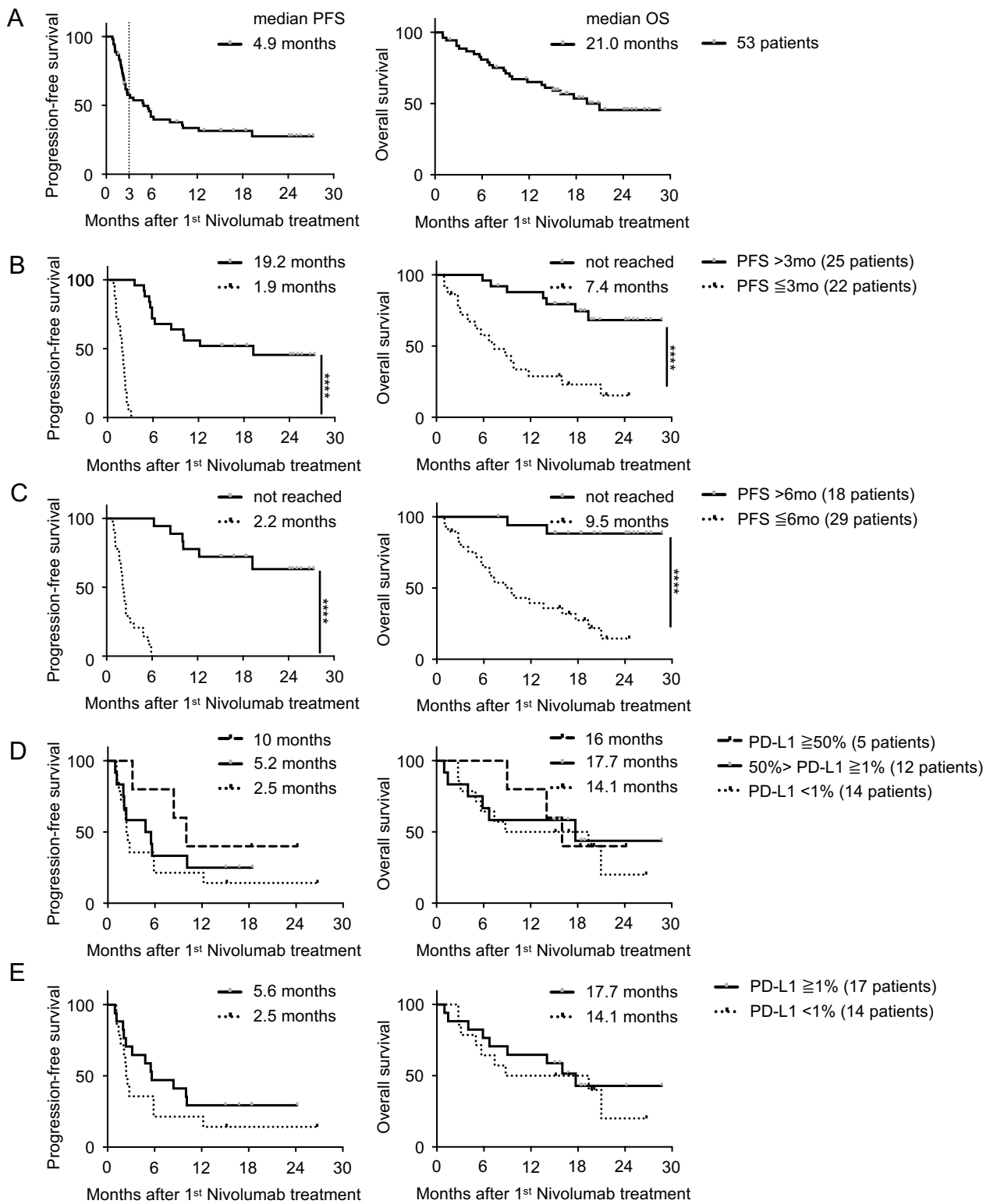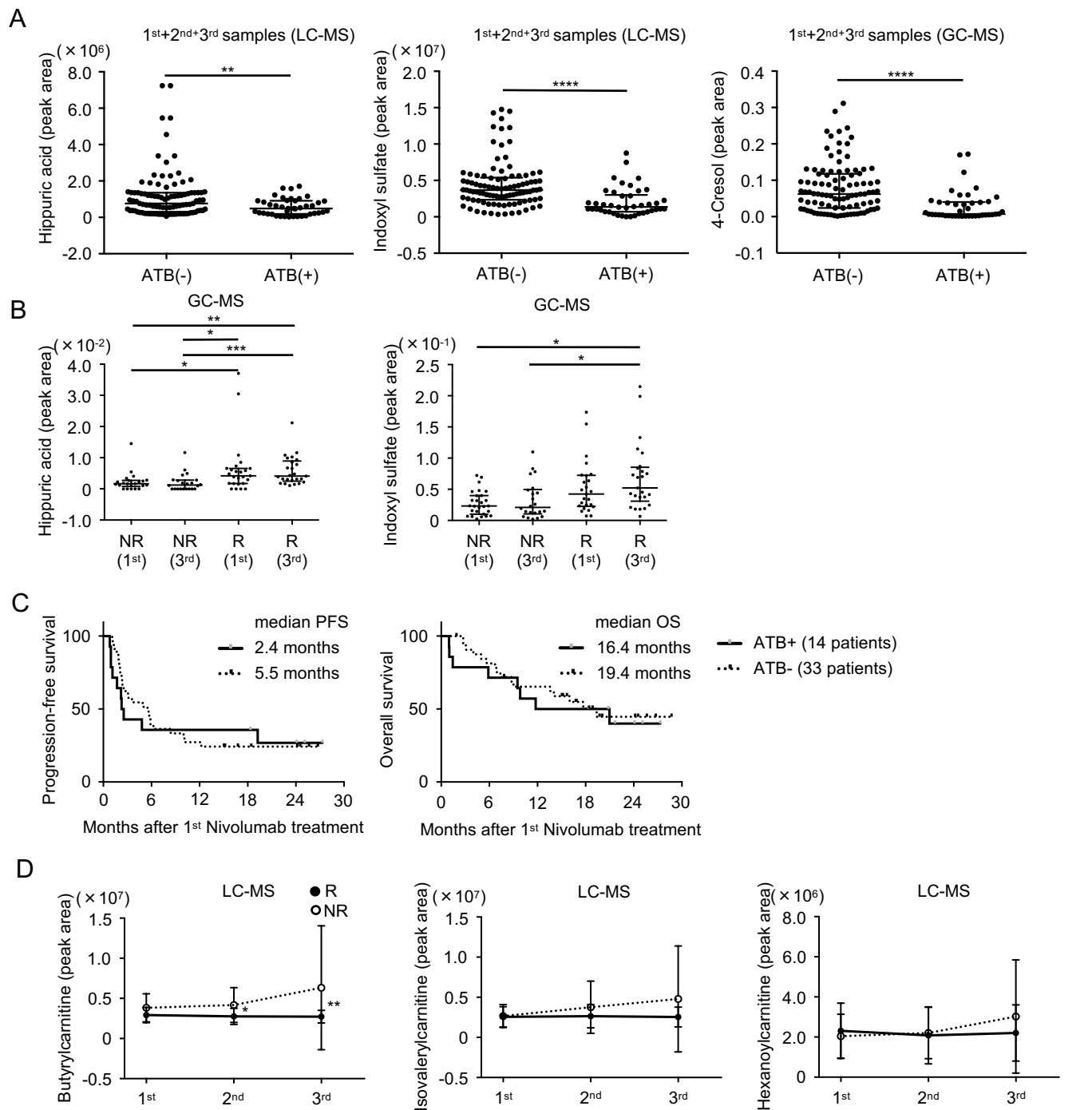# Supplementary Fig. S1

Supplementary Fig. S1. Survival rates are compared between sorted groups based on different criteria. A) Kaplan–Meier plots of PFS and OS of all patients enrolled in this study. **B**) Kaplan–Meier plots of PFS and OS of patients sorted by the criteria of PFS > 3 months (solid line) and PFS ≤ 3 months (dotted line). **C**) The solid line and dotted line show patients with PFS > 6 months and PFS ≤ 6 months, respectively. ****$p < 0.0001$ by Log-rank test. **D**) Kaplan–Meier plots of PFS and OS of patients sorted by frequency of PD-L1 expression on tumors. The dashed line, solid line, and dotted line show patients with high PD-L1 expression (greater than 50%), low PD-L1 expression (1–50%), and rare PD-L1 expression (less than 1%), respectively. **E**) The solid line and dotted line show patients with positive expression (greater than 1%) and negative expression (less than 1%) of PD-L1, respectively.
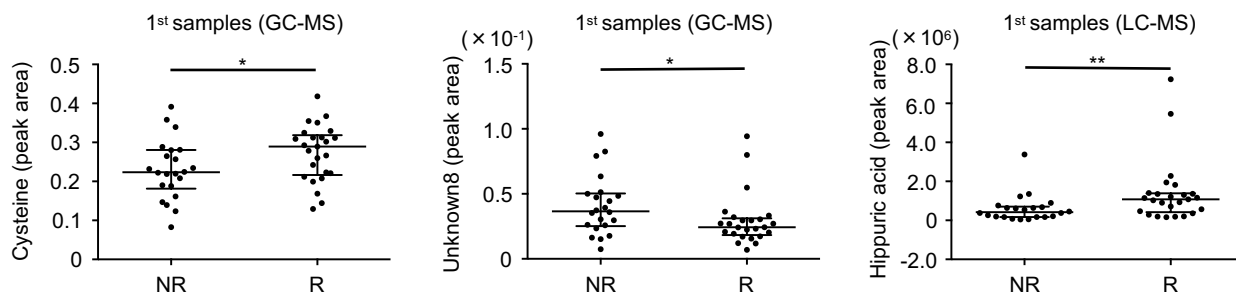
Supplementary Fig. S2

A

B

C

D

**Supplementary Fig. S2. Behaviors of microbiota-derived metabolites and acylcarnitine species.**
A) The peak area measured by GC- or LC-MS of each microbiome related metabolites in patients
without pre-antibiotics treatment (ATB(-)) and with pre-antibiotics treatment (ATB(+)) are shown. These
graphs display the data of the $1^{st} + 2^{nd} + 3^{rd}$ samples. $**p < 0.01$, $****p < 0.0001$ by Wilcoxon rank sum
test. **B)** The peak areas measured by GC-MS of hippuric acid and indoxyl sulfate in non-responders
(NR) and responders (R) are shown. Each dot represents one patient. Error bars show median and
interquartile range. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ by Kruskal–Wallis test followed by Dunn's multiple
comparisons test. **C**) Kaplan–Meier plots of PFS and OS of patients with (solid line) and without ATB
(dotted line) within 3 months prior to the first nivolumab injection. **D**) The peak area detected by LC-MS
of acylcarnitines between 1st, 2nd and 3rd samples are shown. The solid line and dotted line represent
responders and non-responders, respectively. $*p < 0.05$, $**p < 0.01$ by Wilcoxon rank sum test.

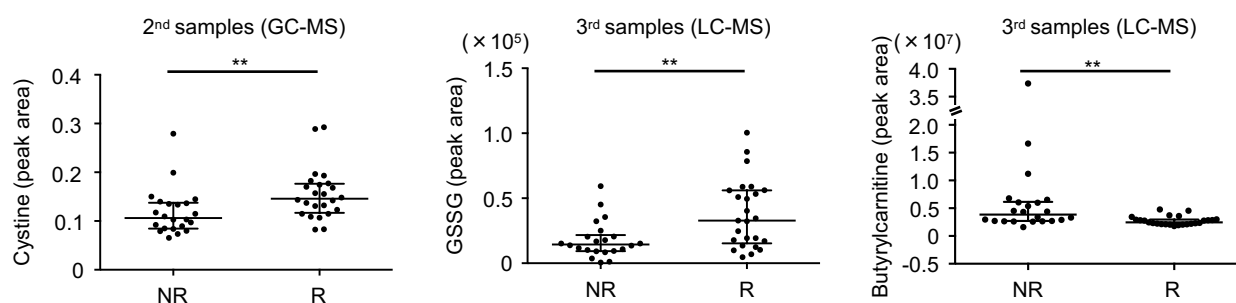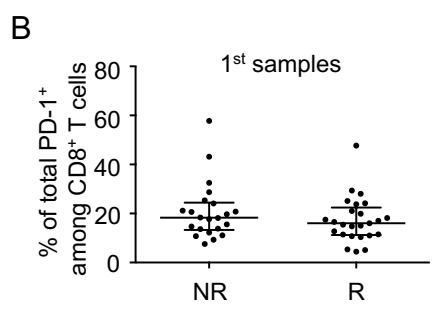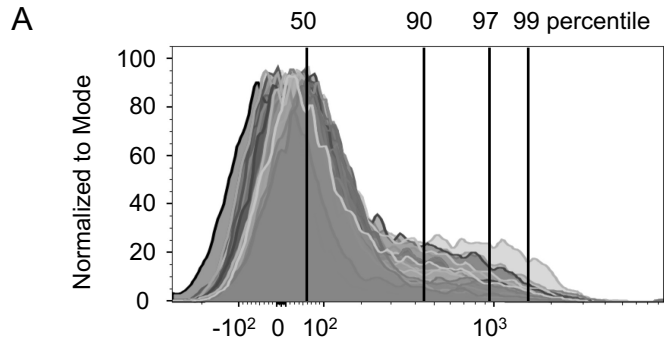# Supplementary Fig. S3
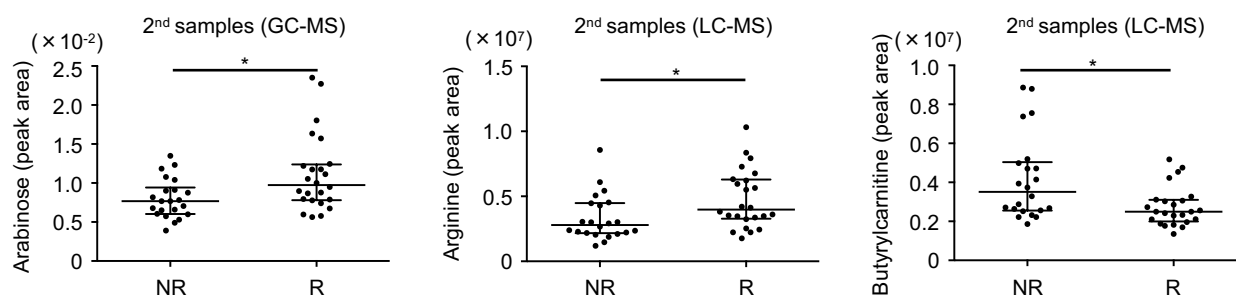
Supplementary Fig. S3. Detailed data of metabolite markers selected by stepwise analysis.
A–C) Graphs show comparison of peak areas of metabolic markers selected by stepwise analysis between non-responders and responders. Each dot represents one patient. Error bars indicate the median and interquartile range. *$p < 0.05$, **$p < 0.01$ by Wilcoxon rank sum test.
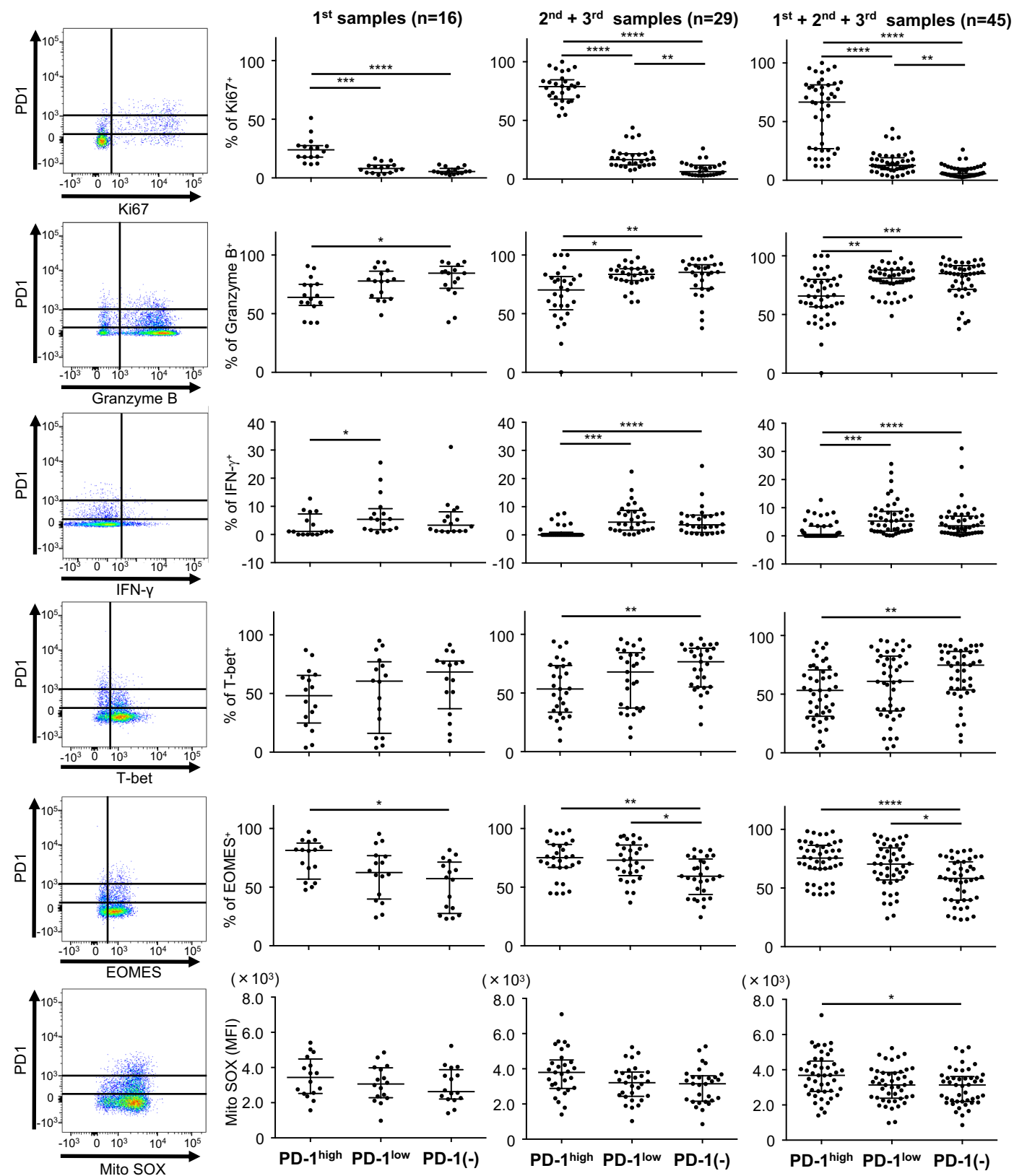
Supplementary Fig. S4

A

PD-1^{high} cut-off	CTLA-4	Tim-3	Lag-3
90 percentile	*0.59	0.73	0.53
97 percentile	**0.65**	**0.76**	**0.53**
99 percentile	0.63	0.69	0.47

* r values by Pearson

B

Supplementary Fig. S4. Definition of PD-1^{high}. A) Overlay histograms of PD-1 intensity gated on CD8$^+$ T cells from age-matched 30 healthy donors. Lines show the 50th, 90th, 97th, and 99th percentile of PD-1 intensity averaged with the donors. The correlation r values between % of PD-1^{high} based on each percentile and gene expression of exhaustion markers (CTLA-4, Tim-3 and Lag-3) in the patients' CD8$^+$ T cells are shown in the table. Since r values are highest in 97th percentile, the 97th percentile was used as cut-off in this study. **B)** Graph shows total PD-1 positive frequency among peripheral CD8$^+$ T cells in non-responders (NR) and responders (R) at the pre-treatment (1st) point.

Supplementary Fig. S5

Supplementary Fig. S5. Detailed phenotypes of PD-1 positive subsets in CD8+ T cells. FACS data show the expression levels of PD-1, Ki-67, Granzyme B, IFN-γ, T-bet, EOMES, and Mito SOX among CD8+ T cell in PBMC. Representative FACS plots of NSCLC samples are depicted (left panels). Indicated parameters between PD-1hi, PD-1low, and PD-1 negative CD8+ T cells are shown. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ by Kruskal–Wallis test followed by Dunn's multiple comparisons test.
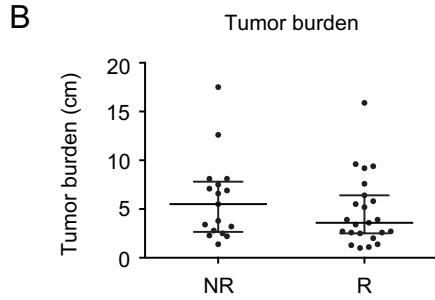
Supplementary Fig. S6

A
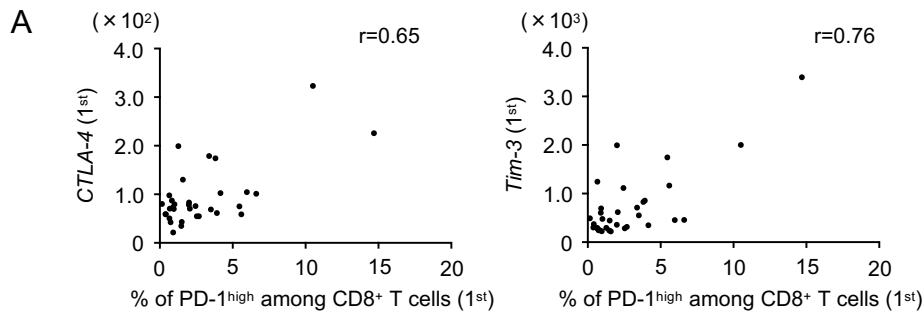
B

Marker name among CD8+ PD-1high	p-value*	Time point	Number of patients analyzed
Ki67+ (%)	0.31	1st	16
Ki67+ (%)	0.19	2nd	16
Ki67+ (%)	0.91	3rd	13
Granzyme B+ (%)	0.18	1st	16
Granzyme B+ (%)	0.85	2nd	16
Granzyme B+ (%)	0.25	3rd	13
IFN-γ+ (%)	0.43	1st	16
IFN-γ+ (%)	0.51	2nd	16
IFN-γ+ (%)	0.65	3rd	13
T-bet+ (%)	0.32	1st	16
T-bet+ (%)	0.32	2nd	16
T-bet+ (%)	0.71	3rd	13
EOMES+ (%)	0.09	1st	16
EOMES+ (%)	0.83	2nd	16
EOMES+ (%)	0.15	3rd	13

*: p-value for distinction between R and NR (Wilcoxon rank sum test).

Supplementary Fig. S6. Characteristics of PD-1high CD8+ T cells. A) Scatter plots between frequency of PD-1high (X-axis) and exhaustion marker gene expression in purified CD8+ T cells from the same cohort (Y-axis). r: Pearson correlation coefficients. Generally, |r| of more than 0.6 in Pearson are considered to have strong correlation. **B)** p-values of each frequency among CD8+ PD-1high T cells between non-responders and responders are listed in the table.

Supplementary Fig. S7

A

B
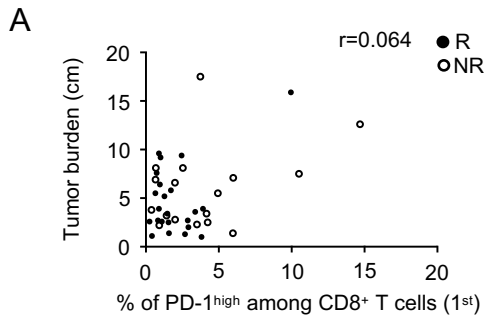
Supplementary Fig. S7. Correlation analysis between PD-1^{high} CD8$^+$ T cells and tumor burden. A) Scatter plots between frequency of PD-1^{high} (X-axis) and tumor barden (Y-axis). The dots and the circles represent the responders and the non-responders, respectively. r: Spearman correlation coefficients. **B)** Graphs show comparison of tumor burden between non-responders and responders. Each dot represents one patient. Error bars indicate the median and interquartile range.

Supplementary Fig. S8
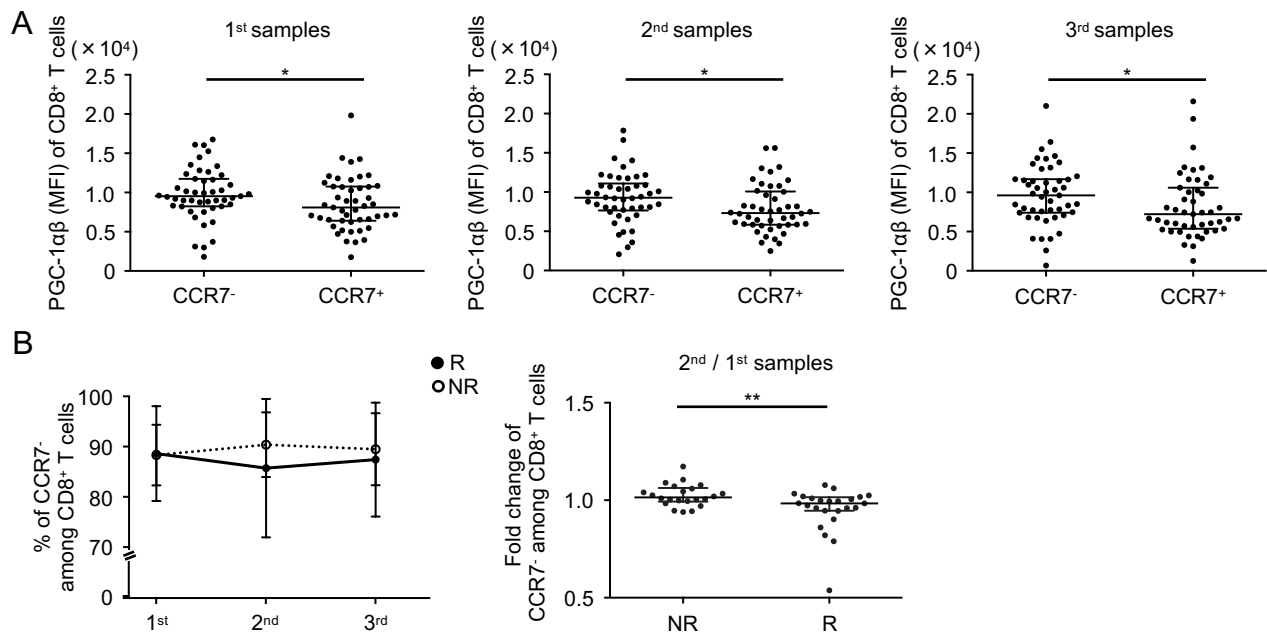
Supplementary Fig. S8. CCR7- CD8+ T cells with higher PGC-1αβ expression decreased by PD-1 blockade.

A) PGC-1αβ expression (MFI) between CCR7- and CCR7+ T cells in the 1st (left), 2nd (middle), and 3rd samples (right) were shown. Each dot represents one patient. Error bars indicate the median and interquartile range. *$p < 0.05$ by Wilcoxon rank sum test. **B)** Frequency of CCR7- among peripheral CD8+ T cells in the 1st, 2nd, and 3rd samples (left). The solid line and dotted line represent responders and non-responders, respectively. Fold change (2nd/1st) of CCR7- frequency among CD8+ T cells in non-responders and responders (right). **$p < 0.01$ by Wilcoxon rank sum test.

Supplementary Fig. S9
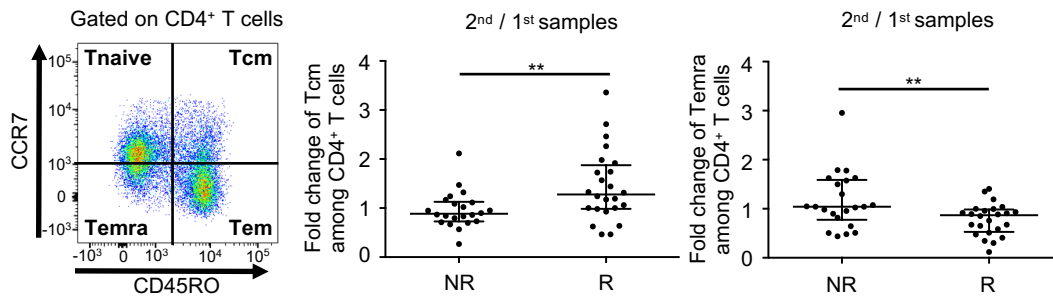
Supplementary Fig. S9. Change of CD4+ T cell subset ratios by PD-1 blockade. CD4+ T cells are divided into naïve, Tcm, Tem, and Temra subsets according to the expression of CD45RO and CCR7 (left panel). The frequency of CD4+ Tcm and CD4+ Temra in non-responders and responders is shown (middle and right panels). **$p < 0.01$ by Wilcoxon rank sum test.

Supplementary Fig. S10

Cellular marker combination II
(NSCLC patients with EGFR mutation, error rate: 0%)
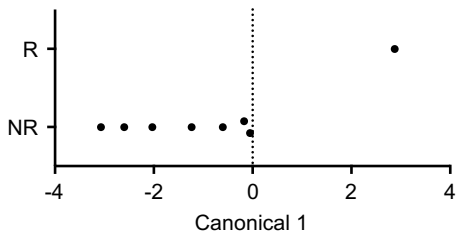
Supplementary Fig. S10. Results of cellular marker combination II in patients with EGFR mutation.
Canonical plot for NSCLC patients with EGFR mutation based on cellular marker combination II.

Supplementary Fig. S11

Pyroglutamic acid ⟶ L-Glutamate ⟶ Cysteine ⇌ Cystine

L-γ-Glutamylcysteine

Glycine ⟶

GSSG ⟵ ROS ⟵ GSH
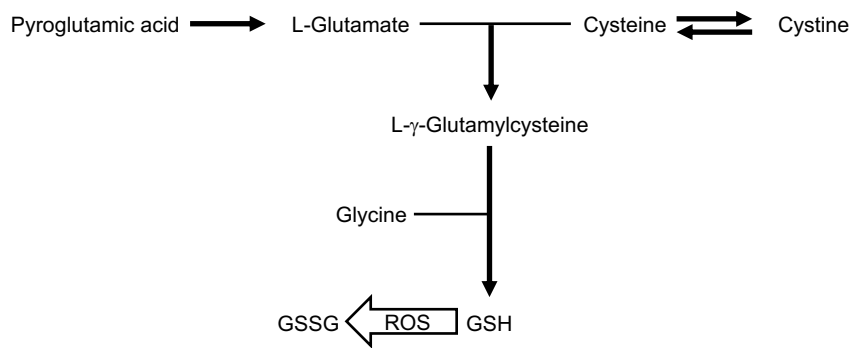
Supplementary Fig. S11. Schema showing biosynthesis and metabolism of glutathione.
L-Glutamate and Cysteine are combined to form L-γ-Glutamylcysteine, which is then combined with glycine to yield GSH. GSH is oxidized into its oxidized form (GSSG) after reaction with reactive oxygen species (ROS).

Supplementary Fig. S12
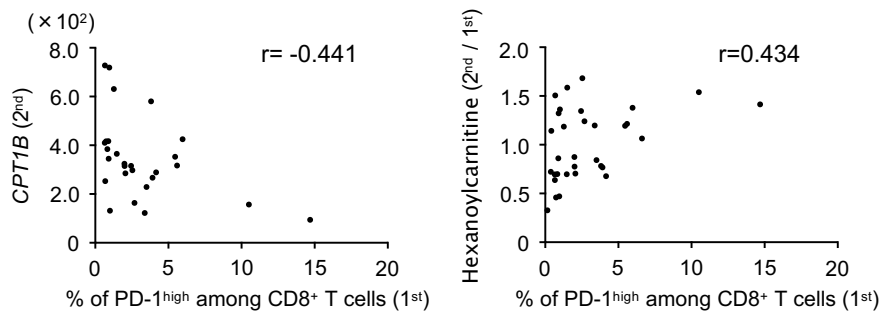
Supplementary Fig. S12. Modest correlation between % of PD-1^{high} among CD8^{+} T cells and FAO-related factors. Scatter plots between frequency of PD-1^{high} (X-axis) and the FAO-related gene (left panel) or metabolite marker (right panel) (Y-axis). r: Spearman correlation coefficients. |r| of more than 0.4 in spearman are considered to have modest to strong correlation.

Supplementary Table S1

Patient demographics (discovery cohort).

	All patients	responders	non-responders	dropout
Number	55	25	22	8
Age (range)	31-83	48-83	33-81	31-79
Age (median)	68	70	64.5	70
Male	39 (70.9%)	19 (76.0%)	15 (68.2%)	5 (62.5%)
Adenocarcinoma	43 (78.2%)	18 (72.0%)	18 (81.8%)	7 (87.5%)
EGFR mutation	9 (17.3%)	1 (7.0%)	7 (35.0%)	1 (12.5%)
ALK translocation	3 (6.8%)	0 (0%)	2 (7.1%)	1 (14.3%)
Antibiotics treatment	17 (30.9%)	6 (24.0%)	8 (36.4%)	3 (37.5%)

Supplementary Table S2

Metabolites measured by GC-MS.

Name	Kegg ID	HMDB ID	Methyloxime/TMS derivative	m/z	Retention time(min)
1,5-Anhydro-D-sorbitol	C07326	HMDB0002712	1,5-Anhydro-D-sorbitol-4TMS	259.0	14.77
1,6-Anhydroglucose	-	HMDB0000640	1,6-Anhydro-beta-D-glucose-3TMS	204.0	13.70
1-Hexadecanol	C00823	HMDB0003424	1-Hexadecanol-TMS	299.0	15.37
2-Aminoadipic acid	C00956	HMDB0000510	2-Aminoadipic acid-3TMS	260.0	13.62
2-Aminobutyric acid	C02356	HMDB0000452	2-Aminobutyric acid-2TMS	130.0	8.69
2-Aminoethanol	C00189	HMDB0000149	2-Aminoethanol-3TMS	174.0	9.77
2-Deoxytetronic acid	-	HMDB0000337	2-Deoxytetronic acid-3TMS	321.0	11.20
2-Hydroxybutyric acid	C05984	HMDB0000008	2-Hydroxybutyric acid-2TMS	131.0	8.17
2-Hydroxyisobutyric acid	-	HMDB0000729	2-Hydroxyisobutyric acid-2TMS	131.0	7.46
2-Hydroxyisovaleric acid	-	HMDB0000407	2-Hydroxyisovaleric acid-2TMS	145.0	8.61
2-Hydroxypyridine	C02502	HMDB0013751	2-Hydroxypyridine-TMS	152.0	7.22
2-Oxobutyric acid	C00109	HMDB0000005	2-Oxobutyric acid-methyloxime-TMS	188.0	7.86
2-Oxoglutaric acid (α-Ketoglutaric acid)	C00026	HMDB0000208	2-Oxoglutaric acid-methyloxime-2TMS	198.0	12.47
2-Oxoisocaproic acid	C00233	HMDB0000695	2-Oxoisocaproic acid-methyloxime-TMS	200.0	9.10
3-(3-Hydroxyphenyl)-3-hydroxypropionic acid	-	HMDB0002643	3-(3-Hydroxyphenyl)-3-hydroxypropionic acid-3TMS	267.0	14.68
3-Aminoisobutyric acid	C05145	HMDB0003911	3-Aminoisobutyric acid-3TMS	304.0	11.67
3-Hydroxybutyric acid	C01089	HMDB0000357	3-Hydroxybutyric acid-2TMS	191.0	8.51
3-Hydroxyisobutyric acid	C06001	HMDB0000023	3-Hydroxyisobutyric acid-2TMS	177.0	8.51
3-Hydroxyisovaleric acid	-	HMDB0000754	3-Hydroxyisovaleric acid-2TMS	131.0	9.09
3-Indolepropionic acid	-	HMDB0002302	3-Indolepropionic acid-2TMS	202.0	16.50
3-Methyl-2-oxobutyric acid	C00141	HMDB0000019	3-Methyl-2-oxobutyric acid-methyloyime-TMS	89.0	7.96
3-Methyl-2-oxovaleric acid	C00671	HMDB0000491	3-Methyl-2-oxovaleric acid-methyloxime-TMS	200.0	8.97
3-Methylhistidine	C01152	HMDB0000479	3-Methylhistidine-2TMS	168.0	15.01
4-Cresol	C01468	HMDB0001858	4-Cresol-TMS	180.0	8.56
4-Hydroxyproline	C01157	HMDB0000725	4-Hydroxyproline-3TMS	140.0	12.15
Acetoacetic acid	C00164	HMDB0000060	Acetoacetic acid-methyloxime-TMS	203.0	8.01
Aconitic acid	C02341	HMDB0000958	Aconitic acid-3TMS	375.0	13.81
Alanine	C00041	HMDB0000161	Alanine-2TMS	218.0	7.91
Alanine	C00041	HMDB0000161	Alanine-3TMS	188.0	10.77
Allose	C01487	HMDB0001151	Allose-methyloxime-5TMS	205.0	15.00
Arabinonic acid	-	HMDB0000539	Arabinonic acid-5TMS	292.0	14.23
Arabinose	C00259	HMDB0000646	Arabinose-methyloxime-4TMS	307.0	13.26
Arabitol	C01904	HMDB0000568	Arabitol-5TMS	217.0	13.75
Asparagine	C00152	HMDB0000168	Asparagine-3TMS	231.0	13.32
Aspartic acid	C00049	HMDB0000191	Aspartic acid-3TMS	232.0	12.03
Benzoic acid	C00180	HMDB0001870	Benzoic acid-TMS	179.0	9.60
beta-Alanine	C00099	HMDB0000056	beta-Alanine-3TMS	290.0	11.31
Boric acid	C12486	HMDB0035731	Boric acid-3TMS	263.0	6.49
Caffeine	C07481	HMDB0001847	Caffeine	194.0	15.05
Caproic acid	C01585	HMDB0000535	Caproic acid-TMS	173.0	7.52
Citric acid	C00158	HMDB0000094	Citric acid-4TMS	363.0	14.45
Citrulline	C00327	HMDB0000904	Citrulline-4TMS	256.0	14.48
Creatinine	C00791	HMDB0000562	Creatinine-3TMS	115.0	12.58
Cysteine	C00097	HMDB0000574	Cysteine-3TMS	218.0	12.44
Cysteinylglycine	C01419	HMDB0000078	Cysteinylglycine-3TMS	220.0	16.14
Cystine	C00491	HMDB0000192	Cystine-4TMS	411.0	17.67
Decanoic acid	C01571	HMDB0000511	Decanoic acid-TMS	229.0	11.47
Elaidic acid	C01712	HMDB0000573	Elaidic acid-TMS	93.0	17.11
Erythritol	C00503	HMDB0002994	Erythritol-4TMS	217.0	11.92
Fructose	C00095	HMDB0000660	Fructose-methyloxime-5TMS	307.0	14.92
Fucose	C01019	HMDB0000174	Fucose-methyloxime-5TMS	117.0	13.89
Fumaric acid	C00122	HMDB0000134	Fumaric acid-2TMS	245.0	10.37
Gluconic acid	C00257	HMDB0000625	Gluconic acid-methyloxime-5TMS	292.0	15.94
Glucose	C00031	HMDB0000122	Glucose-methyloxime-5TMS	229.0	15.32
Glucuronic acid	C00191	HMDB0000127	Glucuronic acid-methyloxime-5TMS	423.0	15.47
Glutamic acid	C00025	HMDB0000148	Glutamic acid-3TMS	246.0	12.85
Glutamine	C00064	HMDB0000641	Glutamine-3TMS	362.0	14.11
Glutaric acid	C00489	HMDB0000661	Glutaric acid-2TMS	261.0	10.94
Glyceric acid	C00258	HMDB0000139	Glyceric acid-3TMS	292.0	10.28
Glycerol	C00116	HMDB0000131	Glycerol-3TMS	218.0	9.69
Glycine	C00037	HMDB0000123	Glycine-3TMS	174.0	10.20
Glycolic acid	C00160	HMDB0000115	Glycolic acid-2TMS	205.0	7.53
Hippuric acid	C01586	HMDB0000714	Hippuric acid-TMS	236.0	14.75
Histidine	C00135	HMDB0000177	Histidine-3TMS	371.0	15.28
Homocysteine	C00155	HMDB0000742	Homocysteine-3TMS	234.0	13.33
Hypotaurine	C00519	HMDB0000965	Hypotaurine-3TMS	188.1	12.92
Hypoxanthine	C00262	HMDB0000157	Hypoxanthine-2TMS	265.0	14.50
Indoleacetic acid	C00954	HMDB0000197	Indol-3-acetic acid-2TMS	202.0	15.68
Indolelactic acid	C02043	HMDB0000671	Indolelactic acid-3TMS	202.0	17.08
Indoxyl sulfate	-	HMDB0000682	Indoxyl sulfate-2TMS	277.0	13.84
Isocitric acid	C00311	HMDB0000193	Isocitric acid-4TMS	245.0	14.41
Isoleucine	C16434	HMDB0000172	Isoleucine-2TMS	232.0	9.99
Kynurenine	C00328	HMDB0000684	Kynurenine-3TMS	307.0	17.07
Lactic acid	C00186	HMDB0000190	Lactic acid-2TMS	219.0	7.39
Lauric acid	C02679	HMDB0000638	Lauric acid-TMS	257.0	13.11
Leucine	C00123	HMDB0000687	Leucine-2TMS	232.0	9.74
Linoleic acid	C01595	HMDB0000673	Linoleic acid-TMS	337.0	17.06
Lysine	C00047	HMDB0000182	Lysine-4TMS	174.0	15.23
Malic acid	C00711	HMDB0000156	Malic acid-3TMS	233.0	11.73
Maltose	C00208	HMDB0000163	Maltose-methyloxime-8TMS	204.0	20.64
Mannose	C00159	HMDB0000169	Mannose-methyloxime-5TMS	319.0	15.05
Margaric acid	-	HMDB0002259	Margaric acid-TMS	117.0	16.58
Methionine	C00073	HMDB0000696	Methionine-2TMS	250.0	12.14
myo-Inositol	C00137	HMDB0000211	myo-Inositol-6TMS	432.0	16.54
Myristic acid	C06424	HMDB0000806	Myristic acid-TMS	285.0	14.60
Octanoic acid	C06423	HMDB0000482	Octanoic acid-TMS	201.0	9.62
Oleic acid	C00712	HMDB0000207	Oleic acid-TMS	339.0	17.08
O-Phosphoethanolamine	C00346	HMDB0000224	O-Phosphoethanolamine-4TMS	299.0	14.29
Ornithine	C00077	HMDB0000214	Ornithine-4TMS	420.0	14.52
Oxalic acid	C00209	HMDB0002329	Oxalic acid-2TMS	219.0	8.13
Palmitic acid	C00249	HMDB0000220	Palmitic acid TMS	313.0	15.95
Palmitoleic acid	C08362	HMDB0003229	Palmitoleic acid-TMS	311.0	15.85
Paraxanthine	C13747	HMDB0001860	Paraxanthine-TMS	237.0	15.77
Phenol	C00146	HMDB0000228	Phenol-TMS	166.0	7.45
Phenylalanine	C00079	HMDB0000159	Phenylalanine-2TMS	192.0	13.12
Phosphoglycerol	C03189	HMDB0000126	Phosphogycerol-4TMS	445.0	14.00
Phosphoric acid	C00009	HMDB0002142	Phosphoric acid-3TMS	225.0	9.76
Pipecolinic acid	C00408	HMDB0000070	Pipecolinic acid-TMS	156.0	10.44
Proline	C00148	HMDB0000162	Proline-2TMS	216.0	10.12
Pyroglutamic acid	C01879	HMDB0000267	5-Oxoproline-2TMS	258.0	12.21
Pyrophosphate	-	HMDB0000250	Pyrophosphate-4TMS	451.0	13.32
Pyruvic acid	C00022	HMDB0000243	Pyruvic acid-methyloxime-TMS	174.0	7.23
Ribitol	C00474	HMDB0000508	Ribitol-5TMS	319.0	13.81
Ribose	C00121	HMDB0000283	Ribose-methyloxime-4TMS	307.0	13.38
Ribulose	C00309	HMDB0000621	Ribulose-methyloxime-4TMS	263.0	13.38
scyllo-Inositol	C06153	HMDB0006088	scyllo-Inositol-6TMS	318.0	16.11
Serine	C00065	HMDB0000187	Serine-3TMS	306.0	10.58
Stearic acid	C01530	HMDB0000827	Stearic acid-TMS	341.0	17.18
Succinic acid	C00042	HMDB0000254	Succinic acid-2TMS	247.0	10.10
Sucrose	C00089	HMDB0000258	Sucrose-8TMS	361.0	19.79
Taurine	C00245	HMDB0000251	2-Aminoethanesulfonic acid-3TMS	326.0	13.50
Threitol	C16884	HMDB0004136	Threitol-4TMS	217.0	11.84
Threonic acid	C01620	HMDB0000943	Threonic acid-4TMS	292.0	12.38
Threonine	C00188	HMDB0000167	Threonine-3TMS	291.0	10.86
Tryptophan	C00078	HMDB0000929	Tryptophan-3TMS	291.0	17.34
Tyrosine	C00082	HMDB0000158	Tyrosine-3TMS	382.0	15.39
Unk12*	-	-	-	196.0	14.50
Unk3*	-	-	-	170.0	8.98
Unk4*	-	-	-	319.0	9.56
Unk6*	-	-	-	320.0	11.66
Unk8*	-	-	-	116.0	13.96
Unk9*	-	-	-	217.0	14.04
Urea	C00086	HMDB0000294	Urea-2TMS	204.0	9.29
Uric acid	C00366	HMDB0000289	Uric acid-4TMS	367.0	16.40
Uridine	C00299	HMDB0000296	Uridine-4TMS	245.0	18.54
Valine	C00183	HMDB0000883	Valine-2TMS	218.0	9.19
Xanthine	C00385	HMDB0000292	Xanthine-3TMS	353.0	15.92
Xylitol	C00379	HMDB0002917	Xylitol-5TMS	217.0	13.66
Xylose	C00181	HMDB0000098	Xylose-methyloxime-4TMS	307.0	13.21

* 'Unk' means that these metabolites were not yet identified at the time of analysis.

Supplementary Table S3

Metabolites measured by LC-MS.

Name	Kegg ID	HMDB ID	Ion transition	Retention time(min)
4-Hydroxyproline	C01157	HMDB0000725	132.10>68.05	1.89
Acetylalanine	-	HMDB0000766	132.10>90.10	4.00
Acetylglycine	-	HMDB0000532	118.00>76.10	3.53
Adenosine	C00212	HMDB0000050	268.10>136.10	8.35
Adenosylhomocysteine	C00021	HMDB0000939	385.10>136.10	8.23
Adenosylmethionine	C00019	HMDB0001185	399.10>250.10	2.18
2-Aminoadipic acid	C00956	HMDB0000510	162.10>98.20	2.35
Argininosuccinic acid	C03406	HMDB0000052	291.10>70.10	2.08
Asparagine	C00152	HMDB0000168	133.10>74.05	1.85
Aspartic acid	C00049	HMDB0000191	134.10>88.00	1.85
Betaine	C00719	HMDB0000043	118.10>42.05	2.07
Creatine	C00300	HMDB0000064	132.10>44.05	2.30
Creatinine	C00791	HMDB0000562	114.10>86.10	2.18
Cystathionine	C00542	HMDB0000099	223.10>88.00	1.84
Cysteine	C00097	HMDB0000574	122.00>59.00	2.04
Cystine	C00491	HMDB0000192	241.00>74.10	1.83
GSH	C00051	HMDB0000125	308.10>179.20	4.32
GSSG	C00127	HMDB0003337	613.20>231.00	8.17
Glutamic acid	C00025	HMDB0000148	148.10>102.05	1.98
Glutamine	C00064	HMDB0000641	147.10>130.05	1.92
Glycine	C00037	HMDB0000123	76.00>30.10	1.84
Guanosine	C00387	HMDB0000133	284.10>152.10	8.39
Histidine	C00135	HMDB0000177	156.10>83.20	1.84
Indoleacetic acid	C00954	HMDB0000197	176.10>130.10	13.67
Indolelactic acid	C02043	HMDB0000671	206.10>118.10	12.62
Indolepyruvic acid	C00331	HMDB0060484	204.10>130.10	12.41
Inosine	C00294	HMDB0000195	269.10>110.10	8.40
Kynurenic acid	C01717	HMDB0000715	190.10>144.00	10.13
Kynurenine	C00328	HMDB0000684	209.10>192.05	9.00
3-Hydroxykynurenine	C02794	HMDB0000732	225.10>110.15	7.49
N'-Formylkynurenine	C02700	HMDB0001200	237.20>146.05	9.02
Leucine	C00123	HMDB0000687	132.10>43.10	7.06
Isoleucine	C16434	HMDB0000172	132.10>69.10	6.50
Lysine	C00047	HMDB0000182	147.10>84.10	1.64
Methionine	C00073	HMDB0000696	150.10>104.00	3.93
S-Methylmethionine	C05319	HMDB0038670	165.10>59.20	9.54
N-Acetyl-Asp-Glu	C12270	HMDB0001067	305.10>148.00	8.17
N-Acetylaspartic acid	C01042	HMDB0000812	176.10>134.00	4.08
N-Acetylhistidine	C02997	HMDB0032055	198.10>110.10	2.81
Nicotinamide	C00153	HMDB0001406	123.10>80.05	5.13
Phenylalanine	C00079	HMDB0000159	166.10>103.10	8.94
Phosphocholine	C00588	HMDB0001565	184.10>125.00	1.85
Proline	C00148	HMDB0000162	116.10>70.10	2.28
Pyroglutamic acid	C01879	HMDB0000267	130.00>56.10	5.16
Riboflavin	C00255	HMDB0000244	377.10>243.20	10.59
Serine	C00065	HMDB0000187	106.10>60.00	1.85
Serotonin	C00780	HMDB0000259	177.10>160.20	8.73
Putrescine	C00134	HMDB0001414	89.10>72.10	1.60
Spermidine	C00315	HMDB0001257	146.20>129.20	1.39
Spermine	C00750	HMDB0001256	203.20>112.20	1.33
Taurine	C00245	HMDB0000251	126.00>44.05	1.87
Threonine	C00188	HMDB0000167	120.10>84.10	1.94
Trigonelline	C01004	HMDB0000875	138.00>94.10	2.39
Trimethyllysine	C03793	HMDB0001325	189.20>60.25	1.82
Tryptophan	C00078	HMDB0000929	205.10>115.20	9.84
Tyrosine	C00082	HMDB0000158	182.10>136.20	7.30
Urea	C00086	HMDB0000294	61.00>44.05	2.05
Uric acid	C00366	HMDB0000289	169.00>126.05	5.26
Uridine	C00299	HMDB0000296	245.10>113.10	7.51
Valine	C00183	HMDB0000883	118.10>55.10	3.15
Xanthosine	C01762	HMDB0000299	285.10>153.20	8.61
Xanthurenic acid	C02470	HMDB0000881	206.00>160.00	9.75
asy-Dimethylarginine	C03626	HMDB0001539	203.10>46.10	2.47
sym-Dimethylarginine	-	HMDB0003334	203.10>172.20	2.57
trans-urocanic acid	C00785	HMDB0000301	139.10>93.10	4.70
2-Hydroxy-3-methylvaleric acid	-	HMDB0000317	131.10>85.15	11.52
2-Hydroxybutyric acid	C05984	HMDB0000008	103.00>57.20	7.95
2-Hydroxyglutaric acid	C01087	HMDB0000606	147.00>129.05	4.27
2-Hydroxyisovaleric acid	-	HMDB0000407	117.10>71.10	9.89
2-Oxoglutaric acid (α-Ketoglutaric acid)	C00026	HMDB0000208	145.10>57.10	3.24
3-Hydroxybutyric acid	C01089	HMDB0000357	103.00>59.10	6.64
3-Hydroxyisovaleric acid	-	HMDB0000754	117.10>59.10	8.89
3-Methylglutaconic acid	-	HMDB0000522	143.00>99.05	10.07
4-Hydroxybenzoic acid	C00156	HMDB0000500	137.00>93.00	10.98
AMP	-	HMDB0000045	346.10>79.05	4.14
Aconitic acid	C02341	HMDB0000958	173.00>85.15	8.17
Arabinonic acid	-	HMDB0000539	165.00>75.05	2.01
Citric acid	C00158	HMDB0000094	191.00>87.10	4.59
Fumaric acid	C00122	HMDB0000134	115.00>71.10	5.93
Glutaric acid	C00489	HMDB0000661	131.00>87.15	8.71
Hippuric acid	C01586	HMDB0000714	178.10>134.05	11.14
Indoxyl sulfate	-	HMDB0000682	212.00>132.20	10.58
Isocitric acid	C00311	HMDB0000193	191.00>73.10	2.96
Lactic acid	C00186	HMDB0000190	89.00>43.10	3.67
Malic acid	C00711	HMDB0000156	133.00>115.00	2.89
Phenyllactic acid	C05607	HMDB0000748	165.10>147.05	12.32
Pyridoxic acid	C00847	HMDB0000017	182.00>108.05	8.56
Succinic acid	C00042	HMDB0000254	117.00>73.05	5.90
cAMP	C00575	HMDB0000058	328.00>134.20	8.36
Thyroxine	C01829	HMDB0000248	777.70>731.50	15.07
Pipecolic acid	C00408	HMDB0000070	130.10>84.30	3.41
3-Indolepropionic acid	-	HMDB0002302	190.10>130.10	14.73
Quinolinic acid	C03722	HMDB0000232	168.10>78.05	4.40
3-Methylhistidine	C01152	HMDB0000479	170.10>81.10	1.85
Carnitine	C00318	HMDB0000062	162.10>85.10	2.13
Acetylcarnitine	C02571	HMDB0000201	204.10>85.10	4.16
Propionylcarnitine	C03017	HMDB0000824	218.10>85.10	8.49
Isobutyrylcarnitine	-	HMDB0000736	232.20>85.15	9.38
Butyrylcarnitine	C02862	HMDB0002013	232.20>85.05	9.51
2-Methylbutyrylcarnitine	-	HMDB0000378	246.20>85.10	10.44
Isovalerylcarnitine	C20826	HMDB0000688	246.20>85.15	10.58
Hexanoylcarnitine	-	HMDB0000705	260.20>85.15	11.88
Glutarylcarnitine	-	HMDB0013130	276.10>85.05	8.48
Adipoylcarnitine	-	HMDB0061677	290.20>85.00	8.96
2-Aminobutyric acid	C02356	HMDB0000452	104.10>41.05	2.19
Choline	C00114	HMDB0000097	104.10>60.00	1.99
3-Aminoisobutyric acid	C05145	HMDB0003911	104.10>86.10	2.31
Acetylcarnosine	-	HMDB0012881	269.10>110.10	4.25
Alanine	C00041	HMDB0000161	90.00>44.10	1.91
Ornithine	C00077	HMDB0000214	133.10>116.05	1.65
Arginine	C00062	HMDB0000517	175.10>70.10	1.83
Citrulline	C00327	HMDB0000904	176.10>159.05	2.05
Glucuronic acid	C00191	HMDB0000127	193.00>103.00	1.95
Gluconic acid	C00257	HMDB0000625	195.10>129.00	1.96
N-Acetylglucosamine	C00140	HMDB0000215	222.10>138.00	2.33
N-Acetylneuraminic acid	C00270	HMDB0000230	310.10>274.10	2.17
4-Guanidinobutanoic acid	C01035	HMDB0003464	146.10>86.15	3.82
N-Acetylornithine	C00437	HMDB0003357	175.10>70.05	2.67

Supplementary Table S4

Cellular markers measured by flow cytometry.

Cellular markers
% of CCR6$^+$ cells among CD4$^+$ T cells
% of CD25$^+$ cells among CD4$^+$ T cells
% of CD4$^+$ T cells among PBMC
% of CD8$^+$ T cells among PBMC
% of CTLA4$^+$ cells among CD4$^+$ T cells
% of CXCR3$^+$ cells among CD4$^+$ T cells
% of FoxP3$^+$ cells among CD4$^+$ T cells
% of FoxP3^{high} cells among CD4$^+$ T cells
% of FoxP3^{low} cells among CD4$^+$ T cells
% of FoxP3$^+$ CD25$^+$ cells among CD4$^+$ T cells
% of FoxP3^{low} CD45RA$^+$ cells among CD4$^+$ T cells
% of FoxP3$^+$ CTLA4$^+$ cells among CD4$^+$ T cells
% of IFNγ^+ cells among CD4$^+$ T cells
% of IFNγ^+ cells among CD8$^+$ T cells
% of KLRG1$^+$ cells among CD4$^+$ T cells
% of KLRG1$^+$ CCR6$^+$ cells among CD4$^+$ T cells
% of KLRG1$^+$ cells among CD8$^+$ T cells
% of PD-1$^+$ cells among CD4$^+$ T cells
% of PD-1$^+$ CD45$^+$ cells among CD4$^+$ T cells
% of PD-1$^+$ FoxP3$^+$ cells among CD4$^+$ T cells
% of PD-1$^+$ cells among CD8$^+$ T cells
% of PD-1^{high} cells among CD8$^+$ T cells
% of T-bet$^+$ cells among CD4$^+$ T cells
% of T-bet^{high} cells among CD4$^+$ T cells
% of T-bet$^+$ KLRG1$^+$ cells among CD4$^+$ T cells
% of T-bet$^+$ cells among CD8$^+$ T cells
% of T-bet^{high} cells among CD8$^+$ T cells
% of EOMES$^+$ cells among CD8$^+$ T cells
% of T-bet$^+$ EOMES$^-$ cells among CD8$^+$ T cells
% of T-bet$^+$ EOMES$^+$ cells among CD8$^+$ T cells
% of T-bet$^-$ EOMES$^+$ cells among CD8$^+$ T cells
% of Tnaive among CD4$^+$ T cells
% of Tcm among CD4$^+$ T cells
% of Tem among CD4$^+$ T cells
% of Temra among CD4$^+$ T cells
% of Tnaive among CD8$^+$ T cells
% of Tcm among CD8$^+$ T cells
% of Tem among CD8$^+$ T cells
% of Temra among CD8$^+$ T cells
% of Tim3$^+$ cells among CD4$^+$ T cells
% of Tim3$^+$ cells among CD8$^+$ T cells
Cell ROX Green (MFI) of CD4$^+$ T cells
Cell ROX Green (MFI) of CD8$^+$ T cells
Mito SOX Red (MFI) of CD4$^+$ T cells
Mito SOX Red (MFI) of CD8$^+$ T cells
Mito Tracker Deep Red (MFI) of CD4$^+$ T cells
Mito Tracker Deep Red (MFI) of CD8$^+$ T cells
Mito Tracker Green (MFI) of CD4$^+$ T cells
Mito Tracker Green (MFI) of CD8$^+$ T cells
p-Akt (MFI) of CD8$^+$ T cells
p-mTOR (MFI) of CD8$^+$ T cells
PGC-1$\alpha\beta$ (MFI) of CD8$^+$ T cells

Supplementary Table S5

Metabolites showing a significant difference between responders (R) and non-responders (NR).

Metabolites	Time point	Changes in R relative to NR	Modality	p-value*
Alanine	1st	higher	GC	0.0474
4-Cresol	1st	higher	GC	0.0238
Cysteine	1st	higher	GC	0.0474
Hippuric acid	1st	higher	GC / LC	0.0062 / 0.0056
Oleic acid	1st	higher	GC	0.0281
Indoxyl sulfate	1st	higher	GC / LC	0.0313 / 0.0225
Ribose	1st	higher	GC	0.0081
Unk8	1st	lower	GC	0.0273
Indoleacetate	1st	higher	LC	0.018
Uric acid	1st	higher	LC	0.0126
Trans-urocanic acid	1st	higher	LC	0.0498
Pipecolic acid	1st	lower	LC	0.0252
N-Acetylglucosamine	1st	higher	LC	0.0281
Uric acid	2nd	higher	GC / LC	0.0114 / 0.0176
Indolelactic acid	2nd	higher	GC	0.0387
Arabinose	2nd	higher	GC	0.0186
Arabitol	2nd	higher	GC	0.0089
Hippuric acid	2nd	higher	GC	0.0323
Cystine	2nd	higher	GC	0.0043
Indoxyle sulfate	2nd	higher	GC / LC	0.0454 / 0.0348
Gluconic acid	2nd	higher	GC	0.0454
Citrulline	2nd	higher	GC / LC	0.0312 / 0.0122
Creatinine	2nd	higher	LC	0.0198
N-Acetylaspartic acid	2nd	higher	LC	0.0311
Pyroglutamic acid	2nd	higher	LC	0.0064
Trimethyyllysine	2nd	higher	LC	0.0408
Asy-Dimethylarginine	2nd	higher	LC	0.0028
Sym-Dimethylarginine	2nd	higher	LC	0.0078
Pipecolic acid	2nd	lower	LC	0.0329
Methylhistidine	2nd	higher	LC	0.0176
Butyrylcarnitine	2nd	lower	LC	0.0101
3-Aminoisobutyric acid	2nd	higher	LC	0.0122
Acethykcarnosine	2nd	higher	LC	0.0367
Alanine	2nd	higher	LC	0.0348
Arginine	2nd	higher	LC	0.0114
N-accetylorinitine	2nd	higher	LC	0.0387
4-Cresol	3rd	higher	GC	0.0023
3-Hydroxyisovaleric acid	3rd	lower	GC / LC	0.0213 / 0.0119
Pyruvic acid	3rd	lower	GC	0.0348
α-ketoglutaric acid	3rd	lower	GC	0.0238
Hippuric acid	3rd	higher	GC / LC	0.0002 / 0.0003
Cystine	3rd	higher	GC	0.0201
Indoxyl sulfate	3rd	higher	GC / LC	0.0049 / 0.0011
Unk13	3rd	higher	GC	0.0451
GSSG	3rd	higher	LC	0.0068
Uric acid	3rd	higher	LC	0.0348
2-Hydrobutyric acid	3rd	lower	LC	0.0281
Pipecolic acid	3rd	lower	LC	0.0348
Butyrylcarnitine	3rd	lower	LC	0.0015

Metabolites	Ratio of two time points	Changes in R relative to NR	Modality	p-value*
Creatinine	2nd/1st	lower	GC	0.0329
1,5-Anhydro-D-sorbitol	2nd/1st	higher	GC	0.0367
Cystine	2nd/1st	higher	GC	0.0419
Glutamine	2nd/1st	higher	LC	0.0114
Glycine	2nd/1st	higher	LC	0.0095
Lysine	2nd/1st	higher	LC	0.0329
Pyroglutamic acid	2nd/1st	higher	LC	0.0095
Taurine	2nd/1st	lower	LC	0.0348
asy-Dimethylarginine	2nd/1st	higher	LC	0.0176
AMP	2nd/1st	lower	LC	0.0198
Isovalerylcarnitine	2nd/1st	lower	LC	0.0454
Hexanoycarnitine	2nd/1st	lower	LC	0.0367
Acetylcarnosine	2nd/1st	higher	LC	0.0264
Arginine	2nd/1st	higher	LC	0.0329
Citrulline	2nd/1st	higher	LC	0.0348
N-accetylorinitine	2nd/1st	higher	LC	0.0037
3-Hydroxybutyric acid	3rd/1st	lower	GC / LC	0.0330 / 0.0281
2-Hydroxyisovaleric acid	3rd/1st	lower	GC / LC	0.0040 / 0.0028
Creatinine	3rd/1st	lower	GC	0.0099
Hippuric acid	3rd/1st	higher	GC	0.0016
Oleic acid	3rd/1st	lower	GC	0.0252
Acetoacetic acid	3rd/1st	lower	GC	0.0483
Ribose	3rd/1st	lower	GC	0.0008
GSSG	3rd/1st	higher	LC	0.0367
Tryptophan	3rd/1st	higher	LC	0.0367
2-Hydroxyglutaric acid	3rd/1st	lower	LC	0.0099
Malic acid	3rd/1st	lower	LC	0.0266
Quinolinic acid	3rd/1st	lower	LC	0.0407
Butyrylcarnitine	3rd/1st	lower	LC	0.0043
Caproic acid	3rd/2nd	higher	GC	0.0408
4-Cresol	3rd/2nd	higher	GC	0.0138
Isoleucine	3rd/2nd	higher	GC	0.0408
Arabinose	3rd/2nd	lower	GC	0.0114
Ribose	3rd/2nd	lower	GC	0.0028
GSH	3rd/2nd	higher	LC	0.0089
GSSG	3rd/2nd	higher	LC	0.0008
3-OH-Kynurenine	3rd/2nd	lower	LC	0.0408
Hippuric acid	3rd/2nd	higher	LC	0.0165
Isobutyrylcarnitine	3rd/2nd	higher	LC	0.0095

*: p-value for distinction beween R and NR (Wilcoxon rank sum test).

Supplementary Table S6

Cellular markers showing a significant difference between responders (R) and non-responders (NR).

Cellular markers	Time point or ratio of two time points	Changes in R relative to NR	p-value*
% of CD4$^+$ T cells among PBMC	2nd	higher	0.0107
% of CD4$^+$ T cells among PBMC	2nd/1st	higher	0.0001
% of CD8$^+$ T cells among PBMC	2nd	higher	0.0478
% of CD8$^+$ T cells among PBMC	2nd/1st	higher	0.0348
% of Tnaive among CD8$^+$ T cells	2nd/1st	higher	0.0176
% of Tcm among CD4$^+$ T cells	2nd/1st	higher	0.0095
% of Tcm among CD8$^+$ T cells	2nd/1st	higher	0.0138
% of Tem among CD8$^+$ T cells	3rd/1st	higher	0.0213
% of Temra among CD4$^+$ T cells	2nd/1st	lower	0.0107
% of Temra among CD4$^+$ T cells	3rd/2nd	higher	0.0081
% of Temra among CD8$^+$ T cells	2nd/1st	lower	0.0009
Mito SOX CD8/CD4	1st	higher	0.0028
Mito SOX CD8/CD4	2nd	higher	0.0089
Mito SOX CD8/CD4	3rd	higher	0.018
Mito SOX CD8/CD4	3rd/1st	lower	0.019
Mito mass CD8/CD4	1st	higher	0.0451
Mito mass CD8/CD4	3rd	higher	0.0348
PGC-1$\alpha\beta$ (MFI) of CD8$^+$ T cells	2nd	lower	0.0176
PGC-1$\alpha\beta$ (MFI) of CD8$^+$ T cells	2nd/1st	lower	0.0052
PGC-1$\alpha\beta$ (MFI) of CD8$^+$ T cells	3rd/2nd	higher	0.0001
% of PD-1^{high} among CD8$^+$ T cells	1st	lower	0.013
% of FoxP3^{low} CD45RA$^+$ among CD4$^+$ T cells	1st	lower	0.027
% of T-bet^{high} among CD4$^+$ T cells	3rd/1st	higher	0.03
% of T-bet^{low} among CD4$^+$ T cells	3rd/1st	higher	0.0214
% of T-bet$^+$ among CD8$^+$ T cells	3rd/2nd	higher	0.0295
% of T-bet$^+$ EOMES$^+$ among CD8$^+$ T cells	3rd/2nd	higher	0.0408

*: p-value for distinction between R and NR (Wilcoxon rank sum test).

Supplementary Table S7

AUC value of each marker in pretreatment.

Marker name	Time point	Area under the curve (AUC)	number of patients analyzed
Mito SOX CD8/CD4	1st	0.75	47
% of PD-1high among CD8+ T cells	1st	0.71	47
PD-L1 TPS	1st	0.66	31

Supplementary Table S8

Equation of LDA for each marker combination.

Marker combination	Before the adaptation	After the adaptation
Metabolite combination I	$9.92 \times$[Cysteine 1st]$-44.7 \times$[Unk8 (1st)] $+5.41 \times 10^{-7} \times$ [Hippuric acid (1st)] -1.50	$9.92 \times$[Cysteine 1st]$-44.7 \times$[Unk8 (1st)] $+5.41 \times 10^{-7} \times$ [Hippuric acid (1st)] -1.46
Metabolite combination II	$207 \times$[Arabinose (2nd)]$+3.17 \times 10^{-7} \times$ [Arginine (2nd)]$-3.77 \times 10^{-7} \times$ [Butyrylcarnitine (2nd)]-1.99	$207 \times$[Arabinose (2nd)] $+3.17 \times 10^{-7} \times$ [Arginine (2nd)]$-3.77 \times 10^{-7} \times$ [Butyrylcarnitine (2nd)]-1.96
Metabolite combination III	$4.43 \times 10^{-7} \times$ [Hippuric acid (1st)]$+12.1 \times$[Cystine (2nd)] $+3.55 \times 10^{-5} \times$ [GSSG (3rd)] $-8.81 \times 10^{-8} \times$ [Butyrylcarnitine 3rd]-2.71	$4.43 \times 10^{-7} \times$ [Hippuric acid (1st)]$+12.1 \times$ [Cystine (2nd)] $+3.55 \times 10^{-5} \times$ [GSSG (3rd)] $-8.81 \times 10^{-8} \times$ [Butyrylcarnitine 3rd]-2.67
Cellular combination I	$-0.302 \times$[% of PD-1high among CD8+ T cells (1st)] $+2.95 \times$[Mito SOX CD8/CD4(1st)]-1.91	$-0.302 \times$[% of PD-1high among CD8+ T cells (1st)] $+2.95 \times$[Mito SOX CD8/CD4(1st)]-1.87
Cellular combination II	$-0.252 \times$[% of PD-1high among CD8+ T cells (1st)] $+3.58 \times$[Mito SOX CD8/CD4(1st)] $-1.22 \times$[PGC-1$\alpha\beta$ of CD8+ T cells (2nd/1st)] $+1.93 \times$[% of CD4+ T cells among PBMC (2nd/1st)]-3.35	$-0.252 \times$[% of PD-1high among CD8+ T cells (1st)] $+3.58 \times$[Mito SOX CD8/CD4(1st)] $-1.22 \times$[PGC-1$\alpha\beta$ of CD8+ T cells (2nd/1st)] $+1.93 \times$[% of CD4+ T cells among PBMC (2nd/1st)]-3.30
Cellular combination III	$-0.281 \times$[% of PD-1high among CD8+ T cells (1st)] $+3.30 \times$[Mito SOX CD8/CD4(1st)] $+2.00 \times$[PGC-1$\alpha\beta$ of CD8+ T cells (3rd/2nd)] $+1.55 \times$[% of CD4+ T cells among PBMC (2nd/1st)]-6.04	$-0.281 \times$[% of PD-1high among CD8+ T cells (1st)] $+3.30 \times$[Mito SOX CD8/CD4(1st)] $+2.00 \times$[PGC-1$\alpha\beta$ of CD8+ T cells (3rd/2nd)] $+1.55 \times$[% of CD4+ T cells among PBMC (2nd/1st)]-5.97