Supplemental Information

IncRNA Inc-TSI Inhibits Metastasis of Clear Cell Renal Cell Carcinoma by Suppressing TGF-β-Induced Epithelial-Mesenchymal Transition

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Figure S2
Figure S5

Supplementary Table 1

Table S1. Characteristics of 150 ccRCC patients at the time of radical nephrectomy

| Clinical information |      |
|----------------------|------|
| Age, years           | 57±12|
| >60                  | 55   |
| ≤60                  | 95   |
| Male, n (%)          | 107 (71.3%) |
| Tumor size           |      |
| >7cm                 | 22   |
| ≤7cm                 | 128  |
| Node metastasis¹     |      |
| N0                   | 147  |
| N1-N3                | 3    |
| Metastasis¹          |      |
| M0                   | 150  |
| M1                   | 0    |
| Clinical stage¹      |      |
| I-II                 | 138  |
| III-IV               | 12   |

¹AJCC grade was defined according to the 7th edition of AJCC ²¹
**Supplementary figure legends**

**Figure S1** Supplementary to Figure 2, lnc-TSI inhibited TGF-β1-induced phosphorylation of Smad3 in 786-O cells

(A) lnc-TSI knockout ccRCC cells were produced using the CRISPR/Cas9 system. A schematic diagram showing the sgRNA target sites. (B) qRT-PCR assay showed that expression of lnc-TSI was reduced in 786-O and Caki-1 cells by CRISPR/Cas9 targeting lnc-TSI. (C) qRT-PCR assay showed that lnc-TSI was upregulated over 10-fold in ccRCC cells transfected with lnc-TSI overexpression lentivirus. (D) Western blot showed that knockout of lnc-TSI promoted Smad3, but not Smad2 phosphorylation in 786-O cells in the presence or absence of exogenous 10 ng/mL of TGF-β1. The data analysis results were showed in D2 and D3. (E) Western blot showed that overexpressing lnc-TSI inhibited Smad3, but not Smad2 phosphorylation in 786-O cells in the presence or absence of exogenous 10ng/ml of TGF-β1. The data analysis results were showed in E2 and E3. (F) Western blot showed that knockout of lnc-TSI promoted Smad3, but not Smad2 phosphorylation in lnc-TSI knockout clone 2 of Caki-1 cells. The data analysis results were showed in F2 and F3. Data were expressed as means ± SD of three independent experiments. *, P<0.05, **, P< 0.01, ***, P < 0.001.

**Figure S2** Supplementary to Figure 4, lnc-TSI inhibited TGF-β1-induced epithelial-mesenchymal transition in ccRCC cells

(A&B) qRT-PCR showed that knockout of lnc-TSI decreased the expression of E-cadherin, and increased the expression of N-cadherin, Snail and Vimentin, particularly
in the presence of exogenous TGF-β1 (10 ng/mL) in 786-O cells (A) and Caki-1 cells (B). Data are expressed as means ± SD of three independent experiments. #, $P<0.05$, ##, $P<0.01$ versus control cells without TGF-β1 stimulation. **, $P < 0.01$, ***, $P < 0.001$ versus control cells with TGF-β1 stimulation. (C) Western blot showed that the knockout of lnc-TSI decrease E-cadherin expression and increased N-cadherin expression, Vimentin, and Snail in lnc-TSI knockout clone 2 of Caki-1 cells. (C1). The data analysis results were shown in C2 to C5. (D) Western blot showed that knockout of lnc-TSI decreased E-cadherin and increased the N-cadherin, Vimentin, and Snail in 786-O cells (D1). The data analysis results were shown in D2 to D5. (E&F) qRT-PCR showed that overexpression of lnc-TSI increased the expression of E-cadherin, and decreased the expression of N-cadherin, Snail and Vimentin, particularly in the presence of exogenous TGF-β1 (10 ng/ml) in 786-O cells (E) and Caki-1 cells (F). Data are expressed as means ± SD of three independent experiments. ##, $P<0.01$ versus control cells without TGF-β1 stimulation. **, $P < 0.01$, ***, $P < 0.001$ versus control cells with TGF-β1 stimulation. (G) Representative Western blot and quantitative data showed that overexpression of lnc-TSI in 786-O cells decreased the expression of N-cadherin, Vimentin and Snail and increased the expression of E-cadherin. Data were expressed as means ± SD of three independent experiments. *, $P<0.05$, **, $P<0.01$, ***, $P < 0.001$.

Figure S3 Supplementary to Figure 5, lnc-TSI did not affect the proliferation and colony formation of ccRCC cells
The representative images of Transwell assays (A) and the data analysis results (A2 and A3) showed that knockout of Inc-TSI enhanced cell migration and invasiveness in Caki-1 cells compared to controls. Caki-1 cells were treated with TGF-β1 (10 ng/mL) for 48 h. (B) Representative images of wound-healing assays (B1) and the data analysis results (B2) showed that knockout of Inc-TSI increased cell migration in Caki-1 cells compared to controls. (C to F) MTS assay showed that knocking out or overexpressing Inc-TSI in ccRCC cells did not affect cell growth. (G&H). Colony formation assay showed that knockout or overexpression of Inc-TSI did not affect the colonies number formed by 786-O (G1 and G2) and Caki-1 cells (H1 and H2). Data are expressed as means ± SD of three independent experiments. *, P < 0.05, ***, P < 0.001.

Figure S4 Supplementary to Figure 7, expression of Inc-TSI between the clinicopathological features in ccRCC. (A-D) Lnc-TSI expression in 150 ccRCC tissues was presented according to AJCC grade (A), tumor diameter (B), age (C) and gender (D).

Figure S5 A schematic feedback loop in which Inc-TSI was transcribed by Smad3 and inhibited Smad3 phosphorylation and the subsequent EMT of ccRCC cells by interfering with the interaction between Smad3 and TβRI.