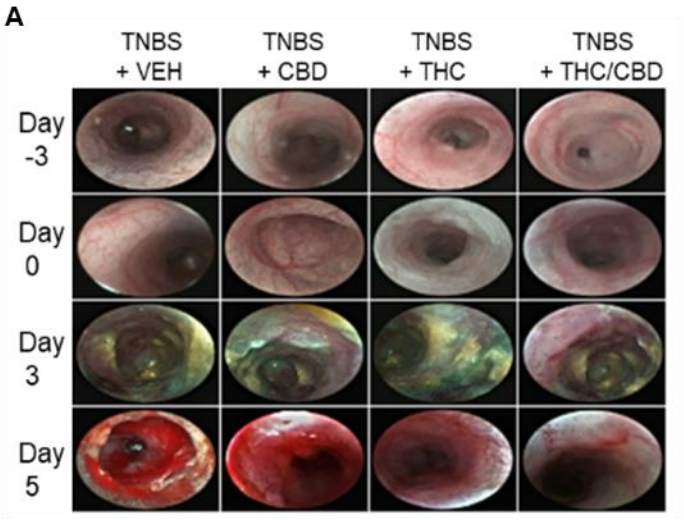
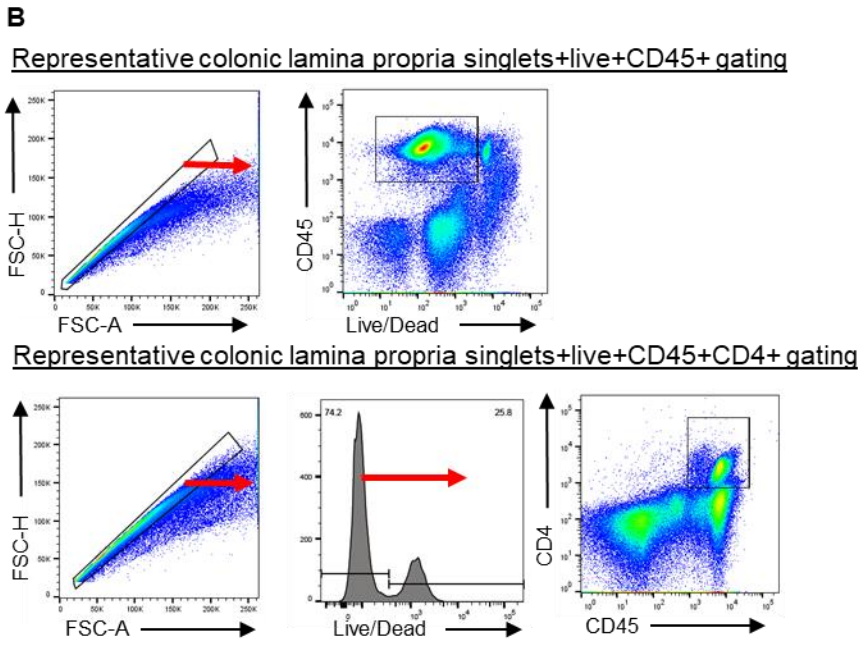


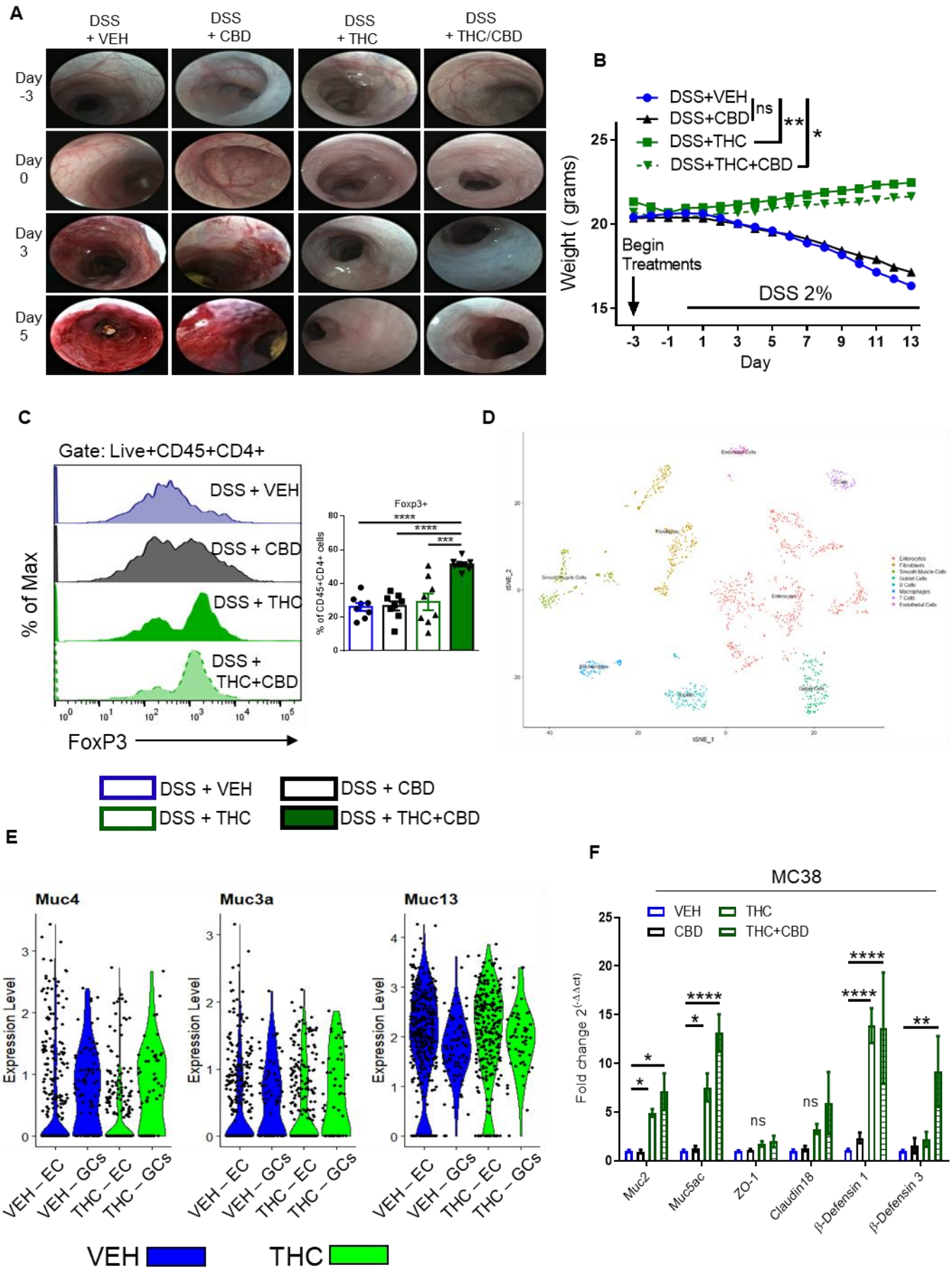


Supplemental Figure 1: TNBS-induced colitis treated with cannabinoids and representative flow gating strategies

BALB/c mice were injected intrarectally with 100 mg/kg TNBS in 50% ethanol. Starting three days before disease induction and continuing daily, mice were gavaged with either: Vehicle (10% EtOH in PBS+Tween-80), CBD (10 mg/kg), THC (10mg/kg) or a combination of THC and CBD (10 mg/kg, both), (n=10).

(A) Representative colonoscopy images from mice at every time point assessed.

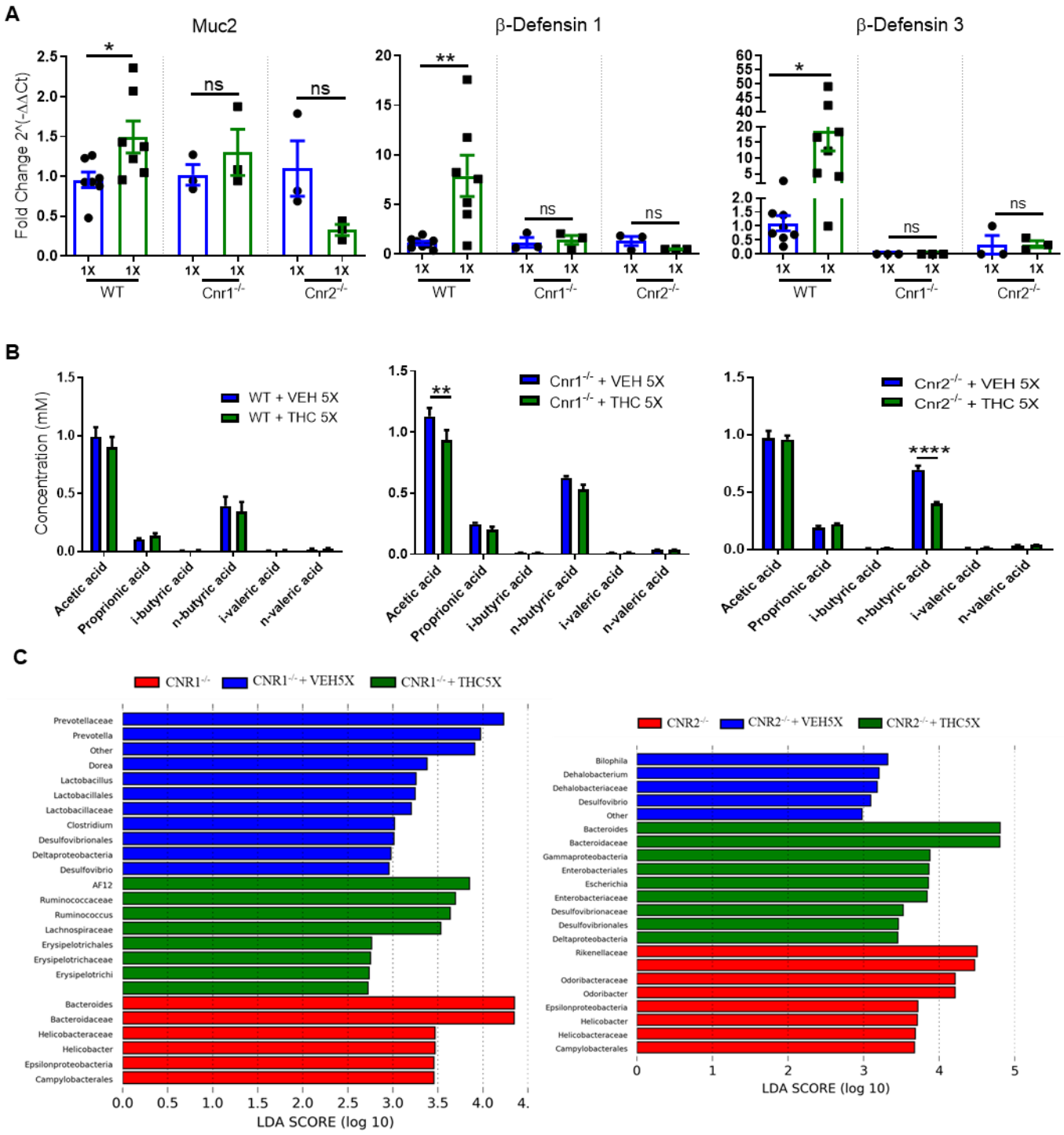
(B) Representative flow cytometry gating strategies.


A
DSS + VEH
DSS + CBD
DSS + THC
DSS + THC/CBD
Day -3
Day 0
Day 3
Day 5
B
DSS+VEH
DSS+CBD
DSS+THC
DSS+THC+CBD
ns
**
*
Weight (grams)
Begin Treatments
DSS 2%
Day
C
Gate: Live+CD45+CD4+
DSS + VEH
DSS + CBD
DSS + THC
DSS + THC+CBD
% of Max
FoxP3
Foxp3+
% of CD45+CD4+ cells
D
tSNE_1
tSNE_2
E
Muc4
Muc3a
Muc13
Expression Level
VEH – EC
VEH – GCs
THC – EC
THC – GCs
VEH
THC
F
MC38
VEH
CBD
THC
THC+CBD
Fold change 2^(-ΔΔct)
Muc2
Muc5ac
ZO-1
Claudin18
β-Defensin 1
β-Defensin 3

Supplemental Figure 2: Cannabinoid effects on colonocytes during disease and at steady state.

To induce DSS-colitis, C57BL/6 mice were treated with either: Vehicle (10% EtOH in PBS+Tween-80), CBD (10 mg/kg), THC (10mg/kg) or a combination of THC and CBD (10 mg/kg, both) for 3 days before 2% DSS was added to their drinking water. DSS remained in the drinking water until termination of the study 14 days later.

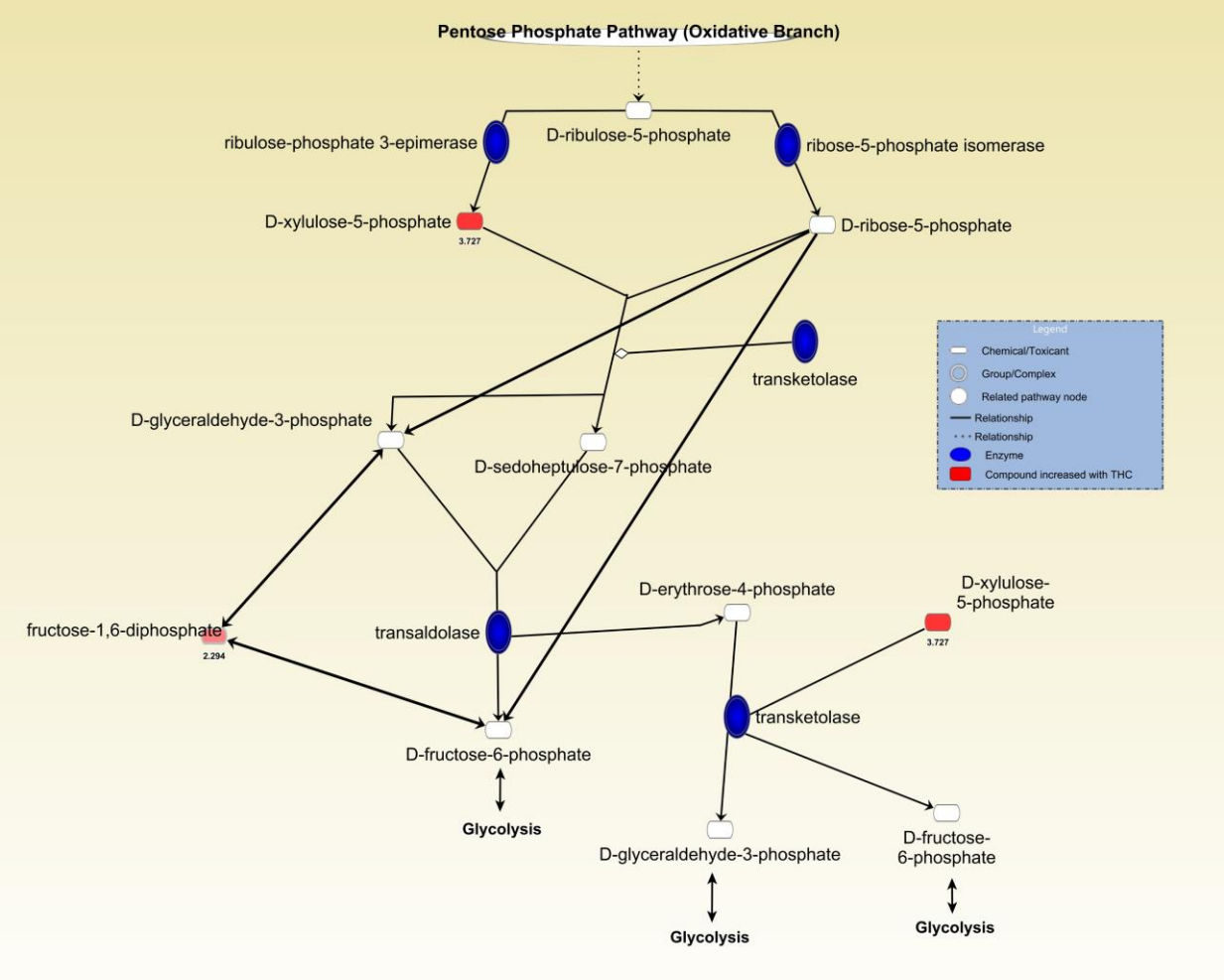
(A) Representative colonoscopy images from mice at every time point assessed.
(B) Actual weight change throughout the experiment (n=5).
(C) Representative overlaid histograms displaying FoxP3 expression in the cLP (gate: Live, CD45+CD4+) and quantification of flow cytometry results (n=8).
(D) scRNA-seq tSNE plot of colon cell clustering from an aggregated sample of colon lamina propria-enriched cells 24 hours after VEH or THC administration.
(E) scRNA-seq violin plot of indicated mRNA expression in the enterocyte (EC) or goblet cell (GC) cluster from the colon of mice treated with VEH or THC for 24 hours.
(F) MC38 cells were treated with VEH, CBD, THC, or a combination of THC and CBD (all 10μM), for six hours before RNA was collected and qRT-PCR was run on indicated genes (n=4).
Each symbol represents an individual mouse or well. Data are presented as mean ± SEM NS, not significant; *p<0.05; **p<0.01, ***p< 0.001, ****p<0.0001 by Two-way ANOVA with Tukey's multiple comparisons test. Data are from one experiment representative of four independent experiments.



Supplemental Figure 3: Short-term cannabinoid administration alters the gut flora and short-chain fatty acid production

(A) qRT-PCR results from PC or SI of WT, Cnr1$^{-/-}$, or Cnr2$^{-/-}$ mice taken 24 hours after one administration of VEH or THC.

(B) Mice of indicated genotype were given five (5X) administrations of VEH or THC and 24 hours later their cecal contents were removed and SCFAs were quantified by GC-MS (n=5-7, all groups).
(C) LDA score of significant bacterial changes occurring after 5 VEH or THC administrations in Cnr1-/- or Cnr2-/- mice (n=6).
Data are presented as mean ± SEM of two independent experiments. NS, not significant; *p<0.05; **p<0.01, ***p< 0.001, ****p<0.0001 by Two-way ANOVA with Tukey's multiple comparisons test or by Students *t*-test.



Supplemental Figure 4: Pentose Phosphate Pathway metabolites are increased with THC

(A) Diagram of the oxidative branch of the pentose phosphate pathway highlighting the role of xylulose-5-phosphate and fructose-1,6-diphosphate.