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Methionine is a metabolic dependency of tumor-initiating cells

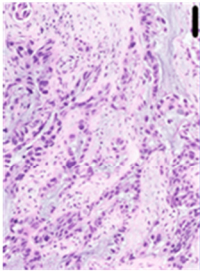
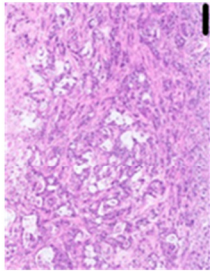
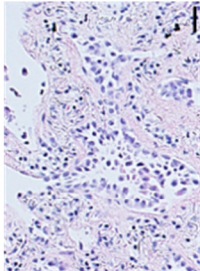
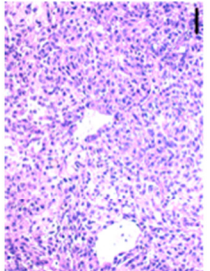
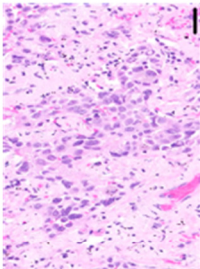
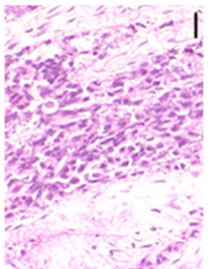
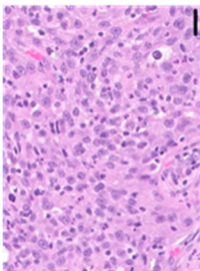
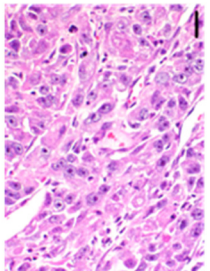
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Supplementary Table 1

Information on patient-derived cell lines, patient-derived xenografts, and primary patient tumor used in the manuscript. The genotypes are identical between patient tumor and the matched cell lines / xenograft. Black scale bars indicate 50 μ m.

Cell Line or Xenograft ID	Patient Tumor Histology	Xenograft Tumor Histology	Sex	Age	NSCLC Tumor Type	Clinical Stage	pTNM	Genotype
TS 10			F	41	Adenocarcinoma	IV	pT2bN2M1b	TP53 (R156 frameshift); RB1 (L694R); EGFR (ELREA746del); EGFR (T790M)
TS 32			F	55	Adenocarcinoma	IA	pT1N0M0	TP53 (R280S); WHSC1L1-FGFR1 fusion
A139			F	76	Adenocarcinoma	IA	pT1bN0M0	TP53 (R280S); ARID1A Deletion
A233			M	81	Adenocarcinoma	IA	pT1bN0M0	EGFR (L858R); MET Amplification, MET Exon 14 Skipping

Supplementary Table 2

Summary of metabolites detected in TS, Adh and GLDC KD cells (**Figure 1E**)

Total number of features in positive and negative ESI modes: 1409

Metabolites with confirmed identities

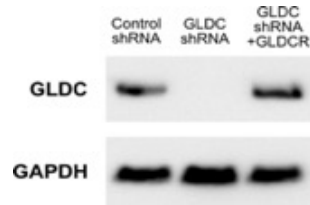
Class/Function	Metabolite ID	Fold-change with respect to Adh	
		Avg GLDC KD	Avg TS
Glycolysis intermediates	Glucose	0.072	0.005
	G6P/F6P	0.313	0.449
	F1,6P	0.689	0.530
	GD3P	0.575	0.655
	Lactate	0.219	1.036
TCA cycle intermediates	Malate	0.285	1.224
	Isocitrate/Citrate	0.714	1.832
Nucleotides	CMP	1.162	2.570
	UMP	1.057	3.305
	AMP	1.633	4.701
	GMP	2.327	11.079
	CDP	1.513	4.882
	ADP	1.007	2.723
	cAMP	1.311	2.309
	Guanosine	0.373	2.253
	Uridine	0.110	3.789
	Inosine	0.319	0.686
	Inosine phosphate	1.903	6.261
Sugars/Nucleotide sugars	N-acetylneuraminate	0.778	1.228
	Glucosamine/Galactosamine	0.771	1.025
	GDP-Fucose	1.172	2.124
	UDP-Glucose/UDP-Galactose	0.461	0.922
	UDP-GlcNAc/UDP-GalNAc	4.385	3.078
Amino acids	Taurine	0.000	0.000
	4-Hydroxy-L-Proline	1.129	0.987
	Aminobutanoate	1.821	4.047
	Arginine	12.024	23.917
	Histidine	1.363	3.558
	Aspartate	0.158	0.367
	Glutamate	2.157	5.701
	Alanine	0.093	0.367
	Valine	0.723	2.467
	Isoleucine	0.830	2.126
	Leucine	2.512	7.634
	Proline	0.808	3.774
	Tryptophan	1.230	4.662
	Phenylalanine	0.761	2.414
	Tyrosine	1.885	8.315
	Serine	0.847	0.691
Methionine cycle and redox metabolism	Methionine	1.303	3.818
	SAH	0.668	1.650
	SAM	1.515	1.412
	Glutamine	1.921	2.656
	Pyroglutamate	0.398	2.543
	Glutathione (Reduced)	1.482	2.679
	Glutathione (Oxidized)	0.897	1.293
Methyl group donors	Cysteine-glutathione disulfide	27.124	0.884
	Choline	3.549	4.784
Co-factors and vitamins	Betaine	15.076	63.656
	Riboflavin	17.623	38.186
	Folic acid	Absent in Adh	Absent in Adh
	Pantothenate	0.054	0.214
	NAD	0.812	1.244
	NADH	0.809	2.051
	NADP	0.949	1.730
Phospholipid synthesis	FAD	0.876	1.300
	CDP-choline	2.166	3.781
	CDP-ethanolamine	0.509	1.441
	Glycerophosphocholine	2.979	3.874
	Glycerophosphoethanolamine	1.887	5.004

Metabolites with putative identities

	Putative metabolite ID*	Fold-change with respect to Adh	
		Avg GLDC KD	Avg TS
*Putative metabolite IDs based on accurate mass matching with HMDB and KEGG databases	2-Phospho-glycerate/3-Phospho-glycerate	1.037	0.455
	7,8-Dihydrobiopterin	0.653	1.573
	10-Formyldihydrofolate	Absent in Adh	Absent in Adh
	Adenylosuccinate	1.001	23.944
	Ala-Ala/Methyl-glutamine	0.013	0.238
	Aminoimidazole ribotide	2.424	2.618
	Asp-Gly	1.106	0.963
	Asp-Phe	5.993	7.340
	beta-Asp-Glu	0.567	0.968
	CMP-N-glycoloylneuramate	2.71E-06	2.61E-06
	cyclic dGMP	0.891	1.170
	Deoxy-ribose phosphate/Deoxy-xylulose phosphate	9.328	30.968
	Dihydroxy-phenylalanine	Absent in Adh	Absent in Adh
	Flavin mononucleotide	0.792	1.488
	Formyldihydrofolate	14.631	52.256
	gamma-Glu-Gln	Absent in Adh	Absent in Adh
	Glu-Glu	86.079	349.636
	Glycerophosphoglycerol	0.243	0.902
	Glycerophosphoinositol	0.048	0.106
	Glycerophosphoric acid	0.365	1.264
	Homovanillate	1.124	0.989
	Lys-Ala-Ala	24.109	201.054
	Methylhippuric acid	13.994	36.452
	N2-Acetyl-L-aminoadipate	3.482	6.350
	N-Acetylasparagine/N-Formimino-glutamate	1.681	6.484
	N-Acetylglucosamine/N-Acetylgalactosamine/Biopterin	1.079	0.833
	N-Acetylglucosamine phosphate/N-Acetylgalactosamine phosphate	1.620	1.641
	N-Acetylglutamine/Glycyl-hydroxyproline	3.230	8.694
	N-glycoloylneuramate	0.001	0.000
	N-Methyl-aspartate	199.239	758.484
	Phenylpyruvate	0.745	1.724
	Phosphocholine	2.750	4.261
	Phosphoribosyl-AMP	0.920	2.190
	Pro-Gly	0.082	0.129
	Propanoyl phosphate/Glycerol cyclic phosphate	0.245	0.988
	Riboflavin cyclic phosphate	0.877	1.204
	S-(Formylmethyl)glutathione	5.971	26.082
	S-Lactoylglutathione	0.355	0.271
	Selenohomocysteine	0.341	0.145
	S-Methyl-thioribose phosphate	14.109	163.343
	Thiamin diphosphate	0.606	0.365

Supplementary Table 3

Comparison of metabolite abundances detected in TS, Control Luc shRNA knockdown and GLDC KD Rescue cells normalized to the abundances in Adh cells. Top panel shows GLDC abundance of indicated cell lines. GAPDH was used as loading control.



Class/Function	Metabolite ID	Fold-change with respect to Adh			p-value with respect to Adh (Welch's t-test)		
		Avg TS	Ave Luc	Ave Rescue	TS	Luc	Rescue
Glycolysis Intermediate	Glucose	0.65	0.61	0.63	0.003	<0.001	0.023
	G6P/F6P	0.80	1.08	1.15	0.011	0.429	0.309
	F1,6P	1.60	2.14	1.72	0.010	<0.001	0.029
	GD3P	0.38	0.31	0.41	0.020	0.018	0.014
	Lactate	1.21	1.31	2.30	0.110	0.019	0.033
TCA Cycle Intermediates	Malate	1.47	1.40	1.76	0.020	0.008	0.007
	Isocitrate/Citrate	2.11	2.09	2.30	0.010	<0.001	<0.001
Nucleotides	CMP	6.13	7.85	9.87	<0.001	<0.001	0.010
	UMP	7.97	8.54	11.68	0.004	<0.001	0.004
	AMP	7.07	10.66	13.29	0.003	<0.001	0.012
	GMP	10.53	14.07	18.56	0.008	0.004	0.027
	CDP	4.52	3.75	4.16	0.002	<0.001	0.012
	ADP	2.63	2.94	3.14	0.007	<0.001	0.012
	cAMP	1.38	1.24	1.79	0.068	0.147	0.009
	Guanosine	0.29	0.41	0.79	0.031	0.047	0.258
	Uridine	0.27	0.48	0.56	0.085	0.147	0.193
	Inosine	0.81	1.47	4.73	0.397	0.120	0.057
Sugars/Nucleotides sugars	Inosine phosphate	2.92	1.62	2.88	0.011	0.008	0.034
	N-acetylneuraminate	3.25	5.11	5.18	0.010	0.002	0.024
	Glucosamine	1.18	1.17	1.10	0.015	0.015	0.042
	GDP-Fucose	1.83	1.74	2.39	0.060	0.004	0.041
	UDP-Glucose/UDP-Galactose	1.14	0.95	1.12	0.314	0.570	0.604
Amino acids	UDP-GlcNAc/UDP-GalNAc	2.39	6.58	4.96	0.031	<0.001	0.077
	Taurine	0.02	0.03	0.03	0.002	0.002	0.002
	4-Hydroxy-L-Proline	1.17	1.16	1.11	0.011	0.011	0.056
	Aminobutanoate	5.78	6.00	7.70	0.005	<0.001	0.023
	Arginine	5.05	6.75	3.45	<0.001	<0.001	0.004
	Histidine	10.90	10.82	13.39	0.012	0.001	0.017
	Aspartate	1.17	1.25	1.06	0.308	0.096	0.769
	Glutamic acid	7.63	7.90	9.78	0.011	<0.001	0.019
	Valine	3.64	4.42	4.69	0.009	<0.001	0.015
	Isoleucine	4.39	5.63	6.15	0.009	<0.001	0.011
	Leucine	5.47	7.07	7.25	0.008	<0.001	0.011
	Proline	4.25	6.56	6.10	0.002	<0.001	0.024
	Tryptophan	3.93	4.67	5.60	0.008	<0.001	0.022
	Phenylalanine	5.15	6.30	7.09	0.007	<0.001	0.017
	Tyrosine	4.48	5.30	6.08	0.005	<0.001	0.007
	Serine	3.17	4.68	4.19	0.059	<0.001	0.085
Methionine cycle and redox metabolism	Methionine	9.32	11.50	12.27	0.007	<0.001	0.021
	SAH	2.29	2.41	3.54	0.013	0.017	0.006
	SAM	2.40	1.76	2.03	0.002	0.001	0.013
	Glutamine	38.60	49.46	46.52	0.003	0.001	0.021
	Pyroglutamic acid	4.93	4.84	6.56	0.005	<0.001	0.018
	Glutathione (Reduced)	5.20	3.63	4.73	0.002	<0.001	0.007
	Glutathione (Oxidised)	3.80	2.83	3.57	0.054	0.006	0.001
Methyl Group Donors	L-Cysteine-glutathione Disulfide	3.37	3.67	3.52	0.106	0.035	0.197
	Choline	1.42	2.66	2.43	0.012	<0.001	0.029
Co-factors and vitamins	Betaine	11.13	16.39	15.34	0.003	<0.001	0.012
	Riboflavin	18.93	19.75	24.22	0.001	<0.001	0.018
	Folic acid	57.17	58.49	78.21	0.014	0.005	0.010
	Pantothenic acid	1.12	1.12	1.32	0.330	0.233	0.213
	NAD	1.45	1.48	1.61	0.005	0.006	0.053
	NADH	1.02	1.35	2.20	0.897	0.129	0.077
	NADP	0.95	1.16	1.21	0.553	0.266	0.265
Phospholipid synthesis	FAD	0.76	0.98	1.18	0.068	0.833	0.229
	CDP-ethanolamine	0.79	1.18	1.05	0.124	0.141	0.788
	Glycerophosphocholine	0.30	0.90	0.93	0.010	0.318	0.608
	Glycerophosphoethanolamine	0.58	1.11	0.98	0.016	0.275	0.837

Supplementary Table 4

Information on patient data for samples in tumor microarrays.

Matched Tumor vs. Normal TMA (Fig. 4c and Extended Fig. 4b)					
No.	Age	Gender	Anatomic Site	Pathology	Histological Grade
1	77	M	Lung	Squamous cell carcinoma	II
2	64	M	Lung	Adenosquamous carcinoma	Mixed subtype (No grade mentioned)
3	78	F	Lung	Adenocarcinoma	II
4	72	F	Lung	Adenocarcinoma	II~III
5	81	M	Lung	Large cell carcinoma	III
6	66	F	Lung	Adenocarcinoma	II
7	79	F	Lung	Adenocarcinoma	II
8	69	F	Lung	Adenocarcinoma	II
9	83	M	Lung	Adenocarcinoma	I
10	61	F	Lung	Adenocarcinoma	II
11	61	F	Lung	Adenocarcinoma	II
12	65	F	Lung	Adenocarcinoma	II
13	83	F	Lung	Adenocarcinoma	II
14	53	F	Lung	Adenocarcinoma	II
15	71	M	Lung	Adenocarcinoma	III
16	Not Specified	Not Specified	Lung	Adenocarcinoma	No grade mentioned
17	72	M	Lung	Squamous cell carcinoma	II
18	78	M	Lung	Adenocarcinoma	II
19	81	M	Lung	Adenocarcinoma	No grade mentioned
20	69	M	Lung	Squamous cell carcinoma	III
21	78	M	Lung	Squamous cell carcinoma	III
22	80	F	Lung	Adenocarcinoma	I~II
23	83	M	Lung	Adenocarcinoma	No grade mentioned
24	74	F	Lung	Adenocarcinoma	I
25	61	M	Lung	Squamous cell carcinoma	II
26	69	M	Lung	Adenocarcinoma	II
27	68	M	Lung	Squamous cell carcinoma	II
28	81	M	Lung	Adenocarcinoma	II
29	53	M	Lung	Adenocarcinoma	II
30	69	F	Lung	Adenocarcinoma	I
31	62	F	Lung	Adenocarcinoma	No grade mentioned
32	73	F	Lung	Adenocarcinoma	No grade mentioned
33	74	F	Lung	Adenocarcinoma	II~III
34	82	M	Lung	Squamous cell carcinoma	III
35	62	M	Lung	Adenocarcinoma	No grade mentioned
36	64	F	Lung	Adenocarcinoma	No grade mentioned
37	68	F	Lung	Adenocarcinoma	No grade mentioned
38	77	M	Lung	Squamous cell carcinoma	III
39	82	M	Lung	Adenocarcinoma	II
40	69	F	Lung	Adenocarcinoma	II
41	70	F	Lung	Adenocarcinoma	II
42	52	M	Lung	Squamous cell carcinoma	II
43	81	M	Lung	Mucinous adenocarcinoma	No grade mentioned
44	73	M	Lung	Squamous cell carcinoma	II~III
45	75	M	Lung	Squamous cell carcinoma, clear cell variant	III
46	79	M	Lung	Squamous cell carcinoma	II~III
47	81	M	Lung	Squamous cell carcinoma, non-keratinizing	II

Tumor Grade and Metastasis TMA (Fig. 4d and Extended Fig. 4c)					
No.	Age	Gender	Anatomic Site	Pathology	Stage
1	56	M	Lung	Squamous cell carcinoma	I
2	69	M	Lung	Squamous cell carcinoma	I
3	41	M	Lung	Squamous cell carcinoma	I
4	60	M	Lung	Squamous cell carcinoma	I
5	56	M	Lung	Squamous cell carcinoma	I
6	62	M	Lung	Squamous cell carcinoma	I
7	55	M	Lung	Squamous cell carcinoma	I
8	53	M	Lung	Squamous cell carcinoma	I
9	45	M	Lung	Adenocarcinoma	I
10	44	F	Lung	Adenocarcinoma	II
11	59	F	Lung	Adenocarcinoma	II
12	65	F	Lung	Adenocarcinoma	II
13	60	M	Lung	Adenocarcinoma	II
14	52	M	Lung	Adenocarcinoma	II
15	70	M	Lung	Adenocarcinoma	II
16	64	M	Lung	Adenocarcinoma	II
17	39	F	Lung	Adenocarcinoma	II
18	60	M	Lung	Squamous cell carcinoma	II
19	74	M	Lung	Squamous cell carcinoma	II
20	65	M	Lung	Squamous cell carcinoma	II
21	49	M	Lung	Squamous cell carcinoma	II
22	73	M	Lung	Squamous cell carcinoma	II
23	57	M	Lung	Squamous cell carcinoma	II
24	56	M	Lung	Squamous cell carcinoma	II
25	54	M	Lung	Squamous cell carcinoma	II
26	50	F	Lung	Squamous cell carcinoma	II
27	54	M	Lung	Squamous cell carcinoma	II
28	52	M	Lung	Squamous cell carcinoma	II
29	80	M	Lung	Adenocarcinoma	II
30	66	M	Lung	Adenocarcinoma	II
31	57	F	Lung	Adenocarcinoma	II
32	68	M	Lung	Adenocarcinoma	II
33	77	M	Lung	Squamous cell carcinoma	II
34	70	M	Lung	Squamous cell carcinoma	II
35	74	M	Lung	Squamous cell carcinoma	II
36	63	M	Lung	Squamous cell carcinoma	II
37	72	M	Lung	Squamous cell carcinoma	II
38	74	M	Lung	Squamous cell carcinoma	II
39	53	M	Lung	Squamous cell carcinoma	II
40	78	M	Lung	Squamous cell carcinoma	II
41	55	M	Lung	Adenocarcinoma	III
42	60	M	Lung	Adenocarcinoma	III
43	58	F	Lung	Adenocarcinoma	III
44	50	M	Lung	Squamous cell carcinoma	III
45	66	M	Lung	Squamous cell carcinoma	III
46	54	M	Lung	Squamous cell carcinoma	III
47	31	F	Lung	Squamous cell carcinoma	III
48	51	M	Lung	Squamous cell carcinoma	III
49	50	M	Lung	Squamous cell carcinoma	III
50	53	M	Lung	Squamous cell carcinoma	III

No.	Age	Gender	Anatomic Site	Pathology	Stage
51	51	M	Lung	Squamous cell carcinoma	III
52	60	M	Lung	Squamous cell carcinoma	III
53	40	F	Lung	Squamous cell carcinoma	III
54	51	M	Lung	Squamous cell carcinoma	III
55	73	M	Lung	Squamous cell carcinoma	III
56	65	M	Lung	Squamous cell carcinoma	III
57	48	M	Lung	Squamous cell carcinoma	III
58	62	M	Lung	Squamous cell carcinoma	III
59	53	M	Lung	Squamous cell carcinoma	III
60	54	M	Lung	Squamous cell carcinoma	III
61	76	M	Lung	Squamous cell carcinoma	III
62	44	F	Lung	Squamous cell carcinoma	III
63	52	M	Lung	Squamous cell carcinoma	III
64	50	M	Lung	Squamous cell carcinoma	III
65	55	M	Lung	Squamous cell carcinoma	III
66	51	M	Lung	Adenocarcinoma	III
67	67	F	Lung	Adenosquamous carcinoma	I
68	52	M	Lung	Squamous cell carcinoma	I
69	52	M	Lung	Adenocarcinoma	I
70	62	M	Lung	Squamous cell carcinoma	I
71	66	M	Lung	Squamous cell carcinoma	I
72	62	M	Lung	Squamous cell carcinoma	I
73	62	F	Lung	Adenosquamous carcinoma	I
74	70	M	Lung	Squamous cell carcinoma	I
75	44	M	Lung	Adenosquamous carcinoma	II
76	75	F	Lung	Adenosquamous carcinoma	II
77	69	M	Lung	Adenocarcinoma	II
78	51	M	Lung	Squamous cell carcinoma	II
79	60	F	Lung	Adenosquamous carcinoma	II
80	52	M	Lung	Squamous cell carcinoma	II
81	56	M	Lung	Adenocarcinoma	II
82	60	M	Lung	Squamous cell carcinoma	II
83	55	F	Lung	Adenocarcinoma	II
84	57	F	Lung	Adenocarcinoma	II
85	56	M	Lung	Adenocarcinoma	II
86	63	M	Lung	Adenocarcinoma	II
87	63	M	Lung	Squamous cell carcinoma	II
88	57	F	Lung	Adenocarcinoma	II
89	64	M	Lung	Squamous cell carcinoma	II
90	63	M	Lung	Adenocarcinoma	II
91	64	M	Lung	Squamous cell carcinoma	II
92	51	F	Lung	Adenocarcinoma	II
93	52	M	Lung	Adenocarcinoma	II
94	43	F	Lung	Squamous cell carcinoma	II
95	73	M	Lung	Adenosquamous carcinoma	II
96	51	M	Lung	Squamous cell carcinoma	II
97	60	M	Lung	Squamous cell carcinoma	II
98	73	M	Lung	Squamous cell carcinoma	II
99	68	M	Lung	Adenosquamous carcinoma	III
100	62	M	Lung	Squamous cell carcinoma	III
101	40	F	Lung	Squamous cell carcinoma	III
102	58	F	Lung	Adenocarcinoma	III
103	76	M	Lung	Squamous cell carcinoma	III
104	53	M	Lung	Squamous cell carcinoma	III
105	53	M	Lung	Adenocarcinoma	III

No.	Age	Gender	Anatomic Site	Pathology	Stage
106	59	M	Lung	Adenocarcinoma	III
107	72	M	Lung	Adenocarcinoma	III
108	54	M	Lung	Squamous cell carcinoma	III
109	70	M	Lung	Adenocarcinoma	III
110	52	M	Lung	Squamous cell carcinoma	III
111	62	M	Lung	Squamous cell carcinoma	III
112	64	M	Lung	Undifferentiated carcinoma	III~IV
113	51	F	Lung	Large cell carcinoma	IV
114	64	M	Lymph node	Lymph node metastasis	Met
115	51	F	Lymph node	Lymph node metastasis	Met
116	40	F	Lymph node	Lymph node metastasis	Met
117	58	F	Lymph node	Lymph node metastasis	Met
118	76	M	Lymph node	Lymph node metastasis	Met
119	53	M	Lymph node	Lymph node metastasis	Met
120	42	M	Lymph node	Lymph node metastasis	Met
121	43	F	Lymph node	Lymph node metastasis	Met
122	60	F	Lymph node	Lymph node metastasis	Met
123	53	M	Lymph node	Lymph node metastasis	Met
124	59	M	Lymph node	Lymph node metastasis	Met
125	72	M	Lymph node	Lymph node metastasis	Met
126	52	M	Lymph node	Lymph node metastasis	Met
127	56	M	Lymph node	Lymph node metastasis	Met
128	60	M	Lymph node	Lymph node metastasis	Met
129	55	F	Lymph node	Lymph node metastasis	Met
130	54	M	Lymph node	Lymph node metastasis	Met
131	57	F	Lymph node	Lymph node metastasis	Met
132	70	M	Lymph node	Lymph node metastasis	Met
133	56	M	Lymph node	Lymph node metastasis	Met
134	52	M	Lymph node	Lymph node metastasis	Met
135	73	M	Lymph node	Lymph node metastasis	Met
136	63	M	Lymph node	Lymph node metastasis	Met
137	51	M	Lymph node	Lymph node metastasis	Met
138	63	M	Lymph node	Lymph node metastasis	Met
139	57	F	Lymph node	Lymph node metastasis	Met
140	64	M	Lymph node	Lymph node metastasis	Met
141	63	M	Lymph node	Lymph node metastasis	Met
142	52	M	Lymph node	Lymph node metastasis	Met
143	60	M	Lymph node	Lymph node metastasis	Met
144	62	M	Lymph node	Lymph node metastasis	Met
145	73	M	Lymph node	Lymph node metastasis	Met
146	64	M	Lymph node	Lymph node metastasis	Met
147	66	M	Lymph node	Lymph node metastasis	Met
148	62	M	Lymph node	Lymph node metastasis	Met
149	62	M	Lymph node	Lymph node metastasis	Met
150	52	M	Lymph node	Lymph node metastasis	Met
151	62	F	Lymph node	Lymph node metastasis	Met
152	51	F	Lymph node	Lymph node metastasis	Met
153	52	M	Lymph node	Lymph node metastasis	Met
154	70	M	Lymph node	Lymph node metastasis	Met

Supplementary Table 5: Elution conditions for LC/MS analysis.

	Time (min)	Solvent A (%)	Solvent B1 (%)	Solvent B2 (%)	Flow rate (mL/min)
Sample analysis	Initial	99.9	0.1		0.400
	0.50	99.9	0.1		
	8.50	50.0	50.0		
	8.51	2.0	98.0		
	11.50	2.0	98.0		
Column wash	12.50	2.0		98.0	0.500
	15.50	2.0		98.0	
Column equilibration	16.50	99.9	0.1		0.400
	18.00	99.9	0.1		

Solvent A: 0.1% formic acid in water, Solvent B1: 0.1% formic acid in methanol and Solvent B2: 0.1% formic acid in acetonitrile.

Supplementary Table 6: Optimized compound-dependent MS parameters using Xevo TQ-S mass spectrometer.

Analyte	Precursor ion mass (m/z)	Fragment ion mass (m/z)	Dwell time (s)	Cone voltage (V)	Collision Energy (V)	ESI Mode
Methionine	150	105	0.025	40	15	Pos
Methionine ($^{13}\text{C}_5^{15}\text{N}_1$)	156	109	0.025	40	15	Pos
*Regenerated methionine from Homocysteine ($^{13}\text{C}_4^{15}\text{N}_1$)	155	108	0.025	40	15	Pos
S-adenosylmethionine	399	136	0.025	62	42	Pos
*SAM ($^{13}\text{C}_5^{15}\text{N}_1$)	405	136	0.025	62	42	Pos
*Regenerated SAM from Homocysteine ($^{13}\text{C}_4^{15}\text{N}_1$)	404	136	0.025	62	42	Pos
S-adenosylhomocysteine	385	134	0.025	66	33	Pos
*SAH ($^{13}\text{C}_5^{15}\text{N}_1$)	390	139	0.025	66	33	Pos
Homocysteine	136	90	0.025	15	16	Pos
Deuterium Labelled Homocysteine ($^2\text{D}_4$)	140	94	0.025	15	16	Pos
*Methionine ($^2\text{D}_4$)	154	108	0.025	40	15	Pos
*SAM ($^2\text{D}_4$)	404	136	0.025	62	42	Pos
*SAH ($^2\text{D}_4$)	389	137	0.025	66	33	Pos
Hippuric acid (Internal Standard)	185	110	0.025	30	16	Pos

*Intracellularly synthesized metabolites

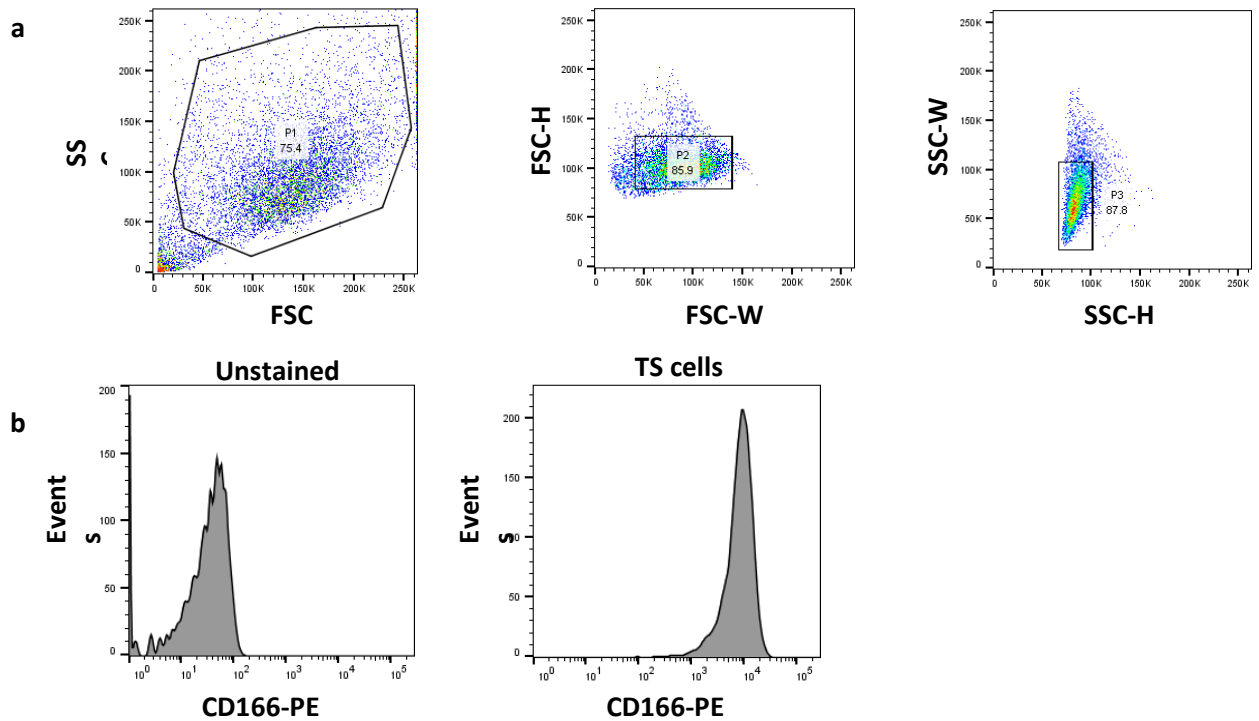
Supplementary Table 7: Metabolite species in ^{13}C -labeled methionine pulse-chase experiment and the corresponding MRM transition pairs (Q1/Q3).

Metabolites		Q1/Q3
Methionine	Endogenous methionine	150/105
	Methionine ($^{13}\text{C}_5^{15}\text{N}_1$)	156/109
	*Regenerated methionine from Homocysteine ($^{13}\text{C}_4^{15}\text{N}_1$)	155/108
S-adenosylmethionine	Endogenous S-adenosylmethionine	399/136
	*SAM ($^{13}\text{C}_5^{15}\text{N}_1$)	405/136
	*Regenerated SAM from Homocysteine ($^{13}\text{C}_4^{15}\text{N}_1$)	404/136
S-adenosylhomocysteine	Endogenous S-adenosylhomocysteine	385/134
	*SAH ($^{13}\text{C}_5^{15}\text{N}_1$)	390/139

*Intracellularly synthesized metabolites

Supplementary Figure 1

Gating strategy



a) Gating strategy from total cell population. b) Histogram of CD166-PE staining of a negative “unstained” sample and a stained freshly sorted TS cell sample.

Supplementary Figure 2

Unprocessed Western Blots in Supplementary Table 3

