Table S1. Primer sequences

Primers for quantitative PCR	
Tgfβ1 **Forward**	**5'-AACCGCACTGTCATTCACC -3'**
Tgfβ1 **Reverse**	**5'-CGCCAA ACTTCTCCA AACC -3'**
Tgfβ2**Forward**	**5'-CTCAACACACCAAAGTCCTC -3'**
Tgfβ2**Reverse**	**5'-ATCAAAACTCCCTCCCTCC -3'**
Tgfβ3 **Forward**	**5'-ACCAAAGCGACAGACCTCAC -3'**
Tgfβ3 **Reverse**	**5'-TTCCCCTAACCAACCCACAC -3'**
Tgfβr1 **Forward**	**5'-AACCGCACTGTCATTCACC -3'**
Tgfβr1 **Reverse**	**5'-CGCCAA ACTTCTCCA AACC -3'**
Tgfβr2 **Forward**	**5'-AAGGAA AAGAAAAGGGCGG -3'**
Tgfβr2 **Reverse**	**5'-GCACACGGTAGCAGTAGA AG -3'**
Thbs1 **Forward**	**5'-TGCCCAAAGGGAGAAAGTCCAGAA -3'**
Thbs1 **Reverse**	**5'-CTGAGCAGCACACACAGAAGCATT -3'**
Acta2 **Forward**	**5'-TTGCTGACAGGATGCAGAAGGAGA -3'**
Acta2 **Reverse**	**5'-TCACAGTTGTGTGCTAGAGGCAGA -3'**
Cnn1 **Forward**	**5'-TTGTGGATGTGACAGCAGCGTTTG -3'**
Cnn1 **Reverse**	**5'-TGTATCACATGGCCTGTGTGTGGA -3'**
D0H4S114 **Forward**	**5'-AGGCCTTGTTCTGAACTGTTTGCG -3'**
D0H4S114 **Reverse**	**5'-GCAGCGCACACTGCAATTCTACAT -3'**
Smad6 **Forward**	**5'-ACCGGGTTACTCCATCAAGGTGTT -3'**
Smad6 **Reverse**	**5'-GCCGCATTGCTATCTGTGGTTGTT -3'**
Ctgf **Forward**	**5'-GACAGCTTGTGGCAAGTGAA -3'**
Ctgf **Reverse**	**5'-TTCCTCGTGGAAATCTGACC -3'**
Hmox1 **Forward**	**5'-AGGTGATGCTGACAGAGGAACACA -3'**
Hmox1 **Reverse**	**5'-ACAGAGAGAAGGCCACATTGGACA -3'**
p21 **Forward**	**5-'TTGTCGCTGTCTTGCACTC-3'**
p21 **Reverse**	**5-'AGGTTTGGAGACTGGGAGAG-3'**
Fn1 **Forward**	**5'-TTTTGACAACGGGAAGCATTATCAGATAA -3'**
Fn1 **Reverse**	**5'-TGATCAAAACATTTCTCAGCTATTGG -3'**
Ccl2 **Forward**	**5'-TCACCTGCTGCTACTCATTCACCA -3'**
Ccl2 **Reverse**	**5'-AAAGGTGCTGAAGACCTTAGGGCA -3'**
Ccl7 **Forward**	**5'-CCCAATGCATCCACATGCTGCTAT -3'**
Ccl7 **Reverse**	**5'-TGCTTCTTGGCTCCTAGGTTGGTT -3'**
Il-6 **Forward**	**5'-ACCACTTCACAAGTCGGAGGCTTA -3'**
Il-6 **Reverse**	**5'-TGGTACTCCAGAAGACCAGAGGAA -3'**
IκBα **Forward**	**5'-ATCCTGACCTGGTTTCGCTCTTGT -3'**
IκBα **Reverse**	**5'-ACACAGTCATCATAGGGCAGCTCA -3'**
RelB **Forward**	**5'-GCGGATTTGCCGAATCAACAAGGA -3'**
RelB **Reverse**	**5'-AACACATTGACAGTCACGGGCTCT -3'**
Mt1 **Forward**	**5'-AGCTTCACCAGATCTCGGAATGGA -3'**
Mt1 **Reverse**	**5'-ACGCTGGGTTGGTCCGATACTATT -3'**
Mt2 **Forward**	**5'-TGGCCATATCCCTTGAGCCAGAAA -3'**
Mt2 **Reverse**	**5'-TTGTCGGAAGCCTCTTTGCAGATG -3'**
Sod1 **Forward**	**5'-TGGCGATGAAAGCGGTGTGC -3'**

***Sod1* Reverse**	**5'-GCGGCTCCCAGCATTTCCAG -3'**
***Sod2* Forward**	**5'-GCACATTAACGCGCAGATCA -3'**
***Sod2* Reverse**	**5'-AGCCTCCAGCAACTCTCCTT -3'**
***Gapdh* Forward**	**5'-AACGACCCCTTCATTGACC -3'**
***Gapdh* Reverse**	**5'-TGAAGACACCAGTAGACTCC -3'**
***Nox1* Forward**	**5'-TGGATGGATCTCTCGCTTCT-3'**
***Nox1* Reverse**	**5'-TCACCCAAGCTCTCCTCTGT-3'**
***Nox2* Forward**	**5'-CTTTCTCAGGGGTTCCAGTG-3'**
***Nox2* Reverse**	**5'-TGCAGTCTATCATCCAAGC-3'**
***Nox4* Forward**	**5'-CCAGAATGAGGATCCCAGAA-3'**
***Nox4* Reverse**	**5'-CAGCAGCAGCATGTAGAAGAC-3'**
***Rac1* Forward**	**5'-TATGGGACACAGCTGGACAA-3'**
***Rac1* Reverse**	**5'-TTGAGTCCTCGCTGTGTGAC-3'**
***Rac2* Forward**	**5'-CATCAGCTACACCACCAACG-3'**
***Rac2* Reverse**	**5'-TCTCGATGGTGTCCTTGTCA-3'**

Primers used in CHIP

***Tgfβ2* promoter -1143 - -813 Forward**	**5'- CTGCAACAACAGACCTCACTTCCT -3'**
***Tgfβ2* promoter -1143 - -813 Reverse**	**5'- TCTTTCTTCCCACTGGTCTTTCCC -3'**
***Tgfβ2* promoter -870 - -370 Forward**	**5'- TCGTGGCCACAGAGTCATCACTAA -3'**
***Tgfβ2* promoter -870 - -370 Reverse**	**5'- ACACACACACACACACACACACAC -3'**
***Tgfβ2* promoter -189 - 94 Forward**	**5'- ACTGCAAATTCCTCATGCCAGTCG -3'**
***Tgfβ2* promoter -189 – 94 Reverse**	**5'- TCTCAGCGCCTTCTCTCTTTCTCT -3'**
***Mt1* promoter -183 - 168 Forward**	**5'- TGGGTTTACGGAGATAGCTGGCTT -3'**
***Mt1* promoter -183 - 168 Reverse**	**5'- ACGTTGAAGTCGTGGTGACGCTTA -3'**
***Sod2* promoter -236 - 1 Forward**	**5'- AATTCTGACCAGCAGCAGAGCCTT -3'**
***Sod2* promoter -236 - 1 Reverse**	**5'- TGTGCCAAATTGGTAGAGGCCG -3'**
***Sod2* intron 2161 – 2498 Forward**	**5'- TGTGGTTGCTGGACCTTTGGAAGA -3'**
***Sod2* intron 2161 - 2498 Reverse**	**5'- AGGAAATGCTTTCCCAACTGGGCT -3'**

Primers used for bisulfite sequencing

***Tgfβ2* BS Forward**	**5'- AAGAGTTATTTTGGAGTAATTTATTTG -3'**
***Tgfβ2* BS Forward**	**5'- ACTACCTACAAAAAAAACCAACCTC -3'**

Primers used for measurements of mouse telomere

Mouse telomere F	**5'-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3'**
Mouse telomere R	**5'-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3'**
Mouse 36B4 F	**5'-ACTGGTCTAGGACCCGAGAAG-3'**
Mouse 36B4 R	**5'-TCAATGGTGCCTCTGGAGATT-3'**

Figure S1. Up-regulation of TGFβ-target genes in the IKKβ-null cells. RNA isolated from wild type and *Ikkβ*$^{-/-}$ cells were examined by real-time qRT-PCR for expression of genes of the TGFβ pathway. Fold change over uninfected cells was calculated based on data from biological triplicates. Results represent the mean values ± SD from at least two independent experiments. **p<0.01 and *** p<0.001 was considered significant.

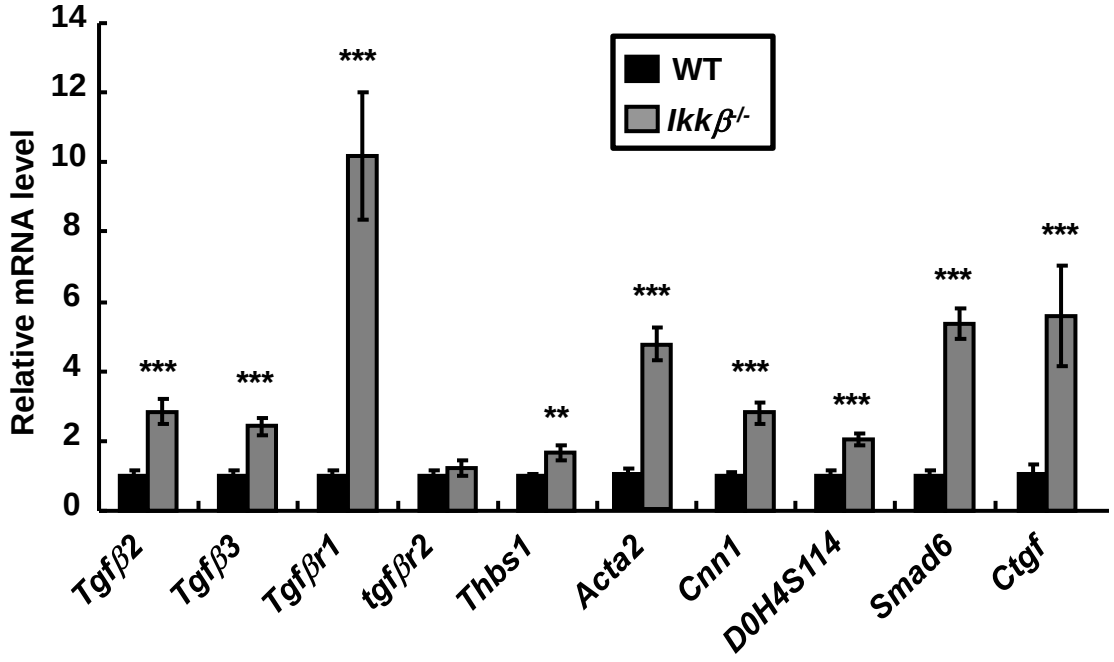
Figure S2. Induction of IKKβ loss *in vitro*. The *Ikkβ*$^{F/F}$ fibroblasts were infected with Ad-GFP and Ad-Cre with or without co-infection with Ad-IKKβ for 48 h. (A) The infected cells were photographed under light and fluorescence microscopy. More than 90% *Ikkβ*$^{F/F}$/Ad-GFP cells were shown to be GFP positive. (B) The *Ikkβ*$^{F/F}$, *Ikkβ*$^{F/F}$/Ad-Cre (infected for 48 h) and *Ikkβ*$^{-/-}$ cells were treated with TNFα for various times; cell lysates were analyzed by Western blotting for IKKβ, IκBα and β-actin. The un-infected or infected *Ikkβ*$^{F/F}$ cells were used for (C) RNA isolation and analyzed by real-time RT-PCR for *Ikkβ* expression, and (D) transfection with κB-Luc reporter and β-gal expression plasmid. The Luc and β-gal activities were examined 24 h after transfection, and the relative Luc activity (versus β-gal activity) was calculated. Fold change over uninfected cells was calculated based on data from biological triplicates. (E) After Ad-Cre infection, the *Ikkβ*$^{F/F}$/Ad-Cre cells were passage *in vitro* for various lengths of time and without or with IKKβ re-expression (Ad-IKKβ infection). RNA was isolated and analyzed by real-time RT-PCR for the expression of NF-κB-target genes. Results represent the mean values ± SD from at least two independent experiments. *** p<0.001 was considered significant.

Figure S3. Genotyping of *Ikkβ*$^{F/F}$*/Tgfβr2*$^{F/F}$/Ad-GFP and *Ikkβ*$^{F/F}$*/Tgfβr2*$^{F/F}$/Ad-Cre fibroblasts. Genomic DNAs were used for PCR genotyping, using primers recognizing the *Ikkβ*^{F}, *Ikkβ*^{Δ}, *Tgfβr2*^{F} and *Tgfβr2*^{Δ} alleles, and primers for *Gapdh*. The PCR products were examined on agarose gel after electrophoresis.

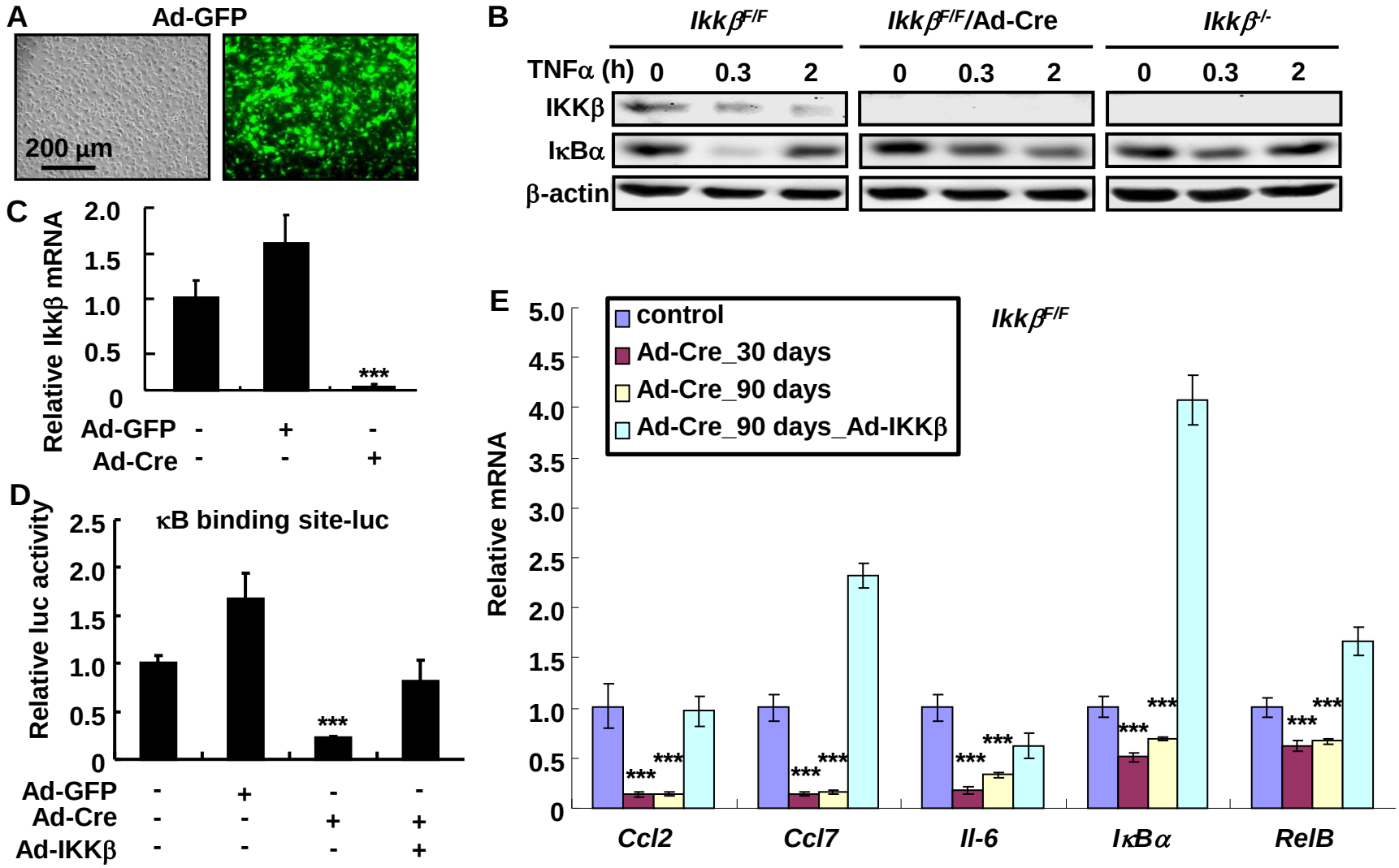
Figure S4. Induction of IKKβ loss leads to progressive ROS elevation. (A) The wild type cells with and without H_2O_2 (100 uM) treatment for 6 h and the *Ikkβ(-/-)* cells with or without Ad-IKKβ infection for 24 h, and (B) the $Ikk\beta^{F/F}$ cells with or without Ad-Cre infection, were labeled with CM-H_2DCFDA and analyzed by flow cytometry at 485/530 nm. y axis, cell numbers, x axis, fluorescence intensity. (C) The cells were divided to two groups based on the level of DCF intensity: the DCF-high (M2) and DCF-low (M1). The percentage of cells in each group was calculated. Results represent the mean values ± SD from at least two independent experiments. *: $p<0.05$ was considered significant.

Figure S5. Increased TGFβ target gene expression and migration of the p65-null cells. (A) RNA isolated from wild type and $p65^{-/-}$ fibroblasts were analyzed by RT-PCR for selected genes of the TGFβ pathways. (B) The cells were subjected to *in vitro* wound healing assays, and healing rate was calculated by comparison of the wound sizes at 0 and 12 h of wounding. Results represent the mean values ± SD from at least two independent experiments. ***: $p <0.001$ were considered significant.

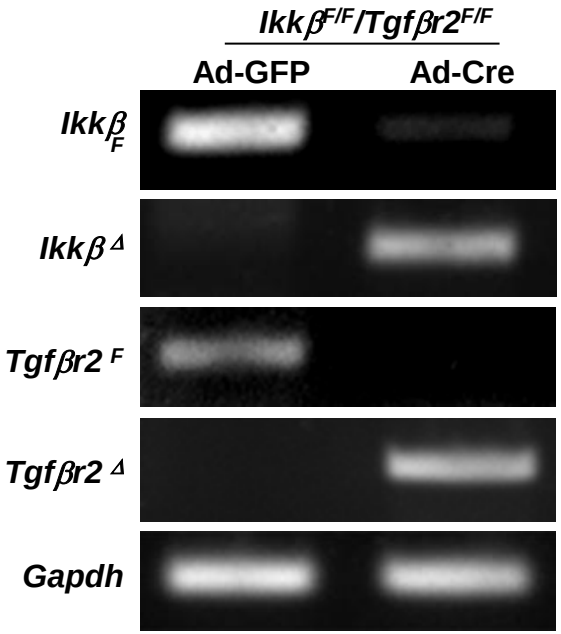
Figure S6. IKKβ loss induces c-Jun binding and H3K4Me3 modification of the *Tgfβ2* promoter (A) Illustration of approximate locations of potential AP-1 binding sites in the *Tgfβ2* promoter. The $Ikk\beta^{F/F}$ and $Ikk\beta^{F/F}$/Ad-Cre cells were subjected to Chromatin-immunoprecipitation (ChIP) assay using antibodies for (B) transcription factors, p65, p50, Nrf2 and c-Jun, and (C) histone markers, H3K27Me3, H3K9Me2 and H3K4Me3. The precipitates were examined by PCR using primers for various regions of the *Tgfβ2* promoter. The data were calculated as percentage of input and represented the mean values ± SD from at least two independent experiments. *** $p <0.001$ were considered significant.



Chen, et. al. Supplemental Figure 1

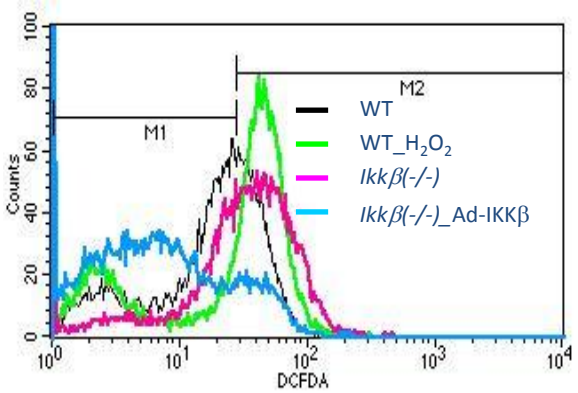


Chen, et. al. Supplemental Figure 2



Chen, et. al. Supplemental Figure 3

A



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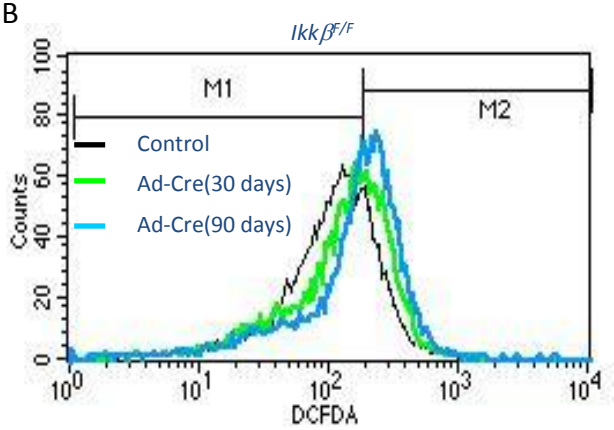
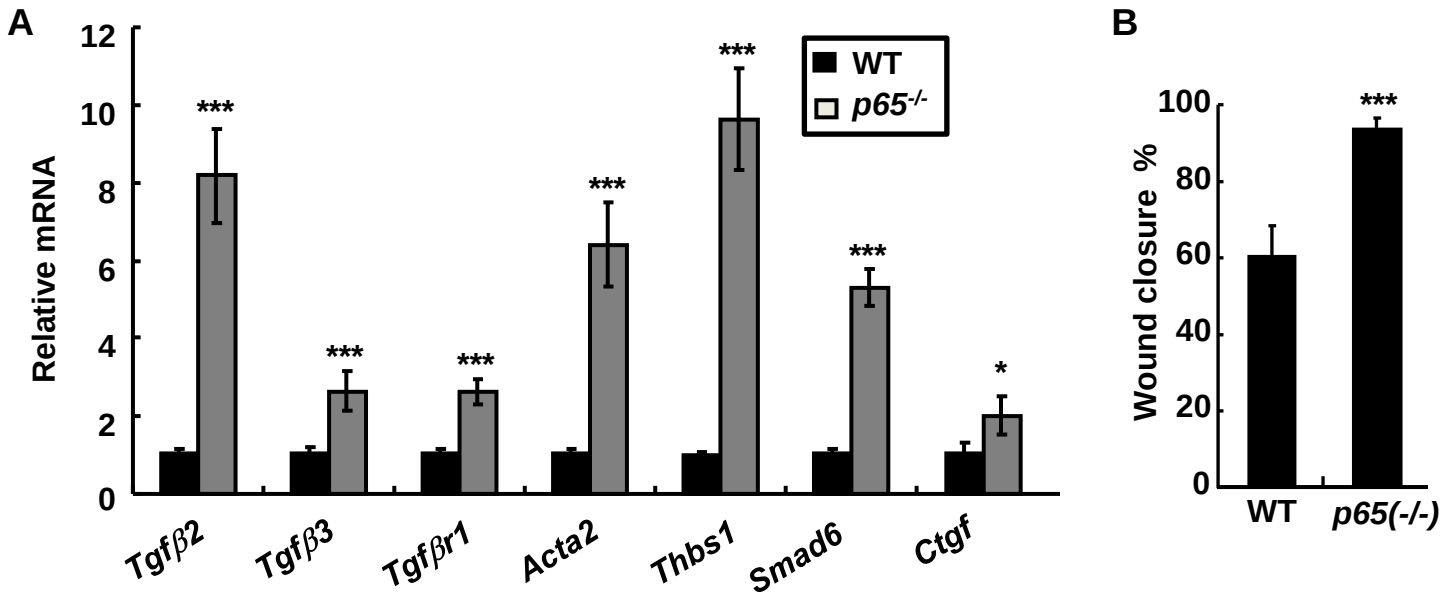


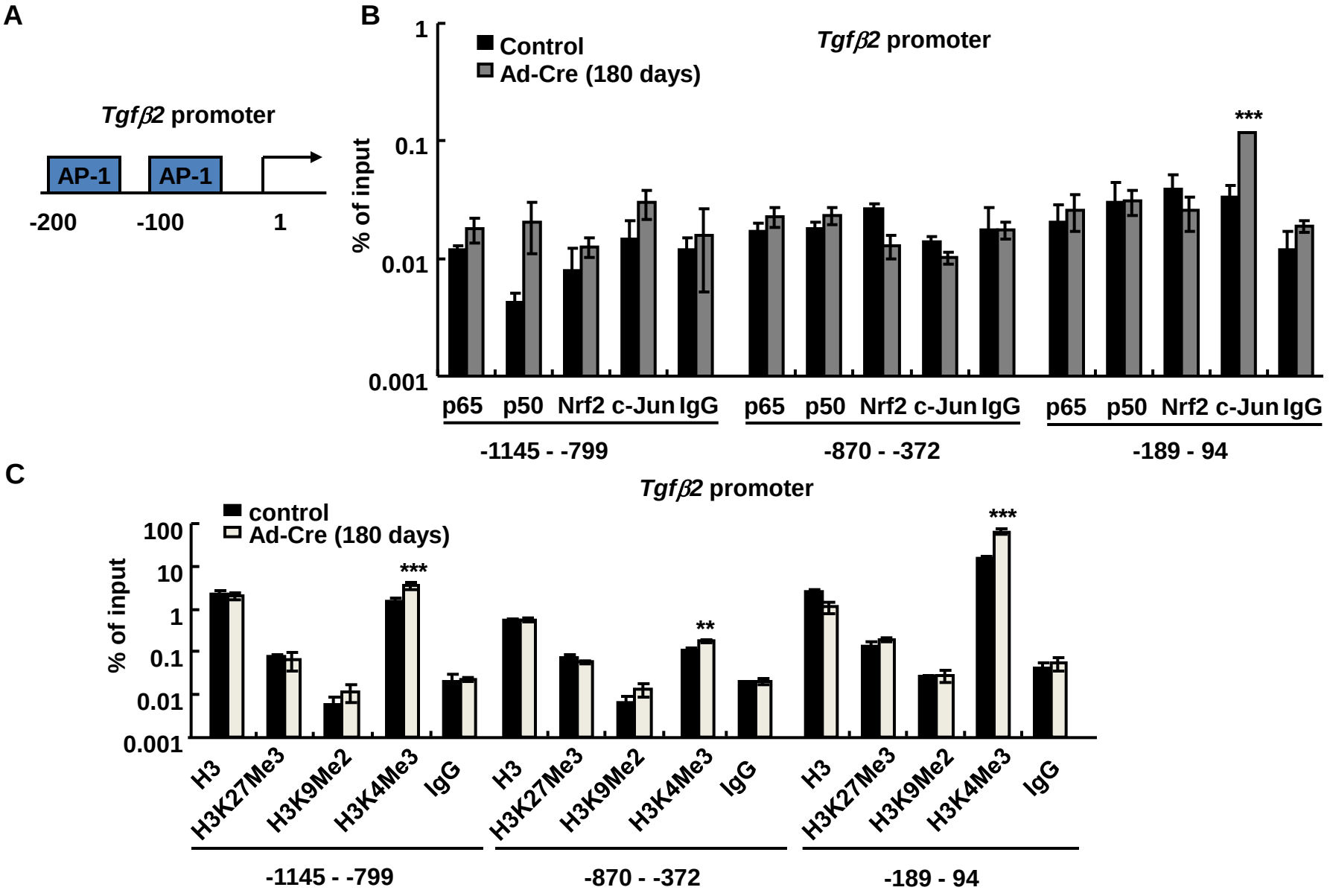
C

	M1 (Low DCFDA)	M2 (High DCFDA)
WT	**73.35%**	**24.12%**
WT_H_2O_2	**44.06%**	**53.32%**
Ikkβ(-/-)	**47.50%**	**50.54%**
***Ikkβ(-/-)*_Ad-IKKβ**	**87.90%**	**11.33%**
Ikkβ$^{F/F}$	**73.41%**	**24.02%**
***Ikkβ$^{F/F}$*_Ad-Cre_30 days**	**61.14%**	**36.07%**
***Ikkβ$^{F/F}$*_Ad-Cre_90 days**	**47.07%**	**49.57%**

Chen, et. al., Supplemental Figure 4



Chen, et. al., Supplemental Figure 5



Chen, et. al. Supplemental Figure 6