Supplemental figures for

Fundc1-dependent mitophagy is obligatory to ischemic preconditioning-conferred renoprotection in ischemic AKI via suppression of Drp1-mediated mitochondrial fission

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Running title: Fundc1 mitophagy attenuates AKI

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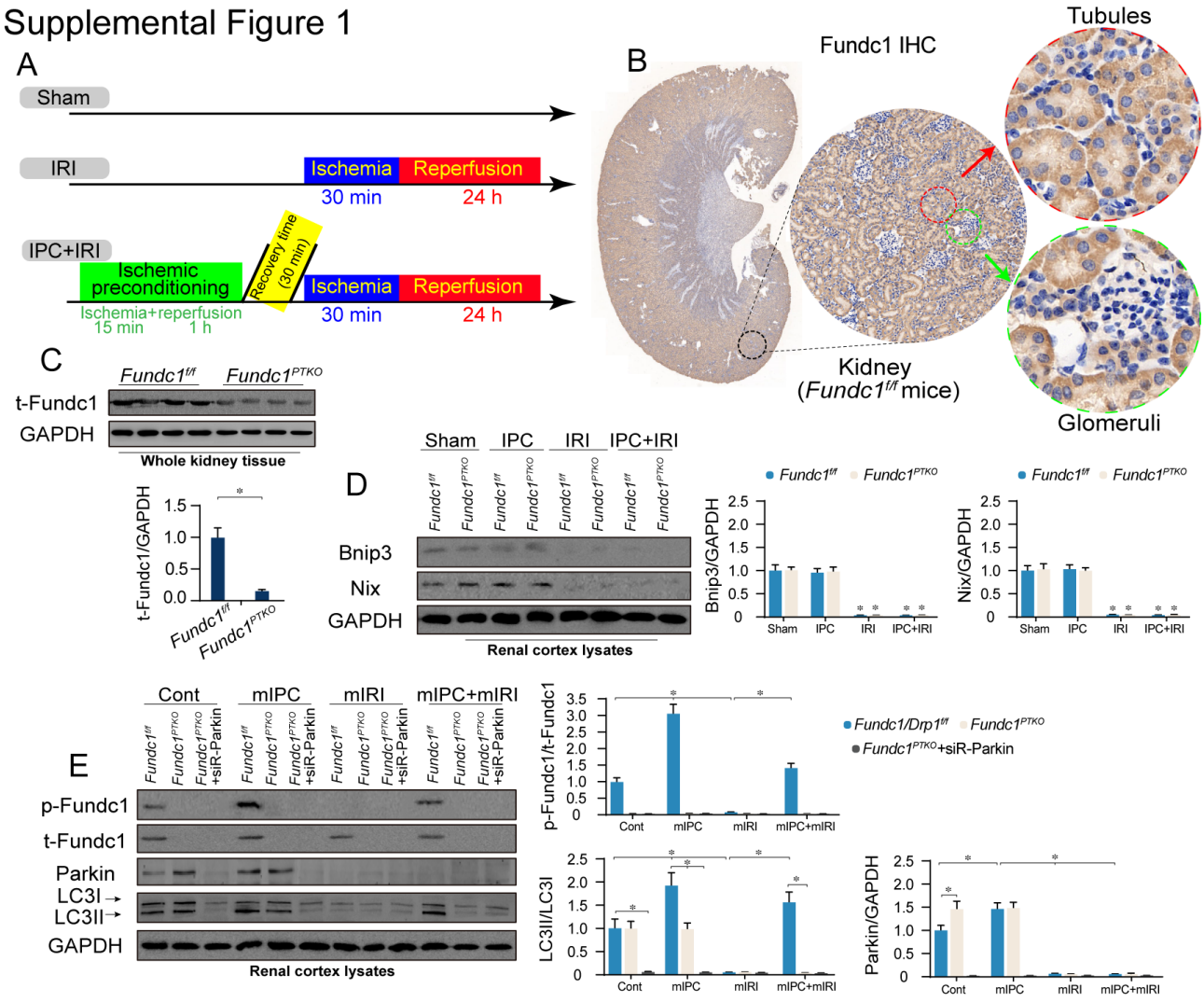
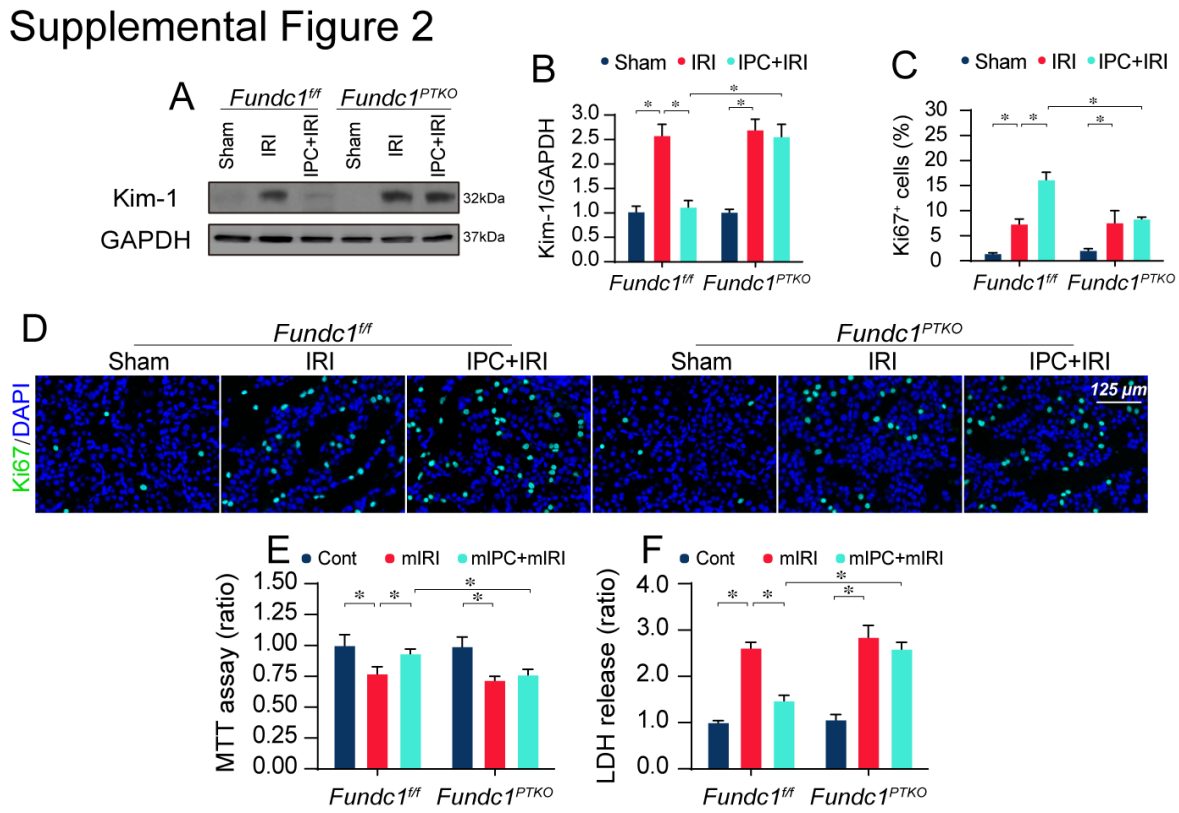


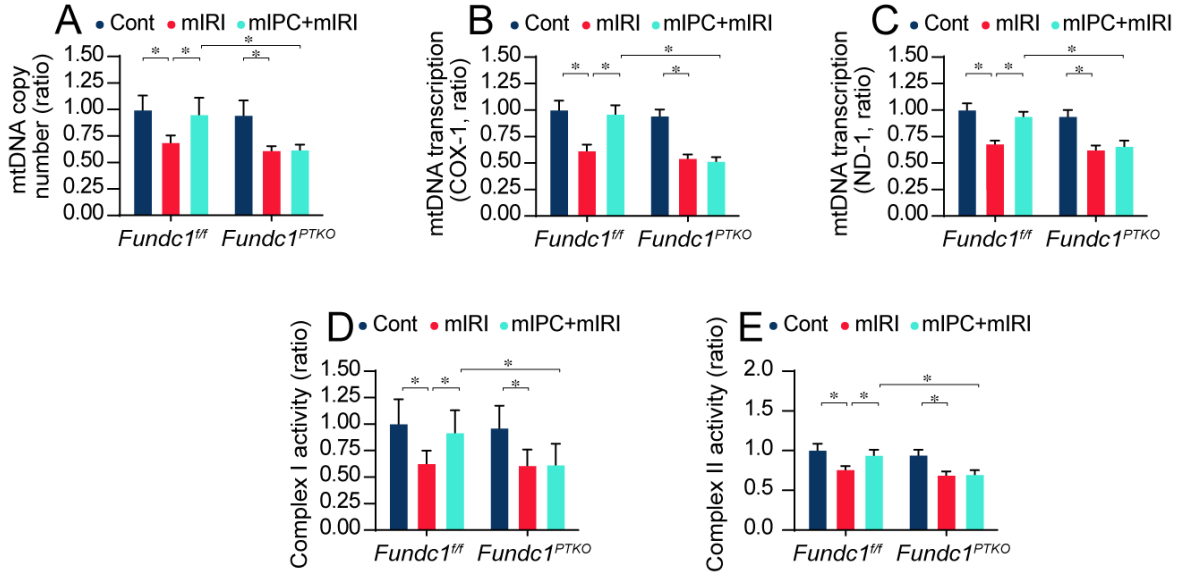
Supplemental Figure 1. Fundc1 deletion primarily impairs IPC-induced mitophagy with little inhibitory effect on baseline mitophagy. (A) Schematic diagram illustrating for surgery procedures of IRI, IPC or both. (B) Immunohistochemistry for Fundc1 in kidney tissues isolated from *Fundc1$^{f/f}$* mice. Expression of Fundc1 in glomeruli and tubules were demonstrated. (C) Whole kidney tissues from *Fundc1$^{f/f}$* and *Fundc1^{PTKO}* mice were used for western blot to determine the expression of Fundc1. (D) *Fundc1$^{f/f}$* and *Fundc1^{PTKO}* mice were subjected to IRI model (30-min ischemia and 24-h reperfusion) with or without IPC treatment. Then, mitophagy regulators such as Bnip3 and Nix were analyzed using western blot. (E) *In vitro*, primary tubule cells were isolated from *Fundc1$^{f/f}$* and *Fundc1^{PTKO}* mice and received mimicked IPC (mIPC) and/or mimicked IRI (mIRI) using rotenone-mediated nutrient

deprivation. Besides, tubule cells isolated from *Fundc1*^{PTKO} mice were also transfected with siRNA against Parkin (siR-Parkin). Then, mitophagy parameters were analyzed via western blot. Experiments were repeated at least three times and data are shown as mean ± SEM (n = 3 independent cell isolations per group). *Fundc1*$^{f/f}$ mice in sham group or *Fundc1*$^{f/f}$ tubule cells in control group were used as the normalizer for all the conditions. **P*<0.05.



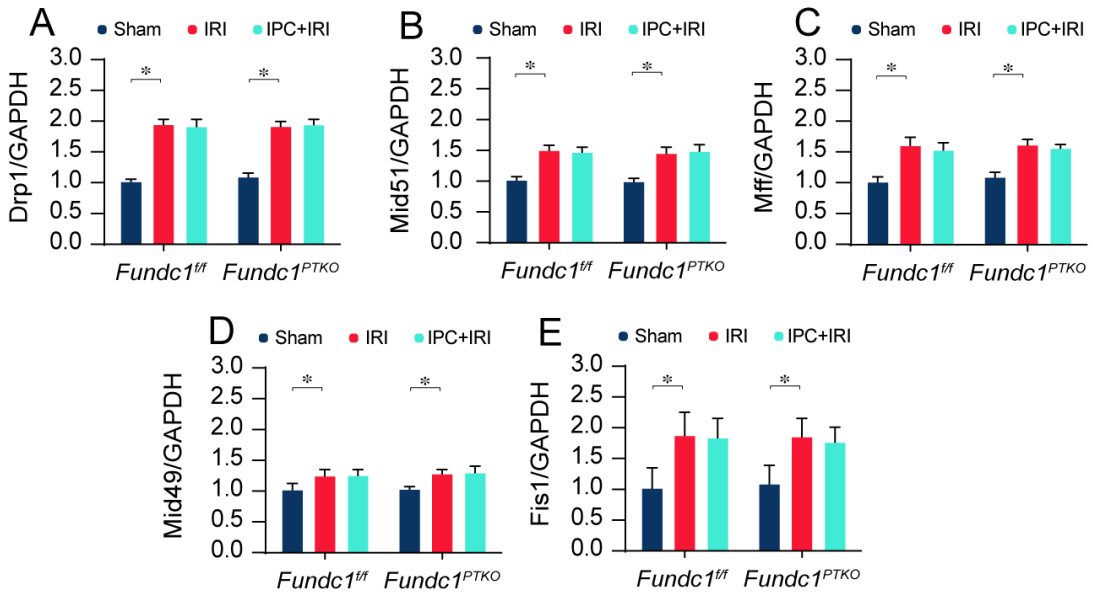
Supplemental Figure 2. IPC-mediated renoprotection is abolished by Fundc1 deficiency. (A-B) Proteins were isolated from *Fundc1*$^{f/f}$ and *Fundc1*^{PTKO} mice and then western blot was used to analyze the content of Kim-1 after renal IRI. (C-D) Ki67 staining was used to observe the proliferative recovery of tubule after IRI. The number of Ki67-positive cell was recorded. (E) Primary tubule cells were isolated from *Fundc1*$^{f/f}$ and *Fundc1*^{PTKO} mice. Cell viability was determined using MTT assay. (F) LDH release assay was employed to evaluate cell death in response to mIRI and mIPC. Experiments were repeated at least three times and data are shown as mean ± SEM (n = 6 mice or 3 independent cell isolations per group). *Fundc1*$^{f/f}$ mice in sham group or *Fundc1*$^{f/f}$ tubule cells in control group were used as the normalizer for all the conditions. **P*<0.05.

Supplemental Figure 3

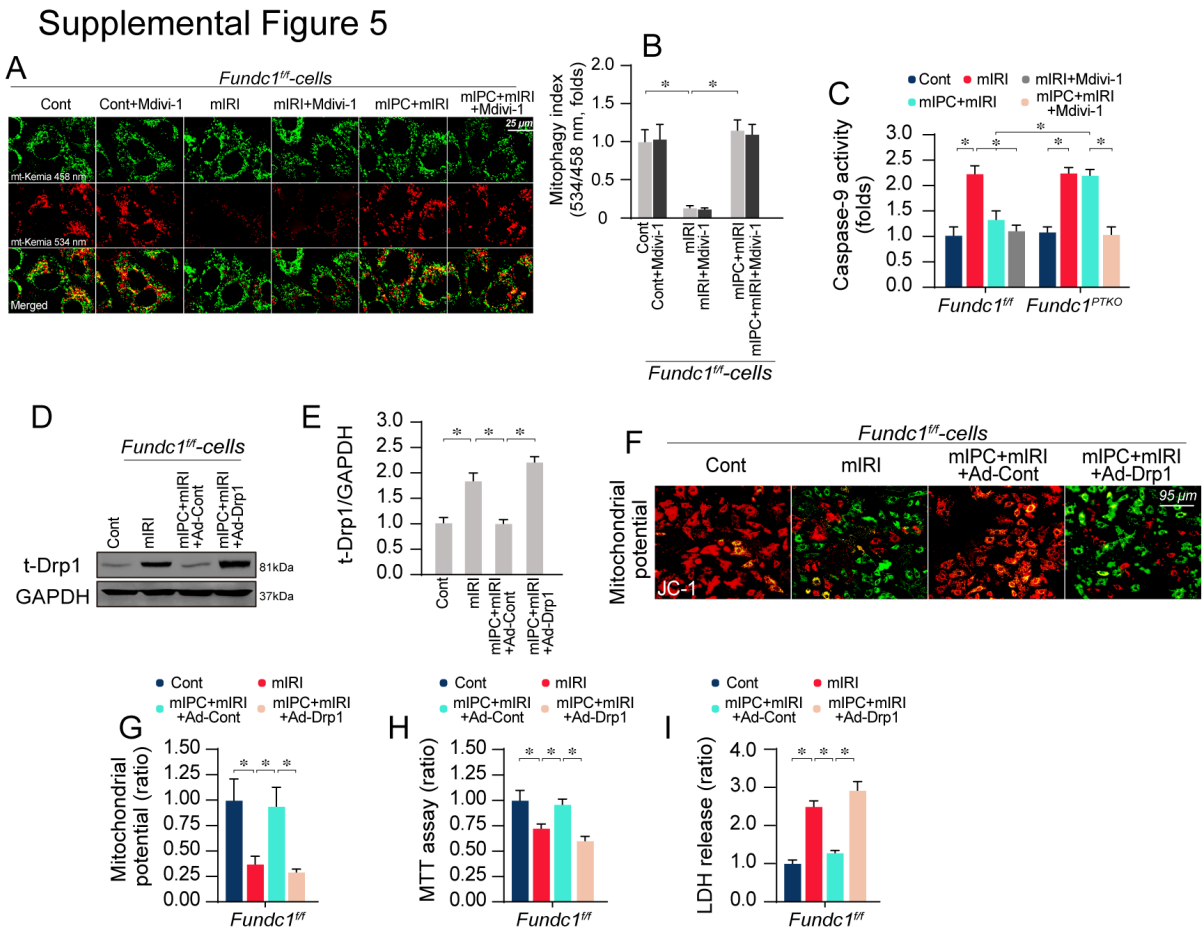


Supplemental Figure 3. Mitochondrial quality control is disturbed by Fundc1 deficiency. (A). mtDNA copy was determined via analyze the ratio of complex IV segment and GAPDH segment using qPCR. (B-C). Primary tubule cells were isolated from *Fundc1*$^{f/f}$ and *Fundc1*^{PTKO} mice. mtDNA transcription was measured via analyze the transcript of COX-1 and ND-1. (D-E) Primary tubule cells were isolated from *Fundc1*$^{f/f}$ and *Fundc1*^{PTKO} mice. ELISA was used to evaluate the activity of complex I and II according to the manufacture's instruction. Experiments were repeated at least three times and data are shown as mean ± SEM (n = 3 independent cell isolations per group). *Fundc1*$^{f/f}$ tubule cells in control group were used as the normalizer for all the conditions. *P<0.05.

Supplemental Figure 4

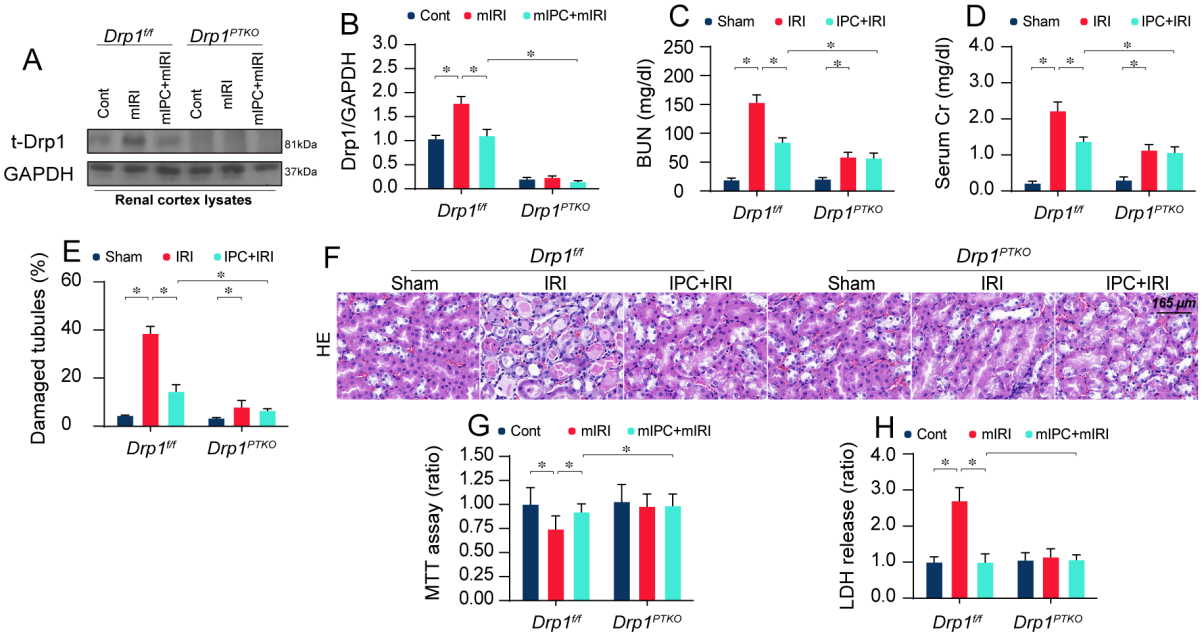


Supplemental Figure 4. Role of Fundc1 deletion in mitochondrial fission-related proteins. (A-E) *In vivo*, RNA was isolated from IRI kidneys and then the transcriptions of Drp1, Mff, Mid49, Fis1 and Mid51 were determined using qPCR. Experiments were repeated at least three times and data are shown as mean ± SEM (n = 6 mice per group). *Fundc1*$^{f/f}$ mice in sham group were used as the normalizer for all the conditions. **P*<0.05.



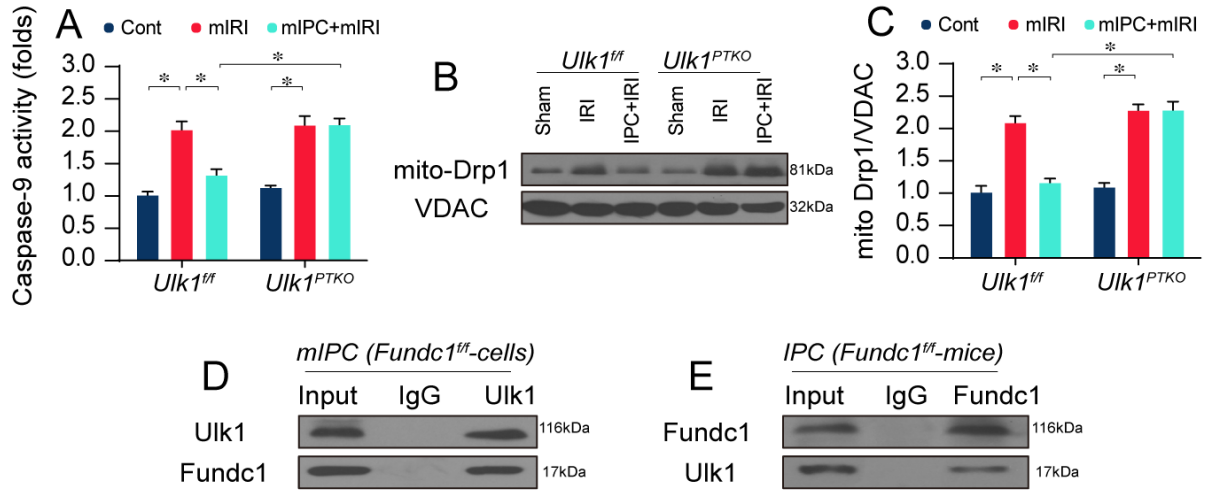
Supplemental Figure 5. Inhibition of Drp1-related mitochondrial fission attenuates Fundc1 deficiency-induced mitochondrial dysfunction and tubule cell damage. (A-B) Primary tubule cells were isolated from *Fundc1*$^{f/f}$ mice. Mt-Kemia assay was used to monitor mitophagy activity *in vitro*. The fluorescence intensity ratio of 534/458 nm was used to quantify mitophagy index after inhibition of mitochondrial fission using Mdivi-1. (C) ELISA assay for caspase-9 activity in response to mitochondrial fission inhibition. (D-E) Drp1 adenovirus (Ad-Drp1) and control adenovirus (Ad-Cont) were transfected into tubule cells isolated from *Fundc1*$^{f/f}$ mice. The overexpression efficiency was determined using western blot. (F-G) JC-1 probe was used to monitor mitochondrial membrane potential. Mdivi-1 was used to inhibit the Drp1 mitochondrial fission. (H) Cell viability was determined using MTT assay. (I) LDH release assay was used to evaluate cell death in response to Drp1 overexpression. Experiments were repeated at least three times and data are shown as mean ± SEM (n = 3 independent cell isolations per group). *Fundc1*$^{f/f}$ tubule cells in control group were used as the normalizer for all the conditions. *P<0.05.

Supplemental Figure 6



Supplemental Figure 6. Drp1 deletion attenuates IRI-mediated kidney damage. (A-B) Proteins were isolated from *Drp1*^{PTKO} mice and Drp1 expression was analyzed. (C-D) After IRI, the levels of BUN and Cr were determined in *Drp1*$^{fl/fl}$ and *Drp1*^{PTKO} mice. (E-F) HE staining for IRI kidneys from *Drp1*$^{fl/fl}$ and *Drp1*^{PTKO} mice. Tubular injury index was determined. (G) Primary tubule cells were isolated from *Drp1*$^{f/f}$ and *Drp1*^{PTKO} mice. MTT assay was used to determine cell viability *in vitro*. (H) LDH release assay was used to evaluate cell death. Experiments were repeated at least three times and data are shown as mean ± SEM (n = 6 mice or 3 independent cell isolations per group). *Drp1*$^{fl/fl}$ mice in sham group or *Drp1*$^{fl/fl}$ tubule cells in control group were used as the normalizer for all the conditions. **P*<0.05.

Supplemental Figure 7



Supplemental Figure 7. Ulk1 is required for IPC-mediated renoprotection. (A) Mitochondrial apoptosis was determined using caspase-9 activity in *Ulk1*$^{f/f}$ and *Ulk1*^{PTKO} mice. (B-C) The expression of mito-Drp1 was determined in tubule cells isolated from *Ulk1*$^{f/f}$ and *Ulk1*^{PTKO} mice using western blots. (D-E) Proteins isolated from tissues and cells were immunoprecipitated with the anti-Fundc1 or anti-Ulk1 antibody, followed by immunoblotting with the anti-Ulk1 or anti-Fundc1 antibody. IgG was employed as a control for the interaction assay between Ulk1 and Fundc1. Experiments were repeated at least three times and data are shown as mean ± SEM (n = 6 mice or 3 independent cell isolations per group). *Ulk1*$^{f/f}$ mice in sham group or *Ulk1*$^{f/f}$ tubule cells in control group were used as the normalizer for all the conditions. **P*<0.05.