Stability of Gut Enterotypes in Korean Monozygotic Twins and Their Association with Biomarkers and Diet

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Supplementary Fig. S1 Estimation of the optimal number of enterotypes. (a) The Calinski– Harabasz pseudo *F*-statistic and (b) Rousseeuw's Silhouette internal cluster quality index for three distance metrics (Jensen–Shannon [JS], Bray–Curtis [BC], Euclidean [EU] distances) of the genus-level relative abundance profiles.



Supplementary Fig. S2 Jensen–Shannon distances of the genus-level relative abundance profiles between individuals over time (self), twin pairs (at the same time point and at two different time points), and unrelated individuals (two sample permutation test; *P < 0.05; mean \pm s.e.m.).



Supplementary Fig. S3 Identification of two functional clusters based on KEGG pathway profiles. (a) Estimation of the optimal number of functional clusters calculated from the Calinski–Harabasz pseudo *F*-statistic and Rousseeuw's Silhouette internal cluster quality index for three distance metrics (Jensen–Shannon [JS], Bray–Curtis [BC], Euclidean [EU] distances) of the KEGG pathway relative abundance profiles, and (b) the first two principal

coordinates of the Jensen–Shannon distances of the KEGG pathway profiles. Samples are colored by functional cluster as identified by the partitioning around medoids (PAM) clustering algorithm. Red is functional cluster 1 and green is functional cluster 2.



Supplementary Fig. S4 Functional differences between two functional clusters based on KEGG pathway profiles. Histogram of the linear discriminant analysis (LDA) scores for differentially abundant KEGG modules between the functional clusters. Negative (red bars) and positive (green bars) LDA scores represent KEGG modules overrepresented in functional cluster 1 and functional cluster 2, respectively. Features with LDA scores >2 are presented.



Supplementary Fig. S5 Heat map of hierarchical clustering for 23 energy-adjusted nutrient values. Clustering was performed using Euclidean distance and an average linkage method. Red indicates enterotype 1 and green indicates enterotype 2.

Supplementary Table S1 Sample assignment to the enterotypes for three distance metrics (Jensen–Shannon [JS], Bray–Curtis [BC], Euclidean [EU] distances) and to the functional clusters based on JS distance.

| SampleID | Twin | Enterotype | | | Functional cluster |
|----------|------|------------|----|----|---------------------------|
| | | JS | EU | BC | JS |
| S183_1 | T92 | 1 | 1 | 1 | 2 |
| S183_2 | T92 | 1 | 1 | 1 | 1 |
| S184_1 | T92 | 1 | 1 | 1 | 1 |
| S184_2 | T92 | 1 | 1 | 1 | 1 |
| S197_2 | T99 | 2 | 2 | 2 | 2 |
| S198_2 | T99 | 2 | 2 | 2 | 2 |
| S205_1 | T103 | 2 | 2 | 2 | 2 |
| S205_2 | T103 | 2 | 2 | 2 | 2 |
| S206_1 | T103 | 2 | 2 | 2 | 2 |
| S206_2 | T103 | 2 | 2 | 2 | 2 |
| S223_1 | T112 | 1 | 1 | 1 | 2 |
| S223_2 | T112 | 1 | 1 | 1 | 2 |
| S224_1 | T112 | 1 | 1 | 2 | 2 |
| S224_2 | T112 | 1 | 1 | 1 | 1 |
| S241_2 | T121 | 1 | 1 | 1 | 1 |
| S242_2 | T121 | 1 | 1 | 1 | 1 |
| S249_1 | T125 | 2 | 2 | 2 | 2 |
| S249_2 | T125 | 1 | 1 | 2 | 2 |
| S250_1 | T125 | 2 | 2 | 2 | 2 |
| S250_2 | T125 | 2 | 2 | 2 | 2 |
| S251_1 | T126 | 2 | 2 | 2 | 2 |
| S251_2 | T126 | 1 | 1 | 2 | 2 |
| S252_1 | T126 | 2 | 2 | 2 | 2 |
| S252_2 | T126 | 2 | 2 | 2 | 2 |
| S267_1 | T134 | 2 | 2 | 2 | 2 |
| S267_2 | T134 | 2 | 2 | 2 | 2 |
| S268_1 | T134 | 2 | 2 | 2 | 2 |
| S268_2 | T134 | 2 | 2 | 2 | 2 |
| S279_1 | T140 | 2 | 2 | 2 | 2 |
| S279_2 | T140 | 2 | 2 | 2 | 2 |
| S280_1 | T140 | 1 | 1 | 1 | 2 |
| S280_2 | T140 | 1 | 1 | 1 | 1 |
| S281_1 | T141 | 1 | 1 | 1 | 1 |
| S281_2 | T141 | 2 | 2 | 2 | 2 |
| S282_1 | T141 | 2 | 2 | 2 | 2 |
| S282_2 | T141 | 2 | 2 | 2 | 2 |