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Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, seeAuthors & Referees and theEditorial Policy Checklist.

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FOI	all statistical analyses, confirm that the following items are present in the figure regend, table regend, main text, or Methods section.
n/a	Confirmed
x	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
x	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
x	The statistical test(s) used AND whether they are one- or two-sided Only common tests should be described solely by name; describe more complex techniques in the Methods section.
x	A description of all covariates tested
x	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
×	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
x	For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
X	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
X	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
X	Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated
	Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

Data analysis

no software was used.

quality trimming; MetaPhlAn v2 was used to perform taxonomic profiling; Jaccard clustering custom code was deposited in https://github.com/ravel-lab/VIRGO; R package iNEXT v2.0 and vegan v2.5-5 was used for rarefaction; IDBA-UD (v1.0) was used for assembly; MetageneMark (v3.25) was for gene calling; CD-HIT-EST (v4.6) was used for gene clustering. The following databases were used to annotate VIRGO: KEGG FTP Release 2013-03-18, EggNOG v4.5, COG v1, CDD v3.14, Pfam v30.0, ProSite/ProDo v20.119, TIGRFAM v15.0. InterPro v60.0, CATH Gene3D v4.1, SMART v7.1, HAMAP v201605.11, PIRSF v 3.01, MobiDB v2.0.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

 $All\ manuscripts\ must\ include\ a\ \underline{data\ availability\ statement}.\ This\ statement\ should\ provide\ the\ following\ information,\ where\ applicable:$

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

All database data and code are freely assessable at https://github.com/ravel-lab/VIRGO. It includes Jaccard index clustering code, VIRGO non-redundant nucleotide gene database, VOG amino acid protein family database, curated taxonomy and functions information, and detailed tutorials. Metagenomes used in the analyses are available at http://virgo.igs.umaryland.edu.

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Sample size	N/A					
Data exclusions	N/A					
Replication	N/A					
Randomization	N/A					
Blinding	N/A					
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Animals and other organisms

Human research participants

Human resea