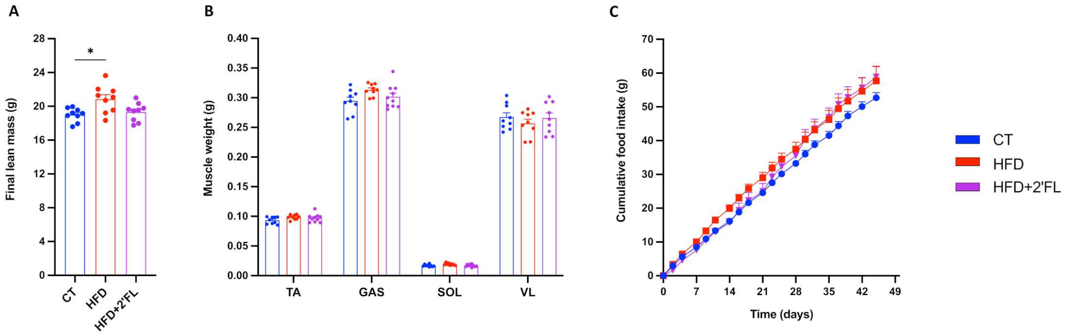
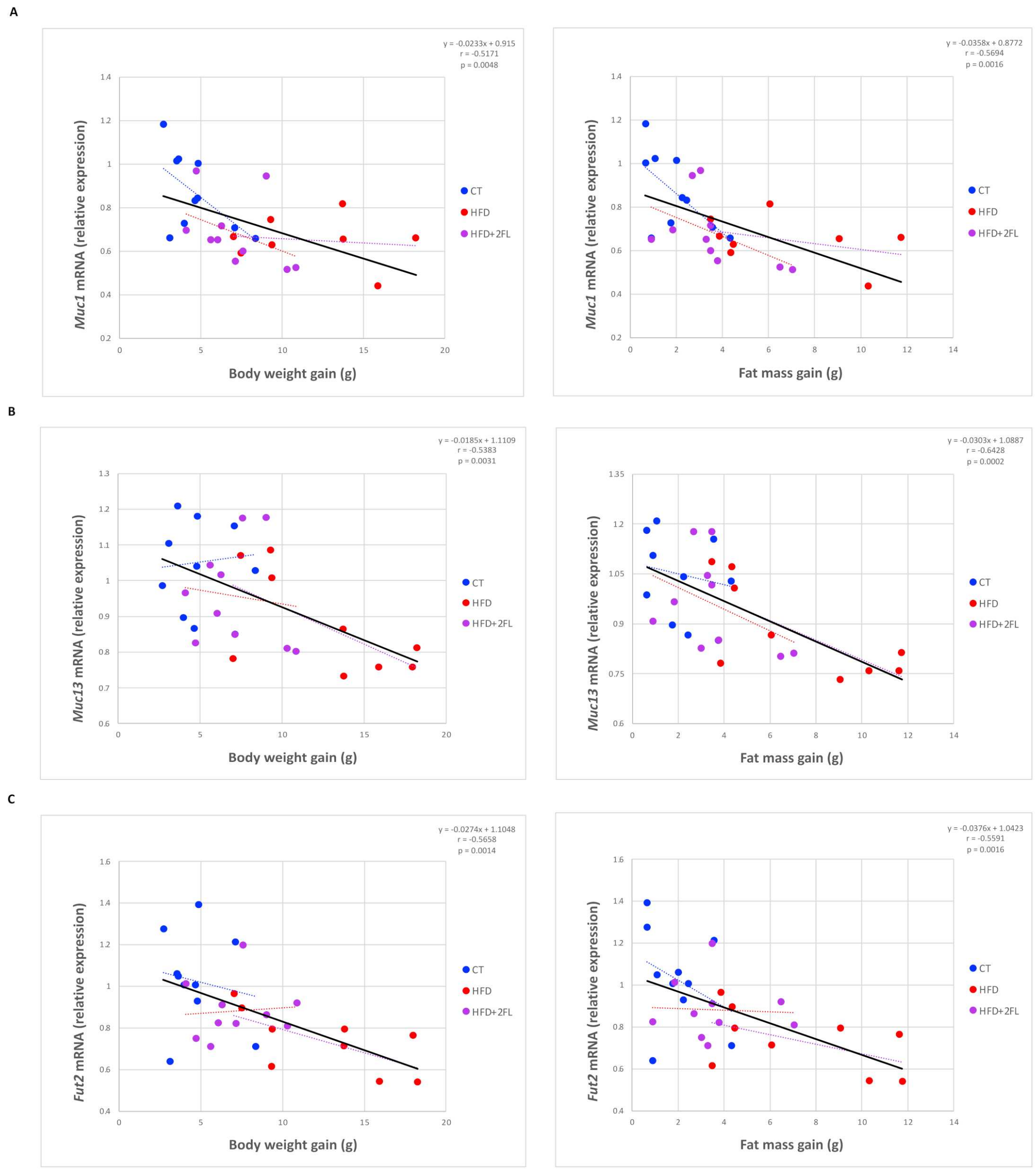
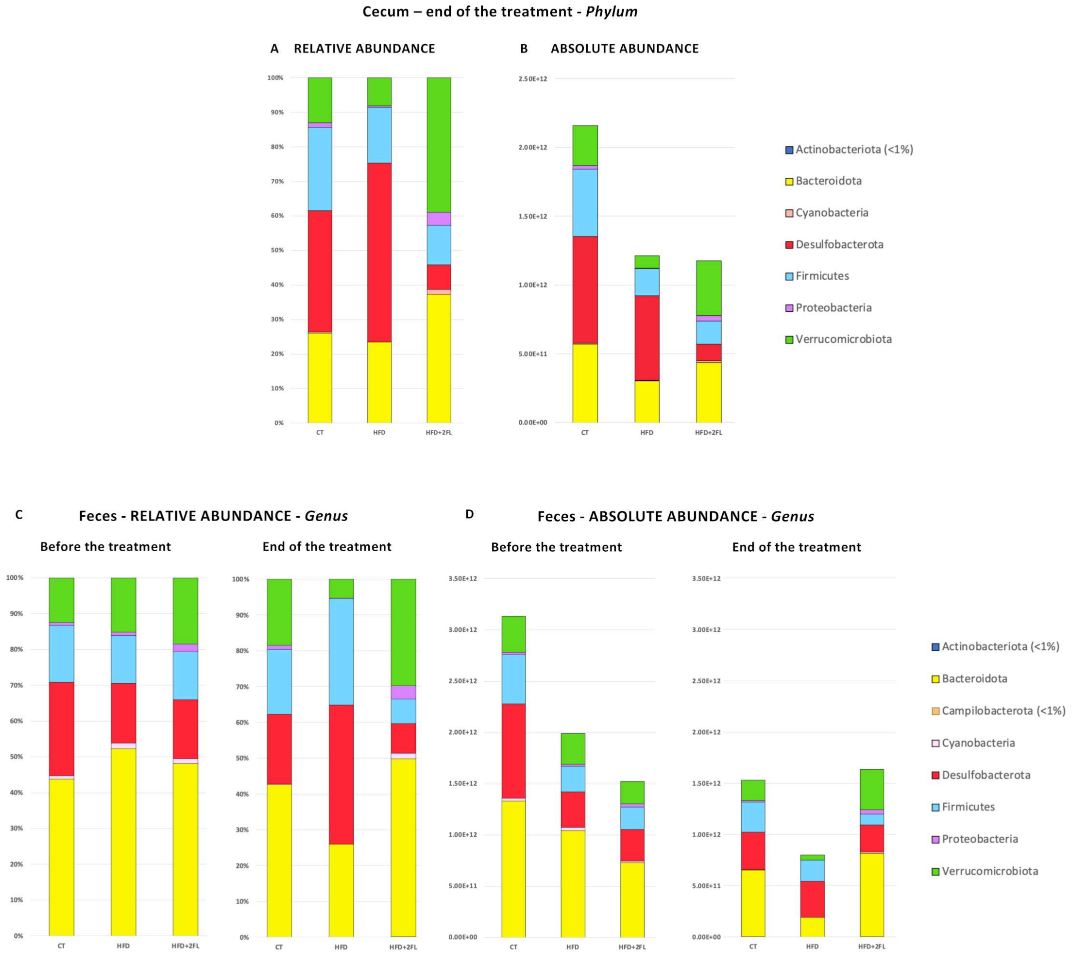


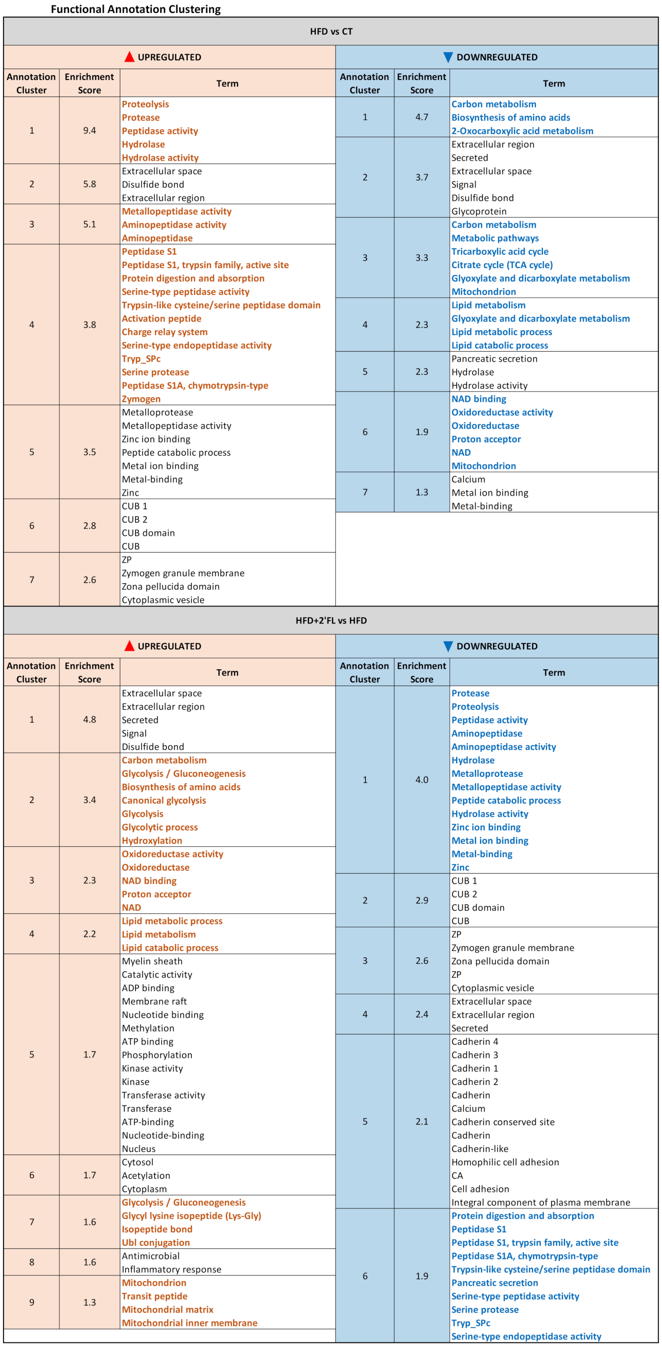
Supplemental Figure 1. (A) Final lean mass and (B) muscle weights (TA = tibialis anterior, VL = vastus lateralis, GAS = gastrocnemius, SOL = soleus). (C) Cumulative food intake. Data are means±s.e.m (n= 9-10/group). Data were analysed using one-way ANOVA for A and B and according to two-way ANOVA for C followed by Tukey post hoc test. *P < 0.05;



Supplemental Figure 2. Pearson correlation between mRNA colonic expression of (A) *Muc1*, (B) *Muc13* and (C) *Fut2* and body weight gain/fat mass gain (n=9-10/group).



Supplemental Figure 3. (A-D) Bar graphs showing grouped taxonomic profiles of the gut bacteria at a phylum level: (A,B) relative and absolute abundance in the cecum, before and at the end of the treatment; (C,D) relative and absolute abundance in the feces, at the end of the treatment. (n= 9-10/group).



Functional Annotation Clustering

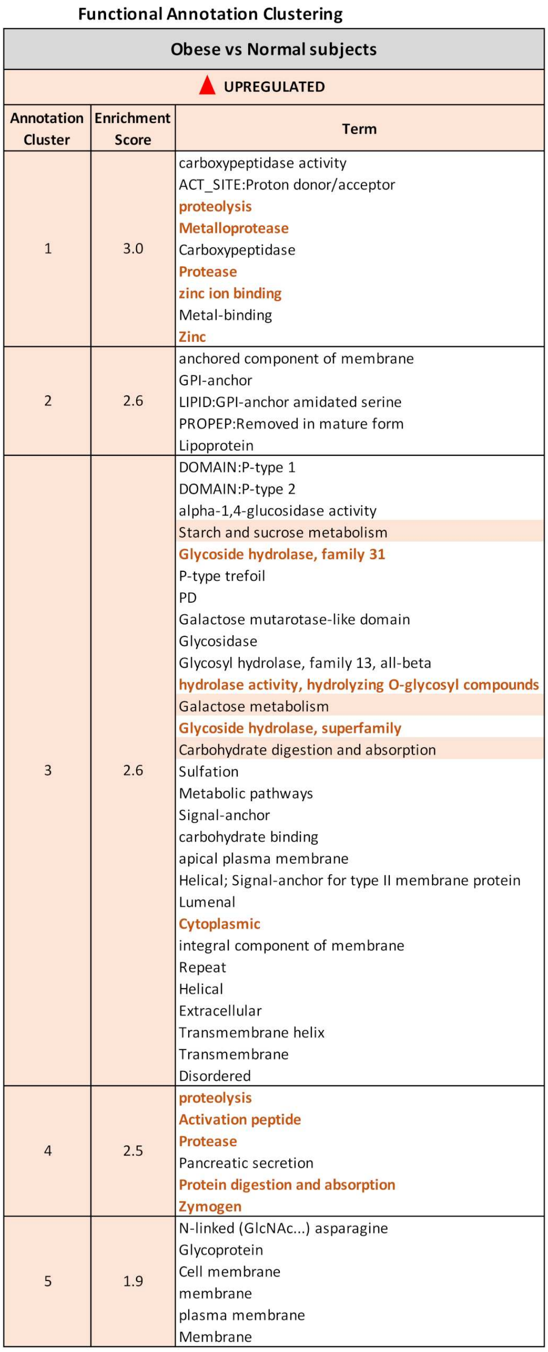
HFD vs CT

▲ UPREGULATED			▼ DOWNREGULATED		
Annotation Cluster	**Enrichment Score**	**Term**	**Annotation Cluster**	**Enrichment Score**	**Term**
1	9.4	**Proteolysis** **Protease** **Peptidase activity** **Hydrolase** **Hydrolase activity**	1	4.7	**Carbon metabolism** **Biosynthesis of amino acids** **2-Oxocarboxylic acid metabolism**
2	5.8	Extracellular space Disulfide bond Extracellular region	2	3.7	Extracellular region Secreted Extracellular space Signal Disulfide bond Glycoprotein
3	5.1	**Metallopeptidase activity** **Aminopeptidase activity** **Aminopeptidase**	3	3.3	**Carbon metabolism** **Metabolic pathways** **Tricarboxylic acid cycle** **Citrate cycle (TCA cycle)** **Glyoxylate and dicarboxylate metabolism** **Mitochondrion**
4	3.8	**Peptidase S1** **Peptidase S1, trypsin family, active site** **Protein digestion and absorption** **Serine-type peptidase activity** **Trypsin-like cysteine/serine peptidase domain** **Activation peptide** **Charge relay system** **Serine-type endopeptidase activity** **Tryp_SPc** **Serine protease** **Peptidase S1A, chymotrypsin-type** **Zymogen**	4	2.3	**Lipid metabolism** **Glyoxylate and dicarboxylate metabolism** **Lipid metabolic process** **Lipid catabolic process**
5	3.5	Metalloprotease Metallopeptidase activity Zinc ion binding Peptide catabolic process Metal ion binding Metal-binding Zinc	5	2.3	Pancreatic secretion Hydrolase Hydrolase activity
6	2.8	CUB 1 CUB 2 CUB domain CUB	6	1.9	**NAD binding** **Oxidoreductase activity** **Oxidoreductase** **Proton acceptor** **NAD** **Mitochondrion**
7	2.6	ZP Zymogen granule membrane Zona pellucida domain Cytoplasmic vesicle	7	1.3	Calcium Metal ion binding Metal-binding

HFD+2'FL vs HFD

▲ UPREGULATED			▼ DOWNREGULATED		
Annotation Cluster	**Enrichment Score**	**Term**	**Annotation Cluster**	**Enrichment Score**	**Term**
1	4.8	Extracellular space Extracellular region Secreted Signal Disulfide bond	1	4.0	**Protease** **Proteolysis** **Peptidase activity** **Aminopeptidase** **Aminopeptidase activity** **Hydrolase** **Metalloprotease** **Metallopeptidase activity** **Peptide catabolic process** **Hydrolase activity** **Zinc ion binding** **Metal ion binding** **Metal-binding** **Zinc**
2	3.4	**Carbon metabolism** **Glycolysis / Gluconeogenesis** **Biosynthesis of amino acids** **Canonical glycolysis** **Glycolysis** **Glycolytic process** **Hydroxylation**	2	2.9	CUB 1 CUB 2 CUB domain CUB
3	2.3	**Oxidoreductase activity** **Oxidoreductase** **NAD binding** **Proton acceptor** **NAD**	3	2.6	ZP Zymogen granule membrane Zona pellucida domain ZP Cytoplasmic vesicle
4	2.2	**Lipid metabolic process** **Lipid metabolism** **Lipid catabolic process**	4	2.4	Extracellular space Extracellular region Secreted
5	1.7	Myelin sheath Catalytic activity ADP binding Membrane raft Nucleotide binding Methylation ATP binding Phosphorylation Kinase activity Kinase Transferase activity Transferase ATP-binding Nucleotide-binding Nucleus	5	2.1	Cadherin 4 Cadherin 3 Cadherin 1 Cadherin 2 Cadherin Calcium Cadherin conserved site Cadherin Cadherin-like Homophilic cell adhesion CA Cell adhesion Integral component of plasma membrane
6	1.7	Cytosol Acetylation Cytoplasm	6	1.9	**Protein digestion and absorption** **Peptidase S1** **Peptidase S1, trypsin family, active site** **Peptidase S1A, chymotrypsin-type** **Trypsin-like cysteine/serine peptidase domain** **Pancreatic secretion** **Serine-type peptidase activity** **Serine protease** **Tryp_SPc** **Serine-type endopeptidase activity**
7	1.6	**Glycolysis / Gluconeogenesis** **Glycyl lysine isopeptide (Lys-Gly)** **Isopeptide bond** **Ubl conjugation**			
8	1.6	Antimicrobial Inflammatory response			
9	1.3	**Mitochondrion** **Transit peptide** **Mitochondrial matrix** **Mitochondrial inner membrane**			

Supplemental Figure 4. Functional annotation clustering performed with DAVID, showing annotation clustering, enrichment scores and terms significantly up/down-regulated by HFD and HFD+2’FL in mice. Only annotation clusters with enrichment scores ≥ 1.3 (corresponding to P-values <0.05) are shown. Terms that changed in an opposite way in HFD-fed mice compared to HFD+2’FL mice are highlighted in bold and red/blue.



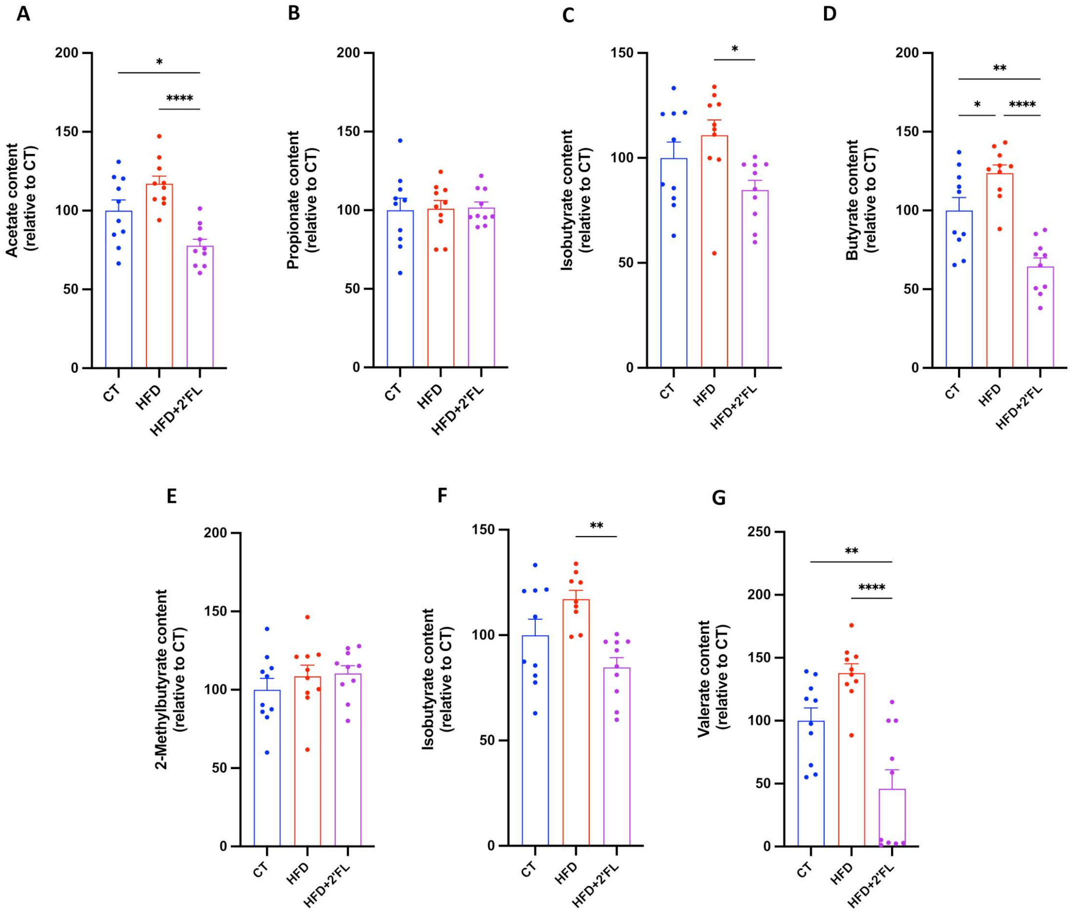
Functional Annotation Clustering

Obese vs Normal subjects

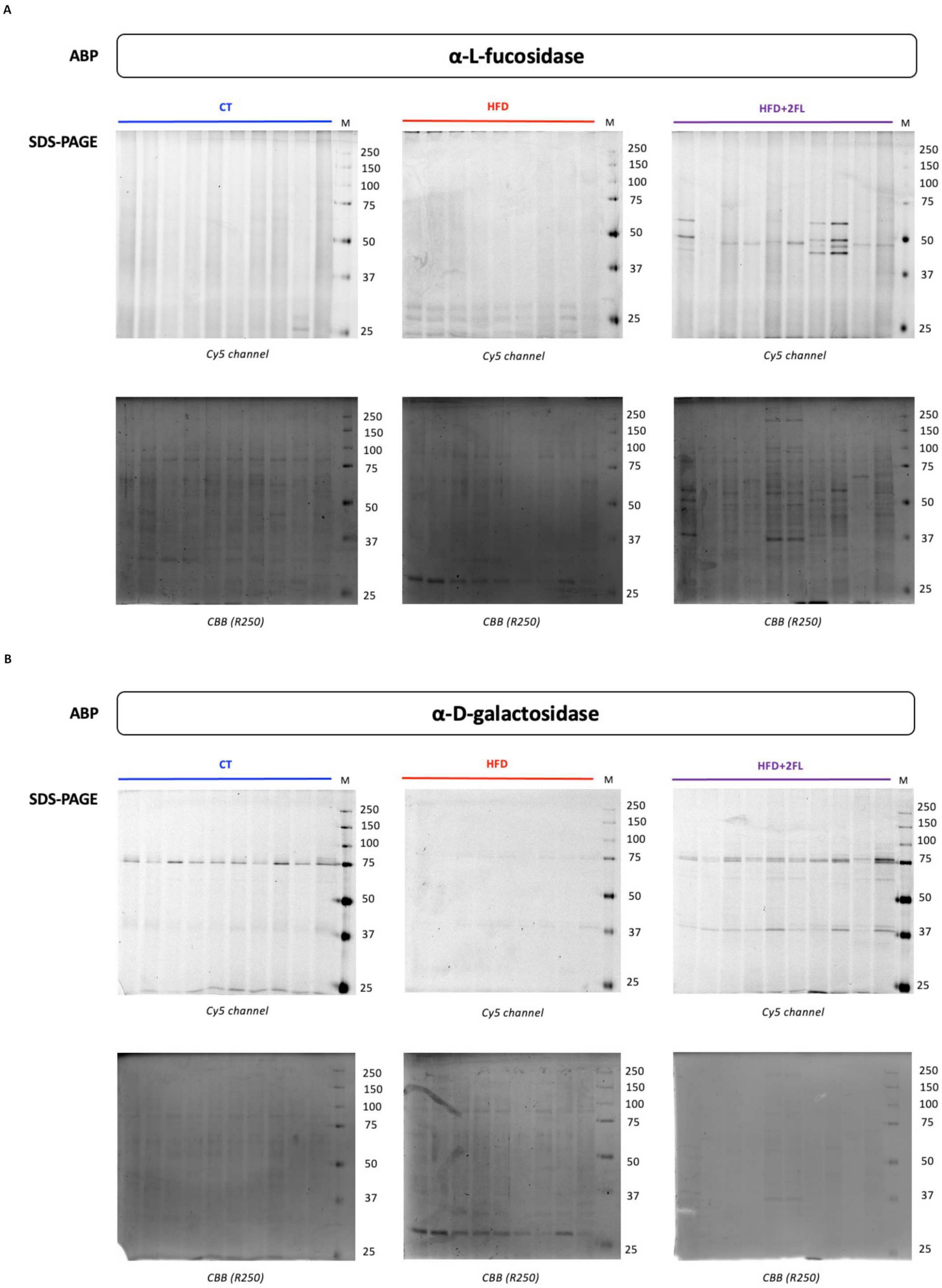
▲ UPREGULATED

Annotation Cluster	Enrichment Score	Term
1	3.0	carboxypeptidase activity
		ACT_SITE:Proton donor/acceptor
		proteolysis
		Metalloprotease
		Carboxypeptidase
		Protease
		zinc ion binding
		Metal-binding
		Zinc
2	2.6	anchored component of membrane
		GPI-anchor
		LIPID:GPI-anchor amidated serine
		PROPEP:Removed in mature form
		Lipoprotein
3	2.6	DOMAIN:P-type 1
		DOMAIN:P-type 2
		alpha-1,4-glucosidase activity
		Starch and sucrose metabolism
		Glycoside hydrolase, family 31
		P-type trefoil
		PD
		Galactose mutarotase-like domain
		Glycosidase
		Glycosyl hydrolase, family 13, all-beta
		hydrolase activity, hydrolyzing O-glycosyl compounds
		Galactose metabolism
		Glycoside hydrolase, superfamily
		Carbohydrate digestion and absorption
		Sulfation
		Metabolic pathways
		Signal-anchor
		carbohydrate binding
		apical plasma membrane
		Helical; Signal-anchor for type II membrane protein
		Lumenal
		Cytoplasmic
		integral component of membrane
		Repeat
		Helical
		Extracellular
		Transmembrane helix
		Transmembrane
		Disordered
4	2.5	**proteolysis**
		Activation peptide
		Protease
		Pancreatic secretion
		Protein digestion and absorption
		Zymogen
5	1.9	N-linked (GlcNAc...) asparagine
		Glycoprotein
		Cell membrane
		membrane
		plasma membrane
		Membrane

Supplemental Figure 5. Functional annotation clustering performed with DAVID, showing annotation clustering, enrichment scores and terms significantly upregulated in obese human subjects compared to normal ones. Only annotation clusters with enrichment scores ≥ 1.3 (corresponding to P-values <0.05) are shown. Terms that are similar to those enriched in HFD-fed mice are in red and some of the terms related to metabolism are highlighted in light red.



Supplemental Figure 6. Short-chain fatty acids (SCFAs) content in the cecal content. (A) acetate, (B) propionate, (C) isobutyrate, (D) butyrate, (E) 2-methylbutyrate, (F) isovalerate, (G) valerate. Data are means±s.e.m (n= 11-12/group). Data were analysed using one-way ANOVA followed by Tukey post hoc test. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.



Supplemental Figure 7. In-gel fluorescent ABP labelling. (A) 1 µM for alpha-L-fucosidase labeling (JJB381) and (B) 0.5 µM for alpha-D-galactosidase (TB474) and their relative Coomassie Brilliant Blue (CBB) staining (n=9-10/group).