Ribose Accelerates Gut Motility and Suppresses Mouse Body Weight Gaining

Supplementary Materials

Fig.S1. Generation of Rbks knock-out mice. (A) Schematic representation of the knock-out strategy for Rbks gene. Panel 1, genomic DNA fragment of *Rbks* gene containing exon 2-4. Panel 2, schematic structure of the *Rbks* targeting vector. Panel 3, genomic structure of $Rbks^{galeo}$ allele after homologuous recombination. Panel 4, genomic structure of $Rbks^{F}$ allele after removal of the *E coli. β-gal* reporter gene and the neomycin expression cassette by Flpe recominase. Panel 5, genomic structure of $Rbks^{\Delta 2}$ allele after removal of *Rbks* exon2 by cre-loxP recombination. The exons are numbered and represented by solid boxes. LoxP and FRT sequences are indicated by black and open triangles, respectively. The grey and black boxes before *β-gal* denote an En2 splice acceptor and an ECMV IRES, respectively. (B) PCR analysis of genomic DNA extracted from $Rbks^{galeo/+}$ mice. Upper panel, three primers PCR (*5'A* & *3'A* & *LAR3*) was applied to distinguish $Rbks^{galeo/+}$ mice from the wild type litter mates. The *β-gal* reporter gene and the neomycin expression cassette were detected by primer pairs *5'A* & *LAR3*, while the *Rbks* wild type allele was detected by primer pairs *5'A* & *3'A*. Lower panel, the loxP locus after exon2 was identified by primer pairs *rbks-F* & *R*. (C) PCR analysis to distinguish $Rbks^{\Delta exon2}$ mutants after cre-loxP recombination. *Rbks* wild type allele was detected by primer pairs *5'A* and *3'A*, while $Rbks^{\Delta 2}$ allele was detected by *2F* and *2R*. All primer pairs are indicated as arrows in (A). The depletion of exon2 was confirmed also at the RNA level by RT-PCR (D).The primer pairs for RT-PCR located on exon2 and exon7.

Fig.S2. **No overt morphological changes were observed after *Rbks* gene depletion.** Gut development was assessed by the small intestine length (A) and intestinal histological morphology (B). There was not a noticeable change in the small intestine length, which was normalized by the body weight, when compare *Rbks* knock-out mice and their litter mate controls. In addition, no overt differences were observed in intestinal hematoxylin and eosin staining sections. Black bar: 100μm.

Figure.S3. Ribose 5 weeks treatment didn't affect the fecal water content and the blood glucose level. (A and B) The fecal water content and the blood glucose level were measured after 5 weeks ribose drinking trial. There were no overt changes between ribose groups and controls. Significances indicated are based on Student's t-test. Data represent the average ± s.e.m.

Figure.S4. Liver dysfunction was not detected after *Rbks* gene depletion or with ribose treatments. (A and B) The liver impairment, indicated by the serum albumin/globulin ratio, was not detected in *Rbks* mutant mice either with normal chow (A) or high fat diet feeding (B). Male *Rbks* mutants (8 weeks old for normal chow, 7 months old for high fat diets) were used for the above blood chemistry tests (n=4). (C-D) After 72 hours ribose treatments in the drinking water and high fat diet challenging, the triglyceride level in the liver was not disturbed in wild type male mice (C). Also, the expression levels of the lipid metabolism related genes were not obviously changed between ribose groups and distilled water controls (D). (E-F) After 3 weeks ribose drinking and high fat diet challenging, the liver weight normalized by body weight (E) and the liver triglyceride level (F) was not altered when compared to the distilled water controls in wild type male mice. Significances indicated are based on Student's t-test. Data represent the average ± s.e.m.

Figure.S5. Kidney dysfunction was not detected after *Rbks* gene depletion. The kidney impairments, suggested by the blood urine nitrogen (BUN) level, were not detected in *Rbks* mutant mice either with normal chow (A) or high fat diet feeding (B). Male *Rbks* mutants (8 weeks old for normal chow, 7 months old for high fat diets) were used for the above blood chemistry tests (n=4). Significances indicated are based on Student's t-test. Data represent the average ± s.e.m.

Fig. S1. Generation of *Rbks* knock-out mice.

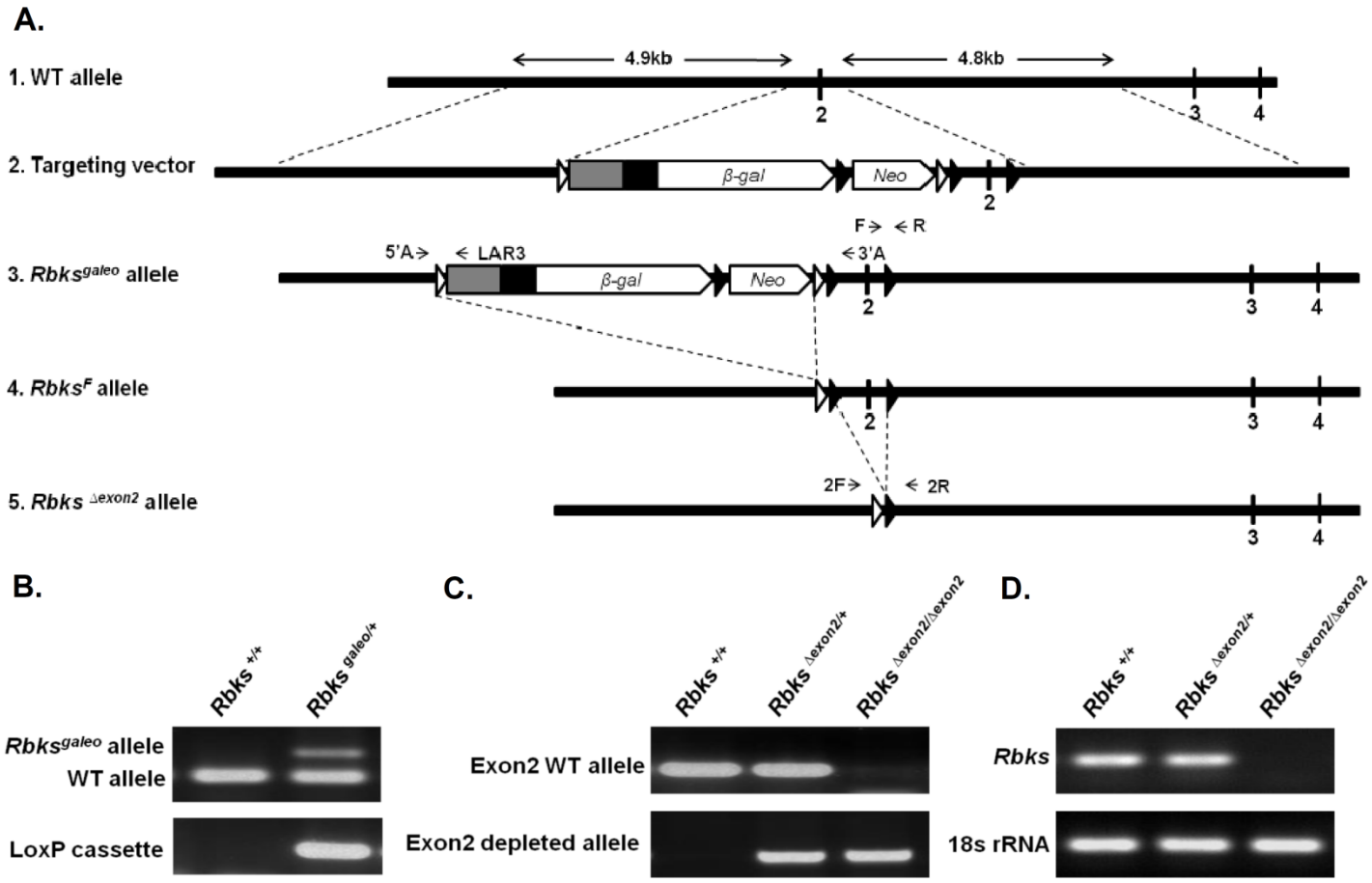

Fig. S2. No overt morphorlogical changes were observed after *Rbks* gene depletion.

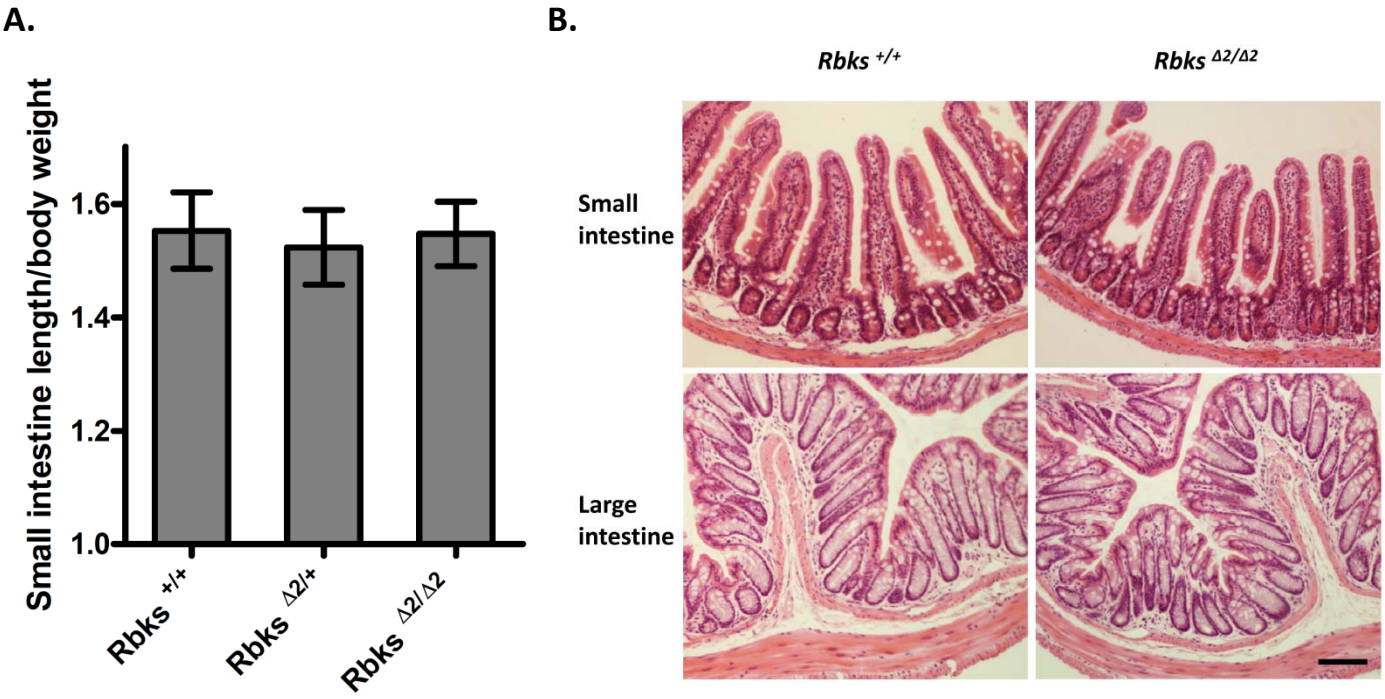

Fig. S3. Ribose 5 weeks treatment didn't affect fecal water content or blood glucose level.

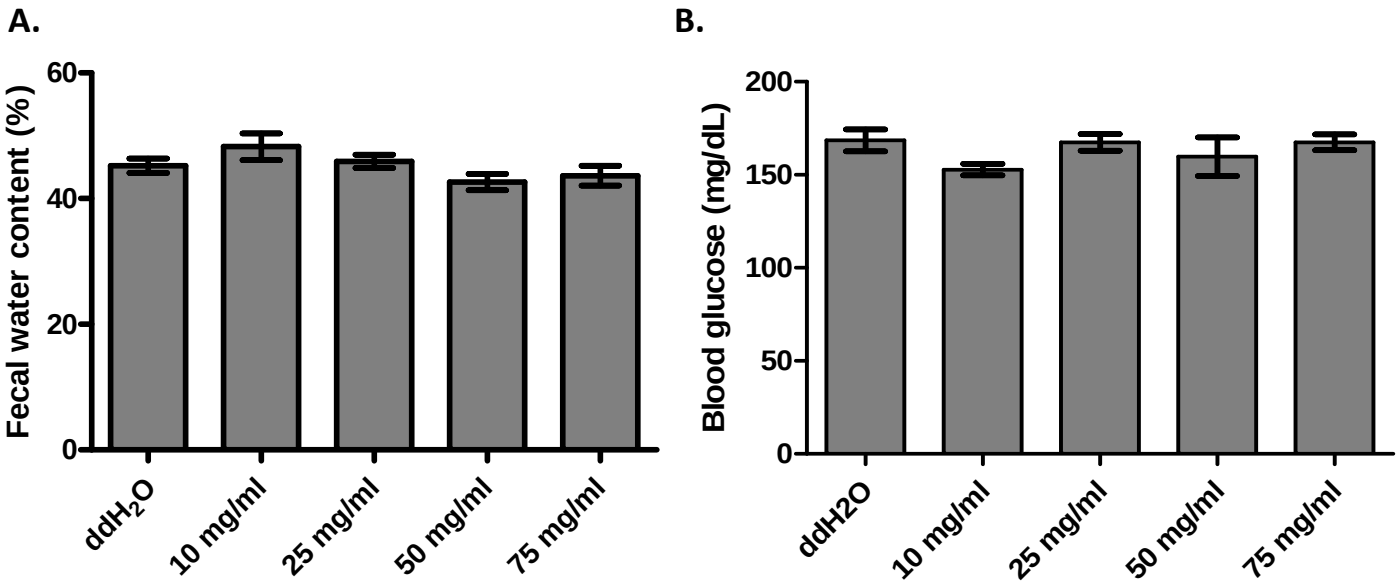

Fig. S4. Liver function was not disturbed after *Rbks* gene depletion or with ribose treatment

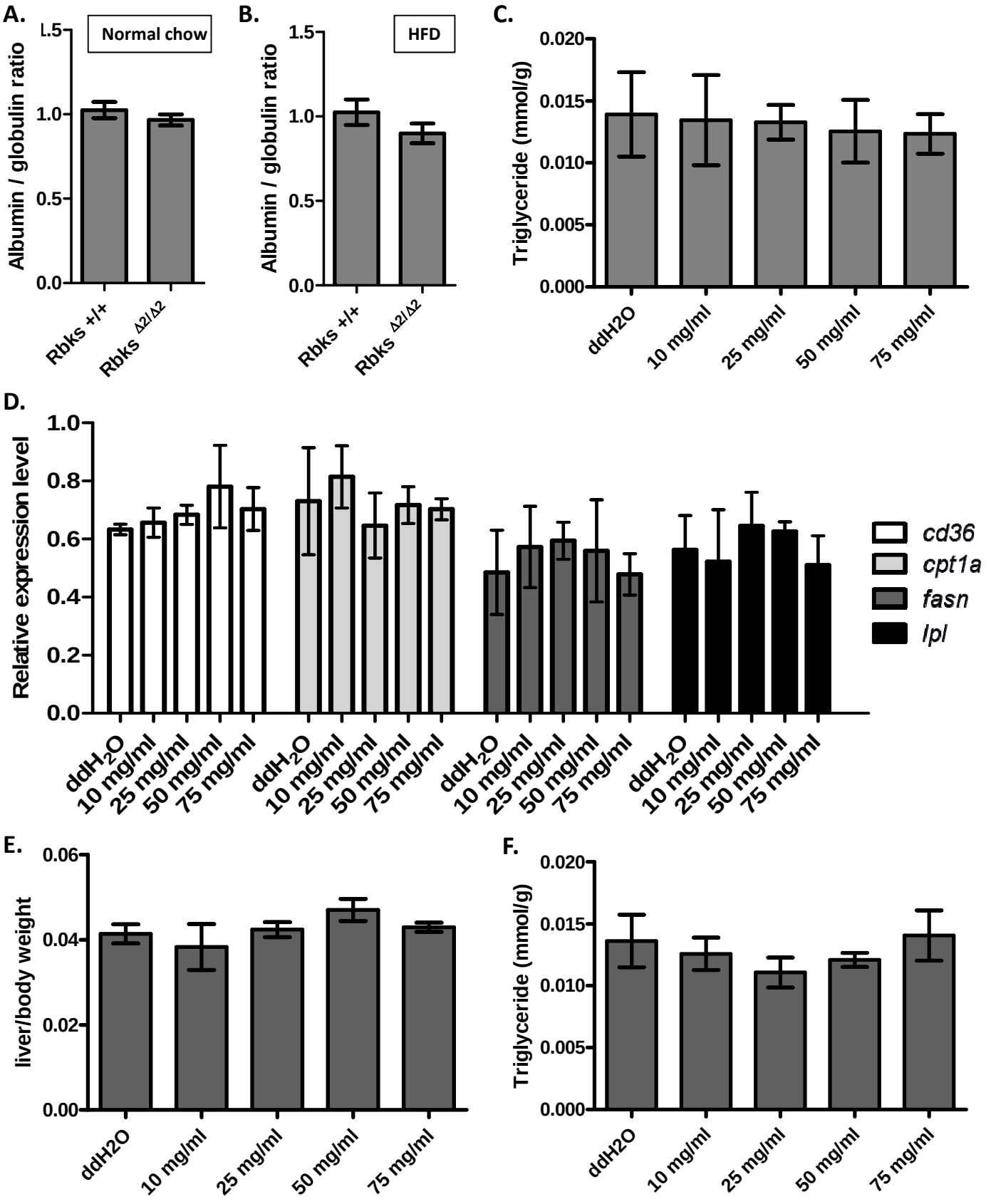

Fig. S5. Kidney function was not disturbed after *Rbks* gene depletion or with ribose treatment