Supplemental figure S1. Similar mutational detection rates in matched tissue and ctDNA samples.

(A) No difference in the distribution of TMB in matched tissue and ctDNA samples. (B) TMB assessed in matched tissue and ctDNA samples are highly correlated. (C) Significant overlap of POL variants detected in tissue and ctDNA samples.
**Supplemental figure S2. Sex distributions of POL variants across different cancers.**

(A) Sex distributions for all 12,266 patients (left) and 526 POL+ patients (right) across different cancer types. (B,C) Comparisons of Sex distributions between POL- and POL+ populations for (B) all patients and (C) lung cancer patients.
Supplemental figure S3. Association between TMB and age.

\[ R = -0.032, \ p = 0.47 \]
Supplemental figure S4. Distribution of somatic and germline POL variants across the polymerase coding regions.

(A) Landscape of somatic (top) and germline (bottom) variants in (A) POLE, (B) POLD1, and (C) POLH.
Supplemental figure S5. Distribution of TMB in patients with germline POL variants.

A

% of all non-synonymous germline variants

POL+ POLD1 POLH

Missense Truncating Splicing

B

TMB (Mutation/Mb)

gPOL+ sample with known driver VUS

C

gPOL+ MS Evaluable Cohort (n=58)

POL+ 35

MSI 1

TMB- ultraH 4 4 9

TMB-H

D

% of Patients

Germline Somatic Germline Somatic

P=0.09 P=0.12

E

TMB (Mutation/Mb)

P=0.003 P=0.046

POL- Germline POL+ Somatic POL+

F

TMB (Mutation/Mb)

P=0.20 P=0.12 P=0.49

Colorectal Gastric Lung

MSS sub-group

POL- Germline POL+ Somatic POL+

(A) Distribution of variant types across the three polymerase genes. (B) TMB in each germline POL+ patients. Dashed line showing threshold for high TMB. (C) Venn diagram showing the relationships among POL germline variants, microsatellite status and TMB in patients with evaluable microsatellite stability. (D) Comparisons of TMB and microsatellite status between patients with germline and somatic POL variants. (E) Distribution of TMB comparing MSS patients with germline POL variants versus those with somatic POL variants or POL- patients. (F) Comparisons of the distribution of TMB between somatic and germline POL+ cases in three major cancer types.
Supplemental figure S6. Functional characterization of POL germline variants.

(A) Distribution of TMB associated with missense and non-missense POL variants. (B) Comparisons of TMB between cases with germline variants affecting the exonuclease domain of POLE and POLD1 and those with non-exonuclease domain variants. (C) Higher proportions of MSI tumor had variants affecting the exonuclease domain of POLE and POLD1. (D) Genetic features associated with germline POL variants (Fisher’s exact tests followed multiple testing correction using the FDR adjustment method).
Supplemental figure S7. Associations of DDR pathway gene alterations with POLE driver mutations.

(A) Top altered genes in the TCGA CRC cases with POLE driver mutations. (B) Comparisons of variant classes between DDR pathway and non-DDR genes in POLE driver-positive samples. (C) Distributions of TMB in POLE drivers, MSI_TMB-H, and MSI_TMB-ultraH samples.
Supplemental figure S8. Functional characterization of somatic POL variants of unknown significance.

(A-C) Distribution of TMB comparing samples with only one VUS across various domains of (A) POLE, (B) POLD1 and (C) POLH. Note the increased TMB associated with VUS affecting the exonuclease domains of POLE and POLD1.
**Supplemental figure S9. Additive effects of POL VUS on TMB.**

A. MSS samples (P<0.001)

B. Driver-negative samples

C. Driver-negative samples

D. Driver-negative MSS samples

E. Driver-negative MSS samples

The figures illustrate the additive effects of POL VUS on tumor mutation burden (TMB) across different categories of MSS samples and driver-negative samples, with statistical significance indicated by P-values.
(A) Changes in TMB levels comparing MSS samples with increased number of POL variants (Jonckheere-
Terpstra test P<.001). (B,C) Changes in TMB levels (B) and average VUS per polymerase gene (C) comparing
driver-negative samples with increased number of polymerase genes altered. (D,E) Changes in TMB levels (E)
and average VUS per polymerase gene (F) comparing driver-negative MSS samples with increased number of
polymerase genes altered.
Supplemental figure S10. No clear association of TMB with number of variants per gene in POL-negative samples.

(A-E) Changes in TMB levels comparing POL- samples with increased number of variants in (A) TP53, (B) LRP1B, (C) APC, (D) FAT1 and (E) PKHD1. (F) Permutation analysis showing the comparison of mutation rates in POL genes and background mutation rates (calculated using 500 randomly selected gene set with similar size to POL genes) in TMB-H samples.
Supplemental figure S11. Association of TMB with survival.

Kaplan-Meier estimates of overall survival in the TCGA cohort comparing patients with different TMB status.
Supplemental figure S12. Association of different variant types in the DDR pathway genes with TMB and survival.

(A) Kaplan-Meier estimates of overall survival in the TCGA cohort comparing patients with different DDR alteration types (DDR_loss: patients with only loss of function (i.e., frameshift or nonsense) DDR mutations; DDR_missense: patients with only otherwise missense DDR mutations. (B) Distributions of patients with varying numbers of POL/DDR pathway genes mutated (all variant types considered). (C) Distributions of TMB comparing patients with the two types of DDR pathway alterations as indicated.