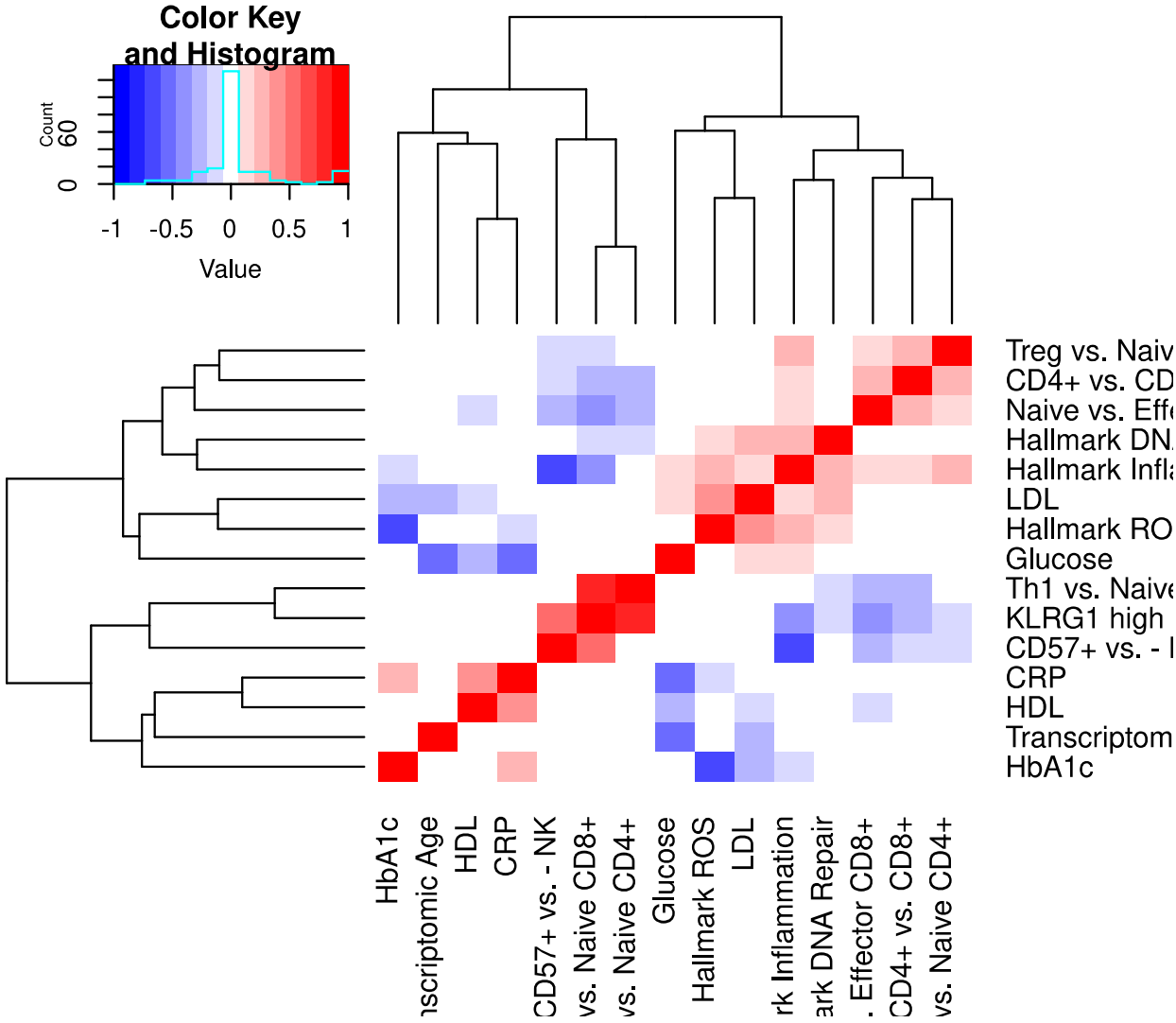
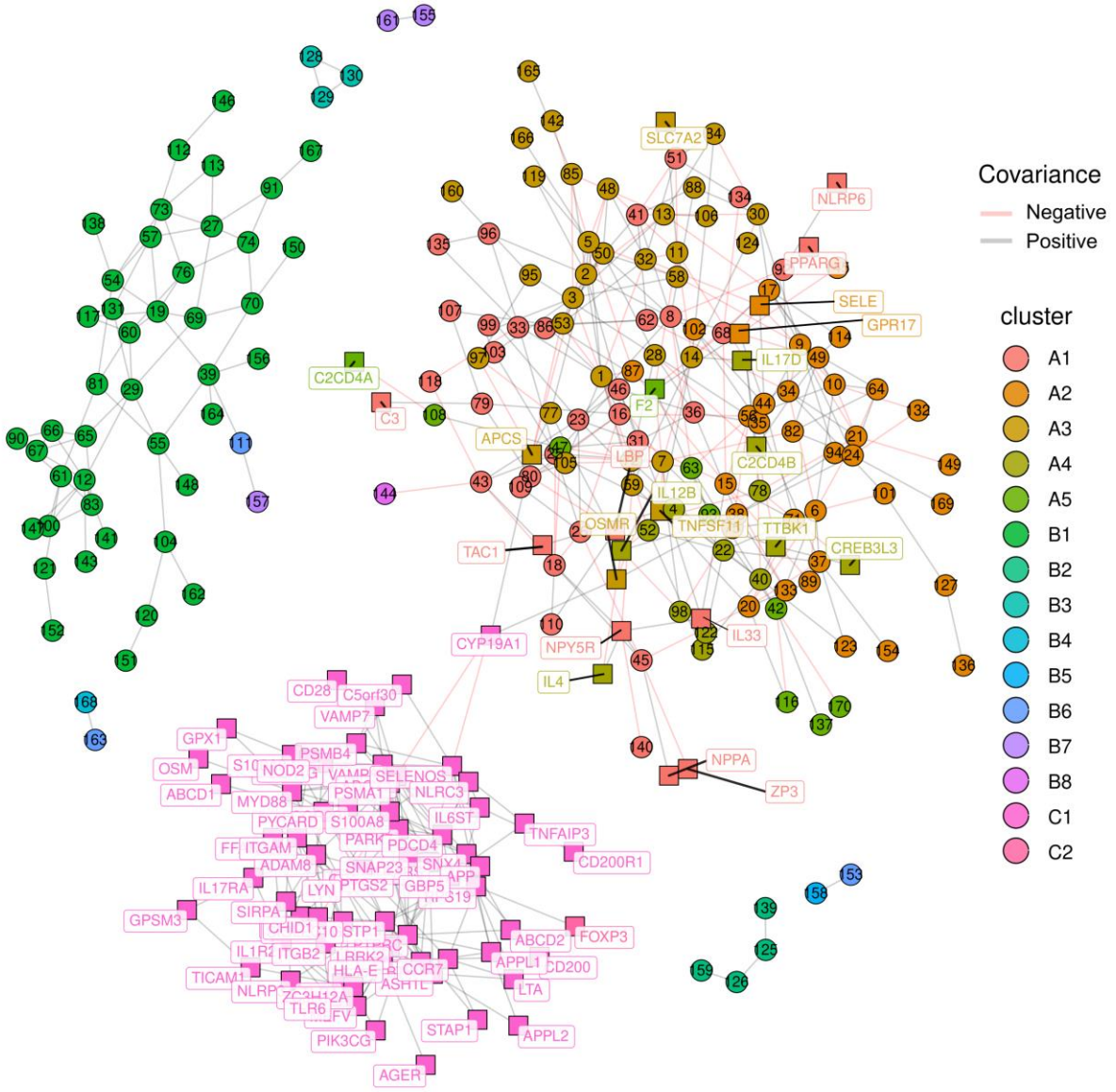
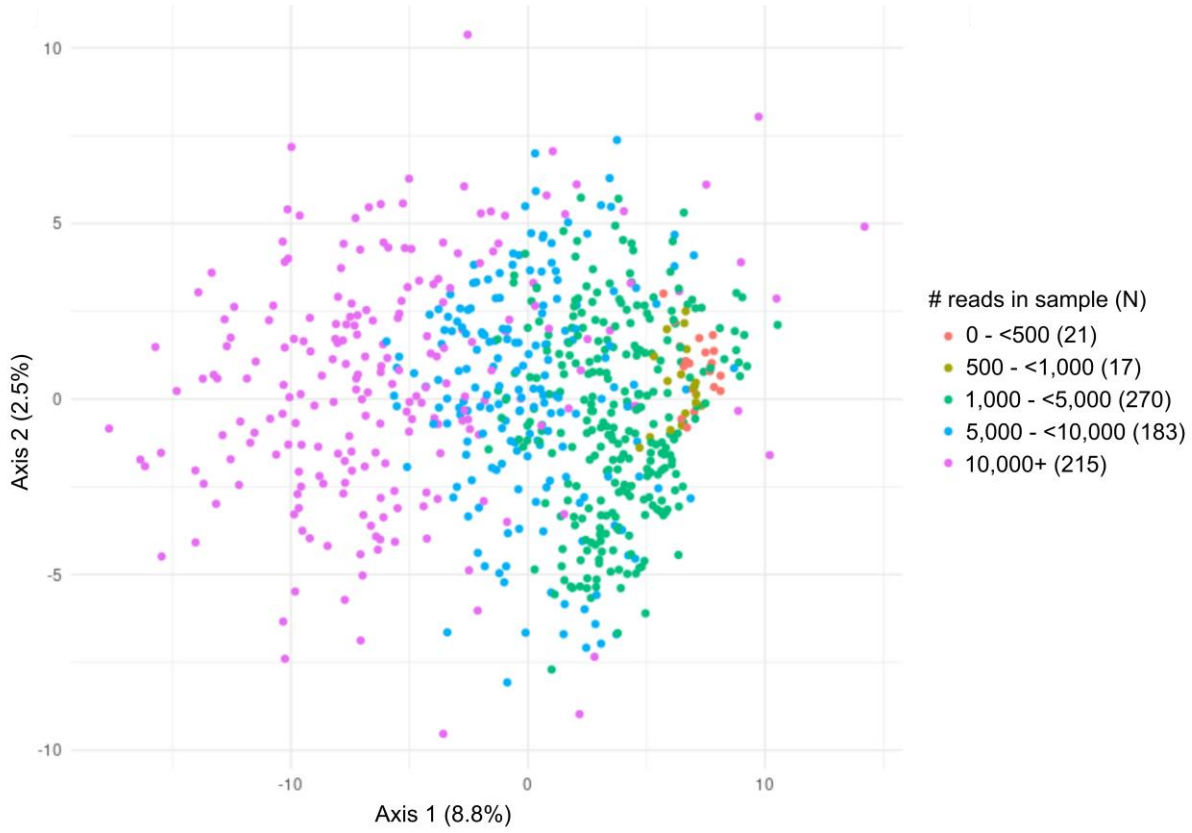


eFigure 1. Pairwise correlations between microbiome signals for each biomarker. Each square represents the correlation between the log2-fold change estimates between biomarker A and every taxon, and the log2-fold change estimates between biomarker B and every taxon, where biomarker A and B are on the X and Y axis, respectively.



eFigure 2. Final network of inflammation-related genes and bacterial taxonomic abundances estimated using a Gaussian graphical model. Only those connections identified in both the discovery (n=177) and replication (n=168) samples are shown. Clusters were identified using Louvain's community detection algorithm. See eTable 4 for a full key.



eFigure 3. Principal component analysis using centered log-ratio transformed microbiome count data, according to number of reads in sample, for all (n=706) samples with sufficient material for sequencing.

eMethods

Measurement of Biomarkers and medications

As part of Wave IV, trained and certified field examiners collected dried blood spots from voluntarily fasting (≥8 hours) and non-fasting participants via finger prick on seven-spot, Whatman 903® Protein Saver cards. Cards were dried on location, then shipped to the University of Washington Department of Laboratory Medicine (Seattle, WA) for assay of glucose (mg/dl), low-density lipoprotein (LDL), high-density lipoprotein (HDL), and high-sensitivity c-reactive protein (CRP); and to FlexSite Diagnostics, Inc. (Palm City, FL) for assay of HbA1c (%). Because LDL, HDL, and glucose are provided in deciles, and because gene expression biomarkers other than transcriptomic age are derived from a standardized principal

component scale, these markers are not directly interpretable as clinically relevant changes. Further information about the dried blood spot collection, assays, and medications are available at (1).

Measurement of Gene Expression

After participation in the Wave V survey, respondents were visited by a field examiner/phlebotomist to collect biological specimens including a sample of venous blood. Using RNA isolated from Pax gene, 200 ng of total RNA were converted to cDNA (Lexogen QuantSeq) and sequenced using an Illumina HiSeq 4000 instrument in the University of California Los Angeles, Neuroscience Genomics Core Laboratory. Each sample yielded >10 million 65nt single-strand sequencing reads, which were mapped to the RefSeq human genome sequence reference (ENSEMBL hg38) and quantified as transcript counts per million mapped reads using STAR aligner v 2.6.(2)

Fecal microbiome sample collection and processing

All microbial DNA was prepared, stored, and sequenced at CU Boulder. Microbial DNA was prepared with a QIAGEN high-throughput system (MagAttract Power Soil Kit EP). Approximately 50ng of DNA from each sample was subjected to PCR with barcoded sequencing primers specific for V4 of the 16S rDNA in triplicate. The following primers were used: 515F (Parada)–806R (Apprill), forward-barcoded: FWD:GTGYCAGCMGCCGCGGTAA; REV:GGACTACNVGGGTWTCTAAT. The triplicates were then pooled, quantitated using picogreen, mixed in equimolar amounts and subjected to paired-end DNA sequence analysis on an Illumina MiSeq Personal Sequencer. Illumina base quality scores of greater than 30 were required. We used QIIME2 (3) v. 2019.4 for all the following data processing steps. Filtered

reads were demultiplexed to assign reads to individual samples, using the Deblur pipeline (4) to determine operational taxonomic units (OTUs) at single-nucleotide resolution. Clustering of OTUs was performed at the 97% similarity level and taxonomy was assigned using closed-reference picking from GreenGenes v. 13-8, which has been shown to be highly effective at identifying taxa from constructed complex samples.(5) Previous work by the American Gut Project has characterized the taxonomic changes expected due to specimen tubes being shipped at room temperature with transit times up to four days, which consist of "blooms" of particular species.(6) As an additional quality control step, we used an algorithm developed by the Knight lab to remove reads identified as reflecting microbial blooms occurring during room temperature shipping using Deblur.(7) Finally, we removed samples with less than 1,000 reads prior to analysis. We selected this cutoff as a compromise between including as many samples as possible and preserving as much variability as possible in the multivariate distribution of the data in compositional PCA (shown in eFigure 3).

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