# Effective establishment of donor gut microbiota in gnotobiotic mice



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## Abstract

Background: Determining whether associations between gut microbiota characteristics and host physiology represent causal relationships is a fundamental challenge for microbiome research. One way these associations can be investigated is to instil donor faecal material into gnotobiotic mice and to assess the extent to which donor phenotype is recapitulated. However, the manner in which this process is performed varies considerably between studies, and assessment of microbiota re-establishment in recipient animals is not always carried out. We report a detailed investigation of microbiome assembly in germ-free mice and compare the effects of single and multiple rounds of faecal gavage, using both native and antibiotic-disrupted donor material.

Results: Levels of bacteria within the faeces of recipient animals increased rapidly following the instillation of donor material. However, considerable instability in microbiota composition continued during the first two weeks post-gavage, with substantial changes in taxon relative abundance occurring in parallel to declining faecal pH. Relative compositional stability was not achieved until day 28 and persistent differences between recipient and donor microbiota remained. These included an increased relative abundance of Bacteroidetes, and a reduced relative abundance of Firmicutes. Of taxa detected in donor material, 52% were represented in stable recipient microbiota following transplantation with native faecal material (single gavage), increasing to 66% following three rounds of gavage. These taxa accounted for 95% and 91% of total donor bacterial abundance, respectively. Performing multiple rounds of gavage significantly increased microbiota similarity between donor and recipient, and significantly reduced within-group dispersion ($P<0.05$). Instillation of antibiotic-associated microbiota resulted in substantially lower temporal and inter-animal variance, with multiple rounds of gavage providing no substantial benefit.

Conclusions: Microbiome assembly in recipient animals is not immediate and several weeks are required for microbiota stability to be achieved. Multiple rounds of faecal gavage result in greater similarity to donor microbiota and reduced inter-animal variance. The process of donor microbiota re-establishment, and therefore the interval required prior to investigations using recipient animals, is influenced by donor microbiota characteristics.

## Full-text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed.

However, the manuscript can be downloaded and accessed as a PDF.

## Figures

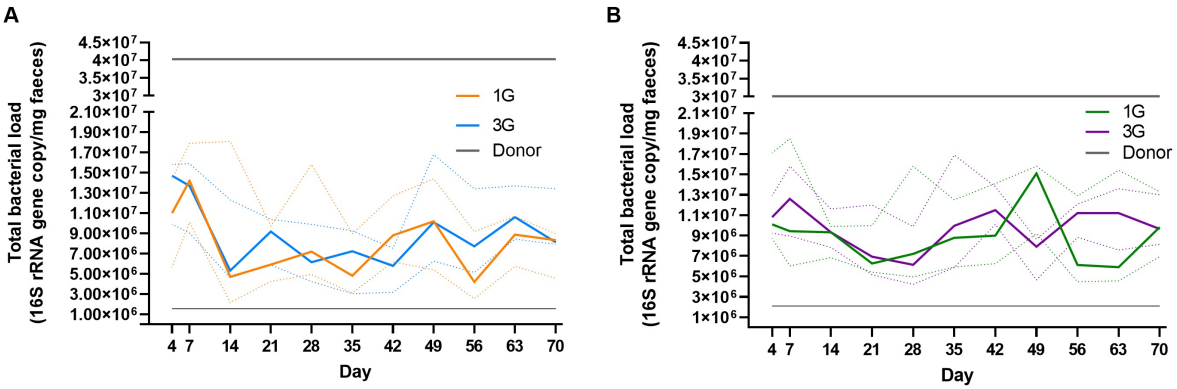



Figure 1

Bacterial load in faecal samples following establishment of gut microbiota in recipient germ-free mice. Determination of faecal total bacterial load in recipient mice that received (A) intact native microbiota or (B) microbiota derived from antibiotic-exposed mice as a single (1G) or three rounds (3G) of gavage. Bacterial load was determined using quantitative PCR of the 16S rRNA gene. The top and bottom grey lines denote the maximum and minimum bacterial load of donor faecal samples. Solid lines and dotted lines denote median values and interquartile ranges, respectively. Statistical comparison between groups were performed using the Mann-Whitney test at a level of $P < 0.05$ for significance.

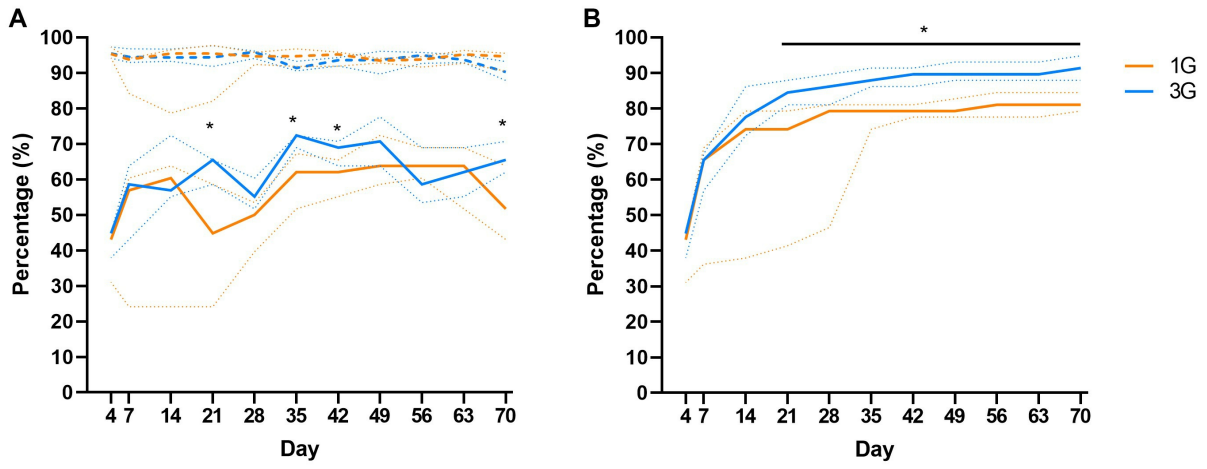



Figure 2

Representation and relative abundance of donor bacterial taxa in mice that received intact native microbiota. (A) Percentage of donor taxa represented in recipient germ-free mice that received one (1G) or three (3G) rounds or faecal gavage with intact microbiota. Representation of the donor taxa (solid line) and their total relative abundances (dashed line) in the recipient mice at each timepoints were determined. (B) The cumulative detection of donor bacterial taxa percentage) in recipient mice. Solid and dashed lines denote median values, dotted lines denote interquartile ranges. Statistical comparison between groups were performed using the Mann-Whitney test at a level of $P < 0.05$ for significance.

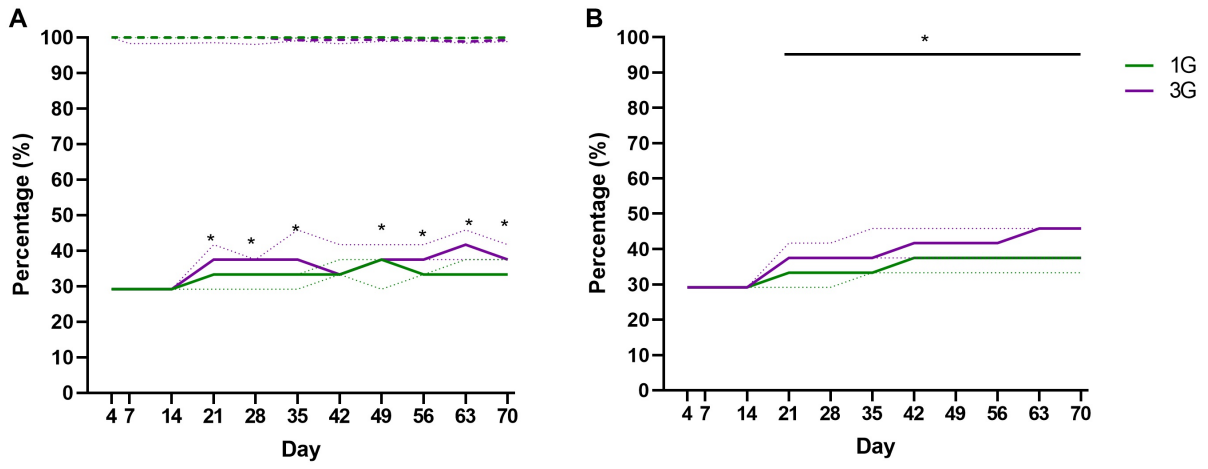



Figure 3

Representation and relative abundance of donor bacterial taxa and in mice receiving material from antibiotic-exposed mice. (A) Percentage of donor taxa represented in recipient germ-free mice that received one (1G) or three (3G) rounds of faecal gavage with antibiotic-exposed microbiota. Solid lines denote representation of the donor taxa and dashed lines denote their total relative abundance. (B) Cumulative representation of donor bacterial taxa (percentage) in recipient mice. Solid and dashed lines denote median values, dotted lines denote interquartile ranges. Statistical comparison between groups were performed using the Mann-Whitney test at a level of $P < 0.05$ for significance.

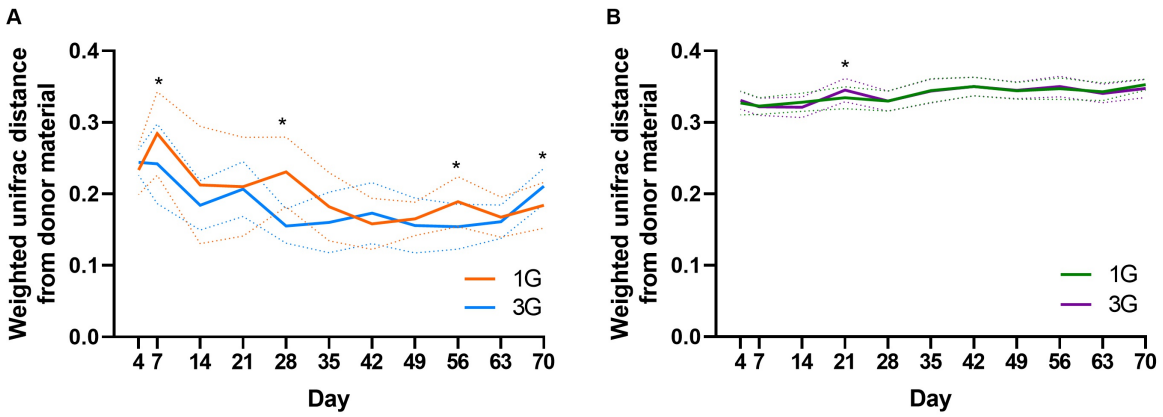



Figure 4

Weighted UniFrac distances of the donor microbiota and recipient microbiota. Weighted UniFrac distance between the microbiota of recipient mice that received (A) intact native microbiota or (B) microbiota derived from antibiotic-exposed mice, and their respective donor microbiota composition throughout the 70 day study period. Recipient mice received either one round (1G) or three rounds (3G) of donor microbiota. Statistical 652 comparison between the groups at each timepoint was performed using the Mann-Whitney test ($P < 0.05$).

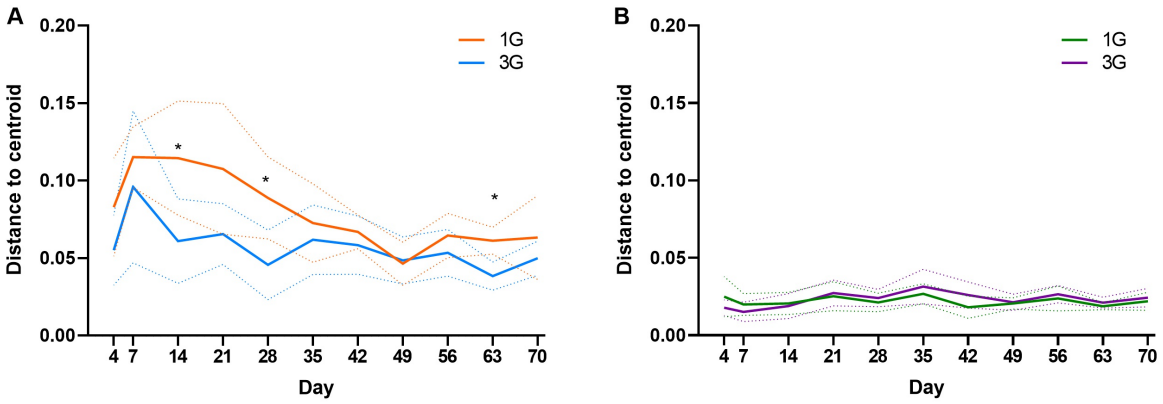
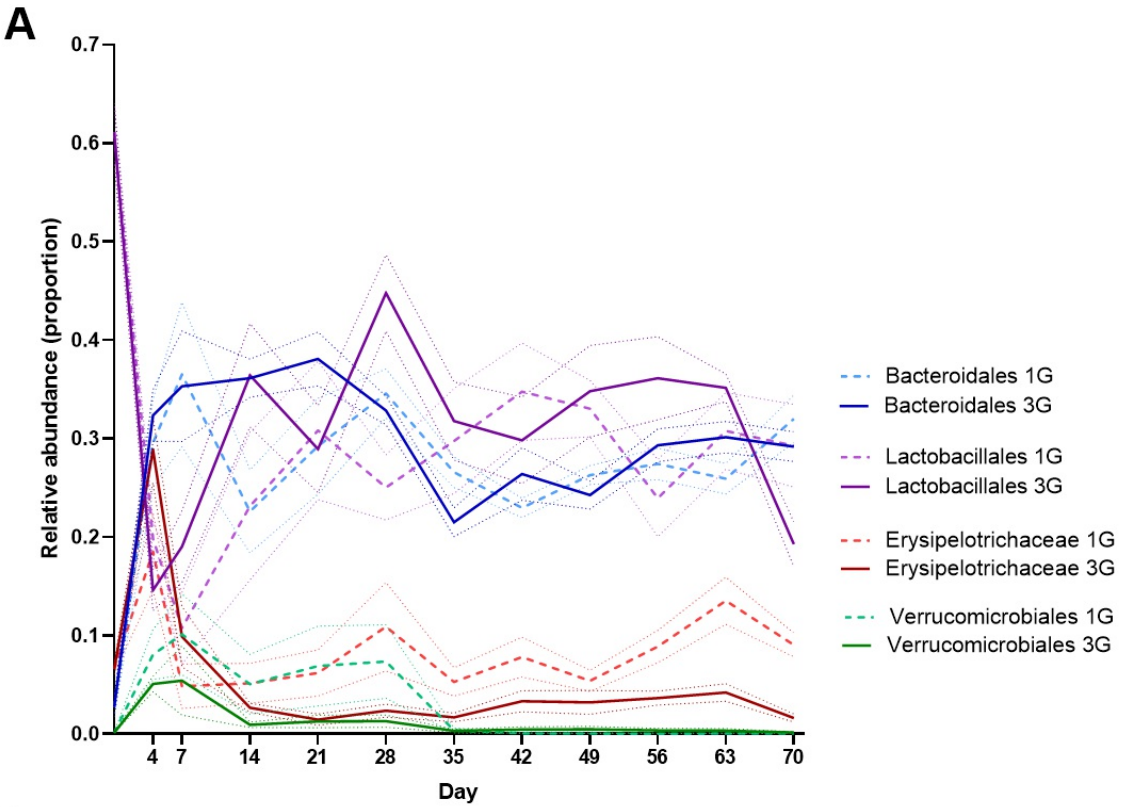



Figure 5

Distance to group centroid of the recipient microbiota. Compositional dispersion of microbiota among recipient mice that received (A) intact native microbiota or (B) microbiota derived from antibiotic-exposed mice were determined based on distances to their respective group centroid throughout the 70 day study period. Recipient mice received either one round (1G) or three rounds (3G) of donor microbiota. Statistical comparison between the groups at each time point was performed using the Mann-Whitney test ($P < 0.05$).



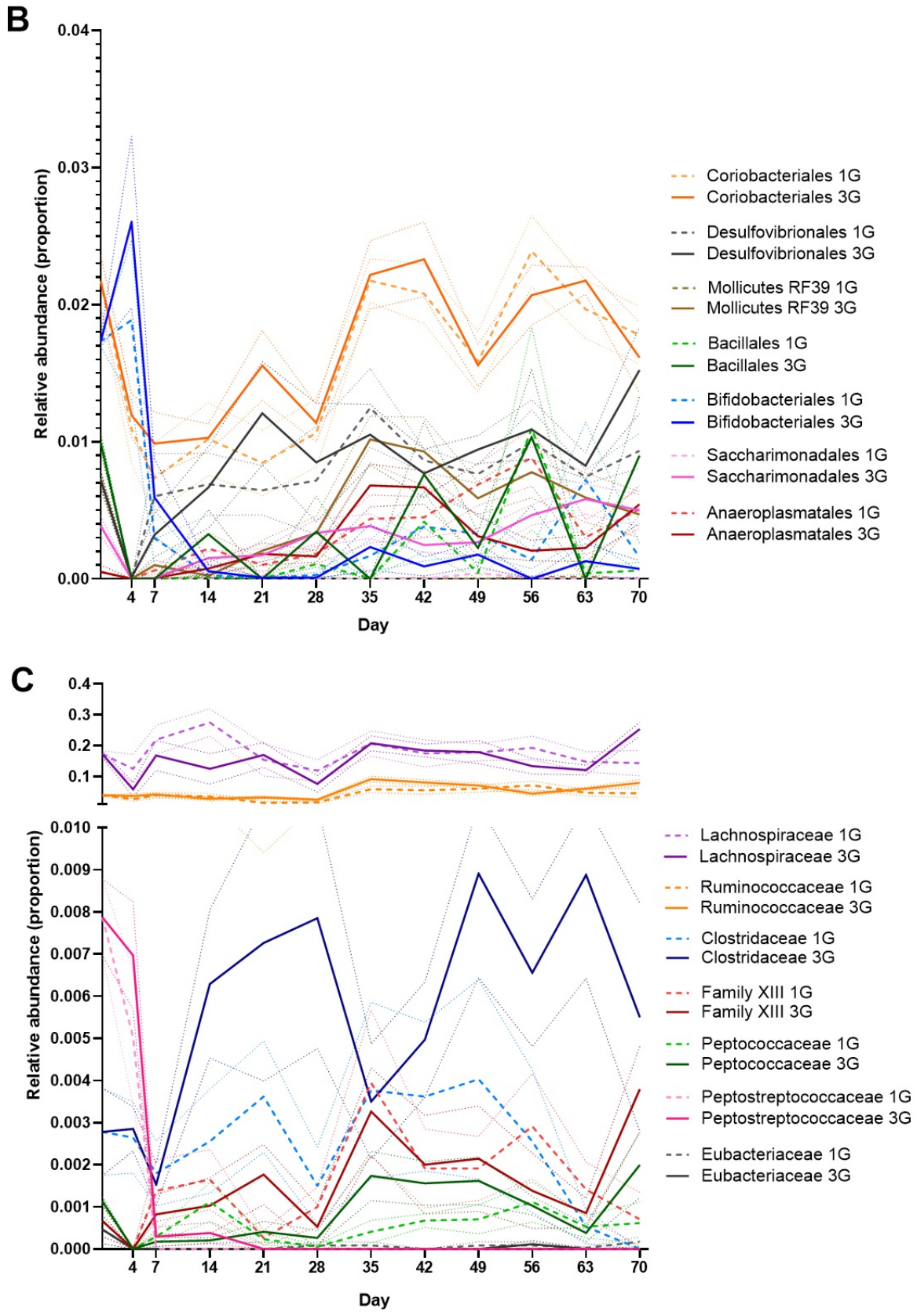



Figure 6

Relative abundance of donor taxa in recipient mice receiving the native microbiota. Donor

taxa observed in recipient that received one or three gavages of intact native microbiota were plotted at the order level based on (A) high relative abundance taxa (>0.03 relative abundance), (B) low relative abundance taxa (<0.03 relative abundance). (C Bacterial taxa within the Clostridiales order were plotted at the family level. Recipient mice received either one round (1G) or three rounds (3G) of donor microbiota. Solid and dotted lines denote the mean ± SEM values.

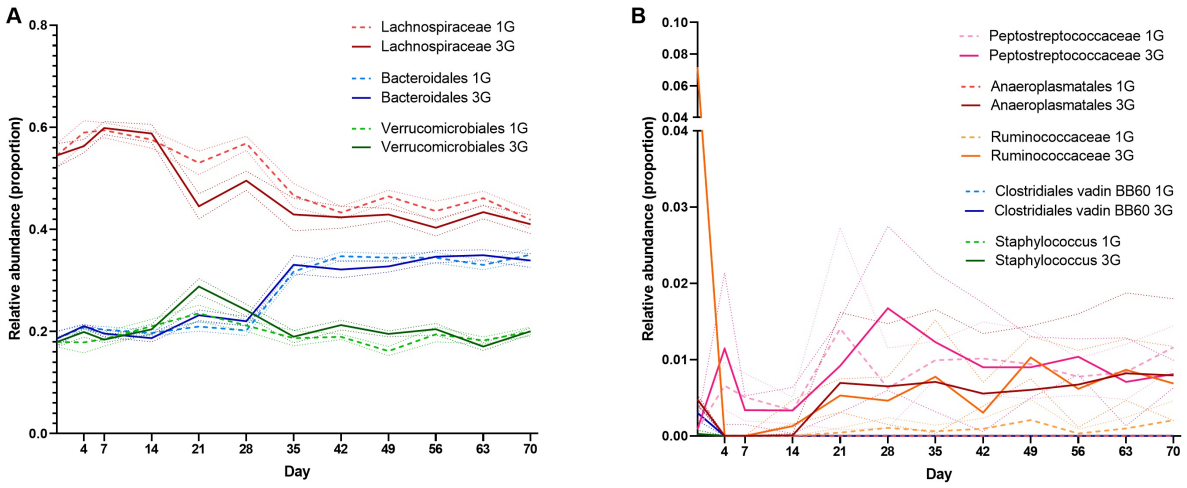



Figure 7

Relative abundance of donor taxa in recipient mice receiving the antibiotic- exposed microbiota. Donor taxa observed in recipient that received one or three gavages of the antibiotic-exposed microbiota were plotted at the order level, except for bacterial taxa in the Clostridiales order, which were plotted at the family level. Bacterial taxa were plotted according to (A) high relative abundance (>0.03 relative abundance) and (B) low relative abundance taxa at the order level (<0.03 relative abundance). Recipient mice received eithermone round (1G) or three rounds (3G) of donor microbiota. Solid and dotted lines denote the mean ± SEM values.

## Supplementary Files

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