

Supplementary Figure 5

Supplementary Figure 5. DLK1 induces self-renewal independently of Notch activation (a) Fold increase in primary neurosphere number in wild-type or  $Dlk1^{-/-}$  NSCs cultures treated with DLK1 (50 ng/ml), PEDF (40 ng/ml) or Jagged1 (1 µg/ml) relative to untreated wild-type NSCs (indicated with a dashed line). (b) Immunocytochemistry for Notch intracellular domain (NICD) in NSCs after DLK1 treatment. DLK1 does not modify NICD localization in NSCs. (c) Activity quantification of an artificial CBF1-dependent reporter that contains consensus CBF1 sequences (4xwtCBF1-luc), directly targeted by NICD, in the absence or presence of DLK1. PEDF was used as a control of activation. The effects of PEDF or DLK1 on CBF1-mediated transcription after Notch signaling (NotchIC overexpression) are shown. No changes in luciferase activity were observed after DLK1 treatment. (d) Quantitative PCR of primary neurospheres untreated or treated with DLK1 for 48h. No differences in expression of *Notch1* and *Notch3* or their targets *Hes1* and *Hes5* were observed. \*p<0.05, \*\*p<0.01. Error bars, s.e.m of three independent experiments with three cultures per genotype. Scale bars: in b, 10 µm.