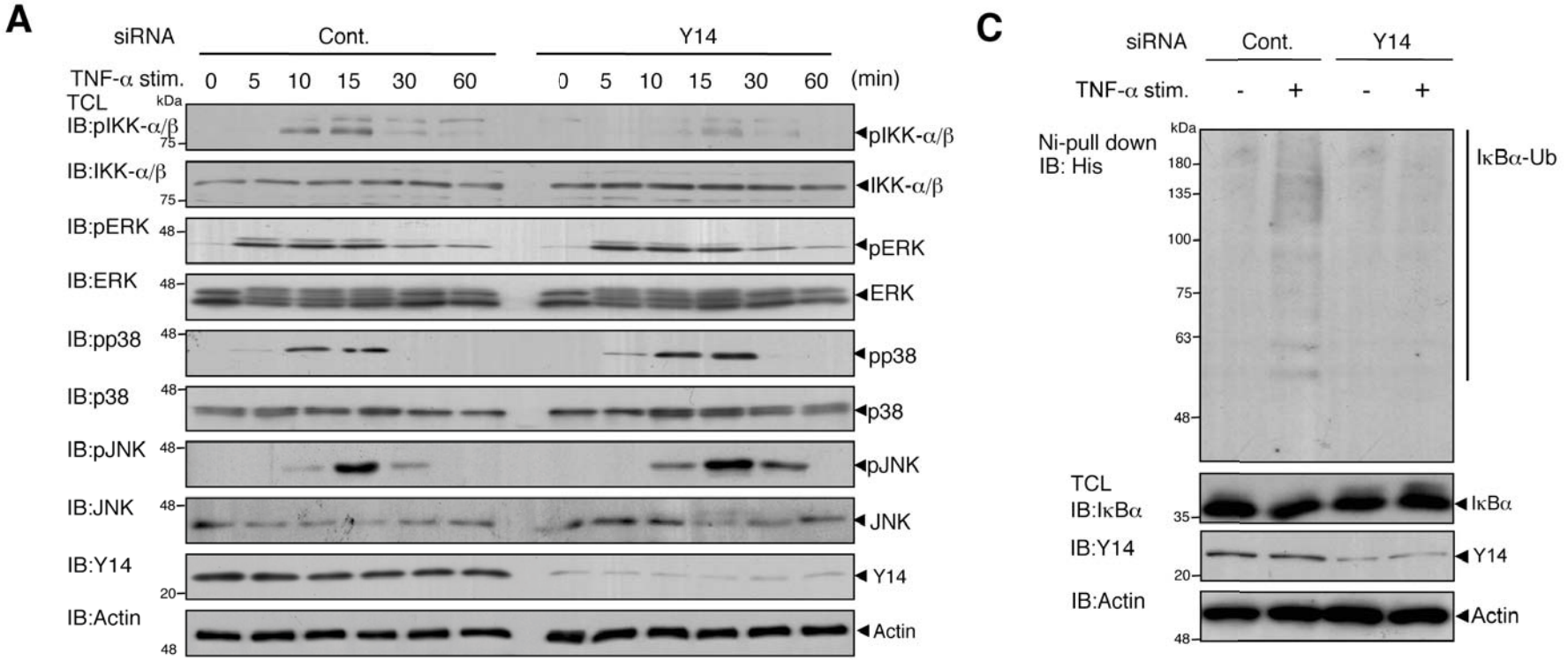
Supplemental Figure 1

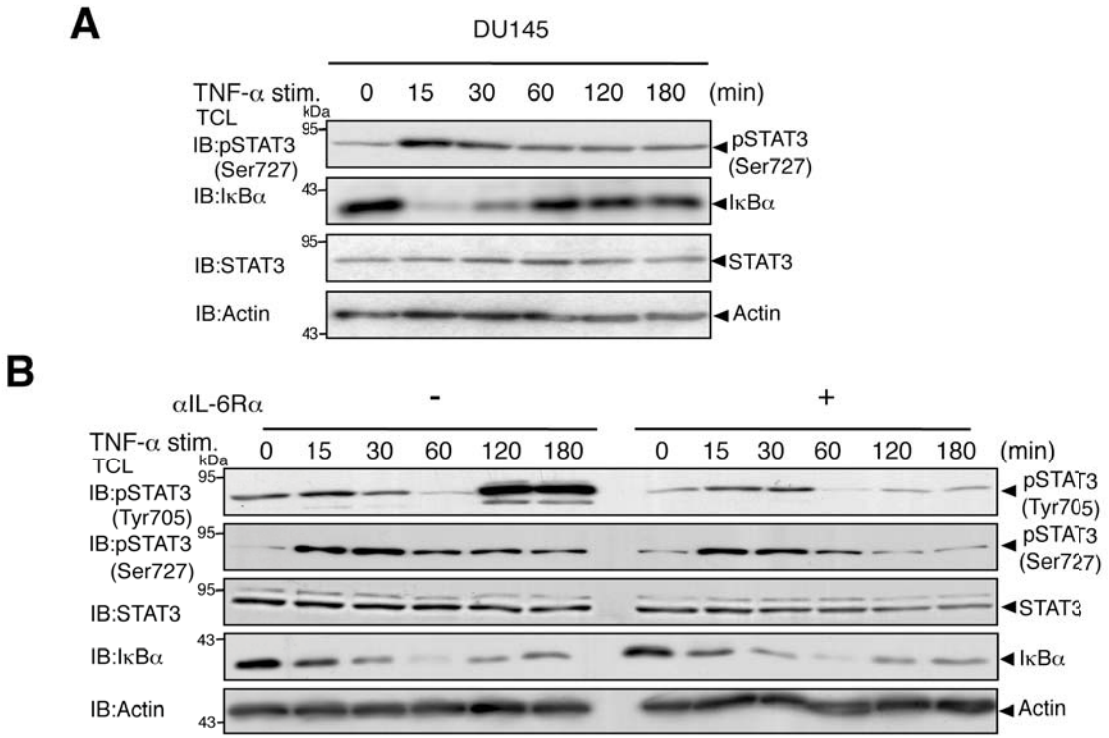


Supplemental Figure 1 A) HeLa cells in a 24-well plate were transfected with control or Y14 siRNA. The cells were then left untreated or treated with TNF-α (10 ng/ml) for the indicated periods. The cells were lysed and an aliquot of each total cell lysates (TCL) were immunoblotted with anti-pIKK-α/β (Cell Signaling Technologies), anti-IKK-α/β (Santa Cruz), anti-pERK (Santa Cruz), anti-ERK (Santa Cruz), anti-pp38 (Cell Signaling Technologies), anti-p38 (Cell Signaling Technologies), anti-pJNK (BD Biosciences), anti-JNK (Santa Cruz), anti-Y14, or anti-Actin antibody.

B) HeLa cells in a 24-well plate were transfected with control or Y14 siRNA and then transfected with FLAG-tagged TAK1 (a kind gift from Dr. Akira, Osaka University). At 36 h after transfection, the cells were then left untreated or treated with TNF-α (10 ng/ml) for the indicated periods. The cells were lysed and an aliquot of each TCL were immunoblotted with anti-pTAK1 (Cell Signaling Technologies) or anti-FLAG, anti-Y14, or anti-Actin antibody.

C) HeLa cells in a 6-cm dish were transfected with control or Y14 siRNA and then transfected with histidine-tagged ubiquitin. The cells were pretreated with MG132 (20 μM) (Wako Chemicals) for 2 h and then left untreated or treated with TNF-α (10 ng/ml) for 10 min. His-tagged proteins were purified by Ni-NTA resin (Ni-pull down) (Life Technologies). Polyubiquitination of IκBα (IκBα-Ub) was detected by blotting with anti-His antibody (Qiagen). An aliquot of each TCL were immunoblotted with anti-IκBα, anti-Y14, or anti-Actin antibody.

Supplemental Figure 2



Supplemental Figure 2 A) A human prostate cancer cell line, DU145 was maintained in DMEM containing 10% FCS. DU145 cells in a 24-well plate were then left untreated or treated with TNF-α (10 ng/ml) for the indicated periods. The cells were lysed, and an aliquot of each total cell lysate (TCL) was immunoblotted with anti-pSTAT3 (Ser727), anti-IκBα, anti-STAT3, or anti-Actin antibody.

B) HeLa cells in a 24-well plate were then left untreated or treated with TNF-α (10 ng/ml) in the absence or presence of anti-IL-6 receptor antibodies (20 ng/ml) (a kind gift from Dr. T. Kishimoto, Osaka University) for the indicated periods. The cells were lysed, and an aliquot of each TCL was immunoblotted with anti-pSTAT3 (Ser727), anti-IκBα, anti-STAT3, or anti-Actin antibody.