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Glutaredoxin1 knockout promotes high-fat diet-induced obesity in male mice but not in female ones

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Table S1. Ingredient composition and nutritional content of diet

Diets	Low-fat diet		High-fat diet	
	gm%	**Kcal%**	**gm%**	**Kcal%**
Protein	19.2	20.0	24.0	20.0
Carbohydrate	67.3	70.0	41.0	35.0
Fat	4.3	10.0	24.0	45.0
Total		100.0		100.0
kcal/gm	3.9		4.7	
	gm	**kcal**	**gm**	**kcal**
Soybean, 80 Mesh	200.0	800.0	200.0	800.0
L-Cystine	3.0	12.0	3.0	12.0
Corn Starch	506.2	2024.8	72.8	291.0
Maltodextrin 10	125.0	500.0	100.0	400.0
Sucrose	68.8	275.2	172.8	691.0
Cellulose, BW200	50.0	0.0	50.0	0.0
Soybean Oil	25.0	225.0	25.0	225.0
Lard	20.0	180.0	177.5	1598.0
Mineral Mix S10026	10.0	0.0	10.0	0.0
DiCalcium Phosphate	13.0	0.0	13.0	0.0
Calcium Carbonate	5.5	0.0	5.5	0.0
Potassium Citrate, 1 H2O	16.5	0.0	16.5	0.0
Vitamin Mix V10001	10.0	40.0	10.0	40.0
Choline Bitartrate	2.0	0.0	2.0	0.0
Total	1055.1	4057.0	858.2	4057.0

Table S2. Primers for genes involving lipid metabolism, cholesterol metabolism and Nrf2 signaling pathway in liver tissue

Gene	F	R	bp
HMGCR	AGGCCATGCATCCGGAAAA	GAGCCCCATGCATGCTAAGT	100
Srebf-1c	CGAGGCCCTGCTGTTGGCAT	TGGCAGTCCAGCAGTAGCCG	135
CD36	ATGACTGGGGTGGCCCCTTTG	GCCTTGCTGTAGCCAAGAACTCC	170
Srebf-2	CCAAAGAAGGAGAGAGGCGG	CGCCAGACTTGTGCATCTTG	125
FASN	CCAGCCGCGTAGTGGGGTT	GGAGAAACGACCAGCGTGGC	128
SCD1	TCCAAAGCCCAGGCCACCTT	GCGGGGACTTGCTCTATCCCT	119
Nrf2	CCTCCGCTGCCATCAGTCAGT	TCGGCTGGGACTCGTGTTCA	161
β -actin	TCCCTGTATGCCTCTGGTCGT	CCAGACGCAGGATGGCGTGA	115

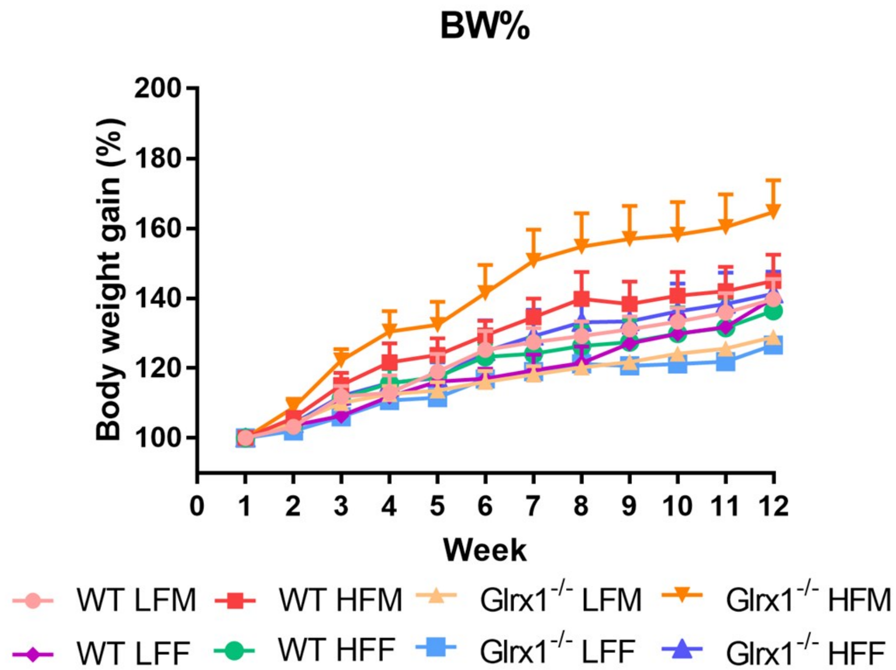


Figure S1. The highest weekly growth rate was in HF-fed Glrx1$^{-/-}$ male mice. Wild-type (WT) and Glrx1$^{-/-}$ mice in both genders were fed a high-fat diet (HF) or low-fat diet (LF). M and F represented male and female, respectively. Data are expressed as means ± SD.

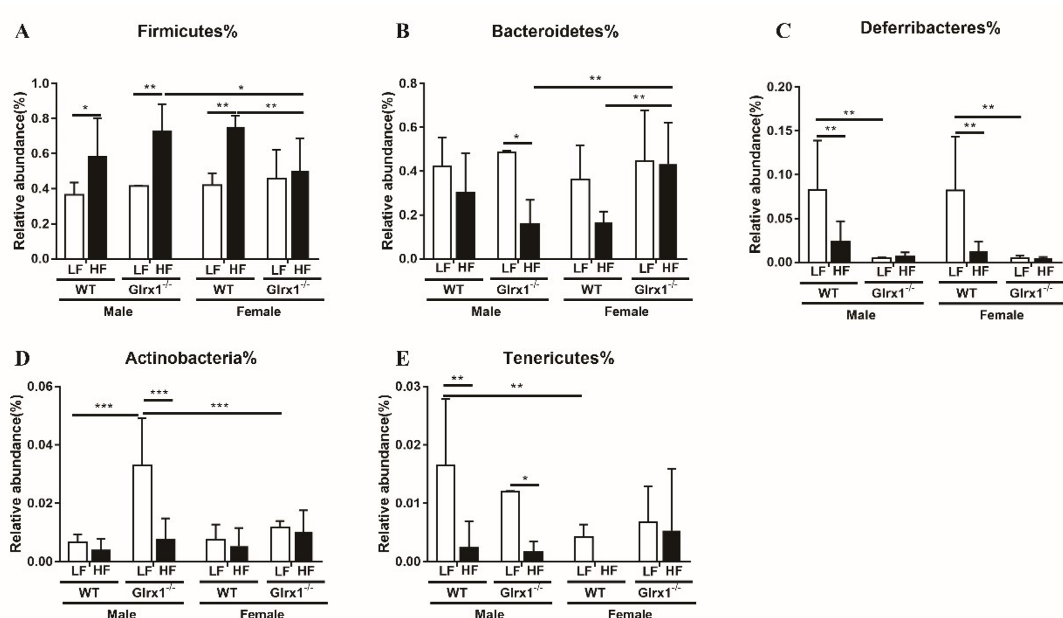


Figure S2. Relative abundance of core bacteria at phylum level in fecal samples. (A) *Firmicutes*, (B) *Bacteroidete*s, (C) *Deferribacteres*, (D) *Actinobacteria*, (E) *Tenericutes*. Effects of diet, genotype and gender on measured variables were evaluated by factorial analysis of variance. Least significant means were compared by Tukey's post hoc t test. The differences were considered significant at $P < 0.05$. Data are expressed as means ± SD. *P < 0.05, **P < 0.01, ***P < 0.001.

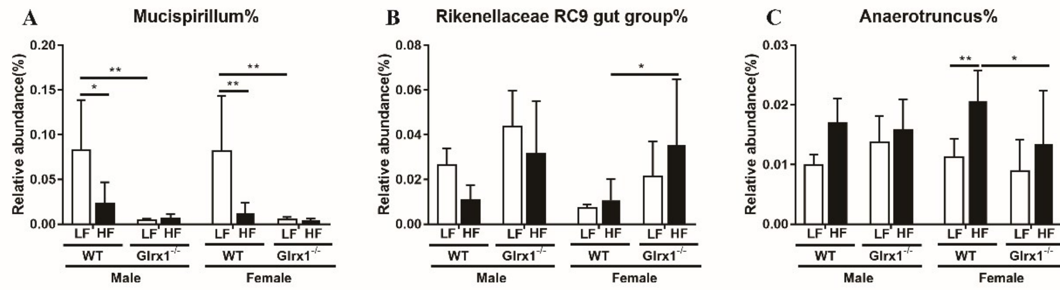


Figure S3. Bacterial community composition of the fecal samples at genus level. The relative abundance of *Mucispirillum* (A), *Rikenellaceae RC9 gut group* (B), *Anaerotruncus* (C) was affected by diet, genotype or gender. Effects of diet, genotype and gender on measured variables were evaluated by factorial analysis of variance. Least significant means were compared by Tukey's post hoc t test. The differences were considered significant at $P < 0.05$. Data are expressed as means ± SD. *P < 0.05, **P < 0.01, ***P < 0.001.

Supplementary file 1 Construction of Glrx1$^{-/-}$ mouse model

To create a Glrx1 knockout mice model by CRISPR/Cas9-mediated genome engineering, we created a strategy (Figure S4). Firstly, Cas9 mRNA and sgRNA were co-injected into zygotes. Glrx1 gene has 3 exons, with the ATG start codon in exon1 and TAA stop codon in exon2. gRNA directed Cas9 endonuclease cleavage of Glrx gene and created a DSB (double-strand break). Such breaks would be repaired and resulted in deletion of all domains. The pups would be genotyped by PCR, followed by sequence analysis.

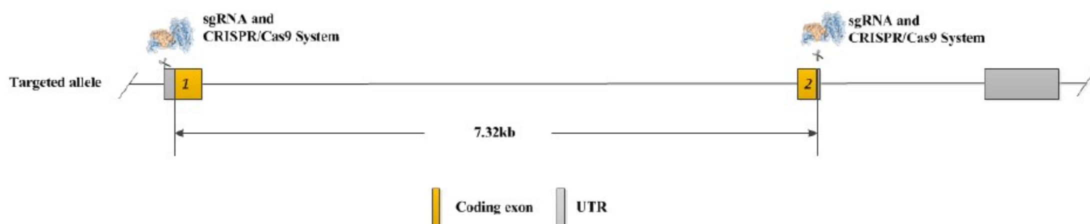


Figure S4. A strategy for constructing Glrx-1-Cas9-KO mice

Selection and design of gRNA of target Glrx1 gene

According to this strategy, sgRNA sequence was designed (Table S3). The sgRNA sequence was inserted in the intron of Glrx1 gene.

Table S3. sgRNA sequences

sgRNA name	Sequences	PAM
Glrx-3S1(forward)	CGGAGATGACACTTACTGATGGG	GGG
Glrx-5S1(forward)	GCTAAGCGCCGCTGCATTACCGG	CGG

Construction of SgRNA vector

A pUC57-sgRNA vector was cut by BsaI enzyme in a 37°C water bath for 1 h. The products were checked on 1% agarose gel and recovered. The sgRNA primers were then annealed. The annealed products were connected with the recovered digestion products, and then transformed into E. coli was. Monoclonal genes were selected for PCR. If the PCR results were positive, the products were sequenced for verification. The sgRNA vector was obtained (Figure S5).

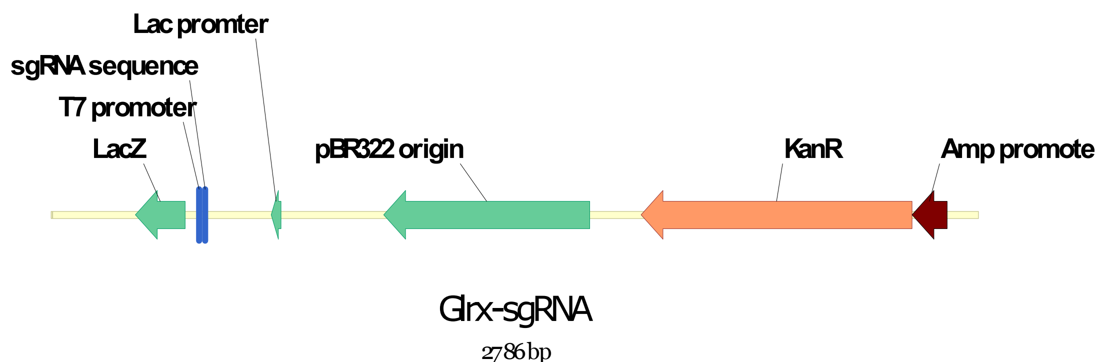


Figure S5. sgRNA vector

In vitro transcription of sgRNA and Cas9 mRNA

The sgRNA and Cas9 mRNA were transcribed in vitro using a commercial kit (AM1354, AM1908, purchased from Ambion). And the strategy was shown in Figure S6 and Table S4.

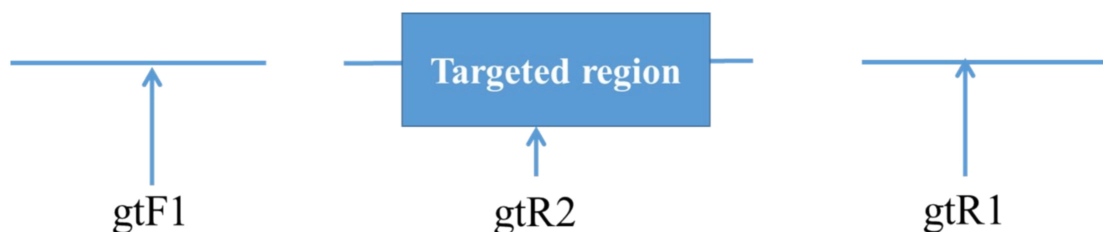


Figure S6. Cas9 knockout genotyping strategy (Fragment deletion)

Table S4. Primers of PCR strategy

Primer Name	Primer Sequences
2074-Glrx-gtF1	GTGGCAAAGTTCAGTCACAA
2074-Glrx-gtR1	TCCTCTTCTGGGCAACTGTC

Microinjection of fertilized eggs

Preparation of single cell fertilized eggs Equine chorionic gonadotropin (5 IU) was intraperitoneally injected in mice on the first day, and then human chorionic gonadotropin was injected after 46-48 hours. Then 2 female mice were mixed with a male mouse in a cage. Thrombolysis was detected on the morning of the 4th day. The fertilized eggs were obtained at 0.5 day of thrombolysis by sacrifice after cervical dislocation. The fallopian tubes were cut out, and the ovum was taken out with microscopic tweezers. After hyaluronidase digestion, plump embryos with uniform

cytoplasm were selected and cultured in M16.

Microinjection of fertilized eggs The selected fertilized eggs were transferred into M2 strip in a row (30 to 50 pieces). An injection dish was placed on the loading table of an inverted microscope so that the direction of the M2 droplet was perpendicular to the operator, that is, on the Y-axis. The injection tube was inserted into the cytoplasm and the Cas9 sgRNA system (sgRNA and Cas9 mRNA) was injected. The plasmid Cas9 D10A (plasmid # 42335, Addgene) was expressed. When loose cytoplasm was observed, the needle was rapidly withdrawn. After the injection, embryo was transferred to a petri dish containing M16 nutrient solution and recovered in a 37 °C and 5% CO2 incubator for 0.5 to 1.0 h. The fertilized eggs were transplanted into E0.5-day pseudopregesis recipients. F0 generation mice were born about 19-21 days after transplantation.

Birth and identification of F0 generation mice

A total of 39 pups were born and 38 survived. Tail cutting identification was conducted on mice of F0 generation 1 week after birth, and 7 positive mice of F0 generation were obtained, with black hair color and gender of 5 females and 2 males. The PCR conditions were listed in Table S5&S6.

Table S5. Regular PCR Program

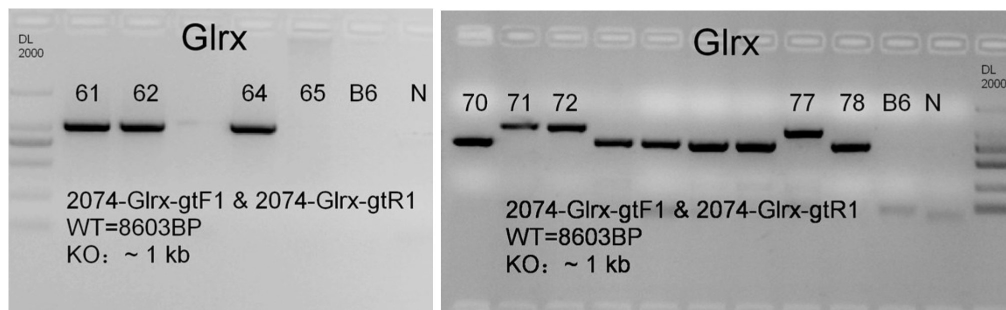
Step	Temp .	Time	Cycles
1	95°C	5min	
2	95°C	30sec	
3	58°C	30sec	
4	72°C	45sec	2-4,35×
5	72°C	5min	
6	10°C	Hold	

Table S6. Touchdown PCR Program

Step	Temp .	Time	Cycles	±Temp./Cycles
1	95°C	5min		
2	98°C	30sec		
3	65°C	30sec		-0.5
4	72°C	45sec	2-4,20×	
5	98°C	30sec		
6	55°C	30sec		
7	72°C	45sec	5-7,20×	
8	72°C	5min		
9	10°C	Hold		

F0 mice were sexually mature and bred, and F1 mice were identified

F0 generation mice were sexually mature at about 8 weeks old and bred with C57BL/6J backcross. F1 generation mice were identified by tail clippings at 1 week-old, and 6 positive F1 generation heterozygotes were obtained (Figure S7 & Table S7).



Note: B6:Negative control of which template is genomic DNA of C57BL/6J; N: Blank control without template; Bands of DL2000: 2000bp/1000bp/750bp/500bp/250bp/100bp.

Figure S7. F1 Identification of F1 generation mice

61#，62#，64#：

GCCCTTTAAAACTGAAGCATCCTACTTGGTAACTCCTCCTCCAAGGAGGTT

CCTTATTAAATGAGAGCTGCTGGCTAAGCGCC--------------7588bp---------ATACACATAGTTCTAGACATAAATACACAAAAAGATAACGT

73#，74#，75#：

CCAGTGTGCAATGGTAGGCCTAGGAAGTACTGACTCATACCAA--------------7898bp--------TAGCTAAGGATGGAAATTTGGGAAGTAT

Table S7. Glrx$^{+/-}$ F1 heterozygotes

ID	Gender	Color	Genotype	Female/Male	Generation
61	♂	Black	-7588bp/wt,E1-E2 (whole coding region) deleted	♂14	F1
62	♂	Black	-7588bp/wt,E1-E2 (whole coding region) deleted	♂14	F1
64	♀	Black	-7588bp/wt,E1-E2 (whole coding region) deleted	♂14	F1
73	♀	Black	-7588bp/wt,E1-E2 (whole coding region) deleted	♀7	F1
74	♀	Black	-7588bp/wt,E1-E2 (whole coding region) deleted	♀7	F1
75	♀	Black	-7588bp/wt,E1-E2 (whole coding region) deleted	♀7	F1

Propagation

Breeding these mice together leads to the production of F2 mice normally comprising 50% of heterozygotes, 25% of mutated homozygotes and 25% of WT mice. From perspective of Mendelian genetics, starting with 2 WT x het breeder pairs, the 20 homozygous mice were ready in about 9 months (from 3 rounds of breeding). The mouse lineage was established and maintained for phenotypic characterization and further functional work (Figure S8).

Figure S8. 7-week-old male Glrx1$^{-/-}$ mice