

# 1 The Telomere Length Landscape of Prostate 2 Cancer

3  
4 Julie Livingstone<sup>1,2,3,4</sup>, Yu-Jia Shiah<sup>5</sup>, Takafumi N. Yamaguchi<sup>1,2,3,4</sup>, Lawrence E.  
5 Heisler<sup>5</sup>, Vincent Huang<sup>5</sup>, Robert Lesurf<sup>5</sup>, Tsumugi Gebo<sup>1,2,3,4</sup>, Benjamin Carlin<sup>1,2,3,4</sup>,  
6 Stefan Eng<sup>1,2,3,4</sup>, Erik Drysdale<sup>5</sup>, Jeffrey Green<sup>5</sup>, Theodorus van der Kwast<sup>6,7</sup>, Robert G.  
7 Bristow<sup>6,8,9</sup>, Michael Fraser<sup>6</sup>, Paul C. Boutros<sup>1,2,3,4,8,10</sup>

8 <sup>1</sup> Department of Human Genetics, University of California, Los Angeles, CA 90095, USA

9 <sup>2</sup> Department of Urology, University of California, Los Angeles, CA 90024, USA

10 <sup>3</sup> Jonsson Comprehensive Cancer Centre, University of California, Los Angeles, CA 90024, USA

11 <sup>4</sup> Institute for Precision Health, University of California, Los Angeles, CA 90024, USA

12 <sup>5</sup> Ontario Institute for Cancer Research, Toronto, ON M5G 0A3, Canada

13 <sup>6</sup> Princess Margaret Cancer Centre, University Health Network, Toronto, ON M5G 2M9, Canada

14 <sup>7</sup> Department of Pathology, Laboratory Medicine Program, University Health Network, Toronto, ON M5G  
15 2C4, Canada

16 <sup>8</sup> Department of Medical Biophysics, University of Toronto, Toronto, ON M5G 1L7, Canada

17 <sup>9</sup> Manchester Cancer Research Centre, Manchester, United Kingdom

18 <sup>10</sup> Department of Pharmacology and Toxicology, University of Toronto, Toronto, ON M5S 1A8, Canada

19

20 **Corresponding Author:** Dr. Paul C. Boutros, 12-109 CHS; 10833 Le Conte Avenue;  
21 Los Angeles, CA 90095. Phone: 310-794-7160; Email: [pboutros@mednet.ucla.edu](mailto:pboutros@mednet.ucla.edu)

22 **Keywords:** Telomere length; prostate cancer; genomic instability; integration

23 **Word Count of the text:** 6,955 (excluding references)

24 **Word Count of the abstract:** 198

## 25 **Abstract**

26 Replicative immortality is a hallmark of cancer, and can be achieved through telomere  
27 lengthening and maintenance. We report telomere lengths (TLs) of 392 localized  
28 prostate cancer tumours and characterize their relationship to genomic, transcriptomic  
29 and proteomic features. Shorter tumour TLs were associated with elevated genomic  
30 instability, including single-nucleotide variants, indels and structural variants. Genes  
31 involved in cell proliferation and signaling were correlated with tumour TL at all levels of  
32 the central dogma. TL was also associated with multiple clinical features of a tumour.  
33 Longer TLs in non-tumour samples were associated with a lower rate of biochemical  
34 relapse after definitive local therapy. Our analysis integrates multi-omics data to  
35 illuminate the relationship of specific genomic alterations in a tumour and TL in prostate  
36 cancer. Although the role of telomere length in cancer has been well studied, its  
37 association to genomic features is less well known. We describe the multi-level  
38 integration of telomere length, genomics, transcriptomics and proteomics in localized  
39 prostate cancer. **Patient Summary** We examined the association between telomere  
40 length and multiple omics-level data in prostate cancer. We observed that traditional  
41 telomere mutations are rare in prostate cancer and that telomere length is associated  
42 with multiple measure of genomic instability.

## 43 Introduction

44 Telomeres, which make up the ends of chromosomes, consist of a repeat TTAGGG  
45 sequence<sup>1</sup> along with bound proteins known as shelterin<sup>2</sup>. Telomeres protect  
46 chromosomal ends from degradation by the DNA double-strand break (DSB) response  
47 pathway. Due to the linearity of chromosomes and chromosomal replication, telomeres  
48 are shortened by approximately 50 bp during mitosis<sup>3</sup>. When telomeres become  
49 substantially shortened, cell cycle progression halts and cells enter replicative  
50 senescence; further replication leads to cellular crisis and eventually cell death<sup>4</sup>.  
51 Telomere maintenance and lengthening is essential for cancer cell proliferation and  
52 enables replicative immortality: a fundamental hallmark of cancer<sup>5</sup>. Telomere regulation  
53 occurs through two known mechanisms: activation of telomerase or alternative  
54 lengthening of telomeres (ALT) which relies on homology-directed DNA replication<sup>6</sup>.

55 Despite the pan-cancer studies analyzing the telomere length from various tumour  
56 types<sup>7,8</sup>, the role of telomere maintenance in individual tumour types is poorly  
57 understand. Moreover, the relationship between telomere length and biologically-  
58 relevant genomic indices, such as percentage of the genome altered (PGA; <sup>9,10</sup>, and  
59 other measures of mutational density has not been assessed, nor has the association  
60 between telomere length and clinical outcome in prostate cancer.

61 We and others have described the genomic, transcriptomic and proteomic landscape of  
62 localized, non-indolent prostate cancer<sup>11–18</sup>: the most frequently diagnosed non-skin  
63 malignancy in North American men (~250,000 new cases per year). Localized prostate  
64 cancer is a C-class tumour<sup>19</sup>, characterized by a paucity of driver single nucleotide  
65 variants (SNVs) and a relatively large number of structural variants (SVs), including  
66 copy number aberrations (CNAs) and genomic rearrangements (GRs). Several of these  
67 aberrations, including mutations in *ATM* and amplifications of *MYC* – which drive DSB  
68 repair and cell proliferation, respectively – are associated with significantly reduced time  
69 to biochemical and metastatic relapse after local therapy<sup>20</sup>. Intriguingly, both of these  
70 mutations have also been associated with telomere maintenance<sup>21,22</sup> and telomere  
71 shortening – relative to adjacent epithelium<sup>23</sup>. Similarly an interaction between hypoxia,  
72 dysregulated *PTEN*, *TERT* abundance and telomere shortening was recently  
73 illustrated<sup>15</sup>. Despite this, no well-powered study exists evaluating the association  
74 between telomere length, somatic features and clinical outcome in prostate cancer.

75 To fill this gap, we quantify the telomere length and somatic mutational landscapes of  
76 392 localized prostate tumours. We explore associations between telomere length and  
77 the tumour methylome, transcriptome and proteome. Using rich clinical annotation, we  
78 further assessed the relationship between telomere length and outcome. Taken  
79 together, these data establish the role and regulation of telomere length in localized  
80 prostate cancer, and establish clear links between telomere maintenance and drivers of  
81 prostate cancer development and clinical aggression.

## 82 Results

### 83 Association of telomere length with somatic nuclear driver events

84 To investigate the impact of telomere length (TL) on the clinico-genomics of prostate  
85 tumours, we exploited whole genome sequencing (WGS) of 392 published tumour-  
86 normal pairs<sup>11–14,24</sup>. We estimated both tumour and non-tumour (blood or adjacent  
87 histologically normal tissue) TLs for each sample using TelSeq v0.0.1<sup>25</sup> and  
88 TelomereHunter (v1.0.4)<sup>26</sup>. After quality control, 381 samples were retained for further  
89 analysis (see **Methods**). All tumours were treatment-naive, and detailed clinical  
90 information was collected and is available in **Supplementary Table 1**. The cohort  
91 consisted of 11% ISUP Grade Group (GG) 1, 52% GG2, 33.5 % GG3, 6.8% GG4 and  
92 3.4% GG5. For the majority of samples, the tumour was confined to the prostate (6.5%  
93 T1, 53.0% T2, 40.0% T3, 0.5% T4). The mean tumour coverage was  $73.1x \pm 20.6x$ ; the  
94 mean non-tumour coverage was  $44.1x \pm 13.4x$ . Median clinical follow-up time was 7.46  
95 years. TLs for each sample, along with clinical and genomic summary data are in  
96 **Supplementary Table 1**. Non-tumour TLs varied dramatically across individuals,  
97 ranging from 2.10 kbp to 15.0 kbp, with a median of  $4.52 \pm 1.35$  kbp. Blood TLs ( $n =$   
98 341) were shorter than those in adjacent normal ( $n = 40$ ; Mann Whitney U test;  $P = 2.80$   
99  $\times 10^{-10}$ ; **Supplementary Fig. 1A**). By contrast, tumour TLs varied less but were  
100 significantly shorter, ranging from 1.03 to 6.45 kbp with a median of  $3.36 \pm 0.87$  kbp.  
101 Non-tumour TLs were not associated with sequencing coverage (**Supplementary Fig.**  
102 **1B**). Tumour TLs were independent of tumour purity but there was a weak negative  
103 correlation between coverage and TL driven by some samples sequenced with over  
104 100x coverage (**Fig. 1D**; **Supplementary Figs. 1B-C**). Tumour and non-tumour TLs  
105 estimates from TelSeq and TelomereHunter were highly correlated (**Supplementary**  
106 **Fig. 1D**) so we decided to use TelSeq estimates throughout. To account for batch  
107 effects and the differences in blood and normal adjacent tissue, a linear model was fit  
108 and TLs were adjusted (**Supplementary Figs. 1E-F**). TL ratios (tumour TL / non-tumour  
109 TL) were calculated to further reduce any effects caused by co-founding of sequencing  
110 method. Tumour and non-tumour TLs were positively correlated with one another ( $\rho =$   
111 0.37,  $P = 7.30 \times 10^{-14}$ , **Fig. 1A**). As expected, TL ratio was positively correlated with  
112 tumour TL ( $\rho = 0.63$ ,  $P < 2.2 \times 10^{-16}$ ; **Fig. 1B**) but negatively correlated with non-tumour  
113 TLs ( $\rho = -0.40$ ,  $P < 2.2 \times 10^{-16}$ ; **Fig. 1C**). There was no difference in TL ratio between  
114 localized and metastatic samples ( $n = 101$ ;  $P = 0.95$ ; Mann Whitney U test;  
115 **Supplementary Fig. 1G**).

116 To assess whether tumour TL was related to any specific genomic property of a tumour,  
117 we evaluated a set of driver mutations previously identified in prostate cancer<sup>14</sup>. The  
118 relationship of each of these features with tumour TL is shown in **Fig. 1D**. While tumour  
119 TL was not associated with any known prostate cancer-related genomic rearrangement  
120 (GR) or single nucleotide variant (SNV) at current statistical power, samples with *CHD1*,

121 *RB1* or *NKX3-1* deletions had shorter tumour TL (**Fig. 1D**; **Fig. 5A**). By contrast, TL was  
122 closely associated with multiple measures of genomic instability. Tumours with shorter  
123 TLs had an elevated number of SNVs ( $\rho = -0.27$ ,  $P = 5.78 \times 10^{-8}$ ; **Fig. 2A**), indels ( $\rho = -$   
124  $0.32$ ,  $P = 2.83 \times 10^{-10}$ ; **Fig. 2C**) and GRs ( $\rho = -0.12$ ,  $P = 1.6 \times 10^{-2}$ ; **Fig. 2E**), as well as  
125 higher PGA ( $\rho = -0.21$ ,  $P = 3.95 \times 10^{-5}$ ; **Fig. 2G**), suggesting tumours with shorter  
126 telomeres accrue more mutations of all types without strong selective pressures for  
127 specific ones.

128 To determine whether these associations with somatic features were also related to an  
129 individual's non-tumour cells, we related each somatic feature against the TL ratio  
130 (tumour TL / non-tumour TL). Similar to tumour TL, the TL ratio did not significantly  
131 differ between samples with any of the recurrent prostate cancer-related GRs or CNAs  
132 but samples with a somatic SNV in the gene *SPOP* had smaller TL ratios  
133 (**Supplementary Fig. 2**). We identified significant correlations between somatic  
134 genomic instability measures and TL ratio. Tumours with an elevated number of SNVs  
135 ( $\rho = -0.15$ ,  $P = 4.20 \times 10^{-3}$ ; **Fig. 2B**), indels ( $\rho = -0.18$ ,  $P = 2.97 \times 10^{-4}$ ; **Fig. 2D**), GRs ( $\rho$   
136  $= -0.22$ ,  $P = 1.08 \times 10^{-5}$ ; **Fig. 2F**) and PGA ( $\rho = -0.13$ ,  $P = 1.69 \times 10^{-2}$ ; **Fig. 2H**) had  
137 smaller TL ratios.

138 We also assessed the association of telomere length with chromothripsis using  
139 published ShatterProof<sup>27</sup> scores from a subset of samples in this cohort ( $n = 170$ )<sup>14</sup>.  
140 There was no correlation between scores representing chromothripsis events in either  
141 tumour TL ( $\rho = 0.06$ ,  $P = 0.43$ ) or TL ratio ( $\rho = 0.02$ ,  $P = 0.80$ ).

## 142 **Fusion events are associated with telomere length**

143 When telomeres shorten beyond a certain length, double strand break repair is  
144 activated and cell cycle progression is arrested *via* the *TP53* pathway<sup>28</sup>. Failure to block  
145 cell growth can lead to telomere crisis and subsequent translocations, chromothripsis or  
146 chromosome fusions<sup>29</sup>. We explored the association of TL and the number of gene  
147 fusions present in a tumour. There was a negative correlation between the number of  
148 gene fusions and tumour TL ( $\rho = -0.26$ ;  $P = 2.18 \times 10^{-3}$ ) but no correlation with TL ratio  
149 (**Figs. 2I-J**). In a previous study, 47 recurrent gene fusions were discovered from  
150 matched RNA-Sequencing data<sup>18</sup>. Differences in tumour TL and TL ratio between  
151 samples with a gene fusion and those without were investigated for each of these  
152 recurrent fusions. No gene fusions were associated with TL ratio, but the  
153 *PCAT1:CASC21* gene fusion was significantly associated with tumour TL (Mann  
154 Whitney U test;  $Q = 2.07 \times 10^{-4}$ ; **Supplementary Fig. 3** and **Supplementary Table 2**).  
155 Tumours with this fusion had shorter tumour telomeres (mean = 3.3 kbp) than those  
156 without (mean = 3.8 kbp). These data suggest that the number of fusions and  
157 specifically the long non-coding RNA *PCAT1*, which promotes cell proliferation, is  
158 related to tumour TL.

## 159 **Proliferation rate is not associated to telomere length**

160 The rapid reproduction or proliferation of a cell should reduce the telomere length in  
161 dividing tumour cells. To test this, we investigated the correlation of TL with MKI67  
162 abundance levels and a previously published proliferation score<sup>30</sup>. Surprisingly, there  
163 was no association between either tumour TL ( $\rho = -0.14$ ;  $P = 0.11$ ) or TL ratio ( $\rho = -$   
164  $0.09$ ;  $P = 0.30$ ) and MKI67 RNA abundance (**Supplementary Fig. 3D-E**). Similarly,  
165 there was no association between proliferation scores and tumour TL ( $\rho = 0.01$ ;  $P =$   
166  $0.91$ ) or TL ratio ( $\rho = -0.05$ ;  $P = 0.54$ ; **Supplementary Fig. 3F-G**). This suggests that  
167 there is a more complex relationship between proliferation and TL at play.

## 168 **The role of *TERT* in prostate cancer**

169 A pan-cancer study reported that *TERT* alterations including promoter mutations,  
170 amplifications and structural variants were seen in approximately 30% of all cancers<sup>7</sup>. In  
171 our cohort, 10% of samples had *TERT* amplifications, 11% had *TERC* amplifications,  
172 ~1% had *TERT* structural variants and no samples had *TERT* SNVs or gene fusions.  
173 *TERT* mutations were seen less frequently in other localized prostate cancer datasets,  
174 1.7% (17/1,013; <sup>31</sup> and 0.6% (2/333; <sup>13</sup>), and in a metastatic dataset 3% (5/150; <sup>20</sup>, likely  
175 reflecting the early-stage of our cohort. Mutations in *ATRX* and *DAXX*, which have been  
176 correlated with longer telomeres<sup>32</sup>, were rare in our cohort: only two samples harboured  
177 a CNA in *DAXX*, and only four samples had an alteration in *ATRX*.

178 Tumour *TERT* RNA abundance was not correlated with tumour TL or TL ratio (**Fig. 3A**).  
179 Samples with higher *TERT* RNA abundance had fewer GRs ( $\rho = -0.17$ ;  $P = 4.79 \times 10^{-2}$ ;  
180 **Fig. 3B**), but there was no correlation between *TERT* abundance and SNV count ( $\rho = -$   
181  $0.04$ ,  $P = 0.67$ ; **Fig. 3C**), indel count ( $\rho = -0.04$ ,  $P = 0.132$ ; **Fig. 3D**) or PGA ( $\rho = -0.13$ ,  $P$   
182  $= 0.679$ ; **Fig. 3E**). The abundance of *TERC*, the telomerase RNA component, was  
183 negatively correlated with tumour TL ( $\rho = -0.24$ ;  $P = 4.55 \times 10^{-3}$ ; **Supplementary Fig.**  
184 **4A**) but there was no correlation with TL ratio or GR count ( $\rho = 0.12$ ;  $P = 0.145$ ;  
185 **Supplementary Fig. 4B**). *TERC* abundance was positively correlated with SNV count  
186 ( $\rho = 0.23$ ;  $P = 7.34 \times 10^{-3}$ ; **Supplementary Fig. 4C**), indel count ( $\rho = 0.34$ ;  $P = 4.88 \times$   
187  $10^{-5}$ ; **Supplementary Fig. 4D**) and PGA ( $\rho = 0.26$ ;  $P = 1.90 \times 10^{-3}$ ; **Supplementary Fig.**  
188 **4E**). *TERT* and *TERC* abundances were not correlated ( $\rho = 0.02$ ;  $P = 0.794$ ). These  
189 data suggest that *TERT* signaling is not significantly abrogated in localized prostate  
190 cancer either by somatic aberrations or through gene expression changes.

191 To explore the relationship of *TERT* RNA abundance and tumour TL further, we  
192 considered known activating transcription factors. Transcription of *TERT* can be  
193 activated by *MYC* and *SP1* and repressed by *AR*<sup>33</sup>. *MYC* amplifications occur in 14.5%  
194 of our samples (51/351; **Fig. 1D**), while *SP1* CNAs are rare (3/351). *TERT* and *MYC*  
195 mRNA abundance was positively correlated ( $\rho = 0.27$ ;  $P = 1.46 \times 10^{-3}$ ) but *MYC*  
196 abundance was unrelated to tumour TL (**Supplementary Fig. 5A**). Contrastingly, there  
197 was a positive correlation between tumour TL length and *SP1* abundance ( $\rho = 0.23$ ;  $P =$

198  $6.84 \times 10^{-3}$ ) but no significant correlation between *SP1* and *TERT* abundance  
199 (**Supplementary Fig. 5B**). We did not observe any statistically significant correlations  
200 between *AR* and *TERT* abundance, or tumour TL (**Supplementary Fig. 5C**). The direct  
201 relationship of these transcription factors on *TERT* is hard to elucidate because of the  
202 low measured abundance of *TERT*. Nonetheless, the abundance of *SP1* and *AR*  
203 appear to positively and negatively affect tumour TL, respectively.

204 To determine whether *TERT* was being regulated epigenetically, we first investigated  
205 the correlation between its methylation status and its RNA abundance using 91  
206 annotated sites. We identified one CpG site with a significant negative correlation and  
207 two with significant positive correlations (Spearman's correlation;  $Q < 0.05$ ;  $|\rho| > 0.2$ ;  
208 **Fig. 3F**). Further, 31% (28/91) of *TERT* CpGs sites were significantly correlated to  
209 telomere length: 7 positively and 21 negatively (Spearman's correlation;  $Q < 0.05$ ;  $|\rho| >$   
210  $0.2$ ; **Fig. 3F**). This strongly suggests that methylation of *TERT* may impact *TERT*  
211 abundance and tumour TL.

## 212 **Candidate regulators of prostate tumour telomere length**

213 Evidence of correlation between methylation and tumour TL in *TERT* led us to  
214 investigate the role of methylation on TL genome-wide. For each gene, we considered  
215 the CpG site most associated to its mRNA abundance (see **Methods**) and related that  
216 to tumour TL ( $n = 241$ ). Methylation of almost half of all genes (46%; 7,088/15,492) was  
217 significantly correlated with tumour TL (Spearman's correlation;  $Q < 0.05$ ;  
218 **Supplementary Table 3**). Similarly, almost a third of genes showed transcriptional  
219 profiles associated with tumour TL (32%; 4,520/13,956). No proteins were significantly  
220 associated with tumour TL after FDR adjustment although 9.3% proteins showed  
221 correlation to tumour TL before adjustment ( $n = 548/5,881$ ; Spearman's correlation;  $P <$   
222  $0.05$ ). There were 112 genes with methylation, transcription and proteome correlations  
223 to telomere length. Remarkably, these showed no functional enrichment. Several genes  
224 showed methylation positively correlated with tumour TL but negatively correlated with  
225 RNA and protein abundance (**Fig. 4A**), suggesting suppression of tumour TL  
226 elongation. One such gene is the oncogene *AKT1*, which regulates processes including  
227 cell proliferation, survival and growth<sup>34</sup>. High *AKT1* abundance may indicate an elevated  
228 proliferation and therefore shorter telomeres.

229 We also identified genes whose methylation was negatively correlated with tumour TL  
230 but positively correlated with RNA and protein abundance suggesting promotion of  
231 telomere elongation (**Fig. 4B**). These included *SLC14A1*, a membrane transporter that  
232 mediates urea transport, and *ITGA3*, an integrin that functions as a cell surface  
233 adhesion molecule. We used gprofiler2<sup>35</sup> to identify pathways enriched in genes with  
234 methylation or transcriptomic profiles that are correlated with tumour TL using KEGG  
235 pathways<sup>36</sup>. We identify 16 pathways enriched in genes with methylation profiles and 16  
236 pathways that were enriched in genes with transcriptomic profiles that were correlated

237 with tumour TL (**Supplementary Fig. 6A**). To reduce false positives and account for  
238 crosstalk between pathways, we applied a crosstalk correction method<sup>37,38</sup>. The  
239 crosstalk matrix (**Supplementary Fig. 6B**) identified overlap between the cancer related  
240 pathways, and after crosstalk adjustment only one pathway remained enriched in genes  
241 with transcriptomic profiles that were correlated to tumour TL: hsa04519 (Focal  
242 adhesion; **Supplementary Fig. 6C**).

243 We similarly investigated whether TL ratio was associated with methylation and found  
244 that the methylation levels of 33.7% (5,218/15,492) of genes were significantly  
245 correlated with TL ratio (Spearman's correlation;  $Q < 0.05$ ; **Supplementary Table 4**).  
246 Surprisingly, fewer than 1% ( $n = 53/13,958$ ) of genes with overlapping data also had a  
247 significant correlation between RNA abundance and TL ratio and none between protein  
248 abundance and TL ratio (Spearman's correlation; unadjusted  $P < 0.05$ ). These results  
249 suggest that tumour TL, not TL ratio, is associated with tumour gene expression.

### 250 **Association of telomere length and specific copy number aberrations**

251 Since prostate tumour gene-expression and clinical behaviour is predominantly driven  
252 by CNAs<sup>14,19</sup> we next investigated their role in TL. As noted above (**Fig. 1D**), driver  
253 CNAs were largely unassociated with tumour TL (**Fig. 5A**; white background) or TL ratio  
254 (**Fig. 5B**; white background). We therefore considered copy number changes genome-  
255 wide for associations with TL. We identified 24 loci encompassing 35 genes in which  
256 there was a significant difference in tumour TL in samples with a copy number change  
257 compared to those without (Mann-Whitney U test;  $Q < 0.05$ ; **Supplementary Table 5**  
258 and **Fig. 5A**). We also identified 128 loci encompassing 319 genes in which there was  
259 an association between copy number status and TL ratio (Mann-Whitney U test,  $Q <$   
260  $0.05$ ; **Supplementary Table 7**). For example, tumours with deletions in DNA  
261 methyltransferase 1, *DNMT1*, had smaller TL ratios ( $Q = 0.028$ , effect size = 0.11, **Fig.**  
262 **5B**). An opposing trend was seen in the chromatin organization gene, *PRDM16* ( $Q =$   
263  $0.027$ , effect size = 0.15) and the membrane metallo-endopeptidase gene, *MMEL1* ( $Q =$   
264  $0.027$ , effect size = 0.14; **Fig. 5B**), where amplifications resulted in smaller TL ratios.  
265 This analysis highlights that copy number aberrations are more associated with TL ratio  
266 (change in length from non-tumour TL to tumour TL) than absolute tumour TL.

267 We also explored CNAs in genes comprising the telomere complex (*TERF1*, *TERF2*,  
268 *TERF2IP*, and *POT1*), shelterin interacting proteins (*PINX1* and *RTEL1*), and the  
269 components of telomerase (*TERT* and *TERC*). There were no differences in the tumour  
270 TL (**Supplementary Fig. 7A**) or TL ratio (**Supplementary Fig. 7B**) between samples  
271 with and without a CNA in these genes.

272 Next, we compared TL across previously identified CNA subtypes. There was no  
273 difference in tumour TL ( $P = 0.53$ ; one-way ANOVA) or TL ratio ( $P = 0.78$ ; one-way  
274 ANOVA) in the four CNA subtypes identified from aCGH arrays and associated with  
275 prognosis<sup>9</sup> (**Supplementary Fig. 8A-B**). There was an association between TL ratio



276 and the six CNA subtypes ( $P = 2.12 \times 10^{-2}$ ; one-way ANOVA) identified from 284  
277 OncoScan SNP arrays<sup>14</sup> but not with tumour TL (**Supplementary Fig. 8C-D**). Samples  
278 in subtype C5, which was defined by amplifications in genes near the end of  
279 chromosomes had smaller TL ratios than C3 (defined by an 8p deletion and an 8q  
280 amplification) and C4 (defined as having a quiet CNA profile). A smaller TL ratio in the  
281 samples from subtype C5 indicates that the non-tumour TL length was longer than in  
282 the tumour TL (**Supplementary Fig. 8E**): the consequences of this remain to be  
283 elucidated.

## 284 **Clinical correlates of telomere length**

285 The clinical features of a tumour can have prognostic value, and have been associated  
286 with the genomic features of tumours<sup>14</sup>. Higher serum abundance of prostate specific  
287 antigen (PSA), higher ISUP Grading and tumour size and extent are all associated with  
288 worse outcome. Therefore, we considered whether there was interplay between TL and  
289 the clinical features of a tumour. Tumour TL was not significantly correlated to age, ( $\rho =$   
290  $-0.10$ ,  $P = 5.8 \times 10^{-2}$ ; **Fig. 6A**) but there was a significant positive correlation between  
291 age at diagnosis and TL ratio ( $\rho = 0.11$ ,  $P = 2.53 \times 10^{-2}$ ; **Fig. 6B**). Tumour TL was  
292 shorter than non-tumour TL in younger patients. This could be related to the  
293 aggressiveness of early onset prostate cancers, which is characteristic of tumours in  
294 younger men<sup>24</sup>. There was a negative correlation between pre-treatment PSA levels  
295 between both tumour TL ( $\rho = -0.16$ ,  $P = 2.23 \times 10^{-3}$ ) and TL ratio ( $\rho = -0.19$ ,  $P = 1.70 \times$   
296  $10^{-4}$ ; **Figs. 6C-D**). Neither tumour TL nor TL ratio was associated with ISUP Grade  
297 (**Figs. 6E-F**). Surprisingly, tumour TL was shorter in smaller tumours (T1) than larger  
298 tumours (T2 or T3; one-way ANOVA,  $P = 2.2 \times 10^{-2}$ ; **Fig. 6G**) but this can be explained  
299 by the higher average age of patients with T1 tumours (mean = 71.3) compared to other  
300 T categories (mean = 62.0). Accordingly, there was no association between TL ratio,  
301 which controls for patient age, and T category ( $P = 0.29$ ; **Fig. 6H**).

302 Telomerase activity and TL has been proposed to have clinical utility at three different  
303 stages; diagnosis, prognosis and treatment<sup>33</sup>. TL from biopsies has been correlated with  
304 progression to metastasis and disease specific death<sup>39</sup>. As well, TL from leukocytes has  
305 been associated with poor survival<sup>40,41</sup>. We explored if tumour TL, non-tumour TL or TL  
306 ratio were associated with biochemical relapse (BCR), an early surrogate endpoint in  
307 intermediate-risk prostate cancer. Cox proportional hazards (Cox PH) models were fit,  
308 splitting patients ( $n = 290$ ) into two groups based on their TL with increasing cutoff  
309 thresholds (50 bp each time; **Supplementary Figs. 9A-C**). From this outcome-oriented  
310 optimal cut-point analysis we discovered that samples with non-tumour TL less than 3.9  
311 kbp had a higher rate of BCR than samples with longer TLs (HR = 2.02,  $P = 1.6 \times 10^{-3}$ ;  
312 **Fig. 6I**). Non-tumour TL is associated with survival independent of PGA (Cox PH model,  
313  $P = 0.02$ ). There was no association between tumour TL and BCR (**Fig. 6J**), but there  
314 was an association between TL ratio and BCR, where samples with a TL ratio greater  
315 than 0.65 had a lower rate of BCR (HR = 0.42,  $P = 2.6 \times 10^{-3}$ ; **Fig. 6K**). We also

316 considered TL as a continuous measurement and fit Cox PH models using tumour TL,  
317 non-tumour TL and TL ratio. Again, there was no association between continuous  
318 tumour TL and BCR but there was an association between non-tumour TL (HR = 0.768,  
319  $P = 0.014$ ) and TL ratio (HR = 1.71,  $P = 0.031$ ; **Supplementary Fig. 9D**). These results  
320 suggest that non-tumour TL and TL ratio are weakly prognostic, and thus may reflect  
321 host factors that may influence patient risk categorization.

## 322 Discussion

323 These data emphasize the relationship of genomic instability and TL. Genomic  
324 instability has previously been linked with poor outcome in prostate cancer<sup>9,14</sup> and TL  
325 shortening could be the cause of some of this instability. Telomere shortening has been  
326 implicated as an early event in prostate cancer due to evidence of shortened telomeres  
327 observed in a precursor histopathology, high-grade prostatic intraepithelial  
328 neoplasia<sup>42,43</sup>. Since cellular proliferation in prostate cancer is increased by seven fold  
329 compared to normal prostatic epithelial cells<sup>33</sup>, telomeres in these dividing cells will  
330 shorten with each cell division. There is no evidence that primary prostate cancer  
331 exhibits ALT lengthening<sup>23</sup> therefore the vast majority, if not all tumours, activate  
332 telomerase for telomere maintenance. We did not observe any *TERT* promoter  
333 mutations in our cohort but there are strong negative correlations between methylation  
334 probes in the promoter of *TERT* and tumour TL. This may be a proxy for telomerase  
335 activity since DNA methylation impedes transcription.

336 We see an unexpected divergence between somatic molecular features associated with  
337 TL ratio and those with tumour TL. Specifically, measures of genomic instability are  
338 linked to TL ratio (which represents the ratio between non-tumour TL and tumour TL)  
339 while specific CNAs, GRs, and SNVs are not (**Fig. 1** and **Supplementary Fig. 2**). This  
340 suggests that during the progression of cells from normal to cancerous, non-tumour TL  
341 may influence tumour genomics, where tumours with shorter TL experience more  
342 genomic instability. Alternatively, a common factor may be influencing during this epoch  
343 of the tumour's evolution. Once tumours are formed, it is the specific mutations within  
344 the cell that are more associated with tumour TL. This may be due to mutations in cell  
345 division and growth regulating genes such as *ATK1* and *SPOP*, which increases the  
346 number of divisions in the tumour and thereby shortens tumour telomeres. Further  
347 evidence of this hypothesis is seen in tumours with *PCAT1* fusions, where tumours with  
348 this fusion had shorter tumour TL than samples without it<sup>44</sup>.

349 One limitation in the estimation of TL using short-read whole genome sequencing is the  
350 difficulty in estimating chromosome specific telomere lengths. Junction spanning reads  
351 from paired-end experiments, in which one read maps within the first or last band of the  
352 chromosome and the other read maps within the telomere region, are scarce. Further  
353 studies should be performed using long read sequences, in which these regions may  
354 have more coverage and can be used to determine chromosome specific shortening  
355 and its association to specific genomic events or biochemical relapse.

356 These data highlight the complicated relationship between telomere length in both  
357 tumour and non-tumour cells, and molecular and clinical tumour phenotypes. They  
358 highlight the need for increased study of telomere length across cancer types, and for  
359 long-read sequencing to introduce chromosome-specific analyses.

## 360 **Acknowledgments**

361 The authors thank all members of the Boutros lab for insightful commentary and  
362 technical support.

## 363 **Funding**

364 This study was conducted with the support of the Ontario Institute for Cancer Research  
365 to PCB through funding provided by the Government of Ontario. This work was  
366 supported by Prostate Cancer Canada and is proudly funded by the Movember  
367 Foundation - Grant #RS2014-01. Dr. Boutros was supported by a Terry Fox Research  
368 Institute New Investigator Award and a CIHR New Investigator Award. This work was  
369 funded by the Government of Canada through Genome Canada and the Ontario  
370 Genomics Institute (OGI-125). This work was supported by the NIH/NCI under award  
371 number P30CA016042, by an operating grant from the National Cancer Institute Early  
372 Detection Research Network (U01CA214194) and by support from the ITCR  
373 (U24CA248265).

## 374 **Author contributions**

375 Formal Analysis: JL. Methodology: JL, SE, ED. Data curation: JL, YSY, TNY, LEH, VH,  
376 RL, TG, BC. Visualization: JL, JG. Supervision: MF., TvdK, RGB, PCB.  
377 Conceptualization, Supervision: PCB, MF, RGB. Pathology Reviews: TvdK. Writing -  
378 original draft: JL, PCB. Writing – review & editing: JL, TNY, VH, RL, MF, PCB. Approved  
379 the Manuscript: All Authors

## 380 **Declaration of Interest and Financial Disclosures**

381 All authors declare that they have no conflicts of interest.

## 382 Figure Legends

383 **Figure 1. Tumour telomere length (TL) is associated with genomic features. A,**  
384 Correlation between tumour TL and non-tumour TL. **B,** Correlation between tumour TL  
385 and TL ratio (tumour TL / non-tumour TL). **C,** Correlation between non-tumour TL and  
386 TL ratio. **D,** Tumour TL is ranked in descending order of length (kbp; top bar plot). The  
387 association of tumour TL and measures of mutational burden, TMPRSS2:ERG (T2E)  
388 fusion status, as well as known prostate cancer genes with recurrent CNAs, coding  
389 SNVs, and GRs are shown. Bar plots to the right indicate the statistical significance of  
390 each association (see **Methods**).

391 **Figure 2. Mutational landscape differs with telomere length. A-B,** Correlation  
392 between the number of SNVs and **A,** tumour TL and **B,** TL ratio. **C-D,** Correlation  
393 between the number of indels and **C,** tumour TL and **D,** TL ratio. **E-F,** Correlation  
394 between the number of GRs and **E,** and tumour TL and **F,** TL ratio. **G-H,** Correlation of  
395 percentage of the genome altered (PGA) and **G,** tumour TL and **H,** TL ratio. **I-J,**  
396 Correlation between the number of fusions and **I,** tumour TL and **J,** TL ratio. Orange  
397 dots indicate tumour TL while green dots indicate TL ratio. Spearman's  $\rho$  and  $P$  values  
398 are displayed.

399 **Figure 3. The genomic correlates of TERT abundance. A,** Correlation of *TERT* RNA  
400 abundance with tumour TL and TL ratio. Orange dots indicate tumour TL while green  
401 dots indicate TL ratio. Spearman's  $\rho$  and  $P$  values are displayed. **B-E,** Correlation of  
402 *TERT* abundance and **B,** the number of GRs, **C,** number of SNVs, **D,** number of indels,  
403 and **E,** PGA. Spearman's  $\rho$  and  $P$  values are displayed. **F,** Spearman's correlation of  
404 significantly associated methylation probes with RNA abundance and tumour TL. Blue  
405 dots indicate a positive correlation while orange dots indicate a negative correlation.  
406 Probes within the promoter are labeled in red while the rest are located in the gene  
407 body. Dot size indicated the magnitude of correlation. Background colour indicates  
408 unadjusted  $P$  values. Methylation probes are ordered by their correlation between *TERT*  
409 RNA abundance from negative to positive.

410 **Figure 4. Association of methylation, RNA abundance, protein abundance and**  
411 **telomere length. A,** Positive correlation of methylation and tumour TL, but negative  
412 correlation of RNA and protein abundance. Top panels in light blue represent  
413 methylation, middle panels in blue-grey represent RNA abundance and the bottom  
414 panels in purple represent protein abundance. Darker purple dots represent undetected,  
415 imputed abundance measures. Spearman's  $\rho$  and  $P$  values are displayed. **B,** Negative  
416 correlation of methylation and tumour TL, but positive correlation of RNA and protein  
417 abundance. Top panels in light blue represent methylation, middle panels in blue-grey  
418 represent RNA abundance and the bottom panels in purple represent protein  
419 abundance. Darker purple dots represent undetected, imputed protein abundance  
420 measures. Spearman's  $\rho$  and  $P$  values are displayed.

421 **Figure 5. Telomere length differs by copy number status. A,** Difference in tumour  
422 TL between samples with a copy number aberration and those without in prostate  
423 cancer related genes and associated genes. **B,** Difference in TL ratio between samples  
424 with a copy number aberration and those without in prostate cancer related and  
425 associated genes.  $Q$  values are from a Mann-Whitney U test and are bolded when  
426 significant ( $FDR < 0.05$ ). Colour of the points indicate copy-number status of the gene:  
427 amplification (red), deletion (blue), or neutral (black).

428 **Figure 6. Telomere length is associated with clinical features and biochemical**  
429 **relapse. A-B,** Correlation of age at diagnosis with **A,** tumour TL and **B,** TL ratio.  
430 Spearman's  $\rho$  and  $P$  values are displayed. **C-D,** Correlation of pre-treatment PSA with  
431 **C,** tumour TL and **D,** TL ratio. Spearman's  $\rho$  and  $P$  values are displayed. **E-F,**  
432 Association of ISUP grade with **E,** tumour TL and **F,** TL ratio.  $P$  value is from an one-  
433 way ANOVA. **G-H,** Association of T category with **G,** tumour TL and **H,** TL ratio.  $P$  value  
434 is from an one-way ANOVA. On all plots, green indicates TL ratio, while orange  
435 indicates tumour TL. **I-K,** Cox proportional hazard models were created for **I,** non-  
436 tumour TL, **J,** tumour TL and **K,** TL ratio with BCR as the endpoint. Samples were split  
437 into two groups based on the optimal cut point analysis (**see Methods**).

## 438 **Methods**

### 439 **Patient cohort**

440 Published whole-genome sequences of tumour and matched non-tumour samples were  
441 downloaded from public repositories (phs000447.v1.p1<sup>11</sup>, phs000330.v1.p1<sup>12</sup>,  
442 EGAS00001000900<sup>14</sup>, phs000178.v11.p8<sup>13</sup>, EGAS00001000400<sup>24</sup>, phs001648.v2.p1<sup>45</sup>).  
443 For RadP patients, BCR was defined as two consecutive post-RadP PSA  
444 measurements of more than 0.2 ng/ml (backdated to the date of the first increase). If a  
445 patient has successful salvage radiation therapy, this is not BCR. If PSA continues to  
446 rise after radiation therapy, BCR is backdated to first PSA > 0.2. If patient gets other  
447 salvage treatment (such as hormones or chemotherapy), this is considered BCR.  
448 Tumour cellularity and Gleason grades were evaluated independently by two  
449 genitourinary pathologists on scanned haematoxylin- and eosin-stained slides. Serum  
450 PSA concentrations (ng/mL) are reported according to the reading at the time of  
451 diagnosis. Cellularity was also determined *in silico* from OncoScan SNP arrays via  
452 qpure (v1.1)<sup>46</sup>.

### 453 **Whole-genome sequencing data analysis**

454 Raw sequencing reads were aligned to the human reference genome, GRCh37, using  
455 BWA-mem (version > 0.7.12; <sup>47</sup> at the lane level. Picard (v1.92;  
456 <http://broadinstitute.github.io/picard/>) was used to merge the lane-level BAMs from the  
457 same library and mark duplicates. Library level BAMs from each sample were also  
458 merged without marking duplicates using Picard. Local realignment and base quality  
459 recalibration was carried out on tumour/non-tumour pairs together using the Genome  
460 Analysis Toolkit (GATK; > version 3.4.0; <sup>48</sup>. Tumour and non-tumour sample level BAMs  
461 were extracted, headers were corrected using SAMtools (v0.1.9; <sup>49</sup>, and files were  
462 indexed with Picard (v1.92).

### 463 **Computational telomere length estimation**

464 Tumour and non-tumour telomere lengths were estimated using TelSeq (v0.0.1; <sup>25</sup> and  
465 TelomereHunter (v1.0.4)<sup>26</sup> on BAM files generated using bwa-mem (version > 0.7.12; <sup>47</sup>  
466 and GATK (version > 3.4.0; <sup>48</sup>. TelSeq estimates telomere length with the following  
467 formula  $l = t_k s c$ , where  $t_k$  is the abundance of telomeric reads (reads that contain  $k$  or  
468 more TTAGGG repeats;  $k = 7$ ),  $c$  is a constant for the genome length divided by the  
469 number of chromosome ends and  $s$  is the fraction of all reads with GC composition  
470 between 48-52%. TelomereHunter also identifies telomeric reads based on the number  
471 to repeat sequences with a read and normalizes by the number of reads with GC  
472 content between 48-52%. This value is multiplied by  $10^6$  to calculate TRPM (telomeric  
473 reads per GC content-matched million reads) values. As a quality measure, TelSeq  
474 estimates for each sample were generated per sequencing lane. Reads from lanes that  
475 contained too few reads to calculate an estimate (marked as UNKNOWN), and outlier  
476 lanes as identified by grub's test, were removed from input BAMs using BAMQL v1.6)<sup>50</sup>.

477 After outliers were removed, TelSeq was run again ignoring read groups with the -u  
478 parameter. Samples with telomere estimates less than one were removed from further  
479 analysis. To account for differences in TL due to sequencing center, a linear model was  
480 fit with TL as the response variable and sequencing center as the predictor variable. A  
481 separate model was fit for tumour and non-tumour length.

### 482 **Somatic variant calling**

483 Single nucleotide variants (SNVs) and genomic rearrangements (GRs) were called  
484 using pipelines that have been described in detail elsewhere<sup>14</sup>. Briefly, SomaticSniper  
485 (v1.0.5; was used to call SNVs on bases with at least 17x coverage in tumours and 10x  
486 in non-tumours. Coding versus non-coding SNVs were determined using Annovar<sup>52</sup>.  
487 Genomic rearrangements were identified using Delly (version 0.7.8; <sup>53</sup>). Gene fusion  
488 events involving *ERG* or *ETV* were collectively referred to as ETS events. Genomic  
489 rearrangement calls were examined to determine if breakpoints led to a *TMPRSS2:ERG*  
490 fusion or if breakpoints were found in both 1 Mbp bins surrounding the following gene  
491 pairs: *ERG:SLC45A3*, *ERG:NDRG1*, *ETV1:TMPRSS2*, *ETV4:TMPRSS2*,  
492 *ETV1:SLC45A3*, *ETV4:SLC45A3*, *ETV1:NDRG1*, and *ETV4:NDRG1*. *ERG*  
493 immunohistochemistry and deletion calls between *TMPRSS2* and *ERG* loci in  
494 OncoScan SNP array data provided further support for these fusions.

### 495 **mRNA abundance data generation and analysis**

496 Generation and analysis of RNA abundance data has been previously described in  
497 detail<sup>18</sup>. Briefly, 200 ng of total RNA was used to construct a TruSeq strand specific  
498 library with the Ribo-Zero protocol (Illumina), and all samples were sequenced on a  
499 HiSeq2000v3 to a minimal target of 180 million paired-end reads. Reads were mapped  
500 using the STAR aligner (v2.5.3a; <sup>54</sup>) to GRCh37 with GENCODE v24lift37<sup>55</sup>. RSEM  
501 (v1.2.29) was used to quantify gene abundance<sup>56</sup>.

### 502 **Methylation microarray data generation**

503 Illumina Infinium HumanMethylation 450k BeadChip kits were used to assess global  
504 methylation, using 500 ng of input genomic DNA, at McGill University and the Genome  
505 Quebec Innovation Centre (Montreal, QC). All samples used in this study were  
506 processed from fresh-frozen prostate cancer tissue. The IDAT files were loaded and  
507 converted to raw intensity values with the use of watermelon package (v1.15.1; <sup>57</sup>.  
508 Quality control was conducted using the minfi package (v1.22.1; <sup>58</sup>; no outlier samples  
509 were detected). Raw methylation intensity levels were then pre-processed using Dasen.  
510 Probe filtering was conducted after normalization, as previously described<sup>14</sup>. Annotation  
511 to chromosome location, probe position, and gene symbol was conducted using the  
512 IlluminaHumanMethylation450kanno.ilmn12.hg19 package (v0.6.0).

### 513 **Association of telomere length with fusions**

514 The association between gene fusion status and tumour TL and TL ratio was tested  
515 using a Mann-Whitney U-test in 47 previously identified recurrent gene fusions.



## 516 **Association of telomere length and proliferation**

517 A proliferation score per sample was generated using a previously published signature<sup>30</sup>  
518 where tumours with a RNA abundance value greater than the mean for each gene in  
519 the signature were given a score of +1, and tumours with a RNA abundance value less  
520 than the mean for that gene were given a score of -1. All values were summed to  
521 generate a proliferation score. Spearman's correlations between TL, TL ratio and the  
522 proliferation score was calculated. The correlation between TL, TL ratio and MKI67  
523 abundance was also calculated.

## 524 **Association of telomere length with chromothripsis**

525 Chromothripsis scores were previously generated using ShatterProof (v0.14; <sup>14,27</sup> with  
526 default settings. Spearman's correlation between the maximum ShatterProof score per  
527 sample and telomere length was calculated using samples with both available metrics  
528 (n = 170).

## 529 **Association of telomere length with clinical and genomic features**

530 Telomere length estimates were associated with genomic and clinical features. Clinical  
531 features, including ISUP Grade, pre-treatment PSA, T category and age at diagnosis,  
532 were categorized and tested for association using an one-way ANOVA. Pathological T  
533 category was used for surgery samples and diagnostic T category was used for  
534 radiotherapy samples. Binary features including the presence of specific GRs, CNAs  
535 and SNVs were tested for association using a Mann-Whitney U test. Summary features  
536 including PGA, GR count, SNV count and indel count were correlated to TL using  
537 Spearman's correlation.

## 538 **Association of telomere length with methylation**

539 The correlation matrix of methylation and mRNA abundance levels from TCGA was  
540 downloaded from <https://gdac.broadinstitute.org/>. For each gene, the probe showing the  
541 highest Spearman's correlation with mRNA abundance levels was used in our  
542 correlation analysis.

## 543 **Association of telomere length with transcriptome and proteome 544 abundance**

545 Spearman's correlations between TL and RNA (n = 139; <sup>14</sup>) and protein abundance (n =  
546 70; <sup>17</sup>) and TL were calculated.

## 547 **Over-representation analysis pathway analysis**

548 Pathway analysis was performed with the gprofiler2<sup>35</sup> R package using genes in which  
549 there was a significant association between TL and methylation or RNA separately  
550 using the KEGG collection of pathways<sup>36</sup>.

## 551 **Crosstalk effects in pathway analysis**

552 To account for crosstalk effects caused by gene overlap in pathway analysis, we  
553 implemented the principle component analysis method proposed by <sup>38</sup>. Briefly, for genes

554 that are overlap among pathways, each gene is only allowed membership in one of the  
555 pathways. This membership is determined by the highest correlation between the gene  
556 and the PC1 of the other genes in the pathway. A fisher's exact test was then used to  
557 determine enrichment of TL correlated genes in the reduced pathway membership.

### 558 **Association of telomere length with copy number aberrations**

559 SNP microarray data generation and analysis has been previously described in detail<sup>14</sup>.  
560 Briefly, SNP microarrays were performed with 200 ng of DNA on Affymetrix OncoScan  
561 FFPE Express 2.0 and 3.0 arrays. Analysis of the probe assays was performed using  
562 .OSCHP files generated by OncoScan Console (v1.1) using a custom reference.  
563 BioDiscovery's Nexus Express™ for OncoScan 3 Software was used to call copy  
564 number aberrations using the SNP-FASST2 algorithm. Gene level copy number  
565 aberrations for each patient were identified by overlapping copy number segments from  
566 OncoScan SNP 3.0 data, with RefGene (2014-07-15) annotation using BEDTools  
567 (v2.17.0; <sup>59</sup>). Genes with the same copy number profile across patients were then  
568 collapsed into contiguous regions. Contiguous gene segments with aberrations in less  
569 than 5% of patients were removed from the analysis. To find associations between TL  
570 and copy number segments, a Mann-Whitney U test was used to compare the mean TL  
571 between samples with a copy number aberration and those without. The copy number  
572 aberration state (either amplified or deleted) was determined as the status with the  
573 largest proportion of samples. Samples with aberrations in the other class was merged  
574 into the without group. For example, three samples have an amplification in *CHD1*,  
575 while 49 samples have a deletion. The three samples would be grouped with copy  
576 number neutral samples and the Mann-Whitney test performed comparing the two  
577 groups. *P* values were FDR adjusted to account for multiple testing.

### 578 **Association with biochemical relapse**

579 Cox proportional hazards models were fit with the R package survival (v3.2-7) using TL  
580 as a continuous variable. Age at diagnosis was controlled for in the model. Kaplan  
581 Meier plots were generated by dichotomizing samples based on the optimal cut point  
582 analysis, in which samples were dichotomized using increasing thresholds of 50 bp.

### 583 **Statistical analyses and data visualization**

584 All statistical analyses were performed within the R statistical environment (v3.3.1).  
585 Visualization in R was performed through the BoutrosLab Plotting General package  
586 (v5.6.1; <sup>60</sup>). *P* values from Spearman's correlations were calculated using the AS-89  
587 algorithm<sup>61</sup>.

### 588 **Data availability**

589 OncoScan SNP array data and whole genome DNA sequencing can be found on EGA  
590 under the accession EGAS00001000900. Processed variant calls are available through  
591 the ICGC Data Portal under the project PRAD-CA (<https://dcc.icgc.org/projects/PRAD->

592 [CA](#)). mRNA data is available in the Gene Expression Omnibus under the accession  
593 GSE84043. Methylation data is available under the accession GSE107298.

## 594 **Supplementary Table Legends**

### 595 **Supplementary Table 1 | Clinical and genomic features of tumours**

596 Clinical data for 382 samples used in analysis after applying quality control metrics.

### 597 **Supplementary Table 2 | Association between Tumour TL and** 598 **recurrent gene fusions**

599 Statistical summary of 47 recurrent fusions pairs tested for association with TL using a  
600 Wilcoxon signed-rank test.

### 601 **Supplementary Table 3 | Genomic and transcriptomic correlations** 602 **with Tumour TL**

603 Results from Spearman's correlation between tumour TL and methylation beta values,  
604 RNA abundance and protein abundance. Q values are FDR adjusted *P* values. NAs  
605 indicate missing values where tests could not be performed.

### 606 **Supplementary Table 4 | Genomic and transcriptomic correlations** 607 **with TL ratio**

608 Results from Spearman's correlation between tumour TL and methylation beta values,  
609 RNA abundance and protein abundance. Q values are FDR adjusted *P* values. NAs  
610 indicate missing values where tests could not be performed.

### 611 **Supplementary Table 5 | Associations between CNAs and Tumour TL**

612 Associations between CNAs and Tumour TL, ordered by FDR adjusted *P* values. Each  
613 row represents collapsed segments containing multiple genes. Contiguous gene  
614 segments with aberrations in less that 5% of patients were removed.

### 615 **Supplementary Table 6 | Associations between CNAs and TL ratio**

616 Statistically significant associations between CNAs and TL ratio, ordered by FDR  
617 adjusted *P* values. Each row represents collapsed segments containing multiple genes.  
618 Contiguous gene segments with aberrations in less that 5% of patients were removed.

## 619 **References**

- 620 1. Moyzis RK, Buckingham JM, Cram LS, et al. A highly conserved repetitive DNA  
621 sequence, (TTAGGG)<sub>n</sub>, present at the telomeres of human chromosomes. *Proc*  
622 *Natl Acad Sci USA*. 1988;85(18):6622-6626.
- 623 2. Arnoult N, Karlseder J. Complex interactions between the DNA-damage response  
624 and mammalian telomeres. *Nat Struct Mol Biol*. 2015;22(11):859-866.  
625 doi:10.1038/nsmb.3092
- 626 3. Samassekou O, Gadji M, Drouin R, Yan J. Sizing the ends: normal length of human  
627 telomeres. *Ann Anat*. 2010;192(5):284-291. doi:10.1016/j.aanat.2010.07.005
- 628 4. Hayflick L, Moorhead PS. The serial cultivation of human diploid cell strains. *Exp*  
629 *Cell Res*. 1961;25:585-621.
- 630 5. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell*. 2000;100(1):57-70.
- 631 6. Dunham MA, Neumann AA, Fasching CL, Reddel RR. Telomere maintenance by  
632 recombination in human cells. *Nat Genet*. 2000;26(4):447-450. doi:10.1038/82586
- 633 7. Barthel FP, Wei W, Tang M, et al. Systematic analysis of telomere length and  
634 somatic alterations in 31 cancer types. *Nat Genet*. 2017;49(3):349-357.  
635 doi:10.1038/ng.3781
- 636 8. Sieverling L, Hong C, Koser SD, et al. Genomic footprints of activated telomere  
637 maintenance mechanisms in cancer. *Nature Communications*. 2020;11(1):733.  
638 doi:10.1038/s41467-019-13824-9
- 639 9. Lalonde E, Ishkanian AS, Sykes J, et al. Tumour genomic and microenvironmental  
640 heterogeneity for integrated prediction of 5-year biochemical recurrence of prostate  
641 cancer: a retrospective cohort study. *Lancet Oncol*. 2014;15(13):1521-1532.  
642 doi:10.1016/S1470-2045(14)71021-6
- 643 10. Lalonde E, Alkallas R, Chua MLK, et al. Translating a Prognostic DNA Genomic  
644 Classifier into the Clinic: Retrospective Validation in 563 Localized Prostate  
645 Tumors. *European Urology*. 2017;72(1):22-31. doi:10.1016/j.eururo.2016.10.013
- 646 11. Baca SC, Prandi D, Lawrence MS, et al. Punctuated evolution of prostate cancer  
647 genomes. *Cell*. 2013;153(3):666-677. doi:10.1016/j.cell.2013.03.021
- 648 12. Berger MF, Lawrence MS, Demichelis F, et al. The genomic complexity of primary  
649 human prostate cancer. *Nature*. 2011;470(7333):214-220.  
650 doi:10.1038/nature09744
- 651 13. The Cancer Genome Atlas Research Network. The molecular taxonomy of primary  
652 prostate cancer. *Cell*. 2015;163(4):1011-1025. doi:10.1016/j.cell.2015.10.025

- 653 14. Fraser M, Sabelnykova VY, Yamaguchi TN, et al. Genomic hallmarks of localized,  
654 non-indolent prostate cancer. *Nature*. 2017;541(7637):359-364.  
655 doi:10.1038/nature20788
- 656 15. Bhandari V, Hoey C, Liu LY, et al. Molecular landmarks of tumor hypoxia across  
657 cancer types. *Nature Genetics*. Published online January 14, 2019:1.  
658 doi:10.1038/s41588-018-0318-2
- 659 16. Espiritu SMG, Liu LY, Rubanova Y, et al. The Evolutionary Landscape of Localized  
660 Prostate Cancers Drives Clinical Aggression. *Cell*. 2018;173(4):1003-1013.e15.  
661 doi:10.1016/j.cell.2018.03.029
- 662 17. Sinha A, Huang V, Livingstone J, et al. The Proteogenomic Landscape of Curable  
663 Prostate Cancer. *Cancer Cell*. 2019;35(3):414-427.e6.  
664 doi:10.1016/j.ccell.2019.02.005
- 665 18. Chen S, Huang V, Xu X, et al. Widespread and Functional RNA Circularization in  
666 Localized Prostate Cancer. *Cell*. 2019;176(4):831-843.e22.  
667 doi:10.1016/j.cell.2019.01.025
- 668 19. Ciriello G, Miller ML, Aksoy BA, Senbabaoglu Y, Schultz N, Sander C. Emerging  
669 landscape of oncogenic signatures across human cancers. *Nat Genet*.  
670 2013;45(10):1127-1133. doi:10.1038/ng.2762
- 671 20. Robinson D, Van Allen EM, Wu Y-M, et al. Integrative clinical genomics of  
672 advanced prostate cancer. *Cell*. 2015;161(5):1215-1228.  
673 doi:10.1016/j.cell.2015.05.001
- 674 21. Kim H, Chen J. c-Myc interacts with TRF1/PIN2 and regulates telomere length.  
675 *Biochem Biophys Res Commun*. 2007;362(4):842-847.  
676 doi:10.1016/j.bbrc.2007.08.064
- 677 22. Lee SS, Bohrsen C, Pike AM, Wheelan SJ, Greider CW. ATM Kinase Is Required  
678 for Telomere Elongation in Mouse and Human Cells. *Cell Rep*. 2015;13(8):1623-  
679 1632. doi:10.1016/j.celrep.2015.10.035
- 680 23. Sommerfeld HJ, Meeker AK, Piatyszek MA, Bova GS, Shay JW, Coffey DS.  
681 Telomerase activity: a prevalent marker of malignant human prostate tissue.  
682 *Cancer Res*. 1996;56(1):218-222.
- 683 24. Weischenfeldt J, Simon R, Feuerbach L, et al. Integrative genomic analyses reveal  
684 an androgen-driven somatic alteration landscape in early-onset prostate cancer.  
685 *Cancer Cell*. 2013;23(2):159-170. doi:10.1016/j.ccr.2013.01.002
- 686 25. Ding Z, Mangino M, Aviv A, Spector T, Durbin R, UK10K Consortium. Estimating  
687 telomere length from whole genome sequence data. *Nucleic Acids Res*.  
688 2014;42(9):e75. doi:10.1093/nar/gku181

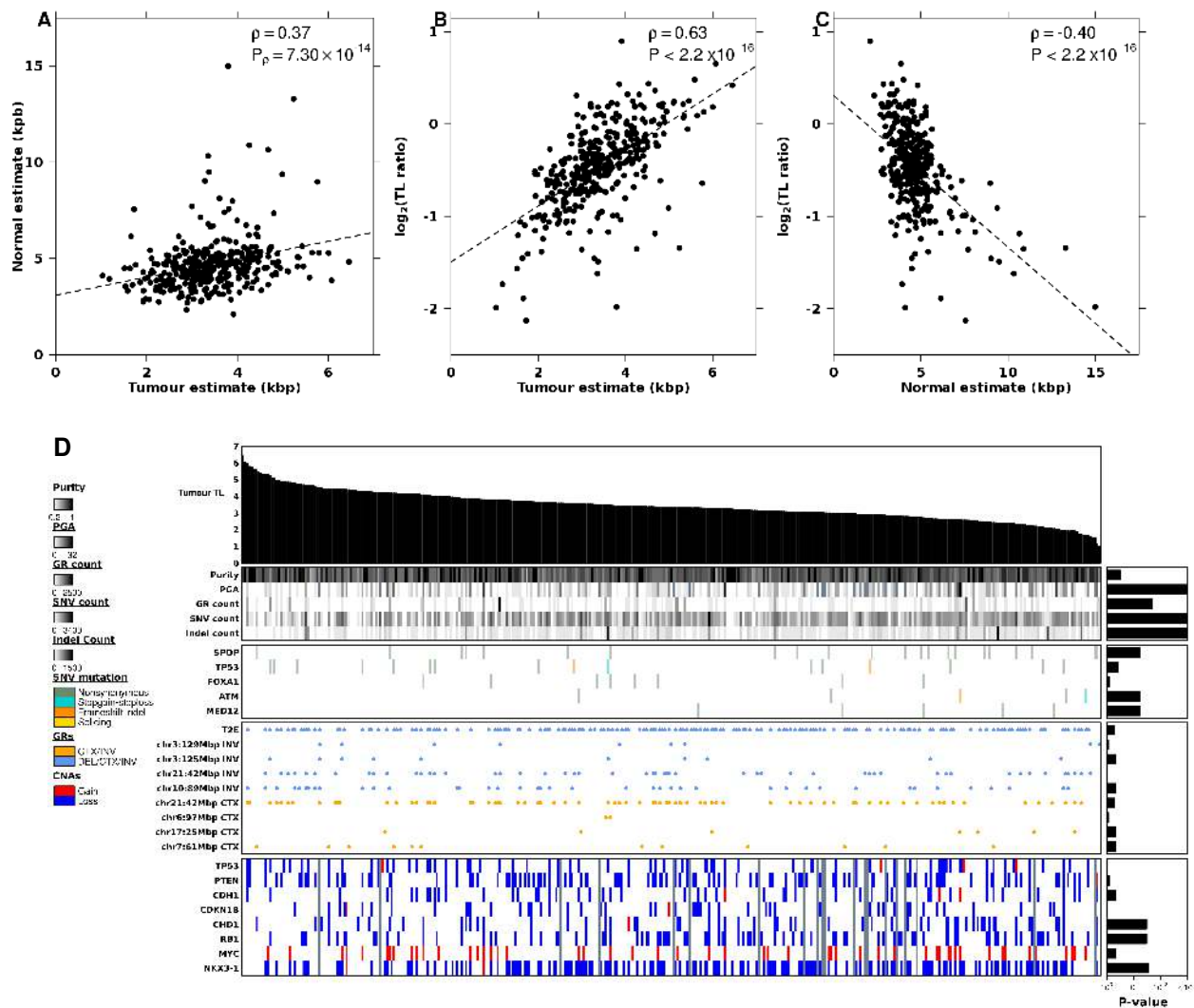
- 689 26. Feuerbach L, Sieverling L, Deeg KI, et al. TelomereHunter – in silico estimation of  
690 telomere content and composition from cancer genomes. *BMC Bioinformatics*.  
691 2019;20(1):272. doi:10.1186/s12859-019-2851-0
- 692 27. Govind SK, Zia A, Hennings-Yeomans PH, et al. ShatterProof: operational  
693 detection and quantification of chromothripsis. *BMC Bioinformatics*. 2014;15:78.  
694 doi:10.1186/1471-2105-15-78
- 695 28. Chin L, Artandi SE, Shen Q, et al. p53 deficiency rescues the adverse effects of  
696 telomere loss and cooperates with telomere dysfunction to accelerate  
697 carcinogenesis. *Cell*. 1999;97(4):527-538.
- 698 29. Maciejowski J, Li Y, Bosco N, Campbell PJ, de Lange T. Chromothripsis and  
699 Kataegis Induced by Telomere Crisis. *Cell*. 2015;163(7):1641-1654.  
700 doi:10.1016/j.cell.2015.11.054
- 701 30. Starmans MHW, Lieuwes NG, Span PN, et al. Independent and functional  
702 validation of a multi-tumour-type proliferation signature. *Br J Cancer*.  
703 2012;107(3):508-515. doi:10.1038/bjc.2012.269
- 704 31. Armenia J, Wankowicz SAM, Liu D, et al. The long tail of oncogenic drivers in  
705 prostate cancer. *Nat Genet*. 2018;50(5):645-651. doi:10.1038/s41588-018-0078-z
- 706 32. Heaphy CM, de Wilde RF, Jiao Y, et al. Altered telomeres in tumors with ATRX and  
707 DAXX mutations. *Science*. 2011;333(6041):425. doi:10.1126/science.1207313
- 708 33. Graham MK, Meeker A. Telomeres and telomerase in prostate cancer development  
709 and therapy. *Nat Rev Urol*. 2017;14(10):607-619. doi:10.1038/nrurol.2017.104
- 710 34. Majumder PK, Sellers WR. Akt-regulated pathways in prostate cancer. *Oncogene*.  
711 2005;24(50):7465-7474. doi:10.1038/sj.onc.1209096
- 712 35. Kolberg L, Raudvere U, Kuzmin I, Vilo J, Peterson H. gprofiler2 -- an R package for  
713 gene list functional enrichment analysis and namespace conversion toolset  
714 g:Profiler. *F1000Res*. 2020;9. doi:10.12688/f1000research.24956.2
- 715 36. Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. *Nucleic  
716 Acids Res*. 2000;28(1):27-30. doi:10.1093/nar/28.1.27
- 717 37. Donato M, Xu Z, Tomoiaga A, et al. Analysis and correction of crosstalk effects in  
718 pathway analysis. *Genome Res*. 2013;23(11):1885-1893.  
719 doi:10.1101/gr.153551.112
- 720 38. Zhou Y, Gao Y, Xu C, Shen H, Tian Q, Deng H-W. A novel approach for correction  
721 of crosstalk effects in pathway analysis and its application in osteoporosis  
722 research. *Sci Rep*. 2018;8(1):668. doi:10.1038/s41598-018-19196-2

- 723 39. Heaphy CM, Yoon GS, Peskoe SB, et al. Prostate cancer cell telomere length  
724 variability and stromal cell telomere length as prognostic markers for metastasis  
725 and death. *Cancer Discov.* 2013;3(10):1130-1141. doi:10.1158/2159-8290.CD-13-  
726 0135
- 727 40. Svenson U, Roos G, Wikström P. Long leukocyte telomere length in prostate  
728 cancer patients at diagnosis is associated with poor metastasis-free and cancer-  
729 specific survival. *Tumour Biol.* 2017;39(2):1010428317692236.  
730 doi:10.1177/1010428317692236
- 731 41. Renner W, Krenn-Pilko S, Gruber H-J, Herrmann M, Langsenlehner T. Relative  
732 telomere length and prostate cancer mortality. *Prostate Cancer Prostatic Dis.*  
733 2018;21(4):579-583. doi:10.1038/s41391-018-0068-3
- 734 42. Koeneman KS, Pan CX, Jin JK, et al. Telomerase activity, telomere length, and  
735 DNA ploidy in prostatic intraepithelial neoplasia (PIN). *J Urol.* 1998;160(4):1533-  
736 1539.
- 737 43. Zhang W, Kapusta LR, Slingerland JM, Klotz LH. Telomerase activity in prostate  
738 cancer, prostatic intraepithelial neoplasia, and benign prostatic epithelium. *Cancer*  
739 *Res.* 1998;58(4):619-621.
- 740 44. Prensner JR, Chen W, Iyer MK, et al. PCAT-1, a long noncoding RNA, regulates  
741 BRCA2 and controls homologous recombination in cancer. *Cancer Res.*  
742 2014;74(6):1651-1660. doi:10.1158/0008-5472.CAN-13-3159
- 743 45. Quigley DA, Dang HX, Zhao SG, et al. Genomic Hallmarks and Structural Variation  
744 in Metastatic Prostate Cancer. *Cell.* 2018;174(3):758-769.e9.  
745 doi:10.1016/j.cell.2018.06.039
- 746 46. Song S, Nones K, Miller D, et al. qpure: A tool to estimate tumor cellularity from  
747 genome-wide single-nucleotide polymorphism profiles. *PLoS ONE.*  
748 2012;7(9):e45835. doi:10.1371/journal.pone.0045835
- 749 47. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler  
750 transform. *Bioinformatics.* 2009;25(14):1754-1760.  
751 doi:10.1093/bioinformatics/btp324
- 752 48. McKenna A, Hanna M, Banks E, et al. The Genome Analysis Toolkit: a MapReduce  
753 framework for analyzing next-generation DNA sequencing data. *Genome Res.*  
754 2010;20(9):1297-1303. doi:10.1101/gr.107524.110
- 755 49. Li H, Handsaker B, Wysoker A, et al. The Sequence Alignment/Map format and  
756 SAMtools. *Bioinformatics.* 2009;25(16):2078-2079.  
757 doi:10.1093/bioinformatics/btp352



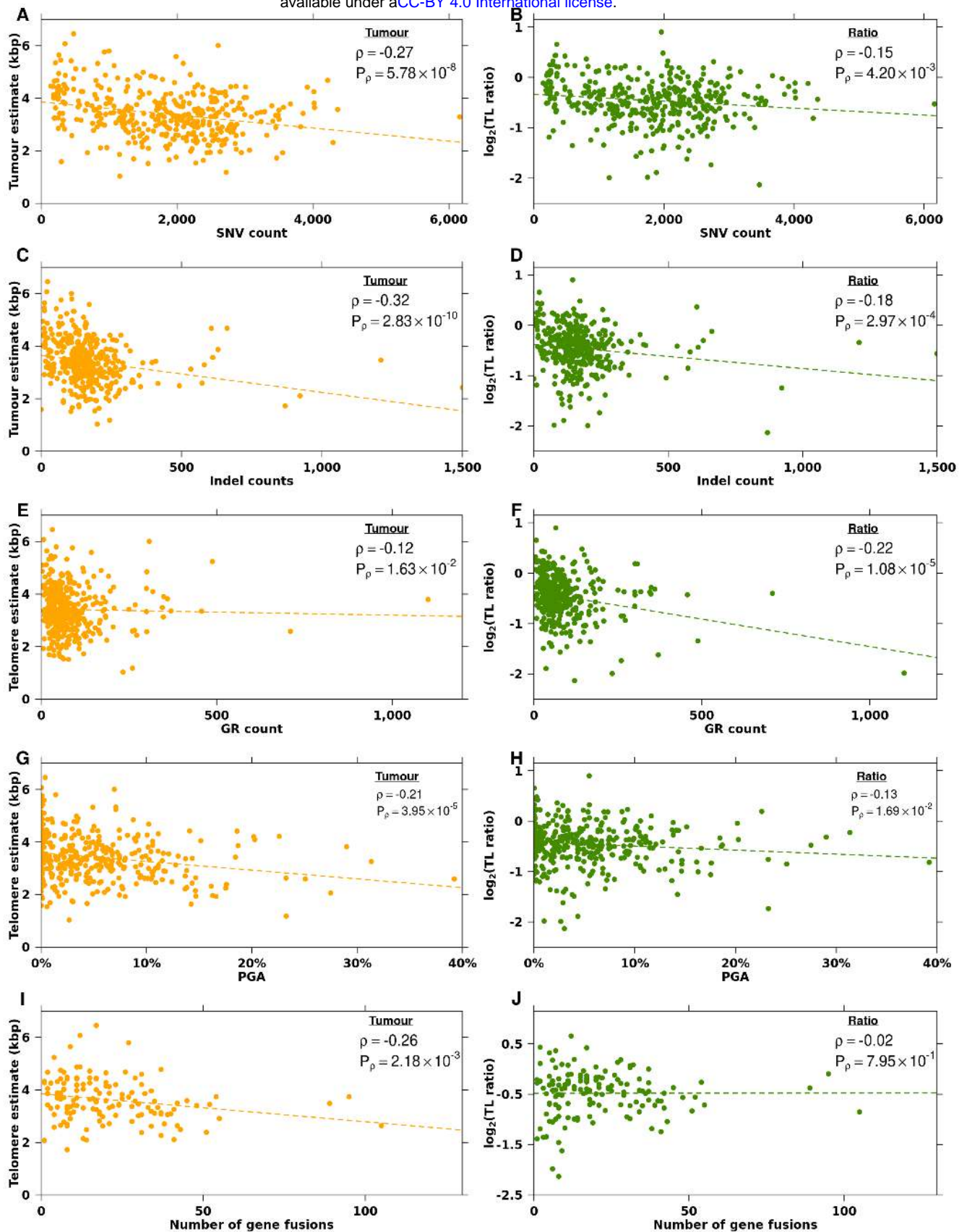
- 758 50. Masella AP, Lalansingh CM, Sivasundaram P, Fraser M, Bristow RG, Boutros PC.  
759 BAMQL: a query language for extracting reads from BAM files. *BMC*  
760 *Bioinformatics*. 2016;17:305. doi:10.1186/s12859-016-1162-y
- 761 51. Larson DE, Harris CC, Chen K, et al. SomaticSniper: identification of somatic point  
762 mutations in whole genome sequencing data. *Bioinformatics*. 2012;28(3):311-317.  
763 doi:10.1093/bioinformatics/btr665
- 764 52. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants  
765 from high-throughput sequencing data. *Nucleic Acids Res*. 2010;38(16):e164.  
766 doi:10.1093/nar/gkq603
- 767 53. Rausch T, Zichner T, Schlattl A, Stütz AM, Benes V, Korbel JO. DELLY: structural  
768 variant discovery by integrated paired-end and split-read analysis. *Bioinformatics*.  
769 2012;28(18):i333-i339. doi:10.1093/bioinformatics/bts378
- 770 54. Dobin A, Davis CA, Schlesinger F, et al. STAR: ultrafast universal RNA-seq aligner.  
771 *Bioinformatics*. 2013;29(1):15-21. doi:10.1093/bioinformatics/bts635
- 772 55. Frankish A, Diekhans M, Ferreira A-M, et al. GENCODE reference annotation for  
773 the human and mouse genomes. *Nucleic Acids Res*. 2019;47(D1):D766-D773.  
774 doi:10.1093/nar/gky955
- 775 56. Li B, Dewey CN. RSEM: accurate transcript quantification from RNA-Seq data with  
776 or without a reference genome. *BMC Bioinformatics*. 2011;12:323.  
777 doi:10.1186/1471-2105-12-323
- 778 57. Pidsley R, Y Wong CC, Volta M, Lunnon K, Mill J, Schalkwyk LC. A data-driven  
779 approach to preprocessing Illumina 450K methylation array data. *BMC Genomics*.  
780 2013;14:293. doi:10.1186/1471-2164-14-293
- 781 58. Aryee MJ, Jaffe AE, Corrada-Bravo H, et al. Minfi: a flexible and comprehensive  
782 Bioconductor package for the analysis of Infinium DNA methylation microarrays.  
783 *Bioinformatics*. 2014;30(10):1363-1369. doi:10.1093/bioinformatics/btu049
- 784 59. Quinlan AR, Hall IM. BEDTools: a flexible suite of utilities for comparing genomic  
785 features. *Bioinformatics*. 2010;26(6):841-842. doi:10.1093/bioinformatics/btq033
- 786 60. P'ng C, Green J, Chong LC, et al. BPG: Seamless, automated and interactive  
787 visualization of scientific data. *BMC Bioinformatics*. 2019;20(1):42.  
788 doi:10.1186/s12859-019-2610-2
- 789 61. Best DJ, Roberts DE. Algorithm AS 89: The Upper Tail Probabilities of Spearman's  
790 Rho. *Journal of the Royal Statistical Society Series C (Applied Statistics)*.  
791 1975;24(3):377-379. doi:10.2307/2347111

792



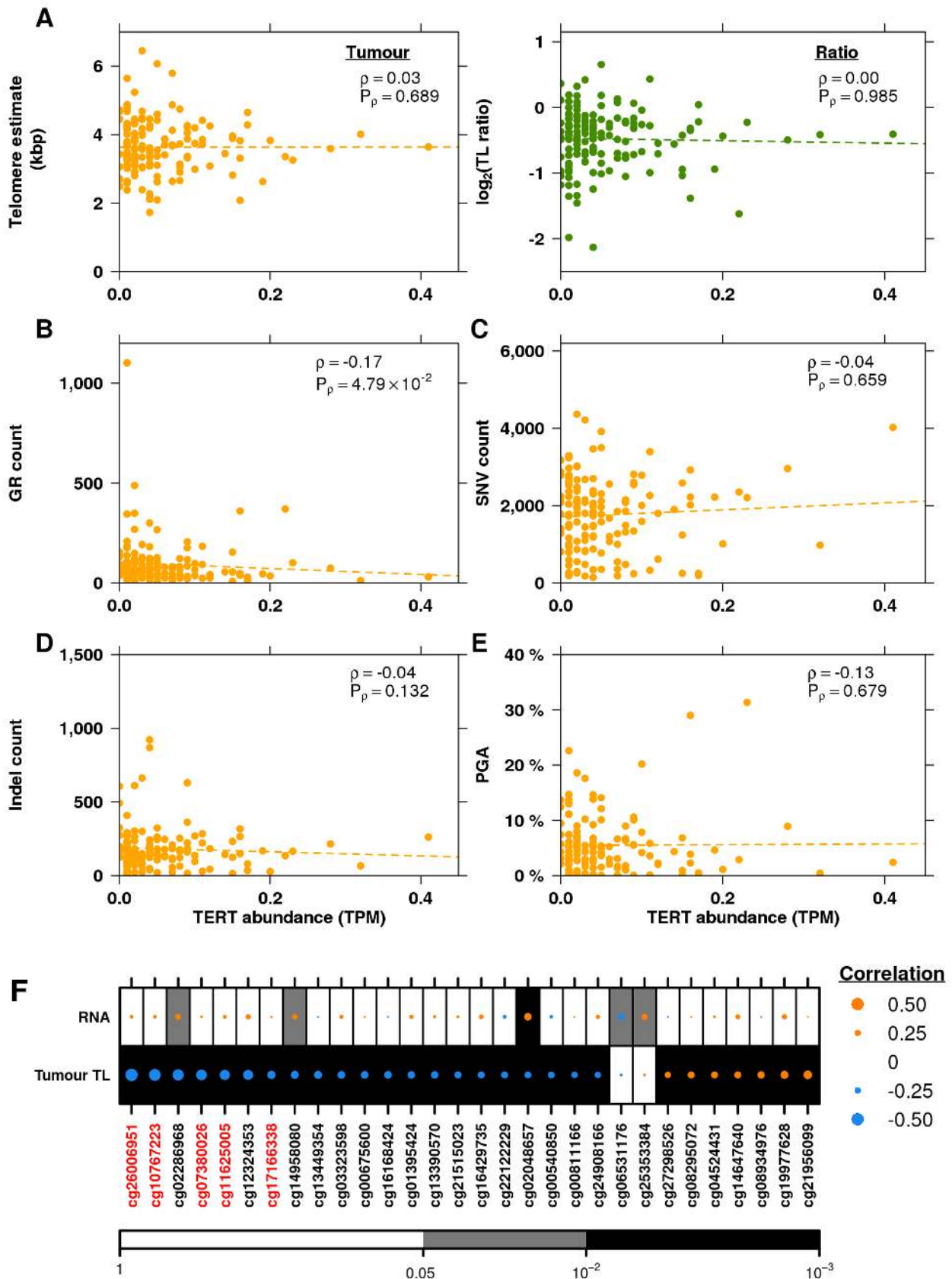
**Figure 1 — Tumour telomere length (TL) is associated with genomic features**

**A-B**, Correlation between tumour TL and **A** non-tumour (blood) TL and **B**, TL ratio (tumour TL / non-tumour (blood) TL). **C**, Correlation between non-tumour (blood) TL and TL ratio. **D**, Tumour TL is ranked in descending order of length (kbp; top bar plot). The association of tumour TL and measures of mutational burden, TMPRSS2:ERG (T2E) fusion status, as well as known prostate cancer genes with recurrent CNAs, coding SNVs, and GRs are shown. Bar plots to the right indicate the statistical significance of each association (**see Methods**).



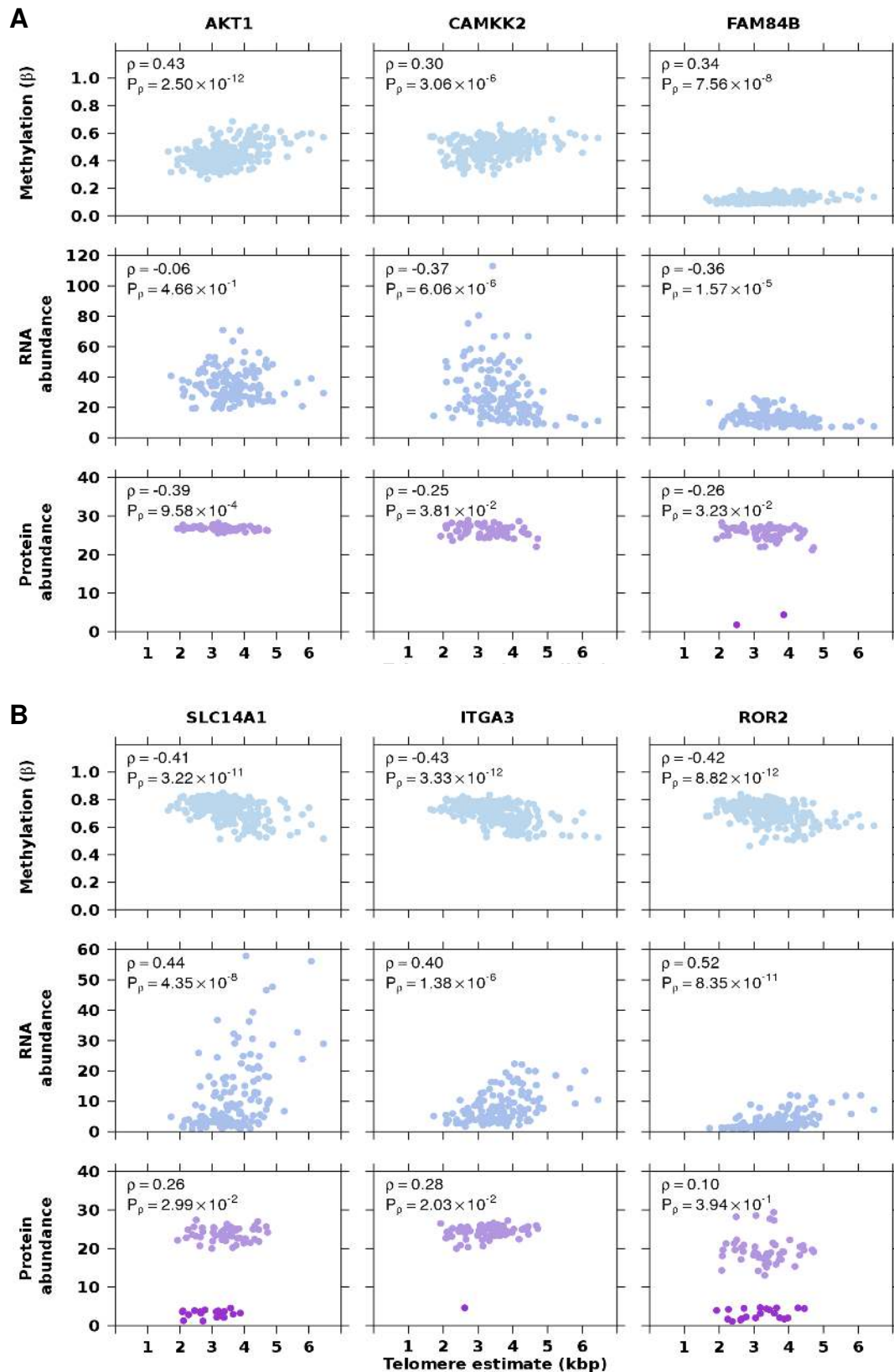
**Figure 2 — Mutational landscape differs with telomere length**

**A-B**, Correlation between the number of SNVs and **A**, tumour TL and **B**, TL ratio. **C-D**, Correlation between the number of indels and **C**, tumour TL and **D**, TL ratio. **E-F**, Correlation between the number of GRs and **E**, and tumour TL and **F**, TL ratio. **G-H**, Correlation of percentage of the genome altered (PGA) and **G**, tumour TL and **H**, TL ratio. **I-J**, Correlation between the number of fusions and **I**, tumour TL and **J**, TL ratio. Orange dots indicate tumour TL while green dots indicate TL ratio. Spearman's  $\rho$  and P-values are displayed.



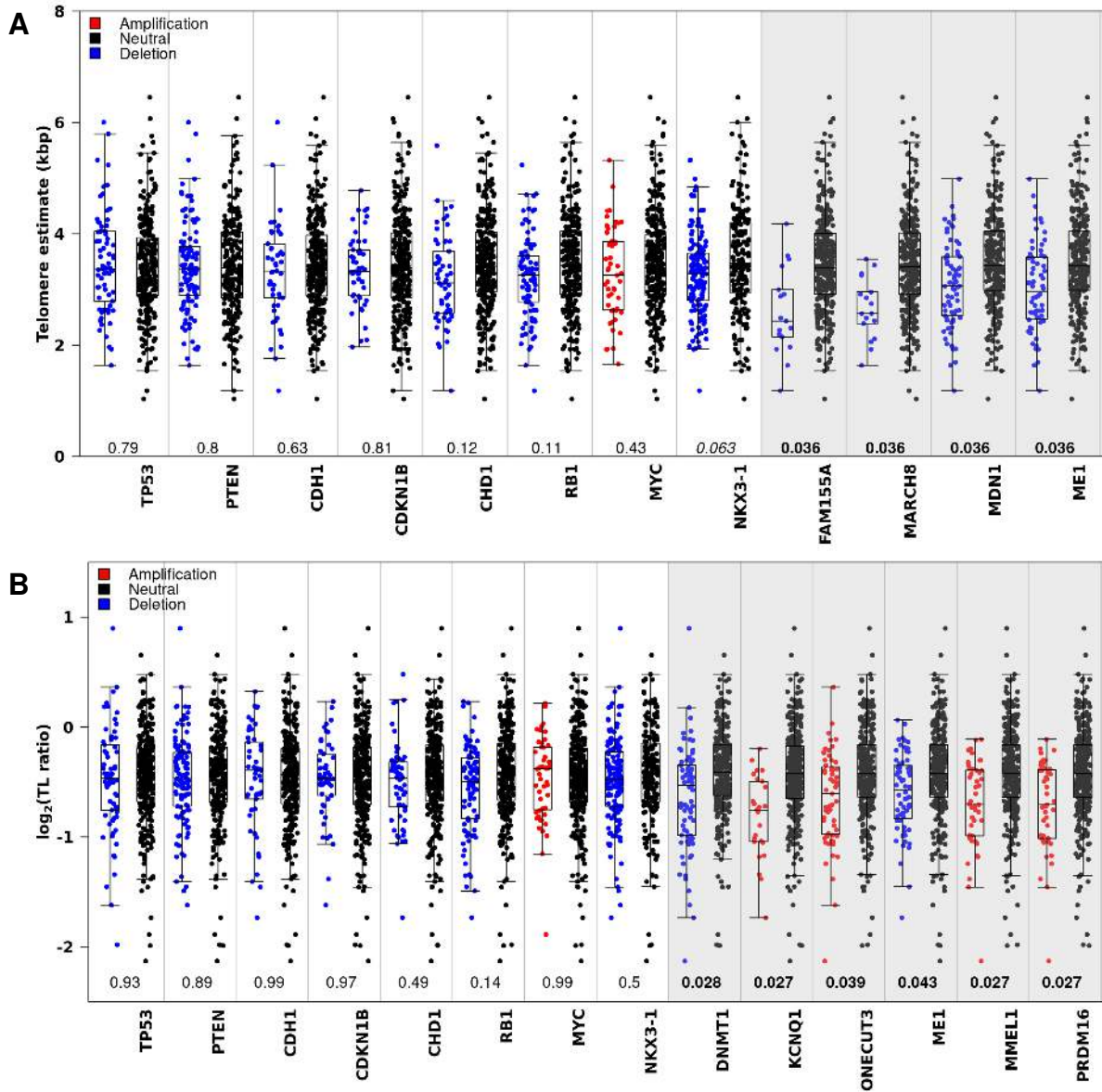
**Figure 3 — The genomic correlates of TERT abundance**

**A**, Correlation of *TERT* RNA abundance with tumour TL and TL ratio. Orange dots indicate tumour TL while green dots indicate TL ratio. Spearman  $\rho$  and P-values are displayed. **B-E**, Correlation of *TERT* abundance and **B**, the number of GRs, **C**, number of SNVs, **D**, number of indels, and **E**, PGA. Spearman  $\rho$  and P-values are displayed. **F**, Spearman's correlation of significantly associated methylation probes with RNA abundance and tumour TL. Orange dots indicate a positive correlation while blue dots indicate a negative correlation. Dot size indicated the magnitude of correlation. Background colour indicates unadjusted P-values. Methylation probes are ordered by their correlation between *TERT* RNA abundance from negative to positive.



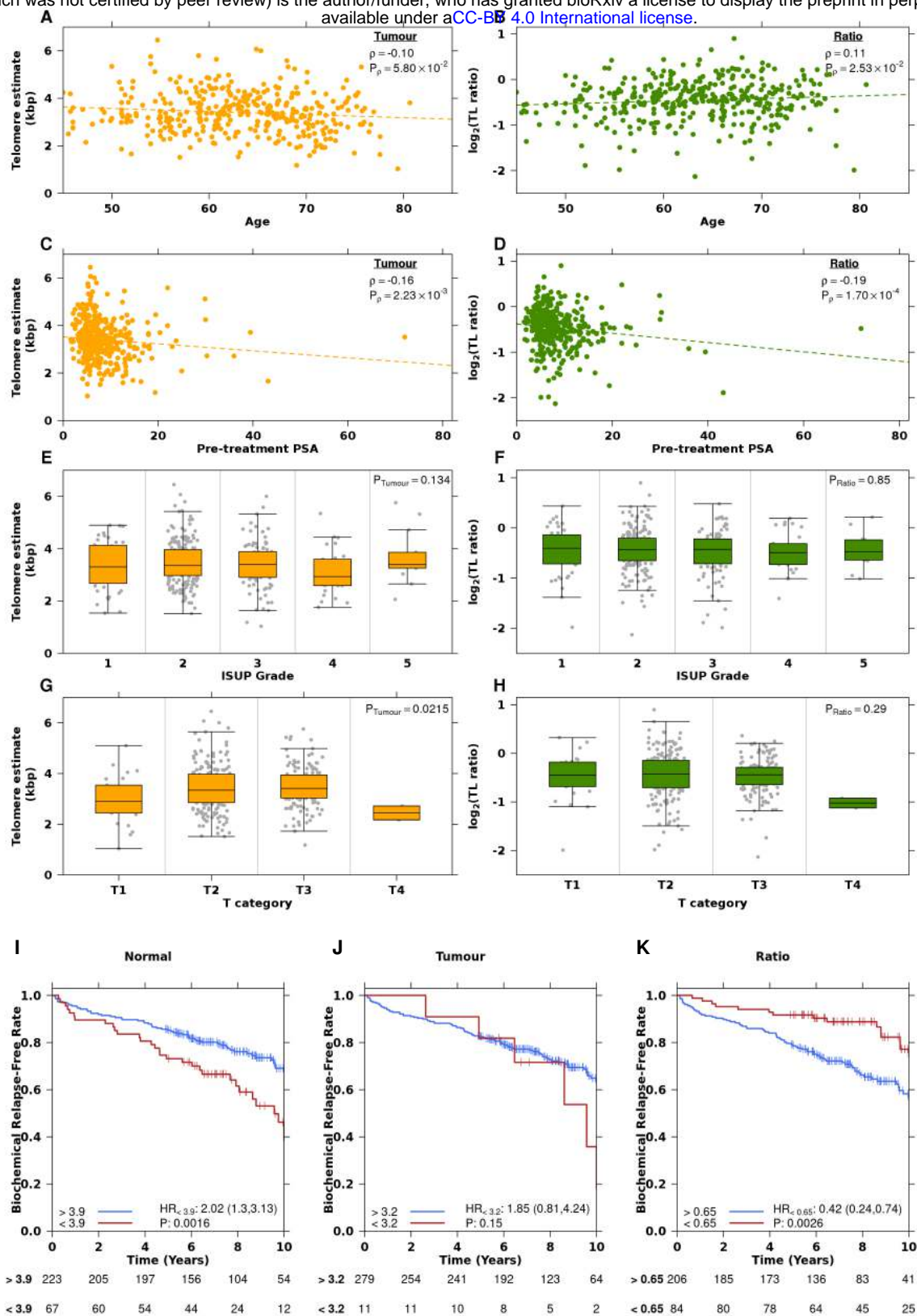
**Figure 4 — Association of methylation, RNA abundance, protein abundance and telomere length**

**A**, Positive correlation of methylation and tumour TL, but negative correlation of RNA and protein abundance. **B**, Negative correlation of methylation and tumour TL, but positive correlation of RNA and protein abundance. Top panels in light blue represent methylation beta values, middle panels in blue-grey represent RNA abundance and the bottom panels in purple represent protein abundance. Darker purple dots represent undetected, imputed protein abundance measures. Spearman  $\rho$  and P-values are displayed.



**Figure 5 — Telomere length differs by copy number status**

**A**, Difference in tumour TL between samples with a copy number aberration and those without in prostate cancer related genes and associated genes. **B**, Difference in TL ratio between samples with a copy number aberration and those without in prostate cancer related and associated genes. Q-values are from a Mann-Whitney U test and are bolded when significant ( $< 0.05$ ). Colour of the dots indicate copy number status of the gene: amplification (red), deletion (blue), or neutral (black). Boxes with a white background are known prostate cancer genes, while boxes with a gray background were identified by a genome wide search.



**Figure 6 — Telomere length is associated with clinical features and biochemical relapse**

**A-B**, Correlation of age at treatment with **A**, tumour TL and **B**, TL ratio. Spearman  $\rho$  and P-values are displayed. **C-D**, Correlation of pre-treatment PSA with **C**, tumour TL and **D**, TL ratio. Spearman  $\rho$  and P-values are displayed. **E-F**, Association of ISUP grade with **E**, tumour TL and **F**, TL ratio. P-value is from an one-way ANOVA. **G-H**, Association of T category with **G**, tumour TL and **H**, TL ratio. P-value is from an one-way ANOVA. On all plots, green indicates TL ratio, while orange indicates tumour TL. **I-K**, Cox proportional hazard models were created for **I**, non-tumour (blood) TL, **J**, tumour TL and **K**, TL ratio with BCR as the endpoint. Samples were split into two groups based on the optimal cut point analysis (see **Methods**).