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Protective Role of the Podocyte IL-15 / STAT5 Pathway in Focal Segmental Glomerulosclerosis

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Introduction: During glomerular diseases, podocyte-specific pathways can modulate the intensity of histological disease and prognosis. The therapeutic targeting of these pathways could thus improve the management and prognosis of kidney diseases. The Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) pathway, classically described in immune cells, has been recently described in detail in intrinsic kidney cells.

Methods: We describe STAT5 expression in human kidney biopsies from patients with focal segmental glomerulosclerosis (FSGS) and studied mice with a podocyte-specific *Stat5* deletion in experimental glomerular diseases.

Results: Here, we show, for the first time, that STAT5 is activated in human podocytes in FSGS. In addition, podocyte-specific *Stat5* inactivation aggravates the structural and functional alterations in a mouse model of FSGS. This could be due, at least in part, to an inhibition of autophagic flux. Finally, interleukin 15 (IL-15), a classical activator of STAT5 in immune cells, increases STAT5 phosphorylation in human podocytes, and its administration alleviates glomerular injury *in vivo* by maintaining autophagic flux in podocytes.

Conclusion: Activating podocyte STAT5 with commercially available IL-15 represents a potential new therapeutic avenue for FSGS.

Kidney Int Rep (2024) **9,** 1093–1106; https://doi.org/10.1016/j.ekir.2024.01.010
KEYWORDS: autophagy; cell signaling; cytokines; focal segmental glomerulosclerosis; IL-15; podocyte

Podocytes, specialized glomerular epithelial cells, are one of the main targets of injury—hypertensive, immune, toxic—in kidney diseases, driving the progression to chronic kidney disease and end-stage kidney disease, both of which are increasing in global frequency. Current specific therapies for chronic kidney disease are limited and end-stage kidney disease treatment is based mostly on replacement therapies such as dialysis or transplantation that are suboptimal and associated with several side effects and a decrease in quality of life. The importance of tissue-specific effectors, acting in kidney epithelial cells, vessels, or the renal interstitium, has been highlighted by several studies over the past few years.[1–3] Their modulation can significantly alter the course and evolution of kidney injury toward chronic kidney disease and end-stage kidney disease. Thus, the therapeutic targeting of these pathways could improve kidney disease management and prognosis.

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Received 26 July 2023; revised 28 December 2023; accepted 2 January 2024; published online 10 January 2024

Classically described in immune cells, the JAK/STAT pathway, has been shown to be important in the kidney in recent years.[4] The JAK/STAT system regulates cell differentiation, growth, and survival. It plays an important role in immune cells, where it is activated by several cytokines. However, it is also expressed in nonimmune cells.[5] For example, STAT3 in podocytes contributes to glomerular injury in experimental glomerulonephritis[6,7] and to podocyte proliferation in experimental HIV-associated nephropathy (HIVAN).[8] In addition, STAT3 activation in tubular cells leads to lipocalin-2 and PDGFβ production with subsequent matrix expansion and fibrosis.[9]

We have previously shown that the common gamma chain (γC, encoded by the *Il2rg* gene), an interleukin subunit receptor that activates the JAK/STAT pathway, is expressed *de novo* by podocytes during human crescentic glomerulonephritis.[1] γC kidney-deficiency aggravates experimental glomerulonephritis in a lymphocyte-independent manner, suggesting a protective role of the γC/JAK/STAT system in kidney cells. Moreover, IL-15 is able to activate downstream JAK/STAT signaling in cultured podocytes in a γC-dependent manner. STAT5 is a transcription factor with 2 isoforms (STAT5A and STAT5B), activated by γC/JAK signaling and expressed by epithelial cells.[10] STAT5 is transactivated by tyrosine phosphorylation and classically regulates proliferation and apoptosis pathways.[11,12] In mice, complete *Stat5* inactivation causes perinatal lethality and reduced development of B cells and T cells.[13] Given the protective role of renal γC during experimental glomerulonephritis, we hypothesized that activation of podocyte STAT5 would protect in glomerular diseases.

We observed an upregulation of glomerular STAT5 in FSGS. Deficiency of STAT5 in podocytes aggravated kidney injury in inflammatory and noninflammatory glomerular disease mouse models. This was due, in part, to an inhibition of autophagic flux in podocytes. Therapeutic stimulation of STAT5 *in vivo* by IL-15 alleviated experimental FSGS and maintained podocyte autophagic flux. Our findings provide a potentially novel target for glomerular disease.

METHODS

Human Tissues

Paraffin-embedded renal biopsy specimens were obtained from the Tenon hospital (Assistance Publique - Hôpitaux de Paris, Paris, France). Human tissue was used after informed consent by all patients and signature of an approval form. The kidney biopsy collection was approved by the Ethics Committee of the Assistance Publique - Hôpitaux de Paris. Kidney biopsy specimens with sufficient tissue for immunohistochemical evaluation after the completion of diagnosis workup were included. We analyzed kidney biopsies from patients with the following diagnosis: HIV-associated nephropathy ($n = 3$), non-HIV–associated nephropathy collapsing FSGS ($n = 4$), and non-collapsing FSGS ($n = 3$). Three-months posttransplant kidney protocol biopsies without any histological abnormality according to 2017 Banff classification were used as controls ($n = 3$).

Animals

All procedures regarding animal experimentation were conducted in accordance with the European Union Guidelines for the Care and Use of Laboratory Animals and approved by the French Ministry of Higher Education and Research (APAFIS#12470-2018022614324645). Animals were housed at a constant temperature with free access to water and food. We used males and females for the experiments.

Mice with a podocyte-specific disruption of *Stat5a* and *Stat5b* (*Nphs2-Cre-*;*Stat5*$^{lox/lox}$) were generated by crossing *Nphs2-Cre* mice[14] with *Stat5*$^{lox/lox}$ (kindly provided by L.Hennighausen[15]) on the C57Bl/6J background. *Nphs2-Cre*;*Stat5*$^{lox/lox}$ mice and control littermates, aged 10 to 12 weeks, were used in this study.

BALB/C mice were obtained from Janvier Labs (Le Genest-Saint-Isle, France). Mice aged 10 to 12 weeks were used for the IL-15-adriamycin experiments.

Experimental Adriamycin Nephropathy

Adriamycin (doxorubicin) (Adriblastine 50 mg, Pfizer, France) or saline was injected intravenously in 10 to 12 weeks-old mice on day 0. As previously described,[16] we used a 10 mg/kg dose for experiments with BALB/C mice and a 25 mg/kg dose for experiments with C57Bl/6J mice, because that genetic background is naturally resistant to adriamycin. To prevent C57Bl/6J mice from excessive weight loss and malnutrition, 1 ml of glucose peritoneal dialysis fluid (Physioneal, Baxter Healthcare Ltd, US) was injected intraperitoneally daily to the mice, as previously described.[16] The mice were weighed on days 0, 2, 3, 4, 6, and 7. Urine samples were collected on day 0 and 7. On day 7, mice were anesthetized with a mix of ketamine and xylazine (10 and 0.1 mg/kg) and blood samples were collected from the retro-orbital sinus. Mice were then euthanized, and kidneys and spleen were collected for analysis.

Experimental Anti-Glomerular Basement Membrane (GBM) Glomerulonephritis

Passive experimental anti-GBM glomerulonephritis was induced using decomplemented sheep antirat GBM

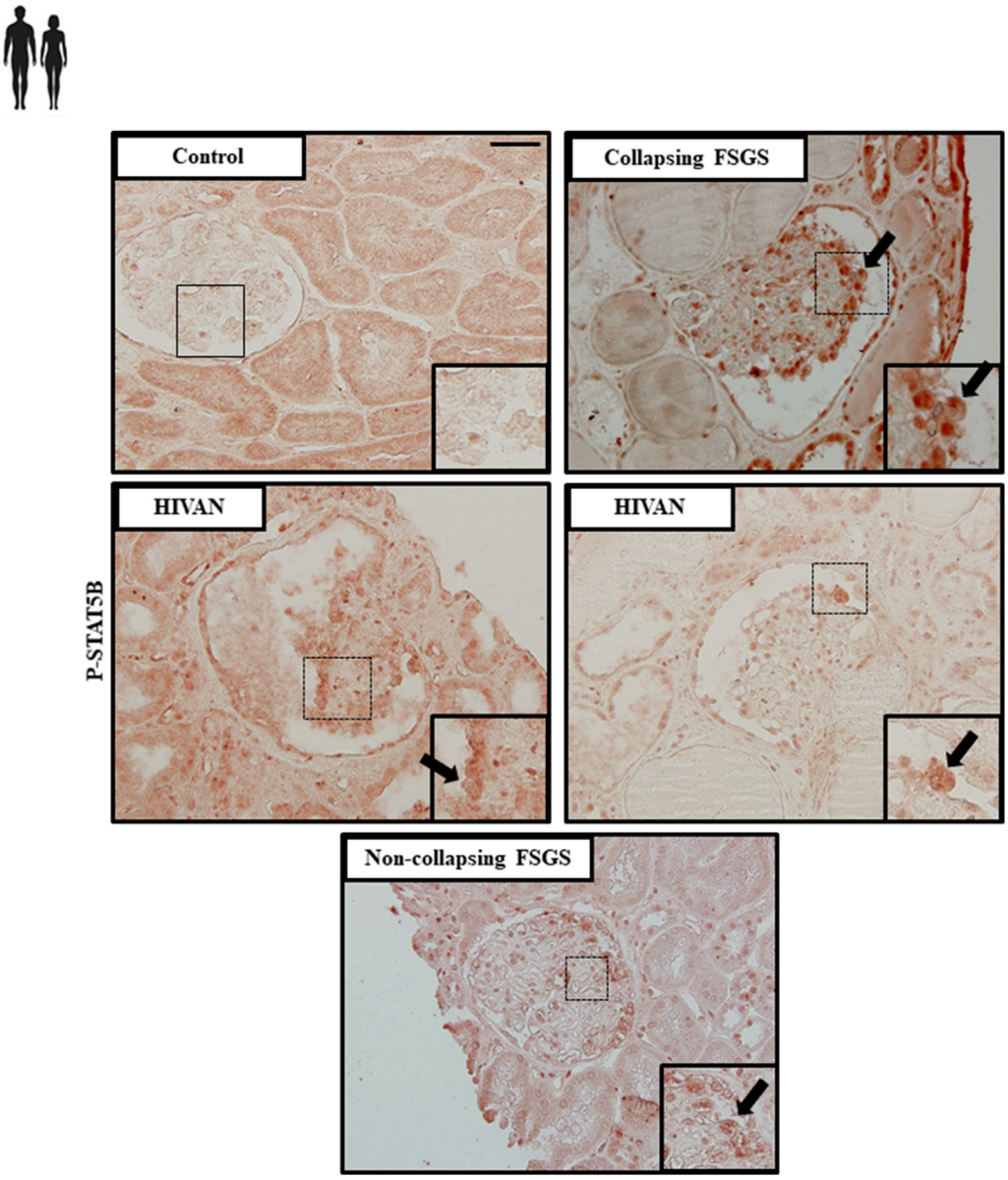

Figure 1. STAT5 activation in podocytes in human focal segmental glomerulosclerosis. Representative images of phospho-STAT5B immunostaining in human kidney biopsies from patients with HIVAN, non-HIVAN collapsing, noncollapsing FSGS and pristine 3 months posttransplant kidney biopsy as a control. Scale bar, 50μm. Higher magnifications are shown in the insets. Each microphotograph corresponds to an individual patient's biopsy. Arrows represent specific nuclear staining. FSGS, focal segmental glomerulosclerosis.

serum prepared as previously reported.[17] Passive nonaccelerated anti-GBM glomerulonephritis was induced by intravenous administration of 1.5 mg total protein/g body weight over 3 consecutive days. Renal injury was evaluated on day 9 following the first injection of anti-GBM serum. As controls, mice were injected with phosphate buffered saline.

Renal Function and Proteinuria Assessment

Plasma urea as well as urine protein and creatinine levels were measured with a Konelab enzymatic spectrophotometric analyzer (ThermoFischer Scientific, Waltham, MA). Urine albumin-to-creatinine ratio was measured using a Beckman Coulter AU 480 analyzer.

Hemograms

Blood samples were analyzed with IDDEX ProCyte Dx hematology analyzer kindly provided by Dr Ivan Moura and Michael Dussiot (Inserm U1163 / CNRS ERL 8254, Institut Imagine, Paris, France).

Quantitative Reverse Transcription–Polymerase Chain Reaction

RNA was extracted from whole kidneys using TRIzol solution (Life Technologies). Residual genomic DNA was removed by DNase I treatment (Thermo Scientific). One μg of total RNA was reverse transcribed using the Maxima First Strand cDNA Synthesis Kit for quantitative reverse transcription–polymerase chain reaction

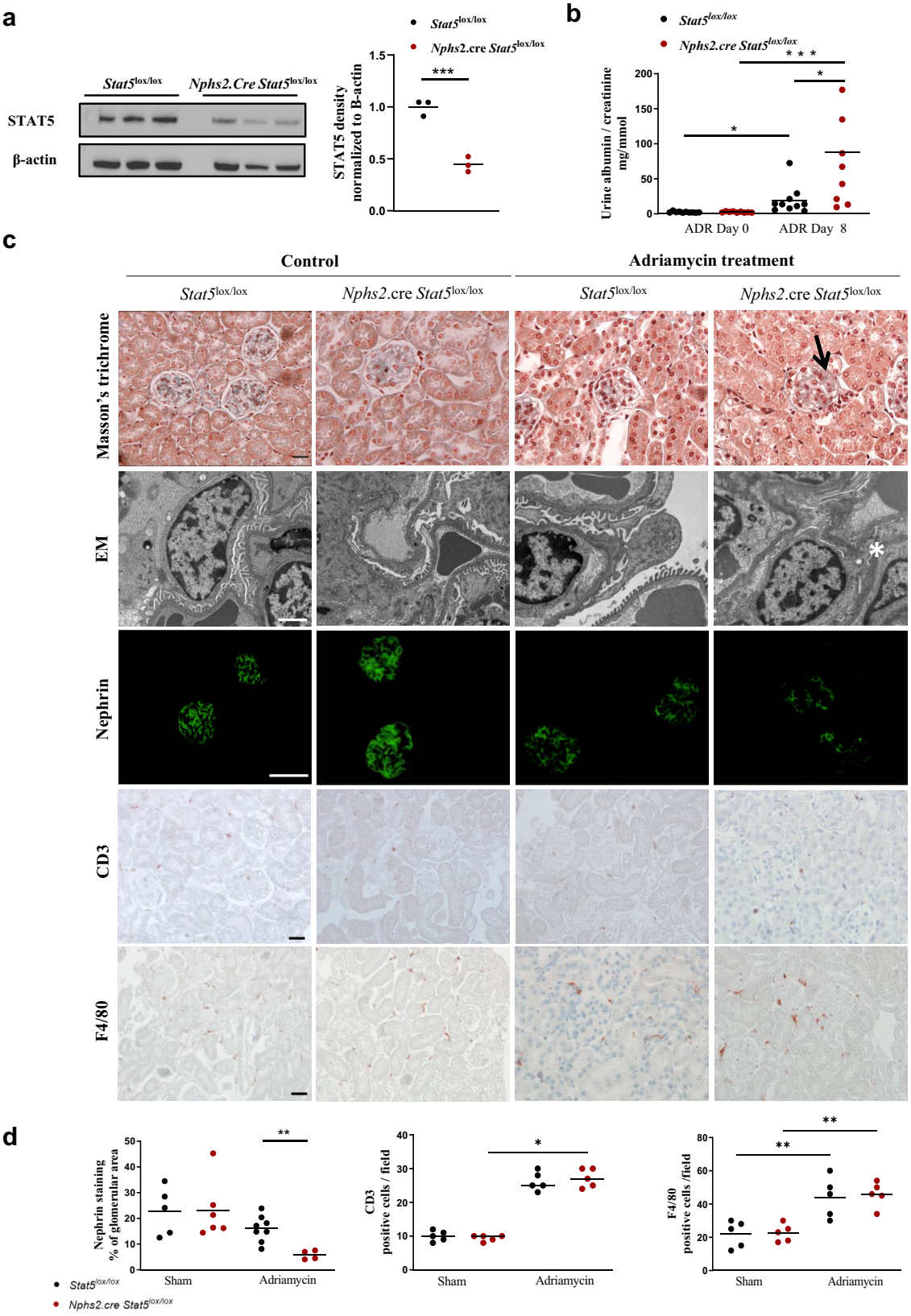

Figure 2. Podocyte STAT5 deficiency aggravates adriamycin-induced albuminuria and glomerular injury. (a) Western blot analysis of STAT5 expression in primary cultured podocytes from glomeruli isolated from 10-week-old *Stat5*$^{lox/lox}$ and *Nphs2*.cre-*Stat5*$^{lox/lox}$ mice. Beta-actin was used as loading control. Quantification of western blot bands for STAT5 normalized to Beta-actin band intensity. ***$P < 0.001$ (b) Urinary albumin-to-creatinine ratio in *Stat5*$^{lox/lox}$ and *Nphs2*.cre-*Stat5*$^{lox/lox}$ mice at basal state and 7 days after adriamycin injection. Individual values are shown, and the bars correspond to means. *$P < 0.05$ and ***$P < 0.001$ (c) Representative images of Masson's trichrome stained sections (upper panels, scale bar: 20μm), transmission electron micrographs (second row panels, scale bar 2μm), nephrin staining (third row panels, scale bar, 20 μM), CD3 staining (fourth row panels, scale bar 20μm) and F4/80 staining (fifth row panels, scale bar 20 μm) (continued)

(Thermo Scientific). *HPRT* was used as endogenous reference housekeeping gene. The sequences of quantitative polymerase chain reaction primer used are listed in Supplementary Table S1. The quantitative polymerase chain reaction was performed using a LightCycler 480 (Roche Diagnostics). The analysis was performed using the $2^{-\Delta Ct}$ method. Results are expressed as arbitrary units, which represent the ratio of the target gene to the internal control gene (HPRT).

Histological and Immunohistochemical Studies

Kidneys were fixed in formalin solution (10%) and embedded in paraffin. Sections (3 μm thick) were processed for histopathology study or immunohistochemistry. Sections were stained with Masson's trichrome, periodic acid–Schiff or Martius Scarlet Blue (Microm Microtech). Tubular lesions (tubular dilation, loss of brush border, and cast formation) were evaluated semiquantitatively using Masson's trichrome-stained or periodic acid–Schiff-stained sections as follows: 0, no lesion; 1, lesions in 1% to 25% of the tubules analyzed; 2, lesions in 26% to 50% of the tubules analyzed; 3, lesions in 51% to 75% of the tubules analyzed; 4, lesions in >76% the tubules analyzed. This tubular injury score was evaluated in 10 randomly selected, nonoverlapping fields in each section by 2 blinded observers.

The proportion of glomeruli with fibrin deposits was evaluated by examination of at least 30 glomeruli per cortical section for each mouse after Martius Scarlet Blue staining, by an examiner (AN) who was blinded to the experimental conditions.

For immunohistochemistry on mouse kidneys, 3 μm paraffin-embedded sections were stained with the following primary antibodies: rabbit anti-CD3 (Abcam, ab5690, 1:150), rat anti-F4/80 (Biorad, MCA97R, 1:200), recombinant anti-Cre (Merck Millipore, 69050, 1:1000), and revealed using Histofine reagent (Nichirei Biosciences). Sections from human kidney biopsies were stained with rabbit antiphospho-STAT5 B (Abcam) or rabbit antiphospho-STAT5A (Cell Signaling) and revealed using Histofine reagent (Nichirei Biosciences).

Nephrin immunofluorescence was performed on 3 μm-thick frozen sections with the antinephrin antibody (AF3159, R & D Systems, 1:200).

Widefield and confocal microscopy were performed using an inverted microscope IX83 (Olympus, Tokyo, Japan) equipped with DSD2 system attached to an Andor Zyla camera (Andor Technology, Belfast, UK). The ImageJ software (National Institutes of Health) was used for the measurement of the surface of glomerular nephrin-positive staining over total glomerular area and the number of CD3 or F4/80 positive cells over section area.

P62, PODXL, and WT1 immunofluorescences were performed on paraffin-embedded mouse kidneys (3 μm sections) with the following antibodies: guinea pig anti-SQSTM1/P62 (1:1000, Progen, GP-62C), goat anti-Podocalyxin (PODXL; 1:1000, Bio-Techne, AF-1556), and rabbit anti-WT1 (1:100, Abcam, ab89901). Secondary antibodies were Alexa 488-conjugated and Alexa 568-conjugated antibodies (Life technologies). Nuclei were stained in blue using Hoechst. Slides were mounted using fluorescent mounting medium (Dako, S3023). Photomicrographs were taken with a Zeiss Axiophot photomicroscope and Axiovision software. Semiautomatic quantifications on Fiji were used for quantifications of P62-positive and PODXL-positive areas per glomerular section on at least 30 glomeruli per mouse. The QuPath software[18] was used to count the number of WT1-positive nuclei per glomerulus on a whole mouse kidney section.

Electron Microscopy

Kidneys were cut into small pieces and immersed in 2.5% glutaraldehyde containing 1% tannic acid in 0.1 mol/l PBS for 2 hours at 4 °C. Samples were postfixed with 1% OsO4, dehydrated, and embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate and then examined under a Philips CM10 electron microscope (Philips Innovation Services, Eindhoven, the Netherlands).

Isolation of Glomeruli and Primary Cultured Podocytes

Freshly isolated renal cortexes from *Stat5*$^{lox/lox}$ and *Nphs2-Cre;Stat5*$^{lox/lox}$ mice were minced and digested by collagenase I (1 mg/ml; Invitrogen) in RPMI 1640 (Invitrogen) for 2 minutes at 37 °C. Next, collagenase I was inactivated by addition of RPMI 1640 plus 10% FBS (Hyclone). Digested tissues were passed through a 100-μm cell strainer. The strainer was rinsed with 15 ml of PBS (Euromedex) containing 0.5% BSA (Euromedex). The flow-through was then passed through a 40-μm cell strainer (BD Falcon). Glomeruli, adherent to the 40-μm cell strainer, were removed with PBS-0.5%

Figure 2. (continued) of the renal cortex from *Stat5*$^{lox/lox}$ and *Nphs2*.cre-*Stat5*$^{lox/lox}$ mice 7 days after the injection of adriamycin. Arrow indicates glomerulosclerosis observed in optical microscopy and * indicates podocyte feet effacement and glomerular basement membrane enlargement observed with EM in *Nphs2*.cre-*Stat5*$^{lox/lox}$ mice. (d) Quantification of nephrin immunostaining, CD3 and F4/80 positive cells in kidneys from *Nphs2*.cre-*Stat5*$^{lox/lox}$ mice compared to *Stat5*$^{lox/lox}$ mice at day 7 after adriamycin injection. Individual values are shown, and the bars correspond to means. *$P < 0.05$. **$P < 0.01$.

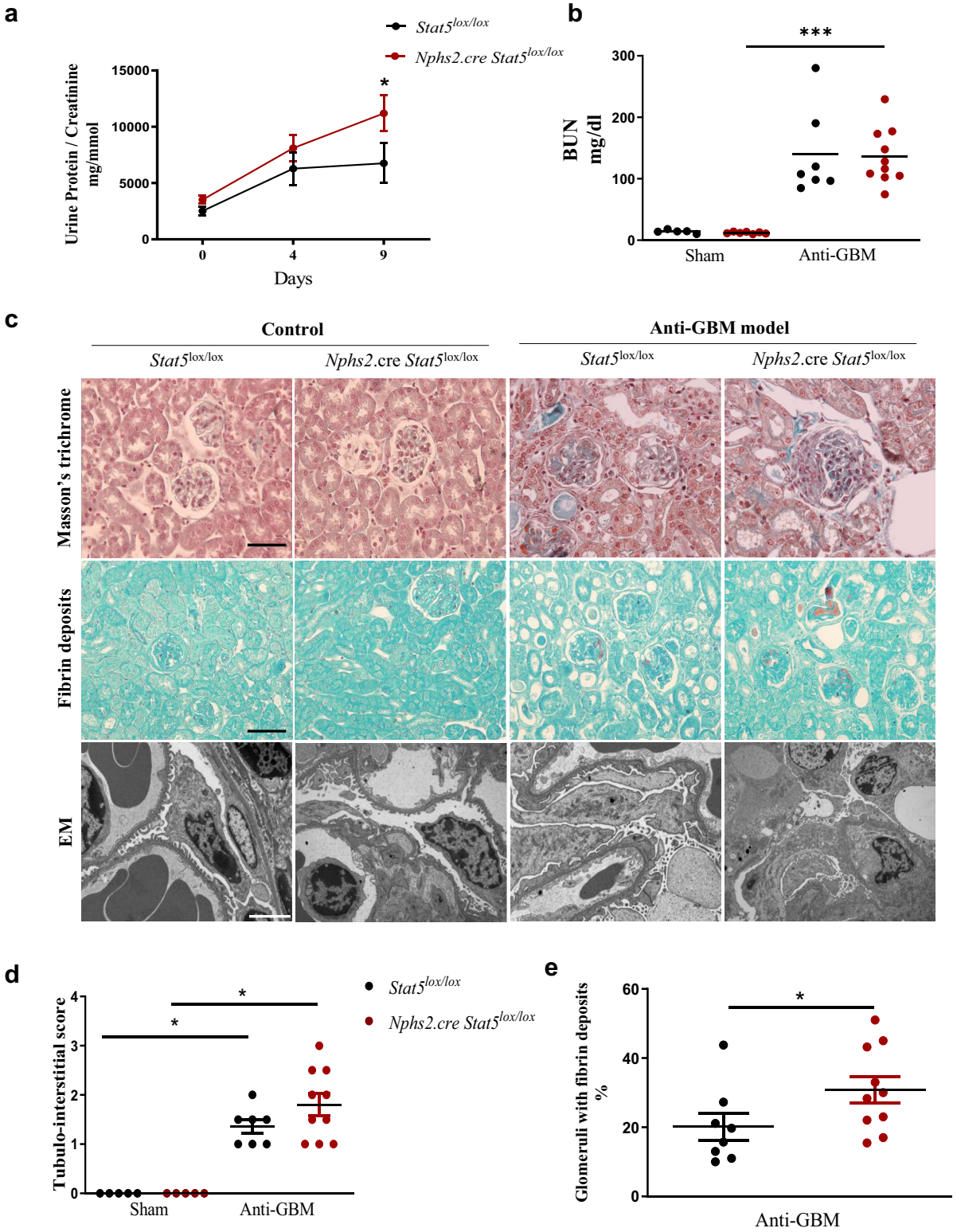

Figure 3. Podocyte STAT5 deficiency aggravates proteinuria and glomerular lesions in anti-glomerular basement membrane experimental glomerulonephritis. (a) Urine protein-to-creatinine ratio at day 0, 4, and 9 after anti-GBM serum injection in *Stat5*$^{lox/lox}$ and *Nphs2*.cre-*Stat5*$^{lox/lox}$ ($n = 7$ to 9 mice per group). Data are mean±SEM. *$P < 0.05$. (b) Plasma BUN in *Stat5*$^{lox/lox}$ and *Nphs2*.cre-*Stat5*$^{lox/lox}$ mice after vehicle or anti-GBM injection. Individual values are shown, and the bars correspond to the means. ***$P < 0.001$, (c) Representative images of Masson's trichrome- (upper panel), Martius Scarlett Blue-stained sections showing glomerular fibrin deposits (middle panel) and transmission electron micrographs (lower panel) of renal cortex from 10–12-week-old anti-GBM-glomerulonephritis-induced *Stat5*$^{lox/lox}$ and *Nphs2*.cre-*Stat5*$^{lox/lox}$ mice and controls. Scale bar, 20 μM for upper panel, 20 μm for middle panel and 2 μm for lower panel (d) Quantification of tubulointerstitial damage in kidneys from *Stat5*$^{lox/lox}$ and *Nphs2*.cre-*Stat5*$^{lox/lox}$ mice at day 9 after anti-GBM serum injection. Individual values are shown, and (continued)

BSA injected under pressure, and finally washed twice in PBS. Freshly isolated glomeruli purity was analyzed by light microscopy and then plated in 6-well plates in RPMI-1640 containing 10% FBS, 2% HEPES buffer (Gibco), and 1% penicillin/streptomycin (Gibco). Two days after seeding, glomeruli became adherent to the dish, and podocytes spread out from glomeruli. Primary podocytes were then lysed in RIPA buffer (Santa Cruz) with orthovanadate, PMSF, protease inhibitor cocktail (Santa Cruz) and NaF for protein extraction.

Statistics

All data are expressed as means±SEM. Statistical analysis was performed using the GraphPad Prism software. The Mann-Whitney test was used to compare 2 groups. The Kruskal-Wallis test was used to analyze the distribution of 3 or more groups. A value <0.05 was considered significant (Supplementary Methods).

RESULTS

STAT5 Activation in Podocytes in Human FSGS

We explored the pathways activated by γC in human glomerular kidney diseases (Supplementary Table S2). STAT5A and STAT5B are activated by phosphorylation and nuclear translocation. Therefore, we performed an immunostaining against phosphorylated STAT5A and STAT5B on kidney biopsies from patients with both collapsing and noncollapsing FSGS. The former is a severe form characterized by apoptosis and proliferation of injured podocytes. We found no significant STAT5A activation in glomerular epithelial cells in FSGS (Supplementary Figure S1). In contrast, there was a significant accumulation of phosphorylated STAT5B in the nucleus of podocytes of patients (Figure 1). Interestingly, STAT5B activation was more intense in collapsing forms of FSGS than in noncollapsing forms. In addition, nuclei expressing higher amounts of phosphorylated STAT5B appeared enlarged.

STAT5 Deficiency in Podocytes Does Not Affect Renal Development in Mice

To further examine the role of STAT5 in podocytes, we generated a mouse model of podocyte-specific knockout of *Stat5* (*Nphs2-Cre;Stat5*$^{lox/lox}$) (Supplementary Figure S2A). We confirmed podocyte-specific STAT5 deficiency by immunoblotting of primary mouse podocytes isolated from control and *Nphs2-Cre;Stat5*$^{lox/lox}$ kidneys (Figure 2a) and podocyte-specific Cre recombinase expression in *Nphs2-Cre;Stat5*$^{lox/lox}$ mice using immunohistochemistry (Supplementary Figure S2B). *Nphs2-Cre;Stat5*$^{lox/lox}$ mice were viable and fertile. Renal structure and function were similar to those of *Stat5*$^{lox/lox}$ littermates (Supplementary Figure S2B–D), suggesting that STAT5 is not essential for podocyte development and baseline function.

Podocyte STAT5 Deficiency Aggravates Adriamycin-Induced Albuminuria and Glomerular Injury

To determine the role of podocyte STAT5 in glomerular injury, we induced adriamycin (doxorubicin) nephropathy, a model of FSGS, in *Nphs2-Cre;Stat5*$^{lox/lox}$ and *Stat5*$^{lox/lox}$ mice. In both groups, adriamycin induced similar and significant weight loss (Supplementary Figure S3A) with a dramatic decrease in circulating leukocytes and platelets, but not hemoglobin (Supplementary Figure S3B). Seven days after adriamycin administration, podocyte STAT5 deficiency was associated with a 5-fold increase in proteinuria (Figure 2b) and more severe glomerular lesions (Figure 2c). Electron microscopy demonstrated podocyte flattening, foot process effacement and GBM thickening in *Nphs2-Cre;Stat5*$^{lox/lox}$ mice (Figure 2c). In addition, nephrin expression was reduced in STAT5-deficient mice compared to controls, with no effect on WT-1 expression (Figure 2c and d; Supplementary Figure S4). These data suggest podocyte dedifferentiation without significant alterations in podocytes number. Adriamycin increased renal T-cell and macrophage infiltration; however, these were unaffected by podocyte STAT5 deficiency (Figure 2c and d). Taken together, these results suggest that podocyte STAT5 protects the glomeruli from injury.

Podocyte STAT5 Deficiency Aggravates Proteinuria and Glomerular Lesions in Anti-GBM Experimental Glomerulonephritis

Next, we induced anti-GBM glomerulonephritis in podocyte-specific STAT5-deficient mice. Although podocyte STAT5 deficiency was associated with increased proteinuria, glomerular injury, and ultrastructural abnormalities (Figure 3a and c) with a trend of decrease in the podocyte number showed by WT-1 expression (Supplementary Figure S5), degree of acute kidney injury and tubulo-interstitialdisease were similar between control and mice with a podocyte STAT5 deficiency (Figure 3b–e). Taken together, these 2 models demonstrate that podocyte STAT5 activation protects against glomerular injury.

Figure 3. (continued) the bars correspond to the means. *$P < 0.05$. (e) Quantification of fibrin glomerular deposits in kidneys from *Stat5*$^{lox/lox}$ and *Nphs2*.cre-*Stat5*$^{lox/lox}$ mice at day 9 after anti-GBM serum injection. Individual values are shown, and the bars correspond to the means. *$P < 0.05$.

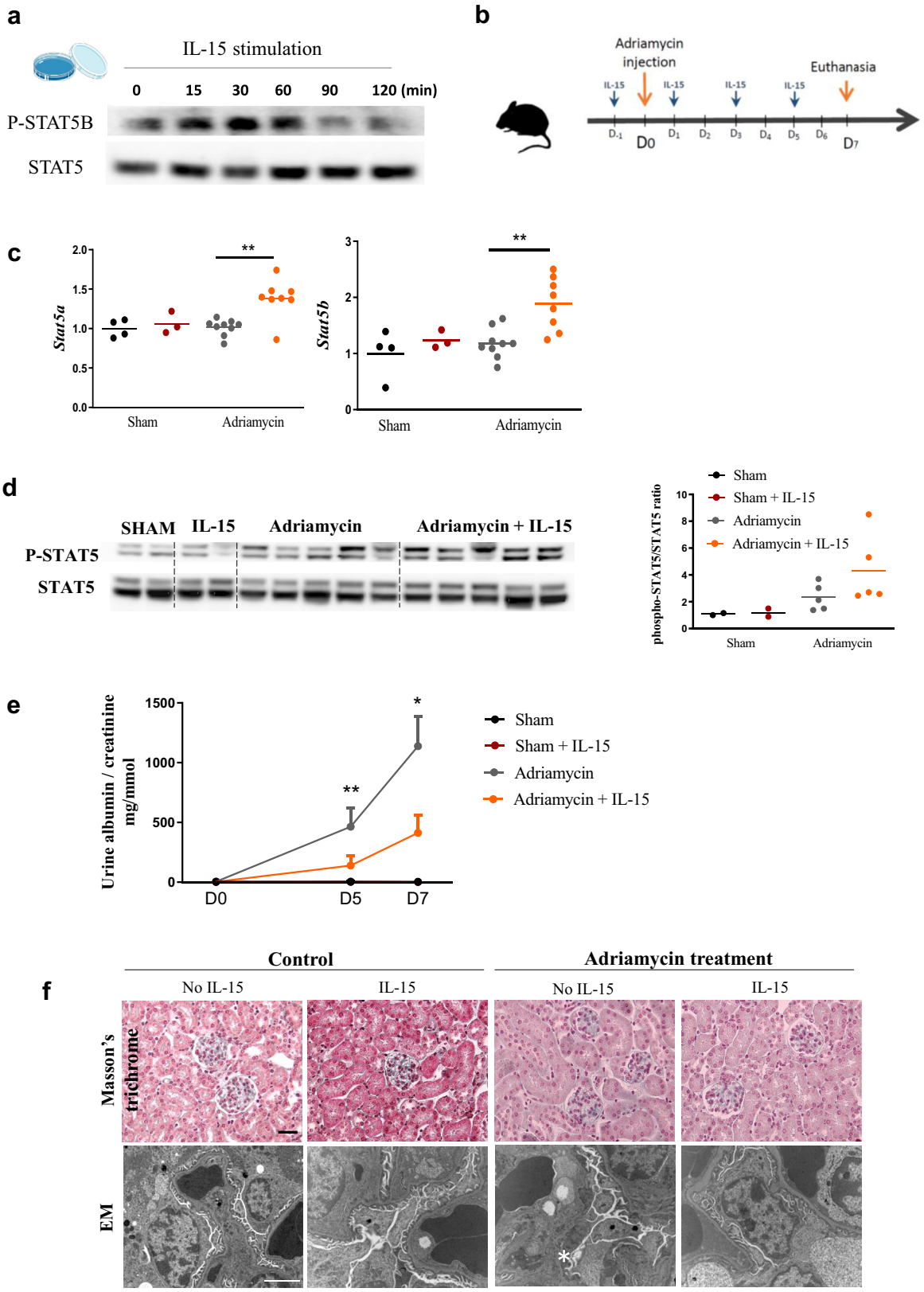

Figure 4. Interleukin-15 activates STAT5 in podocytes and alleviates glomerular injury. (a)Western blot analysis of the expression of phospho-STAT5B and STAT5 in human podocyte cell line after IL-15 stimulation at different time points. (b) Scheme representing experiment details (c) RT-qPCR quantification of the expression of *Stat5a* and *Stat5b* in the kidneys from 12-week-old BALB/C mice on day 7 after adriamycin injection with or without IL-15 treatment. Individual values are shown, and the bars correspond to the means. $^{**}P < 0.01$. (d) Western blot analysis of the expression of phospho-STAT5 and STAT5 in the kidneys from 12-week-old BALB/C mice at day 7 after adriamycin injection with or without IL-15 treatment and its quantification. Individual values are shown, and the bars correspond to the means. (e) Urine albumin-to-creatinine (continued)

IL-15 Administration Alleviates Experimental FSGS in Mice

IL-15 activates STAT5 through γC signaling in immune cells.[19] However, IL-15 is also produced by the renal epithelium,[20] and we have previously shown that IL-15 activates JAK signaling in podocytes.[1] Therefore, we tested if IL-15 could activate STAT5 in human podocytes *in vitro*. Thirty minutes of exogenous IL-15 stimulation increased STAT5B phosphorylation in cultured human podocytes (Figure 4a).

We went on to study the therapeutic effects of IL-15 administration in glomerular disease. The covalent binding of IL-15 to soluble IL-15Rα mimics trans-presentation and increases the bio-stability of IL-15 and its efficacy *in vivo*.[21] In adriamycin-induced FSGS, the administration of the IL-15/IL-15Rα complex (24h prior to, and at 1, 3 and 5 days following adriamycin, Figure 4b) increased renal STAT5A and STAT5B mRNA expression (Figure 4c), with a trend toward increased STAT5 phosphorylation (Figure 4d). In accordance with the hypothesis that STAT5 activation could be protective, we observed that IL-15/IL-15Rα administration significantly reduced adriamycin-induced albuminuria and podocyte ultrastructural abnormalities in mice (Figure 4e and f).

IL-15 administration alone was not associated with any renal effects, although we did observe splenomegaly, in keeping with a role for IL-15 in leucocyte proliferation (Supplementary Figure S6A). Although IL-15/IL-15Rα administration did not modify MCP1 mRNA expression and renal macrophage infiltration, the number of T cell present in the kidney increased (Supplementary Figure S6b–d).

Modulation of the Autophagic Flux and Mitochondrial Activity by STAT5 and IL-15

Autophagy is a critical homeostatic pathway of podocytes, characterized by the removal of dysfunctional organelles and misfolded proteins by enclosure within double membrane vesicles, called autophagosomes, and their subsequent lysosomal fusion and degradation. Autophagy has been shown to be protective in mouse models of glomerular diseases and acute kidney injury.[22–24] Importantly, it is downregulated in several human glomerular pathological conditions.[23–25]

In order to characterize the potential regulation of podocyte autophagic flux by STAT5, we generated human immortalized podocytes[26] with a genetic deletion of *STAT5B* using the Crispr-Cas9 system (Figure 5a) validated in Western blot (Figure 5b). Autophagic flux was measured by quantifying the level of expression of LC3B-II, following serum deprivation and exposure to bafilomycin. As shown in Figure 5c, autophagic flux in $STAT5^{-/-}$ podocytes was decreased compared to $STAT5^{+/+}$ podocytes, suggesting that inhibition of autophagy could be, at least in part, responsible for the severe adriamycin-induced renal alterations observed in mice when STAT5 is inactivated in podocytes. Given that autophagy is important in maintaining mitochondrial function[27–29] and STAT5 is expressed in these organelles,[30] we evaluated the mitochondrial mass *in vitro*. We found a trend toward reduced mitochondrial mass—measured using TOM20 protein abundance—in STAT5 deficient podocytes (Supplementary Figure S7).

Finally, we evaluated the level of autophagy in mouse glomeruli using P62 immunostaining *in vivo*. As expected, we observed an increase in P62 staining, reflecting a decrease in autophagy, in the glomeruli of adriamycin-exposed mice. Interestingly, this staining decreased in the glomeruli of IL-15 treated mice (Figure 5d and e), suggesting that IL-15 administration improves the glomerular autophagic flux in the context of adriamycin-FSGS.

DISCUSSION

STAT5 proteins are classically described in immune cells. Here, we show that the STAT5B transcription factor is induced in podocytes in FSGS. We demonstrate that STAT5 has a protective effect in glomeruli in 2 experimental models of glomerular disease. Moreover, exogenous IL-15 can activate STAT5, maintaining podocyte autophagy and conferring glomerular protection. Therefore, IL-15 offers a potential new therapy for glomerular disease.

The tissue-specific role of STAT5 was first recognized in 1994. This study demonstrated that STAT5 can be activated by prolactin in mammary glands.[11] Since then, several studies have further described the critical role of STAT5 proteins in adipogenesis, cellular differentiation, immune function, and oncogenesis.[12,31] STAT5 also controls cell survival through the regulation of pro-survival genes such as *BCL-XL*.[32] In addition, STAT5 is activated by numerous cytokines such as IL-15, erythropoietin, growth hormone, and prolactin. A protective role for STAT5 proteins has been shown in neuronal cells and in experimental hepatotoxicity, effects which

Figure 4. (continued) ratio at day 0, day 5, and day 7 after adriamycin injection in BALB/C mice treated or not with IL-15. The data represent means±SEM. $*P < 0.05$. $**P < 0.01$. (f) Representative images of Masson trichrome (upper panel; scale bar: 20μM) and transmission electron micrographs (lower panel; scale bar: 2 μm) of kidneys from IL-15 treated and nontreated mice at day 7 after adriamycin treatment. *shows podocyte feet effacement and glomerular basement membrane thickening.

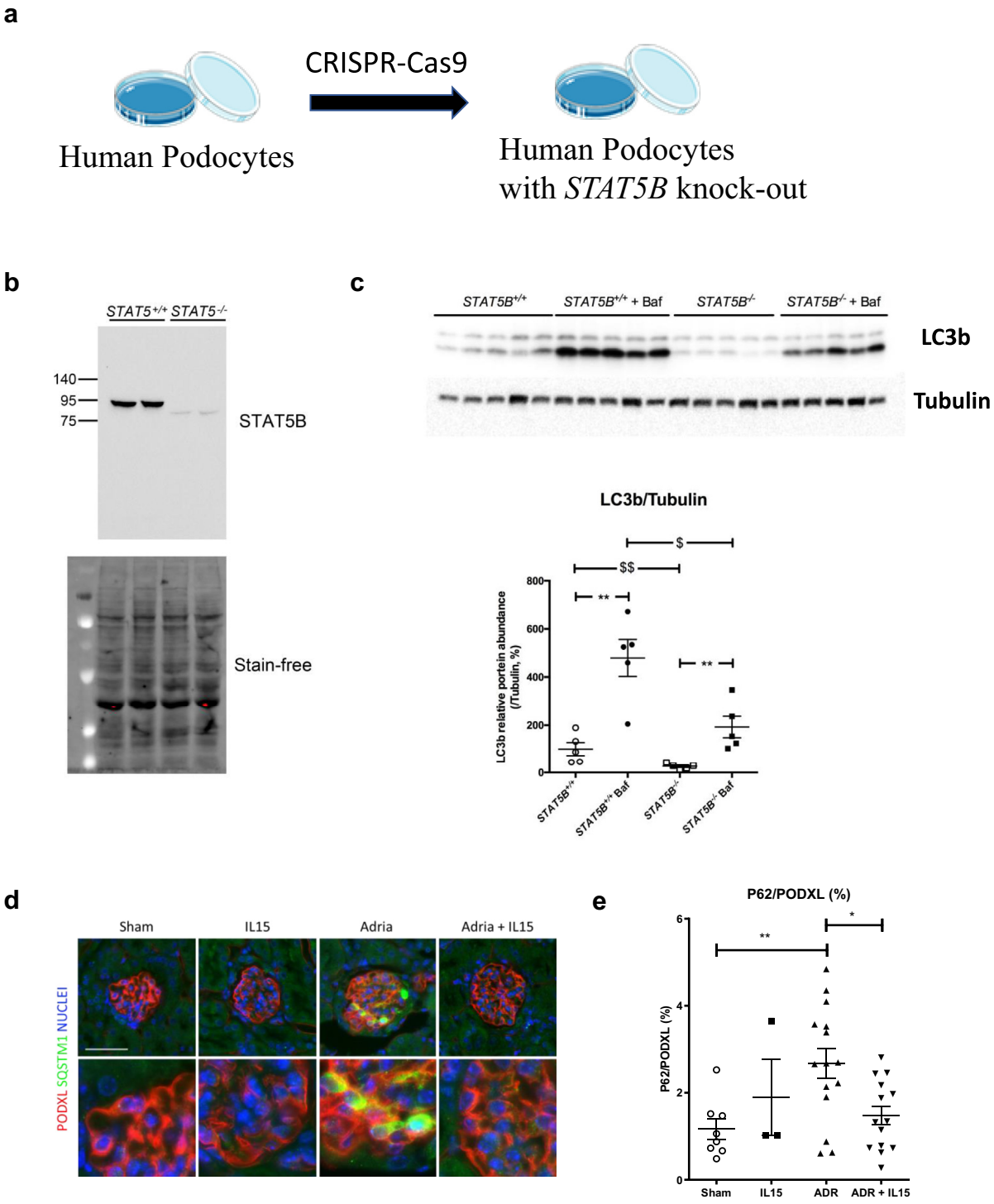

Figure 5. Modulation of the autophagic flux by STAT5 and IL-15 in vitro and *in vivo*. (a) Scheme of CRISPR-Cas9 mediated *STAT5B* knock out in human podocytes. (b) Western blot analysis of the abundance of STAT5B in human podocytes with or without STAT5B deficiency. The stain-free gel serves as normalization. (c) Western blot analysis of the abundance of LC3B-II in human podocytes with or without STAT5B deficiency. Tubulin expression serves as normalization. Podocytes were treated or not treated with bafilomycin A1 (BafA1; 100 nM) for 4 hours before culture arrest. (d) Immunofluorescence of Podocalyxin (PODXL; red), a podocyte marker, and P62/SQSTM1 (green) in glomeruli from wild-type mice at day 7 after adriamycin injection with or without IL-15 treatment showing the accumulation of P62 in podocytes during adriamycin model and decreased by IL-15 treatment. Nuclei were counterstained with Hoechst (blue). Figure subparts with prime indicate higher magnification. Bars = 50 mm. (e) Associated quantification of the P62+ area expressed as the percentage of the P62/PODXL positive area. Values are presented as individual plots and mean±SEM.

are mediated through antiapoptotic factors such as Bcl-xL.[32] With respect to the kidney, Fragiadaki *et al*. demonstrated that tubular STAT5 activation promotes cell proliferation and cyst formation in a model of polycystic kidney disease.[33] In addition, recent work defined STAT5 as a key regulator of repair following experimental ischemic acute kidney injury.[34]

The finding that STAT5 is activated in human podocytes is novel. In glomerular disease, the degree of STAT5 activation appeared to relate to disease severity, because biopsies of patients with more severe collapsing variants of FSGS showed greater STAT5 activation within enlarged nuclei. Interestingly, aberrant podocyte mitotic activity with enlarged nuclei has been described in FSGS.[35-37] STAT5 proteins could thus play a role in cellular proliferation, as suggested by Fragiadaki *et al*. in tubular cells. Our results also show an upregulation of STAT5 in injured human tubular cells, which displayed nuclear enlargement which might be explained by tubular proliferation.

There are few prosurvival pathways described in podocytes. These include peroxisome proliferator activated receptor-gamma, Kruppel-like factors and BMP7.[3,7,38] However, the vast majority do not have commercially available agonists and often exert opposing effects on glomerular and tubular cells, leading to potential side effects if used in kidney diseases. For example, peroxisome proliferator activated receptor-gamma can be activated by thiazolidinediones once used in diabetes.[39] Unfortunately, thiazolidinediones are no longer commercially available due to their hepatotoxicity and cardiovascular side effects promoted by salt retention from the kidney.[40,41]

Recent work on STAT proteins in kidney disease has focused mainly on STAT3. STAT3 activation promotes deleterious effects both on podocyte (experimental glomerulonephritis and HIVAN)[7,8] and on tubular cells by stimulating fibrosis.[9] Compared to STAT3, STAT5 activation has an opposite (positive) effect in glomerular epithelium suggesting that a STAT3/STAT5 balance might exist in epithelial cells similar to that seen in immune cells. In lymphocytes, STAT3 promotes Th17 differentiation and represses regulatory T cell function, whereas STAT5 promotes regulatory T cell differentiation and suppresses Th17 differentiation.[42] STAT3 and STAT5 also have opposing effects on apoptosis in B lymphocytes.[43] Therefore, we propose that a similar STAT3/STAT5 balance might exist in the kidney, and that this balance is disrupted in kidney disease.

We show here that STAT5 participates in the activation or maintenance of autophagy and mitochondrial mass, both essential prosurvival pathways in podocytes. Furthermore, IL-15 activates STAT5 in podocytes and alleviates glomerular injury by maintaining the autophagic flux, at least in part. Autophagy activation by an exogenous compound *in vivo* is an unmet need that could be overcome by IL-15. IL-15 or its super agonist ALT-803, is commercially available with several ongoing clinical trials in cancer.[44,45] Previously, we demonstrated that podocytes express IL-15 receptors during human crescentic glomerulonephritis.[1] Further studies are needed to determine the minimal effective dose of IL-15 in a range of experimental models of kidney disease. Importantly, the kidney is a potential producer of IL-15 following injury, and this endogenous IL-15 can limit kidney damage. This is supported by data from global *Il15* knockout mice, which display accentuated tubular injury in experimental glomerulonephritis.[20]

We acknowledge some limitations of our study. First, the respective roles of STAT5A and STAT5B in the kidney epithelium are not fully understood. Although we observed a strong activation of STAT5B in human kidney, additional studies are needed to resolve this point. Our *in vivo* studies cannot differentiate between the respective roles of STAT5A and STAT5B because the loci for both were deleted in the original construct designed by Hennighausen *et al*.[15] Besides, the efficiency of Cre-mediated deletion in *Nphs2-Cre* mice is not complete because it can be seen with other Cre recombinase-mediated genetic deletion *in vivo*. However, our results show that a complete podocyte deletion is not needed to induce a deleterious phenotype during glomerular disease models. The role of other STAT proteins when STAT5 is absent in the podocyte is not fully understood and would need further studies. Finally, our data cannot exclude that IL-15 has other effectors than STAT5 to provide its protective role to the podocyte.

Altogether, our data demonstrate that STAT5 is activated in podocytes during FSGS and has an important protective role in activating autophagy. Activating podocyte STAT5 represents a new therapeutic avenue with the potential for a range of beneficial effects in glomerular disease.

DISCLOSURE

All the authors declared no competing interests.

ACKNOWLEDGMENTS

This work was supported by Institut National de Santé et de la Recherche Médicale (Inserm), Sorbonne Université and a research grant from the French Agence Nationale de la Recherche (PodoGammaC grant ANR JC to YL). We are grateful to Assistance Publique - Hôpitaux de Paris (Médaille d'argent et Fonds d'Etudes et de Recherche du Corps Médical) and the Société Francophone de Néphrologie,

Dialyse et Transplantation (SFNDT) for supporting Y.L. We thank Liliane Louedec, Jessy Renciot, Claude Kitou, and Gaetan Girault for assistance in animal care and handling, Nicolas Sorhaindo (CRI – Plateforme de Biochimie UMR 1149 Université Paris Diderot, Paris, France) and Gaelle Brideau (Renal Metabolism and Physiology Laboratory, Centre de Recherche des Cordeliers, UMRS 1138, ERL 8228, Paris, France) for biochemical measurements. We acknowledge excellent administrative support from Mélanie Charlery and Emmanuelle Bénard. XX, HD, CS, were funded by Société Francophone de Néphrologie Dialyse et Transplantation (SFNDT). This work has benefited from the facilities and expertise of the CoRaKiD histology and imaging core facilities (Common and Rare Kidney Diseases Unit, INSERM UMR_S1155, Paris, France).

Funding for this study was provided by ANR JC (Agence Nationale de la Recherche, Jeune Chercheur) PODOGAMMAC - ANR-17-CE14-0004; and Francophone Society of Nephrology, Dialysis and Transplantation (SFNDT) grant for translational research project on podocytic diseases (2017)

DATA AVAILABILITY STATEMENT

The authors declare that the data supporting the findings of this study are available within the paper and its supplementary information files. All data are available from the corresponding authors upon reasonable request.

AUTHOR CONTRIBUTIONS

AN contribute to conceptualization, methodology, validation, investigation, formal analysis, writing, and visualization. OL contributed to investigation, methodology, and writing. CS contributed to methodology, investigation, and writing. KL, XX, ACou, HD, Acor, SP, SV, and PG contributed to methodology and investigation. LH contributed to resources. PF contributed software, investigation, and formal analysis. BS, SO, TM, and NY contributed to investigation. LL contributed to resources and investigation. M-CV an Y-CX-D contributed to investigation and visualization. DB contributed to resources and methodology. HF contributed to conceptualization and methodology. ER contributed to conceptualization, supervision, resources, and writing. LM contributed to conceptualization, methodology, supervision, and writing. JH contributed to investigation, methodology, design, supervision, and writing. YL contributed to investigation, conceptualization, methodology, supervision, writing, project administration, and funding acquisition.

SUPPLEMENTARY MATERIALS

Supplementary File (PDF)

Supplementary Methods.

Figure S1. Phospho-STAT5A is not detected in glomerular epithelial cells in FSGS.

Figure S2. STAT5 deficiency in podocytes does not affect renal development in mice.

Figure S3. STAT5 deficiency in podocytes has no influence on depletion of blood cells induced by adriamycin treatment.

Figure S4. WT-1 expression in glomeruli during adriamycin nephropathy.

Figure S5. WT-1 expression in glomeruli during anti-GBM nephropathy.

Figure S6. Interleukin-15 immune effect in adriamycin-induced nephropathy.

Figure S7. Mitochondrial activity is affected by STAT5B deficiency in human podocytes *in vitro*.

Table S1. Primers used for RT-qPCR.

Table S2. Patient's characteristics.

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