Figure S1. Decreased exosome secretion and down-regulated RAB27B in high glucose-treated HK-2 cells. HK-2 cells were cultured in DMEM/F12 containing 30 mM glucose (HG) or 5.5 mM glucose with 24.5 mM D-mannitol (NG) for 8 days as detailed in Methods. Cell culture medium and cell lysates were collected at the 8th day for NTA and immunoblot analysis, respectively. A. High glucose incubation reduced exosome secretion. Exosomes were quantified by NTA and the exosome number was then normalized with the cell numbers in NG and HG groups (n=3). **P<0.01. B. HG treatment did not change the size of exosomes. Peak diameter of exosomes was analyzed by NTA in NG and HG groups (n=3). C. Decreased protein level of RAB27B in HG-treated cells. Representative immunoblots of ALIX, TSG101, RAB27A, and RAB27B from cell lysates in NG and HG groups. D-G. Densitometric analyses of ALIX, TSG101, RAB27A, and RAB27B from cell lysates in NG and HG.
groups. β-ACTIN was used as the loading control. After normalization with β-ACTIN, the protein level in NG group was arbitrarily set as 1 and the protein level in HG group was compared with that one to calculate the fold change. *$P<0.05$ (n=3).

**Decreased mRNA level of Rab27b in HG-treated cells.** The mRNA level of Rab27b was detected by real-time PCR with β-actin as the reference gene. After normalization with β-actin, the mRNA level in NG group was arbitrarily set as one and the mRNA level in HG group was compared with that one to calculate the fold change. **$P<0.01$ (n=3).**
Figure S2. Parameters of diabetic kidney disease in streptozotocin-induced diabetic mice. C57BL/6J (male, 8-weeks) were treated with STZ to induce diabetes or with vehicle solution as control (Con). Body weight (g) and fasting blood sugar (mg/dl) were measured at 20 weeks of age. After that, the mice were sacrificed to collect kidney tissues. A. Decreased body weight of diabetic mice. Body weight was measured in control (n=4) and STZ (n=8) groups. ***P<0.001. B. Increased fasting blood sugar of diabetic mice. Fasting blood sugar was measured in control (n=4) and STZ (n=8) groups. ***P<0.001. C. PAS staining showing worse tubular damage in diabetic mouse kidneys. Magnification, 10x. Scale bar, 0.1 mm. D, E. Sirius Red/Fast Green collagen staining showing more interstitial collagen in diabetic mouse kidneys.
D. Representative Sirius Red/Fast Green collagen staining images (10x). Scale bar, 0.1 mm. E. Quantitative analysis of Sirius Red/Fast Green collagen staining by Image-Pro Plus in control (n=4) and STZ (n=8) groups. ***P<0.001.

Figure S3. FOXO1 phosphorylation and RAB27B downregulation in renal cortical tissues of diabetic Akita mice. Akita male mice were sacrificed at 20 weeks of age to collect renal cortical tissues. A-G. Increased FOXO1 phosphorylation and decreased RAB27B expression in renal cortical tissues of diabetic Akita mice. A. Representative immunoblots of ALIX, TSG101, FOXO1, phospho-FOXO1(Ser256), RAB27A, and RAB27B. B, C. Densitometric analyses of ALIX and TSG101 in control (Con) (n=3) and diabetic kidney disease (DKD) (n=5) groups. Cyclophilin B
was used as the loading control. After normalization with Cyclophilin B, the protein level of control group was arbitrarily set as one and the protein level of other mice was compared with it to calculate the fold change. D. Densitometric analysis of total FOXO1 in control (n=3) and DKD (n=5) groups. β-ACTIN was used as the loading control. After normalization with β-ACTIN, the protein level of the control group was arbitrarily set as one and the protein level of other mice was compared with it to calculate the fold change. E. Densitometric analysis of phospho-FOXO1(Ser256) in control (n=3) and DKD (n=5) groups. P-FOXO1 was normalized with total FOXO1. After normalization, the protein level of the control group was arbitrarily set as one and the protein level of other mice was compared with it to calculate the fold change. *P<0.05. F, G. Densitometric analyses of RAB27A and RAB27B in control (n=3) and DKD (n=5) groups. α-TUBLIN was used as the loading control. After normalization with α-TUBLIN, the protein level of the control group was arbitrarily set as one and the protein level of other mice was compared with it to calculate the fold change. *P<0.05. H. Decreased mRNA level of Rab27b in renal cortical tissues of diabetic Akita mice. The mRNA level of Rab27b was detected by real-time PCR in control (n=3) and DKD (n=5) groups. β-actin was used as the reference gene. After normalization with β-actin, the mRNA level of the control group was arbitrarily set as one and the mRNA levels of other mice were compared with it to calculate the fold change. **P<0.01.
Figure S4. FOXO1 phosphorylation, RAB27B downregulation and decreased exosome secretion in renal cortical tissues of diabetic db/db mice. Mice (db/m and db/db) were sacrificed at 22 weeks of age to collect renal cortical tissues. A. Diabetic db/db mice produced fewer exosomes in renal cortical tissues than non-diabetic db/m mice. The relative number of exosomes in db/m (n=3) and db/db (n=4) groups was based on quantification by NTA from equal weight of renal cortical tissues.
***P<0.001. B. No significant difference in the sizes of exosomes isolated from renal cortical tissues of non-diabetic db/m mice and diabetic db/db mice. Peak diameter of exosomes was analyzed by NTA in db/m (n=3) and db/db (n=4) mice. C-I. Increased FOXO1 phosphorylation and decreased RAB27B expression in renal cortical tissues of diabetic db/db mice. C. Representative immunoblots of ALIX, TSG101, FOXO1, phospho-FOXO1(Ser256), RAB27A, and RAB27B. D, E. Densitometric analyses of ALIX and TSG101 in db/m (n=3) and db/db (n=4) groups. Cyclophilin B was used as the loading control. After normalization with Cyclophilin B, the protein level of control group was arbitrarily set as one and the protein level of other mice was compared with it to calculate the fold change. F. Densitometric analysis of total FOXO1 in db/m (n=3) and db/db (n=4) groups. β-ACTIN was used as the loading control. After normalization with β-ACTIN, the protein level of the control group was arbitrarily set as one and the protein level of other mice was compared with it to calculate the fold change. G. Densitometric analysis of phospho-FOXO1(Ser256) in db/m (n=3) and db/db (n=4) groups. P-FOXO1 was normalized with total FOXO1. After normalization, the protein level of the control group was arbitrarily set as one and the protein level of other mice was compared with it to calculate the fold change. *P<0.05. H, I. Densitometric analyses of RAB27A and RAB27B in db/m (n=3) and db/db (n=4) groups. α-TUBLIN was used as the loading control. After normalization with α-TUBLIN, the protein level of the control group was arbitrarily set as one and the protein level of other mice was compared with it to calculate the fold change.

*P<0.05. J. Decreased mRNA level of Rab27b in renal cortical tissues of diabetic db/db mice. The mRNA level of Rab27b was detected by real-time PCR in db/m (n=3) and db/db (n=4) groups. β-actin was used as the reference gene. After normalization with β-actin, the mRNA level of the control group was arbitrarily set as one and the
mRNA levels of other mice were compared with it to calculate the fold change.

**P<0.01.

**Figure S5. Rab27b is down-regulated in human diabetic kidneys and negatively associated with eGFR.** Data were obtained from Nephroseq database (https://www.nephroseq.org). A. Decreased mRNA level of Rab27b in the glomerulus of DKD patients. Healthy donor (n=21), diabetic kidney disease (DKD) (n=12). **P<0.01. B. Decreased mRNA level of Rab27b in the tubulointerstitium of DKD patients. Healthy donor (n=12), DKD (n=10). **P<0.01. C. The mRNA level of Rab27b in the tubulointerstitium of DKD patients is negatively associated with estimated glomerular filtration rate (eGFR) based on the Modification of Diet in Renal Disease (MDRD) equations (n=8). R=-0.787. P<0.05.

**Figure S6. Effect of TGFβ1 on RAB27B expression in BUMPT cells**

A

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-25 KD

-37 KD

B

![Relative protein expression of RAB27B](#)

Con  TGFβ1
Figure S6. Effect of TGFβ1 on RAB27B expression in BUMPT cells. BUMPT cells were cultured in serum-free DMEM (Con) or 5 ng/ml TGFβ1 added to serum-free DMEM for 48 hours. Cell lysates were collected for immunoblot analysis. A. No significant difference in RAB27B protein levels in TGFβ1-treated cells and untreated controls. Representative immunoblot of RAB27B from cell lysates in control and TGFβ1 groups. β-ACTIN was used as the loading control. B. Densitometric analysis of RAB27B from cell lysates in control and TGFβ1 groups. After normalization with β-ACTIN, the protein level in control group was arbitrarily set as 1 and the protein level in TGFβ1 group was compared with that one to calculate the fold change (n=3).