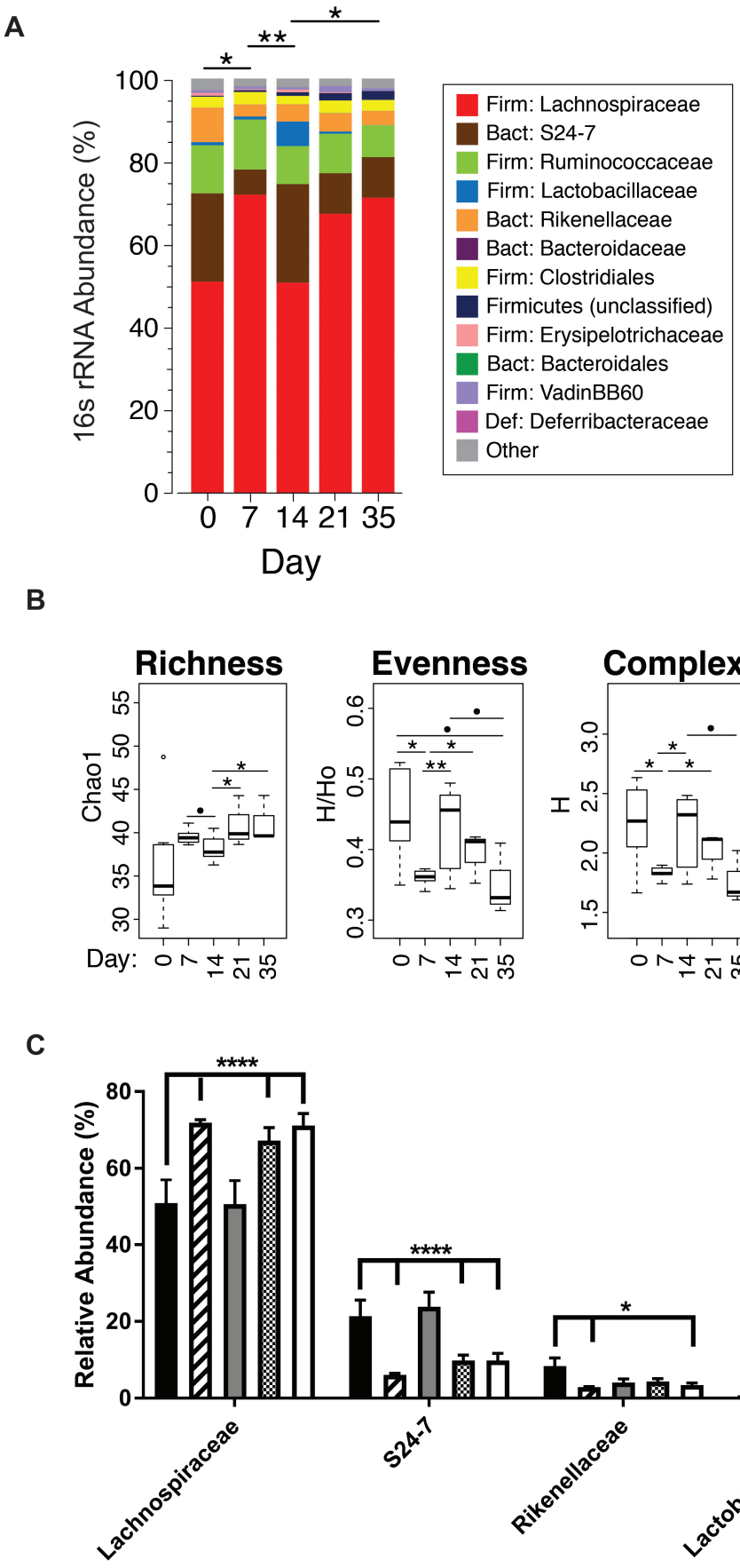
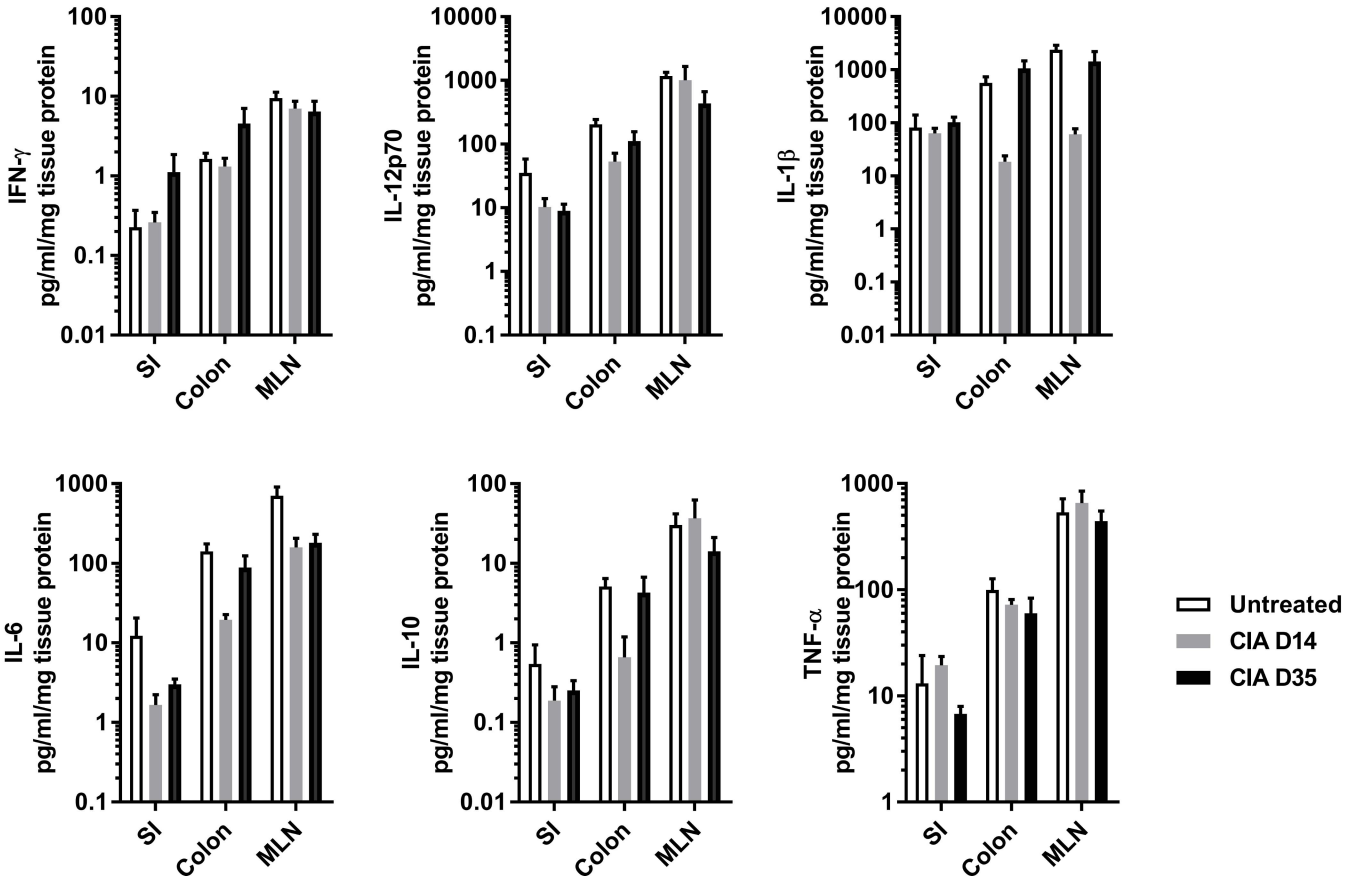
Supplemental Figure S1



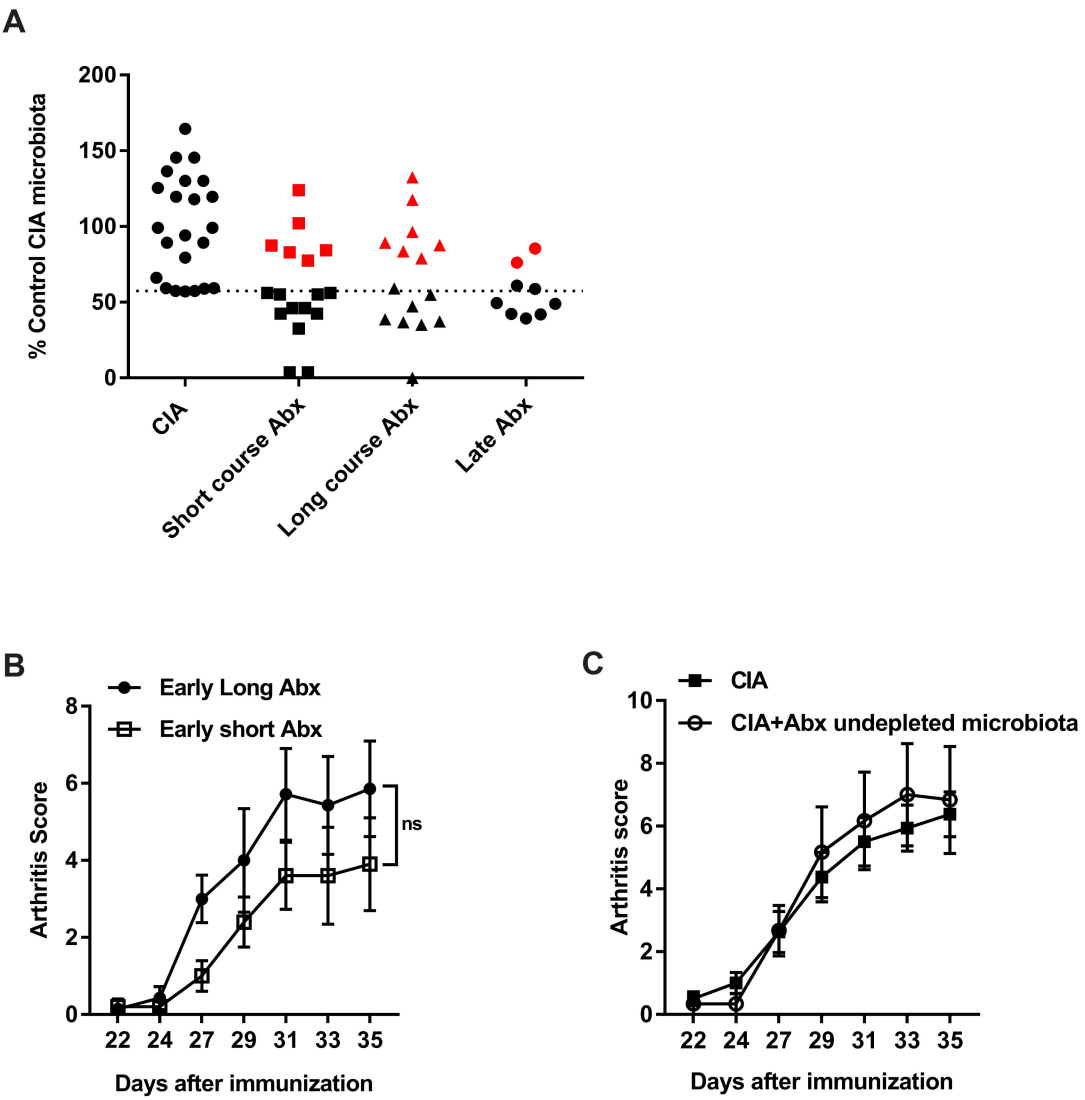
Supplemental Figure S1. Microbial dysbiosis after immunization with CFA alone. Six week old male DBA/1J mice were immunized with CFA on days 0 and 21. Fecal pellets were collected from mice every 7 days from day 0 through 21 and on day 35 after the initial immunization. Fecal bacterial DNA was sequenced for 16S rRNA and analyzed. N=6 for time points 0-14 and N=3 for days 21 and 35. (A) The percent abundance of the top operational taxonomic units (OTU) families were compared across time points. Differences in the overall composition of microbial communities were determined by PERMANOVA. *P<0.05, **P<0.01. (B) Alpha-diversity measures for richness (Chao1), evenness, and complexity (ShannonH) across time points are shown as box-and-whisker plots. Statistical analysis was performed with ANOVA as described in Methods. *P<0.05, **P<0.01 and •P<0.001. (C) Statistically significant changes in family level OTU mean relative abundance ± SEM in were determined by Kruskal-Wallis with Dunn's post-test. *,P<0.05, **P<0.01; ****P<0.0001.

Supplemental Figure S2.



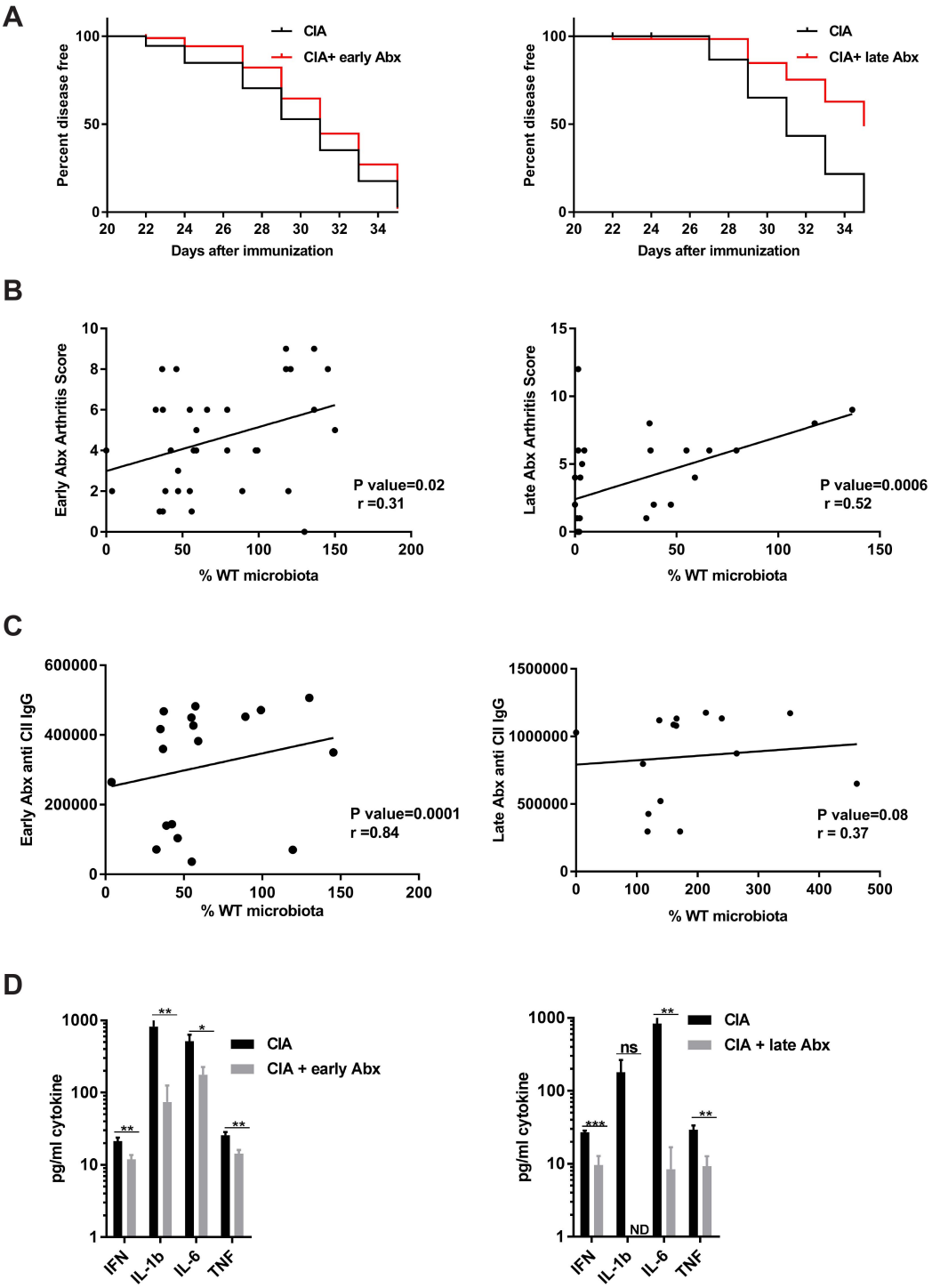
Supplemental Figure S2. Additional intestinal cytokines over time. IFN-γ, IL-12p70, IL-1β, IL-6, IL-10, and TNF-α in SI, colon, and MLNs were measured by ELISA in DBA1/j unimmunized controls and in mice with CIA at days 14 and 35 after initial immunization. Cytokine concentrations were normalized to total protein and reported as pg/ml/mg protein. N=3-9 in each group. Data are the mean ±SEM. No significant changes were detected as determined using Kruskal-Wallis test with Dunn's post-test.

Supplemental Figure S3.



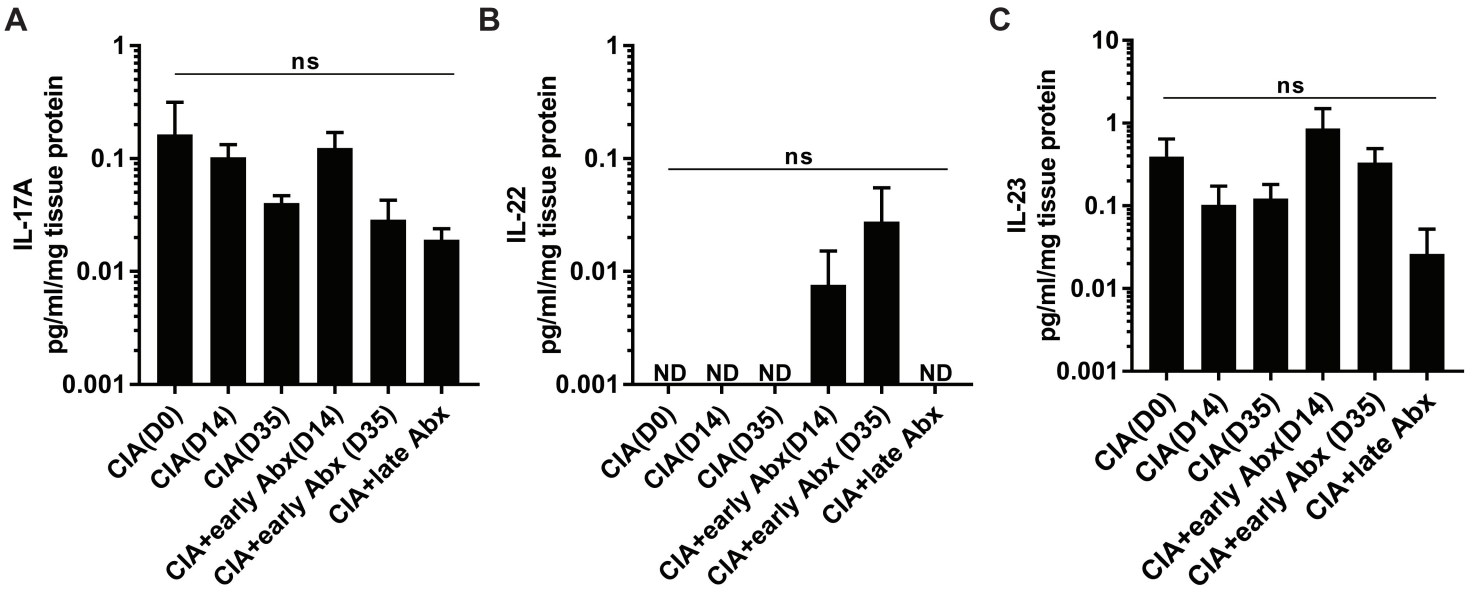
Supplemental Figure S3. Efficacy of antibiotic-treatment for the reduction of intestinal microbiota.
(A) Fecal samples from mice CIA + control water, CIA + early short course Abx, CIA + early long course Abx, and CIA + late course Abx were collected at day 35 and analyzed by qPCR using universal 16S primers for total microbial content. For each reaction, individual samples were compared to the mean concentration of bacteria in control water treated mice to report the percent of control microbiota. Samples from antibiotic treated groups that were above the 10% confidence interval of the mean for the control-treated group were excluded, as shown in red.
(B) Arthritis severity in mice whose microbiota were successfully depleted was compared between the early short and early long antibiotic treatments. A two-way ANOVA with Bonferroni's multiple comparisons test failed to demonstrate significant differences between the two groups. Therefore, both groups were combined for further analyses.
(C) Arthritis severity in mice whose microbiota was **not** successfully depleted was compared to mice given control water. A two-way ANOVA with Bonferroni's multiple comparisons test failed to demonstrate significant differences between the two groups.

Supplemental Figure S4.



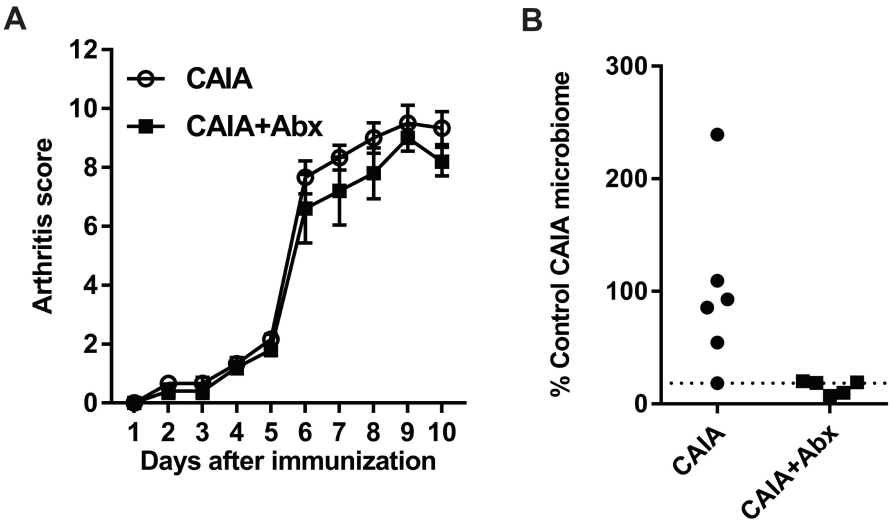
Supplemental Figure S4. Microbiota alter CIA prevalance and correlate with disease severity. (A) Percent of mice remaining disease-free in CIA, CIA + early Abx and CIA + late Abx groups. Mantel-Cox method was used for curve analysis. (B) Non-parametric Spearman correlation of early and late microbial depletion and arthritis scores. Each dot represents one mouse. Lines represent linear regression with 95% confidence intervals. (C) Non-parametric Spearman correlation of early and late microbial depletion and anti-CII IgG. Each dot represents one mouse. Solid lines represent linear regression with 95% confidence intervals as dotted lines. (D) Serum samples from day 35 were tested for IFN-γ, IL-1β, IL-6 and TNF-α, using a multi-analyte ELISA and reported as pg/ml. N=6-14 in each group. Data are the mean ± SEM for each cytokine. *, $p<0.05$; **, $p<0.01$; and ***, $p<0.001$ as determined by an unpaired, two-tailed Student's t-test.

Supplemental Figure S5.



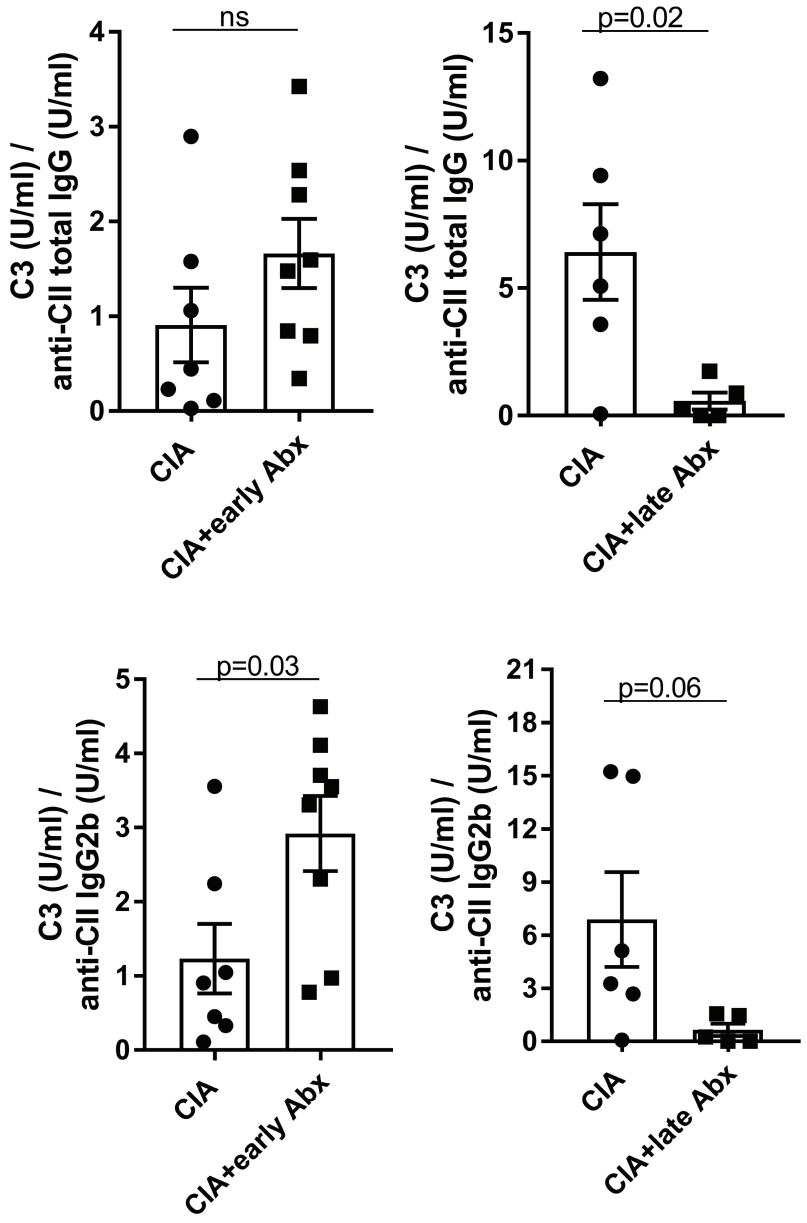
Supplemental Figure S5. Th17-related cytokine production in draining inguinal lymph nodes (ILNs) do not change during CIA. Six week old male DBA/1j mice were treated with antibiotics or control water and immunized with CII as described in Methods and Figure 3a. ILNs were collected either at day 14 or day 35, homogenized and tested for cytokines by multi-analyte ELISA. The final cytokine concentrations in each tissue were normalized to total protein and reported as pg/ml/mg protein. (A) IL-17A (B) IL-22 (C) IL-23 levels. N=6-12 in each group. Data are the mean ± SEM of the tissue concentrations of each cytokine. A Kruskal-Wallis with Dunn's post-test failed to demonstrate statistical significance.

Supplemental Figure S6.



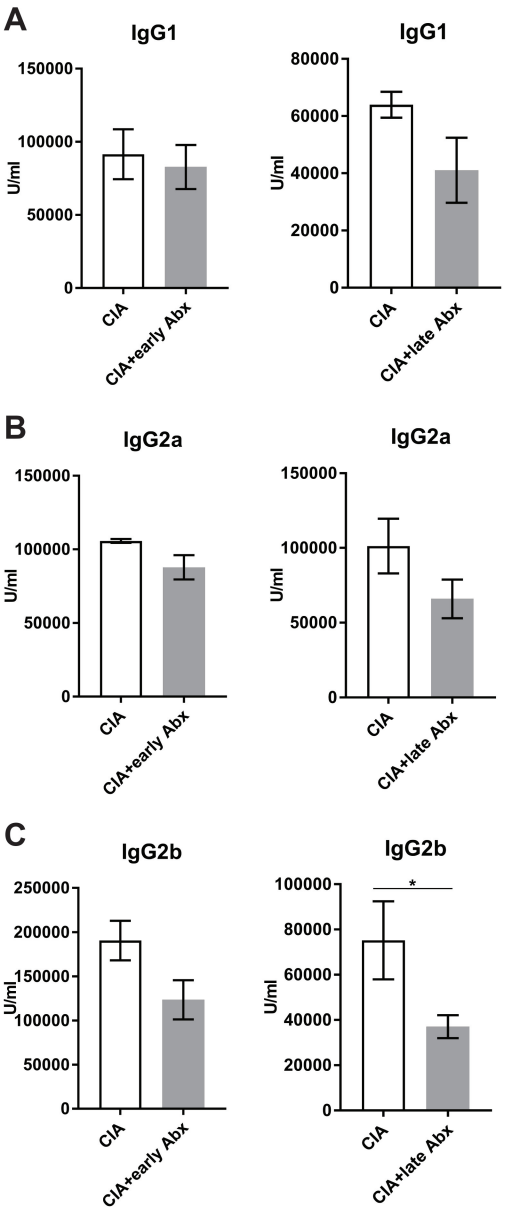
Supplemental Figure S6.Arthritis severity is not affected by antibiotic treatment in collagen antibody-induced arthritis (CAIA). (A) Mice were treated with antibiotics or control drinking water one week before antibody transfer and then throughout the study. Arthritis scores were assessed every day starting after day 3 when LPS was administered until euthanasia at day 10. N= 6 mice/group. Data are the mean arthritis score ± SEM. A two-way ANOVA with Bonferroni's multiple comparisons test failed to demonstrate significant differences between the two groups.
(B) Adequate depletion of microbiota was determined by measuring the microbial load as described in Methods. Dots represent individual mice.

Supplemental Figure S7.



Supplemental Figure S7. Complement activation normalized to serum anti-CII antibody levels. The amount of C3 activation (U/ml) was divided by the concentration of either anti-CII total IgG or IgG2a (U/ml). Each dot represents an idividual mouse with the bars as mean ± SEM. An unpaired, two-tailed Student's t-test was used to determine statistical significance.

Supplemental Figure S8.



Supplemental Figure S8. CII-specific (A) IgG1 (B) IgG2a (C) IgG2b in serum of D35 CIA, CIA + early Abx, and CIA + late Abx mice were measured by ELISA and are shown as mean U/ml ± SEM. Statistical significance was determined by an upaired, two-tailed Student's t-test. *, $p<0.05$.