Table S1, related to Figure 1.

Name	Size	ES	NES	p-value	FDR, q-value	FWER, p
CITRATE_CYCLE_TCA_CYCLE	20	-0.6826643	-2.0174627	0	0.026660765	0.041
HSA00280_VALINE_LEUCINE_AND_ISOLEUCINE_DEGRADATION	42	-0.5626841	-1.9446014	0	0.051109686	0.148
HSA00020_CITRATE_CYCLE	25	-0.63026077	-1.9404854	0	0.037463266	0.16
OXIDATIVE_PHOSPHORYLATION	55	-0.5113584	-1.8906622	0	0.052854355	0.287
HSA00190_OXIDATIVE_PHOSPHORYLATION	100	-0.45311812	-1.8446951	0	0.077939466	0.453
CAMPTOTHECIN_PROBCELL_UP	17	-0.6505533	-1.8020581	0.003590664	0.109991774	0.644
FLAGELLAR_ASSEMBLY	19	-0.6141013	-1.7995689	0	0.097633764	0.653
PHOTOSYNTHESIS	20	-0.618073	-1.7965853	0	0.089019455	0.664
CANCERDRUGS_PROBCELL_UP	15	-0.65977335	-1.7908636	0.003724395	0.08555614	0.691
PENG_GLUTAMINE_UP	227	-0.38959652	-1.7774848	0	0.09194197	0.764
BRENTANI_TRANSPORT_OF_VESICLES	23	-0.6039639	-1.7719051	0.003496504	0.0894655	0.791
ATP_SYNTHESIS	19	-0.6141013	-1.7495784	0.007366483	0.105693154	0.866
TYPE_III_SECRETION_SYSTEM	19	-0.6141013	-1.7393444	0.005190312	0.109290734	0.891
HSA00640_PROPANOATE_METABOLISM	29	-0.54715717	-1.7328124	0	0.10970077	0.905
VALINE_LEUCINE_AND_ISOLEUCINE_DEGRADATION	35	-0.52305466	-1.7323055	0.003514939	0.10280863	0.905

Gene expression sets enriched after treatment of melanoma cell lines (N = 6) with vemurafenib. FDR, false-discovery rate; FWER, family-wise error rate; ES, enrichment score; NES, normalized enrichment score; Size refers to gene set size. Details of statistical methods are described in (Mootha et al., 2003).

Table S2, related to Figure 1.

Name	Size	ES	NES	p-value	FDR, q-value	FWER, p
OXIDATIVE_PHOSPHORYLATION	55	-0.5313751	-1.7654682	0.019455252	1	0.506
BRENTANI_CYTOSKELETON	22	-0.55218005	-1.7624917	0.015533981	0.71292704	0.546
TYROSINE_METABOLISM	28	-0.56091315	-1.6678647	0.026974952	1	0.876
TIS7_OVEREXP_DN	17	-0.6059337	-1.6660074	0.025590552	1	0.876
ATP_SYNTHESIS	19	-0.58863074	-1.6509762	0.015238095	1	0.921
TYPE_III_SECRETION_SYSTEM	19	-0.58863074	-1.6509762	0.015238095	0.8565484	0.921
FLAGELLAR_ASSEMBLY	19	-0.58863074	-1.6509762	0.015238095	0.7341844	0.921
PHENYLALANINE_METABOLISM	21	-0.56528217	-1.643656	0.02734375	0.6949159	0.938
SARCOMAS_LEIOMYOSARCOMA_CALP_UP	15	-0.5939497	-1.6180929	0.02851711	0.7866653	0.974
PHOTOSYNTHESIS	20	-0.56751925	-1.595545	0.026717557	0.8664833	0.979
TPA_RESIST_MIDDLE_DN	107	-0.39462328	-1.5864242	0	0.8576306	0.991
HPV31_DN	44	-0.51095927	-1.5835407	0.045816734	0.808037	0.991
LIAN_MYELOID_DIFF_GRANULE	24	-0.61149573	-1.564989	0.013944224	0.8887546	1
VANASSE_BCL2_TARGETS	76	-0.39814156	-1.5503082	0.034883723	0.9394353	1
GLUCONEOGENESIS	49	-0.45502195	-1.547516	0.02970297	0.9010121	1

Gene expression sets enriched after treatment of melanoma cell lines (N = 5) with MEK inhibitor PD0325901. FDR, false-discovery rate; FWER, family-wise error rate; ES, enrichment score; NES, normalized enrichment score; Size refers to gene set size. Details of statistical methods are described in (Mootha et al., 2003).

Table S3, related to Figure 1.

Name	Size	ES	NES	p-value	FDR, q-value	FWER, p
HSA04370_VEGF_SIGNALING_PATHWAY	66	-0.49320316	-1.9460483	0	0.091074355	0.055
LOTEM_LEUKEMIA_UP	21	-0.6144097	-1.9349252	0.001945525	0.054897256	0.064
INSULIN_NIH3T3_UP	16	-0.65346473	-1.926296	0	0.04251103	0.077
HSA00591_LINOLEIC_ACID_METABOLISM	27	-0.6261818	-1.8495042	0.003809524	0.120073654	0.24
HSA00565_ETHER_LIPID_METABOLISM	27	-0.60989857	-1.8358979	0.002028398	0.1151601	0.274
MUNSHI_MM_UP	65	-0.45032862	-1.8108876	0.002008032	0.14449073	0.364
MPRPATHWAY	22	-0.5725521	-1.7240024	0	0.41018453	0.719
HDACI_COLON_CLUSTER9	53	-0.45657748	-1.7158966	0.002	0.39486316	0.752
HSA04664_FC_EPSILON_RI_SIGNALING_PATHWAY	73	-0.45155552	-1.7113812	0.016528925	0.37106347	0.765
HDACPATHWAY	29	-0.524003	-1.6945506	0.003802281	0.40905857	0.839
ST_GA12_PATHWAY	21	-0.55117834	-1.6834962	0.001930502	0.42459038	0.865
SCHURINGA_STAT5A_DN	17	-0.6395701	-1.6696184	0.003960396	0.45600265	0.899
PASSERINI_OXIDATION	19	-0.53578943	-1.6567032	0.014522822	0.48720393	0.919
HSA00010_GLYCOLYSIS_AND_GLUCONEOGENESIS	55	-0.4307166	-1.6512356	0.012295082	0.47958305	0.928
ERK5PATHWAY	17	-0.48306164	-1.6387306	0.00990099	0.50899017	0.942

Gene expression sets enriched after treatment of non-melanomas with PD0325901 (N = 7). FDR, false-discovery rate; FWER, family-wise error rate; ES, enrichment score; NES, normalized enrichment score; Size refers to gene set size. Details of statistical methods are described in (Mootha et al., 2003).

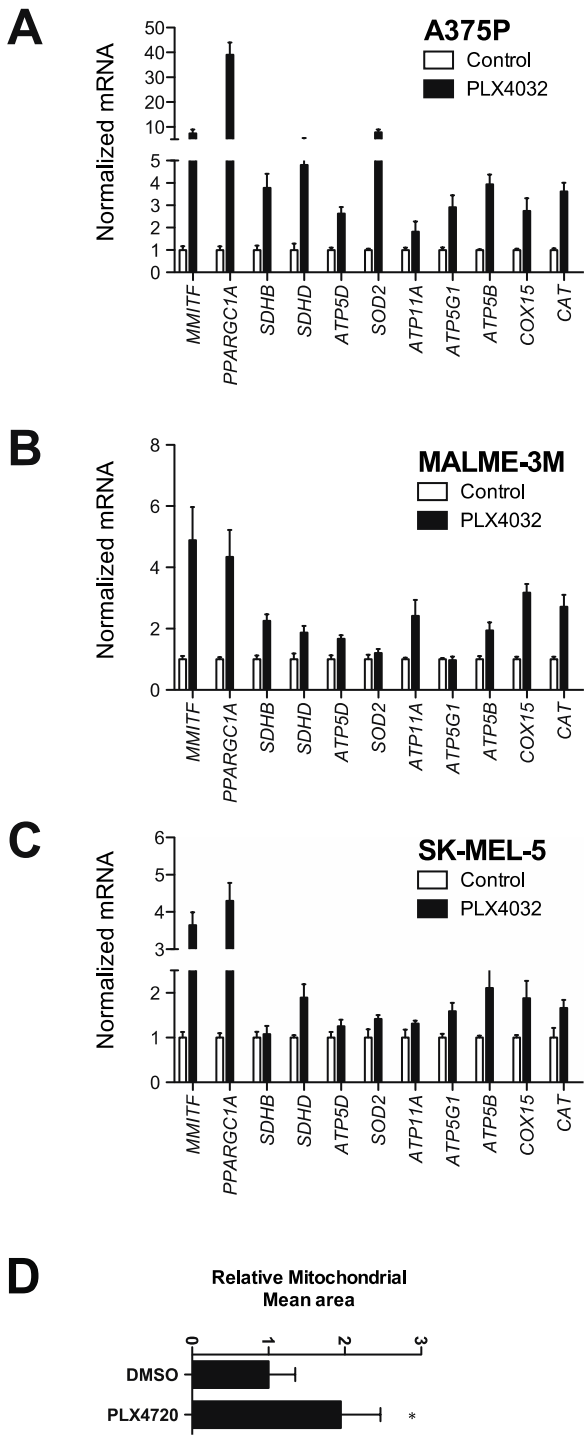

Figure S1, related to Figure 1. (A, B, C) Induction of OXPHOS genes upon treatment with BRAF inhibitors. Indicated mRNAs were quantified by quantitative PCR 24h after PLX4032 (1µM) treatment. (D) Mitochondrial mean in UACC-62 cells treated with PLX4720 for 72 hrs. *, $p < 0.05$ compared to control treated cells. Error bars represent SEM of at least three independent replicates.

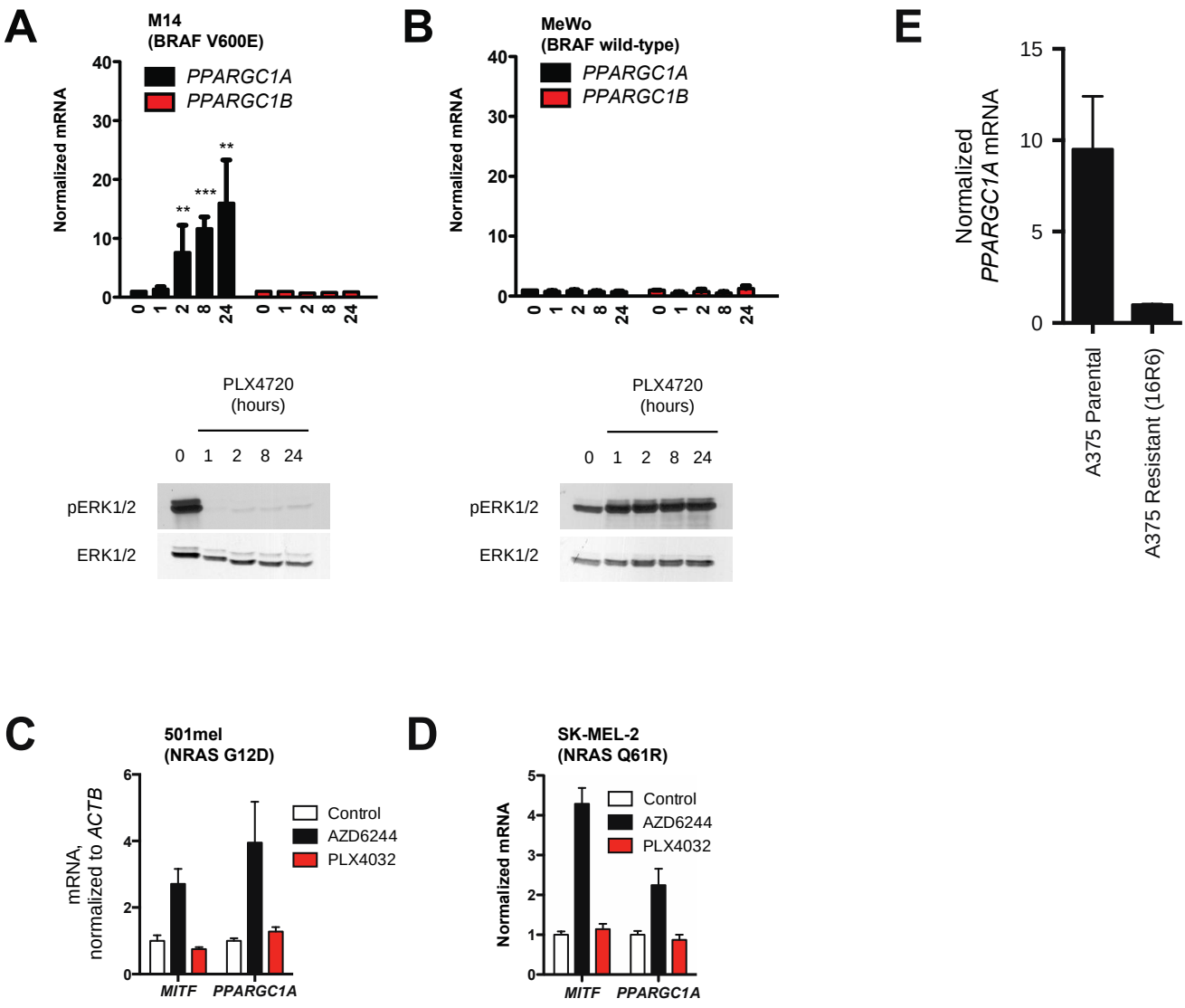

Figure S2, related to Figure 2. Time course of *PGC1α* and *PGC1β* mRNA induction (upper panel) and inhibition of phospho-ERK (lower panel) in BRAF mutant melanoma M14 (A) or BRAF-wild-type melanoma MeWo (B). (C,D) Expression of *MITF* and *PGC1α* in NRAS-mutant cell lines upon 24h treatment with PLX4032 (1μM) or AZD6244 (1μM). (E) Expression of *PGC1α* in A375 parental cells and in BRAF inhibitor resistant clone (16R6) extracted from Greger JG et al. (2012). Error bars represent SEM of at least three independent replicates.

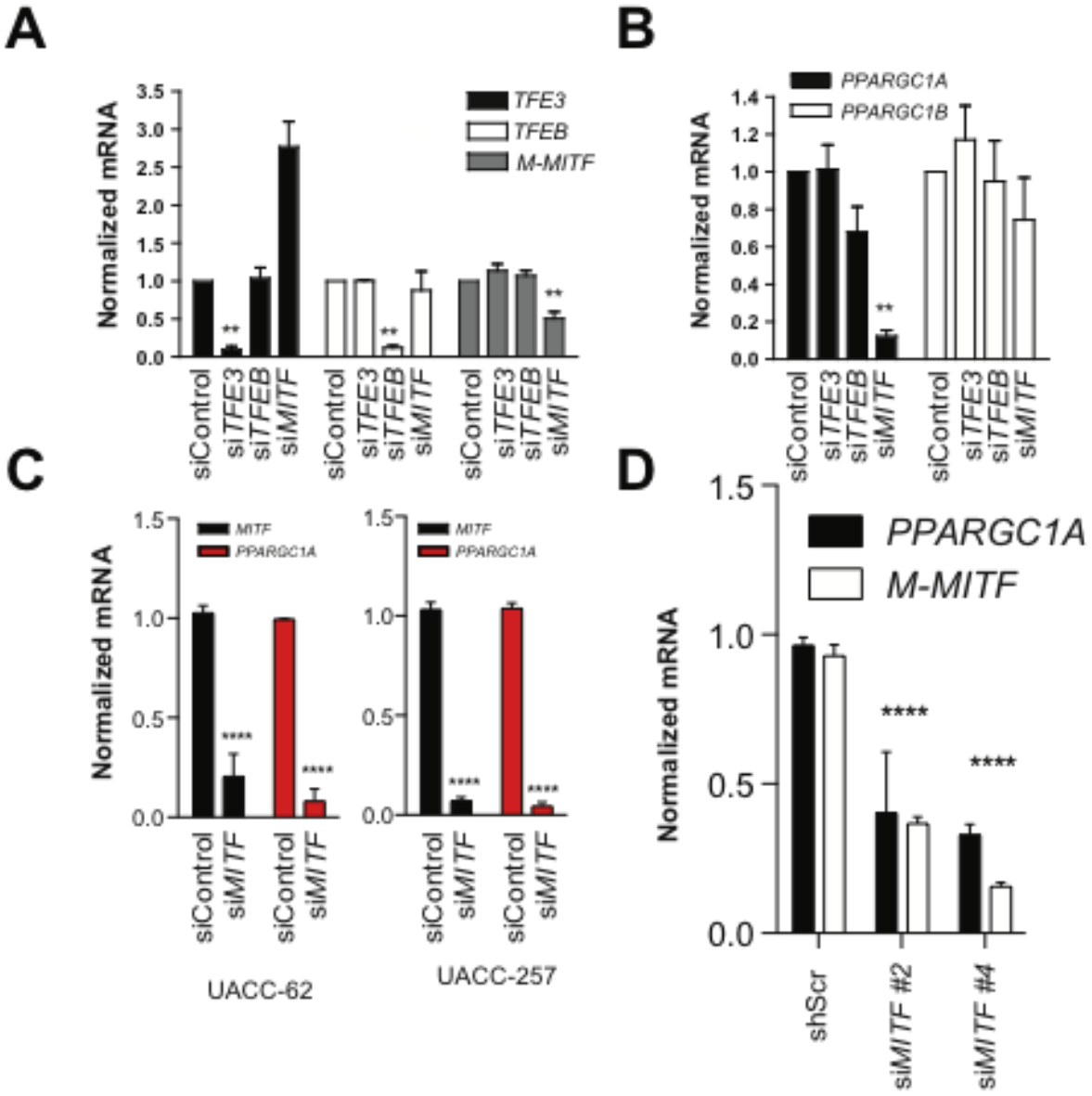

Figure S3, related to Figure 3. (A,B) Effect of knockdown of MiT family members (A) on expression of *PPARGC1A* and *PPARGC1B* (B) in primary human melanocytes. (C) Effect of *MITF* knockdown in two additional melanoma cell lines. (D) Effect of *MITF* knockdown with two distinct shRNAs in MALME melanoma cells on *PPARGC1A* and *MITF* mRNA. Error bars represent SEM of at least three independent replicates.

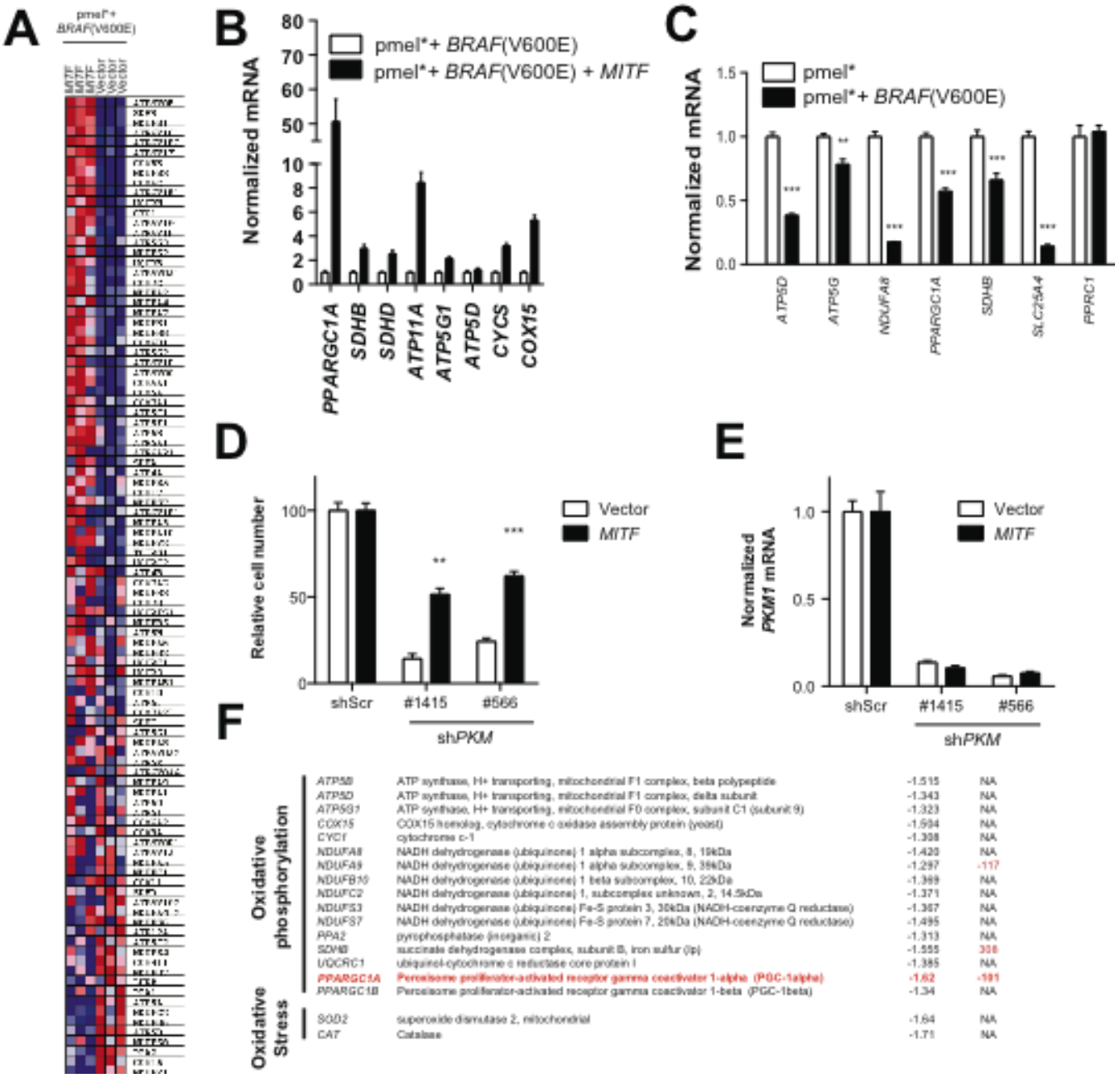

Figure S4, related to Figure 6. (A) Effect of *MITF* overexpression on oxidative phosphorylation gene set. Low expression is depicted in blue, whereas higher expression is colored red. Results were validated by qtPCR (B). (C) Expression of oxidative phosphorylation genes in pmel* and pmel* + *BRAF*(V600E) cells by qtPCR. (D) Effect of suppression of muscle-isoform of pyruvate kinase M (*PKM*), using independent shRNA sequences, on cell number 96h after infection of *BRAF*(V600E)+vector and *BRAF*(V600E)+*MITF* cells. **, $p < 0.01$; ***, $p < 0.001$. (E) Knockdown efficiency of *PKM* shRNAs in *BRAF*(V600E)+vector and *BRAF*(V600E)+MITF cells by quantitative PCR 96 hours after infection. (F) Effect of sh*MITF* on oxidative phosphorylation or oxidative stress genes by microarray (Li et al.,

2012). Fold change after expression of sh*MITF* are shown. Binding of MITF to the proximal promoter of each gene was determined by ChIP-on-ChIP (Li et al., 2012). Error bars represent SEM of at least three independent replicates.

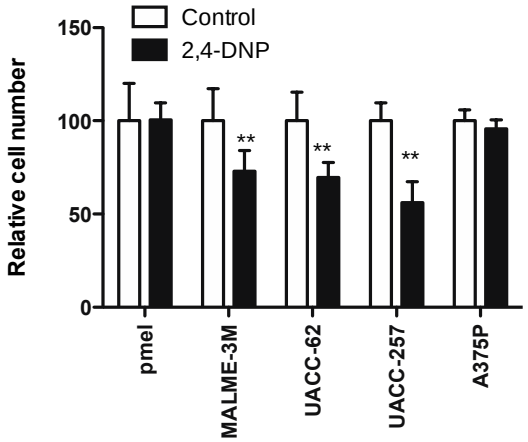

Figure S5, related to Figure 7. Number of melanoma cells or primary human melanocytes following treatment with 2,4-DNP (50 µg/mL) for 72h. **, $p < 0.01$ compared to primary melanocytes treated with 2,4-DNP. Error bars represent SEM of at least three independent replicates.

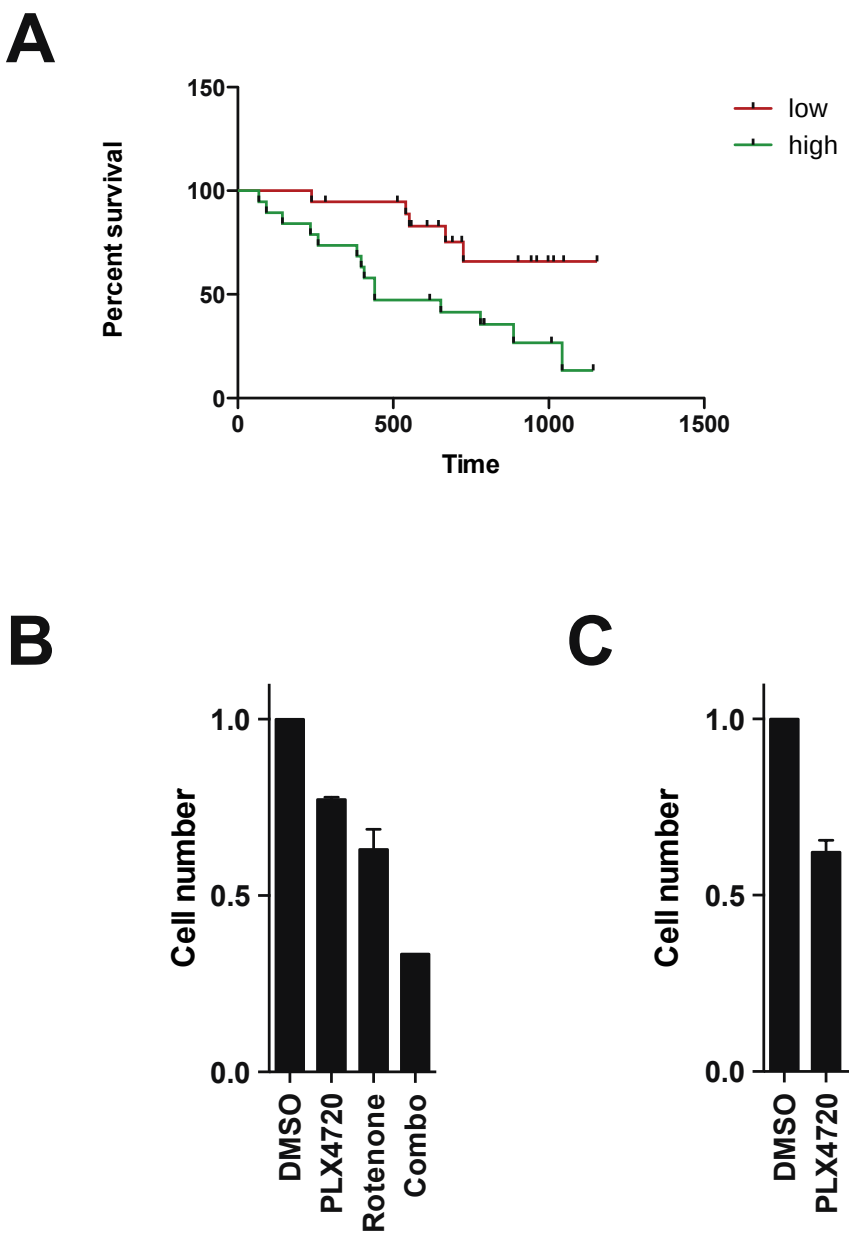

Figure S6, related to Figure 8. (A) Prognosis of Stage III melanoma patients with high and low levels of *PPARGC1A* mRNA derived from GSE19234. (B) Effects of Rotenone (75µM, A) and TTFA (175µM, B) on cytotoxicity of PLX4720 (1µM) in the M14 melanoma cell line. Melanoma cell were treated for 24h with PLX4720 then mitochondrial uncoupler for 48h, prior to estimation of cell number. Error bars represent SEM of at least three independent replicates.

Supplemental Experimental Procedures

Gene expression and bioinformatics

The gene expression datasets have been submitted to the GEO repository under accession GSE38007. Gene profiles were analyzed by GenePattern (Broad Institute of Harvard and MIT) or dChip. PGC1α mRNA expression was compared to the expression of all known and sequence-predicted human transcription factors (http://dbd.mrc-lmb.cam.ac.uk/DBD/index.cgi?Home) by Pearson correlation analysis using the GeneNeighbors module of Gene Pattern. Gene set enrichment analysis was performed using GSEA module of GenePattern. Publically available datasets used in this paper were: GSE20051 (PLX4032 treated melanomas), GSE10086 (PD0325901 treated cell lines), and MALME3M cells treated with shMITF (Li et al., 2012).

Affymetrix HT-HGU133A gene expression data for 88 melanoma samples and five normal melanocytes were obtained from the Broad Melanoma Portal (http://www.broadinstitute.org/melanoma/branding/browseDataHome.jsf). The data were normalized using RMA (Irizarry et al., 2003) and the latest mapping of probes to RefSeq genes (Dai et al., 2005). PGC1α-responsive genes and oxidative phosphorylation gene sets were obtained from (Mootha et al., 2003). RefSeq genes corresponding to different isoforms of the same gene and having identical expression values were merged. The *k*-means algorithm was then used to separate the melanoma samples into two classes based on the expression indices of either the PGC1α gene set or the oxidative phosphorylation gene set. 100 random starts with 1000 maximum iterations were used to find the optimal clustering in each case. We then performed the t-test on the two classes in each clustering analysis. GSEA statistics of oxidative phosphorylation in engineered melanomas was assessed by 7000 iterations of the gene set permutations.

For the measurement of specific gene expression differences attributed to BRAF(V600E) expression, the pmel* and pmel* BRAF(V600E) cells were switched to the identical media conditions (DMEM with 10% fetal bovine serum) for 16 hours prior to RNA harvest and qPCR analysis.

Biochemicals

PLX4072 was obtained from Sai Advantium Pharma Limited (Pune, India). AZD6244 and PLX4032 were obtained from Selleck Chemicals (Houston, Texas).

Western blotting and immunohistochemistry and biopsies

Whole cell lysates were prepared in lysis buffer supplemented with protease and phosphatase inhibitors (Roche). Equal amounts of protein were resolved by electrophoresis on gradient gels (Bio-Rad). Antibodies used were as follows: MITF (C5 hybridoma); PGC1α (Calbiochem); α-tubulin (clone DM1A, Sigma); GAPDH, phospho-ERK1/2, and ERK1/2 (Cell Signaling Technology). Melanoma biopsies prior and during treatment were sectioned, embedded in paraffin and stained with the phospho-ERK1/2 antibody (Cell Signaling Technology). PLX4720 was used at 1μM concentration in all assays, whereas control samples were treated with an equal volume of DMSO.

Evaluation of gene expression in patient biopsies

Biopsies where obtained from patients with metastatic melanoma with the BRAF(V600E) mutation enrolled in clinical trials for treatment with a BRAF inhibitor or a combination of a RAF inhibitor and a MEK inhibitor. Biopsies of tumor material consisted of discarded tissue obtained with informed consent as described (Johannessen et al., 2010). On-treated samples were collected 10-14 days after initiation of vemurafenib treatment. Formalin-fixed tissue was

analyzed by haematoxylin and eosin staining. Sequences of primers used for PCR are listed below.

siRNA delivery and lentivirus infection

siRNAs SMARTpools for *MITF* (M-008674-00-005), *PPARGC1A* (L-005111-00-0010), *TFEB* (L-009798-00) or *TFE3* (L-009363-00-0005) or control (D-001810-10-05) were from Dharmacon. shRNAs targeting *MITF* or non-template control were from the RNAi Consortium (Broad Institute, Cambridge, MA USA). GFP and MITF were respectively cloned into the pCW45 lentiviral expression vector (Dana-Farber/Harvard Cancer Center DNA Resource Core). Lentivirus was prepared by transfection of packaging constructs and lentivirus plasmid in 293T cells per standard protocols (The RNAi Consortium, The Broad Institute). Amount of virus was titrated for near quantitative infection with <5% toxicity of non-template virus.

Cell viability, metabolic assays and flow cytometry

Cell number was calculated using the CyQuant assay (Invitrogen) or crystal violet staining. Lactate measurements were done using the Lactate Assay (BioVision or Sigma) per manufacturer's protocols. Indicated cell lines were plated in complete media overnight. Media was changed to serum free-media to avoid interference with the assay for 16 h with either PLX4720 (3μM) or PLX4032 (1μM) or vehicle control prior to harvesting cells and measurement of lactate. Results were normalized to cell number. All assays were performed in logarithmically growing cells.

MitoTracker Red, Green and MitoSOX were obtained from Invitrogen using manufacturer's recommended protocols. Mean fluorescence was determined by FlowJo software and normalized to vehicle-treated cells.

Glucose uptake (relative to total cell numbers) was measured over 24h as trace [^{3}H]-deoxyglucose uptake in cells growing in glucose-containing media followed by plate harvest and

subsequent analysis on a Wallac 1450 Microbeta scintillation counter. Oxygen consumption was measured using XF Flux Analyzer (Seahorse Biosciences) using standard protocols. ATP measurements were conducted using Enliten ATP assay (Promega) according to standard trichloroacetic acid (TCA) extraction protocols.

Xenograft tumor studies

Engineered melanoma cells with BRAF(V600E)+vector or +MITF were inoculated subcutaneously at three flank positions (100,000 cells in 100µL PBS per site) in NCR-FoxNu (male, 6 weeks) animals. Tumor establishment was monitored and experiment was terminated after 10 weeks. For longitudinal tumor treatment studies, melanoma cell lines (A375P and UACC257, both carrying pFUW-mCherry-puro-LUC) were injected subcutaneously into NCR-FoxNu mice (5,000,000 cells in 100µL PBS with growth factor reduced matrigel). Initial tumor establishment and growth visualized by *in vivo* imaging by comparing signal obtained seven (7) days apart. Treatment was started after tumors reached mean tumor volume of 100mm^3. Treatment with vemurafenib (75mg/kg/day) or 2,4-DNP (20mg/kg/day) was administered by oral gavage in 0.5% methyl cellulose and tumor size measured by calipers twice weekly. Mean response measured based on cohorts of 6-7 mice for each arm (and each cell line).

Electron microscopy

Cells were fixed for 1 hr at room temp in 2.0% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4 (Electron Microscopy Sciences, Hatfield, PA). Cells were rinsed in buffer, scraped and pelleted. The pellets were treated with 1.0% osmium tetroxide (EMS) in cacodylate buffer for 1 hr at room temp. They were rinsed and then resuspended in 2.0% agarose for ease of handling. Small pieces were dehydrated through a graded series of

ethanol, then infiltrated with eponate resin (Ted Pella, Redding, CA) overnight at room temp. They were then embedded in resin overnight at 60°C. Thin sections were cut on a Leica UC6 ultramicrotome, collected onto formvar-carbon coated slot grids and post-stained with uranyl acetate and lead citrate. They were examined in a JEOL 1011 TEM at 80 kV. Images were collected with an AMT digital imaging system (Advanced Microscopy Techniques, Danvers, MA). Mitochondrial density was calculated by morphometrics by standard protocols.

Primer sequences used for quantitative PCR.

Gene	**Forward Primer**	**Reverse Primer**
ACTB	GTTGTCGACGACGAGCG	GCACAGAGCCTCGCCTT
ATP5D	CAGGTCCAACATGTCCAGC	ACGGCACCACCTCCAAATAC
ATP5G1	GCCTGATTAGACCCCTGGTA	GGCTAAAGCTGGGAGACTGA
NDUFA8	CTCCTTGTTGGGCTTATCACA	GCCCACTCTAGAGGAGCTGA
ATP5B	CAAGTCATCAGCAGGCACAT	TGGCCACTGACATGGGTACT
PPARGC1A	CTGCTAGCAAGTTTGCCTCA	AGTGGTGCAGTGACCAATCA
SDHB	AAGCATCCAATACCATGGGG	TCTATCGATGGGACCCAGAC
SLC25A4	CCCTGCTCCTTAGGGATTCT	AGAGGGTCAAACTGCTGCTG
PPRC1	TGACAAAGCCAGAATCACCC	GTGGTTGGGGAAGTCGAAG
CAT	ACGGGGCCCTACTGTAATAA	AGATGCAGCACTGGAAGGAG
SOD2	TAGGGCTGAGGTTTGTCCAG	GGAGAAGTACCAGGAGGCGT
M-MITF	CCGTCTCTCACTGGATTGGT	TACTTGGTGGGGTTTTCGAG
TFEB	GGGAGTTGGATGATGTCATTG	GGCATCTGCATTTCAGGATTG
TFE3	GCAGGCGATTCAACATTAACG	ATAATCCACAGAGGCCTTCAG
PPARGC1B	CAGACAGAACGCCAAGCATC	TCGCACTCCTCAATCTCACC
TRPM1	CAAAGATACATTCCCGTTTGC	GCTGAAAGAGCCTGAGCTGT
PKM1	CTGAAGGCAGTGATGTGGCC	CTCCGTCAGAACTATCAAAGC

Primers sequences used for mutagenesis of *PPARGC1A* promoter

Mutation	**Forward Primer**	**Reverse Primer**
E-box 1	CCTACTTTTTAATAGCTTTGTGAAGT GACTGGGGACTGTAGTAAG	CTTACTACAGTCCCCAGTCACTTCA CAAAGCTATTAAAAAGTAGG
E-box 2	TCTCATGAAAATGTATCACTTCAGGA GCGCTTGCTTCAGTTC	GAACTGAAGCAAGCGCTCCTGAAG TGATACATTTTCATGAGA

Primer sequences used for chromatin immunoprecipitation

Gene	**Forward Primer**	**Reverse Primer**
PPARGC1A	GACGCCAGTCAAGCTTTTTC	CGTCACGAGTTAGAGCAGCA
TYR	GTGGGATACGAGCCAATTCGAAAG	GTGGGATACGAGCCAATTCGAAAG
PPARGC1A (alt)	TCCCGGGATAAAGTGTCATC	TCGAGGGAGCTCTCTGACAT
GAPDH	ATGGTTGCCACTGGGGATCT	TGCCAAAGCCTAGGGGAAGA

Lentiviral shRNAs in pLKO.puro

Gene	**Target Sequence**	**TRC clone name**
PKM	GCTGTGGCTCTAGACACTAAA	NM_182471.1-566s21c1 (#566)
	CGCAAGCTGTTTGAAGAACTT	NM_182471.1-1415s1c1 (#1415)

Supplemental References

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