The phosphorylation of the Smad2/3 linker region by Nemo-like kinase regulates TGF-β signaling

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Supporting Information
**Supplemental Figures**

**Figure S1.** NLK inhibits TGF-β-induced cell migration and transcriptional responses in HaCaT cells. (A-D) Quantitative real-time PCR (qRT-PCR) analysis of mRNA. NLK-OE (stably expressing FLAGHA-NLK) cells, K155M (stably expressing HA-NLK(K155M)) cells and parental HaCaT cells were stimulated with TGF-β at the indicated times before total RNA was extracted. The data were measured in triplicate. Values and bars represent the means and the standard deviation. (E) Wound healing assay. HaCaT cells were infected with retrovirus expressing NLK or NLK (K155M). Wounds were performed by seeding cells into the Culture-Insert 2 Well. Cells were treated with 100 pM TGF-β, and allowed to migrate for 36h. The data were measured in triplicate. Values and bars represent the means and the standard deviation.
Figure S2. Depletion of NLK enhanced TGF-β-induced transcriptional responses in HaCaT cells. (A-C) qRT-PCR analysis of mRNA. HaCaT cells were transfected with NLK siRNA (KD-1 and KD-2) or nontargeting siRNA (NT), then stimulated with TGF-β at the indicated times before total RNA was extracted. The data were measured in triplicate. Values and bars represent the means and standard deviation values. (D) NLK expression in the HaCaT cells stably expressing NLK shRNAs was detected by anti-NLK antibody. The relative band intensity was shown under each panel (GAPDH: relative values of band intensity; NLK: relative values of the band intensity ratio of target protein to GAPDH). (E) Wound healing assay. HaCaT cells were transfected with NLK siRNA (KD-1 and KD-2) or nontargeting siRNA (NT). 24h after siRNA transduction, wounds were performed by seeding cells into the Culture-Insert 2 Well. Cells were treated with 100 pM TGF-β, and allowed to migrate for 36h. The data were measured in triplicate. Values and bars represent the means and the standard deviation.
**Figure S3.** Purified proteins for the *in vitro* kinase assay. (A) GST-Smad2 and GST-Smad3 were expressed and purified as described in the Materials and Methods section. Purified GST-Smad2 and GST-Smad3 were resolved by SDS-PAGE and detected by Coomassie blue staining with a defined amount of GST as a homogeneity control. (B) Flag-NLK and the catalytically inactive form of Flag-NLK (K155M) were obtained as described in the Materials and Methods section. Purified Flag-RFP, Flag-NLK and Flag-NLK K155M were resolved by SDS-PAGE and visualized by Coomassie blue staining.

**Figure S4.** Purified point-mutated Smad3 for use in the *in vitro* kinase assay. Smad3 point mutations were generated with a QuickChange site-directed mutagenesis kit (Stratagene) according to the manufacturer’s instructions. GST-Smad3 proteins with various point mutations were purified as described in the Materials and Methods section. Purified proteins were separated by SDS-PAGE and stained by Coomassie blue with a defined amount of GST used as a homogeneity control.
Supplemental Tables

**Table S1.** The list of all the peptides identified by mass spectrometry in GST-Smad2 protein. GST-Smad2 protein phosphorylated by NLK were gathered form gel described in Figure 4A and used in mass spectrometry analysis.

**Table S2.** The structures of all the peptides phosphorylated in GST-Smad2 as shown in Table S1.

**Table S3.** The list of all the peptides identified by mass spectrometry in GST-Smad3 protein. GST-Smad3 protein phosphorylated by NLK were gathered form gel described in Figure 4A and used in mass spectrometry analysis.

**Table S4.** The structures of all the peptides phosphorylated in GST-Smad3 as shown in Table S3.