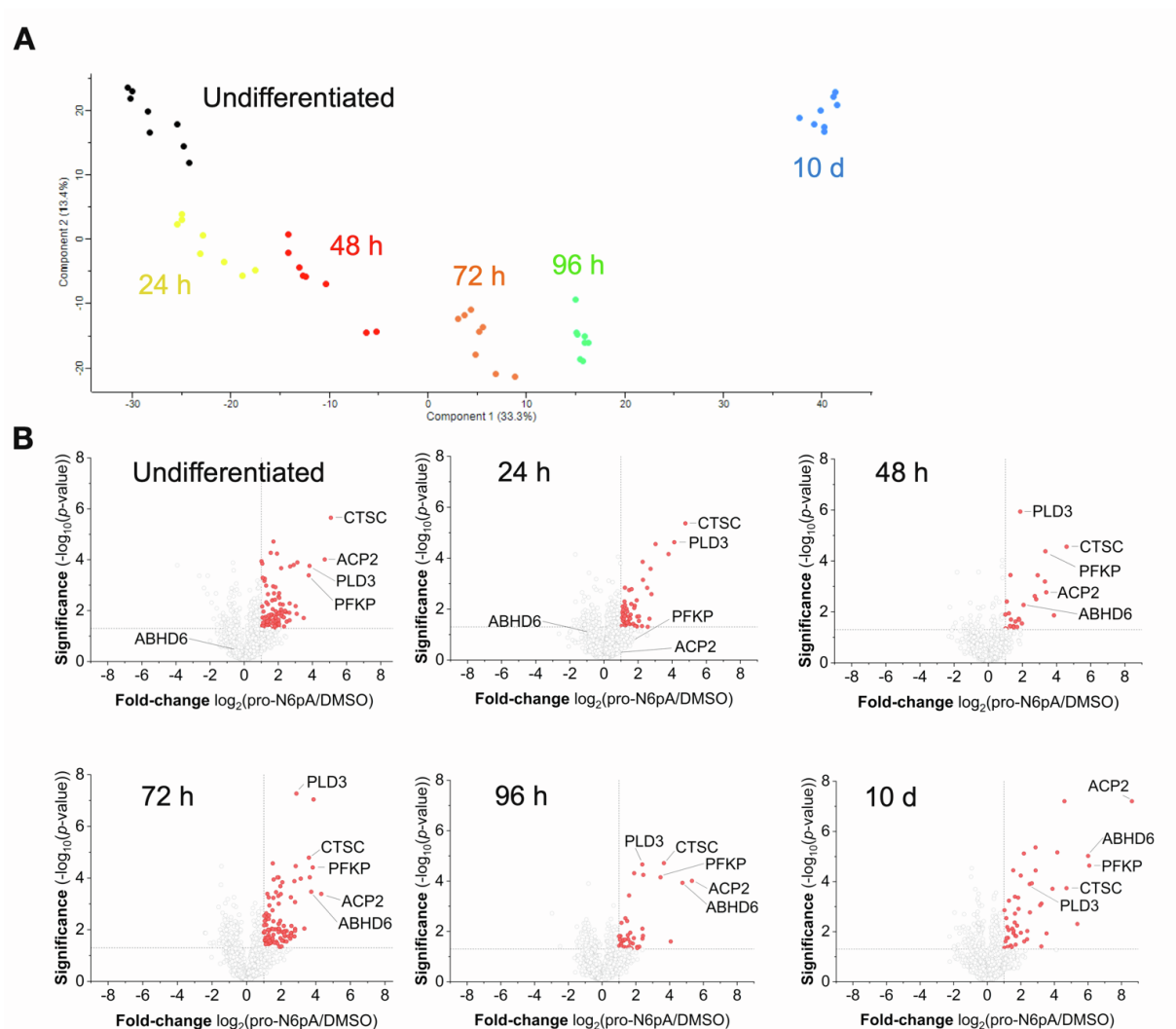


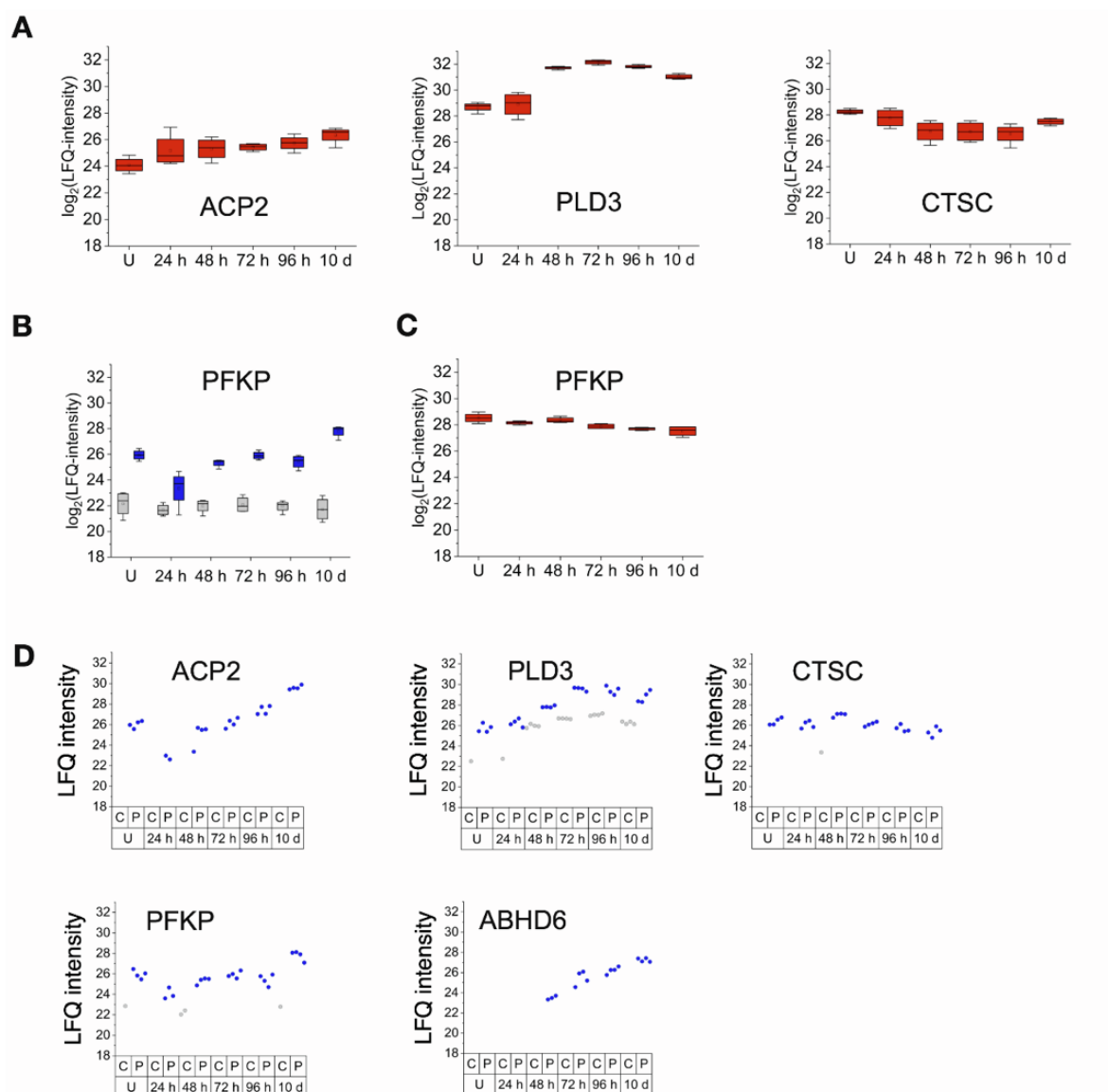
Supplemental information

AMPylation profiling during neuronal differentiation reveals extensive variation on lysosomal proteins

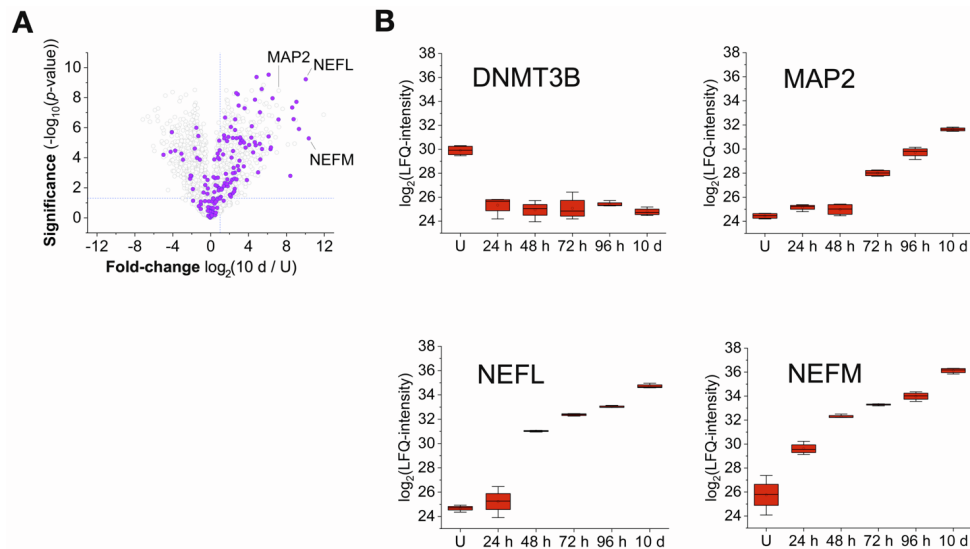
Tobias Becker, Cedric Cappel, Francesco Di Matteo, Giovanna Sonsalla, Ewelina Kaminska, Fabio Spada, Silvia Cappello, Markus Damme, and Pavel Kielkowski



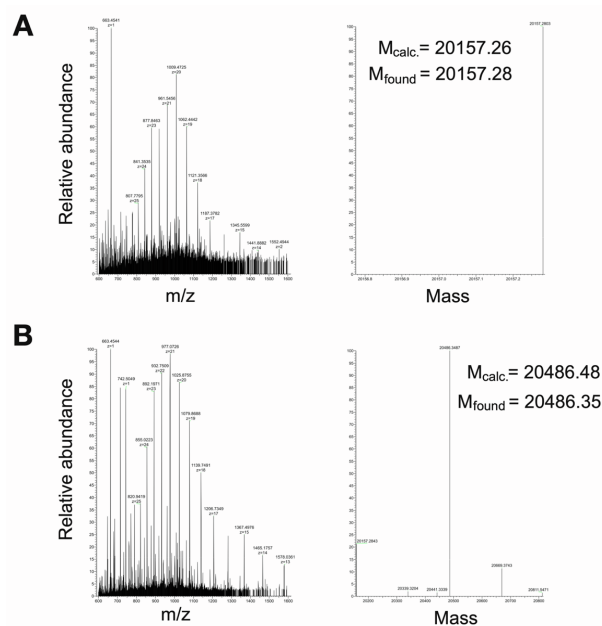
Supplemental Figure 1. Chemical proteomics of neuronal differentiation and maturation. Related to **Figure 2.** **A)** Principal component analysis (PCA) of the chemical proteomic LFQ MS data. **B)** Volcano plots showing the enrichment of AMPylated proteins at different times during differentiation. Red circles highlight the significantly enriched proteins. ($n = 4$, cut-off lines are at $p\text{-value} > 0.05$ and at least 2-fold enrichment).



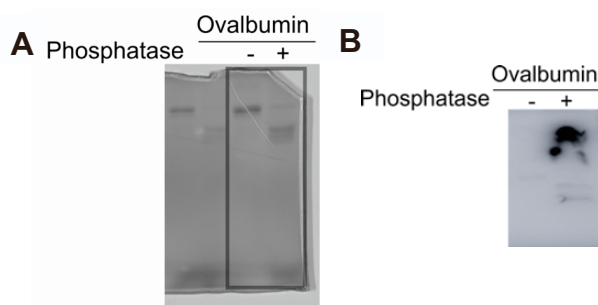
Supplemental Figure 2. Profile plots of chemical proteomics of neuronal differentiation and maturation. Related to **Figure 2**. **A)** Profile plots showing total protein expressions estimated from the whole proteome analysis during the iNGN differentiation and maturation. **B)** Profile plot visualizing the enrichment (blue boxes) of the PFKP in the chemical proteomic experiment. Grey boxes stand for PFKP background binding to the agarose-beads found in the DMSO treated control. **C)** Profile plot showing total expression of PFKP estimated from the whole proteome analysis during the course of differentiation. **D)** Profile plots of the selected hits before imputation of the missing values to visualize the efficiency of the enrichment process and utility of the chemical proteomic workflow. Blue dots represent the probe treated samples (P), gray dots represent the DMSO treated samples (C).



Supplemental Figure 3. iNGN differentiation and maturation. Related to **Figure 2**. **A)** Volcano plot representing the difference in protein expression between undifferentiated iNGNs and 10 d old neurons. Purple circles label protein annotated as neuronal processes by GO terms. **B)** Selected protein markers of neural maturation.

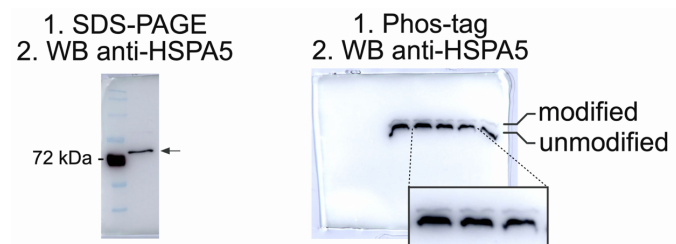


Supplemental Figure 4. Top-down mass spectrometry of Rab1b. Related to **Figure 3**. **A)** Unmodified Rab1b. **B)** AMPylated Rab1b. ΔM is 329.07 Da corresponding to the AMP moiety.

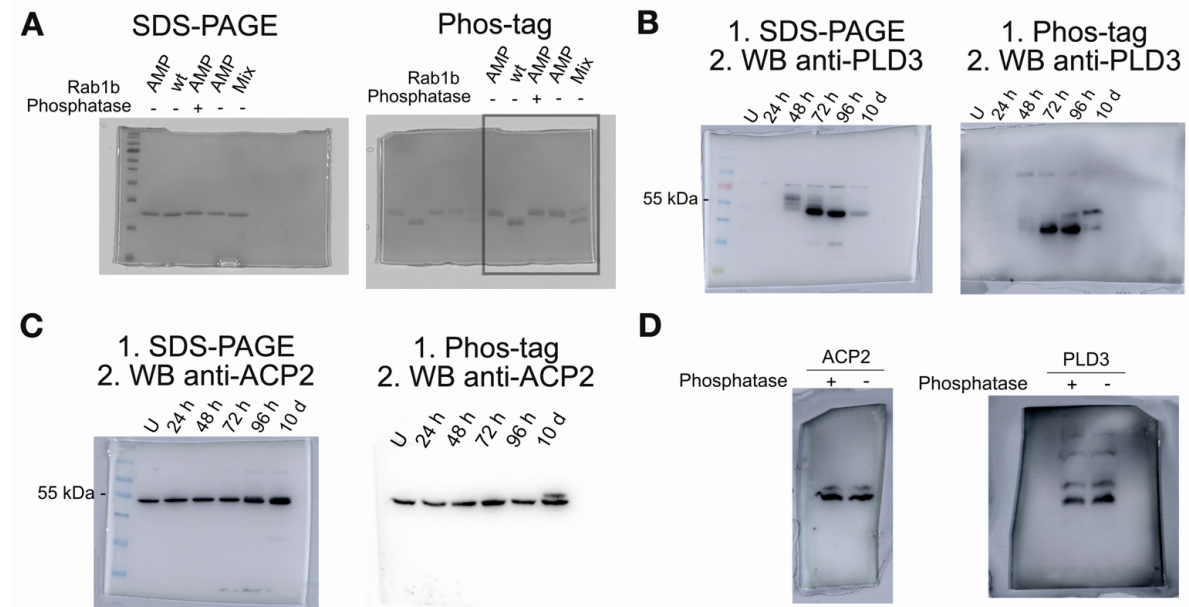


Supplemental Figure 5. Dephosphorylation of Ovalbumin. Related to **Figure 3**. **A)** Phos-tag gel separation of Ovalbumin with and without the phosphatase treatment, Coomassie stain. **B)** Phos-tag

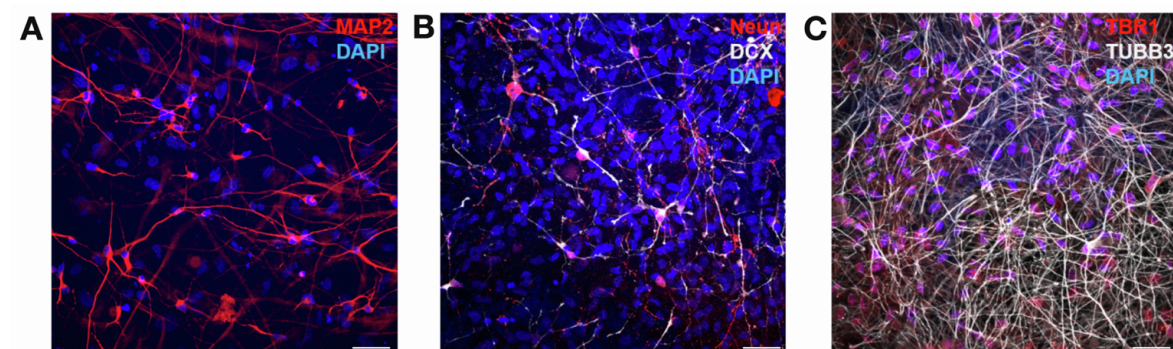
gel separation of Ovalbumin with and without the phosphatase treatment, 5 ng Ovalbumin added to the lysis buffer. Staining using anti-Ovalbumin antibody.



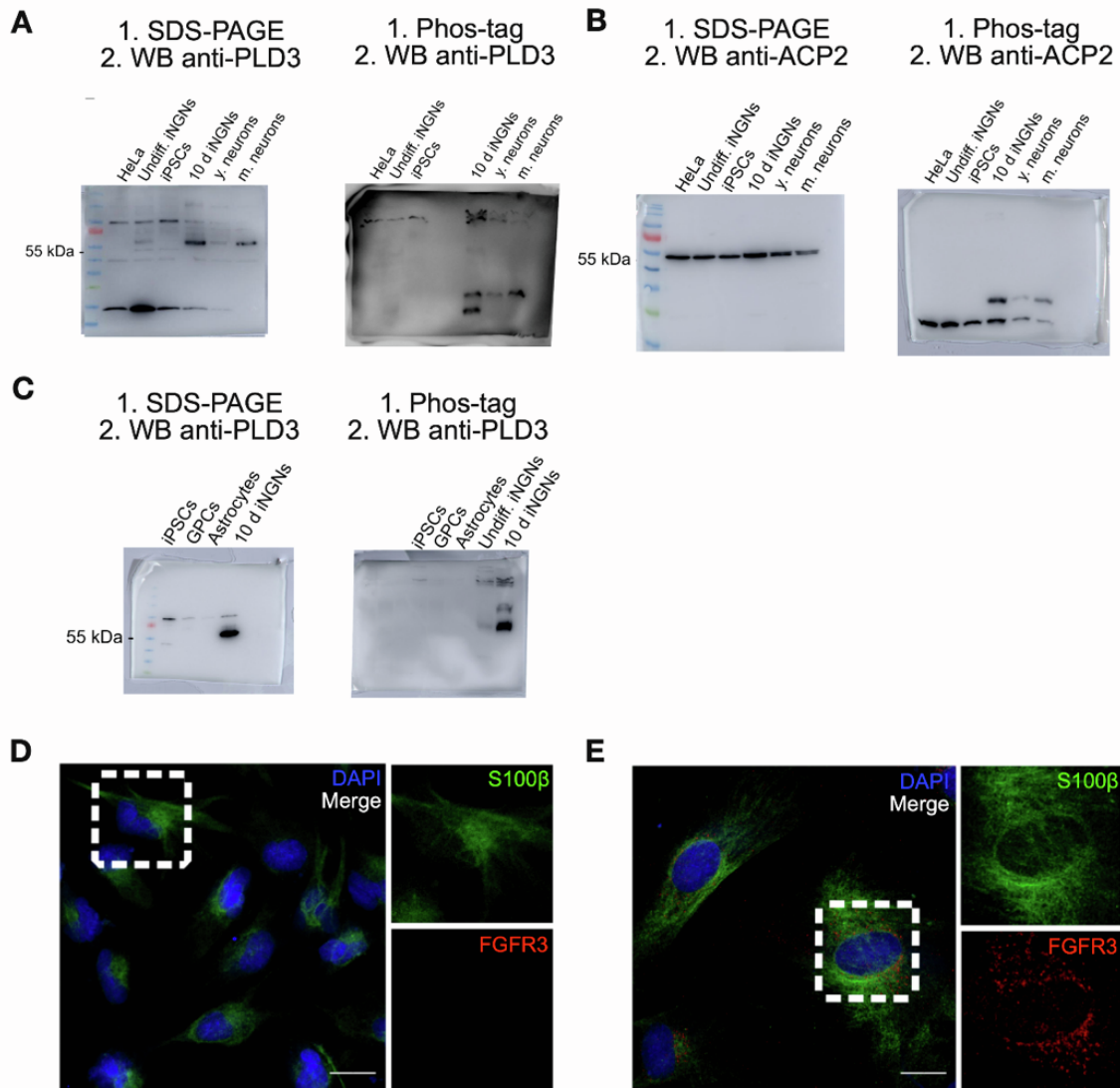
Supplemental Figure 6. HSPA5 separation. Related to **Figure 3**. All lines are from HeLa lysates.



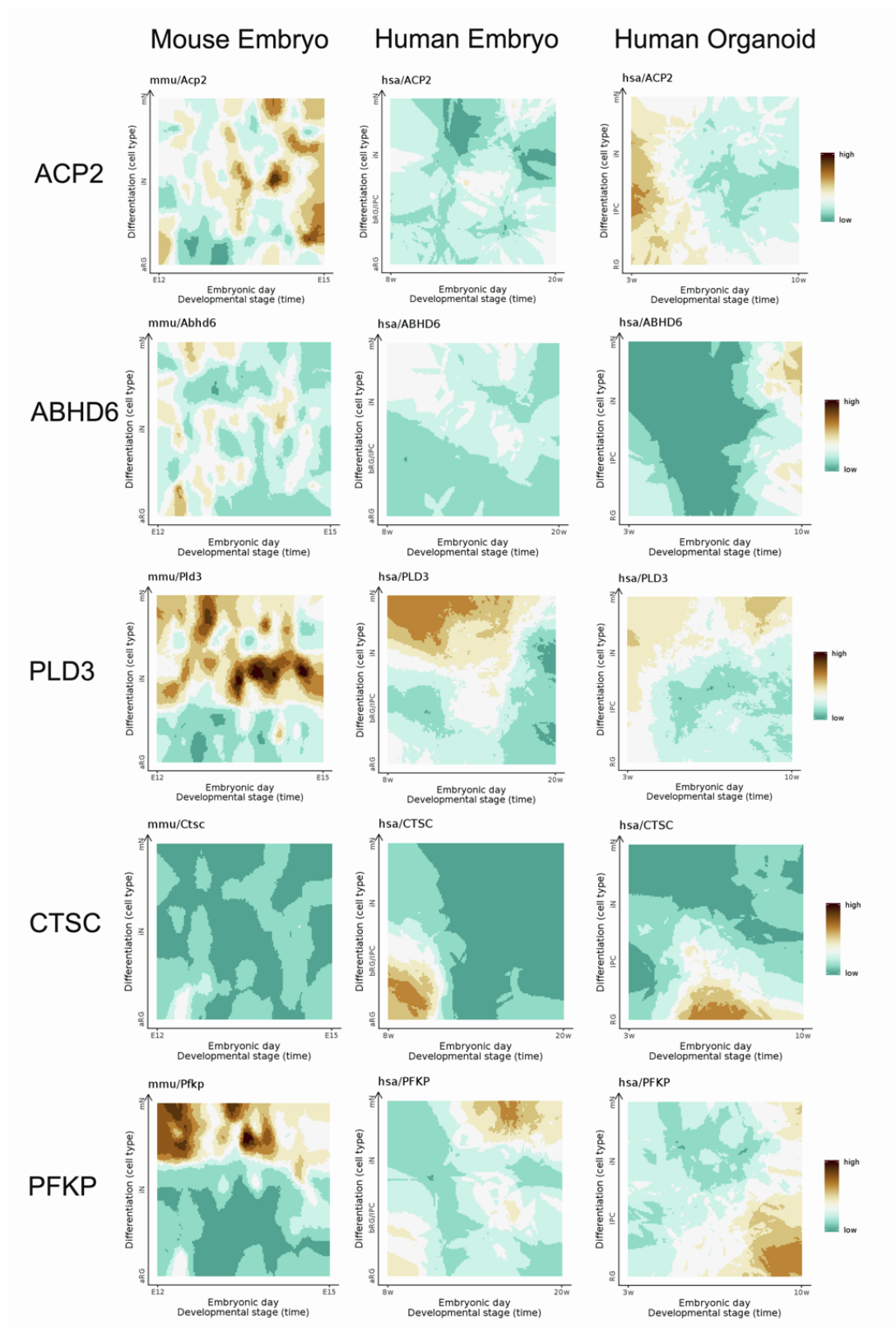
Supplemental Figure 7. Uncut SDS-PAGE and Phos-tag gels used in **Figure 3**. **A)** Coomassie stained SDS-PAGE and Phos-tag gel separation of AMPylated and non-modified Rab1b. **B)** Western blots of the SDS-PAGE and Phos-tag gel separation of PLD3 during differentiation course of iNGN cells. **C)** Western blots of the SDS-PAGE and Phos-tag gel separation of ACP2 during differentiation course of iNGN cells. **D)** Western blots of the SDS-PAGE and Phos-tag gel separation of PLD3 and ACP2 in 10 days differentiated iNGN cells treated with shrimp alkaline phosphatase.



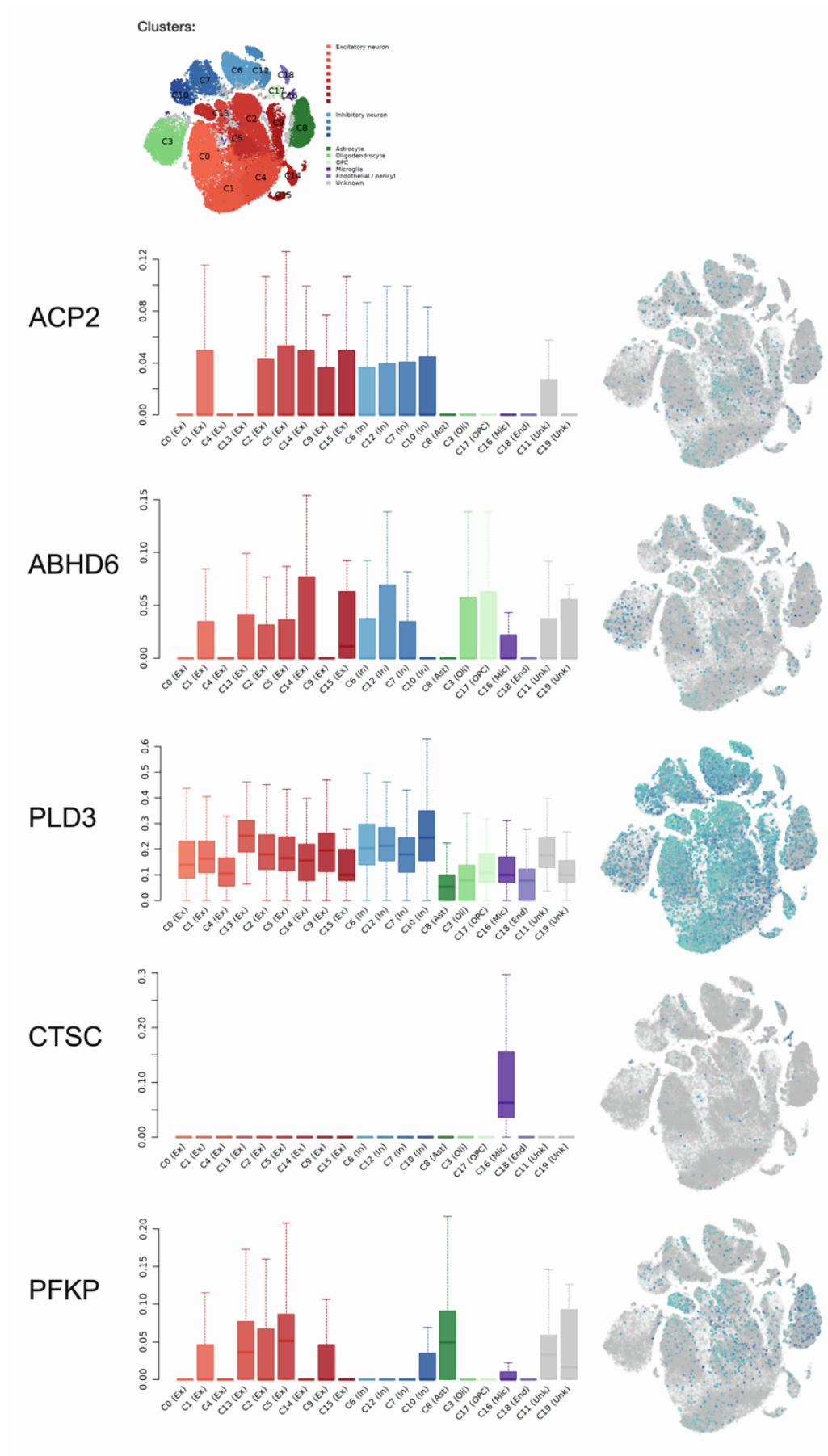
Supplemental Figure 8. Characterization of differentiation and maturation of physiological neurons. Related to **Figure 4**. **A - C)** Micrographs of 2D neuronal cultures after 10 weeks of differentiation. Cells were immunostained for **A)** MAP2, **B)** Neun, Doublecortin (DCX), **C)** TBR1 and TUBB3. Nuclei (blue) are stained with DAPI. Scale bars: 50 μ m.



Supplemental Figure 9. Uncut SDS-PAGE and Phos-tag gels used in **Figure 4**. **A)** Western blots of the SDS-PAGE and Phos-tag gel separation of PLD3 comparing HeLa, iNGNs and dopaminergic neurons. **B)** Western blots of the SDS-PAGE and Phos-tag gel separation of ACP2 comparing HeLa, iNGNs and dopaminergic neurons. **D)** and **E)** Characterization of astrocytes differentiation. Nuclei (blue) are stained with DAPI. Scale bars: 20 μ m. **D)** GPCs. **E)** Astrocytes.



Supplemental Figure 10. Protein expression changes during embryo development. Related to discussion.



Supplemental Figure 11. Analysis of protein expression levels in various cell types. Related to discussion.