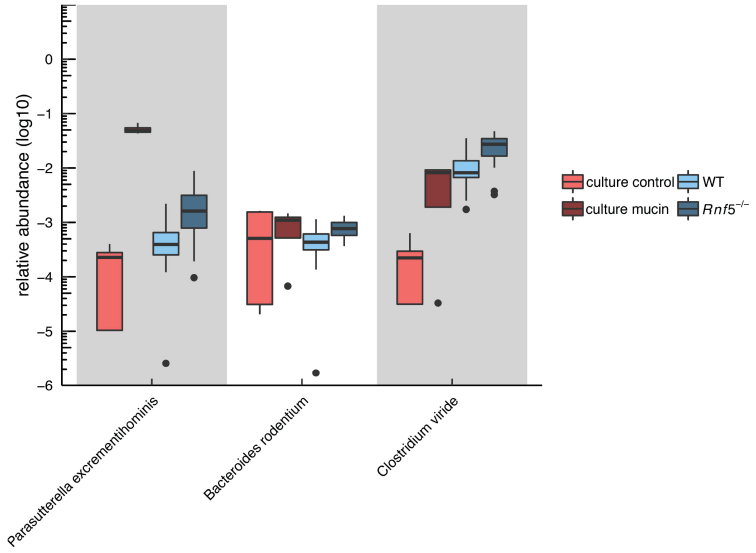
Cell Reports, Volume *30*

Supplemental Information

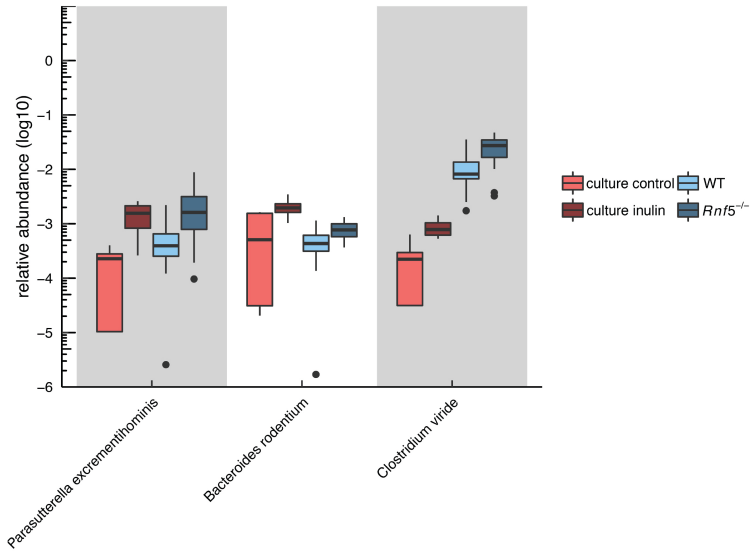
Prebiotic-Induced Anti-tumor Immunity Attenuates Tumor Growth

Yan Li, Lisa Elmén, Igor Segota, Yibo Xian, Roberto Tinoco, Yongmei Feng, Yu Fujita, Rafael R. Segura Muñoz, Robert Schmaltz, Linda M. Bradley, Amanda Ramer-Tait, Raphy Zarecki, Tao Long, Scott N. Peterson, and Ze'ev A. Ronai

a


relative abundance (log10)
0
−1
−2
−3
−4
−5
−6
culture control
culture mucin
WT
Rnf5−/−
Parasutterella excrementihominis
Bacteroides rodentium
Clostridium viride

b


relative abundance (log10)
0
−1
−2
−3
−4
−5
−6
culture control
culture inulin
WT
Rnf5−/−
Parasutterella excrementihominis
Bacteroides rodentium
Clostridium viride

c

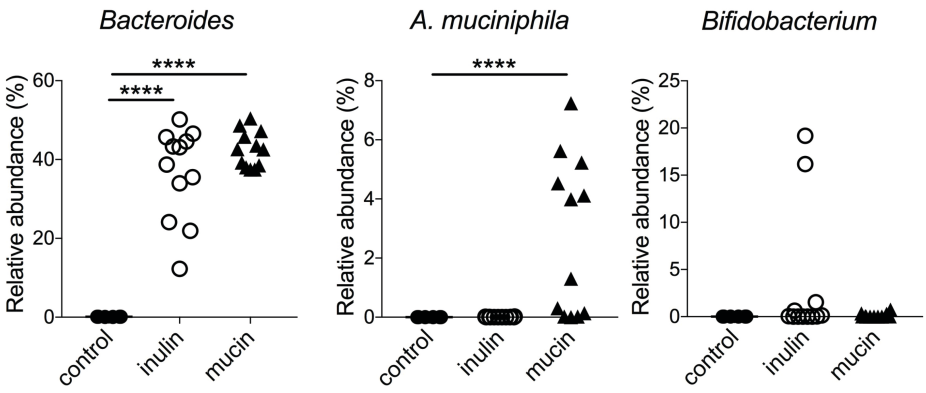

Bacteroides
A. muciniphila
Bifidobacterium
Relative abundance (%)
****
****
****
control
inulin
mucin

Figure S1. Prebiotics that enrich for anti-tumor promoting taxa in vitro. Related to Figure 1.

a, Boxplot of the relative abundance of the common taxa between the ones induced by mucin (1% w/v) in mouse fecal sample culture and the ones enriched in *Rnf5*$^{-/-}$ mouse gut microbiota (culture control n = 3; culture mucin n =3 ; WT n = 30; *Rnf5*$^{-/-}$ n = 30). **b,** Boxplot of the relative abundance of the common taxa between the ones induced by inulin (1% w/v) in mouse fecal sample culture and the ones enriched in *Rnf5*$^{-/-}$ mouse gut microbiota (culture control n = 3; culture mucin n = 3; WT n = 30 ; *Rnf5*$^{-/-}$ n =30). **c**, Fecal samples derived from 12 healthy human subjects cultivated in the presence or absence of 1% prebiotic (control n=6; mucin n=12; inulin n=12). 16S rDNA sequences corresponding to *Bifidobacterium, Bacteroides* and *Akkermansia muciniphila*. Data are one experiment (**a**, **b**) representative of two independent experiments (**c**). Graphs show the mean ± s.e.m. *$P < 0.05$, **$P < 0.005$, ***$P < 0.001$, ****$P < 0.0001$ by one-way ANOVA with Tukey's correction.

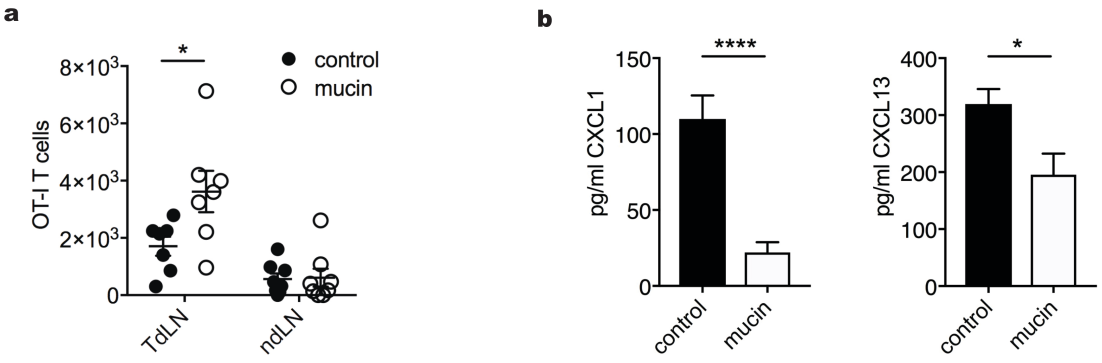


Figure S2. Enhanced anti-tumor immune response in the mucin-treated mice. Related to Figure 2.

a, Quantification of CD45.1$^+$ OT-I CD8$^+$ T cells in the tumor-draining lymph nodes (TdLN) and non-draining lymph nodes (ndLN) of WT C57BL/6 mice CD45.2$^+$ mice that were injected with B16-OVA melanoma cells (TdLN n = 7; ndLN n = 8). **b**, Serum chemokines in WT mice with or without mucin treatment at 10 days after tumor inoculation (n = 10). Data are representative of two independent experiments. Graphs show the mean ± s.e.m. *$P < 0.05$, **$P < 0.005$, ***$P < 0.001$, ****$P < 0.0001$ by two-tailed *t*-test or Mann–Whitney *U* test.

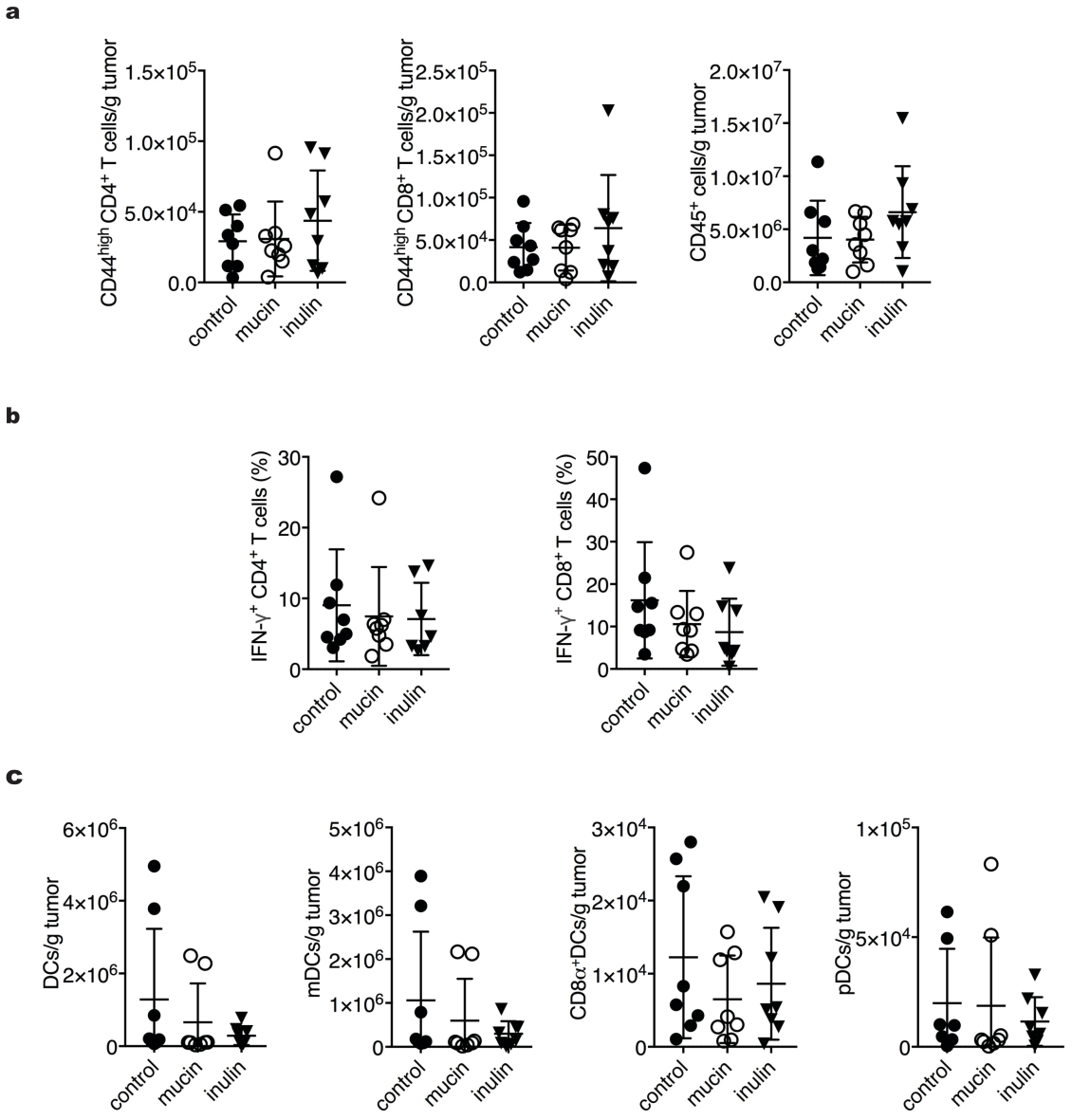


Figure S3. TILs analysis of mucin or inulin-treated C57BL/6 mice injected MC-38 tumor. Related to Figure 4.

a, Quantification of MC-38 tumor-infiltrating effector ($CD44^{hi}$) $CD4^+$ and $CD8^+$ T cells and $CD45^+$ cells in mucin or inulin-treated C57BL/6 mice (n = 8); **b,** Quantification of MC-38 tumor-infiltrating IFN- γ -producing $CD4^+$ and $CD8^+$ T cells in mucin or inulin-treated C57BL/6 mice (n = 8); **c** Quantification of MC-38 tumor-infiltrating total DCs and DC subsets in mucin or inulin-treated C57BL/6 mice (n = 8). Data are representative of two independent experiments. Graphs show the mean ± s.e.m. $*P < 0.05$, $**P < 0.005$, $***P < 0.001$, $****P < 0.0001$ by one-way ANOVA with Tukey's correction.

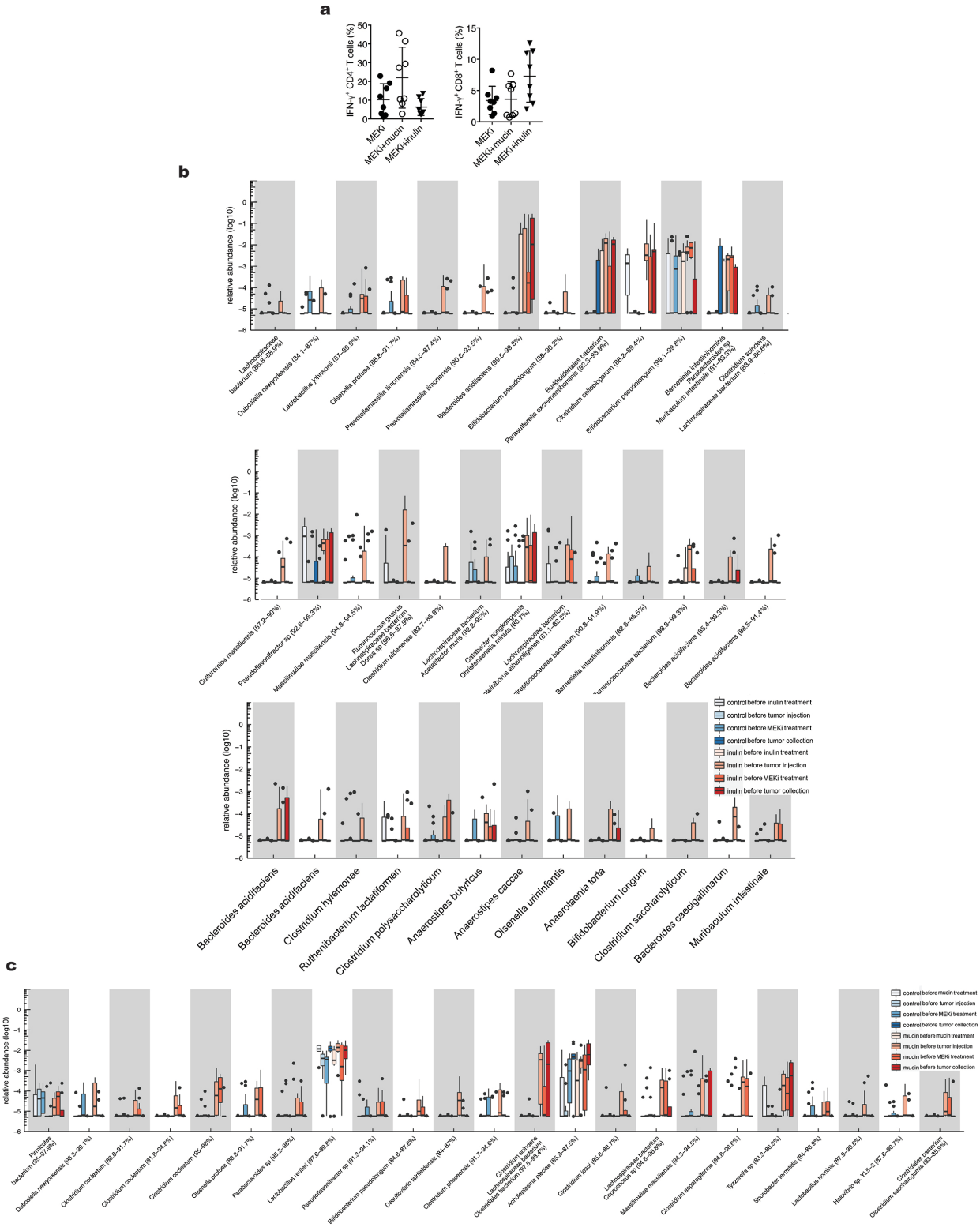

a
IFN-γ+ CD4+ T cells (%)
IFN-γ+ CD8+ T cells (%)
MEKi
MEKi+mucin
MEKi+inulin
b
relative abundance (log10)
Lachnospiraceae bacterium (86.8–88.6%)
Dubosiella newyorkensis (84.1–87%)
Lactobacillus johnsonii (87–89.9%)
Olsenella profusa (88.8–91.7%)
Prevotellamassilia timonensis (84.5–87.4%)
Prevotellamassilia timonensis (90.6–93.5%)
Bacteroides acidifaciens (99.5–99.8%)
Bifidobacterium pseudolongum (88–90.2%)
Burkholderiales bacterium
Parasutterella excrementihominis (92.3–93.9%)
Clostridium cellobioparum (89.2–89.4%)
Bifidobacterium pseudolongum (99.1–99.6%)
Barnesiella intestinihominis
Parabacteroides sp
Muribaculum intestinale (81–83.3%)
Clostridium scindens
Lachnospiraceae bacterium (83.9–86.6%)
Culturomica massiliensis (87.2–90%)
Pseudoflavonifractor sp (92.6–95.3%)
Massilimelitae massiliensis (94.3–94.5%)
Ruminococcus gnavus
Lachnospiraceae bacterium
Dorea sp (96.6–97.9%)
Clostridium aldenense (83.7–85.9%)
Lachnospiraceae bacterium
Acetatifactor muris (92.2–95%)
Catabacter hongkongensis
Christensenella minuta (88.7%)
Barnesiella intestinihominis (92.6–95.5%)
Bacteroides acidifaciens (95.4–98.9%)
Bacteroides acidifaciens (86.5–91.4%)
control before inulin treatment
control before tumor injection
control before MEKi treatment
control before tumor collection
inulin before inulin treatment
inulin before tumor injection
inulin before MEKi treatment
inulin before tumor collection
Bacteroides acidifaciens
Bacteroides acidifaciens
Clostridium hylemonae
Ruthenibacterium lactatiformans
Clostridium polysaccharolyticum
Anaerostipes butyricus
Anaerostipes caccae
Olsenella uriniinfantis
Anaerotaenia torta
Bifidobacterium longum
Clostridium saccharolyticum
Bacteroides caecigallinarum
Muribaculum intestinale
c
relative abundance (log10)
Firmicutes bacterium (86.7–87.8%)
Dubosiella newyorkensis (96.3–99.1%)
Clostridium cocleatum (88.8–91.7%)
Clostridium cocleatum (91.8–94.8%)
Clostridium cocleatum (85–88%)
Olsenella profusa (88.8–91.7%)
Parabacteroides sp (95.2–98%)
Lactobacillus reuteri (97.8–99.8%)
Pseudoflavonifractor sp (91.3–94.1%)
Bifidobacterium pseudolongum (84.8–87.8%)
Desulfovibrio fairfieldensis (84–87%)
Clostridium phoceensis (91.7–94.6%)
Clostridium scindens
Lachnospiraceae bacterium
Clostridiales bacterium (97.5–98.4%)
Acholeplasma pleciae (95.2–97.5%)
Clostridium piriformis (85.8–88.7%)
Lachnospiraceae bacterium
Coprococcus sp (84.8–86.8%)
Massilimelitae massiliensis (94.3–94.5%)
Clostridium asparagiforme (94.8–96.8%)
Tyzzerella sp (93.3–96.3%)
Sporobacter termitidis (94–96.9%)
Lactobacillus hominis (87.9–90.8%)
Halovibrio sp. YLS-2 (97.8–98.7%)
Clostridiales bacterium
Clostridium saccharogumia (85–85.8%)
control before mucin treatment
control before tumor injection
control before MEKi treatment
control before tumor collection
mucin before mucin treatment
mucin before tumor injection
mucin before MEKi treatment
mucin before tumor collection

Figure S4. Microbiome analysis of fecal samples from MEKi + prebiotics treated-mice. Related to Figure 5.

a, Quantification of $NRAS^{Q61K}$ tumor-infiltrating IFN-γ-producing $CD4^+$ and $CD8^+$ T cells from C57BL/6 mice treated with MEKi + mucin or inulin (n=8). **b,** Boxplot of the relative abundance of the taxa enriched in inulin treated-mice gut microbiota that are negatively correlated with tumor size (n = 10). The fecal samples were taken at different time points (before inulin treatment, before tumor injection, before MEKi treatment and before tumor collection). **c,** Boxplot of the relative abundance of the taxa enriched in gut microbiota of mucin treated-mice, which are negatively correlated with tumor size (n = 10). The fecal samples were taken at different time points (before mucin treatment, before tumor injection, before MEKi treatment and before tumor collection) following the innoculation of YUMM1.5 tumor cells. Data are representative of two independent experiments. Graphs show the mean ± s.e.m. *$P < 0.05$, **$P < 0.005$, ***$P < 0.001$, ****$P < 0.0001$ by one-way ANOVA with Tukey's correction.

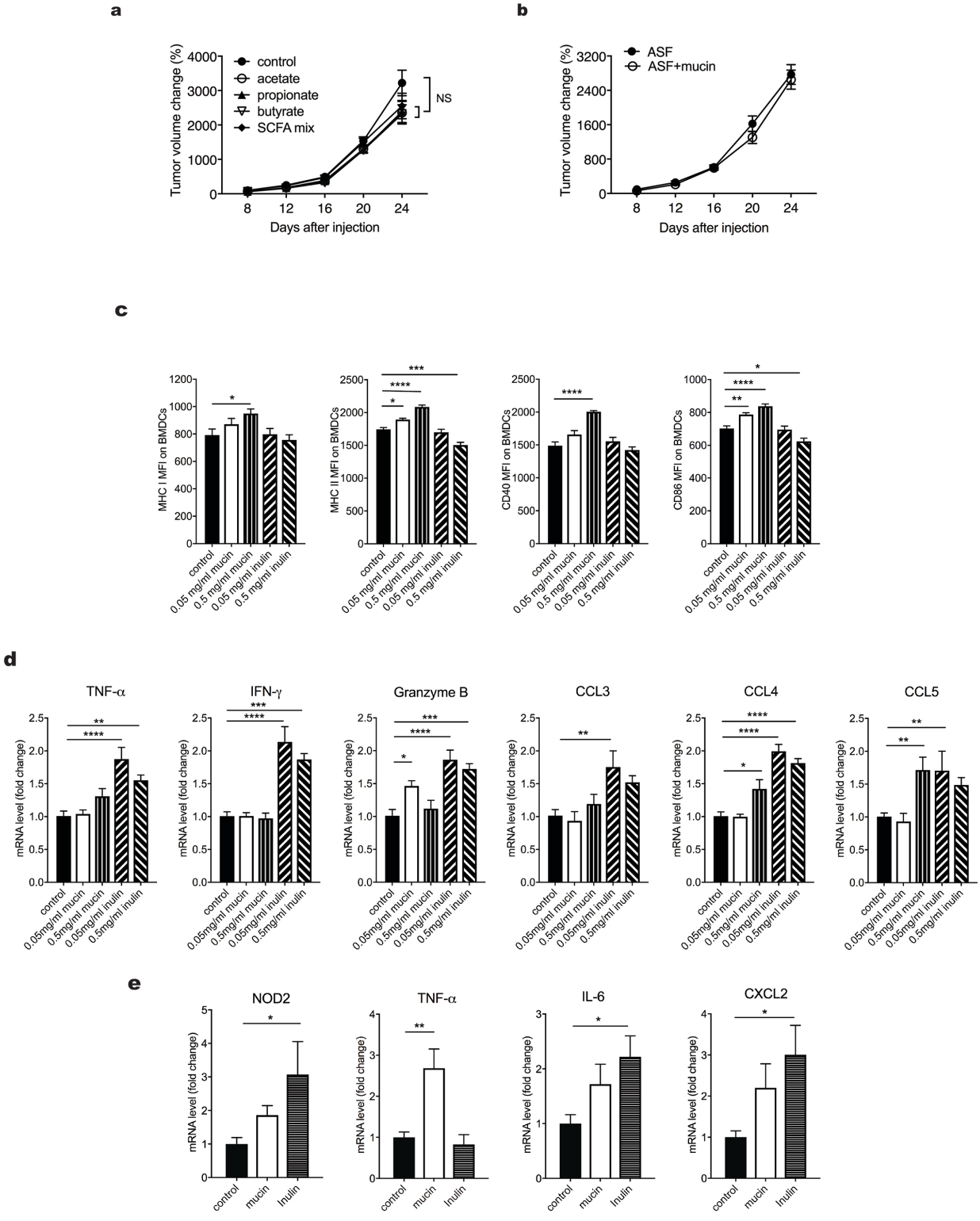

a
Tumor volume change (%)
4000
3000
2000
1000
0
control
acetate
propionate
butyrate
SCFA mix
NS
8
12
16
20
24
Days after injection
b
Tumor volume change (%)
3200
2400
1600
800
0
ASF
ASF+mucin
8
12
16
20
24
Days after injection
c
MHC I MFI on BMDCs
MHC II MFI on BMDCs
CD40 MFI on BMDCs
CD86 MFI on BMDCs
control
0.05 mg/ml mucin
0.5 mg/ml mucin
0.05 mg/ml inulin
0.5 mg/ml inulin
d
TNF-α
IFN-γ
Granzyme B
CCL3
CCL4
CCL5
mRNA level (fold change)
control
0.05mg/ml mucin
0.5mg/ml mucin
0.05mg/ml inulin
0.5mg/ml inulin
e
NOD2
TNF-α
IL-6
CXCL2
mRNA level (fold change)
control
mucin
Inulin

Figure S5. Effect of mucin and inulin on the activation of DCs, T cells and IECs. Related to Figure 6.

a, Growth of Yumm1.5 tumors in C57BL/6 mice provided with butyrate, propionate and acetate (150 mM) and the combination of all three (50 mM each) in drinking water 14 days prior to and during tumor inoculation (n =12). **b,** SW1 tumor growth in ASF-bearing C3H/HeN mice undergoing oral gavage with or without mucin prior to tumor inoculation (n = 15). **c,** MHC I, MHC II, CD40 and CD86 expression (MFI) on BMDCs left untreated (control) or stimulated with 0.05 mg/ml and 0.5 mg/ml mucin and inulin in vitro (n = 6). **d,** qRT-PCR analysis of the indicated cytokine and chemokine mRNAs in $CD8^+$ T cells left untreated (control) or stimulated with 0.05 mg/ml and 0.5 mg/ml mucin and inulin in vitro (n = 4). **e,** qRT-PCR analysis of proinflammatory gene mRNA levels in IECs in mucin or inulin-treated mice (n = 6). Data are representative of two independent experiments. Graphs show the mean ± s.e.m. $*P < 0.05$, $**P < 0.005$, $***P < 0.001$, $****P < 0.0001$ by two-way ANOVA with Tukey's correction(**a** and **b**) or one-way ANOVA with Tukey's correction (**c, d and e**).

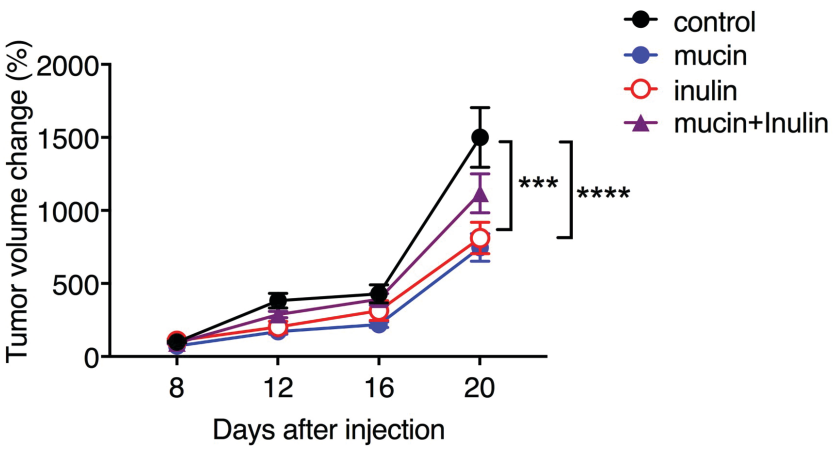


Figure S6. Effect of mucin and inulin combination in C57BL/6 mice. Related to Figure 7.

Growth of Yumm1.5 mouse melanoma cells in C57BL/6 mice that were fed with 0 or 3% mucin in drinking water and (or) a diet enriched 15% inulin, starting 14 days prior to and during tumor inoculation (control n = 7; mucin n=5; inulin n=7; mucin+inulin n=8). Data are representative of two independent experiments.Graphs show the mean ± s.e.m. *$P < 0.05$, **$P < 0.005$, ***$P < 0.001$, ****$P < 0.0001$ by two-way ANOVA with Tukey's correction.