Section S1: Detailed methods

16s rRNA sequencing and analysis

Microbial DNA was extracted using the QIAamp DNA Stool Mini Kit (Qiagen, UK) following manufacturer's instructions. Non-template or blank controls were added and processed to detect any potential contamination from reagents or the extraction process. The V3–V4 hypervariable region of the 16S rRNA gene was amplified using a two-step PCR method, and sequencing was performed on a MiSeq (Illumina Inc., San Diego, CA, USA) in accordance with the manufacturer's protocols. Briefly, sequencing was performed using the V3 forward primer with appropriate overhangs (TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG) and the V4 reverse primer with appropriate overhangs (GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATC). PCR amplifications were conducted using a single step 25 cycle PCR using the KAPA HiFi HotStart ReadyMix (KAPA Biosystems, Boston, MA, USA) under the following conditions: an initial denaturation of 95°C for 3mins, followed by 25 cycles of 95°C for 30s, 55°C for 30s, and 72°C for 30s, after which a final elongation step at 72°C for 5mins was performed. Negative PCR controls were included in all of the amplification reactions. After amplification, PCR products were checked in DNA chips with an Agilent 2100 BioAnalyzer (Agilent Technologies, Palo Alto, CA). Amplicon products were subsequently purified using Agencourt AMPure beads (Agencourt Bioscience Corporation, Beverly, MA, USA), followed by Index PCR, purification with AMPure beads, and library normalization. Libraries were sequenced using the MiSeq 600 cycles reagent kit v3.

Illumina 300-bp paired-end sequencing generated 249,593 and 183,029 sequencing reads. Paired-end fastq files were joined using the Quantitative Insights Into Microbial Ecology (QIIME v1.9) pipeline using join_paired_ends.py. Joined reads having less than 75% of their original lengths were removed, and barcodes were removed using split_libraries.py with default filtering parameters (http://qiime.org/scripts/split_libraries.html). 16S rRNA gene sequences were sorted based on sample ID using QIIME script extract_seqs_by_sample_id.py. Operational taxonomic units (OTUs) were selected using pick_open_reference_otus.py workflow. For more detailed methods on the QIIME pipeline used, please see reference by Caporaso et al[1]. 16s rRNA taxonomy up to the family level was defined by ≥97% similarity to reference sequences in the Greengenes database. Data were rarefied to minimize the effect of disparate sequence number on the results. A suit of diversity analyses were performed using the script core_diversity_analyses.py using default parameters. Following quality control steps, a total of 3,268,224 16S rRNA gene sequences were used; the average read length was 159 base pairs and the average Phred score was 38, indicating a base call accuracy approaching 99.99% [2].

1. Caporaso JG, Kuczynski J, Stombaugh J, et al. QIIME allows analysis of high-throughput community sequencing data. *Nat Methods*. 2010;7(5):335-336. doi:10.1038/nmeth.f.303.
2. Bokulich NA, Subramanian S, Faith JJ, et al. Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. *Nat Methods*. 2013;10(1):57-59. doi:10.1038/nmeth.2276.

Detailed Methods continued:

Isoflurane vs. Naïve groups: All mice (n=13) were littermates and cagemates until the day of GA exposure. Experimental mice were allowed unrestricted access to food and water until the start of isoflurane exposure, whereas PO intake of naïve mice was uninterrupted. For exposure, experimental mice were transferred to an induction chamber with 3% isoflurane. After loss of righting reflex, mice were transferred to rodent nose cones for exposure of 1.5% isoflurane over 4h, with 100% supplemental O_2 admixture at 5L/min. Experimental mice remained *n.p.o* for the duration of anesthesia (4h), after which they were again given unrestricted access to food and water. All experimental mice resumed normal intake between 1h to 5h after GA.

Sample collection: Fecal samples were collected by lifting the mice and gently stroking the abdomen until extrusion of fecal contents. Samples were collected from isoflurane (n=10) and naïve (n=3) groups at three time points: 24h prior to exposure ("pre-"), 1d after exposure ("24h") and 7d after exposure "7d"). In the experimental group, samples were collected from all mice at every time point. For naïve mice, samples from 2 naïve mice were collected at time "pre", and from all 3 naïve mice at "24h" and "7d."