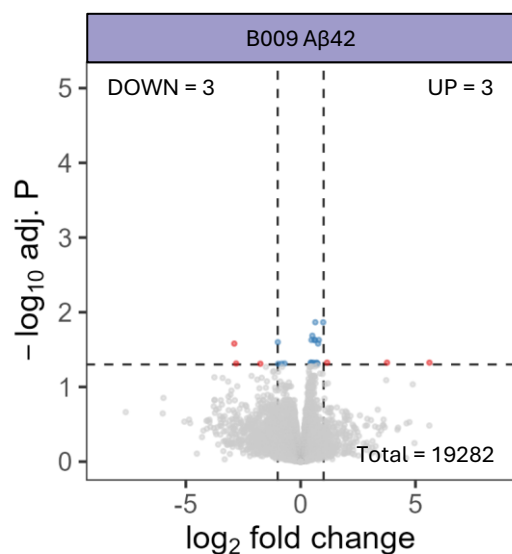
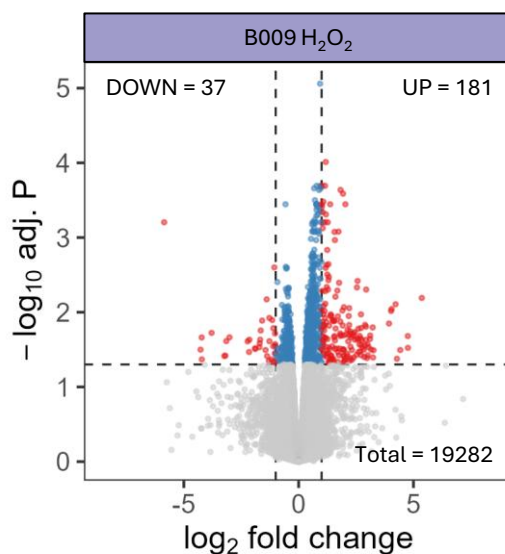


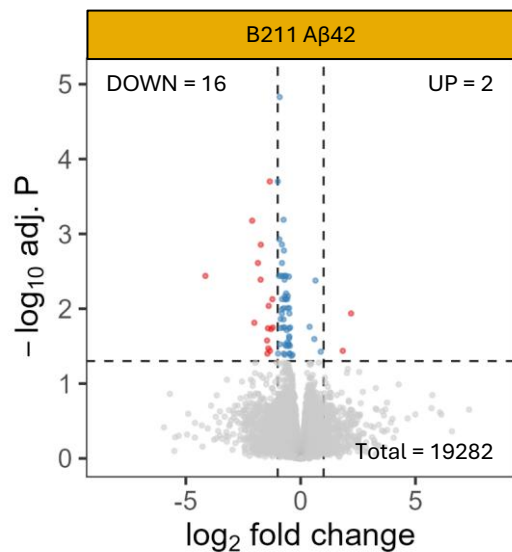
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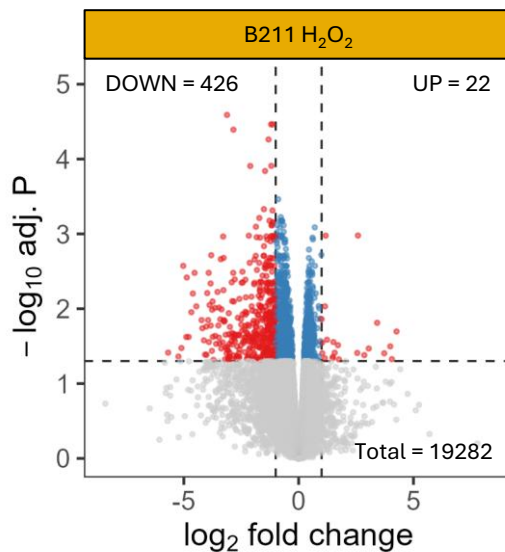
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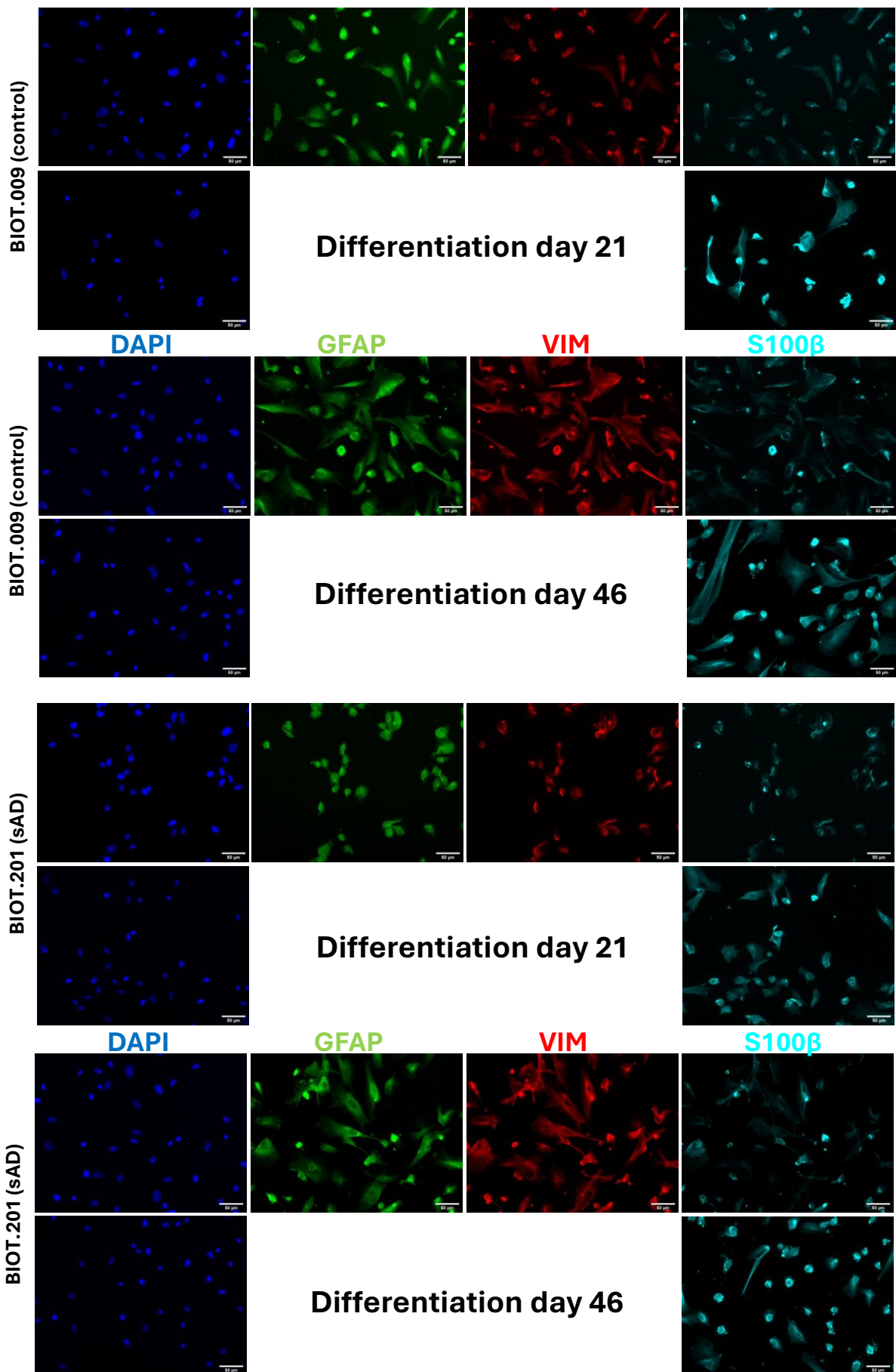
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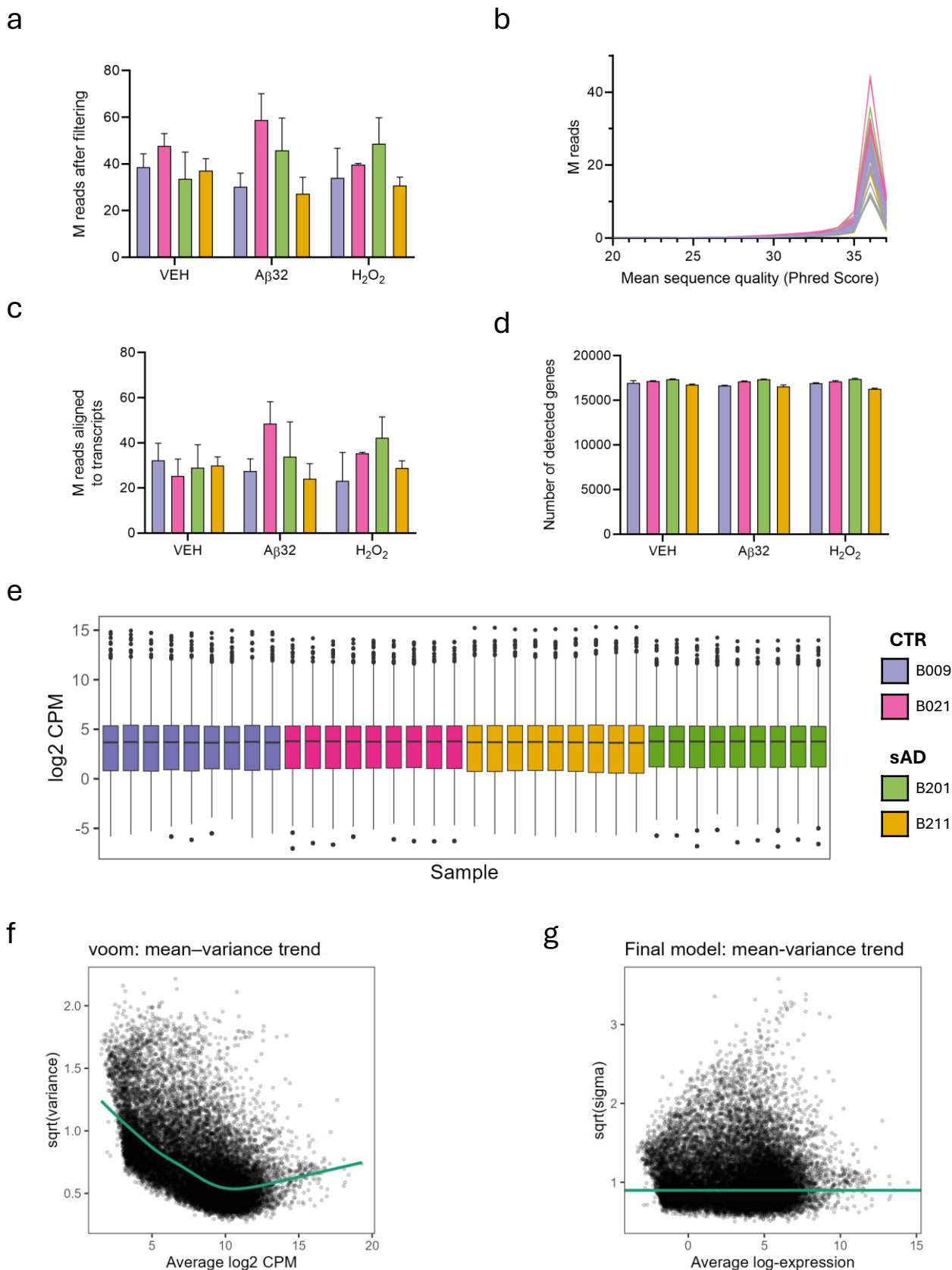
d



Supplementary Figure S1. *Treatment-induced differential gene expression in the higher-purity astrocyte lines.* Volcano plots show differentially expressed genes identified by RNA sequencing using limma-voom, comparing (a) B009 A β 42 vs. vehicle, (b) B009 H₂O₂ vs. vehicle, (c) B211 A β 42 vs. vehicle, and (d) B211 H₂O₂ vs. vehicle. Genes with $|\log_2$ fold change| > 1 and adjusted p-value < 0.05 are highlighted.



Supplementary Figure S2. Immunocytochemical validation of astrocyte-associated marker expression in representative differentiated lines. Representative immunocytochemistry images showing expression of astrocyte-associated markers GFAP (green), VIM (red) and S100β/AQP4 (cyan) in differentiated cultures from lines B009 and B201 at two differentiation time points (Day 21 and 46). These two lines were selected because they represent distinct transcriptional/maturation clusters observed in the RNA-seq-based PCA and deconvolution analyses. Scalebar: 50 μm, Nuclei were stained by DAPI.



Supplementary Figure S3. RNA-seq quality control and limma-voom diagnostics. (a) Number of reads passing fastp filtering. (b) Per-sequence quality score distribution from FastQC, showing the frequency of reads at each mean Phred quality score. (c) Number of reads assigned to transcripts by Salmon. (d) Number of detected genes per sample (CPM > 0.5). (e) Box plot of log₂ counts per million (CPM) after TMM normalization, showing comparable distributions across samples. (f) Mean-variance trend from limma-voom before precision-weight fitting. (g) Mean-variance trend after voom precision-weighting, showing a flat relationship consistent with homoscedastic residuals. Together, these diagnostics indicate uniform sequencing quality and appropriate normalization for downstream differential expression analysis.