Supplemental Figure Legend

Supplemental Figure 1.

Transcriptomic analysis of TAMs, related to figure 1. $CD45^+CD11b^+F4/80^+$ macrophages were enriched by flow cytometry sorting from normal pancreas (i.e. resident macrophages, n=4 mice) or PDAC tumors from control (n=3)) or *Lyz2*$^{cre/+}$*Ahr*$^{fl/fl}$ (n=3) mice 14 days after tumor implantation. The samples were then analyzed by RNA sequencing. **a)** Volcano plot shows differential expression comparing resident macrophages to control tumor-bearing mice. Red dotted line marks significance threshold (FDR <0.01, logFC>2). **b)** Bar graph of iGSEA analysis of upstream regulators for control TAM versus resident macrophages samples in (***a***). All columns shown have a pval>0.05. **c)** Flow cytometry analysis for dtTomato expression in d14 intratumoral cell populations indicated in tumor-bearing *Lyz2*$^{cre/+}$ x Cg-*Gt(ROSA)26Sor*$^{tm9(CAG-tdTomato)Hze}$/J mice. Pval determined by unpaired students T test. **d)** Volcano plot for differential expression based on transcriptome analysis of TAMs from control versus *Lyz2*$^{cre/+}$*Ahr*$^{fl/fl}$ tumor bearing mice. Red dotted line marks FDR < 0.05. **e)** Venn diagram showing significantly differentially expressed genes (FDR < 0.05, logFC > ± 1) for the comparisons indicated. **f** and **g**) Heatmaps showing significantly differential expression (FDR <0.01, logFC>2) of selected genes involved in cell cycling **(*f*)** or extracellular matrix synthesis **(*g*)**. For heat maps each column represents an individual mouse. **h**) Day 14 orthotopic tumor weight.

Supplemental Figure 2.

Assessment of the immune infiltrate in *Lyz2*$^{cre/+}$*Ahr*$^{fl/fl}$ mice by CyTOF and single cell RNA sequencing analysis, related to figure 3. a) Heatmap showing relative protein expression for each PhenoGraph generated cluster. Arcsinh transformed MSI for each marker was normalized by z-score and heatmap was generated using ggplots application in R. **b)** Graph showing relative abundance of each PhenoGraph cluster in *Lyz2*$^{cre/+}$*Ahr*$^{fl/fl}$ immune infiltrate relative to Control baseline. NS= Pval not significant as calculated using the DiffCYT package. **c)** UMAP plots showing PhenoGraph clustering of immune infiltrate subpopulations in control vs. *Lyz2*$^{cre/+}$*Ahr*$^{fl/fl}$ mice. UMAP and PhenoGraph were run in R for concatenated CYTOF data from live CD45+ cells (4 control

and 5 *Lyz2*$^{cre/+}$*Ahr*$^{fl/fl}$) that were down sampled to 5000 events before analysis. Twenty-four distinct immune clusters were obtained. The experiment was repeated twice with similar results. **d)** Normalized expression and per cluster percentage expression of top 10 marker genes for each cell cluster for the scRNA sequencing analysis described in **Fig 3d**. Both relative expression and percent expression were identified with the Seurat package as described in Methods. Cell labels were assigned to automatically detected clusters based on the top 10 marker genes. **e)** Normalized gene expression per cell of different macrophage marker genes projected onto UMAPs of the concatenated scRNA sequencing of day 14 tumors from *Lyz2*$^{cre/+}$*Ahr*$^{fl/fl}$ mice and littermate controls. **f)** iGSEA analysis showing enrichment for indicated pathways in macrophage 3 cluster as activated (positive Z-score; red) or inhibited (negative Z-score; blue).

Supplemental Figure 3.

Analysis of the role of IDO on tumor growth and AhR function and 16S sequencing analysis of the fecal microbiome, related to figure 4. **a)** Mice were treated with 1-methyl-tryptophan containing drinking water as previously described (Ravishankar *et al.*, 2015) for 3 days prior to tumor implantation. Tumors weights were determined at day 14 post-implantation as described in methods and compared to B6 and B6.*Ido1-/-* mice on control drinking water. **b)** CD45^{+}CD11b^{+}F4/80^{+} TAMs were sorted from the tumors in **(*a*),** and expression of the indicated genes was measured by qPCR as described in methods normalized for the housekeeping gene *Bactin.* **c)** Mice (n=4/group) were placed on antibiotic containing drinking water or control water as indicated. 3 days later tumors were implanted. 14 days after tumor implantation CD45^{+}CD11b^{+}F4/80^{+} TAMs were isolated by flow cytometric sorting and samples from each group were pooled and measured for expression of the genes indicated by qPCR. **d)** Boxplots showing alpha diversity measures calculated at the OTU level using the Simpson index (left panel), the Shannon index (middle panel), or the observed number of OTUs (right panel). Black diamonds overlaying the boxes indicate means; boxes show medians and interquartile ranges. Data points, each of which corresponds to the gut microbiome of one mouse, are colored according to treatment as shown below each panel. P-values shown above each

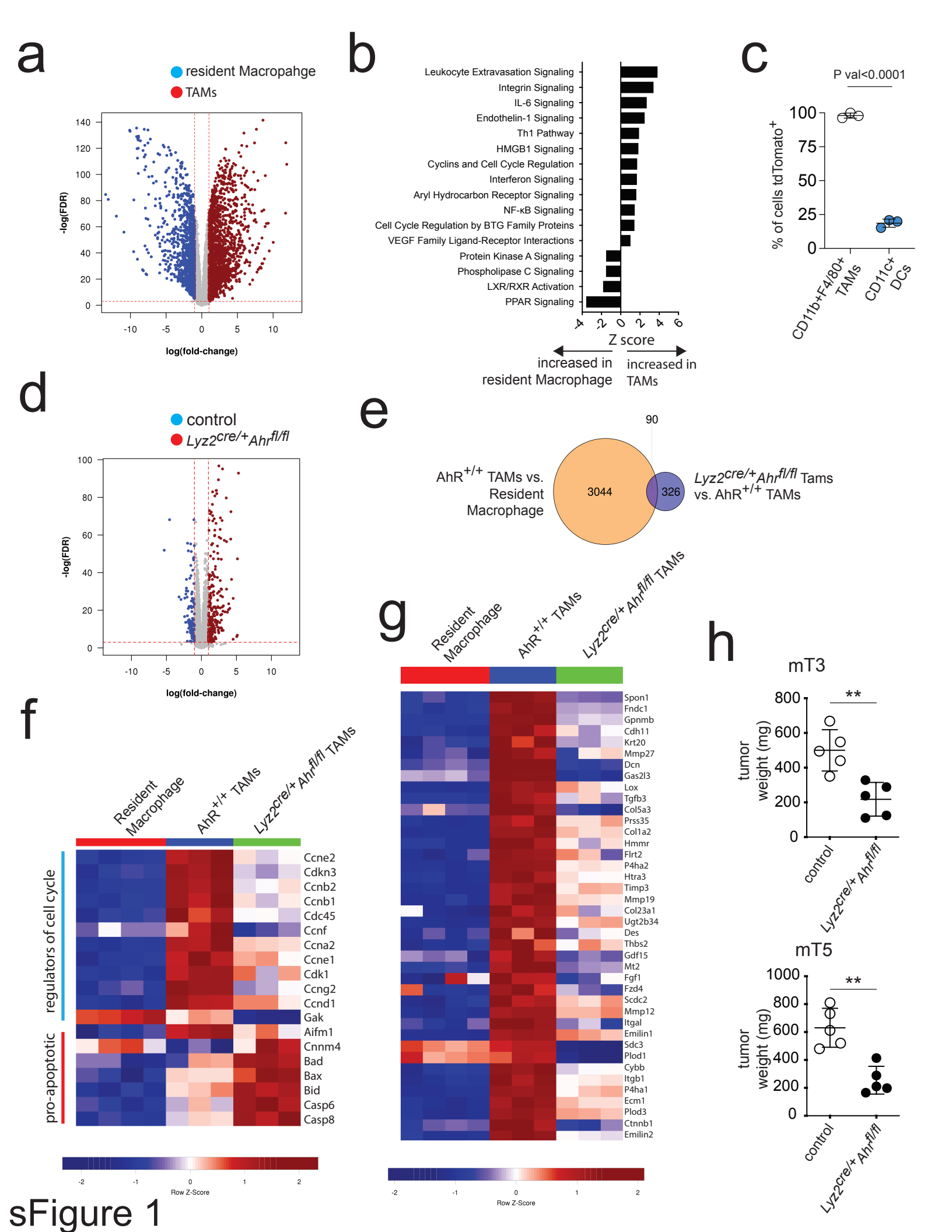
panel were obtained from Kruskal-Wallis tests. **e)** Relative abundances of the top 10 most abundant bacterial families (left panel) or genera (right panel). “Others” in both panels comprises all bacterial taxa that were present but were less abundant. “V6” (left panel) and “V2” (right panel) includes OTUs whose taxonomies could only be resolved at a higher level than that shown, e.g., at the order or family level, respectively.

Supplemental Figure 4.

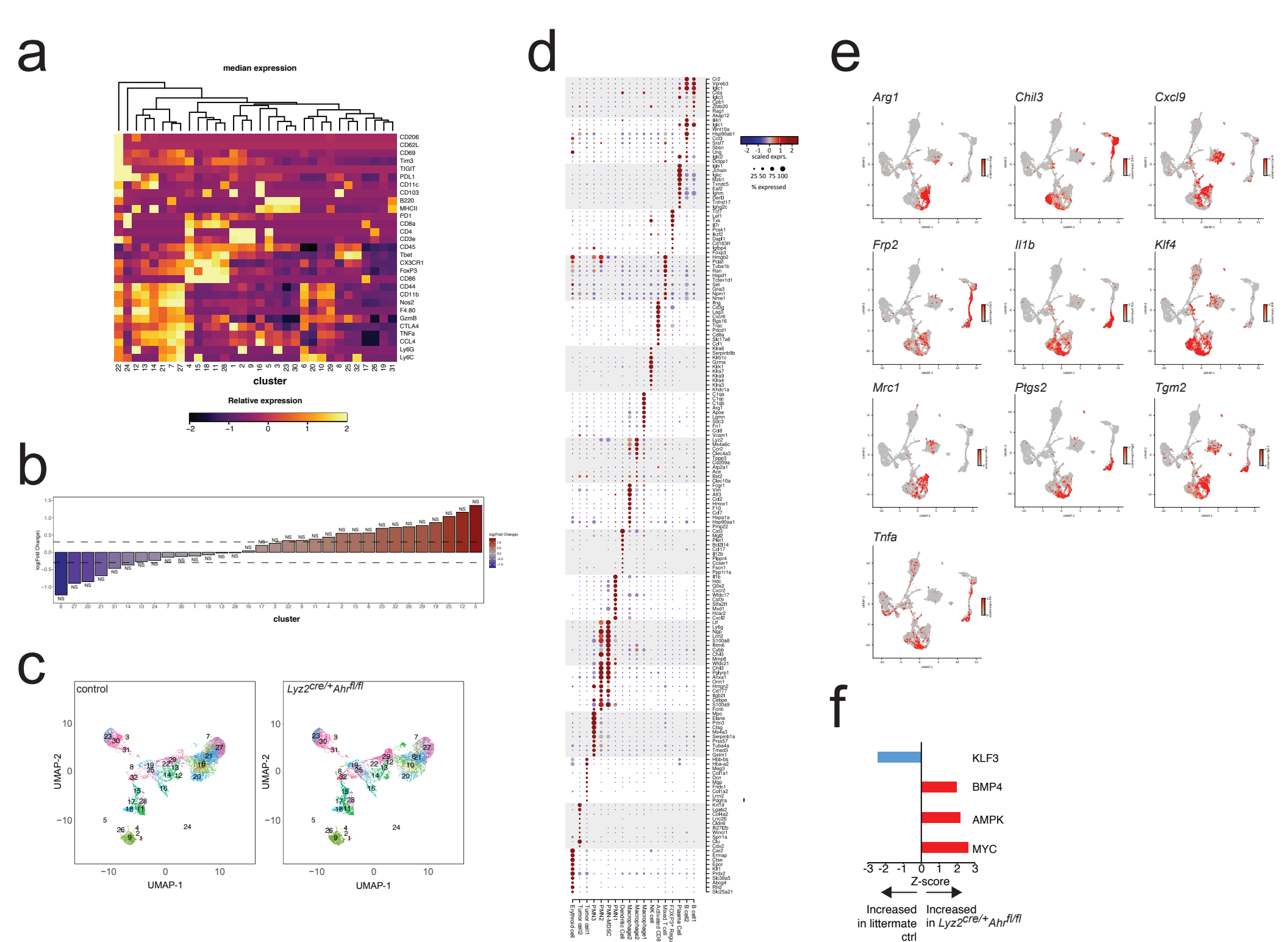
Assessment of the effect antibiotics has on *Lactobacillus* presence in the fecal microbiome and the ability of *Lactobacillus* spp to produce indoles, **related to figure 5. a)** B6 mice were placed on antibiotic-containing drinking water as indicated for 4 days prior to collection of fecal material from the large intestine. 16S sequencing was performed, and the proportion of total *Lactobacillus* reads detected as well as reads for specific *Lactobacillus* species as indicated was determined. Relative frequency refers to the % of total sequencing reads identified belonging to the bacteria indicated. The experiment was repeated twice with similar results. **b)** *Lactobacilli* spp. indicated were cultured overnight in triplicate in the media indicated and culture supernatants were measured for the presence of Trp, lactic acid and the indoles indicated by mass spectroscopy analysis. **c)** Heat map showing hierarchical clustering depicting detected metabolites and amino acids indicated by mass spectroscopy analysis. Each column represents an individual culture. **d)** Principal component analysis of the data described in ***(c)***. Open circles identify *L. murinus* and *L. reuteri* culture data under the 3 culture conditions. **e)** Germ free B6 mice were gavaged with *L. murinus* or control saline as indicated and described in Methods. 30 days later fecal material was collected from the large intestine and the presence of *L. murinus* was determined by PCR as described in methods. The experiment was repeated twice with similar results. **f)** For the mice described in **(*e*)** fecal material was collected from the large intestine and measured for the indoles indicated by mass spectroscopy. **g)** Fecal measurement of indoles indicated was done by mass spectroscopy on mice with the microbiome enrichment indicated. P values were determined by unpaired students T test. Experiment was repeated twice with similar results.

Supplemental Figure 5.

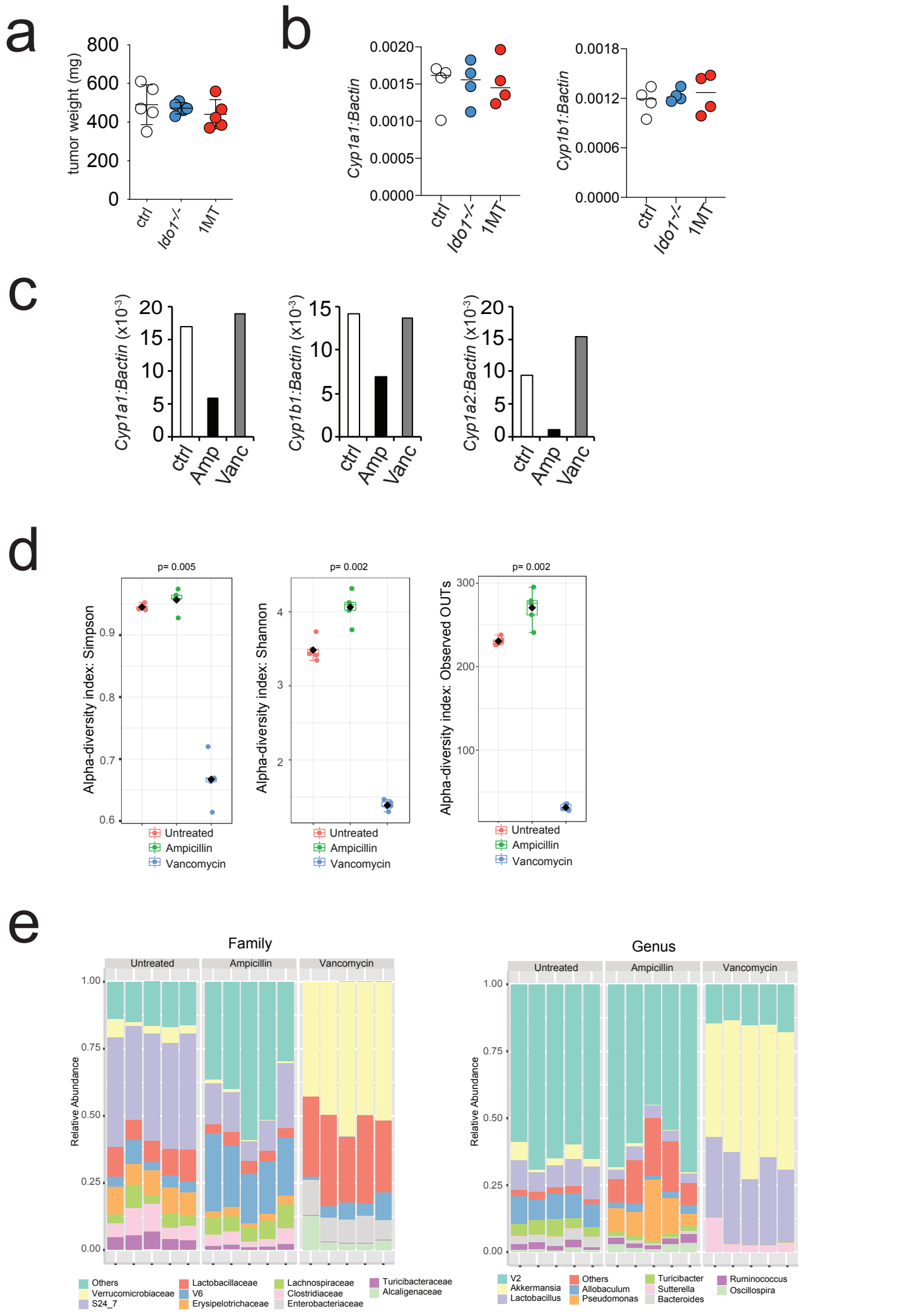
Cox hazard analysis of *AHR* and OS in the TCGA PAAD cohort and analysis of genes with expression patterns most similar to *AHR,* related to figure 6. a and **b)** Forest plots of Cox proportional hazard analyses in TCGA-PAAD data set. **a)** The top 50% and bottom 50% stratified by median *AHR* expression. **b)** The TCGA-PAAD patient data set stratified by quartiles based on *AHR* expression: lowest 25% (Q1), up to second quartile (Q2), next quartile (Q3), and highest 25% (Q4). **c)** Normalized expression and per cluster percentage expression of *AHR* and the top 25 most similarly expressed genes across cell clusters for the scRNA sequencing analysis described in **Fig 6d**.


a
resident Macropahge
TAMs
-log(FDR)
log(fold-change)
b
Leukocyte Extravasation Signaling
Integrin Signaling
IL-6 Signaling
Endothelin-1 Signaling
Th1 Pathway
HMGB1 Signaling
Cyclins and Cell Cycle Regulation
Interferon Signaling
Aryl Hydrocarbon Receptor Signaling
NF-κB Signaling
Cell Cycle Regulation by BTG Family Proteins
VEGF Family Ligand-Receptor Interactions
Protein Kinase A Signaling
Phospholipase C Signaling
LXR/RXR Activation
PPAR Signaling
Z score
increased in resident Macrophage
increased in TAMs
c
P val<0.0001
% of cells tdTomato+
CD11b+F4/80+ TAMs
CD11c+ DCs
d
control
Lyz2cre/+Ahrfl/fl
-log(FDR)
log(fold-change)
e
90
AhR+/+ TAMs vs. Resident Macrophage
3044
326
Lyz2cre/+Ahrfl/fl Tams vs. AhR+/+ TAMs
f
Resident Macrophage
AhR+/+ TAMs
Lyz2cre/+Ahrfl/fl TAMs
regulators of cell cycle
pro-apoptotic
Ccne2
Cdkn3
Ccnb2
Ccnb1
Cdc45
Ccnf
Ccna2
Ccne1
Cdk1
Ccng2
Ccnd1
Gak
Aifm1
Cnnm4
Bad
Bax
Bid
Casp6
Casp8
Row Z-Score
g
Resident Macrophage
AhR+/+ TAMs
Lyz2cre/+ Ahrfl/fl TAMs
Spon1
Fndc1
Gpnmb
Cdh11
Krt20
Mmp27
Dcn
Gas2l3
Lox
Tgfb3
Col5a3
Prss35
Col1a2
Hmmr
Flrt2
P4ha2
Htra3
Timp3
Mmp19
Col23a1
Ugt2b34
Des
Thbs2
Gdf15
Mt2
Fgf1
Fzd4
Scdc2
Mmp12
Itgal
Emilin1
Sdc3
Plod1
Cybb
Itgb1
P4ha1
Ecm1
Plod3
Ctnnb1
Emilin2
Row Z-Score
h
mT3
tumor weight (mg)
**
control
Lyz2cre/+ Ahrfl/fl
mT5
tumor weight (mg)
**
control
Lyz2cre/+ Ahrfl/fl

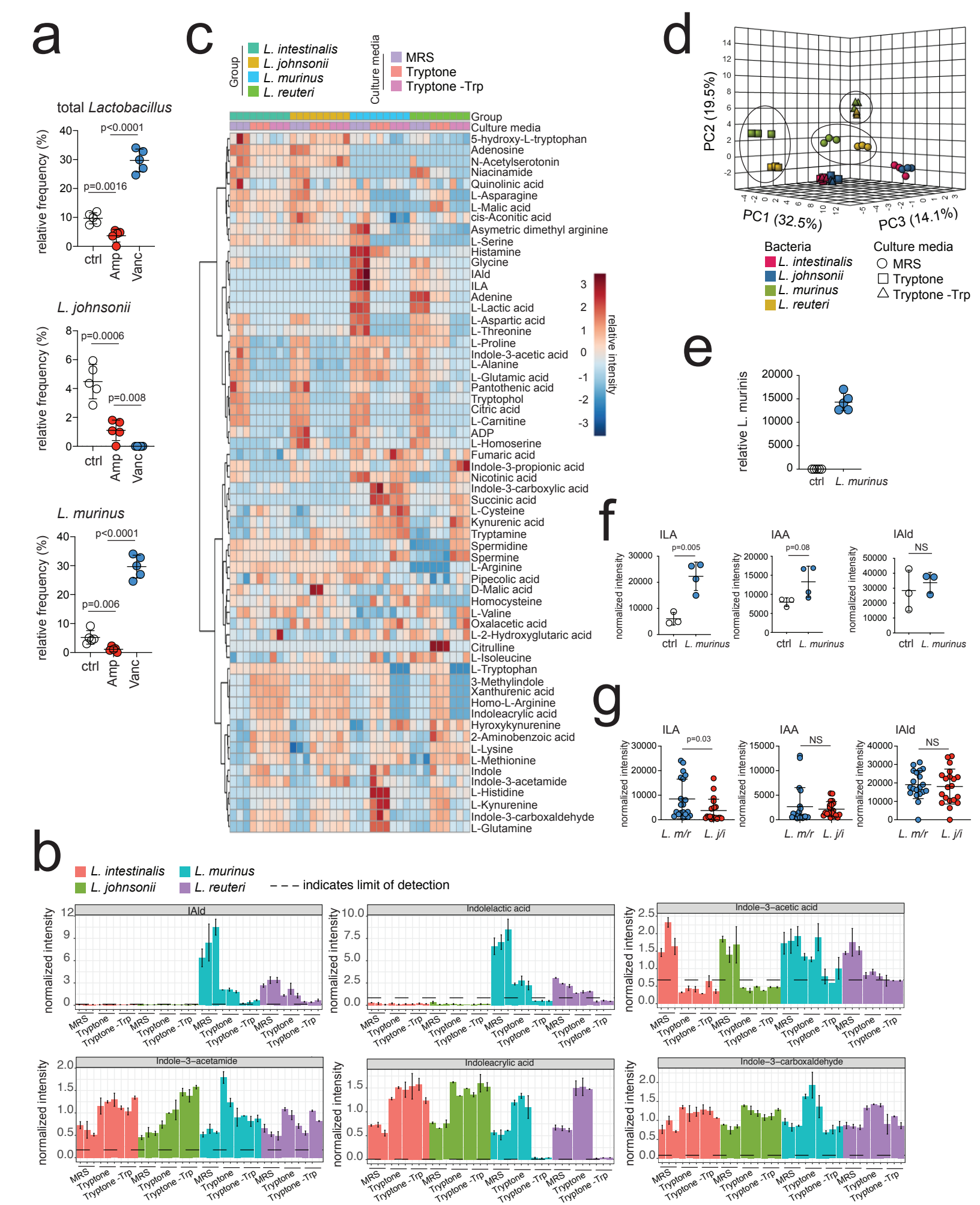
sFigure 1


a
median expression
CD206
CD62L
CD69
Tim3
TIGIT
PDL1
CD11c
CD103
B220
MHCII
PD1
CD8a
CD4
CD3e
CD45
Tbet
CX3CR1
FoxP3
CD86
CD44
CD11b
Nos2
F4.80
GzmB
CTLA4
TNFa
CCL4
Ly6G
Ly6C
cluster
Relative expression
b
log(Fold Change)
cluster
c
control
Lyz2cre/+Ahrfl/fl
UMAP-2
UMAP-1
d
scaled exprs.
% expressed
Plasma Cell
FOXP3+ Regulatory T cell
Activated CD8+ T cell
NK cell
Macrophage1
Macrophage2
Monocytic Cell
PMN1
PMN-MDSC
PMN2
PMN3
Tumor cell2
Tumor cell1
Erythroid cell
B cell2
B cell1
e
Arg1
Chil3
Cxcl9
Frp2
Il1b
Klf4
Mrc1
Ptgs2
Tgm2
Tnfa
f
KLF3
BMP4
AMPK
MYC
Z-score
Increased in littermate ctrl
Increased in Lyz2cre/+Ahrfl/fl

sFigure 2

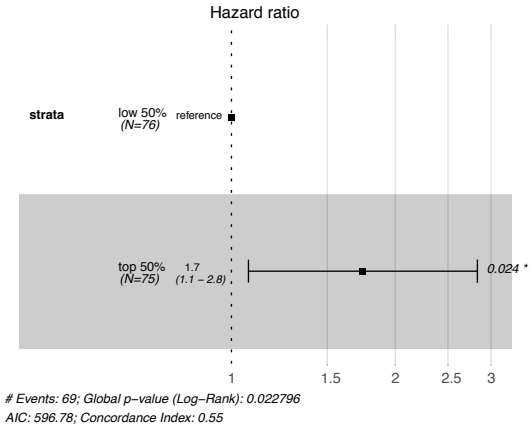

a
tumor weight (mg)
800
600
400
200
0
ctrl
Ido1-/-
1MT
b
Cyp1a1:Bactin
0.0020
0.0015
0.0010
0.0005
0.0000
Cyp1b1:Bactin
0.0018
0.0012
0.0006
0.0000
c
Cyp1a1:Bactin (x10-3)
Cyp1b1:Bactin (x10-3)
Cyp1a2:Bactin (x10-3)
20
15
10
5
0
ctrl
Amp
Vanc
d
p= 0.005
p= 0.002
p= 0.002
Alpha-diversity index: Simpson
Alpha-diversity index: Shannon
Alpha-diversity index: Observed OUTs
Untreated
Ampicillin
Vancomycin
e
Family
Genus
Untreated
Ampicillin
Vancomycin
Relative Abundance
1.00
0.75
0.50
0.25
0.00
Others
Verrucomicrobiaceae
S24_7
Lactobacillaceae
V6
Erysipelotrichaceae
Lachnospiraceae
Clostridiaceae
Enterobacteriaceae
Turicibacteraceae
Alcaligenaceae
V2
Akkermansia
Lactobacillus
Others
Allobaculum
Pseudomonas
Turicibacter
Sutterella
Bacteroides
Ruminococcus
Oscillospira

sFigure 3

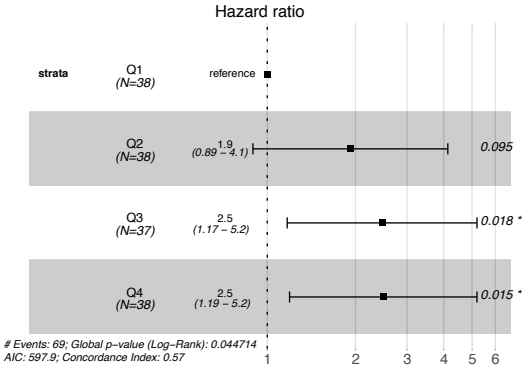

a
total Lactobacillus
p<0.0001
p=0.0016
relative frequency (%)
ctrl
Amp
Vanc
L. johnsonii
p=0.0006
p=0.008
L. murinus
p<0.0001
p=0.006
c
Group
L. intestinalis
L. johnsonii
L. murinus
L. reuteri
Culture media
MRS
Tryptone
Tryptone -Trp
relative intensity
5-hydroxy-L-tryptophan
Adenosine
N-Acetylserotonin
Niacinamide
Quinolinic acid
L-Asparagine
L-Malic acid
cis-Aconitic acid
Asymetric dimethyl arginine
L-Serine
Histamine
Glycine
IAld
ILA
Adenine
L-Lactic acid
L-Aspartic acid
L-Threonine
L-Proline
Indole-3-acetic acid
L-Alanine
L-Glutamic acid
Pantothenic acid
Tryptophol
Citric acid
L-Carnitine
ADP
L-Homoserine
Fumaric acid
Indole-3-propionic acid
Nicotinic acid
Indole-3-carboxylic acid
Succinic acid
L-Cysteine
Kynurenic acid
Tryptamine
Spermidine
Spermine
L-Arginine
Pipecolic acid
D-Malic acid
Homocysteine
L-Valine
Oxalacetic acid
L-2-Hydroxyglutaric acid
Citrulline
L-Isoleucine
L-Tryptophan
3-Methylindole
Xanthurenic acid
Homo-L-Arginine
Indoleacrylic acid
Hyroxykynurenine
2-Aminobenzoic acid
L-Lysine
L-Methionine
Indole
Indole-3-acetamide
L-Histidine
L-Kynurenine
Indole-3-carboxaldehyde
L-Glutamine
d
PC2 (19.5%)
PC1 (32.5%)
PC3 (14.1%)
Bacteria
L. intestinalis
L. johnsonii
L. murinus
L. reuteri
Culture media
MRS
Tryptone
Tryptone -Trp
e
relative L. murinis
ctrl
L. murinus
f
ILA
p=0.005
IAA
p=0.08
IAld
NS
normalized intensity
g
ILA
p=0.03
IAA
NS
IAld
NS
L. m/r
L. j/i
b
L. intestinalis
L. johnsonii
L. murinus
L. reuteri
– – – indicates limit of detection
IAld
Indolelactic acid
Indole–3–acetic acid
Indole–3–acetamide
Indoleacrylic acid
Indole–3–carboxaldehyde
normalized intensity
MRS
Tryptone
Tryptone -Trp

sFigure 4

a



b



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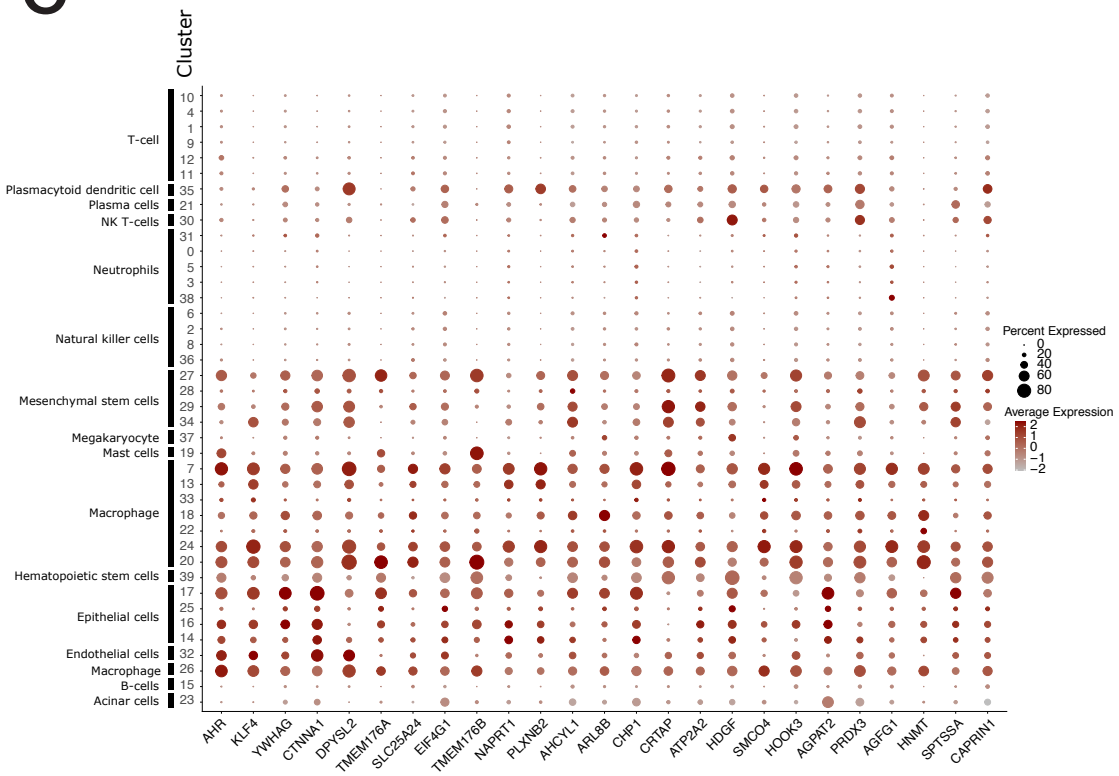

sFigure 5

Bacteria
Bacteroides fragilis
Bacteroides thetaiotaomicron
Bifidobacterium adolescentis
Bifidobacterium bifidum
Clostridium botulinum
Clostridium paraputrificum
Clostridium saccharolyticum
Clostridium sporogenes
Faecalibacterium prausnitzii
Lactobacillus acidophilus
Lactobacillus murinus
Lactobacillus reuteri
Parabacteroides distasonis
Stenotrophomonas maltophilia
Escherichia coli

S Table 2, related to figure 6j. Bacterial taxa target for analysis in figure 6j.