**Supplementary Methods**

**Sample Collection.** Fecal samples were collected at home in 3 parts: (1) in a collection tube containing 70% ethanol, (2) in an empty tube, (3) on sterile Q-tip swab. All three containers were placed in a plastic biohazard bag with a frozen icepack inside the sleeve to keep the samples cold. The samples were picked up by study personnel or returned to the study clinic by the participant within 24 hours of the time the stool was collected. Stool samples were stored at the clinic site in a -200C freezer for up to one week until transfer to a -800C freezer at the University of North Carolina at Chapel Hill (UNC). Blood for serum and plasma was collected and processed at the clinic site during the T4 visit and also transferred for storage at -800C.

**Fecal Microbiome Analysis.** For DNA isolation, 0.3 ml of Qiagen ATL buffer (Valencia, CA) supplemented with 60 mg/ml lysozyme (Thermo Fisher Scientific, Grand Island, NY) was added to approximately 200 mg of stool samples. The suspensions were transferred to 2 ml tubes containing 106/500μm glass beads (Sigma, St. Louis, MO) and incubated for 1 hour at 37°C with occasional agitation. The suspensions were then bead beated on a Digital Vortex Mixer and supplemented with 20uL of Qiagen proteinase K and 0.3 ml of Qiagen AL buffer and incubated at 55°C overnight. DNA then was purified using a standard on-column purification method with Qiagen buffers AW1 and AW2 as washing agents and eluted in 27.5 µl of DNase free water and quantified using QuantIT® PicoGreen®. Sequencing libraries were prepared from 12.5 ng of total DNA amplified using universal primers targeting the V4 region of the bacterial 16S rRNA gene (1). Primer sequences contained overhang adapters appended to the 5’ end of each primer for compatibility with Illumina sequencing platform. Each 16S amplicon was purified using the AMPure XP reagent (Beckman Coulter, Indianapolis, IN). In the next step each sample was amplified using a limited cycle PCR program, adding Illumina sequencing adapters and dual‐index barcodes (index 1(i7) and index 2(i5)) (Illumina, San Diego, CA) to the amplicon target. The final libraries were again purified using the AMPure XP reagent, quantified and normalized prior to pooling. The DNA library pool was then denatured with NaOH, diluted with hybridization buffer and heat denatured before loading on the MiSeq reagent cartridge (Illumina) and on the MiSeq instrument (Illumina). Automated cluster generation and paired–end sequencing with dual reads were performed according to the manufacturer’s instructions.

MiSeq runs were converted to fastq format and demultiplexed using bcl2fastq v.2.20.0. Paired reads were merged using QIIME 1.9.0 (2) invocation of fastq-join with the default options. Index and linker primer sequences were trimmed and the reads were subsequently filtered for quality removing any read where the percentage of quality scores below the quality threshold of 24 fell below 70%. Quality control of both raw and processed sequencing reads was verified by FastQC. Sequencing of 16S rRNA genes from the 92 samples resulted in 7,717,717 non-chimeric sequences. The fraction of non-zero values was 0.084. Each sample had at least 60,000 reads; the average was 83,888. Reads were clustered de novo into operational taxonomic unit (OTUs) at the 97% similarity level using UCLUST (3) algorithm.

Chimeras were identified and removed with VSEARCH (4) using ChimeraSlayer "gold" reference database (5) as a reference. Singletons were removed using QIIME’s filter\_otus\_from\_otu\_table.py. The remaining OTUs were assigned taxonomic identifiers with respect to the Greengenes database (6), their sequences were aligned using template alignment through PyNAST (2), and a phylogenetic tree was built with FastTree 2.1.3 (7). An OTU was retained if it was observed in at least 2 samples.

**Cytokine analysis.** Cytokine analysis was performed using G5 series arrays to profile 80 markers following the manufacturer’s instructions (RayBiotech Life, Peachtree Corners, GA) in plasma samples (n=73). Array slides were scanned using two-color fluorescent detection and the raw data extracted to yield relative fluorescence units (RFUs) for each cytokine per sample. Positive control spots on each array were evaluated to ensure they fell within a quality control range of ≤ 15 % variation to positive control RFUs on a reference-designated array. The data were preprocessed using the RayBiotech Analysis Tool, for background-subtraction and normalization to the positive control values on the reference array. Relative fold-changes were calculated by normalizing the RFU for each cytokine in all groups by the average RFU for all controls. According to the manufacturer’s thresholds, cytokines with a fold difference of ≥ 1.5-fold were accepted as significantly upregulated and denoted by bold letteringand red highlight in Supplementary Table S1, while cytokines with a fold difference of ≤ 0.65-fold were accepted as significantly downregulated and denoted by italicized lettering and green highlight*.*

**Fecal transplantation to germ-free mice.** Human fecal samples (1.6 grams per donor) were prepared with pre-reduced sterile PBS in an anaerobic chamber and vortexed in 50ml pre-reduced PBS for 5 minutes to disrupt all fibrous chunks. Fecal slurries were then centrifuged at 9xgravity for 3 minutes at 40 C to remove all solid material and supernatants were aliquoted and stored at -80. Mice were gavaged with 10ml/kg (250ul maximum) of the fecal slurry. At week 0, mice ranged from 23-28g and received 230-250ul. For all remaining timepoints, all mice were over 25g and received 250ul. After gavage, mice were placed in IsoRacks (double HEPA filtered cages) in a cubicle in the mouse facility such that mice receiving the OA fecal pool were housed separately from those receiving the control pool.

**Histologic evaluation.** Coronal sections of 5-micron thickness were generated and two mid-coronal sections were used for hematoxylin and eosin (H&E) and Safranin O staining. Another section 80 microns posterior was also collected and stained with Safranin O. Articular cartilage damage was graded 0-12 using the Articular Cartilage Structure (ACS) score, proteoglycan loss was graded 0-12 by the Safranin O staining score, osteophytes (0-3), and synovial hyperplasia (0-3) were scored as previously described (8, 9). All four quadrants of the joint (medial and lateral tibial plateaus and femoral condyles) were used for ACS and Saf-O grading on each of the two sections and then added to produce the sum scores. Osteophyte scores were medial plus lateral tibial osteophytes from the mid-coronal section and synovial hyperplasia was the maximal score from the mid-coronal section.

**Statistical analysis**

**Person level analysis.** We derived the “original” WOMAC pain score from the KOOS questionnaires completed by participants http://www.koos.nu/KOOSusersguide2012.pdf. The “original” WOMAC pain score (10) for each knee was calculated as a sum of the 5 items from the KOOS pain subscale. For person level analyses, we used the average WOMAC pain score calculated as a sum of “original” WOMAC pain score for left and right knees divided by 2. Because the distribution was right skewed, average WOMAC pain scores were categorized as a dichotomous variable such that participants were grouped as those who did not have any symptoms or had only mild symptoms (responded “1” for at least one item) versus those who had at least moderate symptoms. Therefore, participants in the first category have average WOMAC pain <= 5, and all participants in the second category have average WOMAC pain > 5. AUSCAN pain scores were also categorized as a dichotomous variable with participants grouped as those who did not have any symptoms versus those who had at least mild symptoms. To determine if there were statistically significant differences between pain categories, the Kruskal-Wallis or Mann-Whitney U tests (following the Benjamini and Hochberg adjustment for multiple comparison) were applied to individual taxa.

**Analysis of LPS/LBP and cytokine levels.** First, LPS and LPB data were checked for normality using numerical (Shapiro-Wilk tests) and visual (box plots) methods. Then, comparisons between OA cases and controls were performed using Student’s t-tests. Spearman’s rank-based correlation coefficients were used to quantify correlations between LPS, LBP and AUSCAN pain score, as well as between LPS, LBP and average WOMAC pain score calculated as a sum of WOMAC pain score for left and right knees divided by 2.

Two-sided Student’s t-tests with the Satterthwaite correction for unequal variances were used to determine statistical difference of each cytokine level between OA cases and controls. P-values were not adjusted for multiple testing due to the exploratory nature of the study as described (11, 12).

**Analysis of mouse data.** Linear mixed effects model with q2-longitudinal plugin in QIIME2(13) was used to determine any effects of the diet on the microbiota and to determine which microbes were associated with the severity of histologic OA. Specifically, we explored whether Shannon diversity changed over time and in response to group (obese OA and obese non-OA fecal transplants). Random effects were included to reflect variation of these effects across mice. Time in weeks, case/control status and their interaction were the fixed effects of the model. Bray-Curtis dissimilarity matrix was calculated, and PCoA plots were generated using QIIME2. An individual clade analysis of samples at the week 0 baseline time point (start of diet 2 weeks after fecal transplant) was performed using non-parametric tests as described above. Box plots of relative abundance stratified by group were created for taxa whose relative abundance were significantly different across groups. Analysis of the histologic measures was performed using the non-parametric Mann Whitney test to compare differences between the two groups.

**Supplementary Methods References**

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