Figure S1

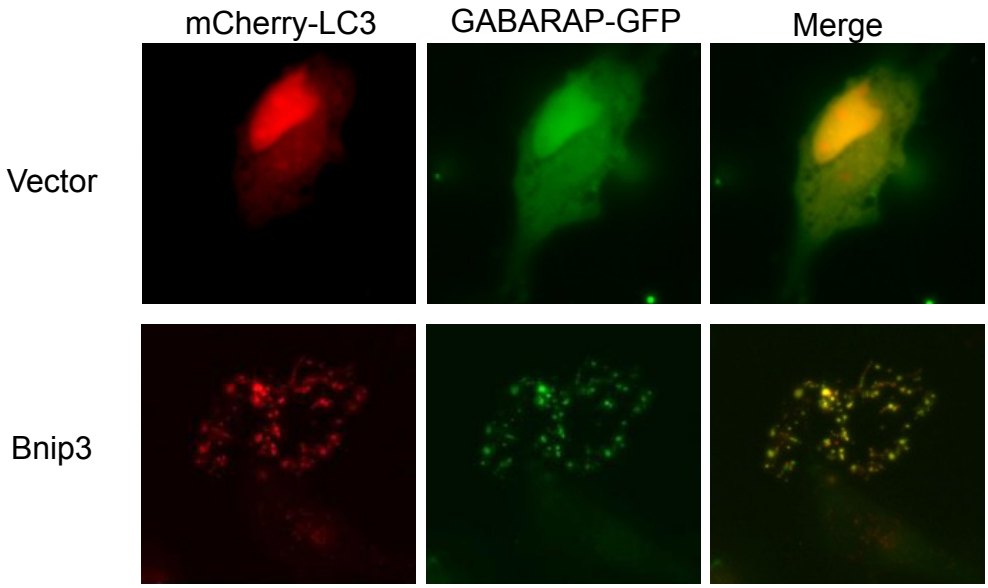

Figure S1. GABARAP-GFP co-localizes with mCherry-LC3-positive autophagosomes. HeLa cells were co-transfected with vector or Bnip3 plus mCherry-LC3 and GABARAP-GFP. Cells were analyzed by fluorescence microscopy 24 h post-transfection for formation of autophagosomes.

Figure S2

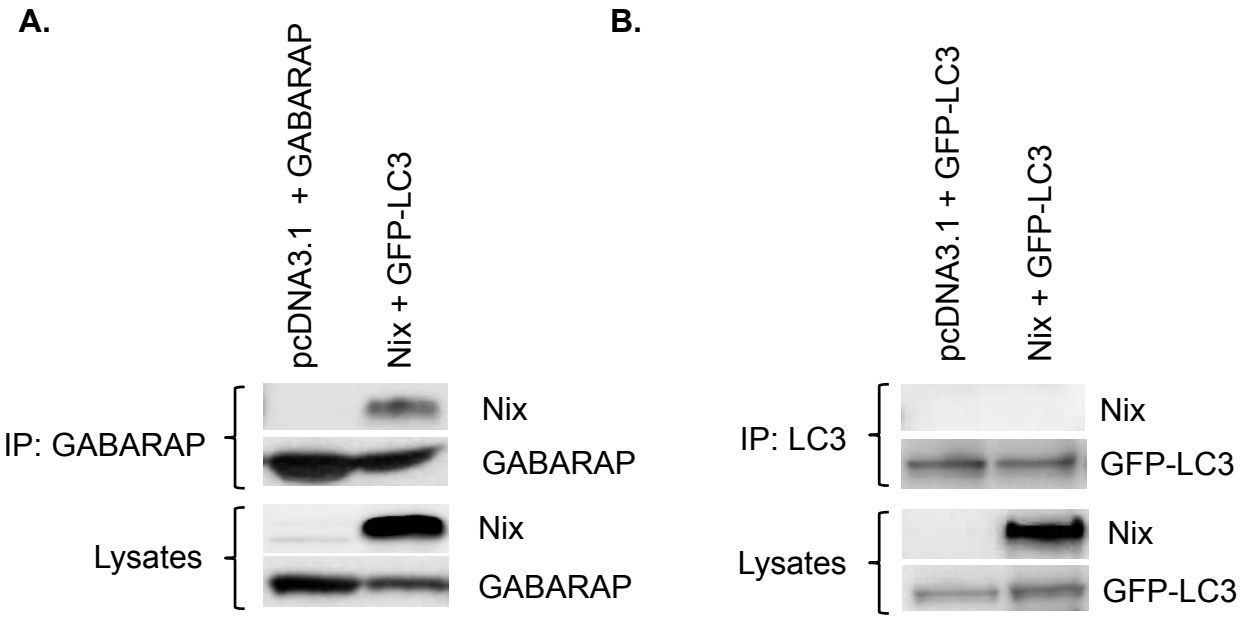

Figure S2. Nix interacts with GABARAP but not LC3. **A.** Nix co-immunoprecipitates with GABARAP in HeLa cells. **B.** Nix does not co-immunoprecipitate with LC3.

Figure S3

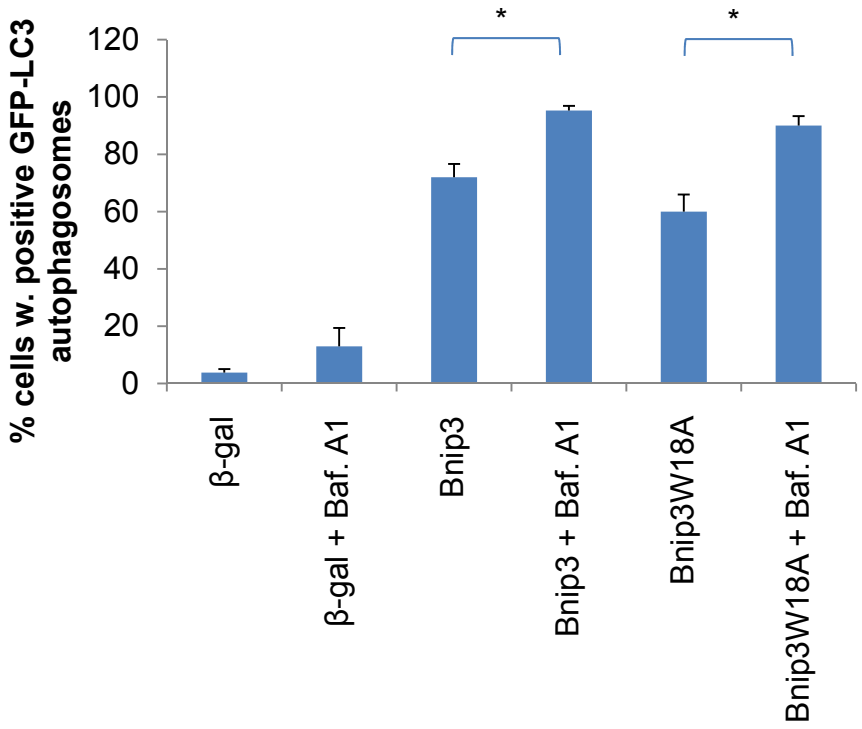

Figure S3. Bnip3W18A does not inhibit autophagic flux in HeLa cells. HeLa cells were transiently transfected with vector, Bnip3, or Bnip3W18A plus GFP-LC3. After 48 hrs, the cells were treated with 50nM Bafilomycin A1 (Baf. A1) for 2 h before fixation. Quantitation of autophagy in the presence and absence of Baf showed an increase in autophagy in the presence of Baf. A1 (*$p<0.05$, $n=3$).

Figure S4

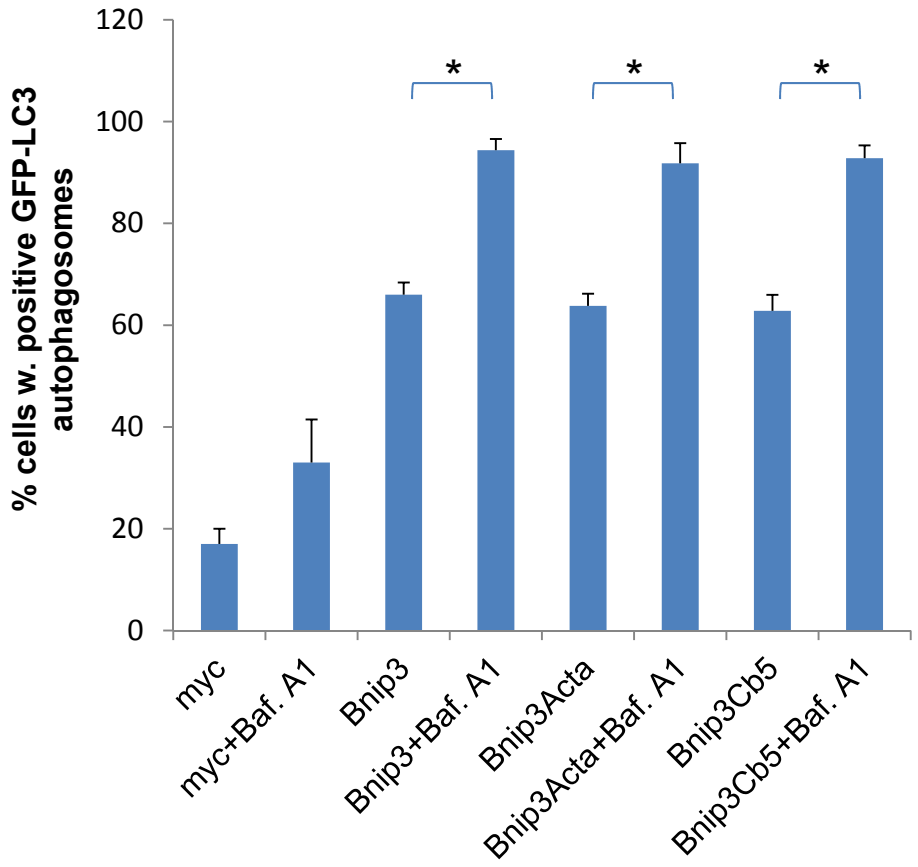

Figure S4. Mito and ER-targeted Bnip3 do not inhibit autophagic flux in HeLa cells. HeLa cells were transfected for 48 h and treated with 50nM Bafilomycin A1 (Baf. A1) for 2 h before fixation. Quantitation of autophagy in the presence and absence of Baf. A1 showed an increase in autophagy in the presence of Baf. A1 (*$p<0.05$, n=3).

Figure S5

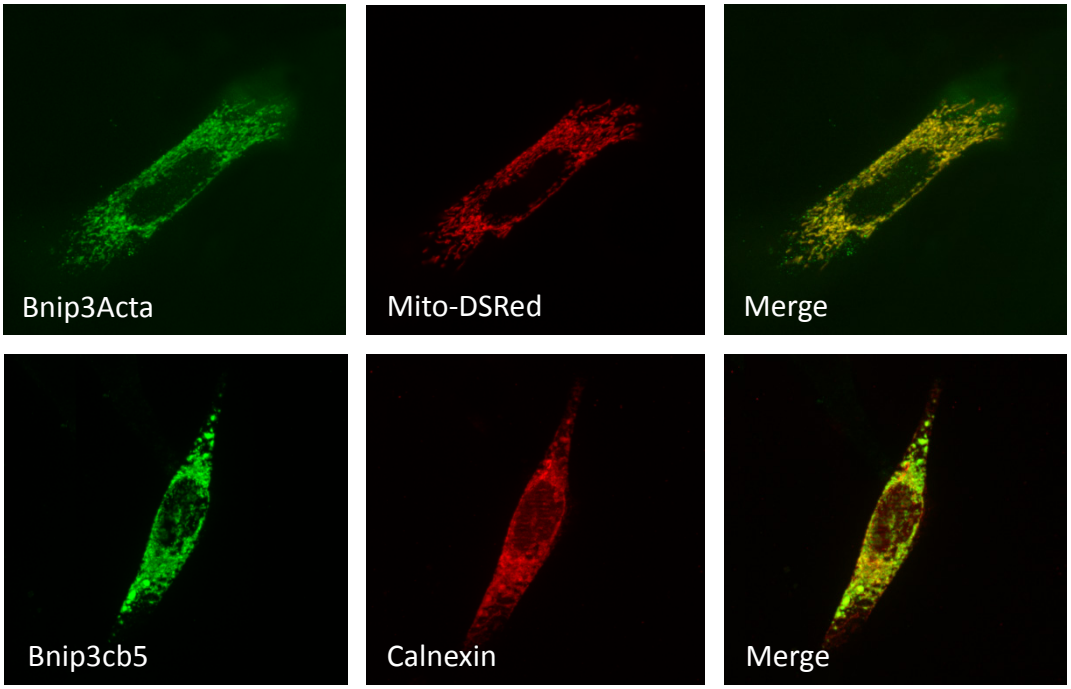

Figure S5. Bnip3Acta and Bnip3cb5 localize to the mitochondria and endoplasmic reticulum, respectively. HeLa cells were transfected with Bnip3Acta plus Mito-DsRed or Bnip3cb5. After 24 h, cells were fixed and stained with anti-Bnip3 and anti-calnexin.

Figure S6

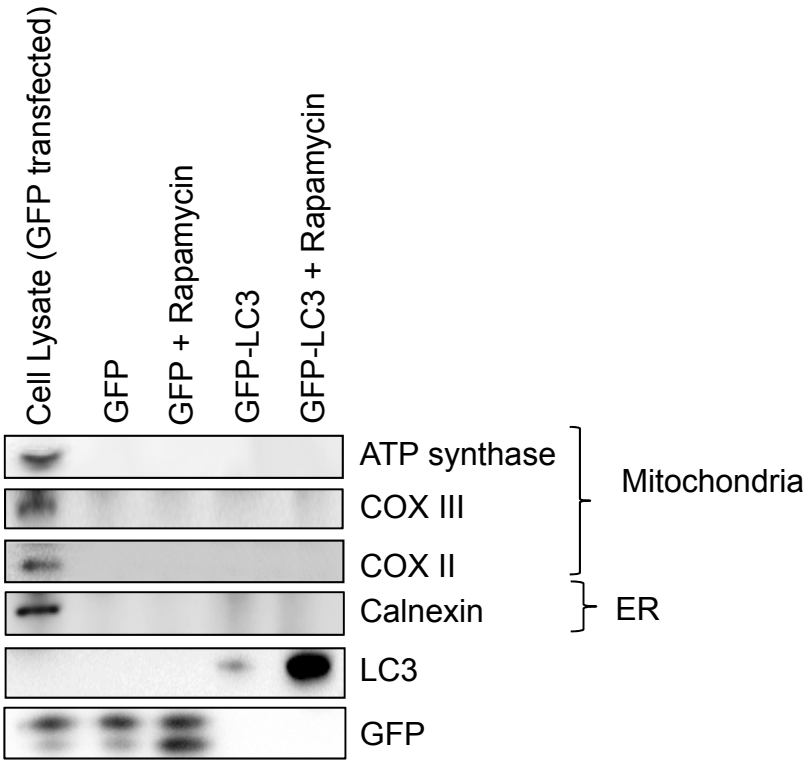

Figure S6. Isolation of autophagosomes from HeLa cells using anti-GFP linked to magnetic beads. Cells transfected with GFP or GFP-LC3 were treated with 5 μM rapamycin for 3 hrs. Western blot analysis of isolated autophagosomes showed an increase in the number of GFP-LC3 positive autophagosomes in rapamycin treated cells. Mitochondrial and ER proteins were not detected in the isolated autophagosomes. Cell lysates transfected with GFP served as a positive control for the Western blot.

Figure S7

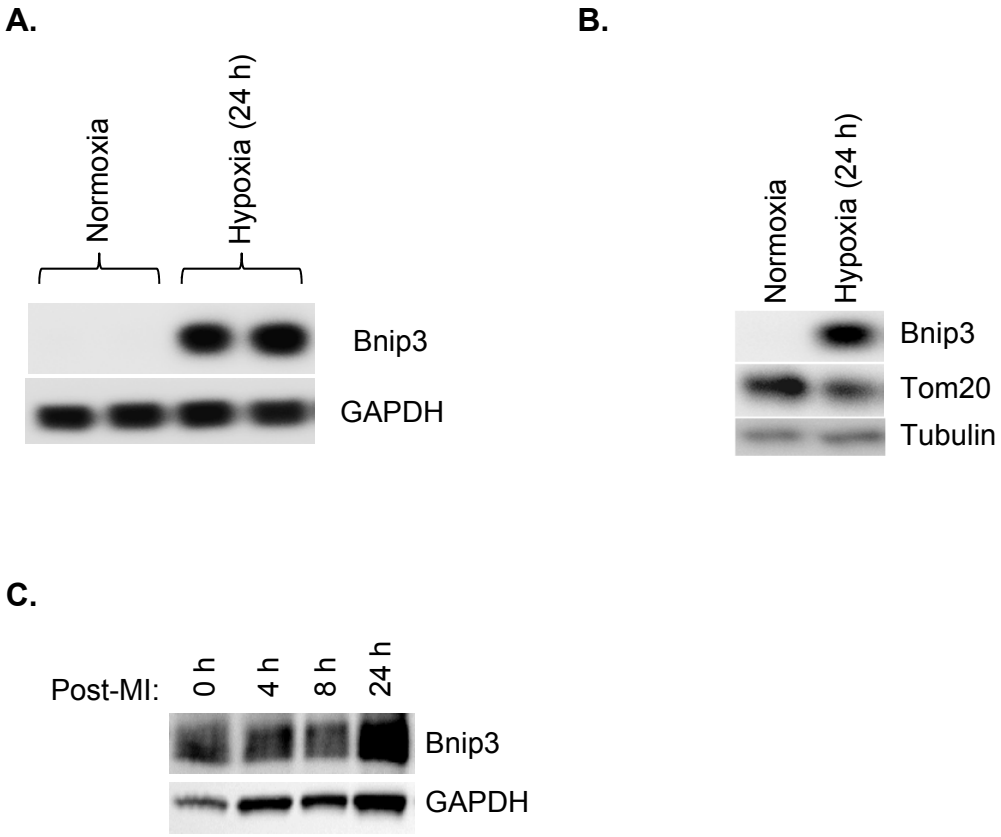

Figure S6. Upregulation of Bnip3 in response to hypoxia or myocardial infarction. **A.** Bnip3 is upregulated in HeLa cells in response to hypoxia. **B.** Hypoxia-mediated upregulation of Bnip3 correlates with a reduction in the mitochondrial protein Tom20. **C.** Bnip3 is upregulated in the border zone of an infarct in a mouse heart after permanent ligation of the left descending coronary artery.