

Supplementary information

Modulated DISP3/PTCHD2 expression influences neural stem cell fate decisions

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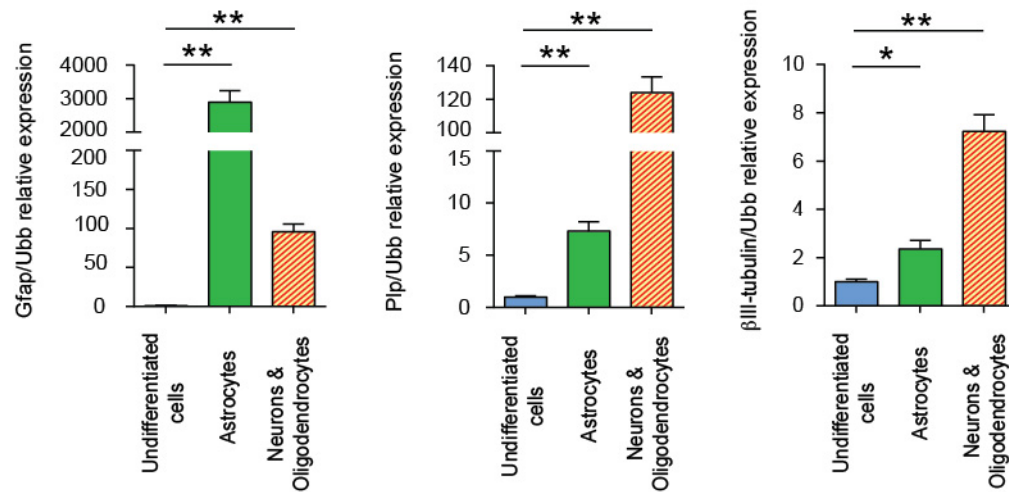
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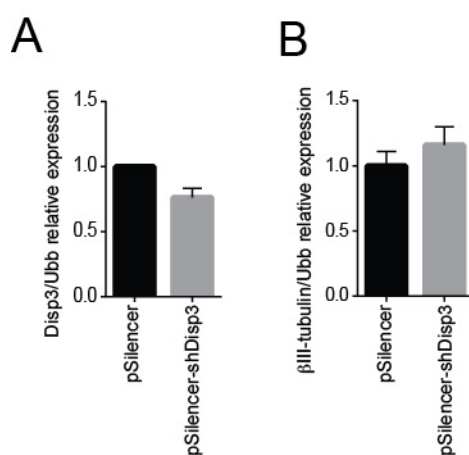
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Supplementary information



Supplementary Figure 1: Quantitative RT-PCR analysis of *Gfap*, *Plp* and β III-tubulin mRNA expression in undifferentiated and differentiated NS-5 cells. *Ubb* was used as a reference gene. Bars represent the mean of three independent samples with error bars indicating standard deviation and the level of statistical significance (* $P < 0.05$, ** $P < 0.01$). Mean values - *Gfap* relative expression: undifferentiated cells=1.00, astrocytes=2900, neurons and oligodendrocytes=96; - *Plp* relative expression: undifferentiated cells=1.00, astrocytes=7.30, neurons and oligodendrocytes=124; - β III-tubulin relative expression: undifferentiated cells=1.00, astrocytes=2.36, neurons and oligodendrocytes=7.23.

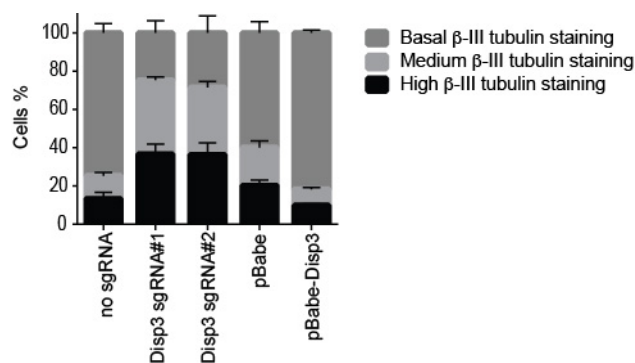
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Supplementary Figure 2: Downregulation of DISP3 by shRNA leads to altered expression levels of β III-tubulin.

(A) qRT-PCR analysis of *Disp3* expression in control (pSilencer) and shDisp3-treated NS-5 cells. *Ubb* was used as a reference gene. Bars represent the mean of three biological replicates with error bars indicating standard deviation. Mean values: pSilencer=1.00, pSilencer-shDisp3=0.76.

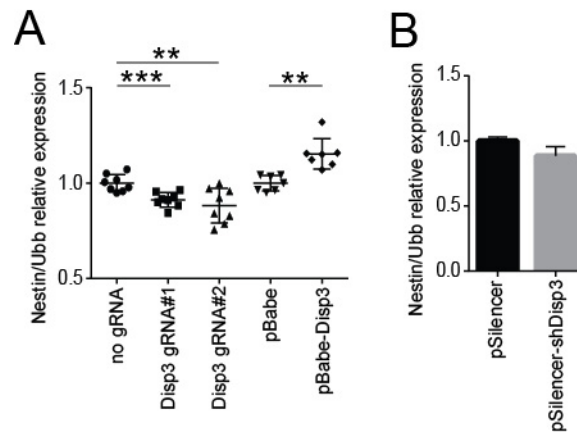
(B) qRT-PCR analysis of β III-tubulin mRNA expression in control (pSilencer) and shDisp3-treated NS-5 cells. *Ubb* was used as a reference gene. Bars represent the mean of three biological replicates with error bars indicating standard deviation. Mean values: pSilencer=1.00, pSilencer-shDisp3=1.16.



Supplementary Figure 3: Distribution of undifferentiated NS-5 cells based on β -III tubulin staining.

β III-tubulin staining of undifferentiated NS-5 cells quantified by the Operetta High-Content Imaging System followed by Columbus software analysis. According to the intensity of β III-tubulin staining, cells were divided into three groups: basal, medium and high intensity of staining. Data represent the percentage of cells in each particular group and the mean of biological replicates with error bars indicating standard deviation. Mean values - high β III-tubulin staining: no sgRNA=13.47, Disp3 sgRNA#1=36.76, Disp3 sgRNA#2=36.40, pBabe=20.29, pBabe-Disp3=9.91; - medium β III-tubulin staining: no sgRNA=11.92, Disp3 sgRNA#1=38.56, Disp3 sgRNA#2=35.18, pBabe=19.93, pBabe-Disp3=8.50; - basal β III-tubulin staining: no sgRNA=74.61, Disp3 sgRNA#1=24.68, Disp3 sgRNA#2=28.42, pBabe=59.78, pBabe-Disp3=81.59.

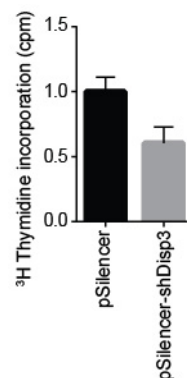
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Supplementary Figure 4: Altered expression of nestin in Disp3 sgRNA (pSilencer-shDisp3) and pBabe-Disp3 cells.

(A) qRT-PCR analysis of nestin mRNA expression in Disp3 sgRNA, pBabe-Disp3 and control cells. *Ubb* was used as a reference gene. Data represent the mean of biological replicates with error bars indicating standard deviation and the level of statistical significance (** $P < 0.01$, *** $P < 0.001$). Mean values: no sgRNA=1.00, Disp3 sgRNA#1=0.91, Disp3 sgRNA#2=0.88, pBabe=1.00, pBabe-Disp3=1.15.

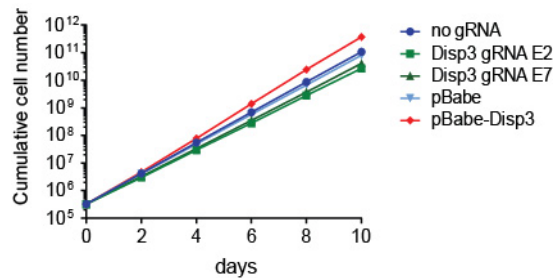
(B) qRT-PCR analysis of nestin mRNA expression in control (pSilencer) and shDisp3-treated NS-5 cells. *Ubb* was used as a reference gene. Data represent the mean of three biological replicates with error bars indicating standard deviation. Mean values: pSilencer=1, pSilencer-shDisp3=0.88.



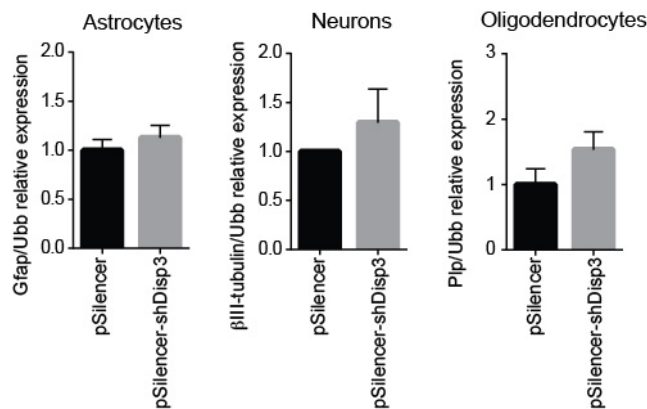
Supplementary Figure 5: Downregulation of DISP3 expression levels by shRNA leads to reduced NS-5 cell proliferation.

Proliferation of control (pSilencer) and shDisp3-treated NS-5 cells was measured by a ³H-thymidine incorporation assay. Bars represent the mean of three biological replicates with error bars indicating standard deviation. Mean values: pSilencer=1.00, pSilencer-shDisp3=0.60.

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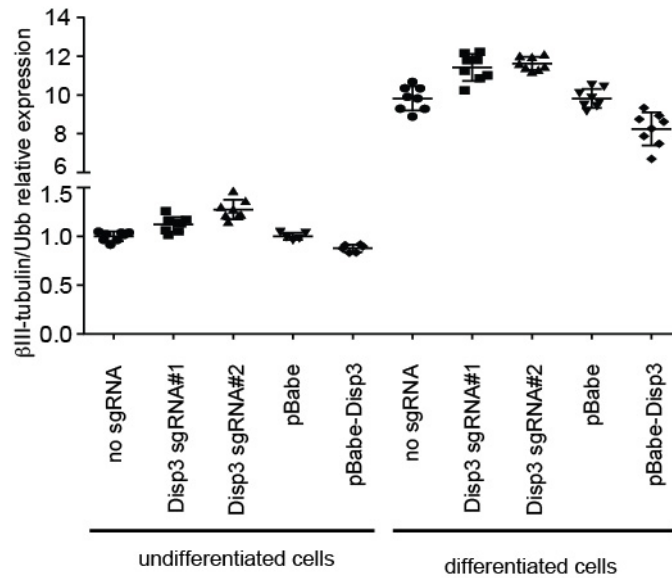
Supplementary Figure 6: Cell proliferation is altered in Disp3 sgRNA and pBabe-Disp3 NS-5 cells. Disp3 sgRNA, pBabe-Disp3 and control cells were counted every other day. The growth rate was plotted as cumulative cell numbers.



Supplementary Figure 7: Downregulation of DISP3 expression by shRNA promotes differentiation of NS-5 cells.

qRT-PCR analysis of *Gfap*, β III-tubulin and *Plp* mRNA expression in control (pSilencer) and shDisp3 cells at days 4 (astrocytes) and 9 (neurons and oligodendrocytes) of differentiation. *Ubb* was used as a reference gene. Bars represent the mean of three biological replicates with error bars indicating standard deviation. Mean values - *Gfap* relative expression: pSilencer=1.00, pSilencer-shDisp3=1.13; - β III-tubulin relative expression: pSilencer=1.00, pSilencer-shDisp3=1.30; - *Plp* relative expression: pSilencer=1.00, pSilencer-shDisp3=1.54.

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Supplementary Figure 8: Changes in DISP3 expression levels affect β III-tubulin expression under both growth/proliferation and differentiation conditions. β III-tubulin expression was quantified by qRT-PCR analysis (*Ubb* was used as a reference gene). Data represent the mean of biological replicates with error bars indicating standard deviation. Mean values - undifferentiated cells: no sgRNA=1.00, Disp3 sgRNA#1=1.12, Disp3 sgRNA#2=1.27, pBabe=1.00, pBabe-Disp3=0.88; - differentiated cells: no sgRNA=9.82, Disp3 sgRNA#1=11.42, Disp3 sgRNA#2=11.63, pBabe=9.82, pBabe-Disp3=8.24.