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Genome-guided design of a defined mouse microbiota that confers colonization resistance against *Salmonella enterica* serovar Typhimurium

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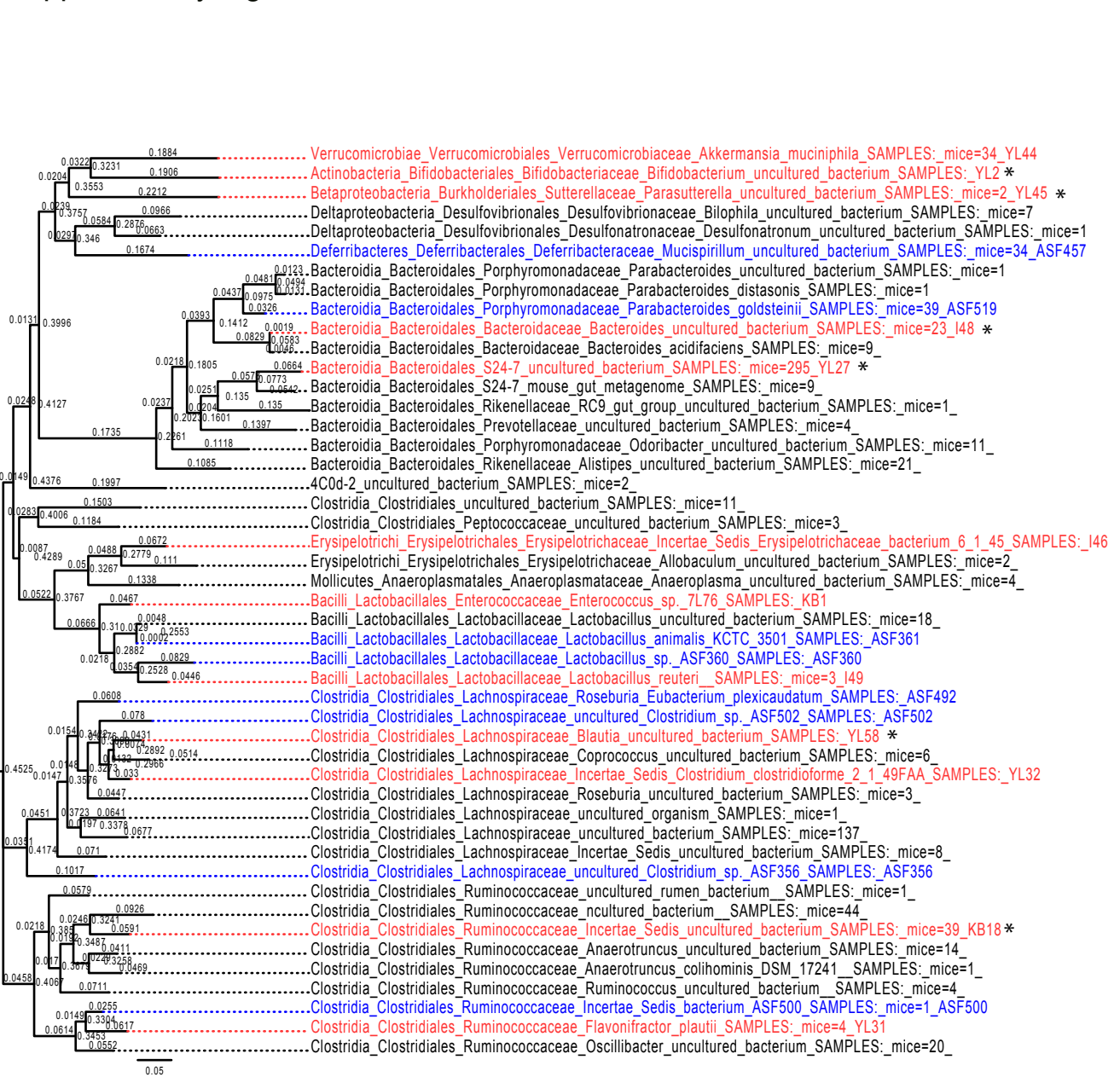
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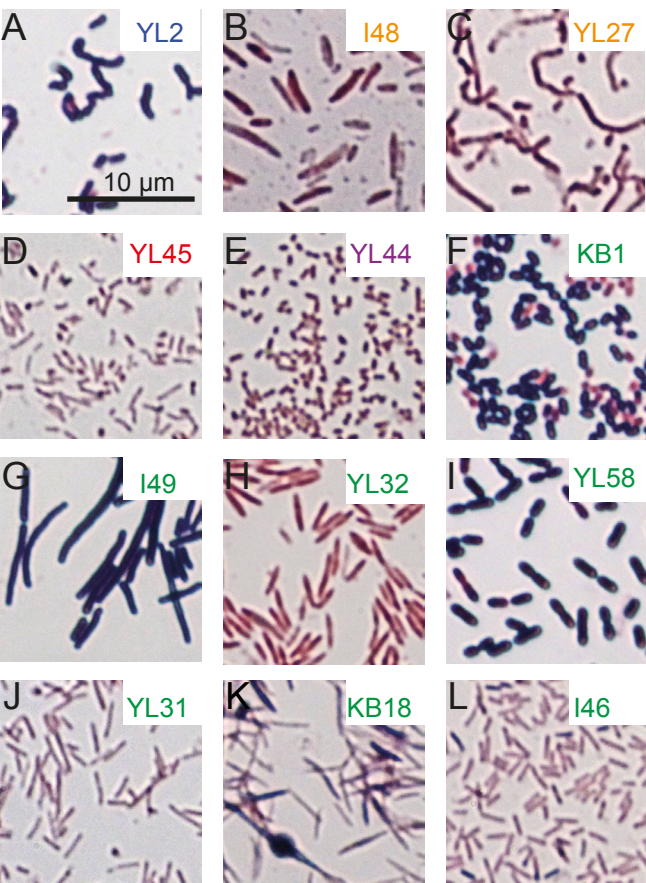
Supplementary Figures

Supplementary Figure 1



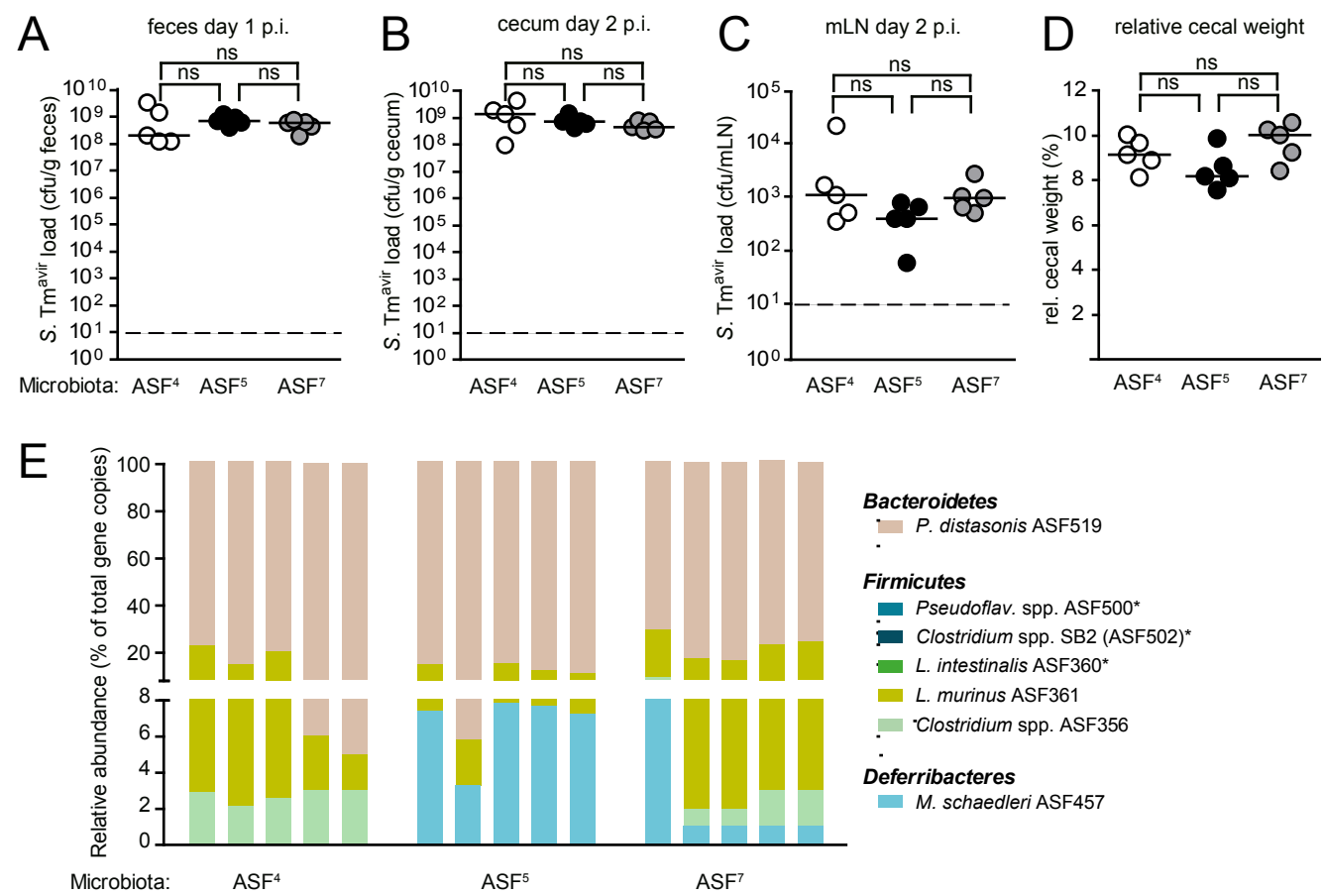
Supplementary Figure 1. Phylogenetic placement and representation of the Oligo-MM strains in conventional mice. The 16S rRNA sequences of the 12 Oligo-MM strains (red) as well as the 8 strains of the Altered Schaedler Flora (blue; ASF356, ASF360, ASF361, ASF457, ASF492, ASF500, ASF502 and ASF519) ¹ were compared against a set of 865 full-length high quality 16S rRNA gene sequences from 2 types of conventional mice ². All sequences were aligned against the SILVA database version 111 NR ³ using MEGABLAST (version 2.2.28+) on a 97% identity level, yielding 47 different taxonomic identities. The best hit of each of the 47 taxonomies from the database was used for the generation of a multiple sequence alignment using Infernal. A phylogenetic tree was generated using fasttree ⁴. The number of sequences per taxonomic identity in the conventional mice is indicated (e.g. mice=20). The Oligo-MM¹² strains represent 10 of the 19 bacterial families (and candidate families), detected in this conventional (CON) microbiota. Families represented only in CON mice include the Desulfovibrionaceae, Desulfonatronaceae (both δ -Proteobacteria), Deferribacteraceae (Deferribacteres phylum), Porphyromonadaceae, Rikenellaceae, Prevotellaceae (Bacteroidetes phylum), Peptococcaceae and Anaeroplasmataceae (Firmicutes phylum). It should be noted that ASF457 is a representative of the Deferribacteraceae and ASF519 is a representative of the Porphyromonadaceae. Potential novel taxa of the Oligo-MM¹² strains (previously uncultured) are highlighted with asterisks.

Supplementary Figure 2



Supplementary Figure 2. Cell morphology of the Oligo-MM strains. Strains were grown individually in liquid cultures to late-logarithmic/early stationary growth phase. Bacteria were Gram-stained and imaged by light microscopy (100-fold magnification, oil immersion). Representatives of the Bacteroidetes, Proteobacteria and Verrucomicrobia phyla ('*Bacteroides caecimuris*' I48, '*Muribaculum intestinale*' YL27, '*Turicimonas muris*' YL45 and *Akkermansia muciniphila* YL44) stained Gram-negative. *Bifidobacterium longum* subsp. *animalis* YL2 (Actinobacteria) as well as *Enterococcus faecalis* KB1, *Lactobacillus reuteri* I49 and *Blautia coccoides* YL58 (Firmicutes) appear Gram-positive. In contrast, the other Firmicutes strains (*Flavonifractor plautii* YL31, *Clostridium clostridioforme* YL32, '*Acutalibacter muris*' KB18 and *Clostridium innocuum* I46) are stained Gram-negative or Gram-variable. Gram-negative staining behavior has been reported for other members of the Clostridiales and might be attributable to a Gram-negative type of cell wall structure or ageing in the stationary growth phase ⁵. Strains are color-coded based on their phylogeny: blue: Actinobacteria; orange: Bacteroidetes; red: Proteobacteria; purple: Verrucomicrobia and green: Firmicutes. Scale bar: 10 µm.

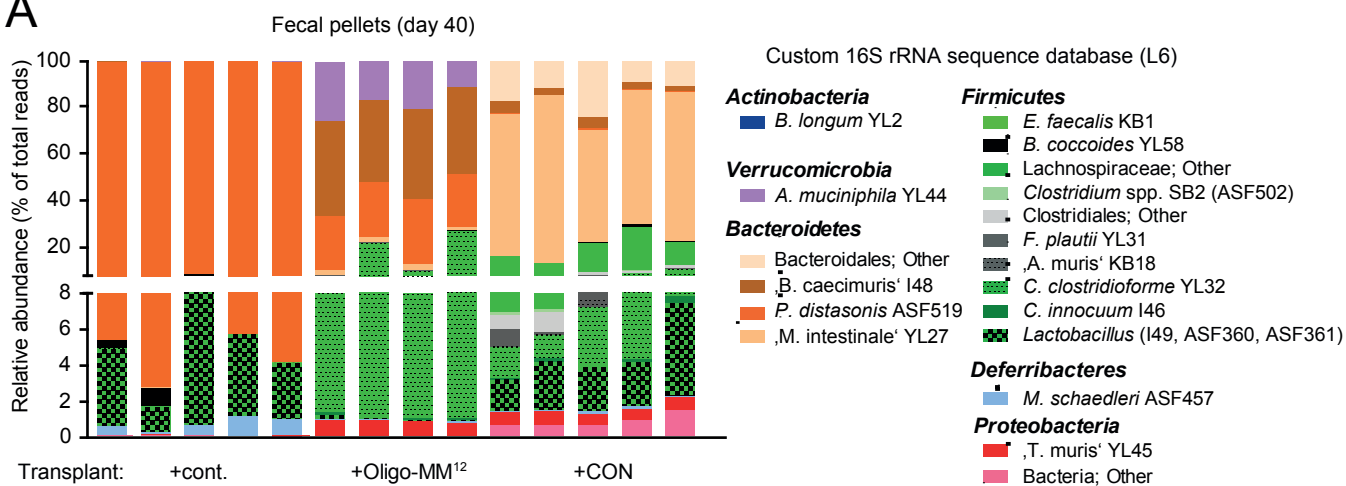
Supplementary Figure 3



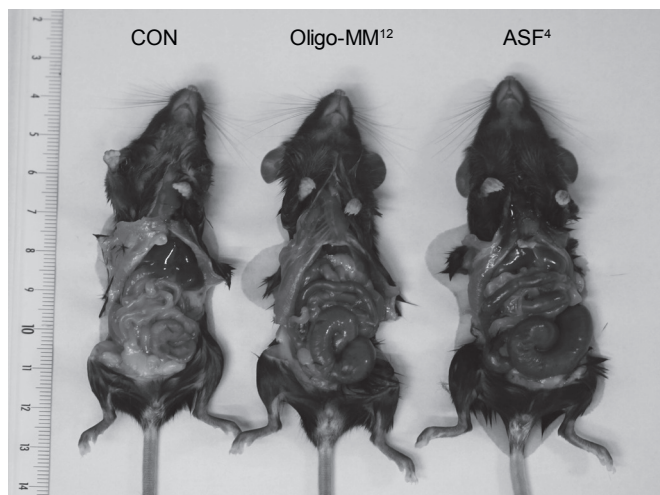
Supplementary Figure 3. Colonization resistance of gnotobiotic mice colonized with members of the ASF mice. Mice colonized with 4 ASF strains (ASF360, ASF361, ASF356 and ASF519; ASF⁴), 5 ASF strains (ASF360, ASF361, ASF457, SB2 [ASF502] and ASF519; ASF⁵) or 7 ASF strains (ASF360, ASF361, ASF356, ASF457, ASF500, SB2 (ASF502), and ASF519; ASF⁷; these mice were generated by transplanting ASF⁴ mice with the additional strains and kept under germ-free conditions for 40 days) were orally gavaged with *S. Tm*^{avir}. Feces was sampled at day 1 p.i. and mice were sacrificed at day 2 p.i. *S. Tm*^{avir} loads at day 1 p.i. in the feces **(A)** and at day 2 p.i. in cecum content **(B)** and the mLN **(C)**. **(D)** Relative cecal weight (cecal weight/body weight) at day 2 p.i. Dotted line: detection limit. Bar indicates median. Statistical analysis was performed using Mann Whitney U test. ns=no significant difference ($p \geq 0.05$), * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **(E)** Fecal microbiota composition at the day of *S. Tm*^{avir} infection. Absolute abundance of each strain was determined by strain-specific qPCR assay and is plotted as relative abundance of the individual strains. *below detection limit. 3 strains of the Altered Schaedler Flora (ASF360, ASF500 and SB2 [ASF502]) were below detection limit. The reason for this remains unclear and was not further investigated here.

Supplementary Figure 4

A



B



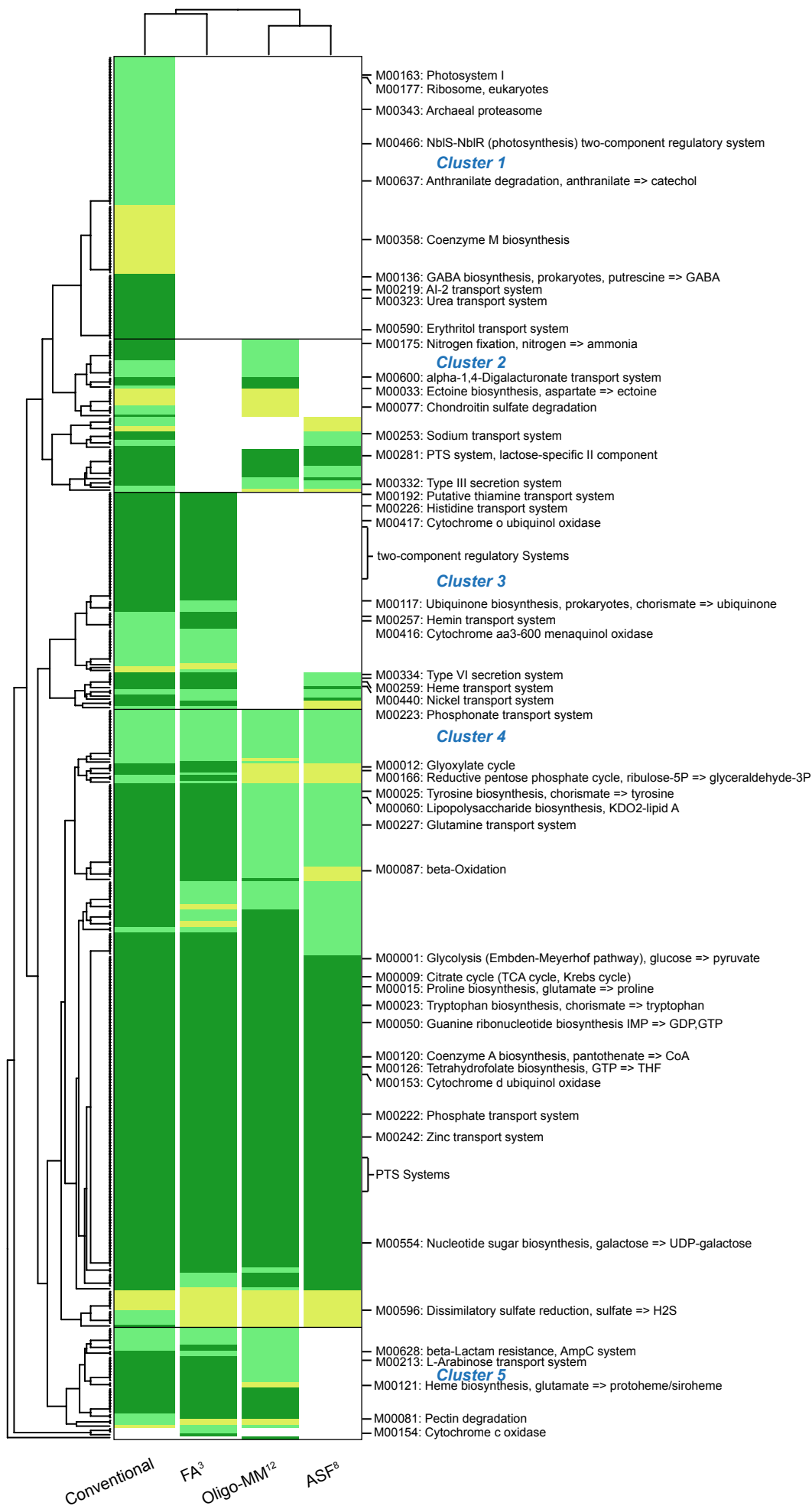
C



Supplementary Figure 4. (A) 16S rRNA gene amplicon sequencing analysis of mice transplanted with Oligo-MM¹². Experiment shown in main Figure 2. Mice stably colonized with ASF strains ASF360, ASF361, ASF457, SB2 [ASF502] and ASF519 (ASF⁵) were transplanted with freshly thawed cryostocks containing the 12 Oligo-MM strains 'Acutalibacter muris' KB18, *Flavonifractor plautii* YL31, *Clostridium clostridioforme* YL32, *Blautia coccoides* YL58, *Clostridium innocuum* I46, *Lactobacillus reuteri* I49, *Enterococcus faecalis* KB1, 'Bacteroides caecimuris' I48, 'Muribaculum intestinale' YL27, *Bifidobacterium longum* subsp. *animalis* YL2, 'Turicimonas muris' YL45 and *Akkermansia muciniphila* YL44 (Oligo-MM¹²), frozen cecum content harvested from a conventional mouse (CON) or sterile culture media as a control (control). Afterwards, mice were kept under germ-free conditions. At 40 days post-transplantation, fecal samples were taken and analyzed by 16S rRNA gene amplicon-sequencing. Amplicons were processed by the QIIME pipeline and taxonomy was assigned against a custom database (see materials and methods). Assignment at genus level (L6) is shown. **Supplementary Table 6. (B) Cecal morphology of OligoMM¹² mice compared to ASF and CON mice.** 8-10 week old female C57Bl/6J mice stably colonized with ASF⁴ (ASF360, ASF361, ASF356 and ASF519), Oligo-MM¹² (strains 'Acutalibacter muris' KB18, *Flavonifractor plautii* YL31, *Clostridium clostridioforme* YL32, *Blautia coccoides* YL58, *Clostridium innocuum* I46, *Lactobacillus reuteri* I49, *Enterococcus faecalis* KB1, 'Bacteroides caecimuris' I48, 'Muribaculum intestinale' YL27, *Bifidobacterium longum* subsp. *animalis* YL2, 'Turicimonas muris' YL45 and *Akkermansia muciniphila* YL44) or a conventional mouse microbiota (CON) were sacrificed and the cecum was exposed to visualize relative cecal size and morphology **(C)**.

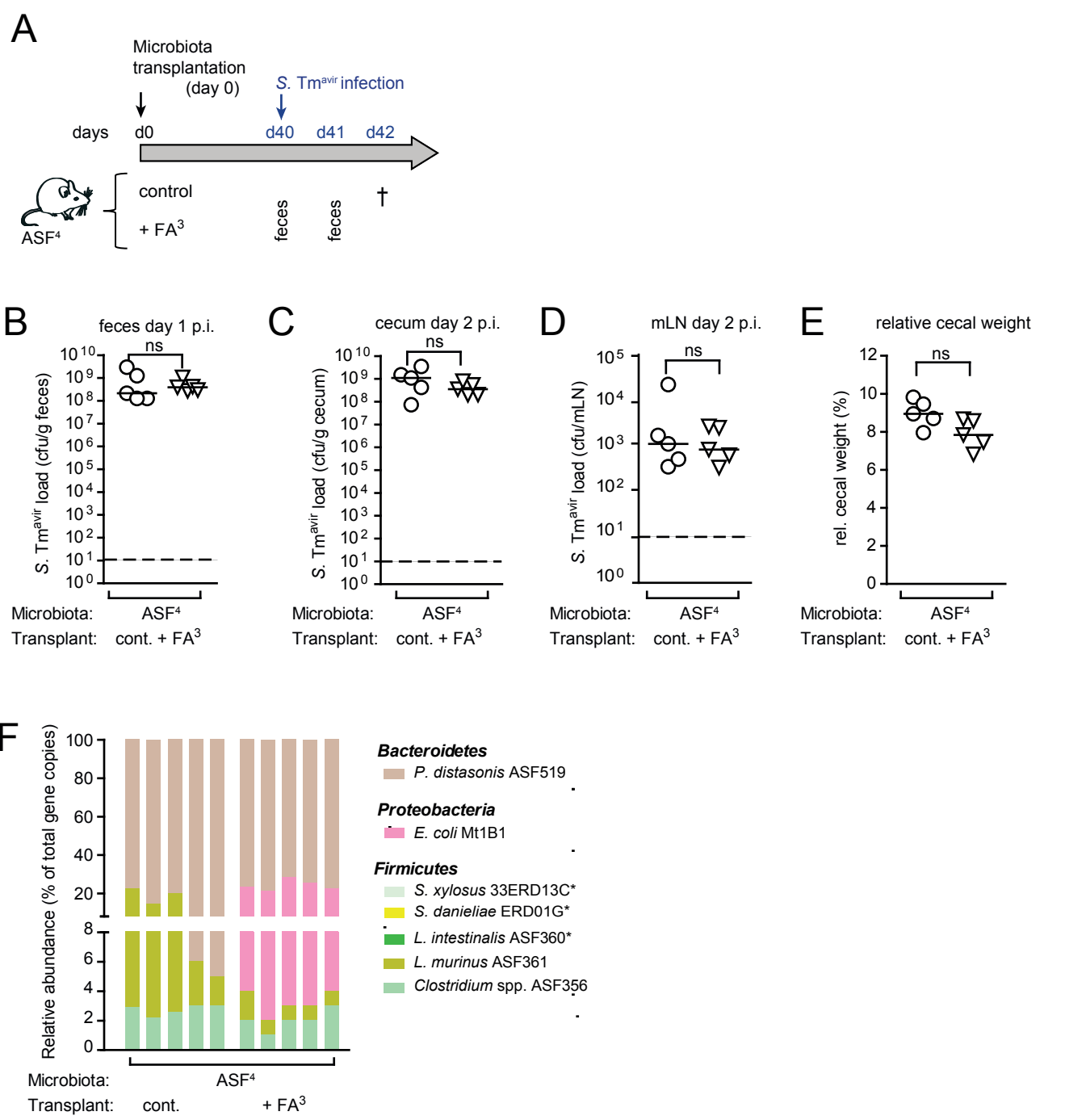
Supplementary Figure 5. Clustering analysis of KEGG modules represented in ASF, FA and Oligo-MM draft genomes. To investigate the functional potential of each strain, genes were predicted from the assembled genomes of 12 Oligo-MM, 3 FA and 8 ASF strains and predicted proteins were classified to modules according to the KEGG ontology. Heat-map of hierarchical clustering of KEGG module distribution in the draft genomes. The color code indicates the presence and completeness of each KEGG module, expressed as value between 0 (module complete) and 4 (module absent). Dark green: module complete; light green: 1 block missing; yellow: 2 blocks missing; white: module absent. Prominent clusters of KEGG modules are highlighted in boxes. Legend of KEGG modules and boxes is shown in **Supplementary Table 9.**

Supplementary Figure 6



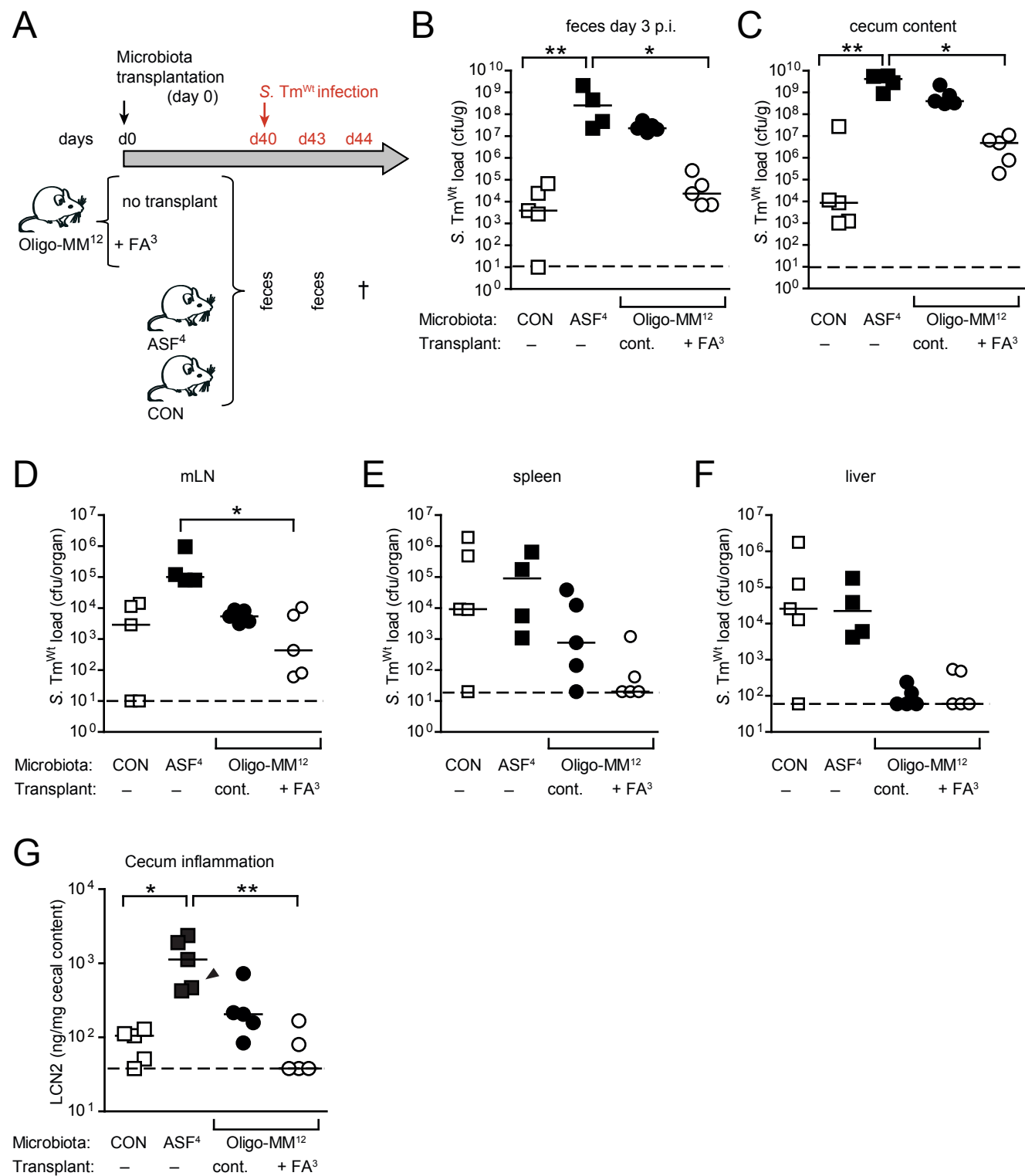
Supplementary Figure 6. Clustering analysis of KEGG modules represented in artificial ASF⁸, FA³ and Oligo-MM¹² metagenomes and conventional metagenomes. To investigate the functional potential of the different bacterial consortia, artificial metagenomes were created by merging assembled individual genomes (ASF⁸, FA³ and OligoMM¹²). In addition, published metagenomes from conventional and wild mice (CON) were included to the analysis. Genes were predicted from the artificial and conventional metagenomes and predicted proteins were classified to modules according to the KEGG ontology. Heat map of hierarchical clustering of KEGG module distribution in the metagenomes. The color code indicates the presence and completeness of each KEGG module, expressed as value between 0 (module complete) and 4 (module absent). Dark green: module complete; light green: 1 block missing; yellow: 2 blocks missing; white: module absent. Prominent clusters of KEGG modules are highlighted in boxes. Legend of KEGG modules and boxes is shown in **Supplementary Table 10**.

Supplementary Figure 7



Supplementary Figure 7. Transplantation of 3 facultative anaerobic bacteria does not restore CR of ASF mice. (A) Experimental design : Mice colonized with ASF356, ASF360, ASF361 and ASF519 (ASF⁴) were transplanted with 3 facultative anaerobic bacteria *E. coli* Mt1B1, *Streptococcus danieliae* ERD01G and *Staphylococcus xylosus* 33-ERD13C (FA³) or sterile culture media as a control (cont.). Afterwards, mice were kept under germ-free conditions. At 40 days post-transplantation (p.t.), fecal samples were taken and mice were orally gavaged with *S. Tm*^{avir}. Feces was sampled at day 1 p.i. and mice were sacrificed at day 2 p.i.. *S. Tm*^{avir} loads at day 1 p.i. in the feces **(B)** and at day 2 p.i. in cecum content **(C)** and the mLN **(D)**. **(E)** Relative cecal weight (cecal weight/body weight) at day 2 p.i.. Dotted line: detection limit. Bar indicates median. Statistical analysis was performed using Mann Whitney U test. ns=no significant difference ($p \geq 0.05$), * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **(F)** Fecal microbiota composition at day 40 p.t.. Absolute abundance of each strain was determined by strain-specific qPCR assay and is plotted as relative abundance of the individual strains. *below detection limit. ASF360, *Streptococcus danieliae* ERD01G and *Staphylococcus xylosus* 33-ERD13C were below detection limit. The reason for this remains unclear and was not further investigated here.

Supplementary Figure 8



Supplementary Figure 8. Transplantation of 3 facultative anaerobic bacteria to Oligo-MM¹² protects against Wild type *S. Tm* colitis. (A) Experimental design : Mice colonized with 'Acutalibacter muris' KB18, *Flavonifractor plautii* YL31, *Clostridium clostridioforme* YL32, *Blautia coccoides* YL58, *Clostridium innocuum* I46, *Lactobacillus reuteri* I49, *Enterococcus faecalis* KB1, 'Bacteroides caecimuris' I48, 'Muribaculum intestinale' YL27, *Bifidobacterium longum* subsp. *animalis* YL2, 'Turicimonas muris' YL45 and *Akkermansia muciniphila* YL44 (Oligo-MM¹²) were transplanted with 3 facultative anaerobic bacteria *E. coli* Mt1B1, *Streptococcus danieliae* ERD01G and *Staphylococcus xylosus* 33-ERD13C (FA³) or PBS as a control (cont.). Afterwards, mice were kept under germ-free conditions. At 40 days post-transplantation (p.t.), fecal samples were taken and mice were orally gavaged with 5×10^7 cfu *S. Tm*^{WT}. As controls, gnotobiotic ASF⁴ mice and conventional C57Bl/6 mice (Janvier, CON) were also orally gavaged with 5×10^7 cfu *S. Tm*^{WT} as controls. Feces was sampled at day 3 p.i.. ASF⁴ mice had to be sacrificed at day 2 (1 mouse; only data for LCN2 levels; arrow) and day 3 p.i. (4 mice) as they exhibited terminal signs of disease. All remaining mice were sacrificed at day 4 p.i.. *S. Tm*^{WT} loads at day 3 p.i. in the feces **(B)** and day 4 p.i. in the cecum content **(C)** and the mLN **(D)**, the spleen **(E)** and the liver **(F)** were determined. **(G)** Lipocalin-2 (LCN2) levels were determined in the cecum content after sacrifice. Dotted line: detection limit. Bars indicate median. Statistical analysis was performed by ANOVA using Kruskal-Wallis and Dunn's Multiple Comparison test. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Supplementary Tables

Supplementary Table 1. Taxonomic classification of the Oligo-MM strains

Taxonomic Classification	Strain ID	DSM number	Classification	Eztaxon best hit
phylum Actinobacteria class Actinobacteria order Bifidobacteriales family Bifidobacteriaceae	YL2	26074	<i>Bifidobacterium longum</i> <i>subsp. animalis</i>	
phylum Bacteroidetes class Bacteroidia order Bacteroidales family Muribaculaceae	YL27	28989	'Muribaculum intestinale'	86.16% <i>B. intestinhominis</i> YIT11860(T) (ADLE01000001)
family Bacteroidaceae	I48	26085	'Bacteroides caecimuris'	96.86% <i>B. xylanisolvens</i> XB1A(T) (AM230650)
phylum Proteobacteria class Betaproteobacteria order Burkholderiales family Sutterellaceae_f	YL45	26109	'Turicimonas muris'	93.92% <i>Parasutterella</i> <i>excrementihominis</i> YIT 11859(T) (AFBP01000029)
phylum Verrucomicrobia class Verrucomicrobiae order Verrucomicrobiales family Verrucomicrobiaceae	YL44	26127	<i>Akkermansia muciniphila</i>	99.86% <i>A.</i> <i>muciniphila</i> _ATCC_BAA-835(T) (CP001071)
phylum Firmicutes class Bacilli order Lactobacillales family Enterococcaceae family Lactobacillaceae	KB1 I49	32036 32035	<i>Enterococcus faecalis</i> <i>Lactobacillus reuteri</i>	
class Clostridia order Clostridiales family Lachnospiraceae	YL32 YL58	26114 26115	<i>Clostridium clostridioforme</i> <i>Blautia coccoides</i>	
family Ruminococcaceae	YL31 KB18	26117 26090	<i>Flavonifractor plautii</i> 'Acutalibacter muris'	
class Erysipelotrichia order Erysipelotrichales family Allobaculum_f	I46	26113	<i>Clostridium innocuum</i>	92.09% <i>C. leptum</i> DSM 753T (ABCB02000019)

Supplementary Table 2. Genome sequencing statistics

Sample	N50 contig size (bp)	Largest contig size (bp)	Genome size (bp)	Number of contigs	Percentage of positive KEGG BLAST genes	SRA Accession Number
I46	116,934	370,189	4,367,037	99	18.0%	SRX1092348
I48	100,406	399,054	4,692,653	154	31.0%	SRX1092357
I49	86,492	157,657	1,999,073	69	55.0%	SRX1092347
KB1	129,660	375,941	2,984,811	47	52.0%	SRX1092355
YL27	491,668	1,113,591	3,180,632	10	27.0%	SRX1092353
YL2	70,565	204,483	2,327,489	110	57.0%	SRX1092362
YL31	225,899	408,893	3,774,556	55	24.0%	SRX1092358
YL32	62,298	248,094	6,842,331	371	25.0%	SRX1092359
YL44	284,982	486,826	2,716,792	27	44.0%	SRX1092354
YL45	58,879	285,653	2,807,473	92	28.0%	SRX1092361
YL58	484,105	987,787	5,099,146	27	27.0%	SRX1092352
KB18*	n.a.	n.a.	3,802,813	1	20.0%	SRX1092360
ERD13C	469,312	970,003	2,760,688	15	57.0%	ERS1032682
ERD01G	8,294	33,745	1,685,213	280	44.0%	ERS1032670
Mt1B1	747,844	1,238,539	5,109,783	20	66.0%	ERS1032680

*The genome of 'A. muris KB18' was sequenced on the PacBio platform yielding one replicon.

Supplementary Table 3. Primers used in this study

Designation	Sequence (5' - 3')	Specificity	Detection limit (qPCR)	Reference
fD1-EcoRV-XbaI	CCGATATCTCTAGAAGAG TTTGATCCTGGCTCAG	Universal bacteria		Modified from ⁶
fD2-EcoRV-XbaI	CCGATATCTCTAGAAGAG TTTGATCATGGCTCAG	Universal bacteria		Modified from ⁶
rP1-EcoRV-BamHI	CCGATATCGGATCCACG GTTACCTTGTTACGACTT	Universal bacteria		Modified from ⁶
fD1	CGATATCTCTAGAAGAGT TTGATCCTGGCTCAG	Universal bacteria		Modified from ⁶
fD2	CGATATCTCTAGAAGAGT TTGATCATGGCTCAG	Universal bacteria		Modified from ⁶
rP1	GATATCGGATCCACGGTT ACCTTGTTACGACTT	Universal bacteria		Modified from ⁶
16S-27f	AGAGTTTGATCMTGGCT CAG	Universal bacteria		GATC Biotech
pJet1-FP	ACTACTCGATGAGTTTTC GG	pJET 1.2		Thermo Fisher
pJet1-RP	TGAGGTGGTTAGCATAGT TC	pJET 1.2		Thermo Fisher
338F-M13	GTAAACGACGGCCAGTG CTCCTACGGGWGGCAGC AGT	Universal bacteria		⁷
1044R-rM13	GGAAACAGCTATGACCAT GACTACGAGCTGACGAC ARCCATG	Universal bacteria		⁷
A-M13	CCATCTCATCCCTGCGTG TCTCCGACTCAG/MID sequence/GTAAACGACGG CCAGTG	M13		⁷
B-rM13	CCTATCCCCTGTGTGCCT TGGCAGTCTCAGGGAAA CAGCTATGACCATGA	rM13		⁷
Isol46_Exonucl.2_fwd	CGGATCGTAAAGCTCTGT TGTAAG	I46	140 copies/5ng template gDNA or 7,7x10 ⁵ bacteria/g intestinal content	This study
Isol46_Exonucl.3_rev	GCTACCGTCACTCCCATA GCA			
Probe3_Isol46	FAM- AAGAACGGCTCATAGAG G-BHQ1			
Isol49_Exonucl._fwd	GCACTGGCTCAACTGATT GATG	I49	338 copies/5ng template gDNA or 1,8x10 ⁶ bacteria/g intestinal content	This study
Isol49_Exonucl._rev	CCGCCACTCACTGGTGA TC			
Probe_Isol49	HEX- CTTGACCTGATTGACGA -BHQ1			
YL58_Exonucl._fwd	GAAGAGCAAGTCTGATG TGAAAGG	YL58	195 copies/5ng template gDNA or	This study

Designation	Sequence (5' - 3')	Specificity	Detection limit (qPCR)	Reference
YL58_Exonucl._rev	CGGCACTCTAGAAAAACA GTTTCC		1,1x10 ⁶ bacteria/g intestinal content	
Probe_YL58	FAM- TAACCCCAGGACTGCAT- BHQ1			
YL27_Exonucl.2_fwd	TCAAGTCAGCGGTAAAAA TTCG			
YL27_Exonucl.2_rev	CCCACTCAAGAACATCAG TTTCAA	YL27	9 copies/5ng template gDNA or 5x10 ⁴ bacteria/g intestinal content	This study
Probe2_YL27	HEX- CAACCCCGTCGTGCC- BHQ1			
YL31_Exonucl.2_fwd	AGGCGGGATTGCAAGTC A			
YL31_Exonucl.3_rev	CCAGCACTCAAGAACTAC AGTTTCA	YL31	4 copies/5ng template gDNA or 2,2x10 ⁴ bacteria/g intestinal content	This study
Probe2_YL31	FAM- CAACCTCCAGCCTGC- BHQ1			
YL32_Exonucl.2_fwd	AATACCGCATAAGCGCA CAGT			
YL32_Exonucl.2_rev	CCATCTCACACCACCAAA GTTTT	YL32	17 copies/5ng template gDNA or 9,4x10 ⁴ bacteria/g intestinal content	This study
Probe2_YL32	HEX- CGCATGGCAGTGTGT- BHQ1			
KB1_Exonucl._fwd	CTTCTTTCTCCCGAGTG CTT			
KB1_Exonucl._rev	CCCCTCTGATGGGTAGG TTACC	KB1	2 copies/5ng template gDNA or 1,1x10 ⁴ bacteria/g intestinal content	This study
Probe_KB1	FAM- CACTCAATTGGAAAGAG GAG-BHQ1			
YL2_Exonucl._fwd	GGGTGAGTAATGCGTGA CCAA			
YL2_Exonucl._rev	CGGAGCATCCGGTATTA CCA	YL2	40 copies/5ng template gDNA or 2,2x10 ⁵ bacteria/g intestinal content	This study
Probe_YL2	HEX- CGGAATAGCTCCTGGAA A-BHQ1			
KB18_Exonucl.2_fwd	TGGCAAGTCAGTAGTGA AATCCA			
KB18_Exonucl.2_rev	TCACTCAAGCTCGACAGT TTCAA	KB18	25 copies/5ng template gDNA or 1,4x10 ⁵ bacteria/g intestinal content	This study
Probe2_KB18	FAM- CTTAACCCATGAACTGC- BHQ1			
YL44_Exonucl._fwd	CGGGATAGCCCTGGGAA A	YL44	13 copies/5ng template gDNA or	This study

Designation	Sequence (5' - 3')	Specificity	Detection limit (qPCR)	Reference
YL44_Exonucl._rev	GCGCATTGCTGCTTTAATCTTT		7,1x10 ⁴ bacteria/g intestinal content	
Probe_YL44	HEX-TGGGATTAATACCGCATA GTA-BHQ1			
YL45_Exonucl._fwd	AGACGGCCTTCGGGTTGTA			
YL45_Exonucl._rev	CGTCATCGTCTATCGGTTATCAA	YL45	2 copies/5ng template gDNA or 1,1x10 ⁴ bacteria/g intestinal content	This study
Probe_YL45	FAM-ACCACTTTTGTAGAGAAC GA-BHQ1			
Isol48_Exonucl._fwd	GGCAGCATGGGAGTTTGCT			
Isol48_Exonucl._rev	TTATCGGCAGGTTGGATACGT	I48	37 copies/5ng template gDNA or 2x10 ⁵ bacteria/g intestinal content	This study
Probe_Isol48	HEX-CAAACCTCCGATGGCGA C-BHQ1			
ASF356_Exonucl.2_fwd	CGGCAAGGTAAGCGATATGTG			
ASF356_Exonucl.2_rev	CGCTTTCCTCTCCTGTACTCTAGCT	ASF356	5 copies/5ng template gDNA or 2,7x10 ⁴ bacteria/g intestinal content	This study
Probe2_ASF356	FAM-TAACTTAAGGATAGCATA ACGAACT-BHQ1			
ASF360_Exonucl.4_fwd	TGAGTGCTAAGTGTTGGGAGGTT			
ASF360_Exonucl.4_rev	CGGAGTGCTTAATGCGTTAGCT	ASF360	25 copies/5ng template gDNA or 1,4x10 ⁴ bacteria/g intestinal content	This study
Probe4_ASF360	FAM-CCGCCTCTCAGTGCT-BHQ1			
ASF361_Exonucl._fwd	TCGGATCGTAAAACCCTGTTG			
ASF361_Exonucl._rev	ACCGTCGAAACGTGAACAGTT	ASF361	11 copies/5ng template gDNA or 6x10 ⁴ bacteria/g intestinal content	This study
Probe_ASF361	HEX-TAGAGAAGAAAGTGCGTGAGAG-BHQ1			
ASF457_Exonucl._fwd	GACTGGAACAACCTTACCGAAAGGT			
ASF457_Exonucl._rev	CAGGTCTCCCCAACTTTTCT	ASF457	9 copies/5ng template gDNA or 5x10 ⁴ bacteria/g intestinal content	This study
Probe_ASF457	FAM-TAATGCCGGATGAGTTATA-BHQ1			
ASF500_Exonucl._fwd	AGGCGGGACTGCAAGTCA	ASF500	31 copies/5ng template gDNA or	This study

Designation	Sequence (5' - 3')	Specificity	Detection limit (qPCR)	Reference
ASF500_Exonucl._rev	CAAATGCAGGCCACAGGTT		1,7x10 ⁵ bacteria/g intestinal content	
Probe_ASF500	HEX-ATGTGAAAACCACGGGC-BHQ1			
ASF502_Exonucl.3_fwd	GACCCAGTACCGCATGTA	ASF502	5 copies/5ng template gDNA or 2,8x10 ⁴ bacteria/g intestinal content	This study
ASF502_Exonucl.3_rev	TCAGACGCGGGCCTATCTTA			
Probe3_ASF502(SB2)	HEX-AGAGGTAAAACTGAGGTGGT-BHQ1			
ASF519_Exonucl._fwd	TGTGGCTCAACCATAAAATTGC	ASF519	25 copies/5ng template gDNA or 1,4x10 ⁵ bacteria/g intestinal content	This study
ASF519_Exonucl._rev	GCATTCCGCCTACCTCAATAT			
Probe_ASF519	HEX-TTGAAACTGGTTGACTTAG-BHQ1			
Salmo_Exonucl._fwd	TGGGAAACTGCCTGATGGA	S. Tm	25 copies/5ng template gDNA or 1,4x10 ⁵ cfu/g intestinal content	This study
Salmo_Exonucl._rev	CTTGCGACGTTATGCGGTATT			
Probe_Salmo	FAM-ATAACTACTGGAAACGGTGGC-BHQ1			
Univ_Exonucl.3_fwd	TGCAYGGYYGTCGTCAGC	All strains		This study
Univ_Exonucl.3_rev	CRTCRTCCYCRCCTTCCTC			
Probe2_Univ.	HEX-AACGAGCGCAACCC-BHQ1			
E.coli_Exonucl._fwd	GGACCTTCGGGCCTCTTG	<i>E. coli</i> (Mt1B1)	25 copies/5ng template gDNA or 1,4x10 ⁵ bacteria/g intestinal content	This study
E.coli_Exonucl._rev	CCTTTACCCACCTACTAGCTAATCC			
Probe_E.coli(Mt1B1)	FAM-ATCGGATGTGCCCAGAT-BHQ1			
Strep._Exonucl._fwd	CAACTGCATCACTACCAGATGGA	<i>S. danieliae</i> (ERD01G)	3 copies/5ng template gDNA or 1,7x10 ⁴ bacteria/g intestinal content	This study
Strep._Exonucl._rev	CGCCTAGGTGAGCCTTTACCT			
Probe_Strep.daniel.	HEX-CTGCGTTGTATTAGCTAGTAG-BHQ1			
Staph._Exonucl._fwd	GGAGCTAATACCGGATAACATTTAGAA	<i>S. xylosus</i> (33ERD13C)	44 copies/5ng template gDNA or	This study

Designation	Sequence (5' - 3')	Specificity	Detection limit (qPCR)	Reference
Staph._Exonucl._rev	CCATCTATAAGTGATAGC AAAACCATCT	)	2,4x10 ⁵ bacteria/g intestinal content	
Probe_Staph.xylosus	HEX- CGCATGGTTCTAAAGTG- BHQ1			

Supplementary Table 4. Plasmids harboring 16S rRNA genes

Plasmid	Backbone	Origin of the insert	Restriction site used for linearization	Reference
pSAB3	pJET 1.2	YL27	NotI	This study
pSAB4	pJET 1.2	YL58	HindIII	This study
pSAB6	pJET 1.2	I46	NotI	This study
pSAB7	pJET 1.2	I48	HindIII	This study
pSAB8	pJET 1.2	I49	HindIII	This study
pSAB9	pJET 1.2	KB1	NotI	This study
pSAB10	pJET 1.2	ASF502	NotI	This study
pSAB12	pJET 1.2	KB18	HindIII	This study
pSAB13	pJET 1.2	ASF500	NotI	This study
pMB1	pJET 1.2	<i>S. Tm</i>	HindIII	This study
pM1452	pCR [®] 2.1-TOPO [®]	YL2	HindIII	This study
pM1456-1	pCR [®] 2.1-TOPO [®]	YL31	HindIII	This study
pM1457-1	pCR [®] 2.1-TOPO [®]	YL32	HindIII	This study
pM1459-1	pCR [®] 2.1-TOPO [®]	YL44	HindIII	This study
pM1460-1	pCR [®] 2.1-TOPO [®]	YL45	NcoI	This study
pM1411-1	pSB-Bluescript	ASF356	NotI	This study
pM1412-4	pSB-Bluescript	ASF360	NotI	This study
pM1413-1	pSB-Bluescript	ASF361	NotI	This study
pM1414-1	pSB-Bluescript	ASF457	NotI	This study
pM1417-1	pSB-Bluescript	ASF519	NotI	This study
pDK4-12	pJET 1.2	<i>E.coli</i> (Mt1B1)	NotI	This study
pDK5-1	pJET 1.2	<i>S. xylosus</i> (33ERD13C)	NotI	This study
pDK6-1	pJET 1.2	<i>S. danieliae</i> (ERD01G)	NotI	This study

Supplementary Table 5. Taxonomic composition of fecal microbiota expressed as relative abundances (Figure 2C)

Supplementary Table 6. Taxonomic composition of fecal microbiota expressed as relative abundances (Supplementary Figure 4)

Supplementary Table 7. KEGG module analysis for Oligo-MM¹² and ASF⁸

Supplementary Table 8. KEGG module analysis for CON metagenomes and artificial metagenomes of Oligo-MM¹² and ASF⁸

Supplementary Table 9. KEGG module analysis for Oligo-MM¹², ASF⁸ and FA³

Supplementary Table 10. KEGG module analysis for CON metagenomes and artificial metagenomes of Oligo-MM¹², FA³ and ASF⁸

Supplementary Table 11. MID barcodes for 454 multiplexing ⁷.

Designation	MID sequence	Designation	MID sequence
MID1	ACGAGTGCGT	MID27	ATCTCTGTGC
MID2	ACGCTCGACA	MID28	CTGTGCGCTC
MID3	AGACGCACTC	MID29	CGACTATGAT
MID4	AGCACTGTAG	MID30	GCGTATCTCT
MID5	ATCAGACACG	MID31	TCTGCATAGT
MID6	ATATCGCGAG	MID32	ATCGAGTCAT
MID7	CGTGTCTCTA	MID33	GTGATGATAC
MID8	CTCGCGTGTC	MID34	CATAGAGAGC
MID9	TAGTATCAGC	MID35	ATGCAGCATA
MID10	TCTCTATGCG	MID36	ATCATGCACT
MID11	TGATACGTCT	MID37	TGAGCTAGCT
MID12	TACTGAGCTA	MID38	GATGACTGAC
MID13	CATAGTAGTG	MID39	CACAGTCACA
MID14	CGAGAGATAC	MID40	TGCTAGCATG
MID15	ATACGACGTA		
MID16	TCACGTACTA		
MID17	TCGATCGAGT		
MID18	CAGTCAGTAG		
MID19	ACACTGACAC		
MID20	GTACGATCGT		
MID21	TGCGTGAGCA		
MID22	ACAGCTCGCA		
MID23	CTCACGCAGA		
MID24	GATGTCACGA		
MID25	GCACAGACTA		
MID26	GAGCGCTATA		

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