STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
p-α-synuclein (Ser129) (1:500)	Cell Signaling Technology	Cat#23706: RRID:AB_2798868
α-synuclein oligomeric (1:500)	Merck Millipore	Cat#ABN2265; RRID:AB_2910172
PGC-1α (1:100)	Santa Cruz Biotechnology	Cat#sc-518025; RRID:AB_2890187
NDUFS1 (1:5000)	Abcam	Cat#AB169540; RRID:AB_2687932
NRF2 (1:1000)	Cell Signaling Technology	Cat#12721; RRID:AB_2715528
PARKIN (1:500)	Santa Cruz Biotechnology	Cat#sc-32282; RRID:AB_628104
BAX (1:500)	Santa Cruz Biotechnology	Cat#sc-20067; RRID:AB_626726
BCL-XL (1:1000)	Cell Signaling Technology	Cat#2762; RRID:AB_10694844
GAPDH (1:1000)	Bioss, ThermoFisher Scientific	Cat#BS-10900R; RRID:AB_3661955
Peroxidase AffiniPure Donkey Anti-Rabbit IgG (1:10000)	Jackson ImmunoResearch	Cat#711-035-152; RRID:AB_10015282
NANOG (rabbit) (1:200)	Invitrogen, ThermoFisher Scientific	Cat#PA1-097; RRID:AB_2539867
OCT4 (1:200)	Cell Signaling Technology	Cat#75463; RRID:AB_2799870
SOX2 (1:500)	Abcam	Cat#Ab97959; RRID:AB_2341193
TRA1-60 (1:100)	Invitrogen, ThermoFisher Scientific	Cat#41-1000; RRID:AB_2533494
BRAT (1:20)	R&D System	Cat#AF2085; RRID:AB_2200235
NESTIN (1:1000)	STEMCELL Technologies	Cat#60091; RRID:AB_2650581
SOX17 (1:20)	R&D System	Cat#AF1924; RRID:AB_355060
FOXA2 (1:1000)	Santa Cruz Biotechnology	Cat#sc-101060; RRID:AB_1124660
LMX1A (1:200)	Invitrogen, ThermoFisher Scientific	Cat#PA5-115517; RRID:AB_2900153
ZO1 (1:300)	Invitrogen, ThermoFisher Scientific	Cat#33-9100; RRID:AB_87181
GIRK2 (1:100)	Abcam	Cat#Ab65096; RRID:AB_1139732
CALB (1:200)	Cell Signaling Technology	Cat#BK13176S; RRID: AB_2687400
TH (1:100)	Invitrogen, ThermoFisher Scientific	Cat#MA1-24654; RRID:AB_795666
DDC (1:200)	Invitrogen, ThermoFisher Scientific	Cat#PA547512; RRID:AB_2609231
NFL (1:100)	Cell Signaling Technology	Cat#2837; RRID: AB_823575
MAP2 (1:1000)	Invitrogen, ThermoFisher Scientific	Cat#PA-10005; RRID: AB_1076848
TH (1:2000)	Antibodies	Cat#A104316-100
TOM20 (1:400)	Proteintech	Cat#66777; RRID:AB_2919694
Anti-Rabbit IgG Alexa Fluor 488 PLUS (1:2000)	Life Technologies, ThermoFisher Scientific	Cat#A32731; RRID:AB_2633280
Anti-Rabbit IgG Alexa Fluor 594 (1:500)	Life Technologies, ThermoFisher Scientific	Cat#A-11012; RRID:AB_141359
Anti-Chicken IgY (H + L) Alexa Fluor 594 (1:200)	Life Technologies, ThermoFisher Scientific	Cat#A-32759; RRID:AB_2762829
Anti-Mouse IgG Alexa Fluor 488 (1:2000)	Life Technologies, ThermoFisher Scientific	Cat#A-11001; RRID:AB_2534069
Anti-Goat IgG Alexa Fluor 594 (1:500)	Life Technologies, ThermoFisher Scientific	Cat#A-11058; RRID:AB_142540
Anti-Mouse IgG Alexa Fluor 594 (1:200)	Life Technologies, ThermoFisher Scientific	Cat#A-11005; RRID:AB_141372
Deposited data		
GEO	Series accession number GSE294029	
Oligonucleotides		
OCT4	GGAGGAAGCTGACAACAATGAA	GGCCTGCACGAGGGTTT
NANOG	TGCAAGAACTCTCCAACATCCT	ATTGCTATTCTTCGGCCAGTT
SOX2	ATGCACCGCTACGACGTGA	CTTTTGCACCCCTCCCATTT
GATA4	GGCCTGTCATCTCACTACGG	ATGGCCAGACATCGCACT
HAND1	CCAGCTACATCGCCTACCTG	CCGGTGCGTCCTTTAATCCT
TUBB3	AACGAGGCCTCTTCTCACAA	TTTTCACACTCCTTCCGCAC

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
CNPY1	GGAAGACCCTGTGACGAAGG	TCCTGGGCGATAAGTGAGGA
CORIN	CCCCGGGAAACTGCAATGTA	GCGATGCTCTGTTGTGGGAT
EN1	GCCCGTGGTCAAAACTGACT	GGAACTCCGCCTTGAGTCTC
NGN2	ACATGGACTATTGGCAGCCC	AGTGAGATGGTTTCCAGGGC
FOXA2	CTGGTCGTTTGTTGTGGCTG	CGTGTTCATGCCGTTCATCC
LMX1A	AACATGCTGGACGGCCTAAA	ATGCCAGAAGCTGTCGTTGA
LMX1B	CGGACTGCGCCAAGATGTT	TTGACTCGCATCAGGAAGCG
SHH	TGGACATCACCACGTCTGAC	GGAAGCAGCCTCCCGATTT
TH	TGTACTGGTTCACGGTGGAGT	TCTCAGGCTCCTCAGACAGG
OTX1	CGTTCACAGCTGGACGTG	CTTTCGGAGCCCGAGCTC
OTX2	CTGTTTGCCAAGACCCGGTA	TGGCCACTTGTTCCACTCTC
NURR1	TGCCGATTTCAGAAGTGCCT	CGAGGGCACTGATCAGACTC
DDC	GCCGCTATCATGGAGAAGCT	AGAAAGGAATCAGGCCAGCC
GIRK2	CACATCAGCCGAGATCGGAC	GGTAGCGATAGGTCTCCCTCA
CALB1	ATCCCTCATCACAGCCTCAC	TTTGCCCATACTGATCCACA
DAT	GTCTGTTTGGATTGACGCGG	AAGGAGAAGACGACGAAGCC
CCNB1	GTTGGTGTCACTGCC	TGGCCAAAGTATGTT
POLQ	GGTCTGATCAATCGC	AGCAGATACCCTCGG
SLC7A11	CTGACCATCTGGACGGTGTG	TGAGGAGTTCCACCCAGACT
QPCTL	CTCACCCTTGCCTGCCATTA	CGCCTCTTCACCATCCAAGA
REST	CTCATACAGGAGAACGCCCA	TGCATGGCGGGTTACTTCAT
RPL7L1	ATGATTCCTGGCGGCAGAAA	TTGCAATGGTTCTCTGCACC
VPS13A	GCTGGCTATGGTCTTGGTCA	GTTGGATCAACTGCTGTTTCACT
VIM	TGAAGGAGGAAATGGCTCGT	CCTCAGGTTCAGGGAGGAAA
SOX9	AGCTCTGGAGACTTCTGAACGAGA	CGTTCTTCACCGACTTCCTCCGC
CDK6	CCGTTTCGTGGCGTTGAAG	TCTGTTCGTGACACTGTGCA
GJA1	AGAACTCAAGGTTGCCCAAA	GATGATGTAGGTTCGCAGCA
ZNF215	AGGAACCTGAATTCATTGCGT	AGGCATCTGAAGAAGGGTGT
ZZZ3	ACCCTCAGAGGCAAGACTCA	CTGAGCCTCGAGTACAGCAA
SESN3	TGAAGAGGCGTCTCAAGAAGAA	CTCTTCTCCTCGTCTGGCAA
CXCR4	CTGGCCTTCATCAGTCTGGA	TCATCTGCCTCACTGACGTT
RB1CC1	CTTGAAGATCGGCTCTACGC	TCATCAACTGATTTGCGTGAC
HIF1A	GCCAGATCTCGGCGAAGTAA	ACCATAACAAAACCATCCAAGGC
ATF6	TGCTAGGGTTAGAGGCGAGA	CACAGACAACTCTTCGCTTTGG
GSK3B	CCACAGAACCTCTTGTTGGA	CGTGTAATCAGTGGCTCCAA
RPS8	GGCTATGTGCTAGAGGGCAA	CAGGCAACATAAATGTGGGAACA
RPS15A	CTTATTAGGCCGTGCTCCAA	TTCTGCCATTTTTCCAGGTC
BRCA1	TGGATTTATCTGCTCTTCGCGT	GGACACTGTGAAGGCCCTTT
USP1	AGAGGACTTGGGGAAGTGTGA	TTCACATTCCAAGCAACGCG
HNRNPU	CACTTCGATGACACAGTGGT	GTGACCAGCCAATACGAACT
CDK3	TCAAGGAACTGAAGCACCCC	TGAGTGGCAGAAACTCACCC
EN2	TGGGTCTACTGTACGCGCTA	CCGTCAGGTACCTGTTGGTC
ADAR	CAGACCCGCGGAGTTTCC	TGGTACCTGAGCTGTCTGTG
ZNF480	CCCTGGTCTGGTGAGAGTGA	CCCTTTCATCTGGAAATAGCTCCA
SPB1	CCCCAATGTGGTGACTCTCC	ACAAGCCAAACGTAGAGCCA
CANX	ATTGAGGACCCAGAAGACCG	CTGCGTCTGGATCAGGTACG
CCNE2	GGGGGATCAGTCCTTGCATT	TGTCATGAACATATCTGCTCTCCT
SOD2	CACTAGCAGCATGTTGAGCC	CCTGGTACTTCTCCTCGGTG
GPX1	TATCGAGAATGTGGCGTCCC	CCGGACGTACTTGAGGGAAT

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
NRF2	CATCCAGTCAGAAACCAGTGG	AATGAAGACTGGGCTCTCGA
MFN2	TGCTAAGGAGGTGCTCAACG	CGTGTGCTGCTCAAACTTGG
FIS1	GAGCACGCAGTTTGAGTACG	ACCCGCGGACGTACTTTAAG
OPA1	TGCCTGACATTGTGTGGGAA	AGAGAAGTAGGTCAGAAGCTCCT
DRP1	ACCCGTGGATGATAAAAGTGCT	AGGTTCGCCCAAAAGTCTCA
MFN1	CCCTCTTGAGAGATGACCTGG	GGAAAGCCGCTCATTCACCT
BAX	GGACGAACTGGACAGTAACATGG	GCAAAGTAGAAAAGGGCGACAAC
FAS	GGAGTACACAGACAAAGCCCA	TTTGGTGCAAGGGTCACAGT
CELF2	GCCAGATAGAAGAATGCCGGA	GTGCCATTGCCCTTGTAGA
PEG3	GGGCCACTCATCAAGATCCA	TTCCCGATTTGGAACTGCGT
GAPDH	TCCTCTGACTTCAACAGCGA	GGGTCTTACTCCTTGGAGGC
Critical commercial assay		
Assay ID	475360	hsa-miR 5683
Assay ID	464290	hsa-miR 3085-3p
Assay ID	003188	hsa-miR 124-3p
Assay ID	462668	hsa-miR 219b-5p
Assay ID	002144	hsa-miR 138-2-3p
Assay ID	002356	hsa-miR 873-5p
Assay ID	002390	hsa-miR 219a-2-3p
Assay ID	000437	hsa-miR 100-5p
Assay ID	002222	hsa-miR 1-3p
Assay ID	002315	hsa-miR 10b-3p
Assay ID	001187	hsa-miR 140-5p
Assay ID	000200	cell-miR 39-3p
Assay ID	001973	snRNA U6
Software and algorithms		
GraphPadPrism (version 9.3.1)		N/A
ImageJ Fiji		N/A
Biorender		N/A

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

All experimental protocols were approved by the Institutional Review Board (IRB) of the "*Comitato Etico Territoriale Regione Calabria"* (**approval number #143, 13-05-2024**), in accordance with ethical guidelines and regulatory standards. Informed consent was obtained from all participants prior to sample collection. The study was conducted in compliance with the principles outlined in the Declaration of Helsinki. The use of human induced pluripotent stem cells (iPSCs) was approved by the Ethics Committee of the University of Catanzaro, with strict adherence to protocols ensuring donor anonymity and data protection.

METHOD DETAILS

Generation of PD patient-induced pluripotent stem cells (iPSCs)

The generation and characterization of healthy donor and PSP-RS patient-derived hiPSC lines were previously described,[21] with the exception of the HC_001-line, detailed previously.[43] For this study, we additionally recruited four sporadic PD patients, carefully matched by sex and age to existing iPSC donors (Ethnicity of all lines: Caucasian). Blood samples were collected in accordance with protocols approved by the University 'Magna Graecia' of Catanzaro and the Azienda Ospedaliero-Universitaria "Renato Dulbecco" of Catanzaro, Italy. PD iPSC lines were generated from peripheral blood mononuclear cells (PBMCs) using the CytoTune^{TM}-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific), following the manufacturer's instructions with minor modifications. A detailed protocol is available in.[21] The characterization of iPSC lines PD-1, PD-2, and PD-4 has been reported previously.[23] In this study, we further characterized the PD-3 iPSC line, confirming its expression of pluripotency markers and trilineage differentiation capacity, validating its use for downstream disease modeling (Figure S1).

Generation of midbrain organoids (MOs)

Midbrain organoids (MOs) were generated based on a previously established protocol,[44] with modifications in the initial phase to enable the formation of multi-donor organoids by pooling healthy and patient-derived iPSCs, as described in.[21] iPSCs were cultured on Matrigel-coated dishes (Corning, Corning, NY, USA) in mTeSR1 medium (StemCell Technologies, Vancouver, BC, Canada) and dissociated using Gibco™ StemPro™ Accutase™ (Thermo Fisher Scientific, Waltham, MA, USA). Cell suspensions from three healthy controls, four PSP-RS patients, and four PD patients were mixed in equal ratios. A total of 10,000 cells per well were seeded in ultra-low attachment 96-well U-bottom plates (Corning, NY, USA) in mTeSR1 medium with 10 μM Y-27632 (Miltenyi Biotec, Bergisch-Gladbach, Germany) to initiate MO formation. After three days, embryoid bodies (EBs) were transferred to a differentiation medium consisting of a 1:1 mix of DMEM/F12 and Neurobasal medium, supplemented with 1:100 N2, 200 mM L-glutamine, and 10,000 U/mL penicillin/streptomycin (Thermo Fisher Scientific). The medium was further enriched with 10 μM SB431542, 100 ng/mL recombinant human Noggin (rhNoggin), 300 ng/mL SHH-C24II, and 1.5 μM CHIR99021 (Miltenyi Biotec). On day 8, the medium was supplemented exclusively with 100 ng/mL FGF-8b (Miltenyi Biotec). From days 11–14, organoids were maintained in Neurobasal medium with 1:50 B27 without vitamin A, 200 mM L-glutamine, and antibiotics, along with 100 ng/mL FGF-8b, 20 ng/mL brain-derived neurotrophic factor (BDNF), and 200 μM L-ascorbic acid. On day 14, organoids were embedded in Matrigel Matrix (Corning) and transferred to long-term differentiation medium containing B27 supplement, 20 ng/mL BDNF, 10 ng/mL glial cell line-derived neurotrophic factor (GDNF) (R&D Systems, Bio-Techne), 200 μM L-ascorbic acid, 500 μM cyclic AMP (Sigma-Aldrich, St. Louis, MO, USA), and 1 μM DAPT (Tocris, Bio-Techne). This multi-donor MO approach enhances the modeling of inter-individual variability, offering a more representative platform for investigating neurodegenerative disease mechanisms.

RNA extraction, reverse transcription, and quantitative real-time PCR

Total RNA was extracted using TRIzol Reagent (Thermo Fisher Scientific), following the manufacturer's instructions. Reverse transcription was performed using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific). Quantitative real-time PCR (qPCR) was carried out using the SensiFAST SYBR Hi-ROX Kit (Meridian Bioscience) on a QuantStudio™ 7 Pro Real-Time PCR System (Applied Biosystems). Gene expression levels were analyzed using the comparative Ct (cycle threshold) method, with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) used as the endogenous control for normalization. For miRNA validation in individual organoids, reverse transcription was performed using the TaqMan™ MicroRNA Reverse Transcription Kit (Thermo Fisher Scientific), followed by quantitative PCR using the TaqMan™ Fast Advanced Master Mix on a StepOnePlus™ Real-Time PCR System (Applied Biosystems). Primer sequences and TaqMan MicroRNA Assay used in this study are listed in the key resources table in the STAR Methods section.

Immunofluorescence

For immunofluorescence analysis, organoids were fixed in 4% paraformaldehyde (PFA) at room temperature. After fixation, they were incubated overnight in a 30% (w/v) sucrose solution at 4°C on an orbital shaker for cryoprotection, then embedded in Optimal Cutting Temperature (OCT) compound (Avantor). Organoids were sectioned at 20 μm thickness and post-fixed in 4% PFA for 10 min. Sections were then blocked for 1 h at room temperature in a solution containing 5% (v/v) goat serum (Thermo Fisher Scientific) and 0.3% (v/v) Triton X-100 (Sigma-Aldrich) in 1× PBS. Following blocking, sections were incubated overnight at 4°C with primary antibodies targeting specific proteins of interest. After primary incubation, sections were treated with Alexa Fluor 488-, 594-, or 647-conjugated secondary antibodies (Thermo Fisher Scientific) for 1 h at room temperature. Nuclear staining was performed using DAPI (Thermo Fisher Scientific), and coverslips were mounted with Fluormount Aqueous Mounting Medium (Sigma-Aldrich). Images were acquired using a MIKA Leica fluorescence microscopy system. A complete list of antibodies used is provided in the key resources table in the STAR Methods section.

Protein extraction and immunoblotting

Proteins were extracted from organoids by mechanical disruption through pipetting in RIPA buffer, composed of 150 mM sodium chloride, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS) (all from Sigma-Aldrich), and 50 mM Tris-HCl (pH 7.5; Gibco), supplemented with Halt™ Protease and Phosphatase Inhibitor Cocktail (Thermo Fisher Scientific). Extracts were sonicated using a Diagenode Bioruptor (10 cycles of 30 s ON/30 s OFF) and then incubated on ice for 30 min. Supernatants were collected by centrifugation at 21,000 × g for 1 h at 4°C to remove insoluble debris. Protein concentrations were determined using the Bradford assay (Bio-Rad). Equal amounts of protein (25 μg) were diluted in 1× Bolt™ LDS Sample Buffer with Bolt™ Reducing Agent (Thermo Fisher Scientific) and denatured at 70°C for 10 min. Proteins were resolved using either Bolt™ 4–12% Bis-Tris Plus gels with Bolt™ MES SDS running buffer (20×) or NuPAGE™ 3–8% Tris-Acetate gels with NuPAGE™ Tris-Acetate SDS buffer (20×), specifically for detecting oligomeric α-synuclein (Thermo Fisher Scientific). Proteins were transferred onto nitrocellulose membranes (Bio-Rad) using the Trans-Blot Turbo™ Transfer System (Bio-Rad). Membranes were blocked for 1 h at room temperature in 5% non-fat dry milk (PanReac AppliChem) to prevent non-specific binding. Primary antibodies were incubated overnight at 4°C, followed by 1 h incubation at room temperature with HRP-conjugated secondary antibodies (Jackson ImmunoResearch). Signals were detected using Clarity™ ECL substrate (Bio-Rad), and images were acquired with the Alliance™ Q9-Atom imaging system (Uvitec). Band intensities were quantified using ImageJ software, with GAPDH used as the internal loading control. The specific antibodies used for immunoblotting are listed the key resources table in the STAR Methods section.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analyses were performed using unpaired *t*-tests with Welch's correction or one-way ANOVA test with Tukey's correction in GraphPad Prism (version 9.3.1). Data are presented as the mean ± standard error of the mean (SEM), based on two or three biological replicates. Statistical significance is indicated as follows: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$. All experiments—including Western blotting, immunofluorescence, and RT-qPCR—were conducted with technical replicates using at least three organoids from three independent differentiation batches.

miRNA sequencing

Library construction, quality control, and sequencing

Total RNA was used as the input material for small RNA library preparation. Adapters were first ligated to the 3′ and 5′ ends of the small RNA molecules. Following adapter ligation, first-strand complementary DNA (cDNA) synthesis was performed via reverse transcription primer hybridization. The resulting cDNA was then PCR-amplified to generate double-stranded cDNA libraries. Libraries were purified and size-selected to enrich for fragments with insert sizes between 18 and 40 base pairs. Final libraries were prepared for single-end sequencing (SE50) on an Illumina platform, with a read length of 50 bases. Library quality and concentration were assessed using a Qubit fluorometer (Thermo Fisher Scientific) and real-time PCR. Fragment size distribution was verified using a Bioanalyzer (Agilent Technologies). Qualified libraries were pooled based on effective concentration and desired sequencing depth, then sequenced on Illumina platforms for downstream analysis.

Bioinformatics analysis

Data preprocessing and quality control

Raw sequencing data in FASTQ format were processed using custom Perl and Python scripts. Clean reads were obtained by filtering out sequences containing poly-Ns, 5′ adapter contaminants, incomplete 3′ adapters or insert tags, as well as low-quality reads. Reads consisting of homopolymeric stretches (poly A, T, G, or C) were also excluded. Quality metrics, including Q20 and Q30 scores and GC content, were calculated to assess the overall data quality.

Mapping and miRNA identification

Cleaned small RNA tags were aligned to the reference genome using Bowtie,[45] allowing for zero or one mismatch. Known microRNAs (miRNAs) were identified using miRBase v22.0 as a reference. To detect novel miRNAs and examine the secondary structure of unannotated tags, we employed modified versions of *mirDeep2*[46] and *srna-tools-cli*. Custom scripts were used to quantify miRNA read counts and assess nucleotide bias at both the first base and across all positions of identified miRNAs. To eliminate non-miRNA tags, reads were also mapped to databases such as RepeatMasker and Rfam, and against species-specific genome annotations, filtering out tags derived from protein-coding genes, repeat elements, rRNAs, tRNAs, snRNAs, and snoRNAs.

Novel miRNA prediction

Novel miRNAs were predicted based on the characteristic hairpin structures of miRNA precursors. Software tools including *miREvo*[47] and *mirDeep2* were used to evaluate secondary structures, Dicer cleavage sites, and the minimum free energy of novel miRNA candidates. Additional custom scripts facilitated miRNA count generation and base bias analysis.

Annotation summary

All alignments and annotations were integrated and summarized. Because some small RNA tags could map to multiple categories, a hierarchical priority rule was applied: known miRNA > rRNA > tRNA > snRNA > snoRNA > repeat > gene > NAT-siRNA > novel miRNA > ta-siRNA. The proportion of rRNA was used as a sample quality metric, with values below 40% considered acceptable.

miRNA quantification and differential expression analysis

miRNA expression levels were quantified as transcripts per million (TPM) using the following formula: Normalized expression = (mapped read count/total reads) × 1,000,000 Differential expression analysis between experimental groups was performed using *DESeq2*. *p*-values were adjusted using the Benjamini–Hochberg method, with an adjusted p-value <0.05 considered statistically significant.

Target prediction and enrichment analysis

Target genes for differentially expressed miRNAs were predicted using the get_multimir function from the *multiMiR* R package,[48] focusing on experimentally validated targets in *miRTarBase*.[49]

Venn diagram analysis

To identify disease-associated miRNAs consistently upregulated across all three time points, Venn diagram analysis was performed using *Venny* (https://bioinfogp.cnb.csic.es/tools/venny/).

GO enrichment analysis

Gene Ontology (GO) enrichment analysis was performed on predicted target genes of differentially expressed miRNAs using the *ClusterProfiler* R package (v4.12.2[50]), considering all three GO domains: biological process, cellular component, and molecular function. Initially, we conducted enrichment analysis on the combined set of target genes from all selected miRNAs across the three comparison groups (PD vs. HC, PSP-RS vs. HC, and PSP-RS vs. PD). Subsequently, GO enrichment was performed for each selected miRNA individually, based on its predicted target genes, to uncover functional categories specifically associated with individual miRNA signatures.