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Supplemental Information

TGF-β Mediates Renal Fibrosis via the Smad3-Erbb4-IR Long Noncoding RNA Axis

Min Feng, Patrick Ming-Kuen Tang, Xiao-Ru Huang, Si-Fan Sun, Yong-Ke You, Jun Xiao, Lin-Li Lv, An-Ping Xu, and Hui-Yao Lan

5’-
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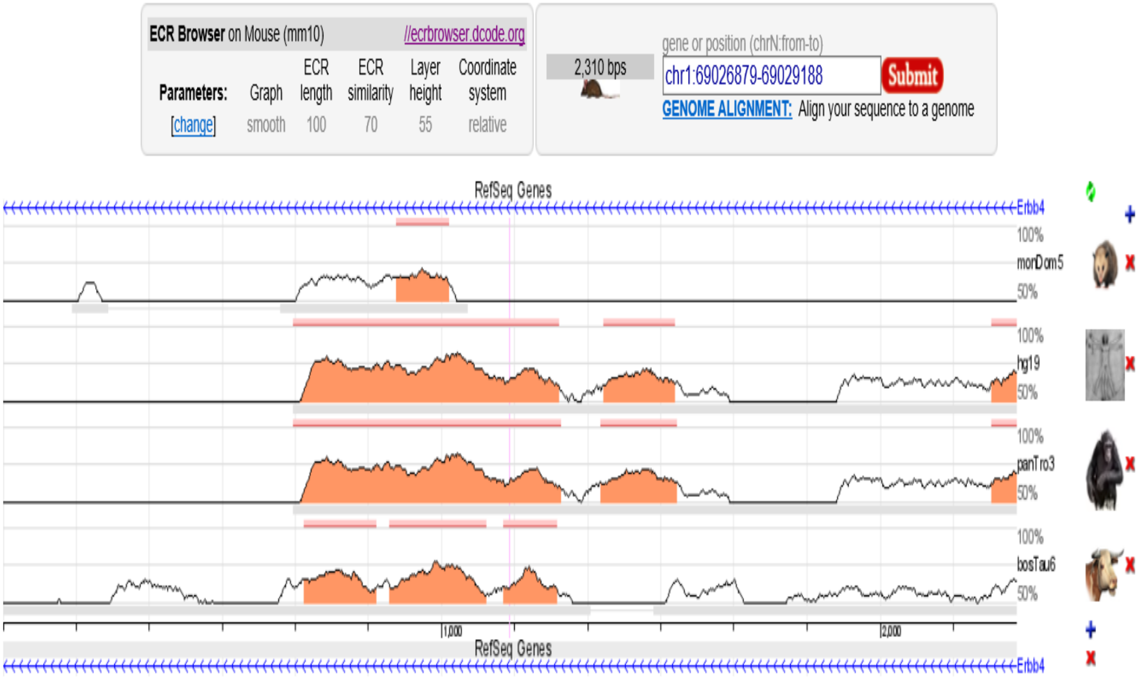
GGAGAGGGTTGGGGGAGGGGGTGACTGGGAGGAGGACAGTGAGTTGGTTGTAAAGTGAATAAGAAAAAA
A-3’

Supplementary Figure S1. Full-length sequence of Erbb4-IR. The 2310 nucleotides full-length sequence of Erbb4-IR is detected by sequencing the 5’ and 3’ RACE products.



ECR Browser on Mouse (mm10) //ecrbrowser.dcode.org

Parameters:	Graph	ECR length	ECR similarity	Layer height	Coordinate system
[change]	smooth	100	70	55	relative

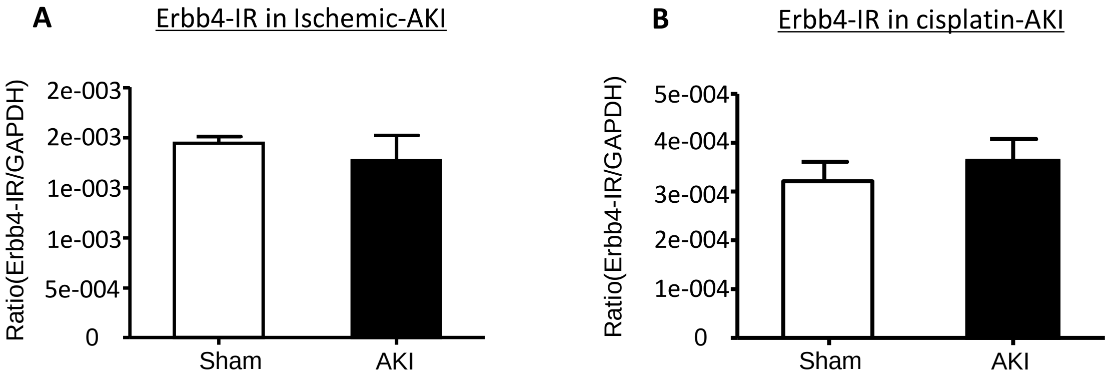
2,310 bps

gene or position (chrN:from-to)

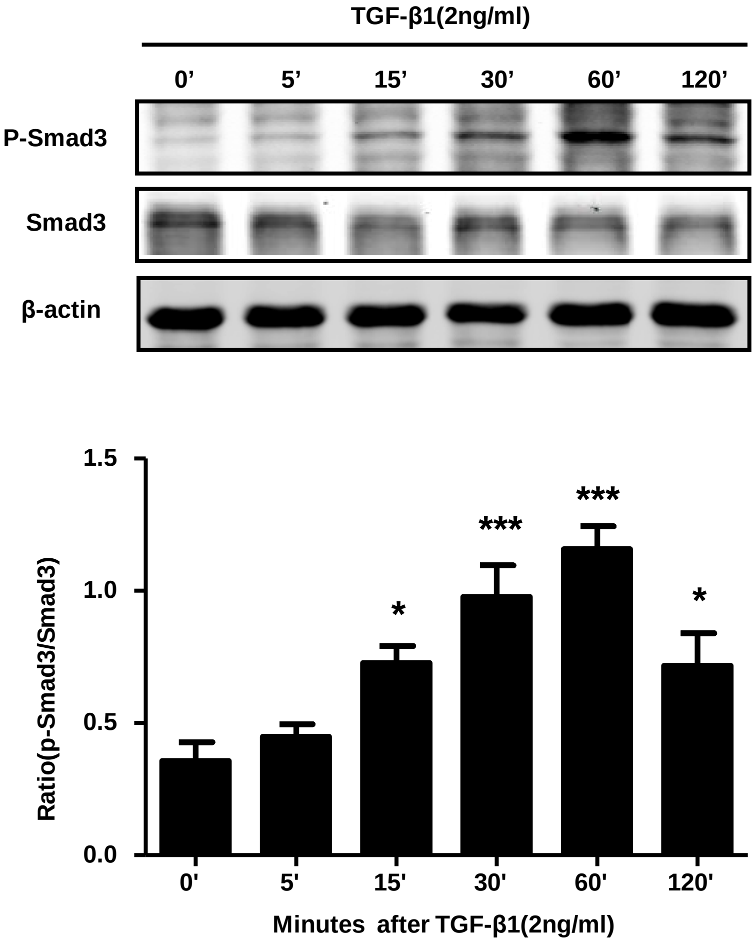
chr1:69026879-69029188

GENOME ALIGNMENT: Align your sequence to a genome

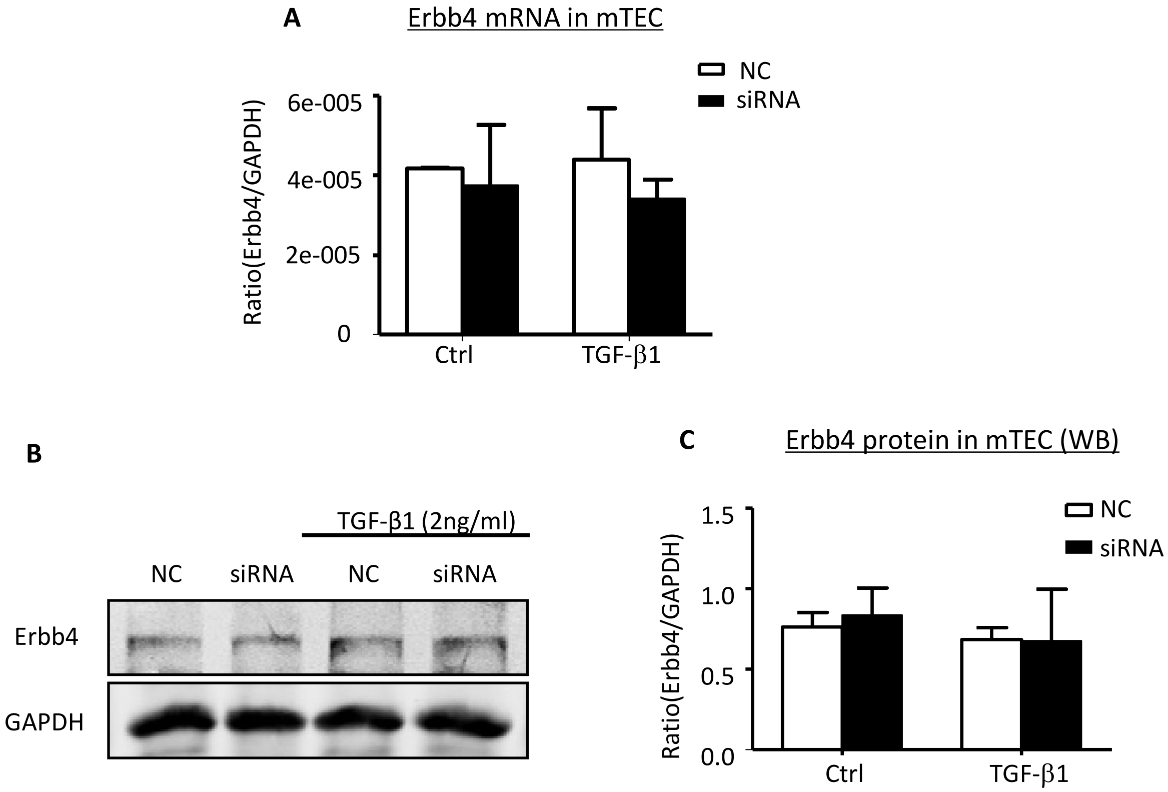
Supplementary Figure S2. Evolutionary conserved regions of Erbb4-IR among genomes of different vertebrate species. The sequence of Erbb4-IR is partially conserved among species.



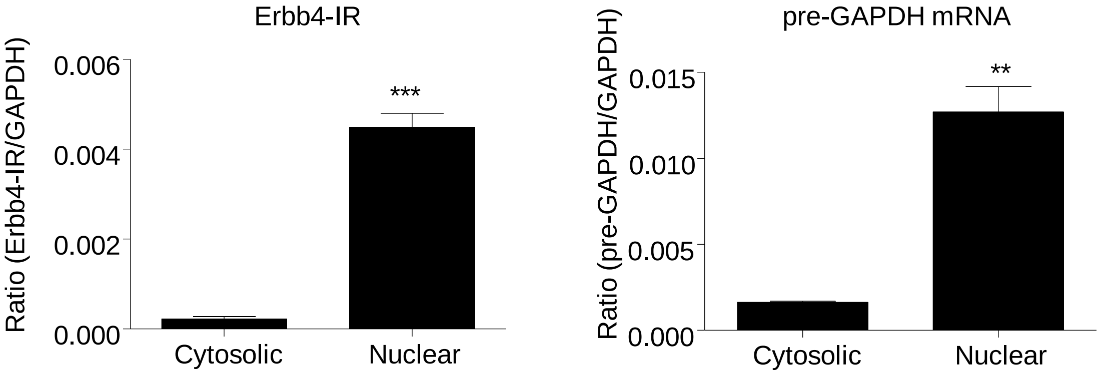
Supplementary Figure S3. Erbb4-IR is not induced in the AKI-kidney. Real-time PCR analysis of Erbb4-IR expression in day 3 kidneys after ischemic reperfusion (IR) induced AKI kidney (A) or cisplatin-induced AKI kidney (B). Results show that Erbb4-IR is not upregulated in both types of AKI-kidneys. Each bar represents the mean ± SEM for groups (N=3).



Supplementary Figure S4. Time course study of Smad3 activation in mTEC under TGF-β1 stimulation. Addition of 2ng/ml of TGF-β1 was able to induce a rapid activation of Smad3 as early as 15 minutes with the peak at 1 hour, showing by western blot and its quantification. Each bar represents the mean±SEM for at least 3 independent experiments. *P<0.05, ***P<0.001 compared with control at time 0 min.

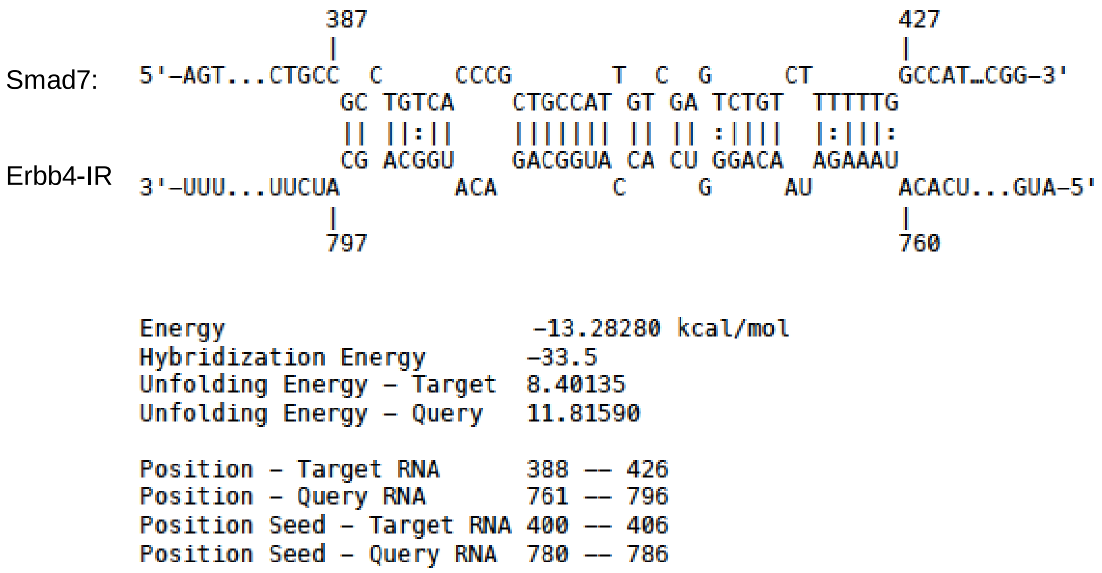


Supplementary Figure S5. Host gene Erbb4 was not induced by TGF-β1 and knockdown of Erbb4-IR had not effect on expression of Erbb4 in mTECs *in vitro*. (A) Real-time PCR analysis shows that transient transfection of siRNA against Erbb4-IR does not alter the expression of Erbb4 in mTECs with or without TGF-β1 (2ng/ml) stimulation at 6h when compared with their nonsense-treated controls (NC). (B, C) Western blot analysis demonstrates that knockdown of Erbb4-IR does not alter the Erbb4 protein levels in mTECs with or without TGF-β1(2ng/ml) treatment at 24h when compared with the NCs. Each bar represents the mean±SEM for at least 3 independent experiments.



Supplementary Figure S6. Subcellular localization of Erbb4-IR in mTEC after TGF-β1 stimulation. The subcellular RNAs in mouse tubular epithelial cells(mTECs) were collected after 24h of TGF-β1 (2ng/ml) stimulation by using PRAIS^{TM} Kit (Invitrogen AM1921). The expression levels of Erbb4-IR and pre-GAPDH (a nuclear positive control; forward primer: 5' GGCTCATGGTATGTAGGCAGT 3' Reverse primer: 5' GAAAACACGGGGGCAATGAGT 3') were measured by real-time PCR. Our result showed that Erbb4-IR is mainly expressed as a nuclear RNA. Each bar represents the mean±SEM for at least 3 independent experiments. $^{**}P<0.01$, $^{***}P<0.001$ compared with cytosolic RNA fraction.

Predicted Interaction between Erbb4-IR and 3’UTR of Smad7 gene



```
                       387                                     427
                       |                                       |
Smad7:    5'-AGT...CTGCC  C     CCCG       T  C  G     CT      GCCAT…CGG-3'
                        GC TGTCA    CTGCCAT GT GA TCTGT  TTTTTG
                        || ||:||    ||||||| || || :||||  |:|||:
                        CG ACGGU    GACGGUA CA CU GGACA  AGAAAU
Erbb4-IR  3'-UUU...UUCUA       ACA        C     G     AU      ACACU...GUA-5'
                       |                                       |
                       797                                     760


Energy                      -13.28280 kcal/mol
Hybridization Energy        -33.5
Unfolding Energy - Target   8.40135
Unfolding Energy - Query    11.81590

Position - Target RNA       388 -- 426
Position - Query RNA        761 -- 796
Position Seed - Target RNA  400 -- 406
Position Seed - Query RNA   780 -- 786
```

Predicted interacting site of Erbb4-IR on 3’UTR of Smad7 gene (blue underlined)

AGTACCTGGGCAGACTGGCAGCAGGTGCCAAGCTCTGCTCCAGCCTGCCGAAGCTGCCCC
GCCCCGCCCCGCCCCCGCCCCCACAGGACACGGGCCTATCCACAGGCTTCTGAGAAGCCA
GCCTGCTAGAAGGCTGAACCAGAACCAATTGTTTTCATCCCTGTCTTACTGCCGCCTGTCAC
CCGCTGCCATTGTCGAGTCTGTCTTTTTTGGCCATCTGCTCCTGGATCTCTCTCTTGAGATGG
GCTTCCCAAGGGCTGCCGG

Supplementary Figure S7. Binding site of Erbb4-IR on 3’UTR of Smad7 gene. Bioinformatic analysis predicts the binding site of Erbb4-IR on 3’UTR of Smad7 genomic sequence, highlighted in blue.