

Hematopoietic telomerase delays premature aging through a telomere-independent immune–microbiota axis

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Abstract

Aging is driven by the interplay between immune dysfunction, microbiome dysbiosis and telomere attrition, yet how these hallmarks interact in vivo, and whether they can be therapeutically uncoupled, remains poorly understood. Here we use Rag1-deficient zebrafish, a vertebrate model of adaptive immune deficiency and premature inflammaging, to show that the gut microbiota and hematopoietic telomerase act as key, targetable modulators of immune-driven aging. Loss of adaptive immunity triggers premature intestinal degeneration, barrier dysfunction, systemic inflammation and progressive dysbiosis dominated by Pseudomonadota expansion. Depleting the microbiota rescues survival and attenuates intestinal and systemic inflammatory signatures, while transfer of *rag1*^{-/-} microbiota into immunocompetent hosts is sufficient to transmit mutant-like molecular features, establishing dysbiosis as a causal amplifier of the phenotype. Telomerase activity also shapes disease progression: genetic telomerase loss accelerates mortality, whereas tert overexpression specifically in hematopoietic cells extends lifespan, reprograms the hematopoietic transcriptome, and partially restores gut, liver and microbiota homeostasis. Notably, this rescue occurs without major changes in telomere length, indicating that hematopoietic telomerase acts through telomere-independent reprogramming of immune cell states rather than telomere elongation per se. Together, these findings define a functional immune-microbiota–telomerase axis controlling premature aging and identify hematopoietic telomerase as a non-canonical regulator of immune and organismal homeostasis, with potential relevance for human immunosenescence and inflammaging.

INTRODUCTION

Aging is accompanied by a progressive loss of tissue homeostasis and increased vulnerability to chronic diseases, reflecting the combined action of interconnected molecular, cellular and systemic alterations. Among the canonical hallmarks of aging, chronic low-grade inflammation (also known as inflammaging), telomere attrition and microbiome dysbiosis have emerged as central, mutually reinforcing drivers of organismal decline^{1,2}. Rather than acting as isolated processes, these hallmarks are increasingly viewed as components of a self-amplifying network in which immune dysfunction, microbial imbalance, DNA damage and impaired regenerative capacity converge to erode healthspan. Understanding how these processes interact in vivo remains a major challenge, particularly in vertebrate models that allow longitudinal manipulation of host immunity, microbiota composition and telomere biology.

The immune system is profoundly remodeled with age. Adaptive immune output declines through thymic involution, reduced lymphocyte production and contraction of antigen-receptor diversity, whereas innate immune activity becomes chronically engaged, giving rise to inflammaging^{3–5}. This imbalance is not simply a consequence of aging but may actively accelerate it. Immunodeficient states, including chronic viral infection and defects in adaptive immunity, are frequently associated with persistent inflammation, increased tissue damage and premature onset of age-related pathologies^{6,7}. However, how the loss of adaptive immune surveillance reshapes systemic aging trajectories, and which downstream mechanisms sustain this decline, remain incompletely understood.

One candidate mechanism is the intestinal microbiota, a highly dynamic ecosystem that is both shaped by host immunity and capable of modulating inflammatory, metabolic and regenerative pathways. Microbial communities contribute to immune maturation, epithelial barrier function and systemic homeostasis through pattern-recognition receptor signaling and the production of metabolites such as short-chain fatty acids, bile acids and tryptophan derivatives^{8–10}. Conversely, immune dysfunction can destabilize microbial ecology. In Rag1-deficient mice, which lack mature T and B lymphocytes, loss of adaptive immunity is associated with altered microbial diversity, expansion of pathobiont-associated taxa and increased inflammatory susceptibility^{11–13}. During aging, similar dysbiotic shifts — including reduced microbial diversity, loss of beneficial anaerobes and expansion of facultative or pro-inflammatory bacteria — have been linked to inflammaging, impaired barrier integrity and systemic immune activation^{14–16}. Moreover, microbiota-transfer experiments indicate that aged microbial communities can transmit inflammatory and functional decline to young hosts, whereas young microbiota can improve age-associated phenotypes in several animal models^{17–20}. These observations suggest that microbiota dysbiosis may be both a consequence and a driver of immune-mediated aging.

Telomerase and telomere biology provides a second mechanistic axis linking immune dysfunction to organismal decline. Progressive telomere shortening limits cellular replicative capacity and can trigger DNA-damage responses, apoptosis or senescence, thereby impairing tissue renewal and promoting inflammatory signaling through senescence-associated secretory programs^{21–24}. Telomerase, through its catalytic subunit TERT, counteracts telomere erosion and is required for long-term maintenance of proliferative compartments, including hematopoietic tissues^{25,26}. Experimental telomerase activation can delay or reverse degenerative phenotypes and extend lifespan in mouse models when appropriately controlled^{27–29}, whereas telomerase deficiency accelerates tissue decline and premature aging across vertebrate systems^{30–32}. Importantly, telomere dysfunction and inflammation are tightly interconnected: DNA damage and senescence promote inflammatory mediators, while inflammatory and oxidative stress can further accelerate telomere attrition, creating a feed-forward loop that may be especially detrimental in chronically activated immune environments^{33–36}.

The zebrafish provides a uniquely tractable vertebrate model to dissect this immune–microbiota–telomere axis in vivo. Zebrafish share conserved immune pathways, telomerase dynamics and microbiota-dependent regulation of intestinal and systemic physiology with mammals, while enabling genetic manipulation and longitudinal aging studies within a practical timeframe^{37–40}. In particular, Rag1-deficient zebrafish lack functional adaptive immunity but survive under conventional conditions, allowing the long-term consequences of adaptive immune loss to be studied without the severe infection burden typically observed in mammalian immunodeficient models^{41–43}. These mutants develop chronic innate immune activation, oxidative stress, DNA damage, telomere shortening, cellular senescence, tissue degeneration and reduced lifespan, thereby providing a vertebrate model of premature inflammaging⁴³.

Here, we use the Rag1-deficient zebrafish model to investigate whether intestinal microbiota dysbiosis and impaired telomere maintenance contribute causally to immune-driven premature aging. We show that loss of adaptive immunity is associated with profound remodeling of the intestinal microbiota, and that manipulation of this microbial ecosystem partially rescues aging-associated phenotypes. We further demonstrate that telomerase overexpression in the hematopoietic compartment extends lifespan and ameliorates the premature-aging phenotype, while also restoring features of the intestinal microbiota. Together, our findings identify microbiota dysbiosis and hematopoietic telomerase as functionally connected regulators of immune-mediated aging, with potential implications for understanding how immune dysfunction, microbial ecology and telomere biology interact during human aging.

RESULTS

1. Rag1 deficiency is associated with premature intestinal degeneration

To determine whether the premature aging phenotype observed in *rag1*^{-/-} zebrafish is associated with alterations in intestinal homeostasis, we first analyzed intestinal architecture in wild-type (WT) and Rag1-deficient fish at 8 months of age. Histological examination revealed marked structural alterations in *rag1* mutants compared with WT controls. The most evident change was a significant reduction in mucosal fold length (Fig. 1a,b), accompanied by a decrease in the estimated absorptive surface area (Fig. 1c). In addition, *rag1*^{-/-} intestines displayed a marked reduction in the number of goblet cells per mucosal fold, together with a tendency towards reduced goblet cell size (Fig. 1d-e). Consistently, both the mucus layer covering the intestinal epithelium and the lamina propria thickness were diminished in Rag1-deficient fish (Fig. 1f-h).

We next assessed whether these architectural changes were associated with altered immune-cell distribution within the intestine. Immunohistochemistry for Lcp1, a pan-leukocyte marker, revealed a reduction in intraepithelial immune cells in *rag1* mutants compared with WT fish (Fig. 1i,j). In contrast, Rag1-deficient intestines showed increased leukocyte infiltration within the lamina propria (Fig. 1k). Immunostaining for Mpx, a neutrophil-specific marker, indicated that the Lcp1-positive infiltrating cells were largely Mpx-negative (Fig. 1l), consistent with non-neutrophil leukocyte infiltration, potentially involving monocyte/macrophage populations⁴³.

Given the loss of intestinal integrity observed in *rag1* mutants, we next assessed whether these alterations were associated with increased cell death. TUNEL staining revealed a significantly higher number of TUNEL-positive cells in the intestinal mucosa and submucosa of Rag1-deficient fish compared with WT controls (Fig. 1m,n). Together, these results indicate that Rag1 deficiency is associated with premature degeneration of intestinal architecture, altered mucosal immune-cell distribution and increased intestinal cell death.

2. Rag1 deficiency causes systemic features of premature aging

We next asked whether the intestinal alterations observed in *rag1* mutants were associated with systemic pathology. Since the histological analysis suggested epithelial barrier disruption, we first assessed intestinal permeability using the Smurf assay, a vital-dye assay in which the dye remains confined to the intestinal lumen under homeostatic conditions but spreads throughout the body when barrier integrity is compromised⁴⁴. At 5 months of age, *rag1* mutants showed a significant increase in whole-body blue signal compared with WT controls, indicating increased intestinal permeability (Fig. 2a-b).

Consistent with impaired barrier function and possible systemic exposure to gut-derived microbial products, liver histology revealed pronounced vacuolization in *rag1* mutants compared with WT fish (Fig. 2c-d). Moreover, qPCR analysis of bacterial 16S rRNA genes in liver genomic DNA detected bacterial sequences in a subset of *rag1*^{-/-} individuals, whereas no signal was detected in WT controls (Fig. 2e). These findings support the presence of barrier dysfunction and suggest that Rag1 deficiency may promote microbial translocation or systemic exposure to microbial components.

We next examined whether these alterations were accompanied by systemic inflammatory activation. Using the *Tg(nfkb:eGFP)* reporter line, we observed heterogeneous but locally increased NF-κB activity in *rag1* mutants at 3 months of age, with focal activation detected in several body regions (Fig. 2f-g). Although whole-body NF-κB activity varied among individuals, these data are consistent with a state of inflammatory activation in a subset of Rag1-deficient fish. Additionally, automated behavioral tracking at 8 months of age. *rag1* mutants displayed a marked reduction in total distance travelled and rotation frequency, together with a downward trend in mean swimming velocity (Fig. 2h and Supplementary Fig. 1), consistent with systemic functional decline.

Finally, to study the systemic immune consequences of Rag1 deficiency at the molecular level, we performed bulk RNA-seq of whole-kidney marrow (WKM) from 4-month-old zebrafish. Principal component analysis showed clear transcriptional separation between *rag1* mutants and WT controls (Fig. 2i), and differential expression analysis confirmed broad remodeling of the hematopoietic compartment (Fig. 2j), in agreement with previous observations in this model⁴³. Gene Ontology enrichment analysis revealed a prominent antiviral and immune-response signature, including upregulation of interferon-stimulated genes such as *mx*a, *mx*b, *mx*f, *mx*h, *ifi*44a8, *ifi*44e and *ifit*8, together with inflammatory mediators including *cas*p*b* and *il*1*b* (Fig. 2k-l). KEGG pathway analysis further highlighted enrichment of base-excision repair and metabolic pathways (Supplementary Fig. 2). Together, these results indicate that the intestinal degeneration observed in *rag1*^{-/-} mutants is accompanied by barrier dysfunction, systemic tissue pathology, inflammatory activation, behavioral decline and broad hematopoietic transcriptional remodeling.

3. Rag1 deficiency induces broad transcriptional remodeling of the aging intestine

To identify the molecular programs underlying the intestinal alterations observed in Rag1-deficient zebrafish, we performed bulk RNA-sequencing of whole-intestine tissue from 4-month-old WT and *rag1*^{-/-} fish. Principal component analysis showed a clear separation between genotypes (Fig. 3a). Differential expression analysis identified 2,351 differentially expressed genes, DEGs, adjusted $P < 0.05$, $|\log_2 \text{fold change}| \geq 1$, including 1,103 downregulated and 1,248 upregulated genes in *rag1* mutants (Fig. 3b).

rag1^{-/-} intestines showed increased expression of chemotactic and pro-inflammatory mediators, including *lta4h*, *ltb4r*, *cxcl11* paralogues, *cxcl14*, *saa*, *crp2*, *mrc1b* and complement components, whereas several antiviral transcripts, including *ifit* genes, *ifitm1*, *irf7*, *irf1b*, *mxs*, *mxs* and *ifnphi2*, were reduced. Genes associated with extracellular matrix organization, cell adhesion and tissue remodeling were also strongly altered, including laminins, collagens, integrins, *fn1a*, *vwf*, *mmp9*, *mmp13a* and *loxl1*. In addition, *rag1*^{-/-} mutants displayed dysregulation of tight-junction and adherens-junction components, including claudins, *jam2b*, *jam3b*, *tjp1b* and *vcla*, consistent with impaired epithelial barrier organization (Fig. 3c and Supplementary Fig. 3a-n).

Gene Ontology enrichment analysis highlighted immune-related processes, complement activation, antigen receptor-mediated signaling and extracellular matrix remodeling (Fig. 3d). KEGG pathway analysis further revealed enrichment of ECM-receptor interaction, focal adhesion, cytoskeleton-related pathways and metabolic processes (Supplementary Fig. 3o). Gene set enrichment analysis showed that *rag1*^{-/-} intestines were enriched for programs associated with arachidonic acid metabolism, cellular senescence and p53 signaling, whereas WT intestines were enriched for fatty acid metabolism, ribosome biogenesis and autophagy (Supplementary Fig. 3p-u). These data indicate that Rag1 deficiency induces an intestinal transcriptional state characterized by inflammation, tissue remodeling, barrier disruption and aging-associated stress.

To define the cellular origin of these changes, we generated a single-cell RNA-seq atlas of intestines from 4-month-old WT and *rag1*^{-/-} zebrafish. After quality control and integration, we identified 23 transcriptionally distinct clusters encompassing epithelial, progenitor/stem-like and immune populations (Fig. 3e and Supplementary Fig. 4a-c). Single-cell analysis revealed a marked remodeling of the epithelial compartment in *rag1* mutants, including loss of an early absorptive enterocyte-like cluster and reduction of stem/progenitor populations (Supplementary Fig. 4d), suggesting that Rag1 deficiency impairs epithelial renewal.

In addition, two clusters present almost exclusively in *rag1* mutants - termed as Unknown1 and Unknown2 - showed high mitochondrial transcript content while retaining an enterocyte-like signature (Fig. 3f-g and Supplementary Fig. 4e). Their position close to the absorptive lineage suggests that they may represent stressed or dying enterocyte-like cells, consistent with the increased TUNEL staining observed in mutant intestines.

Cell-type-specific differential expression analysis showed that the strongest transcriptional remodeling occurred in the absorptive epithelium. Enterocytes from *rag1* mutants upregulated genes involved in antigen presentation, arachidonic acid metabolism, interferon signaling and metabolic stress, while downregulating genes associated with survival, proliferation, differentiation and lipid handling (Fig. 3h and Supplementary Fig. 5a-b). Within the stem/progenitor compartment, mutant cells showed repression of epithelial renewal-associated genes, including *irf6* and *ppdpf*. Among immune populations, macrophages displayed enrichment of phagocytosis, apoptotic-cell clearance and innate immune activation programs, consistent with increased epithelial damage and cell death.

Together, these bulk and single-cell transcriptomic analyses show that Rag1 deficiency remodels the intestine through coordinated epithelial immune activation, barrier disruption, impaired epithelial renewal and accumulation of stressed enterocyte-like cells. These molecular changes provide a mechanistic basis for the intestinal degeneration, permeability defects and increased cell death observed in *rag1* mutants.

4. Rag1 deficiency disrupts intestinal microbiota

To determine whether Rag1 deficiency alters the intestinal microbiota during the progression of premature aging, we performed 16S rRNA sequencing of gastrointestinal samples from WT and *rag1*^{-/-} zebrafish at 2, 4 and 9 months of age. Beta-diversity analysis showed no clear genotype-dependent separation at 2 months, whereas differences became apparent at 4 months and were most pronounced at 9 months, when PCoA analysis revealed a clear segregation between WT and *rag1*^{-/-} samples (Fig. 4a–c). At this later stage, *rag1* mutants also showed a significant reduction in alpha diversity, as measured by the Shannon index (Fig. 4d), together with a decreased total bacterial load quantified by 16S qPCR (Fig. 4e). These data indicate that Rag1 deficiency drives a progressive disruption of intestinal microbial ecology.

Taxonomic analysis revealed that this dysbiosis was characterized by a marked expansion of Pseudomonadota in *rag1* mutants, particularly at 9 months of age (Fig. 4f and Supplementary Fig. 6). At this stage, Pseudomonadota represented the dominant phylum in mutant fish, whereas Fusobacteriota, Bacteroidetes and Bacillota were markedly reduced. Within Pseudomonadota, Gammaproteobacteria were strongly enriched, including members of the Enterobacteriaceae and Aeromonadaceae families (Fig. 4g,h). In contrast, Alcaligenaceae were reduced (Fig. 4j). Within Bacillota, Streptococcaceae, including *Streptococcus* spp., increased in *rag1* mutants (Fig. 4i). Conversely, Fusobacteriaceae, including *Cetobacterium* and *C. somerae*, were strongly depleted (Fig. 4k), as were Bacteroidaceae and the genus *Bacteroides* within Bacteroidetes (Fig. 4l). Thus, the aging *rag1*^{-/-} gut microbiota undergoes a shift towards Pseudomonadota-dominated communities, accompanied by loss of bacterial groups commonly associated with intestinal homeostasis in zebrafish.

To investigate whether these compositional changes were accompanied by functional alterations in the microbial community, we reconstructed the bacterial metatranscriptome from whole-intestine bulk RNA-

seq data using SqueezeMeta. In agreement with the 16S rRNA-seq data, bacterial transcripts mapping to Pseudomonadota were increased in *rag1*^{-/-} intestines, including transcripts assigned to Shewanellaceae, Aeromonadaceae, Comamonadaceae, Burkholderiales and other unclassified Pseudomonadota groups (Supplementary Fig. 7a-b). Principal component analysis based on bacterial open reading frames showed a clear separation between WT and *rag1*^{-/-} samples (Fig. 4m) and differential expression analysis identified 462 bacterial ORFs that were significantly altered in *rag1* mutants, of which 411 were upregulated and 51 were downregulated compared with WT controls (Supplementary Fig. 7c). Functional enrichment analysis of KEGG-annotated bacterial ORFs revealed significant remodeling of microbial metabolic pathways in *rag1*^{-/-} intestines (Fig. 4n). The most strongly upregulated pathways included glyoxylate, fatty-acid, butanoate and tryptophan metabolism, and taxonomic assignment indicated that these changes were largely driven by Pseudomonadota genera such as *Escherichia*, *Shewanella* and *Aeromonas* (Supplementary Fig. 7d-f). Together, these results show that Rag1 deficiency induces a progressive, age-associated dysbiosis characterized by reduced diversity and bacterial load, expansion of Pseudomonadota-dominated communities and altered microbial metabolic activity.

5. The intestinal microbiota contributes to premature aging in zebrafish

5. The intestinal microbiota contributes to premature aging in *rag1*^{-/-} zebrafish

After defining the intestinal and microbial alterations of *rag1* mutants, we next asked whether dysbiosis functionally contributes to their premature-aging phenotype. Initial fecal microbiota transplantation, FMT, experiments showed that WT microbiota failed to stably reconfigure the *rag1*^{-/-} gut ecosystem (Supplementary Fig. 8a-d). These observations suggested that the Rag1-deficient host environment constrains microbial engraftment and prompted us to test whether depletion of the resident microbiota could attenuate disease progression.

Starting at 3 months of age, *rag1*^{-/-} fish received a weekly broad-spectrum antibiotic treatment, ABX, and were analyzed at 5 and 8 months of age, while a parallel cohort was monitored for survival (Fig. 5a). ABX-treated *rag1* mutants showed a visibly healthier appearance by 7 months of age (Fig. 5b), and 16S rRNA qPCR confirmed a significant reduction in total bacterial load compared with untreated *rag1*^{-/-} siblings (Fig. 5c). Importantly, antibiotic treatment significantly extended survival of *rag1* mutants (Fig. 5d), indicating that microbial depletion attenuates the premature-aging phenotype.

To determine whether this improvement was associated with intestinal molecular rescue, we performed bulk RNA-seq of intestinal tissue from untreated and antibiotic-treated *rag1* mutants. Principal component analysis separated ABX-treated fish from untreated mutants along the first principal component (Fig. 5e), and differential expression analysis identified 363 genes altered by antibiotic treatment, including 279 downregulated and 84 upregulated genes, adjusted $P < 0.05$, $|\log_2FC| \geq 1$ (Fig. 5f). KEGG enrichment analysis highlighted pathways related to amino-acid, folate, lipid and steroid

metabolism (Fig. 5g). Notably, several genes previously elevated in *rag1*^{-/-} intestines shifted downward after antibiotic treatment, including complement components, extracellular matrix-remodeling genes and lipid-oxidation genes (Fig. 5h). Conversely, genes previously suppressed in *rag1* mutants, including methylation- and mitochondria-associated transcripts, increased toward WT levels. RT-qPCR confirmed reduced expression of the *rag1*^{-/-}-associated intestinal markers *mmp9* and *tp53inp1* after antibiotic treatment (Fig. 5i,j), supporting partial rescue of the mutant intestinal transcriptional state.

We next assessed whether microbiota depletion also affected systemic pathology. Bulk RNA-seq of WKM revealed marked transcriptional remodeling in ABX-treated *rag1* mutants compared with untreated siblings, with 483 downregulated and 305 upregulated genes (Fig. 5k). Enrichment analysis identified pathways related to proteasome function, protein folding, cell-cycle regulation and cellular senescence (Fig. 5l and Supplementary Fig. 9a). Strikingly, the antiviral and inflammatory signature characteristic of *rag1* mutants was attenuated after antibiotic treatment, including reduced expression of *mx* genes, *ifit* genes, *ifi44* paralogues and inflammatory mediators such as *gbp1*, *cxcl11* paralogues, *cxcl19*, *cxcl8b.1* and *caspb* (Supplementary Fig. 9b-c). Thus, microbial depletion partially normalizes both intestinal and systemic transcriptional alterations in *rag1* mutants.

Histological analysis at 8 months showed that antibiotic treatment did not fully restore intestinal architecture (Supplementary Fig. 10a-b). However, epithelial stress was partially reduced, as shown by a decrease or downward trend in eosinophil density and TUNEL-positive cells in ABX-treated *rag1*^{-/-} intestines (Fig. 5m-n and Supplementary Fig. 10c). These results indicate that microbiota depletion mitigates epithelial damage and systemic inflammatory remodeling, although it does not completely reverse intestinal degeneration.

Finally, we asked whether the dysbiotic microbiota of *rag1* mutants is sufficient to perturb intestinal homeostasis in an immunocompetent host. To this end, fecal material from *rag1*^{-/-} donors was transferred into WT recipients resulting in an efficient colonization and induction of a mutant-like microbial configuration (Fig. 5o and Supplementary Fig. 8a-d) together with marked intestinal transcriptional remodeling (Fig. 5p). Differential expression analysis identified 978 DEGs in WT recipients of *rag1*^{-/-} microbiota, including 491 downregulated and 487 upregulated genes (Fig. 5q). GO enrichment analysis revealed alterations in metabolic pathways, lipid catabolism, circadian regulation and response to xenobiotic stimulus (Fig. 5r). Importantly, a subset of genes altered in WT recipients of *rag1*^{-/-} microbiota shifted in the same direction as in *rag1* mutants (Fig. 5s). These included upregulation of complement components, cytoskeletal and cell-adhesion genes, prostaglandin-related transcripts and insulin-like growth factor signaling components, together with repression of developmental regulators. Moreover, the effects of *rag1*^{-/-} microbiota were not restricted to the intestine. WT recipients of *rag1*^{-/-} microbiota showed increased expression of systemic inflammatory genes previously associated with the *rag1*^{-/-} WKM phenotype, including *mmp9* and *caspa* (Fig. 5t-u). These findings indicate that the dysbiotic microbiota of *rag1* mutants can transmit intestinal and systemic inflammatory features to immunocompetent hosts.

Together, these results demonstrate that dysbiosis is not merely a consequence of Rag1 deficiency, but a functional amplifier of immune-driven premature aging. Microbiota depletion partially rescues survival, intestinal stress and systemic inflammatory transcriptional programs, whereas transfer of *rag1*^{-/-} microbiota to WT fish is sufficient to induce mutant-like molecular features.

6. Telomerase modulates Rag1 deficiency-induced premature aging

To determine the potential role of telomerase in *rag1*^{-/-} zebrafish phenotype, we first crossed the *rag1* mutant line with a previously characterized *tert* knockout line. Longitudinal survival analysis showed that loss of telomerase significantly reduced lifespan in the *rag1*^{-/-} background, accompanied by increased expression of the inflammatory markers (Supplementary Fig. 11). These data indicate that telomerase deficiency exacerbates inflammation and accelerates premature aging in Rag1-deficient zebrafish.

Because *rag1*^{-/-} premature aging is associated with chronic activation of the innate immune system, we next asked whether increasing telomerase expression specifically in blood cells could modify disease progression. To this end, *rag1* mutants were crossed with the *drl:tert* transgenic line, which expresses full-length *tert* under the hematopoietic *drl* promoter (Fig. 6a). *rag1*^{-/-} *drl:tert*^{+/-} fish showed robust *tert* overexpression in WKM, whereas *tert* levels were not increased in the intestine, confirming tissue-restricted transgene expression (Fig. 6c,d). Regarding telomerase activity in WKM extracts, only a modest increase was detected upon telomerase overexpression, possibly reflecting the relatively small proportion of *tert*-overexpressing cells within the tissue and/or post-transcriptional regulation limiting the formation of enzymatically active telomerase complexes (Supplementary Fig. 12). Phenotypically, *rag1*^{-/-} *drl:tert*^{+/-} fish displayed improved external appearance, including brighter pigmentation and fewer lordotic curvatures, compared with *rag1*^{-/-} siblings (Fig. 6b). Most importantly, hematopoietic *tert* overexpression markedly extended survival, increasing mean lifespan by approximately 8 months relative to *rag1*^{-/-} controls (Fig. 6e).

Analysis of telomere parameters revealed that telomerase overexpression did not markedly alter telomere homeostasis (Fig. 6f-g). No differences were observed in the proportion of very short telomeres across the genotypes analyzed (Fig. 6h), whereas a trend towards an increased proportion of very long telomeres was detected in telomerase-overexpressing mutants (Fig. 6i). Consistent with these findings, assessment of DNA damage showed a tendency towards reduced γ-H2AX foci in telomerase-overexpressing mutants, both at telomeres (Telomere Dysfunction Induced Foci - TIFs) and at non-telomeric sites (non-TIFs), although these differences did not reach statistical significance (Fig. 6j-l).

To investigate the molecular basis of this rescue, we performed bulk RNA-seq of WKM from WT, *rag1*^{-/-} and *rag1*^{-/-} *drl:tert*^{+/-} zebrafish. Principal component analysis clearly separated *rag1*^{-/-} *drl:tert*^{+/-} samples from untreated *rag1*^{-/-} mutants (Fig. 6m), indicating broad transcriptional remodeling of the hematopoietic compartment. Differential expression analysis identified 1,184 genes altered by

hematopoietic *tert* overexpression relative to *rag1*^{-/-} siblings, including 798 downregulated and 386 upregulated genes, adjusted $P < 0.05$, $|\log_2\text{FC}| \geq 1$ (Fig. 6n).

Importantly, many genes previously dysregulated in *rag1* mutants shifted toward WT-like expression after hematopoietic *tert* overexpression. These included pro-inflammatory mediators such as *il1b*, *ptges*, *il6*, *il11a* and *il11b*, *anxa1* paralogues, *mmp9*, adhesion-related genes including *cldnf*, *cldne*, *cldn23.1*, *cldn23.2* and *evpla*, hedgehog pathway regulators such as *hhp* and *shha*, and developmental regulators including *hoxa13a* and *hoxd3a* (Fig. 6o). Conversely, several genes downregulated in *rag1* mutants, including *tdo2b*, *ntrk3a* and *dusp3a*, recovered expression after *tert* overexpression. Selected transcriptional reversions were validated by RT-qPCR (Supplementary Fig. 13). These results indicate that hematopoietic *tert* overexpression partially restores the aberrant WKM transcriptional state of *rag1* mutants.

To distinguish simple reversion of the *rag1*^{-/-} phenotype from transcriptional programs specifically induced by hematopoietic *tert* overexpression, we compared DEGs from *rag1*^{-/-} versus WT and *rag1*^{-/-} *drl:tert*^{+/-} versus *rag1*^{-/-} contrasts using Venn analysis (Fig. 6p). Focusing on genes uniquely altered upon *tert* overexpression, this revealed activation of chromatin- and transcription-associated regulators, including *twist1b*, *snai1a*, *pogza*, *nr2f2*, *sall3b* and *ikzf2* (Fig. 6q). This suggests that hematopoietic *tert* overexpression engages regulatory programs linked to chromatin state, transcriptional control and cellular identity. In parallel, hematopoietic *tert* overexpression repressed several transcriptional programs associated with stress, inflammatory alert states and myeloid/erythroid differentiation. These included plasticity-associated transcription factors such as *klf3*, *klf6a*, *klf9* and *gata2a* (Fig. 6r), genes linked to hematopoietic and erythroid programs including *alas2*, *fech*, *sptb* and *slc25a37*, and mitochondrial or heme metabolism-associated genes such as *abcb10*, *slc25a38a* and *flvcr1* (Fig. 6s). Stress- and inflammation-associated factors, including *cish*, *cishb*, *nr4a3* and *hif1a*, were also significantly repressed (Fig. 6t). Thus, *tert* overexpression does not merely reverse the *rag1*^{-/-} transcriptional profile, but induces a distinct hematopoietic state characterized by increased chromatin regulatory activity and suppression of stress-responsive, myeloid, mitochondrial and heme-associated programs.

Finally, because these transcriptional changes suggested altered immune-cell states, we assessed hematopoietic cell composition by flow cytometry. The relative proportions of erythroid, myeloid, lymphoid and precursor populations were not substantially changed by *drl:tert* expression (Fig. 6u-y). This indicates that the rescue induced by hematopoietic *tert* overexpression is unlikely to result from major changes in immune-cell proportions, but rather from functional or transcriptional reprogramming of the hematopoietic compartment.

Together, these findings identify telomerase activity as a key modifier of Rag1 deficiency-induced premature aging. While telomerase loss accelerates mortality and inflammatory activation in *rag1* mutants, specific hematopoietic *tert* overexpression extends lifespan and reprograms the WKM transcriptional landscape toward a less inflammatory and more homeostatic state.

7. Hematopoietic overexpression restores gut and systemic homeostasis in zebrafish

7. Hematopoietic *tert* overexpression restores gut and systemic homeostasis in *rag1*^{-/-} zebrafish
Having shown that hematopoietic *tert* overexpression extends lifespan and reprograms the WKM transcriptional state of *rag1* mutants, we next investigated whether this intervention also ameliorates gut and systemic pathology. Histological analysis of 8-month-old fish showed that *rag1*^{-/-} *drl:tert*^{+/-} intestines displayed a reduction in TUNEL+ nuclei in the intestinal mucosa of *rag1*^{-/-} *drl:tert*^{+/-} fish (Fig. 7a-b), indicating decreased epithelial cell death. Also, a non-significant trend toward increased goblet cell numbers compared with *rag1*^{-/-} siblings was observed, whereas eosinophil counts were not substantially altered (Fig. 7c-d and Supplementary Fig. 14). In addition, liver sections showed markedly reduced vacuolization in *rag1*^{-/-} *drl:tert*^{+/-} fish, approaching WT morphology (Fig. 7e,f). Thus, hematopoietic *tert* overexpression ameliorates both intestinal epithelial stress and systemic tissue pathology in *rag1*^{-/-} mutants.

We next assessed whether these histological improvements were accompanied by transcriptional remodeling of the intestine. Bulk RNA-seq of intestinal tissue separated *rag1*^{-/-} *drl:tert*^{+/-} samples from *rag1*^{-/-} controls and positioned them closer to WT samples by principal component analysis (Fig. 7g). Differential expression analysis identified 627 genes altered by hematopoietic *tert* overexpression in the intestine, including 436 downregulated and 191 upregulated genes compared with *rag1*^{-/-} siblings, adjusted $P < 0.05$, $|\log_2\text{FC}| \geq 1$ (Fig. 7h). Several genes previously dysregulated in *rag1*^{-/-} intestines shifted toward WT-like levels after *tert* overexpression, including inflammation-related genes, *ptges*, *ltb4r*, *il21r.1* and *osmr*; extracellular matrix-remodeling factors, *adamts1* and *mmp14b*; insulin-signaling components, *irs2a* and *igfbp1a*; and stress-response genes, *bbc3*, *tp53inp1* and *tdo2b* (Fig. 7i). Functional enrichment analysis showed that genes altered by hematopoietic *tert* overexpression were associated with organic-acid transport, lipid metabolism and hematopoietic homeostasis-related programs (Fig. 7j). KEGG pathway analysis further highlighted FoxO signaling among the enriched pathways (Fig. 7k), suggesting modulation of stress-resistance and metabolic regulatory networks. RT-qPCR validation confirmed significant reduction of *tp53inp1* expression in *rag1*^{-/-} *drl:tert*^{+/-} intestines, whereas *mmp9* showed a downward trend (Supplementary Fig. 15a-b). Other selected markers, including *ifitm1* and *c6.2*, were not significantly changed, while *fn1a*, *cxc11.5* and *saa* tended to increase (Supplementary Fig. 15c-g), indicating that the intestinal transcriptional rescue induced by hematopoietic *tert* overexpression is partial and gene-specific rather than a complete reversion of the *rag1*^{-/-} state.

Finally, we evaluated whether hematopoietic *tert* overexpression modified the dysbiotic gut microbiota of *rag1* mutants. 16S rRNA amplicon sequencing showed that the microbiota of *rag1*^{-/-} *drl:tert*^{+/-} fish separated from that of *rag1*^{-/-} controls in beta-diversity analyses and clustered closer to WT samples (Supplementary Fig. 16a). Alpha diversity tended to increase in *rag1*^{-/-} *drl:tert*^{+/-} fish and no longer differed significantly from WT controls (Fig. 7l). In parallel, total bacterial load was significantly restored

in *rag1*^{-/-} *drl:tert*^{+/-} intestines compared with *rag1*^{-/-} controls (Supplementary Fig. 16b). At the phylum level, hematopoietic *tert* overexpression reduced the relative abundance of Pseudomonadota and Actinobacteriota and increased Fusobacteriota and Bacillota/Firmicutes (Fig. 7m). Family-level analysis further showed a shift away from the *rag1*^{-/-} dysbiotic profile, including reduced abundance of Dermacoccaceae, Dietziaceae, Pseudomonadaceae and Aeromonadaceae, together with increased abundance of several families depleted in *rag1* mutants, including Prevotellaceae, Veillonellaceae, Barnesiellaceae and Fusobacteriaceae (Fig. 7n).

Together, these findings show that hematopoietic *tert* overexpression has effects beyond the blood compartment. It reduces intestinal apoptosis and liver pathology, partially normalizes inflammatory, metabolic and stress-related intestinal transcripts, and restores key features of the gut microbiota. These results identify blood-cell telomerase activity as a systemic regulator of the gut–microbiota axis and a potent modifier of Rag1 deficiency-driven premature aging.

DISCUSSION

Our study identifies the intestinal microbiota and hematopoietic telomerase as functionally connected regulators of immune-driven premature aging. Using Rag1-deficient zebrafish, a vertebrate model lacking functional adaptive immunity but viable under conventional conditions, we show that adaptive immune loss is associated with intestinal degeneration, barrier dysfunction, progressive dysbiosis, systemic inflammatory remodeling and shortened lifespan. Importantly, these alterations are not merely parallel consequences of Rag1 deficiency. Microbiota depletion extends survival and attenuates intestinal and systemic inflammatory signatures, whereas transfer of *rag1*^{-/-} microbiota to immunocompetent hosts induces mutant-like molecular features. Conversely, hematopoietic *tert* overexpression extends lifespan, reprograms the inflammatory blood compartment and partially restores gut, liver and microbiota homeostasis. These findings place adaptive immunity, gut microbiota and telomerase within a shared regulatory axis controlling organismal aging.

A major implication of our work is that adaptive immune deficiency can precipitate an inflammaging-like cascade centered in the intestine. Rag1-deficient zebrafish were previously shown to develop chronic innate immune activation, oxidative stress, DNA damage, telomere attrition, cellular senescence and reduced lifespan⁴³. Here, we further characterize this model by showing that the intestine is both a prominent target and amplifier of this syndrome. *rag1* mutants displayed shortened mucosal folds, reduced absorptive surface, fewer goblet cells, thinning of the mucus layer and increased epithelial apoptosis, resembling several aspects of age-associated intestinal decline described in humans and animal models^{31,45–50}. Single-cell transcriptomics further revealed loss of an early absorptive enterocyte-like population, reduction of stem/progenitor compartments and accumulation of mitochondrial-high enterocyte-like cells, consistent with impaired epithelial renewal and stress-associated epithelial loss. The repression of renewal-associated genes such as *irf6* and *ppdpf* supports

this interpretation, in line with roles for IRF6 and PPDPF in epithelial stemness and proliferative signaling 51–53.

Barrier dysfunction appears to connect local intestinal damage with systemic pathology. *rag1* mutants showed dysregulation of tight-junction and adhesion genes, increased intestinal permeability, bacterial 16S rRNA detection in liver DNA, hepatic vacuolization and focal NF-κB activation. These findings are consistent with age-related weakening of intestinal barrier function and with the established link between microbial translocation and systemic inflammation^{54–57}. The intestinal transcriptome also showed activation of chemotactic, complement, eicosanoid, extracellular-matrix and antigen-presentation programs, including genes and pathways associated with inflammatory bowel disease and RAG-associated immune pathology, such as *mmp9*, *saa*, *crp*-family genes, *Ita4h* and *cxc11*-like chemokines^{58–61}. Thus, the *rag1*^{-/-} intestine is not simply damaged; it adopts an inflammatory and remodeling-prone state that may actively propagate systemic decline.

A second major conclusion is that dysbiosis is a functional amplifier of the *rag1*^{-/-} aging syndrome. Longitudinal microbiota profiling showed that WT and *rag1*^{-/-} communities diverged before advanced histological deterioration, suggesting that dysbiosis is not merely a terminal consequence of tissue collapse. The *rag1*^{-/-} microbiota progressively lost alpha diversity and bacterial load and became dominated by Pseudomonadota, formerly Proteobacteria, particularly Gammaproteobacteria, while Fusobacteriota, Bacteroidetes and Bacillota were depleted. Proteobacterial blooms, especially Enterobacteriaceae expansion, are strongly associated with inflammation, aging and oxygen-rich inflammatory niches^{62–67}. In parallel, metatranscriptomic analysis revealed remodeling of bacterial fatty-acid metabolism, glyoxylate, butanoate and tryptophan pathways, suggesting that the dysbiotic community is not only taxonomically altered but also functionally rewired. Such metabolic changes may reinforce inflammation, alter epithelial nutrient availability and favor pathobiont persistence^{68–71}.

The microbiota-intervention experiments support a causal contribution of this dysbiosis. WT microbiota failed to durably engraft in *rag1*^{-/-} recipients, suggesting that the lymphocyte-deficient host imposes strong ecological constraints on microbial composition. In contrast, weekly antibiotic treatment reduced bacterial load, extended survival and partially rescued intestinal and systemic transcriptional programs, including complement, extracellular-matrix remodeling, lipid-oxidation and inflammatory signatures. These pathways are plausibly microbiota-responsive, as microbial products can regulate MMP expression and epithelial permeability, SCFAs can dampen MMP activity, and complement is both microbiota-regulated and capable of fueling intestinal inflammation when overactivated^{72–75}. Conversely, transfer of *rag1*^{-/-} microbiota into WT recipients induced intestinal transcriptional remodeling and upregulation of mutant-like inflammatory and systemic markers. Together, these results indicate that dysbiosis acts both as a consequence of adaptive immune deficiency and as a functional amplifier of immune-driven aging.

The telomerase arm of our study reveals a second, interacting layer of regulation. Telomere attrition is a canonical hallmark of aging and a key trigger of DNA damage, senescence and inflammatory signaling^{24,76}. *rag1*^{-/-} zebrafish were previously shown to accumulate DNA-damage foci and exhibit telomere shortening in WKM during later stages of aging⁴³. Here, genetic reduction of telomerase activity shortened survival in the *rag1*^{-/-} background in a dose-dependent manner and increased inflammatory markers. These findings suggest that telomerase insufficiency sensitizes the lymphocyte-deficient host to inflammatory decline. Chronic innate activation and ROS production may accelerate telomere erosion, whereas telomere dysfunction may further promote inflammation through senescence-associated secretory programs and DNA-sensing pathways such as cGAS–STING and ZBP1-dependent responses^{76–79}.

Most strikingly, restoration of *tert* expression specifically in blood cells was sufficient to extend lifespan by approximately eight months and ameliorate systemic pathology. This identifies the hematopoietic compartment as a critical node controlling organismal decline in *rag1*^{-/-} zebrafish. Telomerase is known to be important for immune homeostasis, and telomerase deficiency causes disorders such as dyskeratosis congenita, frequently associated with bone marrow failure and immune dysfunction^{80,81}. In our model, hematopoietic *tert* overexpression reprogrammed the immune compartment without substantially changing major blood-cell proportions. Many inflammatory and tissue-remodeling genes elevated in *rag1* mutants, including *il1b*, *ptges*, *il6*, *il11* paralogues and *mmp9*, shifted toward WT-like expression, while *tert* overexpression induced chromatin- and transcription-associated regulators and repressed stress-responsive, myeloid, mitochondrial and heme-associated programs. To determine whether this reprogramming reflected canonical telomere lengthening, we assessed telomere length and telomere-associated DNA damage in hematopoietic cells. Hematopoietic *tert* overexpression did not significantly alter telomere length or reduce either telomere-associated DNA-damage foci (TIFs) or non-telomeric γ -H2AX foci, despite a non-significant trend toward longer telomeres and reduced DNA damage. These findings argue against telomere elongation as the primary driver of phenotypic rescue, although a minor contribution cannot be excluded. Several of the transcriptional changes induced by hematopoietic *tert* overexpression are compatible with non-canonical TERT activities described in other systems. First, TERT has been shown to modulate NF- κ B-dependent gene expression⁸², and to regulate MMP expression independently of telomere elongation⁸³. The coordinated normalization of NF- κ B-target genes, including *il1b*, *il6* and *mmp9*, induced upon hematopoietic *tert* overexpression is consistent with this axis. However, whereas TERT promotes NF- κ B activity in some experimental settings, hematopoietic *tert* overexpression suppressed these targets in *rag1*^{-/-} fish, suggesting that the direction and functional consequences of TERT–NF- κ B crosstalk are context-dependent. Second, TERT can upregulate DNMT1 expression and activity, thereby stabilizing DNA methylation patterns and limiting aging-associated changes in gene expression⁸⁴. This epigenetic function offers a plausible framework for the induction of chromatin- and transcription-associated regulators, including *twist1b*, *snai1a* and *pogza*, following *tert* overexpression. Third, TERT localizes to mitochondria and protects against excessive ROS production and oxidative stress in several cellular systems^{85–87}, which may explain the

repression of mitochondrial- and heme-associated transcriptional programs in *tert*-overexpressing hematopoietic cells. Finally, non-canonical TERT activity has been repeatedly linked to protection against apoptosis, independently of its telomeric function^{88–90}. Together, these parallels are consistent with a model in which hematopoietic *tert* overexpression rescues the *rag1*^{-/-} phenotype primarily through telomere-independent transcriptional, epigenetic, redox and potentially cell-survival activities, rather than through canonical telomere elongation.

A particularly important finding is that blood-cell *tert* overexpression improved tissues in which the transgene was not detectably overexpressed. *rag1*^{-/-} *drl:tert* fish showed reduced intestinal apoptosis, reduced hepatic vacuolization, partial normalization of intestinal inflammatory and metabolic transcripts, and a shift of the gut microbiota toward a more WT-like configuration, revealing a non-cell-autonomous effect of hematopoietic *tert* expression. Tissue-restricted *tert* expression was previously shown to counteract systemic aging in telomerase-deficient zebrafish following gut-specific restoration of telomere homeostasis⁹¹. Our findings extend this principle to a mechanistically distinct model of inflammation-driven premature aging, in which hematopoietic *tert* overexpression improves distant tissues and gut microbiota homeostasis without significant telomere elongation or reduction of telomere-associated DNA damage.

Our data point to a hematopoietic–gut axis in which immune-derived cytokines, lipid mediators and barrier-regulating signals shape epithelial fitness and microbial ecology. The reduction of *ptges*- and *ltb4r*-related inflammatory programs is consistent with decreased eicosanoid-mediated inflammation, whereas the decline of Pseudomonadota-associated pathobionts and recovery of Fusobacteriaceae, Prevotellaceae and Barnesiellaceae suggest restoration of a more homeostatic intestinal niche. Thus, hematopoietic telomerase activity emerges as a systemic regulator of the gut–microbiota axis, not merely as a cell-intrinsic determinant of blood-cell aging.

Several limitations should be considered. Antibiotics have pleiotropic effects beyond microbial depletion and did not fully restore all intestinal phenotypes; defined microbial consortia or targeted manipulation of candidate pathobionts will be needed to identify the taxa and functions responsible for pathology. Similarly, FMT from *rag1*^{-/-} donors transmitted mutant-like molecular features but did not reproduce the full *rag1*^{-/-} syndrome, indicating that host genotype and immune context remain essential. Finally, single-cell Q-FISH analysis of disaggregated WKM cells did not reveal significant changes in telomere length or telomere-associated DNA damage following hematopoietic *tert* overexpression. As these analyses encompassed the entire WKM population, future studies focusing on *drl* cells may determine whether telomere dynamics are selectively modulated within the transgene-expressing compartment. Similarly, identifying the specific immune cell population(s) that mediate the beneficial effects of hematopoietic *tert* overexpression represents an interesting direction for future research. Despite these limitations, our findings support a model in which loss of adaptive immune surveillance destabilizes the intestinal ecosystem and drives a self-amplifying loop of epithelial damage, dysbiosis, microbial translocation and systemic innate inflammation. Hematopoietic *tert* overexpression breaks this circuit from the immune

side, extending lifespan and restoring aspects of gut, liver and microbiota homeostasis. By linking adaptive immune loss to dysbiosis and hematopoietic telomerase dysfunction, our study defines a vertebrate framework in which immune fitness, microbial ecology and telomere maintenance jointly determine the pace of organismal aging.

METHODS

Zebrafish husbandry and lines

Zebrafish, *Danio rerio*, were maintained according to standard procedures⁹². The following lines were used: WT/*rag1*^{+/+}, *rag1*^{t26683/t26683}, referred to as *rag1*^{-/-} (Wienholds et al., 2002), *tert*^{hu3430/hu3430}, referred to as *tert*^{-/-30}, *Tg(nfkb:eGFP)*⁹³ and *Tg(drl:tert)*. The *rag1*^{-/-} line was crossed with *tert*^{-/-}, *Tg(nfkb:eGFP)* and *Tg(drl:tert)* lines, and offspring were genotyped before analysis. Adult zebrafish were anesthetized in 0.16 mg ml⁻¹ tricaine and euthanized in 0.32 mg ml⁻¹ tricaine diluted in system water. All experiments complied with the European Union Directive 2010/63/EU and Spanish RD 53/2013 and were approved by CARM, approval number #A13220914.

RNA extraction and RT-qPCR

Total RNA was extracted from snap-frozen tissues using TRIzol reagent, Invitrogen, followed by purification with either the RNAqueous-Micro Total RNA Isolation Kit or PureLink RNA Mini Kit, Invitrogen, according to sample size and manufacturer instructions. RNA was treated with DNase I, Invitrogen, and cDNA was synthesized from 1 µg total RNA using SuperScript IV VILO Master Mix, Invitrogen. RT-qPCR was performed on a QuantStudio Real-Time PCR system, Thermo Fisher Scientific, using SYBR Green PCR Core Reagents, Applied Biosystems. Gene expression was normalized to *rps11* and calculated using the Pfaffl method⁹⁴. Reactions were performed in technical triplicate. Primer sequences are listed in Supplementary Table 1.

Bulk RNA sequencing and analysis

Total RNA from whole intestine or WKM was extracted as described above. RNA quality control, library preparation, Illumina sequencing, mapping and gene-expression quantification were performed by Novogene or the ACTI service at the University of Murcia. Differential expression analysis was performed in R using DESeq2⁹⁵. Genes with adjusted *P* < 0.05 and |log2 fold change| > 1 were considered differentially expressed. Heatmaps were generated using ComplexHeatmap⁹⁶. Gene Ontology, GO, Kyoto Encyclopedia of Genes and Genomes, KEGG, enrichment analyses and gene set enrichment analysis, GSEA, of Hallmark gene sets were performed using clusterProfiler⁹⁷.

Single-cell RNA sequencing and analysis

Whole intestines were dissected into ice-cold PBS, minced and washed five times in DMEM containing penicillin–streptomycin. Samples were treated with dithiothreitol, washed, digested with collagenase IV,

Sigma-Aldrich, for 20 min, mechanically dissociated through a 100 µm strainer and washed. Cell suspensions were adjusted to 1,000 cells µl⁻¹. Single-cell libraries were prepared by the ACTI Genetics and Proteomics service at the University of Murcia using the 10x Genomics Chromium Single Cell 3' v2 chemistry and processed with Cell Ranger. Cell Ranger output files were analyzed in R using Seurat v4.3.0.1⁹⁸. Seurat objects were generated with min.cells = 25 and min.genes = 250. Low-quality cells were removed using thresholds of 250–2,500 detected genes and fewer than 15,000 unique molecular identifiers. Doublets were removed using JackStraw⁹⁹. Data were normalized using SCTransform¹⁰⁰. Pseudotime analysis was performed using Monocle¹⁰¹. Plots were generated with ggplot2¹⁰² and enrichment analyses were performed with clusterProfiler⁹⁷.

Histology, immunohistochemistry and TUNEL assay

Adult zebrafish were fixed for 3 d at 4°C in acetic-zinc formalin, 3% ZnCl₂, 5.6% formaldehyde and 1% acetic acid, decalcified for 3 d at 4°C in 0.25 M EDTA, pH 8.0, dehydrated, cleared in isoamyl acetate and embedded in paraffin. Sections, 4 µm, were deparaffinized, rehydrated and stained with hematoxylin and eosin, periodic acid–Schiff, PAS, PAS/Alcian Blue or Mallory trichrome, as indicated. For immunohistochemistry, antigen retrieval was performed at 95°C, endogenous peroxidase was quenched with 3.3% H₂O₂ in PBS and non-specific binding was blocked with 5% BSA in PBS. Sections were incubated overnight at 4°C with antibodies against L-plastin, Lcp1, or myeloperoxidase, Mpx, followed by biotinylated secondary antibodies and avidin–biotin–peroxidase detection using DAB. Antibody details are provided in Supplementary Table 2. Apoptotic cells were detected using the In Situ Cell Death Detection Kit, Fluorescein, Roche, according to the manufacturer's instructions. Sections were counterstained with hematoxylin, dehydrated, mounted and imaged under identical settings using a Leica microscope. Image analysis was performed in Fiji/ImageJ. RGB images were split into channels, and signal was quantified in the green channel using fixed thresholds and manually defined regions of interest. Positive area fraction was measured for PAS staining and Lcp1 immunohistochemistry. TUNEL-positive cells were quantified using thresholding followed by particle analysis.

Immuno-FISH

Whole kidney marrow (WKM) cells were isolated from adult zebrafish by mechanical dissociation through a 40 µm cell strainer, fixed in methanol:acetic acid (3:1, v/v), deposited onto poly-L-lysine-coated slides and air-dried overnight. For Q-FISH, slides were fixed in 4% paraformaldehyde (PFA), permeabilized with acidified pepsin, dehydrated through a graded ethanol series and hybridized with a Cy3-labelled telomeric PNA probe for 3 min at 80°C followed by 2 h at room temperature in a humidified dark chamber. After washing, immunostaining was performed using an anti-γH2AX primary antibody and an Alexa Fluor 488-conjugated anti-rabbit secondary antibody. Nuclei were counterstained with DAPI. Z-stack images were acquired using a Leica Stellaris confocal microscope operating in Lightning mode. Three-dimensional image analysis was performed in Imaris to quantify telomere fluorescence intensity, γH2AX foci and telomere dysfunction-induced foci (TIFs).

Telomerase activity assay

Telomerase activity was quantified using a real-time quantitative telomeric repeat amplification protocol (Q-TRAP). Briefly, protein extracts were prepared from WKM using ice-cold 1× TRAPeze CHAPS Lysis Buffer (Sigma-Aldrich). Q-TRAP reactions were performed with 0.45 µg of total protein per sample. A standard curve was generated from a 10-fold serial dilution of protein extracts from HeLa cells, which served as the positive control. Negative controls were prepared by incubating protein extracts with 1 µg RNase at 37°C for 20 min before the assay. Telomerase activity was expressed as Relative Telomerase Activity (RTA) and calculated according to the following equation: $RTA = 10^{((Ct - y\text{-intercept})/\text{slope})}$.

Intestinal permeability assay

Gut permeability was assessed using the Smurf assay ⁴⁴. Adult zebrafish were immersed for 30 min in 2.5% w/v erioglaucine disodium salt, Sigma-Aldrich, prepared in system water and filtered through a 0.22 µm filter. Fish were rinsed twice, anesthetized and imaged under identical lighting conditions. Whole-body blue hue was quantified in ImageJ after conversion to HSB color space.

Behavioral analysis

Fish were transferred to a quiet room and acclimated for 10 min before individual recording in 6 L tanks for 10 min. Videos were analyzed using EthoVision XT v15 at 30 frames per second. Total distance moved, mean velocity and rotation frequency were quantified during the final 5 min of each recording.

NF-κB reporter imaging

NF-κB activity was assessed in live 3-month-old *Tg(nfkb:eGFP) rag1^{+/+}* and *rag1^{-/-}* zebrafish under anesthesia using a Leica MZ16 FA fluorescence stereomicroscope. Images were acquired under identical exposure, magnification and illumination settings. GFP fluorescence intensity was quantified using Fiji/ImageJ ¹⁰³.

16S rRNA sequencing and analysis

Whole intestines were collected from adult zebrafish after 24 h fasting, stored in RNAlater at – 80°C and processed for DNA extraction using the Maxwell RSC PureFood GMO and Authentication kit. Library preparation and sequencing were performed at the IMIB Pascual Parrilla Genomics Core. Two 16S rRNA sequencing approaches were used. In the first, the Ion 16S Metagenomics Kit and Ion Torrent platform were used to amplify variable regions V2, V3, V4, V6–7, V8 and V9. Libraries were prepared with the Ion Plus Fragment Library kit, and sequences were analyzed using Torrent Suite v5.12.1 and Ion Reporter v5.16. Taxonomic identification was performed using Greengenes and MicroSEQ ID databases. Operational taxonomic units were assigned using > 97% identity for family and genus and > 99% for species. In the second approach, V3–V4 regions were amplified using the Quick-16S Plus NGS Library Prep Kit, Zymo Research, with primers 341F and 806R. Libraries were sequenced on a NextSeq 2000,

Illumina, using paired-end 2 × 300 bp reads, and primary files were generated with Dragen BCL Convert v12.7.4. Primers are listed in Supplementary Table 3. Beta diversity was estimated using Manhattan distances and visualized by principal coordinate analysis. Alpha diversity was assessed using Chao and Shannon indices. Differential abundance analysis was performed in R using phyloseq and DESeq2^{95,104}. Plots were generated with ggplot2.

Bacterial load quantification

Total bacterial load was measured by qPCR amplification of the bacterial 16S rRNA V3–V4 region from intestinal DNA¹⁰⁵. Values were normalized to zebrafish genomic DNA using *actb2* amplification. Primers are listed in Supplementary Table 4.

Metatranscriptomic sequencing and analysis

Total RNA from whole intestines was used for metatranscriptomic sequencing. After rRNA depletion, RNA was fragmented, converted to cDNA and sequenced as paired-end 150 bp reads on an Illumina platform by the ACTI Genetics and Proteomics service at the University of Murcia. Bacterial cDNA sequences were analyzed using SqueezeMeta v1.5 in co-assembly mode¹⁰⁶. Outputs were imported into R v4.0.2 and processed using SQMtools for taxonomic and functional profiling. Differential abundance of bacterial open reading frames and KEGG pathways was assessed using DESeq2⁹⁵.

Fecal microbiota transplantation

For fecal microbiota transplantation, FMT, experimental groups contained 5–6 recipient fish, with a donor-to-recipient ratio of 1:2. Donors and recipients were fasted for 24 h. Recipients and antibiotic-only controls were exposed for 12 h to an antibiotic cocktail containing vancomycin, 0.01 g L⁻¹, neomycin, 0.1 g L⁻¹, metronidazole, 0.5 g L⁻¹, and ampicillin, 0.5 g L⁻¹, in autoclaved system water. Fish were rinsed twice for 10 min in sterile water and transferred to fresh autoclaved tanks. Donor intestines were dissected, opened longitudinally, cut into approximately 0.5 cm fragments, rinsed in PBS and added to recipient tanks for 24 h. Recipients were then returned to the standard recirculating system.

Antibiotic treatment

Fish were treated once per week overnight with an antibiotic cocktail containing vancomycin, 0.01 g L⁻¹, neomycin, 0.05 g L⁻¹, metronidazole, 0.5 g L⁻¹, and ampicillin, 0.5 g L⁻¹. After treatment, fish were transferred to fresh autoclaved tank water and maintained outside the recirculating system.

Survival analysis

For survival analyses, fish were maintained at a maximum density of 20 individuals per tank and monitored daily. Fish reaching predefined endpoint criteria were euthanized. Kaplan–Meier survival curves were generated in GraphPad Prism v8.0 and compared using log-rank tests, as indicated in the figure legends.

Flow-cytometry analysis of WKM leukocytes

WKM was dissected into ice-cold DPBS containing 5% FBS and dissociated by pipetting. Cell suspensions were filtered through a 40 µm strainer, pelleted at 800g for 5 min at 4°C and resuspended in DPBS containing 5% FBS. Forward- and side-scatter parameters were used to gate erythroid, myeloid, lymphoid-gated and precursor populations. Data were analyzed using FlowJo v10.7.2.

Statistical analysis

Statistical analyses were performed using GraphPad Prism v8.0. RT-qPCR and qPCR values were natural log-transformed before analysis. Normality was assessed using the Shapiro–Wilk test. Two-group comparisons were performed using unpaired Student’s t-tests, with Welch’s correction when variances were unequal, or Mann–Whitney U tests for non-normal data. Multiple-group comparisons were performed using one-way ANOVA followed by Tukey’s post hoc test, Welch’s ANOVA when variances were unequal, or Kruskal–Wallis tests for non-normal data. Kaplan–Meier curves were compared using Mantel–Cox or Gehan–Breslow–Wilcoxon log-rank tests, as indicated.

Declarations

COMPETING INTEREST STATEMENT

The authors declare no competing interests.

DATA AND BIOLOGICAL MATERIALS AVAILABILITY

All generated materials/tools are available from the corresponding authors. The source data generated and/or analyzed during the development of this study will be provided as a Source Data file upon acceptance. The bulk and single cell RNA-seq data generated in this study will be deposited in the Gene Expression Omnibus (GEO) upon acceptance.

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Author Contribution

VM and MLC conceived the study; JFR-V, BB-B and FA-P performed the research; JFR-V, BB-B, FA-P, DG-M, IC, AG-A, VM and MLC analyzed data; JFR-V wrote the original draft; VM and ML-C edited the final version. All authors have read and agreed to the published version of the manuscript

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Data Availability

All generated materials/tools are available from the corresponding authors. The source data generated and/or analyzed during the development of this study will be provided as a Source Data file upon acceptance. The bulk and single cell RNA-seq data generated in this study will be deposited in the Gene Expression Omnibus (GEO) upon acceptance

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Figures

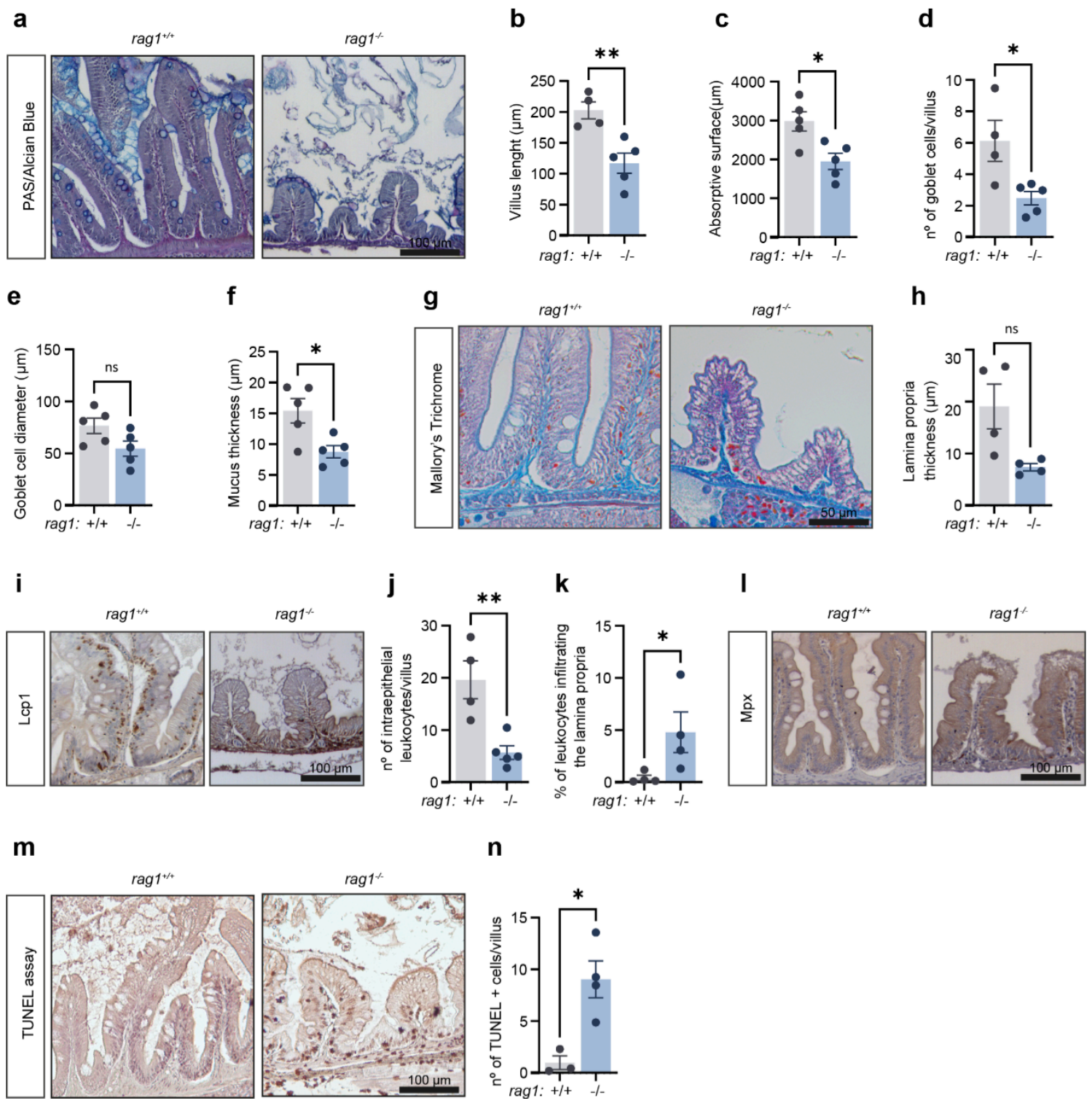


Figure 1

Rag1 deficiency drives premature intestinal degeneration. (a) PAS/Alcian Blue staining of intestinal sections from 8-month-old *rag1*^{+/+} and *rag1*^{-/-} zebrafish. Quantification of (b) mean mucosal fold length, (c) estimated absorptive surface area over 800 μm, (d) number of goblet cells per mucosal fold, (e) mean goblet cell diameter and (f) mean mucus layer thickness. (g) Mallory trichrome staining of intestinal sections from the same groups. (h) Quantification of mean lamina propria thickness in *rag1*^{+/+}

and *rag1*^{-/-} intestines. (i) Lcp1 immunohistochemistry in intestinal sections from 8-month-old *rag1*^{+/+} and *rag1*^{-/-} zebrafish. (j) Quantification of intraepithelial Lcp1+ cells per mucosal fold. (k) Quantification of leukocyte infiltration in the lamina propria, expressed as the percentage of lamina propria area showing Lcp1 signal. (l) Mpx immunohistochemistry in intestinal sections from the same groups. (m) TUNEL assay for detection of apoptotic cells. (n) Quantification of TUNEL+ cells per mucosal fold. Each dot represents one individual; data are shown as mean ± SEM. *P* values were calculated using an unpaired Student's t-test or Mann–Whitney non-parametric test according to data distribution. **P* ≤ 0.05; ***P* ≤ 0.01; ns, not significant.

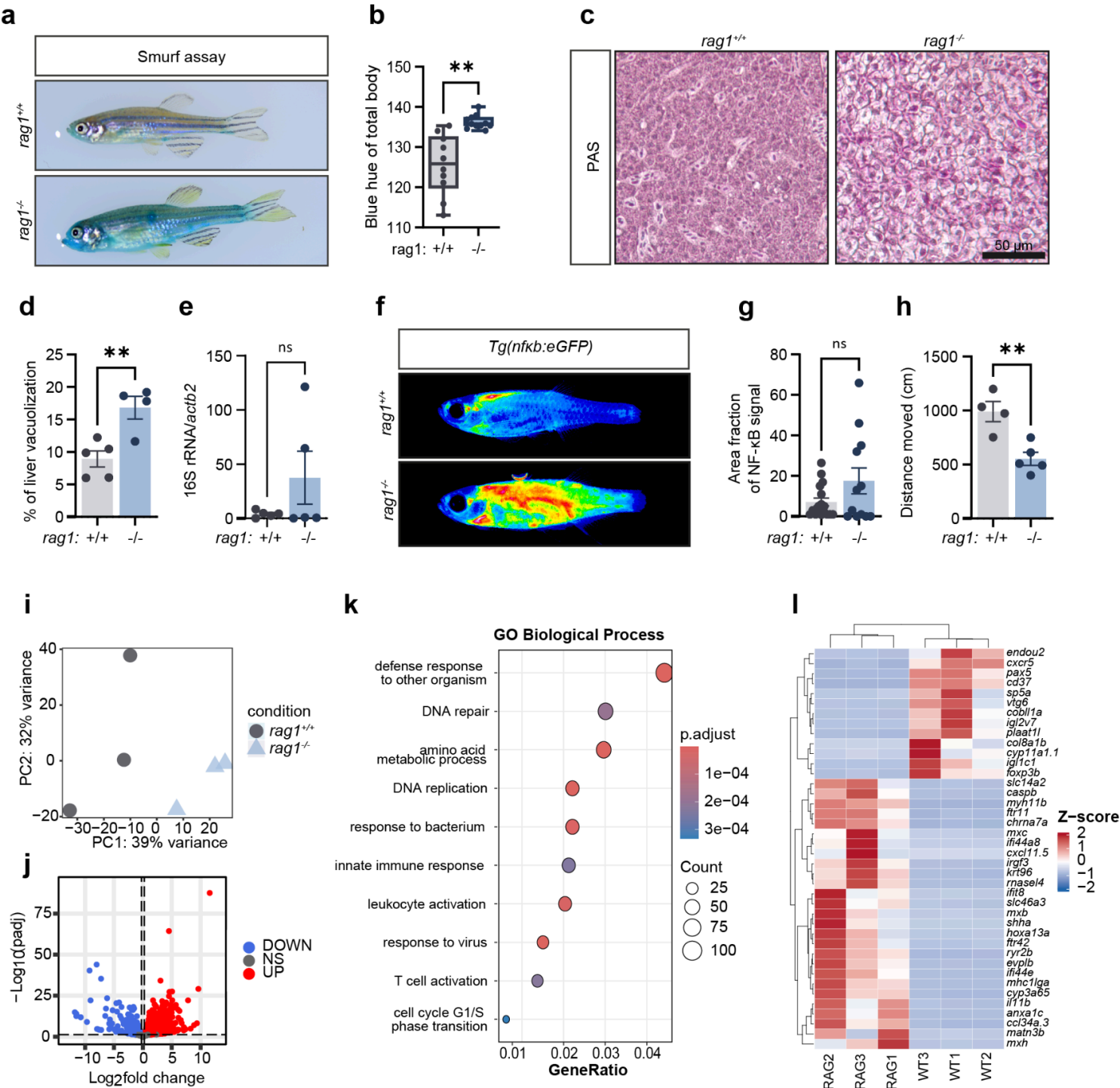


Figure 2

Rag1 deficiency causes systemic features of premature aging. (a) Representative images of the Smurf assay performed in 5-month-old *rag1*^{+/+} and *rag1*^{-/-} zebrafish. (b) Quantification of whole-body blue hue. (c) PAS staining of liver sections from 8-month-old *rag1*^{+/+} and *rag1*^{-/-} fish. (d) Quantification of liver vacuolization from PAS-stained sections, expressed as the percentage of liver area showing negative staining. (e) qPCR quantification of bacterial 16S rRNA genes in whole-liver genomic DNA, normalized to the zebrafish reference gene *actb2*. (f) NF-κB activity visualized in 3-month-old *Tg(nfkb:eGFP)* reporter fish. Representative pseudocolor images are shown after applying a 16-color lookup table in ImageJ. (g) Quantification of GFP fluorescence intensity in *Tg(nfkb:eGFP)* fish. (h) Automated behavioral analysis in 8-month-old fish showing quantification of total distance travelled. (i) Principal component analysis of bulk RNA-seq data from WKM samples of *rag1*^{+/+} and *rag1*^{-/-} zebrafish. (j) Volcano plot showing differentially expressed genes between *rag1* mutants and WT controls. Significantly downregulated genes are shown in blue, significantly upregulated genes in red and non-significant genes in grey. (k) Dot plot showing the top enriched Gene Ontology biological processes among differentially expressed genes. (l) Heatmap displaying selected differentially expressed genes between *rag1* mutants and WT controls. Upregulated genes, positive Z-score, are shown in red, and downregulated genes, negative Z-score, in blue. WT, *rag1*^{+/+}; RAG, *rag1*^{-/-}. Each dot represents one individual; data are shown as mean ± SEM. *P* values were calculated using an unpaired Student's t-test or Mann–Whitney non-parametric test according to data distribution. ***P* ≤ 0.01; ns, not significant.

mutants and WT controls. Upregulated genes, positive Z-score, are shown in red, and downregulated genes, negative Z-score, in blue. WT, *rag1*^{+/+}; RAG, *rag1*^{-/-}. (d) Dot plot showing the top enriched Gene Ontology biological processes among all differentially expressed genes. (e) t-SNE projection showing 23 transcriptional clusters identified by intestinal single-cell RNA-seq of WT and *rag1*^{-/-} zebrafish. (f) UMAP projection of the Monocle pseudotime trajectory after reintegration of the two mitochondrial-high clusters. (g) Percentage of mitochondrial transcripts per cluster, showing markedly elevated levels in the two mitochondrial-high enterocyte-like clusters. (h) Heatmap of representative cell-type-specific differentially expressed genes between *rag1*^{-/-} and WT conditions. Upregulated genes, positive log₂FC, are shown in red, and downregulated genes, negative log₂FC, in blue.

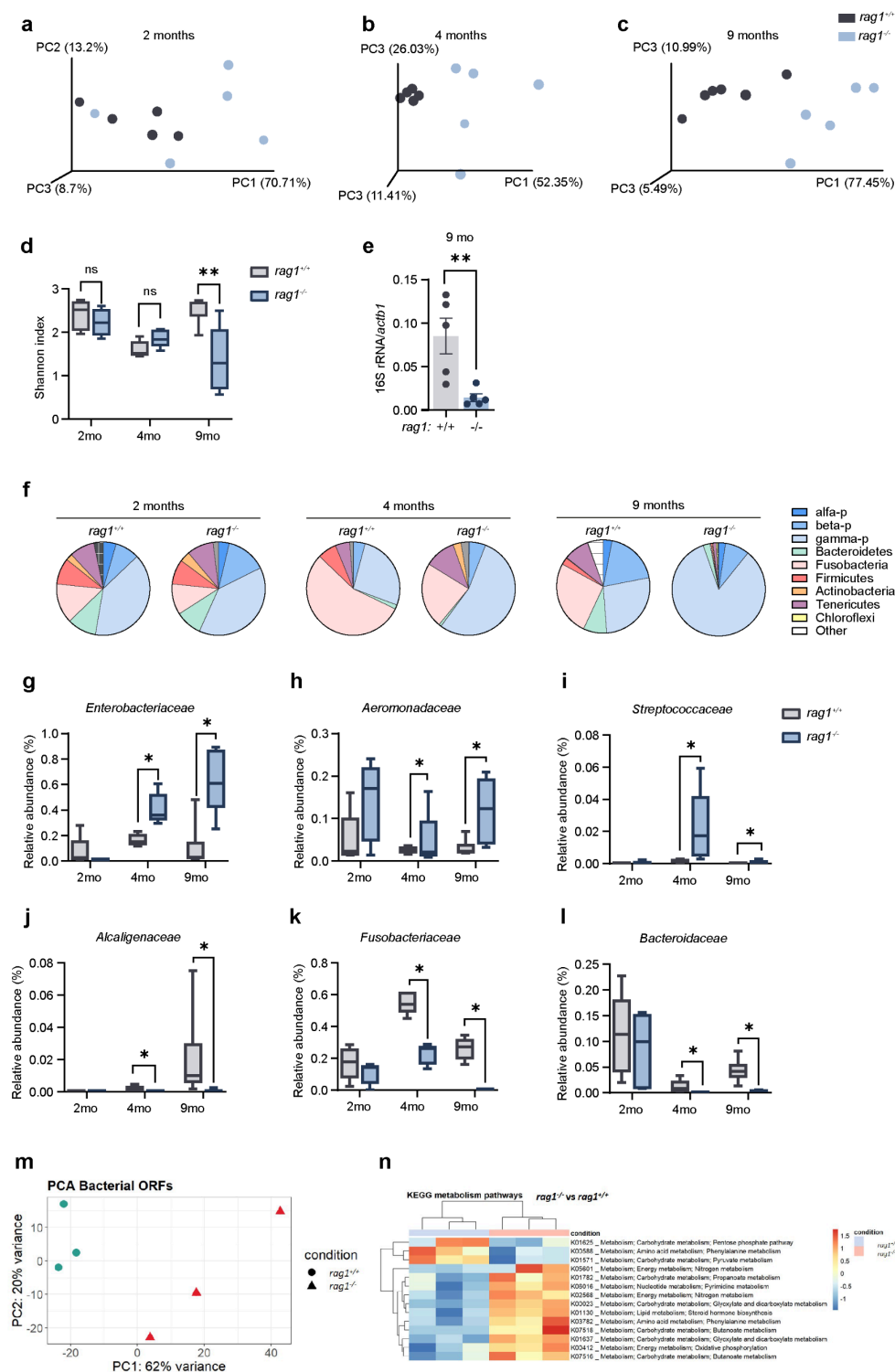


Figure 4

Rag1 deficiency disrupts the intestinal microbiota. (a–c) Principal coordinate analysis, PCoA, plots showing beta diversity of intestinal microbiota from *rag1*^{+/+} and *rag1*^{-/-} zebrafish at (a) 2, (b) 4 and (c) 9 months of age. (d) Alpha diversity, Shannon index, in *rag1*^{+/+} and *rag1*^{-/-} intestinal microbiota at 9 months of age. (e) Total gut bacterial load at 9 months, measured by 16S rRNA qPCR. (f) Phylum-level relative abundance of intestinal microbiota from *rag1*^{+/+} and *rag1*^{-/-} zebrafish at 2, 4 and 9 months of

age. (g–l) Relative abundance of selected bacterial families or genera in *rag1*^{+/+} and *rag1*^{-/-} zebrafish at 2, 4 and 9 months of age, showing genotype-dependent changes over time. (m) Principal component analysis of bacterial open reading frames reconstructed from intestinal bulk RNA-seq data in WT and *rag1*^{-/-} samples. (n) Heatmap showing KEGG metabolic pathways differentially enriched in bacterial metatranscriptomes from *rag1*^{-/-} and WT intestines. Each dot represents one individual; data are shown as mean ± SEM. *P* values were calculated using an unpaired Student's t-test or Mann–Whitney non-parametric test according to data distribution. **P* ≤ 0.05; ***P* ≤ 0.01; ns, not significant.

ABX-treated *rag1*^{-/-} fish at 7 months of age. (c) Total bacterial load quantified by 16S rRNA qPCR. (d) Kaplan–Meier survival curve comparing ABX-treated and untreated *rag1*^{-/-} siblings. (e) Principal component analysis of intestinal RNA-seq data from untreated *rag1*^{-/-}, ABX-treated *rag1*^{-/-} and WT fish. (f) Volcano plot showing differentially expressed genes between ABX-treated and untreated *rag1*^{-/-} intestines. Significantly downregulated genes are shown in blue, significantly upregulated genes in red and non-significant genes in grey. (g) Dot plot showing KEGG pathways enriched among DEGs altered by ABX treatment in *rag1*^{-/-} intestines. (h) Heatmap of selected genes previously altered in *rag1*^{-/-} versus WT intestines and shifted toward WT levels after ABX treatment. Upregulated genes, positive Z-score, are shown in red, and downregulated genes, negative Z-score, in blue. (i,j) RT-qPCR validation of selected genes upregulated in *rag1*^{-/-} intestines and reduced after ABX treatment: (i) *mmp9* and (j) *tp53inp1*. (k) Volcano plot showing differentially expressed genes in WKM between ABX-treated and untreated *rag1*^{-/-} fish. (l) Dot plot showing KEGG pathways enriched among DEGs altered by ABX treatment in WKM. (m) TUNEL staining of intestinal sections from untreated and ABX-treated *rag1*^{-/-} fish. (n) Quantification of TUNEL-positive cells per mucosal fold. (o) Experimental design of fecal microbiota transfer into WT recipients. Four-month-old WT fish were inoculated with fecal microbiota from *rag1*^{-/-} donors. 16S rRNA-seq of gut samples was performed at 1 week and 4 months post-transplant and bulk RNA-seq of the same tissue at 8 months of age. (p) Principal component analysis of intestinal RNA-seq data from untreated WT fish, WT recipients of *rag1*^{-/-} microbiota (*rag1*^{+/+} FMT) and *rag1* mutants. (q) Volcano plot showing differentially expressed genes between WT recipients of *rag1*^{-/-} microbiota and untreated WT controls. Significantly downregulated genes are shown in blue, significantly upregulated genes in red and non-significant genes in grey. (r) Dot plot showing enriched GO Biological Processes among DEGs in WT recipients of *rag1*^{-/-} microbiota. (s) Heatmap of selected genes previously altered in *rag1* mutants that shift toward mutant-like expression patterns in WT recipients of *rag1*^{-/-} microbiota. Upregulated genes, positive Z-score, are shown in red, and downregulated genes, negative Z-score, in blue. (t-u) RT-qPCR analysis of systemic inflammatory genes in WKM after transfer of *rag1*^{-/-} microbiota to WT recipients: (u) *caspa* and (v) *mmp9*. Each dot represents one individual; data are shown as mean ± SEM. Survival differences were analyzed using the log-rank Gehan–Breslow–Wilcoxon test. Other comparisons were analyzed using one-way ANOVA or Kruskal–Wallis non-parametric test according to data distribution. **P* ≤ 0.05; ***P* ≤ 0.01; *****P* ≤ 0.0001; ns, not significant.

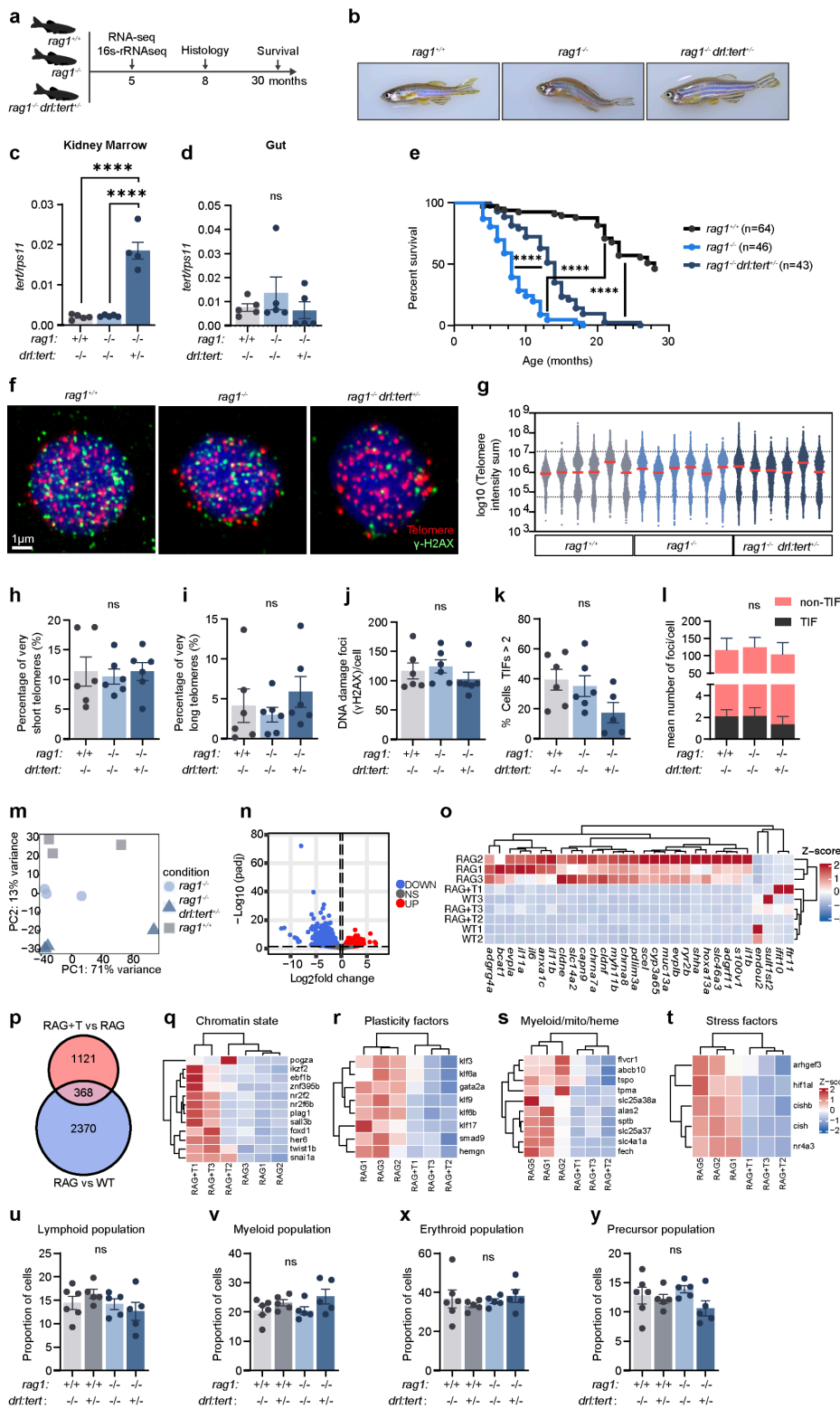


Figure 6

Telomerase activity modulates *rag1* deficiency-induced premature aging. (a) Experimental design. The *rag1*^{-/-} line was crossed with the *drl:tert* transgenic line to induce *tert* overexpression in hematopoietic cells. Gene expression and microbial composition were analyzed at 5 months, histology at 8 months, and survival was monitored longitudinally. (b) Representative images of 8-month-old WT, *rag1*^{-/-} and *rag1*^{-/-} *drl:tert*^{+/+} zebrafish. (c) *tert* mRNA levels in WKM measured by RT-qPCR. (d) *tert* mRNA levels in intestine,

confirming tissue-restricted *tert* overexpression. (e) Kaplan–Meier survival curve comparing WT, *rag1*^{-/-} and *rag1*^{-/-} *drl:tert*^{+/-} zebrafish. (f) Representative immuno-FISH images showing telomeres (red), γ-H2AX (green), and nuclei (blue). (g) Quantification of telomere fluorescence intensity (sum intensity) as a surrogate measure of telomere length. (h) Percentage of very short telomeres. (i) Percentage of very long telomeres. (j) Number of γ-H2AX foci per cell as an indicator of DNA damage. (k) Percentage of cells containing more than two telomere dysfunction-induced foci (TIFs). (l) Mean number of γ-H2AX foci classified as TIFs and non-TIFs. (m) Principal component analysis of WKM RNA-seq data from WT, *rag1*^{-/-} and *rag1*^{-/-} *drl:tert*^{+/-} zebrafish. (n) Volcano plot showing differentially expressed genes between *rag1*^{-/-} *drl:tert*^{+/-} and *rag1*^{-/-} WKM samples. Significantly downregulated genes are shown in blue, significantly upregulated genes in red and non-significant genes in grey. (o) Heatmap of selected genes dysregulated in *rag1*^{-/-} WKM compared with WT and shifted toward WT-like levels after hematopoietic *tert* overexpression. Upregulated genes, positive Z-score, are shown in red, and downregulated genes, negative Z-score, in blue. WT, *rag1*^{+/+}; RAG, *rag1*^{-/-}; RAG+T, *rag1*^{-/-} *drl:tert*^{+/-}. (p) Venn diagram comparing DEGs from *rag1*^{-/-} versus WT and *rag1*^{-/-} *drl:tert*^{+/-} versus *rag1*^{-/-} contrasts. (q–t) Heatmaps showing genes specifically altered upon hematopoietic *tert* overexpression and associated with (q) chromatin state and transcriptional regulation, (r) plasticity-associated factors, (s) myeloid state, mitochondrial and heme-associated programs, and (t) stress and inflammatory alert factors. (u–y) Flow-cytometry quantification of the percentage of total events corresponding to (u) lymphoid, (v) myeloid, (x) erythroid and (y) precursor populations in *rag1*^{+/+} *drl:tert*^{-/-}, *rag1*^{+/+} *drl:tert*^{+/-}, *rag1*^{-/-} *drl:tert*^{-/-} and *rag1*^{-/-} *drl:tert*^{+/-} fish. Each dot represents one individual; data are shown as mean ± SEM. Survival differences were analyzed using the log-rank Mantel–Cox test for (b) and the Gehan–Breslow–Wilcoxon test for (h). Bar plots were analyzed using one-way ANOVA or Kruskal–Wallis non-parametric test according to data distribution. ***P* ≤ 0.01; ****P* ≤ 0.001; *****P* ≤ 0.0001; ns, not significant.

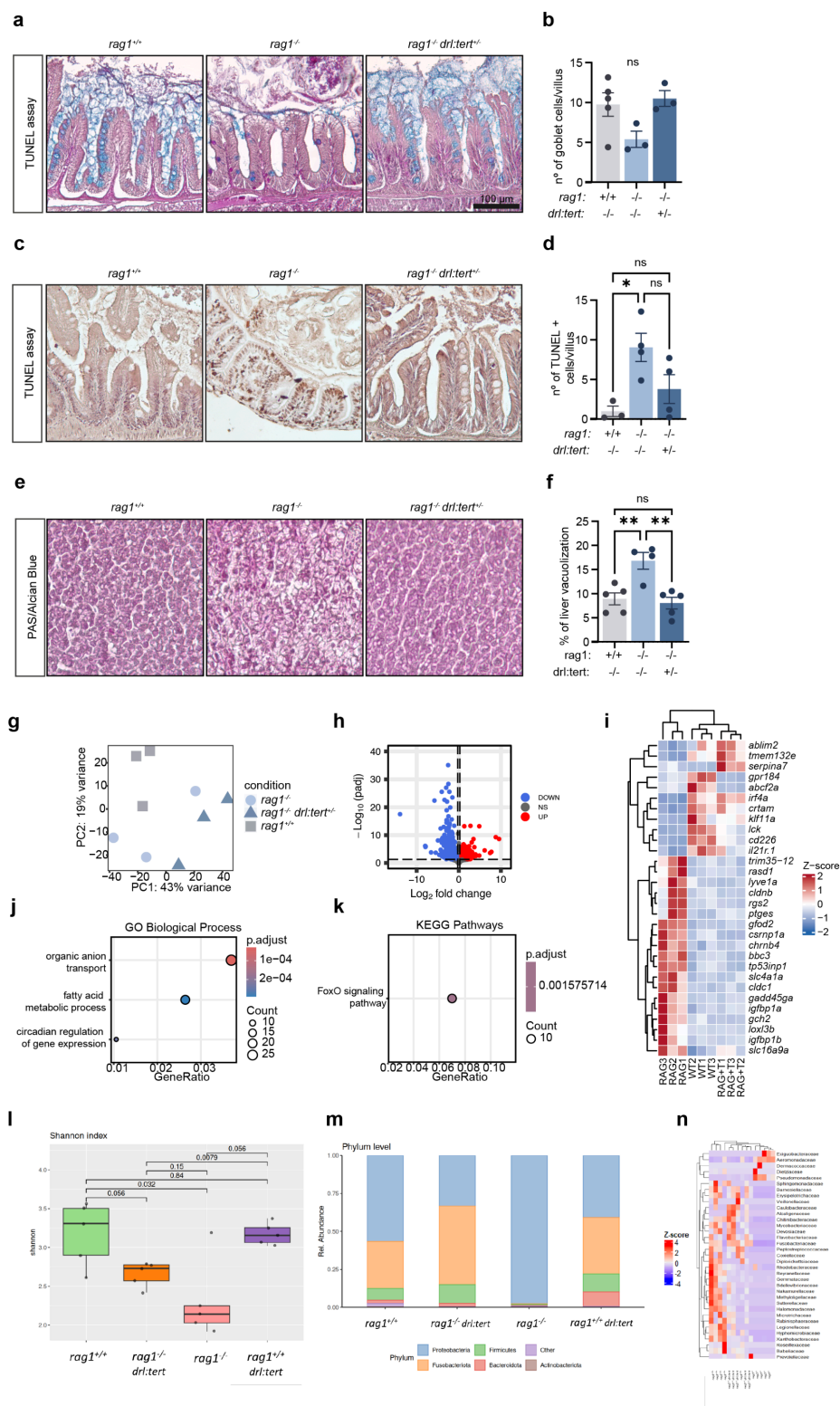


Figure 7

Hematopoietic *tert* overexpression partially restores gut, liver and microbiota homeostasis in *rag1*^{-/-} zebrafish. (a) TUNEL staining of intestinal sections from the same experimental groups. (b) Quantification of TUNEL-positive cells per mucosal fold. (c) PAS/Alcian Blue staining of intestinal sections from 8-month-old WT, *rag1*^{-/-} and *rag1*^{-/-} *drl:tert*^{+/-} zebrafish. (d) Quantification of goblet cells per mucosal fold. (e) PAS staining of liver sections from WT, *rag1*^{-/-} and *rag1*^{-/-} *drl:tert*^{+/-} zebrafish. (f)

Quantification of liver vacuolization from PAS-stained sections, expressed as the percentage of liver area showing negative staining. (g) Principal component analysis of intestinal RNA-seq data from WT, *rag1*^{-/-} and *rag1*^{-/-} *drl:tert*^{+/-} zebrafish. (h) Volcano plot showing differentially expressed genes between *rag1*^{-/-} *drl:tert*^{+/-} and *rag1*^{-/-} intestines. Significantly downregulated genes are shown in blue, significantly upregulated genes in red and non-significant genes in grey. (i) Heatmap of selected genes dysregulated in *rag1*^{-/-} intestines compared with WT and shifted toward WT-like levels after hematopoietic *tert* overexpression. Upregulated genes, positive Z-score, are shown in red, and downregulated genes, negative Z-score, in blue. (j) Dot plot showing the top enriched Gene Ontology Biological Processes among DEGs altered by hematopoietic *tert* overexpression in *rag1*^{-/-} intestines. (k) Dot plot showing the top enriched KEGG pathways among DEGs altered by hematopoietic *tert* overexpression in *rag1*^{-/-} intestines. (l) Box-and-whisker plot of gut microbiota alpha diversity, Shannon index, in the same experimental groups. (m) Phylum-level relative abundance profiles of the gut microbiota. (n) Heatmap showing bacterial families shifted toward WT-like abundance upon hematopoietic *tert* overexpression in *rag1* mutants. OTUs enriched in WT-like conditions, positive Z-score, are shown in red, whereas OTUs enriched in *rag1* mutants, negative Z-score, are shown in blue. Differential abundance was assessed using DESeq2, adjusted $P \leq 0.05$. Each dot represents one individual; data are shown as mean $\pm$ SEM. P values were calculated using one-way ANOVA or Kruskal–Wallis non-parametric test according to data distribution. * $P \leq 0.05$; ** $P \leq 0.01$; ns, not significant.

Supplementary Files

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