SUPPLEMENTAL FIGURE LEGENDS

Supplementary Fig. S1. Hypoxia-induced Bnip3 expression correlates with mitochondrial loss in cancer cells.
(A) Hypoxia/re-oxygenation-induced expression of Bnip3 correlates with decreased detection of mitochondria by Western blot in cancer cells. Panc1-1, HeLa and MCF7 cells were submitted to 42 h of hypoxia and nutrient deprivation, followed by 6 h of re-oxygenation with nutrient deprivation. Control cells were subjected to 48 h of nutrient deprivation. Western blot detection of mitochondrial markers Tim23 and COXIV, Bnip3, and actin.

Supplementary Fig. S2. Entire Western blot scan from Figure 2D.
The full scan to Figure 2D is shown. The dotted line rectangle indicates the region where the IgG heavy chain is predicted to appear in the control. The lack of IgG heavy chain demonstrates efficient crosslinking, and indicates that both detected bands correspond to RFP-Bnip3.

Supplementary Fig. S3. Bnip3 fully localizes to mitochondria and phosphorylation of its LIR determines binding affinity to Atg8 proteins
(A) Bnip3 WT and LIR mutants localize to mitochondria.
(i) RFP-fused Bnip3-WT, and 2SA and 2SE mutants were expressed for 24 h in HeLa cells. Mitochondria were immuno-labelled with Tom20 antibody and cells were imaged at 60X. Colocalization of image stacks was quantified using ImageJ Colormap plug-in (Jaskolski et al, 2005). Bar graphs indicate the mean correlation index (Icorr) ± Std Dev of at least 9 cells (Z-stacks) per condition, expressing WT or mutant Bnip3, and stained for mitochondrial marker Tom20.
(ii) Bnip3-WT, 2SA and 2SE mutants were co-expressed with mito-GFP for 18 h in HeLa cells. Cells were imaged at 60X and representative image stack projections of mitochondrial morphologies are shown.
(B) LIR phosphorylation enhances autophagosomal sequestration of mitochondria.
Quantification of Bnip3 WT, 2SA and 2SE induction of mitophagy by MIFC. Indicated Bnip3 constructs were expressed with GFP-LC3B in HeLa cells for 18h and imaged at 40X using Imagestream X. The subpopulation of WT Bnip3-targeted mitochondria with high GFP- LC3B colocalization was gated, and applied to Bnip3 mutant populations. Population fractions are indicated, as well as the percentage change of the two mutants compared to the wild type.
(C) LIR phosphorylation is responsible for Bnip3 and LC3B binding affinity.
RFP, RFP-Bnip3 WT and indicated mutants were expressed in cardiac HL-1 (i) and HeLa cells (ii) stably -expressing GFP-LC3B. At 48 h of expression, immunoprecipitations were performed with α-GFP. Western blot detection of RFP, GFP and actin.

Supplementary Fig. S4. Bnip3 is phosphorylated downstream of its LIR without impacting LIR activity
(A) Based on reported proteomics data, (http://www.phosphosite.org) human Bnip3 and corresponding mouse Bnip3 phosphorylation events are shown in schematic.
(B) Phosphorylation downstream of the Bnip3 LIR does not alter LC3B binding. Bnip3-5SA (S80A/T84A/S86G/S92A/S93A) RFP-Bnip3 WT and RFP-Bnip3-5SA were expressed in HeLa cells stably-expressing GFP-LC3B. At 48 h of expression, immunoprecipitations were performed with α-GFP. Western blot detection of RFP and GFP.

Supplementary Fig. S5. BH3- binding competent Bcl-x_L promotes mitophagy in the presence of Bnip3.
RFP-fused WT Bcl-xL, Bcl-x_L-F131V (Mut1) deficient in Bax binding, and Bcl-x_L-G138E/R139L/I140N (Mut2), deficient in BH3 binding, were co-expressed with GFP-LC3B and pcDNA-Bnip3 in Hela cells

for 24 h. Plot profiles illustrate colocalizations between Bcl-x_L-targeted mitochondria and autophagosomes.

Supplementary Fig. S6. Hypoxia-induced mitophagy receptors Bnip3 and Fundc1 can have opposite prognostic value in human cancer.

Kaplan Meier curves were generated from publically available colon and neuroblastoma datasets using the R2: microarray analysis and visualization platform (http://r2.amc.nl) resource. These data indicate that different mitophagy receptors, i.e. Bnip3 vs. Fundc1, can exert opposing prognostic indicator values. Furthermore, here high expression of Bnip3 is associated with poorer survival in patients with colon cancer, but better survival in those with neuroblastoma; and vice versa with expression of Fundc1, possibly due to varying, cancer type-specific LIR phosphorylation states.