Figure 1-S

- **RNAi**
  - Control IgG
  - anti-p65

- **IL-1/TNFα**
  - GFP
  - TAB2
  - TAB3
  - TAB2 + TAB3

- **Gel-retardation assay**
  - I–NF-κB supershift complex
  - I–NF-κB complex
  - Free probe

- **IB:**
  - anti-p65
  - p65
  - TAB2
  - TAB3
Supplementary material

Figure 1-S. Effects of TAB2 and TAB3 siRNAs on IL-1- and TNF-α-induced NF-κB activation

HeLa cells were transfected with annealed sense and antisense 21-mer siRNA oligonucleotides directed against Jellyfish GFP (as a control siRNA), TAB2, or TAB3 using Oligofectamine: lanes 1-3; 400 nM GFP siRNA, lanes 4-6; 200 nM TAB2 siRNA + 200 nM GFP siRNA, lanes 7-9; 200 nM TAB3 siRNA + 200 nM GFP siRNA, lanes 10-12; 200 nM TAB2 siRNA + 200 nM TAB3 siRNA. At 72 hrs post-transfection, cells were treated with IL-1 (10 ng/ml) (I) or TNFα (10 ng/ml) (T) for 30 min. Whole cell extracts were prepared from these cells and subjected to a gel retardation assay with labeled oligonucleotide probes containing NF-κB binding sites (top panel). Supershift analysis using antibody was confirmed the existence of p65 in the NF-κB complex (lanes 13 and 14). Western blot analysis was performed on extracts prepared from these cells using antibodies directed against p65 (second panel), TAB2 (third panel), or TAB3 (bottom panel).