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Polo-like kinase 2 activates an antioxidant pathway to promote the survival of cells with mitochondrial dysfunction

Jie Li^{1,*}, Wenzhe Ma^{1,2,*}, Ping-yuan Wang¹, Paula J. Hurley³, Fred Bunz⁴, and Paul M. Hwang^{1,+}

¹Center for Molecular Medicine, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892, USA

²State Key Laboratory for Quality Research in Chinese Medicine, Macau University of Science and Technology, Macau, China

³Department of Urology, The Johns Hopkins School of Medicine, Baltimore, MD 21287, USA

⁴Department of Radiation Oncology and Molecular Radiation Sciences, The Johns Hopkins School of Medicine, Baltimore, MD 21287, USA

Abstract

We previously reported that *Polo-like kinase 2 (PLK2)* is highly expressed in cells with defective mitochondrial respiration and is essential for their survival. Although PLK2 has been widely studied as a cell cycle regulator, we have uncovered an antioxidant function for this kinase that activates the GSK3-NRF2 signaling pathway. Here, we report that the expression of *PLK2* is responsive to oxidative stress and that PLK2 mediates antioxidant signaling by phosphorylating GSK3, thereby promoting the nuclear translocation of NRF2. We further show that the antioxidant activity of PLK2 is essential for preventing p53-dependent necrotic cell death. Thus, the regulation of redox homeostasis by PLK2 promotes the survival of cells with dysfunctional mitochondria, which may have therapeutic implications for cancer and neurodegenerative diseases.

Keywords

Polo-like kinase 2; Antioxidant; Mitochondrial dysfunction; Oxidative stress; GSK3; NRF2; p53; Necrosis

Introduction

Defective mitochondrial respiration can increase the level of reactive oxygen species (ROS) and cause oxidative damage to cells. Growing evidence in disparate fields suggests that oxidative stress accelerates aging and contributes to the pathogenesis of age-associated

[†]Corresponding author. FAX: +1 301 402-0888, hwangp@mail.nih.gov (P.M. Hwang).

^{*}These authors contributed equally to this work

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diseases such as cancer and neuro-degeneration [1, 2]. In order to gain insights into the adaptive processes necessary for cell survival under conditions of oxidative stress, we modeled mitochondrial dysfunction in human cells by the targeted deletion of *Synthesis of Cytochrome c Oxidase 2 (SCO2)*, a gene essential for respiratory complex IV assembly [3, 4]. By gene expression analysis, we identified *Polo-like Kinase 2 (PLK2)* as a gene that is highly expressed and essential for the survival of cells with defective respiration and increased oxidative stress [3, 4].

PLKs are a family of structurally related serine/threonine protein kinases that are defined by the presence of C-terminal “polo box” domains that interact with phosphopeptides and are best known as cell cycle regulators [5–8]. Using a kinase substrate screening assay, we identified the Ser-137 amino acid residue of PLK1 (PLK1-S137), the prototypical PLK family member involved in progression of mitosis, as a target of PLK2 kinase activity and showed that PLK1-S137 phosphorylation promotes the survival of cells under oxidative stress [4, 7, 8]. Notably, these observations are consistent with recent studies demonstrating that PLK1-S137 phosphorylation activates PLK1 and permits cell cycle progression by inactivating the DNA damage checkpoint [9, 10].

PLK2 has also been shown to play a role in post-mitotic cells. The synaptic protein SPAR is a PLK2 substrate involved in the regulation of neuronal plasticity [11]. In addition, PLK2 can phosphorylate and promote selective autophagic clearance of α -synuclein, a synaptic protein that accumulates in the Lewy bodies of Parkinson’s disease, a neurodegenerative condition associated with mitochondrial dysfunction and oxidative stress [12–16]. These different functions of PLK2 in proliferating and post-mitotic cells suggest that the phosphorylation of different substrates represents cell type-specific adaptive processes activated under conditions of stress and is also consistent with the initial identification of PLK2 as an immediate early response gene [17].

In this report, we show that the transcription of the *PLK2* gene is responsive to increased oxidative stress and that PLK2 protein displays a potent antioxidant function. We present evidence that the antioxidant activity of PLK2 is mediated by a signaling pathway involving the phosphorylation of GSK3 and the subsequent nuclear translocation of NRF2, a transcription factor that is well-known to regulate the expression of various redox genes. Furthermore, we show that the antioxidant function of PLK2 prevents p53- and ROS-coordinated necrosis, delineating a new pathway by which cells may adapt to the deleterious effects associated with mitochondrial dysfunction that is observed in various neurodegenerative diseases and cancers.

Materials and methods

Cell culture

Unmodified human colon cancer HCT116 cells (ATCC) and its derivatives *SCO2*^{+/-} and *SCO2*^{-/-} cells [18] were cultured in McCoy’s 5A medium with 10% FBS. To create the *SCO2*^{+/-} *p53*^{-/-} cell line, both alleles of *TP53* (*p53*) were sequentially disrupted in the parental *SCO2*^{+/-} cell line by rAAV-mediated homologous recombination [19]. Western blotting was performed to confirm the absence of p53 protein in the *SCO2*^{+/-} *p53*^{-/-} cell

line (Fig. 5B). For pharmacologic inhibition experiments, cells were treated with 10 mM NAC and/or 3 mM BAPTA/AM for 48 h and 16 h, respectively.

Antibodies and reagents

Antibody sources were as follows: PLK2 SNK N-17 (Santa Cruz), (Abcam); GSK-3 α/β GSK3 and phospho-Ser21/9 GSK-3 α/β (GSK3-S-P) (Cell Signaling); tubulin (Sigma Aldrich); HMGB1, lamin B1, NQO1 and NRF2 (Abcam). As established by Zhang and colleagues, only the NRF2 protein migrating in the ~95–110 kDal range was designated as the specific band [20]. PLK2 antibody specificity was determined by transducing SCO2 $-/-$ cells with PLK2-specific shRNA and demonstrating elimination of its protein band by western blotting (Supplementary Fig. S2B). H₂O₂ and NAC were obtained from Sigma.

In vitro phosphorylation assay

Recombinant GSK3B is known to be highly phosphorylated, therefore 400 ng of the purified recombinant human GSK3 β (Abcam 43626) was pretreated with 16 units of lambda phosphatase (Santa Cruz) for 1 h at 30 °C [21]. The dephosphorylated GSK3B was then incubated with 10 μ M ATP and 400 ng of purified recombinant human PLK2 protein (Abcam 102108) in 400 μ l of kinase buffer (60 mM Hepes, pH 7.5, 3 mM MgCl₂, 3 mM MnCl₂, 1.2 mM DTT, 125 μ g/ml PEG 20,000, 3 μ M sodium orthovanadate, 1% phosphatase inhibitor cocktail (Calbiochem)) for 1 h at 30 °C. The kinase reaction was terminated by mixing with SDS sample buffer and heating for 5 min at 90 °C, and the samples were resolved by SDS-PAGE and immunoblotted.

Lentivirus for gene knockdown and over-expression

Plasmids containing sequences for non-specific, NRF2, PLK2 and NQO1 shRNAs (Sigma-Aldrich) for knockdown and pLEX-MCS PLK2 and NRF2 cDNAs (OpenBiosystems) for over-expression experiments were used to make lentivirus according to manufacturer's protocol (Sigma-Aldrich) [4]. Using the pLEX-MCS PLK2 and NRF2 plasmids, kinase dead PLK2 D223N and degradation resistant NRF2 E79A point mutations were introduced using the QuikChange II kit (Stratagene). Cells were transduced with lentivirus (MOI ~1) for 24 h followed by 2 μ g/ml puromycin selection for at least 3 d prior to experiments. The primer sequences used for gene-specific shRNA and site-directed mutagenesis are provided below.

Sequences for mutant PLK2 D223N primers:

Forward 5'-CATGGAAGTAAAAGTTGGGAAGTTCGGTCTGGCAGCC-3'

Reverse 5'-GGCTGCCAGACCGAAGTTCCCAACTTTTAGTTCCATG-3'

Sequences for mutant NRF2 E79A primers:

Forward 5'-AGATGAAGCGACAGGTGAATTTCTCC-3'

Reverse 5'-ACCTGTCGCTTCATCTAGTTGTAAC-3'

Colony formation assay

shRNA lentivirus transduced cells were plated at varying densities, cultured and stained with crystal violet solution as previously described [4].

Real-time PCR

PLK2 mRNA levels were measured by RT-PCR using an ABI HT7900 thermal cycler and normalized to housekeeping gene *TIF* mRNA [22].

Sequences for PLK2 mRNA quantification primers:

Forward 5'-ACAGATGCTCCTGAGCAATTTATTAGTCAAGTGAC-3'

Reverse 5'-TTTTGGCTTCCCTGTAGATCTCACAGTGG-3'

Oxygen consumption measurement

Whole cell oxygen consumption measurements were performed using the Seahorse XF24 analyzer (Seahorse Bioscience) by plating 4×10^4 cells/well of the indicated isogenic cells as previously described [4].

ROS measurement

Cells were stained with 5 μ M CM-H₂DCFDA (Invitrogen) at 37 °C for 15 min, washed in PBS, trypsinized and resuspended at 1×10^6 cells/ml. DCF signal was measured using FACSCalibur and CELLQuest software (version 3.3, BD Biosciences). Three independently derived *SCO2*^{+/-} cell lines that we have previously described were used to examine ROS levels [18].

Nuclear fractionation

The cells were incubated in buffer A (10 mM HEPES, pH 7.9, 10 mM KCl, 0.1 mM EDTA) supplemented with 1 mM PMSF, 1 mM DTT and Complete Protease Inhibitor Cocktail (Roche) at room temperature for 10 min. After centrifuging at $16,000 \times g$ for 3 min, the pellets were suspended in buffer B (20 mM HEPES, pH 7.9, 0.4 M NaCl, 0.4 M NaCl, 10% glycerol and Protease Inhibitor Cocktail) and rotated for 2 h. The samples were then centrifuged at $16,000 \times g$ for 15 min, and the final supernatant (nuclear fraction) was analyzed by western blotting. Lamin B1 served as nuclear marker and protein loading control.

Confocal immunofluorescent microscopy

Cells were attached overnight on 35 mm glass bottom dishes (MatTek), washed with PBS, fixed in Z-fix (Anatech) at 37 °C for 15 min, permeabilized in 0.2% Triton X-100 PBS at room temperature for 10 min, blocked with 5% serum (of the secondary antibody species) at room temperature for 1 h, and incubated with anti-NRF2 antibody overnight at 4 °C followed by Alexa Fluor 488-labelled secondary antibody (Invitrogen) at room temperature. PBS washes (3 $\times$ 5 min each) were performed between all steps and images were obtained using a Zeiss Axioskop2 microscope. Cells that lacked nuclear clearing relative to cytoplasmic fluorescence were scored as having nuclear NRF2 localization. A total of at

least 300 cells from three independent experiments were quantified for nuclear NRF2 localization in each group.

Necrosis and xenograft assays

HMGB1 protein release into media as marker of necrosis and the xenograft assays were performed as previously described [4].

Statistical analysis

Data shown are expressed as mean $\pm$ SEM and their statistical significance was determined by two-tailed Student's *t* test.

Results

PLK2 expression and GSK-NRF2 signaling are increased in cells with mitochondrial dysfunction and are associated with improved cell survival

We previously identified Ser-21 of GSK3 α (GSK3A) and Ser-9 of GSK3 β (GSK3B) as candidate substrates of PLK2, a kinase that is induced by mitochondrial dysfunction [4]. Phosphorylation of the Ser-21 or Ser-9 residue at the N-terminus of GSK3 is known to create a “competitive pseudo-substrate” that auto-inhibits its kinase activity [23]. GSK3 is known to phosphorylate and prevent the nuclear translocation of NFE2-related factor 2 (NRF2), a major antioxidant transcription factor [24, 25]. Therefore, the predicted effect of increased PLK2 expression would be to inhibit the GSK-mediated phosphorylation of NRF2, resulting in NRF2 translocation into the nucleus and the subsequent transactivation of various downstream antioxidant genes.

The antioxidant nature of this putative PLK2 signaling pathway was notable because decreased mitochondrial function is associated with increased oxidative stress and DNA damage (Supplementary Fig. S1A–B) [26]. We therefore examined the phosphorylation state of GSK3 in isogenic cell lines with defective respiration caused by the disruption of *SCO2* [4, 18]. Consistent with the results of our PLK2 kinase substrate screen [4], we observed higher levels of GSK3 serine residue phosphorylation in association with increased *PLK2* mRNA and protein expression (Fig. 1A and Supplementary Fig. S1C). Serine phosphorylation of the GSK3B isoform in particular showed good correlation with PLK2 protein level (Fig. 1A). We next performed an *in vitro* phosphorylation assay using purified recombinant proteins and confirmed that the Ser-9 residue of GSK3B can be directly phosphorylated by PLK2 (Fig. 1B). Furthermore, consistent with the known inhibitory effect of GSK3B Ser-9 phosphorylation, the activating Tyr-216 autophosphorylation of GSK3B was prevented by recombinant PLK2 (Fig. 1B) [27].

In parallel with the serine phosphorylation of GSK3, there was an increase in the translocation of NRF2 into the nucleus as determined by western blotting of the nuclear fraction and by confocal immunofluorescent imaging of NRF2-positive nuclei (Fig. 1A and 1C, respectively). To determine the significance of NRF2 translocation in cells with respiratory defects, we transduced the three isogenic cells lines with NRF2 shRNA lentivirus and measured their viability by colony formation assay. The knockdown of NRF2 protein

markedly reduced the growth of both *SCO2*^{+/-} and *SCO2*^{-/-} cell lines, but not the wild-type cells, indicating a specific functional contribution of NRF2 signaling to the survival of cells with defective mitochondria (Fig. 2).

PLK2-mediated phosphorylation of GSK3B promotes the nuclear translocation of NRF2

To further substantiate the association between PLK2 activity and nuclear translocation of NRF2, we next examined the effect of primarily modulating the levels of PLK2. Because both *SCO2*^{+/-} and *SCO2*^{-/-} cells have increased levels of PLK2, we transduced these cells with PLK2 shRNA lentivirus and confirmed knockdown of its mRNA and protein by RT-PCR and western blotting, respectively (Supplementary Fig. S2). The knockdown of *PLK2* expression reduced GSK3B serine phosphorylation and nuclear NRF2 levels in both cell lines (Fig. 3A). The attenuated translocation of NRF2 into the nucleus caused by PLK2 depletion could also be visualized by confocal immunofluorescent microscopy in *SCO2*^{-/-} cells (Fig. 3B). Correlating with the NRF2 subcellular localization data, the protein level of *NAD(P)H:quinone oxidoreductase 1 (NQO1)*, a NRF2-transactivated gene that has been shown to protect against oxidative stress, also decreased in response to PLK2 depletion (Fig. 3A) [28]. Conversely, the over-expression of wild-type PLK2, but not the kinase-dead mutant (MUT, PLK2 D223N), increased serine phosphorylation of GSK3, nuclear localization of NRF2, and NQO1 expression in the *SCO2*^{+/-} cell line (Fig. 3C). Confocal immunofluorescent microscopy also showed increased NRF2 translocation into the nucleus by the over-expression of wild-type but not kinase dead mutant PLK2 (Fig. 3D).

PLK2 is induced by oxidative stress and exhibits antioxidant activity that promotes cell survival

If PLK2 is involved in redox homeostasis via the regulation of NRF2 translocation, then *PLK2* expression would be expected to be responsive to cellular redox state. The *PLK2* gene has previously been shown to have a calcium-dependent cAMP response element (CRE) in its promoter region that confers transcriptional regulation in response to intracellular calcium [4]. Notably, adjacent to the CRE sequence in the human *PLK2* promoter region is an antioxidant response element (ARE), suggesting that its expression might also be responsive to oxidative stress. In *SCO2*^{+/-} cells, a model of moderate mitochondrial dysfunction, increasing concentrations of hydrogen peroxide caused a significant rise in *PLK2* mRNA levels that saturated at greater than 100 μ M (Fig. 4A). Consistent with a direct effect on gene transcription, *PLK2* expression increased as early as 1 h after H₂O₂ treatment and reached a plateau at 4 h (Fig. 4B). Treatment of the *SCO2*^{+/-} cells with the antioxidant N-acetylcysteine (NAC) and intracellular calcium chelator (BAPTA) additively inhibited *PLK2* expression, demonstrating that its expression is co-regulated by ROS and calcium, two well-established mitochondrial retrograde signals to the nucleus (Fig. 4C).

We next investigated whether PLK2 can influence cellular redox state. The knockdown of *PLK2* by shRNA in *SCO2*^{+/-} cells markedly increased ROS levels as measured by the H₂O₂-sensitive dye DCF FACS analysis (Fig. 4D). Furthermore, the decreased growth of *PLK2*-depleted cells was rescued by treating with the antioxidant NAC, suggesting a deleterious effect of increased oxidative stress on cell proliferation (Fig. 4E). We speculated that if the antioxidant activities of PLK2 are mediated by NRF2 and play a significant role in

cell survival, then over-expressing NRF2 protein should be sufficient to rescue the growth of PLK2-depleted *SCO2*^{+/-} cells. Indeed, the expression of a mutant (E79A) NRF2, which is resistant to KEAP1-mediated degradation [29], rescued the colony formation of PLK2-depleted *SCO2*^{+/-} cells (Fig. 4F and 4G). These results indicated that PLK2 plays an important antioxidant role through the NRF2 signaling pathway in ameliorating the oxidative stress caused by impaired mitochondrial function.

The antioxidant activity of PLK2 prevents p53-induced necrosis and promotes the xenograft growth of cells with defective mitochondria

To determine whether the antioxidant activity associated with PLK2 has *in vivo* relevance, we knocked down *NQO1* expression using lentiviral shRNA transduction in *SCO2*^{+/-} cells with a moderate degree of respiratory defect and performed xenograft tumor studies. Although multiple genes are likely to be transactivated by NRF2, the shRNA knockdown of a major NRF2 target gene, *NQO1*, in *SCO2*^{+/-} cells was sufficient to significantly inhibit the growth of xenograft tumors. The requirement of the NRF2-NQO1 pathway for tumorigenic growth underscored the important contribution of antioxidant genes to the viability of tumor cells with compromised mitochondrial respiration (Fig. 5A).

ROS has been shown to be necessary for cell necrosis activated by the p53-cathepsin axis [30]. We previously observed that the depletion of PLK2 results in increased necrosis [4] and, thus, hypothesized that p53 may also play a role in this phenomenon. To test this idea, we examined the effect of PLK2 depletion on necrosis in *SCO2*^{+/-} cells that were either p53-proficient or -deficient (Figure 5B). PLK2 knockdown in p53-proficient *SCO2*^{+/-} cells increased the levels of both ROS and p53, which induced necrotic cell death as measured by HMGB1 release into the medium. However, the release of HMGB1 was reduced in the p53-deficient *SCO2*^{+/-} cells despite the higher level of cellular ROS, indicating that oxidative stress alone is not sufficient to cause necrosis. The higher levels of ROS in the absence of p53 under both basal and PLK2 knockdown conditions were entirely consistent with the established antioxidant activities of p53 (Fig. 5B) [31, 32]. While PLK2-depleted *SCO2*^{+/-} cells fail to engraft [4], the absence of p53 was sufficient to dramatically rescue the *in vivo* tumorigenicity of these cells, consistent with the decreased cell death observed *in vitro* (Figure 5C).

Discussion

The current study expands the functional repertoire of PLK2 activities beyond the regulation of the cell cycle. We have shown that the *PLK2* gene is transcriptionally responsive to two mitochondrial retrograde signals, calcium and ROS, as initially suggested by the presence of CRE and ARE in its promoter region. The biological significance of this signaling pathway was demonstrated by the marked increase in oxidative stress and necrosis upon PLK2 depletion in cells with mitochondrial dysfunction and by the opposing effects of PLK2 and p53 on the viability of these cells (Fig. 5D). Under conditions of increased oxidative stress, PLK2 appears to perform a dual function to promote cancer cell growth. PLK2 can reduce ROS levels by increasing NRF2 antioxidant activity, thereby relieving oxidative stress and

cell death. PLK2 can also phosphorylate Ser-137 of PLK1 permitting the bypass of DNA damage checkpoint and promoting cell cycle progression (Figure 5D) [4, 9, 10, 33].

Our observations on PLK2 regulation complement previous reports showing that another PLK family member, PLK3, can respond to oxidative stress and interact with p53 for tumor suppression [34, 35]. However, the role of PLK2 in tumorigenesis appears to be distinct from that of PLK3. PLK2 has been reported to phosphorylate mutant p53 to enhance its oncogenic activities, consistent with its role in improving cell viability that we have observed [36]. We have shown that the antioxidant activity of PLK2 can promote the survival of cancer cells with increased oxidative stress. It will be interesting to learn whether the same activity in normal cells contributes to the maintenance of genomic stability by preventing oxidative DNA damage.

It is becoming apparent that PLK2 may play important roles in post-mitotic neurons during the development of neurodegenerative diseases. The accumulation of α -synuclein, known to be phosphorylated by PLK2 [12, 13], and mitochondrial dysfunction are two notable changes that occur in the brain of patients with Parkinson's disease [14, 37]. Moreover, PLK2 levels have been reported to be increased in the neuronal processes of patients with Alzheimer's disease [13], another neurodegenerative condition associated with mitochondrial dysfunction and increased oxidative stress [38]. Given the homeostatic function of PLK2 in neurons [11], the elevated levels of phosphorylated α -synuclein in the cerebrospinal fluid of patients with Parkinson's disease may be a marker of PLK2 activation as an antioxidant response [37]. Alternatively, it is also possible that the abnormal accumulation of α -synuclein may contribute to Parkinson's disease by inhibiting PLK2 and subsequently disrupting redox homeostasis [39].

In summary, there is emerging evidence that a signaling pathway can have opposite effects depending on whether the disease process involves proliferating or post-mitotic cells that is relevant to tumorigenesis or neurodegeneration, respectively [40, 41]. PLK2-regulated redox homeostasis described in our current study may represent an example of this phenomenon. Our results suggest that treatments directed at restoring or maintaining mitochondrial function and ameliorating oxidative stress can be a double-edged sword. While the antioxidant activity of PLK2 could have detrimental consequences in neoplasia by promoting cancer cell proliferation, the same activity could have beneficial effects in neurodegenerative diseases by promoting neuronal survival.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

ARE	antioxidant response element
CRE	cAMP response element
PLK2	polo-like kinase 2
SCO2	synthesis of cytochrome c oxidase 2
GSK3	glycogen synthase kinase 3
NRF2	NFE2-related factor 2
NQO1	NAD(P)H quinine oxidoreductase
ROS	reactive oxygen species

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Highlights

1. PLK2 expression promotes the survival of cells with dysfunctional mitochondria.
2. PLK2 expression is increased by reactive oxygen species and calcium, two well known mitochondrial retrograde signals.
3. PLK2 phosphorylates GSK3 and activates the NRF2 antioxidant pathway.
4. The antioxidant activity of PLK2 plays an important role in preventing p53-mediated cell death.

Figure 1. Increased PLK2 expression and activation of GSK-NRF2 pathway are associated with mitochondrial dysfunction in SCO2 deficient cells

(A) Protein levels of SCO2, PLK2, and serine phosphorylated GSK3 (GSK3-S-P) (comprised of GSK3A and GSK3B, commonly known as α and β , respectively) in whole cell lysates, and NRF2 in the nuclear fraction (nuc) of the indicated cell lines. Tubulin and lamin B1 (LMNB1) serve as whole cell and nuclear fraction protein loading controls, respectively. (B) *In vitro* phosphorylation of GSK3B by PLK2 using purified recombinant proteins. Note the reciprocal relationship between the levels of the inactivating Ser-9 phosphorylation by PLK2 and the activating Tyr-216 autophosphorylation of GSK3B (GSK3 β -S9-P and GSK3 β -Y216-P, respectively). (C) Subcellular localization of NRF2 by confocal immunofluorescence (upper and middle panels) with DAPI staining of the nuclei (lower panel). The upper panels show enlarged images of the cells enclosed by white rectangles in the middle panels. At least 300 cells were counted in three independent experiments to quantify the fraction of cells with nuclear NRF2 localization. * $P < 0.05$, ** $P < 0.001$, $n=3$.

Figure 2. NRF2 promotes the survival of cells with defective mitochondria

(A) The *SCO2*^{+/+}, *SCO2*^{+/-} and *SCO2*^{-/-} cell lines were transduced with control non-specific (-) or NRF2-specific (+) shRNA lentivirus, and the nuclear (nuc) fraction of NRF2 protein visualized by western blotting. Lamin B1 (LMNB1) serves as protein loading control for the nuclear fractions. (B) After lentiviral transduction with shRNA to deplete NRF2 in the three isogenic cell lines, the indicated number of cells was plated and the resulting colonies were stained with crystal violet and quantified. **P* < 0.01, n=3.

Figure 3. PLK2 promotes the phosphorylation of GSK3 and the nuclear translocation of NRF2 (A) *SCO2*^{+/-} or *SCO2*^{-/-} cells with mitochondrial dysfunction were transduced with non-specific (-) or PLK2-specific (+) shRNA lentivirus and the relative levels of phosphoserine-GSK3A/B (α/β) in whole cell lysate and NRF2 in nuclear fraction (nuc) were evaluated by western blotting. (B) Effect of PLK2 knockdown on the nuclear localization of NRF2 by confocal immunofluorescence (upper panels) with DAPI staining of the nuclei (lower panels) in *SCO2*^{-/-} cells. (C) Lentiviral mediated over-expression of wild type (WT) or kinase-dead mutant (MUT, D223N mutation) PLK2 compared with empty-vector control (-) in *SCO2*^{+/-} cells. Note that MUT PLK2 can be abundantly over-expressed in contrast to WT PLK2 that has kinase activity. The indicated proteins in whole cell lysate and nuclear extract (nuc) samples are shown by western blotting with tubulin and LMNB1 serving as respective protein loading controls. (D) Subcellular localization of NRF2 by confocal immunofluorescence (upper panels) with DAPI staining of the nuclei (lower panels) in *SCO2*^{+/-} cells. Cells with NRF2 staining in their nuclei were quantified. At least 300 cells were counted in three independent experiments to quantify the fraction of cells with nuclear NRF2 localization. * $P < 0.05$, ** $P < 0.001$, $n=3$.

Figure 4. *PLK2* expression is responsive to oxidative stress and it has antioxidant activity that promotes the survival of cells with mitochondrial dysfunction

(A) Dose dependent effect of 4 h of H_2O_2 treatment on *PLK2* mRNA levels measured by RT-PCR in *SCO2*^{+/-} cells as a model of cells with moderate mitochondrial insufficiency. (B) Time course of *PLK2* expression after treatment with 100 μ M H_2O_2 . (C) *SCO2*^{+/-} cells were treated with 10 mM NAC and/or 3 mM BAPTA/AM to evaluate the role of intracellular oxidants and calcium signaling in regulating *PLK2* expression. (D) Endogenous ROS level was quantified by DCF FACS in *SCO2*^{+/-} cells transduced with *PLK2*-specific (+) shRNA compared to non-specific (-) shRNA control. (E) Effect of NAC treatment on colony formation of *SCO2*^{+/-} cells after *PLK2* depletion. (F) Whole cell NRF2 protein levels to confirm over-expression of mutant NRF2 (MUT NRF2) prior to colony formation

assay. Expression of NQO1 is shown as a marker of nuclear NRF2 activity in cells transiently transfected with MUT NRF2 versus control vector (CTL), with and without PLK2 depletion by shRNA lentivirus. **(G)** Effect of MUT NRF2 over-expression on the colony formation of *SCO2*^{+/-} cells with PLK2 depletion. **P* < 0.01, n=3.

Figure 5. The antioxidant activity of PLK2 prevents p53-induced necrosis and promotes the xenograft growth of cells with defective mitochondria

(A) Xenograft growth of *SCO2*^{+/-} cells transduced with NQO1-specific versus non-specific (NS) shRNA lentivirus. Xenograft images of two representative mice are shown, and the graph summarizes tumor growth rate after injection of the indicated cells into contra-lateral flanks of mice. n=10. (B) Effect of p53 on relative ROS levels and necrosis assessed by DCF FACS and HMGB1 release into media, respectively. The *p53* gene was knocked out in *SCO2*^{+/-} cells using somatic cell homologous recombination, and the cells were transduced with non-specific (NS) or PLK2 shRNA. n=4. (C) Knocking out *p53* rescues the xenograft growth of PLK2-depleted *SCO2*^{+/-} cells. Xenograft images of two representative mice injected with *SCO2*^{+/-} cells of the indicated *p53* genotype that were transduced with PLK2-specific shRNA lentivirus are shown and the graph summarizes xenograft tumor growth rate after injection of the indicated cells into contra-lateral flanks of mice. n=10. (D) Oxidative stress (ROS) generated as a consequence of mitochondrial dysfunction can activate both p53 and PLK2. The antioxidant signaling pathway of PLK2 promotes cell

survival by counteracting ROS and p53-mediated cell death. The dashed line indicates that multiple mediators are involved in causing p53 dependent cell death. $*P < 0.01$.