

Supplementary Materials for

Improving the metabolic fidelity of cancer models with a physiological cell culture medium

Johan Vande Voorde, Tobias Ackermann, Nadja Pfetzer, David Sumpton, Gillian Mackay, Gabriela Kalna, Colin Nixon, Karen Blyth, Eyal Gottlieb, Saverio Tardito*

*Corresponding author. Email: s.tardito@beatson.gla.ac.uk

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SUPPLEMENTARY MATERIAL

Table S1. Comparison between the formulations of Plasmax and HPLM.

	Concentration (μM)	
	Plasmax	HPLM
Proteinogenic Amino Acids		
L-Alanine ¹	510	430
L-Arginine ¹	64	110
L-Asparagine ¹	41	50
L-Aspartic acid ¹	6	20
L-Cysteine ²	33	40
L-Glutamate ¹	98	80
L-Glutamine ⁵	650	550
Glycine ¹	330	300
L-Histidine ¹	120	110
L-Isoleucine ¹	140	70
L-Leucine ¹	170	160
L-Lysine ¹	220	200
L-Methionine ¹	30	30
L-Phenylalanine ¹	68	80
L-Proline ¹	360	200
L-Serine ¹	140	150
L-Threonine ¹	240	140
L-Tryptophan ¹	78	60
L-Tyrosine ¹	74	80
L-Valine ¹	230	220
Non-proteinogenic Amino Acids		
α -Aminobutyrate ²	41	20
L-Citrulline ¹	55	40
L-Cystine ¹	65	100
L-Homocysteine ²	9	NA
4-Hydroxy-L-proline ²	13	20
L-Ornithine ¹	80	70
L-Pyroglutamate ²	20	NA
Amino Acids Derivatives		
L-Acetyl glycine ²	70	90
L-Carnosine ²	6	NA
Glutathione (reduced) ²	37	25
Taurine ²	130	90
N-Trimethylglycine (betaine) ²	72	70
Other Components		
Acetate ²	42	40
Acetone ²	55	60
Acetyl carnitine ²	5	NA
Citrate ²	114	130
Carnitine ²	46	40
Creatine ²	37	40
Creatinine ²	74	75
Formate ²	33	50
Fructose	NA	40
Galactose	NA	60
D-Glucose ⁷	5560	5000
Glycerol ²	82	120
2-Hydroxybutyrate ²	31	50

3-Hydroxybutyrate ²	77	50
3-Hydroxyisobutyrate ²	20	NA
Hypoxanthine ²	5	10
Lactate ²	500	1600
Malonate	NA	10
Methyl acetoacetate ²	41	NA
Phenol Red ⁷	25	14
Pyruvate ⁸	100	50
Succinate ²	23	20
Uracil ²	2	NA
Urate ⁴	270	350
Urea ²	3000	5000
Uridine ²	3	NA
Inorganic Salts		
Ammonium Chloride ³	50	40
Calcium Chloride ⁷	1800	2350
Calcium Nitrate	NA	40
Magnesium Chloride	NA	480
Magnesium Sulfate ⁷	813	350
Potassium Chloride ⁷	5330	4100
Sodium Bicarbonate ⁷	26191	24000
Sodium Chloride ⁷	118706	105000
Sodium Phosphate monobasic ⁷	1010	870*
Trace Elements		
Ammonium Metavanadate ³	0.0026	NA
Cupric Sulfate ³	0.0052	NA
Ferric Nitrate ³	0.1238	NA
Ferric Sulfate ³	1.0428	NA
Manganous Chloride ³	0.0002	NA
Sodium Selenite ³	0.0289	NA
Zinc Sulfate ³	1.5	NA
Vitamins		
p-Aminobenzoate	NA	7.3
Ascorbate ⁶	62	NA
D-Biotin ⁵	4.1	0.8
Choline ⁵	7.1	21.5
Folate ⁵	2.3	2.3
myo-Inositol ⁵	11.1	194.3
Niacinamide ⁵	8.2	8.2
D-Pantothenic acid hemicalcium ⁵	4.2	1.05
Pyridoxine ⁵	4.9	4.9
Riboflavin ⁵	0.3	0.5
Thiamine ⁵	3	3
Vitamin B12 ⁶	0.005	0.0037

* present as dibasic salt

Formulations of Plasmix and HPLM (Cantor et al. 2017, Cell 169, 258-272). Plasmix components were dissolved and stocked as follow: ¹ 100x solution 1, ² 100x solution 2, ³ 1,000x solution 3 (trace element concentrations as in Advanced DMEM-F12, Thermo Fisher Scientific cat no. 12634028), ⁴ 500x solution 4, ⁵ 100x commercially available BME vitamin mix. ⁶ 100,000x individual stocks, supplemented to BME vitamin mix to obtain a 100x stock solution 5. ⁷ included in the commercially available EBSS. ⁸ individual stock solutions commercially available.

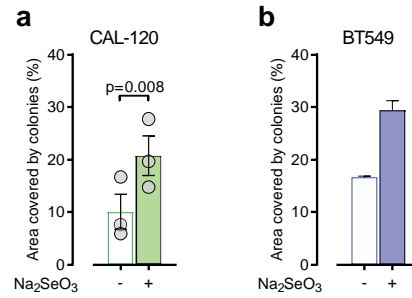


Fig. S1. Selenite-dependent colony formation. Quantification of colony formation assays performed with (a) CAL-120 cells and (b) BT549 seeded 500cells/well and incubated in DMEM-F12 with 28nM Na_2SeO_3 , as indicated. Mean \pm SEM; (a) $n = 3$ (b) $n=1$ independent experiments. Each dot represents an independent experiment. p value refers to a two-tailed t-test for paired homoscedastic samples.

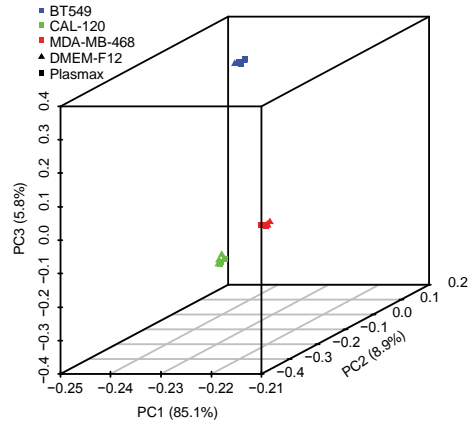


Fig. S2. PCA of gene expression obtained from RNA sequencing data of BT549, CAL-120, and MDA-MB-468 cells cultured in Plasmix or DMEM-F12, in normoxia. Each dot represents an independent experiment.

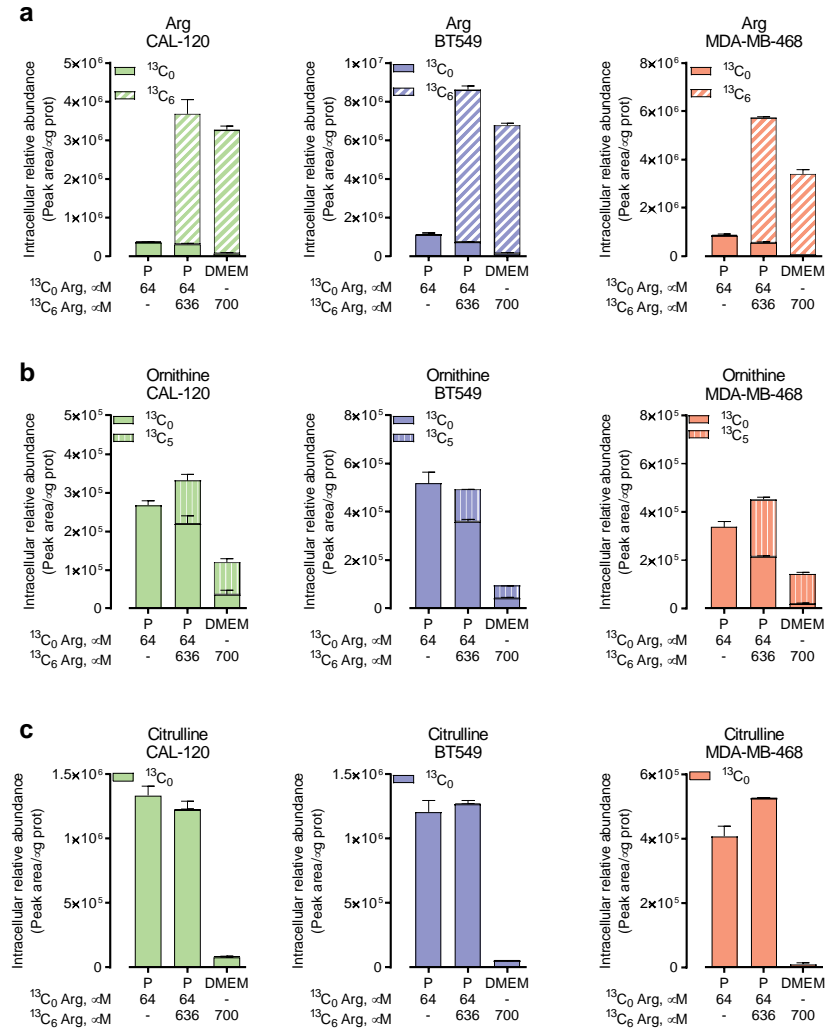


Fig. S3. Isotopologue distribution of urea cycle intermediates. Intracellular levels of (a) ^{13}C arginine (b) ^{13}C ornithine and (c) ^{13}C citrulline in BT549, CAL-120 and MDA-MB-468 cells cultured for 48 hours in Plasmag (P) and DMEM supplemented with $^{13}\text{C}_6$ and $^{13}\text{C}_0$ arginine at the indicated concentrations. Mean \pm SEM; CAL-120 (n = 3 independent experiments); BT549 and MDA-MB-468 (n = 2 independent experiments).