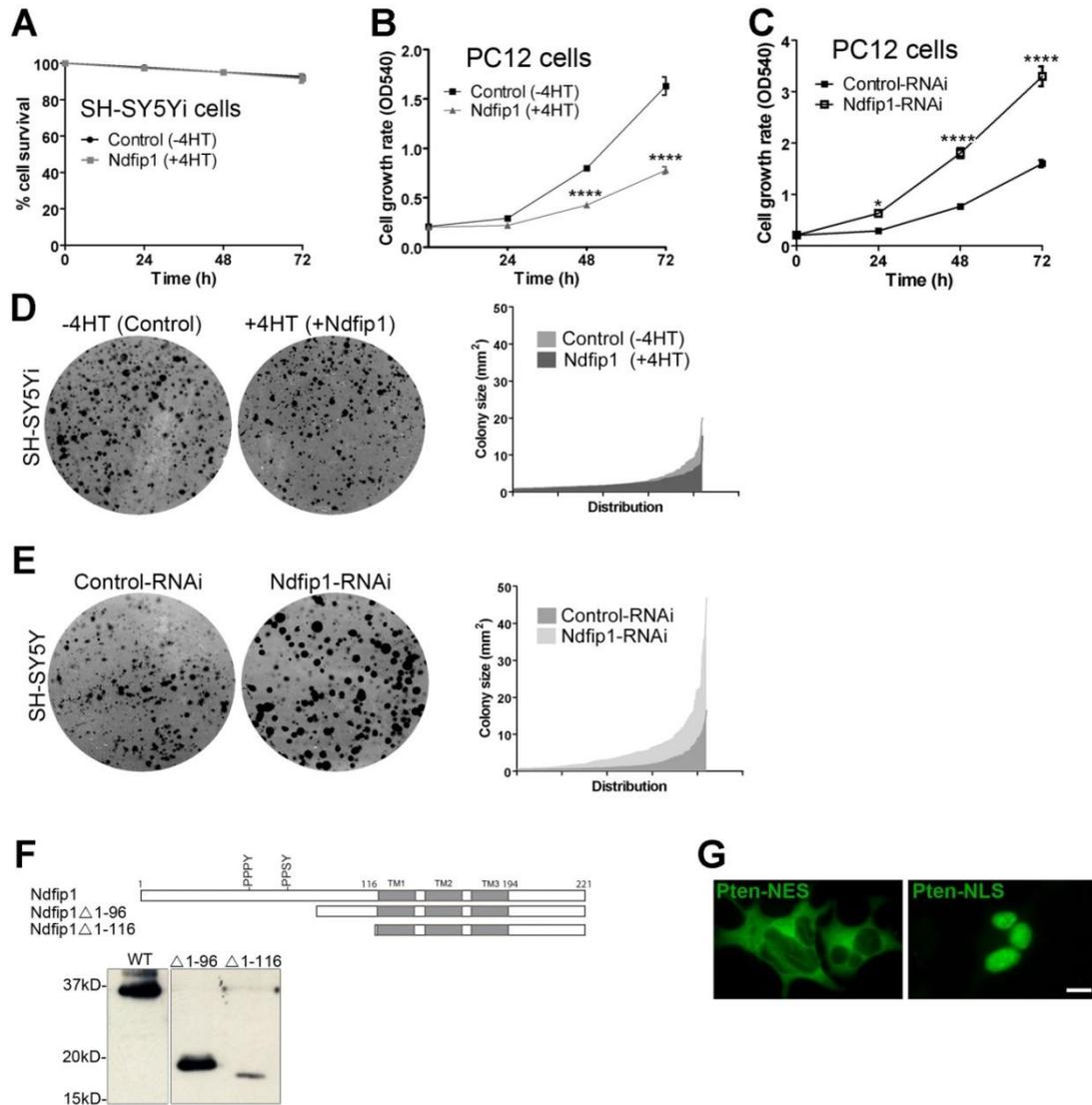


Supplementary material

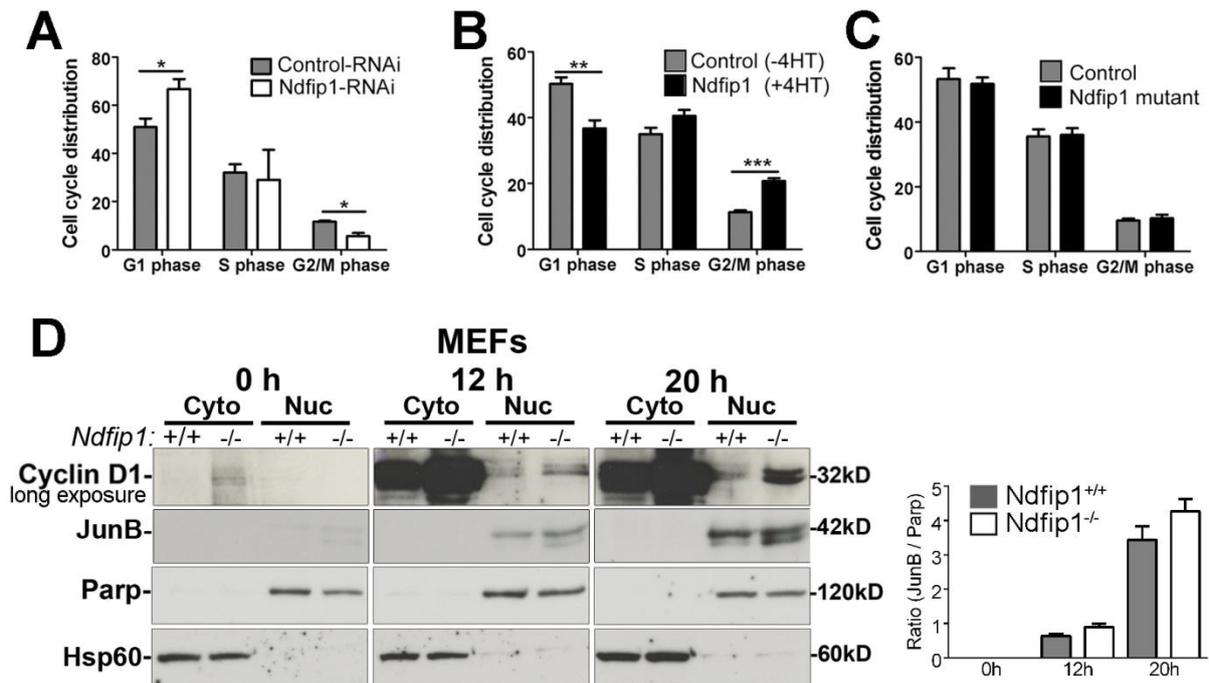
Ndfip1 represses cell proliferation by controlling Pten localization and signaling specificity

Jason Howitt^{1*}, Ley-Hian Low¹, Ulrich Putz¹, Anh Doan¹, Jenny Lackovic¹, Choo-Peng Goh¹, Jenny Gunnensen², John Silke³, Seong-Seng Tan^{1*}.

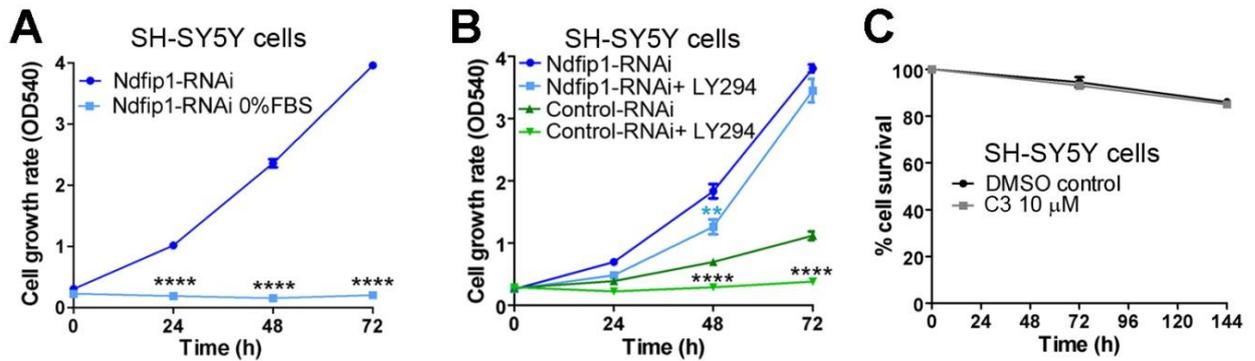
Supplementary Figures S1-S4



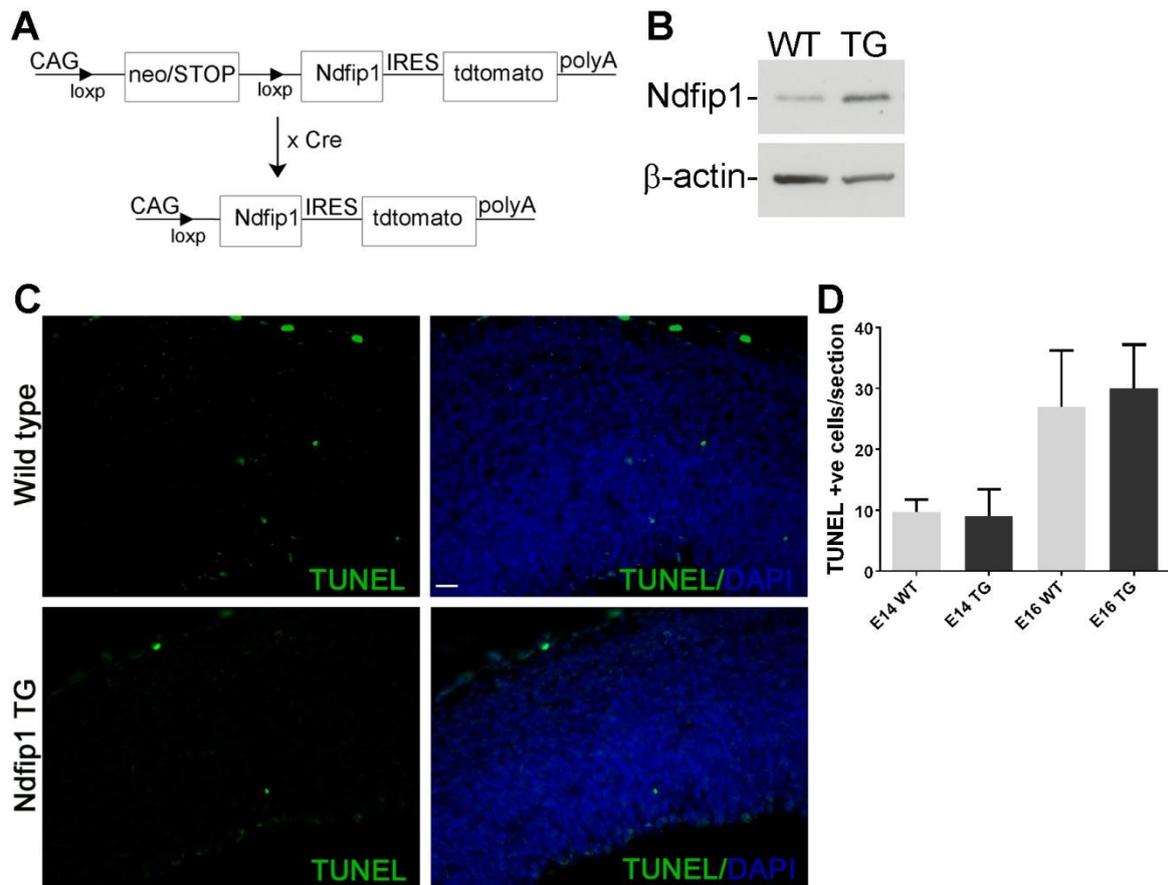
Supplementary Figure S1. Ndfip1 abundance alters the proliferation of cells. (A) To rule out the effects of cell death on decreased cell proliferation observed in Ndfip1 expressing cells (SH-SY5Yi +4HT), propidium iodide staining was performed followed by flow cytometric analysis. Induced expression of Ndfip1 (+4HT) does not result in increased cell death of SH-SY5Yi cells. (B) PC12 cells induced to express Ndfip1 (+4HT) proliferate slower compared to control cells (-4HT) in MTT assays. (C) Knockdown of Ndfip1 (Ndfip1-RNAi) in PC12 cells resulted in increased cell proliferation compared to control-RNAi cells in MTT assays. (D) In colony forming assays expression of Ndfip1 (+4HT) in SH-SY5Yi cells resulted in smaller colonies compared to control cells (-4HT). The distribution of cell size is shown in the histogram. (E) In colony forming assays loss of Ndfip1 (Ndfip1-RNAi) in SH-SY5Y cells resulted in increased colony size compared to control-RNAi cells. The distribution of colony size is shown in the histogram. (F) Schematic and expression of Ndfip1-Strep-Flag tagged mutants used in Figure 2G. Both mutants remove the PPxY motif known to interact with Nedd4 ubiquitin ligases (G) Localisation of Pten-NES and Pten-NLS in MEFs used in Figure 2H and I. Scale bar = 10 μ m. Data points are the average \pm SEM of three independent experiments. p values (A-C) were determined using two-way ANOVA with Bonferroni post-tests.



Supplementary Figure S2. Ndfip1 abundance alters the G2/M phase of the cell cycle. (A) The distribution of SH-SY5Y cell populations in G1, S and G2/M phases of the cell cycle were analysed by flow cytometry. Loss of Ndfip1 (Ndfip1-RNAi) in SH-SY5Y cells resulted in a significant increase in the G1 population and significant decrease in the G2/M population compared to control-RNAi SH-SY5Y cells. **(B)** Flow cytometric analysis of SH-SY5Y cell cycle. Cells expressing Ndfip1 (+4HT) have significantly less cells in the G1 population and significantly increased cells in G2/M population compared to controls. **(C)** No significant change in the population of cells in G1, S or G2/M phase was observed when an Ndfip1 mutant (PPxY motifs mutated to PPxA) that prevents binding to Nedd4 ubiquitin ligases was expressed. This suggests that Nedd4 ubiquitin ligases are required for the affects observed for Ndfip1 control of the cell cycle. **(D)** Western blots from Figure 3B showing a longer exposure for nuclear cyclin D1 and also the levels of JunB during the cell cycle. Quantification of JunB abundance in both *Ndfip1*^{+/+} and *Ndfip1*^{-/-} MEFs is shown. Data points are the average \pm SEM of three independent experiments. p values were determined using unpaired t-test.



Supplementary Figure S3. Proliferation and survival profiles of SH-SY5Y cells. (A) The proliferation of Ndfip1-RNAi SH-SY5Y cells was inhibited by total removal of FBS from growth media. (B) The proliferation of control-RNAi SH-SY5Y cells was significantly reduced when grown in the presence of the PI3K inhibitor LY294002 (20 μ M), in contrast the proliferation of Ndfip1-RNAi SH-SY5Y cells was maintained. (C) SH-SY5Y cells treated with the small molecule C3 do not show any significant increase in cell death compared to untreated cells. Data points are the average \pm SEM of three independent experiments. p values (A and B) were determined using two-way ANOVA with Bonferroni post-tests.



Supplementary Figure S4. Generation of Ndfip1 transgenic mice and cell death analysis during development. (A) Schematic for the generation of Ndfip1 transgenic mice. (B) Western blot of Ndfip1 abundance from the neocortex in Ndfip1 TG and wild type litter mate. (C) TUNEL labelling for cell death in the neocortex of Ndfip1 TG and wild type litter mate at E14.5 showed no increase in cell death in Ndfip1 TG embryos. (D) No significant difference in cell death in the developing cortex between wild type and Ndfip1-TG embryos was observed. Scale bar = 50 μ m.