SUPPLEMENTAL MATERIAL



Fig. S1. *QIIME analysis confirms gut microbial composition and diversity differ between housing conditions, but not genotype.* A: Principal coordinates analysis of stool and ileal microbial composition of WT and *Irgm1* KO mice in conventional (CV) and specific pathogen free (SPF) conditions based on <u>QIIME UniFrac distances</u>. Stool CV versus SPF, FDR < $6.4x10^{-10}$; ileum CV versus SPF, FDR < 0.007. For WT versus KO mice in both tissue compartments, FDR > 0.09. B: Observed OTUs calculated from <u>QIIME close reference</u>

<u>pipeline</u>. C: Chao1 α -diversity index calculated from <u>QIIME close reference pipeline</u>. N = 11 CV WT, 11 CV KO, 7 SPF WT, 10 SPF KO. *FDR = 0.021.

Supplemental Tables

Genus	Compartment	P-value	FDR
Akkermansia	Stool	2.3617E-06	8.26593E-05
Bifidobacterium	Stool	7.89538E-06	0.000138169
Butyricimonas	Stool	0.000466406	0.004081048
Dorea	Stool	0.000461691	0.004081048
Anaeroplasma	Stool	0.001140313	0.007982192
Vagococcus	lleum	1.38967E-12	7.22626E-11
Chelonobacter	lleum	8.79941E-12	2.28785E-10
Erysipelothrix	lleum	2.20262E-11	3.81787E-10
Pseudochrobactrum	lleum	8.97944E-11	1.16733E-09
Marinomonas	lleum	3.59938E-10	3.74336E-09
Halomonas	lleum	5.11173E-10	4.43016E-09
Proteiniclasticum	lleum	8.98642E-10	6.67563E-09
Leucobacter	lleum	5.99842E-09	3.89897E-08
Agrococcus	lleum	7.33039E-09	4.23534E-08
Dysgonomonas	lleum	8.6816E-09	4.51443E-08
Psychrobacter	lleum	2.63154E-07	1.244E-06
Rhodococcus	lleum	7.73384E-07	3.35133E-06
Shewanella	lleum	1.07208E-06	4.28834E-06
Aquimarina	lleum	2.24452E-06	8.33678E-06
Bifidobacterium	lleum	1.22284E-05	4.23919E-05
Anaeroplasma	lleum	1.34341E-05	4.36609E-05
Chryseobacterium	lleum	2.31769E-05	7.08939E-05
Dorea	lleum	2.89641E-05	8.3674E-05
Akkermansia	lleum	0.000197202	0.000539711
Microbacterium	lleum	0.000295184	0.000767478
Coprobacillus	lleum	0.000337282	0.000835173
Blautia	lleum	0.000587913	0.001329194
Clostridium	lleum	0.000568876	0.001329194
Trichococcus	lleum	0.001315745	0.002850781

ButyricimonasIleum0.0018194060.003784364VibrioIleum0.0021939620.004387924HelicobacterIleum0.0046579840.008970933EnterococcusIleum0.0075634280.014046366AdlercreutziaIleum0.0135534570.02430275OscillospiraIleum0.0165147940.028625644DesulfovibrioIleum0.0225233240.036600401StaphylococcusIleum0.0281065720.044289144PseudomonasIleum0.0301432740.046101478FlavobacteriumIleum0.0331122580.047827981				
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EnterococcusIleum0.0075634280.014046366AdlercreutziaIleum0.0135534570.02430275OscillospiraIleum0.0165147940.028625644DesulfovibrioIleum0.0225233240.036600401StaphylococcusIleum0.0281065720.044289144PseudomonasIleum0.0301432740.046101478FlavobacteriumIleum0.032191910.047827981AcinetobacterIleum0.0331122580.047828818	Helicobacter	lleum	0.004657984	0.008970933
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DesulfovibrioIleum0.0225233240.036600401StaphylococcusIleum0.0222338680.036600401AF12Ileum0.0281065720.044289144PseudomonasIleum0.0301432740.046101478FlavobacteriumIleum0.032191910.047827981AcinetobacterIleum0.0331122580.047828818	Oscillospira	lleum	0.016514794	0.028625644
Staphylococcuslleum0.0222338680.036600401AF12lleum0.0281065720.044289144Pseudomonaslleum0.0301432740.046101478Flavobacteriumlleum0.032191910.047827981Acinetobacterlleum0.0331122580.047828818	Desulfovibrio	lleum	0.022523324	0.036600401
AF12Ileum0.0281065720.044289144PseudomonasIleum0.0301432740.046101478FlavobacteriumIleum0.032191910.047827981AcinetobacterIleum0.0331122580.047828818	Staphylococcus	lleum	0.022233868	0.036600401
Pseudomonas Ileum 0.030143274 0.046101478 Flavobacterium Ileum 0.03219191 0.047827981 Acinetobacter Ileum 0.033112258 0.047828818	AF12	lleum	0.028106572	0.044289144
Flavobacterium Ileum 0.03219191 0.047827981 Acinetobacter Ileum 0.033112258 0.047828818	Pseudomonas	lleum	0.030143274	0.046101478
Acinetobacter Ileum 0.033112258 0.047828818	Flavobacterium	lleum	0.03219191	0.047827981
	Acinetobacter	lleum	0.033112258	0.047828818

Table S1. Bacterial taxa differentially abundant in conventional (CV) versus specific pathogen free (SPF) facilities. Genus-level bacterial taxa that are significantly different in CV versus SPF facilities are shown. *P*-values are reported from the mixed linear model using *F*-test, which accounts for the contribution of cage. We controlled for false discovery rate (FDR) by correcting the *P*-values using Benjamini and Hochberg (BH) approach.

	CV	SPF	GF
Feed	Purina 5001	Purina 5053 (irradiated)	Teklad Global 2020SX (autoclaved)
Water	Triple filtered	Reverse osmosis	Autoclaved
Bedding	ALPHA-dri/Cob blend	Corncob (autoclaved)	Teklad 7070C Diamond Dry Cellulose (autoclaved)
Caging	Individual ventilated cages (hot-washed)	Shoebox cages (autoclaved)	Shoebox cages (autoclaved) within flexible film isolators

Table S2. *Husbandry details for individual mouse facilities.* Specific food, water, bedding, enrichment, and caging sources are indicated for conventional (CV), specific pathogen free (SPF), and germ-free (GF) housing facilities. Sterilization techniques are also shown when utilized.

		Conventional	Specific Pathogen Free
		(CV)	(SPF)
Viral Agents	Parvoviruses (MPV-1 & 2, Minute virus)	-	-
	Sendai virus	-	-
	Pneumonia virus of mice	-	-
	Mouse hepatitis virus	-	-
	Mouse norovirus	+	-
	Reovirus	-	-
	Enzootic diarrhea of mice	-	-
	Theiler's murine encephalomyelitis	-	-
Bacterial Agents	Mycoplasma pulmonis	-	-
	Helicobacter spp.	+	-
	Pasturella pneumotropica	Not tested	-

Table S3. Colony health surveillance results from conventional (CV) and specific pathogen free (SPF) facilities. Results of dirty bedding sentinel testing of either exposure (serology) or presence (PCR) of murine pathogens in the CV and SPF colonies during the time course of the study.

	T		
V6F1	5′-	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATAGCGCAACGCGARGAACCTTACC	-3′
V6F2	5′-	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGGGTCAACGCGARGAACCTTACC	-3′
V6F3	5′-	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTTCATCAACGCGARGAACCTTACC	-3′
V6F4	5′-	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGATCGTCAACGCGARGAACCTTACC	-3′
V6F5	5'-	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCCCGTCAACGCGARGAACCTTACC	-3′
V6F6	5'-	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTGTCCAACGCGARGAACCTTACC	-3′
V6F7	5′-	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCACGTCAACGCGARGAACCTTACC	-3′
V6F8	5′-	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCGTACGCAACGCGARGAACCTTACC	-3′
V6F9	5′-	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGACCAACGCGARGAACCTTACC	-3′
V6F10	5′-	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTAGACAACGCGARGAACCTTACC	-3′
V6F11	5′-	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCATCAACGCGARGAACCTTACC	-3′
V6F12	5′-	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACTTCAACGCGARGAACCTTACC	-3′
V6R1	5′-	CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTATAGCGACAACACGAGCTGACGAC	-3′
V6R2	5′-	CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTAGGGTACAACACGAGCTGACGAC	-3′
V6R3	5′-	CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTTTCATACAACACGAGCTGACGAC	-3′
V6R4	5′-	CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTGATCGTACAACACGAGCTGACGAC	-3′
V6R5	5'-	CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTGCCCGTACAACACGAGCTGACGAC	-3′
V6R6	5'-	CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTCTGTCACAACACGAGCTGACGAC	-3′
V6R7	5'-	CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTCACGTACAACACGAGCTGACGAC	-3′
V6R8	5'-	CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTCGTACGACAACACGAGCTGACGAC	-3′
V6R9	5′-	CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTGGACACAACACGAGCTGACGAC	-3′
V6R10	5'-	CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTTAGAACAACACGAGCTGACGAC	-3′
V6R11	5′-	CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTTCATACAACACGAGCTGACGAC	-3′
V6R12	5′-	CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTACTTACAACACGAGCTGACGAC	-3′
PCRF1	5′-	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	-3′
PCRR1	5′-	CAAGCAGAAGACGCCATACGAGATCGGTCTCGGCATTCCGTCTGAACCGCTCTTCCGATCT	-3′

Table S4. Primers used to create V6 16S rRNA library for sequencing.V6F1-12 and V6R1-12were used to initial PCR.PCRF1/PCRR1 were used for second stage PCR.