Supporting Information

Ea and Baltimore 10.1073/pnas.0910439106

SI Materials and Methods

Plasmids and Proteins. Fragments of human p65 cDNA were cloned by polymerase chain reaction (PCR) and then subcloned into pET14b (Novagen) and pGEX-4T1 (GE) for expression in *Escherichia coli*. His_6-tagged and GST-tagged proteins were purified using nickel columns (Qiagen) and glutathione columns (GE) according to manufacturer's instructions. P65 mutants were generated by using the PCR-based mutagenesis and verified by DNA sequencing. The ORFs of wild-type and mutant p65 were subcloned into the retrovirus expression vector pMSCV-Puro (Clontech). Bacterial and mammalian expression Set9 plasmids were kindly provided by Danny Reinberg (NYU).

Cell Culture. HeLa, human embryonic kidney (HEK) 293, and p65-deficient mouse embryonic fibroblast (MEF) cells were cultured in DMEM supplemented with 10% FCS, penicillin G (100 μg/mL), and streptomycin (100 μg/mL).

RT-PCR. HEK293 or HeLa cells were transfected with control or Set9-specific siRNAs by calcium phosphate precipitation. After 48 h, the cells were treated with TNFα for 12 h and TRIZOL LS reagent (Invitrogen) was used to isolate total RNAs. cDNAs were synthesized by using the SuperScript III Reverse Transcriptase (Invitrogen). Quantitative PCR was carried out using the 2X SYBR Green PCR Master mix (Applied Biosystems) and run on the Applied Biosystems ABI 7300 Real-Time–PCR system. All data were normalized with L32. The sequences of the primers are listed in Table S1.

1. Hoffmann A, Levchenko A, Scott ML, Baltimore D (2002) The IkappaB-NF-kappaB signaling module: Temporal control and selective gene activation. *Science* 298(5596):1241–1245.

RNA Interference and Immunoprecipitation. The sequences of siRNAs were as follows: Control siRNA (Santa Cruz Biotechnology: sc-44230); Set9a siRNA (Santa Cruz Biotechnology: sc-44094); Set9b siRNA (Invitrogen: HSS129760); p65 (Santa Cruz Biotechnology: SC-29410). The siRNA were transfected into HEK293 cells by calcium phosphate precipitation at a final concentration of 20 nM. This procedure was repeated the second day to increase the efficiency of gene silencing. The cells were harvested the next day.

For immunoprecipitation of endogenous p65, siRNAs-transfected HEK293 cells were treated with TNFα (10 ng/mL) and harvested at different time points. The cytoplasmic extracts were prepared by using the hypotonic lysis buffer containing 0.2% Nonidet P-40. The cells were incubated on ice for 20 min followed by centrifugation at 500 *g* at 4 °C for 5 min. The supernatants were collected as cytoplamic extracts. The nuclear pellets were resuspended in the whole cell lysis buffer. The nuclear extracts were then collected by centrifugation at 14 k rpm at 4 °C for 10 min. Endogenous p65 was immonoprecipitated by using 20 μL of agarose conjugated anti-p65 (F6) antibodies. After mixing end-to-end at 4 °C for 2 h, the beads were washed 3 times with PBS. Associated proteins were analyzed by immunoblotting with different antibodies as indicated.

Electrophoretic Mobility Shift Assay (EMSA) and ChIP Assay. EMSA was performed as previously described (1). ChIP assay was carried out using EZ-ChIP kit (GE) according to manufacturer's instructions. The sequences of the primers are listed in Table S2.

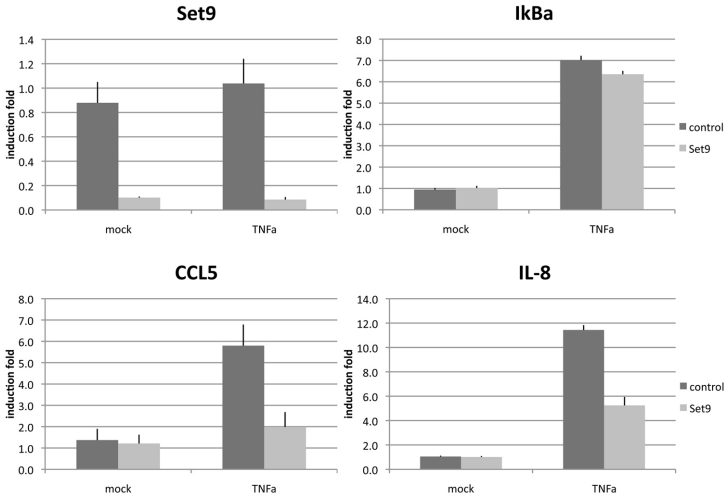

Fig. S1. HeLa cells transfected with control or Set9-specific siRNAs were stimulated with or without TNFα for 12 h. Expression of IκBα, CCL5, and IL-8 was measured by RT-PCR.

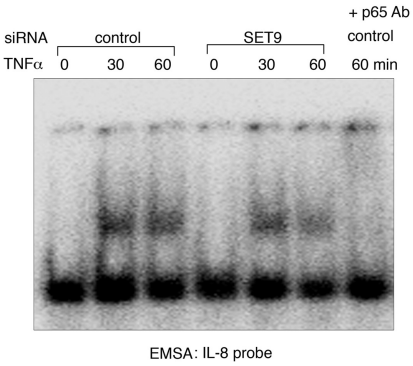

Fig. S2. Five micrograms of the nuclear extracts from Fig. 3*C* were subjected to EMSA using oligos containing the κB-site from IL-8 promoter.

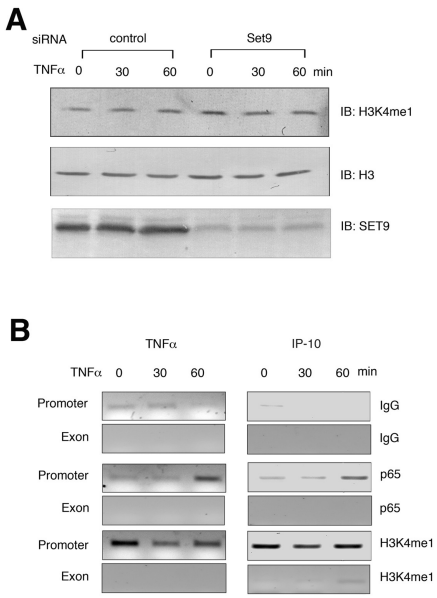

Fig. S3. (*A*) Whole cell extracts were prepared from control or Set9-specific siRNAs transfected HEK293 cells. Cell lysates were immunoblotted with indicated antibodies. (*B*) HeLa cells were treated with TNFα for the indicated time periods, and ChIP assays were performed with control IgG, anti-p65, and H3K4me1 antibodies. ChIP-enriched samples were analyzed by regular PCR using IP-10, TNFα promoter, and exon primers.

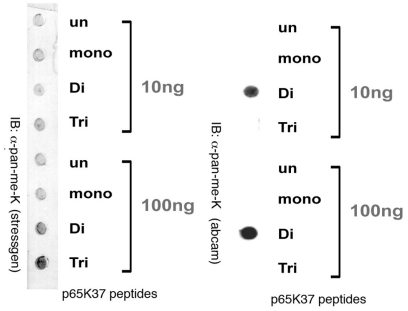

Fig. S4. Dotblot analysis with α-pan-me-K antibodies (Stressgen, *Left* panel; Abcam, *Right* panel) on p65 peptides.

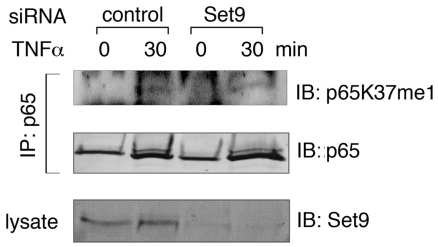

Fig. S5. U2OS cells transfected with control or Set9-specific siRNAs were stimulated with or without TNFα for the indicated time periods. P65 immunoprecipitates from nuclear extracts were immunoblotted with p65K37me1 (*Top*) or p65 (*Middle*) antibodies. The efficiency of RNA interference was verified by immunoblotting (*Bottom*).

Table S1. RT-PCR primer sequences for mRNA analysis

Gene	Forward primer	Reverse primer
hL32	AGCTCCCAAAAATAGACGCAC	TTCATAGCAGTAGGCACAAAGG
hIκBα	CTCCGAGACTTTCGAGGAAATAC	GCCATTGTAGTTGGTAGCCTTCA
hIP-10	CTGACTCTAAGTGGCATT	TGATGGCCTTCGATTCTG
hTNFα	GCCCAGGCAGTCAGATCATCT	TTGAGGGTTTGCTACAACATGG
hp65	TCTGCTTCCAGGTGACAGTG	ATCTTGAGCTCGGCAGTGTT
hSet9	TCCAGTGCTGGAGAAGGACT	GGTTATAGGGCTCAGGCACA
hCCL5	CCCAGCAGTCGTCTTTGTCA	TCCCGAACCCATTTCTTCTCT
hIL-8	AGCTCTGTCTGGACCCCAAG	GAATTCTCAGCCCTCTTCAAAAAC
mL32	AACCCAGAGGCATTGACAAC	ATTGTGGACCAGGAACTTGC
mTNFα	CTACTCCCAGGTTCTCTTCAA	GCAGAGAGGAGGTTGACTTTC
mIP-10	AGGACGGTCCGCTGCAA	CATTCTCACTGGCCCGTCAT
mIκBα	CTGCAGGCCACCAACTACAA	CAGCACCCAAAGTCACCAAGT

Table S2. PCR primer sequences for ChIP analysis

Gene	Promoter/exon	Forward primer	Reverse primer
hIP-10	Pro	AGCCAGCAGGTTTTGCTAAG	TCAGAAAACGTGGGGCTAGT
	Exon	GACCAATGATGGTCACCAAA	GCAGGGTCAGAACATCCACT
hTNFα	Pro	AACCGAGACAGAAGGTGCAG	TGTGCCAACAACTGCCTTTA
	Exon	GGAGCCAGCTCCCTCTATTT	GGCTACATGGGAACAGCCTA
hIκBα	Pro	GCTCAGGGTTTAGGCTTCTT	TATAAACGCTGGCTGGGGAT
	Exon	CCTGGAGCCTCTGCTATTTG	GTCTCGGAGCTCAGGATCAC