate YAP1 through cABL. Together, these studies underscore the importance of the telomere–ROS connection as drivers and amplifiers of inflammation.

Beyond Yap1 inhibition therapy, our study further highlights the beneficial effect of telomerase reactivation in the backdrop of inflammatory disease. This concept of telomerase activation therapy is supported by the mouse model of telomere dysfunction where telomerase reactivation in the epithelium reverses inflammation significantly and suppresses recruitment of immune cells to the intestine (21), as well as the human inflammatory bowel disease patient epithelia where genetic or small molecule-mediated telomerase reactivation leads to the suppression of inflammatory pathways. Evidence supporting such a beneficial effect of telomerase reactivation on suppressing inflammation also comes from a mouse model of experimental liver cirrhosis, where adenoviral-mediated telomerase gene delivery significantly improved liver function and suppressed inflammation (64). However, given the association of telomerase reactivation to the initiation of cancers especially in the context of loss of p53/p21 (34, 36) or RB/p16 senescence checkpoints (65), caution should be taken in the ideation and design of such therapeutic approaches. Along these lines, the safe application of telomerase therapy could be increased through aggressive cancer screening upfront as well as the use of intermittent cycles of telomerase therapy, which may be enough to provide sufficient telomere reserve for normal cell proliferation, yet be inadequate for continuous cancer cell growth.

In conclusion, our study identifies common pathways that are conserved between telomere dysfunctional mice and the IBD epithelium and establishes that telomere dysfunction can directly activate inflammatory pathways in the epithelium to provoke tissue inflammation. The conservation of these signaling pathways in IBD could provide preventive and therapeutic strategies for this inflammatory disease, highlighting YAP1 inhibition as a viable therapeutic target for clinical testing. Although this study focuses specifically on inflammatory bowel disease, it is tempting to speculate that this pathway will be at play in other inflammatory diseases exhibiting telomere shortening with or without germline mutations in telomere-related proteins, such as idiopathic pulmonary fibrosis, liver cirrhosis, kidney fibrosis, coronary diseases, psoriasis, and neurodegenerative diseases.

Materials and Methods

FISH for Telomeres. Human tissue biopsies were processed according to manufacturer's instructions. Briefly, the paraffin sections were deparaffinized in a series of xylene and a gradient of ethanol washes. The slides were incubated with a preheated Cy 5-labeled peptide nucleic acid (PNA) probe from Agilent/DAKO (catalog no. K532711-8) at 82 °C for 5 min before being incubated overnight at 4 °C. The slides were then imaged and specifically the signals from the epithelial cells were quantified in Photoshop CC and tabulated. Telomere intensity was measured from 50 cells from each biopsy using this method and the average plotted in the graph shown in Fig. 1*B*.

Immunofluorescence and TIF Analysis. Human tissue biopsies were processed for immunofluorescence as described previously (21) and incubated overnight with primary antibody against γH2AX (Millipore, 1:1,000). The slides were then processed for telomere FISH with the PNA probe from Agilent/DAKO, as described previously, and imaged (60, 66). Fluorescence intensity was quantified from at least 50 cells per biopsy sample and analyzed. TIF calculations were done by the following method: First we selected the positive nuclei from both the control organoid and TRF2 mutant organoids for damage. We then documented the number of telomere signals that colocalized with the damage signals within these selected nuclei and plotted the numbers. We did this for 50 nuclei from each biopsy section.

Histopathology Scoring of Patient Biopsies. Ten control patient biopsies were obtained from Selvi Thirumurthi at MD Anderson Cancer Center from patients undergoing screening colonoscopies. The other 10 control adult patient biopsies, 20 biopsies for older-onset Crohn's disease patients and 23 older-onset ulcerative colitis patients were obtained from the Digestive Disease Consortium at Baylor College of Medicine, or from the Department of Pathology at The University of Texas, or from the Department of Gastroenterology or the Department of Pathology at the Herlev Hospital, University of Copenhagen. All patient biopsies were deidentified. The hematoxylin/eosin-stained patient biopsies were scored by pathologists in a blinded manner based on the modified Riley histopathological disease activity score (67).

Immunohistochemistry. For immunohistochemistry, the epithelial cells at each intensity of staining were recorded on a scale of 1 (weak staining = low expression), 2 (moderate staining = medium expression), and 3 (strong staining = high expression). Biopsies from each patient were assessed in a blinded manner after examining three different microscopic fields for each biopsy, and scores were assigned. The following antibodies were used for staining: Anti-phosphohistone H2AX (Ser139) (Millipore, clone JBW301, 05-636, 1:1,000), total YAP1 (Novus Biologicals, NB110-58358, 1:1,000), and IL-18 (Sigma, HPA003980, 1:200).

Western Blotting and Antibodies. After removing Matrigel from enteroids, the cell pellets were lysed using RIPA buffer with added proteinase and phosphatase inhibitors and centrifuged. The DC protein assay kit (Bio-Rad) was used to measure protein concentrations and precast 3 to 8% Tris-acetate gradient and bis-Tris 4 to 20% gradient gels (Invitrogen) were used for sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting. The following antibodies were used: ATM (Abcam, ab32420, 1:1,000), anti-phosphohistone H2AX (Ser139) (Millipore, clone JBW301, 05–636, 1:1,000), pATM (Ser1981; Novus Biologicals, NB100-306, 1:500), total YAP1 (Novus Biologicals, NB110-58358, 1:1,000), pYAP1(Y357) (Abcam, ab62751, 1:1,000), pro-IL-18 (Proteintech, 10663-1-AP, 1:1,000), Sirt 1 (Abcam, ab110304, 1:1,000), p53 (Proteintech, 10442-1-AP,1:1,000), acetyl-p53 (Abcam, ab75754, 1:1,000), vinculin (Sigma, V9131, 1:1,000) and actin (Sigma, 1:5,000). Quantification of the Western blots were done with ImageJ 1.8.0_172.

Organoid Culture. Organoids were isolated from human biopsies of either normal subjects or patients with diagnosis of IBD (UC) as described previously in collaboration with the laboratory of E.V. Briefly, the biopsies were treated with 30 mM ethylenediaminetetraacetic acid (EDTA) solution for 30 min at 4 °C. The tissue pieces were then pipetted gently to release the crypts. These crypts were then seeded in Matrigel in the presence of high wingless and Int-1 (WNT) human organoid media in the presence of ROCK inhibitor (Y-27632) for 7 to 10 d as described previously (68). The other organoids specified for CD were obtained from the Digestive Disease Consortium organoid bank and cultured similarly in an undifferentiated state.

Viral Transduction. Enteroids were incubated in high WNT-containing organoid media supplemented with nicotinamide (10 μM, Sigma), CHIR99021 (1 μm, STEMCELL Technologies) and for 48 h for lentiviral transduction. Ultracentrifuged viral pellet was suspended in media supplemented with polybrene (1:1,000, Millipore), Y-27632 (1 μM, STEMCELL Technologies), Jagged-1 peptide (1 μM, Anaspec), *N*-acetylcysteine (1 μm, Sigma), nicotinamide (10 μM, Sigma), and CHIR99021 (1 μM, STEMCELL Technologies). Digested enteroids were viral infected by spinoculation at 600 × *g* for 45 min in a 48-well plate at 32 °C (69). Spinoculated enteroids were incubated for 3 to 6 h at 37 °C and plated with Matrigel. Infected enteroids were incubated in high WNT media with 2 μg/mL puromycin afterward (70). Enteroids were further treated with inhibitor for YAP1 (verteporfin, Selleckchem, 1 μM for 24 h) and assayed for changes.

RNA Isolation. RNA was isolated by direct addition of RLT buffer of the RNeasy Mini Kit (Qiagen) to wells containing ~100 enteroids. DNA was removed by treating with DNase from the kit (Qiagen). Purified RNA was sent for sequencing.

RNA-Seq. The Sequencing and Microarray Facility (SMF) core at MD Anderson Cancer Center performed RNA-seq using an Illumina's TruSeq kit and Illumina HiSeq2000 Sequencer. Converted sequencing data (Fastq) from raw binary base call (BCL) sequence files were aligned to the mouse reference genome (mm10) with STAR software. Raw gene read counts were analyzed by the HTSeq-count program and data normalization and analysis of differential expression were performed using the R package edgeR (log2 fold change ≥ 0.5 or ≤ -0.5 and *P* value ≤ 0.1). *P* values from these analyses were used for pathway enrichment analysis with GSEA software. The R package heat map was used for visualization of clustering. RNA from mouse colonic epithelial cells were isolated and sequenced using the Illumina HiSeq2000 Sequencer. In addition, we obtained a 92 IBD gene signature panel (common between the colons of ulcerative colitis and Crohn's disease patients) from a previous publication (47). These 92 genes were overlapped with the published differential gene expression (21) of G0, LSL-mTERT vs. G4, LSL-mTERT mice.

Cells, Plasmids, and Treatment. TRF2 mutant plasmid (plasmid #16069) was obtained from Addgene and cloned into the doxycycline inducible vector, pINDUCER 2.0 (plasmid #44012) from Addgene. The hTERT overexpression plasmid (plasmid #51637) was obtained from Addgene and cloned into the doxycycline vector pINDUCER 2.0 (plasmid #44012). Doxycycline was added at a concentration of 1 μg/mL to the cell cultures for the indicated amount of time. All the organoid lines were obtained from the Digestive Disease Consortium (DDC) Biobank. Organoids were treated with TA-65 (TA Sciences) at the dose of 10 μM every 24 h for the indicted times as previously described (71). Organoids were treated with NAD^+ free acid (Sigma) at a dose of 10 μM each day for the specified time lengths.

Southern Blot. Southern blot for telomere length assessment was performed according to the manufacturer's instructions and as described previously (72). Briefly, 1.5 μg DNA was separated on a 0.7% agarose gel at 20 V overnight. The gel was then denatured. The DNA was transferred to a nylon membrane and hybridization was done with the probe according to the manufacturer's instruction. Telomeres were visualized with X-ray films.

Statistical Analysis. GraphPad Prism 8.0.0 software was used for data statistical analysis. Data are represented as mean and SEM. All experiments were performed at least two independent times. Student's unpaired *t* test was used for comparing two groups, and $P \leq 0.05$ was considered to be significant. For count data, Fisher's exact test was used to calculate significance for patient biopsy immunohistochemistry. To assess correlation between telomere length and disease activity index, Kendall's rank correlation test was performed to assess the association between telomere length and disease activity index (DAI). Kendall's tau is a statistic measuring the strength and direction of association. The fitted line in boxplot is obtained from the linear model.

Data Availability. All study data are included in the article and/or *SI Appendix*.

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