A novel acetyltransferase p300 inhibitor ameliorates hypertension-associated cardio-renal fibrosis

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Online Supplemental Data
Online Figure 1: Effect of L002 on pro-fibrogenic processes in HCFs

(A) HCFs were transfected with pCI empty vector, wildtype p300 (wtp300) and FAT deleted p300 (mtp300) expression vectors and treated with DMSO or L002 for 48h. Cell lysates were processed for Western blot using antibodies against Type I collagen and α-Tubulin.

(B) For migration study, HCFs were pretreated with L002 or DMSO for 1 hour. Then scratch wounds were made in monolayer cultures, and photographs taken at 0 hour and 24 hours.

(C and D) For proliferation study, HCFs were pretreated with L002 or DMSO for 1 h followed by treatment with TGF-β. Images were taken 48 hours after treatment and cell numbers counted at 24 h and 48 h. Data represented as mean ± SEM. **p<0.05, ***p<0.01

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Online Figure 2: L002 reduces hypertension (HTN) induced murine cardiac fibrosis and hypertrophy

(A) M-mode echocardiographic images showing thickness of left ventricular (LV) wall and LV diameters

(B) Shows endpoint (day 14) diastolic blood pressures (DBP) assessed by tail-cuff methods, n=4-12
Data represented as mean ± SEM.
Online Figure 3: Effect of L002 on pro-fibrogenic processes in renal cells. For migration study, podocytes were pretreated with L002 or DMSO for 1 hour. Then scratch wounds were made in monolayer cultures, and photographs taken at 0 hour and 24 hours. Data represented as mean ± SEM. *p< 0.05
Online Figure 4. Effect of L002 on acetylation of histone H3K9 in renal tissues. Kidney sections from saline, Ang II and Ang II+L002 groups were immunostained with anti-Ac-H3K9 antibody. Representative images are presented. Note: Increased number of Ac-H3K9 positive cells (brown: as marked by arrow heads) are present in Ang II-treated kidneys and L002 treatment partially reduces the number of Ac-H3K9 positive renal cells.
On line Figure 5. FATp300 inhibitors suppress acetylation of histone H3K9. Human cardiac fibroblasts (HCFs) were cultured and treated with L002 (A) or C646 (B) or DMSO in triplicate in the presence or absence of TGF-β. Total proteins were isolated, pooled and processed for Western blot using AcH3K9, H3 and Actin antibodies.
Human cardiac fibroblasts (HCFs) were cultured and pretreated in triplicate with L002 or DMSO for 1h followed by treatment with TGF-β. Total RNA were isolated and processed for qPCR in triplicate using Angiotensin II type I (AT1) and type II (AT2) receptor gene specific primers. *p<0.04, **p<0.008, ***p<0.002