TITLE

Inherited *MUTYH* mutations cause elevated somatic mutation rates and distinctive mutational signatures in normal human cells

AUTHORS

Philip S. Robinson[1,2], Laura E. Thomas[3], Federico Abascal[1], Hyunchul Jung[1], Luke M.R. Harvey[1], Hannah D. West[4], Sigurgeir Olafsson[1], Bernard C. H. Lee[1,5], Tim H.H. Coorens[1], Henry Lee-Six[1], Laura Butlin[4], Nicola Lander[4], Mathijs A. Sanders[1,6], Stefanie V. Lensing[1], Simon J.A. Buczacki[7], Rogier ten Hoppen[8], Nicholas Coleman[9,10], Roxanne Brunton-Sim[11], Simon Rushbrook[11,12], Kourosh Saeb-Parsy[13,14], Fiona Lalloo[15], Peter J. Campbell[1], Iñigo Martincorena[1], Julian R. Sampson[4], Michael R. Stratton[1]*

AFFILIATIONS

1. Cancer, Ageing and Somatic Mutation (CASM), Wellcome Sanger Institute, Hinxton, CB10 1SA, UK.
2. Department of Paediatrics, University of Cambridge, Cambridge, CB2 0QQ, UK.
3. Institute of Life Science, Swansea University, Swansea, SA28PP, UK.
4. Institute of Medical Genetics, Division of Cancer and Genetics, Cardiff University School of Medicine, Cardiff, UK.
5. Hereditary Gastrointestinal Cancer Genetic Diagnosis Laboratory, Department of Pathology, The University of Hong Kong, Queen Mary Hospital, Pokfulam, Hong Kong
6. Department of Haematology, Erasmus University Medical Centre, 3015 CN, Rotterdam, The Netherlands
7. Nuffield Department of Surgical Sciences, Medical Sciences Division, University of Oxford, Oxford, UK.
8. Department of Oncology, University of Cambridge, Cambridge, UK.
9. Department of Pathology, University of Cambridge, Cambridge, UK.
10. Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK.
11. Norfolk and Norwich University Hospital, Norwich, UK.
12. Norwich Medical School, University of East Anglia, Norwich, UK.
13. Department of Surgery, University of Cambridge, Cambridge, UK.
14. Cambridge NIHR Biomedical Research Centre, Cambridge Biomedical Campus, Cambridge, UK.
15. Manchester Centre for Genomic Medicine, Saint Marys Hospital, Oxford Road, Manchester, UK.

* Corresponding author mrs@sanger.ac.uk

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Summary

KEYWORDS

Somatic mutations, normal tissues, genomics, ageing, mutational signatures, DNA repair

ABSTRACT

Cellular DNA damage caused by reactive oxygen species is repaired by the base excision repair (BER) pathway which includes the DNA glycosylase MUTYH. Inherited biallelic *MUTYH* mutations cause predisposition to colorectal adenomas and carcinoma. However, the mechanistic progression from germline *MUTYH* mutations to **M**UTYH-**A**ssociated **P**olyposis (MAP) is incompletely understood. Here, we sequenced normal tissue DNAs from 10 individuals with MAP. Somatic base substitution mutation rates in intestinal epithelial cells were elevated 2 to 5-fold in all individuals, except for one showing a 33-fold increase, and were also increased in other tissues. The increased mutation burdens were of multiple mutational signatures characterised by C>A changes. Different mutation rates and signatures between individuals were likely due to different *MUTYH* mutations or additional inherited mutations in other BER pathway genes. The elevated base substitution rate in normal cells likely accounts for the predisposition to neoplasia in MAP. Despite ubiquitously elevated mutation rates, individuals with MAP do not display overt evidence of premature ageing. Thus, accumulation of somatic mutations may not be sufficient to cause the global organismal functional decline of ageing.

INTRODUCTION

The genomes of all normal human cells are thought to acquire mutations during the course of life. However, the mutation rates of normal cells and the processes of DNA damage, repair and replication that underlie them are incompletely understood[1-8]. A ubiquitous source of potential mutations is DNA damage caused by reactive oxygen species (ROS) which are formed as by-products of aerobic metabolism[9]. ROS cause a variety of DNA lesions, the most common being 8-oxoguanine (8-OG)[10]. As a consequence of mispairing with adenine during DNA replication, 8-OG can cause G:C>T:A (referred to as C>A for brevity) transversion mutations[11]. Under normal circumstances, 8-OG and its consequences are efficiently mitigated by the Base Excision Repair (BER) pathway effected by DNA glycosylases; oxoguanine DNA glycosylase (OGG1) removes 8-OG[12] and MutY DNA glycosylase (MUTYH) removes adenines misincorporated opposite 8-OG[13].

Mutations in *MUTYH* engineered in experimental systems can impair its glycosylase activity, reducing its ability to excise mispaired bases and leading to an increased rate of predominantly C>A mutations[14-18]. *MUTYH* mutations inherited in the germline in humans cause an autosomal recessive syndrome (MUTYH-associated polyposis, MAP) characterised by intestinal adenomatous polyposis and an elevated risk of early onset colorectal and duodenal cancer[19-22]. The age of onset and the burden of intestinal polyps are highly variable between individuals, ranging from 10s to 100s leading to a substantially increased incidence of colorectal cancer.[23-27] Risks of other cancer types are also thought to be increased[28].

Colorectal adenomas and carcinomas from individuals with MAP show a predominance of C>A mutations consistent with the presence of an elevated mutation rate attributed to defective MUTYH function[29-33]. However, whether there is an increased mutation rate in normal cells from individuals with biallelic germline *MUTYH* mutations is unknown. If present in normal cells, understanding the magnitude of the increase in mutation rate, the tissues and cell types in which it occurs, the proportion of cells which show it, the mutational processes responsible and the effects of early neoplastic change would provide insight into the genesis of the elevated cancer risk observed in these individuals. In this study we characterise the somatic mutation rates and mutational signatures of normal intestinal cells, and other normal cells, from individuals with MAP.

RESULTS

Clinical information

Ten individuals aged 16 to 79 years with biallelic germline *MUTYH* mutations were studied. These included five missense mutation homozygotes (four $MUTYH^{Y179C+/+}$, one $MUTYH^{G286E+/+}$), three compound heterozygotes for the same pair of missense mutations ($MUTYH^{Y179C+/-;G396D+/-}$), and two siblings homozygous for a nonsense mutation ($MUTYH^{Y104*+/+}$). These *MUTYH* germline mutations have all been previously recognised as predisposing to MAP[22,23]. All 10 individuals had colorectal polyposis, with between 16 and >100 colonic adenomas, six were known to have duodenal polyps, five had colorectal cancer and one developed jejunal and pancreatic neuroendocrine cancer (Extended Data Table 1).

Mutation rates in normal intestinal stem cells

105 An intestinal crypt is constituted predominantly of a population of epithelial cells arising from a single
106 recent common ancestor[34-36]. The somatic mutations which have accumulated over the course of the
107 individual's lifetime in the ancestral crypt stem cell are present in all its descendant cells[3]. Thus, by
108 sequencing individual crypts, somatic mutations present in the ancestral stem cell can be identified.
109 Using laser-capture microdissection, 144 individual normal intestinal crypts (large intestine n=107 and
110 small intestine n=37) were isolated from the 10 individuals with germline *MUTYH* mutations (Extended
111 Data Table 2). DNA libraries were prepared from individual crypts using a bespoke low-input DNA
112 library preparation method[37] and were whole-genome sequenced at a mean 28-fold coverage.
113
114 The single base substitution (SBS) mutation burdens of individual crypts ranged from a median for
115 each individual of 2,294 to 33,350, equating to mutation rates of 79-1470 SBS/year, 2-33-fold higher
116 than normal crypts from wild-type individuals (~43 SBS/year)[3] (Figure 1b). Therefore, all normal crypts
117 from all MAP individuals studied showed elevated somatic mutation rates (Figure 1a-b).
118
119 Differences in mutation rate were observed between individuals with MAP (Figure 1b). A 33-fold
120 higher rate of SBS accumulation than in wild-type crypts[3] was observed in PD44890, a 16 year old male
121 with $MUTYH^{Y179C+/-\ G396D+/-}$. By contrast, the nine other individuals showed only 2- to 5-fold increases in
122 mutation rate compared to wild type. The reason for this substantial difference is not clear. However,
123 in addition to the *MUTYH* mutations, PD44890 carried two heterozygous germline missense variants
124 in *OGG1* (Extended Data Figure 1), one of which (R46Q) is reported to impair OGG1 activity in
125 experimental systems[38,39] and has been observed as somatically mutated in human cancer[40]. Germline
126 *OGG1* mutations are not currently recognised as causing cancer predisposition in humans[41]. However,
127 if either or both of these mutations results in defective 8-OG excision they could account for the
128 substantially elevated mutation rate in PD44890, particularly in the context of defective MUTYH
129 activity.
130
131 There was also evidence of differences in mutation rates between the various *MUTYH* germline
132 genotypes studied (Fig. 1b). Excluding the outlier individual PD44890, mutation rates were lower in
133 individuals with the compound heterozygous $MUTYH^{Y179C+/-\ G396D+/-}$ (102 SBS/year, range 61-182) than
134 individuals with $MUTYH^{Y179C+/+}$ (214 SBS/year, range 84-356), $MUTYH^{Y104*+/+}$ (204 SBS/year, range 153-
135 309) or $MUTYH^{G286E+/+}$ (152 SBS/year, range 82-238) ($MUTYH^{Y179C+/-\ G396D+/-}$ vs $MUTYH^{Y179C+/+}$ /
136 $MUTYH^{Y104*+/+}$ / $MUTYH^{G286E+/+}$,P=9.5x10^{-8}, P=6.1x10^{-19}, P=9.8x10^{-3} respectively. $MUTYH^{G286E+/+}$ vs.
137 $MUTYH^{Y179C+/+}$ / $MUTYH^{Y104*+/+}$ P=2.7x10^{-2} and P=4.5x10^{-3} respectively). The results, therefore, indicate
138 that different *MUTYH* genotypes confer differentially elevated mutation rates and that the extent of
139 the mutation rate increase can be modified by other factors.
140
141 SBS mutation rates in coding exons in normal intestinal crypts from MAP individuals were also
142 elevated compared to wild-type individuals (Extended Data Fig 2a-b). These increases were, however,
143 slightly smaller than those observed in the genome-wide mutation rate (Extended Data Fig 2a-b).
144 Nonsense, missense and synonymous mutation rates were all increased compared with wild-type
145 crypts, with the greatest increase observed in nonsense mutations (~10-fold more nonsense than
146 wild-type vs ~3.5-fold more missense and ~2.6-fold more synonymous) (Extended Data Fig. 2c). This
147 is attributable to the mutational signatures present (see below) and the tendency of specific mutations
148 at particular trinucleotide contexts to preferentially generate protein-truncating mutations[42,43] .
149

Neoplastic glands from 13 intestinal adenomas from five individuals with MAP showed SBS mutation burdens that were, on average, ~2-fold higher (range 1.2 to 2.5 fold) (Figure 1c) than normal crypts from the same individuals sampled at the same time. Therefore, the elevated mutation rate observed in histologically normal intestinal crypts in individuals with germline *MUTYH* mutations is further increased during the process of neoplastic transformation, as previously observed in wild type individuals[44,45].

Small insertion and deletion (ID) mutations accumulated at a rate of 2.1 ID/yr (linear mixed-effects model, 95% confidence interval (C.I.) 1.2-3.0, $P = <10^{-4}$), which is higher than in wild-type controls (1.3 ID/yr, linear mixed-effects model, C.I. 0.54-2.0, $P = 0.0011$)[3,43]. The cause of this modestly elevated ID rate is not clear. In two MAP individuals additional mutational processes could explain the higher burdens observed in these cases. In PD44890 the high ID mutation rate (ID rate 6/yr) was, at least partially, explained by the presence of an additional ID generating mutational process associated with exposure to the mutagen colibactin produced by a strain of E.*Coli*[3,46,47] present in the colonic microbiome of some people (see below). In PD50747 (ID rate 6/yr), a previously undescribed sporadic ID signature IDA was identified which was not present in other MAP individuals (described below). Structural rearrangements and copy number changes were only observed in a small number of normal intestinal crypts, at similar frequencies to those in wild-type controls (Extended Data Table 2)[3].

Mutational signatures

Mutational signatures were extracted from the combined catalogues of SBS mutations from all normal and neoplastic intestinal crypts and glands using two independent methods. We then decomposed each *de novo* extracted signature into known COSMIC reference mutational signatures. Finally, we used these decompositions to estimate the contribution of each reference signature to each sample (Methods, Supplementary Note). Three *de novo* extracted signatures, N1-N3, accounted for the majority of mutations, all of which were mainly characterised by C>A mutations (Fig. 2a). Two of these closely corresponded to the reference mutational signatures SBS18 and SBS36. The third was abundant only in individual PD44890 (the individual with a high mutation rate carrying *OGG1* germline variants) (Fig. 2b and Supplementary Note) but could also be accounted for by a combination of SBS18 and SBS36 according to the standard parameters used for decomposition (Supplementary Information).

Following decomposition and signature attribution, four reference SBS mutational signatures, SBS1, SBS5, SBS18 and SBS36 were identified in all samples (Fig. 3, Supplementary Note). SBS1, due to deamination of 5-methlycytosine at CG dinucleotides and SBS5, of unknown aetiology, have both been found ubiquitously in normal and cancer cells and accumulate in a more or less linear fashion with age[2-4,8,33,48-50]. SBS18, thought to result from DNA damage due to reactive oxygen species, has previously been reported in normal colorectal cells[3] and many types of cancer[33] and is characterised by C>A mutations predominantly at A<u>C</u>A, C<u>C</u>A, G<u>C</u>A and T<u>C</u>T trinucleotide contexts (mutated base underlined) (Fig. 2a and Fig. 3). SBS36 has previously been found in cancers with germline or somatic *MUTYH* mutations and is also characterised by C>A mutations, albeit with a different profile of preferred trinucleotide contexts from SBS18[30-33](Fig. 2a). SBS88, which is predominantly characterised by T>C and T>G mutations, and is due to early life exposure to the mutagenic agent colibactin produced by some strains of *E.Coli*[3,46,47], was observed in a subset of crypts from PD44890 (Figure 3b).

194 The SBS88 mutation burdens were consistent with those previously seen in wild type individuals
195 indicating that MUTYH is unlikely to be implicated in the genesis of SBS88.
196
197 The increased SBS mutation burdens in normal crypts from individuals with *MUTYH* germline
198 mutations appeared to be due to the contributions of SBS18 and SBS36 mutations (Fig. 3a-e). The
199 proportions of SBS18 and SBS36, however, differed between *MUTYH* germline genotypes. SBS18
200 accounted for a substantially higher proportion of mutations in crypts and glands from individuals
201 with the $MUTYH^{Y179C+/-\ G396D+/-}$ genotype (n=85 crypts) than in individuals with the $MUTYH^{Y179C+/+}$,
202 $MUTYH^{Y104*+/+}$ and $MUTYH^{G286E+/+}$ genotypes (n=59 crypts, Extended Data Fig. 3a). Since $MUTYH^{Y104*}$
203 causes MUTYH protein truncation, it is conceivable that SBS36 is the consequence of complete loss of
204 MUTYH function and therefore that this is also effected by $MUTYH^{Y179C}$ and $MUTYH^{G286E}$. Conversely,
205 $MUTYH^{G396D}$ may retain partial activity[14,21] and thus generates a signature more closely resembling
206 SBS18 which is found in normal tissues with fully active MUTYH.
207
208 The *de novo* extracted mutational signature N2, which primarily contributes to the mutational spectra
209 of crypts from PD44890 ($MUTYH^{Y179C+/-\ G396D+/-}$), resembled reference signature SBS18
210 (https://cancer.sanger.ac.uk/cosmic/signatures) but showed differences, notably with over
211 representation of C>A mutations at GCA and, to a lesser extent, CCA and ACA trinucleotides (mutated
212 base underlined) (Fig. 2a-b and Extended Data Fig. 1). A signature reported in human cells with *in vitro*
213 engineered biallelic *OGG1* deletion is also primarily characterised by C>A mutations at GCA and ACA
214 trinucleotides[51]. It is, therefore, possible that mutagenesis due to the germline *OGG1* variant(s) in
215 PD44890 (see above) is superimposed on the mutational signature produced by the *MUTYH* germline
216 mutations to generate N2 (see Supplementary Information for further analysis and discussion).
217
218 The mutational signatures in adenoma glands were similar to those seen in normal crypts from the
219 same individuals (Fig. 3a,b,d,e). SBS36 and SBS18 were principally responsible for the increased
220 mutation burdens observed in adenomas compared to normal crypts.
221
222 Candidate cancer "driver" mutations, defined as known or likely oncogenic hotspot mutations and
223 truncating mutations in tumour suppressor genes (Methods), were observed in 15% of normal crypts
224 (22/144), more than double the rate observed in wild-type crypts from comparable healthy controls;
225 6% (25/449)[3,43]. A substantial proportion of candidate drivers (16/22) were nonsense mutations,
226 mirroring the broader exome-wide increase in nonsense mutations (Extended Data Fig. 2c), and
227 reflecting the proclivity of certain mutation types to generate truncating mutations[29,42,43]. The
228 mutational spectrum of driver mutations in normal crypts and neoplastic glands resembled the
229 genome-wide spectra with substantial contributions from SBS18 and SBS36 (Extended Data Fig. 4).
230 Hence, the mutational processes resulting from defective MUTYH activity appear to promote the
231 accumulation of putative cancer driver mutations in normal and neoplastic tissues[52,53].
232
233 Three known ID signatures were identified. ID1 & ID2 are characterised predominantly by insertions
234 and deletions of single T bases at T mononucleotide repeats which are associated with strand slippage
235 during DNA replication and are seen in most human cancers and normal tissues[1-4,8,33]. ID18 is
236 associated with colibactin exposure, is found in normal intestinal stem cells and certain cancers, and
237 usually associated with SBS88[3,47]. ID1 was the dominant signature in normal cells whereas ID2
238 predominated in neoplastic cells (Extended Data Fig. 5). ID18 was principally observed in samples from

239 PD44890 and is responsible for the elevated ID rate in this individual (Extended Data Fig. 6). A further
240 ID signature, IDA, identified in PD50747, was characterised by single C insertions at C mononucleotide
241 repeats (Extended Data Fig. 6). IDA was present in both normal crypts (~5% of total ID burden) and to
242 a greater extent in adenoma glands (~20% of total ID burden). The cause of this previously undescribed
243 signature is unclear but may be associated with previous capecitabine treatment and seems unlikely
244 to be related to germline MUTYH mutations.
245
246 **Mutations in other cell types**
247 To investigate whether the elevated mutation rates and mutational signatures observed in intestinal
248 epithelium caused by defective MUTYH are present in other cell types, peripheral white blood cell and
249 tissue lymphocyte DNAs from individuals with biallelic *MUTYH* mutations were whole genome
250 sequenced using a duplex sequencing method (NanoSeq)[50] that allows mutation calling from single
251 DNA molecules and thus accurately discovers somatic mutations in tissues in which multiple clonal
252 lineages are intimately mixed.
253
254 The white blood cell SBS mutation rates of all individuals with *MUTYH* mutations were higher than
255 wild-type controls (n=15 granulocyte samples from 9 healthy individuals aged 20-80yrs)(Fig. 4)(25
256 SBS/yr vs 19 SBS/yr, linear mixed-effects model, R^2=0.89, MUTYH; 95% C.I., 19-31, P=10^{-7} and wild-
257 type; 95% C.I., 14-24, P=10^{-6}). The relative increases in blood mutation rates were lower than in
258 intestinal crypts from each individual (Fig. 4b). Nevertheless, the relative increases paralleled the
259 differential increases observed between individuals in intestinal crypts. SBS mutation rates in tissue
260 lymphocytes were modestly raised compared with wild-type healthy individuals (Fig. 4d) (53 SBS/yr
261 vs 40 SBS/yr, linear mixed-effects model, R^2=0.68, *MUTYH*; 95% C.I., 21-85, P=0.01 and wild-type; 95%
262 C.I., 13-66, P=0.01). The signatures associated with defective MUTYH, SBS18 and SBS36, contributed
263 the excess mutations in all samples (Fig. 4c and Fig. 4e). An additional mutational signature was seen
264 in lymphocytes. SBS9, which is associated with DNA polymerase eta mediated somatic hypermutation
265 and is a key process in the physiological maturation of B-cells, was observed in most lymphocyte
266 samples indicating that the lymphocyte cell populations contained mature B-cells (Fig. 4e).
267
268 **DISCUSSION**
269 This study shows elevated base substitution somatic mutation rates due to SBS18 and/or SBS36 in
270 normal tissues from individuals with *MUTYH* mutations. The results are compatible with all intestinal,
271 and potentially all other cells in the body, showing elevated mutation rates. The relative increases in
272 mutation rate and mutational signature composition differed between individuals, probably due to
273 different *MUTYH* mutations and perhaps to other modifying influences.
274
275 We have previously highlighted the capability of normal human cells to tolerate substantially elevated
276 mutation rates[43]. Carriers of POLE and POLD1 germline exonuclease domain mutations exhibited
277 elevated somatic mutation burdens without evident cellular or organismal consequences, other than
278 an increased cancer risk[43]. This capability is confirmed in *MUTYH* germline mutation carriers. It is
279 further emphasised by the observation of a 33-fold genome-wide elevated base substitution mutation
280 burden in the 16 year old PD44890, which would confer a "mutational age" of ~500 years, without
281 overt evidence of premature ageing. The increase in mutation burden in coding exons is lower than
282 genome-wide in POLE/POLD1 mutation carriers. Similarly, in individuals with *MUTYH* mutations there
283 is a smaller increase of coding exon than genome-wide mutation burdens (Extended Data Fig. 2a-b).

Nevertheless in PD44890 the increase is still ~29-fold, and therefore equivalent to a "mutational age" of ~450 years. Whilst lesser increases in mutation rates compared to wild-type individuals were observed in other tissues from PD44890, ~8-fold in white blood cells and ~7 fold in tissue lymphocytes, these still conferred substantially elevated "mutational ages" in the absence of features of premature ageing. Thus direct deleterious effects of base substitutions accumulated over the course of a lifetime may not be an important cause of ageing.

The elevated mutation rate in normal intestinal epithelium likely contributes to the increased risk of colorectal adenomas and cancers in individuals with *MUTYH* mutations. Indeed, there appears to be a correlation between the extent of elevation of mutation rate and the rate of acquisition of colorectal adenomas. Individuals with the $MUTYH^{Y104*+/+}$ and $MUTYH^{Y179C+/+}$ genotypes exhibited greater increases in somatic mutation rates than individuals with the $MUTYH^{Y179C+/-\ G396D+/-}$ genotype. Previous detailed clinical phenotyping of large series indicates that individuals with biallelic truncating mutations or $MUTYH^{Y179C+/+}$ show higher rates of accumulation of adenomas and earlier age of onset of carcinoma[22,23] than $MUTYH^{Y179C+/-\ G396D+/-}$. The correlation between elevation of mutation rate and severity of clinical phenotype is further highlighted by individual PD44890 (16 years of age, $MUTYH^{Y179C+/-\ G396D+/-}$) who exhibited a substantially higher mutation rate than others of this genotype, and showed a much accelerated rate of colorectal adenoma development (Extended Data Table 1-2). We previously described ~7-fold elevated genome-wide base substitution mutation rates in intestinal cells of POLE germline exonuclease domain mutation carriers[43]. POLE mutation carriers, however, show lower colorectal adenoma rates than *MUTYH* biallelic mutation carriers who generally only show 2-5 fold increased mutation rates. This apparent discrepancy may, however, be explained by the genomic distribution of mutations. In POLE mutation carriers there is relative sparing of coding sequences, with only a three to four fold increase in exonic mutations in intestinal cells, whereas this sparing is less pronounced in *MUTYH* mutation carriers leading to similar increases in exonic mutation rates (Extended Data Fig. 7a-b). These observations lead to the proposition that measurements of somatic coding mutation rates undertaken early in life could, in future, be used to refine individual cancer risk predictions for *POLE/POLD* and *MUTYH* germline mutation carriers.

As for many other cancer predisposition syndromes, it is unclear why *MUTYH* germline mutations lead particularly to intestinal neoplasia. Elevated somatic mutation rates are also found in white blood cells in MAP individuals (and may therefore be present in other tissues) although the increases appear lesser in extent than in intestinal cells. The propensity to generate SBS18 mutations appears greater in wild type intestinal cells than in other cell types[49] and this may also be contributory.

In summary, we report elevated somatic base substitution rates characterised by distinctive mutational signatures in normal tissues from individuals with MAP. These findings underscore previous observations that elevated somatic base substitution rates are largely tolerated by cells and do not overtly accelerate the process of ageing. It is likely, however, that increased mutation rates in normal intestinal cells throughout life lead to increased rates of accumulation of driver mutations and, hence, the procession of neoplastic clones culminating in cancer.

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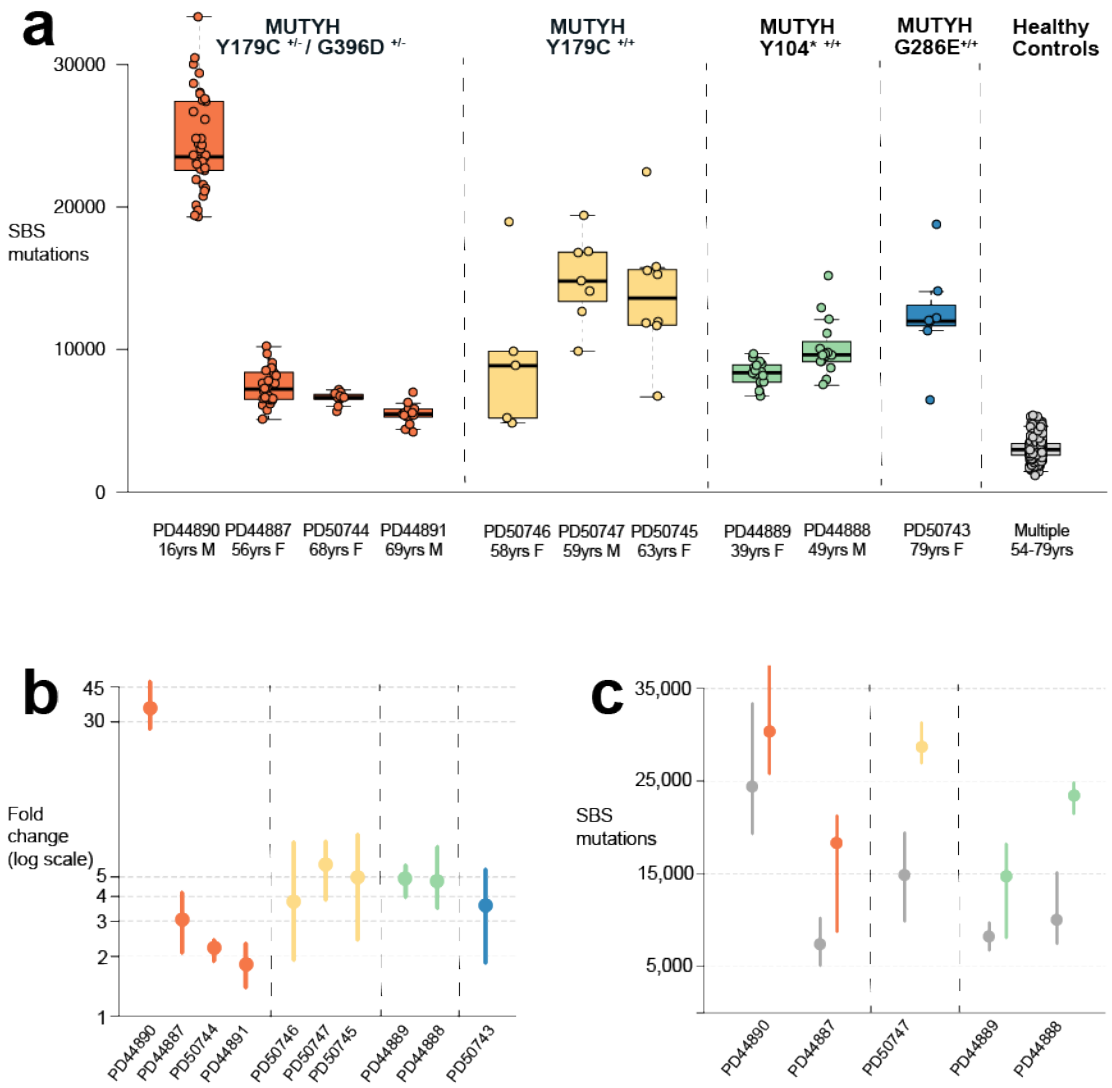
503 **FIGURES**
504



505
506

507 **FIGURE 1 | Somatic mutation burdens in cells with *MUTYH* mutations**

508 Elevated mutation burdens in normal intestinal cells with *MUTYH* mutations (a) Genome-wide single
509 base substitution (SBS) mutation burden of individual intestinal crypts grouped according to patient.
510 Each dot represents an individual intestinal crypt. *MUTYH* genotypes are displayed separately.
511 Boxplots display median, inter-quartile range (IQR) from 1st to 3rd quartiles and whiskers extend from
512 the last quartile to the last data point that is within 1.5x IQR (b) Fold-change in SBS rate compared
513 with wild-type controls[3] coloured according to germline genotype (orange; MUTYH$^{Y179C+/-\ G396D+/-}$,
514 yellow; MUTYH$^{Y179C+/+}$, green; MUTYH$^{Y104*+/+}$, blue; MUTYH$^{G286E+/+}$). Median values are represented by
515 the dot, whiskers represent the range from the minimum to maximum value. P-values for pair-wise
516 comparisons are shown in Supplementary Note. (c) Genome-wide single base substitution burden in
517 histologically normal crypts (grey) and adenoma crypts (orange, yellow and green) arranged by patient

518 and germline mutation. Median values represented by the dot, whiskers represent the range from the
519 minimum to maximum value. Data available for 5 individuals who had adenoma crypts sequenced.
520

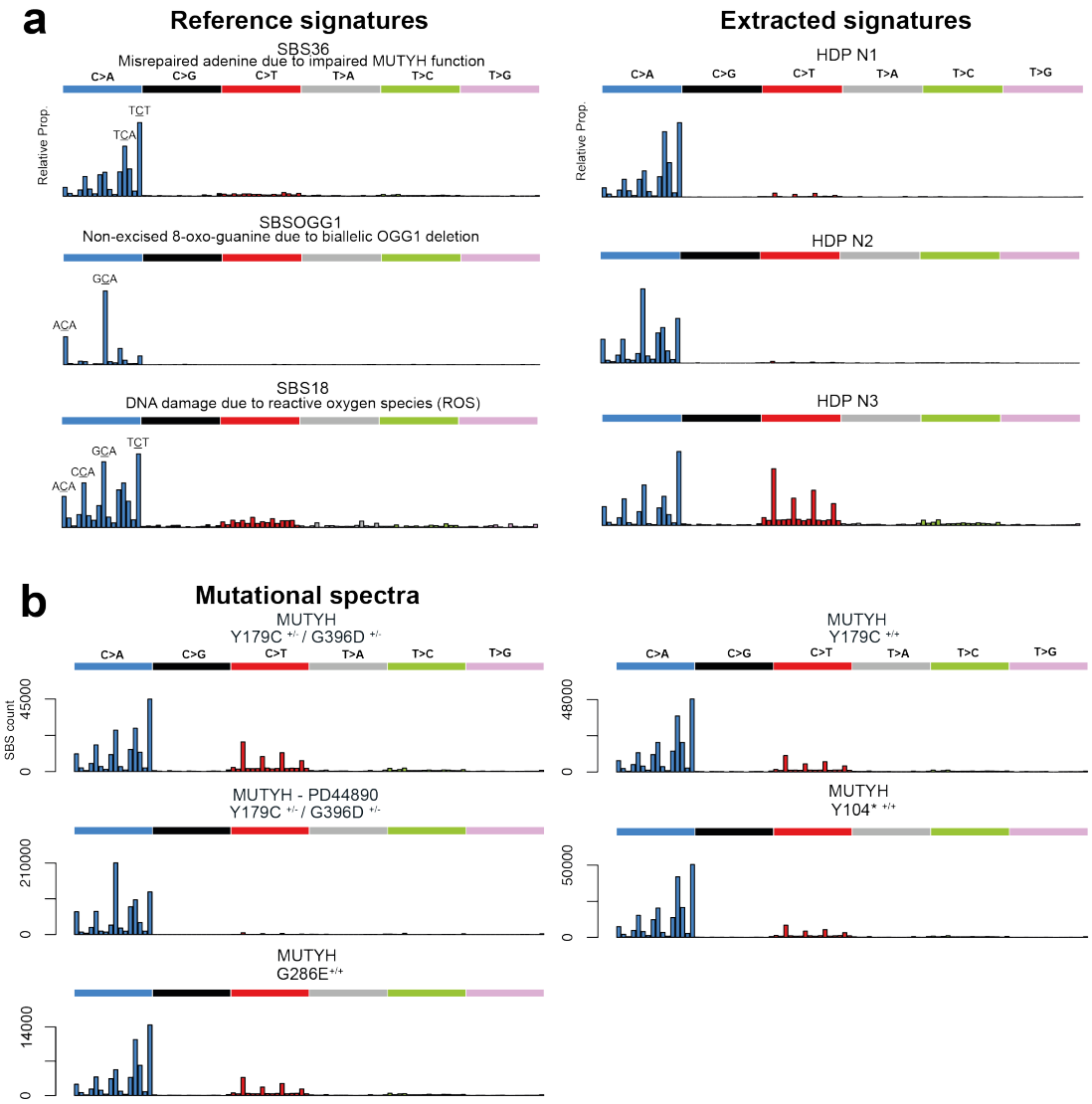


FIGURE 2 | Mutational spectra and signature components from normal cells with MUTYH mutations **(a)** Probability distribution for COSMIC reference signature SBS36[40], recently described *OGG1* deletion signature SBSOGG1[51] and reference signature SBS18[40]. Mutational signature components N1-3 from HDP *de novo* signature extraction (see Supplementary Information for all components and Methods / Supplementary Note for further explanation). **(b)** Mutational spectra in normal tissues displayed by the germline *MUTYH* mutation. Distinctive peaks are annotated with their trinucleotide context (mutated base is underlined). PD44890 is displayed separately to highlight the difference in spectrum observed in this individual.

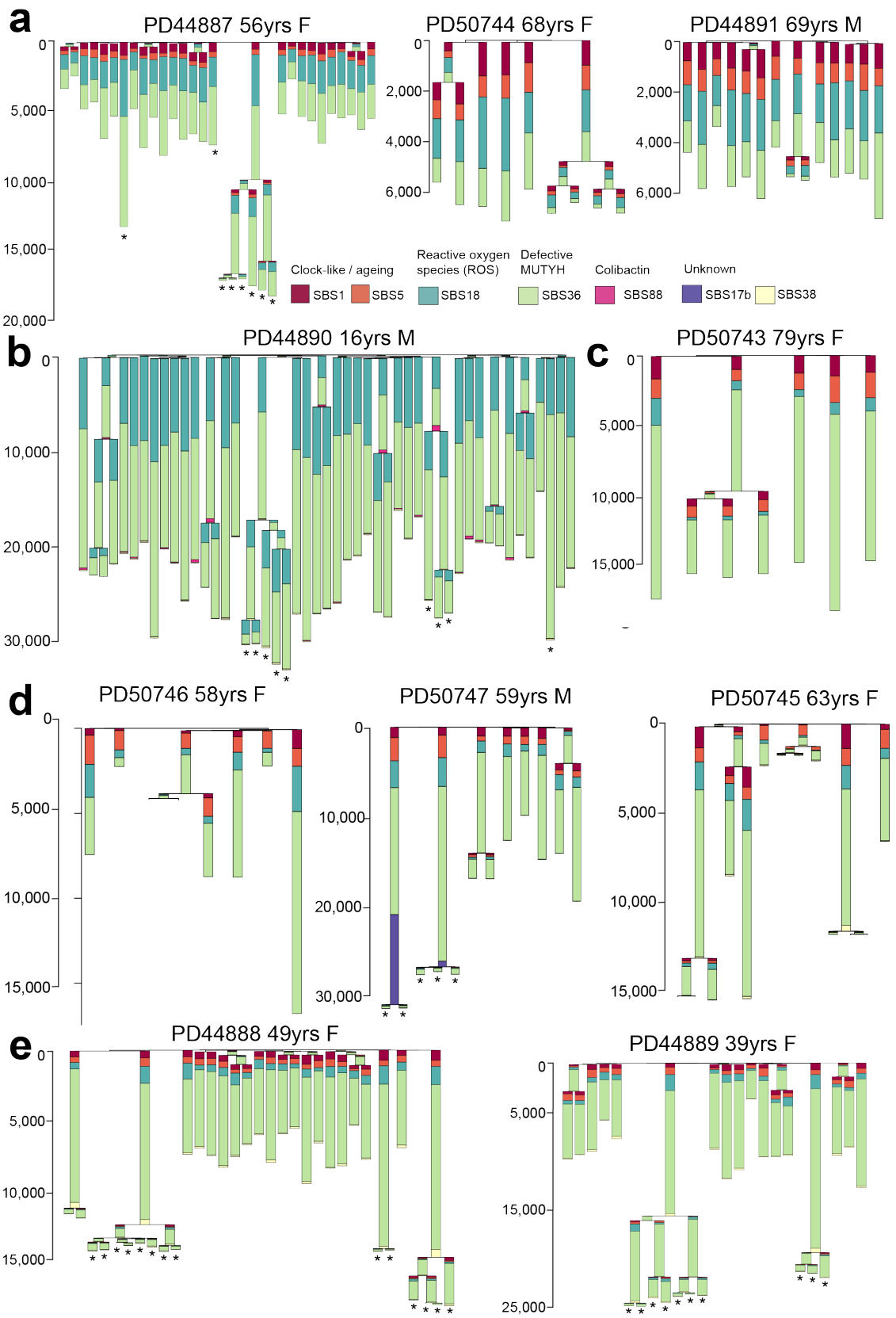


FIGURE 3 | Phylogenetic trees and mutational signatures intestinal cells with germline *MUTYH* mutations

535 Phylogenetic trees per-individual reconstructed from SBS mutations in individual intestinal crypts
536 showing the number of SBS mutations per branch. Stacked barplots are overlaid onto each branch to
537 represent the proportion of each mutational signature contributing to that branch. Phylogenetic trees
538 are arranged by *MUTYH* germline mutation; (a) $MUTYH^{Y179C+/-\ G396D+/-}$ (b) $MUTYH^{Y179C+/-\ G396D+/-}$ with
539 OGG1 germline mutations, (c) $MUTYH^{G286E+/+}$, (d) $MUTYH^{Y179C+/+}$ and (e) $MUTYH^{Y104*+/+}$. Adenoma glands
540 bearing cancer driver mutations are indicated with an asterisk '*'.
541

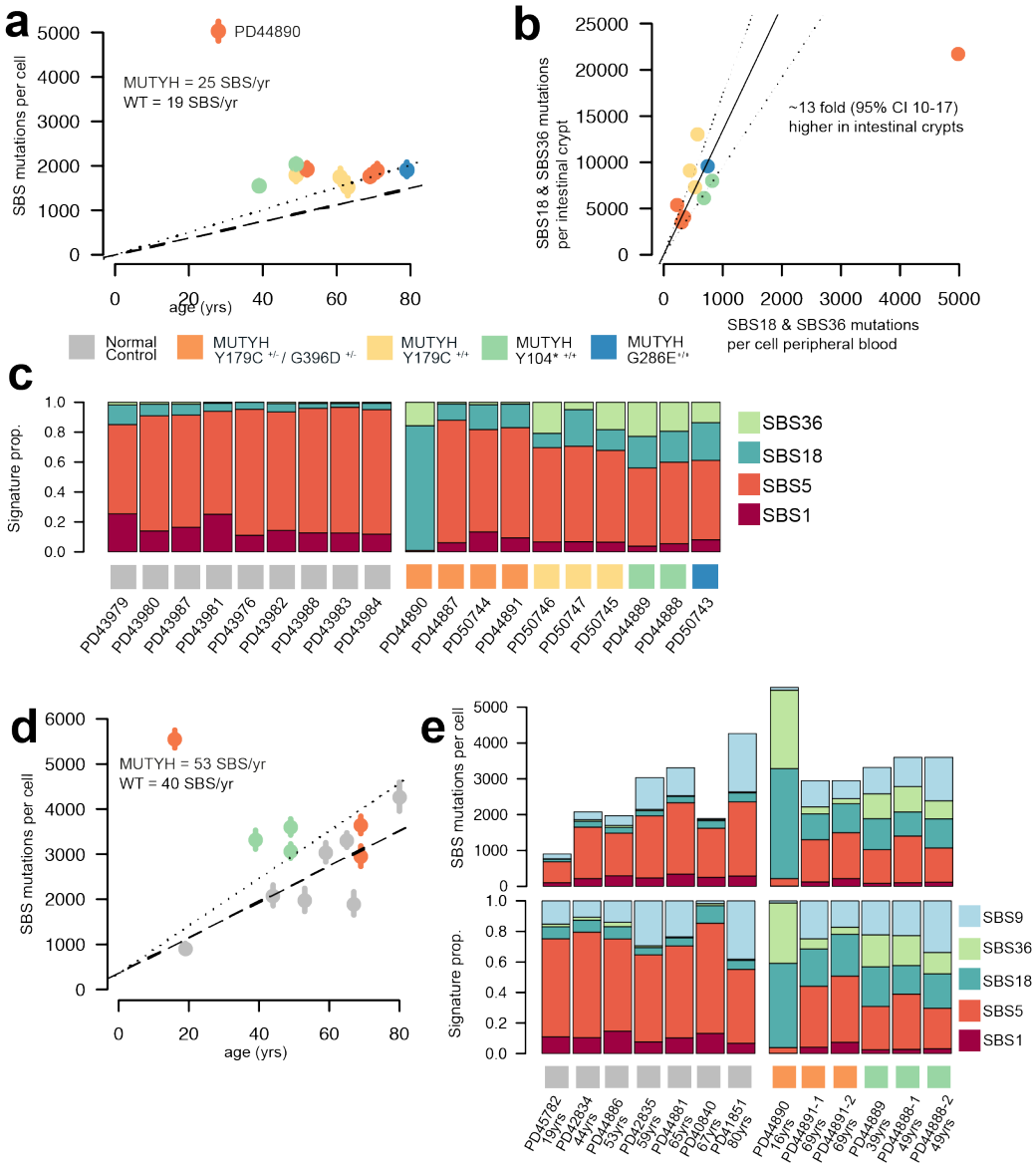
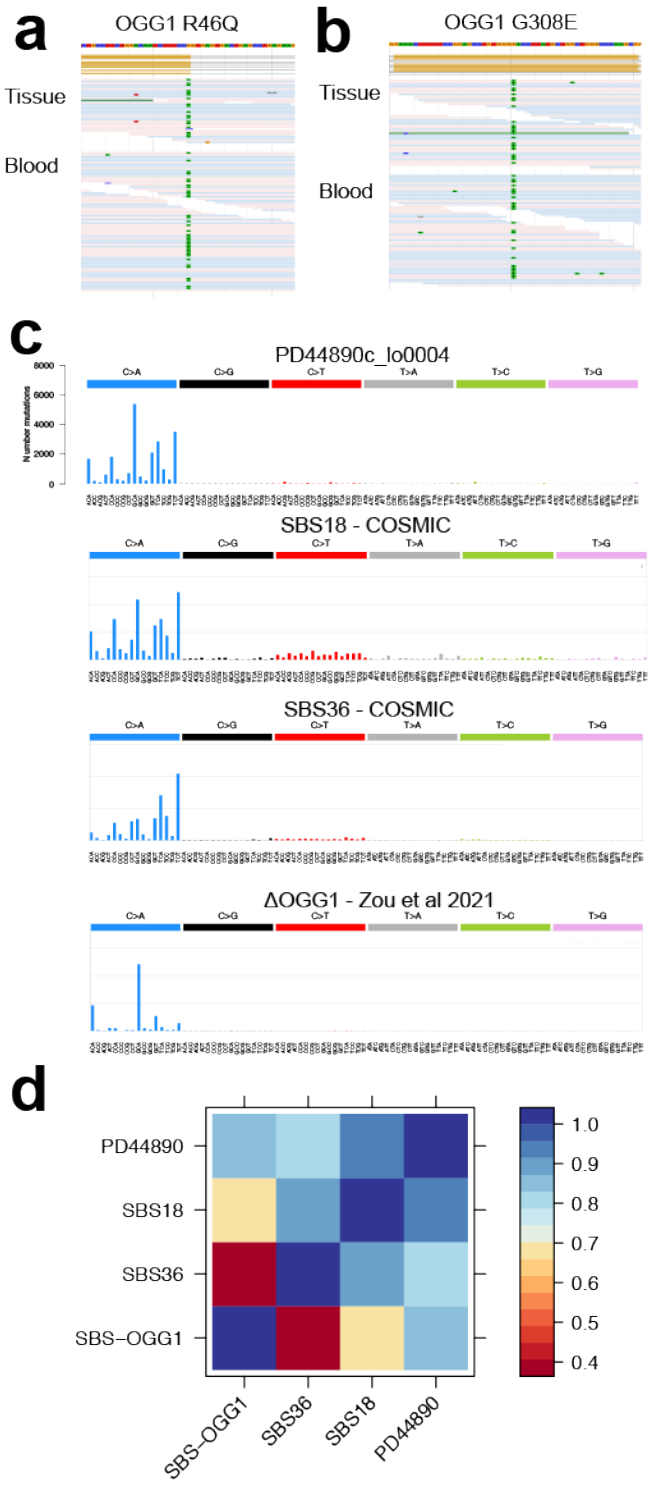


FIGURE 4 | Mutation burdens and mutational signatures in blood and immune cell populations
(a) Mutation burden in peripheral blood. SBS mutation burden per cell plotted against the age of the individual in years coloured according to the individual's germline mutation; orange; MUTYH$^{Y179C+/-G396D+/-}$, yellow; MUTYH$^{Y179C+/+}$, green; MUTYH$^{Y104*+/+}$ and blue; MUTYH$^{G286E+/+}$. Whiskers represent the 95% confidence interval. Black dashed line represents the average mutation rate in normal wild-type control samples[50] **(b)** Mutation burden of *MUTYH* associated mutational signatures; SBS18 and SBS36 per cell for peripheral blood (x-axis) against the SBS18 & SBS36 mutation burden of normal intestinal crypts (y-axis). Each dot represents one individual and they are coloured according to the individual's germline mutation; orange; MUTYH$^{Y179C+/-G396D+/-}$, yellow; MUTYH$^{Y179C+/+}$, green; MUTYH$^{Y104*+/+}$ and blue; MUTYH$^{G286E+/+}$. Ratio MUTYH mutational processes in colon vs blood ~13x fold (linear model, 95% C.I.; 10-17). Black line indicates ratio and dotted lines the 95% confidence intervals. **(c)** Stacked bar plot displaying the mutational signature contribution in each peripheral blood sample organised by patient. Coloured squares indicate the *MUTYH* germline mutation. Normal control data from

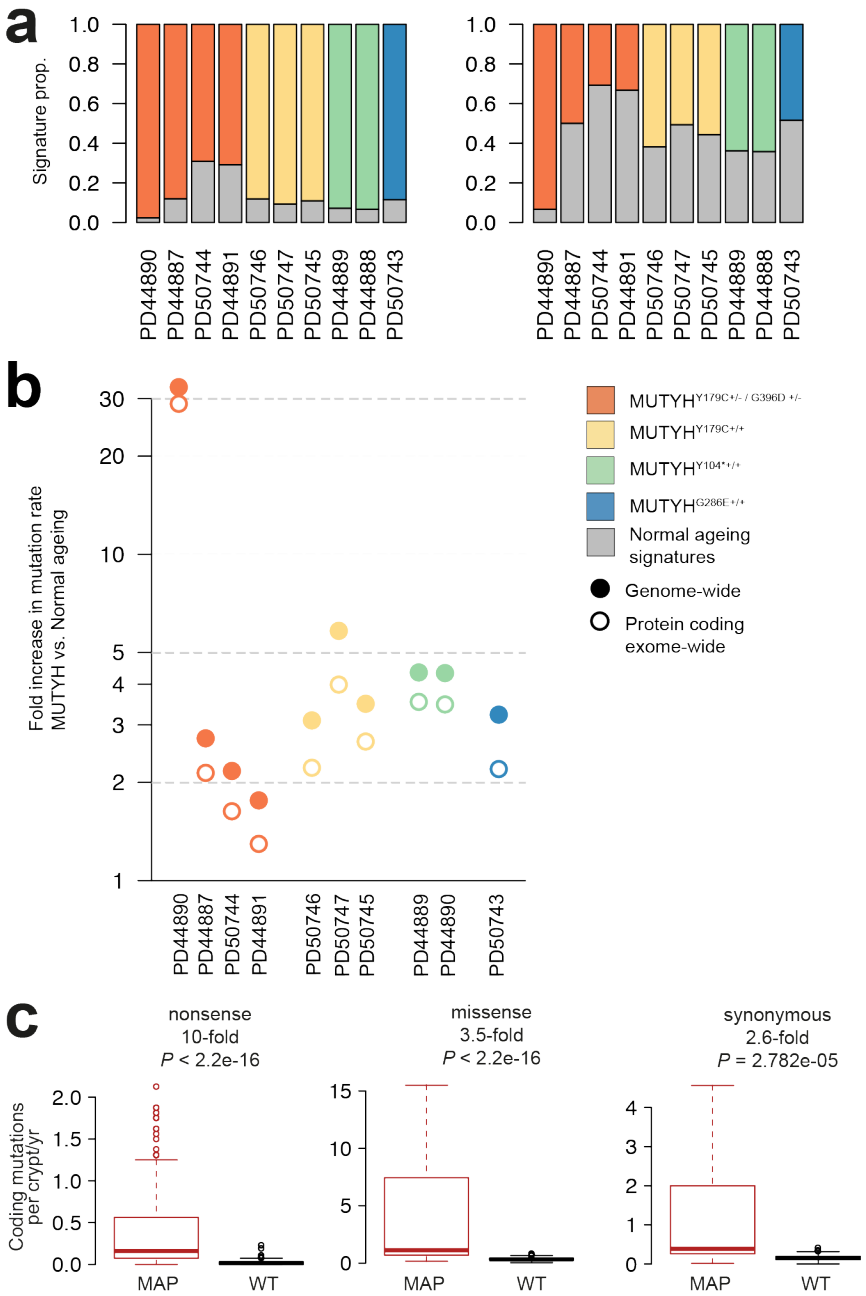
557 granulocytes sequenced using the same method (data from Abascal et al 2021)[50]. Significantly higher
558 proportion of SBS18 and SBS36 are observed in individuals with MUTYH mutations vs normal healthy
559 controls (two-sided Wilcoxon rank sum, *P*=0.00004) **(d)** SBS mutation burden in intestinal lymphocyte
560 cells from wild-type healthy individuals (grey) and individuals with *MUTYH* mutations (coloured
561 according to the germline *MUTYH* genotype) plotted against age (years). Dots represent median
562 values and whiskers represent the 95% confidence interval. Dashed line indicates the rate of increase
563 of SBS burden in wild-type lymphocytes (40 SBS/yr, linear mixed-effects model, R^2=0.68, 95% C.I., 13-
564 66, *P*=0.01) and dotted line indicates the rate of increase in SBS burden in lymphocytes from
565 individuals with *MUTYH* mutations (53 SBS/yr, linear mixed-effects model, R^2=0.68, 95% C.I., 21-85,
566 *P*=0.01). **(e)** Stacked bar plots showing the absolute (above) and relative (below) contributions of each
567 mutational signature in tissue lymphocytes from wild-type healthy individuals (grey squares) and
568 individuals with *MUTYH* mutations (coloured squares).
569
570

571 **EXTENDED FIGURES**



572

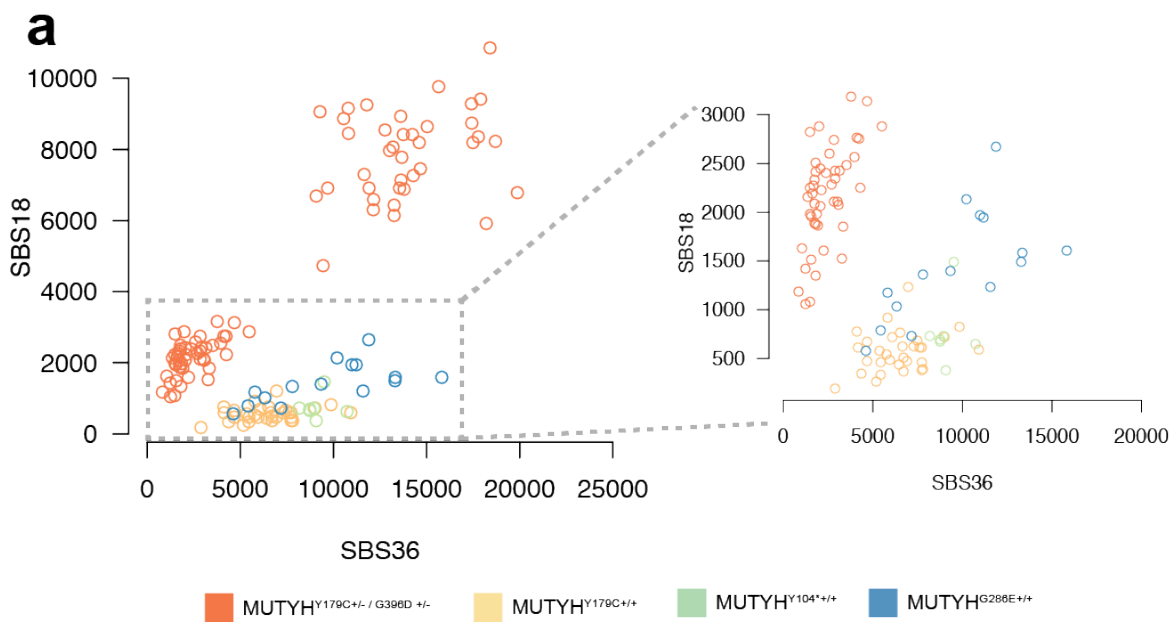
573 **Extended Data Figure 1 | Germline mutations and mutational spectrum of somatic mutations in**
574 **PD44890**
575 Two candidate germline modifier mutations were identified in individual PD44890 both of which are
576 in the oxoguanine glycosylase (OGG1) gene which is a key component of the base excision repair
577 pathway and a partner of MUTYH in the repair of 8-oxo-guanine DNA lesions. Both mutations are
578 heterozygous nonsynonymous mutations. JBrowse images are displayed showing the reads for each
579 mutation; **(a)** OGG1 R46Q **(b)** OGG1 R308E. See Supplementary Note for further discussion of these
580 putative germline modifier mutations. **(c)** Mutational spectrum of SBS mutations from a
581 representative intestinal crypt from individual PD44890, mutational spectra for the COSMIC reference
582 signatures SBS36 and SBS18 are displayed below for comparison (
583 https://cancer.sanger.ac.uk/cosmic/signatures). Mutational spectrum of IPS cells with homozygous
584 OGG1 deletion (Zou et al 2021, Nature Cancer)[51] denoted SBS OGG1. **(d)** Matrix displaying cosine
585 similarity between the observed spectrum in individual PD44890 and reference signatures SSB18,
586 SBS36 and SBS OGG1.
587



Extended Data Figure 2 | Genome-wide and exome-wide mutation burdens in intestinal crypts
(a) Stacked bar plots show the proportion of SBS mutations contributed by MUTYH-related signatures SBS18 and SBS36 (coloured bar) and normal ageing signatures (SBS1 and SBS5) (grey bar). Genome-wide signature proportions (left) protein-coding exome-wide signature proportions (right). **(b)** Fold-increase in the genome- and exome-wide mutation burden of samples from individuals with MUTYH-Associated Polyposis (MAP) compared with normal controls plotted on a logarithmic scale. **(c)** Boxplots showing nonsense, missense and synonymous coding mutation rates (SBS mutations/crypt/year) in histologically normal intestinal crypts from individuals with MUTYH-Associated Polyposis (MAP) (red) and healthy individuals; wild type (WT) (black). Boxplots display median, inter-quartile range (IQR) from 1st to 3rd quartiles and whiskers extend from the last quartile to the last data point that is within 1.5x IQR . Fold changes compared to WT, are shown above each

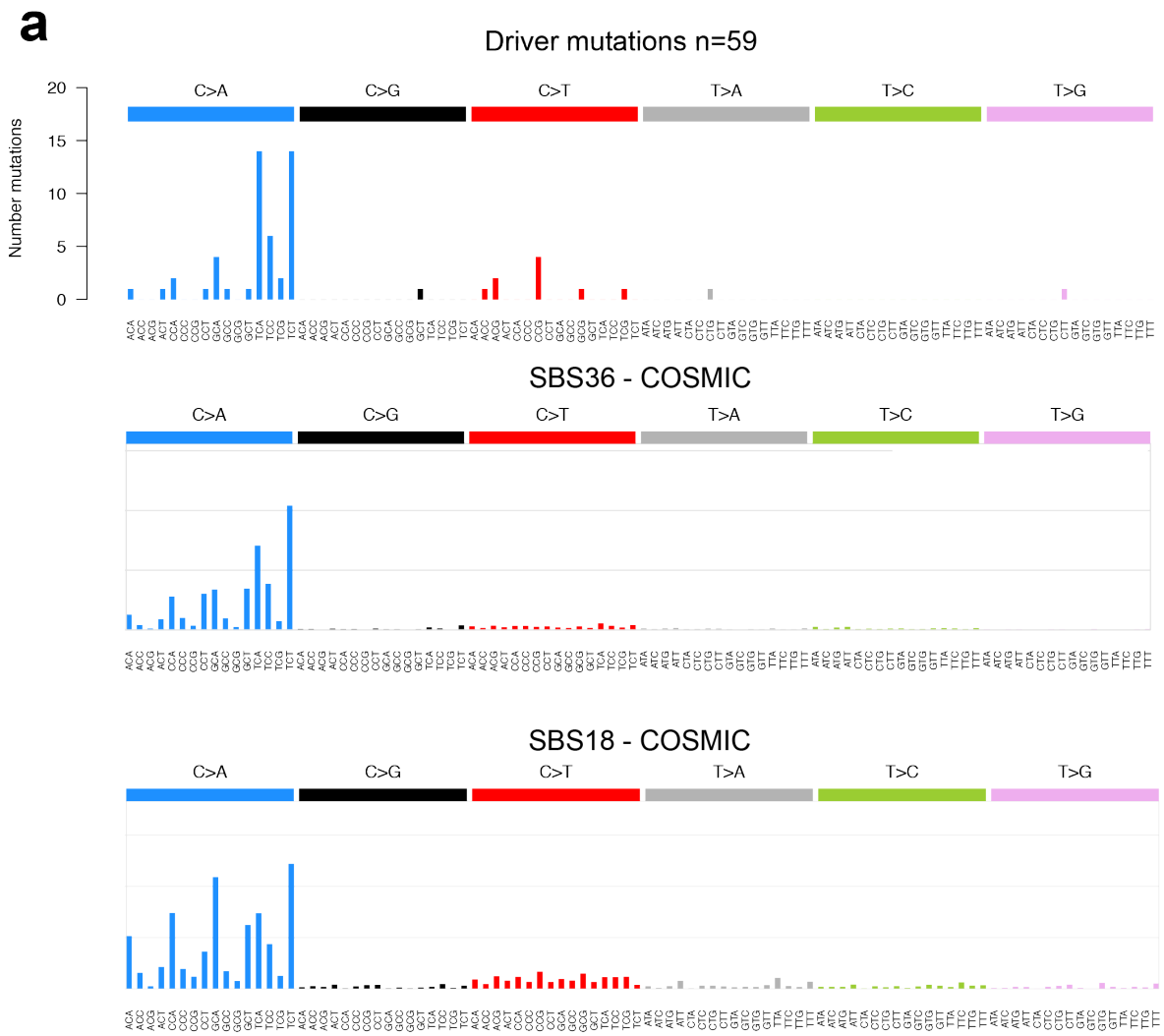
600 pair, *P*-values calculated using a two-sided Wilcoxon test. Data for healthy wild-type individuals from
601 Lee-Six et al 2019[3]
602

603

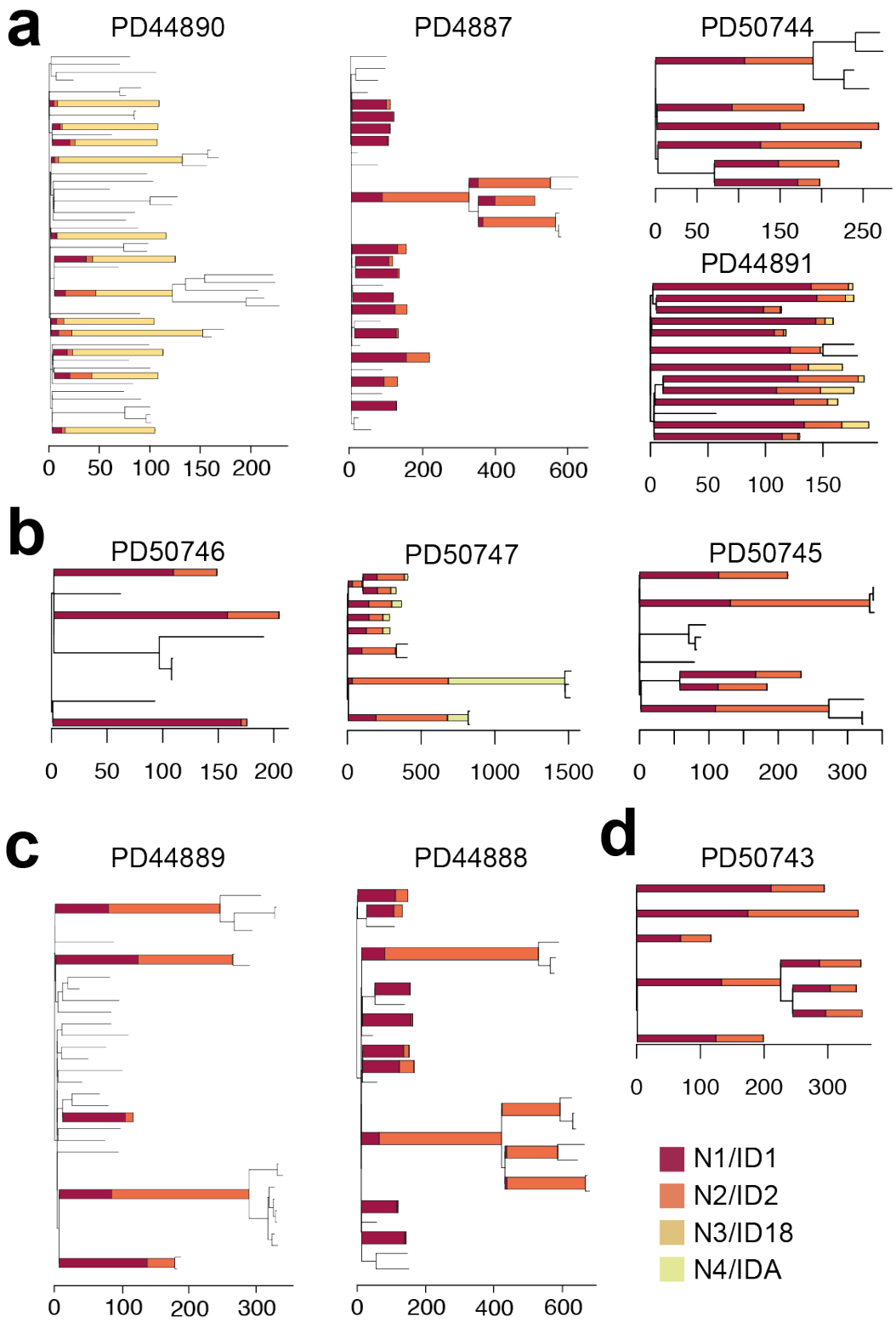


604 **Extended Data Figure 3 | Mutational signatures in normal intestinal crypts and adenoma glands**
605 **(a)** Somatic SBS mutation burden in normal intestinal crypts due to SBS36 (x-axis) and SBS18 (y-axis)
606 coloured according to germline mutation (orange; MUTYH$^{Y179C+/-\ G396D+/-}$, yellow; MUTYH$^{Y179C+/+}$, green;
607 MUTYH$^{Y104*+/+}$, blue; MUTYH$^{G286E+/+}$). Crypts with MUTYH$^{Y179C+/+}$, MUTYH$^{Y104*+/+}$ and MUTYH$^{G286E+/+}$ show
608 greater relative increases in SBS36 whereas those with MUTYH$^{Y179C+/-\ G396D+/-}$ show greater increases in
609 SBS18. Crypts from individual PD44890 are a clear outlier with much greater SBS18 burdens. Magnified
610 plot inset.
611
612

613
614

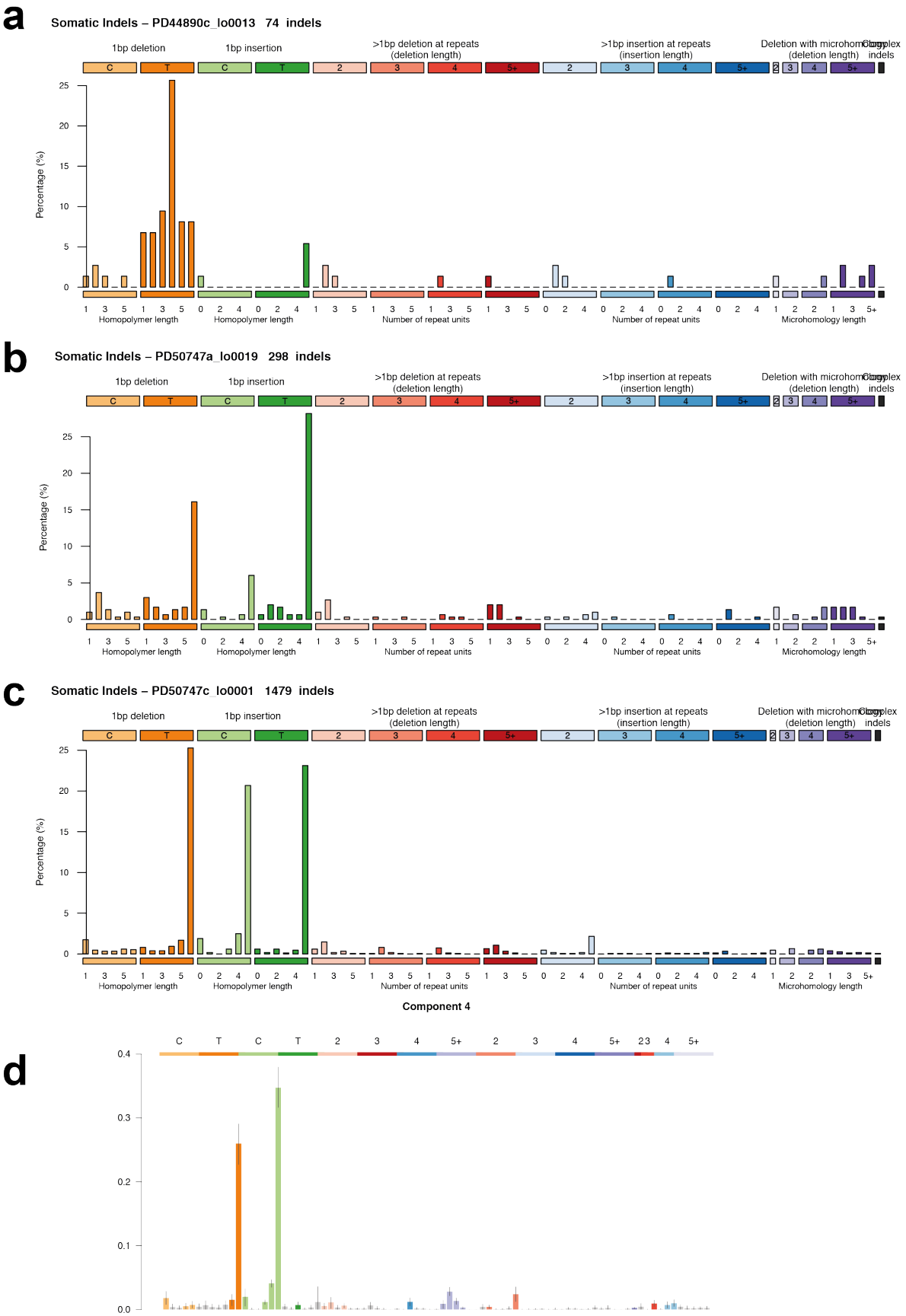


615
616 **Extended Data Figure 4 | Mutational Spectrum of driver mutations in normal and neoplastic**
617 **intestinal crypts**
618 **(a)** Mutational spectrum of drivers mutations identified in normal and neoplastic intestinal stem cells
619 in this cohort (n=59). Mutational spectra for the COSMIC reference signatures **(b)** SBS36 and **(c)** SBS18
620 are displayed below for comparison. The spectra of driver mutations is dominated by SBS36 and
621 SBS18. COSMIC reference signatures available at: https://cancer.sanger.ac.uk/cosmic/signatures.
622
623


a
PD44890
0 50 100 150 200
PD4887
0 200 400 600
PD50744
0 50 150 250
PD44891
0 50 100 150
b
PD50746
0 50 100 150 200
PD50747
0 500 1000 1500
PD50745
0 100 200 300
c
PD44889
0 100 200 300
PD44888
0 200 400 600
d
PD50743
0 100 200 300
N1/ID1
N2/ID2
N3/ID18
N4/IDA

Extended Data Fig 5 | Phylogenetic trees showing ID signature exposure

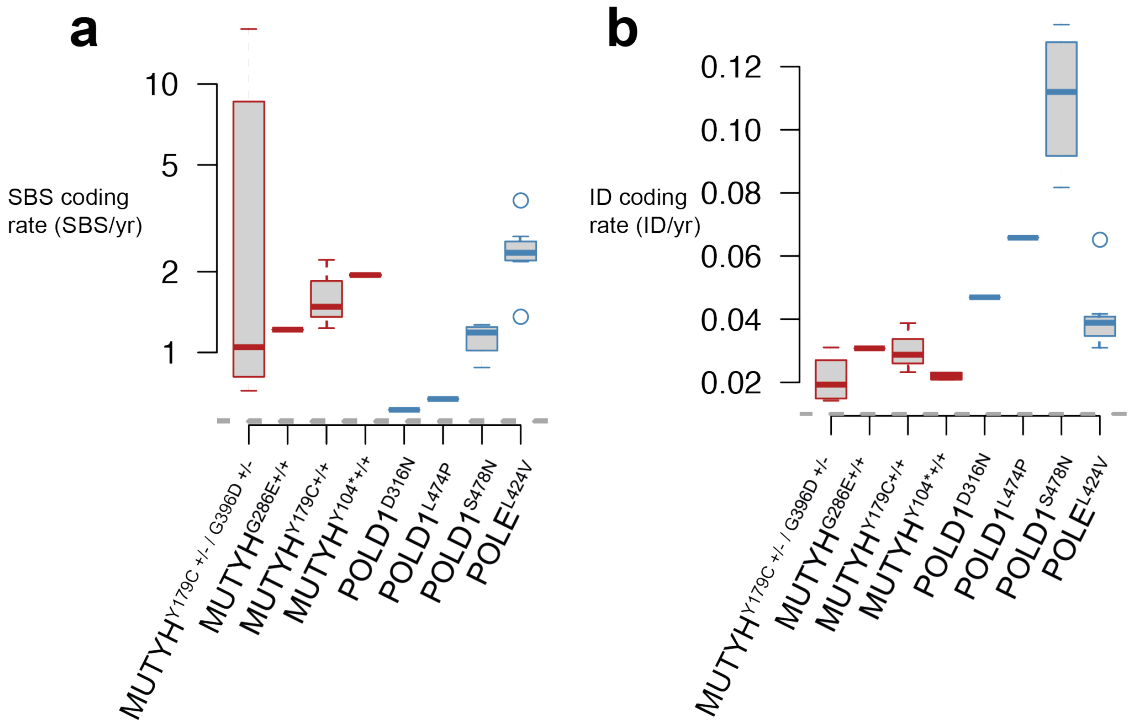
Phylogenetic trees with ID signature exposure overlaid. Branches of the phylogenetic tree with >100 somatic ID mutations have been converted into stacked bar plots displaying the contribution of each HDP signature component / mutational process to that branch: N1/ID1 shown in dark red, N2/ID2 in orange, N3/ID18 in mustard yellow and N4/IDA in lime green. Phylogenetic tress organised by germline genotype, **(a)** MUTYH$^{Y179C+/-\ G396D+/-}$, **(b)** MUTYH$^{Y179C+/+}$, **(c)** MUTYH$^{Y104*+/+}$ and **(d)** MUTYH$^{G286E+/+}$



Extended Data Figure 6 | ID mutational spectra

ID mutational spectra for crypts with sporadic ID signature exposures. **(a)** Representative sample from PD44890 with high ID18 exposure. Representative normal crypt from PD50747 showing IDA exposure

637 in **(b)** normal crypt and **(c)** adenoma gland. **(d)** ID signature component arising from unconditioned *de*
638 *novo* HDP signature extraction (Methods) corresponding to signature IDA.
639
640



641
642 **Extended Data Figure 7 | Coding mutation burdens in intestinal crypts from individuals with**
643 **germline MUTYH and POLE/D1 mutations**
644 Coding mutation burdens in individuals with MUTYH and POLE/D1.**(a)** SBS coding mutation rate
645 (SBS/year) coloured according to cohort; MUTYH; red, POLE/D1; blue. (b) ID coding mutation rate
646 (ID/year).
647

Extended Data Table 1 | Clinical summary and phenotypic characteristics of individuals with germline *MUTYH* mutations

Summary of phenotypic features and disease burden in all individuals in this cohort.

Extended Data Table 2 | Summary of samples sequenced including germline *MUTYH* mutation, sequencing method and mutation burdens

Extended Data Table 3 | Cancer driver mutations identified in this cohort

Cancer driver mutations identified across all samples in this cohort

660 **METHODS**
661
662 **Ethical approval and study participants**
663 This research complies with all relevant ethical regulations. MAP patients were recruited as part of Wales
664 Research Ethics Committee (REC) 12-WA0071 and 15-WA0075 and samples collected were approved for use in
665 this project by REC 18/ES/0133. Normal healthy controls were recruited as part of the following UK Research
666 Ethics Committee (REC) studies; 15/WA/0131, 15/EE/0152, 18/ES/0133 and 08/h0304/85+5.
667
668 Informed consent was obtained from all participants and no monetary compensation was offered for their
669 participation. A complete list of study participants and tissue samples is summarised in Extended Data Table 1
670 and 2.
671
672 **DNA extraction from bulk samples**
673 Frozen whole blood underwent DNA extraction using the Gentra Puregene Blood Kit (Qiagen). Briefly, 1-2ml of
674 frozen blood were thawed, lysed in RBC lysis solution and centrifuged. Cell pellet was resuspended in cell lysis
675 solution and incubated at 37 ℃ for 2 hours. RNA and protein was degraded using RNase A solution and protein
676 precipitation solution. DNA was precipitated with isopropanol.
677
678 **Tissue Preparation**
679 Tissues were embedded in Optimal Cutting Temperature (OCT) compound, frozen histological sections were cut
680 at 25-30µm and mounted on polyethylene naphthalate (PEN) slides and fixed in 70% ethanol for 5 minutes
681 followed by two washes with phosphate buffered saline for 1 minute each. Slides were manually stained in
682 haematoxylin and eosin using a conventional staining protocol. A subset of samples were fixed in RNAlater
683 (Sigma Aldrich) according to manufacturer's instructions. Fixed tissue samples were embedded in paraffin using
684 a Tissue-Tek tissue processing machine (Sakura). No formalin was used in the preparation, storage, fixation or
685 processing of samples. Processed tissue blocks were embedded in paraffin wax, sectioned to 10µm thickness
686 and mounted onto PEN slides (Leica). Tissue slides were stained using a standard haematoxylin and eosin (H&E)
687 protocol. Slides were temporarily cover-slipped and scanned on a NanoZoomer S60 Slide Scanner (Hamamatsu),
688 images were viewed with NDP.View2 software (Hamamatsu).
689
690 **Laser Capture Microdissection**
691 Laser capture microdissection was undertaken using a LMD7000 microscope (Leica) into a skirted 96-well PCR
692 plate. Cell lysis was undertaken using 20µl proteinase-K PicoPure® DNA Extraction kit (Arcturus®), samples
693 were incubated at 65 ℃ for 3 hours followed by proteinase denaturation at 75 ℃ for 30 minutes. Thereafter
694 samples were stored at -20 ℃ prior to DNA library preparation.
695
696 **Low-input DNA library preparation and sequencing**
697 DNA library preparation of micro-dissected tissue samples was undertaken as previously described using a
698 bespoke low-input enzymatic-fragmentation-based library preparation method[2-4,37]. This method was employed
699 as it allows for high quality DNA library preparation from very low starting quantity of material (from 100-500
700 cells). DNA library concentration was assessed after library preparation and used to guide choice of samples to
701 take forward to DNA sequencing, minimum library concentration was 5ng/µL and libraries with >15ng/µL were
702 preferentially chosen. 150bp paired-end Illumina reads were prepared with Unique Dual Index barcodes
703 (Illumina).
704
705 DNA sequencing was undertaken on a NovaSeq 6000 platform using an XP kit (Illumina). Samples were
706 multiplexed in pools of 6-24 samples. Pools were sequenced to achieve a coverage of ~30x.
707
708 **Mutation calling and post-processing filters**

709 Sequencing reads were aligned to NCBI human genome GRCh37 and aligned using the Burrow-Wheeler
710 Alignment (BWA-MEM). Single Base Substitutions (SBS) were called using the 'Cancer Variants through
711 Expectation Maximization' algorithm (CaVEMan)[54]. Mutations were called using an unmatched normal synthetic
712 bam file to retain early embryonic and somatic mutations. Post-processing filters were applied to remove low-
713 input library preparation specific artefacts and germline mutations using a previously described method[1,2,37,55].
714 Filters applied were: (1) common single nucleotide polymorphisms were removed by filtering against a panel of
715 75 unmatched normal samples[56] (2) to remove mapping artefacts, mutations were required to have a minimum
716 median read alignment score of mutant reads (ASMD ≥ 140) and fewer than half of the reads supporting the
717 mutation should be clipped (CLPM =0); (3) a filter to remove overlapping reads that result from the relatively
718 short insert size which could lead to double counting of variant reads; and (4) a filter to remove cruciform DNA
719 structures that can arise during the low-input library preparation method.
720
721 Next, we applied multiple filters to remove germline variants and potential artefacts whilst retaining *bona fide*
722 embryonic and somatic variants. This approach has been detailed in previous publications and the code for these
723 filters can be found at https://github.com/TimCoorens/Unmatched_NormSeq. Mutations were aggregated per
724 patient and a read pile-up was performed using an in-house algorithm (cgpVAF) to tabulate the read count of
725 mutant and reference reads per sample for each mutation locus. Germline mutations were filtered out using an
726 exact binomial test. The exact binomial test is used to distinguish germline from somatic variants and uses the
727 aggregate read counts from all samples of the same patient[1,55]. In brief, the read depth across all samples from
728 that individual was calculated (median in this study 496-fold). This high coverage yields a very precise estimate
729 of the true VAF of each mutation. While the VAF estimates of the earliest embryonic SNVs and germline variants
730 from samples sequenced at 30x might overlap, the VAFs from the aggregate coverage from that individual will
731 be distinguishable using statistical testing. To achieve this, the beta-binomial test was applied. The
732 overdispersion parameter (rho) threshold for genuine variants of rho>0.1 was used.
733
734 Phylogenetic trees were created using MPBoot (version 1.1.0 bootstrapped - 1000) and mutations were mapped
735 to branches using maximum likelihood assignment.
736
737 Indels (ID) were called using Pindel[57] using the same synthetic unmatched normal sample employed in SBS
738 mutation calling. ID calls were filtered to remove calls with a quality score of <300 ('Qual'; sum of mapping
739 qualities of the supporting reads) and a read depth of less than 15. Thereafter, ID filtering was performed in a
740 similar manner as SBS to remove germline variants and library preparation / sequencing artefacts.
741
742 **Copy-number alteration calling**
743 Somatic copy-number variants (CNVs) were called using the Allele-Specific Copy number Analysis of Tumours
744 (ASCAT) algorithm[58], https://github.com/Crick-CancerGenomics/ascat) in the ascatNGS package[59]. Bulk blood
745 samples or phylogenetically unrelated normal samples were used as matched normals. ASCAT was initially run
746 with default parameters. To reduce the number of false-positive calls that arise when analysing normal tissue
747 samples using ASCAT, a bespoke algorithm ascat-PCA was applied. ascat-PCA extracts a noise profile by
748 aggregating the LogR ratio from across a panel of normal unrelated samples and subtracts this signature from
749 that observed in the sample being analysed using principal component analysis (INSERT GITHUB).
750
751 **Structural variant calling**
752 Whole-genome sequences were analysed for somatic structural variants (SVs) using the Genomic
753 Rearrangement Identification Software Suite (GRIDSS). In preparation for this analysis, genomes were remapped
754 to Human Genome Version 38 and GRIDSS was run using the same matched normal as used for CNV analysis.
755 Coordinates for SV calls were subsequently converted back to GRCh37. SV calls in L1 transposon donor regions
756 and fragile sites were excluded from the final SV analysis.
757

758 **Mutational signature analysis**
759 The R package HDP (https://github.com/nicolaroberts/hdp), based on the hierarchical Dirichlet process[60], was
760 used to extract mutational signatures. Analysis of mutational signatures using this package has been applied to
761 normal tissues previously[1-4]. In brief, this nonparametric Bayesian method models categorical count data using
762 the hierarchical Dirichlet process. A hierarchical structure is established using patients as the first tier (parent
763 nodes) and individual samples as the second tier (dependent nodes). Uniform Dirichlet priors were applied
764 across all samples. The algorithm creates a mutation catalogue for each sample and infers the distribution of
765 signatures in any one sample using a Gibbs sampler. We performed mutational signatures analysis per-branch,
766 counting each branch of the phylogenetic tree as a distinct sample to avoid double counting of mutations. Since
767 the MCMC process scales linearly with the number of counts, we randomly subsampled each branch to a
768 maximum of 2500 total substitutions. Branches with fewer than 100 mutations were excluded from the
769 mutational signature extraction. No reference signatures were included as priors.
770
771 To assess the contribution of each mutational process, mutational signatures were refitted to all mutation
772 counts of branches of phylogenies using the R package sigfit (https://github.com/kgori/sigfit)[61]. To avoid
773 overfitting, a limited subset of reference mutational signatures were included per patient corresponding to the
774 HDP signatures that have been identified in that individual.
775
776 Ageing signatures SBS1 and SBS5 are present in all normal intestinal crypts[3]. Lower than expected burdens of
777 SBS1 and SBS5 were observed in most individuals in this study due to; 1. the inherent challenges of accurately
778 estimating mutation burden in hypermutated samples and 2. the appreciable contamination of reference
779 signatures with SBS1 and SBS5. To partially address this, we used the extracted HDP component corresponding
780 to SBS36 in the refitting stage which has lower SBS1 and SBS5 contamination than the COSMIC reference SBS36
781 signature. Nevertheless, in individual PD44890 where SBS18 and SBS36 exposures are many tens of times
782 greater than the normal mutation rate, the estimates of SBS1 and SBS5 are substantially lower than would be
783 expected.
784
785
786
787 **Cancer driver mutations**
788 Cancer driver mutations were identified using two methods aiming to identify genes and mutations in this cohort
789 that are subject to positive selection. Firstly, to identify mutations in cancer genes under positive selection in an
790 unbiased manner, we ran a modified dNdS method[62]. To avoid double-counting of mutations, only unique
791 mutations (SBS and ID) which were mapped to branches of the phylogenetic trees were analysed. dNdScv was
792 run using the following parameters; max_coding_muts_per_sample=5000 and
793 max_muts_per_gene_per_sample=20. Genes with a qval of <0.05 were considered to be under positive
794 selection.
795
796 A second phase of cancer gene mutation analysis was undertaken; identifying mutations in this cohort which
797 are codified in cancer mutation databases and exhibit characteristic traits of cancer driver mutations; an
798 approach previously employed in the study of normal tissues[1,2]. In this phase of the analysis we sought to identify
799 the spectrum and frequency of cancer driver mutations in this cohort. Somatic mutations (SBS and ID) were
800 collated per-sample from all tissues. Analysis was restricted to protein coding regions and mutations were
801 filtered using lists of known cancer genes; mutations in samples from intestinal epithelium were filtered using a
802 list of 90 genes associated with colorectal cancer and includes variants that are commonly identified in small
803 bowel adenocarcinoma[3]; samples from all other tissues including blood were filtered using a pan-cancer list of
804 369 driver genes[62]. Genes were then characterised according to their predominant molecular behaviour;
805 dominant, recessive or intermediate (those demonstrating aspects of both types of behaviour) using the COSMIC
806 Cancer Gene Census[63]. All candidate mutations were annotated using the cBioportal MutationMapper database

(https://www.cbioportal.org/mutation_mapper). Mutations meeting the following criteria were considered to be driver mutations; truncating mutations (those that cause a shortened RNA transcript, nonsense, essential splice-site, splice region and frameshift ID) in recessively acting genes, known activating hotspot mutations in dominant (and recessive) genes and lastly mutations that were in neither of the above categories but characterised by the MutationMapper database as being 'likely oncogenic' were also included in the final driver mutation catalogue. We also sought to compare the frequency of driver mutations in histologically normal crypts with MUTYH mutations to those from individuals who do not carry DNA polymerase mutations. Somatic mutations from 445 normal intestinal crypts[3] were annotated and filtered using the above criteria. Comparison was made with normal intestinal crypts from this cohort of individuals with MUTYH germline mutations.

Modified duplex sequencing (NanoSeq)

DNA from bulk blood samples from individuals with germline *MUTYH* mutations was extracted as outlined above. Samples from normal healthy control was obtained and processed using the following method. Whole blood was diluted with PBS and mononuclear cells (MNC) were isolated using lymphoprepTM (STEMCELL Technologies) density gradient centrifugation. The red blood cell and granulocyte fraction of the blood was then removed. The MNC fraction was depleted of red blood cells by lysis steps involving 3 incubations at room temperature for 20 mins/10 mins/10 mins respectively with RBC lysis buffer (BioLegend). Tissue lymphocytes were isolated from Peyer's patches in intestinal mucosa using laser capture microdissection and subjected to protein lysis as outlined above. Cell lysates were processed and whole genome sequenced using the NanoSeq protocol.

Our modified duplex sequencing method, called NanoSeq, relies on blunt-end restriction enzymes to fragment the genome in order to avoid errors associated to the filling of 5' overhangs and the extension of internal nicks during end repair after sonication. Our modified method has error rates < 5e-9[50].

Given the uneven frequencies of trinucleotides in the digested genome, the strong filtering of common SNPs sites (typically occurring at CpG), and the strong dependence of mutation rates on trinucleotide contexts, our estimates of mutation burdens are normalized and projected onto genomic trinucleotide frequencies.

Let t denote the count of a given trinucleotide of type i = 1...32. The frequency of each trinucleotide is calculated separately for the genome f_i^g and for the NanoSeq experiment f_i^e where:

$$f_i = \frac{t_i}{\sum_{i=1}^{32} t_i}$$

The ratio of genomic to experimental frequencies for a given trinucleotide is:

$$r_i = \frac{f_i^g}{f_i^e}$$

There are j = 1...6 classes of substitution where the mutated base is a pyrimidine. Let s_{ij} denote the count of substitution j in trinucleotide context i, giving a total of 96 substitution classes. Each substitution count is corrected as follows:

$$s'_{ij} = s_{ij} r_i$$

The corrected substitution counts provide a substitution profile projected onto the human genome, and are also used to calculate the corrected mutation burden:

$$\beta' = \frac{\sum_{i=1}^{32} \sum_{j=1}^{6} s'_{ij}}{\sum_{i=1}^{32} t_i}$$

Software used in this study is publicly available at the following locations. Mutation calling algorithms are available at https://github.com/cancerit. Code for filtering mutation calls is available at https://github.com/TimCoorens/Unmatched_NormSeq. Software for mutational signature analysis is available at https://github.com/nicolaroberts/hdp , https://github.com/kgori/sigfit and https://github.com/AlexandrovLab. Software for analysis of duplex / NanoSeq data is provided at https://github.com/cancerit/NanoSeq. Parameters used for these various pieces of software have been included in the manuscript methods section and supplementary information.

Data Availability

DNA sequencing data are deposited in the European Genome-Phenome Archive (EGA) with accession code: EGAD00001007958 and EGAD00001007997.

The cBioPortal MutationMapper database was accessed at:
https://www.cbioportal.org/mutation_mapper?standaloneMutationMapperGeneTab=ATM

Code Availability

Code/software required to reproduce the analyses in this paper are available online and are listed in Methods.

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AUTHOR CONTRIBUTIONS

P.S.R., M.R.S., J.R.S. and L.T. conceived the study design. J.R.S., L.T., F.L., L.B., N.L., H.D.W., R.B-S., S.R, R.t.H., N.C., S.J.A.B. and K.S.P. recruited individuals, collected samples and curated sample and clinical data. P.S.R., B.C.H.L. and S.V.L. undertook laboratory work. F.A., I.M., S.V.L., L.M.R.H., T.H.H.C. and M.A.S. developed bespoke DNA library preparation, sequencing and bioinformatic methods. F.A., I.M., L.M.R.H., H.L.S. and S.O. contributed and analysed control data. P.S.R., F.A., I.M., S.O. and H.J. performed data analysis. M.R.S., P.J.C. and I.M. oversaw statistical analysis. M.R.S. and J.R.S. oversaw the study. All authors were involved in the preparation and review of the manuscript.

COMPETING INTERESTS

P.J.C. is a founder, consultant, and stockholder of Mu Genomics Ltd.