Parkin regulates adiposity by coordinating mitophagy with mitochondrial biogenesis in white adipocytes

Parkin, an E3 ubiquitin ligase, plays an essential role in mitochondrial quality control. However, the mechanisms by which Parkin connects mitochondrial homeostasis with cellular metabolism in adipose tissue remain unclear. Here, we demonstrate that Park2 gene (encodes Parkin) deletion specifically from adipose tissue protects mice against high-fat diet and aging-induced obesity. Despite a mild reduction in mitophagy, mitochondrial DNA content and mitochondrial function are increased in Park2 deficient white adipocytes. Moreover, Park2 gene deletion elevates mitochondrial biogenesis by increasing Pgc1α protein stability through mitochondrial superoxide-activated NAD(P)H quinone dehydrogenase 1 (Nqo1). Both in vitro and in vivo studies show that Nqo1 overexpression elevates Pgc1α protein level and mitochondrial DNA content and enhances mitochondrial activity in mouse and human adipocytes. Taken together, our findings indicate that Parkin regulates mitochondrial homeostasis by balancing mitophagy and Pgc1α-mediated mitochondrial biogenesis in white adipocytes, suggesting a potential therapeutic target in adipocytes to combat obesity and obesity-associated disorders.
knockdown 3T3-L1 preadipocytes (Parkin<sup>−/−</sup>) and a mouse model by deleting the Park2 gene specifically in adipose tissue (Parkin<sup>−/−</sup>). We determined that adipose-specific Park2 deletion prevents both high-fat diet (HFD) and aging-induced obesity. Mechanistic studies revealed that Parkin deletion slightly impaired mitophagy in adipocytes and elevated mitochondrial superoxide levels, which increased the protein level of Nqo1. Moreover, Nqo1 enhances the protein stability of Pgc1α and accelerates mitochondrial biogenesis. Collectively, our findings suggest that Parkin regulates mitochondrial homeostasis in white adipocytes and Parkin is a potential therapeutic target for combating obesity.

**Results**

**Parkin is elevated during adipogenesis**

Mitophagy, the degradation of mitochondria by autophagic machinery, controls beige adipocyte maintenance<sup>21</sup>. However, the role of mitophagy in white adipocytes and how it relates to adiposity are not well elucidated. We identified over 70 single nucleotide polymorphisms (SNPs) near or within the human PARK2 gene (encodes Parkin protein) positively associate with BMI in a series of genome-wide association studies (GWAS) from PhenoScanner (a database of human genotype-phenotype associations)<sup>24,25</sup>. Therefore, we examined whether mitophagy-related genes Park2 and Park6 are altered during murine adipocyte differentiation. We found that both Park2 and Park6 genes were markedly elevated during the differentiation of 3T3-L1 and10T1/2 cells as well as in primary adipocytes of iWAT and brown adipose tissue (BAT) (Fig. 1a,b, Supplementary Fig. 1a,b). Consistently, Parkin protein levels were increased in mature adipose tissues (IWAT and eWAT) and differentiated adipocytes in multiple fat depots (Fig. 1c–e). To determine whether Parkin level is elevated during diet-induced adipogenesis, we fed both male and female wild-type (WT) mice with HFD for 6 weeks. Parkin protein level was only elevated in eWAT of male, but not female WT mice (Fig. 1f; Supplementary Fig. 1c). Increased Park2 gene expression during the differentiation of 3T3-L1 cells indicates potentially enhanced Parkin-mediated mitophagy. However, this finding was not accordant with elevated mitochondrial DNA (mtDNA) content that we and others have found (Supplementary Fig. 1d)<sup>26,27</sup>. These potentially conflicting findings suggest that Parkin

![Image](https://example.com/image.png)

**Fig. 1** | Parkin is elevated in white adipocytes during adipogenesis and by HFD feeding of mice. **a, b** mRNA level of Park2 during differentiation of 3T3-L1 and10T1/2 adipocytes and primary adipocytes from IWAT and BAT of WT mice (n = 3 biological replicate) [Unpaired Student’s t test two-tailed. For 3T3-L1 D0 vs. D10, p < 0.0001, 95%CI = 15.58–19.50, R squared = 0.9936; for 10T1/2 D0 vs. D10 p = 0.0207, 95%CI = 0.8711–3.087, R squared = 0.9922; for IWAT, p = 0.0006, 95%CI = 11.56–20.54, R squared = 0.9610, for BAT D0 vs. D4, p < 0.0001, 95%CI = 1.913–2.452, R squared = 0.9922; for BAT D4 vs. D6, p > 0.0015, 95% CI = 6.284–13.26, R squared = 0.9379]. Western blot analysis (top of panel) and corresponding densitometric quantification (bottom of panel; normalized to Actin) of Parkin in (e) eWAT, (d) iWAT vs. SVF fractions [Unpaired Student’s t test two-tailed, for iWAT SVF vs. iWAT, p = 0.0113, 95%CI = 0.7122–3.087, R squared = 0.8334]; (e) differentiated primary adipocytes from eWAT SVF fractions at day 0, 6, and 10 (n = 3 WT mice per group). Western blot analysis of Parkin in eWAT from normal chow-fed vs. HFD-fed male WT C57BL6/J mice. The bar graph on the right is the densitometric quantification of Parkin protein level normalized to Actin (n = 5 mice for NC group, n = 6 for HFD group) [Unpaired Student’s t test two-tailed. For NC vs. HFD, p = 0.0254, 95%CI = 1.670–1.997, R squared = 0.4429]. Data are presented as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. ND = not detectable. AU = arbitrary units. Source data are provided as a Source Data file.
male ParkinKO mice showed reduced body weight and fat mass (eWAT, iWAT, and BAT (Supplementary Fig. 2e, f). We found that body and skeletal muscle, although adiponectin cre was highly expressed in Glucose tolerance test on 6 weeks of HFD-fed Control f/f and ParkinAdi mice (c) significantly reduced in the eWAT of ParkinAdi mice but not in iWAT, BAT, liver, Parkin deletion in adipose tissue indicates a reduction in adipocyte size in eWAT, iWAT, and BAT (Fig. 2c, d). Moreover, histological analysis of the may be involved in the dynamic regulation of mitochondrial homeostasis during adipogenesis.

**Parkin deletion in adipose tissue prevents HFD and aging-induced obesity**

To understand the role of Parkin in adipogenesis, we studied both female and male whole-body Parkin knockout mice (ParkinKO). Only male ParkinKO mice showed reduced body weight and fat mass (Supplementary Fig. 2a–d), indicating a sexual dimorphism with respect to the role of Parkin in regulating fat mass. Therefore, we will exert the following experiments on male mice. To further explore the role of Parkin in male adipose tissue, we generated adipose tissue-specific Park2 knockout mice (ParkinKO). Parkin protein levels were dramatically reduced in the eWAT of ParkinKO mice but not in iWAT, BAT, liver, and skeletal muscle, although adiponectin cre was highly expressed in eWAT, iWAT, and BAT (Supplementary Fig. 2e, f). We found that body weight and fat mass were significantly lower than the control f/f mice after HFD feeding, suggesting that Parkin deletion in adipose tissue is protective against diet-induced obesity (Fig. 2a, b; Supplementary Fig. 2g, h). Both glucose tolerance and insulin tolerance were improved in ParkinKO mice (Fig. 2c, d). Moreover, histological analysis of the adipose tissue indicates a reduction in adipocyte size in eWAT, iWAT, and BAT (Fig. 2e).

To understand the impact of Parkin deletion on the transcriptome in white adipose tissue, we performed RNA-seq and enrichment analysis on eWAT from HFD-fed control f/f and ParkinKO mice. The downregulated genes were enriched in immune-related pathways and biological processes, including macrophage migration and leukocyte regulation (Fig. 2f). Indeed, we confirmed reduced macrophage infiltration in the eWAT of ParkinKO mice by immunofluorescent staining of F4/80, Perilipin 1, and DAPI in the eWAT of HFD-fed Control f/f and ParkinKO mice (n = 3 mice per genotype) [Unpaired Student’s t test two-tailed. p < 0.0001, 95% CI = −35.78 to −20.24, R squared = 0.3547]. f Volcano plots and enrichment analysis of RNA sequencing results from eWAT of HFD-fed ParkinKO vs. Control f/f mice (n = 4 mice per genotype). g Immunofluorescent staining of F4/80, Perilipin 1, and DAPI in the eWAT of HFD-fed Control f/f and ParkinKO mice (n = 5 mice per genotype) [Unpaired Student’s t test two-tailed. p = 0.00020, 95% CI = −3.519 to −1.558, R squared = 0.9281]. Data are presented as mean ± SEM. * p < 0.05, ** p < 0.01, *** p < 0.001. Source data are provided as a Source Data file.
For EE night, f/f-NC group, CI = 0.1890. Hsl protein in eWAT of HFD-fed Control f/f and ParkinAdi mice (Western blots and densitometric analysis of Atgl, phospho-Hsl Ser563, and total f/p). VO2 night, CO2 production, (c) energy expenditure (EE) [Unpaired Student’s t test two-tailed. For EE night, p = 0.0366, 95% CI = 0.1279–0.3358, R squared = 0.4397] and (d) respiratory exchange ratio (RER) [Unpaired Student’s t test two-tailed. For RER night, p = 0.0166, 95% CI = 0.04921 to 0.060697, R squared = 0.5327] of male Control f/f and ParkinAdi mice fed an HFD for 4 weeks (n = 5 mice per genotype). e mRNA levels of lipid metabolism genes in eWAT of HFD-fed Control f/f and ParkinAdi mice (n = 7 mice for Control f/f group, n = 5 mice for ParkinAdi group) [Unpaired Student’s t test two-tailed. For Srebptc, p = 0.0045, 95% CI = 0.1890–0.3345, R squared = 0.4744; for Acc1, p = 0.0245, 95% CI = 0.1441–1.772, R squared = 0.3322; for Ppara, p = 0.0391, 95% CI = 0.04892–1.625, R squared = 0.2882; for Mtp, p = 0.0216, 95% CI = 0.07500–2.348, R squared = 0.3101]. f Western blots and densitometric analysis of Atgl, phospho-Hsl Ser563, and total Hsl protein in eWAT of HFD-fed Control f/f and ParkinAdi mice (n = 6 mice per genotype) [Unpaired Student’s t test two-tailed. For p-HSL/t-HSL, p = 0.0094, 95% CI = 0.3094–2.529, R squared = 0.50701]. g mRNA levels of mitochondrial and related genes in the eWAT of WT mice fed with NC and HFD (n = 5 mice per diet) [Unpaired Student’s t test two-tailed. For mtnd1, p = 0.0346, 95% CI = −1.403 to −0.66850, R squared = 0.4470; for mtnd4, p = 0.0258, 95% CI = −1.235 to −0.1119, R squared = 0.4856; for mtnd6, p = 0.0377, 95% CI = −1.417 to −0.05340, R squared = 0.4360; for mtco1, p = 0.0446, 95% CI = −1.045 to −0.01642, R squared = 0.4145]. h Relative mRNA copy number in the eWAT of mice fed with NC and HFD (n = 7 per diet) [Unpaired Student’s t test two-tailed. For Atgl, p = 0.0274, 95% CI = 0.5089 to −0.03584, R squared = 0.34431]. l mRNA levels of mitochondrial and related genes in eWAT of Control f/f and ParkinAdi mice fed with NC and HFD (n = 7 mice for Control f/f-NC group, n = 5 mice for ParkinAdi-NC group, n = 5 mice for Control f/f-HFD group, n = 6 mice for ParkinAdi-HFD group) [Unpaired Student’s t test two-tailed. For TjaA Control f/f-NC vs. ParkinAdi-NC, p = 0.0018, 95% CI = 0.1784–1.1819, R squared = 0.4320; for TjaA Control f/f-HFD vs. ParkinAdi-HFD, p = 0.0282, 95% CI = 0.03684–0.5148, R squared = 0.4310; for mtco3 Control f/f-HFD vs. ParkinAdi-HFD, p = 0.0463, 95% CI = 0.004537–0.4377, R squared = 0.3721; for mtnd1 Control f/f-NC vs. ParkinAdi-NC, p = 0.0204, 95% CI = 0.2213–2.511, R squared = 0.4141, for mtnd1 Control f/f-HFD vs. ParkinAdi-HFD, p = 0.0194, 95% CI = 0.03483–0.3077, R squared = 0.4725; for mtnd6 Control f/f-NC vs. ParkinAdi-NC, p = 0.0397, 95% CI = 0.08034–2.712, R squared = 0.3585; for mtnd6 Control f/f-HFD vs. ParkinAdi-HFD, p = 0.0298, 95% CI = 0.2004–3.138, R squared = 0.3907]. j, k Relative mtDNA copy number in the eWAT of Control f/f and ParkinAdi mice fed with NC and HFD (n = 6 mice per genotype per diet) [Unpaired Student’s t test two-tailed. For Control (Scr) vs. ParkinKD, p = 0.0240, 95% CI = 0.2213–1.772, R squared = 0.4744; for Dmnd1, p = 0.0424, 95% CI = 0.0196–0.7033, R squared = 0.3410]. L Mitochondrial oxygen consumption rate of preadipocytes (dash line) and differentiated adipocytes (solid line) of 3T3-L1 cells (n = 4 biological replicate) [Unpaired Student’s t test two-tailed. For Control (Scr) vs. ParkinKD, p = 0.0144, 95% CI = 0.4457–2.267, R squared = 0.8104]. M Normalized mitochondrial membrane potential (TMRE/mitochondrial tracker green (MTG)) of the differentiated 3T3-L1 Control (Scr) and ParkinAdi adipocytes treated with vehicle (DMSO) and FCCP (n = 3 biological replicate) [Unpaired Student’s t test two-tailed. For Control (Scr) vs. ParkinAdi, p = 0.0424, 95% CI = 0.0599–0.7033, R squared = 0.6835]. Data are presented as mean ± SEM. AU = arbitrary units. Oligo = oligomycin, FCCP = trifluoromethoxy carbonylcyanide phenylhydrazone, R/A = rotenone/antimycin a. * p < 0.05, ** p < 0.01, *** p < 0.001. Source data are provided as a Source Data file.
Parkin-mediated mitophagy is pivotal for metabolic health. Although ParkinAdi mice show reduced fat weight gain during HFD-feeding compared to control f/f mice, the long-term effects of Parkin deletion under normal dietary conditions are unknown. To examine the impact of adipose tissue Parkin deletion throughout life, we investigated whether this genetic manipulation would impact aging-associated weight gain. ParkinAdi mice showed a reduction in body weight throughout adulthood without perturbation of lifespan (Supplementary Fig. 2k). Although adipocyte size was not altered between genotypes, ParkinAdi mice showed improved glucose and insulin tolerance at 9 months old but not 5 months old (Supplementary Fig. 2l–o). Taken together, adipose tissue-specific Parkin deletion prevents both HFD-feeding and aging-induced adiposity and disrupted glucose homeostasis.

**Loss of Parkin in BAT fails to improve glucose homeostasis**

Cold stress stimulates mitophagy in brown adipose tissue (BAT) [22,30,31]. Consistently, we found cold stress strikingly increased *Ucp1* gene expression but decreased *Park6* and *Park2* gene expression in BAT (Supplementary Fig. 3a). Because we found ParkinAdi mice had higher BAT of ParkinBat mice, fat mass and glucose homeostasis were not significantly increased in ParkinAdi mice. Parkin protein levels were reduced in BAT but not in other tissues (Supplementary Fig. 3c). Although mtDNA content was significantly increased in BAT of ParkinBat mice, fat mass and glucose homeostasis were not different between genotypes of mice fed with NC or HFD (Supplementary Fig. 3d–g), suggesting that Parkin expression in BAT does not contribute to the lean phenotype and improved glucose homeostasis observed in ParkinAdi and ParkinKO mice.
Parkin deletion increases energy expenditure and mitochondrial activity

To elucidate the mechanisms by which Parkin controls metabolism, we performed indirect calorimetry and found increased oxygen consumption and energy expenditure but reduced RER during the dark phase in body weight-matched Parkin<sup>−/−</sup> vs. control f/f mice (Supplementary Fig. 4a, b). This occurred independent of food intake, water consumption, and physical activity (Fig. 3a–d; Supplementary Fig. 4c–i).

Reduced RER in Parkin<sup>−/−</sup> mice suggests an increased utilization of lipid for energy production. This observation led us to further analyze the lipid metabolism in the adipose tissues. We found that lipid...
Fig. 6 | Overexpression of Nqo1 enhances mitochondrial biogenesis in vitro and in vivo. a mtRNA level of Park2 and Nqo1 in eWAT of WT and Ob/+ mice (n = 6 mice for each group, except one sample in Ob/+ group with undetectable Nqo1 gene expression) [Unpaired Student’s t test two-tailed. For Nqo1 WT vs. Ob/+, p = 0.0276, 95%CI = -0.1921 to 2.596, R squared = 0.4334]. b Western blot analysis of Nqo1 in eWAT of WT and Ob/+ mice (n = 6 mice for each group). c Oil Red O staining and the quantification of Oil Red O in differentiated 3T3-L1 Control (Scr) and Nqo1OE adipocytes (n = 9 biological replicate, scale bars = 200 μm) [Unpaired Student’s t test two-tailed, p = 0.0001, 95%CI = -0.3572 to 0.2469, R squared = 0.8939]. d mRNA level of mitochondrial and related genes in the differentiated 3T3-L1 Control (Scr) and Nqo1OE adipocytes (n = 3 biological replicate) [Unpaired Student’s t test two-tailed. For Pgc1α, p = 0.0030, 95%CI = 1.988–5.001, R squared = 0.9120; for mtND1, p = 0.0333, 95%CI = 0.09336–1.359, R squared = 0.7175; for mtND4, p = 0.0341, 95% CI = 0.06033–0.9301, R squared = 0.7142; for mtND4L, p = 0.0362, 95% CI = 0.05190–0.9380, R squared = 0.7063]. e Western blot and densitometry analysis of Pgc1α and OXPHOS proteins (normalized to Hsp90 or GAPDH) in the differentiated 3T3-L1 Control (Scr) and Nqo1OE adipocytes (n = 3 biological replicate) [Unpaired Student’s t test two-tailed. For Pgc1α, p = 0.0475, 95%CI = 0.01028–1.129, R squared = 0.6665; for mtCo1, p = 0.0120, 95% CI = 0.1436–0.6442, R squared = 0.8267]. f mtDNA content of the differentiated 3T3-L1 Control (Scr) and Nqo1OE adipocytes (n = 3 biological replicate) [Unpaired Student’s t test two-tailed. p = 0.0108, 95%CI = 0.2180–0.9195, R squared = 0.8351]. g Mitochondrial oxygen consumption rate of the differentiated 3T3-L1 Control (Scr) and Nqo1OE adipocytes (n = 3 biological replicate). [Unpaired Student’s t test two-tailed. For 31 min Control (Scr) vs. Nqo1OE, p = 0.0278, 95%CI = 0.03481–9.814, R squared = 0.4737; For 43 min Control (Scr) vs. Nqo1OE, p = 0.0200, 95%CI = 0.1247–10.99, R squared = 0.5119]. h Experimental design for AAV8 injection in HFD-fed male WT mice. Created with BioRender.com. i Western blot and densitometry analysis of Nqo1 and Pgc1α proteins (normalized to Actin) in eWAT of AAV8-Control or AAV8-Nqo1 injected mice (n = 7 mice per group) [Paired Student’s t test two-tailed, t = 3.058, p = 0.0223, 95%CI = 0.1673–1.580, R squared = 0.6091]. j H&E-stained sections of the eWAT from AAV8-Control or AAV8-Nqo1 injected mice; scale bars = 50 μm; The dot graph on the right is the quantified adipocyte area from H&E-stained sections of the eWAT from HFD-fed Control l/f and ParkinAdi mice (n = 6 mice for AAV8-Control group, n = 7 for AAV8-Nqo1 group, total1603-1932 adipocytes) [Unpaired Student’s t test two-tailed, p = 0.0296, 95%CI = -0.9156 to -0.476, R squared = 0.001345]. k Western blot and densitometry analysis of OXPHOS protein levels (normalized to Hsp90) in eWAT of AAV-Control or AAV8-Nqo1 injected mice (n = 5 mice per group) [Paired Student’s t test two-tailed. For Sdhb, t = 3.916, p = 0.0173, 95% CI = 0.1716–1.008, R squared = 0.7931; for Ndub8, t = 2.886, p = 0.0447, 95% CI = 0.03842–1.988, R squared = 0.6735]. l mtDNA content of the eWAT from AAV8-Control or AAV8-Nqo1 injected mice (n = 5 mice per group) [Paired Student’s t test two-tailed. t = 4.023, p = 0.0118, 95% CI = 0.1896–1.034, R squared = 0.8018]. m Heatmap of mtDNA encoded genes from RNA sequencing results in AAV8-Control or AAV8-Nqo1 injected eWAT (n = 3 mice per group). Data are presented as mean ± SEM. AU = arbitrary units. Oligo = oligomycin, FCCP = trifluoromethoxy carbonylcyanide phenylhydrazone, R/A = rotenone/antimycin a. * p < 0.05, ** p < 0.01. Source data are provided as a Source Data file.

transfer gene Mtpp, lipid synthesis genes (Srebp1c and Acc1), and fatty acid oxidation genes (Ppara, Cptla, Cptlb) were significantly increased in eWAT of Park1hsd vs. control l/f mice (Fig. 3e). In addition, the protein levels of phosphorylated hormone-sensitive lipase (HSL) at Ser563 were robustly elevated in the eWAT of Parkinhsd mice (Fig. 3f). Collectively, Parkin deletion alters the gene expression that impact lipid synthesis and oxidation in eWAT and could suggest alterations in mitochondrial oxidative metabolism.
We and others have shown that mitochondrial mass and function are impaired in the context of obesity and type-2 diabetes in humans and rodents\textsuperscript{13,14} (Fig. 3g,h; Supplementary Fig. 4j). Furthermore, we found that both mtDNA encoded genes and mtDNA copy number were elevated regardless of diet or fat pad depot in Parkin\textsuperscript{KO} compared with control f/f mice (Fig. 3i, j; Supplementary Fig. 4k, l). To explore the impact of Parkin deletion on mitochondrial function, we knocked down the Park2 in 3T3-L1 cells (Parkin\textsuperscript{KD}) and found mtDNA copy number was increased 2.3 folds in Parkin\textsuperscript{KD} vs. control (Scr) adipocytes (Supplementary Fig. 4m, n, 4p). Increased mtDNA copy number was also observed in the eWAT of whole-body Parkin knockout mice (Parkin\textsuperscript{KO}) (Supplementary Fig. 4m, n, 4p).

Consistent with our mouse studies, Parkin\textsuperscript{KD} adipocytes also displayed a reduction in lipid levels (Supplementary Fig. 4q). Moreover, increased mitochondrial oxygen consumption rate in both Parkin\textsuperscript{KD} preadipocyte and adipocytes measured by the Seahorse XF analyzer indicates mitochondrial function was enhanced as a consequence of Parkin reduction (Fig. 3i). We also observed a dramatic increase in mitochondrial membrane potential and aspect ratio (Fig. 3m; Supplementary Fig. 4r), reflecting a healthy mitochondrial population in Parkin\textsuperscript{KD} adipocytes. Taken together, our in vitro and in vivo findings confirm our observation in mice that reduction in Parkin expression promotes mitochondrial function in adipocytes.

**Parkin mediates both mitophagy and mitochondrial biogenesis in adipocytes**

Mitochondrial content is regulated by a balance between mitophagy and mitochondrial biogenesis\textsuperscript{5}. Because mitophagy relies on the autophagic machinery and our data suggests that lacking Parkin impacts mitochondrial abundance and function, we next examined the protein levels of autophagy markers p62 and LC3B/I/II. Only p62 protein was reduced but no change in the protein levels of LC3B and LC3BII in eWAT of HFD-fed Parkin\textsuperscript{KO} vs. control f/f mice (Fig. 4a, b). Although mitophagy heavily relies on macro-autophagy, alterations in macro-autophagy makers do not necessarily mean that mitophagy is impacted. Therefore, we decided to directly monitor and quantify the mitophagy using GFP-mCherry-Fis1 (mito-QC reporter). Red fluorescent protein (RFP) signal is typically maintained despite the acidic lysosomal environment (pH 4–5) whereas green fluorescent protein (GFP) loses fluorescence in this pH range\textsuperscript{33,34}. Using the adenoviral GFP-mCherry-Fis1, we assessed the turnover of mitochondria by lysosome and observed a decreased number of mitolysosome puncta indicating a reduction of mitophagy in Parkin\textsuperscript{KD} vs. control (Scr) adipocytes (Fig. 4c, d). Next, we assessed the protein levels of mitochondrial biogenesis markers. Surprisingly, the protein level of Pgc1\x3c\x203a in both eWAT of HFD-fed Parkin\textsuperscript{KO} mice and Parkin\textsuperscript{KD} adipocytes compared with controls (Fig. 4e, f). The protein levels of voltage-dependent anion selective channel 1 (Vdac1) and subunit b of succinate dehydrogenase (Sdhb) were also significantly elevated, but Parkin-interacting substrate (Paris) was not altered in Parkin\textsuperscript{KO} mice (Fig. 4e; Supplementary Fig. 5a). Because the gene expression of Pgc1\x3c\x203a was not changed in eWAT of both NC and HFD-fed Parkin\textsuperscript{KO} mice (Fig. 3i), we hypothesized that Parkin deletion may promote post-translational modification of Pgc1\x3c\x203a to enhance the protein stability. Next, we blocked protein synthesis with cycloheximide (CHX) and determined that the protein level of Pgc1\x3c\x203a was elevated in Parkin\textsuperscript{KD} adipocytes (Fig. 4g). These findings suggest that Parkin may control mitochondrial homeostasis by coordinating mitophagy with mitochondrial biogenesis in adipocytes.

**Elevated Nqo1 stabilizes Pgc1\x3c\x203a protein in Parkin knockdown adipocytes**

To identify the mechanisms underlying Pgc1\x3c\x203a protein stability in the context of Parkin knockdown, we investigated the impact of Nqo1 expression on Pgc1\x3c\x203a in adipocytes, as Nqo1 was previously shown to modulate Pgc1\x3c\x203a protein\textsuperscript{35}. Both the protein and mRNA levels of Nqo1 were elevated in eWAT of HFD-fed Parkin\textsuperscript{KO} mice and Parkin\textsuperscript{KD} adipocytes (Fig. 5a–d). Similar to Pgc1\x3c\x203a, Nqo1 protein stability was also significantly elevated in Parkin\textsuperscript{KD} adipocytes following treatment with the protein synthesis inhibitor cycloheximide (CHX) (Fig. 5e).

Nqo1 is known to interact with Pgc1\x3c\x203a in an NADH-dependent manner. To confirm the protein association of Pgc1\x3c\x203a and Nqo1 in adipose tissues, we performed co-immunoprecipitation using eWAT protein lysates of WT mice. We determined that Pgc1\x3c\x203a associates with Nqo1 in white adipose tissues (Fig. 6f). To further confirm Nqo1 preserves the protein level of Pgc1\x3c\x203a, we next tested whether pharmacological inhibition of Nqo1 activity or genetical knocking down Nqo1 in adipocytes could decrease the protein level of Pgc1\x3c\x203a. We treated adipocytes with the Nqo1 inhibitors Dicoumarol or ES936 and observed that Pgc1\x3c\x203a protein was decreased in Parkin\textsuperscript{KD} adipocytes compared with untreated (Fig. 5g; Supplementary Fig. 5b). Nqo1 and Parkin double knock down reduced the protein level of Pgc1\x3c\x203a in Parkin\textsuperscript{KD} preadipocytes, although this difference did not reach statistical significance (Supplementary Fig. 5c). Furthermore, we found Nqo1 inhibitor Dicoumarol increased the protein ubiquitination of Pgc1\x3c\x203a in 3T3-L1 adipocytes (Fig. 5h; Supplementary Fig. 5d). Together, our findings suggest Pgc1\x3c\x203a associates with Nqo1 in white adipose tissue and increased Nqo1 protein stabilizes Pgc1\x3c\x203a protein in adipocytes with reduced Parkin expression.

**Mitochondrial superoxide increases Nqo1 and Pgc1\x3c\x203a in Parkin deleted adipocytes**

Because Nqo1 is induced by oxidative stress\textsuperscript{36–38}, we examined oxidative stress in Parkin\textsuperscript{KD} adipocytes and determined that superoxide levels were significantly increased (Fig. 5i; Supplementary Fig. 5e). Since mitochondria are a primary source of superoxide, we treated Parkin\textsuperscript{KD} adipocytes with Menadione, a redox-cycling quinone that produces toxic levels of mitochondrial superoxide. We found that menadione increased the protein levels of both Nqo1 and Pgc1\x3c\x203a in adipocytes (Fig. 5j). Moreover, H2O2 administration could also elevate the protein levels of both Nqo1 and Pgc1\x3c\x203a in 3T3-L1 adipocytes at 50–100 μM (Fig. 3k). However, N-acetyl cysteine (NAC), which generates hydrosulfides with direct antioxidation activity, did not change the protein levels of Nqo1 and Pgc1\x3c\x203a in adipocytes (Supplementary Fig. 5f). MitoQ, a mitochondrial-matrix-targeted antioxidant, suppressed the protein levels of both Nqo1 and Pgc1\x3c\x203a (Fig. 5i; Supplementary Fig. 5g). In response to low-dose of oxidative stress, Nr2f2 induces the expression of cytoprotective genes such as Nqo1\textsuperscript{39}. Indeed, Nr2f2 gene expression is elevated in eWAT of Parkin\textsuperscript{KD} mice (Supplementary Fig. 5h), suggesting elevated superoxide activates Nr2f2-induced Nqo1, which enhances Pgc1\x3c\x203a protein stability in Parkin deleted adipocytes.

Nqo1 overexpression elevates Pgc1\x3c\x203a protein level in adipocytes

We and others have found that Nqo1 gene expression is positively associated with adiposity in humans and mice (Supplementary Figs. 6a and 7a,b)\textsuperscript{40}. However, a recent study shows that whole-body Nqo1 transgenic mice prevented HFD-induced obesity and insulin resistance\textsuperscript{41}, suggesting Nqo1 gene expression may not be concordant with its protein level. We then examined both gene expression and protein level of Nqo1 in Ob/+ mice, a genetically obese mouse model. Indeed, we found that Nqo1 gene expression, but not the protein level, was significantly increased in eWAT of Ob/+ mice (Fig. 6a, b). Therefore, we carefully draw any conclusions from gene association results. To investigate the impact of Nqo1 expression on mitochondrial function and lipid metabolism, we overexpressed Nqo1 in 3T3-L1 adipocytes (Nqo1\textsuperscript{OE}). We observed a reduction of lipid levels that corresponded with an increased expression of mtDNA-encoded genes, Pgc1\x3c\x203a mRNA and protein levels, mitochondrial proteins, mtDNA content, and mitochondrial oxygen consumption rate in Nqo1\textsuperscript{OE}.
Overexpression of NQO1 in human adipocytes increases PGC1α protein and improves mitochondrial respiration.

To determine the role of Nqo1 in human adipocytes, we overexpressed human NQO1 in differentiated human subcutaneous adipocytes with adenoviral NQO1-IRES-GFP (Ad-NQO1). Ad-NQO1 not only robustly elevated NQO1 protein but also increased the protein level of PGC1α (Fig. 7a; Supplementary Fig. 7c). Consistent with the findings from Nqo1−/− adipocyte and AAV8-Nqo1 injected mice, NQO1 overexpression in human adipocytes also significantly elevated mtDNA content and basal mitochondrial activity and mildly decreased lipid levels (Fig. 7b, c; Supplementary Fig. 7d, e). Furthermore, elevated NQO1 also suppressed the adipogenesis gene C/EBPα (Fig. 7d). Altogether, our findings suggest that NQO1 controls mitochondrial function and C/EBPα expression that contribute to the inhibition of adipogenesis in human adipocytes.

Discussion

In this study, we provide evidence that the E3 ubiquitin-protein ligase Parkin controls mitochondrial homeostasis in human and rodent white adipocytes. Beyond its regulatory role in mitophagy, Parkin prevents the turnover of the mitochondrial biogenesis master regulator Pgc1α through Nqo1 in white adipocytes. Stabilized Pgc1α protein drives increased mitochondrial abundance and function which combats HFD and aging-induced obesity.

Mitochondrial homeostasis, regulated by a balance of mitophagy and mitochondrial biogenesis, involves in the pathogenesis of obesity and other metabolic disorders. Consistent with other published work, we obtained the paradoxical result that both mitochondrial number and mitophagy protein Parkin were increased during adipogenesis.
in vitro and in vivo. Herein, we investigated this apparent inconsistent observation by studying Parkin-regulated mitochondrial homeostasis and whole-body metabolism using an adipose tissue-specific Park2 deletion mouse model. We show that Parkin deletion ameliorates HFD-feeding and aging-induced adiposity and improves glucose homeostasis. Supporting the lean phenotype that we observed in the Parkin " knockout mouse model, we consistently found smaller adipocytes, suppressed inflammation, increased energy expenditure, and elevated mitochondrial activity from multiple cohorts.

Parkin-mediated mitophagy has been shown to play an important role in the beige-to-white adipocyte transition. Our findings suggest that Parkin expression in BAT may be dispensable in the regulation of adiposity.

Pgc1α regulates mitochondrial biogenesis by activating a variety of transcription factors, which could benefit adipose tissue and systemic metabolism. Our in vitro and in vivo studies suggest that Parkin deletion elevates Pgc1α protein stability. The increase in Pgc1α led to a direct increase in markers of mitochondrial biogenesis including elevated mtDNA copy number and the level of mitochondrial gene expression. Consistent with previous pre-clinical and clinical findings, we found increased mtDNA content and mtDNA encoded gene expression in Parkin-deleted adipocytes, suggesting that loss of Parkin specifically in adipose tissue prevented diet-induced obesity. Our findings highlight that Parkin, in addition to controlling mitophagy, plays a central role in regulating mitochondrial biogenesis in the prevention of adipocyte hypertrophy. Mitochondrial biogenesis reversely regulates mitophagy signal, where Pgc1α and NRF1 transcriptionally regulate the mitophagy receptor FUNDC1 to enhance mitophagy. Together, these findings indicate the complex interplay between mitochondrial biogenesis and mitophagy in maintaining mitochondrial homeostasis.

Numerous studies have shown that pharmacological stimulation of Nqo1 or whole-body Nqo1 overexpression ameliorates obesity. We confirmed and extended these findings by showing that Nqo1 overexpression selectively in white adipocytes enhances mitochondrial activity and lipid metabolism. Our findings provide an opportunity for further studies to examine the metabolic regulation of Nqo1 in vivo.

Parkin has been shown to ubiquitinate Paris, which acts as a transcriptional repressor of Pgc1α in dopamine neurons. Herein, Parkin deletion did not change the protein level of Paris in white adipose tissues, indicating that Parkin may exert different mechanisms to regulate Pgc1α in different tissues. A previous study suggested that Nqo1 interacts with Pgc1α in a NADH-dependent manner by inhibiting degradation of Pgc1α, but not canonical ubiquitin-mediated pathways. In our study, we found that Dicoumarol administration increased Pgc1α ubiquitination in adipocytes, suggesting Nqo1 may inhibit Pgc1α degradation through the ubiquitin-dependent pathway. However, we did not rule out that the Dicoumarol treatment may be working through alternative mechanisms to inhibit Pgc1α degradation. A further thorough examination of the mechanisms of Nqo1-mediated Pgc1α degradation is needed.

We showed that Parkin deletion from adipocytes enhanced mitochondrial function by elevating both mitochondrial respiration and mitochondrial membrane potential. A consequence of increased mitochondrial metabolism in Parkin-deleted adipocytes was an elevation of mitochondrial reactive oxygen species (mtROS). The cellular effects of ROS are largely dependent on the species itself, its concentration, and likely time of exposure. ROS are important physiological molecules to regulate intracellular signaling pathways. Studies have shown that NADPH oxidase (NOX) derived ROS are important modulators of adipogenesis and adipose tissue function. Our findings suggest that a low concentration of mitochondrial superoxide in Parkin-deleted adipocytes may activate cytoprotective signals to maintain adipocyte health.

In conclusion, our research shows that Parkin not only regulates mitophagy but also mitochondrial biogenesis in white adipocytes through inhibiting Nqo1 increased protein stabilization of Pgc1α. These findings suggest that Parkin regulates mitochondrial hemostasis and energy metabolism in WAT, suggesting that Parkin is a viable therapeutic target to combat obesity and obesity-associated disorders.

Methods

Animals

All mouse experimentation and animal care were approved by the University of California, Los Angeles Institutional Animal Care and Use Committee (IACUC). Mice were maintained on a 12 h light/dark cycle from 6 am to 6 pm at ambient temperature (~72 °F) with controlled humidity (~45%) in pathogen-free conditions. Food consumption, mouse activity and health were monitored daily by the Division of Laboratory Animal Medicine at UCLA. Prior to organ harvest, mice were euthanized by isoflurane overdose followed by cervical dislocation. This is an approved method according to the recommendations of the panel on Euthanasia of the American Veterinary Medical Association. Parkin floxed mice (a gift from Ted Dawson) were crossed with adiponectin Cre mice (The Jackson Laboratory, #010803) or Ucp1 Cre mice (The Jackson Laboratory, #024670) to generate animals with Parkin deletion in adipose tissue specifically (Parkin"/" mice) or in brown adipose tissue separately (Parkin"/" mice). Whole-body parkin null mice were obtained from the Jackson Laboratory (The Jackson Laboratory, #006382). Mice were studied under normal chow (NC, LabDiet 5053) and 45 % high-fat diet (HFD, Research Diets, D12451)-fed conditions. All experiments were performed on the male mouse except the results in Supplementary Fig. 1c and Supplementary Fig. 2c,d were collected from female mice. All these mice were in C57BL/6J background about 4–6 months old. Blood was drawn from 6 h-fasted male mice and analyzed for circulating factors: glucose (HemoCue), inflammatory cytokines and chemokines (Meso Scale Discovery, CA, Cat #K15048D). Intraperitoneal glucose tolerance tests (IPGTT, 1 g/kg dextrose) were performed on mice after fasting (6 h for normal chow-fed mice, overnight for HFD-fed mice). After one week of recovery intraperitoneal insulin tolerance tests (IPITT, 0.7 U/kg for NC-fed mice, 0.75 U/kg for HFD-fed mice) were performed on these mice with 6 h fasting.

METSIM studies

Genetic association and gene expression analyses were conducted on data collected from the metabolic syndrome in men (METSIM) study as previously published. Gene-trait relationships presented here were obtained from 770 male participants (age of 45–70 years). No new human samples were acquired for the generation of this manuscript.

Metabolic analysis

Body fat and lean mass were determined by NMR (Bruker). Oxygen consumption, carbon dioxide production, and respiratory exchange ratio were determined in male mice fed with HFD for 4 weeks using the Promethion metabolic screening system (Sable Systems).

Cell culture and transfections

The stromal vascular fraction (SVF) cells from inguinal white adipose tissue (iWAT), eWAT, or BAT of male C57BL/6J wild-type mice (The Jackson Laboratory, #000664, 5 weeks old) were cultured in DMEM/F12 medium with 10 % FBS as described. Then the cells were cultured...
2 days in DM1 medium (DMEM medium, 10% FBS, 5 μg/ml insulin, 1 μM Dexemethasone, 0.5 mM IBMX, and 1 μM rosiglitazone), 2 days in DM2 medium (DMEM/F12 medium with 10% FBS plus 5 μg/ml insulin), and 6 additional days in DMS medium. The primary adipocytes from BAT were differentiated in (DMEM/F12 medium with 10% FBS, 5 μg/ml insulin, 0.5 μM Dexemethasone, 0.125 mM Indomethacin, 1 μM T3, 35 mM IBMX, and 1 μM GW1929). 3T3-L1 preadipocytes were cultured cells in DM1 after 100% confluent for 2 days and in DM2 (DMEM medium, 10% FBS, and 5 μg/ml insulin) for 2 days before replacement with DMS (DMEM medium and 10% FBS) for 2–3 days. Primary human subcutaneous pre-adipocytes were purchased from ATCC (PCS-210-010) and differentiated to adipocytes according to the protocol provided (ATCC, PCS-500-006). Briefly, pre-adipocytes were incubated for 48 h before initiating differentiation via adipocyte differentiation initiation medium for 96 h. After this period, cells were cultured in adipocyte differentiation maintenance medium for an extra 3 days and then incubated with or without 2 × 10^5 PFU/ml adenovirus carrying human Nqo1 (Vector Biolabs, ADV-217023) for another 5 days. To achieve Park2 knockdown (KD), lentiviral particles carrying shRNA targeted to Park2 or scramble shRNA (Sigma-Aldrich) (multiplicity of infection = 3) were used to transduce 3T3-L1 pre-adipocytes. To inhibit Nqo1 activity, differentiated adipocytes were treated with the Nqo1 inhibitor (100 μM Dicoumarol, Santa Cruz Biotechnology, CAS66-76-2) or (400 nM ES936, Santa Cruz Biotechnology, CAS192829-78-3) for 24 h. To overexpress Nqo1 in adipocytes (Nqo1(ok)), 3T3-L1 pre-adipocytes were transfected with pcDNA3-Nqo1-flag (Addgene, #61729) and selected with 500 μg/ml G418 for 4 weeks. Cells were harvested and Nqo1 overexpression was confirmed by immunoblot.

Fluorescence microscopy
All imaging was performed using a Zeiss LSM880 confocal microscope. Super-resolution imaging was performed with a 63× apochromat oil-immersion lens and an Airyscan super-resolution detector. Image analysis was performed using Fiji (ImageJ, NIH). To quantify mitochondrial content, adipocytes were transduced with adenoviral mCherry-GFP-Fis1 (a generous gift from Orian Shirihai) for 3 days. The fluorescent signals were captured and analyzed. In studies assessing membrane potential, tissues were harvested 4 weeks after virus injection. The wound clips were removed. Tissues were harvested and the wound was closed with wound clips. Animals recovered on a heating pad. To prevent infection, sulfamethoxazole (0.48 mg/ml) and trimethoprim (0.096 mg/ml) via an oral suspension was supplied.

ROS detection
Hydrogen peroxide and superoxide were detected using ROS/superoxide detection assay kit (Abcam, ab139476). Briefly, 3T3-L1 adipocytes were cultured in 96 well plate and incubated in ROS/Superoxide detection solution with or without ROS inducer for 1h in the dark. The fluorescent signals were detected by fluorescent microplate reader and standard fluorescein (excitation/emission = 488/520 nm) and rhodamine (excitation/emission = 550/610 nm). The signals were normalized to protein content in each well.

Mitochondrial DNA
Total DNA was extracted from cells using DNaseasy Blood and Tissue kit (Qiagen). For mouse adipocytes, primers for mitochondrial encoded cytochrome c oxidase III (mtCO3) for mtDNA and 18 S for nuclear DNA were used to assess mtDNA content. The ratio of mtCO3 to 18 S was used to determine mtDNA content. For human adipocytes, primers for mitochondrially encoded tRNA leucine 1 for mtDNA and beta-2-microglobulin (B2M) for nuclear DNA were used to determine mtDNA content. The ratio of mtCO3 to 18 S was used to determine mtDNA content.

Mitochondrial respiration
Mitochondrial respiration in 3T3-L1 preadipocytes and differentiated adipocytes was measured using an XF96 Extracellular Flux Analyzer (Seahorse Biosciences). Briefly, cells were plated and grown to confluence on a 96-well plate. Measurements of oxygen consumption were made continuously while cells were sequentially treated with oligomycin (ATP synthase inhibitor), FCCP (an uncoupling agent), and rotenone/miyothenozol (inhibitors of complex I /III of the electron transport chain).

Immunoblot analysis
Mouse tissue samples were pulverized in liquid nitrogen and homogenized in RIPA lysis buffer containing freshly added complete EDTA-Free protease (Roche) and Phosphatase Inhibitor Cocktail 2 (Sigma-Aldrich). All lysates were centrifuged and resolved by SDS-PAGE. Samples were transferred to PVD membranes and subsequently probed with the following antibodies for protein detection: Parkin (Cell Signaling #2132), Pink1 (Cayman Chemicals, #10006283), p62 (Progen Biotechnik GmbH, #03-GP62-C), actin (Santa Cruz Biotechnology, sc-47778), Lc3b (Novus, NB100), GAPDH (Invitrogen, AM4300), F4/80 (Santa Cruz Biotechnology, sc-377009), Pgc1α (EMD Millipore, AB3242; Abcam, ab191838; and Abcam, ab106814), Hsp90 (Cell Signaling #4877), XBP1 (Cell Signaling, #34394), AlexaFluor 488 goat anti rabbit IgG (Invitrogen, A11088), or AlexaFluor 568 goat anti mouse IgG (Invitrogen, A11041). After transfer, PVD membranes were cut in half and imaged separately by the BIORAD ChemiDoc XRS imaging system. The exposure time was adjusted by using the transform settings in the BioRad Quantity One image software (BIO-RAD). The cropped images were organized to form a figure in GraphPad Prism9.4.1 software. Densitometric analysis was performed using BioRad Quantity One image software.

Co-immunoprecipitation
3T3-L1 adipocytes (-1.7 × 10^6 cells/each) were lysed in the lysis buffer (10 mM Heps-KOH, pH 7.9, 0.5% NP-40, 140 mM NaCl, 10 mM KCl, 1.5 mM MgCl2 and protease inhibitors) by dounce on ice. The lysate was centrifuged at 16,000 g for 15 min at 4 °C, and the resulting supernatant was used for immunoprecipitation. After preincubation of each supernatant with 20 μl of Protein A/G magnetic beads (Thermo Scientific, Cat #: 88802) at 4 °C for 1 h the pre-cleared supernatant was incubated with 4 μg of normal IgG or anti-Fsig1 antibody and 30 μl Protein A/G magnetic beads at 4 °C overnight.

Nature Communications | (2022) 13:6661
https://doi.org/10.1038/s41467-022-34468-2
with rotation. After washing 4 times with lysis buffer, the bound proteins were eluted from the beads by boiling them in SDS sample buffer. The eluted proteins were analyzed by Western blotting as described above.

**Protein synthesis assay**

Protein synthesis was determined by using Protein Synthesis Assay Kit (Cayman chemical, Cat # 601100). Briefly, differentiated adipocytes were treated with O-Propargyl-Puromycin (OPP) working solution and followed with a cell-based assay fixative buffer. Washed adipocytes were incubated with 5 FAM-Azide staining solution for 30 min at room temperature. Nuclei were stained with Hoechst 33342 for 10 min. FITC (excitation/emission = 485/535 nm) signal was detected by a fluorescent plate reader. Nuclei were counted by the Operetta high-content imaging system (PerkinElmer).

**Immunohistochemistry**

Formalin-fixed adipose tissues were sectioned and stained for H&E. Following deparaffinization, rehydration, heat-induced epitope retrieval, and permeabilization, slides were blocked with 5% BSA and incubated with the Perilipin 1 antibody overnight followed by the goat anti-rabbit IgG-Alexa Fluor 488 overnight inside a humidified chamber at 4 °C. After BSA blocking, the slides were incubated with F4/80 antibody overnight and followed by the goat anti-mouse IgG-Alexa Fluor 568 overnight at 4 °C again. Images were captured under a fluorescent microscope (Zesis) and BioTek Lionheart LX automated microscope (Agilent).

**Quantitative RT-PCR**

Mouse tissue samples were homogenized using Trizol reagent and RNA was isolated using RNAeasy Mini QIAcube Kit with DNAse digestion (Qiagen). For RNA isolation from cells, lysates occurred using RLT buffer (Qiagen) with 2-Mercaptoethanol. RNA was isolated from lysed cells using the RNAeasy Plus kit per the manufacturer’s instructions. cDNA synthesis was performed using 1 μg of RNA with iScript reverse transcriptase (Bio-Rad). PCR reactions were prepared using PowerUp SYBR Green Master Mix (Applied Biosystems). All PCRs are performed in a QuantStudio 5 Real-Time PCR system (Applied Biosystems). Quantification of a given gene, expressed as relative mRNA level compared with control, was calculated after normalization to a standard housekeeping gene (18 S or ACTIN). Primer pairs were designed using Primer 3 Input software (Version 0.4.0) or previously published sequences. Primer sets were selected spanning at least one exon-exon junction when possible and were checked for specificity using BLAST (Basic Local Alignment Search Tool, NCBI). The specificity of the PCR amplification was confirmed by melting curve analysis ensuring that a single product with its characteristic melting temperature was obtained. See Table S1 for a list of the primers used.

**RNA sequencing and analysis**

A homogenous portion of frozen adipose samples were homogenized using a tissue homogenizer in Trizol. RNA was isolated using the Qiagen RNAeasy Mini QIAcube Kit following the manufacturer’s instructions. Isolated RNA was checked for concentration using a NanoDrop 2000c (ThermoFisher Scientific) and purity using the Agilent TapeStation. Only samples with RIN > 7.0 were used. Libraries were prepared using the KAPA mRNA Hyper Prep Kit following the manufacturer’s instructions. The resulting libraries were combined into two pools and sequenced on the Illumina HiSeq 3000 within the UCLA TCGB core facility following in-house established protocols. Raw reads were checked for quality using FastQC, aligned to the Mus musculus GRCm38, and then counted using MuTator (GRCm38 version 97). Alignment and counting occurred using Rsubread v 2.4.2. Raw counts were then analyzed for differential gene expression using the DESeq2 v1.30.0.

**Statistics**

Values presented are expressed as means ± SEM unless otherwise indicated. Statistical analyses were performed using Student’s t test when comparing two groups of samples or Two-way analysis of variance (ANOVA) for identification of significance within and between groups using GraphPad Prism9.4.1 (GraphPad Software). Significance was set a priori at P < 0.05. For RNA Sequencing analysis, the significance was set as FDR < 0.05.

**Reporting summary**

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

**Data availability**

Source data are provided as a Source Data file. METSIM adipose array data are available from in the Gene Expression Omnibus database under accession code GSE70333, and additional clinical data were obtained from previously published papers.[9,14]. The mouse RNA-seq results from eWAT of HFD-fed Parkin[9,16] vs. Control f/f mice and AAV8-Nqo1 vs. AAV8-Control injected eWAT have been deposited in the Gene Expression Omnibus database under accession code GSE207496. Human PARK2 gene (encodes Parkin protein) association is available from Phenoscan v2 (http://www.phenoscan.medschl.cam.ac.uk). The authors declare that all data supporting the findings of this study are available within the paper and its Supplementary Information files.

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Acknowledgements

We would like to thank Dr. Ted M. Dawson at Johns Hopkins University for providing us Parkin flox mice. We thank undergraduate students Julia Zhou at the University of California San Diego for analyzing the adipocyte size. We thank Wenjuan Ren for immunoblotting and Calvin Pan for HMDP data analysis. Z.Z. was supported by an NIH grant (DK125354). A.L.H. was supported by NIH grants (DK109724, P30DK063491). A.J.L. was supported by NIH grants (US4 DK120342 and DK117850). T.M.M. was supported by the UCLA Intercampus Medical Genetics Training Program (T32GM008243), NRSA predoctoral fellowship (F31DK108657), Carl V. Gisolfi Memorial Research grant from the American College of Sports Medicine, and a predoctoral graduate student award from the Dornsife College at the University of Southern California. D.M.W. is supported by an EMBO long-term fellowship ALTF 828-2021.

Author contributions

Z.Z., T.M.M., and A.L.H conceived and designed the experiments. Z.Z., T.M.M., A.R.S., and X.Z. performed all in vivo studies. Z.Z., T.M.M., X.Z., A.R.S., Y.C., A.M., and P.S. performed the sample collection and subsequent experimental analysis. B.L.C. and T.Q.d.A.V. performed indirect calorimetry studies. Z.Z. and T.M.M. performed all adipocyte culture studies and mtDNA content analysis. L.C. performed all AAV injection studies. D.M.W., J.N., M.S. conducted studies related to mitochondrial respirometry and confocal microscopy. Z.Z., T.M.M., L.C., X.Z., A.R.S., Y.C., B.L.C., A.M., P.S. T.Q.d.A.V., M.L., O.S.S., A.L.H, and A.J.L. analyzed the data. T.M.M., L.C., A.L.H, A.J.L., and Z.Z. wrote the paper.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41467-022-34468-2.

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Peer review information Nature Communications thanks Qiong Wang and the other, anonymous, reviewers for their contribution to the peer review of this work. Peer reviewer reports are available.

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