Supplemental figure legends

Supplemental FIGURE 1. IL-1β stimulation increases the binding of transcription factors to the 5' and 3' region.

A, Quantitative levels of ChIP assays (Fig. 1*C*) were analyzed by real-time PCR and were expressed relative to control (without IL-1β treatment) after normalizing with the input DNA. After treatment with IL-1β (5 ng/ml) for 1 h, ChIP assays were performed with RelA or phospo-c-Jun antibody. PCR was performed with primers specific for the conserved NF-κB binding site of COX2, or the AP1 binding site of MMP13. **B**, Quantitative levels of ChIP assays (Fig. 1*D*) were analyzed by real-time PCR. After IL-1β treatment for 1 h, ChIP assays were performed with Lef1 antibody. PCR was performed with primers specific for the conserved Lef1 binding site of COX2 or MMP13. The data shown are representative of three independent experiments with similar results. Error bars show the standard deviations of the ChIP PCR reactions performed in triplicate.

Supplemental FIGURE 2. Control experiments for the 3C assay.

A-B, The control experiments for cross-linking or ligation in the 3C assay were performed with IL-1β-stimulated chondrocytes with or without formaldehyde cross-linking (A) or ligation (B). The PCR products (with primers for A+D in Fig. 3*A*) from the 3C assay (restricted by NcoI for COX2 or PvuII for MMP13, respectively) were resolved on 2 % agarose gels. Input represents products of PCRs using primers specific for the conserved NF-κB binding site of COX2, performed to establish that equal amounts of template DNA were present in the samples. The data shown are representative of three independent experiments with similar results.

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Supplementary FIGURE 3. Lef1 plays a crucial role in the gene looping.

The effect of Lef1 over-expression or knock-down by Lef1 siRNA on target gene expression (A) and loop formation (B) or its binding on the target gene locus (C) was analyzed by real-time PCR. Levels were expressed relative to control after normalizing with mouse L-32. **A,** Upper, chondrocytes were transfected with Lef1 siRNA or control siRNA and then stimulated with IL-1β. Lower, chondrocytes were transfected with empty or a Lef1 expression vector for 24 h. The data shown are mean of three independent experiments, and error bars indicate standard deviations. Significance was determined by Student's *t*-test. * $P<0.05$, ** $P<0.01$. **B**, Quantitative levels of 3C assay (Fig. 4*B*) were analyzed by real-time PCR and were expressed relative to control after normalizing with input. Input represents PCR products of conserved NF-κB binding site of COX2. Upper, chondrocytes were transfected with control or Lef1 siRNA for 24 h, and IL-1β was further added for 1 h. The 3C assay was performed with AseI restriction for COX2 or PvuII restriction for MMP13. Lower, chondrocytes were transfected with empty or Lef1 expression vector for 24 h, and the 3C assay was performed with AseI restriction for COX2 or PvuII restriction for MMP13. **C**, Quantitative levels of ChIP assays (Fig. 4*C*) were analyzed by real-time PCR and were expressed relative to control (without IL-1β treatment) after normalizing with the input DNA. Input denotes the PCR products obtained with genomic DNA without immunoprecipitation. Left, RelA or Lef1 was immunoprecipitated and PCR was performed with primers specific for the conserved Lef1 or NF-κB binding site of COX2. Right, phospho-c-Jun or Lef1 was immunoprecipitated and PCR was performed with primers specific for the conserved Lef1 or AP1 binding site of MMP13. The data shown in B-C are representative of three independent

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experiments with similar results. Error bars show the standard deviations of the PCR reactions performed in triplicate.

Supplemental FIGURE 4. Knock-down of RelA or c-Jun reduces Lef1 binding to 5' region of COX2 or MMP13.

A, The amount of enriched DNA from the ChIP assays (Fig. 5*B*) were quantitatively analyzed by real-time PCR and were expressed relative to control siRNA after normalizing with the input DNA. Input denotes the PCR products obtained with genomic DNA without immunoprecipitation. Chondrocytes were transfected with control or Lef1 siRNA and then stimulated with IL-1β. Left, RelA or Lef1 was immunoprecipitated and PCR was performed with primers specific for the conserved Lef1 binding site of COX2. Right, phospho-c-Jun or Lef1 was immunoprecipitated and PCR was performed with primers specific for the conserved Lef1 binding site of MMP13. **B**, The amount of enriched DNA from the ChIP assays (Fig. 5*C*) were quantitatively analyzed by real-time PCR and were expressed relative to control siRNA after normalizing with the input DNA. Input denotes the PCR products obtained with genomic DNA without immunoprecipitation. Chondrocytes were transfected with RelA siRNA, c-Jun siRNA or control siRNA and then stimulated with IL-1β. Left, Lef1 or RelA was immunoprecipitated and PCR was performed with primers specific for the conserved NF-κB binding site of COX2. Right, Lef1 or phospho-c-Jun was immunoprecipitated and PCR was performed with primers specific for the conserved AP1 binding site of MMP13. The data shown are representative of three independent experiments with similar results. Error bars show the standard deviations of the PCR products of ChIP assays performed in triplicate.

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Supplemental FIGURE 5. Knock-down of Lef1, RelA or c-Jun suppresses expression of COX2 or MMP13.

A, Chondrocytes were transfected with Lef1 or control siRNA, and then protein levels of Lef1, COX2 or MMP13 were determined by immunoblot analysis. β-tubulin was analyzed as loading controls. **B**, Chondrocytes were transfected with RelA or control siRNA, and then protein levels of RelA and COX2 were determined by immunoblot analysis. **C**, Chondrocytes were transfected with c-Jun or control siRNA and then protein levels of c-Jun and MMP13 were determined by immunoblot analysis. The data shown in A-C are from one of at least three independent experiments, all of which yield similar results.

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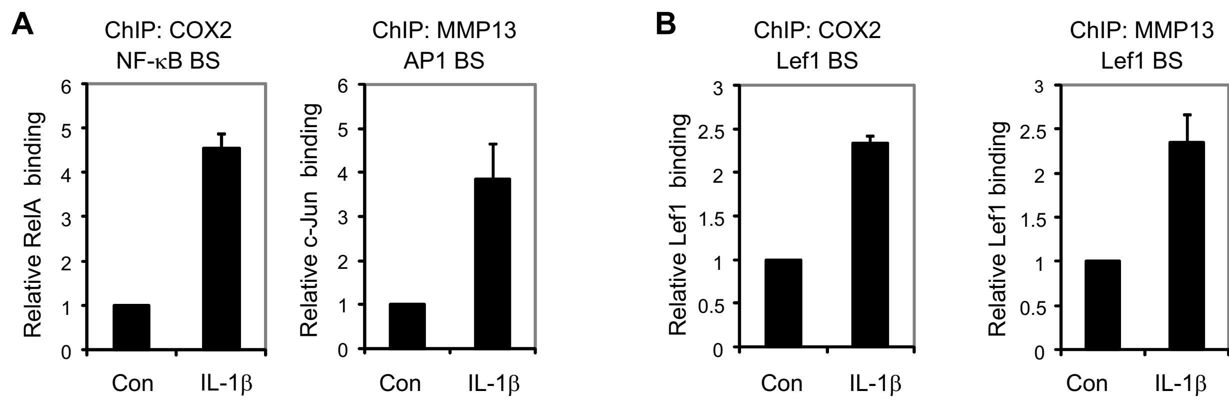
Supplemental Fig. 1, Yun et al



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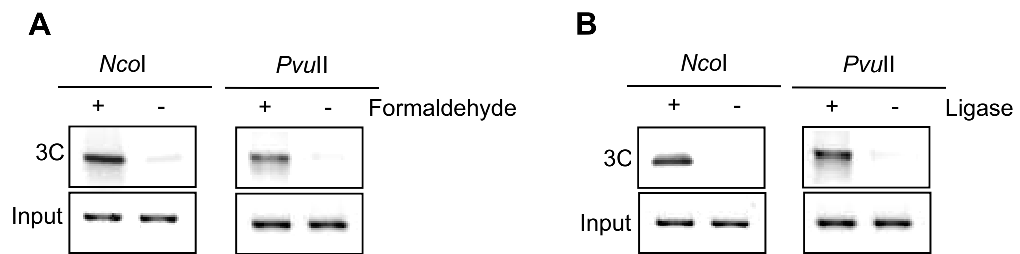

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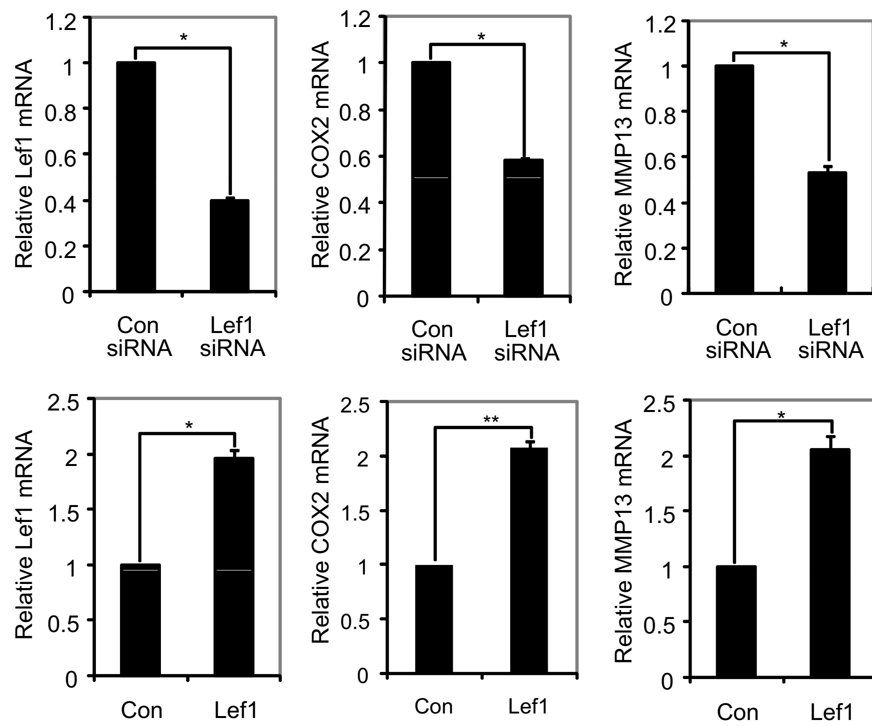

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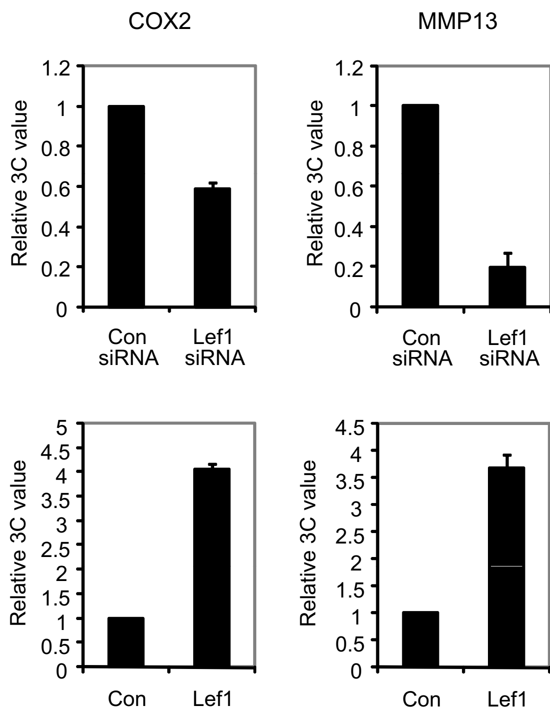

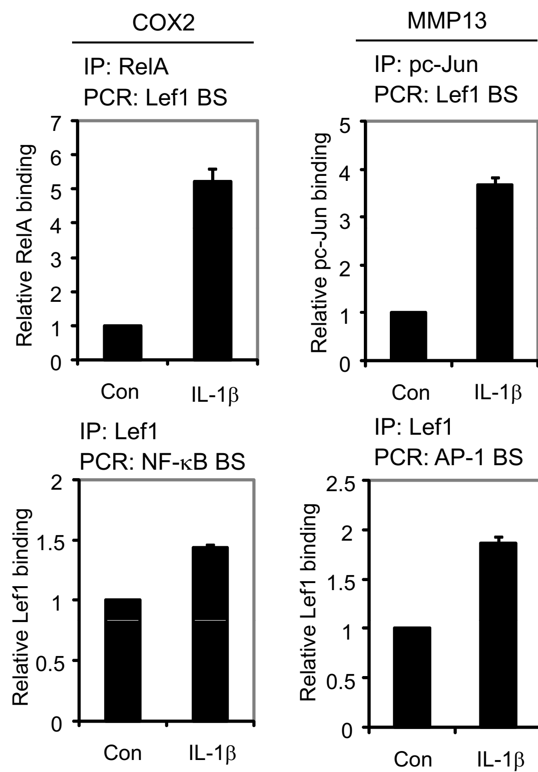

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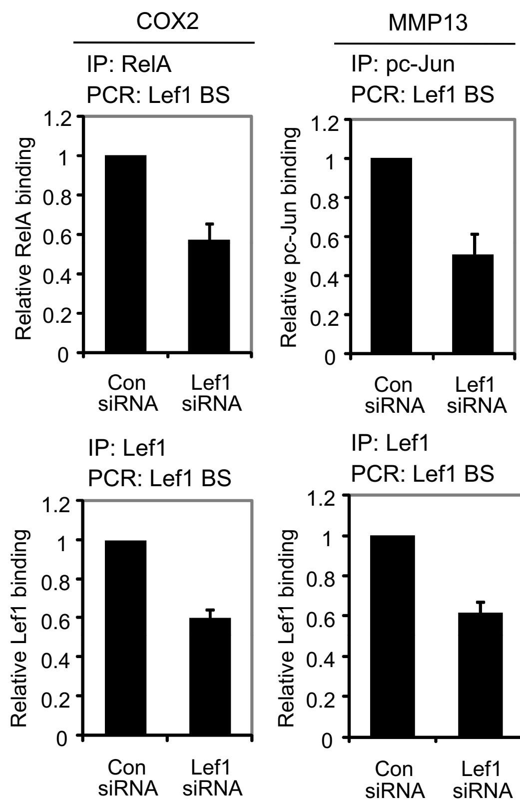

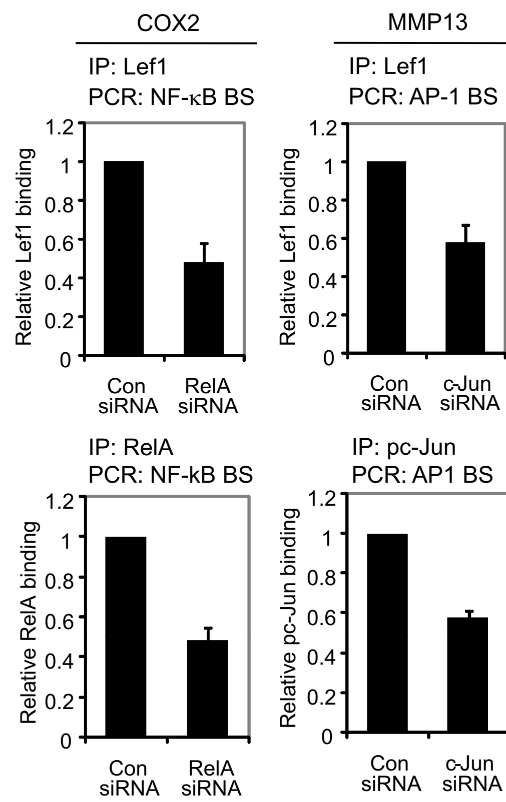

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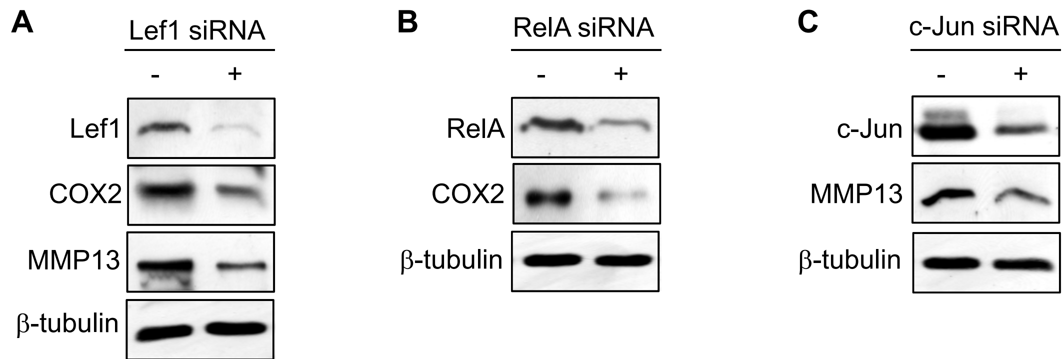

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