Supplementary Material

Measurement of Serum Cytokines

The Simoa Corplex Planar Array Cytokine Assays and Simoa Planar Array Developer Assays are for the quantitative determination of cytokines in human serum and EDTA plasma. Commercially available Simoa Corplex Planar Array Assay-Corplex Cytokine Panel 1 10-plex (interferon-gamma, IL-1β, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-22, and TNFα) and Simoa Planar Array Developer Single Plex Assays (IL-12p40/IL-23, IL-17α, IL-7, IL-15, VEGF-A, IL-13, IL-1α) were used according to the manufacturer's instructions. The assays were run on the Simoa SP-X semi-automated analyzer.

All measurements were conducted on the semi-automated SP-X analyzer (Quanterix, Billerica, MA). For plasma cytokine levels, samples (n = 68) were diluted 4× and assayed in duplicate. The mean of the replicates for each sample was calculated and represented graphically. Results were included in the analysis if the coefficient of variation across replicates was <20%.

Two controls (low and high) were used with every run. Mean (SD) was calculated for each control. Control ranges were generated by running the controls on multiple runs in duplicate. Data generated were reviewed to ensure the coefficient of variation is <20%. Ranges were established by using mean + 3 SD values.

Microbiome Analysis

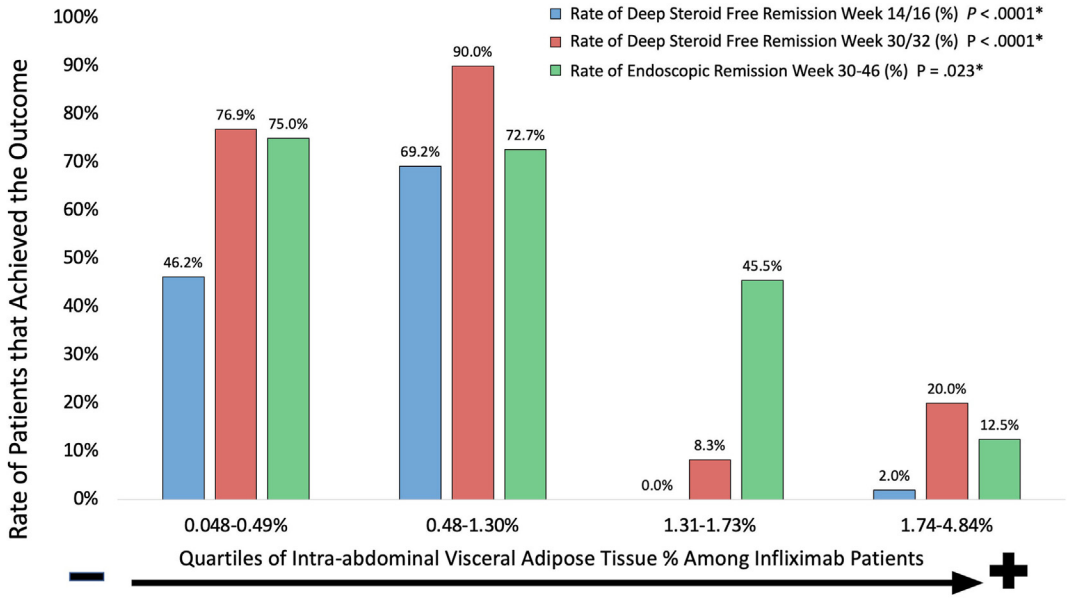
Purified genomic DNA was submitted to the University of Wisconsin-Madison Biotechnology Center. DNA concentration was verified fluorometrically using either the Qubit dsDNA HS Assay Kit or Quant-iT PicoGreen dsDNA Assay Kit (ThermoFisher Scientific, Waltham, MA). Samples were prepared in a process similar to the one described in Illumina's 16s Metagenomic Sequencing Library Preparation Protocol, Part #15044223 Rev. B, with the following modifications: The 16S ribosomal RNA gene V3/V4 variable region was amplified with fusion primers (forward primer 341f: 5′-ACACTCTTTCCCTACACGACGCTCTTCCGATCT(N)3/6CCTACGGGNGGCWGCAG-3′, reverse primer 805r: 5′-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT(N)3/6GACTACHVGGGTATCTAATCC-3′). Region specific primers were described previously in Klindworth et al[e1] (sequences above), and were modified to add 3–6 random nucleotides ((N)3/6) and Illumina adapter overhang nucleotide sequences 5′ of the gene-specific sequences. After the initial amplification, reactions were cleaned using a 0.7× volume of AxyPrep Mag PCR clean-up beads (Axygen Biosciences, Union City, CA). In a subsequent polymerase chain reaction, Illumina dual indexes and sequencing adapters were added using the following primers (Forward primer: 5′-AATGATACGGCGACCACCGAGATCTACAC[55555555]ACACTCTTTCCCTACACGACGCTCTTCCGATCT-3′, Reverse Primer: 5′CAAGCAGAAGACGGCATACGAGAT[77777777]GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT -3′, where bracketed sequences are equivalent to the Illumina Dual Index adapters D501-D508 and D701-D712,N716,N718-N724,N726-N729). After polymerase chain reaction, reactions were cleaned using a 0.7× volume of AxyPrep Mag PCR clean-up beads (Axygen Biosciences). Quality and quantity of the finished libraries were assessed using an Agilent DNA 1000 kit (Agilent Technologies, Santa Clara, CA) and Qubit dsDNA HS Assay Kit (ThermoFisher Scientific), respectively. Libraries were pooled in an equimolar fashion and appropriately diluted before sequencing. Paired end, 300-bp sequencing was performed using the Illumina MiSeq Sequencer and a MiSeq 600 bp (v3) sequencing cartridge. Images were analyzed using the standard Illumina Pipeline, version 1.8.2. Operational taxonomic unit assignments and diversity plots were created using Quantitative Insights Into Microbial Ecology analysis pipeline.

Bioinformatic Analysis

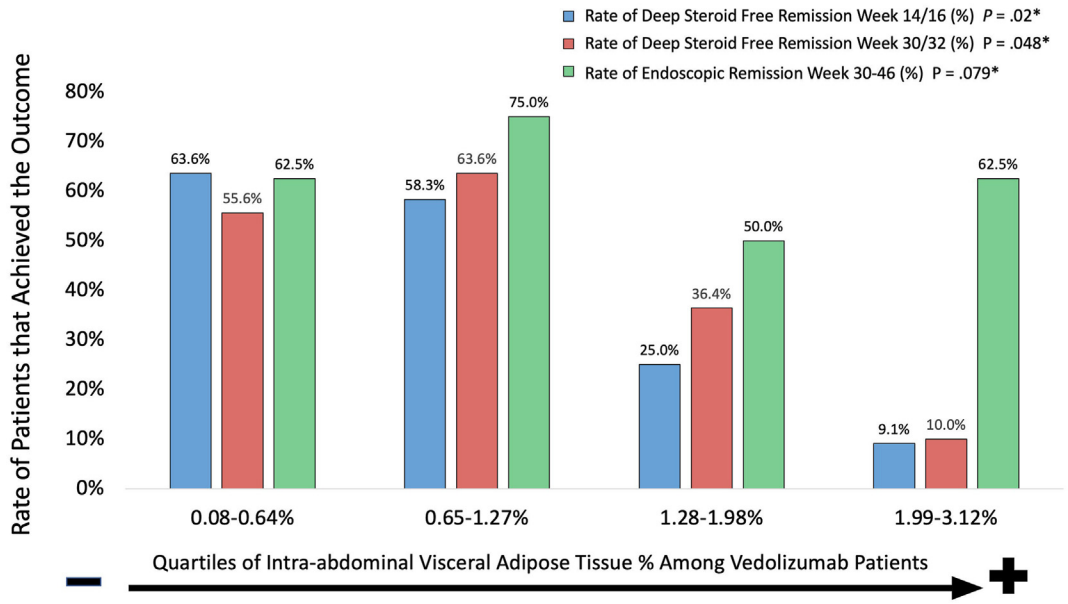
Microbiome analysis was performed by the University of Wisconsin Biotechnology Center using Quantitative Insights Into Microbial Ecology, version 2. Illumina sequencing reads were denoised and quality filtered using the denoising program DADA2. This trimmed low-quality bases, filtered out noisy sequences, corrected errors in marginal sequences, removed chimeric sequences and singletons, and then dereplicated those sequences. The resultant dereplicated sequence was termed as an *Amplicon sequence variant* and is equivalent to an operational taxonomic unit. Sequence variants were aligned and masked using Mafft and the phylogenetic tree of the Amplicon sequence variants was created using FastTree. Taxonomy will be assigned using a Bayesian classifier based on a pretrained silva database within each sample will be generated for further downstream analysis. Low-frequency reads (<0.01%) will be filtered from the Biom-formatted table. α-Rarefaction curves using Shannon, Simpson, and observed were calculated for all samples with a rarefaction upper limit of median depth/sample count and the α-diversity between different groups was compared using Wilcoxon rank sum test. Samples were removed from further characterization if they did not contain succinct reads. β-diversity was calculated and ordination plots were generated using Bray-Curtis and Jaccard (nonphylogenetic), weighted and unweighted Unifrac (phylogenetic) on Amplicon sequence variant data leveled according to the lowest sample depth.

Supplementary Reference

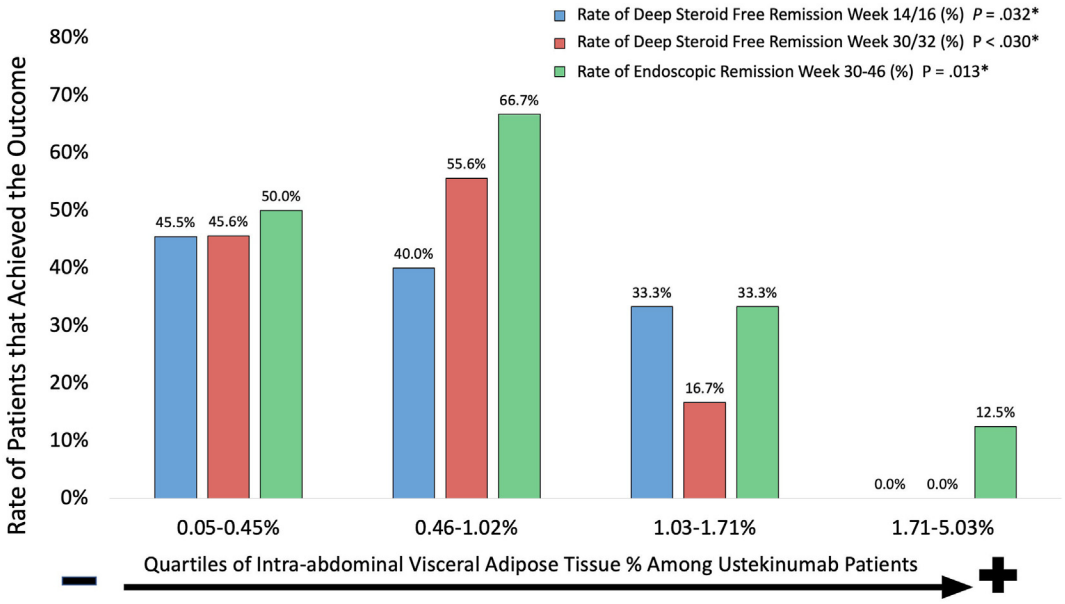
e1. Klindworth A, Pruesse E, Schweer T, et al. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res 2013;41(1):e1.



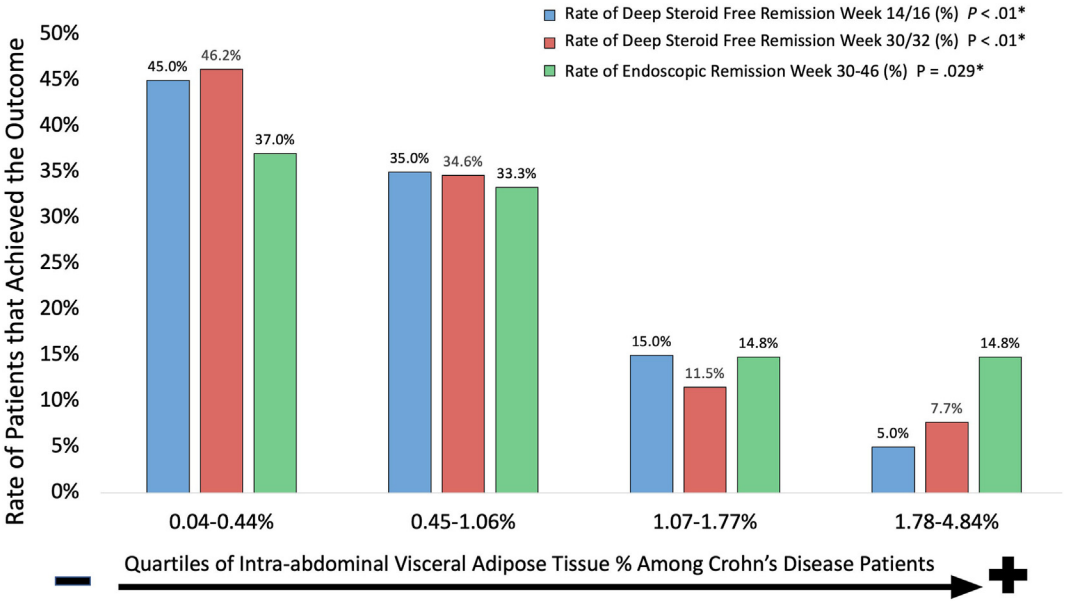
Supplementary Figure 1. Rates of steroid-free deep remission at weeks 14 and 30, and endoscopic remission in patients who started infliximab stratified by intra-abdominal visceral adipose tissue quartiles. **P* value for differences among groups (Kruskal–Wallis test).



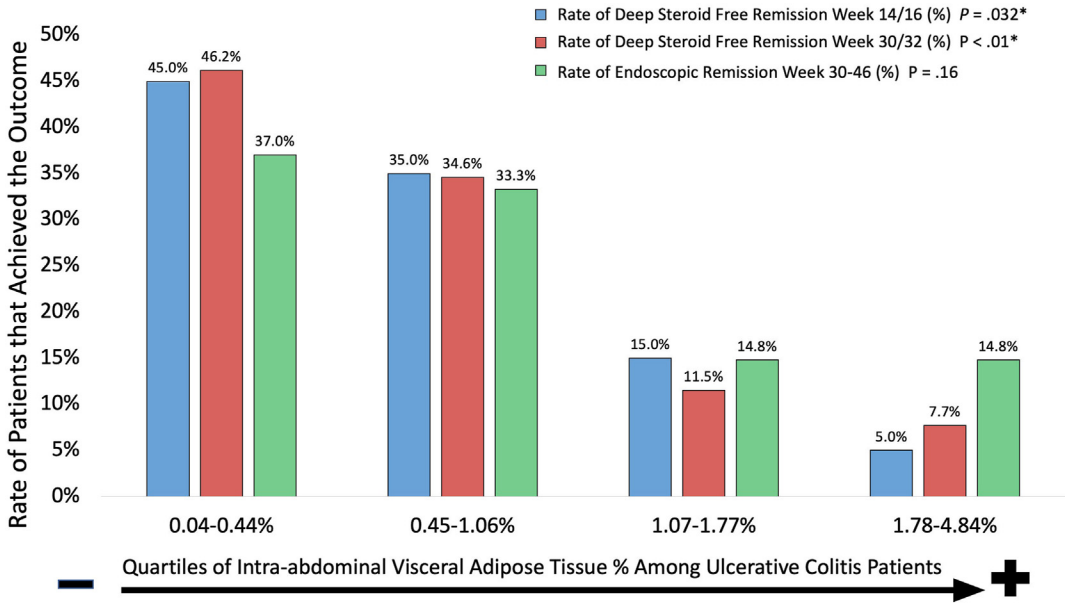
Supplementary Figure 2. Rates of steroid-free deep remission at weeks 14 and 30, and endoscopic remission in patients who started vedolizumab stratified by intra-abdominal visceral adipose tissue quartiles. **P* value for differences among groups (Kruskal–Wallis test).



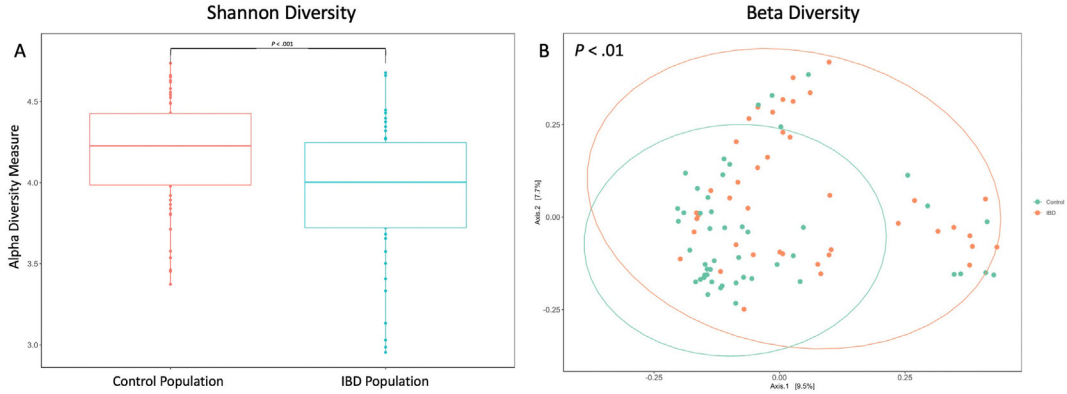
Supplementary Figure 3. Rates of steroid-free deep remission at weeks 14 and 30, and endoscopic remission in patients who started ustekinumab stratified by intra-abdominal visceral adipose tissue quartiles. **P* value for differences among groups (Kruskal–Wallis test).



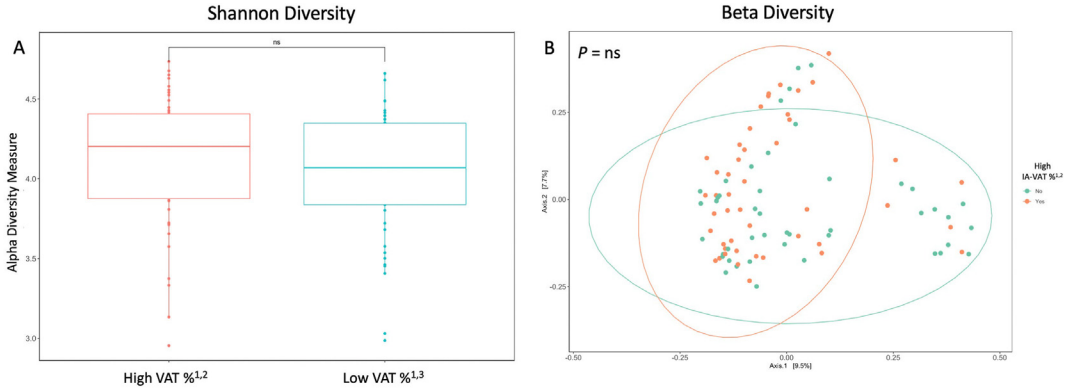
Supplementary Figure 4. Rates of steroid-free deep remission at weeks 14–16 and 30–32, and endoscopic remission in patients who started biologic therapy for CD. **P* value for differences among groups (Kruskal–Wallis test).



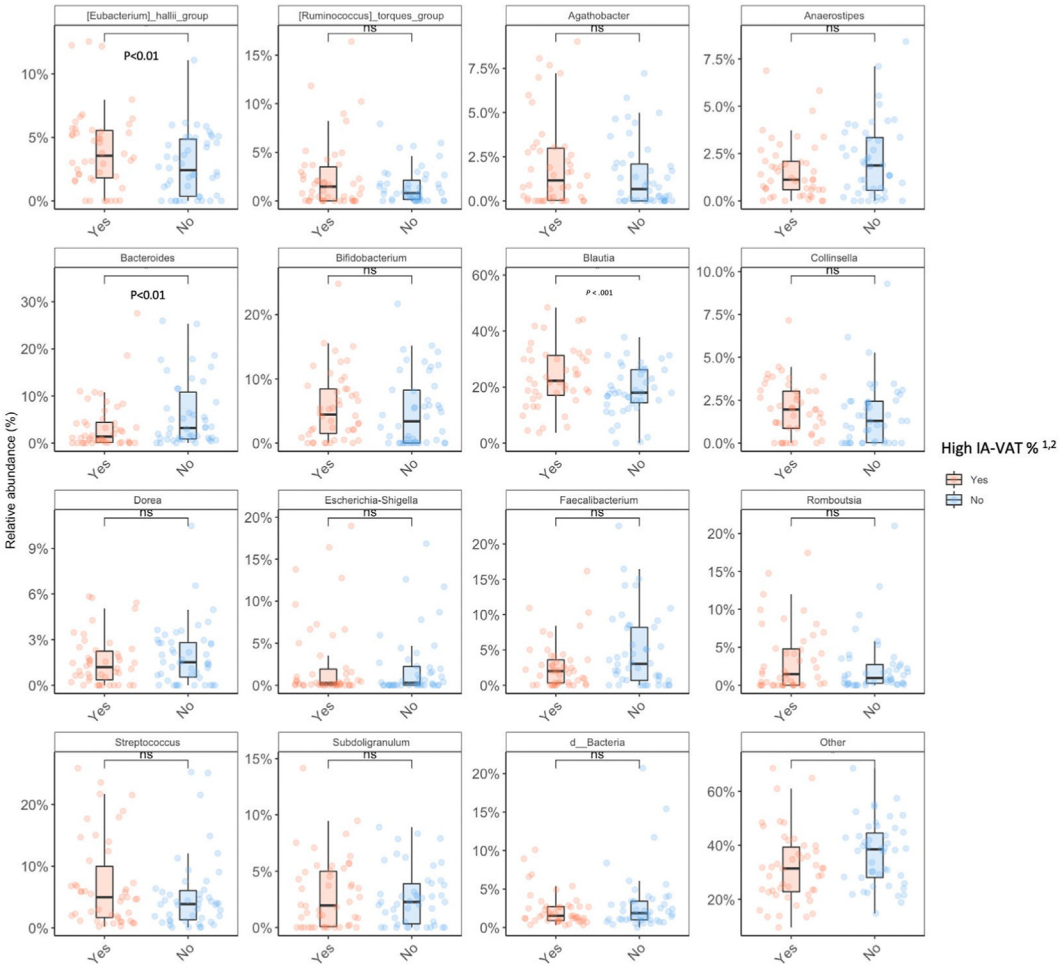
Supplementary Figure 5. Rates of steroid-free deep remission at weeks 14–16 and 30–32, and endoscopic remission in patients who started biologic therapy for ulcerative colitis. **P* value for differences among groups (Kruskal–Wallis test).



Supplementary Figure 6. Patients with IBDs had significantly lower α- (Shannon) (*A*) and β-diversity (*B*) compared with healthy controls.



Supplementary Figure 7. No differences in α- (Shannon) (*A*) or β-diversity (*B*) were seen among patients with active IBD starting therapy with high or low visceral adipose tissue burden. [1]IA-VAT%, visceral adipose tissue. [2]Patients with high IA-VAT % were those on the 2 highest quartiles of the cohort ($\geq$1% of total body mass). [3]Patients with low IA-VAT% were those on the lower 2 quartiles of the cohort (<1% of total body mass).



Supplementary Figure 8. Relative abundance of top taxa in the IBD vs control group.