

Supplemental Figure Legends

Figure S1. Pearson correlation plots of genome-wide poly-A and stranded PSI values for SE events in PT_RYMG3M91 **(A)** and PT_W5GP3F6B **(B)** showing high concordance across RNA library types. **(C)** UpsetR plot showing recurrent differential splicing events ($N \geq 2$) that prefer exon inclusion. Cumulative distribution plots of splicing burden index (SBI) by histology for RI **(D)**, A5SS **(E)**, and A3SS **(F)** events. Correlation plots for SBI vs TMB across the entire cohort including **(G)** and excluding **(H)** hypermutant and ultra-hyper mutant tumors, or within histology **(I)** excluding hypermutant and ultra-hypermutant tumors. Pearson's R and p-values are shown.

Figure S2. **(A)** Stacked barplot showing tumor histology membership in each cluster stratified by molecular subtype for EPN, HGG, LGG and MB histologies. **(B)** Oncoprint displaying mutation frequencies of splicing factors and spliceosome component genes in HGGs sorted by splicing burden index. Additional annotations include gender, molecular subtype, CNS region, and tumor mutation status.

Figure S3. **(A)** Over-representation analysis of mis-spliced kinase genes that result in gain/loss of functional sites stratified by **(A)** exon skipping and **(B)** exon skipping. **(C)** Boxplot of *CLK1* exon 4-containing transcript expression in HGGs compared to GTEx normal brain tissues. **(D)** Oncoprint displaying mutation frequencies of key brain cancer genes in HGGs with annotations for *CLK1* exon 4 PSI, *CLK1-201* expression, total *NF1*, *NF1-215* PSI, *NF1* pS864 and pS2796 phosphoproteomic abundance, and total *NF1* protein abundance z-scores. Additional annotations include gender, molecular subtype, CNS region, and tumor mutation status. All boxplots represent the 25th and 75th percentile and the bar represents the median.

Figure S4. **(A)** Boxplot of dependency scores stratified by high vs low *CLK1* exon 4 containing transcript expression across all available DepMap brain tumor cell lines. Within histology

Wilcoxon p-values are shown. All boxplots represent the 25th and 75th percentile and the bar represents the median. **(B)** Cell viability assay after three days of treatment of KNS-42 cells with increasing concentrations of pan-DYRK/CLK1 inhibitor Cirtuvivint. **(C)** Heatmap displaying single-sample HALLMARK GSVA scores for DS genes affecting functional sites in cells treated with *CLK1* exon 4 morpholino or non-targeting morpholino. **(D)** Barplots illustrating the mean GSVA scores in panel **D** (n = 3 replicates per treatment). **(E)** Heatmap presenting single-sample DNA repair pathway GSVA scores for DS genes affecting functional sites in cells treated with *CLK1* exon 4 morpholino or non-targeting morpholino. **(F)** Barplots displaying mean DNA repair pathway GSVA scores from panel **E** (n = 3 replicates per treatment).

Figure S5. (A) Venn diagram showing the overlap of the total number of DS and DE genes. **(B)** Over-representation analysis of DE genes or **(C)** DS cancer genes that result in gain/loss of functional sites. **(D)** Ranked dotplot of significant CCMA v3 CRISPR gene dependency z-scores in individual CBTN cell lines with *CLK1* expression (red) and splicing-based (blue) target genes highlighted for HGG or **(E)** DMG patient-derived cell lines.

Figure S1

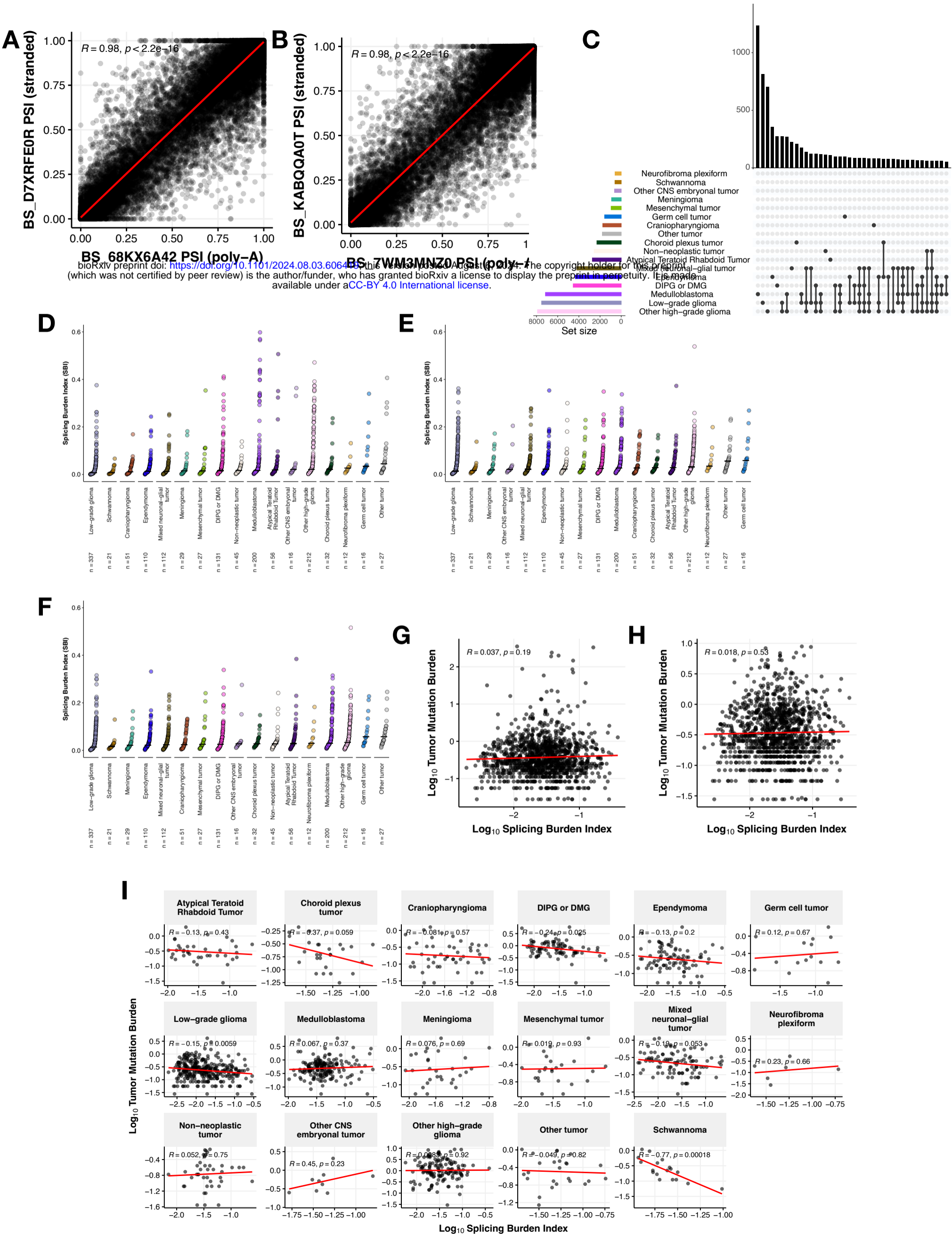
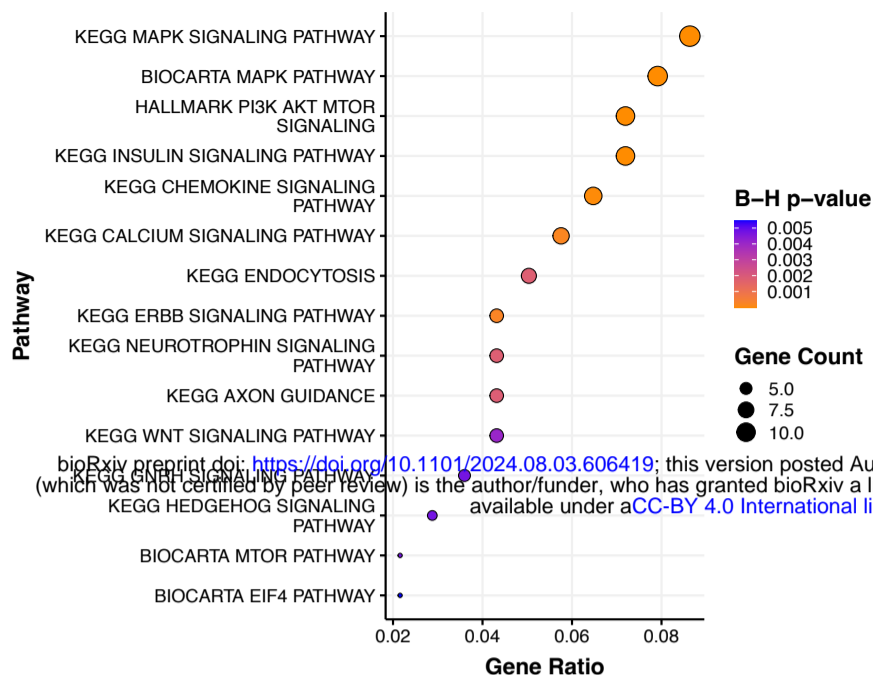
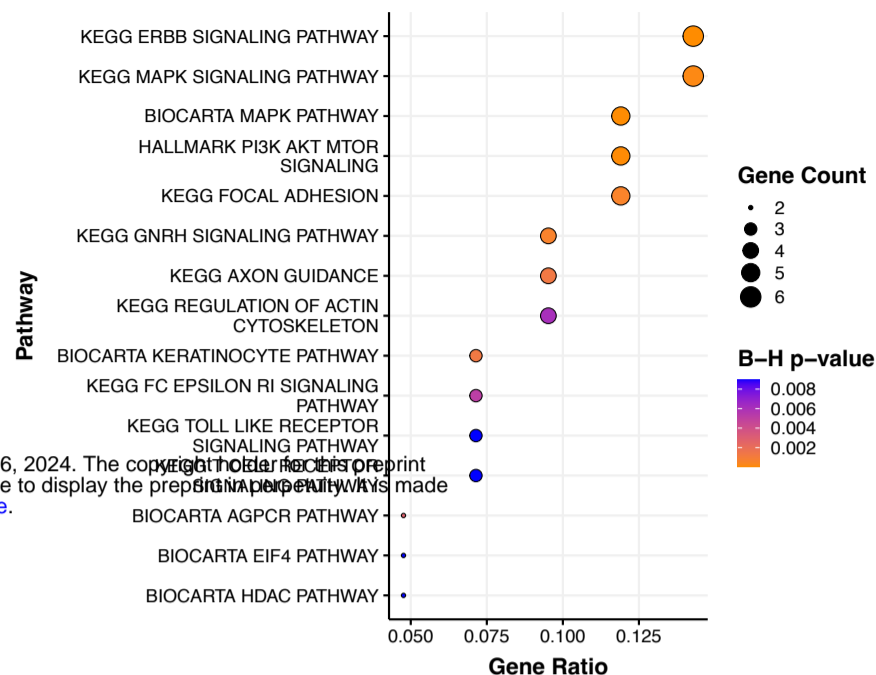


Figure S3

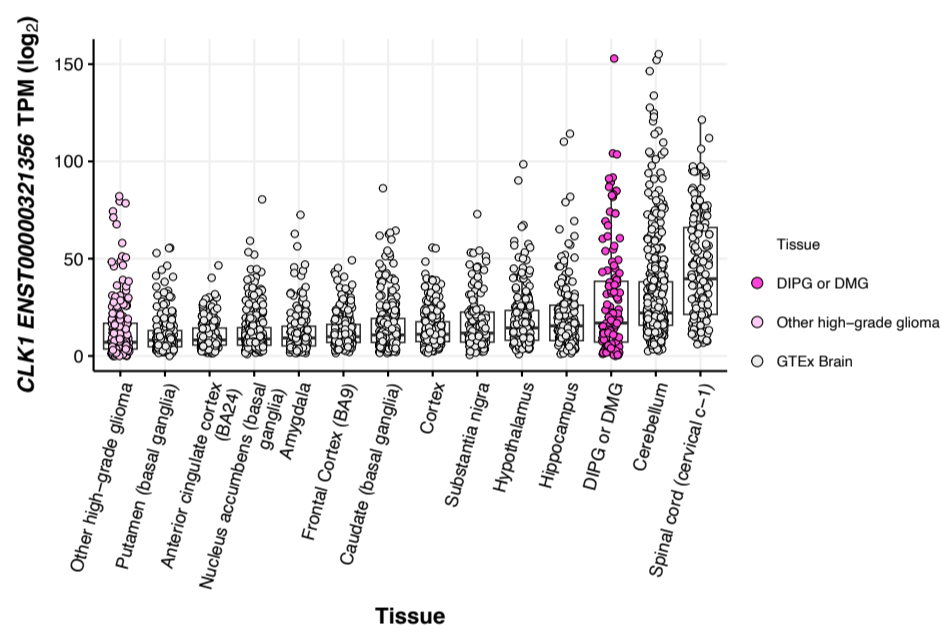
A Exon Skipping



B Exon Inclusion



C



D

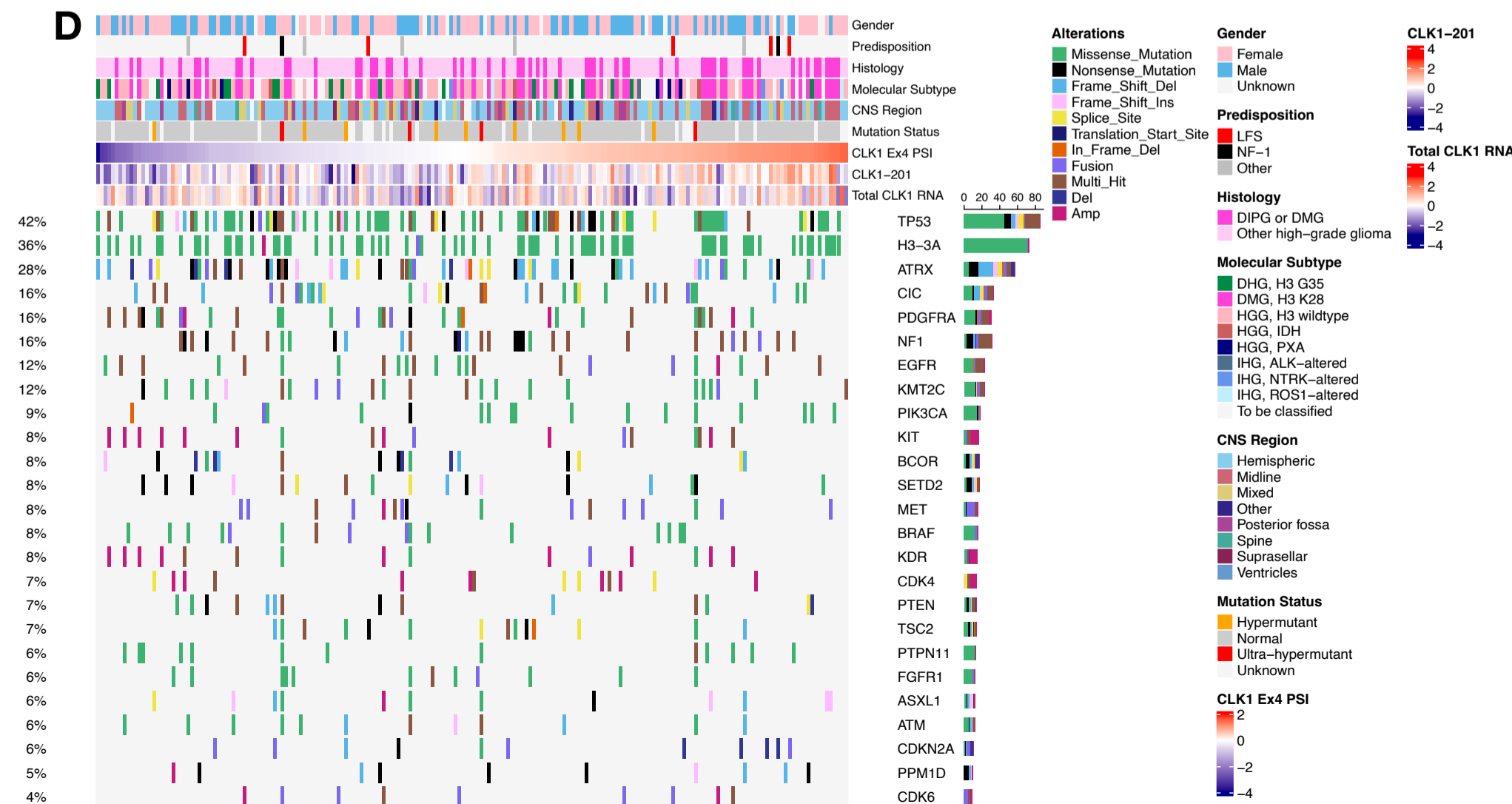
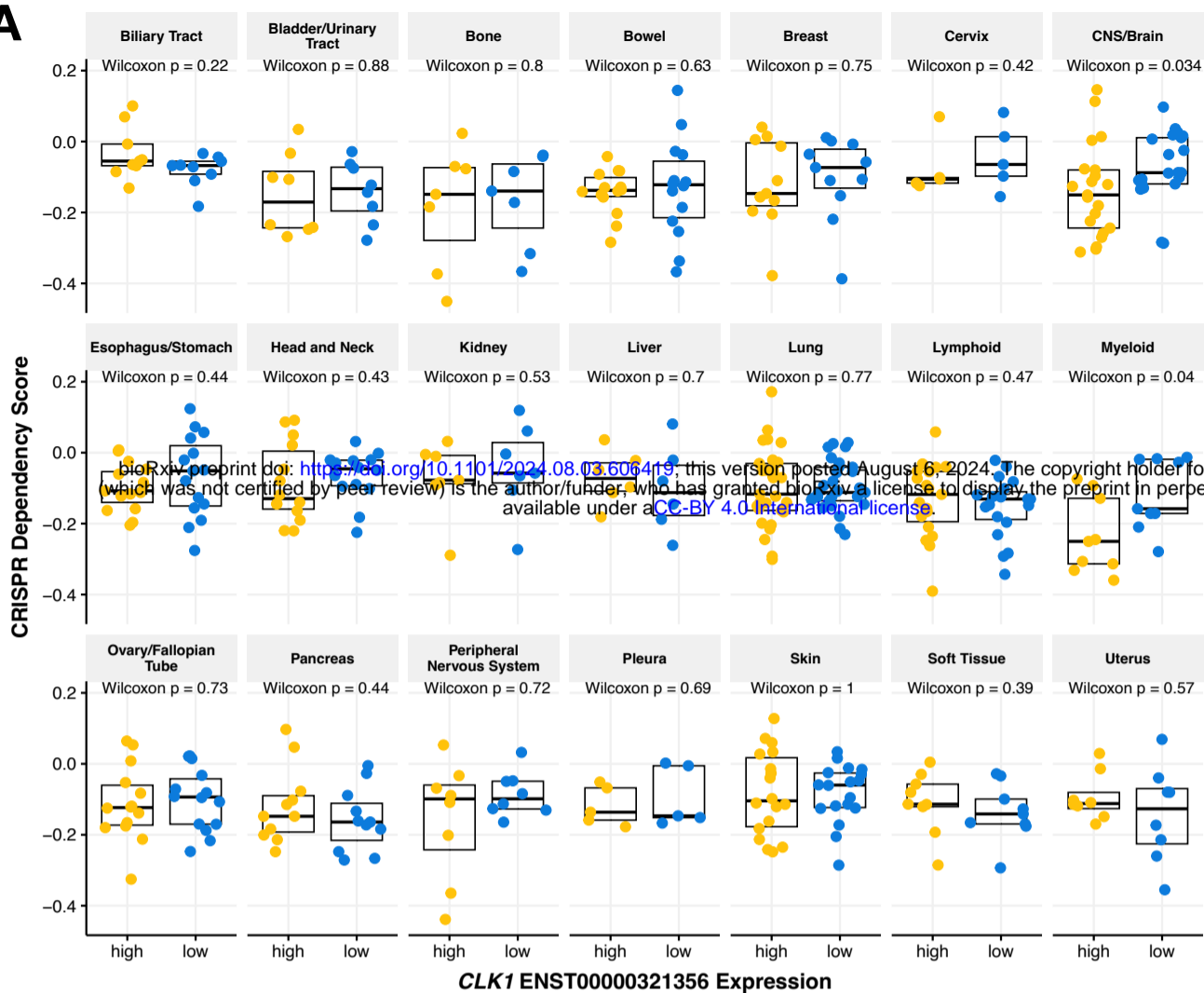
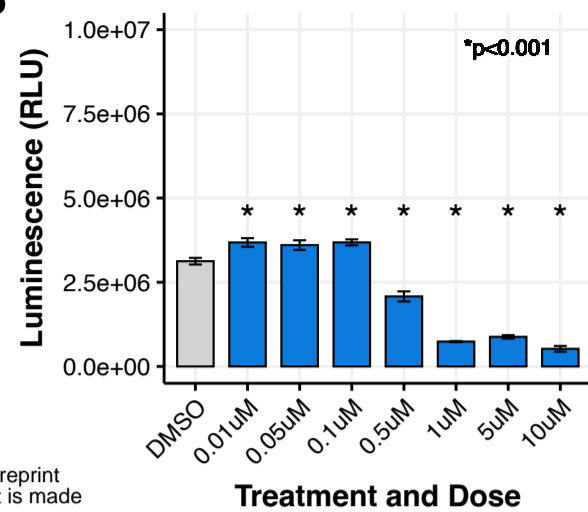


Figure S4

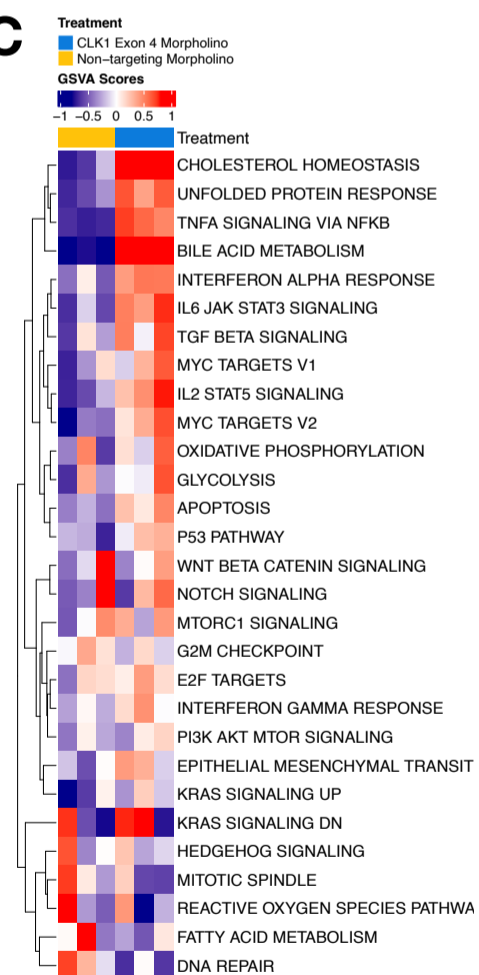
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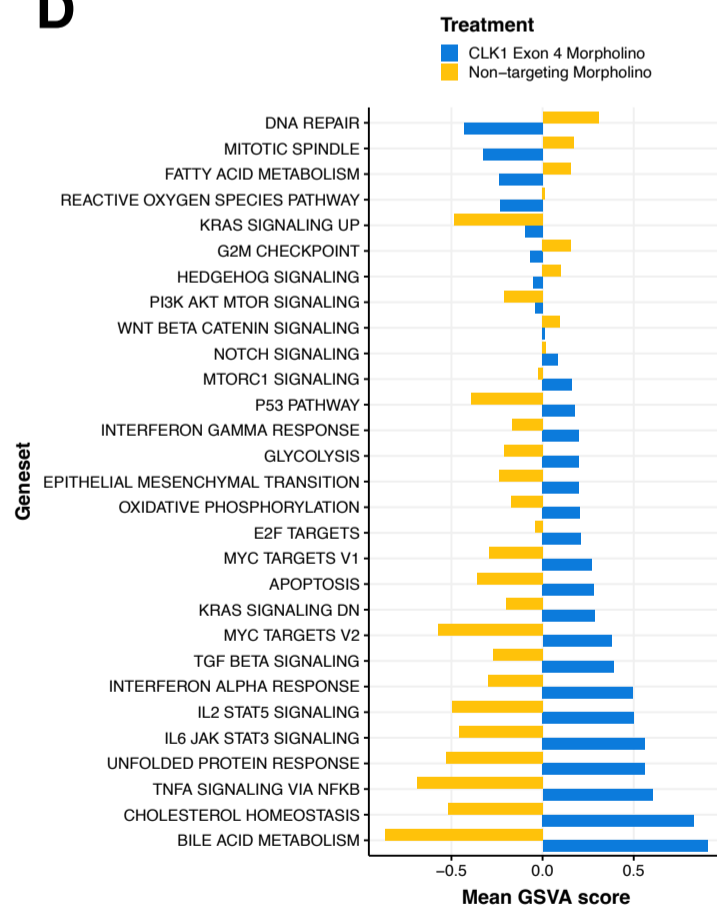
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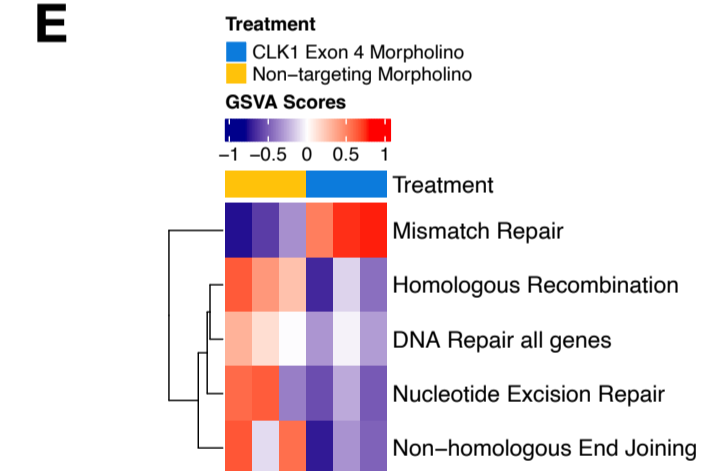
C



D



E



F

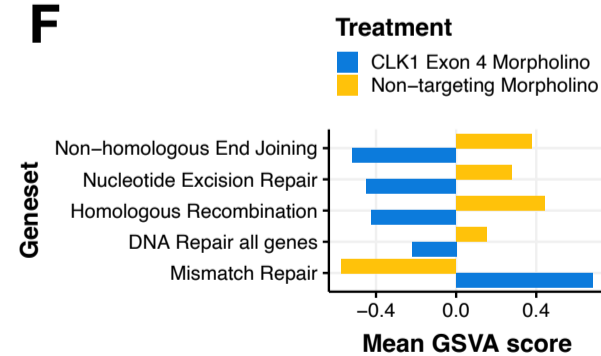
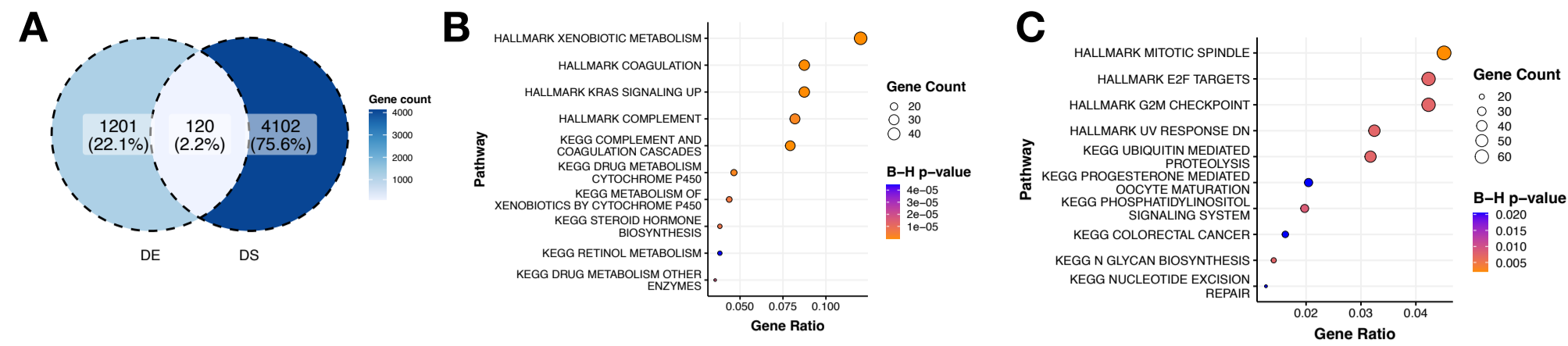


Figure S5



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