Supporting Information to:

Expanding the Interactome of the Noncanonical NF-κB Signaling Pathway

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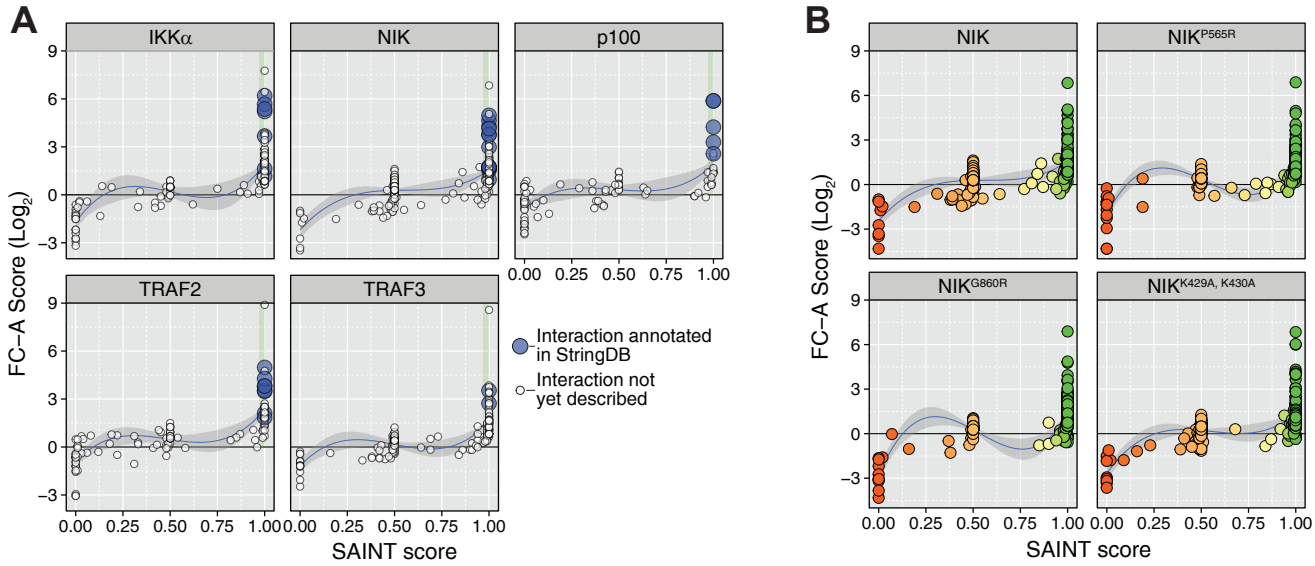
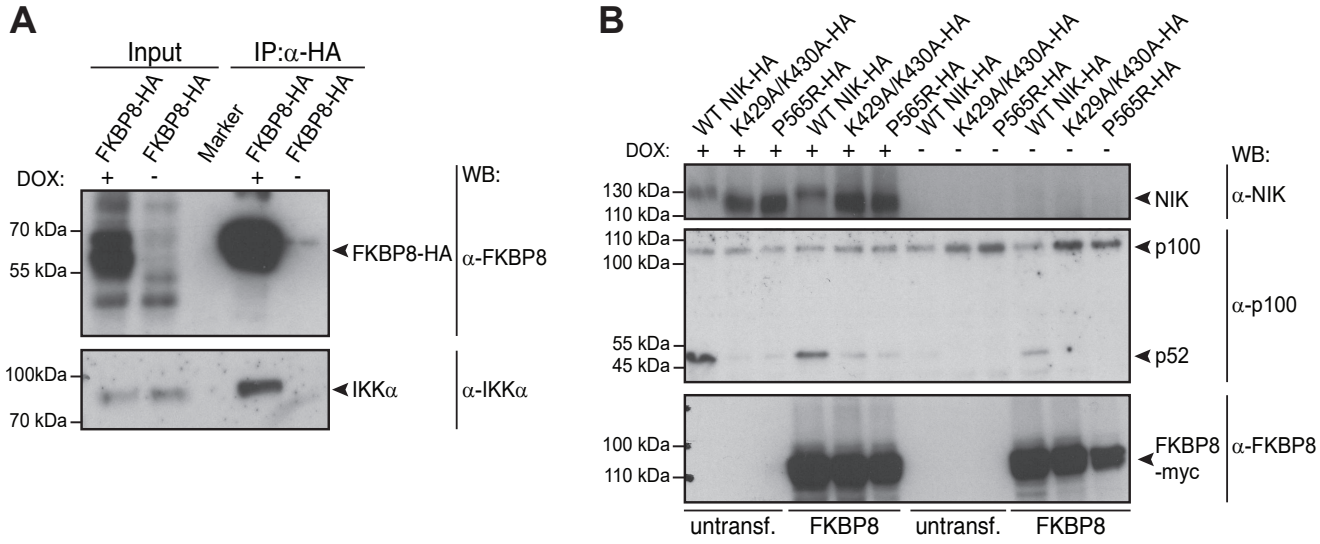
Figure S1



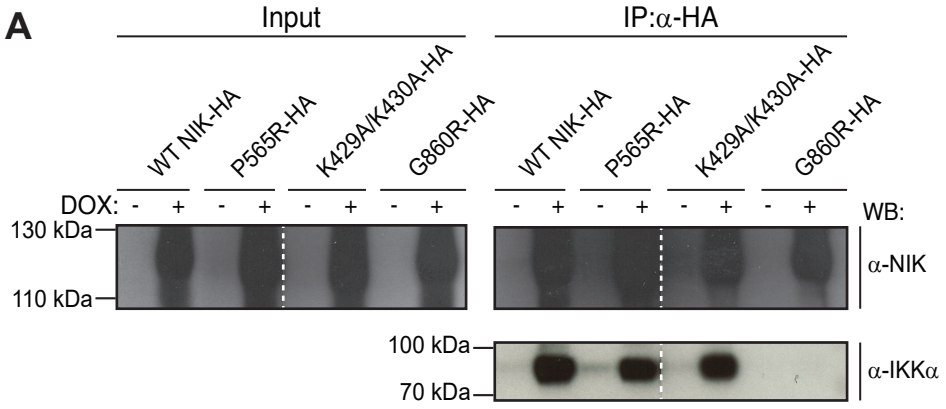
Supplementary Figure 1. Distribution of interactors of SH-tagged proteins plotted by SAINT and CRAPome FC-A score. The areas colored in green contain interactors with a SAINT threshold of 1 and a CRAPome score above 2.5. (A) Plots for SH-tagged proteins IKKα, NIK, NF-κB2/p100, TRAF2 and TRAF3. Blue dots depict interactions annotated in the STRING database (B) Plots for SH-tagged NIK variants NIK^{wt}, NIK^{P565R}, NIK^{G860R}, $NIK^{K429A,K430A}$

Figure S2



Supplementary Figure 2. The interactor FKBP8. (A) Co-IP using doxycycline-inducible cell lines HA-FKBP8. Uninduced samples were used as control. Immunoprecipitation with anti-HA agarose beads indicated an association of HA-FKBP8 with endogenous IKKα. (B) Co-expression of HA-NIK variants (by doxycyclin induction) and myc-tagged FKBP8 (by transient expression vector transfection) in HEK293 FlpIn lines. Unprocessed p100 and processed p52 was monitored in the presence or absence of NIK and FKBP8 overexpression by immunoblot of whole cell lysates. No differential p100 processing was observed when functional NIK was present together with FKBP8. This immunoblot represents an extended version of the blot in Figure 1B.

Figure S3



Supplementary Figure 3. Interaction of NIK variants with IKKα. (A) Co-IP using doxycycline-inducible cell lines HA-NIK and mutant variants of NIK. Uninduced samples were used as control. Immunoprecipitation with anti-HA agarose beads indicated an association of HA-NIK^{wt}, NIK^{P565R}, and NIK^{K429A,K430A} , but not NIK^{G860R} with endogenous IKKα.

Supplementary Material and Methods

Sequences of the plasmid vectors used in this study:

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pTO-SII-HA-GW (NfkappaB2-p100 N terminal)

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pTO-SII-HA-GW (NIK-K429A/K430A N terminal)

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pTO-SII-HA-GW (NIK-G860R N terminal)

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pCS2-6myc-GW (FKBP8 N terminal)

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pCS2-6myc-GW (FBXW7 N terminal)

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