Supplemental Material Table of Contents

Supplemental Figure 1. Cold storage rewarming injury induces apoptosis in cultured tubular cells

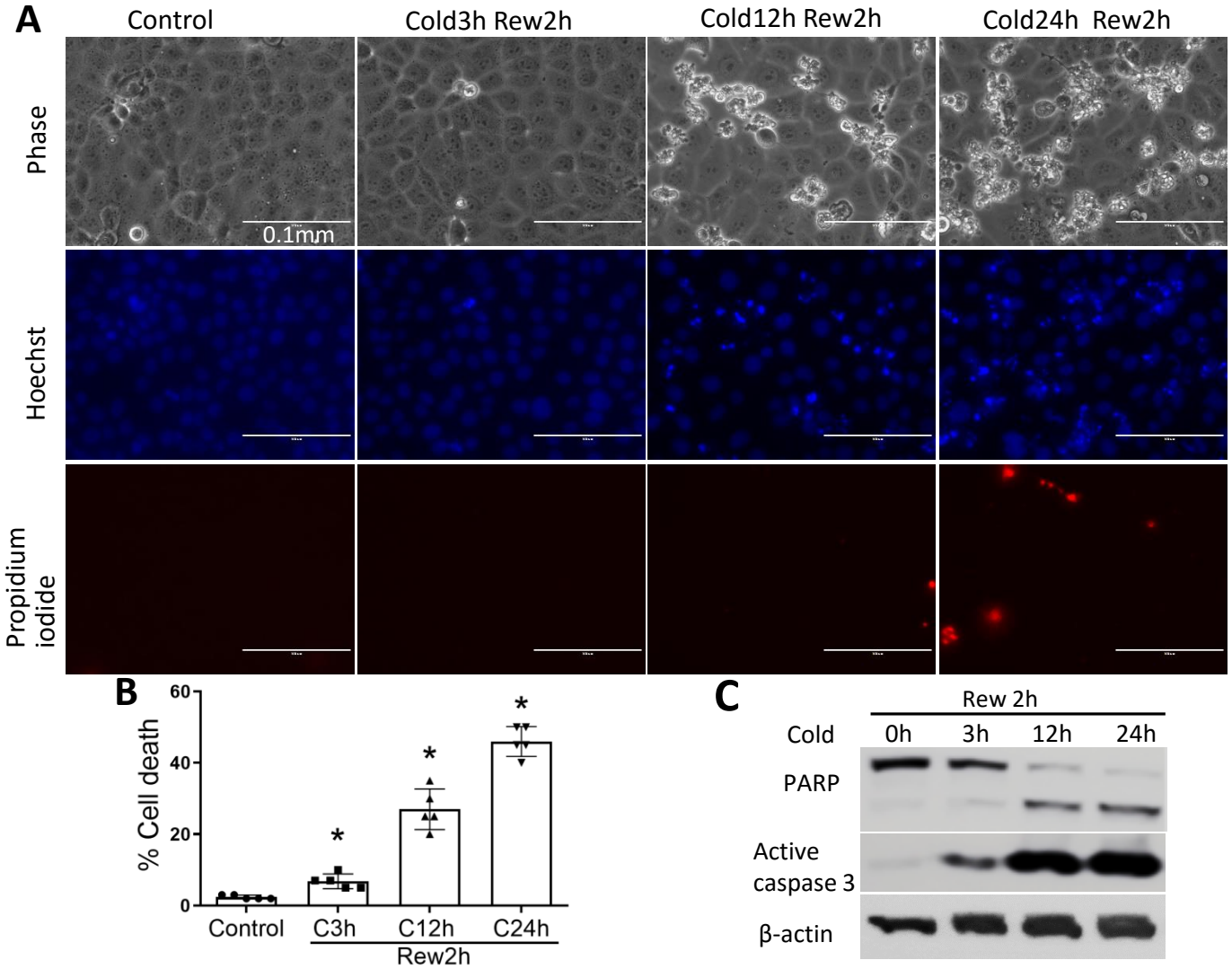

Figure S1. Cold storage rewarming induces apoptosis in RPTC cells. RPTCs were incubated in normal cell culture medium at 37ºC (Control), or stored in 4ºC UW solution for 3, 12, 24 hours with 2 hours of rewarming in normal culture medium at 37ºC. **(A)** Representative images of cells and their nuclei after Hoechst 33342 and PI staining. Apoptotic cells had a bright, shrunken cell body with blebs and condensed, fragmented nucleus. Necrotic cells are positive in PI staining. Scale bar=0.1mm **(B)** Immunoblot analysis of PARP cleavage and cleaved/active caspase-3 with β-actin as loading control. **(C)** Percentage of cell death quantified by counting the cells with typical apoptotic morphology or positive PI staining. Data are expressed as mean ± SD (n=5). *, $P<0.05$ versus Control. RPTC cells, Rat proximal tubular epithelial cells. UW solution, University of Wisconsin solution; PI, Propidium iodide.

Supplemental Figure 2. Cold storage leads mitochondria fragmentation and leakage

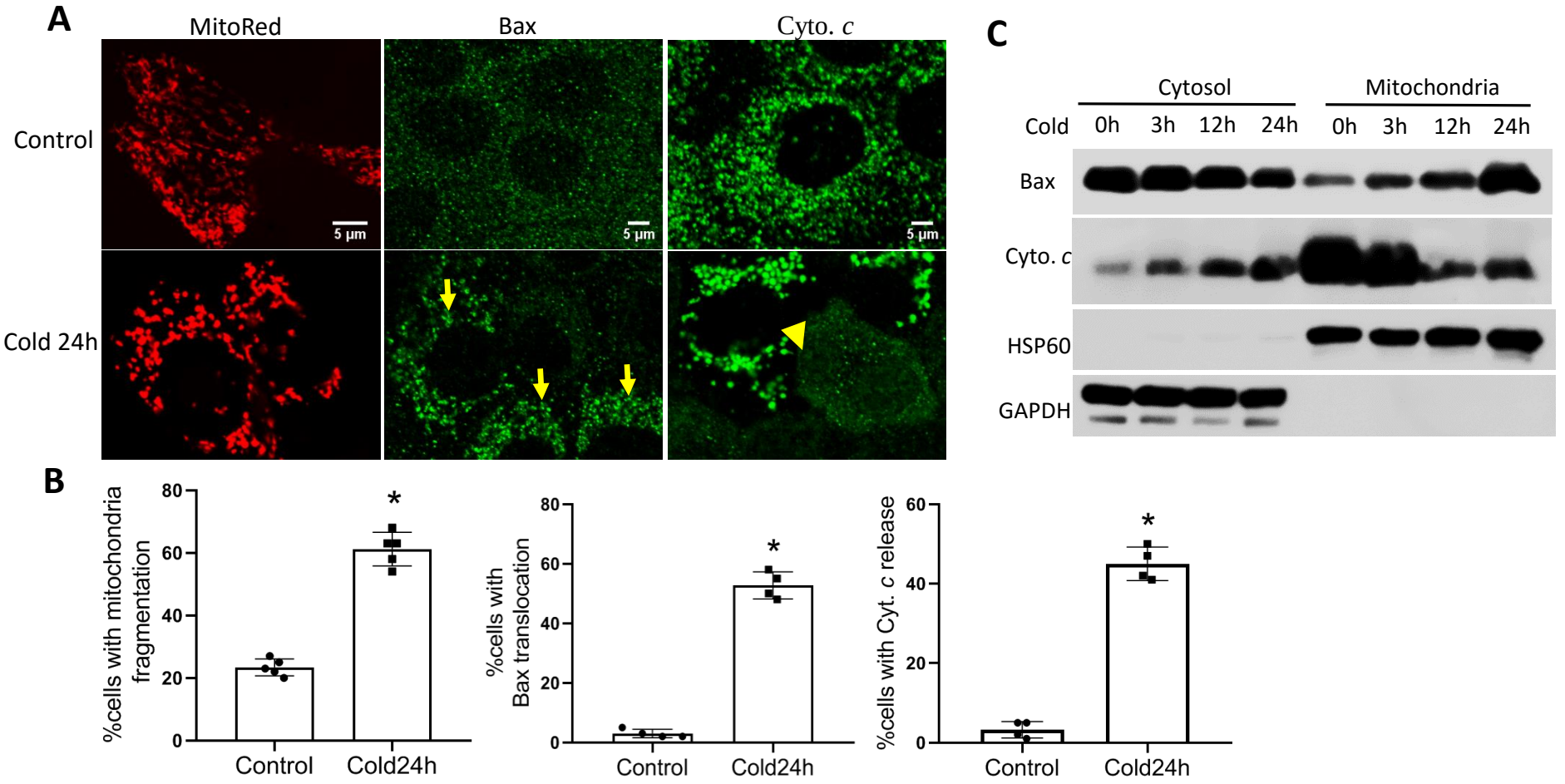

Figure S2. Cold storage induces mitochondrial fragmentation, Bax accumulation and cytochrome c release in RPTC cells. RPTC cells were incubated in normal cell culture medium at 37ºC (Control), or stored in 4ºC UW solution for 3, 12, 24 hours. **(A)** Representative images of mitochondria with MitoRed labeling, Bax and Cyto. *c* immunofluorescence showing mitochondrial fragmentation, Bax accumulation to mitochondria, and Cyto. *c* release from mitochondria into cytosol during cold storage. Arrows point to cells with Bax accumulation in mitochondria; Arrowhead points to a cell with Cyto. *c* release to cytosol. **(B)** Percentage of cells with mitochondria fragmentation, Bax translocation, and Cyto. *c* release. Data are expressed as mean ± SD (n=4-5). *P < 0.01, versus the control group. **(C)** Immunoblots of Bax and Cyto. *c* in cytosolic and mitochondrial fractions with Hsp60 as mitochondrial loading control and GAPDH as cytosolic loading control. Cyto. c, Cytocrome *c*. HSP 60, Heat shock protein 60

Supplemental Figure 3. Cold preservation induces PKCδ activation in cultured tubular cells.

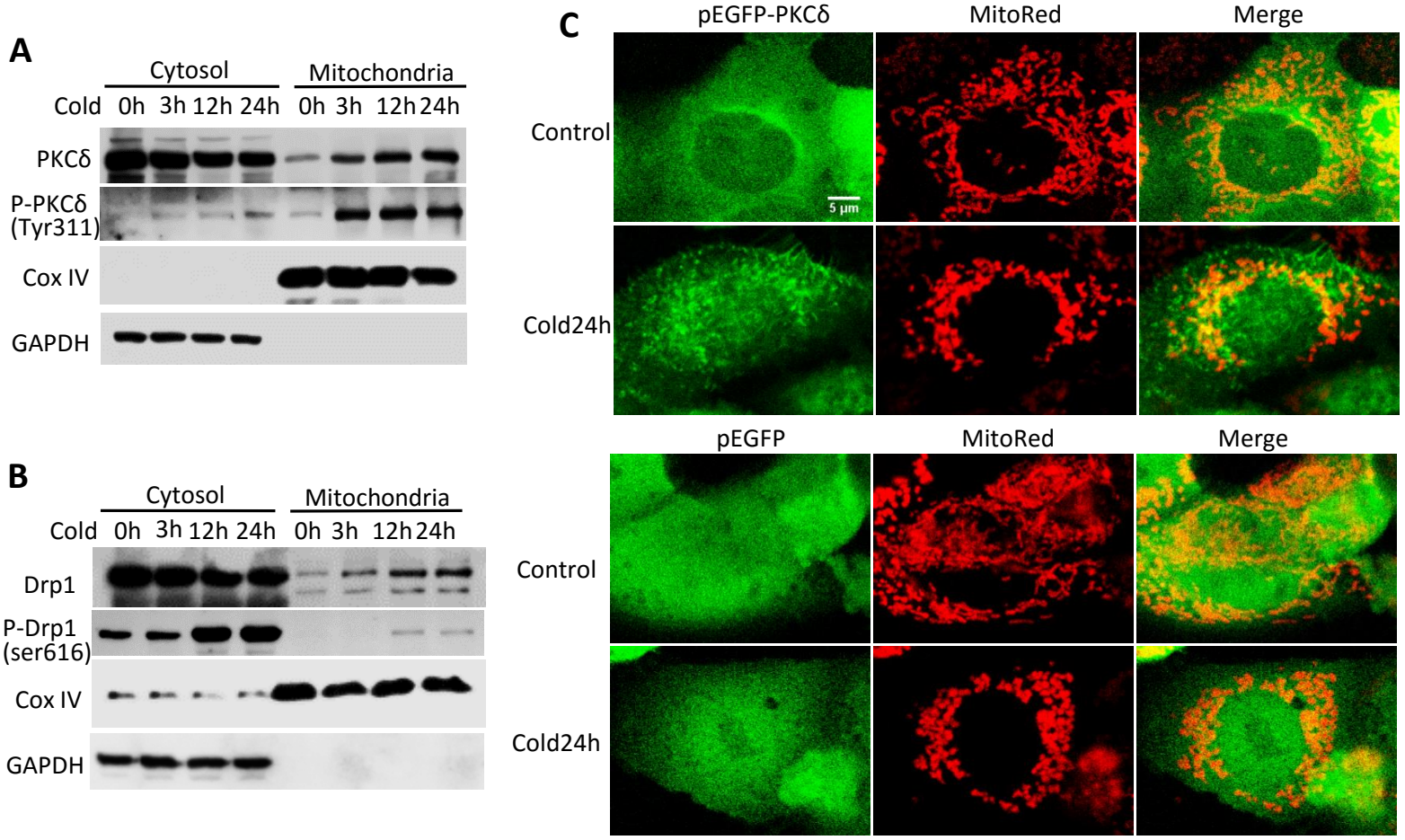

Figure S3. Cold storage induces PKCδ activation in RPTC cells. RPTC cells were incubated in normal cell culture medium at 37ºC (Control), or stored in 4ºC UW solution for 3, 12, 24 hours. **(A)** Immunoblots of PKCδ and pPKCδ-Tyr311 showing the activation of PKCδ and its translocation from the cytosol to mitochondrial fraction during cold storage of RPTC cells. **(B)** Immunoblots of Drp1 and pDrp1-ser616 showing the activation of Drp1 and its translocation from the cytosol to mitochondrial fraction during cold storage of RPTC cells. **(C)** RPTC cells were co-transfected with MitoRed (to label mitochondria) and pEGFP-PKCδ or pEGFP, followed by 24 hours of cold storage in UW solution. The results show the co-localization of pEGFP-PKCδ (but not EGFP) with MitoRed after cold storage.

Supplemental Figure 4. PKCδ promotes mitochondrial fragmentation and leakage during cold storage.

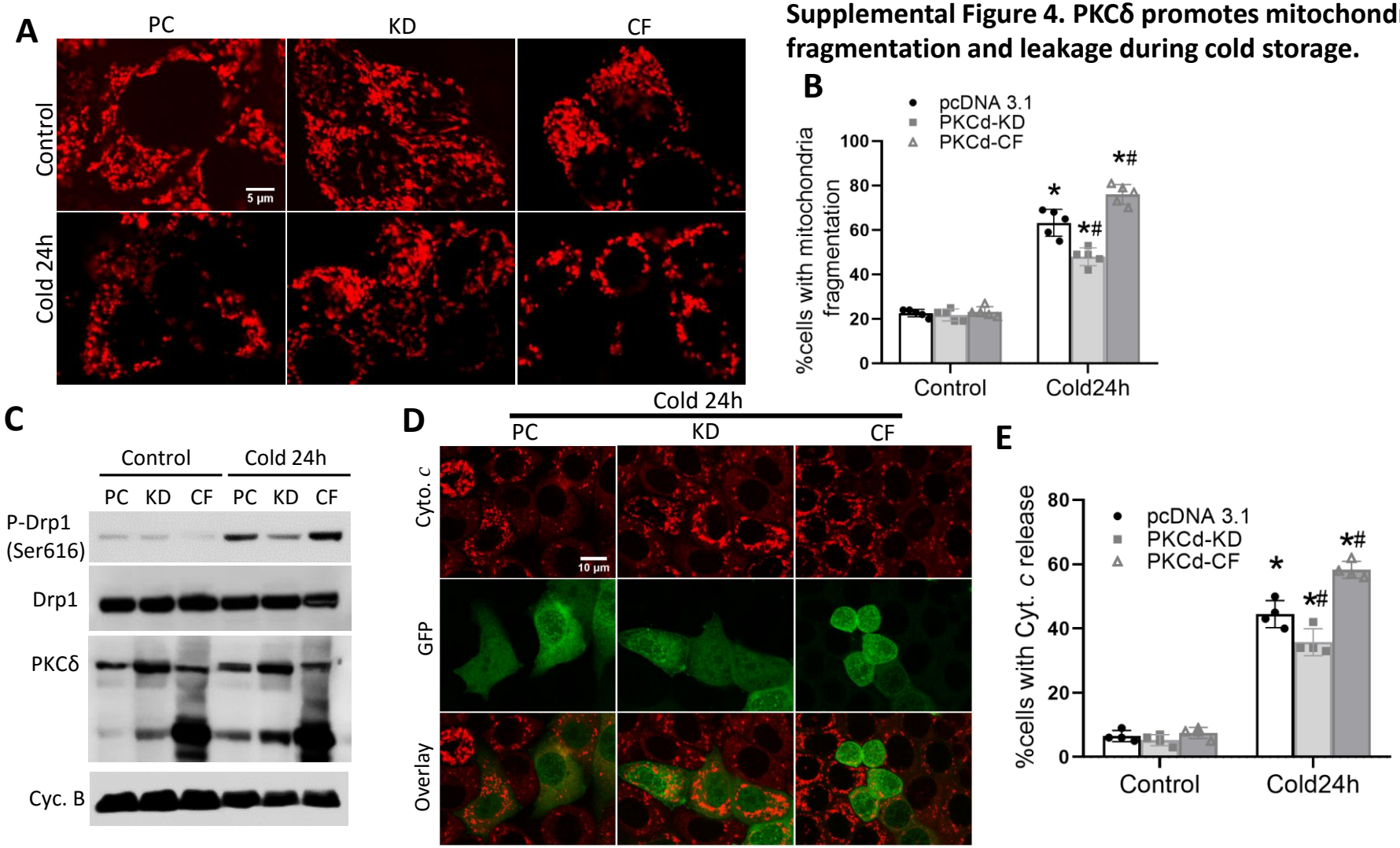

Figure S4. PKCδ promotes mitochondrial fragmentation and leakage during cold storage. (A-C) RPTC cells were co-transfected with Mito Red and PKCδ-KD (KD), PKCδ-CF (CF) or empty vector pcDNA3.1 (PC). The cells were subjected to cold storage for 24 hours or without cold storage as control. The cells were fixed to record mitochondrial morphology (**A**) and quantification of percentage of cells with fragmented mitochondria (**B**) or were lysed for immunoblot analysis of phospho-Drp1(Ser616), PKCδ, and Cyclophilin B (loading control) (**C**). (**D-E**) RPTCs were co-transfected with GFP and PKCδ-CF, PKCδ-KD or empty vector (PC), and then subjected to 24 hours of cold storage. Cells were fixed for cytochrome c immunofluorescence. The transfected (GFP labeled) cells were evaluated for Cyto.*c* release from mitochondria into cytosol. **(D)** Representative images. **(E)** Percentage of GFP-labeled cells with Cyto.*c* release. Quantitative data (B and E) are expressed as mean ± SD (n=4-5); *, $P< 0.05$, versus the respective control. #, $P<0.05$, versus cold preserved cells with pcDNA3.1 transfection**.**

Supplemental Figure 5. Positive control of TUNEL assay

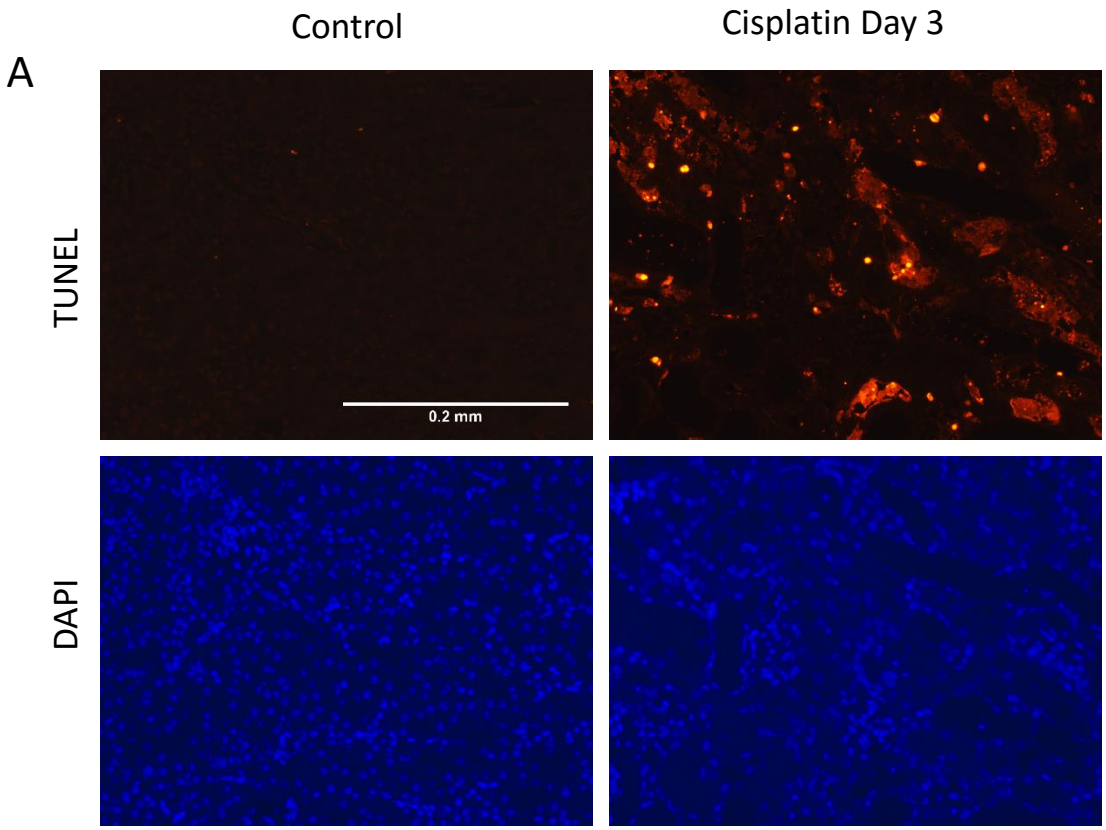

Figure S5. Positive control of TUNEL assay (A) Male C57BL/6 mice were intraperitoneally injected of one dose of cisplatin (30 mg/kg) or saline. Mice were sacrificed at 72 h after cisplatin treatment to collect kidney tissues for TUNEL and DAPI staining. Scale bar=0.2 mm. TUNEL, Terminal deoxynucleotidyl transferase dUTP nick end labeling. DAPI, 4',6-diamidino-2-phenylindole