Developmental Cell

Supplemental Information

CyclinB1/Cdk1 Coordinates Mitochondrial Respiration for Cell Cycle G2/M Progression

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Inventory of Supplemental Information

A. Supplementary Data (including 9 Figures, 9 Tables, and 2 movies)

Dev Cell

A. Supplementary Figures

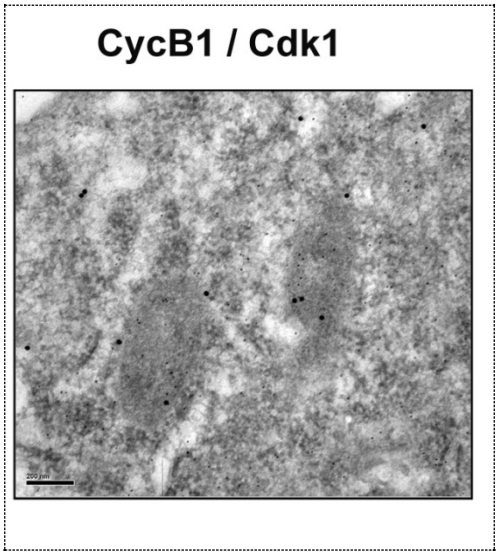

Figure S1. Mitochondrial-localized CyclinB1 and Cdk1. Human breast epithelial MCF10A cells were analyzed by immunoelectron microscopy with gold-labeled antibodies (20 nm gold-particles linked for CyclinB1; 10 nm gold-particles linked for Cdk1; scale bar, 200 nm; borders of two mitochondria are marked by white lines).

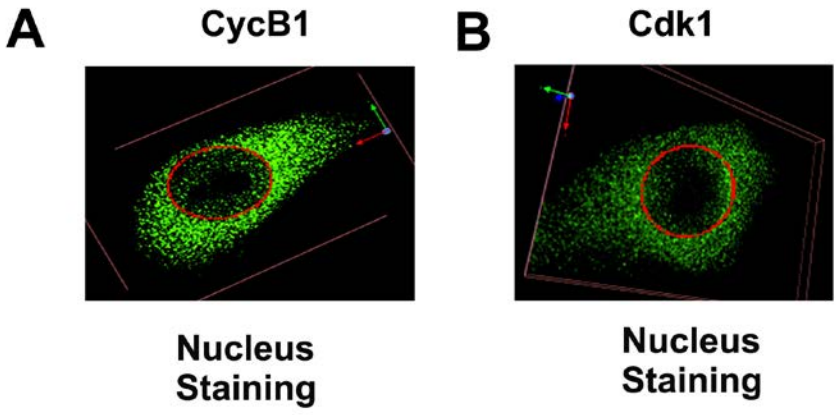

Figure S2. Nucleus staining detected in MCF-10A cells stained with CyclinB1 or Cdk1 antibody by structured illumination microscopy. CyclinB1 (A) and Cdk1 (B) staining in the nucleus (red circle) at z-section of 0.5 µm up from the section shown in the upper panels of Figure 1K & 1L (red circle).

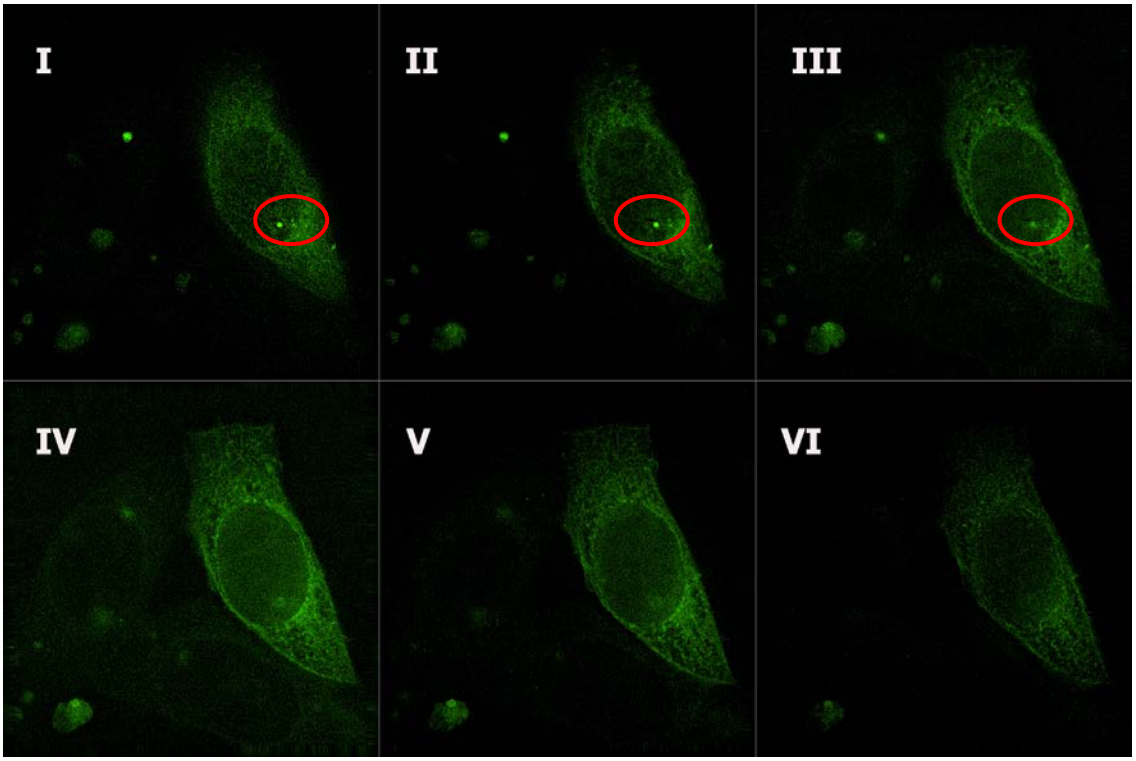

Figure S3. Centrosome-like structures detected in MCF-10A cells stained with CyclinB1 antibody by structured illumination microscopy. **(I)** Cell 1 at Z=0, a bright spot, Centrosome-like structure, is highlighted in the red circle. **(II to VI)** Same cell at different Z value, the brightness of centrosome-like structures (highlighted in the red circle) gradually decreases (II to IV) or disappears (V & VI).

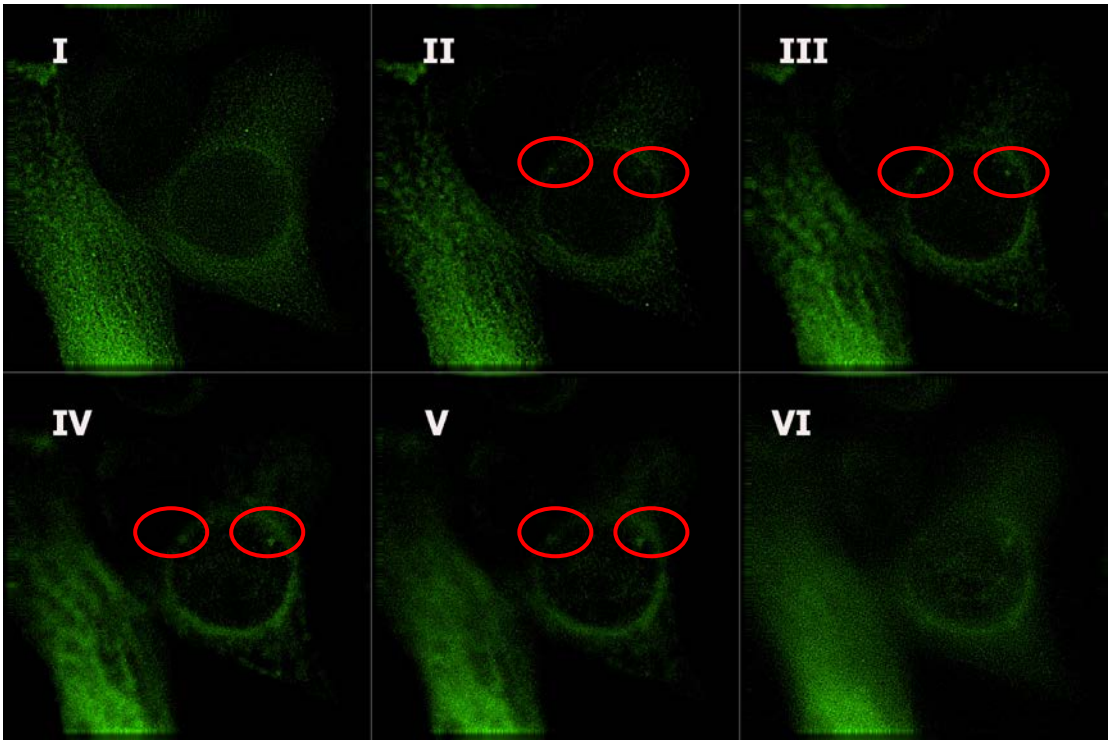

Figure S4. Centrosome-like structures detected in MCF-10A cells stained with Cdk1 antibody by structured illumination microscopy. Cell at different Z value, two bright spots, the centrosome-like structure, are highlighted in the red circles. The brightness of centrosome-like structure gradually decreases or disappears.

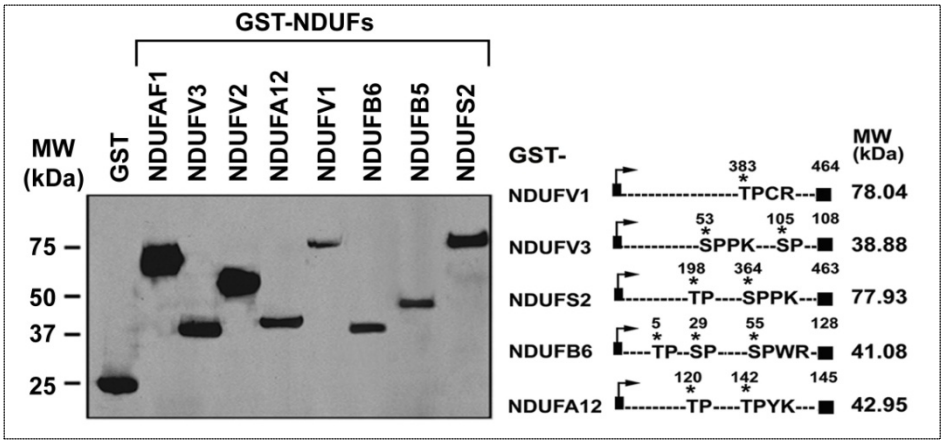

Figure S5. Immunoblotting of synthesized GST-fusion subunits of human complex I (CI). GST protein was included as the positive control. Phosphorylation motifs of some CI subunits are shown in the right panel.

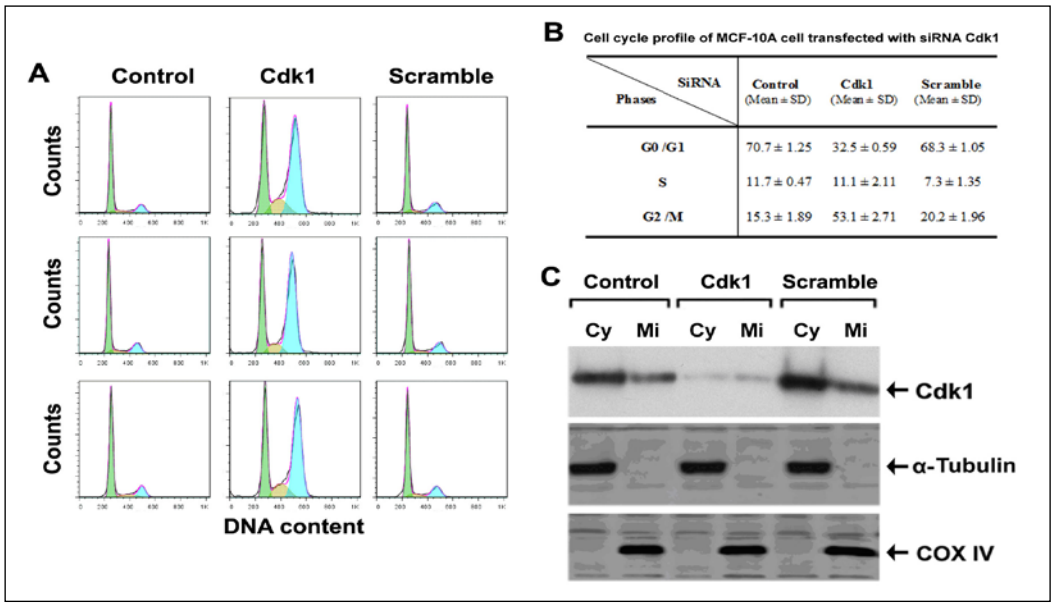

Figure S6. SiRNA against Cdk1 arrests cells at the G2/M phase. (**A, B**) Cell cycle profile of MCF-10A cells transfected with siRNA against Cdk1. (**C**) SiRNA against Cdk1 inhibits Cdk1 proteins in both cytoplasm and mitochondria. Scramble siRNA was used as control.

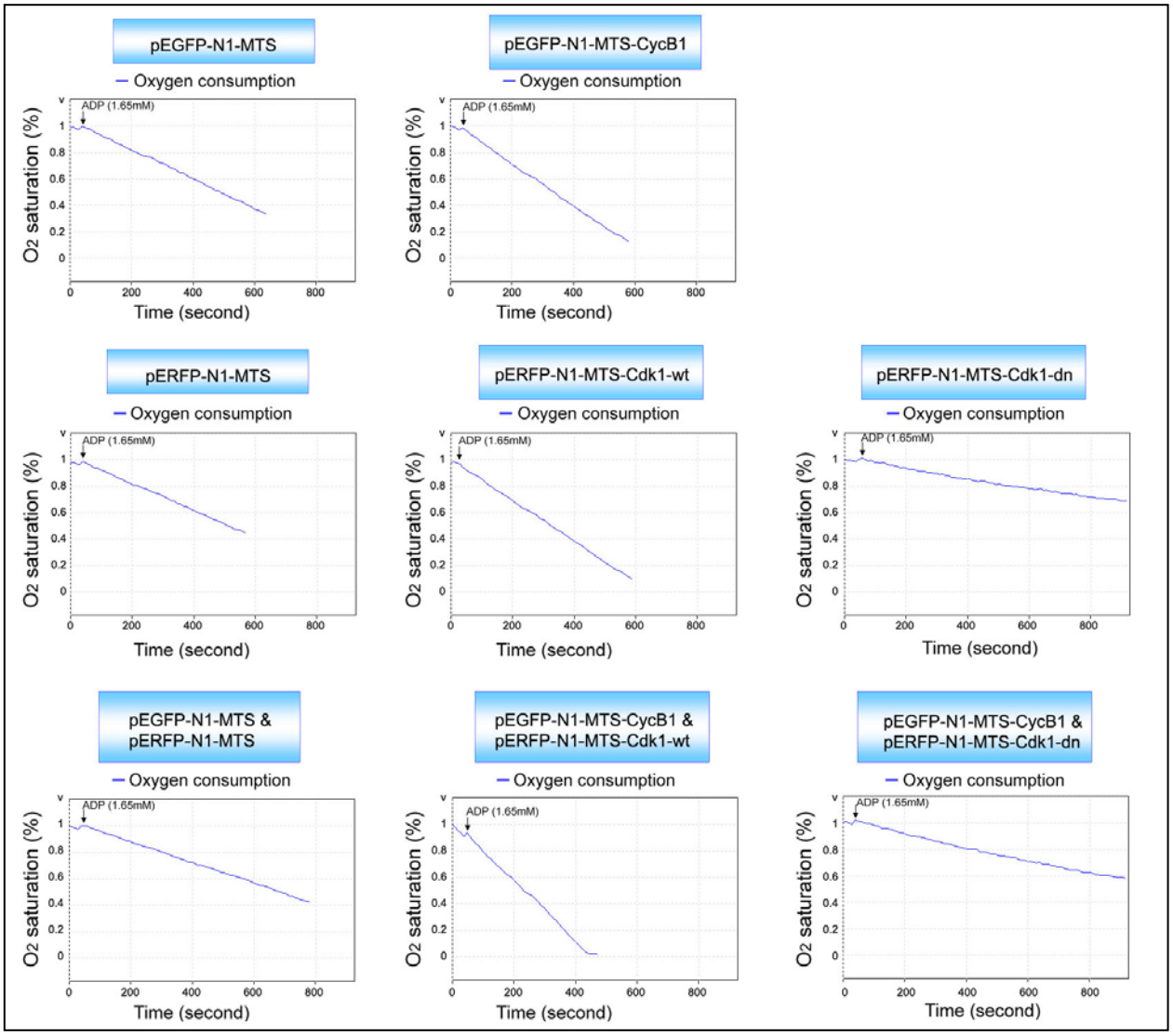

Figure S7. Representative oxygen consumption data from cells transfected with vectors as indicated. MTS, mitochondrial targeting sequence; CycB1, CyclinB1; wt, wild type; dn, dominant negative mutant.

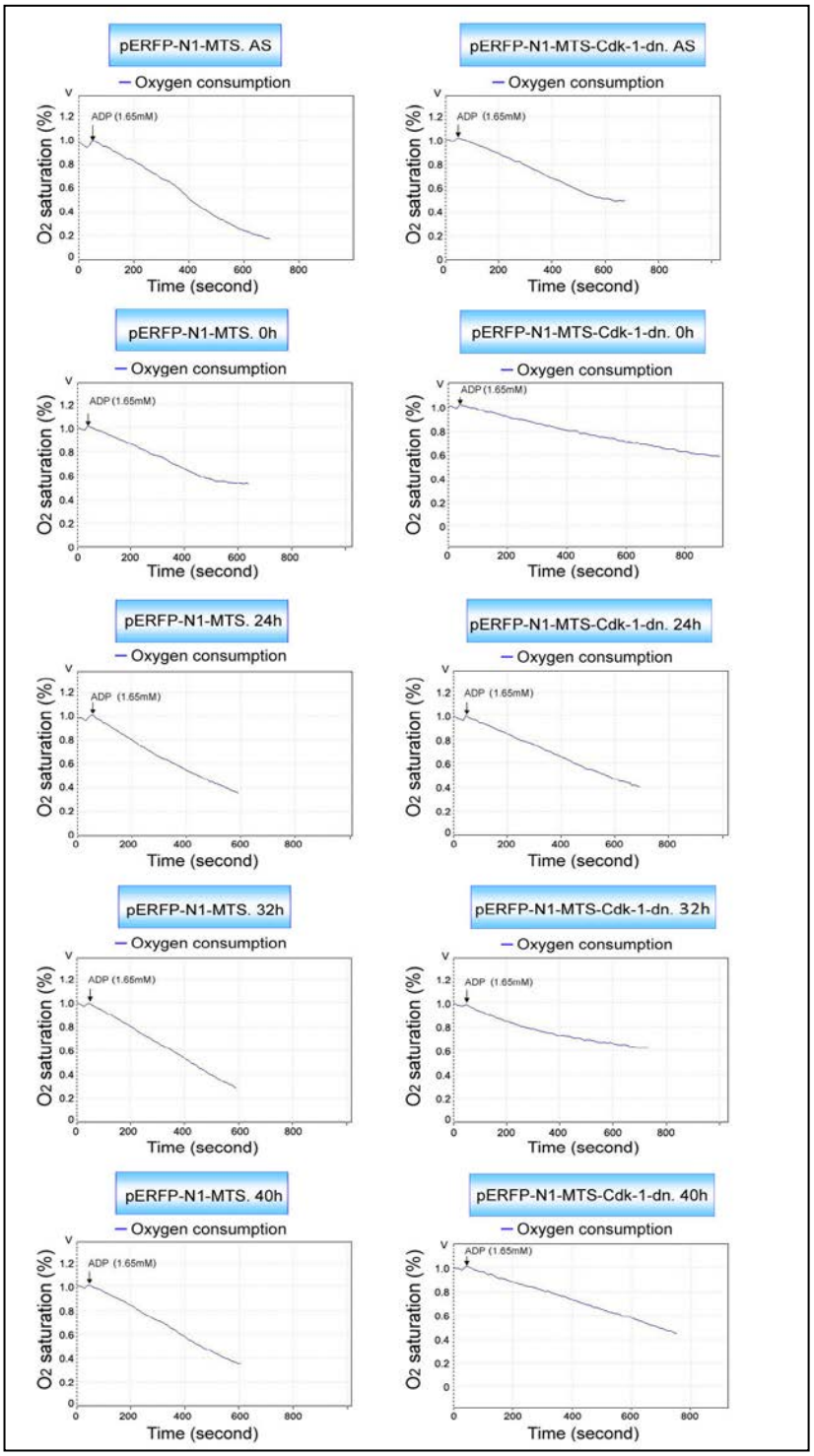

Figure S8. Representative oxygen consumption data from synchronous or asynchronous cells transfected with pERFP-N1-MTS or with pERFP-N1-MTS Cdk1-dn. The plasmid of pERFP-N1-MTS served as a positive control for mitochondrial localization, and as a negative control for Cdk1 inhibition. As: asynchronous cells.

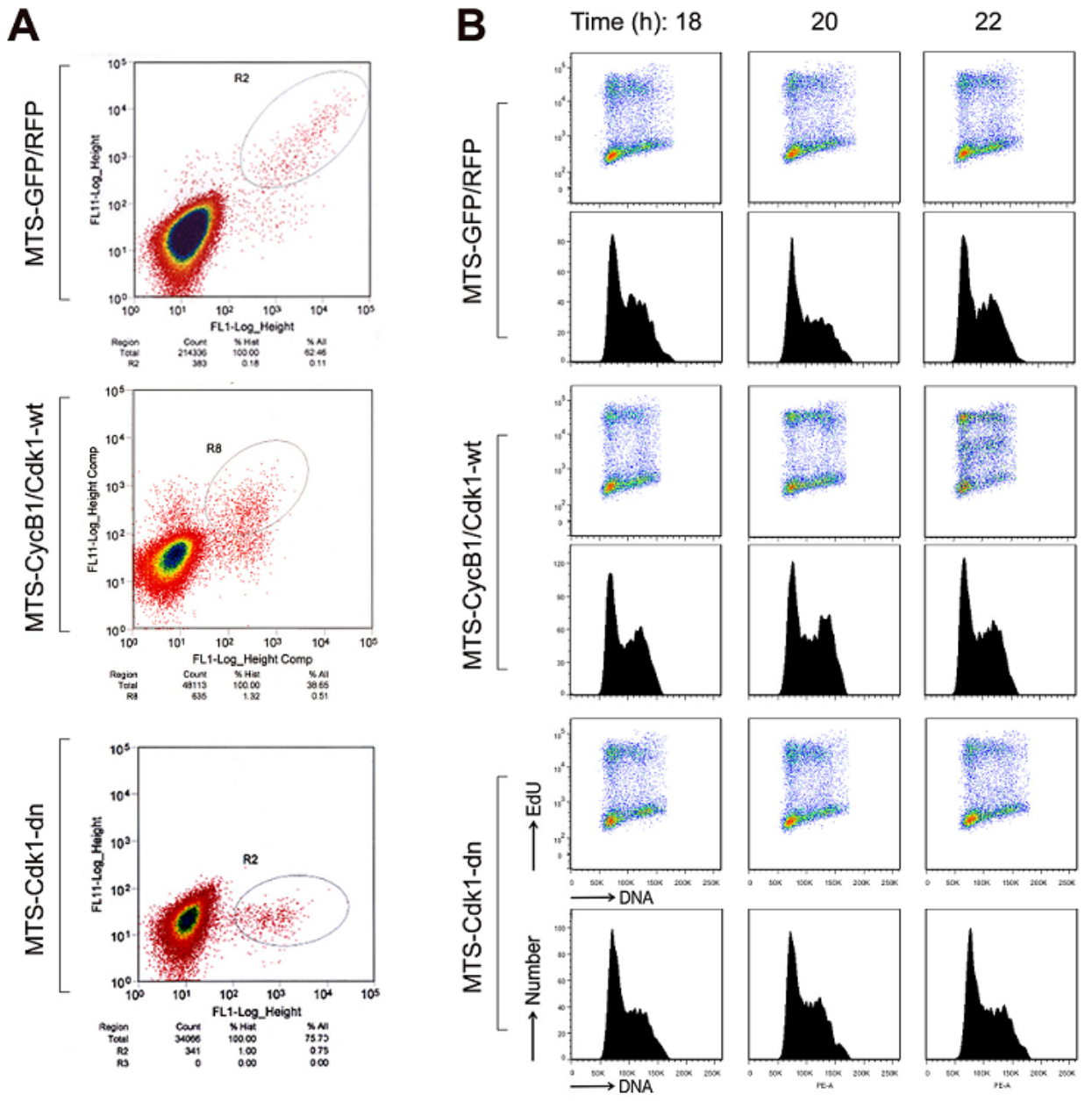

Figure S9. Cell cycle analysis with EdU pulse-chase labeling. (A) Live sorting of MTS-tagged GFP-CyclinB1, MTS-tagged RFP-Cdk1, and MTS-tagged GFP-CyclinB1/RFP-Cdk1 expressing cells. MCF10A cells stably expressing the fluorescence-tagged CyclinB1 and Cdk1 proteins were live sorted from non-expressing cells via FACS and the cells were used for analysis of cell cycle progression with EdU pulse-chase labeling. (B) MCF-10A cells transfected with indicated vectors; positive transfectants were live sorted and then pulsed with EdU for 1 hour. The EdU-positive population is followed over time as it transitions through the phases of the cell cycle.

Scatter plot histograms of EdU-labeled cells stained for DNA content (X-axis) and EdU (Y-axis). The lower figures in each panel show the mean fluorescence intensity of the EdU labeled nuclei. The time points are indicated in hours after the EdU pulse.

Table S1. CyclinB1/Cdk1-mediated Phosphorylation of Mitochondrial Proteins detected by *in vitro* CyclinB1/Cdk1 Kinase Assay

Functional category[a]	Spots [b]	Gene Symbol	M.W.	pI	IPI Acc. [c]	Optimal P-site and position[c]	Minimal P-site number
Amino acid metabolism	98	GLUD1 Glutamate	61359.2	7.66	IPI00016801	T^{144} P C K	2
Amino acid metabolism	81	GATM Isoform	48424.3	8.26	IPI00032103	0	3
Amino acid metabolism	91	GOT2 Aspartate	47445.3	9.14	IPI00018206	0	2
Amino acid metabolism	92	ABAT cDNA FLJ56034	58191.5	8.35	IPI00009532	0	1
Amino acid metabolism	45	BCKDHB 2-oxoisovalerate	43094.9	5.89	IPI00011276	0	5
Amino acid metabolism	72	AGMAT Agmatinase	37636.2	7.55	IPI00305360	0	4
Amino acid metabolism	84	OTC Ornithine	39909.7	8.75	IPI00295363	0	3
Amino acid metabolism	79	SHMT2 Serine	55957.7	8.76	IPI00002520	S^{266} P F K	3
Carbohydrate metabolism	96	ALDH1B1 Aldehyde	57202.2	6.36	IPI00103467	S^{91} P W R	4
Carbohydrate metabolism	99	ALDH4A1 Aldehyde	59837.7	7.62	IPI00647328	S^{44} P E R	3
Carbohydrate metabolism	97	ALDH6A1	57802.6	8.72	IPI00024990	0	2
Carbohydrate metabolism	21	ALDH2 Aldehyde	56345.6	6.63	IPI00006663	S^{91} P W R	3
Lipid metabolism	76	HMGCS2	56599.4	8.4	IPI00008934	T^{6} P V K	4
Lipid metabolism	73	SUCLG2 Succinyl-CoA	46481.4	6.15	IPI00096066	0	4
Lipid metabolism	74	ACADSB Short/branched	47455.3	6.53	IPI00024623	0	1

Lipid metabolism	29[d]	ACADM medium-chain	46989.8	8.57	IPI00895801	0	1
Lipid metabolism	93	ACOT1 Acyl-coenzyme A	46248.1	6.9	IPI00333838	0	2
Lipid metabolism	82	ACAT1 Acetyl-CoA	45170.6	8.98	IPI00030363	0	5
Lipid metabolism	42	SUCLG2 Succinyl-CoA	46481.4	6.15	IPI00096066	0	4
Lipid metabolism	103	ACADS Short-chain	44268.8	8.13	IPI00027701	0	2
Lipid metabolism	65	HSD17B10 Isoform 1	26906.1	7.66	IPI00017726	0	1
Lipid metabolism	62	HSD17B8 Estradiol	26956.8	6.09	IPI00021890	0	1
Lipid metabolism	18[d]	ECH1	35793.4	8.16	IPI00011416	0	1
Lipid metabolism	16, 17	ACSL1 Isoform 1	77892.9	6.81	IPI00012728	0	1
Redox	59	SOD2 manganese	19718	7.81	IPI00896370	0	1
Redox	61	PRDX3 peroxiredoxin 3	25822.3	7.04	IPI00374151	T^{128} P R K	3
Redox	58	MPST Mercaptopyruvate	24966.5	7.14	IPI00877618	0	2
Redox	12, 13, 14, 15	TST Thiosulfate	33407.8	6.77	IPI00216293	0	2
Redox	86	NNT NAD(P) transhydrogenase	113895.59	8.31	IPI00337541.3	S^{400} P D K T^{467} P F R	4
OXPHOS (complex V)	32, 33	ATP5B ATP synthase	56524.6	5.26	IPI00303476	0	3
OXPHOS (complex III subunit 1)	30, 31	UQCRC1 Cytochrome	52612.4	5.94	IPI00013847	T^{266} P C R	6
OXPHOS (complex III subunit 5)	66	UQCRFS1 Cytochrome	29649.4	8.55	IPI00026964	0	1
OXPHOS (complex V)	85	ATP5A1 23 kDa protein	22943.4	9.68	IPI00549805	0	1
OXPHOS (complex I)	28[d]	NDUFV1 NADH dehydrogenase [ubiquinone] flavoprotein 1	50817.09	8.51	IPI00028520.2	T^{383} P C R	0
OXPHOS (complex I)	24[d]	NDUFV2 NADH dehydrogenase [ubiquinone] flavoprotein 2	27349.47	8.22	IPI00291328.3	T^{164} P D K	4

OXPHOS (complex I)	36[d]	NDUFV3 NADH dehydrogenase [ubiquinone] flavoprotein 3	11940.53	9.72	IPI00008214.4	S^{530} P P K	1
OXPHOS (complex I)	25	NDUFS2 NADH dehydrogenase [ubiquinone] iron-sulfur protein 2	52545.65	7.21	IPI00025239.2	S^{364} P P K	1
OXPHOS (complex I)	21[d]	NDUFB6 NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 5	21750.27	9.62	IPI00013459.1	S^{550} P W R	2
OXPHOS (complex I)	34[d]	NDUFB6 NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 6	15489.19	9.63	IPI00219385.3	S^{550} P W R	2
OXPHOS (complex I)	33[d]	NDUFA12 13kDa differentiation-associated protein variant	17114.44	9.63	IPI00005966.6	T^{142} P Y K	1
OXPHOS (complex I)	87	NDUFAF1 Complex I intermediate-associated protein	37763.75	7.11	IPI00032560.2	S^{450} P G K	1
Protein targeting	49	HSPD1 60 kDa heat	61016.4	5.7	IPI00784154	0	4
Protein targeting	6, 7, 8	14-3-3 protein epsilon	29155.4	4.63	IPI00000816.1	S^{187} **P** D R	0
Protein targeting	29	HSPD1 cDNA FLJ51046	55049	5.45	IPI00917575	0	3
TCA cycle	102	PCK2 mitochondrial	70684.6	7.57	IPI00797038	0	7
TCA cycle	90	MDH2 Malate	35480.7	8.92	IPI00291006	0	4
TCA cycle	22[d]	FH Isoform Mitochondrial	54602.2	8.85	IPI00296053	0	1
Protein synthesis	94	TUFM Tu translation	49843.3	7.26	IPI00027107	0	2

[a,] Protein function cited from the NCBI protein database: www.ncbi.nlm.nih.gov.
[b,] A list of *in vitro* CyclinB1/Cdk1-phosphorlated mitochondrial proteins from Figure 2A
[c,]IPI Acc., International Protein Index accession number; IPI was cited from the database: www.ebi.ac.uk/IPI/IPIhelp.html
[d,] Spots and protein ID information were obtained from Figure 2C.

Table S2. Primers for Vector Construction of MTS-GFP-CyclinB1, MTS-RFP-Cdk1 and MTS-RFP-Cdk1-dn

Name of primer	Sequence	Gene ID*	Name of plasmid
MTS (Sense)	5'-ATATGCTAGCATGTCCGTCCTGACGCCGCTGCTGCTG CGGGGCTTGACAGGCTCGGCCCGGCGGCTCCCAGTGCCGC GCGCCAAGATCCATTCGTTGGGGGATCCATAT-3'	NM_004074	pEGFPN1-or pERFPN1-MTS
MTS (Antisense)	5'-ATATGGATCCCCCAACGAATGGATCTTGGCGCGCGG CACTGGGAGCCGCCGGGCCGAGCCTGTCAAGCCCCGCAGCA GCAGCGGCGTCAGGACGGACATGCTAGCATAT-3'		
Cyclin B1 (Forward)	5'-ATATGGATCCAATGGCGCTCCGAGTCA-3'	NM_031966	pEGFPN1-MTS-Cyclin B1
Cyclin B1 (Reverse)	5'-ATATGGATCCTGCACCTTTGCCACAGCC-3'		
Cdk1 (Forward)	5'-CAGTGGATCCAATGGAAGATTATACCAAAAT-3'	NM_001786	pERFPN1-MTS-Cdk1
Cdk1 (Reverse)	5'-CTG TGG ATC CTG CAT CTT CTT AAT CTG ATT-3'		
Cdk1-dn D146 to N (Forward)	5'- AAA GGA ACA ATT AAA CTG GCT AAT TTT GGC CTT GCC AGA GCT TTT -3'	NM_001786	pERFPN1-MTS-Cdk1-dn
Cdk1-dn D146 to N (Reverse)	5'- AAA AGC TCT GGC AAG GCC AAA ATT AGC CAG TTT AAT TGT TCC TTT -3'		

*, Gene identity (ID) number was acquired from the NCBI database: www.ncbi.nlm.nih.gov.

Table S3. CyclinB1/Cdk1-mediated Phosphorylation of Mitochondrial Proteins *in vivo*[a]

Spots	Functional category [a]	Gene Symbol	M.W.	pI	IPI Acc. [b]	Optimal P-site and position[c]	Minimal P-site number
1	Redox	NNT NAD(P) transhydrogenase	113895.59	8.31	IPI00337541.3	S^{400} P D K T^{467} P F R	4
2	OXPHOS (Complex I subunit)	NDUFAF1 Complex I intermediate-associated protein	37763.75	7.11	IPI00032560.2	S^{450} P G K	1
3	Amino acid metabolism	BCKDHB 2-oxoisovalerate	43094.9	5.89	IPI00011276	0	5
4	OXPHOS (complex V)	ATP5B ATP synthase	56524.6	5.26	IPI00303476	0	3
6	Lipid metabolism	SUCLG2 Succinyl-CoA	46481.4	6.15	IPI00096066	0	4
7	Protein targeting	14-3-3 protein epsilon	29155.4	4.63	IPI00000816.1	S^{187} **P** D R	0
8	Carbohydrate metabolism	ALDH2 Aldehyde	56345.6	6.63	IPI00006663	S^{91} P W R	3
10	Redox	TST Thiosulfate	33407.8	6.77	IPI00216293	0	2
11	Redox	PRDX3 peroxiredoxin 3	25822.3	7.04	IPI00374151	T^{128} P R K	3
12	Lipid metabolism	ACSL1 Isoform 1	77892.9	6.81	IPI00012728	0	1
13	Redox	MPST Mercaptopyruvate	24966.5	7.14	IPI00877618	0	2
14	Carbohydrate metabolism	ALDH2 Aldehyde	56345.6	6.63	IPI00006663	S^{91} P W R	3
15	Carbohydrate metabolism	ALDH1B1 Aldehyde	57202.2	6.36	IPI00103467	S^{91} P W R	4

[a,] Protein ID information were obtained from Figure 2F.
[b,] Function cited from the NCBI protein database: www.ncbi.nlm.nih.gov.
[c,] IPI Acc., International Protein Index accession number; IPI was cited from the database

Table S4. Human Mitochondrial Complex I Subunits with Cdk1 Consensus Motif

Human protein name	Number of AA[a]	N-terminal modification[b]	MW (kDa) of theoretical mitochondrial protein	Optimal P-site and position[c]	Minimal P-site number	MW (kDa) of GST fusion protein	MW (kDa) of GFP fusion protein	Function
NDUFV1	464	ΔMTS 1-20	51.04 / 48.84	T^{383} P C R	0	78.04	75.84	NADH/FMN binding
NDUFV2	249	ΔMTS 1-32	27.39 / 23.87	T^{164} P D K	4	54.39	50.87	NADH/FMN binding
NDUFV3	108	ΔMTS 1-34	11.88 / 8.14	S^{530} P P K	1	38.88	35.14	NADH/FMN binding
NDUFS2	463	ΔMTS 1-33	50.93 / 47.3	S^{364} P P K	1	77.93	74.3	Assembly of complex I
NDUFAF1	327		35.97	S^{450} P G K	1	62.97	62.97	Assembly of complex I
NDUFA12	145	N-acetyl	15.95	T^{142} P Y K	1	42.95	42.95	Assembly of complex I
NDUFB5	189	ΔMTS 1-46	20.79 / 15.73	S^{128} P E K	2	47.79	42.73	Assembly of complex I
NDUFB6	128	ΔMet, N-acetyl	14.08	S^{550} P W R	2	41.08	41.08	Assembly of complex I

Abbreviations for amino-acid residues are: S, Ser; T, Thr; P, Pro; K, Lys; R, Arg; D, Asp; C, Cys; E, Glu; G, Gly; W, Trp; Y, Tyr; x, Any amino acid; *, Phosphorylation site.

[a] Number of amino acids from human sequences acquired from the NCBI protein database: www.ncbi.nlm.nih.gov.

[b] Δ, cleavage of mitochondrial targeting sequence, MTS; N-acetyl, acetylation of N-terminal residue; Met, cleavage of N-terminal acetylation.

[c] P-site, Phosphorylation site.

Table S5. Primers for Construction of Wild Type Human Mitochondrial Complex I Subunits with GST Tag

Name of Primer	Sequence	Gene ID*	Name of Plasmid
NDUFV1 (Forward)	5'- ATATGTCGACTCATGCTGGCAACACGG -3'	NM_007103	pGEX-5X-1-NDUFV1
NDUFV1 (Reverse)	5'- ATATGCGGCCGCTACTAAGAGGCAGCCT -3'		
NDUFV3 (Forward)	5'- ATATGGATCCAAATGGCTGCCCCGTGTTT -3'	NM_001001503	pGEX-5X-1-NDUFV3
NDUFV3 (Reverse)	5'- ATATGTCGACCTCAGTGTCGAGGTGACT -3'		
NDUFB6 (Forward)	5'- ATATGGATCCAAATGACGGGGTACACTCC -3'	NM_002493	pGEX-5X-1-NDUFB6
NDUFB6 (Reverse)	5'-ATCGCTCGAGTCAATGATGTTGATCAGGAAA -3'		
NDUFA12 (Forward)	5'- ATCGGAATTCATGGAGTTAGTGCAGGTCCTG -3'	NM_018838	pGEX-5X-1-NDUFA12
NDUFA12 (Reverse)	5'- ATCGCTCGAGTTACTTGTAAGGTGTTGAAGG -3'		
NDUFS2 (Forward)	5'- ATATGAATTCATGGCGGCGCTGAGGGCTTT -3'	NM_004550	pGEX-5X-1-NDUFS2
NDUFS2 (Reverse)	5'- ATCGGTCGACCTCACCGATCTACTTCTCCAAA -3'		
NDUFAF1 (Forward)	5'- ATCGGGATCCCCATGGCTTTGGTTCACAAA -3'	NM_016013	pGEX-5X-1-NDUFAF1
NDUFAF1 (Reverse)	5'- ATCGCTCGAGTTATTTAAAAAGCCTTGGGTT -3'		
NDUFB5 (Forward)	5'- ATATGGATCCAAATGGCGGCCATGAGTTT -3'	NM_002492	pGEX-5X-1-NDUFB5
NDUFB5 (Reverse)	5'- ATCGCTCGAGTTAATTGTCAGGAGTTGCTTT -3'		
NDUFV2 (Forward)	5'- ATATGGATCCCCATGTTCTTCTCCGCGGC -3'	NM_021074	pGEX-5X-1-NDUFV2
NDUFV2 (Reverse)	5'- ATCGCTCGAGTTAAAGGCCTGCTTGTACACC -3'		

*, Gene identity (ID) number was acquired from the NCBI database: www.ncbi.nlm.nih.gov.

Table S6. Primers for Construction of Wild Type Human Mitochondrial Complex I Subunits with GFP Tag

Name of primer	Sequence	Gene ID*	Name of Plasmid
NDUFV1 (Forward)	5’-ATATAAGCTTATGCTGGCAACACGGCGGCTG -3’	NM_007103	pEGFP-N1-NDUFV1
NDUFV1 (Reverse)	5’-ATATGTCGACTGAGAGGCAGCCTGCCGGGC -3’		
NDUFV3 (Forward)	5’-ATATAAGCTTATGGCTGCCCCGTGTTTGCTG -3’	NM_001001503	pEGFP-N1-NDUFV3
NDUFV3 (Reverse)	5’-ATATGGATCCCGGTGTCGAGGTGACTCCCGGC -3’		
NDUFS2 (Forward)	5’-ATATAAGCTTATGGCGGCGCTGAGGGCTTT -3’	NM_004550	pEGFP-N1-NDUFS2
NDUFS2 (Reverse)	5’- ATCGGTCGACTGCCGATCTACTTCTCCAAA -3’		
NDUFB6 (Forward)	5’- ATATCTCGAGATGACGGGGTACACTCC -3’	NM_002493	pEGFP-N1-NDUFB6
NDUFB6 (Reverse)	5’- ATCGGGATCCCGATGATGTTGATCAGGAAA -3’		
NDUFA12 (Forward)	5’- ATATCTCGAGATGGAGTTAGTGCAGGTCCTG -3’	NM_018838	pEGFP-N1-NDUFA12
NDUFA12 (Reverse)	5’- ATCGGAATTCGCTTGTAAGGTGTTGAAGG -3’		

*, Gene identity (ID) number was acquired from the NCBI database: www.ncbi.nlm.nih.gov.

Table S7. Primers for Construction of Mutant Human Mitochondrial CI Subunits with GST/GFP Tag

Name of primer	Sequence	Gene ID*	Name of plasmid
NDUFV1 T383→D (Forward)	5'-CTGTGGCCAGTGTGACCCATGCCGTGAGGG-3’	NM_007103	pEGFPN1- or pGEX 5X-1-NDUFV1T383/D
NDUFV1 T383→D (Reverse)	5’-CCCTCACGGCATGGGTCACACTGGCCACAG-3’		
NDUFV1 T383→A (Forward)	5'-CTGTGGCCAGTGTGCACCATGCCGTGAGGG-3’		pEGFPN1- or pGEX 5X-1-NDUFV1T383/A
NDUFV1 T383→A (Reverse)	5’-CCCTCACGGCATGGTGCACACTGGCCACAG-3’		
NDUFV3 S53→D (Forward)	5’-CAGAATTCCAAGAAGCAAGACCCACCAAA AAAGCCAGCC-3’	NM_001001503	pEGFPN1- or pGEX 5X-1-NDUFV3S53/D
NDUFV3 S53→D (Reverse)	5’-GGCTGGCTTTTTTGGTGGGTCTTGCTTCTTGG AATTCTG-3’		
NDUFV3 S53→A (Forward)	5’-CAGAATTCCAAGAAGCAAGCACCACCAAAA AAGCCAGCC-3’		pEGFPN1- or pGEX 5X-1-NDUFV3S53/A

NDUFV3 (Reverse)	S53→A	5’-GGCTGGCTTTTTTGGTGGTGCTTGCTTCTTGGAATTCTG-3’		
NDUFS2 (Forward)	S364→D	5’-GTTGATGATGCCAAAGTGGACCCACCTAAGCGAGCAGAG-3’	NM_004550	pEGFPN1- or pGEX 5X-1-NDUFS
NDUFS2 (Reverse)	S364→D	5’-CTCTGCTCGCTTAGGTGGGTCCACTTTGGCATCATCAAC-3’		
NDUFS2 (Forward)	S364→A	5’-GTTGATGATGCCAAAGTGGCACCACCTAAGCGAGCAGAG-3’		pEGFPN1- or pGEX 5X-1-NDUFS2S364/A
NDUFS2 (Reverse)	S364→A	5’-CTCTGCTCGCTTAGGTGGTGCCACTTTGGCATCATCAAC-3’		
NDUFB6 (Forward)	S55→D	5’-AAATTTTTGGAGAATAAAGACCCTTGGAGGAAAATGGTC-3’	NM_002493	pEGFPN1- or pGEX5X-1-NDUFB6 S55/D
NDUFB6 (Reverse)	S55→D	5’-GACCATTTTCCTCCAAGGGTCTTTATTCTCCAAAAATTT-3’		
NDUFB6 (Forward)	S55→A	5’-AAATTTTTGGAGAATAAAGCACCTTGGAGGAAAATGGTC-3’		pEGFPN1- or pGEX 5X-1-NDUFB6S55/A
NDUFB6 (Reverse)	S55→A	5’-GACCATTTTCCTCCAAGGTGCTTTATTCTCCAAAAATTT-3’		

NDUFB6 T5→D (Forward)	5’- ATGACGGGGTACGACCCGGATGAGAAA CTGCGGCTGCA -3’		pEGFPN1- or pGEX 5X-1-NDUFB6T5/D S55/D
NDUFB6 T5→D (Reverse)	5’- TGCAGCCGCAGTTTCTCATCCGGGTCGTACC CCGTCAT -3’		
NDUFB6 T5→A (Forward)	5’- ATGACGGGGTACGCACCGGATGAGAAACTG CGGCTGCA -3’		pEGFPN1- or pGEX 5X-1-NDUFB6T5/A S55/A
NDUFB6 T5→A (Reverse)	5’- TGCAGCCGCAGTTTCTCATCCGGTGCGTACC CCGTCAT -3’		
NDUFB6 S29→D (Forward)	5’- AAGGACCAGGAGCTG GACCCTCGGGAGCCG G -3’		pEGFPN1- or pGEX 5X-1-NDUFB6T5/D S29/D, S55/D
NDUFB6 S29→D (Reverse)	5’- CCGGCTCCCGAGGGTCCAGCTCCTGGTCCTT-3’		
NDUFB6 S29→A (Forward)	5’- AAGGACCAGGAGCTG GCA CCTCGGGAGCC GG -3’		pEGFPN1- or pGEX 5X-1-NDUFB6T5/A S29/A, S55/A
NDUFB6 S29→A(Reverse)	5’-CCGGCTCCCGAGGTGCCAGCTCCTGGTCC TT-3’		
NDUFA12T142→D (Forward)	5’- TGGATCCCACCTTCAGACCCTTACAAGTAACT CGAG-3’	NM_018838	pEGFPN1- or pGEX 5X-1-NDUFA12T 142/D

NDUFA12T142→D (Reverse)	5’- CTCGAGTTACTTGTAAGGGTCTGAAGGTGGG AT CCA -3’		
NDUFA12T142→A (Forward)	5’- TGGATCCCACCTTCAGCACCTTACAAGTAACT CGAG-3’		pEGFPN1- or pGEX 5X-1- NDUFA12T 142/A
NDUFA12T142→A (Reverse)	5’- CTCGAGTTACTTGTAAGGTGCTGAAGGTGGG AT CCA -3’		
NDUFA12T120→D (Forward)	5’- CAACGTGACTGGCACCGACGAACAATATGTA CC -3’		pEGFPN1- or pGEX 5X-1- NDUFA12T120/ DT142/D
NDUFA12T120→D (Reverse)	5’- GGTACATATTGTTCGTCGGTGCCAGTCACGT TG -3’		
NDUFA12T120→A (Forward)	5’- CAACGTGACTGGCACCGCAGAACAATATGTA CC -3’		pEGFPN1- or pGEX 5X-1- NDUFA12 T120/ AT142/A
NDUFA12T120→A (Reverse)	5’- GGTACATATTGTTCTGCGGTGCCAGTCACGT TG -3’		

*, Gene identity (ID) number was acquired from the NCBI database: www.ncbi.nlm.nih.gov.

Table S8. SiRNA Sequences

Name of Oligos	Sequence	Gene ID*	Name of SiRNA
CyclinB1 (Sense)	5’- AATTTCTGGAGGGTACATTTCCCTGTCTC-3’	NM_031966	CyclinB1 SiRNA
CyclinB1 (Antisense)	5’- AAGAAATGTACCCTCCAGAAACCTGTCTC-3’		
Cdk1(Sense)	5’- AAGGGGTTCCTAGTACTGCAACCTGTCTC -3’	NM_001786	Cdk1 SiRNA
Cdk1 (Antisense)	5’- AATTGCAGTACTAGGAACCCCCCTGTCTC -3’		
NDUFV1 (Sense)	5’- AACTCGCTTTATTGTCCAGCACCTGTCTC-3’	NM_007103	NDUFV1 SiRNA
NDUFV1(Antisense)	5’- AATGCTGGACAATAAAGCGAGCCTGTCTC-3’		
NDUFV3 (Sense)	5’- AACTTGTGACTCTTGAGTGGACCTGTCTC-3’	NM_001001503	NDUFV3 SiRNA
NDUFV3 (Antisense)	5’- AATCCACTCAAGAGTCACAAGCCTGTCTC-3’		
NDUFB6 (Sense)	5’- AAAAACTTAGGCTCATAAGCCCCTGTCTC-3’	NM_002493	NDUFB6 SiRNA
NDUFB6 (Antisense)	5’- AAGGCTTATGAGCCTAAGTTTCCTGTCTC-3’		
NDUFA12 (Sense)	5’- AATTGCATGTTTCAACTGTTCCCTGTCTC-3’	NM_018838	NDUFA12 SiRNA
NDUFA12 (Antisense)	5’- AAGAACAGTTGAAACATGCAACCTGTCTC-3’		
NDUFS2 (Sense)	5’- AACTTCTTGGCTTCTCACCTACCTGTCTC-3’	NM_004550	NDUFS2 SiRNA
NDUFS2 (Antisense)	5’- AATAGGTGAGAAGCCAAGAAGCCTGTCTC-3’		

*, Gene identity (ID) number was acquired from the NCBI database: www.ncbi.nlm.nih.gov.

Table S9. Estimation of Durations of Cell Cycle Phases in MCF-10A Cells Expressing Mitochondrial-targeted CyclinB1/Cdk1

Plasmids \ Time (h)	T_{G1}	T_S	T_{G2+M}	T_C
pEGFPN1-MTS/pEGRFPN1-MTS	11.2 ± 0.39	10.2 ± 0.34	5.9 ± 0.15	27.3 ± 0.59
pEGFPN1-MTS-CycB1/pERFPN1-MTS-Cdk1-wt	6.1 ± 0.16**	7.9 ± 0.25**	4.1 ± 0.13*	18.1 ± 0.65**
pERFPN1-MTS-Cdk1-dn	13.1 ± 0.29*	10.6 ± 0.23	6.5 ± 0.12	30.2 ± 0.66*

Wt, wild type; dn, dominant negative mutant; $T_{G1,}$ G1 phase time; T_S, S phase time; T_{G2+M}, G2 and M phase time; and T_C , cell cycle time. *p < 0.05, **p < 0.01.

Supplemental Experimental Procedures

Reagents

[γ-32P] ATP (6000 Ci/mmol) was purchased from Perkin Elmer; Histone H1 was from EMB Biosciences; CDC2-Cyclin B1 kinase was from New England BioLabs. Silencer siRNA construction kit and the scramble siRNA duplex were from Ambion. Mito Tracker Red, Mito Tracker Green, Goat anti-mouse Alexa Fluor 488, Goat anti-rabbit Rhodamine, ATP determination kit, MitoSOX red, propidium iodide, and Pro-Q diamond phosphoproteins gel stain solution were purchased from Molecular Probes. Protease inhibitor, Glutathione-sepharose 4B bead, phosphatase inhibitor cocktail, DNase-free RNase A, and mitochondria isolation kit were from Thermo Scientific. JC-1 (5, 5', 6, 6'-tetrachloro-1, 1', 3, 3'- tetraethylbenzimida- zolyl carbocyanine iodide), Trizol reagent and transfection reagents were from Invitrogen. Reagents of two-dimensional (2D) gel electrophoresis were from GE Healthcare. Protein A/G PLUS-Agarose was from Abcam. Ubiquinone, antimycin A, and soybean Trypsin were from Sigma. Pfu Turbo DNA polymerase was from Stratagene. AMV reverse transcriptase was from Promega. Cdk1 selective inhibitor, RO-3306, was from Enzo Life Sciences International. EdU (5-ethynyl-2′-deoxyuridine) was from Invitrogen.

Antibodies

Antibodies against CyclinB1, Cdk1, α-tubulin, NDUFV1 (H-23), Hsp60, TOM 40, NDUFV3 (D-16), and GFP were from Santa Cruz. COX IV, NDUFB6, NDUFS2, NDUFA12, Giantin, Calnexin was from Abcam. Antibody against β-actin was from Sigma. The phophoserine and phosphothreonine antibodies were from Cell signaling/EMD Millipore. The Timm13 antibody was from Proteintech Group Inc. Secondary antibody of goat anti-rabbit IgG conjugated with 10 nm or 20 nm of gold particles was from Ted Pella.

Cell Lines

The human breast epithelial cell line MCF10A (Gajewski et al., 2007) was obtained from American Type Culture Collection (ATCC) and maintained in DMEM supplemented with 5% horse serum, 20 ng/ml of EGF, 0.5 µg/ml of Hydrocortisone, 100 ng/ml of Cholera Toxin, 10 µg/ml of Insulin. The breast cancer cell line MDA-MB-231 (ATCC) was maintained in EMEM with 10% of FBS and 0.1 mM of nonessential amino acid (NEAA). The MCF7 breast cancer cell line (ATCC) was maintained in EMEM medium with 5% FBS, 0.1 mM NEAA and 1 mM Sodium Pyruvate. The human skin keratinocytes HK18 cells (Chen et al., 2002) were maintained in DMEM medium with 10% FBS. The mouse skin epithelial JB6 cells (Li et al., 1996) were maintained in EMEM medium with 5% FBS.

Immunoelectron Microscopy

Cells were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer. Preservation and contrast were enhanced by incubating for 30 min in 0.1% tannic acid in phosphate buffer followed by an ethanol dehydration series with 4% uranyl acetate in 70% ethanol for 1 h during the series. Cells were embedded in LR white using a clinical microwave for polymerization. Sections were picked up on gold coated grids. The grids were blocked, incubated for 2 h with anti-Cdk1, and then overnight with anti-CyclinB1 antibody. Secondary labeling was done with anti-mouse 10 nm gold-conjugated and anti-rabbit 20 nm gold-conjugated antibody. The grids were stained in uranyl acetate and lead citrate before viewing (Tait et al., 2000). The images were acquired using transmission microscope (Philips CM120, Biotwin Lens, model 794/20, digital camera of 2K × 2K).

Co-immunoprecipitation (Co-IP)

After various treatments, mitochondrial proteins (100-150 μg) were pre-cleared using normal mouse or rabbit IgG and Protein A/G PLUS-Agarose. The pre-cleaned supernatants were incubated with primary antibodies overnight at 4°C with shaking. The reaction was further incubated with Protein A/G PLUS-Agarose for 3 h at 4°C. The immunoprecipitates were rinsed three times with washing buffer (1 M NaCl, 1% NP-40, 50 mM Tris–HCl, pH 8.0) and analyzed by immunoblotting analysis. Normal IgG was used as the negative control for immuno-precipitation.

CyclinB1/Cdk1 *in vitro* Kinase Assay

In vitro CyclinB1/Cdk1 kinase assay was performed according to the established protocols (Atherton-Fessler et al., 1993; Pan et al., 1993; Pan and Hurwitz, 1993). Equal amounts of mitochondrial proteins were immunoprecipitated from synchronized cells at indicated releasing time point by 2 μg of Cdk1 antibody. The immuno-captured mitochondrial CyclinB1/Cdk1 kinase was incubated with 10 μM Histone H1 in 60 μl of kinase reaction buffer (50 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM EGTA, 2 mM DTT, 0.01% Brij 35, pH 7.5) supplemented with 100 μM of cold ATP and 100 μCi/μmol of [γ-^{32}P] ATP at 30°C for 2 h. Commercial CyclinB1/Cdk1 kinase was included as the positive control. Samples were separated via SDS-PAGE and visualized by autoradiography.

Construction and Transfection of Mitochondrial-targeted CyclinB1 and Cdk1 Plasmids

The pEGFP-N1-MTS-CyclinB1 and pERFP-N1-MTS-Cdk1-wt (Table S7) plasmids were constructed by in frame insertion of an 87 base MTS (mitochondria targeting sequence) fragment (Rizzuto et al., 1995) before CyclinB1 or Cdk1 start codons. The pERFP-N1-MTS-Cdk1-dn (changing D146 to N) plasmid (van den Heuvel and Harlow, 1993) was generated by Pfu Turbo

DNA polymerase from a mutagenesis kit (Stratagene) and plasmid pERFP-N1-MTS-Cdk1-wt was used as template. Transfection was conducted using Lipofectamine 2000. Subsequent analysis, including mitochondrial isolation, mitochondrial function and cell cycle distribution, were performed 48 h after transfection. High resolution fluorescence images were acquired with a Personal DV microscope (Applied Precision Inc, a GE Healthcare Company, Issaquah, WA). The raw fluorescence images were deconvoluted with a proprietary software package (SoftWoRx v5.0, Applied Precision Inc., a GE Healthcare Company, Issaquah, WA) to enhance the resolutions.

Protein Identification with Mass Spectrometry Analysis (MALDI-TOF/TOF)

Significant protein spots determined by 2-D DeCyder analysis were excised, de-stained and subjected to in-gel trypsin digestion, followed by multiple trifluoroacetic acid extractions. The tryptic peptides were analyzed by MALDI-TOF-MS/MS and the MS and MS/MS spectra were searched against the International Protein Index (IPI) human database using GPS Explorer™ Version 3.0 and MASCOT 2.0. Proteins with protein scores greater than 59 ($P < 0.05$) and individual ions scores greater than 21 ($P < 0.05$) were identified as significant (Chen et al., 2008).

Construction of GST- and GFP-tagged Mitochondrial CI Subunit Plasmids

Human mitochondria CI subunits cDNAs generated by RT-PCR were cloned into pGEX-5X-1 (Amersham Biosciences) or pEGFP-N1 (Clontech) vectors. All primers used in the cloning were shown in Table S5 and S6. Mutant CI subunits in which the Serine or Threonine (S or T) residues in Serine/Threonine-Proline (S/T-P) sequences were changed to Alanine (A, phosphorylation-defective) or Aspartate (D, phosphorylation-mimic) were generated by Pfu Turbo DNA polymerase from a mutagenesis kit (Stratagene; primers are shown in Table S7). For

selection of stable pEGFP-N1-CI subunits transfectants, 800 μg/mL of G418 was added to the culture medium.

Expression and Purification of GST-tagged Proteins

Escherichia coli BL-21 containing GST-tagged CI subunit was cultured in *Luria-Bertani* (LB) broth with 50 μg/ml of ampicillin until an optical density (OD) of 0.6 was reached. To induce fusion protein production, 0.1 mM isopropyl-*b*-D-thio-galactopyranoside (IPTG) was added and incubated for an additional 3 h. The induced culture was collected and suspended in PBS buffer containing 5 mM of dithiothreitol, proteinase inhibitor cocktail, and 0.1% lysozyme. Cells were lysed by sonication, cell debris was removed by centrifugation and whole cell protein extract was collected in the supernatant. This protein extract was incubated with glutathione-sepharose 4B beads at a volume ratio of 4:1 (supernatant: bead) for 1 h. The GST-fusion proteins were eluted from the beads with glutathione elution buffer (10 mM reduced glutathione in 50 mM Tris-HCl, pH 8.0) and the eluted proteins were subjected to dialysis in molecular porous membrane tubing (Spectrum labs) with 1×PBS /1 mM EDTA. The purified protein was analyzed by SDS-PAGE and immunoblotting.

Gene Silencing with siRNA

SiRNAs were synthesized using the siRNA construction kit from Ambion. The siRNAs against CyclinB1 and Cdk1 were designed to target their coding regions. The siRNAs against CI subunits, *i.e.*, NDUFV1, NDUFV3, NDUFS2, NDUFB6 and NDUFA12, were designed to target a region on their 5'- or 3'-UTR's that results in the specific inhibition of endogenous CI subunit RNA's, without affecting exogenous transcripts (Supplementary Table 8). Lipofectamine^{TM} RNAi MAX and 10-30 nM of siRNA were used for transfection following the manufacturer's

instructions. After 6 h, cells were released from G0/G1 synchronization, and were transfected with CyclinB1 and Cdk1 siRNA for 26 h.

Identification of Intramitochondrial Localization of CyclinB1/Cdk1

To distinguish between mitochondrial membrane protein, soluble matrix and intermembrane space protein, an alkaline extraction was performed as previously reported (Lu et al., 2007). Briefly, the prepared mitochondrial fraction was incubated in 0.1 M Na_2CO_3 (pH 11) for 20 min at 4^{o}C. The membrane was then pelleted by centrifugation at 100,000 × g. Proteins in the supernatants were precipitated using a final volume of 10% trichloroacetic acid (TCA). The pellets were resuspended in dissolving buffer with pH 8.5 (7 M urea, 3 M thiourea, 2% CHAPS, 30 mM Tris) and analyzed by immunoblotting. Mitoplasting was performed by diluting mitochondria in hypotonic sucrose buffers (1 mM EDTA, 10 mM MOPS-KOH, pH 7.2; sucrose concentration ranging from 25 mM to 200 mM) on ice for 30 min with or without 50 ug/ml soybean trypsin. Proteolysis was stopped by adding with 1 mM PMSF. The mitoplasts were separated by centrifugation at 16,000 × g at 4°C for 10 min. Pellets were resuspended in mitochondria-buffer and all supernatant fractions were precipitated by 10% TCA and resuspended in dissolving buffer. All fractions were analyzed by immunoblotting.

Double Staining Immunofluorescence Analysis

Cells growing on poly-L-Lysine coated glass cover slips were fixed in 4% paraformaldehyde (pH 7.4) for 15 min at room temperature. Following the general procedure including permeabilization and blocking, the slides were co-incubated with antibodies of both Cdk1 (mAb, mouse antibody) and COX IV (rAb rabbit antibody), or co-incubated with antibodies of both CyclinB1 (mAb, mouse antibody) and COX IV overnight at 4°C. After three times washing with PBS, the slides were incubated with the mixture of two secondary antibodies, *i.e.*, anti-mouse-

Alexa Fluor 488 and anti-rabbit-Rhodamin Red in dark for 1 h. Super-resolution fluorescence images were acquired using a DeltaVision OMX V3.0 Blaze system (Applied Precision Inc, a GE Healthcare Company).

Three-dimensional (3D) Structured-illumination Microscopy (SIM)

Three-dimensional (3D) super-resolution fluorescence images and movies were acquired with a commercial model of structured-illumination microscopy: a DeltaVision OMX V3.0 Blaze system (Applied Precision Inc, a GE Healthcare Company) that has been described in details previously (Kner et al., 2009). Briefly the system uses 488 and 532 nm lasers as the light source for illumination. A grating in the beam path is used to generate three coherent beams that create three dimensional structured illumination patterns in the sample. Fluorescence emission is collected with a 60 × 1.42NA objective immersed with immersion oil (1.514 index). Fluorescence of different colors are separated by a dichroic mirror and filtered by filters before being collected by two scientific fast sCMOS cameras (PCO-TECH Inc.). To acquire 3D images, the sample is moved along the *z*- direction at a step size of 125 nm. For each slice, the illumination pattern is rotated three times and shifted five times, resulting in a total of 15 exposures per channel.

Three-dimensional (3D) Image Acquisition and Data Processing

Acquired raw images were processed with a proprietary software package (SoftWoRx v5.0, Applied Precision Inc.) to reconstruct super-resolution 3-D images. Reconstructed images of different colors are then registered using a build in software to correct chromatic aberrations and image distortions. Prior to experiment, the system and the color registration software had been calibrated using multi-color polymeric beads of 0.1 micron (tetra-spectra beads, Molecular

Probes). The resolution of the system is found to be ~ 110 nm for *x*-, *y*- and ~ 250 nm for *z*-directions. The color registration error is smaller than a pixel, *i.e.*, 40 nm. Colocalization of proteins was calculated using a commercial software package (Volocity with Quantitation, Perkin–Elmer) with automated threshold settings. Three-dimensional rendering was performed using Volocity + Visualization package.

Deconvolved Fluorescence Image Acquisition

Deconvolved fluorescence images were acquired with a PersonalDV microscope (Applied Precision Inc.). An oil immersion objective of 60 × 1.42NA was used with 1.514 index immersion oil to collect fluorescence emitted from the sample that is excited by a xenon lamp. The raw fluorescence images were then deconvolved with SoftWoRx v5.0 (Applied Precision Inc.) to enhance the image resolution. The sample was moved along the *z*- direction at a step size of 400 nm to collect images at different planes (Kner et al., 2009).

Semiquantitative RT-PCR

Total RNA was isolated using Trizol reagent and subjected to DNase I treatment to eliminate genomic DNA contamination. Synthesis of cDNAs was carried out with the AMV reverse transcriptase, followed by 30 cycles of PCR reactions. Primers for each CI subunit were as same as those used in construction of the pEGFP-N1-subunit plasmids shown in Table S5. The primers of internal control (human GAPDH) were: 5’-GGACTCATGACCACAGTCCAT-3’ (forward) and 5’-GTTCAGCTCAGGGATGACCTT-3’ (reverse).

Cell Cycle Length Determination

Asynchronous MCF-10A cells were transfected with plasmids as indicated in the legend of Figure 7 for 48 h. For in vitro pulse-labeling with EdU, we added EdU at a final concentration of

20 µM to the cells and incubated for 1 h at 37°C to pulse label the cells. At indicated time points after the EdU pulse, the cells were harvested and fixed in ice-cold 70% ethanol at 4°C overnight. Then the cells were centrifuged and re-suspended in approximately 50-100 µL of 70% ethanol. 1 mL of 2N HCl/Triton X-100 was slowly added to denature the DNA at room temperature for 30 minutes. Then the cells were centrifuged and re-suspended in 1 mL of 0.1 M $Na_2B_4O_7$, pH 8.5 to neutralize the sample, followed by centrifugation and re-suspension in 1 mL of PBS containing 5 µg/mL propidium iodide. The samples were then analyzed by flow cytometry (FACScan, BD Biosciences Immunocytometry Systems). Data analysis was performed with ANALYSIS MODFIT LT 2.0 software (Verity Software, Topsham, ME). Cell-cycle times were determined as described (Sasaki et al., 1987).

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