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## 15 **Topography of mutational signatures in human cancer**

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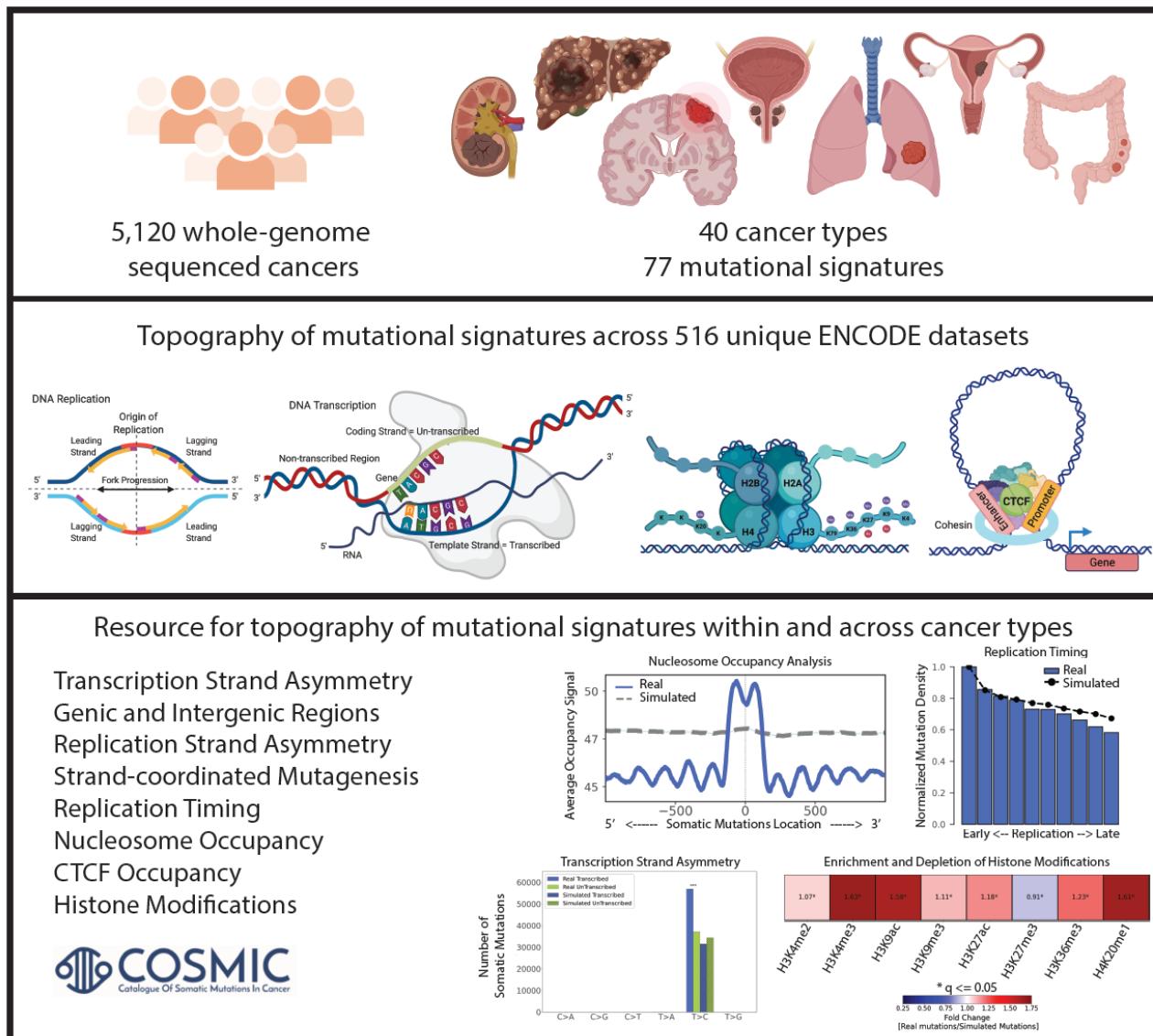
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28 **SUMMARY**

29 The somatic mutations found in a cancer genome are imprinted by different mutational  
30 processes. Each process exhibits a characteristic mutational signature, which can be affected by  
31 the genome architecture. However, the interplay between mutational signatures and  
32 topographical genomic features has not been extensively explored. Here, we integrate mutations  
33 from 5,120 whole-genome sequenced tumours from 40 cancer types with 516 topographical  
34 features from ENCODE to evaluate the effect of nucleosome occupancy, histone modifications,  
35 CTCF binding, replication timing, and transcription/replication strand asymmetries on the  
36 cancer-specific accumulation of mutations from distinct mutagenic processes. Most mutational  
37 signatures are affected by topographical features with signatures of related aetiologies being  
38 similarly affected. Certain signatures exhibit periodic behaviours or cancer-type specific  
39 enrichments/depletions near topographical features, revealing further information about the  
40 processes that imprinted them. Our findings, disseminated via COSMIC, provide a  
41 comprehensive online resource for exploring the interactions between mutational signatures and  
42 topographical features across human cancer.

## 43 GRAPHICAL ABSTRACT



44

45

46 **HIGHLIGHTS**

47 • Comprehensive topography analysis of mutational signatures encompassing 82,890,857  
48 somatic mutations in 5,120 whole-genome sequenced tumours integrated with 516 tissue-  
49 matched topographical features from the ENCODE project.

50

51 • The accumulation of somatic mutations from most mutational signatures is affected by  
52 nucleosome occupancy, histone modifications, CTCF binding sites, transcribed regions, and  
53 replication strand/timing.

54

55 • Mutational signatures with related aetiologies are consistently characterized by similar  
56 genome topographies across tissue types.

57

58 • Topography analysis allows both separating signatures from different aetiologies and  
59 understanding the genomic specificity of clustered somatic mutations.

60

61 • A comprehensive online resource, disseminate through the COSMIC signatures database,  
62 that allows researchers to explore the interactions between somatic mutational processes and  
63 genome architecture within and across cancer types.

64

65 **Keywords:** somatic mutations; topography; mutational processes; mutational patterns;  
66 mutational signatures; human cancer

67

## 68 INTRODUCTION

69 Cancer genomes are peppered with somatic mutations imprinted by the activities of different  
70 endogenous and exogenous mutational processes (Martincorena and Campbell, 2015; Stratton et  
71 al., 2009). Due to their intrinsic biophysical and biochemical properties, each mutational process  
72 engraves a characteristic pattern of somatic mutations, known as a mutational signature  
73 (Alexandrov et al., 2013). Our previous analyses encompassing more than 5,000 whole-genome  
74 and 20,000 whole-exome sequenced human cancers have revealed the existence of at least 78  
75 single base substitution (SBS), 11 doublet-base substitution (DBS), and 18 indel (ID) mutational  
76 signatures (Alexandrov et al., 2020; Islam et al., 2022; Moody et al., 2021; Zhang et al., 2021).  
77 Through statistical associations and further experimental characterizations, aetiology has been  
78 proposed for approximately half of the identified signatures (Alexandrov et al., 2016;  
79 Alexandrov et al., 2020; Huang et al., 2017; Jager et al., 2019; Kucab et al., 2019; Meier et al.,  
80 2018; Petljak et al., 2019; Phillips, 2018; Zhivagui et al., 2019). Prior studies have also explored  
81 the interactions between somatic mutations imprinted by different mutational processes and the  
82 topographical features of the human genome for certain cancer types and a small subset of  
83 topographical features. However, previously, there has been no comprehensive evaluation that  
84 examined the effect of genome architecture and topographical features on the accumulation of  
85 somatic mutations from different mutational signatures across human cancer.

86

87 Early studies have shown that late replicating regions and condensed chromatin regions  
88 accumulate more mutations when compared to early replicating regions, actively transcribed  
89 regions, and open chromatin regions (Lawrence et al., 2013; Polak et al., 2015; Schuster-Bockler  
90 and Lehner, 2012; Stamatoyannopoulos et al., 2009). Subsequent analyses of hundreds of cancer

91 genomes have revealed that differential DNA repair can explain variations in mutation rates  
92 across some cancer genomes (Supek and Lehner, 2015) as well as that chromatin features  
93 originating from the cell of origin, which gave rise to the tumour, can affect mutation rate and  
94 the distribution of somatic mutations (Polak et al., 2015). Recently, Morganella *et al.* examined  
95 the effect of the genomic and the epigenomic architecture on the activity of 12 SBS signatures in  
96 breast cancer (Morganella et al., 2016). These analyses demonstrated that mutations generated by  
97 different mutational processes exhibit distinct strand asymmetries and that mutational signatures  
98 are differently affected by replication timing and nucleosome occupancy (Morganella et al.,  
99 2016). Pan-cancer exploration of strand asymmetries was also conducted for different mutation  
100 types across multiple cancer types (Haradhvala et al., 2016) as well as for different mutational  
101 signatures (Tomkova et al., 2018). In particular, pan-cancer analyses of more than 3,000 cancers  
102 have revealed the strand asymmetries and replication timings of the 30 SBS mutational  
103 signatures from COSMICv2 signatures database (Tomkova et al., 2018). Similarly, more than  
104 3,000 cancer genomes were used to elucidate the mutation periodicity of the 30 SBS COSMIC  
105 signatures database v2 signatures in regard to nucleosome occupancy (Pich et al., 2018). More  
106 recently, a study has also shown the interplay between the three-dimensional genome  
107 organization and the activity of different mutational signatures (Akdemir et al., 2020; Vohringer  
108 et al., 2021).

109

110 Here, we report the most comprehensive evaluation of the effect of nucleosome occupancy,  
111 histone modifications, CCCTC-binding factor (CTCF) binding sites, replication timing,  
112 transcription strand asymmetry, and replication strand asymmetry on the cancer-specific  
113 accumulation of somatic mutations from distinct mutational signatures. Our analysis leverages

114 the complete set of known COSMICv2 mutational signatures (78 SBS, 11 DBS, and 18 ID) and  
115 it examines 5,120 whole-genome sequenced cancers while simultaneously utilizing 516 unique  
116 tissue-matched topographical features from the ENCODE project (**Table S1**) (Consortium et al.,  
117 2007). In all analyses, the observed patterns of somatic mutations are compared to background  
118 simulation models of mutational signatures that mimic both the trinucleotide pattern of these  
119 signatures as well as their mutational burden within each chromosome in each examined sample  
120 (**Methods**). Our results confirm many of the observations previously reported for strand  
121 asymmetry, replication timing, and nucleosome periodicity for the original 30 COSMICv2 SBS  
122 signatures. Further, the richer and larger dataset allowed us to elucidate novel biological findings  
123 for some of these 30 SBS signatures revealing previously unobserved pan-cancer and cancer-  
124 specific dependencies. Additionally, this report provides the first-ever map of the genome  
125 topography of indel, doublet-base, and another 24 substitution signatures in human cancer.  
126 Moreover, our study examines, for the first time, the tissue-specific effect of CTCF binding and  
127 11 different histone modifications on the accumulation of somatic mutations from different  
128 mutational signatures. As part of the results, we provide a global view of the topography of  
129 mutational signatures across 5,120 whole-genome sequenced tumours from 40 types of human  
130 cancer. As part of the discussion, we zoom into two distinct case studies: *(i)* the topography of  
131 different types of clustered somatic mutations; and *(ii)* using the topography of mutational  
132 signatures to separate mutational signatures with similar patterns. Lastly, the reported results are  
133 released as part of the COSMIC signatures database, <https://cancer.sanger.ac.uk/signatures>,  
134 providing an unprecedented online resource for examining the topography of mutational  
135 signatures within and across human cancer types.  
136

137 **RESULTS**

138 **Transcription Strand Asymmetries of Mutational Signatures**

139 Transcription strand asymmetries have been generally attributed to transcription-coupled  
140 nucleotide excision repair (TC-NER) since bulky adducts (*e.g.*, ones due to tobacco carcinogens)  
141 in actively transcribed regions of the genome will be preferentially repaired by TC-NER (Sancar,  
142 2016). Additionally, transcription-coupled damage may also lead to transcription strand  
143 asymmetry due to one of the strands being preferentially damaged during transcription  
144 (Haradhvala et al., 2016).

145

146 Mutational signatures with similar aetiologies generally exhibited consistent patterns of  
147 transcription strand asymmetries. Specifically, signatures attributed to exogenous mutational  
148 processes, including ones due to environmental mutagens or chemotherapy, in most cases,  
149 showed transcription strand bias with mutations usually enriched on the transcribed strand  
150 (**Figure 1A&C**). Mutational signatures due to tobacco smoking had strong transcription strand  
151 bias for C>A and T>A mutations (SBS4) as well as CC>AA mutations (DBS2) on the  
152 transcribed strand. Similarly, signature SBS29 (tobacco chewing) exhibited enrichment on the  
153 transcribed strand for C>A. SBS22 (aristolochic acid) had strong transcription strand bias for  
154 T>A on the transcribed strand, while SBS24 (aflatoxin) showed C>A transcription asymmetry  
155 with enrichment on the transcribed strand. Signatures SBS31, SBS35, DBS5 (platinum drugs),  
156 SBS32 (prior treatment with azathioprine), and SBS25 (likely due to a chemotherapy agent) also  
157 showed transcription strand bias with strong enrichment of mutations on the transcribed strand.  
158 SBS16 (alcohol consumption) had extreme transcription strand bias with almost all mutations  
159 occurring on the transcribed strand (**Figure 1A**). In contrast, mutational signatures due to direct

160 damage from ultraviolet light (*viz.*, SBS7a/b/c/d and DBS1) were the only known exogenous  
161 mutational processes to exhibit transcription strand asymmetry with strong enrichment of  
162 mutations on the untranscribed strand, consistent with damage from ultraviolet light on cytosine  
163 (**Figure 1A&C**).

164  
165 Transcription strand asymmetry with consistent enrichment of mutations on the transcribed  
166 strand was also observed for clock-like signature SBS5 (mainly for T>C mutations at ApTpN),  
167 as well as for multiple mutational signatures with unknown aetiology, including: SBS12 (biliary,  
168 kidney, and liver cancers), SBS19 (liver cancer), and ID14 (oesophageal, colorectal, and stomach  
169 adenocarcinomas; **Figure 1A&C**). Strand bias with preferences for the untranscribed strand was  
170 observed for SBS33 (unknown aetiology) in oesophageal as well as head and neck cancers.  
171 Similarly, ID11 (oesophageal, liver, and head and neck cancers) had transcription strand  
172 asymmetry on the untranscribed strand. Lastly, other mutational signatures exhibited  
173 transcription strand asymmetry in only a small subset of cancer types (**Figure 1A&C**).  
174

## 175 **Distribution of Mutational Signatures in Genic and Intergenic Regions**

176 Except for signatures SBS16 and ID11, all other mutational signatures showed statistically  
177 significant enrichment of mutation in intergenic regions across most cancer types  
178 (**Supplementary Figure S1A-C**). Excluding SBS16 and ID11, this enrichment ranged from  
179 1.30-fold, for example, for signature SBS24, to more than 2-fold, for example, for signatures  
180 SBS17a/b. To quantify whether the observed depletion of mutations in genic regions can be  
181 attributed to transcription strand asymmetries, we nullified the asymmetry by assigning the  
182 number of mutations on both transcribed and untranscribed strands to their highest value.

183 Effectively, this removed the transcription strand asymmetries and inflated the number of  
184 mutations in genic regions without affecting the number of mutations in intergenic regions.  
185 Nevertheless, this inflation resulted in only a minor change from 1.37 average odds ratio of  
186 mutations in intergenic regions for real somatic mutations (0.30 standard deviation) to 1.31  
187 average odds ratio of mutations in intergenic regions after inflating the number of mutations in  
188 genic regions by removing strand bias (0.30 standard deviation; **Supplementary Figure S1D-E**).  
189 Overall, these results suggest that transcription strand asymmetry, usually attributed to the  
190 activity of TC-NER, do not account for the high enrichment of somatic mutations in intergenic  
191 regions.

192

193 SBS16 and ID11 showed statistically significant enrichment of mutation in genic regions in liver  
194 and oesophageal cancers, while ID11 was also enriched in genic regions in cancers of the liver.  
195 SBS16 has been previously associated with exposure to alcohol (Chang *et al.*, 2017; Letouze *et*  
196 *al.*, 2017; Li *et al.*, 2018) and attributed to the activity of transcription-coupled damage  
197 (Haradhvala *et al.*, 2016). Prior studies have also associated ID11 to alcohol consumption in  
198 oesophageal cancers (Moody *et al.*, 2021). Re-examining ID11 in the current cohort of whole-  
199 genome sequenced liver cancers, by comparing the mutations attributed to ID11 in 32 heavy  
200 drinkers to the mutations attributed to ID11 in 94 light drinkers, reveals a 2-fold enrichment in  
201 heavy drinkers (p-value:  $1.31 \times 10^{-3}$ ; Mann-Whitney U test). This and the prior associations in  
202 oesophageal cancers (Moody *et al.*, 2021) strongly suggest a similar exogenous mutational  
203 processes, related to alcohol consumption, accounting for the enrichment of mutation in genic  
204 regions for both signatures SBS16 and ID11.

205

206 **Replication Strand Asymmetries of Mutational Signatures**

207 Replication strand bias was consistently observed in most signatures attributed to aberrant or  
208 defective endogenous mutational processes with strand bias either on the leading or on the  
209 lagging strand (**Figure 1B&D**). Strong replication strand asymmetries with enrichment of  
210 mutations on the leading strand was observed for signatures SBS10a, SBS10b, and DBS3 which  
211 are exclusively found at extremely high levels in samples with exonuclease domain mutations in  
212 DNA polymerase epsilon (POLE). This strand bias is consistent with recent observations  
213 suggesting that POLE plays a major role in leading strand DNA synthesis (Daigaku et al., 2015;  
214 Pursell et al., 2007; Shinbrot et al., 2014). Interestingly, SBS28 (unknown aetiology) exhibited a  
215 strong replication strand bias when found at high levels in POLE deficient samples. Additionally,  
216 replication strand asymmetries were also observed for SBS9, attributed to infidelity of  
217 polymerase eta (POLH), and SBS10c, due to defective polymerase delta (POLD1).

218

219 Mutational signatures associated with defective DNA mismatch repair exhibited statistically  
220 significant replication strand bias either on the leading or the lagging strand (**Figure 1B**).  
221 Signatures SBS14, SBS20, SBS21, and SBS26 (all attributed to mismatch repair deficiencies)  
222 caused mutations preferentially on the lagging strand. Signatures SBS6, SBS15, and SBS44 (also  
223 attributed to mismatch repair deficiencies) exhibited C>A and T>C substitutions on the lagging  
224 strand as well as C>T preferentially found on the leading strand. ID1 and DBS2 also exhibited  
225 replication strand bias on the lagging and leading strands, respectively.

226

227 Somatic mutations due to signatures SBS2 and SBS13, both attributed to the aberrant behaviour  
228 of the APOBEC3 family of deaminases, were found enriched on the lagging strand in all cancer

229 types. This result is consistent with the observation that single-stranded DNA formed during  
230 DNA replication on the lagging strand is a major substrate for the APOBEC3 family of  
231 deaminases (Roberts *et al.*, 2012; Saini and Gordenin, 2020). Lastly, several other mutational  
232 signatures, most with unknown aetiology, exhibited replication strand bias within a small set of  
233 cancer types (**Figure 1B&D**).

234

### 235 **Mutational Signatures with Strand-coordinated Mutagenesis**

236 Prior analyses have shown that certain types of mutations on the same reference allele were  
237 observed on the same strand more frequently than expected by chance (Morganella *et al.*, 2016;  
238 Nik-Zainal *et al.*, 2012; Roberts *et al.*, 2012). This strand-coordinated clustered mutations  
239 usually arise due to damage on single-stranded DNA, and they are often indicative of the  
240 formation of hypermutable loci in the genome (Roberts *et al.*, 2012; Saini and Gordenin, 2020).

241

242 SBS7a (UV light) attained the highest strand-coordinated mutagenesis with lengths of  
243 subsequent mutations up to 40 consecutive mutations (**Figure 1E**). In contrast, other mutational  
244 signatures attributed to ultraviolet light, mainly, SBS7b/c/d, either did not exhibit or exhibited  
245 much lower strand-coordinated mutagenesis. APOBEC3-attributed SBS2 and SBS13 showed  
246 strand-coordinated mutagenesis with as many as 21 consecutive strand-coordinated mutations  
247 (**Figure 1E**). SBS17b (unknown aetiology) also exhibited processive groups with as many eight  
248 strand-coordinated mutations. In ultra-hypermutated samples with deficiency in the *POLE*  
249 proofreading domain, SBS10a and SBS10b also showed strand-coordinated mutagenesis with up  
250 to 14 consecutive strand-coordinated mutations. Similarly, consecutive strand-coordinated  
251 mutations were observed for SBS4 (associated with tobacco smoking; up to seven consecutive

252 mutations), SBS26 (defective mismatch repair; up to eight mutations), and SBS28 (unknown  
253 aetiology; up to 11 mutations; **Figure 1E**).

254

255

## 256 **The Effect of DNA Replication Timing on Somatic Mutagenesis**

257 Consistent with prior reports (Chen et al., 2010; Koren et al., 2012; Stamatoyannopoulos et al.,  
258 2009; Watanabe et al., 2002), the aggregated set of somatic mutations was shown to be enriched  
259 in late replicating regions for most cancer types (**Figure 2**). Specifically, from the examined 40  
260 cancer types, SBSs were found more common in regions of the genome that undergo late  
261 replication in 39/40 cancer types (indicated as ↘:39 in **Figure 2**). In one cancer type, SBSs were  
262 not associated with replication time (indicated as →:1) and there were no cancer types in which  
263 SBSs were enriched in early replication regions (indicated as ↗:0). Similarly, DBSs and IDs  
264 were enriched in late replicating regions in 18/18 and 30/32 cancer types, respectively. Note that  
265 due to their lower mutational burdens, we could confidently evaluate DBSs only in 18 of the 40  
266 cancer types and IDs only in 32 of the 40 cancer types. In contrast to the aggregated analysis,  
267 examining somatic mutations attributed to different mutational signatures revealed distinct  
268 replication time dependencies.

269

270 At least six mutational signatures were predominately enriched in early replicating regions:  
271 SBS6 attributed to mismatched repair (enriched in early replicating regions in 2 out of 3 cancer  
272 types; 2/3), SBS11 attributed to temozolomide therapy (1/1), SBS15 due to DNA mismatch  
273 repair deficiency (1/1), SBS16 (2/2) and ID11 (3/5) both associated with alcohol consumption,  
274 and SBS84 (1/1) due to aberrant activities of activation-induced (AID) cytidine deaminases.

275 ID17 signature, most probably due to *TOP2A* mutations (Boot et al., 2022), was also enriched in  
276 early replicating regions in oesophageal squamous cell carcinoma.

277

278 All mutational signatures that were previously associated with age of diagnosis in at least one  
279 cancer type had a predominately increased normalized mutational density from early to late  
280 replicating regions: SBS1 (23/36), SBS5 (36/37), SBS40 (31/31), ID1 (24/24), ID2 (21/21), and  
281 ID5 (19/20). SBS3 (14/14) and ID6 (12/12), both attributed to defective homologous  
282 recombination, as well as mutational signatures attributed to defective polymerase epsilon  
283 (SBS10a, SBS10b, SBS14, and DBS3) were also enriched in late replicating regions in all  
284 examined cancer types (**Figure 2**). Similarly, signatures attributed to reactive oxygen species  
285 (SBS18 and SBS36) were enriched in late replicating regions in all examined cancer types.

286 Additionally, most mutational signatures due to environmental and chemotherapeutic exposures  
287 were enriched in late replicating regions in all examined cancer types including signatures of  
288 tobacco smoking (SBS4, DBS2, ID3), tobacco chewing (SBS29), ultraviolet light (SBS7a/c/d,  
289 SBS38, DBS1, and ID13), aristolochic acid (SBS22), aflatoxin (SBS24), prior treatment (SBS31,  
290 SBS32, SBS35, and DBS5), and non-canonical AID activity (SBS85). Many of the mutational  
291 signatures with unknown aetiologies were also enriched in late replicating regions: SBS8,  
292 SBS12, SBS17a/b, SBS19, SBS28, SBS33, SBS34, SBS37, and SBS41. Two of the signatures  
293 attributed to the APOBEC3 family of deaminases, SBS2 (15/17) and DBS11 (2/3), had an  
294 increased normalized mutation density from early to late replicating regions.

295

296 Importantly, SBS13, attributed to the APOBEC3 family of deaminases, showed no dependence  
297 with replication timing in 7 of the 17 examined cancer types (*viz.*, bladder, breast, uterus, cervix,

298 ovary, and thyroid, acute lymphocytic leukaemia). This is consistent with prior reports where  
299 SBS13 was attributed to uracil excision of deaminated cytosine followed by processing by DNA  
300 translesion polymerases in breast cancer (Helleday et al., 2014; Petljak and Maciejowski, 2020).  
301 Surprisingly, in 10/17 cancer types, SBS13 was enriched in late replicating regions indicating  
302 that other mechanisms may also give rise to this mutational signature. Interestingly, signature  
303 SBS7b, attributed to ultraviolet light, showed no dependencies with replication timing in  
304 melanoma in contrast to all other signatures attributed to ultraviolet light (*viz.*, SBS7a/c/d,  
305 SBS38, DBS1, and ID13); this indicates that processing of UV-induced DNA damage by base  
306 excision repair and DNA translesion polymerases may give rise to signature SBS7b. SBS30, a  
307 signature of deficient base excision repair due to mutations in the bifunctional DNA glycosylase  
308 *NTHL1*, showed flat replication timing in 3 of the 3 examined cancer types. Mutations due to  
309 signatures SBS20 (2/4), SBS21 (2/3), and SBS44 (3/5), all attributed to failure of mismatch  
310 repair, as well as mutations due to signatures SBS39 (1/1), ID4 (5/8), and ID8 (7/18), all with  
311 unknown aetiologies, were also generally unaffected by replication timing (**Figure 2**).  
312

### 313 **The Effect of Nucleosome Occupancy on Mutational Signatures**

314 Nucleosomes are the basic packing units of chromatin with each nucleosome consisting of ~147  
315 base-pair (bp) DNA wrapped around a histone octamer with 60 to 80 bp linker DNA between  
316 consecutive nucleosomes (Davey et al., 2002; Richmond and Davey, 2003). Previous analyses  
317 have revealed dependencies between mutational signatures operative in breast cancer and  
318 nucleosome occupancy (Morganella et al., 2016) as well as a pan-cancer periodicity of mutation  
319 rates within nucleosomes due to multiple substitution signatures (Pich et al., 2018). However,

320 beyond breast cancer, there has been no cancer-specific examination of the effect of nucleosome  
321 occupancy on the accumulation of somatic mutations due to different mutational signatures.

322

323 All types of somatic mutations and most mutational signatures were depleted near nucleosomes  
324 compared to simulated data mimicking the mutational landscapes of the examined cancer  
325 genomes (**Figure 3**). Similar to simulated data, aggregated small insertions and deletions  
326 exhibited a consistent behaviour across most tumour types with aggregated indels predominantly  
327 located at linker DNA in 31 of the 32 examined cancer types (**Figure 3**). Conversely, aggregated  
328 single base and doublet-base substitutions had dissimilar behaviour across cancer types with only  
329 a subset of cancer types exhibiting similar behaviour (25/40 for substitutions; 13/18 for doublet-  
330 base substitutions; **Figure 3**). Remarkably, the majority of SBS, DBS, and ID mutational  
331 signatures were similarly affected by nucleosome occupancy across most cancer types. Further,  
332 the effect of nucleosome occupancy tended to be consistent for many signatures with a shared  
333 aetiology. Different types of periodicities of mutation rates around the nucleosome structure  
334 were observed for most signatures associated with tobacco smoking, ultraviolet light,  
335 aristolochic acid, reactive oxygen species, and defective mismatch repair (**Figure 3**).  
336

337 Mutational signatures attributed to tobacco smoking (SBS4, DBS2, and ID3) exhibited similar  
338 patterns around nucleosome positions across multiple cancer types (**Figure 3; Supplementary**  
339 **Figure S2**). ID3 was the only indel mutational signatures with expected and observed  
340 enrichment of mutations near nucleosomes (**Figure 3**). Similarly, the substitution mutational  
341 signatures of alkylating agents (SBS11), aflatoxin (SBS24), tobacco chewing (SBS29), platinum  
342 therapies (SBS31, SBS35), and azathioprine treatment (SBS32) were preferentially found in

343 nucleosome positions in all cancer types in which they were detected. Most mutational signatures  
344 due to direct or indirect damage from ultraviolet light (SBS7b/d, SBS38, and DBS1) had a higher  
345 number of mutations at nucleosome sites. In contrast, signatures SBS7c and ID13, also attributed  
346 to UV-light exposure, were depleted at nucleosomes and enriched at linker DNA sequences.

347

348 Mutations due to SBS9, associated with polymerase eta driven replication errors, and signatures  
349 SBS10a/b/c, SBS28 and DBS3, attributed to exonuclease mutations in *POLE* and/or *POLD1*,  
350 strikingly appeared at linker DNA. Some mutational signatures associated with microsatellite  
351 instability (*viz.*, SBS21, SBS26 and DBS7) were preferably located at linker DNA. In contrast,  
352 other microsatellite instability associated signatures, namely, SBS6, SBS14, SBS15, SBS20, and  
353 SBS44, as well as SBS30, due to deficiency in base excision repair, were found to match  
354 simulated data with expected high number of mutations at nucleosome occupied regions (**Figure**  
355 **3**).

356

357 Signatures SBS16 (alcohol) and SBS22 (aristolochic acid) were depleted at nucleosome  
358 positions and enriched at linker DNA sequences in all cancer types in which these signatures  
359 were detected. Similar behaviour was observed for multiple signatures with unknown  
360 aetiologies, including: SBS12, SBS34, SBS37, and SBS41. In contrast, consistent propensity for  
361 elevated mutation burden at nucleosome regions was shown by multiple other mutational  
362 signatures with unknown aetiology, including: SBS19, SBS39, DBS4, DBS6, and DBS9.

363

364 Only one of the clock-like signatures, SBS1, behaved consistently with higher number of  
365 mutations at nucleosomes in 36/36 cancer types. In contrast, signature SBS5 behaved similarly in

366 only 18/37 examined cancer types. The behaviour of SBS40 was also inconsistent across most  
367 cancer types (**Figure 3**). Signature SBS3, attributed to defective homologous recombination, was  
368 slightly elevated in 11/14 cancer types in which it was found. The inconsistent behaviour of these  
369 flat signatures (SBS3, SBS5, and SBS40) may reflect tissue-specific differences but it could also  
370 be due to technical issues as, in some cases, there is a high-level ambiguity in assigning flat  
371 signatures to individual samples (Alexandrov et al., 2020; Li et al., 2020; Maura et al., 2019).  
372 Interestingly, nucleosome occupancy had a similar effect on the APOBEC3 mutational  
373 signatures (SBS2, SBS13, and DBS11) in most cancer types with little effect on the  
374 accumulation of somatic mutations from these signatures (**Figure 3**).  
375

376 Signatures SBS18 and SBS36, attributed to mutations due to DNA damage induced by reactive  
377 oxygen species, exhibited higher number of mutations at nucleosome regions and strong  
378 periodicity of approximately ~192 bp (**Figure 3**). Interestingly, signatures SBS17a/b showed  
379 similar behaviour providing further circumstantial evidence for the hypothesis that SBS17a/b  
380 may also be due to ROS damage of the deoxyribonucleoside triphosphate (dNTP) pools  
381 (Christensen et al., 2019; Dvorak et al., 2007; Focaccetti et al., 2015; Hidaka et al., 2008; Inoue  
382 et al., 1998; Tomkova et al., 2018). Except for ID3, attributed to tobacco smoking, and ID17,  
383 associated with *TOP2A* mutations, all other signatures of small insertions and deletions were  
384 preferentially located at linker DNA sequences and depleted at nucleosome positions (**Figure 3**).  
385

386 Lastly, prior analyses have revealed pan-cancer periodicity of mutation rates within nucleosomes  
387 due to signatures SBS4, SBS7, SBS8, SBS9, SBS10, SBS14, SBS16, SBS17, and SBS18 (Pich  
388 et al., 2018). Here, we both confirm and elaborate on the cancer- and signature-specific

389 periodicity of these signatures. For example, SBS4 shows strong periodicity in cancers of the  
390 lung and head and neck but not in cancers of the liver or cancers of the oesophagus  
391 (**Supplementary Figure S2A**). Similarly, signatures SBS10a and SBS10b behaved differently  
392 with SBS10a exhibiting no periodicity and SBS10b exhibiting strong periodicity of mutation  
393 rates within nucleosomes across all tissue types (**Figure 3**). Analogously, the UV-light  
394 associated signatures SBS7a/b/c/d showed different level of nucleosome periodicity. In contrast,  
395 signatures SBS17a/b behaved consistently in almost all examined cancer types (**Figure 3**).  
396 Lastly, the current examination provides the first report for periodicities of mutation rates near  
397 nucleosomes for another three signatures: SBS22, SBS36, and SBS38 (**Figure 3**).  
398

### 399 **The Effect of CTCF Binding on Mutational Signatures**

400 CCCTC-binding factor (CTCF) is a multi-purpose sequence-specific DNA-binding protein with  
401 an essential role in transcriptional regulation, somatic recombination, and chromatin architecture  
402 (Ghirlando and Felsenfeld, 2016; Kentepozidou et al., 2020; Kim et al., 2015; Merkenschlager  
403 and Odom, 2013; Ong and Corces, 2014). The human genome harbours many CTCF binding  
404 sites with prior studies reporting that mutations due to ultraviolet light are enriched in CTCF  
405 binding sites (Poulos et al., 2016).  
406

407 Somatic mutations exhibited clear patterns of both enrichment and/or periodicity for multiple  
408 mutational signatures and CTCF binding sites (**Figure 4**). While some signatures were  
409 consistently depleted at CTCF binding sites across the majority of cancer types when compared to  
410 simulated data (SBS1, SBS9, SBS10a/b, SBS15, SBS37, SBS84, and SBS85), others were

411 commonly enriched (SBS3, SBS5, SBS7a/b/d, SBS12, SBS17a/b, SBS18, SBS22, and SBS40;  
412 DBS1; ID5, ID6, ID8, and ID9; **Figure 4A**).

413  
414 Aggregated single base substitutions exhibited an inconsistent behaviour across cancer types  
415 with enrichment in some cancers (e.g., liver cancers) and depletions in others (e.g., lymphomas).  
416 In contrast, indels were enriched at CTCF binding sites in the majority of cancer types (**Figure**  
417 **4A**). Remarkably, the effect of CTCF occupancy tended to be also consistent for many signatures  
418 with similar aetiologies. Strong periodicities of mutation rates around CTCF binding sites were  
419 observed for UV-associated signature SBS7a but not for UV-associated signatures DBS1 and  
420 SBS7b/c/d (**Figure 4B**).

421  
422 Mutations due to SBS9, associated with defective polymerase eta driven replication errors, and  
423 signatures SBS10a/b, found in samples with mutations in *POLE* and/or *POLD1*, were strikingly  
424 depleted at CTCF binding sites. Signatures SBS15, associated microsatellite instability, was  
425 strongly depleted at CTCF binding sites (**Figure 4A**).

426  
427 Only one of the clock-like signatures, SBS1, exhibited a depletion of mutations at CTCF binding  
428 sites (**Figure 4A**) while simulated data indicated that SBS1 should be enriched at these sites  
429 (**Figure 4B**). Signature SBS3, attributed to defective homologous recombination, was highly  
430 elevated in CTCF binding sites for breast, ovarian, stomach, and oesophageal cancers. Signatures  
431 SBS17a/b exhibited a striking enrichment at CTCF binding sites in all cancer types with  
432 sufficient number of mutations from each signature (**Figure 4A**). SBS17a showed enrichment in  
433 stomach and oesophageal cancers, while SBS17b shows enrichment for stomach, oesophageal,

434 breast, pancreatic cancers, and non-Hodgkin's lymphomas. In contrast, simulated data indicate  
435 that CTCF binding should have no effect on the accumulation of mutations from signatures  
436 SBS17a/b (**Figure 4B**).

437

#### 438 **The Effect of Histone Modifications on Mutational Signatures**

439 Each nucleosome consists of four pairs of core histones: H2A, H2B, H3, and H4. Post-  
440 translational modifications of histone tails play a key role in regulating DNA replication, gene  
441 transcription, and DNA damage response (Allis and Jenuwein, 2016; Kouzarides, 2007; Mendez-  
442 Acuna et al., 2010; Sun et al., 2020). For example, histone acetylation generally enables DNA  
443 accessible for transcription, replication, and repair (Bar-Ziv et al., 2016; Dhar et al., 2017; Gong  
444 and Miller, 2013; Hunt et al., 2013; Ruan et al., 2015; Sterner and Berger, 2000; Struhl, 1998;  
445 Unnikrishnan et al., 2010; Vogelauer et al., 2002), while histone methylation has diverse  
446 functions associated with both transcription activation and repression (Allis and Jenuwein, 2016;  
447 Hyun et al., 2017). To evaluate the effect of histone modifications on the accumulation of  
448 mutations from different mutational signatures, we mapped the depletion or enrichment of  
449 mutations compared to simulated data in the context of the tissue specific positions of 11 histone  
450 modifications: *(i)* H2AFZ, a replication-independent member of the histone H2A family that  
451 renders chromatin accessible at enhancers and promoters regulating transcriptional activation and  
452 repression (Giaimo et al., 2019; Lamaa et al., 2020; Subramanian et al., 2015; Zhang et al.,  
453 2017); *(ii)* H3K4me1, histone mark often associated with enhancer activity (Kang et al., 2021a;  
454 Kang et al., 2021b); *(iii)* H3K4me2, a histone post-translational modification enriched in *cis*-  
455 regulatory regions, including both enhancers and promoters (Bernstein et al., 2005; Koch et al.,  
456 2007; Wang et al., 2014); *(iv)* H3K4me3, post-translational modification enriched in active

457 promoters near transcription start sites (Calo and Wysocka, 2013); (v) H3K9ac, associated with  
458 active gene promoters and active transcription (Gates et al., 2017); (vi) H3K9me3, silencer,  
459 typical mark of constitutive heterochromatin (Cai et al., 2021; Saksouk et al., 2015); (vii)  
460 H3K27ac, histone modification generally contained at nucleosomes flanking enhancers (Calo  
461 and Wysocka, 2013; Heinz et al., 2015); (viii) H3K27me3, repressive, associated with silent  
462 genes (Cai et al., 2021; Nestorov et al., 2013; Saksouk et al., 2015); (ix) H3K36me3, associated  
463 with transcribed regions and playing a role in regulating DNA damage repair (Sun et al., 2020);  
464 (x) H3K79me2, detected in the transcribed regions of active genes (Wang et al., 2008); and (xi)  
465 H4K20me1, found in gene promoters and associated with gene transcriptional elongation and  
466 transcription activation (Li et al., 2011; Wang et al., 2008).

467  
468 Aggregated substitutions, dinucleotides, and indels exhibited dissimilar behaviour for different  
469 histone modifications across cancer types. Aggregated substitutions were predominately depleted  
470 around H2AFZ, H3K4me2, H3K4me3, and H3K27ac in approximately half of the examined  
471 cancer types with generally no effect observed in the other half of cancer types (**Figure 5A-C**).  
472 Aggregated doublets and indels did not have any clear pan-cancer preference but showed cancer-  
473 type specific enrichments and depletions. In contrast, many mutational signatures had generally  
474 similar behaviour in vicinity of different histone modifications.

475  
476 Clock-like signature SBS1 was consistently depleted across cancer-types for multiple histone  
477 marks. In contrast, clock-like signatures SBS5 and SBS40 were generally unaffected by histone  
478 marks except for cancers of the brain and lymphatic system. APOBEC3 signatures, SBS2 and  
479 SBS13, were both enriched at activator histone mark H3K27ac for majority of cancer types.

480 SBS2 and SBS13 mutations were also enriched at H2AFZ locations for breast cancer. DBS11, a  
481 doublet-base substitution signature attributed to APOBEC3, was enriched in the vicinity of  
482 multiple activator histone marks in non-Hodgkin's lymphoma samples, including: H3K4me1,  
483 H2K4me3, H3K9ac, H327ac, H3K36me3, and H4K20me1 (**Figure 5B**).  
484 Ultraviolet-light signatures SBS7a/b/c/d and SBS38 were consistently depleted at H3K4me3,  
485 H3K9ac, and H3K27me3 locations (**Figure 5A**). Interestingly, the doublet-signature attributed to  
486 ultraviolet-light, DBS1, was depleted only at H3K9ac and H3K27me3 locations, while the indel  
487 signature attributed to ultraviolet-light, ID13, was not depleted near any histone marks but it was  
488 enriched near H3K27ac modifications.

489

490 Signature SBS9, attributed to the activity of polymerase eta and found exclusively in B-cell  
491 malignancies, was highly enriched by the presence of multiple activator histone marks,  
492 including: H3K4me1, H3K4me2, H3K4me3, H3K9ac, H3K27ac, and H4K20me1 (**Figure 5A**).

493 In contrast, signatures SBS10a/b and DBS3, attributed to the failed activity of *POLE*, were all  
494 depleted near H3K4me3 and H3K27ac locations. Signature SBS10b was also depleted near  
495 H3K9ac, and H3K27me3, while signature SBS10a and DBS3 were also depleted near  
496 H3K4me1. SBS84 and SBS85, due to aberrant activities of activation-induced cytidine  
497 deaminases, were significantly enriched in the vicinity of most histone modifications.

498

499 Signatures SBS18 (reactive oxygen species) showed depletions across most cancer types at  
500 multiple histone marks, including: H2AFZ, H3K4me1, H3K4me2, H3K4me3, H3K9ac,  
501 H3K27ac, H3K27me3, and H3K79me2 (**Figure 5A**). Further, SBS18 was enriched near  
502 H3K9me3 and H4K20me1 in medulloblastomas. In contrast SBS36, attributed to reactive

503 oxygen species accumulation due to *MUTYH* deficiencies, was depleted only at H3K27ac  
504 locations. Interestingly, mutations due to signatures SBS17a/b (unknown aetiology) were  
505 significantly depleted at activator histone marks, H3K4me1, H3K4me3, H3K9ac and H3K27ac;  
506 and repressive histone marks H3K9me3 and H3K27me3 in multiple cancer types (**Figure 5A**).  
507 Tobacco smoking signatures, SBS4, DBS2, and ID3 exhibited consistent depletions near histone  
508 modifications H3K4me3 and H3K27ac in cancers of the lung, liver, oesophageal, and head and  
509 neck. Similarly, tobacco chewing signature, SBS29, showed depletions of mutations near  
510 H3K9ac and H3K27ac histone marks in liver cancer (**Figure 5A**). Moreover, signature SBS22  
511 (aristolochic acid) was depleted near H3K4me3 and H3K27ac in liver, kidney, and biliary  
512 cancers. In contrast, histone marks had little effect on SBS24 (aflatoxin) except enrichment at  
513 H3K36me3 histone mark in liver cancer (**Figure 5A**). Signatures SBS31, SBS35, and DBS5 (due  
514 to chemotherapy with platinum drugs) mutations were all depleted near H3K27ac modifications,  
515 while SBS31 and SBS35 were both depleted at H3K4me3 modification sites and SBS31 was also  
516 found depleted near H3K4me1. Other mutational signatures were either unaffected by histone  
517 modifications or exhibited minor changes in a cancer-specific manner. Lastly, while enrichments  
518 and depletions of somatic mutations in the vicinity of histone marks were commonly observed  
519 for different mutational signatures (**Figure 5A-C**), there was no specific pattern of mutations  
520 within 1,000 base-pairs for any of the examined histone modifications (e.g., there was no  
521 periodicity like the one observed for CTCF binding sites or for nucleosomes). Exemplars of  
522 typically observed patterns of enrichments, depletions, or no changes around different histone  
523 modifications are provided for signatures SBS7a and ID1 across several histone modifications  
524 (**Figure 5D**).

525 **DISCUSSION**

526 Our analysis provides a comprehensive resource that maps the effects of topographical genomic  
527 features on the cancer-specific accumulation of somatic mutations from distinct mutational  
528 signatures. The reported results confirmed many of the prior observations for strand asymmetry,  
529 replication timing, and nucleosome periodicity for some of the original 30 COSMICv2 SBS  
530 signatures (Morganella *et al.*, 2016; Pich *et al.*, 2018; Tomkova *et al.*, 2018). The examined  
531 larger dataset provided us with a greater resolution to identify previously unobserved pan-cancer  
532 and cancer-specific dependencies for some of these 30 signatures as well as to reveal the effect  
533 of genome architecture on the accumulation of another 47 mutational signatures across human  
534 cancer. Importantly, this report also provides the first-ever examination of the tissue-specific  
535 effect of CTCF binding and 11 different histone modifications on the accumulation of somatic  
536 mutations from different mutational signatures. In addition to the global view, in this discussion,  
537 we zoom into two specific case studies to further illustrate the power of examining topography of  
538 mutational signatures.

539

540 First, analysis of SBS28 in *POLE* deficient samples (*POLE*<sup>-</sup>) and *POLE* proficient samples  
541 (*POLE*<sup>+</sup>) revealed a distinct behaviour (**Figure 6**). While the trinucleotide patterns of SBS28 in  
542 *POLE*<sup>+</sup> and *POLE*<sup>-</sup> samples were similar (cosine similarity: 0.96), SBS28 in *POLE*<sup>-</sup> samples  
543 accounted for 97.7% mutations of all SBS28 mutations and it exhibited a clear enrichment in late  
544 replicating regions as well as depletions at nucleosomes and at CTCF binding sites (**Figure 6B-**  
545 **D,F**). Moreover, SBS28 in *POLE*<sup>-</sup> samples showed a strong replication strand bias on the leading  
546 strand and exhibited a strand-coordinated mutagenesis with as many as 11 consecutively mutated  
547 substitutions (**Figure 6E,G**). In contrast, SBS28 in *POLE*<sup>+</sup> samples were enriched in early

548 replication regions, lacked depletion of mutations at nucleosomes or CTCF binding sites, had  
549 weak replication strand bias on lagging strand, and did not exhibit much of a strand-coordinated  
550 mutagenesis (**Figure 6**). Based on these topographical differences, we have now split SBS28 into  
551 two distinct signatures: (*i*) SBS28a due to *POLE* deficiency found in ultra-hypermutate  
552 colorectal and uterine cancers; and (*ii*) SBS28b with unknown aetiology found in lung and  
553 stomach cancers.

554

555 Second, our analyses revealed striking difference in topographical features of clustered and non-  
556 clustered somatic mutations in 288 whole-genome sequenced B-cell malignancies (Alexandrov  
557 *et al.*, 2020). In particular, the topographical behaviours of single base substitutions were  
558 examined after separating them into non-clustered mutations, diffuse hypermutation of  
559 substitutions termed *omikli* (Mas-Ponte and Supek, 2020), and longer clusters of strand-  
560 coordinated substitutions termed *kataegis* (Bergstrom *et al.*, 2022a; Nik-Zainal *et al.*, 2012;  
561 Roberts *et al.*, 2012). In contrast to most cancer types, where *omikli* and *kataegis* are  
562 predominately generated by APOBEC3 deaminases (Bergstrom *et al.*, 2022b), in B-cell  
563 malignancies, these clustered events are almost exclusively imprinted by the activity of AID  
564 (Bergstrom *et al.*, 2022b). Further, the overall pattern of non-clustered mutations was very  
565 different than the ones of *omikli* or *kataegis*. A representative example is provided using a single  
566 malignant B-cell lymphoma (**Figure 7A**) where non-clustered and clustered mutations have very  
567 different trinucleotide patterns (**Figure 7B-D**). Non-clustered mutations exhibited different  
568 topographical features when compared to *omikli* or *kataegis*. Specifically, while non-clustered  
569 mutations had some minor periodicity in regard to nucleosome occupancy, such periodicity was  
570 not observed for any type of clustered events (**Figure 7E**). Similarly, non-clustered mutations

571 were slightly depleted around CTCF binding sites while *omikli* and *kataegis* were very highly  
572 depleted (**Figure 7F&H**). Further, non-clustered and *omikli* events were clearly enriched in late  
573 replication regions while *kataegis* was highly enriched in early replication regions (**Figure 7G**).  
574 Distinct patterns of enrichments were also observed for both *omikli* and *kataegis* mutations in the  
575 vicinity of promoter and enhancer sites delineated by histone marks of H3K4me3, H3K9ac,  
576 H3K27ac, H3K36me3, and H4K20me1(**Figure 7H**). Only very minor differences were observed  
577 for transcription or replication strand asymmetries between clustered and non-clustered somatic  
578 mutations across the 288 whole-genome sequenced B-cell malignancies (**Supplementary Figure**  
579 **S3**).

580

581 In summary, in this report we have performed a comprehensive topography analysis of  
582 mutational signatures encompassing 82,890,857 somatic mutations in 5,120 whole-genome  
583 sequenced tumours integrated with 516 tissue-matched topographical features from the  
584 ENCODE project. Our evaluation encompassed examining the effects of nucleosome occupancy,  
585 histone modifications, CTCF binding sites, replication timing, transcription strand asymmetry,  
586 and replication strand asymmetry on the accumulation of somatic mutations from more than 70  
587 distinct mutational signatures. The results from these analyses have been provided as an online  
588 resource as a part of COSMIC signatures database, <https://cancer.sanger.ac.uk/signatures/>, where  
589 researchers can explore each mutational signature as well as each topographical feature in a  
590 cancer-specific manner.

591

592 **FIGURE LEGENDS**

593 **Figure 1. Strand asymmetries and strand-coordinated mutagenesis of mutational**  
594 **signatures. (A)** Transcription strand asymmetries of signatures of single base substitutions  
595 (SBSs). Rows represent the signatures, where  $n$  reflects the number of cancer types in which  
596 each signature was found. Columns display the six substitution subtypes based on the mutated  
597 pyrimidine base: C>A, C>G, C>T, T>A, T>C, and T>G. SBS signatures with transcription  
598 strand asymmetries on the transcribed and/or untranscribed strands with  $q\text{-value} \leq 0.05$  are  
599 shown in circles with blue and green colours, respectively. The colour intensity reflects the odds  
600 ratio between the ratio of real mutations and the ratio of simulated mutations, where each ratio is  
601 calculated using the number of mutations on the transcribed strand and the number of mutations  
602 on the untranscribed strand. Only odds ratios above 1.10 are shown. Circle sizes reflect the  
603 proportion of cancer types exhibiting a signature with specific transcription strand asymmetry.  
604 **(B)** Replication strand asymmetries of SBS signatures. Rows represent the signatures, where  $n$   
605 reflects the number of cancer types in which each signature was found. Columns display the six  
606 substitution subtypes based on the mutated pyrimidine base: C>A, C>G, C>T, T>A, T>C, and  
607 T>G. SBS signatures with replicational strand asymmetries on the lagging strand or leading  
608 strand with  $q\text{-value} \leq 0.05$  are shown in circles with red and yellow colours, respectively. The  
609 colour intensity reflects the odds ratio between the ratio of real mutations and the ratio of  
610 simulated mutations, where each ratio is calculated using the number of mutations on the lagging  
611 strand and the number of mutations on the leading strand. Circle sizes reflect the proportion of  
612 cancer types exhibiting a signature with specific replication strand asymmetry. **(C)** Transcription  
613 strand asymmetries of signatures of doublet-base substitutions (DBSs) and of small  
614 insertions/deletions (IDs). Data are presented in a format similar to the one in panel **(A)**. **(D)**

615 Replication strand asymmetries of DBS and ID mutational signatures. Data are presented in a  
616 format similar to the one in panel *(B)*. **(E)** Strand-coordinated mutagenesis in SBS signatures.  
617 Rows represent SBS signatures and columns reflect the lengths, in numbers of consecutive  
618 mutations, of strand-coordinated mutagenesis groups. SBS signatures with statistically  
619 significant strand-coordinated mutagenesis ( $q\text{-value} \leq 0.05$ ) are shown as circles under the  
620 respective group length with a minimum length of 5 consecutive mutations. The size of each  
621 circle reflects the number of consecutive mutation groups for the specified group length  
622 normalized for each signature. The colour of each circle reflects the statistical significance of the  
623 number of subsequent mutation groups for each group length with respect to the simulated  
624 mutations using  $-\log_{10}(q\text{-value})$ .

625

626 **Figure 2. Interplay between replication timing and mutational signatures.** Top three panels  
627 reflect results for all single base substitutions (SBSs), all dinucleotide substitutions (DBSs), and  
628 all small insertions/deletions (IDs) across all examined cancer types with each cancer type  
629 examined separately. Bottom panels reflect all somatic mutations attributed to a particular  
630 signature across all cancer types. Replication time data are separated into deciles, with each  
631 segment containing exactly 10% of the observed replication time signal (x-axes). Normalized  
632 mutation densities per decile (y-axes) are presented for early (left) to late (right) replication  
633 domains. Real data for SBS signatures are shown as blue bars, for DBS signatures as red bars,  
634 and for ID signatures as green bars. In all cases, simulated somatic mutations are shown as  
635 dashed lines. The total number of evaluated cancer types for a particular mutational signature is  
636 shown on top of each plot (*e.g.*, 36 cancer types were evaluated for SBS1). For each signature,  
637 the number of cancer types where the mutation density increases with replication timing is

638 shown next to ↗ (e.g., 23 cancer types for SBS1). Similarly, the number of cancer types where  
639 the mutation density decreases with replication timing is shown next to ↘ (e.g., 0 cancer types  
640 for SBS1). Lastly, the number of cancer types where the mutation density is not affected by  
641 replication timing is shown next to → (e.g., 13 cancer types for SBS1).

642

643 **Figure 3. Relationship between mutational signatures and nucleosome occupancy.** Top three  
644 panels reflect results for all single base substitutions (SBSs), all doublet-base substitutions  
645 (DBSs), and all small insertions/deletions (IDs) across all examined cancer types with each  
646 cancer type examined separately. Bottom panels reflect all somatic mutations attributed to a  
647 particular signature across all cancer types. In all cases, solid lines correspond to real somatic  
648 mutations with blue solid lines reflecting SBSs, red solid lines DBSs, and green solid lines  
649 reflecting IDs. Solid lines and dashed lines display the average nucleosome signal (y-axes) along  
650 a 2 kilobase window (x-axes) centred at the mutation start site for real and simulated mutations,  
651 respectively. The mutation start site is annotated in the middle of each plot and denoted as 0. The  
652 2 kilobase window encompasses 1,000 base-pairs 5' adjacent to each mutation as well as 1,000  
653 base-pairs 3' adjacent to each mutation. For each mutational signatures, the total number of  
654 similar and considered cancer types using an  $X/Y$  format, with  $X$  being the number of cancer  
655 types where a signature has similar nucleosome behaviour (Pearson correlation  $\geq 0.5$  and q-value  
656  $\leq 0.05$ ) and  $Y$  representing the total number of examined cancer types for that signature. For  
657 example, signature SBS3 annotated with 11/14 reflects a total of 14 examined cancer types with  
658 similar nucleosome behaviour observed in 11 of these 14 cancer types.

659

660 **Figure 4. Relationship between mutational signatures and CTCF binding sites. (A)**

661 Enrichments and depletions of somatic mutations within CTCF binding sites. Heatmaps display  
662 only mutational signatures and cancer types that have at least one statistically significant  
663 enrichment or depletion of somatic mutations attributed to signatures of either single base  
664 substitutions (SBSs), doublet-base substitutions (DBSs), or small insertions/deletions (IDs). Red  
665 colours correspond to enrichments of real somatic mutations when compared to simulated data.  
666 Blue colours correspond to depletions of real somatic mutations when compared to simulated  
667 data. The intensities of red and blue colours reflect the degree of enrichments or depletions based  
668 on the fold change. White colours correspond to lack of data for performing statistical  
669 comparisons (e.g., signature not being detected in a cancer type). Statistically significant  
670 enrichments and depletions are annotated with \* (q-value  $\leq 0.05$ ). (B) The top three panels  
671 reflect average CTCF occupancy signal for all SBSs, DBS, and IDs across all examined cancer  
672 types. Bottom panels reflect all somatic mutations attributed for several exemplar mutational  
673 signatures across all cancer types. In all cases, solid lines correspond to real somatic mutations  
674 with blue solid lines reflecting SBSs, red solid lines reflecting DBSs, and green solid lines  
675 reflecting IDs. Solid lines and dashed lines display the average CTCF binding signal (y-axes)  
676 along a 2 kilobase window (x-axes) centred at the mutation start site for real and simulated  
677 mutations, respectively. The mutation start site is annotated in the middle of each plot and  
678 denoted as 0. The 2 kilobase window encompasses 1,000 base-pairs 5' adjacent to each mutation  
679 as well as 1,000 base-pairs 3' adjacent to each mutation.

680

681 **Figure 5. Relationships between mutational signatures and histone modifications. (A-C)**

682 Relationships between 11 histone modifications and signatures of single base substitutions

683 (SBSs) in panel (A), doublet-base substitutions (DBSs) in panel (B), and small  
684 insertions/deletions (IDs) in panel (C). The examined histone modifications encompass H2AFZ,  
685 H3K4me1, H3K4me2, H3K4me3, H3K9ac, H3K9me3, H3K27ac, H3K27me3, H3K36me3,  
686 H3K79me2, and H4K20me1. Rows and columns reflect the mutational signatures and histone  
687 modifications, respectively. The circle in each cell is separated in red, blue, and grey segments  
688 proportionate to the cancer types in which the signature has a specific behaviour. A red segment  
689 in a circle reflects the signature being enriched in the vicinity of a histone modification (q-value  
690  $\leq 0.05$  and at least 5% enrichment). A blue segment in a circle reflects the signature being  
691 depleted in the vicinity of a histone modification (q-value  $\leq 0.05$  and at least 5% depletion). A  
692 grey segment in a circle corresponds to neither depletion nor enrichment of the signature in the  
693 vicinity of a histone modification. Cells without a circle correspond to insufficient data to  
694 perform any statistical comparisons. (D) Exemplars of enrichment, depletions, or no effect for  
695 several histone modifications and signatures SBS7a and ID1. Solid lines and dashed lines display  
696 the average signal for a particular histone modification (y-axes) along a 2 kilobase window (x-  
697 axes) centred at the mutation start site for real and simulated mutations, respectively. The  
698 mutation start site is annotated in the middle of each plot and denoted as 0. The 2 kilobase  
699 window encompasses 1,000 base-pairs 5' adjacent to each mutation as well as 1,000 base-pairs  
700 3' adjacent to each mutation.

701

702 **Figure 6. Topography of signature SBS28 in *POLE* deficient (*POLE*<sup>-</sup>) and *POLE* proficient  
703 (*POLE*<sup>+</sup>) samples. (A)** Mutational patterns of signature SBS28 in *POLE*<sup>-</sup> and *POLE*<sup>+</sup> samples  
704 displayed using the conventional 96 mutational classification schema for single base  
705 substitutions. (B) Nucleosome occupancy of SBS28 in *POLE*<sup>-</sup> and *POLE*<sup>+</sup> samples. Blue solid

706 lines and grey dashed lines display the average nucleosome signal (y-axes) along a 2 kilobase  
707 window (x-axes) centred at the mutation start site for real and simulated mutations, respectively.  
708 The mutation start site is annotated in the middle of each plot and denoted as 0. The 2 kilobase  
709 window encompasses 1,000 base-pairs 5' adjacent to each mutation as well as 1,000 base-pairs  
710 3' adjacent to each mutation. **(C)** CTCF occupancy of SBS28 in *POLE*<sup>-</sup> and *POLE*<sup>+</sup> samples.  
711 Blue solid lines and grey dashed lines display the average CTCF binding signal (y-axes) along a  
712 2 kilobase window (x-axes) centred at the mutation start site for real and simulated mutations,  
713 respectively. The mutation start site is annotated in the middle of each plot and denoted as 0. The  
714 2 kilobase window encompasses 1,000 base-pairs 5' adjacent to each mutation as well as 1,000  
715 base-pairs 3' adjacent to each mutation. **(D)** Replication timing of SBS28 mutations in *POLE*<sup>-</sup>  
716 and *POLE*<sup>+</sup> samples. Replication time data are separated into deciles, with each segment  
717 containing exactly 10% of the observed replication time signal (x-axes). Normalized mutation  
718 densities per decile (y-axes) are presented for early (left) to late (right) replication domains.  
719 Normalized mutation densities of real somatic mutations and simulated somatic mutations from  
720 early to late replicating regions are shown as blue bars and dashed lines, respectively. **(E)**  
721 Replication strand asymmetry of SBS28 mutations in *POLE*<sup>-</sup> and *POLE*<sup>+</sup> samples. Bar plots  
722 display the number of mutations accumulated on the lagging strand and leading strand for six  
723 substitution subtypes based on the mutated pyrimidine base: C>A, C>G, C>T, T>A, T>C, and  
724 T>G in red and yellow colours, respectively. Simulated mutations on lagging and leading strands  
725 are displayed in hatched bar plots. Statistically significant strand asymmetries are shown with  
726 stars: \* q-value  $\leq 0.05$ ; \*\* q-value  $\leq 0.01$ ; \*\*\* q-value  $\leq 0.001$ . **(F)** Enrichments and depletions  
727 of SBS28 somatic mutations in *POLE*<sup>-</sup> and *POLE*<sup>+</sup> samples within CTCF binding sites, histone  
728 modifications, and nucleosome occupied regions. Red colours correspond to enrichments of real

729 somatic mutations when compared to simulated data. Blue colours correspond to depletions of  
730 real somatic mutations when compared to simulated data. The intensities of red and blue colours  
731 reflect the degree of enrichments or depletions based on the fold change. White colours  
732 correspond to lack of data for performing statistical comparisons. Statistically significant  
733 enrichments and depletions are annotated with \* (q-value  $\leq 0.05$ ). **(G)** Strand-coordinated  
734 mutagenesis of SBS28 mutations in *POLE*<sup>-</sup> and *POLE*<sup>+</sup> samples. Rows represent SBS28  
735 signature in *POLE*<sup>-</sup> and *POLE*<sup>+</sup> samples across all cancer types and columns reflect the lengths,  
736 in numbers of consecutive mutations, of strand-coordinated mutagenesis groups. Statistically  
737 significant strand-coordinated mutagenesis (q-value  $\leq 0.05$ ) are shown as circles under the  
738 respective group length with a length starting from 2 to 11 consecutive mutations. The size of  
739 each circle reflects the number of consecutive mutation groups for the specified group length  
740 normalized for each SBS28 signature in *POLE*<sup>-</sup> and *POLE*<sup>+</sup> samples. The colour of each circle  
741 reflects the statistical significance of the number of subsequent mutation groups for each group  
742 length with respect to the simulated mutations using  $-\log_{10}$  (q-value).  
743

744 **Figure 7. Topography of non-clustered, *omikli*, and *kataegis* substitutions across 288 whole-  
745 genome sequenced B-cell malignancies. (A)** A rainfall plot of an example B-cell malignancy  
746 sample, MALY-DE\_SP116612, depicting the intra-mutational distance (IMD) distributions of  
747 substitutions across genomic coordinates. Each dot represents the minimum distance between  
748 two adjacent mutations. Dots are coloured based on their corresponding classifications.  
749 Specifically, non-clustered mutations are shown in grey, doublet-base substitutions (DBSs) in  
750 red, multi-base substitutions (MBSs) in black, *omikli* events in green, *kataegis* events in orange,  
751 and all other clustered events in blue. The red line depicts the sample-dependent IMD threshold

752 for each sample. Specific clustered mutations may be above this threshold due to corrections for  
753 regional mutation density. **(B-D)** The trinucleotide mutational spectra for the different catalogues  
754 of non-clustered, *omikli*, and *kataegis* mutations for the exemplar sample (DBSs and MBSs are  
755 not shown). **(E)** Nucleosome occupancy of non-clustered, *omikli*, and *kataegis* mutations of B-  
756 cell malignancies. Blue solid lines and grey dashed lines display the average nucleosome signal  
757 (y-axes) along a 2 kilobase window (x-axes) centred at the mutation start site for real and  
758 simulated mutations, respectively. The mutation start site is annotated in the middle of each plot  
759 and denoted as 0. The 2 kilobase window encompasses 1,000 base-pairs 5' adjacent to each  
760 mutation as well as 1,000 base-pairs 3' adjacent to each mutation. **(F)** CTCF occupancy of non-  
761 clustered, *omikli*, and *kataegis* mutations of B-cell malignancies. Blue solid lines and grey  
762 dashed lines display the average CTCF signal (y-axes) along a 2 kilobase window (x-axes)  
763 centred at the mutation start site for real and simulated mutations, respectively. The mutation  
764 start site is annotated in the middle of each plot and denoted as 0. The 2 kilobase window  
765 encompasses 1,000 base-pairs 5' adjacent to each mutation as well as 1,000 base-pairs 3'  
766 adjacent to each mutation. **(G)** Replication timing of non-clustered, *omikli*, and *kataegis*  
767 mutations of B-cell malignancies. Replication time data are separated into deciles, with each  
768 segment containing exactly 10% of the observed replication time signal (x-axes). Normalized  
769 mutation densities per decile (y-axes) are presented for early (left) to late (right) replication  
770 domains. Normalized mutation densities of real somatic mutations and simulated somatic  
771 mutations from early to late replicating regions are shown as blue bars and dashed lines,  
772 respectively. **(H)** Enrichments and depletions of non-clustered, *omikli*, and *kataegis* mutations of  
773 B-cell malignancies within CTCF binding sites and histone modifications. Red colours  
774 correspond to enrichments of real somatic mutations when compared to simulated data. Blue

775 colours correspond to depletions of real somatic mutations when compared to simulated data.

776 The intensities of red and blue colours reflect the degree of enrichments or depletions based on

777 the fold change. White colours correspond to lack of data for performing statistical comparisons.

778 Statistically significant enrichments and depletions are annotated with \* (q-value  $\leq 0.05$ ).

779

780 **SUPPLEMENTARY FIGURE LEGENDS**

781 **Figure S1. Somatic mutations in genic and intergenic regions imprinted by different**  
782 **mutational signatures.** **(A)** Somatic mutations in genic and intergenic regions for signatures of  
783 single base substitutions (SBSs). Rows represent the signatures, where  $n$  reflects the number of  
784 cancer types in which each signature was found. Columns display the six substitution subtypes  
785 based on the mutated pyrimidine base: C>A, C>G, C>T, T>A, T>C, and T>G. SBS signatures  
786 with genic and intergenic regions asymmetries with  $q\text{-value} \leq 0.05$  are shown in circles with  
787 cyan and grey colours, respectively. The colour intensity reflects the odds ratio between the ratio  
788 of real mutations and the ratio of simulated mutations, where each ratio is calculated using the  
789 number of mutations in the genic regions and the number of mutations in the intergenic regions.  
790 Only odds ratios above 1.10 are shown. Circle sizes reflect the proportion of cancer types  
791 exhibiting a signature with specific genic versus intergenic regions asymmetry. **(B)** Somatic  
792 mutations in genic and intergenic regions for signatures of doublet-base substitutions (DBSs).  
793 Data are presented in a format similar to the one in panel **(A)**. **(C)** Somatic mutations in genic and  
794 intergenic regions for small insertions/deletions (IDs). Data are presented in a format similar to  
795 the one in panel **(A)**. **(D)** Histogram of fold enrichment as odds ratio between the ratio of real  
796 mutations and the ratio of simulated mutations, where each ratio is calculated using the number  
797 of mutations in the genic regions and the number of mutations in the intergenic regions.  
798 Frequency of fold enrichments (y-axis) are presented for discreet bins of fold enrichments (x-  
799 axis). Each fold enrichment reflects the odds ratio between real and simulated mutations where  
800 each ratio is the number of mutations in intergenic regions divided by the number of mutations in  
801 genic regions. Total number of fold enrichments, mean, and standard deviation of fold  
802 enrichments are shown in the upper right corner of the histogram. **(E)** Same format as panel **(D)**

803 with the underlaying data reflecting fold enrichments after inflating the number of somatic  
804 mutations in genic regions to remove any transcription strand asymmetry.

805

806 **Figure S2. Topography of signature SBS4 across all cancer types. (A)** Nucleosome  
807 occupancy of SBS4 across all cancer types and within individual cancer type. In all cases, blue  
808 solid lines and grey dashed lines display the average nucleosome signal (y-axes) along a 2  
809 kilobase window (x-axes) centred at the mutation start site for real and simulated mutations of  
810 SBS4, respectively. The mutation start site is annotated in the middle of each plot and denoted as  
811 0. The 2 kilobase window encompasses 1,000 base-pairs 5' adjacent to each mutation as well as  
812 1,000 base-pairs 3' adjacent to each mutation. **(B)** CTCF occupancy of SBS4 across all cancer  
813 types and for each cancer type. In all cases, blue solid lines and grey dashed lines display the  
814 average CTCF binding signal (y-axes) along a 2 kilobase window (x-axes) centred at the  
815 mutation start site for real and simulated mutations, respectively. The mutation start site is  
816 annotated in the middle of each plot and denoted as 0. The 2 kilobase window encompasses  
817 1,000 base-pairs 5' adjacent to each mutation as well as 1,000 base-pairs 3' adjacent to each  
818 mutation. **(C)** Replication timing of SBS4 across all cancer types and for each cancer type.  
819 Replication time data were separated into deciles, with each segment containing exactly 10% of  
820 the observed replication time signal (x-axes). Normalized mutation densities per decile (y-axes)  
821 are presented for early (left) to late (right) replication domains. In all cases, blue bars and dashed  
822 lines show the normalized mutation densities of real and simulated somatic mutations,  
823 respectively. **(D)** Enrichments and depletions of SBS4 somatic mutations within CTCF binding  
824 sites and histone modifications. Only histone modifications H3K4me3, H3K9me3, and H3K27ac  
825 are shown as they were the only ones with statistically significant results (q-value  $\leq 0.05$ ).

826 Heatmap displays SBS4 signature for each cancer type in which SBS4 is found. Red colours  
827 correspond to enrichments of real somatic mutations when compared to simulated data. Blue  
828 colours correspond to depletions of real somatic mutations when compared to simulated data.  
829 The intensities of red and blue colours reflect the degree of enrichments or depletions based on  
830 the fold change. Statistically significant enrichments and depletions are annotated with \* (q-  
831 value  $\leq 0.05$ ). **(E)** Transcription strand asymmetries of SBS4 across cancer types. Rows  
832 represent SBS4 combined across all cancer types as well as SBS4 within each individual cancer  
833 type in which SBS4 has been detected. Columns display the six substitution subtypes based on  
834 the mutated pyrimidine base: C>A, C>G, C>T, T>A, T>C, and T>G. SBS4 signature with  
835 transcription strand asymmetries on the transcribed and/or untranscribed strands with q-value  $\leq$   
836 0.05 are shown in circles with blue and green colours, respectively. The colour intensity reflects  
837 the odds ratio between the ratio of real mutations and the ratio of simulated mutation, where each  
838 ratio is calculated using the number of mutations on the transcribed strand and the number of  
839 mutations on the untranscribed strand. Only odds ratios above 1.10 are shown. Circle sizes  
840 reflect the proportion of cancer types exhibiting SBS4 with specific transcription strand  
841 asymmetry. **(F)** Somatic mutations in genic and intergenic regions for SBS4 across cancer types.  
842 Rows represent SBS4 combined across all cancer types as well as SBS4 within each individual  
843 cancer type in which SBS4 has been detected. Columns display the six substitution subtypes  
844 based on the mutated pyrimidine base: C>A, C>G, C>T, T>A, T>C, and T>G. SBS4 signature  
845 with genic and intergenic regions asymmetries with q-value  $\leq 0.05$  are shown in circles with  
846 cyan and grey colours, respectively. The colour intensity reflects the odds ratio between the ratio  
847 of real mutations and the ratio of simulated mutations, where each ratio is calculated using the  
848 number of mutations in the genic regions and the number of mutations in the intergenic regions.

849 Only odds ratios above 1.10 are shown. Circle sizes reflect the proportion of cancer types  
850 exhibiting SBS4 with specific genic versus intergenic regions asymmetry.

851

852 **Figure S3. Strand asymmetries of non-clustered, *omikli*, and *kataegis* substitutions across**  
853 **288 whole-genome sequenced B-cell malignancies.** Transcription strand asymmetries are  
854 shown in the left panels where bars display the six substitution subtypes based on the mutated  
855 pyrimidine base: C>A, C>G, C>T, T>A, T>C, and T>G (depicted on the x-axes). Y-axes  
856 correspond to the numbers of single base substitutions. Blue bars reflect real transcribed  
857 substitutions, while shaded blue bars correspond to simulated transcribed substitutions.  
858 Similarly, green bars reflect real untranscribed mutations, whereas shaded green bars correspond  
859 to simulated untranscribed substitutions. Replication strand asymmetries are shown in the middle  
860 panels where bars display the six substitution subtypes based on the mutated pyrimidine base:  
861 C>A, C>G, C>T, T>A, T>C, and T>G (depicted on the x-axes). Y-axes correspond to the  
862 numbers of single base substitutions. Red bars reflect real substitutions on the lagging strand,  
863 while shaded red bars correspond to simulated substitutions on the lagging strand. Similarly,  
864 yellow bars reflect real substitutions on the leading strand, whereas shaded yellow bars  
865 correspond to simulated substitutions on the leading strand. Comparisons of genic and intergenic  
866 regions are shown in the right panels where bars display the six substitution subtypes based on  
867 the mutated pyrimidine base: C>A, C>G, C>T, T>A, T>C, and T>G (depicted on the x-axes). Y-  
868 axes correspond to the numbers of single base substitutions. Cyan bars reflect real substitutions  
869 in genic regions, while shaded cyan bars correspond to simulated substitutions in genic regions.  
870 Similarly, grey bars reflect real substitutions in intergenic regions, whereas shaded grey bars  
871 correspond to simulated substitutions in intergenic regions. Results for non-clustered mutations

872 are shown in panel **(A)**, *omikli* mutations in panel **(B)**, and *kataegis* mutations in panel **(C)**.

873 Statistically significant strand asymmetries are shown with stars: \* q-value  $\leq 0.05$ ; \*\* q-value  $\leq$

874 0.01; \*\*\* q-value  $\leq 0.001$ .

875

## 876 SUPPLEMENTARY TABLES

877 **Table S1.** ENCODE datasets utilized for locations of CTCF binding sites, nucleosome occupancy,

878 histone modification sites, and replication timing.

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891

892 **DECLARATION OF INTERESTS**

893 LBA is a compensated consultant and has equity interest in *io9*, LLC. His spouse is an employee  
894 of Biotheranostics, Inc. LBA is also an inventor of a US Patent 10,776,718 for source  
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896 applications for “Clustered mutations for the treatment of cancer” (U.S. provisional application  
897 serial number 63/289,601) and “Artificial intelligence architecture for predicting cancer  
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901

902 **AUTHOR CONTRIBUTIONS**

903 BO and LBA conceived the performed computational analyses and wrote the manuscript with  
904 assistance from MDG. BO developed the Python code and performed the bioinformatics  
905 analyses with assistance from MDG, ENB, and MB. The online COSMIC signatures topography  
906 database was designed by BO, IV, and LBA with assistance from MDG and MB. The COSMIC  
907 signatures topography database was implemented by IV with feedback from all authors. LBA  
908 supervised the overall development of the code, website, analysis, and writing of the manuscript.  
909 All authors read and approved the final manuscript.

910

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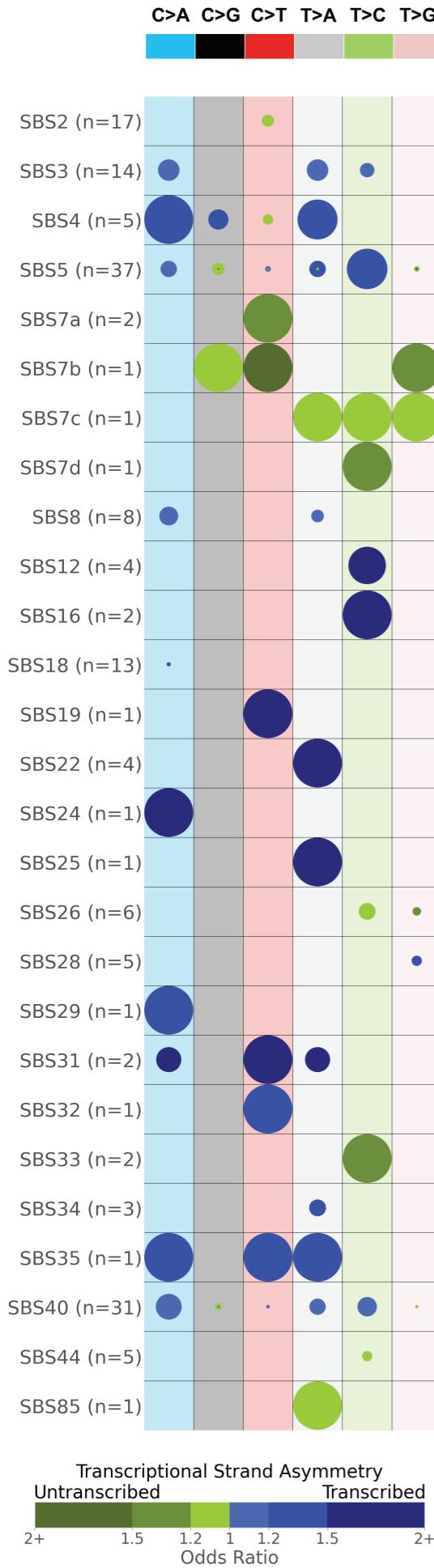
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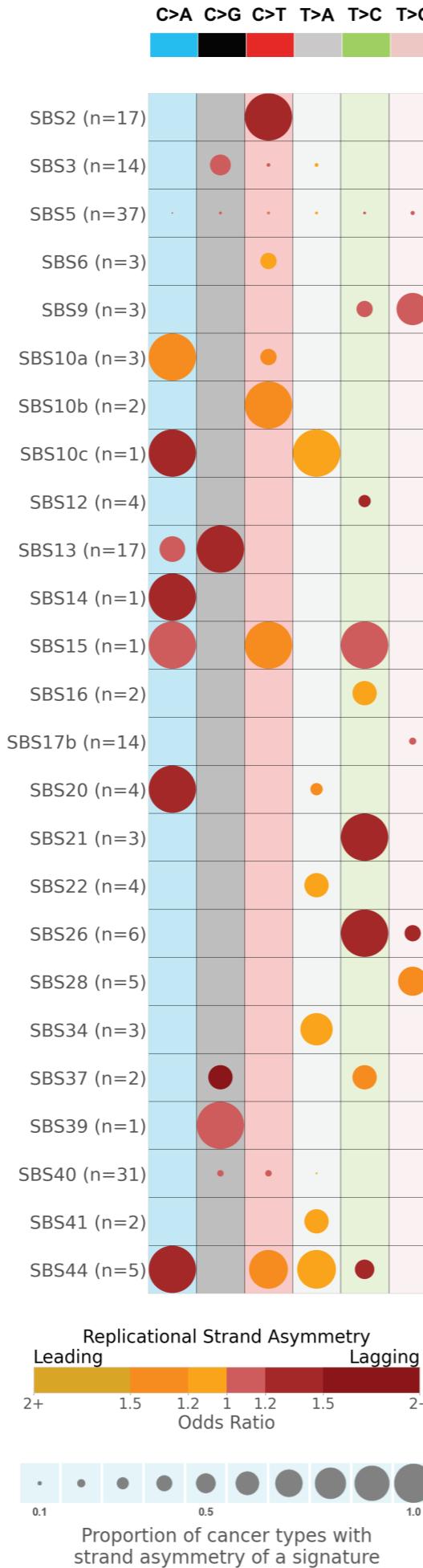
# Figure 1. Strand asymmetries and strand-coordinated mutagenesis of mutational signatures.

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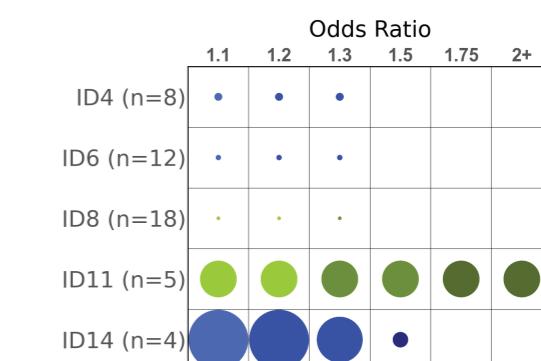
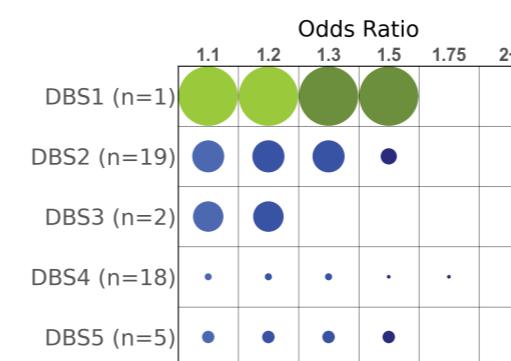
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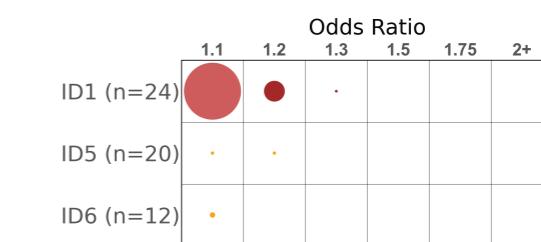
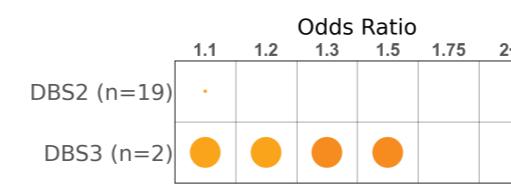
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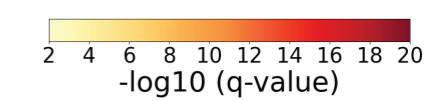
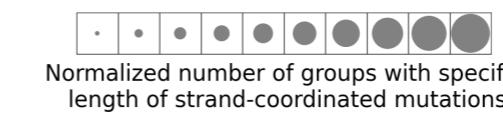
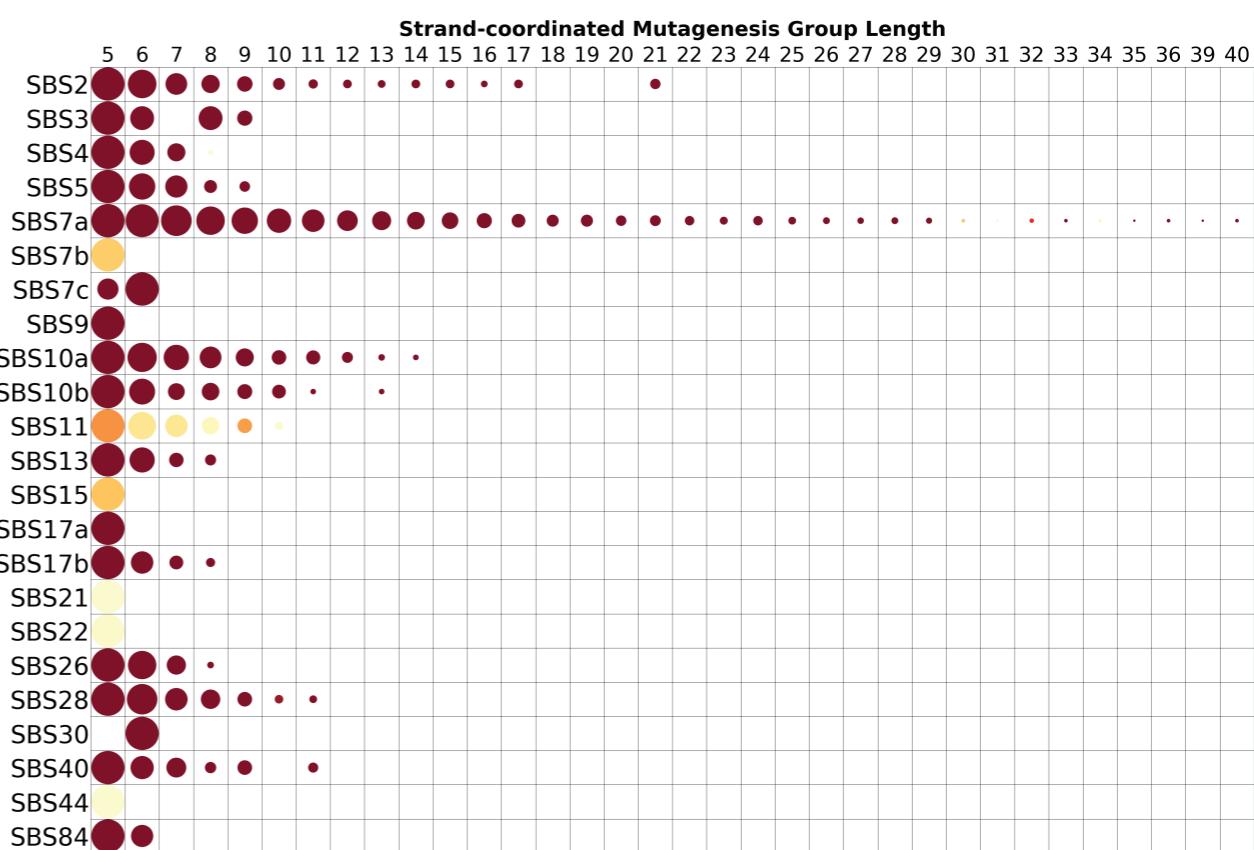
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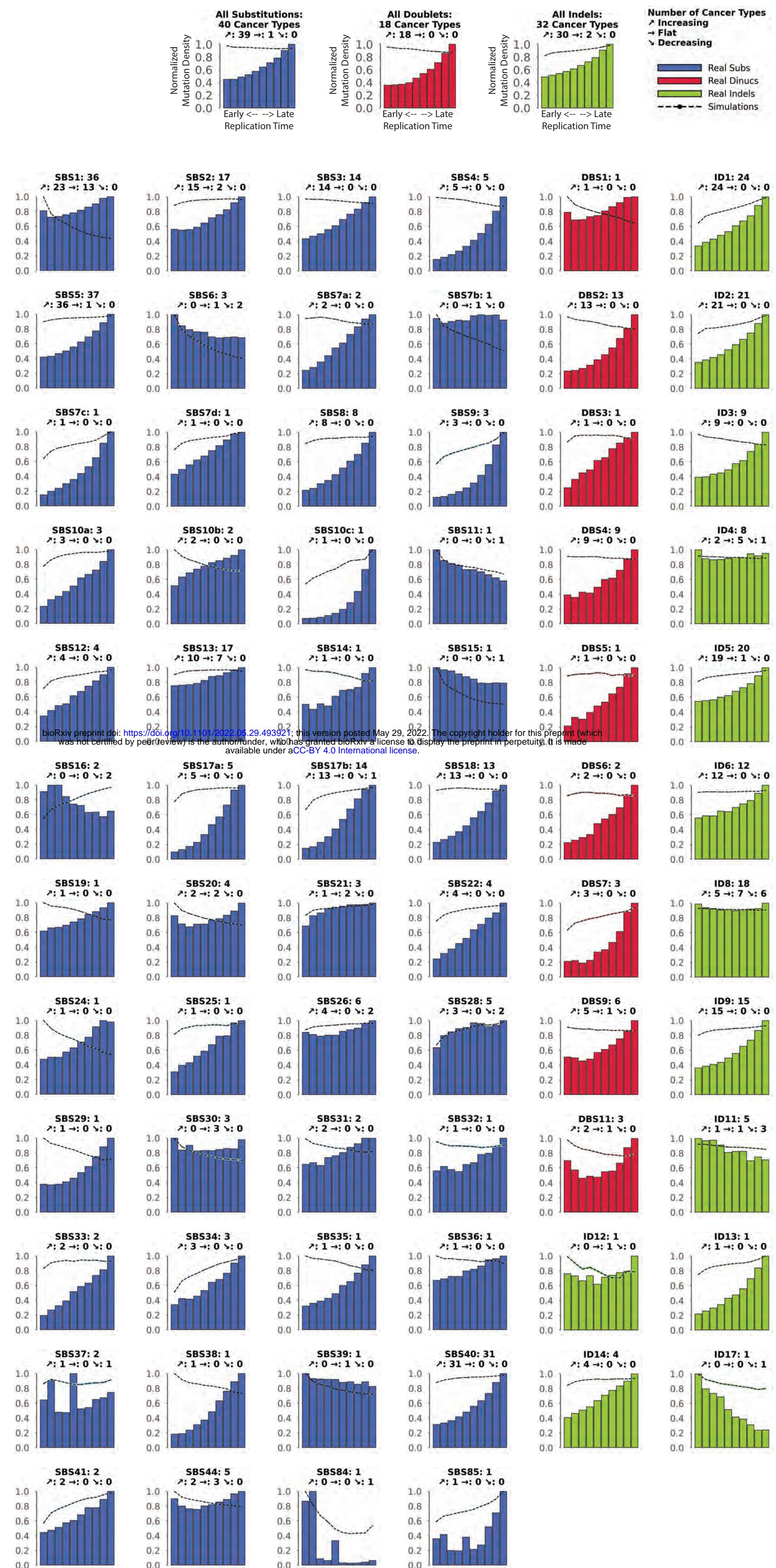
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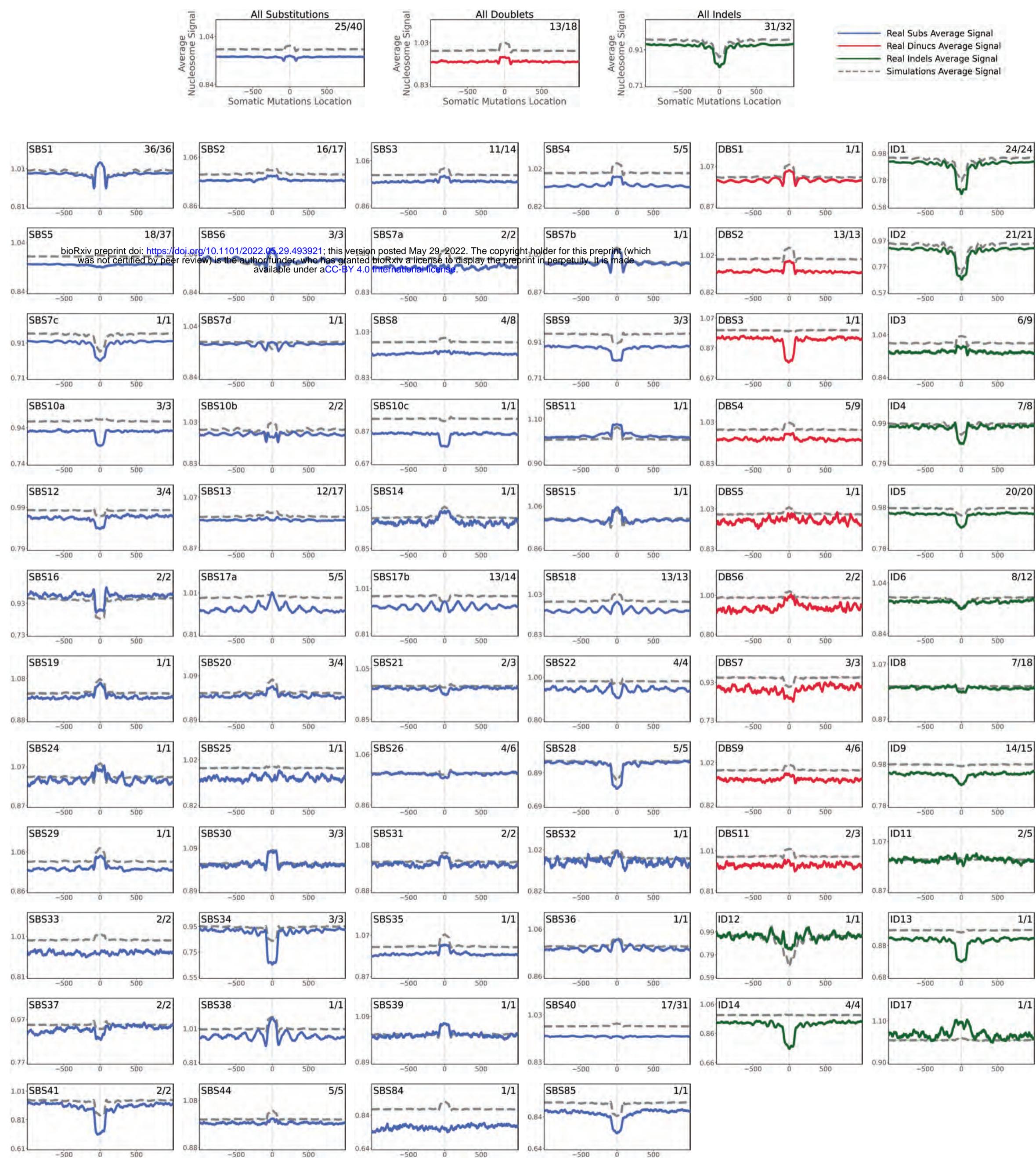
E



**Figure 2. Interplay between replication timing and mutational signatures.**

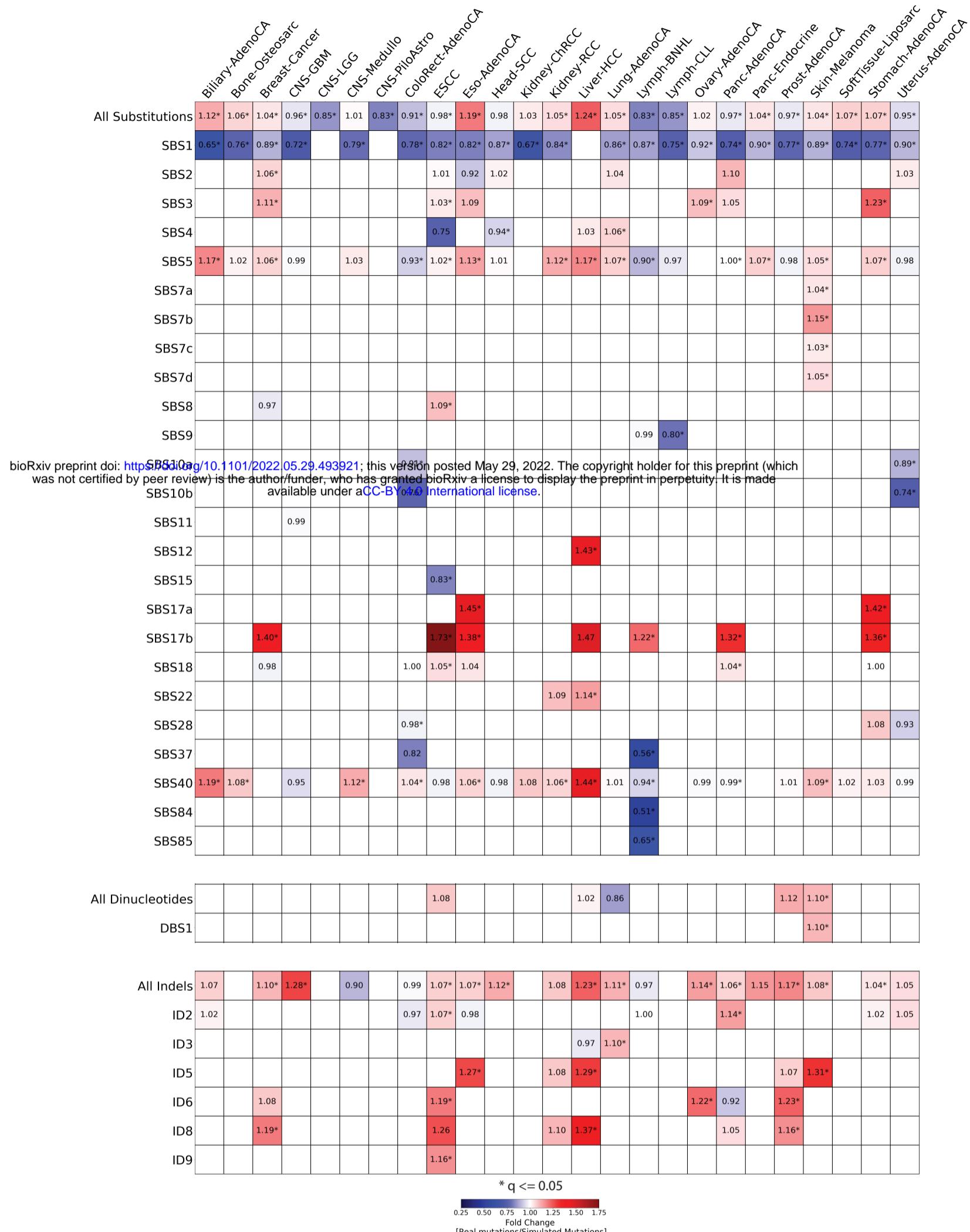


**Figure 3. Relationship between mutational signatures and nucleosome occupancy.**

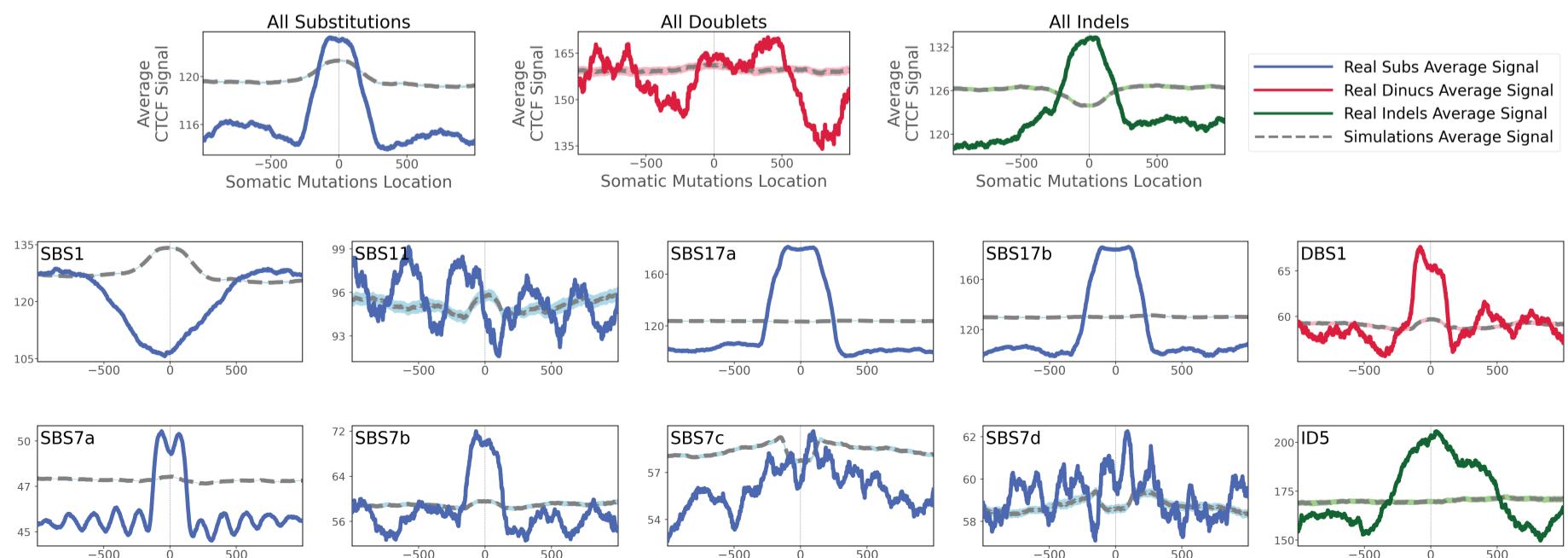


# Figure 4. Relationship between mutational signatures and CTCF binding sites.

A

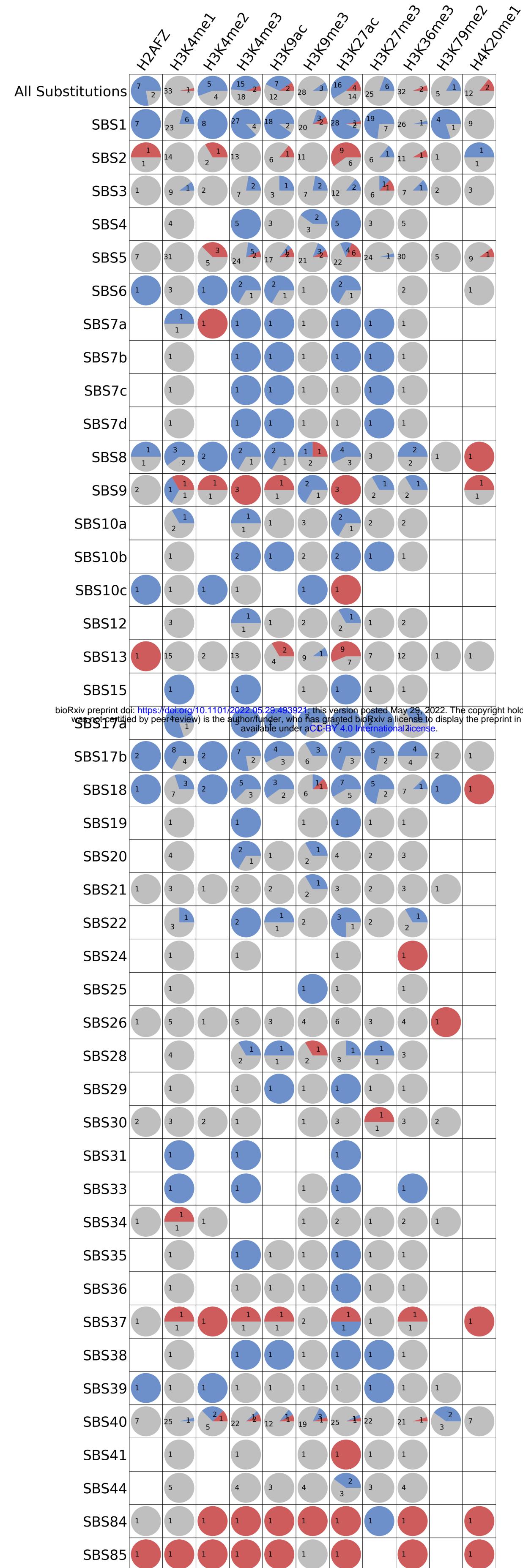


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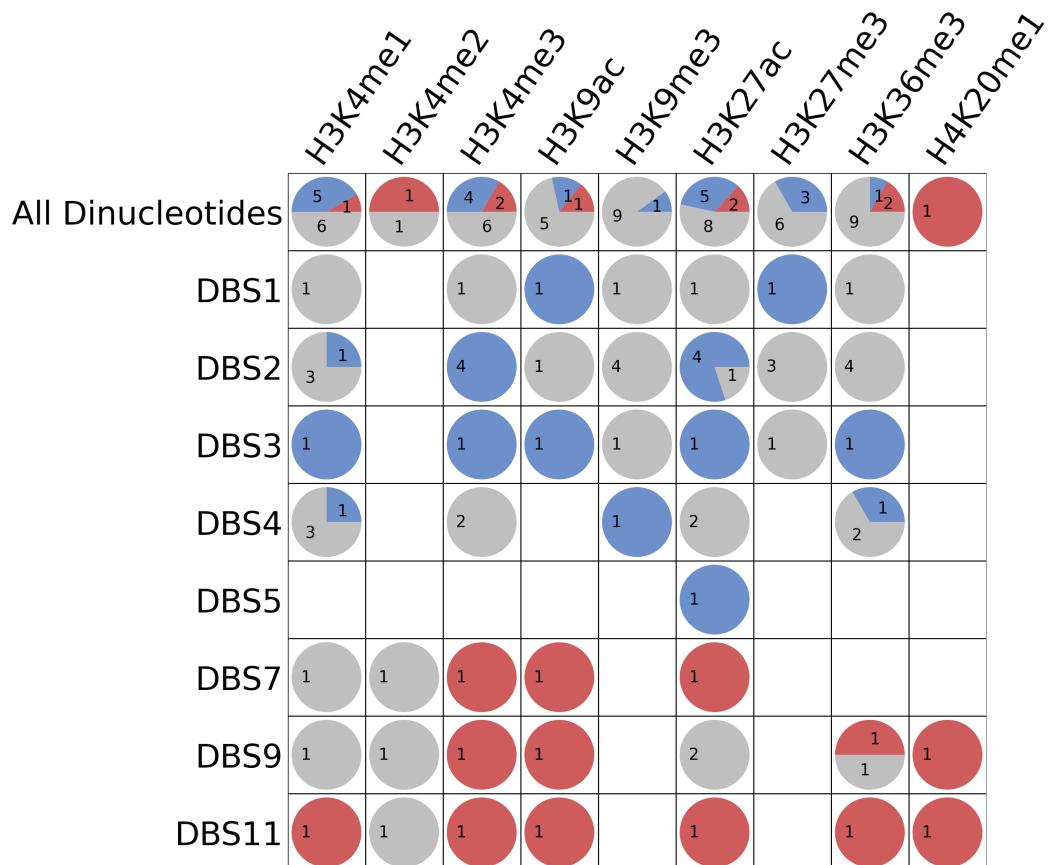


**Figure 5. Relationships between mutational signatures and histone modifications.**

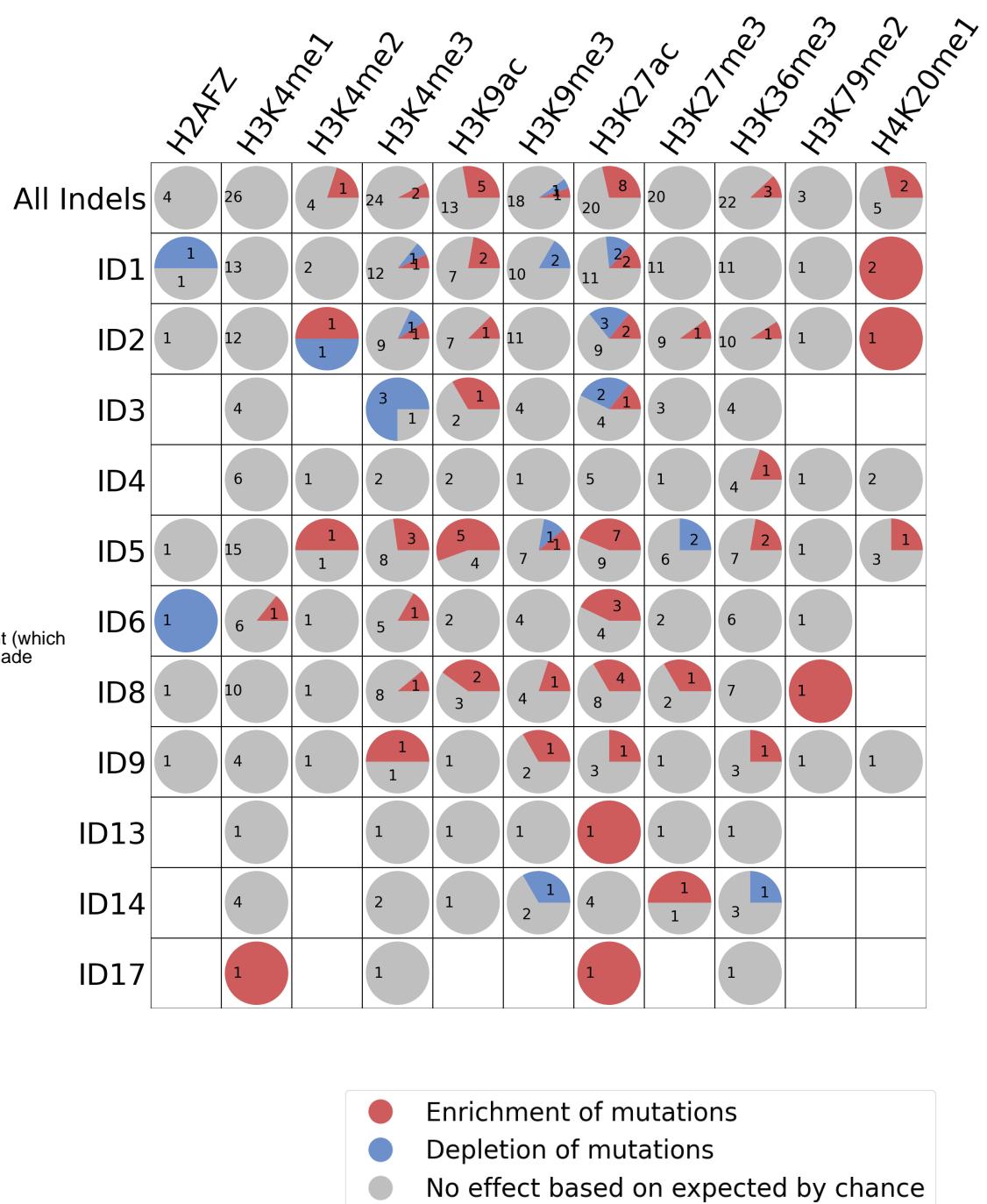
**A**



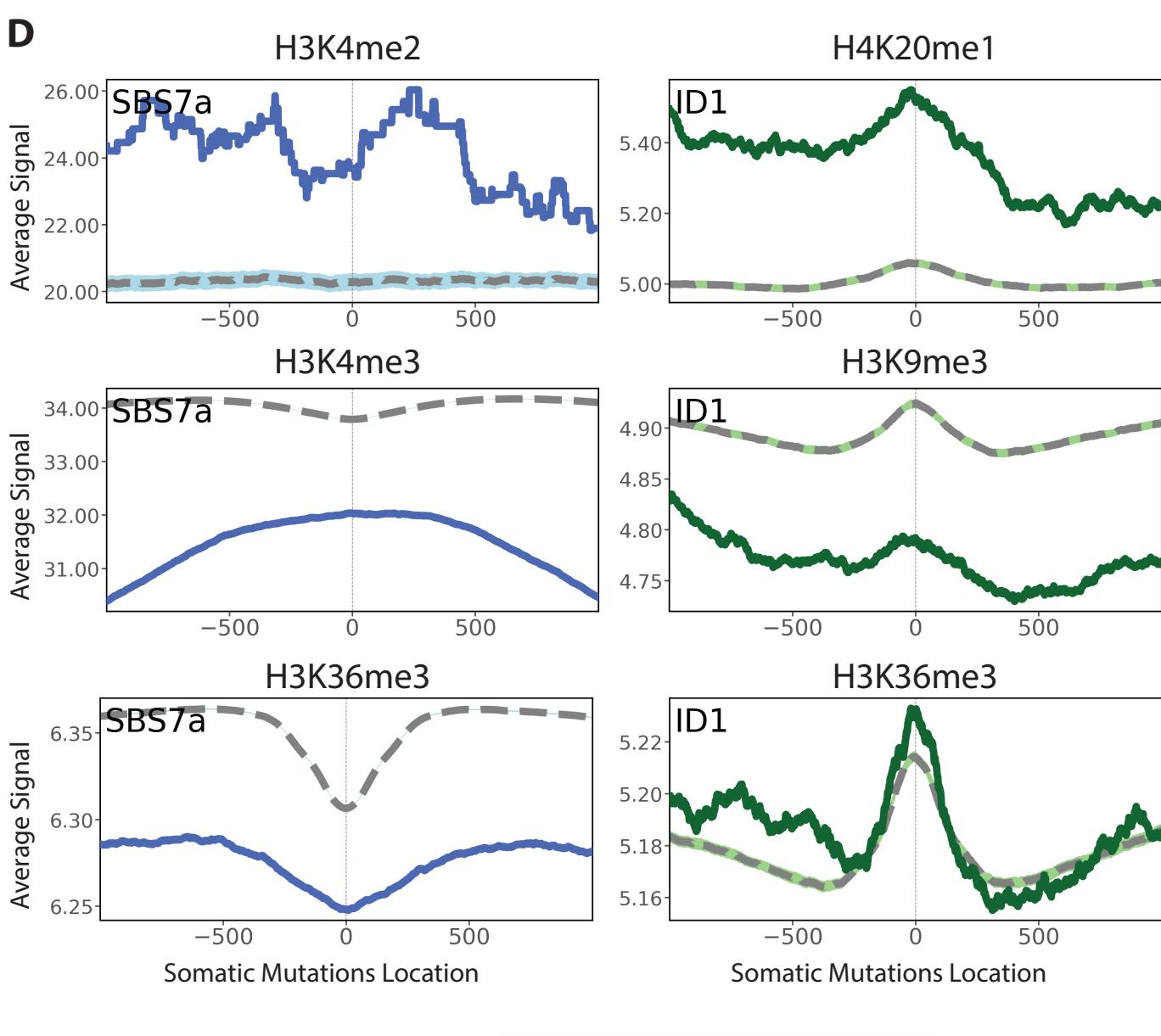
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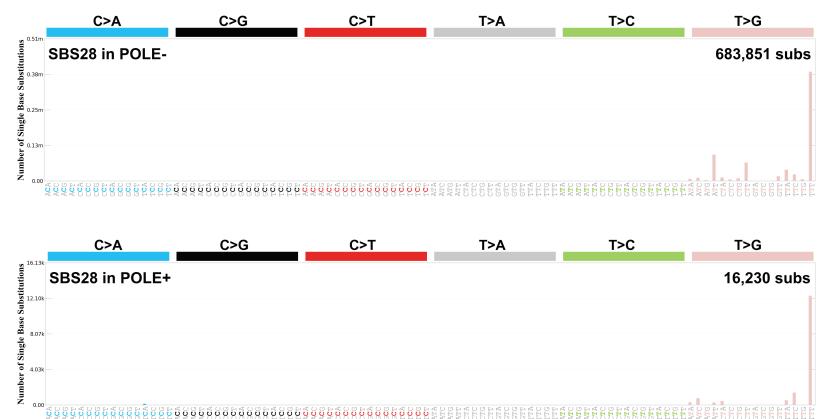


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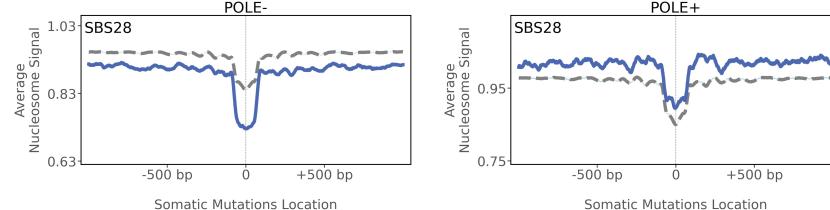


**Figure 6. Topography of signature SBS28 in POLE deficient (POLE-) and POLE proficient (POLE+) samples.**

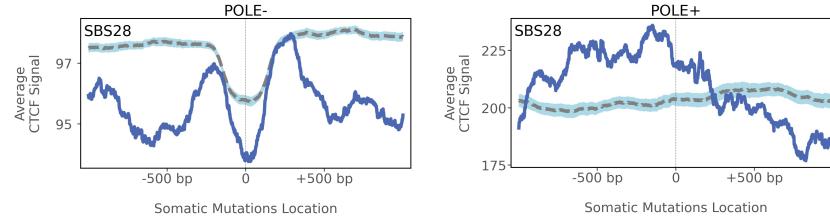
**A**



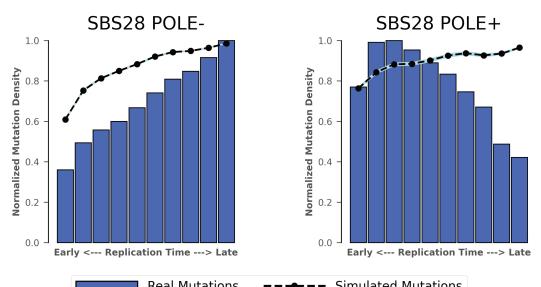
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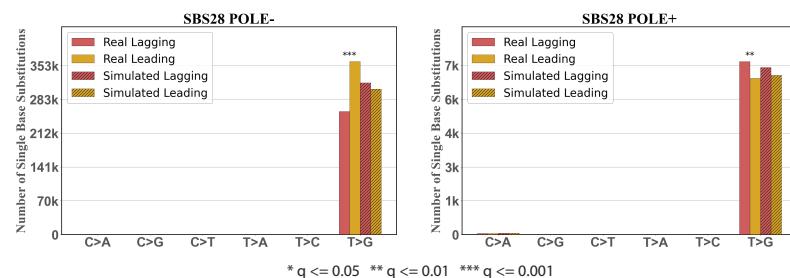
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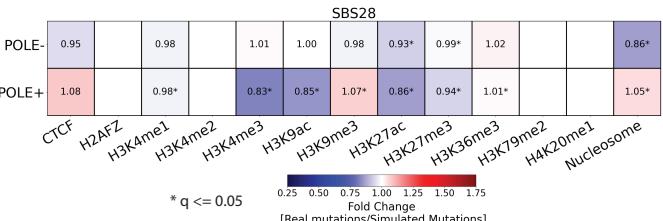
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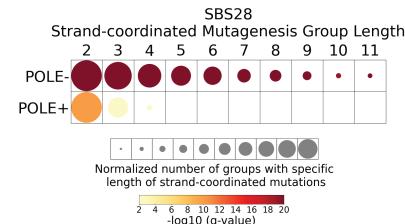
**E**



**F**



**G**



**Figure 7. Topography of non-clustered, omikli, and kataegis substitutions across 288 whole-genome sequenced B-cell malignancies.**

