Cell

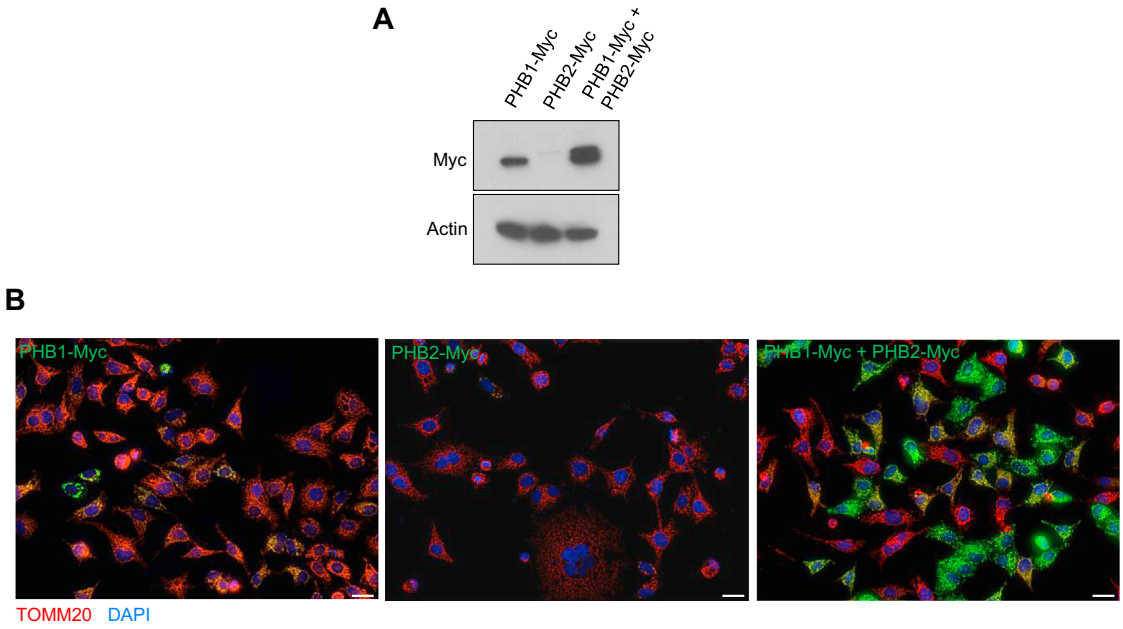

Figure S1. Detection of Exogenously Expressed PHB1-Myc and PHB2-Myc in HeLa/Parkin Cells, Related to Figure 1

(A and B) HeLa/Parkin cells were transfected with equal amounts of PHB1-Myc, PHB2-Myc or PHB1-Myc + PHB2-Myc and then subjected to (A) western blot analysis or (B) immunostaining with a monoclonal anti-Myc antibody. Scale bars, 30 μM.

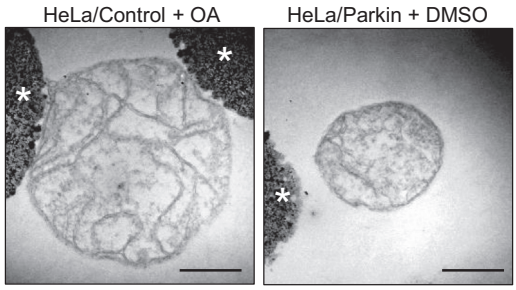

Figure S2. Representative Electron Micrographs of Immunoprecipitated Mitochondria from Cells in the Absence of Mitophagy Induction, Related to Figure 3

HeLa/Control cells were treated for 4 hr with OA (oligomycin, 2.5 μM; antimycin A, 250 nM) and HeLa/Parkin cells were treated for 4 hr with DMSO. Asterisk, Dynabead. Scale bar, 500 nm.

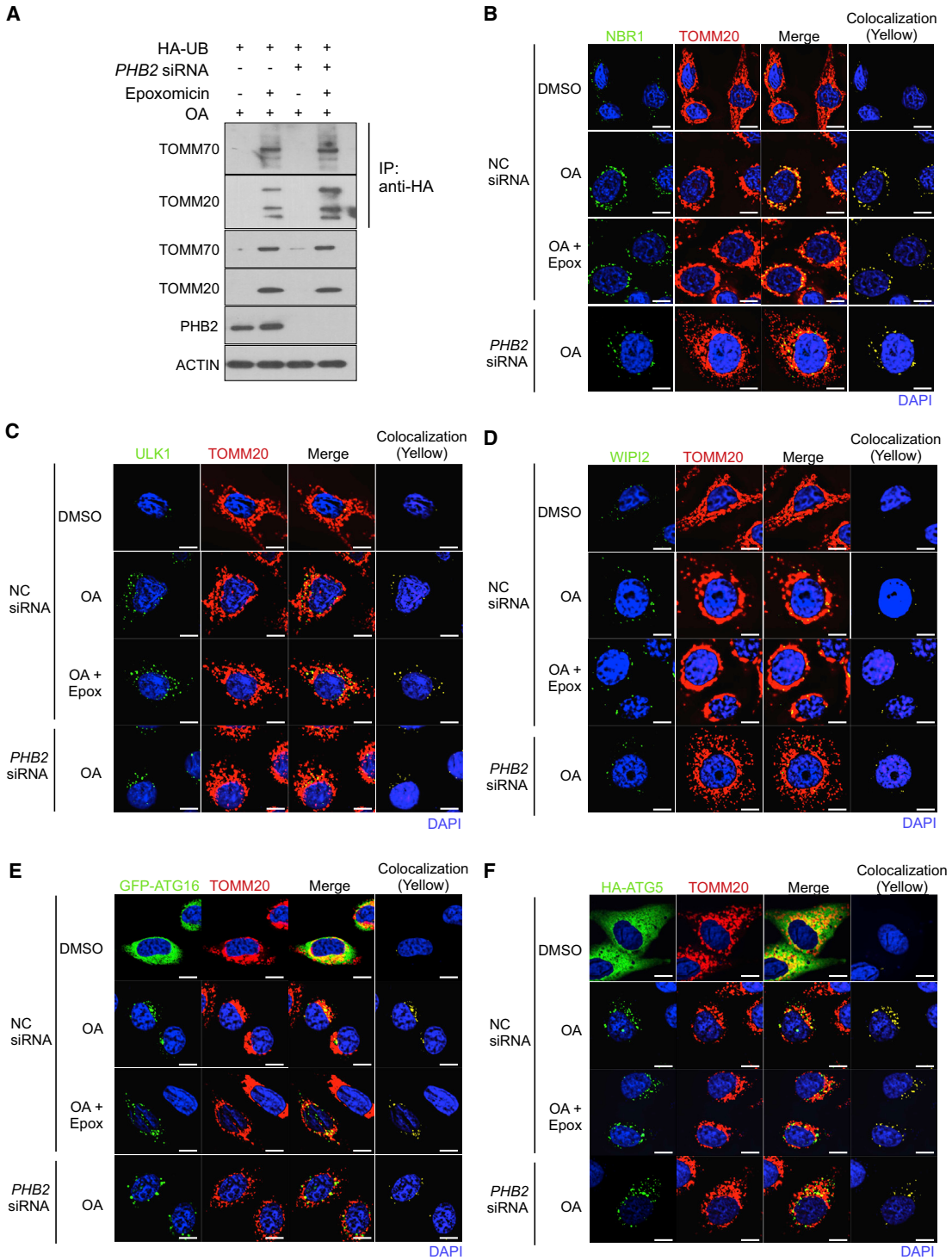

Figure S3. PHB2 Knockdown and Proteasomal Inhibition May Not Block Early Stages of Mitophagy, Related to Figure 4

(A) Western blot analysis of ubiquitination of indicated mitochondrial outer membrane proteins. HeLa/Parkin cells were co-transfected with control siRNA or *PHB2* siRNA and HA-Ubiquitin for 48 hr and then treated with OA in the presence or absence of 100 nM epoxomicin for 8 hr. Anti-HA immunoprecipitates were blotted with indicated antibodies. WCL, whole cell lysates.

(B–F) Representative light micrographs images showing co-localization of endogenous NBR1 (B), ULK1 (C), WIPI2 (D), or overexpressed GFP-ATG16 or HA-ATG5 with TOMM20 in HeLa/Parkin cells. Cells were transfected with control or *PHB2* siRNA for 48 hr and then treated with DMSO, OA or OA + epoxomycin for 2 hr. The DAPI channel was used to create nuclear masks as described in the STAR Methods. NC, non-coding siRNA. Epox, epoxomycin. Scale bars, 10 μM.