PNAS
www.pnas.org

Supplementary Information for

CD8 T cells drive anorexia, dysbiosis and blooms of a commensal with immunosuppressive potential after viral infection

Lara Labarta-Bajo[1], Anna Gramalla-Schmitz[1†], Romana R. Gerner[2,6], Katelynn R. Kazane[1], Gregory Humphrey[2], Tara Schwartz[2], Karenina Sanders[2], Austin Swafford[3], Rob Knight[2,3,4,5], Manuela Raffatellu[2,3,6,7], Elina I. Zúñiga[1*].

*Lead Contact and correspondence to Elina I. Zúñiga

Email: eizuniga@ucsd.edu

This PDF file includes:

Supplementary Materials and Methods
Supplementary Figures 1-8
Supplementary References

Other supplementary materials for this manuscript include the following:

Supplementary Dataset S1-6

www.pnas.org/cgi/doi/10.1073/pnas.2003656117

Supplementary Materials and Methods

Mice, infections and antibody treatments

All of our studies were performed with six to ten-week-old female C57BL/6 mice bred at the Jackson Laboratories. For microbiome studies, purchased mice were randomly distributed in cages containing 5 mice each and housed in that way for 3 weeks before the day of infection per guidelines of the Earth Microbiome Project (1). Mice were infected intravenously (i.v.) with $2x10^6$ plaque forming units (PFU) of LCMV ARM or Cl13. Viral stocks were grown, identified and quantified as reported previously (2). Viral loads were determined by standard plaque assay (2) of serum or tissue homogenates. For CD8 T cell depletion studies, mice were intraperitoneally (i.p.) injected with anti-CD8α (53-6.72, BioXcell) or IgG2a (2A3, BioXcell) antibodies on day -2, -1, and 5 p.i. (250 µg/mouse) as well as on the day of infection (200 µg/mouse). For CD4 T cell depletion studies we used anti-CD4 (GK1.5, BioXcell) or IgG2b (LTF-2, BioXcell) antibodies at the same doses and times as described for CD8 T cell depletion. For IFNAR *in vivo* blockade, mice were i.p. injected with IFNAR neutralizing antibody (MAR1-5A3; BioXCell) or mouse IgG1 isotype control (MOPC-21; BioXcell) on days -1 and 0 p.i. (500 µg/mouse) as well as on days 2, 4 and 6 p.i. (250 µg/mouse) with Cl13 (3, 4).

For food consumption measurements, food pellets from cages containing four or five mice were weighed every 24 hrs. Grams of food consumed in each cage were then divided by the number of mice housed within the cage, normalized to the body weight of each mouse and multiplied by 100. In this way, we obtained the amount of food consumed per gram of body weight in the form of a percentage. Note that we chose to normalize by body weight to account for the differences in body weight among uninfected, ARM-infected and Cl13-infected mice (Fig. S3A). To establish a 'healthy range' of daily food consumption, food consumption measurements were taken every 24 hrs, for 30 consecutive days and from 10 uninfected female mice that were housed in two different cages containing 5 mice each. To estimate the healthy range, we computed the average daily food consumption ± one standard deviation from all measurements taken during the 30 days (i.e. 17 g of food/g of body weight ± 5). For acute starvation experiments, food pellets were removed from cages containing three uninfected mice for a total of 24 hrs. Mice were maintained in a closed breeding facility located at the Biomedical Sciences Building or Pacific Hall at UCSD, where mice were housed in ventilated cages containing HEPA filters. Mouse handling conformed to the requirements of the National Institutes of Health and the Institutional Animal Care and Use Guidelines of UCSD.

Lymphocyte isolation from spleen and small intestinal IEL

Spleens were harvested, digested for 20 min at 37 ºC in 1mg/ml collagenase D (Roche) and mashed through a 100 µm filter. Cells were then centrifuged at 1500 rpm at 4^0C for 5 min. Red blood cells (RBC) were lysed by incubating pellets in 1ml of RBC-lysis buffer (150mM NH_4Cl, 10mM $KHCO_3$, 0.1mM Na_2EDTA in deionized water, at pH 7.4) for 4 min at room temperature (RT). After RBC lysis, 10ml of FACS buffer (PBS1x + 3%FBS) were added followed by centrifugation as stated above. Pellets were resuspended in 5ml of complete Roswell Park Memorial Institute (RPMI, Gibco), which consisted of RPMI supplemented with 10% heat-inactivated Fetal Bovine Serum (FBS, Atlanta Biologicals), 2% Penicillin/Streptavidin (P/S, BioWhittaker), 1 mM Sodium Pyruvate (Na-pyr, Thermo Scientific), 1mM L-Glutamine (L-Gln, BioWhittaker), 20 mM HEPES

(Thermo Scientific) and 55 mM beta-mercaptoethanol (Life Technologies). Absolute leukocyte numbers were determined via FSC/SSC gating in a Guava Easycyte automated cell counter (MilliporeSigma, MA).

Small intestines were harvested, cleaned from adipose tissue, flushed and macroscopically visible Peyer's Patches were excised. To isolate the epithelial layer, intestines were longitudinally and transversely cut into 1-2 cm pieces, placed in Pyrex conical flask containing a stirring magnet and 30 mL of 'IEL buffer', and stirred on a stirring plate (Scilogex) set at 37 ^{o}C for a total of 15 min. IEL buffer consisted of 1xPBS supplemented with 3% FBS, 2% P/S, 1mM Na-pyr, 20 mM HEPES and 10 mM Ethylenediaminetriacetic Acid (EDTA, Thermo Fisher Scientific). The soluble fraction was then collected, diluted in RPMI supplemented with 3% FBS and pelleted by centrifugation at 1500 rpm for 5min at 4^{0}C. Epithelial pellets were resuspended in 5 ml of '44% Percoll solution' and 2.5 ml of '67% Percoll solution' were added underneath with a Pasteur pipette. Tubes were then spun down at 2000 rpm (with no brake set) at RT for 20 min. Percoll solution was prepared by adding 1 part of 10X PBS (Gibco) to 9 parts of Percoll (GE Healthcare Biosciences) and further mixed with IEL buffer to obtain a 44% or a 67% solution. The interphase layer containing IEL was collected after centrifugation, resuspended in 1ml of complete RPMI and leukocyte counts were determined with a Guava Easycyte (MilliporeSigma, MA) as indicated above.

Quantitative PCR

To quantify *A. muciniphila* genomes, previously frozen colonic and caecal contents were thawed, weighed, and DNA was extracted with DNeasy PowerSoil Kit (Qiagen) by following the manufacturer's protocol. Quantitative PCR (qPCR) was performed using Fast SYBR Green Master Mix (Thermo Fisher Scientific) and the following *A. muciniphila*-specific primers: F 5'-CAGCACGTGAAGGTGGGGAC-3', R 5'-CTTGCGGTTGGCTTCAGAT-3' with 10 ng of input DNA in triplicate. The qPCR reaction was ran following this protocol: 95 °C for 15 min, 40 cycles of 95 °C for 15 s, 66°C for 40 s, and 72°C for 30 s, as in (5), followed by melt curve analysis on a CFX96 Touch Detection System (Bio-Rad). DNA extracted from a food pellet was used as a negative control. To obtain an *A. muciniphila* qPCR standard, we extracted DNA from colonic and caecal contents from an uninfected mouse, as described above, and performed a regular PCR on a Veriti Thermal Cycler (Thermo Fischer Scientific) with the same *A. muciniphila*-specific primers as the ones used for qPCR and following this protocol: 95 °C for 15 min, 25 cycles of 95 °C for 15 s, 66°C for 40 s, and 72°C for 30 s. The resulting PCR reaction was ran on a 1% agarose gel and the 328 base pair (bp)-long PCR product was purified with a QIAquick Gel Extraction kit (Qiagen). The DNA concentration of the purified PCR product was determined with a NanoDrop One (Thermo Fisher Scientific) and serial dilutions of the standard were ran in parallel to all qPCR reactions described above and resulting Cq were used to create a standard curve. To estimate the amount of *A. muciniphila* genomes we applied the following formula: number of molecules (genomes) = [DNA weight (ng) * $6.022x10^{23}$ (molecules/mole)] / [328bp * 650 (g/mol/bp) * $1x10^{9}$ (ng/g)], where $6.022x10^{23}$ corresponds to Avogadro's Number and 650 corresponds to the average mass of 1 bp double-stranded DNA.

For quantification of transcripts in liver, tissues were collected from sacrificed mice, snap frozen and stored at -80 °C. Later, tissues were thawed, bead homogenized in RLT buffer (Qiagen) and centrifuged for 10 min 10,000 rpm to pellet debris. Supernatants were used for total RNA extraction using RNeasy kits (Qiagen), digested with DNase I (RNase-free DNase set; Qiagen) and reverse-

transcribed into cDNA using M-MLV RT (Promega). The expression of LCMV nucleoprotein was quantified, in triplicates, using Fast SYBR Green Master Mix (Thermo Fisher Scientific) followed by CFX96 Touch Real-Time PCR Detection System (Bio-Rad). Relative transcript levels were normalized against murine *Actb*. Graphs depicting qRT-PCR analysis of murine genes represent biological replicates of individual mice from one representative experiment or pooled experiments as indicated in figure legend. Q-PCR primer sequences are the following: LCMV nucleoprotein F 5′-GCATTGTCTGGCTGTAGCTTA-3′, R 5′-CAATGACGTTGTACAAGCGC-3′; *Actb* F 5'-AGGGAAATCGTGCGTGACAT-3', 5'-GAACCGCTCGTTGCCAATAG-3'.

Flow cytometry

Cells were stained at a maximal concentration of $2x10^8$ cells/ml in FACS buffer or as indicated below. For surface staining of splenocytes and IEL cells were first stained with a fixable viability dye (Ghost dye, Tonbo) in 1xPBS for 10 min at 4 ºC followed by staining with MHC-I tetramers H2-D^b/$GP_{33–41}$-BV421 and H2-D^b/$NP_{396-404}$-PE provided by NIH Tetramer Core Facility (Atlanta, GA) in FACS buffer for 1h at RT followed by staining for 20min at 4ºC with remaining surface antibodies. For intracellular staining after *ex vivo* peptide restimulation, cells were fixed in 1% paraformaldehyde (PFA) for 10min at RT and stained with antibodies in 1x perm/wash buffer (Thermo Scientific) for 30 min at 4 ºC. Alternatively, for intranuclear staining, cells were fixed with the Foxp3 Transcription Factor Staining Buffer Set Kit (Thermo Scientific) per vendor's recommendation and stained with antibodies in 1x perm/wash buffer for 1h at RT. Antibodies used in this study were purchased from Thermo Fisher Scientific (Waltham, MA), BD Biosciences or Biolegend (San Diego, CA): GrzB PE-TR (GB11), T-BET PE (4B10), CD103 FITC (2E7), CD44 BV570 (IM7), CD127 Alexa Fluor 488 (A7R34), Foxp3 FITC (FJK-16s), CD8a BV786 or APC efluor 780 (53-6.7), CD4 BV650 or BV711 (RM4-5), IFNγ APC (XMG1.2), TNFα FITC (MP6-XT22), IL-2 PE (JES6-5H4), KLRG1 PeCy7 or PE-TR (2F1). Anti-H3K27me3 conjugated to Alexa Fluor 488 (C36B11) and anti-TCF1 (C63D9) conjugated to Alexa Fluor 647 were purchased from Cell Signaling Technologies (Danvers, MA). Cells were acquired using a Digital LSR II flow cytometer (Becton Dickinson, San Jose, CA) or a ZE5 Cell Analyzer (Bio-Rad, Hercules, CA). Flow cytometric data were analyzed with FlowJo software v9.9.6 and v10.

Ex vivo T cell stimulation

Splenocytes were cultured at $1x10^7$ cells/ml in round-bottom 96-well plates for 5 h in complete RPMI supplemented with Brefeldin A (1 μg/mL; Sigma) and 1 μg/mL of the MHC class-I-restricted LCMV $NP_{396–404}$ or $GP_{33–41}$ peptides (all >99% pure; Synpep). Cells were then stained with a fixable viability dye (Tonbo Ghost Dye) and surface staining with the anti- CD8 mAbs described above, fixed in 1% PFA, permeabilized and stained with the aforementioned antibodies against IFNγ, TNFα and IL-2 in 1x perm/wash buffer for 30 min at 4^0C. Unstimulated controls in which cells were cultured without peptide were performed in parallel and showed background levels of cytokine staining.

Culture and inoculation of *Akkermansia muciniphila*

Akkermansia muciniphila (ATTC BAA-835) was purchased from ATCC. To make glycerol stocks, lyophilized *A. muciniphila* was re-suspended in liquid medium composed of Brain Heart Infusion (BHI) (Sigma) supplemented with 0.25% v/v type III mucin from porcine stomach (Sigma) and

1mM L-Cysteine (BHI+mucin media) that was previously sterilized and removed of oxygen, as in (5), and cultured in an anaerobic chamber (H_2 7.5%, CO_2 10%, O_2 < 10 ppm and N_2 as balance, Coy Lab) for 7 days, after which cultures were mixed with sterile glycerol at a final concentration of 25% w/v. To prepare mucin stocks, lyophilized mucin was solubilized in 0.1 M acetate buffer at pH 5 to make a 5% w/v mucin solution, autoclaved, aliquoted and kept at -80ºC until further use. To sterilize and to remove oxygen from the liquid medium, we injected CO_2 at 35 psi and N_2 at 50 psi for 35 min into the liquid medium while it was being stirred at 1500 rpm with a magnet. After that, we injected CO_2 at 50 psi and N_2 at 60 psi into the air space between the medium and the top of the media bottle for 5 min, after which the bottle was tightly sealed with a rubber stopper (DWK Life Sciences) and autoclaved for 15 min.

To initiate *A. muciniphila* cultures for oral inoculation, glycerol stocks were brought into the anaerobic chamber on dry ice, scraped with a sterile tip that was then placed in 3-5 mL of BHI+mucin media in 15 ml stopper cap falcon tubes and cultured at 37 ^{0}C in the anaerobic chamber for three to four days. Control tips with no *A. muciniphila* and media alone were cultured in parallel as a control for contamination and to be inoculated as vehicle control, respectively. Initially, it was determined that after three to four days of *A. muciniphila* glycerol stock culture in these conditions, the optical density at 600nm was 0.5-0.6, which after plating at serial dilutions corresponded to 5.5-6 x 10^8 colony forming units (cfu)/ml. 200µl of these cultures (i.e. ~1.2x10^8 cfu) or vehicle control were inoculated per mouse at indicated time-points. Note that very similar amounts of bacteria per dose have been used previously (6). *A. muciniphila* cultures were routinely plated in BHI+mucin media and 1% agar to confirm the homogeneity of the culture and to rule out potential contamination.

Bacterial Fluorescence in Situ Hybridization (FISH)

Caecal and colonic tissue samples from respective experimental groups were harvested and fixed in Carnoy's fixative (60% ethanol, 30% chloroform, 10% glacial acetic acid) and processed further as previously described (7). Tissue sections (5µM) were deparaffinized followed by hybridization for 4 hours at 46°C in the presence of 20% formamide utilizing the following probes as reported earlier (8): pan-bacterial EUB338 (5′-GCTGCCTCCCGTAGGAGT-3′), non-EUB (5′-ACATCCTACGGGAGGC-3′) and species-specific A.MUC-1437 (5′-CCTTGCGGTTGGCTTCAGAT-3′). Probes were labeled at the 5′ and 3′ ends with fluorescein isothiocyanate (FITC), Cy5 or Cy3 Berlin (BioTez, Berlin, Germany). After washing, slides were counterstained and cover-slipped using ProLong® Gold Antifade Reagent including 4',6-diamidino-2-phenylindole (DAPI, Life Technologies, Grand Island, NY). Samples were viewed and imaged on an Olympus IX81 inverted microscope in combination with a FV1000 confocal laser scanning system.

Sample collection for microbiome studies

Mice were sacrificed, and peritoneal cavity opened with one set of tools. A new sterile set of tools was then used to retrieve colonic and caecal contents, which were placed in sterile tubes, frozen on dry ice and stored at -80°C. Both sets of tools were submerged in 70% ethanol between animals belonging to the same group and a new set of sterile tools was used when changing experimental groups.

DNA extraction for 16S rRNA V4 amplicon sequencing and shotgun metagenomic sequencing

We followed the protocol described in (9). Briefly, to extract DNA from colonic and caecal contents, frozen samples were thawed and transferred into 96-well plates containing garnet beads. DNA was extracted using Qiagen PowerSoil DNA kit adapted for magnetic bead purification and eluted in 100 µL of Qiagen elution buffer. DNA quantification was performed via Quant-iT PicoGreen Assay (Invitrogen).

Library generation and sequencing

Sequencing was done following standard protocols from the Earth Microbiome Project (10, 11). Extracted DNA was amplified by using barcoded primers. Each sample was PCR-amplified in triplicate and V4 pair-end sequencing or whole-genome sequencing using a miniaturized version of the KAPA HyperPlus kit was performed using Illumina MiSeq or HiSeq (La Jolla, CA).

16S rRNA gene amplicon data analysis

Processing of our 16S rRNA raw data involved splitting of FASTQ libraries, demultiplexing, trimming of sequences to 150bp of length (13) followed by deblur 1.1.0 (14) with Qiita default parameters as follows: indel probability of 0.01, mean per nucleotide error rate of 0.005, minimum per- sample read threshold of 2, Greengenes_13.8 as reference phylogeny for SEPP and 1 thread per sample). Features with a minimum frequency of 10 were retained and reference hit table obtained was rarefied to 10,000 counts, which was in the plateau region of alpha-rarefaction curves (15). To assign taxonomy, we used the pre-fitted sklearn-based taxonomy classifier ('classify_sklearn') with the Green Genes classifier (gg-13-8-99-515-806-nb-classifier) (16, 17). Raw 16S rRNA sequencing data from the stools of mice fed a calorie-restricted diet for 3 weeks (18) was obtained from NCBI Short Read Archive (SRA: PRJNA480387), uploaded to Qiita and processed as described above. Beta-diversity distance matrices were generated with the 'beta_phylogenetic'module using Unweighted UniFrac or the 'beta' module using Jaccard and Bray-Curtis metrics. This was followed by principal coordinates analysis ('pcoa' module) and significance and differential dispersion were calculated for 'infection' with the 'beta_group_significance' module using PERMANOVA and PERMDISP tests, respectively, with 999 permutations. When more than 2 conditions were compared, the q-values obtained after PERMANOVA or PERMDISP analysis were adjusted for multiple tests with the false discovery rate (Benjamini & Hochberg) correction. To obtain R-squared values, we ran the 'adonis PERMANOVA test for beta group significance' using the previously generated distance matrices and 'infection' as the model formula. Alpha diversity was computed using the 'alpha' module for Shannon Diversity Index. All the aforementioned analyses were done with Qiita platform version 052020 5f09f46. To identify differentially abundant genera among groups in our 16S rRNA sequencing data as well as in caloric-restriction data (18), we collapsed our rarefied .biom tables to the genus level and used Songbird multinomial regression analysis (19) with default parameters (epochs of 10000, differential prior of 0.5) (https://github.com/biocore/songbird). We applied cut-off rank values of 1 and -1 to focus our analysis on highly perturbed genera. Validation of the model was performed for all analyses with tensor board.

Shotgun metagenomic data analysis

To pre-process these data we performed adapter trimming with Atropos v1.1.15 (20), followed by quality control filtering (QC_Filter) to bowtie2/Mus_musculus, followed by genome alignment of adapter-trimmed files with bowtie2 (with Rep94 database) (21) by running SHOGUN 0.1.5.(22) on Qiita (12). A table in .biom format with taxonomic predictions at the species level was then rarefied to a depth of 200,000 or 450,000. Alpha diversity was computed using the Shannon Diversity Index and beta-diversity was computed with the 'beta' module using Jaccard distance metric. PCoA representation and PERMANOVA were calculated as described above for the 16S rRNA gene amplicon data. To identify differentially abundant species between groups, we collapsed our rarefied .biom tables to the species level and used Songbird multinomial regression analysis (Morton et al., 2019) with parameters described above for 16S rRNA gene amplicon data analysis. In this case, we applied cut-off rank values of 0.5 and -0.5 to include moderately and highly perturbed genera. Validation of the model was performed for all analyses with tensor board.

Data availability

All 16S rRNA gene amplicon and shotgun metagenomics sequencing data are publicly available on the European Nucleotide Archive (https://ebi.ac.uk/ena, accession number ERP123227) and on Qiita (https://qiita.ucsd.edu, study ID 11043). On Qiita, processed 16S .fastq corresponding to 2 experimental repeats on day 8 p.i. and the first experimental repeat on day 20 p.i. can be found under preparation ID 3348. Processed 16S .fastq corresponding to the second experimental repeat on day 20 p.i. can be found under preparation ID 8906. Processed metagenomics .fastq files corresponding to the data shown in Figure 2 and Figure S2 can be found under preparation ID 7150 and 9028, respectively. Detailed metadata for all samples can be found under the 'Sample Information' tab.

Statistical analysis

Unpaired two-tailed t-Student test was used to compare two groups. If variances were not equal by F-test, data were tested using the non-parametric Mann Whitney-U test. Significant differences among more than two groups were determined based on one-way ANOVA with Tukey's multiple comparison correction or, in the case of unequal variances, non-parametric Kruskal-Wallis test with Dunn's multiple comparison correction. Statistical tests were performed using GraphPad Prism v8.

A

Unweighted UniFrac	PERMANOVA Day 8p.i. Exp1	PERMANOVA Day 8p.i. Exp2	PERMANOVA Day 20p.i. Exp1	PERMANOVA Day 20p.i. Exp2	PERMDISP Day 8p.i. Exp1	PERMDISP Day 8p.i. Exp2	PERMDISP Day 20p.i. Exp1	PERMDISP Day 20p.i. Exp2
Un vs. ARM (q-val)	0.002	0.001	0.007	0.001	0.54	0.264	0.524	0.117
Un vs. Cl13 (q-val)	0.002	0.001	0.003	0.001	0.96	0.162	0.524	0.856
Cl13 vs. ARM (q-val)	0.002	0.001	0.003	0.001	0.54	0.162	0.524	0.119

R-square values	Day 8p.i. Exp1	Day 8p.i. Exp2	Day 20p.i. Exp1	Day 20p.i. Exp2
Factor				
Infection status	0.2	0.219	0.195	0.234

B

Bray-Curtis dissimilarity	PERMANOVA Day 8p.i. Exp1	PERMANOVA Day 8p.i. Exp2	PERMANOVA Day 20p.i. Exp1	PERMANOVA Day 20p.i. Exp2	PERMDISP Day 8p.i. Exp1	PERMDISP Day 8p.i. Exp2	PERMDISP Day 20p.i. Exp1	PERMDISP Day 20p.i. Exp2
Un vs. ARM (q-val)	0.085	0.029	0.023	0.002	0.559	0.321	0.876	0.603
Un vs. Cl13 (q-val)	0.002	0.002	0.015	0.002	0.559	0.033	0.876	0.426
Cl13 vs. ARM (q-val)	0.002	0.002	0.003	0.002	0.684	0.105	0.876	0.533

R-square values	Day 8p.i. Exp1	Day 8p.i. Exp2	Day 20p.i. Exp1	Day 20p.i. Exp2
Factor				
Infection status	0.254	0.249	0.184	0.274

C

Jaccard similarity index	PERMANOVA Day 8p.i. Exp1	PERMANOVA Day 8p.i. Exp2	PERMANOVA Day 20p.i. Exp1	PERMANOVA Day 20p.i. Exp2	PERMDISP Day 8p.i. Exp1	PERMDISP Day 8p.i. Exp2	PERMDISP Day 20p.i. Exp1	PERMDISP Day 20p.i. Exp2
Un vs. ARM (q-val)	0.001	0.001	0.001	0.001	0.715	0.825	0.612	0.006
Un vs. Cl13 (q-val)	0.001	0.001	0.001	0.001	0.715	0.185	0.612	0.9
Cl13 vs. ARM (q-val)	0.001	0.001	0.001	0.001	0.715	0.033	0.689	0.069

R-square values	Day 8p.i. Exp1	Day 8p.i. Exp2	Day 20p.i. Exp1	Day 20p.i. Exp2
Factor				
Infection status	0.185	0.198	0.203	0.206

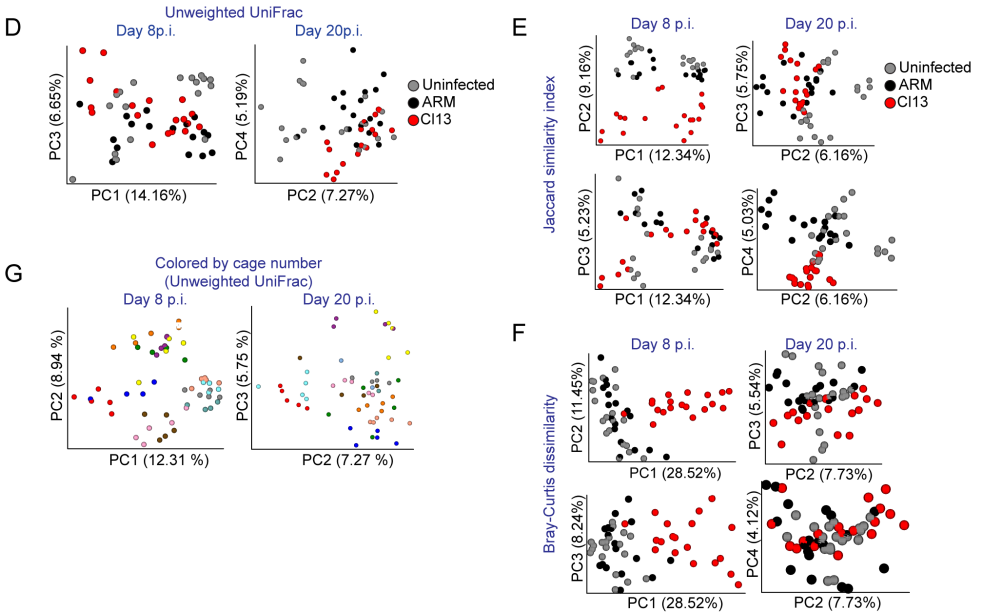

Figure S1. Intestinal microbiomes of ARM infected and Cl13-infected mice (but not mice housed in different cages) formed significantly distant clusters compared to uninfected mice. Related to Figure 1. C57BL/6 mice were infected with LCMV ARM, Cl13 or left uninfected (Un) and 16S rRNA gene amplicon sequencing was performed on colonic and caecal contents on day 8 and 20 p.i. (A-C) Beta-diversity with unweighted UniFrac distance (A), Bray-Curtis dissimilarity (B) or Jaccard similarity index (C) followed by beta group significance were calculated with PERMANOVA (999 permutations) for each experimental repeat (Exp1&Exp2). Differences in dispersion were calculated with PERMDISP (999 permutations). Q-values (q-val) were adjusted for multiple tests with the false discovery rate (Benjamini & Hochberg) correction. R-square values were computed with Adonis PERMANOVA (999 permutations). (D,G) PCoA plot generated with unweighted UniFrac distances and comparing PC1 vs PC3 (left) and PC2 vs PC4 (right) (D) or with each color representing individual cages (G). (E,F) PCoA plots comparing PC1,2 & 3 (left) or PC2, 3 and 4 (right) generated with Jaccard (E) or Bray-Curtis (F) metrics. Data represent individual experimental repeats (A-C) or are pooled from two experiments with n=8-10 mice/group (D-G).

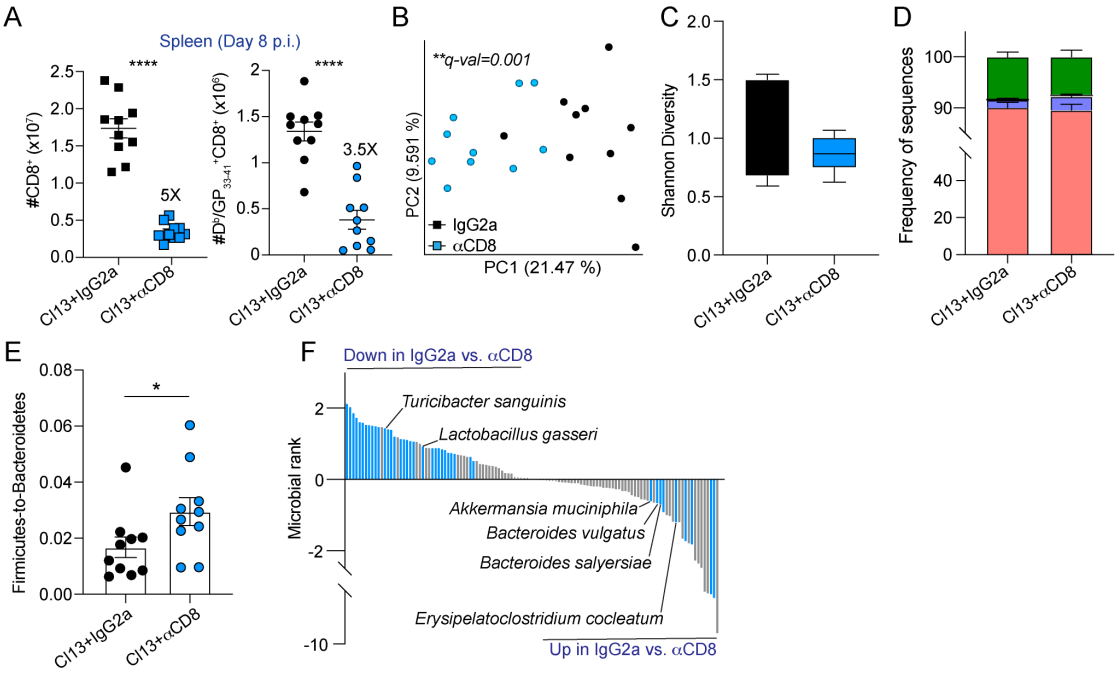

Figure S2. Anti-CD8 antibodies efficiently depleted CD8 T cells and shifted the composition of the intestinal microbiome after LCMV Cl13 infection. Related to Figure 2. See also Dataset S5. C57BL/6 mice were infected with LCMV Cl13, injected intraperitoneally (i.p.). with isotype control (IgG2a) or CD8-depleting antibodies (αCD8). (A) Numbers of splenic total $CD8^+$ and $D^b/GP_{33\text{-}41}{}^+CD8^+$ cells. Numbers indicate the fold-change between groups. (B-F) Shotgun metagenomics sequencing was performed on colonic and caecal contents on day 8 p.i. (B) Beta-diversity PCoA with Jaccard distance. (C) Alpha diversity by the Shannon diversity index. (D) Frequency of sequences at the phylum level. (E) Frequency of phylum Firmicutes over Bacteroidetes for each mouse. (F) Perturbed species in IgG2a- vs. αCD8-treated Cl13-infected mice via Songbird MR analysis. Taxa with rank cut-off values of -0.5 and 0.5 that were perturbed in the same direction in two independent experimental repeats are highlighted in blue and indicated by name. (A,C-E) Averages ± S.E.M. (A,D,E) or min or max (C). (A-F) Representative data from one independent experiment with n=9-10 mice/group. (A,E) Mann-Whitney test, (B) PERMANOVA with 999 permutations, (C) Kruskal-Wallis test; *p or q-val<0.05, **<0.01, ***<0.001, ****<0.0001.

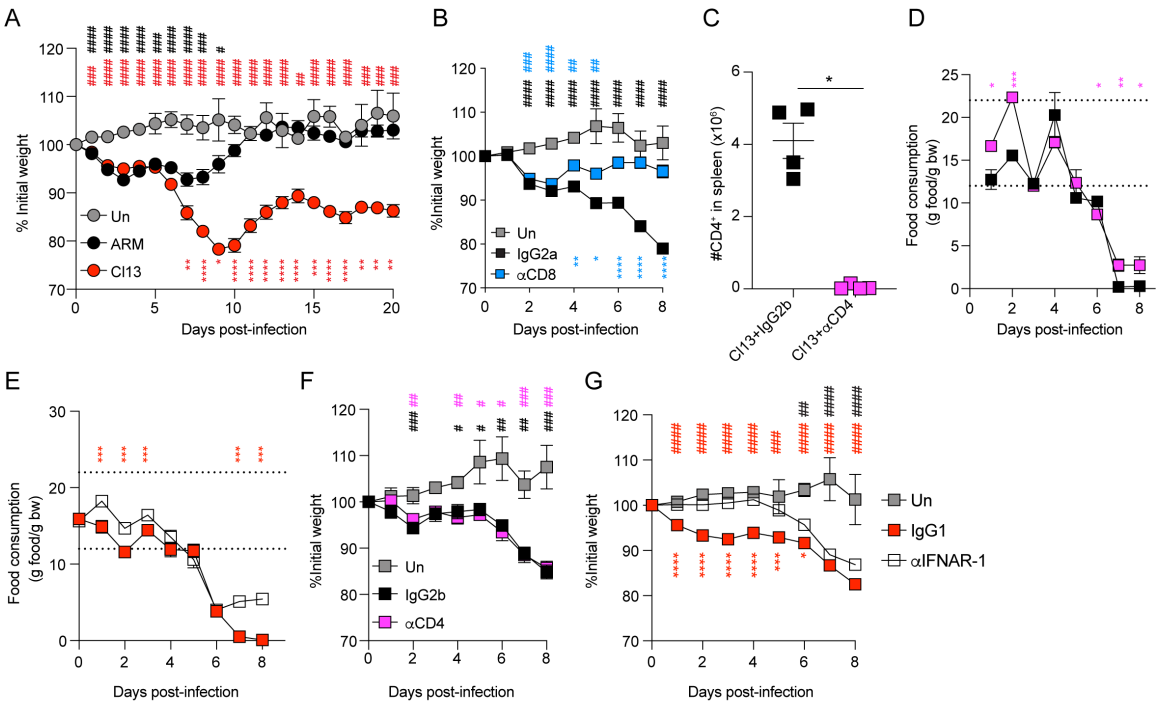

Figure S3. Weight loss after LCMV Cl13-infection was profoundly reverted by anti-CD8 antibody treatment while weight loss and anorexia were partially or not attenuated by anti-IFNAR or anti-CD4 antibodies, respectively. Related to Figure 3. C57BL/6 mice were left uninfected (Un), infected with LCMV ARM (A) or Cl13 (A-G) and treated with isotype control IgG2a or CD8-depleting antibodies (αCD8, B), with isotype control IgG2b or CD4-depleting antibodies (αCD4, C,D,F) or with isotype control IgG1 or anti-IFNAR1 antibodies (αIFNAR-1, E,G) i.p. (A,B,F,G) Percentage of initial weight over-time. (C) Numbers of splenic $CD4^+$ cells at day 8 p.i. (D,F) Grams (g) of food consumed per g of body weight (bw) over-time in indicated groups. Dotted lines mark the healthy range of food consumption determined in uninfected mice as described in the methods section. (A-G) Average ± S.E.M. (A-G) Data are representative of three (B), two (C) or pooled from two (A, D-G) independent experiments with n=4-10 mice per group. (A,B,F,G) One-way ANOVA with Tukey's multiple comparisons correction for each timepoint with Cl13 vs. ARM (red *), Cl13 vs. Un (red #) and ARM vs. Un (black #) (A); αCD8 vs. IgG2a (blue *), αCD8 vs. Un (blue #) and IgG2a vs. Un (black #) (B); αCD4 vs. Un (pink #) and IgG2b vs. Un (black #) (F); αIFNAR-1 vs. IgG1 (red *), αIFNAR-1 vs. Un (black #) and IgG1 vs. Un (red #) (G). (C) Mann-Whitney test, (D,E) unpaired two-tailed Student's t-test. (D) Increased food consumption by the αCD4 group was significantly increased in one out of two experiments and after pooling. * or # p-val<0.05, ** or ## p-val<0.01, ***or ### p-val<0.001, **** or #### p-val <0.0001.

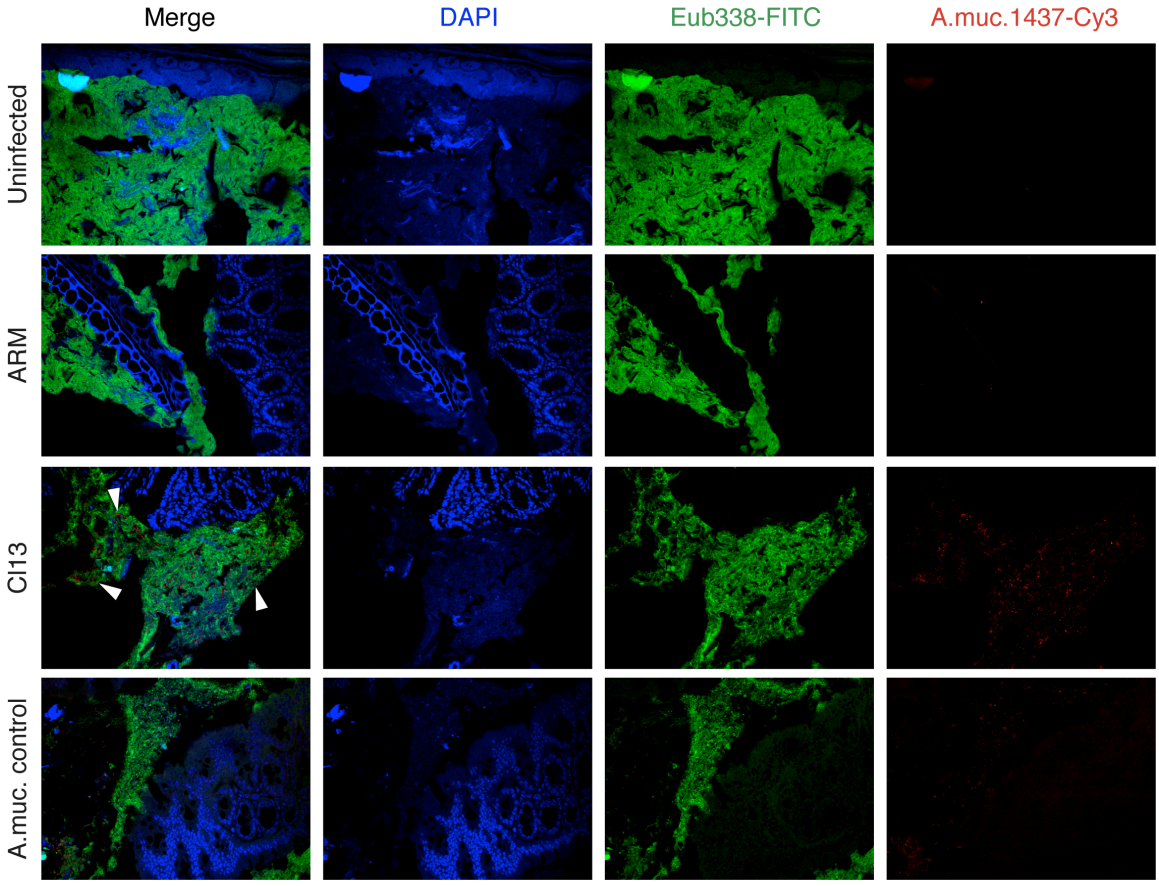

Figure S4. Increased *A. muciniphila* detection by FISH in caecums from Cl13-infected vs. ARM-infected and uninfected mice on day 8 p.i. Related to Figure 3. C57BL/6 mice were infected with LCMV ARM, Cl13 or left uninfected. Representative fluorescence In Situ Hybridization (FISH) images using specific probes for *Eubacteria* (Eub338, green) and *A. muciniphila* (A.muc.1437, red) along with nuclear DAPI staining, from caecums of respective mice on day 8 p.i. One uninfected mouse was intra-orally inoculated with ~$1.2x10^8$ cfu of live *A. muciniphila* 24 hrs prior to necropsy and served as positive control (A.muc. control). Images are representative of 2 experiments with n=3 mice per group.

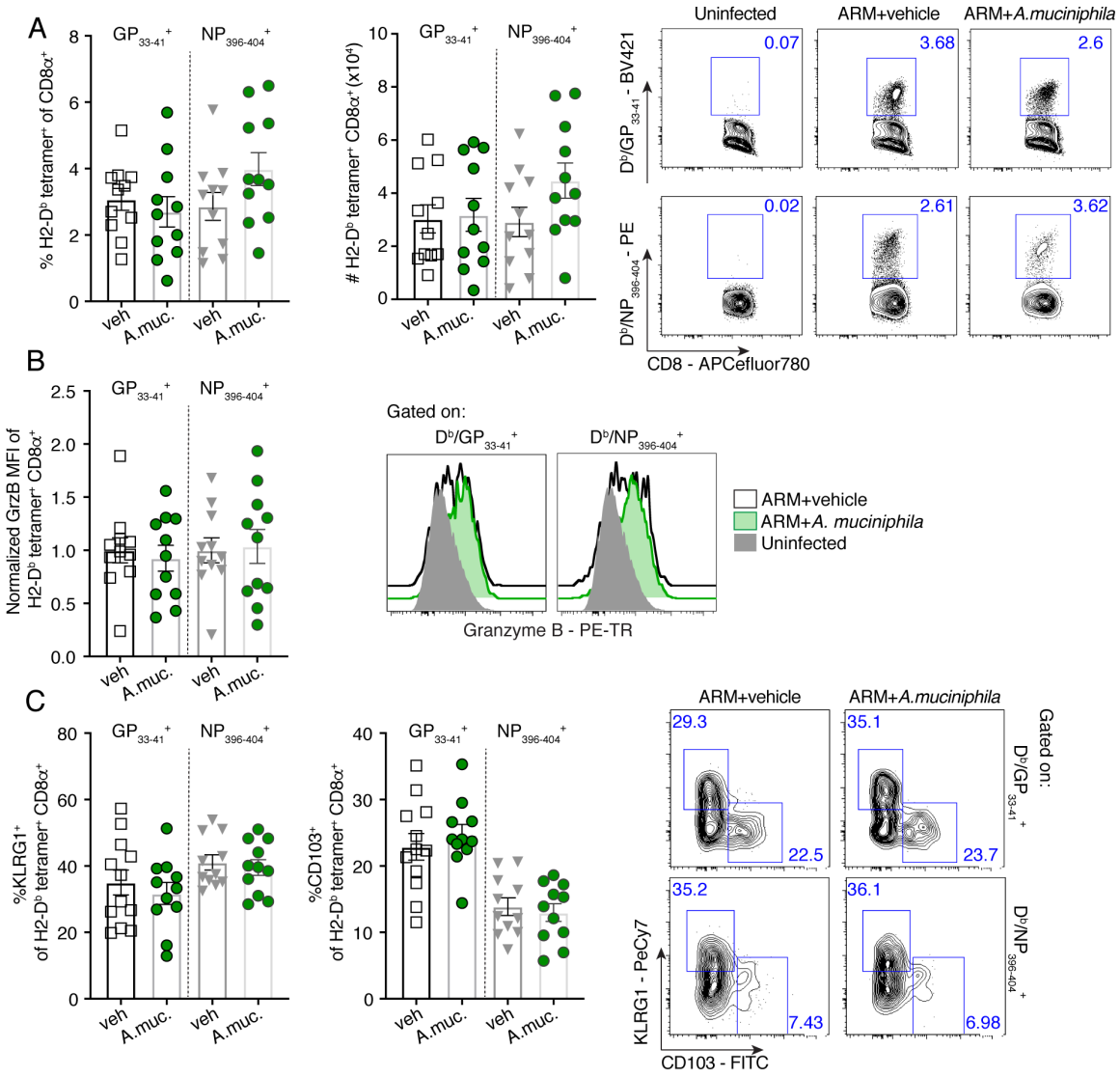

Figure S5. *A. muciniphila* inoculation into LCMV ARM-infected mice did not alter accumulation, effector features or differentiation of small intestinal IEL CD8 T cells. Related to Figure 4. C57BL/6 mice were infected with LCMV ARM and intraorally inoculated with vehicle (veh) or ~1.2x10^8 cfu of live *A. muciniphila* (A.muc.) once daily on days 3.5 through 7.5 p.i. Small intestine IEL FACS analysis was done on day 8 p.i. (A) Frequencies, numbers and representative FACS plots of D^b/GP$_{33\text{-}41}^+$ and D^b/NP$_{396\text{-}404}^+$ CD8 T cells. (B) Granzyme B (GrzB) MFI normalized to the average of the vehicle control and representative FACS plots within gates for D^b/GP$_{33\text{-}41}^+$ and D^b/NP$_{396\text{-}404}^+$ CD8 T cells. (C) Frequencies of KLRG1$^+$ (left) or CD103$^+$ (right) of D^b/GP$_{33\text{-}41}^+$ and D^b/NP$_{396\text{-}404}^+$ CD8 T cells and representative FACS plots within gates for D^b/GP$_{33\text{-}41}^+$ and D^b/NP$_{396\text{-}404}^+$ CD8 T cells. (A-C) Average ± S.E.M. (A-C) Data are pooled from three independent experimental repeats with 3-5 mice/group. (A-C) Unpaired two-tailed Student's t-test.

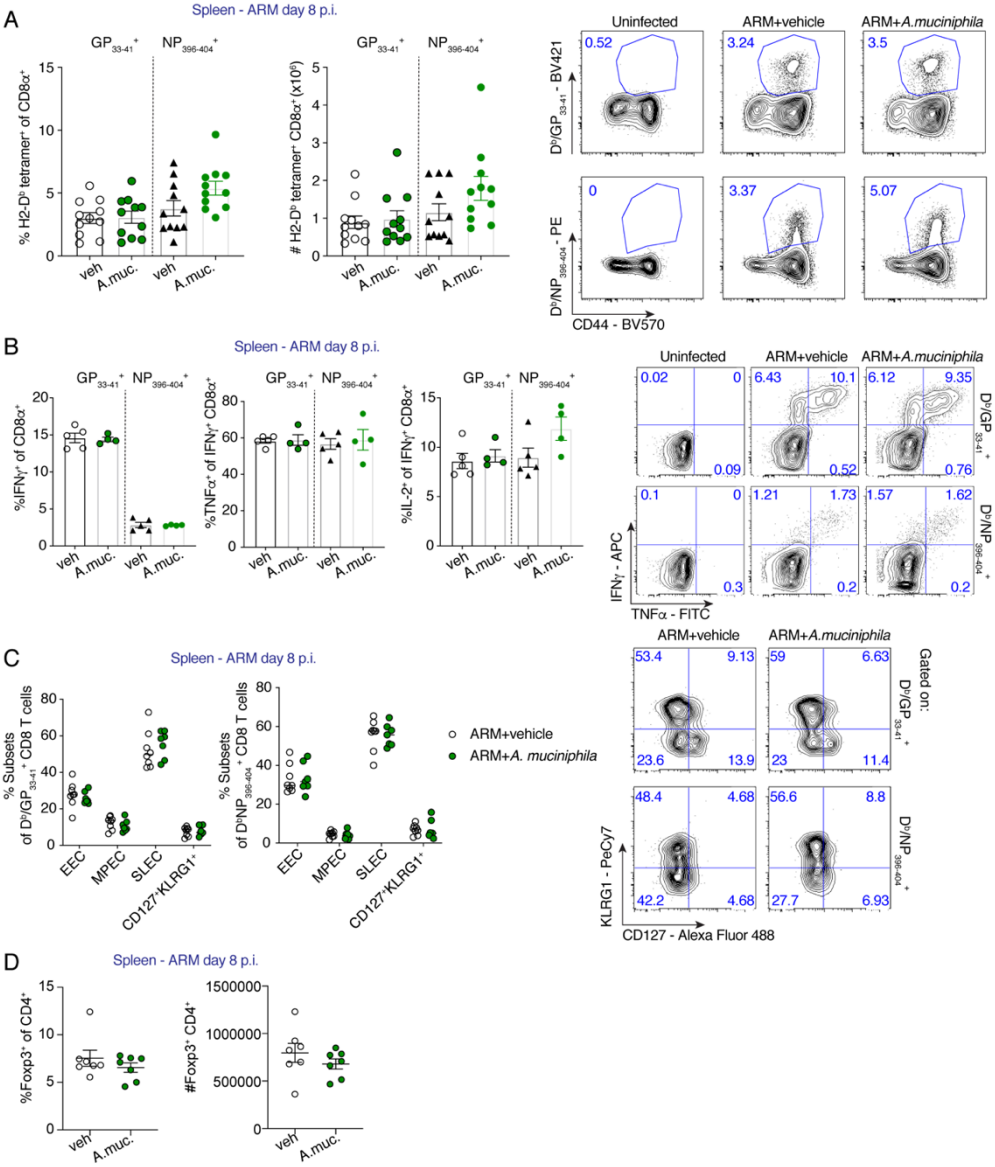

Figure S6. *A. muciniphila* inoculation into ARM-infected mice did not alter accumulation, cytokine-production or differentiation of spleen CD8 T cells nor accumulation of CD4$^+$Foxp3$^+$ T cells. Related to Figure 4. C57BL/6 mice were infected with LCMV ARM and administered vehicle (veh) or ~1.2x10^8 cfu of live *A. muciniphila* (A.muc.) i.o. once daily on days 3.5 through 7.5 p.i. Spleen FACS analysis was done on day 8 p.i. (A) Frequencies, numbers and representative FACS plots of D^b/GP$_{33\text{-}41}^+$ and D^b/NP$_{396\text{-}404}^+$ CD8 T cells. (B) Frequencies of IFNγ-producing, IFNγ and TNFα- or IFNγ and IL-2- co-producing CD8 T cells upon *ex vivo* restimulation with indicated peptides; and representative FACS plots for IFNγ and TNFα staining. (C) Frequencies of EEC, MPEC, SLEC or CD127$^+$KLRG1$^+$ subsets defined by KLRG1 and CD127 expression among D^b/GP$_{33\text{-}41}^+$ and D^b/NP$_{396\text{-}404}^+$ CD8 T cells; and representative FACS plots. (D) Percentages and numbers of CD4$^+$ Foxp3$^+$ Treg. (A-D) Average ± S.E.M. (A-D) Data are pooled from two (D), three (A, C) independent experimental repeats with 3-5 mice/group or are representative of one out of three independent experiments that showed equivalent results (B). (A-D) Unpaired two-tailed Student's t-test.

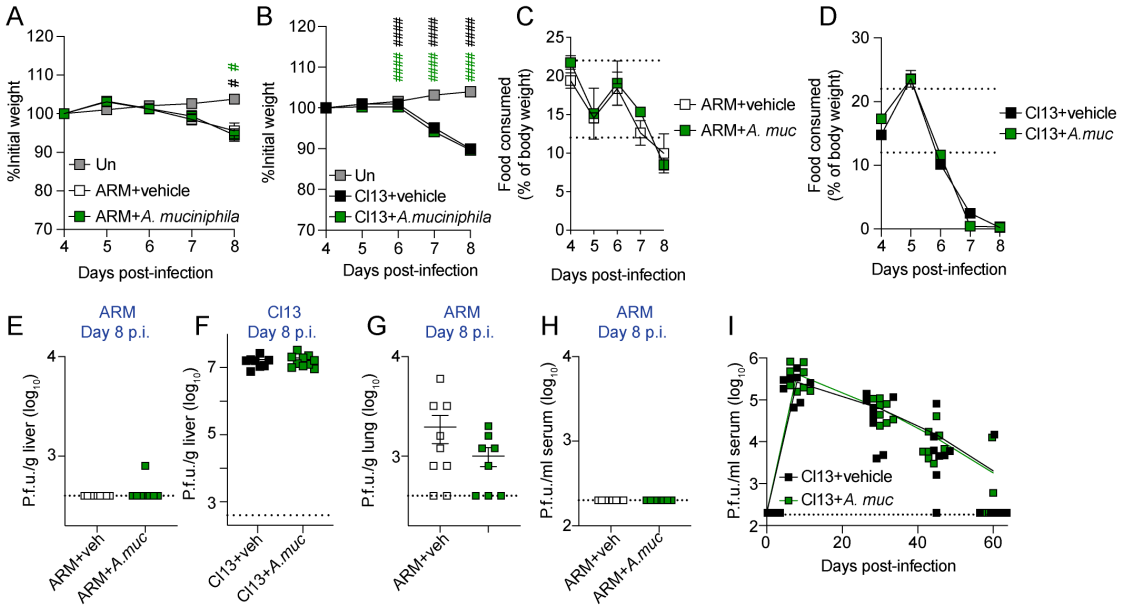

Figure S7. *A. muciniphila* inoculation into LCMV ARM- or Cl13-infected mice did not alter weight loss, eating behavior or titers of infectious viral particles at the time points assessed. Related to Figure 4. C57BL/6 mice were left uninfected (Un) (A-B), infected with ARM (A,C,E-G) or with Cl13 (B,D,H,I) and administered vehicle (veh) or ~$1.2x10^8$ cfu of live *A. muciniphila* (A.muc.) i.o. once daily on days 3.5 through 7.5 p.i. (A,B) Percentage of initial weight over-time. (C,D) Grams (g) of food consumed per g of body weight (bw) over-time in indicated groups. Dotted lines mark the healthy range of food consumption determined in uninfected mice as described in the methods section. (E-I) Infectious viral particles were quantified by plaque assay in liver (E,F), lung (G) and serum (H,I) at time points indicated. (A-I) Average ± S.E.M. Data are pooled from three (A,C) or two (B,D,E-I) independent experiments with n=4-8 mice/group. (A,B) One-way ANOVA with Tukey's multiple comparisons correction with vehicle vs. Un (black #) and *A. muciniphila* vs. Un (green #). (C-I) Unpaired two-tailed Student's t-test; # p-val <0.05, ### p-val <0.001.

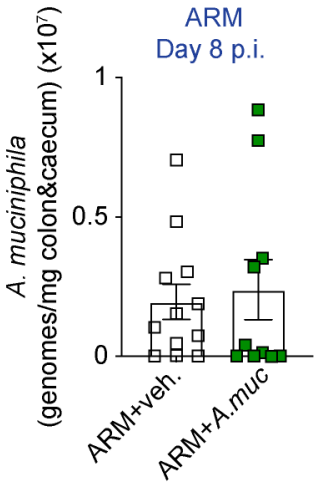

Figure S8. Intestinal *A. muciniphila* levels were not significantly increased after oral inoculation of live cultures of this commensal into LCMV ARM-infected mice. Related to Figure 4. C57BL/6 mice were infected with ARM and administered vehicle (veh) or ~$1.2x10^8$ cfu of live *A. muciniphila* (A.muc.) i.o. once daily on days 3.5 through 7.5 p.i. Absolute *A. muciniphila* genomes in colonic and caecal contents were quantified by qPCR at day 8 p.i. Average ± S.E.M. Data are pooled from three independent experiments with n=3-5 mice/group. Unpaired two-tailed Student's t-test.

Supplementary References

1. L. R. Thompson *et al.*, A communal catalogue reveals Earth's multiscale microbial diversity. *Nature* **551**, 457-463 (2017).
2. R. Ahmed, A. Salmi, L. D. Butler, J. M. Chiller, M. B. Oldstone, Selection of genetic variants of lymphocytic choriomeningitis virus in spleens of persistently infected mice. Role in suppression of cytotoxic T lymphocyte response and viral persistence. *J Exp Med* **160**, 521-540 (1984).
3. J. R. Teijaro *et al.*, Persistent LCMV infection is controlled by blockade of type I interferon signaling. *Science* **340**, 207-211 (2013).
4. E. B. Wilson *et al.*, Blockade of chronic type I interferon signaling to control persistent LCMV infection. *Science* **340**, 202-207 (2013).
5. M. Derrien, E. E. Vaughan, C. M. Plugge, W. M. de Vos, Akkermansia muciniphila gen. nov., sp. nov., a human intestinal mucin-degrading bacterium. *Int J Syst Evol Microbiol* **54**, 1469-1476 (2004).
6. A. Everard *et al.*, Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. *Proc Natl Acad Sci U S A* **110**, 9066-9071 (2013).
7. A. Swidsinski *et al.*, Viscosity gradient within the mucus layer determines the mucosal barrier function and the spatial organization of the intestinal microbiota. *Inflamm Bowel Dis* **13**, 963-970 (2007).
8. M. Derrien, M. C. Collado, K. Ben-Amor, S. Salminen, W. M. de Vos, The Mucin degrader Akkermansia muciniphila is an abundant resident of the human intestinal tract. *Appl Environ Microbiol* **74**, 1646-1648 (2008).
9. C. Marotz *et al.*, DNA extraction for streamlined metagenomics of diverse environmental samples. *Biotechniques* **62**, 290-293 (2017).
10. J. G. Caporaso *et al.*, Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J* **6**, 1621-1624 (2012).
11. J. A. Gilbert, J. K. Jansson, R. Knight, The Earth Microbiome project: successes and aspirations. *BMC Biol* **12**, 69 (2014).
12. A. Gonzalez *et al.*, Qiita: rapid, web-enabled microbiome meta-analysis. *Nat Methods* **15**, 796-798 (2018).
13. J. G. Caporaso *et al.*, QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* **7**, 335-336 (2010).
14. A. Amir *et al.*, Deblur Rapidly Resolves Single-Nucleotide Community Sequence Patterns. *mSystems* **2** (2017).
15. N. J. Gotelli, R. K. Colwell, Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. *Ecology Letters* **4**, 379-391 (2001).
16. N. A. Bokulich *et al.*, Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. *Microbiome* **6**, 90 (2018).
17. T. Z. DeSantis *et al.*, Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl Environ Microbiol* **72**, 5069-5072 (2006).
18. S. Fabbiano *et al.*, Functional Gut Microbiota Remodeling Contributes to the Caloric Restriction-Induced Metabolic Improvements. *Cell Metab* **28**, 907-921 e907 (2018).
19. J. T. Morton *et al.*, Establishing microbial composition measurement standards with reference frames. *Nat Commun* **10**, 2719 (2019).
20. J. P. Didion, M. Martin, F. S. Collins, Atropos: specific, sensitive, and speedy trimming of sequencing reads. *PeerJ* **5**, e3720 (2017).
21. B. Langmead, S. L. Salzberg, Fast gapped-read alignment with Bowtie 2. *Nat Methods* **9**, 357-359 (2012).
22. B. Hillmann *et al.*, Evaluating the Information Content of Shallow Shotgun Metagenomics. *mSystems* **3** (2018).