Telomerase reverse transcriptase is required for resistance to mycobacterial infection

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Abstract

Age is an important risk factor for infections such as tuberculosis (TB). Telomerase is expressed in immune cells yet leukocyte telomere length declines during ageing suggesting an age-dependent loss of telomerase activity in the immune system. Leukocyte telomere length has been correlated with worse outcomes in TB patients, however the mechanisms linking telomere biology to TB susceptibility and response to therapy are unexplored. Here we use the zebrafish-*Mycobacterium marinum* model to investigate the role of telomerase in TB resistance. We find depletion and inhibition of Tert, the catalytic subunit of telomerase, increases bacterial burden in zebrafish embryos while small molecule activation of Tert decreases bacterial burden. Depletion of shelterin components did not recapitulate the infection susceptibility phenotype, and infection susceptibility could not be rescued by p53 or STING depletion. Consistent with a previously described role for Tert in developmental hematopoiesis, we find Tert is necessary for demand-driven emergency myelopoiesis to support containment of extended mycobacterial infection. Our findings establish a previously undescribed role for host telomerase in supporting infection demand-driven hematopoiesis to control infection.

Introduction

Ageing is one of the most important risk factors for tuberculosis (TB) susceptibility and severity (*1*). Animal models and clinical measurements have identified inflammageing, a persistent low grade interferonopathy triggered by accumulated DNA damage, and immune anergy, caused qualitatively by declining lymphocyte function and quantitatively by the biased production of myeloid cells, as potential mechanisms underlying age-dependent TB susceptibility (*2, 3*). Recent work across guinea pig and human systems have demonstrated that TB accelerates ageing suggesting that *Mycobacterium*

tuberculosis may actively benefit from subverting host ageing pathways and that interception of this process could yield novel targeted adjunctive therapies for an important at-risk group (*4*).

Leukocyte telomere length (LTL) decreases during ageing and influences immune function, specifically affecting susceptibility to infectious diseases driven by host immunopathology including TB (*5-12*). Some aspects of the relationship between LTL and TB biology may be ethnicity specific as while South African patients with longer LTL are more likely to have a successful response to TB treatment, South East Asian and East Asian patients with longer LTL experienced more treatment side effects (*13-15*). As telomerase activity is required for maintenance of LTL, we hypothesized decreased leukocyte expression of the telomerase reverse transcriptase (Tert) may drive the increased susceptibility of the elderly and LTL individuals to mycobacterial infection.

Expression of *tert* by hematopoietic stem cells is necessary for telomere elongation and likely protects chromosomes during hematopoiesis. There is an increasing body of evidence that Tert and the RNA template *TERC* execute important non-canonical functions in immunity as an inflammation-responsive gene aiding the expression of key hematopoietic differentiation genes (*16-18*). Telomerase-expressing macrophages (T-MACS) have been previously described and loss of telomerase expression has been associated with reduced immune function (*19*).

Here we utilize the zebrafish-*M. marinum* model of human TB to study the role of Tert in mycobacterial infection. We find that Tert is expressed at the site of infection and loss of Tert is causes reduced control of infection. Tert-dependent infection demand-driven hematopoiesis can be supplemented by *tert* mRNA providing evidence for a role for Tert in the resilience of the hematopoietic system during chronic infections.

Results

Telomerase reverse transcriptase is expressed at the host-mycobacterial interface

To determine if T-MACs are present in mycobacterial granulomas we analyzed a previously published scRNAseq dataset of dissected zebrafish-*M. marinum* granulomas (*20*). We found *tert* is most expressed in proliferative cells, as expected from canonical function in telomere elongation of proliferating cells and matching the expression of the DNA polymerase subunit *pola2* (Figure 1A). Expression of *tert* was detected in myeloid-derived macrophage subsets, many of which lacked *pola2* expression suggesting a non-proliferative role of *tert* expression. There appeared to be comparable levels of *tert* expression across epithelialized macrophages (Epmacs, Cluster 3, and cdh1+ve) which make up the ring-like structure of mature granulomas compared to the “non-structural” myeloid cells (monocytes, inflammatory macrophages, DCs). The overall level of *tert* expression in the myeloid cell clusters was less than half of the level of *tert* expression proliferating cell clusters.

We analyzed the expression of *tert* in adult zebrafish-*M. marinum* granulomas by *in situ* hybridization and found expression in both the flat inner layer of primarily macrophage-derived epithelialized cells and in round cells around the periphery of the granuloma, presumably the proliferating cells identified in the scRNAseq dataset or monocyte-derived cells (Figure 1B). As expected from the heterogenous nature of previously described T-MACs, there were fewer *tert* positive cells detected within granulomas than were seen in previously published images of macrophages visualized with the pan monocyte marker *mpeg1* illustrating expression only in a subset of macrophages (*21*).

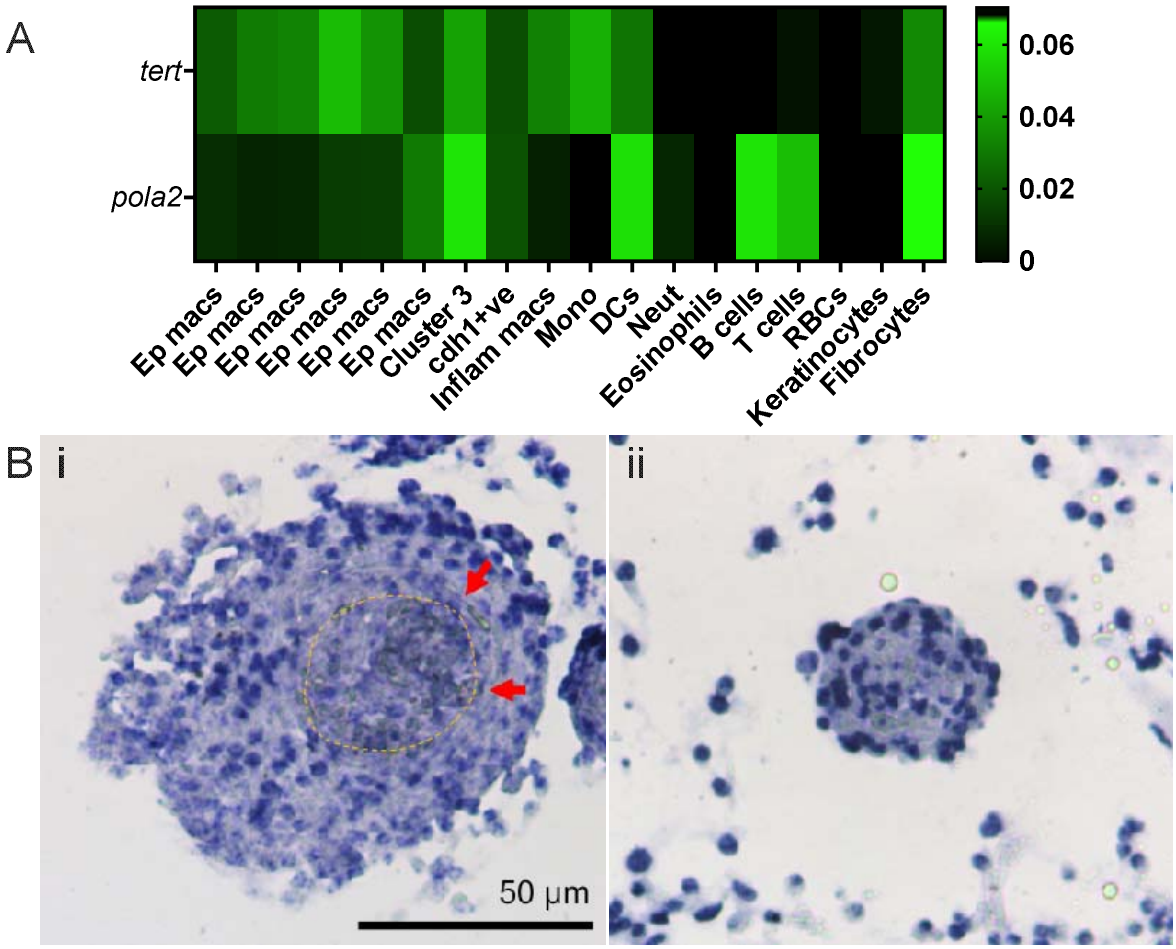

Figure 1: A subset of zebrafish macrophages express *tert* in mycobacterial granulomas. (A) Mean expression value of *tert* and *pola2* across clusters of granuloma cells identified by scRNAseq of adult zebrafish-*M. marinum* granulomas from (*20*). (B) Detection of *tert* expression (blue staining) in (i) necrotic and (ii) non-necrotic adult zebrafish-*M. marinum* granulomas by *in situ* hybridization. Yellow dashed line indicates edge of necrotic core and red arrows indicate epithelioid macrophage layer.

Telomerase deficiency increases the severity of pathogenic mycobacterial infection

We next examined the role of *tert* in the immune response to mycobacterial infection by knocking down expression with a pooled gRNA-CRISPR approach (Supplementary Figure 1A). Knockdown of *tert* increased *M. marinum* burden in zebrafish larvae at 5 days post infection (dpi) (Figure 2A).

In contrast, infection with two relatively non-pathogenic mycobacteria, *Mycobacterium smegmatis* and smooth colony variant *Mycobacterium abscessus*, was comparable between scramble control and *tert* crispant embryos (Figure 2B and 2C). Infection with the more virulent rough colony variant *M. abscessus* resulted in higher bacterial burden in *tert* crispant embryos (Figure 2D). These data suggest the mycobacterial infection susceptibility is specific to infections capable of forming persistent inflammatory lesions.

We next tested the ability of *tert* crispants to control Chikungunya virus (CHIKV) infection, a well characterized wide host range RNA virus capable of replicating in zebrafish embryos (*22*). Quantification of infected cell area by fluorometry revealed comparable CHIKV

infection levels between scramble control and *tert* crispant embryos, demonstrating a specific immune defect to infection by pathogenic bacteria (Figure 2E).

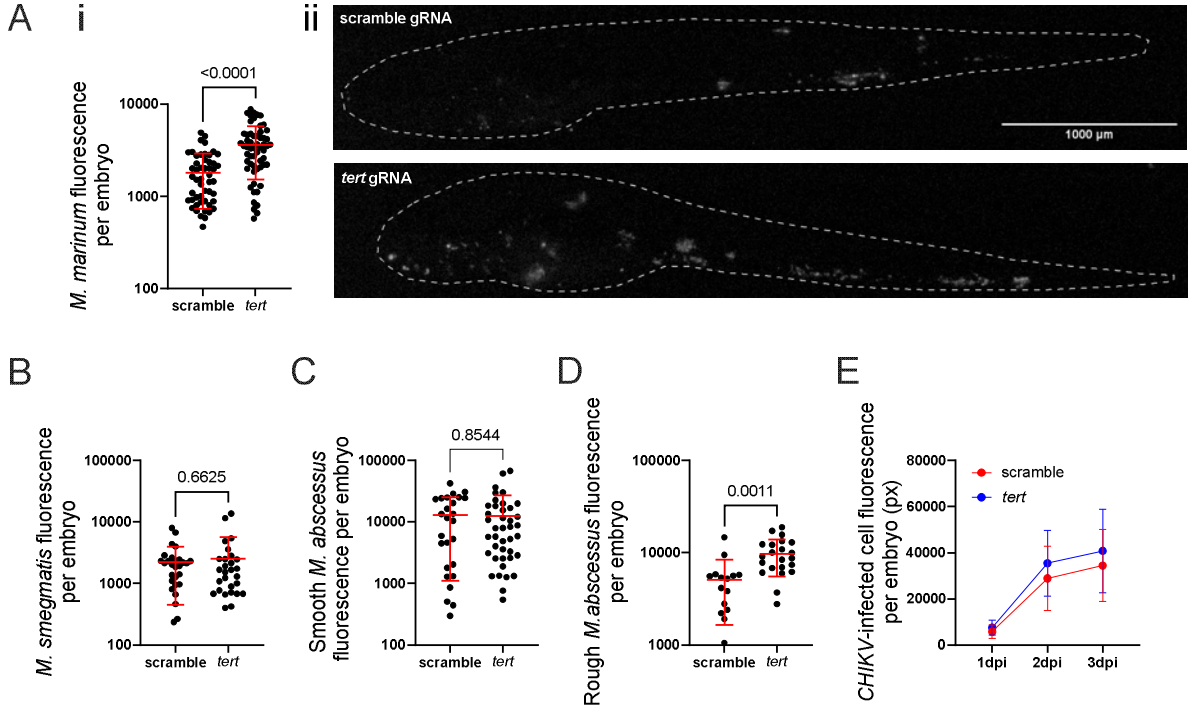

Figure 2: Knockdown of *tert* increases zebrafish susceptibility to pathogenic mycobacterial infection
(A) i. Quantification of *M. marinum* burden in 5 dpi *tert* crispant embryos. ii. Representative images of fluorescent *M. marinum* signal in 5 dpi *tert* crispant embryos. Dashed lines indicate outline of embryos.
(B) Quantification of *M. smegmatis* burden in 5 dpi *tert* crispant embryos.
(C) Quantification of smooth *M. abscessus* burden in 5 dpi *tert* crispant embryos.
(D) Quantification of rough *M. abscessus* burden in 5 dpi *tert* crispant embryos
(E) Quantification of CHIKV burden in 1-3 dpi *tert* crispant embryos (n= 29 scramble, n= 35 *tert* crispants).

Inhibition of telomerase reverse transcriptase phenocopies the knockdown mycobacterial infection susceptibility phenotype and activation reverses susceptibility

We complemented our CRISPR-Cas9 knockdown approach with small molecule TERT inhibitors BIBR1532 and MST-312 (*23, 24*). Treatment of infected embryos increased *M. marinum* burden consistent with our genetic approach data (Figure 3A and 3B).

We next targeted the RNA biogenesis factor *naf1* by CRISPR-Cas9 mutagenesis as it is necessary for TERT function in other models (*25*). Knockdown of *naf1* by CRISPR-Cas9 mutagenesis also increased the susceptibility of zebrafish embryos to *M. marinum* infection (Figure 3C, Supplementary Figure 1B).

Conversely, to determine if telomerase activation could protect against mycobacterial infection, we treated infected zebrafish embryos with cycloastragenol, a small molecule with potential telomerase activating properties in zebrafish (*26*). Cycloastragenol treatment of infected embryos reduced *M. marinum* burden (Figure 3D).

Genetic supplementation of endogenous *tert* by microinjection with mRNA encoding zebrafish *tert* also reduced *M. marinum* burden (Figure 3E and 3F). Interestingly, supplementation of catalytically dead “VAA” *tert* mRNA reduced *M. marinum* burden, suggesting a non-canonical function of Tert could be protective against infection by pathogenic mycobacteria.

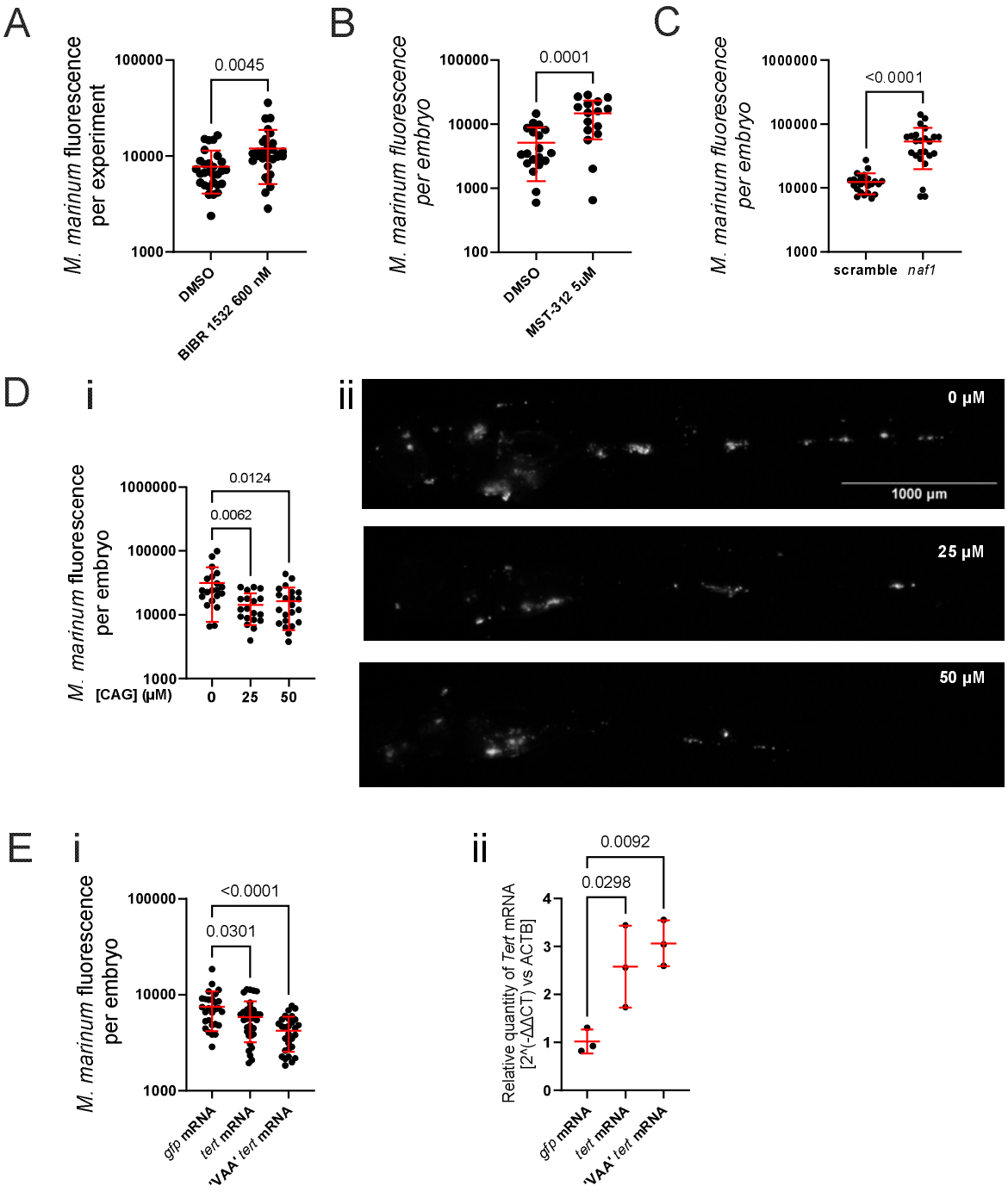

Figure 3: Small molecule and genetic inhibition of Tert increases susceptibility to mycobacterial infection while activation increases resistance to mycobacterial infection. (A) Quantification of *M. marinum* burden in 5 dpi embryos treated with BIBR1532.

(B) Quantification of *M. marinum* burden in 5 dpi embryos treated with MST-312.
(C) Quantification of *M. marinum* burden in 5 dpi *naf1* crispant embryos.
(D) i. Quantification of *M. marinum* burden in 5 dpi embryos treated with cycloastragenol (CAG). ii. Representative images of fluorescent *M. marinum* signal in 5 dpi embryos treated with the indicated concentrations of cycloastragenol.
(E) i. Quantification of *M. marinum* burden in 5 dpi embryos injected with control *gfp*, *tert*, or VAA *tert* mRNA. ii. Quantification of *tert* transcript abundance in 1 dpf embryos injected with control *gfp*, *tert*, or VAA *tert* mRNA.

Telomere protection is not associated with resistance against mycobacterial infection

Chronic infections reduce LTL and DNA damage-induced interferonopathy plays a critical role in TB pathogenesis (*27, 28*), however previous reports have demonstrated Tert-dependent telomere shortening is only observed in adult zebrafish and not at the embryo stage (*16, 29, 30*). We next investigated the hypothesis that canonical telomere-protective functions of Tert were necessary for resistance to *M. marinum* infection in the context of an increased genotoxic microenvironment caused by infection. Consistent with the presence of an inflammatory DNA damage response we observed SPiDER-βGal staining around *M. marinum* granulomas demonstrating the presence of the senescent cell marker beta galactosidase activity (Figure 4A).

Expression of the DNA damage response gene *tp53* was increased in infected *tert* crispant embryos compared to uninfected *tert* crispants indicating a small global increase in DNA damage in the infection-susceptible *tert* crispants (Figure 4B). However, functional examination of the DNA damage response in *tp53* mutants and *sting* crispants failed to rescue the *tert* knockdown-induced susceptibility to *M. marinum* infection (Figure 4C and 4D, Supplementary Figure 1C).

Knockout of zebrafish shelterin genes induces premature ageing in adult animals and provides an alternative experimental approach to examine the canonical role of Tert in telomere protection (*31*). Knockdown of the shelterin genes *acd*, *pot1*, and *trf1* did not affect control of *M. marinum* infection in zebrafish embryos (Figure 4E and Supplementary Figure 1D). Together these data suggest the increased susceptibility to infection seen in Tert-deficient embryos could be due to non-canonical Tert functions.

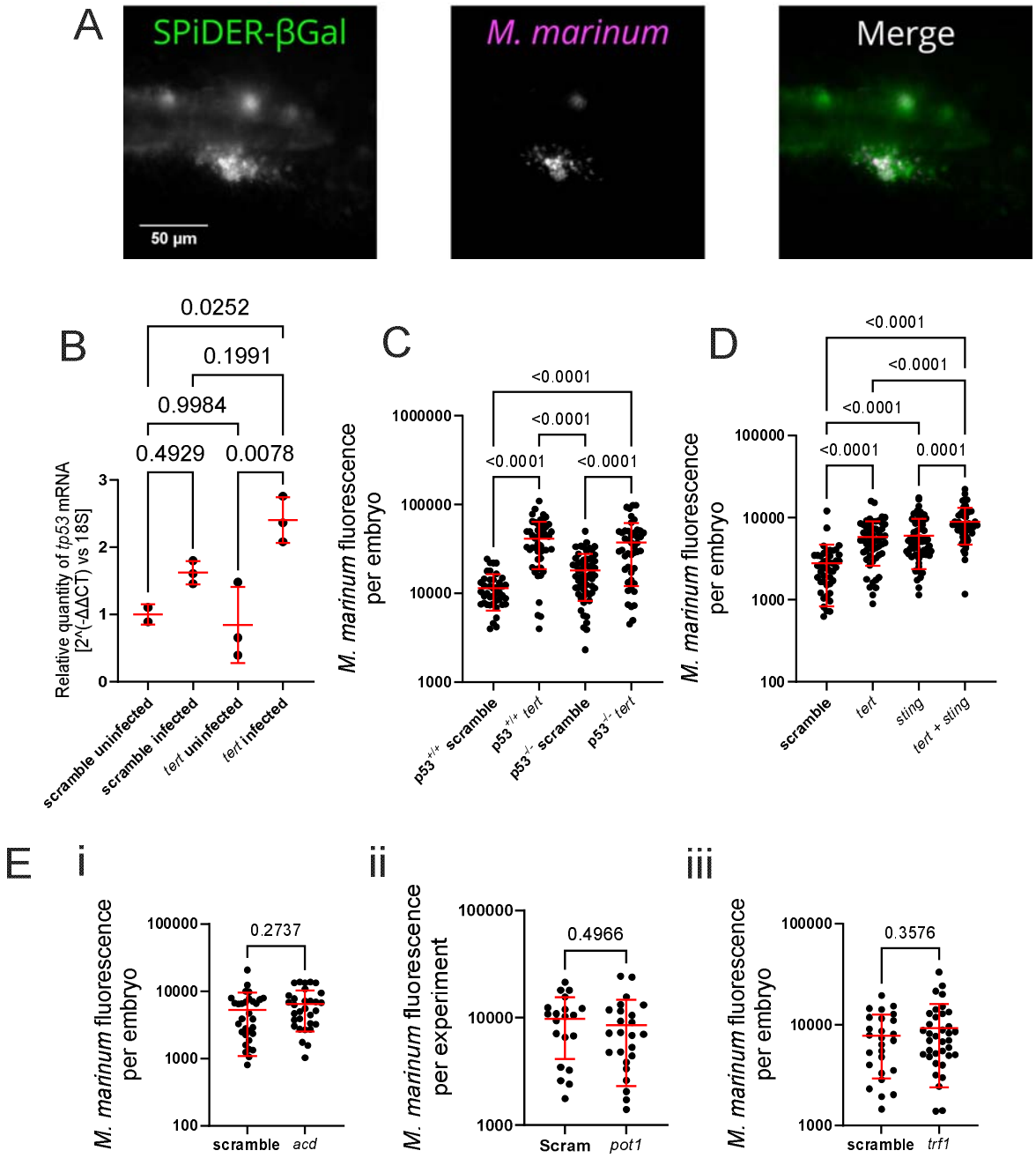

Figure 4: Increased susceptibility to infection in *tert* crispants is not driven by DNA damage response pathways.
(A) Fluorescent SPiDER-βGal staining (green) around *M. marinum* granuloma (magenta) in a 5 dpi embryo.
(B) Quantification of *tp53* transcript abundance in 5 dpi *tert* crispant embryos infected with *M. marinum*.
(C) Quantification of *M. marinum* burden in 5 dpi *tp53* mutant embryos.
(D) Quantification of *M. marinum* burden in 5 dpi *sting* crispant embryos.
(E) Quantification of *M. marinum* burden in 5 dpi i. *acd*, ii. *pot1*, and iii. *trf1* crispant embryos.

Tert supports infection-induced myelopoiesis

Zebrafish *tert* and *terc* are known to be necessary for developmental myelopoiesis (*16, 17, 32, 33*), and *M. marinum* infection control requires replenishment of the myeloid cell compartment (*34*). We next analyzed infection-induced myelopoiesis as a potential mechanism of susceptibility in *tert* crispants using *Tg(lyzc:GFP)*^{nz117} and *Tg(lyzc:dsred)*^{nz50} lines, where neutrophils are fluorescently labelled (*35*), and the *TgBAC(mpeg1.1:EGFP)*^{vcc7} line where macrophages are fluorescently labelled (*36*). Caudal hematopoietic tissue-resident neutrophil and macrophages were reduced in *tert* crispants late in infection (Figure 5A and 5B).

To establish if the loss of *lyzc* and *mpeg1.1* positive cells was due to reduced haematopoietic stem cell (HSC) numbers or a lack of differentiation of myeloid cells, we enumerated *Tg(Mmu.Runx1:NLS-mCherry)*^{cz2010} positive HSCs (Figure 5C) (*37*). Tert crispants exhibited a decline in HSC counts at 5 dpi, corresponding with the observed loss of myeloid cells.

Genetic supplementation of endogenous *tert* by microinjection with mRNA encoding full length and catalytically dead zebrafish *tert* increased neutrophil numbers late in infection (Figure 5D). This increase in leukocyte number corresponded with protection from infection (Figure 3E) placing Tert in the control pathogenic mycobacterial infection via supporting infection-induced myelopoiesis.

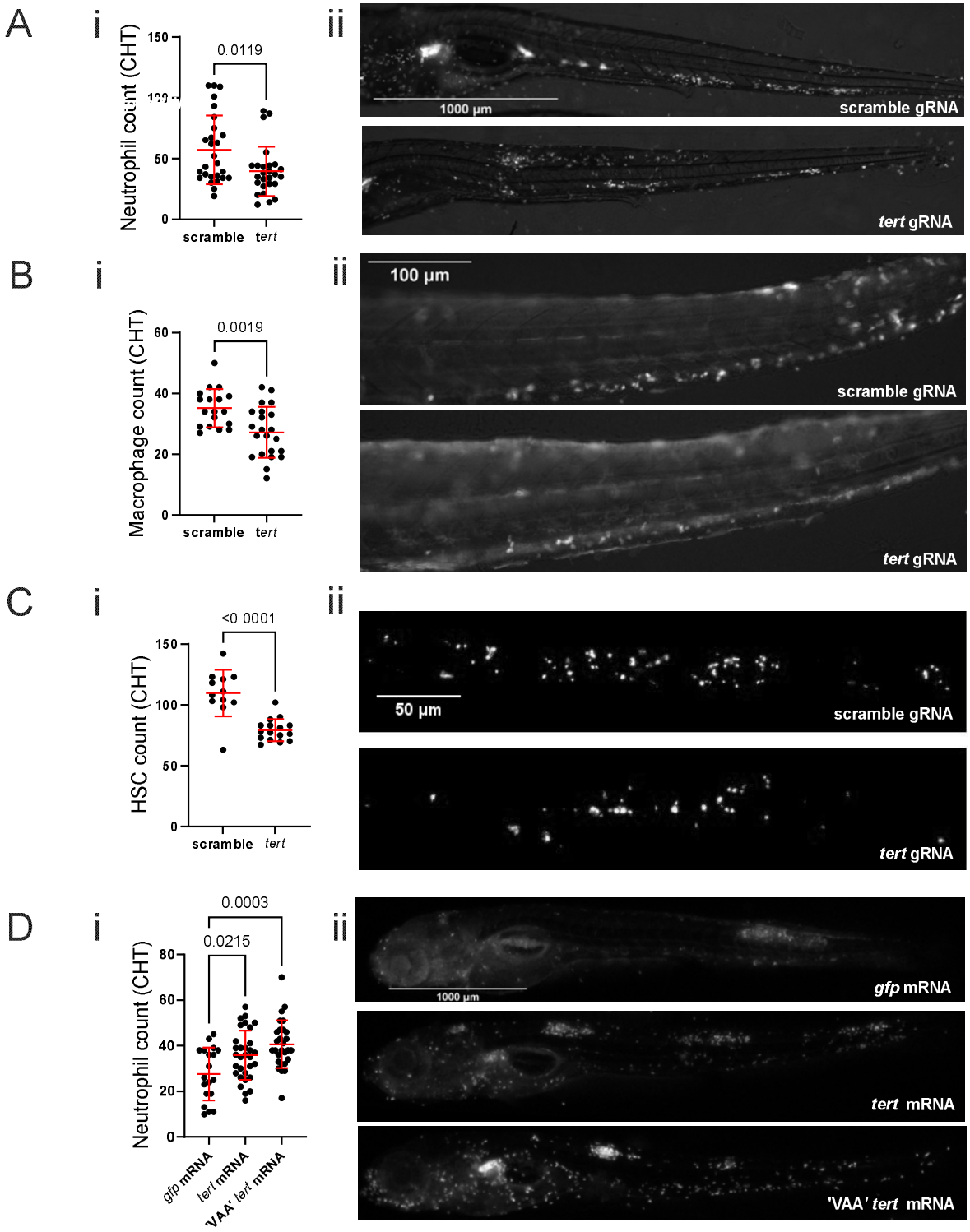

Figure 5: Expression of *tert* correlates with infection demand-driven leukocyte abundance. (A) i. Quantification of CHT-resident neutrophils in 5 dpi *Tg(lyzC:dsred) tert* crispant embryos infected with *M. marinum*. ii. Representative images of fluorescent neutrophils. Bracket demarcates CHT region used for counting.

(B) i. Quantification of CHT-resident macrophages in 5 dpi *TgBAC(mpeg1.1:gfp) tert* crispant embryos infected with *M. marinum*. ii. Representative images of fluorescent macrophages in the CHT region.
(C) i. Quantification of CHT-resident HSCs in 5 dpi *Tg(Mmu.Runx1:NLS-mCherry)* embryos infected with *M. marinum*. ii. Representative images of fluorescent HSCs in the CHT region.
(D) i. Quantification of CHT-resident neutrophils in 5 dpi *Tg(lyzC:dsred)* embryos injected with control eGFP, *tert*, or VAA *tert* mRNA and infected with *M. marinum*. ii. Representative images of fluorescent neutrophils.

Discussion

Our study used depletion of zebrafish telomerase reverse transcriptase to find a novel role for telomerase in the control of mycobacterial infection via supporting infection demand-driven myelopoiesis. These data provide a mechanism that may contribute to the increasing susceptibility to TB during ageing and explain the more general association of short LTL with infection susceptibility. The data further suggest supplementation of telomerase or myelopoietic factors could be used to bolster anti-TB immunity in the elderly (*34*).

While disruption of telomerase activity by small molecule inhibition and *naf1* depletion suggest a canonical telomere-shortening mechanism of infection susceptibility, our use of the VAA active site mutant *tert* to curtail *M. marinum* infection and the lack of effect of shelterin mutation on *M. marinum* infection outcome point towards a non-canonical function of Tert in infection demand-driven hematopoiesis. We have previously reported a non-canonical role of zebrafish Tert in supporting gut macrophage proliferation in adult animals (*19*), an organ with constant turnover and immune stimulation, and consistent with the defect in infection demand-driven hematopoiesis found in this study. Non-canonical telomerase, non-coding RNA component (TERC), and shelterin functions span a wide range of cellular activities, while there are similarities in the DNA-based themes of these functions there is also clear mechanistic independence of their non-canonical activities (*38, 39*).

Disseminated *M. tuberculosis* drives epigenetic reprogramming of HSCs to impair the myelopoietic response of infection (*40*). Our zebrafish-*M. marinum* infection model data adds to this concept by demonstrating loss of HSCs sensitized by the loss of host telomerase, suggesting TB infection may accelerate the onset of clonal hematopoiesis in the elderly. Our finding that the premature aging model of telomerase deficiency is a susceptibility factor for pathogenic mycobacterial infection ties together with prior human TB and guinea pig-*M. tuberculosis* infection model data finding *M. tuberculosis* infection prematurely ages the host (*4*). Together, these data suggest TB actively ages the host as a virulence mechanism to trigger premature decline of hematopoietic function.

A commonality of hematopoietic diseases is an association with DNA-associated genes demonstrating the importance of safeguarding HSC DNA for maintaining the cellular components of the immune system (*41*). The loss of HSCs in our chronic mycobacterial infection model suggests TERT may safeguard HSCs from infection-induced damage in addition to performing previously described roles as an inflammatory transcription factor (*42*). Additionally, we must include the possibility that loss of canonical telomerase activity renders HSCs susceptible to premature replicative senescence in context of chronic infection, which provides an alternative explanation for the infection susceptibility phenotype seen in small molecule telomerase inhibitor-treated and *naf1* depleted embryos (Supplementary Figure 2). Further phenotyping of the HSC compartment in the context of

infection is required to determine the range of roles played by telomerase in protecting HSCs.

Multiple studies have linked short telomere length as a proxy for reduced telomerase expression to viral infection susceptibility in species as diverse as humans and rock oysters (*5, 10-12, 43*), however little is known about the mechanistic basis for this association. The only prior mechanistic data connecting telomerase to infection susceptibility is the cell-intrinsic hijacking of host telomerase to support viral replication during lytic human cytomegalovirus infection (*44*). Our bacterial infection experiments add a new dimension to the literature documenting interactions between telomerase and infection across host and pathogen species. We hypothesize the relative contributions of HSC and T-MAC telomerase expression will manifest in a disease-specific manner and encourage more investigation of telomerase function in diverse disease settings.

Methods

Animal husbandry

Zebrafish experiments were approved under the A*STAR IACUC protocols 211667 and 221694; Shanghai Public Health and Clinical Center, Fudan University GB/T 35823-2018 and 2022-A002-01; UK Home Office Project License PPL PP5900632. Breeding adults were housed under 14 hour light / 10 hour dark cycles in a 28°C recirculating aquarium. Eggs were produced by natural spawning and embryos were raised in E3 media at 28°C and supplemented with PTU from 24 hours post fertilization.

Bacterial culture

M. abscessus, M. marinum, and *M. smegmatis* were cultured in 7H9 or on 7H10 supplemented with OADC and hygromycin to select for pTEC fluorescent protein plasmids (L. Ramakrishnan, plasmids are available through Addgene https://www.addgene.org/Lalita_Ramakrishnan/). Single cell suspensions of mycobacteria were prepared and frozen at -70°C in aliquots for individual experiments as previously described (*45*).

Viral culture

Chikungunya virus (CHIKV) virus was produced from an infectious clone plasmid containing a duplicated subgenomic promoter driving ZsGreen (*Anthozoa* reef coral fluorescent protein) expression between the non-structural and structural genes. The viral genome was flanked by a CMV and a HDV ribozyme to launch transcription. BHK cells (RRID: CVCL_1914) were transfected with 500 ng of plasmid per well in a 6 well plate using lipofectamine 2000 (Thermofisher). At 3 days post transfection when CPE reaches approximately 40%, supernatant was cleared of cell debris and used to expand CHIKV culture on VeroE6 cells (RRID: CVCL_0574) for 44 hours. Cleared supernatant was tittered by standard plaque assay on VeroE6 cells. VeroE6 and BHK were grown in DMEM high glutamine (Gibco) with 10% FBS (Hyclone).

Infection

Zebrafish embryos were infected by microinjection with approximately 200 CFU *M. marinum*, 1000 CFU DESX1 *M. marinum* or *M. abscessus*. Primary infection was performed by injection into the caudal vein or neural tube above the yolk sac extension at 2 dpf. Infection was assayed by stereomicroscopy of immobilized zebrafish embryos on a Nikon SMZ25 stereoscope or an inverted Nikon Eclipse Ti2 at 5 dpi unless otherwise mentioned. Image analysis was performed in ImageJ/FIJI with bacterial fluorescent pixel count (FPC) carried out as previously described (*46*).

HSC counts were carried out on a Zeiss Celldiscoverer 7 (CD7) widefield microscope equipped with a 20x/0.95 plan-apochromat objective and 0.5x tube lens to acquire a 3 Z-stacks every 5 µm.

In situ hybridization

DIG-labeled RNA antisense probes were synthesized by *in vitro* transcription with DIG-labeled mix (Roche 11277073910) and the MEGA shortscript T7 Transcription kit (Thermo Fisher AM1354) from *tert* cDNA cloned into a pBluescript SK vector (primers referred to as ISH-tert in Table 1). Probe preparation and *in situ* hybridization of paraffin sections were carried out as described (*21*).

CRISPR-Cas9 mutagenesis

Gene-specific oligos for *in vitro* transcription of gRNAs were designed with IDT or selected from Wu *et al*. (*47*). HiScribe T7 High Yield RNA Synthesis Kit (NEB E2040) was used for *in vitro* transcription and Alt-R CRISPR-Cas9 crRNA was purchased commercially (IDT). Alt-R S.p. Cas9 Nuclease V3 (IDT 1081059) was combined with gRNA/crRNA and injected into the yolk of 1-2 cell stage zebrafish embryos.

Small molecule treatment

BIBR1532 (Selleckchem S1186), MST-312 (Sigma M3949), and cycloastragenol (Sigma SML1448) were dissolved in DMSO. Small molecules were added to the media of infected embryos at final concentrations of 600 nM BIBR1532, 2.5 µM MST-312 and 25 or 50 µM cycolastragenol. Small molecules were refreshed in complete media changes every second day.

In vitro mRNA synthesis

A full length *tert* cDNA (referred to as FL-tert in Table 1) was cloned into pGEMTeasy using Q5 polymerase (New England Biolabs). This was subcloned into a pSP64 Poly(A) plasmid (Promega P1241) using NEBuilder HiFi DNA Assembly (NEB E2621). Site-directed mutagenesis was carried out with the Q5 Site-Directed Mutagenesis Kit (NEB E0554) to mutate the ATGAT bases to CCGCC resulting in a predicted change in aspartate to alanine recreating the VAA catalytically dead TERT mutation (*48*). *In vitro* transcription of capped mRNA was performed with mMESSAGE mMACHINE Sp6 Transcription Kit (Invitrogen AM1340). 300 pg of mRNA was injected into the yolk of 1-2 cell stage zebrafish embryos.

DNA damage assays

SPiDER-βGal staining was carried out with the Cellular Senescence Detection Kit - SPiDER-βGal (Dojindo SG03-10) as per manufacturer's instructions on fixed zebrafish embryos equilibrated to McIlvain buffer pH6 and stained for 30 minutes at 37°C.
TUNEL staining was carried out with the In Situ Cell Death Detection Kit, TMR red (Roche 12156792910) as per manufacturer's instruction on fixed zebrafish embryos with incubation for 1 hour at 37°C.

Gene expression analysis

Quantitative PCR was performed with reverse transcription using the High Capacity cDNA Kit (Applied Biosystems 4368813) and PCR amplification with PowerUp SYBR Green Master Mix (Thermo Fisher A25742).

Statistics

Analyses of quantitative data were carried out with Graphpad Prism using T-tests for pairwise comparisons or ANOVA for multiple comparisons. All data are representative of at

least 3 biological replicates unless otherwise stated in the captions. Error bars on graphs represent standard deviation.

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Pamela S Ellis: provided tools, approved draft
Catarina M Henriques: provided tools, designed experiments, approved draft
Bo Yan: experimentation, approved draft
Serge Mostowy: experimentation, approved draft
Stefan H Oehlers: conceived study, experimentation, wrote draft

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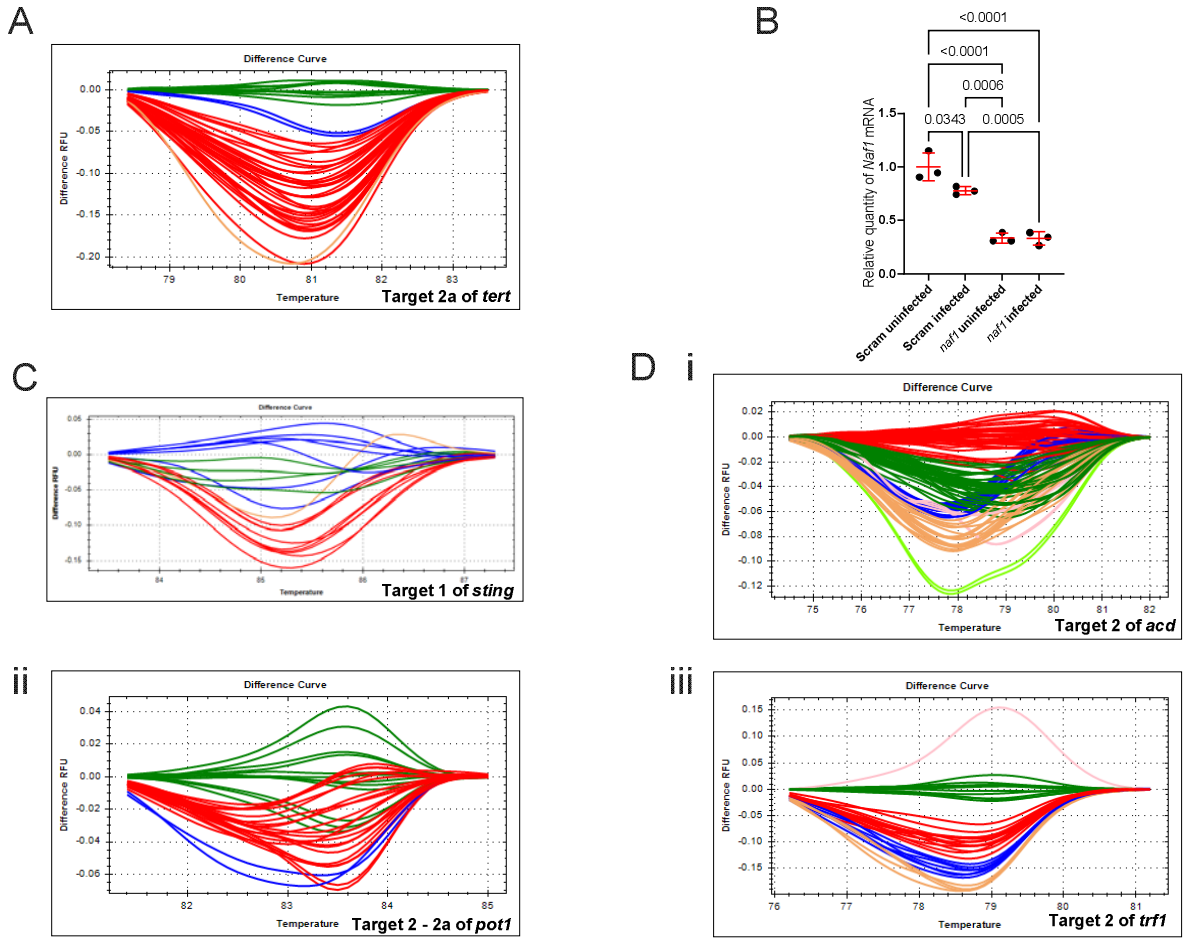
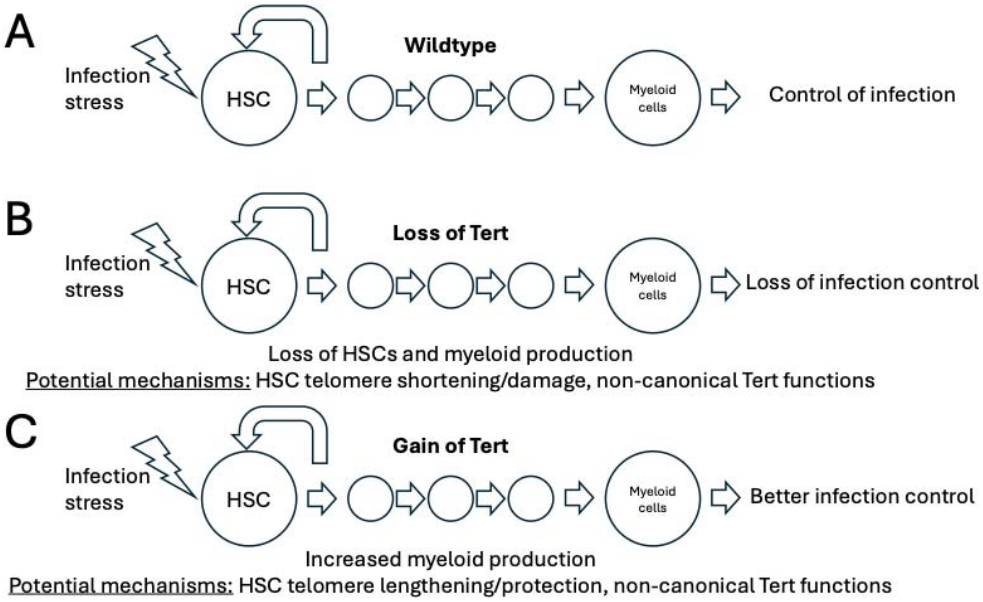


Supplementary Figure 1: Editing efficiencies of pooled gRNA-Cas9 gene editing.
(A) High resolution melt analysis of *tert* gRNA2 target site, n=35, 100% efficiency, control clusters: Green & Blue.
(B) Quantification of *naf1* transcripts in 5 dpi embryos infected with *M. marinum*.
(C) High resolution melt analysis of *sting* gRNA1 target site, n=18, 77.77% efficiency, control clusters: Green & Blue.
(D) i. High resolution melt analysis of *acd* gRNA2 target site, n=32, 84.3% efficiency, control clusters: Green & Blue.
ii. High resolution melt analysis of *pot1* gRNA2 target site, n=24 100% efficiency, control cluster: Green.
iii. High resolution melt analysis of *trf1* gRNA2 target site, n= 25 100% efficiency, control clusters: Green & Pink.



Supplementary Figure 2: Hypothesis for modulation of infection susceptibility by *tert* loss- and gain-of-function

(A) In the control wildtype setting, infection stress drives HSC proliferation to produce myeloid cells that control infection.

(B) In the Tert loss-of-function setting, loss of telomere elongation and/or protection from infection-associated genotoxic stress, and Tert-dependent hematopoietic transcription precipitates hematopoietic collapse and subsequent loss of infection control.

(C) In the Tert gain-of-function setting, increased telomere elongation and/or protection from infection-associated genotoxic stress, and Tert-dependent hematopoietic transcription increases myelopoiesis which protects against infection.

Table 1: Oligonucleotides

Use	Name	Sequence 5′-3′	Reference/notes
Cloning eGFP	eGFP Fw	acacatataccttcatacttaattgtcaagTTATTTGTATAGTTCATCCATGCCATGTG	This study, upper case letters indicate coding sequence
Cloning eGFP	eGFP Fw	ctctgcaaagcaagagattaataacccatcATGAGTAAAGGAGAAGAACTTTTCACTG	This study, upper case letters indicate coding sequence
Cloning full length zebrafish *tert* transcript for IVT	FL-Tert Fw	TAAGCATGTCTGGACAGTACTCGACAGATG	This study
Cloning full length zebrafish *tert* transcript for IVT	FL-Tert Rv	CAGGTTTTTTTTACACCCGC	This study
Cloning full length zebrafish *tert* transcript for ISH	ISH-Tert Fw	caagagattaataacccatcATGTCTGGACAGTACTCGACAGAT	This study, upper case letters indicate coding sequence
Cloning full length	ISH-Tert Rv	cttcatacttaattgtcaagTTACACCCGCATGCTGAAATCCAG	This study, upper

zebrafish *tert* transcript for ISH			case letters indicate coding sequence
Gene expression *actb*	Bactin 1 qFW	CGAGCAGGAGATGGGAACC	McCurley and Callard (*49*)
Gene expression *actb*	Bactin 1 qRv	CAACGGAAACGCTCATTGC	McCurley and Callard (*49*)
Gene expression *tert*	tert qFw	GCGCTCCTTATATCCAGTCG	This study
Gene expression *tert*	tert qRv	GGCGCATACAATAAGTCCACT	This study
Gene expression *tp53*	tp53 qFw	GATGGTGAAGGACGAAGGAA	Novoa *et al.* (*50*)
Gene expression *tp53*	tp53 qRv	AAATGACCCCTGTGACAAGC	Novoa *et al.* (*50*)
Gene expression *naf1*	naf1 qFw	GAAAGAGCAAAAGAGGCGCC	This study
Gene expression *naf1*	naf1 qRv	GGAAGTTGCTGTGGAAACCG	This study
gRNA *acd*	acd_Target_1	TAATACGACTCACTATAGGTGAAACGTCGCCGAATGGGTTTTAGAGCTAGAAATAGC	Wu *et al*. (*47*)
gRNA *acd*	acd_Target_2	TAATACGACTCACTATAGGTTTCTGAACCCCCCCCGCGTTTTAGAGCTAGAAATAGC	Wu *et al*. (*47*)
gRNA *acd*	acd_Target_3	TAATACGACTCACTATAGGTCTAGTGCAAACACGACCGTTTTAGAGCTAGAAATAGC	Wu *et al*. (*47*)
gRNA *acd*	acd_Target_4	TAATACGACTCACTATAGGGCTAAACTGGGTCGGCTTGTTTTAGAGCTAGAAATAGC	Wu *et al*. (*47*)
gRNA *naf1*	naf1_Target_1	TAATACGACTCACTATAGGTTGGTCTCATCTTGATCCGTTTTAGAGCTAGAAATAGC	Wu *et al*. (*47*)
gRNA *naf1*	naf1_Target_2	TAATACGACTCACTATAGGAGTCTGGCACTGGATCGTGTTTTAGAGCTAGAAATAGC	Wu *et al*. (*47*)
gRNA *naf1*	naf1_Target_3	TAATACGACTCACTATAGGTGCTTGATATTGTGCCGAGTTTTAGAGCTAGAAATAGC	Wu *et al*. (*47*)

gRNA *naf1*	naf1_Target_4	TAATACGACTCACTATAGGACCAGGAACCTCCCCCTGGTTTTAGAGCTAGAAATAGC	Wu *et al*. (*47*)
gRNA scaffold	sgRNA Scaffold Primer	AAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC	Wu *et al*. (*47*)
gRNA Scrambled	scramble_target_1	TAATACGACTCACTATAGGCAGGCAAAGAATCCCTGCCGTTTTAGAGCTAGAAATAGC	Wu *et al*. (*47*)
gRNA Scrambled	scramble_target_2	TAATACGACTCACTATAGGTACAGTGGACCTCGGTGTCGTTTTAGAGCTAGAAATAGC	Wu *et al*. (*47*)
gRNA Scrambled	scramble_target_3	TAATACGACTCACTATAGGCTTCATACAATAGACGATGGTTTTAGAGCTAGAAATAGC	Wu *et al*. (*47*)
gRNA Scrambled	scramble_target_4	TAATACGACTCACTATAGGTCGTTTTGCAGTAGGATCGGTTTTAGAGCTAGAAATAGC	Wu *et al*. (*47*)
gRNA *sting*	sting_Target_1	TAATACGACTCACTATAGGGACTGGGTTTTCTTACTCGTTTTAGAGCTAGAAATAGC	Wu *et al*. (*47*)
gRNA *sting*	sting_Target_2	TAATACGACTCACTATAGGCATGTTGCTCAATAGCCTGTTTTAGAGCTAGAAATAGC	Wu *et al*. (*47*)
gRNA *sting*	sting_Target_3	TAATACGACTCACTATAGGCAGAAGGATATGTAGACGGTTTTAGAGCTAGAAATAGC	Wu *et al*. (*47*)
gRNA *sting*	sting_Target_4	TAATACGACTCACTATAGGAGTTTCTCACCGTTGAGCGTTTTAGAGCTAGAAATAGC	Wu *et al*. (*47*)
gRNA *tert*	tert_Target_1	TAATACGACTCACTATAGGAACCGGCTTTCGGCCGTCGTTTTAGAGCTAGAAATAGC	Wu *et al*. (*47*)
gRNA *tert*	tert_Target_2	TAATACGACTCACTATAGGACATTGCGAGGGTCGAGGGTTTTAGAGCTAGAAATAGC	Wu *et al*. (*47*)
gRNA *tert*	tert_Target_3	TAATACGACTCACTATAGGGCAGCCCCAGAGCTCGTGGTTTTAGAGCTAGAAATAGC	Wu *et al*. (*47*)
gRNA *tert*	tert_Target_4	TAATACGACTCACTATAGGTTCAGGCCTGCCATTGGCGTTTTAGAGCTAGAAATAGC	Wu *et al*. (*47*)
gRNA *trf1*	trf1_Target_1	TAATACGACTCACTATAGGTTAGAGAAAGGTCTTCGGGTTTTAGAGCTAGAAATAGC	Wu *et al*. (*47*)
gRNA *trf1*	trf1_Target_2	TAATACGACTCACTATAGGAACAGACGGTTCTCTGAGGTTTTAGAGCTAGAAATAGC	Wu *et al*. (*47*)
gRNA *trf1*	trf1_Target_3	TAATACGACTCACTATAGGAAGAAACCTTGCAGTGGCGTTTTAGAGCTAGAAATAGC	Wu *et al*. (*47*)
gRNA *trf1*	trf1_Target_4	TAATACGACTCACTATAGGTTTCTTACTCGAAGTATGGTTTTAGAGCTAGAAATAGC	Wu *et al*. (*47*)
HRM *acd*	HRM_Acd_Fw	TGCGTTTAACGGGTAGTGTTAAT	This study
HRM *acd*	HRM_Acd_Rv	CGTTTACAGTTCAGTGCAAGTGTA	This study
HRM *sting*	HRM_Sting_Fw	CTGTGATGGGAGAAGACGCT	This study
HRM *sting*	HRM_Sting_Rv	TCACTGAACTTGTCTGAGTCCA	This study
HRM *tert*	HRM_Tert_Fw	GTTGGAAACCAGCGGATCAG	This study

HRM *tert*	HRM_Tert_Rv	AGGAAGTTCTTCATGCCCCG	This study
HRM *trf1*	HRM_Trf1_Fw T2	TGTGTTTAGGTTTAGTGGATGAGC	This study
HRM *trf1*	HRM_Trf1_Rv T2	CGTCCATCACTCTGGCTAGG	This study
HRM *pot1*	HRM_Pot1_Fw T2/2a	AAGCCGATAAGCGCAGAGTT	This study
HRM *pot1*	HRM_Pot1_Rv T2/2a	CTATGCATGACAGCCTTGGC	This study
ISH probe *tert*	ISH_Tert_Fw	TGGCGGATTCAGGCCGGTTTTG	This study
ISH probe *tert*	ISH_Tert_Rv	TATTGAACATTGCGAGGGTCGAG	This study
Site directed mutagenesis of zebrafish *tert* transcript	SDM Tert 'AA' Fw	GAGGCTGATTG[CCGCC]TTTTTGCTCATTACTCC	This study, bracketed sequence indicates mutant sequence
Site directed mutagenesis of zebrafish *tert* transcript	SDM Tert 'AA' Rv	ATCAGACACCCTCCTTTAG	This study