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Supplementary Materials for

Negative Feedback in Noncanonical NF-κB Signaling Modulates NIK Stability Through IKKα-Mediated Phosphorylation

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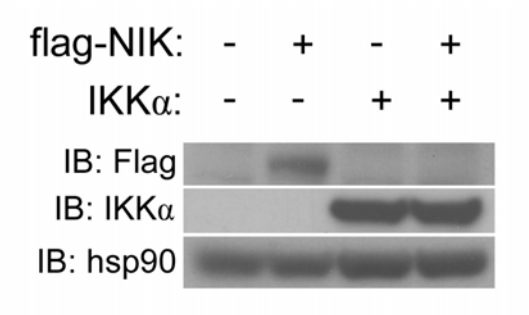

Fig. S1. Ectopic expression of IKKα results in destabilization of ectopically expressed NIK. *IKKα*$^{-/-}$ fibroblasts were retrovirally transduced with viruses derived from pBABE-empty vector (-) or pBABE-IKKα (IKKα), selected with puromycin, and subsequently retrovirally transduced with viruses derived from pBABE-empty vector (-) or pBABE-NIK (NIK). Data shown are representative of three experiments.

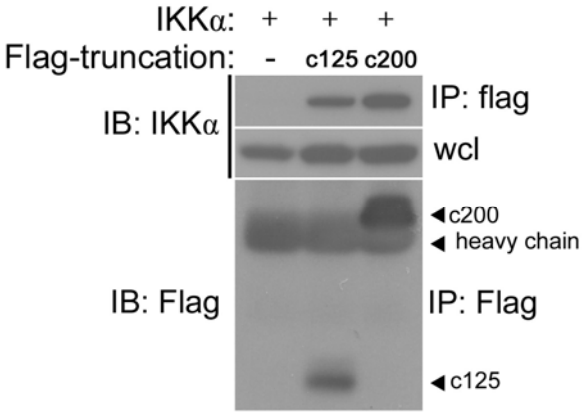

Fig. S2. Truncated mutants of NIK continue to interact with IKKα. HEK 293T cells were cotransfected with plasmids encoding FLAG-tagged NIK truncation mutants c125 (residues 817 to 942) or c200 (residues 742 to 942) and IKKα. Samples were subjected to immunoprecipitation reactions with M2-conjugated agarose and analyzed by Western blotting as indicated. Data shown are representative of three experiments.

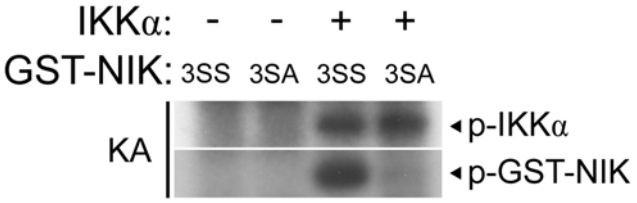

Fig. S3. IKKα phosphorylates Ser^{809}, Ser^{812}, and Ser^{815} of NIK. Immunopurified IKKα was subjected to in vitro kinase assay (KA) with GST fusion proteins containing fragments of NIK corresponding to amino acid residues 700 to 828 in which either serine residues 809, 812, and 815 were left unchanged (3SS) or were mutated to alanine residues (3SA). To enhance the radioactive signal associated with phosphorylation of residues Ser^{809}, Ser^{812}, and Ser^{815}, we mutated the six N-terminal and five C-terminal serine or threonine residues adjacent to residues 809, 812, and 815 to alanine in both fusion proteins. Data shown are representative of three experiments.

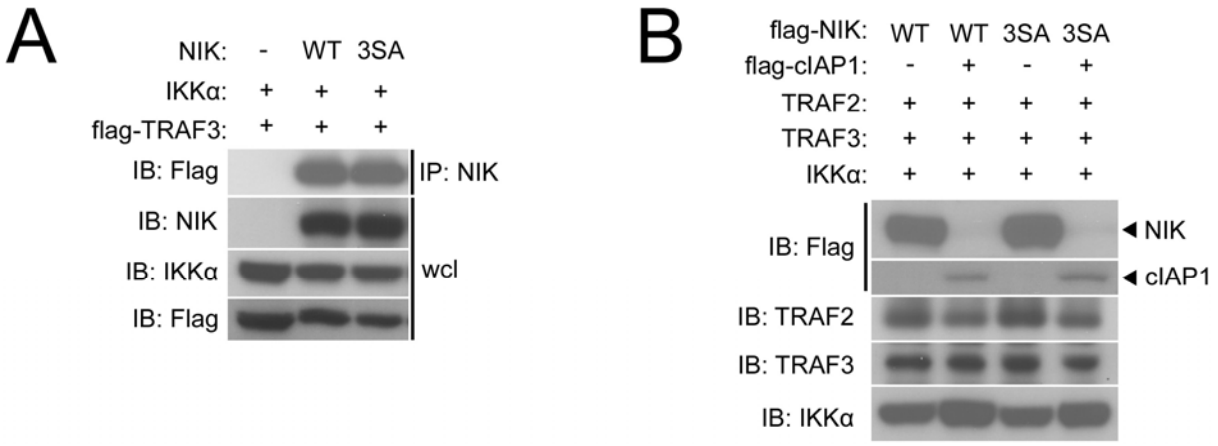

Fig. S4. NIK-3SA does not affect the interaction between wild-type NIK and TRAF3 or its degradation by the TRAF-cIAP complex. (**A**) NIK-WT and NIK-3SA interact comparably with TRAF3 in the presence of IKKα, as evidenced by their respective immunoprecipitation with TRAF3 from HEK 293T cells cotransfected with plasmid encoding IKKα. (**B**) NIK-WT and NIK-3SA are degraded comparably by the TRAF-cIAP complex in the presence of IKKα as evidenced by their respective degradation when all components of the TRAF-cIAP complex (TRAF2, TRAF3, cIAP1) are present, together with IKKα, in transfected HEK 293T cells. Data shown are representative of three experiments.

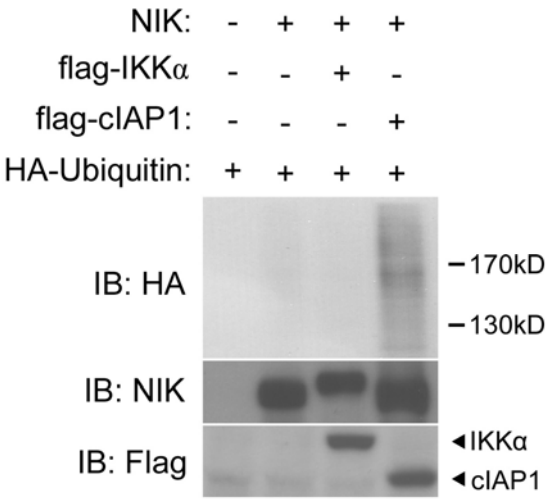

Fig. S5. Ubiquitination of NIK is not enhanced by the presence of IKKα in HEK 293T cells. HEK 293T cells were cotransfected with plasmids encoding NIK and HA-tagged ubiquitin (HA-Ub) with or without plasmids encoding IKKα or cIAP1. Samples were then subjected to immunoprecipitation with an antibody against NIK, washed extensively, and analyzed by Western blotting to detect the HA tag. Coexpression of cIAP1 was used as a positive control for the ubiquitination of NIK. Data shown are representative of three experiments.