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The effects of omega-3 fatty acids on Wnt activity in iPSC-derived neural stem cells from adult ADHD patients

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Supplementary Information

Supplementary Table 1. Demographic information for the subjects included in this study.

Cell line	Diagnosis	Age	Sex	ADHD-PRS (z-score)	iPSC QC can be found on
CTL1	aControl	25	Male	0.442	Suppl. Fig. 1
CTL2	aControl	28	Male	-1.674	McNeill et al. 2025
CTL3	aControl	45	Male	0.550	Suppl. Fig. 1
CTL4	aControl	27	Female	0.051	McNeill et al. 2025

CTL5	aControl	39	Female	-0.209	Palladino et al. 2020
ADHD1	aADHD	53	Male	-0.133	Suppl. Fig. 1
ADHD2	aADHD	39	Male	-1.696	McNeill et al. 2023
ADHD3	aADHD	27	Male	1.109	Suppl. Fig. 1
ADHD4	aADHD/MDD	46	Male	1.175	Suppl. Fig. 1
ADHD5	aADHD	30	Female	0.385	Palladino et al. 2020

Supplementary Table 2. List of all Wnt-reporter analyses conducted in this study.

Cell line	Readout						
	Calculation of EC ₅₀ values after Wnt3a	Calculation of IC ₅₀ values after DKK1	Determination of Wnt activity after MPH 10 nM	Determination of Wnt activity after EPA (25 and 50 µM)	Determination of Wnt activity after DHA (25 and 50 µM)	Determination of Wnt activity after MPH 10 nM + DHA 25 µM	Determination of Wnt activity after EPA 16.7 µM + DHA 8.3 µM
CTL1	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓
CTL2	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓
CTL3	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓
CTL4	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓
CTL5	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓
ADH D1	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓
ADH D2	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓
ADH D3	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓
ADH D4	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓
ADH D5	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓

Each check mark (✓) equals to one independent experiment.

Supplementary Table 3. EC₅₀ and IC₅₀ values after treatment with Wnt3a and DKK1, respectively.

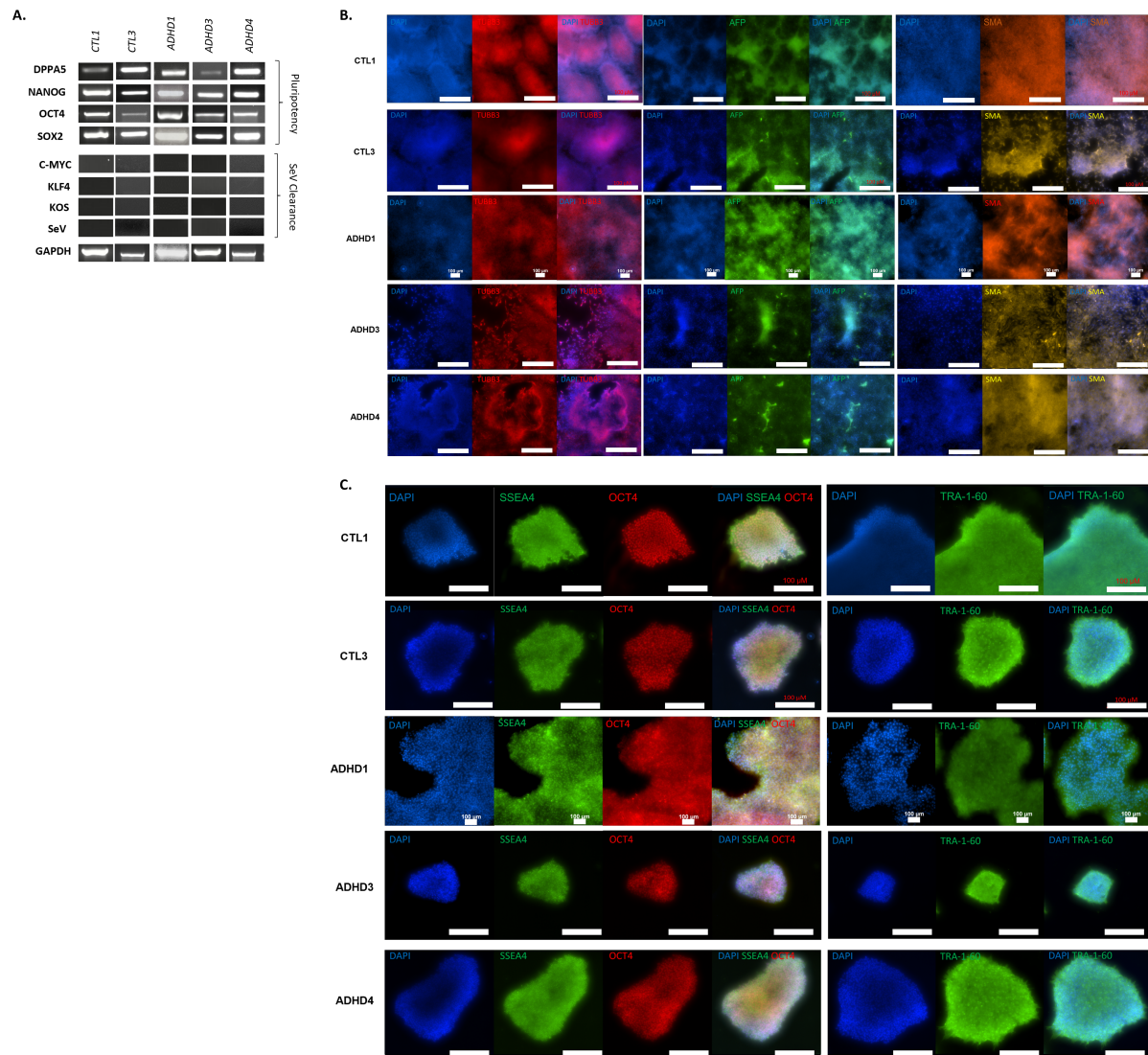
Cell line	Diagnosis	Wnt3a's EC ₅₀ (ng/mL)	DKK1's IC ₅₀ (ng/mL)
CTL1	Healthy adult	209.7	29.8
CTL2	Healthy adult	269.9	7.279
CTL3	Healthy adult	249.9	9.09
CTL4	Healthy adult	235.7	5.34
CTL5	Healthy adult	251.8	4.213
ADHD1	Adult ADHD	349.9	10.11
ADHD2	Adult ADHD	276.3	8.37
ADHD3	Adult ADHD	405.9	21.44
ADHD4	Adult ADHD	445.7	15.13
ADHD5	Adult ADHD	203.7	18.74

Supplementary Table 4. Effect sizes (Hedge's *g*) for pairwise comparisons between the aADHD and aControl groups.

Parameter	Hedge's <i>g</i>	Magnitude
Wnt3a EC₅₀	1.311	Large
<i>DKK1 IC₅₀</i>	<i>0.426</i>	<i>Medium</i>
EPA 25μM	0.373	Small
EPA 50μM	0.049	Negligible
DHA 25μM	0.073	Negligible
DHA 50μM	0.098	Negligible
EPA + DHA	0.081	Negligible
<i>MPH 10nM</i>	<i>0.59</i>	<i>Medium</i>
MPH + DHA	1.574	Large

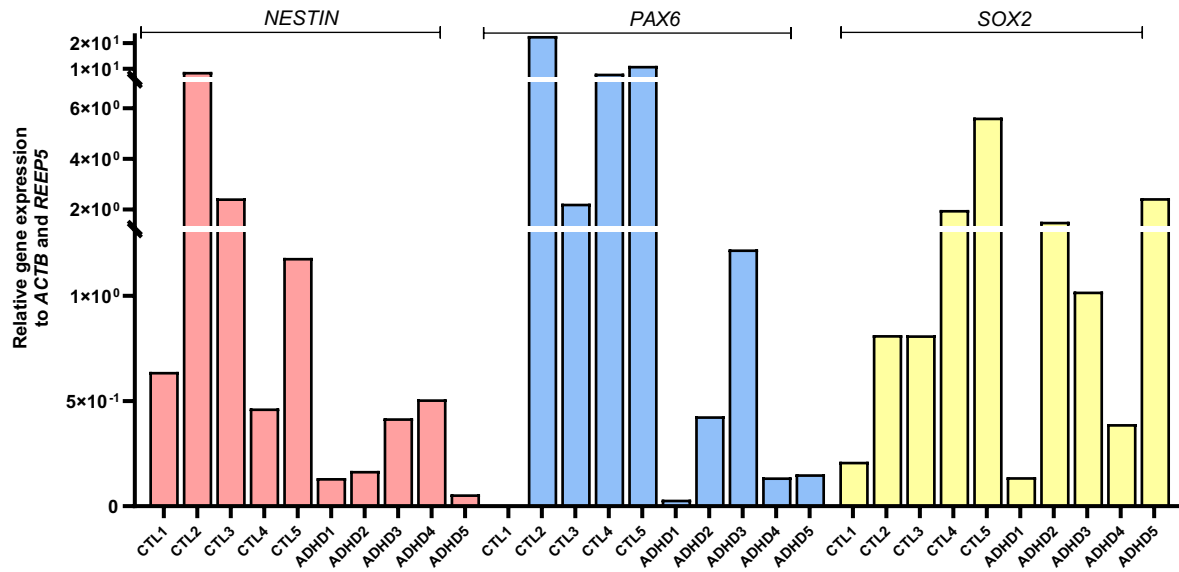
Supplementary Table 5. Effect sizes (Hedge's *g*) for pairwise comparisons between treated conditions following PUFAs, MPH and combined treatments.

Parameter	Group	Hedge's <i>g</i>	Magnitude
EPA 25µM x Vehicle	aADHD	0.188	Negligible
	<i>aControl</i>	<i>0.564</i>	<i>Medium</i>
EPA 50µM x Vehicle	aADHD	0.443	Small
	aControl	0.394	Small
EPA 25µM x EPA 50µM	aADHD	0.255	Small
	aControl	0.171	Negligible
DHA 25µM x Vehicle	aADHD	0.46	Small
	<i>aControl</i>	<i>0.535</i>	<i>Medium</i>
DHA 50µM x Vehicle	aADHD	1.01	Large
	aControl	1.111	Large
DHA 25µM x DHA 50µM	<i>aADHD</i>	<i>0.551</i>	<i>Medium</i>
	<i>aControl</i>	<i>0.576</i>	<i>Medium</i>
EPA + DHA x Vehicle	<i>aADHD</i>	<i>0.673</i>	<i>Medium</i>
	<i>aControl</i>	<i>0.592</i>	<i>Medium</i>
MPH 10nM x Vehicle	<i>aADHD</i>	<i>0.601</i>	<i>Medium</i>
	aControl	0.068	Negligible
MPH + DHA x Vehicle	<i>aADHD</i>	<i>0.763</i>	<i>Medium</i>
	<i>aControl</i>	<i>0.788</i>	<i>Medium</i>

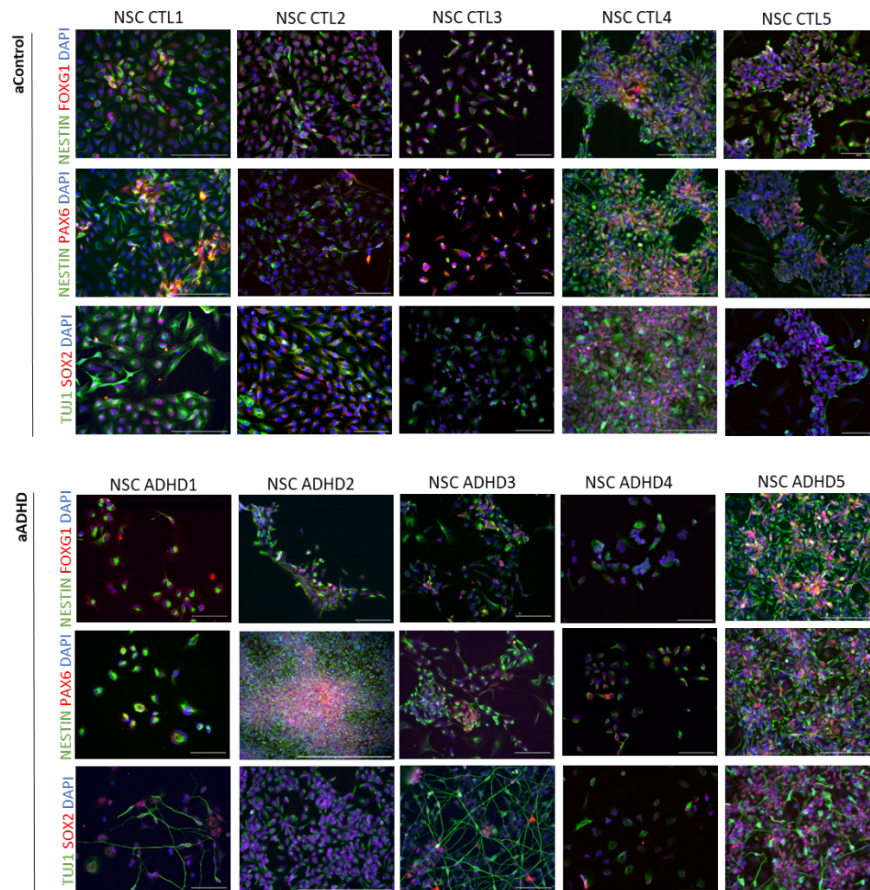


Supplementary Figure 1. Quality control of iPSCs derived from adult ADHD patients and matched healthy controls. A) RT-qPCR amplicon bands demonstrate positive gene expression of pluripotency genes (*DPPA5*, *NANOG*, *OCT4* and *SOX2*) and absence of Sendai virus (SeV)-related genes. *GAPDH* was used as reference gene. Individual bands were cropped from the original images for better visualization. B) Immunocytochemistry (ICC) was used to confirm the ability of differentiation into the three germ layers (endoderm, mesoderm and ectoderm) through positive expression of AFP, SMA and TUBB3, respectively, in iPSCs CTL1, CTL3, ADHD1, ADHD3 and ADHD4. Scale bar: 100 μ m. C) The same technique was applied to demonstrate the positive protein expression of pluripotency markers SSEA4, OCT4 and TRA-1-60. Scale bar: 100 μ m.

A.

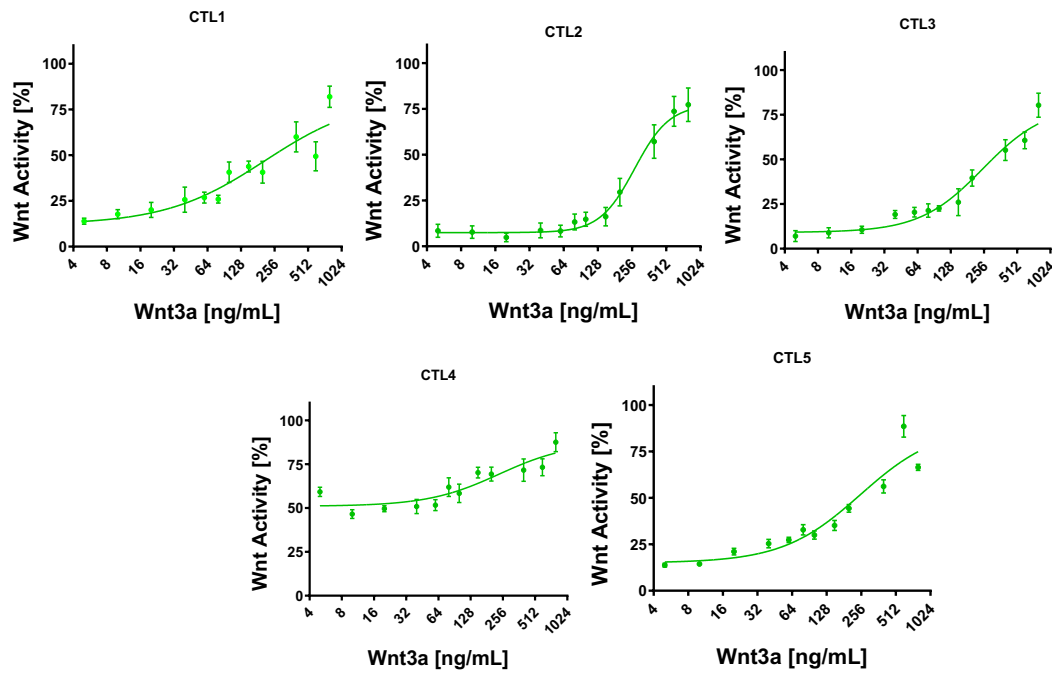


B.

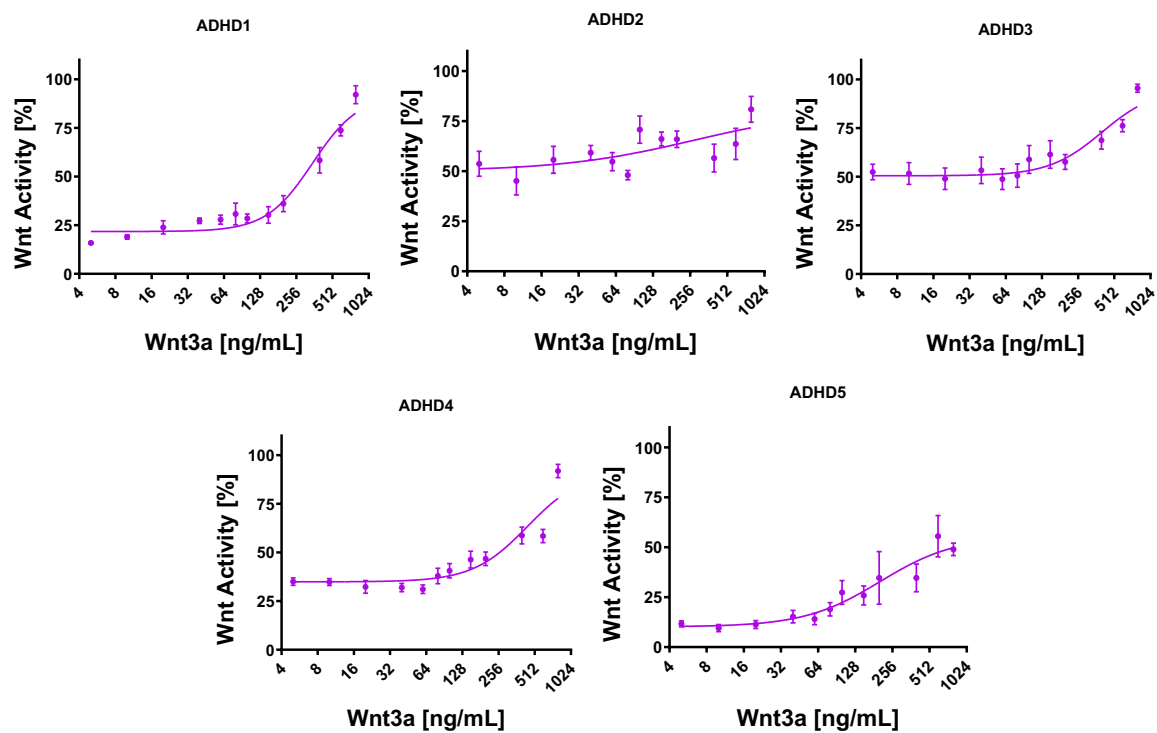


Supplementary Figure 2. Quality control of iPSC-derived NSCs. A) Heatmap showing the relative gene expression of *SOX2*, *Nestin* and *PAX6* after RT-qPCR. *ACTB* and *REEP5* served as reference genes. B) Representative immunocytochemistry images from all cell lines included in this study showing positive protein expression of classical NSC markers (FOXG1, NESTIN, SOX2, PAX6 and TUBB3). Scale bar: 100 μm. aControl: adult control; aADHD: adult ADHD.

A.

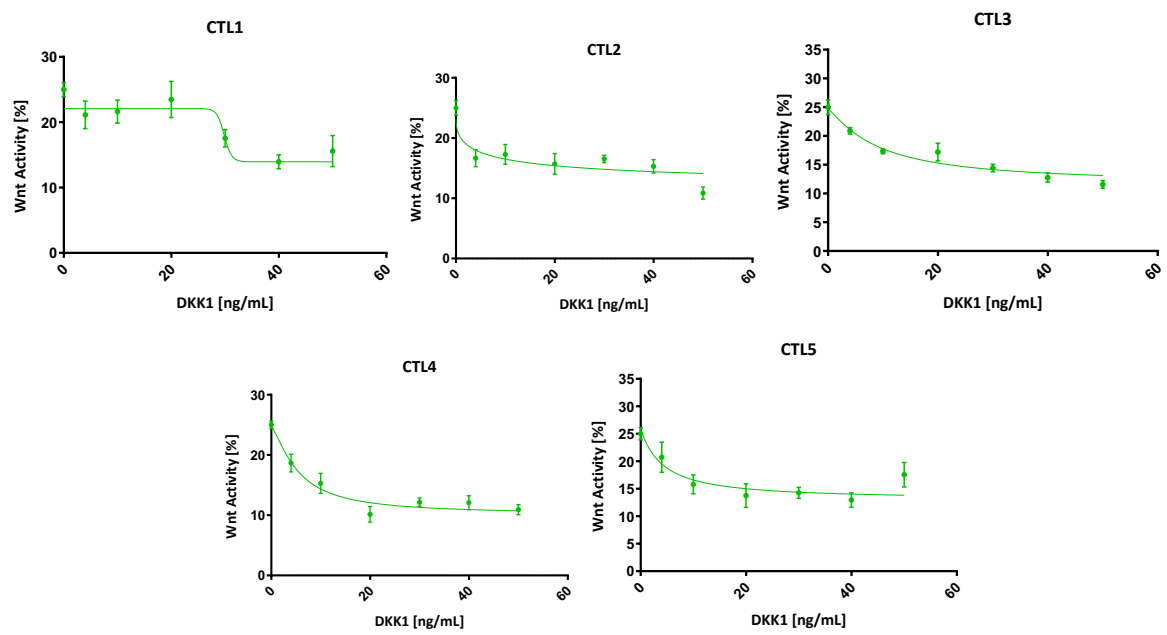


B.

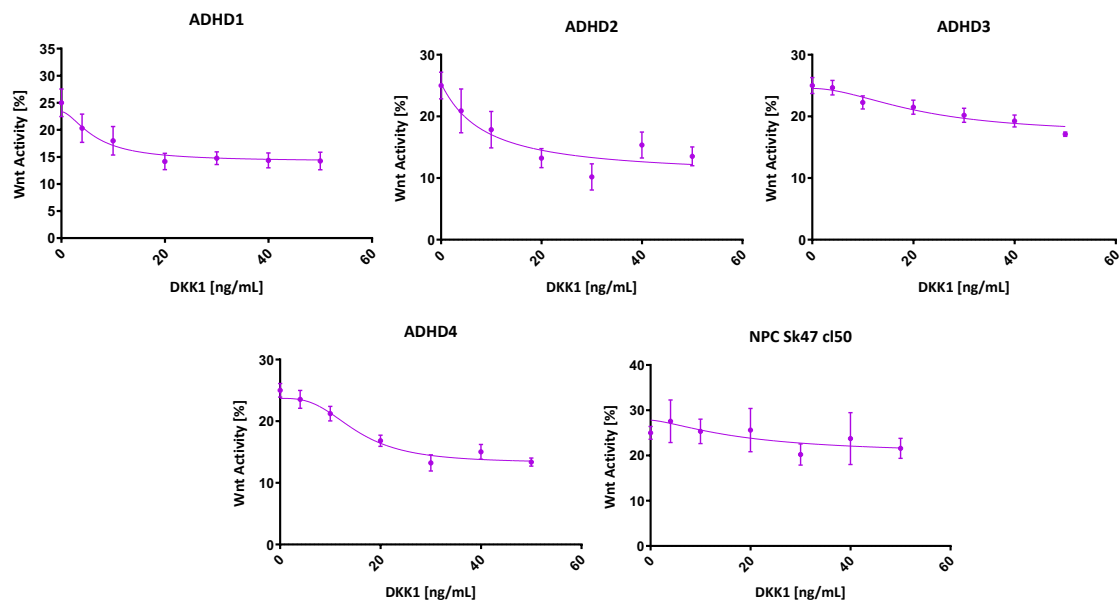


Supplementary Figure 3. Individual dose-response curves after treatments with increasing concentrations of Wnt3a in control (A) and ADHD (B) cell lines. The curves represent mean $\pm$ SEM considering triplicates of two independent experiments per NSC line.

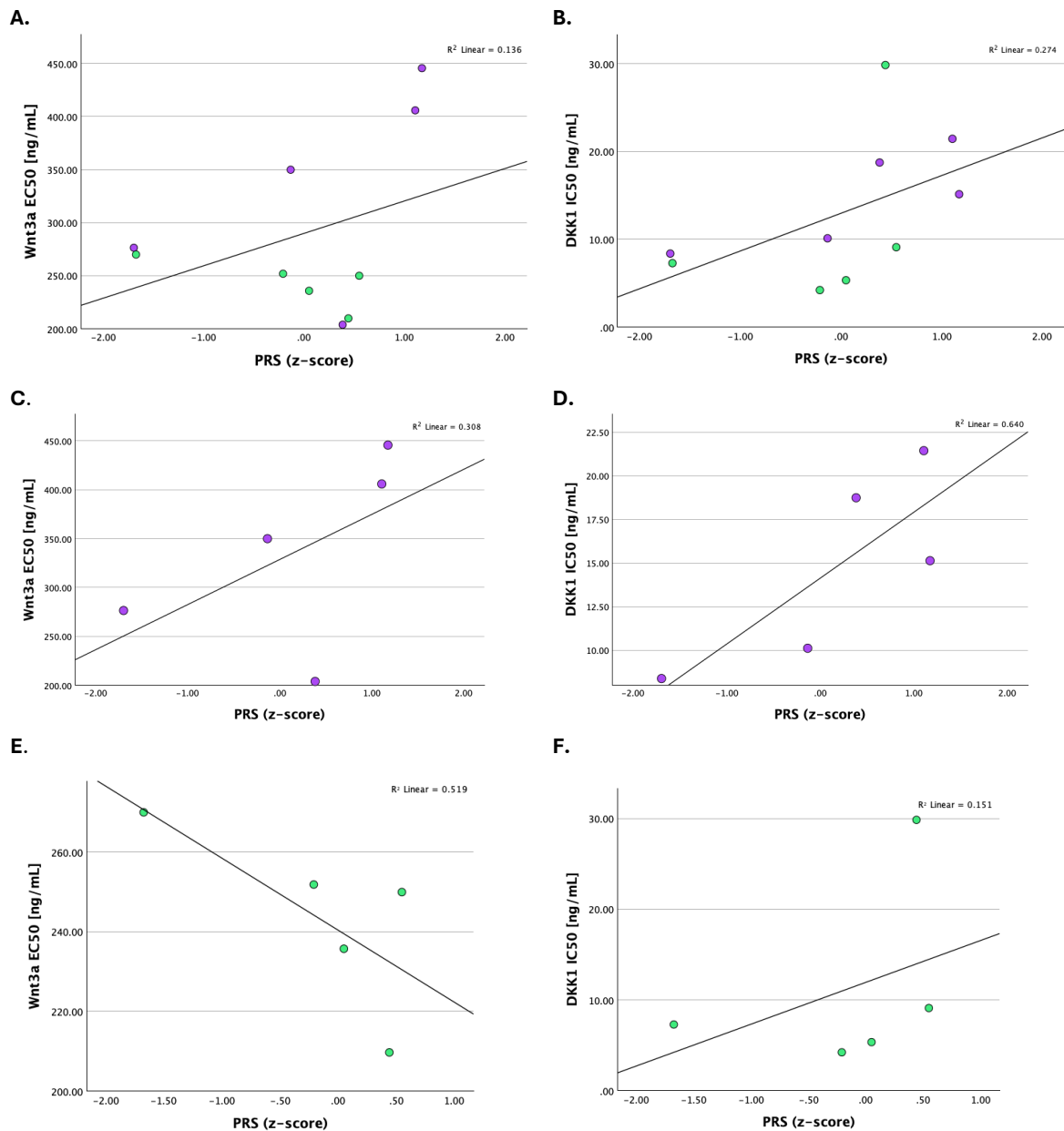
A.



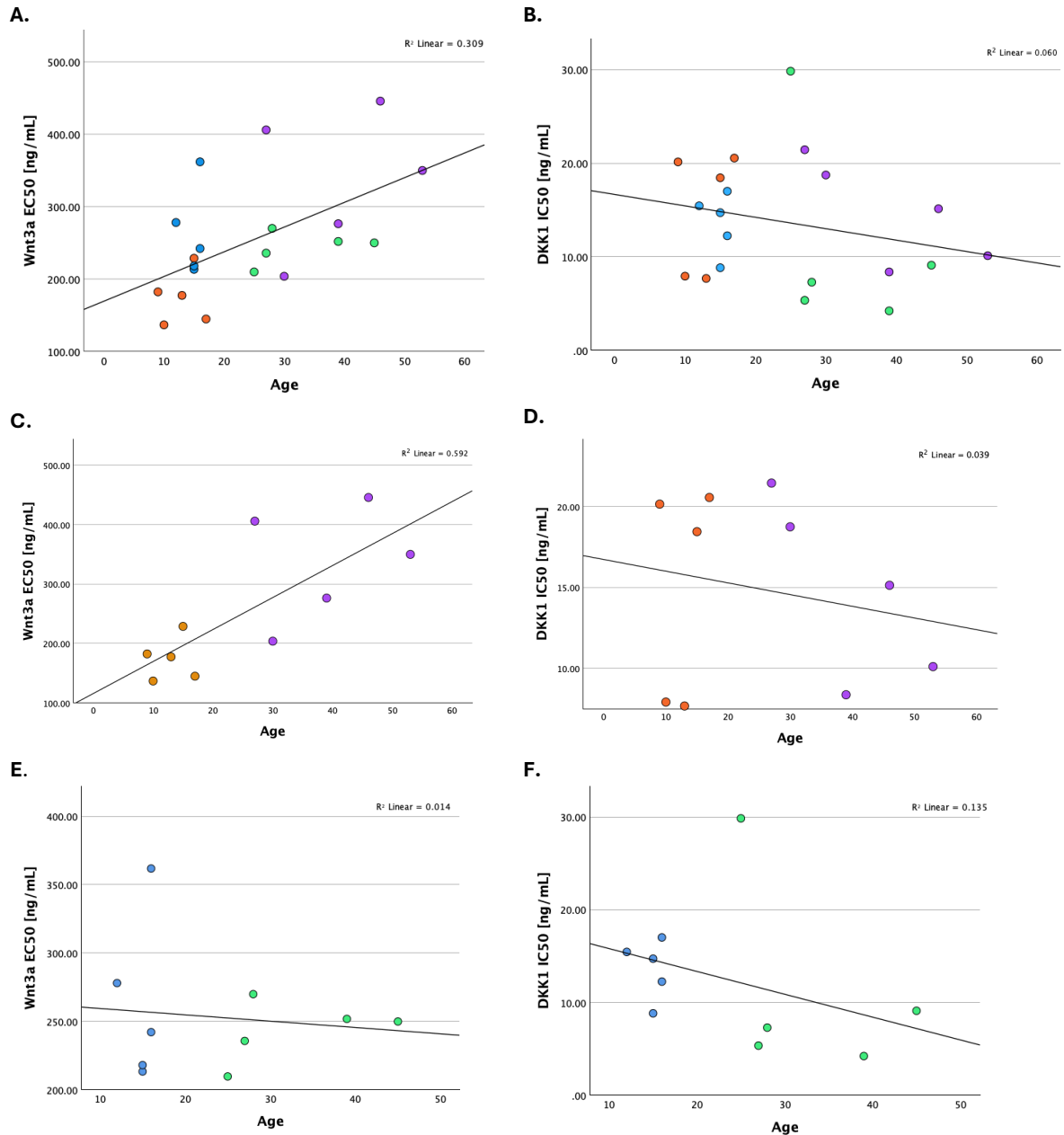
B.



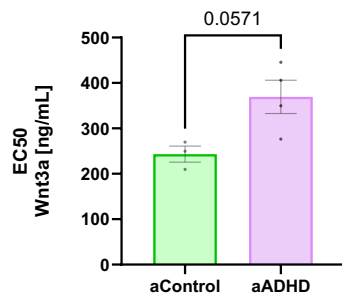
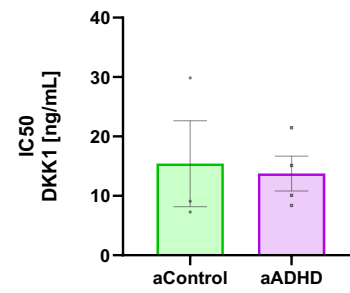
Supplementary Figure 4. Individual dose-response curves after treatments with increasing concentrations of DKK1 in control (A) and ADHD (B) cell lines. The curves represent mean $\pm$ SEM considering triplicates of two independent experiments per NSC line.



Supplementary Figure 5. Correlation analyses between Wnt-related parameters and genetic predisposition to ADHD. A) No correlation was observed between ADHD-PRS and EC₅₀ values of Wnt3a was seen. Spearman's $\rho = 0.139$; $p = 0.701$. B) A significant positive correlation was observed between ADHD-PRS and DKK1 IC₅₀ values (Spearman's $\rho = 0.648$; $*p = 0.043$). When adult ADHD cell lines were analyzed excluding the controls, no correlation was observed between ADHD-PRS and EC₅₀ (C) or IC₅₀ (Spearman's $\rho = 0.700$; $p = 0.188$ for both EC₅₀ and IC₅₀) (D). The same pattern of response was observed for the control group (Spearman's $\rho = -0.700$; $p = 0.188$ for EC₅₀ and Spearman's $\rho = 0.600$; $p = 0.285$ for IC₅₀) (E-F). Purple dots represent aADHD while the green dots represent aControl.



Supplementary Figure 6. Correlation analyses between Wnt-related parameters in NSCs and age of the subjects from the present study and from Walter *et al.* 2024. A) Significant positive correlation between Wnt3a EC₅₀ values and age. Spearman's $\rho = 0.556$; $*p = 0.011$. B) An opposite, although non-significant, tendency was observed for the correlation between DKK1 IC₅₀ values and age. Spearman's $\rho = 0.164$; $p = 0.469$. When pediatric and adult ADHD cell lines were analyzed without the controls, a significantly positive correlation between age and Wnt3a EC₅₀ values was seen (Spearman's $\rho = 0.745$, $*p = 0.013$) (C), but not between DKK1 IC₅₀ (Spearman's $\rho = -0.006$; $p = 0.987$) (D). Non-statistically significant and opposing tendencies of correlation were seen for the control cell lines (E, F) in comparison to ADHD (Spearman's $\rho = 0.091$; $p = 0.802$ for EC₅₀ and Spearman's $\rho = -0.518$; $p = 0.125$ for IC₅₀). Purple = adult ADHD, green = adult control, orange = ADHD children and adolescents (Walter *et al.* 2024), blue = children and adolescents from the control group (Walter *et al.* 2024). Wnt-related findings represent one clone per donor.

A.**B.**

Supplementary Figure 7. Analysis of basal Wnt activity in NSCs derived from only male adult participants. A nominal increase was observed for EC₅₀ values of the aADHD group (A) but not for IC₅₀ values (B), according to Mann-Whitney tests. *P*-values are displayed in the graphs. Each dot represents the average of two independent experiments from each individual cell line (N = 3 and N = 4 for aControl and aADHD, respectively).