**Supplementary Methods**

**Constructs**

CRISPR constructs: For our CRISPR-based glioma models, oligo primers were used to clone the following sgRNAs into the pX330-Cas9 vector. PiggyBac constructs: pGLAST-PBase and pPBCAG-eGFP (gifts from Dr. LoTurco) were used to mark the electroporated glial lineage cells and tumors.

Trp53: 5’-GCCTCGAGCTCCCTCTGAGCC-3’,

Pten: 5’- GAGATCGTTAGCAGAAACAAA-3’,

Nf1: 5’-GCAGATGAGCCGCCACATCGA-3’.

Pot1a: 5’-GTACCTTCCTTGAGTAGATTC-3’,

Pot1b: 5’-GCCTTCCTTAAGTAGATTTAG-3’

Wildtype POT1 coding sequence was cloned from an ORF library into pPBCAG-eGFP-2a Gateway vector. POT1 mutant versions were generated using a standard targeted mutagenesis protocol and their sequences were confirmed using Sanger sequencing

Lentivirus constructs and virus generation: Mutant POT1 sequences or mCherry (Control) were cloned into pHAGE-EF1a-IRES-Puro Gateway vector and lentivirus was generated in 293T cells and collected over 48 hours and concentrated via PEG precipitation.

**Mice**

To generate mouse gliomas, we used pregnant CD1 wild type mice for IUE surgeries. The mice were purchased from The Center for Comparative Medicine (CCM) at Baylor College of Medicine (BCM). All procedures were approved by the Institutional Animal Care and Use Committee and conform to the US Public Health Service Policy on Humane Care and Use of Laboratory Animals.

**IHC Details and antibodies**

For immunohistochemistry (IHC), slides were dehydrated followed by antigen retrieval at 99°C in pH 6.0 sodium citrate buffer. Endogenous peroxidases were then blocked using 3% H2O2. After serum blocking, slides were incubated with primary antibody overnight at 4°C followed by secondary antibody for 1 hour at room temperature. DAB and hematoxylin were then applied for chromogenic stain and counterstain. For immunofluorescence (IF), the H2O2 step was replaced with Sudan Black treatment to reduce autofluorescence, and the DAB and hematoxylin steps were also replaced by Hoechst nuclear counter staining. **Antibodies** The following primary antibodies were used at the listed titers: Iba1 (Abcam ab5076, 1:100), Ki67 (BD Pharmingen B56, 1:100), TP53BP1 (Abcam ab36823, 1:250), BrdU (Abcam ab6326, 1:200), GFP (Thermo Fisher Scientific A-11122, 1:1000), POT1 (Santa Cruz Biotechnology sc-33789, 1:200). We used species-specific secondary antibodies (1:1000) tagged with HRP (for IHC and WB) or Alexa Fluor 488 or 568 (for IF).

**Image acquisition and quantification**

All IHC and IF images were obtained using Zeiss Axio Imager M2 Fluorescent microscope. IHC images were first subjected to batch automated filtering for brown pigmentation (DAB product) by Adobe Photoshop (parameters available upon request). Filtered IHC and raw IF images were then subjected to batch automated quantification of particle count or area by ImageJ. Parameters for thresholding, size, and circularity are available upon request.

**BrdU labeling**

For the non-cancer model, IUE mouse pups were subjected to daily intraperitoneal injection of BrdU (50 μg/g body weight) at P6-P10 prior to sacrifice at P13. Brains were then harvested and fixed in 4% paraformaldehyde and 70% ethanol prior to paraffin embedding. Sections of medial prefrontal cortex were used for BrdU quantification. Normalized BrdU+ count was defined as the ratio of BrdU+ count on the electroporated (marked by GFP) and non-electroporated sides. Results were quantified from 3 mouse brains in each group and sex-determination (F:female, M:male) showed the following breakdown: Control: F/F/F, POT1(WT): F/M/M, POT1-G95C: M/M/M, Pot1a/b CRISPR: F/F/F.

**Telomere FISH assay**

In short, PNA probe (PNA Bio Inc, F1002 TelC-Cy3) at 300 ng/mL in PNA buffer was added to slides and heated at 84°C for 5 minutes for denaturation followed by hybridization at room temperature for 2 hours. After wash steps, GFP immunofluorescence and Hoechst counterstain was performed. Images were acquired using an Olympus IX-71 at 100X followed by deconvolution using a conservative restorative algorithm. Projected gray-scale TIFFs were used for quantification of telomere content relative to total nuclear DNA using Telometer (FIJI plugin).

**DNA damage quantification**

We used TP53BP1, a marker of DNA double-strand break, as a surrogate for DNA damage. Briefly, we performed IHC on glioma tumor sections using TP53BP1 antibody along with a DAPI nuclear counterstain. Images were obtained on a confocal microscope (Airyscan 2 LSM 900) with a 63X oil objective. Z-stacks were convertaed into composite gray-scale TIFF images which were subsequently used for quantification of TP53BP1 signal relative to total nuclear DNA using Telometer (FIJI plugin). Results showing 0% or greater than 25% relative signal (rare) were discarded.

**Human glioma data**

The clinical and genetic data were updated using The Broad Institute’s Firehose pipeline. Raw RNA sequencing counts for these tumors processed through Rsubread and featureCounts were obtained from Gene Expression Omnibus (GSE62944). Tumors missing IDH or codeletion status, grade, survival information, or RNA-seq data were excluded. Only IDH-wildtype tumors were included for subsequent analyses, and Grade II and III tumors were counted as one factor level against grade IV tumors in linear modeling of IDH-wildtype glioma.

**Statistical analysis**

The number of biological replicates for each experiment are listed in the corresponding Results section. For experiments on brain sections, between 3-5 sections per animal were analyzed and averaged, and for brain sections containing tumors, 3 areas of tumor per section were analyzed and averaged. For the BrdU assay, brain sections from each mouse where BrdU counts in the EP side and non-EP side varied by >2 folds were discarded. For all sex-tumor interaction analyses, any sample value varying >2 standard deviations from the group mean was discarded (a very rare occurrence). One-way ANOVA was used to compare multiple means in the limiting dilution assay and the BrdU assay. Means of two groups were compared using Student’s t test and two proportions were compared using Fisher’s exact test. All Kaplan-Meier analyses used the log-rank test to compare groups. Cox proportional hazard model for human IDH-wildtype glioma consisted of 3 parameters: age, grade, and POT1 expression (Log2 transformed). All linear models used to assess sex-tumor interaction were of the same form: effect = a + b(sex) + c(tumor) + d(sex:tumor), where effect was one of log of hazard ratio (Cox model), telomere content, Log2(expression), Iba1+ IHC area, or Ki67+ cell density.