

A genome-wide atlas of recurrent repeat expansions in human cancer

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1 Abstract

2 **Expansion of a single repetitive DNA sequence, termed a tandem repeat (TR), is**
3 **known to cause more than 50 diseases. However, repeat expansions are often not explored**
4 **beyond neurological and neurodegenerative disorders. In some cancers, mutations**
5 **accumulate in short tracts of TRs (STRs), a phenomenon termed microsatellite instability**
6 **(MSI); however larger repeat expansions have not been systematically analyzed in cancer.**
7 **Here, we identified TR expansions in 2,622 cancer genomes, spanning 29 cancer types. In 7**
8 **cancer types, we found 160 recurrent repeat expansions (rREs); most of these (155/160)**
9 **were subtype specific. We found that rREs were non-uniformly distributed in the genome**
10 **with an enrichment near candidate cis-regulatory elements, suggesting a role in gene**
11 **regulation. One rRE located near a regulatory element in the first intron of *UGT2B7* was**
12 **detected in 34% of renal cell carcinoma samples and was validated by long-read DNA**
13 **sequencing. Moreover, targeting cells harboring this rRE with a rationally designed,**
14 **sequence-specific DNA binder led to a dose-dependent decrease in cell proliferation.**
15 **Overall, our results demonstrate that rREs are an important but unexplored source of**
16 **genetic variation in human cancers, and we provide a comprehensive catalog for further**
17 **study.**

18

19 Introduction

20 Expansions of tandem DNA repeats (TRs) are known to cause more than 50 devastating
21 human diseases including Huntington's disease and Fragile X syndrome^{1,2}. TR tracts that cause

22 human disease are typically large (more than 100 base pairs)¹. However, identifying large TRs
23 with short-read DNA sequencing methods is difficult because they are ubiquitous in the genome,
24 and many are too large—larger than the typical sequencing read length—to uniquely map to the
25 reference genome³. Thus, many large TRs go undetected with current genomic technologies, and
26 despite their importance to monogenic disease, the frequency and function of recurrent repeat
27 expansions (rREs) are unknown in complex human genetic diseases, such as cancer⁴.

28 Previous studies have profiled the landscape of alterations in STRs in cancer genomes⁵⁻⁷.
29 In particular, microsatellite instability (MSI)⁸⁻¹⁰, defined by an alteration in the lengths of short
30 TRs, is prevalent in various types of cancer including endometrial (30%), stomach (20%), and
31 colorectal cancers (15%)^{5,6,11-12}. However, the systematic analysis of the frequency of genome-
32 wide large TR expansions has not been studied in cancer even though it was posited more than
33 25 years ago¹³.

34 Recently, new bioinformatic tools to identify repeat expansions in short-read whole-
35 genome sequencing (WGS) datasets¹⁴⁻¹⁷ have led to the identification of both known and novel
36 repeat expansions in human disease, primarily in the area of neurological disorders where repeat
37 expansions have historically been studied¹⁴⁻²². Here, we analyzed 2,622 human cancer genomes
38 with matching normal samples for the presence of somatic repeat expansions. We identified 160
39 recurrent repeat expansions (rREs) in seven types of cancer, including many rREs located in or
40 near known regulatory elements. One of these rREs is observed in 34% of kidney cancers, and
41 targeting this repeat expansion with sequence-specific DNA binders led to a dose-dependent
42 decrease in cellular proliferation. Overall, our approach reveals a new class of recurrent changes
43 in cancer genomes and provides an initial resource of these changes. Our results also suggest an
44 opportunity for a new class of oncologic TR-targeted therapeutics^{23,24}.

45

46 **Results**

47 **Recurrent repeat expansions in cancer**

48 We collected uniformly-processed alignments of whole-genome sequencing (WGS) of
49 tumor-normal pairs in the International Cancer Genome Consortium (ICGC), The Cancer
50 Genome Atlas (TCGA), both a part of the pan-cancer analysis of whole genomes (PCAWG)
51 datasets²⁵. After filtering, these data consist of 2,622 cancer genomes from 2509 patients across
52 29 different cancer types (**Extended Data Figure 1**). Each cancer type was treated as its own
53 cohort and analyzed independent of other cancer types. We called somatic recurrent repeat
54 expansions (rREs) with ExpansionHunter Denovo (EHdn) (see **Methods**), which measures TRs
55 whose length exceeds the sequencing read length in short-read sequencing datasets^{26,27}. That is,
56 EHdn performs case-control comparisons using a non-parametric statistical test to determine
57 whether repeat length is longer in tumor genomes compared to matching normal genomes. This
58 approach is analogous to joint population-level genotyping.

59 We first confirmed the accuracy of EHdn by performing whole-genome short- and long-
60 read sequencing on 786-O and Caki-1 cancer lines. We found that EHdn captured 72% of the
61 repeat expansions observed in long-read sequencing (**Extended Data Figure 2**). We also tested
62 the effect of sequencing coverage on the detection of rREs, and found that EHdn was robust
63 down to 30x coverage (**Extended Data Figure 2**). We then analyzed 2,622 matching tumor and
64 normal genomes with EHdn (285,363 TRs). We identified 578 candidate rREs (locus-level false
65 discovery rate (FDR) < 10%).

66 EHdn is expected to be sensitive to the copy number variations observed in cancer
67 genomes. We therefore devised and implemented a local read depth filtering method to account
68 for copy number variants, which normalizes the signal originating from repeat reads using the
69 read depth in the vicinity of the TR (see **Methods** and **Extended Data Figure 3**). We
70 benchmarked the local read depth normalization approach with simulated chromosomal
71 amplifications ranging from two (diploid) to 10 copies. We found that this filter accounts for
72 changes in chromosomal copy number in a manner superior to the standard global read depth
73 normalization (**Fig. S5**). Overall, we conclude that local read depth normalization is valuable to
74 identify *bona fide* rREs in cancer genomes and that many of the rREs that pass the filter are
75 expanded in cancer. For example, without local read depth normalization, we could only detect
76 31% of candidate rREs in independent cohorts of matching tumor-normal tissue samples for
77 breast, prostate, and kidney cancer (15, 18, and 12 patients, respectively). Our local read depth
78 filtering approach removed >75% (418/578) false-positive candidate rREs (**Extended Data**
79 **Figure 3**). Importantly, several rRE candidates that were removed are situated in hotspots for
80 chromosomal amplification, such as chromosomal 8q amplifications that
81 increase *MYC* production in breast cancer (**Extended Data Figure 3**)²⁸. Our analysis suggests
82 that the standalone EHdn method may have selected these loci due to amplification rather than
83 repeat expansions, and thus their removal is important.

84 After implementing our local read depth filtering strategy, we increased our detection rate
85 to 57% (8/14) in independent cohorts (**Extended Data Figure 3**). Importantly, the loci we could
86 not validate had lower expansion frequencies (5–12%). These rREs may be real but more
87 difficult to validate in the small validation cohorts (**Table S7**). Thus, we believe this number is
88 likely an underestimate of the independent detection rate. Of the 14 candidate rREs that failed

89 our local read depth filter, 29% (4/14) were detected in independent cohorts of samples
90 indicating that the filtering removes most loci that cannot be validated (**Extended Data Figure**
91 **3**), but also removes some true positives as well.

92 After accounting for local read depth, we detected 160 rREs in 7 human cancers (**Fig.**
93 **1b**). We expected high concordance with ExpansionHunter given that this tool is related to
94 EHdn, and indeed we observed a 91% confirmation rate with ExpansionHunter (**Extended Data**
95 **Figure 4**). We found that most (80%) of these loci are rarely expanded in the general population
96 (<5% of the time, $n = 6,514$ genomes, **Extended Data Figure 2**). rREs were primarily observed
97 in prostate and liver cancer, but we also detected rREs in ovarian, pilocytic astrocytoma, renal
98 cell carcinoma (RCC), chromophobe RCC, and squamous cell lung carcinoma. Thus, rREs are
99 found in tissues derived from each of the three primary germ layers (ectoderm, mesoderm, and
100 endoderm), suggesting these expansions are a phenomenon inherent to the human genome rather
101 than any tissue-specific process. In prostate and liver cancer, most cancer genomes (93% and
102 95%, respectively) contain at least one rRE, with some genomes harboring several rREs (**Fig.**
103 **1c**). For some pathogenic repeats, a larger TR length at birth predisposes an individual to somatic
104 repeat expansions later in life^{1,2}, but we did not generally observe that with rREs (**Table S8**).
105 Overall, rREs are found in 7 of 29 human cancers examined and are largely cancer subtype-
106 specific.

107 We next examined whether rREs correlate with changes in MSI^{5,6}. We determined
108 whether samples harboring an rRE had a higher mutation rate in STRs, which is a hallmark of
109 MSI^{5,29}. We did not observe any significant difference in STR mutation rate for genomes with an
110 rRE compared to those lacking an rRE (two-tailed Wilcoxon rank sum test, $P = 0.27$, **Fig. 1d**).
111 We also compared cancer genomes harboring rREs with cancer genomes previously identified as

112 MSI, using recent results from the PCAWG consortium²⁹. We did not observe any enrichment in
113 MSI for samples harboring an rRE, and instead found a weak but significant preference for rREs
114 in microsatellite stable (MSS) samples, not MSI samples (Two-tailed Wilcoxon rank-sum test, P
115 = 0.04, **Fig. 1e**, see also **Extended Data Figure 5**). Thus, our findings might suggest a model
116 where rREs are formed by a process that is distinct from MSI.

117 In addition to MSI, different mutational processes lead to a signature of somatic
118 mutations. We tested whether rREs are associated with known mutational signatures by
119 comparing them to 49 signatures of single base substitutions (SBS) and 11 doublet base
120 substitutions (DBS)³⁰. We performed a multiple linear regression to predict the number of rREs
121 in a sample based on SBS and DBS signatures, respectively. Only one DBS signature, DBS2,
122 showed a very weak association with rREs ($r^2 = 0.12$) (**Extended Data Figure 5**).

123

124 **rREs overlap regulatory elements**

125 Among the 160 rREs, we observed a variety of different motifs (**Table S1**) whose repeat
126 unit length follows a bimodal distribution, consistent with REs identified in other diseases (**Fig.**
127 **2a**, **Extended Data Figures 6 and 7**)²⁷. rREs are distributed across a range of GC content;
128 approximately half (76/160) have GC content less than 50% (**Table S1**). Six rREs contained a
129 known pathogenic motif, all of which were GAA³¹. We examined if any motifs were enriched in
130 the rRE catalog compared to the tandem repeat finder (TRF) catalog. Although this enrichment
131 could arise from a biological and/or technical process, we found that one of the three enriched
132 motifs was GAA (**Fig. 2b**). As an example, Friedreich's ataxia is caused by a repeat expansion of
133 a GAA motif in the intron of the frataxin gene. This expansion leads to DNA methylation and the

134 deposition of repressive chromatin marks, leading to robust repression of the gene and
135 development of disease³¹. Because of this, we suspect some of the rREs found in cancer might
136 alter the epigenome and affect gene regulatory networks.

137 rREs were distributed non-uniformly across the genome, with a bias towards the ends of
138 chromosome arms (**Fig. 2c, Extended Data Figure 6**). This observation is consistent with
139 previous reports of TRs and structural variants^{15,32}. We also examined the distribution of rREs
140 relative to gene features with `annotatr` (**Fig. 2d**)³³. The 7% of rREs labeled as exonic appeared
141 proximal to, but not within, exons, but others were in introns, untranslated regions (UTRs), and
142 splice sites. These results suggest rREs may play different functional roles in the regulation of
143 gene expression.

144 We measured the distance between rREs and candidate cis-regulatory elements
145 (cCREs)³⁴; cCREs comprise approximately one million functional elements including promoters,
146 enhancers, DNase-accessible regions, and insulators bound by CCCTC-binding factor (CTCF).
147 An rRE near a regulatory element could alter the function of that regulatory element, as is
148 observed in Fragile X syndrome and Friedreich's ataxia¹. Interestingly, rREs are located closer to
149 cCREs than expected by chance, and we find that 47 of 160 rREs directly overlap with a known
150 cCRE (Welch's *t*-test, $P = 6.00e-45$, **Fig. 2e & Extended Data Figure 7**). Thus, rREs are often
151 found in or near functional regions of the genome.

152

153 **rREs with links to cancer**

154 We mapped each rRE to the nearest genes and found that nine rREs map to Tier 1 genes
155 present in the census of somatic mutations in cancer (COSMIC) database (**Fig. 3, Table S1**). We

156 also observed a strong correlation with cancer-related genes (Jensen disease-gene associations³⁵).
157 That is, four of the top five diseases associated with the collection of 160 rRE are cancers (**Fig.**
158 **3b, Table S4**).

159 To examine whether some rREs play a role in oncogenesis, we looked at their association
160 with previously-identified cancer risk loci. Many rREs were identified in prostate cancer, and 63
161 loci have previously been associated with susceptibility to prostate cancer from available
162 genome-wide association studies³⁶. When we examined the co-localization of rREs and cancer
163 risk loci in prostate cancer, we found that rREs are located closer to prostate cancer susceptibility
164 loci than standard STRs or by chance (Student's *t*-test, FDR $q = 0.08$, **Fig. 3c & Extended Data**
165 **Figure 7**).

166 We next studied the relationship between the occurrence of the census of somatic
167 mutations in cancer (COSMIC) genes to the occurrence of rREs (**Fig. 3d**). Interestingly, we
168 found that there are five COSMIC genes whose somatic mutations are found to occur
169 significantly more in patients' genomes with no rREs, after correcting for multiple hypothesis
170 testing. Among them, *TP53* was particularly striking, as wildtype *TP53* is critical for mediating
171 the pathogenic effects of repeat expansions in both Amyotrophic Lateral Sclerosis (ALS) and
172 Huntington's Disease^{37,38}. Consistent with these findings, a DNA damage repair gene in yeast,
173 *Rad53*, is phosphorylated and activated in the presence of an expanded repeat³⁹.

174 MSI-high cancers are often correlated with higher levels of immune cell infiltration⁴⁰. We
175 hypothesized that some rREs might also be associated with higher immune cell infiltration, but
176 we did not observe a correlation between cytotoxic activity⁴¹ and the presence of an rRE
177 (**Extended Data Figure 9**). Because there are matching RNA-seq data for only 4 of 160 rREs,

178 this analysis warrants further investigation as more matching WGS and RNA-seq datasets
179 become available.

180

181 **An rRE in the *UGT2B7* gene observed in RCC**

182 A GAAA expansion located in the intron of *UGT2B7* was observed in 34% of RCC
183 samples. *UGT2B7* is a glucuronidase that clears small molecules—including
184 chemotherapeutics—from the body and is selectively expressed in the kidney and liver⁴².

185 With gel electrophoresis, we identified the expected TR size of ~26 GAAA repeats in the
186 normal kidney cell line, HK-2, corresponding closely to the length observed in the reference
187 genome (**Fig. 4a**). In contrast, we identified an expansion between ~63 and ~160 GAAA repeat
188 units in 5 of 8 clear cell RCC cell lines. Most expansions were heterozygous (**Fig. 4a**). Long-
189 read DNA sequencing with highly-accurate PacBio HiFi reads confirmed the PCR results and
190 revealed the precise structure of this repeat expansion at single base pair resolution for both 786-
191 O and Caki-1 (**Fig. 4b**). We also detected this repeat expansion in five out of 12 primary kidney
192 tumor tissue samples from patients with clear cell RCC (**Extended Data Figure 8**), which
193 showed more heterogeneity than the RCC cell lines; more heterogeneity might be expected for
194 human tumor samples compared to the clonal cell lines.

195 Given that *UGT2B7* is selectively expressed in the liver and kidney, and that it plays a
196 role in clearing small molecules from the body, we examined whether this rRE may be located
197 near any functional elements that could regulate its expression. Analysis of the chromatin
198 environment surrounding the rRE in *UGT2B7* revealed a nearby enhancer, raising the possibility
199 that this rRE alters the expression of *UGT2B7* (**Fig. 4c**). The repeat motif of this rRE, GAAA,

200 appears similar to the pathogenic repeat motif found in Friedreich's ataxia, which is GAA. The
201 pathogenic GAA repeat expansion blocks *FXN* expression³¹. We therefore hypothesized that the
202 intronic GAAA repeat expansion might repress the expression of *UGT2B*; we found a modest
203 decrease in expression that was not statistically significant (**Extended Data Figure 8**). While
204 this rRE is also not associated with a difference in survival (**Extended Data Figure 8**), it is
205 associated with a significant decrease in a transcript isoform in *UGT2B7* (Wald test with FDR
206 correction, $P = 0.0048$) (**Fig. 4e**). Interestingly, a shift in isoform usage of *UGT2B7* has been
207 noted in cancer⁴³.

208

209 **Repeat-targeting anti-proliferative agents**

210 Do GAAA repeat expansions contribute to cell proliferation? We previously showed that
211 targeting a related TR motif, GAA, with synthetic transcription elongation factors (Syn-TEF1)
212 reverses pathogenesis in several models of Friedreich's ataxia²³. Therefore, if the GAAA rRE in
213 RCC behaves similarly, then a Syn-TEF targeting GAAA may have anti-proliferative activity.
214 We rationally designed Syn-TEF3, which contains a GAAA-targeting polyamide (PA), and a
215 bromodomain ligand, JQ1, designed to recruit part of the transcriptional machinery (**Fig. 5a** and
216 **Fig. S2**). We also included a control molecule, Syn-TEF4, which targets GGAA TRs, as well as
217 polyamides (PAs) PA3 and PA4 that lack the JQ1 domain. We have previously shown that Syn-
218 TEFs and PAs localize to repetitive TRs in living cells^{23,44}.

219 The effect of Syn-TEFs on cell proliferation was examined (**Fig. 5b**). Caki-1 and 786-O were
220 selected because they have the largest (161) and smallest (24) GAAA tracts within the first
221 intron of *UGT2B7*, respectively. In a dose-dependent manner, we observed that Syn-TEF3 led to

222 a significant decrease in the proliferation of Caki-1 cells but had little effect on 786-O cells. Syn-
223 TEF4, which does not target a GAAA TR, did not significantly decrease proliferation in either of
224 the cell lines tested, demonstrating the requirement for GAAA-specific targeting (**Fig. 5b**). Two
225 additional GAAA repeat expansion cell lines as well as two additional control non-expanded
226 lines showed a similar association between Syn-TEF sensitivity and the presence of the repeat
227 expansion (**Extended Data Figure 10**). Consistent with this finding, Caki-1 cells treated with
228 Syn-TEF3 exhibited a significant increase in cell death compared to DMSO control, as measured
229 by propidium iodide staining (**Fig. 5c,d** and **Extended Data Figure 10**). In contrast, 786-O cells
230 treated with Syn-TEF3 showed no significant difference in propidium iodide-positive cells
231 compared to DMSO (**Fig. 5c,d** and **Extended Data Figure 10**). Importantly, the Syn-TEF4,
232 PA3, and PA4 control agents exhibited no significant effect on cell death in either cell line
233 compared to vehicle control (**Fig. 5c,d** and **Extended Data Figure 10**). These results suggest
234 that GAAA repeat expansions may represent a genetic vulnerability in RCC and provide a proof-
235 of-principle study for the functional role of rREs in cancer.

236

237 **Discussion**

238 Here, for the first time, we conduct a genome-wide survey of recurrent repeat expansions
239 (rREs) across cancer genomes, distinct from MSI. Our data identified (i) 160 rREs in 7 human
240 cancers and revealed that (ii) most (155 of 160) rREs are cancer subtype-specific; (iii) amongst
241 diseases, rREs are enriched in human cancer loci and tended to occur near regulatory elements;
242 (iv) recurrent repeat expansions do not correlate with MSI status; and (v) targeting a GAAA
243 repeat expansion in RCC with a small molecule leads to cancer cell killing. Taken together, our

244 results uncover an unexplored genetic alteration in cancer genomes with important mechanistic
245 and therapeutic implications.

246 Cancer cells evolve and adapt in response to environmental or pharmacological
247 perturbations, but the mechanisms supporting these changes are still being uncovered. One
248 source of genetic variation that may enable genetic adaptations is TR DNA sequences. Mutations
249 in repeat length of TRs can occur up to 10,000 times more frequently than single nucleotide
250 variants (SNVs) or insertions and deletions (INDELs)¹. Repeat expansions may provide a source
251 of genetic variation to enable cancer cells to adapt to changes in the environment⁴⁵. Indeed,
252 colorectal cancers acquire mutations in STRs in response to targeted therapy just 24 hours
253 following treatment, suggesting that mutations in these regions may associate with rapid
254 evolution⁴⁶. In future studies, it will be particularly valuable to study repeat expansions in the
255 genomes of cancer cells that face changing environments, including metastasis and
256 chemotherapy.

257 Historically, MSI has been the focus of efforts to profile changes in STRs in cancer
258 genomes because specific cancer-causing genetic alterations in repair genes can promote
259 widespread STR alterations. Interestingly, we find little to no correlation between rREs and MSI.
260 These results are consistent with previous findings in which the correlation between MSI and
261 repeat instability at larger TRs is not definitive⁴⁷. MSI may contribute to a subtype of rREs that
262 we have not yet uncovered, or rREs may arise from a mutation process that is distinct from that
263 of MSI. There are several different DNA cellular repair systems, and presumably the rREs that
264 we observed are due to very specific loci-associated mechanisms or activities. Some of these
265 repeat expansions may be due to cis-regions with interesting DNA or chromatin configurations

266 that are prone to expansion at distinct loci, rather than gene mutations that cause global trans
267 effects, as occurs in MSI.

268 There are numerous mechanisms by which a repeat expansion can alter cellular function.
269 Known pathogenic repeat expansions can alter the coding sequence of a protein, such as in the
270 case of Huntington's disease. However, there are several repeat expansions that occur in non-
271 coding regions that alter gene expression¹. In other instances, the repeat expansion can lead to a
272 pathogenic RNA molecule (myotonic dystrophy) or protein (ALS)¹. Finally, repeat expansions in
273 MSI-associated cancers, which are too small to detect by EHDn, can disrupt DNA replication⁴⁸.
274 Thus, our catalog represents a powerful resource to explore the mechanisms by which rREs alter
275 cellular function in cancer.

276 The identification of repeat expansions would benefit from improved sequencing
277 coverage and increased cohort sizes. Like other tools that identify repeat expansions, we cannot
278 distinguish zygosity from sample heterogeneity or obtain precise lengths of repeats. Our
279 independent experimental validation showed that some repeat expansions are heterogeneous
280 (**Extended Data Figure 8**). We suspect that tumor heterogeneity may lead to an underreporting
281 of rREs. Furthermore, this study focuses on somatic mutations, but repeat expansions that occur
282 in the context of normal development will be another important area of study⁴. Furthermore,
283 germline events that predispose an individual to cancer would also be worthwhile to study; there
284 is evidence that a TR in the androgen receptor gene is associated with prostate cancer onset,
285 tumor stage, and tumor grade⁴⁹. Finally, we only detected changes in repeat length that were
286 greater than sequencing read length. In future studies, it will be important to explore recurrent
287 changes that are smaller in length. Finally, it is important to acknowledge that rREs could be
288 mediators of phenotypes or passengers that result from genetic instability and clonal selection. In

289 the one instance where we targeted the rRE in RCC, cell proliferation was reduced, consistent
290 with a mediator role for this rRE. Distinguishing between these two possibilities for each rRE is
291 an important line of work in the future.

292 To our knowledge, this is the first genome-wide survey of repeat expansions beyond a
293 neurological or neurodegenerative disorder. Thousands of high-quality whole genome sequences
294 exist for many diseases, and our data provide evidence that repeat expansions should be explored
295 beyond the classical bounds of neurodegenerative diseases where they have been most
296 investigated.

297 A careful dissection of repeat expansions in human disease may reveal their role as
298 causative or contributory. We show here that repeat expansions can be targeted by tandem
299 repeat-targeting precision molecules²³. Thus, our results set the stage for a new class of
300 therapeutics to be deployed in cancer and other diseases.

301

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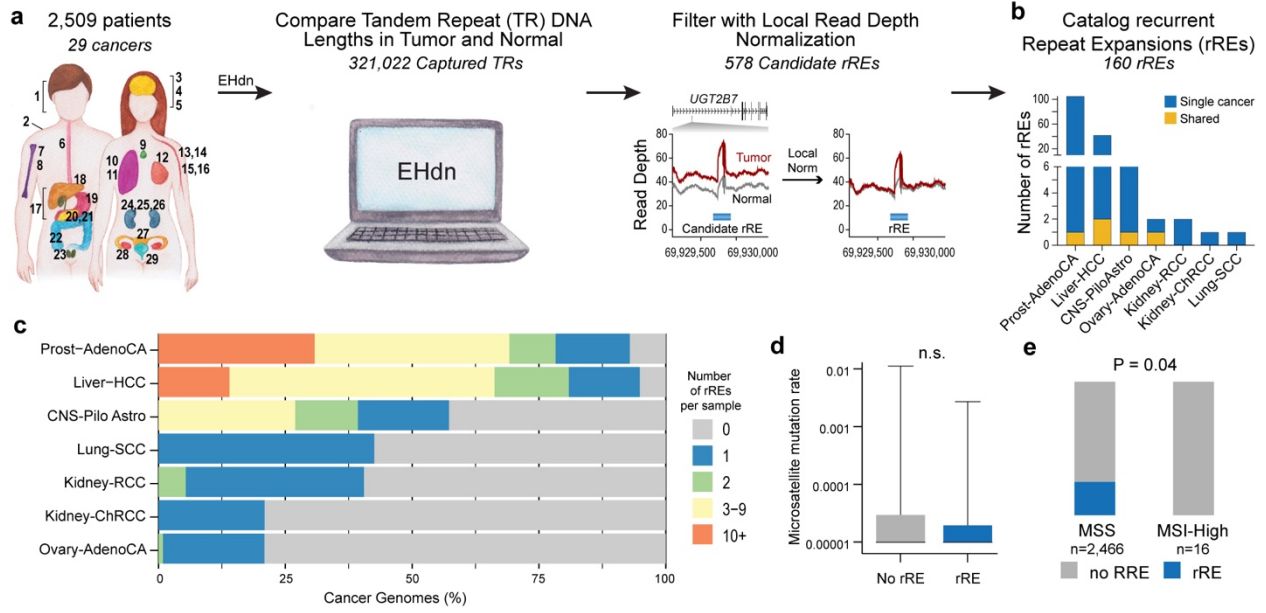
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- 410

411 **Figure 1. Genome-wide detection of recurrent repeat expansions (rREs) in cancer genomes.**

412 a) Scheme of method to identify rREs in 2,509 patients across 29 human cancers. 1, head and
413 neck squamous cell carcinoma (Head-SCC); 2, Skin-Melanoma; 3, glioblastoma (CNS-GBM);
414 4 medulloblastoma (CNS-Medullo); 5, pilocytic astrocytoma (CNS-PiloAstro); 6, esophageal
415 adenocarcinoma (Eso-AdenoCA), 7, osteosarcoma (Bone-Osteosarc); 8, leiomyosarcoma
416 (Bone-Leiomyo); 9, thyroid adenocarcinoma (Thy-AdenoCA); 10, lung adenocarcinoma
417 (Lung-AdenoCA); 11, lung squamous cell carcinoma (Lung-SCC); 12, mammary gland
418 adenocarcinoma (Breast-AdenoCA); 13, B-cell non-Hodgkin lymphoma (Lymph-BNHL); 14,
419 chronic lymphocytic leukemia (Lymph-CLL); 15, acute myeloid leukemia (Myeloid-AML); 16,
420 myeloproliferative neoplasm (Myeloid-MPN); 17, biliary adenocarcinoma (Biliary-AdenoCA);
421 18, hepatocellular carcinoma (Liver-HCC); 19, stomach adenocarcinoma (Stomach-AdenoCA);
422 20, pancreatic adenocarcinoma (Panc-AdenoCA), 21, pancreatic neuroendocrine tumor
423 (Panc-Endocrine); 22, colorectal adenocarcinoma (ColoRect-AdenoCA); 23, prostatic
424 adenocarcinoma (Prost-AdenoCA); 24, chromophobe renal cell carcinoma (Kidney-ChRCC);
425 25, renal cell carcinoma (Kidney-RCC); 26, papillary renal cell carcinoma (Kidney-pRCC); 27,
426 uterine adenocarcinoma (Uterus-AdenoCA); 28, ovarian adenocarcinoma (Ovary-AdenoCA);
427 29, transitional cell carcinoma of the bladder (Bladder-TCC). b) Distribution of rREs across
428 cancer types. c) Proportion of cancer genomes with rREs. d) STR mutation rate for cancer
429 genomes with and without a rRE. Two-tailed Wilcoxon rank sum test. e) Distribution of rREs
430 across microsatellite stable (MSS) and microsatellite instability high (MSI-high) cancers. Chi-
431 square test with Yates' correction.



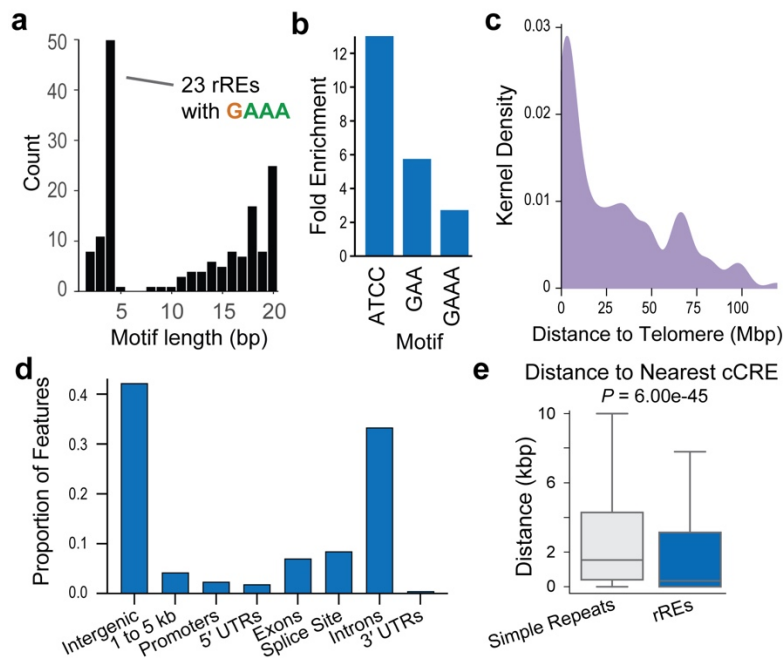
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436 **Figure 2. Features of rREs.** a) Distribution of the repeat unit (motif) for rREs. b) Motifs
437 enriched in the catalog of rREs. c) Distance of rREs to the end of the chromosome arm. d)
438 Proportion of genic features that overlap with rREs. UTR, untranslated region. e) Distance of
439 simple repeats and rREs to the nearest ENCODE candidate cis-regulatory element (cCRE).
440 Center values represent the median. Welch's *t*-test.



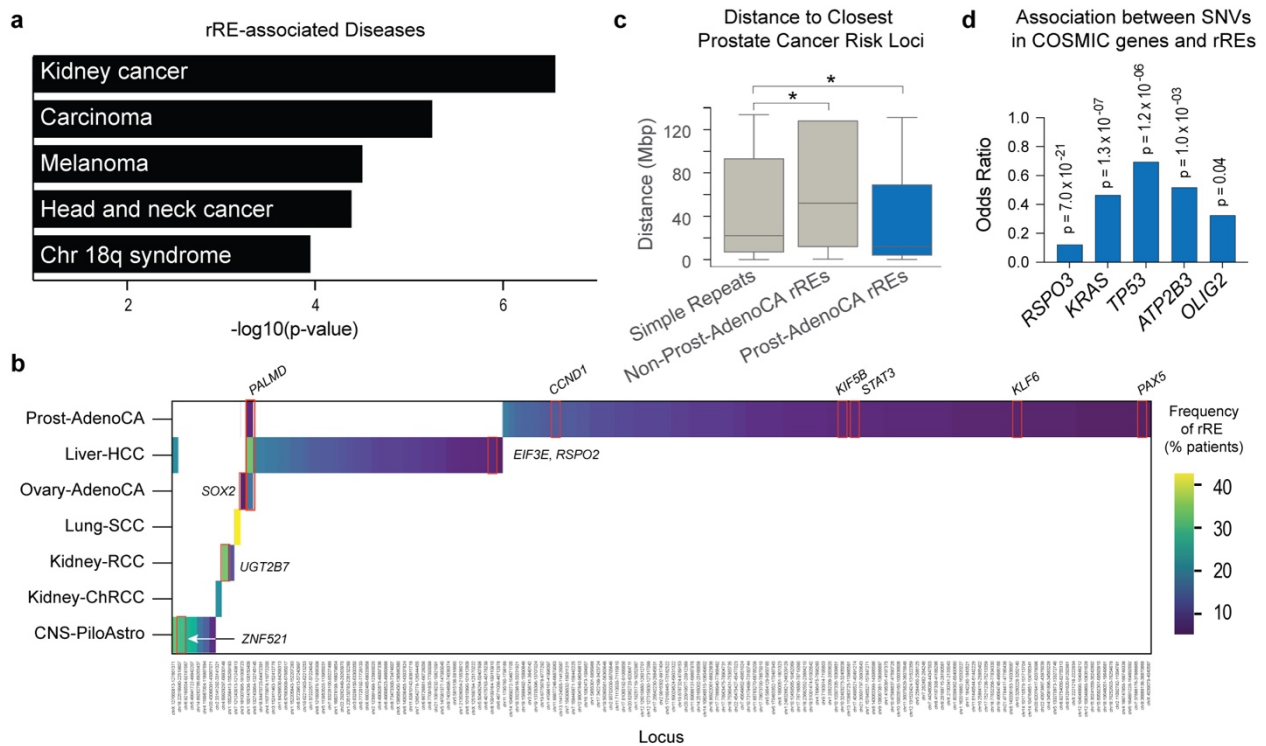
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445 **Figure 3. Association of rREs with cancer features.** a) Association of rREs with human
 446 diseases. b) Frequency of rREs in genes of interest, including the nine COSMIC genes, are
 447 highlighted. c) Distance of simple repeats, non-prostate cancer rREs, and prostate-cancer rREs to
 448 the nearest prostate cancer risk locus. Center values represent the median. Statistical significance
 449 was measured with Welch's *t*-test (* $q < 0.10$). d) Association between SNVs in genes in the
 450 census of somatic mutations in cancer (COSMIC) Tier 1 genes and the presence of rREs.
 451 Student's *t*-test with FDR correction by Benjamini-Hochberg.

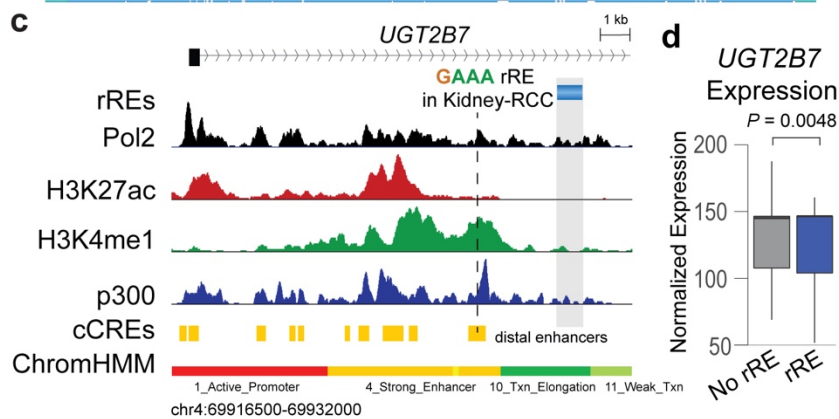
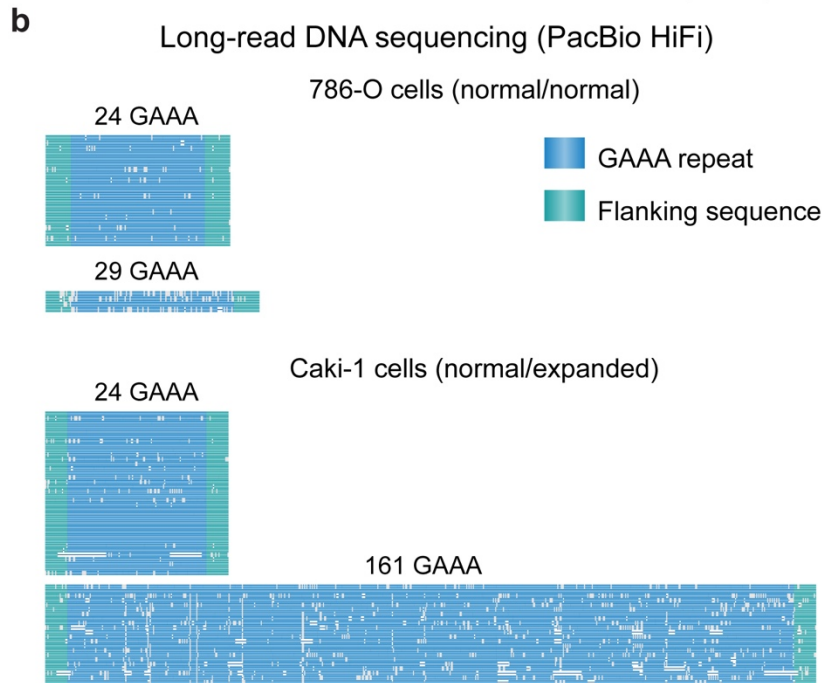
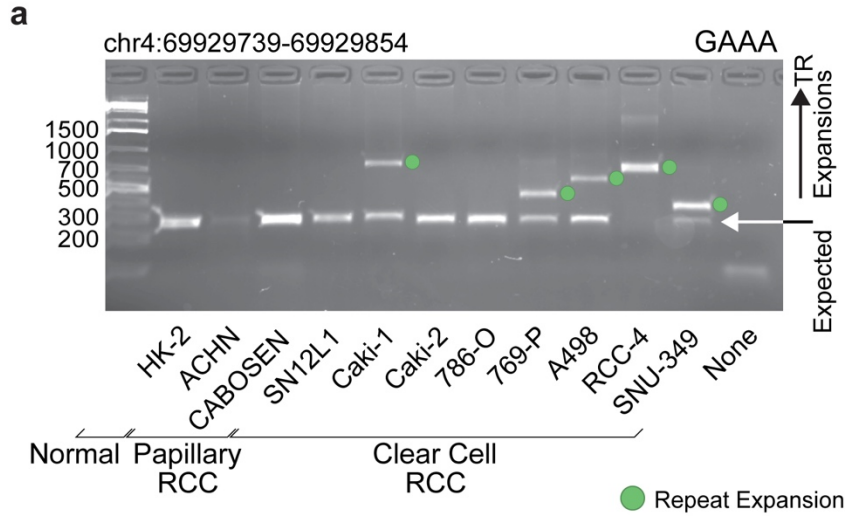


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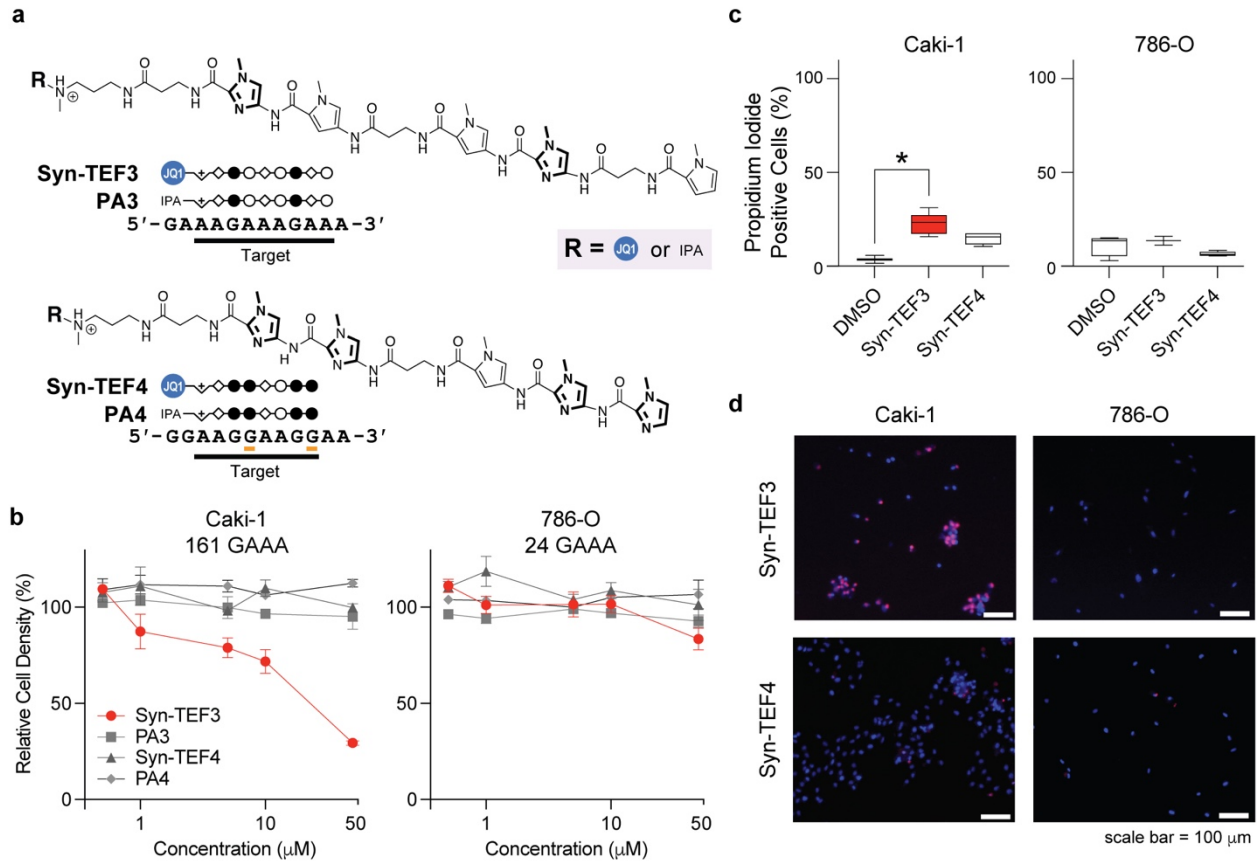
455 **Figure 4. An rRE in Renal Cell Carcinoma (RCC).** a) Gel electrophoresis of the GAAA
456 tandem repeat in RCC samples. This analysis was performed in duplicate and the gel is
457 representative of the results. For gel source data, see **Fig. S1**. b) Visualization of long-read
458 sequencing of GAAA rRE in the intron of *UGT2B7*. Data are from PacBio HiFi sequencing. c)
459 The locus surrounding the rRE detected in the intron of *UGT2B7*. Signal traces of Pol2,
460 H3K27ac, H3K4me1, and p300 in HepG2 cells are shown. Candidate cis-regulatory elements
461 (cCREs) and chromatin states (ChromHMM) are also depicted. d) Expression of *UGT2B7*
462 isoform ENST00000508661.1 in RCC samples as a function of the detection of the rRE in
463 *UGT2B7* (Normalized Expression, Counts). Center values represent the median. Significance
464 was measured by Wald test with FDR correction (Benjamini-Hochberg).



465

466

467 **Figure 5. The design and characterization of GAAA-targeting molecules in RCC. a)**
468 Chemical structures of Syn-TEF3, PA3, Syn-TEF4 and PA4. Syn-TEF3, and PA3 target 5'-
469 AAGAAAGAA-3'. Syn-TEF4* and PA4 target 5'-AAGGAAGG-3'. The structures of *N*-
470 methylpyrrole (open circles), *N*-methylimidazole (filled circles), and β -alanine (diamonds) are
471 shown. *N*-methylimidazole is bolded for clarity. The structure of JQ1 linked to polyethylene
472 glycol (PEG₆) is represented as a blue circle. The structure of isophthalic acid and linker is
473 represented as IPA. Complete chemical structures are depicted in **Fig. S2**. The asterisk indicates
474 the site where the R group attaches to the polyamide. Mismatches formed with Syn-TEF4 and
475 PA4 are indicated with orange. b) Relative cell density of RCC cell lines Caki-1 and 786-O
476 following treatment (72 h) with compounds, as indicated. Relative cell density was measured
477 with CCK-8 assay (see **Methods**). Results are mean \pm SEM ($n = 4$). c) Quantitation of the
478 percentage of propidium iodide-positive cells. Whiskers are minimum and maximum values. * p
479 < 0.05 . P values are from a one-way ANOVA with multiple comparisons. d) Live cell
480 microscopy of Caki-1 and 786-O cells stained with propidium iodide (red) and Hoechst 33342
481 (blue). Scale bars, 100 μ m. See also **Extended Data Figure 10**.



482

483

484 **Methods**

485 **Data curation**

486 We obtained white-listed data from the International Cancer Genome Consortium (ICGC) and
487 The Cancer Genome Atlas (TCGA) pan-cancer analysis of whole genomes (PCAWG) dataset.
488 Data were accessed through the Cancer Genome Collaboratory. We used the aligned reads
489 (BAM files), which were aligned to GRCh37 as described previously²⁵. These data are available
490 through the PCAWG data portal (<https://docs.icgc.org/pcawg>). A list of samples included in the
491 analysis is available in **Table S2**.

492

493 **Identification of somatic recurrent repeat expansions**

494 We analyzed tumor and matching normal samples for each cancer type independently.
495 We executed ExpansionHunter Denovo (EHdn) (v0.9.0)¹⁵ with the following parameters: --min-
496 anchor-mapq 50 --max-irr-mapq 40. To prioritize loci, we developed a workflow termed Tandem
497 Repeat Locus Prioritization in Cancer (TROPIC). We included loci from chr1-22, X, and Y for
498 downstream analysis. We removed loci where >10% of Anchored in-repeat read (IRR) values
499 were >40, which is the theoretical maximum value. The p-value (a non-parametric one-sided
500 Wilcoxon rank sum test) for each locus was used to calculate a false discovery rate (FDR) q-
501 value. Loci with FDR < 0.10 are reported. We selected loci where >5% of samples had an
502 Anchored in-repeat read (IRR) Quotient > 2.5. For a repeat expansion to be detected by EHdn,
503 the tandem repeat must be larger than the sequencing read length. A somatic repeat expansion
504 was defined as having an FDR q-value < 0.05 between tumor and normal samples. To call repeat
505 expansions in individual cancer samples, we analyzed the distribution of tumor and normal

506 Anchored IRR values and selected a conservative threshold for the Anchored IRR Quotient
507 $((\text{Tumor Anchored IRR} - \text{Normal Anchored IRR}) / (\text{Normal Anchored IRR} + 1)) > 2.5$ (**Extended**
508 **Data Figure 4**).

509

510 **Local read depth normalization**

511 EHdn normalizes the number of Anchored IRRs for a given locus to the global read
512 depth. To account for chromosomal amplifications and other forms of genetic variation that
513 could alter local read depth, we performed the following normalization. For each rRE locus and
514 sample in its corresponding cancer, samtools v1.13 was used with the parameter depth -r to find
515 the read depth at each base pair within the locus and a 500 bp region surrounding the start and
516 stop positions of the TR. We calculated the average read depth at each base pair and defined this
517 as the local read depth. Finally, we calculated the local read depth-normalized Anchored IRR
518 value specific to a sample and rRE combination by dividing the unnormalized Anchored IRR
519 value from EHdn by the local read depth at the locus.

520

521 **Generation of CABOSEN cells**

522 CABOSEN cells were generated from a cabozantinib-sensitive (CABOSEN) human papillary
523 RCC xenograft tumor grown in RAG2^{-/-} gammaC^{-/-} mice, as described previously⁵⁰. Tumor tissue
524 was minced with a sterile blade and the cell suspension cultured in DMEM/F-12 medium
525 (Corning) supplemented with 10%(v/v) Cosmic Calf Serum (ThermoFisher). Cells were
526 expanded and cryopreserved in growth medium supplemented with 10%(v/v) DMSO and cells
527 from passage 8 were used for analysis.

528

529 **Analysis of rREs by gel electrophoresis**

530 We performed PCR with CloneAmp HiFi PCR Mix (Takara Biosciences, Mountain
531 View, CA) and added DMSO to a final concentration of 5-10% (v/v) as needed. All cell lines
532 were tested negative for mycoplasma contamination with the MycoAlert Mycoplasma Detection
533 Kit (Lonza). Cell line identities were authenticated by STR profiling by the Genetic Resources
534 Core Facility at Johns Hopkins University, with the exception of SNU-349, which did not match
535 the reported STR profile of SNU-349 or any other catalogued cell line, but has a mutated *VHL*
536 gene and expresses high levels of *PAX8* and *CA9*, consistent with ccRCC origin. A list of
537 primers used to analyze the loci is available in **Table S6**.

538

539 **Visualization of repeat expansions with ExpansionHunter and REViewer**

540 To inspect the reads supporting a repeat expansion, we annotated the repeat as described
541 on the GitHub page for ExpansionHunter. We then profiled the region with ExpansionHunter
542 (v4.0.2) using the default settings¹⁴. The resulting reads were visualized with REViewer (v0.1.1)
543 using the default settings. REViewer is available at <https://github.com/Illumina/REViewer>. A
544 repeat expansion was called when the repeat tract length for one allele of the tumor sample was
545 greater than 100 bp and exceeded the repeat tract length of either normal allele. A locus was
546 considered validated if at least 10 cancer genomes had a repeat expansion.

547

548 **Validation of rREs in independent cohorts of samples**

549 Twelve pairs of matching normal and tumor samples from patients with clear cell renal
550 cell carcinoma were obtained with the patients' informed consent *ex vivo* upon surgical tumor
551 resection (Stanford IRB-approved protocols #26213 and #12597) and analyzed. Eighteen and 15
552 pairs of matching normal and tumor samples for prostate and breast cancer, respectively, were
553 obtained from the Tissue Procurement Shared Resource facility at the Stanford Cancer Institute
554 and analyzed. Nucleic acid was isolated with either the Quick Microprep Plus kit (Catalog
555 D7005) or the Zymo Quick Miniprep Plus kit (Catalog D7003) (Zymo Research, Irvine, CA).
556 Gel electrophoresis was performed as described above. A locus was considered detected if a
557 somatic repeat expansion was identified in at least one patient tumor sample compared to a
558 matching normal sample.

559

560 **Downsampling Analysis**

561 For the downsampling analysis, tumor genomes from renal cell carcinoma samples were
562 downsampled from their mean (52x) sequencing depth to 40, 30, 20, and 10x with the samtools
563 view command. EHDn was run, as described above for each of the sequencing depths, and the
564 Bonferroni-corrected p-value was plotted for the recurrent repeat expansion in *UGT2B7* (GAAA,
565 chr4:69929297-69930148).

566

567 **Benchmarking the Local Read-Depth Normalization (LRDN) filter**

568 We benchmarked the local read depth filter *in silico* by observing its behavior with
569 simulated reads. First, we created a reference genome containing artificially expanded repeats.
570 We randomly selected 10 TRs located in chromosome 1 that were less than the sequencing read

571 length of 100 bp. We artificially expanded these TRs in chromosome 1 of GRCh37 with the
572 BioPython python package (version 1.79). Next, we used wgsim (version 0.3.1-r13) to simulate
573 reads from the reference file with the command “wgsim -N 291269925 -1 100 -2 100
574 reference_file.fasta output.read1.fastq output.read2.fastq”. The number of reads (specified by the
575 -N option) was calculated to achieve 30x coverage of chromosome 1. The resulting pair of files,
576 hereinafter referred to as the base fastq files, contained a copy number of 2 for all of the
577 expansions.

578 To simulate copy number amplification, the read simulation process was repeated using
579 reference files that contained only the artificially expanded repeats and their surrounding 1,000
580 bp flanks. We created 10 pairs of fastq files, each with an increasing copy number. We specified
581 the copy number by multiplying the number of reads to generate (wgsim -N option) by the
582 required number. To generate the final set of fastq files, we concatenated each pair of copy
583 number-amplified fastq files with the base fastq files. The end result is 8 pairs of fastq files that
584 contain reads of chromosome 1 and a copy number amplification varying from 2 to 10 of the
585 expanded repeats.

586 The base fastq file with a copy number of 2, in addition to the eight copy number-
587 amplified fastq files, were aligned to chromosome 1 of GRCh37 with bwa-mem (v 0.6) with the
588 default options. The resulting SAM files were converted to BAM format with samtools (v 1.15)
589 with the default options. Finally, we ran the EHDn profile command (v 0.9.0) with the minimum
590 anchor mapping quality set to 50 and maximum IRR mapping quality set to 40. Finally, the
591 Anchored IRR values were extracted by overlapping the STR coordinates with the *de novo*
592 repeat expansion calls.

593

594 **Short-read and long-read DNA sequencing**

595 We sequenced Caki-1 and 786-O with both short-read sequencing (60x sequencing
596 coverage, 150 bp paired-end sequencing on a NovaSeq 6000 instrument) and long-read DNA
597 sequencing (50x sequencing coverage, PacBio HiFi sequencing on a Sequel IIe instrument). We
598 aligned the long reads to GRCh37 with pbmm2 v1.7.0, using the parameters --sort --min-
599 concordance-perc 70.0 --min-length 50. We aligned the short reads to GRCh37 with Sentieon
600 (v202112.01) with parameters -K 10000000 -M, an implementation of BWA-MEM, and
601 analyzed the samples with EHdn, as described above. We included loci containing at least one
602 sample with an Anchored IRR value >0 for further analysis. Anchored IRR values >0 arise when
603 the repeat length exceeds the sequencing read length. To benchmark EHdn against long-read
604 sequencing data, we manually determined the TR length of a given locus in the long-read
605 sequencing data. If the TR length in the long-read sequencing data exceeded the short-read
606 sequencing read length of 150 bp, we considered that locus confirmed.

607 The PacBio HiFi data were aligned to GRCh37 with pbmm2 v1.7.0 and visualized at the
608 *UGT2B7* locus with Tandem Repeat Genotyper v0.2.0
609 (<https://github.com/PacificBiosciences/trgt>).

610

611 **Analysis of rRE loci**

612 To determine if rREs were associated with any human diseases, rREs were mapped to
613 genes with GREAT (v4.0.4, default settings)⁵¹. The resulting genes were analyzed with Enrichr
614 using Jensen Diseases⁵². To determine whether repeat expansions were associated with
615 microsatellite instability-high (MSI-High) cancers, we obtained data from Hause et al⁵. The

616 percentage of MSI-high cancers was obtained from colon adenocarcinoma (COAD), stomach
617 adenocarcinoma (STAD), kidney renal cell carcinoma (KIRC), ovarian serous
618 cystadenocarcinoma (OV), prostate adenocarcinoma (PRAD), head and neck squamous cell
619 carcinoma (HNSC), liver hepatocellular carcinoma (LIHC), bladder urothelial carcinoma
620 (BLCA), glioblastoma multiforme (GBM), skin cutaneous melanoma (SKCM), thyroid
621 carcinoma (THCA), and breast invasive carcinoma (BRCA) and compared to the number of
622 repeat expansions and the percentage of patients with at least one repeat expansion in the
623 corresponding cancer type from the PCAWG dataset. We also overlapped cancer genomes
624 containing rREs with the microsatellite mutation rate, which we term the STR mutation rate, and
625 MSI calls from Fujimoto et al²⁹. The association of rREs with STR mutation rate was assessed
626 with the two-tailed Wilcoxon rank sum test. The association of rREs with MSI calls was assessed
627 with Chi-square test with Yates' correction.

628 To determine whether rREs are associated with known mutational signatures, we
629 downloaded mutational signatures from the ICGC DCC
630 (https://dcc.icgc.org/releases/PCAWG/mutational_signatures/Signatures_in_Samples). We
631 performed a multiple linear regression for each single-base-substitution (SBS) and doublet-base-
632 substitution (DBS) signatures to identify predictors of the number of rREs present in a sample.
633 To choose the predictors, we performed best subset selection on DBS and SBS signatures and
634 included age as a possible confounding factor. We used the statsmodels v0.12.2 in Python and,
635 specifically, the ordinary least squares model found in the statsmodels.api.OLS module to
636 estimate the coefficients of the selected predictors in their corresponding multiple linear
637 regression model⁵³.

638 To determine whether repeat expansions were associated with a difference in cytotoxic
639 activity, we calculated cytotoxic activity as previously described for four cancers that had
640 matching RNA-seq and WGS⁴¹. For each locus, we compared cytolytic activity for patients with
641 a repeat expansion to patients without a detected repeat expansion using a Welch's *t*-test with
642 correction for multiple hypothesis testing (Benjamini-Hochberg FDR q -value < 0.05). rREs were
643 annotated with genic elements using annotatr (v1.18.1)³³.

644 To determine if rREs were associated with regulatory elements, we downloaded
645 candidate cis-regulatory elements (cCREs)³⁴ and mapped them to GRCh37 with LiftOver
646 (UCSC)⁵⁴. We determined the distance between rREs and cCREs with the bedtools closest
647 command (v2.27.1)⁵⁵, and compared this distance to the simple repeats catalog⁵⁶. To compare the
648 distance to ENCODE cCREs, a Welch's *t*-test was performed.

649 To determine if prostate cancer rREs were associated with prostate cancer susceptibility
650 loci³⁶, we calculated the distance to three sets of loci using the "bedtools closest" command. We
651 calculated the distance between (1) rREs present in prostate cancer samples and prostate cancer
652 susceptibility loci, (2) rREs not present in cancer samples and cancer susceptibility loci, and (3)
653 simple repeats and cancer susceptibility loci. To compare the distances between these three
654 associations, we performed a Welch's *t*-test with FDR correction (Benjamini-Hochberg).

655 To determine whether rREs were associated with replication timing, we downloaded
656 Repli-seq replication timing data from seven cell lines from the ENCODE website (NCI-H460,
657 T470, A549, Caki2, G401, LNCaP, and SKNMC)⁵⁷. We selected regions for which all cell lines
658 had concordant signals for analysis (early or late replication designations agreed for each cell
659 line at a given locus). We determined whether there was a difference in the distribution of rREs
660 across early- and late-replicating regions compared to the simple repeats catalog with

661 bootstrapping ($n=10,000$). We sampled 54 loci (the number of rREs that are present in a
662 concordant replication region) from rREs and simple repeats. A Welch's t -test was performed on
663 the bootstrapped samples to estimate a p -value. We applied FDR correction (Benjamini-
664 Hochberg) to the estimated p -values. To determine whether rRE status in *UGT2B7* was
665 associated with survival outcome in clear cell RCC patients (TCGA abbreviation: KIRC), we
666 used Welch's t -test quartile.

667 To identify motifs enriched and depleted in the rRE catalog, we followed the same
668 method used in the Motif-Scan python module (v1.3.0)⁵⁸. We compared our rRE catalog to
669 Simple Repeats (Tandem Repeat Finder, TRF) as a control. For each unique motif present, we
670 built a contingency table specifying the count of rREs and Simple Repeats with and without the
671 motif. Two one-tailed Fisher's exact tests were applied to the table to test for significance in both
672 directions, enrichment and depletion. The "stats" module in the Scipy python package (v1.7.0)
673 was used to conduct the significance test. Since multiple hypothesis tests were performed, we
674 applied FDR correction (Benjamini-Hochberg) for multiple hypothesis testing to the p -values,
675 with a cutoff (FDR) of 0.01.

676 For the comparison of SNVs in COSMIC genes to rREs, we first divided cancer genomes
677 into two categories: rRE cohort and non-rRE cohort. The rRE cohort contains all of the genomes
678 that have at least one rRE detected ($n = 615$) and the non-rRE cohort contains all of the genomes
679 that have no rREs detected ($n = 1897$). We then looked at the number of donors in the rRE cohort
680 that have at least one mutation on a given gene (COSMIC Tier 1 genes) i and the number of
681 donors in the non-rRE cohort that have at least one mutation on a given gene i with a
682 contingency table. We then calculated the p -value (Fisher's exact test) for the significance of
683 associating genes to either rRE or non-rRE cohort. This p -value calculation is repeated for all

684 COSMIC genes and then an FDR at 0.05 significance level (Benjamini-Hochberg) was
685 employed to correct for multiple hypothesis testing.

686

687 **Estimation of expansions in the general population**

688 To estimate the frequency of the rREs in the general population, ExpansionHunter
689 Denovo (version 0.9.0) was run on 1000 Genomes Project samples⁵⁹ (n = 2,504) (GRCh38) and
690 Medical Genome Reference Bank⁶⁰ samples (n = 4,010) (GRCh37 lifted over to GRCh38).

691 The genomic coordinates of the 160 rREs (GRCh37) were padded with 1,000 bp and
692 translated to GRCh38 coordinates with the UCSC LiftOver. Then, these rRE coordinates
693 (GRCh38) were overlapped with loci from the population samples containing the Anchored IRR
694 calls. rREs that overlapped with matching motifs in the population samples were selected for
695 further analysis. We next sought to identify expanded rREs in the population samples to quantify
696 their prevalence. To do so, we converted their global-normalized Anchored IRR values to be
697 comparable to ICGC values. This step was necessary because sequencing read lengths from the
698 PCAWG dataset are generally 100 bp while the read lengths from 1000Genomes and Medical
699 Genome Reference Bank are 150 bp. The conversion follows the formula (Anchored IRR, 100
700 bp) = $0.5 + 1.5 * (\text{Anchored IRR, 150 bp})^{15}$. A sample in the population samples was counted as
701 expanded if its Anchored IRR value was greater than the 99th percentile of Anchored IRR values
702 in the normal samples from the PCAWG dataset, a threshold that is comparable to the threshold
703 used to call expansions in tumor samples (**Extended Data Figure 4**). In future rRE catalogs, for
704 the rare instance where the estimated frequency of repeat expansions in the population samples is

705 higher than expected, these data could be used to further filter rREs to improve the detection of
706 cancer-specific repeat expansions.

707 To compare the length of TRs in normal samples with and without a matching rRE in a
708 tumor sample, donors in the Prost-AdenoCA and Kidney-RCC cohorts whose data are available
709 for download through the Cancer Collaboratory were included (n=253). We used
710 ExpansionHunter (v5.0.0) with the default options to genotype prostate and kidney cancer rREs
711 in the normal samples of the selected donors. When there were two alleles of an rRE in a sample,
712 both alleles were included and treated as distinct data points. For each rRE, we tested whether
713 the distribution of genotypes from donors who have an expansion in their tumor samples differed
714 from donors who did not have an expansion. Student's t-test was used to compute p-values, and
715 FDR-correction (Benjamini-Hochberg) to adjust for multiple hypothesis testing.

716

717 **Association of rREs with gene expression**

718 Matching RNA-seq and WGS data were available for Kidney-RCC, Ovary-AdenoCA,
719 Panc-AdenoCA, and Panc-Endocrine. RNA-seq data from these samples were obtained from
720 DCC (<https://dcc.icgc.org/>) and values were converted to transcripts per million (TPM).
721 Normalized gene expression (TPM) values were compared for samples with and without an rRE
722 (Welch's *t*-test, with FDR correction). For isoform analysis, normalized gene expression counts
723 were compared for samples with and without a repeat expansion using the DESeq2 (v1.32.0)
724 package in R v4.0.5. We used the DESeq function to calculate the log₂ fold change values for 3
725 isoforms of the *UGT2B7* gene (ENST00000305231.7, ENST00000508661.1,

726 ENST00000502942.1) and performed a Wald test with FDR correction using the Benjamini-
727 Hochberg procedure (threshold q -value < 0.01).

728

729 **Design, synthesis, and characterization of Syn-TEFs and PAs**

730 Synthetic transcription elongation factors (Syn-TEFs) and polyamides (PAs) were
731 designed to target a GAAA repeat (Syn-TEF3 and PA3) or a control GGAA repeat (Syn-TEF4
732 and PA4). Syn-TEF3, Syn-TEF4, PA3, and PA4 were synthesized and purified to a minimum of
733 95% compound purity by WuXi Apptec and used without further characterization. HPLC
734 conditions for chemical characterization: 1.0 mL/min, Solvent A: 0.1% (v/v) trifluoroacetic acid
735 (TFA) in H₂O, Solvent B: 0.075% (v/v) TFA in acetonitrile, Gemini, Column: C18 5 μ m 110A
736 150*4.6mm. Full results of characterization can be found in **Fig. S2**.

737

738 **Treatment of RCC cell lines with synthetic transcription elongation factors (Syn-TEFs)**

739 Caki-1, and 786-O, and Caki-2 cells were obtained from ATCC and grown in RPMI 1640 media
740 with L-glutamine (Gibco Catalog 11875093), supplemented with 10% (v/v) FBS. A498 and
741 ACHN cells were obtained from ATCC and grown in DMEM media with glucose, L-glutamine,
742 and sodium pyruvate (Corning Catalog 10-013-CV), supplemented with 10% (v/v) FBS. RCC-4
743 cells were obtained from Amato Giacca (Stanford University) and grown in DMEM media with
744 glucose, L-glutamine, and sodium pyruvate (Corning Catalog 10-013-CV), supplemented with
745 10% (v/v) FBS. Cell lines were confirmed by STR profiling (Genetic Resource Core Facility,
746 Johns Hopkins University) and tested negative for mycoplasma. Cells were seeded in 96-well
747 plates on day 0. On day 1, cells were treated with the indicated molecules. Molecules were

748 dissolved in DMSO (vehicle) and added to cells (0.1% (v/v) DMSO final concentration). On day
749 4 (72 h later), relative metabolic activity was measured as a proxy for relative cell density, with
750 the Cell Counting Kit (CCK-8; Dojindo Molecular Technologies) per the manufacturer's
751 instructions. Absorbance (450 nm) of cells treated with molecules was normalized to DMSO
752 (0.1%(v/v)) or no treatment. Absorbance was measured with an Infinite M1000 microplate
753 reader (Tecan, Mannedorf, Switzerland).

754 For microscopy, Caki-1 and 786-O cells were plated on glass-bottom 96-well plates
755 under standard culture conditions. One day after plating, media containing either no drug,
756 0.1%(v/v) DMSO, 50 μ M Syn-TEF3, or 50 μ M Syn-TEF4 was added, and the cells were
757 incubated for 72 hours at 37°C. As a control, wells that received no treatment were incubated
758 with 70%(v/v) ethanol for 30 seconds prior to staining. Cells were then stained with propidium
759 iodide and Hoechst 33342 from the Live-Dead Cell Viability Assay Kit (Millipore Sigma,
760 Catalog CBA415) according to manufacturers' instructions and immediately imaged at 10x
761 magnification with a 0.17 numerical aperture CFI60 objective on a Keyence BZ-X710
762 microscope. Four replicates were measured for each treatment condition, and the experiment was
763 repeated three times. Quantitation was conducted using FIJI software. For statistical analyses, a
764 one-way ANOVA with multiple comparisons was conducted with GraphPad Prism.

765

766 **Methods References**

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791 phenotype data of 2570 healthy elderly. *Nat. Commun.* **11**, 435 (2020).

792

793 **Data availability**

794 Whole-genome sequencing data (both short- and long-read DNA sequencing) from 786-

795 O and Caki-1 cell lines are deposited in NCBI with accession PRJNA868795.

796

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805

806 **Author Contributions**

807 G.S.E. conceived the study. G.S.E., G.G., A.C.F., J.T.L., M.A.E., M.P.S., and M.G. supervised
808 research. G.S.E., G.G., R.A., A.S, E.D., J.P., C.M.B., K.Z., R.K.C.Y., and A.A.E. analyzed data.
809 G.S.E., C.R.H., L.R., A.A., A.A., K.V.K., R.A.K., D.A.S., S.M.W., and T.J.M. conducted wet
810 lab experiments. G.S.E. and M.P.S. wrote the manuscript with input from all the authors.

811

812 **Competing Interests declaration**

813 G.S.E. and M.P.S. are inventors on a patent application describing anti-proliferative agents. E.D.
814 and M.E. are shareholders and currently or formerly employed by Illumina and Pacific
815 Biosciences.

816

817 **Additional Information**

818 **Supplementary Information** is available for this paper.

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