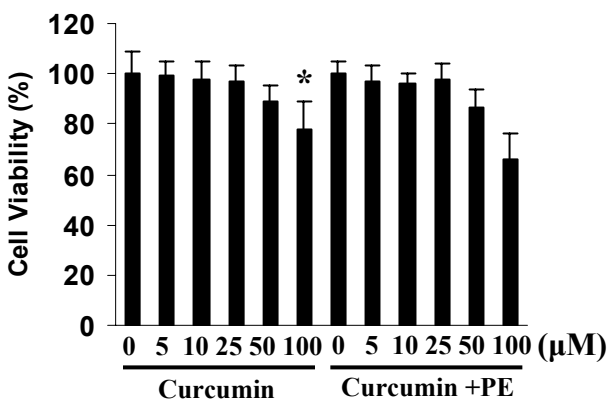
## Li et al., Supplemental Table. 1

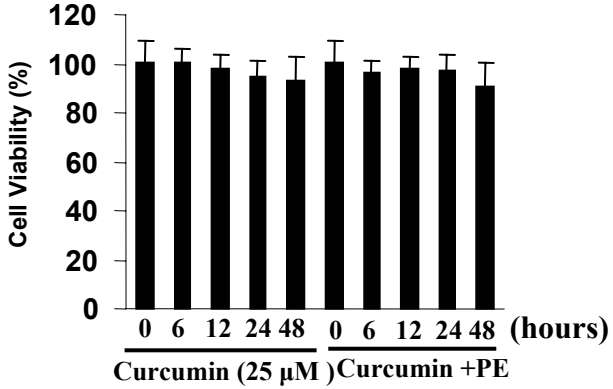
**The dose-dependent effects of curcumin on cardiac hypertrophy induced by aortic banding model.**

| | Sham | | | | AB 3 week | | | |
|---|---|---|---|---|---|---|---|---|
| **Curcumin (mg)** | **Vehicle** | **50** | **75** | **100** | **Vehicle** | **50** | **75** | **100** |
| **Number** | n=12 | n=10 | n=8 | n=8 | n=12 | n=11 | n=7 | n=8 |
| **BW (g)** | 27.4±1.1 | 27.6±1.4 | 28.7±1.2 | 27.6±1.5 | 27.4 ±1.5 | 27.7 ±1.3 | 27.2±0.4 | 28.1±1.4 |
| **HW/BW (mg/g)** | 5.10±0.06 | 5.16±0.03 | 5.08±0.09 | 5.06±0.07 | 6.78 ±0.03$^{A}$ | 5.51 ±0.06$^{B}$ | 5.21±0.02$^{B}$ | 5.18±0.05$^{B}$ |
| **LW/BW(mg/g)** | 6.14±0.05 | 6.11±0.02 | 6.13±0.07 | 6.06±0.04 | 6.12 ±0.05 | 6.07 ±0.03 | 6.11±0.02 | 6.08±0.04 |
| **SBP (mmHg)** | 106.0±3.3 | 107.4±5.3 | 108.0±6.7 | 106.0±1.7 | 134.2±2.6$^{A}$ | 127.5±4.3$^{A}$ | 129.8±3.7$^{A}$ | 125.7±6.1$^{A}$ |
| **HR (bpm)** | 454±26 | 456±31 | 476±12 | 478±7 | 454±40 | 469±19 | 484±11 | 475±14 |
| **LVEDD(mm)** | 3.53±0.05 | 3.46±0.03 | 3.51±0.01 | 3.42±0.06 | 4.09±0.02$^{A}$ | 3.92±0.06 | 3.65±0.03$^{B}$ | 3.66±0.04$^{B}$ |
| **LVESD(mm)** | 1.70±0.02 | 1.74±0.02 | 1.68±0.06 | 1.65±0.02 | 2.45±0.02$^{A}$ | 1.97±0.03$^{B}$ | 1.82±0.03$^{B}$ | 1.70±0.03$^{B}$ |
| **IVSD(mm)** | 0.73±0.02 | 0.72±0.04 | 0.75±0.02 | 0.72±0.05 | 1.16±0.02$^{A}$ | 0.89±0.04 | 0.78±0.05$^{B}$ | 0.76±0.04$^{B}$ |
| **LVPWD (mm)** | 0.62±0.01 | 0.61±0.02 | 0.57±0.02 | 0.66±0.03 | 1.07±0.02$^{A}$ | 0.81±0.07 | 0. 68±0.04$^{B}$ | 0.62±0.03$^{B}$ |
| **FS (%)** | 56.6±3.2 | 54.4±4.1 | 53.0±2.1 | 55.2±1.2 | 34.2±1.8$^{A}$ | 44.6±1.0$^{AB}$ | 52.7±1.4$^{B}$ | 54.3±0.8$^{B}$ |

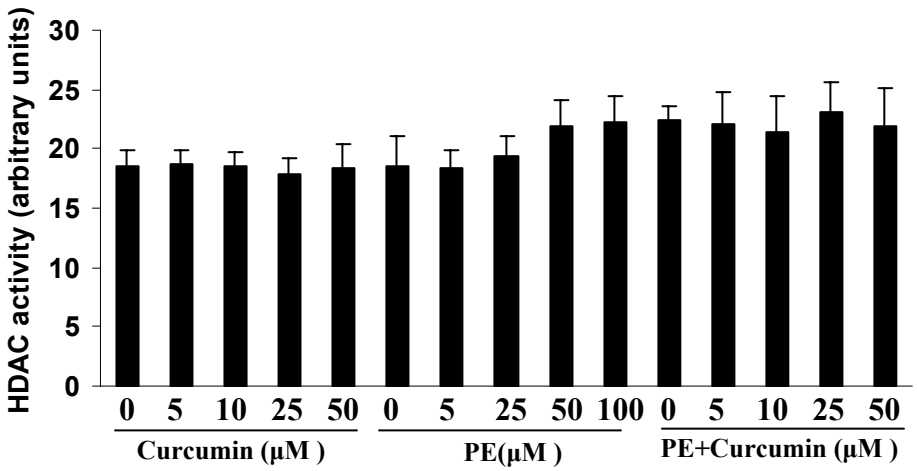
**BW, body weight; HW, heart weight; SBP, systolic blood pressure; HR, heart rate; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; IVSD, left ventricular septum, diastolic; LVPWD, left ventricular posterior wall, diastolic; FS, fractional shortening. All values are mean ± SEM. $^{A}$*P*<0.05 aortic banding versus sham-operation; $^{B}$ *P*<0.05 Curcumin treatment versus Vehicle treatment after aortic banding.**

**Li et al., Supplemental Figure. 1**



**Li et al., Supplemental Figure. 2**



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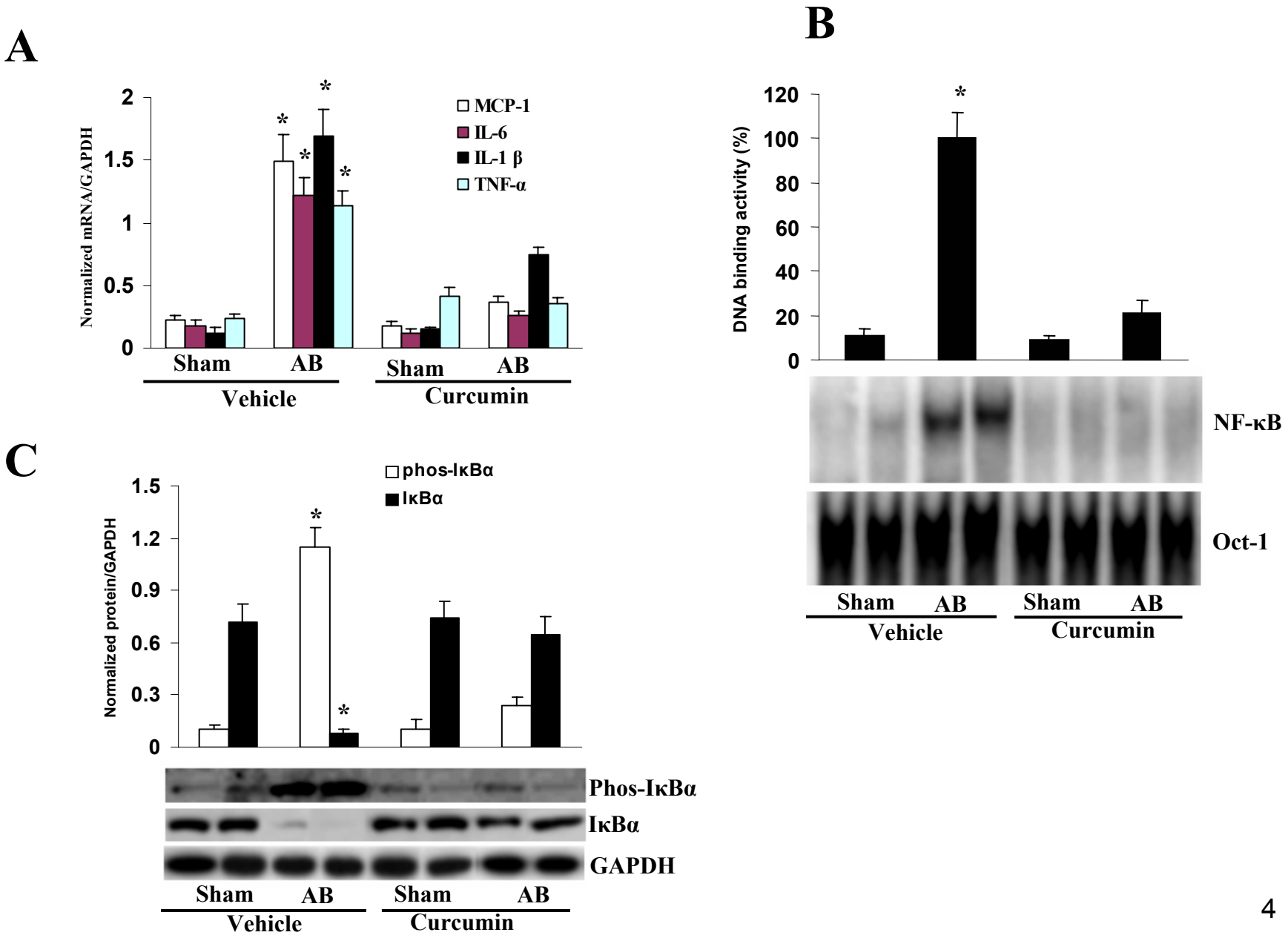
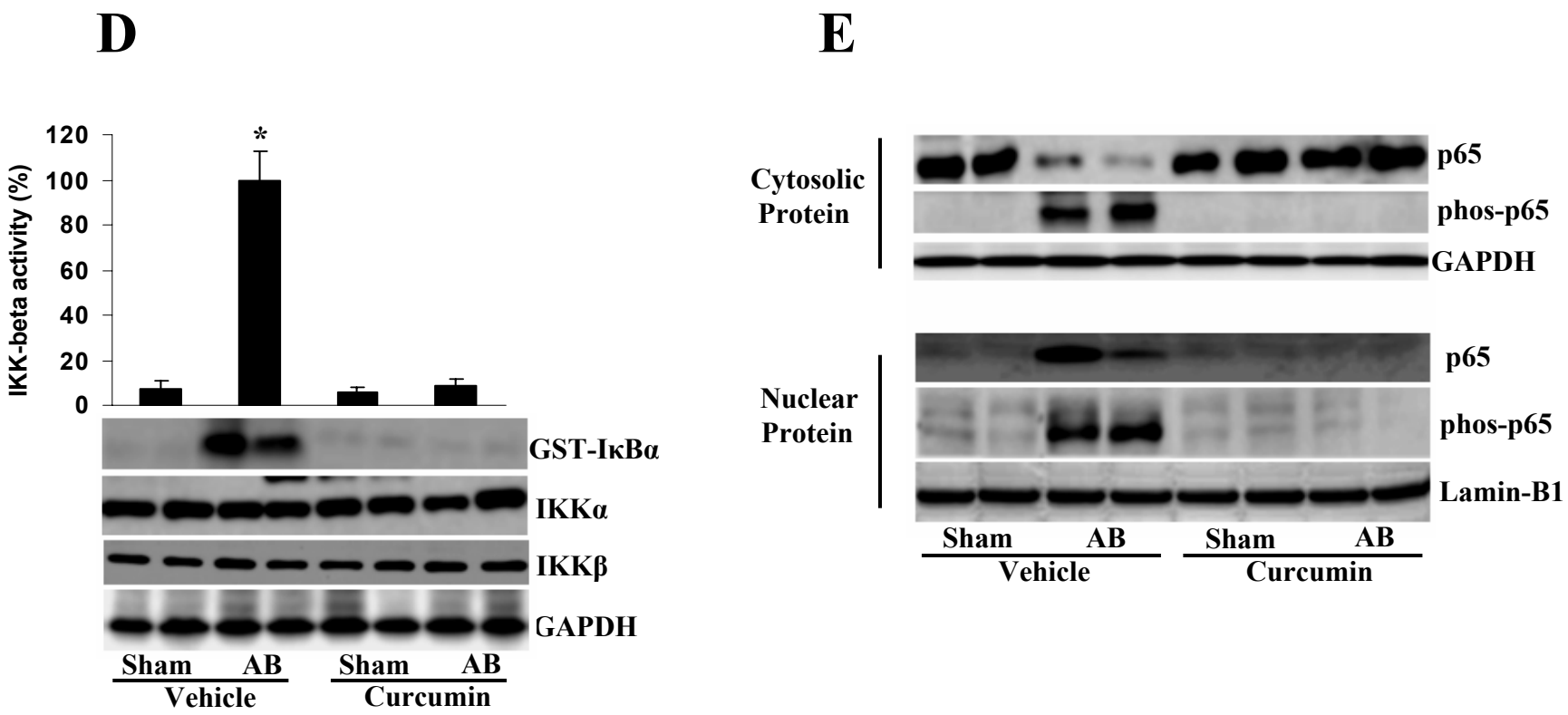
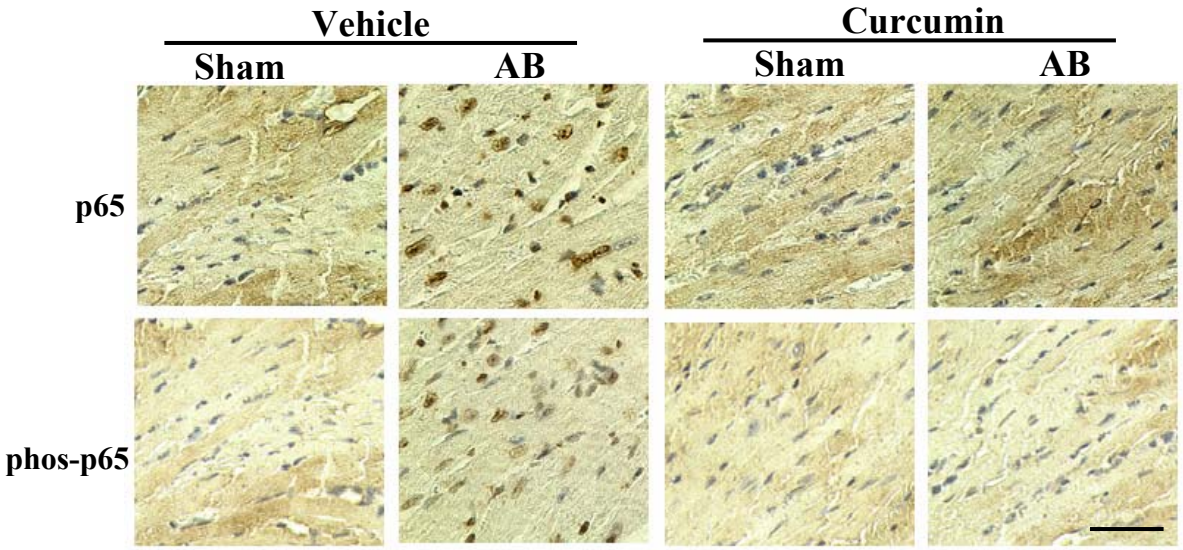
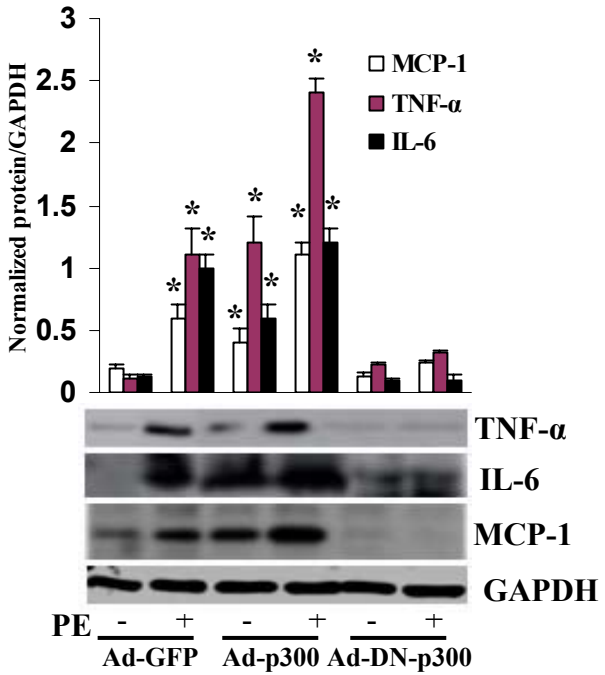


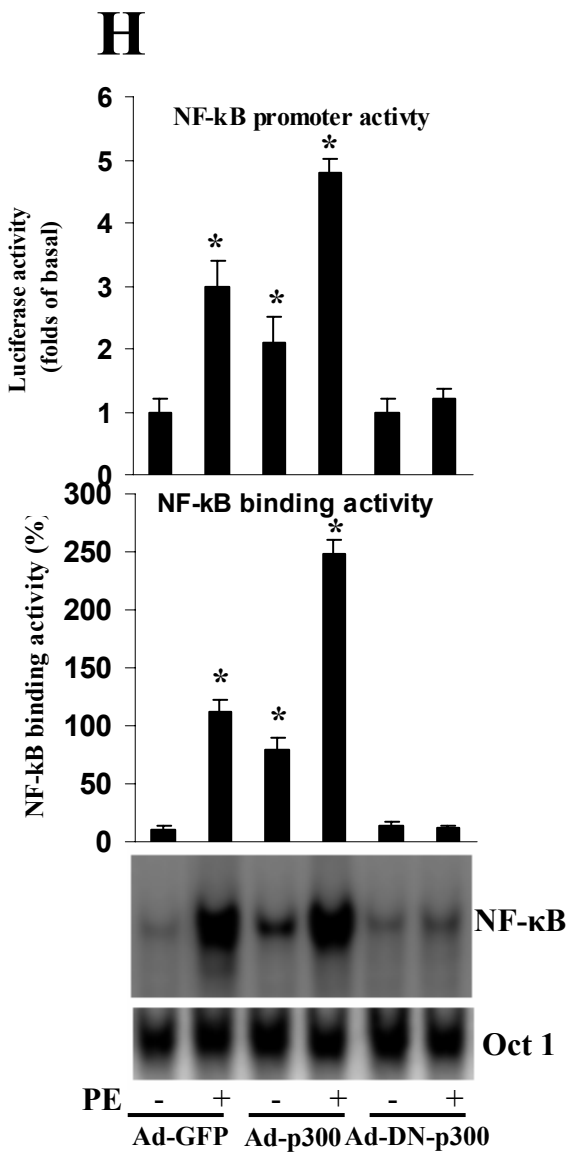

D
E
IKK-beta activity (%)
120
100
80
60
40
20
0
*
GST-IκBα
IKKα
IKKβ
GAPDH
Sham
AB
Sham
AB
Vehicle
Curcumin
Cytosolic Protein
Nuclear Protein
p65
phos-p65
GAPDH
p65
phos-p65
Lamin-B1
Sham
AB
Sham
AB
Vehicle
Curcumin

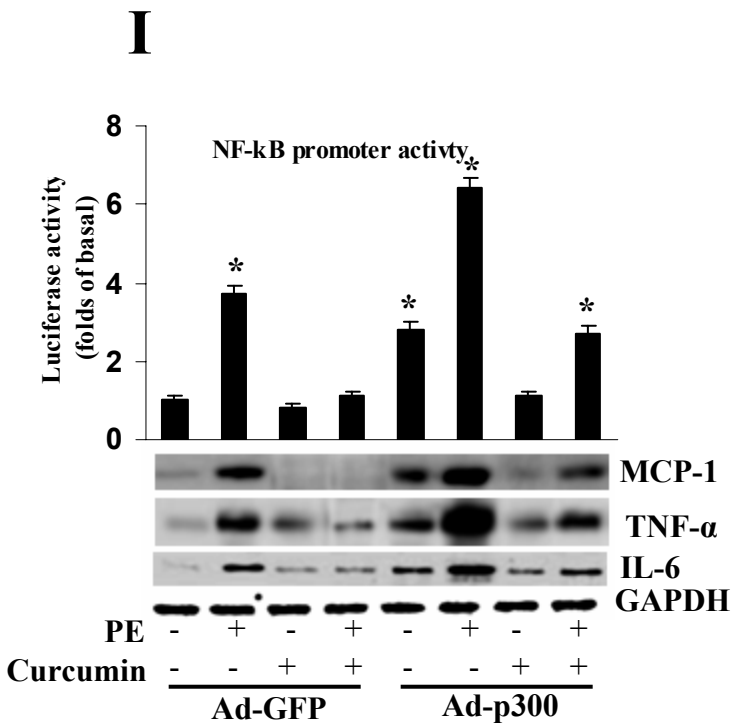
F


Vehicle
Curcumin
Sham
AB
Sham
AB
p65
phos-p65

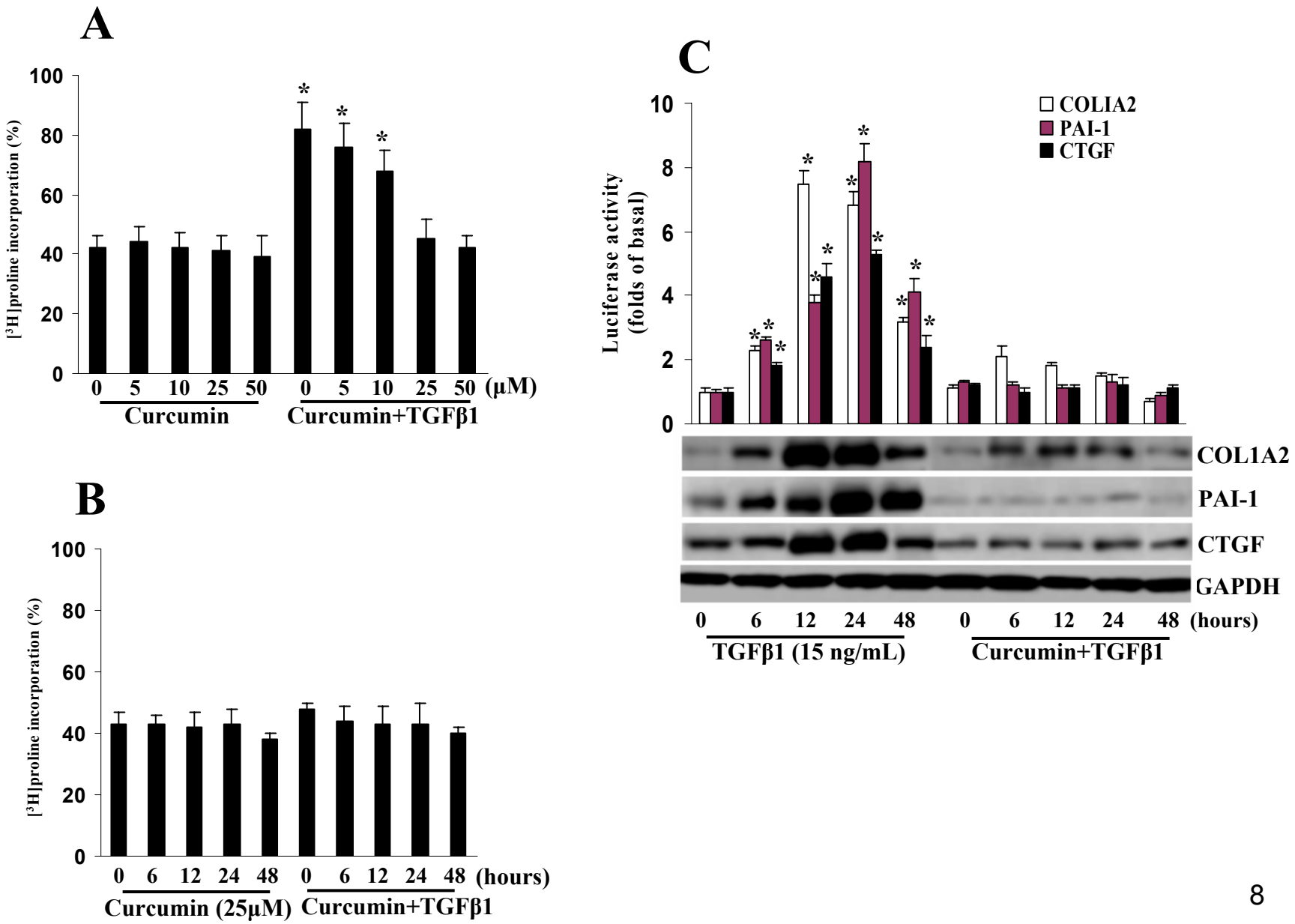
G

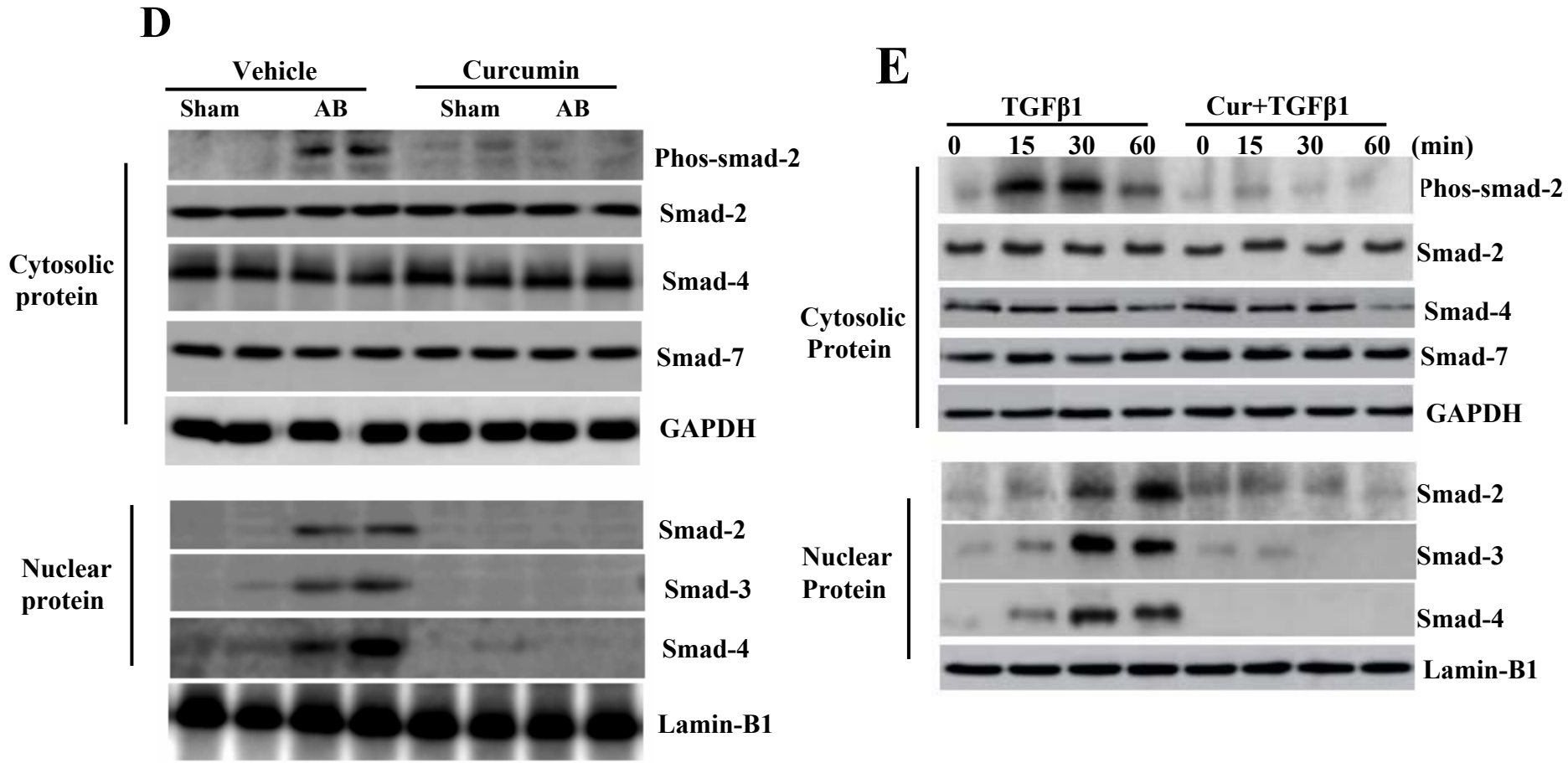

Normalized protein/GAPDH
3
2.5
2
1.5
1
0.5
0
MCP-1
TNF-α
IL-6
TNF-α
IL-6
MCP-1
GAPDH
PE - + - + - +
Ad-GFP
Ad-p300
Ad-DN-p300

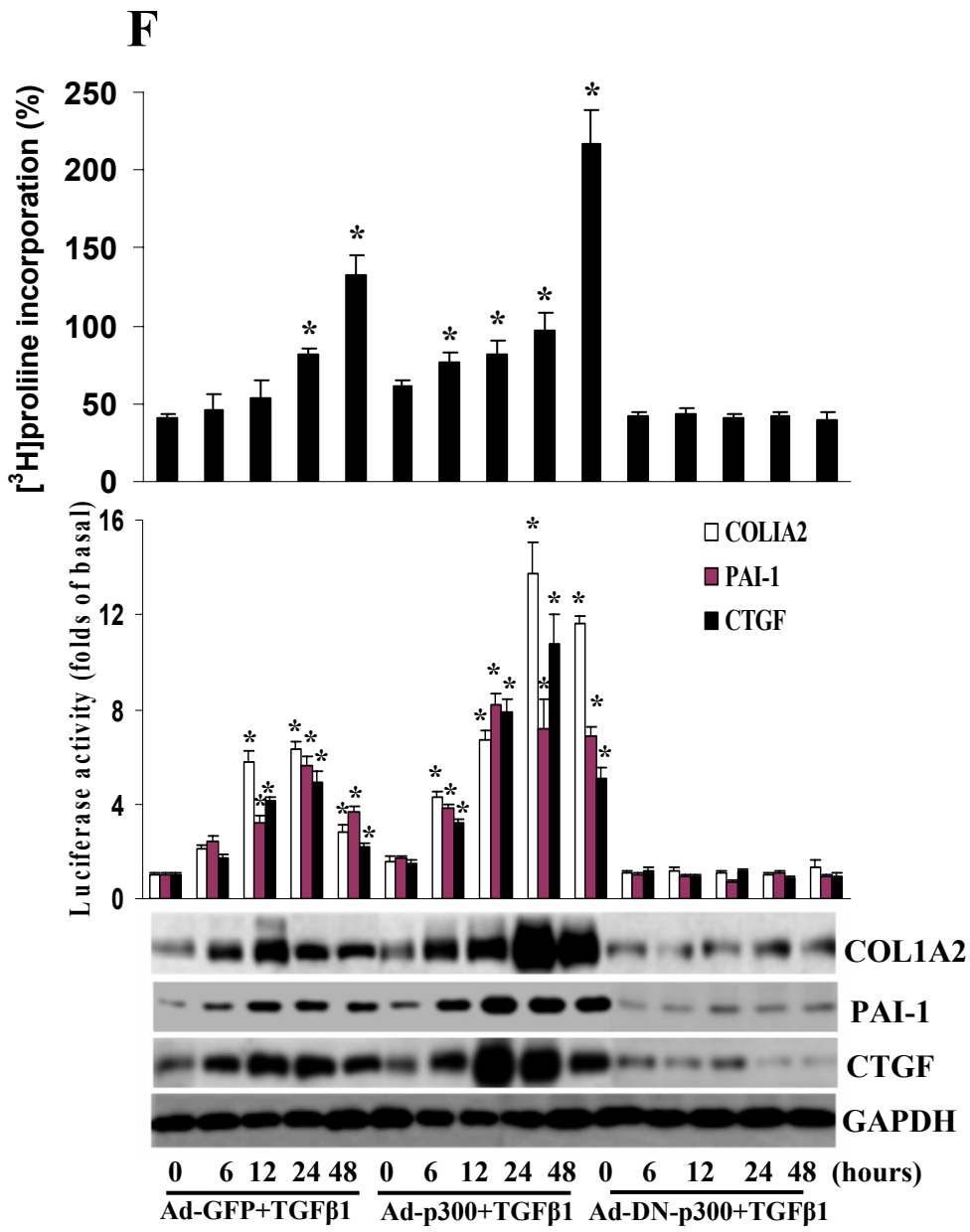

H
NF-kB promoter activty
Luciferase activity (folds of basal)
NF-kB binding activity
NF-kB binding activity (%)
NF-κB
Oct 1
PE
Ad-GFP
Ad-p300
Ad-DN-p300

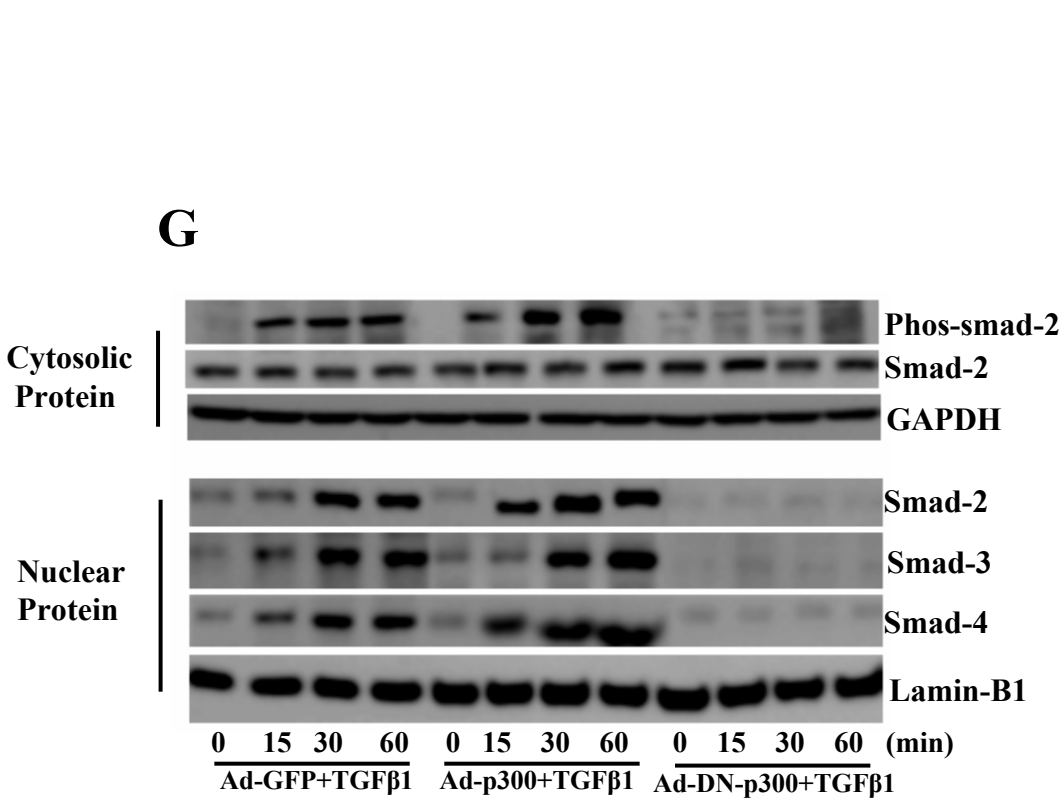

I
NF-kB promoter activty
Luciferase activity (folds of basal)
MCP-1
TNF-α
IL-6
GAPDH
PE
Curcumin
Ad-GFP
Ad-p300

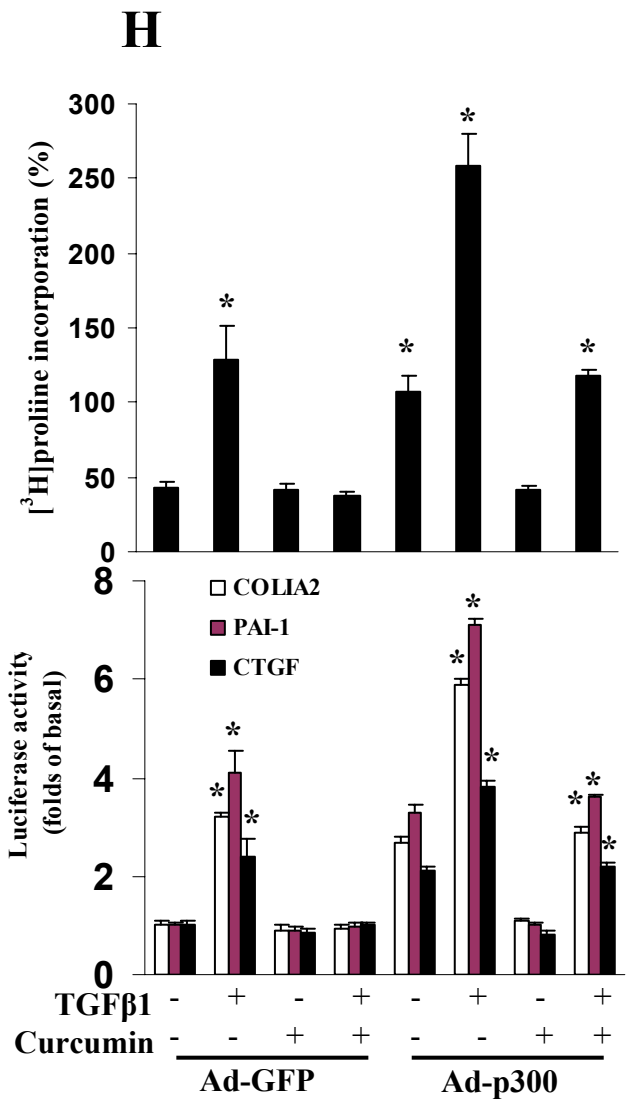
**Li et al., Supplemental Figure. 4**



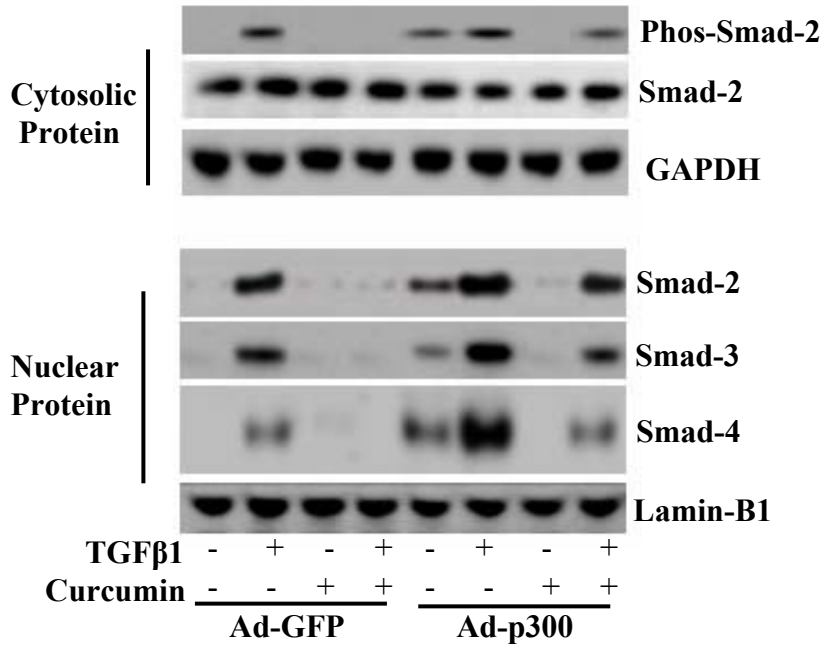

D
Vehicle
Curcumin
Sham
AB
Sham
AB
Phos-smad-2
Smad-2
Smad-4
Smad-7
GAPDH
Cytosolic protein
Smad-2
Smad-3
Smad-4
Lamin-B1
Nuclear protein
E
TGFβ1
Cur+TGFβ1
0
15
30
60
0
15
30
60
(min)
Phos-smad-2
Smad-2
Smad-4
Smad-7
GAPDH
Cytosolic Protein
Smad-2
Smad-3
Smad-4
Lamin-B1
Nuclear Protein


F
[3H]proline incorporation (%)
250
200
150
100
50
0
Luciferase activity (folds of basal)
16
12
8
4
0
COLIA2
PAI-1
CTGF
COL1A2
PAI-1
CTGF
GAPDH
0 6 12 24 48 0 6 12 24 48 0 6 12 24 48 (hours)
Ad-GFP+TGFβ1
Ad-p300+TGFβ1
Ad-DN-p300+TGFβ1

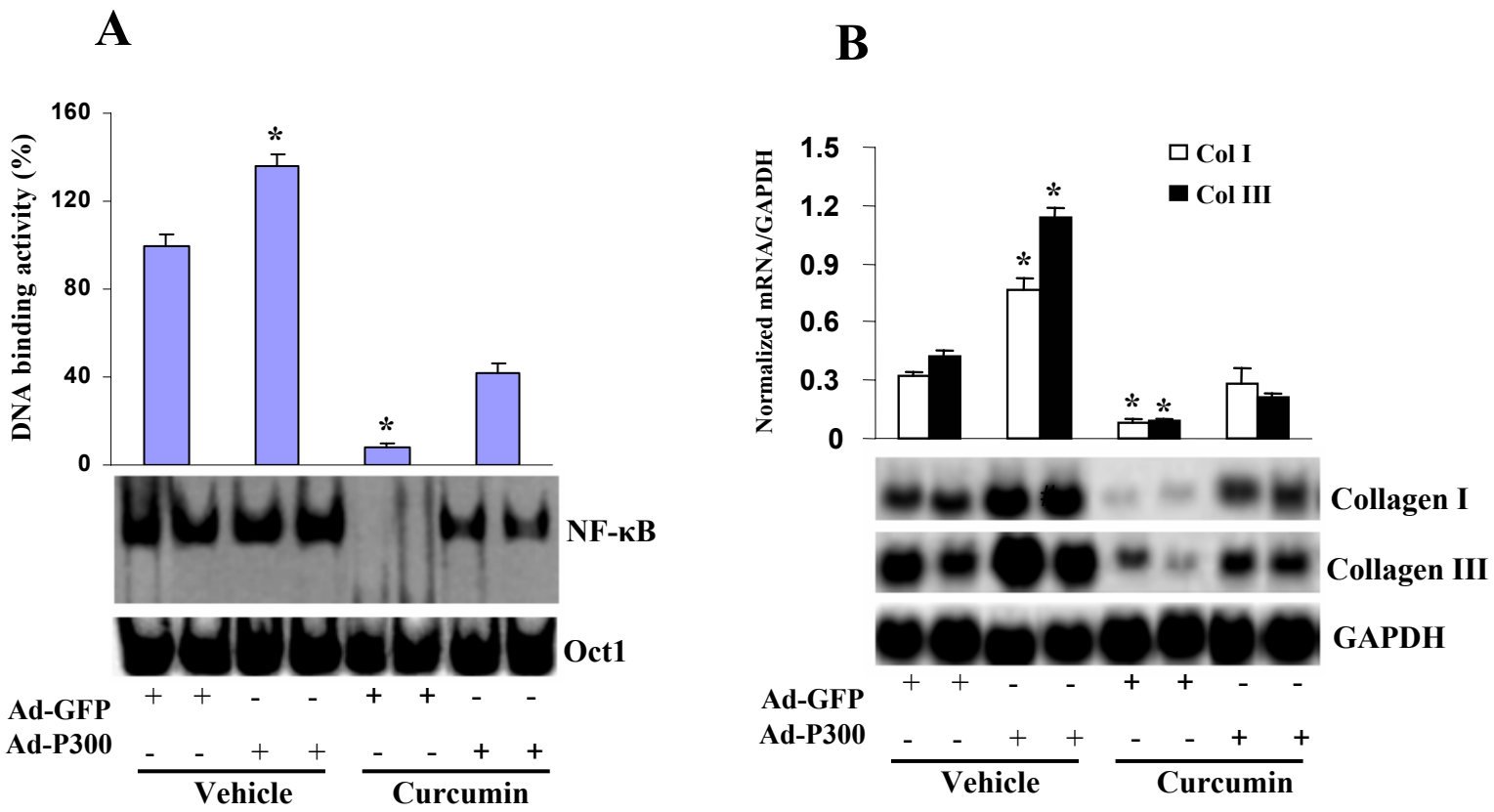

G
Cytosolic Protein
Nuclear Protein
Phos-smad-2
Smad-2
GAPDH
Smad-2
Smad-3
Smad-4
Lamin-B1
0 15 30 60 0 15 30 60 0 15 30 60 (min)
Ad-GFP+TGFβ1
Ad-p300+TGFβ1
Ad-DN-p300+TGFβ1


H
[3H]proliine incorporation (%)
300
250
200
150
100
50
0
COLIA2
PAI-1
CTGF
Luciferase activity (folds of basal)
8
6
4
2
0
TGFβ1 - + - + - + - +
Curcumin - - + + - - + +
Ad-GFP
Ad-p300

**I**


Phos-Smad-2
Smad-2
GAPDH
Cytosolic Protein
Smad-2
Smad-3
Smad-4
Lamin-B1
Nuclear Protein
TGFβ1 - + - + - + - +
Curcumin - - + + - - + +
Ad-GFP
Ad-p300

**Li et al., Supplemental Figure. 5**



# Supplemental Figure Legend

## Supplemental Figure 1

Effect of curcumin and/or PE on cell viability in cardiac myocyte. The results were reproducible in three separate experiments.*$P$<0.05 vs. exposed to control.

## Supplemental Figure 2

**(A)** Effect of curcumin and/or PE on the in vitro activity of HDAC. HDAC activity in the pellet was assayed and reported as arbitrary units as described in *Methods*. **(B)** Effect of p300 on the inhibitory effect of curcumin on cardiac hypertrophy. Cells were treated with or without 25 μM curcumin for 60 minutes and then incubated with 100 μM PE for 48 hours. [$^3$H]leucine incorporation, luciferase activity and Northern blot were described in *Materials and Methods*. Each assay was performed at least three times. *$P$<0.05 vs. exposed to control.

## Supplemental Figure 3

**(A)** Real Time-PCR analysis of TNF-α, IL-1β, IL-6 and MCP-1 mRNA expression in the myocardium obtained from indicated groups (n=6). Each assay was performed in triplicate. *$P$<0.05 for difference from vehicle/sham values. **(B)** EMSA for NF-κB activity in the myocardium from 4 groups of animals (n=5). Top, optical density in region of NF-κB was quantified with PhosphorImager system. Bottom, representative bands. Oct-1 binding activity was used as a control for EMSA assay. (**C**) Western blot analysis of IκBα degradation and phosphorylation of the myocardium was obtained from indicated animals (n=5). Top, quantitative results. Bottom, representative blots. (**D)** IKK activity (n=3). Top, quantitative results. Bottom, the extracts were assayed for IKK by the immunocomplex kinase assay (upper panel) and for IKK-α (middle panel) and IKK-β (lower panel) protein expression by Western blot analysis as described in *Materials and Methods*. (**E)** Curcumin inhibited p65 phosphorylation and translocation induced by AB (n=3). Western blot analysis of p65 and phosphorylated p65 in the cytosolic and nuclear fractions of the myocardial cell obtained from each respective group. Anti-lamin B1 and anti-GAPDH were used as the loading controls for nuclear protein and cytosolic protein content, respectively. (**F)** Tissue sections from each group were immunostained with anti-

p65 or anti-phosphorylated p65 antibodies (n=4; Scale bar: 20 μm). From A to F, each assay was performed in triplicate. *$P$<0.05 for difference from vehicle/sham values. **(G and H)** Effect of p300 on TNF-α, IL-6 and MCP-1 protein expression (G) and NF-κB activation (H) induced by PE. Cells were infected with Ad-p300, Ad-DN-p300 or Ad-GFP for 24 hours, and then treated with 100 μM PE for 36 hours for cytokines or for 24 hours for NF-κB activation. **(I)** P300 significantly reversed the inhibitory effect of curcumin on the NF-κB transcriptional activity and cytokines protein expression induced by PE. Cells were infected with Ad-p300 or Ad-GFP for 24 hours, treated with 25 μM curcumin for 60 minutes, and then incubated with 100 μM PE for 24 hours. Luciferase activity and Western blot were performed as described in *Materials and Methods*. From G to I, the results were reproducible in three separate experiments. *$P$<0.05 vs. exposed to Ad-GFP infection alone group.

**Supplemental Figure 4**

**(A and B)** The dose and time course of curcumin on TGF-β1-induced [$^3$H] proline incorporation is depicted in these graphs. In these experiments cells were pretreated with either different doses of curcumin, or with 25μM curcumin, for 60 minutes and then incubated with either 15 ng/ml of TGF-β1 for 48 hours, or with curcumin for up to 48 hours, respectively. Each assay was performed in triplicate. *$P$<0.05 compared to control group. **(C)** Curcumin was shown to inhibit the promoter activities and protein expression of COL1A2, PAI-1 and CTGF. Luciferase assay and Western blot analysis were performed as described in *Materials and Methods*. **(D)** Immunoblot analysis of Smad-2 phosphorylation and Smad 2/4/7 expression in the cytosol along with Smad-2/4/7 expression in the nucleus from each group 8 weeks after AB (n=3). *$P$<0.05 was obtained for the vehicle/sham values. **(E)** Curcumin blocked Smad-2 phosphorylation and Smad-2/3/4 translocation induced by TGF-β1 in cardiac fibroblasts. **(F)** The effect of p300 on collagen synthesis along with promoter activities and protein expression of COL1A2, PAI-1 and CTGF. Cells were infected with or without Ad-p300, Ad-DN-p300 or Ad-GFP for 24 hours and then incubated with 15 ng/ml TGF-β1 for up to 48 hours. Luciferase activity and Western blot were performed as described in *Materials and Methods*. **(G)** The effect of p300 on Smad-2 phosphorylation and Smad-2/3/4 translocation. **(H and I)** P300 significantly reversed the inhibitory effects of curcumin on collagen synthesis, promoter activities of COL1A2, PAI-1 and CTGF as well as Smad-2 phosphorylation and Smad-2/3/4 translocation. Cells were infected with Ad-p300 or Ad-GFP for 24 hours, treated with 25 μM curcumin for 60 minutes, and then

incubated with 15 ng/ml TGF-β1 for either 36 hours to observe [$^3$H] proline incorporation and promoter activity (H) or 60 minutes to observe Smad-2 phosphorylation and Smad-2/3/4 translocation (I). Each assay was performed in triplicate. *$P$<0.05 compared to Ad-GFP infection alone.

**Supplemental Figure 5**

(**A**)Top, quantitative results from EMSA analysis for NF-κB; Bottom, representative bands of NF-κB obtained through EMSA; **(B)** Analysis of Collagen I and III mRNA expression level in each group (n=5). Top, quantitative analysis, Bottom, representative Northern blots. Each assay was performed in triplicate. *$P$<0.05 compared to vehicle/Ad-GFP values.