Figure S5. MIB1 mediates ubiquitination of IκBα.
(A) The amount of p65-bound IκBα was increased in *MIB1*^{-/-} cells. The indicated cells (4x10^{5}) were treated with MG132 (50 μM) for the indicated times or left untreated. Cell lysates were immunoprecipitated with anti-p65. The immunoprecipitates were analyzed by immunoblot with anti-p65 or anti-IκBα. The expression levels of the endogenous proteins were detected with the indicated antibodies.
(B) MIB1 is associated with IκBα. 293 cells (1x10^{6}) were transfected with the indicated plasmids (5 μg each). Twenty-four hr after transfection, the cell lysates were immunoprecipitated with control mouse IgG or anti-MIB1. The immunoprecipitates were analyzed by immunoblot with anti-IκBα (upper panel) or anti-MIB1 (lower panel).
(C) MIB1 causes the ubiquitination of IκBα. 293 cells (1x10^{6}) were transfected with the indicated plasmids. Twenty-four hr after transfection, immunoprecipitation and immunoblots were performed with the indicated antibodies. The expression levels of the transfected proteins were examined with anti-HA or anti-Flag.
(D) Knockout of MIB1 impairs the ubiquitination of endogenous IκBα. Wild-type or *MIB1*^{-/-} HCT116 cells (5x10^{7}) were treated with MG132 (50 μM) for 4 hr or left untreated. Cell lysates were immunoprecipitated with anti-IκBα. The immunoprecipitates were analyzed by immunoblots with anti-ubiquitin. The expression levels of the endogenous proteins were detected by immunoblots with the indicated antibodies.