**Supplementary tables**

Table S1. Information of cell lines used in the present study

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Internal cell line identifier** | **Cell line name** | **Origin (Healthy, WT; Patient, PD)** | **Sex (Male, M; Female, F)** | **Age of sampling** | **Age of onset** | **Source** | **Reference** | **Karyotype** |
| 362 | Ctrl1 | WT | M | 30 |  | Coriell institute | GM25256\*I | Normal |
| 232 | Ctrl2 | WT | F | 53 | - | *Reinhardt et al., 2013* |  | Normal |
| 336 | 3xSNCA | PD | F | 55 | 50 | EBISC | EDi001-A | Normal |
| 320 | SNCAKO | WT | M | 67 | - | *Barbuti et al.,* 2020 |  |  |

Table S2. List of primers used for quantitative PCR

|  |  |  |  |
| --- | --- | --- | --- |
| **Gene** | **Sequence** | **Sense** | **Amplicon size** |
| LMX1B | ATCGTGGCCATGGAACAGAG | Forward | 177bp |
| GTCTGAGGAGCCGAGGAAG | Reverse |
| LMX1A | CAACTCAACAGAGGCGAGCATT | Forward | 128bp |
| GTTTTGGAACCACACCTGGAC | Reverse |
| EN1 | AACCGCTACATCACGGAGCA | Forward | 102bp |
| GATCTTGGCGCGCTTGTTCT | Reverse |
| ACTB | TCAAGATCATTGCTCCTCCTGAG | Forward | 87bp |
| ACATCTGCTGGAAGGTGGACA | Reverse |
| HOXA2 | CGTCGCTCGCTGAGTGCCTG | Forward | 92bp |
| TGTCGAGTGTGAAAGCGTCGAGG | Reverse |
| NKX2.1 | CGTACCAGGACACCATGAGG | Forward | 168bp |
| GGGCCATGTTCTTGCTCAC | Reverse |
| NKX6.1 | GCCTCGGAGAACGAGGAAGA | Forward | 106bp |
| CGCTGCTGGACTTGTGCTTC | Reverse |
| GBX2 | GTTCCCGCCGTCGCTGATGAT | Forward | 118bp |
| GCCGGTGTAGACGAAATGGCCG | Reverse |
| NKX2.2 | GAAGCGCCGAGTGCTCTTCTCC | Forward | 375bp |
| GCCGAGCTGTACTGGGCGTTGT | Reverse |
| HOXB4 | CTACTGCCGCTGCTGGAAGA | Forward | 122bp |
| TGTGTGTGTGTTACCGTGACCAA | Reverse |
| PAX7 | ACCCCTGCCTAACCACATC | Forward | 121bp |
| GCGGCAAAGAATCTTGGAGAC | Reverse |
| HOXC4 | GGCAGCTACCCCGGGTACT | Forward | 101bp |
| TGTGAGTTATGTTTTATAACCTGGTAATGTC | Reverse |
| OTX2 | GCCAATCCTTGGTTGAATCTTAGG | Forward | 120bp |
| CAATCAGTCACACAATTCACACAGC | Reverse |
| PAX6 | CCAGGGCAATCGGTGGTAGT | Forward | 84bp |
| ACGGGCACTCCCGCTTATAC | Reverse |
| RPL37A | GTGGTTCCTGCATGAAGACAGTG | Forward | 84bp |
| RPL37A | TTCTGATGGCGGACTTTACCG | Reverse |

Table S3. Antibody list and application

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Antibody** | **Species** | **Concentration** | **Source** | **Catalog number** | **RRID** | **Used in** |
| GABA | Guinea pig | 1:400 | Abcam | ab17413 | AB\_443865 | Immunostaining |
| GAD67 | Mouse | 1:400 | Sigma | G5419 | AB\_261978 | Immunostaining |
| Serotonin | Rat | 1:100 | Millipore | MAB352 | AB\_11213564 | Immunostaining |
| TPH2 | Rabbit | 1:500 | Abcam | ab111828 | AB\_10862137 | Immunostaining |
| TH | Rabbit | 1:1000 | Abcam | ab112 | AB\_297840 | Immunostaining |
| MAP2 | Chicken | 1:1000 | Abcam | ab5392 | AB\_2138153 | Immunostaining |
| MAP2 | Rabbit | 1:100 | Abcam | ab32454 | AB\_776174 | Immunostaining |
| ChAT | Rabbit | 1:100 | Thermo Fisher Scientific | PA5-29653 | AB\_2547128 | Immunostaining |
| OTX2 | Goat | 1:200 | Neuromics | GT15095 | AB\_2157174 | Immunostaining |
| GBX2 | Rabbit | 1:500 | Thermo Fisher Scientific | PA5-66953 | AB\_2662957 | Immunostaining |
| vGLUT2 | Mouse | 1:600 | Abcam | ab79157 | AB\_1603114 | Immunostaining |
| NET | Rabbit | 1:500 | NOVUS Biologicals | NBP1-60120 | AB\_11014401 | Immunostaining |
| nNOS | Guinea pig | 1:500 | Synaptic Systems | 432 005 | AB\_2832223 | Immunostaining |
| Neuropeptide Y | Chicken | 1:500 | Synaptic Systems | 394 006 | AB\_2814940 | Immunostaining |
| α-Synuclein (2A7) | Mouse | 1:1000 | NOVUS Biologicals | NBP1-05194 | AB\_1555287 | Immunostaining |
| α-Synuclein (211) | Rabbit | 1:1000 | Santa Cruz | sc-12767 | AB\_628318 | Western Blot |
| α-Synuclein [MJFR1] | Rabbit | 1:1000 | Abcam | ab138501 | AB\_2537217 | Dot Blot |
| Secondary AB Anti- rabbit IgG ECL | Donkey | 1:1000 | GE Healthcare | NA934 | AB\_772206 | Dot Blot |
| Phospho- α-Synuclein (Ser129) (D1R1R) | Rabbit | 1:500 | Cell Signaling  Technology | 23706S | AB\_2798868 | Immunostaining/  Western Blot |
| ß-actin | Mouse | 1:10000 | Cell Signaling  Technology | 3700 | AB\_2242334 | Western Blot |
| Tyrosine Hydroxylase | Mouse | 1:600 | Millipore | MAB5280 | AB\_2201526 | Immunostaining |
| Tyrosine Hydroxylase TH (H-196) | Rabbit | 1:1000 | Santa Cruz | sc-14007 | AB\_671397 | Western Blot |
| ßIII Tubulin | Mouse | 1:20000 | BioLegend | 801201 | AB\_2313773 | Western Blot |
| Anti-chicken 488 | Goat | 1:1000 | Invitrogen | A-11039 | AB\_142924 | Immunostaining |
| Anti-chicken 488 | Donkey | 1:1000 | Jackson Immuno  research | 703-545-155 | AB\_2340375 | Immunostaining |
| Anti-chicken 568 | Goat | 1:1000 | Invitrogen | A-11041 | AB\_2534098 | Immunostaining |
| Anti-rabbit 568 | Goat | 1:1000 | Invitrogen | A11036 | AB\_2534094 | Immunostaining |
| Anti-rabbit 568 | Donkey | 1:1000 | Invitrogen | A10042 | AB\_2534017 | Immunostaining |
| Anti-rabbit 647 | Goat | 1:1000 | Invitrogen | A-21244 | AB\_141663 | Immunostaining |
| Anti-mouse IgG1 647 | Goat | 1:1000 | Invitrogen | A-21240 | AB\_2535809 | Immunostaining |
| Anti-mouse IgG2a 647 | Goat | 1:1000 | Invitrogen | A-21241 | AB\_2535810 | Immunostaining |
| Anti-rat 568 | Goat | 1:1000 | Invitrogen | A-11077 | AB\_2534121 | Immunostaining |
| Anti-goat 647 | Donkey | 1:1000 | Invitrogen | A21447 | AB\_141844 | Immunostaining |
| Anti-guinea pig 647 | Goat | 1:1000 | Invitrogen | A21450 | AB\_141882 | Immunostaining |

Table S4. Percentages of different cell populations within the different models: midbrain organoids after assembly (Healthy-Healthy, Healthy-PD), Ctrl\_2-HBO model and PD-MO model, considered in the snRNAseq dataset

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Cell types** | **Healthy-Healthy (%)** | **Healthy-PD (%)** | **Ctrl\_2-HBO (%)** | **PD-MO (%)** |
| Oligodendrocytes | 0.733390854 | 0.016641704 | 33.27364439 | 0.063572791 |
| Astrocytes | 27.04918033 | 31.1699118 | 16.85498108 | 12.39669421 |
| Neural Progenitors | 7.722174288 | 0.93193543 | 20.81715006 | 0.445009536 |
| Dopaminergic Neurons | 48.66264021 | 46.19737061 | 6.625472888 | 38.33439288 |
| Dopaminergic Progenitors | 0.992234685 | 0.798801797 | 7.41740227 | 43.99237127 |
| GABAergic Neurons | 13.89128559 | 18.48893327 | 0.035308953 | 4.640813732 |
| Neural Progenitors 2 | 0.086281277 | 2.313196871 | 3.442622951 | 0 |
| Oligodendrocyte Progenitors | 0.819672131 | 0.033283408 | 3.286254729 | 0 |
| Neural Progenitors 3 | 0 | 0 | 2.549810845 | 0 |
| Reactive Astrocytes | 0 | 0 | 2.292559899 | 0 |
| Dopaminergic Progenitors 2 | 0 | 0.049925112 | 2.14627995 | 0 |
| Dopaminergic Neurons 2 | 0.043140638 | 0 | 1.25851198 | 0.127145582 |