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## Supplemental information

# Genomic analysis of human brain metastases treated with stereotactic radiosurgery reveals unique signature based on treatment failure

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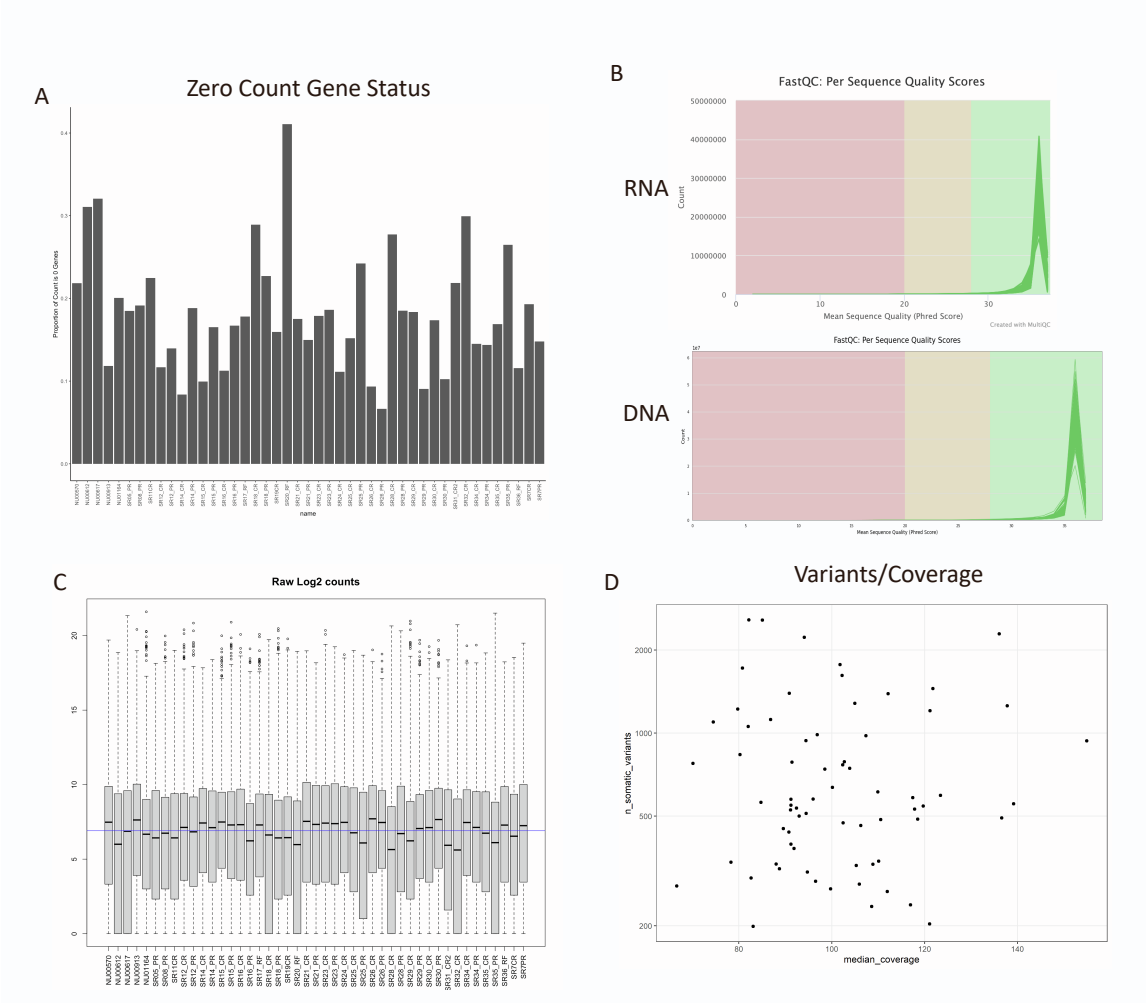
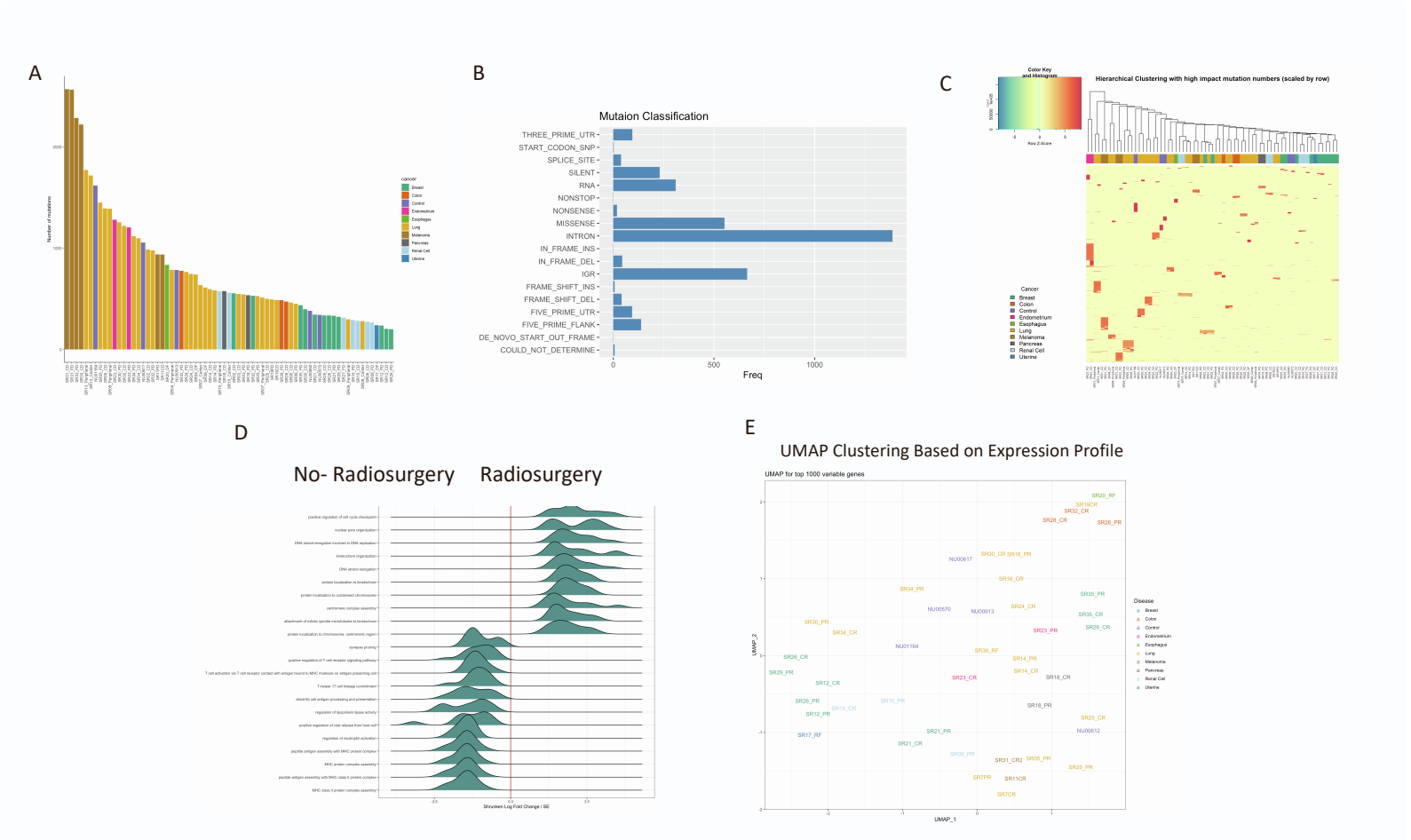
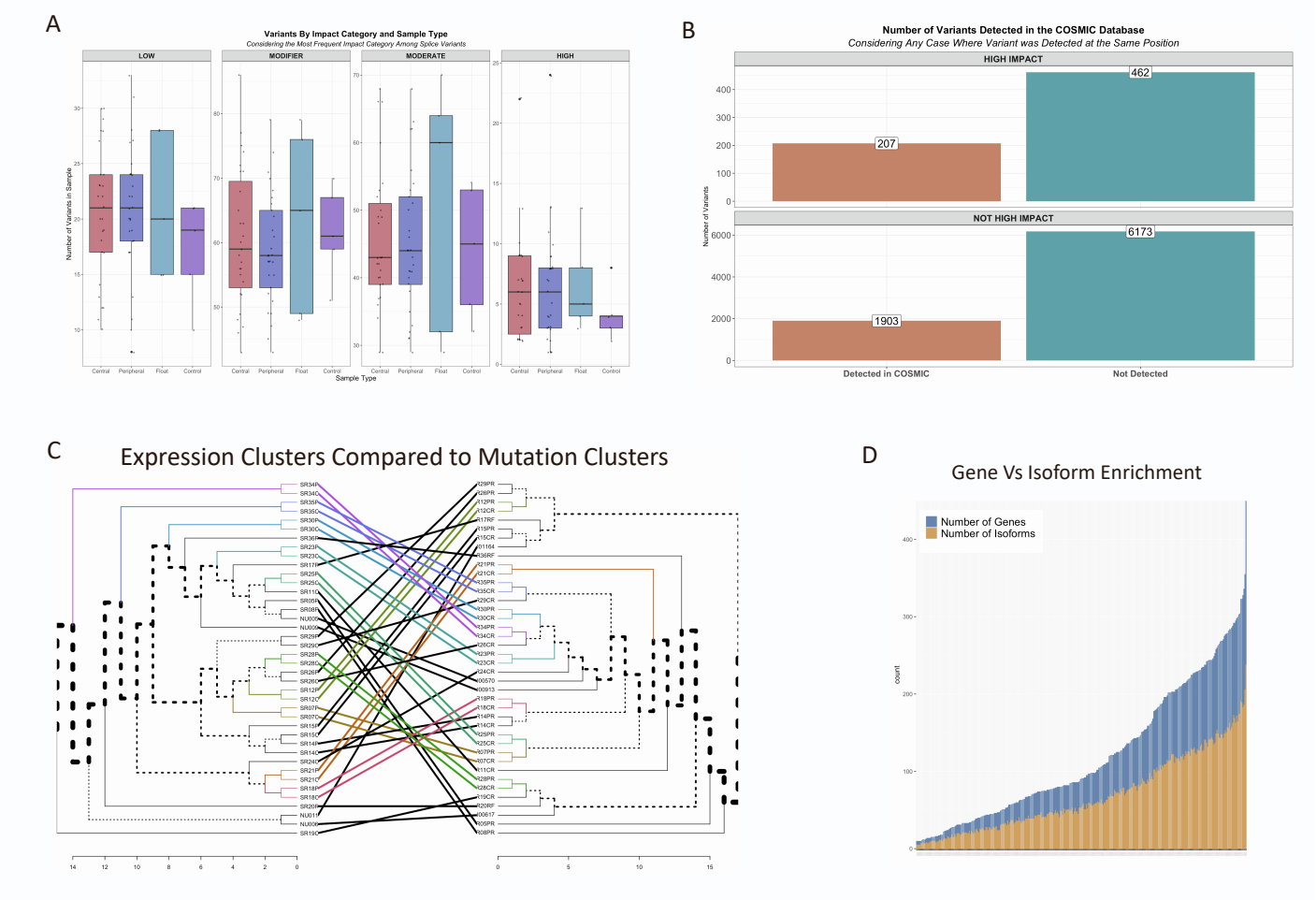


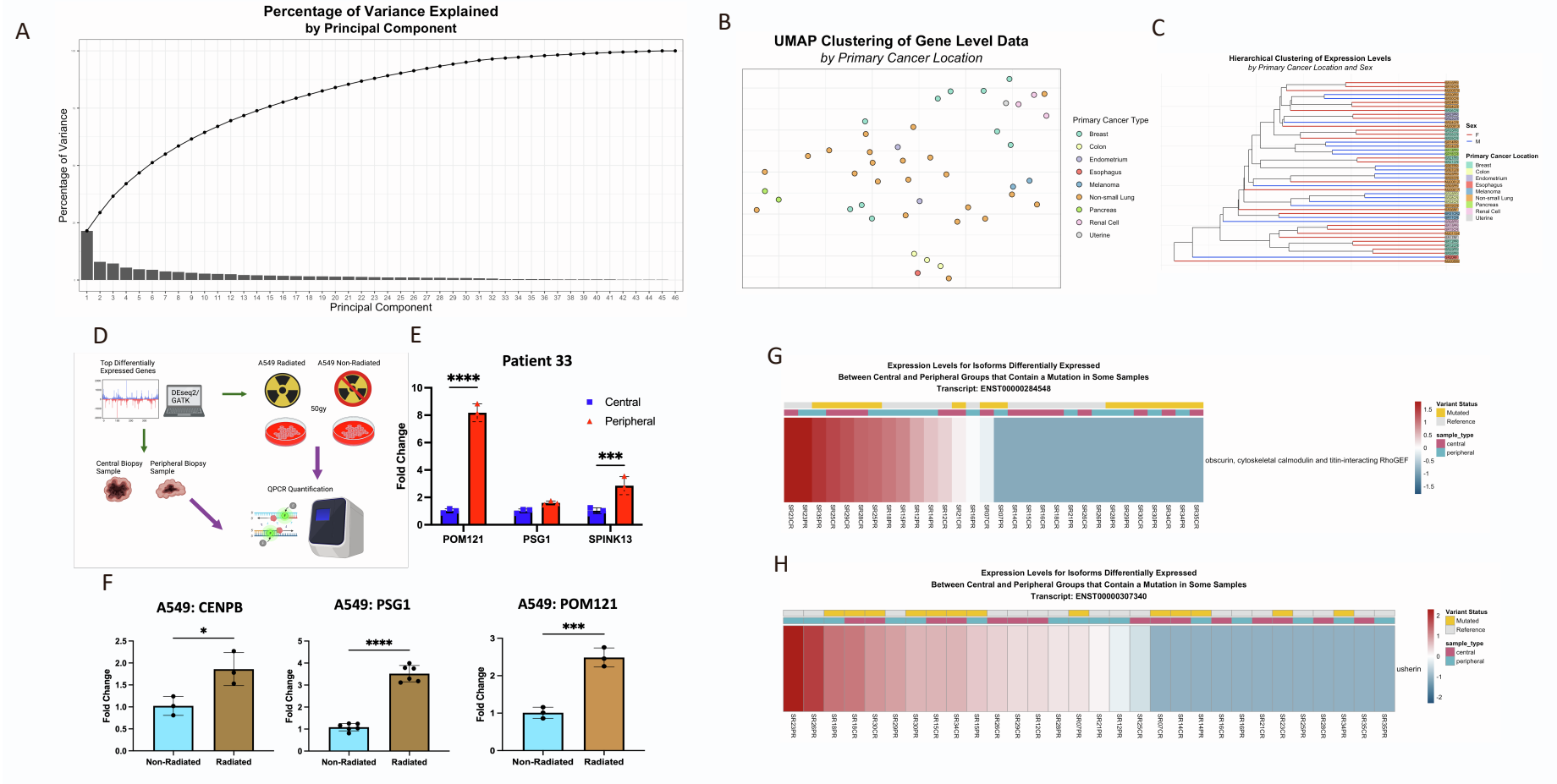

**Supplementary Figure 1:** *Quality control of sequenced samples.* [Related to Figure 1] **A)** Infographic depicting the primary tumor type of clinical trial participants and components of center or peripheral DNA and/or RNA contributed to the analyses. **B)** FASTQC per sequence quality scores across all samples for WES and RNA-Seq. **C)** RAW log2 counts per sample. **D)** Plot of variants detected per coverage obtained across samples.




**Supplementary Figure 2:** *Radiosurgery vs No-Radiosurgery comparisons among all sample types.* [Related to Figure 2] **A)** Plot of number of variants detected per sample annotated by primary tumor location. **B)** Classification of type of mutation found within samples and its frequency. **C)** Hierarchical clustering applied to variants detected across samples. **D)** ALLEZ GSEA of all significant terms between radiosurgery and no-radiosurgery groups (not restricted to lung primary tumor location). **E)** UMAP clustering applied to top 1000 variable genes and annotated with tumor type.




**Supplementary Figure 3:** *Gene annotation and isoform enrichment between central and peripheral biopsy samples.* [Related to Figure 3] **A)** Impact of variants called among all sample types with float representing samples where central or peripheral location could not be determined. **B)** Results of annotation of variants using the COSMIC dataset. **C)** Chaos plot of clustering between WES data and RNA-seq data. **D)** Visualization of gene vs isoform enrichment among all samples.




**Supplementary Figure 4:** *Clustering among all patient samples along with RNA expression levels of genes with detected mutations.* [Related to Figure 5] **A)** Visualization of principal components used for dimensional reduction clustering. **B/C)** UMAP and hierarchical clustering of samples by tumor primary location and primary location and patient sex, respectively. **D)** Schematic depicting the in-vitro validation of bioinformatic gene hits using QPCR on both isolated peripheral and central biopsy samples as well as radiated A549 primary tumor cells. **E)** QPCR quantification of relative expression of POM121, PSG1, and SPINK13, in isolated central or peripheral tumor biopsies. **F)** QPCR quantification of relative expression of CENPB, PSG1, and POM121, in radiated and non-radiated A549

tumor cells. **G/H)** Visualization of gene expression levels (RNA-Seq) among genes that were annotated to have a variant (WES). * $p<0.05$, *** $p<0.001$, **** $p<0.0001$