Figure S1. Mitochondrial dysfunction causes CDDP-resistance in A549 cells. (A) A scheme showing the methods for generating of ρ A549 cells. (B) Number of mtDNA copies was determined by qPCR of MT-COI DNA relative to nuclear β-actin DNA in EtBr-treated A549 cells. (C) Cells were incubated in culture medium containing various concentrations of CDDP for 72 h. Cell viability was measured using crystal violet assay. Each value represents mean ± SE (n=4). Unpaired t-test.; **P<0.01. CDDP, cisplatin; mtDNA, mitochondrial DNA.

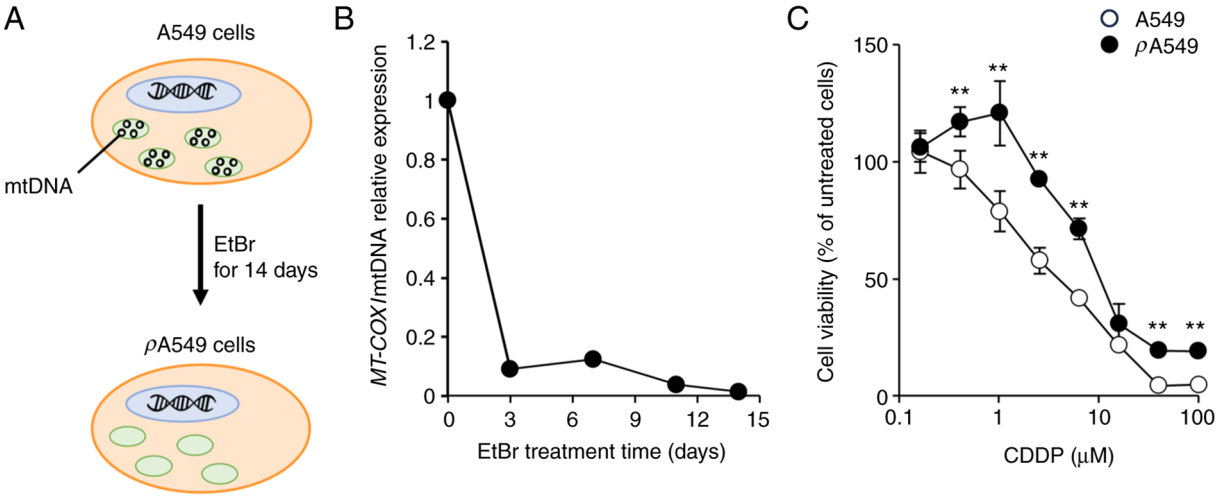

Figure S2. ROS levels in A549 and ACR20 cells after treatment with CDDP. Cells were treated with 20 μM CDDP for 24 h, washed with PBS and incubated with CM-H_2DCFDA for 30 min. Green fluorescence was observed by fluorescence microscopy (EVOS® FL). Representative images under the fluorescence microscope are shown. ROS, reactive oxygen species; CDDP, cisplatin.

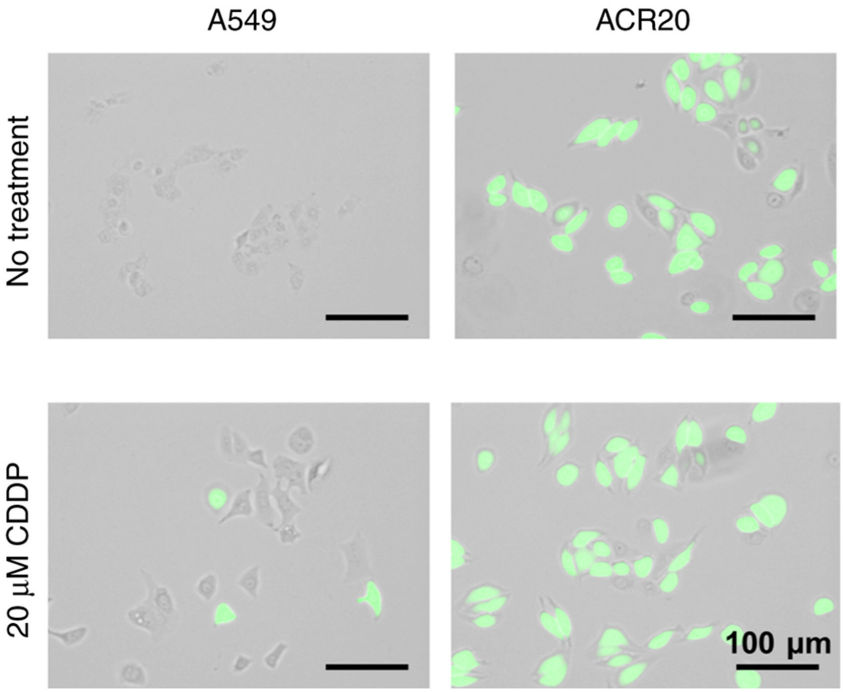

Figure S3. Four identified mtDNA mutations with varying percentage levels in ACR20 cells. Gel electrophoresis, showing PCR-RFLP results after digestion with restriction enzymes on A549 and ACR20 cells. (A) 4587T>C mutation: -4587T allele contained a *Dra*I restriction enzyme cutting site, but the -4587C allele did not contain a *Dra*I restriction enzyme cutting site. A total of 144 bp segments were detected in only A549 cells, while 173 bp segments were detected in A549 and ACR20 cells. (B) 11384C>A mutation: -11384C allele contained an *Mnl*I restriction enzyme cutting site, but the -11384A allele did not contain an *Mnl*I restriction enzyme cutting site. A total of 203 and 99 bp segments were detected in only A549 cells, 302 bp segments were detected in A549 and ACR20 cells. (C) 13148C>A mutation: -13148A allele contained a *Bal*I restriction enzyme cutting site, but the -13148C allele did not contain a *Bal*I restriction enzyme cutting site. A total of 187 bp segments were detected in only ACR20 cells, while the 214 bp segment was detected in A549 and ACR20 cells. (D) 14612G>T mutation: -14612T allele contained a *Psi*I restriction enzyme cutting site, but the -14612A allele did not contain a *Psi*I restriction enzyme cutting site. A total of 141 and 159 bp segments were detected in only ACR20^{cyb} cells, while 300-bp segments were detected in A549^{cyb} cells. mtDNA, mitochondrial DNA.

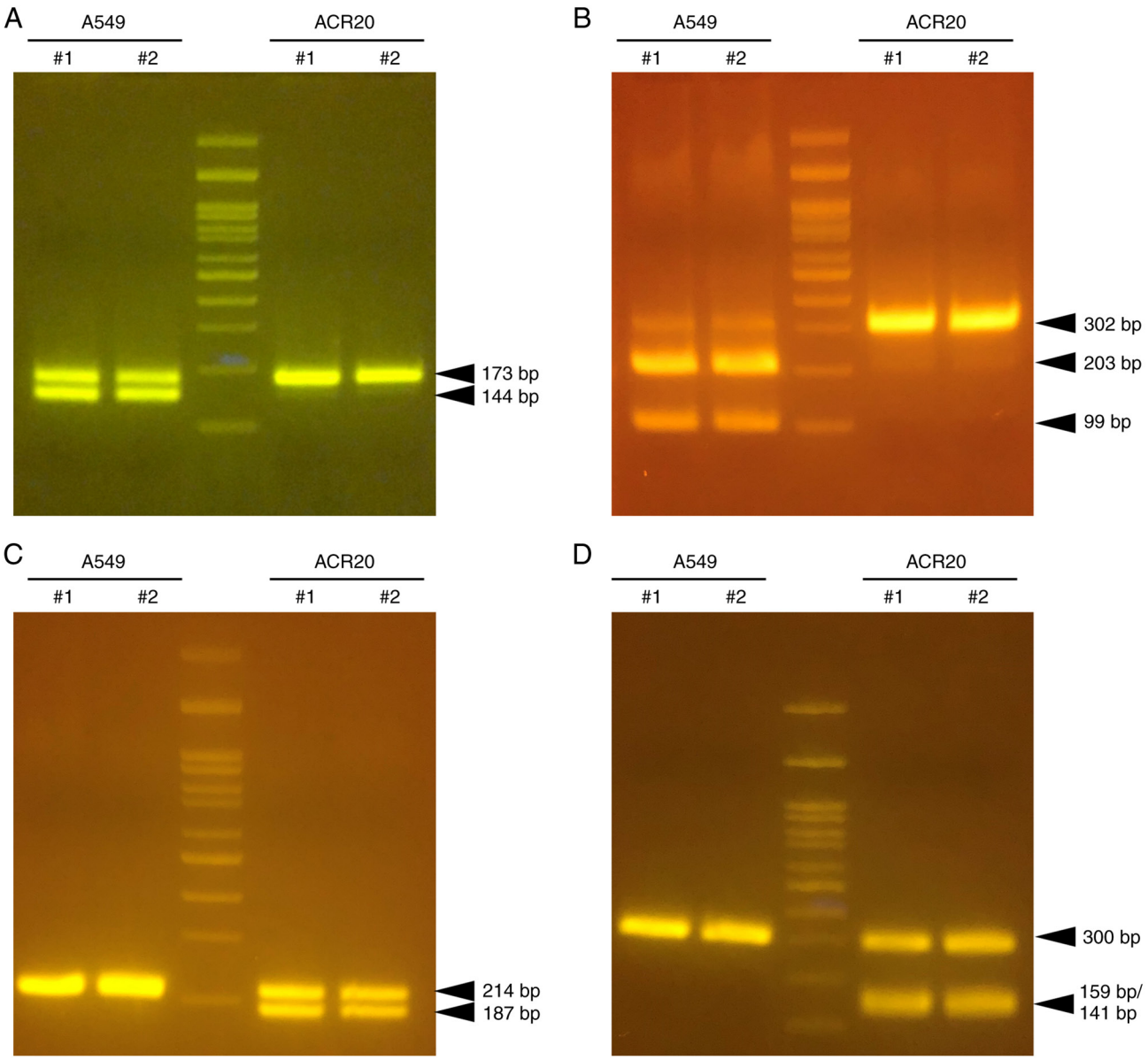

Figure S4. A549^{cyb} and ACR20^{cyb} cells incorporate mtDNA derived from A549 or ACR20 cells, respectively. Gel electrophoresis, showing PCR-RFLP results after digestion with the restriction enzyme *Acl*I (10044A>G) and *Psi*I (14612G>T) on A549^{cyb} and ACR20^{cyb} cells. (A) 10044A>G mutation: -10044G allele contained an *Acl*I restriction enzyme cutting site, but the -10044A allele did not contain an *Acl*I restriction enzyme cutting site. A total of 209 and 263 bp segments were detected in A549^{cyb} and ACR20^{cyb} cells. (B) 14612G>T mutation: -14612T allele contained a *Psi*I restriction enzyme cutting site, but the -14612A allele did not contain a *Psi*I restriction enzyme cutting site. A total of 141 and 159 bp segments were detected in only ACR20^{cyb} cells, while 300 bp segments were detected in A549^{cyb} cells. mtDNA, mitochondrial DNA. The numbers of the lanes here represent the clone number, respectively. M, molecular weight.

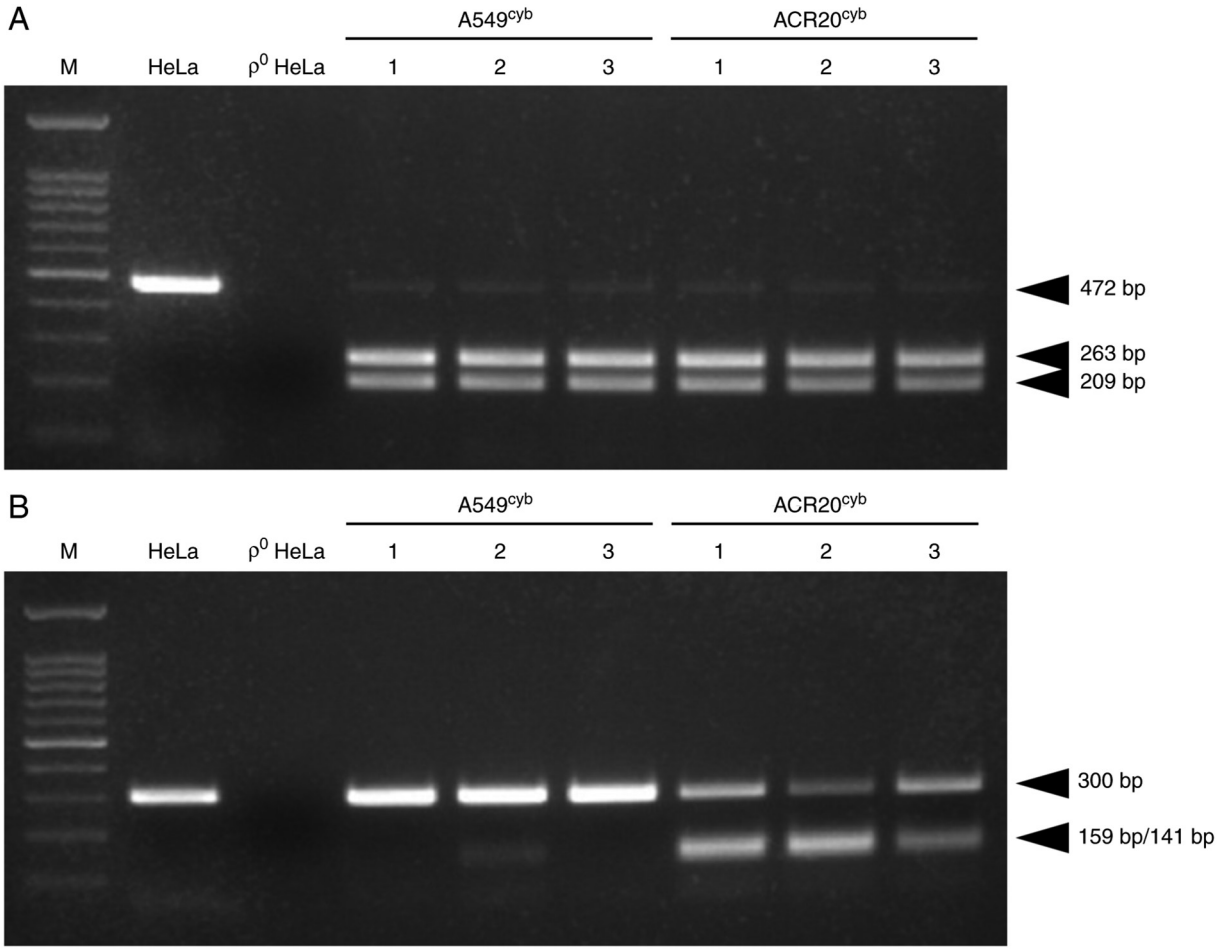

Table SI. Primary antibodies used in western blotting.

Primary antibody	Commercial source	Catalog no.	RRID	Working concentration
Caspase-3 (D3R6Y) rabbit monoclonal antibody (mAb)	Cell Signaling	14220	AB_2798429	1:500
IκBα (L35A5) mouse mAb	Cell Signaling	4814	AB_390781	1:1,000
Phospho-IκBα (Ser32) (14D4) rabbit mAb	Cell Signaling	2859	AB_561111	1:1,000
NF-κB p65 (D14E12) XP® rabbit mAb	Cell Signaling	8242	AB_10859369	1:1,000
Phospho-NF-κB p65 (Ser536) (93H1) rabbit mAb	Cell Signaling	3033	AB_331284	1:1,000
c-IAP1 (D5G9) rabbit mAb	Cell Signaling	7065	AB_10890862	1:1,000
c-IAP2 (58C7) rabbit mAb	Cell Signaling	3130	AB_10693298	1:1,000
XIAP (3B6) rabbit mAb	Cell Signaling	2045	AB_2214866	1:5,000
NDUFB8 rabbit mAb	Abcam	ab192878	AB_2847808	1:5,000
GAPDH (D16H11) XP® rabbit mAb	Cell Signaling	5174	AB_10622025	1:5,000

Table SII. Primer sequences for mtDNA sequencing.

No.	Primer Seq 5'-3'		Position	Product size (bp)
	Forward	Reverse		
1	CACCCTATTAACCACTCACG	TGAGATTAGTAGTATGGGAG	15-484	470
2	ACAAAGAACCCTAACACCAGC	ACTTGGGTTAATCGTGTGACC	391-921	561
3	CATCAAGCACGCAGCAATG	AATCCACCTTCGACCCTTAAG	756-1425	670
4	CTCACCACCTCTTGCTCAGC	GCCAGGTTTCAATTTCTATCG	1234-1769	536
5	CTTGACCGCTCTGAGCTAAAC	TGTTGAGCTTGAACGCTTTC	1657-2216	560
6	GAGGAACAGCTCTTTGGACAC	AGAGACAGCTGAACCCTCGTG	2105-2660	556
7	CACTGTCAACCCAACACAGG	ATGTCCTGATCCAACATCGAG	2417-3006	590
8	CCCAACCTCCGAGCAGTACATG	AGAAGAGCGATGGTGAGAGC	2834-3557	724
9	ACTACAACCCTTCGCTGACG	TGAAGCCTGAGACTAGTTCGG	3441-3940	500
10	GCCTAGCCGTTTACTCAATCC	TGAGTTGGTCGTAGCGGAATC	3636-4162	527
11	TCAGGCTTCAACATCGAATACG	TTATGGTTCATTGTCCGGAGAG	3931-4728	798
12	ATGAGAATCGAACCCATCCC	TAAGATTTTGCGTAGCTGGGT	4337-5006	670
13	GACATCCGGCCTGCTTCTT	GAGTGGGGTTTTGCAGTCC	4841-5608	768
14	CACCATCACCCTCCTTAACC	GCTGAGTGAAGCATTGGACTG	5318-5884	567
15	TAAGCACCCTAATCAACTGGC	GCCTCCACTATAGCAGATGCG	5700-6262	563
16	TCTAAGCCTCCTTATTCGAGC	ATAGTGATGCCAGCAGCTAGG	5999-6526	528
17	GCCATAACCCAATACCAAACG	TGGGCTACAACGTAGTACGTG	6426-7030	605
18	GGCTTCCTAGGGTTTATCGTG	TTTCATGTGGTGTATGCATCG	6744-7255	512
19	GAGGCTTCATTCACTGATTTCC	GGGCAGGATAGTTCAGACGG	7075-7792	718
20	TATCACCTTTCATGATCACGC	GACGATGGGCATGAAACTG	7645-8215	571
21	CGGTCAATGCTCTGAAATCTGTG	CATTGTTGGGTGGTGATTAGTCG	8164-8669	506
22	CTGTTCGCTTCATTCATTGCC	GTGGCGCTTCCAATTAGGTG	8539-9059	521
23	CCCACTTCTTACCACAAGGC	GTGCTTTCTCGTGTTACATCG	8903-9403	501
24	TTTCACTTCCACTCCATAACGC	GAAAGTTGAGCCAATAATGACG	9309-9848	540
25	AGTCTCCCTTCACCATTTCCG	AAAGGAGGGCAATTTCTAGATC	9754-10275	522
26	CCATCTATTGATGAGGGTCTT	ATAATTAGGCTGTGGGTGGTT	9970-10860	891
27	CTAGTATATCGCTCACACCTCA	GCTTCGACATGGGCTTTAGGGA	10527-11428	902
28	CTACTCACTCTCACTGCCCAAG	GGGAATTAGGGAAGTCAGGGT	11291-12388	1098
29	CTCACAAGAACTGCTAACTCATGCC	CTAGTAGTGGGGTGAGGCTTGGATTA	12224-12961	738
30	CATCGGCTGAGAGGGCGTAGGAAT	GAGAGTAATAGATAGGGCTCAGGCG	12759-13575	817
31	CTCCGGGTCCATCATCCACAACCT	GATCCTATTGGTGCGGGGGCTTTG	13362-14262	901
32	CAATTTCACAGCACCAAATCTCCA	TATTAGGGGGTTAGTTTTGCG	14021-14779	759
33	ACTAAACCCACACTCAACAGAAAC	GTCATTAAGGAGAGAAGG	14636-15421	786
34	ATACATTGGGACAGACCTAGTTC	CTTTGGGTGCTAATGGTGGAGT	15208-15995	788
35	CGCCTACACAATTCTCCGATC	CGGTTGTTGATGGGTGAGTC	15574-16084	511
36	TTAACTCCACCATTAGCACC	GCGAGGAGAGTAGCACTCTTG	15971-16451	481
37	TACATTACTGCCAGCCACCATG	TTAAGTGCTGTGGCCAGAAG	16097-336	746

Table SIII. Primer sequence restriction enzymes for PCR-RFLP.

Mutation	Primer Seq 5'-3'		Restriction enzyme	Annealing temperature
	Forward	Reverse		
4587T>C	GTTGGTTATACCCTTCCCGTACTA ATT	GGGTTTATTTTTTTGGTTAGAACT GGTTTA	DraI	64°C
10044A>G	ATTGGCTCAACTTTCCTCACTATC	TAGTTGTTTGTAGGGCTCATGGTA	AclI	58°C
11384C>A	AACGCAGCACATACTTCCTATTC	ATTATACCATAGCCGCCTAGTTTT	MnlI	61°C
13148C>A	CTTCCACCCCCTAGCAGAAAATGG	GTGGGTACAGATGTGCAGGAATGC	BalI	69°C
14612G>T	GCCATCGCTGTAGTATATCCAAAG	GGTCATTGGTGTTCTTGTAGTTGA	PsiI	58°C

Table SIV. Primer sequences for PCR.

Gene	Primer Seq 5'-3'	
	Forward	Reverse
COX1	TTCGCCGACCGTTGACTATTCTCT	AAGATTATTACAAATGCATGGGC
TUBA1A	TGAGCAACACCACAGCCATT	CTTCCCTGTAAAAGCAGCACCT
TFAM	CGCTCCCCCTTCAGTTTTGT	CCAACGCTGGGCAATTCTTC
POLRMT	GGGACCATCGAAAGGTGTCT	CTTCAGAACAGTGGCCCGAT
NDUFB8	ATGGCGACTACCCGAAGCTC	GGATGTATCCACACGGTTCCTG
BCL2	TCCGCATCAGGAAGGCTAGA	AGGACCAGGCCTCCAAGCT
BIRC5	TCCACTGCCCCACTGAGAAC	TGGCTCCCAGCCTTCCA
GAPDH	TTCTTTTGCGTCGCCAGCCGA	GTGACCAGGCGCCCAATACGA