Supplemental Figures and Figure Legends

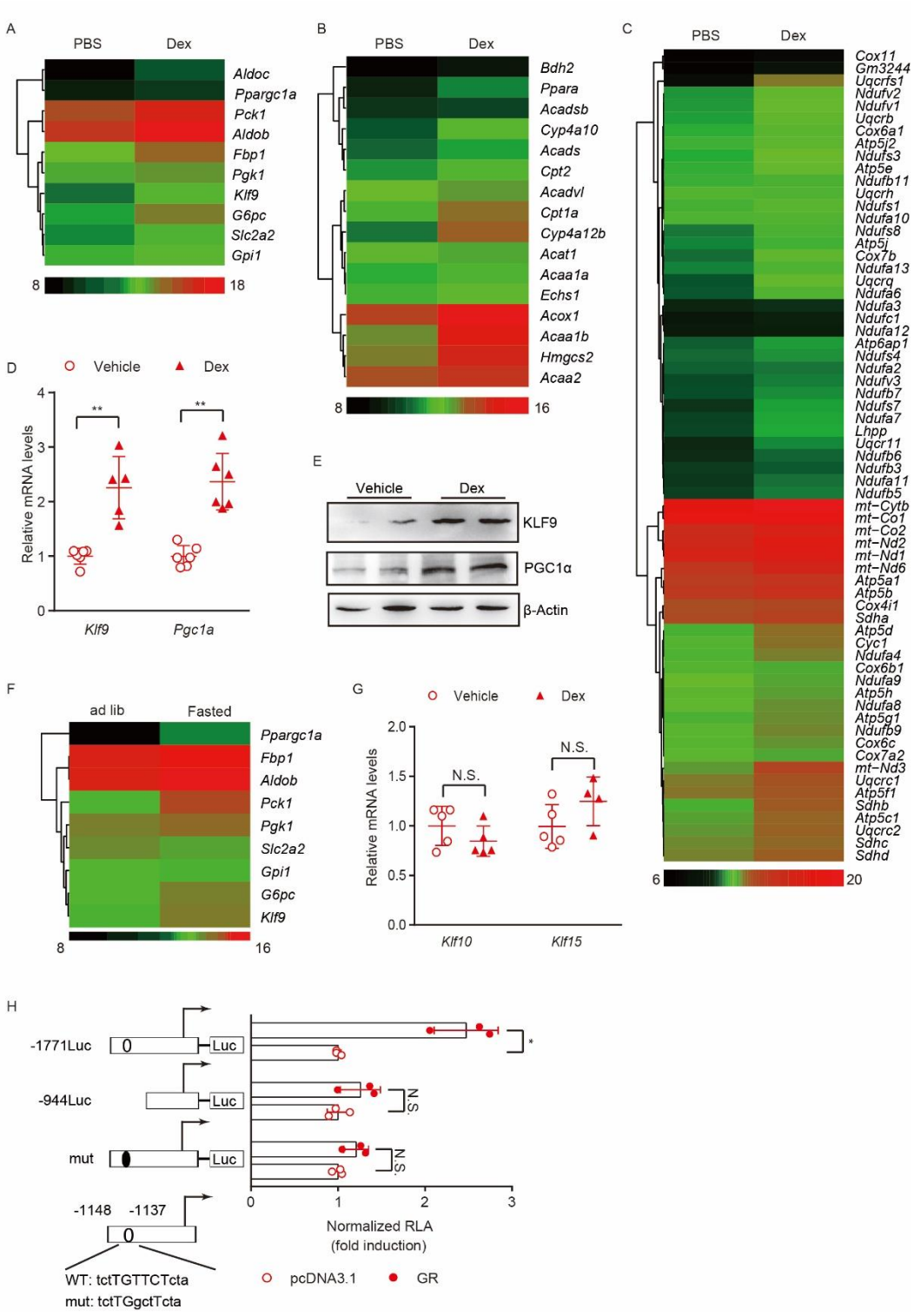

Figure S1: (Related to Figure 1)

Dexamethasone regulates the expression of KLF9 and other genes involved in glucose, lipid and energy metabolism in the livers of C57 BL/6J mice.

(A-C) Heat maps showing differentially expressed genes involved in glucose

942 metabolism (A), fatty acid oxidation (B) and energy metabolism (C) by RNA-seq
943 analysis of livers of C57BL/6J mice treated with Dex (1 mg/kg) every other day for two
944 months or saline. (D, E) Quantitative PCR and Western blot analysis of *Pgc1a* and *Klf9*
945 in the livers of C57BL/6J mice described in A. (F) Heat map showing differentially
946 expressed gluconeogenic genes by Affymetrix microarray analysis of hepatic RNA of
947 wild-type mice under feeding condition (control) or fasted for 24h. (G) Quantitative
948 PCR analysis of *Klf10* and *Klf15* in mouse primary hepatocytes treated with 100 nM
949 DEX. (H) The wild-type putative GR-binding element and its mutant sequence in the
950 *Klf9* promoter region. A series of *Klf9* promoters fused to the luciferase reporter gene
951 (-1771Luc: wild-type promoter; -944Luc: 5'-deletion promoter without GRE; mut: -
952 1771Luc with GRE mutated) were co-transfected into HepG2 cells together with
953 pcDNA3.1 (control) or GR expression plasmids. DEX (100 nM) was added for the final
954 24 hr. At 48 hr after transfection, the cells were harvested for luciferase assays, and the
955 relative luciferase activity (RLA) was corrected for Renilla luciferase activity and
956 normalized to the control activity. Throughout, data are presented as the mean ± s.e.m.
957 *P < 0.05, **P < 0.01 by the two-tailed Student's t-test (D, G, H).

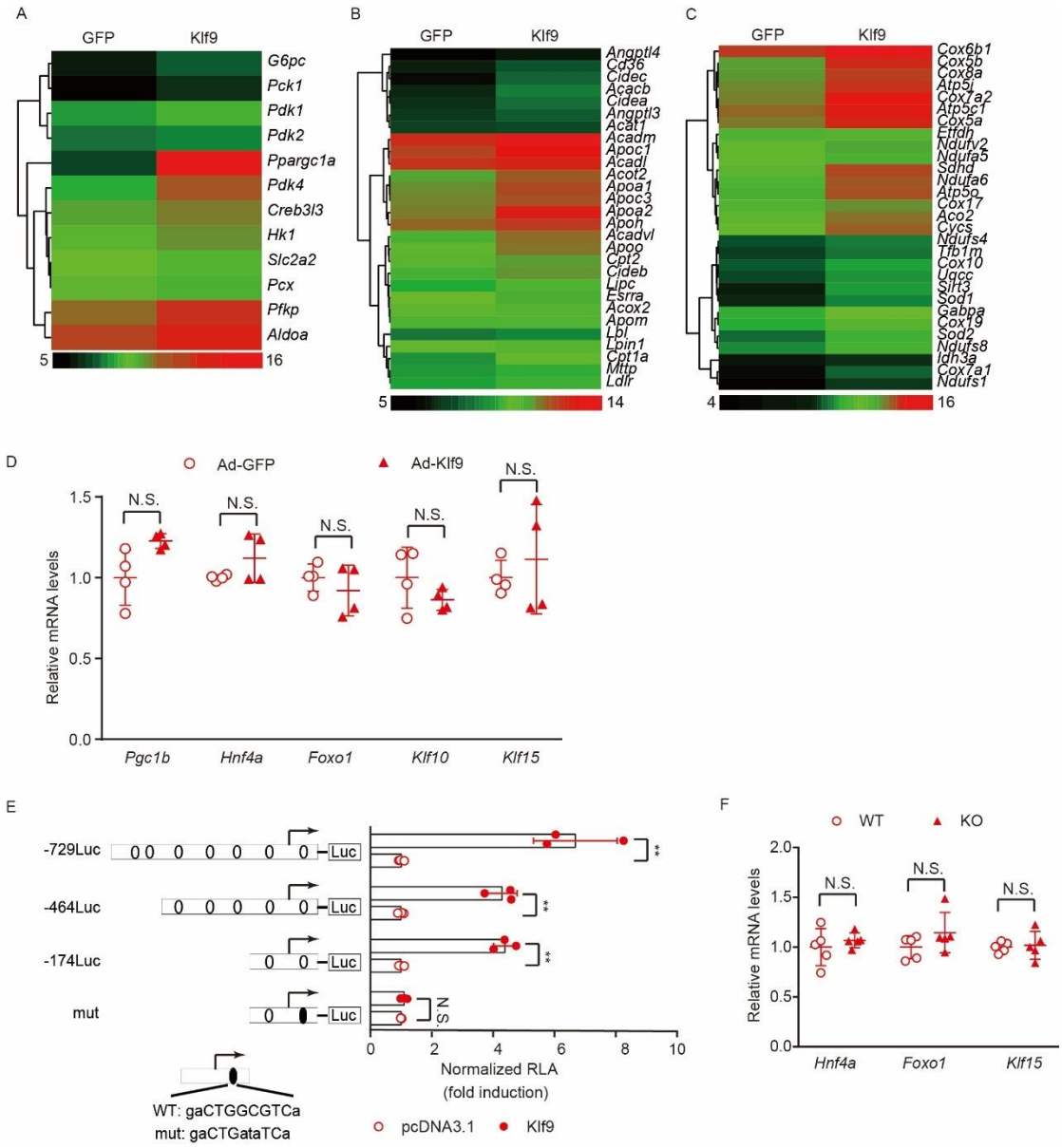

Figure S2: (Related to Figure2)

KLF9 activates the expression of *Pgc1a* and its downstream target genes involved in gluconeogenesis, fatty acid oxidation, and energy metabolism in primary hepatocytes.

(A-C) Heat maps showing differentially expressed genes involved in glucose metabolism (A), lipid metabolism (B), and energy metabolism (C) by Affymetrix microarray analysis of hepatocytes infected with Ad-Klf9 or Ad-GFP. At 24 hr after infection, cells were collected for RNA extraction. (D) Quantitative PCR analysis of *Pgc1b*, *Hnf4a*, *Foxo1*, *Klf10* and *Klf15* in primary hepatocytes infected with Ad-GFP, Ad-Klf9. (E) (Top) 5'-Deletion series of the mouse *Pgc1α* promoters fused to luciferase reporter constructs (-729Luc, -464Luc, -174Luc, mut) were co-transfected into HepG2

cells, together with pcDNA3.1 (control) or *Klf9* expression plasmids. After 48 hr, the cells were harvested, and the RLA was corrected for Renilla luciferase activity and normalized to the control activity. (Bottom) The wild-type putative KLF9-binding site and its mutant sequence in the *Pgc1a* promoter region. (F) Quantitative PCR analysis of *Hnf4a*, *Foxo1* and *Klf15* in primary hepatocytes isolated from liver of WT mice and global *Klf9* KO mice.Throughout, data are presented as the mean ± s.e.m. *P < 0.05 by the two-tailed Student's t-test (D-F). Throughout, data are presented as the mean ± s.e.m. **P < 0.01 by the two-tailed Student's t-test (D-F).

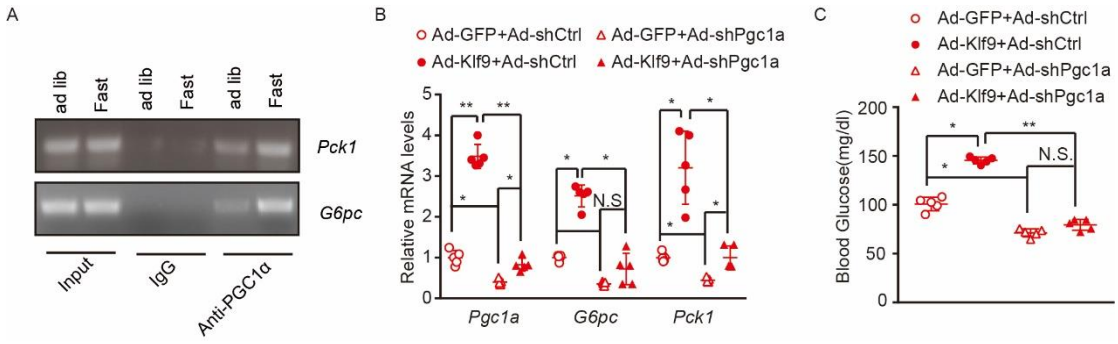

Figure S3: (Related to Figure3)

PGC1α mediates KLF9 stimulatory effects on hepatic gluconeogenesis

(A) ChIP assay performed as described in Methods showing that fasting ehances endogenous PGC1α protein binding to the proximal promoter region of the *Pck1* and *G6pc* gene harbouring a Foxo1 binding element. (B) Quantitative PCR analysis of *Pgc1a*, *G6pc* and *Pck1* in the liver of mice infected with indicated adenoviruses. (C) Blood glucose levels in 6 hr-fasted mice treated as in (B) (*n*=5/group). Throughout, data are presented as the mean ± s.e.m. *P < 0.05, **P<0.01 by the two-way ANOVA (B, C).

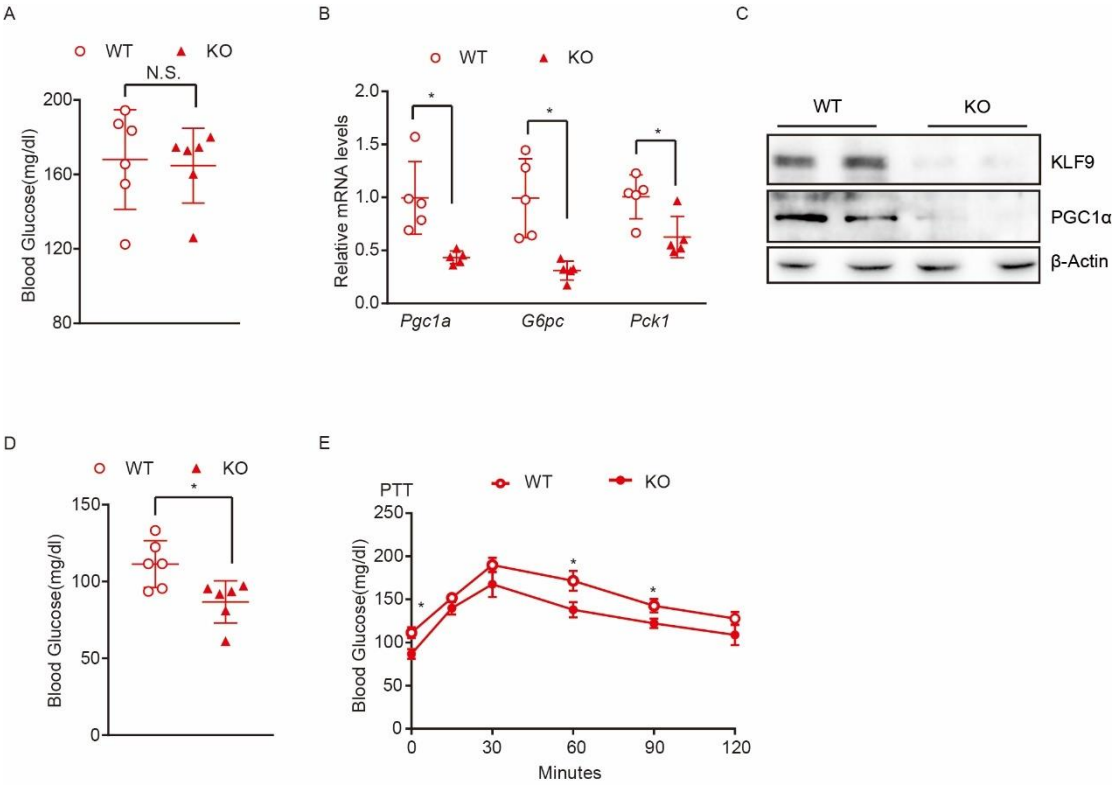

Figure S4: (Related to Figure4)

Global *Klf9*-mutant mice display decreased blood glucose levels.

(A) Blood glucose in 6 hr-fasted WT mice and global *Klf9*-mutant mice (*Klf9* KO mice) at 5 months of age (*n*=5/group). (B) Quantitative PCR analysis of *Pgc1a*, *G6pc* and *Pck1* in the livers described in (A). (C) Representative Western blot analysis of hepatic KLF9 and PGC1α in the mice described in (A). (D) Blood glucose in 16 hr-fasted WT mice and *Klf9* KO mice at 5 months of age (*n*=6/group). (E) Plasma Blood glucose during the PTT of WT mice and *Klf9* KO mice (*n*=6/group). Throughout, data are presented as the mean ± s.e.m. *P < 0.05 by the two-tailed Student's t-test (A, B, D, E).

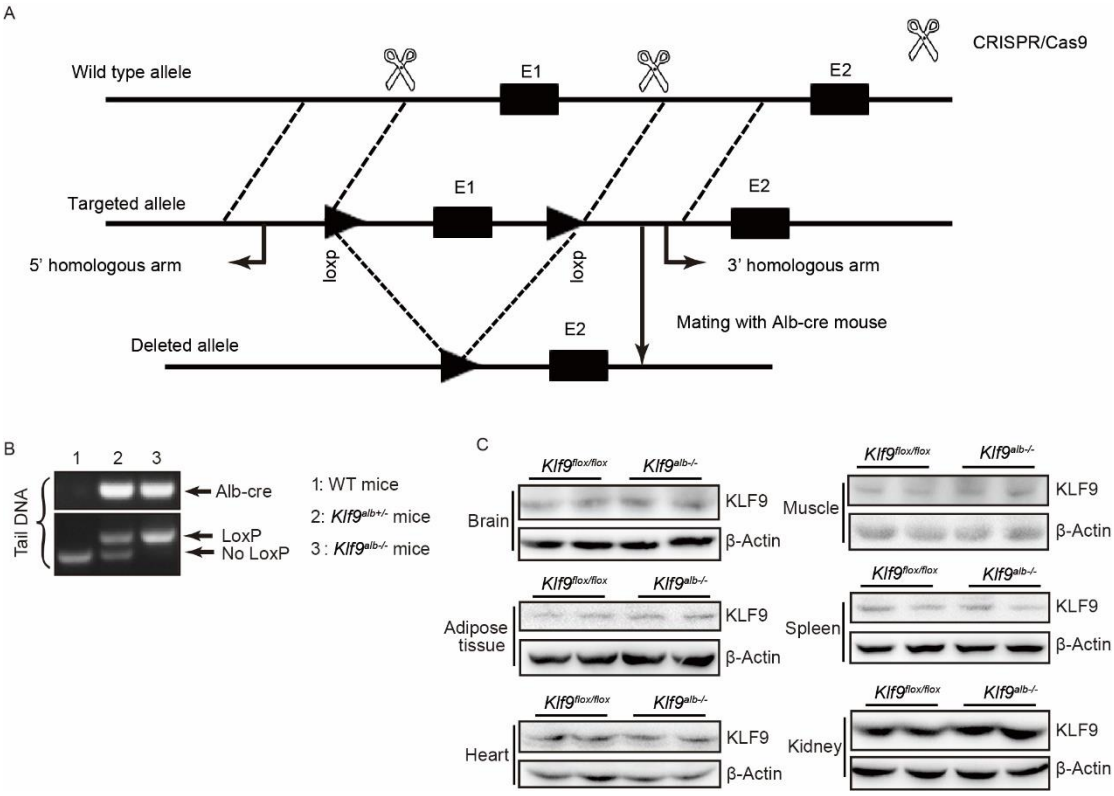

Figure S5: (Related to Figure4)

Generation and characterization of liver-specific *Klf9*-deficient mice.

(A) Generation of liver-specific *Klf9*-deficient mice. *Klf9*$^{flox/flox}$ mice were generated by the Crispr/Cas9 system with two loxP sites flanking exon 1 of the *Klf9* gene. These mice were subsequently bred to Albumin-Cre transgenic mice to obtain *Klf9*$^{alb-/-}$ mice, leading to excision of exon 1 of *Klf9* within the liver. (B) PCR-based genotyping confirmed DNA recombination in the liver of *Klf9*$^{alb-/-}$ mice. (C) Western blot analysis showing that the KLF9 expression remained unchanged in the tissues examined.

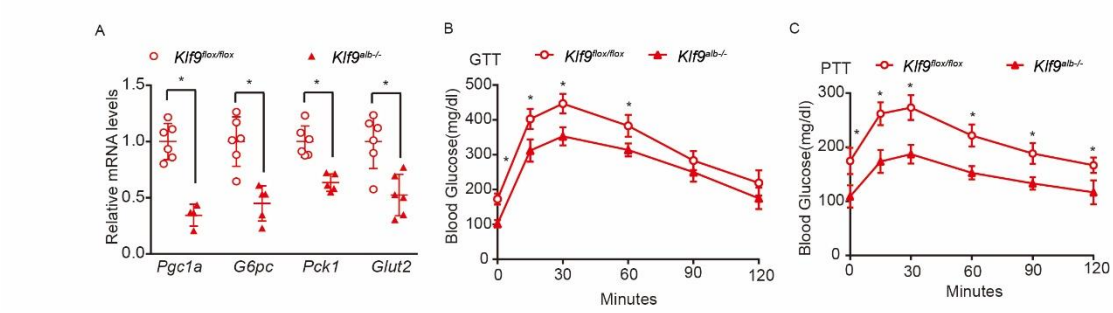

Figure S6: (Related to Figure4)

The glucose metabolic phenotype of *Klf9*$^{alb-/-}$ *mice* fed a high fat diet

(A) Quantitative PCR analysis of *Pgc1a*, *G6pc*, *Pck1* and *Glut2* in the livers of *Klf9*$^{flox/flox}$ mice and *Klf9*$^{alb-/-}$ mice fed a high fat diet for 8 weeks (*n*=6/group). (B) Blood glucose levels during the GTT (B) and PTT (C) of *Klf9*$^{flox/flox}$ mice and *Klf9*$^{alb-/-}$ mice described in (A). Throughout, data are presented as the mean ± s.e.m. *P < 0.05 by the two-tailed Student's t-test (A-C).

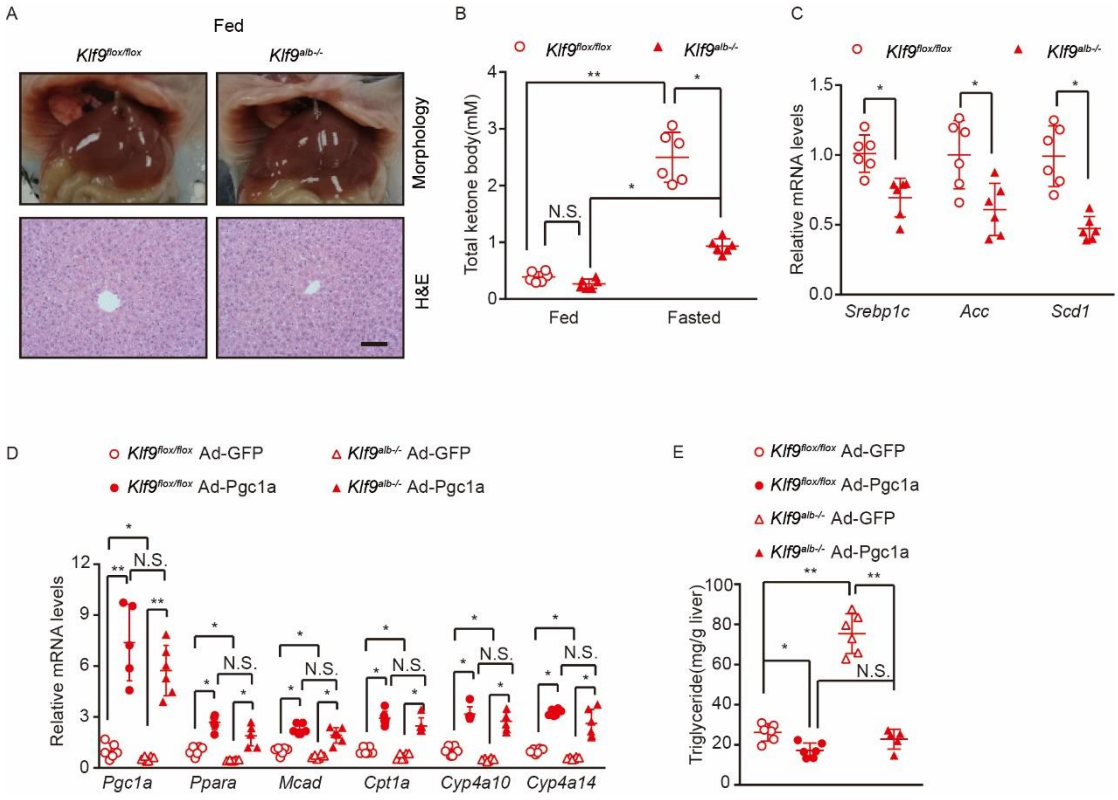

Figure S7: (Related to Figure5)

***Pgc1a* overexpression reverses hepatic steatosis phenotype in *Klf9*$^{alb-/-}$ mice.**

(A) Representative gross morphology and H&E staining of livers from ad libitum fed *Klf9*$^{flox/flox}$ mice and *Klf9*$^{alb-/-}$ mice. (B) Total serum ketone body levels in *Klf9*$^{flox/flox}$ mice and *Klf9*$^{alb-/-}$ mice under the feeding and fasting conditions (*n* =6/group). (C) Quantitative PCR analysis of *Srebp1c*, *Acc*, *Scd1* in livers of the 24-fasted mice. (D) Quantitative PCR analysis of *Pgc1a*, *Ppara*, *Mcad*, *Cpt1a*, *Cyp4a10* and *Cyp4a14* in livers of 24-fasted *Klf9*$^{flox/flox}$ mice and *Klf9*$^{alb-/-}$ mice infected with Ad-GFP or Ad-Pgc1a (*n*=6/group). (E) Biochemical analysis showing hepatic TG in 24-fasted *Klf9*$^{flox/flox}$ mice and *Klf9*$^{alb-/-}$ mice treated as in (D) Scale bars, 20 μm (H&E). Throughout, data are presented as the mean ± s.e.m. **P < 0.05, **P<0.01 by the two-tailed Student's t-test (C) or by the two-way ANOVA (B, D-E).

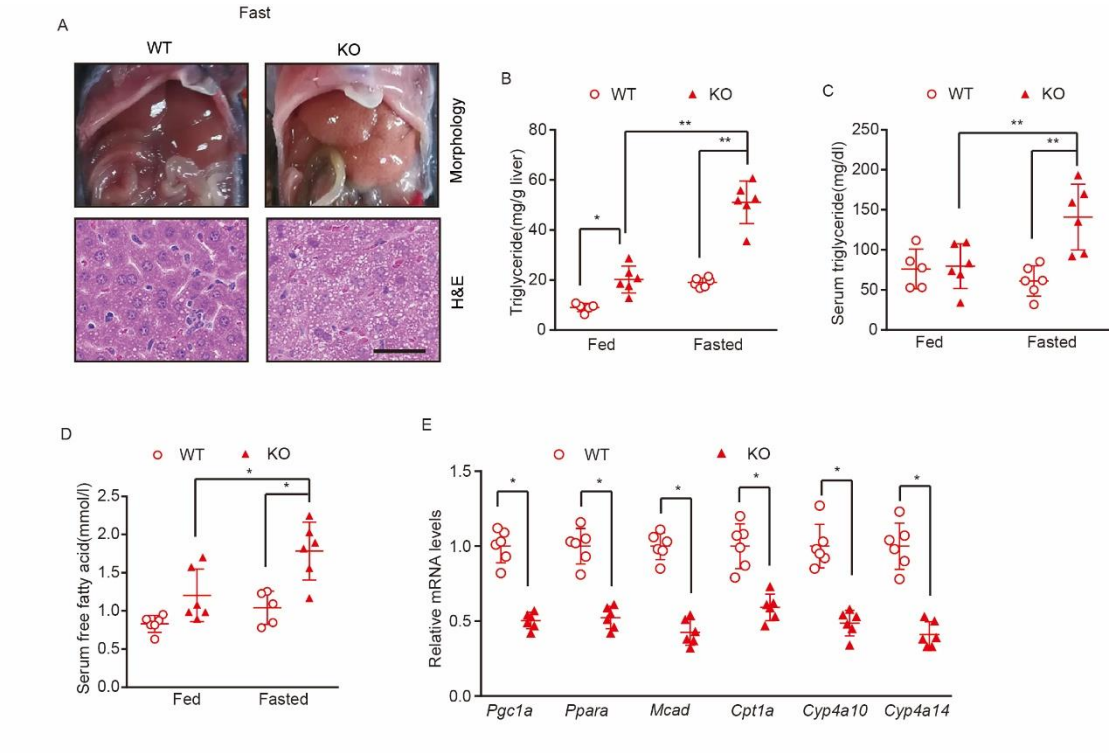

Figure S8: (Related to Figure5)

Global *Klf9*-mutant mice display fasting-induced hepatic steatosis(A) Representative gross morphology and H&E staining of livers from 24 hr-fasted WT mice and global *Klf9* KO mice. (B-D) Biochemical analysis showing hepatic TG (B), serum TG (C) and FFA content (D) in ad libitum-fed or 24 h-fasted WT mice and *Klf9* KO mice. (E) Quantitative PCR analysis of *Pgc1a*, *Ppara*, *Mcad*, *Cpt1a*, *Cyp4a10* and *Cyp4a14* in 24-fasted WT mice and *Klf9* KO mice (*n*=6/group) Scale bars, 20 μm (H&E). Throughout, data are presented as the mean ± s.e.m. *P < 0.05, **P<0.01 by the two-tailed Student's t-test (E) or by the two-way ANOVA. (B-D）.

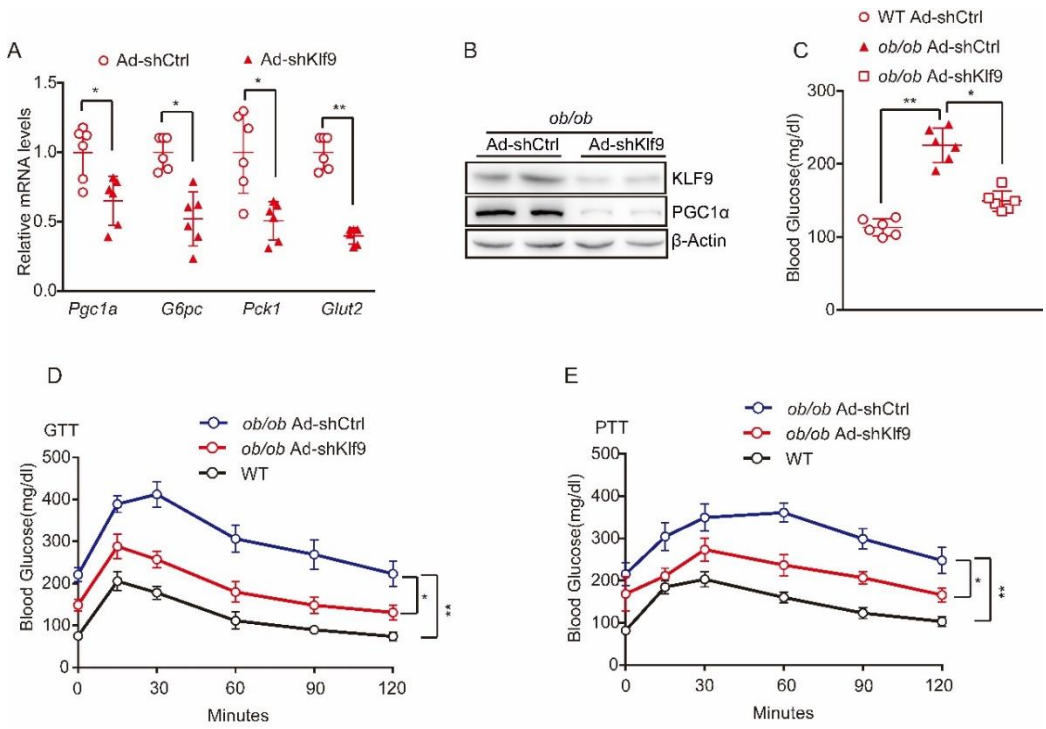

Figure S9: (Related to Figure7)

Hepatic *Klf9* knockdown decreases blood glucose in ob/ob mice.

(A) Quantitative PCR analysis of *Pgc1a*, *G6pc*, *Pck1* and *Glut2* in the livers of *ob/ob* mice injected with Ad-shCtrl or Ad-shKlf9 (*n*=6/group). Seven days after injection, the mice fasted for 6 hr were sacrificed. (B) Representative Western blot analysis of KLF9 and PGC1α from the livers of *ob/ob* mice treated as in (A). (C) Blood glucose in the 6 hr-fasted *ob/ob* or WT mice treated as in (A) (*n*=6/group). (D, E) Plasma Blood glucose during the GTT (D) and PTT (E) of *ob/ob* mice injected with Ad-shCtrl or Ad-shKlf9 or WT mice (*n*=5/group). Throughout, data are presented as the mean ± s.e.m. *P < 0.05, **P<0.01 by the two-tailed Student's t-test (A) or by the one-way ANOVA（C-E）.

Supplemental Table 1. Quantitative-PCR primer sequences.

Primers used in quantitative-PCR		
gene	Primer name	Sequence
Mouse *Klf9*	Forward	5'-*CGAGCGGCTGCGACTACCTG* -3'
Mouse *Klf9*	Reverse	5'-*GGGCTGTGGGAAGGACTCGAC* -3'
Mouse *Pgc1a*	Forward	5'-*GACATAGAGTGTGCTGCTCTG* -3'
Mouse *Pgc1a*	Reverse	5'- *CATTGTTGTACTGGTTGGATATG* -3'
Mouse *Pck1*	Forward	5'- *CAGGATCGAAAGCAAGACAGT* -3'
Mouse *Pck1*	Reverse	5'- *AAGTCCTCTTCCGACATCCAG* -3'
Mouse *G6pc*	Forward	5'- *GACTGGTTCAACCTCGTCTTC* -3'
Mouse *G6pc*	Reverse	*5'- GTCTCACAGGTGACAGGGAAC -3'*
Mouse *Glut2*	Forward	*5'- GGCTAATTTCAGGACTGGTT-3'*
Mouse *Glut2*	Reverse	5'- *TTTCTTTGCCCTGACTTCCT* -3'
Mouse *Mcad*	Forward	5'-*AACACTTACTATGCCTCGATTGCA*-3'
Mouse *Mcad*	Reverse	5'-*CCATAGCCTCCGAAAATCTGAA*-3'
Mouse *Pparg*	Forward	5'-*ACAAGGCCTCAGGGTACCA*-3'
Mouse *Pparg*	Reverse	5'-*GCCGAAAGAAGCCCTTACAG*-3'
Mouse *Cpt1a*	Forward	5'-*GAACCCCAACATCCCCAAAC*-3'
Mouse *Cpt1a*	Reverse	5'-*TCCTGGCATTCTCCTGGAAT*-3'
Mouse *Cyp4a10*	Forward	5'-*TCCAGCAGTTCCCATCACCT*-3'
Mouse *Cyp4a10*	Reverse	5'-*TTGCTTCCCCAGAACCATCT*-3'
Mouse *Cyp4a14*	Forward	5'-*ACCTGTTTCCCATCTCGCTT*-3'
Mouse *Cyp4a14*	Reverse	5'- *ACCAGATGGCAACATGCTTC*-3'
Mouse *Srebp1c*	Forward	5'-*GGAGCCATGGATTGCACATT*-3'
Mouse *Srebp1c*	Reverse	5'-*GGCCAGGGAAGTCACTGT*-3'
Mouse *Acc*	Forward	5'-*AGGAAGATGGCGTCCGCTCTG*-3'
Mouse *Acc*	Reverse	5'-*GGTGAGATGTGCTGGGTCAT*-3'
Mouse *Scd1*	Forward	5'-*AGCTCTACACCTGCCTCTTCG*-3'
Mouse *Scd1*	Reverse	5'-*AGCCGTGCCTTGTAAGTTCTG*-3'
Mouse *36b4*	Forward	5'-*GAGGAATCAGATGAGGATATGGGA*-3'
Mouse *36b4*	Reverse	5'- *AAGCAGGCTGACTTGGTTGC*-3'

Supplemental Table 2. ChIP PCR primer sequences.

Primers used in ChIP		
Pgc1a-proximal region	forward	5'-*AAGACAGGTGCCTTCAGTTC*-3'
Pgc1a-proximal region	reverse	5'-*CCAGGAATCATTGCATCTGA* -3'
Pgc1a-distal region	forward	5'-*AAGTGCTGAGAGTTGGTTATGTC*-3'
Pgc1a-distal region	reverse	5'-*CAAGAATGTCCAGGGAATGAAG*-3'
Klf9- proximal region	forward	5'-*AGAGTCAGGAATCGGGAACC*-3'
Klf9- proximal region	reverse	5'-*CCCATAAACTGAGACCAATAA*-3'
Klf9- distal region	forward	5'-*CTCACTCTGTAGACCAGGCT*-3'
Klf9- distal region	reverse	5'-*CTGAGATGAGTGCTGGGTTG*-3'
Pck1 (-438 to -326)	forward	5'-*GTGGGAGTGACACCTCACAGC*-3'
Pck1 (-438 to -326)	reverse	5'-*AGGGCAGGCCTAGCCGAGACG*-3'
G6pc (-251 to -31)	forward	5'-*GCCTCTAGCACTCAAGCAGTG*-3'
G6pc (-251 to -31)	reverse	5'-*TGTGCCTTGCCCCTGTTTTATATG*-3'

Supplemental Table 3. Plasmids construction primer sequences.

Primers used in plasmids construction		
GR	forward	5'*GATCGTAGGATCCCCACCATGGACTACAAAGACGATGACGACAAGATG GACTCCAAAGAATCATTA*-3'
GR	reverse	5'-*GATCGTACTCGAGTCACTTTTGATGAAACAGAA*-3'
Klf9	forward	5'-*GAATTCCCACCATGTCCGCGGCCGCCTACA*-3'
Klf9	reverse	5'-*CTCGAGTCACAAGGGGCTGGC*-3'
Klf9 promoter -1771Luc	forward	5'-*ACGCGTCCCCAGAGAACCCGGTGGAGGTATC*-3'
Klf9 promoter -1771Luc	reverse	5'-*CTCGAGAGCCACGAAGTCCATGTAGGCGG*-3'
Klf9 promoter -944Luc	forward	5'-*ACGCGTAAGATGGCGGTGCTTTTGTGT*-3'
Klf9 promoter -944Luc	reverse	5'-*CTCGAGAGCCACGAAGTCCATGTAGGCGG*-3'
Klf9 mut	forward	5'-*GTAGACCAGTCT TGGCTTCTACAATTGTGAA*-3'
Klf9 mut	reverse	5'-*TTCACAATTGTAGAAGCCAAGACTGGTCTAC*-3'
Pgc1a promoter -729Luc	forward	5'-*TACTACGCGTGGTTTTGTTGACTAAACATGG*-3'
Pgc1a promoter -729Luc	reverse	5'-*TACTCTCGAGCCAGCTCCCGAATGACGC*-3'
Pgc1a promoter -464Luc	forward	5'-*TACTACGCGTTGAGTCTGGGGCTACTTGGA*-3'
Pgc1a promoter -464Luc	reverse	5'-*TACTCTCGAGCCAGCTCCCGAATGACGC*-3'
Pgc1a promoter -174Luc	forward	5'-*TACTACGCGTACTTCACTGAGGCAGAGGGC*-3'
Pgc1a promoter -174Luc	reverse	5'-*TACTCTCGAGCCAGCTCCCGAATGACGC*-3'
Pgc1a mut	forward	5'-*GAAAAAGCTTGACTGataTCATTCGGGAGC*-3'
Pgc1a mut	reverse	5'-*GCTCCCGAATGAtatCAGTCAAGCTTTTTC*-3'

Full unedited gel for Figure 1

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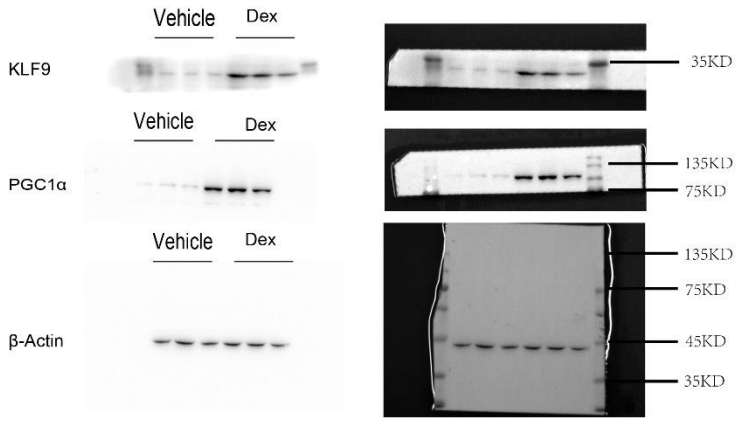

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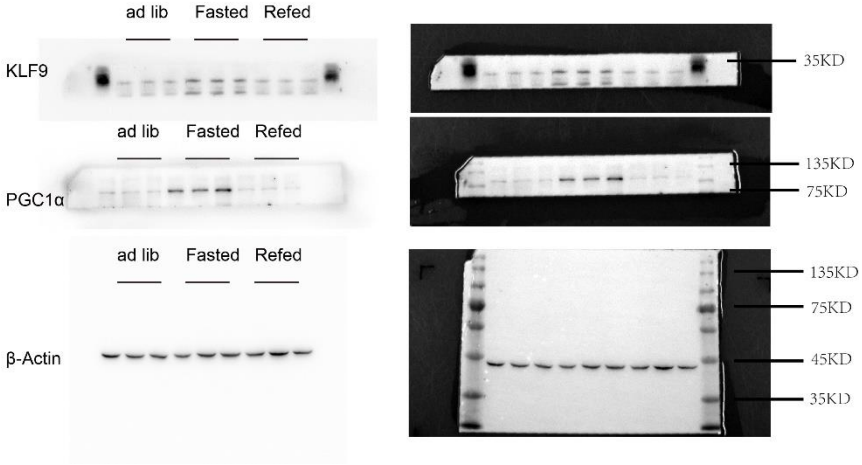

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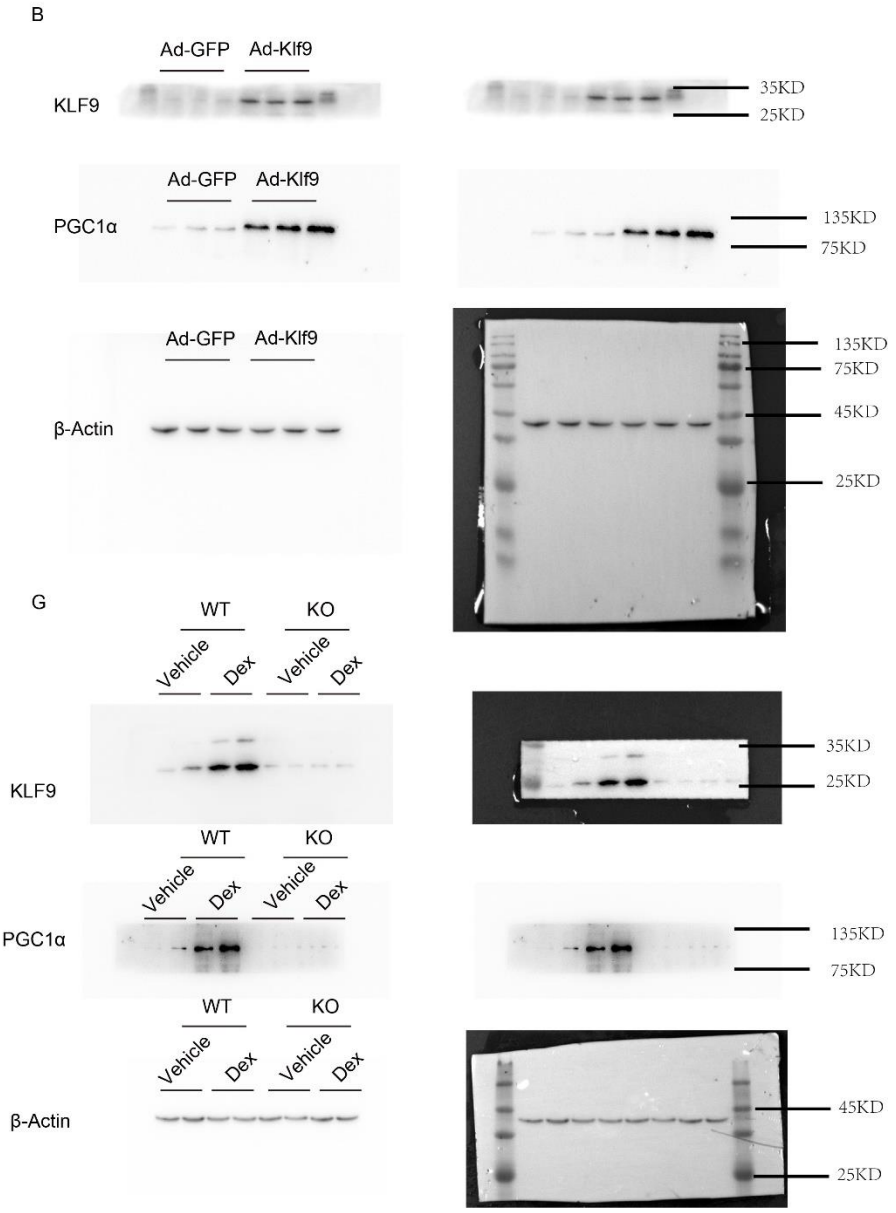

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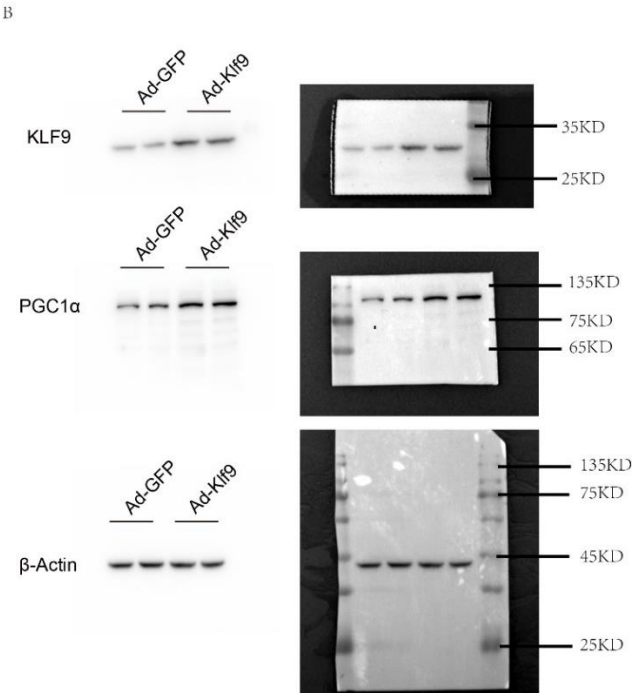

Full unedited gel for Figure 4

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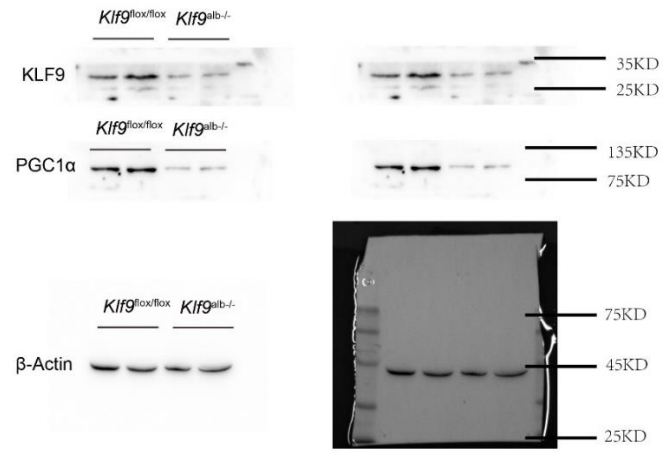

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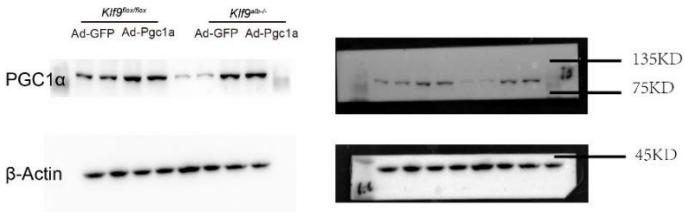

Full unedited gel for Figure 6

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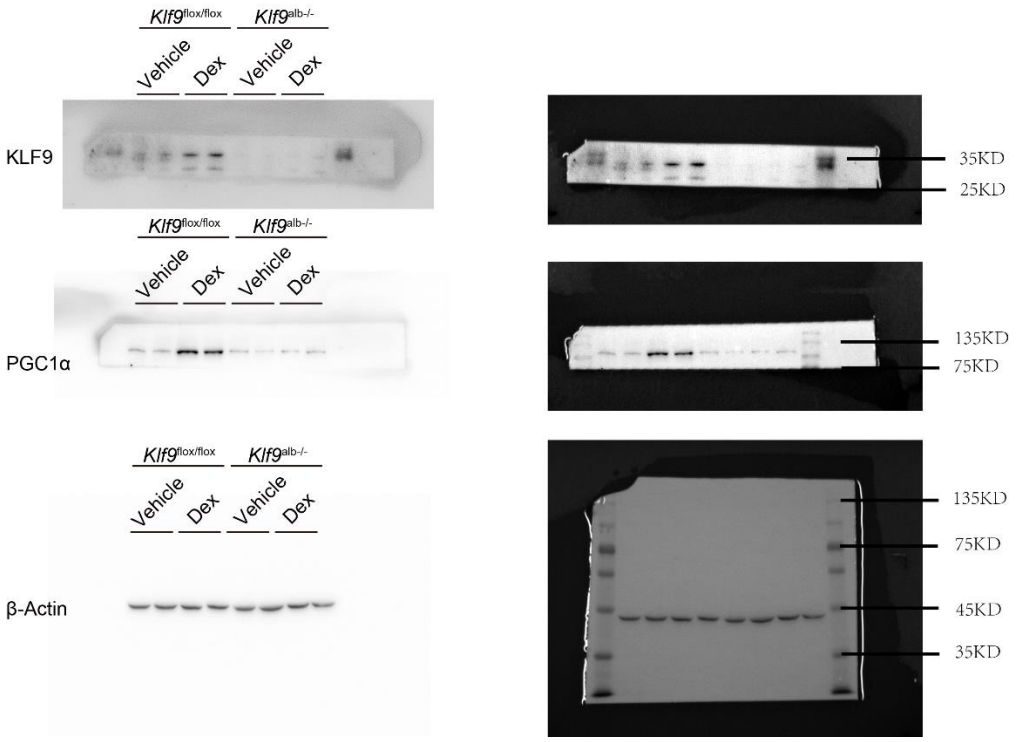

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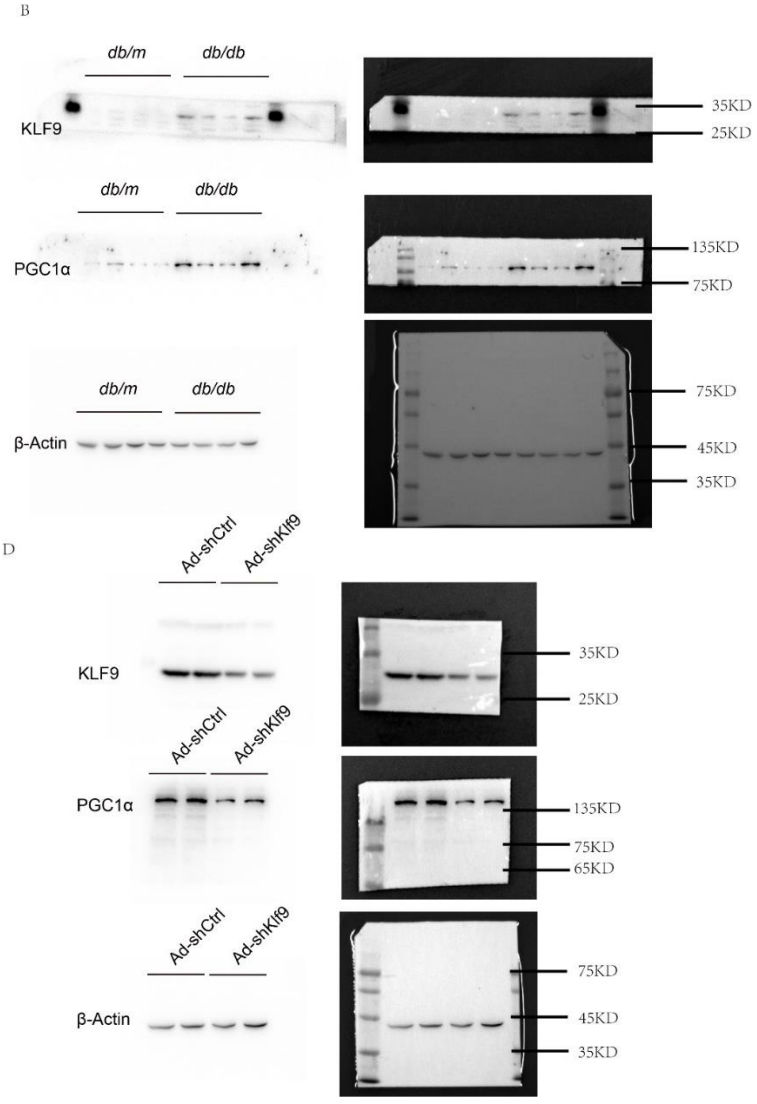

Full unedited gel for Figure S1

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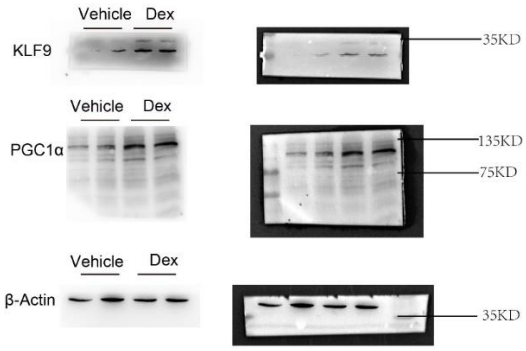

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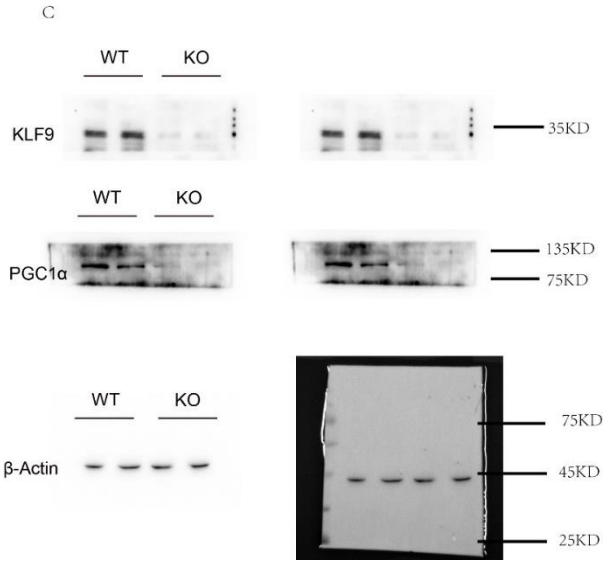

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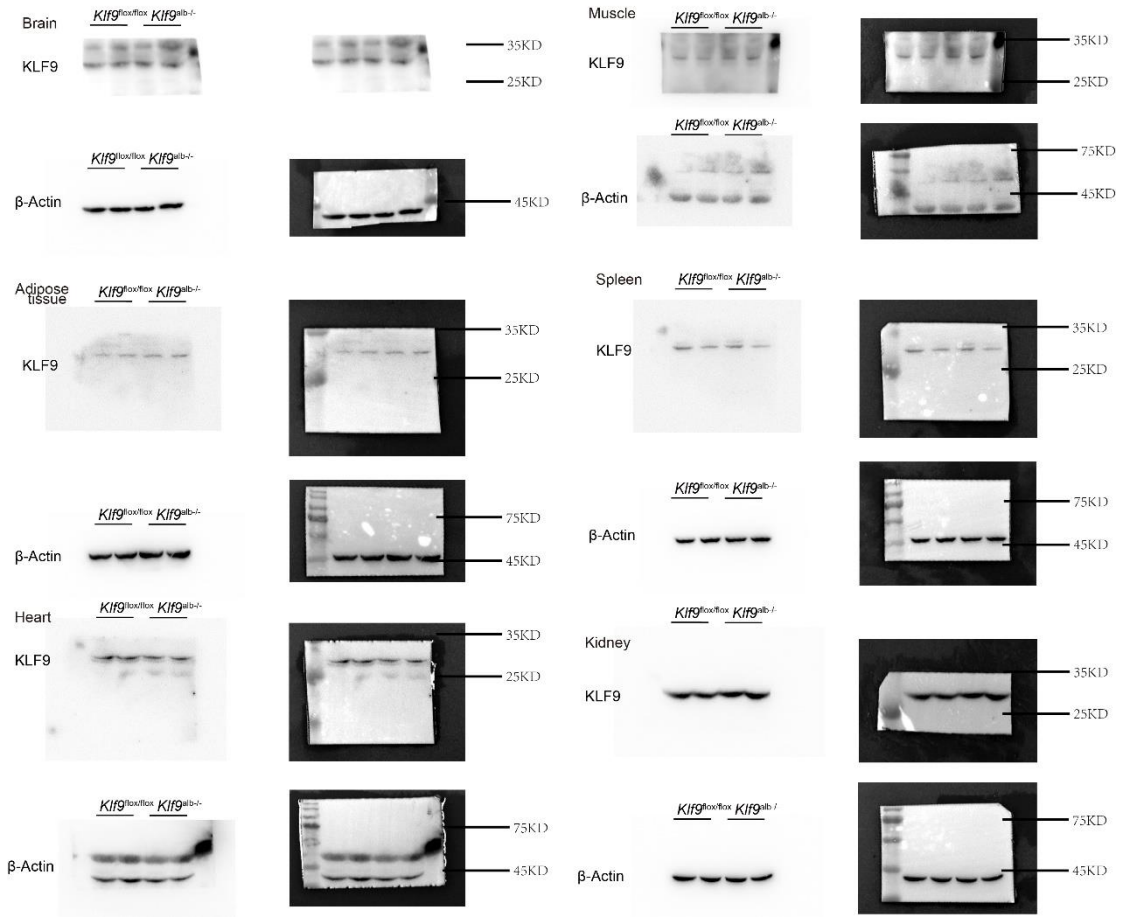

Full unedited gel for Figure S9

B