

## SUPPLEMENTAL FIGURE AND TABLE LEGENDS

### **Figure S1. *F. nucleatum* does not affect total bacterial load in intestinal tumors.**

Quantitative PCR measurement of actin and 16S rRNA gene relative abundance using cDNA generated from adenomas of *Apc*<sup>Min/+</sup> mice from (A) colon or (B) small intestine. “n.s.” indicates not significant. Symbols represent data from individual mice with horizontal bars showing the mean and error bars indicating SEM. Related to Figure 2.

**Figure S2. Intratumoral immune cell analyses.** Functionality of intratumoral MDSCs and TAMs. (A) Representative histograms of Arginase-1 and iNOS on M-MDSC (solid line), G-MDSC (dashed line) granulocytes (dotted line) and isotype control (shaded) by flow cytometry (left panel) and cumulative data from five mice analyzed as individuals (7 tumors were pooled per mouse) are shown (right panel). MFI, mean fluorescence intensity. Data are represented as mean +/- SEM. (B) Representative histogram of Arginase-1 on M2-like TAM (solid line), TAM (dashed line) and isotype control (shaded) by flow cytometry (left panel) and cumulative data from five mice are shown, data are represented as mean +/- SEM (right panel). (C) CD4 T cell suppressive activity of tumor-permissive myeloid cells. CD4 T cells were labeled with Cell Trace violet and incubated alone (shaded) or co-cultured with sorted MDSCs, TAMs, or M2-like TAMs (solid line) for 3 days (left panel). The proliferation of labeled CD4 T cells was analyzed by flow cytometry. The cell division index was calculated from three experiments by

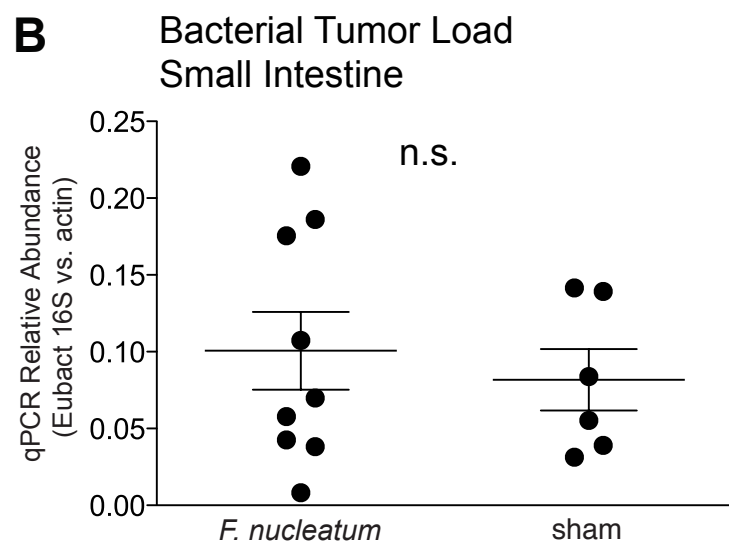
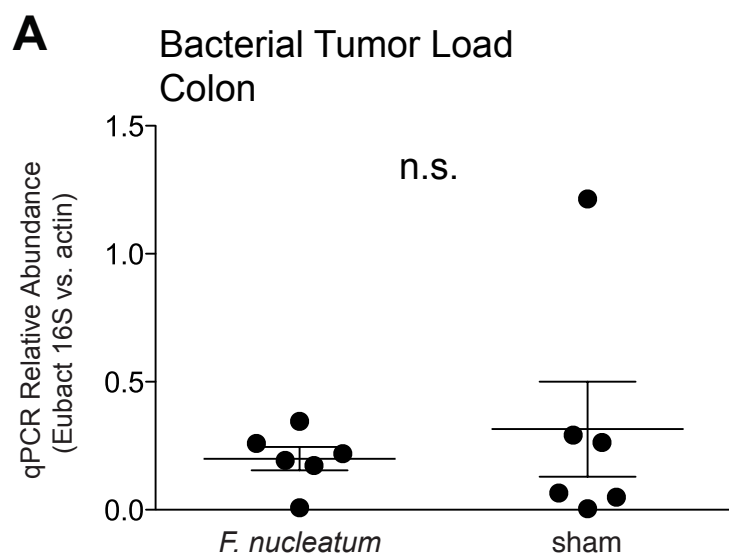
using Flow Jo (right panel). *P* values are shown where significant. *F. nucleatum* does not affect intratumoral Th17 nor Foxp3<sup>+</sup> Treg cell numbers. Flow cytometric analyses: Cell number/gram tumor of **(D)** CD3<sup>+</sup>CD4<sup>+</sup> IL-17<sup>+</sup> T cells (left panel) and CD3<sup>+</sup>CD4<sup>+</sup> Foxp3<sup>+</sup> T cells (right panel). Symbols represent data from individual mice with horizontal bars indicating the mean. Related to Figure 3.

**Figure S3. Human colon tumor RNA-Seq analysis shows enrichment for inflammatory functions in genes correlated with *Fusobacterium* abundance but not the abundance of other genera.**

Spearman's rank correlations of genus-level microbial relative abundance with host gene expression was used to weight Gene Ontology enrichment using Ingenuity Pathway Analysis software for the top 5 most abundant genera. Gene-set intersect indicates the number of genes that are shared in the enriched gene sets between *Fusobacterium* and each of the other genera. Related to Figure 4.

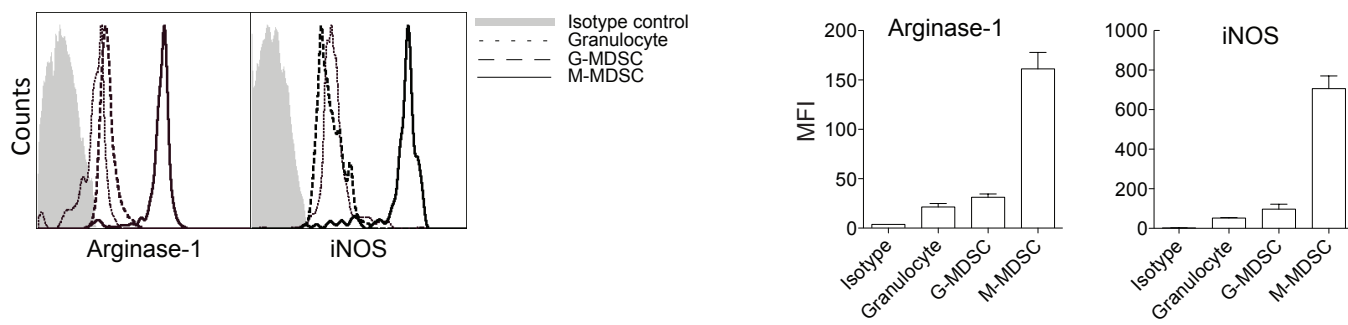
**Table S1. Human sample data (A)** Sample list of all colonic adenomas assessed for *Fusobacterium* abundance. Related to Figure 1. **(B)** *Fusobacterium* is detected at a higher abundance in stool from CRC and adenoma cases than from healthy controls. Related to Figure 1.

# Figure S1

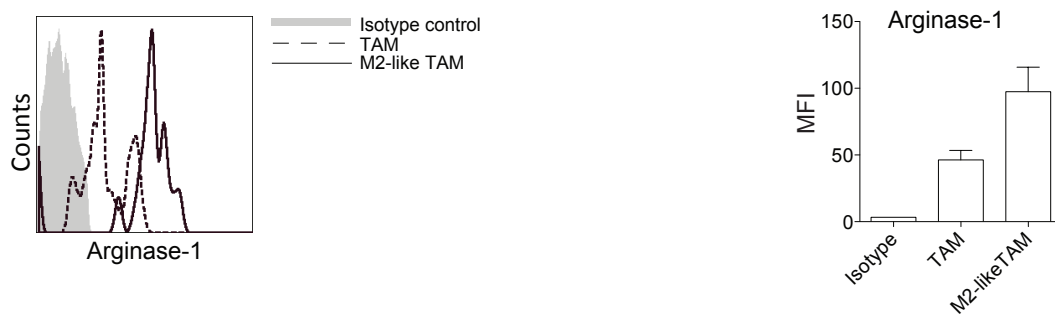


## Figure S2

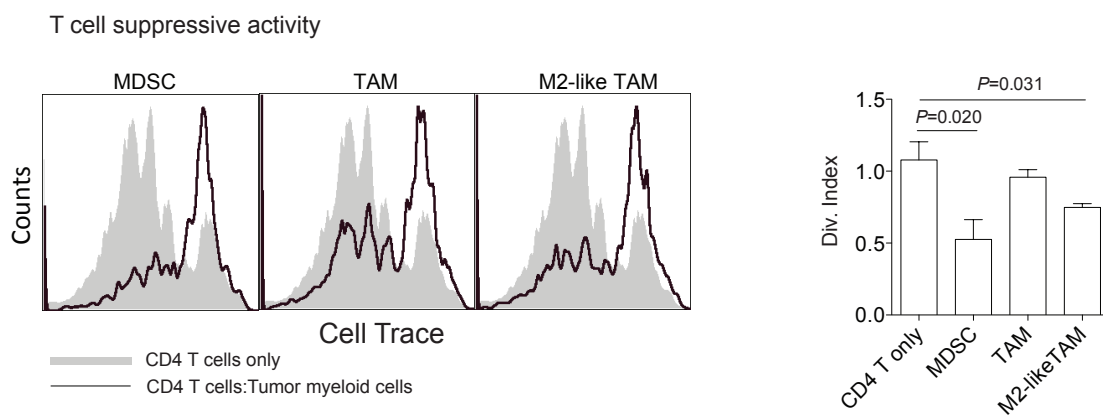
A



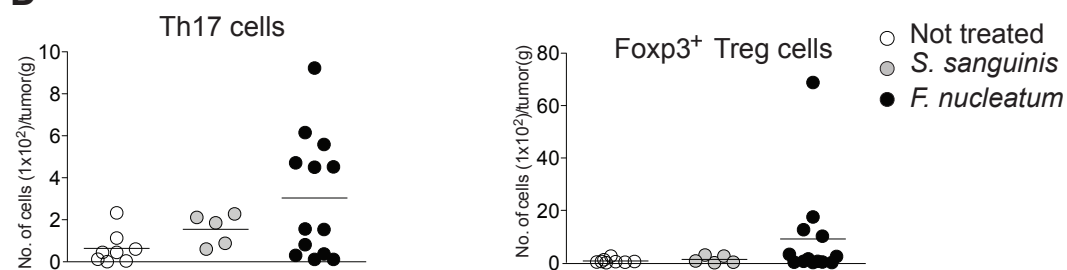
B



C



D



# Figure S3

*Escherichia*

*Propionibacterium*

*Fusobacterium*

*Streptococcus*

*Bacteroides*

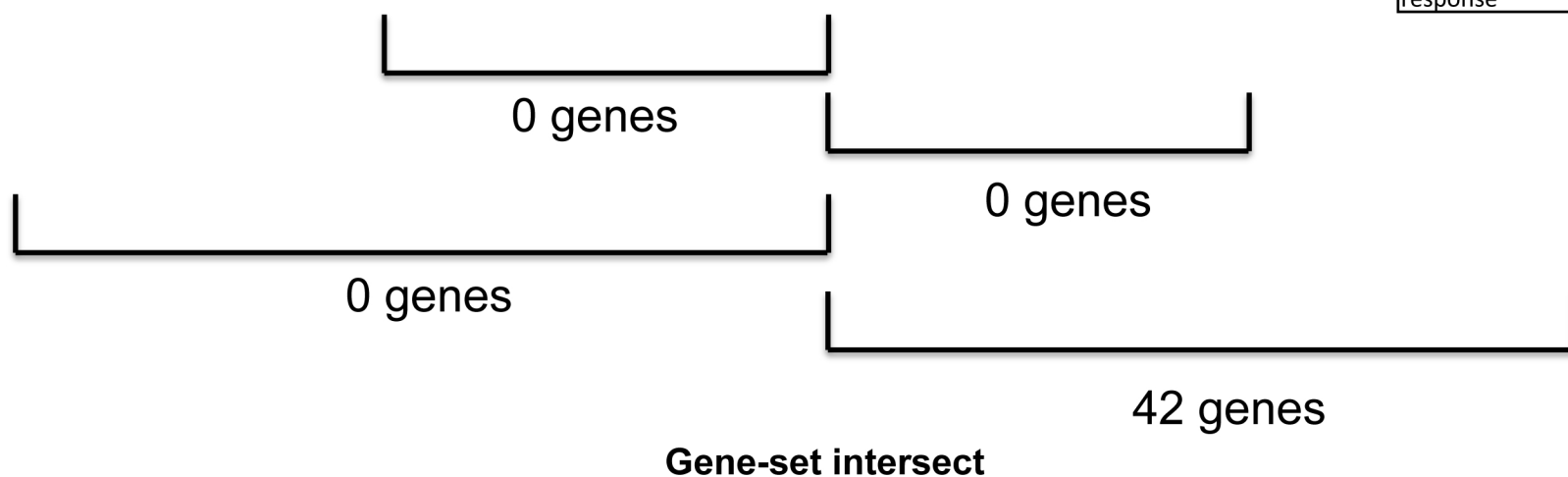
Biological Function	Corrected P-val
Infection mechanism	6.44E-07
Cell cycle	1.86E-04
Nervous system development and function	2.25E-04
Organ morphology	2.25E-04

Biological Function	Corrected P-val
Carbohydrate metabolism	3.36E-04
Genetic disorder	3.04E-03
Cell cycle	3.04E-03

Biological Function	Corrected P-val
Infammatory response	2.91E-34
Cell-to-cell signaling and interaction	1.98E-27
Immune cell trafficking	2.43E-24
Cellular growth and proliferation	1.14E-24
Cell death	1.30E-21
Immunological disease	3.11E-17
Inflammatory disease	3.11E-17
Lymphoid tissue structure and development	5.47E-16

Biological Function	Corrected P-val
Infection mechanism	7.62E-04
Gene expression	3.73E-04
Genetic disorder	1.55E-03
Cardiovascular disease	3.79E-03

Biological Function	Corrected P-val
Cellular growth and proliferation	4.79E-09
Cell-to-cell signaling and interaction	1.30E-08
Antigen presentation	1.25E-08
Inflammatory response	1.19E-08
Antimicrobial response	1.39E-06



**Table S1****A**

Sample	Origin	$\Delta\text{Ct}$ (Fuso - Eubact)
n01	U. of Pennsylvania	Fuso (-)
a01	U. of Pennsylvania	Fuso (-)
n02	U. of Virginia Health System	Fuso (-)
a02	U. of Virginia Health System	Fuso (-)
n03	U. of Virginia Health System	Fuso (-)
a03	U. of Virginia Health System	17.36
n04	U. of Virginia Health System	Fuso (-)
a04	U. of Virginia Health System	Fuso (-)
n05	U. of Virginia Health System	Fuso (-)
a05	U. of Virginia Health System	Fuso (-)
n06	Mass. Gen. Hosp.	18.29
a06	Mass. Gen. Hosp.	17.65
n07	Mass. Gen. Hosp.	Fuso (-)
a07	Mass. Gen. Hosp.	Fuso (-)
n08	Mass. Gen. Hosp.	Fuso (-)
a08	Mass. Gen. Hosp.	Fuso (-)
n09	Mass. Gen. Hosp.	Fuso (-)
a09	Mass. Gen. Hosp.	20.35
n10	Vanderbilt U. Med. Center	26.62
a10	Vanderbilt U. Med. Center	15.35
n11	Vanderbilt U. Med. Center	Fuso (-)
a11	Vanderbilt U. Med. Center	12.29
n12	Vanderbilt U. Med. Center	14.41
a12	Vanderbilt U. Med. Center	14.08
n13	Vanderbilt U. Med. Center	Fuso (-)
a13	Vanderbilt U. Med. Center	Fuso (-)
n14	Vanderbilt U. Med. Center	19.42
a14	Vanderbilt U. Med. Center	10.75
n15	Vanderbilt U. Med. Center	Fuso (-)
a15	Vanderbilt U. Med. Center	Fuso (-)

n16	U. of Aberdeen	Fuso (-)
a16	U. of Aberdeen	Fuso (-)
n17	U. of Aberdeen	17.96
a17	U. of Aberdeen	14.43
n18	U. of Aberdeen	17.97
a18	U. of Aberdeen	17.04
n19	U. of Aberdeen	Fuso (-)
a19	U. of Aberdeen	21.30
n20	U. of Aberdeen	11.44
a20	U. of Aberdeen	11.59
n21	U. of Aberdeen	Fuso (-)
a21	U. of Aberdeen	Fuso (-)
n22	U. of Aberdeen	25.53
a22	U. of Aberdeen	23.98
n23	U. of Aberdeen	Fuso (-)
a23	U. of Aberdeen	Fuso (-)
n24	U. of Aberdeen	21.34
a24	U. of Aberdeen	Fuso (-)
n25	U. of Aberdeen	24.19
a25	U. of Aberdeen	21.91
n26	U. of Aberdeen	21.36
a26	U. of Aberdeen	20.86
n27	U. of Aberdeen	22.72
a27	U. of Aberdeen	18.91
n28	U. of Aberdeen	Fuso (-)
a28	U. of Aberdeen	Fuso (-)
n29	U. of Aberdeen	Fuso (-)
a29	U. of Aberdeen	Fuso (-)
n30	U. of Aberdeen	Fuso (-)
a30	U. of Aberdeen	Fuso (-)
n31	U. of Aberdeen	Fuso (-)
a31	U. of Aberdeen	Fuso (-)

**B**

Stool	# indiv.	Fuso (-)	Fuso (+)	<i>P</i> value
Normal	31	16	15	
Adenoma	28	4	24	$P < 0.005$
Carcinoma	27	0	27	$P < 0.00005$