EGFR drives the progression of AKI to CKD through upregulation of HIPK2

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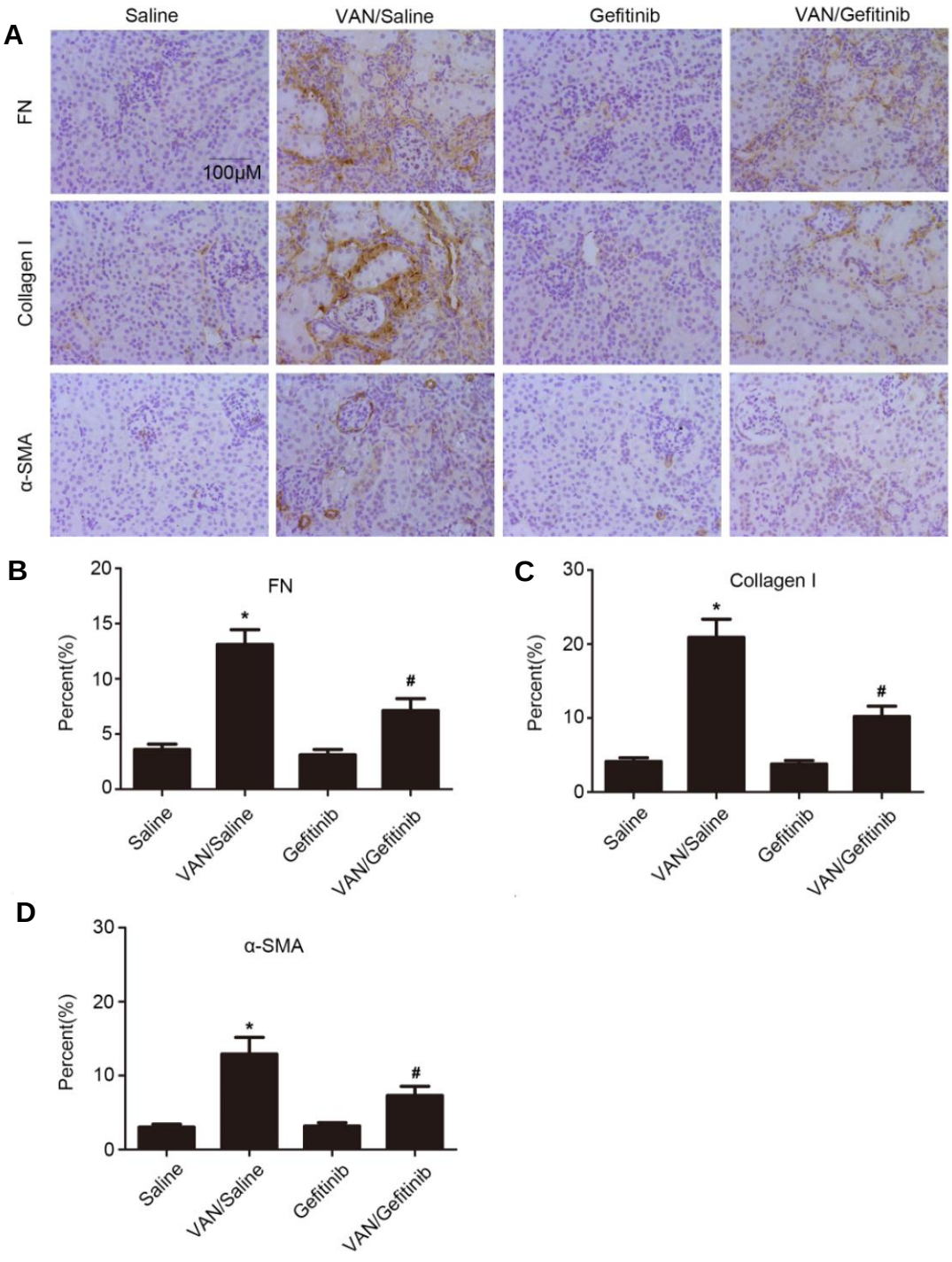
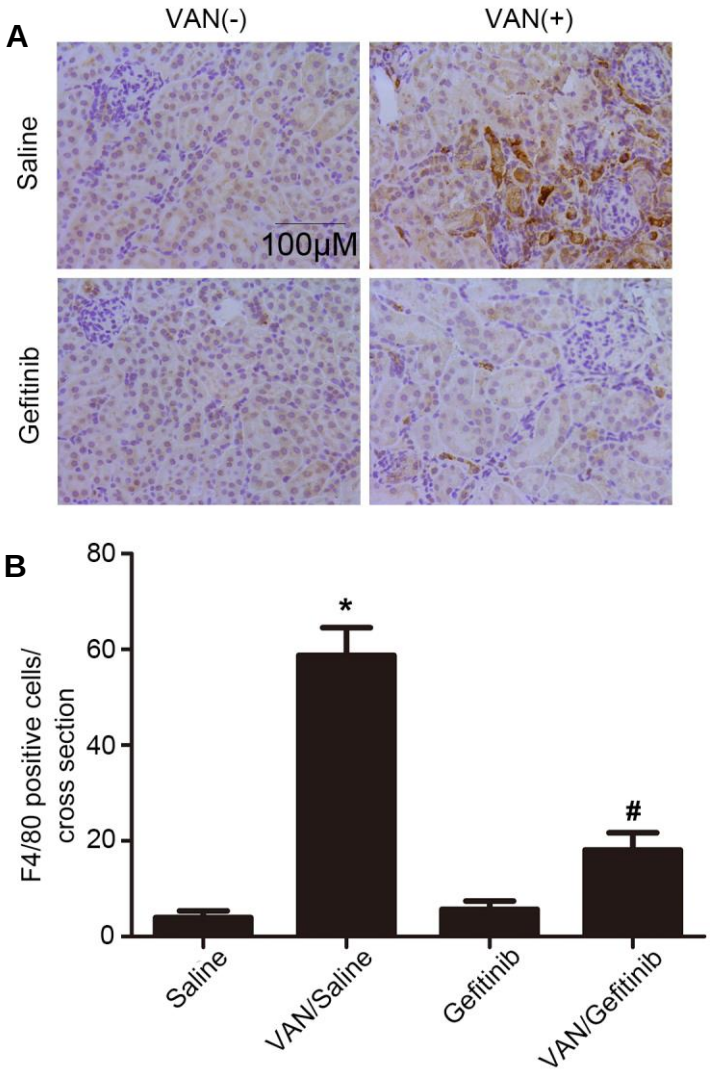
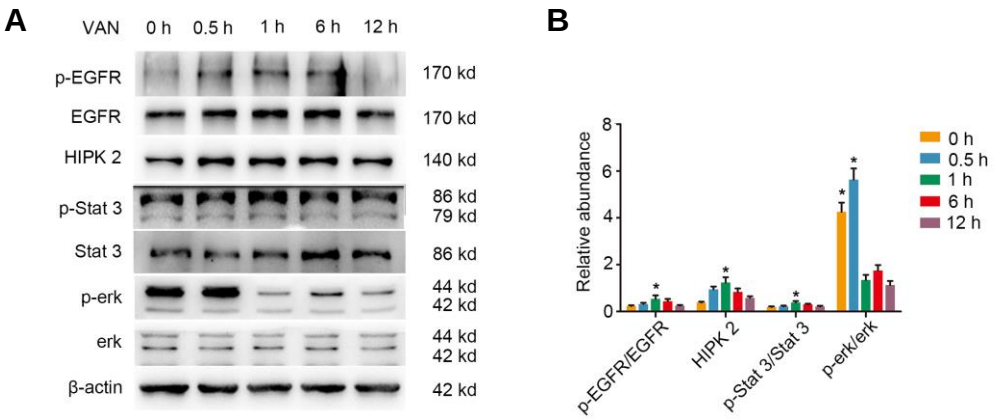


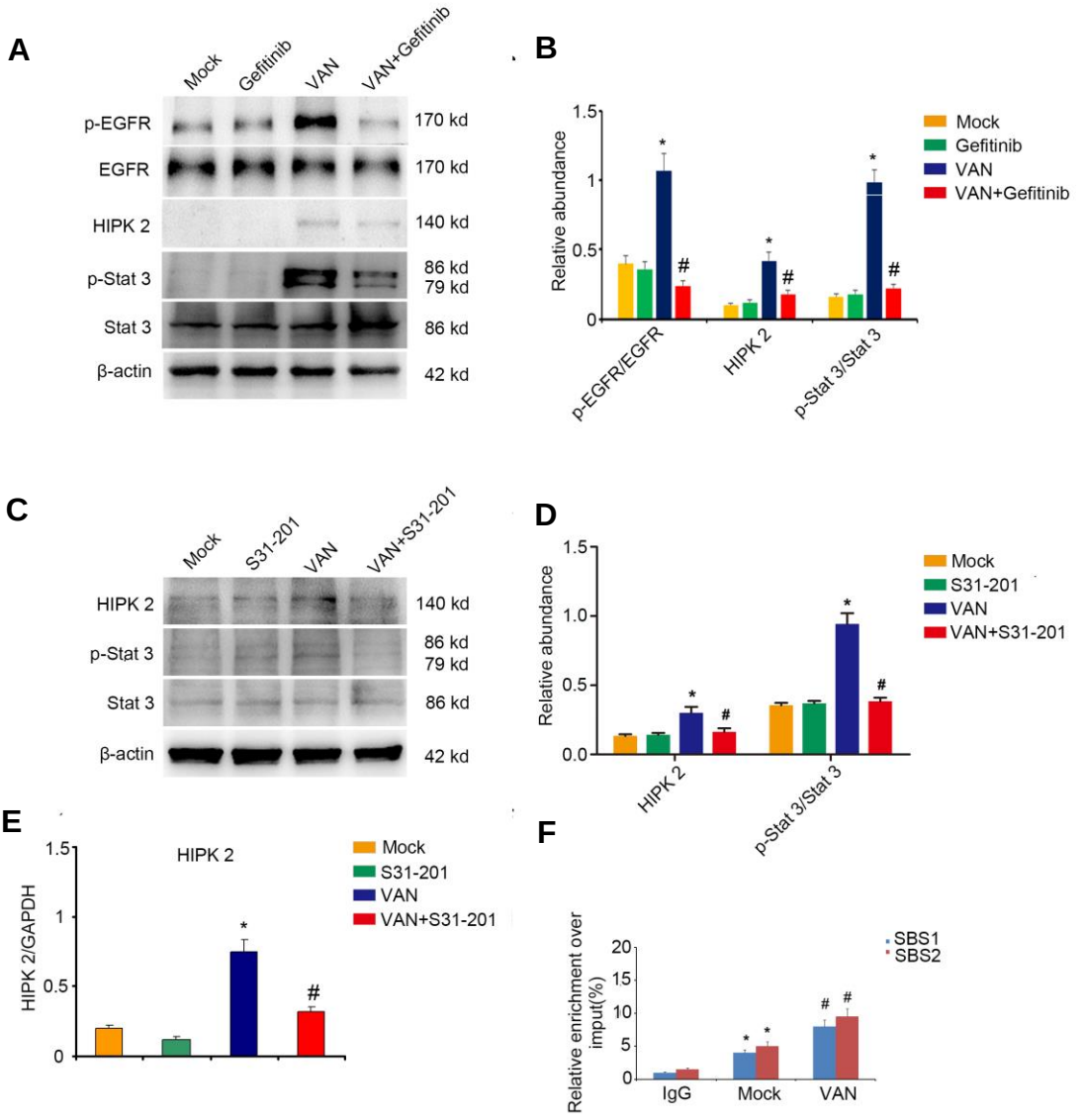
Supplementary Figure 1. The expression of fibronectin, collagen I, and α-SMA **was reduced by continued gefitinib treatment after VAN-induced AKI.** Male C57/B6 mice were intraperitoneal injected with gefitinib at dose of 100mg/kg/day for three weeks after seven days of continuous injection of VAN injected with 600 mg/kg VAN. The tissue samples of kidney were collected for the immunohistochemistry staining (A) and the quantitative analysis (B-D) of the expression of fibronectin, collagen I, and α-SMA. Bar：100μM. Data are shown as means ± SD. (n=8); * *P<0.05* versus Saline group; # *P<0.05* versus VAN group. Original magnification, x 400 in A.



Supplementary Figure 2. **The infiltration of macrophage was suppressed by continued gefitinib treatment after VAN-induced AKI.** Male C57/B6 mice were intraperitoneal injected with gefitinib at dose of 100mg/kg/day for three weeks after seven days of continuous injection of VAN injected with 600 mg/kg VAN. The tissue samples of kidney were collected for the immunohistochemistry staining (A) and the quantitative analysis (B) ofthe expression of F4/80. Bar：100μM. Data are shown as means ± SD. (n=8); * *P<0.05* versus Saline group; # *P<0.05* versus VAN group. Original magnification, x 400 in A.

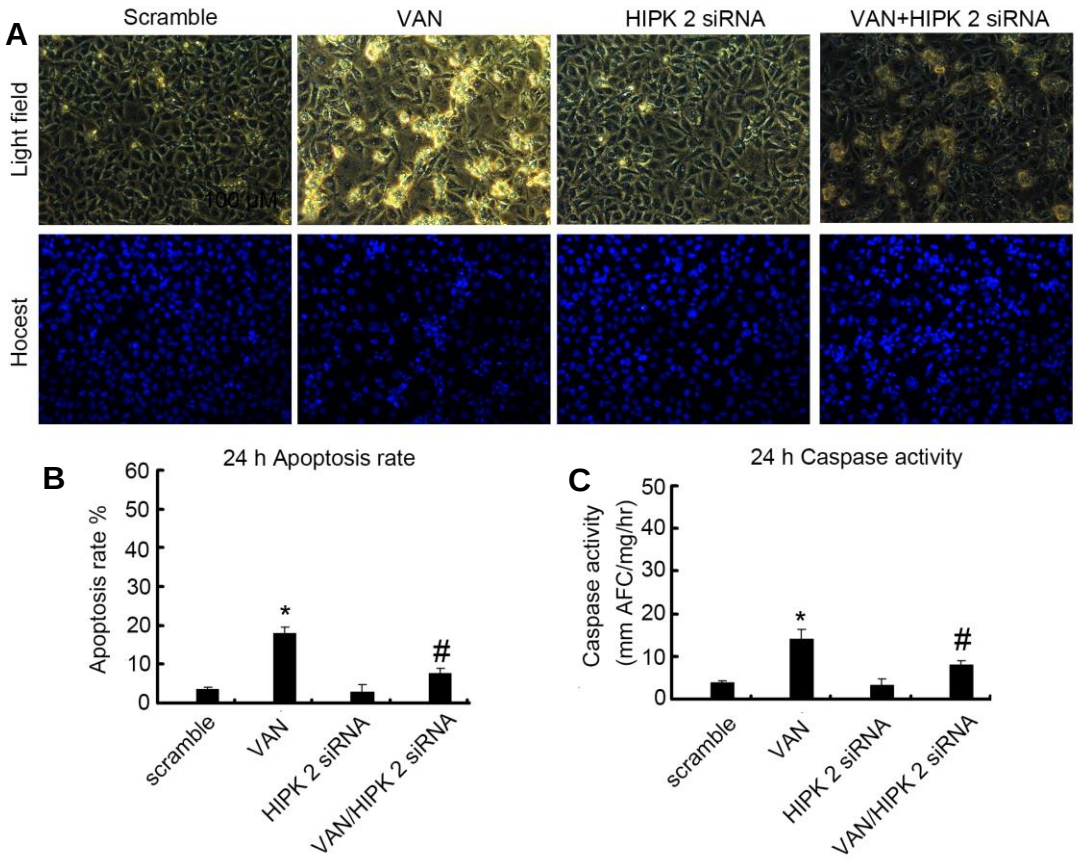


Supplementary Figure 3. **VAN induced activation of EGFR and STAT3, inactivation of ERk1/2, and the expression of HIPK2 in HK-2 cells**. VAN at 4 mm/L was used to treat HK-2 cells for 0-12 h. (A) The immunoblot analysis of p-EGFR, EGFR, p-STAT3, STAT3, p-ERK1/2, ERK1/2, and HIPK2. (B) Densitometric analysis of the p-EGFR/EGFR, p-ERK1/2/ERK1/2, and p-STAT3/STAT3, and HIPK2/GAPDH ratio. * $P<0.05$ versus the other group.



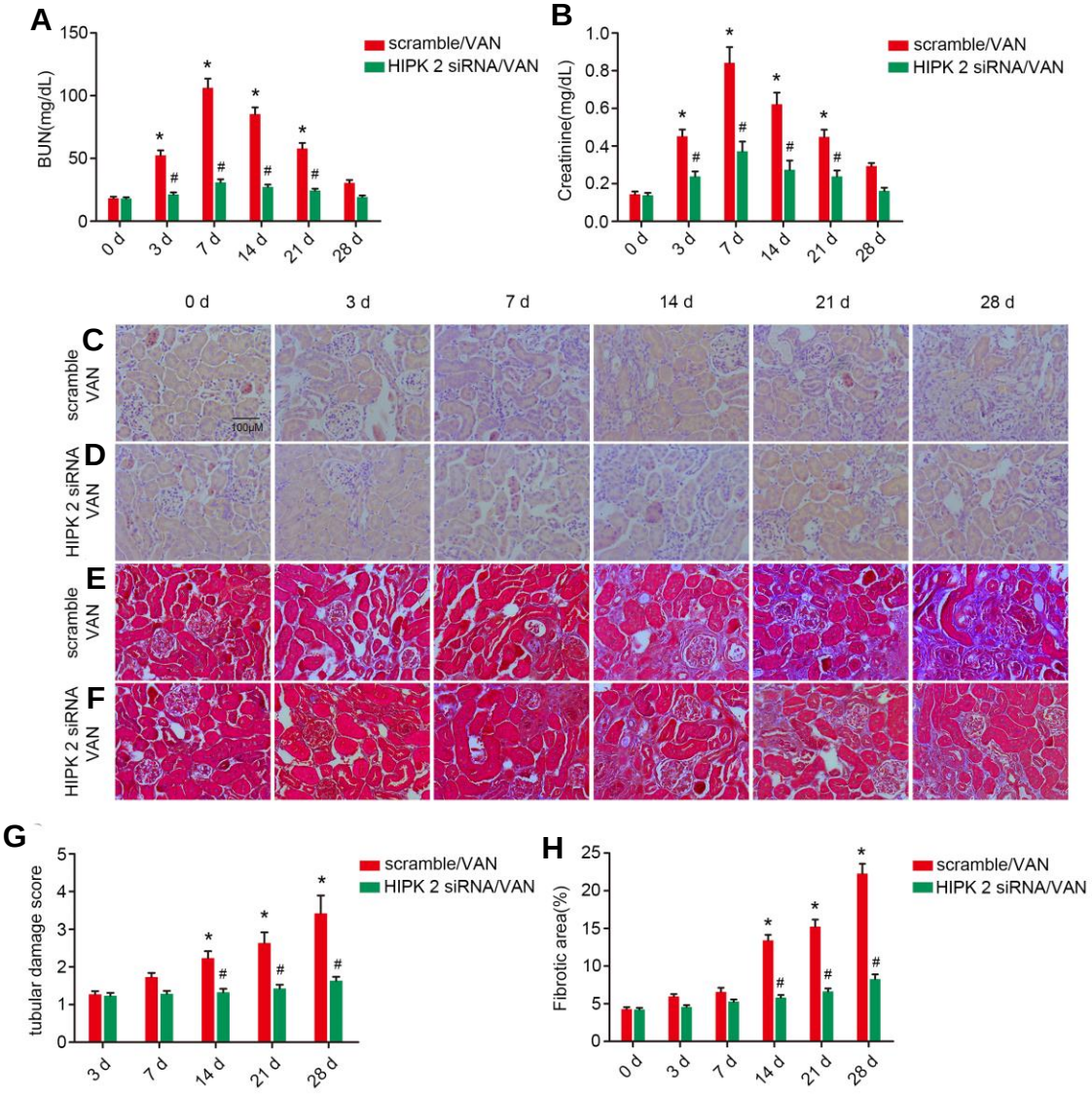
Supplementary Figure 4. Gefitinib suppressed VAN induced HIPK2 by inactivation of EGFR/STAT3 signaling in HK-2 cells.HK-2 cells were treated with 4 mm/L VAN in presence or absence of 5nM gefitinib or 50μM S3I-201 for 1 h. (A) The immunoblot analysis of p-EGFR, EGFR, p- STAT3, STAT3, and HIPK2. (B) Densitometric analysis of the p-EGFR/EGFR, p-STAT3/STAT3 , and HIPK2/GAPDH ratio. (C) The immunoblot analysis of p- STAT3, STAT3, and HIPK2. (D) Densitometric analysis of the p-STAT3/STAT3 and HIPK2/GAPDH ratio. (E) Real time PCR analysis of HIPK2. (F) ChIP assays for detection of the potential STAT3

binding sites (SBS1 and 2) by the PCR. * *P<0.05* versus the Mock group or IgG group, # *P<0.05* versus the VAN group.

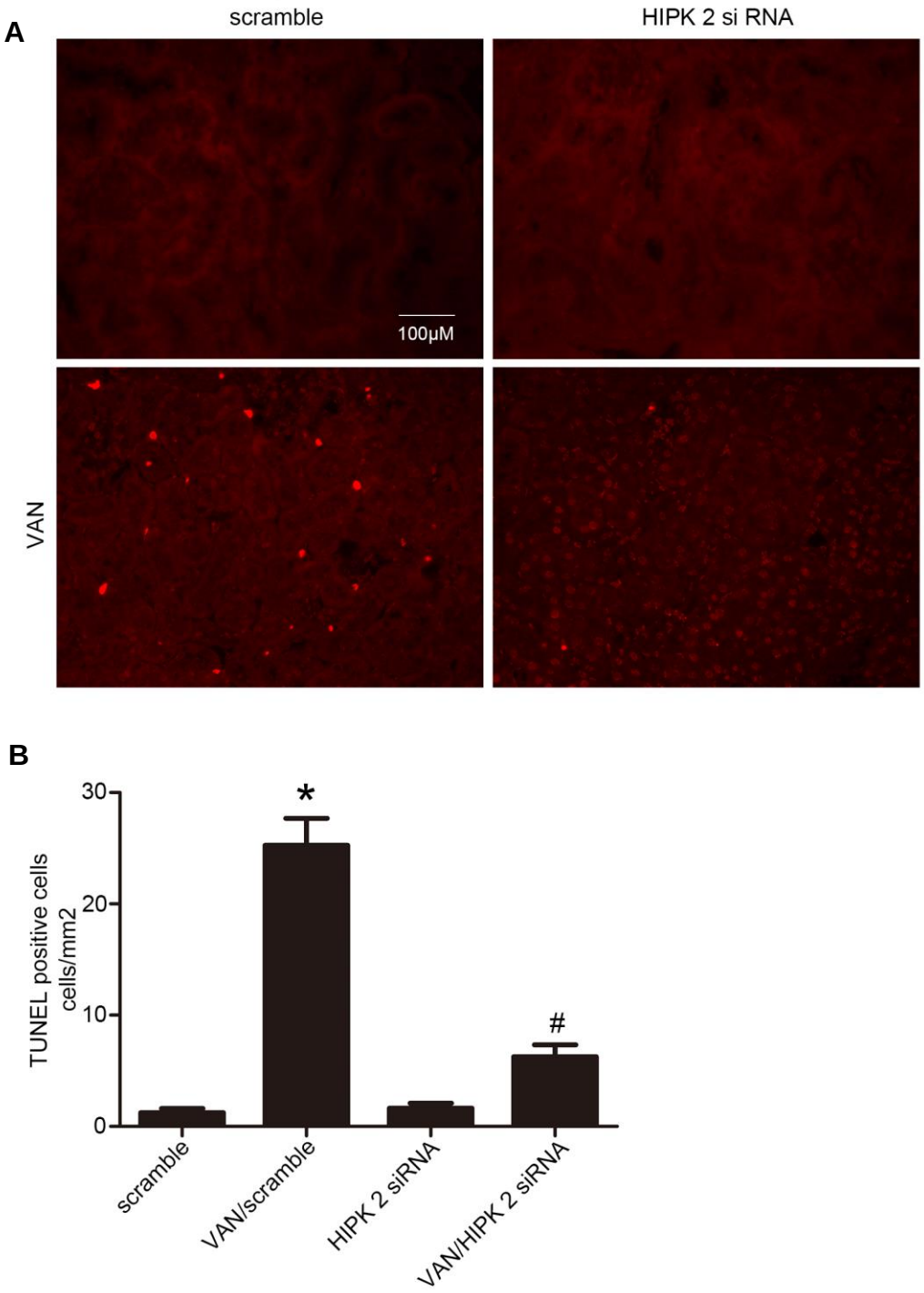


Supplementary Figure 5. Effects of siRNA HIPK2 on VAN induced apoptosis in HK-2 cells. HK-2 cells were transfected with 50 nmol/L HIPK2 siRNA or the scrambled sequence (scramble). Cells were then left untreated or treated for 24 h with

4 mm/L VAN. (A) Morphology. Scale bar, 100 μM. (B) Apoptosis rate. (C) Caspase activity. Data are shown as means ± SD. (n=8); * *P<0.05* versus the Scramble group, # *P<0.05* versus the VAN group. Original magnification, x400. Data are the representative of at least four separate experiments.

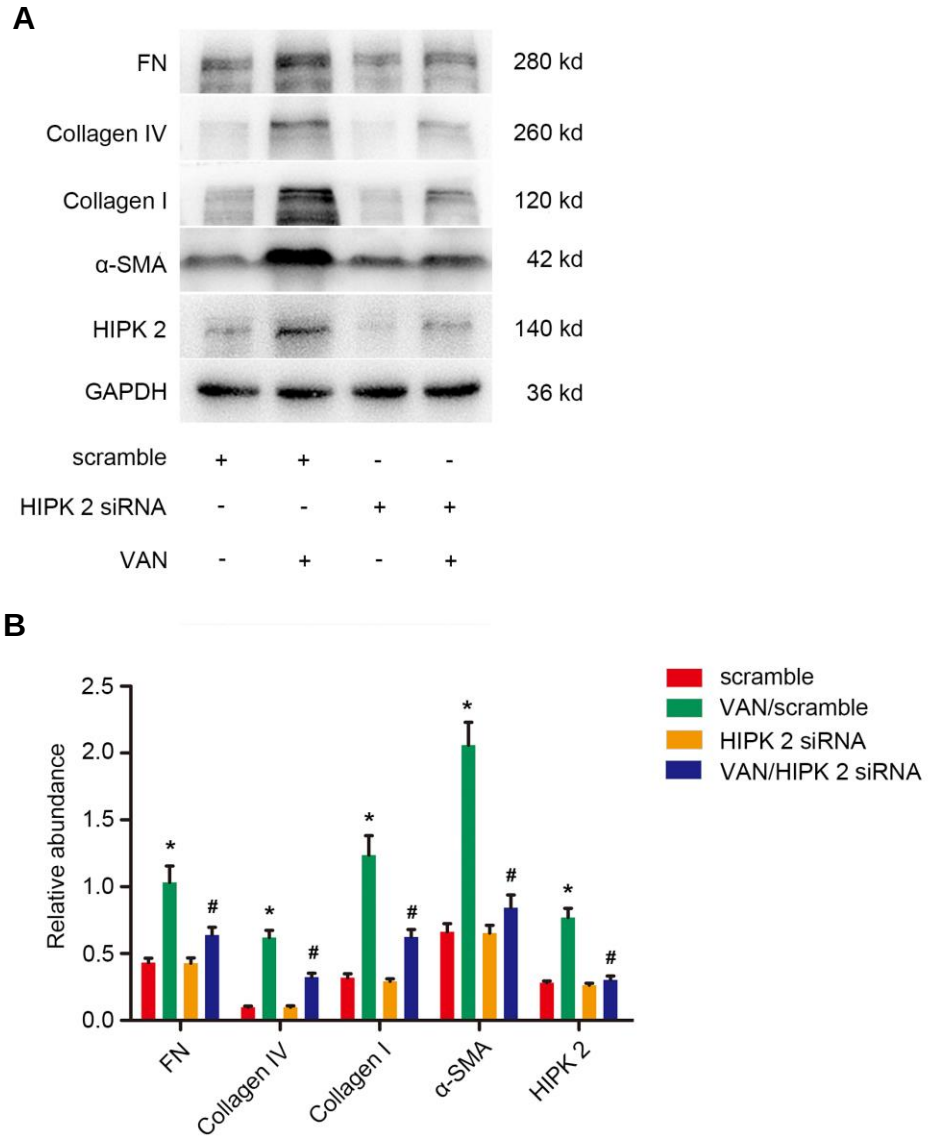


Supplementary Figure 6. **SiRNA HIPK2 attenuated VAN-induced transition of AKI to CKD in C57/B6 mice.** Male C57BL/6 mice were injected with 600 mg/kg VAN for 7 days, and also injected with or without 15 mg/kg siRNA HIPK2 or scramble for 28 days of examination. Kidney tissues and blood samples were collected on day 0-28. Evaluation of renal function via BUN (A) and serum creatinine (B) at different time points. (C&D) Tubular damage analyzed by HE staining. (E&F) Masson's trichrome staining. (G) The values of tubular damage score. (H) Histochemical analysis of tubulointerstitial fibrosis. Bar: 100 μM. Data are presented as mean ± SD (n=8); * $P<0.05$ versus Scramble group; # $P<0.05$ versus VAN group. Original magnification, x400 in C-F.



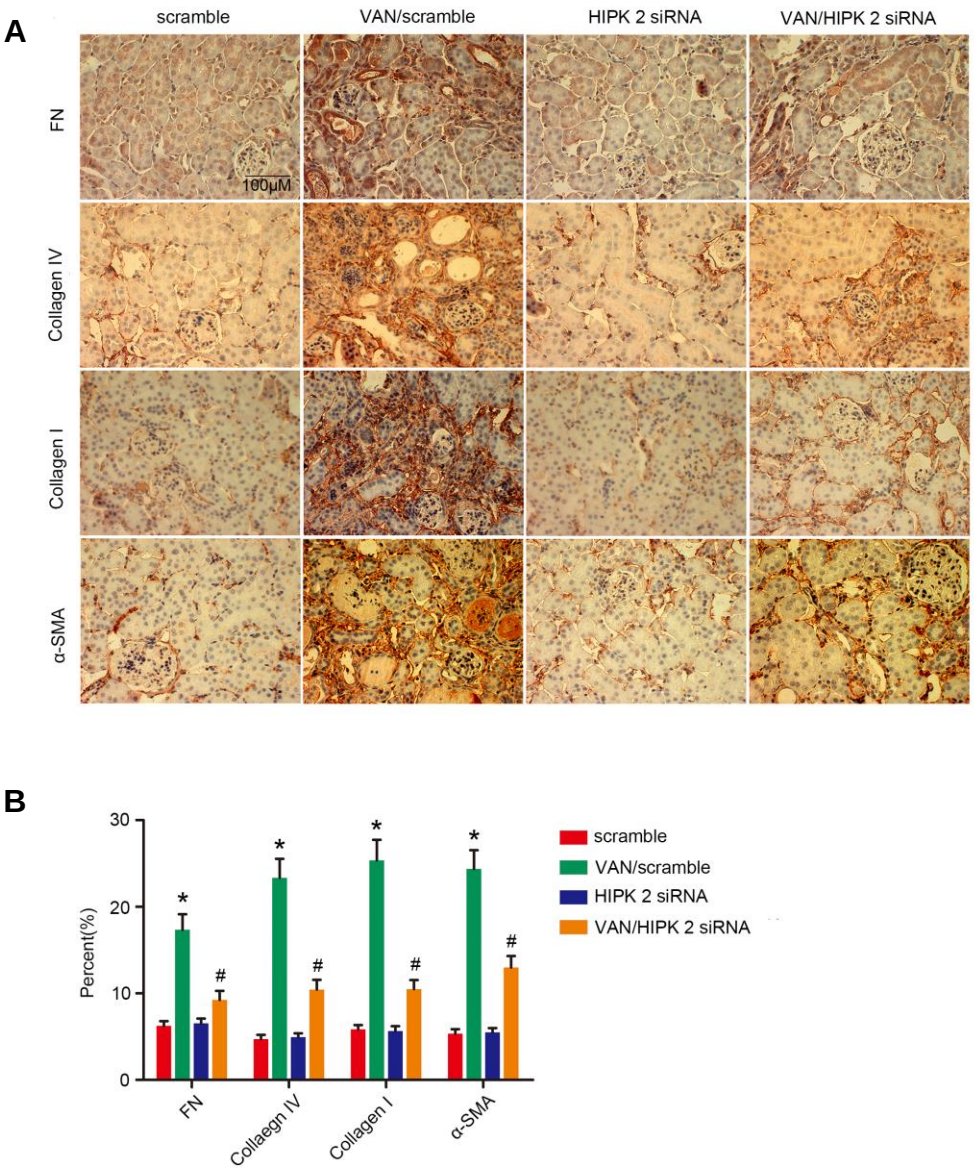
Supplementary Figure 7. **SiRNA HIPK2 attenuated VAN-renal cell apoptosis in C57/B6 mice.** Male C57BL/6 mice were injected with 600 mg/kg VAN with or without 15 mg/kg siRNA HIPK2 or scramble for 7 days of examination. Kidney

tissues and blood samples were collected on day 7. (A) The kidney samples were subjected to TUNEL assay. (B) The percentage of TUNEL-positive cells (cells/mm^2) were calculated. Bar: 100μM. Data are presented as mean ± SD (n=8); * *P<0.05* versus Scramble group; # *P<0.05* versus VAN group. Original magnification, x400 in A.



Supplementary Figure 8. **SiRNA HIPK2 reduced the expression of fibronectin, collagen IV, collagen I and α-SMA by downregulation of HIPK2 in C57/B6 mice**

Male C57BL/6 mice were injected with 600 mg/kg VAN for 7 days, and also injected with or without 15 mg/kg siRNA HIPK2 or scramble for 28 days of examination. The kidney tissue samples were collected for immunoblot analysis of fibronectin, collagen IV, collagen I, α-SMA, HIPK2, and GAPDH expression(A), followed by densitometric analysis of these proteins(B). Data are shown as mean ± SD (n=8); * $P<0.05$ Scramble group; # $P<0.05$ versus VAN group.



Supplementary Figure 9. **SiRNA HIPK2 reduced the expression of fibronectin, collagen IV, collagen I and α-SMA in C57/B6 mice.** Male C57BL/6 mice were injected with 600 mg/kg VAN for 7 days, and also injected with or without 15 mg/kg siRNA HIPK2 or scramble for 28 days of examination. Immunohistochemical staining (A) and quantitative image analysis (B) of fibronectin, collagen IV, collagen I and α-SMA expression. Bar: 100 μM. Data are shown as mean ± SD (n=8); * *P<0.05* Scramble group; # *P<0.05* versus VAN group. Original magnification, x 400 in A.