Influence of washing and quenching in profiling the metabolome of adherent mammalian cells: A case study with the metastatic breast cancer cell line MDA-MB-231

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Table S1 Assay parameters employed for the estimation of ATP content by luciferase bioluminescence assay in MDA-MB-231 cells. The amount of ATP determined was then normalised to protein content estimated by BCA microplate assay

Assay Parameters	
Scale	24 well plate (Corstar®)
Seeding density	50000 cells per well (cpw)
Incubation	36hr - 500µl full * RPMI 1640 (37°C + 5% CO_2)
Luciferase assay mix dilution factor	1:25 (FLASC Kit dilution buffer)
Amount of ATP releasing reagent (ARR)	150 µl ATP Releasing Reagent, 100µl Luciferase
ATP standard range (n=3) for preliminary experiments	0, 0.2, 0.3, 0.44, 0.67, 1.0, 1.25, 1.5 (nM)
ATP standard range (n=3) for other experiments.	0, 0.44, 0.67, 1.0, 1.25, 1.5, 2 (nM)
Base line ATP MDA-231	1.2-1.3 nM
Additional test std. dilution (0.87nM) (n=3)	6.5µl (1.5nM ATP) + 1493µl dH_2O
Protein quantification	BCA Microplate assay (ab. 570nm) (Pierce)
*Full = supplemented: 1% fungazone, 1% strep/P, 1% glutamine and 10% fetal bovine serum (FBS)	

Table S2 Raw data and calculation part for estimation of nM amounts of free ATP produced in response to various washing solutions/steps. ATP levels were normalised to the protein levels. (Sample calculation part for Figure 3 a))

Sample (MDA-MB-231) Figure 4.5 results							
Samples Code	ATP RLU	ATP nmole	Protein Absorbance	Protein mg	ATP nmole/mg protein	Mean	SD	SEM
C	2812.00	0.79	1.37	1.19	0.66			
C	2205.00	0.60	1.13	1.00	0.60	0.59	0.08	0.05
C	2213.00	0.61	1.39	1.20	0.50			
PB1	2299.00	0.63	1.14	1.01	0.63			
PB1	2951.00	0.83	1.03	0.93	0.90	0.74	0.14	0.08
PB1	2429.00	0.67	1.09	0.97	0.69			
PB2	2729.00	0.76	0.84	0.77	0.99			
PB2	2380.00	0.66	0.88	0.80	0.83	0.93	0.09	0.05
PB2	2788.00	0.78	0.88	0.80	0.98			
W1	3354.00	0.95	1.00	0.90	1.06			
W1	3012.00	0.85	0.98	0.88	0.96	1.03	0.06	0.04
W1	3368.00	0.95	0.98	0.88	1.08			
W2	5012.00	1.45	1.03	0.92	1.57			
W2	4218.00	1.21	0.91	0.83	1.46	1.51	0.05	0.03
W2	4588.00	1.32	0.97	0.88	1.50			

Initially the ATP standard curve and BCA standard curves were generated. Later, the nM amounts of free ATP produced in response to various washing steps/solutions were normalised to the protein levels as detailed below.

Concentration of free ATP (nM) in response to each treatment was calculated using equation:

$y = 0.0003x - 0.571$

Concentration of protein (mg) in response to each treatment was calculated using equation:

$y = -0.0934x2 + 1.0069x - 0.0143$

The nM amounts of free ATP produced in response to various washing solutions/steps were normalised to the protein levels within each whole-cell lysate, the results are summarised in figure 3a.
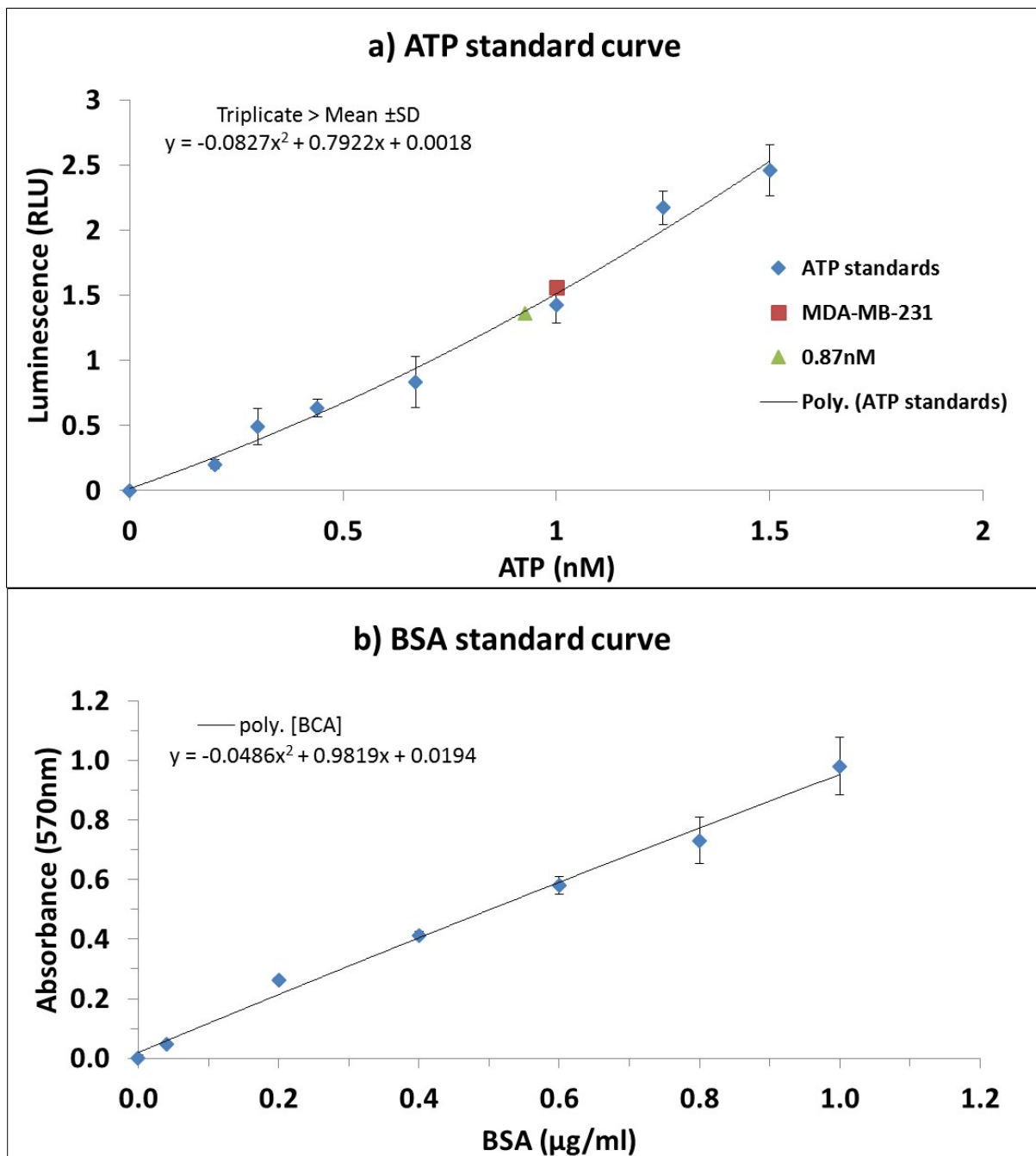
Fig. S1 Standard curves generated for both luciferase bioluminescence ATP assay (a) and BCA protein assay (b) using mean values of ATP and protein content obtained with set of standard solutions (n=3).
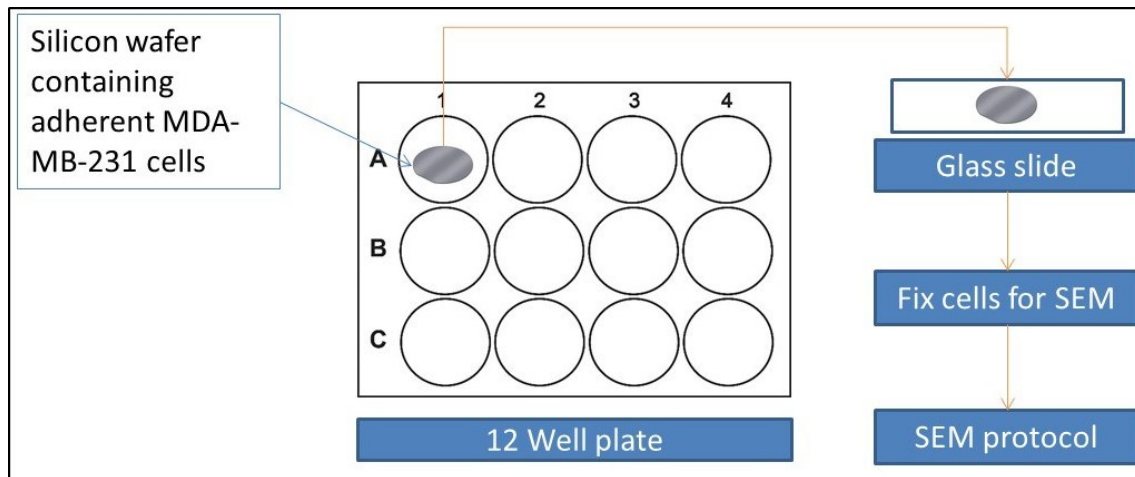
Fig. S2 Schematic displaying workflow for SEM analysis of adherently growing cells. Adherent cells were grown on silicon wafers, which can be removed from the well plates by simply lifting the silicon wafers with forceps and cells along with the silicon wafers can be fixed for SEM analysis.

Table S3 List of 112 identified and 28 unknown metabolites across all treatments in adherently growing metastatic cancer cell line MDA-MB-231. All the identified metabolites were classified based on their physicochemical properties into 11 different metabolite classes (class ID). **1** Organic acids (non-fatty) and derivatives; **2** Sugars/sugar alcohols and derivatives; **3** Amino acids and derivatives; **4** Nucleotides, nucleosides, nucleobases; **5** Fatty acids/fatty alcohols and derivatives; **6** Biogenic amines/polyamine; **7** Phosphates; **8** Alkanes; **9** Alcohols (others); **10** Ketones and ethers and **11** Others.

Met ID	Corrected Met ID's	Class ID
1	2-Piperidinecarboxylic acid	1
2	3-Methyl-2-oxopentanoic-acid	1
3	Adipic acid	1
4	Butanoic acid	1
5	Citric acid	1
6	Erythronic acid	1
7	Fumaric acid	1
8	Glutaric acid	1
9	Glyoxylic acid	1
10	Gulonic acid	1
11	Iminodiacetic acid	1
12	Indole-3-acetic acid	1
13	Lactic acid	1
14	Malic acid	1
15	Mandelic acid	1
16	Oxalic acid	1
17	Pyruvic acid	1
18	Threonic acid	1
19	Allose	2
20	Arabitol	2
21	Erythritol	2
22	Fructose	2
23	Galactitol	2
24	Galactose	2
25	Gentiobiose	2
26	Glucose	2
27	Inositol	2
28	Kestose	2
29	Laminaribiose	2
30	Lyxose	2
31	Mannitol	2
32	Mannose	2
33	Psicose	2
34	Ribitol	2
35	Ribose	2
36	similar to Fructose Derivate	2
37	similar to Inositol	2
38	Sorbitol	2
39	Sorbose	2
40	Sucrose	2
41	Tagatose	2
42	Threitol	2
43	Threose	2

Met ID	Corrected Met ID's	Class ID
44	Xylitol	2
45	Xylose	2
46	Alanine	3
47	Aminomalonic acid	3
48	Asparagine	3
49	Aspartic acid	3
50	Cysteine	3
51	Glutamic acid	3
52	Glutamine	3
53	Glycine	3
54	Homoserine	3
55	Isoleucine	3
56	Leucine	3
57	Lysine	3
58	Methionine	3
59	N-Carboxyglycine	3
60	Norleucine	3
61	Ornithine	3
62	Phenylalanine	3
63	Proline	3
64	Prolyl-glycine	3
65	Pyroglutamic acid	3
66	Serine	3
67	Threonine	3
68	Tryptophan	3
69	Tyrosine	3
70	Valine	3
71	Nicotinamide	4
72	Purine	4
73	Pyridoxamine	4
74	Cholesterol	5
75	Cholesterol-5beta	5
76	Dodecanoic acid	5
77	Dodecanol	5
78	Eicosan-1-ol	5
79	Eicosatetraenoic acid	5
80	Heptadecan-1-ol	5
81	Hexadecanoic acid	5
82	Isocaproic acid	5
83	Isocitric acid	5
84	Octadecan-1-ol	5
85	Octadecanoic acid	5
86	Octadecenoic acid	5
87	Pantothenic acid	5

88	Pentadecan-1-ol	5
89	Pentadecanoic acid	5
90	Tetradecanoic acid	5
91	Tridecan-1-ol	5
92	Ethanolamine	6
93	Hypotaurine	6
94	Phenethylamine	6
95	Putrescine	6
96	Sphingosine	6
97	Triethanolamine	6
98	Urea	6
99	Ethanolaminephosphate	7
100	Glycerol-3-phosphate	7
101	Phosphoric acid	7
102	Pyrophosphate	7
103	Glycerol	9
104	Propane-1,2-diol	9
105	Flavone	10
106	Glucoheptonic acid-1,4-lactone	10
107	Phenylacetaldehyde	10
108	Creatinine	11
109	Maleimide	11
110	Quinazoline	11
111	similar to Ditertbutylphenol	11
112	Valero-1,5-lactam	11
113	UK1	11
114	UK2	11
115	UK3	11
116	UK4	11
117	UK5	11
118	UK6	11
119	UK7	11
120	UK8	11
121	UK9	11
122	UK10	11
123	UK11	11
124	UK12	11
125	UK13	11
126	UK14	11
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131	UK19	11

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134	UK22	11
135	UK23	11
136	UK24	11
137	UK25	11
138	UK26	11
139	UK27	11
140	UK28	11