Histone deacetylase 6 inhibition mitigates renal fibrosis by suppressing TGFβ and EGFR signaling pathways in obstructive nephropathy

Xingying Chen[1#], Chao Yu[1#], Xiying Hou[1], Jialu Li[1], Tingting Li[1], Andong Qiu[2], Na Liu[1], and Shougang Zhuang[1,3*]

[1]Department of Nephrology, Shanghai East Hospital, Tongji University School of Medicine, Shanghai 200120, China; [2]School of Life Sciences and Technology, Tongji University, Shanghai, China [3]Department of Medicine, Rhode Island Hospital and Alpert Medical School, Brown University, Providence, RI 02903.

# These authors contributed to this manuscript equally

***Correspondence to** Dr. Shougang Zhuang, MD, PhD, Department of Nephrology, Shanghai East Hospital, Tongji University School of Medicine, Shanghai 200120, China.

Email: szhuang@lifespan.org

Running title: HDAC6 mediates renal fibrosis

Abstract

We have recently shown that histone deacetylase 6 (HDAC6) is critically involved in the pathogenesis of acute kidney injury. Its role in renal fibrosis, however, remains unclear. In this study, we examined the effect of ricolinostat (ACY-1215), a selective inhibitor of HDAC6, on the development of renal fibrosis in a murine model induced by unilateral ureteral obstruction (UUO). HDAC6 was highly expressed in the kidney following UUO injury, which was coincident with deposition of collagen fibrils and expression of α-smooth muscle actin, fibronectin, and collagen III. Administration of ACY-1215 reduced these fibrotic changes and inhibited UUO-induced expression of transforming growth factor β1 (TGFβ1) and phosphorylation of Smad3, while increasing expression of Smad7. ACY-1215 treatment also suppressed phosphorylation of epidermal growth factor receptor (EGFR) and several signaling molecules associated with renal fibrogenesis, including AKT, signal transducer and activator of transcription 3 and nuclear factor kappa light chain enhancer of activated B cells in the injured kidney. Furthermore, ACY-1215 was effective in inhibiting dedifferentiation of renal fibroblasts to myofibroblasts and the fibrotic change of renal tubular epithelial cells in culture. Collectively, these results indicate that HDAC6 inhibition can attenuate development of renal fibrosis by suppression of TGFβ1 and EGFR signaling, and suggest that HDAC6 would be a potential therapeutic target for the treatment of renal fibrosis.

Key Words: histone deacetylase 6; renal fibrosis; ACY-1215; unilateral ureteral obstruction; transforming growth factor β1; epidermal growth factor receptor

INTRODUCTION

Chronic kidney disease (CKD) is a major public health problem, affecting nearly 10% of the world's population (24). Tubular interstitial fibrosis is considered to be the most common pathway leading to end-stage renal disease (ESRD) (23). The pathogenesis of renal fibrosis is characterized by renal interstitial fibroblast activation, and abnormal accumulation of extracellular matrix (ECM)(23, 24). So far, there are no effective approaches to prevent and halt the progression of CKD to ESRD. Understanding the molecular basis of renal fibrosis will aid in the development of therapeutic strategies to treat kidney diseases.

Renal fibrosis is a complicated process associated with activation of multiple signaling pathways and numerous genes. TGFβ/Smad signaling is considered the key regulator in renal fibrosis. Upon TGFβ1 binding to the TGFβ receptor, Smad3 is recruited and phosphorylated. Phosphorylated Smad3 is translocated to the nucleus where it drives the expression of profibrotic genes like collagen I (12, 24). Smad7 counters the activation of TGFβ receptor and Samd3 to inhibit renal fibrosis (24). In addition to TGFβ/Smad signaling, epidermal growth factor receptor (EGFR) is also a critical mediator of profibrotic signals initiated by its ligands and other biological substances (14). The activation of EGFR by substances other than its own ligands is called transactivation, which mediates the profibrotic responses induced by many cytokines and vascular substances such as TGFβ1, angiotensin II and endothelin (19). Activation of EGFR and other cellular membrane receptors can induce phosphorylation of multiple intracellular signaling molecules such as AKT, signal transducer and activator of transcription 3 (STAT3) and nuclear factor kappa light chain enhancer of activated B cells (NF-κB), which act as mediators in gene expression (14, 19).

Increasing evidence indicate that epigenetic modification plays an important role in the regulation of gene expression (33, 34). Among several types of epigenetic modifications, histone acetylation has been widely studied. Histone acetylation is positively regulated by histone acetyltransferases (HATs) and negatively regulated by histone deacetylases (HDACs)(33). At

present, 18 histone deacetylases (HDAC) have been identified in mammals and divided into four categories: Class I HDAC (HDAC1, 2, 3, and 8), Class II HDAC, subdivided into Class IIa Class (HDAC4, 5, 7 and 9) and IIb (HDAC6 and 10), Class III HDAC (SIRT1-7) and Class IV HDAC (HDAC11). Unlike other HDAC isoforms, whose deletion in mice leads either to death in utero or severe developmental defects, HDAC6 can be deleted in mice, which still develop normally without major organ dysfunction. This unique feature of HDAC6 may have important implications for the safety of potential therapeutic inhibition of HDAC6.

In the past several years, HDAC6 inhibitors have been developed and used in preclinical and clinical studies. Specific HDAC6 inhibitors have shown anti-cancer properties in several tumors including multiple myeloma (3), chronic lymphocytic leukemia (3), and acute myeloid leukemia (13). Tubastatin A was also effective in improving polycystic kidney disease (ADPKD) (17), hypertensive nephropathy (7), acute kidney injury (AKI)(30, 32) and peritoneal fibrosis (38) in animal models. However, Tubastatin A may have limited success in clinical trials because of its poor pharmacokinetic properties and potential genotoxicity (4, 38). Recently, other HDAC6 inhibitors have been developed, and ACY-1215 (ricolinostat) and ACY-241 have reached clinical trial to treat tumors (27, 35). Studies have shown that ACY-1215 is a potent and selective HDAC6 inhibitor with IC 50 at 5 nM (29) and can attenuate several diseases, including neurodegenerative diseases, acute liver injury, and tumors disease in animal models (36, 39-41). However, ACY-1215 has not been studied to treat renal fibrosis yet.

In this study, we assessed the effect of ACY-1215 on renal fibrosis and the mechanism involved in a murine model of renal fibrosis induced by unilateral ureteral obstruction (UUO) in order to provide evidence for future clinical trials in chronic fibrotic kidney disease.

MATERIALS AND METHODS

Chemical and antibodies

ACY-1215 (Ricolinostat) and aristolochic acid were purchased from Selleckchem (Houston, TX, USA). Antibodies to Smad3, p-Smad3, Smad7, acetyl-H3, acetyl-α-tubulin, GAPDH, EGFR, p-EGFR, p-NF-κB, NF-κB, p-STAT3, STAT3, p-AKT, AKT were purchased from Cell signaling Technologies (Danvers, MA, USA). Antibodies to collagen III was purchased from Servicebio (Wuhan, China). Antibodies to α-SMA, Fibronectin, HDAC6 were purchased from Absin Bioscience Inc. (Shanghai, China).

Cell culture and treatments

Rat renal interstitial fibroblasts (NRK-49F) were obtained from the ATCC (Manassas, VA); murine renal tubular epithelial cells (mTECs) are a gift from Dr. Jeffrey B. Kopp (National Institutes of Health, Bethesda, MD), which were shown to be of proximal tubular origin by a combination of morphological, biochemical, and transport characteristics (15). NRK-49F and mTECs were cultured in DMEM containing 5% FBS, 1% penicillin in an atmosphere of 5% CO_2, and 95% air at 37 °C. To determine the effect of HDAC6 inhibition on renal fibroblast activation induced by serum, ACY-1215 was directly cultured NRK-49F with 5% FBS at different concentrations. To determine the effect of HDAC6 inhibition on TGFβ1-induced renal fibroblast activation, NRK-49F cultured with DMEM containing 5% FBS were exposed to TGFβ1 (5 ng/ml) for 36 hours in the presence or absence of ACY-1215. To determine the effect of HDAC6 inhibition on the expression of TGFβ1 and fibrotic responses to injury, mTECs were cultured for 24 hours in the DMEM without FBS and then exposed to TGFβ1 (5 ng/ml) or aristolochic acid (10 μM) or for an additional 24 hours before harvesting cells for immunoblot analysis.

Unilateral ureteral obstruction (UUO) model and ACY-1215 treatment

The UUO model was established in male C57BL/6J mice that weighed 20-25 g (Shanghai SLAC Laboratory Animal Co., Ltd) as described in our previous study (26). Briefly, the abdominal cavity was exposed via a midline incision and the left ureter was isolated and ligated. The contralateral

kidney was used as a control. To examine the effects of ACY-1215 on renal fibrosis after UUO injury, 25 mg/kg ACY-1215 in 50 μl of DMSO was intraperitoneally administered immediately and then given every day at the same dose for 6 days. Selection of this dose of 25 mg/kg was based on a previous report (42). For the UUO alone group, mice were injected with an equivalent amount of DMSO. Five mice were used in each group. The animals were sacrificed, and the kidneys were removed at day 7 for protein analysis and histological examination. All the experiments were conducted in accordance with the animal experimentation guideline of Tongji University School of Medicine, China.

Immunoblot analysis

Immunoblot analysis of kidney tissue samples was conducted as described previously (26).The densitometry analysis of immunoblot results was conducted by using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Immunofluorescent and histochemical staining

Immunofluorescent and immunohistochemical staining was performed according to the procedure described in our previous studies (21). Renal tissue was fixed in 4.5% buffered formalin, dehydrated, and embedded in paraffin. For immunofluorescent staining, primary antibodies and fluorescent-conjugated secondary antibodies were applied to the sections. For assessment of renal fibrosis, Masson trichrome staining was performed according to the protocol provided by the manufacture (Sigma, St. Louis, MO). The collagen tissue area (blue color) was quantitatively measured using Image Pro-Plus software (Media-Cybernetics, Silver Spring, MD, USA) by drawing a line around the perimeter of positive staining area. The average ratio to each microscopic field (200×) was calculated and graphed.

Statistical analysis

All the experiments were conducted at least three times. Data depicted in graphs represent the means ± SD. for each group. Intergroup comparison was made using one-way analysis of variance. Multiple means were compared using Turkey's test. The differences between two groups were determined by Student's t test. The statistically significant difference between mean values was marked in each graph. $P<0.05$ is considered significant. The statistical analyses were conducted by using IBM SPSS Statistics 20.0 (Beijing, China).

RESULTS

Administration of ACY-1215 inhibits HDAC6 expression and renal fibrosis in a murine model induced by UUO

To demonstrate the role of HDAC6 in renal fibrosis, we established a murine model of renal fibrosis induced by UUO and then administered ACY-1215, a highly selective HDAC6 inhibitor (28) immediately after UUO. At 7 days after injection, we collected the kidney tissue to analyze renal fibrosis by Masson staining. As shown in Figure 1A, UUO injury resulted in renal fibrosis (blue area), which was significantly attenuated by administration of ACY-1215, but no renal fibrosis was seen in the sham-operated mice with and without drug treatment (Figure 1A, B). In parallel with the fibrotic changes, expression levels of HDAC6 were upregulated and in the kidney after injury, and ACY-1215 treatment reduced this response (Figure 1C, D). In contrast, ACY-1215 increased expression levels of acetyl-Histone H3 and acetyl a-tubulin in the sham-operated and injured kidneys, indicating the effectiveness of this inhibitor (Figure 1C, E, F). These data suggest that HDAC6 can induce acetylation of proteins located in both the nucleus and cytosol of kidney cells after UUO injury but can only induce acetylation of its substrates in the cytosol of sham-operated kidneys.

HDAC6 is expressed in renal tubules in the UUO model

To examine the distribution of HDAC6 in the injured kidney, we conducted immunofluorescent staining. Figure 2 showed that the expression of HDAC6 in the UUO group was significantly higher than that in the Sham group, and HDAC6 was mainly expressed in the cytoplasm of the renal tubules; the expression of α-SMA in the kidney of the UUO model was also significantly increased compared to the Sham group, but HDAC6 was rarely co-stained with α-SMA. Since α-SMA is mainly expressed in myofibroblasts, and HDAC6 is expressed in renal tubular epithelial cells, these results suggested that HDAC6 may act in renal tubular cells to mediate development of renal fibrosis after UUO injury.

Inhibition of HDAC6 reduces fibroblast activation and ECM deposition in renal fibrosis induced by UUO

Deposition of excessive extracellular matrix (ECM) and renal myofibroblast activation are two major pathologic processes of renal fibrosis (23). To investigate the effect of ACY-1215 in the UUO model, we examined by immunoblot analysis the expression of α-SMA, a hallmark of myofibroblasts (active fibroblasts) as well as expression of ECM proteins collagen III and fibronectin. As indicated in Figure 3A-D, α-SMA, collagen III, and fibronectin were detected in sham-operated kidneys with and without ACY-1215 administration; their expression levels were dramatically increased, however, in the kidneys of mice subjected to UUO. Administration of ACY-1215 largely blocked UUO-induced α-SMA, collagen III, fibronectin expression. These results suggested that pharmacological targeting of HDAC6 can prevent the development of renal fibrosis and inhibit differentiation of renal interstitial fibroblasts into myofibroblasts.

HDAC6 is required for activation of the TGFβ/Smad3 signaling pathway in the kidney after UUO injury

TGFβ1 signaling pathway plays a predominant role in promoting development of renal interstitial fibrosis (12). To explore whether HDAC6 is involved in the activation of TGFβ1/Smad signaling

pathway, we examined the effect of ACY-1215 on the phosphorylation of Smad3 (p-Smad3) and expression of TGFβ1 and Smad7 in the UUO injured kidney. As shown in Figure 4, A-D, a small amount of TGFβ1 was expressed in sham-operated kidneys; it was increased after UUO. p-Smad3 is minimally detectable in normal kidneys, but UUO damage significantly increased its phosphorylation. Smad7 is abundantly expressed in normal kidneys, but its levels declined in UUO injured kidneys. ACY-1215 treatment significantly reduced TGFβ1 expression and Smad3 phosphorylation, while partially restored Smad7 expression in the injured kidney. These results show that ACY-1215 may alleviate renal fibrosis by inhibiting the TGFβ1/Smad3 signaling pathway through a mechanism associated with preservation of Smad7 expression.

Inhibition of HDAC6 suppresses phosphorylation of EGFR and AKT in the kidney after UUO injury

Activation of the EGFR/AKT signaling pathway promotes the progression of renal fibrosis. In neuronal cells, HDAC6 is involved in the activation of the AKT signaling pathway (44). As shown in Figure 5, A-B, the expression of p-EGFR in the injured kidney was increased, but largely suppressed by ACY-1215, while the expression level of total EGFR remained the same in all the groups. Corresponding to this observation, AKT phosphorylation also increased in the injured kidney, while ACY-1215 treatment reduced this response. The expression level of total AKT was the same in the injured kidney and in the control kidney. Thus, these data suggest that HDAC6 may also contribute to renal fibrosis by activation of the EGFR/AKT signaling pathway.

Blocking HDAC6 inhibits activation of NF-κB and STAT3 signaling pathway in the kidney after UUO injury

NF-κB is a key transcription factor involved in the inflammatory response. Its activation can trigger the release of various inflammatory factors. The activation of the STAT3 signaling pathway is also related to the inflammatory response (25). To investigate the effect of NF-κB and STAT3 signaling

pathway in renal fibrosis, we examined the protein expression of p-NF-κB (p65), NF-κB (p65), p-STAT3 and STAT3. Figure 6, A-C showed that in the injured kidney, p-NF/κB (p65) and p-STAT3 increased; administration of ACY-1215 significantly decreased phosphorylation of NF-κB (p65) and STAT3 but did not affect expression of their total levels. Taken together, these results indicated that blocking HDAC6 partially inhibits activation of NF-κB and STAT3 signaling pathways in the kidney after UUO injury.

ACY-1215 inhibits activation of renal interstitial fibroblasts in culture. It has been reported that HDAC6 is expressed in fibroblasts (43). To understand whether HDAC6 mediates renal fibroblast activation, we examined the effect of ACY-1215 on the expression of α-SMA in renal interstitial fibroblasts (NRK-49F) cultured with 5% FBS. We demonstrated that ACY-1215 reduced expression of α-SMA in a dose dependent manner with the maximum at 50 μM (Figure 7A, B). As the concentration of ACY-1215 increased, the expression level of HDAC6 gradually decreased. In contrast, the expression level of acetylated histone 3 was gradually increased with increasing doses of ACY-1215, indicative of its effective inhibition of HDAC6. These inhibitory effects were not significantly different at 25 μM and 50 μM (Figure 7A, C, D). We thus suggest that HDAC6 mediates dedifferentiation of renal fibroblasts to myofibroblasts. Given that 25 μM of ACY-1215 reached the maximum inhibitory effect on HDAC6 expression and activation, this dose of ACY-1215 was used in the following in vitro experiments.

ACY-1215 inhibits TGFβ1-induced activation of renal interstitial fibroblasts

TGFβ1 is a major cytokine/growth factor, and serum is a mixture of growth factors, both of which can induce renal interstitial fibroblast activation and renal fibrosis. As such, we asked whether TGF-β1 would further stimulate activation of renal fibroblasts in the presence of 5% serum and whether ACY-1215 would affect activation of renal fibroblasts. To do this, we added TGFβ1 (5 ng/ml) to the culture of NRK-49F cells with 5% FBS in the presence or absence of 25 μM of ACY-1215 and then continued culturing for 36 hours. The collected cell lysates were subjected to

immunoblot analysis. Figure 8, A-G showed that compared with the control group (5% FBS), TGFβ1 addition further increased the expression levels of α-SMA, which was accompanied by a slight increase of HDAC6. Treatment with ACY-1215 suppressed expression of α-SMA and HDAC6 in cells treated with and without TGFβ1, which was coincident with increased expression of acetylated histone 3. These data suggest that combined treatment with serum and TGFβ1 has an additive effect on renal fibroblast activation and HDAC6 also mediate this process.

ACY-1215 inhibits TGFβ1-induced profibrotic phenotype changes of cultured renal epithelial cells

It has been reported that upon injury or stimulation with growth factors or cytokines such as TGFβ1, renal tubular epithelial cells display a profibrotic phenotype that expresses α-SMA and ECM proteins (10, 23). Given that HDAC6 is highly expressed in renal tubular epithelial cells, we further examined the effect of ACY-1215 on the transition of renal epithelial cells to a profibrotic phenotype by examining expression of α-SMA, fibronectin and collagen III in cultured mTECs. As shown in Figure 9A-D, the basal levels of α-SMA, fibronectin and collagen III were detected in mTECs and ACY-1215 treatment did not significantly alter their expression. Exposure of cells to TGFβ1 resulted in increased expression of these three proteins, and presence of ACY-1215 markedly reduced their expression; this was coincident with downregulation of HDAC6 and upregulation of acetyl-histone H3 (Figure 9E-G). These data suggest that HDAC6 also mediates TGF-β1-induced profibrotic phenotype changes of renal epithelial cells.

ACY-1215 inhibits expression of TGFβ1 in the kidney after UUO and in cultured renal epithelial cells after aristolochic acid exposure

Recent research has demonstrated that injury-induced profibrotic phenotype of renal tubular cells acquires the ability to produce a variety of profibrotic factors and cytokines, including TGFβ1 (10, 23). Given that Figure 4A and B shows that ACY-1215 treatment reduced expression of TGFβ1,

we proceeded to examine specifically whether HDAC6 would mediate expression of TGFβ1 in renal epithelial cells. Immunohistochemical staining indicated that TGFβ1 was abundantly expressed in the renal tubular cells of UUO injured kidney, and significantly declined after treatment with ACY-1215 (Figure 10A-B). Notably, TGFβ1 was minimally expressed in this cell type of sham-operated kidney. Similar to this observation, TGFβ1 expression levels were also increased in mTECs upon exposure to aristolochic acid compared with the control culture; ACY-1215 treatment also reduced this response (Figure 10 C-D). As expected, ACY-1215 was effective in the inactivation of HDAC6 as indicated by increased expression of acetyl-histone H3 (Figure 10C, E). This inhibitor also slightly reduced HADC6 expression (Figure 10 C, F). On this basis, we suggest that HDAC6 contributes to the expression and production of TGF-β1 in the renal epithelial cells after injury.

DISCUSSION

Our recent studies have demonstrated that HDAC6 plays a critical role in AKI (30, 32), and peritoneal fibrosis (38)in animal models. In the current study, we found that inhibition of HDAC6 with ACY-1215 also reduced the accumulation of extracellular matrix components and inhibited TGFβ/Smad3 and EGFR, two key signaling pathways associated with fibrosis in the kidney after UUO injury. Moreover, ACY-1215 was effective in inhibiting activation of renal interstitial fibroblasts in culture. These data indicate that HDAC6 is a critical mediator in renal fibrosis and suggest that pharmacological inactivation of HDAC6 could offer therapeutic effects for renal fibrosis.

Unlike most HDAC isoforms, which are located in the nucleus, HDAC6 contains a cytoplasmic retention signal and a nuclear localization signal (NLS)(2).This structural feature enables it to shuttle between the nucleus and the cytoplasm and deacetylate proteins both in the nucleus (i.e. histone H3) and cytoplasm (i.e. α-tubulin)(17). In this study, we observed that UUO injury results in increased expression of renal HDAC6, which was mainly expressed in the cytosol of renal tubular cells in the injured kidney. This suggests that profibrotic actions of HDAC6 may be primarily

306 initiated in renal epithelial cells. Although it remains controversial whether renal epithelial cells
307 become renal fibroblasts through a complete or partial process of epithelial-mesenchymal
308 transition, recent studies have shown that partial EMT can occur in tubular epithelial cells that then
309 arrests at the G2/M phase of the cell cycle (10, 23). This type of cells acquires the ability to produce
310 pro-fibrotic factors leading to renal fibrosis (10, 23). In support of this hypothesis, the present study
311 found that blocking HADC6 with ACY-1215 inhibited transition of renal tubular epithelial cells to a
312 profibrotic phenotype in cultured mTECs and injury–induced expression of TGFβ1 in renal
313 epithelial cells in vitro and in vivo. Our previous studies also demonstrate that HDAC6 is involved
314 in the EMT response of peritoneal mesothelial cells (38). Moreover, Shan et al. have shown that
315 HDAC6 activation is essential for induction of EMT in lung cancer cell lines (A549) and breast
316 epithelial cells (11). Nevertheless, we cannot exclude the possibility that HDAC6 may also
317 contribute to renal fibrosis through direct activation of renal interstitial fibroblasts. This is evident
318 by our observations that increased HDAC6 in the cultured renal interstitial fibroblasts exposed to
319 serum and TGF-β1, and inhibition of HDAC6 significantly reduced expression levels of α-SMA, a
320 hallmark of myofibroblasts in vivo and in vitro.

321 The mechanisms by which HDAC6 mediates renal fibrosis remain elusive but may be
322 associated with activation of TGFβ1/Smad3 signaling. In the UUO injured kidney, treatment with
323 ACY-1215 reduced TGFβ1 expression and Smad3 phosphorylation levels, suggesting that
324 HDAC6 is required for the activation of TGFβ1/Smad3 signaling. How HDAC6 promotes Smad3
325 activation remains unclear, but it may be related to regulation of Smad7. As Smad7 is a negative
326 feedback regulator of the TGFβ1/Smad3 pathway, its down-regulation can reciprocally promote
327 the recruitment of Smad3 to phosphorylated TGFβ1 receptor to induce its phosphorylation (8).
328 Thus, HDAC6-induced upregulation of Smad7 may counteract the action of TGFβ1/Smad3.
329 Indeed, our results show that UUO injury increased the expression level of TGFβ1 and p-Smad3
330 and reduced Smad7 expression, while administration of ACY-1215 significantly inhibited
331 expression of TGFβ1 and p-Smad3 while partially restoring Smad7 expression. Similarly, ACY-

1215 also effectively reduced expression of TGFβ1 in cultured renal epithelial cells stimulated by aristolochic acid. Since HDAC6 is mainly distributed in the cytoplasm and can acetylate many cytoplasmic proteins (16, 29), it is also possible that HDAC6 may directly modify Smad3 and then change its phosphorylation levels. Further work is needed to test this hypothesis.

HDAC6 may also attenuate renal fibrosis by inhibiting the EGFR signaling pathway. Increasingly, studies reveal that activation of the EGFR signaling pathway not only regulates kidney development and regeneration, but that the pathway also participates in chronic kidney disease caused by different etiologies such as diabetic nephropathy (18), uric acid nephropathy (22), and obstructive nephropathy (20). The primary pathological change of these various forms of kidney disease is renal interstitial fibrosis. Our previous research indicates that the fibrotic kidney contains persistently high expression of phosphorylated EGFR (31), suggesting excessive activation of EGFR. Excessive activation of EGFR signaling can promote the expression of TGFβ1, Smad3 activation, epithelial cell arrest in the G2/M stage of the cell cycle and inflammatory cytokine release (37). In addition, EGFR also plays a key role in mediating Ang II-induced renal fibrosis (5). Therefore, EGFR can be used as a convergent point of signaling pathways to promote the occurrence and development of fibrosis (37). In this study, we found that blocking HDAC6 significantly reduced levels of UUO-induced phosphorylated EGFR and also inhibited the phosphorylation of its downstream signaling protein molecule AKT. As such, HDAC6 may also promote renal fibrosis by regulating the activation of EGFR signaling pathway.

HDAC6 activation may also be required for the inflammatory response during the process of fibrogenesis in the kidney. Renal inflammation is characterized by expression of cytokines/chemokines and macrophage infiltration, and STAT3 and NF-κB are two major transcription factors involved in promoting the release of proinflammatory cytokines and chemokines (1, 6). We found that targeted inhibition of HDAC6 significantly reduced the phosphorylation level of STAT3 and NF-κB (p65), suggesting that HDAC6 intervention can reduce the expression of various inflammatory cytokines / chemokines by inhibiting STAT3 and NF-κB

and other inflammation-related transcription factor, thus alleviate renal fibrosis. In line with this speculation, our recent study revealed that dephosphorylation of STAT3 and NF-κB as a result of HDAC6 inhibition is coincident with the suppression of multiple pro-inflammatory cytokines (38). HDACs are overexpressed in many kidney diseases, in particular renal fibrosis, and are thus proposed as promising therapeutic targets. While pan-HDAC inhibitors have shown excellent efficacy in the treatment of many forms of kidney disease, including diabetic nephropathy, polycystic kidney disease and lupus nephritis (17), their significant adverse effects largely limited their clinical application in chronic indications. On this basis, developing HDAC isoform selective inhibitors may have more clinical value than pan-HDAC inhibitors. Only HDAC6 knockout mice develop normally and have no life limiting defects, suggesting that HDAC6 inhibitors could exert therapeutic effects with no apparent toxicity (43). Currently, ACY-1215 is undergoing Phase I / II clinical evaluation for the treatment of multiple myeloma and lymphoid malignancies (9). Our results showing that ACY-1215 effectively reduced renal fibrosis, thus providing a theoretical basis for future clinical trials of that HDAC6 inhibitor to prevent and treat renal fibrosis.

In conclusion, we used HDAC6 inhibitors for the first time to successfully alleviate the occurrence and development of renal fibrosis. The anti-fibrotic effects of HDAC6 inhibition are related to the inactivation of TGF-β1/Smad3, EGFR/AKT, NF-κB and STAT3 signaling pathways. These results provide evidence that HDAC6 could be a feasible target for the prevention and treatment of renal fibrosis.

ACKNOWLEDGMENTS

We thank Dr. George Bayliss for his critical editing of this manuscript.

GRANTS

This study was supported by the National Natural Science Foundation of China grants (81670623 and 81830021 to S.Z.), National key R&D Program of China (2018YFA0108802 to S.Z.), US National Institutes of Health (2R01DK08506505A1 to S.Z.).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

X.C and S.Z conceived and designed research; X.C.,C.Y., X.H.,J.L and T.L conducted experiments; X.C. analyzed data, prepared figures and interpreted results of experiments; N.L. and A.Q. interpreted results of experiments. X.C., S.Z drafted manuscript; S.Z edited and revised manuscript; X.C., C.Y., X.H., J.L., T.L., N.L., A.Q. and S.Z approved final version of manuscript.

REFERENCES

1. **Andrade-Oliveira V, Foresto-Neto O, Watanabe IKM, Zatz R, and Câmara NOS**. Inflammation in Renal Diseases: New and Old Players. *Front Pharmacol* 10: 1192, 2019. doi:10.3389/fphar.2019.01192.
2. **Bertos NR, Gilquin B, Chan GK, Yen TJ, Khochbin S, and Yang XJ**. Role of the tetradecapeptide repeat domain of human histone deacetylase 6 in cytoplasmic retention. *J Biol Chem* 279: 48246-48254, 2004. doi:10.1074/jbc.M408583200.
3. **Brindisi M, Saraswati AP, Brogi S, Gemma S, Butini S, and Campiani G**. Old but Gold: Tracking the New Guise of Histone Deacetylase 6 (HDAC6) Enzyme as a Biomarker and Therapeutic Target in Rare Diseases. *J Med Chem* 63: 23-39, 2020. doi:10.1021/acs.jmedchem.9b00924.
4. **Butler KV, Kalin J, Brochier C, Vistoli G, Langley B, and Kozikowski AP**. Rational design and simple chemistry yield a superior, neuroprotective HDAC6 inhibitor, tubastatin

A. *J Am Chem Soc* 132: 10842-10846, 2010. doi:10.1021/ja102758v.

5. **Chen L, Yang T, Lu DW, Zhao H, Feng YL, Chen H, Chen DQ, Vaziri ND, and Zhao YY**. Central role of dysregulation of TGF-β/Smad in CKD progression and potential targets of its treatment. *Biomed Pharmacother* 101: 670-681, 2018. doi:10.1016/j.biopha.2018.02.090.

6. **Chen W, Yuan H, Cao W, Wang T, Chen W, Yu H, Fu Y, Jiang B, Zhou H, Guo H, and Zhao X**. Blocking interleukin-6 trans-signaling protects against renal fibrosis by suppressing STAT3 activation. *Theranostics* 9: 3980-3991, 2019. doi:10.7150/thno.32352.

7. **Choi SY, Ryu Y, Kee HJ, Cho SN, Kim GR, Cho JY, Kim HS, Kim IK, and Jeong MH**. Tubastatin A suppresses renal fibrosis via regulation of epigenetic histone modification and Smad3-dependent fibrotic genes. *Vascul Pharmacol* 72: 130-140, 2015. doi:10.1016/j.vph.2015.04.006.

8. **Fleischmajer R, Perlish JS, Burgeson RE, Shaikh-Bahai F, and Timpl R**. Type I and type III collagen interactions during fibrillogenesis. *Ann N Y Acad Sci* 580: 161-175, 1990. doi:10.1111/j.1749-6632.1990.tb17927.x.

9. **Gao X, Shen L, Li X, and Liu J**. Efficacy and toxicity of histone deacetylase inhibitors in relapsed/refractory multiple myeloma: Systematic review and meta-analysis of clinical trials. *Exp Ther Med* 18: 1057-1068, 2019. doi:10.3892/etm.2019.7704.

10. **Grande MT, Sánchez-Laorden B, López-Blau C, De Frutos CA, Boutet A, Arévalo M, Rowe RG, Weiss SJ, López-Novoa JM, and Nieto MA**. Snail1-induced partial epithelial-to-mesenchymal transition drives renal fibrosis in mice and can be targeted to reverse established disease. *Nat Med* 21: 989-997, 2015. doi:10.1038/nm.3901.

11. **Gu S, Liu Y, Zhu B, Ding K, Yao TP, Chen F, Zhan L, Xu P, Ehrlich M, Liang T, Lin X, and Feng XH**. Loss of α-Tubulin Acetylation Is Associated with TGF-β-induced Epithelial-Mesenchymal Transition. *J Biol Chem* 291: 5396-5405, 2016. doi:10.1074/jbc.M115.713123.

12. **Gu YY, Liu XS, Huang XR, Yu XQ, and Lan HY**. Diverse Role of TGF-β in Kidney Disease. *Front Cell Dev Biol* 8: 123, 2020. doi:10.3389/fcell.2020.00123.

13. **Hackanson B, Rimmele L, Benkißer M, Abdelkarim M, Fliegauf M, Jung M, and Lübbert M**. HDAC6 as a target for antileukemic drugs in acute myeloid leukemia. *Leuk Res* 36: 1055-1062, 2012. doi:10.1016/j.leukres.2012.02.026.

14. **Harskamp LR, Gansevoort RT, van Goor H, and Meijer E**. The epidermal growth factor receptor pathway in chronic kidney diseases. *Nat Rev Nephrol* 12: 496-506, 2016. doi:10.1038/nrneph.2016.91.

15. **Haverty TP, Kelly CJ, Hines WH, Amenta PS, Watanabe M, Harper RA, Kefalides NA, and Neilson EG**. Characterization of a renal tubular epithelial cell line which secretes the autologous target antigen of autoimmune experimental interstitial nephritis. *J Cell Biol* 107: 1359-1368, 1988. doi:10.1083/jcb.107.4.1359.

16. **Hyndman KA**. Histone Deacetylases in Kidney Physiology and Acute Kidney Injury. *Semin Nephrol* 40: 138-147, 2020. doi:10.1016/j.semnephrol.2020.01.005.

17. **Ke B, Chen Y, Tu W, Ye T, Fang X, and Yang L**. Inhibition of HDAC6 activity in kidney diseases: a new perspective. *Mol Med* 24: 33, 2018. doi:10.1186/s10020-018-0027-4.

18. **Li Z, Li Y, Overstreet JM, Chung S, Niu A, Fan X, Wang S, Wang Y, Zhang M-Z, and Harris RC**. Inhibition of Epidermal Growth Factor Receptor Activation Is Associated With Improved Diabetic Nephropathy and Insulin Resistance in Type 2 Diabetes. *Diabetes* 67: 1847-1857, 2018. doi:10.2337/db17-1513.

19. **Liu F, and Zhuang S**. Role of Receptor Tyrosine Kinase Signaling in Renal Fibrosis. *Int J Mol Sci* 17: 2016. doi:10.3390/ijms17060972.

20. **Liu N, Guo JK, Pang M, Tolbert E, Ponnusamy M, Gong R, Bayliss G, Dworkin LD, Yan H, and Zhuang S**. Genetic or pharmacologic blockade of EGFR inhibits renal fibrosis. *J Am Soc Nephrol* 23: 854-867, 2012. doi:10.1681/asn.2011050493.

21. **Liu N, Tolbert E, Pang M, Ponnusamy M, Yan H, and Zhuang S**. Suramin inhibits renal

fibrosis in chronic kidney disease. *J Am Soc Nephrol* 22: 1064-1075, 2011. doi:10.1681/asn.2010090956.

22. **Liu N, Wang L, Yang T, Xiong C, Xu L, Shi Y, Bao W, Chin YE, Cheng SB, Yan H, Qiu A, and Zhuang S**. EGF Receptor Inhibition Alleviates Hyperuricemic Nephropathy. *J Am Soc Nephrol* 26: 2716-2729, 2015. doi:10.1681/asn.2014080793.

23. **Lovisa S, LeBleu VS, Tampe B, Sugimoto H, Vadnagara K, Carstens JL, Wu CC, Hagos Y, Burckhardt BC, Pentcheva-Hoang T, Nischal H, Allison JP, Zeisberg M, and Kalluri R**. Epithelial-to-mesenchymal transition induces cell cycle arrest and parenchymal damage in renal fibrosis. *Nat Med* 21: 998-1009, 2015. doi:10.1038/nm.3902.

24. **Meng XM, Tang PM, Li J, and Lan HY**. TGF-β/Smad signaling in renal fibrosis. *Front Physiol* 6: 82, 2015. doi:10.3389/fphys.2015.00082.

25. **O'Brown ZK, Van Nostrand EL, Higgins JP, and Kim SK**. The Inflammatory Transcription Factors NFκB, STAT1 and STAT3 Drive Age-Associated Transcriptional Changes in the Human Kidney. *PLoS genetics* 11: e1005734, 2015. doi:10.1371/journal.pgen.1005734.

26. **Pang M, Kothapally J, Mao H, Tolbert E, Ponnusamy M, Chin YE, and Zhuang S**. Inhibition of histone deacetylase activity attenuates renal fibroblast activation and interstitial fibrosis in obstructive nephropathy. *Am J Physiol Renal Physiol* 297: F996-f1005, 2009. doi:10.1152/ajprenal.00282.2009.

27. **Richardson PG, Moreau P, Laubach JP, Maglio ME, Lonial S, and San-Miguel J**. Deacetylase inhibitors as a novel modality in the treatment of multiple myeloma. *Pharmacol Res* 117: 185-191, 2017. doi:10.1016/j.phrs.2016.11.020.

28. **Santo L, Hideshima T, Kung AL, Tseng J-C, Tamang D, Yang M, Jarpe M, van Duzer JH, Mazitschek R, Ogier WC, Cirstea D, Rodig S, Eda H, Scullen T, Canavese M, Bradner J, Anderson KC, Jones SS, and Raje N**. Preclinical activity, pharmacodynamic, and pharmacokinetic properties of a selective HDAC6 inhibitor, ACY-1215, in combination

with bortezomib in multiple myeloma. *Blood* 119: 2579-2589, 2012. doi:10.1182/blood-2011-10-387365.

29. **Seidel C, Schnekenburger M, Dicato M, and Diederich M**. Histone deacetylase 6 in health and disease. *Epigenomics* 7: 103-118, 2015. doi:10.2217/epi.14.69.

30. **Shi Y, Xu L, Tang J, Fang L, Ma S, Ma X, Nie J, Pi X, Qiu A, Zhuang S, and Liu N**. Inhibition of HDAC6 protects against rhabdomyolysis-induced acute kidney injury. *Am J Physiol Renal Physiol* 312: F502-f515, 2017. doi:10.1152/ajprenal.00546.2016.

31. **Tang J, Liu N, Tolbert E, Ponnusamy M, Ma L, Gong R, Bayliss G, Yan H, and Zhuang S**. Sustained activation of EGFR triggers renal fibrogenesis after acute kidney injury. *Am J Pathol* 183: 160-172, 2013. doi:10.1016/j.ajpath.2013.04.005.

32. **Tang J, Shi Y, Liu N, Xu L, Zang X, Li P, Zhang J, Zheng X, Qiu A, and Zhuang S**. Blockade of histone deacetylase 6 protects against cisplatin-induced acute kidney injury. *Clin Sci (Lond)* 132: 339-359, 2018. doi:10.1042/cs20171417.

33. **Tang J, Yan H, and Zhuang S**. Histone deacetylases as targets for treatment of multiple diseases. *Clin Sci (Lond)* 124: 651-662, 2013. doi:10.1042/cs20120504.

34. **Tang J, and Zhuang S**. Epigenetics in acute kidney injury. *Curr Opin Nephrol Hypertens* 24: 351-358, 2015. doi:10.1097/mnh.0000000000000140.

35. **Vogl DT, Raje N, Jagannath S, Richardson P, Hari P, Orlowski R, Supko JG, Tamang D, Yang M, Jones SS, Wheeler C, Markelewicz RJ, and Lonial S**. Ricolinostat, the First Selective Histone Deacetylase 6 Inhibitor, in Combination with Bortezomib and Dexamethasone for Relapsed or Refractory Multiple Myeloma. *Clin Cancer Res* 23: 3307-3315, 2017. doi:10.1158/1078-0432.ccr-16-2526.

36. **Wang F, Zhong BW, and Zhao ZR**. ACY 1215, a histone deacetylase 6 inhibitor, inhibits cancer cell growth in melanoma. *J Biol Regul Homeost Agents* 32: 851-858, 2018.

37. **Wang L, Liu N, Xiong C, Xu L, Shi Y, Qiu A, Zang X, Mao H, and Zhuang S**. Inhibition of EGF Receptor Blocks the Development and Progression of Peritoneal Fibrosis. *J Am*

Soc Nephrol 27: 2631-2644, 2016. doi:10.1681/asn.2015030299.

38. **Xu L, Liu N, Gu H, Wang H, Shi Y, Ma X, Ma S, Ni J, Tao M, Qiu A, and Zhuang S**. Histone deacetylase 6 inhibition counteracts the epithelial-mesenchymal transition of peritoneal mesothelial cells and prevents peritoneal fibrosis. *Oncotarget* 8: 88730-88750, 2017. doi:10.18632/oncotarget.20982.

39. **Yanda MK, Liu Q, and Cebotaru L**. An inhibitor of histone deacetylase 6 activity, ACY-1215, reduces cAMP and cyst growth in polycystic kidney disease. *Am J Physiol Renal Physiol* 313: F997-f1004, 2017. doi:10.1152/ajprenal.00186.2017.

40. **Zhang L, Liu C, Wu J, Tao JJ, Sui XL, Yao ZG, Xu YF, Huang L, Zhu H, Sheng SL, and Qin C**. Tubastatin A/ACY-1215 improves cognition in Alzheimer's disease transgenic mice. *J Alzheimers Dis* 41: 1193-1205, 2014. doi:10.3233/jad-140066.

41. **Zhang WB, Zhang HY, Jiao FZ, Wang LW, Zhang H, and Gong ZJ**. Histone deacetylase 6 inhibitor ACY-1215 protects against experimental acute liver failure by regulating the TLR4-MAPK/NF-κB pathway. *Biomed Pharmacother* 97: 818-824, 2018. doi:10.1016/j.biopha.2017.10.103.

42. **Zhang WB, Zhang HY, Wang Y, Jiao FZ, Wang LW, and Gong ZJ**. Quantitative Proteomic Analysis Reveals the Sites Related to Acetylation and Mechanism of ACY-1215 in Acute Liver Failure Mice. *Front Pharmacol* 10: 653, 2019. doi:10.3389/fphar.2019.00653.

43. **Zhang Y, Kwon S, Yamaguchi T, Cubizolles F, Rousseaux S, Kneissel M, Cao C, Li N, Cheng HL, Chua K, Lombard D, Mizeracki A, Matthias G, Alt FW, Khochbin S, and Matthias P**. Mice lacking histone deacetylase 6 have hyperacetylated tubulin but are viable and develop normally. *Mol Cell Biol* 28: 1688-1701, 2008. doi:10.1128/mcb.01154-06.

44. **Zhu T, Zhao D, Song Z, Yuan Z, Li C, Wang Y, Zhou X, Yin X, Hassan MF, and Yang L**. HDAC6 alleviates prion peptide-mediated neuronal death via modulating PI3K-Akt-mTOR pathway. *Neurobiol Aging* 37: 91-102, 2016. doi:10.1016/j.neurobiolaging.2015.09.021.

538 **Figure legends**

539 **Figure 1. Inhibition of HDAC6 with ACY-1215 attenuates renal fibrosis. Mice were**
540 **subjected to UUO and daily treated with ACY-1215 for 7 days before harvesting for analysis.**
541 *A*: Photomicrographs illustrating Masson trichrome staining of kidney tissue. *B*: The percentage
542 of Masson trichrome-positive tubulointerstitial area (blue) relative to the whole area was quantified.
543 (original magnification × 200). Scale bar = 50 μm. *C*: Kidney tissue lysates were subject to
544 immunoblot analysis with specific antibodies against HDAC6, Acetyl-H3，Acetyl-α-tubulin or
545 GAPDH. *D*- *F*: The protein expression levels of HDAC6 (*D*), Acetyl-H3 (*E*) or Acetyl-α-tubulin (*F*)
546 were qualified by densitometry and normalized with GAPDH. Values are means ± SD of at least
547 three independent experiments. Bars with different letters (a-c) for each molecule are significantly
548 different from one other (P <0.05).

549 **Figure 2. Expression of HDAC6 in the kidney.** Mice were subjected to UUO and daily treated
550 with ACY-1215 for 7 days before harvesting for analysis. Photomicrograph illustrating protein
551 expression of HDAC6 (red) and α-SMA (green) after immunofluorescent co-staining of them and
552 counterstaining with DAPI (blue). (original magnification × 400). In the injured kidney, HDAC6 is
553 most abundant in the cytoplasm of renal tubular cells, but also observed in the nucleus of this cell
554 type. Scale bar = 50 μm.

555 **Figure 3. Inhibition of HDAC6 with ACY-1215 reduces renal fibroblast activation and ECM**
556 **protein deposition in the renal interstitium.** Mice were subjected to UUO and daily treated with
557 ACY-1215 for 7 days before harvesting for analysis. *A*: Whole kidney tissue lysates from
558 obstructed (UUO) and contralateral non-obstructed (Sham) ureters were processed for
559 immunoblotting analysis with antibodies specific to α-SMA, Fibronectin, Collagen III, GAPDH.
560 Expression levels of α-SMA (*B*), Fibronectin (*C*) and Collagen III (*D*) were qualified by densitometry

and normalized with GAPDH. Values are means ± SD of at least three independent experiments. Bars with different letters (a-c) for each molecule are significantly different from one other (P <0.05).

Figure 4. HDAC6 blockade inhibits UUO-induced activation of TGFβ/Smad3 signaling in the kidney. Mice were subjected to UUO and daily treated with ACY-1215 for 7 days before harvesting for analysis. *A*: Whole kidney tissue lysates from obstructed (UUO) and contralateral non-obstructed (Sham) ureters were processed for immunoblotting analysis. Whole kidney tissue lysates from obstructed (UUO) and contralateral non-obstructed (Sham) were processed for immunoblotting analysis with antibodies specific to TGFβ1, p-Smad3, Smad3, Smad7 and GAPDH. Expression levels of TGFβ1 (*B*), p-Smad3 (*C*) Smda3 (*D*) and Smad7 (*E*) were qualified by densitometry and normalized with GAPDH. Values are means ± SD of at least three independent experiments. Bars with different letters (a-d) for each molecule are significantly different from one other (P <0.05).

Figure 5. HDAC6 blockade inhibits UUO-induced activation of EGFR/AKT signaling pathway in the kidney. Mice were subjected to UUO and daily treated with ACY-1215 for 7 days before harvesting for analysis. *A*: Whole kidney tissue lysates from obstructed (UUO) and contralateral non-obstructed ureters (Sham) were processed for immunoblotting analysis with antibodies specific to p-EGFR, EGFR, p-AKT, and AKT. *B*: p-EGFR expression levels were qualified by densitometry and normalized EGFR. *C*： p-AKT expression levels were qualified by densitometry and normalized AKT. Values are means ± SD of at least three independent experiments. Bars with different letters (a-c) for each molecule are significantly different from one other (P <0.05).

Figure 6. HDAC6 blockade inhibits UUO-induced activation of STAT3/ NF-κB (p65) signaling pathway in the kidney. Mice were subjected to UUO and daily treated with ACY-1215 for 7 days before harvesting for analysis. *A*: Whole kidney tissue lysates from obstructed (UUO) and contralateral non-obstructed (Sham) were processed for immunoblotting analysis with antibodies

specific to p-NF-κB(p65), NF-κB (p65), p-STAT3, STAT3 and GAPDH. *B*: p-NF-κB(p65) expression levels were qualified by densitometry and normalized NF-κB(p65). *C*: p-STAT3 expression levels were qualified by densitometry and normalized with STAT3. Values are means ± SD of at least three independent experiments. Bars with different letters (a-d) for each molecule are significantly different from one another (P <0.05).

Figure 7. Inhibition of HDAC6 with ACY-1215 reduces activation of renal interstitial fibroblasts in cultured NRK-49F. NRK-49F were cultured with 5% FBS and treated with various concentrations of ACY-1215 (0-50 μM) for 36 hours. *A*: Western blot analysis of cell lyses with various antibodies as indicated. The expression levels of α-SMA (*B*), HDAC6 (*C*) and Acetyl-H3 (*D*) were qualified by qualified by densitometry and normalized with GAPDH. The values shown in the graph are the means ± SD of at least three independent experiments. Each letter (a-d) indicates that different bars are significantly different from each other (P <0.05).

Figure 8. Inhibition of HDAC6 with ACY-1215 reduces activation of renal interstitial fibroblasts in cultured NRK-49F. Normally cultured NRK-49F was exposed to 5 ng / ml TGFβ1 and then cultured for 36 h in the absence or presence of ACY-1215 (25 μM). *A* and *E*: Western blot analysis of cell lysates with various antibodies as indicated. The protein expression levels of Fibronectin (*B*), Collagen III (*C*), α-SMA (*D*), HDAC6 (F), Acetyl-H3 (*G*) were qualified by densitometry and normalized with GAPDH. The values shown in the graph are the means ± SD of at least three independent experiments. Each letter (a-c) indicates that different bars are significantly different from each other (P <0.05).

Figure 9. ACY-1215 inhibits profibrotic phenotype changes of renal epithelial cells. Serum-starved murine renal tubular epithelial cells (mTECs) were treated with TGF-β1 (5 ng/ml) in the presence or absence of ACY-1215 (25 μM) for 24 hours and then harvested. Western blot analysis of cell lysates with various antibodies as indicated (*A, E*). The protein expression levels of

Fibronectin (*B*), Collagen III (*C*), α-SMA (*D*), HDAC6 (*F*), Acetyl-histone H3 (G) were qualified by densitometry and normalized with GAPDH. The values shown in the graph are the means ± SD of at least three independent experiments. Each letter (a-c) indicates that different bars are significantly different from each other (P <0.05).

Figure 10. ACY-1215 inhibits expression of TGF-β1 in the kidney after UUO and in cultured renal tubular epithelial cells after aristolochic acid (AA) exposure. Mice were subjected to UUO and daily treatment with ACY-1215 for 7 days before harvesting for analysis. *A*: Photomicrographs illustrating TGFβ1 staining of kidney tissue. *B*: The percentage of TGFβ1 positive area (yellow) relative to the whole area was quantified. (original magnification × 200). Scale bar = 100 μm. *C*: Murine renal tubular epithelial cells were treated as indicated in Materials and Methods. The prepared cell lysates were subjected to immunoblot analysis using antibodies against TGFβ1, Acetyl-Histone H3 or HADC6. The protein expression levels of TGFβ1 (D), Acetyl-Histone H3 (E) or HDAC6 (F) were qualified by densitometry and normalized with GAPDH. The values shown in the graph are the means ± SD of at least three independent experiments. Each letter (a-c) indicates that different bars are significantly different from each other (P <0.05).

Figure 1

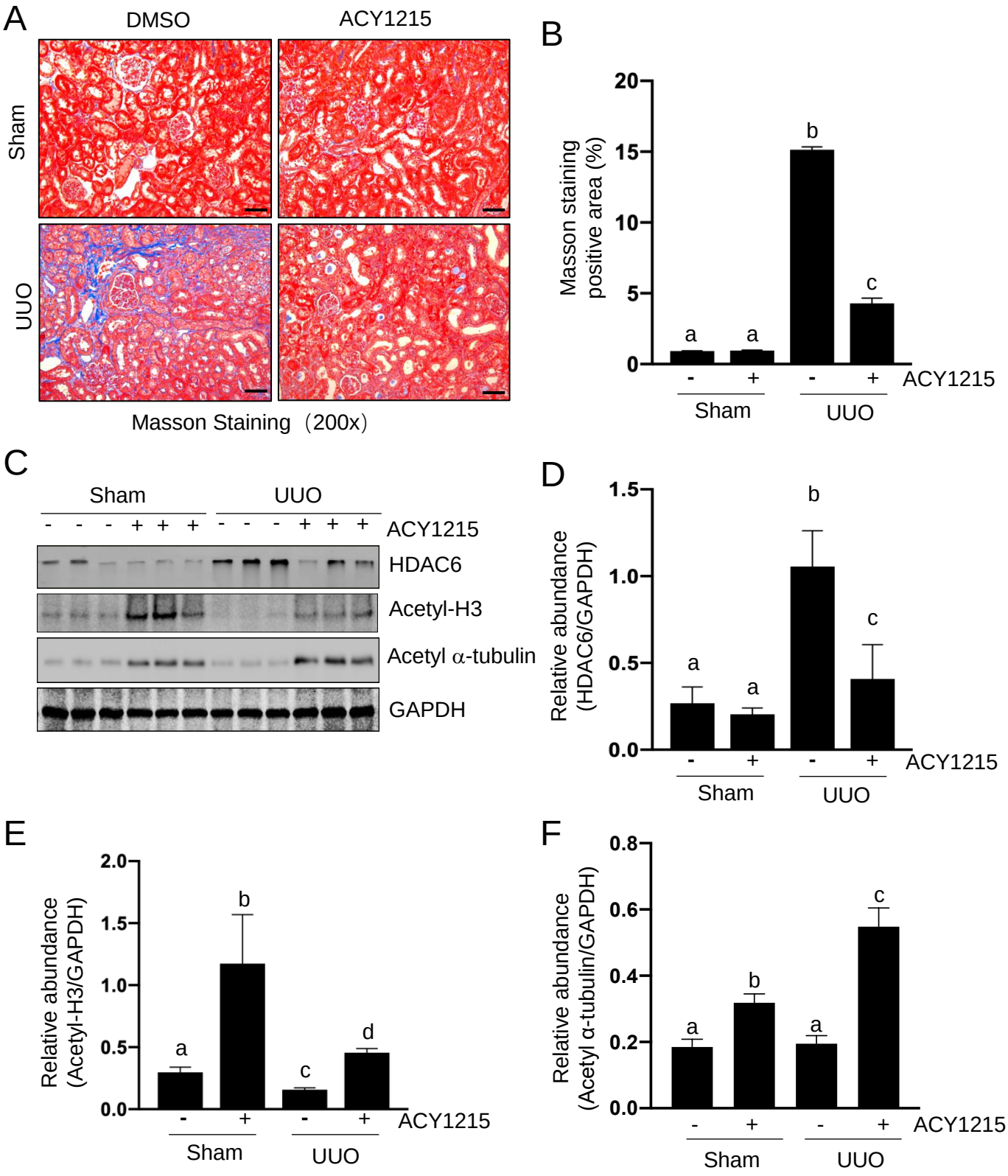
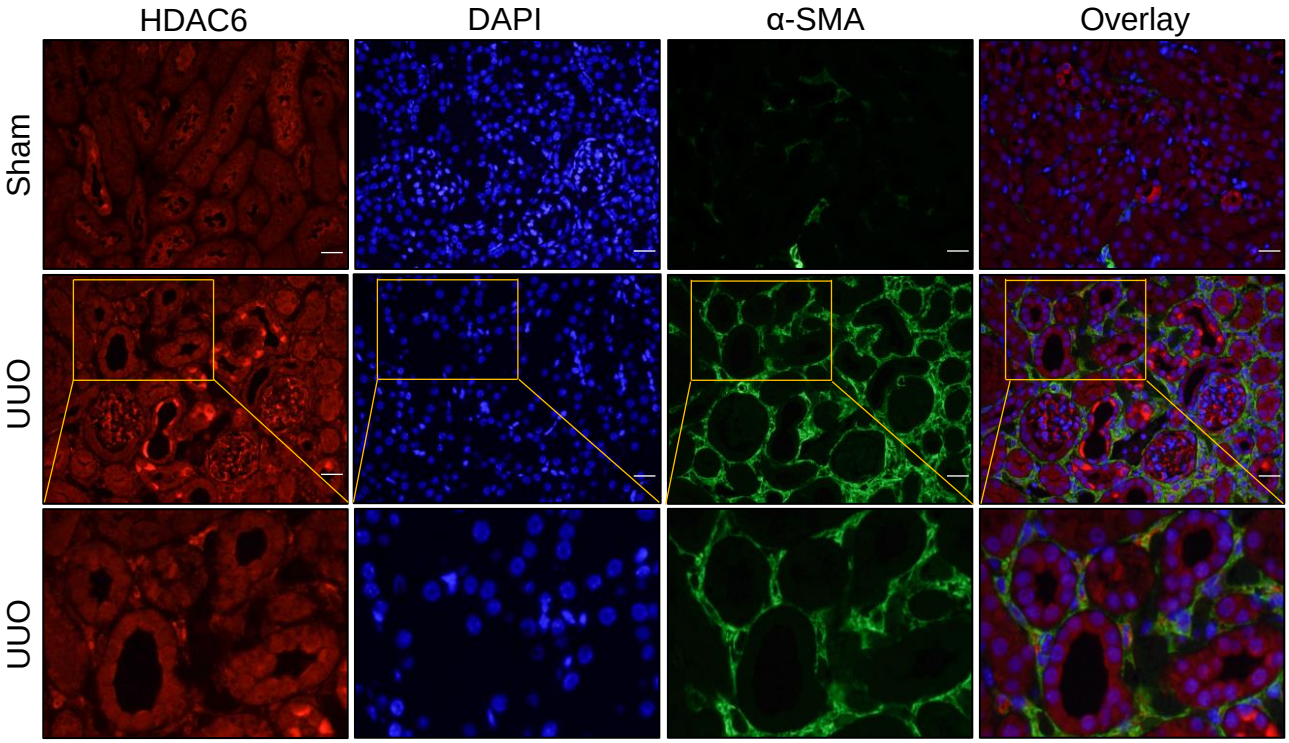


Figure 2



Immunofluorescence Staining(400x)

Figure 3

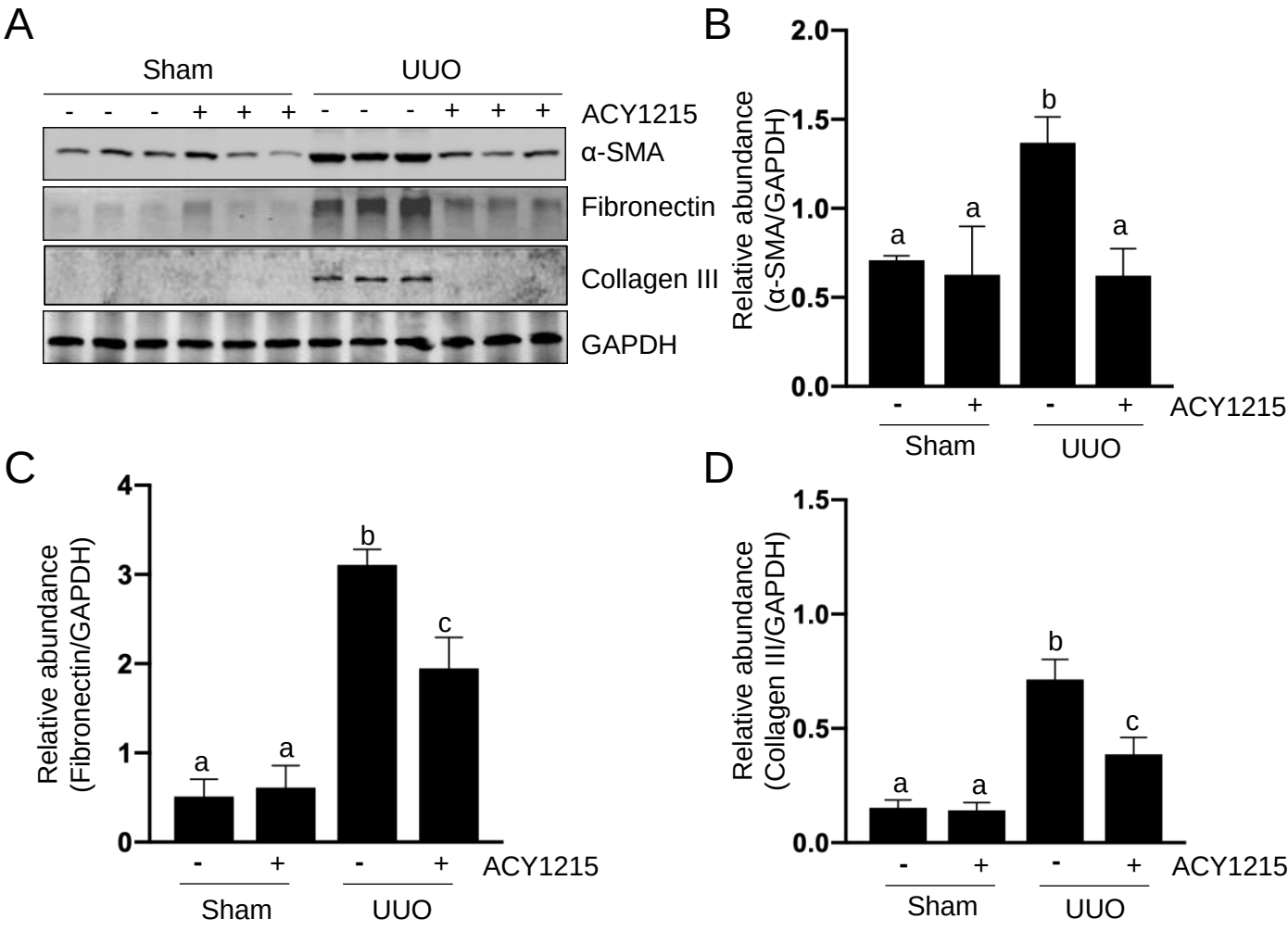


Figure 4

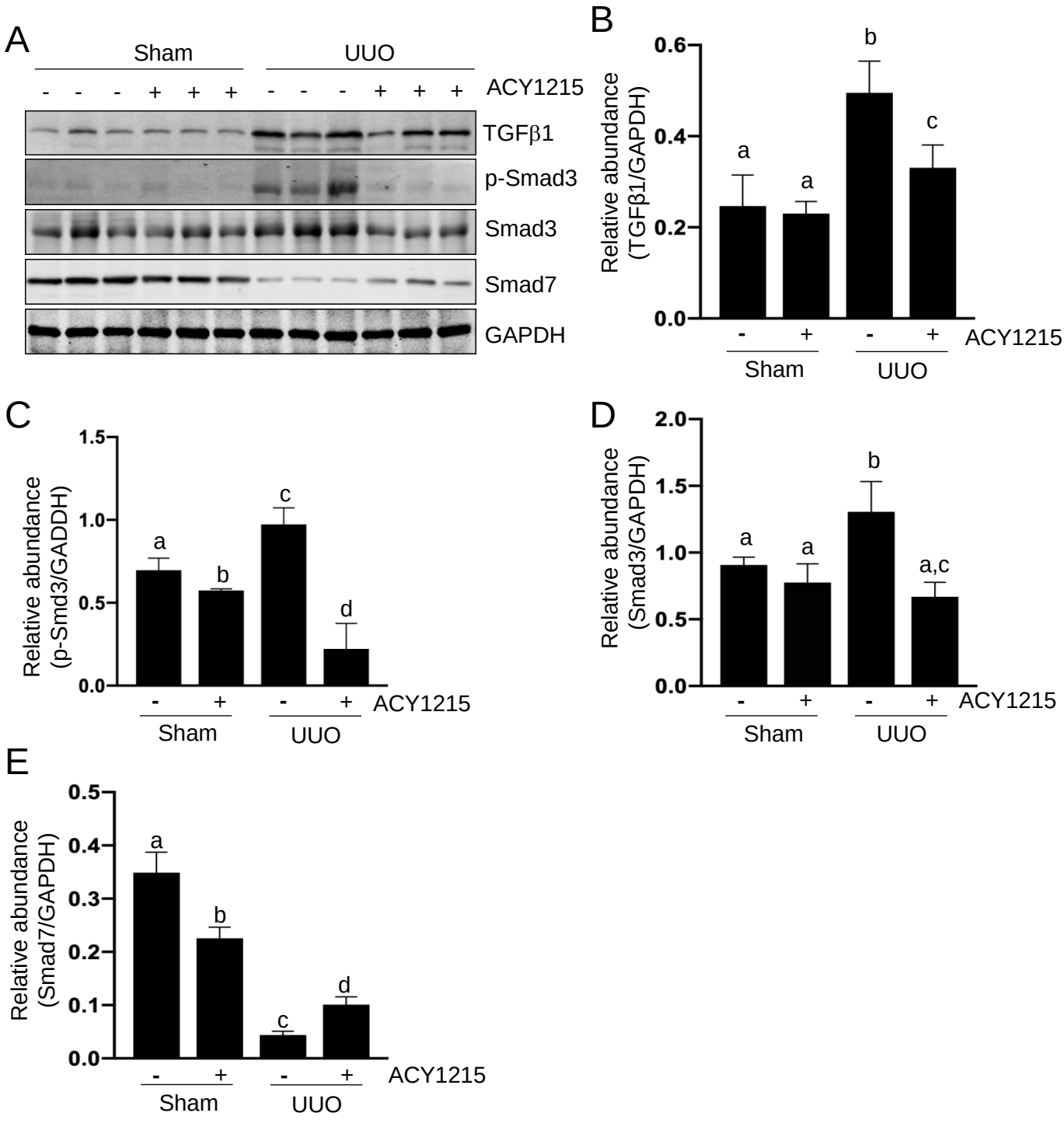


Figure 5

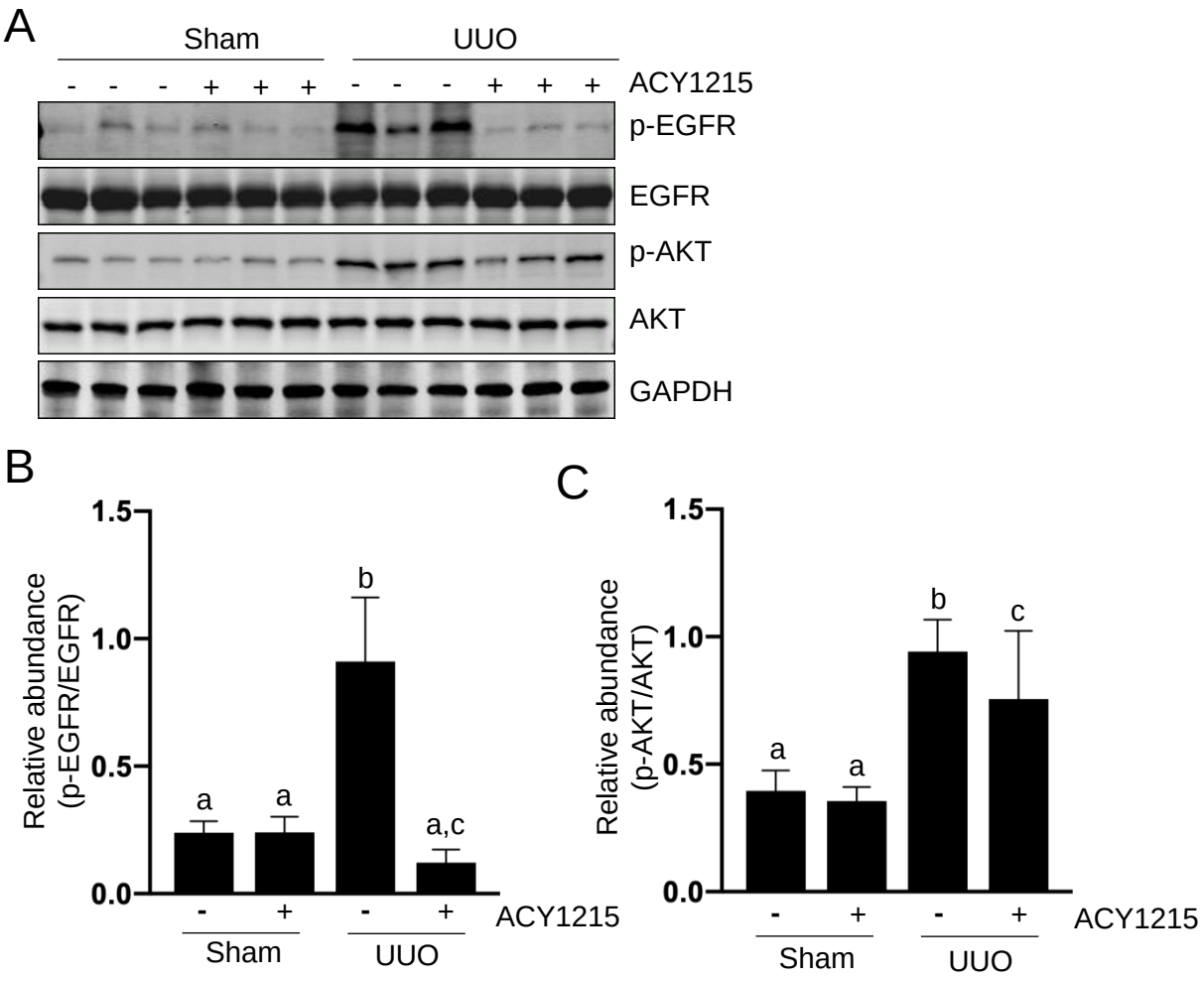


Figure 6

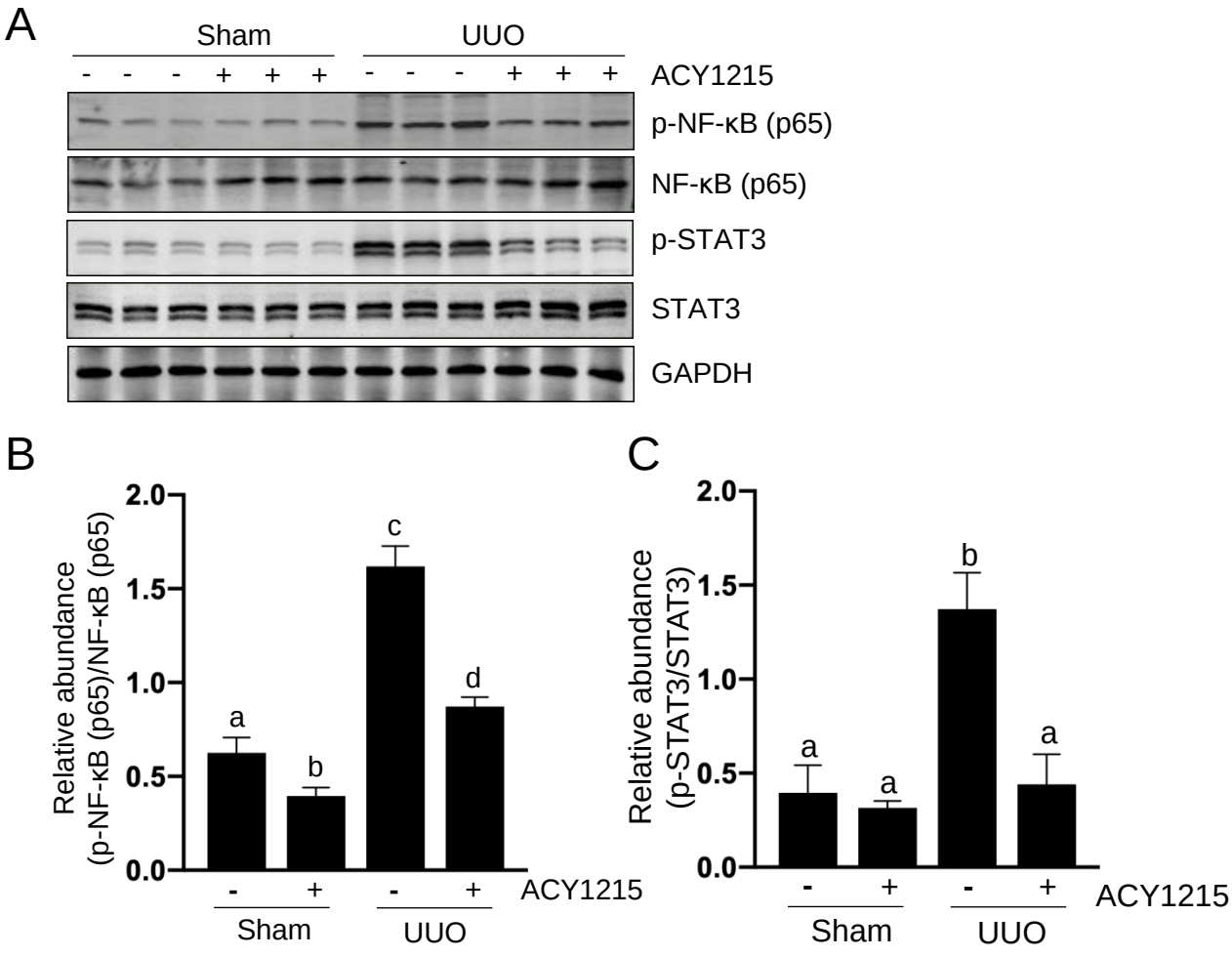


Figure 7

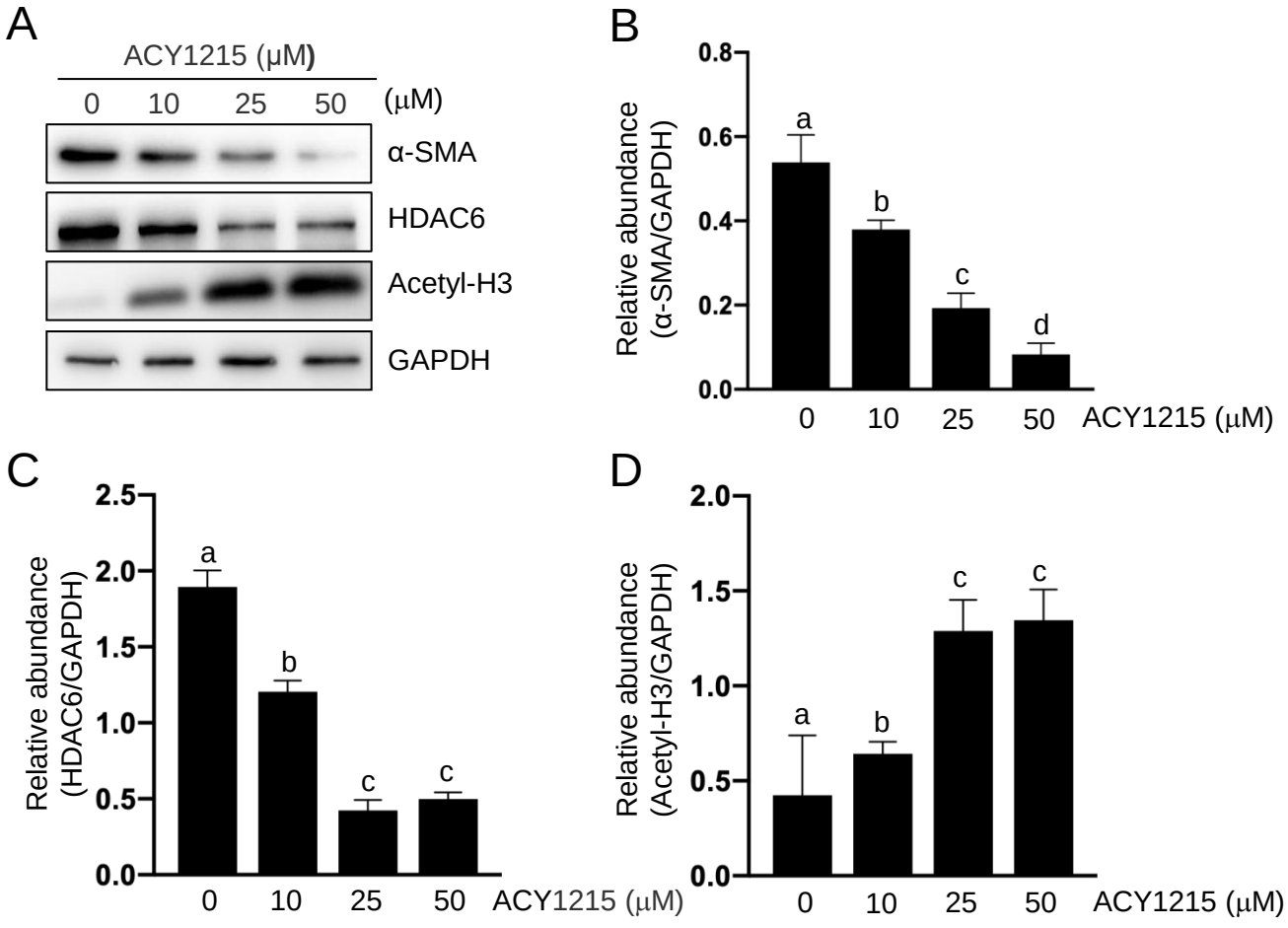


Figure 8

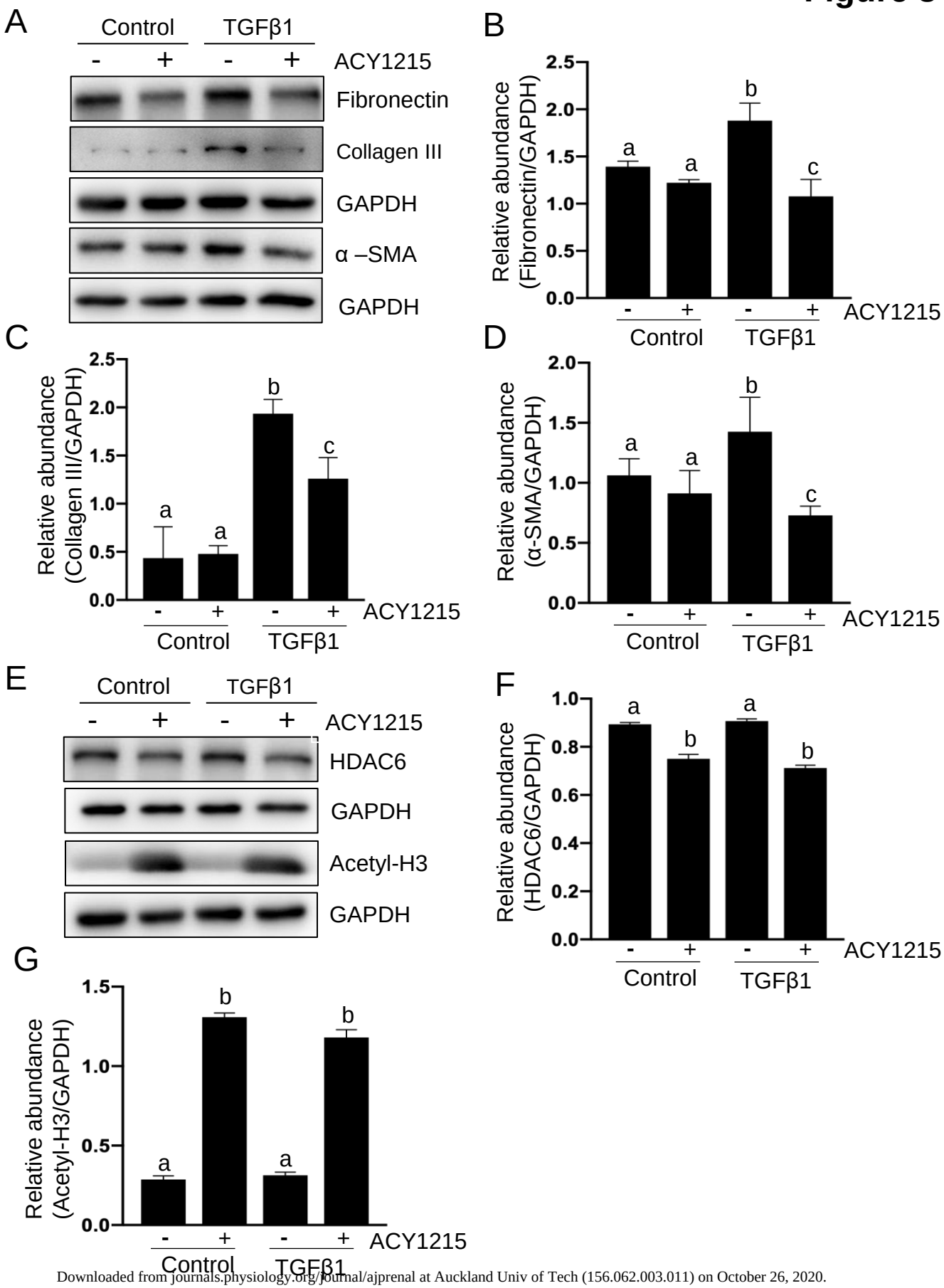


Figure 9

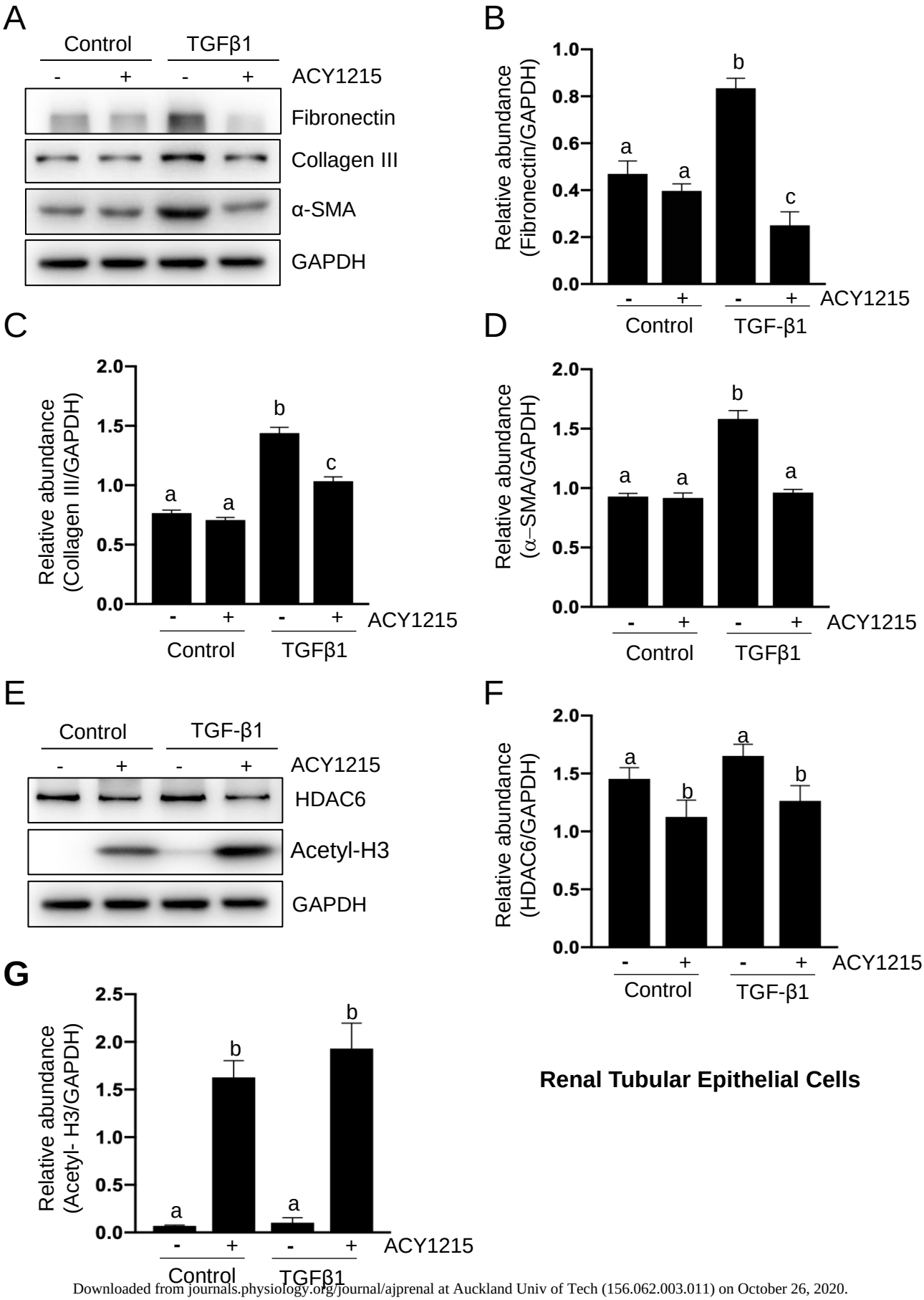
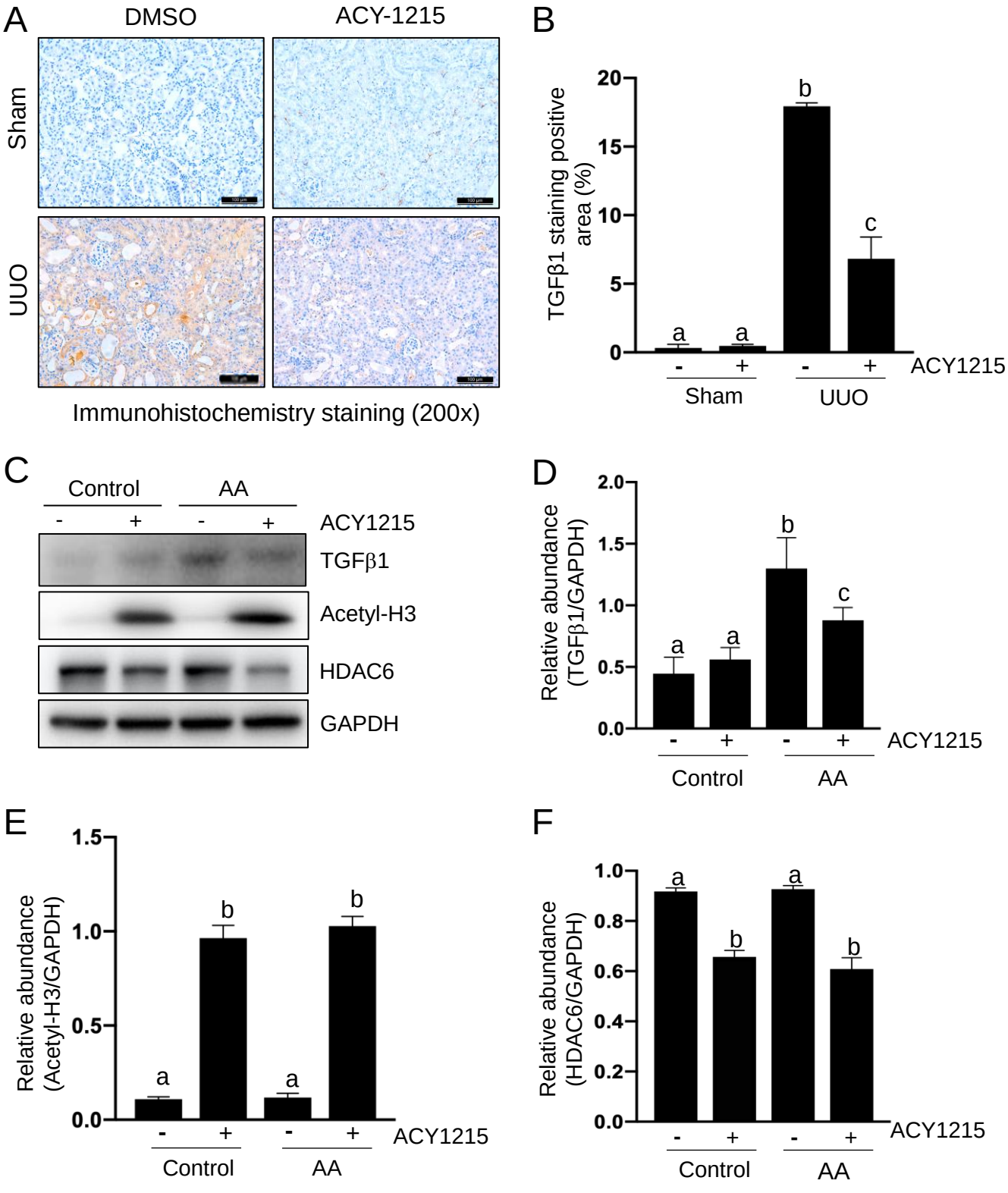


Figure 10

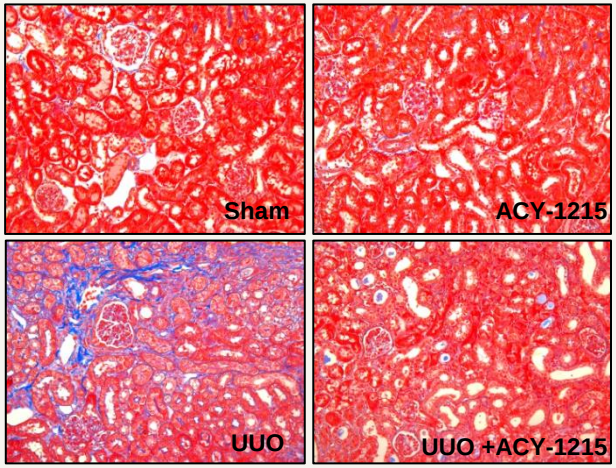


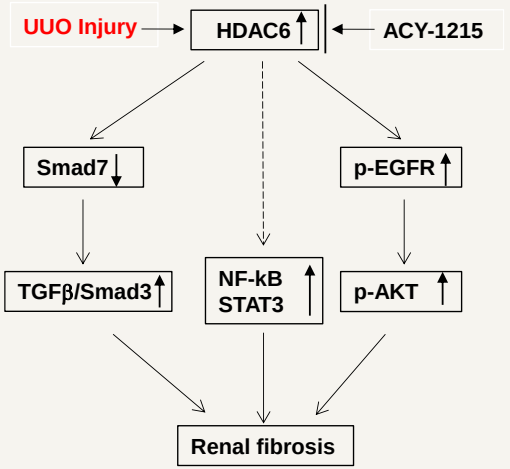
Histone deacetylase 6 inhibition mitigates renal fibrosis by suppressing TGFβ and EGFR signaling pathways in obstructive nephropathy

METHODS

- A murine model of ureteral unilateral obstruction was used to assess the effect of a HDAC6 inhibitor, ACY-1215, on the development of renal fibrosis.
- Cultured rat renal interstitial fibroblasts and mouse renal tubular epithelial cells were used to examine HDAC6-mediated profibrotic response.
- To examine the effect of HDAC6 inhibition on activation of several profibrotic signaling pathways including TGFβ1/Smad3, EGFR/AKT, STAT3 and NF-κB.

OUTCOME





CONCLUSION: HDAC6 inhibition attenuates renal fibrosis by suppression of multiple profibrotic signaling pathways, including TGFβ1/Samd3, EGFR/AKT, STAT3,and NF-κB, and suggests that HDAC6 would be a potential therapeutic target for the treatment of renal fibrosis.