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Supplementary Information for

Telomere shortening produces an inflammatory environment that increases tumor incidence in zebrafish

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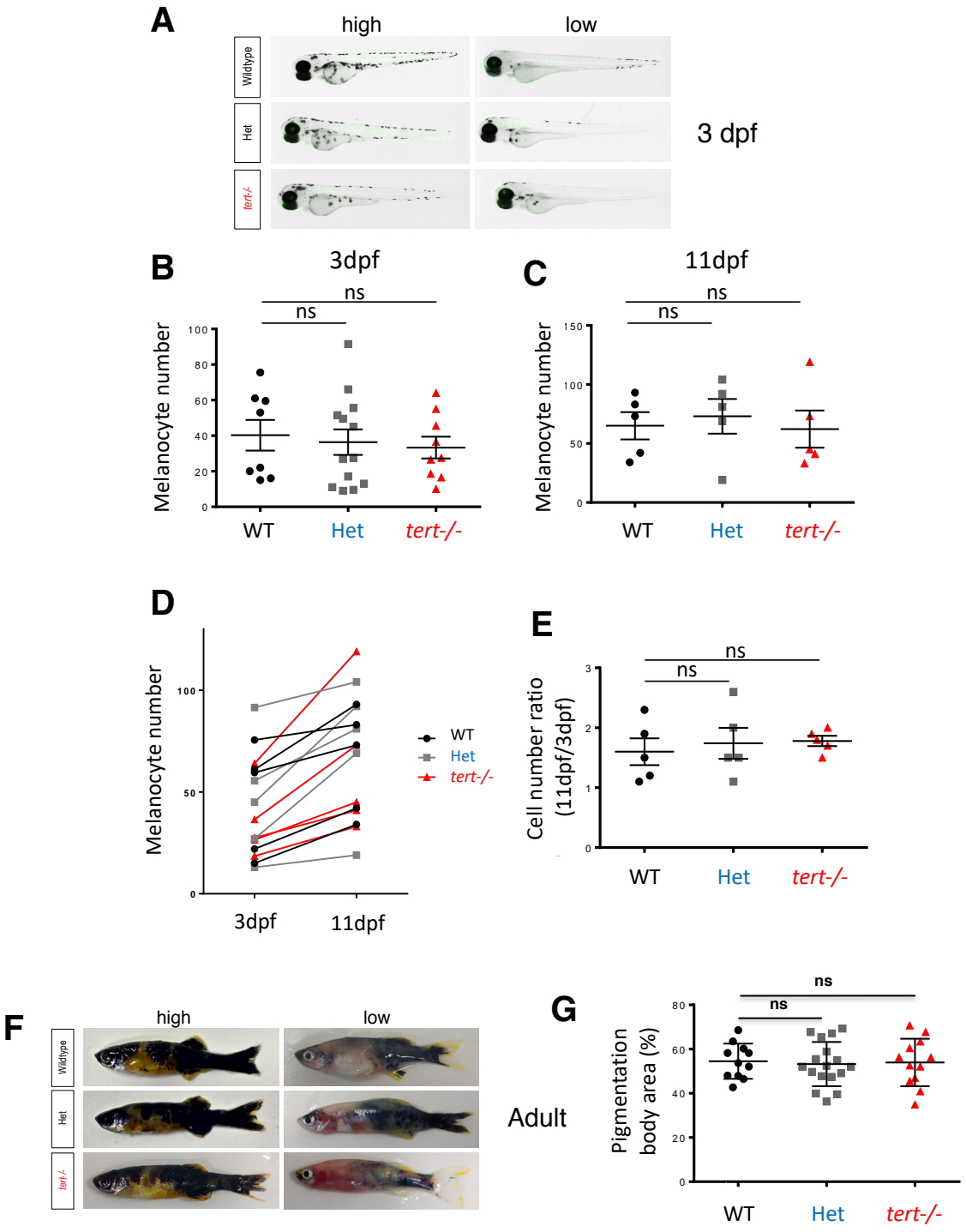
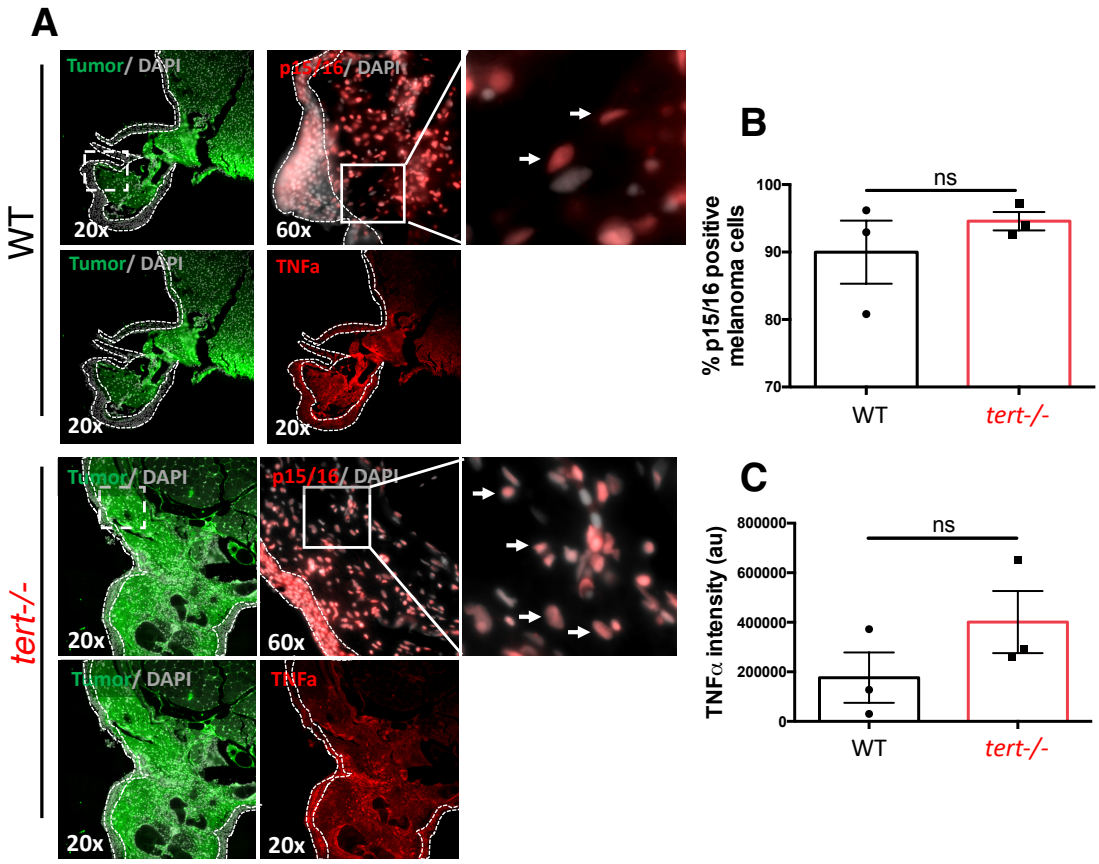


Supplementary Figure 1. *tert* genetic status of chimera recipients does not influence melanocyte number in larvae or adults. A) Representative images of 3dpf chimeras

exhibiting high (left) and low (right) number of melanocytes. Blastula mitfa:HRAS; β-actin:GFP cells were injected into the same stage embryos resulting from an incross of *tert*+/-; Casper. Larvae were genotyped at 3dpf and followed individually until 11dpf. B and C) No significant differences between melanocyte numbers in hosts of different *tert* genotype, both at 3dpf and 11dpf. Each point in the graph represents an individual animal. Data are represented as mean +/- SEM. D) Melanocyte proliferation for individual animals represented between 2 timepoints was not significantly influenced by host genotype. E) Cell proliferation in individual animals, represented as the ratio of cell numbers between the 2 timepoints was not significantly altered by host genotype. F) Chimeras harboring a tumor were analyzed for extent of pigmentation in adults. Left side animals with high pigmentation, right side: low pigmentation. G) Quantification of pigmented area in percent of total surface. Each datapoint represents one animal (both sides). Pigmented area did not significantly differ depending on the host genotype. Data are represented as mean +/- SEM. ns: not significant.



Supplementary Figure 2: The level of senescence and TNFα appears to be similar in melanoma of wildtype and *tert-/-* chimeras. A) Representative immunofluorescence images of p15/16 and TNFα in melanoma from wildtype and telomerase mutant chimeras. Dashed lines locate the skin (no green fluorescence) and arrows indicate p15/16 positive cells. B) Quantification of p15/16 positive cells and levels of TNFα in melanoma of chimeric wildtype and *tert-/-* zebrafish (N=3). Squares with dashed lines show place of amplification in a sequential section and squares with solid lines show place of amplification on that section. Data are represented as mean +/- SEM. ns: not significant.

Table S1 – List of primers used in RT-qPCR expression analysis and *tert* genotyping.

Gene name	Primer sequences
cdkn2a/b	forward – 5' GGATGAACTGACCACAGCAGCA 3' reverse – 5' CGGCTGCGGAAAGAGTCTCAG 3'
cdkn1a	forward – 5' ATGCAGCTCCAGACAGATGA 3' reverse – 5' CGCAAACAGACCAACATCAC 3'
tnfa	forward – 5' AGGCAATTTCACTTCCAAGGC 3' reverse – 5' GGTCCTGGTCATCTCTCCAGT 3'
rpl13	forward – 5' TTCACCACCACAGCCGAAAGA 3' reverse – 5' TACCGCAAGATTCCATACCCA 3'