Supplemental information

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Supple. Fig. 14. Effect of 17β-estradiol on AMPK phosphorylation in time- and dose-dependent *in vitro*.

Supple. Fig. 15. Estradiol ameliorates HG-induced mTORC1 activation and matrix protein accumulation in MCT cells.

Supple. Fig. 16. Effect of palmitate on mTORC1-matrix accumulation in MCT cells.

Supple. Fig. 17.. Pathways which are increased in diabetic WT male and KAMPKγ2KO female mice by ingenuity pathway analysis.

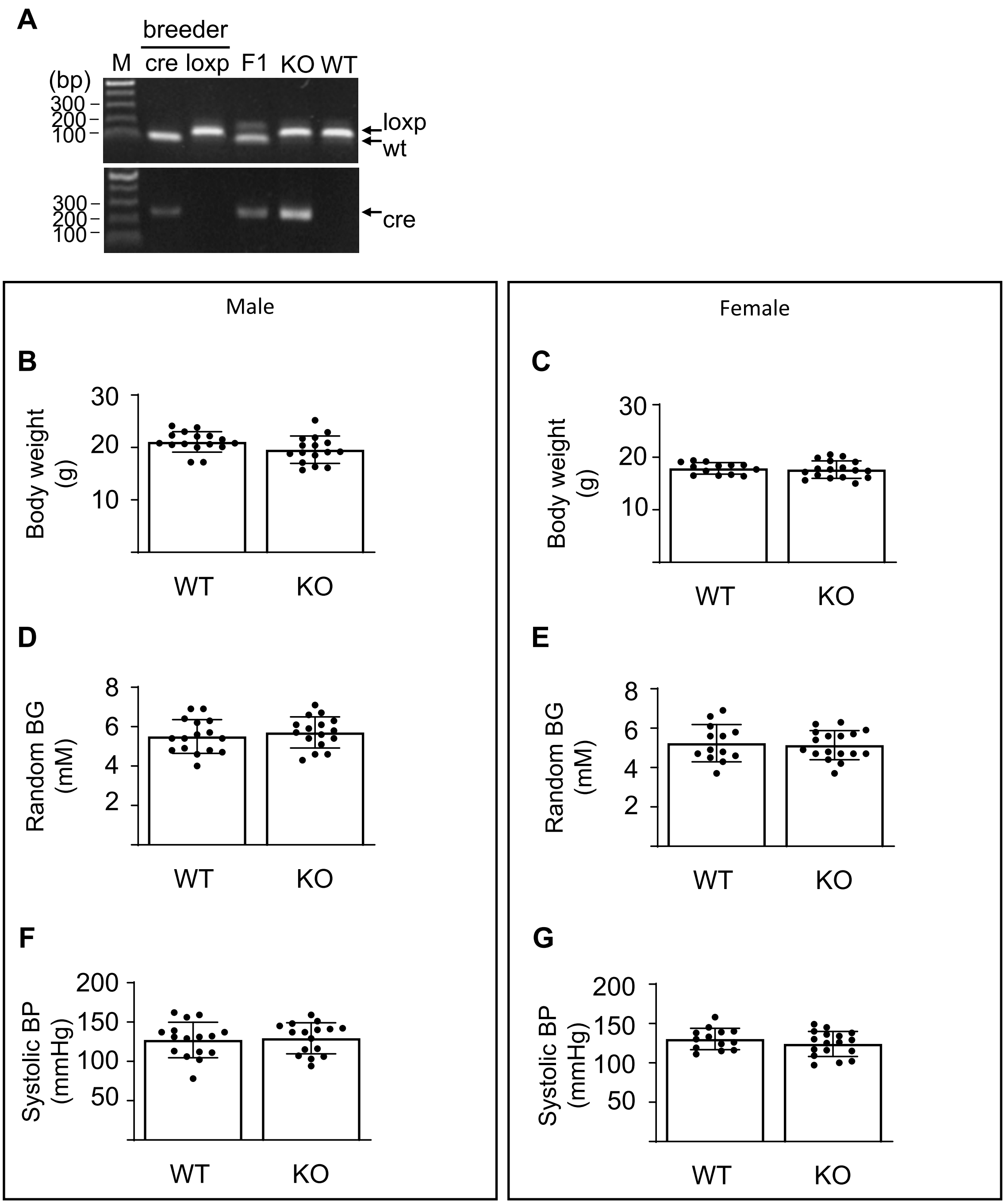
Supple. Fig. 18. 4-hydroxyphenylpyruvate dioxygenase (HFD) is decreased in diabetic WT male and KAMPKγ2KO female mice.

Supple. Table 1. Plasma 17β-estradiol levels in male and female mice.

Supple. Table 2. Visualization of DEGs from group comparisons.

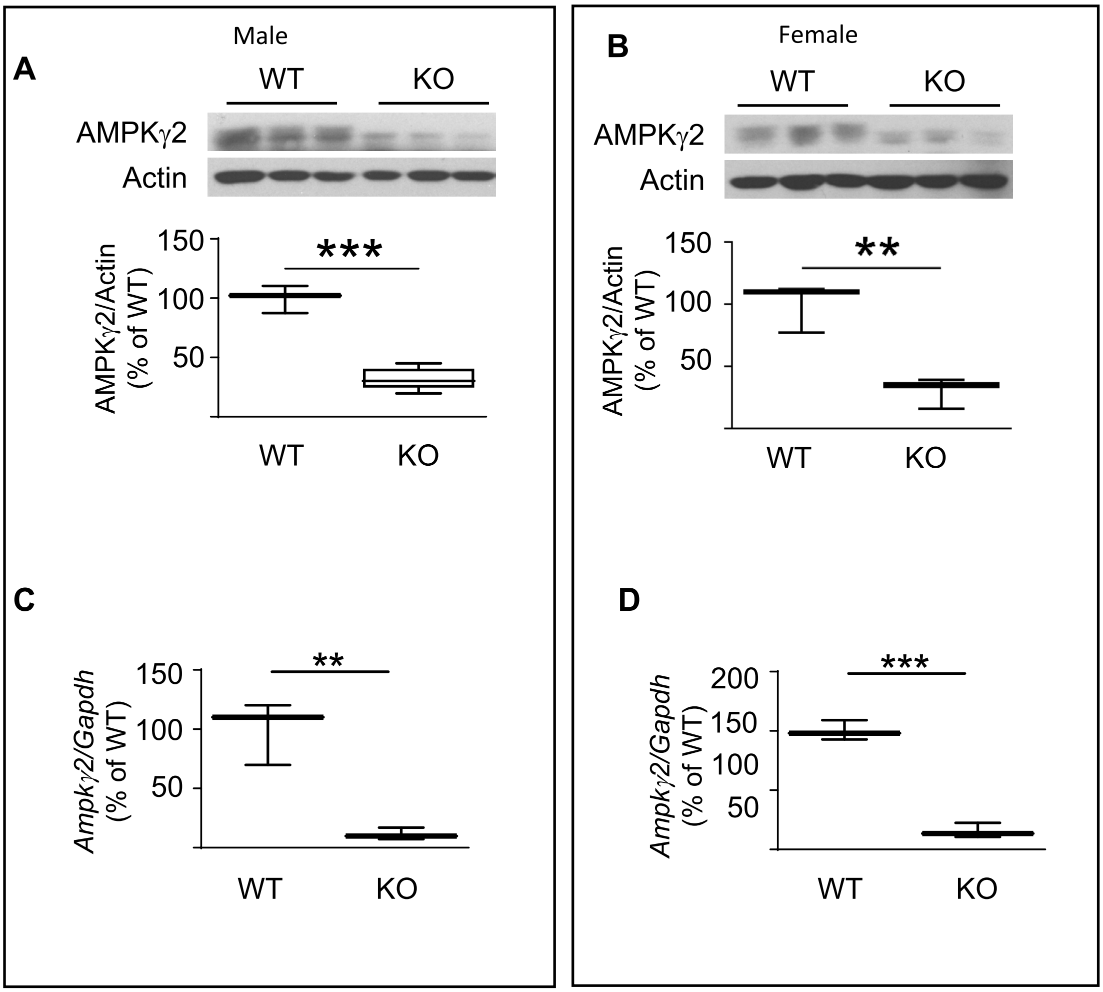
Supple. Table 3. Urine metabolites analysis

Suppl. Fig. 1



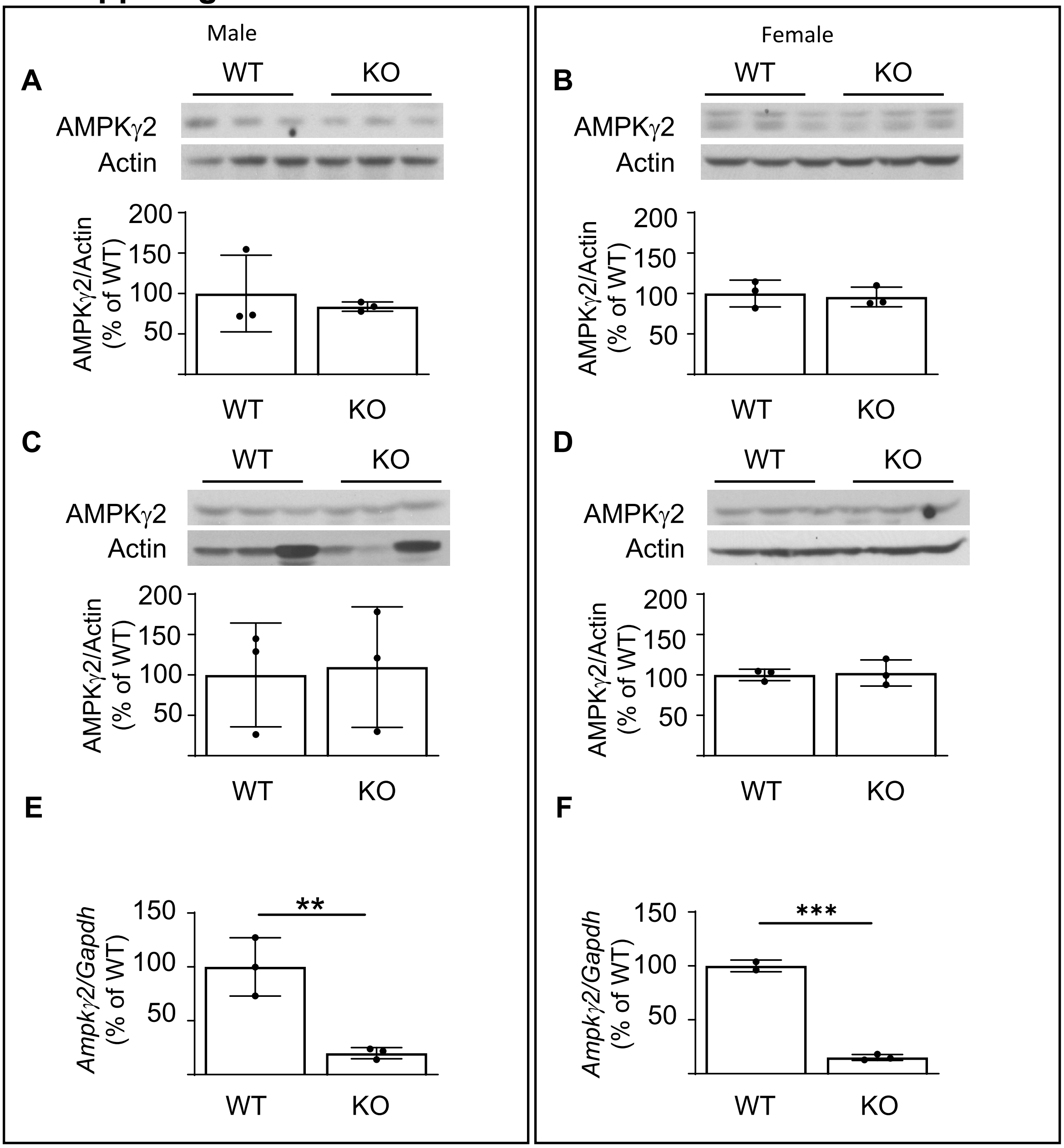
Supple. Fig. 1. Generation of KTAMPKγ2KO mice. **A.** Representative genotyping data. M: Standard marker, cre: pax8-cre, loxp: AMPKγ2-loxp, F1: F1 generation, WT: wild type, KO: AMPKγ2KO. **B-E.** Body weight, random blood glucose level and systolic blood pressure were similar between WT and AMPKγ2KO male and female mice (n= 13-16 per group).

Suppl. Fig. 2



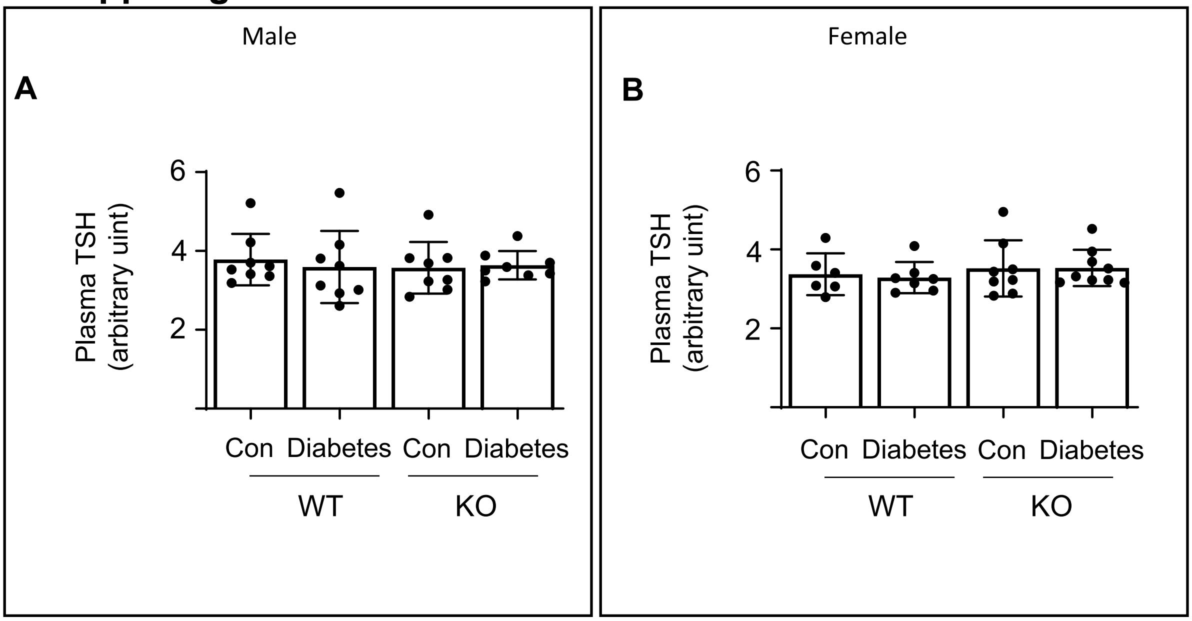
Supple. Fig. 2. A, B. AMPKγ2 expression in the kidney. **C, D.** AMPKγ2 mRNA expression in the kidney. $^{**}p<0.01$, *** $p<0.001$ by two-tailed t-test.

Suppl. Fig. 3



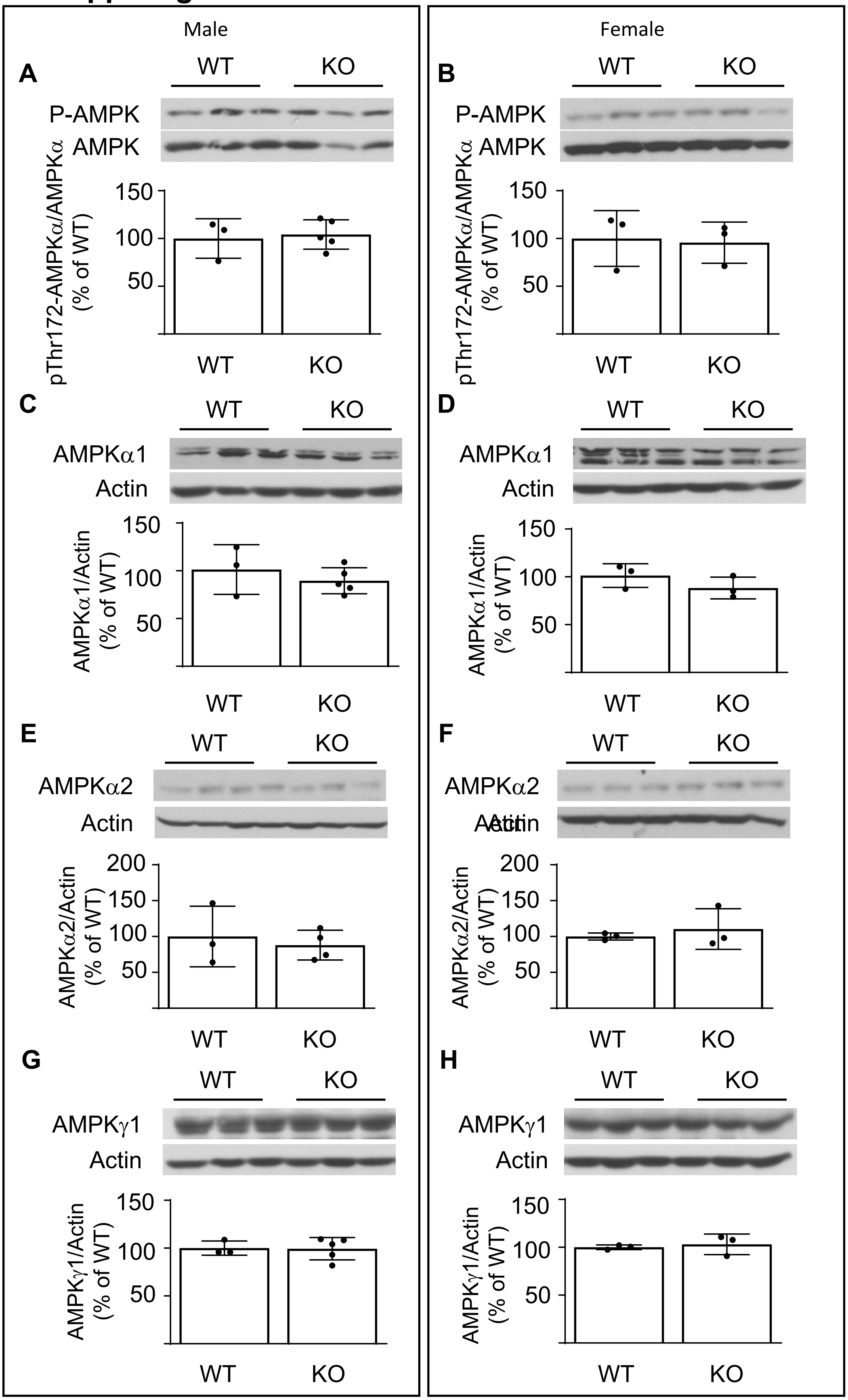
Supple. Fig. 3. A, B. AMPKγ2 expression in the liver. **C, D.** AMPKγ2 expression in the muscle. **E, F.** mRNA expression of AMPKγ2 in the thyroid. **p<0.01, *** p<0.001 by two-tailed t-test.

Suppl. Fig. 4



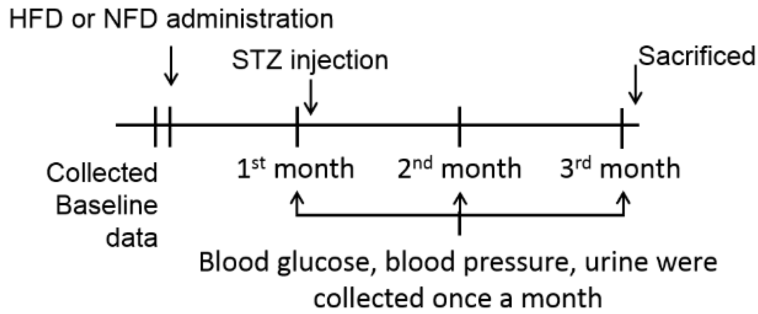
Supple. Fig. 4. A, B. Plasma TSH level in the experimental animals. TSH ELISA kit (catalog MBS9716662, MyBioSource) were used to measure the plasma TSH.

Suppl. Fig. 5



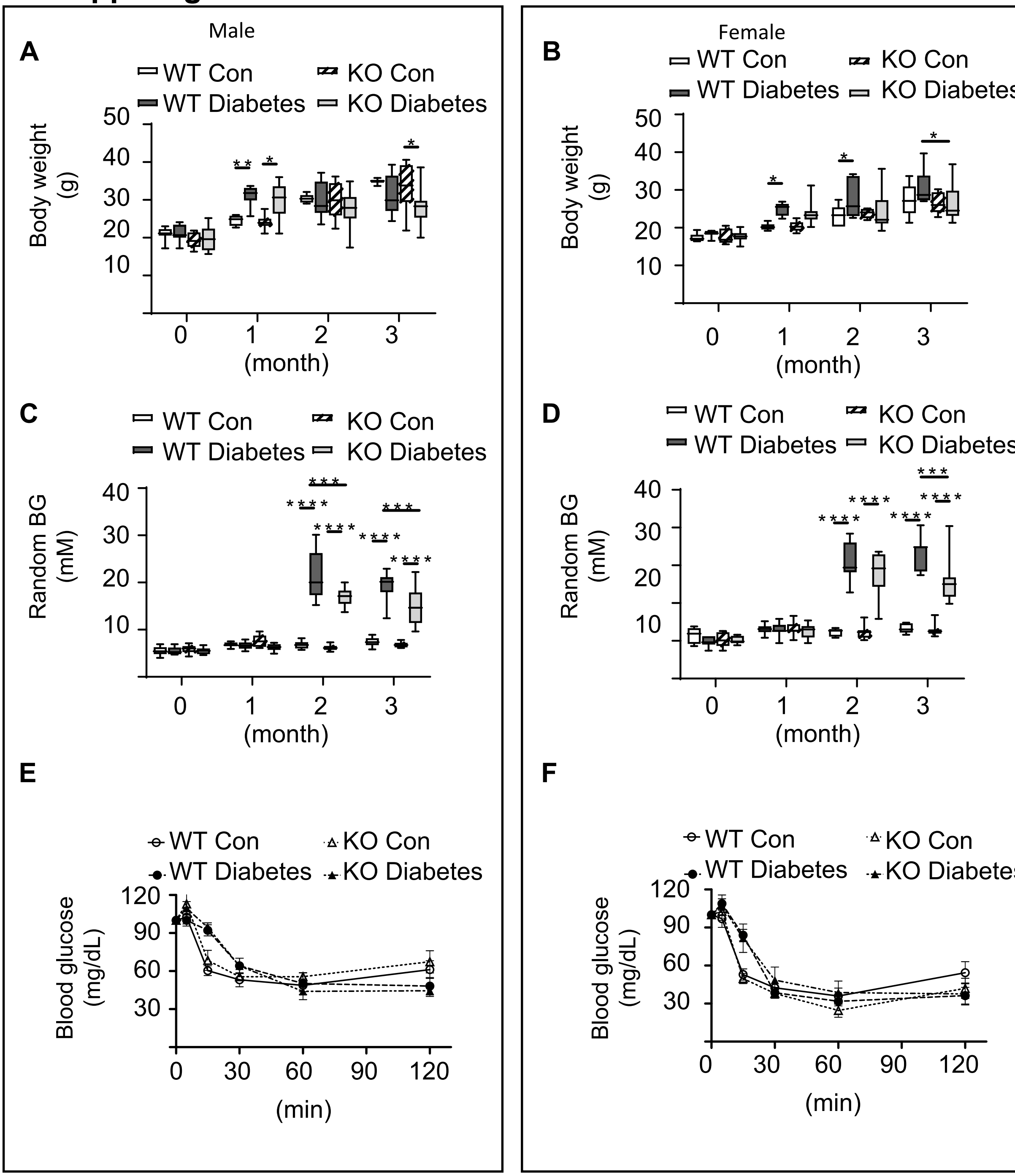
Supple. Fig. 5. A, B. AMPK phosphorylation in the kidney. **C-H.** Expression of AMPK α1, α2 and γ1 in the kidney.

Suppl. Fig. 6



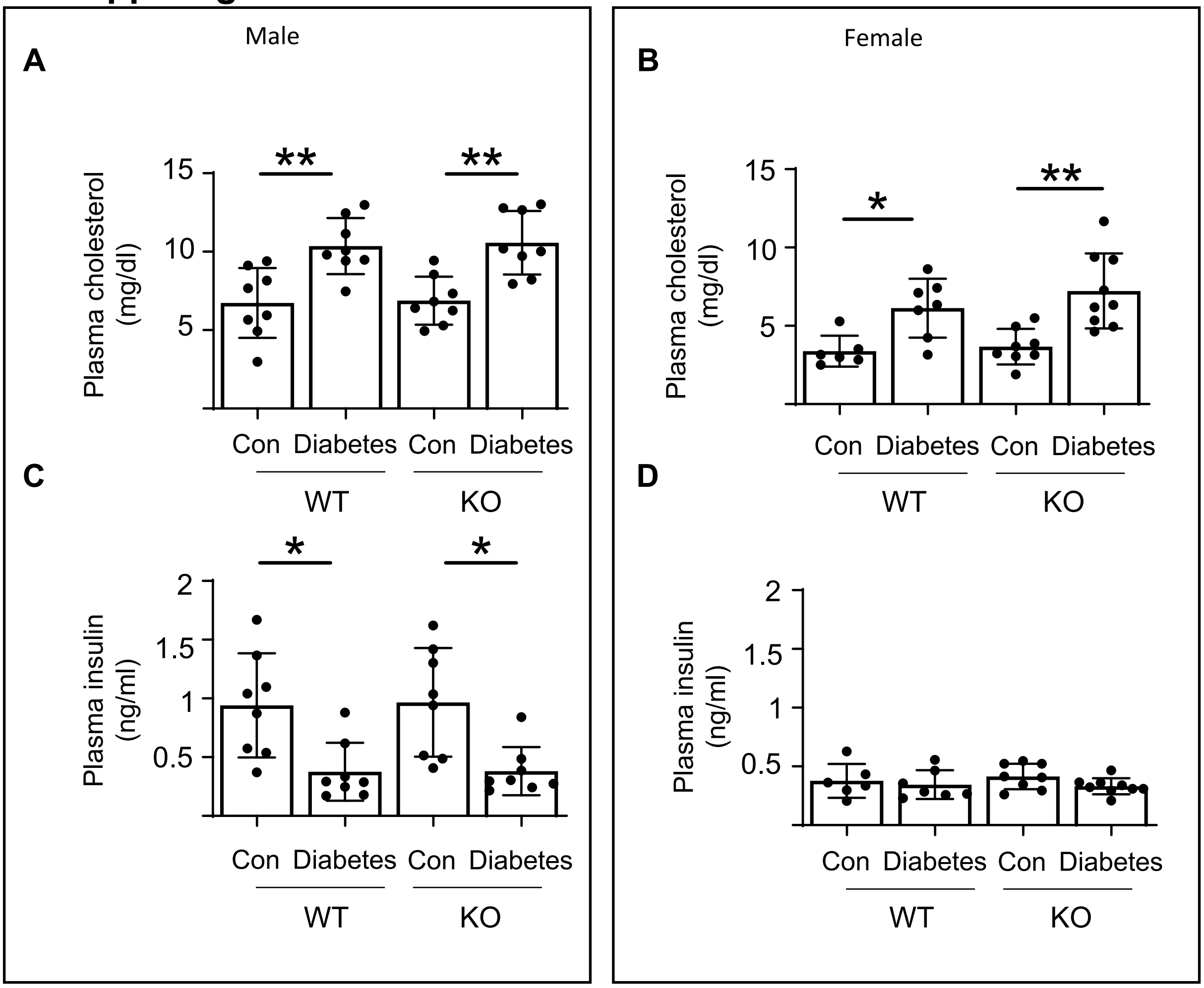
Supple. Fig. 6. Diagram of establishment of diabetes in WT and KTAMPKγ2KO mice.

Suppl. Fig. 7



Supple. Fig. 7. A, B. Body weight. **C, D.** Random blood glucose level. **E, F.** Insulin tolerance test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 by two-way ANOVA.

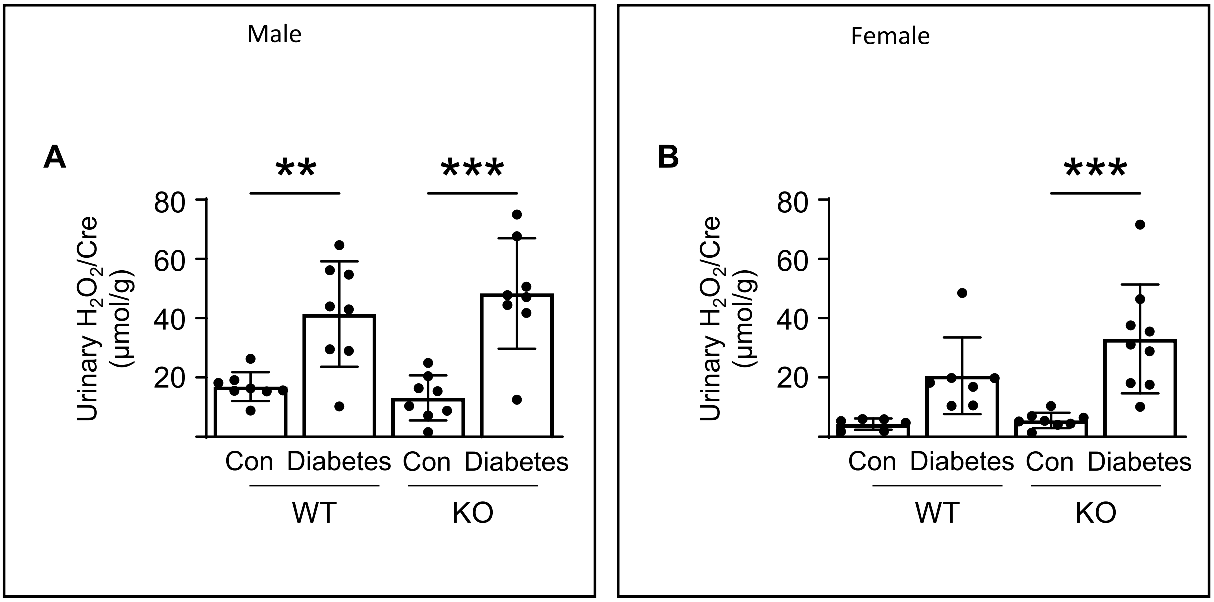
Suppl. Fig. 8



Supple. Fig. 8. A, B. Plasma cholesterol level. **C, D.** Plasma fasting insulin level. *p<0.05, **p<0.01 by one-way ANOVA.

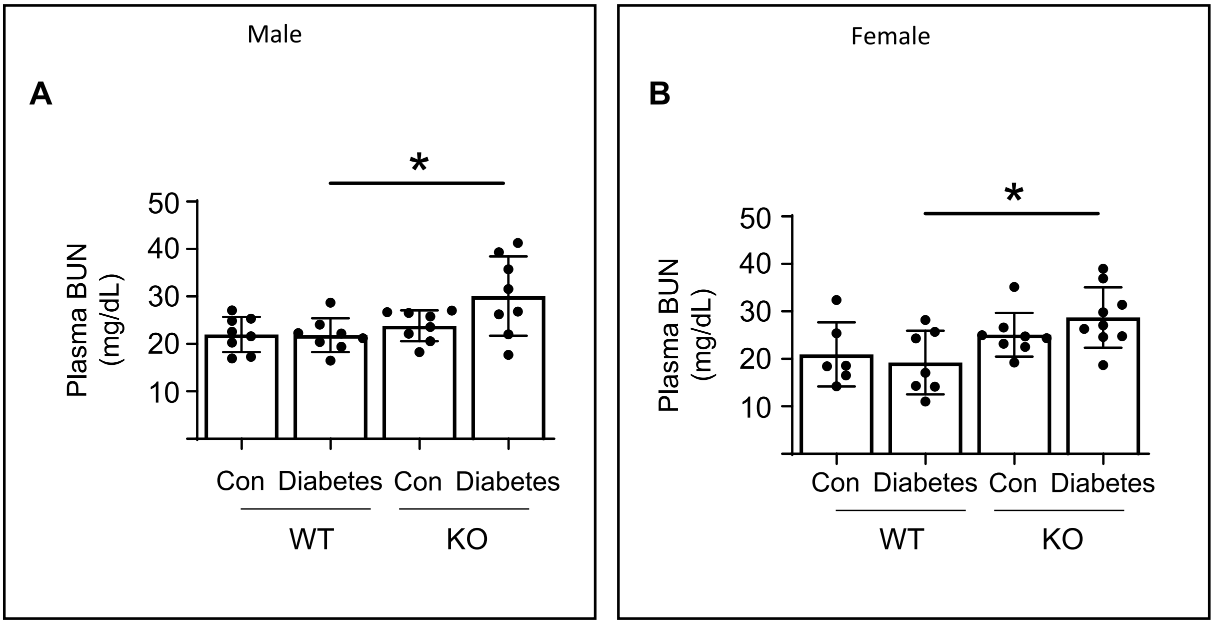
Cholesterol fluorometric assay kit (catalog 1007640, Cayman) and mouse Insulin ELISA Kit (catalog 90080, Crystal Chem) were used to measure the plasma cholesterol levels and insulin levels (6 h fasting), respectively.

Suppl. Fig. 9



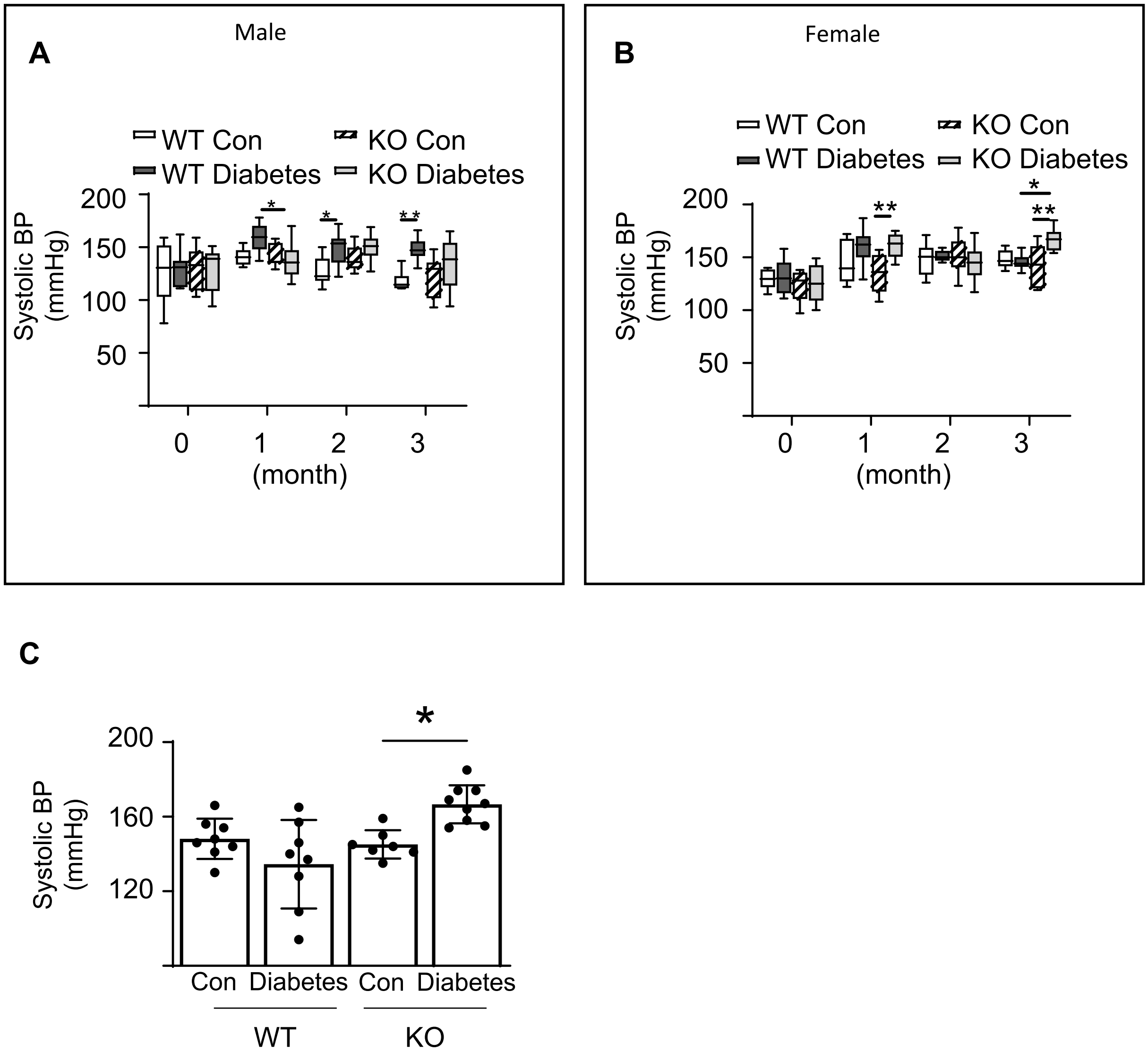
Supple. Fig. 9. A, B. Urinary H_2O_2. **p<0.01, ***p<0.001 by one-way ANOVA**.** Amplex Red H_2O_2 Assay Kit (catalog A22188, Invitrogen) was used to measure urinary H_2O_2.

Suppl. Fig. 10



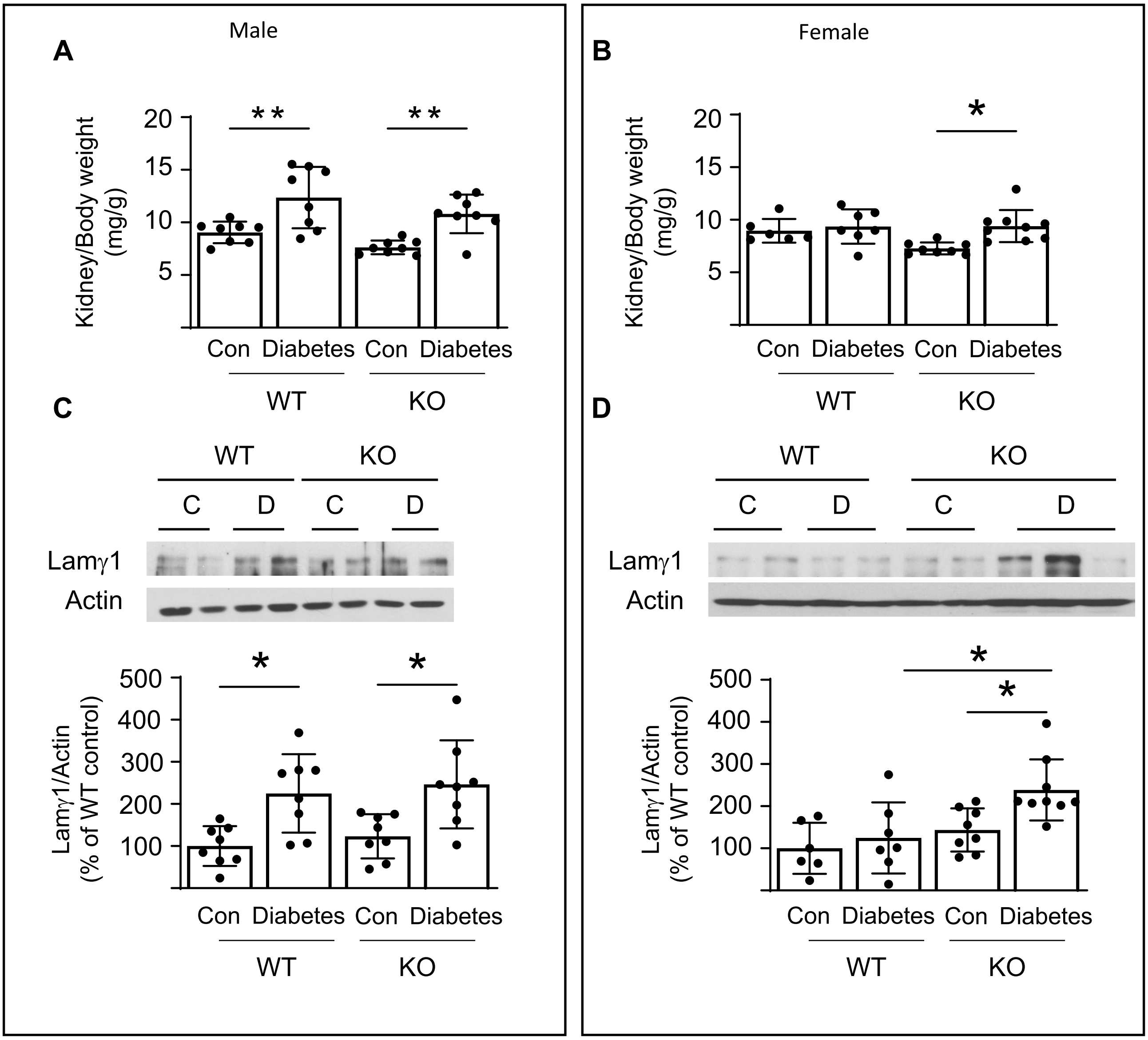
Supple. Fig. 10. A, B. Plasma BUN. *p<0.05 by two-way ANOVA. Plasma BUN was measured by BUN colorimetric detection kit (catalog numberK024-H1, Arbor Assays)

Suppl. Fig. 11



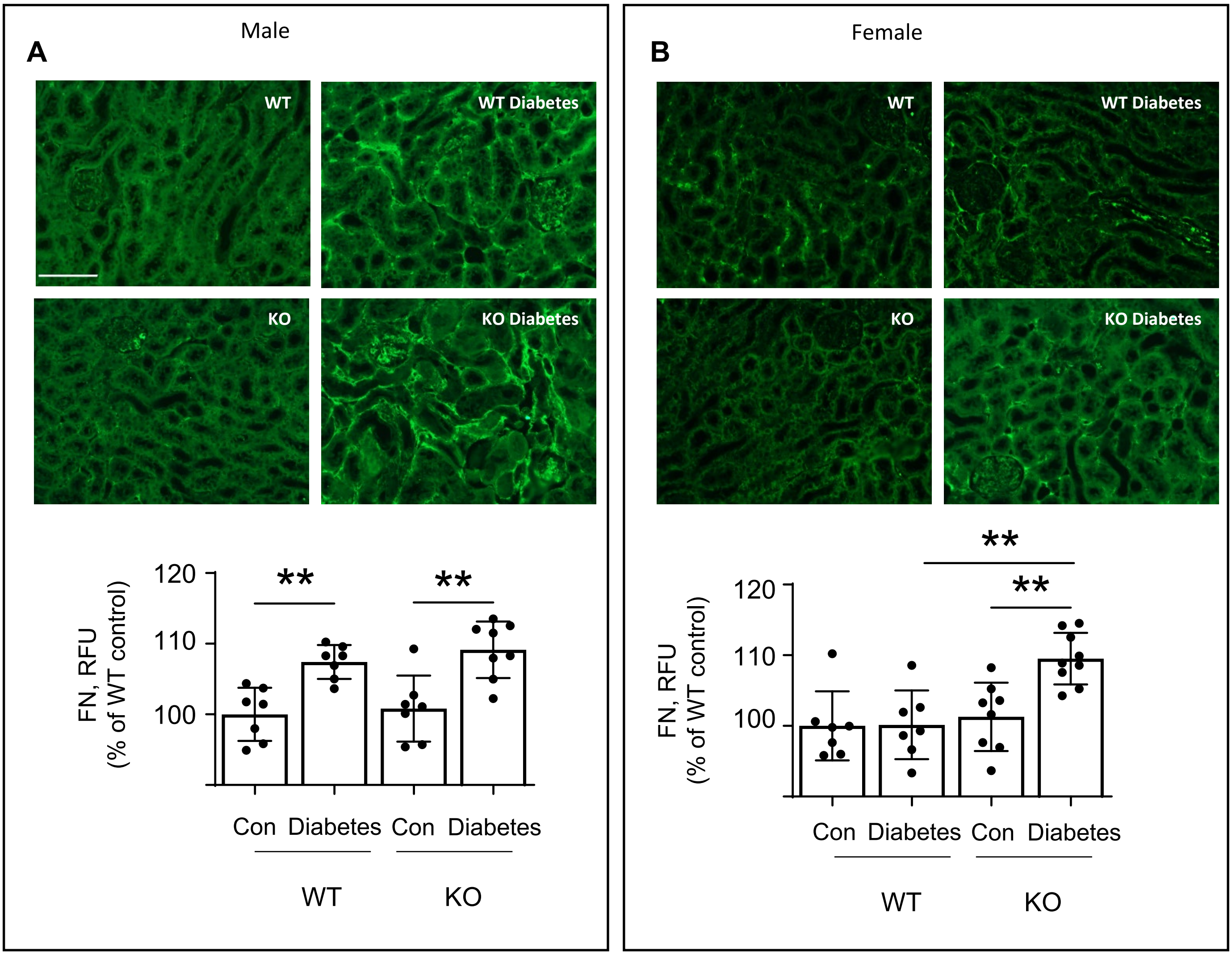
Supple. Fig. 11. A, B. Systolic blood pressure. *p<0.05, **p<0.01 by two-way ANOVA (A, B) and one-way ANOVA (C).

Suppl. Fig. 12



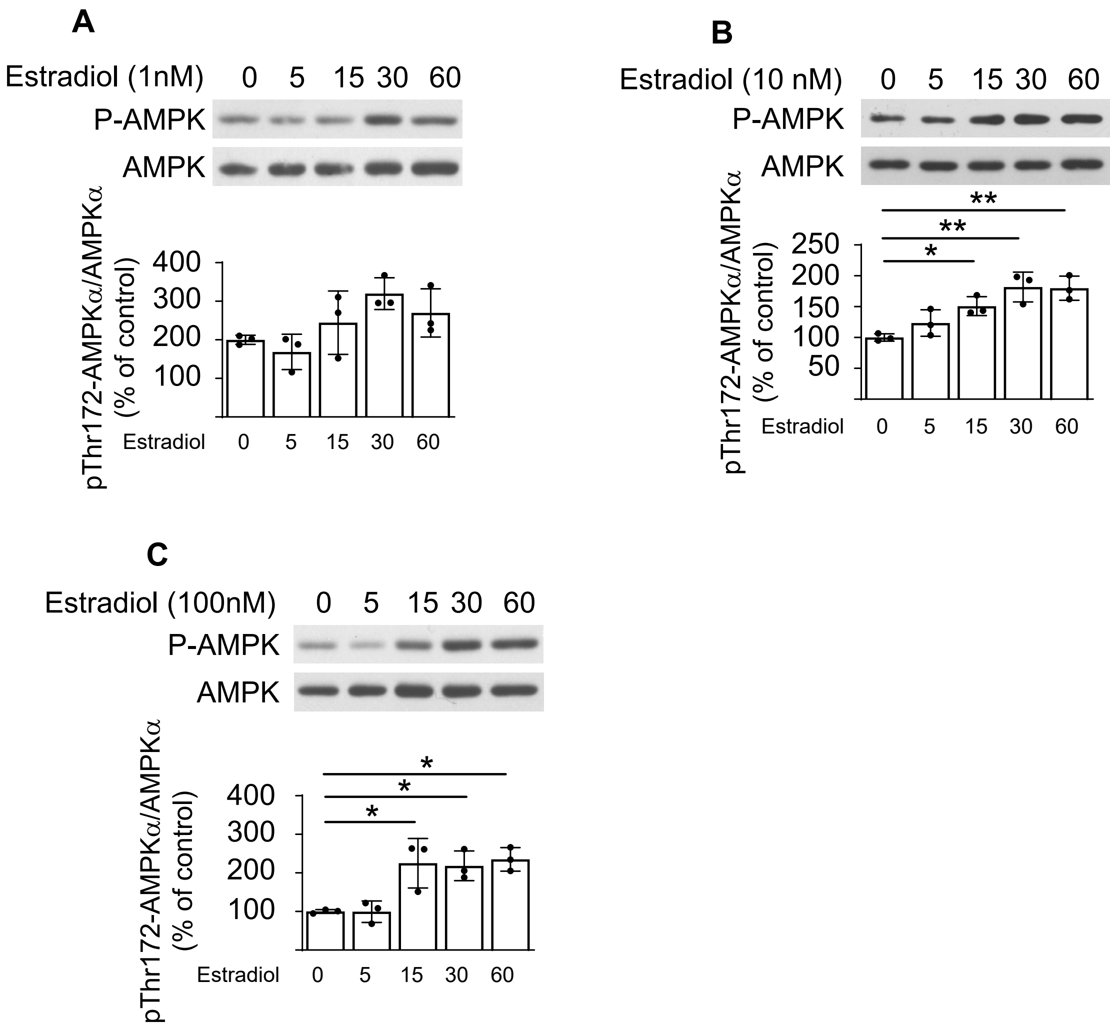
Supple. Fig. 12. A, B. kidney to body weight ratio. **C, D.** Laminin gamma1 expression in the kidney. *p<0.05, **p<0.01 by one-way ANOVA.

Suppl. Fig. 13



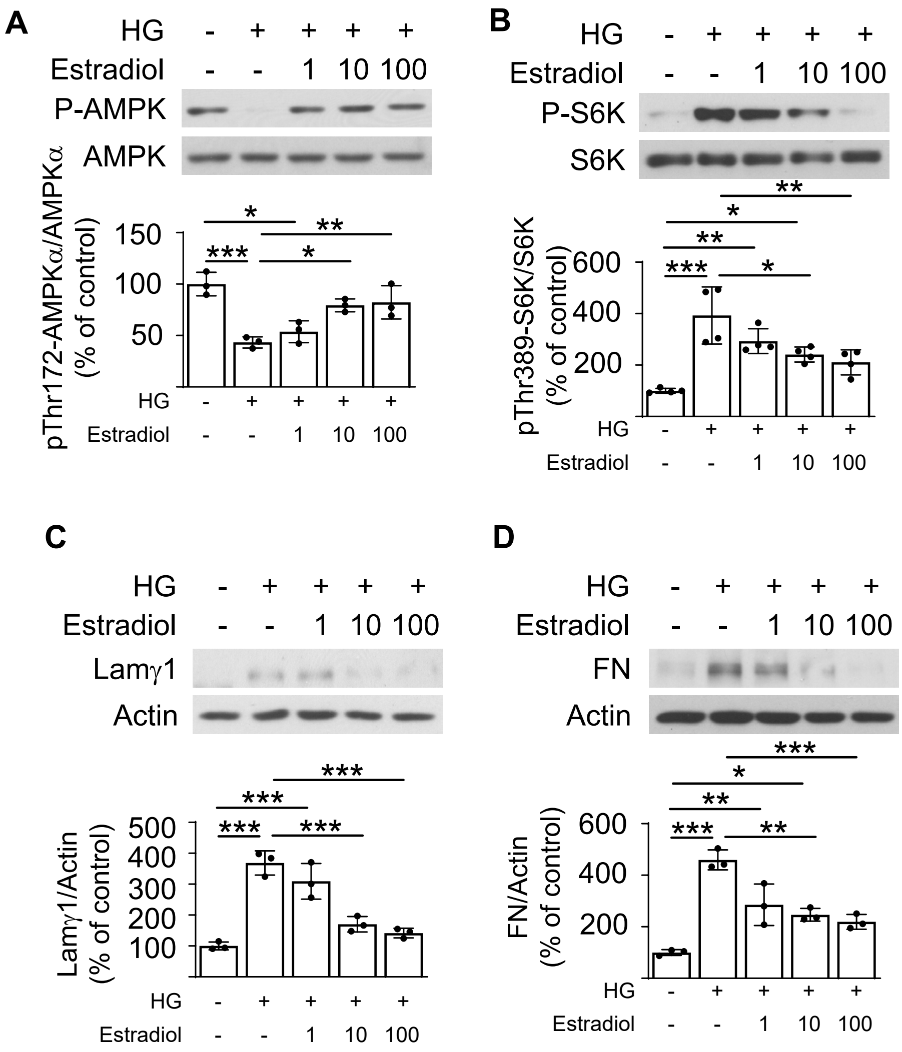
Supple. Fig. 13. A, B. Kidney fibronectin expression. $^{**}p<0.01$ by one-way ANOVA. Immunofluorescence was performed as described below. The kidney sections were deparaffinized in xylene, hydrated in decreasing concentrations of ethanol, and washed with Tris-buffered solution (TBS). To unmask antigens, slides were heated in antigen retrieval solution (catalog H3300, Vectors lab). After the fixation, the sections were hydrated in TBS. To detect fibronectin in the kidney and the liver, anti-fibronectin antibody (catalog ab2413, Abcam) was diluted 1:100 in TBS containing 1% BSA and applied overnight at 4º C in a humidity chamber. After incubation with primary antibodies, all the sections were washed in TBS containing 0.01% Triton X-100. Alexa Fluor 488-conjugated secondary antibodies (in 1:150 dilution) were applied for 1 h at room temperature. Then the sections were washed, and mounted with Prolong Gold Antifade Reagent. At least 7-9 fields of each section were captured. Representative images were photographed using Axio Imager A1 microscope (Carl Zeiss, Melville, NY). ImageJ software was used to count fluorescence intensity on the images. To verify specificity of all immunostainings, primary antibodies were omitted in negative controls.

Suppl. Fig. 14



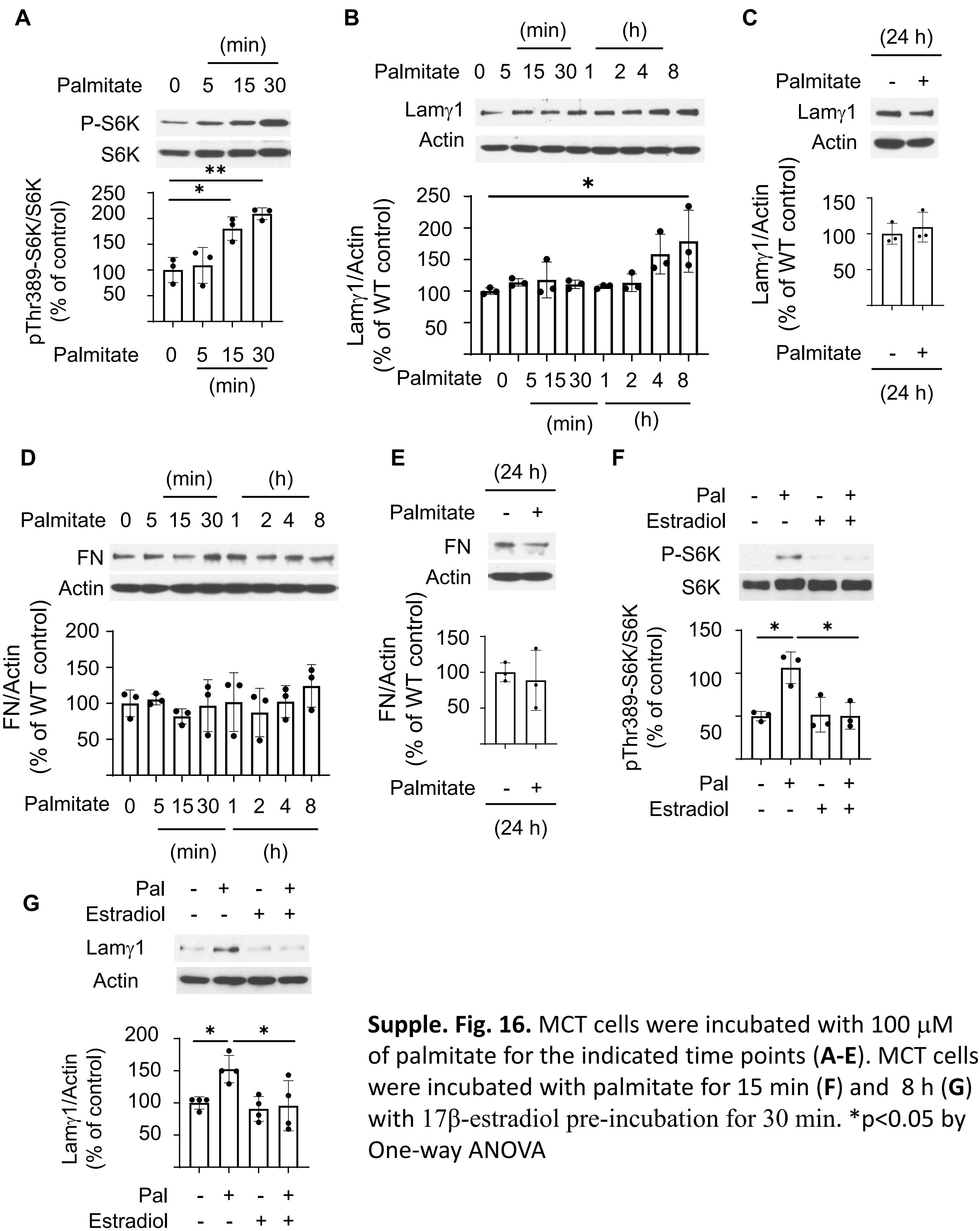
Supple. Fig. 14. A-C. MCT cells were incubated with 1 nM, 10 nM or 100 nM of estradiol for the indicated time points. *$p<0.05$, $p<0.01$ by One-way ANOVA

Suppl. Fig. 15



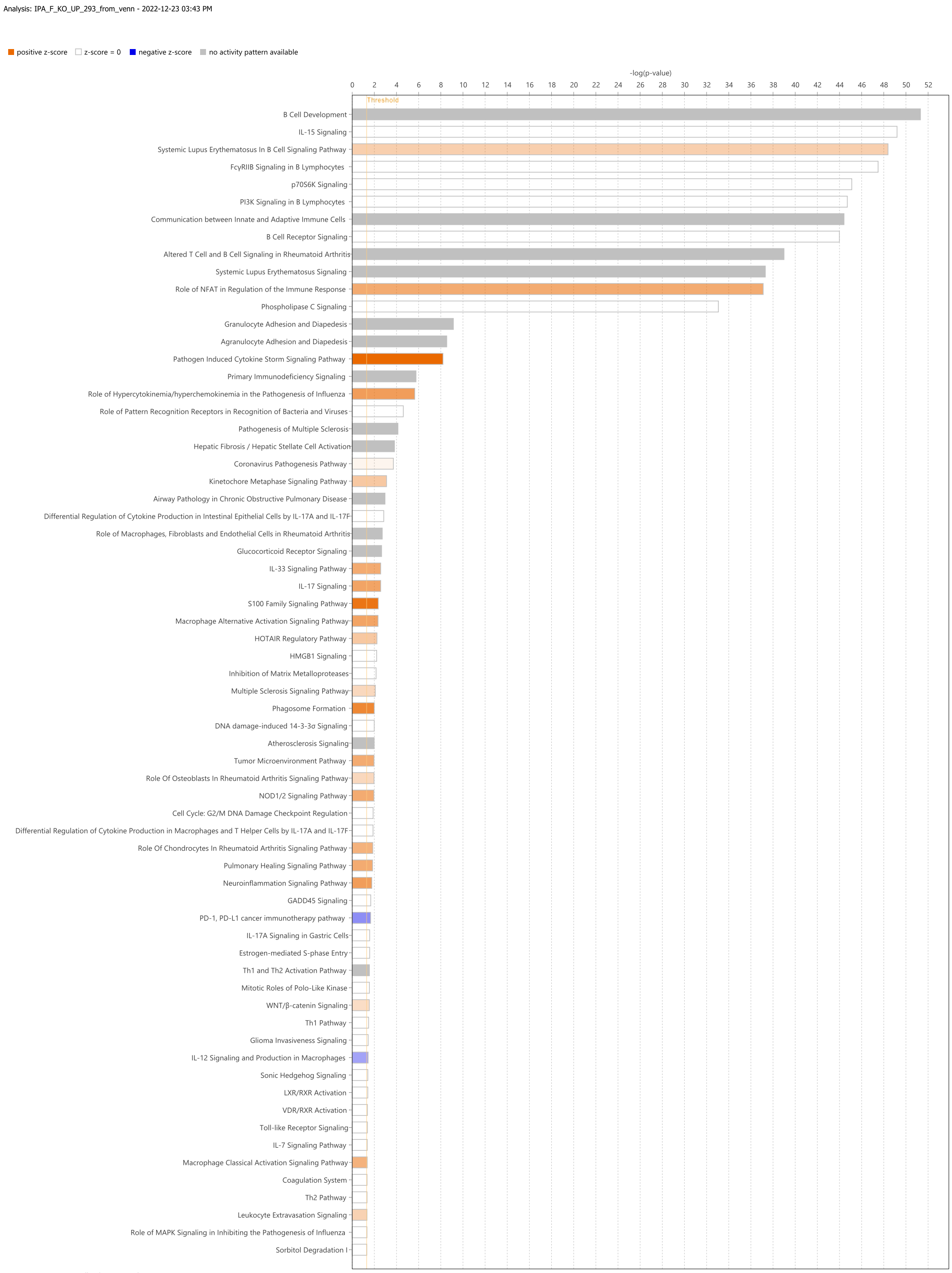
Supple. Fig. 15. Estradiol ameliorate HG-induced mTORC1 activation and matrix protein accumulation in MCT cells. A. B. HG decreased AMPK phosphorylation and increased p70S6K phosphorylation which were ameliorated by estradiol. **C, D.** HG-induced expression of laminin-γ1 and fibronectin was ameliorated by estradiol. The composite graphs shown mean ± SD (3-4 experiments). *p<0.05, **p<0.01, ***p<0.001 by One-way ANOVA.

Suppl. Fig. 16



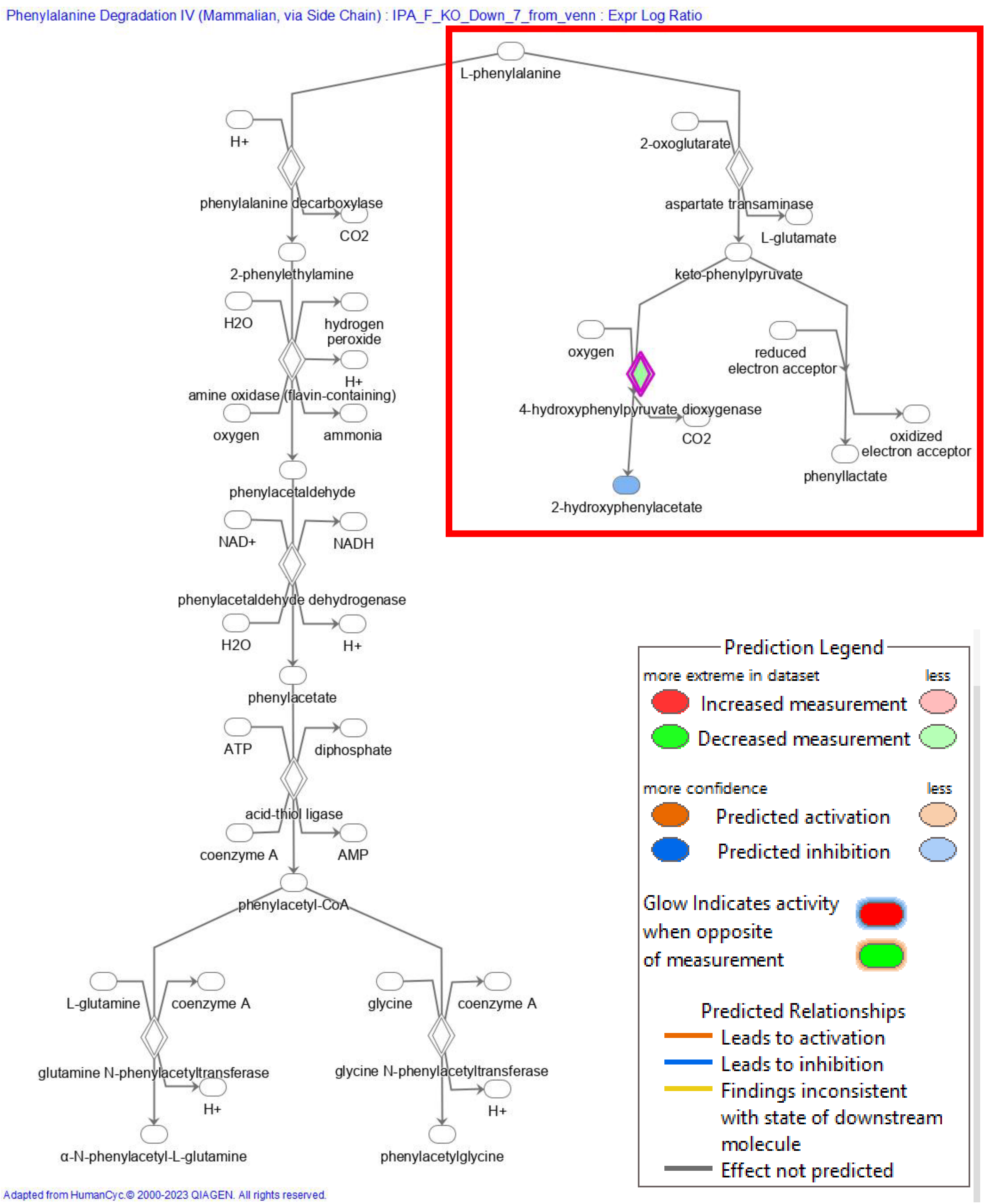
Supple. Fig. 16. MCT cells were incubated with 100 μM of palmitate for the indicated time points (**A-E**). MCT cells were incubated with palmitate for 15 min (**F**) and 8 h (**G**) with 17β-estradiol pre-incubation for 30 min. *p<0.05 by One-way ANOVA

Suppl. Fig. 17



Supple. Fig. 17. Pathways which are increased in diabetic WT male and KAMPKγ2KO female mice by ingenuity pathway analysis. 293 genes were increased in the kidney.

Suppl. Fig. 18



Supple. Fig. 18. 4-hydroxyphenylpyruvate dioxygenase (HFD) is decreased in diabetic WT male and KAMPKγ2KO female mice . HFD is involved in phenylalanine degradation pathway.

Suppl. Table 1. Plasma 17β-estradiol levels in male and female mice

Male					Female				
17β-estradiol (pg/ml)	WT		AMPKγ2KO		17β-estradiol (pg/ml)	WT		AMPKγ2KO	
	Con	Diabetes	Con	Diabetes		Con	Diabetes	Con	Diabetes
AVE	46.3	137.5	119.6	38.1	AVE	37.2	73.1	59.7	60.7
STDEV	53.3	126.7	81.8	81.8	STDEV	41.4	41.9	98.7	79.5

Suppl. Table 1. Plasma 17β-estradiol levels in male and female mice. Mouse 17β-estradiol ELISA kit was used to measure plasma 17β-estradiol (catalog, ADI-900-174, Enzo Life Science, Farmingdale, NY)

Suppl. Table 2. Visualization of DEGs from group comparisons

Sex	Genotype	Group	Sample	DEGs
Male	WT	Control	5206	443 upregulated 80 downregulated
			5208	
			5209	
		Diabetes	591	
			5207	
			5215	
	AMPK G2 KO	Control	5201	1054 upregulated 53 downregulated
			5203	
			5225	
		Diabetes	5202	
			5204	
			5205	
Female	WT	Control	595	120 upregulated 4 downregulated
			5210	
			5219	
		Diabetes	596	
			5211	
			5213	
	AMPK G2 KO	Control	588	832 upregulated 45 downregulated
			592	
			594	
		Diabetes	590	
			593	
			5212	

Suppl. Table 3

	Urine metabolites							
	Male				Female			
	WT		KTAMPKg2KO		WT		KTAMPKg2KO	
(μM/mM)	Control	Diabetes	Control	Diabetes	Control	Diabetes	Control	Diabetes
Alanine/Creatinine	21.7 ±3.3	73.1 ±106.4	16.2 ±4.8	33.4 ±11.0	32.0 ±8.4	94.0 ±128.2	21.0 ±3.8	1017.7 ±1390.0
Arginine/Creatinine	7.7 ±1.4	16.6 ±8.6	8.9 ±2.4	22.2 ±10.9**	4.4 ±0.7	11.1 ±4.3	5.7 ±1.8	44.7 ±44.7*
Asparagine/Creatinine	14.2 ±1.5	17.0 ±7.2	12.0 ±2.6	21.0 ±6.1*	14.2 ±1.8	32.8 ±39.7	14.4 ±1.9	319.3 ±406.0
Aspartic acid/Creatinine	3.1 ±0.9	8.3 ±10.9	5.1 ±4.6	3.4 ±0.8	2.6 ±0.5	3.3 ±1.5	2.3 ±0.9	4.5 ±2.4
Cysteine/Creatinine	15.2 ±1.7	44.8 ±29.3*	19.2 ±7.5	52.8 ±24.1*	11.7 ±6.7	32.5 ±25.9	10.4 ±2.6	91.3 ±77.2
Glutamic acid/Creatinine	14.5 ±4.3	61.2 ±60.9*	18.5 ±9.9	33.6 ±10.0	14.1 ±2.3	29.9 ±12.9	14.4 ±4.2	91.6 ±60.5**
Glutamine/Creatinine	37.4 ±5.1	292.5 ±372.5	39.2 ±5.9	304.8 ±255.2	43.8 ±15.6	208.0 ±257.4	40.4 ±4.5	2318.0 ±2171.7**
Glycine/Creatinine	95.3 ±9.0	220.6 ±100.5***	104.3 ±26.2	153.4 ±35.3	71.5 ±7.9	141.0 ±72.1	60.9 ±4.7	652.4 ±676.5*
Histidine/Creatinine	6.1 ±0.6	58.0 ±89.1	5.3 ±0.6	33.6 ±21.7	5.5 ±1.2	44.9 ±75.8	5.7 ±1.1	485.4 ±504.1*
Isoleucine/Creatinine	3.4 ±0.4	23.0 ±15.07***	3.4 ±1.4	17.4 ±7.6*	4.6 ±0.8	27.8 ±32.5	5.0 ±1.8	221.9 ±244.6*
Leucine/Creatinine	12.0 ±0.2	50.8 ±30.9**	7.2 ±1.7	42.5 ±17.9**	8.9 ±2.2	57.0 ±73.0	7.3 ±2.5	458.0 ±556.2*
Lysine/Creatinine	8.7 ±1.6	15.4 ±7.6	9.4 ±2.2	17.3 ±5.9*	11.2 ±2.4	24.0 ±10.6	12.4 ±2.7	145.9 ±171.1
Methionine/Creatinine	2.5 ±0.4	14.8 ±14.7*	2.3 ±0.8	10.0 ±5.4	3.8 ±1.3	15.4 ±17.7	3.4 ±1.0	133.5 ±174.4
Phenylalanine/Creatinine	5.4 ±0.5	15.5 ±5.2****	4.3 ±0.9	12.9 ±3.6***	5.4 ±1.0	17.7 ±13.2	5.0 ±1.4	107.1 ±122.6*
Proline/Creatinine	3.9 ±0.7	57.0 ±112.1	4.2 ±2.9	11.0 ±4.3	5.2 ±1.8	76.5 ±146.5	5.2 ±1.8	189.3 ±379.9
Serine/Creatinine	9.6 ±1.1	32.1 ±22.3*	8.4 ±2.1	35.4 ±16.4**	10.4 ±3.2	62.0 ±93.2	10.3 ±2.3	634.1 ±789.2
Threonine/Creatinine	18.3 ±3.0	155.6 ±203.1	16.4 ±2.4	123.6 ±88.8	22.3 ±4.6	181.7 ±331.1	24.3 ±6.6	1593.3 ±1815.1*
Tryptophan/Creatinine	1.7 ±0.1	6.6 ±8.2	1.5 ±0.2	4.3 ±1.6	2.1 ±0.4	8.1 ±10.7	2.0 ±0.3	65.4 ±72.9
Tyrosine/Creatinine	5.6 ±1.0	48.5 ±54.7	5.2 ±1.8	46.3 ±30.8	10.3 ±5.5	70.2 ±109.4	8.9 ±2.8	803.0 ±851.2*
Valine/Creatinine	7.6 ±1.2	35.5 ±22.3***	6.2 ±1.6	28.6 ±10.9*	9.8 ±2.3	43.0 ±46.4	11.6 ±3.7	441.5 ±530.0*
Adenine/Creatinine	0.80 ±0.22	0.69 ±0.43	1.72 ±2.1	0.39 ±0.15	0.41 ±0.15	0.42 ±0.24	0.46 ±0.21	0.57 ±0.30
Betaine/Creatinine	31.0 ±7.2	145.4 ±105.1**	30.1 ±8.7	59.6 ±13.1	60.6 ±28.0	141.9 ±68.1	32.1 ±11.8	145.2 ±84.2**
GABA/Creatinine	26.1 ±4.0	65.4 ±26.6***	38.5 ±5.7	52.0 ±11.7	22.8 ±12.9	20.7 ±6.0	14.0 ±2.9	28.6 ±8.3**
L-α-aminobytyric acid/Creatinine	0.3 ± 0.1	4.7 ±4.0**	0.3 ±0.1	2.8 ±2.2	0.7 ±0.2	2.5 ±2.8	0.8 ±0.2	61.1 ±62.7*
Ornithine/Creatinine	2.1 ±0.7	9.9 ±4.4***	3.4 ±1.0	8.7 ±3.7*	2.8 ±1.8	6.3 ±2.6	2.5 ±0.6	32.4 ±30.5*
Pipecolate/Creatinine	44.0 ±6.2	45.0 ±5.1	43.3 ±1.7	51.4 ±7.1	52.4 ±6.7	52.7 ±7.6	48.8 ±6.9	59.8 ±14.8
Serotonine/Creatinine	0.9 ±0.1	0.7 ±0.1*	0.9 ±0.1	0.6 ±0.1****	0.9 ±0.2	0.6 ±0.1	0.8 ±0.1	0.5 ±0.4
(nM/mM)	Control	Diabetes	Control	Diabetes	Control	Diabetes	Control	Diabetes
3-hydroxykynurenine/Creatinine	150.1 ±35.3	577.9 ±406.8**	56.8 ±38.2	303.0 ±83.0	49.9 ±18.5	103.7 ±42.6	38.0 ±35.0	220.9 ±120.8***
DL-homocysteine/Creatinine	27.4 ±13.2	12.2 ±7.0	33.4 ±33.8	12.0 ±5.7	38.4 ±27.6	7.1 ±2.9*	45.8 ±18.6	16.2 ±15.7*
Kynurenine/Creatinine	28.5 ±28.1	70.5 ±113.8	7.0 ±1.2	46.7 ±41.7	33.8 ±11.4	82.2 ±131.2	46.6 ±12.4	1470.2 ±1711.9*
Nicotinic acid/Creatinine	35.6 ±56.5	887.8 ±1663.0	436.2 ±907.1	188.8 ±251.5	131.0 ±197.6	555.6 ±727.8	16.1 ±26.9	324.6 ±464.1
Sulpiride/Creatinine	6.2 ±1.3	12.2 ±7.0	7.0 ±1.2	12.0 ±5.7	5.1 ±2.9	7.1 ±2.9	5.5 ±2.4	10.7 ±6.4
Glycyl-histidine/Creatinine	21.0 ±16.2	56.7 ±41.8	24.0 ±15.2	42.6 ±31.1	29.5 ±12.9	44.9 ±14.6	28.8 ±16.1	95.1 ±58.0

*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 vs non-diabetic control