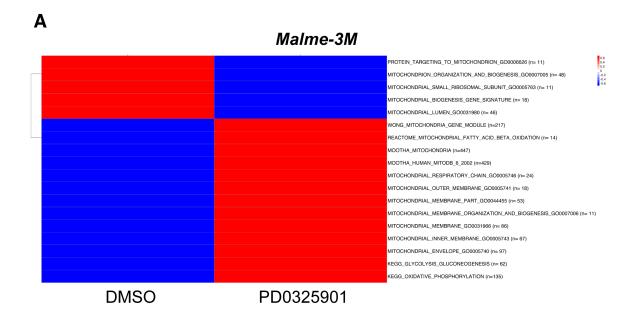
Supplemental data

Supplemental data include extended experimental procedures, 7 supplemental figures and 9 supplemental tables.

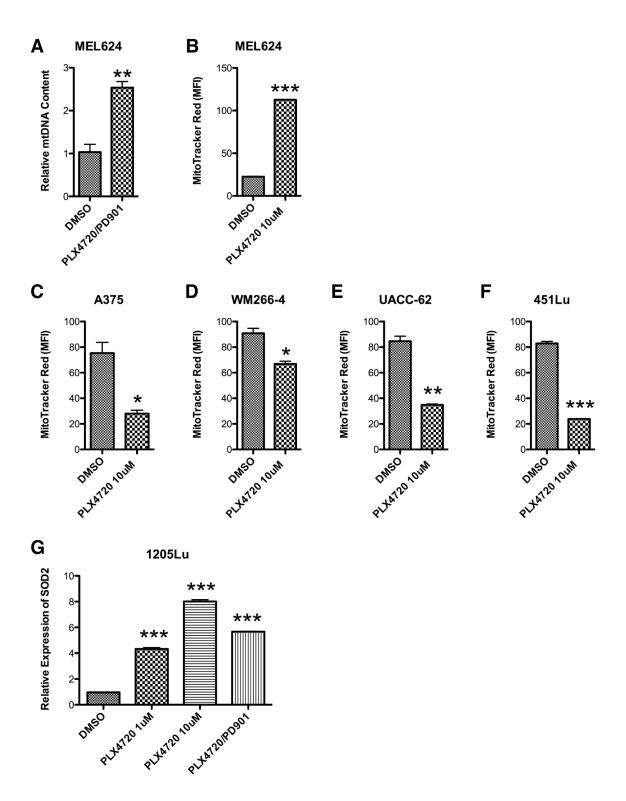
Rank	Gene Set	NES	NOM p-val	FDR q-val
1	MEK TARGETS PNAS JOSEPH	3.04	0	0
2	BRAF TARGETS PNAS JOSEPH	3	0	0
3	KEGG LYSOSOME	2.81	0	0
4	MEK TARGETS PRATILAS PNAS2009	2.59	0	0
5	KEGG ECM RECEPTOR INTERACTION	2.21	0	0
6	KEGG AMINO SUGAR AND NUCLEOTIDE SUGAR METABOLISM	2.19	0	0.001
7	KEGG VIBRIO CHOLERAE INFECTION	2.05	0	0.005
8	KEGG GLYCOSAMINOGLYCAN DEGRADATION	1.91	0.002	0.017
9	KEGG MELANOGENESIS	1.88	0	0.022
10	KEGG TYROSINE METABOLISM	1.87	0	0.020

Supplemental Figure 1. Related to Figure 1. The Top 10 ranked pathways associated with the "melanoma" phenotype. CCLE melanoma cell lines were compared to CCLE non-melanoma cell lines followed by GSEA.



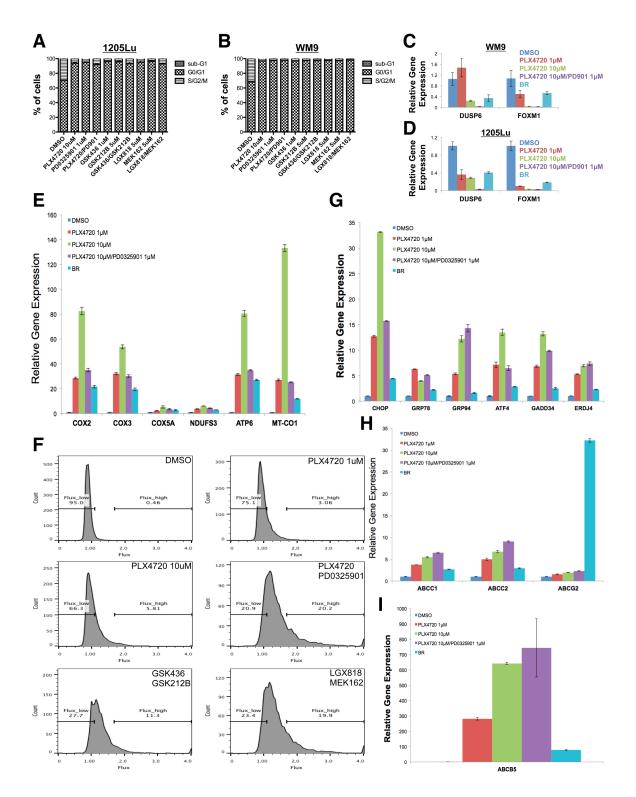
В **COLO829** 0.6 0.4 0.2 0 -0.2 -0.4 -0.6 ONG_MITOCHONDRIA_GENE_MODULE (n=217) PROTEIN TARGETING TO MITOCHONDRION GO0006626 (n= 11) MOOTHA_MITOCHONDRIA (n=447) MOOTHA_HUMAN_MITODB_6_2002 (n=429) MITOCHONDRION_ORGANIZATION_AND_BIOGENESIS_GO0007005 (n= 48) MITOCHONDRIAL_SMALL_RIBOSOMAL_SUBUNIT_GO0005763 (n= 11) MITOCHONDRIAL OUTER MEMBRANE GO0005741 (n= 18) MITOCHONDRIAL_MEMBRANE_ORGANIZATION_AND_BIOGENESIS_GO0007006 (n= 11) MITOCHONDRIAL_MEMBRANE_GO0031966 (n= 86) MITOCHONDRIAL LUMEN GO0031980 (n= 46) MITOCHONDRIAL_INNER_MEMBRANE_GO0005743 (n= 67) MITOCHONDRIAL_ENVELOPE_GO0005740 (n= 97) MITOCHONDRIAL BIOGENESIS GENE SIGNATURE (n= 18) KEGG_GLYCOLYSIS_GLUCONEOGENESIS (n= 62) REACTOME_MITOCHONDRIAL_FATTY_ACID_BETA_OXIDATION (n= 14) MITOCHONDRIAL_RESPIRATORY_CHAIN_GO0005746 (n= 24) KEGG_OXIDATIVE_PHOSPHORYLATION (n=135) MITOCHONDRIAL MEMBRANE PART GO0044455 (n= 53) DMSO PD0325901

Supplemental Figure 2. Related to Figure 3. (A and B) The heat maps of enrichment scores of 20 gene sets in Malme-3M cells (A) and COLO829 cells (B) treated with the control and PD0325901.



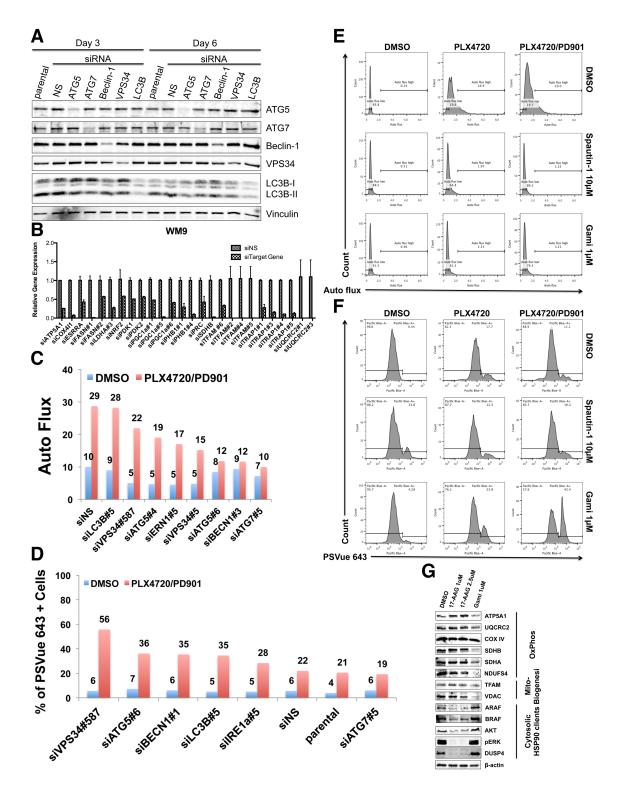
Supplemental Figure 3. Related to Figure 5. (A) Relative mitochondrial DNA copy number in MEL624 melanoma cells treated for 72 h with DMSO or indicated MAPK inhibitors. Technical replicates (n=3) for each condition were included. Data represented 2 independent experiments. (B-F) The MFI of MitoTracker Red in MEL624 (B), A375 (C), WM266-4 (D), UACC-62 (E) and 451Lu (F) treated for 72 h with DMSO or PLX4720 at 10 μ M. 2 biological replicates for

each condition were included. Data represented 2 independent experiments. (G) Relative gene expression of SOD2 determined by qRT-PCR in 1205Lu melanoma cells treated for 72 h with DMSO or indicated MAPK inhibitors. 3 technical replicates for each condition were included. Data represent 2 independent experiments. (A-G) An unpaired two-tailed t-test was used.



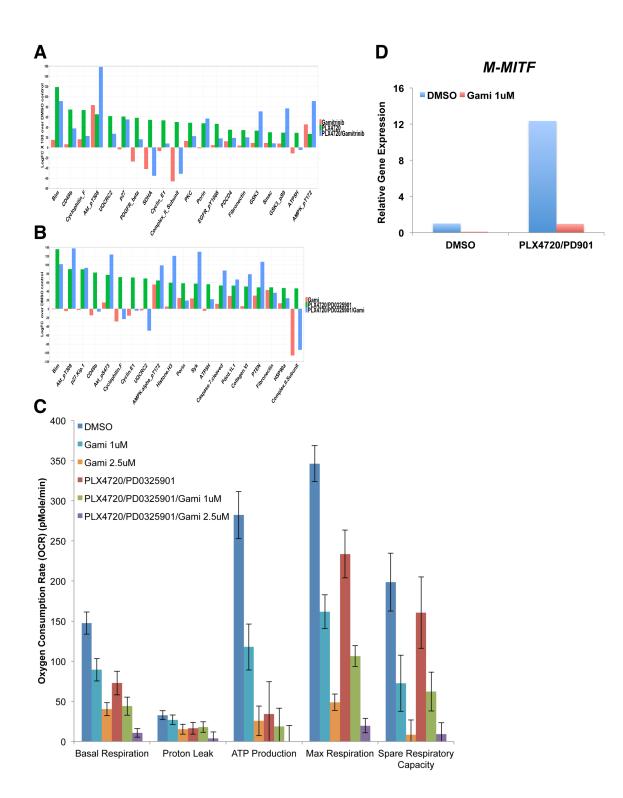
Supplemental Figure 4. Related to Figure 6. (A and B) Percentages of sub- G_1 , G_0/G_1 and S/G₂/M phase of the cell cycle were assessed by the Propidium Iodide Staining Cell Cycle FACS assay conducted in 1205Lu (A) and WM9 (B) cells that were treated with DMSO or indicated MAPK inhibitors for 3 days. (C and D) Relative gene expression of *FOXM1* and *DUSP6* assessed by qRT-PCR in WM9 (C) and 1205Lu (D) melanoma cells treated for 72 h with DMSO or

indicated MAPK inhibitors. BRAF inhibitor resistant cells (BR) were included as the control. (E) Relative gene expression of 6 representative mitochondrial respiratory chain subunits assessed by qRT-PCR in WM9 cells that were treated for 72 h with DMSO or indicated inhibitors. BRAF inhibitor resistant cells (BR) are included as the control. (F) The autophagic flux assessed by FACS analysis in WM9 cells expressing the mCherry-eGFP-LC3B construct treated for 72 h with DMSO or indicated MAPK inhibitors. Data represented the average of 2 biological replicates. (G-I) Relative gene expression of ER stress response genes (G) and ABC transporter genes (H and I) assessed by qRT-PCR in WM9 cells that were included in (E). (C, D, E, G, H and I) 3 technical replicates for each condition are included. Data represented 2 independent experiments.



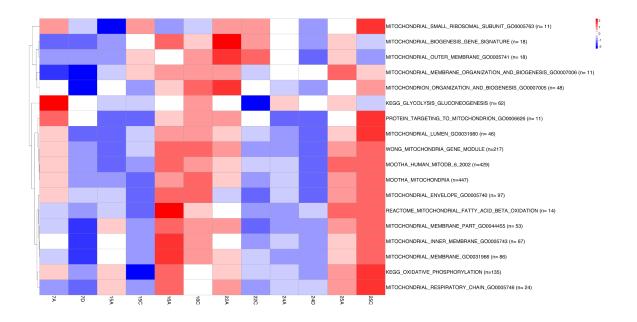
Supplemental Figure 5. Related to Figure 8. (A) Immunoblotting of autophagy proteins in WM9 cells transfected with siATG5, siATG7, siBeclin-1, siVPS34 or siLC3B at 20 nM for 3 and 6 days. Parental cells and cells transfected with siNS are included as controls. (B) Relative gene expression of MitoB, glycolysis and OxPhos genes assessed by qRT-PCR in WM9 cells transfected with siRNAs targeting MitoB, glycolysis and OxPhos genes at 20 nM for 3 days. 2

technical replicates for each condition were included. Data represented 2 independent experiments. (C) The autophagic flux assessed by FACS analysis of WM9 cells expressing the mCherry-eGFP-LC3B construct that were transfected with the indicated siRNAs targeting autophagy and ER stress response and treated for 72 h with DMSO or MAPK inhibitors. The average of 2 biological replicates was plotted and data represented 2 independent experiments. (D) The percentage of PSVue 643 positive WM9 cells expressing the mCherry-eGFP-LC3B construct transfected with the indicated siRNAs targeting autophagy and ER stress response and then treated for 72 h with DMSO or indicated MAPK inhibitors. The average of 2 biological replicates was plotted and data represented 2 independent experiments (E) and the percentage of PSVue 643 positive (F) WM9 cells expressing the mCherry-eGFP-LC3B construct that were treated for 72 h with Gamitrinib at 1 μ M or Spautin-1 at 10 μ M in combination with indicated MAPK inhibitors. Cells treated with DMSO were used as the control for the set-up of the gating. Data represented 2 biological replicates. (G) Immunoblotting of proteins related to OxPhos, MitoB and cytosolic Hsp90 clients in WM9 cells treated with DMSO, 17-AAG or Gamitrinib for 2 days.



Supplemental Figure 6. Related to Figure 10. (A and B) Relative expression of 20 proteins assessed by RPPA in WM9 melanoma cells that were most significantly up-regulated by the treatment with PLX4720 at 10 μ M (A) or the combination of PLX4720 at 10 μ M and PD0325901 at 1 μ M for 72 h. WM9 cells were also treated with Gamitrinib at 1 μ M, and the combination PLX4720 and Gamitrinib. WM9 cells treated with DMSO were used as the control. Data represented the average of 3 biological replicates. (C) Quantification of five parameters of

oxygen consumption rates of samples included in Figure 10E. (**D**) Relative gene expression of *M*-*MITF* assessed by qRT-PCR in WM9 cells treated for 48 h with DMSO or the combination of PLX4720 at 10 μ M and PD0325901 at 1 μ M, with or without the addition of Gamitrinib at 1 μ M. Data represented 2 independent experiments.



Supplemental Figure 7. Related to Figure 12. The heatmap of enrichment scores of 20 gene sets in paired pre- and post-treatment tumor biopsies that are included in EGAS00001000992.

Supplemental Table 1. The List of 5 Additional MitoB Genes (Related to Figure 1 and Table	1
1)	

Category	Symbol	Synonyms
Mitochondrial Outer Membrane Channel	VDACI	Outer mitochondrial membrane protein porin 1
Mitochondrial Protein Import	HSP60	60 kDa heat shock protein, mitochondrial
Mitochondria Protein Folding	TRAPI	TNF receptor-associated protein 1, mitochondrial
Mitochondria Protein Translation	TUFM	Tu translation elongation factor, mitochondrial
Antioxidant	SOD2	Superoxide dismutase 2, mitochondrial

Supplemental Table 2. The List of Sinificantly Enriched Gene Sets (Related to Figure 4)

Up-regulated Rank	12h	24h	48h	60h	72h	96h
1	STEROID BIOSYNTHESIS		LYSOSOME		OXIDATIVE PHOSPHORYLATION	OXIDATIVE PHOSPHORYLATION
2	JAK STAT SIGNALING PATHWAY	METABOLISM OF XENOBIOTICS BY CYTOCHROME			LYSOSOME	PARKINSONS DISEASE
3			AUTOIMMUNE THYROID DISEASE	PARKINSONS DISEASE	PARKINSONS DISEASE	LYSOSOME
4	CELL ADHESION MOLECULES CAMS	PARKINSONS DISEASE	GRAFT VERSUS HOST DISEASE	ALZHEIMERS DISEASE	ALZHEIMERS DISEASE	HUNTINGTONS DISEASE
5	ECM_RECEPTOR_INTERACTION	DRUG_METABOLISM_CYTOCHROME_P450	PARKINSONS_DISEASE	HUNTINGTONS_DISEASE	HUNTINGTONS_DISEASE	ALZHEIMERS_DISEASE
6	INTESTINAL_IMMUNE_NETWORK_FOR_IGA_PRODU	HUNTINGTONS_DISEASE	ALZHEIMERS_DISEASE	GRAFT_VERSUS_HOST_DISEASE	GRAFT_VERSUS_HOST_DISEASE	GRAFT_VERSUS_HOST_DISEASE
7	SMALL_CELL_LUNG_CANCER	ALZHEIMERS_DISEASE	GLYCOSYLPHOSPHATIDYLINOSITOL_GPI_ANCHOR	AUTOIMMUNE_THYROID_DISEASE	ABC_TRANSPORTERS	AUTOIMMUNE_THYROID_DISEASE
8	COMPLEMENT_AND_COAGULATION_CASCADES	CITRATE_CYCLE_TCA_CYCLE	ABC_TRANSPORTERS	ANTIGEN_PROCESSING_AND_PRESENTATION	AUTOIMMUNE_THYROID_DISEASE	PORPHYRIN_AND_CHLOROPHYLL_METABOLISM
9	PPAR_SIGNALING_PATHWAY	PORPHYRIN_AND_CHLOROPHYLL_METABOLISM	ALLOGRAFT_REJECTION	GLYCOSYLPHOSPHATIDYLINOSITOL_GPI_ANCHOR_	GLYCOSAMINOGLYCAN_BIOSYNTHESIS_CHONDRO	GLYCOSAMINOGLYCAN_BIOSYNTHESIS_CHONDROI
10	GLYCOSAMINOGLYCAN_BIOSYNTHESIS_HEPARAN_	STEROID_HORMONE_BIOSYNTHESIS	HUNTINGTONS_DISEASE	PORPHYRIN_AND_CHLOROPHYLL_METABOLISM	TYPE_I_DIABETES_MELLITUS	TYPE_I_DIABETES_MELLITUS
11	ALLOGRAFT_REJECTION	PROTEASOME	TYPE_I_DIABETES_MELLITUS	TYPE_I_DIABETES_MELLITUS	ALLOGRAFT_REJECTION	CITRATE_CYCLE_TCA_CYCLE
12	VIRAL_MYOCARDITIS	ABC_TRANSPORTERS	ANTIGEN_PROCESSING_AND_PRESENTATION	GLYCOSAMINOGLYCAN_BIOSYNTHESIS_CHONDRO	PORPHYRIN_AND_CHLOROPHYLL_METABOLISM	ANTIGEN_PROCESSING_AND_PRESENTATION
13	FOCAL_ADHESION	VALINE_LEUCINE_AND_ISOLEUCINE_DEGRADATION	INTESTINAL_IMMUNE_NETWORK_FOR_IGA_PRODU	ALLOGRAFT_REJECTION	CITRATE_CYCLE_TGA_CYCLE	ALLOGRAFT_REJECTION
14	AUTOIMMUNE_THYROID_DISEASE	CALCIUM_SIGNALING_PATHWAY	VALINE_LEUCINE_AND_ISOLEUCINE_DEGRADATION	CITRATE_CYCLE_TCA_CYCLE	NOTCH_SIGNALING_PATHWAY	RETINOL_METABOLISM
15	BLADDER_CANCER	GLUTATHIONE_METABOLISM	STARCH_AND_SUCROSE_METABOLISM	GLYCOSAMINOGLYCAN_DEGRADATION	GLYCOSYLPHOSPHATIDYLINOSITOL_GPI_ANCHOR	ABC_TRANSPORTERS
16	BIOSYNTHESIS_OF_UNSATURATED_FATTY_ACIDS	GLYCOSAMINOGLYCAN_DEGRADATION	PORPHYRIN_AND_CHLOROPHYLL_METABOLISM	DRUG_METABOLISM_CYTOCHROME_P450	APOPTOSIS	GLYCOSYLPHOSPHATIDYLINOSITOL_GPI_ANCHOR_
17	THYROID_CANCER		CITRATE_CYCLE_TCA_CYCLE	INTESTINAL_IMMUNE_NETWORK_FOR_IGA_PRODU		HEMATOPOIETIC_CELL_LINEAGE
18	LEISHMANIA_INFECTION	TYPE_II_DIABETES_MELLITUS	ALDOSTERONE_REGULATED_SODIUM_REABSORP	VIRAL_MYOCARDITIS	ANTIGEN_PROCESSING_AND_PRESENTATION	INTESTINAL_IMMUNE_NETWORK_FOR_IGA_PRODU
19	NON_SMALL_CELL_LUNG_CANCER		HEMATOPOIETIC_CELL_LINEAGE		CARDIAC_MUSCLE_CONTRACTION	CARDIAC_MUSCLE_CONTRACTION
20	MELANOGENESIS	ADIPOCYTOKINE_SIGNALING_PATHWAY	ALANINE_ASPARTATE_AND_GLUTAMATE_METABOL	STARCH_AND_SUCROSE_METABOLISM	VIRAL_MYOCARDITIS	NOTCH_SIGNALING_PATHWAY
Down-regulated						
Rank	12h	24h	48h	60h	72h	96h
1	MEK_TARGETS_PNAS_JOSEPH	MEK_TARGETS_PNAS_JOSEPH	RIBOSOME	MEK_TARGETS_PNAS_JOSEPH	MEK_TARGETS_PNAS_JOSEPH	MEK_TARGETS_PNAS_JOSEPH
2	BRAF_TARGETS_PNAS_JOSEPH	BRAF_TARGETS_PNAS_JOSEPH	MEK_TARGETS_PNAS_JOSEPH	BRAF TARGETS PNAS JOSEPH	BRAF TARGETS PNAS JOSEPH	CELL CYCLE
3	MEK_TARGETS_PRATILAS_PNAS2009					
4		CELL_CYCLE	MEK_TARGETS_PRATILAS_PNAS2009	RIBOSOME	CELL_CYCLE	BRAF_TARGETS_PNAS_JOSEPH
	STEROID_BIOSYNTHESIS		MEK_TARGETS_PRATILAS_PNAS2009 BRAF_TARGETS_PNAS_JOSEPH		CELL_CYCLE MEK_TARGETS_PRATILAS_PNAS2009	
5	STEROID_BIOSYNTHESIS JAK_STAT_SIGNALING_PATHWAY	MEK_TARGETS_PRATILAS_PNAS2009		MEK_TARGETS_PRATILAS_PNAS2009		BRAF_TARGETS_PNAS_JOSEPH
5		MEK_TARGETS_PRATILAS_PNAS2009 DNA_REPLICATION	BRAF_TARGETS_PNAS_JOSEPH	MEK_TARGETS_PRATILAS_PNAS2009 CELL_CYCLE	MEK_TARGETS_PRATILAS_PNAS2009	BRAF_TARGETS_PNAS_JOSEPH MEK_TARGETS_PRATILAS_PNAS2009
5 6 7	JAK_STAT_SIGNALING_PATHWAY CELL_ADHESION_MOLECULES_CAMS	MEK_TARGETS_PRATILAS_PNAS2009 DNA_REPLICATION COCYTE_MEIOSIS	BRAF_TARGETS_PNAS_JOSEPH CELL_CYCLE	MEK_TARGETS_PRATILAS_PNAS2009 CELL_CYCLE DNA_REPLICATION	MEK_TARGETS_PRATILAS_PNAS2009 DNA_REPLICATION	BRAF_TARGETS_PNAS_JOSEPH MEK_TARGETS_PRATILAS_PNAS2009 DNA_REPLICATION
8	JAK_STAT_SIGNALING_PATHWAY CELL_ADHESION_MOLECULES_CAMS	MEK_TARGETS_PRATILAS_PNAS2009 DNA_REPLICATION OOCYTE_MEIOSIS MISMATCH_REPAIR	BRAF_TARGETS_PNAS_JOSEPH CELL_CYCLE DNA_REPLICATION	MEK_TARGETS_PRATILAS_PNAS2009 CELL_CYCLE DNA_REPLIGATION MISMATCH_REPAIR	MEK_TARGETS_PRATILAS_PNAS2009 DNA_REPLICATION RIBOSOME	BRAF_TARGETS_PNAS_JOSEPH MEK_TARGETS_PRATILAS_PNAS2009 DNA_REPLICATION MISMATCH_REPAIR
8 9	JAK_STAT_SIGNALING_PATHWAY CELL_ADHESION_MOLECULES_CAMS GLYCOSAMINOGLYCAN_DEGRADATION	MEK_TARGETS_PRATILAS_PNAS2009 DNA_REPLICATION OCCYTE_MEIOSIS MISMATCH_REPAIR RIBOSOME	BRAF_TARGETS_PNAS_JOSEPH CELL_CYCLE DNA_REPLICATION MISMATCH_REPAIR	MEK_TARGETS_PRATILAS_PNAS2009 CELL_CYCLE DNA_REPLICATION MISMATCH_REPAR NUCLEOTIDE_EXCISION_REPAIR	MEK_TARGETS_PRATILAS_PNAS2009 DNA_REPLICATION RIBOSOME MISMATCH_REPAIR	BRAF_TARGETS_PNAS_JOSEPH MEK_TARGETS_PRATILAS_PNAS2009 DNA_REPUCATION MISMATCH_REPAIR NUCLEOTIDE_EXCISION_REPAIR
8 9 10	JAK_STAT_SIGNALING_PATHWAY CELL_ADHESION_MOLECULES_CAMS GLYCOSAMINOCLYCAN_DEGRADATION ECM_RECEPTOR_INTERACTION COMPLEMENT_ANG_CONGULATION_CASCADES INTESTINAL_IMMUNE_NETWORK_FOR_IGA_PRODU	MEK_TARGETS_PRATLAS_PNA52009 DNA_REPLICATION OCCYTE_UEDSIS MISMATCH_REPAIR RIBOSOME JAK_STAT_SIGNALING_PATHWAY PSS_SIGNALING_PATHWAY	BRAF_TARGETS_PNAS_JOSEPH CEL_CYCLE DNA_REPLICATION MISMATCH_REPAIR NUCLEOTIDE_EXCISION_REPAIR BASE_EXCISION_REPAIR OCCYTE_MEIOSIS	MEK_TARGETS_PRATILAS_PNA52009 CELL_CYCLE DWA_REPLICATION MISMATCH_REPWR NUCLEOTIDE_EXCISION_REPAIR OCCVTE_WEIDISIS VINT_SIGNALUNG_PATHWAY	MEK_TARGETS_PRATILAS_PNAS2009 DNA_REPLICATION RIBOSOME MISMATCH_REPAIR NUCLEOTIDE_EXCISION_REPAIR BASE_EXCISION_REPAIR OCCYTE_MEIOSIS	BRAF_TARGETS_PHALS_DOEPH MEK_TARGETS_PRATUAS_PHAS2009 DMU_REPLACINN MISMATCH_REPAR NOLLCOTTE_REVARIN NOLLCOTTE_REVARING NOLLCOTTE_REVARING COCYTE_MEIOSIS BOSYNTHESIS_OF_JUNATURATED_FATTY_ACIDS
8 9 10 11	JAK_STAT_SIGNALING_PATHWAY CELL_DOMESION_MOLECULES_CAMS GUYCOSANMODUCYCAN_DEGRADATION ECM_RECEPTOR_INTERACTION COMPLEMENT_AND_COAGULATION_CASCADES INTESTINAL_IMMURE_NETWORK_FOR_IGA_PROOU SMAL_CELL_LUNG_CANCER	MEX_TARGETS_PRATILAS_PA452009 DIA_REPLICATION OCOTTE_MEDOSIS MISMATCH_REPAIR REDOSIALE JAK_STAT_SIGNALING_PATHWAY J PSS_SIGNALING_PATHWAY NOLLGEDTDE_EXCISION_REPAIR	BRAF_TARGTS_PHAS_JOSEPH CEIL_CYCLE DMS_REPLICATION MISMATCH_REPAIR BLACEECKISION_REPAIR BASE_EXCISION_REPAIR OOCYTE_MEIGHS SHUCEOSOME	MEK_TARGETS_PRATILAS_PHA82009 CELL_CYCLE DWA_REFUICATION MISMATCH_REPAIR NOLCOTDE_EXCSIGN_REPAIR OCCYTE_REFORMS WITT_SIGNALING_PATHWAY MITT_SIGNALING_PATHWAY	MEX_TARGETS_PRATILAS_PAAS000 DM_REFLICATION REDOCIME MEMATCH_REPAR BASE_EXCISION_REPAR BASE_EXCISION_REPAR OCCYTE_MEIOSIS UNT_SIGNALING_NTHWAY	ISAUE_TANGETS_PHAS_DOEPH MEX_TANGETS_PHATUA_SPHAS2000 DNA_DEFUCATION MESIATCH_(REPAIR NEIGODIE NOCITOTIE_MEDIS BOOSTIMESSI BOOSTIMESSI DOCTE_MEDIS BOOSTIMESSI DOCTE_MEDIS BOOSTIMESSI DOCTEMENTS DOC
8 9 10 11 12	JAK_STAT_BIGNALING_PATHWAY CELL_ADRESION_MOLECULS_CAMS GUYCOSAMNOCICAN_DEGRAADATON ECM_RECEPTOR_INTERACTION COMPLEMENT_ADD_COADULATION_CASCADES INTESTINAL_IMMUNE_INTYNORF_FOR_IGA_PRODU SMALL_GELL_LUNG_CAMCER PARK_SIGNALUNG_PATHWAY	MEY, TANGETS, PRATILAS, PANG2009 DUM, REPURZING DOCTE, MIDDOS MISMATCH, REPAR REGISTAR JANG, TAT, SIGNALING, PATHWAY PAS, SIGNALING, PATHWAY NUCLEOTDE, EXCISION, REPAR NUCLEOTDE, EXCISION, REPAR	BRAF_TARGETS_PHAS_JOSEPH CEL_CYCLE DML_REPLICATION MIGMATCH_REPAIR NUCLEOTIDE_EXCISION_REPAIR BASE_EXCISION_REPAIR OCOYTE_MEIOSIS SPLICEOSOME MA_STAT_SIGNALING_PATHWAY	MEY, TARGETS, PRATLAG, PHA32009 CELL, CYCLE DNA, BERUGATION MISMATCH, REPAR DOCYTE, BENDSIN, REPAR DOCYTE, BENDSIN, PATHWAY UM, STAT, SIGNALING, PATHWAY UM, STAT, SIGNALING, PATHWAY	MEK_TARGETS_PRATILAS_PMA5009 DM_REVICATION REDSOME MISIMATCH_REPAR NUCLEOTIDE_EXCISION_REPAR BAGE_EXCISION_REPAR OCCYTE_REMOSIS WHT_SIGNALING_PATHWAY	IBAUE_TANGETS_PHALS_DOEPH MEX_TANGETS_PRATLAS_PHAS2009 DMU_REPLICATION MISMATCH_REPAR NUCLEOTINE_REPAR NUCLEOTINE_REPARTN NUCLEOTINE NUCLEOTINE_REPARTN NUCLEOTINE_REPARTN NUCLEOTINE NUCL
8 9 10 11 12 13	JAK, STAT, SIGNAUNG, PATHWAY CELL, ADHESION MA EDULES, CAMS GLYCOGAMINOCI/CAN, DEGRADATION ECM, REGEPTOR, INTERNATION COMPLIMENT, JANO, COAGUATIONE, JORADO SMALL, CELL, LUNG, CANCER PRAR, SIGNALING, PATHWAY ALLOGRAFT, REELECTION	HER, TARGET, SPARILLAE, PAUS2009 DIM, REPLICATION ODIM, REPLICATION DIM, BERLESS MISILATOR, REPLIK MISILATOR, REPLIK MISILATOR, REPLIK PROSENTENDE, MISILATOR DATIONAL PROGESTERIONE, MISILATOR DATIONAL PROGESTERIONE, MISILATOR DATIONAL PROGESTERIONE, MISILATOR DATIONAL PROVIDENTIAL DATIONAL PROVIDED ACCORD REPLICATION OF PROVIDED ACCORDANCE REPLICATION OF PROVIDED ACCORDANCE REPLICATIONAL ACCORDANCE REPLICATION OF PROVIDED ACCORDANCE REPLICATION OF PROVIDATIONAL ACCORDANCE	BRAE_TARGETS_PINAS_LOBEPH OELL_CYCLE DAM.REPLICATION MISMATCH_REPUR NUCLEOTOE_EXCISION, REPAR BASE_EXCISION, REPAR BASE_EXCISION, REPAR BASE_EXCISION, REPAR DOOTTE_MEDDBS SPLICEOSOME SPLICEOSOME	MER, TARGETS, PARTULAS, PHAS2009 CELL, CYCLE DAM, REPLICATION MISMATCH, REPAR AUX, REPLICATION MISMATCH, REPAR AUX, SCHOLAND, PARTHWAY PARA, SIGNALING, PARTHWAY PARA, SIGNALING, PARTHWAY MARE, EXCISION, REPAR	NEC, TARGET, PARLIAG, PARSOO DIAL, REPLICATION REGOOME MEMATORI (REPAR NASELECTORE, EXCISION, REPAR BASE, EXCISION, REPAR BASE, EXCISION, REPAR BASE, EXCISION, REPAR MIT, SIGNALING, PATHWAY JAK, STAT, SIGNALING, PATHWAY GUIDMA	ISNE TAGETE FINAL JOBETH ISNE TAGETE FINAL JOBETH DNA REPLACINGN INSUECTIONE EXCERDIN JERVIN NUCLEOTIDE EXCERDIN JERVIN NUCLEOTIDE EXCERDIN JERVIN DOCTTE JEROSE BOSTIM-IESOS GUIDA PARA ERGULAR JATIWAY MELANDIAN
8 9 10 11 12 13 14	JAK STAT, SENALING (PATHWAY CELL, ADHESION, MOLEGULES, CANS GUICOGANINGOLY, DEGRAPATON ECOM, REGEVERTON, INTERACTION ECOM, REGEVERTON, INTERACTION MUL, CELL, LUNG, CANCER PPAR, SIGNALING, PATHWAY ALLOBRAY, RELECTION GUICOGANINGOLY, BIOSTITHESIS, JERPARAN,	INC, TARGET, PARTULA, PAASOO DOOTTE, MEDSIB MEMBORIE, MENDER MEMBORIE, EXCERNIC AM, STAT, STONAL NO, PATHWAY NOLLOTOE, EXCERNIC JEAN MA, STAT, STONAL NO, ATHWAY NOLLOTOE, EXCERNIC JEAN MODESTETROEM, MEMBRIE, DOOTTE, MITURATIO SMALL, GLIL, LUNG, ONDER	RAPE_TARGETS_PINAS_JOSEPH CELL_CYCLE UNA REPLACTION MEMOTOR_REPAR NUCLEOTIDE_EXCISION_REPAR AGAE_DEXCISION_REPAR SPLCEOSOME MAX_STAT_SIGNALING_PATHWAY WHT_SIGNALING_PATHWAY HIT_SIGNALING_PATHWAY	MEY, TARGETS (PARTLAG, PAAS2009 CULL, VYCLE DNA, JEPLOLTION MEDMOTIV, IERDAR OCOTTE, JEKOSION, REPAR OCOTTE, JEKOSION, JERDAR MAY, SIGNALING, JANTHWY MAY, SIGNALING, JANTHWY MAY, SIGNALING, JANTHWY MARE, SIGNALING, SYNTHWY MARE, SIGNALING, SYNTHWY MARE, SIGNALING, SYNTHWY	NET, TAROTTE, PARALAS, PARASOO DAN, REFLICATION NEMOSOME	IBAUE TANGETT, PINAL JOBETH IBAUE TANGETT, PINAL JOBETH IBAU, REPLACINGN IBAU, REPLACINGN IBAUMATCH, REPINA IBAUMATCH, REPINA IBAUMATCH, REPLACE IBAUMATCH, REPLACE I
9 10 11 12 13 14 15	JAK STAT SORALING, PATHWY CILL, ADRESION, ADRECHLES, CAMS CUCCOSMINGOLYCAN, DEGRARATION COMPLEMENT, AND, COAGULATION, CASCADES INTESTINA, MANARE, JERTWORK, CASCADES MITESTINA, MANARE, JERTWORK, CASCADES MALL, CILL, LUNG, COAGULATION, CASCADES MALL, CILL, LUNG, CASCADES MALL, CAS	МС, ТАРОСТ, РАЛЬА, РАККОО ОООТТ, РИСЛИ, РАККОО ОООТТ, РИСЛИ, РИСЛИ ПОВОЛИ ПОВОЛИ И СТАТ, СТАТ, СТАТИКА И СТАТ, СТАТИКА И СТАТИ, СТАТИ, СТАТИКА И СТАТИКО И СТАТИКА И СТАТИКО И СТАТИКА И	BRAT_TARGETS_PINAS_JOSEPH CELL_CYCLE DNR_REPLICATION MINIMOTOL_REPAR MUNICLEDTOCE_EXCISION_REPAR DAGE_EXCISION_REPAR DAGE_EXCISION_REPAR SUICESSME MUN_SIGNALINAS_PATHWAY MIN_SIGNALINAS_PATHWAY MIN_SIGNALINAS_PATHWAY	MEC, TARGET, PRAFLAG, PRASDO CULL, OYCLE DNA, FERLUCATION MANGTU, REVAR MACLEOTOE, EXOSION, REPAR OOTTE, MEDIS VATI, SIGALAND, PATHWAY MAT, SIGALAND, PATHWAY MAR, SIGALAND, PATHWAY MAR, SIGAL, SIGALAND, PATHWAY MARK, SIGAL, SIGALAND, PATHWAY PRAR, SIGALAND, PATHWAY MARK, SIGALAND, SIGALA	NEC, TANGET, PARATUAL, PARASORO DAN, REFUCION REDSOME MANGTAL, REPAR NUCLEOTOE, EXOSION, REPAR DASE, EXOSION, CHERAR DOCTT, BUROSIS VIT, SIGNALING, PATHAW JAK, STAT, SIGNALING, PATHAW GUGMA GUGMA GUGMA	BINE_TAGGETE_PINAL_DOBEPH MULTEDTEL_PINAL_DOBEPH BINUTCY_DETUNAL_PINALBONG BINUTCY_DETUNAL BINUTCY_DETUNAL BINUTCY_DETUNAL BINUTCY_DETUNAL BINUTCY_DETUNAL BINUTCY_DETUNAL BINUTCY_DETUNALBONG BINUTCY_DETUNALBON
9 9 10 11 12 13 14 15 15 16	JAK STAT, SERVALKO, PATHWY CILL, ADRESION, ADECULSE, CMS GUYCOSAMNOGUYCAN, DECRADATON ECOMINELIEM, JAND. CONGLATON, CASCADE COMINELIEM, JAND. CONGLATON, CASCADE SHALL, CILL, LUNO, CONGLATON, CASCADE SHALL, CILL, LUNO, CANCER AMPR. SIGNALARO, SHATON ALLCORAT, FLERECTICH GUYCOSAMNOGUYCAN, BIOSYNTHESB, HEPPANN, ALTOMANKE, THYPOD, DIEAE	MIC TARGET PARTICLE PARSON MIC TARGET PARTICLE PARTICLE PARSON MIC TARGET PARTICLE PARTICLE PARTICLE PARSON MIC TARGET PARTICLE PARTICLE PARTICLE PARSON MIC TARGET PARTICLE P	BRAE_TARGETS_PINAS_JOSEPH CULL_CYCLE DWA_REPLICATION MISMOTOL/REINAR MADELEDICE_SIONEN_REPAR BASE_EXCISION_REPAR BASE_EXCISION_REPAR SPLCEOSEME MAN_STM_SIGNALING_PATHWAY WHT_SIGNALING_PATHWAY PROGESTERDE_MEDIATED_SOLFTE_MATURATION PROGESTERDE_MEDIATED_SOLFTE_MATURATION MONTROPHIC_LEARLA_SOLEDOSE_ALS	MEY, TARGETS PARALAG, PAAS2009 CILL, OYOLE DNA, JEPLUCTION MISMICH, IKENRA NGLEOTOE, EXCISION, REPAR OCOTTE, MEDIAIS ANS, SIGNALINO, JADHIWY MAY, SIGNALINO, JADHIWY MAY, SIGNALINO, JADHIWY MAR, SIGNALINO, SIGNIHIAY MAR, SIGNALINO, SIGNIHIAY MAR, SIGNALINO, SIGNIHIAY MINICABRU, SIGNERINA, ONL JAPECTON PHOGENETSENGE, MEDIAIED, JOOUTE, MATURATIO SINCEDOWE	NET, TARGETS, PARALLAS, PARASOOO DAW, AIRENCOLTON RIBOSOME NUCLEOTONE EXCERCIN, REPAIR BASE, EXCERCIN, REPAIR BASE, EXCERCIN, REPAIR BASE, EXCERCIN, REPAIR BASE, EXCERCIN, REPAIR MIT, SIGNALLAS, PATHANY ALCHER, MIT, STANLAND, PATHANY GLOMA MICHENDER, METABOLISM MELIANOMA	BAUE_TANGETE, PINAL_JOBEPH BAUE_TANGETE, PINAL_JOBEPH BAUE_TANGETE, PINAL_JOBEPH BAUMACTO, LERINA BAUSACTO, ELRINA
9 9 10 11 12 13 14 15 16 16 17	JAK STAT SERAULAR PATHWY CILL, ADR-BION, MOLECILES, CAMS BUYCOSMINGOUYCAN, DEGRADATION ECOM, PERSPECTION COMINESIENT, JANG COSAULTATION, CASCADES INTESTINAL, JAMANE, JERVIORY, CAR, JA, PROCU SMALL, CILL, LUNG, COMER IPMA, SIGNALING, JANIHAW ALLOBART, FLEETCON GUYCOSMINOGUYCAN, BIOSTHEBS, JERMANA, ALTOMANIE, THYNOD, DISEASE FOCK, JORESION VIRAL_MYCOCABITIS	МС, ТАРОСТ, РИЛАД, РИАБХОО ОООТТ, ВИСЛИ, РИАБХОО ОООТТ, ВИСОВ МИХИСЧ, ЦЕРИК НИБОСМЕ ИК, STAT, БИЛАИЛО, РАТНИКУ РРЗ, БИСИЛИЯ, РАТНИКУ РИСОВСТВИИТ, ВИСОК, ВИРИК РИСОВСТВИИТ, ВИСОК, ВИРИК РИСОВСТВИИТ, ВИСОК, ВИРИК ОКС, САКОЛИ, ОКОРК ИТ, БИСОКИ, ОКОРК НИТ, БИСОКИИ, ОКОРК НИТОКИИ, ОКОРК НИТОКИИ, ОКОРКАНИИ, ОКОРК НИТОКИИ, ОКОРК НИТОКИИ, ОКОРКАНИИ, О	BRAT_TARGETS_PINAS_JOSEPH CELL_CYCLE DNR_REPLICATION MINIMOTIL_REPAIR MINIMOTIL_REPAIR MINIMOTIL_REPAIR MINIMOTIL_REPAIR MINIMOTIL_REPAIR MINIMOTIL_MINIMOTILANELANI MINIMANNIMANINA MINIMANINA MINIMANINA MINIMATINA MINIMATINA MINIMATINA MINIMATINA MINIMATINA MINIMATINA MINIMATINA	MEX, TARGET, PRAFLAG, PRASDO CELL, CYCLE DNA, FERLUCATION MARCHI, LEYAR MACLEOTOE, EXOSION, REPAR OOTTE, MEDIS MAT, SIGALAND, PATHWAY MAT, SIGALAND, PATHWAY MAT, SIGALAND, PATHWAY MAT, SIGALAND, PATHWAY MAT, SIGALAND, PATHWAY PRAR, SIGALAND, SIGALAND	NEC, TANGET, PARALAS, PARASX09 DAN, REFUCATION REDSCIME MANGTAL, REPAR NUCLEOTOE, EXOSION, REPAR DASE, EXOSION, DERPAR DASE, EXOSION, REPAR DOCTT, BURDING, SAMIMAY ANS, STAT, SANULARG, PATHAWY GLOBAL GUOMA GUOMA CHICABOLIPO, METABOLISM PROGESTERDER, MEDIATED, COCYTE, MATURATIC PARAL, SIGNALAR, SAMIMAY	ISNE TAGETE FINAL JOBETH INIT, RADETE FINAL JOBETH INIT, RADETE FINAL FINAL SALESSON INIT, REPLACING INIT, REPLACE INIT, REPLACE INIT, REPLACE INIT, INIT, INIT, AND IN INIT, INIT, INIT, INIT, INIT, AND IN INIT, INIT, IN
8 9 10 11 12 13 14 15 16 17 18	JAK, STAT, SONALIKO, PATHWY CILL, ADRESION, ANELOCILIS, CANA GUYCOSAMINOGUYCAN, DEGRADATION COMPLENT, AND CONGLATION, CASCADE COMPLENT, AND CONGLATION, CASCADE SINAL, CELL, LUNO, CONGLATION, CASCADE SINAL, CELL, LUNO, CANCER AND CONSTRUCTIVE, STATUS ALLOGRAFT, FERECTION GUYCOSAMINGOCYAN, BIOSYNTHESB, HEPRANI, ALTORAMAE, THYROD, DIELASE FOCAL, ADRESSI D, FUNKATUATED, JATTY, ACIDS BIOSYNTHESB, C. J., UNATUANED, JATTY, ACIDS	MIC TARGET PRATULA PAAS000 DOOTE LENSIS MANAGENERSIS MANA	RIAE_TARGETS_PINAS_JOSEPH CULL_CYCLE DWA_REPLICATION MISMOTOL/REINAR MULEOTORE_SIONSON_REPAR BAGE_SIONSON_REPAR BAGE_SIONSON_REPAR SINCEGOSEME MIC_SIONSON_REPARTMINY MIC_SIONALING_PATHWAY MIC_SIONALING_PATHWAY MICROSCHERAE_SIONALING_INTERNATION ANOTROPHIC_LEARCH_SIONES_ALS THRIRDO_CANCER PROSESTENCE_MEDIATE_SIGNALING_INTERNATION	MEY, TARGETS PARALAG, PARASOON GULL, YOVLE DNA, JEPLUCTION MILLOTION MILLOTION MILLOTIONE EXCISION, REPAR OCOTTE JECISION, PARAN MILLOTIG, SIGNALINO, JANTHWY MAY, SIGNALINO, JANTHWY MILLOTION, SIGNALINO, JANTHWY MILLOTIONE, SIGNAL PARAN MILLOTIONE, MILLOTION, MILLOTION MILLOTION, MILLOTION, MILLOTION MILLOTIONE, MILLOTION, MILLOTION BIOSYNTHIESS, OF, UNRATURATIO BIOSYNTHIASIS, OF, UNRATURATIO, JATY, ACOS GUCFEROLP, DJ. MILLOTION, SIGNAL	NET, TARGETS, PARTLAS, PARS2009 DNA, REFLICATOR REGISTANT REGISTANT NUCLESTORE EXCERNIN, REFNIR SAGE, EXCERNIN, REFNIR SAGE, EXCERNIN, REFNIR MIT, SIGNALING, PATHWAY AL, STAT, SSGNALAN, JATHWAY GLOMA ACCERNING, JATHWAY RELANDIA RECINATIONALING, PATHWAY THRODESTERODE, MEDIATED, OCOTTE, MATURATIC PARA SISTAL, SAGA, MEDIATED, OCOTTE, MATURATIC PARA SISTALING, PATHWAY	BAUE_TANGET, PANA_JOBEPH BAUE_TANGET, PANA_JOBEPH BAUE_TANGET, PANA_JOBEPH BAUMACH_EXCANDR BAUMACH_EXCANDR BAUMACH_EXCANDR BAUSTOL, PERMA
8 9 10 11 13 14 15 16 17 18 19	JAL STAT SERVILING PATHWY CILL, ADR-BION, MOLECULES, CANS GUYCOSMINGOUYCAN, DEGRADATON ECO, HECEFTON, MERSANTON COMINESIEM, JANDE, DEVINORY, COR, GA, PROCU SMALL, CILL, LUNG, DAVEER IPMR, SDAULAND, JANIHWY ALL, CORU, LUNG, DAVEER IPMR, SDAULAND, JANIHWY ALL, DAVID, HELECTON GUYCOSMINDI, CANS, BOSTHERSE, HEPRAN, AJTOMANE, THOROD, DISEASE FOCK, JORGSON WILL, MYCOGANTIS BIOSYNTHESS, OF UNSATURATED JATY, ACIDS BLODER, CANCER	МС, ТАРОСТ, РАЛЬА, РАККОО ООУТЕ, ИКОВК МОМЛЕЧ, ЕКОКК МИКЛОЧ, КЕРКИ КИБОКИ РАЗ, БТАТ, БИКАКИ, РАЛЧИИУ РАЗ, БТАТ, БИКАКИ, РАЛЧИИУ РАЗ, БТАТ, БИКАКИ, РАЛЧИИУ РАЗ, БТАТ, БИКАКИ, РАЛЧИИУ РАЗ, БТАТ, БИКАКИ, РАЛЧИИУ МОЦСТОНЕ, СКОБОК, КЕРКИ РИОВСКИТСКИ, СТАРСКИ, СМОЕТ ВИСКОВАЦИЯ, РАЛЧИИУ МИКЛИКА ОКЕ, СКОВОК, ОРОСЯ, ВУ-ГАЛТЕ МАКДЕ КОСИВОК, ОКОЕТ ВИСКОВАЦИЯ, РАЛЧИИУ МИКЛИКА ЛИСКОВА, СКОВОК, ОКОЕТ ВИСКОВАНИЯ, РАЛЧИИУ ВИСКОВАНИЯ, РАЛЧИИУ МИКЛИКА ОКЕ, СКОВОК, ОКОЕТ ВИСКОВАНИЯ, РАЛЧИИУ ВИСКОВАНИЯ, РАЛЧИИУ ВИСКОВАНИЯ, РАЛЧИИУ ВИСКОВАНИЯ, РАЛИКИ, ОКОЕТ ВИСКОВАНИЯ, РАЛИКИ, ОКОЕТ ВИСКОВАНИЯ, РАЛИКИ, СКОВАНИЯ, РАЛИКИ, ОКОЕТ ВИСКОВАНИЯ, РАЛИКИ, ОКОЕТ ВИСКОВАНИЯ, РАЛИКИ, ОКОЕТ ВИСКОВАНИЯ, РАЛИКИ, ОКОЕТ ВИСКОВАНИЯ, РАЛИКИ, ОКОЕТ ВИСКОВАНИЯ, РАЛИКИ, ОКОЕТ ВИСКОВАНИЯ, РАЛИКИ, РАЛИКИ, ОКОЕТ ВИСКОВАНИЯ, РАЛИКИ, РАЛИКИ, ОКОЕТ ВИСКОВАНИЯ, РАЛИКИ, РАЛИКИ, ОКОЕТ ВИСКОВАНИЯ, РАЛИКИ, ОКОЕТ ВИСКОВАНИЯ, РАЛИКИ, ОКОЕТ ВИСКОВАНИЯ, РАЛИКИ, ОКОЕТ ВИСКОВАНИЯ, РАЛИКИ, ОКОЕТ ВИСКОВАНИЯ, РАЛИКИ, РАЛИКИ, ОКОЕТ ВИСКОВАНИЯ, РАЛИКИ, РАЛИКИ, ОКОЕТ ВИСКОВАНИЯ, РАЛИКИ, РАЛИКИ, РАЛИКИ, ОКОЕТ ВИСКОВАНИЯ, РАЛИКИ, РА	BRAT_TARGETS_PINAS_JOSEPH CELL_CYCLE DWA_REPLACTION MILMINGTOL, REPAR MULCEDODE EXCISION, REPAR ABAE EXCISION, LEPAR ABAE EXCISION, LEPAR BAUCEOBOR BAUCEOBOR MULTISONALING, PATHWAY PRPAS_BIOLAND, PATHWAY PRPAS_BIOLAND, PATHWAY PRPAS_BIOLAND, PATHWAY PRPAS_BIOLAND, CASENDER, ALS THRODO_CANCER_ PAS_SIGNALING, RATHWAY PROXESIONE	MEX, TARGET, PRATLAG, PRASDO CELL, CYCLE DNA, JEPLUCHTON MARCH, JERPAR MACLEOTOE, EXCISION, REPAR OCTTE, MEDIS MAT, GIADALMO, PATHWAY MAT, GIADALMO, PATHWAY MAT, STAT, SIGNALMO, PATHWAY MALE, XXXIDO, MERAN RAMAGEK, ESCHERICHA, COLL FARTY, ACDS GIV/CFRAD, UNIXATED, JATY, ACDS	NEC, TANGET, PARATUA, PARASX09 DAN, REFUCTION REDSCRIE MINITOR, REPAR NUCLEOTOE, EXCISION, REPAR ASE, EXXISION, REPAR ASE, EXXISION, REPAR OCOTT, BURCHER, SAMINAY AW, STAT, SAMULING, PATHAWY GUIDAN GUIDAN AUX, STAT, SAMULING, PATHAWY GUIDAN GUIDAN MINITORIO, SAMINAY PROGESTERONE, MEDIATED_CONTE_ MATURATIC PROGRESTERONE, MEDIATED_CONTE_ MATURATIC THYRODE_AMARKER	BIALE_TAGGETE_PINAL_DOBEPH DIAL_EPIZATION DIAL_EPIZATION DIAL_EPIZATION DIAL_EPIZATION DIAL_EPIZATION DIAL_EPIZATION DIALEDESS BIOSTIMESSOS DIGONAL ANG_TATI_SIGNALING_INTIMAY DIGOSTENICAL-BIOLOGICAL DIALEDIATION DIGOSTENICAL-BIOLOGICAL DIALEDIATION DISCOMULTA DIALEDIATION DISCOMULTA DIALEDIATIONICAL DIALEDIATION DISCOMULTA DIALEDIATIONICAL DIALEDIATIONICALUNA DIALEDIATIONICAL DIALEDIATIONICALUNA DIALEDIATIONICALUNA DIALEDIATIONICALUNA DIALEDIATIONICON DIALEDIATIONI DIALEDIATIONICONICIDIATIO
8 9 10 11 12 13 14 15 16 17 18	JAK, STAT, SONALIKO, PATHWY CILL, ADRESION, ANELOCILIS, CANA GUYCOSAMINOGUYCAN, DEGRADATION COMPLENT, AND CONGLATION, CASCADE COMPLENT, AND CONGLATION, CASCADE SINAL, CELL, LUNO, CONGLATION, CASCADE SINAL, CELL, LUNO, CANCER AND CONSTRUCTIVE, STATUS ALLOGRAFT, FERECTION GUYCOSAMINGOCYAN, BIOSYNTHESB, HEPRANI, ALTORAMAE, THYROD, DIELASE FOCAL, ADRESSI D, FUNKATUATED, JATTY, ACIDS BIOSYNTHESB, C. J., UNATUANED, JATTY, ACIDS	МС, ТАРОСТ, РАЛЬА, РАККОО ООУТЕ, ИКОВК МОМЛЕЧ, ЕКОКК МИКЛОЧ, КЕРКИ КИБОКИ РАЗ, БТАТ, БИКАКИ, РАЛЧИИУ РАЗ, БТАТ, БИКАКИ, РАЛЧИИУ РАЗ, БТАТ, БИКАКИ, РАЛЧИИУ РАЗ, БТАТ, БИКАКИ, РАЛЧИИУ РАЗ, БТАТ, БИКАКИ, РАЛЧИИУ МОЦСТОНЕ, СКОБОК, КЕРКИ РИОВСКИТСКИ, СТАРСКИ, СМОЕТ ВИСКОВАЦИЯ, РАЛЧИИУ МИКЛИКА ОКЕ, СКОВОК, ОРОСЯ, ВУ-ГАЛТЕ МАКДЕ КОСИВОК, ОКОЕТ ВИСКОВАЦИЯ, РАЛЧИИУ МИКЛИКА ЛИСКОВА, СКОВОК, ОКОЕТ ВИСКОВАНИЯ, РАЛЧИИУ ВИСКОВАНИЯ, РАЛЧИИУ МИКЛИКА ОКЕ, СКОВОК, ОКОЕТ ВИСКОВАНИЯ, РАЛЧИИУ ВИСКОВАНИЯ, РАЛЧИИУ ВИСКОВАНИЯ, РАЛЧИИУ ВИСКОВАНИЯ, РАЛИКИ, ОКОЕТ ВИСКОВАНИЯ, РАЛИКИ, ОКОЕТ ВИСКОВАНИЯ, РАЛИКИ, СКОВАНИЯ, РАЛИКИ, ОКОЕТ ВИСКОВАНИЯ, РАЛИКИ, ОКОЕТ ВИСКОВАНИЯ, РАЛИКИ, ОКОЕТ ВИСКОВАНИЯ, РАЛИКИ, ОКОЕТ ВИСКОВАНИЯ, РАЛИКИ, ОКОЕТ ВИСКОВАНИЯ, РАЛИКИ, ОКОЕТ ВИСКОВАНИЯ, РАЛИКИ, РАЛИКИ, ОКОЕТ ВИСКОВАНИЯ, РАЛИКИ, РАЛИКИ, ОКОЕТ ВИСКОВАНИЯ, РАЛИКИ, РАЛИКИ, ОКОЕТ ВИСКОВАНИЯ, РАЛИКИ, ОКОЕТ ВИСКОВАНИЯ, РАЛИКИ, ОКОЕТ ВИСКОВАНИЯ, РАЛИКИ, ОКОЕТ ВИСКОВАНИЯ, РАЛИКИ, ОКОЕТ ВИСКОВАНИЯ, РАЛИКИ, РАЛИКИ, ОКОЕТ ВИСКОВАНИЯ, РАЛИКИ, РАЛИКИ, ОКОЕТ ВИСКОВАНИЯ, РАЛИКИ, РАЛИКИ, РАЛИКИ, ОКОЕТ ВИСКОВАНИЯ, РАЛИКИ, РА	RIAE_TARGETS_PINAS_JOSEPH CULL_CYCLE DWA_REPLICATION MISMOTOL/REINAR MULEOTORE_SIONSON_REPAR BAGE_SIONSON_REPAR BAGE_SIONSON_REPAR SINCEGOSEME MIC_SIONSON_REPARTMINY MIC_SIONALING_PATHWAY MIC_SIONALING_PATHWAY MICROSCHERAE_SIONALING_INTERNATION ANOTROPHIC_LEARCH_SIONES_ALS THRIRDO_CANCER PROSESTENCE_MEDIATE_SIGNALING_INTERNATION	MEX, TARGET, PRATLAG, PRASDO CELL, CYCLE DNA, JEPLUCHTON MARCH, JERPAR MACLEOTOE, EXCISION, REPAR OCTTE, MEDIS MAT, GIADALMO, PATHWAY MAT, GIADALMO, PATHWAY MAT, STAT, SIGNALMO, PATHWAY MALE, XXXIDO, MERAN RAMAGEK, ESCHERICHA, COLL FARTY, ACDS GIV/CFRAD, UNIXATED, JATY, ACDS	NET, TARGETS, PARTLAS, PARS2009 DNA, REFLICATOR REGISTANT REGISTANT NUCLESTORE EXCERNIN, REFNIR SAGE, EXCERNIN, REFNIR SAGE, EXCERNIN, REFNIR MIT, SIGNALING, PATHWAY AL, STAT, SSGNALAN, JATHWAY GLOMA ACCERNING, JATHWAY RELANDIA RECINATIONALING, PATHWAY THRODESTERODE, MEDIATED, OCOTTE, MATURATIC PARA SISTAL, SAGA, MEDIATED, OCOTTE, MATURATIC PARA SISTALING, PATHWAY	BAUE_TANGET, PANA_JOBEPH BAUE_TANGET, PANA_JOBEPH BAUE_TANGET, PANA_JOBEPH BAUMACH_EXCANDR BAUMACH_EXCANDR BAUMACH_EXCANDR BAUSTOL, PERMA

 Supplemental Table 3. Percentages of Tumor Volumes in Each Treatment Group (Related to Figure 7E).

Treatment	%T/C
Vehicle control	100
PLX4720 200mg/kg diet	51.41
2,4-DNP 20mg/kg QD p.o.	101.96
PLX4720 + 2,4-DNP	42.7

Supplemental Table 4. Percentages of Tumor Volumes in Each Treatment Group (Related to Figure 7F).

Treatment	%T/C
Vehicle control	100
PLX4720 200mg/kg diet	56.88
Gamitrinib 5mg/kg QD i.p.	124.59
Gamitrinib 15mg/kg QD	
i.p.	57.3
PLX4720 + Gami 5mg/kg	25.9
PLX4720 + Gami	
15mg/kg	6.99

Supplemental Table 5. Percentages of Tumor Volumes in Each Treatment Group (Related

to Figure 7G).

Treatment	%T/C
Vehicle control	100
PLX4720 200mg/kg diet	56.14
Gamitrinib 15mg/Kg QD i.p.	44.63
PLX4720 + Gami 15mg/Kg	16.36

Supplemental Table 6. The Hospital of University of Pennsylvania Patients' Clinical Information (Related to Table 2)

MP#	Biopsy Date	Biopsy Type	Tissue Type	Sp to Rx Time (Pre/Post)	Progression Sp (Yes/No)	Study Drug	Date on C1D1	Date of PD	Evidence of progression	Date of Last Dose
578	1/5/10	L arm excision	In transit Mets	Pre	NA	RO5185426	2/18/10	3/2/11	new brain mets with cerebral edema	3/3/11
578	3/3/11	Left parietal craniotomy	Brain Mets	Post	Yes	RO5185426	2/18/10	3/2/11	new brain mets with cerebral edema	3/3/11
610	5/27/05	R. axillary	LN Mets	Pre	NA	RO 5185426	12/16/09	10/7/10	progression of nontarget disease - other lung nodules and at wrist on most recent imaging	10/14/10
		R wrist/R							progression of nontarget disease - other lung nodules and at wrist on most	
610	10/15/10	breast	SubQ Mets	Post	Yes	RO 5185426	12/16/09	10/7/10	recent imaging	10/14/10
11_3	7/15/08	R axillary	R axilla mets	Pre	NA	Arm A / RO5185426	9/24/10	6/6/11	C13, new skin lesions	still on drug
11_3	6/6/11	(B)R.axillary, (C)L. shoulder,(D) L. flank lesions Excisional in VA	metastatic	Post	Yes	Arm A / RO5185426	9/24/10	6/6/11	C13, new skin lesions	still on drug
503	1/7/09	Excisional, small bowel, retroperatonea l mets resection	Visceral Mets	Pre	NA	PLX 4032	5/5/09	NA	NA	off of drug due to CHF on 11/16/12 (best estimate for date off of drug). Sometime between 11/01/12 and 01/29/13. off of drug due to CHF on 11/16/12 (best
503	9/22/10	R back excision	SubQ Mets	Post	No	PLX 4032	5/5/09	NA Patient is	NA	estimate for date off of drug). Sometime between 11/01/12 and 01/29/13.
11_35	6/21/11	4mm punchx2	SubQ Mets	Pre	NA	R05185426	7/12/11	being treated by a local oncologist at the University of Maryland	NA	NA
11_35	7/26/11	4 mm punch	SubQ Mets	Post	NA	R05185426	7/12/11	Patient is being treated by a local oncologist at the University of Maryland	NA	NA
11_47	6/25/10	Skin and L inguinal LN	LN and SubQ Mets	Pre	NA	R05185426	6/14/11	2/3/12	progressive CNS disease	2/9/12
11_47	2/9/12	R Parietal lobe Excisional	brain mets	Post	Yes	R05185426	6/14/11	2/3/12	progressive CNS disease Mets to liver,	2/9/12
12_161	4/9/10	R. Scalp WE R parotid	SubQ Primary	Pre	NA (neoadjuvant)	Zelboraf	10/11/11	4/27/12	abdominal wall Mets to liver,	1/22/12
12_161	2/8/12	gland Exicisional	LN Mets	Post	NA (neoadjuvant)	Zelboraf	10/11/11	4/27/12	abdominal wall	1/22/12
11_20	6/4/2010	L inguinal	LN Mets	Pre	NA	Part C Combo 1 Arm	3/1/11	8/30/11	scans show progression to last scans but better than baseline scans show progression to last occans but	8/29/11
11_20	10/14/11	R chest wall exitional	SubQ Mets	Post	Yes	Part C Combo 1 Arm	3/1/11	8/30/11	last scans but better than baseline	8/29/11
11_20	3/31/11	R Chest	SubQ Mets	Pre	NA	Part C Combo 1 Arm	4/7/11	1/12/12	more lesions grew	2/8/12
11_6	4/21/11	R chest wall skin excisional	In transit Mets	Post	No	Part C Combo 1 Arm	4/7/11	1/12/12	more lesions grew	2/8/12

outcome	Gene-Pre	Hazard ratio	min95	max95	p-value
OS	C10orf2	1.08	0.336	3.472	0.897
OS	DNM1L	1.635	0.732	3.649	0.2302
OS	ESRRA	1.201	0.421	3.427	0.7324
OS	FIS1	0.593	0.232	1.512	0.2736
OS	HSPD1	1.821	0.748	4.436	0.1867
OS	MFN1	2.401	0.46	12.539	0.2991
OS	MFN2	0.811	0.315	2.087	0.6645
OS	NFE2L2	0.797	0.512	1.242	0.3168
OS	OPA1	1.331	0.659	2.687	0.4257
OS	PHB	1.255	0.488	3.228	0.6368
OS	PHB2	1.535	0.575	4.099	0.3925
OS	POLG	1.891	0.485	7.379	0.359
OS	POLG2	1.193	0.31	4.598	0.7976
OS	PPARGC1A	1.438	0.972	2.128	0.0691
OS	PPARGC1B	1.408	0.617	3.21	0.416
OS	PPRC1	1.561	0.275	8.859	0.6151
OS	SOD2	0.766	0.455	1.291	0.3168
OS	TFAM	1.519	0.635	3.63	0.3473
OS	TFB1M	0.985	0.334	2.906	0.9775
OS	TFB2M	1.017	0.467	2.213	0.966
OS	TRAP1	2.338	0.784	6.971	0.1276
OS	TUFM	2.01	0.709	5.7	0.1892
OS	VDAC1	8.135	1.103	59.978	0.0397

Supplemental Table 7. Analysis of the Association of Expression of MitoB Genes in Pretreatment Tumor Biopsies with Patients' Overall Survival (Related to Figure 7)

Abbreviations:

OS: overall survival; Pre: pre-treatment.

outcome	Gene-Prog	Hazard ratio	min95	max95	p-value
OS	C10orf2	1.047	0.391	2.805	0.9271
OS	DNM1L	1.011	0.522	1.959	0.9731
OS	ESRRA	1.021	0.464	2.248	0.9581
OS	FIS1	0.787	0.415	1.495	0.4645
OS	HSPD1	1.178	0.726	1.913	0.507
OS	MFN1	0.877	0.19	4.054	0.8663
OS	MFN2	1.039	0.407	2.655	0.9356
OS	NFE2L2	1.012	0.772	1.327	0.9298
OS	OPA1	1.155	0.658	2.027	0.6159
OS	PHB	1.098	0.482	2.502	0.8242
OS	PHB2	1.643	0.615	4.386	0.3219
OS	POLG	1.643	0.464	5.815	0.4416
OS	POLG2	1.079	0.458	2.541	0.8618
OS	PPARGC1A	1.292	0.915	1.825	0.1453
OS	PPARGC1B	1.172	0.594	2.313	0.6469
OS	PPRC1	1.007	0.383	2.648	0.9886
OS	SOD2	0.634	0.392	1.025	0.0628
OS	TFAM	1.053	0.562	1.974	0.8718
OS	TFB1M	0.173	0.042	0.717	0.0156
OS	TFB2M	1.097	0.703	1.71	0.6836
OS	TRAP1	2.054	0.605	6.969	0.2484
OS	TUFM	3.65	1.105	12.053	0.0337
OS	VDAC1	1.468	0.572	3.765	0.4246

Supplemental Table 8. Analysis of the Association of the Expression of MitoB Genes in Post-treatment Tumor Biopsies with Patients' Overall Survival (Related to Figure 7)

Abbreviations:

OS: overall survival; Prog: progressive.

outcome	Gene-Pre	Hazard ratio	min95	max95	p-value
PFS	C10orf2	0.518	0.198	1.35	0.1783
PFS	DNM1L	2.121	1.021	4.405	0.0438
PFS	ESRRA	0.708	0.338	1.484	0.3608
PFS	FIS1	0.591	0.274	1.276	0.1804
PFS	HSPD1	2.666	1.051	6.764	0.039
PFS	MFN1	2.704	0.576	12.7	0.2074
PFS	MFN2	0.793	0.395	1.593	0.5148
PFS	NFE2L2	1.13	0.788	1.619	0.5068
PFS	OPA1	1.377	0.795	2.384	0.2534
PFS	PHB	1.165	0.569	2.389	0.6759
PFS	PHB2	0.7	0.292	1.677	0.4239
PFS	POLG	0.866	0.314	2.385	0.7806
PFS	POLG2	1.789	0.597	5.363	0.299
PFS	PPARGC1A	1.229	0.867	1.744	0.2471
PFS	PPARGC1B	0.774	0.39	1.534	0.463
PFS	PPRC1	0.602	0.17	2.13	0.4315
PFS	SOD2	0.938	0.608	1.447	0.7737
PFS	TFAM	1.68	0.725	3.893	0.2264
PFS	TFB1M	1.149	0.415	3.181	0.7894
PFS	TFB2M	1.14	0.685	1.897	0.6142
PFS	TRAP1	1.056	0.515	2.166	0.8823
PFS	TUFM	0.869	0.414	1.822	0.7099
PFS	VDAC1	5.077	1.014	25.415	0.048

Supplemental Table 9. Analysis of the Association of Expression of MitoB Genes in Pretreatment Tumor Biopsies with Patients' Progression-Free Survival (Related to Figure 7)

Abbreviations:

PFS: progression-free survival; Pre: pre-treatment.

outcome	Gene-prog	Hazard ratio	min95	max95	p-value
PFS	C10orf2	1.289	0.5	3.322	0.5988
PFS	DNM1L	0.623	0.398	0.974	0.0379
PFS	ESRRA	1.304	0.603	2.82	0.5
PFS	FIS1	0.999	0.514	1.94	0.9979
PFS	HSPD1	0.754	0.506	1.123	0.1646
PFS	MFN1	0.359	0.136	0.947	0.0384
PFS	MFN2	1.576	0.565	4.393	0.3847
PFS	NFE2L2	0.958	0.747	1.228	0.7337
PFS	OPA1	0.809	0.516	1.268	0.3551
PFS	PHB1	1.023	0.543	1.929	0.9438
PFS	PHB2	1.796	0.713	4.522	0.2139
PFS	POLG	1.263	0.5	3.193	0.6217
PFS	POLG2	0.761	0.364	1.591	0.4676
PFS	PPARGC1A	1.105	0.702	1.74	0.6659
PFS	PPARGC1B	0.837	0.268	2.607	0.7582
PFS	PPRC1	0.879	0.346	2.233	0.7864
PFS	SOD2	1.138	0.646	2.006	0.654
PFS	TFAM	0.669	0.394	1.137	0.1371
PFS	TFB1M	0.559	0.226	1.382	0.2079
PFS	TFB2M	0.918	0.637	1.323	0.6459
PFS	TRAP1	1.165	0.507	2.679	0.7185
PFS	TUFM	1.484	0.704	3.127	0.2994
PFS	VDAC1	0.767	0.275	2.141	0.6129

Supplemental Table 10. Analysis of the Association of Expression of MitoB Genes in Posttreatment Tumor Biopsies with Patients' Progression-Free Survival (Related to Figure 7)

Abbreviations:

PFS: progression-free survival; Prog: progressive.

Supplemental Methods

Cell lines established from human melanomas and patient-derived xenografts

All human metastatic melanoma cell lines that were established at the Wistar Institute have been previously described (44). UACC-62 and UACC-903 cells were kind gifts from Dr. Marianne B. Powell (Stanford University, Stanford, CA 94305, USA). A375 cells were purchased from ATCC. LOX-IMVI cells were kindly provided by Dr. Lin Zhang (University of Pennsylvania, Philadelphia, PA 19104, USA). All resistant cell lines that acquired drug resistance to PLX4720 (hereafter referred to as "BR" cell lines) or the combination of PLX4720 and PD0325901 (hereafter referred to as "CR" cell lines) were established after continuous exposure to PLX4720 at 10 µM or the combination of PLX4720 at 10 µM and PD0325901 at 1 µM. Fine needle aspiration (FNA) tumor samples derived from melanoma patients were directly transplanted and grown in mice. Tumors were harvested, fragmented and re-transplanted in mice to establish melanoma patient-derived xenografts (PDX). Short-term primary cultures established from resistant PDXs (hereafter referred to as "RPDX" cell lines) were maintained in the presence of PLX4720 at 1µM. All cell lines were maintained in RPMI-1640 media (Mediatech, Inc.) supplemented with 10% fetal bovine serum (Tissue Culture Biologicals) and cultured in a 37° C humidified incubator supplied with 5% CO2. All cell lines were authenticated by DNA fingerprinting.

Chemicals

The BRAF inhibitor PLX4720 and the MEK inhibitor PD0325901 were provided by Plexxikon Inc. The BRAF inhibitor GSK2118436 and the MEK inhibitor GSK1120212B were provided by GlaxoSmithKline. The BRAF inhibitor LGX818, the MEK inhibitor MEK162 and the mTOR inhibitor BEZ235 were provided by Novartis. The ERK inhibitor SCH772984 was provided by Merck & Co. Rapamycin was purchased from LC Laboratories. Gamitrinib has been previously described (41). Spautin-1 was purchased from EMD Millipore. Phenformin and 2,4-DNP were purchased from Sigma. All siRNA clones were synthesized by Qiagen.

Reagents

CellTraceTM Violet (cat. # C34557), CellROX Deep Red (cat. # C10422), CyQUANT (cat. # 7026) and MitoTracker® Red CM-H2Xros (cat. # M-7513) were purchased from Life Technologies. Propidium iodide solution (cat. # P4864-10ML) was purchased from Sigma. PSVue® 643 (cat. # P-1006) was purchased from Molecular Targeting Technologies. 2-NBDG (cat. # 11046) was purchased from Cayman Chemical. CellTiter-Glo® Assay (cat. # G7570) was purchased from Promega. Extracellular O₂ probe (cat. # ab140097) was purchased from Abcam. All antibodies were purchased from Cell Signaling. MT-CO1, MitoProfile® Total OXPHOS Human WB and MitoBiogenesisTM Western Blot Cocktail were purchased from Abcam.

Plasmids, Lentiviral Production and Infection

The pLVO-Puro-mCherry-eGFP-LC3 plasmid has been previously described (45). 293T cells were co-transfected with packaging and envelope plasmids, pCMV and pVsvg with pLVO-Puro-mCherry-eGFP-LC3 and Lipofectamine 2000 (Life Technologies). Supernatants containing lentiviral particles were collected every 24 h for 3 days, and were then pooled, filtered and aliquoted. Vials of lentiviral particles were stored in a -80°C freezer. Adherent WM9 and 1205Lu melanoma cells were infected overnight with lentiviral particles supplemented with polybrene (Sigma) at 8 μ g/ml. Stably infected clones were established by an initial selection with puromycin at 1 μ g/ml for 48 h, followed by FACS sorting.

Drug Sensitivity IC₅₀ Data

 IC_{50} data of human melanoma cell lines with $BRAF^{V600}$ mutations to selective BRAF and MEK inhibitors were curated from the Cancer Cell Line Encyclopedia (CCLE).

Cell Growth/Viability and Assessment of Cell Clonogenicity

Cell viability was measured by the MTT assay or CellTiter-Glo luminescent cell viability assay (Promega). For the assessment of cell clonogenicity, cells were seeded into 12-well tissue culture plates at a density of 500 cells/well as biological triplicates in drug-free medium. Medium was refreshed every 3 or 4 days for 14 days. Colonies were then stained overnight with methanol containing 0.05% crystal violet. After extensive washing with distilled H₂O, cells were air-dried and subjected to image acquisition using a Nikon D200 DLSR camera.

CellTrace Violet Labeling and CellROX Deep Red and MitoTracker Red Staining

Adherent melanoma cells were harvested with 0.05% Trypsin-EDTA and were washed once with 1X DPBS. Cells were labeled with CellTraceTM Violet according to the manufacturer's protocol and were allowed to adhere to tissue culture plates overnight following the drug treatment. At indicated time points following the drug treatment, floating and adherent cells were pooled, washed with Hank's Buffered Salt Solution (HBSS) and then stained with CellROX Deep Red at 2.5 μ M or MitoTracer Red at 0.25 μ M for 1 h in a 37°C humidified incubator supplied with 5% CO₂. Cells were then pelleted, washed with DPBS, and stained with PSVue® 643 at 5 μ M diluted in TES buffer for 5 min in the dark. Cells were then immediately subjected to FACS analysis using a BD LSR II flow cytometer and at least 5,000 cells per sample were acquired.

Measurement of 2-(N-(7-nitrobenz-2-oxa-l,3-diazol-4-yl)amino)-2-deoxyglucose (2-NBDG) Uptake

Cells were washed with HBSS and were then pre-incubated with Opti-MEM (Life Technologies) for 15 min. Cells were then incubated with DMEM deprived of glucose, glutamine and phenol red (Life Technologies, cat. # A14430-01) and Fetal Bovine Serum that was supplemented with 2-NBDG (Cayman Chemical, cat. # 11046) at 10 mg/ml for 1 h. Cells were washed with HBSS and were immediately harvested for FACS analysis.

Measurement of Autophagic Flux

WM9 cells stably expressing pLVO-Puro-mCherry-eGFP-LC3 were analyzed with a BD LSR II flow cytometer. Fluorescence of mCherry and eGFP was recorded to derive the ratio of mCherry-height over eGFP-height. This ratio indicates the autophagic flux.

Measurement of ATP Production

Cells were seeded into Black Plate, Clear Bottom 96 Well Assay Plates (Corning Inc., cat. # 3603) at a density of 5,000 cells/well. Immediately following the drug treatment, culture media were removed. Cells in each well were incubated with a mixture of reagents containing 50 μ l DPBS, 50 μ l reconstituted CellTiter-Glo® Assay and 0.5 μ l CyQUANT for 10 min in the dark. Both ATP luminescence and CyQUANT fluorescence were recorded with a BioTek Multi-Mode Microplate Reader. The ATP production per cell was calculated as the ratio of ATP luminescence over CyQUANT fluorescence.

Measurement of Basal Oxygen Consumption

Cells were seeded into Black Plate, Clear Bottom 96 Well Assay Plates at a density of 5,000 cells/well. Immediately following the drug treatment, the culture medium in each well was removed and replaced with 140 μ l 10% DMEM deprived of glucose, glutamine and phenol red that was supplemented with 10 μ l reconstituted extracellular O₂ probe. Cells were incubated for 2 h at 37°C in a humidified incubator supplied with 5% CO₂. Fluorescence of the extracellular O₂ probe was recorded with a BioTek Multi-Mode Microplate Reader. Cell numbers were determined using identical plates in the same experimental setting. The basal oxygen consumption per cell was calculated as the ratio of the extracellular O₂ probe fluorescence over CyQUANT fluorescence.

Quantification of Mitochondrial DNA Copy Number

Mitochondria DNA copy number was measured by the ratio of a mitochondrial gene, mtDNA-3, and a nuclear gene, 18S rRNA. The mtDNA-3/18S rRNA ratio was quantified by qRT-PCR on an Applied Biosystems 7500 Fast Real-Time PCR System. The reaction mixtures (25 μ L final volume) contained 12.5 μ L Fast SYBR Green Master Mix, 500 nM of each primer and 10 ng genomic DNA. The reaction conditions were 95°C for 20 s, followed by 45 cycles of 95°C for 3 s and 60°C for 30 s. The relative mtDNA copy number was analyzed by the 2^{- $\Delta\Delta$ Ct} method.

Quantitative Real-Time PCR

One µg total mRNA was reverse transcribed using a Maxima First Strand cDNA Synthesis Kit for qRT-PCR (Thermo Fisher). Fast SYBR® Green Master Mix (Life Technologies) was used with cDNA template and primers to evaluate the expression of target genes and GAPDH. Primers used were purchased from Integrated DNA Technologies. Amplifications were performed using an Applied Biosystems® 7500 Real-Time PCR System (Life Technologies). All experiments were performed in triplicate. Expression ratios of controls were normalized to 1. Please see Extended Experimental Procedures for the list of PCR primer sequences.

Time-Course Illumina Gene Expression Microarray and Data Analysis

WM9 melanoma cells treated with PLX4720 at 10 µM were washed twice with HBSS and harvested directly with TRI Reagent® (Sigma) at various time points (0, 12, 24, 48, 60, 72 and 96 h) after drug treatment. WM9 melanoma cells were treated with DMSO and harvested directly with TRI Reagent® (Sigma) at 96 h. RNA isolation and cDNA synthesis was performed in the Wistar Genomics Facility. Illumina HumanHT-12 v4 Expression BeadChips were hybridized, labeled and processed according to the manufacturer's protocol. Microarray data were obtained from 2 independent biological replicates per time point.

Gene Expression Microarray Data and TCGA Melanoma Patients' RNA-seq Data

Normalized CCLE gene expression microarray data were directly downloaded from Broad CCLE (http://www.broadinstitute.org/ccle). Gene expression microarray data of A375 and SK-MEL-28 melanoma cells were downloaded from GEO under accession numbers GSE42872 and GSE37441, respectively. Gene expression microarray data of Malme-3M and COLO829 melanoma cells were downloaded from GEO under the accession number GSE51113. Normalized RNA-seq data of TCGA melanoma patients were downloaded from TCGA Data Portal (https://tcga-data.nci.nih.gov/tcga/). Single sample gene set enrichment analysis (ssGSEA) was performed in each data set to calculate an enrichment score of each gene set.

Analysis of Illumina Gene Expression Microarray Data and Reverse Phase Protein Array (RPPA) Data

The raw data of gene expression microarrays generated from Illumina Chips were normalized, background-corrected and summarized using the R package "lumi". Probes below background level (detection P-value <0.01) were excluded and differential expression was identified with Bayes-adjusted variance analysis using the Bioconductor Limma package. To reduce false positives, the unexpressed probes were removed. The R package "limma" was employed for gene differential expression analysis, followed by multiple test correction by the Benjamini and Hochberg procedure. Genes with adjusted p values < 0.05 and fold change > 2 were claimed as significantly differentially expressed and were subjected to the hypergeometric test for gene set enrichment analysis (GSEA). We also conducted GSEA as previously reported. For GSEA, we analyzed gene sets obtained from the Molecular Signatures Database (www.broadinstitute.org/gsea/msigdb/). The same differential expression analysis method was applied to RPPA data.

Relapsed Melanoma Patients' Gene Expression Microarray and RNA-seq data

Gene expression microarray data and RNA-sequencing data of paired pre- and post-treatment tumor biopsies derived from melanoma patients were downloaded from GEO under accession number GSE50509, GSE50535, GSE61992 and GSE65185 and from EGA under accession number EGAS00001000992. Data were normalized, background-corrected and summarized using the R package "lumi". We then selected patients whose 30% quantile of genes in the OxPhos gene set under the Prog condition was positive. We then conducted one-way clustering of the OxPhos genes over the selected samples.

Kaplan-Meier Survival Analysis of TCGA Melanoma Patient Data

We clustered TCGA melanoma patient RNA-seq data into 2 groups using Cox regression analysis based on expression of 18 MitoBiogenesis genes. We also clustered TCGA melanoma patient RNA-seq data into 4 groups using k-means based on expression of 62 "Glycolysis" genes and 117 "OxPhos" genes. We clustered TCGA melanoma patient RNA-seq data into 2 groups based on expression of PPARGC1A. We then performed a log-rank test to test the survival rate difference between these subgroups.

Immunohistochemistry

Tissues were stained with TFAM (Cell Signaling; Catalog# 8076), TRAP-1 (Thermo Scientific[™] Pierce; Catalog# PIPA527596) and MT-CO1 (Abcam; Catalog# 14705) according to the manufacturer's instructions. Melanoma samples were stained with a red chromogen to distinguish true staining from melanin. All stained tissues were blindly evaluated by a pathologist at Hospital of the University of Pennsylvania.

Western Blotting and Antibodies

Cells were washed with ice-cold PBS containing $100 \ \mu M \ Na_3 VO_4$ and scraped off culture dishes. After centrifugation, cell pellets were lysed in buffer containing 10 mM Tris-HCl, pH 7.8, 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40, 1 mM Na₃VO₄ and protease inhibitors (Roche complete protease inhibitor tablets). Lysates were cleared by micro-centrifugation and protein concentrations were determined with Protein Assay Dye Reagent Concentrate (Bio-Rad). For western blots, 25 µg of each lysate were run on 15-well 10% or 12% SDS-PAGE gels and transferred onto nitrocellulose membranes using a dye fast Trans-Blot^R TurboTM transfer system (Bio-Rad). Blots were blocked in SEA BLOCK Blocking Buffer (Thermo Scientific) diluted with 1X TBS at 1:1 ratio at room temperature for 1 h, incubated overnight with primary antibody at 4°C, stained with secondary antibodies conjugated to IRDye® Infrared Dyes (LI-COR Biosciences) and then visualized using an Odyssey flat bed scanner (LI-COR Biosciences).

Time-course Gene Expression Microarray Data Analysis and GSEA

Time-course gene expression microarray raw data were normalized, background corrected and summarized using the R package "lumi. To reduce false positives, unexpressed probes were removed, leaving 23,569 probes that were examined in all experiments described herein. When multiple probe IDs interrogated the same gene, the maximum value was taken as the gene expression level, which was used in the downstream data analysis. Fold changes were calculated by comparing the difference in the mean of gene expression levels of 3 biological replicates between pre-treatment and post-treatment derived at different time points. Student's T-test was used to obtain the parametric p values for measuring the significance of gene expression level differences. Genes with p value < 0.01 and fold change >1.2 (up- or down-regulated) were claimed to be significantly up- or down-regulated, which were then subjected to hypergeometric test based GSEA. 1,453 canonical pathways were obtained from the Molecular Signatures Database (MSigDB) (http://www.broadinstitute.org/gsea/msigdb/index.jsp) for GSEA. We only considered pathways that contain at least 10 genes and at most 150 genes profiled and expressed in the experiment. Two-way hierarchical clustering was performed and plotted using the "heatplot" function in the R package "made4", with the basis of Euclidean distance, centering log-

transformed gene expression by mean and single linkage clustering. Genes that are up- or downregulated at one or more time points were subjected to the two-way clustering analysis to generate the heatmaps.

Prognostic Analysis of Mitochondrial Biogenesis Biomarkers using Relapsed Melanoma

Patients' Gene Expression Microarray Data

Univariate Cox regression models were used to test the associations between those mitochondrial biogenesis biomarkers in relapsed melanoma patients' pre-treatment and early on-treatment tumor biopsies with progression-free survival and overall survival.

qRT-PCR Primer Sequences

Gene	Forward Socuence	Deverse Seguence
Name	Forward Sequence	Reverse Sequence
ALDH1A1	TGT TAG CTG ATG CCG ACT TG	TTC TTA GCC CGC TCA ACA CT
ATP5B	CAAGTCATCAGCAGGCACAT	TGGCCACTGACATGGGTACT
ATP5D	TTTGTGAGCAGCGGTTCCAT	GGCCTTCTCCAAGTTTGCCT
ATP5G1	GCCTGATTAGACCCCTGGTA	GGCTAAAGCTGGGAGACTGA
ATP5L	AAGAAATTGAGCGGCATAAG	GGAAGCACACAGGTTGATTT
COX II	CCATCCCTACGCATCCTTTAC	GTTTGCTCCACAGATTTCAGAG
COX5A	CGAGCATCAAACTCCTCAT	GAGGCCTCCTGCACTCC
COX6A1	ATGGCGGTAGTTGGTGTGTC	GTGAGAGTCTTCCACATGCGA
COX6C	CAAAGAAAGAAGGCATACGCAGAT	CTGAAAGATACCAGCCTTCCTCAT
COX7B	CTTGGTCAAAAGCGCACTAAATC	CTATTCCGACTTGTGTTGCTACA
HBB	GTGAAGGCTCATGGCAAGA	AGCTCACTCAGGTGTGGCAAAG
HIF-1α	TATTGCACTGCACAGGCCACATTC	TGATGGGTGAGGAATGGGTTCACA
M-MITF	CCGTCTCTCACTGGATTGGT	TACTTGGTGGGGTTTTCGAG
MTCO1_4	GAGCTGCTGTTCGGTGTCC	TGCCAGTGGTAGAGATGGTTG
MTCO1_5	ACCC TAGA CCAA ACCT ACGC CAAA	TAGG CCGA GAAA GTGT TGTG GGA
MTCO1_6	GACGTAGACACACGAGCATATTTCA	AGGACATAGTGGAAGTGAGCTACA
mtDNA	cga aag gac aag aga aat aag g	ctg taa agt ttt aag ttt tat gcg
NDUFA1	TGGGCGTGTGCTTGTTGAT	CCCGTTAGTGAACCTGTGGATGT
NDUFA11	CAATCCTCCGGGCACCTT	TGCAGTGAACGTGTATTGTCCAA
NDUFA12	GGCATCGTTGGCTTCACAGT	TTACGAGCAGTAAGTGGTTTTGTTG
NDUFA13	CGCGCTGTTGCCACTGTTA	CCCGAAGCATCTGCAAGGT
NDUFA3	CAAGGCCACGCCCTACAAC	TCGGGCATGTTCCCATCAT
NDUFA4	ATGCTCCGCCAGATCATCG	GCAAGAGATACAGTGTTGCTCCA
NDUFA7	CTGTGCCCCCTTCCATCAT	TCTGCTGGCTTGCCTGACA
NDUFA8	CTCCTTGTTGGGCTTATCACA	GCCCACTCTAGAGGAGCTGA
NDUFAF2	TGGGTTGGTCTCAGGATTTGTT	TGCTCCTTCACTTCCCTTGACA

NDUFAF4	TTATGAGGAGATGGGAGCACTAGTG	CCGCTCGGTTCTCTAGGTTGA
NDUFB11	TCCTTGGCAGCACCTTTGTG	CGGCGGGACCACTCTTTC
NDUFB3	GCTGGCTGCAAAAGGGCTA	CTCCTACAGCTACCACAAATGC
NDUFB7	ATGTGATGCGCATGAAGGAGTT	CTTCTCCCGCCGCTTCTT
NDUFC1	GGCCCTTCAGTGCGATCA	CCAGTCAGGTTTGGCATTCG
NDUFS3	GCTGACGCCCATTGAGTCTG	GGAACTCTTGGGCCAACTCC
NDUFS8	GGCTGAGCCAAGAGCTGATG	GATGCACTTGGTCATGTCGATGT
PDK1	CCAAGACCTCGTGTTGAGACC	AATACAGCTTCAGGTCTCCTTGG
PDP2	ACCACCTCCGTGTCTATTGG	CCAGCGAGATGTCAGAATCC
PGC1a	CTGCTAGCAAGTTTGCCTCA	AGTGGTGCAGTGACCAATCA
POLGA	CAC ACC TAA ACT CAT GGC AC	GTC CAC GTC GTT GTA AGG TC
POLGB	GTT TGC CAT GAG TCC ATC TAA C	CTC TGT CAG CTG GAA AGA ATC
SDHB	AAGCATCCAATACCATGGGG	TCTATCGATGGGACCCAGAC
SOD2	TAGGGCTGAGGTTTGTCCAG	GGAGAAGTACCAGGAGGCGT
TFAM	CCATCTACCGACCGGATGTTA	CAGACCTTCCCAGGGCACTCA
TFB1M	ATGGCTCAGTACCTCTGCAATG	TGGGCTGTATCAAGGGAGTGA
TFB2M	ATCCCGGAAATCCAGACTTGT	GACCAAGGCTCCATGTGCA
TWINKLE	GCA CAA GTC CAT CGT ATC TTT C	CAT ACT CAC TGA TGA ATG TCG T
UCRC	GTGGGCGTCATGTTCTTCGA	ACAGCTTCCCCTCGTTGATGT
UQCRB	ACTGGGGTTAATGCGAGATG	GTCCAGTGCCCTCTTAATGC
β-GLOBIN	caa ctt cat cca cgt tca cc	gaa gag cca agg aca ggt ac