SDC, MATERIALS AND METHODS

16S sequencing and analysis

Fecal samples were collected from mice, either before transplantation or at day 7 after transplantation. Amplification and DNA sequencing of the 16S rRNA gene was performed at the Genome Science Centre in Vancouver, British Columbia, Canada. The V3-V4 region of the 16S gene was amplified using degenerate primers tailed with linker sequences to facilitate nested PCR (Fwd – CGCTCTTCCGATCTCTGCCTACGGGNGGCWGCAG, Rev – TGCTCTTCCGATCTGACGACTACHVGGGTATCTAATCC). PCR was performed with Phusion DNA polymerase (Fisher Scientific, Boston, MA) and amplicons were indexed using a nested PCR and cleaned up with AMPure XP beads. Amplicon libraries were evaluated using an Agilent High Sensitivity Bioanalyzer DNA kit (Agilent Technologies, Palo Alto, CA) before sequencing on the Illumina MiSeq platform using paired-end 250bp reads.

Read pairs were quality filtered and stitched together using PEAR. Stitched reads were discarded if shorter than 300 or if 80% of the nucleotides were of low quality (score less than Phred 30). Operational taxonomic units (OTUs) were created through QIIME v1.9 open-reference clustering using SortMeRNA and SUMACLUST at 97% identity clustering. OTUs were taxonomically classified against Greengenes v13.8. OTUs with less than 0.1% of reads and OTUs classified as coming from chloroplasts were removed. OTU abundance tables were subsampled to 2383 reads per sample.

All analyses were performed in R v3.3.2, except differential abundance of phyla and classes were assessed for significance using LEfSe with a LDA cutoff of 4. Boxplots were created using ggplot v2.2.1. Principal coordinate analysis (PCoA) was performed based on weighted UniFrac distances calculated from subsampled OTU abundances calculated using the phyloseq R package.
REFERENCES


Figure S1. Effect of antibiotic treatment on bacterial and fungal content in fecal samples. 

A. Quantification of DNA content in fecal samples by DAPI fluorescence in untreated (UT) and antibiotic treated (ABT) mice. Mean ± SEM of fluorescence area. N=3. *** p<0.001. 

B. qPCR of 18S fungal DNA in fecal samples. Mean cycle threshold ± SEM. N=5
Figure S2. Luminal and total cross-sectional area of syngraft artery segments. Aortic interposition grafts were performed from C57Bl/6 donors into C57Bl/6 recipients that were untreated (UT, N=3) or treated with antibiotics for their entire life (ABT, N=3). Artery segments were harvested at day 30 post-transplantation, stained with H&E and total cross-sectional area quantified. Mean ± SEM.
Figure S3. Analysis of B and NK cells in aortic interposition grafts. Aortic interposition grafts were performed from Balb/c donors into C57Bl/6 recipients that were untreated (UT, N=7), treated with antibiotics for their entire life (ABT, N=7), or treated with antibiotics for the first 3 weeks of life (ABT until 3 wo, N = 6). Syngrafts served as controls. Artery segments were harvested at day 7 post-transplantation and immunohistochemically stained for A. Pax5 to detect B cells and B. CD335 (NKp46) to detect NK cells. Spleens were stained as positive controls and insets are photomicrographs of isotype staining controls.
Figure S4. Circulating neutrophils in peripheral blood of untreated mice and mice treated with antibiotics until 3 weeks old. Peripheral blood cells were stained with antibodies to CD11b and GR-1. Quantification of neutrophils (CD11b+GR-1+) in peripheral blood by flow cytometry. Mean ± SEM. UT, N=3; ABT until 3wo, N=4.