**SUPPLEMENTAL INFORMATION**

Cancers from *Pole* mutant mice provide insights into polymerase-mediated hypermutagenesis and immune checkpoint blockade

**SI TITLES AND LEGENDS**

**Supplementary Figure S1 (related to Figure 1): *PoleP286R* mouse model design, validation, and tumor findings.**

(A) Schematic showing a portion of the mouse *Pole* gene used for CRISPR-Cas9 targeting for the P286R mutation. Altered bases are underlined in black and amino acid changes are bolded. Numbers indicate amino acid position. Green line indicates the sequence of the sgRNA and red line indicates the PAM site.

(B) Validation of mutant *Pole* allele expression in *PoleP286R/+* mice via Sanger sequencing of RT-PCR products from *Pole+/+* and two *PoleP286R/+* mice.

(C) Genotyping *Pole+/P286R* and *PoleP286R/P286R* mouse E13.5 embryos. (*Top*) The indicated 312bp PCR-amplified genomic DNA sequence surrounding the Pro286 codon is shown. The *Pole*WT allele contains three DdeI restriction sites. CRISPR editing knocked in the P286R codon change (red) and a silent T:A>C:G change (blue) that inactivates on DdeI site. Diagnostic DdeI restriction fragment sizes are shown. (Bottom) Agarose gel showing DdeI digests of *Pole+/+*, *Pole+/P286R* controls and *PoleP286R/P286R* embryo. Fragment sizes (bp) shown on left.

(D-H) *(Left)* Necropsy and *(Right)* histological findings from *PoleP286R* and *PoleS459F* mice. White bar indicates 50μm.

**Supplementary Figure S2 (related to Figure 1): *PoleS459F* mouse model design, validation, and tumor findings.**

(A) Schematic showing a portion of the mouse *Pole* gene used for CRISPR-Cas9 targeting for the S459F mutation. Altered bases are underlined in black and amino acid changes are bolded. Numbers indicate amino acid position. Green line indicates the sequence of the sgRNA and red line indicates the PAM site.

(B) Validation of mutant *Pole* allele expression in *PoleS459F/S459F* and *PoleS459F/+* mice via Sanger sequencing of RT-PCR products from *PoleS459F/S459F, PoleS459F/+,* and *Pole+/+* mice.

(C-L) *(Left)* Necropsy and *(Right)* histological findings from *PoleS459F* mice. Black bar indicates 50μm.

**Supplementary Figure S3 (related to Figure 2)**: **WES analysis reveals a stochastic accumulation of mutations in** ***Pole* mutant tumors.**

Mutation frequencies, as calculated by the number of mutations per target region covered by whole exome sequencing, are plotted per chromosome for each *Pole* mutant tumor.

**Supplementary Figure S4 (related to Figure 2)**: **Tumors from** ***Pole* mutant mice exhibit mutational signatures found in *POLE* driven human cancers.**

Whole-exome sequencing (WES) derived mutational signatures in *PoleS459F/S459F, PoleS459F/+,* and *PoleP286R/+* mouse tumors. The 96 possible mutation types based on trinucleotide context are shown for each sample that underwent WES in this study. Cosmic signatures as called by deconstructSigs are shown on the right for each sample.

**Supplementary Figure S5 (related to Figure 2):** Exome data from tumor fractions from a single mouse were compared for 3 additional mice.

**Supplementary Figure S6. Determination of cell of origin for *PoleS459F/S459F* and *PoleS459F/+* mice lymphomas (Related to Figure 3).**

**(A)** Representative 10% probability contour plots of TCRβ versus B220 or CD8b on live single cells (left 2 columns) or CD4 versus CD8β expression by and TCRβ+ (3rd column) and TCRβ- CD11b- B220- cells (last column) WT SPL (top row) Group A (middle row; 3031), and Group B (bottom row; 3158) samples. Quadrant gates (blue) show percentage of cells in each population.

**(B)** Scatter plots show the frequency of B cells (CD19+ CD22+) and myeloid cells (CD11b+) among total live cells from each group.

**(C)** Representative immunohistochemistry (IHC) staining with anti-CD3 (*top*) and anti-B220 (*bottom*) on WT SPL (*left*), Group A mediastinal mass (*middle*), and Group B enlarged SPL and mediastinal mass (*right*). scale bar = 50μm; inlet scale bar = 500μm.

**Supplementary Figure S7. Characterization of T cell malignancies in *PoleS459F/S459F* and *PoleS459F/+* mice (Related to Figure 3).**

**(A)** Contour plots show TCRβ versus the indicated markers on total live cells on 2 thymic lymphomas from *Atm-/-* mice stained with the same mass cytometry panel as the *Pole* lymphomas.

**(B)** Contour plots show CD4 versus the indicated markers on TCRβ+ cells from the samples shown in Supplementary Fig. S6A.

**(C)** Contour plots show IdU versus the indicated markers on TCRβ+ cells from the samples shown in Supplementary Fig. S6A.

**Supplemental Figure S8. Extrinsic effect of ‘Group B’ malignant T cells on B cell population; (Related to Figure 3).**

**(A)** Representative 10% probability contour plots of CD4 (y-axis) vs the indicated T cell activation markers (x-axis) expressed by TCRβ+ cells from WT spleen (top row) versus three Group B samples (18, 3124, and 3158). Quadrant gates (blue) show percentage of cells in each population.

**(B)** Heatmap of Germinal Center markers (columns) among B cells present in the listed samples (rows). Marker intensity is shown as the raw median value for each sample. Samples are manually grouped as control WT (n= 4) and Group B (n= 7) samples. Sample ID to the right indicates genotype and tissue code, wherein PP=Peyer’s patch, S=spleen, and L=lymph node.

**(C)** Contour plots of IgD vs CD95, CD150, BCL6, and IdU expression on CD22+ (B cells) between WT Peyer’s patch and indicated Group B samples. Quadrant gates (blue) show percentage of IgD- GC-like B cells amongst total B cell population.

**(D)** Probability contour plots (10%) of CD4 vs PD-1 (left), or ICOS (right) expression by TCRβ+ cells from thymus (top) versus spleen (bottom) isolated from three different mice with Group B lymphomas (*Top*, 18; *middle*, 3124; *Bottom*, 4219). Quadrant gates show percentages of CD4-expressing PD-1+ and ICOS+ T cells in blue.

**SUPPLEMENTAL INFORMATION TABLES**

**Supplementary Table S1:** Spontaneoustumor incidence across *PoleS459F/S459F, PoleS459F/+, and PoleP286R/+* moribund mice. Moribund mice were euthanized and necropsied, and tumors were diagnosed by histology. **\***Incidences (%) among 31 *PoleS459F/S459F*, 33 PoleS459F/+, and 27 *PoleP286R*/+ mice. ¥For lymphomas mice were divided into two groups: 1) mice with mediastinal masses (thymic lymphomas), which typically included infiltration and enlargement of the spleen and other lymph nodes, and 2) mice with spleen and/or lymph node enlargement (without macroscopic thymic enlargement).

**Supplementary Table S2:** Summary ofPOLE germline mutations in human cancer

**Supplementary Table S3:** Mass cytometry panel