

Supplementary file S1. The details of the method parameters for proteins identification and quantification used for melanocytes analyses following their UVA (18 J/cm²) irradiation and 24h incubation with cannabigerol (CBG, 1 µM), 3-O-ethyl ascorbic acid (EAA, 150 µM) and both compounds used together (CBG + EAA).

- A. Method of UHPLC Dionex UltiMate3000 RSLCnano System** (Dionex, Idstein, Germany) with a 150 mm x 75 µm PepMap RSLC capillary analytical C18 column (Dionex, LC Packings)

Instrument Setup

UV.TimeConstant	0.12 [s]
UV.Data_Collection_Rate	10.0 [Hz]
Sampler.Temperature.Nominal	5.0 [°C]
PumpModule.LoadingPump.Pressure.LowerLimit	0 [bar]
PumpModule.LoadingPump.Pressure.UpperLimit	500 [bar]
PumpModule.NC_Pump.Pressure.LowerLimit	2 [bar]
PumpModule.NC_Pump.Pressure.UpperLimit	800 [bar]
PumpModule.NC_Pump.MaximumFlowRampUp/Down	0.300 [µl/min ²]
ColumnOven.Temperature.Nominal	40.0 [°C]
UV.UV_VIS_1.Wavelength	214 [nm]

Time 0.000

Inject Preparation

Sampler.Inject

Start Run

Duration	60.000 [min]
PumpModule.LoadingPump.Flow.Nominal	5.000 [µl/min]
PumpModule.NC_Pump.Flow.Nominal	0.300 [µl/min]
PumpModule.NC_Pump.%B.Value	4.0 [%]

Time 3.000

PumpModule.NC_Pump.Flow.Nominal	0.300 [µl/min]
PumpModule.NC_Pump.%B.Value	4.0 [%]

Time 40.000

PumpModule.NC_Pump.Flow.Nominal	0.300 [µl/min]
PumpModule.NC_Pump.%B.Value	55.0 [%]

Time 44.000

PumpModule.NC_Pump.Flow.Nominal	0.300 [µl/min]
PumpModule.NC_Pump.%B.Value	90.0 [%]

Time 50.000

PumpModule.NC_Pump.Flow.Nominal	0.300 [µl/min]
PumpModule.NC_Pump.%B.Value	4.0 [%]

Time 60.000

PumpModule.LoadingPump.Flow.Nominal	5.000 [µl/min]
PumpModule.NC_Pump.Flow.Nominal	0.300 [µl/min]
PumpModule.NC_Pump.%B.Value	4.0 [%]

Stop Run

End

B. Method of O Exactive HF with an electrospray ionization source (ESI) (Thermo Fisher Scientific, Bremen, Germany)

Overall method settings

Global Settings

Chrom. peak width (FWHM) 15 s

Time

Method duration 60.00 min

Full MS/dd-MS² (TopN)

General

Runtime 0 to 60 min

Polarity positive

In-source CID 0.0 eV

Default charge state 2

Full MS

Microscans 1

Resolution 120,000

AGC target 3e6

Maximum IT 100 ms

Number of scan ranges 1

Scan range 200 to 2000 m/z

Spectrum data type Profile

dd-MS²/dd-SIM

Microscans 1

Resolution 30,000

AGC target 1e5

Maximum IT 50 ms

Loop count 5

MSX count 1

TopN 5

Isolation window 4.0 m/z

Isolation offset 0.0 m/z

Scan range 200 to 2000 m/z

Spectrum data type Profile

dd Settings

Minimum AGC target 1.00e3

Intensity threshold 2.0e4

Multiple charge states all

Peptide match preferred

Exclude isotopes on

Dynamic exclusion 10.0 s

C. Method of data processing using Proteome Discoverer 2.0 (Thermo Fisher Scientific, Bremen, Germany)(searched against the UniProtKB - SwissProt database (taxonomy: Homo sapiens, release 2025-04))

Workflow of processing step

Processing node 1: Spectrum Selector

General Settings:

- Precursor Selection: Use MS1 Precursor
- Use New Precursor Reevaluation: True
- Use Isotope Pattern in Precursor Reevaluation: True

Spectrum Properties Filter:

- Lower RT Limit: 0
- Upper RT Limit: 0
- First Scan: 0
- Last Scan: 0
- Lowest Charge State: 0
- Highest Charge State: 0
- Min. Precursor Mass: 350 Da
- Max. Precursor Mass: 5000 Da
- Total Intensity Threshold: 0
- Minimum Peak Count: 1

Scan Event Filters:

- MS Order: Is Not MS1
- Min. Collision Energy: 0
- Max. Collision Energy: 1000
- Scan Type: Is Full

Peak Filters:

- S/N Threshold (FT-only): 1.5

Replacements for Unrecognized Properties:

- Unrecognized Charge Replacements: Automatic
- Unrecognized Mass Analyzer Replacements: ITMS
- Unrecognized MS Order Replacements: MS2
- Unrecognized Activation Type Replacements: CID
- Unrecognized Polarity Replacements: +
- Unrecognized MS Res@200 Replacements: 60000
- Unrecognized MSn Res@200 Replacements: 30000

Precursor Pattern Extraction:

- Precursor Clipping Range Before: 2.5 Da
- Precursor Clipping Range After: 5.5 Da

Processing node 2: MS Amanda

Input Data:

- Protein Database: Homo sapiens, release 2018-04
- Enzyme Name: Trypsin
- Missed Cleavages: 2
- MS1 tolerance: 5 ppm
- MS2 tolerance: 0.02 Da

Dynamic Modifications:

- Dynamic Modification: Carbamidomethyl
Substitution= "H(3) C(2) N O" PositionType="Any" />
- Dynamic Modification: Carbamyl
Substitution= "H C N O" PositionType="Any" />
- Dynamic Modification: 4-HNE
Substitution= "H(16) C(9) O (2)" PositionType="Any" />

Additional Settings:

- Max No. of same mods: 3

- Max No. of dynamic mods: 4
- Ion Settings: b,y
- Max. Rank: 5
- Max number of same neutral losses (H₂O, NH₃): 1
- No. of considered NLs (modifications): 2
- Perform deisotoping: True
- Use monoisotopic mass: True

Processing node 3: Percolator

Input Data:

- Maximum Delta Cn: 0.05
- Decoy Database Search:
- Target FDR (Strict): 0.01
- Target FDR (Relaxed): 0.05
- Validation based on: q-Value

Processing node 5: Event Detector

General Settings:

- Mass Precision: 2 ppm
- S/N Threshold: 1

Processing node 6: Precursor Ions Area Detector

No parameters

Workflow of consensus step

Processing node 1: PSM Grouper

Peptide Group Modifications:

- Site Probability Threshold: 75
- Display Options:
- Modification Sites Shown: Best Position

Processing node 2: Peptide Validator

General Validation Settings:

- Validation Mode: Automatic (Control peptide level error rate if possible)
- Target FDR (Strict) for PSMs: 0.01
- Target FDR (Relaxed) for PSMs: 0.05
- Target FDR (Strict) for Peptides: 0.01
- Target FDR (Relaxed) for Peptides: 0.05

Specific Validator Settings:

- Validation Based on: q-Value
- Use Concatenated FDR Calculation for PSM Level FDR Calculation Based on Score: False
- Reset Confidences for Nodes without Decoy Search (Fixed score thresholds): False

Processing node 3: Peptide and Protein Filter

Peptide Filters:

- Peptide Confidence At Least: Medium
- Keep Lower Confident PSMs: False
- Minimum Peptide Length: 6
- Remove Peptides Without Protein Reference: False

Protein Filters:

- Minimum Number of Peptide Sequences: 1
- Count Only Rank 1 Peptides: False
- Count Peptides Only for Top Scored Protein: False

Processing node 4: Protein Scorer

No parameters

Processing node 5: Protein Grouping

Protein Grouping:

- Apply strict parsimony principle: True

Processing node 6: Protein FDR Validator

Confidence Thresholds:

- Target FDR (Strict): 0.01

- Target FDR (Relaxed): 0.05

Processing node 7: Peptide and Protein Quantifier

Ratio Calculation:

- Minimum Quan Value Threshold: 0.0001

- Replace Missing Quan Values With Minimum Intensity: False

- Reject All Quan Values If Not All Quan Channels Are Present: False

- Maximum Allowed Fold Change: 100

- Use Ratios Above Maximum Allowed Fold Change for Quantification: False

- Create Separate Quan Columns: False

Ratio Calculation for Precursor Quan:

- Use Single-Peak Quan Channels: False

Ratio Calculation for Reporter Quan:

- Apply Quan Value Corrections: True

- Co-Isolation Threshold: 100

Protein Quantification:

- Use Only Unique Peptides: True

- Consider Proteins Groups for Peptide Uniqueness: True

- Top N Peptides Used for Area Calculation: 3

Normalization:

- Experimental Bias Correction: None

- Minimum Ratio Count for Median Normalization: 20

- Manual Normalization Factor: 1

Display Options:

- Show the Raw Quan Values: True

- Show Standard Error: True

- Show Ratio Variabilities: False

- Show Ungrouped Ratios: False

- Show Ratio Counts: False

Quan Ratio Distributions:

- 1st Fold Change Threshold: 2

- 2nd Fold Change Threshold: 4

- 3rd Fold Change Threshold: 6

- 4th Fold Change Threshold: 8

- 5th Fold Change Threshold: 10