

Figure S1

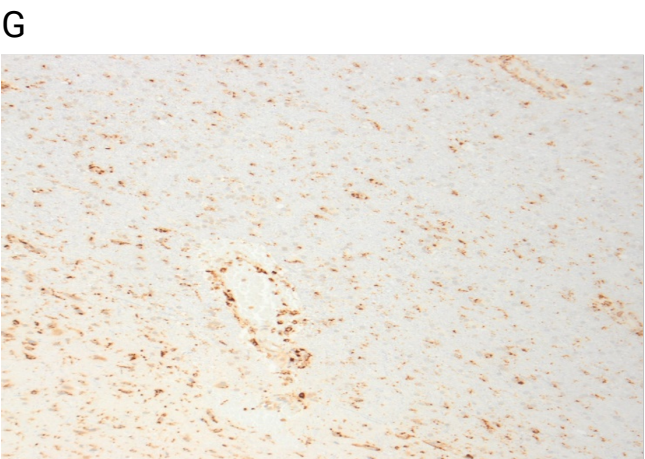
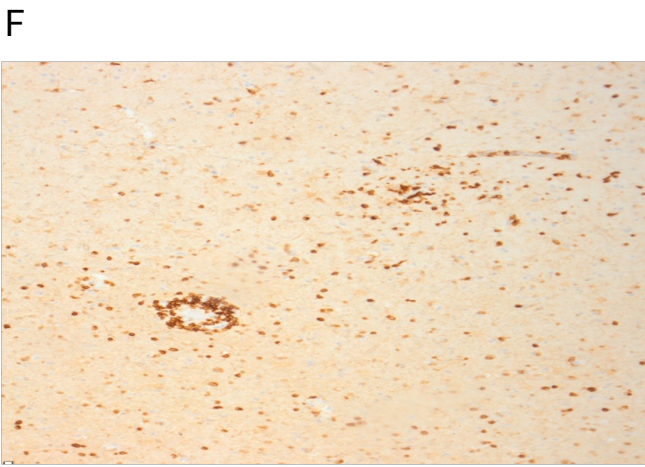
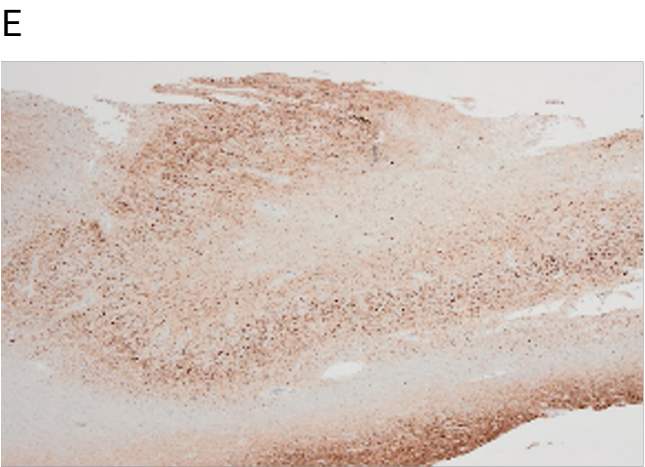
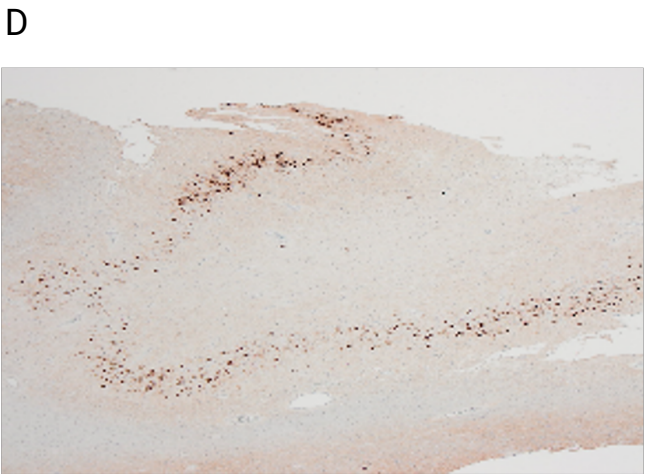
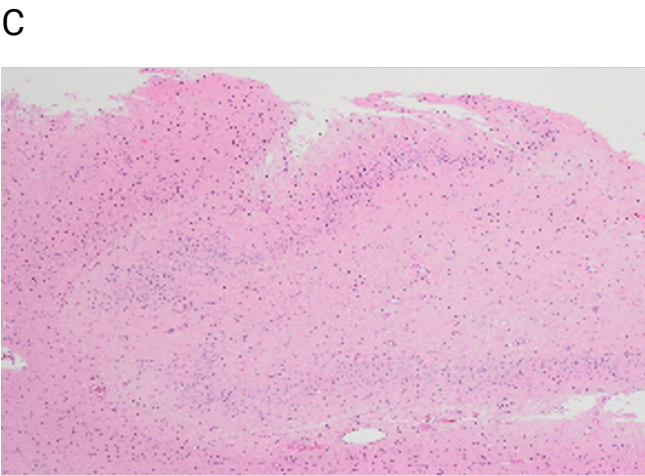
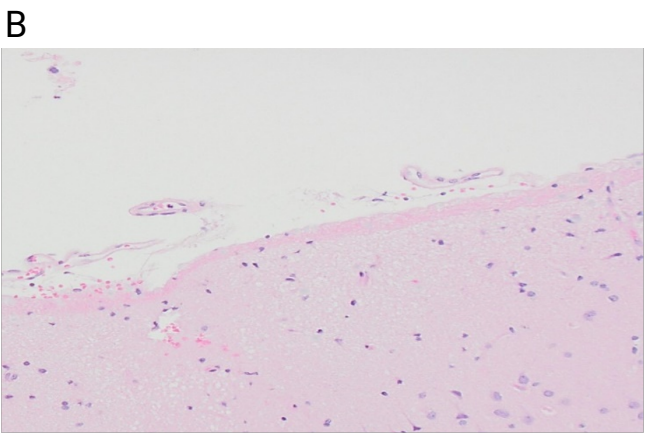
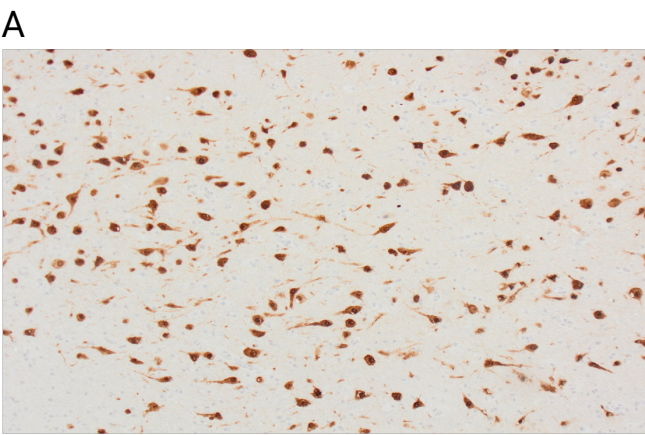
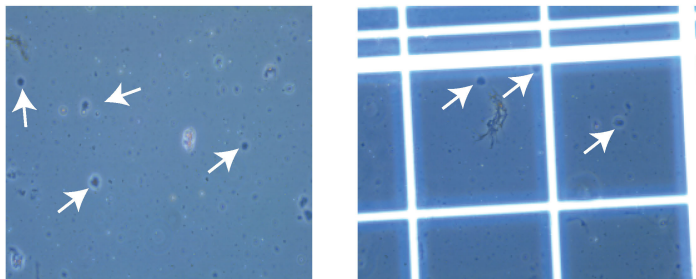


Figure S2

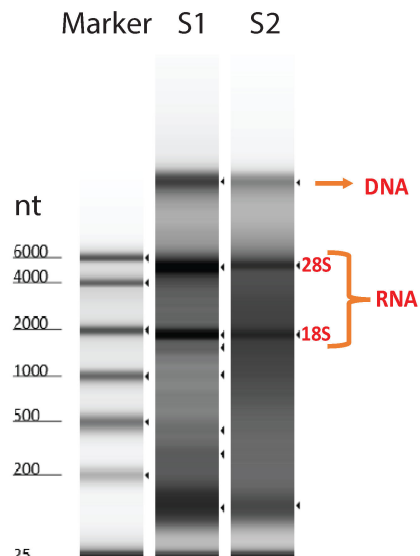
A.



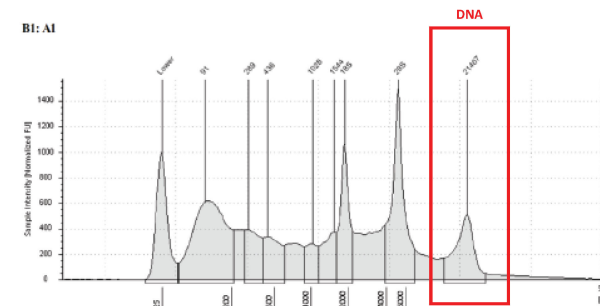
B.

Number of cut pieces	Concentration (ng)	A260/280	A260/230
12	84	1.98	1.08
6	31.1	1.86	0.74
4	34.8	1.87	0.23
3	35	1.83	1.03
2	13.9	1.58	0.35

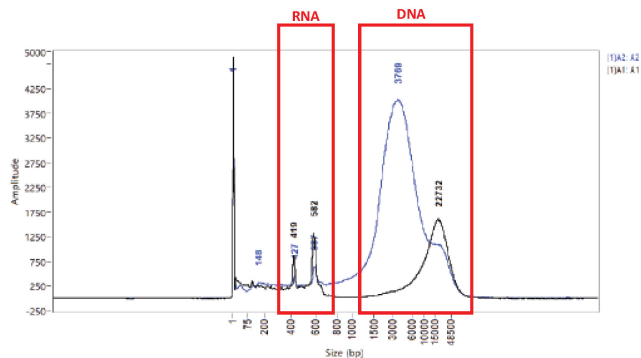
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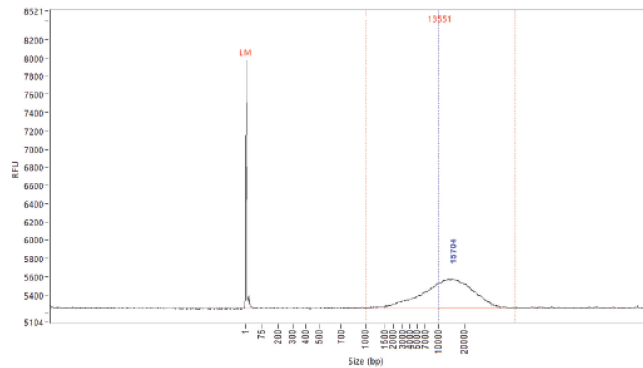
D.



E.



F.



G.

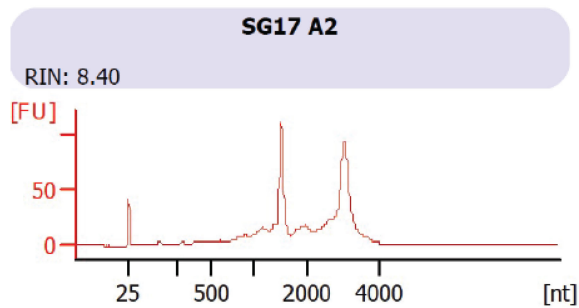


Figure S3

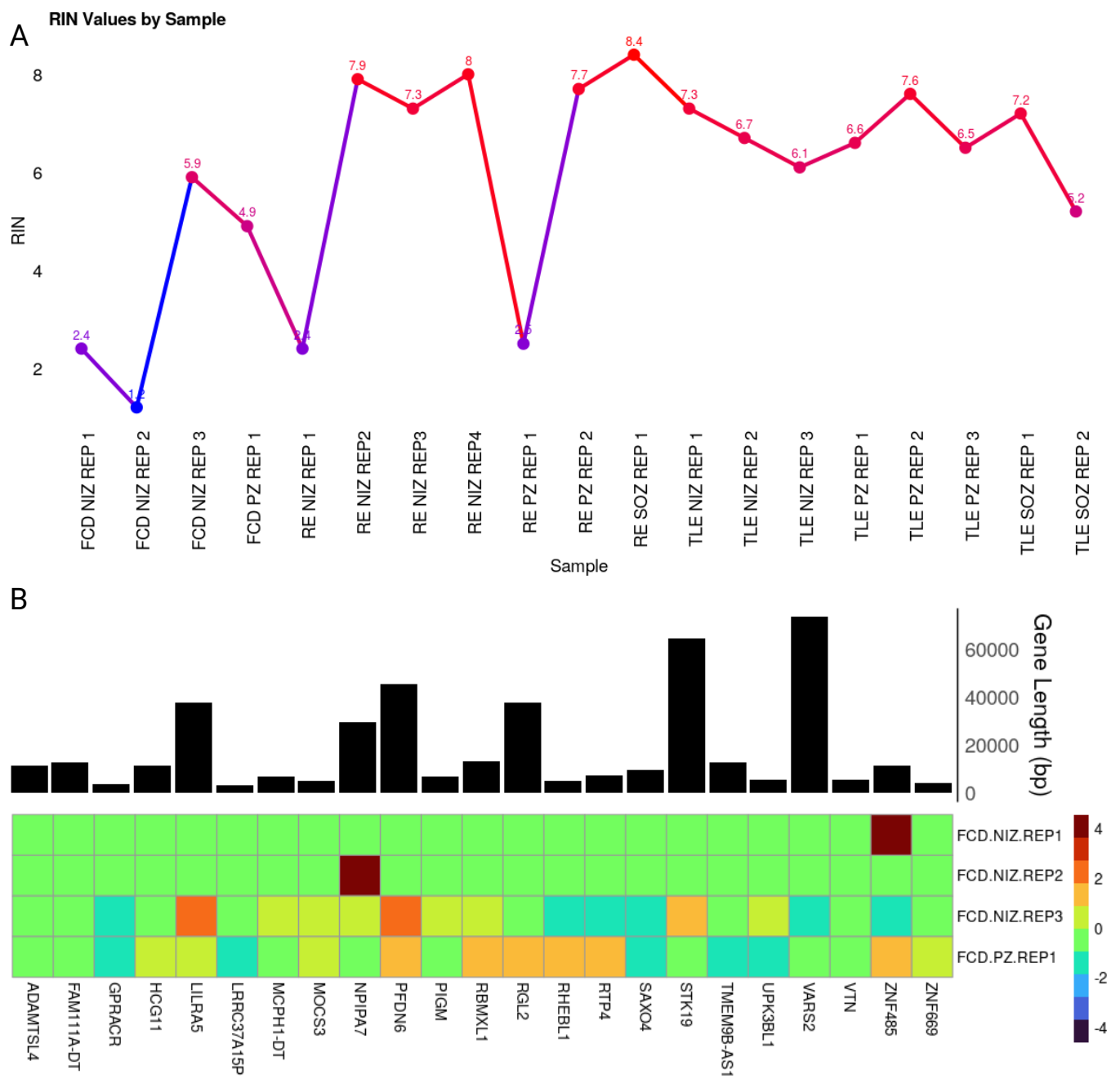


Figure S4

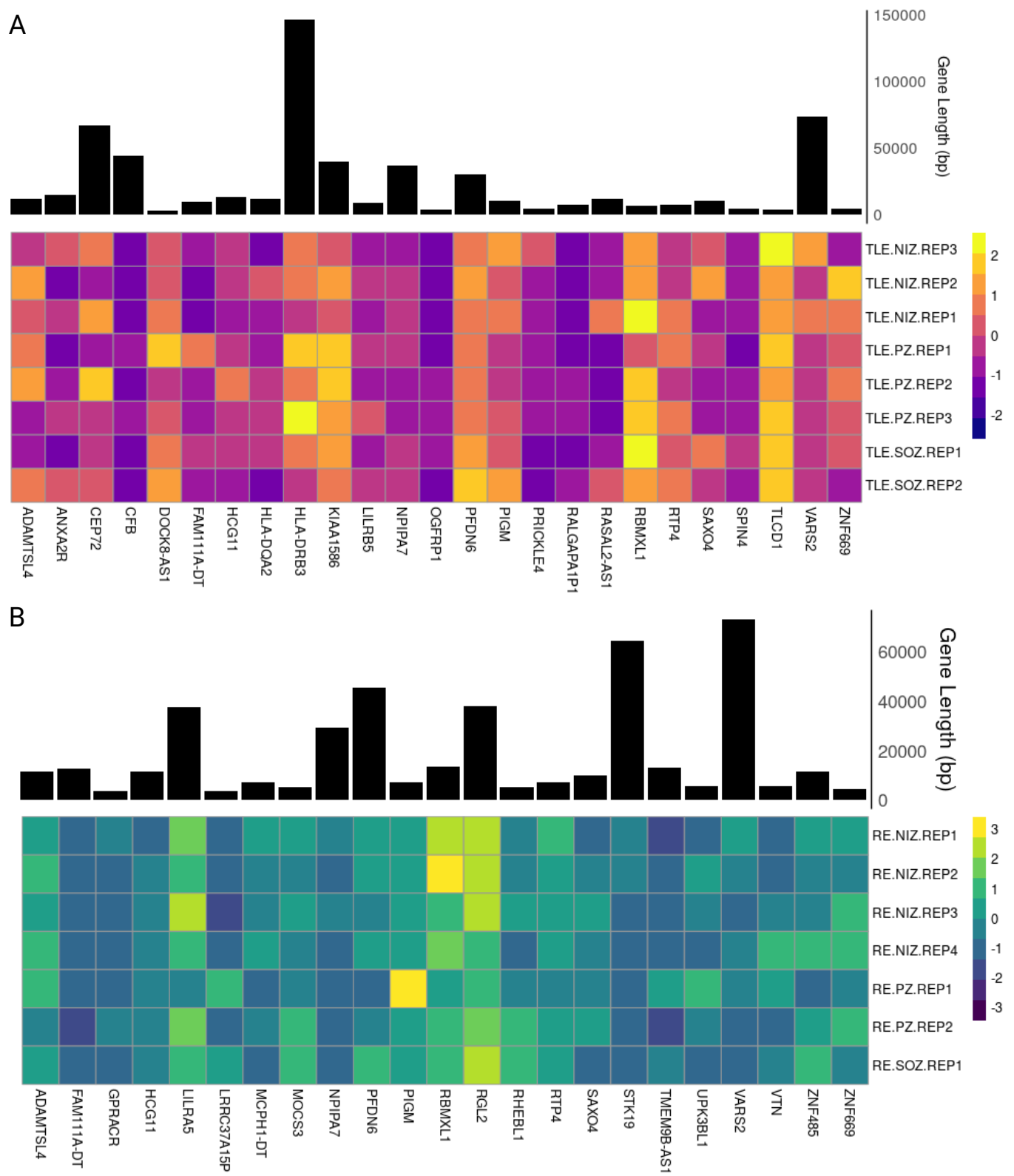


Figure S5

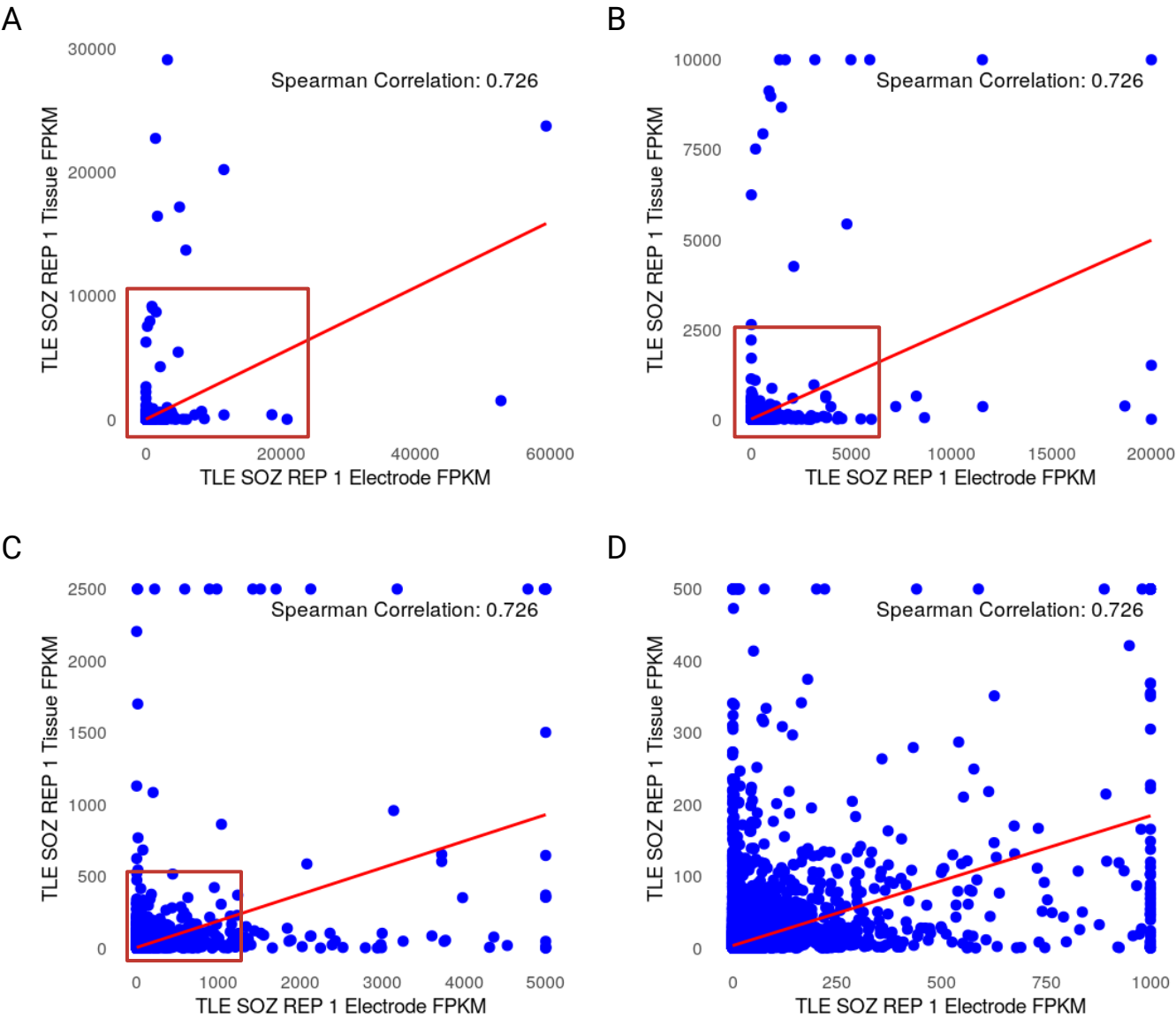


Figure S6

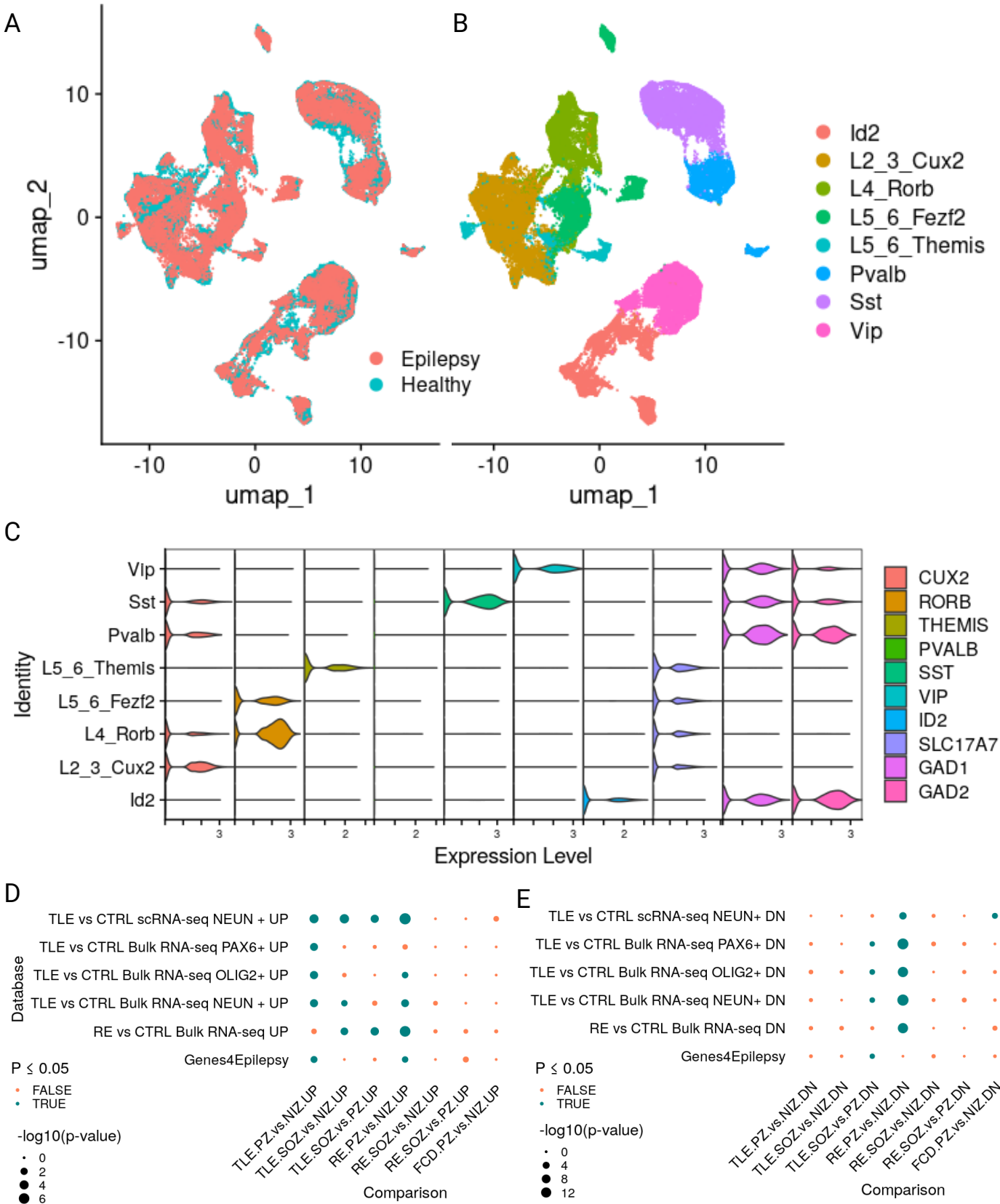


Figure S7

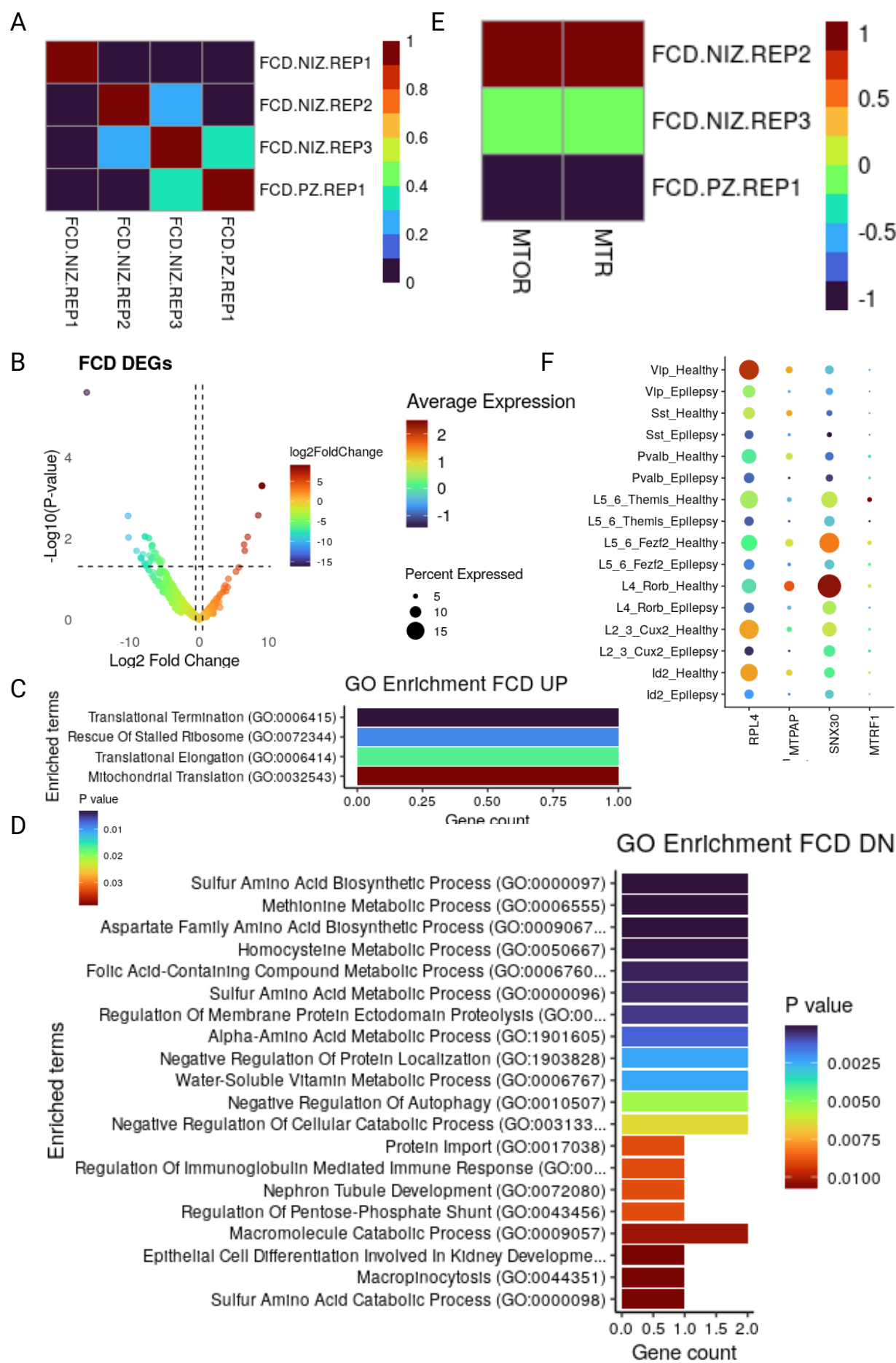


Figure S8

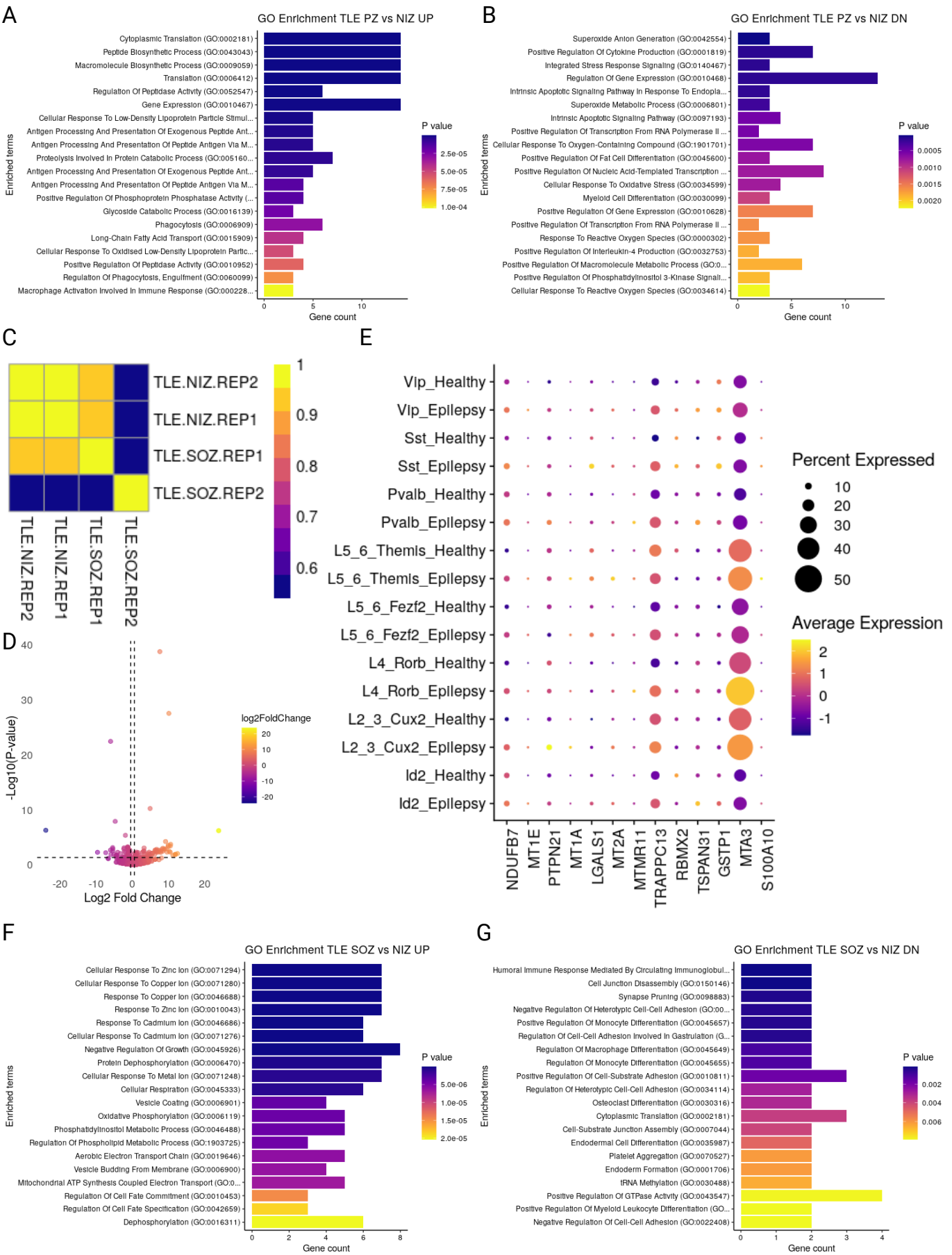


Figure S9

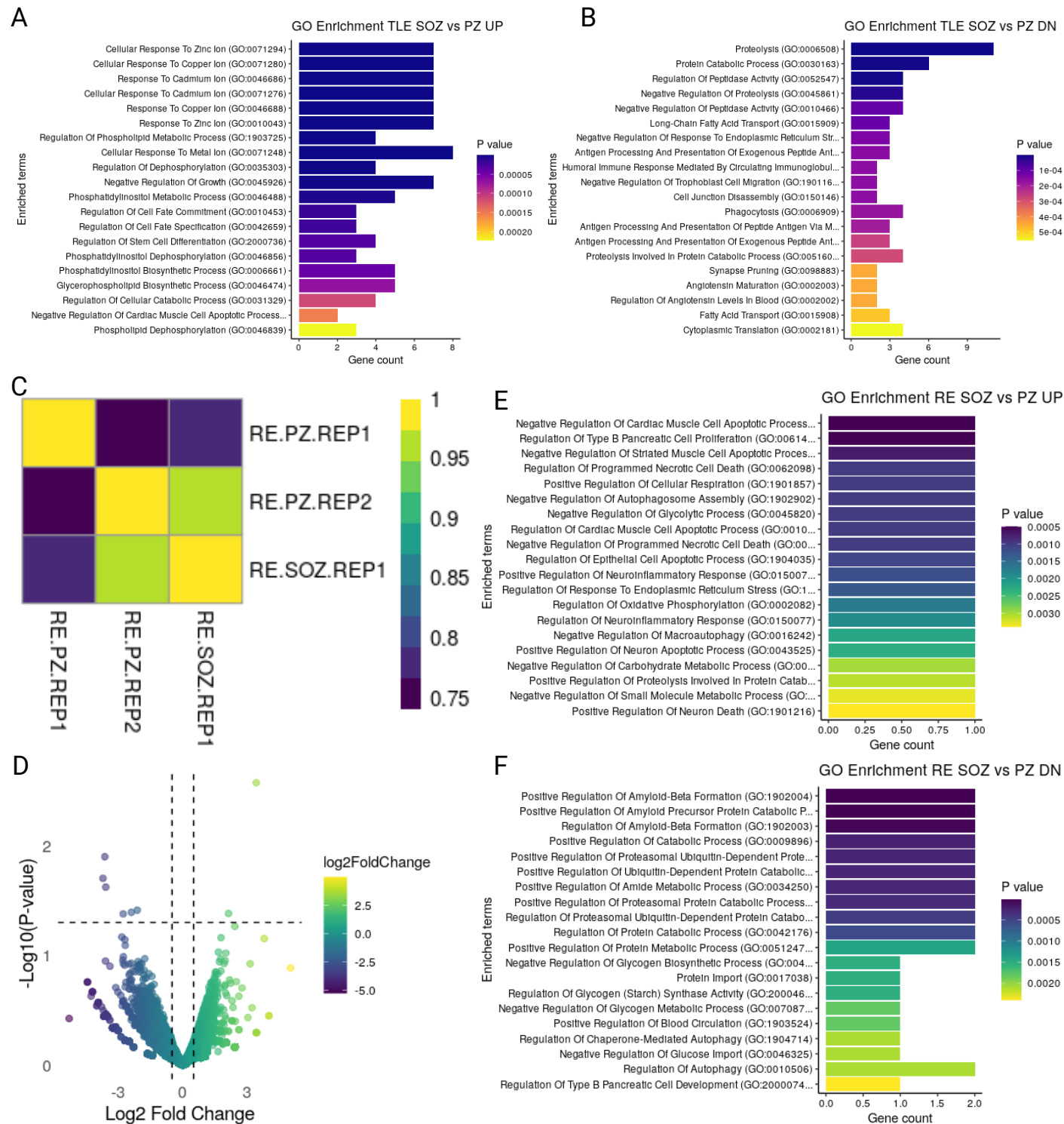


Figure S10

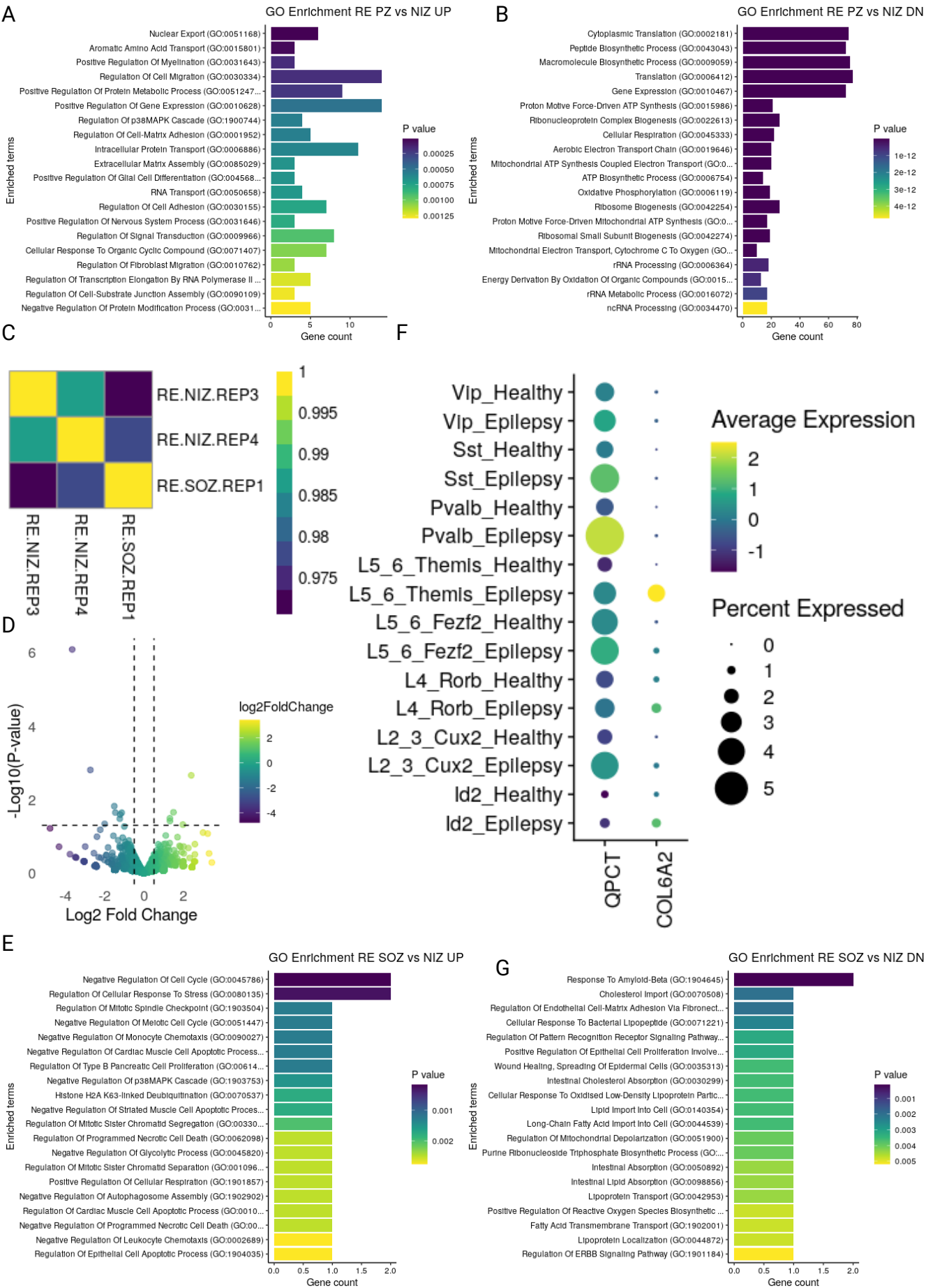


Figure S11

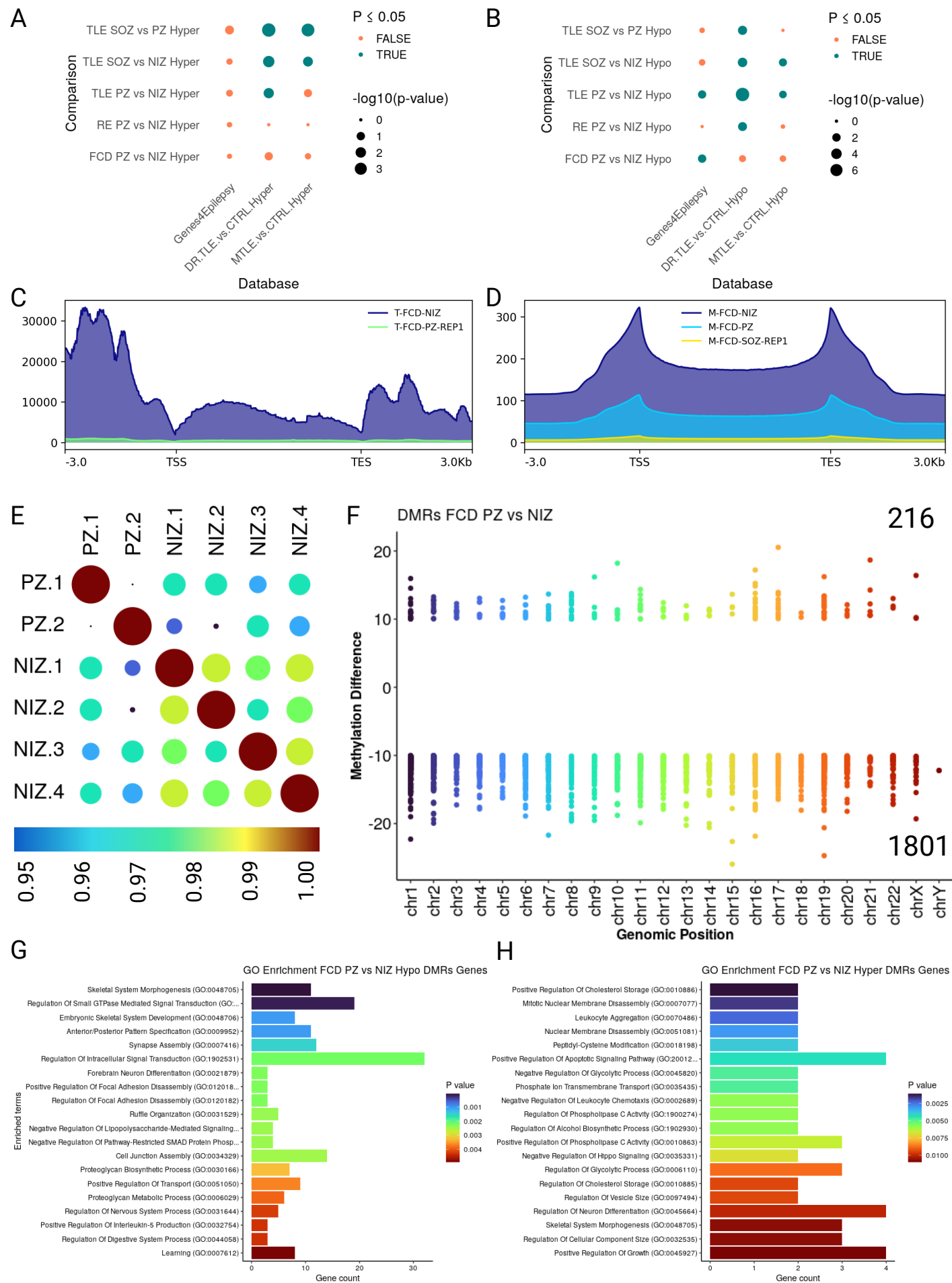


Figure S12

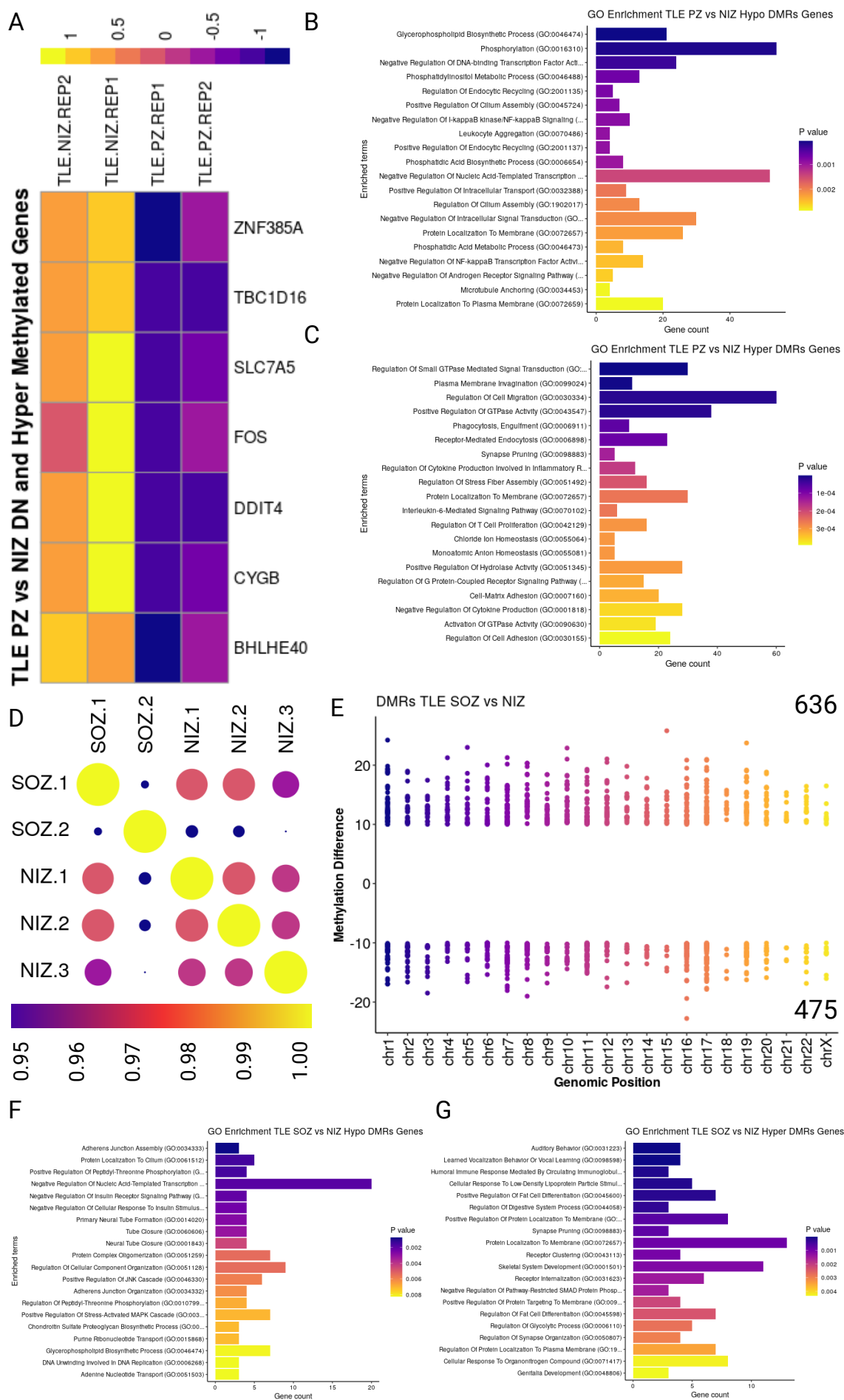


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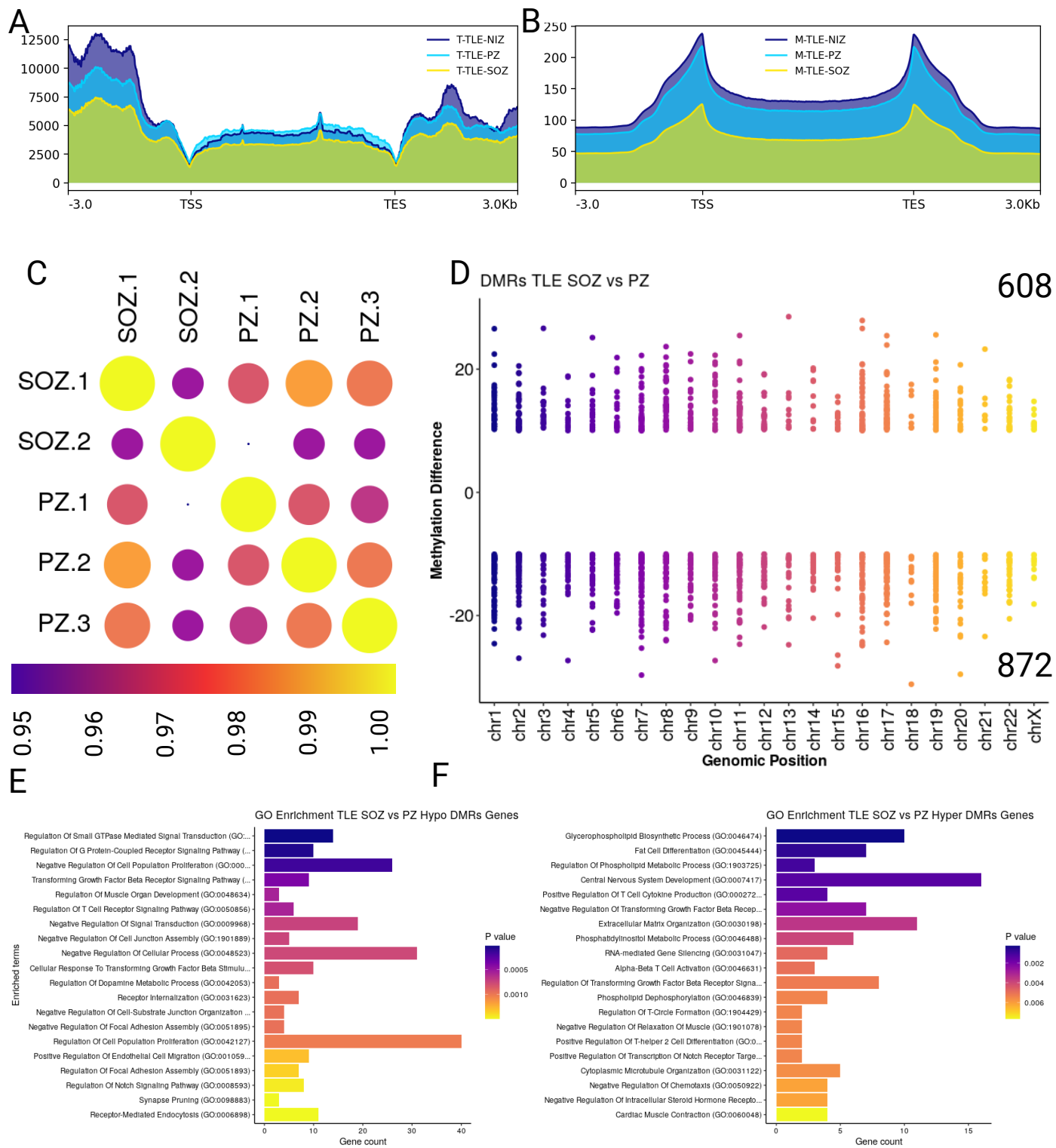


Figure S14

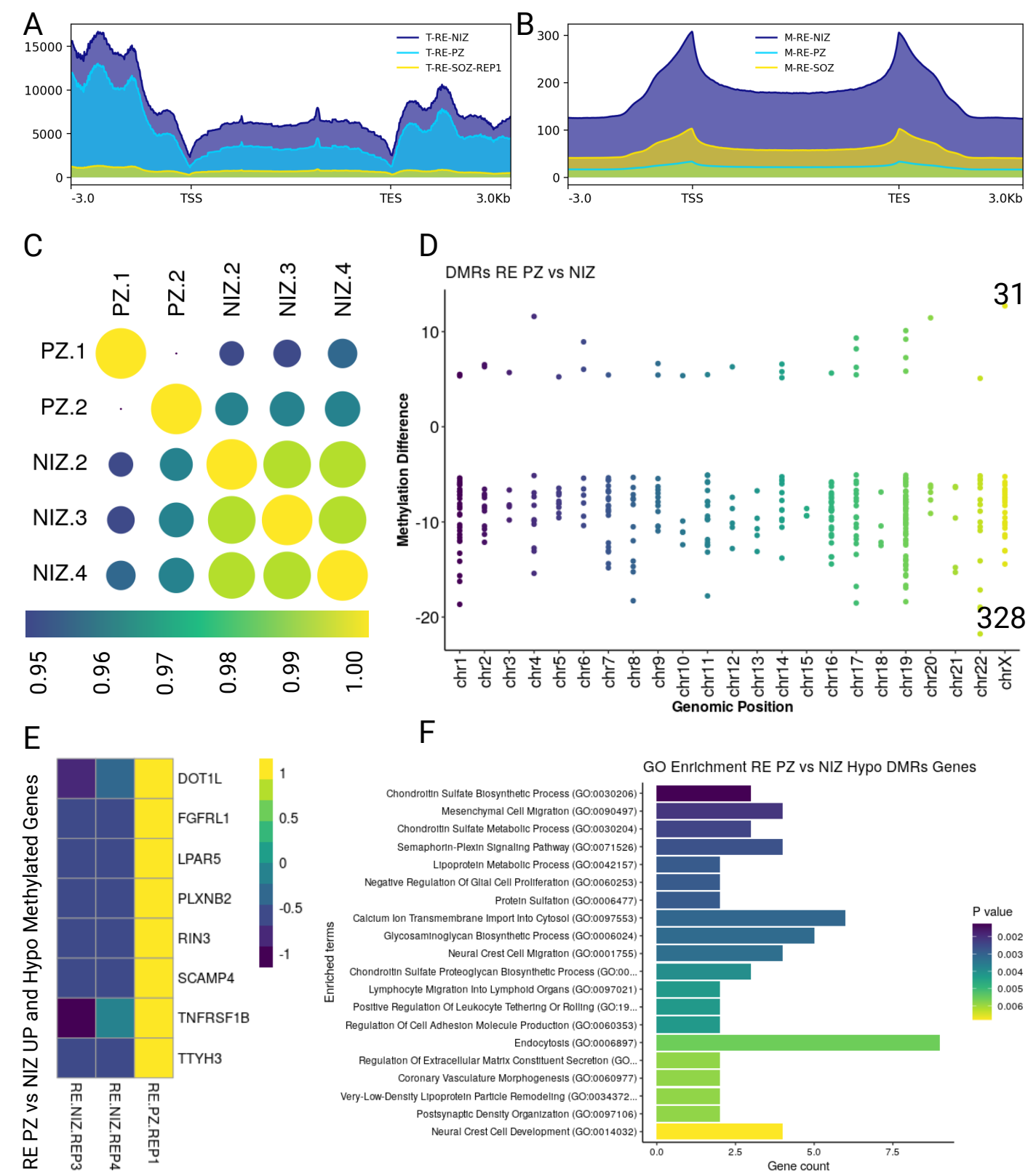


Figure S15

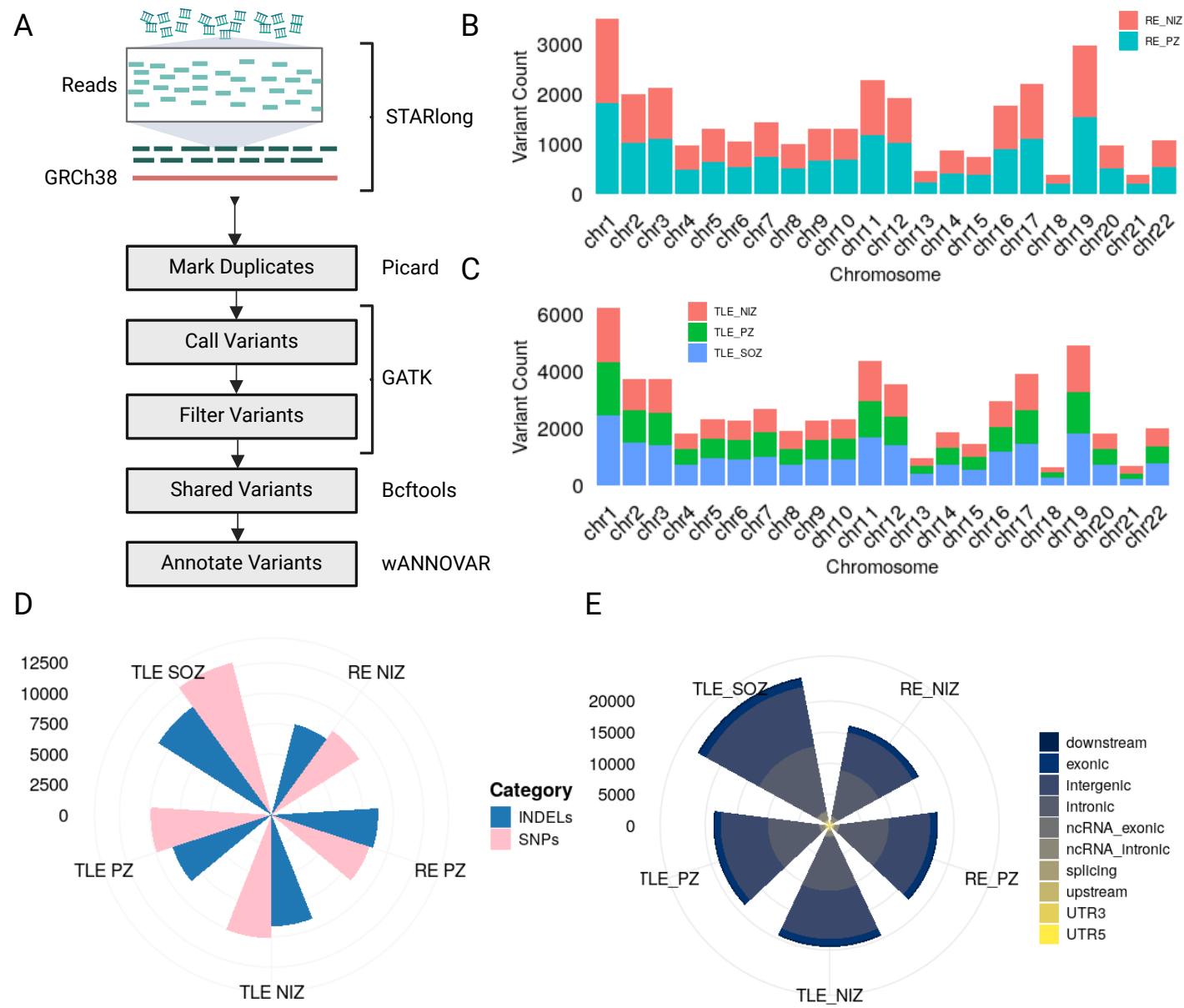


Figure S16

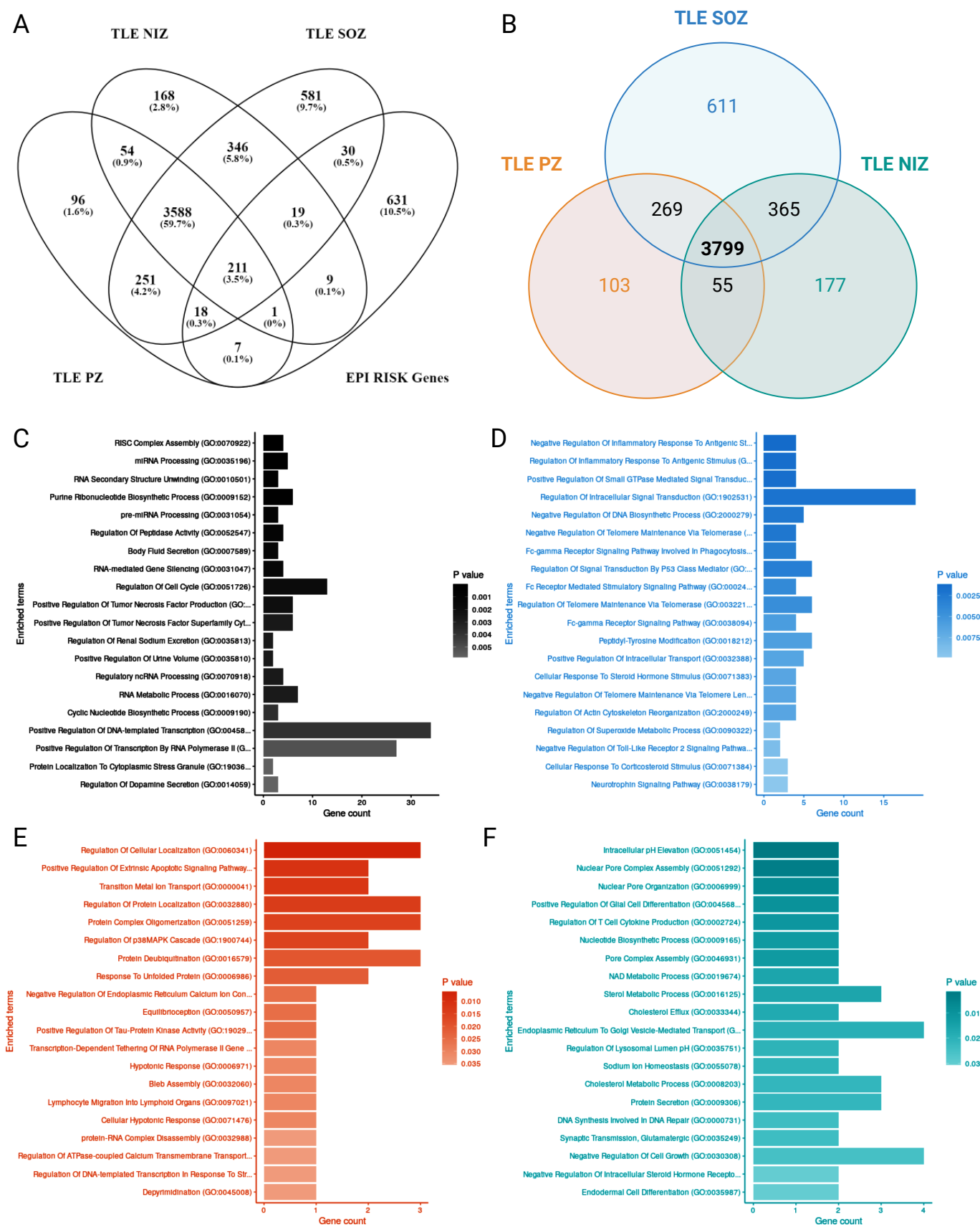


Figure S17

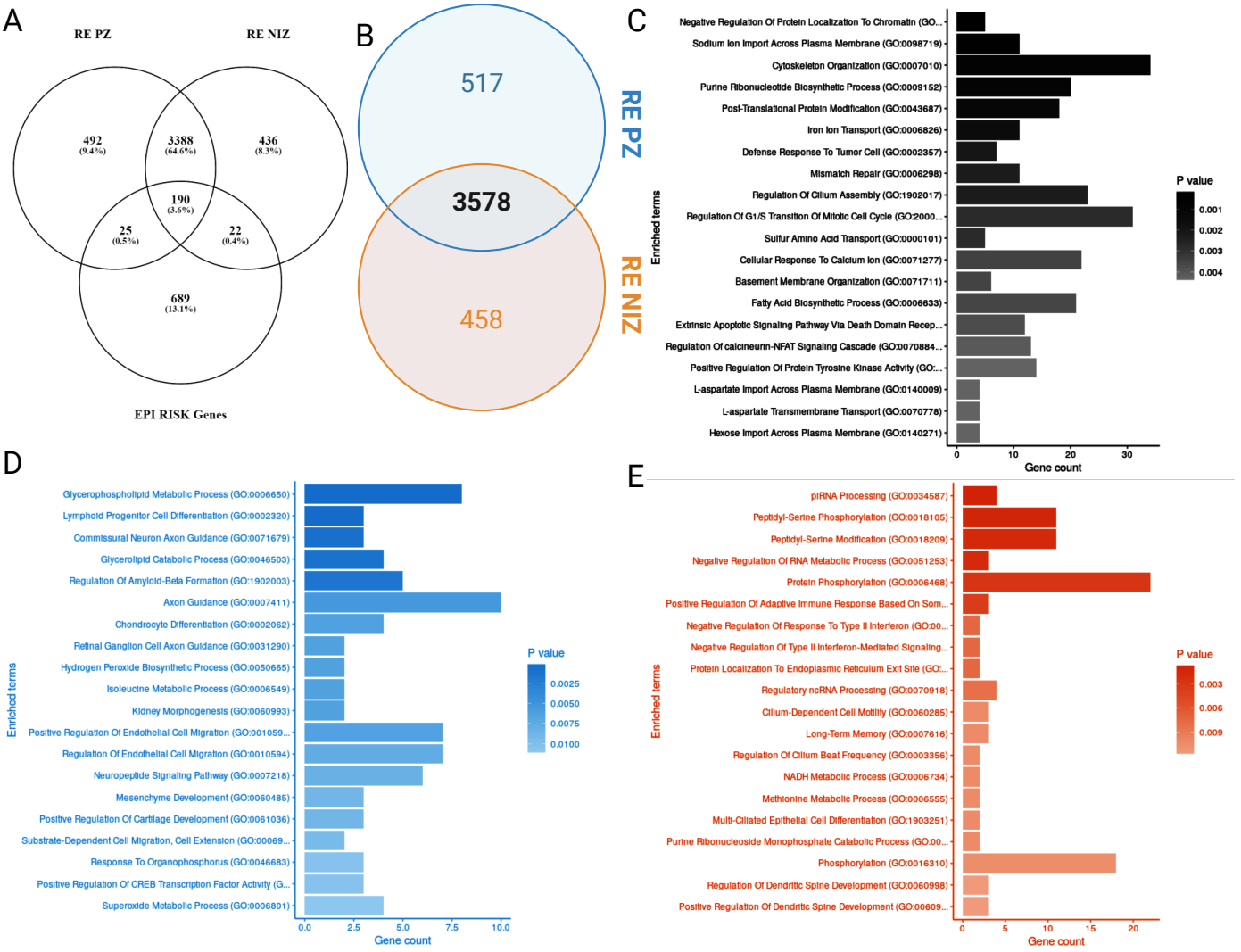


Table S1: Full Neuropathology findings in the operative tissue specimens

Patient A
In many areas cortical neuronal lamination appears normal without significant neuronal loss or gliosis. In one area however, NeuN immunohistochemical staining shows slightly disrupted neuronal lamination with scattered dysmorphic and irregularly clustered neurons. Occasional cells with the morphology suggestive of balloon cells are seen (not definite, and not resolved by immunohistochemistry). A neoplastic infiltrate is not identified and mitoses, Rosenthal fibres, eosinophilic granular bodies, calcification are not seen. Features are consistent with malformation of cortical development, Focal Cortical Dysplasia type IIA.
Patient B
On microscopic examination of the temporal pole cortical grey matter, subcortical white matter and overlying leptomeninges, there are several foci of leptomeningeal mixed inflammatory infiltrate composed of lymphocytes, macrophages and granulocytes including eosinophils. Subjacent superficial cortical subacute infarction (pallor and dissolution of normal tissue architecture, acute eosinophilic neuronal necrosis and prominence of capillary blood vessels) is consistent with SEEG insertion site. Elsewhere there is prominent Chaslin's subpial gliosis. Where optimally orientated, cortical neurology lamination appears normal without significant neuronal loss or gliosis. Dysmorphic or malorientated neurons not seen, and balloon cells are not identified. There is no evidence of parenchymal inflammation or mineralisation. The boundary between grey and white matter is well defined. Heterotopic white matter neurons are not present to excess. There is a mild increase in subcortical white matter cellularity, interpreted as a degree of non-neoplastic oligodendrocyte hyperplasia. The conclusion is Chaslin's subpial gliosis, with no evidence of focal cortical dysplasia and no diagnostic features of hippocampal sclerosis. There is no evidence of inflammation, vascular malformation or neoplasm.
Patient C
Right frontal biopsy (six years prior to SEEG) Perivascular and parenchymal lymphocytic inflammation is present and mild to moderate in intensity. Mononuclear inflammatory infiltrate surrounding leptomeningeal vessels is also noted. Vessel wall infiltration by lymphocytes, fibrinoid necrosis or thrombosis, is not identified. Microglial activation is striking, and multiple microglial nodules are identified throughout the grey matter. Small lymphocytes and microglia are focally closely apposed to neurons, but definite neuronophagia is not seen. Subpial gliosis is moderate and extends into cortex and subcortical white matter. Perivascular pigment and macrophages are noted. CD3 highlights large number of T-lymphocytes within the leptomeninges, perivascular spaces and parenchymal brain tissues, as do CD4 and CD8, the former also highlighting very large numbers of microglia. The conclusion is of chronic encephalitis with T-cell rich perivascular and parenchymal inflammation, microglial nodules and widespread microglial activation, subpial and parenchymal gliosis. SEEG resection specimen In the lateral temporal pole, a significant inflammatory infiltrate is not seen. Microglial nodules are not identified. There is a single foci of perivascular chronic inflammation. In one

specimen there is a linear transversely orientated focus of cortical gliosis and rarefaction without amounting to insipient parenchymal cavitation. In one specimen there is occasional leptomeningeal and perivascular lymphocytes, and rare parenchymal lymphocytes. In the hippocampal head, there is depletion of and dispersion of granular neurons and pyramidal neurons. In the frontal lobe there are occasional foci of perivascular lymphocytic inflammation. A diffuse parenchymal inflammatory infiltrate or microglial nodules are not seen. The conclusion is that minimal inflammation is present in the current specimen, restricted largely to perivascular aggregates of T-lymphocytes. Significant parenchymal inflammation is not seen. There are however foci of cortical gliosis and evidence of severe hippocampal sclerosis with aberrant mossy fibre sprouting patterns.

LEGEND OF SUPPLEMENTARY TABLES

Table S1: Full Neuropathology findings in the operative tissue specimens

Table S2. Nanodrop quantifications for DNA isolated from SEEG electrodes.

Table S3. Nanodrop quantifications for RNA isolated from SEEG electrodes.

Table S4. DNA QC before library preparation.

Table S5. RNA QC before library preparation.

Table S6. RNA QC after library preparation.

Table S7. Up regulated genes in FCD PZ vs NIZ comparison.

Table S8. Down regulated genes in FCD PZ vs NIZ comparison.

Table S9. Enriched GO terms in up regulated genes in FCD PZ vs NIZ comparison.

Table S10. Enriched GO terms in down regulated genes in FCD PZ vs NIZ comparison.

Table S11. Up regulated genes in TLE PZ vs NIZ comparison.

Table S12. Down regulated genes in TLE PZ vs NIZ comparison.

Table S13. Enriched GO terms in up regulated genes in TLE PZ vs NIZ comparison.

Table S14. Enriched GO terms in down regulated genes in TLE PZ vs NIZ comparison.

Table S15. Up regulated genes in TLE SOZ vs NIZ comparison.

Table S16. Down regulated genes in TLE SOZ vs NIZ comparison.

Table S17. Enriched GO terms in up regulated genes in TLE SOZ vs NIZ comparison.

Table S18. Enriched GO terms in down regulated genes in TLE SOZ vs NIZ comparison.

Table S19. Up regulated genes in TLE SOZ vs PZ comparison.

Table S20. Down regulated genes in TLE SOZ vs PZ comparison.

Table S21. Enriched GO terms in up regulated genes in TLE SOZ vs PZ comparison.

Table S22. Enriched GO terms in down regulated genes in TLE SOZ vs PZ comparison.

Table S23. Up regulated genes in RE PZ vs NIZ comparison.

Table S24. Down regulated genes in RE PZ vs NIZ comparison.

Table S25. Enriched GO terms in up regulated genes in RE PZ vs NIZ comparison.

Table S26. Enriched GO terms in down regulated genes in RE PZ vs NIZ comparison.

Table S27. Up regulated genes in RE SOZ vs PZ comparison.

Table S28. Down regulated genes in RE SOZ vs PZ comparison.

Table S29. Enriched GO terms in up regulated genes in RE SOZ vs PZ comparison.

Table S30. Enriched GO terms in downregulated genes in RE SOZ vs PZ comparison.

Table S31. Up regulated genes in RE SOZ vs NIZ comparison.

Table S32. Down regulated genes in RE SOZ vs NIZ comparison.

Table S33. Enriched GO terms in up regulated genes in RE SOZ vs NIZ comparison.

Table S34. Enriched GO terms in down regulated genes in RE SOZ vs NIZ comparison.

LEGENDS FOR SUPPLEMENTARY FIGURES

Figure S1. Neuropathology details of study participants. (A) NeuN (MilliporeSigma, USA) immunohistochemical staining for patient A demonstrating dysmorphic, haphazardly arranged and abnormally clustered cortical neurons consistent with Focal Cortical Dysplasia, ILAE Type 2A. No clear balloon cells were identified. (B) Hematoxylin and eosin (H&E) staining for patient B showing prominent Chaslin's subpial gliosis. Cortical neurology lamination appears normal without significant neuronal loss or gliosis. Dysmorphic or malorientated neurons not seen, and balloon cells are not identified. Heterotopic white matter neurons are not present to excess. There is a mild increase in subcortical white matter cellularity, interpreted as a degree of non-neoplastic oligodendrocyte hyperplasia. H&E (C), NeuN (D) and Zinc transporter 3 (Proteintech, USA) (E) staining for patient C showing severe hippocampal sclerosis with granular neuron depletion and mossy fibre sprouting. In the hippocampal head, there is depletion of and dispersion of granular neurons and pyramidal neurons. CD3 (Agilent Dako, US) (F) and CD68 (Agilent Dako, USA) (G) staining for patient C highlighting the presence of T-lymphocytes and macrophages respectively. Minimal inflammation is present in the current specimen, restricted largely to perivascular aggregates of T-lymphocytes. Significant parenchymal inflammation is not seen.

Figure S2. Nucleic acid extraction from SEEG electrodes from epilepsy patients. (A). Trypan blue staining shows the presence of cells in the SEEG electrodes from epilepsy patients. (B). The amount of total nuclear fractions was directly proportional to a number of cut pieces (Metal contact points) from one electrode. (C). High sensitivity fragment analyser shows the presence of both DNA

and RNA in the samples (S1 and S2). (D-E). Electropherograms from the fragment analyzer confirm the RNA and DNA with their respective molecular weight. (F). Electropherograms from the fragment analyzer confirm the effective separation of DNA from total nucleic acid fraction. (G). Tape station analysis of purified RNA fraction confirmed the quality of (RIN) extracted RNA.

Figure S3. Quality check of the transcriptome datasets. (A) The distribution of RNA integrity number (RIN) across all samples from the NIZ, PZ, and SOZ regions of FCD, TLE, and RE brains. The plot illustrates the variability in RNA quality within each region and across different brain conditions. (B) A heatmap depicting the consistent expression of genes across all replicates of FCD brain samples, regardless of gene length and the RIN values of the samples. The heatmap highlights the robustness of gene expression patterns in FCD brains.

Figure S4. Quality assessment for TLE and RE brain transcriptomes. (A) Heatmap illustrating uniform gene expression across all TLE brain sample replicates, indicating stable expression profiles independent of gene length and RIN values. (B) Heatmap depicting consistent gene expression across all RE brain sample replicates, similarly indicating reliable expression patterns regardless of gene length and RIN values.

Figure S5. Reproducibility of electrode transcriptome in tissue samples from matched brain regions with similar seizure activity. (A-D) The transcriptome from electrodes and corresponding tissue samples from TLE patients in SOZ region shows a strong correlation (correlation coefficient: 0.7). The correlation was evaluated at various FPKM thresholds to assess the consistency of results.

Figure S6. Enrichment of differentially expressed genes (DEGs) identified in our study in publicly available scRNA-seq and bulk RNA-seq datasets. (A) UMAP shows the integration of Epilepsy (TLE) and control (healthy) scRNA and (B) their cell type annotation using the (C) marker

expression in violin plot. (D) Dotplot shows the enrichment of our DEGs in previously reported TLE and RE bulk and single cell transcriptome (RNA-seq).

Figure S7. Gene expression differences between NIZ and PZ areas of FCD brains. (A) Replicate-wise correlation of NIZ and PZ regions transcriptome. (B) Volcano plot of differentially expressed genes between PZ and NIZ regions. (C-D) GO enrichment of up and down regulated genes, respectively. (E) Heatmap of down regulated known epilepsy risk genes in PZ as compared with NIZ region. (F) Dotplot showing the expression of down regulated genes (PZ vs NIZ) in Epilepsy vs Healthy scRNA data.

Figure S8. Gene expression differences between NIZ and PZ areas of TLE brains. This figure examines gene expression changes in different regions of non-lesional temporal lobe epilepsy (TLE) brains. Panels (A-B) show GO enrichment for upregulated and downregulated genes in the PZ vs. NIZ comparison in TLE brains, respectively. (C-D) Display the replicate-wise correlation of NIZ and SOZ regions and a volcano plot of differentially expressed genes for these regions. (F-G) Present GO enrichment for upregulated and downregulated genes in the SOZ vs. NIZ comparison in TLE brains, respectively. (E) Dot plot showing the expression of upregulated genes in SOZ vs. NIZ and Epilepsy vs. Healthy samples from public data.

Figure S9. Transcriptome response in PZ and SOZ areas of TLE and RE brains. This figure explores gene expression changes in the PZ and SOZ regions of TLE and RE brains. Panels (A-B) show GO enrichment analysis of upregulated and downregulated genes in the SOZ vs. PZ comparison within the TLE brain. (C-D) Present replicate-wise correlations and differential gene expression analysis between the SOZ and PZ regions in the RE brain. Panels (E-F) display GO enrichment analysis of upregulated and downregulated genes in the SOZ vs. PZ comparison within the RE brain.

Figure S10. RE transcriptome response in NIZ, PZ and SOZ regions. (A-B) GO enrichment of up- and down regulated genes in PZ vs NIZ region comparison of RE brain. (C-D) Replicate- wise correlations and differential expression of genes in SOZ and NIZ regions of RE brain. (E and G) GO enrichment of up- and down regulated genes in SOZ vs NIZ region comparison of RE brain. (F) Dotplot showing the expression of up regulated genes (SOZ vs NIZ) in Epilepsy vs Healthy scRNA from public data.

Figure S11. Enrichment of differentially methylated genes (DMGs) identified in our study in publicly available bulk methylome data and DNA methylome landscape in FCD. (A-B) Dotplot shows the enrichment of our DMGs in previously reported drug resistant TLE (DR TLE) and Mesial TLE (MTLE) methylome. (C-D) Transcriptome and methylome density plot in gene body and flanking regions. (E) Replicated wise correlation plot of PZ and NIZ regions methylome in FCD brain. (F) A comparable number of hypermethylated (216) and hypomethylated (1801) DMRs are identified when comparing the PZ to the NIZ region. (G-H) GO enrichment of hypomethylated and hypermethylated genes found in PZ regions.

Figure S12. Transcriptome and methylome comparison for TLE brain. (A) Heatmap of down regulated and hyper methylated genes in PZ vs NIZ comparison. (B-C) GO enrichment of hypomethylated and hypermethylated genes in PZ vs NIZ regions. (D) Replicate-wise correlation plot of SOZ and NIZ regions methylome in TLE brain. (E) A comparable number of hypermethylated (636) and hypomethylated (475) DMRs are identified when comparing the SOZ to the NIZ region. (F-G) GO enrichment of hypomethylated and hypermethylated genes found in SOZ vs NIZ regions.

Figure S13. Transcriptome and methylome analogy in SOZ and PZ regions in TLE brain. (A-B) Transcriptome and methylome density plot in gene body and flanking regions. (C) Replicated wise correlation plot of SOZ and PZ regions methylome in TLE brain. (D) A comparable number of

hypermethylated (608) and hypomethylated (872) DMRs are identified when comparing the SOZ to the PZ region. (E-F) GO enrichment of hypomethylated and hypermethylated genes found in SOZ vs PZ regions.

Figure S14. Transcriptome and methylome landscape in SOZ and NIZ regions in RE brain. (A-B) Transcriptome and methylome density plot in gene body and flanking regions. (C) Replicated wise correlation plot of PZ and NIZ regions methylome in RE brain. (D) A comparable number of hypermethylated (31) and hypomethylated (328) DMRs are identified when comparing the PZ to the NIZ region. (E) Up regulated and hypo methylated genes in PZ as compared to NIZ regions. (F) GO enrichment of hypomethylated and hypermethylated genes found in PZ vs NIZ regions.

Figure S15. An attempt to identify short variants (SNPs + Indels) from transcriptome (RNA-seq) data in TLE and RE brains. (A) We identified short variants using GATK best practices workflows for RNAseq short variant discovery (SNPs + Indels) and annotated the high quality variants through wANNOVAR. (B-C). Chromosome wise distribution of variants in RE (NIZ and PZ) and TLE (NIZ, PZ and SOZ) brains. (D) Number of variants falling in different categories in TLE and RE brains. (E) Functional annotations of variants harbouring at different genomic regions.

Figure S16. Functional enrichment analysis of variants in NIZ, PZ, and SOZ regions of TLE brain. (A-B) Venn diagrams depicting the overlap between genes with variants in the NIZ, PZ, and SOZ regions of TLE brains and those associated with epilepsy risk. (C) GO enrichment analysis of genes carrying variants across all three regions (NIZ, PZ, and SOZ). GO enrichment specific to genes with variants found exclusively in the (D) SOZ, (E) PZ, and (F) NIZ regions.

Figure S17. Functional enrichment analysis of variants in NIZ and PZ regions of RE brains. (A-B) Venn diagrams illustrating the intersection of genes with variants in the NIZ and PZ regions of RE brains and known epilepsy risk genes. (C) GO enrichment analysis for genes containing variants

across NIZ and PZ regions. GO enrichment results for genes with variants specific to the (D) PZ and (E) NIZ regions.