

Table S1. Diet Composition

	LF/CON		LF/2'-FL		HF/CON		HF/2'-FL	
	gm	kcal	gm	kcal	gm	kcal	gm	kcal
Protein	19.5	20.0	17.6	20.0	24.4	20.0	22.0	20.0
Carbohydrate	73.3	70.0	76.0	70.0	47.7	35.0	52.9	35.0
Fat	4.3	10.0	3.9	10.0	24.4	45.0	22.0	45.0
Total		100.0		100.0		100.0		100.0
kcal/gm	3.90		3.51		4.88		4.39	
Ingredient	gm	kcal	gm	kcal	gm	kcal	gm	kcal
Casein	207	826	207	826	207	826	207	826
L-Cystine	3	12	3	12	3	12	3	12
Corn Starch	535	2141	535	2141	64	255	64	255
Maltodextrin 10	125	500	125	500	125	500	125	500
Sucrose	56	223	56	223	161	642	161	642
2'-fucosyllactose	0	0	119.3	0	0	0	95.4	0
Cellulose	53.7	0	53.7	0	43.0	0	43.0	0
Soybean Oil	25	225	25	225	25	225	25	225
Lard	22	194	22	194	185	1661	185	1661
AIN-93G Mineral Mix	35	30.9	35	30.9	35	30.9	35	30.9
AIN-93 Vitamin Mix	10	39.2	10	39.2	10	39.2	10	39.2
Choline Bitartrate	2.5	0	2.5	0	2.5	0	2.5	0
TBHQ, antioxidant	0.014	0	0.014	0	0.014	0	0.014	0
FD&C Yellow Dye #5	0.04	0	0	0	0	0	0.025	0
FD&C Red Dye #40	0	0	0	0	0.05	0	0.025	0
FD&C Blue Dye #1	0.01	0	0.04	0	0	0	0	0
Total	1073	4192	1193	4192	859	4192	954	4192
% Sucrose	9.4	7.0	8.5	7.0	23.9	17.0	21.5	17.0
% Cellulose	5.0	0.0	4.5	0.0	5.0	0.0	4.5	0.0

HF, high fat; HF/CON, HF without 2'-FL; HF/2'-FL, HF with 2'-FL (w/w) in diet; LF, low fat; LF/CON, LF without 2'-FL; LF/2'-FL, LF with 2'-FL (w/w) in diet; 2'-FL, 2-fucosyllactose.

Table S2. Primer Sequences used for RT-PCR

Gene	Accession no.	Forward primers (5' to 3')	Reverse primers (5' to 3')
ACC-2	NM_133904	CGC TCA CCA ACA GTA AGG TGG	GCT TGG CAG GGA GTT CCT C
CD36	NM_007643	CGC TTT CTG CGT ATC GTC TG	GAT GCA CGG GAT CGT GTC T
CLDN-2	NM_016675.4	GGC TGT TAG GCT CAT CCA T	TGG CAC CAA CAT AGG AAC TC
CLDN-3	NM_009902.4	AAG CCG AAT GGA CAA AGA A	CTG GCA AGT AGC TGC AGT G
CLDN-7	NM_016887.6	AGG GTC TGC TCT GGT CCT T	GTA CGC AGC TTT GCT TTC A
CLDN-15	NM_021719.4	GCT TCT TCA TGT CAG CCC TG	TTC TTG GAG AGA TCC ATG T
Col1a2	NM_007917	GGC AGG TCT GGG CTT TAT TA	GCT GAG CAT GTC TAC CAC TT
Eif4e	NM_031189.2	GTG TGT GGA GCT GTT GTT AAT G	CGC TCT TTG TAG CTG TGT CT
FABP4	NM_011989.5	AAG GTG AAG AGC ATC ATA ACC CT	TCA CGC CTT TCA TAA CAC ATT CC
FOXO1	NM_019739.3	GGAAGCCCTGAAACCTAACA	CCAGCTGAAACCACAGAGAA
FOXO3	NM_019740.3	GCA GAC CAG CTC ACA AGA TAA	CTG GTG CCT GAA ACC TAA CA
GAPDH	NM_008084.3	ACG GTC AGG TCA TCA CTA TC	GAT GCC ACA GGA TTC CAT AC
Igf1	NM_010512.5	GGG ATG CCA CCA CAA TAT C	CTT TCT CCT CTC TCC CTT CT
IL-1 β	NM_008361.4	ACA GAT CGG CTC CTA CTT	CGG GTC TGC TCA TAG TAA TG
IL-22	NM_016971.2	GGA CCT GCT GTT TAT GTC T	TTC AAC TGA GCC AGG TTT C
JAM-A	NM_172647.2	CTG ATC TTT GAC CCC GTG AC	ACC AGA CGC CAA AAA TCA AG
Klf15	NM_023184.4	GCA GTG GAG GTA TTG GAG ATA G	CTC AGC CTC CAT GTT CTC TT
MCP-1	NM_011333.3	CGA TGT CTA AGA GAG AAA GGG	GGA AAC AGG TAC CCA CAA A
MuRF-1	NM_001039048.2	TGCCAAGCAGCTCATCAA	CAGCCTCCTTCTGTAAACTC
Myod1	NM_010866.2	CGG AGT GGC AGA AAG TTA AG	CGA AAC ACG GGT CAT CAT AG
PPAR γ	NM_011146.3	GAG CAT CTC CCT CAC AAT TC	GGG TGC AGC GAA CTT TAT
Reg3 γ	NM_011260.2	CAC TCC AGC TGT TGG AAG TTT A	GCT CTG ACA CAG TAC CCT CC
SREBP-1c	NM_016971.2	GCA GCC ACC ATC TAG CCT G	CAG CAG TGA GTC TGC CTT GAT
TNF α	NM_013693.3	TCT ACT CCC AGG TTC TCT TC	GCT GAC TTT CTC CTG GTA TG
ZO-1	NM_001163574.1	GCC GCT AAG AGC ACA GCA A	TCC CCA CTC TGA AAA TGA GGA
ZO-3	NM_013769.3	ATC CGA GGA CCT TGA CCT GAC GG	GTC GCT GTT GGT CCG ACT GTC AC

ACC-2, acetyl-CoA carboxylase 2; CD36, cluster of differentiation 36; CLDN, claudin; COL1A1, collagen Type I Alpha 1; Eif4e, eukaryotic translation initiation factor 4E; FABP4, fatty acid binding protein 4; FOXO, Forkhead box protein O; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; Igf1, insulin-like growth factor 1; IL, interleukin; JAM-A, junctional adhesion molecule-A; Klf15, Kruppel Like Factor 15; MCP-1, macrophage chemoattractant protein 1; MuRF-1, muscle RING-finger protein-1; Myod1, myoblast determination protein 1; PPAR γ , peroxisome proliferator-activated receptor gamma; Reg3 γ , regenerating islet-derived protein 3 gamma; SREBP-1c, sterol regulatory element-binding protein-1c; TNF α , tumor necrosis factor alpha; ZO, zonula occludens-1.

Table S3. Relative Gene Expression of Muscle Atrophy Function Genes

Muscle	Gene	LF/CON		LF/2'-FL		HF/CON		HF/2'-FL	
		RE	SEM	RE	SEM	RE	SEM	RE	SEM
Gastro	MuRF1	1.000	0.163	0.831	0.031	0.978	0.062	0.886	0.100
Gastro	Igf1	1.000	0.083	1.015	0.081	1.335	0.127	1.084	0.077
Gastro	Klf15	1.000	0.071	0.953	0.089	1.063	0.048	0.943	0.073
Gastro	Foxo1	1.000	0.074	0.814	0.073	0.937	0.078	0.916	0.138
Gastro	Foxo3	1.000	0.062	1.038	0.051	1.074	0.093	0.943	0.142
Gastro	Eif4e	1.000	0.059	0.875	0.027	1.193	0.104	1.041	0.046
Gastro	Colla1	1.000	0.130	1.376	0.186	1.665	0.336	1.295	0.215
Gastro	Myod1	1.000	0.115	1.020	0.067	1.313	0.106	1.110	0.089
Soleus	MuRF1	1.000	0.094	0.832	0.133	0.866	0.074	0.970	0.100
Soleus	Igf1	1.000	0.106	0.944	0.060	1.207	0.118	1.155	0.119
Soleus	Klf15	1.000	0.082	0.980	0.074	0.957	0.048	1.003	0.078
Soleus	Foxo1	1.000	0.111	0.878	0.066	0.939	0.108	0.899	0.084
Soleus	Foxo3	1.000	0.103	0.929	0.102	1.226	0.096	1.165	0.089
Soleus	Eif4e	1.000	0.042	0.994	0.044	1.047	0.054	0.992	0.031
Soleus	Colla1	1.000	0.238	1.014	0.102	1.305	0.247	1.287	0.122
Soleus	Myod1	1.000	0.173	0.821	0.071	1.222	0.198	1.220	0.149
TA	MuRF1	1.000	0.082	0.767	0.081	0.863	0.046	0.766	0.043
TA	Igf1	1.000	0.115	1.146	0.049	1.167	0.112	1.191	0.100
TA	Klf15	1.000	0.063	0.839	0.077	0.828	0.036	0.800	0.044
TA	Foxo1	1.000	0.076	0.960	0.106	1.148	0.107	0.909	0.069
TA	Foxo3	1.000	0.059	0.855	0.037	1.010	0.055	0.886	0.061
TA	Eif4e	1.000	0.052	1.091	0.060	1.284	0.064	1.123	0.051
TA	Colla1	1.000	0.132	0.986	0.106	1.198	0.116	1.041	0.059
TA	Myod1	1.000	0.060	0.963	0.111	1.109	0.102	1.150	0.091

COL1A1, collagen Type I Alpha 1; Eif4e, eukaryotic translation initiation factor 4E; FOXO, Forkhead box protein O; Gastro, gastrocnemius; Igf1, insulin-like growth factor 1; Klf15, Kruppel Like Factor 15; MuRF-1, muscle RING-finger protein-1; Myod1, myoblast determination protein 1; RE, relative expression; SEM, standard error of mean; TA, tibialis anterior.

Table S4. Relative Gene Expression of Cecal Barrier Function Genes

Gene	RE	SEM	RE	SEM	RE	SEM	RE	SEM
Reg3g	1.000	0.382	0.889	0.196	1.187	0.275	1.158	0.264
Occludin	1.000	0.090	1.056	0.149	0.887	0.064	0.908	0.067
JAM-A	1.000	0.076	1.221	0.177	1.034	0.057	1.115	0.042
ZO-1	1.000	0.094	1.224	0.231	0.921	0.119	1.253	0.121
ZO-3	1.000	0.043	0.804	0.075	0.962	0.081	0.848	0.048
CLDN-2	1.000	0.133	0.853	0.142	0.731	0.064	0.574	0.069
CLDN-3	1.000	0.048	1.056	0.120	0.953	0.092	0.930	0.108
CLDN-4	1.000	0.121	0.676	0.124	0.854	0.085	0.595	0.056
CDLN-7	1.000	0.093	1.134	0.152	1.060	0.096	1.050	0.098
CLDN-15	1.000	0.089	1.355	0.302	1.148	0.116	1.323	0.145
IL-22	1.000	0.184	1.127	0.167	0.634	0.117	0.965	0.185

CLDN, claudin; IL-22, interleukin-22; JAM-A, junctional adhesion molecule-A; RE, relative expression; Reg3 γ , regenerating islet-derived protein 3 gamma; SEM, standard error of mean; ZO, zonula occludens-1.

Table S5. Relative Gene Expression of Ileal Inflammatory Genes

Gene	LF/CON		LF/2'-FL		HF/CON		HF/2'-FL	
	RE	SEM	RE	SEM	RE	SEM	RE	SEM
IL-1 β	1.000	0.166	1.082	0.252	0.794	0.180	1.101	0.275
IL-22	1.000	0.189	2.227	0.477	0.490	0.059	1.537	0.389
TNF α	1.000	0.292	1.124	0.130	1.071	0.243	1.156	0.170
MCP-1	1.000	0.166	1.082	0.252	0.794	0.180	1.101	0.275
Reg3 γ	1.000	0.233	2.504	0.313	3.427	0.431	2.657	0.365

Significant Differences (One-Way ANOVA)

Reg3g	Adj. p value
LF/CON vs LF/2'-FL	0.0166
LF/CON vs HF/CON	<0.0001
HF/CON vs HF/2'-FL	0.3188

HF, high fat; HF/CON, HF without 2'-FL; HF/2'-FL, HF with 2'-FL (w/w) in diet; Igf1, insulin-like growth factor 1; IL, interleukin; JAM-A; LF, low fat; LF/CON, LF without 2'-FL; LF/2'-FL, LF with 2'-FL (w/w) in diet; ; MCP-1, macrophage chemoattractant protein 1; RE, relative expression; Reg3 γ , regenerating islet-derived protein 3 gamma; SEM, standard error of mean; TNF α , tumor necrosis factor alpha.

Table S6. Identification of 2'FL in plasma of mice fed low or high fat diet with or without 2' FL supplementation by using LC-QToF MS/MS.

Mouse ID	Diet	Presence or absence of 2' FL
3	Low Fat	Absent
14	Low Fat	Absent
17	Low Fat	Absent
20	Low Fat	Absent
26	Low Fat	Absent
32	Low Fat	Absent
33	Low Fat	Absent
41	Low Fat	Absent
8	Low Fat/2'-FL	Present
12	Low Fat/2'-FL	Absent/Not identified
16	Low Fat/2'-FL	Present
29	Low Fat/2'-FL	Present
31	Low Fat/2'-FL	Present
34	Low Fat/2'-FL	Partial identification
35	Low Fat/2'-FL	Present
2	High Fat	Absent
11	High Fat	Absent
21	High Fat	Absent
30	High Fat	Absent
36	High Fat	Absent
39	High Fat	Absent
40	High Fat	Absent
5	High Fat/2'-FL	Present
15	High Fat/2'-FL	Present
22	High Fat/2'-FL	Present
28	High Fat/2'-FL	Present
37	High Fat/2'-FL	Present
38	High Fat/2'-FL	Present

2'-FL, 2-fucosyllactose