

**Supplementary information.**

**Chloroquine and amodiaquine enhance AMPK phosphorylation and improve mitochondrial fragmentation in diabetic tubulopathy**

**(Chloroquine and amodiaquine have effects on diabetic tubulopathy)**

Hye Yun Jeong<sup>1</sup>, Jun Mo Kang<sup>1</sup>, Hak Hoon Jun<sup>2</sup>, Dong-Jin Kim<sup>3</sup>, Seon Hwa Park<sup>3</sup>, MinJi Sung<sup>1</sup>, Jin Hyung Heo<sup>4</sup>, Dong Ho Yang<sup>1</sup>, Sang Ho Lee<sup>3\*</sup>, So-Young Lee<sup>1\*</sup>

<sup>1</sup>Division of Nephrology, Department of Internal Medicine, CHA Bundang Medical Center, CHA University, Seongnam, <sup>2</sup>Department of Surgery, CHA Bundang Medical Center, CHA University, Seongnam, <sup>3</sup>Division of Nephrology, Department of Internal Medicine, Kyung Hee University Hospital at Gangdong, Kyung Hee University, Seoul, <sup>4</sup>Department of Pathology, CHA Bundang Medical Center, CHA University, Seongnam, South Korea

**Corresponding Authors:**

Dr. So-Young Lee, Division of Nephrology, Department of Internal Medicine, CHA University School of Medicine, CHA Bundang Medical Center, 59 Yatap-ro, Bundang-gu, Seongnam-si, 13496, South Korea. Phone: +82-31-780-5025, Fax: +82-31-780-5219, E-mail: ysy0119@cha.ac.kr

Or Dr. Sang Ho Lee, Division of Nephrology, Department of Internal Medicine Kyung Hee Univ. Hospital at Gangdong, 892 Sangil-ro, Gangdong-gu, Seoul, 05278, South Korea. Phone +82-1-440-6121, E-Mail: lshkidney@khu.ac.kr

H. Y Jung, J. M. Kang, H. H. Jun contributed equally to the work.

S-Y. Lee and S. H. Lee contributed equally to the work.

**Supplementary Fig. S1.** pAMPK $\alpha$  expression in hRPTCs under HG conditions. (a) Western blot analyses showed that pAMPK $\alpha$  expression decreased according to the time of incubation in 30 mM D-glucose. (b) pAMPK $\alpha$  expression in hRPTCs after incubation with 5 mM or 30 mM D-glucose or 25 mM mannitol (\* p < 0.05 vs 5 mM D-glucose, #p <0.05 vs 30 mM D-glucose). The original images of (c) pAMPK $\alpha$  and (d) GAPDH in Supplementary Fig. S1a. The original images of (e) pAMPK $\alpha$  and (f) GAPDH in Supplementary Fig.S1b.

**Supplementary Fig. S2.** CQ and AQ increased AMPK $\alpha$  phosphorylation in hRPTCs under HG conditions. (a) pAMPK $\alpha$  expression in hRPTCs under HG conditions after treatment with different concentrations of CQ. (b) pAMPK $\alpha$  expression in hRPTCs under HG conditions after treatment with different concentrations of AQ. The original images of (c) pAMPK $\alpha$ , (d) AMPK $\alpha$  and (e) GAPDH in Supplementary Fig. S2a. The original images of (f) pAMPK $\alpha$ , (g) GAPDH, (h) AMPK $\alpha$  and (i) GAPDH in Supplementary Fig. S2b.

**Supplementary Fig. S3.** CQ and AQ normalized the levels of AMP/ATP ratio in hRPTCs under HG condition. Results are means  $\pm$  SEM. for experiments in triplicate. (\*p < 0.05 vs 5 mM, #p < 0.05 vs 30 mM)

**Supplementary Fig. S4.** CQ and AQ induce LKB1 phosphorylation under HG conditions (a) Western blot analyses showed that pLKB1 expression increased after treatment with CQ and AQ in hRPTCs subject to high glucose. The original images of (b) pLKB1, (c) LKB1, and (d)  $\beta$ -actin in Supplementary Fig S4. (\*\*p < 0.01 vs 5 mM, #p < 0.05 vs 30 mM)

**Supplementary Fig. S5.** Effects of CQ and AQ on the expression of pAMPK $\alpha$  and pPGC1 $\alpha$  in diabetic kidneys. (a) CQ and AQ restored pAMPK $\alpha$  and pPGC1 $\alpha$  expression in diabetic kidneys. The original images of (b) pAMPK $\alpha$ , (c) AMPK $\alpha$ , (d) pPGC1 $\alpha$ , (e) PGC1 $\alpha$  and (f)  $\beta$ actin in Supplementary Fig. S5.

**Supplementary Fig. S6.** Western blot of cell lysate in Fig.1. The original images of (a) pAMPK $\alpha$ , (b) AMPK $\alpha$ , and (c) pPGC1 $\alpha$  in Fig. 1A. The original images of (d) PGC1 $\alpha$ , (e) pPGC1 $\alpha$ , and (f)  $\beta$ actin in Fig. 1B.

**Supplementary Fig. S7.** Western blot of cell lysate in Fig. 2. The original images of (a) pAMPK $\alpha$ , (b) AMPK $\alpha$ , (c) pPGC1 $\alpha$ , (d) PGC1  $\alpha$ , and (e)  $\beta$ actin in Fig 2A. The original images of (f) pAMPK $\alpha$ , (g) AMPK $\alpha$ , (h) pPGC1 $\alpha$ , (i) PGC1 $\alpha$ , and (j)  $\beta$ actin in Fig. 2B.

**Supplementary Fig. S8.** Western blot of cell lysate in Fig. 3. The original images of (a) Tom20, and (b) GAPDH in Fig. 3B. The original images of (c) Drp1, (d) Mfn1, and (e) GAPDH in Fig. 3C.

**Supplementary Fig. S9.** Western blot of cell lysate in Fig. 4. The original images of (a) Tom20, (b) Drp1, (c) Mfn1 and (d)  $\beta$ actin in Fig. 4A. The original images of (e) Tom20, (f) Drp1, (g) Mfn1, and (h)  $\beta$ actin in Fig. 4B.

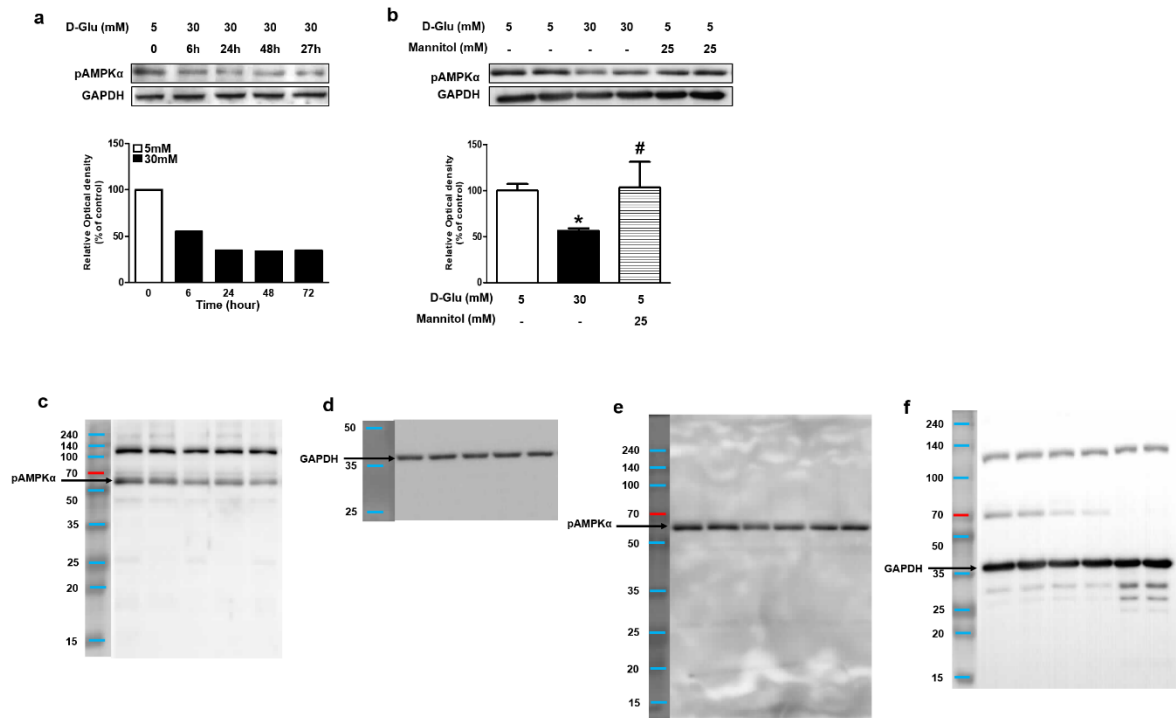
**Supplementary Fig. S10.** Western blot of cell lysate in Fig. 5. The original images of (a) Bcl-2, (b) Bax, and (c) GAPDH in Fig. 5B. The original images of (d) Cyt.C, and (e) GAPDH in Fig. 5C.

**Supplementary Fig. S11.** Western blot of cell lysate in Fig. 6. The original images of (a) TGF- $\beta$ 1, and (b) GAPDH in Fig. 6A. The original images of (c) E-cad, (d)  $\alpha$ -SMA, (e) fibronectin and (f) GAPDH in Fig.6B.

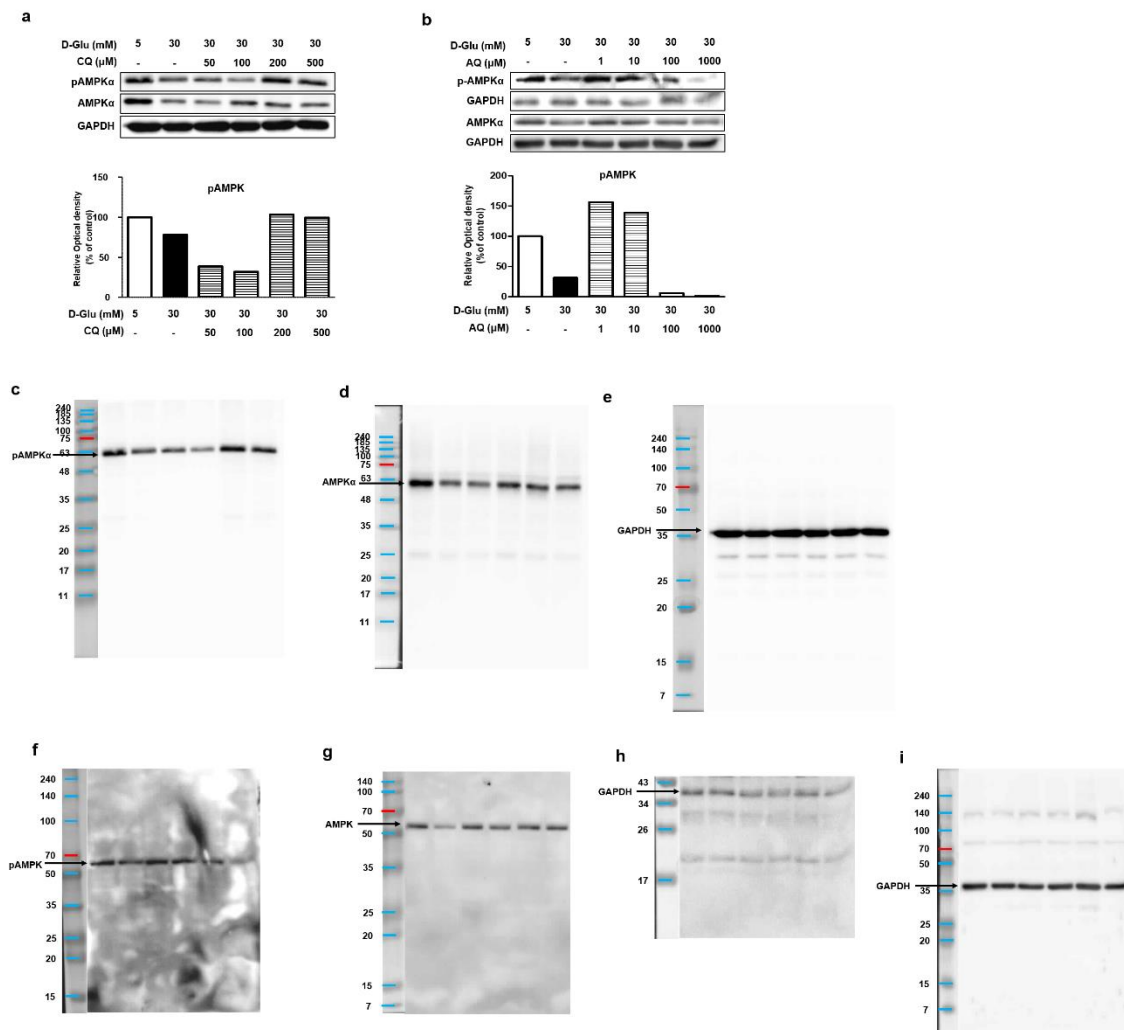
**Supplementary Fig. S12.** Western blot of tissue lysate in Fig. 8. The original images of (a) Tom20 in Fig. 8A. The original images of (b) Drp1, and (c) Mfn1 in Fig. 8B. The original images of (d)  $\beta$ actin in Fig. 8A and 8B.

**Supplementary Fig. S13.** Western blot of tissue lysate in Fig. 9. The original images of (a) Bcl-2, (b) Bax, and (c) Cyt.C in Fig. 9A. The original images of (d) TGF- $\beta$ 1, (e) E-cad, (f)  $\alpha$ -SMA, and (g) fibronectin in Fig. 9D. The original images of (h)  $\beta$ actin in Fig. 9A and 9D.

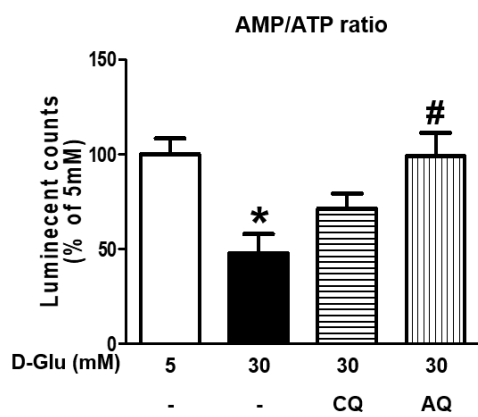
# Supplementary Fig. S1.



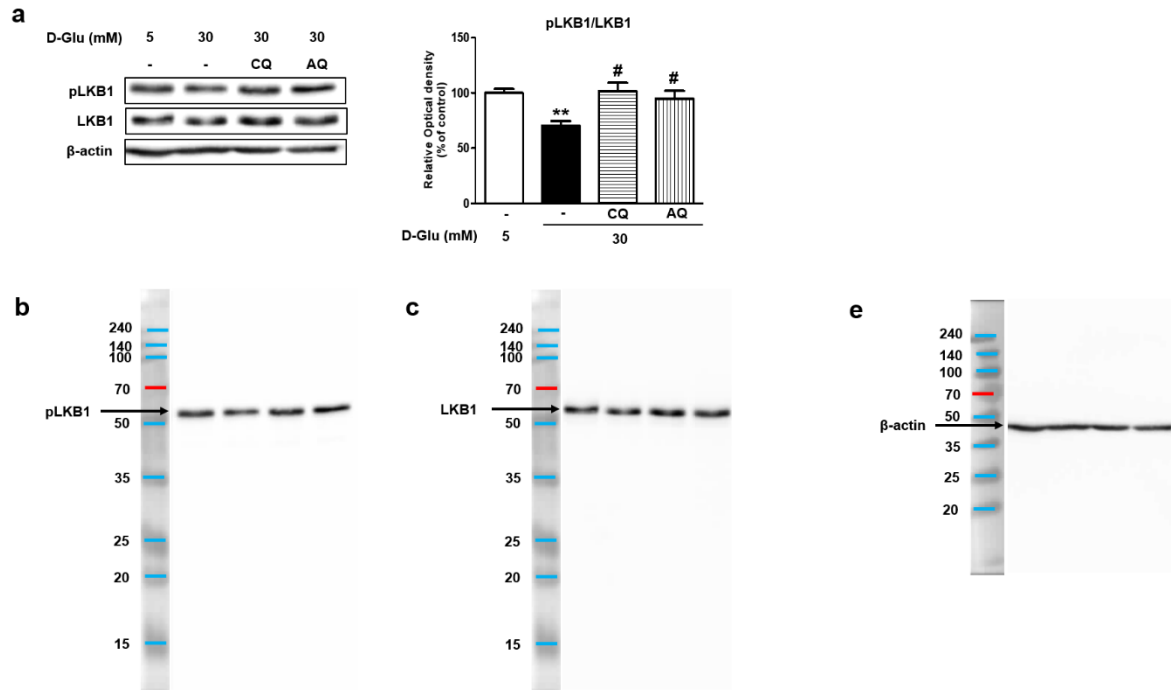
Supplementary Fig. S2.



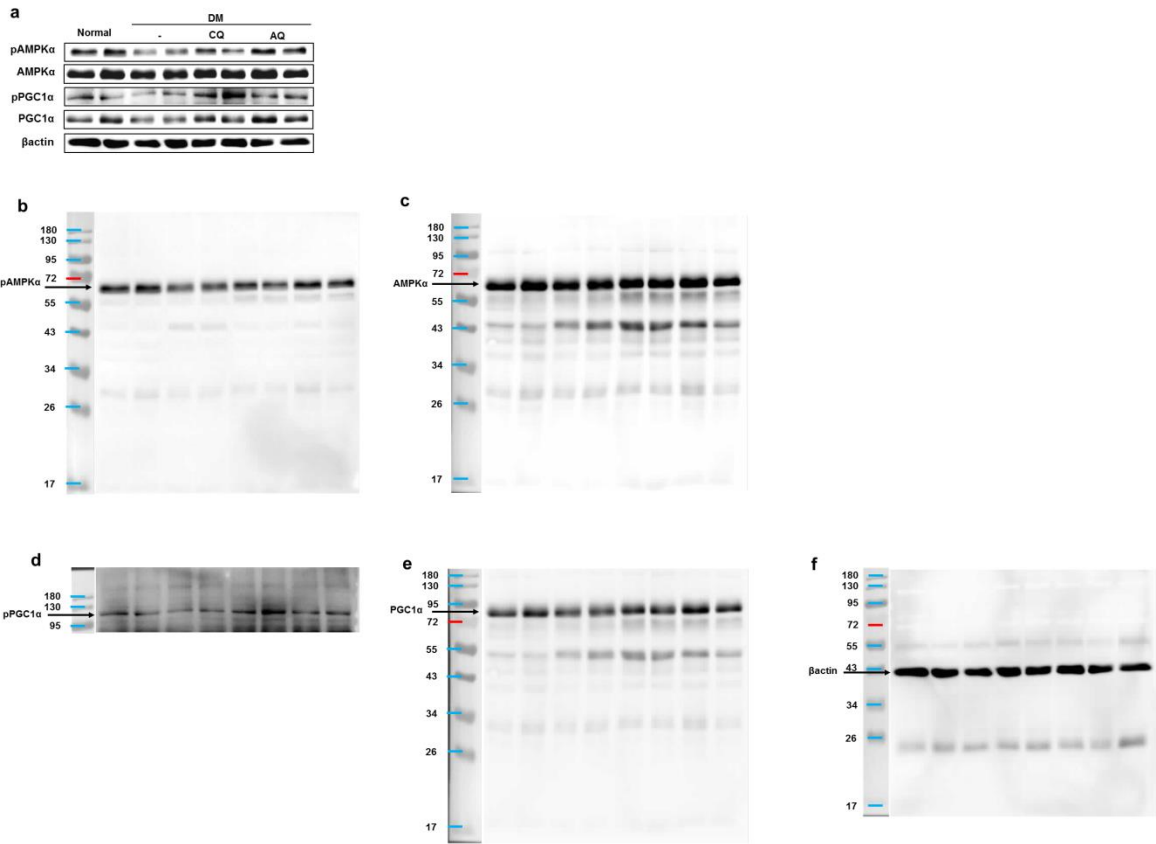
Supplementary Fig. S3.



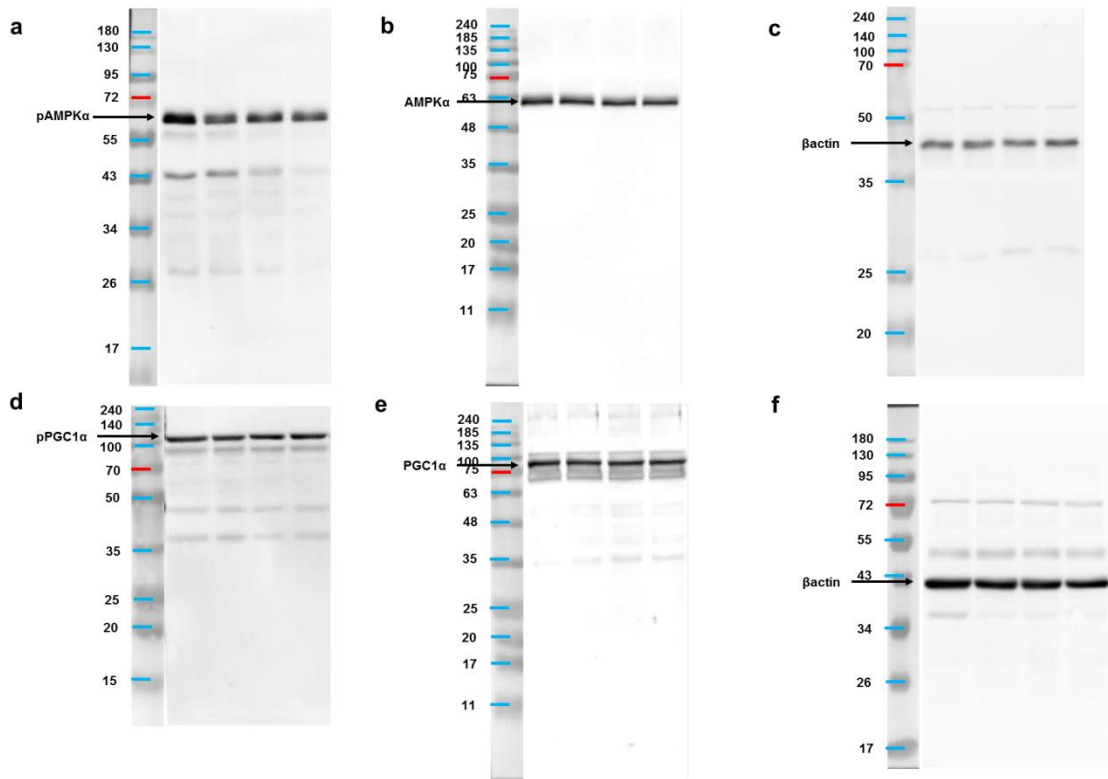
## Supplementary Fig. S4.



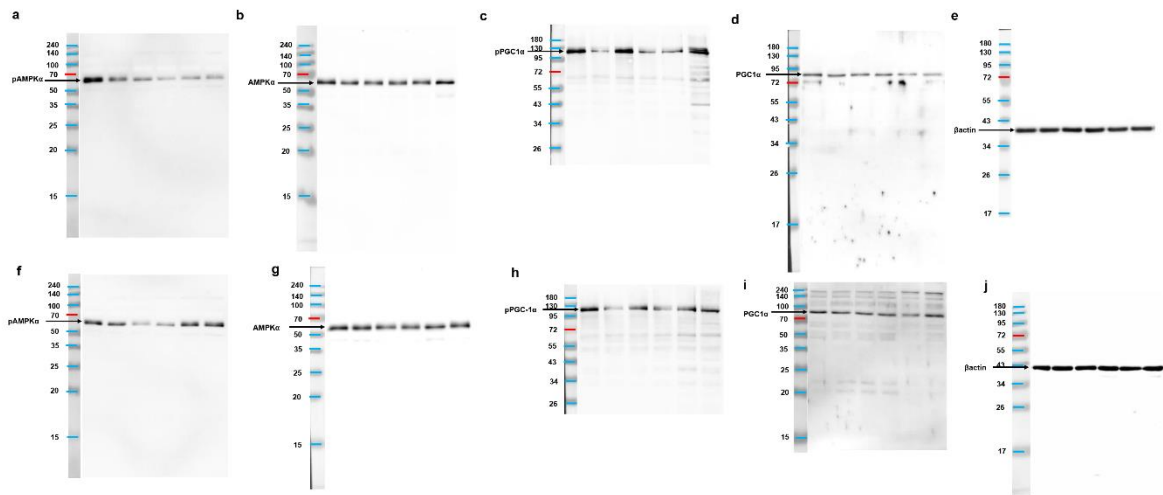
# Supplementary Fig. S5.



Supplementary Fig. S6.

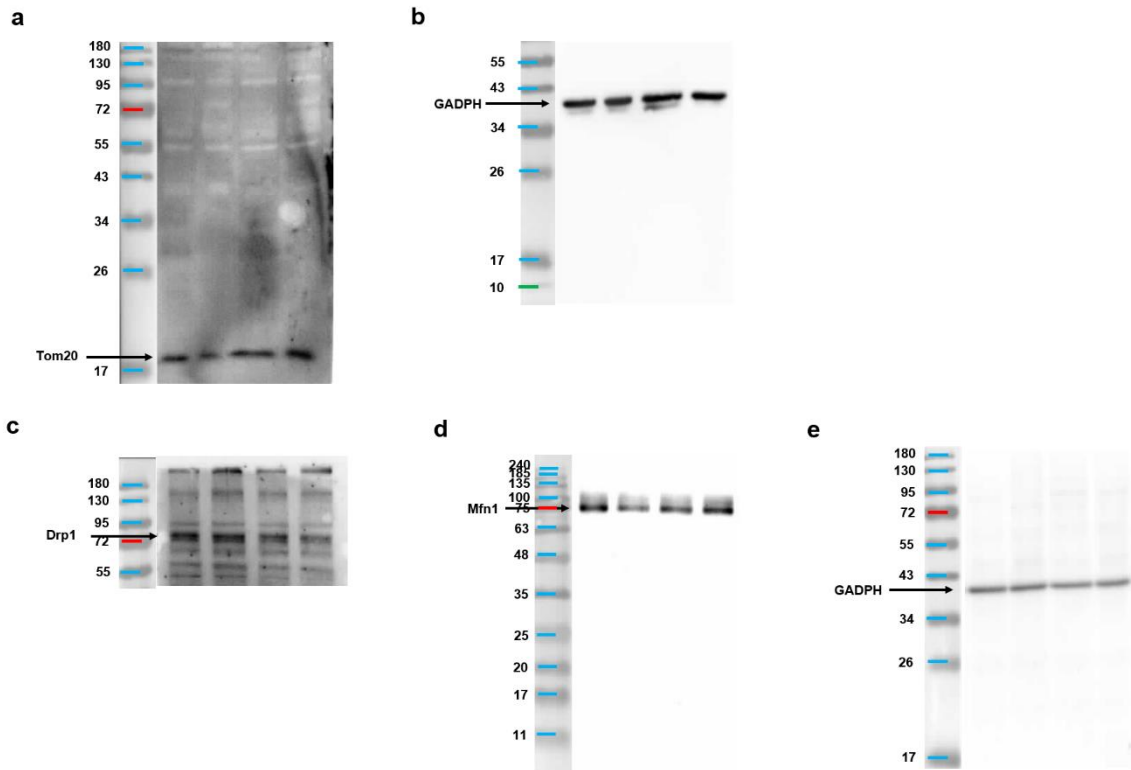


Supplementary Fig. S7.

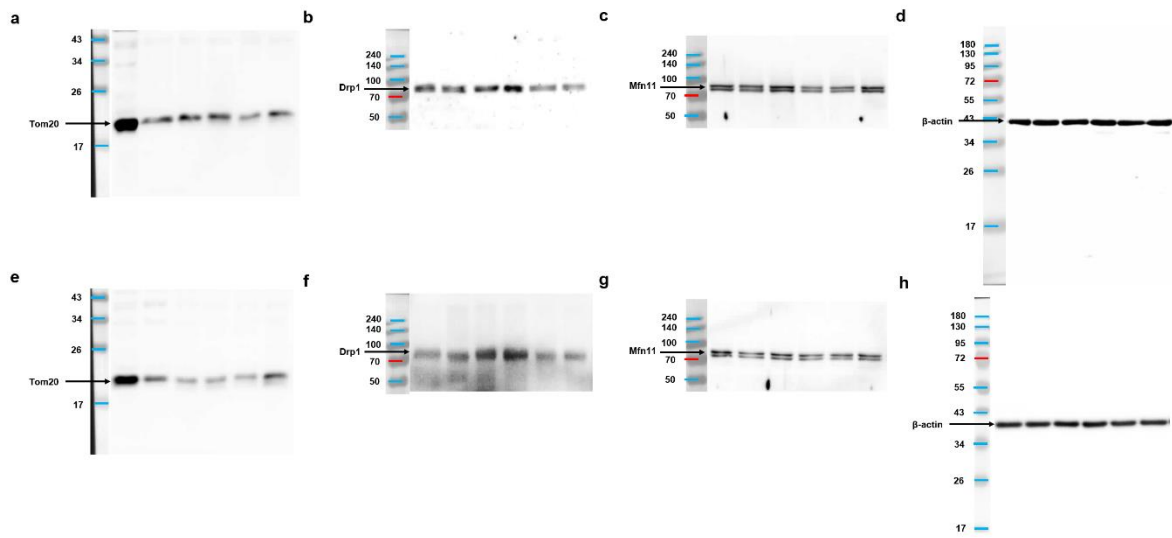




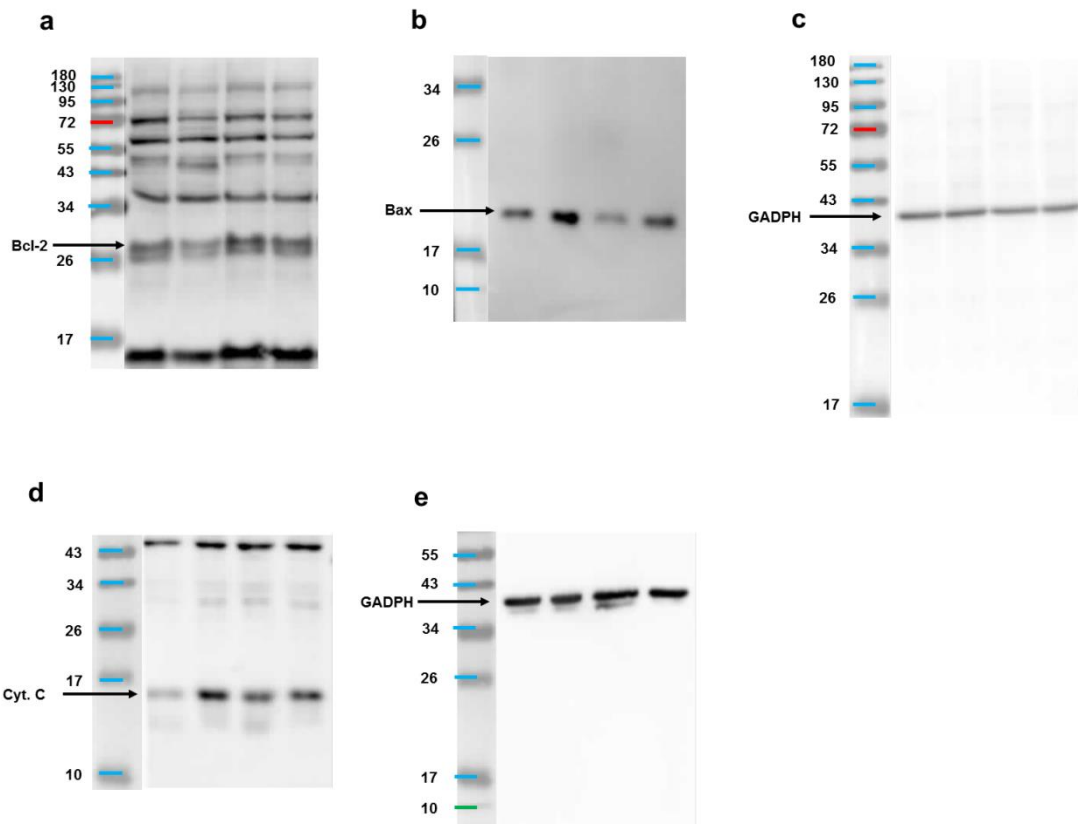
# Supplementary Fig. S8



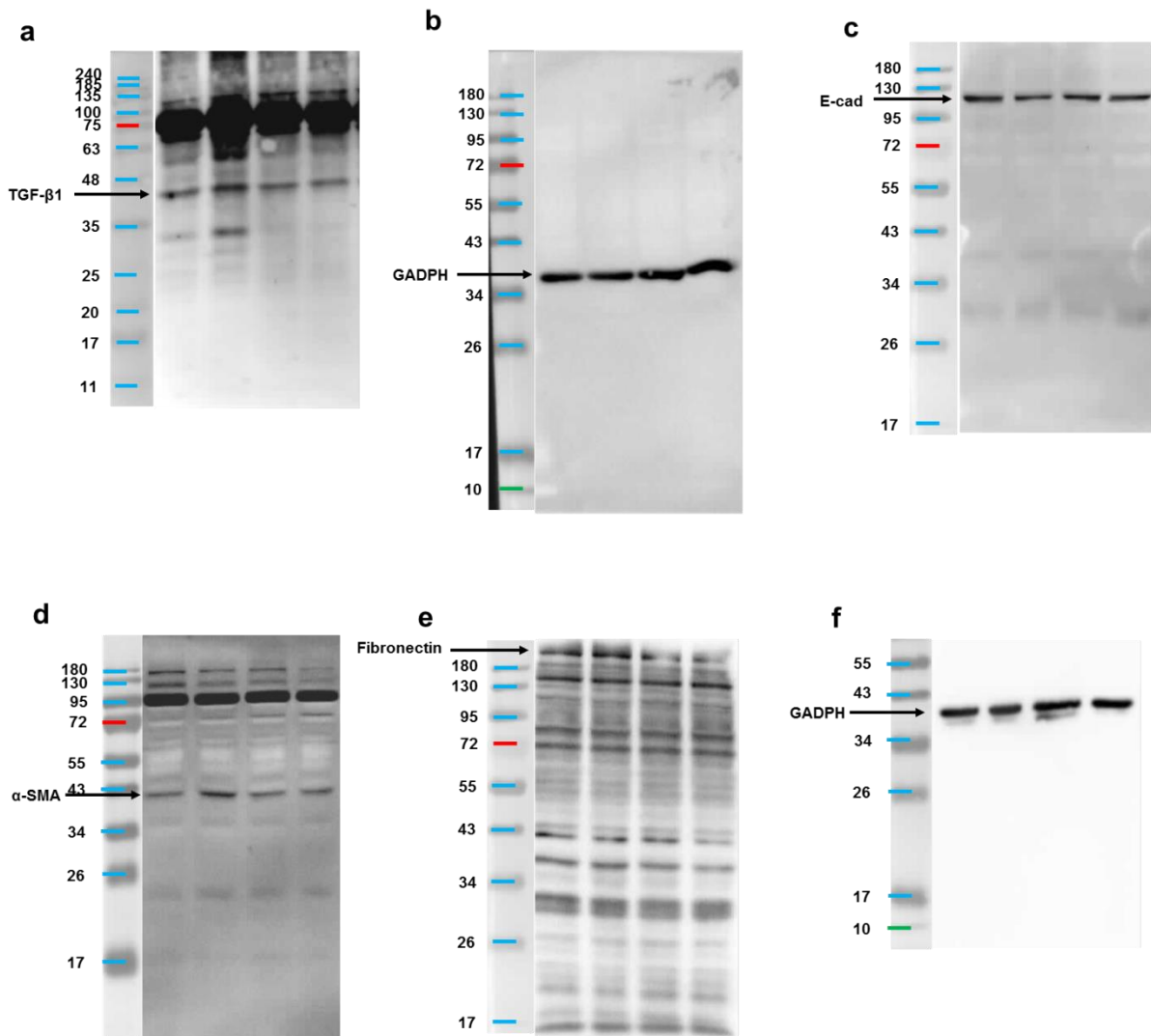
# Supplementary Fig. S9



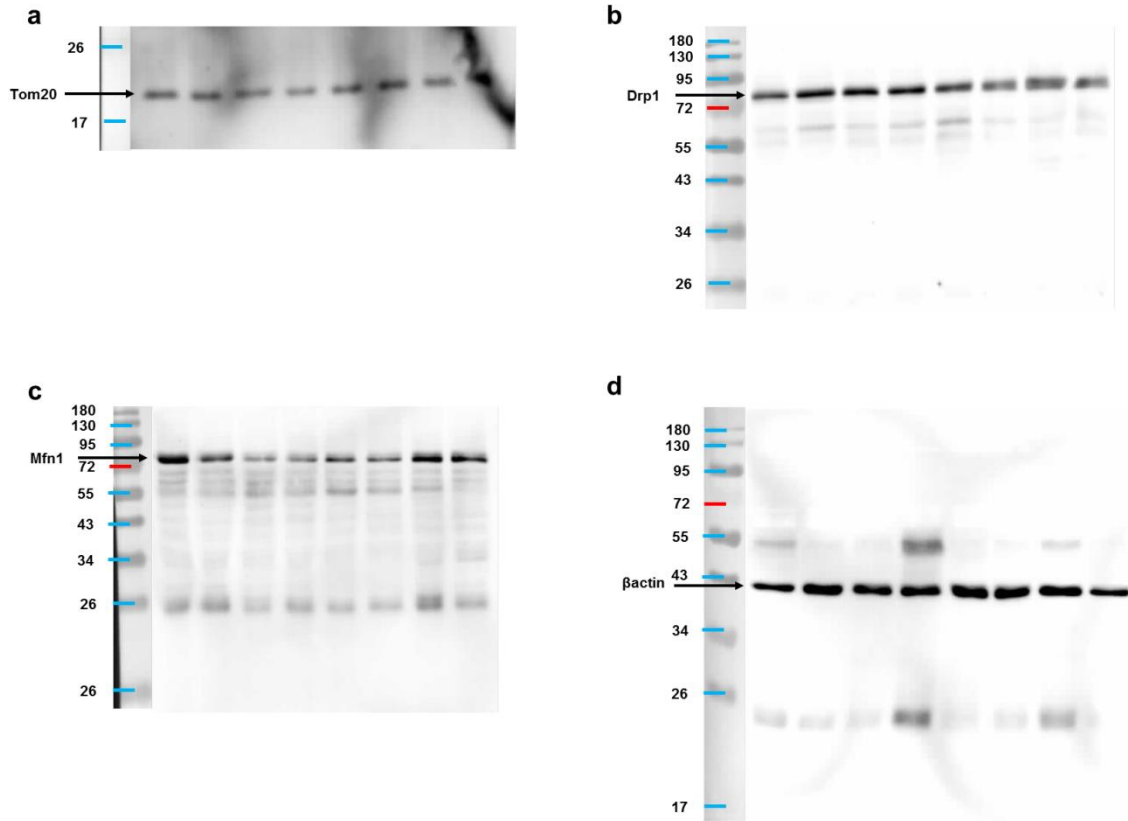
Supplementary Fig. S10.



# Supplementary Fig. S11



## Supplementary Fig. S12



## Supplementary Fig. S13.

