**Table S1. Clinical characteristics and cancer prevalence among 226 participants in the Johns Hopkins Telomere Syndrome Study (2003-2022), supplementary to Figure 1**

| | **Whole Cohort** N = 226 | **Females** N = 94 (41.6%) | **Males** N = 132 (58.4%) | **P**** |
|---|---|---|---|---|
| **Age at Enrollment - mean (SD)** | 45.44 (19.47) | 44.1 (18.17) | 46.4 (20.37) | ns |
| **Age at Enrollment - median (range)** | 50 (0.58, 81) | 49 (1, 76) | 51 (0.58, 81) | ns |
| | | | | |
| **Deceased as of data cutoff** | | | | |
| No | 105 (46.5) | 55 (58.5) | 50 (37.9) | **0.008** |
| Unknown | 60 (26.5) | 20 (21.3) | 40 (30.3) | |
| Yes | 61 (27) | 19 (20.2) | 42 (31.8) | |
| | | | | |
| **Sex** | | | | |
| Female | 94 (41.6) | 94 (100) | 0 (0) | < 0.001 |
| Male | 132 (58.4) | 0 (0) | 132 (100) | |
| | | | | |
| **Mutant Gene** | | | | |
| *DKC1* - | 17/226 (7.5) | 0/94 (0) | 17/132 (12.9) | **< 0.001** |
| *RTEL1* | 25/226 (11.1) | 11/94 (11.7) | 14/132 (10.6) | ns |
| *TERT* | 99/226 (43.8) | 49/94 (52.1) | 50/132 (37.9) | **0.041** |
| *TINF2* | 3/226 (1.3) | 2/94 (2.1) | 1/132 (0.8) | ns |
| *TR* | 27/226 (11.9) | 18/94 (19.1) | 9/132 (6.8) | **0.006** |
| *ZCCHC8* | 3/226 (1.3) | 0/94 (0) | 3/132 (2.3) | ns |
| *NAF1* | 5/226 (2.2) | 4/94 (4.3) | 1/132 (0.8) | ns |
| *PARN* | 8/226 (3.5) | 2/94 (2.1) | 6/132 (4.5) | ns |
| | | | | |
| **Transplant status** | | | | |
| Any Transplant | 51/226 (22.6) | 16/94 (17) | 35/132 (26.5) | ns |
| Received BMT | 17/226 (7.5) | 8/94 (8.5) | 9/132 (6.8) | ns |
| Received Solid Organ Transplant | 36/226 (15.9) | 9/94 (9.6) | 27/132 (20.5) | **0.028** |
| | | | | |
| **Cancer diagnoses*** | | | | |
| Any Cancer | 35/226 (15.5) | 7/94 (7.4) | 28/132 (21.2) | **0.005** |
| Solid Tumor | 14/226 (6.2) | 1/94 (1.1) | 13/132 (9.8) | **0.009** |
| MDS or AML | 24/226 (10.6) | 6/94 (6.4) | 18/132 (13.6) | ns |

**Cancer diagnoses did not include resected non-melanoma cutaneous malignancies, breast or prostate cancers which were not curated

**P-values were calculated using Student's *t*-test for comparisons of means, Wilcoxon rank sum test for comparisons of medians, and Fisher's exact test for comparison of categorical variables.

**Table S2. Cumulative incidence of cancer by ages 50, 60 and 70, with 95% confidence intervals (supplementary to Figure 1)**

| | N | N events | Age 50 | Age 60 | Age 70 | HR (95% CI) | P |
|---|---|---|---|---|---|---|---|
| **Solid Tumors: Whole Cohort** | 226 | 14 | 0.03 (0.01, 0.06) | 0.08 (0.03, 0.12) | 0.09 (0.04, 0.14) | | |
| **Sex** | | | | | | | |
| Females | 94 | 1 | 0.01 (0, 0.03) | 0.01 (0, 0.03) | 0.01 (0, 0.03) | | |
| Males | 132 | 13 | 0.05 (0.01, 0.09) | 0.11 (0.05, 0.18) | 0.13 (0.06, 0.21) | 8.01 (1.07, 60.26) | **0.0432** |
| **Received Solid Organ Transplant** | | | | | | | |
| No | 190 | 9 | 0.03 (0, 0.06) | 0.06 (0.01, 0.1) | 0.07 (0.02, 0.12) | | |
| Yes | 36 | 5 | 0.03 (0, 0.08) | 0.14 (0.01, 0.27) | 0.14 (0.01, 0.27) | 2.13 (0.74, 6.1) | 0.1583 |
| ***DKC1* Mutation-By-Sex** | | | | | | | |
| No *DKC1* Mutation (Males) | 115 | 9 | 0.01 (0, 0.03) | 0.08 (0.02, 0.15) | 0.1 (0.03, 0.18) | 1.0 (Ref) | 1.0 (Ref) |
| *DKC1* Mutation | 17 | 4 | 0.37 (0.06, 0.67) | 0.37 (0.06, 0.67) | | 7.02 (2.12, 23.3) | **0.0014** |
| No *DKC1* Mutation (Females) | 94 | 1 | 0.01 (0, 0.03) | 0.01 (0, 0.03) | 0.01 (0, 0.03) | 0.17 (0.02, 1.31) | 0.089 |
| **MDS/AML: Whole Cohort** | 226 | 24 | 0.04 (0.01, 0.08) | 0.13 (0.08, 0.19) | 0.18 (0.11, 0.26) | | |
| **Sex** | | | | | | | |
| Females | 94 | 6 | 0.02 (0, 0.06) | 0.08 (0, 0.16) | 0.15 (0.03, 0.26) | | |
| Males | 132 | 18 | 0.06 (0.01, 0.11) | 0.16 (0.08, 0.24) | 0.2 (0.11, 0.29) | 1.69 (0.68, 4.23) | 0.2603 |
| **MDS/AML: *DKC1* Mutation-By-Sex** | | | | | | | |
| No *DKC1* Mutation (Males) | 115 | 16 | 0.04 (0, 0.09) | 0.15 (0.07, 0.24) | 0.2 (0.1, 0.29) | 1.0 (Ref) | 1.0 (Ref) |
| *DKC1* Mutation | 17 | 2 | 0.23 (0, 0.59) | 0.23 (0, 0.59) | | 1.97 (0.41, 9.5) | 0.4007 |
| No *DKC1* Mutation (Females) | 94 | 6 | 0.02 (0, 0.06) | 0.08 (0, 0.16) | 0.15 (0.03, 0.26) | 0.63 (0.25, 1.58) | 0.3197 |

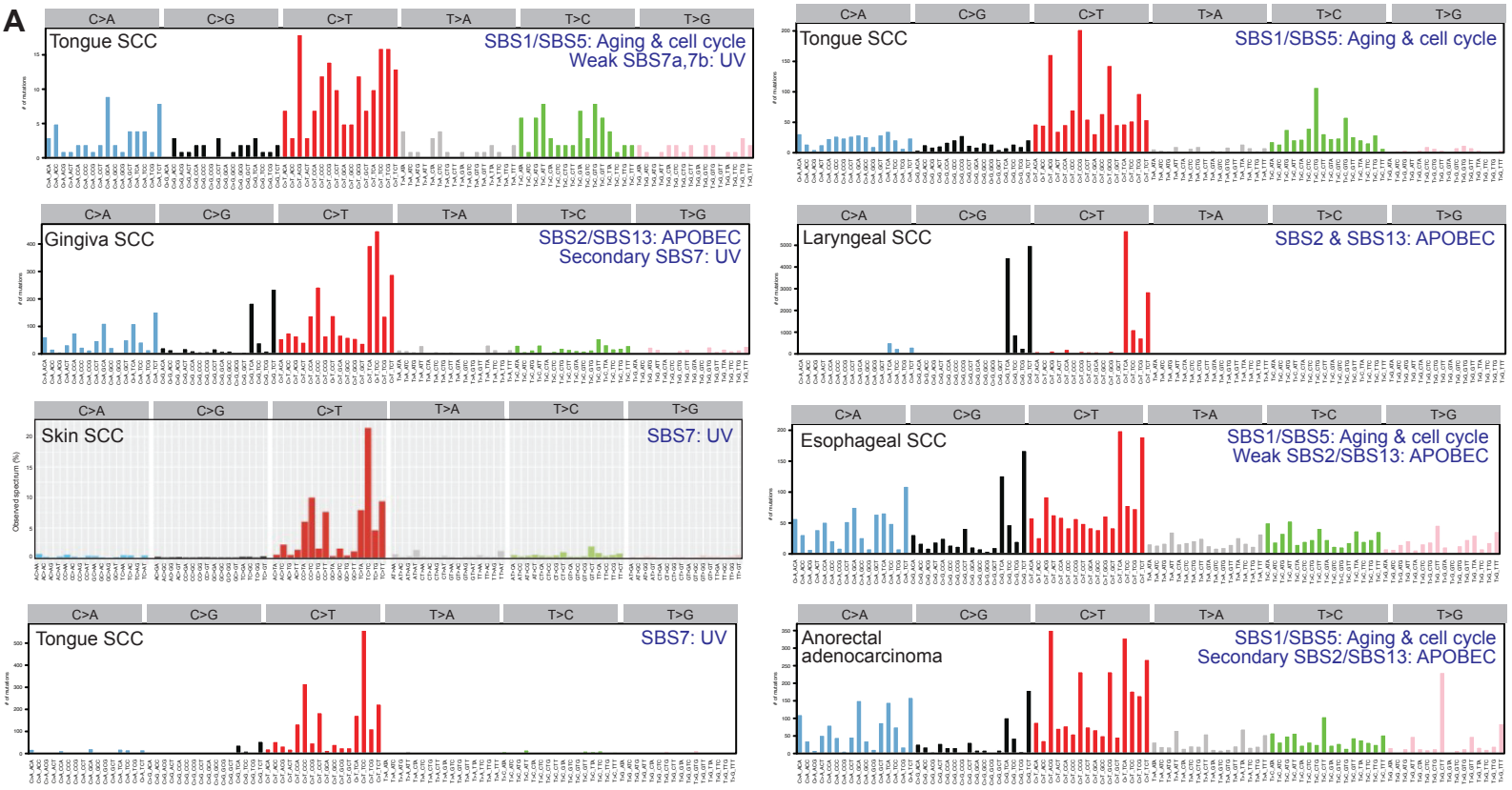
Hazard ratios (HR) and P-values were estimated for differences in cancer incidence between patient groups using Fine and Gray's method. Cumulative incidence of cancer was estimated using age as a time scale, accounting for the competing risk of death in univariate analysis.

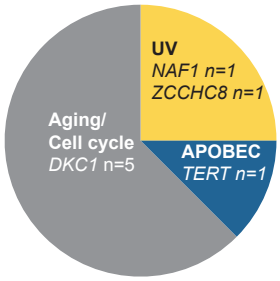
**Table S3. Risk of solid tumors estimated by hazard ratio using two multi-variate competing risks regression models (supplementary to Figure 1)**

| | HR [95% CI] | P |
|---|---|---|
| *Model 1* | | |
| Males v Females | 7.55 (0.94, 60.43) | **0.0568** |
| Rec'd Solid Organ Transplant vs. Did Not | 1.85 (0.63, 5.45) | 0.264 |
| *Model 2* | | |
| Rec'd Solid Organ Transplant vs. Did Not | 1.84 (0.55, 6.16) | 0.3255 |
| *DKC1* Mutation vs. No *DKC1* Mutation (Males) | 6.93 (1.87, 25.68) | **0.0038** |
| No *DKC1* Mutation (Females) vs. No *DKC1* Mutation (Males) | 0.18 (0.02, 1.46) | 0.1089 |

Model 1 includes sex and receipt of a solid organ transplant or not.
Model 2 includes sex-by-*DKC1* mutation groups and receipt of a solid organ transplant or not.

# Figure S1


A
C>A
C>G
C>T
T>A
T>C
T>G
Tongue SCC
SBS1/SBS5: Aging & cell cycle
Weak SBS7a,7b: UV
Tongue SCC
SBS1/SBS5: Aging & cell cycle
Gingiva SCC
SBS2/SBS13: APOBEC
Secondary SBS7: UV
Laryngeal SCC
SBS2 & SBS13: APOBEC
Skin SCC
SBS7: UV
Esophageal SCC
SBS1/SBS5: Aging & cell cycle
Weak SBS2/SBS13: APOBEC
Tongue SCC
SBS7: UV
Anorectal
adenocarcinoma
SBS1/SBS5: Aging & cell cycle
Secondary SBS2/SBS13: APOBEC


B
UV
NAF1 n=1
ZCCHC8 n=1
Aging/
Cell cycle
DKC1 n=5
APOBEC
TERT n=1
COSMIC SBS
mutational signature

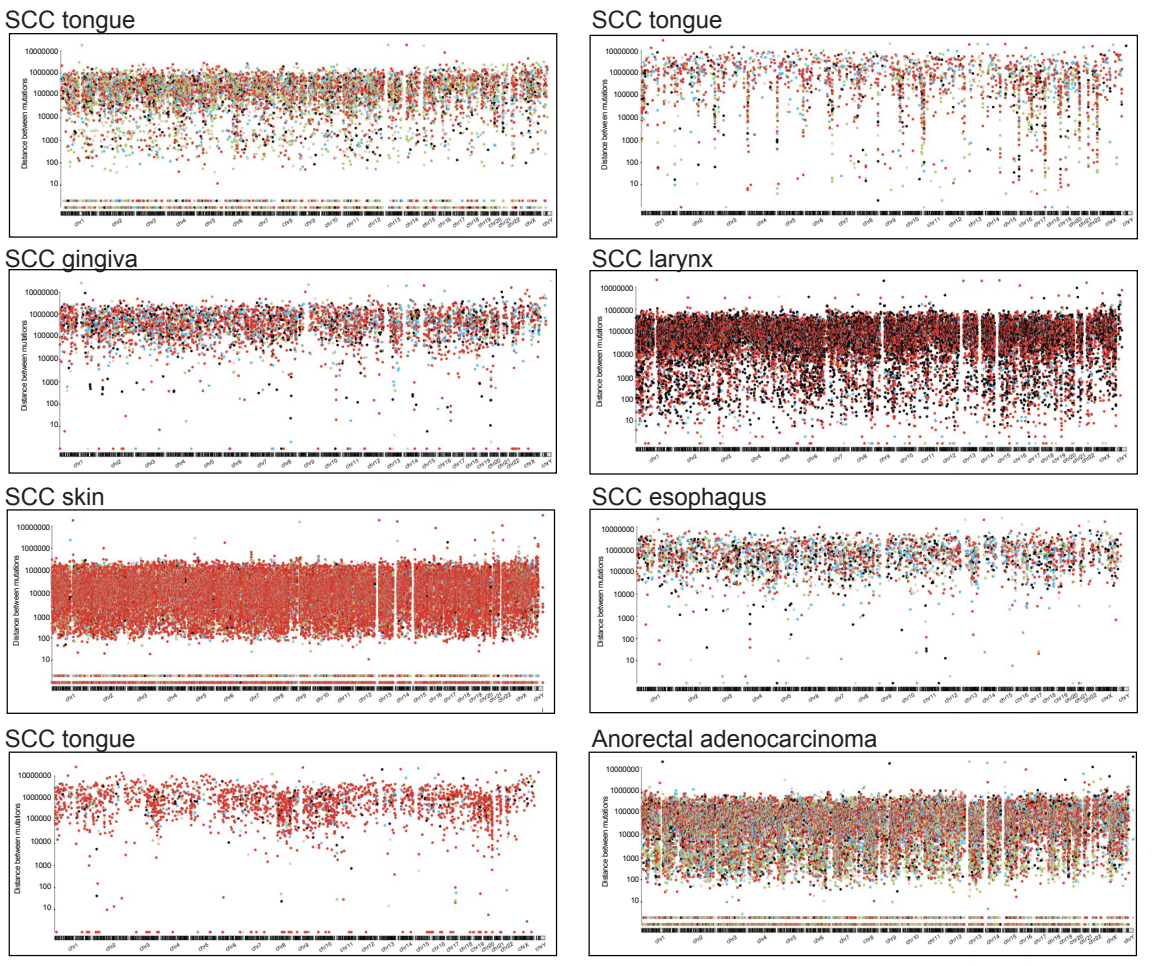

C
SCC tongue
SCC tongue
SCC gingiva
SCC larynx
SCC skin
SCC esophagus
SCC tongue
Anorectal adenocarcinoma
Distance between mutations
C>A
C>G
C>T
T>A
T>C
T>G

**Figure S1. COSMIC single base substitution (SBS) mutational signatures and rainfall plots of 8 solid tumors derived from individuals with short telomere syndromes (supplementary to Figure 2).**

**A.** Each mutational signature plot shows the distribution of mutations across the six potential types of substitutions generated by Mutalisk. Mutational signature analysis resulted in cosine score values above 0.90. Signatures were further analyzed using SigProfilerSingleSample and the concordant, predominant signature is annotated for each tumor in addition to any secondary or weaker signatures, if present. Weaker signatures ere defined as less than 10% of the subtype detected and secondary as greater than or equal to 10% but less than the predominant signature.
**B.** Distribution of SBS mutational signatured by germline mutant gene.
**C.** Rainfall plot analysis of kataegis focal hypermutability pattern analyzed by high pass whole genome sequencing. Each panel represents one tumor's analysis. Each dot represents a somatic single nucleotide variants, and dots are ordered on the horizontal axis according to genomic position. The vertical axis indicates the genomic distance between consecutive variants. None of the plots display a clustering of mutations over a 2 kb-sized DNA region as is characteristic of kataegis. All the plots were generated using a Bambino-based variant calling pipeline, except the squamous cell cancer of the skin plot which was generated using Mutect2 due to high background noise.

**Figure S2**

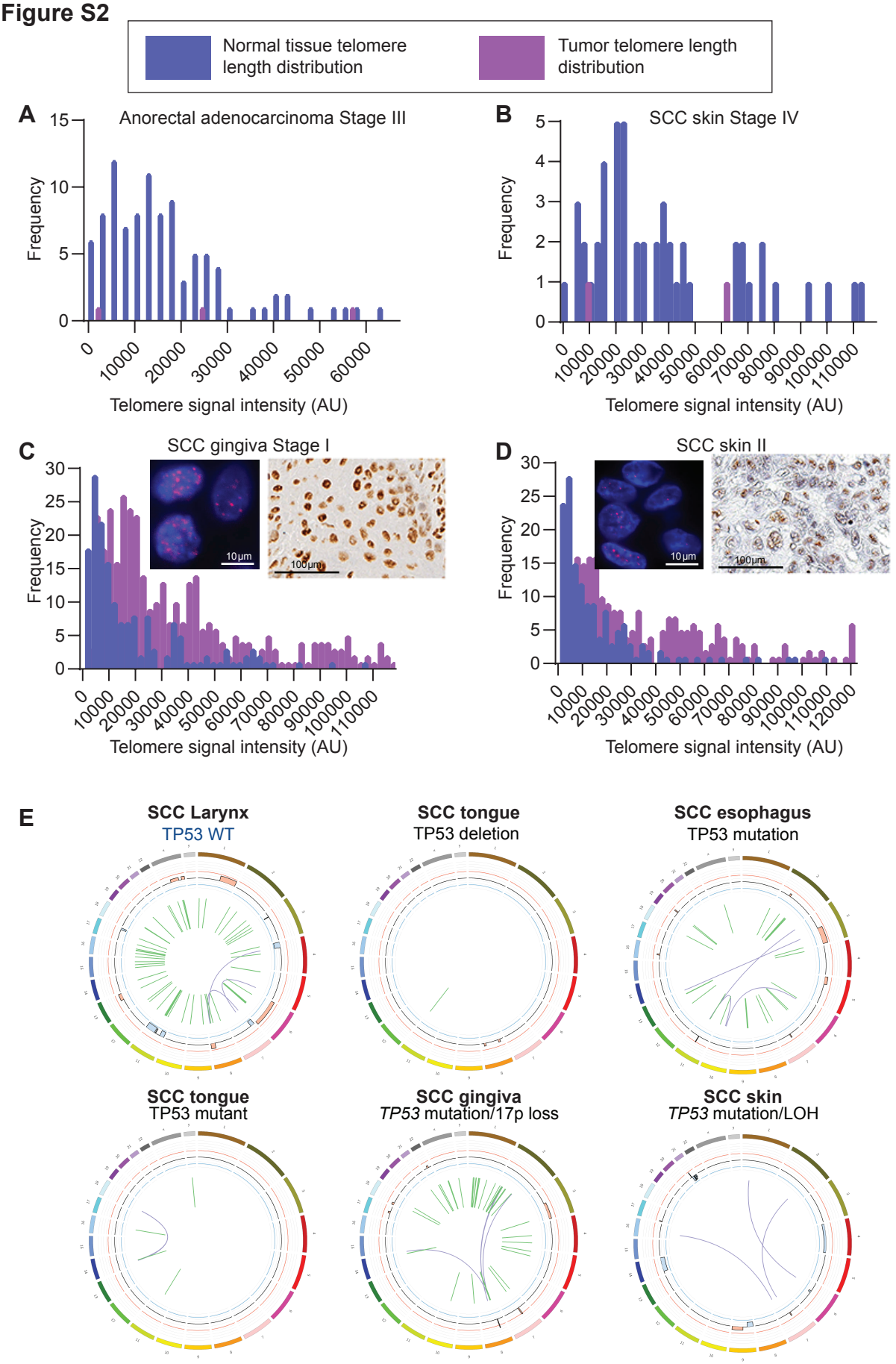
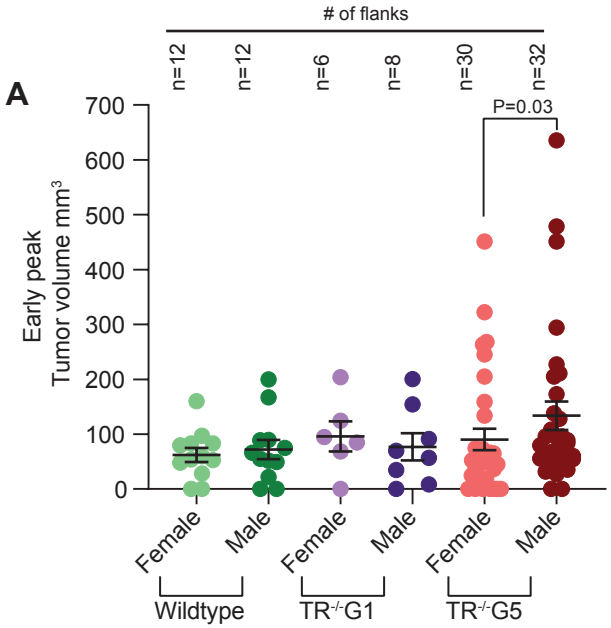



**Figure S2. Telomere length distribution by quantitative in situ hybridization (Q-FISH) and circos plots of genomic alterations (supplementary to Figure 2).**

**A-B**. Distribution of two tumors where the telomere length in the tumor had a very low signal, below detection in most cells, relative to adjacent normal tissue. These individuals had advanced stage disease.
**C-D**. Two tumors which have a longer telomere signal relative to adjacent normal tissue. Immunofluorescent inset panels show no evidence of ultrabright telomere foci characteristic of the alternative lengthening of telomeres (ALT) mechanism. In parallel, immunohistochemistry insets of the same respective tumors show retained ATRX staining in both tumors suggesting the longer telomeres were maintained via an ATRX-independent mechanism.
**E.** Genomic circos plots displaying somatic structural variants as well as greater than 3 Mb copy number variants detected using Illumina whole genome sequencing. Each circos plot is labelled by the cancer type and *TP53* status above. Circos plots for 6 tumors are shown with remaining 2 tumors shown in Figure 2I and 2J.

**Figure S3**



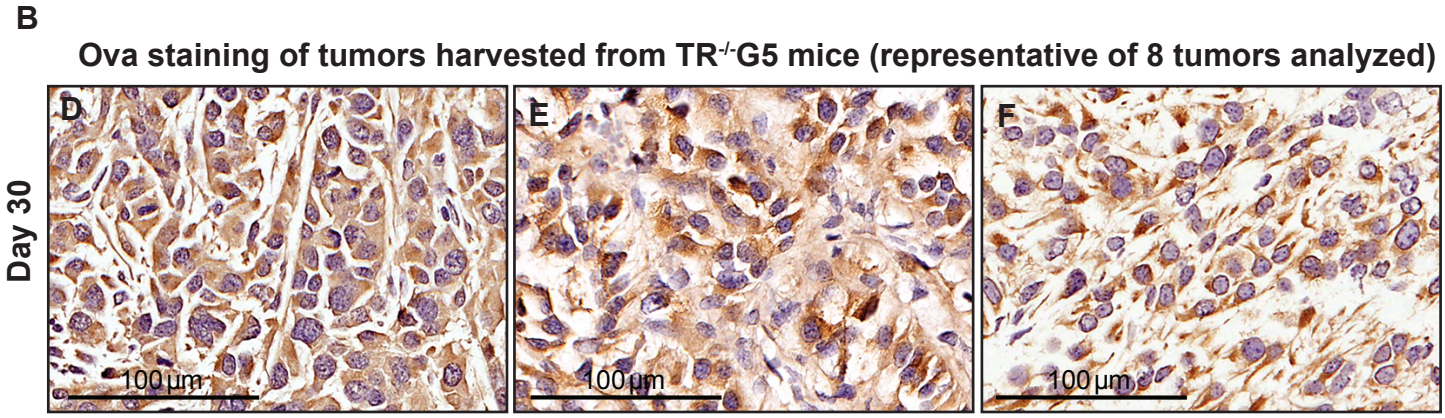




**Figure S3. Tumor size by sex and ovalbumin immunohistochemistry of relapsed tumors (supplementary to Figure 4).**

**A.** Maximum tumor volume in the first 10 days with each datapoint representing a single flank in each of the male and female mice studied. Mean and standard error bars are shown. P-value reflects two-sided Mann-Whitney test calculation.
**B.** Immunohistochemistry of tumors harvested after day 30 post-subcutaneous implantation that were harvested from short telomere mice (*mTR$^{-/-}$G5*). Three representative images show retained cytoplasmic staining of the ovalbumin antigen (all 8 tumors showed a similar pattern). Tumors were harvested at a mean of 32 days post-implantation (range 24-47).