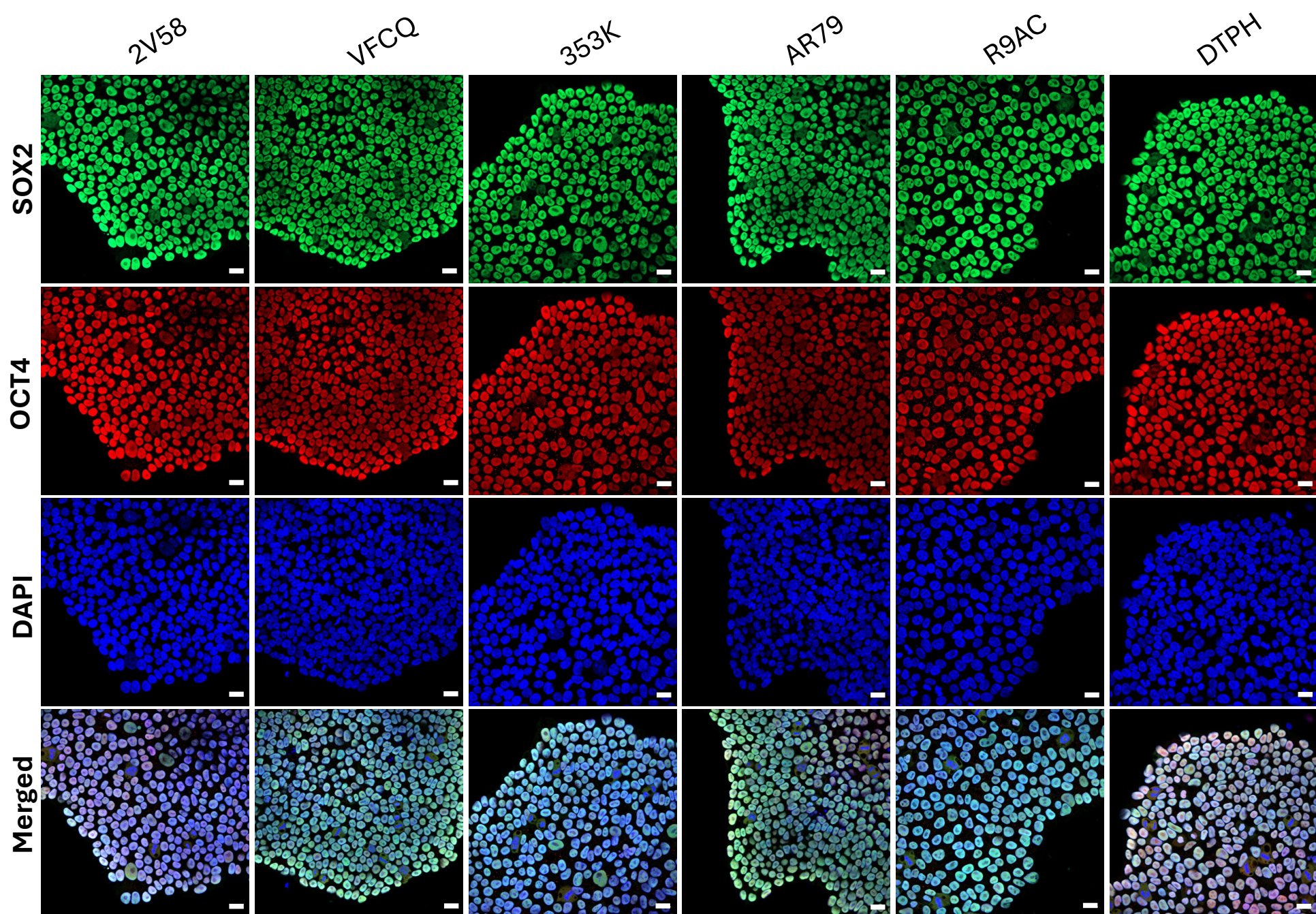
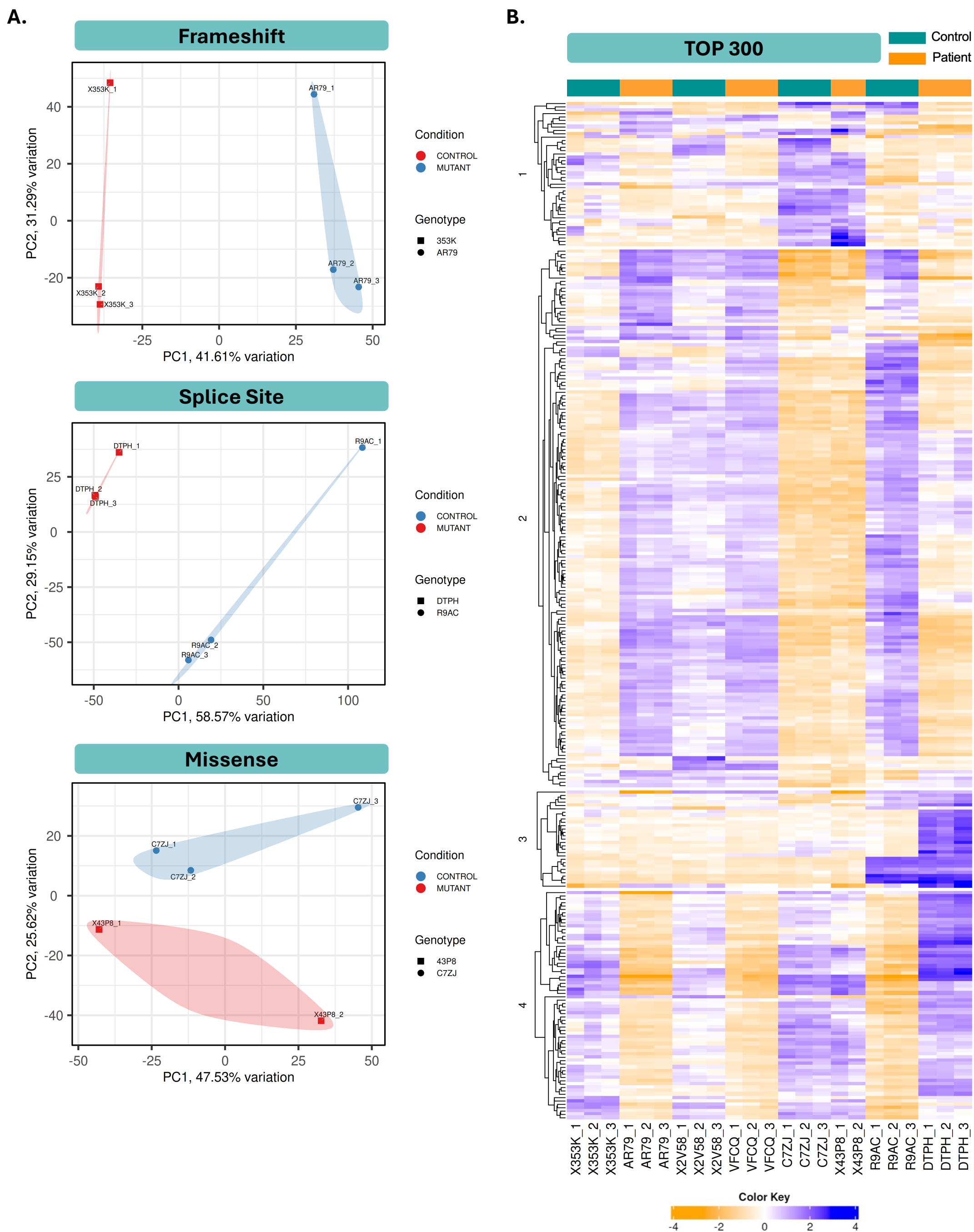


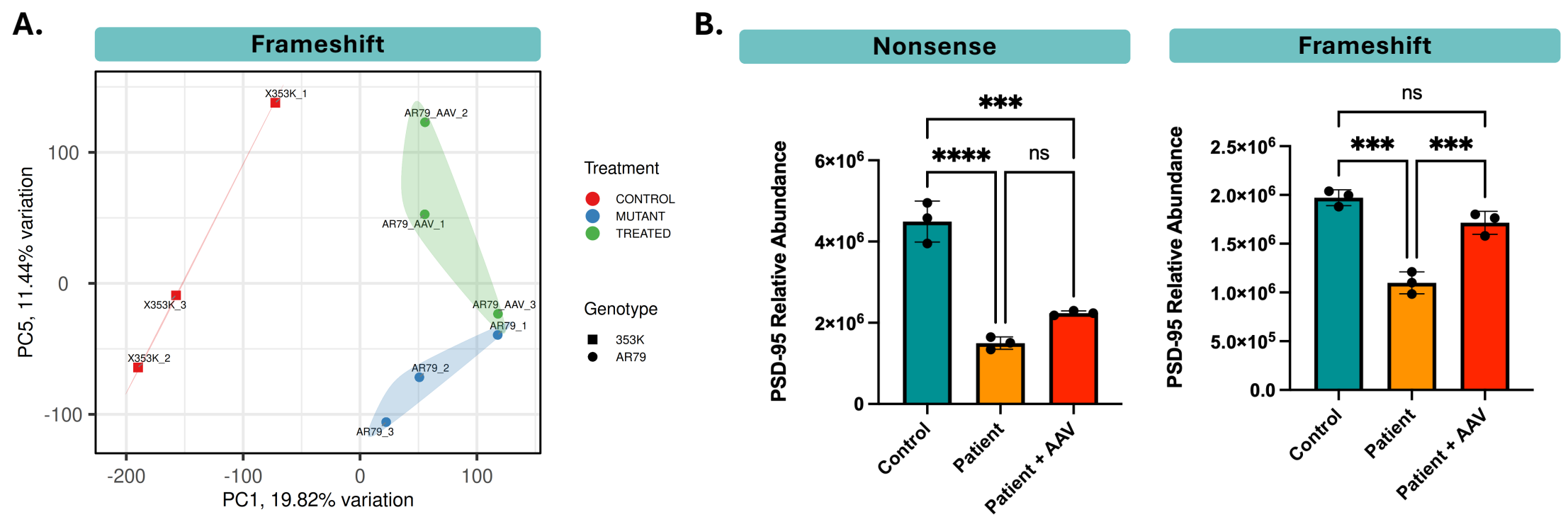


Supplementary Figure S1. Characterization of patient-derived and control iPSC lines.

Representative immunofluorescence images of iPSC lines used in this study, including control 2V58, 353K, and R9AC and DLG4-mutant VFCQ, AR79, and DTPH lines. Cells were stained for the pluripotency markers SOX2 and OCT4 and nuclear DAPI. Merged images demonstrate expression of pluripotency markers across all cell lines. Scale = 20 μ m.



Supplementary Figure S2. Transcriptomic profiling of DLG4-mutant cortical organoids. A) Principal component analysis (PCA) of transcriptomic profiles from cortical organoids carrying individual DLG4 variants and their respective controls, shown separately for each mutation-control pair. **B)** Hierarchical clustering heatmap of the top 300 differentially expressed transcripts across DLG4-mutant cortical organoids and their respective controls. Differentially expressed transcripts were grouped into four major transcriptional clusters according to their expression patterns across genotypes.



Supplementary Figure S3. Molecular characterization of AAV-mediated DLG4 restoration in patient-derived cortical organoids. A) Principal component analysis (PCA) of proteomic profiles from control, untreated DLG4 frameshift, and AAV-treated DLG4 frameshift cortical organoids. **B)** PSD-95 protein abundance in cortical organoids carrying the DLG4 nonsense and frameshift variants and their respective controls, before and after AAV-mediated DLG4 restoration.

Study ID (CRID)	Age	Sex	Race/Ethnicity	Height (inches)	Weight (lbs)	Speciment Type	Variant Type	NM_001321075: CDNA change.	NM_001321075: Protein change.
DTPH	8	M	Mixed	48	47	Fresh Tissue	Splice Site	c.150+4_150+5del	p.?
R9AC	44	M	White	66	160	Fresh Tissue	Control for DTPH	Control	Control
AR79	8	F	Asian	53	69	Fresh Tissue	Frameshift	c.910del	p.(Glu304Argfs*12)
353K	11	F	Asian	63	105	Skin Biopsy	Control for AR79	Control	Control
VFCQ	3	F	White	37	33	Skin Biopsy	Nonsense	c. 1195C>T	P.(Arg399*)
2V58	5	F	White	45	49	Skin Biopsy	Control for VFCQ	Control	Control
43P8	7	F	White	52	54	Fibroblasts	Missense	C.557A>T	p.(Asp186Val)
C7ZJ	31	F	White	67	188	Fibroblasts	Control for 43P8	Control	Control

Supplementary Table S1. Demographic and genetic characteristics of DLG4 patient-derived and control cell lines.

Demographic, sample, and genetic information for the DLG4 patient-derived and corresponding control cell lines used in this study. Information includes study ID, age, sex, race/ethnicity, height, weight, specimen type, variant classification, and DLG4 cDNA and protein changes according to transcript NM_001321075. Controls are identified according to their corresponding DLG4-mutant line.

Gene	Event	rMATS ID	GRCh38 coordinates	Δ PSI	P value	FDR	R9AC control PSI	DTPH PSI
DLG4	A5SS	7456	chr17:7,208,074-7,208,239; short donor start 7,208,173	-0.75	7.31852356495e-11	4.98694290230955e-08	0.233, 0.177, 0.341	1.000, 1.000, 1.000
Predicted consequence								
Long-form extension	+99 nt / +33 aa							
Protein isoforms	724 aa short/control-dominant; 757 aa long/DTPH							
Inserted sequence	KYRYQDEDTPPLEHSPAHLPNQVNAPELVHVAE							
Domain annotation	PEST-like region; PF10608 / IPR019590							

Supplementary Table S3. rMATS analysis of the DLG4 alternative 5' splice-site event in DTPH cortical organoids.

rMATS evidence for differential DLG4 alternative 5' splice-site (A5SS) usage between DTPH and R9AC control samples. The table reports the rMATS event identifier, GRCh38 genomic coordinates, change in percent spliced in (Delta PSI), statistical significance, false discovery rate (FDR), and replicate-level PSI values. The predicted consequence of the splice-site event is also summarized, including a 99-nucleotide extension encoding 33 additional amino acids, the resulting 724- and 757-amino-acid protein isoforms, the inserted amino acid sequence, and annotation of the inserted region as PEST-like.