

Supporting Information

Globally Optimized Targeted Mass Spectrometry (GOT-MS):

Reliable Metabolomics Analysis with Broad Coverage

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A Separate Excel Table: Table S1. The list of GOT-MS MRMs. A few precursor and product ion pairs had similar molecular weight, but they were kept in the table because they had different optimized MS parameters (data not shown).

Page S-3: Table S2. Demographic and clinical information for the patients and healthy controls included in the CRC study.

Page S-4: Table S3. The concentrations of spiked U-¹³C¹⁵N-amino acids in the 1:4 dilution sample.

Page S-5: Figure S1. The general HILIC/RP-LC strategy for GOT-MS.

Page S-6: Figure S2. Typical RP C18-SIMs in the m/z range of 100-190 from a pooled serum sample under a) positive and b) negative ion detection modes in GOT-MS.

Page S-7: Figure S3. a) The intraday (n=3) and interday (n=3x3 consecutive days) CVs for amino acids detected by GOT-MS, b) the intraday (n=3) and interday (n=3x3 consecutive days) CVs of amino acids detected by GOT-MS, after normalization to the corresponding isotope labeled (U-¹³C¹⁵N-) internal standards, and c) the linearity (R²) of amino acids from the 5 dilution samples in GOT-MS.

29 **Page S-8: Figure S4.** a) The intraday (n=3) and interday (n=3x3 consecutive days) CVs
30 of amino acids detected by Q-TOF-MS, b) the intraday (n=3) and interday (n=3x3
31 consecutive days) CVs of amino acids detected by Q-TOF-MS, after normalization to the
32 corresponding isotope labeled (U-¹³C¹⁵N-) internal standards, and c) the linearity (R²) of
33 amino acids detected by Q-TOF-MS using the 5 dilution samples.

34 **Page S-9: Figure S5.** The flow chart of GOT-MS-based metabolomics for CRC
35 diagnosis in this study.

36 **Page S-10: Figure S6.** The PCA score plot (PC1 vs. PC2) for the 155 GOT-MS MRMs
37 collected from CRC and healthy control samples.

38 **Page S-11: Figure S7.** a) SIM scan of m/z 147 in GOT-MS, b) the MS/MS spectrum
39 targeting the ions with m/z 147 at 4.4 min in a), c) MRM scan (147->130.1) of glutamine
40 standard, and d) MRM scan (147->130.1) of lysine standard.

41 **Page S-12: Figure S8.** a) The MS/MS spectrum of lysine standard, and b) the MS/MS
42 spectrum of glutamine standard.

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44 **Table S2.** Demographic and clinical information for the patients and healthy controls
 45 included in the CRC study.

	Healthy Controls	CRC Patients
Subjects	20	18
Age , median (range)	58 (21-78)	52 (27-76)
BMI^a , median (range)	27.4 (20.1-40.0)	27.0 (20.9-32.3)
Gender		
Male	11	12
Female	9	6
Stage		
I/II	-	8
III	-	7
IV	-	3
Ethnicity		
Caucasian	18	8
African American	-	1
NA	2	9

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47 ^a1 control and 9 CRC samples do not have BMI data.

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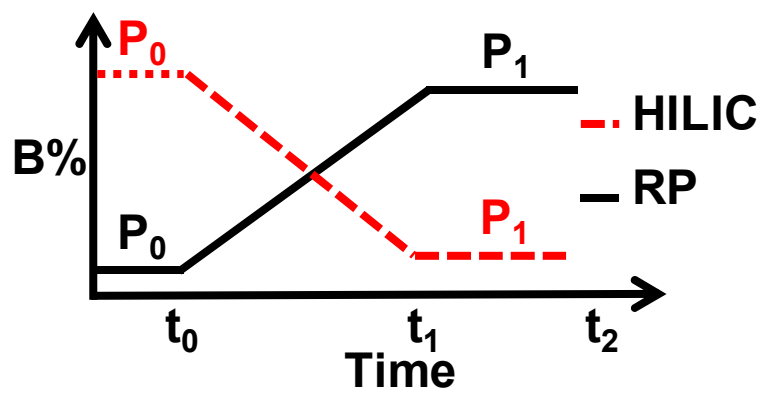
50 **Table S3.** The concentrations of spiked U-¹³C¹⁵N-amino acids in the 1:4 dilution sample.

U- ¹³ C ¹⁵ N-Amino Acids	Concentration (μM)
U- ¹³ C ¹⁵ N-Gly	87.6
U- ¹³ C ¹⁵ N-Ala	93.2
U- ¹³ C ¹⁵ N-Ser	39.2
U- ¹³ C ¹⁵ N-Pro	58.6
U- ¹³ C ¹⁵ N-Val	28.8
U- ¹³ C ¹⁵ N-Thr	32.5
U- ¹³ C ¹⁵ N-Leu	82.3
U- ¹³ C ¹⁵ N-Ile	16.0
U- ¹³ C ¹⁵ N-Asn	67.3
U- ¹³ C ¹⁵ N-Asp	68.2
U- ¹³ C ¹⁵ N-Gln	60.7
U- ¹³ C ¹⁵ N-Lys	93.5
U- ¹³ C ¹⁵ N-Glu	64.7
U- ¹³ C ¹⁵ N-Met	14.2
U- ¹³ C ¹⁵ N-His	6.7
U- ¹³ C ¹⁵ N-Phe	11.2
U- ¹³ C ¹⁵ N-Arg	56.4
U- ¹³ C ¹⁵ N-Tyr	7.1
U- ¹³ C ¹⁵ N-Trp	16.3
U- ¹³ C ¹⁵ N-Cys	5.4

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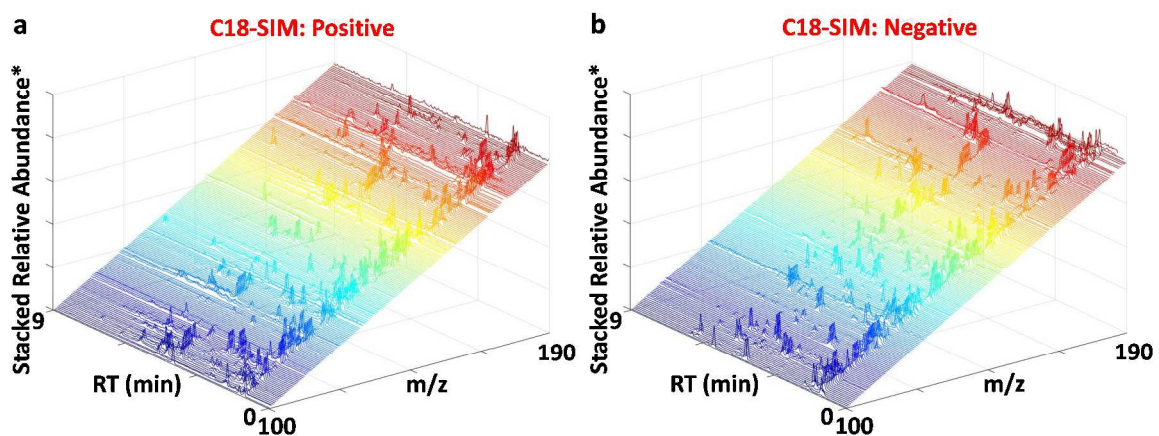


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55 **Figure S1.** The general HILIC/RP-LC strategy for GOT-MS.

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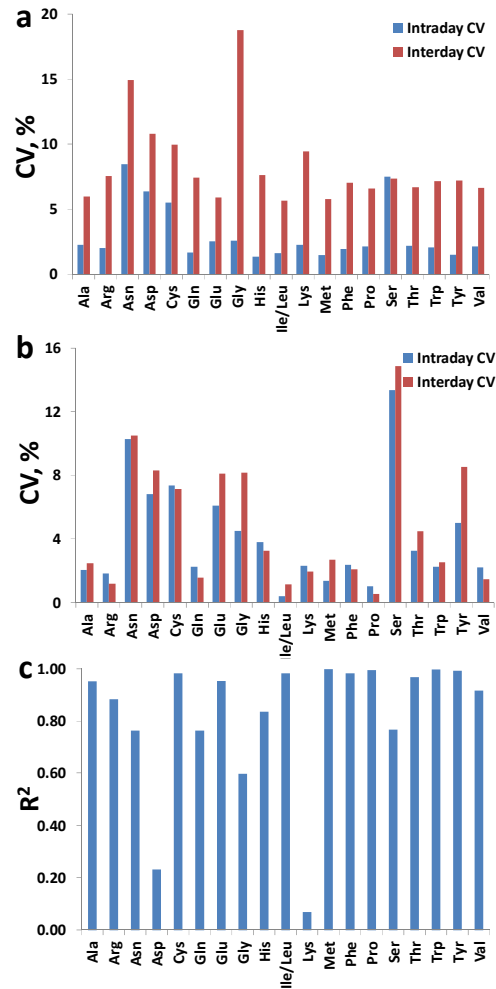
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59 **Figure S2.** Typical RP C18-SIMs in the m/z range of 100-190 from a pooled serum
60 sample under a) positive and b) negative ion detection modes in GOT-MS.

61 * Each SIM data was first linearly scaled, so that the minimum is 0 and the maximum is
62 1. An increment of 0.04 was then added to each SIM across different retention time.

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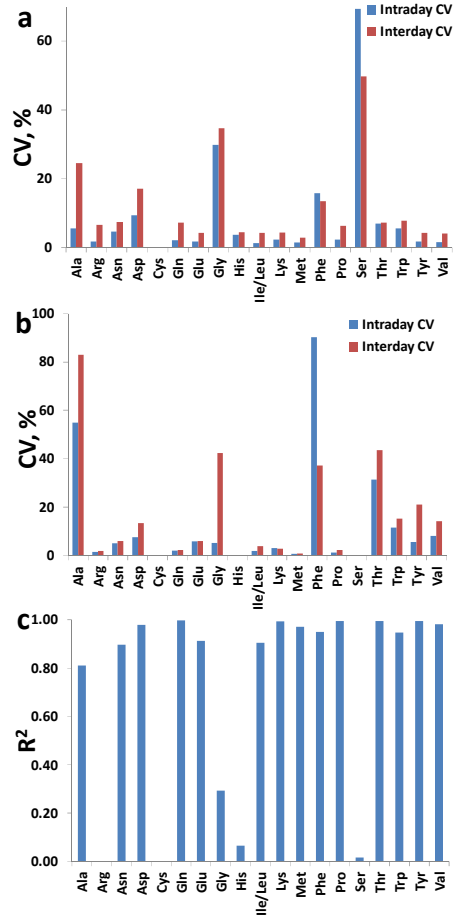


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67 **Figure S3.** a) The intraday (n=3) and interday (n=3x3 consecutive days) CVs for amino
 68 acids detected by GOT-MS, b) the intraday (n=3) and interday (n=3x3 consecutive days)
 69 CVs of amino acids detected by GOT-MS, after normalization to the corresponding
 70 isotope labeled (U-¹³C¹⁵N-) internal standards, and c) the linearity (R²) of amino acids
 71 from the 5 dilution samples in GOT-MS.

72 * Ile and Leu were integrated together since they had the same MRMs and baseline
 73 separation between them was not observed.

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76 **Figure S4.** a) The intraday (n=3) and interday (n=3x3 consecutive days) CVs of amino
 77 acids detected by Q-TOF-MS, b) the intraday (n=3) and interday (n=3x3 consecutive
 78 days) CVs of amino acids detected by Q-TOF-MS, after normalization to the
 79 corresponding isotope labeled (U-¹³C¹⁵N-) internal standards, and c) the linearity (R²) of
 80 amino acids detected by Q-TOF-MS using the 5 dilution samples.

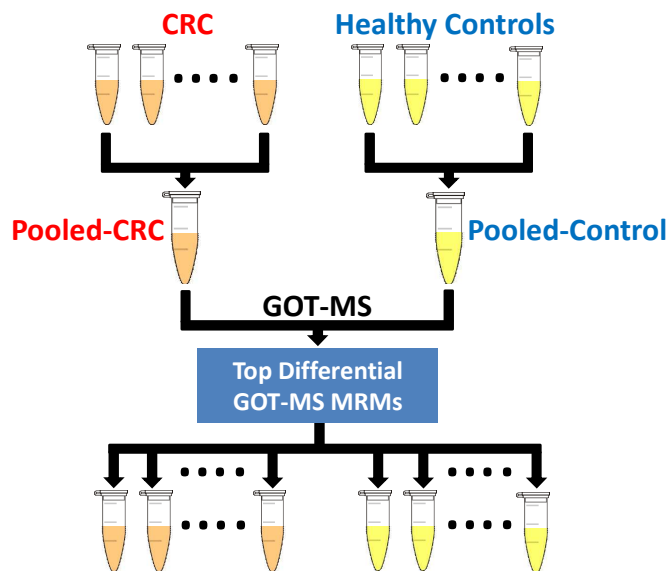
81 * Ile and Leu were integrated together since they had the same MRMs and baseline
 82 separation between them was not observed.

83 ** Cysteine was not detectable in these samples using Q-TOF.

84 *** U-¹³C¹⁵N-cysteine, U-¹³C¹⁵N-serine, and U-¹³C¹⁵N-histidine were not detectable in
 85 these samples using Q-TOF.

86 **** R² for Arginine was very small.

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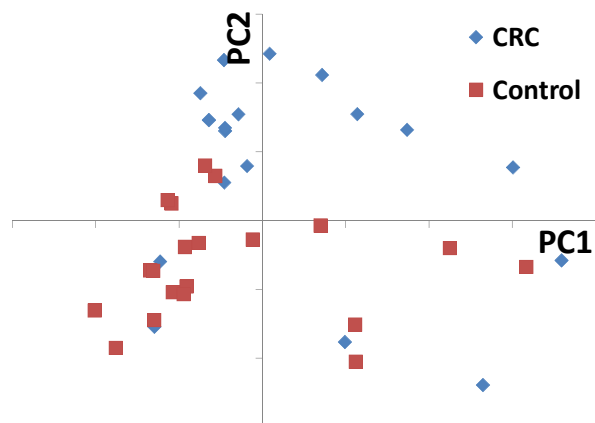


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89 **Figure S5.** The flow chart of GOT-MS-based metabolomics for CRC diagnosis used in
90 this study.

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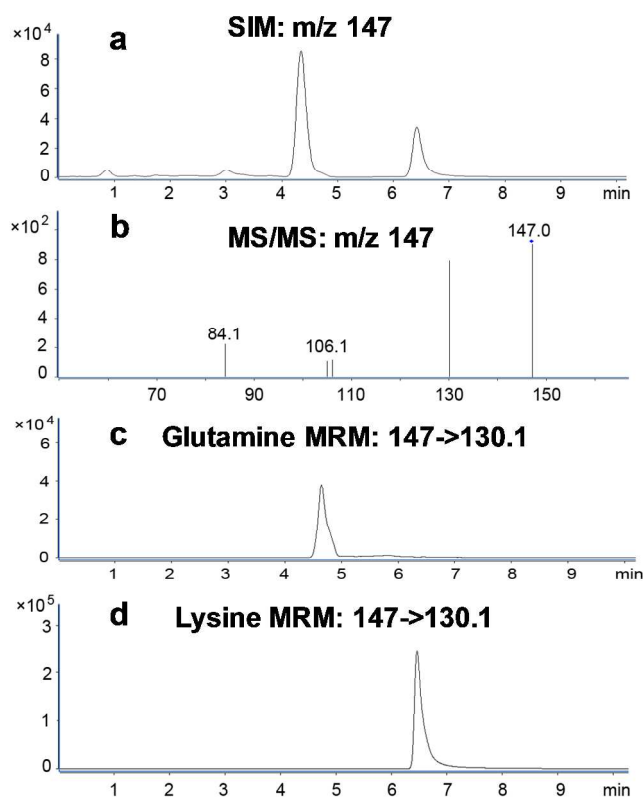


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94 **Figure S6.** The PCA score plot (PC1 vs. PC2) for the 155 GOT-MS MRMs collected

95 from CRC and healthy control samples.

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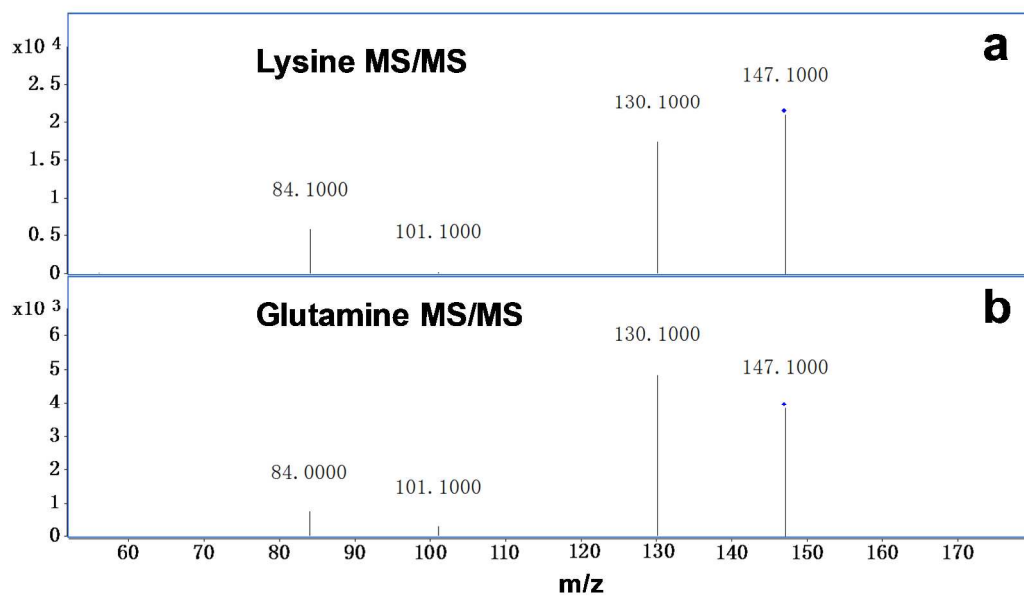
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99 **Figure S7.** a) SIM scan of m/z 147 in GOT-MS, b) the MS/MS spectrum targeting the
100 ions with m/z 147 at 4.4 min in a), c) MRM scan (147->130.1) of glutamine standard, and
101 d) MRM scan (147->130.1) of lysine standard.

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106 **Figure S8.** a) The MS/MS spectrum of lysine standard, and b) the MS/MS spectrum of
107 glutamine standard.

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