

# **Obesity reshapes the microbial population structure along the gut-liver-lung axis in mice**

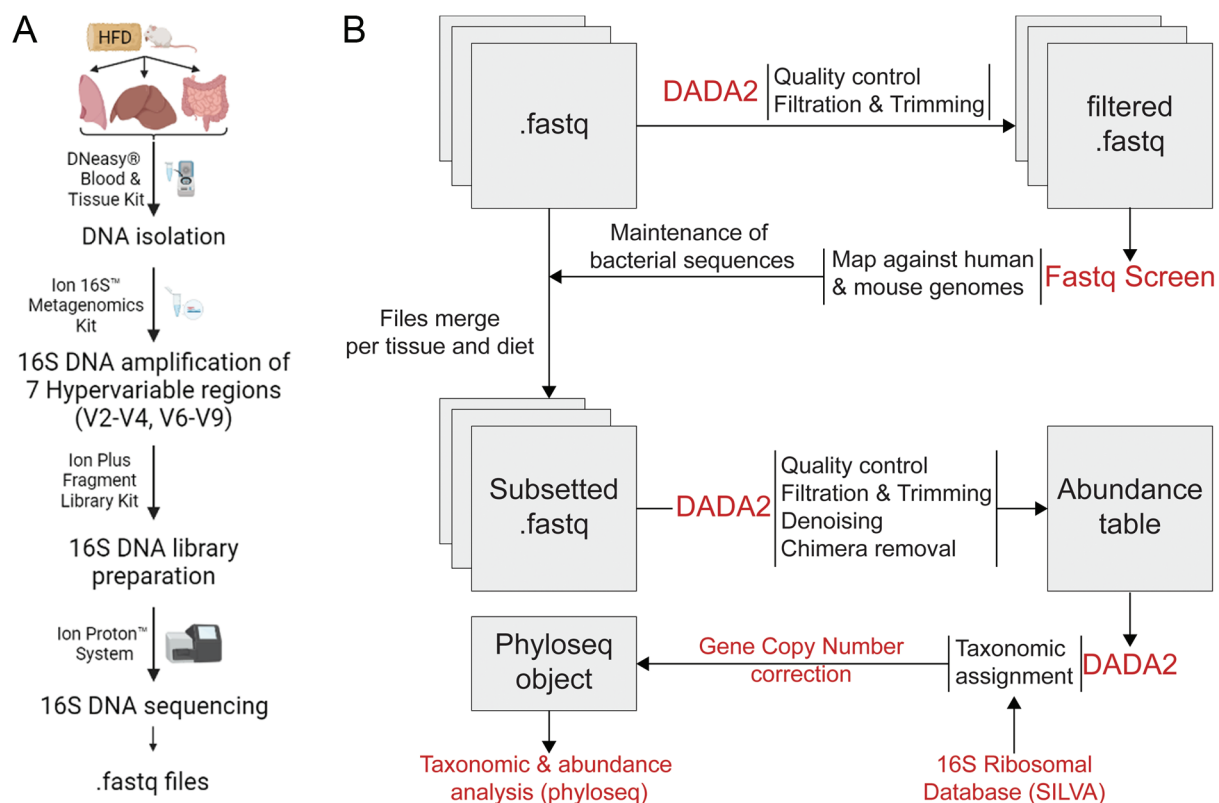
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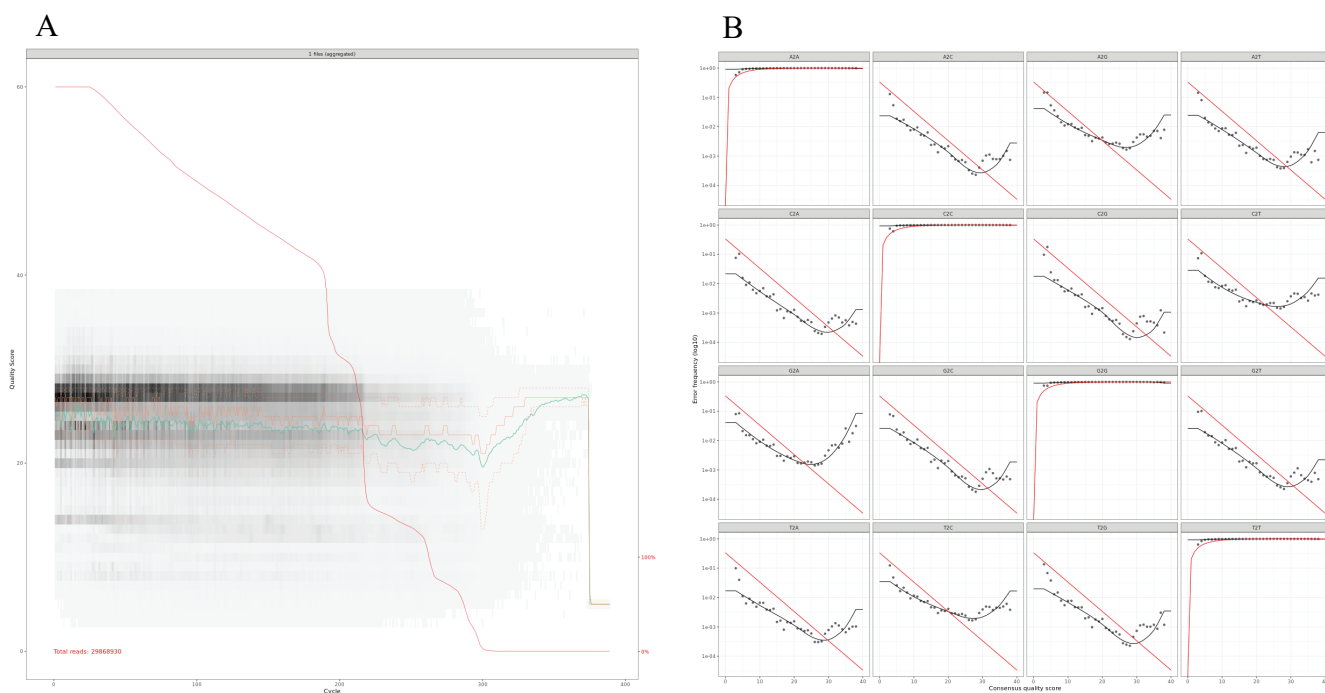
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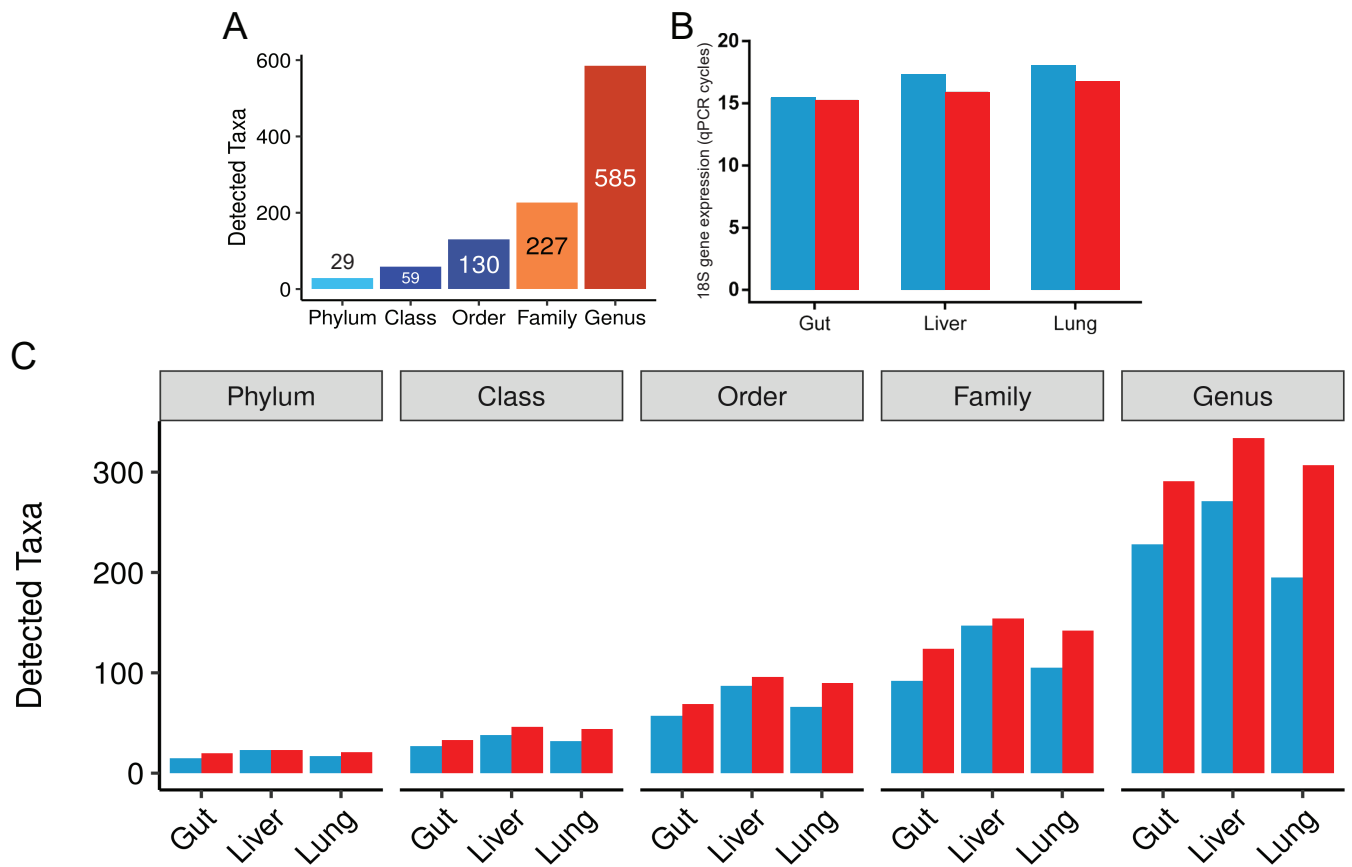
**SUPPLEMENTARY DATA**



**Figure S1. Schematic overview of experimental design and 16S rDNA sequencing and data analysis.** (A) Mice fed with HFD and their relative controls were sacrificed 16 weeks after HFD consumption and their lung, liver, and gut tissues were isolated. Total genomic DNA was extracted, followed by the amplification of seven (V2-V4 and V6-V9) 16S hypervariable regions. 16S DNA libraries were then constructed and sequenced using the Ion Proton™ System. (B) FastQ data exported from the Ion Proton™ System were filtered and trimmed using DADA2 for quality control of the reads. Then, abundance tables were constructed for every sample and taxonomy assignment was performed using the SILVA database, followed by Gene Copy Number (GCN) correction. Phyloseq was then used to perform taxonomic and abundance analysis of all samples.



**Figure S2. Data quality control and error rate estimation. A. Read quality profile inspection.** The dataset contains good quality reads as can be seen by the heatmap, the mean (green line) and the quartiles (orange lines) of quality score at each position. **B. Error rates for each nucleotide transition.** The estimated error rates (black line) are a good fit to the observed ones (points). In addition, it can be seen that generally error rates decrease with quality increase, as expected.



**Figure S3. Obesity increased microbial complexity along the gut-liver-lung axis.** (A) Overall phyla, classes, orders, families, and genera identified. (B) No differences were detected in host tissue 18S rRNA gene, as detected with Q-PCR, ensuring equal sample loading. (C) (HFD)-driven obesity resulted in a tendency for greater number of taxa in all tissues, as compared to the control diet ones.

**Table S1.** Quality control metrics before (grey colored cells) and after (white colored cells) human and bacteria sequences removal.

Fastq File	Total Input	QC Filtered	Denoised	Chimeras Filtered	#Bacteria	%Bacteria	#Human	%Human	#Mouse	%Mouse
GutCtrl_3_2	1770986	1532202	1512880	1268758	1248402	98.4%	8726	0.6%	27105	1.8%
	1731525	1498021	1486729	1246433	1246408	100.0%	536	0.0%	810	0.1%
GutCtrl_3_3	1760510	1521842	1499042	1265975	1240755	98.0%	7354	0.5%	32938	2.2%
	1715354	1482715	1470362	1233805	1233611	100.0%	406	0.0%	689	0.0%
GutHFD_3_1	1928020	1574046	1555039	1320228	1305339	98.9%	7063	0.4%	17912	1.1%
	1900932	1550501	1537875	1309130	1306946	99.8%	572	0.0%	900	0.1%
GutHFD_3_2	1948602	1609327	1589515	1363872	1353998	99.3%	6560	0.4%	10526	0.7%
	1929311	1593182	1578588	1335353	1331687	99.7%	516	0.0%	784	0.0%
GutHFD_3_3	1673227	1433420	1415385	1149494	1123049	97.7%	6110	0.4%	32992	2.3%
	1631187	1395532	1383614	1112892	1112289	99.9%	452	0.0%	722	0.1%
GutHFD_3_4	534150	461772	432985	369036	326957	88.6%	13178	2.9%	55207	12.0%
	455485	395226	387076	320836	320056	99.8%	110	0.0%	184	0.0%
LiverCtrl_3_1	374935	336859	326398	266703	241140	90.4%	5196	1.5%	30444	9.0%
	337707	302906	299886	240129	239941	99.9%	1	0.0%	2	0.0%
LiverCtrl_3_2	1679307	1531637	1498578	1390006	511884	36.8%	46344	3.0%	916165	59.8%
	687747	604892	597515	512209	511013	99.8%	1	0.0%	31	0.0%
LiverCtrl_3_4	1718143	1315061	1294719	1130788	1104193	97.6%	6150	0.5%	33244	2.5%
LiverCtrl_3_4	1674692	1276969	1263353	1100935	1099857	99.9%	690	0.1%	1009	0.1%
LiverHFD_3_1	2547259	2232461	2212419	1816707	1803158	99.3%	7725	0.3%	16971	0.8%
	2519085	2208565	2193784	1790959	1790814	100.0%	626	0.0%	944	0.0%
LiverHFD_3_2	938898	789124	746957	633620	594901	93.9%	42609	5.4%	35647	4.5%
	852138	712640	703984	589527	589376	100.0%	253	0.0%	454	0.1%
LiverHFD_3_3	778814	666642	650869	550628	516459	93.8%	6037	0.9%	40773	6.1%
	727892	621013	613072	514674	514407	99.9%	220	0.0%	385	0.1%
LiverHFD_3_4	912159	803836	788019	659803	592211	89.8%	5933	0.7%	77285	9.6%
	823363	722352	714886	589288	589183	100.0%	243	0.0%	356	0.0%
LungCtrl_3_1	2767079	2259528	2249469	1911019	1907913	99.8%	3884	0.2%	6519	0.3%
	2755550	2250366	2240080	1906006	1902901	99.8%	856	0.0%	1336	0.1%
LungCtrl_3_2	1107349	843096	826303	648015	601917	92.9%	4182	0.5%	52427	6.2%
	1041219	786589	773609	600137	599592	99.9%	443	0.1%	673	0.1%
LungCtrl_3_3	1265273	1002180	996025	858122	856185	99.8%	2348	0.2%	1746	0.2%
	1260665	998144	994103	855733	855568	100.0%	0	0.0%	0	0.0%
LungHFD_3_1	1678219	1470977	1458803	1151079	1075140	93.4%	8572	0.6%	80815	5.5%
	1584575	1384374	1379440	1075217	1075037	100.0%	0	0.0%	1	0.0%
LungHFD_3_2	2271075	1831748	1811799	1557239	1420279	91.2%	8140	0.4%	149511	8.2%
	2100318	1676383	1663373	1424845	1423374	99.9%	704	0.0%	1132	0.1%
LungHFD_3_3	1946944	1704019	1691277	1424228	1395615	98.0%	3686	0.2%	32899	1.9%
	1907175	1667881	1657191	1384509	1383929	100.0%	491	0.0%	827	0.0%
LungHFD_3_4	2294588	1969212	1953321	1657752	1613357	97.3%	5564	0.3%	50680	2.6%
	2233010	1913974	1902153	1600244	1599679	100.0%	592	0.0%	965	0.1%

**Table S2.** Important taxa as presented in Figure 2D, E.

Inter-tissue common taxa unique per dietary regime		
Taxonomic level	Unique in	Unique taxa
Phylum	HFD	Fusobacteria; Acidobacteria
	Ctrl	-
Family	HFD	Peptostreptococcaceae; Muribaculaceae; Rhodobacteraceae; Bacillaceae; Bacteroidaceae; Bifidobacteriaceae; Azospirillaceae; Family_XIII; Listeriaceae; Leuconostocaceae; Flavobacteriaceae; Beijerinckiaceae; Nocardaceae; Dermacoccaceae; Mitochondria; Sphingobacteriaceae; Nocardoidaceae
	Ctrl	-
Inter-tissue common taxa		
Taxonomic level	Found in	Taxa
Phylum	HFD	Bacteroidetes; Proteobacteria; Firmicutes; Actinobacteria; Cyanobacteria
	Ctrl	Firmicutes; Actinobacteria; Proteobacteria; Bacteroidetes; Cyanobacteria; Fusobacteria; Acidobacteria
Family	HFD	Propionibacteriaceae; Staphylococcaceae; Streptococcaceae; Xanthobacteraceae; Peptoniphilaceae; Corynebacteriaceae; Lactobacillaceae; Micrococcaceae; Pasteurellaceae; Caulobacteraceae; Moraxellaceae; Peptostreptococcaceae; Rhizobiaceae; Prevotellaceae; Lachnospiraceae; Carnobacteriaceae; Neisseriaceae; Actinomycetaceae; Muribaculaceae; Veillonellaceae; Porphyromonadaceae; Burkholderiaceae; Weeksellaceae; Aeromonadaceae; Rhodobacteraceae; Erysipelotrichaceae; Enterobacteriaceae; Ruminococcaceae; Atopobiaceae; Aerococcaceae; Bacillaceae; Fusobacteriaceae; Sphingomonadaceae; Microbacteriaceae; Bacteroidaceae; Bifidobacteriaceae; Azospirillaceae; Pseudomonadaceae; Family_XIII; Listeriaceae; Xanthomonadaceae; Leuconostocaceae; Flavobacteriaceae; Beijerinckiaceae; Nocardaceae; Dermacoccaceae; Mitochondria; Sphingobacteriaceae; Nocardoidaceae
	Ctrl	Xanthobacteraceae; Rhizobiaceae; Caulobacteraceae; Staphylococcaceae; Propionibacteriaceae; Streptococcaceae; Peptoniphilaceae; Erysipelotrichaceae; Corynebacteriaceae; Lactobacillaceae; Burkholderiaceae; Pasteurellaceae; Micrococcaceae; Carnobacteriaceae; Lachnospiraceae; Prevotellaceae; Neisseriaceae; Enterobacteriaceae; Moraxellaceae; Porphyromonadaceae; Veillonellaceae; Atopobiaceae; Actinomycetaceae; Weeksellaceae; Ruminococcaceae; Sphingomonadaceae; Microbacteriaceae; Xanthomonadaceae; Aerococcaceae; Pseudomonadaceae; Aeromonadaceae; Fusobacteriaceae

**Table S3.** Number of shared taxa between any pairwise combination of experimental conditions.

		GutCtrl	GutHFD	LiverCtrl	LiverHFD	LungCtrl	LungHFD
Phylum	GutCtrl	-	5	5	5	5	5
	GutHFD	5	-	7	7	6	8
	LiverCtrl	5	7	-	7	6	7
	LiverHFD	5	7	7	-	6	8
	LungCtrl	5	6	6	6	-	6
	LungHFD	5	8	7	8	6	-
Family	GutCtrl	-	41	34	39	34	36
	GutHFD	41	-	45	52	38	50
	LiverCtrl	34	45	-	53	40	55
	LiverHFD	39	52	53	-	41	59
	LungCtrl	34	38	40	41	-	43
	LungHFD	36	50	55	59	43	-

**Table S4.** Detected genera belonging to the Streptococcaceae, Staphylococcaceae, Peptoniphilaceae and Pasteurellaceae families. Marked with red are those that are affected by high fat diet in at least one tissue as defined by HFD-to-control relative abundance difference (non-zero difference).

Phylum	Family	Genus
Firmicutes	Streptococcaceae	Streptococcus
		Lactococcus
	Staphylococcaceae	Staphylococcus
		Salinicoccus
		Jeotgalicoccus
	Peptoniphilaceae	Gemella
		Peptoniphilus
		Anaerococcus
		Finegoldia
		Gallicola
		Parvimonas
		Tissierella
		Ezakiella
		Murdochiella
		W5053
		Sedimentibacter
Proteobacteria	Pasteurellaceae	NA



**Table S5.** Detected species related with superantigen proteins as recorded by UniProt database and/or respective literature.

Phylum	Family	Species	Source
Firmicutes	Peptoniphilaceae	<i>Finegoldia magna</i>	Literature
	Streptococcaceae	<i>Lactobacillus delbruecki</i>	TrEBML
		<i>Lactococcus lactis</i>	TrEBML
	Staphylococcaceae	<i>Staphylococcus aureus</i>	KB; TrEBML; Literature
		<i>Staphylococcus massiliensis</i>	TrEBML
		<i>Staphylococcus epidermis</i>	TrEBML
Proteobacteria	Moraxellaceae	<i>Acinetobacter baumannii</i>	TrEBML