

SUPPLEMENTARY INFORMATION

Acute and short-term administrations of delta-9-tetrahydrocannabinol modulate major gut metabolomic regulatory pathways in C57BL/6 mice

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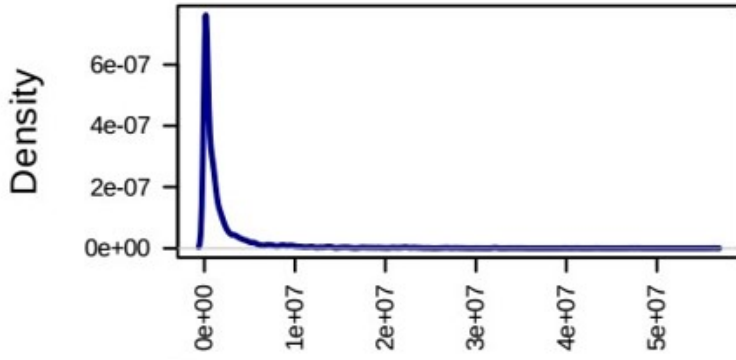
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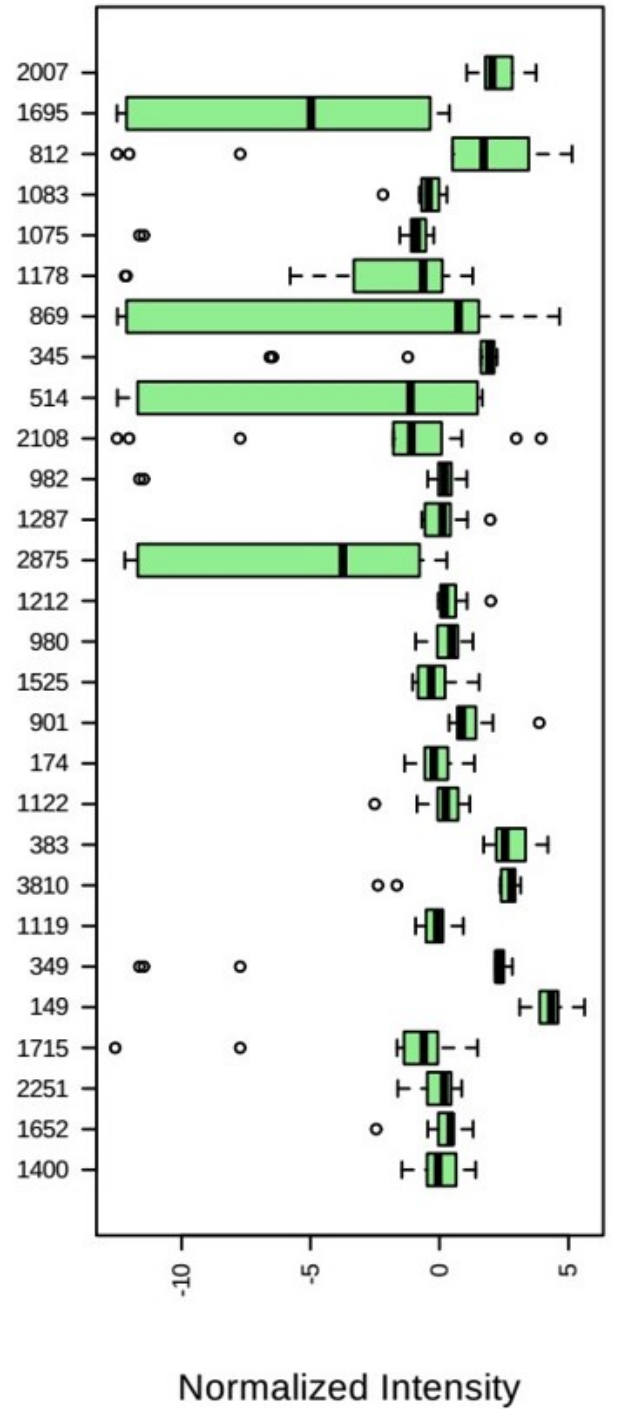
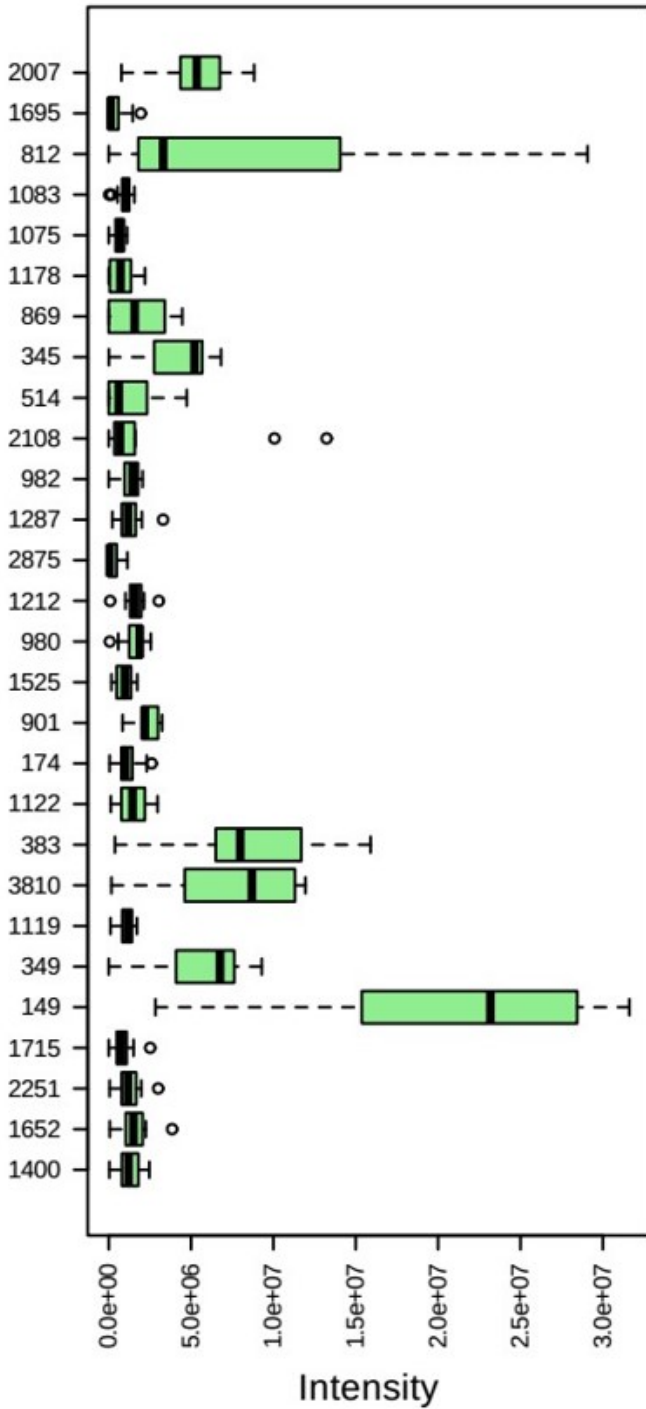
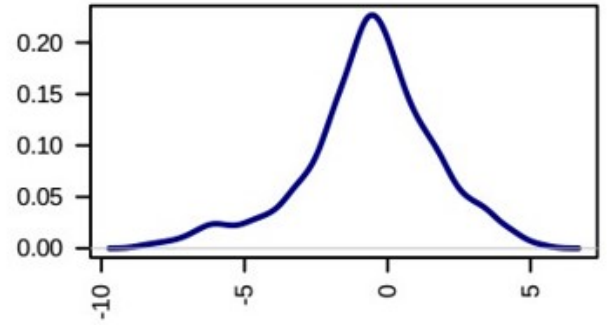
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Before Normalization



After Normalization



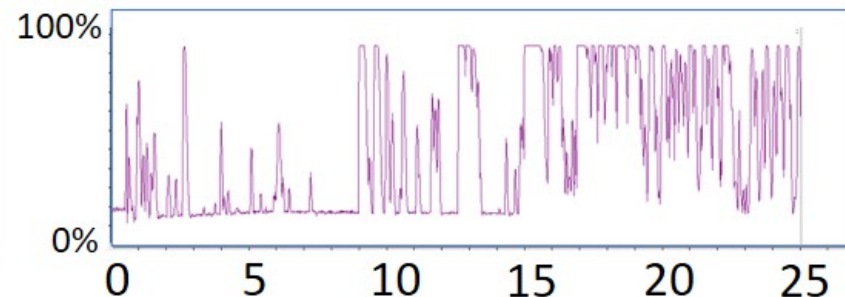
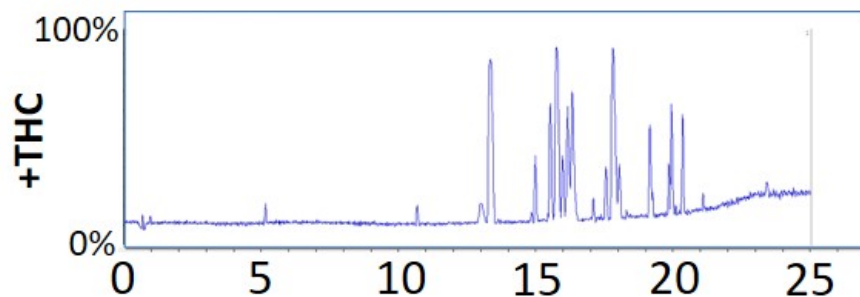
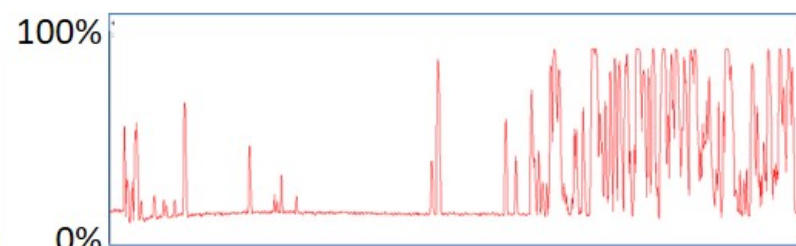
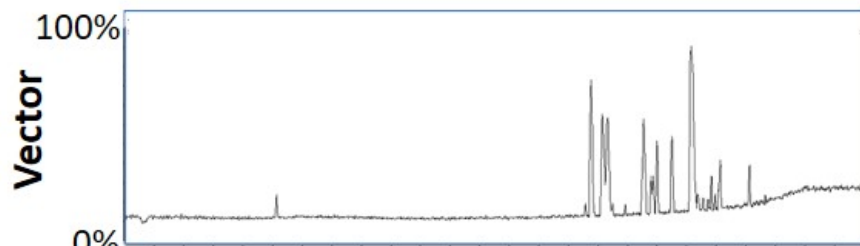
1 **SI Fig 1. Normalization of ion peak intensities** The raw data generated from Mass
2 Hunter workstation was stored as a table with one sample per row and one variable
3 (bin/peak/metabolite) per column. For comparability of metabolite profiles between
4 samples, we implemented a three-category data normalization procedure that included:
5 (a) sample specific normalization involving manual adjustment of concentrations based on
6 biological inputs (i.e. volume, mass), (b) row-wise normalization, a normalization by the
7 sample median for adjustment of differences among samples, and (c) a generalized log
8 transformation of data (gLog2). Density plots and box plots before and after normalization
9 are shown. The boxplots show at most 50 features due to space limit. The density plots
10 are based on all samples.
11

Negative mode

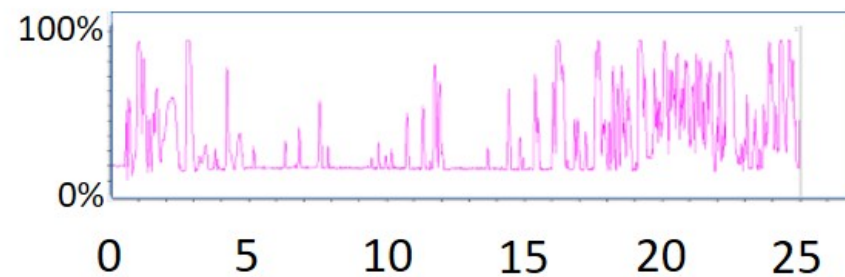
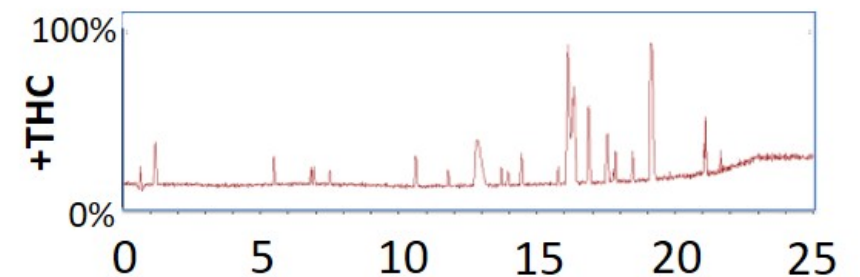
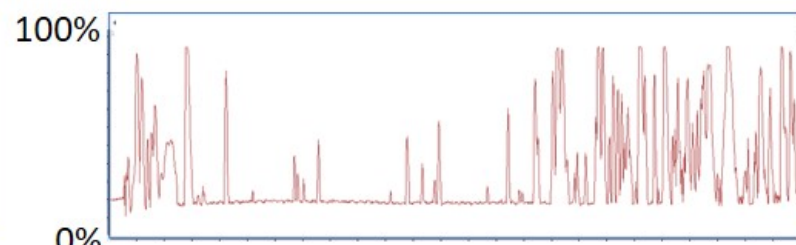
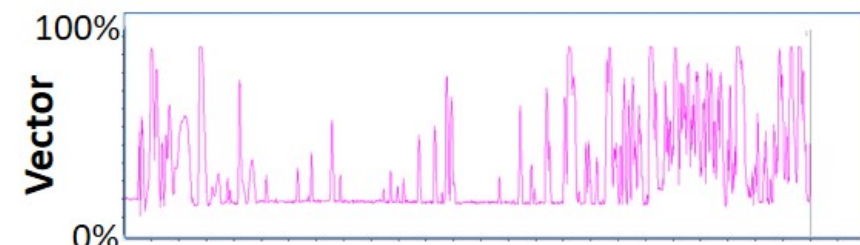
Positive mode

Sampling points

1X

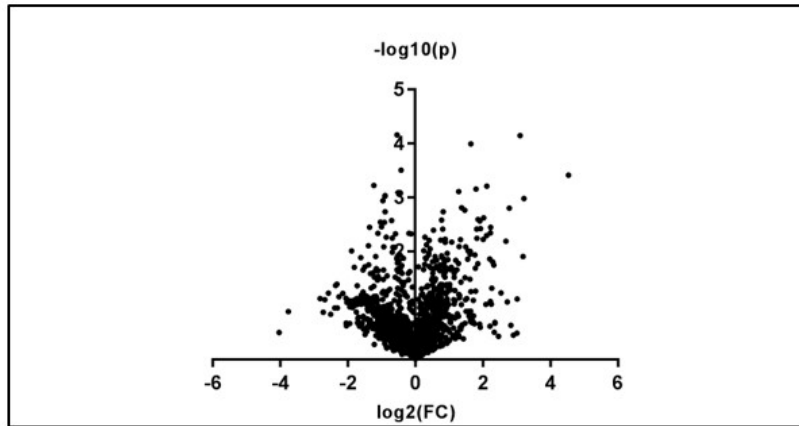
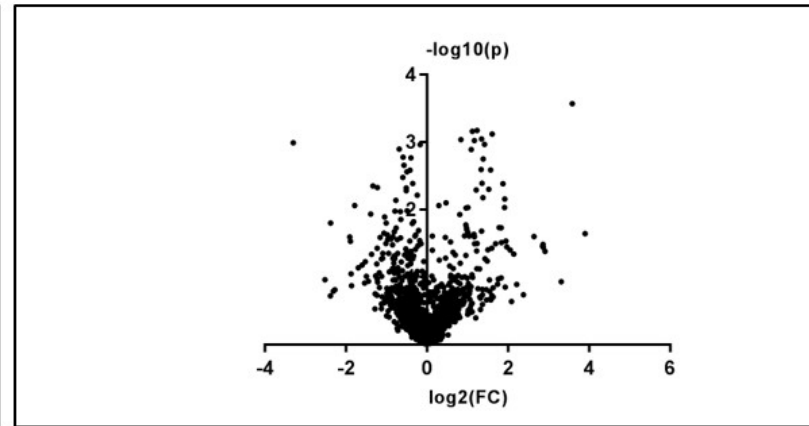
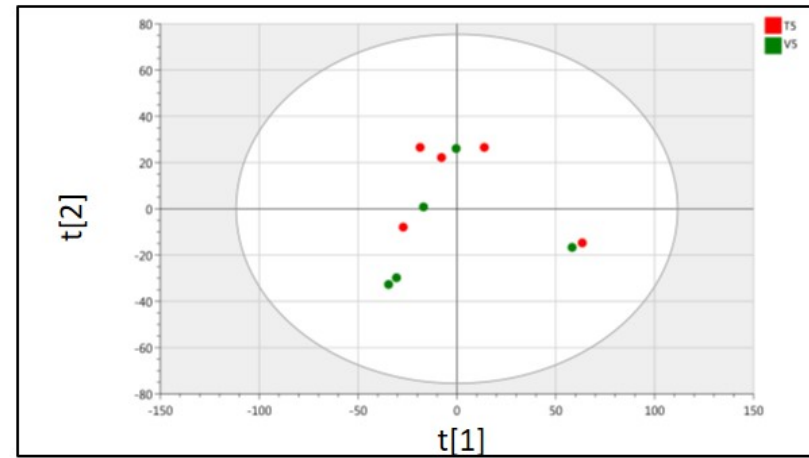
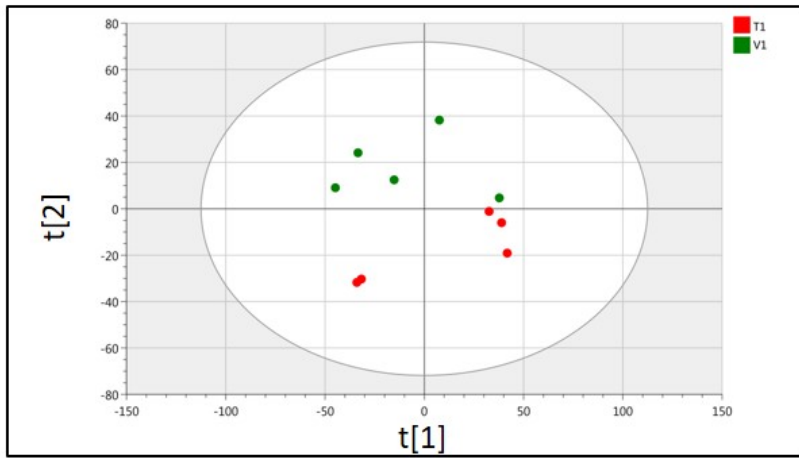
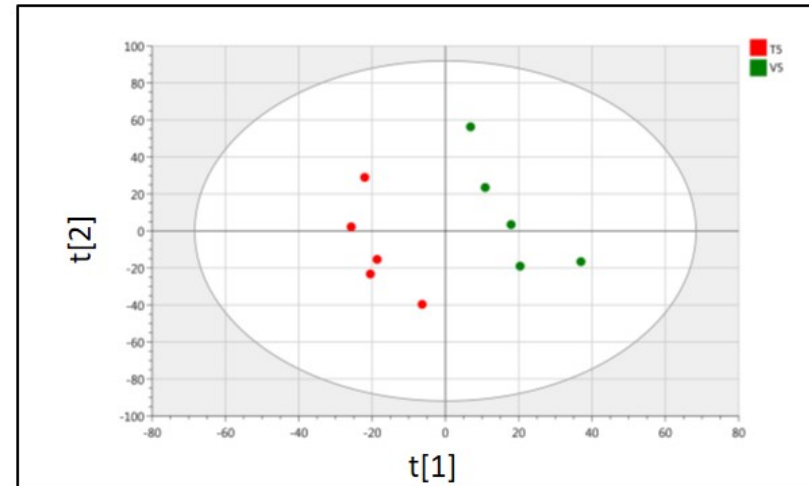
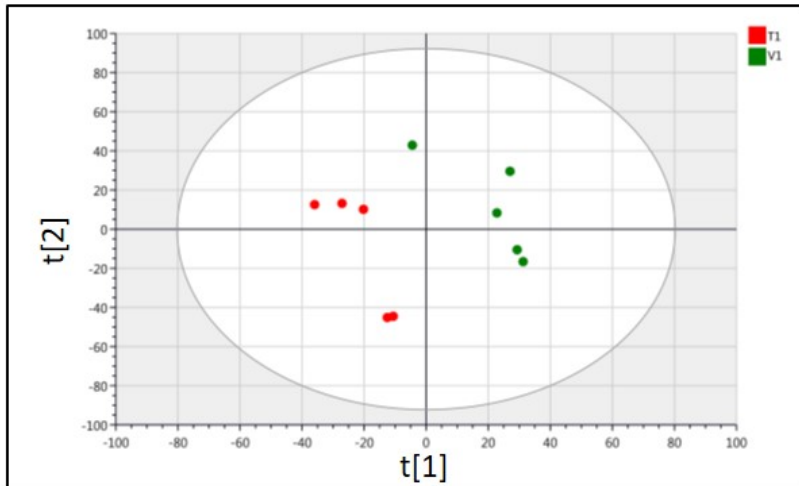


5X



Counts vs. acquisition time (min)

12 **SI Fig 2. Representative Base peak intensity (BPI) chromatograms of fecal extracts**
13 **in positive and negative modes using UPLC-ESI-TOF-MS.** Fecal samples (0.1g) were
14 ultrasonically homogenized in 1mL cold methanol/water (1:1) in for 30mins followed by
15 vortexing on cooled (4°C) mixer for five mins. The homogenized samples were centrifuged
16 (10000xg, 4°C, 10 min). The supernatant (300 µL) was dried in a vacuum concentrator
17 and re-dissolved in 150µL methanol/water (1:1) prior to analysis. Metabolites were
18 separated from injected samples (5 µL aliquots) using Ultra Performance Liquid
19 Chromatograph (1290 Infinity Binary LC System, Agilent Technologies, USA) and
20 screened with ESI-MS (targeted MS/MS mode). The mobile phase consisted of 0.1%
21 formic acid-water (solvent A) and 0.1% formic acid-acetonitrile (solvent B) with a gradient
22 elution (0–1min, 95% A; 1–6min, 95–70% A; 6–20min, 70–5% A). The flow rate of the
23 mobile phase was set at 0.5mL·min⁻¹. The column temperature was maintained at 45°C,
24 and the sample manager temperature was set at 4°C.
25
26

1 X**5 X****b.****c.**

27 **SI Fig 3. Intestinal tissue metabolome comparisons between THC and vector**
28 **administered mice.** a) Volcano plots from 1X and 5X samples showing the metabolites
29 with differential abundance between THC treated mice and the controls. These were
30 selected based on fold change (X-axis) and p-value in (Y-axis). FC, fold change; p, p-
31 value. Metabolites with fold change (increase/decrease) ≥ 2.0 and p-value ≤ 0.05 in THC
32 administered mice compared to the vehicle controls and were selected for further
33 characterization. b) PCA score scatter plots of 1X and 5X samples based on tissue
34 metabolic profiling of THC (n = 5) and vehicle control (n = 5) mice c) PLS-DA score scatter
35 plots of 1X and 5X samples based on tissue metabolic profiling of THC (n = 5) and vehicle
36 control (n = 5) mice. T, THC administered mice, C, vehicle control mice.
37
38