



(51) International Patent Classification:

A61K 31/7076 (2006.01) A61P 13/12 (2006.01)

A61K 38/17 (2006.01)

(21) International Application Number:

PCT/EP2019/075468

(22) International Filing Date:

23 September 2019 (23.09.2019)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

18306233.0 24 September 2018 (24.09.2018) EP

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(81) Designated States (unless otherwise indicated, for every

kind of national protection available): AE, AG, AL, AM,

AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ,

CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO,

DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN,

HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP,

KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME,

MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ,

OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA,

SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN,

TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every

kind of regional protection available): ARIPO (BW, GH,

GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ,

UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

— with sequence listing part of description (Rule 5.2(a))

(54) Title: USE OF EPAC1 ACTIVATORS FOR THE TREATMENT OF CHRONIC KIDNEY DISEASES

(57) Abstract: Chronic Kidney Disease (CKD) is a major burden of public health affecting millions of people around the world. It is rising in prevalence with high public health costs and is associated with a high degree of morbidity and mortality. Here, the inventors determined whether the exchange protein directly activated by cAMP 1 (Epac1) contributed to severe glomerulonephritis (GN) induced by injecting nephrotoxic serum (NTS) in mice. Treatment of GN mice with a pharmacological activator of Epac1, 8-(4-Chlorophenylthio)-2'-O-methyladenosine-3', 5'-cyclic monophosphate (8-pCPT) improved functional and structural renal parameters, and reduced renal inflammation after NTS injection. Taken together, these findings suggest that Epac1 activation is an attractive and promising pharmacological intervention for the therapeutic treatment of CKD. Thus the present invention relates to use of Epac1 activators for the treatment of chronic kidney diseases..

USE OF EPAC1 ACTIVATORS FOR THE TREATMENT OF CHRONIC KIDNEY DISEASES

FIELD OF THE INVENTION:

The present invention relates to use of Epac1 activators for the treatment of chronic kidney diseases.

BACKGROUND OF THE INVENTION:

Chronic Kidney Disease (CKD) is a major burden of public health affecting millions of people around the world. It is rising in prevalence with high public health costs and is associated with a high degree of morbidity and mortality. Diabetes and hypertension are the predominant causes of CKD, accounting for approximately 44% and 30% of incident CKD cases. Even though many aspects of the complex mechanisms orchestrating progression of renal disease have been identified, so far there is no specific treatment to slow down or prevent CKD progression.

Cyclic AMP is a universal second messenger, and its production is initiated upon binding of a wide range of extracellular ligands to G protein-coupled receptors and subsequent activation of membrane-bound adenylyl cyclases (ACs), which generate cAMP (Robichaux and Cheng, 2018). Historically, the biological effects of cAMP have been attributed to phosphorylation events catalyzed by the protein kinase A (PKA). Recently, a family of proteins directly activated by cAMP, named Epac was discovered as an important new regulator of cAMP mediated signaling (Robichaux and Cheng, 2018). The Epac family members, Epac1 and Epac2, act as guanine exchange factors (GEFs) for the small G-proteins Rap1 and Rap2, and function in a PKA-independent manner (de Rooij et al., 1998; Kawasaki et al., 1998; Breckler et al., 2011). In human and rodents Epac1 is abundantly expressed in the kidney and is the major Epac isoform expressed in this tissue (de Rooij et al., 1998; Kawasaki et al., 1998; Robichaux and Cheng, 2018).

Although the role of PKA in kidney pathophysiology has been largely studied, the impact of Epac remains relatively enigmatic.

SUMMARY OF THE INVENTION:

The present invention relates to use of Epac1 activators for the treatment of chronic kidney diseases. In particular, the present invention is defined by the claims.

DETAILED DESCRIPTION OF THE INVENTION:

Here, the inventors determined whether the exchange protein directly activated by cAMP 1 (Epac1) contributed to severe glomerulonephritis (GN) induced by injecting nephrotoxic serum (NTS) in mice. They showed that the glomerular expression of Epac1 was decreased in mice treated with NTS. Notably, Epac1 genetic ablation accelerated the decline of renal function compared with Wild-Type (WT) NTS treated animals. Crescent formation and tubular dilation were increased in Epac1 knock-out (KO) versus WT animals indicating that decreased Epac1 expression also potentiated the deterioration of renal structure during the progression of GN. Additionally, Epac1 KO mice showed exacerbated renal infiltration of inflammatory cells and interstitial renal fibrosis. Of particular importance, treatment of GN mice with a pharmacological activator of Epac1, 8-(4-Chlorophenylthio)-2'-O-methyladenosine-3', 5'- cyclic monophosphate (8-pCPT) improved functional and structural renal parameters, and reduced renal inflammation after NTS injection. Taken together, these findings suggest that Epac1 activation is an attractive and promising pharmacological intervention for the therapeutic treatment of CKD.

Accordingly, the first object of the present invention relates to a method of treating a chronic kidney disease in a patient in need thereof comprising administering to the patient a therapeutically effective amount of at least one Epac1 activator.

As used herein, the term "chronic kidney disease" or "CKD" has its general meaning in the art and refers to a progressive loss in renal function over a period of months or years. CKD is used to classify numerous conditions that affect the kidney, destruction of the renal parenchyma and the loss of functional nephrons or glomeruli. It should be further noted that CKD can result from different causes, but the final pathway remains renal fibrosis. CKD is defined as kidney damage or glomerular filtration rate (GFR) $<60 \text{ mL/min/1.73 m}^2$ for 3 months or more, irrespective of cause. Kidney damage in many kidney diseases can be ascertained by the presence of albuminuria, defined as albumin-to-creatinine ratio $>30 \text{ mg/g}$ in two of three spot urine specimens. GFR can be estimated from calibrated serum creatinine and estimating equations, such as the Modification of Diet in Renal Disease (MDRD) Study equation or the Cockcroft-Gault formula. Kidney disease severity is classified into five stages according to the level of GFR. Examples of etiology of CKD include, but are not limited to, cardiovascular diseases, hypertension, diabetes, glomerulonephritis, polycystic kidney diseases, and kidney graft rejection. In some, the patient in need thereof suffers from a disease selected from the group consisting of nephropathy (e.g. membranous nephropathy (MN), diabetic nephropathy and hypertensive nephropathy), glomerulonephritis (e.g. membranous glomerulonephritis and membranoproliferative glomerulonephritis (MPGN) such as rapidly progressive

glomerulonephritis (RPGN)), interstitial nephritis, lupus nephritis, idiopathic nephrotic syndrome (INS) (e.g. minimal change nephrotic syndrome (MCNS) and focal segmental glomerulosclerosis (FSGS)), obstructive uropathy, polycystic kidney disease (e.g. Autosomal Dominant Polycystic Kidney Disease (ADPKD) and Autosomal Recessive Polycystic Kidney Disease (ARPKD)), cardiovascular diseases, hypertension, diabetes, and kidney graft rejection (e.g. acute and chronic kidney rejection).

As used herein the term “Epac” has its general meaning in the art and refers to the guanine exchange factor (GEF) Epac (Exchange Protein directly Activated by Cyclic AMP). There are two isoforms of Epac, Epac1 and Epac2, both consisting of a regulatory region binding directly cAMP and a catalytic region that promotes the exchange of GDP (Guanosine diphosphate) for GTP (Guanosine-5'-triphosphate) on the Ras-like small GTPases Rap1 and Rap2 isoforms). The two isoforms of Epac differ in that Epac1 has a single cyclic nucleotide-binding (CNB) domain, whereas Epac2 has two CNB domains, called CNB-A and CNB-B, which are located on both sides of the DEP domain (Dishevelled, Egl-10 and Pleckstrin domain). The additional N-terminal CNB domain in Epac2 has a low affinity for cAMP, and its deletion does not affect the regulation of Epac2 in response to agonists (*J. de Rooij, H. Rehmann, M. van Triest, et al., Mechanism of regulation of the Epac family of cAMP-dependent RapGEFs, J. Biol. Chem. 275 (2000) 20829-20836*). An exemplary human amino acid sequences is represented by SEQ ID NO:1.

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SEQ ID NO:1 >sp|095398|RPGF3_HUMAN Rap guanine nucleotide exchange
factor 3 OS=Homo sapiens OX=9606 GN=RAPGEF3 PE=1 SV=6
MKVGWPGESCWQVGLAVEDSPALGAPRVGALPDVVPEGTLLNMVLRMRHPRSCSYQLLL
EHQRPSCIQGLRWTPLTNSEESLDFSESLEQASTERVLRAGRQLHRHLLATCPNLIIRDK
YHLRLYRQCCSGRELVDGILALGLGVHSRSQVVGICQVLLDEGALCHVKHDWAFQDRDAQ
FYRFPGPEPEPVRTHEMEEELAEAVALLSQRGPDALLTVALRKPPGQRTDEELDLIFEEL
LHIKAVAHLSNSVKRELAALLFEPHSHKAGTVLFSQDGKTSWYIIWKGSVNVVTHGKGL
VTTLHEGDDFGQLALVNDAPRAATIIILREDNCHFLRVDKQDFNRIIKDVEAKTMRLEEKG
KVVLVLERASQGAGPSRPPTPGRNRYTVMSGTPEKILELLLEAMGPDSSAHDPPTETFLSD
FLLTHRVFMPSAQLCAALLHHFHVEPAGGSEQERSTYVCNKRQQILRLVSQWVALYGSML
HTDPVATSFLQKLSDLVGRDTRLNLLREQWPERRRCHRENGCGNASPQMKARNLPVWL
PNQDEPLPGSSCAIQVGDKVPYDICRPDHSVLTLLQLPVTASVREVMALAQEDGWTGQV
LVKVNSAGDAIGLQPDARGVATSLGLNERLFVVNPQEVHELIPHPDQLGPTVGSAGGLDL
VSAKDLAQQLTDHDWSLFNSIHQVELIHVYLGPHLRDVTANLERFMRRFNEQYVWAT
ELCLCPVPGPRAQLLRKFIKLAHLKEQKNLSFFAVMFGLSNSAISRLAHTWERLPHKV
RKLYSALERLLDPSWNHRVYRLALAKLSPPVIPFMPLLLKDMTFIHEGNHTLVENLINFE
KMRMMARAARMLHCRSHNPVPLSPLRSRVSHLHEDSQVARISTCSEQSLSTRSPASTWA
YVQQLKVIDNQRELSRLSRELEP

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As used herein the term “Epac1 activator” refers to any compound that is able to activate or increase the activity or expression of Epac1. Epac1 activators are known in the art. Typically, the Epac1 activator is a cAMP analog, more preferably a cAMP analog which has no effect on the activity of protein kinase A (PKA) and moderate or no inhibitory effect on Epac2 isoform. Suitable compounds for use in the present invention are the cAMP analogs disclosed in WO2003104250. In some embodiments, the Epac1 activator is 8-(4-Chlorophenylthio)-2'-O-methyladenosine-3',5'-cyclic monophosphate (also known as 8-pCPT-2'-O-Me-cAMP). Of course, other known or yet to be identified (small molecule) agents capable of activating Epac1, preferably without affecting PKA activity, may also be used.

In some embodiments, the Epac1 activator is an activator of Epac1 expression. An “activator of expression” refers to a natural or synthetic compound that has a biological effect to increase the expression of a gene or to prevent its degradation. Therefore, an “activator of Epac1 expression” denotes a natural or synthetic compound that has a biological effect to increase the expression of Epac1 gene or prevent the degradation of Epac1 mRNA or protein. In some embodiments, the activator is a nucleic acid that encodes for a Epac1 polypeptide (e.g. SEQ ID NO:1) that can be administered to the patient. In some embodiments, the nucleic acid molecule of the present invention is included in a suitable vector, such as a plasmid, cosmid, episome, artificial chromosome, phage or a viral vector. So, a further object of the invention relates to a vector comprising a nucleic acid encoding for a mutated FX polypeptide of the invention. Typically, the vector is a viral vector which is an adeno-associated virus (AAV), a retrovirus, bovine papilloma virus, an adenovirus vector, a lentiviral vector, a vaccinia virus, a polyoma virus, or an infective virus. In some embodiments, the vector is an AAV vector. As used herein, the term “AAV vector” means a vector derived from an adeno-associated virus serotype, including without limitation, AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, and mutated forms thereof. Typically, the nucleic acid molecule or the vector of the present invention include “control sequences”, which refers collectively to promoter sequences, polyadenylation signals, transcription termination sequences, upstream regulatory domains, origins of replication, internal ribosome entry sites (“IRES”), enhancers, and the like, which collectively provide for the replication, transcription and translation of a coding sequence in a recipient cell. Not all of these control sequences need always be present so long as the selected coding sequence is capable of being replicated, transcribed and translated in an appropriate host cell. Another nucleic acid sequence, is a “promoter” sequence, which is used herein in its ordinary sense to refer to a nucleotide region comprising a DNA regulatory

sequence, wherein the regulatory sequence is derived from a gene which is capable of binding RNA polymerase and initiating transcription of a downstream (3'-direction) coding sequence. Transcription promoters can include "inducible promoters" (where expression of a polynucleotide sequence operably linked to the promoter is induced by an analyte, cofactor, regulatory protein, etc.), "repressible promoters" (where expression of a polynucleotide sequence operably linked to the promoter is induced by an analyte, cofactor, regulatory protein, etc.), and "constitutive promoters".

As used herein, the term "effective amount" refers to a quantity sufficient of the Epac1 activator to achieve treatment of CKD. In the context of therapeutic or prophylactic applications, the amount of a composition administered to the subject will depend on the type and severity of the disease and on the characteristics of the individual, such as general health, age, sex, body weight and tolerance to drugs. It will also depend on the degree, severity and type of disease. The skilled artisan will be able to determine appropriate dosages depending on these and other factors. In some embodiments, an effective amount of the Epac1 activator for achieving a therapeutic or prophylactic effect, range from about 0.000001 mg per kilogram body weight per day to about 10,000 mg per kilogram body weight per day. Typically, the dosage ranges are from about 0.0001 mg per kilogram body weight per day to about 100 mg per kilogram body weight per day. For example dosages can be 1 mg/kg body weight or 10 mg/kg body weight every day, every two days or every three days or within the range of 1-10 mg/kg every week, every two weeks or every three weeks. In some embodiments, a single dosage of peptide ranges from 0.1-10,000 micrograms per kg body weight. In some embodiments, aromatic- cationic peptide concentrations in a carrier range from 0.2 to 2000 micrograms per delivered milliliter.

Typically, the Epac1 activator is combined with pharmaceutically acceptable excipients, and optionally sustained-release matrices, such as biodegradable polymers, to form pharmaceutical compositions. "Pharmaceutically" or "pharmaceutically acceptable" refer to molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when administered to a mammal, especially a human, as appropriate. A pharmaceutically acceptable carrier or excipient refers to a non-toxic solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. Typically, the pharmaceutical compositions contain vehicles, which are pharmaceutically acceptable for a formulation capable of being injected. These may be in particular isotonic, sterile, saline solutions (monosodium or disodium phosphate, sodium, potassium, calcium or magnesium chloride and the like or mixtures of such salts), or dry, especially freeze-dried compositions

which upon addition, depending on the case, of sterilized water or physiological saline, permit the constitution of injectable solutions. The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil or aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. Sterile injectable solutions are prepared by incorporating the Epac1 activator in the required amount in the appropriate solvent with several of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

The invention will be further illustrated by the following figures and examples. However, these examples and figures should not be interpreted in any way as limiting the scope of the present invention.

FIGURES:

Figure 1. Epac1 is downregulated in GN and Epac1 gene deletion worsens the progression of NTS-induced GN, monitored by body weight increase. (A) Upper panel. Representative immunoblots of Epac1 and Epac2 isoforms. The GAPDH (glyceraldehyde-3-phosphate dehydrogenase) is the loading control. Graph. Quantification of Epac1 protein in wild-type (WT) glomeruli mice subjected or not to NTS injection. Epac1 expression was determined at day 15. (B) Body weight increase. n=6 to 7 per group. *: $p < 0.05$, **: $p < 0.01$, vs indicated values (ANOVA). n=7 mice per group. D, number of days after NTS injection.

Figure 2. Epac1 genetic inhibition accelerates deterioration of renal function and has a deleterious effect on maintenance of renal structure in the experimental model of NTS-induced GN. Renal function was evaluated by proteinuria (A) and BUN (B). NTS-induced alterations of renal structure was more important in Epac1 KO mice because they developed more glomerular crescents (C) and tubular dilation compared with WT treated NTS mice (D). Crescents are expressed as the percentage of glomeruli presenting cellular crescents.

n=7 mice per group. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, vs indicated values (ANOVA). D, number of days after NTS injection.

Figure 3. Infiltration of macrophage and T cells are highly increased in the kidney of Epac1 KO mice. (A) Evaluation of the percentage of positive area for F4/80 and (B) CD3ε staining at day 15 in WT and Epac1 KO kidney treated or not with NTS. n=7 mice per group. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, vs control or WT+NTS as indicated (ANOVA).

Figure 4. Epac1 gene deletion dramatically increases expression of renal pro-inflammatory markers. (A-C) Mice were treated and measurements of mRNA of pro-inflammatory markers were made at day 15. n=7 mice per group. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, vs control or WT+NTS as indicated (ANOVA).

Figure 5. Fibrotic and renal cell stress markers are increased in Epac1 KO mice after induction of NTS GN. (A, B) Fibrotic and (C, D) renal cell stress markers were determined by quantitative PCR as described in methods. n=7 mice per group. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, vs control or WT+NTS as indicated (ANOVA).

Figure 6. Deletion of Epac1 increases renal fibrosis assessed by Sirius red coloration. Quantification of fibrillar collagen at day 15. n=7 mice per group. *: $p < 0.05$, **: $p < 0.01$, vs control or WT+NTS as indicated (ANOVA).

Figure 7. Epac1 pharmacological activation protects against NTS-induced GN. (A) Body weight evolution and (B, C) renal function parameters (proteinuria and serum creatinine). n=8 or 4 mice for NTS or PBS groups, respectively. *: $p < 0.05$, **: $p < 0.01$, vs control or 8-pCPT+NTS as indicated (ANOVA).

Figure 8. Activation of Epac1 with 8-pCPT preserves renal structure and reduces renal macrophage infiltration. (A) Renal structure was preserved in NTS injected mice treated with 8-pCPT, because they developed fewer glomerular crescents. (B) Percentage of positive area for F4/80. n=4 mice per group. *: $p < 0.05$.

* Mini pumps were implanted 1 day after the final injection of NTS; these pumps are active and start delivering their content 24h after implantation.

EXAMPLE:

Methods

Reagents and antibodies. The Epac1 agonist 8-(4-Chlorophenylthio)-2'-O-methyladenosine-3', 5'-cyclic monophosphate (8-pCPT) was purchased from BioLog (Bremen, Germany). Antibodies and their suppliers were: anti-GAPDH from Cell Signaling, anti-Epac1 from Abcam, anti-F4/80 from AbD Serotec, and anti-CD3 from DakoFrance.

Animal and protocols. All mice were handled in strict accordance with good animal practice as defined by the relevant national animal welfare bodies of France, and all animal work was approved by the appropriate committee of the National Institute for Health and Medical Research and the Pierre and Marie Curie University (Paris, France). Animals were housed at constant temperature with access to water and food ad libitum. Epac1-deficient mice (Epac1 KO) have been engineered in our laboratory as previously described (Laurent et al., 2015). Glomerulonephritis (GN) was induced by intravenous injection of decomplexed nephrotoxic serum (NTS) prepared as previously described (Guerrot et al., 2011). Three-month-old Epac1 female mice (C57BL/6-SV129 background) and their wild-type littermates were used to induce a passive NTS-GN by intravenous administration of 20 μ l NTS per gram of body weight over 2 consecutive days. Mice were euthanized 15 days after NTS administration. Control mice were injected with PBS. In a separate study, 3 month-old C57Bl6 female mice were injected with NTS (17 μ l/gBW for 2 consecutive days). The day after the last injection, osmotic mini-pumps (Alzet model 1002) were implanted subcutaneously in mice anaesthetized with isoflurane (1%). Pumps were filled with either 8-pCPT or sterile PBS and were set to deliver 8-pCPT at 3 mM (1.5 mg)/mouse/day. Mice were then subsequently sacrificed.

Renal Function. Urine samples were collected on days 0, 4, 8, 12, and 15, and blood samples were collected before euthanasia. Proteinuria was measured with a Konelab automater (Thermo Fisher Scientific, Waltham, MA) and normalized to urine creatinine. Blood urea nitrogen (BUN) and plasma creatinine levels were measured with an enzymatic method (Konelab automater) and expressed in millimoles per liter and micromolar, respectively.

Histology. Kidneys were fixed in 4% formalin solution and embedded in paraffin; 4- μ m sections were stained with Masson Trichrome for histologic evaluation. Interstitial fibrosis was assessed semi-quantitatively on Sirius red-stained paraffin sections at magnification of 200 using computer-based morphometric analysis software (Analysis; Olympus) as previously described (Abed et al., 2014). Crescent formation and tubular dilation were evaluated blinded on coded slides as already reported (Kerhoch et al., 2012). Immunohistochemistry for F4/80 and CD3 was performed as previously described (Kerhoch et al., 2016).

Western Blot analysis. Proteins were separated by SDS-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes (Millipore). The membranes were incubated with the primary antibody and followed by incubation with peroxidase-conjugated secondary antibodies (Bio-Rad). Membranes were revealed with a ECL Plus Prime (Amersham Biosciences). Signals were quantified by densitometry using the Image Lab software (Bio-Rad).

Total RNA isolation and real-time quantitative RT-PCR. Total RNA was extracted from kidneys using TRIzol reagent (Euromedex). RNA quality was checked by control of OD at 260 and 280 nm. cDNA was synthesized from 1 mg RNA using the Fermentas H Minus First-Strand cDNA Synthesis Kit according to the manufacturer's instructions. Quantitative PCR experiments were performed as previously described (Abed et al., 2014). Each sample was run in triplicate, and analysis of relative gene expression was done by using the 2- $\Delta\Delta$ CT method. Results are expressed in graphs as arbitrary units, which represent the ratio of the target gene to the internal control gene (HPRT).

Data analysis. Values are expressed as mean $\pm$ SEM. Data were analyzed using oneway ANOVA followed by the protected least significant difference Fisher test of the Statview software package.

Results

Loss of Epac1 expression accelerates the progression of renal disease

Epac1 was mainly expressed in the glomeruli of wild type (WT) mice and was downregulated after NTS treatment whereas Epac2 was barely detectable in basal and disease conditions (**Figure 1A**). To investigate the functional relevance of Epac1 downregulation in experimental GN, we used Epac1 KO mice that were subjected or not to NTS injections. Epac1 deletion did not generate renal defects in mouse in basal conditions (**Figure 1B**) with no compensation with Epac2 (**Figure 1A**). As expected, NTS administration progressively degraded renal function as evidenced by the values of body weight increase (**Figure 1B**), proteinuria and BUN (**Figures 2A and 2B**). The sharp decline of renal function was accompanied with renal structure abnormalities as shown by increased crescent formation and tubular dilation (**Figures 2C and 2D**) in WT animals. Importantly, Epac1 genetic ablation accelerated the decline of renal function compared with WT NTS treated animals (**Figures 1B, 2A**). In accordance, at day 15, BUN, crescent formation and tubular dilation were significantly increased in Epac1 KO versus WT mice (**Figures 2B-D**). Thus, Epac1 deficiency does not cause baseline renal abnormalities but remarkably potentiates the deterioration of renal structure and function during the progression of NTS-GN.

Epac1 KO mice show increased inflammation and fibrosis after NTS Injection

Macrophages and T-lymphocyte play a crucial role in the onset and the progression of crescentic GN (Maria and Davidson, 2017). F4/80 and CD3 immunostainings showed a dramatic increase in macrophage and T lymphocyte infiltration in the renal cortex of Epac1 KO mice compared with WT mice after 15 days of NTS administration (**Figures 3A and 3B**). Consistently, upregulation of VCAM-1, crucial for monocyte adhesion, was largely increased

in Epac1 KO NTS mice (**Figure 4A**). Furthermore, other important pro-inflammatory genes including MCP-1 and TNF- α was highly increased in Epac1 KO NTS mice 15 days post-NTS injection (**Figures 4B and 4C**). As expected, a pro-fibrotic agent (TGF- β , **Figure 5A**), a fibrotic marker (Collagen I, **Figure 5B**) and a typical marker of renal tubular damage, KIM1 (**Figure 5C**) were highly increased in Epac1 KO NTS mice. These effects were associated with podocyte injury as evidenced by the down-regulation of the podocyte-specific marker, WT1 (**Figure 5D**). Finally, quantification of Sirius red colorations confirmed that interstitial collagen accumulation was potentiated (**Figure 6**). Thus, decreased Epac1 expression markedly potentiated renal inflammation and interstitial fibrosis in NTS-GN.

Pharmacological activation of Epac1 delays the development of NTS-GN

We next sought to determine whether Epac1 activation could have beneficial effects on renal function after the onset of the disease. To this end, at day 3 of the protocol WT C57BL/6 mice were treated with either the Epac1 selective agonist, 8-pCPT (1.5 mg/mouse/day), or vehicle until the end of experiments (day 15). 8-pCPT treatment attenuated NTS-induced increase of body weight, proteinuria and creatininemia (**Figures 7A–C**). This functional protection was accompanied with a preservation of renal structure and decreased numbers of crescents (**Figure 8A**). F4/80 immunostaining showed limited macrophage infiltration in the renal cortex of 8-pCPT treated mice (**Figure 8B**) and further confirmed the beneficial effect of Epac1 activation. Thus, activating Epac1 protects against the progression of the NTS-GN.

Conclusion:

In conclusion, Epac1 appears as an interesting target for therapy of CKD. Here, we have shown that genetic inhibition of Epac1 is critical for and accelerates the progression of nephropathy. Inversely, Epac1 pharmacological activation blunts the progression of experimental GN. Our study represents a “proof-of-concept” for the therapeutic effectiveness of activating Epac1 in CKD using small-molecule pharmacotherapy.

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Throughout this application, various references describe the state of the art to which this invention pertains. The disclosures of these references are hereby incorporated by reference into the present disclosure.

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CLAIMS:

1. A method of treating a chronic kidney disease (CKD) in a patient in need thereof comprising administering to the patient a therapeutically effective amount of at least one Epac1 activator.
- 5 2. The method of claim 1 wherein the patient suffers from a disease selected from the group consisting of nephropathy (e.g. membranous nephropathy (MN), diabetic nephropathy and hypertensive nephropathy), glomerulonephritis (e.g. membranous glomerulonephritis and membranoproliferative glomerulonephritis (MPGN) such as rapidly progressive glomerulonephritis (RPGN)), interstitial nephritis, lupus nephritis,
10 idiopathic nephrotic syndrome (INS) (e.g. minimal change nephrotic syndrome (MCNS) and focal segmental glomerulosclerosis (FSGS)), obstructive uropathy, polycystic kidney disease (e.g. Autosomal Dominant Polycystic Kidney Disease (ADPKD) and Autosomal Recessive Polycystic Kidney Disease (ARPKD)), cardiovascular diseases, hypertension, diabetes, and kidney graft rejection (e.g. acute
15 and chronic kidney rejection).
3. The method of claim 1 wherein the Epac1 activator is a cAMP analog.
4. The method of claim 1 wherein the Epac1 activator is 8-(4-Chlorophenylthio)-2'-O-methyladenosine-3',5'-cyclic monophosphate.
5. The method of claim 1 wherein the Epac1 activator is an activator of Epac1 expression.
- 20 6. The method of claim 5 wherein the activator is a nucleic acid that encodes for a Epac1 polypeptide.

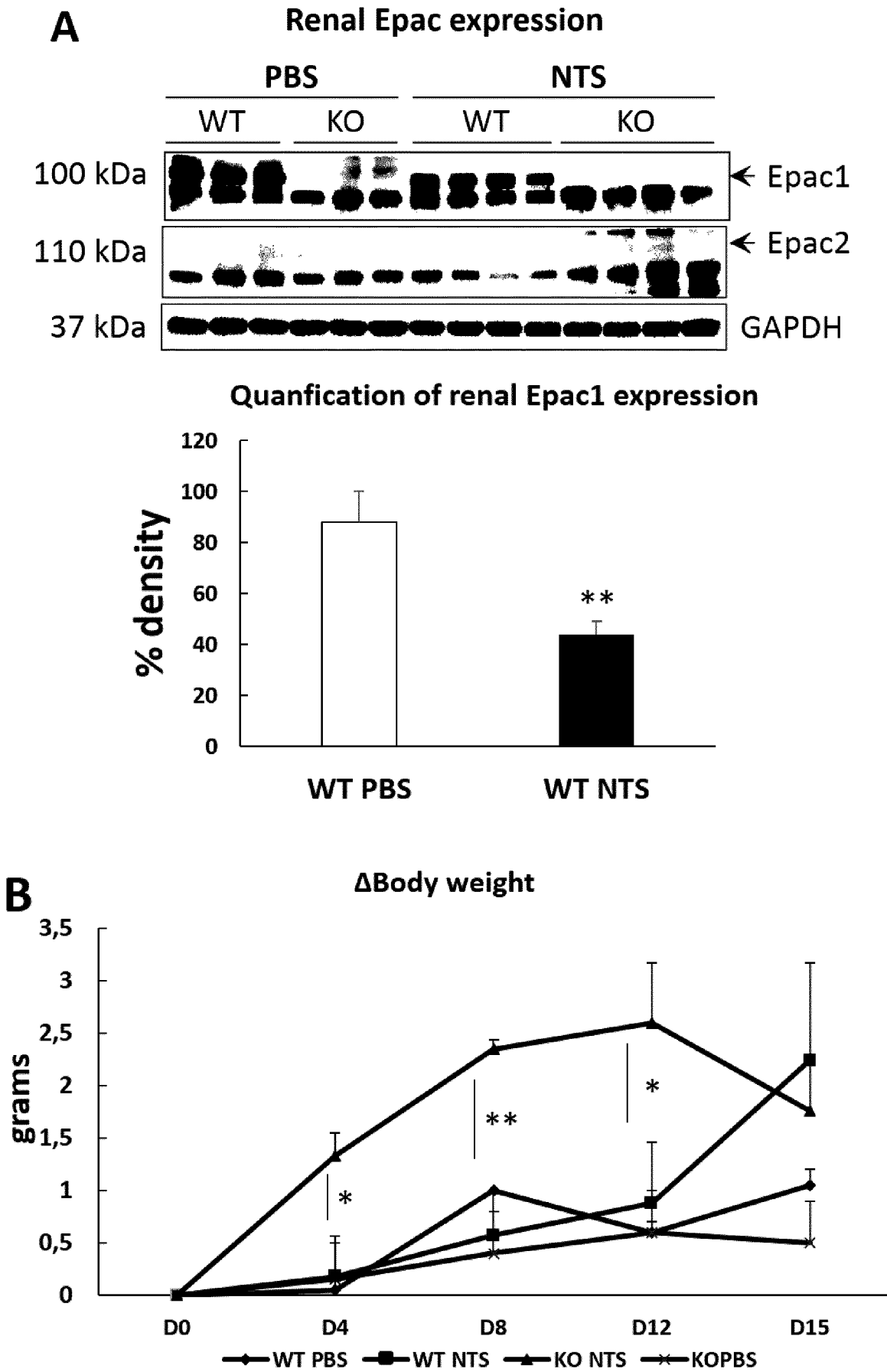
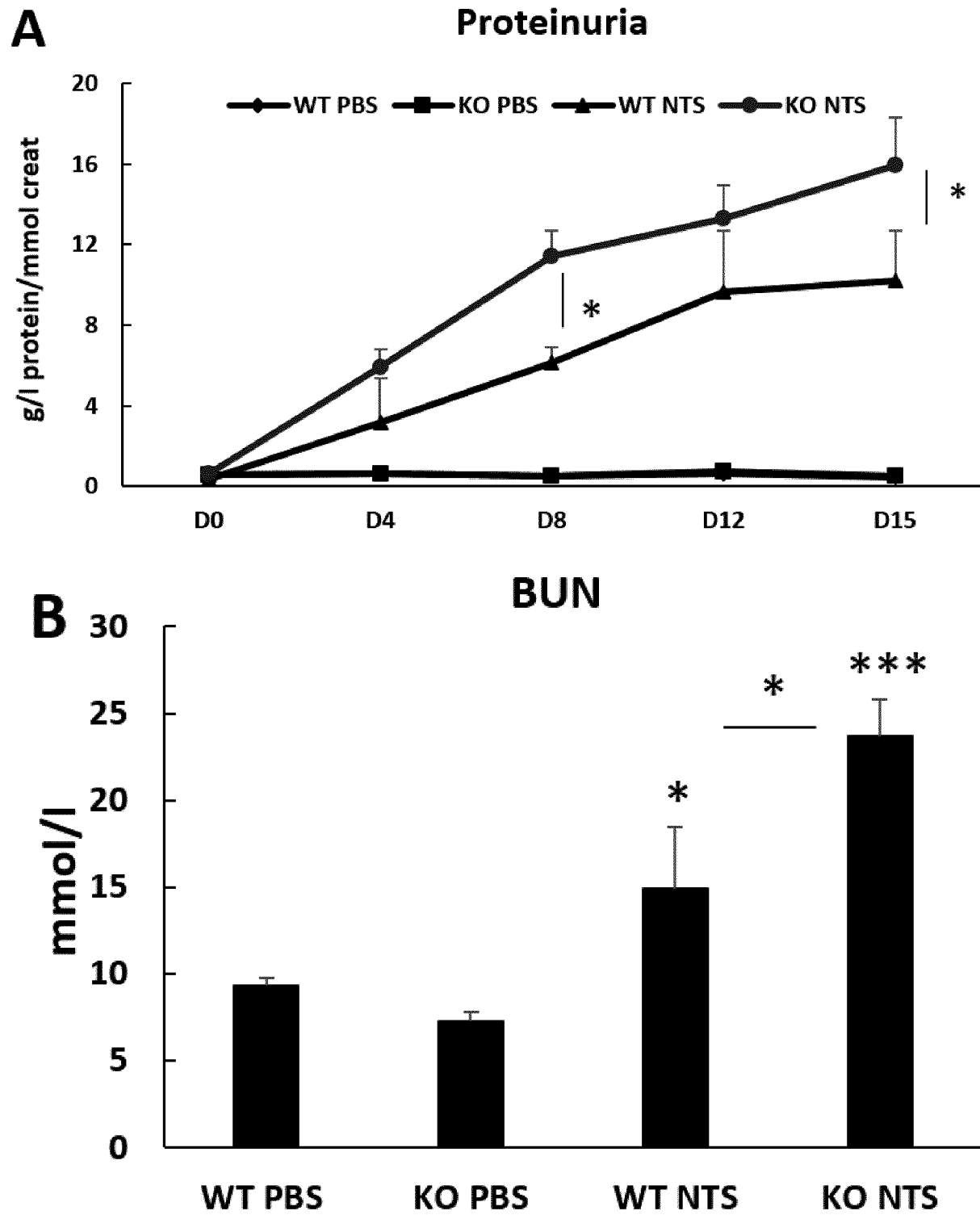


Figure 1A and 1B



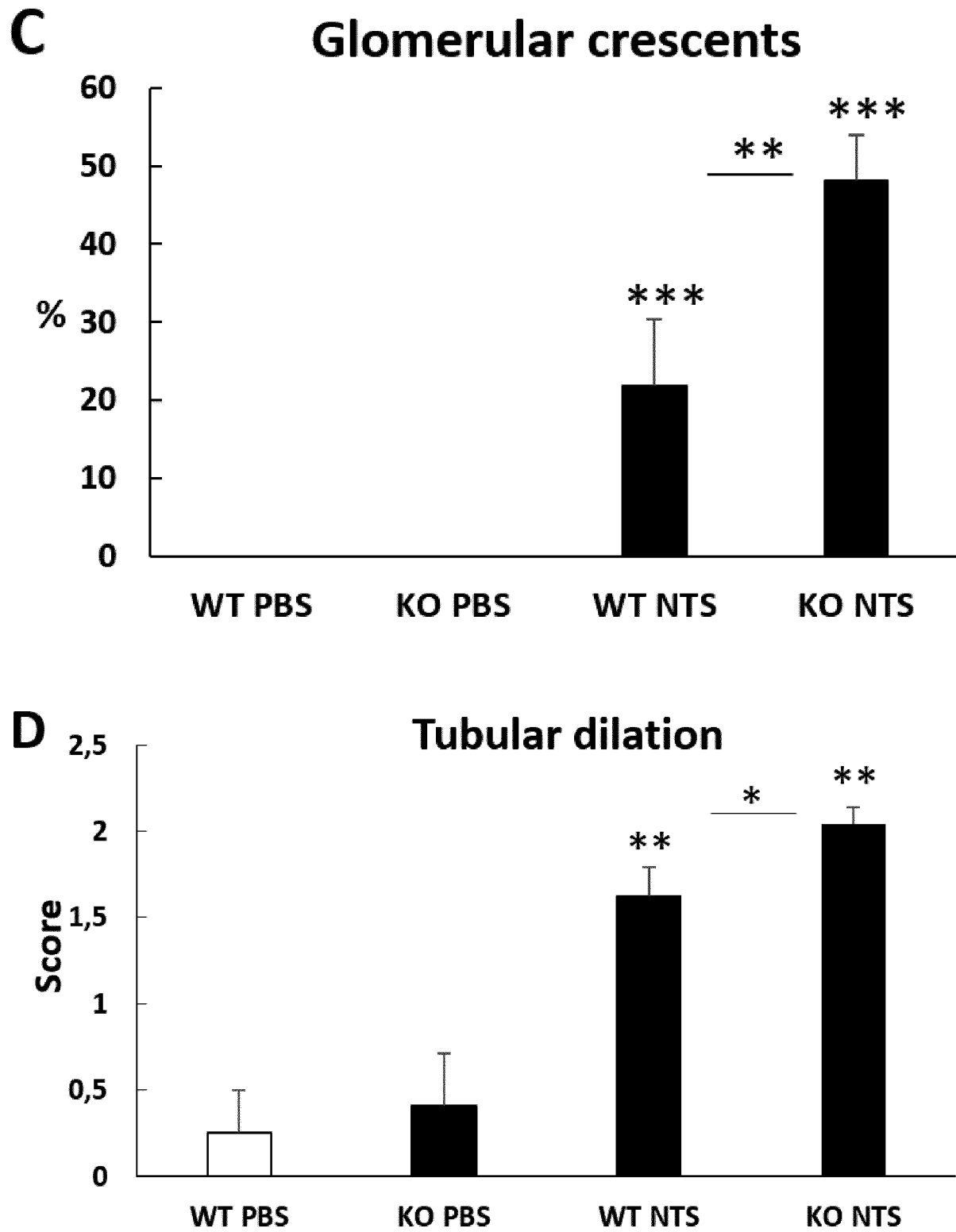


Figure 2C and 2D

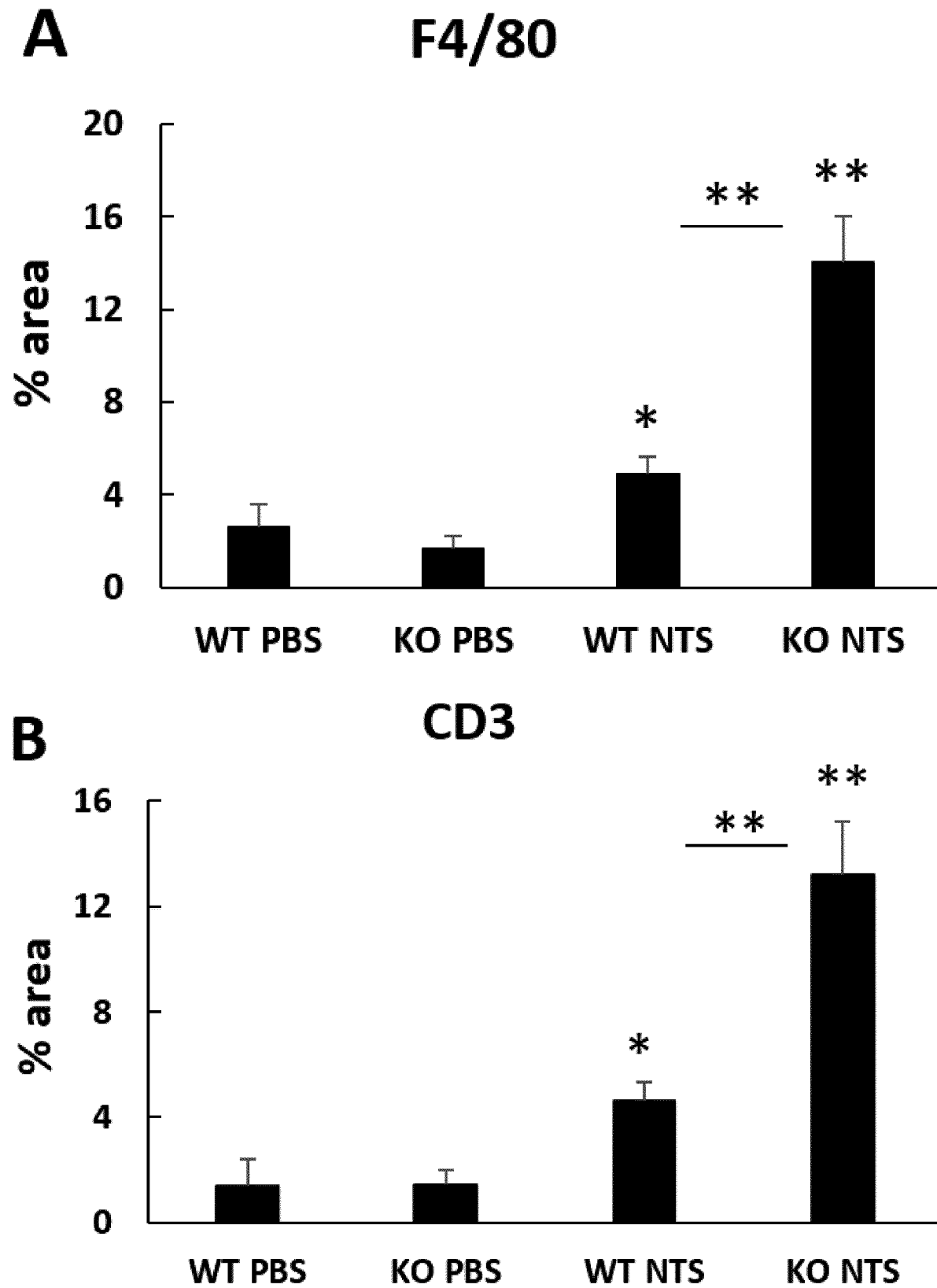


Figure 3A and 3B

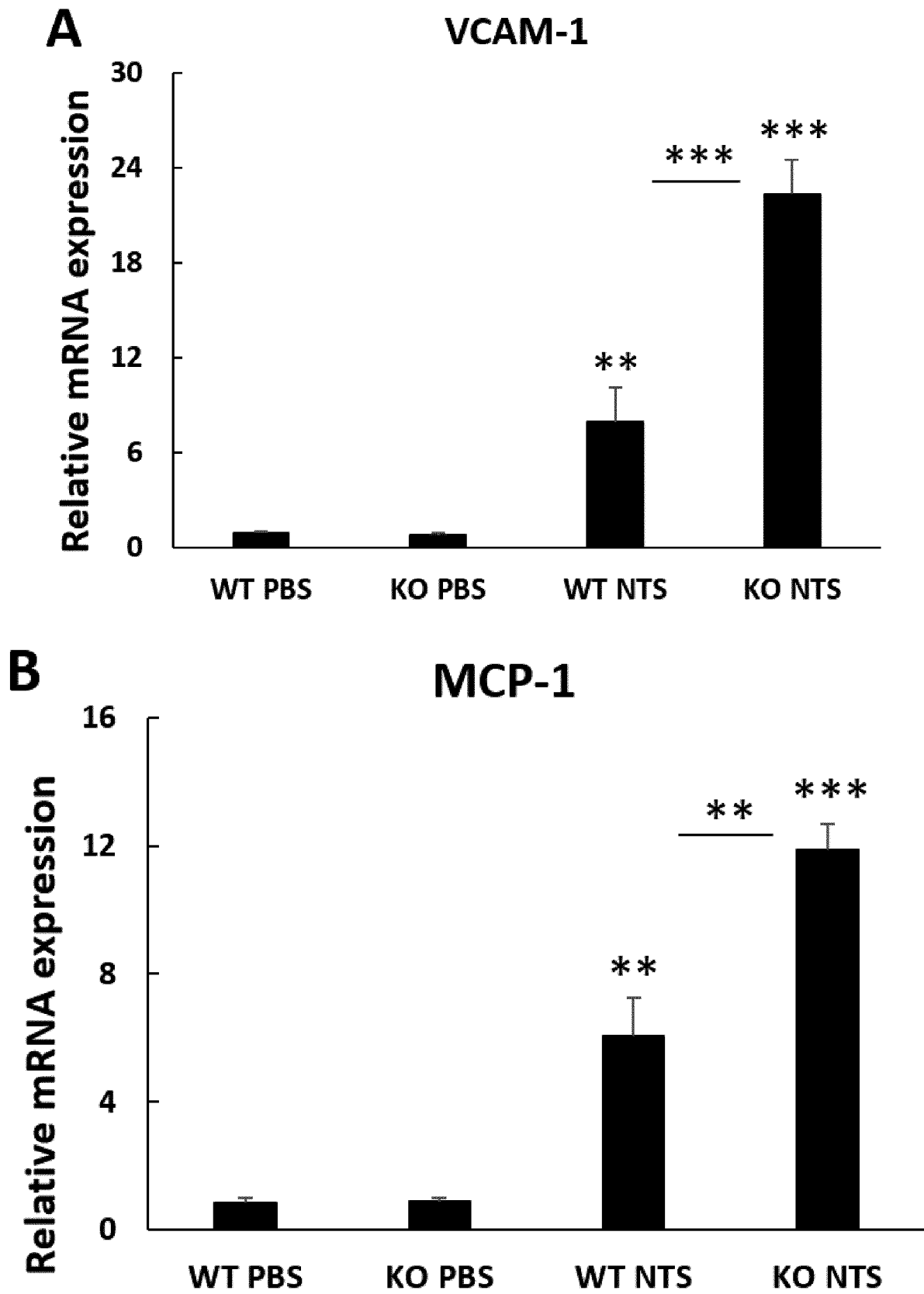


Figure 4A and 4B

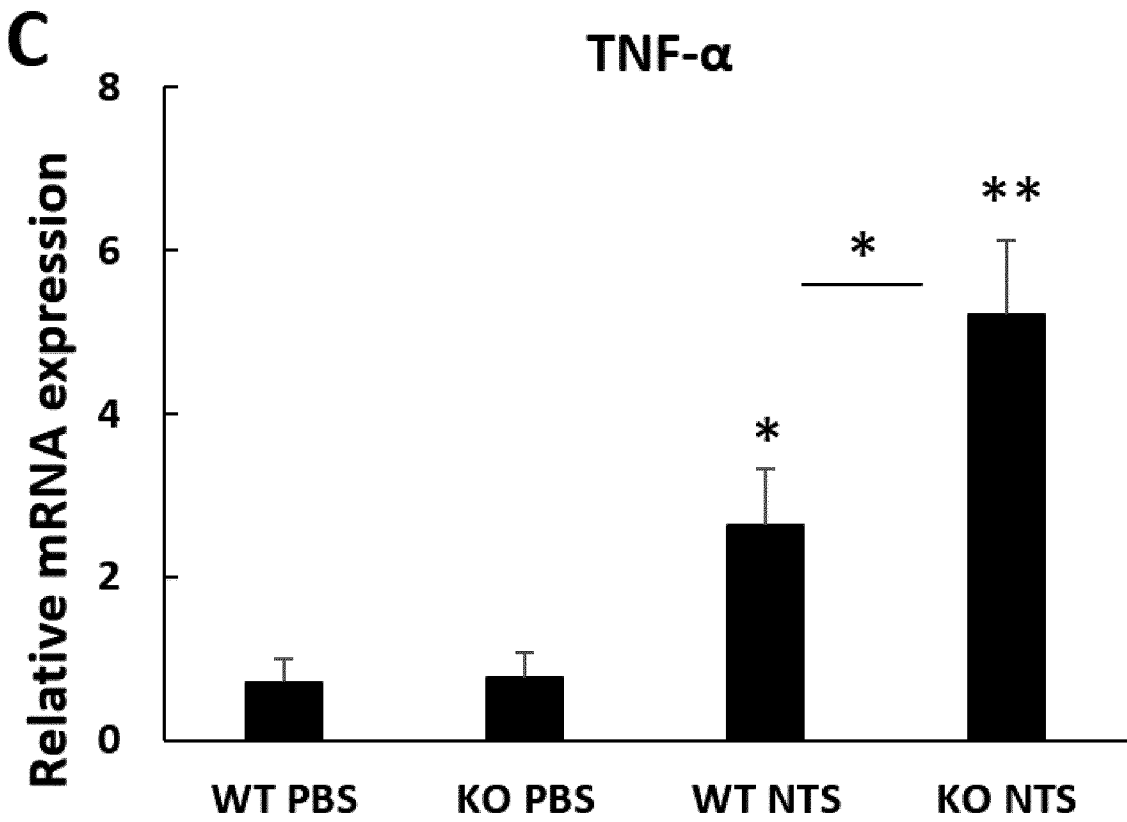


Figure 4C

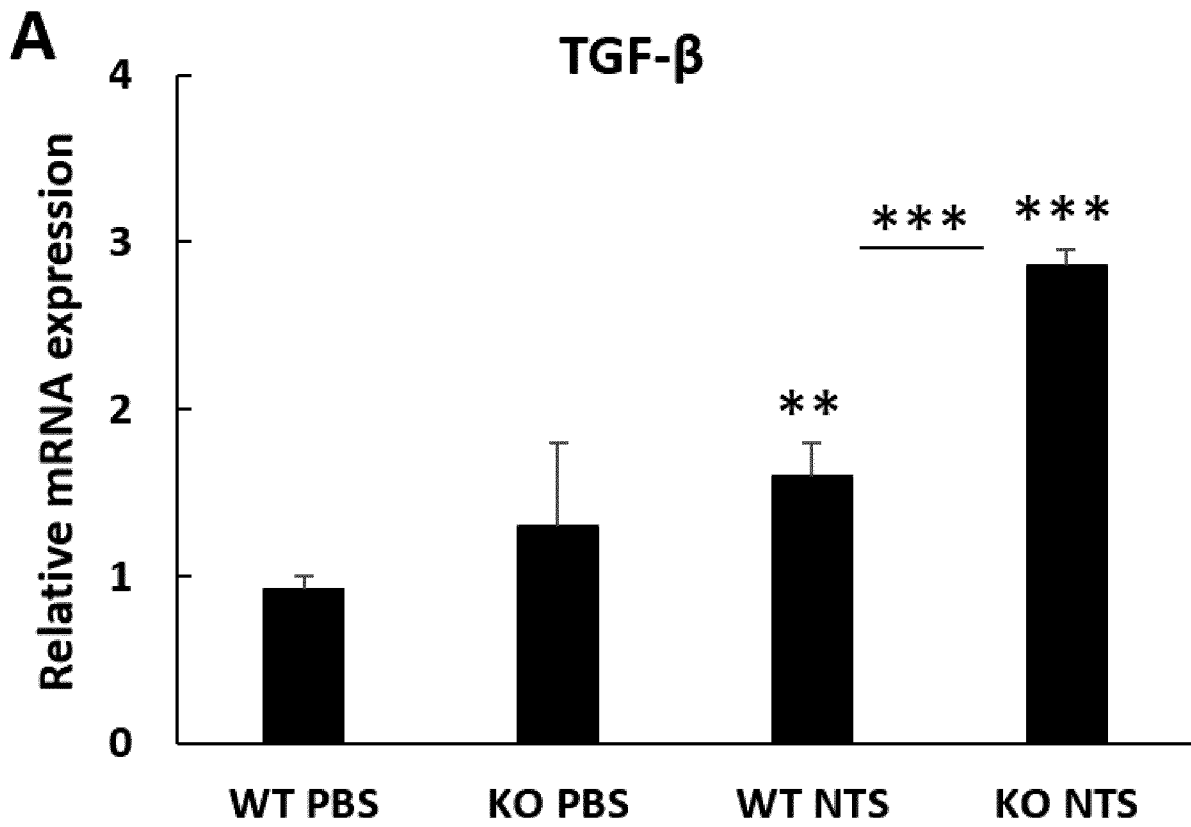


Figure 5A

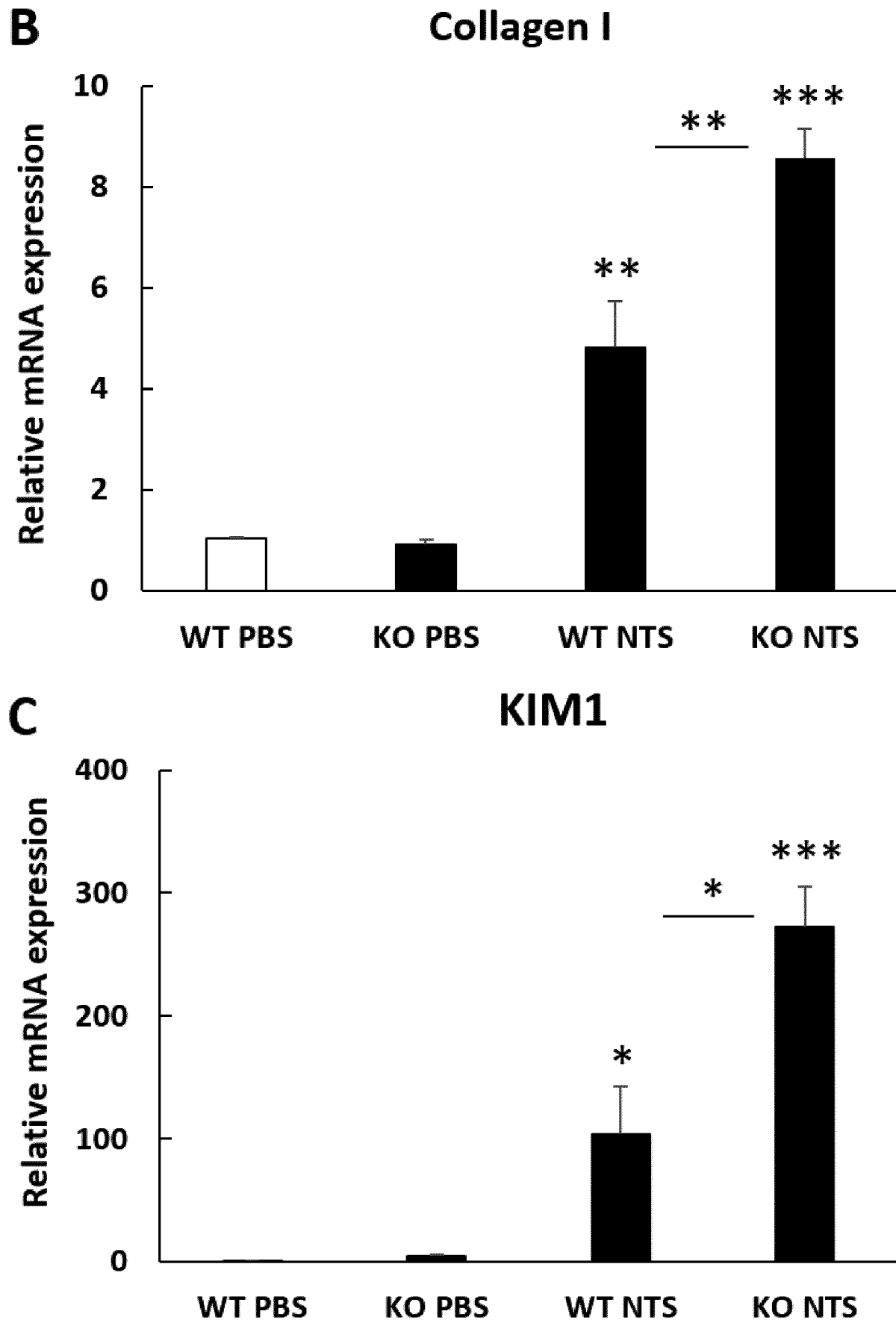


Figure 5B and 5C

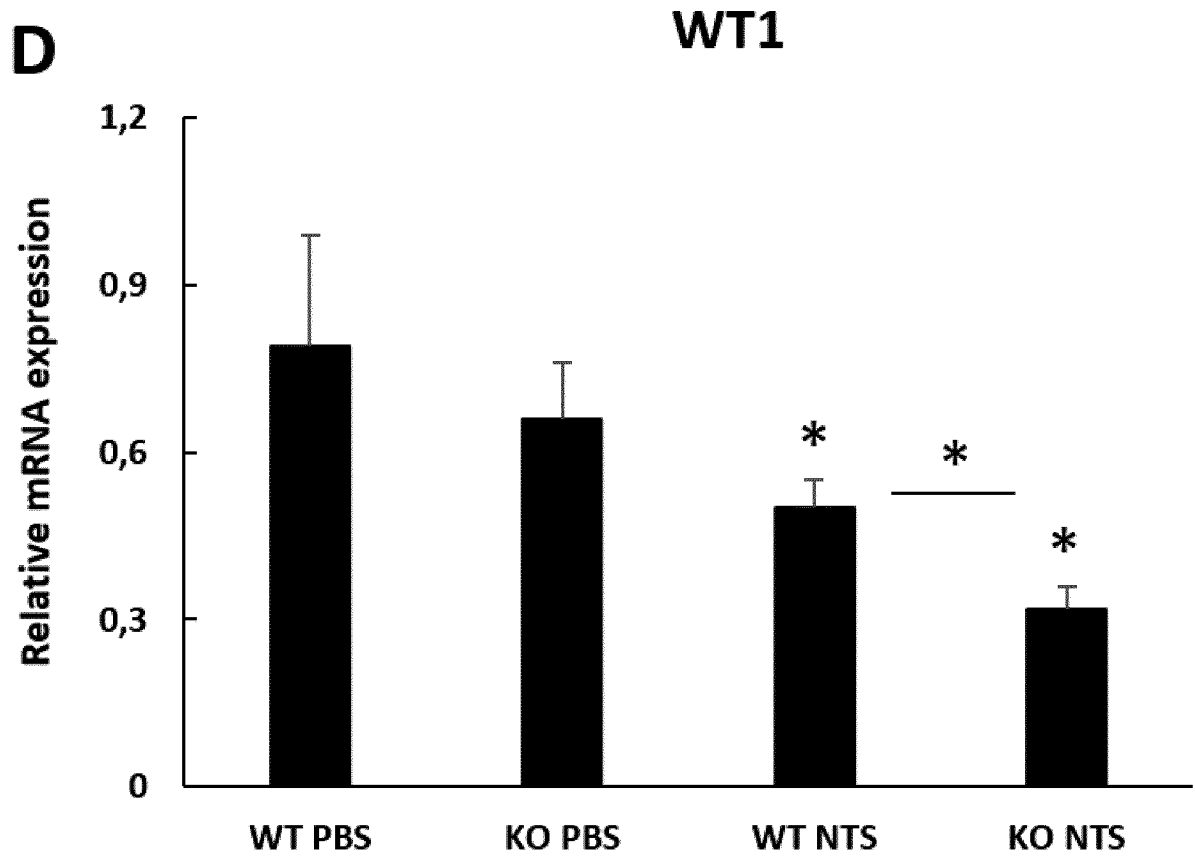


Figure 5D

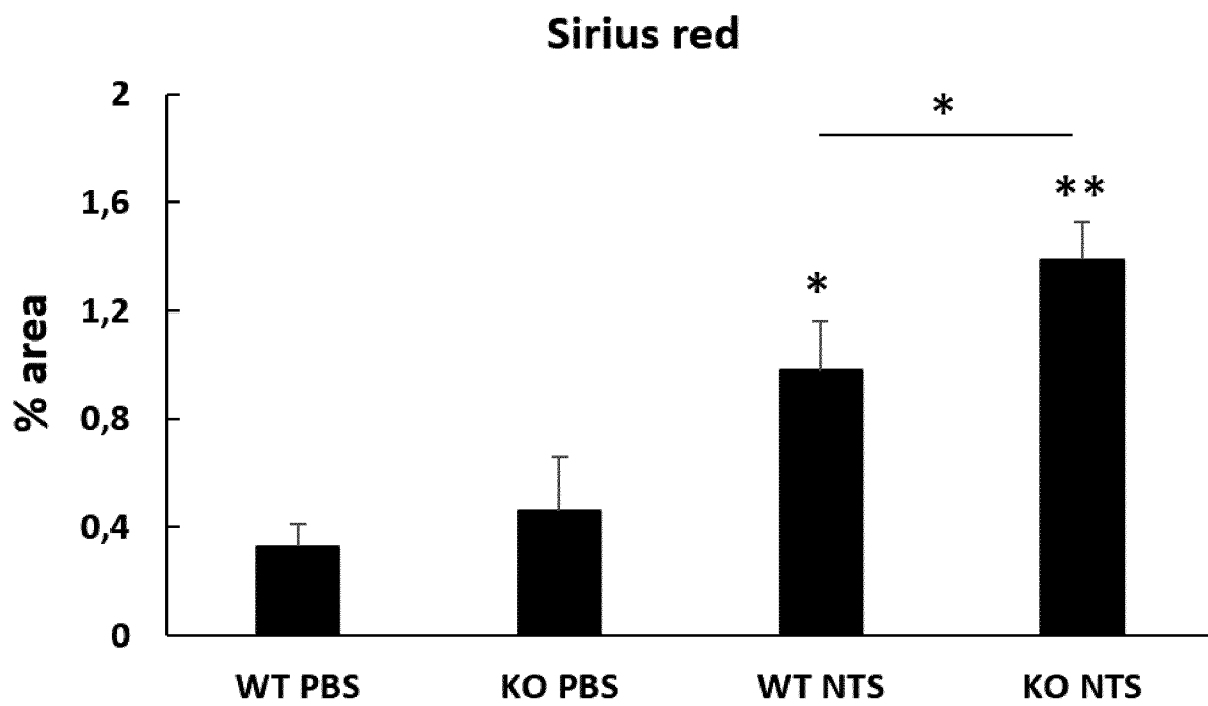


Figure 6

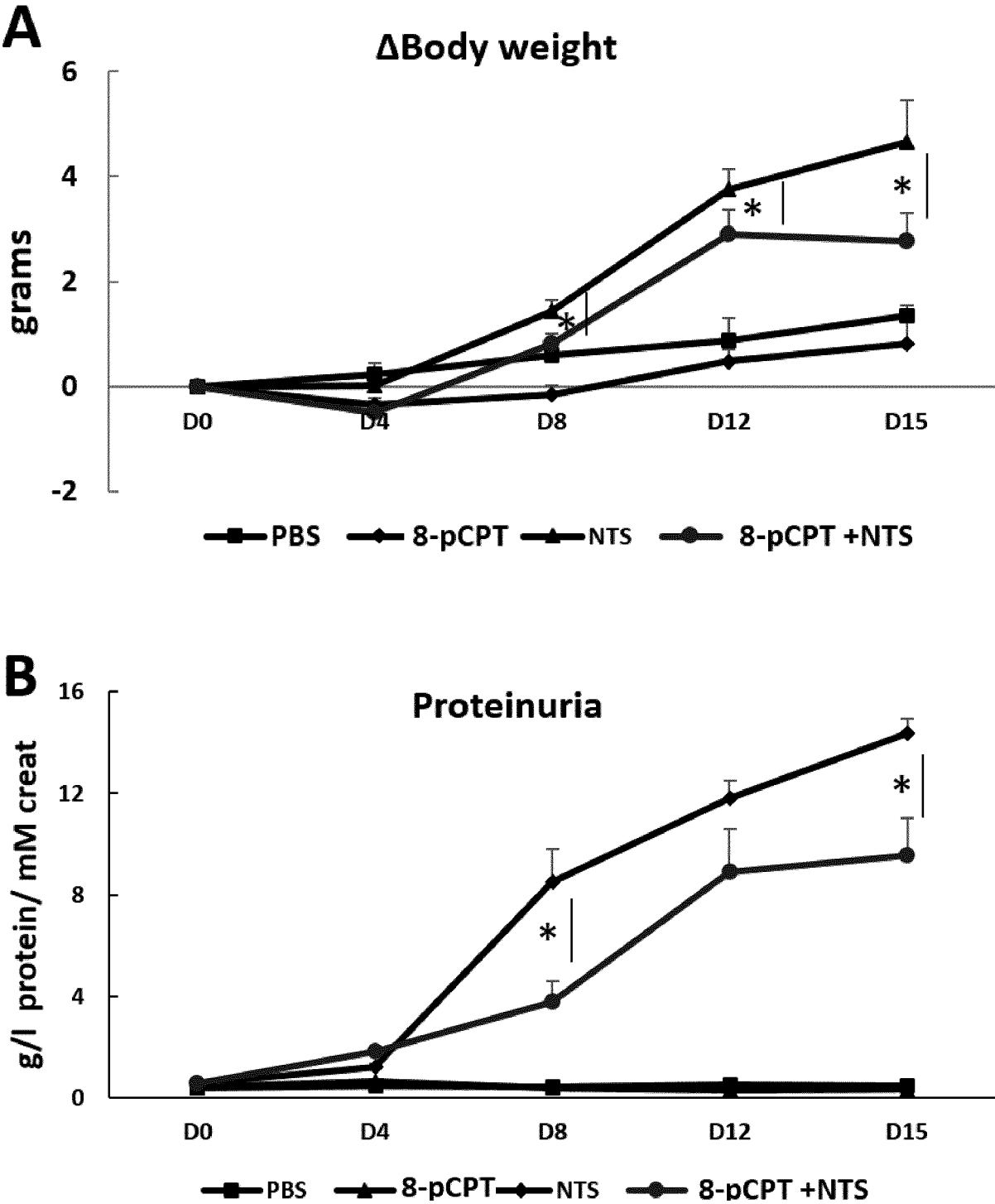


Figure 7A and 7B

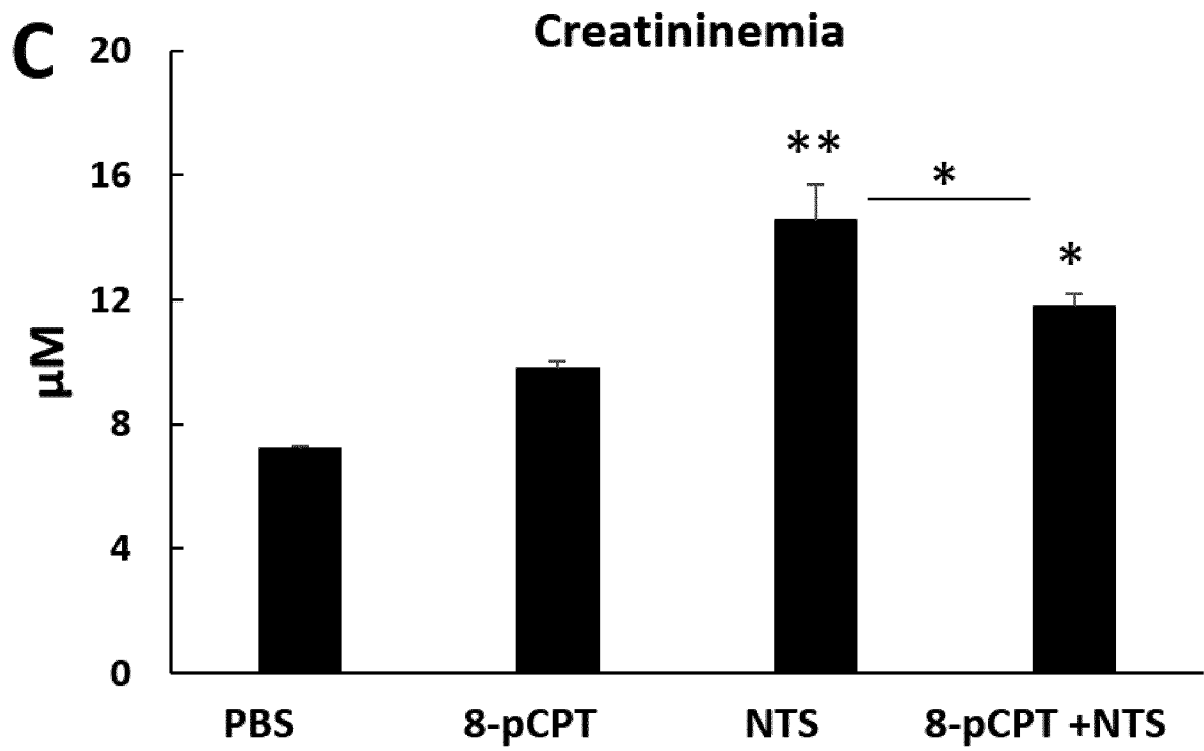


Figure 7C

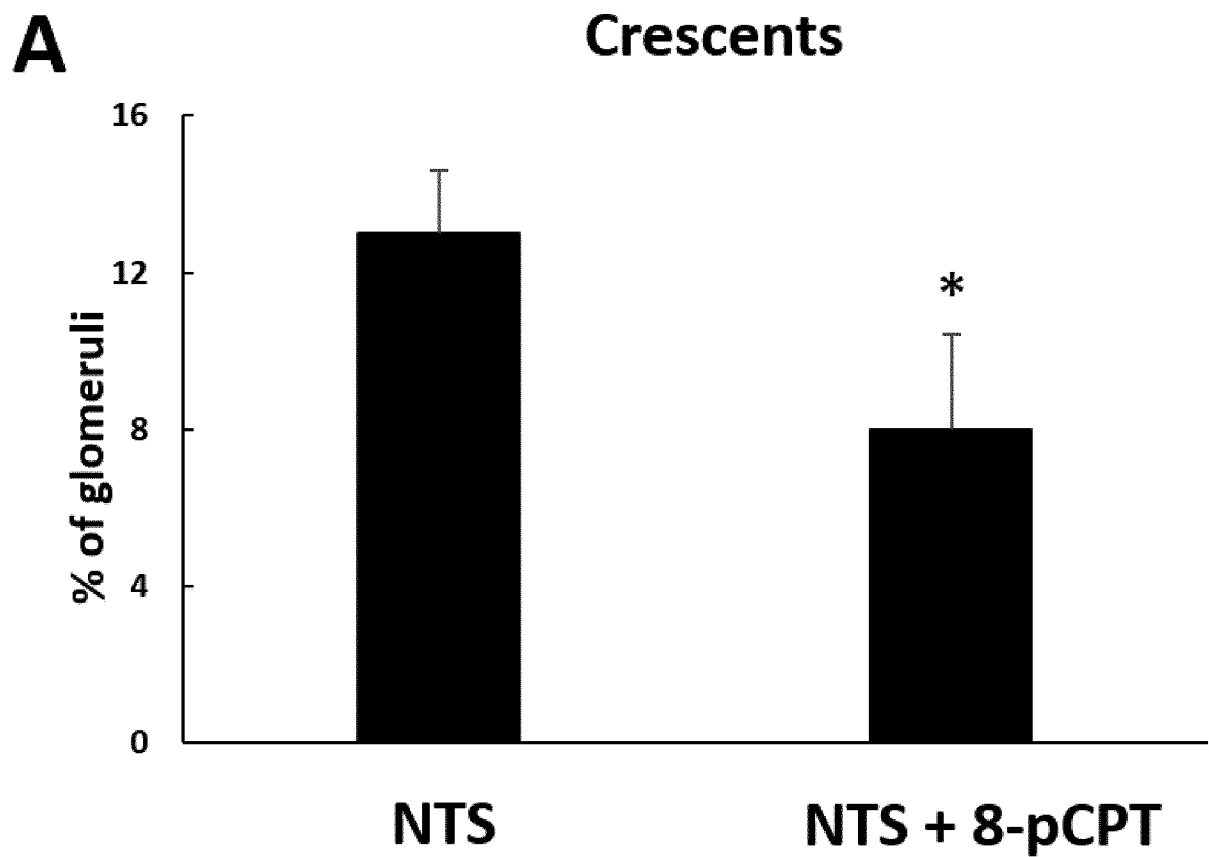


Figure 8A

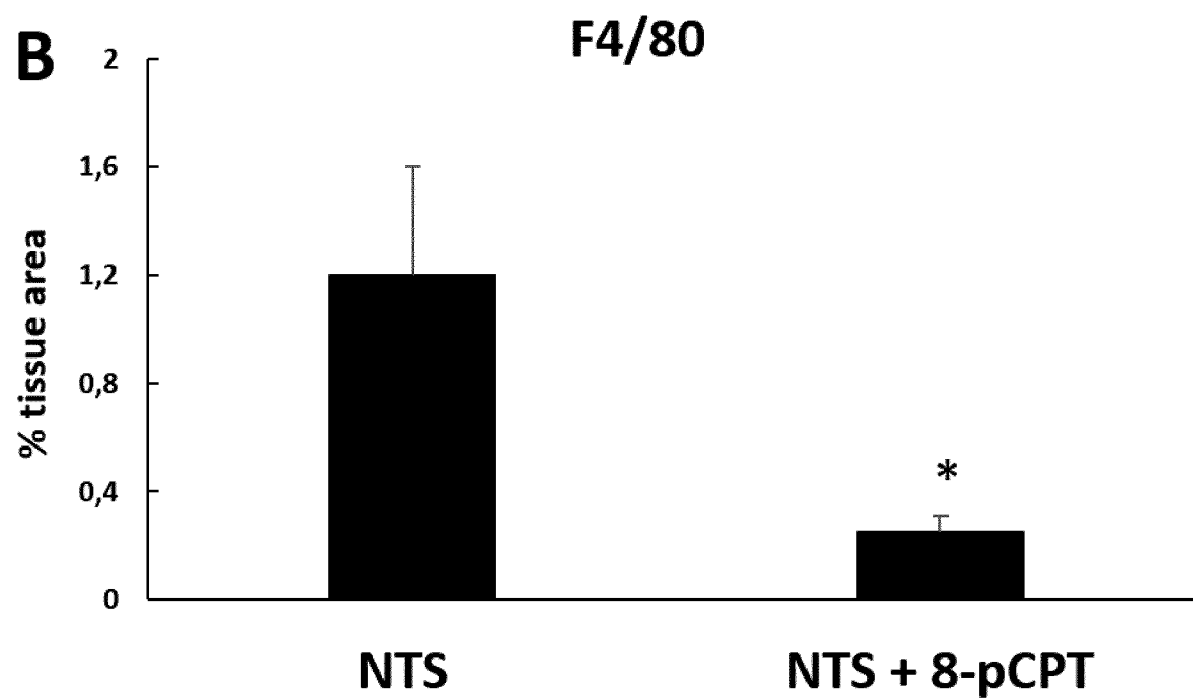


Figure 8B

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/075468

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K31/7076 A61K38/17 A61P13/12
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2007/006544 A2 (UNIV BRUXELLES [BE]; KOOIJMAN RON [BE]; GERLO SARAH [BE]; VERDOOD PEGG) 18 January 2007 (2007-01-18)	1-5
Y	example 3	1-6
Y	----- WO 2006/094703 A1 (HERCHE MEDICALE INSERM INST NA [FR]; LEZOUALC H FRANK [FR]; MOREL ERIC) 14 September 2006 (2006-09-14) page 1, line 20 - line 26; claims 1-3 ----- -/-	1-6



Further documents are listed in the continuation of Box C.



See patent family annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

12 December 2019

Date of mailing of the international search report

02/01/2020

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
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Authorized officer

Winger, Rudolf

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/075468

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PING XIE ET AL: "Modulation of angiotensin II-induced inflammatory cytokines by the Epac1-Rap1A-NHE3 pathway: implications in renal tubular pathobiology", AMERICAN JOURNAL OF PHYSIOLOGY: RENAL PHYSIOLOGY, vol. 306, no. 11, 1 June 2014 (2014-06-01) , pages F1260-F1274, XP055575649, United States ISSN: 1931-857X, DOI: 10.1152/ajprenal.00069.2014 figure 10 -----	1-6
A	WO 03/104250 A1 (KYLIX B V [NL]; DE KONING JOHN [NL]; CHRISTENSEN ANNE [NO]; SCHWEDE FR) 18 December 2003 (2003-12-18) cited in the application page 27, line 29 - page 28, line 2; claims -----	1-6

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2019/075468

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2007006544	A2	18-01-2007	NONE

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			JP 2008531631 A 14-08-2008
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