Supplementary Figures

Bif-1 interacts with prohibitin-2 to regulate mitochondrial inner membrane during cell stress and apoptosis

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Figure S1. Bif-1-knockdown suppresses azide-induced apoptosis in RPTC cells.

Figure S2. Bif-1 knockdown suppresses cytochrome c release and Bax activation in mitochondria during azide-treatment of RPTC cells.

Figure S3. Effects of Bif-1 deficiency on mitochondrial dynamics proteins.

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Figure S5. Verification of the specificity of the antibodies used for immunoprecipitation.

Figure S6. Bif-1-knockout mice have less TUNEL and cleaved caspase-3 staining in kidney tissues during IR injury.

Figure S7. Schematic diagram of the Bif-1/PHB2/OMA1/OPA1 pathway in mitochondrial inner membrane cleavage.

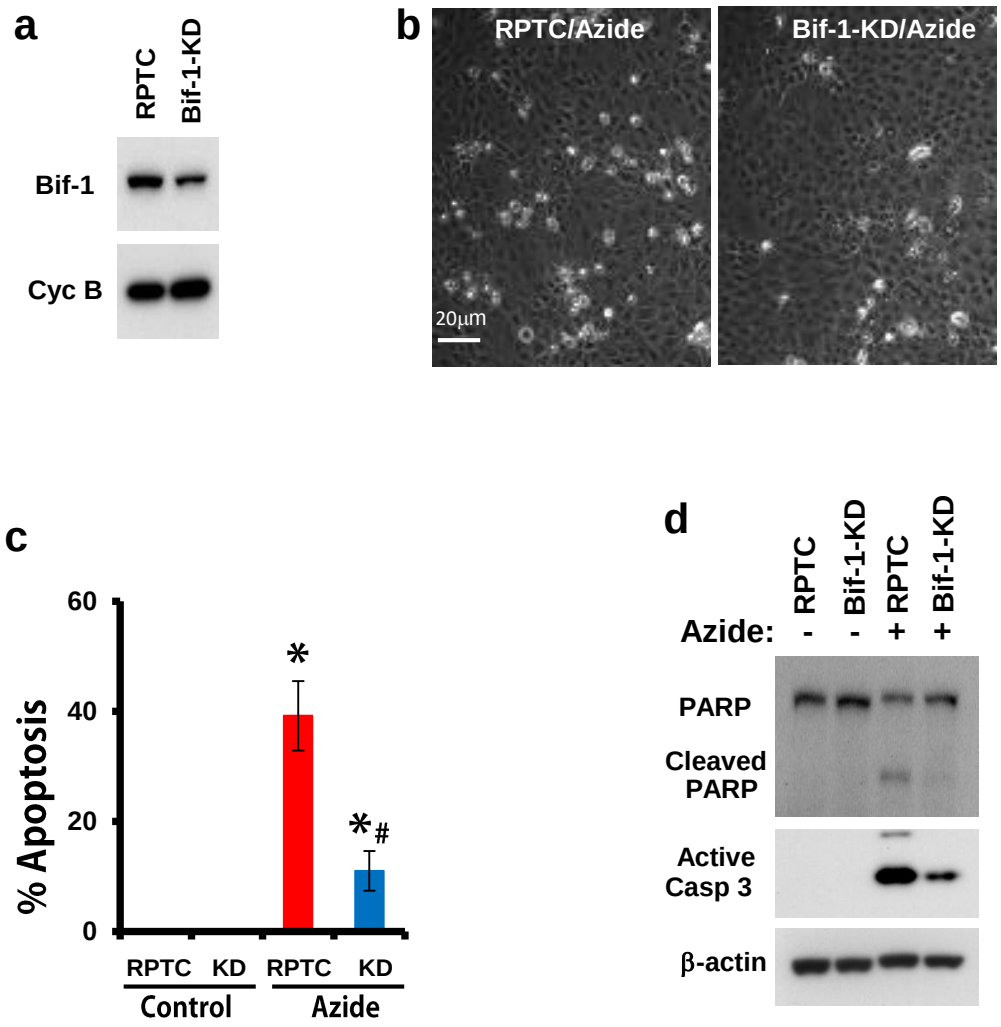

Figure S1. Bif-1-knockdown suppresses azide-induced apoptosis in RPTC cells. Bif-1 specific shRNAs were transfected into RPTC to generate stable Bif-1-knockdown (KD) cells. RPTC and Bif-1-KD cells were incubated with 10 mM azide in glucose-free buffer for 3 h followed by 2 h of recovery in normal culture medium **(a-d)**. **(a)** Immunoblot analysis confirming Bif-1 knockdown in Bif-1-KD cells. Cyclophilin B (Cyc B) was probed as loading control. **(b)** Cell morphology. The bright, condensed and fragmented cells are apoptotic. **(c)** % of apoptosis assessed by counting the cells with apoptotic morphology. Data are mean±SD (n=3); * $p<0.05$ vs. Control; # $p<0.05$ vs. HeLa or RPTC. **(d)** Caspase-3 activation and PARP cleavage. Whole cell lysate was analyzed by immunoblotting of indicated proteins. β-actin was probed as loading control.

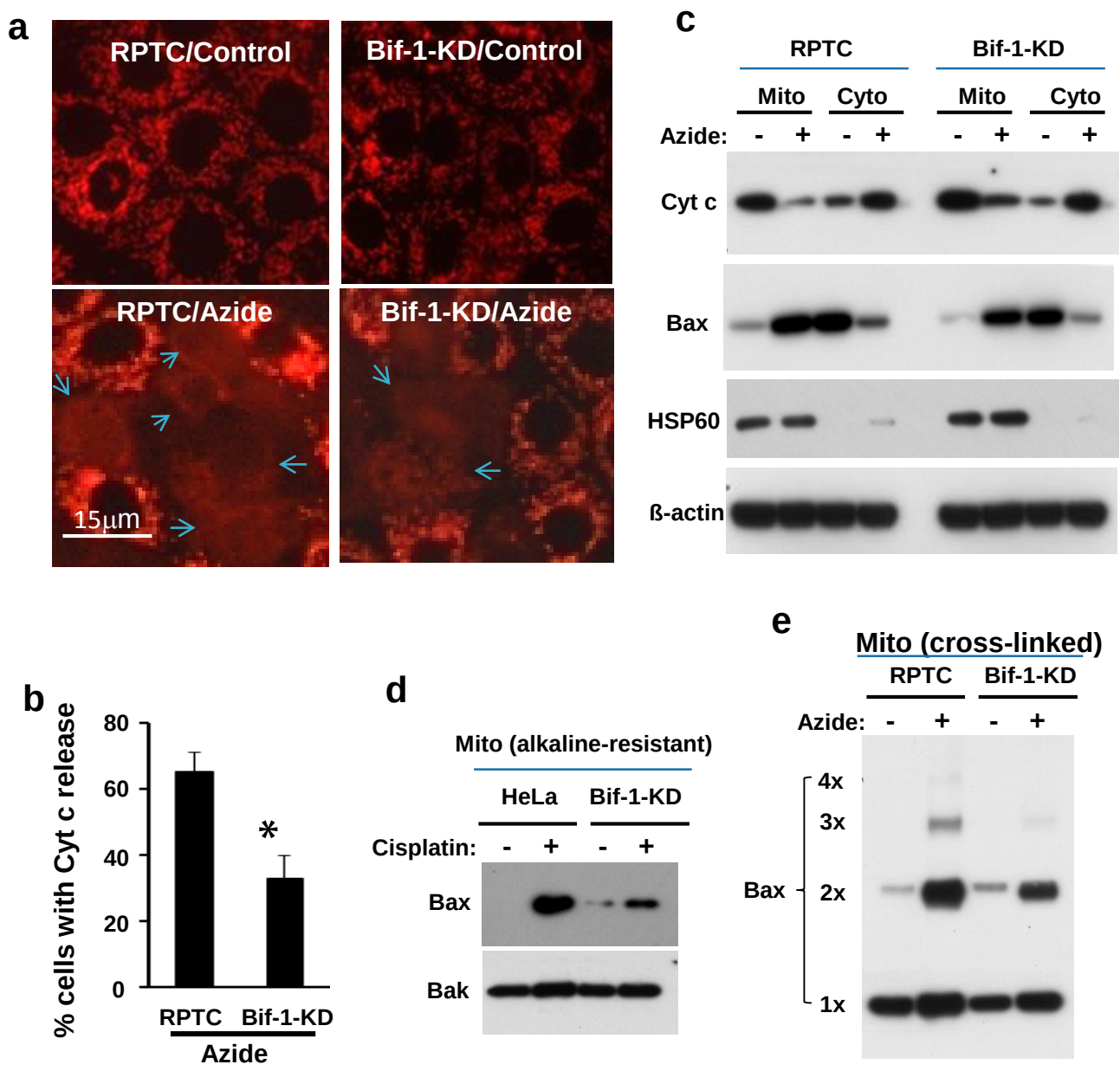

Figure S2. Bif-1 knockdown suppresses cytochrome c release and Bax activation in mitochondria during azide-treatment of RPTC cells. Wild-type and Bif-1-knockdown cells were treated with azide. The cells were fixed for immunofluorescence of Cyt (**a**, **b**), or fractionated into mitochondria-enriched fraction (Mito) and cytosolic fraction (Cyto) for immunoblot analysis of Cyt c and Bax (**c**). Arrows in (**a**): Cyt c released cells. Data in (**b**) are mean±SD (n=3); * $p<0.05$ vs. RPTC. To analyze Bax in mitochondria, mitochondrial fractions were subjected to alkaline treatment to remove loosely attached proteins for immunoblot analysis of membrane-inserted Bax (**d**) or subjected to chemical cross-linking with DSP for immunoblot analysis of Bax oligomerization (**e**).

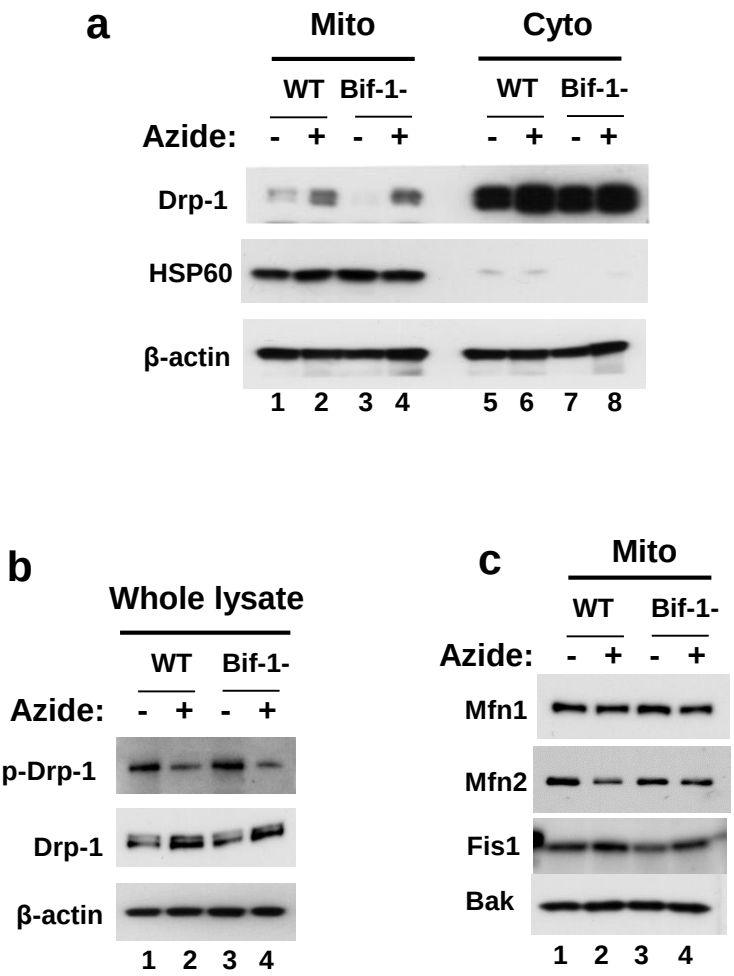

Figure S3. Effects of Bif-1 deficiency on mitochondrial dynamics proteins. Wild-type and Bif-1-null MEFs were treated with azide for 3 h or left untreated. The cells were then fractionated into membrane-bound fraction with mitochondria (Mito) and cytosolic fraction (Cyto) for immunoblot analysis of Drp-1 (**a**), or lysed to collect whole lysate for analysis of total and serine-637 phosphorylated Drp-1 (**b**), or fractionated to collect Mito fraction for analysis of indicated proteins (**c**).

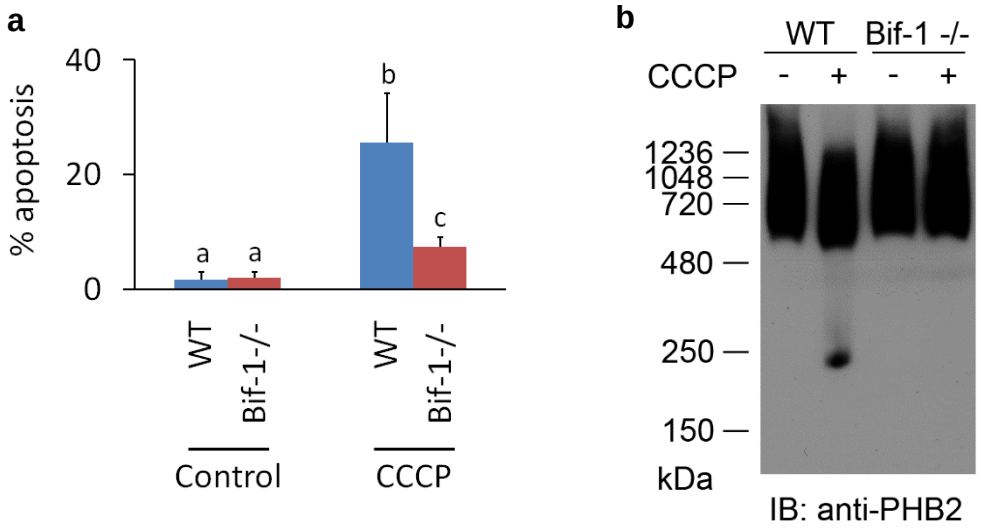

Figure S4. Bif-1-null cells are resistant to CCCP-induced apoptosis and prohibitin complex disruption. Wild-type and Bif-1-null MEFs were incubated with or without 20mM CCCP for 3h. (**a**) To evaluate apoptosis, the cells were returned to normal medium for 2-3h and the cells with apoptotic morphology were counted. (**b**) To analyze prohibitin complexes, the cells were fractionated after CCCP treatment to collect the mitochondria-enriched fraction for chemical cross-linking with 1 mM DSP. After re-suspension, the samples were resolved by BN-PAGE for immunoblot analysis of PHB2 .

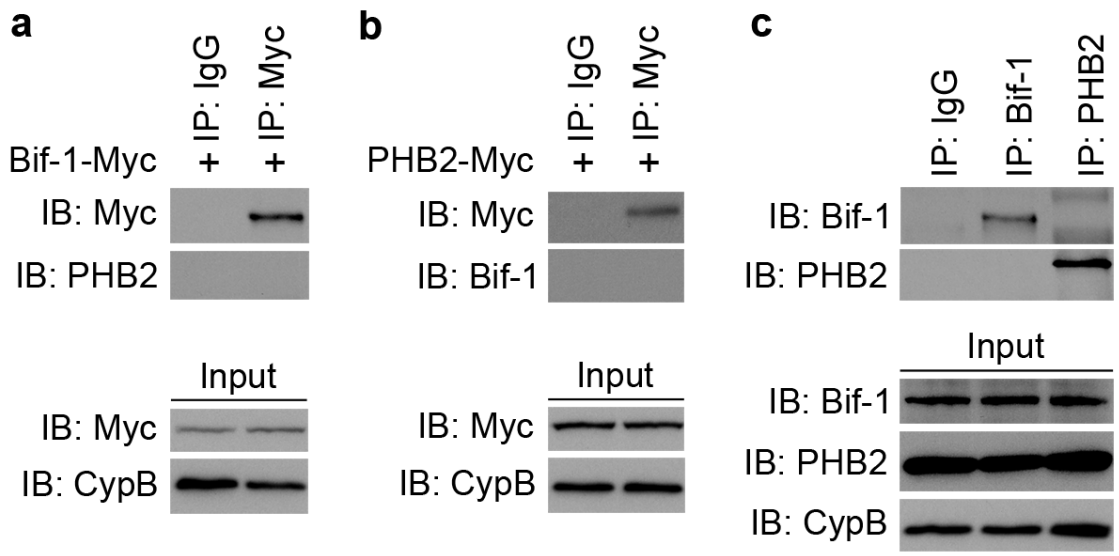

Figure S5. Verification of the specificity of the antibodies used for immunoprecipitation. (a, b) HEK293 cells were transfected with Bif-1-Myc or PHB2-Myc plasmids to collect lysate for immunoprecipitation with anti-Myc antibody or non-immune IgG, followed by immunoblot analysis with indicated antibodies. (**c**) HEK293 cell lysate was collected for for immunoprecipitation with anti-Bif-1, anti-PHB2, or non-immune IgG, followed by immunoblot analysis with indicated antibodies. Input was analyzed to verify that comparable amounts of proteins were used for immunoprecipitation with specific antibodies or non-immune IgG.

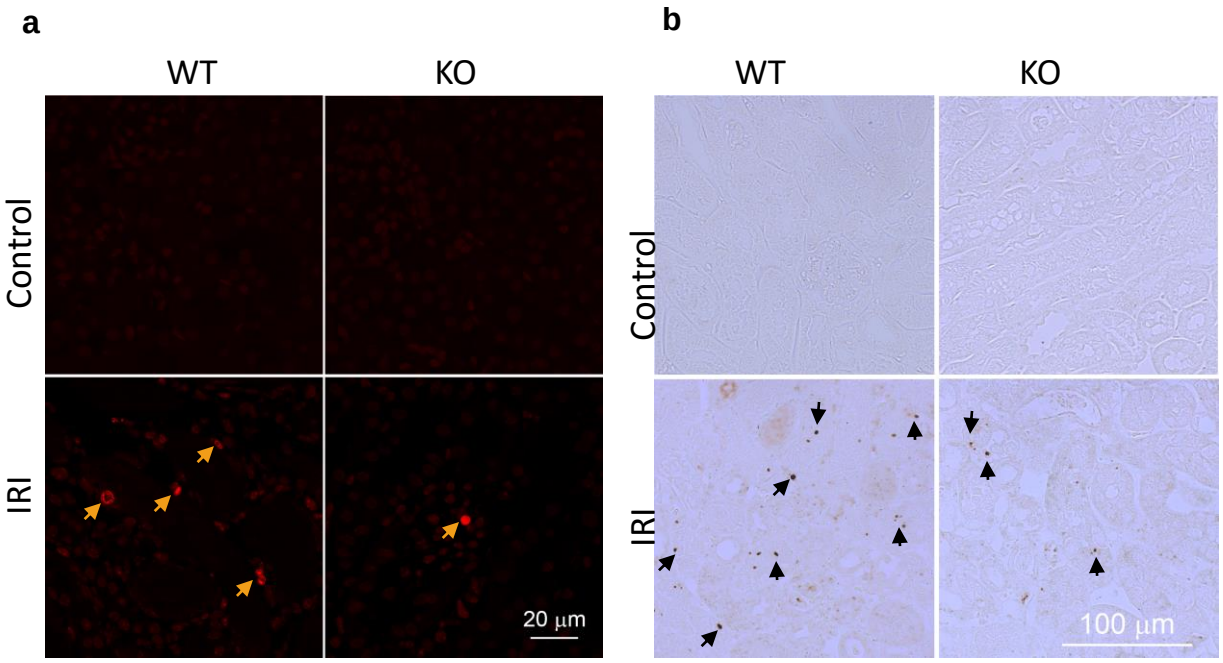

Figure S6. Bif-1-knockout mice have less TUNEL and cleaved caspase-3 staining in kidney tissues during IR injury. Bif-1-knockout (KO) and wild-type (WT) littermate mice were subjected to 30 minutes of bilateral renal ischemia with 48 hours of reperfusion to collect kidney tissues for TUNEL assay and immunohistochemical staining of cleaved/active casase-3 of apoptosis(d). (**a**) Representative images of TUNEL assay. (**b**) Representative images of cleaved caspase-3 staining with quantification of positive staining cells. Arrows point to positive staining cells.

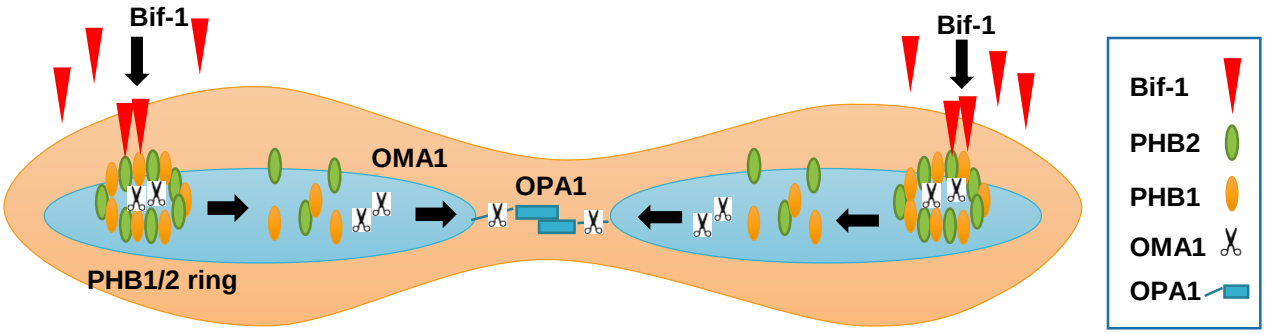

Figure S7. Schematic diagram of the Bif-1/PHB2/OMA1/OPA1 pathway in mitochondrial inner membrane cleavage. Upon cell stress, Bif-1 translocates to mitochondria and interacts with PHB2, resulting in the disruption of the prohibitin ring complexes and the release of OMA1. OMA1 then gains access to OPA1 for proteolysis and inactivation of this inner membrane fusion protein, contributing to inner membrane cleavage.