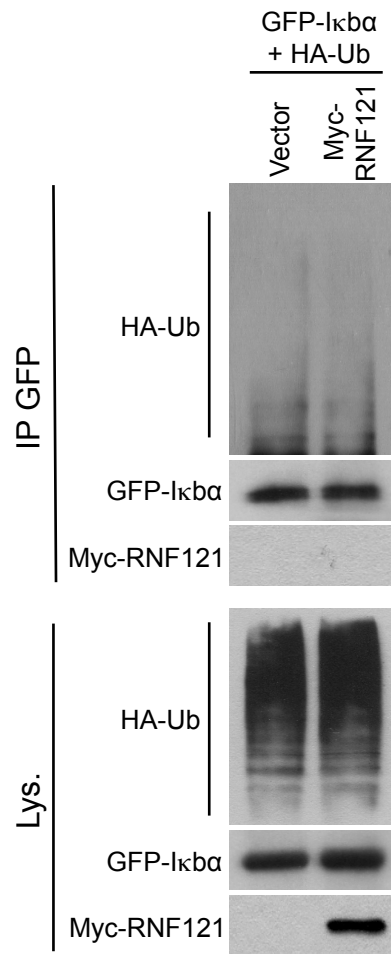
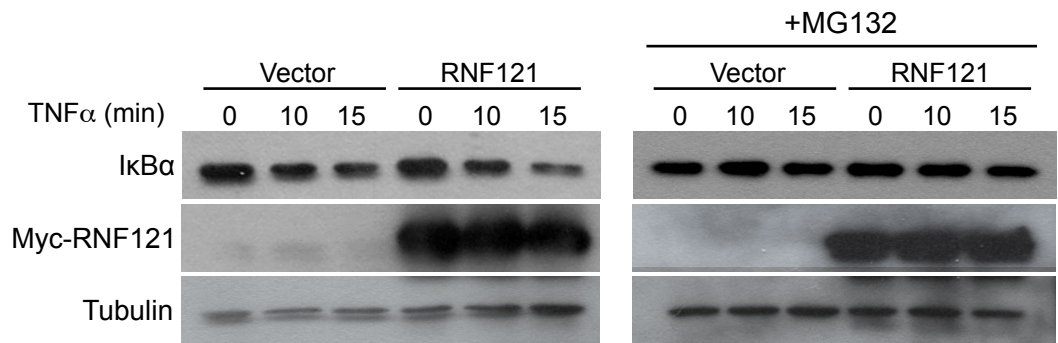
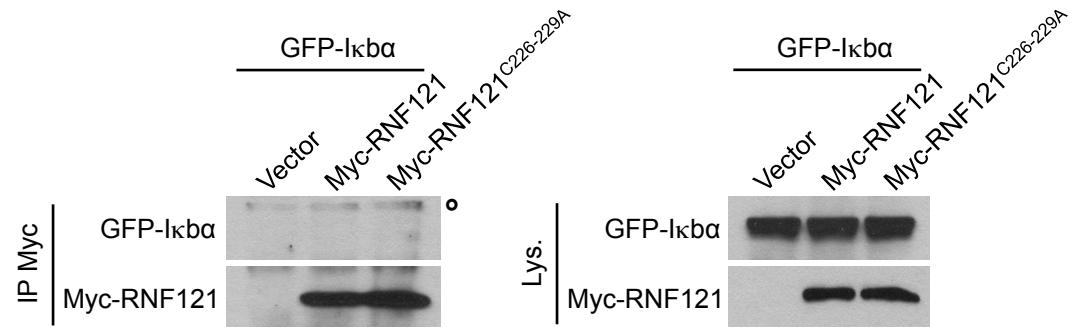


A**B****C**

Additional File 7

Additional file 7. RNF121 does not directly ubiquitinate IκBα.

(A) HEK293 cells were transfected with a plasmid encoding myc-tagged RNF121 or with an empty vector as a control and with plasmids encoding GFP-tagged IκBα and HA-tagged ubiquitin (Ub). Twenty-four hours after transfection, IκBα was immunoprecipitated with an anti-GFP and its ubiquitination was assessed with an anti-HA (upper panel). Expression of the proteins was examined by immunoblotting with the indicated antibodies in the lysate (Lys.) (lower panel). (B) HEK293 cells were transfected with a plasmid encoding myc-tagged RNF121 or with an empty vector as a control. 24 hrs later, cells were stimulated with TNFα (10 ng/ml) for the indicated times in the presence or the absence of the proteasome inhibitor MG132 (10 μM) and cell extracts were analyzed by immunoblotting as indicated. (C) HEK293 cells were transfected with a plasmid encoding myc-tagged RNF121 or with an empty vector as a control and with a plasmid encoding GFP-tagged IκBα. Twenty-four hours after transfection, myc-RNF121 was immunoprecipitated with an anti-myc antibody. Samples were then analyzed by immunoblotting. ° indicates a non specific band.