Supplementary information for:

Synapsin III controls early phases of dopaminergic neurons development in fishes and mammals by acting upstream of BDNF and Cdk5 signaling

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Supplementary information

Online resource 1

Light sheet microscopy-based 3D imaging of the brain of *Syn III*-MO-injected Tg(*neurod1*:eGFP) zebrafish larvae 48 hpf.

Online resource 2

Light sheet microscopy-based 3D imaging of the brain of ST-MO-injected Tg(*neurod1*:eGFP) zebrafish larvae 48 hpf.

Online resource 3

Light sheet microscopy-based time-lapse imaging of the *Syn III*-MO-injected Tg(*neurod1*:eGFP) zebrafish larvae from 19 to 24 hpf.

Online resource 4

Light sheet microscopy-based time-lapse imaging of the ST-MO-injected Tg(*neurod1*:eGFP) zebrafish larvae from 19 to 24 hpf.

Online resource 5 Phylogenetic sequence conservation of Syn III

a. Multiple alignments of mammalian and zebrafish Syn III protein. The amino acid sequences of *Homo Sapiens SYN3* and *Danio Rerio syn3* were aligned using AlignX of Vector NTI Advance 10.1.1 based on the Clustal W algorithm. Subsequently, the alignment was edited with GeneDoc using the conservation mode for shading. Consensus residues were assigned based on the number of occurrences of the character in the column. Different colors were set to 100% conserved (blue shading), 80% or greater conserved (green shading), 60% or greater conserved (red shading), and less than 60% conserved (yellow, orange and white shading).

b. Percentage of identity between *Homo Sapiens*, *Rattus Norvegicus*, *Mus Musculus* and *Danio Rerio* Syn III genes. The Fig shows the percentage of amino acid identity between mammalian and zebrafish Syn III gathered from multiple sequence alignment of Syn III proteins using ClustalW2 software.

c. Gene trace synteny around the *SYN3* locus between *Homo Sapiens*, *Mus Musculus* and *Danio Rerio* chromosome generated by Synteny Database (50-gene sliding window).

Online resource 6 Analysis of Syn III, II, Syn I and BDNF levels in the nigrostriatal system of wt and Syn III ko mice

a,b. Representative images showing Syn III, Syn II and Syn I immunopositive bands deriving from the western blot analysis of midbrain and striatal protein extracts from newborn (nb), 2 or 10-month-old wt or Syn III ko mice.

c,d. Absence of Syn III expression in Syn III ko mice.

e,f. No difference in Syn II levels was observed at 0, 2 and 10 months of age in both areas.

g,h. Syn I levels were significantly decreased in 10-month-old Syn III ko mice when compared to wt littermates.

i. Representative images and graphs showing prepro-BDNF and mature BDNF levels in the midbrain of newborn wt and Syn III ko mice. Please note the significant decrease of both prepro-BDNF and mature BDNF in the Syn III ko animals. Data are expressed as protein levels normalized vs. α-tubulin expression (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; two-way ANOVA + Bonferroni's multiple comparisons test. $n = 3$ animals per each condition).

Online resource 7

Two-photon microscopy-based Z-stack tile-scan imaging allowing the 3D visualization of the TH-positive neurons of the SN in the brain of Syn III ko newborn mice subjected to X-Clarity-based tissue clearing and deep tissue immunolabeling.

Online resource 8

Two-photon microscopy-based Z-stack tile-scan imaging allowing the 3D visualization of the TH-positive neurons of the SN in the brain of C57BL/6J wt newborn mice subjected to X-Clarity-based tissue clearing and deep tissue immunolabeling.

Online resource 9 mDN derived from Syn III ko mice showed early delayed axonal growth

a. Schematic representation of Sholl test analysis. The distance between concentric circle is fixed at 10 µm.

b. Representative images of mDN from wt and Syn III ko mice at div 1, 2, 10. Scale bars: 10 µm for 2 and 10 div; 20 µm for 10 div.

c. Representative images of mDN from wt and Syn III ko mice at div 1 receiving BDNF treatment. Scale bar, 10 µm.

d. Representative images of mDN from wt and Syn III ko mice at div 1 receiving DA treatment. Scale bar, 10 µm.

e. Representative images of mDN from wt and Syn III ko mice at div 10 receiving roscovitine treatment. Scale bar, 10 µm.

Online resource 10 iPSCs-derived mDN showed early neurodevelopmental deficits

a,b. Representative images of mDN from CTRL-1 (a) and CTRL-2 (b)-derived iPSCs at div 25, 40, 50. Scale bar 100 µm.

c-f. Representative western blot results showing the reduced Syn III expression at div 25, 40 and 50 in AAV-shSynIII infected neurons of CTRL-1 and CTRL-2 when compared to AAV-shNSC-infected neurons (* $p < 0.05$, two-way ANOVA + Bonferroni's multiple comparisons test. $n = 3$ replicates per each experimental condition).