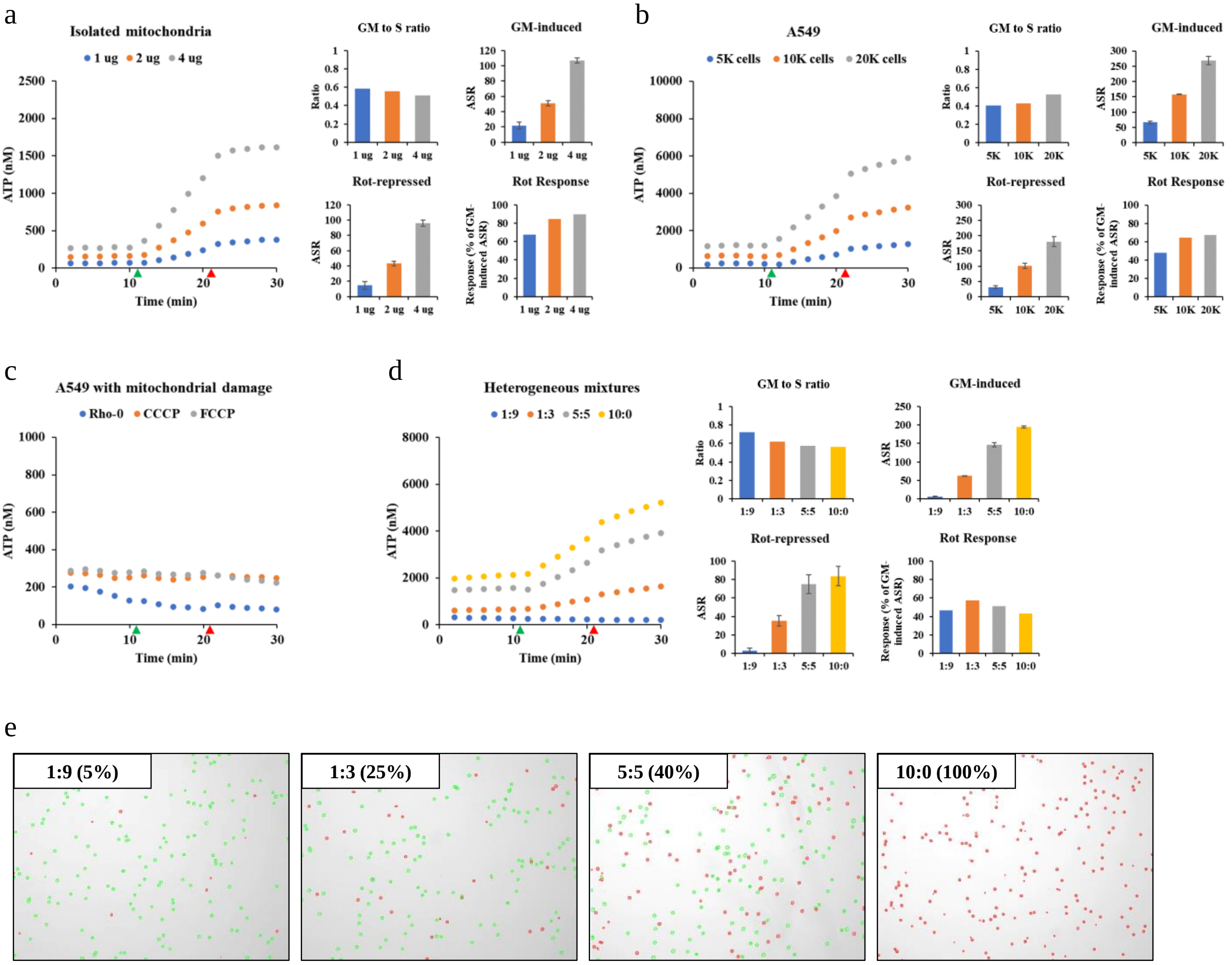
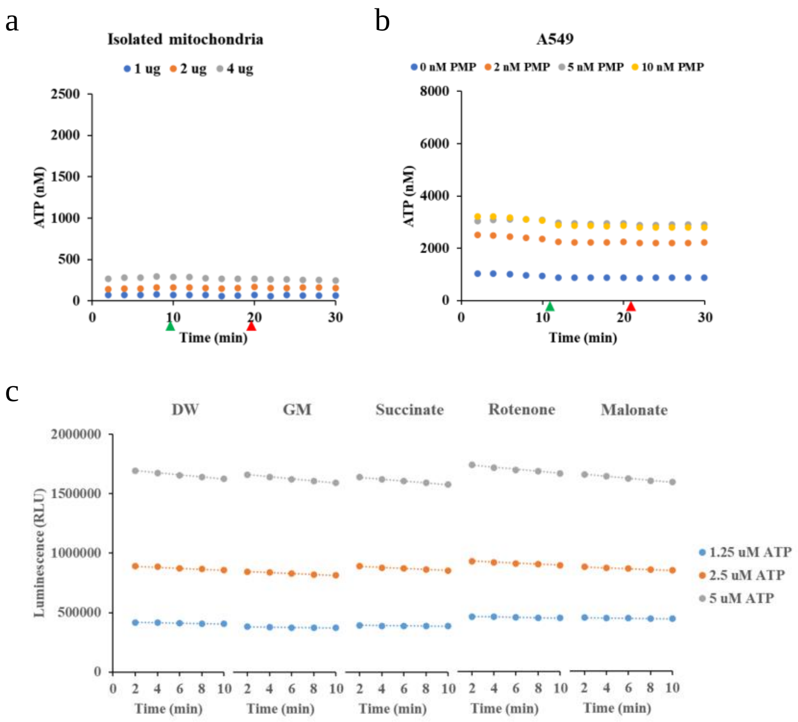
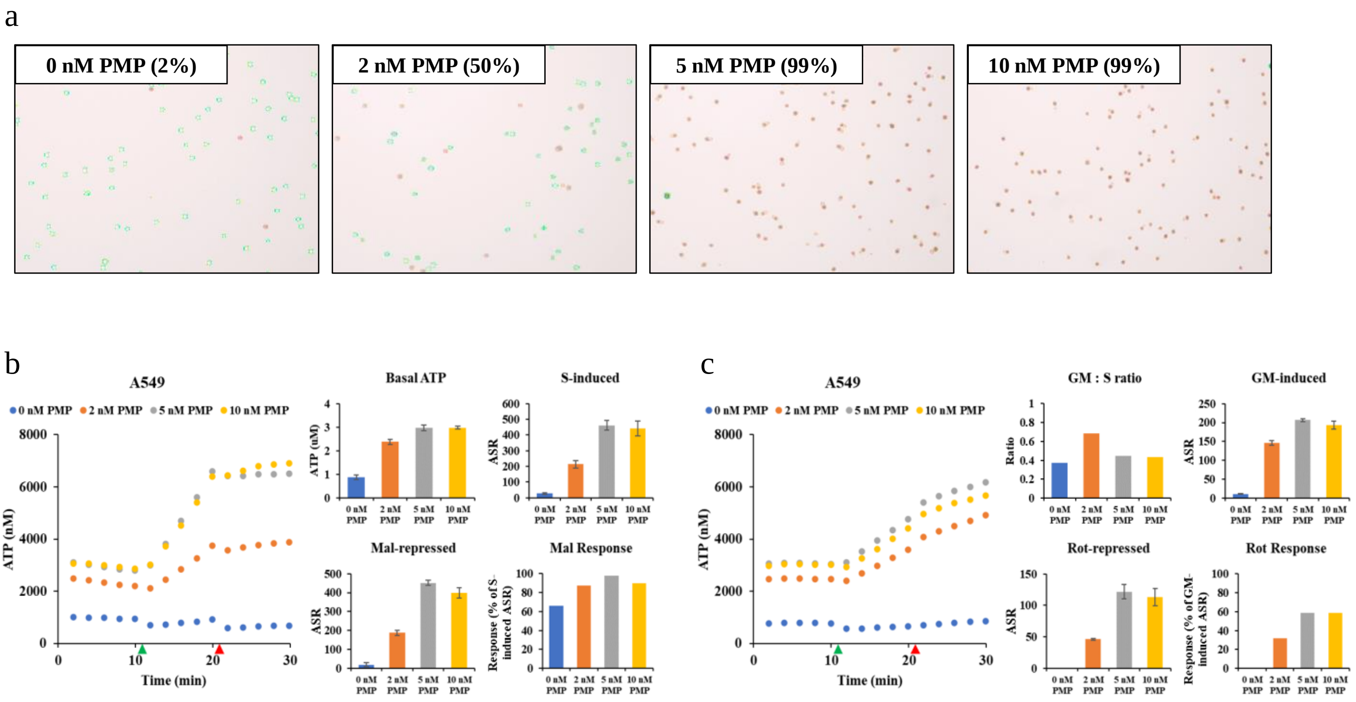


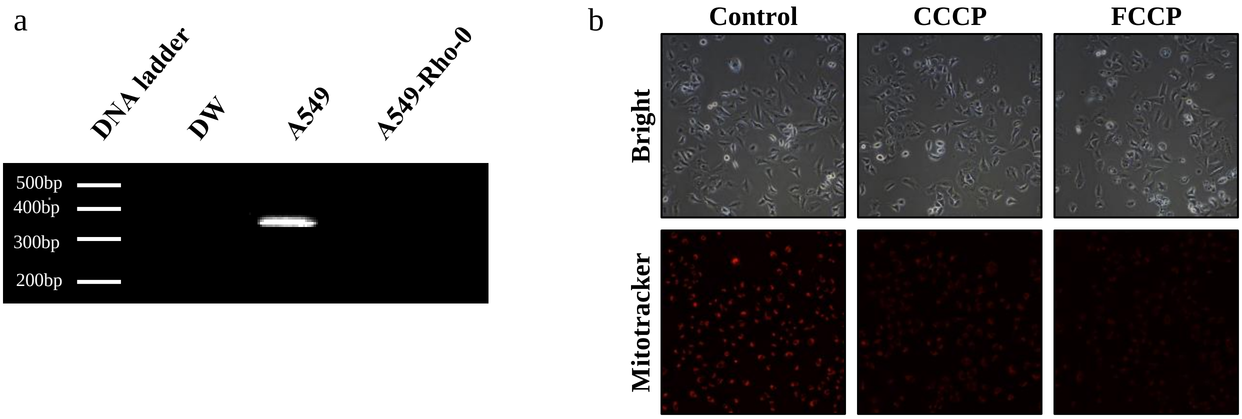
Supplementary Fig. 1. Validation of MitoRAISE assay using isolated mitochondria and viable cells. Dot plots of the real-time accumulation of ATP level from (a) isolated mitochondria, (b) varying cell numbers, (c) cells with mitochondrial damage, and (d) heterogeneous mixtures. Bar graphs of the change in slope of relative ATP synthesis rate after glutamic acid and malic acid injection (GM-induced), the change in slope of relative ATP synthesis rate after rotenone injection (Rot-repressed), the Rot-repressed of GM-induced ratio (Rot Response), and the GM-induced by S-induced (GM to S ratio). The green triangle indicates the time at which glutamic acid and malic acid was injected, while the red triangle indicates the time at which rotenone was injected. (e) Represent images of trypan blue staining of heterogeneous mixtures. Green cells represent cells with intact cellular membranes, while red cells represent cells with permeabilized cellular membranes.



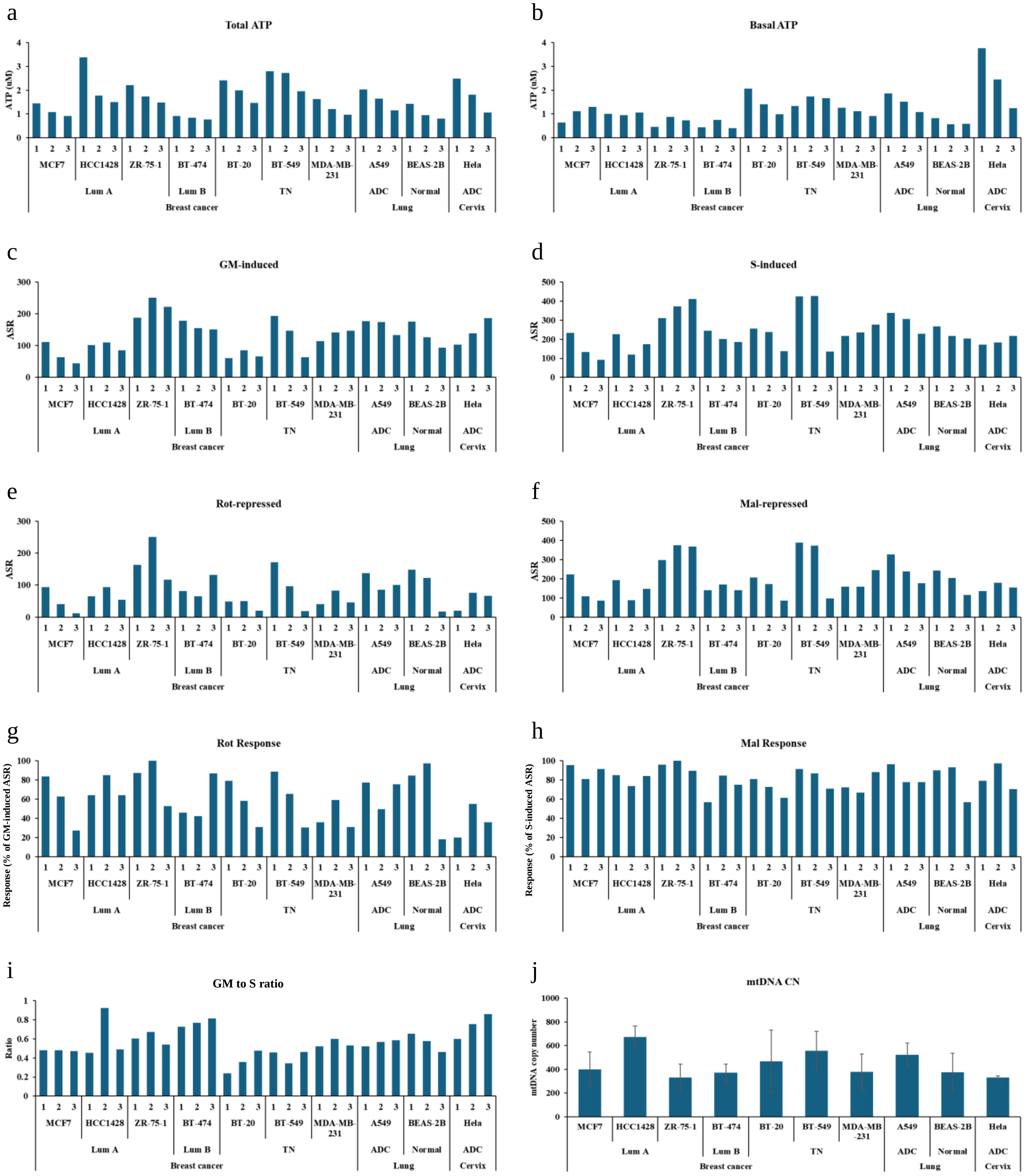
Supplementary Fig. 2. False-positive test result of the MitoRAISE assay. Dot plots of the real-time accumulation of ATP level from (a) isolated mitochondria and (b) A549 cells treated with 0 nM to 10 nM of PMP after distilled water injection instead of substrate. The green and red triangle indicate the time at which distilled water was injected. (c) Dot plots of the real-time accumulation of ATP level from standard ATP solution after substrate injection.



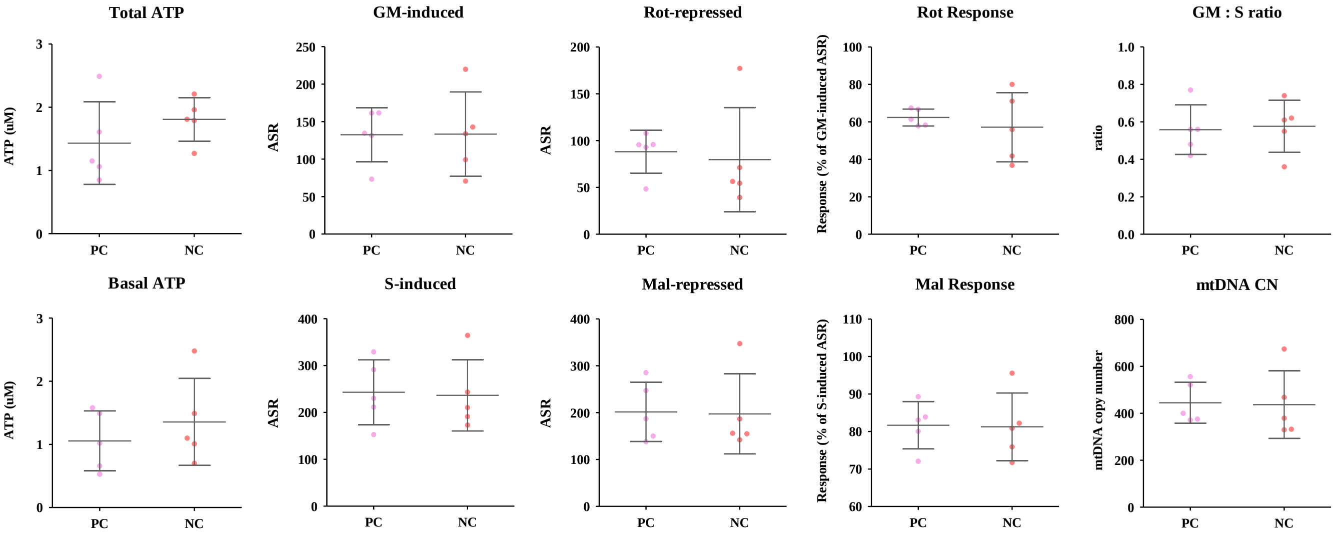
Supplementary Fig. 3. Validation of MitoRAISE assay using cells with varying degrees of permeabilization. (a) Represent images of trypan blue staining of A549 cells treated with 0 nM to 10 nM of PMP. Green cells represent cells with intact cellular membranes, while red cells represent cells with permeabilized cellular membranes. Dot plots of the real-time accumulation of ATP level from A549 cells treated with 0 nM to 10 nM of PMP after injection such as (b) succinate and malonate, and (c) glutamic acid and malic acid and rotenone. Bar graphs show MitoRAISE data.



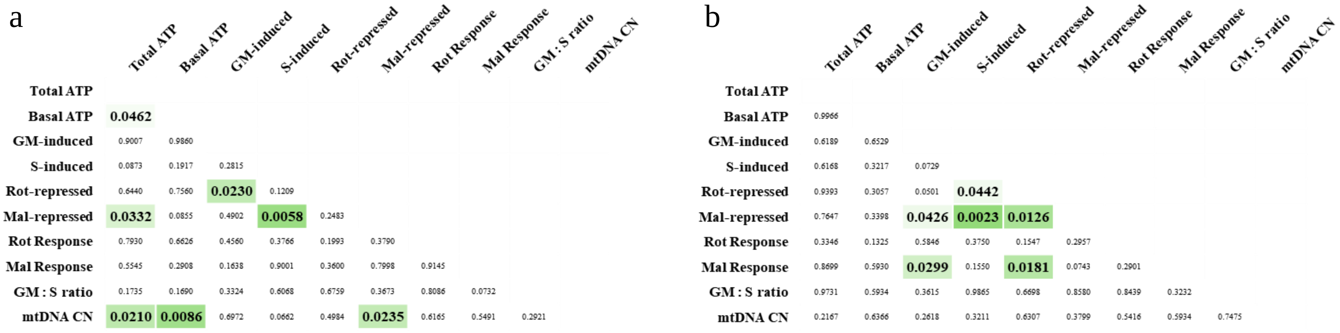
Supplementary Fig. 4. Validation of A549 cells with mitochondria damage. (a) Gel electrophoresis shows no mitochondrial DNA in A549-Rho-0 cells. (b) Fluorescence microscope image shows the uncoupling of A549 cells after treatment with CCCP and FCCP. CCCP; 2-[2-(3-Chlorophenyl)hydrazinylyidene]propanedinitrile. FCCP; carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone.



Supplementary Figure 5. Cell lines screening using MitoRAISE assay. Bar graphs show that 10 cell lines were screened using (a) conventional ATP detection kit, (b-i) MitoRAISE assay, and (j) mitochondrial DNA copy number analysis.



Supplementary Fig. 6. A comprehensive analysis of mitochondria in cell lines using the MitoRAISE assay. Scatter dot plot of MitoRAISE data comparing the PC group with the NC group. The PC group includes MCF7, BT-474, BT-549, A549, and BEAS-2B cells. The NC group includes MDA-MB-231, ZR-75-1, HeLa, BT-20, and HCC1428 cells.



a

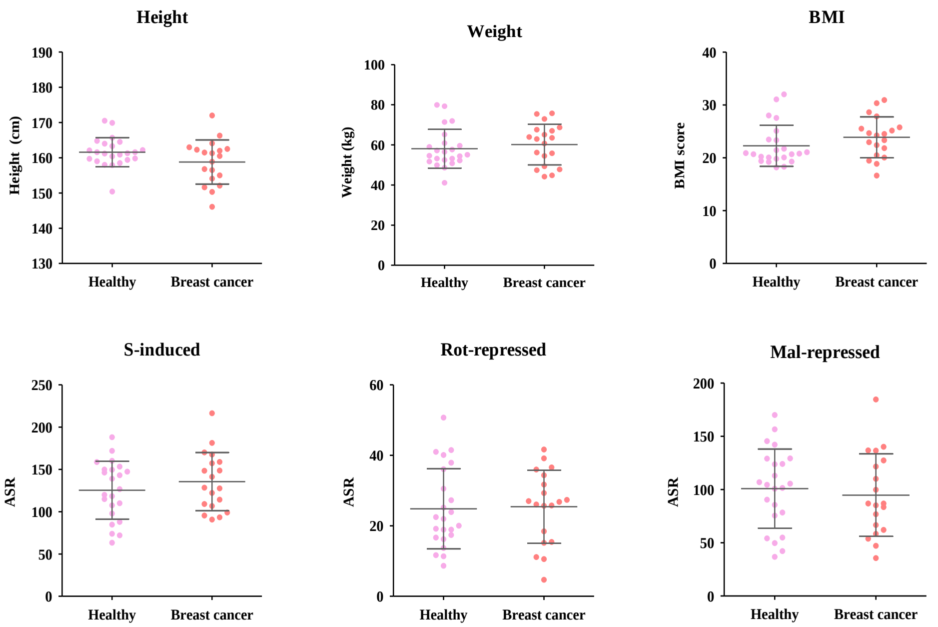
	Total ATP	Basal ATP	GM-induced	S-induced	Rot-repressed	Mal-repressed	Rot Response	Mal Response	GM : S ratio	mtDNA CN
Total ATP										
Basal ATP	**0.0462**									
GM-induced	0.9007	0.9860								
S-induced	0.0873	0.1917	0.2815							
Rot-repressed	0.6440	0.7560	**0.0230**	0.1209						
Mal-repressed	**0.0332**	0.0855	0.4902	**0.0058**	0.2483					
Rot Response	0.7930	0.6626	0.4560	0.3766	0.1993	0.3790				
Mal Response	0.5545	0.2908	0.1638	0.9001	0.3600	0.7998	0.9145			
GM : S ratio	0.1735	0.1690	0.3324	0.6068	0.6759	0.3673	0.8086	0.0732		
mtDNA CN	**0.0210**	**0.0086**	0.6972	0.0662	0.4984	**0.0235**	0.6165	0.5491	0.2921	

b

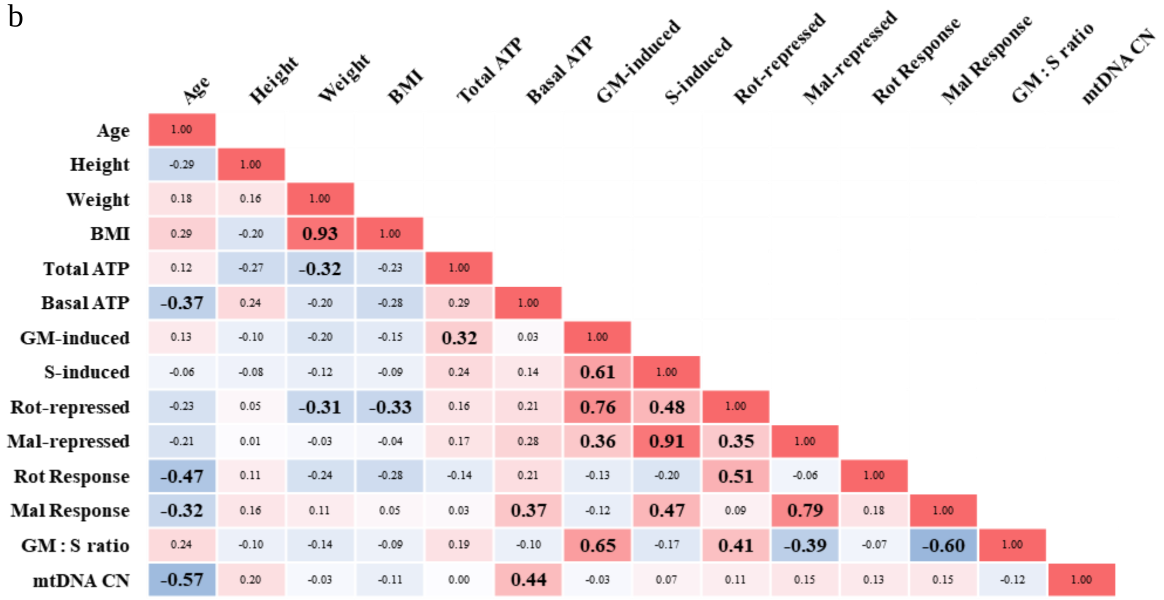
	Total ATP	Basal ATP	GM-induced	S-induced	Rot-repressed	Mal-repressed	Rot Response	Mal Response	GM : S ratio	mtDNA CN
Total ATP										
Basal ATP	0.9966									
GM-induced	0.6189	0.6529								
S-induced	0.6168	0.3217	0.0729							
Rot-repressed	0.9393	0.3057	0.0501	**0.0442**						
Mal-repressed	0.7647	0.3398	**0.0426**	**0.0023**	**0.0126**					
Rot Response	0.3346	0.1325	0.5846	0.3750	0.1547	0.2957				
Mal Response	0.8699	0.5930	**0.0299**	0.1550	**0.0181**	0.0743	0.2901			
GM : S ratio	0.9731	0.5934	0.3615	0.9865	0.6698	0.8580	0.8439	0.3232		
mtDNA CN	0.2167	0.6366	0.2618	0.3211	0.6307	0.3799	0.5416	0.5934	0.7475	

Supplementary Fig. 7. p-value table for Pearson correlation analysis of cell lines. (a) p-value table for Pearson correlation analysis of the group shows a positive correlation between total ATP levels and GM- and S-induced MASC. (b) p-value table for Pearson correlation analysis of the group shows a negative or no correlation between total ATP levels and GM- or S-induced MASC.

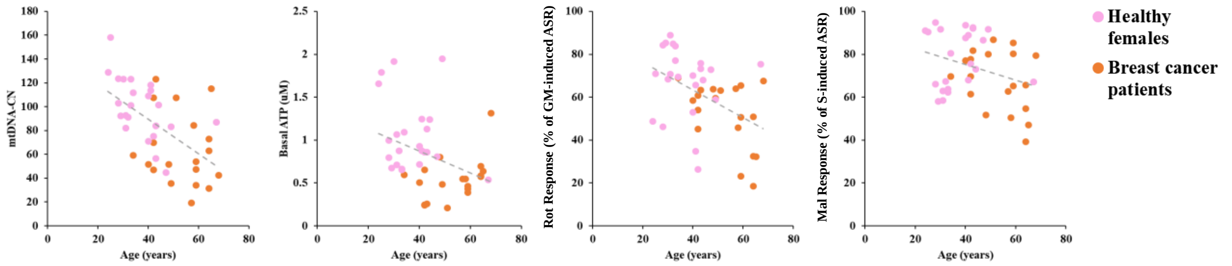
a



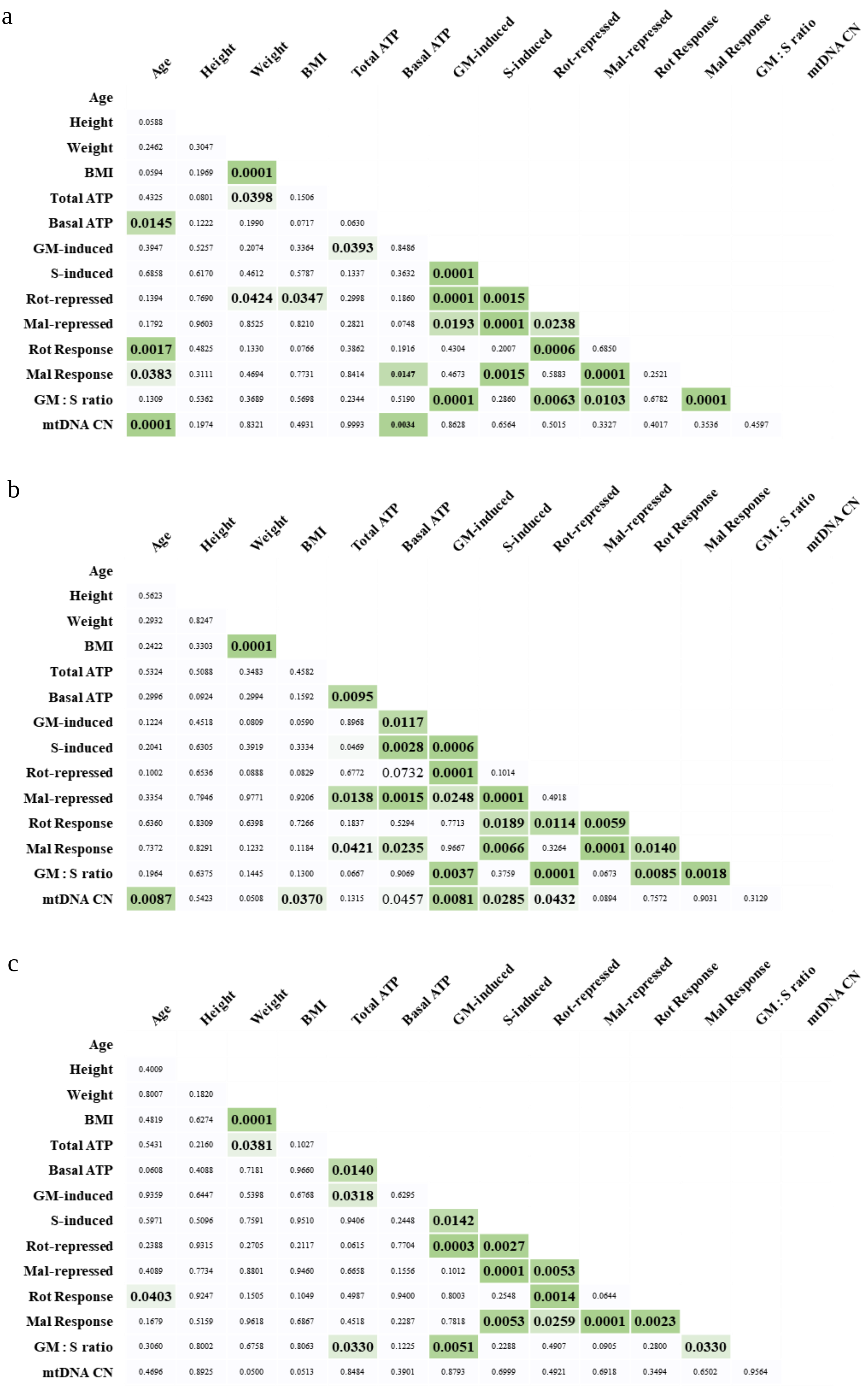
b



	Age	Height	Weight	BMI	Total ATP	Basal ATP	GM-induced	S-induced	Rot-repressed	Mal-repressed	Rot Response	Mal Response	GM : S ratio	mtDNA CN
Age	1.00													
Height	-0.29	1.00												
Weight	0.18	0.16	1.00											
BMI	0.29	-0.20	**0.93**	1.00										
Total ATP	0.12	-0.27	**-0.32**	-0.23	1.00									
Basal ATP	**-0.37**	0.24	-0.20	-0.28	0.29	1.00								
GM-induced	0.13	-0.10	-0.20	-0.15	**0.32**	0.03	1.00							
S-induced	-0.06	-0.08	-0.12	-0.09	0.24	0.14	**0.61**	1.00						
Rot-repressed	-0.23	0.05	**-0.31**	**-0.33**	0.16	0.21	**0.76**	**0.48**	1.00					
Mal-repressed	-0.21	0.01	-0.03	-0.04	0.17	0.28	**0.36**	**0.91**	**0.35**	1.00				
Rot Response	**-0.47**	0.11	-0.24	-0.28	-0.14	0.21	-0.13	-0.20	**0.51**	-0.06	1.00			
Mal Response	**-0.32**	0.16	0.11	0.05	0.03	**0.37**	-0.12	**0.47**	0.09	**0.79**	0.18	1.00		
GM : S ratio	0.24	-0.10	-0.14	-0.09	0.19	-0.10	**0.65**	-0.17	**0.41**	**-0.39**	-0.07	**-0.60**	1.00	
mtDNA CN	**-0.57**	0.20	-0.03	-0.11	0.00	**0.44**	-0.03	0.07	0.11	0.15	0.13	0.15	-0.12	1.00



Supplementary Fig. 8. Assessing the Clinical Relevance of relative ATP Synthesis rate. (a) Scatter dot plot of MitoRAISE data comparing healthy females with breast cancer patients. (b) Pearson correlation analysis table of mixed sample of healthy females and breast cancer patients.



a

	Age	Height	Weight	BMI	Total ATP	Basal ATP	GM-induced	S-induced	Rot-repressed	Mal-repressed	Rot Response	Mal Response	GM : S ratio	mtDNA CN
Age														
Height	0.0588													
Weight	0.2462	0.3047												
BMI	0.0594	0.1969	**0.0001**											
Total ATP	0.4325	0.0801	**0.0398**	0.1506										
Basal ATP	**0.0145**	0.1222	0.1990	0.0717	0.0630									
GM-induced	0.3947	0.5257	0.2074	0.3364	**0.0393**	0.8486								
S-induced	0.6858	0.6170	0.4612	0.5787	0.1337	0.3632	**0.0001**							
Rot-repressed	0.1394	0.7690	**0.0424**	**0.0347**	0.2998	0.1860	**0.0001**	**0.0015**						
Mal-repressed	0.1792	0.9603	0.8525	0.8210	0.2821	0.0748	**0.0193**	**0.0001**	**0.0238**					
Rot Response	**0.0017**	0.4825	0.1330	0.0766	0.3862	0.1916	0.4304	0.2007	**0.0006**	0.6850				
Mal Response	**0.0383**	0.3111	0.4694	0.7731	0.8414	**0.0147**	0.4673	**0.0015**	0.5883	**0.0001**	0.2521			
GM : S ratio	0.1309	0.5362	0.3689	0.5698	0.2344	0.5190	**0.0001**	0.2860	**0.0063**	**0.0103**	0.6782	**0.0001**		
mtDNA CN	**0.0001**	0.1974	0.8321	0.4931	0.9993	**0.0034**	0.8628	0.6564	0.5015	0.3327	0.4017	0.3536	0.4597	

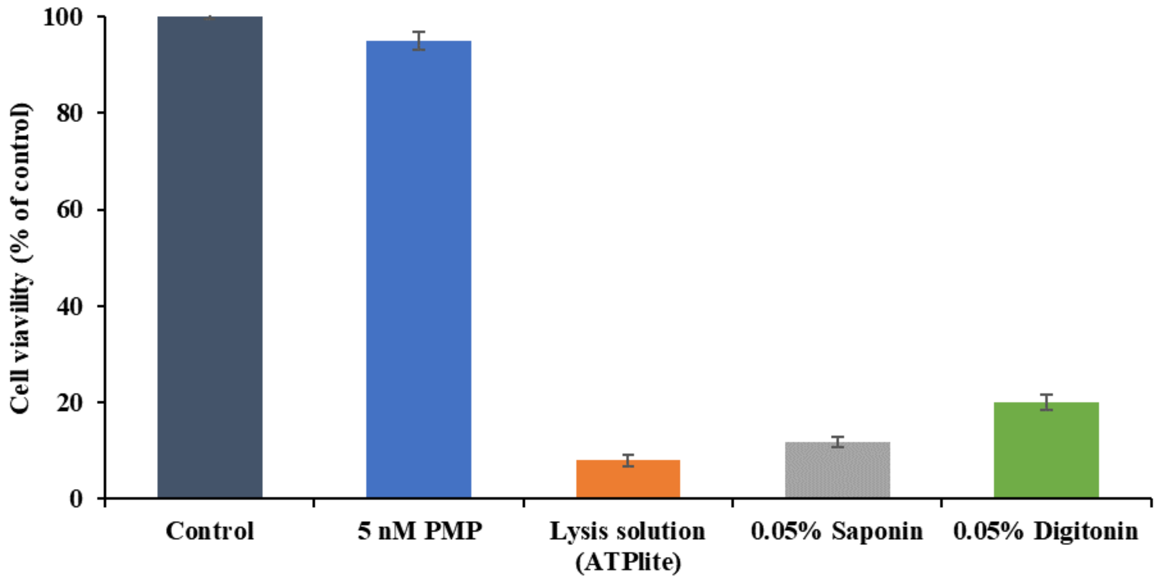
b

	Age	Height	Weight	BMI	Total ATP	Basal ATP	GM-induced	S-induced	Rot-repressed	Mal-repressed	Rot Response	Mal Response	GM : S ratio	mtDNA CN
Age														
Height	0.5623													
Weight	0.2932	0.8247												
BMI	0.2422	0.3303	**0.0001**											
Total ATP	0.5324	0.5088	0.3483	0.4582										
Basal ATP	0.2996	0.0924	0.2994	0.1592	**0.0095**									
GM-induced	0.1224	0.4518	0.0809	0.0590	0.8968	**0.0117**								
S-induced	0.2041	0.6305	0.3919	0.3334	0.0469	**0.0028**	**0.0006**							
Rot-repressed	0.1002	0.6536	0.0888	0.0829	0.6772	0.0732	**0.0001**	0.1014						
Mal-repressed	0.3354	0.7946	0.9771	0.9206	**0.0138**	**0.0015**	**0.0248**	**0.0001**	0.4918					
Rot Response	0.6360	0.8309	0.6398	0.7266	0.1837	0.5294	0.7713	**0.0189**	**0.0114**	**0.0059**				
Mal Response	0.7372	0.8291	0.1232	0.1184	**0.0421**	**0.0235**	0.9667	**0.0066**	0.3264	**0.0001**	**0.0140**			
GM : S ratio	0.1964	0.6375	0.1445	0.1300	0.0667	0.9069	**0.0037**	0.3759	**0.0001**	0.0673	**0.0085**	**0.0018**		
mtDNA CN	**0.0087**	0.5423	0.0508	**0.0370**	0.1315	0.0457	**0.0081**	**0.0285**	**0.0432**	0.0894	0.7572	0.9031	0.3129	

c

	Age	Height	Weight	BMI	Total ATP	Basal ATP	GM-induced	S-induced	Rot-repressed	Mal-repressed	Rot Response	Mal Response	GM : S ratio	mtDNA CN
Age														
Height	0.4009													
Weight	0.8007	0.1820												
BMI	0.4819	0.6274	**0.0001**											
Total ATP	0.5431	0.2160	**0.0381**	0.1027										
Basal ATP	0.0608	0.4088	0.7181	0.9660	**0.0140**									
GM-induced	0.9359	0.6447	0.5398	0.6768	**0.0318**	0.6295								
S-induced	0.5971	0.5096	0.7591	0.9510	0.9406	0.2448	**0.0142**							
Rot-repressed	0.2388	0.9315	0.2705	0.2117	0.0615	0.7704	**0.0003**	**0.0027**						
Mal-repressed	0.4089	0.7734	0.8801	0.9460	0.6658	0.1556	0.1012	**0.0001**	**0.0053**					
Rot Response	**0.0403**	0.9247	0.1505	0.1049	0.4987	0.9400	0.8003	0.2548	**0.0014**	0.0644				
Mal Response	0.1679	0.5159	0.9618	0.6867	0.4518	0.2287	0.7818	**0.0053**	**0.0259**	**0.0001**	**0.0023**			
GM : S ratio	0.3060	0.8002	0.6758	0.8063	**0.0330**	0.1225	**0.0051**	0.2288	0.4907	0.0905	0.2800	**0.0330**		
mtDNA CN	0.4696	0.8925	0.0500	0.0513	0.8484	0.3901	0.8793	0.6999	0.4921	0.6918	0.3494	0.6502	0.9564	

Supplementary Fig. 9. p-value table for Pearson correlation analysis of PMBCs. *p*-value table for Pearson correlation analysis of (a) mixed sample of healthy females and breast cancer patients, (b) healthy females, and (c) breast cancer patients.



Supplementary Fig. 10. Cell viability after treatment with well-known permeabilization agents. Bar graph shows that cell viability after treatment with well-known permeabilization agents.

Supplementary table 1. PCR primers for mtDNA copy number

mtDNA copy number	Sequence
MT-ND1_forward	5′-CCTAAAACCCGCCACATCTA-3′
MT-ND1_reverse	5′-CATAGTAGAAGAGCGATGGTGAGA-3′
ALB_forward	5′-TTAGCATACCCTTTTTGGAGACA-3′
ALB_reverse	5′-GCACAGCAGTCAGCCATTT-3′

Supplementary table 2. PCR primers for confirming A549-Rho-0 cells

Gel electrophoresis	Sequence
MT-ND1_forward	5′-CAA CAT CGA ATA CGC CGC AG-3′
MT-ND1_reverse	5′-GTT TGA GGG GGA ATG CTG GA-3′