

iPSC-based modeling of THD recapitulates disease phenotypes and reveals neuronal malformation

APPENDIX

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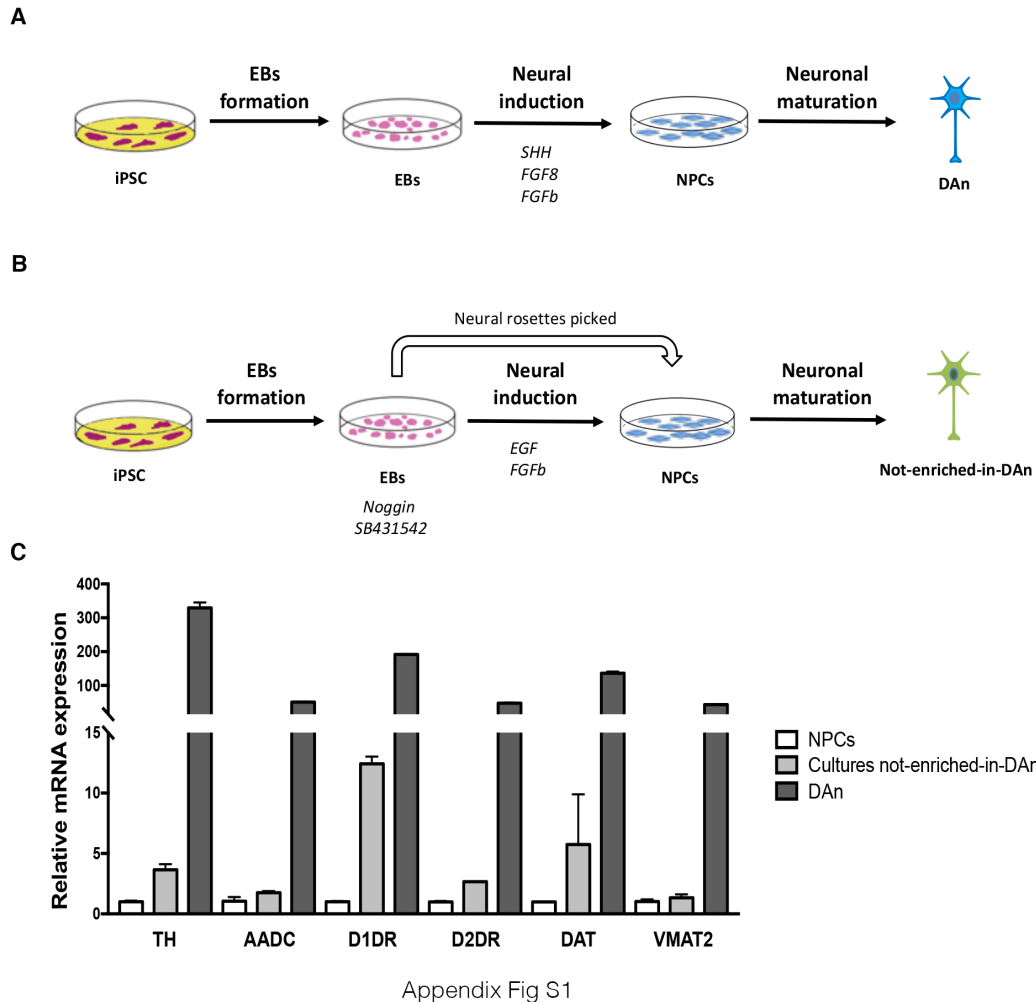
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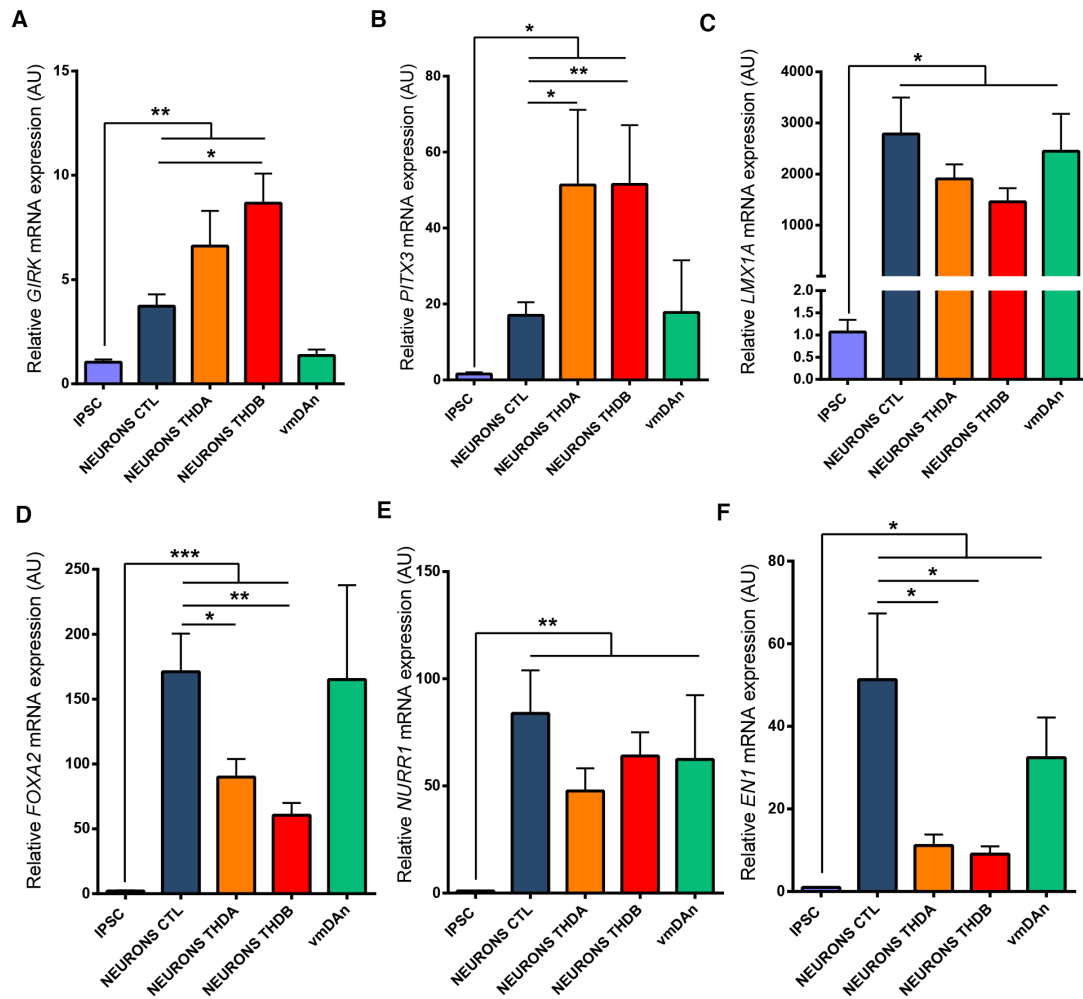
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Appendix table S1: Primers used for gene expression analysis.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
<i>Tyrosine hydroxylase (TH)</i>	TGTAAGCAGAACGGGGAGGT	AGCTTGTCCTTGGCGTCACT
<i>Vesicular monoamine transporter 2 (VMAT2)</i>	ATGAGTTTGTGGGGAAGACG	TGTTTGCAAAGCAGATGGAG
<i>Aromatic L-amino acid decarboxylase (AADC)</i>	GAACAGACTTAACGGGAGCCTTT	AATGCCGGTAGTCAGTGATAAGC
<i>Dopaminergic receptor 1 (D1DR)</i>	CTTAGGATGCTACAGACTTTGCCCTG	CATGTGGGATCAGGTAAACCAGATTG
<i>Dopaminergic receptor 2 (D2DR)</i>	TCTTCGGACTCAATAACGCAGACC	GATGTAGACCAGCAGGGTGACAAT
<i>Dopamine transporter (DAT)</i>	ATCCTGCAATGGGAGAGACACGAA	ATTACAGCAACACAAGACACGGCG
<i>G Protein-Activated Inward Rectifier Potassium Channel 2 (GIRK2)</i>	CATGGATCAGGACGTCGAAA	GCCTGCTTAGGCAACTTTGG
<i>Paired Like Homeodomain 3 (PITX3)</i>	CAGAGGACGGTTCGCTGAAAA	AGCTGCCTTTGCATAGCTCG
<i>LIM Homeobox Transcription Factor 1 Alpha (LMX1A)</i>	ACGTCCGAGAACCATCTTGAC	CACCACCGTTTGTCTGAGC
<i>Forkhead Box A2 (FOXA2)</i>	CCACCACCAACCCACAAAATG	TGCAACACCGTCTCCCCAAAGT
<i>Nuclear Receptor Subfamily 4 Group A Member 2 (NR4A2/NURR1)</i>	ACCACTCTTCGGGAGAATACA	GGCATTGGTACAAGCAAGGT
<i>Engrailed Homeobox 1 (EN1)</i>	GCACACGTTATTCGGATCG	GCTTGTCTCCTTCTCGTTCT
<i>Beta-actin</i>	AGGCCAACCGCGAGAAG	ACAGCCTGGATAGCAACGTACA



Appendix Fig S1. Expression of dopaminergic markers in iPSC-derived DAn and iPSC-derived neural cultures not-enriched-in-DAn. A Scheme of iPSC dopaminergic neuron differentiation. B Schematic diagram showing the iPSC differentiation into neural lineages (not-enriched-in-DAn). C Relative mRNA expression (assessed by qRT-PCR) of different dopaminergic genes such as *TH*, *AADC*, *D1DR*, *D2DR*, *DAT*, and *VMAT2*, relative to actin expression ($n=2$ independent experiments for all genes analyzed except for *TH* that includes $n=3$ experiments).



Appendix Fig S2

Appendix Fig S2. Relative mRNA expression of other DA markers. **A** Relative *GIRK* mRNA expression. **B** Relative *PITX3* mRNA expression. **C** Relative *LMX1A* mRNA expression. **D** Relative *FOXA2* mRNA expression. **E** Relative *NURR1* mRNA expression. **F** Relative *EN1* mRNA expression. Ventral midbrain dopaminergic neurons (vmDAn) were used as positive control and iPSCs were used as negative control. $N=6$ independent experiments except for iPSC samples ($n=3$). Data are expressed as mean $\pm$ SEM. ANOVA or Kruskal-Wallis tests were performed for multiple comparisons. Unpaired two-tailed Student's t test or Mann-Whitney U test was used for pairwise comparisons. *** $p<0.001$ ** $p<0.01$ * $p<0.05$.