# Supplemental Material

## Intermediate progenitors are increased by lengthening of cell cycle through calcium signalling and p53 expression in human Neural Progenitors

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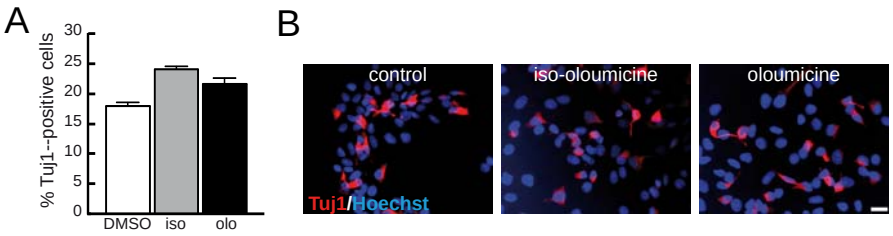
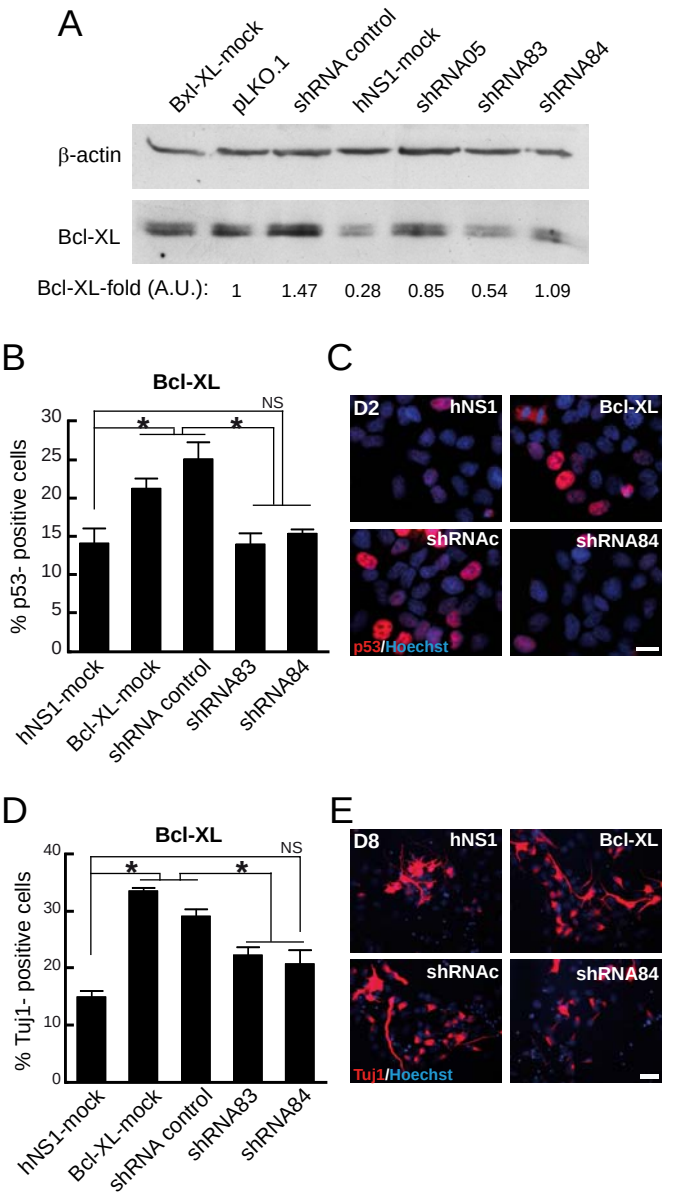
**Supplemental Material contains:**




**FigureS1**. Lengthening of cell cycle has no effect on neuron production on Bcl-XL cells. (A) Bcl-XL cells were treated with Oloumicine (15μM) and its inert form iso-oloumicine (15μM) on day1 of differentiation for 24 hours, cells were fixed on day 8 of differentiation and stained for Tuj1 (red) and Hoechst (blue). (B) Scale bar 25 μm. Data represent means±SEM, there is no significant differences ($p>0.05$, n=4, one-way ANOVA), two independent experiments were run.




**FigureS2**. Recovery of hNS1 phenotype after Bcl-XL trangene down regulation. Bcl-XL cells were infected with lentiviral particle transducing for different control vectors and shRNA against rat Bcl-XL at MOI 1. The infection efficiency was 95%. (A) After 48 hours of infection, cell lysates were collected, blotted and membranes were probed against Bcl-XL and β-actin. shRNA83 and 84 were used chosen for efficiently down regulating rat Bcl-XL(transgene RNA) but not human Bcl-XL (endogenous RNA). Densitometry data are expressed as the Bcl-XL normalised by β-actin ratio of each sample between Bcl-XL/β-actin ratio of pLKO.1 sample. AU, arbitrary units. (B) Bcl-XL cells were infected with indicated shRNAs at the same time of factor withdrawal. After 24 hours, cells were fixed and stained for p53 (red) and Hoescht (blue). (C) Representative IF of p53 and Hoescht in Bcl-XL cells after infection. Scale bar 10μm. (D) and (E) Bcl-XL cells were infected and allowed to differentiate for 8 days, cultures are fixed and stained for Tuj1 and Hoescht. Cultures infected with shRNA83 and shRNA84 have the same amount of neurons are hNS1 mock cells. Data represent means±SEM, *indicates significant difference, $p<0.05$ and NS stands for significant difference, $p>0.05$, n=4, one-way ANOVA followed by a Tuckey post hoc test. Scale bar 25μm.

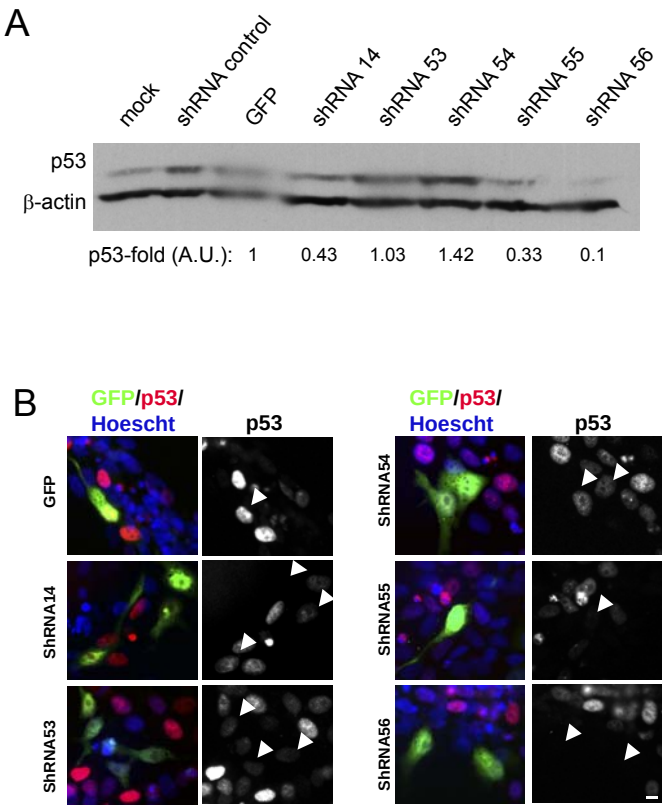
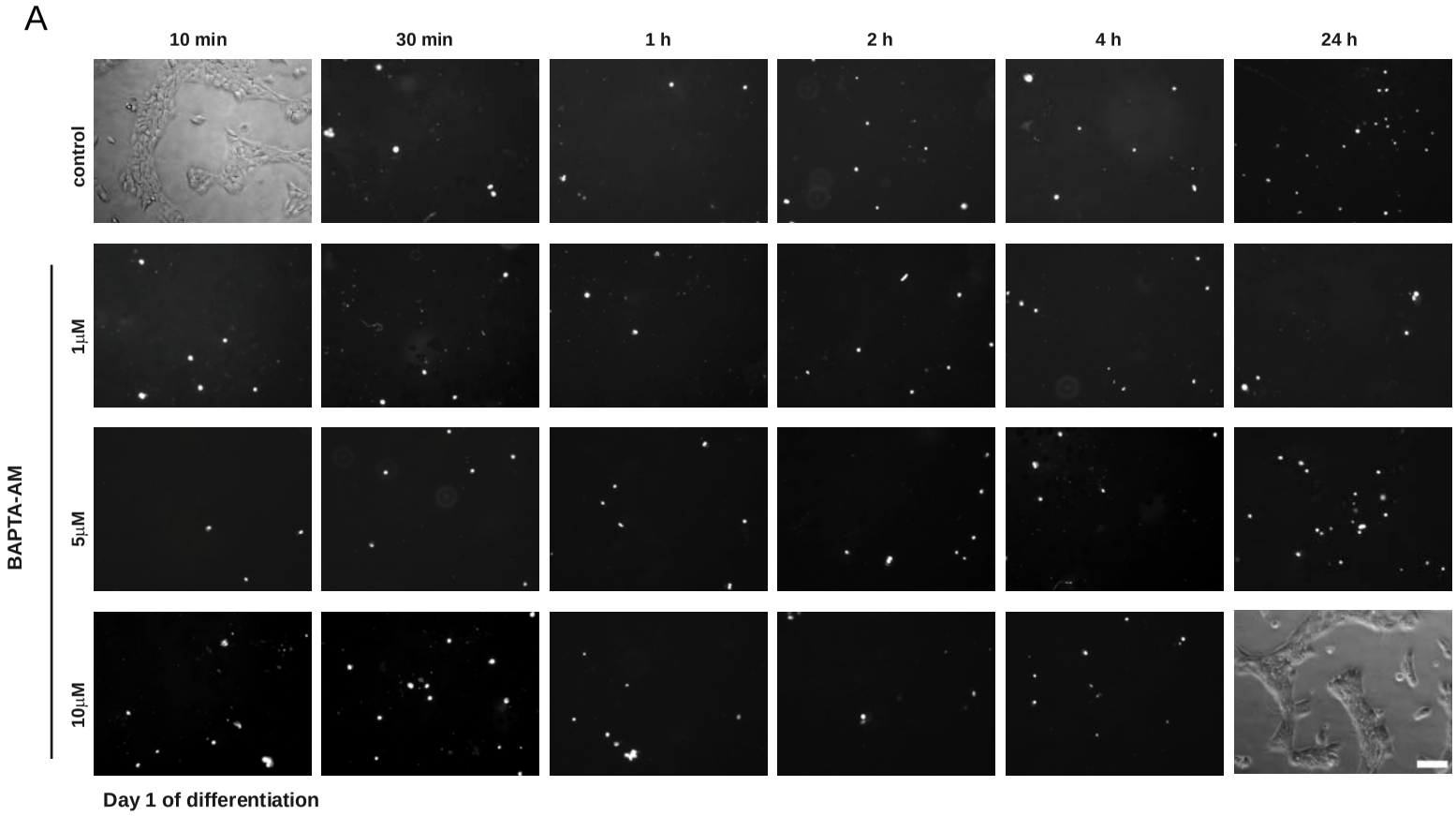
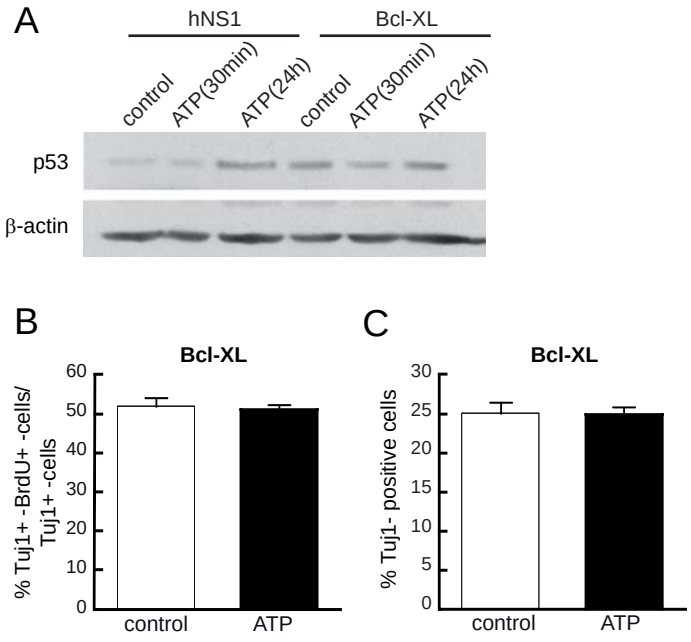



**Figure S3**. Lowering p53 protein levels by shRNA directed against p53 mRNA. Bcl-XL over-expressing cells were infected with lentiviral particles at MOI 2 immediately after growth factor withdrawal. (A) WB of cell lysates collected on day 2 of differentiation, 48 hours post infection. Membranes were probed with antibodies against p53 and β-actin. Densitometry data are expressed as the ratio to the value of cells transduced with control vector coding for GFP only. AU, arbitrary units. (B) Bcl-XL over-expressing cells were transfected with vectors expressing GFP and different shRNA or GFP alone, immediately after of growth factor withdrawal. Representative images show the decrease of p53 fluorescence in GFP cells transfected with the indicated shRNA vectors as compared with GFP control, 48 hours after transfection. Arrowheads denote p53 staining of transfected cells. Scale bar 10μm.




**FigureS4**. 10μM BAPTA-AM treatment for 24 hours is not toxic for hNS1 cells and is effective to low cytosolic calcium of Bcl-XL cells as the same level of hNS1 cells. (A) Bcl-XL cells were treated with different concentration of BAPTA-AM for 24 hours. Cells were stained with propidium Iodide at different time points to assay cell death in comparison to not treated cells. Scale bar 25μm. (B) Cytosolic calcium was measured with Fura-2 on day 2 of differentiation in hNS1 cells, Bcl-XL cells and Bcl-XL cells treated with 10μM of BAPTA-AM for 24 hours. Data represent means±SEM, * indicates significant differences ($p<0.05$, n=4, one-way ANOVA followed by Fisher post hoc), and NS means no significant differences, two independent experiment were performed.




**FigureS5**. ATP treatment does not have effect on p53, intermediate progenitors and neuron when supplied to Bcl-XL cells. Bcl-XL cells were treated with 250μM ATP on day1 of differentiation. Then cells were allowed to differentiate until: (A) day 2 of differentiation, then cell lysates were collected and WB was performed to detect p53 protein. As panel A shows only hNS1 cells increases the levels of p53 after 24 hours treatment. (B) day 8 of differentiation, previous 1mM BrdU pulse was supplied fr 24 hours, then cells were fixed and double Tuj1+-BrdU+ cells were quantified, and (C) day 8 of differentiation, where cells were fixed and stained for Tuj1 and Hoescht. Data represent means±SEM, Studen

## Supplemental Methods

### Infection and transfection of shRNA

Lentiviral particles, approximately with $4x10^5$ TU/ml for every shRNA vector, were used to infect at a multiple of infection (MOI) of: i) two particles per cell for shRNA interfering p53and ii) one for shRNA interfering human and rat Bcl-XL. 48 hours later cells are collected and lysate.

Transfection of hNS1 cells with and pLKO.1-CMV-TurboGFP based vectors was performed with Lipofectamine2000 24 hours after of seeding on OPTIMEM medium (Gibco) for 4 hours, then previous conditioned media was restored and in the next 24 hours, the growth factors were withdrawn. After 24 hours of growth factor withdrawal, the cultures were rinsed with 0.1M phosphate buffered saline (PBS) and fixed for 15 min in freshly prepared 4% PFA/PHEM/4% sucrose. Samples were blocked for 1h in 10% goat serum, 0.25% Triton X-100 in PBS and incubated overnight at 4ºC with mouse monoclonal antibodies p53 (DO1) (1:1000, Santa Cruz) dissolved in 1% goat serum, 0.25% Triton X-100 in PBS. On the next day, samples were incubated with: goat anti-mouse Cy3-conjugated (1:500, Jackson Immunoresearch). Cell nuclei were counterstained with Hoescht 33258 (Molecular Probes) at 0.2 mg/ml in PBS.

For WB, 20µg of protein was loaded into a 10-12% polyacrylamide gel, electrophoresed and transferred to a nitrocellulose membrane. Membranes were blocked with 5% skimmed milk, 0.05% Tween20 in 50mM Tris buffered saline (TBS). Then, membranes were incubated at 4ºC overnight with mouse p53 (DO1) (1:2000, Santa Cruz), rabbit Bcl-XL (Bcl-XL (1:500, Transduction Lab) and β-actin (1:5000, Sigma) antibodies in 1% skimmed milk, 0.05% Tween20 in 50mM TBS. Horse anti-mouse peroxidase (1:10000, Vector) secondary antibodies were incubated for 1 hour. The blots were developed using the ECL system (Amersham Biosciences)