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Supplemental Information

Antibodies Set Boundaries Limiting Microbial Metabolite Penetration and the Resultant Mammalian Host Response

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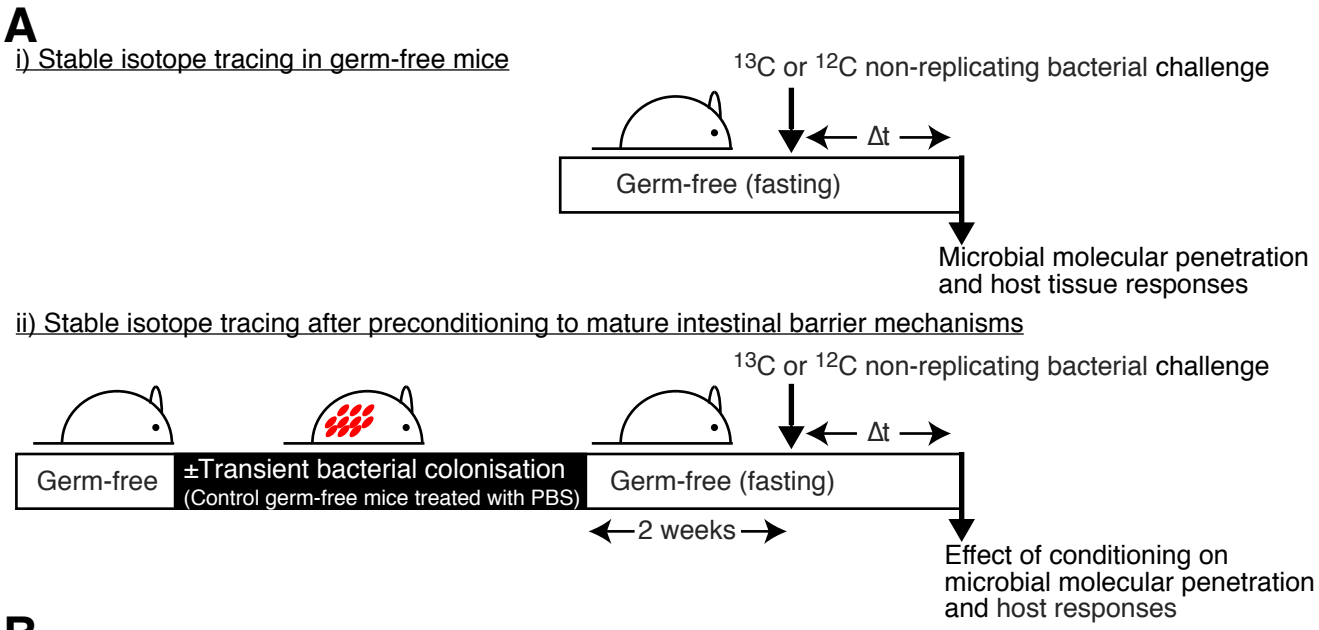
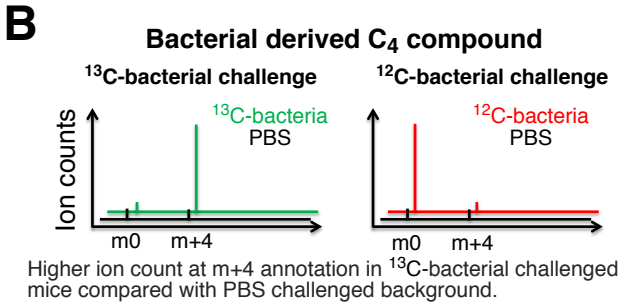
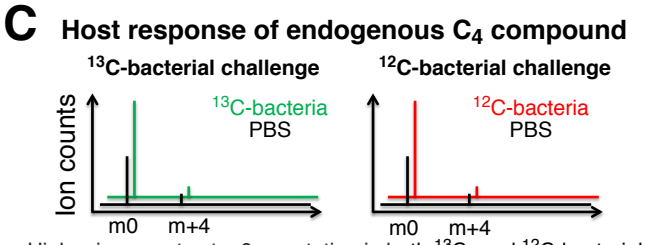
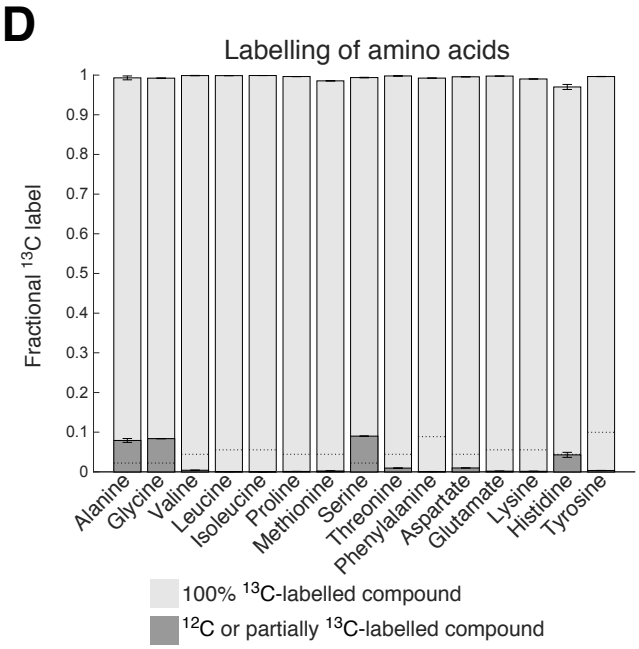
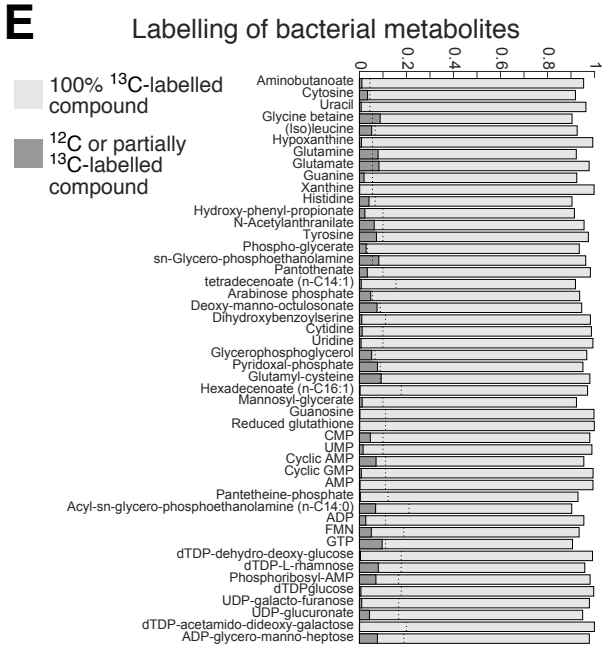
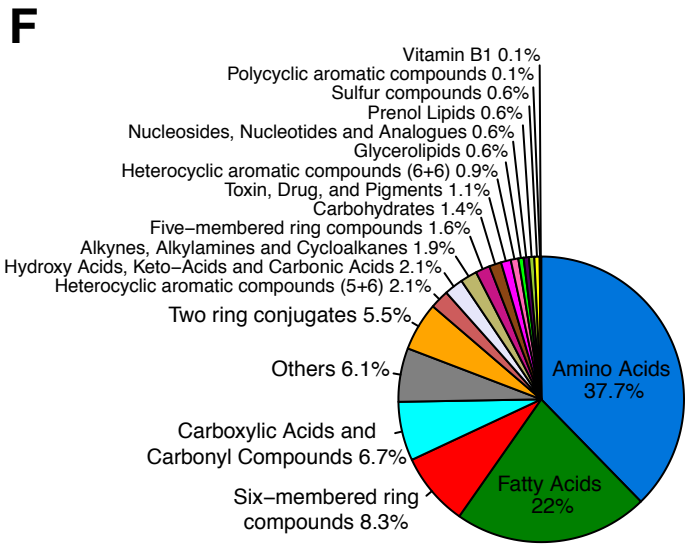








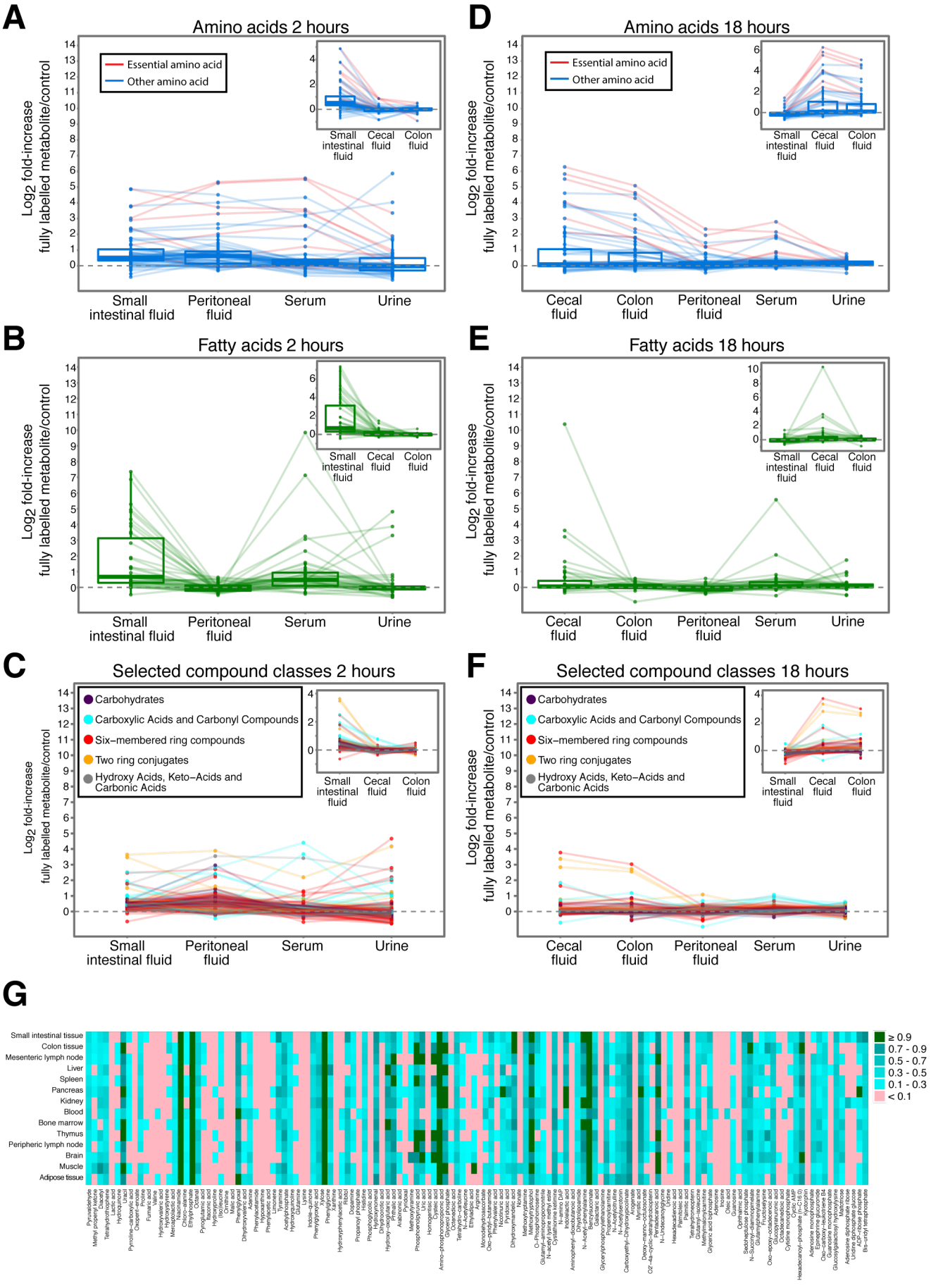




Uchimura et al., Figure S1
(color figure, related to Figure 1)

Figure S1. Stable isotope tracing design for concurrent measurement of penetration of microbial metabolites and the host response (related to Figure 1).

(A) i) The schematic representation shows the experimental design of germ-free control mice challenged with ^{13}C or ^{12}C non-replicating bacteria and subsequent sequential sampling.
ii) The extended schematic representation shows the experimental design using mice that were preconditioned with transitory bacterial colonization in order to mature immune and non-immune consolidated intestinal barrier functions and comparison with non-conditioned controls. Two weeks later they received ^{13}C or ^{12}C non-replicating bacterial challenge or control PBS gavage with subsequent sequential sampling to determine ^{13}C bacterial metabolite penetration into the host and the ^{12}C host response.
(B) A schematic representation of the mass spectrometric readout of a hypothetical bacterial-derived $^{13}C_4$ compound in the analysis of fluids or tissues in panel A.
(C) A schematic representation of the mass spectrometric readout of a hypothetical host-derived $^{12}C_4$ compound in the analysis of fluids or tissues in panel A.
(D) Fractional ^{13}C-labelling of amino acids of the ^{13}C non-replicating bacterial gavage cocktail (*E. coli* HA107) was analyzed with gas chromatography-mass spectrometry (GC-MS).
(E) as in (D), but showing the fractional ^{13}C-labelling of a range of compound classes obtained from a 6550 Agilent Q-TOF mass spectrometer with non-targeted flow injection.
(F) Piechart showing relative representation of different microbial compound classes with ≥ 2-fold increased metabolites and ≥ 75% ^{13}C-labeling at 2-hours and 18-hours after ^{13}C-HA107 gavage in fluid samples corresponding to Figure 2A and B.



Uchimura et al., Figure S2
(color figure, related to Figure 2)

Figure S2. Penetration of ^{13}C-labelled bacterial metabolites from the intestine to systemic sites (related to Figure 2).

Germ-free C57BL/6 mice were gavaged with ^{13}C-labelled HA107 or PBS as control (n = 3 - 4 mice per group). Fluid samples were collected 2 or 18 hours later and analyzed with Q-TOF mass spectrometry.
(A) The plot shows annotated ions representing metabolites containing ≥75% ^{13}C of the amino acid compound class from small intestinal fluids, peritoneal fluids, serum and urine at 2 hours, with lines connecting identical metabolites. The red coloured lines highlighted essential amino acids from other amino acids (blue).The inset shows intestinal samples from small intestinal fluids, cecal fluids and colon fluids also at 2 hours.
(B) As for panel A, but showing the fatty acid compound class at 2 hours.
(C) As for panel A, but showing carbohydrates (purple), carboxylic acids and carbonyl compounds (cyan), six-membered ring compounds (red), two ring conjugates (orange), and hydroxy acids, keto-acids and carbonic acids (grey) at 2 hours.
(D-F) are as in (A-C) respectively, but showing 18 hour samples.
(G) Heatmap indicating fractional labelling degree of bacterial-derived metabolites estimated as relative abundance of the fully (uniformly, U) labelled isoform (U-13C / (U-13C+12C)), ≥ 90%; dark green 10-90% pale to dark cyan and potentially exclusively host-originated metabolites <10% light red, in different host samples at 2 hours.

Uchimura et al., Figure S2
(related to Figure 2)

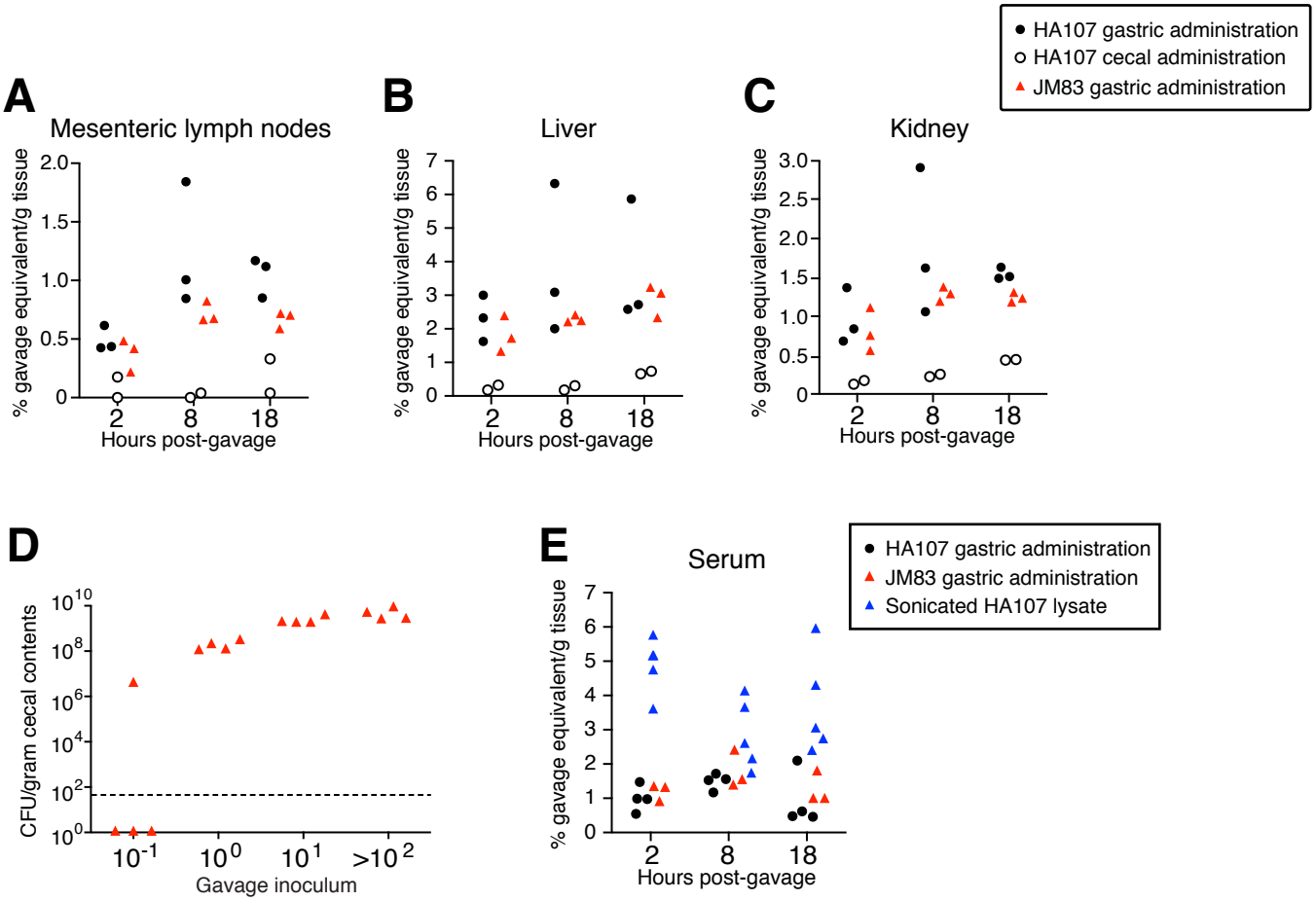
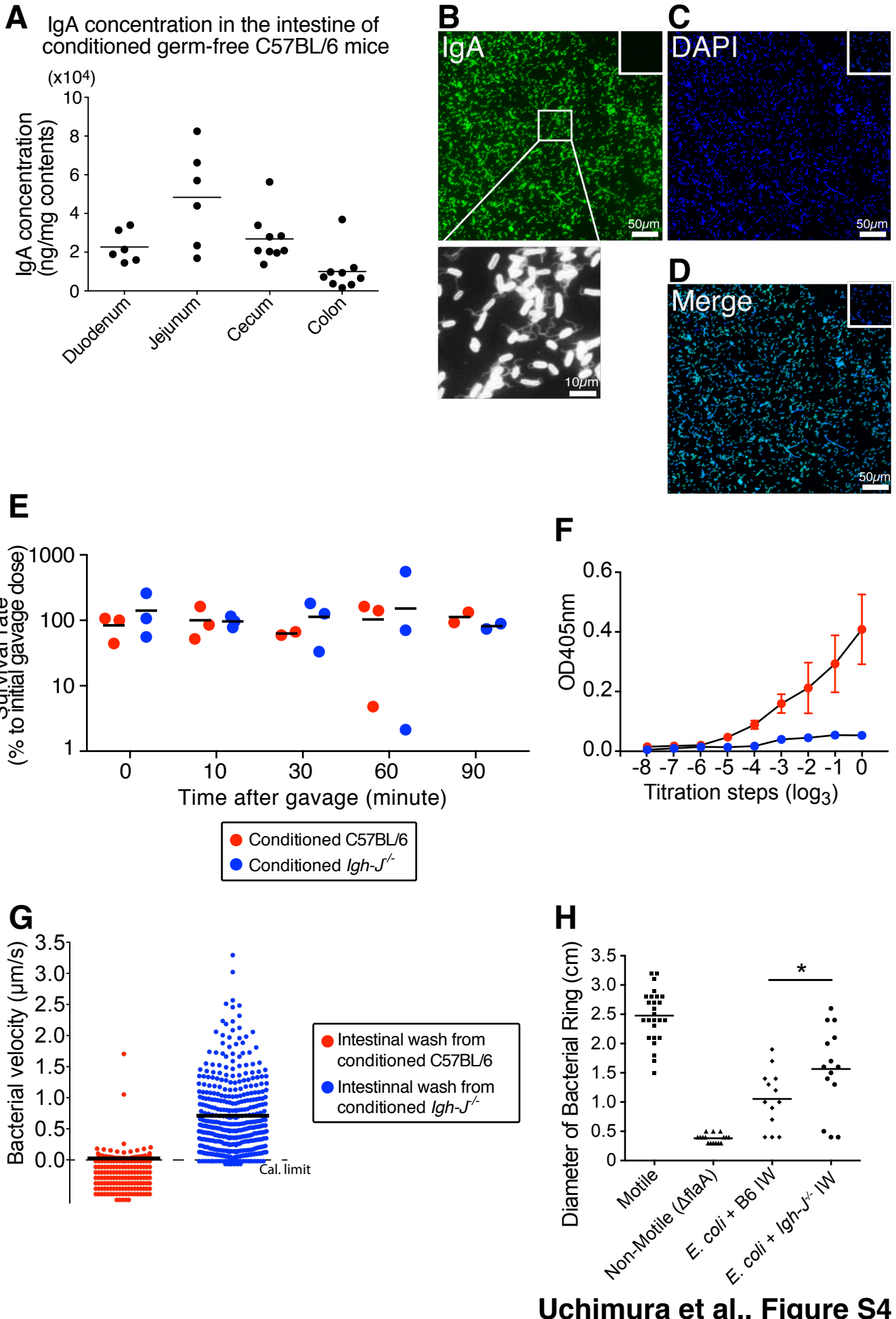


Figure S3. Penetration of bacterial products into systemic sites after treatment with *E.coli* HA107 or its replication-competent parent strain and determination of sensitivity of colonisation without significant gastric microbial killing (related to Figure 3).

(A-C) ^{14}C-labelled *E. coli* HA107 (after growth on ^{14}C-glucose; closed circles) was administered directly into the stomach or mixed directly with the cecal contents (open circles) of germ-free C57BL/6 mice. For comparison, ^{14}C-labelled *E. coli* JM83 (the replication competent parent strain of HA107; red triangles) was administered into the stomach of a separate group of mice. ^{14}C-radioactivity in (A) mesenteric lymph nodes, (B) liver and (C) kidney were monitored at 2, 8, and 18 hours after oral gavage or cecal administration. Background radioactivity from the respective solid or liquid samples of control germ-free mice was subtracted and results shown as a percentage of gavage equivalent of the bacterial cocktail used for the oral gavage or cecal administration. The results were pooled from two independent experiments.
(D) Serial dilutions (10^{-1}, 10^{0}, 10^{1}, and $\geq 10^{2}$ CFU) of *E. coli* JM83 (HA107 parent strain) were orally administrated to germ-free C57BL/6 mice. After 18 hours of colonisation, cecal contents was cultured on LB plates, and the total CFU/gram contents were plotted. Each point represents one mouse.
(E) Live ^{14}C-labelled HA107, live ^{14}C-labelled JM83 and sonication-generated ^{14}C-labelled *E. coli* HA107 lysis were gavaged to germ-free C57BL/6 mice. Serum samples were collected from mice at 2, 8 and 18 hours post gavage. Radioactivity of serum samples were analyzed as in Figure 3A.

Uchimura et al., Figure S3
(color figure, related to Figure 3)

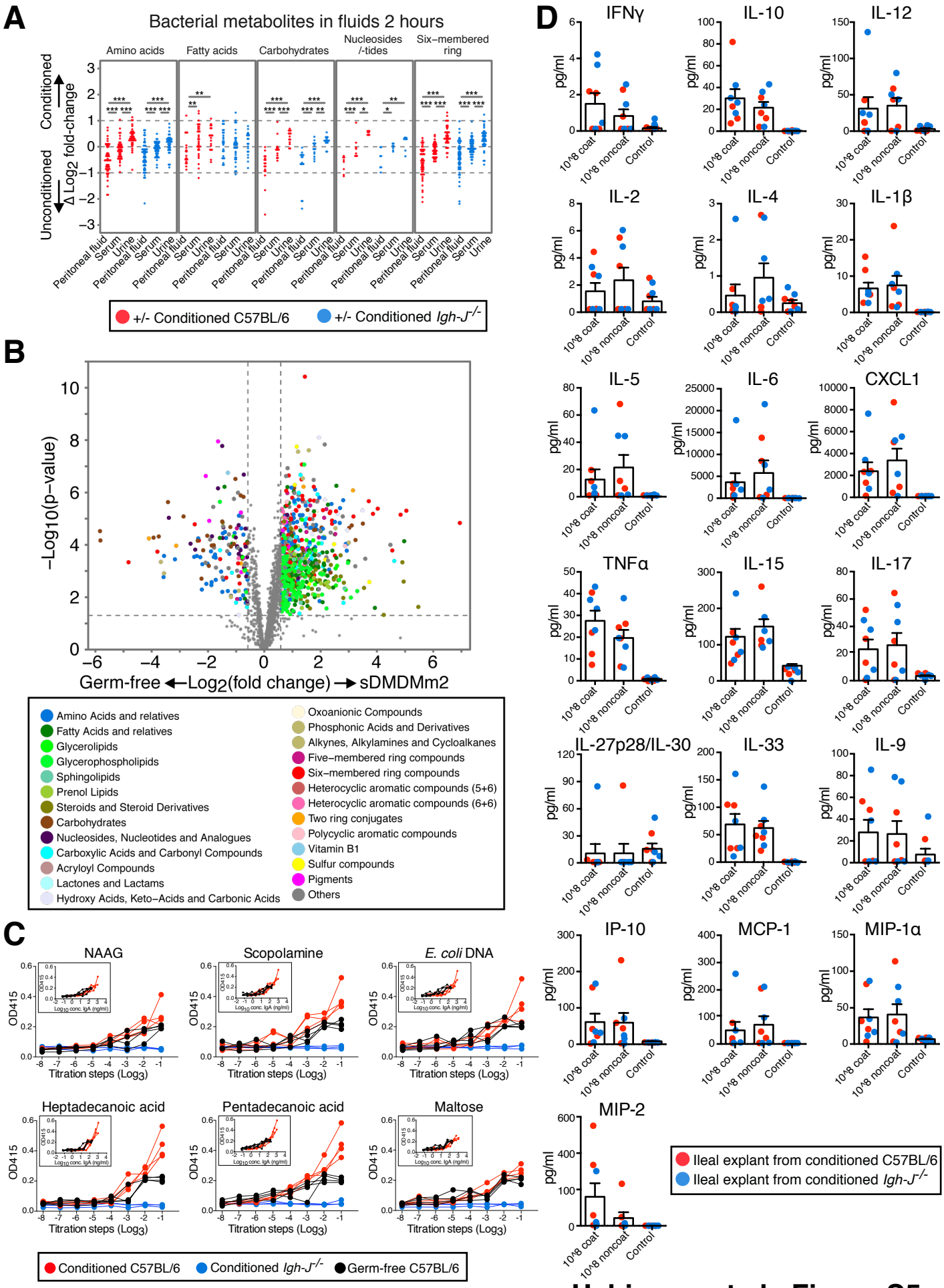


Uchimura et al., Figure S4
(color figure, related to Figure 4)

Figure S4. Intestinal conditioning with transient HA107 colonisation generates a bacterial-specific secretory IgA response in the small intestine of germ-free mice with reduced bacterial motility (related to Figure 4).

(A) IgA concentration in intestinal contents obtained from HA107 preconditioned germ-free C57BL/6 mice. Two weeks after the last HA107 gavage, intestinal contents from each indicated gut sections were collected and IgA levels were measured by ELISA. Each dot indicates the value obtained from an individual mouse.
(B-D) *E. coli* HA107 was incubated with native intestinal wash fluid collected from HA107 preconditioned germ-free C57BL/6 mice. The IgA antibodies bound to HA107 were visualized with FITC-conjugated anti-mouse IgA antibody (B). Bacterial DNA was stained with DAPI (C). The merged image is shown in (D). The upper right insets show negative controls without intestinal wash fluid. One Area in panel B was enlarged to highlight the flagella coated by IgA. Flash Slide Scanner images are shown using x40 magnification.
(E) The survival of HA107 was determined over time in small intestinal blind-loops. Surgically created small bowl loops of HA107 conditioned or untreated germ-free C57BL/6 or *Igh-J*$^{-/-}$ mice were injected with 500 μl of 10^{10} HA107. At indicated time points, samples were collected and plated to calculate the bacterial survival.
(F) Antigen specific ELISA was used to determine the direct binding of IgA to purified flagellin. Intestinal fluid prepared from either HA107 conditioned C57BL/6 or conditioned *Igh-J*$^{-/-}$ mice was used as primary antibody. Data shown are mean±SD, n = 4.
(G and H) *E. coli* was incubated with freshly prepared intestinal washes from either HA107-conditioned C57BL/6 or *Igh-J*$^{-/-}$ mice. The motility of GFP labelled bacteria were tracked by Zeiss LSM 700 confocal microscopy (supplementary videos 1 and 2). Bacterial velocities were quantified using Volocity software to semi-automatedly track bacterial motility (G). Bacteria were seeded on semi-solid motility agar after incubation with intestinal fluid as in G, and diameters of bacterial rings were measured after incubation at 37˚C for overnight (H).

Uchimura et al., Figure S4
(color figure, related to Figure 4)

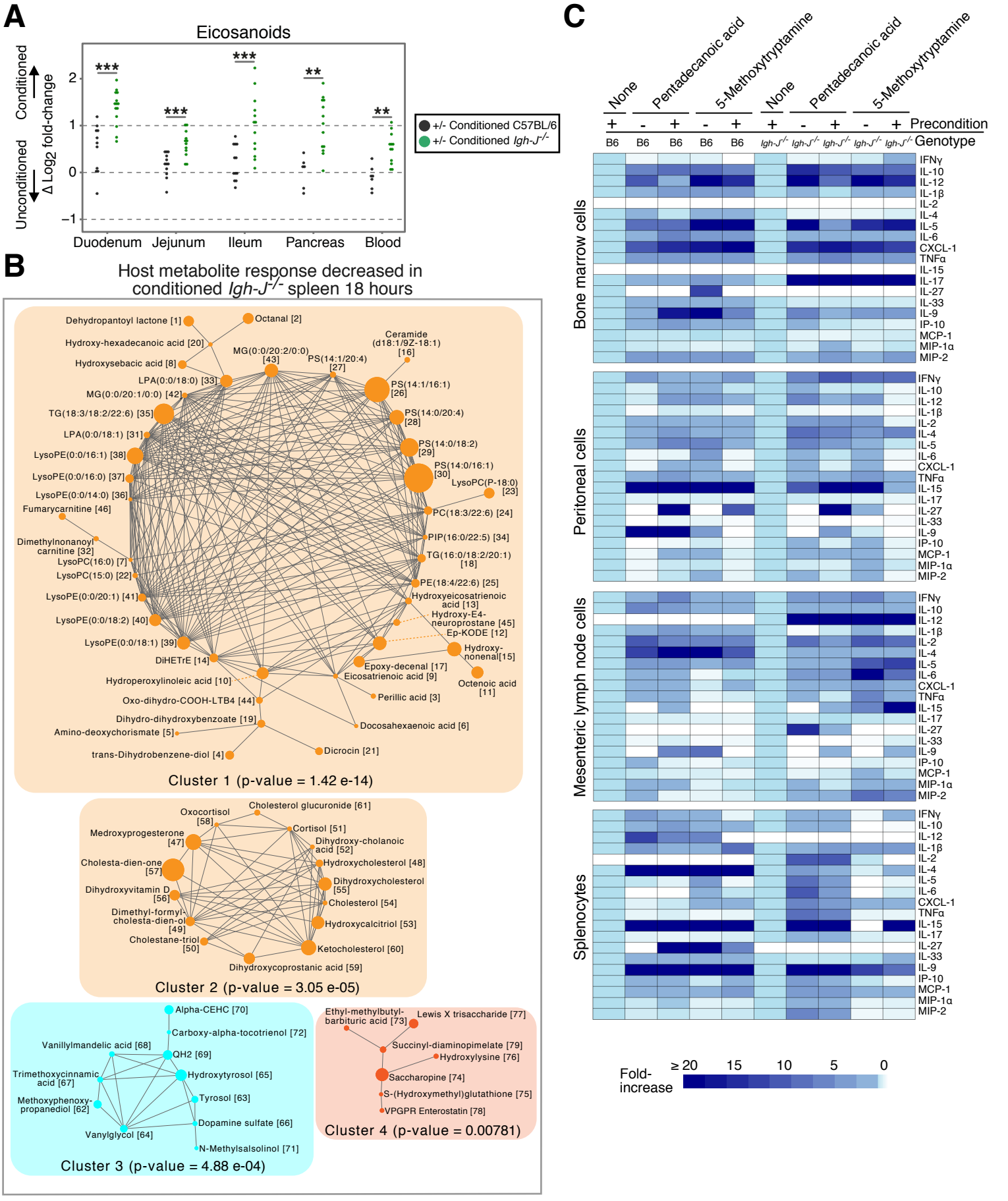


Uchimura et al., Figure S5
(color figure, related to Figure 5)

Figure S5. Migration of microbial metabolites of specific compound classes from intestinal and systemic tissues after intestinal challenge in germ-free conditioned or unconditioned mice at 2 hours and demonstration of the steady-state lipid load in germ-free and permanently colonised mice (related to Figure 5).

Germ-free C57BL/6 mice (red symbols) or antibody-deficient *Igh-J*$^{-/-}$ mice (blue) were preconditioned with HA107 or control-treated, returning to germ-free status twelve days prior to re-challenge with a gastric dose of fully ^{13}C-labelled HA107 or PBS as described in the legend to Figure 4.
(A) Penetration of different compound classes in host fluids at 2 hours (peritoneal fluid, serum and urine) are shown. Mann-Whitney U test was used and **= $p < 0.01$; *** = $p < 0.001$.
(B) Cecal fluid was collected from sDMDMm2 C57BL/6 (n=6) mice colonised with a standardised microbiota of 12 organisms (Brugiroux et al., 2016) and control germ-free C57BL/6 mice (n=6).
Metabolites above 1.5 fold-difference between sDMDMm2 colonized and germ-free control, p value < 0.05 were color-coded.
(C) The binding of intestinal IgA to bacterial derived metabolites was determined by antigen-specific ELISA. Intestinal IgA were collected from HA107 precoditioned C57BL/6 mice (red symbols), antibody-deficient *Igh-J*$^{-/-}$ mice (blue) or untreated germ-free C57BL/6 mice (black). Insets show results normalised for intestinal IgA concentrations after different treatments.
(D) Ileal explants (0.1 cm^2) collected from HA107 preconditioned germ-free C57BL/6 mice (red symbols) or preconditioned antibody-deficient *Igh-J*$^{-/-}$ mice (blue) were seeded in 96-well cell culture plates. 10^8/ml IgA-coated or -uncoated HA107 were used to trigger the explants for 6 hours. Culture supernatants from ileal explants were harvested to address cytokine release using MSD cytokine 19-plex plates. Bar plots (mean±SD) show the pooled data of C57BL/6 and *Igh-J*$^{-/-}$ for each condition.·

Uchimura et al., Figure S5
(color figure, related to Figure 5)



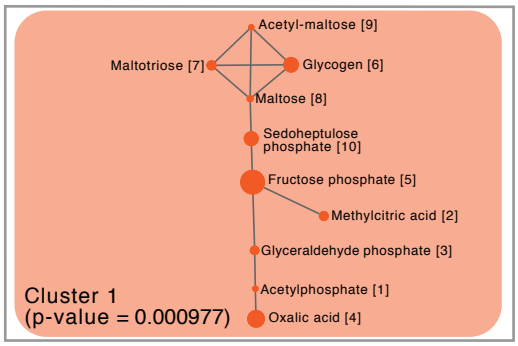
Uchimura et al., Figure S6
(related to Figures 6 and 7)

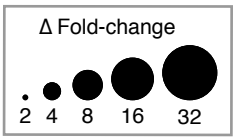
Figure S6. Host intestinal and systemic eicosanoid responses following bacterial challenge in conditioned and unconditioned wild-type and antibody-deficient mice. Tissue-specific host metabolite responses in spleen following intestinal bacterial challenge in antibody-deficient mice (related to Figures 6 and 7).

(A) Germ-free C57BL/6 mice (black circles) or antibody-deficient *Igh-J*$^{-/-}$ mice (green circles) were preconditioned with HA107 or control-treated, returning to germ-free status two weeks prior to re-challenge with a gastric dose of fully ^{13}C-labelled or ^{12}C HA107. Tissue samples were collected after 18 hour HA107 oral-gavage and analyzed with Q-TOF mass spectrometry. Annotated ions representing eicosanoid metabolites containing concordant changes of metabolites from ^{13}C and ^{12}C pulsed mice, without mass shift of the annotated ions were plotted. Log_2-fold change differences between conditioned and unconditioned mice for each metabolite are shown for the duodenum, jejunum, ileum, pancreas and blood 18 hours after HA107 gavage.
(B) Increased ^{12}C-annotated host metabolites in conditioned *Igh-J*$^{-/-}$ spleen 18 hours after challenge were applied to MetaMapp to obtain chemical similarity networks (Tanimoto chemical similarity ≥ 0.7). The size of nodes is proportional to absolute value of the Δ fold-change. Mann Whitney U test (paired) was used to calculate the p value.
(C) Germ-free C57BL/6 mice or antibody-deficient *Igh-J*$^{-/-}$ mice were preconditioned with HA107 or left untreated. After two weeks, mice were either re-challenged with pentadecanoic acid or 5-methoxytryptamine. The cytokines released by cells from different lymphoid tissues were measured as described in the legend to Figure 6D. Results show the mean of all data of each group compared with unchallenged controls with same genetic background (C57BL/6 or *Igh-J*$^{-/-}$) n = 4 mice of each group.

Uchimura et al., Figure S6
(related to Figures 6 and 7)

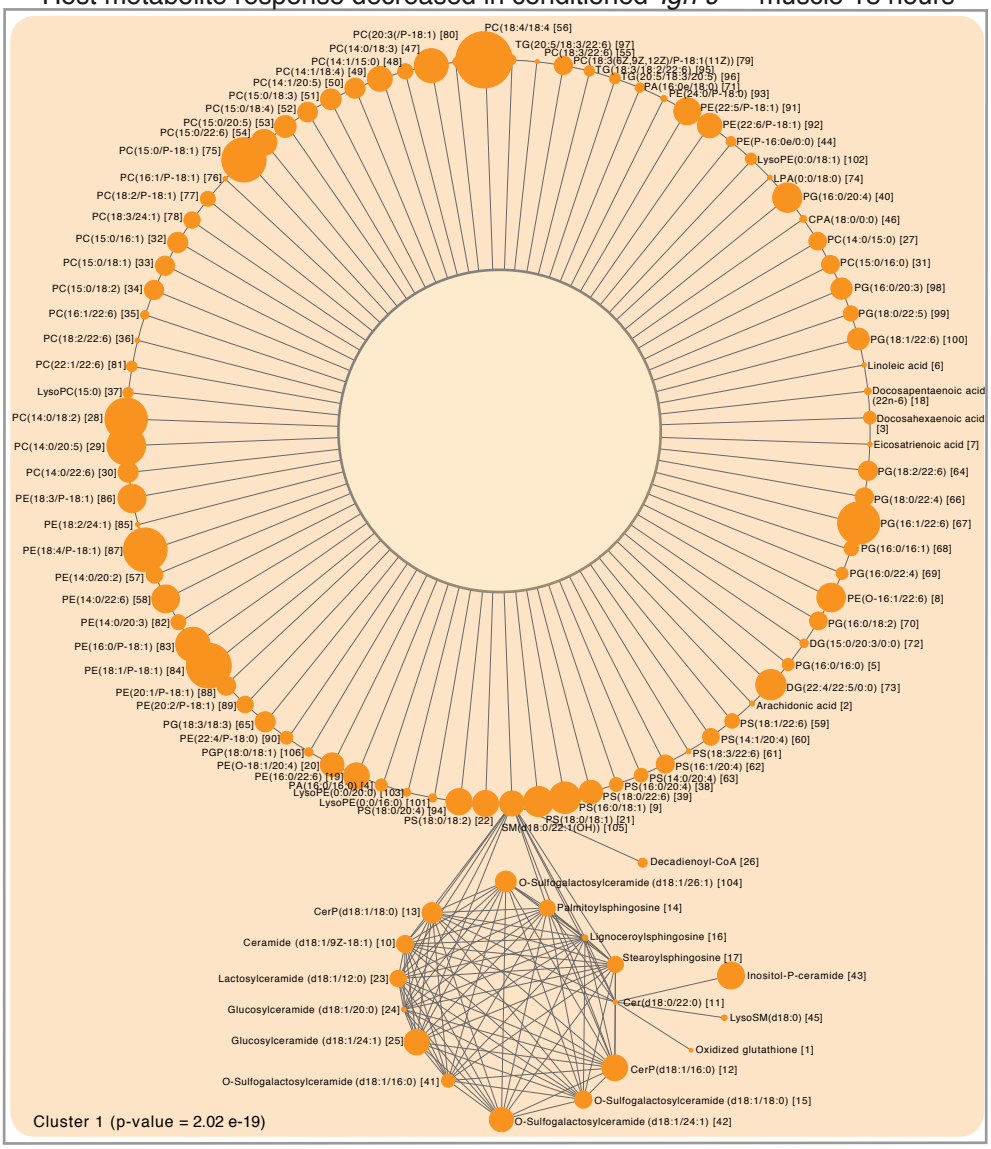
A Host metabolite response increased in conditioned *Igh-J*$^{-/-}$ muscle 18 hours



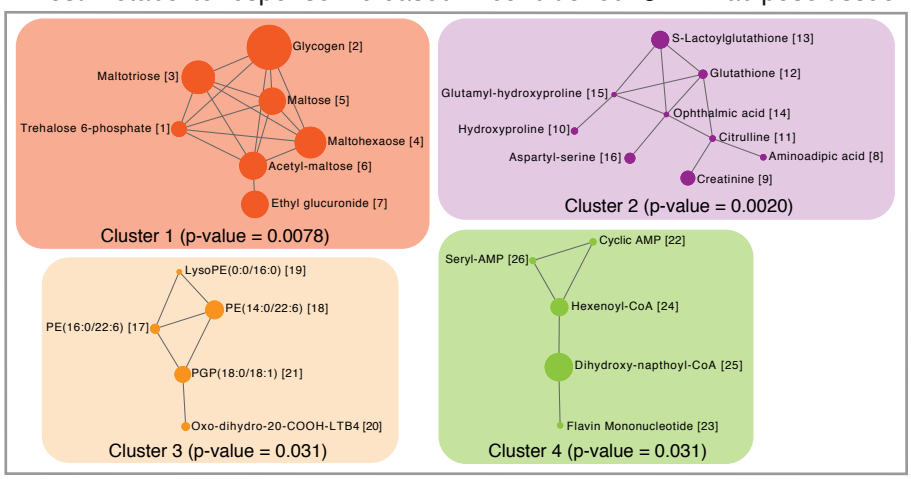


Numbers in squared brackets refer to the metabolite annotation (TableS3).

B Host metabolite response decreased in conditioned *Igh-J*$^{-/-}$ muscle 18 hours



C Host metabolite response increased in conditioned *Igh-J*$^{-/-}$ adipose tissue 18 hours



Uchimura et al., Figure S7
(related to Figures 6 and 7, continued overleaf)

Figure S7. Tissue-specific host metabolite responses in muscle and adipose tissue following intestinal bacterial challenge in antibody-deficient mice (related to Figures 6 and 7).

Chemical similarity index networks of metabolites increased in skeletal muscle **(A)**, decreased in skeletal muscle **(B)** and increased in adipose tissue **(C)** in antibody-deficient mice 18 hours after challenge as described in the legend to Figure S6B.