

Topography of mutational signatures in human cancer

Burcak Otlu¹⁻³, Marcos Díaz-Gay¹⁻³, Ian Vermes⁴, Erik N Bergstrom¹⁻³, Mark Barnes¹⁻³, and Ludmil B. Alexandrov^{1-3*}

Affiliations

¹Department of Cellular and Molecular Medicine, UC San Diego, La Jolla, CA, 92093, USA

²Department of Bioengineering, UC San Diego, La Jolla, CA, 92093, USA

³Moore's Cancer Center, UC San Diego, La Jolla, CA, 92037, USA

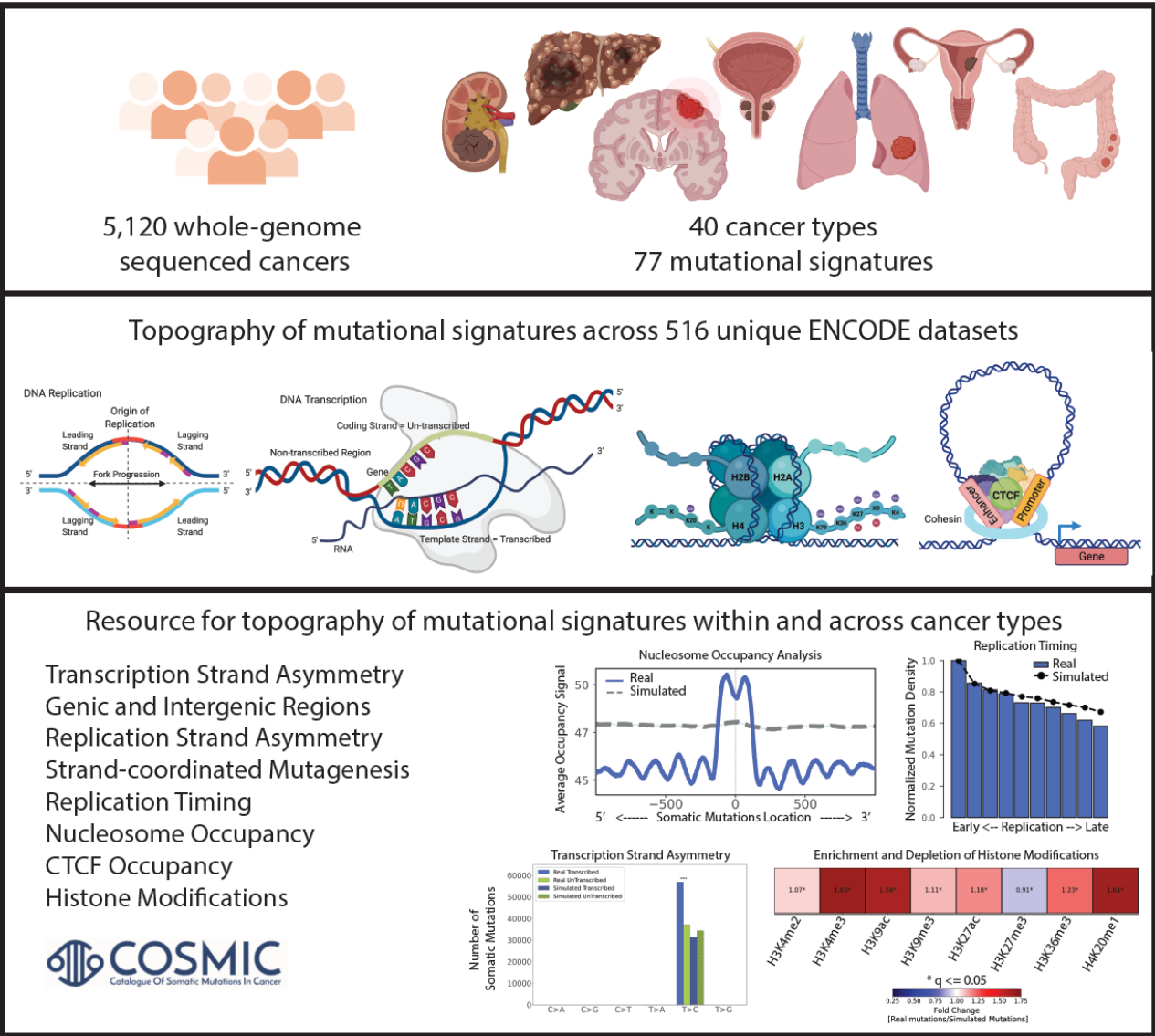
⁴COSMIC, Wellcome Sanger Institute, Hinxton, Cambridgeshire, CB10 1SA, UK

*Correspondence should be addressed to L2alexandrov@health.ucsd.edu.

SUMMARY

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

43 **GRAPHICAL ABSTRACT**



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HIGHLIGHTS

- Comprehensive topography analysis of mutational signatures encompassing 82,890,857 somatic mutations in 5,120 whole-genome sequenced tumours integrated with 516 tissue-matched topographical features from the ENCODE project.
- The accumulation of somatic mutations from most mutational signatures is affected by nucleosome occupancy, histone modifications, CTCF binding sites, transcribed regions, and replication strand/timing.
- Mutational signatures with related aetiologies are consistently characterized by similar genome topographies across tissue types.
- Topography analysis allows both separating signatures from different aetiologies and understanding the genomic specificity of clustered somatic mutations.
- A comprehensive online resource, disseminate through the COSMIC signatures database, that allows researchers to explore the interactions between somatic mutational processes and genome architecture within and across cancer types.

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

INTRODUCTION

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

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

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

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

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

RESULTS

Transcription Strand Asymmetries of Mutational Signatures

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

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

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

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

Distribution of Mutational Signatures in Genic and Intergenic Regions

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

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

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

Replication Strand Asymmetries of Mutational Signatures

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

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

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

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

Mutational Signatures with Strand-coordinated Mutagenesis

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

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

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

The Effect of DNA Replication Timing on Somatic Mutagenesis

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

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

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

All mutational signatures that were previously associated with age of diagnosis in at least one cancer type had a predominately increased normalized mutational density from early to late replicating regions: SBS1 (23/36), SBS5 (36/37), SBS40 (31/31), ID1 (24/24), ID2 (21/21), and ID5 (19/20). SBS3 (14/14) and ID6 (12/12), both attributed to defective homologous recombination, as well as mutational signatures attributed to defective polymerase epsilon (SBS10a, SBS10b, SBS14, and DBS3) were also enriched in late replicating regions in all examined cancer types (**Figure 2**). Similarly, signatures attributed to reactive oxygen species (SBS18 and SBS36) were enriched in late replicating regions in all examined cancer types. Additionally, most mutational signatures due to environmental and chemotherapeutic exposures were enriched in late replicating regions in all examined cancer types including signatures of tobacco smoking (SBS4, DBS2, ID3), tobacco chewing (SBS29), ultraviolet light (SBS7a/c/d, SBS38, DBS1, and ID13), aristolochic acid (SBS22), aflatoxin (SBS24), prior treatment (SBS31, SBS32, SBS35, and DBS5), and non-canonical AID activity (SBS85). Many of the mutational signatures with unknown aetiologies were also enriched in late replicating regions: SBS8, SBS12, SBS17a/b, SBS19, SBS28, SBS33, SBS34, SBS37, and SBS41. Two of the signatures attributed to the APOBEC3 family of deaminases, SBS2 (15/17) and DBS11 (2/3), had an increased normalized mutation density from early to late replicating regions.

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

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

The Effect of Nucleosome Occupancy on Mutational Signatures

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

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

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

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

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

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

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

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

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

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

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

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

The Effect of CTCF Binding on Mutational Signatures

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

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

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

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

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

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

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

The Effect of Histone Modifications on Mutational Signatures

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

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

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

Clock-like signature SBS1 was consistently depleted across cancer-types for multiple histone marks. In contrast, clock-like signatures SBS5 and SBS40 were generally unaffected by histone marks except for cancers of the brain and lymphatic system. APOBEC3 signatures, SBS2 and 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

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

DISCUSSION

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

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

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

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

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

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

FIGURE LEGENDS

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

signatures. (A) Transcription strand asymmetries of signatures of single base substitutions

(SBSs). Rows represent the signatures, where n reflects the number of cancer types in which

each signature was found. Columns display the six substitution subtypes based on the mutated

pyrimidine base: C>A, C>G, C>T, T>A, T>C, and T>G. SBS signatures with transcription

strand asymmetries on the transcribed and/or untranscribed strands with q -value ≤ 0.05 are

shown in circles with blue and green colours, respectively. The colour intensity reflects the odds

ratio between the ratio of real mutations and the ratio of simulated mutations, where each ratio is

calculated using the number of mutations on the transcribed strand and the number of mutations

on the untranscribed strand. Only odds ratios above 1.10 are shown. Circle sizes reflect the

proportion of cancer types exhibiting a signature with specific transcription strand asymmetry.

(B) Replication strand asymmetries of SBS signatures. Rows represent the signatures, where n

reflects the number of cancer types in which each signature was found. Columns display the six

substitution subtypes based on the mutated pyrimidine base: C>A, C>G, C>T, T>A, T>C, and

T>G. SBS signatures with replicational strand asymmetries on the lagging strand or leading

strand with q -value ≤ 0.05 are shown in circles with red and yellow colours, respectively. The

colour intensity reflects the odds ratio between the ratio of real mutations and the ratio of

simulated mutations, where each ratio is calculated using the number of mutations on the lagging

strand and the number of mutations on the leading strand. Circle sizes reflect the proportion of

cancer types exhibiting a signature with specific replication strand asymmetry. **(C)** Transcription

strand asymmetries of signatures of doublet-base substitutions (DBSs) and of small

insertions/deletions (IDs). Data are presented in a format similar to the one in panel (A). **(D)**

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

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

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

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

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

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

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

Relationships between 11 histone modifications and signatures of single base substitutions

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

Figure 6. Topography of signature SBS28 in *POLE* deficient (*POLE*⁻) and *POLE* proficient (*POLE*⁺) samples. (A) Mutational patterns of signature SBS28 in *POLE*⁻ and *POLE*⁺ samples displayed using the conventional 96 mutational classification schema for single base substitutions. **(B)** Nucleosome occupancy of SBS28 in *POLE*⁻ and *POLE*⁺ 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*⁻ and *POLE*⁺ 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*⁻
716 and *POLE*⁺ 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*⁻ and *POLE*⁺ 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 ≤ 0.05; ** q-value ≤ 0.01; *** q-value ≤ 0.001. **(F)** Enrichments and depletions
727 of SBS28 somatic mutations in *POLE*⁻ and *POLE*⁺ samples within CTCF binding sites, histone
728 modifications, and nucleosome occupied regions. Red colours correspond to enrichments of real

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

Figure 7. Topography of non-clustered, *omikli*, and *kataegis* substitutions across 288 whole-genome sequenced B-cell malignancies. (A) A rainfall plot of an example B-cell malignancy sample, MALY-DE_SP116612, depicting the intra-mutational distance (IMD) distributions of substitutions across genomic coordinates. Each dot represents the minimum distance between two adjacent mutations. Dots are coloured based on their corresponding classifications. Specifically, non-clustered mutations are shown in grey, doublet-base substitutions (DBSs) in red, multi-base substitutions (MBSs) in black, *omikli* events in green, *kataegis* events in orange, and all other clustered events in blue. The red line depicts the sample-dependent IMD threshold

for each sample. Specific clustered mutations may be above this threshold due to corrections for regional mutation density. **(B-D)** The trinucleotide mutational spectra for the different catalogues of non-clustered, *omikli*, and *kataegis* mutations for the exemplar sample (DBSs and MBSs are not shown). **(E)** Nucleosome occupancy of non-clustered, *omikli*, and *kataegis* mutations of B-cell malignancies. Blue solid lines and grey dashed lines display the average nucleosome signal (y-axes) along a 2 kilobase window (x-axes) centred at the mutation start site for real and simulated mutations, respectively. The mutation start site is annotated in the middle of each plot and denoted as 0. The 2 kilobase window encompasses 1,000 base-pairs 5' adjacent to each mutation as well as 1,000 base-pairs 3' adjacent to each mutation. **(F)** CTCF occupancy of non-clustered, *omikli*, and *kataegis* mutations of B-cell malignancies. Blue solid lines and grey dashed lines display the average CTCF signal (y-axes) along a 2 kilobase window (x-axes) centred at the mutation start site for real and simulated mutations, respectively. The mutation start site is annotated in the middle of each plot and denoted as 0. The 2 kilobase window encompasses 1,000 base-pairs 5' adjacent to each mutation as well as 1,000 base-pairs 3' adjacent to each mutation. **(G)** Replication timing of non-clustered, *omikli*, and *kataegis* mutations of B-cell malignancies. Replication time data are separated into deciles, with each segment containing exactly 10% of the observed replication time signal (x-axes). Normalized mutation densities per decile (y-axes) are presented for early (left) to late (right) replication domains. Normalized mutation densities of real somatic mutations and simulated somatic mutations from early to late replicating regions are shown as blue bars and dashed lines, respectively. **(H)** Enrichments and depletions of non-clustered, *omikli*, and *kataegis* mutations of B-cell malignancies within CTCF binding sites and histone modifications. Red colours 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 ≤ 0.05).
 779

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Somatic mutations in genic and intergenic regions imprinted by different

mutational signatures. (A) Somatic mutations in genic and intergenic regions for signatures of

single base substitutions (SBSs). Rows represent the signatures, where n reflects the number of

cancer types in which each signature was found. Columns display the six substitution subtypes

based on the mutated pyrimidine base: C>A, C>G, C>T, T>A, T>C, and T>G. SBS signatures

with genic and intergenic regions asymmetries with q -value ≤ 0.05 are shown in circles with

cyan and grey colours, respectively. The colour intensity reflects the odds ratio between the ratio

of real mutations and the ratio of simulated mutations, where each ratio is calculated using the

number of mutations in the genic regions and the number of mutations in the intergenic regions.

Only odds ratios above 1.10 are shown. Circle sizes reflect the proportion of cancer types

exhibiting a signature with specific genic versus intergenic regions asymmetry. **(B)** Somatic

mutations in genic and intergenic regions for signatures of doublet-base substitutions (DBSs).

Data are presented in a format similar to the one in panel (A). **(C)** Somatic mutations in genic and

intergenic regions for small insertions/deletions (IDs). Data are presented in a format similar to

the one in panel (A). **(D)** Histogram of fold enrichment as odds ratio between the ratio of real

mutations and the ratio of simulated mutations, where each ratio is calculated using the number

of mutations in the genic regions and the number of mutations in the intergenic regions.

Frequency of fold enrichments (y-axis) are presented for discreet bins of fold enrichments (x-

axis). Each fold enrichment reflects the odds ratio between real and simulated mutations where

each ratio is the number of mutations in intergenic regions divided by the number of mutations in

genic regions. Total number of fold enrichments, mean, and standard deviation of fold

enrichments are shown in the upper right corner of the histogram. **(E)** Same format as panel (D)

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

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

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

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

Figure S3. Strand asymmetries of non-clustered, *omikli*, and *kataegis* substitutions across 288 whole-genome sequenced B-cell malignancies. Transcription strand asymmetries are shown in the left panels where bars display the six substitution subtypes based on the mutated pyrimidine base: C>A, C>G, C>T, T>A, T>C, and T>G (depicted on the x-axes). Y-axes correspond to the numbers of single base substitutions. Blue bars reflect real transcribed substitutions, while shaded blue bars correspond to simulated transcribed substitutions. Similarly, green bars reflect real untranscribed mutations, whereas shaded green bars correspond to simulated untranscribed substitutions. Replication strand asymmetries are shown in the middle panels where bars display the six substitution subtypes based on the mutated pyrimidine base: C>A, C>G, C>T, T>A, T>C, and T>G (depicted on the x-axes). Y-axes correspond to the numbers of single base substitutions. Red bars reflect real substitutions on the lagging strand, while shaded red bars correspond to simulated substitutions on the lagging strand. Similarly, yellow bars reflect real substitutions on the leading strand, whereas shaded yellow bars correspond to simulated substitutions on the leading strand. Comparisons of genic and intergenic regions are shown in the right panels where bars display the six substitution subtypes based on the mutated pyrimidine base: C>A, C>G, C>T, T>A, T>C, and T>G (depicted on the x-axes). Y-axes correspond to the numbers of single base substitutions. Cyan bars reflect real substitutions in genic regions, while shaded cyan bars correspond to simulated substitutions in genic regions. Similarly, grey bars reflect real substitutions in intergenic regions, whereas shaded grey bars 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 ≤ 0.05 ; ** q-value \leq
 874 0.01; *** q-value ≤ 0.001 .

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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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DECLARATION OF INTERESTS

LBA is a compensated consultant and has equity interest in *io9*, LLC. His spouse is an employee of Biotheranostics, Inc. LBA is also an inventor of a US Patent 10,776,718 for source identification by non-negative matrix factorization. ENB and LBA declare provisional patent applications for “Clustered mutations for the treatment of cancer” (U.S. provisional application serial number 63/289,601) and “Artificial intelligence architecture for predicting cancer biomarker” (serial number 63/269,033). All other authors declare no competing interests.

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.

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

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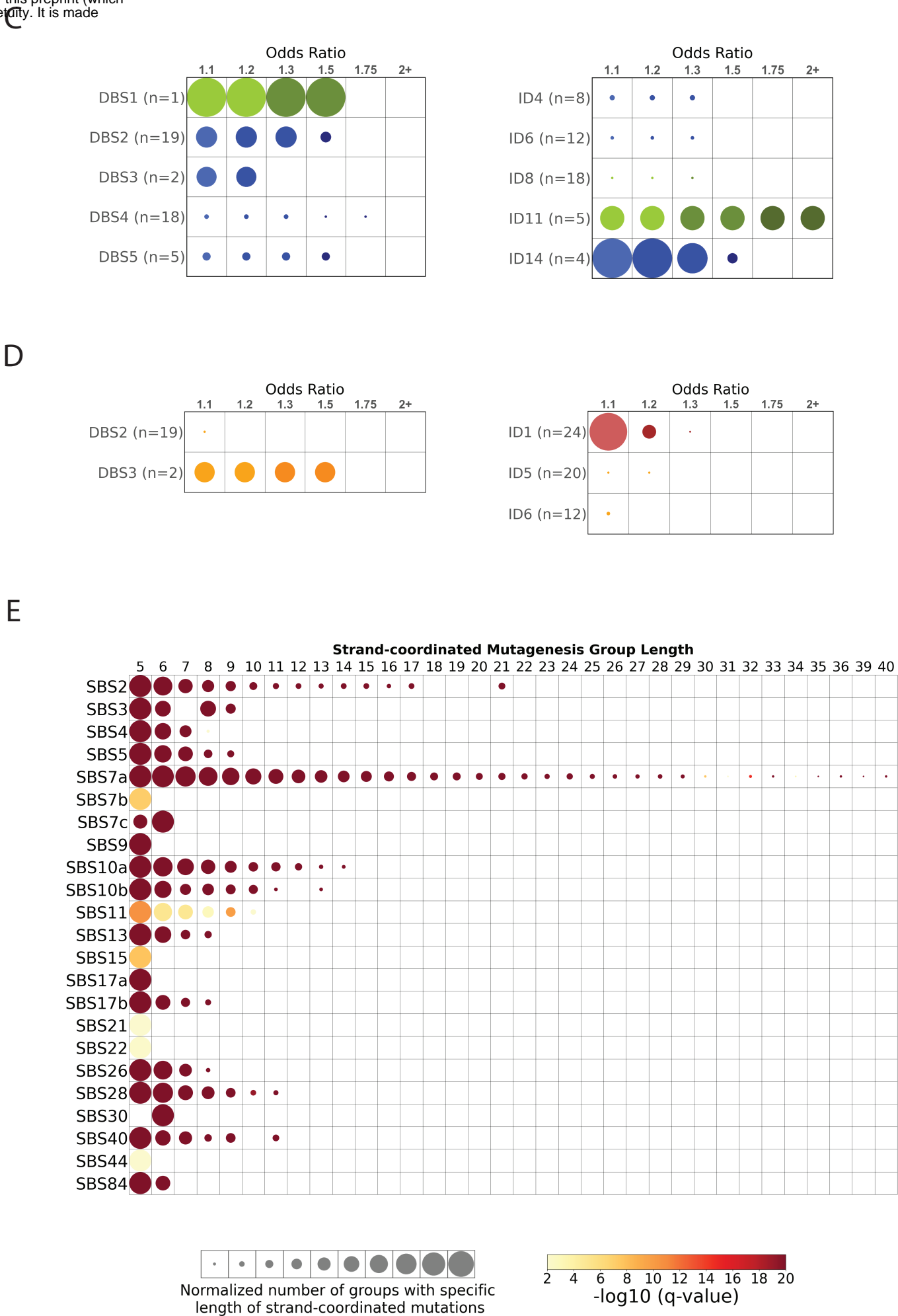
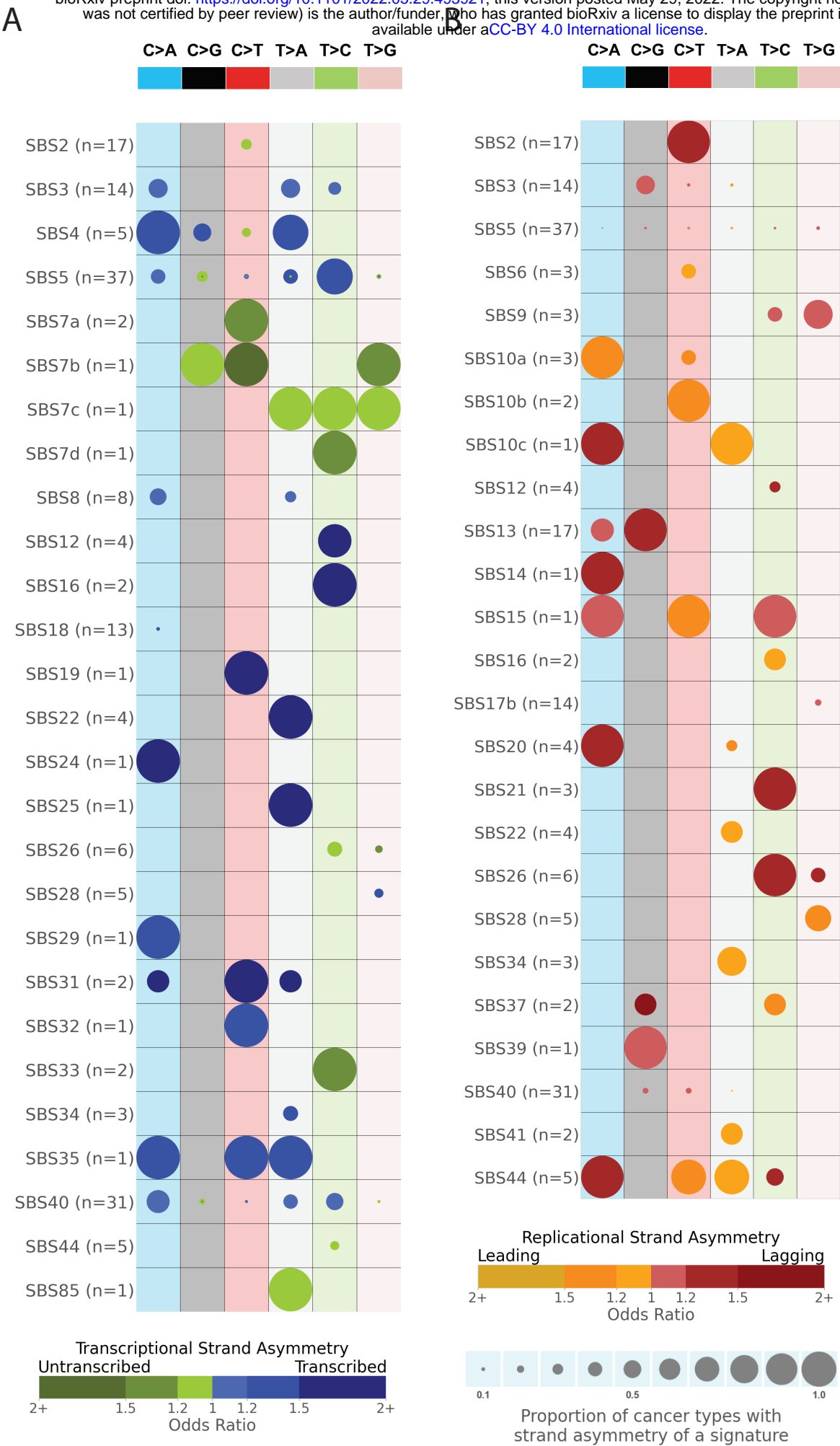


Figure 2. Interplay between replication timing and mutational signatures.

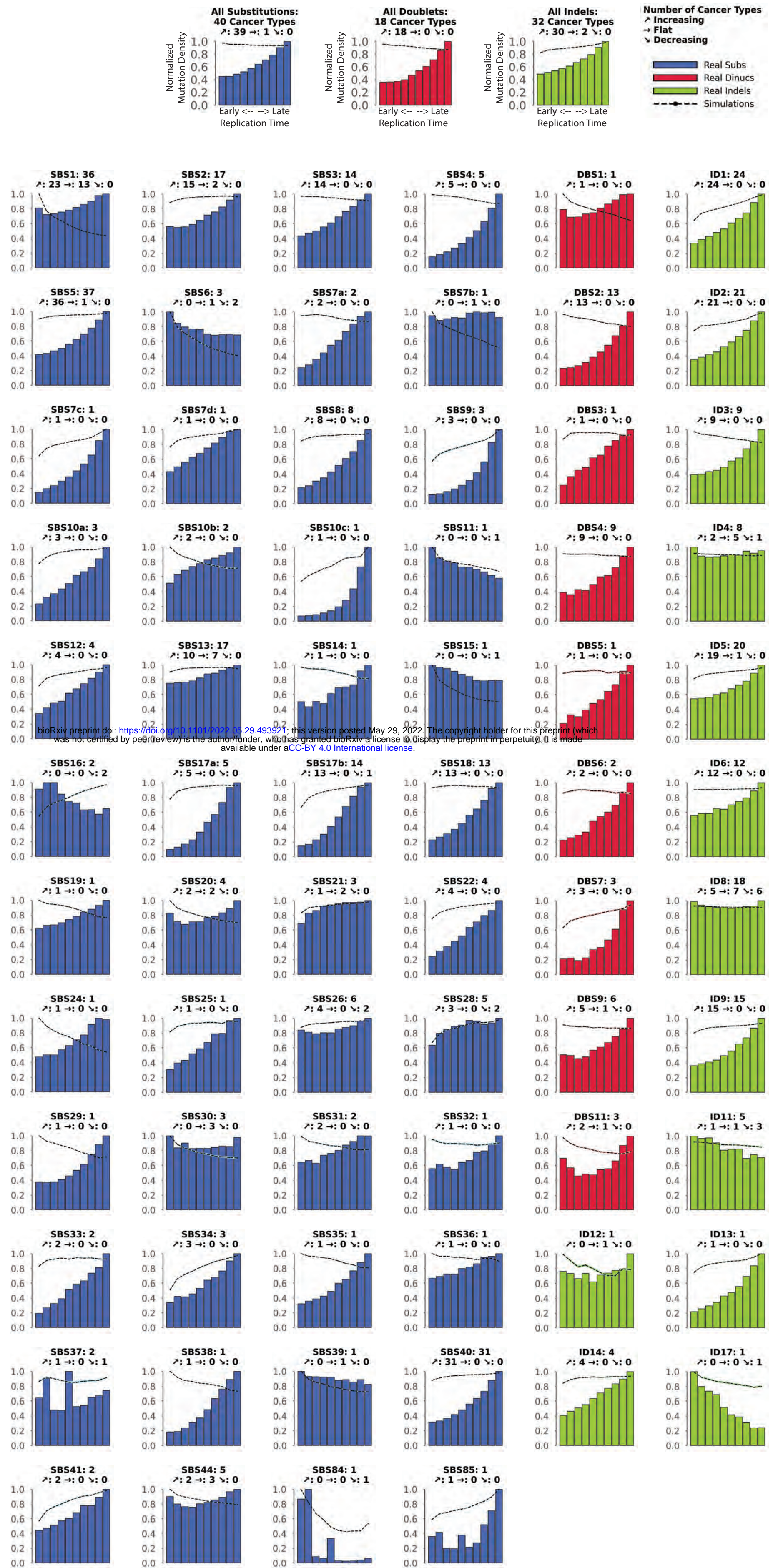


Figure 3. Relationship between mutational signatures and nucleosome occupancy.

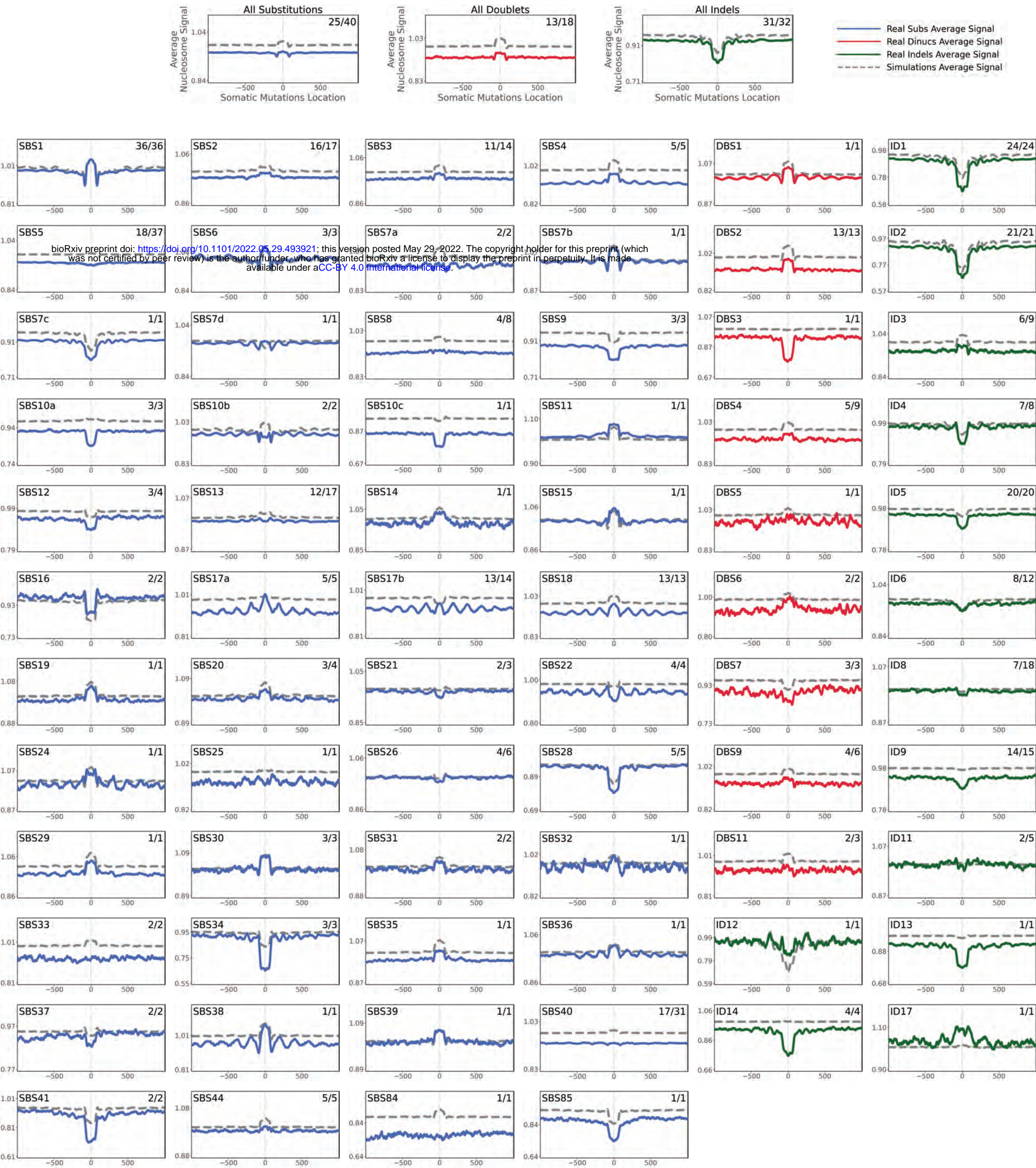
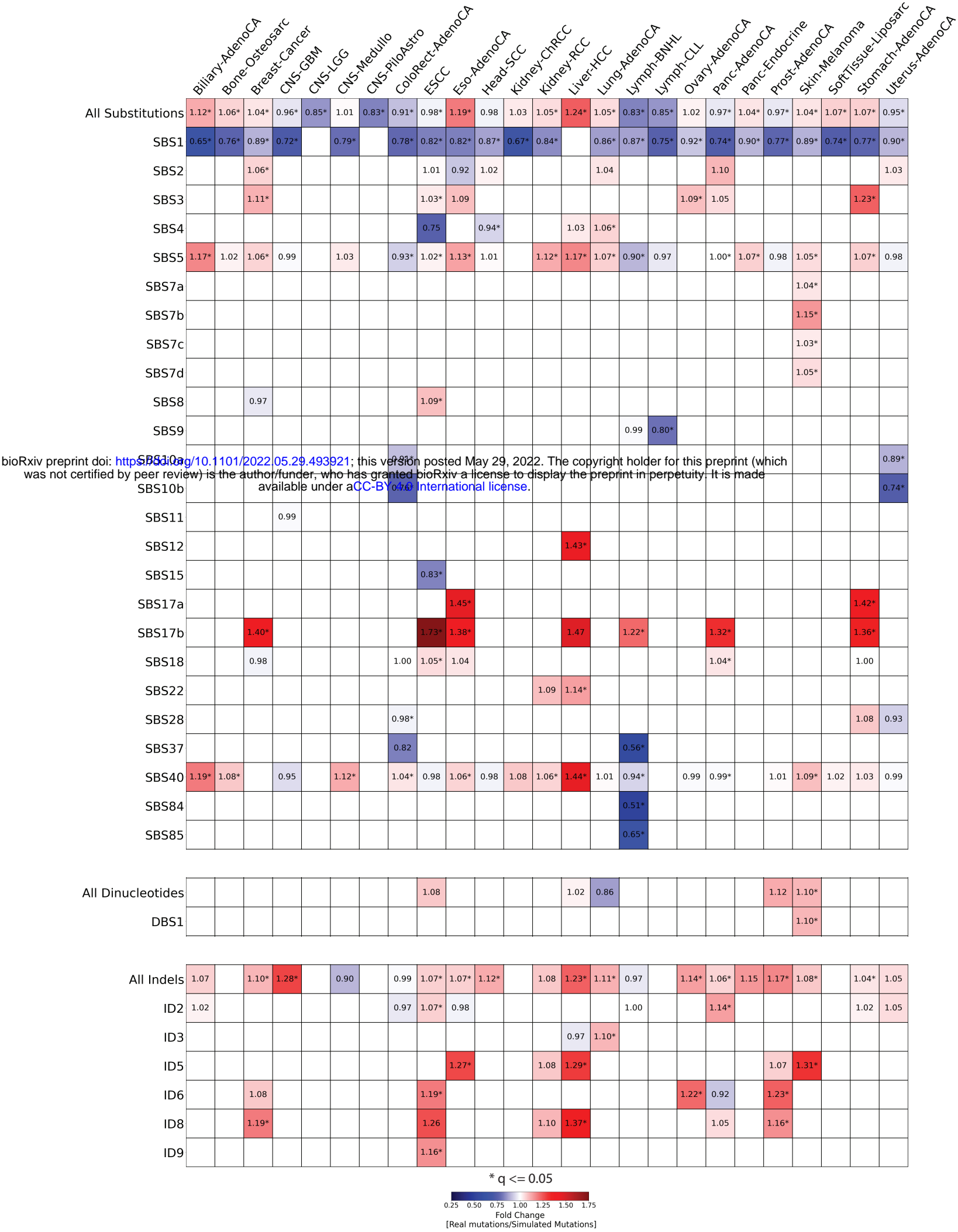


Figure 4. Relationship between mutational signatures and CTCF binding sites.

A



B

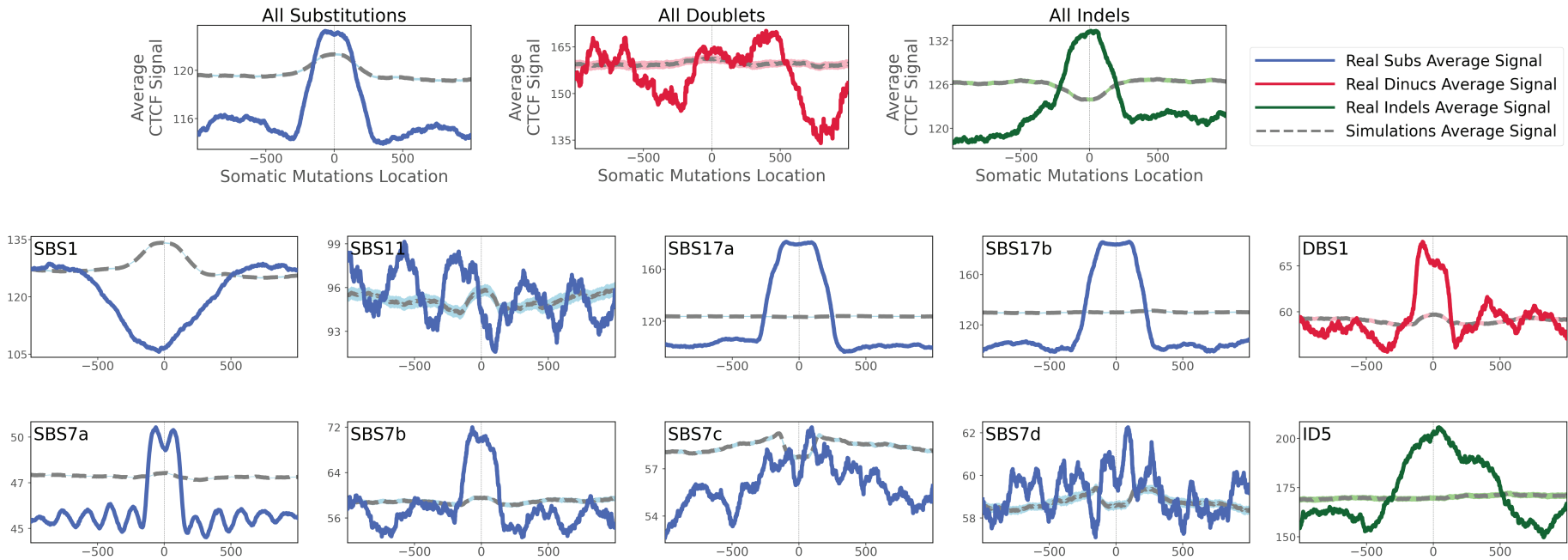
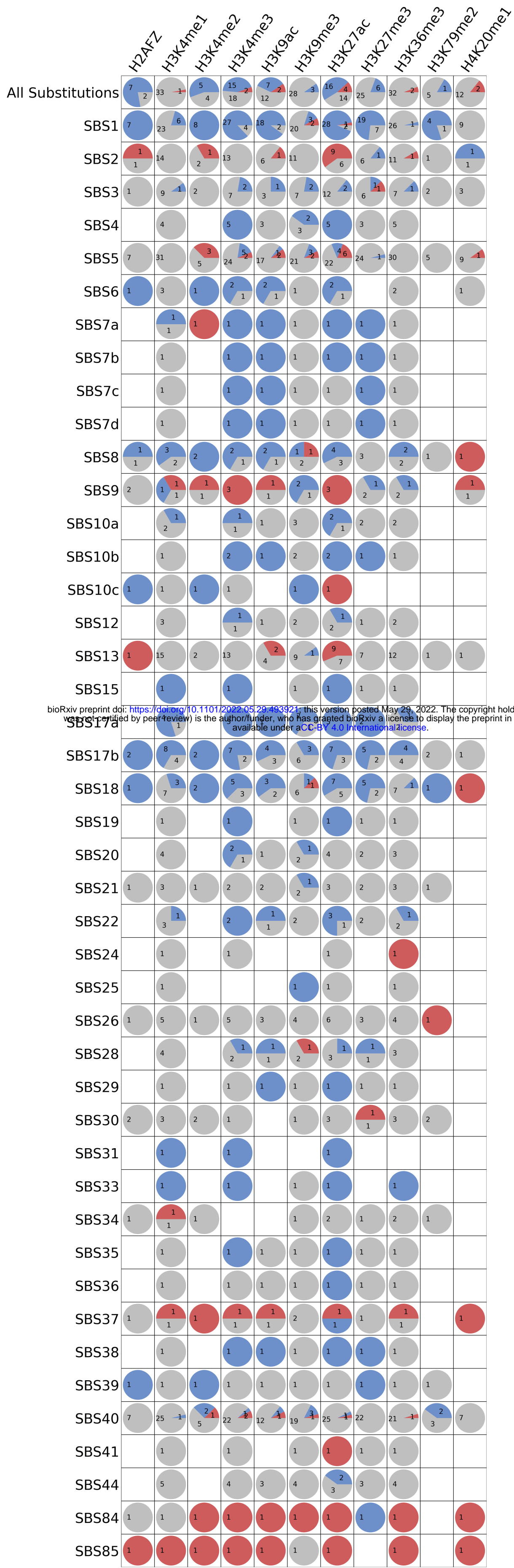
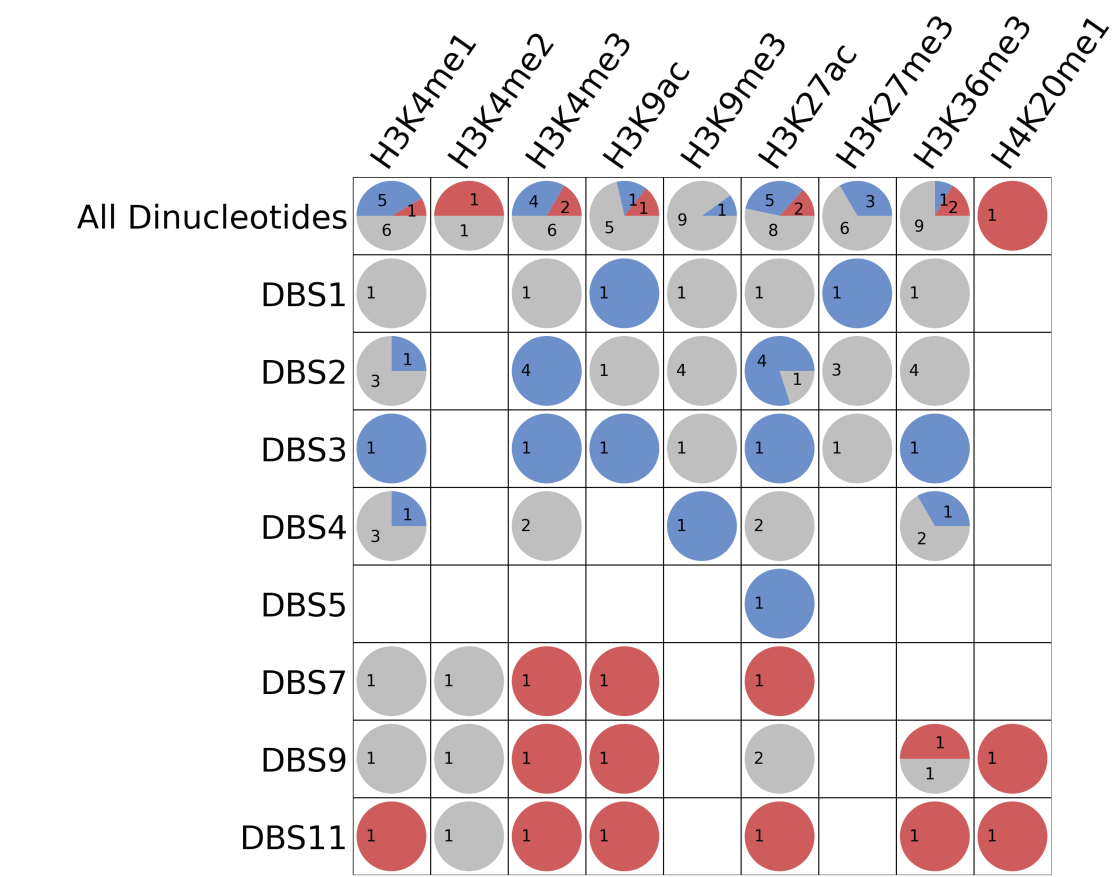


Figure 5. Relationships between mutational signatures and histone modifications.

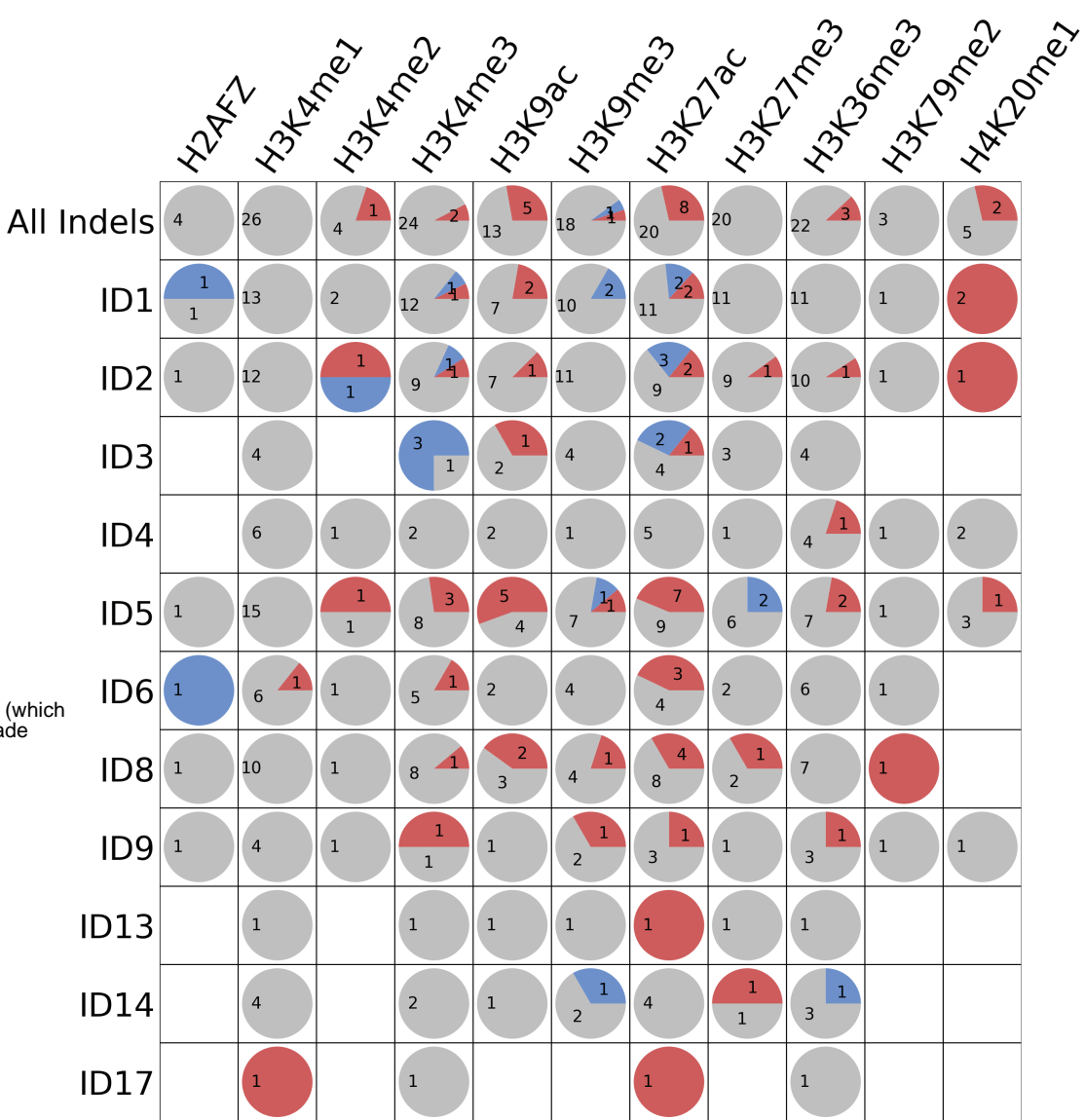
A



B



C



D

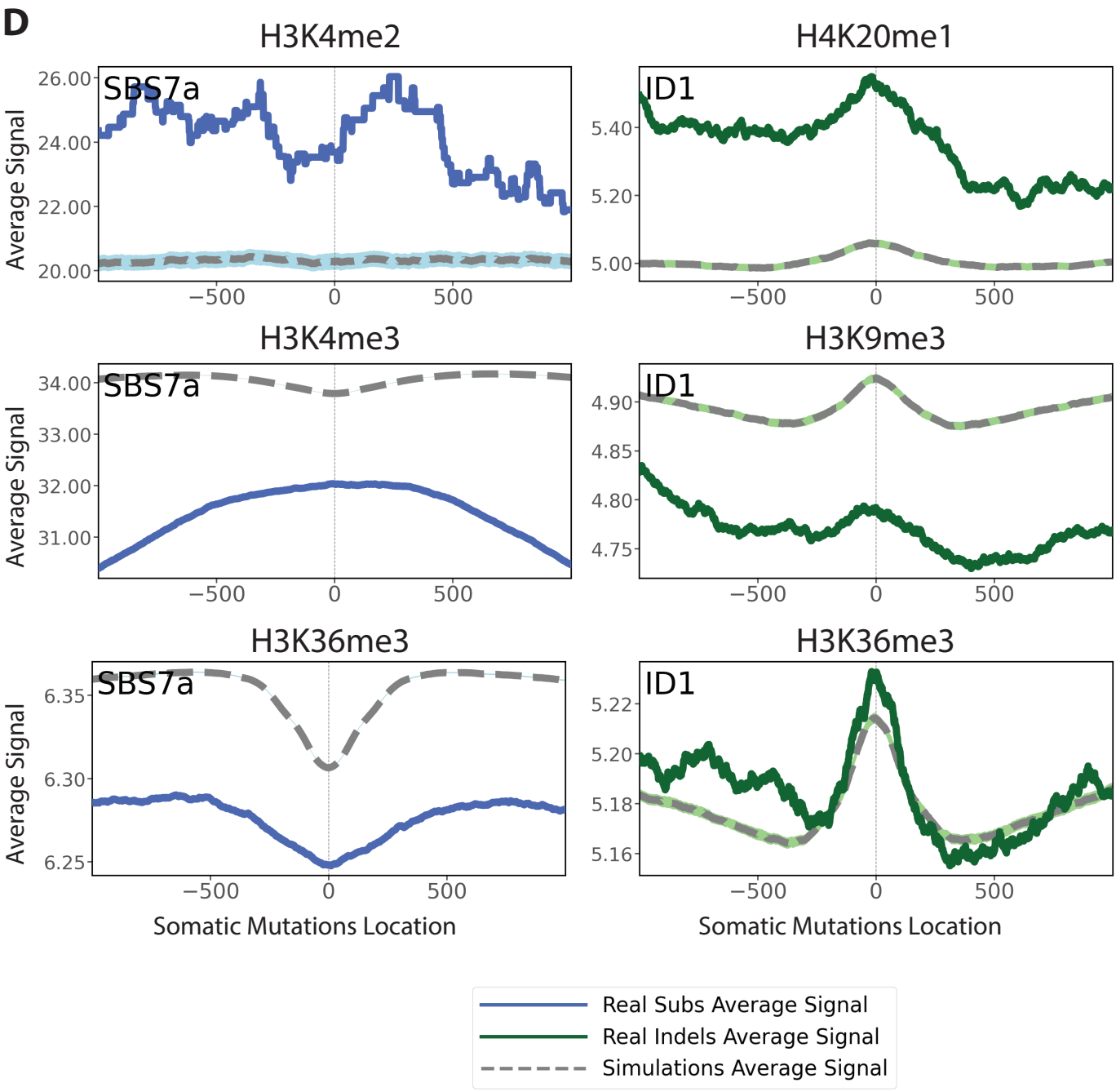
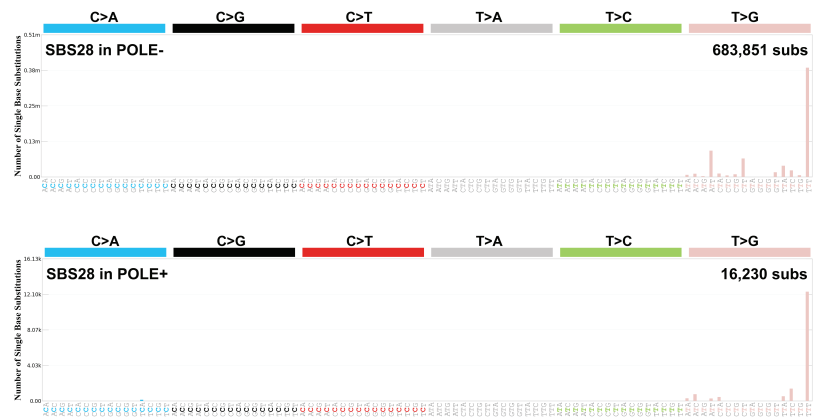
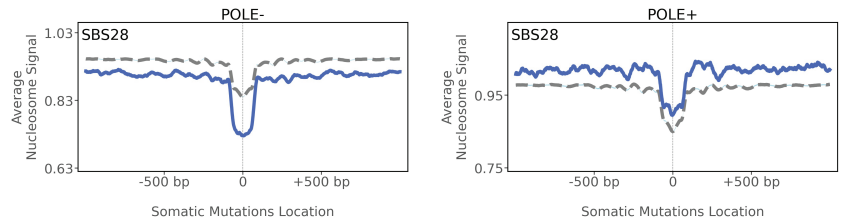


Figure 6. Topography of signature SBS28 in POLE deficient (POLE-) and POLE proficient (POLE+) samples.

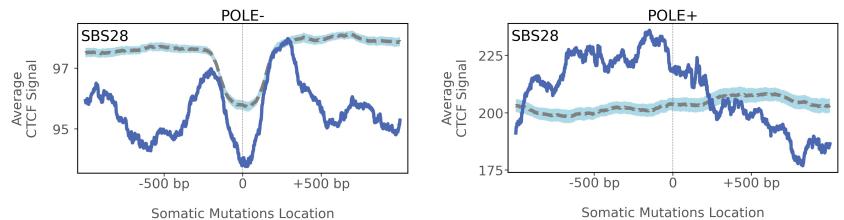
A



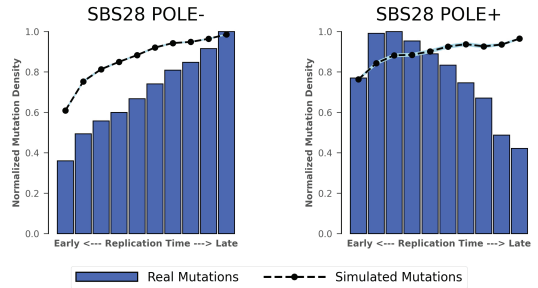
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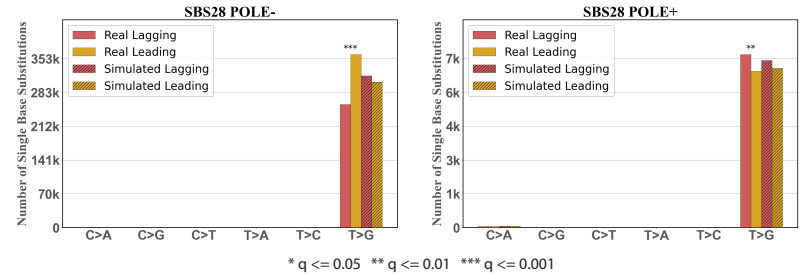
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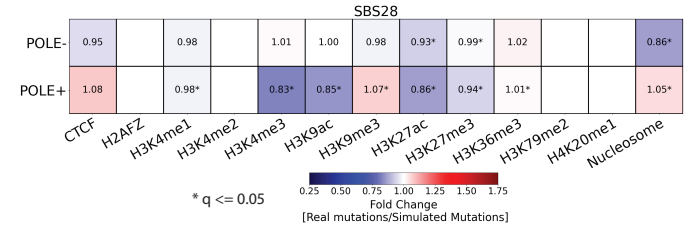
D



E



F



G

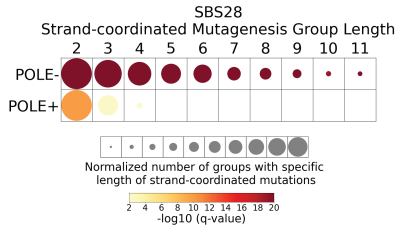


Figure 7. Topography of non-clustered, omikli, and kataegis substitutions across 288 whole-genome sequenced B-cell malignancies.

