

Supplementary Information for Assessing the dark field of metaproteome

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Figure S1

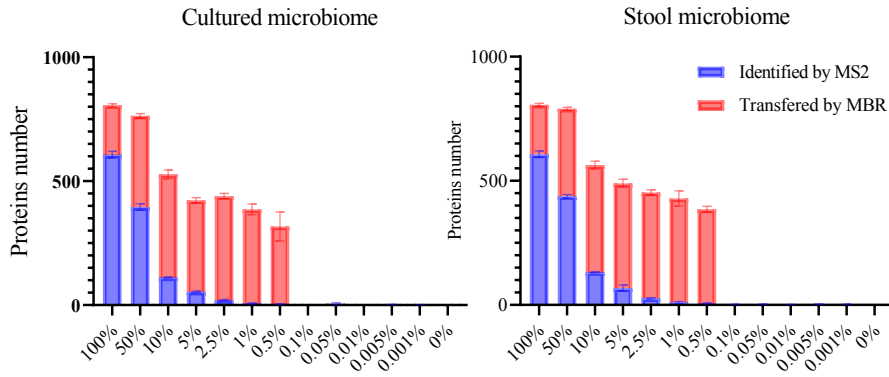


Figure S1. The number of identified ^{15}N protein groups along *E. coli* concentration with or without match-between-run in cultured microbiome group (a) and stool microbiome group (b).

Figure S2

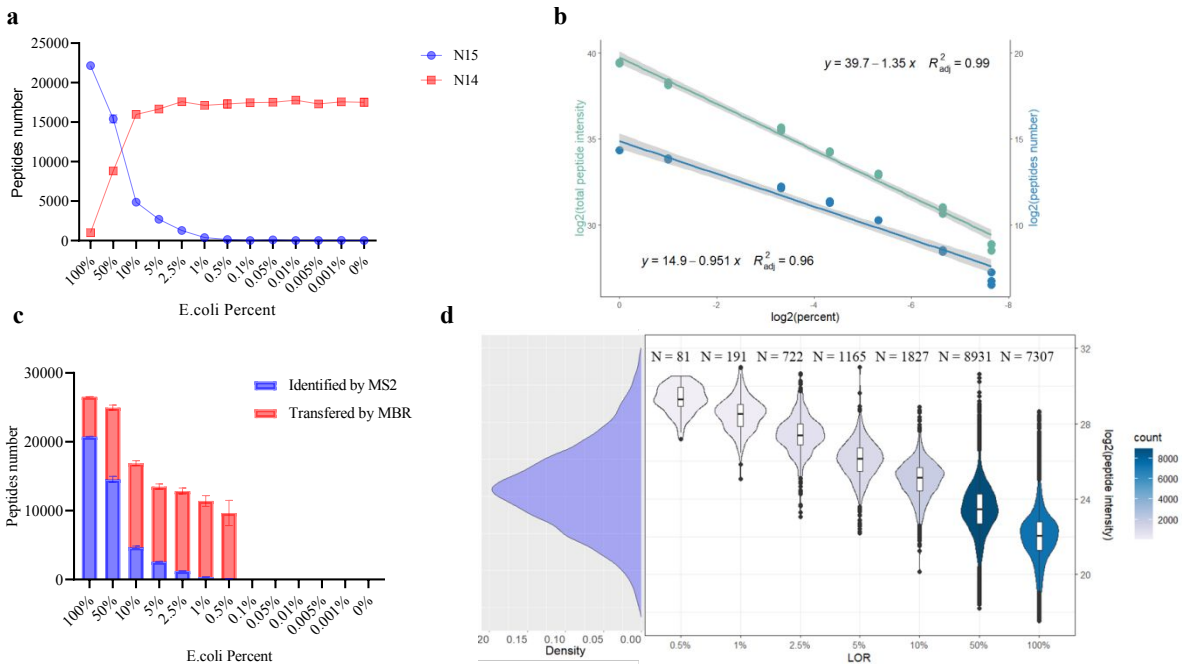


Figure S2. Performance of DDA method on peptide identification and quantification with the cultured protein as background. a. The number of identified peptides in each sample (Stool). b. The number of quantified peptides in each sample. Peptides identified directly by database searching (Pfind 3.0) are shown in red. Peptides transferred by

MBR (FlashLFQ) are shown in blue. c. Linear regression between log₂ quantified peptide number and log₂ percentage (blue). Linear regression between log₂ total peptide intensity and log₂ percentage (green). d. Density plot of all quantified ¹⁵N peptides on peptide intensity (left.). Distribution of LODs on peptide intensity (right). The figures above each LOD group represent the number of peptides in each group, which is also displayed by the color scale.

Figure S3

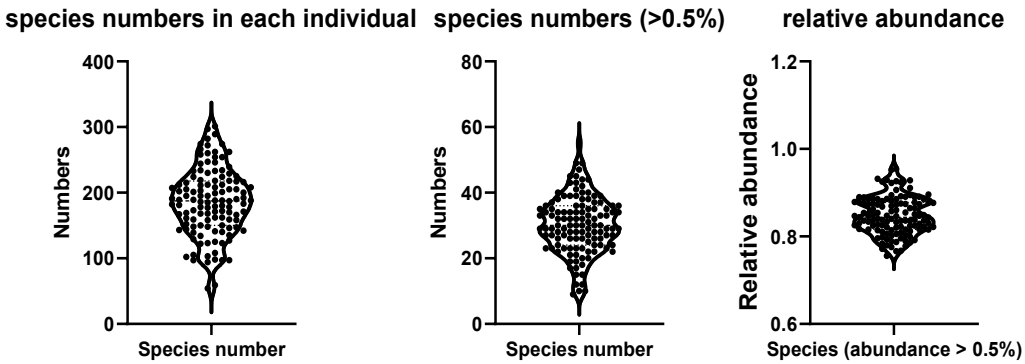


Figure S3. Abundance distribution of gut microbiome. a. Species number in each individual. b. The number of the species whose abundance over 0.5%. c. Relative abundance of species whose abundance over 0.5%. The analysis was based on the previous published metagenomic data.

Figure S4

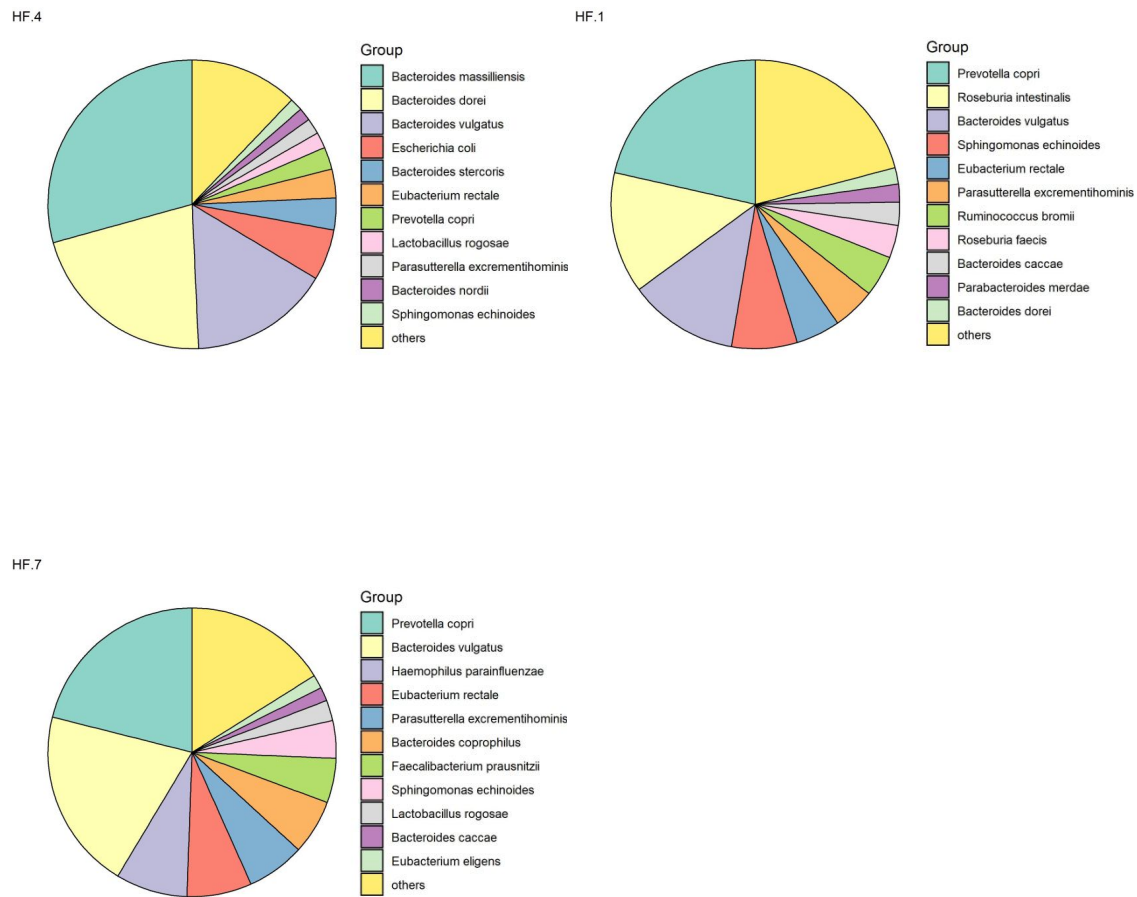


Figure S4. The three representative microbiome composition plots are based on the previously published metagenomic data. The pie charts were built by the species-level taxonomies. Sample indexes from original data are shown in the top left corner.

Figure S5

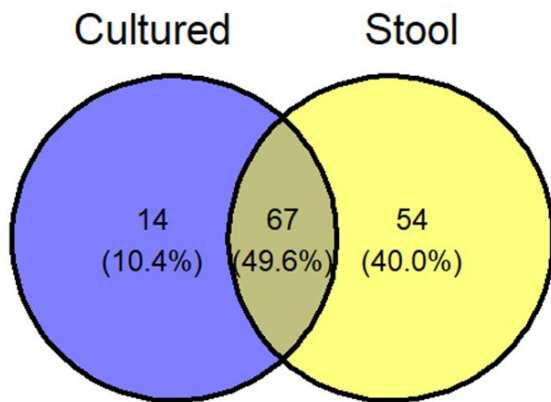


Figure S5. Intersected peptides with LOR at 0.5% between two groups.

Figure S6

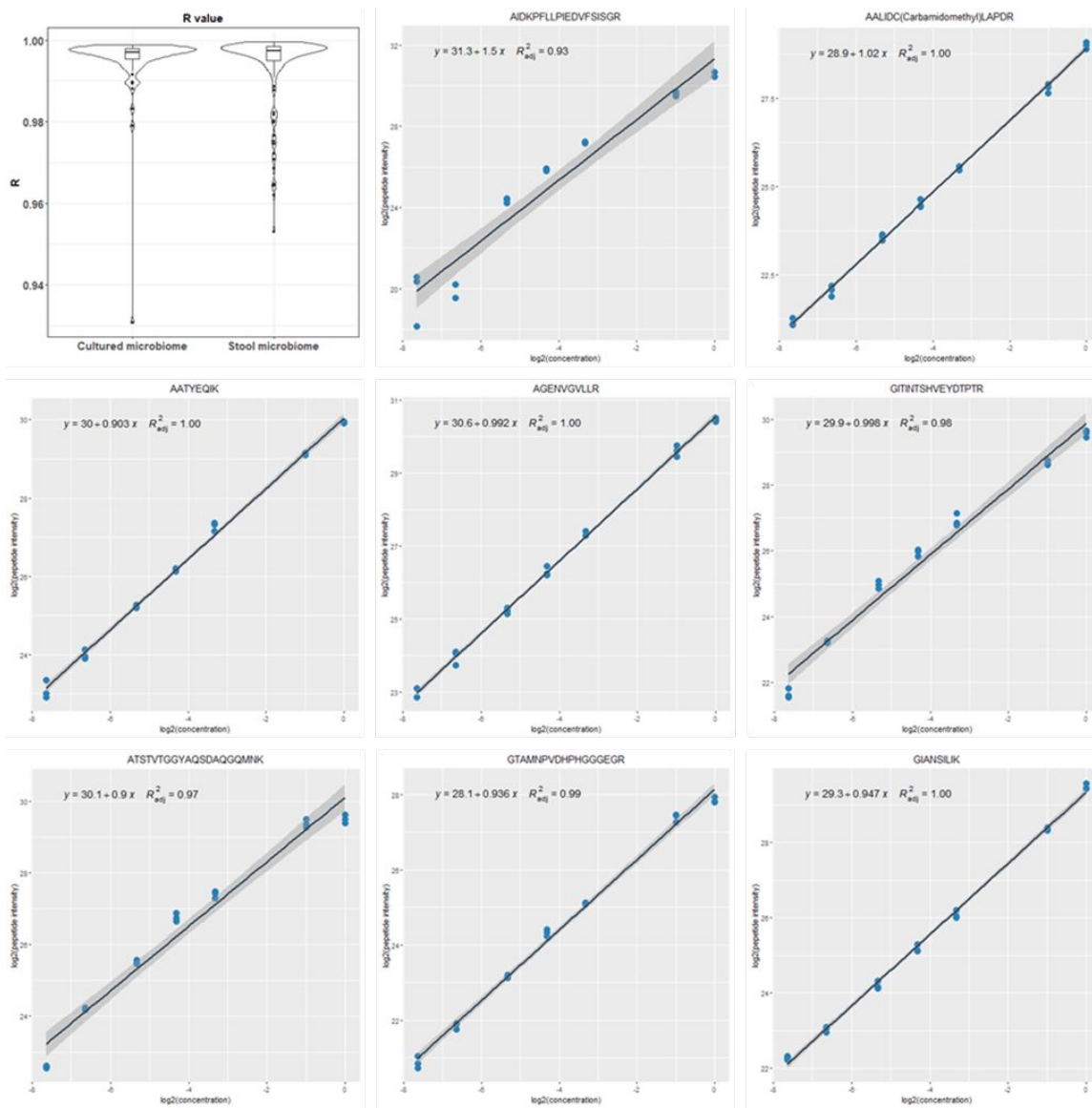


Figure S6. R^2 value from robust regression between log2 intensity of peptides whose LORs were at 0.5% and *E. coli* percentage in mixtures (The first figure) and representative linear regression plot when not performing MBR. (The other eight figures, the first four are from the cultured microbiome group; the last four are from the stool microbiome group).

Figure S7

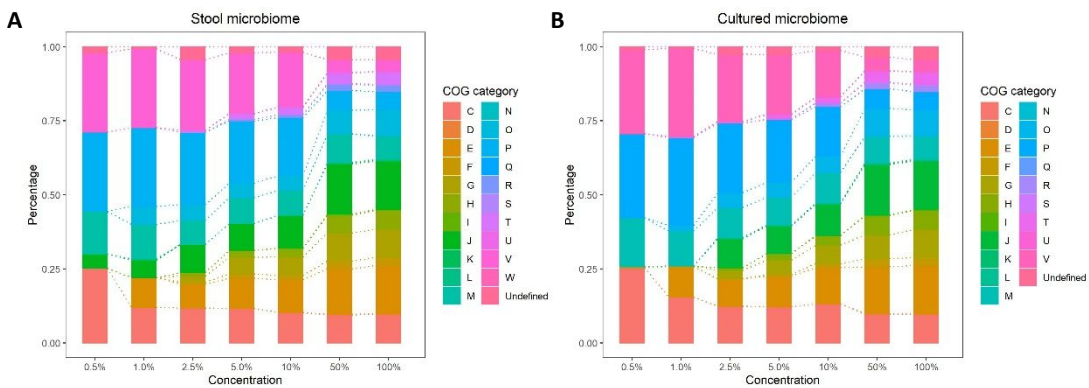


Figure S7. Evaluation of taxonomy and function analysis. The COG category composition along with *E. coli* concentration (Stool microbiome: A; Cultured microbiome: B).

Figure S8

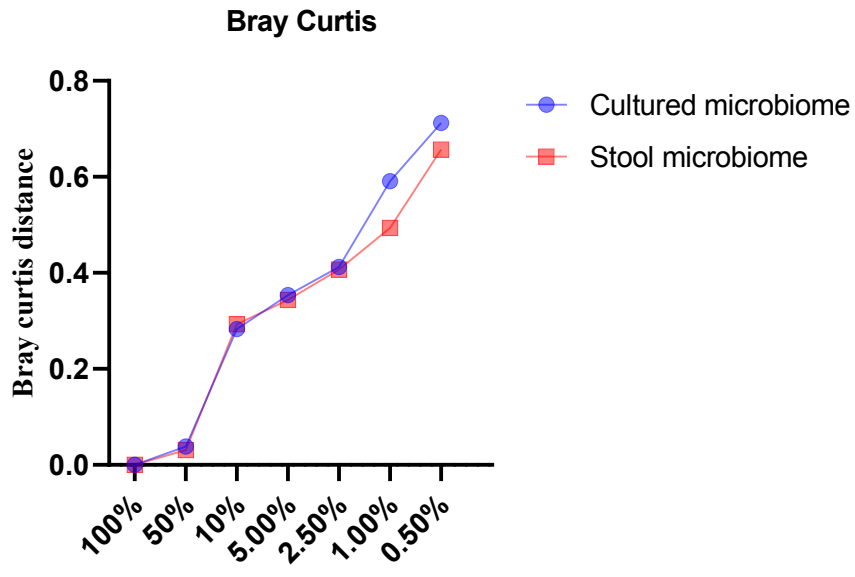


Figure S8. Bray-Curtis distance between each and 100% *E. coli* on COG composition.

Figure S9

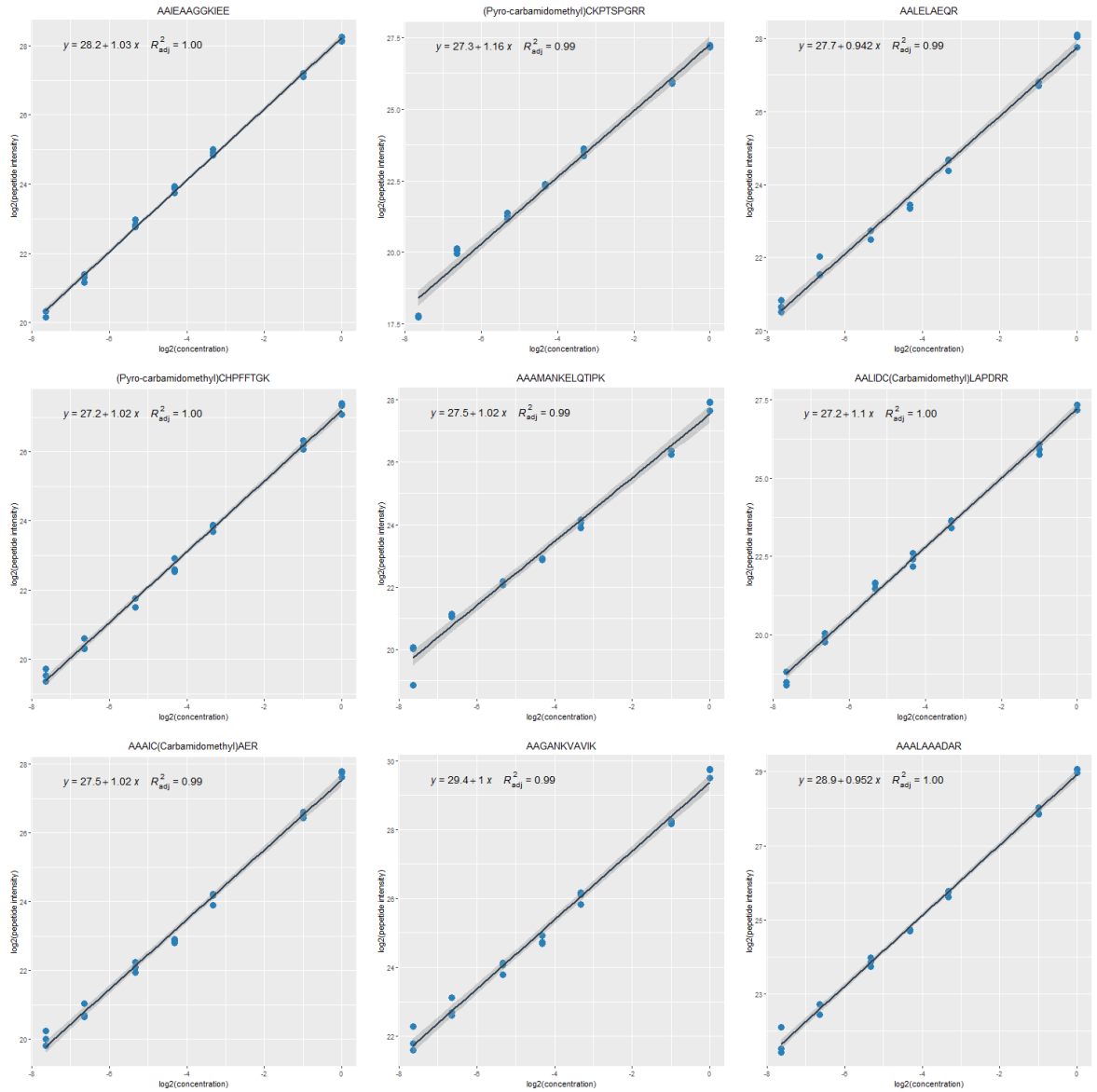


Figure S9. Representative well-fitted linear regression plot from cultured microbiome group when using MBR.

Figure S10

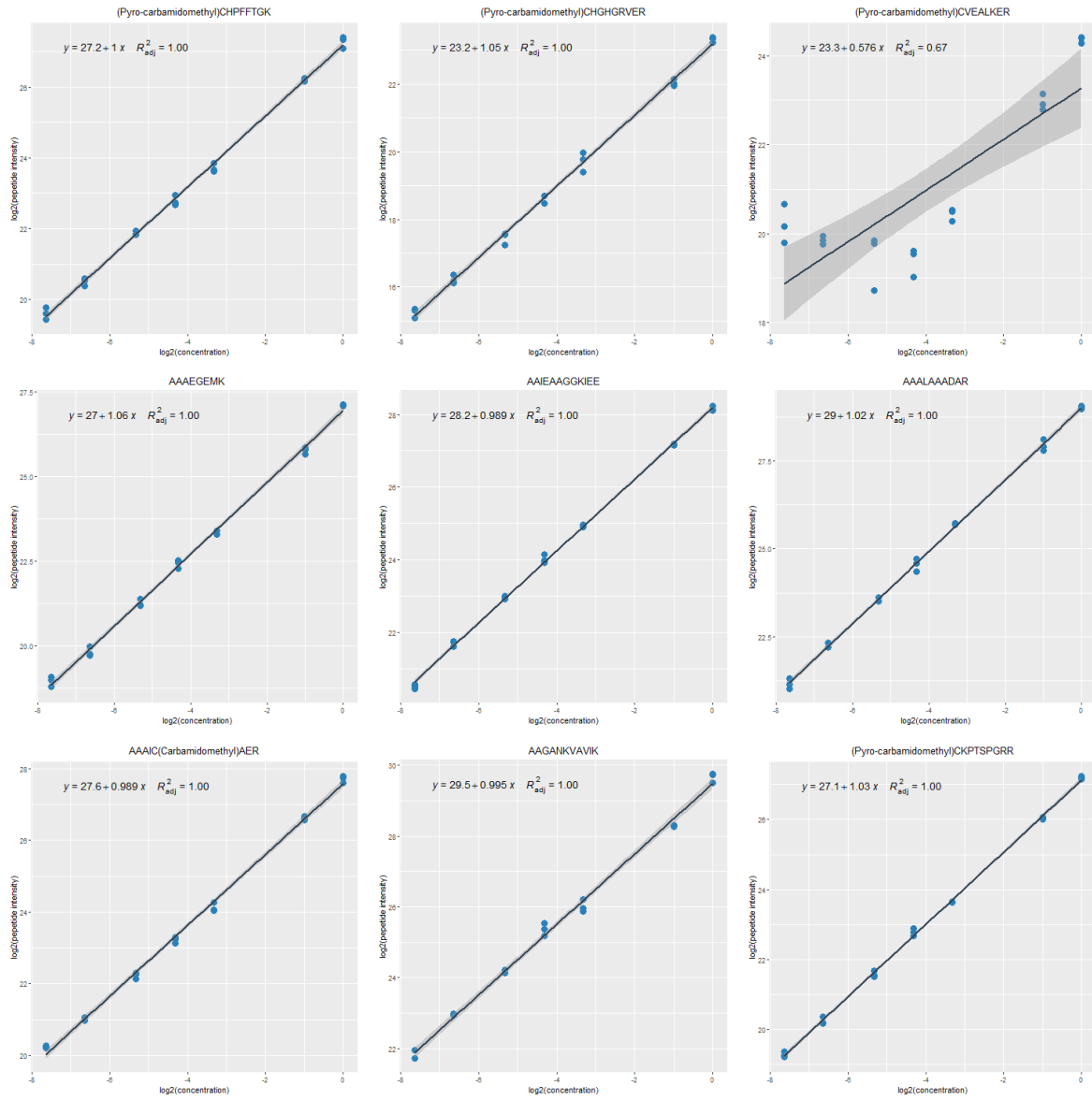


Figure S10. Representative well-fitted linear regression plot from stool microbiome group when using MBR.

Figure S11

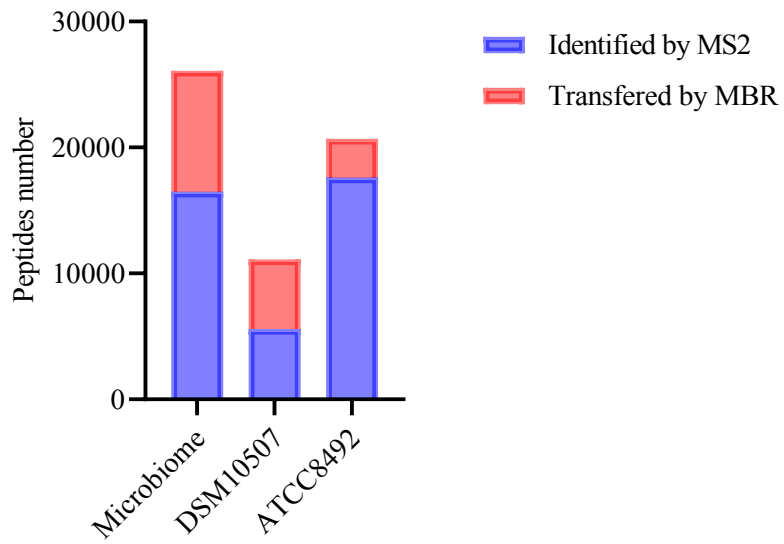


Figure S11. The number of quantified peptides. Peptides identified directly by database searching are shown in red. Peptides transferred by MBR are shown in blue.