**OMTN, Volume *12***

## Supplemental Information

# lncRNA ZEB1-AS1 Was Suppressed by p53 for Renal Fibrosis in Diabetic Nephropathy

**Juan Wang, Jian Pang, Huiling Li, Jie Long, Fang Fang, Junxiang Chen, Xuejin Zhu, Xudong Xiang, and Dongshan Zhang**

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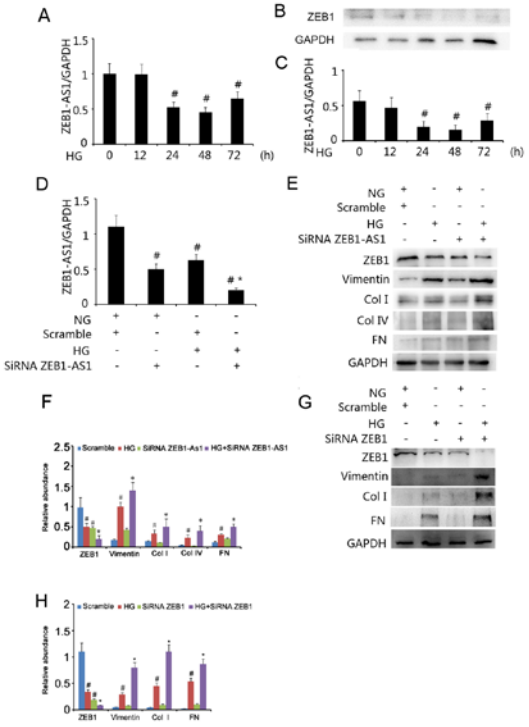
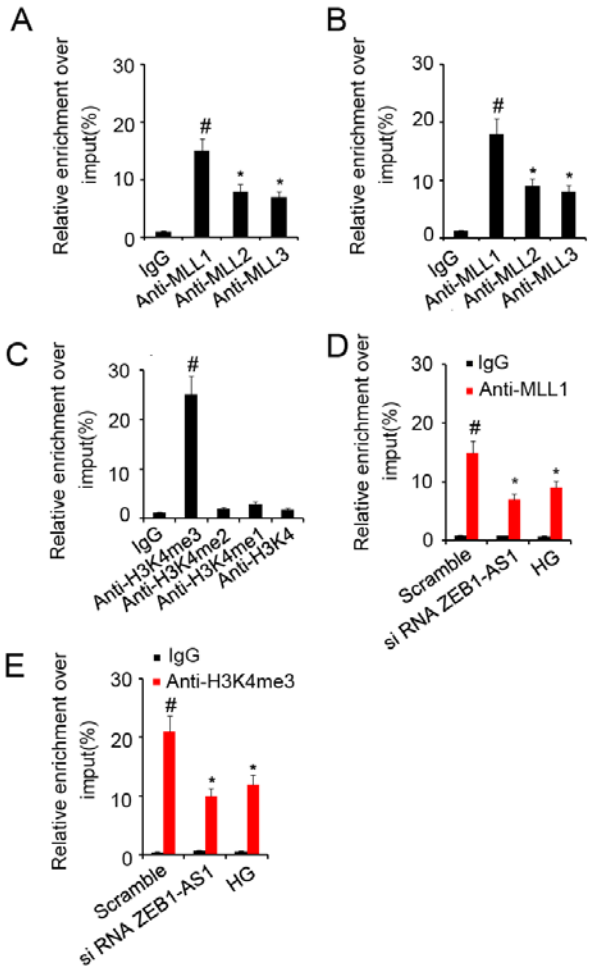
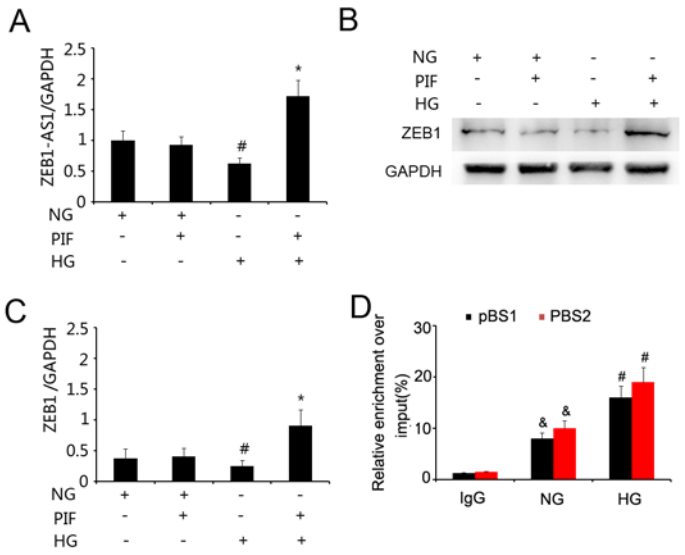


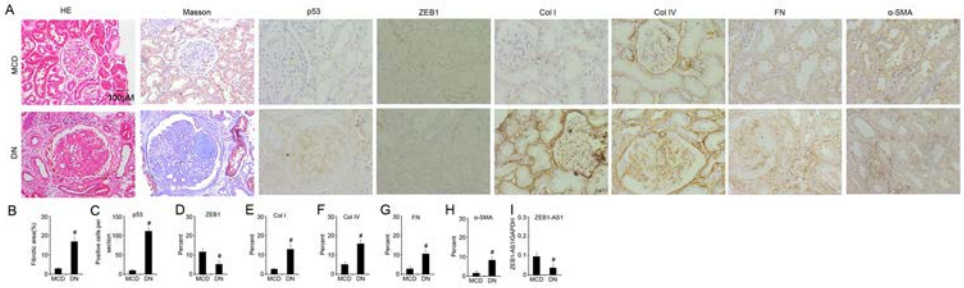

**Supplementary Figure 1. LncRNA ZEB1-AS1 inhibited high glucose-induced the expression of collagen I, collagen IV, fibronectin, and vimentin by upregulation of ZEB1.** (A) Relative mRNA expression levels of LncRNA ZEB1-AS1. (B) Relative protein expression levels of ZEB1 and GAPDH at indicated time points after HG treatment. (C) Densitometry of ZEB1 signals on immunoblots. (D) Transfection of siRNA ZEB1-AS1 (100nM) or scramble, relative mRNA expression levels of LncRNA ZEB1-AS1, (E) relative protein expression levels of ZEB1, vimentin, collagen I, collagen IV, fibronectin, and GAPDH, and densitometry (F) of proteins signals on immunoblots.(G) Representive immunoblots of ZEB1, vimentin, collagen I, fibronectin, and GAPDH. (H) Densitometry of proteins signals on immunoblots. Data were expressed as means ± sd (n=6); # $p<0.05$: 24h or 48 h or 72 h vs 0 h or 12h, or HG group vs NG group; * $p<0.05$: HG+si RNA ZEB1-AS1 or HG+ siRNA ZEB1 group vs HG group.



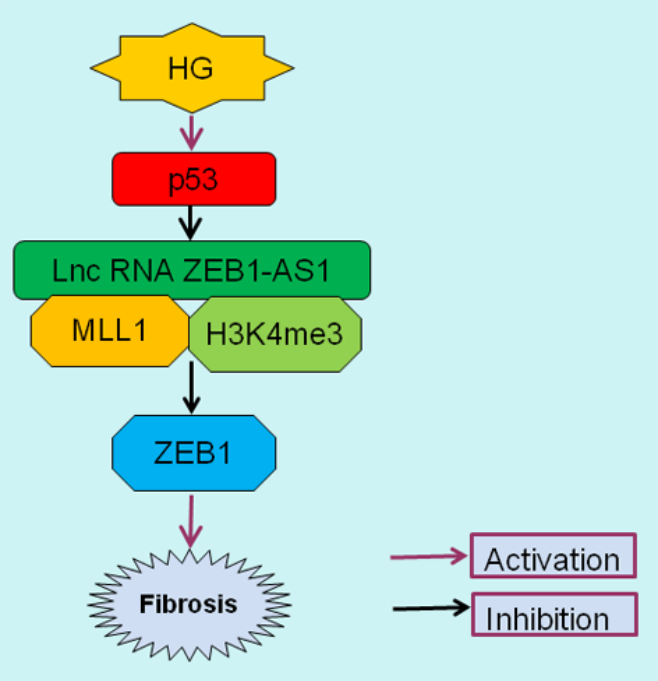

**Supplementary Figure 2. ZEB1-AS1 induces epigenetic changes at the ZEB1 promoter. (**A) The RIP assay was performed to demonstrate whether ZEB1-AS1 could directly bind with MLL1,MLL2, and MLL3 in HK-2 cells. The ChIP assay was conducted to verify whether MLL1,MLL2, and MLL3 (B) or H3K4me3, H3K4me2, H3K4me1, and H3K4(C) could directly bind to ZEB1 promoter region in HK-2 cells. ChIP-qPCR of MLL1 occupancy and H3K4me3 binding in the promoter of ZEB1 in HK-2 cells transfected with siRNA ZEB1 or scrambled siRNA or HG treatment (D and E). Data are shown as percent of input. # $p<0.05$: MLL1 or H3K4me3 group vs IgG group; * $p<0.05$: MLL2 or MLL3 group vs MLL1group； siRNA ZEB1 or HG group vs Scramble group




**Supplementary Figure 3. LncRNA ZEB1-AS1 was markedly suppressed by p53 during HG treatment in HK-2 cells.** (A) Real-time PCR analysis of LncRNA ZEB1-AS1. (B) Immunoblot analysis for ZEB1. (C) Densitometry of ZEB1 signals on immunoblots. (D) ChIP assays for p53 were performed with chromatin material isolated from HK2 cells treated with HG and NG. Precipitates with p53 or without antibody (input) were used as template for PCR detection of the potential p53 binding sites (pBS1 and pBS2).Data were expressed as means ± sd (n=6); # $p<0.05$: HG group vs NG group; * $p<0.05$: HG+PIF group vs HG group.& $p<0.05$: NG group vs IG group.




**Supplementary Figure 4**. **p53-induced down stream gene expression in patients with DN** (A) Histology, Masson staining for fibrosis analysis, and immunohistochemistry staining for p53, ECM related gene expression, and ZEB1. (B) Quantification of the tubulointerstitial fibrosis in the kidney cortex. (C-H) Quantify immunohistoche mistry staining. (I) Real time PCR analysis of Lnc ZEB1-AS1. Bar：100μM. Data were expressed as means ± sd (n=6); *#P<0.05* versus MCD group. Original magnification, x 200 or 400.




**Supplementary Figure 5. The role and molecular mechanism of p53 in HG-induced renal fibrosis.** HG suppressed Lnc RNA ZEB1-AS1 expression by induction of p53. Furthermore, down regulation of ZEB1-AS1 reduced the binding and recruiting of histone methyltransferase MLL1 to the promoter region of ZEB1, and suppressed H3K4me3 modification therein, which led to the inactivation of ZEB1 and upregulation of the production of probrotic proteins.