

Supplementary Information

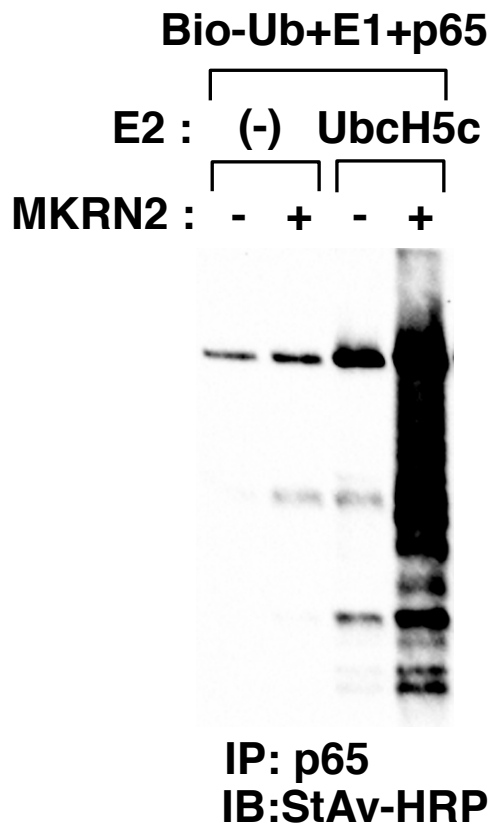
MKRN2 is a novel ubiquitin E3 ligase for the p65 subunit of NF- κ B and negatively regulates inflammatory responses

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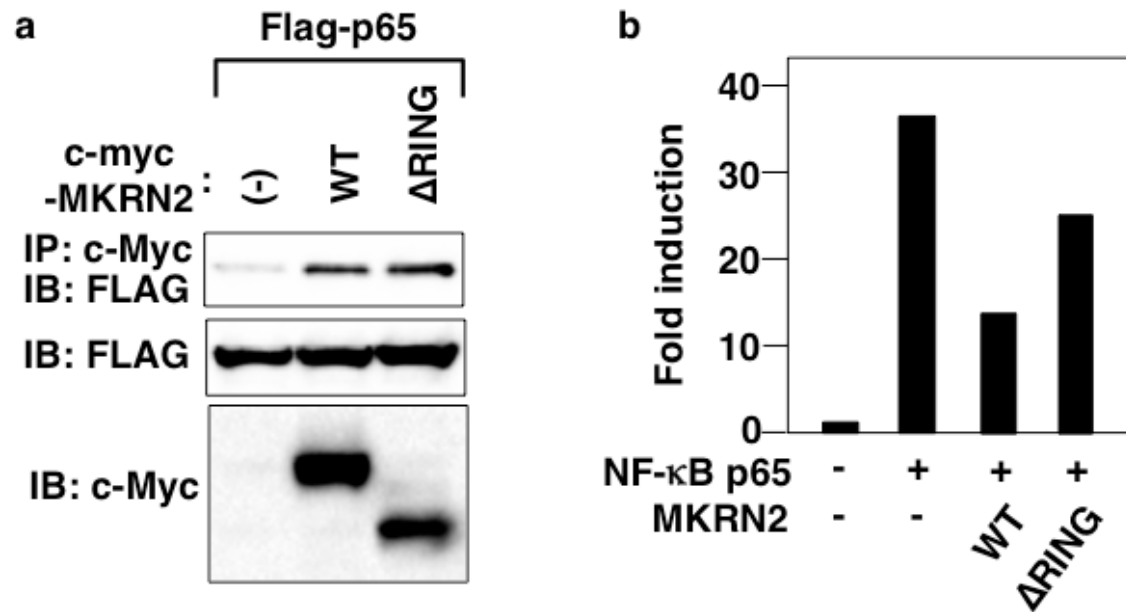
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Supplementary Fig. 1. MKRN2 is a ubiquitin E3 ligase targeting p65.

MKRN2 promotes ubiquitination of p65 in vitro. MKRN2 proteins, immunoprecipitated with anti-FLAG from 293T cells transfected with a FLAG-tagged MKRN2, then eluted by 3xFLAG peptide, were incubated in vitro with ubiquitin components as indicated including recombinant p65. Ubiquitinated p65 was detected by immunoprecipitation with anti-p65 and immunoblot with HRP-Conjugated Streptavidin.



Supplementary Fig. 2. MKRN2 mutant lacking RING finger domain binds to p65 and suppresses p65 transactivation.

(a) 293T cells were transfected with a FLAG-tagged p65 expression plasmid along with or without c-Myc-tagged MKRN2 or the MKRN2 mutant lacking the RING finger domain (ΔRING). Whole cell extracts were immunoprecipitated with anti-c-Myc, and immunoblotted with anti-Flag. Western blots are representative of at least three independent experiments. (b) Luciferase activity in MEF cells transfected with an ELAM-1-luc with or without plasmids encoding p65 in the absence or presence of expression plasmids encoding wild-type or ΔRING MKRN2. Data are representative of at least three independent experiments.