



# The IFN $\gamma$ -PDL1 Pathway Enhances CD8T-DCT Interaction to Promote Hypertension

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**BACKGROUND:** Renal T cells contribute importantly to hypertension, but the underlying mechanism is incompletely understood. We reported that CD8Ts directly stimulate distal convoluted tubule cells (DCTs) to increase NCC (sodium chloride co-transporter) expression and salt reabsorption. However, the mechanistic basis of this pathogenic pathway that promotes hypertension remains to be elucidated.

**METHODS:** We used mouse models of DOCA+salt (DOCA) treatment and adoptive transfer of CD8<sup>+</sup> T cells (CD8T) from hypertensive animals to normotensive animals in in vivo studies. Co-culture of mouse DCTs and CD8Ts was used as in vitro model to test the effect of CD8T activation in promoting NCC-mediated sodium retention and to identify critical molecular players contributing to the CD8T-DCT interaction. Interferon (IFN $\gamma$ )-KO mice and mice receiving renal tubule-specific knockdown of PDL1 were used to verify in vitro findings. Blood pressure was continuously monitored via radio-biotelemetry, and kidney samples were saved at experimental end points for analysis.

**RESULTS:** We identified critical molecular players and demonstrated their roles in augmenting the CD8T-DCT interaction leading to salt-sensitive hypertension. We found that activated CD8Ts exhibit enhanced interaction with DCTs via IFN $\gamma$ -induced upregulation of MHC-I and PDL1 in DCTs, thereby stimulating higher expression of NCC in DCTs to cause excessive salt retention and progressive elevation of blood pressure. Eliminating IFN $\gamma$  or renal tubule-specific knockdown of PDL1 prevented T cell homing into the kidney, thereby attenuating hypertension in 2 different mouse models.

**CONCLUSIONS:** Our results identified the role of activated CD8Ts in contributing to increased sodium retention in DCTS through the IFN $\gamma$ -PDL1 pathway. These findings provide a new mechanism for T cell involvement in the pathogenesis of hypertension and reveal novel therapeutic targets.

**GRAPHIC ABSTRACT:** A [graphic abstract](#) is available for this article.

**Key Words:** blood pressure ■ hypertension ■ immunity ■ interferon ■ sodium chloride

## Meet the First Author, see p 1489

**M**ore than one billion people worldwide are afflicted with hypertension and over half of them exhibit salt sensitivity,<sup>1,2</sup> which increases the risk for cardiovascular events and mortality.<sup>3,4</sup> Although many drug families are used to treat hypertension, fewer than 50% of patients achieve blood pressure control.<sup>5-7</sup> Thus, it is important to identify unknown mechanisms involved in

the pathogenesis of salt-sensitive hypertension and develop improved medications for its treatment. In recent years, an important role has been proposed for T cells in hypertension.<sup>8-11</sup> Unfortunately, there are no related treatment options because the mechanisms underlying T cell involvement in blood pressure regulation are largely unknown. Therefore, new research focused on

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## Novelty and Significance

### What Is Known?

- Hypertension is a major public health problem, contributing to a plethora of cardiovascular diseases and related death.
- The kidney plays a key role in the pathogenesis of hypertension, especially in augmenting salt-sensitivity of hypertension, which increases the risk for cardiovascular events and mortality.
- Recently, an important role has been proposed for T cells in hypertension, yet without related treatment options, because the mechanisms underlying T-cell involvement are largely unknown.

### What New Information Does This Article Contribute?

- Data from the current study determined a mechanism for T-cell homing into the kidney and interacting with

kidney cells to promote salt retention and development of hypertension.

- The conclusion of this study elucidated immune disorder as a pathogenic event contributing to salt-induced hypertension, justifying a critical need to consider immunologic strategies to treat this disease.
- We also identified new molecular targets as new players contributing to salt-induced hypertension and provide novel insights for new therapeutic strategies against this form of hypertension, especially those resistant to current treatments.

Our study identified critical molecular players and demonstrated their roles in augmenting the interactions between CD8 T cells and distal convoluted tubule cells, leading to excessive salt-retention and consequent elevation of blood pressure. These findings provide a new mechanism for T-cell involvement in the pathogenesis of salt-induced hypertension.

## Nonstandard Abbreviations and Acronyms

<b>Ang II</b>	angiotensin II
<b>CD8T</b>	CD8+ T cells
<b>DCT</b>	distal convoluted tubule
<b>DOCA</b>	deoxycorticosterone acetate
<b>IFN<math>\gamma</math></b>	interferon $\gamma$
<b>IFN<math>\gamma</math>-KO</b>	knockout of IFN $\gamma$
<b>mDCT</b>	mouse DCT
<b>MHC-I</b>	major histocompatibility complex class I
<b>MHC-II</b>	major histocompatibility complex class II
<b>mIFN<math>\gamma</math></b>	mouse IFN $\gamma$
<b>NCC</b>	sodium chloride co-transporter
<b>PD1</b>	programmed cell death protein 1, also known as CD279
<b>PDL1</b>	programmed death-ligand 1, also known as CD274 or B7-H1
<b>TNF<math>\alpha</math></b>	tumour necrosis factor $\alpha$

high salt (DOCA)-treated hypertensive mice more effectively stimulated DCTs to express NCC compared with the same number of CD8Ts isolated from normotensive mice<sup>12</sup>; however, the mechanisms underlying this observation remain elusive. Recent clinical and preclinical studies have indicated that CD8Ts in hypertensive subjects exhibit greater activity, as evidenced by higher production of the cytokine IFN $\gamma$  (interferon  $\gamma$ ) compared with those isolated from normotensive subjects.<sup>13,14</sup> Whether and how this inappropriate activation of CD8Ts contributes to excessive NCC-mediated salt retention are critical questions, that if answered, will not only provide insight into the pathogenesis of salt-sensitive hypertension but also potentially identify new therapeutic targets.

Several reports have shown that the immunosuppressive drug mycophenolate mofetil, which suppresses T cell activity and disrupts cytokine production, attenuates hypertension in different animal models of the disease.<sup>15–18</sup> Although the underlying molecular mechanisms are unclear, IFN- $\gamma$  is evidently involved in the upregulation of NCC and the development of hypertension, because deletion of IFN- $\gamma$  blunts NCC upregulation and blood pressure elevation in an Ang II (angiotensin II) infusion mouse model.<sup>19</sup> These findings led us to hypothesize that IFN- $\gamma$  may be a component of the molecular pathway by which activated CD8Ts induce NCC upregulation in DCTs, thereby contributing to the pathogenesis of excessive salt retention.

In our previous report, we also proposed that an immunologic synapse-like direct contact between CD8Ts and DCTs is necessary for CD8Ts to stimulate NCC expression and salt retention in DCTs.<sup>12</sup> However, in addition to the CD8T-specific antigen-presenting molecule MHC-I

the cellular targets of T cells and their pathogenic signaling pathways are critically needed to improve our understanding of the pathogenesis of hypertension.

A previous study from our group demonstrated a potential mechanism for T cell-mediated sodium handling in the kidney.<sup>12</sup> We found that CD8<sup>+</sup> T cells (CD8Ts) directly interact with renal distal convoluted tubule cells (DCTs), upregulating the expression and activity of the sodium chloride co-transporter (NCC), leading to excessive salt retention.<sup>12</sup> Interestingly, we also observed that CD8Ts isolated from deoxycorticosterone acetate and

(major histocompatibility complex class I), a co-signaling molecule is often required to form synapses between CD8T cells and target cells.<sup>20,21</sup> The critical co-signaling molecules that mediate the synapse interface between DCTs and CD8Ts in the kidney are unknown. PDL1 (programmed death-ligand 1, also known as CD274 or B7-H1) belonging to the B7 immune regulatory family, is a potent co-signaling molecule that reduces activated CD8T-induced cytotoxicity in target cells.<sup>22–25</sup> Renal transplant studies reveal that IFN $\gamma$  produced by activated T cells stimulates the expression of PDL1 in renal tubular epithelial cells. Subsequently PDL1 protects these cells from T cell-induced death by binding to its PD1 (programmed cell death protein 1) receptor on the surface of activated T cells.<sup>26–28</sup> Despite its protective role in renal transplant, we investigated whether this PDL1-PD1 interaction also may fulfill the requisite co-signaling role for the kidney homing of CD8Ts in hypertension.

To our knowledge, no earlier studies determined the role of IFN $\gamma$ -mediated renal expression of PDL1 in the development of salt-sensitive hypertension. Thus, in the current study, we propose a novel mechanism contributing to the pathogenesis of hypertension: as a response to renal infiltration of activated T cells, DCTs in the kidney are primed by excessive IFN $\gamma$  to express a high level of PDL1, which provides co-signaling to its receptor PD1 on CD8Ts, leading to enhanced synapse-like direct interaction between activated CD8Ts and DCTs, pathologically stimulating NCC expression and function, exacerbating salt retention, and elevating blood pressure.

## METHODS

### Data Availability

All relevant data are available from the authors upon request.

### Animals

The animal use protocol was approved by the Institutional Animal Care and Use Committee at University of Arkansas for Medical Sciences. In the DOCA-salt mouse model, 10 to 11 weeks old male C57B6 mice were uninephrectomized and randomly assigned to either the sham group, or the DOCA-salt group that received a DOCA pellet subcutaneously followed by 1% NaCl in the drinking water for 3 weeks. The kidneys and splenic T cells were harvested at the end of day 20. In the CD8<sup>+</sup> T cell adoptive transfer mouse model, which we described previously,<sup>12</sup> uninephrectomized 12 weeks old male C57B6 mice were randomly assigned to the sham group that received a saline injection, or assigned to the adoptive transfer group that was injected with fresh isolated CD8<sup>+</sup> T cells (from spleens of DOCA-salt mice) via a single tail vein injection at the dose of  $1 \times 10^7$  cells/100  $\mu$ L saline/mouse. High-salt drinking water (1%) was available to sham mice and mice receiving CD8<sup>+</sup> T cells as indicated in Figure 7. Blood pressure in this mouse model was measured directly by radio-biotelemetry. The current study only used male mice because biological sex is

a significant factor that affects the regulation of blood pressure and immunity. Specifically, a previous report indicated that adoptive transfer of T cells to T cell deficient mice restores Ang II-induced hypertension in male recipient mice but not in female recipients,<sup>29</sup> suggesting the role of T cells in mediating hypertension is greater in males than females.

### T-Cell Isolation and Analysis

Mouse spleens or kidneys were dissociated using a GentleMACs tissue dissociator and C-tubes followed the manufacturer's protocol. Dynabeads T-cell isolation kits were used to isolate T cells from splenocytes. The kidney T-cell isolation followed a previously reported method.<sup>10</sup> Briefly, kidney homogenate was filtered using a 70  $\mu$ m sterile filter and leukocytes enriched by centrifugation in 36% and 72% percoll followed by CD45 selection using MagniSort beads. Subtypes of collected T cells were confirmed and analyzed using flow cytometry.

### Ex-Vivo Stimulation

Intracellular cytokine assays to evaluate cytokine production in CD8T cells used freshly isolated CD8T cells, which were either untreated or stimulated with cell activation cocktail (BioLegend No. 423304, phorbol myristate acetate/Ionomycin) in the presence of brefeldin A for 3 to 4 hours at 37°C.<sup>30–32</sup>

### Renal Tubule Specific Knockdown Using Nanoparticles

Ambion in vivo siRNAs against mouse PDL1 or scramble sequence (as negative control) were purchased from Thermo Fisher and encapsulated into renal tubule-specific nanoparticles at the dose of 5 ng siRNA per 1 mg nanoparticles. Nanoparticles loaded with siPDL1 (or scrambled control) were formulated similarly to a previous description using an emulsion-nanoprecipitation method.<sup>33–35</sup> Briefly, 100 mg diblock poly (lactic-co-glycolic acid)-carboxy-terminated polyethylene glycol was dissolved in 2 mL acetonitrile, into which 100 ng of siPDL1 (or scrambled control) in RNase-free water were added dropwise and bath sonicated for 2 minutes. The resulting emulsification was added dropwise to 100 mL water with 100  $\mu$ L Pluronic F-68 as a surfactant. After stirring for 2 hours, the particle suspension was recovered by centrifugation and immediately washed and centrifuged again. The particles were suspended in 2% sucrose solution and lyophilized to dryness. The hydrodynamic diameter of nanoparticles was evaluated via dynamic light scattering and determined to be  $402.5 \pm 7.4$  nm with a polydispersity index of 0.34 for siPDL1-nanoparticles and  $399.3 \pm 16.8$  nm with a polydispersity index of 0.28 for scrambled control siRNA nanoparticles. Immediately before use, nanoparticles were suspended at 16 mg/mL in saline and injected into mice at the dose of 1.6 mg nanoparticles/mouse via tail vein injection. Our preliminary tests indicate that the treatment with siPDL1 containing nanoparticles does not alter baseline blood pressure or renal T-cell infiltration as shown in Figure S1.

### Cell Culture Treatment and Harvest

The mouse DCTs (mDCT15) cells (referred to in the text simply as mDCTs) and the CD8<sup>+</sup>T cells (TK1 cells) were introduced in our previous report.<sup>12</sup> In preactivation studies, CD8Ts were preincubated with cell activation cocktail (phorbol myristate acetate/Ionomycin, Biolegend) for 4 hours before being washed and co-cultured with mDCTs. All cells were maintained at 37°C and 5% CO<sub>2</sub>, and no contaminations were found in cultured cells during our experiments. All treatments of mDCTs

were overnight ( $\approx 12$  hours). Co-culture and harvest methods also followed the same protocol as we previously reported.<sup>12</sup> The quantification of area covered by T cells co-cultured with mDCT cells was estimated by a semi-automated segmentation script using iVision 4.5.4 (BioVision Technologies, Exton, PA). The segmentation was based on intensity threshold, mean intensity, area, and circularity to exclude artifacts. Segmented images were inspected for accuracy and fine adjustments to parameters were made as necessary. Representative images of CD8T-DCT adhesion after co-culture were selected to represent the average of quantification data. Cell co-culture (adhesion) images were acquired using an inverted routine microscopy and Cannon EOS camera with eye-piece adaptor. The scale bars of images were estimated by calculating pixels in an 1 mm length image acquired using the same system.

### Transfection of siRNAs

All siRNAs were purchased from ABI. Transfection of siRNAs was accomplished using lipofectamine RNAiMax following the manufacturer's protocol. siRNA-mediated knockdown effects in mDCTs are shown in Figure S2.

### Flow Cytometry

For surface staining, cells were stained with antibodies for 30 to 45 minutes in cell isolation buffer in dark tubes before analysis. For the NCC functional study using CoroNa Green (cell permeable, Molecular Probes), details are described in our previous publication.<sup>12</sup> Briefly, mDCTs were treated with ouabain, bumetanide and amiloride for 30 minutes at room temperature followed by loading of CoroNa Green at a concentration of 10  $\mu\text{mol/L}$  for 1 hour at room temperature. Cells were washed and re-suspended in PBS containing the same blockers mentioned above for 45 minutes and analyzed in a BD Accuri C6 flow cytometer immediately. Intracellular cytokine staining of CD8Ts was performed using BD Cytotfix/Cytoperm Plus (Cat. 555028) according to manufacturer protocols. In brief, stimulated cells were incubated in the presence of the BD GolgiPlug Protein Transport Inhibitor (with Brefeldin A, 1 microliter/mL cell culture). After 3 hours, cells were harvested and fixed in BD fixation/permeabilization solution, then washed twice in perm/wash buffer and resuspended for intracellular cytokine staining. After 30 minutes of incubation, cells were washed and resuspended in cell isolation buffer for analysis. Flow cytometry data were analyzed using FlowJo software (version v10.7.1). For all flow cytometry studies, cells lacking incubation with fluorescent antibodies (or corona-green) were used as negative controls to determine autofluorescence and gating. Single cells were selected using the FSC-H/FSC-A gating method for all studies, and live cells were selected using 7-AAD staining, as shown in Figure S3. Representative flow cytometry images were selected to exemplify quantification data.

### Immunostaining

Four percent PFA fixed kidneys were processed as 8  $\mu\text{m}$  thick paraffin sections in the DNA Damage and Toxicology Core at UAMS. Following antigen retrieval using antigen unmasking solution (Vector), sections were washed and blocked in 5% nonfat milk containing 4% goat serum at room temperature for 1 hour before overnight incubation with primary antibodies. After secondary antibody staining, sections were sealed with ProLong Gold mounting media (Molecular Probes). Images were captured using Keyence XZ-800 microscope. Cell counting

was conducted using ImageJ software. Representative images were selected to exemplify the average of quantification data.

### Mean Intensity Quantification of NCC

All kidney sections in the experimental group and the corresponding controls were stained, imaged, and analyzed under the same conditions. The whole kidney section image was constructed by stitching thousands of images taken on Keyence XZ-800 with a 10 $\times$  PlanApo NA 0.45 at low power and exposure time to avoid saturation. Intensity cutoffs for tissue and NCC-positive pixels were determined by a blinded investigator and applied to the experimental and control groups uniformly. The mean intensity for each kidney was calculated by (total NCC-positive pixel intensity—mean background tissue intensity $\times$ total NCC-positive pixel area)/total tissue area. Image segmentation and analysis were performed using iVision 4.5 (BioVision Technologies).

### Western Blot

Detailed methods and materials have been published.<sup>12</sup> Briefly, bis-tris gel (from Genscript) was used for electrophoresis, and gels were transferred to PVDF (polyvinylidene difluoride) membranes on ice. All membranes were blocked in 5% nonfat milk for 1 hour before incubation with primary antibodies overnight. After washing and incubation with HRP (horseradish peroxidase) conjugated secondary antibodies, Western blot images were obtained using a ChemiDoc XRS+ system and analyzed using ImageLab software (BioRad). Representative images were selected that exemplified the average of quantification data.

### Statistical Analysis

Data are presented as means $\pm$ SEM. All statistical analysis were preformed using GraphPad 7.05. Data were tested for normality using D'Agostino & Pearson test (for sample  $n \geq 8$ ) or Shapiro-Wilk test (for sample  $n < 8$ ). The threshold for normality tests was set to 0.05, and  $P > 0.05$  were considered normally distributed. Unpaired  $t$  tests were used to compare parametric data between 2 independent groups, whereas Mann-Whitney tests were used to compare nonparametric data between 2 independent groups. Two-sided  $P$  values are indicated in the figures. For multiple comparisons, statistical analysis was performed by ANOVA followed by Tukey or Dunnett post hoc tests.  $P$  values  $< 0.05$  were considered to be significant, nonsignificant were labeled as ns in figures. No sample size estimate was performed, but sample sizes were selected based on previous experiments. All assays were repeated in independent experiments, and displayed figures are representative.

### Major Resources

Please see the Major Resources Table in the Supplemental Materials.

## RESULTS

### CD8Ts in Hypertensive Mice Are Excessively Activated and Promote Stimulation of DCTs

We reported earlier that CD8Ts increased expression of NCC in mDCTs.<sup>12</sup> Here, we found that compared with Sham CD8Ts (CD8Ts isolated from sham normotensive

mice), DOCA CD8Ts (CD8Ts isolated from DOCA hypertensive mice) stimulated higher proportion of mDCTs to exhibit elevated NCC-mediated sodium retention (Figure 1A). NCC-mediated sodium uptake was assessed by the fluorescent intracellular sodium indicator Corona Green as we previously reported.<sup>12</sup> A comparison between CD8Ts isolated from DOCA hypertensive mice and sham normotensive mice indicated that DOCA CD8Ts exhibited higher activity than sham CD8Ts, as evidenced by higher expression of the cytokines IFN $\gamma$  and TNF (tumor necrosis factor  $\alpha$ ; Figure 1B). This new finding was supported by cell surface staining with PD1 (Figure S4), a check-point molecule that is upregulated in activated CD8Ts.<sup>36,37</sup> Higher proportions of PD1-expressing CD8Ts were found in DOCA mice compared with sham mice (Figure S4). To determine whether activation of CD8Ts augments their interaction with DCTs, we preactivated CD8Ts (act CD8Ts) by stimulating them with phorbol myristate acetate and ionomycin for 4 hours before co-culture with mDCTs. After overnight co-culture and washing off nonadherent CD8Ts using PBS, we observed significantly higher numbers of act-CD8Ts adhering to mDCTs compared with naive CD8Ts (nonact CD8Ts, Figure 1C). Whereas CD8Ts are known to stimulate NCC expression in mDCTs,<sup>12</sup> we found that CD8T-induced NCC upregulation in mDCTs was remarkably augmented by preactivating the CD8Ts (Figure 1D). These findings indicate that the higher activity of CD8Ts in salt-sensitive hypertensive subjects may promote inappropriate upregulation of NCC in their kidneys.

### IFN $\gamma$ Mediates the Stimulatory Effect of Preactivated CD8Ts on Progressive Elevation of Blood Pressure

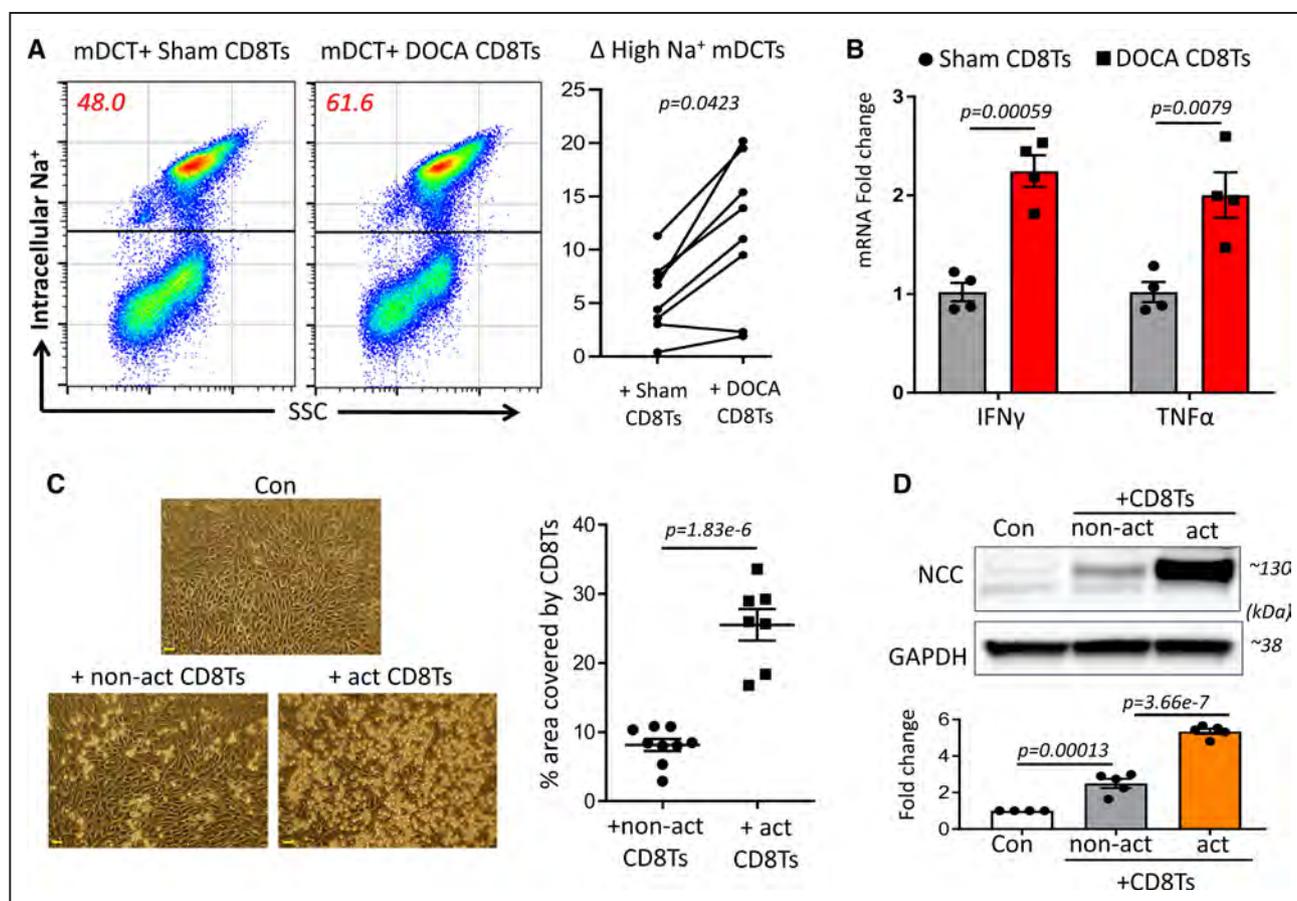
One major function of activated T cells is to produce cytokines and chemokines, particularly the cytokines IFN $\gamma$  and TNF $\alpha$ , which are reported as important contributing factors to the development of hypertension and subsequent kidney injury.<sup>11,13,14,38,39</sup> To explore a potential difference in cytokine-producing capacity between sham-CD8Ts and DOCA-CD8Ts, we stimulated these cells ex-vivo and stained for intracellular cytokines using specific antibodies.<sup>30–32</sup> Results in Figure 2A show that stimulated DOCA CD8Ts produce significantly more IFN $\gamma$  compared with sham CD8Ts, whereas no difference was found in the production of TNF $\alpha$  between 2 groups of cells (Figure 2A). These results agree with recent clinical and preclinical observations of higher production of IFN $\gamma$  but not TNF $\alpha$  by CD8Ts obtained from hypertensive patients compared with normotensive individuals,<sup>13,14</sup> and support the finding that IFN $\gamma$  contributes to upregulation of renal NCC and elevated blood pressure in Ang II-treated hypertensive mice.<sup>19</sup> These results led us to speculate a plausible role for IFN $\gamma$  in augmenting CD8T-DCT interaction and NCC upregulation, thereby exacerbating hypertension.

To further explore this hypothesis in in vitro studies, we introduced neutralizing antibody against IFN $\gamma$  at 15  $\mu$ g/mL into co-cultures of both types of cells. As expected, preactivation of CD8Ts-induced additional expression of NCC in DCTs, which was prevented by neutralizing IFN $\gamma$  in the co-culture environment (Figure 2B). It is worth noting that neutralizing IFN $\gamma$  only blocked the additional effect of NCC upregulation by preactivated CD8Ts, without further normalizing the expression of NCC in DCTs to control level (Figure 2B). This result is consistent with our previous report that neutralizing IFN $\gamma$  does not affect NCC expression in DCTs treated with nonact CD8Ts.<sup>12</sup> As a corollary, we found that adding recombinant mIFN $\gamma$  (mouse IFN $\gamma$ ) into the co-culture augmented adherence of DCTs and nonact CD8Ts (Figure S5A) and profoundly enhanced the effect of nonact CD8T-induced NCC upregulation in DCTs (Figure S5B). However, IFN $\gamma$  per se did not affect NCC expression in DCTs without co-culture (Figure S5B).

Results consistent with the in vitro findings were obtained from in vivo studies. We found that IFN $\gamma$ -KO (knockout of IFN $\gamma$ ) lowers blood pressure in DOCA-salt treated mice (Figure 2C). However, we noticed that this effect only occurred in the later phase of progressive elevation of blood pressure, but not in the early phase of the initial blood pressure rise (Figure 2C). This in vivo result resembles those in previous studies using DOCA-salt treated thymus-deficient nude mice or DOCA-salt treated mice administered the immunosuppressant mycophenolate mofetil,<sup>18,40–42</sup> implying a possible contributing role of IFN $\gamma$  to adaptive immunity-induced suppression of pressure-natriuresis, which leads to progression of hypertension. This concept was supported by our new finding that T cell homing induced by DOCA-salt treatment is attenuated in the kidneys of IFN $\gamma$ -KO mice compared with the kidneys of DOCA-salt treated wild-type mice (Figure 2D). Taken together, these data suggest that IFN $\gamma$  is a critical factor in mediating activated CD8T-induced interaction between CD8Ts and DCTs, which contributes to excessive NCC upregulation and exacerbates hypertension.

### IFN $\gamma$ Primes DCTs to Express MHC-I and Co-Signaling Molecule PDL1 Via IFN $\gamma$ Rs

A large amount of IFN $\gamma$  is released from activated immune cells and plays an important role in modulating immune responses.<sup>43–45</sup> In the kidney, one important function of IFN $\gamma$  is to prime renal tubule cells to express the checkpoint molecule PDL1, which binds to its receptor PD1 on CD8Ts to protect renal tubule cells from CD8T-induced cytotoxicity.<sup>26–28</sup> This mechanism is common in kidney transplant recipients, a population suffering from a high prevalence of salt-sensitive hypertension.<sup>46–48</sup> Therefore, we speculated that this protective mechanism of IFN $\gamma$  may enhance



**Figure 1. Activation of CD8Ts in deoxycorticosterone acetate (DOCA) mice and impact of activated CD8Ts on distal convoluted tubules (DCTs).**

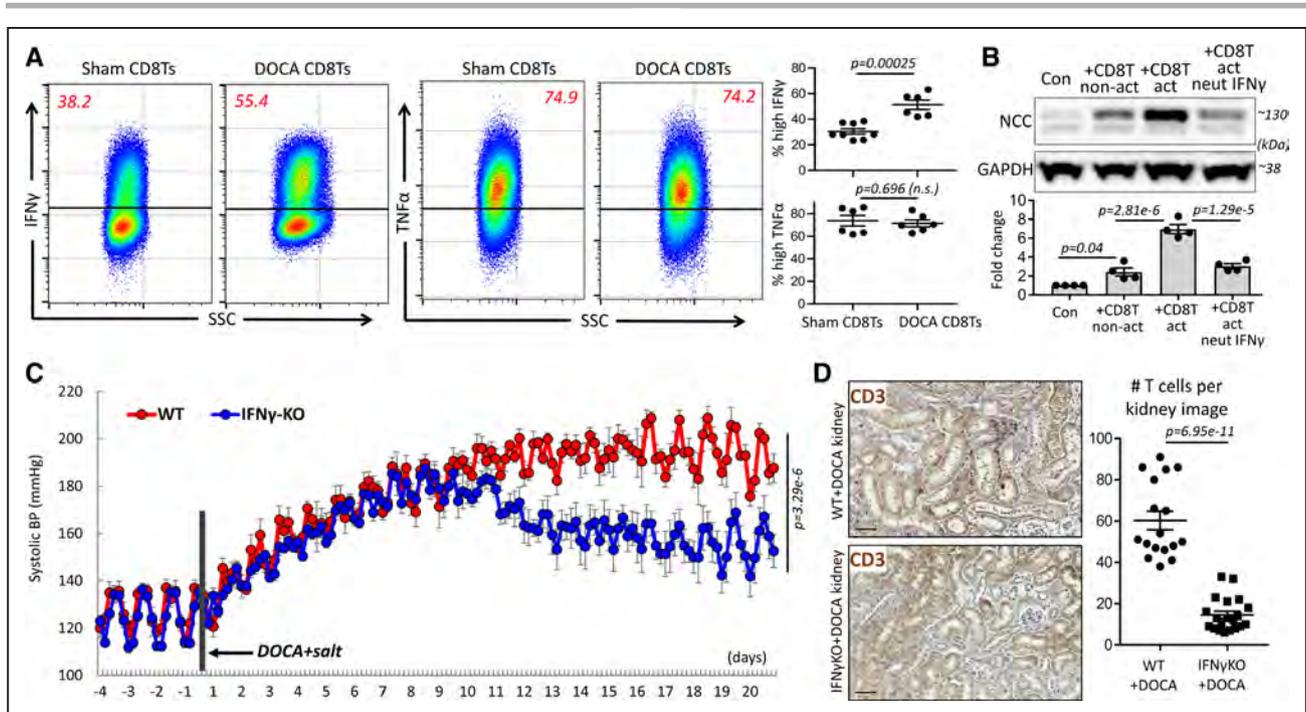
**A**, Flow cytometry study using the intracellular sodium indicator Corona Green to detect sodium chloride co-transporter (NCC)-mediated sodium uptake in mouse DCTs (mDCTs). Upper population represent DCTs with higher NCC-mediated sodium uptake (number inside the square indicate proportion of cells); lower population represent cells with lower sodium uptake. All studies were performed in the presence of ouabain, bumetanide and amiloride. Data are representative of 8 independent tests. Quantifications determined the CD8T-induced  $\Delta$  proportion of high-sodium retaining mDCTs compared with baseline (mDCTs only, without co-culture). **B**, mRNA expression of activation markers IFN $\gamma$  (interferon  $\gamma$ ) and TNF $\alpha$  (tumour necrosis factor  $\alpha$ ) in CD8Ts isolated from spleens of DOCA hypertensive mice and sham normotensive mice.  $n=4$  mice in each group. **C**, Effects of preactivating CD8Ts on their adhesion with DCTs. After overnight co-culture, cells were washed with PBS  $\times 2$  times. Scale=30  $\mu\text{m}$ . Data are representative of  $n=7-9$  images in each group. **D**, Effects of preactivating CD8Ts on stimulating NCC expression in DCTs. Quantitative Western blot data were normalized using GAPDH as a loading control.  $n=4-5$  samples in each group. Data are means $\pm$ SEM. Statistical significance was assessed by  $t$  test for **A**, **B**, **C**, and ANOVA for **D**.

direct contact between DCTs and CD8Ts, suppressing cytotoxic effects, but at the cost of boosting CD8T-induced upregulation of NCC in DCTs. To test this hypothesis, we administered mIFN $\gamma$  to mDCTs with or without knockdown of IFN $\gamma$  receptor subunits 1 and 2. Adding IFN $\gamma$  to mDCTs significantly increased their surface expression of the CD8-specific antigen-presenting molecule MHC-I (Figure 3A, left panel), but not the CD4-specific antigen presenting molecule MHC-II (Figure 3A, right panel), which may result in mDCTs attracting CD8Ts instead of CD4Ts. As expected, knockdown of IFN $\gamma$  receptor attenuated the IFN $\gamma$ -induced increase of MHC-I in mDCTs, but this effect in MHC-II was not detectable (Figure 3A). Moreover, IFN $\gamma$  induced high expression of PDL1 on DCTs at both the mRNA and protein level (Figure 3B and 3C), which may provide the co-signaling

molecule for enhanced interaction between CD8Ts and DCTs. Knockdown of the IFN $\gamma$  receptor subunits attenuated IFN $\gamma$  treatment-induced upregulation of MHC-I and PDL1 in DCTs (Figure 3A and 3C), further confirming that these effects are mediated by the IFN $\gamma$  signaling pathway.

### Increased PDL1 in DCTs Is Associated With Enhanced CD8T-DCT Interaction and NCC Upregulation

Similar to findings using IFN $\gamma$ -treated mDCTs (Figure 3), we also observed increased expression of PDL1 in mDCTs treated with preactivated CD8Ts as determined using flow cytometry (Figure 4A). Next, we introduced the intracellular sodium indicator Corona Green into mDCTs to examine NCC-mediated sodium



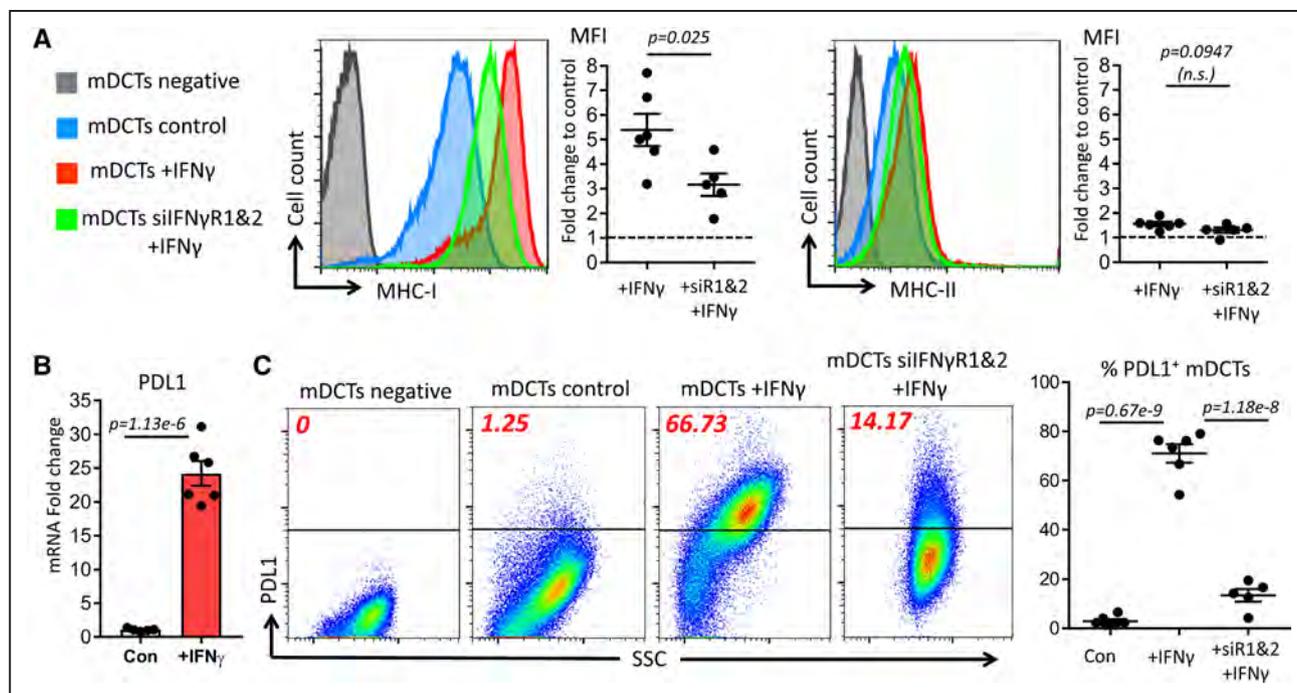
**Figure 2. Effects of IFN (interferon)  $\gamma$  deficiency on mouse distal convoluted tubule (mDCT) cell phenotype, and blood pressure and renal CD8T cell abundance in hypertensive mice.**

**A**, Flow cytometry was used to determine cytokine production by CD8Ts using intracellular staining of IFN $\gamma$  (interferon  $\gamma$ ) and TNF $\alpha$  (tumour necrosis factor  $\alpha$ ) in PMA (phorbol myristate acetate) and ionomycin-stimulated CD8Ts. Numbers in upper square indicate the percentage of isolated CD8Ts with high production of IFN $\gamma$  or TNF $\alpha$ .  $n=6-8$  mice each group. **B**, Effects of neutralizing IFN $\gamma$  on sodium chloride co-transporter (NCC) expression in DCTs treated with preact CD8Ts. Quantitative Western blot data were normalized using GAPDH as loading control.  $n=4$  samples in each group. **C**, Averaged radio-biotelemetry recording of systolic blood pressure in 4 wild-type mice (WT, red line) and 7 IFN $\gamma$ -knockout mice (IFN $\gamma$ -KO, blue line). All mice were given deoxycorticosterone acetate (DOCA) salt treatment as indicated. Blood pressure was recorded for 10 seconds every 15 min. **D**, Immunostaining of T-cell marker CD3 (brown) in kidneys from DOCA-salt-treated WT and IFN $\gamma$  KO mice. Scale-50  $\mu$ m. Data are representative of  $n>15$  images in each group. Data are means $\pm$ SEM. Statistical significance was assessed by *t* test for **A**, **D**, 1-way ANOVA for **B**, and 2-way ANOVA for **C**.

retention in mDCTs with and without administration of preactivated CD8Ts. As we expected, mDCTs treated with nonact CD8Ts demonstrated increased sodium retention (Figure 4B) consistent with our previous study.<sup>12</sup> However, treatment with preact CD8Ts resulted in a much greater proportion of mDCTs exhibiting high NCC-mediated sodium retention (Figure 4B). Importantly, most of these preact CD8T-induced NCC-hyperactive mDCTs expressed high levels of PDL1 on their surface (Figure 4B), leading us to consider the possibility that NCC hyperactivity in act CD8T-treated DCTs may be related to the surface expression of PDL1 on those DCTs. This association was further tested by adding siRNA to knockdown PDL1 in the mDCTs. We found that knockdown of PDL1 in mDCTs diminished preact CD8T-induced enhanced interaction between the 2 cell types (Figure 4C), and moreover, prevented preactivation of CD8T-induced additional upregulation of NCC and excessive sodium reabsorption in co-cultured mDCTs (Figure 4D and 4E). Taken together, these *in vitro* studies revealed a critical role for PDL1 in mediating the enhanced interaction between preact CD8Ts and DCTs. Next, this mechanism was further evaluated *in vivo* to test its role in hypertension.

### Renal Tubule Specific Knockdown of PDL1 Ameliorates T-Cell Accumulation in the Kidneys of DOCA Mice

Numerous earlier studies demonstrated chronic inflammation and cytokine accumulation in the kidneys of hypertensive subjects.<sup>38,39,49,50</sup> Similarly, we observed higher expression of IFN $\gamma$  in the kidneys of DOCA mice compared with sham mice (Figure 5A). Moreover, staining the kidney sections with PDL1 specific antibodies revealed that PDL1 is expressed around tubules in the DOCA kidney (Figure 5B) where CD8Ts attach, as demonstrated in our previous report.<sup>12</sup> An obvious next step was to knockout or block PDL1, which may serve as a potential co-signaling ligand for the CD8Ts. However, knockout of PDL1 or administration of blocking antibodies systemically may cause systemic complications, which could further affect blood pressure, as evidenced by the fact that both hypotension and hypertension are observed as side effects in cancer patients treated with PDL1 inhibiting antibodies.<sup>51-56</sup> Therefore, we employed renal tubule specific nanoparticles<sup>33-35</sup> to introduce siRNAs designed to specifically knockdown PDL1 in renal tubules. Kidney-targeted polymeric mesoscale nanoparticles containing siPDL1 were injected intravenously into mice via tail



**Figure 3. Effects of IFN $\gamma$  (interferon- $\gamma$ )–IFN $\gamma$  receptor signaling on priming distal convoluted tubules (DCTs).**

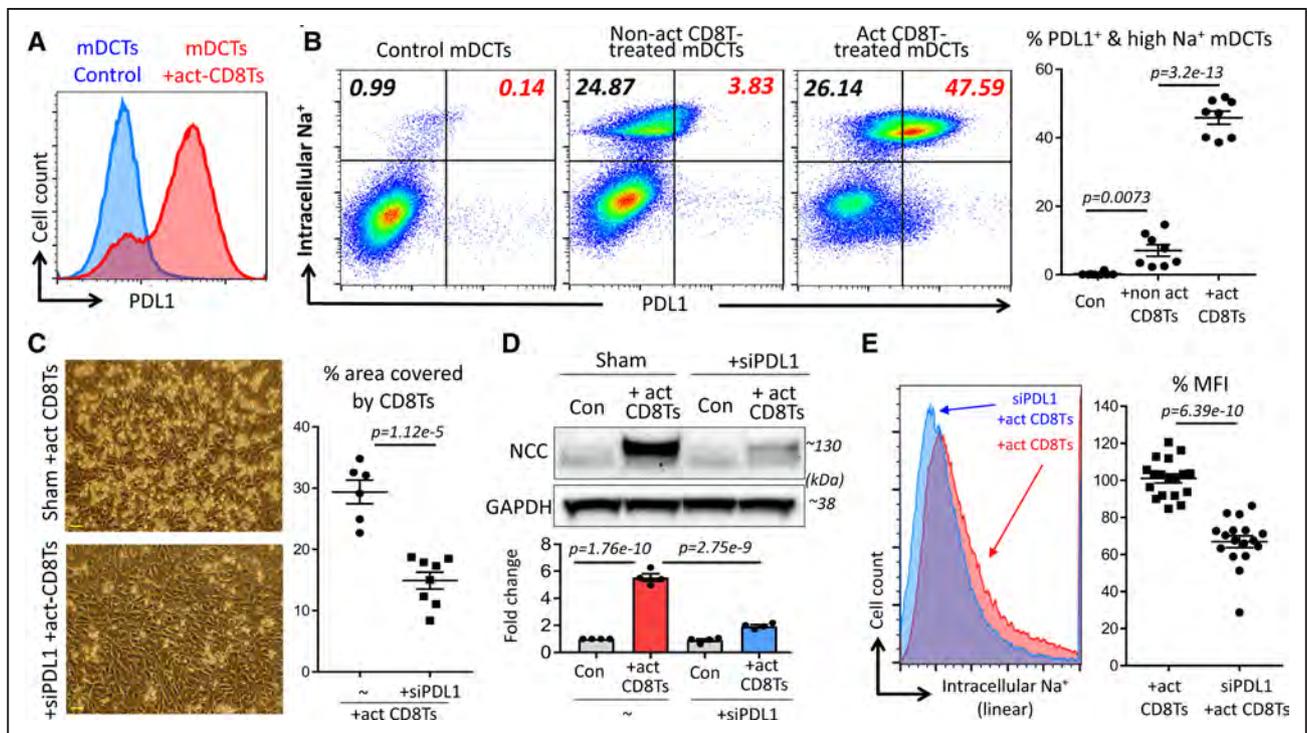
**A**, Flow cytometry study to examine the effects of IFN $\gamma$  signaling on regulating the surface expression of major histocompatibility complex (MHCs) on mouse DCT (mDCT) cells. Groups of cells are indicated by different colors as shown in **A**.  $n=5-6$  each group. Fold changes of geometric mean fluorescence intensity (MFI) in treated groups are relative to mDCT control cells in each independent test. **B**, mRNA expression of PDL1 (programmed death-ligand 1) in untreated mDCTs or mDCTs treated with either IFN $\gamma$  only, or IFN $\gamma$  + siIFN $\gamma$ R1&2.  $n=4$  samples each group. **C**, Flow cytometry study to examine the effects of IFN $\gamma$  signaling on regulating the surface expression of PDL1 on mDCTs. Numbers in upper square indicate the proportion of mDCTs with high expression of PDL1.  $n=5-6$  samples each group. Data are means $\pm$ SEM. Statistical significance was assessed by *t* test for **A**, **B**, and ANOVA for **C**.

vein 1 day after the start of DOCA-salt treatment. At the end of the DOCA-salt treatment period, we detected higher levels of PDL1 in the kidneys of DOCA mice, as expected (Figure 5C). In addition, we found that renal tubule-specific nanoparticles+siPDL1 suppressed renal PDL1 expression in DOCA mice through the end of DOCA-salt treatment (18 days after a single injection) without affecting PDL1 expression in other organs (Figure 5C and Figure S6). In the absence of excessive expression of PDL1 in the kidneys, DOCA-salt treatment failed to cause T-cell accumulation in the renal cortex (Figure 5D). Quantification of renal accumulation of CD8Ts in mice was performed by counting CD8Ts isolated from kidneys using percoll density gradient centrifugation, CD45<sup>+</sup> bead selection and flow cytometry analysis (example shown in Figure 5E left panel). As expected, DOCA-salt treatment led to enhanced infiltration of CD8Ts into the kidneys and this effect was absent after knockdown of PDL1 using specific siRNA (Figure 5E, right panel).

### Knockdown of PDL1 in Renal Tubules Normalizes NCC Expression and Lowers Blood Pressure in DOCA Mice

We reported earlier that increased infiltration of CD8Ts into the kidney contributes by an unrecognized

mechanism to NCC upregulation and excessive salt retention in DOCA mice.<sup>12</sup> Here, we speculate that since renal-specific knockdown of PDL1 prevented CD8T-accumulation in the kidneys, this intervention also would attenuate upregulation of NCC expression in DCTs and consequently attenuate the development of hypertension in these animals. To assess NCC expression in the kidneys of DOCA mice with or without knockdown of PDL1, we immunostained the kidney sections using antibodies against NCC and performed semi-automated quantification analysis of total fluorescence intensities of NCC positive pixels in stitched images of whole kidney sections (Figure 6A). Compared with the kidneys of DOCA mice not receiving nanoparticle treatment, those from mice treated with DOCA plus nanoparticles to knockdown PDL1 expressed significantly less NCC in their kidneys (Figure 6B). Consistently, DOCA treatment-induced hypertension in mice was ameliorated by renal-specific knockdown of PDL1 using nanoparticles (Figure 6C), where nanoparticles containing scrambled siRNA did not affect DOCA treatment-induced elevation of blood pressure in mice (Figure 6C). However, it is worth noting that although knockdown of PDL1 in the kidney almost completely abolished the effect of DOCA-induced accumulation of CD8Ts in the kidney, it did not restore blood pressure of DOCA-treated mice



**Figure 4. Impact of PDL1 (programmed death-ligand 1) on distal convoluted tubules (DCTs) co-cultured with act-CD8Ts.**

**A**, Expression of PDL1 in mouse DCTs (mDCTs) treated with or without preactivated CD8Ts. Data are representative of 3 independent experiments with  $n > 6$  in each group. **B**, Flow cytometry study using intracellular sodium indicator Corona Green to determine sodium chloride co-transporter (NCC)-mediated sodium retention in PDL1 positive (top right) and negative (top left) mDCTs.  $n = 8$  in each group. **C–E**, Effects of knocking down PDL1 in mDCTs on their adhesion with preactivated CD8Ts (**C**, Scale = 30  $\mu\text{m}$ , data are representative of  $n > 10$  images in each group), NCC expression (**D**,  $n = 4$  samples each group) and sodium retention determined by geometric mean fluorescence intensity (MFI) of intracellular sodium indicator Corona Green (**E**, color defined in the figure,  $n > 15$  samples in each group). Data are means  $\pm$  SEM. Statistical significance was assessed by ANOVA for **B**, **D**, and t test for **C** and **E**.

to the baseline level (Figure 6C), indicating possible involvement of other mechanisms in DOCA treatment-induced hypertension.

### Effects of Renal Tubule-Specific Knockdown of PDL1 in Mice After Adoptive Transfer of CD8Ts From DOCA Mice

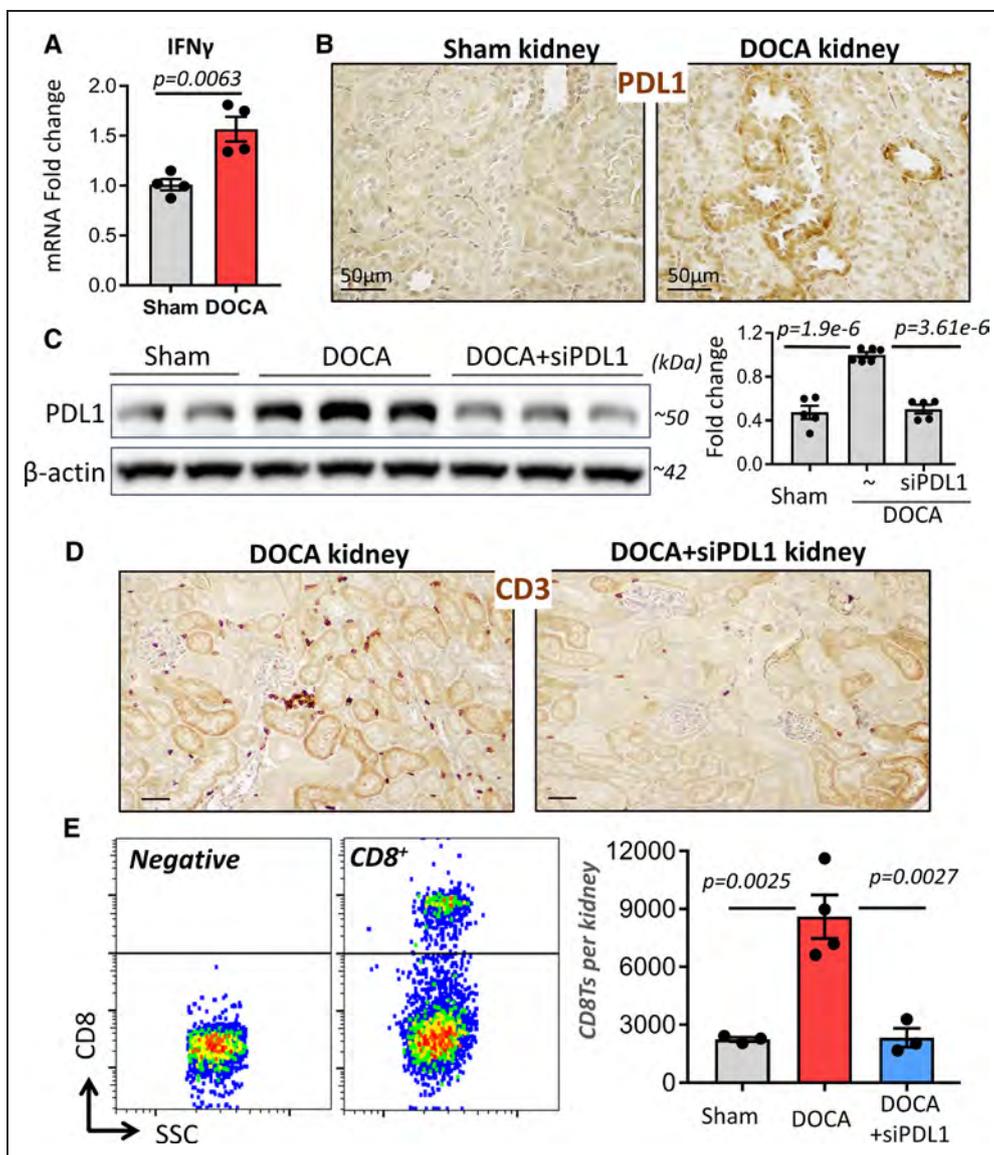
The effects of renal tubule-specific knockdown of PDL1 were further tested in a more direct model of CD8T-induced hypertension, in which mice received adoptive transfer of CD8Ts ( $1 \times 10^7$ /mouse) isolated from DOCA treated hypertensive mice, as demonstrated by us earlier.<sup>12</sup> Analysis of kidneys from nanoparticle treated CD8T-recipient mice revealed similar results as obtained from the kidneys of nanoparticle treated DOCA mice, namely that kidney-specific knockdown of PDL1 diminished accumulation of CD8Ts around renal tubules (Figure 7A) and attenuated the renal expression of NCC mediated by adoptive transfer of DOCA-CD8Ts (Figure 7B). Mice that received adoptive transfer of DOCA-CD8Ts demonstrated salt-sensitive hypertension (Figure 7C), consistent with our previous report.<sup>12</sup> Here, we further found that this effect was abolished by renal tubule-specific knockdown of PDL1 (Figure 7C). Interestingly, unlike

the end point blood pressure in DOCA mice, the blood pressure in adoptive transfer mice with kidney specific knockdown of PDL1 returned to a similar level as their baseline (Figure 7C).

Taken together, our results have demonstrated an important mechanism for the accumulation of CD8Ts in the kidneys of salt-sensitive hypertensive animals, namely that higher production of IFN $\gamma$  primes DCTs to express MHC-I and PDL1, which enhances the interaction between CD8Ts and DCTs, leading to accentuated upregulation of NCC and consequently, aggravating hypertension.

## DISCUSSION

In recent years, a growing body of evidence has revealed a pivotal role of immune cells, particularly T cells, in the pathogenesis of hypertension.<sup>8,10,11,16,57,58</sup> However, the molecular and cellular mechanisms for T-cell contributions to blood pressure elevation remained unrecognized. Earlier we reported one potential molecular event linking T cells to excessive salt retention in the kidney, namely that CD8Ts directly stimulate DCTs to increase NCC expression and salt reabsorption.<sup>12</sup> In the present study, we further elucidated a mechanism that drives the



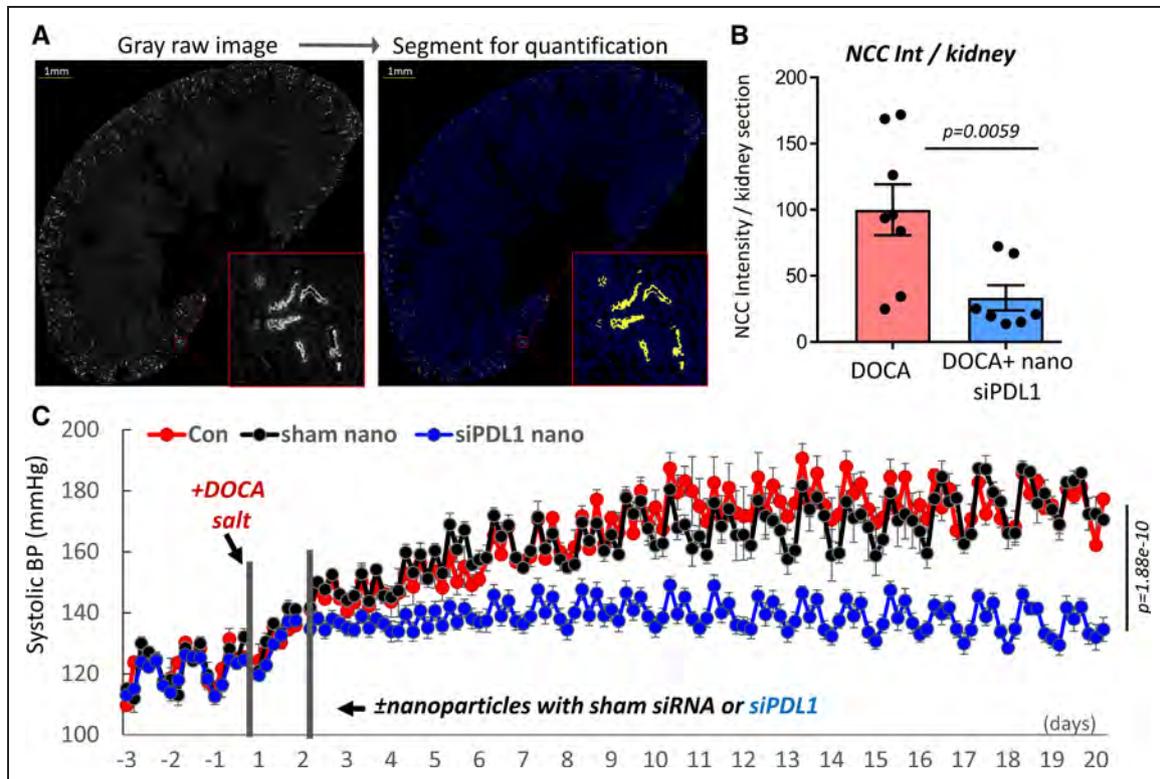
**Figure 5. Effects of renal tubule specific knockdown of PDL1 (programmed death-ligand 1) in the kidneys of deoxycorticosterone acetate (DOCA) mice.**

**A** and **B**, The expression of IFN $\gamma$  (interferon  $\gamma$ ; **A**, mRNA level,  $n=4$  mice each group) and PDL1 (**B**, immunostaining, data are representative of  $n>5$  images in each group) in the kidneys of mice with or without DOCA treatment. **C**, Efficacy of siPDL1 delivered by kidney-specific polymeric mesoscale nanoparticles in the kidneys of DOCA mice.  $n=5-6$  mice in each group. **D** and **E**, Renal infiltration of T cells and CD8Ts in DOCA mice with or without targeted siPDL1 as demonstrated by immunostaining of CD3 (**D**, data are representative of  $n>10$  images in each group) and flow cytometry of CD8 $^+$  T cells (**E**,  $n=3-4$  mice in each group). **Left** indicates gating strategy for CD8 positive cells, **right** shows quantitative numbers of CD8 $^+$  T cells from each kidney. Data are means $\pm$ SEM. Statistical significance was assessed by *t* test for **A**, and ANOVA for **C**, **E**.

interaction between DCTs and activated CD8Ts, leading to increased CD8T homing to kidneys and contributing to the pathogenesis of hypertension.

Recent clinical and preclinical studies have found inappropriate activation of CD8Ts in hypertensive humans and experimental animals,<sup>13,14</sup> and multiple factors may contribute to this inappropriate activation of CD8Ts.<sup>10,59-62</sup> For example, it has been reported that a unique T-cell receptor (TCR) subtype of CD8Ts are activated by hypertension-specific neoantigens, including isolevuglandin-modified proteins and heat shock proteins.<sup>10,62</sup> Another proposed mechanism is altered

gut microbiota, which may play a critical role in altering the activity status of immune cells, as evidenced by high-salt intake altering the microbiome profile, and the imbalance of gut microbiomes stimulating activation of CD8Ts.<sup>61,63-65</sup> While ongoing studies are still investigating the causes of the activation of CD8Ts in hypertension, whether and how this activation of CD8Ts contributes to the pathological CD8T-DCT interaction and participates in the pathogenesis of hypertension are critical questions that need to be addressed. In the current study, we first determined that activation of CD8Ts in salt-sensitive hypertensive animals exhibit a higher



**Figure 6. Effect of renal tubule-specific knockdown of PDL1 (programmed death-ligand 1) on sodium chloride co-transporter (NCC) expression and blood pressure in deoxycorticosterone acetate (DOCA) mice.**

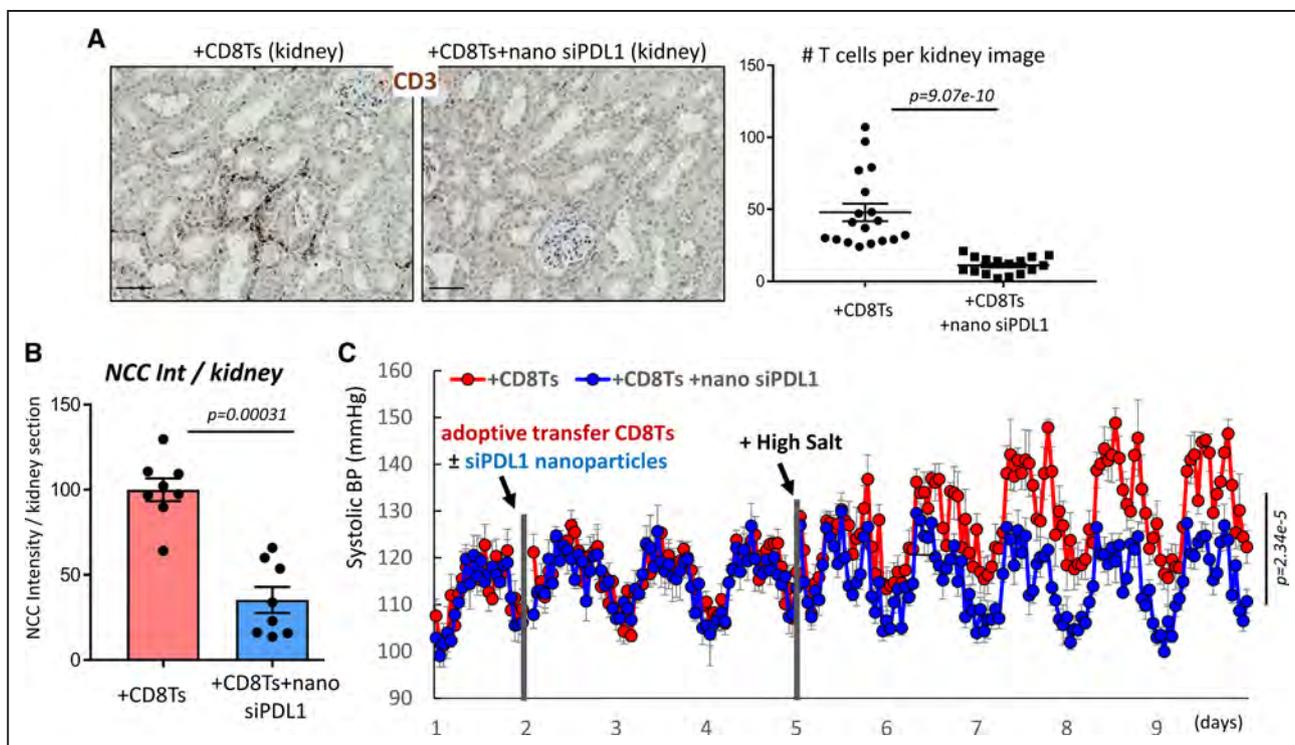
**A**, Raw fluorescent image of the whole kidney section (left) and intensity-based segmentation (right) of the kidney tissue (blue) and NCC-positive pixels (yellow). Inset: zoomed-in image of the red square. No pixels were saturated, and brightness was adjusted for display purposes only. **B**, Intensity quantification of NCC positive segments in stitched images of whole kidney sections from DOCA mice treated with or without siPDL1. *n* = 7–8 mice in each group. **C**, Radio-biotelemetry recording of systolic blood pressure in DOCA mice treated with or without nanoparticles containing sham siRNA or siPDL1. Blood pressure was recorded for 10 seconds every 15 min. *n* = 6 mice for DOCA only, *n* = 6 mice for DOCA+sham nano, *n* = 10 mice for DOCA+siPDL1 nano. Data are means ± SEM. Statistical significance was assessed by Mann-Whitney test for **B** and 2-way ANOVA for **C**.

ability to interact with DCTs and stimulate NCC upregulation. Moreover, during continued exploration of this new mechanism, we identified key molecules contributing to the direct interaction between activated CD8Ts and DCTs. Eliminating these molecules attenuated the progressive elevation of blood pressure in salt-sensitive hypertensive mice, which further elucidated their important role in the pathogenesis of hypertension.

One important finding of the present study is that the large amount of IFN $\gamma$  produced by immune cells primes DCTs to accommodate activated CD8Ts; subsequently, the accentuated interaction between CD8Ts and DCTs results in upregulation of NCC and excessive salt retention. In this regard, our findings provide a mechanism for an earlier report that IFN $\gamma$  increases NCC expression and blood pressure in Ang II-induced hypertension,<sup>19</sup> an effect that we show relies on mediation by activated CD8Ts. We additionally show that knockout of IFN $\gamma$  ameliorates blood pressure in another classic animal model of hypertension, DOCA-salt treated mice. Interestingly, we noticed that in this same model, IFN $\gamma$  deficiency only normalized the later phase of progressive blood pressure elevation, which was termed thymus dependent blood

pressure elevation by an earlier study.<sup>41</sup> These findings led us to consider the possibility that IFN $\gamma$  contributes to the impairment of pressure-natriuresis by adaptive immunity and thereby the pathogenesis of salt-sensitive hypertension. Indeed, we found that knockout of IFN $\gamma$  remarkably reduced CD8T cell accumulation in the kidneys of DOCA-salt treated mice and antibody neutralization of IFN $\gamma$  diminished the preactivated CD8T-induced additional upregulation of NCC in DCTs. Collectively, our findings argue for a critical role of IFN $\gamma$  in activated CD8T-induced upregulation of NCC in DCTs and progression of hypertension.

A second important finding in this study is that we identified a molecular mechanism for IFN $\gamma$  priming of DCTs, which enhances DCT-interaction with activated CD8Ts. We demonstrated that IFN $\gamma$  induced higher expression of the CD8-specific antigen-presenter MHC-I in DCTs, but not the CD4-specific antigen MHC-II. This result is consistent with our earlier finding that CD8Ts, but not CD4Ts, play a major role in upregulating NCC in DCTs.<sup>12</sup> Moreover, we also found that IFN $\gamma$  stimulates DCTs to express high levels of PDL1, which is a well-known co-signaling molecule required for cells



**Figure 7. Effects of renal tubule-specific knockdown of PDL1 (programmed death-ligand 1) in mice receiving adoptive transfer of CD8Ts.**

**A**, Effects of renal tubule specific siPDL1 on T-cell accumulation in the kidneys of mice received CD8T-adoptive transfer. Scale=50  $\mu$ m. Data are representative of  $n > 15$  images in each group. **B**, Intensity quantification of NCC positive segments in stitched images of whole kidney sections from CD8T-adoptive transfer recipient mice treated with or without siPDL1.  $n = 8$  mice in each group. **C**, Radio-biotelemetry recording of systolic blood pressure in recipient mice of CD8T adoptive transfer with or without injection of nanoparticles containing siPDL1. Blood pressure was recorded for 10 seconds every 15 min.  $n = 5$  mice for +CD8Ts,  $n = 6$  mice for +CD8Ts + siPDL1. Data are means  $\pm$  SEM. Statistical significance was assessed by Mann-Whitney test for **A**, **B**, and 2-way ANOVA for **C**.

to interact with CD8Ts that express receptor PD1.<sup>22,23,55</sup> Some kidney transplantation studies have implied that PDL1 expression in renal tubules may be associated with salt-sensitive hypertension, because high expression levels of PDL1 often are detected on the surface of renal tubular cells in recipients of kidney transplantation.<sup>26–28</sup> In the same population, the prevalence of salt-sensitive hypertension is extremely high compared with the nontransplant population.<sup>46–48</sup> We conjecture that this increased expression of PDL1 on DCTs may provide a critical link to augmented cell-cell interaction with activated CD8Ts, resulting in an accentuated homing of CD8Ts to the kidney, higher NCC expression, and excessive salt-retention. It is known that global knock-out of PDL1 may cause systemic complications, including diabetes and multi-organ inflammation at an early age, as described by the Jackson Laboratory ([www.jax.org/strain/018307](http://www.jax.org/strain/018307)). Therefore, we delivered PDL1-specific siRNA directly to renal tubules using renal tubule-specific nanoparticles<sup>33–35</sup> to overcome this limitation. Our results confirmed high expression of PDL1 in renal tubules of salt-sensitive hypertension animals, and moreover, renal tubule-specific knockdown of PDL1 abolished kidney accumulation of T cells, reduced renal expression of NCC, and attenuated salt-sensitive

hypertension. In addition to identifying a critical role for PDL1 signaling in CD8T homing to kidneys of DOCA mice, the latter findings show the potential of using renal tubule-targeting nanoparticle technology to treat hypertension while minimizing off-target effects.

Although our findings suggest a critical role for PDL1 in mediating the interaction between activated CD8Ts and DCTs, which results in NCC upregulation and development of salt-sensitive hypertension, they do not rule out the possibility of other co-signaling, checkpoint, or adhesion molecules participating in this interaction. In fact, diminishing the IFN $\gamma$ -PDL1 pathway did not block the increase in NCC expression and NCC-mediated sodium retention in mDCTs caused by nonactivated CD8Ts (Figures 2 and 4), suggesting other molecular pathways may promote interaction between CD8Ts and DCTs. Moreover, PDL1 expression in renal tubule cells was anticipated to protect cells from the cytotoxic effects of activated CD8Ts; however, in both cultured DCTs and mouse kidneys with PDL1 knocked down, we observed no obvious cell death (such as loss of cells, damaged renal tubule morphology, etc) at the end of the treatment period, implying potential involvement of other molecules interfering with downstream cytotoxicity. Hence, we anticipate that future studies will identify additional

molecular mechanisms participating in the interaction between immune cells and renal tubule cells.

Finally, our data do not exclude the possibility that other immune cells in addition to CD8Ts also stimulate DCTs and other renal tubule segments by producing IFN $\gamma$  or other cytokines that contribute to the pathogenesis of hypertension. It is well known that hypertensive kidneys are infiltrated with many types of immune cells.<sup>16,38,50</sup> However, the crosstalk between these immune cells and with renal tubule cells is yet to be identified. One unanticipated finding of our studies was that kidney-specific knockdown of PDL1 in DOCA mice resulted in a more rapid and profound antihypertensive effect compared with the knockout of IFN $\gamma$ . Because we did not observe siPDL1-induced changes in viability of CD8Ts in our preliminary studies, it is reasonable to consider that other factors in addition to IFN $\gamma$  may regulate sodium transporter molecules on DCTs or other renal tubule segments. These processes may be clarified by future explorations.

Collectively, our data suggest a novel mechanism by which CD8Ts participate in the pathogenesis of hypertension, namely that activation of CD8Ts magnifies their interaction with DCTs via IFN $\gamma$ -induced MHC-I and PDL1 expression on DCTs, thereby stimulating enhanced homing of CD8Ts into the kidney and greater NCC expression in the kidney, resulting in excessive salt-retention and salt-sensitive hypertension. This mechanism identified in the current study not only adds to our current understanding of the pathogenesis of hypertension, but also provides insight into new therapeutic targets for anti-hypertensive medications based on understanding the crosstalk between hypertension and adaptive immunity.

## ARTICLE INFORMATION

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### Author Contributions

Y. Guo, K.S. Deck, and C.J. Mora contributed to additional experiments during revision; L.-X. Li participated in studies using IFN $\gamma$ -KO mice; L. Huang helped with in vivo isolation of T cells; Z. Qin helped with in vitro flow cytometry studies; B. Ko and R.S. Hoover provided mDCT15 cells and contributed to data discussion; R.M. Williams, D.A. Heller, and E.A. Jaimes prepared renal tubule specific nanoparticles preloaded with in vivo siRNAs. S. Mu designed the research, participated in both in vitro and in vivo experiments, and wrote the article. All co-authors contributed to discussion and editing of the article. We would like to thank Drs Nancy Rusch and Philip Palade in the Department of Pharmacology and Toxicology, University of Arkansas for Medical Sciences (UAMS) for contribution to data discussion and editing the article. L.N. Ben-

son, Y. Liu, X. Wang, and Y. Xiong performed in vitro and in vivo experiments and analyzed data; S.W. Rhee conducted blinded intensity quantification of stitched kidney section images and participated in quantifications of other images.

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### Disclosures

D.A. Heller is a co-founder and officer with an equity interest of Goldilocks Therapeutics Inc, Lime Therapeutics, Inc, and Resident Diagnostics, Inc and a member of the scientific advisory board of Concarlo Holdings LLC, Nanorobotics Inc, and Mediphage Bioceticals, Inc. R.M. Williams is a scientific advisor with equity interest in Goldilocks Therapeutics, Inc. E.A. Jaimes is Chief Medical Officer, co-founder and shareholder of Goldilocks Therapeutics, Inc. The other authors report no conflicts.

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