**Supplementary material**

**Methylphenidate enhances neuronal differentiation and reduces proliferation concomitant to activation of Wnt signal transduction pathways**

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**Supplementary Material and Methods**

1. **Preparation of compound solution**

   Cells were treated with varying concentrations (1 nM – 100 µM) of d/l-threo methylphenidate (MPH) (cat. no. M2892; Sigma Aldrich, Switzerland) or the vehicle (water) as control. MPH was protected from light during all treatments and the dilutions were always prepared freshly prior to use to avoid any auto-degradation. GBR12909 (cat. no. D052; Sigma Aldrich, Switzerland) was diluted in DMSO and cells were treated in varying concentrations (1 nM – 10 µM) or with DMSO as a control. R-spondin 1 (100 ng/ml) and dickkopf-protein 1 (200 ng/ml) (dkk-1; cat. no. cat. no. SRP6487 & SRP3258; Sigma Aldrich, Switzerland) were diluted in water.

2. **Murine neuronal stem cell (mNSC) sphere monolayer culturing and differentiation study**

   2.1. **Immunocytochemistry**

   Fixed and prepared cells were stained for differentiation studies with rabbit monoclonal antibody against glial fibrillary acidic protein (GFAP; 1:200, Sigma aldrich, USA, Cat-No: C4546) and/or with mouse monoclonal antibody against β-tubulin III (Tuj 1; 1:300, Sigma aldrich, USA, Cat-No: T3952) overnight at 4°C. For total cell count, nuclei were visualized using Hoechst 33258 staining (1:100; Invitrogen, Cat-No: H3569) for 15 minutes at room temperature (RT). After overnight incubation, the primary antibodies were visualized with secondary antibodies against rabbit or mouse conjugated to the following fluorochromes: Alexa Fluor®-488 and Alexa Fluor®-555 (ThermoScientific, Switzerland). Staining was visualized under the inverted microscope (Olympus IX81, Germany) with DP72 Digital camera and the xcellence software (Olympus, Germany). In Supplementary Fig. S1 examples for staining and overlays are shown.

   For each well, first the total number of cells within the well was determined manually counting Hoechst positive stained cells. Secondly, GFAP positive or Tuj 1 positive cells were manually counted, respectively. Subsequently, the relative ratio of positive cells (sum of GFAP or Tuj 1 positive cells, respectively) to the total cell count (sum of Hoechst-stained cells) was calculated. In order to present results in comparison to the untreated cells, percentages were calculated for each treatment dose, setting the control as 100%.

3. **Neuronal cell line culture**

   3.1. **Culturing of proliferating SH-SY5Y and PC12 cells**

   Human neuroblastoma SH-SY5Y cells (European Collection of Authenticated Cell Cultures (ECACC), UK) were grown in buffered Dulbecco’s modified Eagle medium (DMEM/F-12) (Sigma Aldrich, Switzerland) supplemented with 10% fetal bovine serum (FBS; Thermo Fisher Scientific, Switzerland) in a humidified incubator (5% CO₂) at 37°C. Rat pheochromocytoma (PC12) cells (ECACC, UK) were grown in buffered 10 mg/ml glucose Dulbecco's modified Eagle medium (DMEM) (Thermo Fisher Scientific, Switzerland) supplemented with 10% FBS (Thermo Fisher Scientific, Switzerland), 5% horse serum (Thermo Fisher Scientific, Switzerland), and 0.3% gentamycin (50 mg/ml) (Thermo Fisher Scientific, Switzerland) in a humidified incubator (5% CO₂) at 37°C.
3.2. Differentiation of SH-SY5Y and PC12 cells

SH-SY5Y cells were seeded in poly-D-lysine/laminin coated 8-well slides (Corning, Switzerland) at a density of 10^4 cells/well. The cells were grown at 37°C, 5% CO₂ for 24 hours in normal growth medium before it was exchanged with a differentiation medium (DM), consisting of a normal growth medium supplemented with 10 μM retinoic acid (Sigma Aldrich, Switzerland) and 50 ng/ml nerve growth factor (Sigma Aldrich, Switzerland). PC12 cells were seeded in Collagen I (ScienCell, USA) coated 4-well slides (Corning, Switzerland) at a density of 10^5 cells/well. The cells were grown at 37°C, 5% CO₂ for 24 hours in normal growth medium before it was exchanged with DM, consisting of a 1:5 mixture of normal growth medium and DMEM supplemented with 100 ng/ml nerve growth factor. DM was exchanged every second day.

3.3. BrdU Staining

Cells were fixed with 4% ice-cold paraform aldehyde (SantaCruz Biotechnologies, Switzerland) for 20 minutes at RT. This was followed by incubation in 1 M HCl on ice for 10 minutes, followed by 2 M HCl at RT for 20 minutes and another 20 minutes in 2 M HCl at 37°C, in order to enable breaking of the nucleus and denaturing the DNA. After neutralizing with 1.5 M sodium borate buffer for 10 minutes, the cells were washed trice with PBS. To prevent unspecific binding, the cells were blocked in a blocking buffer (PBS, 1% goat serum and 0.03 % triton-X-100) for 90 minutes. Alexa Fluor 488®-linked anti-BrdU antibody (Millipore, Switzerland) was added to the cells, which were then incubated in the dark overnight at 4°C. The cells were washed with PBS three times for five minutes. For the mounting a DAPI (nucleus staining reagent)-containing mounting medium (Abcam, UK) was used. The slide was dried overnight before pictures were taken at 20x magnification under the inverted microscope (Olympus IX81, Germany) with DP72 Digital camera. Cells were counted using the xcellence software (Olympus, Germany).

4. Activity detection of canonical Wnt-signaling with luciferase Wnt reporter assay

To confirm our pharmacological analysis of the Wnt-signaling activation by MPH and R-Spondin1 against Wnt-signaling inhibition via dkk1, we used the Leading Light® Wnt Reporter Assay (Enzo Life Sciences, Lausen, Switzerland). This assay, is a cell-based luciferase activity test conducted in a 96-well plate, relaying on an engineered 3T3 mouse fibroblast cell line, which expresses the firefly luciferase reported gene under the control of Wnt-responsive promoters (TCF/LEF). Dose-dependent up-regulation of luciferase activity can be achieved by adding Wnt protein or Wnt agonist, while down-regulation is achieved by addition of Wnt antagonist. Wnt reporter cell lines were grown in 4500mg/L glucose Dulbecco’s modified Eagle medium (DMEM) (Sigma, Switzerland) supplemented with 10% FBS (Thermo Fisher Scientific, Switzerland), 4mM L-Glutamine (Life Technologies) and 1mM Sodium Pyruvate (Life Technologies, Switzerland). 50μl cells (15,000 cells/well) in growth medium were added to the 96-well plate. Plates were incubated overnight in humidified 37°C and 5% CO₂ incubator.
Supplementary Figure S1: Effect of methylphenidate (MPH) on embryonic murine neuronal stem cell (mNSC) differentiation into astrocyte (GFAP⁺ cells) and neurons (Tuj1⁺ cells). Embryonic mNSC were treated with MPH (1 nM up to 100 µM) or with vehicle (control). The cell number was determined via nucleus staining using Hoechst four days after treatment with MPH, while differentiation into astrocytes was determined by positive staining to the glial fibrillary acidic protein (GFAP) and differentiation into immature neurons was determined by positive staining to the neuron-specific class III beta-tubulin (Tuj1) compared to the total number of cells. Representative immunocytochemistry staining's for each of the treatments and control of embryonic mNSC against GFAP (red), Tuj 1 (green) and for total cell count using Hoechst 33258 staining (blue). Scale bar = 50 µm.
Supplementary Figure S2: Influence of methylphenidate (MPH) treatment on PC12 cells proliferation and possible toxicity. A. The time line of cell culturing, treatment and measurements. B. Representative xCELLigence run measuring in real-time impedance of cell proliferation (given as mean cell index on y-axis) before and after MPH (1nm-100µM) or vehicle (water) treatment during 72 h (x-axis). C. A representative statistical analysis conducted in MATLAB showing vehicle vs. MPH 1nM as in (B) with an overlay (green line) of the p-value according to Welch-test (right y-axis). D) Mean apoptosis (ApoTox-Glo) measured as % of control ± SEM at 20 h after MPH treatment showing negligible apoptosis signal. E) Mean cytotoxicity (CellTox Green) measured as % of control ± SEM at 20 h after MPH treatment showing negligible cytotoxicity signal.
Supplementary Figure S3: Effect of Wnt activators (Wnt3a, R-Spondin 1, and LiCl) and an inhibitor (Dkk1) on luciferase Wnt reporter assay activation / inhibition. (A) Dose response of Wnt3a protein on the activation of the Leading Light Wnt reporter cells. n=4-22; (B) Dose response of R-spondin 1 (R-Spo) on the activation of the Leading Light Wnt reporter cells. n=3-7; (C) Dose response of LiCl on the activation of the Leading Light Wnt reporter cells. n=3-19; (D) Dose response of Dkk1 protein in the presence of Wnt3a (100 ng/ml) on the inhibition of the Leading Light Wnt reporter cells. n=9-22. Exponential regression was conducted fitting the regression curve with R^2>0.7.
**Supplementary Figure S4:** Effect of methylphenidate on the interaction of sclerostin (SOST) with LRP5 tested by the Leading Light® Sclerostin-LRP interaction screening kit (Enzo Life Sciences Inc. Lausen, Switzerland). Positive binding between sclerostin and LRP5 following addition of LRP5-Alkaline phosphatase protein induced high luminescence signal (red bar), while addition of the Acid Green 25 (binding inhibitor) resulted in signal reduction (ca. 6% of the binding activation is left) (blue bar). Addition of methylphenidate starting from 1nM up to 1 mM reduced the signal to maximum of 17% (green bars). Bars represent mean RLU ± SEM (n=5-6). Kruskal-Wallis (F=8, H=22.380 p=0.0043), followed by Mann-Whitney-test * p<0.05 ** p<0.01 versus binding activation.
**Supplementary Table S1:** Gene Set Enrichment Analysis (GSEA; using the software Pathway Studio® version 114.0.8) of the available database for gene expression studies involving methylphenidate treatment in mammals.

1) Sadasivan S et al. 2012 ¹: Swiss Webster mice (3 weeks old), i.p. treated with saline, 1 mg/kg or 10 mg/kg methylphenidate once daily- for 12 weeks, 7 days' washout. Substantia nigra mRNA using Affymetrix HT MG-430 PM array.

¹ Database on GEO: GSE33691

### Chronic 1mg/kg MPH Mouse SN

| Name                                         | # of Entities | Expanded # of Entities | # of Measured Entities | Median change | p-value       | Hit type               |
|----------------------------------------------|---------------|------------------------|------------------------|---------------|---------------|------------------------|
| multicellular organismal development         | 1171          | 1171                   | 1073                   | -1.001770694 | 2.25296E-12 | biological_process     |
| plasma membrane                              | 5390          | 5390                   | 3575                   | -1.005534712 | 2.09322E-11 | cellular_component     |
| integral component of plasma membrane        | 1360          | 1360                   | 1101                   | -1.005092317 | 1.00697E-10 | cellular_component     |
| proteinaceous extracellular matrix           | 349           | 349                    | 310                    | 1.003269325  | 3.13082E-10 | cellular_component     |
| Atlas of Signaling                           | 380           | 2028                   | 1245                   | -1.002716707 | 9.4145E-10  | Cell Signaling         |
| cell differentiation                         | 849           | 849                    | 755                    | -1.000402833 | 1.39388E-06 | biological_process     |
| neuropeptide signaling pathway               | 106           | 106                    | 90                     | -1.019128624 | 3.70092E-06 | biological_process     |
| cell surface                                 | 645           | 645                    | 542                    | -1.004494091 | 4.5826E-06  | cellular_component     |
| axon guidance                                | 385           | 385                    | 371                    | -1.005587771 | 5.87638E-06 | biological_process     |
| dendrite membrane                            | 22            | 22                     | 22                     | 1.023333506  | 1.35323E-05 | cellular_component     |
| neural crest cell development                | 15            | 15                     | 13                     | 1.058296741  | 4.13872E-05 | biological_process     |
| neuron fate specification                    | 14            | 14                     | 13                     | -1.034779373 | 5.1894E-05  | biological_process     |
| synaptic transmission                        | 472           | 472                    | 428                    | -1.004584643 | 7.84504E-05 | biological_process     |
| social behavior                              | 52            | 52                     | 46                     | 1.020725612  | 0.000101242 | biological_process     |
| Wnt signaling pathway                        | 231           | 231                    | 193                    | 1.006366167  | 0.000227649 | biological_process     |
| synapse assembly                             | 55            | 55                     | 48                     | -1.005885398 | 0.000328795 | biological_process     |
### Chronic 10mg/kg MPH Mouse SN

| Name                                | # of Entities | Expanded # of Entities | # of Measured Entities | Median change | p-value       | Hit type                  |
|-------------------------------------|---------------|------------------------|------------------------|---------------|---------------|---------------------------|
| Atlas of Signaling                  | 380           | 2028                   | 1245                   | -1.000721214  | 6.33031E-14  | Cell Signaling            |
| extracellular region                | 2250          | 2250                   | 1651                   | -1.002486493  | 1.40597E-11  | cellular_component        |
| proteinaceous extracellular matrix  | 349           | 349                    | 310                    | 1.010965241   | 1.04276E-10  | cellular_component        |
| plasma membrane                     | 5390          | 5390                   | 3575                   | -1.004804104  | 2.81937E-10  | cellular_component        |
| integral component of plasma membrane| 1360          | 1360                   | 1101                   | -1.004003321  | 4.38301E-09  | cellular_component        |
| cell differentiation                | 849           | 849                    | 755                    | -1.004708505  | 1.65844E-05  | biological_process        |
| neuropeptide signaling pathway      | 106           | 106                    | 90                     | 1.00459659    | 1.73489E-05  | biological_process        |
| neuromuscular synaptic transmission | 19            | 19                     | 18                     | 1.03913796    | 6.63467E-05  | biological_process        |
| synaptic transmission               | 472           | 472                    | 428                    | -1.003749126  | 0.000188697  | biological_process        |

2) Dela Pena I et al. 2013: Male Wistar rats (6 weeks old), i.p. treated 2x daily for 7 days: with saline (C1), 5 mg/kg methylphenidate (C1.1). Following CPP-group with self-administration for 8-days or either Saline or MPH (C2). Striatum and Prefrontal cortex (PFC) mRNA using Affymetrix Rat Genome 230 2.0 array.

### 5mg/kg MPH rat striatum C1.1 vs saline C1

| Name                                              | # of Entities | Expanded # of Entities | # of Measured Entities | Median change | p-value       | Hit type                  |
|---------------------------------------------------|---------------|------------------------|------------------------|---------------|---------------|---------------------------|
| extracellular region                              | 2250          | 2250                   | 1331                   | -1.045058812  | 8.05471E-32  | cellular_component        |
| extracellular space                               | 1557          | 1557                   | 1019                   | -1.048719144  | 1.59526E-19  | cellular_component        |
| integral component of plasma membrane             | 1360          | 1360                   | 963                    | -1.080850862  | 6.27611E-16  | cellular_component        |
| sequence-specific DNA binding                     | 645           | 645                    | 453                    | -1.115796201  | 9.14035E-15  | molecular_function        |
| plasma membrane                                   | 5390          | 5390                   | 3130                   | -1.078799144  | 3.38168E-14  | cellular_component        |
| Biofluids assayable substances                    | 4140          | 9263                   | 3392                   | -1.064087529  | 8.5269E-14  | Pathway Studio Ontology   |
| signal transduction                               | 1843          | 1843                   | 1365                   | -1.075349382  | 2.2553E-13  | biological_process        |
| TGFB1-ACVRL1 Expression Targets                   | 221           | 230                    | 208                    | -1.038256566  | 9.21814E-13  | Expression Targets Pathways|
| G-protein coupled receptor signaling pathway      | 2567          | 2567                   | 463                    | -1.098828607  | 4.37575E-12  | biological_process        |
| G-protein coupled receptor activity               | 1768          | 1768                   | 301                    | -1.140718965  | 1.17771E-11  | molecular_function        |
| hormone activity                  | 152 | 152 | 105 | -1.053268852 | 2.85489E-11 | molecular_function |
|-----------------------------------|-----|-----|-----|-------------|-------------|--------------------|
| transcription factor activity, sequence-specific DNA binding | 1125 | 1125 | 731 | -1.07943767 | 3.6269E-11 | molecular_function |
| transcriptional activator activity, RNA polymerase II core promoter proximal region sequence-specific binding | 267 | 267 | 211 | -1.126835414 | 5.15856E-11 | molecular_function |
| external side of plasma membrane | 288 | 288 | 210 | -1.073643514 | 8.56879E-11 | cellular_function |
| Proteins involved in Pathogenesis of Atherosclerosis | 201 | 212 | 165 | 1.014999983 | 9.50404E-11 | Disease Collections |
| signal transducer activity        | 739 | 739 | 474 | -1.102954853 | 1.0978E-10 | molecular_function |
| Insulin/CEBPA/CTNNB/FOXA/FOXO Expression Targets | 145 | 157 | 138 | -1.054490618 | 1.73111E-10 | Expression Targets Pathways |
| immune response                   | 468 | 468 | 264 | 1.039052844 | 1.89774E-10 | biological_process |
| Proteins Involved in Pathogenesis of Arterial Hypertension | 257 | 281 | 234 | -1.06174063 | 5.37103E-10 | Disease Collections |
| multicellular organismal development | 1171 | 1171 | 843 | -1.0678359 | 6.00508E-09 | biological_process |
| cell differentiation              | 849 | 849 | 628 | -1.071770248 | 1.57668E-07 | biological_process |

5mg/kg MPH rat striatum C2 vs saline C2

| Name                                      | # of Entities | Expanded # of Entities | # of Measured Entities | Median change | p-value            | Hit type                      |
|-------------------------------------------|---------------|------------------------|------------------------|---------------|--------------------|--------------------------------|
| extracellular region                      | 2250          | 2250                   | 1331                   | 1.123785391   | 5.19614E-37        | cellular_component             |
| extracellular space                       | 1557          | 1557                   | 1019                   | 1.105057259   | 2.52616E-25        | cellular_component             |
| plasma membrane                           | 5390          | 5390                   | 3130                   | 1.059837403   | 9.81867E-24        | cellular_component             |
| integral component of plasma membrane    | 1360          | 1360                   | 963                    | 1.042840131   | 3.43473E-20        | cellular_component             |
| G-protein coupled receptor signaling pathway | 2567          | 2567                   | 463                    | 1.101812067   | 2.22145E-17        | biological_process             |
| signal transduction                       | 1843          | 1843                   | 1365                   | 1.095857912   | 1.72332E-16        | biological_process             |
| G-protein coupled receptor activity       | 1768          | 1768                   | 301                    | 1.095857912   | 1.03716E-15        | molecular_function             |
| Atlas of Signaling                        | 380           | 2028                   | 1039                   | 1.085267142   | 1.57994E-15        | Cell Signaling                 |
| Biofluids assayable substances           | 4140          | 9263                   | 3392                   | 1.074778808   | 6.96678E-15        | Pathway Studio Ontology        |
| sequence-specific DNA binding             | 645           | 645                    | 453                    | 1.125952007   | 9.13462E-15        | molecular_function             |
| multicellular organismal development     | 1171          | 1171                   | 843                    | 1.11746541    | 1.049E-14          | biological_process             |
### Supplementary

| Secreted proteins | 7555 | 12985 | 6654 | 1.056185806 | 2.05585E-14 | Pathway Studio Ontology |
|-------------------|------|-------|------|-------------|-------------|-------------------------|
| external side of plasma membrane | 288 | 288 | 210 | 1.10983508 | 3.43E-13 | cellular_component |
| Proteins Involved in Pathogenesis of Arterial Hypertension | 257 | 281 | 234 | 1.116239899 | 1.0469E-11 | Disease Collections |
| IGF1/MEF/MYOD/MYOG Expression Targets | 135 | 148 | 132 | 1.151018415 | 1.24432E-11 | Expression Targets Pathways |
| signal transducer activity | 739 | 739 | 474 | 1.093796224 | 2.39635E-11 | molecular_function |
| Transcriptional activator activity, RNA polymerase II core promoter proximal region sequence-specific binding | 267 | 267 | 211 | 1.161696575 | 3.70286E-11 | molecular_function |
| TGFB1-TGFBR1/AP-1 Expression Targets | 123 | 134 | 122 | 1.174460534 | 5.54533E-11 | Expression Targets Pathways |
| TGFB1-ACVRL1 Expression Targets | 221 | 230 | 208 | 1.150224108 | 6.75501E-11 | Expression Targets Pathways |
| TGFB1-TGFBR2 Expression Targets | 116 | 122 | 108 | 1.159089702 | 1.01171E-10 | Expression Targets Pathways |
| Proteins Involved in Pathogenesis of Atherosclerosis | 201 | 212 | 165 | 1.216120358 | 1.52405E-10 | Disease Collections |
| synaptic transmission | 472 | 472 | 418 | 1.03481214 | 2.47204E-10 | biological_process |
| IGF1/STAT Expression Targets | 107 | 113 | 106 | 1.154541884 | 2.5247E-10 | Expression Targets Pathways |
| proteinaceous extracellular matrix | 349 | 349 | 263 | 1.175787989 | 2.69761E-10 | cellular_component |
| cell-cell signaling | 269 | 269 | 218 | 1.151018415 | 3.25009E-10 | biological_process |
| IL1B Expression Targets | 169 | 188 | 162 | 1.13998323 | 3.66446E-10 | Expression Targets Pathways |
| EGF/CTNN Expression Targets | 143 | 152 | 139 | 1.151018415 | 4.64596E-10 | Expression Targets Pathways |
| ion transmembrane transport | 291 | 291 | 239 | 1.098950927 | 9.37338E-10 | biological_process |
| immune response | 468 | 468 | 264 | 1.113115538 | 9.64808E-10 | biological_process |
| cell differentiation | 849 | 849 | 628 | 1.101692894 | 7.9454E-09 | biological_process |
| postsynaptic membrane | 227 | 227 | 203 | 1.096269846 | 1.25526E-06 | cellular_component |

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### 5mg/kg MPH rat PFC C1.1 vs saline C1

| Name | # of Entities | Expanded # of Entities | # of Measured Entities | Median change | p-value | Hit type |
|------|---------------|------------------------|------------------------|---------------|--------|----------|
| extracellular region | 2250 | 2250 | 1331 | -1.046290799 | 4.94976E-26 | cellular_component |
| extracellular space | 1557 | 1557 | 1019 | -1.051940289 | 1.57008E-15 | cellular_component |
| G-protein coupled receptor activity | 1768 | 1768 | 301 | -1.101329228 | 1.47712E-14 | molecular_function |
| Name                                              | # of Entities | Expanded # of Entities | # of Measured Entities | Median change | p-value          | Hit type                  |
|--------------------------------------------------|---------------|------------------------|------------------------|---------------|-------------------|---------------------------|
| integral component of plasma membrane            | 1360          | 1360                   | 963                    | -1.089762753 | 3.89799E-14       | cellular_component         |
| plasma membrane                                  | 5390          | 5390                   | 3130                   | -1.081730797 | 5.81395E-14       | cellular_component         |
| immune response                                  | 468           | 468                    | 264                    | -1.001758461 | 1.13252E-13       | biological_process        |
| G-protein coupled receptor signaling pathway      | 2567          | 2567                   | 463                    | -1.074775622 | 1.17876E-11       | biological_process        |
| signal transduction                              | 1843          | 1843                   | 1365                   | -1.07424468  | 1.37001E-11       | biological_process        |
| positive regulation of transcription from RNA polymerase II promoter | 1041          | 1041                   | 842                    | -1.08614211  | 5.77276E-11       | biological_process        |
| Biofluids assayable substances                   | 4140          | 9263                   | 3392                   | -1.034780535 | 5.99451E-11 Pathway Studio Ontology |
| sequence-specific DNA binding                     | 645           | 645                    | 453                    | -1.067247492 | 1.57115E-10 molecular_function |
| synaptic transmission                            | 472           | 472                    | 418                    | -1.130977952 | 2.23372E-07       | biological_process        |
| cell differentiation                              | 849           | 849                    | 628                    | -1.07141252  | 1.12163E-06       | biological_process        |
| Canonical WNT Signaling Expression Targets        | 47            | 69                     | 55                     | -1.10192998  | 2.31766E-05 Expression Targets Pathways |
| regulation of neuron differentiation              | 39            | 39                     | 33                     | -1.29654646  | 8.83452E-05       | biological_process        |

### 5mg/kg MPH rat PFC C2 vs saline C2

| Name                                              | # of Entities | Expanded # of Entities | # of Measured Entities | Median change | p-value          | Hit type                  |
|--------------------------------------------------|---------------|------------------------|------------------------|---------------|-------------------|---------------------------|
| extracellular region                              | 2250          | 2250                   | 1331                   | 1.073552014  | 1.05216E-35       | cellular_component         |
| extracellular space                               | 1557          | 1557                   | 1019                   | 1.089045295  | 2.37554E-31       | cellular_component         |
| integral component of plasma membrane            | 1360          | 1360                   | 963                    | 1.07933207   | 1.42277E-22       | cellular_component         |
| G-protein coupled receptor activity               | 1768          | 1768                   | 301                    | 1.021735941  | 1.85815E-18       | molecular_function         |
| G-protein coupled receptor signaling pathway      | 2567          | 2567                   | 463                    | 1.054024962  | 5.50181E-18       | biological_process        |
| plasma membrane                                  | 5390          | 5390                   | 3130                   | 1.071083099  | 6.19118E-18       | cellular_component         |
| signal transduction                              | 1843          | 1843                   | 1365                   | 1.083165255  | 1.83353E-16       | biological_process        |
| Biofluids assayable substances                   | 4140          | 9263                   | 3392                   | 1.067314879  | 1.51282E-14 Pathway Studio Ontology |
| external side of plasma membrane                  | 288           | 288                    | 210                    | 1.091184159  | 1.88365E-12       | cellular_component         |
| hormone activity                                  | 152           | 152                    | 105                    | 1.177512318  | 2.47992E-12       | molecular_function         |
| signal transducer activity                        | 739           | 739                    | 474                    | 1.057149949  | 6.93254E-12       | molecular_function         |
### Supplementary

| Name                                                  | # of Entities | Expanded # of Entities | # of Measured Entities | Median change | p-value       | Hit type               |
|-------------------------------------------------------|---------------|------------------------|------------------------|---------------|---------------|------------------------|
| nucleoplasm                                           | 2669          | 2669                   | 2518                   | -1.00722603   | 2.66938E-38   | cellular_component      |
| nucleus                                               | 6877          | 6877                   | 5961                   | -1.002796865  | 5.69797E-32   | cellular_component      |
| cytoplasm                                             | 6831          | 6831                   | 6084                   | -1.001717133  | 3.9469E-22    | cellular_component      |
| nucleotide binding                                    | 1946          | 1946                   | 1792                   | -1.004093337  | 2.59455E-17   | molecular_function      |
| regulation of transcription, DNAtemplated             | 2670          | 2670                   | 2279                   | 1.00486156    | 2.77711E-16   | biological_process      |
| transcription, DNAtemplated                           | 2430          | 2430                   | 2206                   | 1.005069899   | 1.33166E-15   | biological_process      |
| cytosol                                               | 3173          | 3173                   | 3034                   | -1.002656374  | 1.10898E-14   | cellular_component      |
| metal ion binding                                     | 3568          | 3568                   | 3178                   | 1.00150887    | 9.57377E-13   | molecular_function      |
| Golgi apparatus                                       | 1245          | 1245                   | 1151                   | -1.002474234  | 4.82146E-12   | cellular_component      |
| poly(A) RNA binding                                   | 1254          | 1254                   | 1109                   | -1.009325635  | 6.12536E-12   | molecular_function      |
| DNA binding                                           | 2411          | 2411                   | 2103                   | 1.002913502   | 7.75104E-11   | molecular_function      |

3) Schwarz R et al. 2015: Human lymphoblastoid cells (LCL) from adult ADHD (aADHD; 5 males 4 females) and healthy controls (2 males 7 females). 30ng/µl MPH (Mw=269.77 g/mol; 111.2µM) treatment or vehicle for 1h, and chronic after 2 weeks. LCL mRNA pool (all samples together) using Affymetrix Human Genome U133 Plus 2.0 array.

# Database on GEO: GSE52889

### Human LCL aADHD MPH vs. Saline

| Name                                                  | # of Entities | Expanded # of Entities | # of Measured Entities | Median change | p-value       | Hit type               |
|-------------------------------------------------------|---------------|------------------------|------------------------|---------------|---------------|------------------------|
| nucleoplasm                                           | 2669          | 2669                   | 2518                   | -1.00722603   | 2.66938E-38   | cellular_component      |
| nucleus                                               | 6877          | 6877                   | 5961                   | -1.002796865  | 5.69797E-32   | cellular_component      |
| cytoplasm                                             | 6831          | 6831                   | 6084                   | -1.001717133  | 3.9469E-22    | cellular_component      |
| nucleotide binding                                    | 1946          | 1946                   | 1792                   | -1.004093337  | 2.59455E-17   | molecular_function      |
| regulation of transcription, DNAtemplated             | 2670          | 2670                   | 2279                   | 1.00486156    | 2.77711E-16   | biological_process      |
| transcription, DNAtemplated                           | 2430          | 2430                   | 2206                   | 1.005069899   | 1.33166E-15   | biological_process      |
| cytosol                                               | 3173          | 3173                   | 3034                   | -1.002656374  | 1.10898E-14   | cellular_component      |
| metal ion binding                                     | 3568          | 3568                   | 3178                   | 1.00150887    | 9.57377E-13   | molecular_function      |
| Golgi apparatus                                       | 1245          | 1245                   | 1151                   | -1.002474234  | 4.82146E-12   | cellular_component      |
| poly(A) RNA binding                                   | 1254          | 1254                   | 1109                   | -1.009325635  | 6.12536E-12   | molecular_function      |
| DNA binding                                           | 2411          | 2411                   | 2103                   | 1.002913502   | 7.75104E-11   | molecular_function      |
**Supplementary**

**Proteins Involved in Pathogenesis of Melanoma**

| Function                  | # of Entities | Expanded # of Entities | # of Measured Entities | Median change | p-value       | Hit type          |
|---------------------------|---------------|------------------------|------------------------|---------------|---------------|------------------|
| nucleic acid binding      | 1094          | 1094                   | 856                    | -1.001676871 | 1.06298E-10   | molecular_function|
| ATP binding               | 1601          | 1601                   | 1422                   | -1.002953279 | 6.15091E-10   | molecular_function|
| zinc ion binding          | 1359          | 1359                   | 1173                   | 1.003697079  | 8.0144E-10    | molecular_function|

**Proteins Involved in Pathogenesis of Neuroblastoma**

| Function                  | # of Entities | Expanded # of Entities | # of Measured Entities | Median change | p-value       | Hit type          |
|---------------------------|---------------|------------------------|------------------------|---------------|---------------|------------------|
| nucleic acid binding      | 1094          | 1094                   | 856                    | -1.001676871 | 1.06298E-10   | molecular_function|

**Human LCL Control MPH vs. Saline**

| Name                              | # of Entities | Expanded # of Entities | # of Measured Entities | Median change | p-value       | Hit type          |
|-----------------------------------|---------------|------------------------|------------------------|---------------|---------------|------------------|
| nucleoplasm                       | 2669          | 2669                   | 2518                   | 1.001257691  | 1.02974E-25   | cellular_component|
| nucleus                            | 6877          | 6877                   | 5961                   | 1.000046504  | 5.2368E-25    | cellular_component|
| transcription, DNA-templated      | 2430          | 2430                   | 2206                   | 1.005042845  | 1.53769E-22   | biological_process|
| regulation of transcription, DNA-templated | 2670          | 2670                   | 2279                   | 1.00449709   | 4.42731E-22   | biological_process|
| metal ion binding                 | 3566          | 3568                   | 3178                   | -1.000110916 | 2.42997E-18   | molecular_function|
| DNA binding                       | 2411          | 2411                   | 2103                   | 1.004503036  | 1.10098E-15   | molecular_function|
| cytoplasm                         | 6831          | 6831                   | 6084                   | -1.000488189 | 1.54986E-15   | cellular_component|
| positive regulation of transcription, DNA-templated | 623           | 623                    | 579                    | 1.007308911  | 6.68324E-13   | biological_process|
| nucleotide binding                | 1946          | 1946                   | 1792                   | -1.000554726 | 1.42437E-12   | molecular_function|
| nucleic acid binding              | 1094          | 1094                   | 856                    | 1.004358534  | 2.36262E-12   | molecular_function|
| transcription factor activity, sequence-specific DNA binding | 1125          | 1125                   | 961                    | 1.005918872  | 6.0621E-12    | molecular_function|
| negative regulation of transcription from RNA polymerase II promoter | 799           | 799                    | 709                    | 1.006048569  | 7.09537E-12   | biological_process|
| positive regulation of transcription from RNA polymerase II promoter | 1041          | 1041                   | 947                    | 1.006516349  | 2.51457E-11   | biological_process|
| negative regulation of transcription, DNA-templated | 590           | 590                    | 506                    | 1.006228895  | 3.27618E-11   | biological_process|
## Human LCL aADHD vs. Control (both saline)

| Name                    | # of Entities | Expanded # of Entities | # of Measured Entities | Median change | p-value      | Hit type               |
|-------------------------|---------------|------------------------|------------------------|---------------|--------------|------------------------|
| cell junction           | 705           | 705                    | 668                    | 1.000670164   | 3.13357E-13  | cellular_component     |
| plasma membrane         | 5390          | 5390                   | 3785                   | 1.000481876   | 2.72858E-11  | cellular_component     |
| cytoplasm               | 6831          | 6831                   | 6084                   | 1.003976041   | 3.50727E-11  | cellular_component     |
| synapse                 | 466           | 466                    | 431                    | 1.001119376   | 2.62418E-10  | cellular_component     |
| postsynaptic density    | 168           | 168                    | 164                    | 1.005543612   | 4.34769E-10  | cellular_component     |
| dendrite                | 396           | 396                    | 373                    | -1.002282386  | 4.59111E-08  | cellular_component     |
| axon                    | 318           | 318                    | 304                    | 1.000391067   | 4.45183E-07  | cellular_component     |
| neuron projection       | 378           | 378                    | 357                    | 1.00028352    | 5.35847E-07  | cellular_component     |
| axon guidance           | 385           | 385                    | 374                    | 1.000934601   | 1.1837E-06   | biological_process    |
| nervous system development | 523        | 523                    | 484                    | -1.000024763  | 1.71094E-06  | biological_process    |
| synaptic transmission   | 472           | 472                    | 454                    | -1.001928998  | 1.79574E-06  | biological_process    |
| neuronal cell body      | 466           | 466                    | 434                    | -1.002282386  | 4.89876E-06  | cellular_component     |
| dendritic spine         | 135           | 135                    | 127                    | 1.003774527   | 8.03048E-06  | cellular_component     |

## Human LCL aADHD MPH vs. Control MPH

| Name                                      | # of Entities | Expanded # of Entities | # of Measured Entities | Median change | p-value      | Hit type               |
|-------------------------------------------|---------------|------------------------|------------------------|---------------|--------------|------------------------|
| Secreted proteins                         | 7555          | 12985                  | 8259                   | 1.001211728   | 2.11719E-18  | Pathway Studio Ontology |
| plasma membrane                           | 5390          | 5390                   | 3785                   | 1.004115727   | 1.3399E-16   | cellular_component     |
| signal transduction                       | 1843          | 1843                   | 1618                   | 1.003692665   | 1.57567E-13  | biological_process    |
| membrane                                  | 7012          | 7012                   | 6263                   | -1.000478468  | 3.59447E-12  | cellular_component     |
| immune response                           | 468           | 468                    | 369                    | -1.003564706  | 3.95518E-11  | biological_process    |
| Proteins Involved in Pathogenesis of Melanoma | 245          | 264                    | 240                    | 1.00444264    | 6.62609E-11  | Disease Collections   |
| cell adhesion                             | 616           | 616                    | 564                    | 1.006635379   | 8.16755E-11  | biological_process    |
| negative regulation of transcription from RNA polymerase II promoter | 799           | 799                    | 709                    | -1.000651379  | 9.04343E-11  | biological_process    |
| positive regulation of GTPase activity    | 494           | 494                    | 446                    | 1.002430828   | 1.14063E-10  | biological_process    |
| Term (Gene Ontology)                                      | Gene Symbol | Gene Symbol | Gene Symbol | q-value     | p-value      |
|----------------------------------------------------------|-------------|-------------|-------------|--------------|--------------|
| positive regulation of transcription from RNA polymerase II promoter | 1041        | 1041        | 947         | -1.000158886 | 1.53068E-10  |
| cell surface                                             | 645         | 645         | 573         | 1.004718042  | 3.43588E-10  |
| Proteins Involved in Pathogenesis of Glioma              | 304         | 351         | 304         | -1.001578048 | 5.99891E-10  |
| negative regulation of cell proliferation                | 471         | 471         | 433         | -1.003522753 | 6.00697E-10  |
| Proteins Involved in Pathogenesis of Endometriosis       | 257         | 257         | 253         | 1.000672849  | 9.29616E-10  |
| axon guidance                                            | 385         | 385         | 374         | 1.007388538  | 1.8896E-06   |
| postsynaptic density                                     | 168         | 168         | 164         | 1.00902234   | 1.94917E-06  |
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