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## Untargeted MS-based lipidomic analyses

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Maria Jose Perez J.<sup>1</sup>

<sup>1</sup>Institut Imagine

Team Deleidi



Federico Bertoli

ASAP

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**We use this protocol and it's working**

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## Abstract

Untargeted MS-based lipidomic analyses



- 1 Collect iPSC-derived microglia and prepare cell pellets. Flash-freeze cell pellets to be shipped on dry ice (our pellets were sent to Lipotype GmbH, Dresden, Germany)
- 2 Samples were processed using a modified chloroform/methanol extraction protocol with internal standards specific to each lipid class (Ref. main text Surma et al., 2021).
- 3 Lipid extracts were analyzed on a Q Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific) equipped with a Triversa Nanomate automated nano-ESI source.
- 4 Measurements were performed in both positive and negative ion modes.
- 5 External mass calibration was conducted weekly to maintain <5 ppm mass accuracy.
- 6 Lipids were identified using LipotypeXplorer software based on MS1 and MS/MS data.
- 7 Quantification data were normalized to internal standards, and data were reported in both pmol and mol% formats
- 8 Lipid species with signal intensities  $\geq 5$ -fold above blank and noise thresholds were retained.
- 9 Reproducibility was confirmed using quality control samples (mammalian blood) with a median coefficient of variation (CV) of ~4%.