Supporting Information

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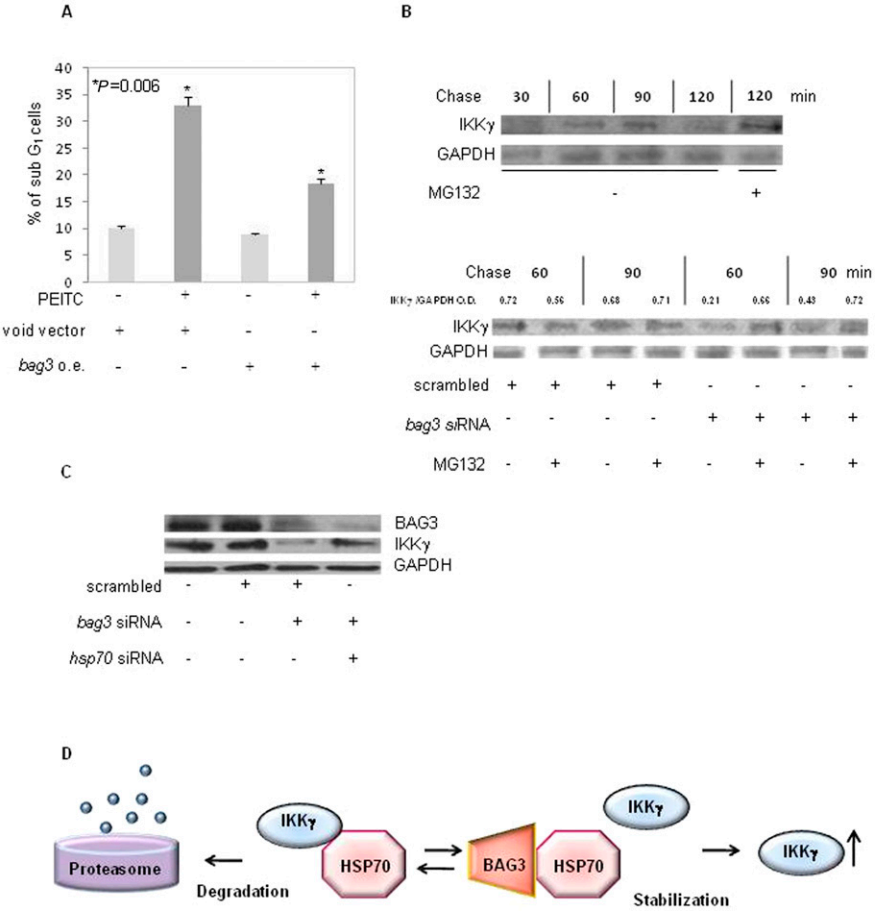

Fig. S1. (*A*) M14 cells were transfected with either a *bag3* overexpressing (o.e.) vector or a void vector. After 48 h, cells were treated with PEITC (25 μM). After an additional 18 h, apoptosis was evaluated as the percentage of sub-G_1 cells by propidium iodide staining in flow cytometry. (*B*) M14 cells were transfected with *bag3* siRNA or a control scrambled RNA (200 nM); after 72 h, cells were labeled for 30 min with 150 μCi/mL [^{35}S]methionine and [^{35}S]cysteine (ICN Biochemicals), washed, and chased for the indicated time with fresh medium containing unlabeled amino acids and cycloheximide (1 μM; Sigma) in presence or absence of the proteasome inhibitor MG132 (1 μM) (Alexis Biochemicals). Cell lysates were obtained and precleared using protein A-Sepharose (Amersham Biosciences), and proteins were immunoprecipitated with anti-IKKγ or anti-GAPDH antibody (Santa Cruz Biotechnology). Immune complexes were resolved by SDS/PAGE, gels were dried and autoradiographed, and band intensity was quantified by densitometry. (*C*) M14 cells were transfected with *bag3* siRNA, a control scrambled RNA (200 nM) and/or an *hsp70*-specific siRNA (target sequence: 5′-AAG AAC CAG GUG GCG CUG AAC-3′). After 96 h, total cell lysates were analyzed for their content of IKKγ and BAG3; an antibody recognizing GAPDH was used to monitor equal loading conditions. (*D*) Proposed mechanism for BAG3 modulation of HSP70 association with IKKγ and its regulation.