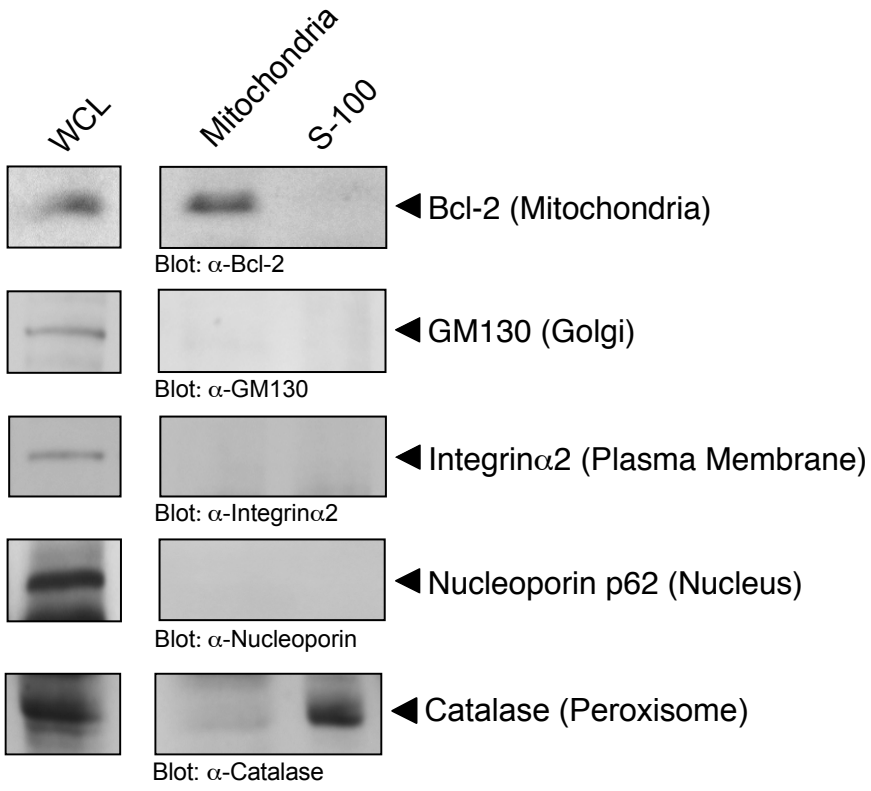
Supplemental Figure S1

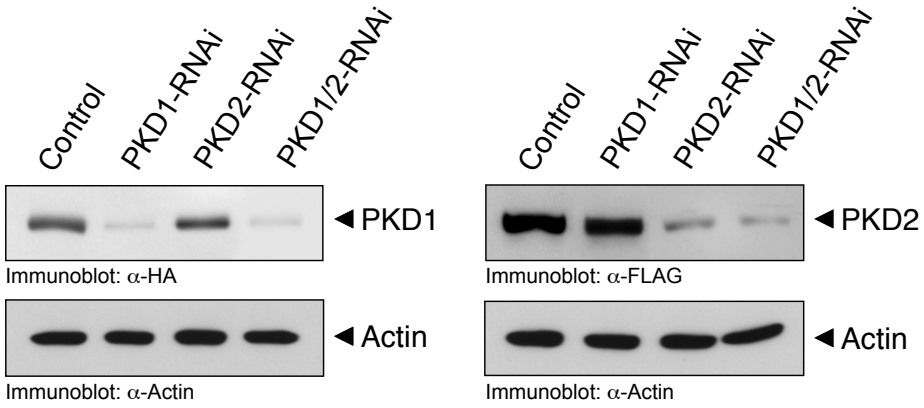
Characterization of mitochondrial preparations



Whole cell lysates (WCL), mitochondrial preparations or the S100 fraction were immunoblotted against markers for mitochondria (anti-Bcl-2), Golgi (anti-GM130), plasma membranes (anti-integrinα2), nuclei (anti-nucleoporin p62) or peroxisomes (anti-catalase).

Supplemental Figure S2

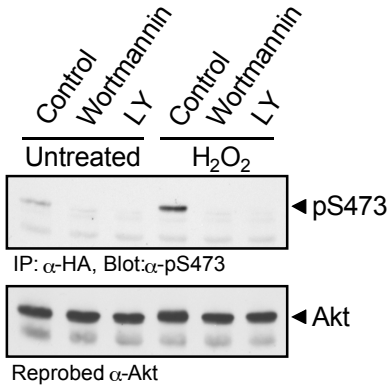
Specificity of the PKD1 and PKD2 RNAi



HeLa cells were transfected with vector control (pSuper), PKD1 RNAi or PKD2 RNAi (pSuper PKD1/pSuper PKD2) as indicated for 24 hr. Cells were transfected in a second transfection with HA-PKD1 or FLAG-PKD2. After 24 hr cells were analyzed for PKD1 or PKD2 expression using α-HA (PKD1) or α-FLAG (PKD2) antibodies. Analysis of actin expression (α-actin) served as a loading control.

Supplemental Figure S3

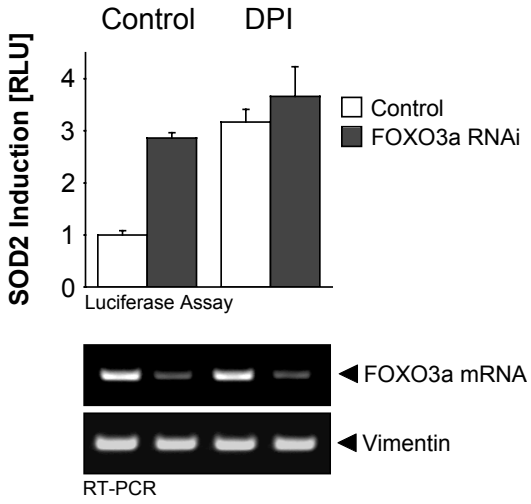
Akt activation by oxidative stress is dependent on PI3K activity



Cells were transfected with HA-tagged Akt, treated with Wortmannin (15 min, 100 nM) or LY294002 (15 min, 30 μM) and then stimulated with H_2O_2 (10 μM, 10 min). Akt was immunoprecipitated. Samples were re-solved by SDS-PAGE, transferred to nitrocellulose and immunoblotted with α-pS473 antibody. Membranes were then stripped and re-probed with α-Akt antibody.

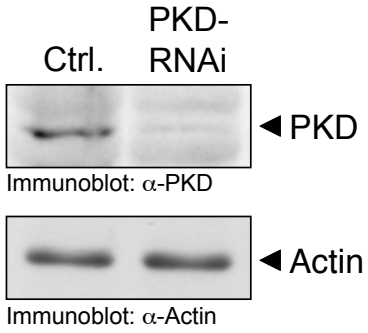
Supplemental Figure S4

SOD2 regulation by FOXO3a in response to DPI treatment



Cells were transfected with vector control (pSuper), or FOXO3a RNAi (pSuper FOXO3a) for 24 hr. Then cells were transfected a second time with reporter constructs and 8 hr after transfection, stimulated with DPI (1μM, 16hr). Reporter gene assays were performed to measure SOD2 gene reporter transcriptional activity (SOD2-luciferase reporter plasmid) or β-galactosidase activity (normalization). Error bars represent standard deviation (SD). FOXO3a down-regulation was measured using RT-PCR (Vimentin served as a control).

Supplemental Figure S5



Cells were transfected with vector control (pSuper), or PKD RNAi (pSuper PKD1/2) for 48 hr. Cells were analyzed by Western Blot for silencing of PKD and seeded in 24 well plates for the experiments depicted in Figure 7A.