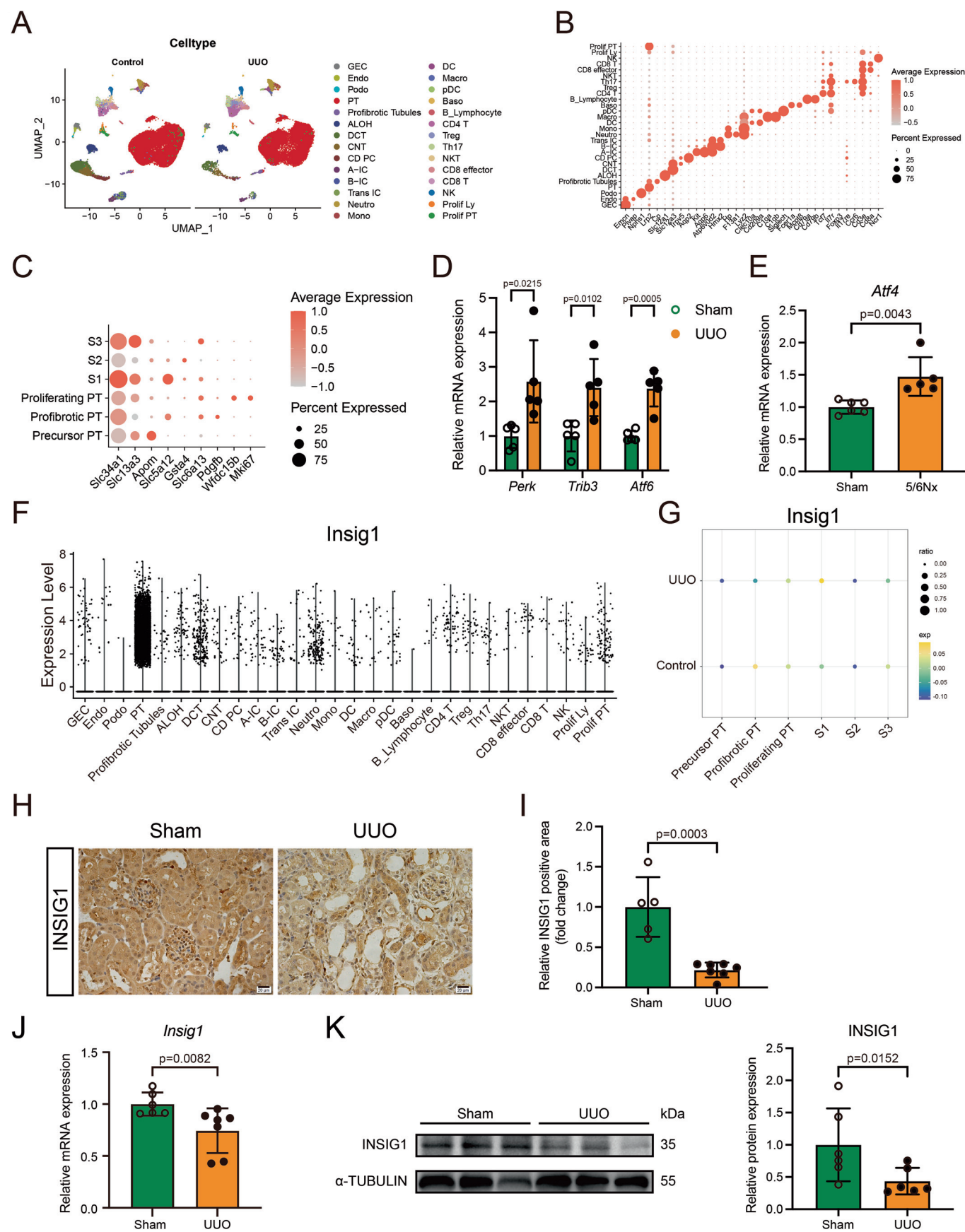


Expanded View Figures

Figure EV1. Re-analysis of scRNA-seq dataset GSE182256 and the expression of ER stress indexes and *Insig1* in the CKD model.

(A) UMAP dimension Reduction showing 28 distinct cell types in Control and UUO kidneys identified by unsupervised clustering. GEC, glomerular endothelial cell; Endo, endothelial; Podo, podocyte; ALOH, ascending loop of Henle; DCT, distal convoluted tubule; CNT, connecting tubule; CD PC, collecting duct principal cell; A-IC, α intercalated cell; B-IC, β intercalated cell; Trans-IC, transitional intercalated cell; Neutro, neutrophil; Mono, monocyte; Macro, macrophage; Baso, basophil; B Lymph, B lymphocyte; Prolif Ly, proliferating lymphocyte; Prolif PT, proliferating PT. (B) Bubble plots showing the expression of cell cluster marker genes in UUO kidney. (C) Bubble plots showing the expression of PTCs cluster marker genes in UUO kidney. (D) qPCR analysis of *Perk*, *Trib3*, and *Atf6* in the kidneys of UUO model ($n = 5$, in each group, biological replicates). (E) qPCR analysis of *Atf4* in the kidneys of 5/6 Nx model ($n = 6$ in Sham group; $n = 5$ in 5/6 Nx group, biological replicates). (F) The violin plot showed the *Insig1* expression levels in each type of normal kidney cells ($n = 6$ in Control group; $n = 2$ in UUO group). (G) The *Insig1* expression changes in each PT subsets in UUO and Control groups. (H, I) Representative images and quantification of IHC (INSIG1) in kidneys of UUO model ($n = 5$ in Sham group; $n = 7$ in UUO group, biological replicates; scale bars, 20 μm). (J) qPCR analysis of *Insig1* in the kidneys of UUO model ($n = 6$ in Sham group; $n = 7$ in UUO group, biological replicates). (K) Representative immunoblot bands and quantification of INSIG1 in the kidneys of UUO model ($n = 6$, in each group, biological replicates). Data information: In (D, I), Data are represented as mean $\pm$ SD. Student's *t* test. (E, J, K) Data are represented as mean $\pm$ SD. Mann-Whitney test. Source data are available online for this figure.



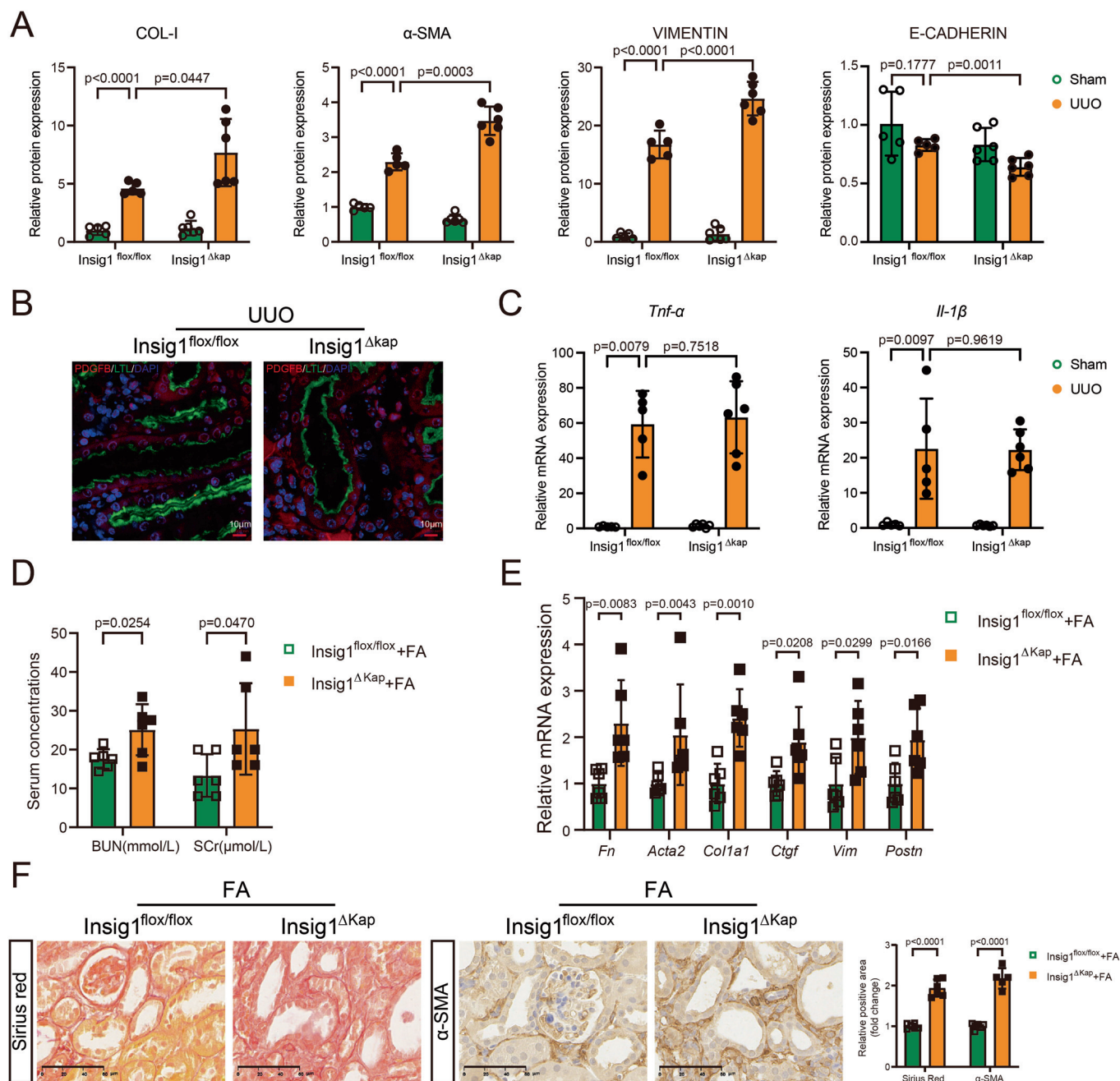
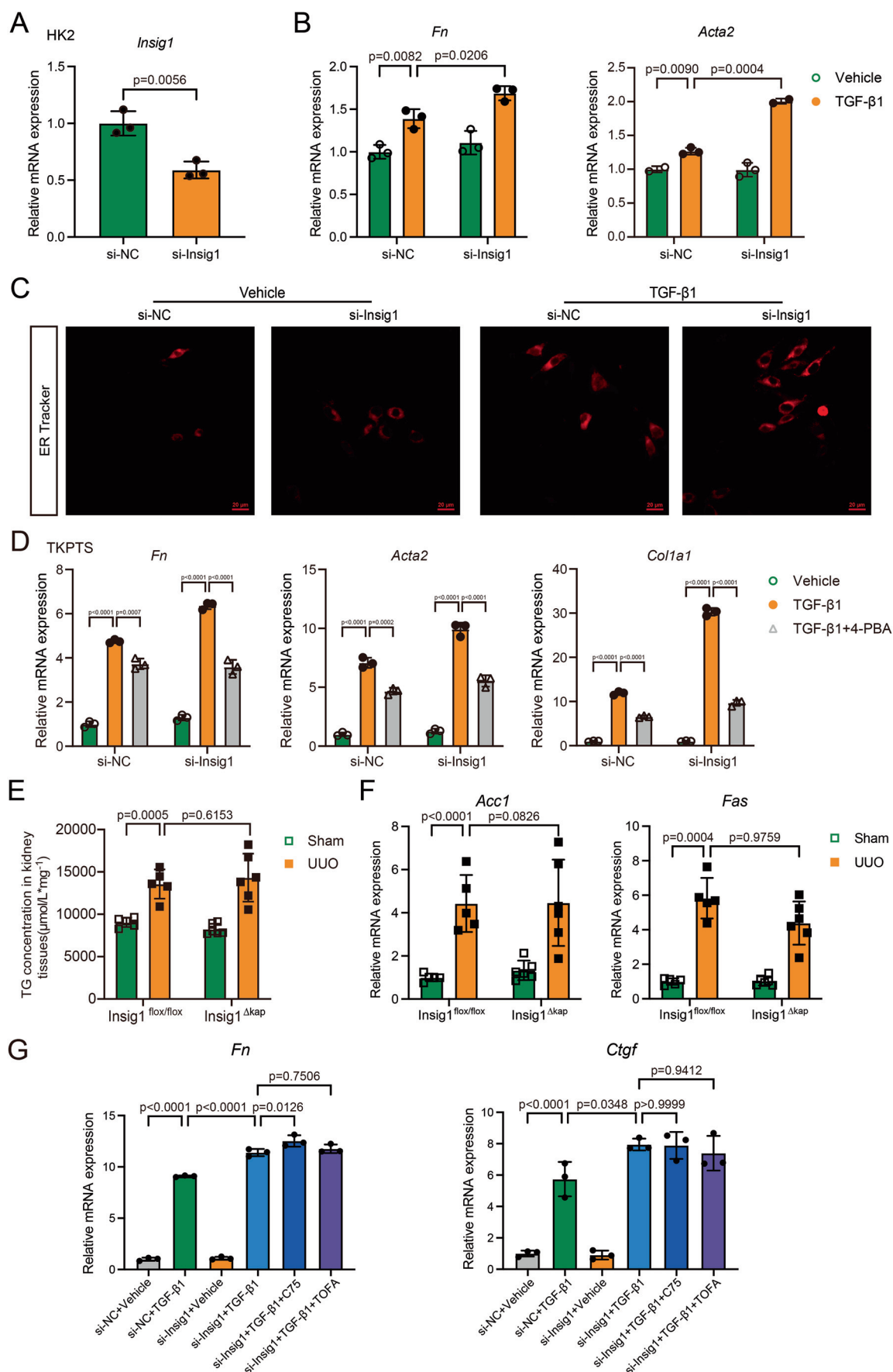


Figure EV2. Insig1 loss in PTCs exacerbated UUO-induced and FA-induced kidney fibrosis.

(A) Quantification of COL-I, α -SMA, VIMENTIN, and E-CADHERIN in Fig. 2F ($n = 5$ in *Insig1*^{flox/flox} group and *Insig1*^{flox/flox} + UUO group; $n = 6$ in *Insig1* ^{Δ Kap} group and *Insig1* ^{Δ Kap} + UUO group, biological replicates). (B) Representative images of PDGFB staining in *Insig1*^{flox/flox} + UUO and *Insig1* ^{Δ Kap} + UUO groups ($n = 3$ in each group, biological replicates; scale bars, 10 μ m; red: PDGFB; green: LTL; blue: DAPI). (C) qPCR analysis of *Tnf- α* and *Il-1 β* in the kidneys of *Insig1*^{flox/flox} and *Insig1* ^{Δ Kap} mice after UUO ($n = 5$ in *Insig1*^{flox/flox} group and *Insig1*^{flox/flox} + UUO group; $n = 6$ in *Insig1* ^{Δ Kap} group and *Insig1* ^{Δ Kap} + UUO group, biological replicates). (D) SCR and BUN concentrations were measured in FA-treated *Insig1*^{flox/flox} and *Insig1* ^{Δ Kap} mice ($n = 6$ in each group, biological replicates). (E) qPCR analysis of *Fn*, *Acta2*, *Col1a1*, *Ctgf*, *Vim*, and *Postn* in the kidneys of FA-treated *Insig1*^{flox/flox} and *Insig1* ^{Δ Kap} mice ($n = 6$ in each group, biological replicates). (F) Representative images and quantification of Sirius red staining and IHC (α -SMA) staining in mouse kidneys (scale bars, 60 μ m; $n = 5$ -6 in each group, biological replicates). Data information: In (A, D, F), Data are represented as mean $\pm$ SD. Student's *t* test. (C, E) Data are represented as mean $\pm$ SD. Student's *t* test or Mann-Whitney test. Source data are available online for this figure.



◀ **Figure EV3. The profibrotic effect of *Insig1* deletion in PTCs was not be correlated with lipogenic activity.**

(A) qPCR analysis of *Insig1* in *Insig1* silencing HK2 cells ($n = 3$ in each group, biological replicates). (B) qPCR analysis of *Fn* and *Acta2* in *Insig1* silencing HK2 cells stimulated by TGF- β 1 ($n = 2-3$ in each group, biological replicates). (C) Representative images of ER tracker staining in *Insig1* silencing HK2 cells stimulated by TGF- β 1 (scale bars, 20 μ m; $n = 3$ in each group, biological replicates). (D) qPCR analysis of *Fn*, *Acta2* and *Col1a1* in *Insig1* silencing TKPTS cells stimulated by TGF- β 1 combined with 4-PBA ($n = 3$ in each group, biological replicates). (E) TG concentration was measured in the kidneys of *Insig1*^{flox/flox} and *Insig1* ^{Δ Kap} mice after UUO ($n = 5$ in *Insig1*^{flox/flox} group and *Insig1*^{flox/flox} + UUO group; $n = 6$ in *Insig1* ^{Δ Kap} group and *Insig1* ^{Δ Kap} + UUO group, biological replicates). (F) qPCR analysis of *Acc1* and *Fas* in the kidneys of *Insig1*^{flox/flox} and *Insig1* ^{Δ Kap} mice subjected to UUO ($n = 5$ in *Insig1*^{flox/flox} group and *Insig1*^{flox/flox} + UUO group; $n = 6$ in *Insig1* ^{Δ Kap} group and *Insig1* ^{Δ Kap} + UUO group, biological replicates). (G) qPCR analysis of *Fn* and *Ctgf* in *Insig1* silencing TKPTS cells stimulated by TGF- β 1 combined with C75 or TOFA ($n = 3$ in each group, biological replicates). Data information: In (A, B, E, F), Data are represented as mean $\pm$ SD. Student's t test. (D, G) Data are represented as mean $\pm$ SD. One-way ANOVA. Source data are available online for this figure.

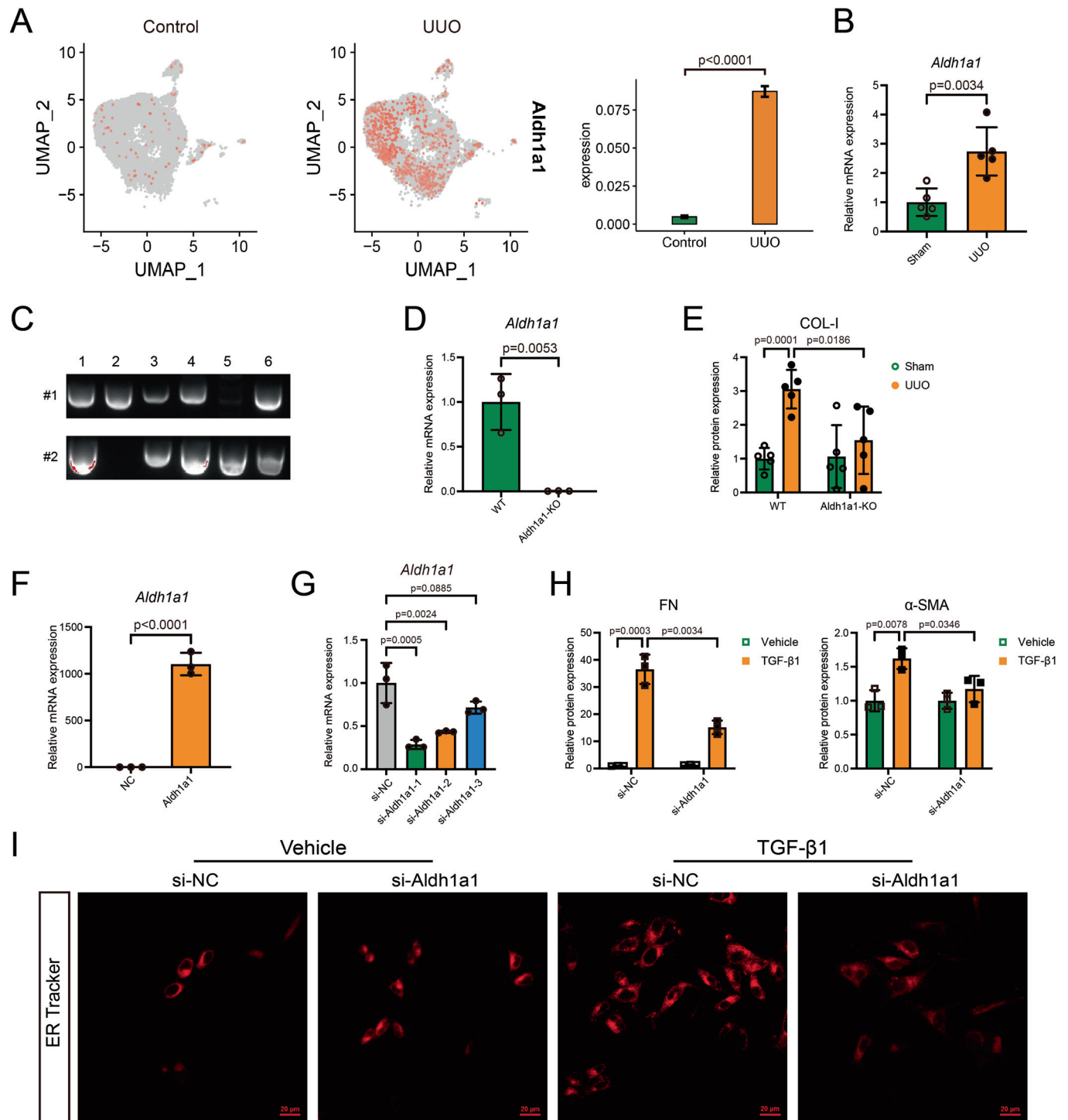
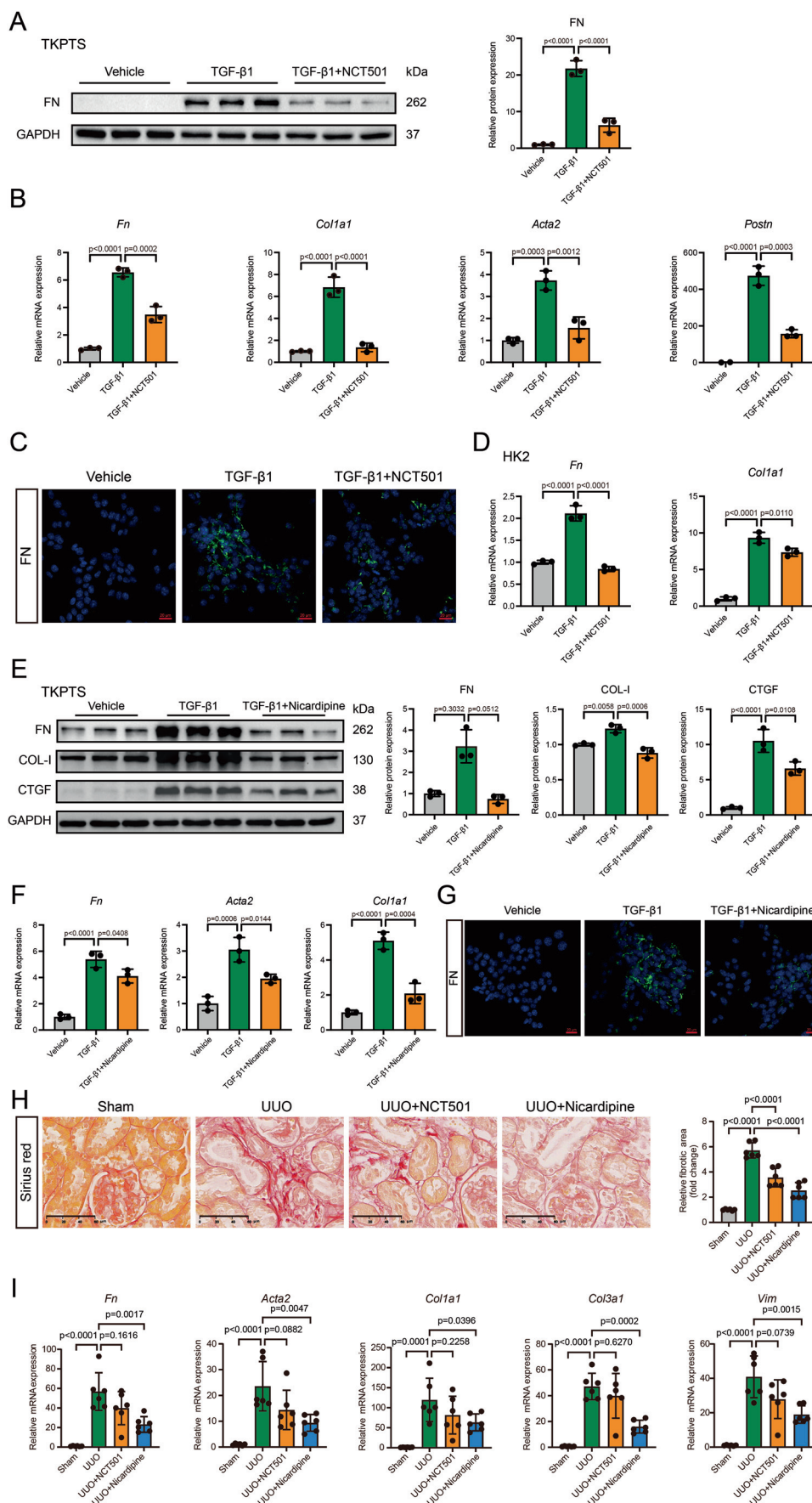


Figure EV4. The expression of Aldh1a1 in UVO model and Aldh1a1 silencing ameliorated TGF-β1-induced fibrotic responses and ER stress in vitro.

(A) UMAP dimension reduction showing Aldh1a1 expression in Control and UVO kidneys ($n = 6$ in Control group; $n = 2$ in UVO group). (B) qPCR analysis of Aldh1a1 in the kidneys of UVO model ($n = 5$ in each group, biological replicates). (C) PCR analysis of Aldh1a1 for the genetic identification of Aldh1a1-KO mice. (HET: 1, 3, 4, 6; HOM: 2; WT: 5). (D) qPCR analysis of Aldh1a1 in the kidneys of WT and Aldh1a1-KO mice ($n = 3$ in each group, biological replicates). (E) Quantification of COL-I in Fig. 7E ($n = 5$ in each group, biological replicates). (F) qPCR analysis of Aldh1a1 in Aldh1a1 overexpressed TKPTS cells ($n = 3$ in each group, biological replicates). (G) qPCR analysis of Aldh1a1 in Aldh1a1 silencing TKPTS cells (si-Aldh1a1-1 was selected for additional experimentation) ($n = 3$ in each group, biological replicates). (H) Quantification of FN and α-SMA in Fig. 7L ($n = 3$ in each group, biological replicates). (I) Representative images of ER tracker staining in Aldh1a1 silencing TKPTS cells stimulated by TGF-β1 (scale bars, 20 μm; $n = 3$ in each group, biological replicates). Data information: In (B, D, E, F, H), Data are represented as mean ± SD. Student's *t* test. (G) Data are represented as mean ± SD. One-way ANOVA. Source data are available online for this figure.



◀ **Figure EV5. NCT501 and nicardipine improved renal fibrosis in vivo and in vitro.**

(A) Representative immunoblot bands and quantification of FN in TGF- β 1-treated TKPTS cells stimulated with NCT501 ($n = 3$ in each group, biological replicates). (B) qPCR analysis of *Fn*, *Acta2*, *Col1a1* and *Postn* in TGF- β 1-treated TKPTS cells stimulated with NCT501 ($n = 2-3$ in each group, biological replicates). (C) Representative images of FN staining in TGF- β 1-treated TKPTS cells stimulated with NCT501 (scale bars, 20 μ m; $n = 3$ in each group, biological replicates). (D) qPCR analysis of *Fn* and *Col1a1* in TGF- β 1-treated HK2 cells stimulated with NCT501 ($n = 3$ in each group, biological replicates). (E) Representative immunoblot bands and quantification of FN, COL-1, and CTGF in TGF- β 1-treated TKPTS cells with or without nicardipine treatment ($n = 3$ in each group, biological replicates). (F) qPCR analysis for *Fn*, *Acta2*, and *Col1a1* in TGF- β 1-treated TKPTS cells with or without nicardipine treatment ($n = 3$ in each group, biological replicates). (G) Representative images of FN staining in TGF- β 1-treated TKPTS cells with or without nicardipine treatment (scale bars, 20 μ m; $n = 3$ in each group, biological replicates). (H) Representative images and quantification of Sirius red staining in mouse kidneys (scale bars, 60 μ m; $n = 6$ in each group, biological replicates). (I) qPCR analysis for *Fn*, *Acta2*, *Col1a1*, *Col3a1* and *Vim* in mouse kidneys ($n = 6$ in each group, biological replicates). Data information: Data are represented as mean $\pm$ SD. One-way ANOVA. Source data are available online for this figure.