REDUNDANT AND EXCLUSIVE ROLES OF CBP AND P300 IN NEURAL PROGENITORS PROLIFERATION AND DIFFERENTIATION

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KAT3 family members CBP and p300 are transcriptional co-activators that play important roles in the maintenance of neuronal identity in the adult brain. These proteins are also essential for neural development and it is known that their hemi-deficiency in humans causes Rubinstein-Taybi syndrome (RSTS). Although the function of these proteins has been extensively studied, the specific events downstream of CBP and p300 deficiencies responsible for neurodevelopmental deficits in RSTS patients remain obscure. For instance, it is still unclear whether these proteins play redundant or specific roles during developmental processes, such as proliferation of neural precursors, differentiation to different neural cell types or both. Here, through the analysis of neurospheres lacking CBP, p300 or both proteins we demonstrate that any of them is sufficient to maintain neural progenitor proliferation. However, the absence of either protein seriously compromises differentiation in both neuronal and astrocytic lineages. Single cell RNA sequencing (scRNA-seq) analysis of neural cell cultures derived from CBP or p300 mutants was consistent with this view and revealed that divergent trajectories of neural differentiation upon CBP or p300 ablation. These data contribute to a better understanding on the individual roles of KAT3 proteins in neural development and the etiopathology of RSTS opening new avenues for therapy.

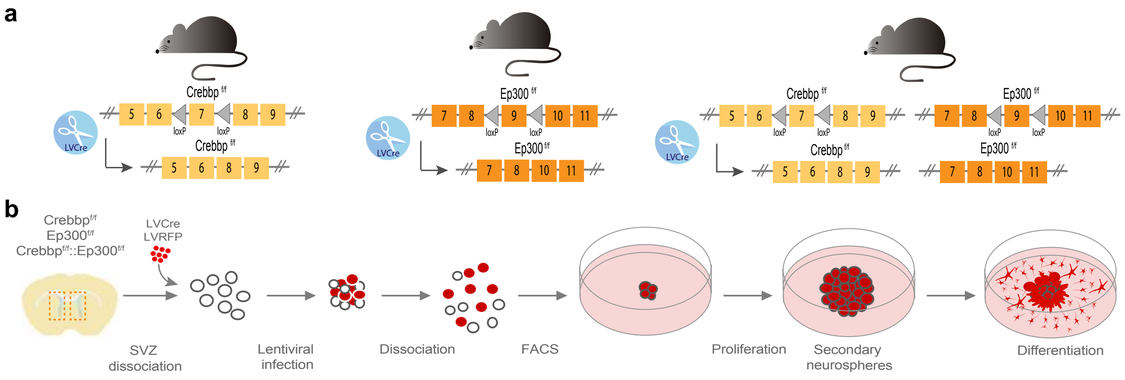
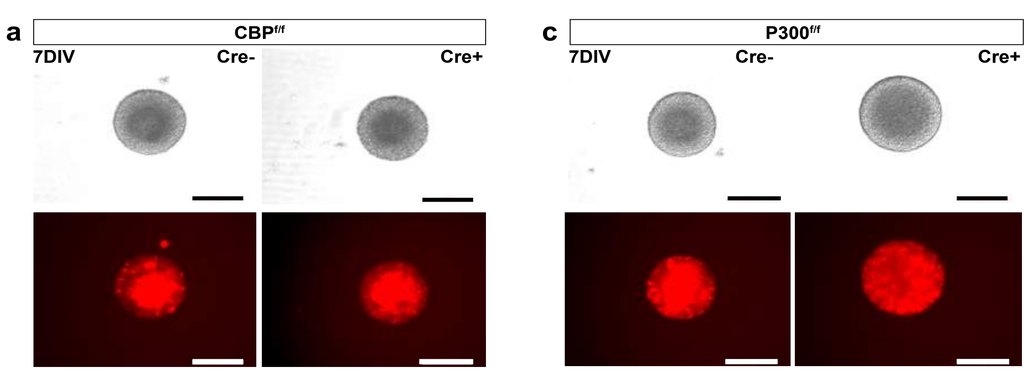


Figure 1. Analysis of the role of KAT3 proteins in neural stem cells (NSC). (**a**) Scheme representing the genetic strategy to precisely study the ablation of KAT3 proteins in the different steps of neural development. (**b**)Experimental design of the approach used to generate neurosphere cultures to investigate the particular role of KAT3 proteins in neural stem cells (NSCs).



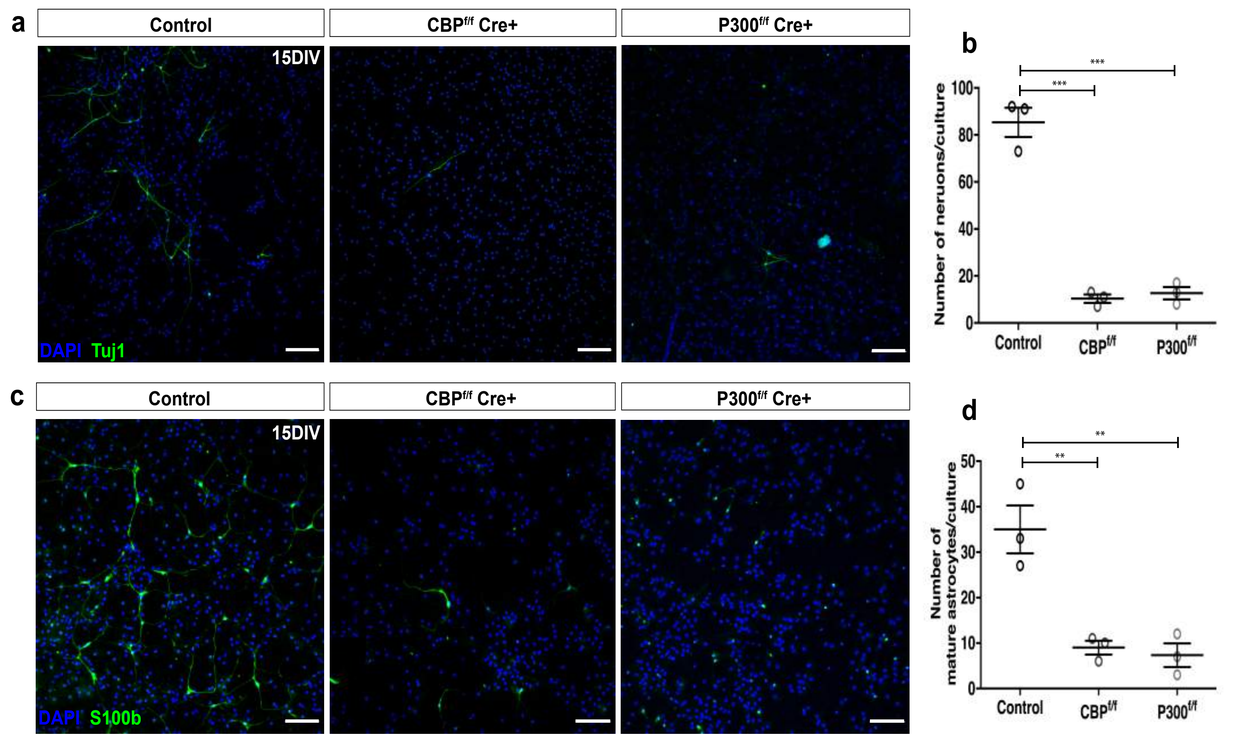


Figure 4. CBP and P300 are individually required for differentiation of NSCs into the three neural lineages. (a,c) Immunofluorescent staining for neurons (Tuj1, a) and mature astrocytes (S100ß, c) showed a dramatic reduction of these markers in differentiated CBP or P300 ablated neurospheres at 15DIV. (b,d) Quantification of neurons (b) and mature astrocytes (d) in neurospheres lacking CBP or P300 after 15DIV of differentiation. **P<0.01; ***p < 0.001 (n=3 independent cultures). Statistic: One-way ANOVA. Error bars denote SEM. Scale bars 100µm.

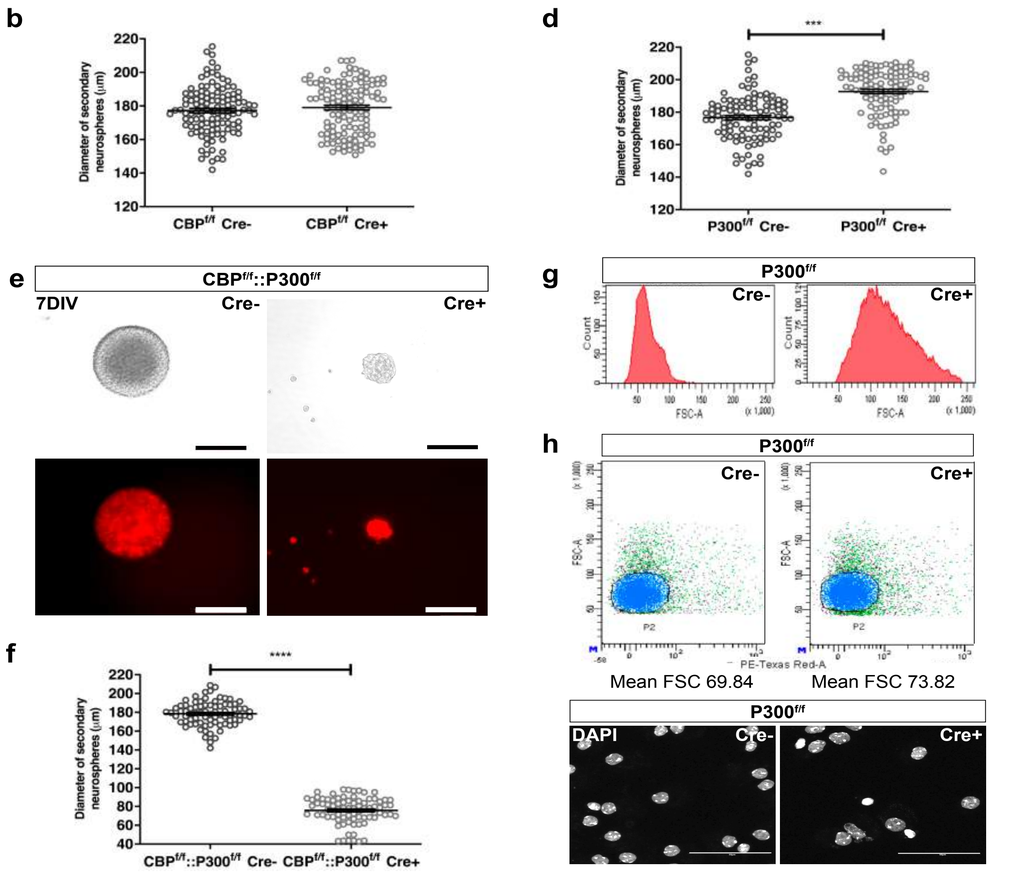


Figure 2. Individual ablation of CBP or P300 does not affect proliferation of NSC. (**a,c,e**) Representative images of infected clonal secondary neurospheres lacking CBP (**a**), P300 (**c**) and both KAT3 proteins (**e**) at 7DIV. **(b,d,f)** Quantitative analysis of the diameter of clonal secondary neurospheres in the absence of CBP (**b**), P300 (**d**) or both proteins (**f**) at 7DIV. ***p < 0.001; ****p<0.0001 (n≈120 neurospheres from four independent experiments). Statistic: t-test (b,f), Mann-Whitney (d). (**g**) Flow cytometry sorting plots for infected cells from controls and P300 ablated NSCs showing counts against forward scatter (FSC) (**h)** (top) Fluorescent-activated sorting analysis plots showing forward scatter (FSC) against red fluorescence of nuclei from controls and P300 ablated NSC. (bottom) High magnification of nuclei counterstained against DAPI of control and P300 ablated NSC. Error bars denote SEM. Scale bar 200µm (a,c,e) and 50µm (g).

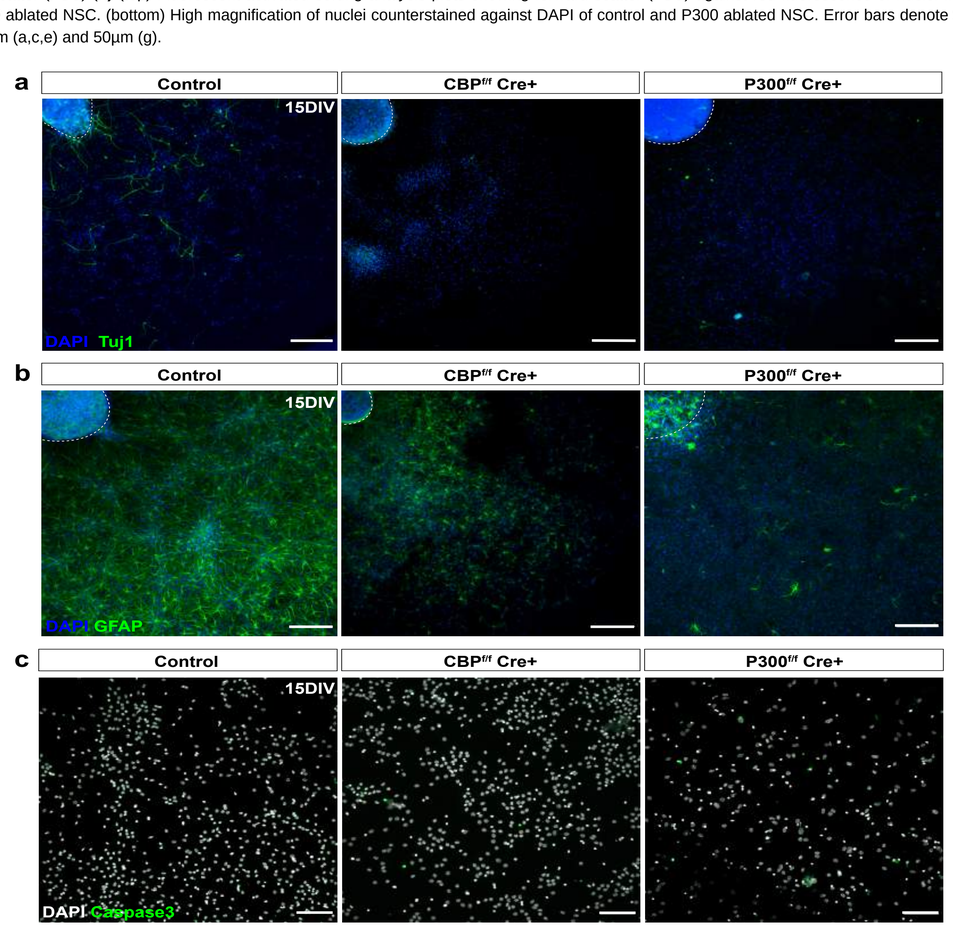


Figure 3. CBP and P300 lacking neurospheres showed alterations in neural differentiation programs whereas cell death is not altered. (a,b) Low magnification of differentiated neurospheres lacking CBP or P300 at 15DIV exhibit alterations in neuronal differentiation (Tuj1 positive cells) (**a**) and glial differentiation (GFAP positive cells) (**b**) compared to differentiated control neurospheres. (**c**) Immunostaining against Caspase 3 showed no differences in cell death after differentiation of NSCs lacking CBP or P300 compared to control NSCs. Scale bars 200µm (a,b) and 100 µm (c).

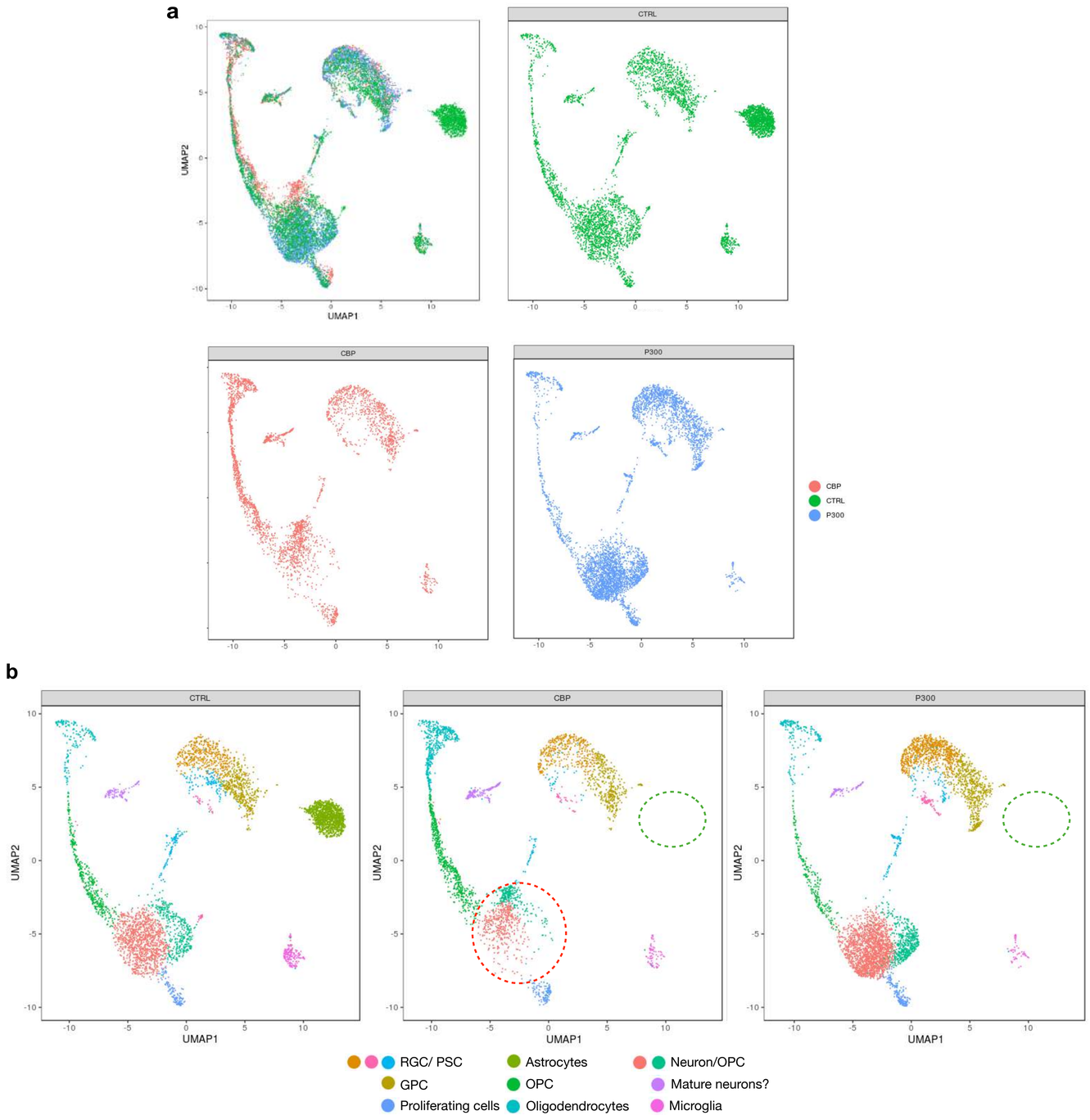


Figure 5. Single-nucleus RNA sequencing analysis of differentiated neurospheres revealed alterations in neural populations after removal of CBP or P300. (**a**) UMAP plot showing the integration of single-cell RNA-seq datasets from differentiated neurospheres at 15DIV. Each dot represent a single nucleus. Nuclei are colored by experimental condition: Control (green) 4884 nuclei, CBP ablated cells (red) 3017 nuclei and P300 ablated cells (blue) 4648 nuclei. (**b**) UMAP plots displaying differential clustering on differentiated neurospheres of control, CBP or P300 ablated nuclei. Nuclei are colored by their classification label as shown in panel. Astrocytic cluster (green dotted line) is nos detected in CBP or P300 ablated nuclei. Neuron-OPC population is reduced in CBP ablated nuclei (red dotted line). RGC: radial glia, PSC: pluripotent stem cells, OPC: oligodendrocytes progenitor cells, GPC: glial progenitor cells.

CONCLUSIONS

- CBP and P300 are essential for neural differentiation but dispensable for proliferation
- Astrocytic population dissapear in the absence of one of the two KAT3 protein
- Neuron-OPC subpopulation is reduced in CBP ablated nuclei