

1 **Supplementary Information**

2 **Supplementary Methods**

3 **Sequence processing and taxonomic assignment**

4 After filtering the sequences in MOTHUR, reads with ambiguous base calls, average
5 quality scores below 25 and homopolymers longer than 10 were eliminated. Bacterial V1-
6 V2 regions of the 16S rRNA gene were verified and extracted using V-XTRACTOR 2.0 [1].
7 High quality sequences were queried against GREENGENES [2] using a naïve Bayesian
8 classifier implemented in MOTHUR and a minimum bootstrap support of 60%. Similar
9 sequences were clustered into operational taxonomic units (OTUs) using an unsupervised
10 Bayesian clustering approach implemented in CROP [3] and the default threshold for
11 species level. The consensus taxonomy of each OTU was determined using MOTHUR, as
12 the taxonomic path represented by at least 51% of the sequences within the OTU.
13 Abundance of OTUs at specific taxonomic ranks, species, genus, family, order, class, and
14 phylum was determined and used to generate rank-specific matrices permitting
15 downstream analyses of indicators at each taxonomic rank.

16

17 **Multivariate analysis of community structures and diversity**

18 Data were standardized by dividing the number of reads in each taxonomic unit by the
19 total number of reads in each sample. A resemblance matrix was calculated using Bray-
20 Curtis similarities [4] based on the standardized data. Principal Coordinate Analysis
21 (PCO, [5]) implemented in the PRIMER6+ was used to display similarities in microbial
22 community structures among all fecal and ileal samples. Tests of the multivariate null
23 hypotheses of no differences among *a priori* defined groups were examined using

24 permutational multivariate analysis of variance (PERMANOVA, [6]). PERMANOVA
25 was performed with 9,999 permutations and run in the PRIMER6+ software package.
26 Residuals of raw data were permuted under a reduced model and partitioning was
27 performed using Type III sums of squares. Pseudo-F values were reported to indicate the
28 effects strength ($F > 1$ indicates an effect) and P(Perm) values were reported to indicate the
29 permutation-based p-value, which is the accurate value based on the 9,999 permutations
30 run as described above. Bray Curtis similarity indices were reported to describe the
31 average pairwise distance between the treatment groups analyzed. PCO ordination was
32 plotted with STATISTICA 8.0 (StatSoft, Tulsa, OK). Simpson's diversity index [7] was
33 used to calculate community diversity between treatment groups.

34

35 **Identification of bacterial indicators**

36 Taxa-treatment association analysis was based on the point biserial correlation coefficient
37 [8] and used to determine the association strength (R) of each taxonomic unit with *a*
38 *priori* groups. The analysis was based on fecal data obtained for each group (control,
39 vancomycin and streptomycin-treated). This analysis was performed at each taxonomic
40 level, from phylum to individual OTU. All possible combinations of *a priori* groups were
41 analyzed to account for different niche breadths of the taxonomic units [9]. Correlation
42 analysis was performed on each taxonomic unit using the diagnostic species analysis
43 routine implemented in GINKGO [10] with 999,999 permutations. False discovery rate
44 (FDR) correction was used to re-calculate these probabilities and draw experiment-wide
45 conclusions [11]. Based on the P value distribution, Q values were determined using the

46 software QUALITY [12] and associations were considered significant with an FDR of 5%
47 ($q < 0.05$).

48

49 **Supplementary references**

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79

80 **Supplementary Figure Legends**

81 **Fig S1.** Adult antibiotic treatment has no effect on allergic asthma. **(A)** Eosinophil
82 numbers in the bronchoalveolar lavage (BAL) fluid of control or antibiotic-treated mice
83 challenged with ovalbumin (OVA) or PBS. **(B)** Serum OVA-specific IgE responses
84 measured by ELISA. **(C)** Total pathological scores and representative H&E stained lung
85 sections. Scale bar, 300 μ m. All assessments were made on day 26. The data are shown as
86 means of 5-7 mice per group \pm SEM and represent two independent experiments. Statistics
87 shown are based on comparisons to OVA-challenged controls. The antibiotic-treated
88 animals were treated with antibiotics at 7 weeks of age. Vanco, vancomycin; Strep,
89 streptomycin; n.s.= not significant, n.d.= none detected.

90

91 **Fig S2.** Antibiotic treatment alone has no effect on lung histopathology. Total
92 pathological scores and representative H&E stained lung sections. Scale bar, 300µm. All
93 assessments were made on day 26. The data are shown as means of 4 mice per
94 group±SEM and represent two independent experiments. Statistics shown are based on
95 comparisons to PBS-treated controls. The antibiotic-treated animals were treated using
96 the neonatal antibiotic regime. Vanco, vancomycin; Strep, streptomycin.

97

98 **Fig S3.** Intestinal communities shift differently after neonatal and adult antibiotic
99 treatments. Bacterial communities from feces of OVA-challenged mice treated with
100 antibiotics neonatally or only as adult were compared using principal coordinate analysis
101 (PCO). Corresponding OVA-challenged controls for each experiment are also shown.
102 Vanco, vancomycin; Strep, streptomycin.

103

104 **Supplementary Data Sets**

105 **Data Set S1.** Representative sequences from OTUs that were classified within a
106 Greengenes OTU identified in Table 1. Sequences that could not be matched to a
107 Greengenes OTU (gg_OTU) and thus remain “unclassified” in Table 1 have been listed
108 under the headings corresponding to the closest-related taxon identified.

109

110 > Adlercreutzia ggOTU 916_OTU089
111 ACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGGAGCACCCCTGA
112 AAGAGTTTTTCGGACAATGGAAGGGAATGCTTAGTGGCGGACTGGTGAGTAAC
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114 TACCGCATGATGTGTTTCGATGGCATCATCGAGACACCAAAGATTTATCGCTG
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116 GCGACGATCAGTAGCCG
117 > Adlercreutzia ggOTU 916_OTU105

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145
146 >Alistipes ggOTU 1053_OTU036
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164 CGCACAGAGCCGCATGGCTCAGTGTGAAAACTCCGGTGGTGTAAAGATGGAT
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174 >Unclassified Bacteroidales ggOTU 991_OTU004
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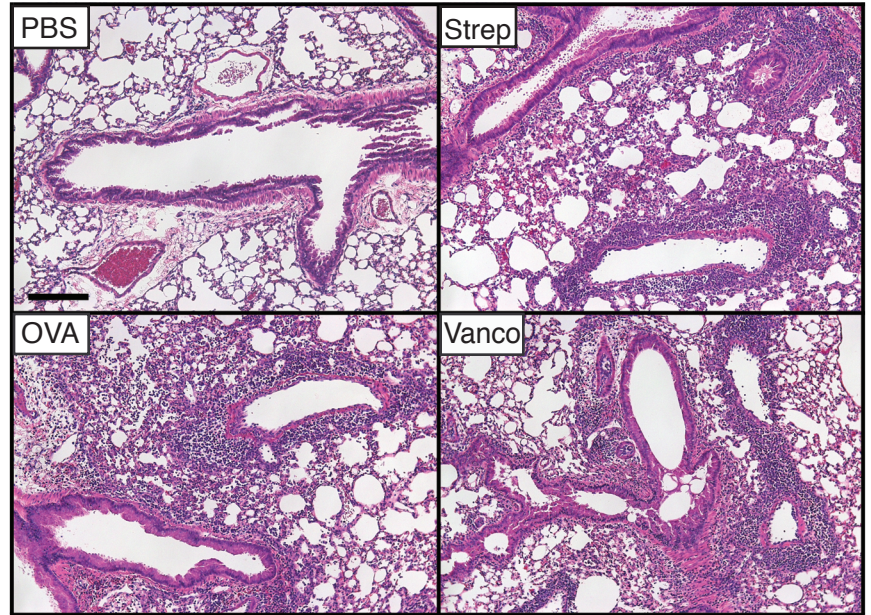
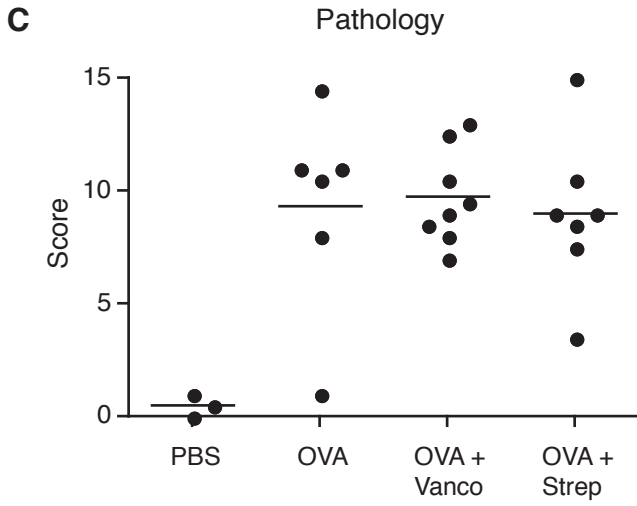
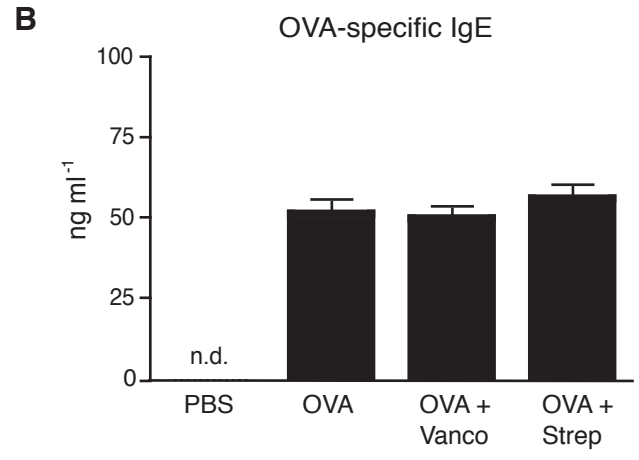
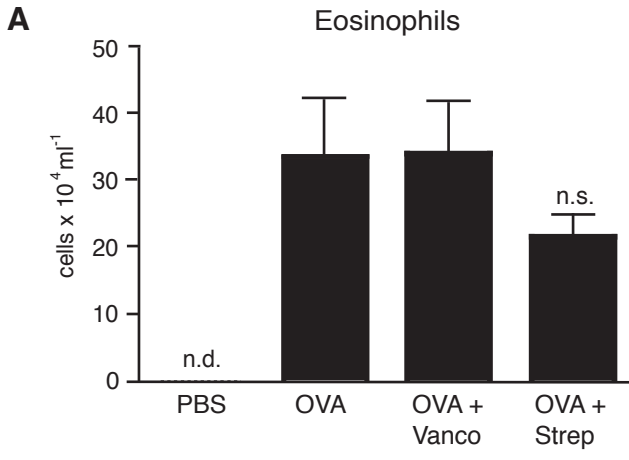
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1339 CACAGTGCTGCATGGCACAGTGTGAAAACTCCGGTGGTATGGGATGGGTCC
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1341 AGCCG
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1343 ATGAACGCTAGCGACAGGCCTAACACATGCAAGTCGAGGGGCATCGGGGCG
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1345 GACCTGCCCCGTTGCAGGGGGATAATCGGGAGAAATCCCGTCTAATACAGCAT
1346 GACGCCGGGAAGGGACATCCCTTTCGGCCAAAGGGGGCGACTCCGGTCACG
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1348 ACGATGCGTAGGGG
1349 > Unclassified Runinococcaceae_OTU293
1350 TTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCGGTAGCACAGAG
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1352 GCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAATGT
1353 CGCAAGACCAAAGTGGGGGACCTTCGGGCCTCATGCCATCAGATGTGCCAG
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1355 CTG
1356 > Unclassified Runinococcaceae_OTU312
1357 ATGAACGCTGGCGGCGTGCCTAACACATGCAAGTCGAACGAAGCGCTTTTCC
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1359 CGTGGGGAACCTGCCCTGTACCGGGGGATAACAGCCGGAAACGGCTGCTAAT

1360 ACCGCATAAGCGCACAGTGCCGCATGGCACGGTGTGAAAAACCACGGTGGTA
1361 CAGGATGGCCCCGCGTCTGATTAGTTGGTTGGCAGGGTAACGGCCTACCAAG
1362 ACGGCGATCAGTAGCCG
1363
1364 >Unclassified Tenericutes_OTU002
1365 ATGAACGCTGGCGGCATGCCTAATACATGCAAGTCGAACGGATATCTTCGGA
1366 TATGAGTGGCGAACGGGTGAGTAACACGTAGGGAACCTGCCTGCATGAGCGG
1367 GAGA ACTTCTGGAAACGGAAGCTGATACCGGATGAGCAAAGAGGAGGCATC
1368 TTCTTTTGGAAAAGGGGACAAGAGTCCC GCATGCAGATGGACCTGCGGTGC
1369 ATTAGCTGGTTGGAGAGGTAACGGCT



Pathology

