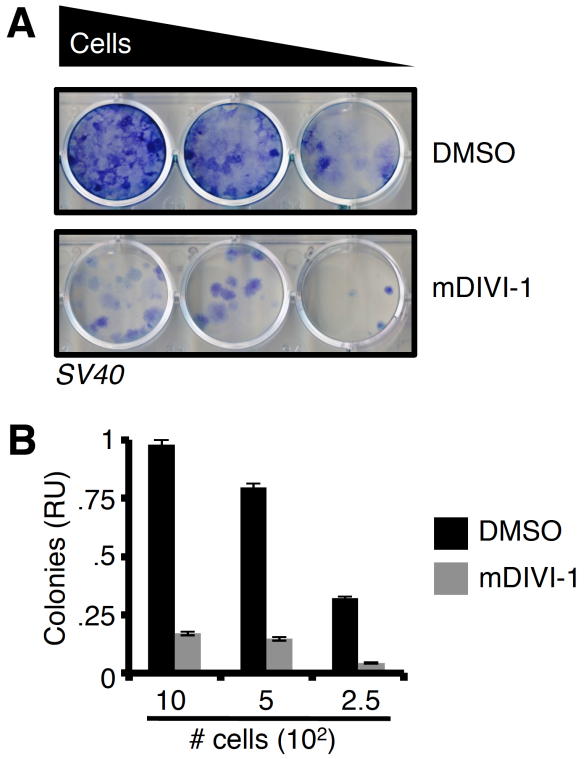


Figure S1 ***(Related to Figure 2).*** **Pharmacological inhibition of DRP1 prevents SV40-induced clonogenic survival.**

(A-B) Dilutions of primary Wt MEFs were treated with mDIVI-1 (25 μM; or DMSO) for 24 h, infected with SV40, and the resulting colonies were stained *(A)* and quantified *(B)*.

All data are representative of at least triplicate experiments, and reported as ± S.D., as required.

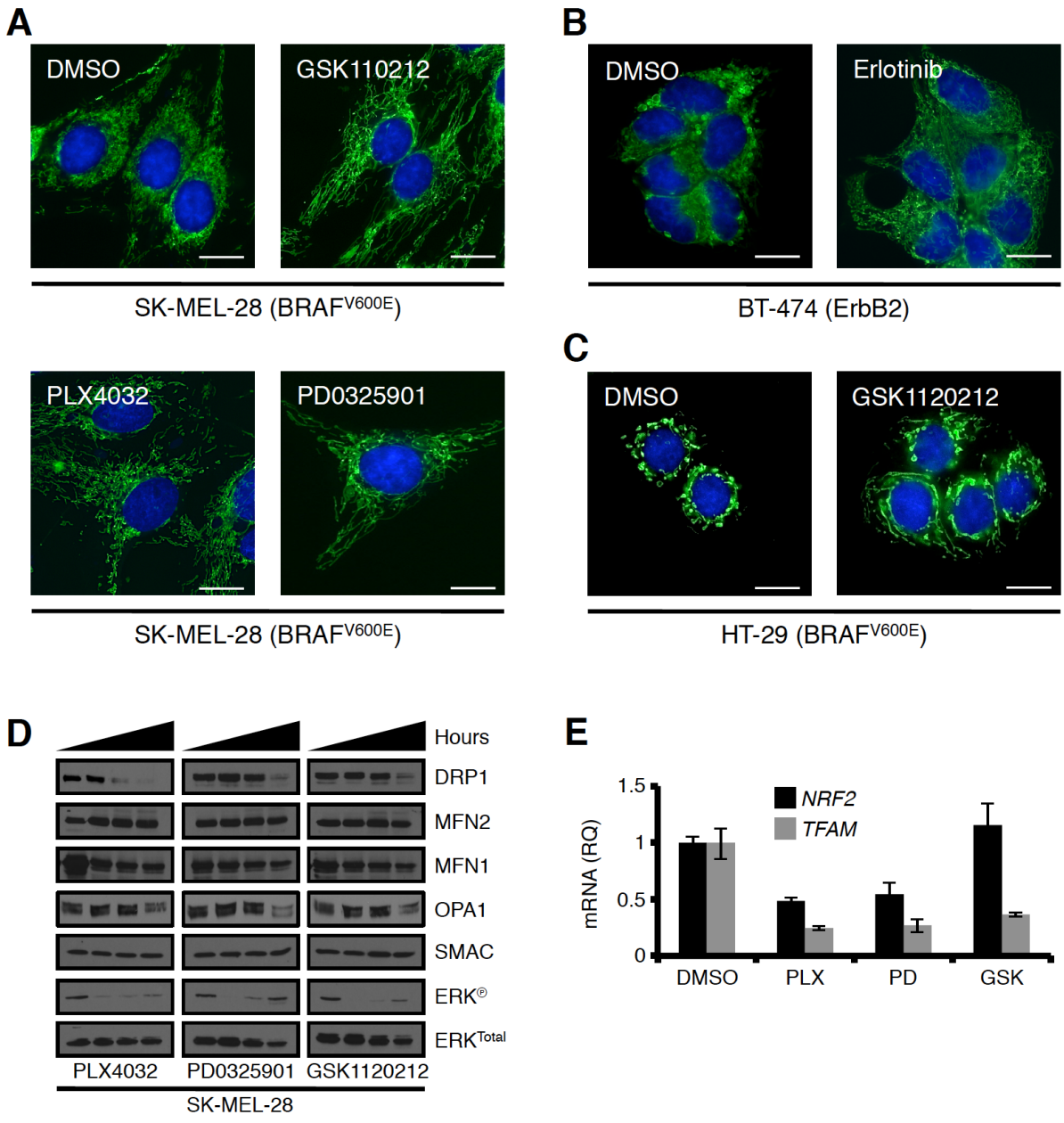


Figure S2. ***(Related to Figure 4).*** **Pharmacological inhibition of oncogenic MAPK signaling results in mitochondrial fusion.**

(A) SK-MEL-28 were treated with PLX4032 (1 μM), PD0325901 (50 nM), or GSK1120212 (10 nM) for 24 h, and loaded with MitoTracker Green and Hoechst 33342 (nuclei) before live cell imaging.

(B) BT-474 were treated with Erlotinib (2 μM) for 24 h, and loaded with MitoTracker Green and Hoechst 33342 (nuclei) before live cell imaging.

(C) HT-29 were treated with PD0325901 (1 μM) for 24 h, and loaded with MitoTracker Green and Hoechst 33342 (nuclei) before live cell imaging.

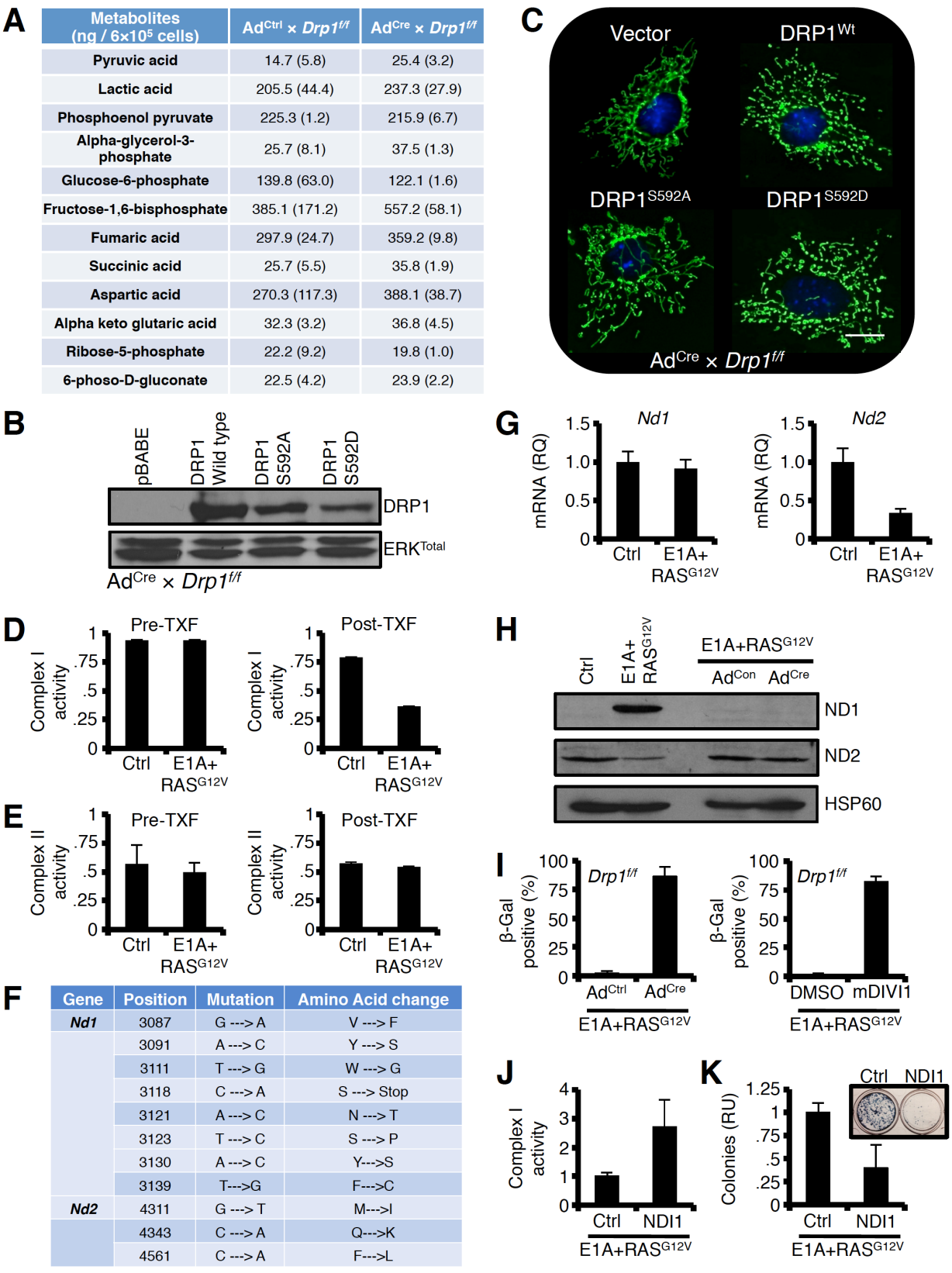
(D) SK-MEL-28 cells were treated with PLX4032 (1 μM), PD0325901 (50 nM), or GSK1120212 (10 nM) for 0, 12, 24, 48 h; and lysates were subjected to western blot analyses for indicated proteins.

(E) A375 cells were treated with PLX4032 (1 μM), PD0325901 (50 nM), or GSK1120212 (10 nM) for 2 h, harvested for total RNA, subjected to qPCR for indicate genes, and normalized against *Actin* and *GAPDH*.

All data are representative of at least triplicate experiments, and reported as ± S.D., as required.

A

Metabolites (ng / 6×10^5 cells)	Ad^{Ctrl} × *Drp1$^{f/f}$*	Ad^{Cre} × *Drp1$^{f/f}$*
Pyruvic acid	14.7 (5.8)	25.4 (3.2)
Lactic acid	205.5 (44.4)	237.3 (27.9)
Phosphoenol pyruvate	225.3 (1.2)	215.9 (6.7)
Alpha-glycerol-3-phosphate	25.7 (8.1)	37.5 (1.3)
Glucose-6-phosphate	139.8 (63.0)	122.1 (1.6)
Fructose-1,6-bisphosphate	385.1 (171.2)	557.2 (58.1)
Fumaric acid	297.9 (24.7)	359.2 (9.8)
Succinic acid	25.7 (5.5)	35.8 (1.9)
Aspartic acid	270.3 (117.3)	388.1 (38.7)
Alpha keto glutaric acid	32.3 (3.2)	36.8 (4.5)
Ribose-5-phosphate	22.2 (9.2)	19.8 (1.0)
6-phoso-D-gluconate	22.5 (4.2)	23.9 (2.2)



F

Gene	Position	Mutation	Amino Acid change
Nd1	3087	G ---> A	V ---> F
	3091	A ---> C	Y ---> S
	3111	T ---> G	W ---> G
	3118	C ---> A	S ---> Stop
	3121	A ---> C	N ---> T
	3123	T ---> C	S ---> P
	3130	A ---> C	Y--->S
	3139	T--->G	F--->C
Nd2	4311	G ---> T	M--->I
	4343	C ---> A	Q--->K
	4561	C ---> A	F--->L

Figure S3. ***(Related to Figure 5).*** **Reconstitution of floxed primary *Drp1$^{f/f}$* MEFs with DRP1 variants; and metabolite profiling to determine the effects of floxing *Drp1*.**

(A) Primary *Drp1*$^{f/f}$ MEFs were treated with Ad^{Cre} or Ad^{Ctrl} for 72 h, and metabolite profiling was performed. The provided values are ng of indicated metabolite per 600,000 cells.

(B) Primary *Drp1*$^{f/f}$ MEFs were infected with E1A+RAS^{G12V}, cultured for 48 h, treated with Ad^{Cre} or Ad^{Ctrl} for 72 h, and reconstituted with vector, DRP1^{Wt}, DRP1^{S592D}, or DRP1^{S592A}. Cell lysates were analyzed by western blot for DRP1 and ERK (loading control). Numerous experiments revealed consistent unequal expression between the variants. However, functionally, the variants behaved as expected *(see S3C)*.

(C) 48 h after reconstitution, the above cells were loaded with MitoTracker Green and Hoechst 33342 (nuclei) for live cell imaging.

(D) Primary Wt MEFs were infected with E1A+RAS^{G12V}, and pre- and post-transformation cultures were harvested and compared for complex I activity.

(E) Primary Wt MEFs were infected with E1A+RAS^{G12V}, and pre- and post-transformation cultures were harvested and compared for complex II activity.

(F) Mitochondrial DNA from transformed Wt MEFs was harvested and sequenced. *Nd1* and *Nd2* sequences are provided.

(G) qPCR analyses for *Nd1* and *Nd2* before and after transformation, normalized to *18S* and *Gapdh*.

(H) Primary Wt MEFs were infected with E1A+RAS^{G12V}, allowed to transform, and lysates were subjected to SDS-PAGE and western blot. Primary *Drp1*$^{f/f}$ MEFs were infected with E1A+RAS^{G12V} and lysates were subjected to SDS-PAGE and western blot. HSP60 is a mitochondrial marker loading control.

(I) Primary *Drp1*$^{f/f}$ MEFs were treated with Ad^{Cre} or Ad^{Ctrl} for 72 h (or, DMSO or 25 μM mDIVI), and then infected with E1A+RAS^{G12V}. The cells were cultured for 3 weeks and assayed for senescence-associated β-galactosidase activity (300 cells per condition).

(J-K) Primary Wt MEFs were infected with control or NDI1 retroviruses, cultured for 96 hours, and then infected with E1A+RAS^{G12V}. Complex I activities and resulting colonies were quantified, and normalized to control infections.

All data are representative of at least triplicate experiments, and reported as ± S.D., as required.

A

Metabolites (ng / 8×10^5 cells)	DMSO	PLX-4032
Pyruvic acid	845.1 (74.8)	100.5 (30.2)
Lactic acid	15798.4 (585.6)	1715.2 (493.8)
Phosphoenol pyruvate	1008.6 (213.2)	495.8 (48.4)
Alpha-glycerol-3-phosphate	386.9 (43.1)	117.6 (17.1)
Glucose-6-phosphate	381.0 (51.2)	268.4 (11.5)
Fructose-1,6-bisphosphate	1197.1 (275.1)	1537.4 (47.0)
Fumaric acid	1617.0 (172.3)	1190.4 (159.4)
Succinic acid	270.9 (19.3)	98.2 (28.3)
Alpha keto glutaric acid	513.6 (122.9)	95.9 (5.8)
Ribose-5-phosphate	68.9 (8.4)	28.5 (3.1)
6-phoso-D-gluconate	173.4 (16.3)	117.5 (6.7)
UDPG	3500.6 (337.1)	1553.7 (206.3)

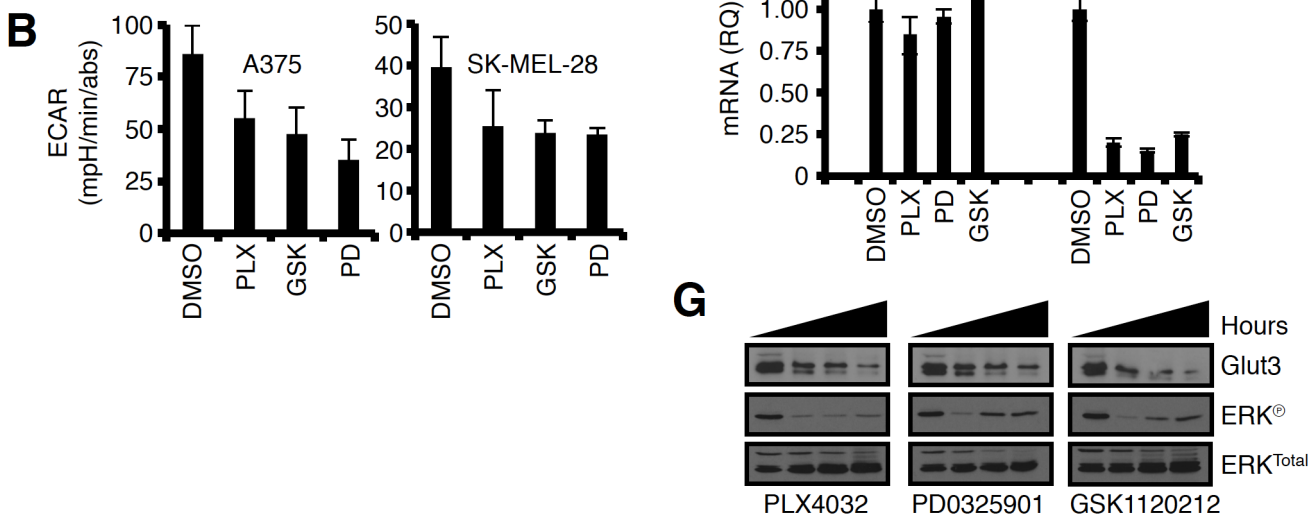


Figure S4. ***(Related to Figure 6).*** **Metabolic changes in response to oncogenic MAPK signaling inhibition.**

(A) A375 cells were treated with PLX4032 for 24 h, and metabolite profiling was performed. The provided values are ng of indicated metabolite per 800,000 cells.

(B) A375 and SK-MEL-28 cells were treated with PLX4032 (1 μM), PD0325901 (50 nM), or GSK1120212 (10 nM) for 24 h before extracellular acidification rates (ECAR) were determined per 50,000 cells.

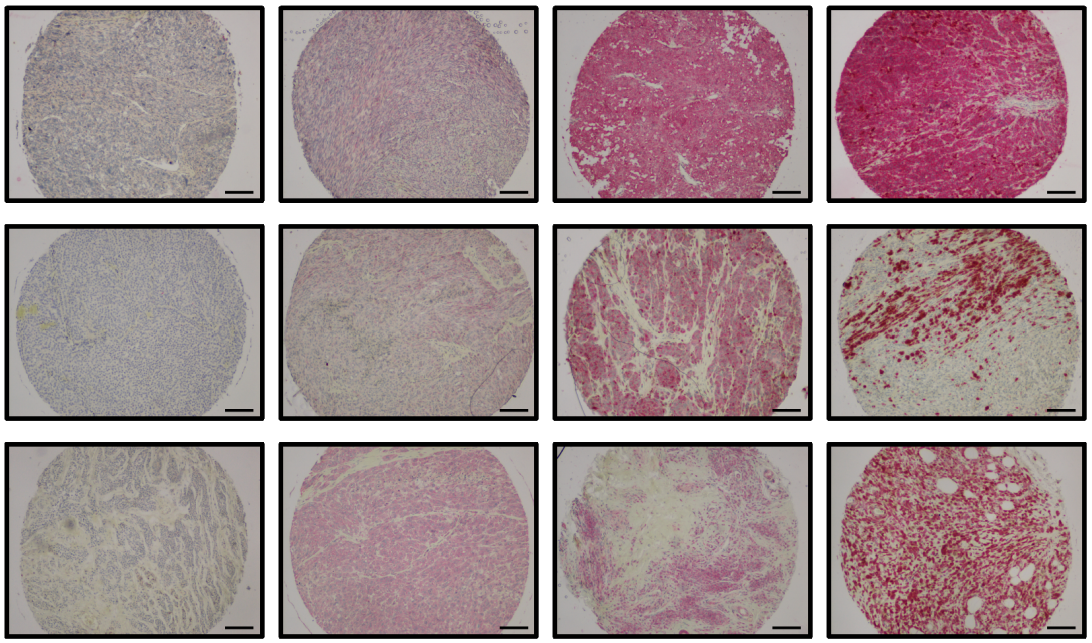
(C-E) Indicated cells were treated with PLX4032 (1 μM), PD0325901 (50 nM), GSK1120212 (10 nM), or Erlotinib (2 μM) for 24 h, and media pH was measured.

(F) A375 cells were treated with indicated drugs for 2 h, harvested for total RNA, subjected to qPCR for indicate genes, and normalized against *GAPDH*.

(G) A375 cells were treated with PLX4032 (1 μM), PD0325901 (50 nM), or GSK1120212 (10 nM) for 0, 12, 24, 48 h; and lysates were subjected to western blot analyses for indicated proteins.

All data are representative of at least triplicate experiments, and reported as ± S.D., as required.

A



B

BRAF status (Wt / V600E)	DRP1 S616Ⓟ (positive / negative)	Number/321 (%)	Fisher's Exact	Chi-Squared (X^2)
BRAF ^{Wt}	DRP1 $^{S616Ⓟ}$ (–)	114 (35.6%)	$p = 0.0001$	$p = 0.0001$
BRAF ^{Wt}	DRP1 $^{S616Ⓟ}$ (+)	9 (2.8%)		
BRAF ^{V600E}	DRP1 $^{S616Ⓟ}$ (–)	62 (19.3%)		
BRAF ^{V600E}	DRP1 $^{S616Ⓟ}$ (+)	136 (42.4%)		
Normal skin	DRP1 $^{S616Ⓟ}$ (+)	0/18 (0%)		

C

	DRP1 $^{S616Ⓟ}$ (positive / negative)	Number (%)
DRP1 ^{Total}	DRP1 $^{S616Ⓟ}$ (–)	35/95 (36.8%)
DRP1 ^{Total}	DRP1 $^{S616Ⓟ}$ (+)	38/95 (40%)
	TOTAL	**73/95 (76.8%)**

Figure S5. ***(Related to Figure 7).*** **Immunohistochemistry and complete statistics of BRAF^{V600E}, DRP1$^{S616Ⓟ}$, DRP1^{Total} status in melanoma.**

(A) Immunohistochemistry was performed to detect the status of BRAF^{V600E}, DRP1$^{S616Ⓟ}$, and DRP1^{Total}; and examples of tumor scoring are shown.

(B) 321 FFPE human melanoma biopsy sections were stained and scored for $BRAF^{V600E}$ and $DRP1^{S616℗}$ status; results were analyzed by a Fisher's Exact Test (p = < 0.0001) and Chi-Squared Test (p = < 0.0001) for statistical significance. Normal skin is shown for negative controls.

(C) 95 FFPE human melanoma biopsy sections were stained and scored for $DRP1^{Total}$ and $DRP1^{S616℗}$ status.

Movie S1 ***(Related to Figure 1A).*** Primary MEFs were labeled with MitoTracker Green (100 nM), and imaged using complete growth media without phenol red.

Movie S2 ***(Related to Figure 1A).*** E1A+RAS^{G12V} transformed MEFs were labeled with MitoTracker Green (100 nM) and imaged using complete growth media without phenol red.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Cell cycle and senescence analyses. Cells were treated as indicated, trypsinized, and pelleted. Cells were simultaneously resuspended and fixed by adding 500 μl of 70% ethanol with gentle vortexing, and incubated overnight at 4ºC. The samples were repelleted, the supernatant removed, and each pellet was resuspended in 1 ml of PBS containing propidium iodide (60 μg/ml) and RNAse A (20 μg/ml), and immediately analyzed by flow cytometry. Cellular senescence was determined with a β-galactosidase staining kit (Cell Signaling) according to the manufacturer's instructions.

Heavy membrane isolations. At least 1×15 cm dish at 90-95% confluency was used per treatment. Cells were harvested by trypsinization, and pelleted by centrifugation at 1000×*g* for 10 min as described (Renault et al., 2013). In brief, cell pellets were washed once with mitochondrial isolation buffer (MIB: 200 mM mannitol, 68 mM sucrose, 10 mM HEPES-KOH pH 7.4, 10 mM KCl, 1 mM EDTA, 1 mM EGTA, 0.1% BSA), and resuspended in MIB supplemented with protease inhibitors (HALT, Pierce Biotechnology). The cell suspension was incubated on ice for 20 min, and homogenized using a 2 ml Potter-Elvehjem dounce. The homogenate was centrifuged for 10 min at 800×*g* at 4ºC, the supernatant collected, and centrifuged again using the same conditions to ensure that no unlysed cells or nuclei were present. The resulting supernatant was centrifuged for 10 min at 8000×*g* at 4ºC, and the pellet was collected as the heavy membrane fraction (*i.e.,* mitochondria).

Western blot analyses. Whole cell protein lysates were made from trypsinized cells, pelleted, resuspended in RIPA buffer supplemented with protease inhibitors (HALT, Pierce Biotechnology), incubated on ice for 10 min and centrifuged for 10 min at 21,000×*g*. Lysates were then adjusted with RIPA buffer to equal the protein concentrations. Proteins (25-50 μg/lane) were subjected to SDS-PAGE before transferring to nitrocellulose by standard western conditions, blocked in 5% milk/TBST and primary antibodies (1:1000 in blocking buffer; incubated overnight at 4°C). The secondary antibody (1:5000 in blocking buffer) was incubated at 25°C for 1 h before standard enhanced chemiluminescence detection.

Live cell imaging. Cells were seeded on rat-tail collagen I coated plates for 24 h before indicated treatments. Mitochondria and nuclei were labeled with MitoTracker Green (100 nM) and Hoechst 33342 (20 μM) for 30 min at 37ºC, respectively. Phenol red free media supplemented with 10% FBS and 2 mM L-glutamine and antibiotics was used for all imaging performed on a Zeiss Imager.Z1 equipped with a N-Achroplan 40 X/0.75 water immersion lens and an AxioCAM MRm digital camera; images were captured using AxioVision 4.8 and Zeiss Zen software. At least 300 cells per condition were quantified. The Z-stack images were processed using Image J software (NIH) and the mitochondrial length was measured using NIS Elements software (Nikon, USA). Scale bars = 25 μm.

Glucose uptake & pH measurement. Cells were plated in phenol red free DMEM (HyClone) supplemented with 10% FBS and 2 mM glutamine and antibiotics, and treated with the indicated drug concentrations. After treatment, the glucose concentration of the media was determined using the Glucose GO assay kit (Sigma-Aldrich) according to the manufacturer's instructions. Glucose consumption was normalized to the number of cells per well. For the measurement of media pH, 37ºC media was analyzed using a standard pH meter 96 h after indicated treatments.

Real-time quantitative PCR. Total cellular RNA was extracted using an RNeasy kit (Qiagen) according to the manufacturer's instructions. Total RNA (1 μg) was used to synthesize first strand cDNA using the SuperScript™ III First-Strand Synthesis System for RT-PCR (Invitrogen). Gene expression was analyzed using the SYBR Green detection system (FastStart Universal SYBR Green Master, Roche) and Applied Biosciences ViiA™ 7 Real-Time PCR system, using the comparative C_T method. The expression of relevant genes was normalized to *18S* and *gapdh*. The following primer pairs (5'-3') were used: *mDrp1* GCGAACCTTAGAATCTGTGGACC & CAGGCACAAATAAAGCAGGACGG; *mMfn1* CCAGGTACAGATGTCACCACAG & TTGGAGAGCCGCTCATTCACCT; *mMfn2* GTGGAATACGCCAGTGAGAAGC & CAACTTGCTGGCACAGATGAGC; *mNd1* CCTATGGATCCGAGCATCTT & GGTGGTACTCCCGCTGTAAA; *mNd2* CACAATATCCAGCACCAACC & GAGGCTGTTGCTTGTGTGAC; *mOpa1* TCTCAGCCTTGCTGTGTCAGAC & TTCCGTCTCTAGGTTAAAGCGCG; *mFis1*

GCTGGTTCTGTGTCCAAGAGCA & GACATAGTCCCGCTGTTCCTCT; *mGapdh* CATCACTGCCACCCAGAAGACTG & ATGCCAGTGAGCTTCCCGTTCAG; *mActβ* CATTGCTGACAGGATGCAGAAGG & TGCTGGAAGGTGGACAGTGAGG; *hDRP1* GATGCCATAGTTGAAGTGGTGAC & CCACAAGCATCAGCAAAGTCTGG; *hMFN1* GGTGAATGAGCGGCTTTCCAAG & TCCTCCACCAAGAAATGCAGGC; *hMFN2* ATTGCAGAGGCGGTTCGACTCA & TTCAGTCGGTCTTGCCGCTCTT; *hOPA1* GTGGTTGGAGATCAGAGTGCTG & GAGGACCTTCACTCAGAGTCAC; *hFIS1* CAAGGAACTGGAGCGGCTCATT & GGACACAGCAAGTCCGATGAGT; *hACTβ* TCACCCACACTGTGCCCATCTACGA & CAGCGGAACCGCTCATTGCCAATGG; *h18S* TAGAGGGACAAGTGGCGTTC & CGCTGAGCCAGTCAGTGT; *hNRF2A* CTGCTGCACTGGAAGGCTATAG & GGTGAGGTCTATATCGGTCATGC; *hTFAM* GTGGTTTTCATCTGTCTTGGCAAG & TTCCCTCCAACGCTGGGCAATT; *hGLUT1* TTGCAGGCTTCTCCAACTGGAC & CAGAACCAGGAGCACAGTGAAG; and *hGLUT3* TGCCTTTGGCACTCTCAACCAG & GCCATAGCTCTTCAGACCCAAG.

Mitochondrial membrane potential ($\Delta\varphi_M$), mtROS measurements, and Complex I & II assays. Cells were seeded for 24 h, and treated as indicated. TMRE (100 nM) or MitoSOX RED (5 μM) was added to the media, and the plates were incubated at 37ºC in the dark for 25 min. The cells were then trypsinized and analyzed by flow cytometry. Complex I and II activities were determined using NADH Dehydrogenase (150 μg cell lysate; 50 μg mitochondria) and Succinate Dehydrogenase (60 μg cell lysate; 10 μg mitochondria) Profiling ELISA kits (MitoSciences/Abcam) according to the manufacturer's instructions.

Seahorse analysis. Cells were seeded in 200 μl DMEM complete media in XF96 plates (Seahorse Bioscience); plating densities: A375 4×10^3, SK-MEL-28 8×10^3, primary MEFs 2×10^3, and E1A+RAS^{G12V} transformed MEFs 8×10^3, and treated as indicated. Oxygen consumption rates (OCR) and extracellular acidification rates (ECAR) were measured using the XF96 Extracellular Flux Analyzer and the XF Cell Mito Stress Test kit (Seahorse Bioscience). At the end of the assay, media was removed and cells were stained with methylene blue, de-stained, and the absorbance was measured at 668 nm using a plate reader (Synergy H1 Hybrid multi-mode micro-plate reader, Biotek). The OCR and ECAR

measurements were normalized against the cell densities. Each experiment contained triplicate data points.

Metabolite profiling. Cells were seeded in 10 cm^2 plates, grown to ~95% confluency, and treated as indicated. Cells were rinsed once with ice cold PBS and collected in cold PBS using a cell scraper. Cells were pelleted by centrifugation at 600×*g* for 5 min, the PBS was aspirated, and the pellets were flash frozen in liquid nitrogen. Pellets were submitted for LC-MS metabolite profiling using modules 3 and 4 at the Stable Isotope & Metabolomics Core (Albert Einstein School of Medicine, NY). All metabolites were assessed using a triple quadrupole Waters Xevo mass spectrometer. For glycolytic, pentose, and TCA metabolites, chromatographic analysis prior to mass spectrometric analyses was performed using an Aquity UPLC using a Waters BEH amide 1.7 μm column 2.1×100 mm, and an acidic mobile phase containing an acetonitrile/water gradient. For adenine nucleotides and redox metabolites, chromatographic analysis prior to mass spectrometric analyses was performed using a SeQuant® ZIC®-cHILIC 3 μm column 2.1×100 mm and a basic mobile phase with acetonitrile/water gradient.

BRAF exon 15 sequencing. Cell lines and FFPE tissue samples were treated using the QuickExtract™ FFPE DNA Extraction Kit using standard protocols. Primers (5'-3') used for PCR amplification of *BRAF* exon 15 were: TCATAATGCTTGCTCTGATAGGA and GGCCAAAAATTTAATCAGTGGA. PCR amplification was performed using *Taq* DNA polymerase (Life Technologies) and resolved in a 0.8% agarose/TAE gel containing GelRed™ Nucleic Acid Gel Stain. PCR products were then isolated using the illustra™ GFX™ PCR DNA and Gel Band Purification Kit (GE Healthcare Life Sciences). Sanger sequencing of *BRAF* exon 15 was performed using primers (5'-3'):
TGTTTTCCTTTACTTACTACACCTC and TAATCAGTGGAAAAATAGCCTC.

Immunofluorescence. 2.5×10^4 cells were plated per 1.8 cm^2 in Nunc™ Lab-Tek™ chamber slides and treated as indicated. Cells were then fixed with 4% formaldehyde, and incubated in blocking buffer (1X PBS supplemented with 5% normal goat serum and 0.3% Triton™ X-100) for 60 min at RT. Primary antibodies for DRP1$^{S616℗}$ (1:3200) and HSP60

(1:300) were diluted in 1X PBS supplemented with 1% BSA and 0.3% Triton™ X-100, and incubated overnight at 4°C. Secondary antibodies Alexa Fluor® 488 (1:1000, Cell Signaling) and Texas Red (1:200, Cell Signaling) were diluted in the above primary antibody buffer and incubated for 90 min at RT. Samples were mounted with ProLong® Gold Antifade Reagent containing DAPI, cured overnight at RT, and imaged with a Zeiss Imager.Z1 and an AxioCAM MRm digital camera; images were captured using AxioVision 4.8 software. Scale bars = 100 μm.

Immunohistochemistry and statistical analyses. 416 FFPE human melanoma biopsy sections were obtained from the Icahn School of Medicine at Mount Sinai Department of Dermatology, the Mount Sinai Biorepository, and tissue microarrays (US Biomax, SK181, ME1004a, ME208b; Imgenex, IMH-369). A Leica BOND RX automated system for immunohistochemistry was utilized; primary antibodies were diluted in BOND Primary Antibody Diluent as indicated: $BRAF^{V600E}$ (1:100, clone VE1, Spring Biosciences), $DRP1^{S616®}$ (1:200, Cell Signaling), and $DRP1^{Total}$ (1:150, BD Biosciences). Bond Epitope Retrieval (ER) solution 2 was used for 30 min; the slides were then mounted with Permount™, and imaged. Slides were scored by intensity of staining (0, 1+, 2+, 3+), and a subset of samples was confirmed by pathologists within the Mount Sinai Division of Dermatopathology. 0 and 1+ scoring were graded as negative, while 2+ and 3+ scorings were positive. Scoring was analyzed via a Fisher's Exact Test and a Chi-Squared Test without Yates correction. Chi-Squared equaled 115.373 with 1 degrees of freedom. The two-tailed p value is < 0.0001. Statistics were performed with GraphPad QuickCalc online software. The Program for the Protection of Human Subjects office determined that the above study is exempt human research (HS#13-00606) as defined by DHHS regulations (45CFR46.101(b)(4). Data are representative of at least triplicate experiments, and reported as ± S.D., as required, for other all other assays.

Mitochondrial DNA sequencing. Total DNA was purified from cultured cells using the DNAeasy Blood and Tissue Kit (Qiagen) according to manufacturer's instructions. The region spanning *Nd1* and *Nd2* was amplified using specific primer pairs targeting the flanking sequences, *Nd1*: 5'-AAACCTTGTTCCCAGAGGTTCAAATCC-3' and 5'-AACCTCTATAATTTACTCTATCAAAGTAATTCT-3'; *Nd2*: 5'-

GGGCCCATACCCCGAAAACGTTGGTTT-3’ and 5’-TTTTCTTAGGGCTTTGAAGGCTCGCGG-3’. The TaKaRa LA PCR ™ Kit was utilized for PCR amplification, and the resulting PCR products were purified (QIAquick PCR purification kit), and then fragmented to 400bp - 600bp in size using a Covaris E210. An Illumina compatible sequencing library was constructed using the DNA fragments with the KAPA Hyper Prep Kit. The manufacturer's recommendations were followed; 8 cycles of PCR was used to amplify libraries. Libraries were sequenced using 150nt paired-end reads using the Illumina MiSeq instrument.

SUPPLEMENTAL REFERENCES

Renault, T.T., Floros, K.V., and Chipuk, J.E. (2013). BAK/BAX activation and cytochrome c release assays using isolated mitochondria. Methods *61*, 146-155.