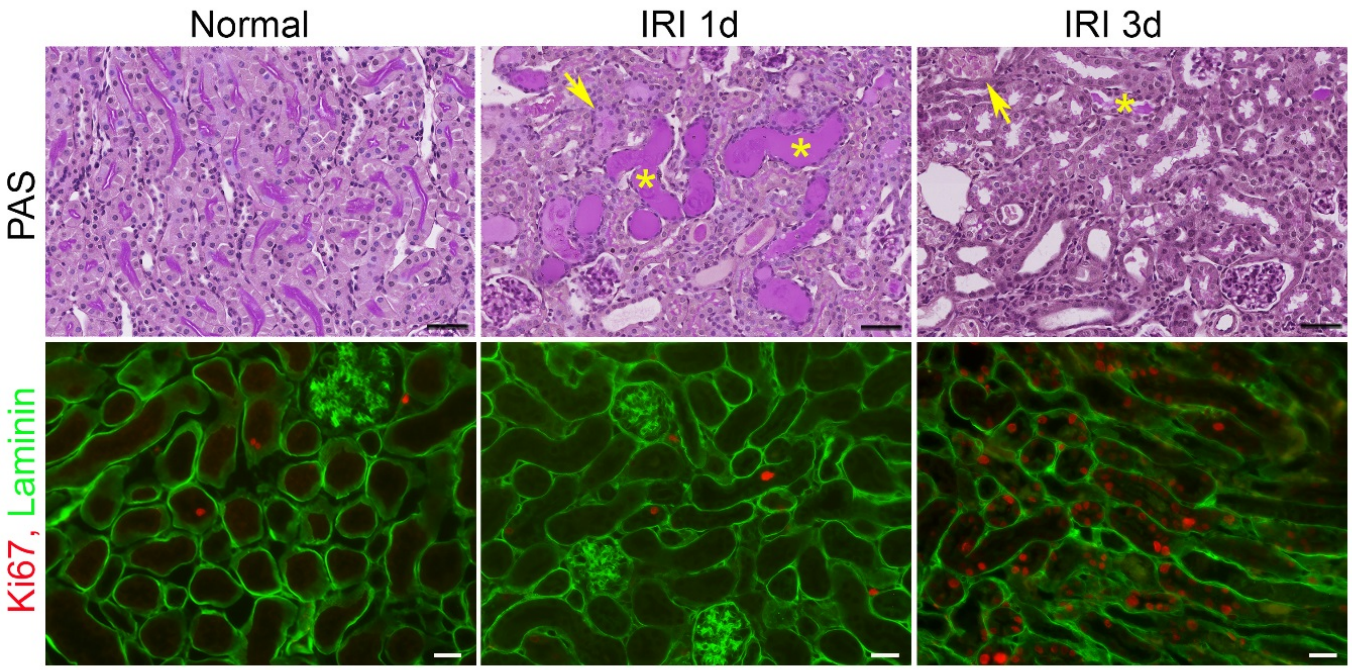
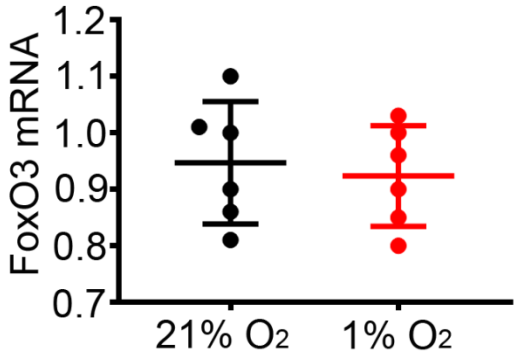
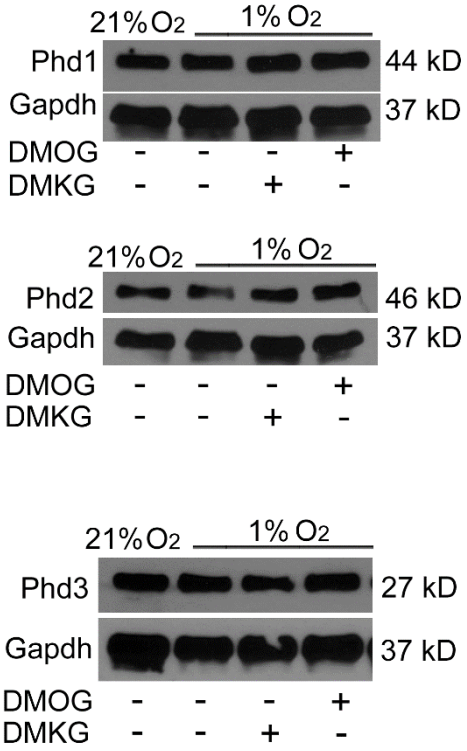
**Supplemental Figures**



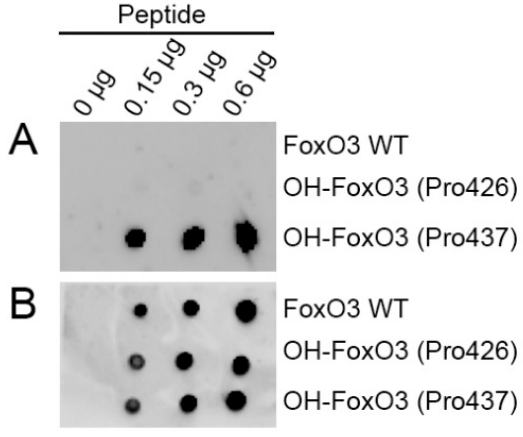

**SF1.** A severe renal ischemic injury results in profound tubular epithelial injury and cell proliferation. Female mice (8 w old) were subjected to a 45 min left ischemia-reperfusion injury (IRI) and kidneys examined at 1 and 3 days post-IRI. Top panel, PAS staining indicates tubular injury and interstitial inflammation. Arrows indicate tubular cell detachment from the basement membrane and asterisks indicate cast formation. Bottom panel shows cell proliferation indicated by the expression of Ki67 (red) in renal tubules labeled with basement membrane marker laminin (green). Scale bar, 100 μm in the top and 50 μm in the bottom panel.



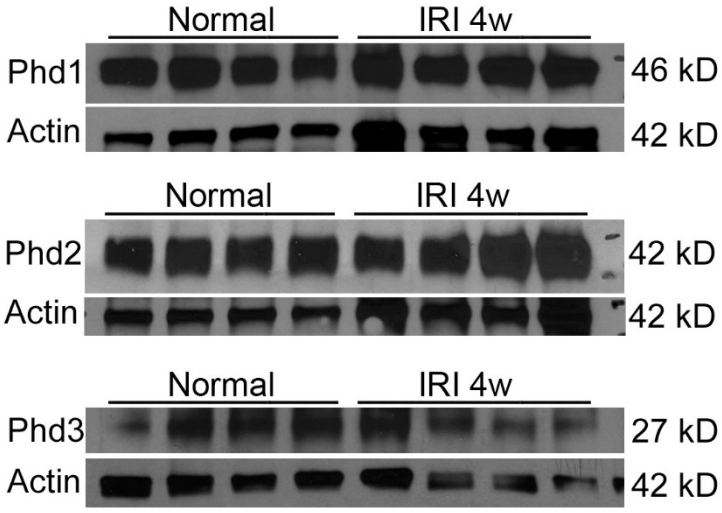

**SF2**. Exposure of primary culture of renal epithelial cells isolated from 3 week-old wild-type mice to 1% $O_2$ for 60 min did not result in changes in FoxO3 mRNA levels measured by renal-time RT-PCR. Cell cultures were obtained from 6 mice and experiments performed in duplicates. A two-tailed Student's *t* test was performed for statistical analysis.



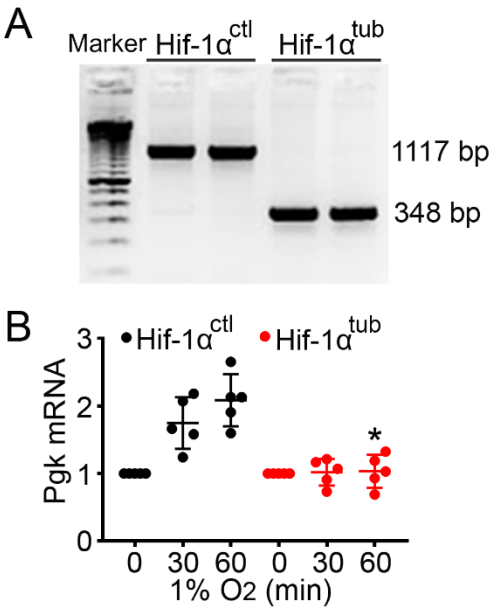

**SF3.** Exposure of primary culture of renal epithelial cells isolated from 3 week-old wild-type mice to 1% $O_2$ or treating cells with Phd enzyme inhibitor DMOG, or DMKG, a cell permeable analogue of 2-oxoglutarate, did not change protein levels of PHD1, 2 and 3 enzymes. Gapdh serves as loading controls. n=4 with duplicates. A two-tailed Student's *t* test was performed for statistical analysis.



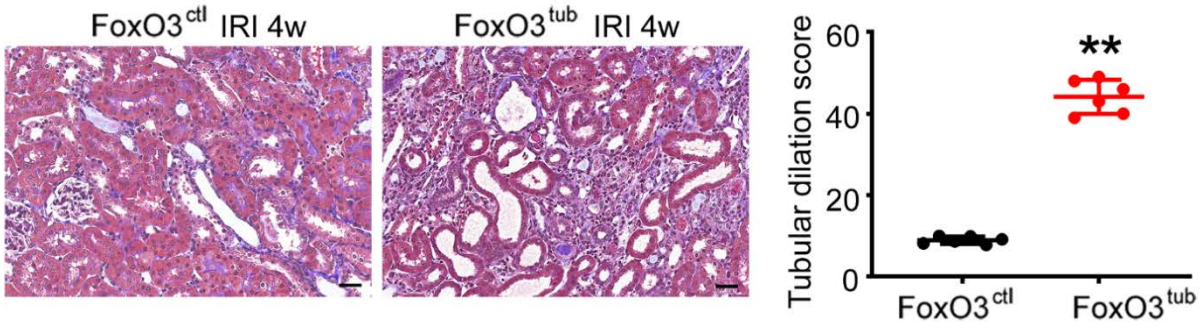

**SF4.** Validation of the specificity of the antibody to OH-FoxO3 at Pro437 (Millipore, ABE1848). Various amount of N-terminal biotinylated FoxO3 peptides (420-444) were spotted on the nitrocellulose membrane and detected by the antibody to the prolyl hydroxylated FoxO3 at Pro437 (A) or anti-biotin antibody (B). Note, the antibody to OH-FoxO3 at Pro437 did not recognize FoxO3 without hydroxylation (WT) or hydroxylated at Pro426. Peptide sequences: WT FOXO3 420-444: GSGLGSPTSSFNSTVFGPSSLNSLR; FOXO3 P426-OH: GSGLGSP (OH) TSSFNSTVFGPSSLNSLR; and FOXO3 P437-OH: GSGLGSPTSSFNSTVFGP(OH)SSLNSLR.



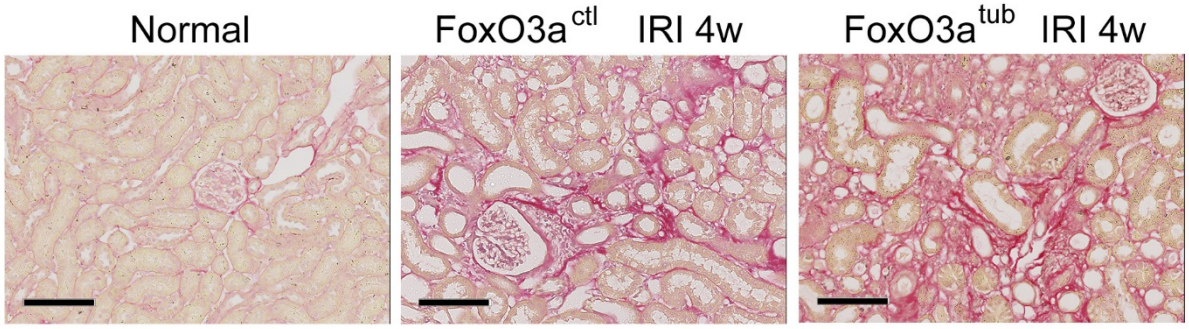

**SF5.** Protein levels of Phd isoforms in normal and injured kidneys. Wild type mice underwent left kidney IRI and right nephrectomy and kidney were obtained 4 w post-IRI. No differences in Phd1, Phd2 and Phd3 protein levels were detected in whole kidney lysates between normal and injured kidneys (4 mice in each group). Actin was used as loading controls. A two-tailed Student's *t* test was performed for statistical analysis.



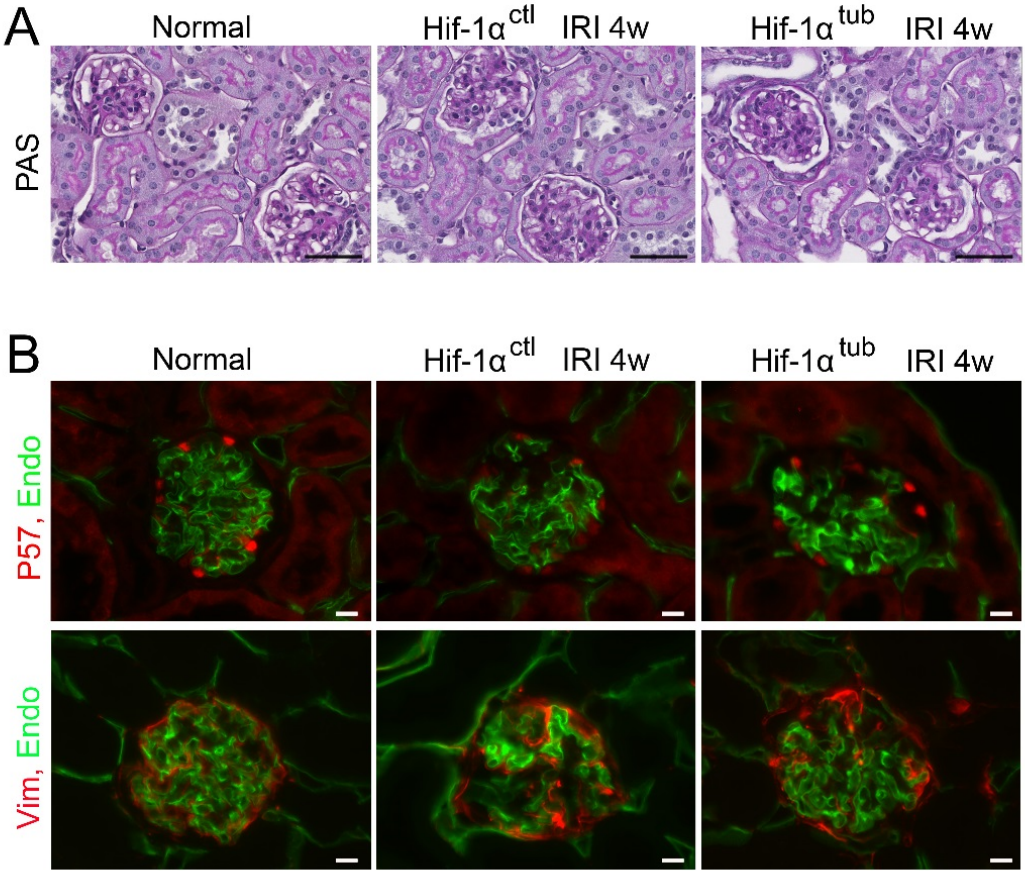

**SF6.** A. Hif-1α gene was deleted at the genomic level in cells isolated from *Hif-1α$^{tub}$* mice. *Hif-1α$^{tub}$* or *Hif-1α$^{ctl}$* mice were given doxycycline or vehicle in the drinking water for 2 w before cells were isolated for cultures. Renal epithelial cells were isolated from 6 of *Hif-1α$^{ctl}$* or 3 *Hif-1α$^{tub}$* mice without renal injury and each DNA sample was analyzed in duplicates. B. A control experiment with a known Hif-1 regulated gene phosphoglycerate kinase 1 (Pgk-1) showed gradual increases of *Pgk-1* mRNA in *Hif-1α$^{ctl}$* cells but not in *Hif-1α$^{tub}$* cells after exposure to 1% $O_2$. n=5 with duplicates, *p<0.05 comparing *Hif-1α$^{ctl}$* with *Hif-1α$^{tub}$* cells at 30 or 60 min with a two-tailed Student's *t* test.



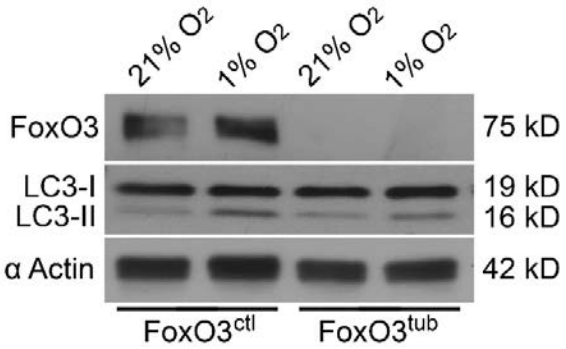

**SF7.** Tubular deletion of FoxO3 resulted in pronounced tubular dilation. FoxO3 was deleted at days 8-21 following a 35 min left kidney IRI and right nephrectomy. Kidneys were analyzed 4 w post-IRI. *FoxO3$^{tub}$* indicates FoxO3 deletion with doxycycline and *FoxO3$^{ctl}$* indicates control with vehicle treatment. Masson's trichrome stain and a grid point count method were used for quantification of tubular dilatation. Scale bar 50 μm, n=6, ** $p<0.01$ using non-parametric t-test (Wilcoxon-Mann-Whitney).




**SF8.** Representative picrosirius red stain indicated more tubulointerstitial fibrosis in kidneys with FoxO3 deletion. FoxO3 was deleted at days 8-21 following a 35 min left kidney IRI and right nephrectomy. Kidneys were analyzed 4 w post-IRI. *FoxO3$^{tub}$* indicates FoxO3 deletion with doxycycline and *FoxO3$^{ctl}$* indicates control with vehicle treatment. Scale bar 100 μm, n=6.




**SF9.** Tubular deletion of Hif-1α (*Hif-1α$^{tub}$*) during the AKI to CKD transition resulted in no apparent alterations in the glomeruli. Tubular Hif-1α was deleted at days 8-21 following a 35 min left kidney IRI and right nephrectomy. Kidneys were analyzed 4 w post-IRI. **A.** PAS staining indicated no increases in glomerular cellularity. **B.** Immunostaining of endothelial cells with endomucin (Endo, green) was combined with podocyte marker P57 (red, top panel) or vimentin (red, bottom panel). No significant changes in the number of podocytes per glomerulus (9.9± 0.4 in normal, 9.9 ± 0.5 in *Hif-1α$^{tub}$* and 11.0 ± 0.6 in *Hif-1α$^{ctl}$*) were detected at 4 w post-IRI. Similarly, quantification of randomly selected glomeruli (50 glomeruli in each mouse kidney) showed no differences in the vimentin-positive area per glomerulus between *Hif-1α$^{tub}$* and *Hif-1α$^{ctl}$* mice at 4 w post-IRI. Scale bar, 50 μm in A and 10 μm in B. n=5




**SF10.** Primary cultures isolated from uninjured kidney of 3 week-old *FoxO3$^{ctl}$* and *FoxO3$^{tub}$* mice were exposed to 1% $O_2$ for 30 min. While *FoxO3$^{ctl}$* cells showed a significant increase in LC3II/I ratio from that of cells exposed to 21% $O_2$ ($p<0.05$), *FoxO3$^{tub}$* cells had insignificant changes in LC3II/I ratio ($p=0.28$). n=4. A two-tailed Student's *t* test was performed for statistical analysis.

**Supplemental Methods**

***Immunohistochemistry and antibody information*** For immunohistochemistry, Kidney sections were fixed with 4% PFA in PBS for 4 h, permeabilized with 0.1% Triton X-100 in PBS for 30 min, then blocked with 10% goat serum and 0.5% bovine serum albumin (BSA) in PBS. Sections for FoxO3 and p-FoxO3 staining were alternatively blocked with 5% goat serum and 0.3% Triton X-100 in PBS. Incubations with primary antibodies were carried out at 4 °C overnight. After washing, the sections were further incubated for 1 h at room temperature with the appropriate secondary antibodies. Nuclei were counterstained with DAPI (Vector, H-1200). Sections were visualized with a Zeiss AxioObserver.Z1 inverted fluorescence microscope, photographed with a digital camera, and analyzed with Axiovision software. The following primary antibodies were used: FoxO3 (75D8) (Cell Signaling Technology, 2497, 1:50), Vimentin (abcam, ab92547, 2:500), Endomucin (Santa Cruz, SC-65495, 1:250), E-cadherin (Invitrogen, 13-1900, 1:200), Ki67 (eBioscience, 14-5698, 1:500), CD45 (Life Tech, MCMD4500, 1:200), Collagen I (Southern Biotech, 1310-01, 1:200), p57 (Santa Cruz, SC8298, 1:50), p-FoxO3 (Cell Signaling, 13129s, 1:100), Kim1 (R&D Systems, AF1817, 1:800), and laminin (Sigma, L9393, 1:1000). For immunocytochemistry of FoxO3, anti-FoxO3 antibody (cell signaling, 12829s, 1:50) was used after primary cultures of renal epithelial cells were fixed with 100% cold methanol for 5 min. Secondary antibodies included Alexa Fluor 488-conjugated goat anti-rabbit, goat anti-rat, or donkey anti-goat IgG, or donkey anti-goat IgG and Alexa Fluor 594-conjugated goat anti-rabbit IgG (Molecular Probes, A-11070, A-11006, A-21206A–11036, 1:200). Proximal tubules were stained using fluorescein-conjugated lectin Lotus tetragonolobus agglutinin (FITC-LTA; FL-1321, Vector, 1:800). For quantification of peritubular microvascular density, tissue sections were immunostained with endomucin (Santa Cruz, SC-65495, 1:200) and photographed at a magnification of ×200. 10 images per kidney were taken randomly in OSOM region. Images were analyzed using Adobe Photoshop CS5.1 by applying an arbitrary common threshold to all images, inverting the images, and determining total area fraction as described. For detection of

renal hypoxia, mice received an intraperitoneal injection of Hypoxyprobe™ (pimonidazole hydrochloride, Hypoxyprobe, Inc., HP3–100 Kit) at 60 mg/kg 90 min prior to kidney harvesting. Kidneys were fixed with 4% PFA and processed as cryosections. Pimonidazole hydrochloride formed protein adducts in cells with a $pO_2$ < 10 mm Hg (1.4% $O_2$) and tissue incorporation of pimonidazole was assessed with immunohistochemistry using anti-pimonidazole antisera (Hypoxyprobe, Inc., Pab2627, 1:100) as described (1).

***Immunoblot analysis.*** For protein extraction from kidneys or primary cultured renal epithelial cells, 2% SDS (62.5 mM Tris-HCl, pH 6.8, 10% glycerol, 2% SDS) or RIPA buffer (Thermo Scientific, 89900) were used, respectively, in conjunction with a protease inhibitor cocktail (Thermo Scientific, 78430), phosphatase inhibitor cocktail (Thermo Scientific, 1862495), and DNase I (Thermo Scientific, 90083, 1:50). Immunoblots were probed with antibodies to FoxO3 (75D8) (Cell Signaling, 2497, 1:1000), LC3 (Novus Biologicals, NB100–2220, 1:1000), Ulk1 (Abcam, ab128859, 1:500), Bnip3 (Abcam, ab10433, 1:800), AKT (Cell Signaling, 4691S, 1:2000), p-AKT (Cell Signaling, 4060S, 1:2000), p-FoxO3 (Cell Signaling, 13129, 1:1000), Trans hydroxyproline (Advanced Targeting Systems, AB-T044, 1:1000), NGAL (R&D Systems, AF1857, 1:1000), SOD2 (R&D Systems, MAB3419, 1:2000), Hif1α (R&D Systems, AF1935, 1:2000), PHD1 (Novus, NB100-139, 1:1000), PHD2 (Cell Signaling, 4835s, 1:1000), PHD3 (Novus, NB100-139, 1:1000), OH-FoxO3 Pro437 (Millipore, ABE1848, 1:1000), and followed by further incubation with the appropriate secondary antibodies conjugated with horseradish peroxidase (HRP, Life Technologies, G21234, 1:5000) and detected by chemiluminescence (Thermo Scientific, 34076). Gapdh (Santa Cruz, SC-25778, 1:1000) was used as a loading control. Levels of protein expression were quantified by NIH Image J 1.47 software. For Hif1α western blot, proteins from kidneys were isolated with 2 × Laemmili buffer containing 4% SDS, 20% glycerol, and 120 mM TrisCl (pH 6.8). 100 mM DTT, 1% proteinase inhibitor cocktail and 5% DNase I were added immediately before use. Briefly, kidneys were homogenized in 2 × Laemmili buffer (12.5 µl per 1mg kidney) and boiled at 95°C for 5 min. After cooling down, urea

powder was dissolved in to a final concentration of 6M. Then kidney lysates were centrifuged at 12,000 rpm at room temperature for 5min and preceded with immunoblot.

***Hif-1α PCR****. Pax8-rtTA;tetO-cre;Hif1α*$^{F/F}$ mice were treated with doxycycline at 2 mg/ml for 2 weeks before cell isolation for primary cultures. Cells were digested with DirectPCR (Tail) (QIAGEN, 102-T) overnight at 55°C, and Hif-1α PCR was performed. Primers were designed with Integrated DNA Technologies Primer Quest Tool. A forward primer P2 and a reverse primer P1 were designed to generate a 1117 bp fragment amplification from the genomic DNA of *Hif-1α*$^{f/f}$ mice, while the 348 bp fragment detected Hif-1α deletion. The sequence of each primer is as follows: P1 (reverse): gaaaactgtctgtaacttcatttcc. P2 (forward): tagtgtcttaacctgcccataaatg.

***Real-time RT-PCR analysis****.* Total RNA was isolated from cells or kidneys using 1 ml of TRIzol reagent (Life Technologies 15596-026). RNA concentration and integrity were measured with an Epoch™ microplate spectrophotometer. DNA was eliminated from 1 μg of RNA with deoxyribonuclease I (DNase I, Invitrogen, 18068-015). Post-DNase I digestion, 1 μg of RNA was applied for reverse transcription with iScript™ cDNA Synthesis Kit (Bio-Rad, 1708890). Each time, 100 ng of cDNA and 500 nM primers were used for real-time PCR in a 10-μl reaction mixture with iQ SYBR Green Supermix (Bio-Rad, 170-8880) using a Bio-Rad™ CFX96TM Real-Time PCR System. Primers were designed with Integrated DNA Technologies Primer Quest Tool. The sequences of each primer are as follows: Foxo3, forward: 5'-GCTAAGCAGGCCTCATCTCA-3' and reverse: 5'-TTCCGTCAGTTTGAGGGTCT-3'; *18s* rRNA forward: 5′-GTAACCCGTTGAACCCCATT-3′ and reverse: 5′-CCATCCAATCGGTAGTAGCG-3′; and mouse Pgk, forward: GGAAGCGGGTCGTGATGA and reverse: GCCTTGATCCTTTGGTTGTTTG. *18s* rRNA was used as controls. The expression of each specific gene relative to *18S* rRNA was calculated using the Pfaffl method (2).

***siRNA transfection.*** Primary cultures of renal epithelial cells were isolated from wild type mice and seeded into 6-well plates. Upon 60-80% confluence, cells were washed thoroughly with Opi-

MEM medium (Giboco, 31985-070) before adding ON-TARGETplus non-targeting siRNA #1 (Dharmacon Inc, D-001810-01-05), or siRNA to PhD isoforms prepared with the Lipofectamine RNAiMax reagent (Invitrogen, 13778-150) at a final concentration of 20 nM. Cells were incubated for 72 h before Western blot analysis for Phd isoforms or OH-FoxO3. The following lists the information for siRNA to Phd isoforms. Phd1 siRNA: CAACAUCGAGCCACUCUUU; Phd2 siRNA smart pool of 4 siRNAs (#1: GCGAUAAGAUCACCUGGAU, #2: GACCUGAUACGCCACUGUA, #3: GCUCAUCGCUGUUCCAGGA, and #4: GAACAAGCACGGCAUCUGU); and Phd3: ON-TARGETplus Mouse Egln3 (112407) siRNA-SMARTpool (Dharmacon Inc. L-040261-00-0005).

**Reference**


1. Basile DP, Donohoe DL, Roethe K, and Mattson DL. Chronic renal hypoxia after acute ischemic injury: effects of L-arginine on hypoxia and secondary damage. *American journal of physiology Renal physiology.* 2003;284(2):F338-48.
2. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic acids research.* 2001;29(9):e45.