The CPLANE protein Intu protects kidneys from ischemia-reperfusion injury by targeting STAT1 for degradation

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Intu is known as a ciliogenesis and planar polarity effector (CPLANE) protein. Although roles for Intu have been reported during embryonic development and in the context of developmental disorders, its function and regulation in adult tissues remain poorly understood. Here we show that ablation of Intu specifically in kidney proximal tubules aggravates renal ischemia-reperfusion injury, and leads to defective post-injury ciliogenesis. We identify signal transducer and activator of transcription 1 (STAT1) as a novel interacting partner of Intu. In vitro, Intu and STAT1 colocalize at the centriole/basal body area, and Intu promotes proteasomal degradation of STAT1. During cell stress, Intu expression preserves cilia length and cell viability, and these actions are antagonized by STAT1 expression. Thus, we propose a role for Intu in protecting cells and tissues after injury by targeting STAT1 for degradation and maintaining primary cilia.
Planar cell polarity (PCP) refers to the coordinated alignment of cell polarity across the tissue plane, which is essential for embryonic development and normal tissue function in animals. PCP is established and maintained by the complex machinery of two modules (PCP core and Fat systems) and effector proteins. The effector proteins are further divided into two groups with Daam, Rac and Rho in group 1, and Intu, Fuzzy and Fritz/Wdpcp in group 2. Recently, a potential relationship between PCP and primary cilia has been suggested. In this regard, several PCP proteins have been localized at the cilium or basal body area in Drosophila embryos led to defects in cilia and neural tube closure. Recent work further discovered several Intu mutations in ciliopathy patients. Based on their dual functions, Intu and related PCP proteins are classified as CPLANE (ciliogenesis and planar polarity effector) proteins.

Almost every kidney tubular cell has a primary cilium protruding toward the lumen. Dysregulation of several cilia and PCP proteins has been implicated in the pathogenesis of renal diseases, such as polycystic kidney disease. Moreover, ciliary defects sensitize kidneys to ischemia-reperfusion injury (IRI). In addition, following renal IRI, there seems to be an adaptive growth of primary cilia in kidney proximal tubules. While these findings suggest a role of primary cilia and probably PCP in kidney injury and repair, the underlying mechanism is poorly understood.

In this study, we localize endogenous Intu at the centriole/basal body area in mouse and rat kidney proximal tubular cells. Functionally, we find that knockout of Intu specifically in kidney proximal tubular cells aggravates ischemic kidney injury in mice. Mechanistically, we show that Intu likely interacts with STAT1 at centriole/basal body area to induce proteasomal degradation of STAT1. Thus, Intu may protect cells by interacting with STAT1 to induce its degradation.

Results
Knockout of Intu from proximal tubules aggravates IRI. We generated kidney proximal tubule-specific Intu knockout (PT-Intu-KO) and wild-type (PT-Intu-WT) mice by crossing PEPCK-Cre mice with Intu-floxed mice (Fig. 1a). Intu expression in kidney tissues from both KO mice and WT mice

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**Fig. 1** Knockout of Intu from kidney proximal tubular cells aggravates renal IRI in mice. a Breeding protocol for generating PT-Intu-KO mouse model. b Induction of Intu in mouse kidneys by 35 min of bilateral renal ischemia with 6 or 48 h of reperfusion. c Intu expression at renal IRI (48 h) was abolished in PT-Intu-KO mice as compared to PT-Intu-WT mice (n = 3). d PT-Intu-KO mice and PT-Intu-WT mice had similarly low levels of BUN and SCr under control condition (n = 9), but renal IRI induced higher BUN and SCr in PT-Intu-KO mice (n = 9). e Renal IRI induced more severe renal tubular damage in PT-Intu-KO mice than in PT-Intu-WT mice as shown by representative histology and quantification of the percentage of damaged tubules (n = 6). Quantitative data are mean ± s.d. (error bars). Paired or grouped t test was used. *p < 0.05, **p < 0.01. Scale bar: 100 µm. AU arbitrary unit.
was low and, upon renal IRI, Intu was induced in WT kidneys but not in KO kidneys (Fig. 1b, c; Supplementary Fig. 1, 2), validating the knockout model. Under control conditions, both KO and WT mice had normal renal function as shown by low levels of blood urea nitrogen (BUN) and serum creatinine (SCr). Upon renal IRI, KO mice had significantly higher BUN and SCr than WT mice at both 24 and 48 h of reperfusion (Fig. 1d). Consistently, KO mice showed higher degrees of renal tubule damage (Fig. 1e).

**Intu regulates kidney tubular cell death.** To study the role of Intu in cell death regulation, we performed Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay on kidney sections. TUNEL staining revealed more death cells in KO mouse kidney tissues (Fig. 2a, Supplementary Fig. 3). To further functionally characterize Intu, we examined the effect of stable Intu knockdown with specific short hairpin RNA (shRNA) in BUMPT cells (shIntu; Fig. 2b, c). Upon azide or CCCP (carbonyl cyanide 3-chlorophenylhydrazone) treatment, Intu-knockdown cells had significantly higher levels of cell death than control shRNA-transfected cells (shControl; Fig. 2d, e). We further established tetracycline-inducible Intu expression cells, which expressed Intu upon doxycycline treatment (Fig. 3a: D-Rex Intu3, 5, 10). Induced Intu expression protected renal tubular cells from azide-induced metabolic stress and cisplatin-induced toxic injury morphologically (Fig. 3b, c); consistently Caspase-3 and Parp cleavage in these cells was also suppressed (Fig. 3d). **STAT1 is a novel Intu-interacting protein.** To understand how Intu protects, we probed its interacting proteins by tandem affinity purification (TAP) assay, which pulled down at least seven distinctive bands on silver-stained SDS-PAGE gel (Supplementary Fig. 4). Liquid-chromatography tandem mass spectrometry (LC-MS/MS) analysis of these bands suggested the presence of 160 proteins (Supplementary Table 1). Among them, two peptides of STAT1 (Fig. 4a) were identified suggesting that STAT1 might be a novel interacting partner of Intu. We further confirmed Intu/STAT1 interaction by streptavidin (STV)-pulldown assay and co-immunoprecipitation (IP) (Fig. 4b, c). The interaction of endogenous Intu and STAT1 was further validated in mouse kidneys (Fig. 4d). Moreover, in STV-pulldown assay, the Intu N-terminus containing PDZ domain precipitated endogenous STAT1, whereas Intu mutants lacking this domain did not.
suggesting a pivotal role of the PDZ domain in mediating Intu/STAT1 interaction (Fig. 4e). To study what modulates the interaction between Intu and STAT1, we treated cells with interferon(IFN)-γ, azide, or cisplatin, three inducers of STAT1 activation and phosphorylation. It was found that all three treatments increased Intu-bound STAT1 (Fig. 4f). We then determined where inside the cell Intu and STAT1 interact. Our immunofluorescence (IF) analysis detected Intu accumulation in the basal body/centriole area labeled by γ-tubulin during both interphase and mitosis in renal tubular cells and 293FT cells (Fig. 4g, Supplementary Fig. 5a). We further revealed primary interphase and mitosis in renal tubular cells and 293FT cells antibody 1155PB427 (Supplementary Fig. 5c). Likewise, trans-staining pattern of Intu was also consistent with STAT1 likely at the centriole or basal body? To address this, we first examined the effect of Intu on STAT1 expression. When co-transfected, Intu reduced the expression of both total STAT1 and its phosphorylation mutants (STAT1αY701F, STAT1αS727A) (Fig. 5a). Moreover, Intu suppressed the expression of both total STAT1 and phospho-STAT1S727 during IFN-γ, azide, or cisplatin treatment in different types of cells (Fig. 5b, Supplementary Fig. 6). We further found that full-length Intu and IntuΔ268–942, but not IntuΔ1–267 or IntuΔ181–942, could suppress STAT1 expression, indicating that the N-terminal 267 aa with the PDZ domain was essential for the suppressive effect of Intu on STAT1 (Fig. 5c). In addition, the proteosome inhibitor MG132 could restore the expression level of STAT1 in the presence of Intu (Fig. 5d), suggesting that Intu may target STAT1 for degradation through the ubiquitin-proteasome system. Intu-KO mouse kidneys showed higher total and phospho-STAT1S727 than WT tissues after renal IRI (Fig. 5e), providing in vivo evidence for Intu-mediated STAT1 degradation. Consistent with kidney injury analysis (Fig. 1), Intu-KO mouse kidneys had higher levels of Caspase-3 and Parp cleavage, but lower levels of Bcl-2 and Bcl-xL, indicative of apoptosis (Fig. 5e). Together, these results suggest that Intu may suppress STAT1 to protect kidney cells and tissues.

**Intu inhibits cell death through STAT1.** To further determine their functional interaction, we co-transfected STAT1 into inducible Intu-expressing cells. In the cells with doxycycline-

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**Fig. 3** Inducible Intu expression suppresses cell death. a, Inducible Intu expression in RPTC cells. Three inducible cell clones (D-REx Intu3, 5, 10) were treated with doxycycline to induce Intu expression shown by immunoblotting in comparison to three empty vector controls (D-REx CTL1, 2, 3). b, Less cell death was observed in Intu-expressing cells after azide treatment. c, Resistance of Intu-expressing cells to cisplatin treatment. d, Immunoblot analysis of Caspase-3 and Parp cleavage to confirm less apoptosis in Intu-expressing cells after azide or cisplatin treatment. Quantitative data are mean ± s.d. (error bars). Grouped t test was used (*p < 0.05, **p < 0.001). Scale bar: 20 μm.

**Intu targets STAT1 for degradation.** Why does Intu interact with STAT1 likely at the centriole or basal body? To address this,
immunointeraction during IFN-treatments. Cell lysate was collected for STV-pulldown assay to show that more STAT1 was pulled down by Intu in treated 293FT cells.

immuno stained with DAPI to show nuclear morphology in merged images. Arrow-head: basal body/centriole. Scale bar: 10 µm.

containing PDZ domain (CBP-SBP-Intu were then analyzed for STAT1 and myc-tagged Intu to show their co-precipitation or interaction.

transfected into 293FT cells to collect lysate for immunoprecipitation of STAT1 with anti-STAT1 antibody or non-immune IgG (control). The precipitates were then analyzed for STAT1 and myc-tagged Intu to show their co-precipitation or interaction.

Co-immunoprecipitation of Intu and STAT1 in kidney tissues of PT-Intu-KO or WT mice after renal IRI.

We initially analyzed the association of apoptosis and cilium length in kidney tubules after renal IRI in WT mice, showing that STAT1 participates in Intu-mediated cilium length regulation.

After demonstrating the physical and functional interactions between Intu and STAT1 during cell stress, we wondered if STAT1 participates in Intu-mediated cilium length regulation.

STAT1 is involved in Intu-modulated ciliogenesis. As a typical CPLANE protein, Intu is known to contribute to ciliogenesis27. After demonstrating the physical and functional interactions between Intu and STAT1 during cell stress, we wondered if STAT1 participates in Intu-mediated cilium length regulation. We initially analyzed the association of apoptosis and cilium length in kidney tubules after renal IRI in WT mice, showing that cilia grew longer in TUNEL-negative/non-apoptotic tubules, and diminished in TUNEL-positive/apoptotic tubules (Fig. 7a, Supplementary Fig. 9). We further examined primary cilia in kidney proximal tubules in PT-Intu-KO and -WT mice. Proximal cilia grew longer in TUNEL-negative/non-apoptotic tubules, and diminished in TUNEL-positive/apoptotic tubules (Fig. 7a, Supplementary Fig. 9). We further examined primary cilia in kidney proximal tubules in PT-Intu-KO and -WT mice.
tubules were labeled by lotus tetragonolobus agglutinin (LTA) or phaseolus vulgaris erythroagglutinin (PHA-E), and cilia by Actin staining. Under sham control condition, KO and WT mice had similar cilia in proximal tubules. However, after renal IRI, WT mice had a significant growth in primary cilia of proximal tubules, whereas KO mice did not (Fig. 7b, Supplementary Fig. 10). We also determined the effects of Intu and STAT1 on primary cilia in cultured renal tubular cells. As expected, knockdown of Intu led to the shortening of primary cilia under control conditions, while inducible overexpression of Intu could preserve cilia during cell injury (Fig. 7c, d). In sharp contrast, knockdown of STAT1 resulted in longer cilia and higher percentage of cells with cilia (Fig. 7e). Moreover, STAT1 knockdown partially restored cillum length in Intu-silenced cells (Fig. 7f). Conversely, induced Intu expression led to the attenuation of STAT1 activation (phospho-STAT1S727) at the basal body/centriole area (Fig. 7g). These results suggest a reciprocal regulation of primary cilia by Intu and STAT1.

Discussion
In this study, we have demonstrated a protective role of the CPLANE protein Intu in renal IRI in vivo and toxic/metabolic stress in vitro. Mechanistically, we have unveiled a novel physical and functional interaction between Intu and STAT1. Specifically, Intu interacts with STAT1 likely at the basal body/centriole area.
Knockdown of STAT1 rescued Intu-silenced cells from azide-induced cell death (by shRNAs in Intu/STAT1 double knockdown cells. STAT1 consists of seven proteins (STAT1, 2, 3, 4, 5a, 5b, 6) partner of Intu. STAT1 is a key member of the STAT family that mediated protection, we con...pathological conditions by inducing STAT1 degradation (Fig.8).

Intu protects cells from death through STAT1. Inducible expression of Intu suppressed STAT1 expression and Caspase-3 cleavage during azide and cisplatin treatment. Inducible expression of Intu suppressed azide-induced cell death in STAT1α-transfected cells (n = 5). After exposure to cisplatin, Intu/STAT1 double overexpression cells showed resistance as compared to STAT1 single overexpression cells (n = 8). Confirmation of STAT1 knockdown by shRNAs in Intu/STAT1 double knockdown cells. Knockdown of STAT1 inhibited azide-induced Caspase-3 cleavage/activation in Intu-silenced cells. Knockdown of STAT1 rescued Intu-silenced cells from azide-induced cell death (n = 12). Quantitative data are mean ± s.d. (error bars). Grouped t test was used. *p < 0.05, ***p < 0.001. Scale bars: 20 µm

to induce STAT1 degradation, ciliogenesis, and cell death resistance, whereas deficiency or blockage of Intu leads to ciliary defects, and STAT1 increase and accumulation in cell nucleus to promote cell death. As such, Intu protects cells and tissues under pathological conditions by inducing STAT1 degradation (Fig. 8).

The TAP assay suggested ~160 Intu-interacting proteins in BUMPT cells, among which ~45% of the proteins were also found by another group. By delineating the mechanism of Intu-mediated protection, we confirmed STAT1 as a new interacting partner of Intu. STAT1 is a key member of the STAT family that consists of seven proteins (STAT1, 2, 3, 4, 5a, 5b, 6)29–31. STAT proteins are known to regulate apoptosis following phosphorylation by Janus kinase proteins32. STAT1 can be phosphorylated at both tyrosine 701 and serine 727 sites, and phosphorylated STAT1 forms homodimers or heterodimers with other STAT proteins, which translocate into the nucleus for gene transcription to regulate apoptosis33. Although tyrosine 701 phosphorylation is important in regulating gene transcription, serine 727 phosphorylation has been reported to maximize the apoptotic function of STAT132,34. Our current study suggests that Intu can bind and target STAT1 for degradation through the proteasome system, resulting in the decrease in both total and phosphorylated STAT1. Renal IRI is associated with the induction of Intu (Fig. 1), which may be an intrinsic protective or adaptive mechanism to antagonize the STAT1 pathway of apoptosis. Our results further support a critical role of serine 727 phosphorylation in STAT1 regulation (Fig. 5), which is consistent with the finding in heart IRI34. These observations are supported by the previous studies showing the role of STAT1 in kidney IRI35–37.

As a CPLANE protein, Intu is known to regulate planar polarization in Drosophila and ciliogenesis19,38. However, ablation of Intu specifically from kidney proximal tubules does not reduce the number or length of primary cilia in mouse kidneys under control conditions (Fig. 7b). In contrast, primary cilia grow longer after kidney injury in WT mice, but not in KO mice (Fig. 7b). Thus, Intu may not be required for cilium maintenance, but it is important for ciliogenesis and ciliary elongation. Based on the current understanding, failure of primary cilia to elongate after renal IRI in KO mice may be caused by the lack of Intu-mediated IFT protein recruitment to the base of primary cilia11.
Fig. 7 Regulation of primary cilia by Intu and STAT1.

a Cilium length and cell death in kidney tubules during renal IRI in mice. WT mice were subjected to sham operation or 35 min of ischemia with 48 h of reperfusion to harvest kidneys for Ac-tubulin immunofluorescence staining and TUNEL assay. TUNEL-negative tubules in injured tissues had longer cilia compared to sham controls, while TUNEL-positive tubules had shorter cilia. White triangle denotes cilia.

b Ac-tubulin and LTA staining showing cilium length and frequency in proximal tubules after renal IRI in WT mice that was attenuated in PT-Intu-KO mice.

c Ac-tubulin staining showing shortened cilia and lower frequency in Intu-knockdown cells (shIntu), compared to control shRNA-transfected cells (shControl).

d Intu overexpression (D-REx Intu3) preserved cilium length during azide treatment.

e Longer cilia and higher cilium frequency in STAT1-knockdown cells as compared to control cells. f Intu/STAT1 double knockdown cells had longer cilia than Intu only knockdown cells.

g Double immunofluorescence of serine 727 phosphorylated STAT1 and γ-tubulin (centriole marker) to show that inducible Intu expression abolished centriole-associated STAT1. Fluorescence signal was quantified with ImageJ (n = 7). n is the number of cilia (a-f) or centrioles (g). Quantitative data are mean ± s.d. (error bars). Grouped t test was used. *p < 0.05, **p < 0.01, ***p < 0.001, ###p < 0.001. Scale bar (μm): 10 (a, b, g), 5 (c-f).
Our current study, however, suggests an alternate mechanism that involves Intu-STAT1 interaction. We show that STAT1 is present in centriole/basal body area and lost from this site after Intu overexpression. Moreover, knockdown of STAT1 increases cilium length and frequency (Fig. 7c). Therefore, it is possible that Intu may stimulate ciliogenesis by reducing STAT1 from centriole/basal body area. The mechanism whereby STAT1 regulates ciliogenesis remains elusive. Nonetheless, it may regulate ciliogenesis directly from the basal body through the Intu-IFT protein system and/or indirectly by transcribing relevant genes. Previous work reported the elongation of primary cilia during kidney repair or recovery after acute injury, but the underlying mechanism remains elusive. Our results support a critical role of Intu in ciliogenesis and elongation following kidney injury. Another interesting observation in our study is that TUNEL-positive or death-associated cells tend to have shorter cilia and lower cilium frequency. This is particularly true for Intu-KO cells (Supplementary Fig. 10), suggesting that Intu may also play a role in preserving cilia during cell injury and death. Together, the results of this study suggest that Intu may promote cell survival and ciliogenesis in disease conditions by binding STAT1 to induce its degradation. Discovery of the Intu-STAT1 pathway offers new therapeutic targets. Future research may identify or develop specific pharmacological agents to modulate Intu-STAT1 interaction for the prevention and treatment of diseases such as ischemic kidney injury.

Methods

Antibodies and reagents. Intu antibody 1155PB4 (immunoblot (IB), 1:2500; IF, 1:100) was generated and characterized previously.27 Intu antibody S4166 (IB, 1:1000–2500; IF, 1:100–500) was generated in this study by Proteintech. Brieﬂy, a single KLH-conjugated mouse Intu peptide (DTDVEPEWLDVQKNGEL) was used to immunize two prescreened rabbits. Harvested antisera from the rabbits were separately afﬁnity-puriﬁed and designated as S41661 and S41662. Sources of other antibodies used in this study: antibodies to total STAT1 (14994; IB, 1:1000; IF, 1:100), phospho-STAT1 at serine 727 (STAT1p727, 9177; IB, 1:1000; IF, 1:100), Parp (9542; 1:1000), caspase-3 (9665; 1:1000), and cleaved caspase-3 (9661; 1:1000) from Cell Signaling Technology; c-Myc antibody (9E10; 1:1000) from Invitrogen; cyclolin B antibody (CypB, ab16045; 1:5000) from Abcam; antibodies to FLAG (F3165; IB, 1:1000; IF, 1:500), acetylated tubulin (T7451; 1:1000), γ-tubulin (T5326; 1:500), and β-actin (actin, A2228; 1:10,000) from Sigma-Aldrich; anti-calmodulin-binding protein epitope tag antibody (CBP, 07-482; 1:1000) from Millipore; Bcl-2 (sc-783; 1:200) and Bcl-xL (sc-8392; 1:200) from Santa Cruz Technology; secondary antibodies for immunoblot (goat antimouse 31430, goat antirabbit 31460) from ThermoFisher Sci and for IF staining (goat antimouse 115-165-003, goat antirabbit 115-055-003) from Jackson Immunoresearch. Fluorescein LT (FL-1321) and PHA-E (FL-1121) were purchased from the Vector Labs. Prolong Gold antifade reagent with DAPI (4′,6-diamidino-2-phenylindole, P36931), doxycycline (D9891), MG132 (M7449), cisplatin (cis-diaminoplatinum dichloride, 479306), azide (S2002), and carbonyl cyanide 3-chloropropylhydrazide (C2759) were purchased from Sigma-Aldrich. Recombinant IFN-γ (F30431), basicidin S HCl (B21001), zocin (R25001), Hoechst 33342 (H1399), and Lipofectamine 2000 (12566014) were obtained from ThermoFisher Sci. Puruynic was purchased from Clontech. FuGENE 6 (E2693) was from Promega. NEMBUTAL (pentobarbital sodium injection, NDC 67386-501-55) was manufactured in Abbott Laboratories. Buprenex (buprenorphine hydrochloride, NDC 12496-0757-1) was produced in Reckitt Benckiser Pharmaceuticals Inc.

Constructs and transfection. The mouse Intu construct Intu-mycDNA was purchased from OriGene and subcloned into pNTAP vector to generate CBP-SBP-Intu construct for TAP assay. Intu was subcloned into pcdNA4/TO to establish doxycycline-inducible construct. CBP-SBP-Intu was subcloned into pNTAP vector to generate CBP-SBP-Intu-Aβ-FLAG. All STAT1 wild-type and mutation constructs, including STAT1α-FLAG, STAT1αp727A, were obtained from Addgene32. shRNA constructs for Intu (shIntu) were purchased from Sigma-Aldrich. The constructs were transfected into cells with Lipofectamine 2000 or FuGENE 6.

Proximal tubule-specific Intu knockout mice. Intu-flxed (IntuΔ5) mice and PEPCK-Cre mice were generated previously.25,27 IntuΔ5 mice were crossed with PEPCK-Cre mice to produce IntuΔ5/Δ5Cre and IntuΔ5/XY mice for further breeding (Fig. 1a). IntuΔ5Δ5Cre mice were Intu-deficient in kidney proximal tubular cells. PT-Intu-KO mice and WT littermates (IntuΔ5/XY, PT-Intu-WT) (male, 8–10-week-old) were used for experiments. Mice were routinely housed in the animal facility of Charlie Norwood VA Medical Center at Augusta, GA. All animal experiments were performed according to a protocol approved by the Institutional Animal Care and Use Committee of Charlie Norwood VA Medical Center. Genomic DNA was extracted from mouse tail biopsy for PCR-based

![Fig. 8 Schematic model of Intu/STAT1 regulation of cell death and cilium in kidney injury. In wild-type cells and tissues, renal IRI upregulates Intu, which binds and sequesters STAT1 in the basal body/centriole area for degradation, reducing phosphorylated STAT1 translocation to the nucleus and alleviating cell death (upper). Intu-KO cells and tissues are devoid of Intu, the STAT1 antagonizing mechanism, and as a result, more phosphorylated STAT1 accumulates in the nucleus to promote cell death (lower). Also, Intu promotes ciliogenesis, whereas STAT1 may have opposite effects. Phosphorylated STAT1 is marked with a circled P in red for Y701 and in black for S727.](image-url)
Renal ischemia-reperfusion. Bilateral renal ischemia reperfusion was induced in mice\(^{39,40}\). Briefly, mice were anesthetized with pentobarbital (60 mg kg\(^{-1}\)) and kept on a heating pad to maintain body temperature at \(-37 ^\circ\)C. Supraren- 

phine (0.05 mg kg\(^{-1}\)) was administered as analgesics for pain relief. Kidneys were subsequently exposed for renal pedicle clamping with arterial clips to induce 

renal ischemia. After 35 min of clamping, the arterial clips were released for reperfusion. Sham control mice were subjected to identical procedure but without renal pedicle clamping. Blood was collected on first and second day after ischemia. During killing, kidneys were collected for histological and biochemical analysis.

Renal function and histology. Renal function was indicated by the levels of BUN and SCR. Briefly, blood samples were collected, kept at room temperature for cloting, and then centrifuged at 12,000 g for 5 min to obtain serum. BUN and SCR in serum samples were measured with the commercial kits from Stanbio Laboratories. The histology of kidney tissues was examined by hematoxylin and eosin (H&E) staining. The histology of kidney tissues was examined by hematoxylin and eosin (H&E) staining. Histological changes were evaluated by the percentage of renal tubules showing tubular lysis, loss of brush border, cast formation, and other signs of injury.

Cell culture, treatments, and morphological analysis of cell death. BUMPT (originally obtained from Drs. Lieberthal and Shwartz at Boston University) and 293FT (Invitrogen) cells were cultured in DMEM with 10% FBS media. RPTC cells (originally obtained from Dr. Hopfer at Case Western Reserve University) were maintained in a specialized medium\(^{39}\). For ciplatin treatment, cells were incubated with 20 µM cisplatin. For azide or CCCP treatment, cells were incubated with 20 mM azide or 20 µM CCCP in glucose-free buffer for 3 h and then returned to full cell culture medium for 3 h of “reperfusion.” For IFN-γ treatment, cells were exposed to 5 ng ml\(^{-1}\) IFN-γ in culture media for 30 min. For morphological analysis of cell death, cells were stained with Hoechst 33342 for 2 min and then examined microscopically with the EVOS cell image system (ThermoFisher Sc). Cellular and nuclear morphologies were recorded by phase contrast and fluorescence microscopy. Typical cell death was indicated by cellular shrinkage and blebbing, and nuclear condensation and fragmentation.

Tandem affinity purification and mass spectrometry. TAP was done with the InterPlay Mammalian TAP System from Agilent Technologies. Briefly, BUMPT cells were transfected with CBP-SBP-Intu or empty vector. After 24 h, the 
cells were washed with PBS to remove cell debris and remaining lipids in lysis buffer for protein extraction. STV was then added into the cell lysate for 2 h of incubation at 4 °C. Subsequently, STV was collected, washed completely, and resuspended in Lysis buffer for protein complexes extraction. After washing with phosphate buffer saline (PBS), the blots were incubated with the blocking buffer containing 5% non-fat milk or 3% bovine serum albumin (BSA), and then primary antibody. After washing with phosphate buffer saline (PBS), the blots were incubated with HRP-conjugated secondary antibody at room temperature. After further washing, proteins on the blot were detected with the SuperSignal West Pico Chemiluminescent Substrate (ThermoFisher Sc) or Clarity Western ECL Substrate (Bio-Rad).

For STV pulldown assay, 40 µl STV resin slurry was washed to remove the ethanol storage buffer, incubated with the protein samples for 2 h at 4 °C, and then collected by cen- 

trifugation at 15000g for 5 min. For co-IP, one specific antibody and agarose 

beads were added to cell lysate for 2 h of incubation at 4 °C under constant mixing and then collected by centrifugation. Following extensive washes, the agarose resin was boiled in sample loading buffer with SDS to release the bound proteins. The protein 

samples were subjected to SDS-PAGE and transferred to Immun-Blot PVDF 

membrane (Bio-Rad). The blot was incubated in the blocking buffer containing 5% non-fat milk or 3% bovine serum albumin (BSA), and then primary antibody. Rehy- 

dration through graded concentrations of ethanol. Antigen retrieval was done by 

boiling slides in the retrieval buffer (10 mM sodium citrate pH 6.0, 0.05% 

TWEEN-20) for 1 h. For cultured cell fixation, cells were fixed in 4% para- 

ormaldehyde/2% sucrose solution and permeabilized with 0.1% Triton X-100 for 5 

min. Alternatively, samples were fixed with methanol at –20 °C for 20 min as 

before\(^{39,41}\). Cells and tissue sections were blocked with BSA for 1 h. After incub- 

ation with primary antibodies for 1–2 h, the sections were washed with PBS, 

and then incubated with secondary antibodies for 1–1.5 h. Slides were mounted on 

the Prolong Gold antifade reagent containing DAPI or Hoechst 33342 for 

nuclear staining. TUNEL assay was done with the In Situ Cell Death Detection Kit 

from Roche. For image analysis, Zeiss Axio fluorescence and confocal microscopes (Carl 

Zeiss Inc.) were used. The confocal microscope was equipped with the LSM Image 

Analysis System that was used for the measurement of the cilium length and 

quantification of cilium frequency. Cilium length was measured on maximum 

intensity projection images obtained from 4 µm kidney sections. Measurements did 

not account for potential curvature in the Z-axis. Briefly, cilium length was shown in 

collected images by drawing a line along the primary cilium. If cilium length was 

no shorter, images would be expanded so that the length was measurable. To 

maximize the accuracy, >50 cilia were measured in each condition.

Doxycycline-inducible expression of Intu in RPTC cells. To establish Intu- 

inducible cell lines, RPTC cells were first transfected with pcDNA6 and incubated 

with blasticidin (10 µg ml\(^{-1}\) to select stably transfected cell clones. A total of 14 

clones were selected and tested by immunostaining after pcDNA4-Intu transient 

transfection with or without doxycycline addition. Five clones were further tested 

by immunoblotting to determine which clone had tightly controlled Intu expres- 

sion. Clone 4 was used for further establishment of doxycycline-inducible cells. 

After selection with zeocin (1 mg ml\(^{-1}\)), we verified nine clones by immunoblotting 

and immunostaining.

Single- and double-gene Knockdown BUMPT cells. For STAT1 knockdown in 

BUMPT cells, we purchased validated STAT1 transduction particles from Sigma- 

Aldrich. Intu-knockdown transduction particles were generated based on the 

standard protocol. For double Intu/STAT1 knockdown, we sequentially transduced 

BUMPT cells with both types of knockdown transduction particles. Cells were 

selected and maintained with 2.5 µg ml\(^{-1}\) and 1 µg ml\(^{-1}\) puromycin, respectively.

RNA isolation, reverse transcription, and quantitative PCR. Total RNA was 

isolated from kidney cortex tissues or cultured BUMPT cells according to the 

instructions in mirVana miRNA Isolation Kit (Life Technologies, Carlsbad, CA). 

Reverse transcription into cDNA was done with 1 µg RNA by using iScript cDNA 

Synthesis Kit (Bio-Rad). qRT PCR was performed by a StepOne Real-time System 

(Applied Biosystems, Foster City, CA) with Taq Universal SYBR Green Supermix (Bio-Rad). Each gene reaction was performed in triplicate and data were nor-

mized by the internal control β-actin. The genes and primers used in the 

experiments are, BuL-2 (5'-GGAGGATTGGCCTCTTTT-3'), 5'-GAGACACGG 

AGGAGAATAC-3'), Caspase-1 (5'-AACAGATGCTGTCGGGAC-3'), 

Intra (5'-CTGAACAGTGGTACCGCT-3'), 5'-GATTGTACCAGCCAGCAT-3'; 

β-actin (5'-GACCT 

GATCTCCTTGCT-3'), 5'-GATTACTGCTGCTTG-3'). Data were expressed as mean ± s.d. Paired or grouped 
test was calculated. \(p < 0.05\) was considered statistically significant.

Data availability. All relevant data of this study are available from the authors.
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Author contributions

S.W. and Z.D. designed the experiments. S.W. performed the experiments. S.W., G.W., H.-F.D., S.H., S.N., and Z.D. analyzed the results. S.W., A.L., G.W., H.-F.D., S.H., S.N., and Z.D. wrote the manuscript.

Additional information

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