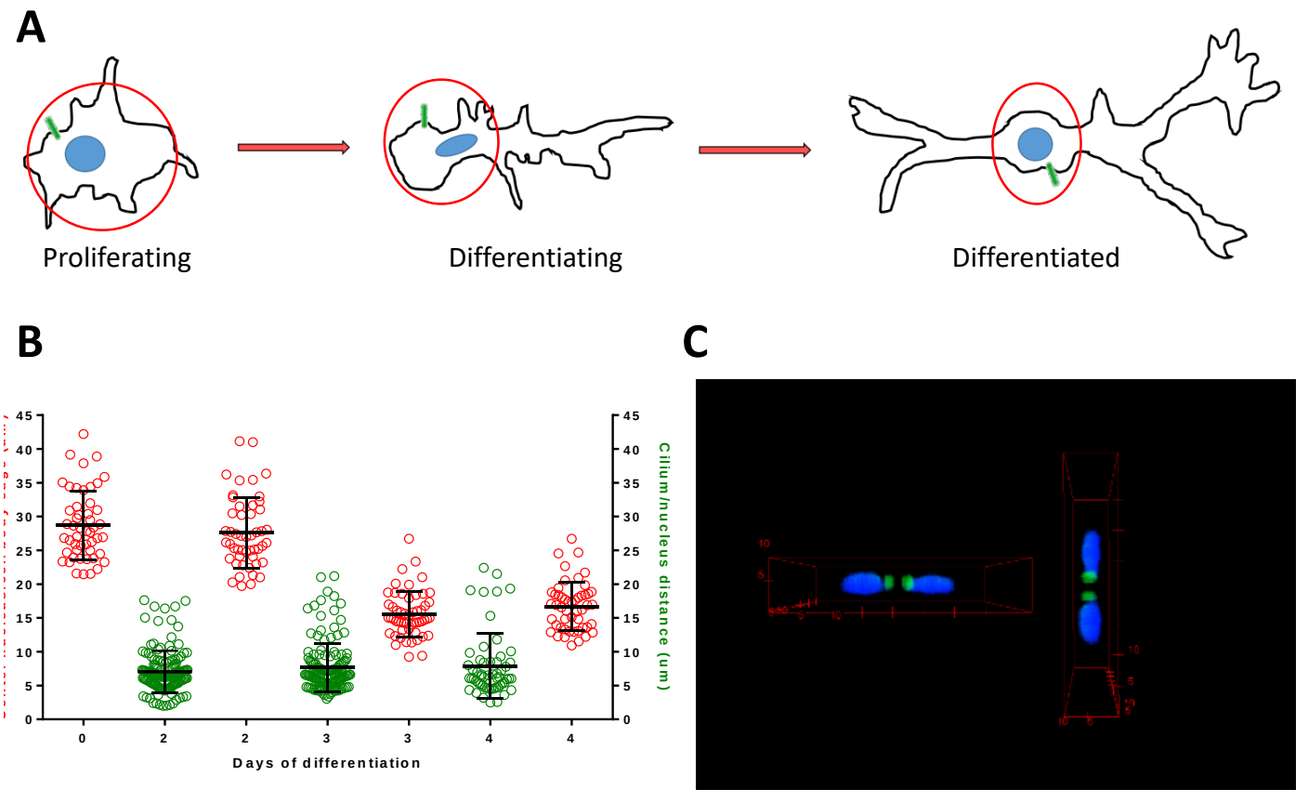
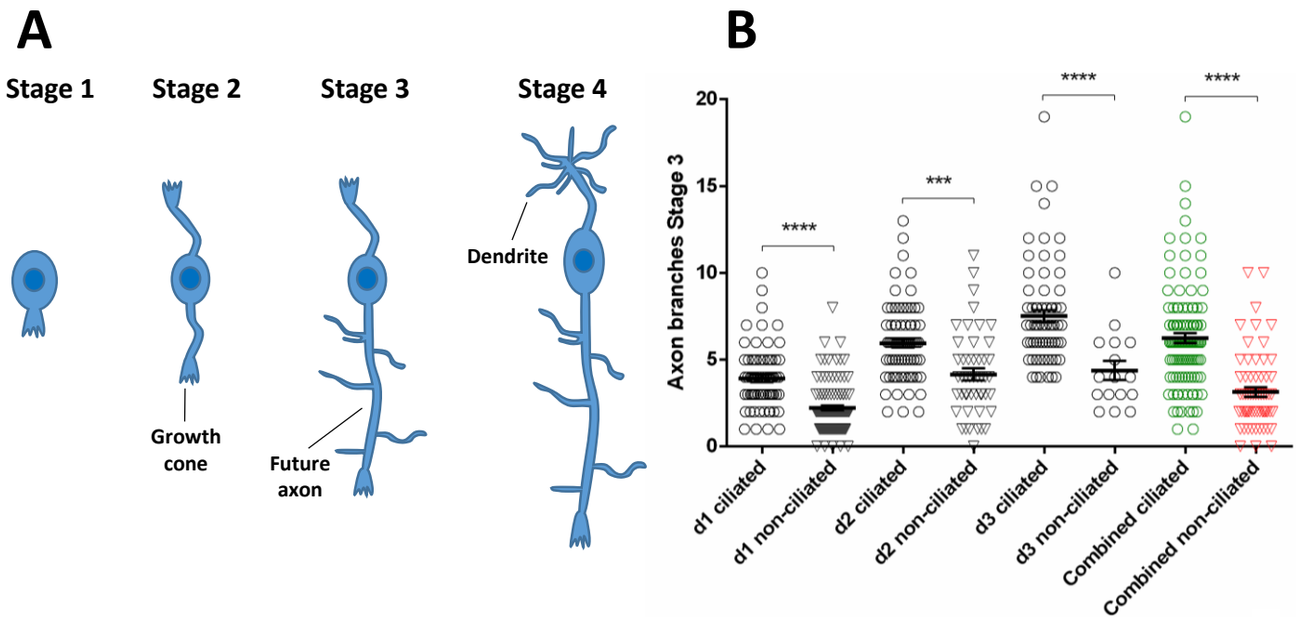
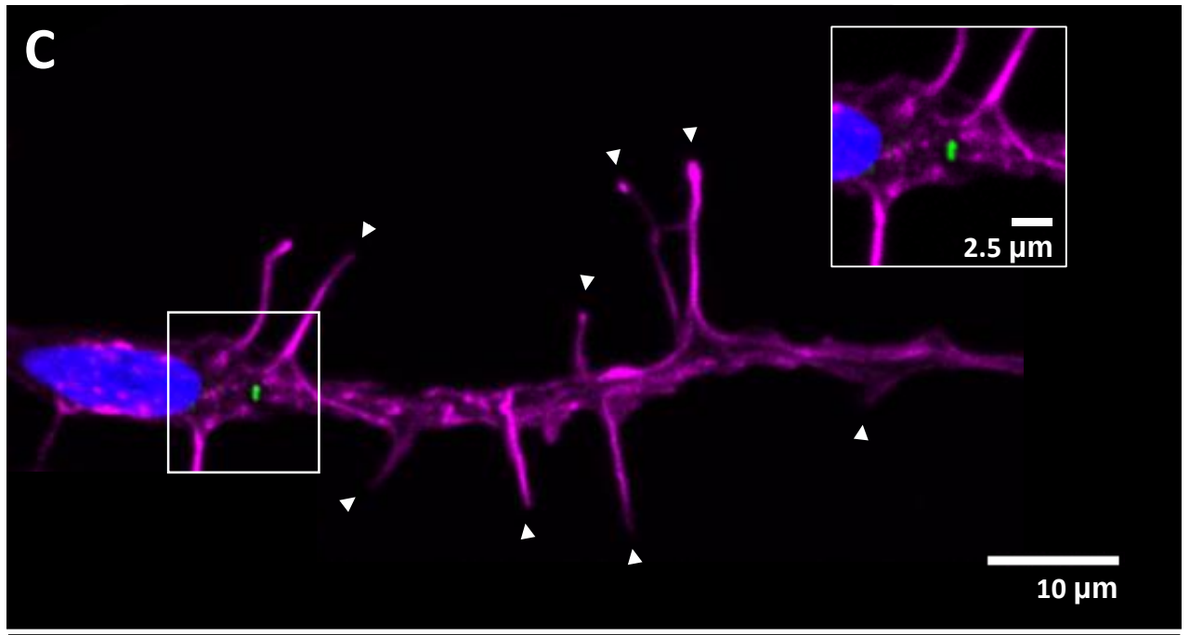


Figure S1: Location of cilia in differentiating LUHMES neurons. (A) Schematic representation of LUHMES cells and neurons at proliferating (d0), differentiating (d3) and differentiated (d6) stages. Primary cilia are depicted in green and nuclei in blue, whereby the cell body is contained within the red circle. **(B)** The distances from the center of the nucleus to the boundaries of the cell body (transition-zone-to-neurites) at d0, d2, d3, d4 (in red) are compared to the distances from the center of the nucleus to the cilium at d2, d3, d4 (in green). Cilia in differentiating LUHMES neurons are located at the cell body. **(C)** A 3-D rendering of cilia location is provided along the z-axis of stacked images. The same rendering is shown from two different viewpoints. Cilia, stained with ARL13B (green), typically appear in the middle sections of a z-stack. Nuclei are counterstained with DAPI (blue). See also **Movie 1**. All distances in **(B-C)** are in µm.





DAPI ARL13B F-actin

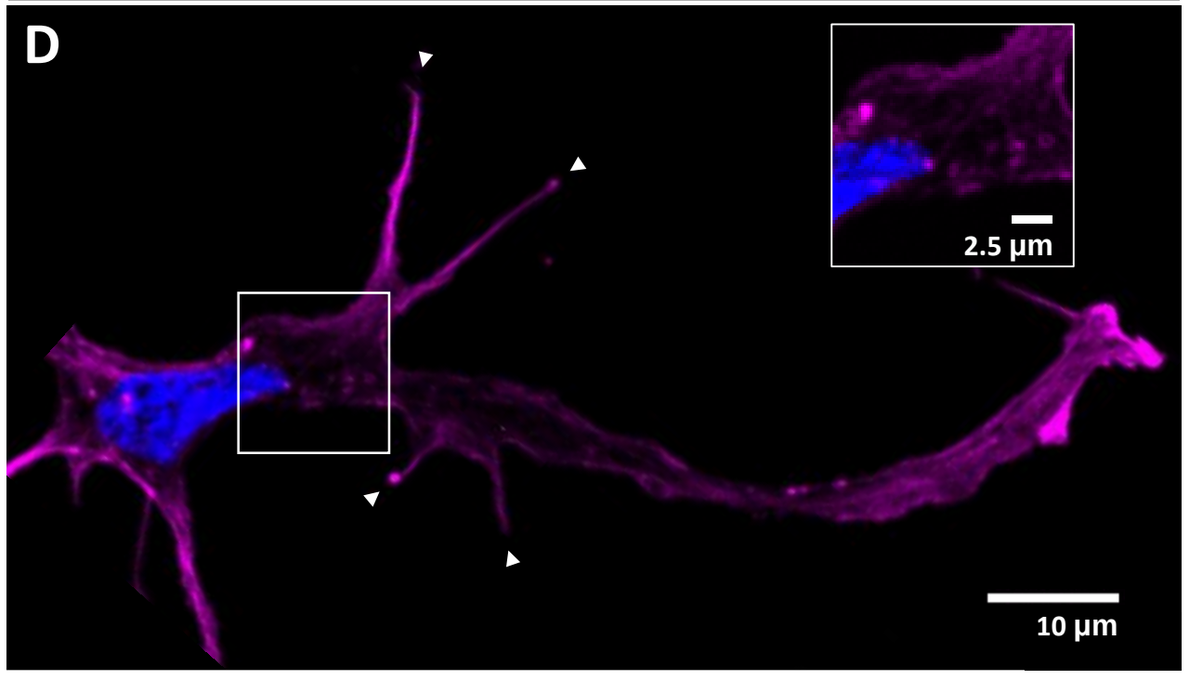


Figure S2: The presence of cilia strongly impacts neuronal differentiation in LUHMES, like axon outgrowth, exemplified by axon branching complexity. (A) Schematic representation of early neuronal differentiation commonly divided by stages. **(B)** The effect of the presence of neuronal cilia on axon branching of stage 3 LUHMES neurons at different time points of differentiation (d1, d2, d3) and all time points combined; as compared to LUHMES neurons where cilia are not detectable. Data are shown as the mean +/- SEM from two independent experiments. ***: $P < 0.0005$; ****: $p < 0.0001$. **(C-D)** Detection by immunocytochemistry of axon branches (arrowheads) in ciliated **(C)** and in non-ciliated **(D)** stage 3 LUHMES neurons, using ARL13B (green) as the ciliary marker and Phalloidin staining F-actin (magenta) as a marker defining overall neuronal anatomy; blue = DAPI/nucleus. Note that in panel C the growth cone at the end of the axon is out of focus (not visible). In panels **(C)** and **(D)** the scale bar represents 10 µm, in the boxed areas 2.5 µm.

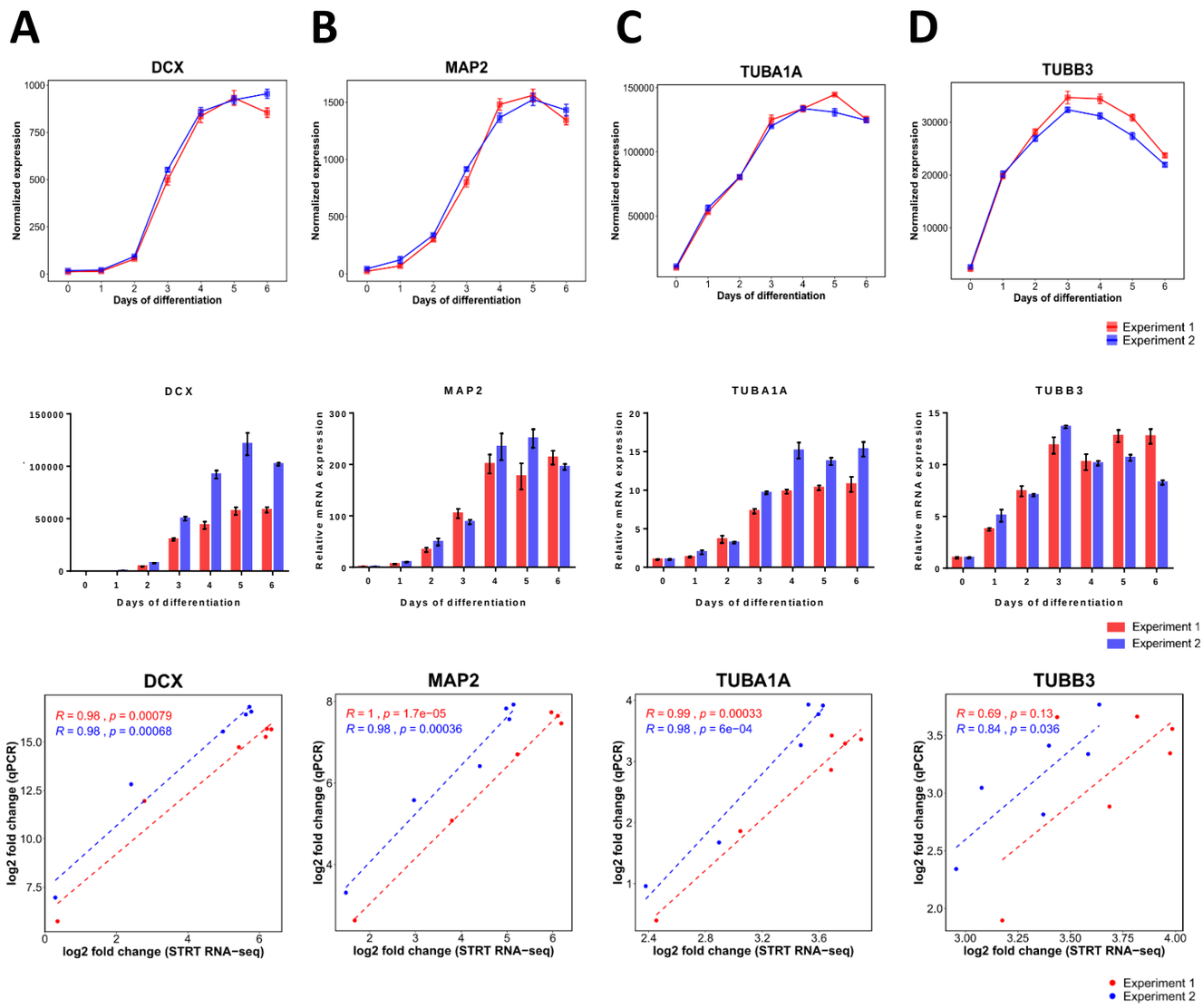


Figure S3: Gene expression profiles of LUHMES cells differentiating into neurons. (A-D) Differential gene expression patterns of neuronal marker genes DCX, MAP2, TUBA1A, and TUBB3 were assessed by STRT RNA-seq from d0 to d6 and validated by quantitative real-time PCR (qRT-PCR). Gene expression data using both methods were correlated as $\log_2$ fold change in STRT RNA-seq versus $\log_2$ fold change in qRT-PCR. R refers to the Pearson correlation coefficient. Data were normalized to the housekeeping gene GAPDH and displayed as the mean +/- SEM from two independent experiments (red and blue). Upper panels show STRT RNA-seq time course data; middle panels show qRT-PCR time course data; lower panels show the correlation plots, respectively.

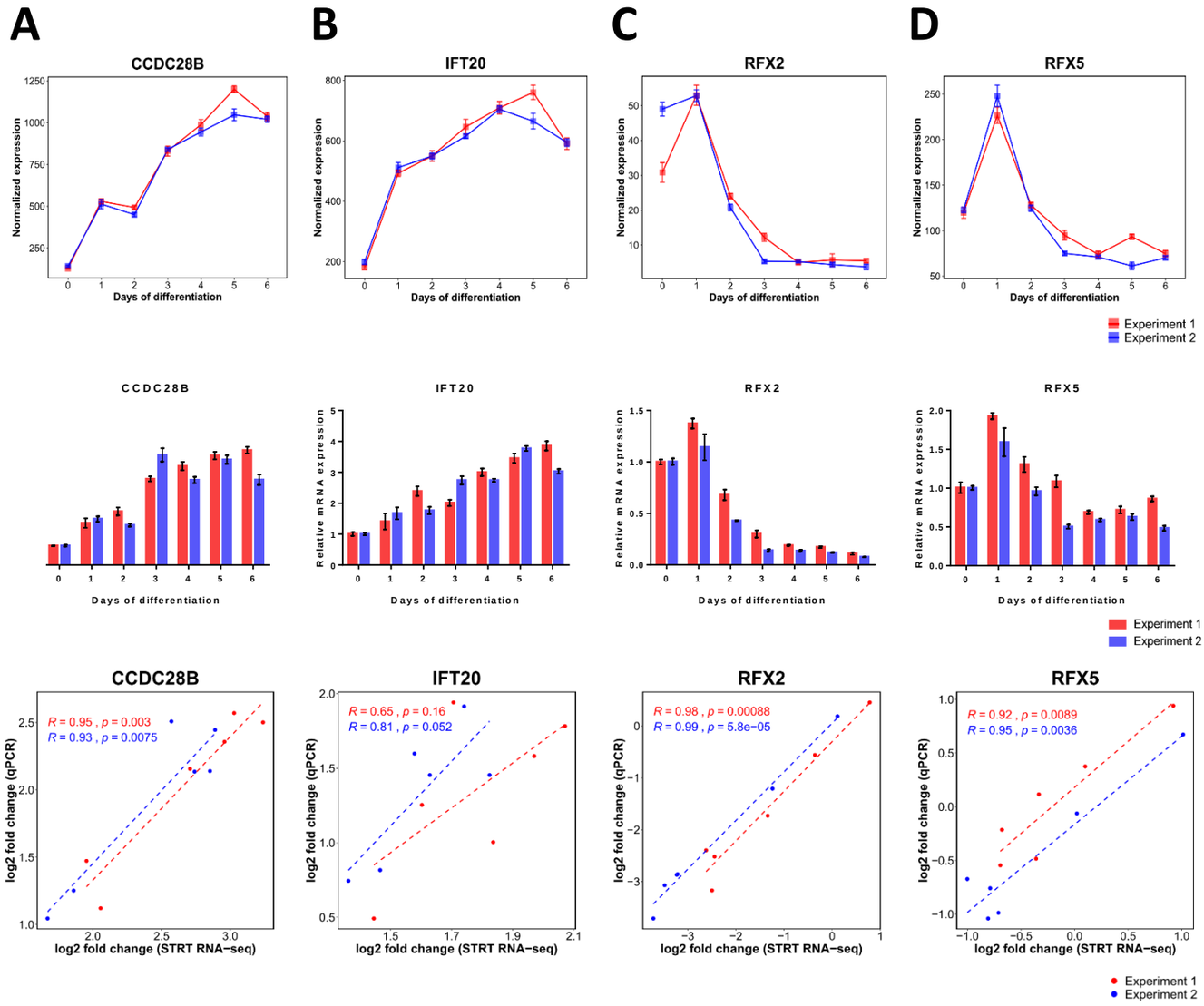


Figure S4: Gene expression profiles of LUHMES cells differentiating into neurons. Differential gene expression patterns of **(A-B)** ciliary and ciliopathy genes CCDC28B and IFT20, and **(C-D)** ciliogenic transcription factor genes RFX2 and RFX5 were assessed by STRT RNA-seq from d0 to d6 and validated by quantitative real-time PCR (qRT-PCR). Gene expression data using both methods were correlated as $\log_2$ fold change in STRT RNA-seq versus $\log_2$ fold change in qRT-PCR. R refers to the Pearson correlation coefficient. Data were normalized to the housekeeping gene GAPDH and displayed as the mean +/- SEM from two independent experiments (red and blue). Upper panels show STRT RNA-seq time course data; middle panels show qRT-PCR time course data; lower panels show the correlation plots, respectively.

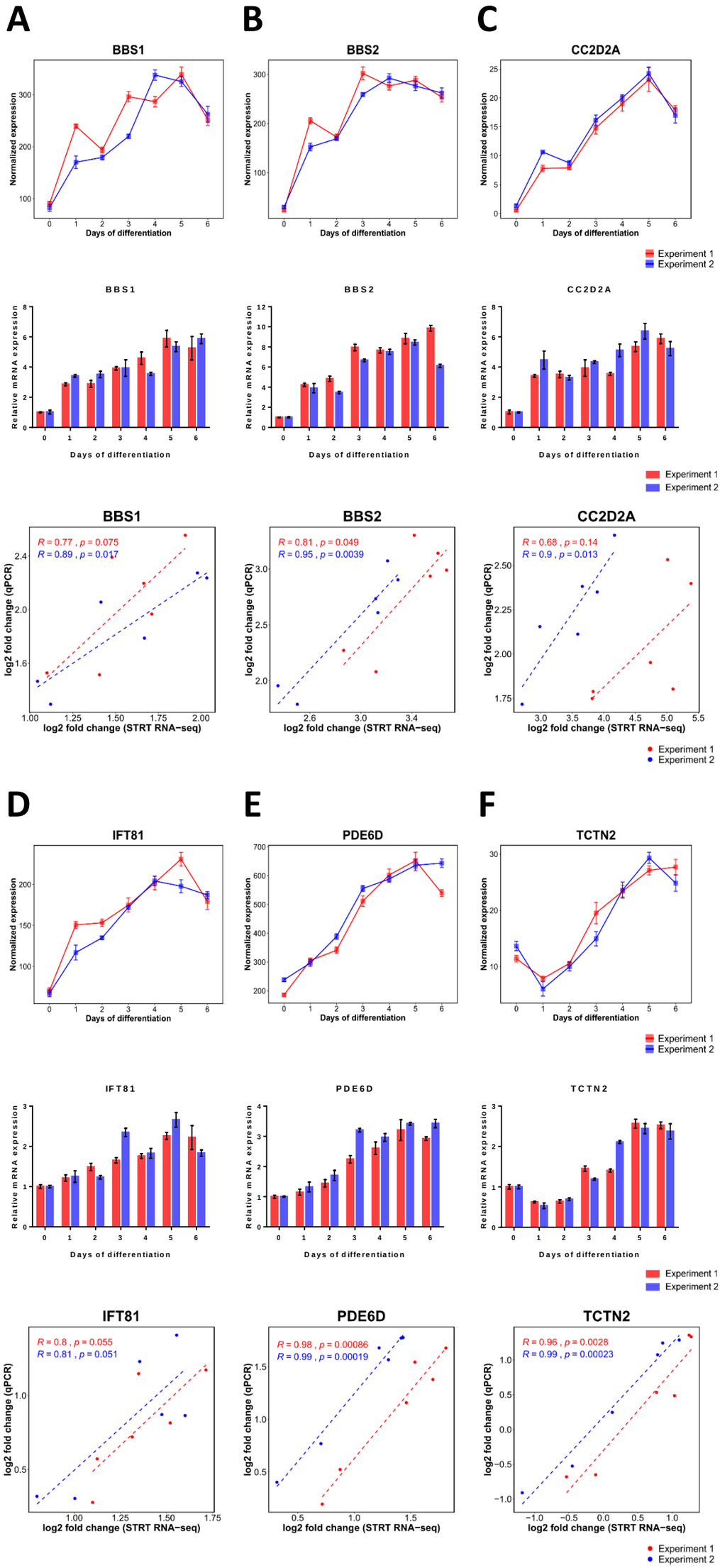
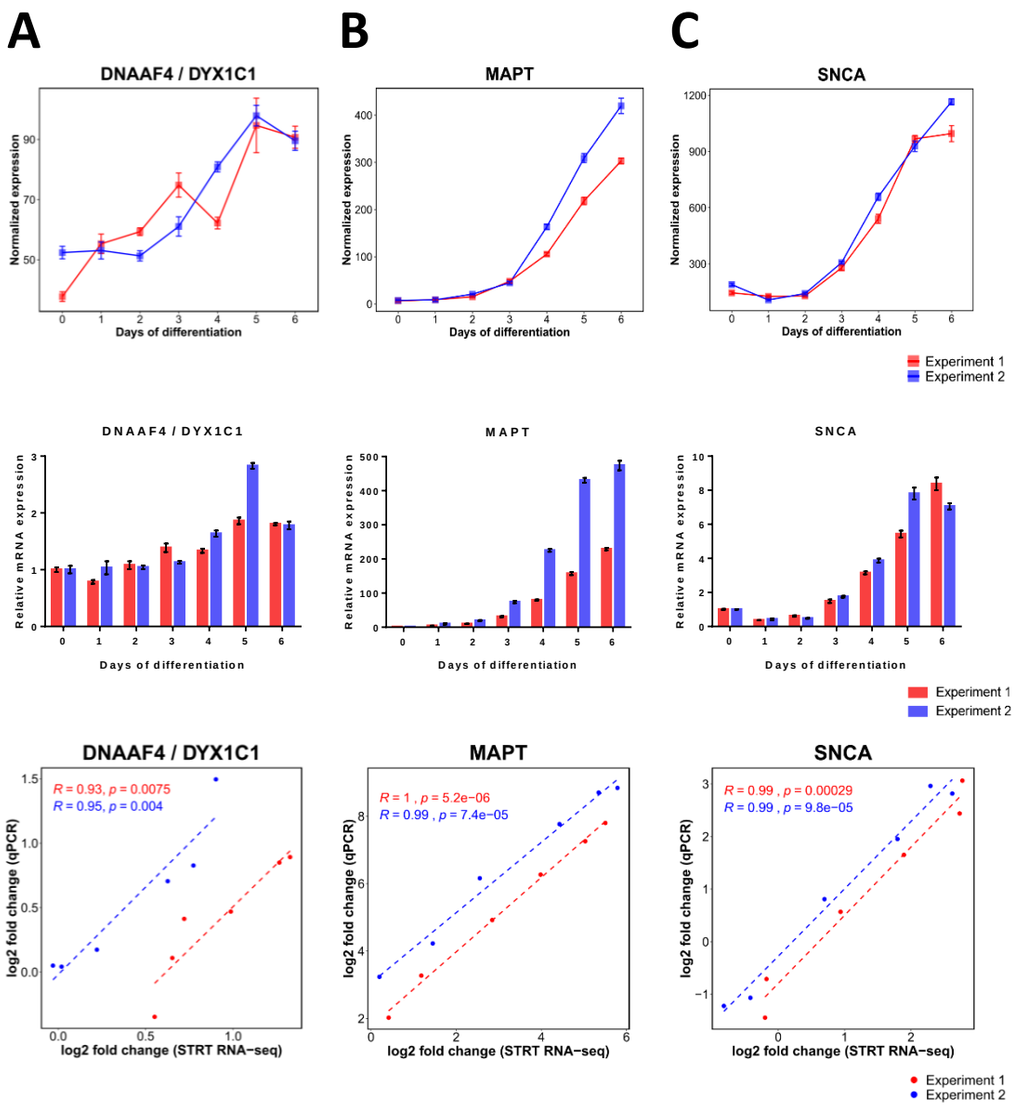


Figure S5: Gene expression profiles of LUHMES cells differentiating into neurons. (A-F) Differential gene expression patterns of ciliary and ciliopathy genes with associated brain phenotypes BBS1, BBS2, CC2D2A, IFT81, PDE6D and TCTN2 were assessed by STRT RNA-seq from d0 to d6 and validated by quantitative real-time PCR (qRT-PCR). Gene expression data using both methods were correlated as $\log_2$ fold change in STRT RNA-seq versus $\log_2$ fold change in qRT-PCR. R refers to the Pearson correlation coefficient. Data were normalized to the housekeeping gene GAPDH and displayed as the mean +/- SEM from two independent experiments (red and blue). Upper panels show STRT RNA-seq time course data; middle panels show qRT-PCR time course data; lower panels show the correlation plots, respectively.



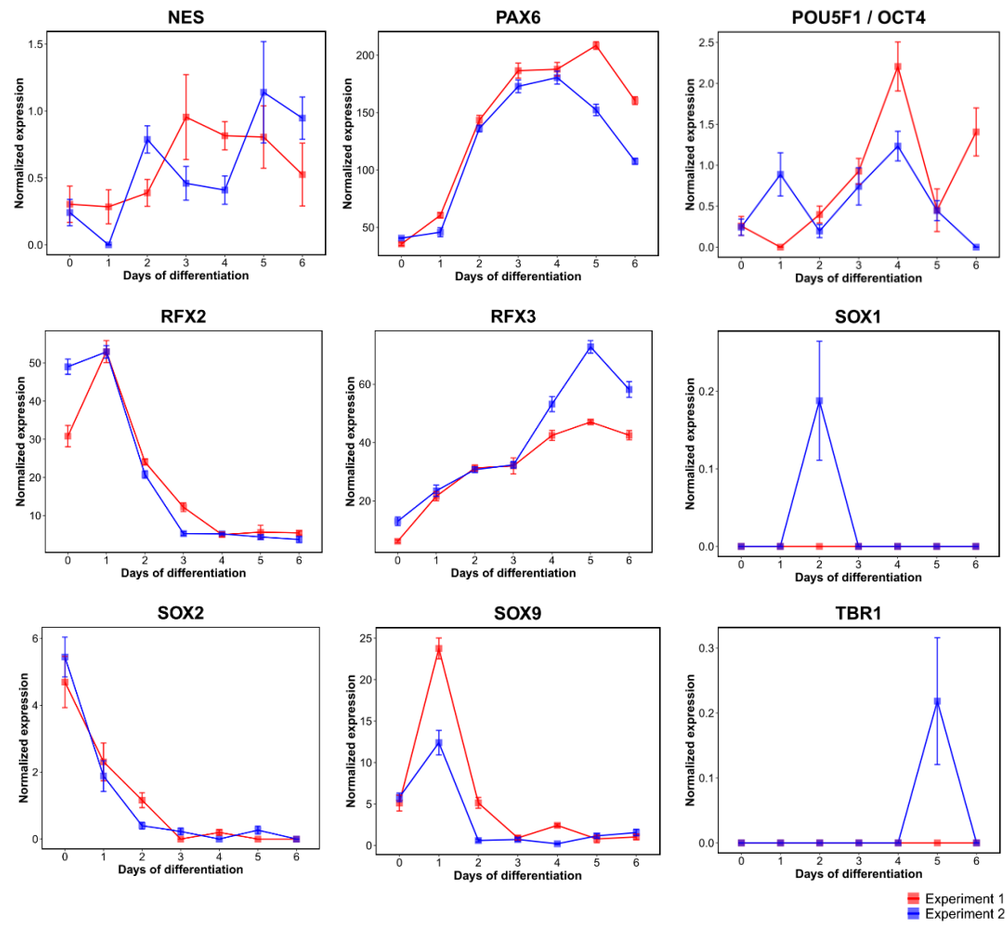


Figure S6: Gene expression profiles of LUHMES cells differentiating into neurons. Differential gene expression patterns of **(A)** the dyslexia candidate gene DNAAF4 (DYX1C1), and **(B-C)** the Parkinson's disease candidate genes MAPT and SNCA were assessed by STRT RNA-seq from d0 to d6 and validated by quantitative real-time PCR (qRT-PCR). Gene expression data using both methods were correlated as $\log_2$ fold change in STRT RNA-seq versus $\log_2$ fold change in qRT-PCR. R refers to the Pearson correlation coefficient. Data were normalized to the housekeeping gene GAPDH and displayed as the mean +/- SEM from two independent experiments (red and blue). Upper panels show STRT RNA-seq time course data; middle panels show qRT-PCR time course data; lower panels show the correlation plots, respectively. **(D)** STRT RNA-seq gene expression patterns of the transcription factor genes NES, PAX6, POU5F1 (OCT4), RFX2, RFX3, SOX1, SOX2, SOX9 and TBR1 during LUHMES cells differentiating into neurons.

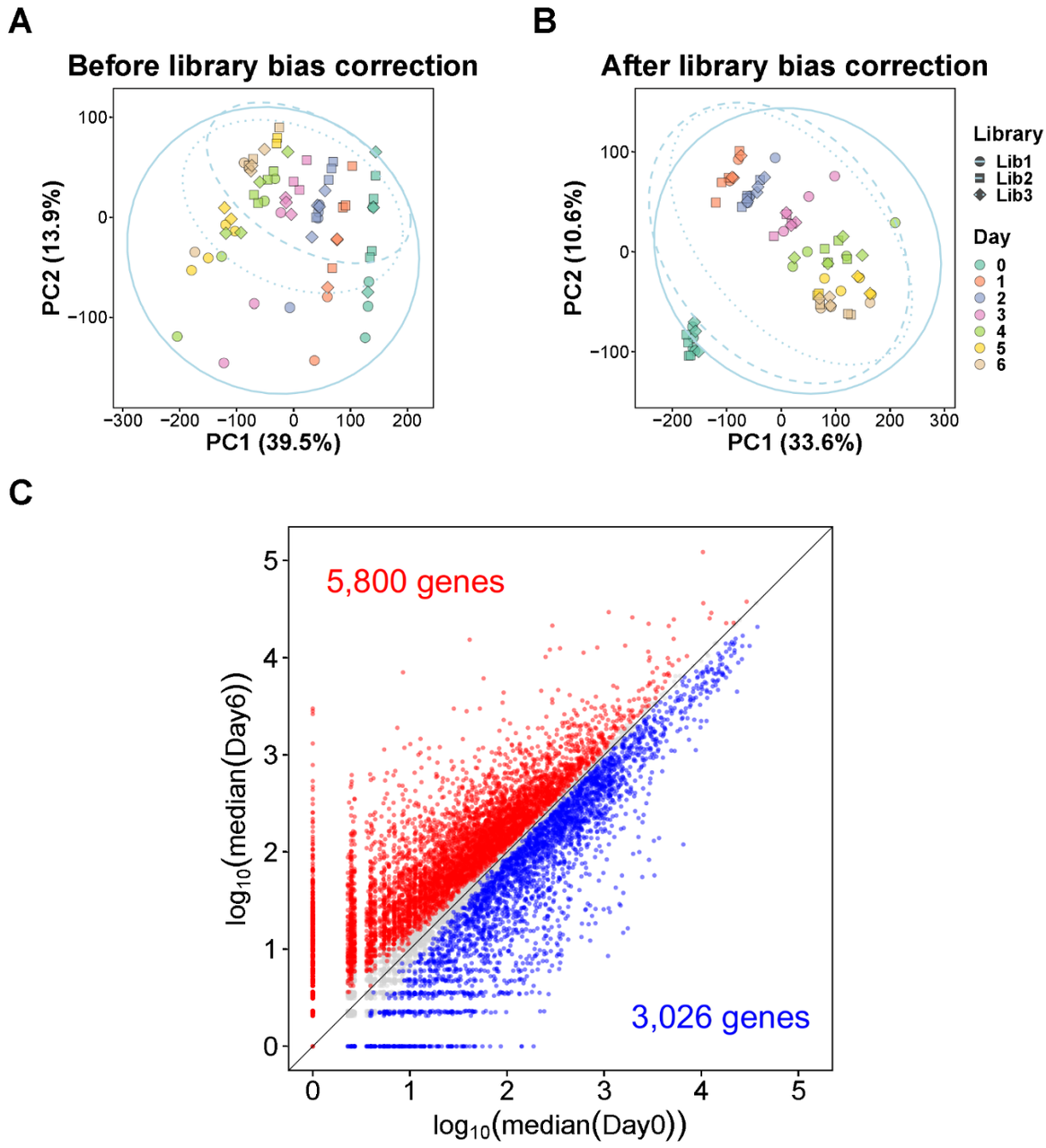


Figure S7: Library bias correction and gene expression changes between day 0 and day 6. (A-B) Principal component analysis (PCA) of LUHMES samples before and after library bias correction. PCA plots covering 32,483 significantly fluctuating transcript far 5´ ends (TFEs) of 70 STRT RNA-seq time course samples, representing LUHMES cells differentiating into neurons (d0 to d6), before **(A)** and after **(B)** library bias correction. Solid, dashed, and dotted lines represent the 95% confidence interval ellipses for each of the three independent libraries used. **(C)** Scatter plot of STRT RNA-seq gene expression levels from LUHMES cells differentiating into neurons (d0 versus d6). Red dots represent significantly upregulated genes, while blue dots represent significantly downregulated genes on d6 (differentiated LUHMES neurons) versus d0 (proliferating LUHMES cells).

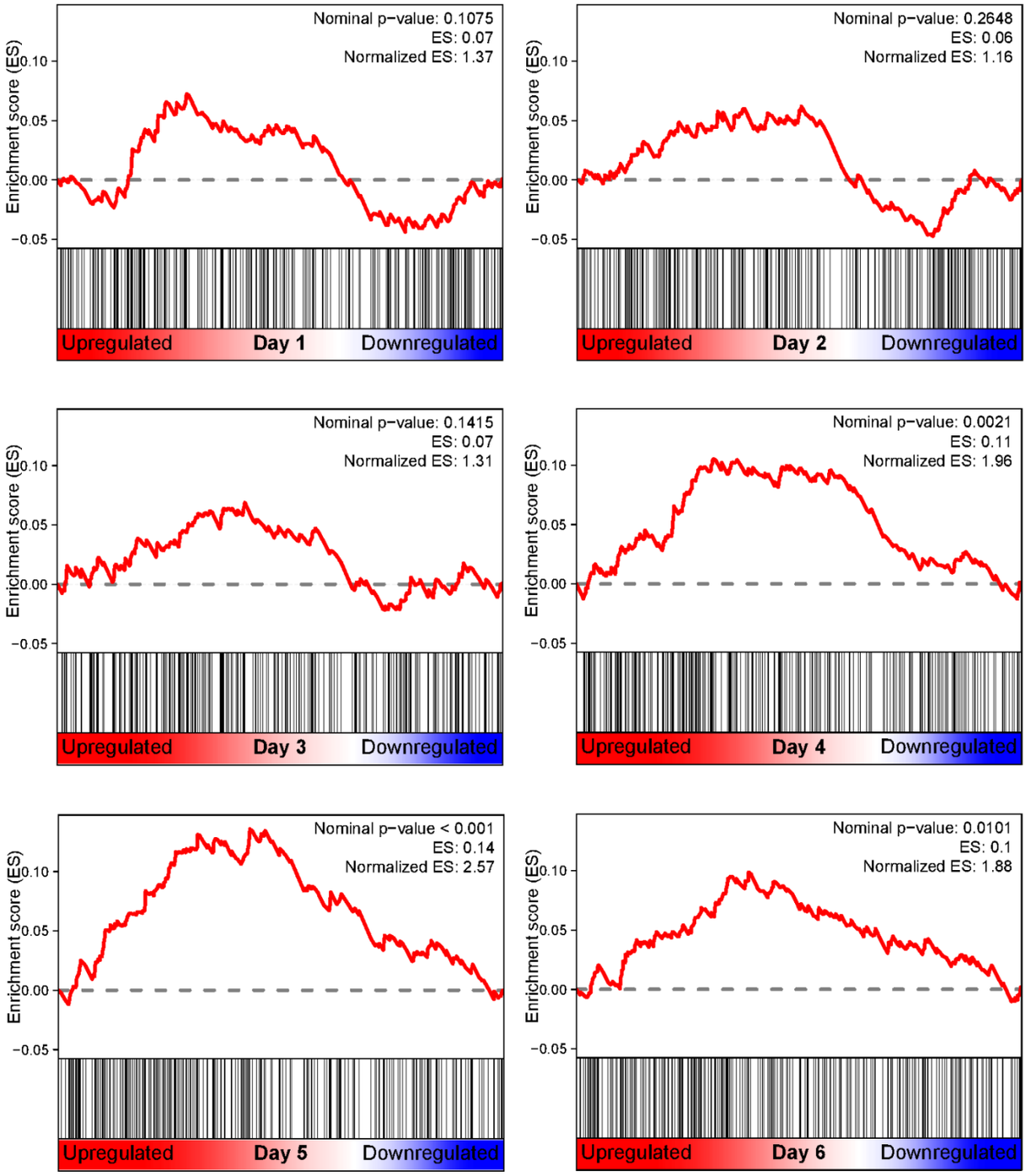


Figure S8: Ciliary gene set enrichment analysis (GSEA) of differentiating LUHMES neurons (d1-d6) compared with proliferating LUHMES cells (d0). Black vertical lines represent ranked ciliary genes based on differential gene expression comparisons between d0 and the respective day indicated in each graph. Enrichment scores (ES) are highest on d5 of differentiation. See also **Figure 8A**.

Table S1: Summary of STRT RNA-seq sequencing output.

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Table S2: Gene ontology (GO) enrichment analysis of significantly upregulated genes between day 0 and day 6 of the LUHMES neuronal differentiation time course.

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Table S3: Gene ontology (GO) enrichment analysis of significantly downregulated genes between day 0 and day 6 of the LUHMES neuronal differentiation time course.

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Table S4: Gene ontology (GO) enrichment analysis of core genes in cluster 1 - based on LUHMES neuronal differentiation time course expression patterns.

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Table S5: Gene ontology (GO) enrichment analysis of core genes in cluster 2 - based on LUHMES neuronal differentiation time course expression patterns.

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Table S6: Gene ontology (GO) enrichment analysis of core genes in cluster 3 - based on LUHMES neuronal differentiation time course expression patterns.

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Table S7: Gene ontology (GO) enrichment analysis of core genes in cluster 4 - based on LUHMES neuronal differentiation time course expression patterns.

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Table S8: Differentially regulated genes during LUHMES neuronal differentiation (d0-d6) as determined by both STRT RNA-seq and qRT-PCR, including functional assignments, descriptions and the list of SYBR Green primers used for qRT-PCR; with the exception of the genes GLI1, HHIP, PTCH1 and PTCH2, whose expression was quantified by qRT-PCR to assess their involvement in the SHH signaling pathway.

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Table S9: List of Z-values, which indicate transcription factor binding motif activity changes during LUHMES differentiation into neurons. Motif hits are listed separately for each of the three independent libraries used.

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Table S10: Transcription factor binding motif enrichment analysis of differentially expressed transcript far 5' ends (TFEs) between neighboring days of LUHMES differentiation into neurons. Only the top five significantly enriched motifs are listed.

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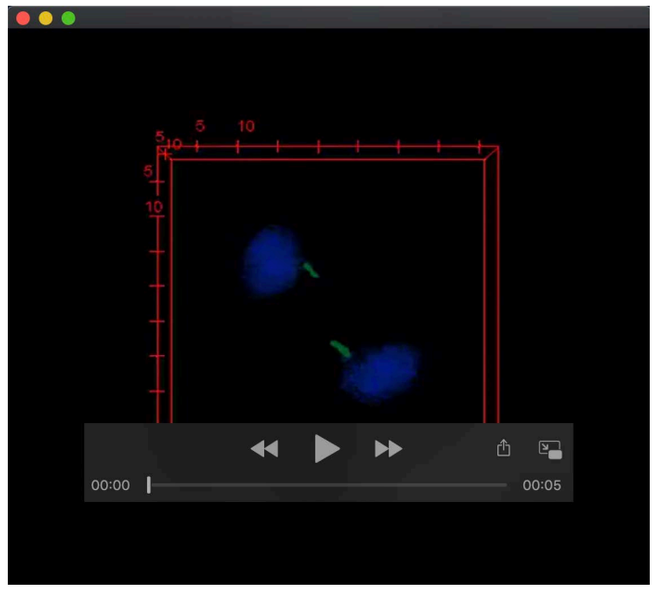

Movie 1 is a complement to **Figure S1** – see there for details.