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DELETION OF THE MITOCHONDRIAL CHAPERONE TRAP-1 UNCOVERS GLOBAL REPROGRAMMING OF METABOLIC NETWORKS

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Abstract

Reprogramming of metabolic pathways contributes to human disease, especially cancer, but the regulators of this process are unknown. Here, we generated mice knockout for the mitochondrial chaperone TRAP-1, a regulator of bioenergetics in tumors. TRAP-1$^{-/-}$ mice are viable and showed reduced incidence of age-associated pathologies, including obesity, inflammatory tissue degeneration, dysplasia and spontaneous tumor formation. This was accompanied by global upregulation of oxidative phosphorylation and glycolysis transcriptomes, causing deregulated mitochondrial respiration, oxidative stress, impaired cell proliferation and a switch to glycolytic metabolism, in vivo. These data identify TRAP-1 as a central regulator of mitochondrial bioenergetics, and this pathway could contribute to metabolic rewiring in tumors.

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The authors declare that no conflict of interest exists.

SUPPLEMENTAL INFORMATION Supplemental information includes Supplemental Experimental Procedures, Supplemental Reference and Supplemental Figures S1–4.

Keywords

TRAP-1; mitochondria; chaperone; oxidative phosphorylation; glycolysis

INTRODUCTION

The control of protein folding in subcellular organelles, including mitochondria (Kang et al., 2007) maintains cellular homeostasis (Ellis, 2007) by buffering proteotoxic stress and ensuring flexible adaptation to environmental cues (Balch et al., 2008). In mitochondria, a network of chaperones of the Heat Shock Protein-90 (Hsp90) gene family (Kang et al., 2007), including Hsp90 (Taipale et al., 2010) and its related homolog TNFR-Associated Protein-1 (TRAP-1) (Lavery et al., 2014), protects against oxidative stress and permeability transition-induced apoptosis (Altieri et al., 2012). This pathway is exploited in cancer, where mitochondrial Hsp90s are dramatically upregulated, compared to normal tissues (Kang et al., 2007).

Recent evidence suggests that mitochondrial Hsp90s may play a more global role in organelle homeostasis. Mechanistically, this involves protein folding quality control of a discrete mitochondrial Hsp90 proteome, overseeing gene expression, protein translation, redox balance and a host of metabolic pathways (Chae et al., 2013). In this context, mitochondrial Hsp90s, including TRAP-1, preserve the association of the first enzyme of glycolysis, hexokinase II (HK-II) (Ward and Thompson, 2012) to the organelle outer membrane (Chae et al., 2012), and ensure the folding/stability of the oxidative phosphorylation Complex II subunit (Wallace, 2012), succinate dehydrogenase, SDHB (Chae et al., 2013). These functions are important for cellular homeostasis, as targeting TRAP-1 by pharmacologic or genetic approaches irreversibly compromises mitochondrial integrity, impairs both glycolysis (Chae et al., 2012) and oxidative phosphorylation (Chae et al., 2013), and may provide a novel anticancer strategy in humans (Altieri et al., 2012).

However, how TRAP-1 regulation of mitochondrial homeostasis fits with general mechanisms of tumor metabolic reprogramming (Ward and Thompson, 2012) has not been clearly delineated. Known as the “Warburg effect” (Ward and Thompson, 2012), most tumors rewire their energy sources towards aerobic glycolysis and at the expense of mitochondrial respiration (Wallace, 2012). Together with the identification of mutations in oxidative phosphorylation subunits that produce “oncometabolites” (Lu et al., 2012; Turcan et al., 2012), or stabilize oncogenes, such as HIF1α (Selak et al., 2005), this has suggested that mitochondrial bioenergetics has limited (if any) role in cancer (Ward and Thompson, 2012), and may actually function as a “tumor suppressor” (Frezza et al., 2011). In this context, the role of mitochondrial Hsp90s in bioenergetics has become controversial, as conflicting reports in the literature have claimed that TRAP-1 actually inhibits SDHB activity in tumor cells, promoting the accumulation of the oncometabolite, succinate (Sciacovelli et al., 2013), or, conversely, acts as a potential “tumor suppressor” through stimulation of glucose metabolism (Yoshida et al., 2013).

To clarify mechanisms of metabolic rewiring in mitochondria, and conclusively elucidate the function of mitochondrial Hsp90s in bioenergetics, we have now generated TRAP-1 knockout mice.

RESULTS

Generation of TRAP-1 knockout mice

Using gene-trap technology, we obtained mouse ES cells in which a β-galactosidase gene disrupts the TRAP-1 locus on chromosome 16, downstream of exon 1 (Figure 1A). Transfer of TRAP-1-targeted zygotes into C57Bl/6 pseudopregnant recipient mice generated TRAP-1 knockout animals. In genotyping experiments, PCR products of 565 nt, or, conversely, 1148 nt identified the wild type (WT) TRAP-1 allele or the β-galactosidase (LacZ)-containing allele, respectively (Figure 1B). TRAP-1 knockout (TRAP-$1^{-/-}$) mice were born viable, fertile, and at expected Mendelian rates for both genders. Analysis of tissues from TRAP-$1^{-/-}$ mice, including liver, kidney, uterus, spleen, brain, heart and testis confirmed the absence of TRAP-1 protein, compared to WT littermates, by Western blotting (Figure 1C). Histologic analysis of 15-month old TRAP-$1^{-/-}$ mice revealed significantly reduced incidence of multiple age-associated pathologies. Compared to age-matched WT littermates, these included lower body weight (Figure 1D) and organ (liver, spleen) hyperplasia (Figure S1A), decreased chronic inflammatory infiltrates in lung, stomach, pancreas, and small intestine (Figure S1B), lower hepatic lipidosis (Figure S1C), and reduced pancreatic fibrotic degeneration (Figure S1B). Two out of three WT mice examined at this age harbored spontaneous tumors or dysplastic lesions, including bronchoalveolar adenoma, dental dysplasia and histiocytic sarcoma (Figure S1D–H). In contrast, no tumors or dysplasia were detected in age-matched TRAP-$1^{-/-}$ mice. In terms of blood chemistry parameters, a complete blood count was unremarkable between the various animal groups (Figure S1I), whereas TRAP-$1^{-/-}$ mice showed decreased blood levels of glucose (see below) and creatinine, compared to WT animals (Figure S1J).

We next profiled signaling pathways in WT or TRAP-$1^{-/-}$ mice by Reverse Phase Protein Array (RPPA). Consistent with a role of mitochondria in “retrograde” signaling and nuclear gene expression (Butow and Avadhani, 2004), TRAP-1 deletion was associated with general reduction in growth factor receptor activity (MIG6, Her3, EGFR, MEK2, PI3K), attenuated TGFβ responses (Smad1, 3), modulation of cell-cell and cell-matrix communication (N-cadherin, E-cadherin, β-catenin, transglutaminase, fibronectin), and stimulation of cell cycle transitions (p21, Rb1, E2F1, Cyclin E1, PCNA) (Figure 1E). TRAP-1 deficiency also profoundly affected bioenergetics pathways. Consistent with recent observations (Chae et al., 2013), TRAP-1 loss resulted in significantly reduced levels of mitochondrial Complex II subunit, SDHB (Figure 1E). This was associated with reduced expression of fatty acid synthase, and mitochondrial Complex IV subunit, Cox-IV, upregulation of Complex V subunit, ATP5H, and higher levels of the glycolytic enzyme, G6PD (Figure 1E). Decreased Cox-IV expression in TRAP-$1^{-/-}$ mice *versus* WT samples was confirmed by Western blotting (Figure 1F). In addition, liver extracts of TRAP-$1^{-/-}$ mice showed increased recruitment of cytoprotective chaperones Hsp90 (Kang et al., 2007) and Hsp27 to

mitochondria, whereas levels of Hsp70, Hsp60 or VDAC were unchanged in WT or TRAP-1$^{-/-}$ mice (Figure 1F).

Requirement of TRAP-1 for mitochondrial oxidative phosphorylation

To examine a role of TRAP-1 in cellular respiration (Chae et al., 2013), we next used purified mitochondria from primary hepatocytes (Figure S2A) and non-transformed mouse embryonic fibroblasts (MEFs) (Figure S2B). In these experiments, citrate synthase-normalized (Figure S2C) mitochondrial Complex I activity was not significantly different between WT and TRAP-1$^{-/-}$ mice (Figure 2A), as assessed in three independent mitochondrial preparations (Figure 2B). Complex II activity, which was proposed to be inhibited by TRAP-1 (Sciacovelli et al., 2013), was instead unchanged between the two animal groups (Figure 2C–D). In addition, treatment with Gamitrinib, a small molecule antagonist that target TRAP-1/Hsp90 selectively in mitochondria (Chae et al., 2012), inhibited Complex II activity in WT mitochondria, but had no effect on TRAP-1$^{-/-}$ samples (Figure 2E), consistent with the absence of its target, TRAP-1, in these cells. Conversely, mitochondria isolated from TRAP-1$^{-/-}$ hepatocytes showed significantly increased activity of Complex III (Figure 2F–G), as well as Complex IV (Figure 2H–I), compared to WT samples. Consistent with these data, mitochondrial respiration was deregulated in TRAP-1$^{-/-}$ mice, and resulted in aberrantly increased oxygen consumption levels, compared to WT cultures (Figure 2J).

The mechanistic underpinning of deregulated cellular respiration in TRAP-1 knockout mice was further investigated. Accordingly, deletion of TRAP-1 resulted in a global compensatory upregulation of an oxidative phosphorylation “transcriptome”, with increased expression of virtually every subunit of mitochondrial respiration complexes (Figure S2D–H). Compared to WT littermates, this included an average fold increase of 1.31±0.03 (n=33) for Complex I subunits, 1.42±0.081 (n=4) for Complex II, 1.36±0.025 (n=7) for Complex III, and 1.3±0.06 (n=24) for Complex V (Figure S2D–F, H). Two subunits in Complex IV, Cox4i2 and Cox6b2, increased by approximately 4- and 20-fold, respectively, in TRAP-1$^{-/-}$ mice, compared to WT littermates, by array analysis (Figure S2G), as well as RT-qPCR (Figure S2I), resulting in a 2.86±1.34 (n=15) average fold increase for all Complex IV subunits. In contrast, overall mitochondrial DNA content was unchanged in WT or TRAP-1$^{-/-}$ mice (Figure S2J).

Glycolytic reprogramming in TRAP-1 knockout mice

In addition to mitochondrial oxidative phosphorylation, TRAP-1 has been linked to the regulation of glycolysis (Chae et al., 2012; Yoshida et al., 2013), and this function was next investigated. Similar to the changes in mitochondrial respiration (Figure S2C–G), but in contrast with a recent report (Yoshida et al., 2013), deletion of TRAP-1 resulted in uniformly upregulated expression of a glycolysis “transcriptome” (Figure S3). This involved an average fold increase of 2.36±0.33 (n=22) for regulators of glycolysis (Figure S3A), 2.28±0.4 (n=6) for glucose metabolism (Figure S3B), 1.38±0.11 (n=29) for the TCA cycle (Figure S3C), and 1.61±0.27 (n=4) for glycogen synthesis (Figure S3D). There was also an average fold increase of 1.78±0.26 (n=6) for molecules involved in glycogen degradation (Figure S3E), 1.52±0.044 (n=7) for gluconeogenesis (Figure S3F), and 1.42±0.19 (n=10) for

the pentose phosphate pathway (Figure S3G). Expression of sodium-independent glucose transporters Slc2a2 (Glut2) and Slc2a10 (Glut10) was also increased in TRAP-1$^{-/-}$ hepatocytes, whereas the levels of other glucose transporters, Slc2a4 (Glut4), Slc2a8 (Glut8) and Slc2a9 (Glut9) was unchanged, compared to WT cultures (Figure S4A, B). Consistent with these changes, TRAP-1$^{-/-}$ hepatocytes or MEFs dramatically switched their metabolism to aerobic glycolysis compared to WT controls, as reflected by higher glucose consumption (Figure 3A), and increased lactate production (Figure 3B).

To quantify glycolytic reprogramming, in vivo, we next carried out ^{18}F-FDG whole body PET/CT scans of WT or TRAP-1$^{-/-}$ mice. In these experiments, TRAP-1$^{-/-}$ mice exhibited a 2- to 3-fold increase in standardized uptake values (SUVs) of the radioactive tracer in the liver, compared to WT littermates, as determined 1 h post-injection of ^{18}F-FDG (Figure 3C, D). Consistent with these data, and together with the increased mitochondrial respiration associated with TRAP-1 deletion (Figure 2), hepatocytes or MEFs isolated from TRAP-1$^{-/-}$ mice exhibited greater ATP production than WT cultures (Figure 3E). Despite their switch to glycolytic metabolism, TRAP-1-deleted cells were as sensitive as WT cultures to apoptosis induced by glucose deprivation (Figure S4C), or treatment with the non-metabolizable glucose analog, 2-deoxyglucose (2-DG) (Figure S4D), potentially reflecting the higher levels of cytoprotective chaperones Hsp90 and Hsp27 in mitochondria (Figure 1F). To independently validate a role of TRAP-1 in glycolytic reprogramming, we next reconstituted WT or TRAP-1$^{-/-}$ MEFs with control vector or a Myc-TRAP-1 cDNA (Figure 3F). Transient re-expression of TRAP-1 was sufficient to normalize the glycolytic phenotype in these cells, lowering glucose consumption to the levels of WT MEFs (Figure 3G). In contrast, transfection of a TRAP-1 cDNA in WT cultures had no effect on glucose consumption (Figure 3G).

We next asked whether targeting TRAP-1 in tumor cells, which have typically a different bioenergetics profile from normal tissues (Ward and Thompson, 2012), also resulted in metabolic reprogramming. Transfection of prostate adenocarcinoma PC3 cells with four independent TRAP-1-directed siRNAs efficiently silenced TRAP-1 expression (Figure S4E), consistent with previous observations (Chae et al., 2012). Within six days of TRAP-1 silencing, these cells already exhibited compensatory increased recruitment of Hsp90 to mitochondria, compared to control transfectants (Figure S4E), thus similar to TRAP-1$^{-/-}$ mice (Figure 1F). Second, we established clones of cervical carcinoma HeLa cells selected for stable shRNA knockdown of TRAP-1 (Figure S4F). Analysis of these cells also revealed compensatory metabolic reprogramming, with increased oxygen consumption (Figure S4G), and higher ATP production (Figure S4H), compared to shRNA controls, in a response that correlated with the degree of TRAP-1 depletion (Figure S4F).

TRAP-1 regulation of oxidative damage

Deregulated mitochondrial function as observed in TRAP-1$^{-/-}$ cells may result in oxidative stress, and this possibility was next investigated. In these experiments, TRAP-1$^{-/-}$ MEFs exhibited a modest, but significant increased production of reactive oxygen species (ROS), compared to WT cultures (Figure 4A, Figure S4I). TRAP-1 deletion also resulted in consistently higher ROS production in response to increasing oxidative stress (H_2O_2),

compared to WT cultures (Figure S4J). Consistent with increased sensitivity to oxidative damage, TRAP-1 KO cells stained positive for markers of a DNA damage response, as reflected by increased reactivity for phosphorylated H2AX (γH2AX) (Figure 4B and C). Under these conditions, TRAP-$1^{-/-}$ MEFs exhibited profound defects in cell proliferation compared to WT cultures (Figure 4D), coinciding with cell cycle arrest at G2/M (Figure 4E), and accumulation of mitotic cyclin B1 (Figure 4F). In contrast, the expression of cyclin A or Cdk2 was unchanged, and p21 levels were reduced in TRAP-$1^{-/-}$ MEFs (Figure 4G), consistent with the data of RPPA profiling (Figure 1D).

DISCUSSION

In this study, we have shown that homozygous deletion of the mitochondrial chaperone, TRAP-1 causes global reprogramming of cellular bioenergetics, with compensatory upregulation of oxidative phosphorylation and glycolysis transcriptomes, increased mitochondrial accumulation of cytoprotective chaperones Hsp90 and Hsp27, and a dramatic switch to glycolytic metabolism, in vivo. This is accompanied by reduced incidence of age-associated pathologies, in vivo, including obesity, dysplasia and tumor formation, decreased cell proliferation, and low level ROS production.

The data presented here refute recent and contradictory claims that TRAP-1 inhibits mitochondrial SDHB-Complex II activity (Sciacovelli et al., 2013), or, conversely, promotes glycolysis (Yoshida et al., 2013). These preliminary suggestions were at odd with a large body of literature, in which pharmacologic or genetic targeting of TRAP-1 inhibited mitochondrial respiration (Butler et al., 2012; Chae et al., 2013), impaired mitochondrial quality control (Costa et al., 2013), caused oxidative damage (Butler et al., 2012; Pridgeon et al., 2007), and suppressed ATP production (Agorreta et al., 2014; Chae et al., 2012). Consistent with this model, we found that homozygous deletion of TRAP-1 resulted in decreased SDHB expression, reflecting loss of protein folding quality control in mitochondria (Chae et al., 2013). Mechanistically, this role of TRAP-1 in organelle protein homeostasis (Chae et al., 2013) emerged here as a critical requirement for mitochondrial bioenergetics. Accordingly, a compensatory upregulation of virtually every effector of oxidative phosphorylation and glycolysis in TRAP-$1^{-/-}$ mice was sufficient to restore Complex II activity, increase mitochondrial respiration through higher activity of Complex III and IV (Wallace, 2012), and impart a “pseudo-Warburg” glycolytic phenotype validated in whole body ^{18}F-FDG PET/CT analysis, in vivo. Together, these findings reinforce a pivotal role of TRAP-1 in maintaining mitochondrial homeostasis and bioenergetics (Chae et al., 2013), not suppressing it (Sciacovelli et al., 2013).

At variance with recent claims of TRAP-1 as a “tumor suppressor” (Yoshida et al., 2013), older TRAP-$1^{-/-}$ mice were instead significantly healthier than their age-matched WT littermates, with significantly reduced obesity, inflammatory and degenerative pathologies, or spontaneous dysplastic lesions, including tumor formation. More work is required to conclusively dissect the observed phenotype. However, increased mitochondrial respiration, as paradoxically induced as compensation for TRAP-1 loss (this study), has been associated with extended lifespan in model organisms (Guarente, 2008), potentially contributing to the increased longevity afforded by calorie restriction (Nisoli et al., 2005). In this context, low

levels of mitochondrial respiration-derived ROS, as demonstrated here in TRAP-1$^{-/-}$ mice, may activate “retrograde” gene expression mechanisms of adaptation and cytoprotection (Merksamer et al., 2013), and exert beneficial effects in aging (Schulz et al., 2007). In addition, the combination of a chronic DNA damage response, which opposes malignant transformation (Gorgoulis et al., 2005), and impaired proliferative capacity, as observed here in TRAP-1$^{-/-}$ cells, may further preserve organ integrity during aging.

A pivotal function of TRAP-1 in mitochondrial homeostasis, as reinforced here, suggests that models of extramitochondrial bioenergetics, i.e. aerobic glycolysis (Ward and Thompson, 2012) may not fully recapitulate the complexity of metabolic reprogramming in tumors (Wallace, 2012). In this context, TRAP-1-directed mitochondrial metabolism may be important to support highly energy-demanding traits of tumor cells, for instance cell invasion (Caino et al., 2013) or cell proliferation (this study), especially under conditions of chronic nutrient deprivation. In parallel, ATP produced under these conditions may blunt tumor suppression mechanisms driven by AMPK activation (Liang and Mills, 2013), escape autophagic cell death (Kimmelman, 2011), and preserve mTOR signaling (Guertin and Sabatini, 2007), all key requirements of tumor progression, in vivo. Consistent with this view, oxidative phosphorylation is being increasingly recognized as a key requirement of aggressive tumor behavior, preserving a cancer stem cell phenotype (Janiszewska et al., 2012), and promoting drug resistant disease (Haq et al., 2013). Together with its broad cytoprotective functions, these properties make TRAP-1 a uniquely attractive therapeutic target in cancer, suitable to disable multiple pathways of bioenergetics, cell survival and adaptation in genetically heterogeneous tumors (Altieri et al., 2012).

EXPERIMENTAL PROCEDURES

Generation of TRAP-1 knockout (KO) mice

All procedures involving animals were approved by an Institutional Animal Care and Use Committee (IACUC) at The Wistar Institute. A murine 129P2 ES clone E150H04, containing an insertional mutation downstream of the first exon of the TRAP-1 gene (rFlipROSAßgeo vector) was obtained from the German Gene Trap Consortium. Splinkerette PCR sequence of the ES clone is available at http://www.ncbi.nlm.nih.gov/nucgss/FI570935.1. Following pronuclear injections, zygotes were transferred to pseudopregnant C57BL/6 female mice and resulting chimeric male mice were crossed with C57BL/6 females to obtain mice heterozygous for the insertional mutation. The colony was maintained on a mixed B6/129 background by brother x sister mating.

Statistical analysis

Data were analyzed using the two-sided unpaired *t* tests using a GraphPad software package (Prism 4.0) for Windows. The Kolmogorov-Smirnov two-sample test was used to analyze standardized uptake values (SUV) in WT and TRAP-1$^{-/-}$ mice in PET/CT experiments using StatXact 9. Data are expressed as mean±SD or mean±SEM of replicates from a representative experiment out of at least 3 independent determinations. A p value of <0.05 was considered as statistically significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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HIGHLIGHTS

- TRAP-1 knockout mice have reduced age-associated pathologies
- Loss of TRAP-1 upregulates oxidative phosphorylation and glycolysis transcriptomes
- TRAP-1-deleted cells have deregulated mitochondrial respiration and enhanced glycolysis
- TRAP-1 deletion induces oxidative stress, DNA damage and reduced cell proliferation

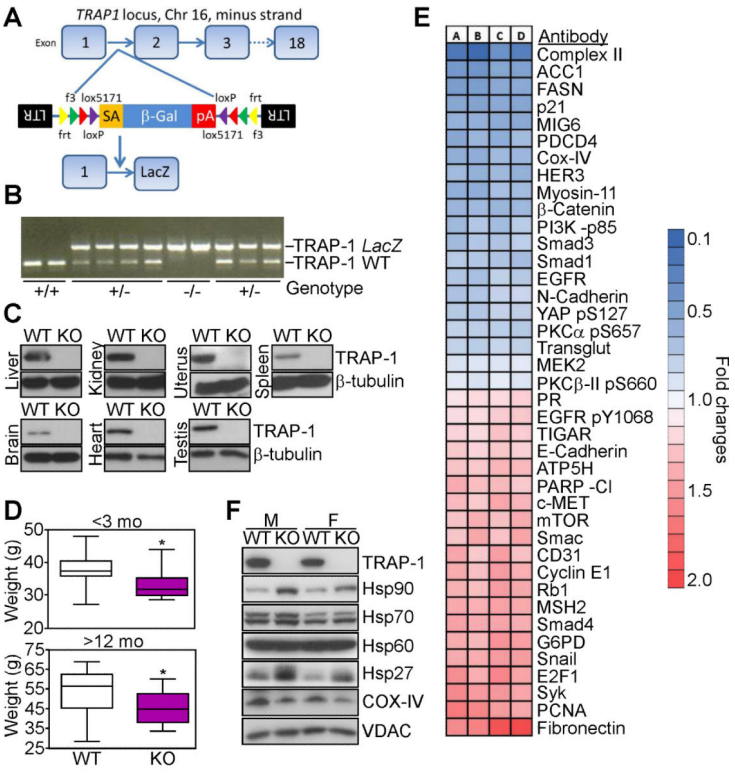

Figure 1. Characterization of TRAP-1$^{-/-}$ mice

(A) Map of the gene trapping construct and position of the β-galactosidase cassette inserted in the mouse TRAP-1 locus.

(B) PCR genotyping of TRAP-1 wild type (WT, +/+), heterozygous (+/−) and homozygous (−/−) mice. The position of WT and LacZ alleles is indicated.

(C) The indicated tissue extracts from WT or TRAP-1 knockout (KO) mice were analyzed by Western blotting.

(D) WT or TRAP-1 KO mice were analyzed for changes in body weight at the indicated ages (mo, months). Data are represented as box plots. Ages <3 mo (*top*), WT, n=16; KO, n=15; Ages>12 mo (*bottom*), WT, n=16; KO, n=26. *, p=0.033–0.022.

(E) Heatmap of changes in protein expression and/or phosphorylation in liver extracts isolated from WT or TRAP-1 KO mice (A–C, replicates; D, average), as determined by Reverse Phase Protein Array (RPPA). *Blue*, downregulated; *red*, upregulated. Only statistically significant changes ($p<0.05$) are shown.

(F) Liver extracts from WT or TRAP-1 KO mice were analyzed by Western blotting. M, male; F, female.

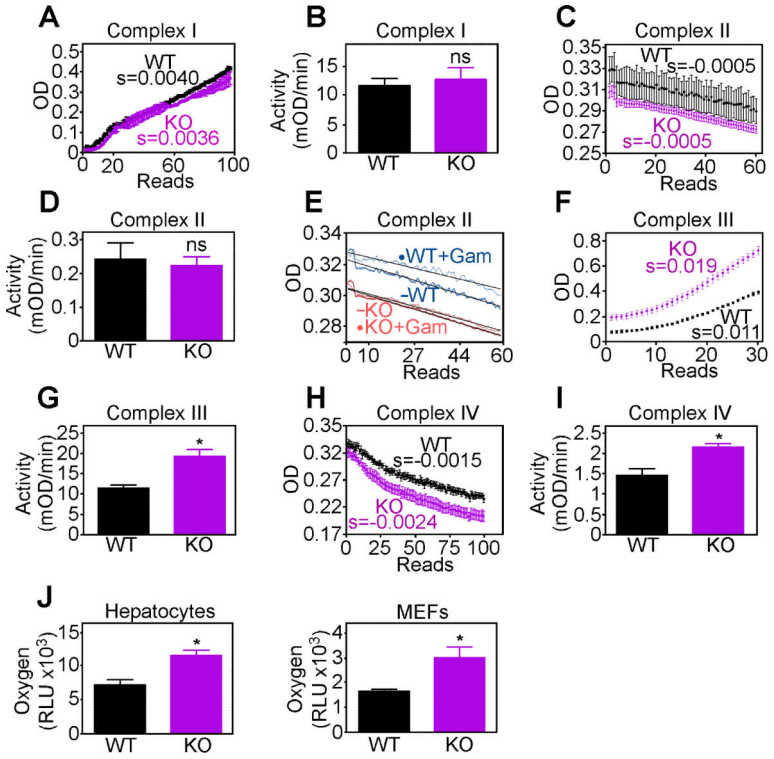

Figure 2. TRAP-1 regulation of mitochondrial oxidative phosphorylation

(A) Mitochondria isolated from WT or KO hepatocytes were analyzed for Complex I activity. The quantification of the slope (s) per each reaction is indicated. Representative experiment. (B) Quantification of Complex I specific activity in WT or KO mitochondria. Mean±SEM (n=3). ns, not significant (p=0.67).

(C, F, H) Representative experiments of mitochondrial Complex II (C), Complex II+III (F) or Complex IV (H) activity in mitochondria isolated from WT or KO hepatocytes. The quantification of the slope (s) per each reaction is indicated.

(D, G, I) Quantification of mitochondrial Complex II (D), Complex II–III (G) or Complex IV (I) specific activity in WT or KO hepatocytes. Data per each mitochondrial Complex activity were normalized against citrate synthase activity. Mean±SD (n=3). ns, not significant (p=0.66). *, p=0.027–0.033.

(E) Representative experiment of mitochondrial Complex II activity in the presence or absence of mitochondrial Hsp90 inhibitor, Gamitrinib (Gam) The slope of individual curves is as follows, WT-Gam, s=−0.0005; WT+Gam, s=−0.0004; KO-Gam, s=−0.0005; KO +Gam, s=−0.0005.

(J) WT or TRAP-1 KO hepatocytes (*top*) or mouse embryonic fibroblasts (MEFs) (*bottom*) were analyzed for oxygen consumption. Data were normalized for cell number by direct counting and cell viability by a fluorescence reporter. Mean±SEM of replicates of a representative experiment. *, p=0.015–0.036.

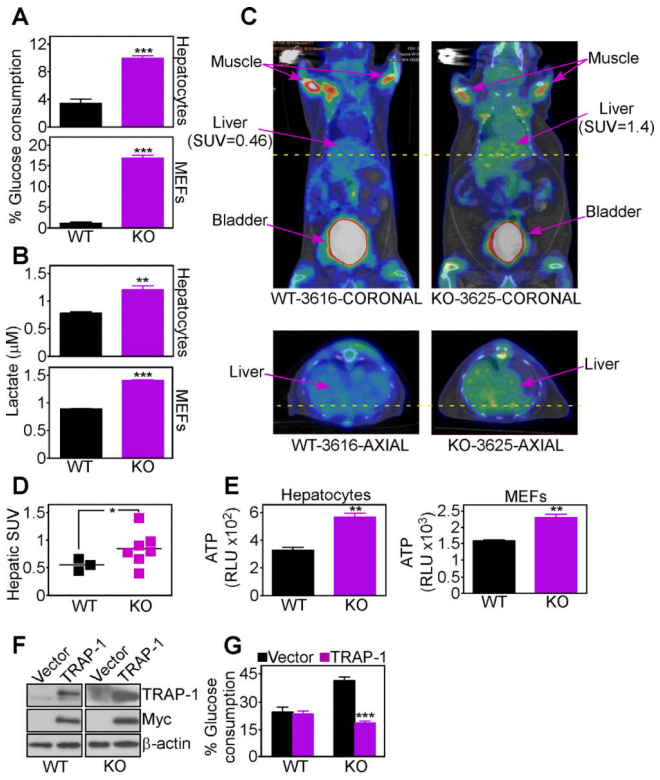

Figure 3. Glycolytic reprogramming induced by TRAP-1 deficiency

(A and B) Mitochondria isolated from WT or TRAP-1 KO hepatocytes (*top*) or MEFs (*bottom*) were analyzed for glucose consumption (A) or lactate generation (B). Data were normalized for cell number by direct cell counting and cell viability by a fluorescence reporter. Mean±SEM of replicates. ***, p=0.0001–0.0009; **, p=0.0057.

(C) Whole body PET/CT images of a WT (left) and TRAP-1 KO (right) mouse obtained 1 h post ^{18}F-FDG injection. Three times greater uptake of radioactivity in the liver of the TRAP-1 KO mouse represents increased glucose consumption. SUV, standardized uptake values.

(D) Quantification of hepatic standardized uptake values (SUVs) of ^{18}F-FDG as determined by PET/CT analysis in WT or TRAP-1 KO mice. Each point corresponds to an individual mouse. *, p=0.03 by Kolmogorov-Smirnov two-sample test.

(E) Mitochondria isolated from WT or TRAP-1 KO hepatocytes (*left*) or MEFs (*right*) were analyzed for ATP production. Mean±SEM of replicates. **, p=0.0025.

(F and G) WT or TRAP-1 KO MEFs were transfected with vector or Myc-TRAP-1 cDNA, and analyzed by Western blotting (F) or glucose consumption (G). Mean±SEM of replicates of a representative experiment. ***, p<0.0001.

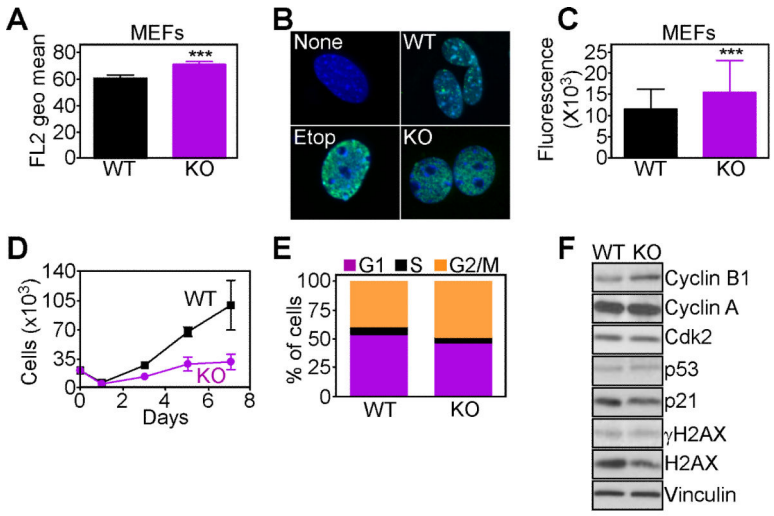

Figure 4. TRAP-1 deficiency induces low level oxidative damage and cell cycle defects

(A) WT or TRAP-1 KO MEFs were analyzed for ROS production by flow cytometry. Data are expressed as mean geometric fluorescence. Mean±SEM of replicates of a representative experiment. ***, p=0.0001.

(B and C) WT or TRAP-1 KO MEFs were analyzed for γH2AX reactivity by fluorescence microscopy (B), and quantified as normalized mean fluorescence (C). Nuclei were stained with DAPI. None, untreated. Etop, etoposide. Magnification, x60. ***, p=0.0003.

(D) WT or TRAP-1 KO MEFs were analyzed for cell proliferation at the indicated time intervals by direct cell counting. Mean±SEM of replicates. Representative experiment.

(E) WT or TRAP-1 KO MEFs were stained with propidium iodide and analyzed for DNA content by flow cytometry. The percentage of cells in the various cell cycle transitions is indicated.

(F) WT or TRAP-1 KO MEFs were analyzed by Western blotting.