DHHC21 deficiency attenuates renal dysfunction during septic injury

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Renal dysfunction is one of the most common complications of septic injury. One critical contributor to septic injury-induced renal dysfunction is renal vascular dysfunction. Protein palmitoylation serves as a novel regulator of vascular function. Here, we examined whether palmitoyl acyltransferase (PAT)-DHHC21 contributes to septic injury-induced renal dysfunction through regulating renal hemodynamics. Multispectral optoacoustic imaging showed that cecal ligation and puncture (CLP)-induced septic injury caused impaired renal excretion, which was improved in DHHC21 functional deficient (Zdhhc21dep/dep) mice. DHHC21 deficiency attenuated CLP-induced renal pathology, characterized by tissue structural damage and circulating injury markers. Importantly, DHHC21 loss-of-function led to better-preserved renal perfusion and oxygen saturation after CLP. The CLP-caused reduction in renal blood flow was also ameliorated in Zdhhc21dep/dep mice. Next, CLP promoted the palmitoylation of vascular α1-adrenergic receptor (α1AR) and the activation of its downstream effector ERK, which were blunted in Zdhhc21dep/dep mice. Vasoreactivity analysis revealed that renal arteries from Zdhhc21dep/dep mice displayed reduced constriction response to α1AR agonist phenylephrine compared to those from wild-type mice. Consistently, inhibiting PATs with 2-bromopalmitate caused a blunted vasoconstriction response to phenylephrine in small arteries isolated from human kidneys. Therefore, DHHC21 contributes to impaired renal perfusion and function during septic injury via promoting α1AR palmitoylation-associated vasoconstriction.
PAT-DHHC21, unlike the majority of other DHHCs, is expressed on the plasma membrane, making it possible for DHHC21 to regulate the activities of cell surface receptors in blood vessels \(^{18,22}\). Our previous findings indicate that DHHC21 functional deficiency demonstrates survival benefits in animals with septic injury \(^{22}\). Moreover, a recent study shows that DHHC21 regulates vascular tone through the palmitoylation of vascular α1-adrenergic receptor (α1AR), a seven-transmembrane G protein-coupled receptor (GPCR) that induces smooth muscle contraction \(^{15}\). Increased release of endogenous α1AR agonist norepinephrine has been reported in patients with septic injury, contributing to intrarenal vasoconstriction and tissue hypoperfusion \(^{24}\). Norepinephrine is also used as the first-line vasopressor to treat septic shock, which could further augment regional ischemia in kidneys \(^{12,25}\). Therefore, in this study, we utilized DHHC21-deficient (Zdhhc21 \(^{21/21\text{dep}}\)) mice and tested the hypothesis that DHHC21 contributes to renal dysfunction and tissue damage in septic injury via promoting α1AR palmitoylation-dependent renal vasoconstriction and tissue hypoperfusion. Our findings indicated that Zdhhc21 \(^{21/21\text{dep}}\) mice were more resistant to septic injury-induced renal dysfunction and structural damage. DHHC21 loss-of-function improved renal perfusion and RBF through inhibiting palmitoylation of α1AR and its ability to mediate renal vasoconstriction. Thus, we suggest DHHC21 as a novel mediator of septic injury-induced renal dysfunction via mechanisms involving α1AR palmitoylation and α1AR activation-associated renal tissue hypoperfusion.

**Results**

DHHC21 loss-of-function reduces renal dysfunction in septic injury. Zdhhc21 \(^{21/21\text{dep}}\) mice were employed to investigate the role of PAT-DHHC21 in renal dysfunction during septic injury. DHHC21 functional deficiency in Zdhhc21 \(^{21/21\text{dep}}\) mice is due to a 3 bp deletion in exon 7 of Zdhhc21 gene, resulting in the loss of a single amino acid phenylalanine (F) at position 23326. Detailed information about the genotype and phenotype in patients with septic injury, contributing to intrarenal vasoconstriction and tissue hypoperfusion \(^{24}\). Norepinephrine-induced renal dysfunction via mechanisms involving α1AR palmitoylation and α1AR activation-associated smooth muscle contraction \(^{15}\). Increased release of endogenous α1AR agonist norepinephrine has been reported in patients with septic injury, contributing to intrarenal vasoconstriction and tissue hypoperfusion \(^{24}\). Norepinephrine is also used as the first-line vasopressor to treat septic shock, which could further augment regional ischemia in kidneys \(^{12,25}\). Therefore, in this study, we utilized DHHC21-deficient (Zdhhc21 \(^{21/21\text{dep}}\)) mice and tested the hypothesis that DHHC21 contributes to renal dysfunction and tissue damage in septic injury via promoting α1AR palmitoylation-dependent renal vasoconstriction and tissue hypoperfusion. Our findings indicated that Zdhhc21 \(^{21/21\text{dep}}\) mice were more resistant to septic injury-induced renal dysfunction and structural damage. DHHC21 loss-of-function improved renal perfusion and RBF through inhibiting palmitoylation of α1AR and its ability to mediate renal vasoconstriction. Thus, we suggest DHHC21 as a novel mediator of septic injury-induced renal dysfunction via mechanisms involving α1AR palmitoylation and α1AR activation-associated renal tissue hypoperfusion.

**DHHC21 functional deficiency attenuates septic injury-induced renal damage.** We then performed Periodic Acid-Schiff staining to visualize kidney structural damage in WT and Zdhhc21 \(^{21/21\text{dep}}\) mice after CLP. As shown in Fig. 3a, septic injury led to severe renal tissue damage, including glomerular abnormality, loss of brush border in proximal tubule, vacuolation of tubule epithelium, tubular cell detachment/necrosis, and neutrophil infiltration; however, these morphological alterations were attenuated in Zdhhc21 \(^{21/21\text{dep}}\) mice. Quantitative analysis showed that septic injury resulted in significantly increased renal injury score, which was greatly inhibited by DHHC21 loss-of-function (Fig. 3b). The levels of circulating creatinine and blood urea nitrogen (BUN) were also measured as indicators of renal dysfunction. Consistent with the histology results, septic injury significantly up-regulated plasma levels of creatinine and BUN in WT mice; however, these changes were ameliorated in Zdhhc21 \(^{21/21\text{dep}}\) mice (Fig. 3c,d).

We also examined the levels of circulating cytokines in WT and Zdhhc21 \(^{21/21\text{dep}}\) mice after sham and septic injury. Cytokine array analysis revealed that CLP led to an increased production of cytokines such as interleukin-1 (IL-1), IL-6, and TNF-α in both WT and Zdhhc21 \(^{21/21\text{dep}}\) mice; yet, there was no remarkable differences in cytokine profiles between the two mouse strains after CLP (Supplementary Fig. S2). These data indicate that the protective effects of DHHC21 functional deficiency on kidney function during septic injury are not due to inhibiting the production of pro-inflammatory cytokines.

**DHHC21 functional deficiency prevents the reduction of kidney perfusion and oxygen saturation during septic injury.** Given the fact that renal tissue hypoperfusion is a major cause of renal dysfunction during septic injury, we compared kidney perfusion between WT and Zdhhc21 \(^{21/21\text{dep}}\) mice after septic injury using MSOT. Detecting intrinsic NIR light absorbing hemoglobin via MSOT has been reported to be a reliable technique to measure organ perfusion in many studies \(^{31,32}\). As shown in Fig. 4a, the intensity of total hemoglobin detected by MSOT was displayed remarkably less oxygenated hemoglobin but greater deoxygenated hemoglobin in kidneys than sham-operated mice, whereas these responses were ameliorated in Zdhhc21 \(^{21/21\text{dep}}\) mice (Fig. 4c). Consistently, quantitative data in Fig. 4d indicated that Zdhhc21 \(^{21/21\text{dep}}\) mice were more resistant to septic injury-induced decrease in renal oxygen saturation.
Figure 1. Representative MSOT images showing the clearance of IRDye800CW from mouse kidneys. IRDye800CW (60 nM dissolved in 0.9% saline) was injected intravenously into WT mice 24 h after sham operation. The clearance/movement of IRDye800CW (green) in the cross section of the right kidney (white square) was recorded. ROIs in cortex and medulla/pelvis region of the kidney are delineated in red and blue, respectively. Representative images of 28 mice (n = 7 in each group). The green bar represents the scale for the mean pixel intensity of the IRDye800CW signal.
Zdhhc21<sup>dep/dep</sup> mice exhibit better-preserved RBF after septic injury. Next, we examined the functional impact of DHHC21 on RBF during septic injury. The levels of RBF of WT and Zdhhc21<sup>dep/dep</sup> mice were compared using transonic flowmeter 24 h after CLP. Our results indicated that the RBF in WT mice was greatly reduced after septic injury; this reduction was significantly blunted by DHHC21 functional deficiency (Fig. 5a,c). We also monitored the changes in mean arterial pressure (MAP) during septic injury. As shown in Fig. 5b,d, septic injury caused a drastic decrease in MAP in WT mice; yet, no significant difference in MAP was
Figure 3. Zdbhc21<sup>dep/dep</sup> mice are resistant to septic injury-induced kidney damage. (a) Representative images of Periodic Acid-Schiff-stained renal tissue collected 24 h after septic injury induction. Pathological alterations are indicated by arrows with different colors: brush border loss (blue), tubular cell detachment (red), neutrophil infiltration (black). Images are representative of 5 mice. (b) Mice with DHHC21 loss-of-function display a reduced renal injury score than WT mice after septic injury. n = 5 independent experiments; 5 views per animal were imaged and analyzed. (c) The level of circulating creatinine. Mouse plasma was collected 24 h after CLP-induced septic injury. n = 12. (d) The level of blood urea nitrogen 24 h after septic injury. n = 11. Results represent mean ± SEM. *p ≤ 0.05 versus WT + Sham, #p ≤ 0.05 versus WT + CLP.
found between WT and Zdhhc21^dep/dep mice after septic injury. These data indicate that the improved RBF in Zdhhc21^dep/dep mice is not secondary to systemic blood pressure changes.

**DHHC21 functional deficiency decreases α1AR palmitoylation and inhibits α1AR-mediated signaling pathway activation and vasoconstriction.** To investigate the mechanism by which DHHC21 regulates RBF and renal tissue perfusion, we determined the roles of DHHC21 in α1AR palmitoyla-
tion and function. Both DHHC21 and α1AR are localized in the plasma membrane, which allows DHHC21 to dynamically interact with α1AR. Moreover, α1AR has previously been reported to co-immunoprecipitate with DHHC21, indicating their direct binding interaction. We compared the levels of α1AR palmitoylation in renal arteries of WT and Zdhhc21dep/dep mice after CLP, using resin-assisted capture (RAC, Supplementary Fig. S3). As illustrated in Fig. 6a, septic injury resulted in a dramatic increase in α1AR palmitoylation in renal arteries of WT mice, which was significantly reduced in Zdhhc21dep/dep mice. Furthermore, we assessed the phosphorylation of extracellular signal-regulated kinase (ERK), a downstream signaling event of α1AR activation, in renal arteries of WT and Zdhhc21dep/dep mice after septic injury. The level of phosphorylated ERK was significantly increased by CLP in renal arteries of WT mice; this response was diminished by DHHC21 loss-of-function (Fig. 6b).

In an effort to further evaluate the functional impacts of DHHC21 on α1AR-mediated vasoconstriction, we isolated renal arteries from WT and Zdhhc21dep/dep mice and challenged the vessels with phenylephrine, an α1AR agonist. Our wire myograph data showed no significant difference in the high potassium (60 mM)-induced vasoconstrictions between WT renal arteries and Zdhhc21dep/dep renal arteries (Supplementary Fig. S4a); yet renal arteries isolated from Zdhhc21dep/dep mice displayed a significant reduction in phenylephrine-induced vasoconstriction compared to those of WT mice (Fig. 6c,d). These results suggest the critical role of DHHC21-mediated α1AR palmitoylation in α1AR signaling activation and α1AR-mediated vasoconstriction.

Figure 5. DHHC21 loss-of-function attenuates septic injury-induced reduction in renal blood flow. (a,b) Representative recordings of RBF (a) and MAP (b) in different groups 24 h after septic injury. Images are representative of 8 mice. (c) The reduced RBF in WT mice upon septic injury is rescued in Zdhhc21dep/dep mice. *p ≤ 0.05 versus WT + Sham, #p ≤ 0.05 versus WT + CLP.
Figure 6. DHHC21 catalyzes α1AR palmitoylation and contributes to α1AR-mediated signaling pathway activation and vasoconstriction. (a) The level of palmitoylated α1AR in renal arteries. Palmitoylated proteins were isolated by RAC in the presence of hydroxylamine and then analyzed via immunoblotting for α1AR. Blots are representative of 8 independent experiments; renal arteries from 12 mice were pooled for each independent immunoblot analysis. Band intensity is quantified and normalized to control. Full-length blots are presented in Supplementary Fig. S5a. (b) Septic injury-induced ERK activation is inhibited in Zdhhc21dep/dep mice. The band intensity of phosphorylated ERK is normalized to that of total ERK. n = 9. *p ≤ 0.05 versus WT + Sham, #p ≤ 0.05 versus WT + CLP. Full-length blots are presented in Supplementary Fig. S5b. (c) Representative myograph recordings of WT and Zdhhc21dep/dep renal arteries. KPSS represents 60 mM potassium physiological saline solution; W represents wash. (d) Myograph results show that renal arteries of Zdhhc21dep/dep mice exhibit reduced tension compared to those of WT mice upon phenylephrine challenge. Results represent mean ± SEM. n = 9 mice. One artery per animal was used. *p ≤ 0.05 versus WT.
Consistent findings were observed in small arteries isolated from viable human kidneys. Our immunofluorescence images showed evidence for co-localization of DHHC21 and α1AR in human renal arteries (Fig. 7a). PAT inhibitor 2-bromopalmitate (2-BP) was applied to block the function of DHHC21 in small arteries isolated from human kidneys. 2-BP treatment showed no effects on high potassium-induced vasoconstriction (Supplementary Fig. S4b); however, 2-BP-treated small arteries displayed blunted constriction responses to phenylephrine compared to vehicle-treated ones (Fig. 7b). Collectively, the above findings demonstrated that DHHC21 contributes to impaired renal perfusion and function during septic injury via promoting α1AR palmitoylation and α1AR-mediated renal vasoconstriction.

**Discussion**

In the present work, we report DHHC21 as a novel regulator of renal perfusion and function during septic injury. Our new findings indicate: (1) Zdhhc21<sup>−/−</sup> mice exhibit better renal function and less renal damage after septic injury compared to WT mice; (2) DHHC21 functional deficiency preserves renal tissue perfusion, oxygen saturation, and RBF in septic injury; and (3) DHHC21-catalyzed α1AR palmitoylation is required for the activation of α1AR signaling pathway and α1AR-induced constriction of renal arteries. This study provides a new mechanistic insight into the regulation of renal perfusion and function during septic injury. We suggest that DHHC21 functional deficiency confers protective effects on kidney function in septic injury and that inhibition of DHHC21 may serve as a therapeutic strategy to combat renal dysfunction during septic injury.

Renal tissue hypoperfusion/hypoxia is one of the leading causes of renal dysfunction during septic injury<sup>3–5</sup>. We detected renal hypoperfusion in mice after CLP, evidenced by weakened MSOT signal intensity of total hemoglobin, decreased oxygen saturation in renal tissue, and reduced RBF after septic injury. Consistent with our findings, the reduction in RBF has been observed in sepsis patients with kidney injury as well as in large-animal models of septic injury<sup>24,33,34</sup>. It is worth noting that several studies report that renal dysfunction can develop in sepsis animals with unchanged or even increased RBF<sup>23,35</sup>. These discrepant findings may be attributed to different animal models and methods used to measure blood flow. For instance, while CLP-induced polymicrobial septic injury has been commonly used, some studies use intravenous injection of *Escherichia coli*<sup>29</sup>.

Protein palmitoylation has recently been reported as a novel regulator of protein function, involved in the pathogenesis and progression of many diseases. It was first identified as a new type of post-transcriptional modification by Schmidt et al.<sup>36,37</sup>. The discovery of the DHHC family has since promoted the rapid expansion of this field<sup>18,19</sup>. The roles of palmitoylation and PATs have been reported in numerous physiological and pathological conditions, including lipid metabolism, cancer, cardiovascular diseases, and neurological disorders<sup>23,40,41</sup>. Although the involvement of palmitoylation in polycystic kidney disease and kidney cancer has been previously reported<sup>23,42</sup>, there have been very limited studies examining the functional role of PATs in kidney diseases, specifically in renal dysfunction during septic injury. To the best of our knowledge, we are the first to investigate the role of DHHC21 in renal dysfunction in septic injury. Our findings indicate that the inhibition of DHHC21 greatly rescues kidney function and preserves kidney structure in septic injury.

Our study utilizing Zdhhc21<sup>−/−</sup> mice demonstrates that the beneficial effects of DHHC21 functional deficiency on kidney function is attributed to its ability to improve renal tissue perfusion during septic injury. Under basal conditions, Zdhhc21<sup>−/−</sup> mice show no signs of salt/water imbalance or renal structural/functional damage<sup>45</sup>. Yet, DHHC21 loss-of-function suppresses the septic injury-induced reduction of RBF, renal perfusion, and renal oxygen saturation. Increased renal vascular resistance caused by excessive renal vasoconstriction is considered as a major reason for impaired renal tissue perfusion in septic injury<sup>6,33</sup>. Our results indicate the involvement of α1AR in mediating septic injury-induced renal tissue hypoperfusion, as evidenced by the increase in α1AR palmitoylation and the activation of α1AR signaling pathway in septic injury. However, DHHC21 functional deficiency inhibits α1AR palmitoylation and results in a blunted ability of α1AR to activate its downstream effector and to mediate phenylephrine-induced vasoconstriction in renal arteries.

The molecular mechanism underlying the regulation of α1AR function by DHHC21 still remains to be fully elucidated. The defect in α1AR function caused by DHHC21 loss-of-function may be attributed to the conformational change of α1AR. Many GPCRs rely on palmitoylation for their appropriate intracellular conformation. The attached palmitate in the C-terminal tails of GPCRs inserts into the plasma membrane to create the fourth intracellular loop, which is essential for the interaction of GPCRs with their partner proteins and the propagation of GPCR signals<sup>17,40</sup>. Previous study has indicated that the palmitoylation of α1bAR also occurs in its C-terminal region<sup>44</sup>. Therefore, the lack of DHHC21-mediated α1AR palmitoylation may alter the intracellular conformation of α1AR, thus blocking the propagation of α1AR signals. This can be supported by our results showing that the activation of ERK, a downstream signaling event of α1AR activation, is inhibited in Zdhhc21<sup>−/−</sup> mice subjected to septic injury. However, the DHHC21-regulated topology of α1AR carboxyl terminal needs to be further confirmed using X-ray crystallography.

Another novel aspect of our study is the utilization of MSOT to evaluate kidney perfusion and function during septic injury. MSOT has been reported as a label-free method to measure the perfusion of different organs and a reliable imaging technique to determine kidney function<sup>26,32,45</sup>. Compared to Doppler flowmeter which does not allow visualization of microvasculature<sup>45</sup>, MSOT generates images with high spatial resolution at 150 μm of DHHC21 may serve as a therapeutic strategy to combat renal dysfunction during septic injury.

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Figure 7. DHHC21 co-localizes with α1AR and palmitoylation regulates the constriction of small arteries isolated from human kidneys. (a) Co-localization of DHHC21 and α1AR in the cross-section of human renal arteries. Representative images of renal arteries isolated from 3 donors. (b) Inhibition of PATs with 2-BP results in impaired α1AR-mediated vasoconstriction in small arteries isolated from human kidneys. Vehicle control or 2-BP (100 μM) was given 1 h prior to phenylephrine challenge. Small arteries are from 3 different donors. Results represent mean ± SEM. *p ≤ 0.05.
Currently, there are no DHHC21-specific inhibitors available. The most commonly used PAT inhibitor is 2-BP which has a broad effect on multiple DHHCs and also interfere with fatty acid metabolism\(^47\). Thus, developing small molecule inhibitors that specifically target DHHC21 would be a promising direction. It is also worth pointing out that α1AR is not the only substrate of DHHC21. Several other DHHC21 substrates have recently been identified, including platelet endothelial cell adhesion molecule (PECAM-1), estrogen receptor, caveolin-1, endothelial nitric oxide synthase (eNOS), Fyn, superoxide dismutase (SOD-1), and PLCβ1\(^22\).\(^{\text{41,48,49}}\). While beyond the scope of the present study, we cannot rule out the possibility that these substrates may also affect renal function during septic injury.

In conclusion, the present study demonstrates for the first time that DHHC21 plays a critical role in regulating renal perfusion during septic injury via mechanism involving α1AR palmitoylation and α1AR-mediated vasodilation. Furthermore, inhibition of DHHC21 exerts protective effects on renal function during septic injury.

Materials and methods

Reagents. All reagents are listed in Supplementary Table S1.

Animals. Zdhhc21<sup>dep/dep</sup> mice and their wild-type control mice (B6C3F1) were purchased from Jackson Laboratory. The genotype of Zdhhc21<sup>dep/dep</sup> mice was confirmed by sequencing (GeneWiz, NJ, USA). Primers used for sequencing were: AGCTGACTGAAAGGCACC (forward) and AAAACCTGTAACCGATTTCGA (reverse)\(^22\). Animals were maintained under a 12/12 h light/dark cycle with free access to food and water. Mice (16–20 weeks) of both genders were used for this study. All animal experiments are approved by the University of South Florida Institutional Animal Care and Use Committee and conform to the NIH Guide for the Care and Use of Laboratory Animals. The study was carried out in compliance with the ARRIVE guidelines.

Cecal ligation and puncture. Mice were anesthetized with isoflurane (3% induction and 1% maintenance). A midline incision was made in the shaved abdominal region, and the cecum was exteriorized, tightly ligated at 5 mm below the ileocecal valve, and perforated twice with a 20-gauge needle distal to the point of ligation. One mm of feces was extruded from each puncture hole. The cecum was then repositioned, and the abdomen was closed in two layers. 37 °C Lactated Ringer solution was applied topically to prevent the cecum from drying. Mice were then given 37 °C 0.9% saline subcutaneously for fluid resuscitation. A preoperative temperature (RT), followed by incubation with Schiff’s solution for 25 min. Sections were then counterstained with hematoxylin. Images were captured using Keyence BZ-X710 (Itasca, IL, USA). Renal structural damage was assessed by calculating the area fraction of pathological features.

Kidney histopathology. Kidneys were fixed, cut along the sagittal plane, and processed for paraffin-embedding. Sections (5 μm) were de-paraffinized, rehydrated, and oxidized with periodic acid for 8 min at room temperature (RT), followed by incubation with Schiff’s solution for 25 min. Sections were then counterstained with hematoxylin. Images were captured using Keyence BZ-X710 (Itasca, IL, USA). Renal structural damage was evaluated based on the following features: glomerular abnormality, loss of brush border of proximal tubule, vacuolation, dilation of tubule epithelium, tubular cell detachment/necrosis, and neutrophil infiltration\(^53\). Each feature was graded on a scale of 0–5 based on the severity.

Measurement of RBF and MAP. Mice were anesthetized using isoflurane. The blood pressure transducer was cannulated into the carotid artery. A midline incision was made in the abdominal area followed by a left transverse incision to expose the renal artery. RBF was measured using an ultrasound transit-time flowmeter (TS-420; Transonic Systems Inc., Ithaca, NY, USA). After placing the flow probe around the exposed renal artery, mice were allowed to be stabilized for at least 30 min. RBF and MAP were recorded simultaneously using...
2-BP-treated arteries were constructed and compared. Phenylephrine followed by acetylcholine (10 μM). The concentration–response curves of vehicle control- or reduced responses to KPSS were discarded. The arteries were then challenged with the same concentrations 1 h; the vessel viability was then tested again with 60 mM KPSS with 100 μM 2-BP. The small arteries with no responses of phenylephrine (10 nM–30 μM). After washing off, the vessels were incubated with 2-BP (100 μM) for 1 h; the vessel viability was then tested again with 60 mM KPSS and treated with increasing concentrations of phenylephrine (10 nM–30 μM). Arteries that did not respond to KPSS were discarded. The arteries were then treated with increasing concentrations of phenylephrine (10 nM–30 μM). Endothelial integrity was then assessed using acetylcholine (10 μM). The concentration–response curves were constructed.

Small arteries from human kidneys. Small arteries (~1000 μm in diameter) were dissected from intact viable human kidneys that were rejected for transplant surgery. Vessel segments (~2 mm long) were mounted onto the wire myograph chamber (Living Systems Instrumentation, VT, USA) between two tungsten wires (30 μm in diameter). The isometric tension was recorded using Living Systems signal conditioner (MYO-SC-1) coupled with LabScribe v4 iWorks software. After equilibrating for 30 min at 37 °C in PSS, the normalization procedure was performed to determine optimal internal circumference that corresponds to transmural pressure of 100 mmHg. Next, the vessel viability was tested with 60 mM KPSS (74.7 mM NaCl, 60 mM KCl, 1.18 mM KH₂PO₄, 14.9 mM NaHCO₃, 5.5 mM glucose, 0.026 mM EDTA, and 1.6 mM CaCl₂, pH 7.4). Arteries that did not respond to KPSS were discarded. The arteries were treated with increasing concentrations of phenylephrine (10 nM–30 μM). Arteries that did not respond to KPSS were discarded. The arteries were then challenged with the same concentrations of phenylephrine followed by acetylcholine (10 μM). The concentration–response curves of vehicle control- or 2-BP-treated arteries were constructed and compared.

Immunofluorescence. Human renal arteries were fixed in 10% formalin for 48 h, paraffin-embedded and sectioned. Slides were then deparaffinized, rehydrated and permeabilized with PBS containing 0.05% Triton X-10022. After blocking, slides were labelled with rabbit anti-DHHC21 and goat anti-α1AR primary antibodies (1:500) overnight at 4 °C. After washing with TBST, the membrane was incubated with IRDye800CW donkey anti-rabbit secondary antibody (1:20,000) for 45 min at RT.

Measurement of palmitoylated α1AR. Samples collected from RAC were loaded on a 4–20% Tris–Glycine gel and transferred onto a nitrocellulose membrane after electrophoresis. After blocking for 1 h at RT, α1AR was probed with rabbit anti-α1AR primary antibody (1:500) overnight at 4 °C. After washing three times with TBST, the membrane was incubated with IRDye800CW donkey anti-mouse and IRDye680RD donkey anti-rabbit secondary antibodies were used for secondary incubation (1:20,000). The membranes were imaged and analyzed using LI-COR Odyssey CLx.
Data availability
The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author contributions
X.Y. performed, analyzed, interpreted the experiments, and drafted the manuscript. E.Z. assisted with most of the experiments. Y.M., V.C., N.V., E.Z., and S.Y.Y. edited and revised the manuscript. N.V. and J.W.B. participated in wire myograph analysis. R.L. participated in RBF and MAP measurement. M.H.W. assisted with the MSOT experiments and palmitoylation assays, as well as data interpretation and manuscript preparation. S.Y.Y. initiated, directed, and sponsored the work throughout all levels of development. All authors approved it for publication.

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