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Supplemental Material to:

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The Down syndrome-related protein kinase DYRK1A phosphorylates p27Kip1 and Cyclin D1 and induces cell cycle exit and neuronal differentiation

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Supplemental material

Primer and conditions of quantitative real-time PCR

cDNA was synthesized from 1 µg of total RNA using oligo(dT) primers and MMLV reverse transcriptase (Promega) at 40°C for 1 h. The DNA SYBR Green I Master and a LightCycler® 480 (Roche) were used for pRT-PCR (5.5 µl SYBR Green I Master, 0.625 µM of each primer, 1 µl cDNA). Primers were ordered RP-HPLC purified. As standard all samples were mixed and a dilution series was generated (1:1, 1:2, 1:8, 1:32 and 1:128). Negative controls were performed with ddH_2O instead of template and with samples lacking reverse transcription. Expression data were normalized to GAPDH using the advanced relative quantification provided by the LightCycler® software 1.5 (Roche). All samples were analyzed as triplicates. Before data analysis, melting curves and the PCR efficiency were verified. PCR products were analyzed for correct amplicon size and unspecific products by agarose gel electrophoresis. Statistical analysis was performed using GraphPad Prism 5.0. The sequences of PCR primers and the PCR conditions are listed below:

qRT-PCR conditions

Target	Denaturation	Annealing	Elongation	Cycles	Product
DYRK1A	10 sec 95°C	10 sec 62°C	15 sec 72°C	45	173 bp
p27Kip1	10 sec 95°C	10 sec 60°C	10 sec 72°C	45	120 bp
TrkB	10 sec 95°C	10 sec 62°C	15 sec 72°C	45	203 bp
MAP2*	10 sec 95°C	10 sec 60°C	10 sec 72°C	45	233 bp
Tau*	10 sec 95°C	10 sec 60°C	10 sec 72°C	45	228 bp
GAPDH	10 sec 95°C	10 sec 66°C	15 sec 72°C	40	300 bp

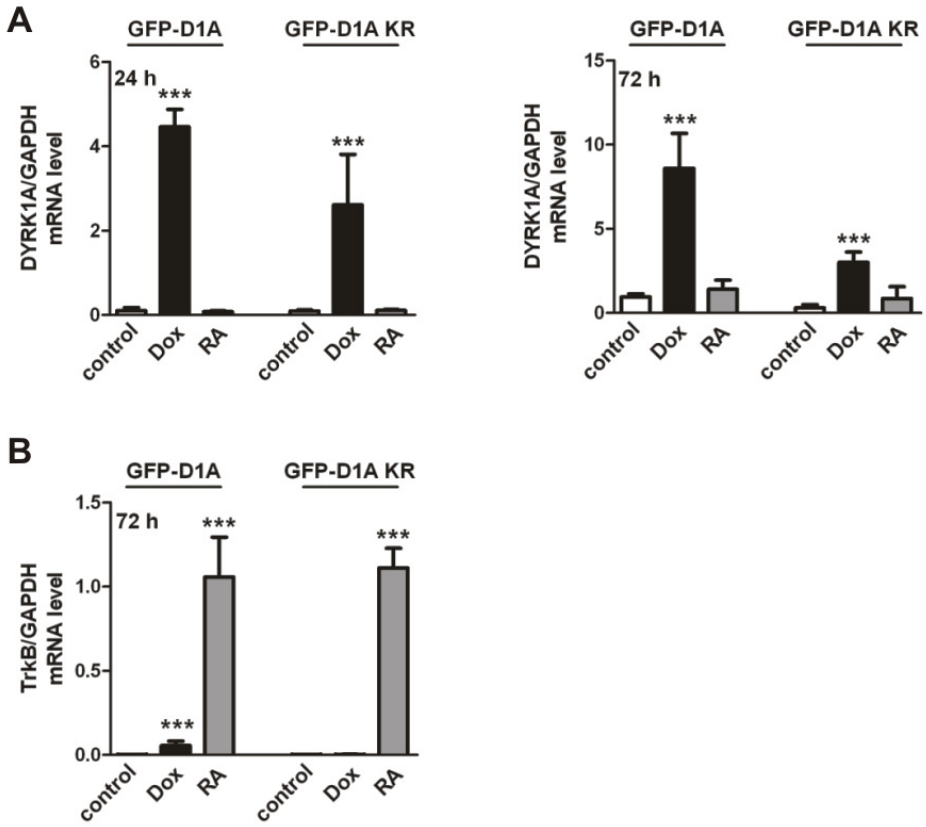
qRT-PCR primers

Target	forward primer (5´-3´)	reverse primer (5´-3´)
DYRK1A	TCTGGGTATTCCACCTGCTC	GTCCTCCTGTTTCCACTCCA
$p27^{Kip1}$	TCCGGCTAACTCTGAGGACA	GAAGAATCGTCGGTTGCAGG
TrkB	GGCCCAGATGCTGTCATTAT	TCCTGCTCAGGACAGAGGTT
*MAP2**	CATGGGTCACAGGGCACCTATTC	GGTGGAGAAGGAGGCAGATTAGCTG
*Tau**	GCGGCAGTGTGCAAATAGTCTACAA	GGAAGGTCAGCTTGTGGGTTTCAAT
GAPDH	CGGGGCTCTCCAGAACATCATCC	CCAGCCCCAGCGTCAAAGGTG

* Primer design and qRT-PCR condition according to Constantinescu et al. (2007)

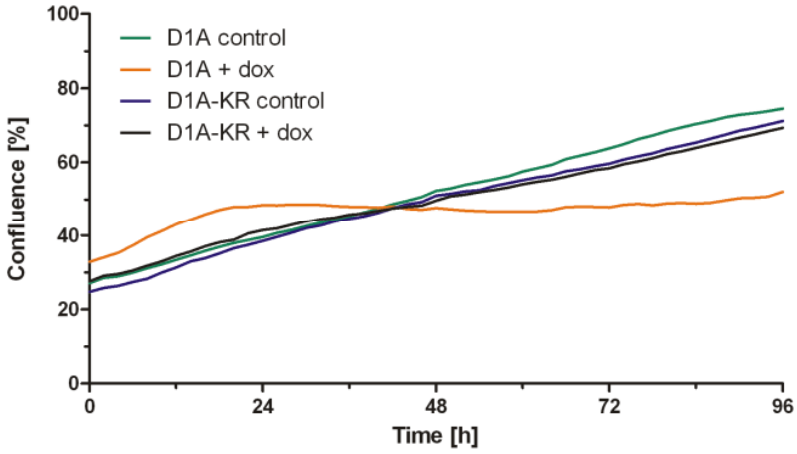
Constantinescu R, Constantinescu AT, Reichmann H, Janetzky B. Neuronal differentiation and long-term culture of the human neuroblastoma line SH-SY5Y. J Neural Transm Suppl 2007; 72:17–28.

Supplemental figure 1



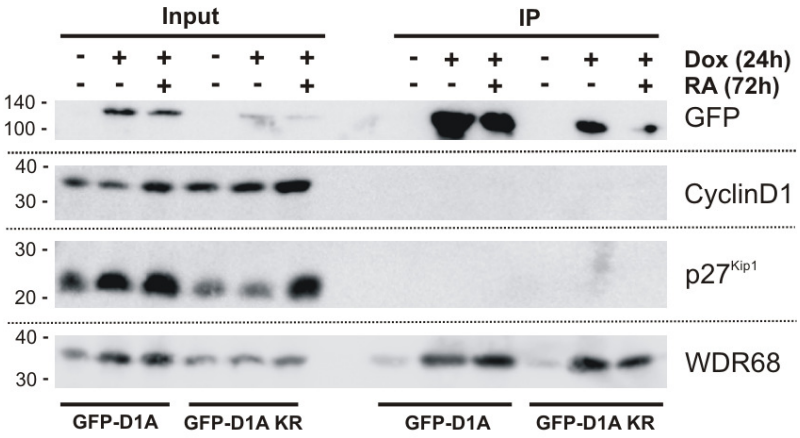
Supplemental figure 1. Analysis of DYRK1A and TrkB mRNA levels by qRT-PCR.

(A) SH-SY5Y cells were treated with doxycycline or RA for 24 h or 72 h before DYRK1A mRNA levels were quantified by qRT-PCR. (B) SH-SY5Y cells were treated with doxycycline or RA for 72 h and TrkB mRNA levels were quantified. mRNA levels are shown as relative quantification to GAPDH mRNA levels, which were used as internal standard. n = 3, means + SD; ***, $p \leq 0.001$; analyzed by One-way ANOVA + Bonferroni post test to compare GFP-DYRK1A or GFP-DYRK1A-K188R data to the respective untreated controls.



Supplemental figure 2. DYRK1A overexpression induces proliferation arrest of SH-SY5Y cells.

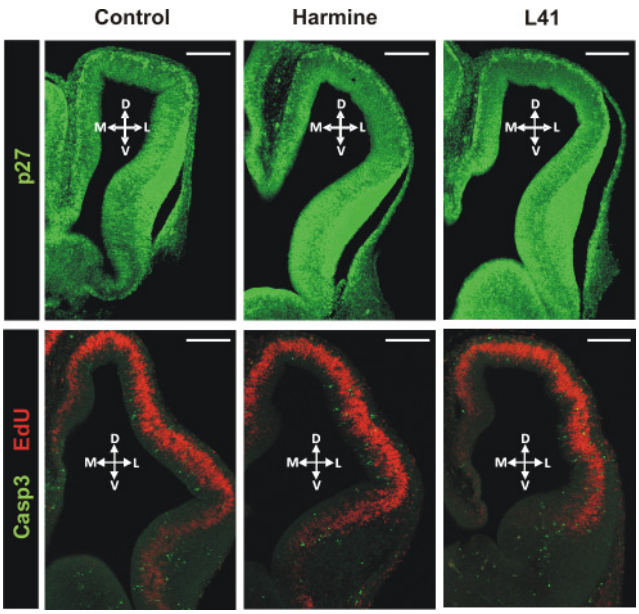
Analysis of SH-SY5Y cell proliferation by continuous live cell imaging using the IncuCyte™ kinetic imaging system. The well confluence of cells overexpressing wild type DYRK1A (D1A + dox) or kinase deficient DYRK1A-K188R (D1A-KR + dox) was automatically evaluated in 1 h intervals using the IncuCyte™ software from the beginning of doxycycline treatment (t = 0 h). Untreated cells served as control. (Graph shows means of four independent experiments).



Supplemental figure 3. DYRK1A does not form stable complexes with endogenous p27^{Kip1} or CyclinD1 in SH-SY5Y cells.

Immunoprecipitation (IP) of GFP-DYRK1A or GFP-DYRK1A-KR from SH-SY5Y cells. To increase endogenous p27^{Kip1} protein levels, cells were treated with RA for 72 h. DYRK1A overexpression was induced after 48 h RA treatment for 24 h. Possible protein-protein interactions were analyzed by western blotting and immunodetection, using the indicated antibodies. WDR68 is known to form stable complexes with DYRK1A and was therefore detected as positive control (Miyata et al. 2011).

Miyata Y, Nishida E. DYRK1A binds to an evolutionarily conserved WD40-repeat protein WDR68 and induces its nuclear translocation. Biochim Biophys Acta 2011; 1813:1728–39.



Supplemental figure 4. Short time inhibition of DYRK1A does not alter proliferation or cell death in the embryonic mouse telencephalon.

Confocal images from one hemisphere showing the telencephalon of E12 mouse embryos cultured for 6 h in the presence of harmine (4 µM), leucettine L41 (8 µM) or without inhibitor (control). EdU was added to the culture media 1 h before stopping the culture. Brain slices were stained for $p27^{Kip1}$, caspase 3 (Casp3) and $p27^{Kip1}$ as indicated. The dorso-ventral (D-V) and medial-lateral (M-L) orientations are indicated. Scale bar = 200µm. One representative experiment is shown from n = 3.