

## Supplementary Information

# Deciphering the protein dynamics and molecular determinants of iPSC-derived neurons.

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**Figure S1. Global proteome analysis.** (A) Bar graph showing the number of proteins identified in each replicate and in each time point. (B) Venn diagram showing the proteins identified in each biological replicate and neuronal sub-type. (C) Distribution of quantified protein abundances in all replicas and all time points, spanning four orders of magnitude. In red tissue enrichment analysis using DAVID, of the 25% most abundant proteins. (D) Pie charts display the distribution of protein classes, based on PANTHER classification from all quantified proteins. (E) Box plots of log2 transformed data normalized on protein peak areas of each individual replicate.

**Figure S2. Proteins only identified in iN cells or MNs.** Proteins exclusively identified in (A) iN cells or (B) MN differentiation. Proteins were selected for a twofold change in the two biological replicates in each group.

**Figure S3. Transcription factors and cytoskeletal proteins.** (A) Transcription factors enriched using the Database for Annotation, Visualization, and Integrated Discovery (DAVID). (B) Cytoskeletal proteins enriched using DAVID. (C) Example of the Transgelin family that undergo different expression during differentiation towards iN cells and MNs.

**Figure S4. Signaling pathways.** (A) Proteins related to TGFβ signaling enriched from KEGG and WIKI Pathways. (B) Proteins related to Wnt signaling. (C) Proteins related to Hedgehog signaling. (D) Proteins related to RA signaling. (E) Homeobox proteins all expressed in MNs and none of the different members expressed in iN cells. (F) Proteins related to Notch signaling. (G) Proteins related to axon guidance

**Figure S5. Neurogenesis.** Neurogenesis associated proteins enriched using the Database for Annotation, Visualization, and Integrated Discovery (DAVID).

**Figure S6. Uncropped blots.** (A, B) Western blot analyses on iPSC, Neuro-2a cells (ATCC), and iPSC-derived MN (DIV14) protein lysates. For (B), membrane from (A) was stripped and reprobed with anti-TUBB3 antibodies. (C) REVERT Total Protein Stain results [700 channel Odyssey CLx scanner (LI-COR)].

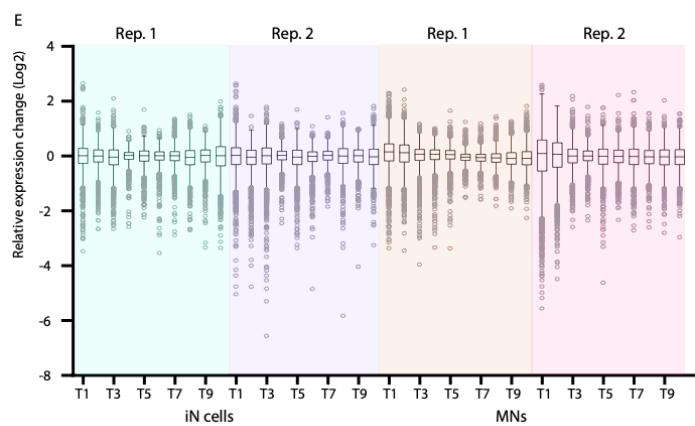
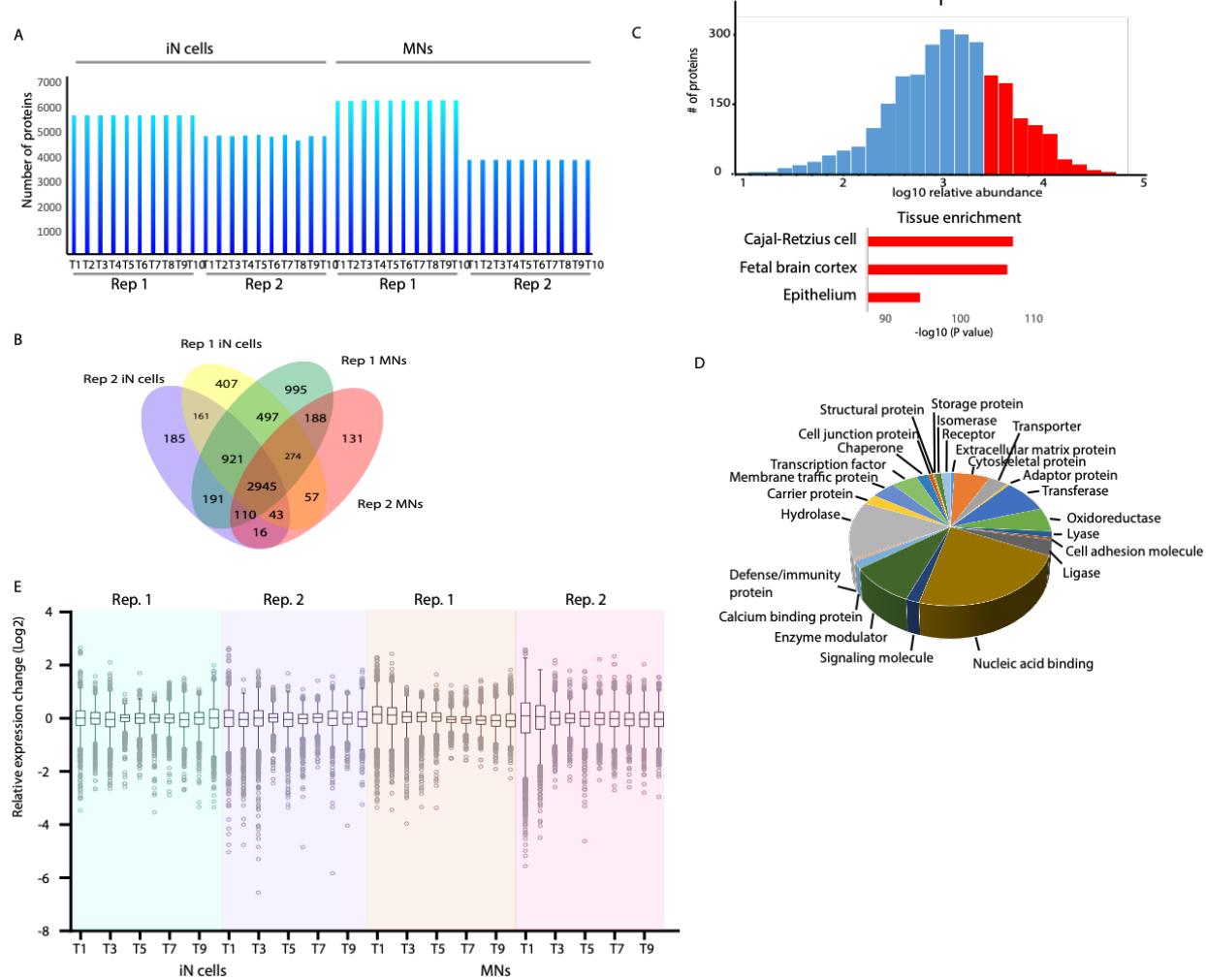
**Table S1. Expression changes of proteins during iN cell differentiation**

**Table S2. Expression changes of proteins during MN differentiation**

**Table S3. Significant differentially expressed proteins in the opposite direction.**

**Table S4. Proteins significantly downregulated in iN cells and in MNs**

Figure S1



**Figure S2**

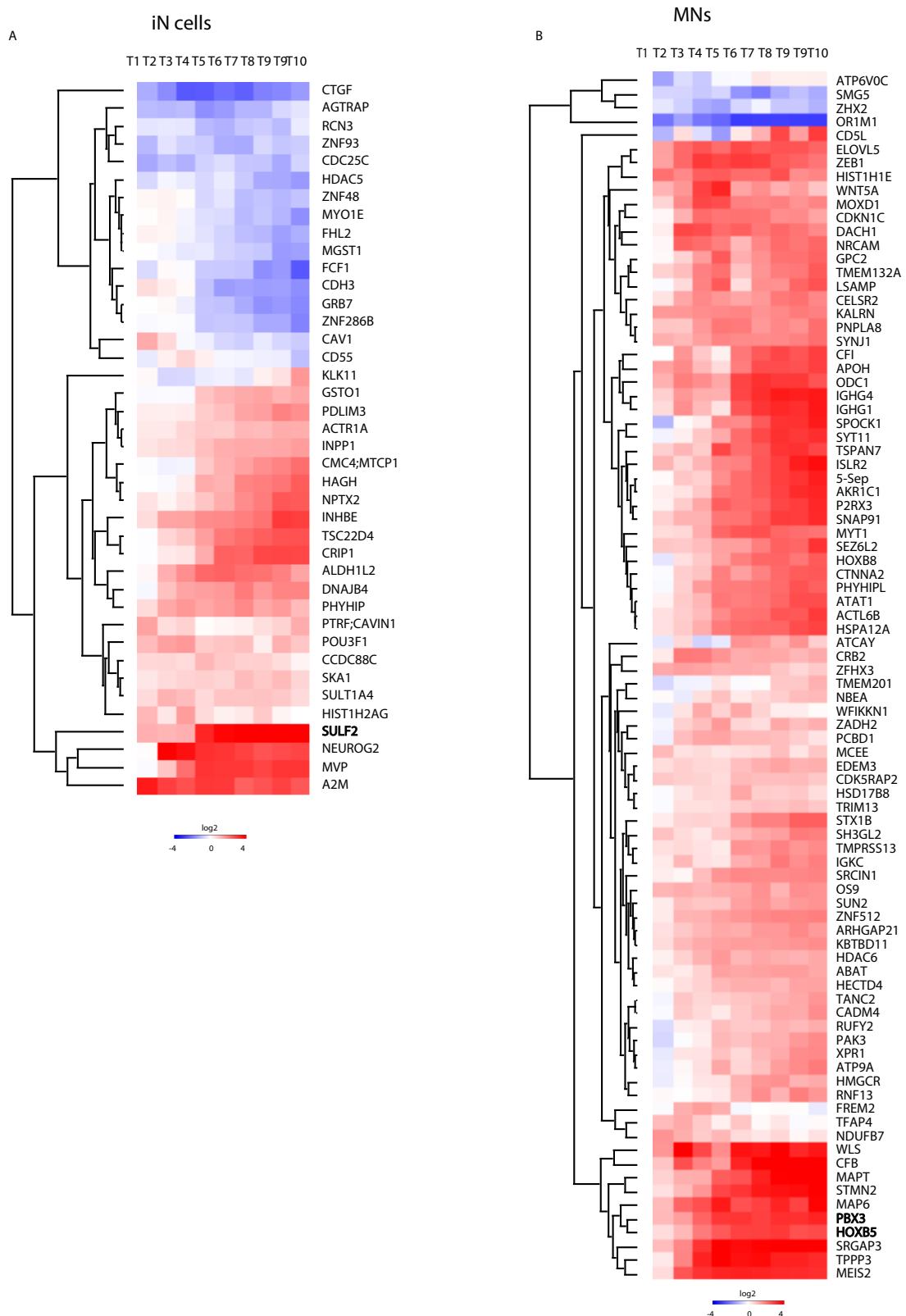


Figure S3

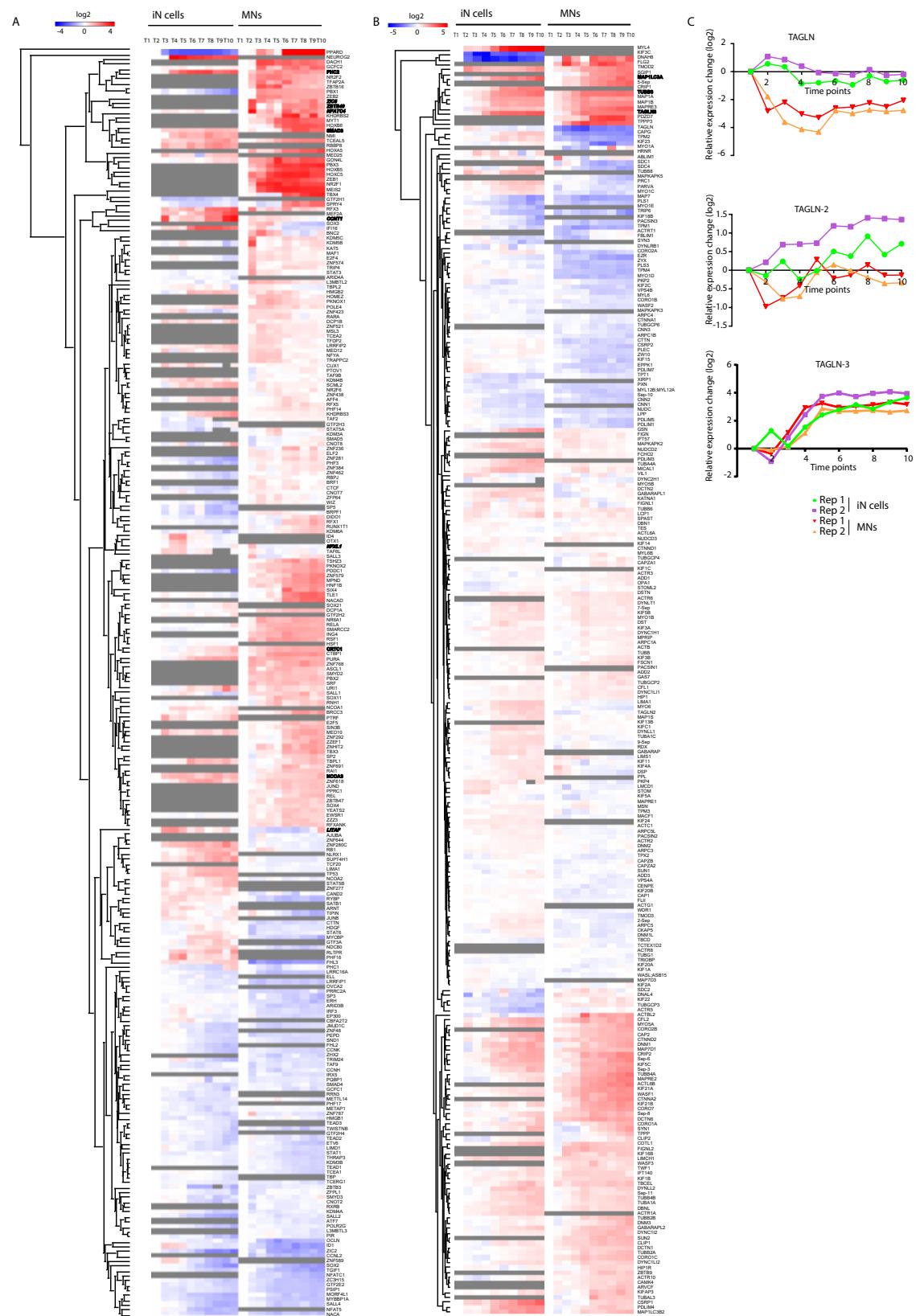
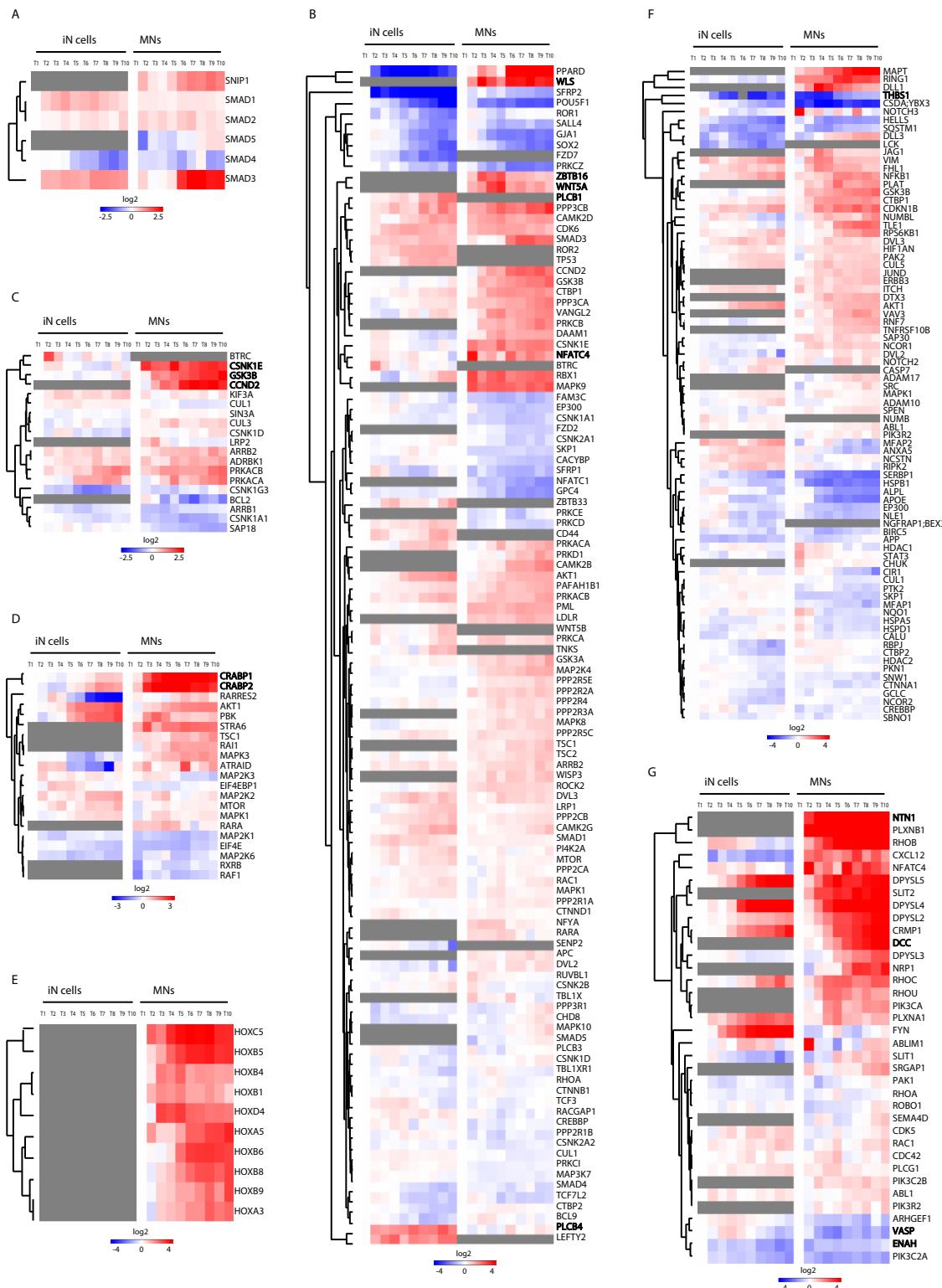


Figure S4



**Figure S5**

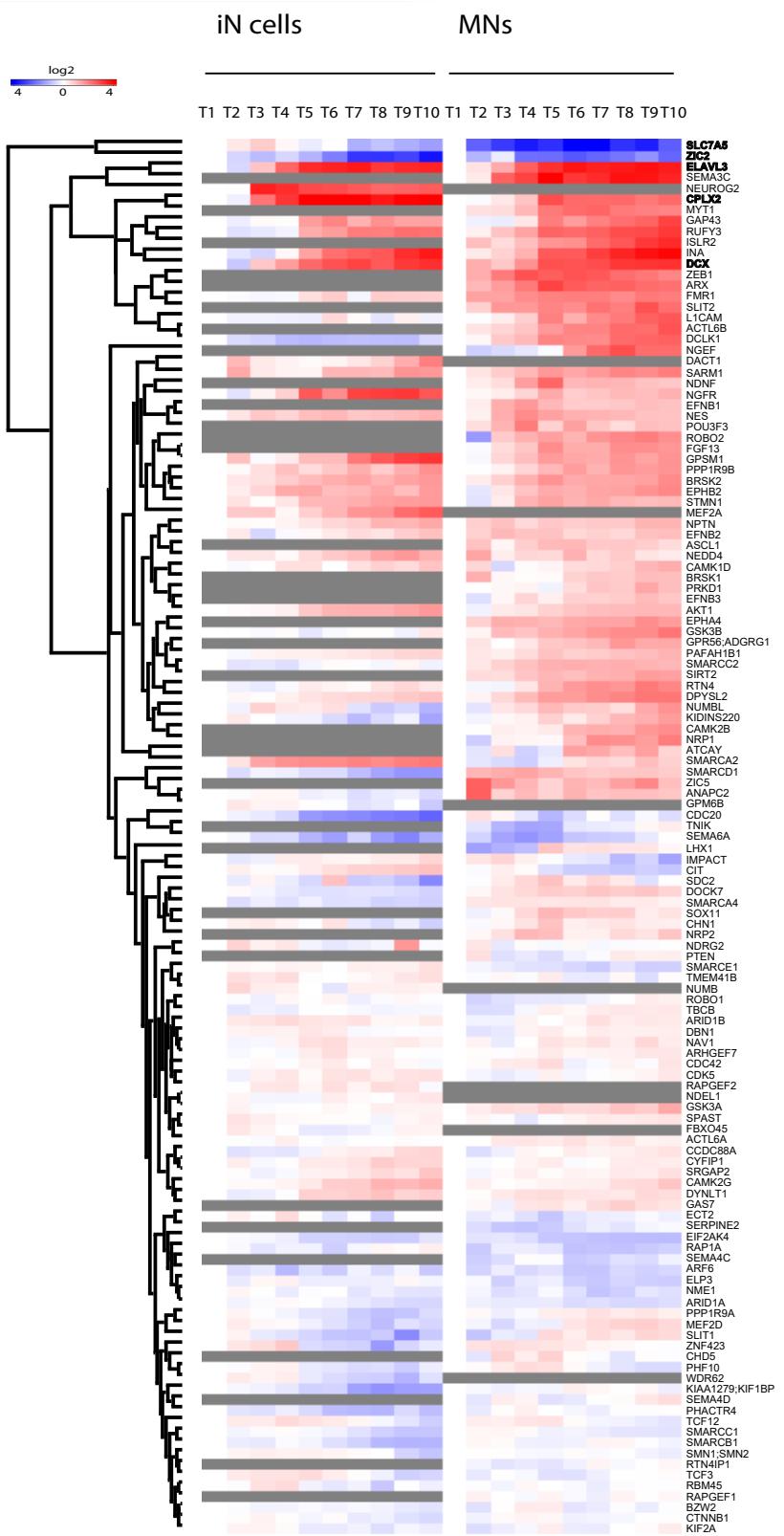


Figure S6

