



Supporting Online Material for

Metabolic Syndrome and Altered Gut Microbiota in Mice Lacking Toll-Like Receptor 5

Matam Vijay-Kumar, Jesse D. Aitken, Frederic A. Carvalho, Tyler C. Cullender,
Simon Mwangi, Shanthi Srinivasan, Shanthi V. Sitaraman,
Rob Knight, Ruth E. Ley, Andrew T. Gewirtz*

*To whom correspondence should be addressed. E-mail: agewirt@emory.edu

Published 4 March 2010 on *Science Express*
DOI: 10.1126/science.1179721

This PDF file includes:

Materials and Methods
Figs. S1 to S19
Tables S1 to S3
References

MATERIALS AND METHODS

Mice: TLR5-deficient mice were generated as previously described (1). Data in this paper were generated from WT littermates and T5KO mice that were back-crossed for 10 generations. T5KO mice were crossed with, respectively, mice lacking TLR2 and TLR4 (T2KO, T4KO) and RAG-1 (RAG1-KO) (purchased from Jackson Laboratories) to generate T2/T5DKO, T4/5DKO and T5/RAG1-DKO mice and WT littermates, RAG1-KO, T2KO, and T4KO. MyD88KO mice and corresponding WT controls were bred at Emory University. All animal experiments were approved by the Emory University ethical committee.

Rederivation: Male T5KO mice were bred with WT female C57BL/6J mice purchased from Jackson Laboratory (Bar Harbor, ME). The embryos were transferred to surrogate C57BL/6J mice and generated TLR5 heterozygotes. These mice were bred to obtain WT and T5KO mice with gut microbiota identical (or similar) to C57BL/6J mice from Jackson Laboratories.

Body fat analysis: The mice were imaged on a Biospec (Bruker) 3-T MRI system 9.4 Tesla using a custom-sized send-receive coil.

Ex-Vivo organ culture: One cm segments of proximal colons washed in HBSS containing penicillin and streptomycin or 100 mg of adipose tissue were cultured in 24-well culture plates in serum-free RPMI 1640 medium supplemented with penicillin and streptomycin, L-glutamine, and non essential amino acids. After 24-h, supernatant was collected and centrifuged at 13,000 g for 10 minutes at 4°C and stored at -20°C until analyzed.

Stool output measurement: Mice were placed in a clean cage individually and stools were collected for 1-h. Dry weights were taken after stool pellets were incubated over night at 65°C.

Serum analysis: Mice were given either regular laboratory chow (fat 4.5%, Lab Diets 5001) or high-fat diet (fat 34.9%, D12492, Research Diets Inc., New Brunswick, NJ). Mice were fasted for 5-h at which time blood was collected by retrobulbar intraorbital capillary plexus. Hemolysis-free serum was generated by centrifugation of blood using serum separator tubes (Becton Dickinson, Franklin Lakes, NJ). Serum cholesterol and triglycerides were quantified by colorimetric kits from BioVision (Mountain View, CA). Serum insulin and leptin were analyzed by ELISA kits purchased from Linco Research Inc (St. Charles, MO). Serum neuropeptide Y levels were quantified (on serum isolated from mice fed *ad libitum*) using a competitive ELISA kit from Ray Biotech (Atlanta, GA). Plasma GLP-1 was assayed by an ELISA kit from Alpco Immunoassays. Serum ghrelin was quantified by an ELISA kit from Millipore Corporation (Billerica, MA). Cytokines and lipocalin-2 was assayed by DuoSet ELISA kits (R&D Systems, Minneapolis, MN).

Blood pressure analysis: Blood pressure was measured on conscious animals via the tail-cuff method (MC4000; Hatteras Instruments, Cary, NC) following at least two weeks of daily training by a single operator. Pulse trace analysis of each reading was necessary to ascertain the stress level of the animal and determine the validity of the measurement.

Assessment of islets: Four 5 µm pancreas sections (separated by 200 µm) per mouse were used to assess islet number and size. Sections were H&E stained and assessed for size and number of islets

by a technician unaware of sample identity. Islet score reflects the average combined score per mouse. Each mouse received the combined score of 4 slides where for each slide each islet was assigned a score of 1-3 based on size.

Immunofluorescence: Pancreata were fixed in 10% formalin solution and embedded in paraffin using standard techniques. Paraffin sections were processed according to suggested protocols (Cell Signaling Technologies, Danvers, MA) and stained with guinea pig anti-insulin antibody (1:50) obtained from Zymed Laboratories (South San Francisco, CA) and probed with Alexa Fluor 488 conjugated to an anti-guinea pig antibody (1:500) (Invitrogen, Carlsbad, CA).

Glucose tolerance test and estimation of insulin sensitivity: Age-matched WT and T5KO mice were fasted for 5-h and baseline blood glucose levels measured with the aid of an Accu-Check Advantage blood glucose meter (Roche) using blood collected from the tail vein. Mice were injected intraperitoneally with 2 g glucose/kg body weight in sterile PBS and blood glucose levels measured 15, 30, 60, 90, and 120 min after injection. For insulin sensitivity test, 5-h fasted mice were injected intraperitoneally with 1.0 U insulin/kg body weight (Eli Lilly Co, Indianapolis, IN). Blood glucose was then measured 15, 30, 60, 90, and 120 min after injection.

Assessment of insulin secretion: For *in vivo* insulin secretion, 5-h fasted mice were injected intraperitoneally with 3 g glucose/kg body weight and serum insulin measured 0 and 2.5 min post-injection via ELISA.

Caloric restriction: Four week old WT and T5KO mice were given the amount of food every 2 days that a separate group of age and gender matched WT mice had eaten during the previous 2 days for a period of 12 weeks. Throughout the experiment, mice were monitored for body weight.

Antibiotic treatment: Four week old WT and T5KO mice were placed on broad spectrum antibiotics ampicillin (1.0g/L) and neomycin (0.5g/L) in drinking water for 12 weeks. As ampicillin and neomycin are poorly absorbed, such treatment primarily affects only intestinal microbiota without direct systemic effects (2). During this period mice were monitored for food intake, body weights, gross cecal appearance, and cecal microbiota quantification.

Tissue myeloperoxidase (MPO) assay: Tissue MPO was measured as described in (1)

Bomb calorimetry: Bomb calorimetry was performed on fecal samples collected from 13 mice (7 WT, 6 T5KO). Fecal samples were collected and frozen at -80°C until processing, then oven dried at 58°C for 48 hours. Gross energy content was measured using an isoperibol bomb calorimeter (Model C 2000, from IKA Works, Inc.). The calorimeter energy equivalent factor was determined using benzoic acid standards.

Short-chain fatty acid analysis: Short-chain fatty acids (SCFAs) were measured from 13 cecal samples (7 WT, 6 T5KO). SCFA levels were quantified according to previously published protocols (3). Briefly, approximately 50 mg of deproteinized cecal contents were suspended in acidified water spiked with isotope-labeled internal SCFA standards. Samples were mixed thoroughly, centrifuged, and supernatant was removed for quadruple extraction with diethyl ether. Derivatization of SCFAs was performed in duplicate with N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA) and the resulting TBDMS derivatives were analyzed by GC-MS.

Cecal microbiota analysis by 16S rRNA sequencing: To avoid the confounding effects of co-housing on the diversity of cecal bacteria (4, 5), we selected mice from multiple litters that were housed separately. Bulk DNA was extracted from frozen extruded cecal contents using a modified phenol-chloroform extraction technique with mechanical disruption (bead-beating) as previously described (4). Briefly, an aliquot (~100mg) of each homogenized sample was, while frozen, suspended in a solution containing 500 µl of DNA extraction buffer [200 mM Tris (pH 8.0), 200 mM NaCl, 20 mM EDTA], 210 µl of 20% SDS, 500 µl of a mixture of phenol:chloroform:isoamyl alcohol (25:24:1), and 500 µl of a slurry of 0.1-mm-diameter zirconia/silica beads (BioSpec Products, Bartlesville, OK). Microbial cells were then lysed by mechanical disruption with a bead beater (BioSpec Products) set on high for 2 min (23°C), followed by extraction with phenol:chloroform:isoamyl alcohol, and precipitation with isopropanol. DNA was quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen) and a plate reader. For each sample, 16S rRNA genes were amplified using a composite forward primer and a reverse primer containing a unique 12-base barcode used to tag each PCR product (6), as previously described (7). Thus, for each sample, we amplified the 16S rRNA gene using the composite forward primer 5'-*GCCTTGCCAGCCCGCTCAGTC-AGAGTTTGATCCTGGCTCAG*-3': the italicized sequence is 454 Life Sciences® primer B, and the bold sequence is the broadly conserved bacterial primer 27F. The reverse primer used was 5'-*GCCTCCCTCGCGCCATCAGNNNNNNNNNNNCA-TGCTGCCTCCCGTAGGAGT*-3': the italicized sequence is 454 Life Sciences' primer A, and the bold sequence is the broad-range bacterial primer 338R. *NNNNNNNN* designates the unique twelve-base barcode used to tag each PCR product (6), with 'CA' inserted as a linker between the barcode and rRNA primer. Three independent PCRs were performed for each

sample along with a no-template control. For each sample, the three replicate PCR products were combined and purified with Ampure magnetic purification beads (Agencourt Bioscience Corp. Beverly, MA), and products quantified described above. A master DNA pool was generated from the purified products in equimolar ratios to a final concentration of 21.5 ng/μl. The pooled products were sequenced using a Roche 454 FLX pyrosequencer at the EnGenCore genome sequencer center at the University of South Carolina. After removal of low-quality sequences and trimming of primer sequences, sequences were assigned to a sample based on the barcodes (7). **Phylotype assignment** - at 97% pair-wise sequence identity was generated from the sequence pool as previously described (8). Sequence identity was calculated using megablast (9). **Taxonomy assignment** - Taxonomy was assigned using the best megablast (10) hit against Greengenes (11) (E-value cutoff of 1×10^{-12} ; minimum %ID of 88% of the length of the shorter sequence; word size 38) and the Hugenholtz taxonomy (http://greengenes.lbl.gov/Download/Sequence_Data/Greengenes_format/greengenes16SrRNAgenes.txt.gz, downloaded Sept 18 2008 and flagged chimeras omitted). To determine the best BLAST hit to mouse-only or human-only derived sequences, custom databases including matches to human or mouse-derived sequences only were constructed from the Greengenes database. **Sample clustering using UniFrac** - One sequence representing each phylotype was aligned using NAST (11) and a phylogenetic tree built using FastTree (12) with Kimura correction. Samples were clustered using the UniFrac metric (13): UniFrac distances between samples are based on the fraction of branch length that is unique to one sample or another in a shared phylogenetic tree composed of sequences from both samples. Principal coordinates analysis was applied to the unweighted UniFrac distance matrix as previously described (8, 14). **Phylogenetic reconstructions** – sequences representative of enriched or depleted phylotypes

were aligned using NAST and the nearest cultured and uncultured neighbors were also retrieved from the Greengenes database using the Simrank online tool (http://greengenes.lbl.gov/cgi-bin/nph-NAST_align.cgi). A maximum likelihood analysis was conducted with RAxML, using four sequences from candidate phylum WS3 as an outgroup, and 100 bootstrap replicates were performed using the online server <http://phylobench.vital-it.ch/raxml-bb/> (15).

Quantification of cecal microbiota by qPCR: Quantitative PCR was performed on genomic DNA extracted from the cecal contents of WT or T5KO mice (n=10) as described above. qPCR was performed and analyzed using SYBR® Green I dye chemistry and 7300 Real-Time PCR Systems and Software (Applied Biosystems). Each 50 µl reaction contained 10-30 ng of cecal DNA, 25 µl of 2x SYBR® Green PCR Master Mix (Applied Biosystems, CA), 40 nM of the bacteria-specific primer 8F (5'-AGAGTTTGATCCTGGCTCAG-3'), and 40 nM of the universal primer 338R (5'-CTGCTGCCTCCCGTAGGAGT-3'), (Invitrogen, Carlsbad, CA). Standard curves were prepared from serial dilution of *Escherichia coli* 25 genomic DNA extracted in the same manner as above and all samples were run in duplicate. Primers were optimized to yield the minimum C_T and maximum ΔR_n . Cycling conditions were 50°C for 2 min, followed by 95°C for 10 min, followed by 40 cycles of 95°C for 30 s and 56°C for 1 min, with a final melting curve period of 95°C for 15 s, 60°C for 30 s, and another 15 s at 95°C.

Microbiota transplantation: Cecal contents were pooled from 3 T5KO mice and age- and gender-matched WT littermates. Cecal extracts were suspended in PBS (2.5 ml per cecum) and were administered (0.1 ml per mouse) immediately to sterilely-packed 4 week old Swiss-Webster

male WT germ free mice that were purchased from Taconic (Hudson, NY). Transplanted mice were maintained in sterile cages and monitored for body weight and food intake.

Statistical analysis: In point plots, horizontal lines indicate means. Data in bar plots are means + S.E.M. Statistical significance was assessed by Student's two-tailed t test, with p-values of <0.05 considered significant. For the comparisons of mean phylotype abundances in WT and T5KO mice, significance levels were adjusted for multiple comparisons using Bonferroni's correction (Tables S2, S3).

References (for methods)

1. M. Vijay-Kumar *et al.*, *J Clin Invest* **117**, 3909 (Dec, 2007).
2. L. Ferrier *et al.*, *Am J Pathol* **168**, 1148 (Apr, 2006).
3. P. J. Turnbaugh *et al.*, *Nature* **444**, 1027 (Dec 21, 2006).
4. R. E. Ley *et al.*, *Proc Natl Acad Sci U S A* **102**, 11070 (Aug 2, 2005).
5. L. Wen *et al.*, *Nature* **455**, 1109 (Oct 23, 2008).
6. M. Hamady, J. J. Walker, J. K. Harris, N. J. Gold, R. Knight, *Nat Methods* **5**, 235 (Mar, 2008).
7. N. Fierer, M. Hamady, C. L. Lauber, R. Knight, *Proc Natl Acad Sci U S A* **105**, 17994 (Nov 18, 2008).
8. P. J. Turnbaugh *et al.*, *Nature* **457**, 480 (Jan 22, 2009).
9. S. McGinnis, T. L. Madden, *Nucleic Acids Res* **32**, W20 (Jul 1, 2004).
10. S. F. Altschul, W. Gish, W. Miller, E. W. Myers, D. J. Lipman, *J Mol Biol* **215**, 403 (Oct 5, 1990).
11. T. Z. DeSantis, Jr. *et al.*, *Nucleic Acids Res* **34**, W394 (Jul 1, 2006).
12. M. N. Price, P. S. Dehal, A. P. Arkin, *Mol Biol Evol* **26**, 1641 (Jul, 2009).
13. C. Lozupone, M. Hamady, R. Knight, *BMC Bioinformatics* **7**, 371 (2006).
14. C. Lozupone, R. Knight, *Appl Environ Microbiol* **71**, 8228 (Dec, 2005).
15. A. Stamatakis, P. Hoover, J. Rougemont, *Syst Biol* **57**, 758 (Oct, 2008).

Figure Legends for Supplemental Figures.

Fig. S1. Rederived T5KO mice exhibit mild colitis. T5KO mice and WT littermates were rederived via embryo transplantation using mice from Jackson Laboratories. Mice were analyzed at age 12 weeks. (A) Mass of spleen. (B) Colon mass. (C) Cecal MPO. (D) Colonic myeloperoxidase (MPO). (E) Serum lipocalin-2. Results are from a single group of mice (n=7-21) and representative of 3 independent analyses. (* indicates $p < 0.05$).

Fig. S2. Elevated proinflammatory cytokine secretion by T5KO adipose tissue. Adipose tissue (100 mg) from 20 week old WT and T5KO mice was cultured for 24-h and cytokines in the supernatants were assayed. (A) IFN γ . (B) IL-1 β . Results are from a single group of mice experiment (n=6). (* indicates $p < 0.05$).

Fig. S3. T5KO mice exhibit dysglycemia and enlarged islets. (A) Blood glucose assayed from mice fed *ad libitum*. (B) H & E stained pancreatic islets. (C) Sections of pancreas were immunostained for insulin. Results are from a single group of mice (n=6) and representative of 2 independent analyses.

Fig. S4. High fat diet aggravates metabolic syndrome in T5KO mice. Four week old WT and T5KO mice were given a high-fat diet for 8 weeks. (A) Body mass. (B) Fat pad mass. (C) Serum triglycerides. (D) Serum cholesterol. (E) Serum leptin. (F) Serum

insulin. Results are from a single experiment (n=5-6) and representative of 2 independent experiments. (* indicates $p < 0.05$).

Fig. S5. Stool output in WT and T5KO mice. Individual mice fed *ad libitum* were placed in cages for 1 h. Stools were collected, weighed and re-weighed following overnight drying at 65°C. (A) Wet stool mass. (B) Dry stool mass. Results are from a single experiment (n=6) and representative of 5 independent experiments. (* indicates $p < 0.05$).

Fig. S6. Fecal Energy and short-chain fatty acid content in WT and T5KO mice. Fecal samples were isolated from 8 week-old WT and T5KO mice (n= 6-7) and the following measured: (A) Energy content measured by bomb calorimetry, expressed as mean Kcal per gram dry mass. (B) Levels of the short-chain fatty acids acetate, propionate, and butyrate expressed as mmoles per gram dry mass. The differences between WT and T5KO mice were not statistically significant.

Fig. S7. T5KO mice hyperphagia is independent of appetite regulating peptide hormones. Sera was isolated from 5h-fasted 20 week old mice (n=15-18) were analyzed for: (A) Ghrelin. (B) Neuropeptide Y (NPY). (C) Leptin. (D) Plasma GLP-1. (* indicates $p < 0.05$).

Fig. S8. Calorie restriction reduces serum indicators of metabolic syndrome in T5KO mice. Sera from WT and T5KO mice that had been subjected to food restriction for 12 weeks (from Fig 4) were analyzed for the following: (A) Cholesterol. (B) Leptin. and (C) Insulin.

Fig. S9. T5KO metabolic syndrome does not require TLR2. Twenty week old mice deficient in TLR2 (T2KO) or TLR2 and TLR5 (T2/5-DKO) mice were analyzed for the following parameters: (A) Body mass. (B) Food intake. (C) 15-h fasting blood glucose. Results are from an individual group of mice (n=8-11). (* indicates $p < 0.05$).

Fig. S10. T5KO metabolic syndrome is independent of TLR4. Twenty week old mice deficient in TLR4 (T4KO) or TLR4 and TLR5 (T4/5-DKO), and age-matched WT mice were analyzed for the following parameters: (A) Food intake. (B) Body mass. (C) 15-h fasting blood glucose. (D) Serum insulin. (E) Insulin sensitivity. (F) Photograph showing abdominal adiposity. (G) Fat pad mass. Results are from an individual group of mice (n=6-12) and representative of 2 groups of mice. (* indicates $p < 0.05$).

Fig. S11. MyD88 deficient mice do not exhibit metabolic syndrome. Twenty week old WT or MyD88-deficient (MyD88KO) mice were analyzed for the following parameters: (A) Body mass. (B) Food intake. (C) 15-h fasting blood glucose. (D) Fat pad mass. Results are from an individual group of mice (n=8) and representative of 2 groups of mice. (* indicates $p < 0.05$).

Fig. S12. T5KO dysglycemia is independent of adaptive immunity. Mice deficient in RAG1 (RAG1-KO) or RAG1 and TLR5 (RAG1/T5-DKO) at indicated age were fasted for 15-h and blood glucose levels were measured. Results are from sequential measurements of a single group of mice (n=10) and representative of measurements performed on 2 distinct groups of mice. (* indicates $p < 0.05$).

Fig. S13. Broad spectrum antibiotics reduce microbiota density. Immediately after weaning WT and T5KO mice (n=4-10) were placed on drinking water containing ampicillin and neomycin. Twelve weeks later, mice were assayed for: (A) Gross cecal appearance. (B) Microbiota quantification by qRT-PCR of 16S rRNA. (* indicates $p < 0.05$).

Fig. S14. Broad spectrum antibiotics prevents T5KO metabolic syndrome. Immediately after weaning WT and T5KO mice were placed on drinking water containing ampicillin and neomycin. Twelve weeks later, mice were assayed for: (A) Fat pad mass. (B) Food intake. (C) Serum triglycerides. (D) 15-h fasting blood glucose. Results are from an individual group of mice (n=6) and representative of 2 independent experiments (other shown in fig 4). (* indicates $p < 0.05$).

Fig. S15. T5KO mice exhibit taxonomical alterations in gut microbiota. Untreated WT and T5KO mouse cecal contents were analyzed via 16S rRNA analysis. Mouse cecal bacterial communities were clustered using principal coordinates analysis (PCoA) of the UniFrac unweighted distance matrix. PC1 and PC2 are plotted. Each symbol

corresponds to a mouse: filled circles are T5KO and open squares are WT. The percentage of the variation explained by the plotted principal coordinates is indicated in the axis labels. Results are from an analysis of 4-5 mice per group. (* indicates mean PC1 values for WT and T5KO are significantly different).

Fig. S16. Altered *Firmicutes* in T5KO mice. Phylogenetic relationships of the significantly enriched or depleted phylotypes in WT (blue labels, sequence name prefix="WT_UP") and T5KO (red labels, sequence name prefix="KO_UP"). The closest relatives that are uncultured (black labels) and cultured (green labels) as determined by searching against the Greengenes database, are included. The best tree from maximum likelihood analysis with 100 bootstrap replicates is shown, filled circles on nodes represent $\geq 70\%$ support, see text for details. Scale bar shows nucleotide substitutions per site. This tree includes all phylotypes except those of the phylum *Bacteroidetes*.

Fig. S17. Altered *Bacteroidetes* in T5KO mice. Phylogenetic tree of the *Bacteroidetes*, see legend for Fig. S16.

Fig. S18. T5KO mice gut microbiota is necessary and sufficient to transfer metabolic syndrome to WT germ-free mice. 4-week old WT germ-free mice were intragastrically administered cecal contents from WT or T5KO mice (n=5-6), monitored for 7 weeks, and analyzed for the following: (A) Serum insulin. (B) 15-h fasting blood glucose. (C) Photograph showing abdominal adiposity. (D) Colon mass. (* indicates $p < 0.05$).

Fig. S19. Proposed mechanism of T5KO metabolic syndrome

Table S1 and S2. Phylotypes depleted (S1) and enriched (S2) in T5KO versus WT

mice. Numbers are means of the percent abundance of a given phylotype in each individual mouse for each mouse type (T5KO, n=5, or WT, n=4) with standard errors.

The p-values were calculated using a Student's t-test (2-tailed, equal variance). Only data for phylotype mean abundance differences with p-values < 0.05 are shown.

“Phylotype ID” refers to the label of each phylotype in the phylogenetic trees (Figures S17, S18). Consensus lineages are derived from the taxonomy consensus of 80% of sequences in a phylotype. The phylotypes highlighted blue have significant p-values when the Bonferroni correction is applied to correct for multiple comparisons. Consensus lineages are derived from the taxonomy consensus of 80% of sequences in a phylotype.

Table S3. Closest matches between phylotypes obtained in this study and phylotypes obtained from studies of human gut microbiotas (upper table) and mouse gut

microbiotas (lower table). Table lists closest bacterial species, based on BLAST analysis, to the phylotypes listed in Table S1 and S2.

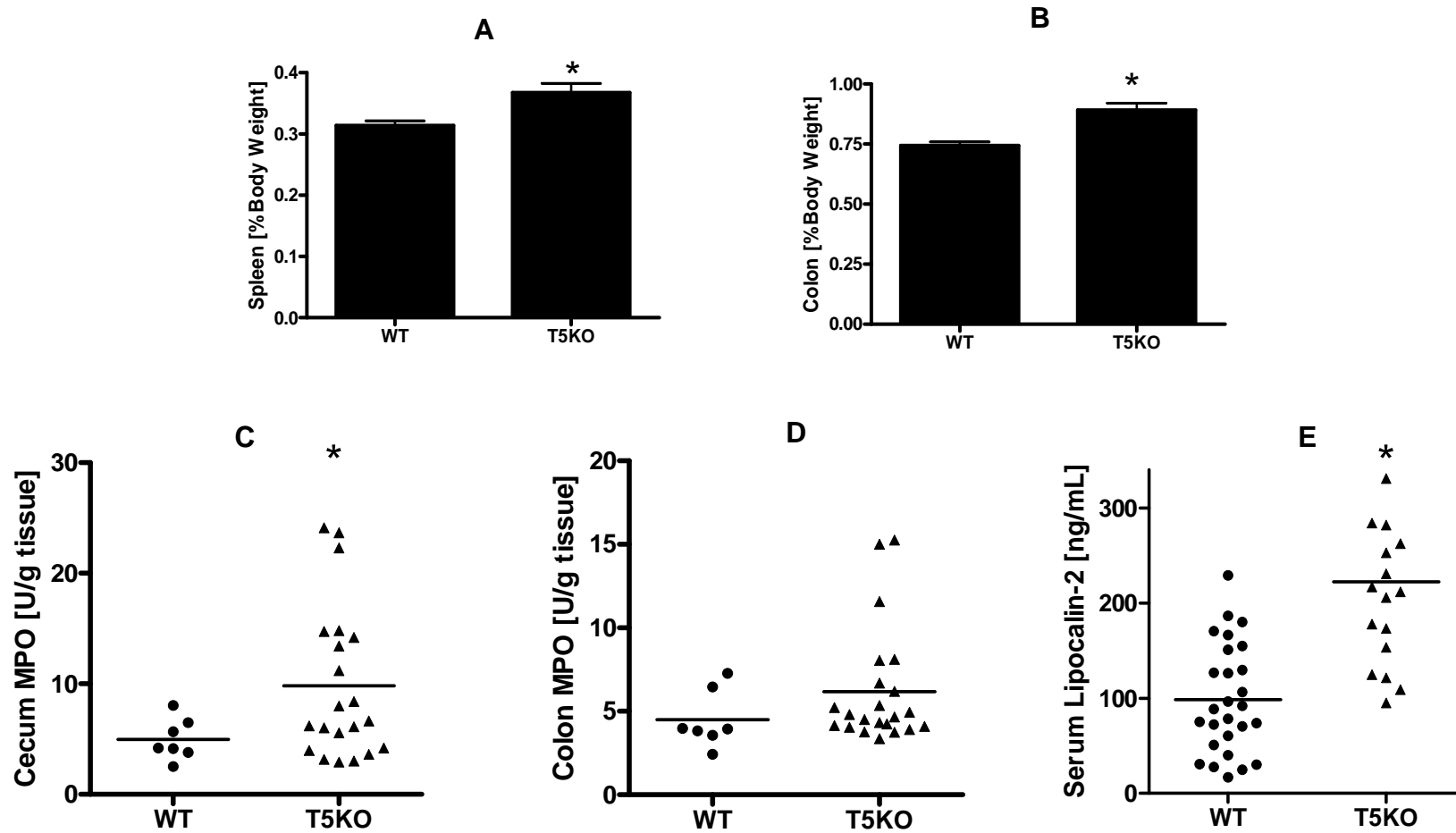


Fig. S1.

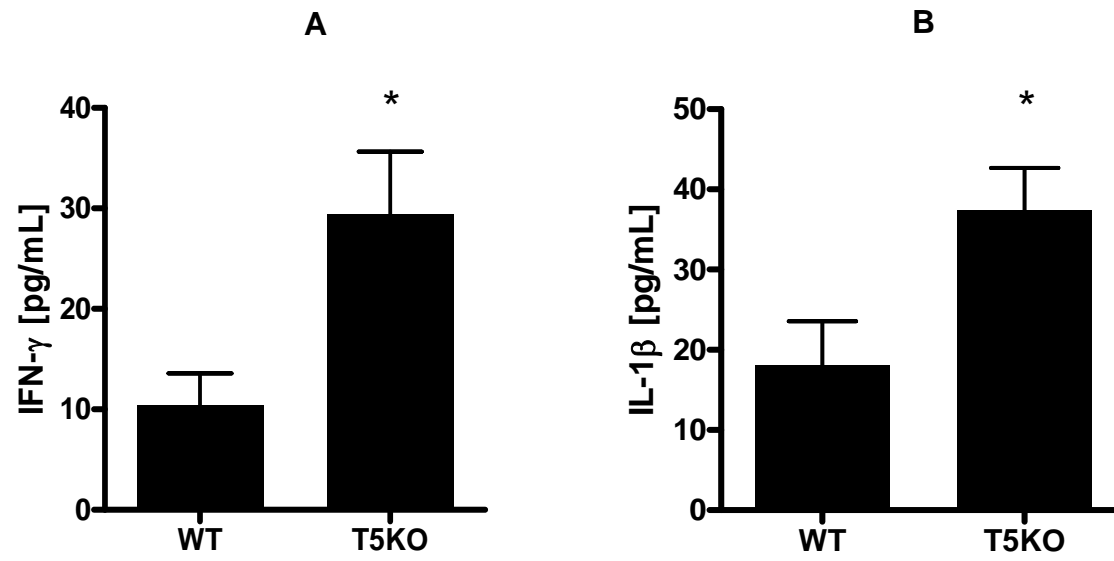


Fig. S2.

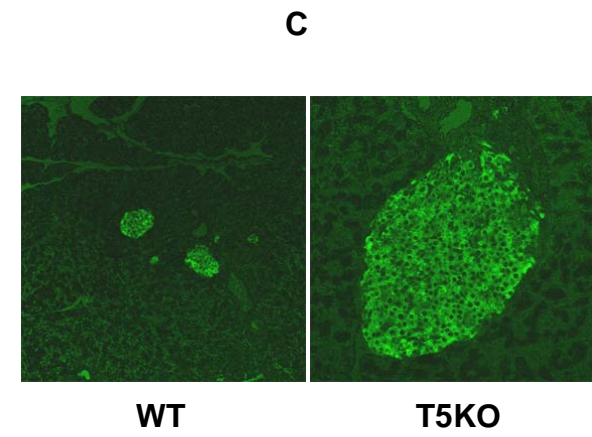
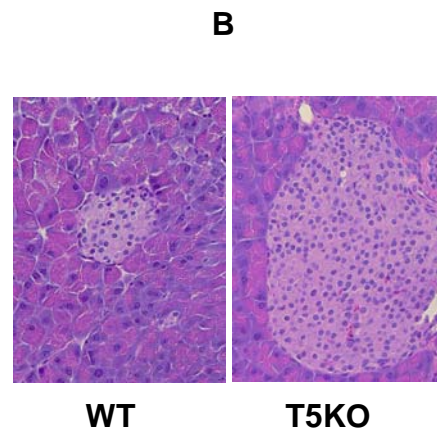
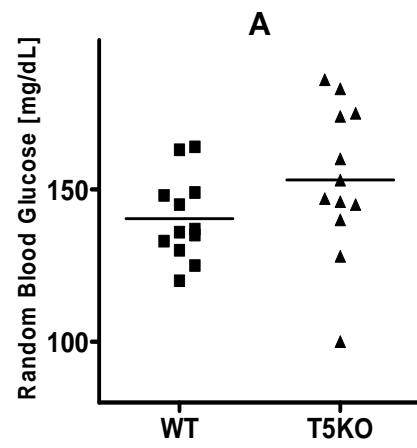


Fig. S3.

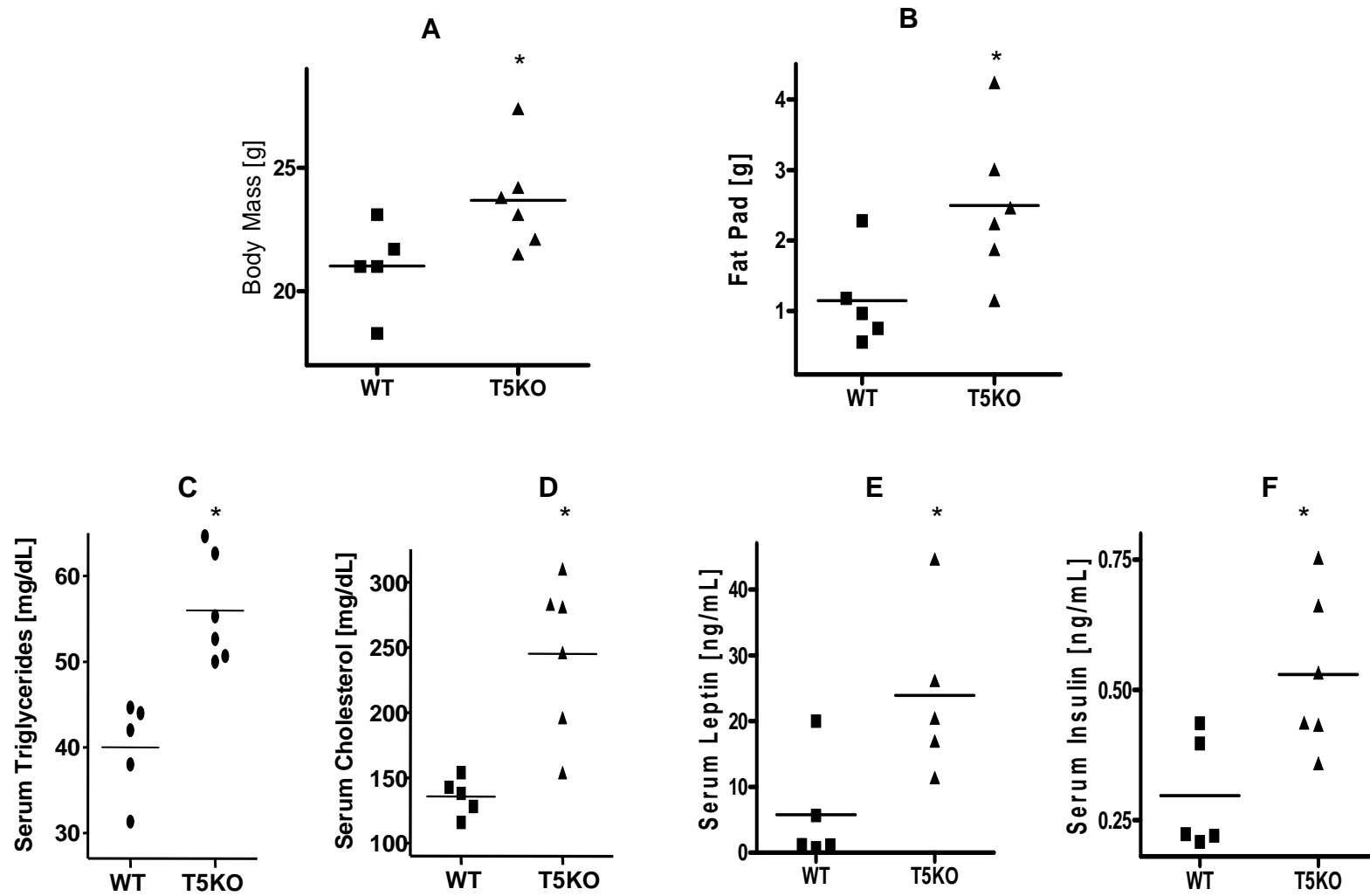


Fig. S4.

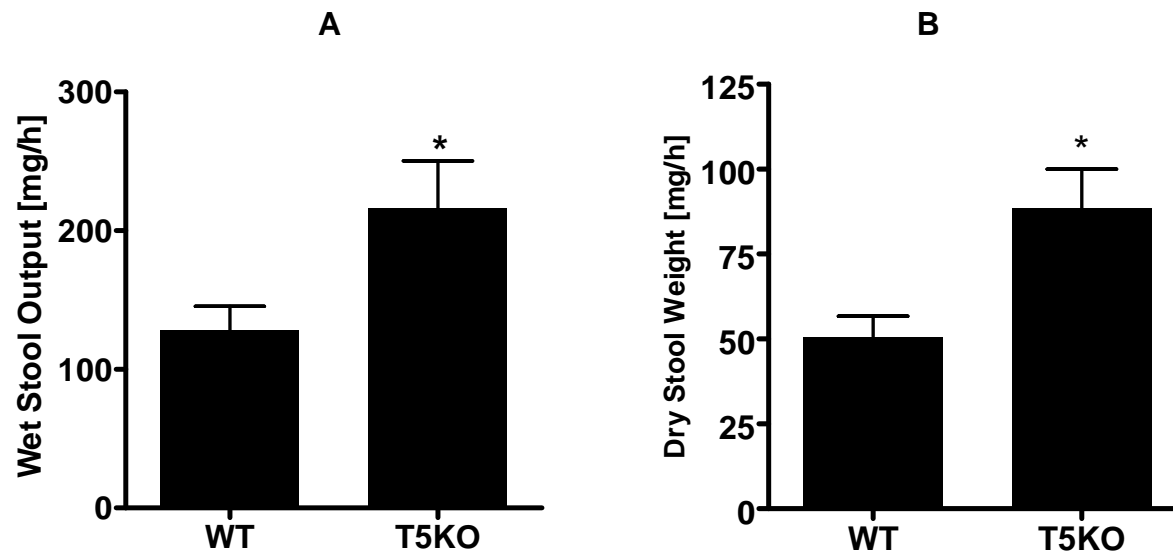


Fig. S5.

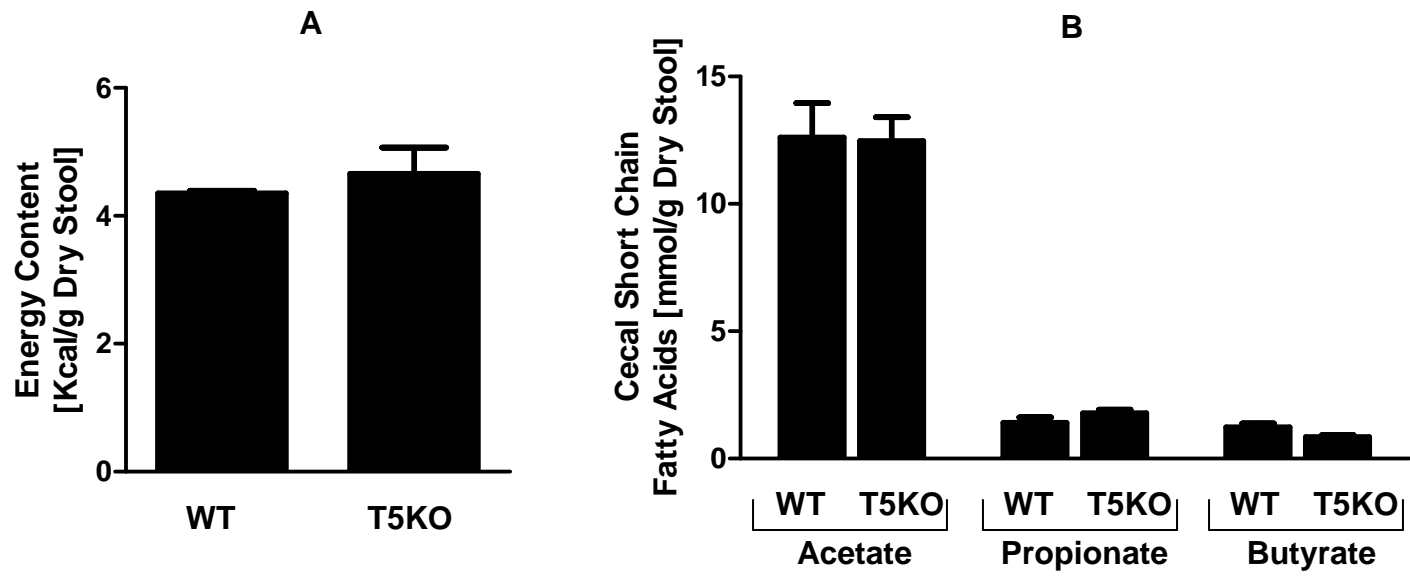


Fig. S6.

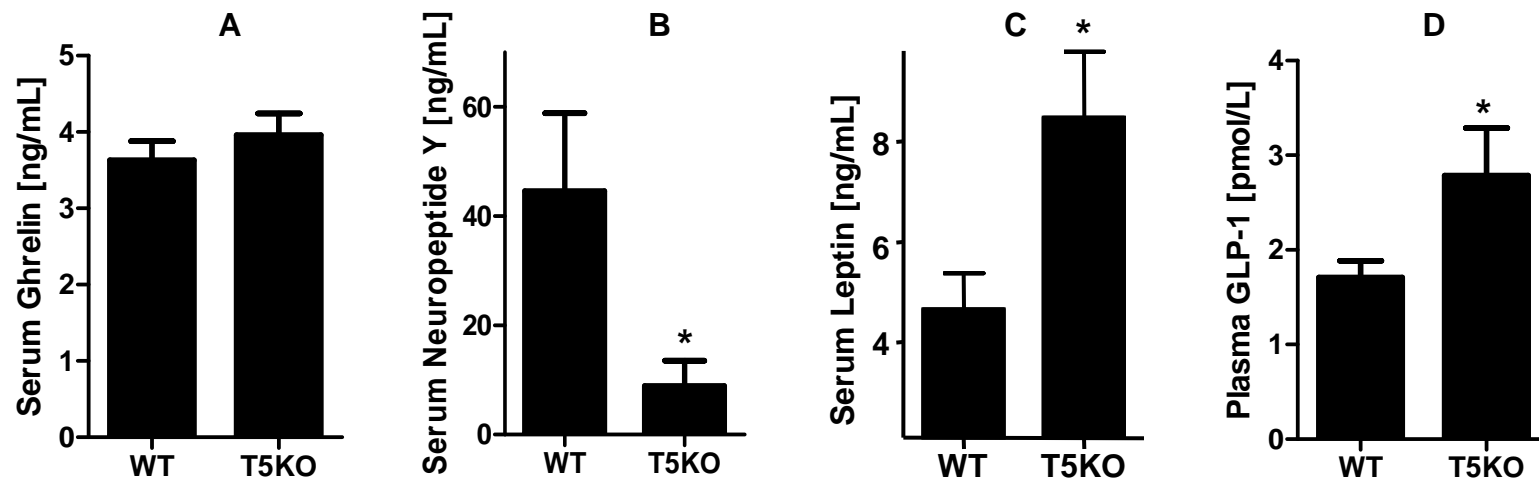


Fig. S7.

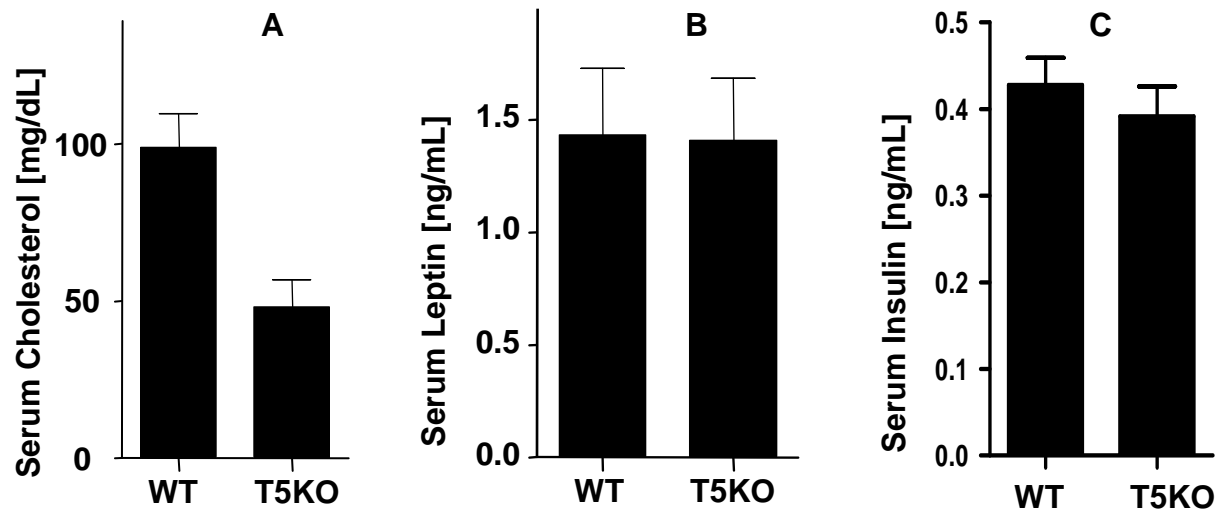


Fig. S8.

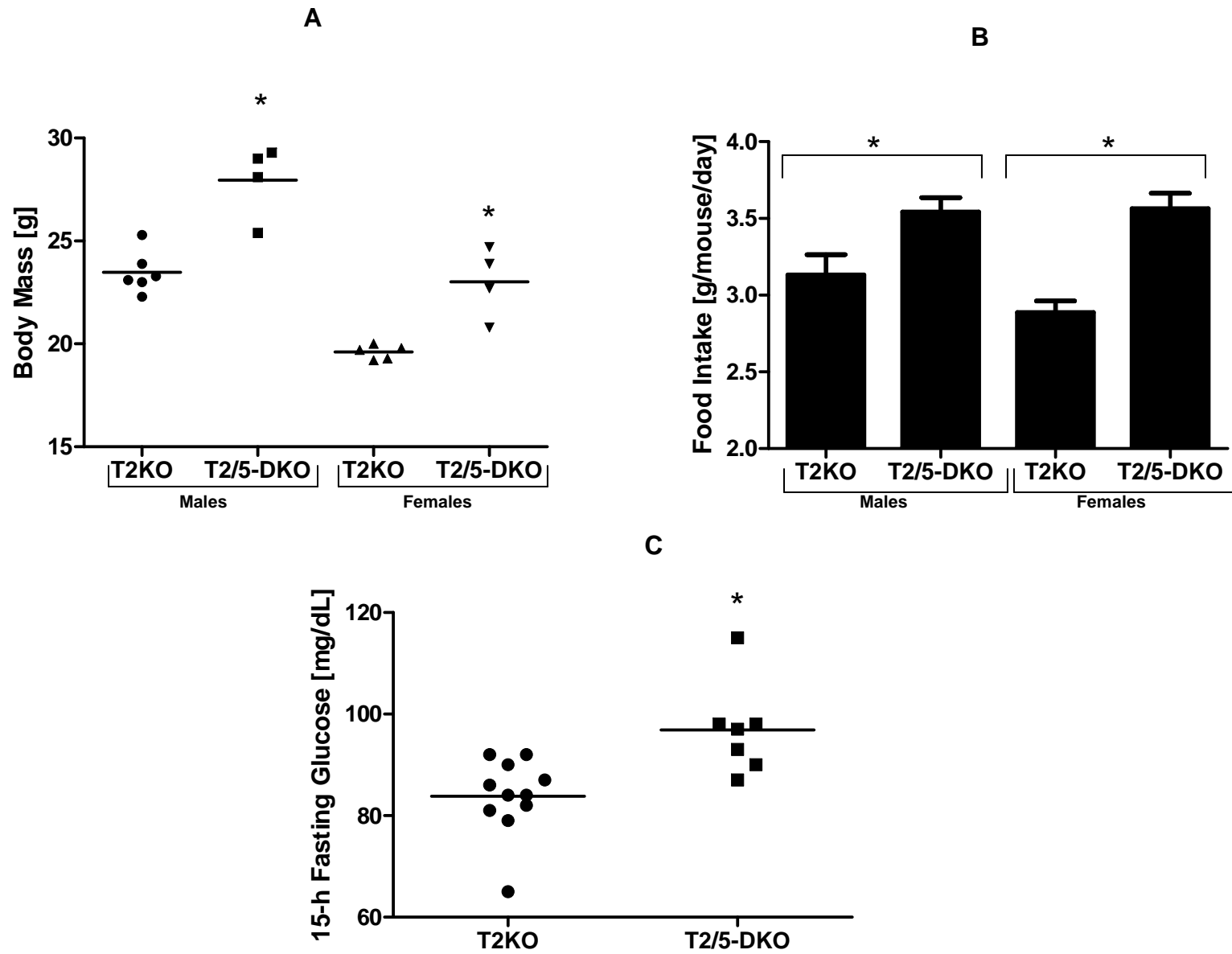


Fig. S9.

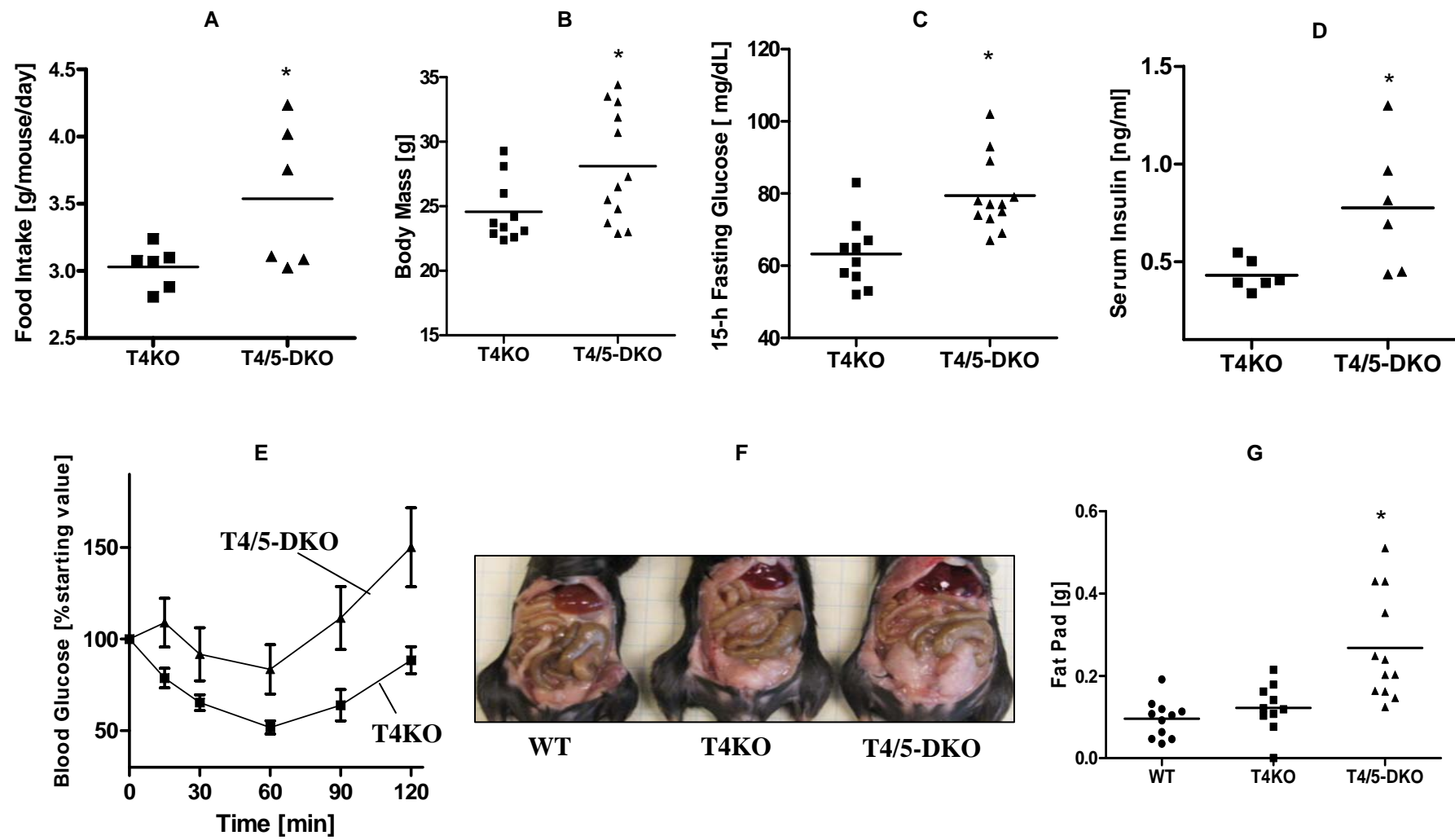


Fig.S10.

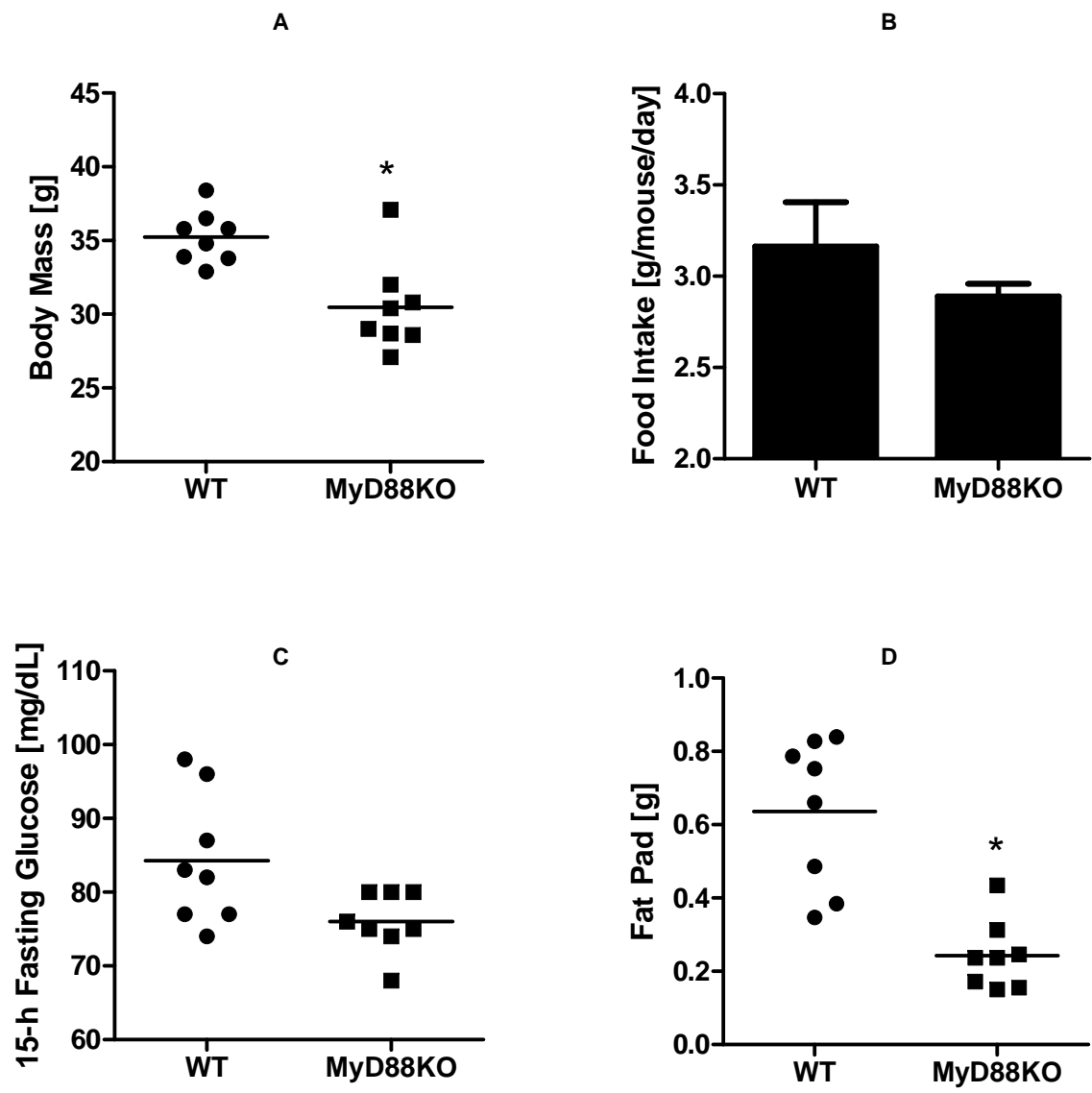


Fig. S11.

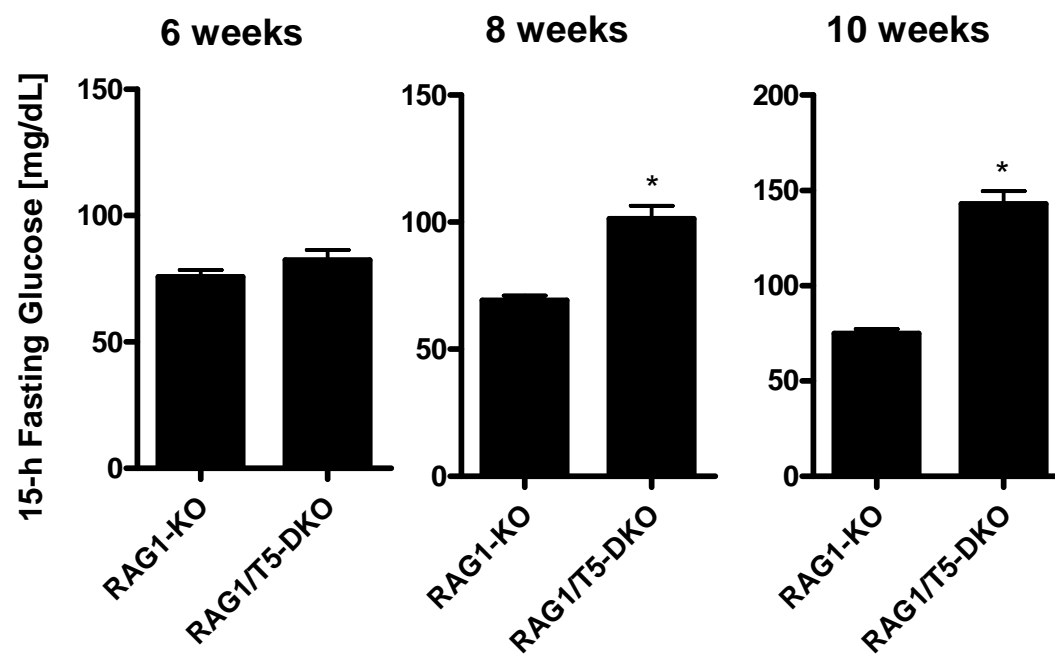


Fig. S12.

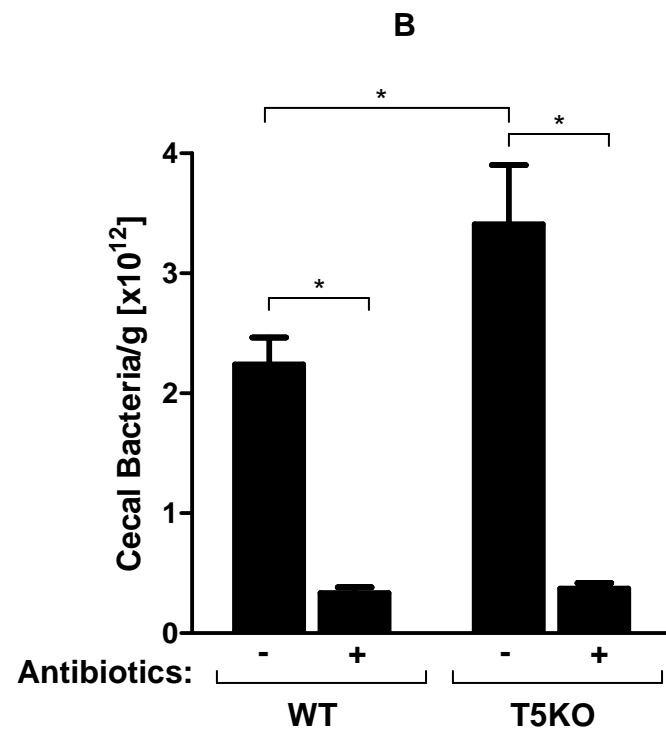
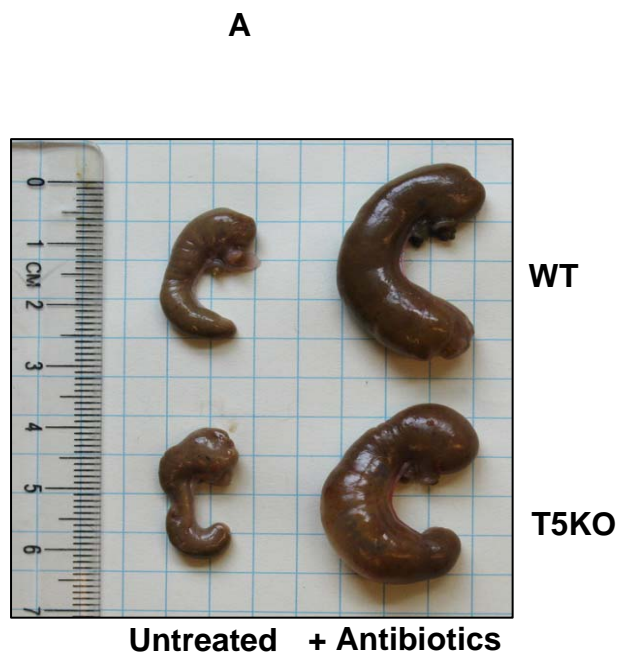


Fig. S13.

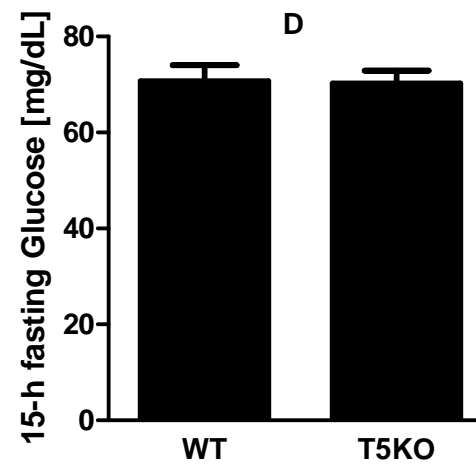
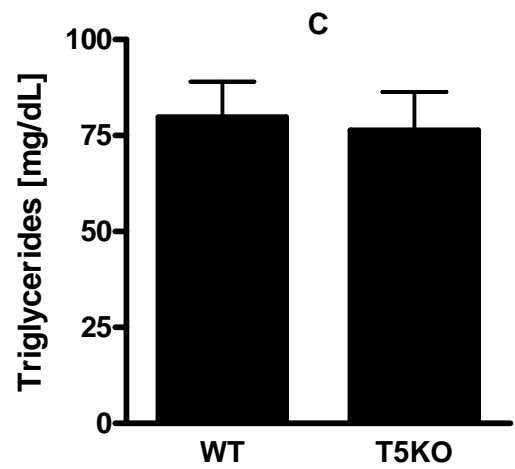
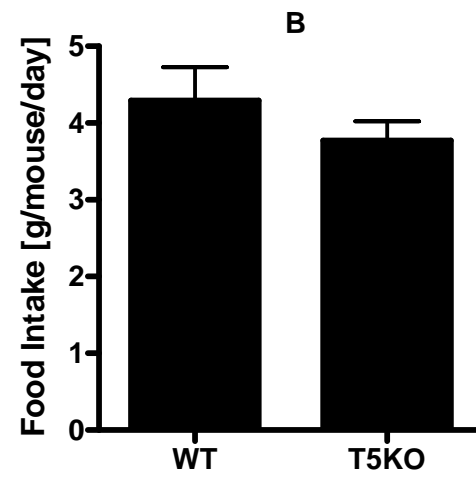
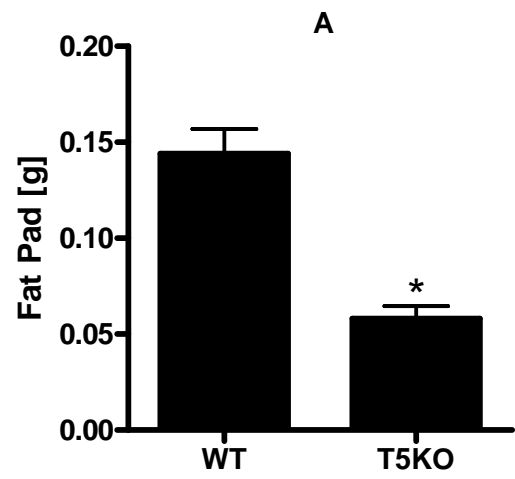


Fig. S14.

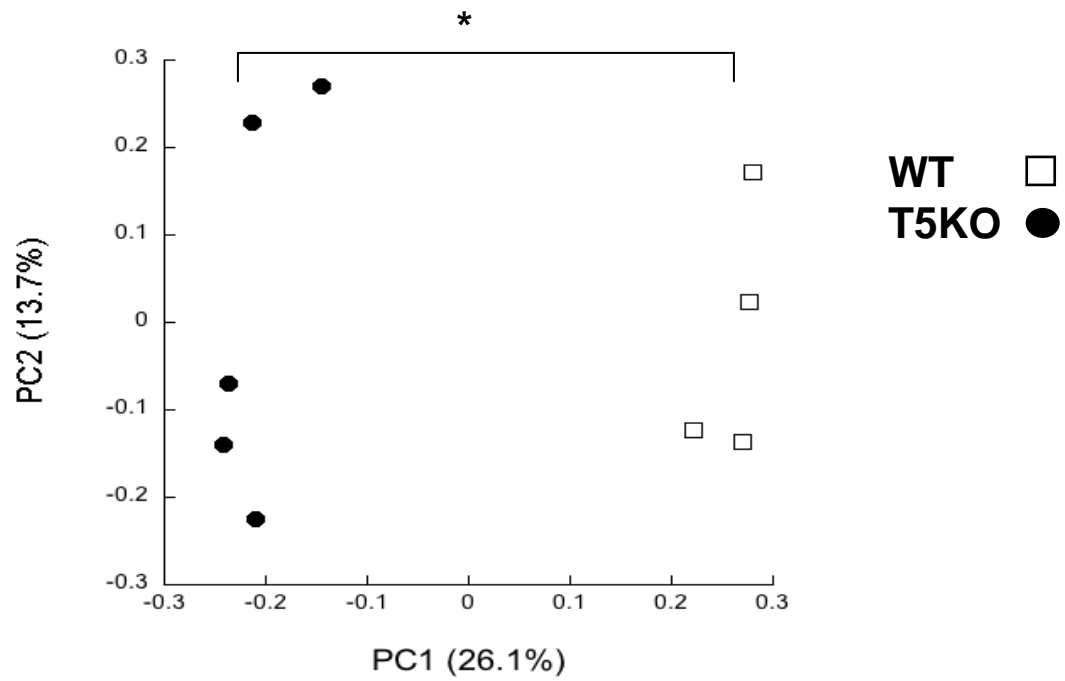


Fig. S15.

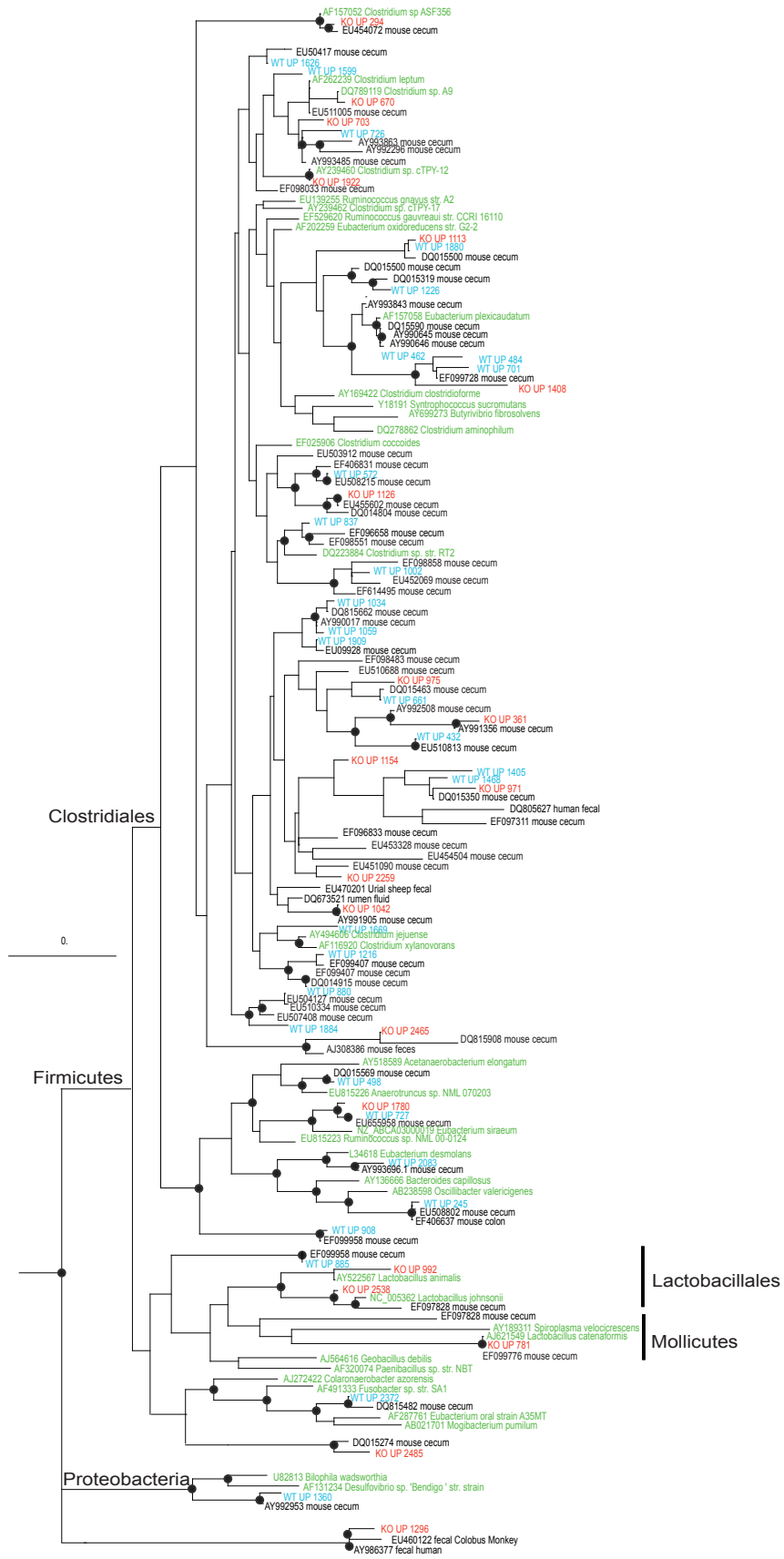


Fig. S16. Altered Firmicutes in T5KO mice.

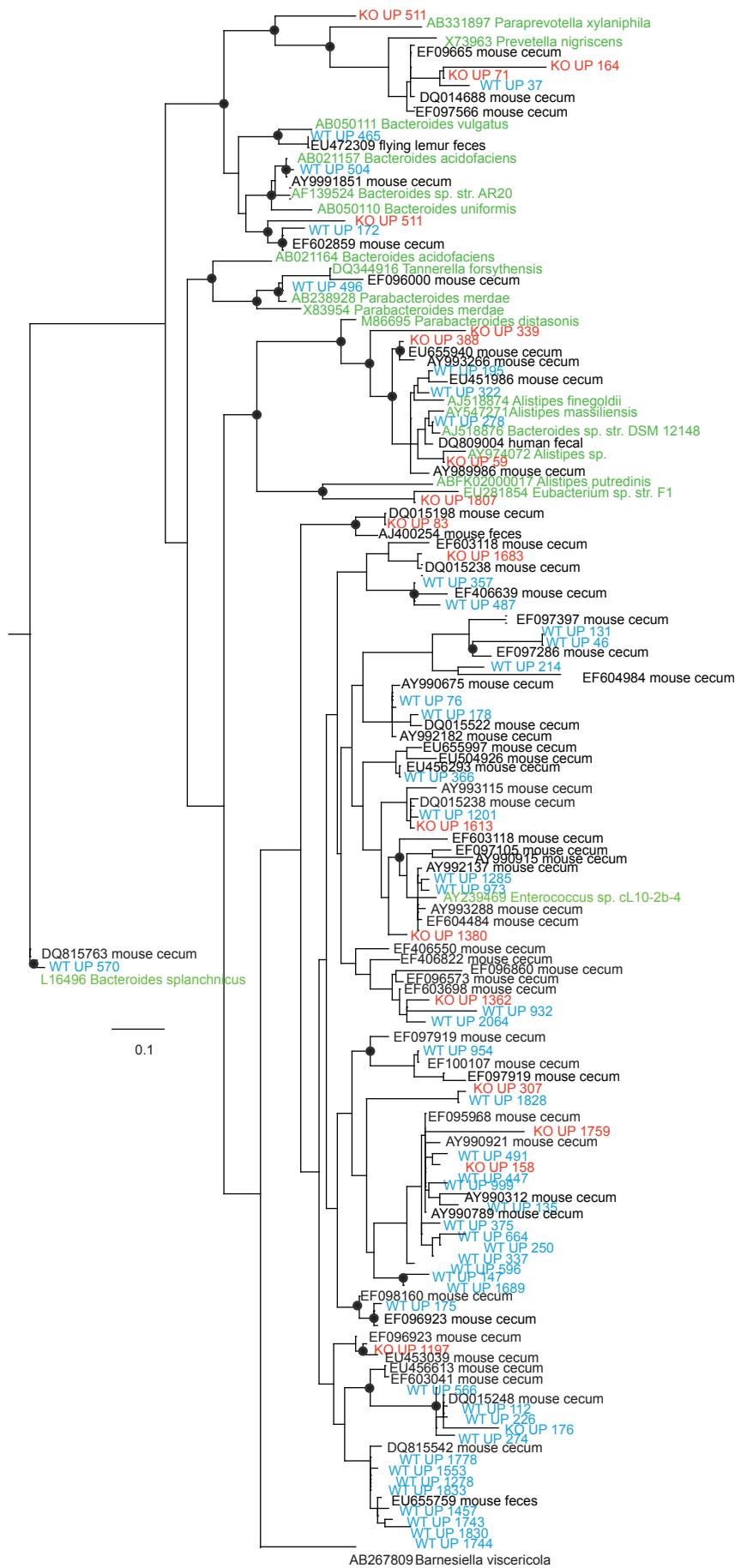


Fig.S17. Altered Bacteroidetes in T5KO mice

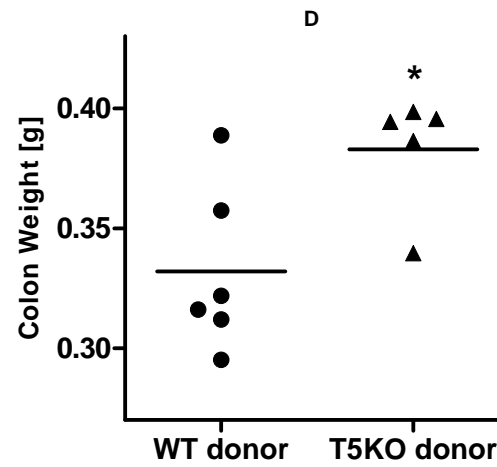
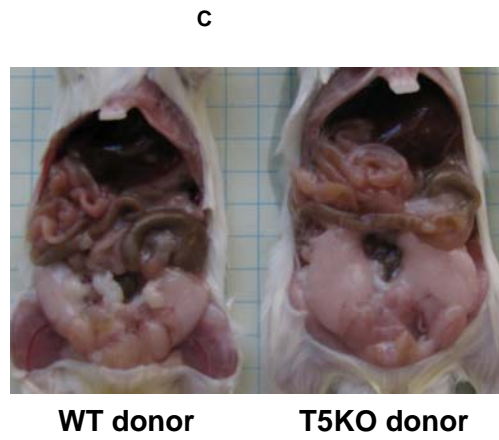
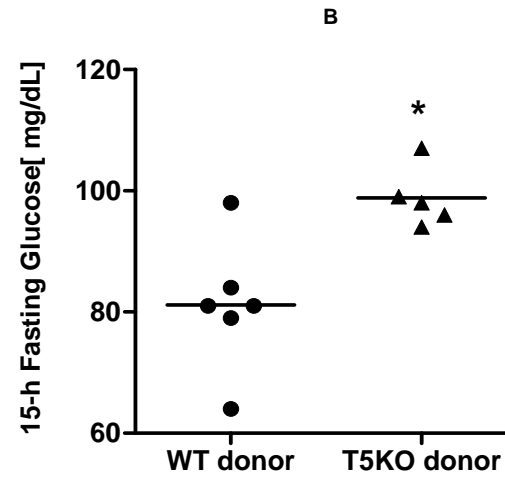
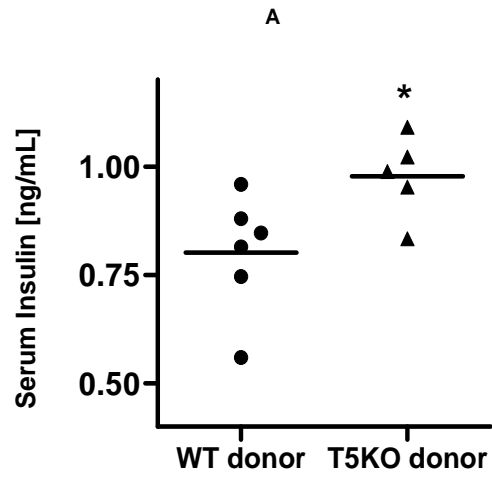


Fig. S18.

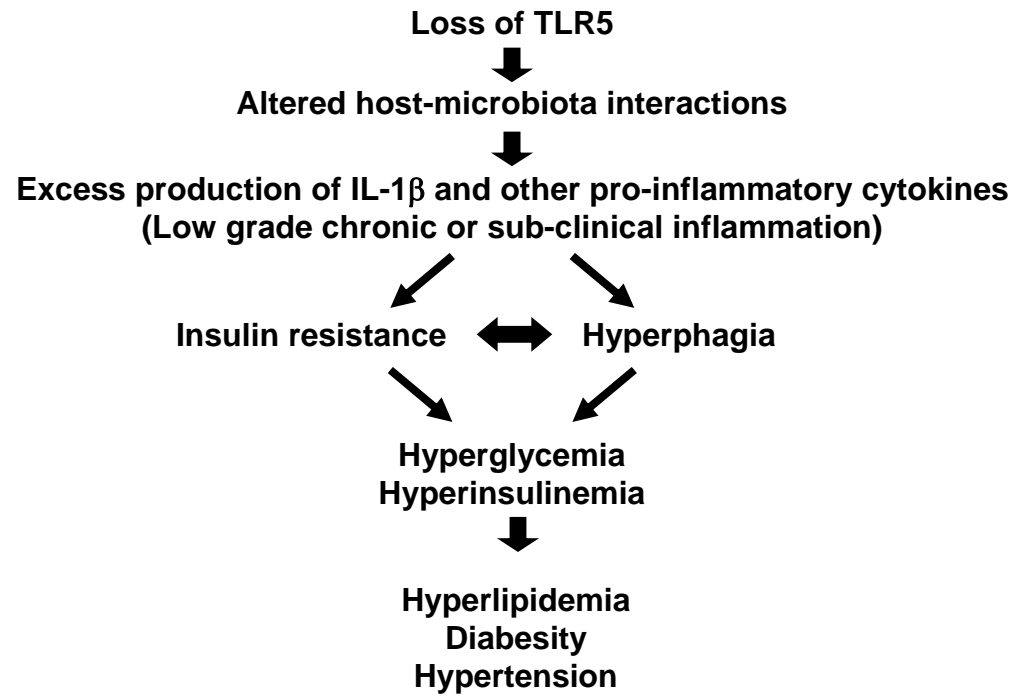


Fig. S19.

Suppl. Table 1: Phylotypes depleted in T5KO versus WT mice.

TLR5-KO	(se)	WT	(se)	p-value	Phylotype	Consensus Lineage
0	0	0.105	0.006	1.3E-07	WT_UP_487	Bacteroidetes
0	0	1.002	0.088	3.6E-06	WT_UP_46	Bacteroidetes
0	0	0.775	0.074	6.9E-06	WT_UP_1278	Bacteroidetes
0	0	0.595	0.074	3.9E-05	WT_UP_1778	Bacteroidetes
0.034	0.017	0.242	0.016	4.6E-05	WT_UP_954	Bacteroidetes
0	0	0.081	0.012	1.4E-04	WT_UP_1689	Bacteroidetes
0	0	0.805	0.123	1.4E-04	WT_UP_1553	Bacteroidetes
0	0	0.286	0.056	6.6E-04	WT_UP_570	Bacteroidetes
0	0	0.186	0.042	1.6E-03	WT_UP_1844	Bacteroidetes
0	0	0.284	0.070	2.4E-03	WT_UP_1201	Bacteroidetes
0.006	0.006	0.093	0.021	3.3E-03	WT_UP_226	Bacteroidetes
0	0	0.086	0.023	3.8E-03	WT_UP_250	Bacteroidetes
0.019	0.019	0.305	0.076	4.8E-03	WT_UP_337	Bacteroidetes
0	0	0.087	0.025	5.0E-03	WT_UP_1468	Bacteroidetes
0	0	0.037	0.012	1.1E-02	WT_UP_178	Bacteroidetes
0	0	0.037	0.012	1.1E-02	WT_UP_999	Bacteroidetes
0	0	0.143	0.049	1.3E-02	WT_UP_1285	Bacteroidetes
0.063	0.047	0.298	0.056	1.4E-02	WT_UP_664	Bacteroidetes
0.009	0.009	0.043	0.006	1.8E-02	WT_UP_112	Bacteroidetes
0	0	0.093	0.035	2.0E-02	WT_UP_375	Bacteroidetes
0.006	0.006	0.100	0.035	2.2E-02	WT_UP_135	Bacteroidetes
0	0	0.050	0.020	2.6E-02	WT_UP_76	Bacteroidetes
0	0	0.050	0.020	2.6E-02	WT_UP_245	Bacteroidetes
0	0	0.050	0.021	2.9E-02	WT_UP_357	Bacteroidetes
0	0	0.086	0.037	3.2E-02	WT_UP_1405	Bacteroidetes
0	0	0.074	0.032	3.2E-02	WT_UP_447	Bacteroidetes
0.065	0.028	0.191	0.040	3.2E-02	WT_UP_2064	Bacteroidetes
0	0	0.204	0.088	3.3E-02	WT_UP_147	Bacteroidetes
0	0	0.125	0.059	4.8E-02	WT_UP_932	Bacteroidetes
0	0	0.390	0.019	8.0E-08	WT_UP_1744	Bacteroidetes; Bacteroidetes
0	0	6.162	0.355	2.1E-07	WT_UP_1833	Bacteroidetes; Bacteroidetes
0	0	1.214	0.127	1.2E-05	WT_UP_596	Bacteroidetes; Bacteroidetes
0	0	0.074	0.015	6.6E-04	WT_UP_973	Bacteroidetes; Bacteroidetes
0	0	0.130	0.028	1.1E-03	WT_UP_1743	Bacteroidetes; Bacteroidetes
0	0	0.118	0.029	2.3E-03	WT_UP_1457	Bacteroidetes; Bacteroidetes
0	0	0.056	0.016	5.7E-03	WT_UP_491	Bacteroidetes; Bacteroidetes
0.055	0.037	0.261	0.043	8.2E-03	WT_UP_175	Bacteroidetes; Bacteroidetes
0	0	0.099	0.035	1.4E-02	WT_UP_1830	Bacteroidetes; Bacteroidetes
0	0	0.062	0.024	2.1E-02	WT_UP_274	Bacteroidetes; Bacteroidetes
0	0	0.050	0.021	2.9E-02	WT_UP_465	Bacteroidetes; Bacteroidetes
0	0	0.074	0.032	3.3E-02	WT_UP_214	Bacteroidetes; Bacteroidetes
0	0	0.050	0.000	6.9E-13	WT_UP_131	Bacteroidetes; Bacteroidetes; Bacteroidales
0	0	0.068	0.019	4.5E-03	WT_UP_366	Bacteroidetes; Bacteroidetes; Bacteroidales
0	0	0.049	0.020	2.7E-02	WT_UP_566	Bacteroidetes; Bacteroidetes; Bacteroidales
0	0	0.197	0.049	2.5E-03	WT_UP_172	Bacteroidetes; Bacteroidetes; Bacteroidales; Bacteroidaceae; Bacteroides
0	0	0.539	0.141	3.4E-03	WT_UP_504	Bacteroidetes; Bacteroidetes; Bacteroidales; Bacteroidaceae; Bacteroides
0	0	0.565	0.072	4.6E-05	WT_UP_496	Bacteroidetes; Bacteroidetes; Bacteroidales; Porphyromonadaceae; Parabacteroides
0	0	2.501	0.452	4.0E-04	WT_UP_1828	Bacteroidetes; Bacteroidetes; Bacteroidales; Porphyromonadaceae; Parabacteroides
0	0	0.253	0.067	3.6E-03	WT_UP_37	Bacteroidetes; Bacteroidetes; Bacteroidales; Prevotellaceae
0	0	0.105	0.029	4.4E-03	WT_UP_322	Bacteroidetes; Bacteroidetes; Bacteroidales; Rikenellaceae; Alistipes
0	0	0.068	0.023	1.3E-02	WT_UP_195	Bacteroidetes; Bacteroidetes; Bacteroidales; Rikenellaceae; Alistipes
0.015	0.009	0.249	0.095	2.7E-02	WT_UP_278	Bacteroidetes; Bacteroidetes; Bacteroidales; Rikenellaceae; Alistipes
0.007	0.007	2.513	0.264	1.3E-05	WT_UP_701	Firmicutes
0.051	0.027	0.346	0.043	5.3E-04	WT_UP_885	Firmicutes
0	0	0.062	0.012	6.1E-04	WT_UP_2372	Firmicutes; Clostridia; Clostridiales
0	0	0.093	0.026	4.8E-03	WT_UP_1880	Firmicutes; Clostridia; Clostridiales
0	0	0.273	0.016	2.0E-07	WT_UP_1002	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae
0.119	0.098	1.868	0.171	3.3E-05	WT_UP_1909	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae
0	0	0.482	0.070	1.1E-04	WT_UP_1599	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae
0	0	0.253	0.044	3.1E-04	WT_UP_1626	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae
0	0	0.161	0.031	5.8E-04	WT_UP_837	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae
0.019	0.012	0.111	0.012	9.5E-04	WT_UP_1669	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae
0.006	0.006	0.254	0.052	1.1E-03	WT_UP_432	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae
0	0	0.160	0.041	3.1E-03	WT_UP_726	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae
0	0	0.205	0.058	5.0E-03	WT_UP_661	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae
0	0	0.056	0.016	5.7E-03	WT_UP_880	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae
0	0	0.729	0.213	5.9E-03	WT_UP_1034	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae
0	0	0.037	0.012	1.1E-02	WT_UP_462	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae
0.016	0.010	0.062	0.012	2.4E-02	WT_UP_572	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae
0.019	0.012	0.094	0.026	2.6E-02	WT_UP_1059	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae
0.073	0.057	0.868	0.319	2.8E-02	WT_UP_1216	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae
0.082	0.026	0.248	0.070	4.4E-02	WT_UP_1226	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae
0	0	0.174	0.015	4.0E-06	WT_UP_727	Firmicutes; Clostridia; Clostridiales; Ruminococcaceae
0	0	0.124	0.032	2.9E-03	WT_UP_2083	Firmicutes; Clostridia; Clostridiales; Ruminococcaceae
0.067	0.030	0.324	0.081	1.4E-02	WT_UP_908	Firmicutes; Clostridia; Clostridiales; Ruminococcaceae
0	0	0.050	0.020	2.6E-02	WT_UP_498	Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; Anaerotruncus
0	0	0.285	0.079	4.6E-03	WT_UP_582	ProteoBetalproteobacteria
0	0	0.074	0.032	3.2E-02	WT_UP_1360	ProteoDeltaproteobacteria
0.015	0.009	0.050	0.000	1.4E-02	WT_UP_484	Unclassified-Screened

Suppl. Table 2: Phylotypes enriched in T5KO versus WT mice.

TLR5-KO	(se)	WT	(se)	p-value	Phylotype	Consensus Lineage
0.732	0.176	0	0	8.0E-03	KO_UP_1362	Bacteroidetes
0.189	0.051	0	0	1.4E-02	KO_UP_1807	Bacteroidetes
0.032	0.009	0	0	1.4E-02	KO_UP_307	Bacteroidetes
1.563	0.465	0	0	2.1E-02	KO_UP_1683	Bacteroidetes
0.051	0.015	0	0	2.1E-02	KO_UP_971	Bacteroidetes
0.053	0.018	0	0	3.5E-02	KO_UP_1759	Bacteroidetes
1.172	0.279	0.390	0.033	4.3E-02	KO_UP_1613	Bacteroidetes
0.079	0.028	0	0	4.3E-02	KO_UP_388	Bacteroidetes
0.350	0.108	0.050	0.020	4.5E-02	KO_UP_158	Bacteroidetes
0.278	0.034	0	0	1.8E-04	KO_UP_1380	Bacteroidetes; Bacteroidetes
0.098	0.018	0.012	0.012	7.0E-03	KO_UP_176	Bacteroidetes; Bacteroidetes
0.044	0.016	0	0	4.3E-02	KO_UP_83	Bacteroidetes; Bacteroidetes
0.973	0.176	0.198	0.034	6.3E-03	KO_UP_1197	Bacteroidetes; Bacteroidetes; Bacteroidales
0.189	0.066	0	0	3.8E-02	KO_UP_511	Bacteroidetes; Bacteroidetes; Bacteroidales; Bacteroidaceae; Bacteroides
0.040	0.014	0	0	4.1E-02	KO_UP_164	Bacteroidetes; Bacteroidetes; Bacteroidales; Prevotellaceae
0.040	0.015	0	0	4.6E-02	KO_UP_71	Bacteroidetes; Bacteroidetes; Bacteroidales; Prevotellaceae
0.071	0.025	0	0	3.9E-02	KO_UP_339	Bacteroidetes; Bacteroidetes; Bacteroidales; Rikenellaceae
0.032	0.009	0	0	1.4E-02	KO_UP_59	Bacteroidetes; Bacteroidetes; Bacteroidales; Rikenellaceae; Alistipes
0.775	0.230	0	0	2.1E-02	KO_UP_2465	Firmicutes
0.120	0.034	0.012	0.012	3.2E-02	KO_UP_1408	Firmicutes
0.072	0.019	0.012	0.012	4.4E-02	KO_UP_781	Firmicutes
0.049	0.017	0	0	4.3E-02	KO_UP_992	Firmicutes; Bacilli; Lactobacillales
0.043	0.014	0	0	3.1E-02	KO_UP_2538	Firmicutes; Bacilli; Lactobacillales; Lactobacillaceae; Lactobacillus
5.933	1.703	0	0	1.8E-02	KO_UP_294	Firmicutes; Clostridia
0.059	0.016	0	0	1.6E-02	KO_UP_361	Firmicutes; Clostridia; Clostridiales
0.089	0.021	0	0	6.9E-03	KO_UP_1126	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae
0.083	0.023	0	0	1.5E-02	KO_UP_1922	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae
2.856	0.849	0	0	2.1E-02	KO_UP_670	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae
0.804	0.244	0	0	2.3E-02	KO_UP_1042	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae
0.064	0.023	0	0	4.0E-02	KO_UP_2259	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae
1.319	0.470	0	0	4.3E-02	KO_UP_703	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae
0.098	0.036	0	0	4.9E-02	KO_UP_975	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae
0.384	0.073	0.086	0.042	1.3E-02	KO_UP_1113	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; LachnospiraceaeIncertaeSedis
0.202	0.042	0	0	3.7E-03	KO_UP_1780	Firmicutes; Clostridia; Clostridiales; Ruminococcaceae
0.939	0.216	0	0	6.4E-03	KO_UP_1296	Proteobacteria
2.274	0.464	0	0	3.5E-03	KO_UP_1154	Unclassified-Screened
0.656	0.153	0.205	0.043	3.9E-02	KO_UP_2485	Unclassified-Screened

Suppl. Table 3: Closest matches between phylotypes obtained in this study and phylotypes obtained from studies of human gut microbiotas (upper table) and mouse gut microbiotas (lower table)

Best BLAST to sequences derived from humans

Phylotype	%ID	Greengenes ID	Description [Genbank accession number]
KO_UP_1042	94	213401	Selective colonization insoluble human faecal faeces clone D1B-6.5-48h-6 [AM237879.1]
KO_UP_1113	97	179742	Microbial ecology: gut microbes obesity human feces; subj02; 52 weeks; fat-r diet clone RL305aal86d02 [DQ799444.1]
KO_UP_1126	92	190961	Microbial ecology: gut microbes obesity human feces; subj10; 12 weeks; carb-r diet clone RL247_aaj22e07 [DQ799971.1]
KO_UP_1154	90	145377	Metagenomic gut microbiome healthy human stool clone EA96 [DQ327520.1]
KO_UP_1197	97	119549	Human stool clone D016 [AY985773.1]
KO_UP_1296	97	121353	Human stool clone D164 [AY985884.1]
KO_UP_1362	91	146438	Reduced Firmicutes intestinal Crohn's disease human intestine clone Cadhufec26ml [AY850513.1]
KO_UP_1380	88	174331	Microbial ecology: gut microbes obesity human feces; subj12; 52 weeks; carb-r diet clone RL304_aal74f01 [DQ824331.1]
KO_UP_1408	100	184905	Microbial ecology: gut microbes obesity human feces; subj07; 12 weeks; carb-r diet clone RL246_aai73f04 [DQ793567.1]
KO_UP_158	94	146438	Reduced Firmicutes intestinal Crohn's disease human intestine clone Cadhufec26ml [AY850513.1]
KO_UP_1613	97	146438	Reduced Firmicutes intestinal Crohn's disease human intestine clone Cadhufec26ml [AY850513.1]
KO_UP_164	94	186661	Microbial ecology: gut microbes obesity human feces; subj08; 12 weeks; carb-r diet clone RL245_aai83a09 [DQ795828.1]
KO_UP_1683	97	173237	Effect bowel preparation and colonoscopy on post-procedure intestinal microbiota Homo sapiens fecal clone D13-a3 [DQ905194.1]
KO_UP_1759	88	174331	Microbial ecology: gut microbes obesity human feces; subj12; 52 weeks; carb-r diet clone RL304_aal74f01 [DQ824331.1]
KO_UP_176	95	119549	Human stool clone D016 [AY985773.1]
KO_UP_1780	94	148925	Human biopsies ulcerative colitis biopsy clone UC7-117 [AJ608247.1]
KO_UP_1807	93	194238	Between Tissue- Intestinal Microfloras Patients Crohn's Disease and Ulcerative Colitis human intestinal biopsies clone CD18 [DQ441269.1]
KO_UP_1922	94	117065	Human stool clone B134 [AY984243.1]
KO_UP_2259	93	182165	Microbial ecology: gut microbes obesity human feces; subj02; 52 weeks; fat-r diet clone RL305aal86e04 [DQ799456.1]
KO_UP_2465	92	190003	Microbial ecology: gut microbes obesity human feces; subj08; 26 weeks; carb-r diet clone RL202_aai50e10 [DQ796036.1]
KO_UP_2485	95	114444	Human ascending colon biopsy clone LW88 [AY916186.1]
KO_UP_2538	97	132527	Microbes on human vaginal epithelium clone rRNA121 [AY958894.1]
KO_UP_294	92	192517	Between Tissue- Intestinal Microfloras Patients Crohn's Disease and Ulcerative Colitis human intestinal biopsies clone CD104 [DQ441355.1]
KO_UP_307	96	146438	Reduced Firmicutes intestinal Crohn's disease human intestine clone Cadhufec26ml [AY850513.1]
KO_UP_339	89	112720	Human transverse colon biopsy clone NH37 [AY916174.1]
KO_UP_361	96	189690	Microbial ecology: gut microbes obesity human feces; subj05; 0 weeks; fat-r diet clone RL180_aah38f04 [DQ807896.1]
KO_UP_388	90	198374	Microbial ecology: gut microbes obesity human feces; subj14; 52 weeks; lean control clone RL388_aao94a06 [DQ801324.1]
KO_UP_511	96	47157	Bacteroides uniformis str. JCM 5828T [AB050110.1]
KO_UP_59	94	194627	Microbial ecology: gut microbes obesity human feces; subj06; 12 weeks; fat-r diet clone RL243_aai88a12 [DQ809004.1]
KO_UP_670	98	187212	Microbial ecology: gut microbes obesity human feces; subj02; 12 weeks; fat-r diet clone RL242_aak00d07 [DQ799014.1]
KO_UP_703	96	180842	Microbial ecology: gut microbes obesity human feces; subj03; 12 weeks; fat-r diet clone RL251_aaj86e06 [DQ803493.1]
KO_UP_71	95	92223	Human oral cavity clone GI030 [AY349395.1]
KO_UP_781	93	150015	Metagenomic gut microbiome healthy human stool clone B551 [DQ325818.1]
KO_UP_83	95	117284	Human stool clone C482 [AY985514.1]
KO_UP_971	97	113069	Human stool clone D761 [AY916386.1]
KO_UP_975	88	13997	Ruminococcus hydrogenotrophicus str. S5a36 [X95624.1]
KO_UP_992	94	149197	Metagenomic gut microbiome healthy human stool clone E756 [DQ327224.1]
WT_UP_1002	92	118072	Human ascending colon mucosal biopsy clone LT31 [AY975998.1]
WT_UP_1034	95	181763	Microbial ecology: gut microbes obesity human feces; subj03; 0 weeks; fat-r diet clone RL187_aah68b02 [DQ804070.1]
WT_UP_1059	94	181706	Microbial ecology: gut microbes obesity human feces; subj03; 0 weeks; fat-r diet clone RL187_aah69a12 [DQ804131.1]
WT_UP_112	96	119549	Human stool clone D016 [AY985773.1]
WT_UP_1201	97	146438	Reduced Firmicutes intestinal Crohn's disease human intestine clone Cadhufec26ml [AY850513.1]
WT_UP_1216	93	199637	Microbial ecology: gut microbes obesity human feces; subj06; 0 weeks; fat-r diet clone RL184_aan81f06 [DQ809619.1]
WT_UP_1226	91	149210	Metagenomic gut microbiome healthy human stool clone E565 [DQ327057.1]
WT_UP_1278	97	119549	Human stool clone D016 [AY985773.1]
WT_UP_1285	98	146438	Reduced Firmicutes intestinal Crohn's disease human intestine clone Cadhufec26ml [AY850513.1]
WT_UP_131	95	117284	Human stool clone C482 [AY985514.1]
WT_UP_135	95	146438	Reduced Firmicutes intestinal Crohn's disease human intestine clone Cadhufec26ml [AY850513.1]
WT_UP_1360	93	172513	Effect bowel preparation and colonoscopy on post-procedure intestinal microbiota Homo sapiens fecal clone 002-e12 [DQ904964.1]
WT_UP_1405	98	113069	Human stool clone D761 [AY916386.1]
WT_UP_1457	93	146438	Reduced Firmicutes intestinal Crohn's disease human intestine clone Cadhufec26ml [AY850513.1]
WT_UP_1468	87	146438	Reduced Firmicutes intestinal Crohn's disease human intestine clone Cadhufec26ml [AY850513.1]
WT_UP_147	93	119549	Human stool clone D016 [AY985773.1]
WT_UP_1553	94	146438	Reduced Firmicutes intestinal Crohn's disease human intestine clone Cadhufec26ml [AY850513.1]
WT_UP_1599	96	177738	Microbial ecology: gut microbes obesity human feces; subj02; 26 weeks; fat-r diet clone RL205_aaj19g03 [DQ799251.1]
WT_UP_1626	95	121393	Human ascending colon mucosal biopsy clone KN24 [AY975195.1]
WT_UP_1669	92	203854	Symbiotic gut microbes modulate metabolic phenotypes human fecal subject MO clone SJTU_G_02_14 [EF405321.1]
WT_UP_1689	90	209529	Gastrointestinal tract microbiota microarray reference pool created pooling products obtained human adult infant stool breast milk and vaginal swabs mothers clone 1099945077996 [EF434334.1]
WT_UP_172	91	116794	Human stool clone C086 [AY985222.1]
WT_UP_1743	93	146438	Reduced Firmicutes intestinal Crohn's disease human intestine clone Cadhufec26ml [AY850513.1]
WT_UP_1744	94	146438	Reduced Firmicutes intestinal Crohn's disease human intestine clone Cadhufec26ml [AY850513.1]
WT_UP_175	94	175793	Microbial ecology: gut microbes obesity human feces; subj12; 52 weeks; carb-r diet clone RL304_aal75d12 [DQ824388.1]

WT_UP_1778	96	146438	Reduced Firmicutes intestinal Crohn's disease human intestine clone Cadhufec26ml [AY850513.1]
WT_UP_178	91	119549	Human stool clone D016 [AY985773.1]
WT_UP_1828	99	123030	Human ascending colon mucosal biopsy clone LS52 [AY975943.1]
WT_UP_1830	94	146438	Reduced Firmicutes intestinal Crohn's disease human intestine clone Cadhufec26ml [AY850513.1]
WT_UP_1833	95	121050	Human stool clone D372 [AY986052.1]
WT_UP_1880	87	174821	Microbial ecology: gut microbes obesity human feces; subj12; 26 weeks; carb-r diet clone RL204_aaj60g07 [DQ824201.1]
WT_UP_1884	94	194697	Microbial ecology: gut microbes obesity human feces; subj02; 0 weeks; fat-r diet clone RL182_aah33h06 [DQ799802.1]
WT_UP_1909	95	187143	Microbial ecology: gut microbes obesity human feces; subj03; 0 weeks; fat-r diet clone RL187_aah68c12 [DQ804088.1]
WT_UP_195	93	112720	Human transverse colon biopsy clone NH37 [AY916174.1]
WT_UP_2064	91	146438	Reduced Firmicutes intestinal Crohn's disease human intestine clone Cadhufec26ml [AY850513.1]
WT_UP_2083	91	173815	Microbial ecology: gut microbes obesity human feces; subj03; 26 weeks; fat-r diet clone RL200_aai60f02 [DQ803975.1]
WT_UP_214	95	117284	Human stool clone C482 [AY985514.1]
WT_UP_226	95	119549	Human stool clone D016 [AY985773.1]
WT_UP_2372	92	43339	Human subgingival plaque clone CK047 [AF287762.1]
WT_UP_245	94	209622	Human infant gastrointestinal tract microbiota stool mother babies 13 and 14 7 months after birth clone C244 [EF434362.1]
WT_UP_250	98	146438	Reduced Firmicutes intestinal Crohn's disease human intestine clone Cadhufec26ml [AY850513.1]
WT_UP_274	95	119549	Human stool clone D016 [AY985773.1]
WT_UP_278	100	213671	Symbiotic gut microbes modulate metabolic phenotypes human fecal subject GF clone SJTU_B_10_51 [EF402636.1]
WT_UP_322	94	179296	Microbial ecology: gut microbes obesity human feces; subj12; 26 weeks; carb-r diet clone RL204_aaj57f11 [DQ823978.1]
WT_UP_337	88	174331	Microbial ecology: gut microbes obesity human feces; subj12; 52 weeks; carb-r diet clone RL304_aal74f01 [DQ824331.1]
WT_UP_357	96	209529	Gastrointestinal tract microbiota microarray reference pool created pooling products obtained human adult infant stool breast milk and vaginal swabs mothers clone 1099945077996 [EF434334.1]
WT_UP_366	96	146438	Reduced Firmicutes intestinal Crohn's disease human intestine clone Cadhufec26ml [AY850513.1]
WT_UP_37	96	92223	Human oral cavity clone GI030 [AY349395.1]
WT_UP_375	88	174331	Microbial ecology: gut microbes obesity human feces; subj12; 52 weeks; carb-r diet clone RL304_aal74f01 [DQ824331.1]
WT_UP_432	96	146579	Metagenomic gut microbiome healthy human stool clone E010 [DQ326577.1]
WT_UP_447	87	174331	Microbial ecology: gut microbes obesity human feces; subj12; 52 weeks; carb-r diet clone RL304_aal74f01 [DQ824331.1]
WT_UP_46	95	117284	Human stool clone C482 [AY985514.1]
WT_UP_462	97	184729	Microbial ecology: gut microbes obesity human feces; subj03; 0 weeks; fat-r diet clone RL187_aah69a08 [DQ804129.1]
WT_UP_465	89	178803	Microbial ecology: gut microbes obesity human feces; subj12; 26 weeks; carb-r diet clone RL204_aaj58d12 [DQ824036.1]
WT_UP_484	100	116804	Human ascending colon mucosal biopsy clone KH22 [AY974867.1]
WT_UP_487	93	173773	Effect bowel preparation and colonoscopy on post-procedure intestinal microbiota Homo sapiens fecal clone 013-b11 [DQ905202.1]
WT_UP_491	88	174331	Microbial ecology: gut microbes obesity human feces; subj12; 52 weeks; carb-r diet clone RL304_aal74f01 [DQ824331.1]
WT_UP_496	95	116688	Human rectum mucosal biopsy clone LL78 [AY980673.1]
WT_UP_498	89	118971	Human stool clone B722 [AY984761.1]
WT_UP_504	97	47157	Bacteroides uniformis str. JCM 5828T [AB050110.1]
WT_UP_566	98	119549	Human stool clone D016 [AY985773.1]
WT_UP_570	91	203997	Symbiotic gut microbes modulate metabolic phenotypes human fecal subject GGM clone SJTU_D_13_11 [EF401651.1]
WT_UP_572	93	182700	Microbial ecology: gut microbes obesity human feces; subj02; 12 weeks; fat-r diet clone RL242_aaj99b09 [DQ798925.1]
WT_UP_596	88	174331	Microbial ecology: gut microbes obesity human feces; subj12; 52 weeks; carb-r diet clone RL304_aal74f01 [DQ824331.1]
WT_UP_661	94	117935	Human stool clone B293 [AY984391.1]
WT_UP_664	88	174331	Microbial ecology: gut microbes obesity human feces; subj12; 52 weeks; carb-r diet clone RL304_aal74f01 [DQ824331.1]
WT_UP_701	96	184905	Microbial ecology: gut microbes obesity human feces; subj07; 12 weeks; carb-r diet clone RL246_aai73f04 [DQ793567.1]
WT_UP_726	94	119218	Human cecum mucosal biopsy clone MV44 [AY977329.1]
WT_UP_727	93	148925	Human biopsies ulcerative colitis biopsy clone UC7-117 [AJ608247.1]
WT_UP_76	91	119549	Human stool clone D016 [AY985773.1]
WT_UP_837	94	208029	Symbiotic gut microbes modulate metabolic phenotypes human fecal subject BB clone SJTU_F_13_20 [EF399598.1]
WT_UP_880	93	119715	Human transverse colon mucosal biopsy clone Z170 [AY979143.1]
WT_UP_885	97	211800	Symbiotic gut microbes modulate metabolic phenotypes human fecal subject BB clone SJTU_F_03_88 [EF398446.1]
WT_UP_908	92	205567	Human infant gastrointestinal tract microbiota stool baby 14 7 months old clone 1099973758462 [EF434338.1]
WT_UP_932	93	146438	Reduced Firmicutes intestinal Crohn's disease human intestine clone Cadhufec26ml [AY850513.1]
WT_UP_954	94	173954	Microbial ecology: gut microbes obesity human feces; subj12; 26 weeks; carb-r diet clone RL204_aaj58d01 [DQ824028.1]
WT_UP_973	87	146438	Reduced Firmicutes intestinal Crohn's disease human intestine clone Cadhufec26ml [AY850513.1]
WT_UP_999	89	174331	Microbial ecology: gut microbes obesity human feces; subj12; 52 weeks; carb-r diet clone RL304_aal74f01 [DQ824331.1]

Average 93.6

Best BLAST to sequences derived from mice

Phylotype	%ID	Greengenes ID	Description [Genbank accession number]
KO_UP_1042	100	130142	Mouse cecum clone C20_f02 [AY991798.1]
KO_UP_1113	96	132416	Mouse cecum clone C9_L04 [AY990737.1]
KO_UP_1126	99	133604	Mouse cecum clone C15_L12 [AY993887.1]
KO_UP_1154	100	167204	Mouse cecum clone (clone) [DQ815411.1]
KO_UP_1197	96	131630	Mouse cecum clone C12_O02 [AY990138.1]
KO_UP_1296	87	149308	Anaplasma phagocytophilum str. Jilin-1 [DQ342324.1]
KO_UP_1362	98	131677	Mouse cecum clone C9_A08 [AY990629.1]
KO_UP_1380	98	129314	Mouse cecum clone C13_B16 [AY991253.1]
KO_UP_1408	98	206456	Pathogen-induced host response provides competitive advantage enteropathogens over intestinal microbiota mouse colon clone infected_28days-C8 [EF406822.1]
KO_UP_158	96	129725	Mouse cecum clone M3_f08 [DQ015402.1]
KO_UP_1613	99	133103	Mouse cecum clone M2_f09_1 [DQ015238.1]

KO_UP_164	99	179810	Obesity- gut microbiome increased capacity energy harvest cecal contents Mus musculus strain C57BL/6J; lean_recipient leptin genotype +/+ clone SWPT20_aaa03e05 [EF097770.1]
KO_UP_1683	97	196733	Obesity- gut microbiome increased capacity energy harvest cecal contents Mus musculus strain C57BL/6J; obob_recipient leptin genotype +/+ clone SWPT2_aaa03c02 [EF099162.1]
KO_UP_1759	99	129725	Mouse cecum clone M3_f08 [DQ015402.1]
KO_UP_176	99	129838	Mouse cecum clone M2_h02_3 [DQ015248.1]
KO_UP_1780	95	166612	Mouse cecum clone (clone) [DQ815739.1]
KO_UP_1807	99	130249	Mouse cecum clone C21_c14 [AY993071.1]
KO_UP_1922	98	132741	Mouse cecum clone M2_g02_2 [DQ015189.1]
KO_UP_2259	97	131637	Mouse cecum clone M3_h08 [DQ015424.1]
KO_UP_2465	99	161771	Mouse cecum clone (clone) [DQ815908.1]
KO_UP_2485	96	132833	Mouse cecum clone C23_n17 [AY992664.1]
KO_UP_2538	97	129387	Mouse cecum clone C12_I15 [AY989956.1]
KO_UP_294	98	129672	Mouse cecum clone C19_P22 [AY993606.1]
KO_UP_307	99	174375	Obesity- gut microbiome increased capacity energy harvest cecal contents Mus musculus strain C57BL/6J; obob_recipient leptin genotype +/+ clone SWPT3_aaa01d08 [EF099304.1]
KO_UP_339	95	162025	Mouse cecum clone (clone) [DQ815696.1]
KO_UP_361	99	131705	Mouse cecum clone C23_g16 [AY992502.1]
KO_UP_388	98	129437	Mouse cecum clone C21_I17 [AY993266.1]
KO_UP_511	97	130153	Mouse cecum clone C20_h11 [AY991851.1]
KO_UP_59	99	162245	Mouse cecum clone (clone) [DQ815433.1]
KO_UP_670	97	132301	Mouse cecum clone M2_1g06 [DQ015322.1]
KO_UP_703	100	131684	Mouse cecum clone C10_B13 [AY991381.1]
KO_UP_71	99	130079	Mouse cecum clone M1_d10_1 [DQ014680.1]
KO_UP_781	100	192375	Obesity- gut microbiome increased capacity energy harvest cecal contents Mus musculus strain C57BL/6J; obob_recipient leptin genotype +/+ clone SWPT4_aaa04b08 [EF099776.1]
KO_UP_83	97	99034	Feces clone F18U [AJ400254.2]
KO_UP_971	97	133315	Mouse cecum clone M2_e05 [DQ015056.1]
KO_UP_975	94	130335	Mouse cecum clone M3_g05_1 [DQ015495.1]
KO_UP_992	97	189189	Obesity- gut microbiome increased capacity energy harvest cecal contents Mus musculus strain C57BL/6J; obob_recipient leptin genotype +/+ clone SWPT4_aaa04f05 [EF099809.1]
WT_UP_1002	98	193103	Obesity- gut microbiome increased capacity energy harvest cecal contents Mus musculus strain C57BL/6J; obob_recipient leptin genotype +/+ clone SWPT1_aaa02b08 [EF098858.1]
WT_UP_1034	97	131631	Mouse cecum clone C9_I14 [AY990665.1]
WT_UP_1059	99	129358	Mouse cecum clone C22_i13 [AY992917.1]
WT_UP_112	99	129838	Mouse cecum clone M2_h02_3 [DQ015248.1]
WT_UP_1201	99	133103	Mouse cecum clone M2_f09_1 [DQ015238.1]
WT_UP_1216	99	175432	Obesity- gut microbiome increased capacity energy harvest cecal contents Mus musculus strain C57BL/6J; obob_recipient leptin genotype +/+ clone SWPT3_aaa02g12 [EF099407.1]
WT_UP_1226	98	136647	Abundant and fungal microbiota murine intestine clone 1252-2 [AY994030.1]
WT_UP_1278	87	181605	Obesity- gut microbiome increased capacity energy harvest cecal contents Mus musculus strain C57BL/6J; obob_recipient leptin genotype +/+ clone SWPT5_aaa01f07 [EF099877.1]
WT_UP_1285	98	129314	Mouse cecum clone C13_B16 [AY991253.1]
WT_UP_131	98	174456	Obesity- gut microbiome increased capacity energy harvest cecal contents Mus musculus strain C57BL/6J; lean_recipient leptin genotype +/+ clone SWPT19_aaa02a10 [EF097397.1]
WT_UP_135	99	173912	Obesity- gut microbiome increased capacity energy harvest cecal contents Mus musculus strain C57BL/6J; obob_recipient leptin genotype +/+ clone SWPT3_aaa04h06 [EF099553.1]
WT_UP_1360	98	129449	Mouse cecum clone C21_I20 [AY993269.1]
WT_UP_1405	94	134050	Mouse cecum clone C4_H031 [AY990863.1]
WT_UP_1457	96	131630	Mouse cecum clone C12_O02 [AY990138.1]
WT_UP_1468	98	129314	Mouse cecum clone C13_B16 [AY991253.1]
WT_UP_147	98	133024	Mouse cecum clone C15_D13 [AY993700.1]
WT_UP_1553	88	175080	Obesity- gut microbiome increased capacity energy harvest cecal contents Mus musculus strain C57BL/6J; obob_recipient leptin genotype +/+ clone SWPT3_aaa04e12 [EF099535.1]
WT_UP_1599	99	131769	Mouse cecum clone C20_b14 [AY991738.1]
WT_UP_1626	98	130326	Mouse cecum clone M3_b04 [DQ015358.1]
WT_UP_1669	95	167204	Mouse cecum clone (clone) [DQ815411.1]
WT_UP_1689	99	183958	Obesity- gut microbiome increased capacity energy harvest cecal contents Mus musculus strain C57BL/6J; obob_recipient leptin genotype +/+ clone SWPT15_aaa02b08 [EF098390.1]
WT_UP_172	99	129321	Mouse cecum clone C12_I01 [AY989949.1]
WT_UP_1743	96	131630	Mouse cecum clone C12_O02 [AY990138.1]
WT_UP_1744	97	131630	Mouse cecum clone C12_O02 [AY990138.1]
WT_UP_175	96	175580	Obesity- gut microbiome increased capacity energy harvest cecal contents Mus musculus strain C57BL/6J; lean_recipient leptin genotype +/+ clone SWPT13_aaa02g07 [EF096923.1]
WT_UP_1778	96	176860	Obesity- gut microbiome increased capacity energy harvest cecal contents Mus musculus strain C57BL/6J; obob_recipient leptin genotype +/+ clone SWPT16_aaa03g05 [EF098708.1]
WT_UP_178	99	131350	Mouse cecum clone M3_b05_2 [DQ015522.1]
WT_UP_1828	99	199414	Obesity- gut microbiome increased capacity energy harvest cecal contents Mus musculus strain C57BL/6J; lean_donor leptin genotype +/+ clone lean2_aaa01a12 [EF095968.1]
WT_UP_1830	97	131630	Mouse cecum clone C12_O02 [AY990138.1]
WT_UP_1833	97	131630	Mouse cecum clone C12_O02 [AY990138.1]
WT_UP_1880	99	134346	Mouse cecum clone M3_g10_1 [DQ015500.1]
WT_UP_1884	94	178817	Obesity- gut microbiome increased capacity energy harvest cecal contents Mus musculus strain C57BL/6J; obob_recipient leptin genotype +/+ clone SWPT15_aaa02g11 [EF098421.1]
WT_UP_1909	95	134135	Mouse cecum clone C23_n15 [AY992662.1]
WT_UP_195	100	129437	Mouse cecum clone C21_I17 [AY993266.1]
WT_UP_2064	98	129961	Mouse cecum clone C21_e08 [AY993105.1]
WT_UP_2083	98	131250	Mouse cecum clone M1_1c10_1 [DQ014946.1]
WT_UP_214	96	131795	Mouse cecum clone C11_L131 [AY991639.1]
WT_UP_226	99	129838	Mouse cecum clone M2_h02_3 [DQ015248.1]
WT_UP_2372	100	169953	Mouse cecum clone (clone) [DQ815482.1]
WT_UP_245	99	209962	Pathogen-induced host response provides competitive advantage enteropathogens over intestinal microbiota mouse colon clone infected_7days-B9 [EF406637.1]
WT_UP_250	99	131413	Mouse cecum clone M2_b05_1 [DQ015088.1]
WT_UP_274	96	129838	Mouse cecum clone M2_h02_3 [DQ015248.1]
WT_UP_278	93	184664	Obesity- gut microbiome increased capacity energy harvest cecal contents Mus musculus strain C57BL/6J; lean_recipient leptin genotype +/+ clone SWPT13_aaa04g11 [EF097057.1]

WT_UP_322	93	129747	Mouse cecum clone C20_k07 [AY991906.1]
WT_UP_337	98	129725	Mouse cecum clone M3_f08 [DQ015402.1]
WT_UP_357	98	206426	Pathogen-induced host response provides competitive advantage enteropathogens over intestinal microbiota mouse colon clone infected_7days-F7 [EF406680.1]
WT_UP_366	99	135674	Mouse cecum clone C21_e18 [AY993115.1]
WT_UP_37	99	130079	Mouse cecum clone M1_d10_1 [DQ014680.1]
WT_UP_375	100	129725	Mouse cecum clone M3_f08 [DQ015402.1]
WT_UP_432	100	131150	Mouse cecum clone C10_H21 [AY991407.1]
WT_UP_447	98	129725	Mouse cecum clone M3_f08 [DQ015402.1]
WT_UP_46	99	130741	Mouse cecum clone M1_e11 [DQ014606.1]
WT_UP_462	99	129981	Mouse cecum clone C20_e02 [AY991778.1]
WT_UP_465	97	192906	Obesity- gut microbiome increased capacity energy harvest cecal contents Mus musculus strain C57BL/6J; lean_recipient leptin genotype +/+ clone SWPT11_aaa04d01 [EF096768.1]
WT_UP_484	98	133024	Mouse cecum clone C15_D13 [AY993700.1]
WT_UP_487	99	130676	Mouse cecum clone C12_C22 [AY990087.1]
WT_UP_491	99	176121	Obesity- gut microbiome increased capacity energy harvest cecal contents Mus musculus strain C57BL/6J; obob_donor leptin genotype ob/ob clone obob1_aaa03a09 [EF096232.1]
WT_UP_496	99	180731	Obesity- gut microbiome increased capacity energy harvest cecal contents Mus musculus strain C57BL/6J; lean_donor leptin genotype +/+ clone lean2_aaa01f09 [EF096000.1]
WT_UP_498	99	130945	Mouse cecum clone M3_f07_1 [DQ015569.1]
WT_UP_504	99	130153	Mouse cecum clone C20_h11 [AY991851.1]
WT_UP_566	98	129570	Mouse cecum clone M1_d09_2 [DQ014765.1]
WT_UP_570	94	134208	Mouse cecum clone C21_m13 [AY993284.1]
WT_UP_572	99	213896	Pathogen-induced host response provides competitive advantage enteropathogens over intestinal microbiota mouse colon clone infected_28days-D5 [EF406831.1]
WT_UP_596	98	129725	Mouse cecum clone M3_f08 [DQ015402.1]
WT_UP_661	99	133779	Mouse cecum clone M3_d06_1 [DQ015463.1]
WT_UP_664	97	129725	Mouse cecum clone M3_f08 [DQ015402.1]
WT_UP_701	97	190375	Obesity- gut microbiome increased capacity energy harvest cecal contents Mus musculus strain C57BL/6J; obob_recipient leptin genotype +/+ clone SWPT4_aaa03e07 [EF099728.1]
WT_UP_726	97	130543	Mouse cecum clone C16_I16 [AY992309.1]
WT_UP_727	95	166612	Mouse cecum clone (clone) [DQ815739.1]
WT_UP_76	99	131350	Mouse cecum clone M3_b05_2 [DQ015522.1]
WT_UP_837	98	174389	Obesity- gut microbiome increased capacity energy harvest cecal contents Mus musculus strain C57BL/6J; obob_recipient leptin genotype +/+ clone SWPT16_aaa01c05 [EF098551.1]
WT_UP_880	99	129960	Mouse cecum clone M1_1d06 [DQ014915.1]
WT_UP_885	99	185341	Obesity- gut microbiome increased capacity energy harvest cecal contents Mus musculus strain C57BL/6J; obob_recipient leptin genotype +/+ clone SWPT15_aaa02h05 [EF098426.1]
WT_UP_908	99	132507	Mouse cecum clone M1_1c05_1 [DQ014991.1]
WT_UP_932	95	129961	Mouse cecum clone C21_e08 [AY993105.1]
WT_UP_954	100	174526	Obesity- gut microbiome increased capacity energy harvest cecal contents Mus musculus strain C57BL/6J; obob_recipient leptin genotype +/+ clone SWPT2_aaa03d01 [EF099169.1]
WT_UP_973	99	129314	Mouse cecum clone C13_B16 [AY991253.1]
WT_UP_999	99	129725	Mouse cecum clone M3_f08 [DQ015402.1]

Average 97.5