SUPPLEMENTAL FIGURE TITLES & LEGENDS

Figure S1. *Allobaculum sp*. 128 does not bloom during inflammation, and a second UC patient *Allobaculum* isolate is colitogenic in gnotobiotic mice, Related to Figure 1. (A) Time course of fecal microbiota profiling of MC+Allo colonized mice across 7 days of 2% DSS administration (n=4 mice/group). (B) Time course of fecal lipocalin (LCN-2) overlaid with *Allobaculum sp*. 128 abundance. (C) H&E-stained colon sections from *Rag1*$^{-/-}$ mice on d7 of DSS administration. Scale bars, 200μm. (D-E) Colon lamina propria CD4^{+}IL-17A^{+} T cells from *Il10*$^{-/-}$ mice after 8 weeks colonization (n=5 mice/group). (F-G) GF mice were monocolonized with either *Ery47* (control bacteria) or *Allobaculum sp*. 128 for 7 days before the induction of acute colitis via 2% DSS administration. Colonic inflammation was assessed by fecal lipocalin on d3 (F) and colon length on d7 (G) (n=5 mice/group). (H) Phylogenetic tree constructed using 16S rRNA gene sequences from members of family *Erysipelotrichaceae*, using maximum likelihood estimation, bootstrapped (BS) to 1,000 replicates. BS values are shown along branches. (I) Experimental schematic. (J) Second *Allobaculum* isolate (Allo2) was used for acute colitis model in WT gnotobiotic mice as in Figure 1C-1H. Three of six mice colonized with Allo2 were found dead (3 F.D.) prior to endpoint. (K) Fecal lipocalin, LCN2, was measured longitudinally by ELISA. Error bars show Mean ± SEM. Data shown in (A-B) from N=1 experiment. Data in (D-E) fron one of N=2 experiments. Data in (F-G) from N=1 experiment. Data shown in (J-K) from one of N=2 independent experiments, n=5-6 mice per group.

Figure S2. Unremarkable histopathology and total serum Ig of untreated *Allobaculum sp*. 128-colonized WT mice, Related to Figure 1. (A) Bouin's-fixed H&E-stained colon sections from WT mice colonized with MC bacteria or MC+*Allobaculum sp*. 128, euthanized 12 weeks later. Scale bars, 200μm. (B) Blinded scoring for colitis. One experiment shown is representative of N=2 independent experiments. (C-D) Total serum IgA and IgG content at 7 weeks (n=3-4 mice/group). (E) FITC-dextran concentration in serum 1h after oral gavage (n=4-8 mice/group). (F) *Allobaculum sp.* 128-specific IgG in fecal water at 7 weeks post-colonization with MC or MC+Allo (n=5 mice/group). (G) Thickness of the colonic inner mucus layer after 2 weeks of colonization (tissues fixed in Carnoy's solution to preserved the inner mucus layer; n=10-12 mice/group). Welch's t-test was used to compare microbiota groups at each time point. ** P<0.01, * P<0.05. Data shown in (A-B) are from one representative of N=3 experiments. Data shown in (C-D) show one of N=2 experiments. Data in (E) are from N=1 experiments. Data in (F) show one of N=2 experiments. Data in (G) are compiled from N=2 experiments.

Figure S3. Microbial diversity cannot explain the relationship between *Allobaculum and A. muciniphila, A. muciniphila* and *Allobaculum* co-colonize the ileal mucosa, and co-colonization has minimal impacts on *A. muciniphila* and *Allobaculum* gene expression, Related to Figures 3 & 4. (A) Genus-level richness, Simpson's diversity index, Shannon's diversity index, and evenness of each microbiome that contained *Allobaculum* (n=14) or lacked *Allobaculum sp*. 128 (n=5). (B) Legend accompanying Fig. 4B. Data shown from N=1 experiment. (C) GF WT mice were mono- or bi-colonized as shown for 10 days. Terminal ilea were fixed and sections stained with bacterial FISH probes EUB338 (to stain *Allobaculum*) and VP403 (to stain *A. muciniphila*). Scale bars, 10μm. (D) *In vivo* bacterial transcriptomes from the

ileum and colon were compared for differential expression of ORFs across single colonization or co-colonization conditions (MC+Allo vs MC+Both, and MC+Akk vs. MC+Both). (E) *Allobaculum sp*. 128 and (F) *A. muciniphila* ORFs that were differentially expressed in the ileum, along with their fold changes, P-values, and annotations (Prokka). Data are from N=1 experiment.

Figure S4. Type strain *A. muciniphila* attenuates *Allobaculum sp.* 128-mediated colitis and *Allobaculum sp.* 128 blunts *A. muciniphila*-induced dendritic cell responses in MLN, Related to Figure 5. (A) Experimental schematic for acute DSS colitis in WT gnotobiotic mice colonized with MC, MC+*Allobaculum sp.* 128, MC+*A. muciniphila*^{T} (type strain ATCC BAA-835), or MC+*Allobaculum sp.* 128+*A. muciniphila*^{T} (ATCC BAA-835) (n=4-6 mice/group). (B) Fecal microbiota profiling, (C) colon length, (D) d2 fecal lipocalin (LCN2), and (E) gross colon pathology. (F) Representative gating strategy for analysis of MLN cells performed in FlowJo after ≥100,000 events per sample were collected. (G) Immunophenotyping of MLN cell populations (% of viable cells) (n=4-6 mice/group). (H) Quantification of DCs (Live B220^{-}TCRb^{-} CD11b^{+}CD11c^{+}MHCII^{+}). Welch's t-test was used to compare microbiota groups. *P<0.05. Data shown in (A-E) are from N=1 experiment. Data shown in (F-H) are from one of N=2 independent experiments.

Figure S5. Approach for profiling microbiota-dependent mucosal immune landscape using single cell RNAseq, Related to Figure 7. (A) Schematic depicting single cell RNAseq (scRNAseq) experiment. (B) Quality control metrics used for filtration of scRNAseq data before proceeding to clustering and differential expression analyses. Only cells with 500-5000 RNA features were retained (between blue dashed lines), as well as cells with <8% genes of mitochondrial (mt) origin. Welch's t-test was used to compare each cell lineage across microbiota groups; * P<0.05, ** P<0.01, *** P<0.001. N=1 experiment.

Figure S6. Expression of marker genes mapped to MLN cell clusters, Related to Figure 7. (A) Violin plots showing expression of marker genes across MLN cell clusters, numbered to match clusters shown in Figure S7.

Figure S7. Epistatic reversal of *A. muciniphila*-induced MLN immune cell clusters by co-colonization with *Allobaculum sp*. 128, and direct assessment of MLN DC function in co-colonized gnotobiotic mice, Related to Figure 7 and Figure S6. (A-E) Graph-based probabilistic analysis of MLN scRNAseq data, comparing two microbiota groups at a time. (A-B) MC+Allo relative to MC+Both or relative to MC. (C-E) MC+Akk relative to MC+Both reveals strong reversal (high MELD score) of cell clusters induced by *A. muciniphila* after co-colonization with *Allobaculum sp*. 128. The marker genes that define each cluster are displayed in Figure S6. (F-G) *Ex vivo* co-cultures of purified MLN DCs from gnotobiotic mice were examined for their capacity to prime CellTrace Violet (CTV)-labeled naïve OT-II T cells, by proliferation and dilution of CTV. Data shown are from N=1 experiment.

SUPPLEMENTAL TABLES

Table S1. *Allobaculum* and *A. muciniphila* abundance in two human cohorts broadly reflects an inverse relationship, Related to Figure 3. (Sheet 1. American Gut Project) Enumeration of sequencing data from American Gut Project (McDonald, et al. 2018; QIITA study IDs: 48742, 51570, 52698, 53379, & 54454). (Sheet 2. Schirmer_UC) Relative abundance data from pediatric ulcerative colitis (Schirmer, et al. 2018).

Table S2. Human microbiota-associated gnotobiotic mouse screen reveals inverse correlation between *Allobaculum sp.* 128 and *A. muciniphila*, Related to Figure 3. (Sheet 1. Relative Abundance) fecal microbiota profiles across n=19 gnotobiotic mice. (Sheet 2. Correlation Metrics, Sorted) Compiled statistics evaluating correlation and logistic regression between each pair of microbiota OTUs.

Figure S1.

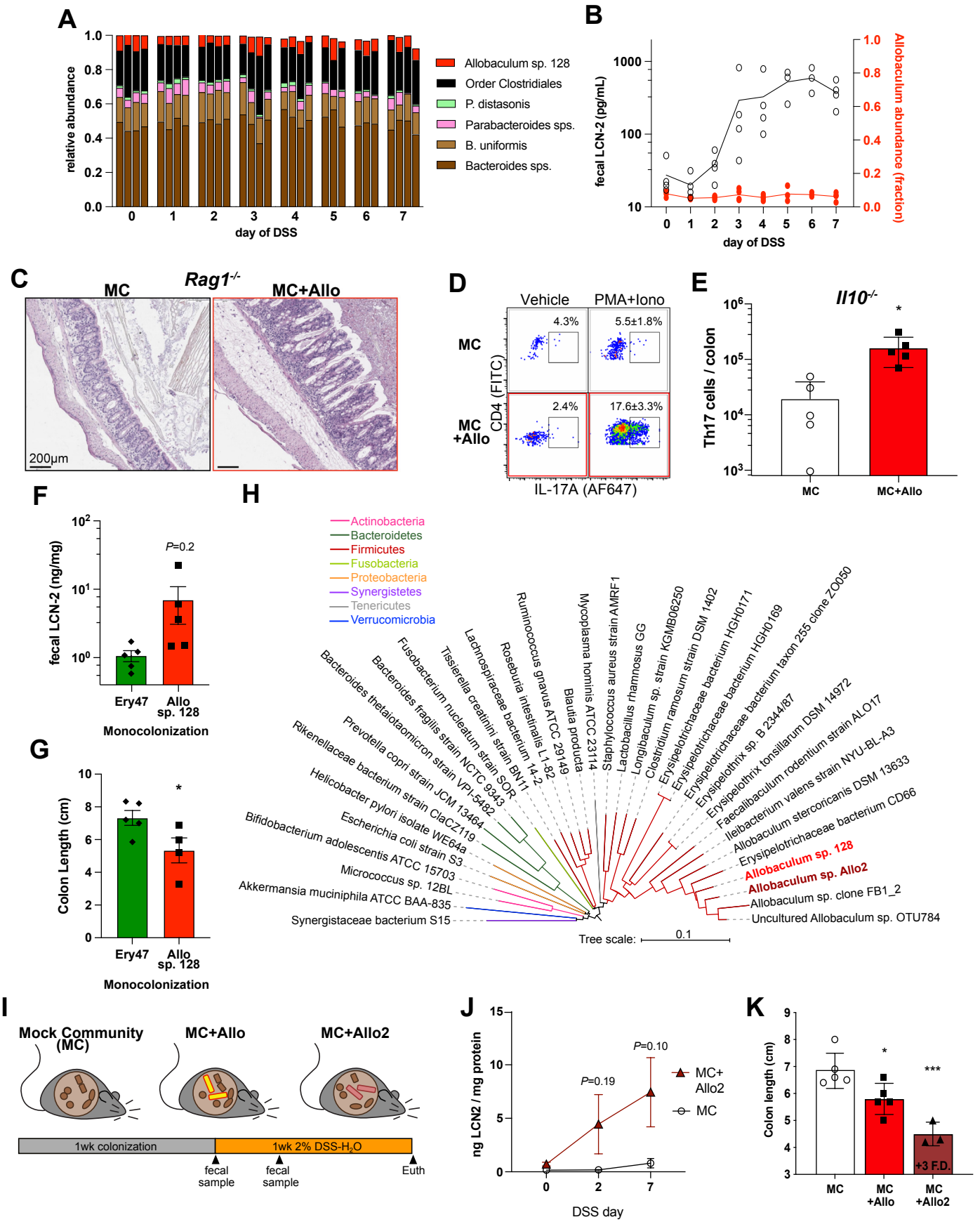

A
relative abundance
day of DSS
Allobaculum sp. 128
Order Clostridiales
P. distasonis
Parabacteroides sps.
B. uniformis
Bacteroides sps.
B
fecal LCN-2 (pg/mL)
Allobaculum abundance (fraction)
day of DSS
C
MC
Rag1-/-
MC+Allo
200µm
D
Vehicle
PMA+Iono
MC
MC +Allo
4.3%
5.5±1.8%
2.4%
17.6±3.3%
CD4 (FITC)
IL-17A (AF647)
E
Il10-/-
Th17 cells / colon
MC
MC+Allo
F
fecal LCN-2 (ng/mg)
P=0.2
Ery47
Allo sp. 128
Monocolonization
G
Colon Length (cm)
Ery47
Allo sp. 128
Monocolonization
H
Actinobacteria
Bacteroidetes
Firmicutes
Fusobacteria
Proteobacteria
Synergistetes
Tenericutes
Verrucomicrobia
Ruminococcus gnavus ATCC 29149
Roseburia intestinalis L1-82
Lachnospiraceae bacterium 14-2
Tissierella creatinini strain BN11
Fusobacterium nucleatum strain SOR
Bacteroides fragilis strain NCTC 9343
Bacteroides thetaiotaomicron strain VPI-5482
Prevotella copri strain JCM 13464
Rikenellaceae bacterium strain ClaCZ119
Helicobacter pylori isolate WE64a
Escherichia coli strain S3
Bifidobacterium adolescentis ATCC 15703
Micrococcus sp. 12BL
Akkermansia muciniphila ATCC BAA-835
Synergistaceae bacterium S15
Mycoplasma hominis ATCC 23114
Staphylococcus aureus strain AMRF1
Lactobacillus rhamnosus GG
Longibaculum sp. strain KGMB06250
Clostridium ramosum strain DSM 1402
Erysipelotrichaceae bacterium HGH0171
Erysipelotrichaceae bacterium HGH0169
Erysipelotrichaceae bacterium taxon 255 clone ZO050
Erysipelothrix sp. B 2344/87
Erysipelothrix tonsillarum DSM 14972
Faecalibaculum rodentium strain ALO17
Ileibacterium valens strain NYU-BL-A3
Allobaculum stercoricanis DSM 13633
Erysipelotrichaceae bacterium CD66
Allobaculum sp. 128
Allobaculum sp. Allo2
Allobaculum sp. clone FB1_2
Uncultured Allobaculum sp. OTU784
Tree scale: 0.1
I
Mock Community (MC)
MC+Allo
MC+Allo2
1wk colonization
1wk 2% DSS-H2O
fecal sample
fecal sample
Euth
J
ng LCN2 / mg protein
P=0.19
P=0.10
MC+ Allo2
MC
DSS day
K
Colon length (cm)
MC
MC +Allo
MC +Allo2
+3 F.D.

Figure S2.

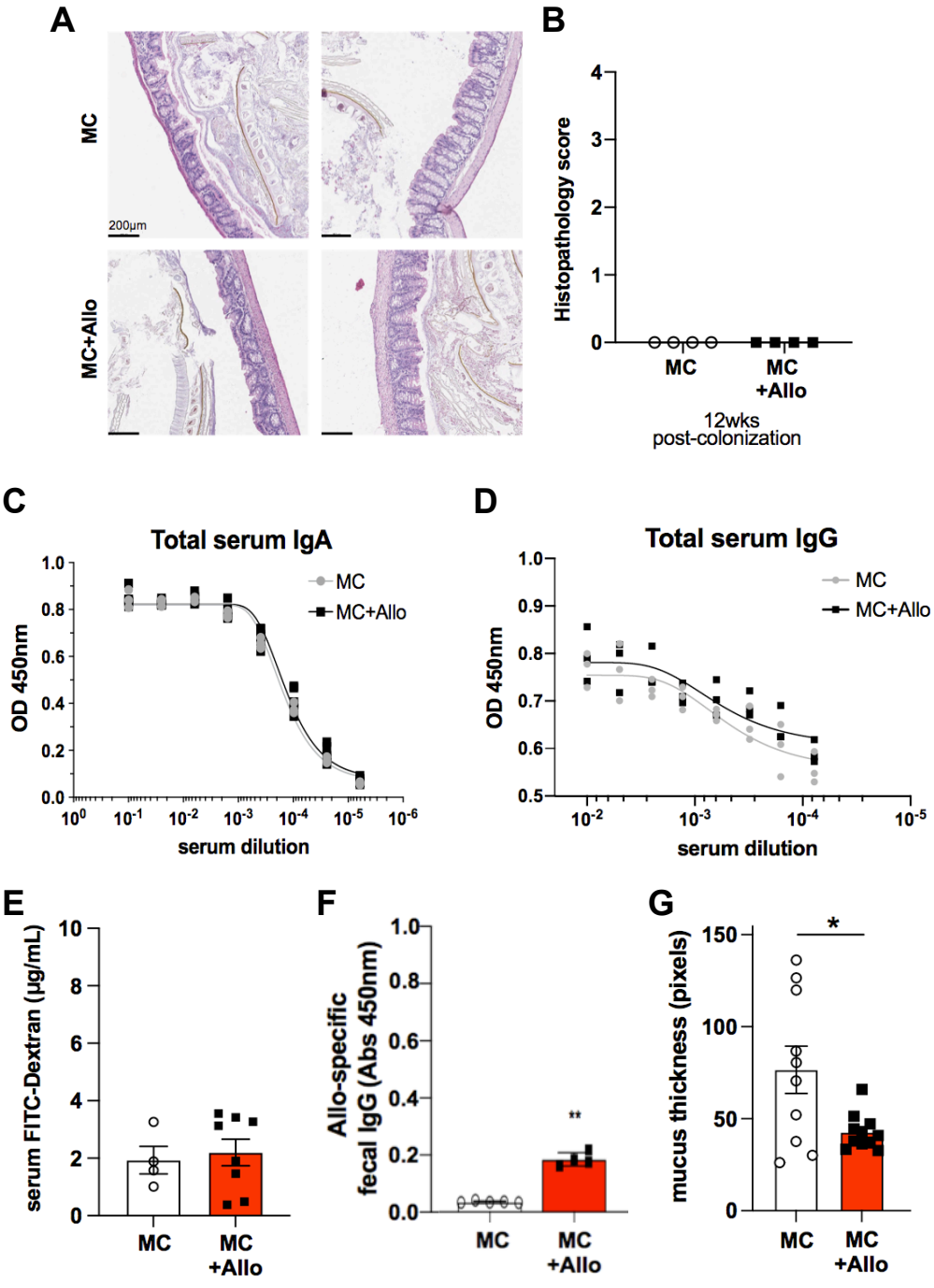
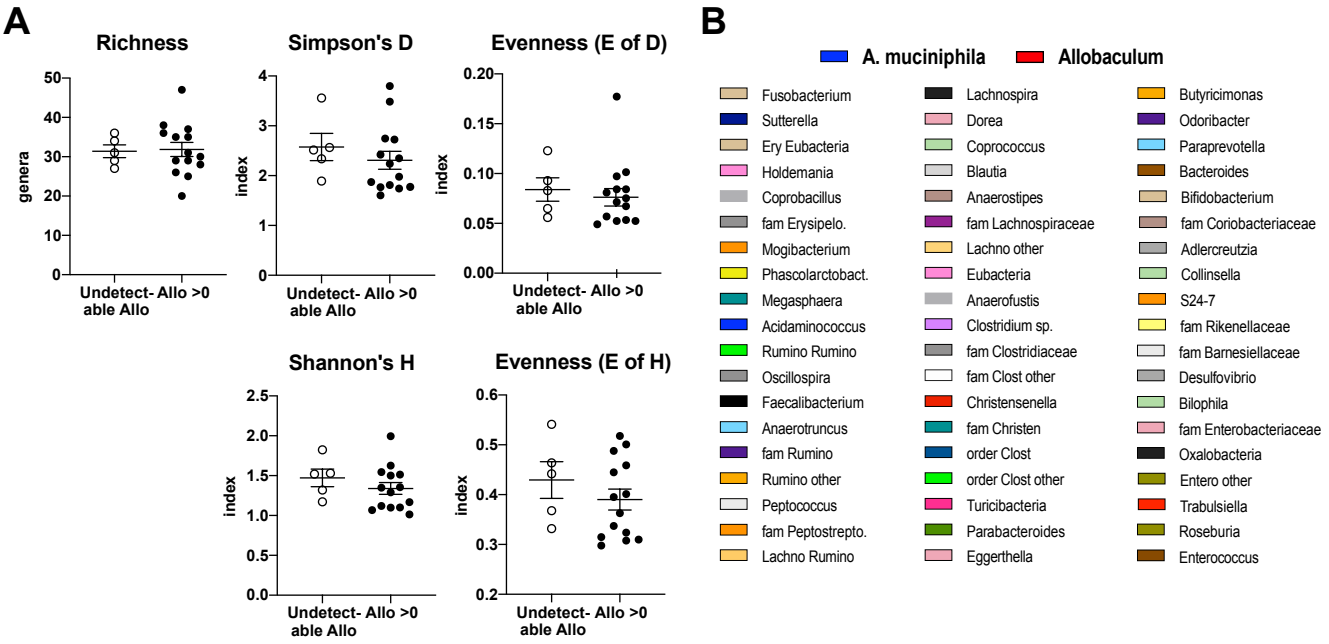

A
MC
MC+Allo
200μm
B
Histopathology score
MC
MC +Allo
12wks post-colonization
C
Total serum IgA
MC
MC+Allo
OD 450nm
serum dilution
D
Total serum IgG
MC
MC+Allo
OD 450nm
serum dilution
E
serum FITC-Dextran (μg/mL)
MC
MC +Allo
F
Allo-specific fecal IgG (Abs 450nm)
**
MC
MC +Allo
G
mucus thickness (pixels)
*
MC
MC +Allo

Figure S3.



E

Allobaculum_Gene_id	log2FC (MC+Both / MC+Allo)	P-val	Annotation
JIPLEDIK_00875	-3.178	9.08E-06	Ferredoxin
JIPLEDIK_01363	-3.332	9.17E-06	hypothetical protein
JIPLEDIK_00615	-2.727	3.08E-04	hypothetical protein
JIPLEDIK_00694	2.861	2.15E-04	hypothetical protein
JIPLEDIK_01703	2.450	2.35E-04	Thiamine transporter ThiT
JIPLEDIK_01939	-2.622	3.31E-04	hypothetical protein
JIPLEDIK_01938	-2.485	5.69E-04	hypothetical protein
JIPLEDIK_00356	-2.638	1.18E-03	putative FMN/FAD exporter YeeO
JIPLEDIK_00877	-2.223	1.51E-03	4Fe-4S ferredoxin FdxA
JIPLEDIK_01427	-2.369	1.77E-03	HTH-type transcriptional regulator Xre

F

Akkermansia_Gene_id	log2FC (MC+Both / MC+Akk)	P-val	Annotation
BJFPNCKO_00183	-2.225	4.94E-06	hypothetical protein
BJFPNCKO_00757	-2.403	7.51E-06	ddlB = D-alanine--D-alanine ligase B
BJFPNCKO_00224	2.196	3.14E-05	hypothetical protein
BJFPNCKO_01031	-1.899	2.96E-05	hpf = Ribosome hibernation promotion factor
BJFPNCKO_01755	-2.080	3.80E-05	ftnA = Bacterial non-heme ferritin
BJFPNCKO_01247	-2.164	5.65E-05	hypothetical protein
BJFPNCKO_00915	-2.009	9.00E-05	hypothetical protein
BJFPNCKO_01746	-2.296	1.15E-04	cydB = Cytochrome bd-I ubiquinol oxidase subunit 2
BJFPNCKO_01836	-1.763	1.53E-04	hypothetical protein
BJFPNCKO_01351	-1.715	2.05E-04	hypothetical protein
BJFPNCKO_00539	2.068	2.43E-04	hypothetical protein

Figure S4.

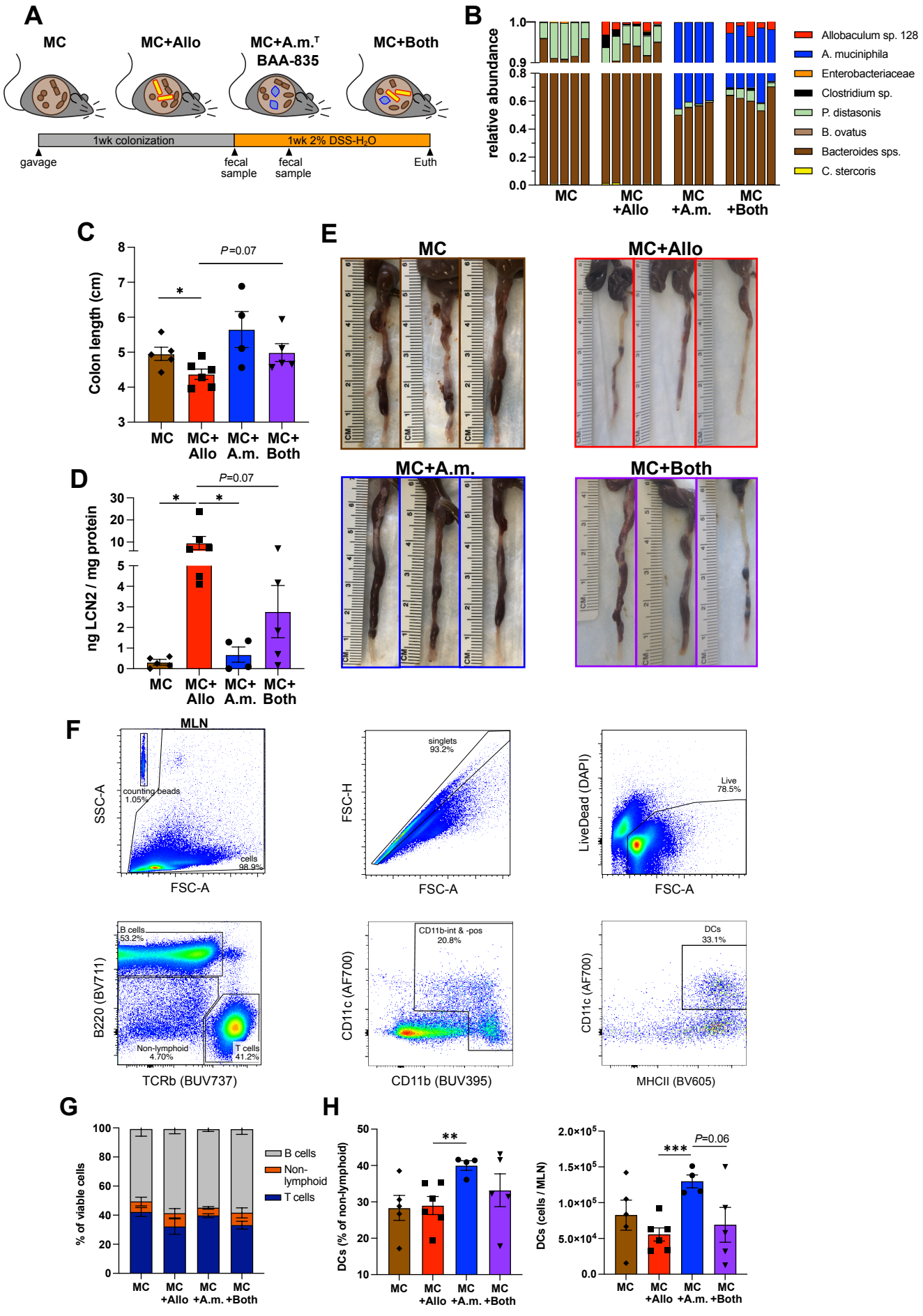

A
MC
MC+Allo
MC+A.m.T BAA-835
MC+Both
1wk colonization
1wk 2% DSS-H2O
gavage
fecal sample
fecal sample
Euth
B
relative abundance
Allobaculum sp. 128
A. muciniphila
Enterobacteriaceae
Clostridium sp.
P. distasonis
B. ovatus
Bacteroides sps.
C. stercoris
C
Colon length (cm)
P=0.07
D
ng LCN2 / mg protein
P=0.07
E
MC
MC+Allo
MC+A.m.
MC+Both
F
MLN
SSC-A
counting beads 1.05%
cells 98.9%
FSC-A
FSC-H
singlets 93.2%
LiveDead (DAPI)
Live 78.5%
B220 (BV711)
B cells 53.2%
Non-lymphoid 4.70%
T cells 41.2%
TCRb (BUV737)
CD11c (AF700)
CD11b-int & -pos 20.8%
CD11b (BUV395)
DCs 33.1%
MHCII (BV605)
G
% of viable cells
B cells
Non-lymphoid
T cells
H
DCs (% of non-lymphoid)
DCs (cells / MLN)
P=0.06

Figure S5.

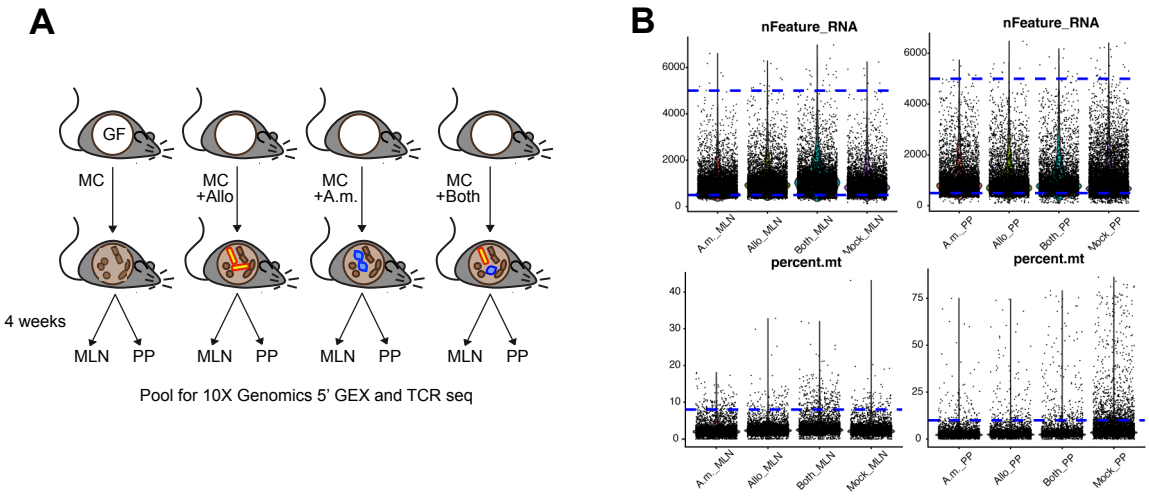

A
GF
MC
MC +Allo
MC +A.m.
MC +Both
4 weeks
MLN
PP
Pool for 10X Genomics 5' GEX and TCR seq
B
nFeature_RNA
percent.mt
A.m._MLN
Allo_MLN
Both_MLN
Mock_MLN
A.m._PP
Allo_PP
Both_PP
Mock_PP

Figure S6.

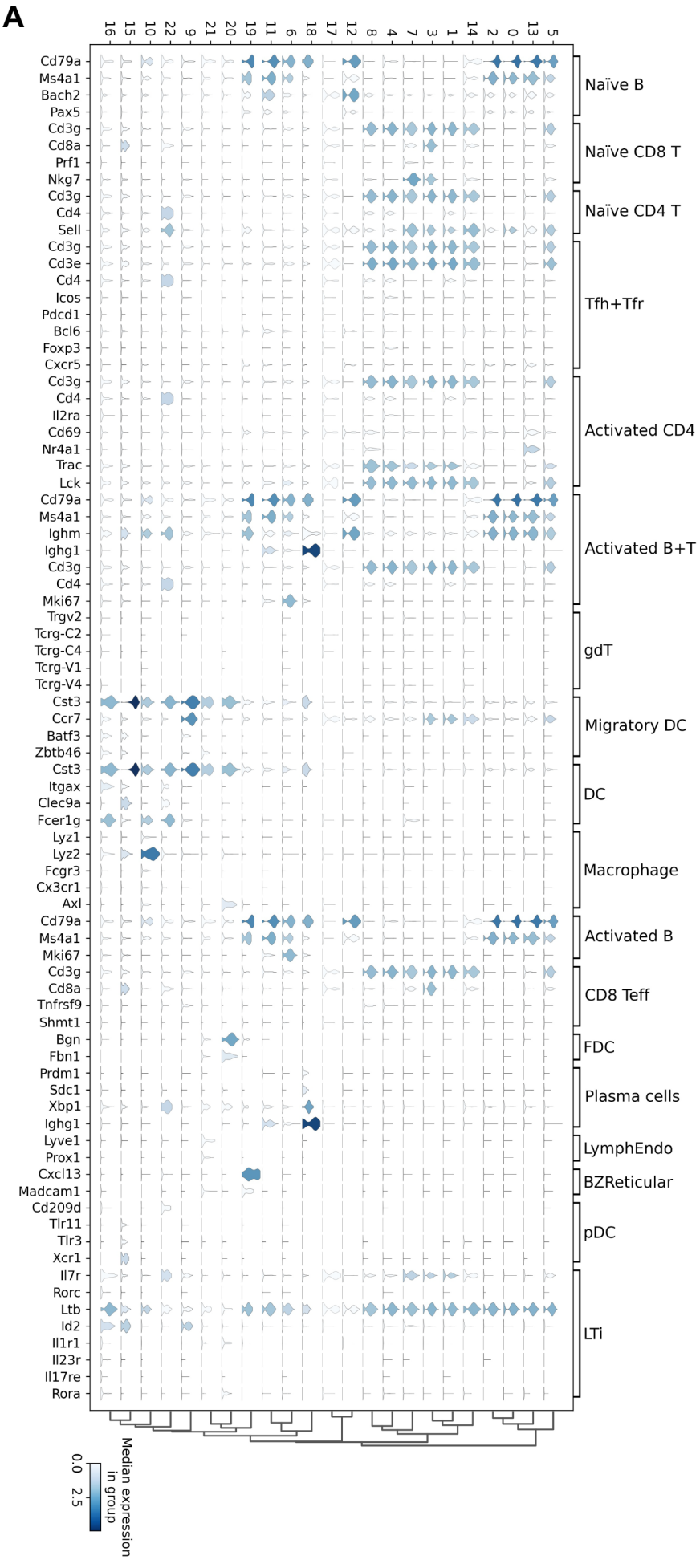
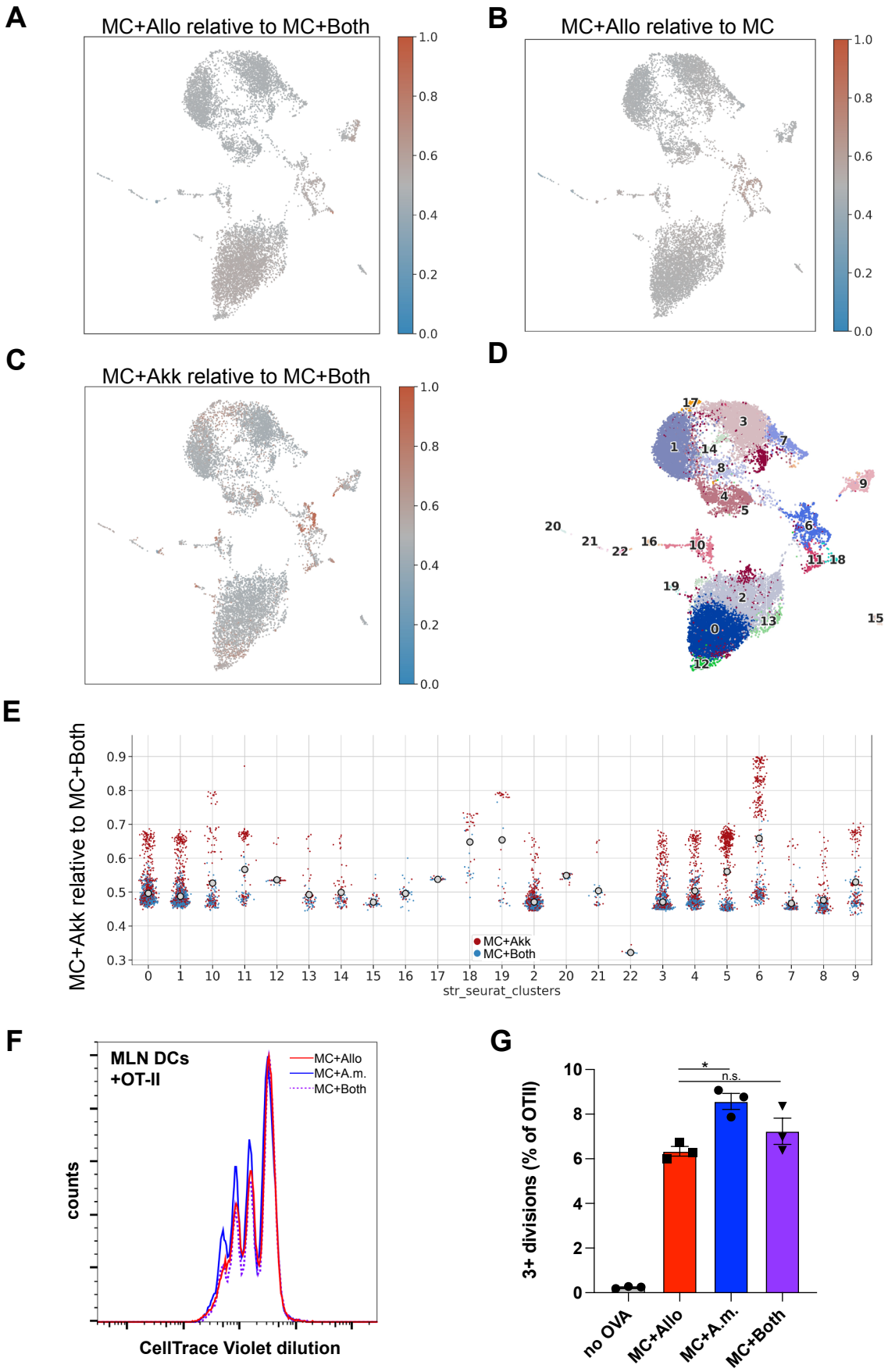

A
16 15 10 22 9 21 20 19 11 6 18 17 12 8 4 7 3 1 14 2 0 13 5
Cd79a
Ms4a1
Bach2
Pax5
Cd3g
Cd8a
Prf1
Nkg7
Cd3g
Cd4
Sell
Cd3g
Cd3e
Cd4
Icos
Pdcd1
Bcl6
Foxp3
Cxcr5
Cd3g
Cd4
Il2ra
Cd69
Nr4a1
Trac
Lck
Cd79a
Ms4a1
Ighm
Ighg1
Cd3g
Cd4
Mki67
Trgv2
Tcrg-C2
Tcrg-C4
Tcrg-V1
Tcrg-V4
Cst3
Ccr7
Batf3
Zbtb46
Cst3
Itgax
Clec9a
Fcer1g
Lyz1
Lyz2
Fcgr3
Cx3cr1
Axl
Cd79a
Ms4a1
Mki67
Cd3g
Cd8a
Tnfrsf9
Shmt1
Bgn
Fbn1
Prdm1
Sdc1
Xbp1
Ighg1
Lyve1
Prox1
Cxcl13
Madcam1
Cd209d
Tlr11
Tlr3
Xcr1
Il7r
Rorc
Ltb
Id2
Il1r1
Il23r
Il17re
Rora
Naïve B
Naïve CD8 T
Naïve CD4 T
Tfh+Tfr
Activated CD4
Activated B+T
gdT
Migratory DC
DC
Macrophage
Activated B
CD8 Teff
FDC
Plasma cells
LymphEndo
BZReticular
pDC
LTi
0.0
2.5
Median expression in group

Figure S7.


A
MC+Allo relative to MC+Both
1.0
0.8
0.6
0.4
0.2
0.0
B
MC+Allo relative to MC
C
MC+Akk relative to MC+Both
D
17
3
7
1
14
8
9
4
5
20
6
21
22
16
10
11
18
19
2
13
15
0
12
E
MC+Akk relative to MC+Both
0.9
0.8
0.7
0.6
0.5
0.4
0.3
MC+Akk
MC+Both
0 1 10 11 12 13 14 15 16 17 18 19 2 20 21 22 3 4 5 6 7 8 9
str_seurat_clusters
F
MLN DCs
+OT-II
MC+Allo
MC+A.m.
MC+Both
counts
CellTrace Violet dilution
G
3+ divisions (% of OTII)
10
8
6
4
2
0
*
n.s.
no OVA
MC+Allo
MC+A.m.
MC+Both