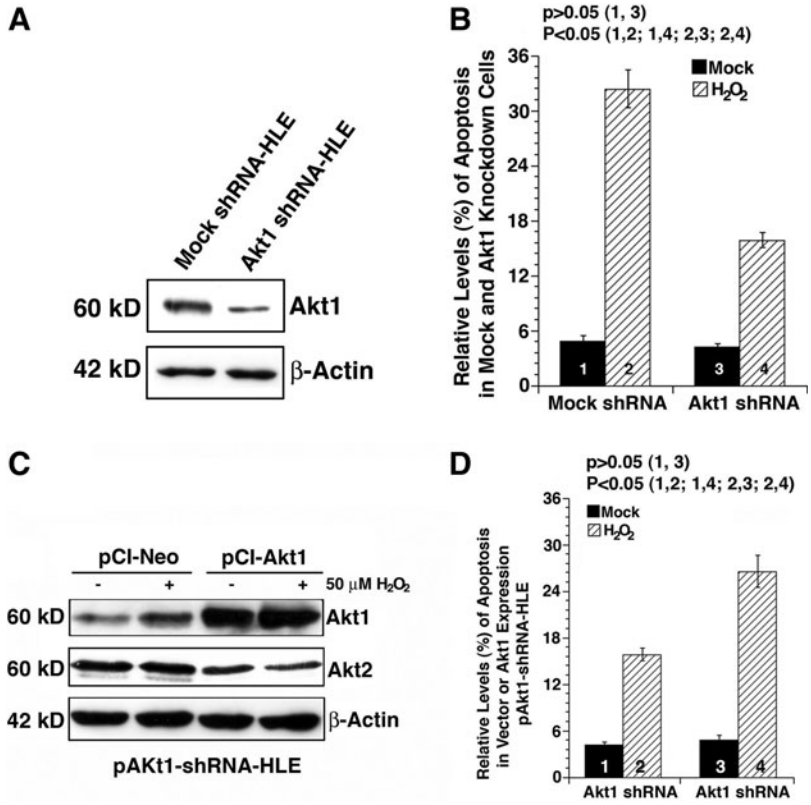
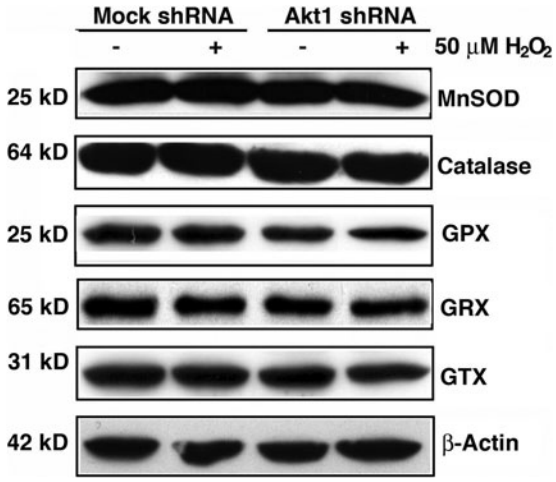
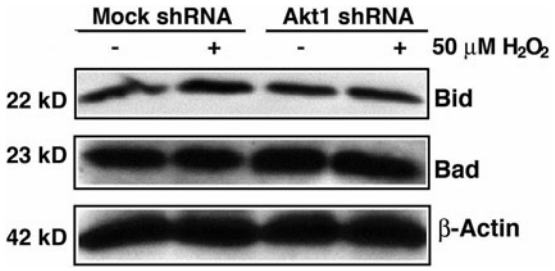
Supplementary Data



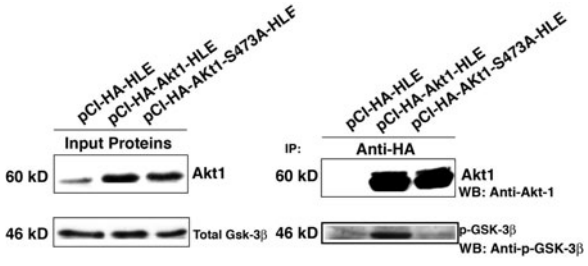
SUPPLEMENTARY FIG. S1. Establishment of mocked and Akt1 knockdown cell lines and their differential response to H_2O_2. The mock and Akt1 small interference RNA (shRNA) plasmids were transfected into human lens epithelial cells (HLECs) and the transfected cells were screened under 0.25 μg/ml puromycin (Sigma) for 4 weeks. After screening, individual stable clones were grown in the same medium containing 0.375 μg/ml puromycin. **(A)** Confirmation of the Akt1 knockdown by western blot analysis. Both mock and Akt1 shRNA plasmids-transfected cell clones were grown to 95% confluence in MEM containing 0.375 μg/ml puromycin and then harvested for analysis of Akt1 and β-actin expression. Note that Akt1 was knocked down more than 80% in the Akt1 shRNA plasmid-transfected cell clone. **(B)** Differential responses of the mock and Akt1 shRNA plasmids-transfected cell clones to 50 μM H_2O_2. Note that the Akt1 shRNA plasmid-transfected cells displayed approximately 50% decreases in H_2O_2-induced apoptosis. Apoptosis was determined through cell flow cytometry. Data were analyzed with the Student-Newman-Keuls (SNK) test. **(C)** Expression of Akt1 in Akt1 knockdown cells. Note that when Akt1 is overexpressed, Akt2 level is no longer upregulated under the treatment by hydrogen peroxide. **(D)** Expression of Akt1 in Akt1 knockdown cells slightly enhanced the oxidative-stress-induced apoptosis.



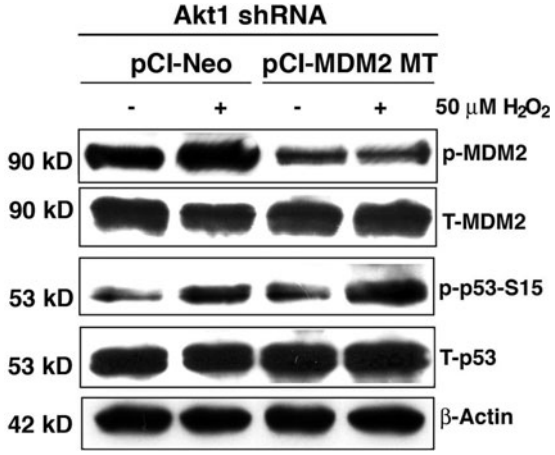
SUPPLEMENTARY FIG. S2. Western blot analysis of antioxidative stress enzymes in the mocked and akt1 knockdown HLECs. Note that in both mock and Akt1 shRNA plasmid-transfected cell clones, oxidative stress did not change the expression levels of the antioxidative stress-enzymes: MnSOD, catalase, glutathione peroxidase, glutathione reductase, and glutathione transferase.



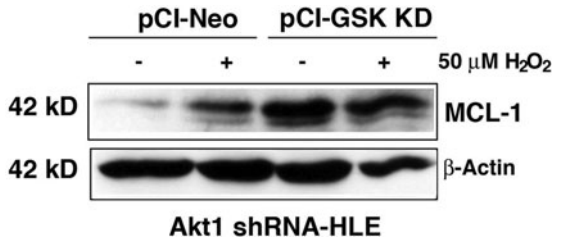
SUPPLEMENTARY FIG. S4. Western blot analysis of Bid and Bad in the mock and Akt1 knockdown HLECs. Note that in both mock and Akt1 shRNA plasmids-transfected cell clones, oxidative stress hardly changed the expression levels of either Bid or Bad.



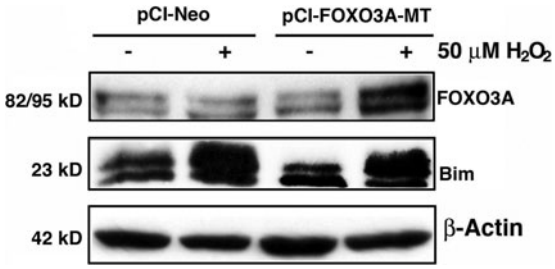
SUPPLEMENTARY FIG. S3. Demonstration that phosphorylation of Akt at Ser-473 is equal to its activity. Wild-type Akt1 expression construct or S473A mutant Akt expression construct were introduced into HLECs, the transfected cells were harvested for extraction of total proteins which were used for immunoprecipitation with anti-HA antibody. The immunoprecipitated Akt1 or mutant Akt1 were used for kinase assay with glycogen synthase kinase 3 beta (GSK-3β) as substrate. Note that the Akt1 mutant has little Akt activity.



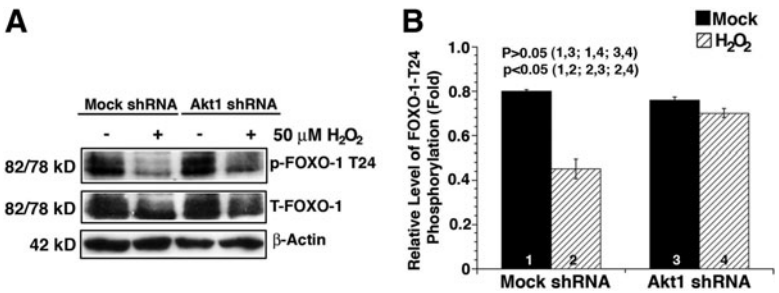
SUPPLEMENTARY FIG. S5. Demonstration that murine double minute 2 (MDM2) regulates p53 in Akt1 shRNA-transfected cells. The vector or mutant MDM2 were introduced into Akt1 shRNA-transfected cells. The MDM2 mutant interferes the interaction between MDM2 and p53, leading to upregulation of p53 expression and phosphorylation.



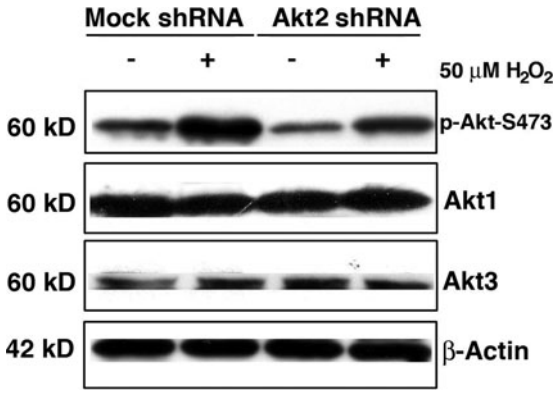
SUPPLEMENTARY FIG. S6. Demonstration that GSK-3β kinase dead mutant regulates MCL-1 in HLECs. The vector or kinase dead GSK-3β mutant were introduced into Akt1 shRNA-HLECs. The kinase dead GSK-3β mutant interferes with the phosphorylation MCL-1 and thus promotes the later stability, leading to upregulation of MCL-1.



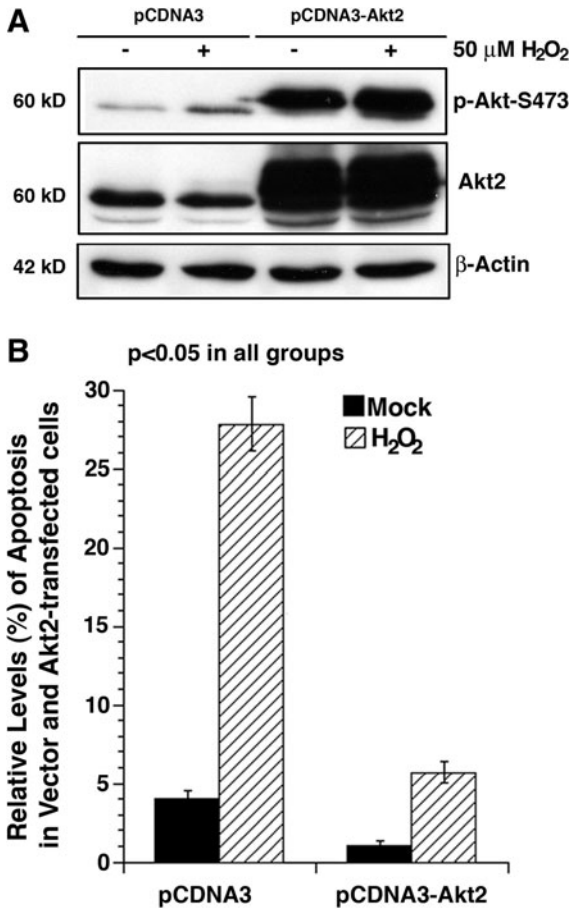
SUPPLEMENTARY FIG. S8. Demonstration that FOXO3A S32A mutant regulates Bim in HLECs. The vector or FOXO3A T32A mutant were introduced into HLECs. The FOXO3A T32A mutant stays in the nucleus and is constitutively active, and thus enhancing Bim expression.



SUPPLEMENTARY FIG. S7. Western blot analysis of the total FOXO1A and phospho-FOXO1A at T24 in the mocked and Akt1 knockdown HLECs. Note that oxidative stress caused much more downregulation of the phospho-FOXO1A at T24 in the mock shRNA plasmid-transfected cells than that in the Akt1 shRNA plasmid-transfected cells. Data were analyzed with analyzed with the SNK test.



SUPPLEMENTARY FIG. S9. Demonstration that knockdown of Akt2 does not affect the expression of Akt1 or Akt3. Both mock and Akt2 shRNA plasmid-transfected HLECs were either mocked treated (by H_2O) or treated with 50 μM H_2O_2 for 2 h. After treatment, the cells were harvested for analysis of Akt1, Akt3 and Akt activity.



SUPPLEMENTARY FIG. S10. Demonstration that overexpression of Akt2 resists on oxidative-stress-induced apoptosis. Both mock and Akt2 overexpression plasmids were transfected into HLECs and the overexpression was confirmed by Western blot analysis **(A)**. The overexpression cells were either mocked treated (by H_2O) or treated with 50 μM H_2O_2 for 2 h **(B)**. Note that overexpression of Akt2 in HLECs provides significant protection from oxidative-stress-induced apoptosis.