Supplementary Figure 1 Purification and identification of NAF-1. Preparative SDS-PAGE of cross-linked LM from H1299 HA-BCL-2b5 cells and identification of NAF-1. **(A)** Large batches of BMH-treated LM from H1299 HA-BCL-2b5 cells were collected, combined, and resolved by preparative gel electrophoresis (Ng et al., 1997). **(B)** Eluted fractions were analyzed by immunoblot for BCL-2 to identify those fractions corresponding to this complex. These fractions (#51-66) were pooled together and the solution modified to support immunoprecipitation with anti-BCL-2 antibody. The immunoprecipitate was resolved by SDS-PAGE and the corresponding BCL-2-containing band was excised, trypsin digested and analyzed by liquid chromatography-tandem mass spectrometry. Eight peptide sequences were found to derive from BCL-2 and three peptide sequences from a hypothetical 15 kDa protein of unknown function. This protein was later denoted NAF-1.

Supplementary Figure 2 Alignment of amino acid sequences of NAF-1 orthologs from five species. Multiple sequence alignment of NAF-1 in five different species was performed using ClustalW (http://www.ebi.ac.uk/clustalw/). An asterisk (*) represents identical amino acids amongst all five species. A colon (:) represents highly conserved amino acids, and a period (.) represents weakly conserved amino acids.

Supplementary Figure 3 Co-immunoprecipitation of NAF-1 and BCL-XL. Lysate collected from H1299 neo cells were subjected to immunopreciptation with anti-NAF-1 and analysis by immunoblot.

Supplementary Figure 4 Targeting and insertion of NAF-1 into dog pancreas microsomes. NAF-1-Flag and HA-BAK were transcribed and translated in rabbit reticulocyte lysate in the presence of purified dog pancreas microsomes (DPM; 7 μg protein) or purified mouse heart mitochondria (HM; 35 μg protein). Microsomes or mitochondria were recovered and analyzed directly or after extraction in 0.1M Na_2CO_3, pH 11, to remove peripheral and loosely bound proteins (McBride et al, 1992; Wang et al, 2008). Proteins were detected by immunoblot using anti-NAF-1 and anti-BAK antibodies. IVT, in vitro translation.

Supplementary Figure 5 Nutrient deprivation does not affect the physical association between endogenous NAF-1 and BCL-2. SK-Mel5 cells were either maintained in normal medium or starved for 4h in EBSS. Cells were collected, lysed and subjected to immunoprecipitation as in Fig 2A.

Supplementary Figure 6 The WFS2 protein does not affect starvation-induced autophagy. H1299 neo cells treated with NAF-1 shRNA were either mock-transfected, transfected with empty plasmid (pcDNA3) or plasmid encoding WFS2-HA and subsequently either maintained in normal medium or starved for 4h in EBSS with DMSO (vehicle) or Baf A1 (100nM). Cell lysates were analyzed by immunoblot.