Supporting Information

Combining Amine Metabolomics and Quantitative Proteomics of Cancer Cells Using Derivatization with Isobaric Tags

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Figure S-1

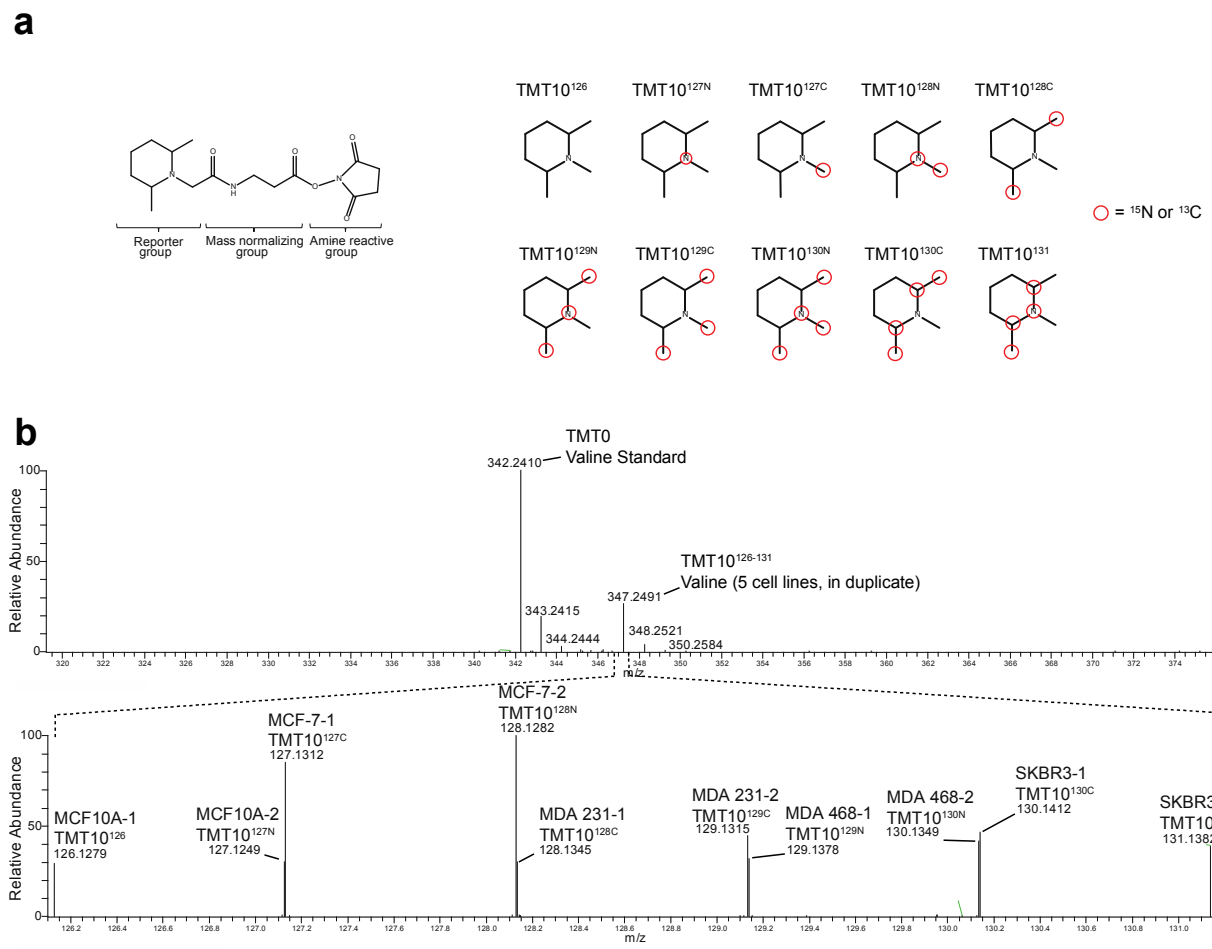

Figure S-1. Schematic for MS2-based amino acid quantification by 10-plex isobaric TMT labeling. **(a)** TMT10 reporter ions produced upon fragmentation of the TMT-labeled amino acid. Unique reporter isotopic masses are achieved by the incorporation of ^{13}C or ^{15}N into the TMT reagents at the positions outlined. To achieve a nominal MS1 mass for all 10 reagents, ^{13}C and ^{15}N are also incorporated into the mass-normalizing group to balance the reporter ion masses (not shown here). **(b)** Typical MS2-based amino acid analysis using TMT0 as an internal standard, exemplified by valine measurement between 5 cell lines (in duplicate). The TMT0-labeled internal standard is used to determine the total amount of valine in all 10 samples (TMT10). Targeted fragmentation of TMT10-labeled valine (*m/z*=347.2492) generates reporter ions (distinguishable by high-resolution; R=3 x 10^4 at 400 *m/z*) to determine the fraction of the total valine originating from each sample.

Figure S-2

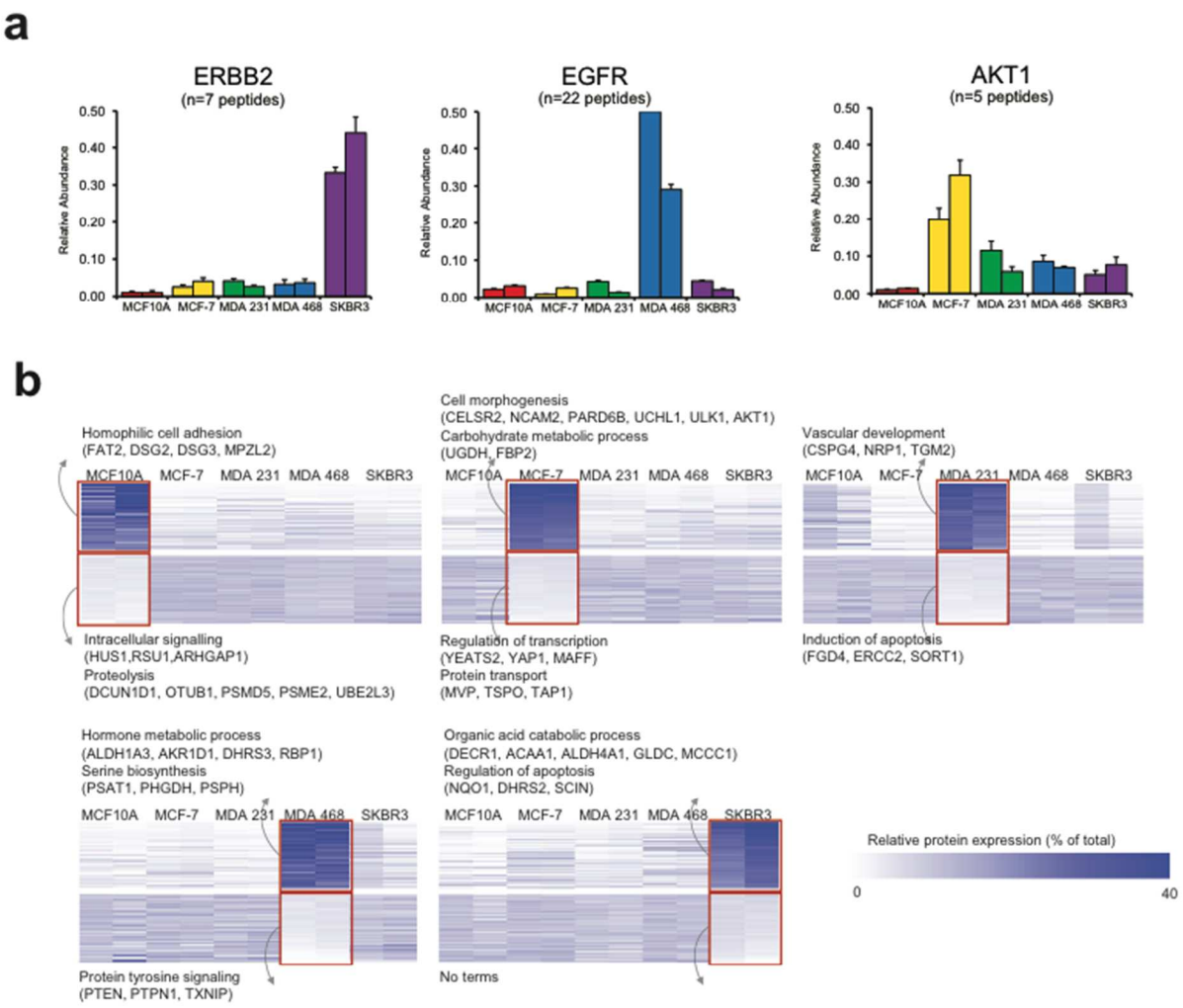

Figure S-2. Proteins and functional terms present at comparatively high or low levels between the 5 breast cancer cell lines used in the study, from the 10-plex quantitative proteomics dataset. **(a)** Relative expression of several proliferation and metabolism-linked kinases detected with variable expression in our dataset (ERBB2, EGFR, and AKT1). **(b)** Gene-annotation enrichment analysis was assessed on the top 50 and bottom 50 comparatively expressed proteins for each cell line using DAVID (http://david.abcc.ncifcrf.gov/). Shown are the enriched terms ($p<0.05$) and the contributing proteins.

Figure S-3

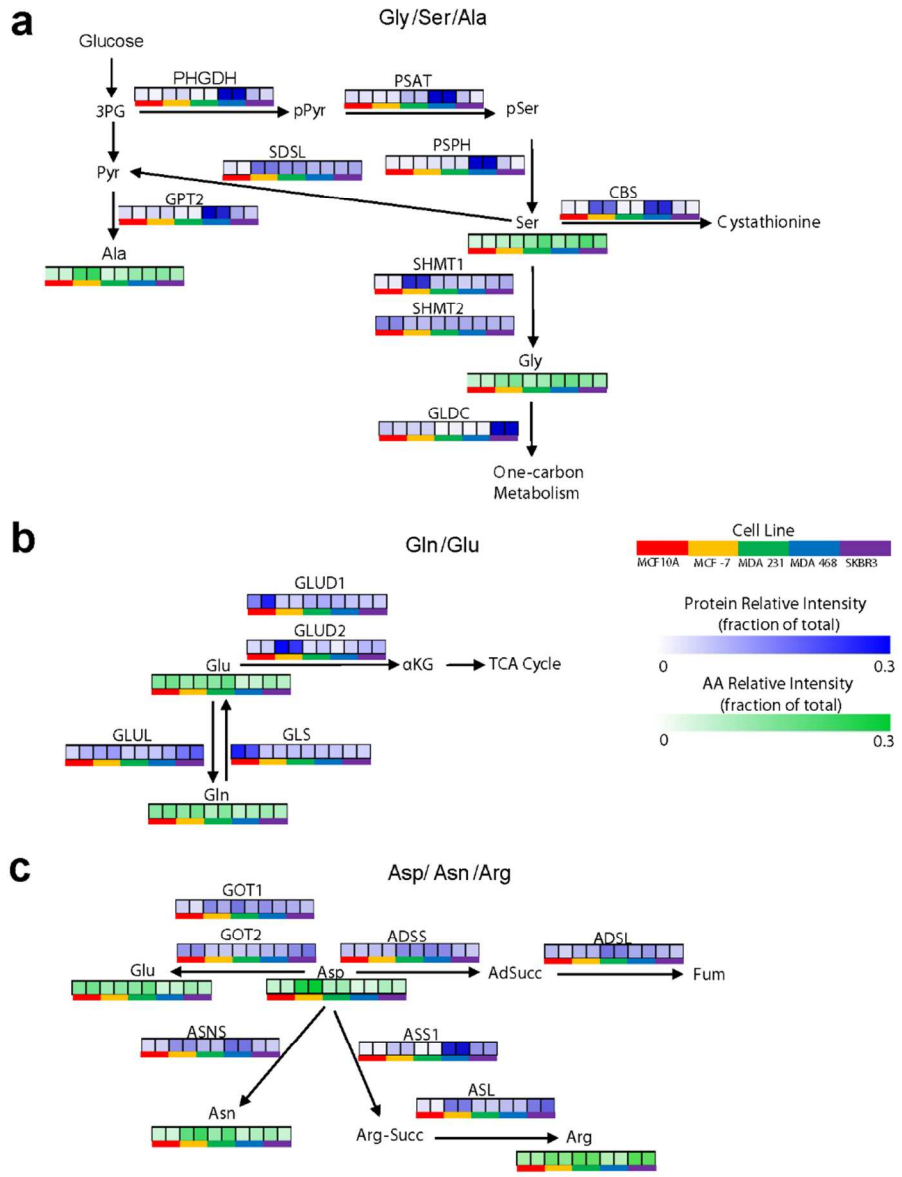

Figure S-3. Protein and amino acid data overlaid onto amino acid metabolism pathways. All data are shown as duplicates for each cell line. (a) Glycine, serine, and alanine amino acid biosynthesis branching from glycolysis. (b) Glutamine and glutamate catabolism leading to the TCA cycle. (c) Aspartate, asparagine, and arginine metabolism.

Figure S-4

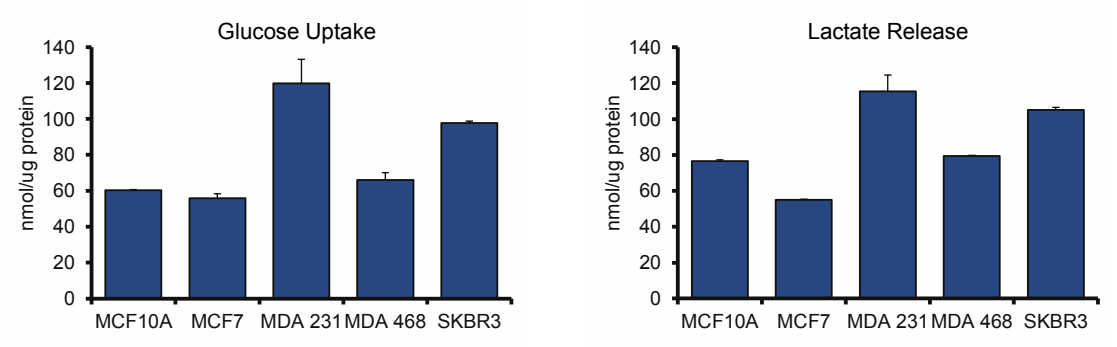

Figure S-4. Comparison of glucose uptake and lactate release between the 5 cell lines used in this study. Media was collected and measured for glucose and lactate by YSI.

Figure S-5

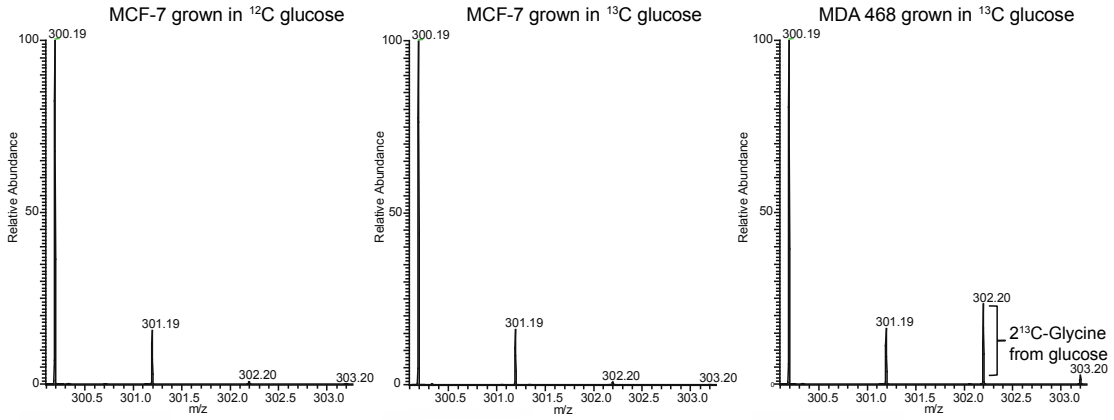

Figure S-5. Naturally abundant and glucose-acquired ^{13}C in TMT0-labeled glycine. The natural abundance of the $2^{13}C$ in glycine is negligible (<5%) such that any major change in TMT0-labeled $2^{13}C$-glycine (m/z=302.20) is attributed to metabolic labeling.

Table S-1

	Mass *(m/z)*		
Compound	**Observed**	**Predicted**	**Peak Area (x 10^6)**
Phenylalanine	390.2380	390.2387	4100
Valine	342.2380	342.2387	3400
Leucine	356.2530	356.2544	2300
Isoleucine	356.2530	356.2544	2100
Glycine	300.1921	300.1918	1400
Glutamine	371.2272	371.2289	940
Tyrosine	406.2328	406.2336	670
Threonine	344.2186	344.2180	570
Proline	340.2240	340.2231	570
Glutamate	372.2135	372.2129	560
Tryptophan	429.2494	429.2496	560
Methionine	374.2107	374.2108	480
Taurine	350.1750	350.1744	450
Lysine (2+), 2 TMT	298.2126	298.2125	270
Alanine	314.2075	314.2074	240
Aspartate	358.1976	358.1973	150
Methionine Sulfoxide	390.2060	390.2057	140
Serine	330.2011	330.2023	96
Asparagine	357.2137	357.2132	41
Glutathione Disulfide	837.3142	837.3117	35
Glutathione	532.2445	532.2436	16
Cystathione	447.2272	447.2272	14
Histidine	380.2292	380.2292	8
Cysteine	346.1797	346.1795	6.6
2-Aminooctanoic acid	384.2858	384.2857	4.9
N-Acetylputrescine	355.2702	355.2704	4.5
Citrulline	400.2557	400.2554	4.3
Arginine	399.2706	399.2714	3.5
Homocysteine/methylcysteine	360.1952	360.1952	2.7
Pyridoxamine	393.2499	393.2496	2.4
Carnitine	386.2632	386.2649	2.2
Ornithine	357.2491	357.2496	1.5

Table S-1. Intracellular amines assigned by LC-MS. Peaks were assigned to metabolites in Maven by matching to a database of TMT0-modified amine-containing metabolites obtained from a comprehensive metabolomics analysis of central carbon metabolites in cells[8]. Peak selection tolerance = 5 ppm, S/N >30.