

**Targeting PI3K and AMPK α Signaling Alone or in Combination to Enhance
Radiosensitivity of Triple Negative Breast Cancer**

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SUPPLEMENTARY MATERIALS

Figures S1-S4 contain graphs and IHC staining that is referenced in the manuscript.

Data Tables S1-S5 contain data that was used for calculations in the manuscript.

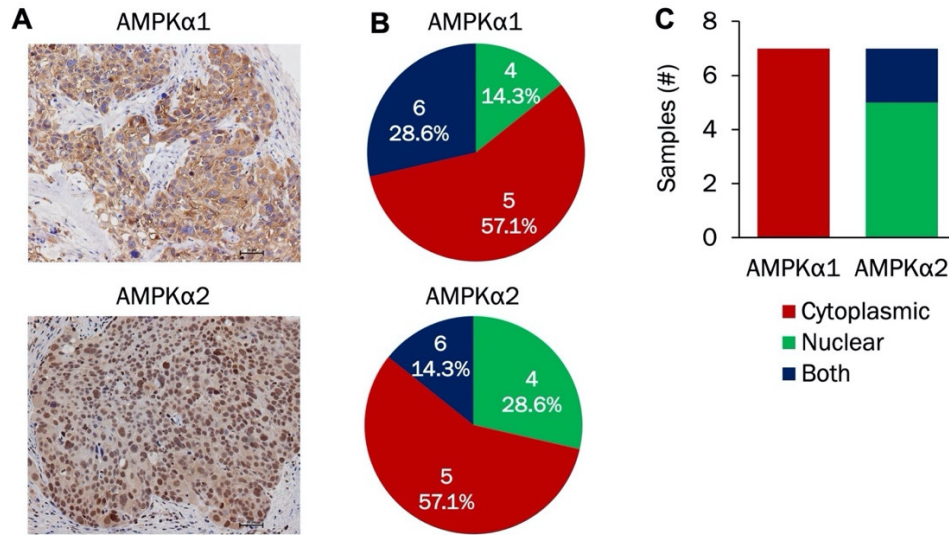


Figure S1. Analysis of AMPK α 1 and AMPK α 2 expression in TNBC PDX samples. (A) Representative IHC staining of AMPK α 1 and AMPK α 2 in TNBC PDX samples. **(B)** Scoring distribution of AMPK α 1 and AMPK α 2 in TNBC PDX samples ($n = 7$). **(C)** Subcellular localization (nuclear, cytoplasmic, or both) of AMPK α 1 and AMPK α 2 in TNBC PDX samples ($n = 7$).

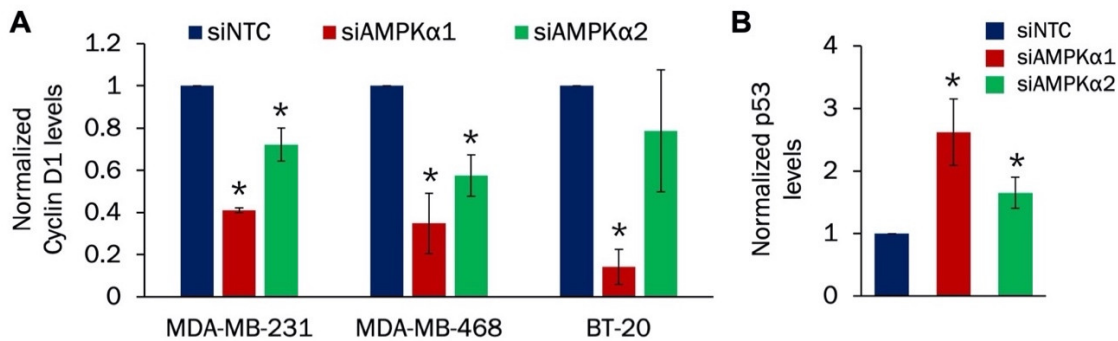


Figure S2. Densitometry measurements of cyclin D1 and p53 after AMPK α 1 or AMPK α 2 knockdown in TNBC cells. (A) MDA-MB-231, MDA-MB-468, and BT-20 cells were transfected with 50 nM of siRNA targeting AMPK α 1 or AMPK α 2. Expression of cyclin D1 was analyzed by western blot densitometry 72h after transfection ($n = 3$). **(B)** MDA-MB-231 cells were transfected with 50 nM of siRNA targeting AMPK α 1 or AMPK α 2. Expression of p53 was analyzed by western blot densitometry 72h after transfection ($n = 3$). *indicates p -value < 0.05 ; NTC, Non-targeting control was used as a negative control.

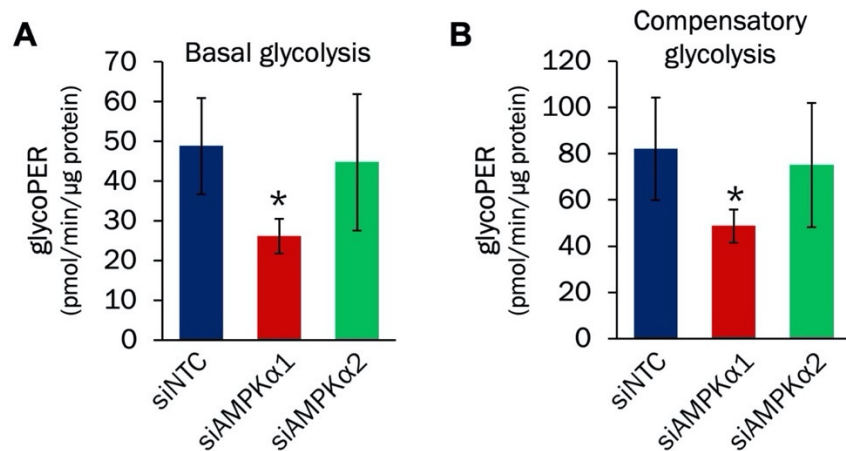


Figure S3. Effect of AMPK α 1 or AMPK α 2 knockdown on basal and compensatory glycolysis in MDA-MB-231 cells. MDA-MB-231 cells were transfected with 50 nM of siRNA targeting AMPK α 1 or AMPK α 2. Non-targeting control was used as a negative control. Cells were seeded at a density of 20,000 cells/well 48h after transfection, and the rates of (A) basal and (B) compensatory glycolysis were determined 24h later ($n = 18$). The results are representative of 2 independent experiments. *indicates p -value < 0.0001 .

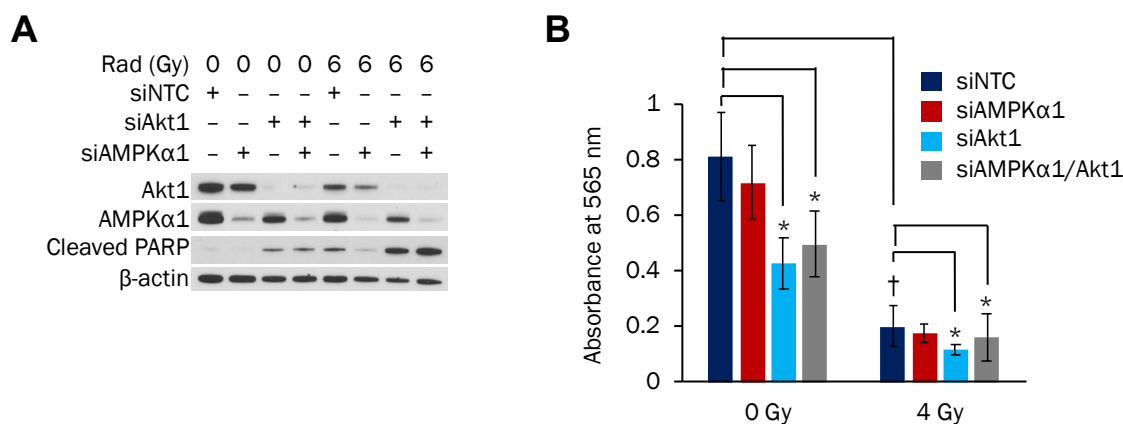


Figure S4. Silencing Akt1 enhances radiation-induced apoptosis in MDA-MB-231 cells. (A) MDA-MB-231 cells were transfected with siRNA to AMPK α 1, Akt1, or AMPK α 1/Akt1. Transfection concentrations were: (1) individual siRNA: 50 nM, (2) combination siRNA: 50 nM each (100 nM total), and (3) NTC: 100 nM. Cells were exposed to 0 or 6 Gy radiation dose 24h after transfection and collected 48h after radiation. Expression of AMPK α 1, Akt1, and cleaved PARP was determined by western blot. (B) MDA-MB-231 cells were transfected with siRNA to AMPK α 1, Akt1, or AMPK α 1/Akt1. Transfection concentrations were: (1) individual siRNA: 50 nM, (2) combination siRNA: 50 nM each (100 nM total), and (3) NTC: 100 nM. Cells were

seeded at a density of 100 cells/well into 96-well plates 48h after transfection. Cells were exposed to 0 or 4 Gy radiation dose 24h later. Colonies were fixed and quantified with the SRB assay protocol 1 week after irradiation ($n = 18$). NTC, Non-targeting control was used as a negative control. *indicates $p < 0.01$ vs. group control; † indicates $p < 0.0001$ vs. no radiation.

Data Table S1. Western blot data used for densitometry calculations in paper.

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MDA-MB-231 Cells				MDA-MB-468 Cells				BT-20 Cells			
Normalized Cyclin D1				Normalized Cyclin D1				Normalized Cyclin D1			
	siNTC	siAMPKα1	siAMPKα2		siNTC	siAMPKα1	siAMPKα2		siNTC	siAMPKα1	siAMPKα2
Rep 1	1	0.42207499	0.80997237	Rep 1	1	0.24249245	0.48132773	Rep 1	1	0.05161267	0.78855112
Rep 2	1	0.407942915	0.69800853	Rep 2	1	0.29001615	0.56460761	Rep 2	1	0.21674908	1.07579107
Rep 3	1	0.399301194	0.6577587	Rep 3	1	0.51194717	0.67725438	Rep 3	1	0.15322177	0.49636264
Normalized p53											
	siNTC	siAMPKα1	siAMPKα2								
Rep 1	1	3.05383243	1.86973381								
Rep 2	1	2.780327811	1.71017351								
Rep 3	1	2.031290802	1.37851921								

Data Table S2. Basal and compensatory glycolysis data used for calculations in paper.

Data Table S2. Basal and compensatory glycolysis data used for calculations in paper.								
Basal Glycolysis (pmol/min) Normalized to Protein			Compensatory Glycolysis (pmol/min) Normalized to Protein					
siNTC	siAMPKα1	siAMPKα2	siNTC	siAMPKα1	siAMPKα2			
39.757	26.270	49.452	75.048	54.395	80.078			
35.704	29.472	43.687	61.667	53.728	69.214			
35.005	36.071	75.691	59.746	65.136	127.037			
52.317	21.988	37.145	85.600	44.852	62.676			
35.670	25.915	34.467	62.588	45.060	67.187			
36.571	27.885	46.620	68.281	52.579	70.114			
56.372	22.188	51.353	95.911	37.083	82.375			
41.575	24.592	31.961	41.101	44.570	50.939			
38.938	26.279	40.141	67.702	48.317	67.357			
55.327	23.724	91.778	96.115	43.631	151.227			
57.050	32.007	23.021	93.248	55.723	38.091			
58.685	33.598	44.753	94.672	58.125	74.312			
48.206	22.931	48.244	86.205	40.909	78.137			
56.313	19.778	25.377	81.919	41.546	47.238			
42.964	27.575	31.796	73.923	51.058	61.436			
42.894	25.092	59.557	77.372	51.531	91.670			
67.971	21.618	33.194	123.878	45.730	66.763			
77.320	23.762	37.468	131.254	41.653	66.342			

Data Table S3. Proliferation assay data used for calculations in paper.

Data Table S3. Proliferation assay data used for calculations in paper.								
	Viable cells	Viable cells		Viable cells	Viable cells		Viable cells	Viable cells
	/ml (x10 ⁶)	relative to siNTC		/ml (x10 ⁶)	relative to siNTC		/ml (x10 ⁶)	relative to siNTC
siNTC	1.04	103.9170776	siAMPKα1	0.59	59.22782154	siAMPKα2	0.44	44.09728361
siNTC	1.05	105.1211074	siAMPKα1	0.64	64.39508242	siAMPKα2	0.40	40.37485197
siNTC	0.98	98.14781374	siAMPKα1	0.57	57.4318239	siAMPKα2	0.37	37.24439708
siNTC	0.94	93.94377435	siAMPKα1	0.63	63.08068955	siAMPKα2	0.44	43.73607705
siNTC	0.99	98.87022687	siAMPKα1	0.63	63.08068955	siAMPKα2	0.46	46.61569737
	Average							
siNTC	1.00							

Data Table S4. SRB assay data used for calculations in paper.

Data Table S4. SRB assay data used for calculations in paper.										
	siNTC	siAMPKα1	siAMPKα2	siAkt1	siP110α	siAMPKα1/Akt1	siAMPKα1/p110α	siAMPKα2/Akt1	siAMPKα2/p110α	
	0.605	0.516	0.442	0.435	0.472	0.446	0.312	0.398	0.443	
	0.661	0.538	0.434	0.481	0.43	0.455	0.337	0.428	0.475	
	0.64	0.55	0.503	0.532	0.491	0.471	0.361	0.437	0.541	
	0.649	0.493	0.481	0.484	0.453	0.494	0.348	0.449	0.5	
	0.656	0.502	0.476	0.529	0.446	0.461	0.337	0.52	0.541	
	0.717	0.52	0.434	0.478	0.449	0.418	0.328	0.452	0.525	
	0.694	0.584	0.481	0.418	0.488	0.43	0.306	0.43	0.451	
	0.71	0.512	0.396	0.453	0.498	0.431	0.339	0.456	0.456	
	0.716	0.55	0.49	0.481	0.564	0.439	0.3	0.441	0.482	
	0.724	0.578	0.453	0.531	0.513	0.442	0.292	0.421	0.458	
Absorbance at 565 nm	0.745	0.595	0.48	0.537	0.464	0.436	0.316	0.453	0.44	
	0.699	0.543	0.433	0.562	0.492	0.394	0.295	0.422	0.516	

Data Table S5. Colony formation SRB assay data used for calculations in paper.

Data Table S5. Colony formation SRB assay data used for calculations in paper.										
	0 Gray				4 Gray					
	siNTC	siAMPKα1	siAkt1	siAMPKα1/Akt1	siNTC	siAMPKα1	siAkt1	siAMPKα1/Akt1		
	0.843	0.635	0.457	0.556	0.143	0.193	0.151	0.124		
	1.017	0.871	0.342	0.496	0.206	0.162	0.148	0.086		
	0.848	0.771	0.5	0.395	0.19	0.106	0.113	0.116		
	0.974	0.648	0.414	0.337	0.157	0.164	0.108	0.1		
	0.729	0.665	0.431	0.353	0.233	0.192	0.14	0.161		
	1.176	0.748	0.599	0.44	0.429	0.215	0.109	0.399		
	0.822	0.619	0.38	0.571	0.172	0.228	0.118	0.143		
	0.592	0.494	0.427	0.447	0.2	0.174	0.128	0.121		
	0.67	0.75	0.386	0.422	0.18	0.165	0.113	0.122		
	0.805	0.48	0.318	0.423	0.237	0.137	0.122	0.221		
	0.929	0.887	0.338	0.563	0.246	0.182	0.092	0.331		
	0.956	0.753	0.473	0.478	0.264	0.153	0.099	0.169		
	0.825	0.964	0.432	0.761	0.196	0.139	0.134	0.15		
	0.597	0.652	0.518	0.494	0.12	0.154	0.087	0.092		
	0.714	0.872	0.304	0.443	0.132	0.227	0.083	0.104		
	0.62	0.564	0.646	0.563	0.122	0.191	0.105	0.116		
Absorbance at 565 nm	0.744	0.772	0.338	0.4	0.152	0.158	0.119	0.089		
	0.679	0.737	0.358	0.768	0.222	0.169	0.09	0.196		