Online Supplemental Data

Metabolic reprogramming by N-acetyl-seryl-aspartyl-lysyl-proline protects against diabetic kidney disease

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Running Title: Metabolic reprogramming by AcSDKP

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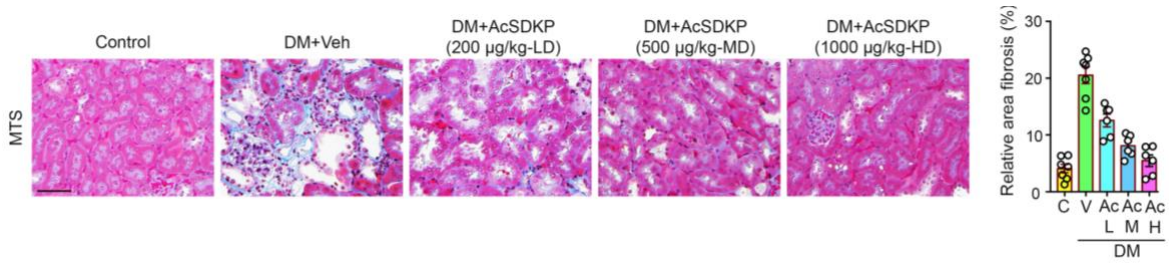

Figure S1 Dose dependent antifibrotic response of AcSDKP in the kidneys of diabetic mice.

Masson trichrome staining analysis in the kidneys of control, diabetic and AcSDKP (low-200 µg, medium-500 µg and high-1000 µg) treated diabetic mice. Representative pictures are shown. Scale bar: 50 µM in each panel. The kidneys of seven mice were analyzed in control and diabetic group, whereas, six mice were analyzed in the low, medium and high dose AcSDKP treated diabetic mice. The data are expressed as the means ± SEM and are included in the graph. Statistical significance *-P<0.05. DM represents diabetic group

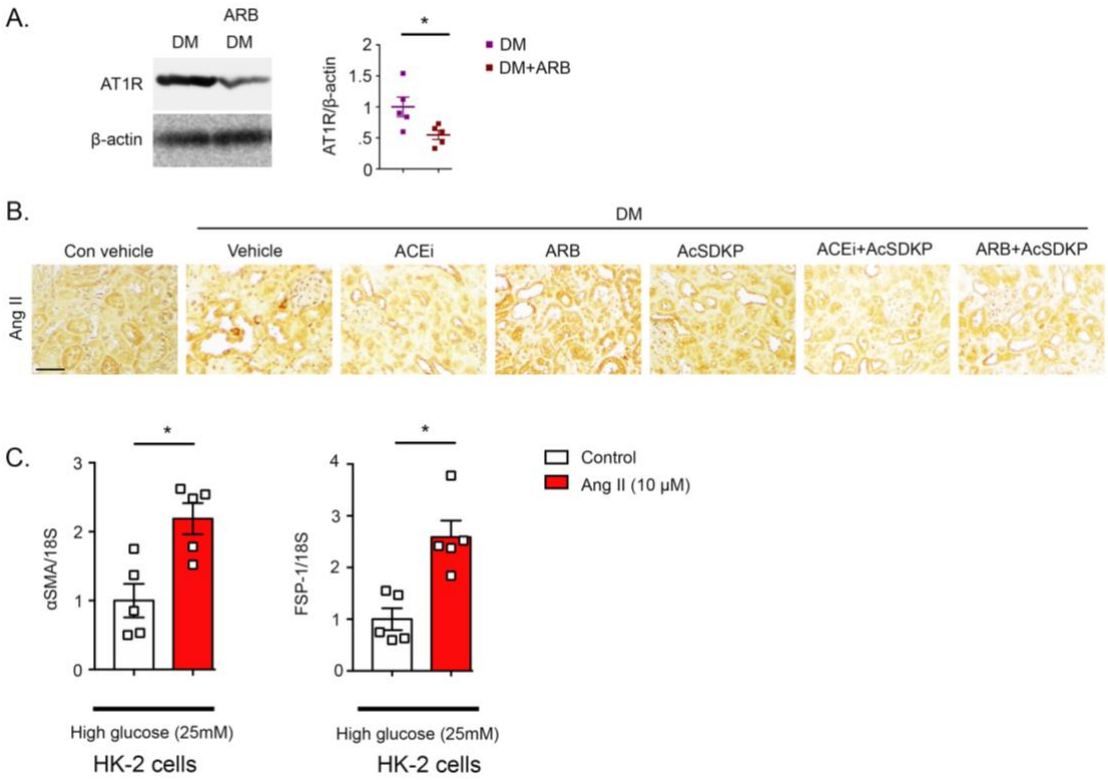

Figure S2 Angiotensin II level in the kidneys.

A. Western blot analysis of AT1R in the kidneys of ARB treated diabetic mice and vehicle treated diabetic mice. Representative blot is shown. Densitometry data were normalized by β-actin. **B.** Immunohistochemistry analysis of Angiotensin II in the kidneys of the control, diabetic, and ACEi, ARB, AcSDKP, ACEi+AcSDKP, ARB+AcSDKP treated diabetic CD-1 mice. Five mice were analyzed in each group. Representative pictures in each panel are shown. Scale bar 50 μm. **C.** Gene expression analysis of αSMA and FSP-1 by qPCR in the Angiotensin II (10 μM) stimulated high glucose (25 mM) treated HK2 cells. 18S was used as internal control to normalize the expression level. Five well in each group were analyzed. The data are expressed as the means ± SEM and are included in the graph. Statistical significance *-*P*<0.05. DM represents diabetic group.

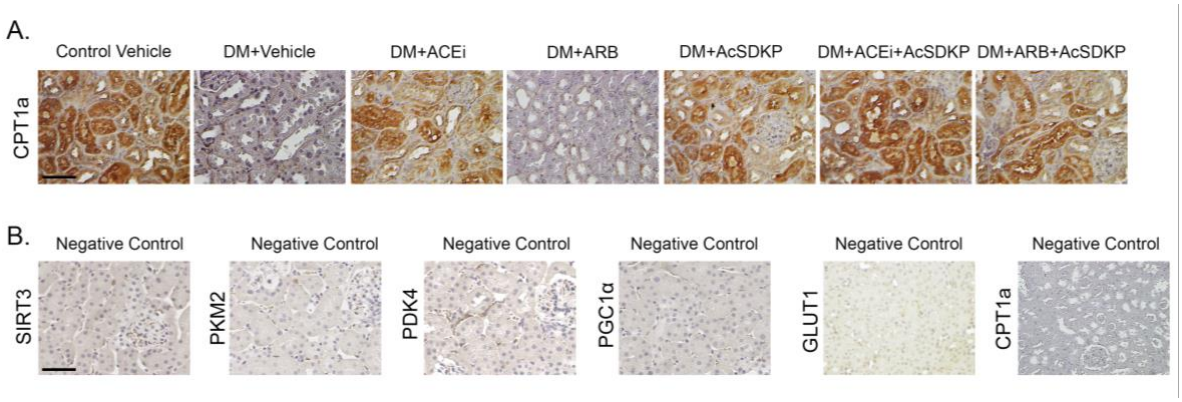

Figure S3 CPT1a level in kidneys

A. Immunohistochemistry analysis of CPT1a in the kidneys of the control, diabetic, and ACEi, ARB, AcSDKP, ACEi+AcSDKP, ARB+AcSDKP treated diabetic CD-1 mice. Five mice were analyzed in each group. Representative pictures in each panel are shown. Scale bar 50 μm. **B.** Negative controls

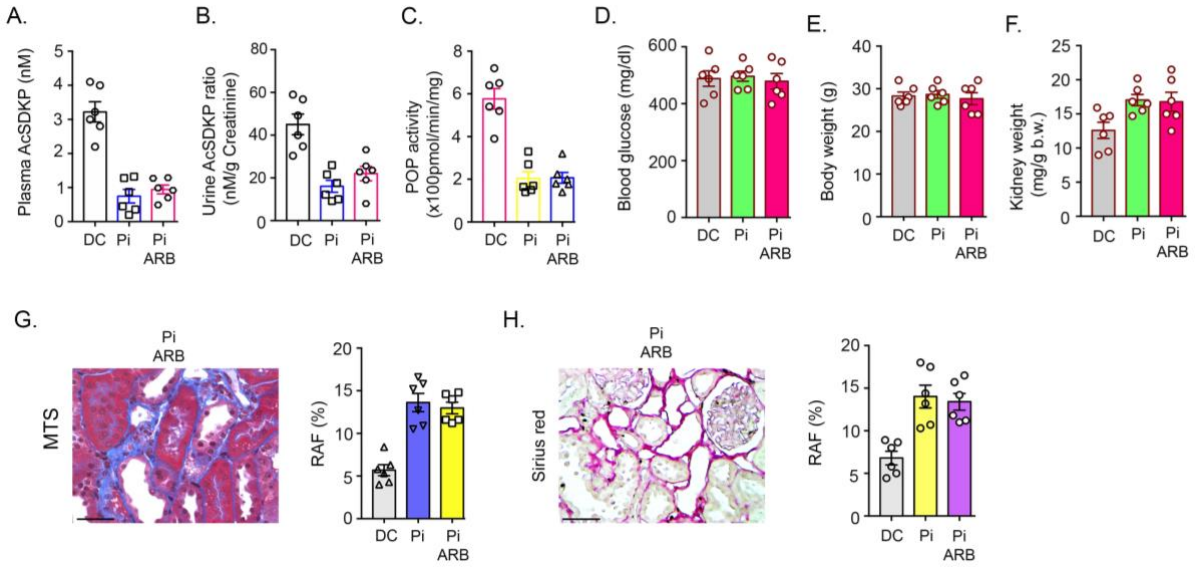

Figure S4 Effect of ARB in the POP inhibitor treated diabetic mice

A. The level of AcSDKP in the plasma, and **(B)** in urine of diabetic control, POPi treated and ARB-intervened POPi treated diabetic C57Bl6 mice, were analyzed. Urine AcSDKP levels were normalized by the urine creatinine level. Six mice were analyzed in each group. **C.** POP enzyme activity analysis by fluorimeter in kidney homogenate of indicated groups. The kidneys of six mice were analyzed in each group. **D-F.** Physiological characteristics (blood glucose, body weight and kidney weight,) of diabetic control, POPi, and ARB-intervened POPi injected diabetic mice. Six mice were analyzed in each group. **G.** Masson's trichrome staining (MTS) in the kidneys of indicated group and the quantification of the relative area fibrosis (RAF) by ImageJ. Scale bar: 50 μM. The kidneys of six mice were analyzed in each group. **H**. Sirius red staining in the kidneys of indicated group and quantification by ImageJ. Scale bar: 50 μM. The kidneys of six mice were analyzed in each group. The data are expressed as the means ± SEM and are included in the graph. Statistical significance *-P<0.05. DC represents diabetic group; Pi represents POPi treatment group

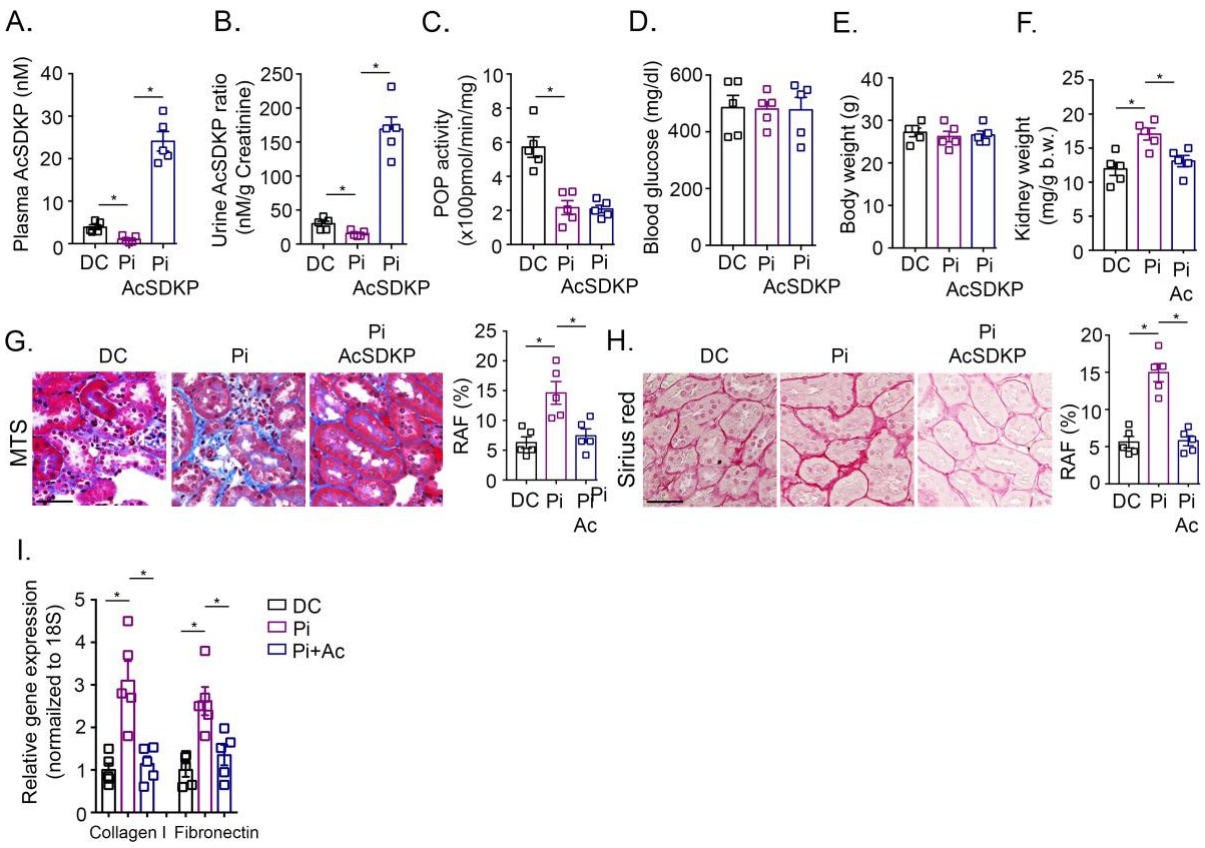

Figure S5 Effect of AcSDKP in the POP-inhibitor treated diabetic mice

A. The level of AcSDKP in the plasma, and **(B)** in urine of diabetic control, POPi treated and AcSDKP-intervened POPi treated diabetic C57Bl6 mice. Urine AcSDKP levels were normalized by the urine creatinine level. Five mice were analyzed in each group. **C.** POP enzyme activity analysis by fluorimeter in kidney homogenate of indicated groups. The kidneys of five mice were analyzed in each group. **D-F.** Physiological characteristics (blood glucose, body weight and kidney weight,) of diabetic control, POPi, and AcSDKP-intervened POPi injected diabetic mice. Five mice were analyzed in each group. **G.** Masson's trichrome staining (MTS) in the kidneys of indicated group and the quantification of the relative area fibrosis (RAF) by ImageJ. Scale bar: 50 μM. The kidneys of five mice were analyzed in each group. **H**. Sirius red staining in the kidneys of indicated group and quantification by ImageJ. Scale bar: 50 μM. The kidneys of five mice were analyzed in each group. **I.** Relative gene expression analysis of collagen I and fibronectin in the indicated groups. The kidneys of five mice were analyzed in each group by qPCR. The data are expressed as the means ± SEM and are included in the graph. Statistical significance *-*P*<0.05. DC represents diabetic group; Pi represents POPi treatment group

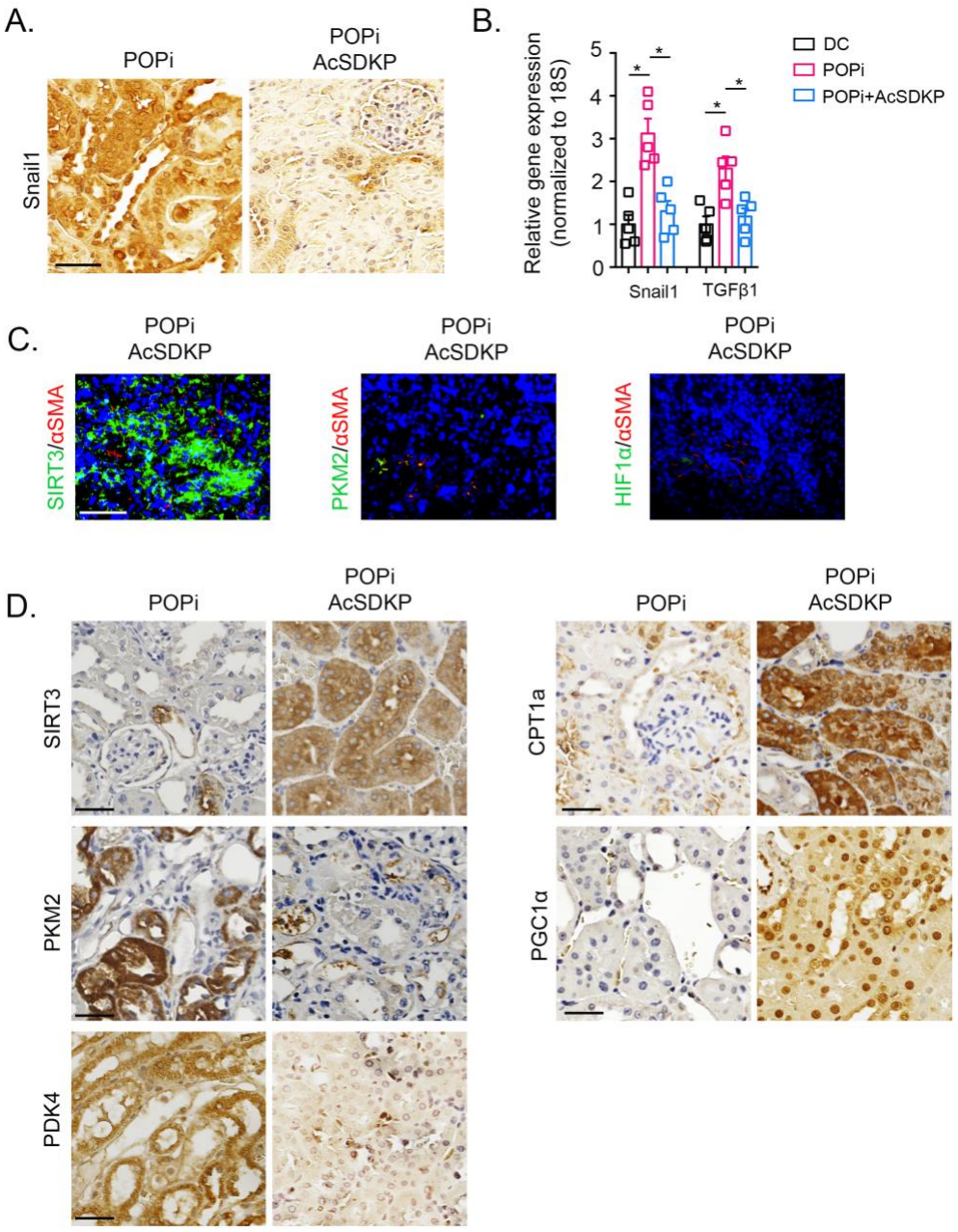

Figure S6 Effect of AcSDKP on the renal metabolism in the POP-inhibitor treated diabetic mice.

A. Immunohistochemistry analysis of Snail1 in the kidney of POPi treated, and AcSDKP intervened POPi-treated diabetic C57Bl6 mice. Representative pictures are shown. Scale bar 50 μm. Five number of mice were analyzed in each group. **B.** Gene expression analysis of Snail1, and TGF-β1 by qPCR in the kidneys of indicated groups. The kidneys of five mice were analyzed in each group. Gene expression data were normalized by 18S. **C.** Co-immunofluorescence analysis of SIRT3, PKM2, and HIF1α, with α-SMA, were analyzed by using fluorescence microscope. The kidneys of five mice were analyzed in each group. Representative pictures in each panel are shown. **D.** Immunohistochemistry analysis of SIRT3, PKM2, PDK4, CPT1a and PGC1α , in the kidneys of the POPi, and AcSDKP intervened POPi-treated diabetic mice. Five mice were analyzed in each group. Representative pictures in each panel are shown. Scale bar 50 μm. Data in the graph are presented as mean±SEM. Statistical significance *-*P*<0.05.

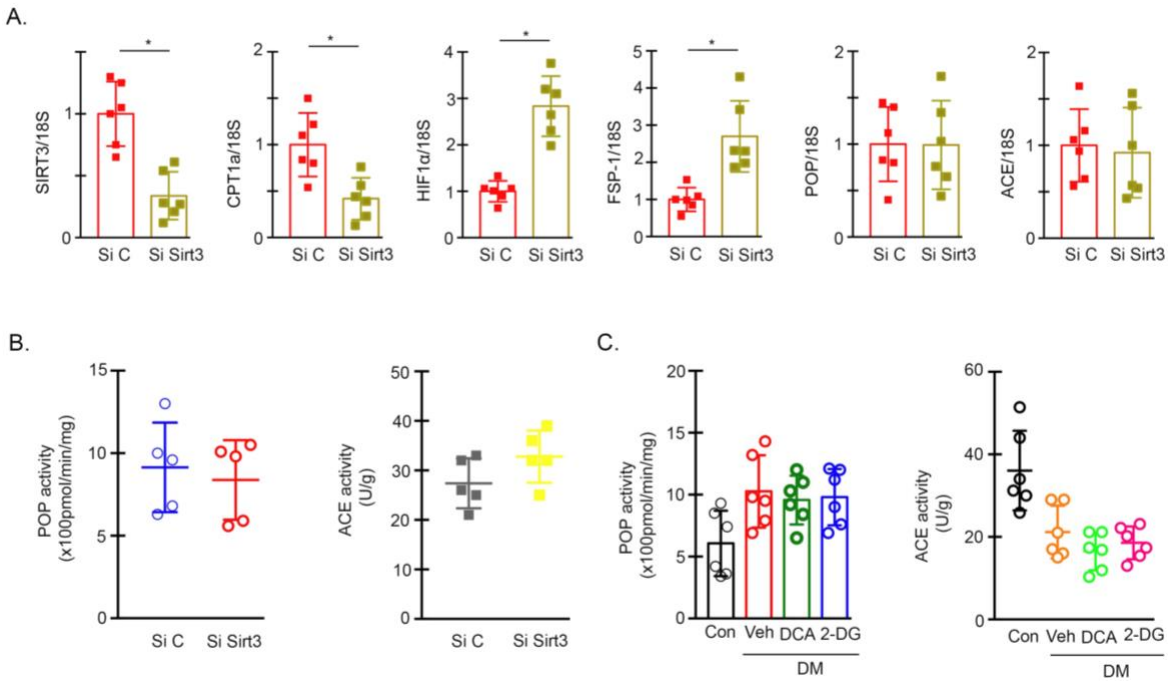

Figure S7 Effect of SIRT3 knockdown in the ACE and POP activity in the kidneys of diabetic mice

A. Gene expression analysis of SIRT3, CPT1a, HIF1α, FSP-1, POP and ACE by qPCR in the kidneys of scramble siRNA and SIRT3 siRNA injected diabetic mice. 18S was used as internal control to normalize the expression level. The kidneys of six mice were analyzed. **B.** POP and ACE enzyme activity analysis by fluorimeter in kidney homogenate of indicated groups. The kidneys of five mice were analyzed in each group. **C.** POP and ACE enzyme activity analysis by fluorimeter in kidney homogenate of indicated groups. The kidneys of six mice were analyzed in each group. The data are expressed as the means ± SEM and are included in the graph. Statistical significance *-P<0.05. Si C represents scramble siRNA; Si Sirt3 represents SIRT3 siRNA, con represents nondiabetic control while, DM represents diabetic group.