4. Scientific Rigor and Reproducibility

This study will use at least three hiPSC lines across two differentiation protocols, with 2-3 organoids or assembloids per line to capture variability in neuronal differentiation[58,59]. For patch-clamp recordings, I will use at least 10 organoids per group (WT and HET), recording 4-10 neurons each (20-50 samples per group, alpha = 0.05, N ~ 36, ES = 0.65 or alpha = 0.01, N ~ 36, ES = 0.8); for MEA recordings, at least 10 organoids per group with 16 electrodes each; and for immunostaining, 10 organoids per group, imaging 3-5 areas per organoid. Axon projections will be assessed in at least 10 assembloids per group, spine density in 50 dendritic branches across 5-8 assembloids, neurotransmitter levels in 10 assembloids per group, and single-cell RNA sequencing in 4-6 assembloids per group. For Aim 3, mice of both sexes (0-8 months) will be used, with results pooled if no sex differences are observed, and 8-20 chimeric brains will be included per group in Experiments 8, 9, and 10.

To control false positives in multiple comparisons, a significance level of 0.01 will be used, with sample sizes of 36 per group to detect an effect size (Cohen's d) of 0.8 with 80% power. If this significance level is too conservative, we will use an **effect size (Cohen's d) of 0.65 to get 36 a sample size of 36 per group under the significance level** 0.05 under 80% power. If needed, the effect size will be re-calculated based on pilot data, which will then determine a new sample size for the experiment. Data will be checked for normality, with the two-sample t-test for most outcomes, and GEE for repeated measures. RNA-seq data will be processed with RUVSeq if batch correction is deemed necessary. edgeR for differential expression with FDR < 0.05 to identify modulated gene, and analyzed for enriched KEGG, Reactome, and GO terms with clusterProfiler and Ingenuity Pathway Analysis. The Benjamini-Hochberg method was applied for multiple testing correction, considering genes with an FDR below 0.05 as differentially expressed. Analyses will be performed in a blinded manner, and Purdue's Statistical Consulting Service directed by Dr. Bruce Craig will provide statistical support.