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RESEARCH ARTICLE

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Deletion of delta-like 1 homologue accelerates renal inflammation by modulating the Th17 immune response

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Abstract

Preclinical studies have demonstrated that activation of the NOTCH pathway plays a key role in the pathogenesis of kidney damage. There is currently no information on the role of the Delta-like homologue 1 (DLK1), a NOTCH inhibitor, in the regulation of renal damage. Here, we investigated the contribution of DLK1 to experimental renal damage and the underlying molecular mechanisms. Using a *Dlk1*-null mouse model in the experimental renal damage of unilateral ureteral obstruction, we found activation of NOTCH, as shown by increased nuclear translocation of the NOTCH1 intracellular domain, and upregulation of *Dlk2/hey-1* expression compared to wild-type (WT) littermates. NOTCH1 over-activation in *Dlk1*-null injured kidneys was associated with a higher inflammatory response, characterized by infiltration of inflammatory cells, mainly CD4/IL17A + lymphocytes, and activation of the Th17 immune response. Furthermore, pharmacological NOTCH blockade inhibited the transcription factors controlling Th17 differentiation and gene expression of the Th17

Abbreviations: ADAM, A disintegrin and metalloproteinase; BCA, bicinchoninic acid assay; DAPT, N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester; DLK, delta-like homologue; Dll, delta-like family; DNER, delta and NOTCH-like epidermal growth factor-related receptor; EGF, epithelial growth factor; EMT, epithelial-to-mesenchymal transition; F3, contactin 1; FA1, fetal antigen 1; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; Hes, hairy and enhancer of split; Hey, hes-related proteins; MCP-1, monocyte chemoattractant protein-1; NB-3, contactin 6; NF-κB, nuclear factor-kappa B; NICD, NOTCH intracellular domain; PAS, periodic acid-schiff; Pref-1, pre-adipocyte factor 1; PVDF, polyvinylidenedifluoride; RBP-Jk, recombination signal-Binding Protein-J kappa; RORγt, RAR (retinoic acid receptor)-related orphan receptor gamma t; STAT3, transducer and activator of transcription protein 3; Th17, lymphocyte T helper 17; Tnf-α, tumor necrosis factor-alpha; UUO, unilateral ureteral obstruction.

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effector cytokine IL-17A and other related-inflammatory factors, linked to a diminution of inflammation in the injured kidneys. We propose that the non-canonical NOTCH ligand DLK1 acts as a NOTCH antagonist in renal injury regulating the Th17-mediated inflammatory response.

KEYWORDS

DLK1, inflammation, NOTCH, renal damage, Th17 response

1 | INTRODUCTION

The NOTCH pathway is an evolutionarily conserved mechanism formed by a family of receptors, ligands and transcription factors. This pathway is essential in controlling spatial patterning, morphogenesis, and homeostasis in embryonic and adult tissues.[1] The NOTCH receptors and ligands are type 1 transmembrane proteins that belong to the Epidermal Growth Factor (EGF)-like family of proteins. In mammals, four NOTCH receptors (NOTCH1/2/3/4) and five canonical NOTCH ligands (Jagged1/2 and Delta-like family [Dll 1, 3, 4]) have been identified. NOTCH signaling activation is characterized by successive proteolytic cleavages triggered by the interaction between membrane-bound NOTCH receptors and ligands expressed on neighboring cells.[2,3] The first activating cleavage is mediated by ADAMs, followed by the action of the γ-secretase complex, which release the NOTCH receptor intracellular domain (NICD) into the cytoplasm. NICD migrates into the nucleus forming a nuclear complex with RBP-Jκ, a transcriptional activator of their downstream target genes, which belongs to a large family of transcription repressors and indirectly regulates the expression of several NOTCH-target genes. These are known as Hes (hairy and enhancer of split) 1, 5, and 7 and Hey (Hes-related proteins) 1 and 2 genes.[3,4]

Interestingly, several non-canonical ligands can regulate NOTCH signaling, including DNER (Delta and NOTCH-like epidermal growth factor-related receptor), F3/Contactin1, NB-3/Contactin6, Delta-like homologue-1 (*Dlk1*, also known as pre-adipocyte factor-1, Pref-1, or human adrenal specific cDNA, pG2)[5] and Delta-like homologue 2 (DLK2).[6] However, the precise function of these ligands remains unclear. DLK1 is the most studied NOTCH non-canonical ligand. DLK1 is structurally similar to the Dll ligands, contains six cysteine-rich EGF-like repeats, but lacks a DSL-binding domain at the N-terminal region of the protein and, therefore, fails to transactivate NOTCH.[7,8] DLK1 is known to regulate several processes, including cell fate decisions, differentiation, adipogenesis, hematopoiesis, and angiogenesis.[8,9] Although DLK1 and DLK2 have been shown to act as NOTCH signaling inhibitors in vitro,[7,10] their function in vivo is incompletely understood. DLK1 is synthesized as a transmembrane protein and its extracellular domain can be cleaved to generate a soluble form, called fetal antigen 1 (FA1), which circulates in blood.[11,12]

The NOTCH pathway is highly active in kidney development, participating in nephrogenesis, tubulogenesis, and glomerulogenesis. However, in the mature kidney, very little active NOTCH-1, and expression of its canonical ligands can be detected.[13] Previous studies have demonstrated that the NOTCH pathway is activated in human and experimental renal damage, and some authors have suggested that NOTCH-1/Jagged-1 levels could be potential biomarkers of human renal damage progression.[14-17] FA-1 is found in serum, urine, and is highly enriched in the amniotic fluid in the second trimester. In an early study in patients with renal failure, a 10-fold increase in DLK1 serum concentration was described[11,18] but data are lacking in more recent human studies. Some research groups, including ours, have found that NOTCH activation blockade using γ-secretase inhibitors ameliorates renal disease progression[13,15,19-21] through the inhibition of fibroblast proliferation, epithelial to mesenchymal transition (EMT), matrix accumulation[13,15,19,22] and, as reported more recently, by the inhibition of nuclear factor kappa-B (NF-κB) pathway activity and its effects in renal inflammation.[20] These data suggest that NOTCH blockade could be a potential therapeutic target for renal diseases. For this reason, in the present study, we have evaluated whether DLK1 could act as an inhibitor of NOTCH signaling in the damaged kidney, using the renal damage experimental model of unilateral ureteral obstruction (UUO) in *Dlk1*-null mice.

2 | MATERIALS AND METHODS

2.1 | Experimental models

All animal procedures were performed according to the guidelines of animal research in the European Community and with prior approval by the Animal Ethics Committee of the Health Research Institute IIS-Fundación Jiménez Díaz.

Description of the unilateral ureteral obstruction (UUO): Surgery was done in mice under isoflurane-induced anesthesia; the left ureter was ligated with silk (5/0) at two locations and cut between ligatures to prevent urinary tract infection

(obstructed kidney), as described.[23] In a parallel experiment, mice were randomly distributed in two groups treated with the γ-secretase inhibitor DAPT (0.1 mg/day, i.p.; Calbiochem), or vehicle, starting 1 day before UUO surgery, At least 4-10 animals per group were used. At the time of sacrifice, animals were anesthetized with 5 mg/kg xylazine (Rompun, Bayer AG) and 35 mg/kg ketamine (Ketolar, Pfizer) and the kidneys were perfused in situ with cold saline before removal. A piece of the kidney (1/4) was fixed, embedded in paraffin, and used for immunohistochemistry, and the rest was snap-frozen in liquid nitrogen for renal cortex RNA and protein studies.

The UUO model was conducted in 3-month-old male 129/SvJ mice (WT) and *Dlk1*-deleted mice (*Dlk1*-null) and studied after 2, 5, 10, and 14 days; and in 3-month-old C57BL/6 mice (untreated or DAPT-treated) studied at 5 days after UUO. Ligation of one ureter causes inflammation and overproduction of several profibrotic mediators contributing to renal injury.[24] The UUO model has been extensively employed to investigate the contribution of gene deletion of different growth factors, receptors, and mediators in renal damage.[23,24] Previous studies have demonstrated activation of the Th17 immune response in the UUO model, most of those studies were done in mice of C57BL/6 genetic background.[23,25,26] The evaluation of renal lesions in UUO mice at different time points showed no difference between 129/SvJ mice (WT) and C57BL/6 mice (including renal gene expression levels of Th17-related cytokines, not shown). Due to 3R animal recommendations and mice availability, C57BL/6 mice were used to study the effect of the pharmacological NOTCH activation inhibitor DAPT in renal damage following UUO.

2.2 | Protein studies

Total protein samples from frozen renal tissue were isolated in lysis buffer as described.[23] Proteins (20-100 μg per lane, quantified using a BCA protein assay kit) were separated on 8%-12% polyacrylamide-SDS gels under reducing conditions, as described.[23] Samples were then transferred on to polyvinylidene difluoride membranes (Millipore), blocked in TBS containing 0.1% Tween 20 and 5% nonfat dry milk for 1 hour at room temperature, and incubated in the same buffer with different primary antibodies overnight at 4°C. After washing, membranes were incubated with the appropriate HRP (horseradish peroxidase)-conjugated secondary antibody (Amersham Biosciences) and developed using an ECL kit (Millipore). The quality of proteins and efficacy of protein transfer were evaluated by Red Ponceau staining and used as loading control in the RORγt protein studies. Results were analyzed by LAS 4000 from GE Healthcare or Amersham Imager 600 from GE Healthcare. The following primary antibodies were employed [dilution]: Jagged-1 ([1:500]; sc-6011, Santa Cruz Biotechnology), RORγT ([1:1000] #14-6981-82, e-bioscience), GAPDH ([1/5000]; CB1001, Millipore), NOTCH2 ([1:500]; (D76A6) #5732T, cell signaling), NOTCH4 ([1:250]; A-1 sc-393893, Sta. Cruz Biotechnology), α-tubulin ([1:5000], T5168, Sigma-Aldrich), ERK1/2 ([1:500]; C-9 sc-514302, Sta. Cruz Biotechnology).

2.3 | Histology and immunohistochemistry

Paraffin-embedded kidney sections were stained using standard histology procedures, as described elsewhere.[23] Periodic acid Schiff staining (PAS) was performed and tubular dilation, interstitial inflammatory infiltration, and fibrosis, expressed as arbitrary units as previously described.[27] Immunostaining was carried out in 3 μm-thick tissue sections. Antigen retrieval was performed using the PTlink system (Dako) with sodium citrate buffer (10 mM) adjusted to pH 6-9, depending on the immunohistochemical marker. Endogenous peroxidase was blocked. Tissue sections were incubated for 1 hour at room temperature with 4% BSA and 10% of a specific serum (depending on the secondary antibody used) in PBS to eliminate non-specific protein binding sites. Primary antibodies were incubated overnight at 4°C. Specific biotinylated secondary antibodies (Amersham Biosciences) were used, followed by streptavidin-horseradish peroxidase conjugate, and 3,3-diaminobenzidine as a chromogen, then sections were counterstained with Carazzi's hematoxylin. The primary antibodies used were: N1ICD ([1:300]; ab8925, Abcam), F4/80 ([1:50]; MCA497, Bio-Rad), IL-17A ([1:250], ab9565, Abcam], CD3 ([1:100], A0452, DAKO), myeloperoxidase ([1X], IS511, DAKO), CD4([1X], IS649, DAKO) STAT3 ([1:100] #8768S, cell signaling) and NOTCH3 ([1:300]; (D11B8) #5276S, cell signaling). Specificity was checked by omission of primary antibodies. Quantitation was made by determining in 5-10 randomly chosen fields (×200 magnification) the total number of positive cells using Image-Pro Plus software (data expressed as the positive-stained area relative to the total area) or quantifying manually the number of positive nuclei.

2.4 | Gene expression studies

RNA from renal cortex (pulverized in a metallic chamber) was isolated with TriPure reagent (Roche). cDNA was synthesized by a High Capacity cDNA Archive kit (Applied Biosystems) using 2 μg of total RNA primed with random hexamer primers following the manufacturer's instructions. Next, quantitative gene expression analysis was performed by real-time PCR on an AB7500 fast real-time PCR

system (Applied Biosystems) using fluorogenic TaqMan MGB probes and primers designed by Assay-on-Demand™ gene expression products. Mouse assays IDs were: *Dlk1*: Mm00494477_m1; *Dlk2*: Mm01281511_g1, *Jag-1* (*Jagged-1*): Mm00496902_m1, *Hes-1* Mm01342805_m1; *Hey-1*: Mm00468865_m1; *Ccl-2*: Mm00441242_m1; *Ccl-5 (Rantes)*: Mm01302428_m1, *Ccl19*: Mm00839967_g1, *Ccl21*: Mm03646971_gH, *Tnf-α*: Mm00443258_m1 *Cxcl10*: Mm00445235_m1, *Notch2:* Mm00803077_m1, *Notch3*: Mm01345646_m1, *Notch4*: Mm00440525_m1. Data were normalized to *Gapdh*: Mm99999915_g1 (Vic). The mRNA copy numbers were calculated for each sample by the instrument software using Ct value ("arithmetic fit point analysis for the lightcycler"). Results were expressed in copy numbers, calculated relative to control after normalization against *Gapdh*.

2.5 | Statistical analysis

Results are expressed as the n-fold increase with respect to the control as the mean and SEM. In the studies with Dlk1-null mice, data were obtained comparing both kidneys (contralateral vs. obstructed) in each mouse. Only when analyzing components of the NOTCH pathway, data were shown comparing all kidneys vs. contralateral kidneys of WT mice. In C57BL/6 studies, mean results from untreated contralateral kidneys were taken as reference values (control). Differences between groups were assessed by the Mann-Whitney test. Statistical significance was concluded for values of $P < .05$. These analyses were done using SPSS version 16.0 (Chicago, IL), or GraphPad Prism version 5.0 (San Diego, CA).

3 | RESULTS

3.1 | The non-canonical NOTCH ligands DLK1 and DLK2 were upregulated in injured kidneys after UUO

Previous studies have shown that elimination of *Dlk1* is not deleterious; but *Dlk1*-null mice present high prenatal mortality, growth retardation, obesity, skeletal malformations, increased angiogenesis, and abnormalities of hematopoiesis.[28-31] Previously, NOTCH-1/Jagged activation has been demonstrated in studies performed with several experimental models of renal damage, including UUO.[15] However, no data are available about gene expression levels of *Dlk1* and *Dlk2*, which code for non-canonical NOTCH ligands. We observed that obstructed kidneys from WT mice showed increased *Dlk1 and Dlk2* gene expression compared to their corresponding contralateral kidneys starting from 5 days after renal damage and remaining elevated thereafter (Figure 1). Activation of the canonical NOTCH-1 pathway, including Jagged-1 expression, was observed as early as 2 days after UUO (Figure S1), persisting upregulated, as previously described.[13,15,20]

3.2 | Dlk1 deletion increases the presence of inflammatory cells in the injured kidney after UUO

To study the involvement of the non-canonical NOTCH ligand DLK1 in experimental renal injury, the progression of kidney damage was evaluated in *Dlk1*-null mice compared to WT animals. UUO was done in both genotypes and mice

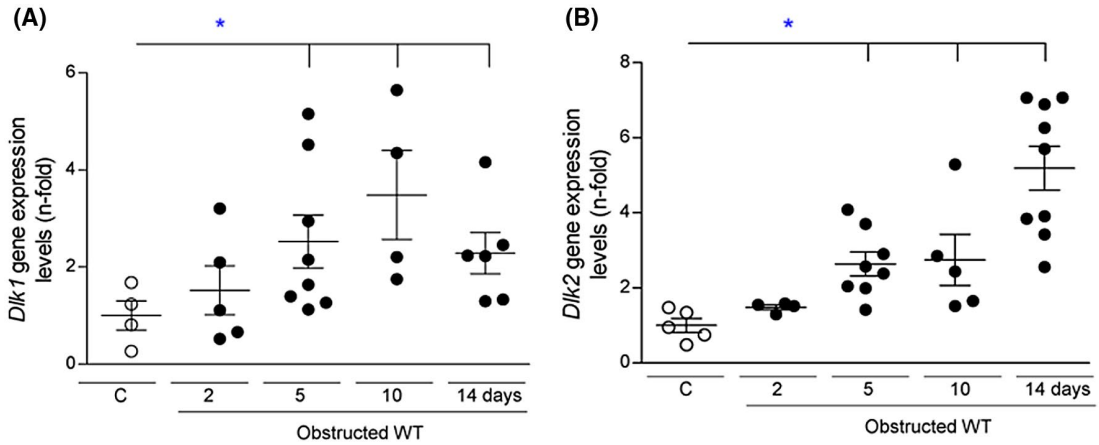

FIGURE 1 Evaluation of NOTCH non-canonical ligands *Dlk1* and *Dlk2* levels after renal injury by UUO. Unilateral ureteral obstruction (UUO) model was performed in wild-type (WT) mice from the 129/SvJ strain. Kidney mRNA expression levels of *Dlk1* (A) and *Dlk2* (B) after 2, 5, 10, and 14 days following UUO were evaluated by qRT-PCR. Gene levels in contralateral kidneys were not significantly different among time points, so only data at day 2 are shown (C = Contralateral). *Gapdh* was used as internal control. Data were normalized vs. contralateral kidneys and expressed as mean ± SEM of 4-10 animals per group. *$P < .05$ vs. Contralateral kidneys. Nonparametric two-tailed Mann-Whitney statistical test was performed

were studied after 2, 5, 10, and 14 days. Morphology of renal lesions was examined by PAS. Contralateral kidneys had no lesions (data not shown). After UUO, infiltration of immune cells in the kidney was observed, starting at 2 days and increasing thereafter (Figure 2). Nevertheless, in *Dlk1*-null mice obstructed kidneys, a marked increase in perivascular interstitial inflammatory cell infiltration compared to WT animals was observed starting after 5 days of obstruction (Figure 2D). At 14 days, a noticeably perivascular inflammatory infiltrate was found in the *Dlk1*-null mice (Figure 2B, D). However, obstructed *Dlk1*-null kidneys did not show more tubular damage or fibrosis than obstructed WT ones at any of the studied time-points (Figure 2A, D).

3.3 | Dlk1 deletion increases renal NOTCH1 pathway activation

The activation of the NOTCH1 pathway was determined by the evaluation of NOTCH1 intracellular domain (N1ICD)

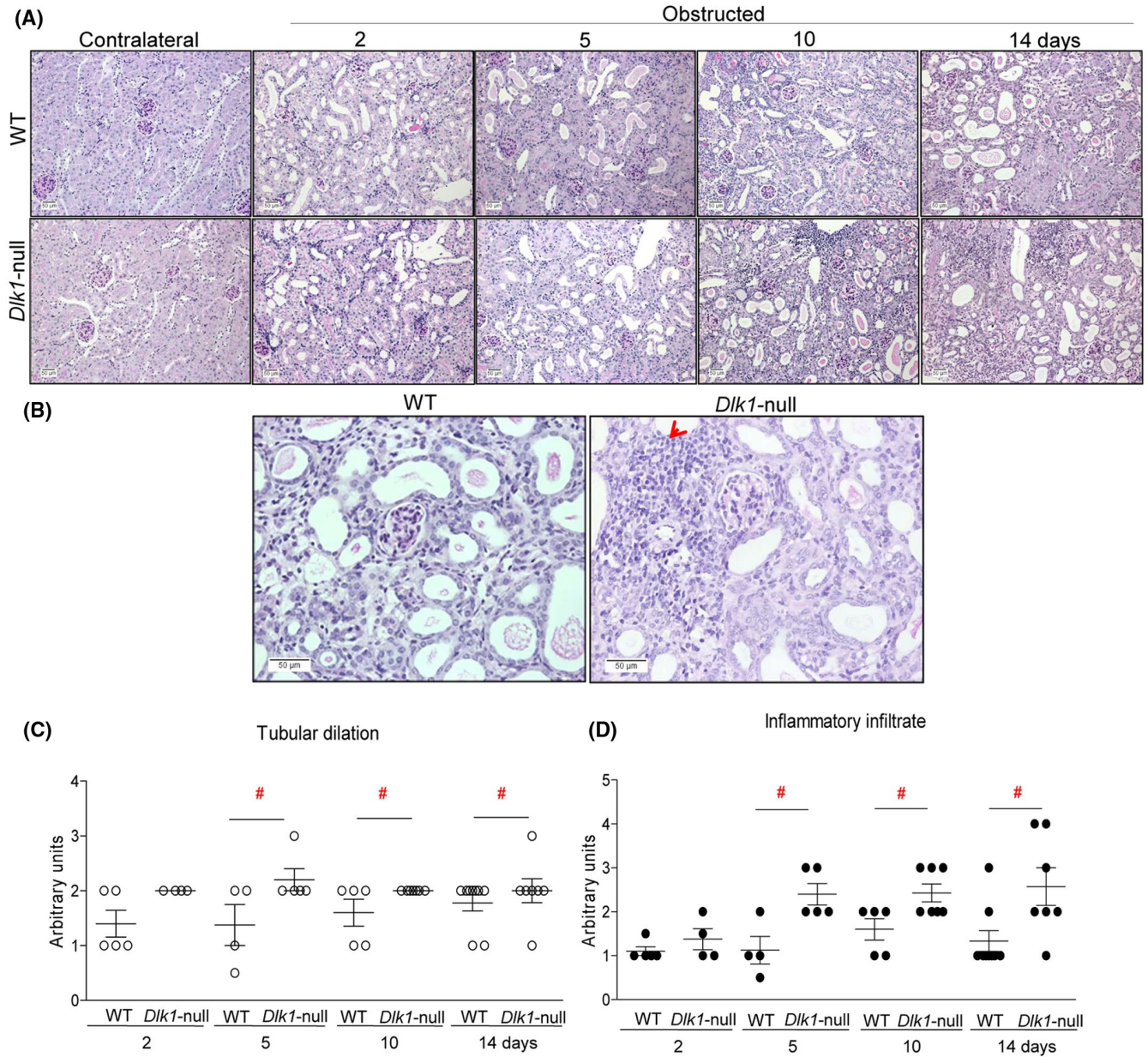

FIGURE 2 Effect of *Dlk1* deletion on experimental renal damage caused by UUO. Unilateral ureteral obstruction (UUO) was carried out in wild-type (WT) and *Dlk1*-null mice from the 129/SvJ strain and studied after 2, 5, 10, and 14 days. To evaluate renal damage, PAS staining was performed and scored. A, Representative micrographs of each group are shown at 200X. B, Obstructed kidneys of WT and *Dlk1*-null mice at 14 days at 400X. The arrow indicates the presence of inflammatory cell infiltration. Scale Bars = 50µm. Data of tubular dilation (C) and inflammatory infiltrate (D) are expressed as arbitrary units of 4-9 animals per group. $^{\#}P < .05$ vs. obstructed WT kidneys. Nonparametric two-tailed Mann-Whitney statistical test was performed

levels and localization. Obstructed kidneys presented increased N1ICD levels compared to contralateral ones, showing a clear nuclear localization of N1ICD (Figure 3A), as previously described in this model of renal damage.[15] However, NOTCH1 activation was markedly increased in obstructed kidneys from *Dlk1*-null mice compared to WT mice, showing a significant difference between both groups at day 14, as evaluated by immunohistochemistry and western blot

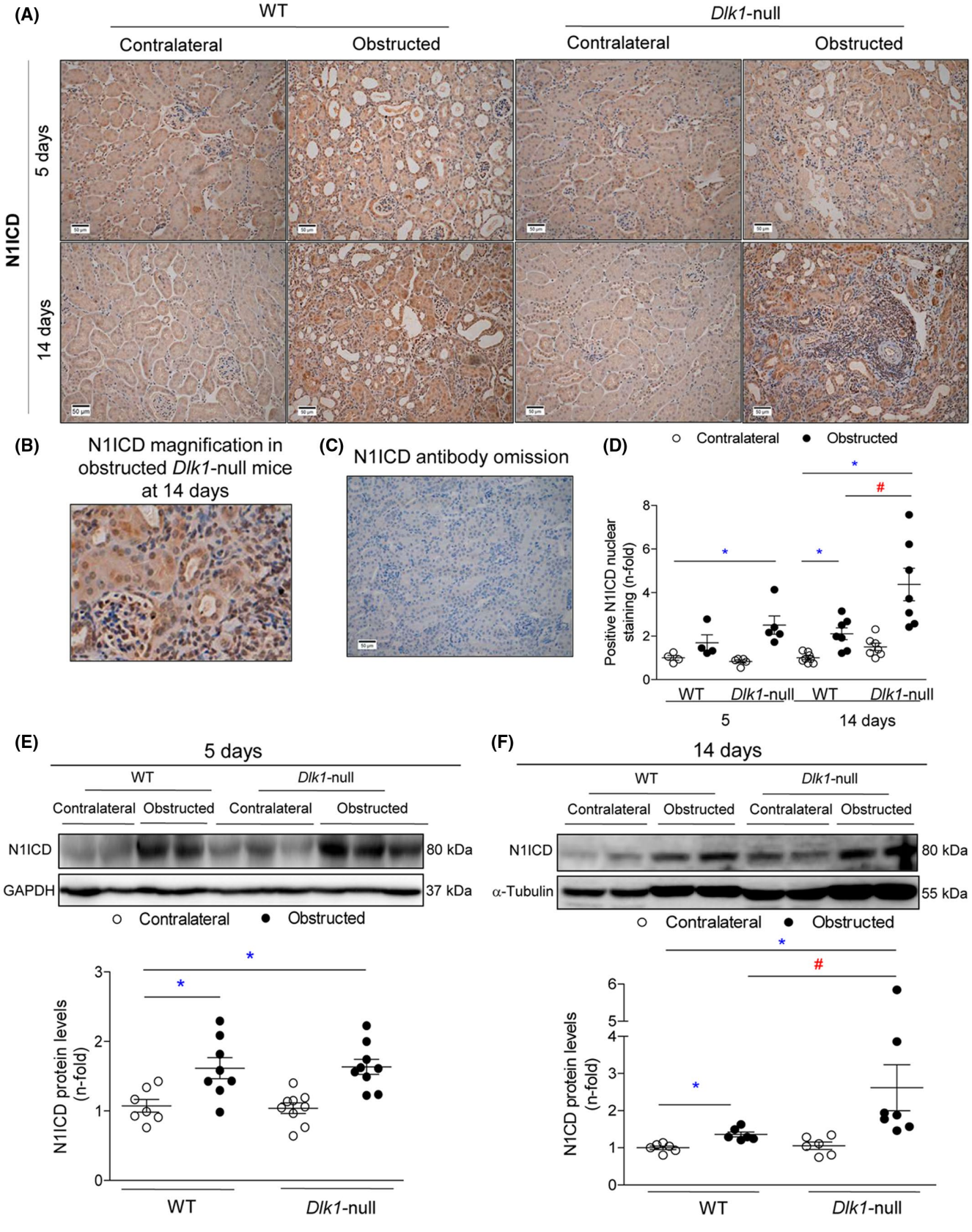

FIGURE 3 Evaluation of NOTCH activation after renal injury by UUO in wild-type and *Dlk1*-null mice. Unilateral ureteral obstruction (UUO) was carried out in wild-type (WT) and *Dlk1*-null mice from the 129/SvJ strain, and NOTCH1 activation was evaluated after 5 and 14 days of UUO. A, Microphotographs depict a representative kidney section of each group (200x magnification). Scale Bars = 50μm. B, Magnification of NOTCH1 Intracellular Domain (N1ICD) positive nuclear staining from a *Dlk1*-null obstructed kidney at 14 days. C, Negative control incubated only with secondary antibody. Scale Bars = 50μm. D, Quantitation was done by counting positive nuclear staining. Data were normalized vs. contralateral WT kidneys. E and F, Representative Western blot of N1ICD (upper panel) and the Quantitation (lower panel) at 5 (E) and 14 days (F) from total protein kidney extracts. GAPDH or α-tubulin were used as loading control and data were normalized vs. contralateral WT kidneys. Results are expressed as mean ± SEM of f4-9 animals per group. $^{*}P < .05$ vs. contralateral WT kidneys. $^{\#}P < .05$ vs. obstructed WT kidneys. Nonparametric two-tailed Mann-Whitney statistical test was performed.

(Figure 3). These data show that *Dlk1*-null mice present an over-activation of the NOTCH-1 pathway in the kidney.

The evaluation of the downstream N1ICD-target genes *Hes-1 and Hey-1* showed upregulation of both genes in obstructed kidneys (Figure 4A,B), but only *Hey-1* was markedly increased in *Dlk1*-null mice compared to WT controls after 5 days of UUO (Figure 4B). Interestingly, the mRNA levels of the non-canonical NOTCH ligand *Dlk2* also showed a significant increase 5 days after obstruction in *Dlk1*-null mice compared to WT animals (Figure 4C). Jagged-1, another upstream mediator of NOTCH activation, was increased in obstructed kidneys but there was no difference between genotypes (Figure S1).

These data indicate that *Dlk1*-null mice exhibit over-activation of the NOTCH1pathway independently of Jagged-1, reinforcing the NOTCH-antagonistic role of DLK1 in the kidney.

3.4 | Effect of Dlk1 deletion on other NOTCH receptors

Next, we evaluated expression levels of three additional NOTCH receptors. In WT obstructed kidneys there was an upregulation of NOTCH 2, 3, and 4, both at gene and protein levels, compared to contralateral ones (Figure S2 and S3). The effect of *Dlk1* deletion was associated with reduced gene expression levels of these receptors (reaching statistical significance only for *Notch4*, Figure S2B). Some differences were also found at the protein level; NOTCH2 levels were decreased in obstructed kidneys from *Dlk1*-null mice compared to obstructed ones from WT mice (Figure S2C,D), whereas no significant differences in NOTCH3 (Figure S3) and NOTCH4 (Figure S2E,F) protein levels were detected between genotypes.

3.5 | Renal inflammation by UUO was markedly increased in the absence of Dlk1

Interstitial inflammatory cell infiltration is one of the earliest features of renal damage following UUO,[24] and contributes to the progression of renal damage. We analyzed the infiltration of immune cells in UUO by immunohistochemistry. After 14 days, *Dlk1*-null mice obstructed kidneys showed a significantly increased number of monocytes/macrophages (F4/80 + cells), infiltrated neutrophils (myeloperoxidase staining) and T lymphocytes (CD3 + and CD4 + cells) compared to obstructed WT kidneys (Figure 5). The inflammatory cell infiltration in the kidney is regulated by the local overexpression of proinflammatory mediators. Hence, the gene expression levels of several chemokines and cytokines were evaluated. Although many of them were upregulated in the obstructed kidneys of both genotypes (Figure 6), only *Ccl-2* gene expression was significantly increased in *Dlk1*-null obstructed kidneys compared to WT mice, at 10 and 14 days (Figure 6A).

3.6 | Activation of the Th17 immune response in Dlk1-null mice following UUO

Dong and Griffin showed the recruitment and infiltration of the IL-17-producing CD4 + T-cell subset in murine kidneys after ureteral obstruction.[25] After this seminal work, numerous studies have demonstrated the importance of the Th17 immune response in renal disease, as well as the key role played by IL-17A as the effector cytokine for the regulation of renal inflammation.[32] Therefore, we evaluated whether the Th17 immune response was involved in the increased presence of inflammatory cells in *Dlk1*-null obstructed kidneys. The presence of IL-17A-producing CD4 + T-cells in *Dlk1*-null obstructed kidneys was demonstrated by specific immunostaining of IL-17A (Figure 7A). The differentiation of CD4 + Th cells into Th17 cells depends on the activation of the transcription factor Signal Transducer and Activator of Transcription protein 3 (STAT3) and Retinoic acid-related Orphan Receptor γt (RORγt).[33] The expression of the latter is directly activated by the binding of the complex NICD/RBPj-κ to its promoter region, as well as to the promoter region of IL-17A.[34,35] Renal RORγt levels were elevated in obstructed kidneys, showing a significant increase in *Dlk1*-null compared to WT mice (Figure 7B,C).

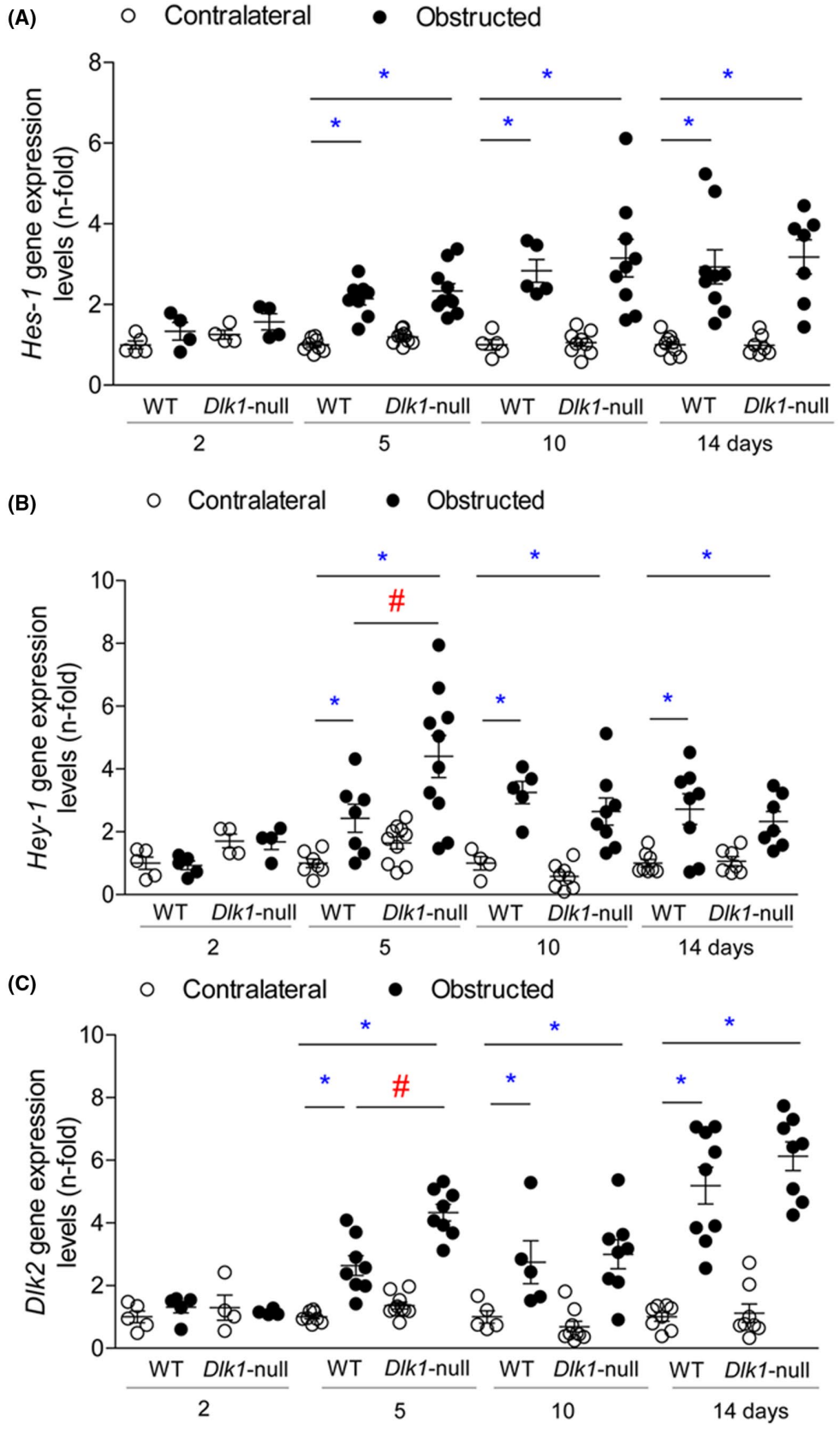

FIGURE 4 Expression of the effector NOTCH *genes Hes-1* and *Hey-1,* and the non-canonical ligand *Dlk2* after renal injury by UUO in wild-type and *Dlk1*-null mice. Unilateral ureteral obstruction (UUO) was carried out in wild-type (WT) and *Dlk1*-null mice from the 129/SvJ strain and gene expression levels of *Hes-1* (A), *Hey-1* (B) and *Dlk2* (C) were studied by qRT-PCR at 2, 5, 10, and 14 days. *Gapdh* was used as internal control. Data were normalized vs. contralateral WT kidneys and expressed as mean ± SEM of 4-10 animals per group. $^{*}P < .05$ vs. contralateral WT kidneys. $^{\#}P < .05$ vs. obstructed WT kidneys. Nonparametric two-tailed Mann-Whitney statistical test was performed.

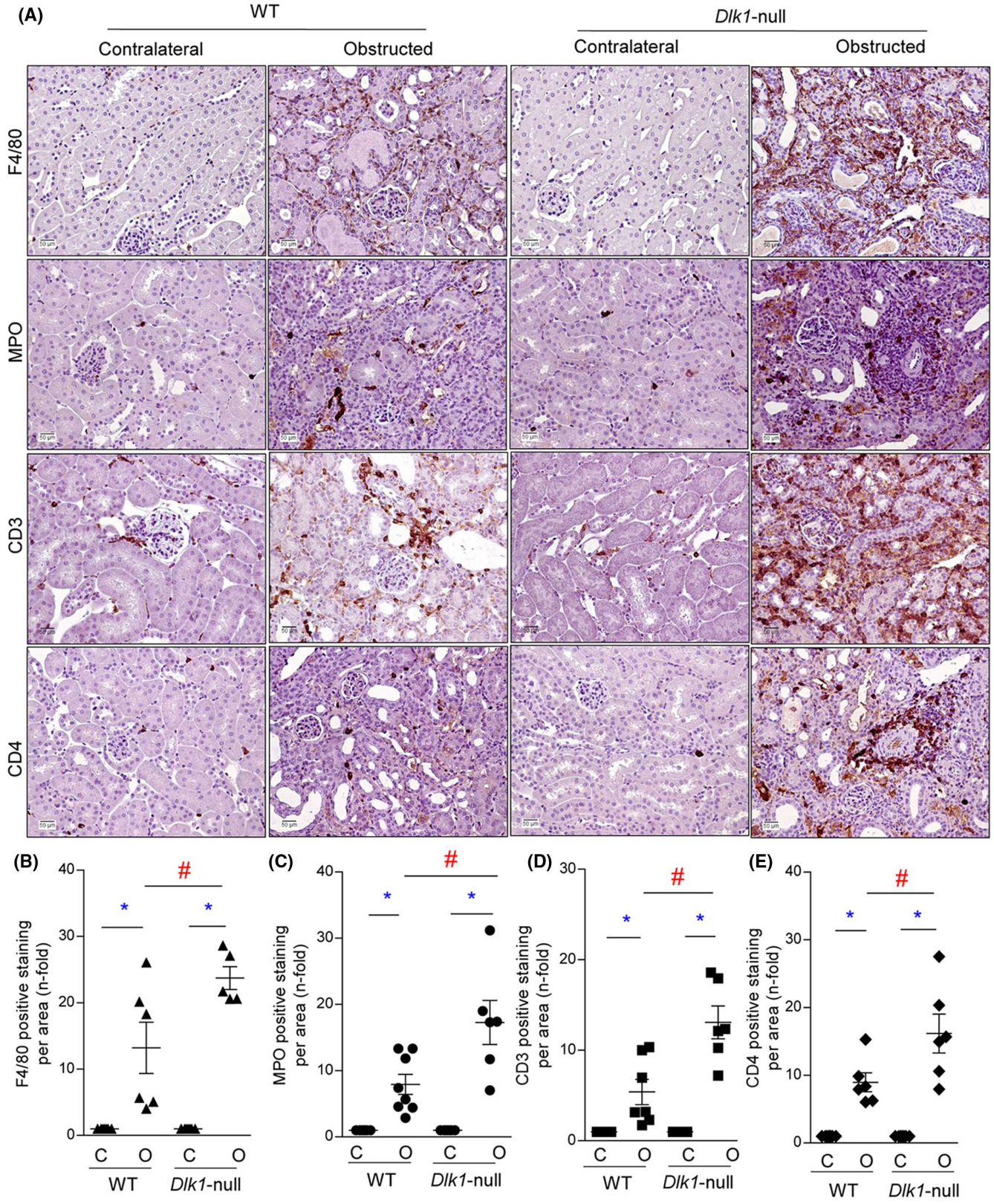

FIGURE 5 Characterization of inflammatory cell infiltration after renal injury by UUO in wild-type and *Dlk1*-null mice. Unilateral ureteral obstruction (UUO) was carried out in wild-type (WT) and *Dlk1*-null mice from the 129/SvJ strain and inflammatory cell infiltration was evaluated after 14 days. Immunohistochemistry was done using antibodies against F4/80 (marker for monocytes/macrophages/dendritic cells), myeloperoxydase (marker for neutrophils), CD3 and CD4 (markers for T lymphocytes). A, A representative kidney section micrograph from each group (magnification 200x) is shown. Scale Bars = 50µm. B-E, Quantitations are represented in the graphics for each marker (staining area per total area). C, contralateral; MPO, myeloperoxidase; O, obstructed. Data were normalized vs. their own contralateral kidneys and expressed as mean ± SEM of 5-8 animals per group. $^{*}P < .05$ vs. contralateral kidneys. $^{\#}P < .05$ vs. obstructed WT kidneys. Nonparametric two-tailed Mann-Whitney statistical test was performed.

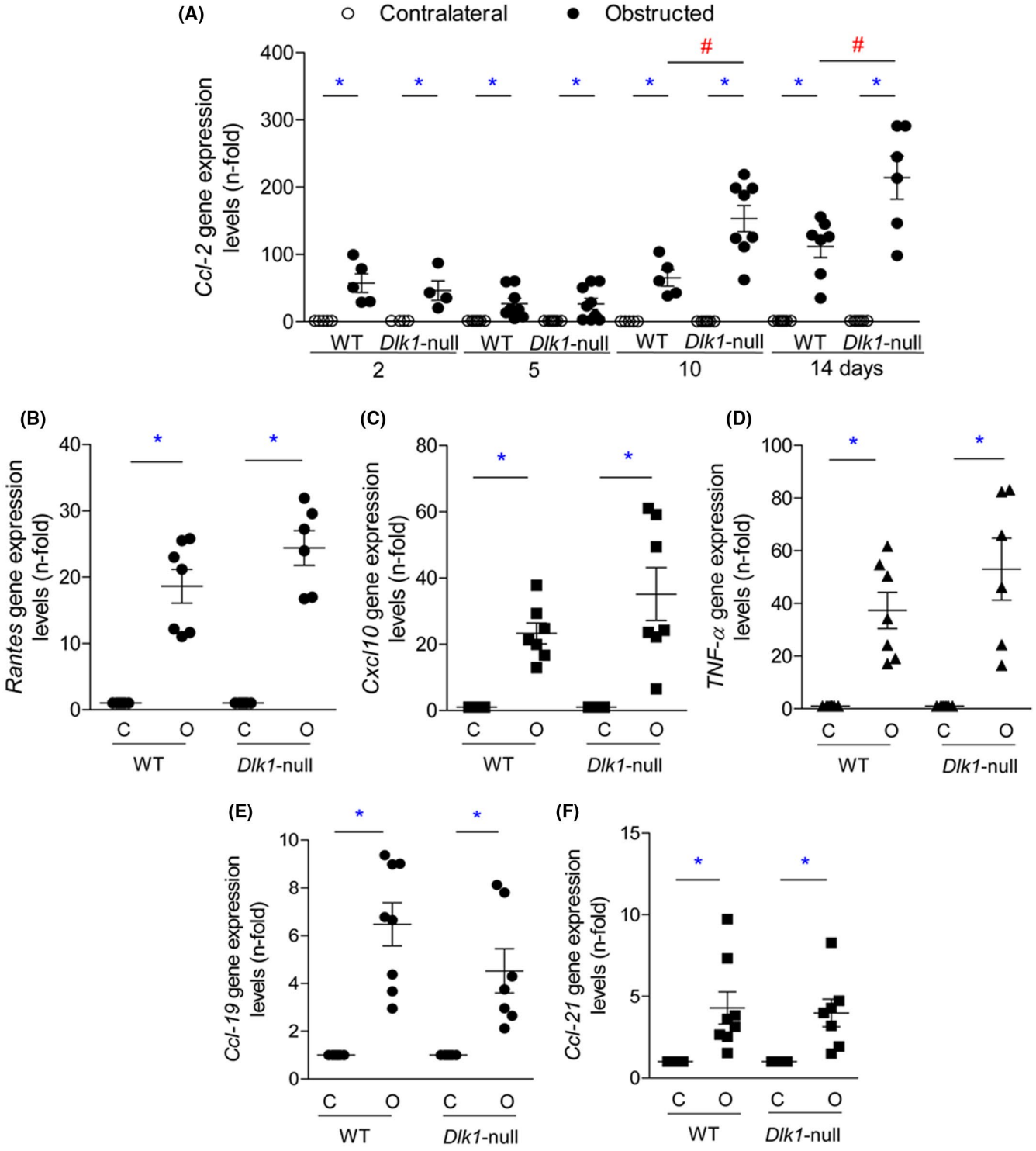

FIGURE 6 Evaluation of proinflammatory gene expression in injured kidneys by UUO. Unilateral ureteral obstruction (UUO) was carried out in wild-type (WT) and *Dlk1*-null mice from the 129/SvJ strain and sacrificed at 2, 5, 10, and 14 days. Gene expression levels of *Ccl-2* (A) at all time-points and *Rantes* (B), *Cxcl10* (C), *Tnf-α* (D), *Ccl19* (E) and *Ccl21* (F) at 14 days were determined by qRT-PCR *Gapdh* was used as internal control. C, contralateral; O, obstructed. Data were normalized vs. their own contralateral kidneys and expressed as the mean ± SEM of 4-9 animals per group. $^{*}P < .05$ vs. contralateral kidneys. $^{\#}P < .05$ vs. obstructed WT kidneys. Nonparametric two-tailed Mann-Whitney statistical test was performed.

Accordingly, in *Dlk1*-null obstructed kidneys, a significant increase of phosphorylated STAT3 levels were found when compared to those of WT animals, indicating an overactivation of nuclear STAT3 in the absence of *Dlk1* (Figure 7D,E). These data support the notion that the lack of *Dlk1* helps to over-activate the Th17 immune response.

3.7 | The γ-secretase inhibitor DAPT decreased the presence of CD4/IL-17A + cells in injured kidneys

Previous studies have shown that blockade of the NOTCH pathway improves experimental renal damage in several murine models, including UUO, by abrogating renal inflammation, among other responses.[15,19,20] However, the effect of the pharmacological blockade of NOTCH on the Th17 immune response observed in experimental renal damage has not been investigated. Interestingly, in autoimmune experimental diseases NOTCH inhibition modulates the Th17

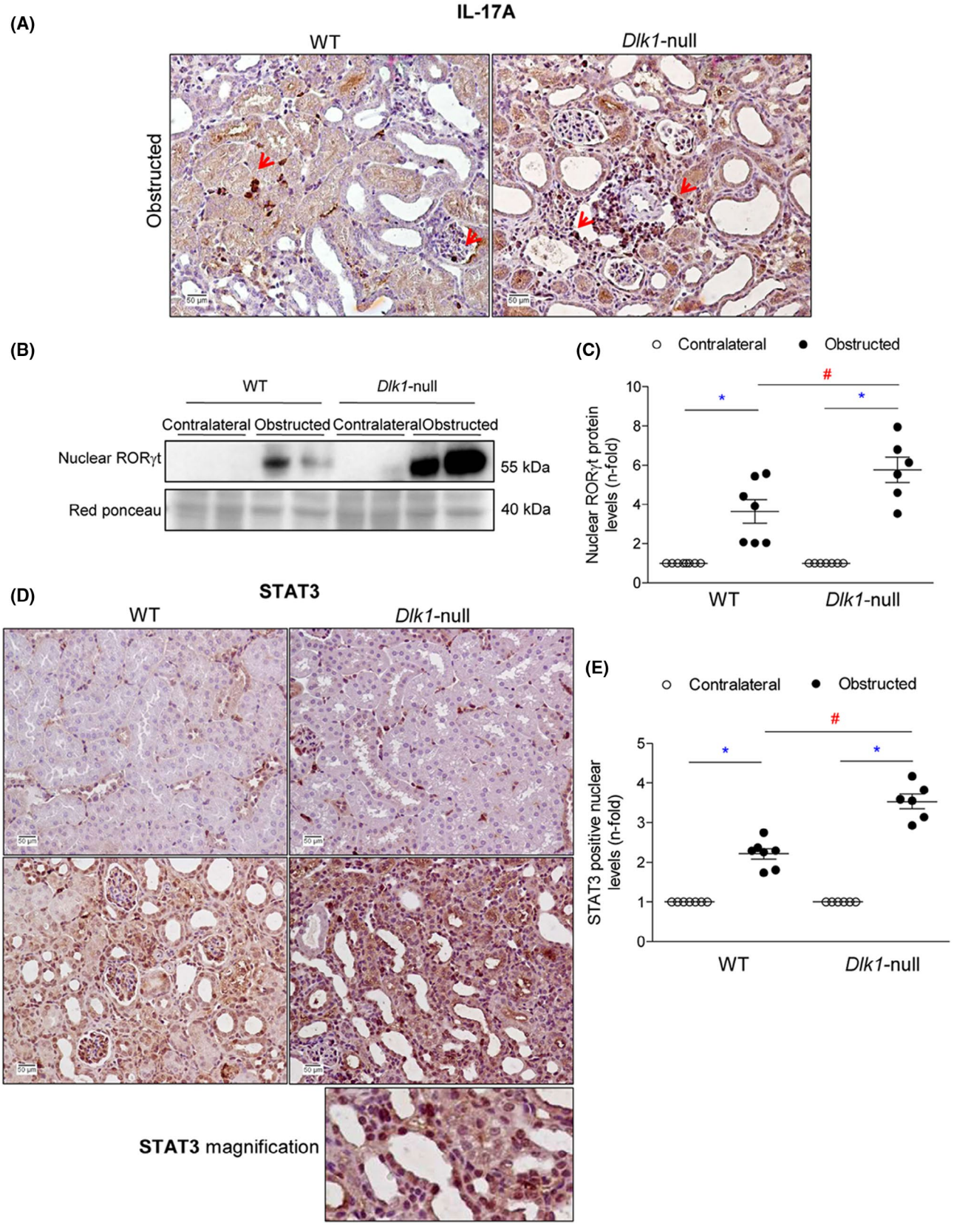

FIGURE 7 Activation of the Th17 immune response in the absence of *Dlk1* after renal injury by UUO. Unilateral ureteral obstruction (UUO) was carried out in wild-type (WT) and *Dlk1*-null mice from the 129/SvJ strain and Th17 response was evaluated after 14 days. A, IL-17A positive cells were detected by immunohistochemistry in obstructed kidneys, as indicated by arrows. B, Representative Western blot (WB) of kidney RORγt nuclear levels in *Dlk1*-null and WT and (C) their corresponding quantitation. Equal protein loading was confirmed by red ponceau. Data were normalized vs. their own contralateral kidneys and expressed as mean ± SEM of 6-7 animals per group. D and E, STAT3 activation was determined by nuclear positive immunostaining. D, Representative micrographs and (E) nuclear quantitation of STAT3. Data were normalized vs. their own contralateral kidneys and expressed as mean ± SEM of 6-7 animals per group. $^{*}P < .05$ vs. contralateral kidneys, $^{\#}P < .05$ vs. obstructed WT kidneys. Nonparametric two-tailed Mann-Whitney statistical test was performed.

immune response.[35-37] To inhibit NOTCH signaling, mice were pre-treated with the γ-secretase inhibitor DAPT, previously to the UUO surgery. NOTCH inhibition decreased the presence of CD4/IL-17A + cells in obstructed kidneys (Figure 8A, B) and also decreased RORγt protein levels (Figure 8C, D), in consistence with an inhibition of the Th17 immune response at 5 days.

4 | DISCUSSION

Several studies have evaluated the levels of the NOTCH pathway components in experimental and human renal diseases[13-17,38,39]; however, data about non-canonical NOTCH ligands are scarce. NOTCH ligands and receptors are expressed in a wide range of renal diseases, including membranous nephritis, lupus glomerulonephritis, IgA nephropathy, and diabetic nephropathy.[13,14,16,17] In experimental renal diseases, podocyte-specific NOTCH expression was correlated with albuminuria and glomerulosclerosis, while expression of cleaved intracellular NOTCH1 in tubules was associated with tubulointerstitial fibrosis.[17] A few studies have evaluated the levels of DLK1 (or its soluble form, FA1) in human pathological conditions. Serum FA1 levels increase with obesity and they are linked to insulin-resistance.[12,18,40] In an early study in 10 patients with renal failure, increased FA-1 serum levels were observed.[11] Now, we have shown that in experimental kidney damage caused by UUO the gene expression of non-canonical NOTCH ligands, *Dlk1*, and *Dlk2*, was upregulated. This attests to a relevant role for of these non-canonical ligands in renal diseases.

NOTCH activation has been observed in renal progenitors and podocytes from patients with glomerular disorders, whereas it is virtually absent from healthy adult glomeruli.[16] In an elegant study by Susztak's group, the involvement of NOTCH in tubulointerstitial fibrosis was demonstrated using transgenic mice with specific NOTCH deletion or activation, both in tubular epithelial and interstitial cells, in several experimental models of renal damage, including UUO.[15] In previous studies performed in cultured cells from *Dlk1*-null mice, we found increased levels of N1ICD and the downstream transcriptional effector *Hey1*, linked to angiogenesis.[41] We now show that deletion of *Dlk1* induced NOTCH pathway activation in the injured kidney, as demonstrated by both higher nuclear levels of N1ICD in *Dlk1*-null mice compared to WT mice and a consistent upregulation of the NOTCH-effector *Hey-1*. Taken together these data support that the absence of *Dlk1* may contribute to de-repress the NOTCH/Hey1 pathway by reducing the presence of a functional NOTCH antagonist, therefore, suggesting that DLK1 is an antagonist of NOTCH signaling in the injured kidney. Several evidences point out that other NOTCH receptors can also participate in renal damage.[42] NOTCH3 receptors transcripts are upregulated in human kidney diseases and experimental mice models,[38] as we have confirmed here in UUO. Studies done in *Notch3*-deficient mice with the UUO model found less tubular injury and significantly reduced interstitial collagen deposition.[38,43,44] Similarly, *Notch2- and Notch4-* deficient mice are protected from several renal-related injuries, such as folic-acid or immunodeficiency virus-associated nephropathy.[39,45] In our study, total NOTCH2 and NOTCH4 receptor protein levels are decreased in *Dlk1*-null mice as compared to WT, indicating that this could be an effect of *Dlk1* deletion or of concomitant *Dlk2* overexpression. Recent findings indicate that both DLK1 and DLK2 can modulate the activity of the four NOTCH receptors,[46,47] albeit in a different manner. This was not dissected in our study and awaits further analysis. NOTCH3 has been suggested as the most important NOTCH receptor related to kidney fibrosis.[38,48] Although by immunohistochemistry we found NOTCH3 activation in obstructed kidneys of both WT and *Dlk1*-null mice, there were no significant differences between genotypes. Accordingly, in *Dlk1*-null mice there was exacerbated inflammation, but no further increase in fibrosis, supporting the main role of NOTCH1 activation in the renal damage observed in *Dlk1*-null mice following UUO.

Numerous data suggest that unresolved inflammation contributes to tissue damage, being chronic inflammation a common feature in progressive kidney diseases.[49] The importance of Th17 cells, and its hallmark cytokine IL-17A, in immune-mediated glomerulonephritis, including experimental antimyeloperoxidase glomerulonephritis, crescentic glomerulonephritis and lupus nephritis, as well as in non-immune renal injury is well established.[23,50-53] Importantly, the blockade of IL-17A is a promising tool for the treatment of human chronic inflammatory diseases, as observed in ankylosing spondylitis, chronic plaque psoriasis and psoriatic arthritis.[54-57] In renal diseases, studies in different models of

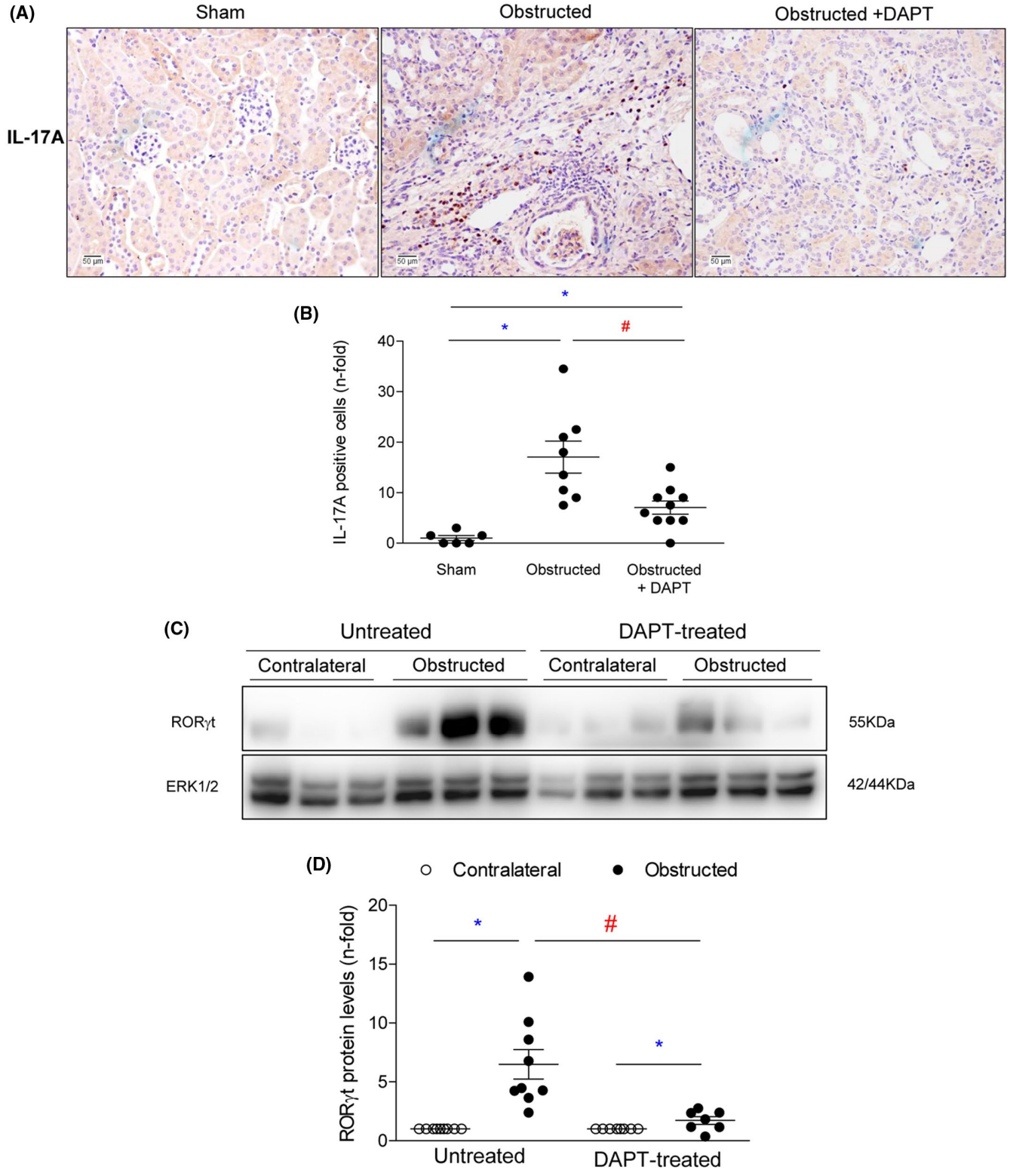

FIGURE 8 Evaluation of the effects of NOTCH1 signaling inhibition in the Th17 response in a 5-days UUO model. Unilateral ureteral obstruction (UUO) was carried out in C57BL/6 mice strain in which one group was treated with the NOTCH activation inhibitor DAPT (0.1 mg/day) and evaluated at day 5. A, Representative micrographs of IL-17A immunohistochemistry staining (Scale Bar = 50µm) and (B) the quantitation (counting positive cells). Data were normalized vs. sham kidneys and expressed as mean ± SEM of 6-10 animals per group. $^{*}P < .05$ vs. sham kidneys, $^{\#}P < .05$ vs. obstructed kidneys. C, Representative Western blot of RORγt and (D) their quantitation. Total ERK1/2 was used as loading control. Data were normalized vs. their own contralateral kidneys and expressed as mean ± SEM of 6-10 animals per group. $^{*}P < .05$ vs. contralateral kidneys, $^{\#}P < .05$ vs. obstructed untreated kidneys. Nonparametric two-tailed Mann-Whitney statistical test was performed

renal damage have reported beneficial effects of Th17 blockade,[23,50-53] as proven using IL-17A neutralizing antibodies in diabetic nephropathy.[53] Our studies in the UUO model in *Dlk1*-deficient mice, clearly demonstrate that *Dlk1* deletion exacerbates renal inflammation associated to increased NOTCH activation. This enhanced inflammatory response was mediated by the activation of the Th17 immune response, as shown by the overexpression of the key Th17 differentiation factors, STAT-3 and RORγt, and the induction of IL-17A. A recent study found that NOTCH1 participates in the differentiation from CD4 + naïve T cells to different Th lymphocytes subsets, including Th17 cells, mainly via Delta-like 4 ligand-receptor interaction,[58] supporting our findings. Moreover, we have observed that treatment with the γ-secretase inhibitor DAPT, which blocks NOTCH activation, inhibited the Th17 immune response and downstream inflammatory response in obstructed kidneys. Our findings extend to renal diseases the previous studies describing that NOTCH inhibition diminished the Th17 immune response in the experimental models of allergic asthma,[59] and autoimmune encephalomyelitis.[35] Interestingly, although in injured kidneys there is a markedly upregulation of many proinflammatory genes, only *Ccl2* (encoding MCP-1) was significantly upregulated in *Dlk1*-null mice compared to WT mice. Several findings suggest that MCP-1 could be a key mediator of IL-17A-mediated inflammatory responses in the kidney. Earlier studies showed that IL-17A increases the production of MCP-1 in tubular epithelial cells.[60] We have recently described that systemic administration of IL-17A in mice induces renal inflammation characterized mainly by MCP-1 upregulation, whereas levels of other relevant cytokines, such as IL-6 and IL1-β, remained unchanged.[61] Moreover, IL-17A blockade diminished *Ccl2* expression and inflammatory cell infiltration in the experimental models of diabetic nephropathy and Angiotensin II-induced renal damage.[53,61] These data support that in *Dlk1*-null mice local NOTCH activation in kidneys could contribute to exacerbated inflammation by the upregulation of IL-17A/MCP-1 signaling.

Preclinical studies have shown that blockade of the NOTCH pathway by γ- secretase inhibitors[15] or antagonists of some NOTCH ligands, including Delta-like 4,[62] could be an important therapeutic option for several diseases, including chronic kidney diseases, cardiovascular pathologies, or proliferative disorders. The γ-secretase inhibitors are being tested in clinical trials as a potential treatment for several diseases, such as leukemia, melanoma, and Alzheimer's disease (NCT00594568, NCT00762411, NCT01193868, NCT01196416, NCT01981551), where the role of inflammation is of vital importance. All these studies support the idea that NOTCH inhibition, may represent a therapeutic option for the treatment of chronic kidney diseases.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors have reviewed the manuscript and approved the final version. L. Marquez-Exposito contributed to the design of the experiments, acquisition, analysis, and interpretation of all data, and drafted the manuscript; R.R. Rodrigues-Diez and M. Fierro-Fernandez contributed to analysis and interpretation of data, and critical review of the manuscript; S. Rayego-Mateos, R. Rodrigues-Diez, M. Orejudo, L Santos-Sanchez, and E. Maria Blanco participated in the development of mouse models and analysis of data; S. Mezzano has been involved in pathological characterization of the model and critical review of the manuscript; J. Laborda and S. Lamas contributed to the critical review of the manuscript and previous phenotype characterization of the *Dlk1*-null mouse model; M. Ruiz-Ortega and C. Lavoz contributed to the design of the experiments, analysis and interpretation of the all data, draft of the manuscript and financial support of the experiments.

REFERENCES

1. Bray SJ. Notch signalling: a simple pathway becomes complex. *Nat Rev Mol Cell Biol*. 2006;7:678-689.
2. Jarriault S, Brou C, Logeat F, Schroeter EH, Kopan R, Israel A. Signalling downstream of activated mammalian Notch. *Nature*. 1995;377:355-358.
3. Fortini ME. Notch signaling: the core pathway and its posttranslational regulation. *Dev Cell*. 2009;16:633-647.

4. Quillard T, Charreau B. Impact of notch signaling on inflammatory responses in cardiovascular disorders. *Int J Mol Sci*. 2013;14:6863-6888.
5. D'Souza B, Meloty-Kapella L, Weinmaster G. Canonical and non-canonical Notch ligands. *Curr Top Dev Biol*. 2010;92:73-129.
6. Nueda M-L, Baladron V, Garcia-Ramirez J-J, et al. The novel gene EGFL9/Dlk2, highly homologous to Dlk1, functions as a modulator of adipogenesis. *J Mol Biol*. 2007;367:1270-1280.
7. Sanchez-Solana B, Nueda ML, Ruvira MD, et al. The EGF-like proteins DLK1 and DLK2 function as inhibitory non-canonical ligands of NOTCH1 receptor that modulate each other's activities. *Biochim Biophys Acta*. 2011;1813:1153-1164.
8. Falix FA, Aronson DC, Lamers WH, Gaemers IC. Possible roles of DLK1 in the Notch pathway during development and disease. *Biochim Biophys Acta*. 2012;1822:988-995.
9. Laborda J. The role of the epidermal growth factor-like protein dlk in cell differentiation. *Histol Histopathol*. 2000;15:119-129.
10. Baladron V, Ruiz-Hidalgo MJ, Nueda ML, et al. dlk acts as a negative regulator of Notch1 activation through interactions with specific EGF-like repeats. *Exp Cell Res*. 2005;303:343-359.
11. Jensen CH, Krogh TN, Stoving RK, Holmskov U, Teisner B. Fetal antigen 1 (FA1), a circulating member of the epidermal growth factor (EGF) superfamily: ELISA development, physiology and metabolism in relation to renal function. *Clin Chim Acta*. 1997;268:1-20.
12. Smas CM, Chen L, Sul HS. Cleavage of membrane-associated pref-1 generates a soluble inhibitor of adipocyte differentiation. *Mol Cell Biol*. 1997;17:977-988.
13. Sirin Y, Susztak K. Notch in the kidney: development and disease. *J Pathol*. 2012;226:394-403.
14. Bonegio R, Susztak K. Notch signaling in diabetic nephropathy. *Exp Cell Res*. 2012;318:986-992.
15. Bielesz B, Sirin Y, Si H, et al. Epithelial Notch signaling regulates interstitial fibrosis development in the kidneys of mice and humans. *J Clin Invest*. 2010;120:4040-4054.
16. Lasagni L, Ballerini L, Angelotti ML, et al. Notch activation differentially regulates renal progenitors proliferation and differentiation toward the podocyte lineage in glomerular disorders. *Stem Cells*. 2010;28:1674-1685.
17. Murea M, Park J-K, Sharma S, et al. Expression of Notch pathway proteins correlates with albuminuria, glomerulosclerosis, and renal function. *Kidney Int*. 2010;78:514-522.
18. Jensen CH, Krogh TN, Hojrup P, et al. Protein structure of fetal antigen 1 (FA1). A novel circulating human epidermal-growth-factor-like protein expressed in neuroendocrine tumors and its relation to the gene products of dlk and pG2. *Eur J Biochem*. 1994;225:83-92.
19. Kretzler M, Allred L. Notch inhibition reverses kidney failure. *Nat Med*. 2008;14:246-247.
20. Lavoz C, Poveda J, Marquez-Exposito L, et al. Gremlin activates the Notch pathway linked to renal inflammation. *Clin Sci (Lond)*. 2018;132:1097-1115.
21. Marquez-Exposito L, Cantero-Navarro E, Lavoz C, et al. Could Notch signaling pathway be a potential therapeutic option in renal diseases? *Nefrologia*. 2018;38:466-475.
22. Marquez-Exposito L, Lavoz C, Rodrigues-Diez RR, et al. Gremlin regulates tubular epithelial to mesenchymal transition via VEGFR2: potential role in renal fibrosis. *Front Pharmacol*. 2018;9:1195.
23. Rodrigues-Diez R, Rodrigues-Diez RR, Rayego-Mateos S, et al. The C-terminal module IV of connective tissue growth factor is a novel immune modulator of the Th17 response. *Lab Invest*. 2013;93:812-824.
24. Ucero AC, Benito-Martin A, Izquierdo MC, et al. Unilateral ureteral obstruction: beyond obstruction. *Int Urol Nephrol*. 2014;46:765-776.
25. Dong X, Bachman LA, Miller MN, Nath KA, Griffin MD. Dendritic cells facilitate accumulation of IL-17 T cells in the kidney following acute renal obstruction. *Kidney Int*. 2008;74:1294-1309.
26. Pindjakova J, Hanley SA, Duffy MM, et al. Interleukin-1 accounts for intrarenal Th17 cell activation during ureteral obstruction. *Kidney Int*. 2012;81:379-390.
27. Zoja C, Corna D, Camozzi D, et al. How to fully protect the kidney in a severe model of progressive nephropathy: a multidrug approach. *J Am Soc Nephrol*. 2002;13:2898-2908.
28. Moon YS, Smas CM, Lee K, et al. Mice lacking paternally expressed Pref-1/Dlk1 display growth retardation and accelerated adiposity. *Mol Cell Biol*. 2002;22:5585-5592.
29. Raghunandan R, Ruiz-Hidalgo M, Jia Y, et al. Dlk1 influences differentiation and function of B lymphocytes. *Stem Cells Dev*. 2008;17:495-507.
30. Waddell JN, Zhang P, Wen Y, et al. Dlk1 is necessary for proper skeletal muscle development and regeneration. *PLoS One*. 2010;5:e15055.
31. Puertas-Avendano RA, Gonzalez-Gomez MJ, Ruvira MD, et al. Role of the non-canonical notch ligand delta-like protein 1 in hormone-producing cells of the adult male mouse pituitary. *J Neuroendocrinol*. 2011;23:849-859.
32. Cortvrindt C, Speeckaert R, Moerman A, Delanghe JR, Speeckaert MM. The role of interleukin-17A in the pathogenesis of kidney diseases. *Pathology*. 2017;49:247-258.
33. Kitching AR, Holdsworth SR. The emergence of TH17 cells as effectors of renal injury. *J Am Soc Nephrol*. 2011;22:235-238.
34. Mukherjee S, Schaller MA, Neupane R, Kunkel SL, Lukacs NW. Regulation of T cell activation by Notch ligand, DLL4, promotes IL-17 production and Rorc activation. *J Immunol*. 2009;182:7381-7388.
35. Keerthivasan S, Suleiman R, Lawlor R, et al. Notch signaling regulates mouse and human Th17 differentiation. *J Immunol*. 2011;187:692-701.
36. Ma L, Xue H, Qi R, Wang Y, Yuan L. Effect of gamma-secretase inhibitor on Th17 cell differentiation and function of mouse psoriasis-like skin inflammation. *J Transl Med*. 2018;16:59.
37. Yin X, Liu B, Wei H, et al. Activation of the Notch signaling pathway disturbs the CD4(+)/CD8(+), Th17/Treg balance in rats with experimental autoimmune uveitis. *Inflamm Res*. 2019;68(9):761-774.
38. Djudjaj S, Chatziantoniou C, Raffetseder U, et al. Notch-3 receptor activation drives inflammation and fibrosis following tubulointerstitial kidney injury. *J Pathol*. 2012;228:286-299.
39. Puri RV, Yerrathota S, Home T, et al. Notch4 activation aggravates NF-κB-mediated inflammation in HIV-1-associated nephropathy. *Dis Model Mech*. 2019;12(12):dmm040642.
40. Chacon MR, Miranda M, Jensen CH, et al. Human serum levels of fetal antigen 1 (FA1/Dlk1) increase with obesity, are negatively associated with insulin sensitivity and modulate inflammation in vitro. *Int J Obes (Lond)*. 2008;32:1122-1129.
41. Rodriguez P, Higueras MA, Gonzalez-Rajal A, et al. The non-canonical NOTCH ligand DLK1 exhibits a novel vascular role as a strong inhibitor of angiogenesis. *Cardiovasc Res*. 2012;93:232-241.
42. Sanchez-Niño MD, Ortiz A. Notch3 and kidney injury: never two without three. *J Pathol*. 2012;228:266-273.

43. Kavvadas P, Keuylian Z, Prakoura N, et al. Notch3 orchestrates epithelial and inflammatory responses to promote acute kidney injury. *Kidney Int*. 2018;94:126-138.
44. El Machhour F, Keuylian Z, Kavvadas P, Dussaule J-C, Chatziantoniou C. Activation of Notch3 in glomeruli promotes the development of rapidly progressive renal disease. *J Am Soc Nephrol*. 2015;26:1561-1575.
45. Huang S, Park J, Qiu C, et al. Jagged1/Notch2 controls kidney fibrosis via Tfam-mediated metabolic reprogramming. *PLoS Biol*. 2018;16:e2005233.
46. Nueda M-L, González-Gómez M-J, Rodríguez-Cano M-M, et al. DLK proteins modulate NOTCH signaling to influence a brown or white 3T3-L1 adipocyte fate. *Sci Rep*. 2018;8:16923.
47. Rodríguez-Cano M-M, González-Gómez M-J, Sánchez-Solana B, et al. NOTCH receptors and DLK proteins enhance brown adipogenesis in mesenchymal C3H10T1/2 cells. *Cells*. 2020;9.
48. Huang M, Zhang J, Xu H, et al. The TGFβ-ERK pathway contributes to Notch3 upregulation in the renal tubular epithelial cells of patients with obstructive nephropathy. *Cell Signal*. 2018;51:139-151.
49. Lee SB, Kalluri R. Mechanistic connection between inflammation and fibrosis. *Kidney Int. Suppl*. 2010;S22-S26.
50. Xue L, Xie K, Han X, et al. Detrimental functions of IL-17A in renal ischemia-reperfusion injury in mice. *J Surg Res*. 2011;171:266-274.
51. Peng X, Xiao Z, Zhang J, Li Y, Dong Y, Du J. IL-17A produced by both gammadelta T and Th17 cells promotes renal fibrosis via RANTES-mediated leukocyte infiltration after renal obstruction. *J Pathol*. 2015;235:79-89.
52. Ramani K, Biswas PS. Interleukin 17 signaling drives Type I Interferon induced proliferative crescentic glomerulonephritis in lupus-prone mice. *Clin Immunol*. 2016;162:31-36.
53. Lavoz C, Matus YS, Orejudo M, et al. Interleukin-17A blockade reduces albuminuria and kidney injury in an accelerated model of diabetic nephropathy. *Kidney Int*. 2019;95:1418-1432.
54. Baeten D, Baraliakos X, Braun J, et al. Anti-interleukin-17A monoclonal antibody secukinumab in treatment of ankylosing spondylitis: a randomised, double-blind, placebo-controlled trial. *Lancet (London, England)*. 2013;382:1705-1713.
55. Baeten D, Sieper J, Braun J, et al. Secukinumab, an interleukin-17A inhibitor, in ankylosing spondylitis. *N Engl J Med*. 2015;373:2534-2548.
56. Leonardi C, Matheson R, Zachariae C, et al. Anti-interleukin-17 monoclonal antibody ixekizumab in chronic plaque psoriasis. *N Engl J Med*. 2012;366:1190-1199.
57. Mease PJ, McInnes IB, Kirkham B, et al. Secukinumab inhibition of interleukin-17A in patients with psoriatic arthritis. *N Engl J Med*. 2015;373:1329-1339.
58. Vijayaraghavan J, Osborne BA. Notch and T cell function - a complex tale. *Adv Exp Med Biol*. 2018;1066:339-354.
59. Huang Q, Han L, Lv R, Ling L. Magnolol exerts anti-asthmatic effects by regulating Janus kinase-signal transduction and activation of transcription and Notch signaling pathways and modulating Th1/Th2/Th17 cytokines in ovalbumin-sensitized asthmatic mice. *Korean J Physiol Pharmacol*. 2019;23:251-261.
60. Van Kooten C, Boonstra JG, Paape ME, et al. Interleukin-17 activates human renal epithelial cells in vitro and is expressed during renal allograft rejection. *J Am Soc Nephrol*. 1998;9:1526-1534.
61. Orejudo M, Rodrigues-Diez RR, Rodrigues-Diez R, et al. Interleukin 17A Participates In Renal Inflammation Associated To Experimental And Human Hypertension. *Front Pharmacol*. 2019;10:1015.
62. Fukuda D, Aikawa M. Expanding role of delta-like 4 mediated notch signaling in cardiovascular and metabolic diseases. *Circ J*. 2013;77:2462-2468.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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