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Article in *Nature* · March 2015

DOI: 10.1038/nature14232

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# Dietary emulsifiers impact the mouse gut microbiota promoting colitis and metabolic syndrome

Benoit Chassaing<sup>1</sup>, Omry Koren<sup>2</sup>, Julia K. Goodrich<sup>3</sup>, Angela C. Poole<sup>3</sup>, Shanthi Srinivasan<sup>4</sup>, Ruth E. Ley<sup>3</sup> & Andrew T. Gewirtz<sup>1</sup>

The intestinal tract is inhabited by a large and diverse community of microbes collectively referred to as the gut microbiota. While the gut microbiota provides important benefits to its host, especially in metabolism and immune development, disturbance of the microbiota-host relationship is associated with numerous chronic inflammatory diseases, including inflammatory bowel disease and the group of obesity-associated diseases collectively referred to as metabolic syndrome. A primary means by which the intestine is protected from its microbiota is via multi-layered mucus structures that cover the intestinal surface, thereby allowing the vast majority of gut bacteria to be kept at a safe distance from epithelial cells that line the intestine<sup>1</sup>. Thus, agents that disrupt mucus-bacterial interactions might have the potential to promote diseases associated with gut inflammation. Consequently, it has been hypothesized that emulsifiers, detergent-like molecules that are a ubiquitous component of processed foods and that can increase bacterial translocation across epithelia *in vitro*<sup>2</sup>, might be promoting the increase in inflammatory bowel disease observed since the mid-twentieth century<sup>3</sup>. Here we report that, in mice, relatively low concentrations of two commonly used emulsifiers, namely carboxymethylcellulose and polysorbate-80, induced low-grade inflammation and obesity/metabolic syndrome in wild-type hosts and promoted robust colitis in mice predisposed to this disorder. Emulsifier-induced metabolic syndrome was associated with microbiota encroachment, altered species composition and increased pro-inflammatory potential. Use of germ-free mice and faecal transplants indicated that such changes in microbiota were necessary and sufficient for both low-grade inflammation and metabolic syndrome. These results support the emerging concept that perturbed host-microbiota interactions resulting in low-grade inflammation can promote adiposity and its associated metabolic effects. Moreover, they suggest that the broad use of emulsifying agents might be contributing to an increased societal incidence of obesity/metabolic syndrome and other chronic inflammatory diseases.

Mice were administered the emulsifiers carboxymethylcellulose (CMC) or polysorbate-80 (P80) via drinking water (1.0% w/v or v/v, respectively) for 12 weeks. P80 has been studied for toxicity and carcinogenic potential<sup>2,4,5</sup> and is approved by the US Food and Drug Administration for use in select foods at up to 1.0%. CMC has not been extensively studied but is deemed ‘generally regarded as safe (GRAS)’ and used in various foods at up to 2.0%<sup>3,6</sup>. We used wild-type C57Bl/6 mice and two engineered strains of mice, namely *Il10*<sup>-/-</sup> and *Tlr5*<sup>-/-</sup>, that are prone to developing shifts in microbiota composition and inflammation<sup>7,8</sup>. While observations that loss of Toll-like receptor (TLR) function alters microbiota composition and promotes inflammation were suggested to be an artefact of mouse husbandry practices<sup>9</sup>, the fact that epithelial-cell-specific deletion of TLR5 altered microbiota composition relative to wild-type siblings and resulted in low-grade inflammation/metabolic syndrome indicates that, at least for TLR5, this is not the case<sup>10</sup>.

Confocal microscopy, using mucus-preserving Carnoy fixation<sup>11,12</sup>, indicated that the closest bacteria resided, on average, about 25 µm from

epithelial cells with no bacteria observed within 10 µm (Fig. 1a, d). In contrast, emulsifier-treated mice exhibited some bacteria in apparent contact with the epithelium while the average distance was reduced by more than 50% (Fig. 1b–d). Such microbiota encroachment correlated with reduced mucus thickness. This altered mucus thickness was not correlated with altered expression of MUC2 (Extended Data Fig. 1a–d). *Il10*<sup>-/-</sup> and *Tlr5*<sup>-/-</sup> mice exhibited basal microbiota encroachment that was enhanced by both CMC and P80 (Fig. 1e–h and Extended Data Fig. 1e–h). Emulsifiers did not affect total levels of faecal bacteria in wild-type, *Il10*<sup>-/-</sup> or *Tlr5*<sup>-/-</sup> mice but resulted in more than a twofold increase in bacteria adherent to the colons of wild-type and *Il10*<sup>-/-</sup> mice (Fig. 1i, k and Extended Data Fig. 1i–l). Moreover, interrogation of microbiota composition with 16S RNA sequencing, followed by phylogenetic analysis, and use of the unweighted UniFrac algorithm to compare community structure<sup>12</sup> revealed that both CMC and P80 dramatically altered microbiota composition in both faecal and intestinal-adherent bacteria of wild-type, *Il10*<sup>-/-</sup> and *Tlr5*<sup>-/-</sup> mice (Fig. 1j, l and Extended Data Fig. 2a–o and Supplementary Tables 1 and 2).

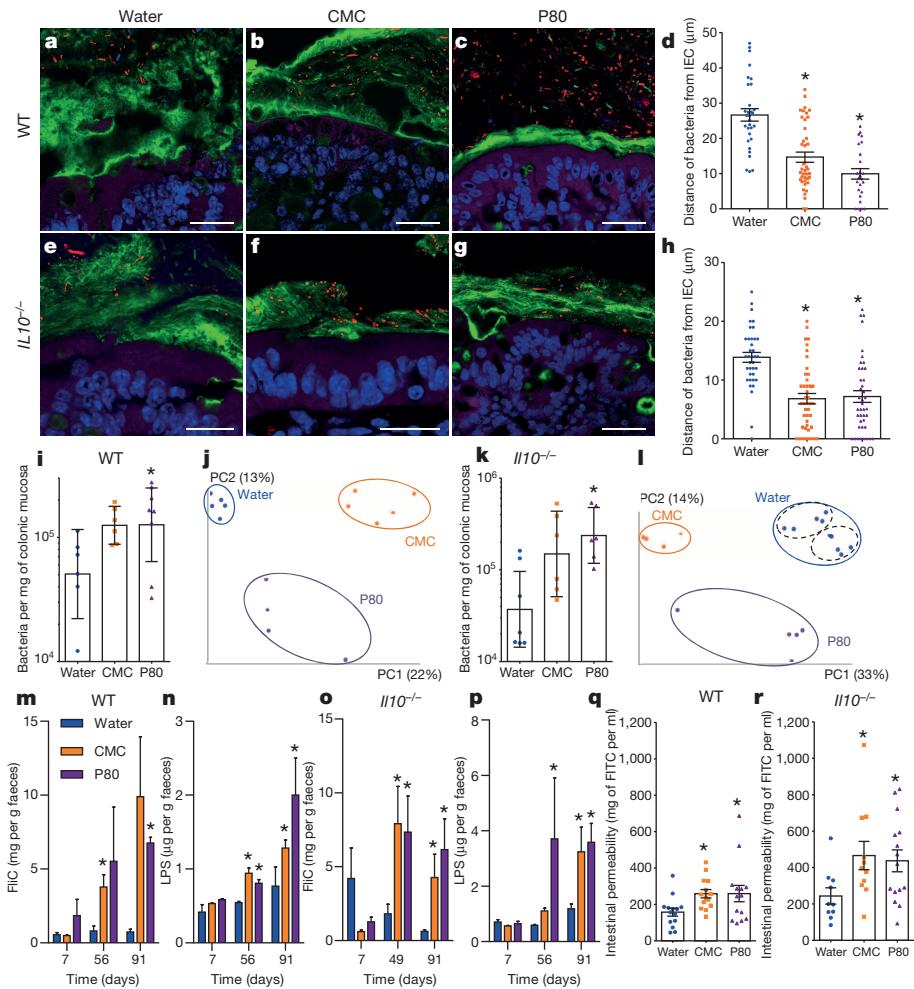
In order to verify that differences in microbiota composition were a consequence of emulsifier treatment rather than simply reflecting that mice shared cages during treatment, which results in microbiota composition clustering in coprophagous animals, multiple litters were split equally into groups that were to receive water, CMC or P80 (three separate cages per condition as shown in Extended Data Fig. 3). As expected, this experimental design avoided pre-treatment clustering. However, microbiota composition clustered strongly following treatment irrespective of cage clustering in both male and female mice with wild-type and *Tlr5*<sup>-/-</sup> genotypes (Extended Data Fig. 3), confirming that emulsifiers alter microbiota composition.

Alterations in microbiota composition included reduced levels of Bacteroidales, associated with health<sup>13–15</sup>, and increased levels of several mucolytic operational taxonomic units (OTUs) including *Ruminococcus gnavus*<sup>16</sup> (Extended Data Figs 2p–t and 4 and Supplementary Tables 1 and 2). In *Il10*<sup>-/-</sup> mice, both CMC and P80 induced a marked reduction in microbial diversity, bloom in Verrucomicrobia phyla (especially *Akkermansia muciniphila*; Extended Data Fig. 2p–t and Supplementary Tables 1 and 2)<sup>17</sup>, and enriched mucosa-associated inflammation-promoting Proteobacteria<sup>18,19</sup>. Emulsifier-induced changes in microbiota composition were sufficiently uniform such that a low number of OTUs (<15) afforded a reliable prediction of emulsifier treatment (Extended Data Fig. 4 and Supplementary Tables 1 and 2). Such distinguishing OTUs were spread across the Bacteria domain and several were common to all three host genotypes.

While most experiments herein used young mice (4 weeks old at the start of the experiment) based on the notion that microbiota are more prone to disturbance at an early age<sup>18,20</sup>, administration of emulsifiers beginning at 4 months of age also destabilized and altered microbiota composition (Extended Data Fig. 5h–p). Specifically, both CMC and P80 resulted in decreased alpha diversity and reduced stability, as evidenced by an increased extent of week-to-week changes in principal

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coordinates, and a greater extent of change over the course of the experiment that outweighed effects of cage clustering (Extended Data Fig. 5h–p). Thus, emulsifiers can alter the gut microbiota composition of hosts of a broad age range.

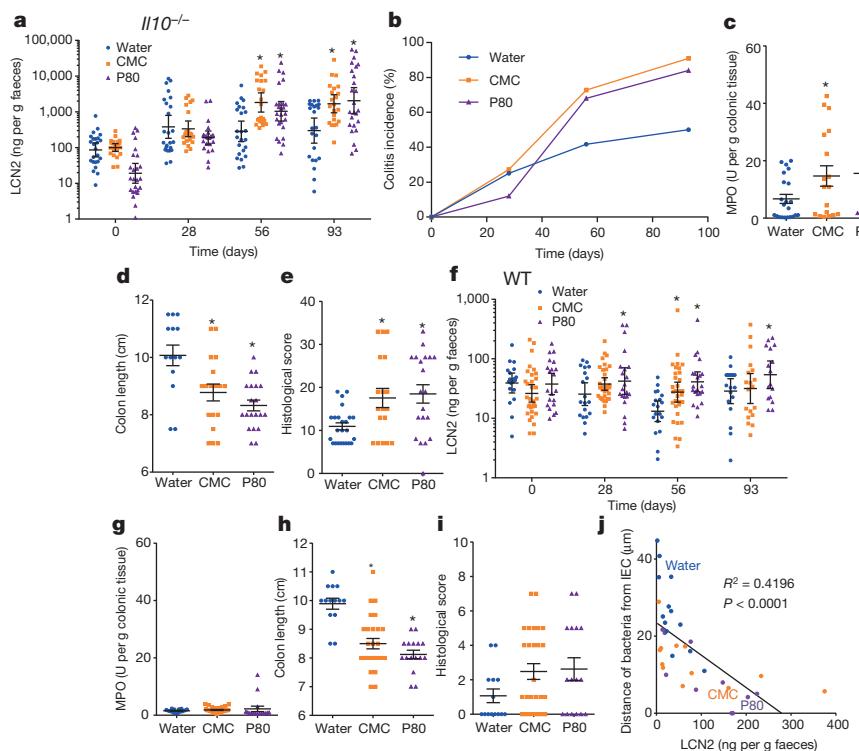
Microbiota composition influences the ability of a microbiota to activate innate immune signalling<sup>21</sup>. Thus, we measured the capacity of faeces from control and emulsifier-treated mice to activate pro-inflammatory gene expression via the lipopolysaccharide (LPS) and flagellin receptors TLR4 and TLR5, respectively. Exposure to emulsifiers increased faecal levels of bioactive LPS and flagellin in wild-type, *Il10*<sup>−/−</sup> and *Tlr5*<sup>−/−</sup> mice (Fig. 1m–p and Extended Data Fig. 5q–r). Emulsifier treatment also increased gut permeability in wild-type and *Il10*<sup>−/−</sup> mice (Fig. 1q, r), which correlated with increased levels of serum antibodies to flagellin and LPS (Extended Data Fig. 5s–v), thought to reflect gut permeability<sup>22</sup>. Thus, chronic exposure to dietary emulsifiers results in erosion of the protective function of the mucus, increased bacterial adherence and a more pro-inflammatory microbiota.

The hallmark of active colitis is the presence of immune cell infiltrates, which is typically paralleled by changes in gross colon morphology and elevated levels of the leukocyte enzyme myeloperoxidase and pro-inflammatory markers. Based on such criteria, emulsifiers promoted the extent and incidence of colitis in both *Il10*<sup>−/−</sup> and *Tlr5*<sup>−/−</sup> mice (Fig. 2a–e and Extended Data Figs 6a, b, h and 7a–g). Emulsifiers did not induce overt colitis in wild-type mice but did result in subtle histopathologic and gross evidence of chronic intestinal inflammation, including epithelial damage and shortened colons (Fig. 2f–i and Extended Data Fig. 6c–g). Faecal lipocalin 2 (LCN2) is a sensitive and broadly dynamic marker of intestinal inflammation in mice<sup>23</sup>. Emulsifier-treated wild-type mice exhibited modestly elevated faecal LCN2 levels 4 weeks

after initial exposure (Fig. 2f). In *Il10*<sup>−/−</sup> and *Tlr5*<sup>−/−</sup> mice, basally elevated faecal LCN2 levels were further elevated (approximately ten-fold) by 12 weeks of exposure to CMC and P80 (Fig. 2a and Extended Data Fig. 7a). Such robust colitis in *Il10*<sup>−/−</sup>, but not *Tlr5*<sup>−/−</sup>, correlated with *Bilophila* and *Helicobacter* enrichment, analogous to previous observations in *Il10*<sup>−/−</sup> mice (Supplementary Tables 2 and 3)<sup>24,25</sup>. The extent of inflammation inversely correlated with bacterial–epithelial distance in both wild-type and *Il10*<sup>−/−</sup> mice (Fig. 2j and Extended Data Fig. 6i–k). Thus, emulsifiers may promote robust colitis in susceptible hosts and induce low-grade inflammation in wild-type hosts.

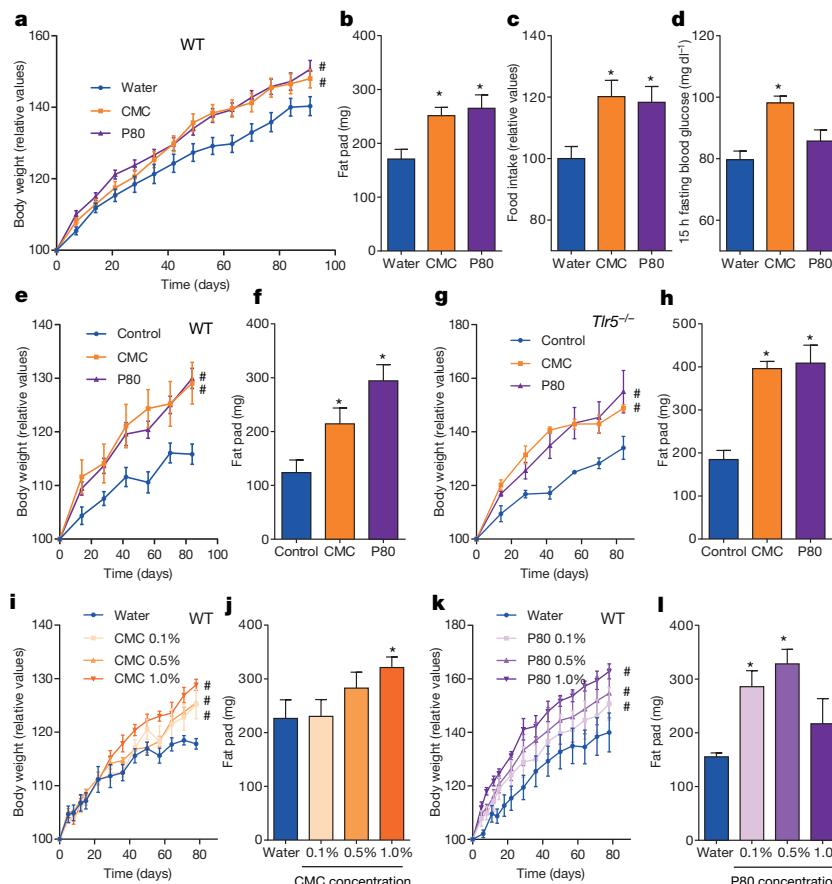
Metabolic syndrome is associated with, and may be promoted by, low-grade inflammation<sup>26</sup>. Thus, we examined whether emulsifier-induced microbial dysbiosis and low-grade inflammation might promote this disorder in wild-type mice. Both CMC and P80 resulted in modest but significant gains in overall weight and a marked increase in adiposity as measured by fat mass (Fig. 3a, b). Such increased adiposity was associated with increased food consumption (Fig. 3c) that probably drove the increased adiposity. Emulsifier treatment also impaired glycaemic control as assessed by fasting blood glucose concentration (Fig. 3d) and glucose/insulin tolerance testing (Extended Data Fig. 7h, i).

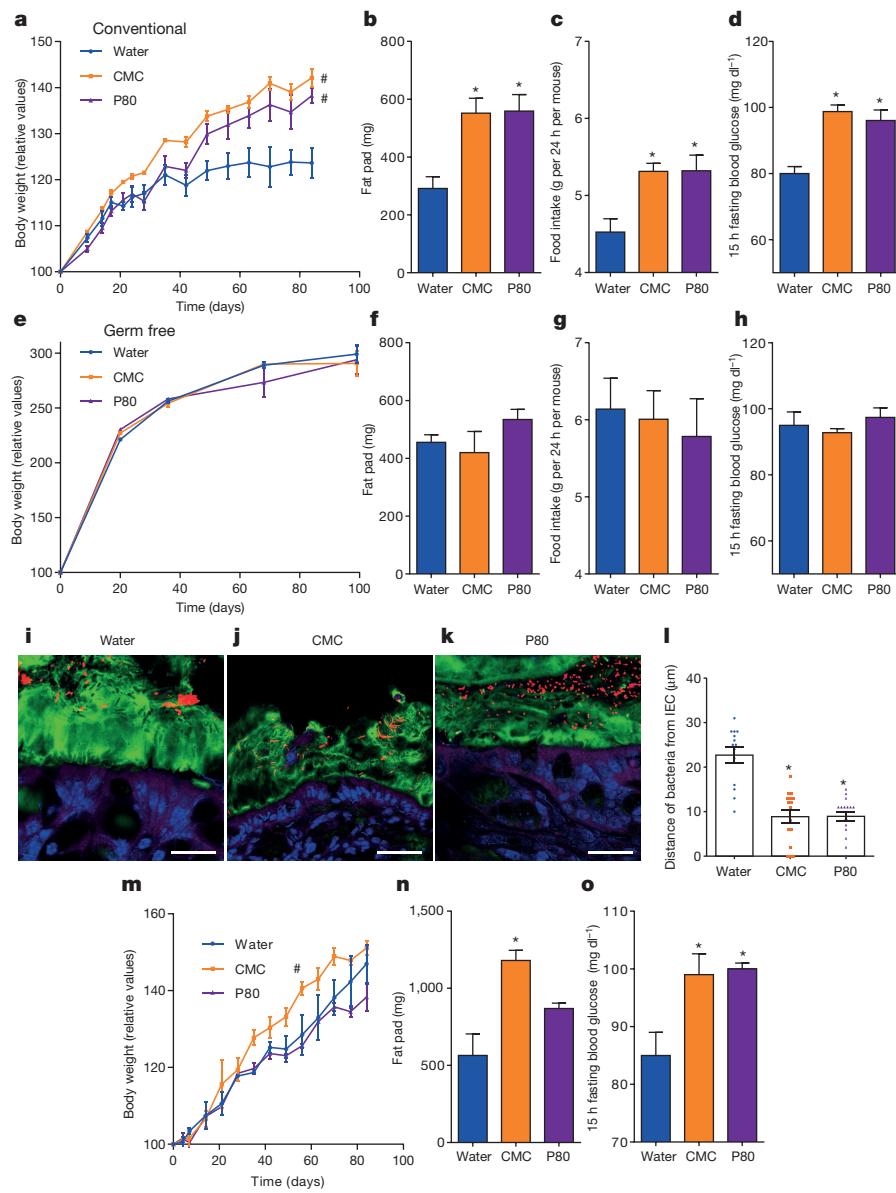
Human exposure to dietary emulsifiers occurs in liquid and solid foods. Hence, we next examined whether incorporating emulsifiers into mouse chow would drive similar phenotypes. Supplementation of chow with 1.0% CMC or P80 fully mimicked the pro-inflammatory effects and metabolic changes induced by emulsifiers in drinking water (Fig. 3e, f and Extended Data Fig. 7l–t). While human emulsifier consumption is not a widely tracked parameter, their pervasive use in many foods exceeds the 1.0% level (in food or water) used herein<sup>6</sup>. Nonetheless, we next sought to define the minimum dose of emulsifiers that would produce



evidence of low-grade inflammation/metabolic syndrome. As little as 0.1% CMC resulted in modest increases in body weight and fasting glucose, while 0.5% resulted in clear evidence of low-grade inflammation (shortened colon, enlarged spleen) and increased adiposity (Fig. 3*i*, *j* and Extended Data Fig. 7*a*–*a'*). For P80, as little as 0.1% resulted in

evidence of low-grade inflammation and increased adiposity, while 0.5% resulted in mild dysglycaemia (Fig. 3*k*, *l* and Extended Data Fig. 7*b*–*b'*). Emulsifier-induced metabolic syndrome was also observed in older mice (Extended Data Fig. 5*a*–*g*) and persisted for at least 6 weeks after stopping emulsifier consumption (Extended Data Fig. 8*a*–*k*).





**Figure 4 | Altered microbiota is necessary and sufficient for emulsifier-induced metabolic syndrome.** **a-h**, Conventionally housed (**a-d**) and germ-free (**e-h**) Swiss Webster mice were exposed to drinking water containing CMC or P80 (1.0%) for 12 weeks. **a, e**, Body weight over time; **b, f**, fat-pad mass; **c, g**, food intake; and **d, h**, 15 h fasting blood glucose concentration. **i-o**, Germ-free Swiss Webster mice were conventionalized via microbiota transplant from mice that received standard drinking water or drinking water containing CMC or P80 (1.0%). **i-k**, Confocal microscopy analysis of microbiota localization: MUC2, green; actin, purple; bacteria, red; and DNA, blue. Scale bar, 20  $\mu$ m. Pictures are representative of 10 biological replicates. **l**, Distances of closest bacteria to intestinal epithelial cells (IEC) per condition over five high-powered fields per mouse. **m**, Body weight over time; **n**, fat-pad mass; and **o**, 15 h fasting blood glucose concentration. Data are the means  $\pm$ s.e.m. ( $n = 5$  for **a-l**,  $n = 3$  for **m-o**). Points are from individual mice. Significance was determined using one-way ANOVA corrected for multiple comparisons with a Sidak test (\* $P < 0.05$ ) or two-way group ANOVA corrected for multiple comparisons with a Bonferroni test (# $P < 0.05$ ) compared to control group.

Promotion of metabolic syndrome was not seen upon exposure to sodium sulfite, which is a common food additive but not an emulsifier (Extended Data Fig. 8l-s). *Tlr5*<sup>-/-</sup> mice are prone to developing metabolic syndrome<sup>27</sup>, which results from poor microbiota management<sup>18</sup>. Emulsifiers markedly promoted multiple parameters of metabolic syndrome in *Tlr5*<sup>-/-</sup> mice (Extended Data Figs 7j-k and 8t-w), including hyperphagia, increased adiposity and glucose dysregulation, and was observed upon supplementation of chow or drinking water with as little as 0.1% P80 (Fig. 3g, h and Extended Data Fig. 8x-o'). A trend towards the development of metabolic syndrome in emulsifier-treated non-colitic *Il10*<sup>-/-</sup> mice was also observed, which was particularly evident upon exclusion of colitic mice, which exhibited weight loss (Extended Data Fig. 9a-f). Emulsifier-induced metabolic syndrome was observed in mice from multiple vivaria and strains, including Swiss Webster mice, which, in contrast to C57BL/6 mice, are considered obesity-resistant<sup>28</sup> (Fig. 4a-d and Extended Data Fig. 9g-k, x-a').

Emulsifier-induced low grade inflammation and metabolic syndrome required the presence of a microbiota in that administration of emulsifiers to germ-free mice resulted in neither low-grade inflammation, as assessed by faecal LCN2, colon length and splenomegaly, nor all measured parameters of metabolic syndrome including body mass, fat mass, food intake and fasting glucose levels (Fig. 4e-h and Extended Data

Fig. 9g-p). This result could reflect direct action of CMC and P80 on gut bacteria composition or metabolism. In accord with both of these possibilities, emulsifiers altered faecal levels of short-chain fatty acids (Extended Data Fig. 9q-w), including decreased levels of butyrate, which is thought to play a key role in dampening inflammation<sup>29</sup>. Emulsifiers also altered bile acid levels (Extended Data Fig. 9b'-t'), which influence microbiota composition<sup>24</sup>. However, in germ-free mice, emulsifiers did not alter bile acids, reduce mucus thickness nor alter penetrance of 0.5  $\mu$ m beads into the mucus (Extended Data Figs 9b'-t' and 10a-l), suggesting that these changes are not purely a direct effect of emulsifiers on mucus structures. Collectively, these data suggest that alterations in mucus in emulsifier-treated mice might result, at least in part, from altered gut microbiota composition but do not exclude direct effects on the host.

Transfer of microbiota from emulsifier-treated mice to germ-free mice (not exposed to emulsifiers) transferred microbiota encroachment, low-grade inflammation, increased adiposity and dysglycaemia (Fig. 4i-o and Extended Data Fig. 10s-y). Microbiota transplant from CMC-treated mice resulted in a longer-lasting increase in adiposity, paralleling that adiposity was less rapidly reversible following stoppage of CMC exposure (Extended Data Fig. 8a-k). Such transplanted phenotypes correlated with the acquisition of elevated levels of faecal LPS and flagellin

(Extended Data Fig. 10m–r) and altered microbiota composition (Extended Data Fig. 10z–e'). Thus, emulsifier-induced changes in the microbiota have a role in driving the inflammation and metabolic changes promoted by these food additives.

The last half-century has witnessed a steady increase in the consumption of food additives, many of which have not been carefully tested as they were given GRAS status at the time that government entities charged with regulating food safety were created and/or expanded. Moreover, the testing of food additives that has been performed has generally used animal models designed to detect acute toxicity and/or promotion of cancer. Our data suggest that such testing may be inadequate—a notion supported by the recent observation that artificial sweeteners induce dysglycaemia in humans<sup>30</sup>. More specifically, our data suggest that one ubiquitous class of food additives, namely emulsifiers, can disturb the host–microbiota relationship resulting in a microbiota with enhanced mucolytic and pro-inflammatory activity that promotes intestinal inflammation. Such chronic gut inflammation can manifest as colitis or metabolic syndrome. While additional studies will be needed to determine if CMC, P80 and/or other emulsifiers impact human health, our observations in mice suggest the possibility that dietary emulsifiers may have contributed to the post-mid-twentieth-century increase in incidence of inflammatory bowel disease, metabolic syndrome, and perhaps other chronic inflammatory diseases. Notably, this hypothesis does not dispute the commonly held assumption that excess caloric consumption is a predominant factor driving the metabolic syndrome epidemic. Rather, it suggests such hyperphagia may be driven, in part, by food additives and other factors that might alter gut microbiota and promote low-grade intestinal inflammation.

**Online Content** Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

Received 5 December 2013; accepted 14 January 2015.

Published online 25 February 2015.

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**Supplementary Information** is available in the online version of the paper.

**Acknowledgements** This work was supported by NIH grant DK099071 and DK083890. B.C. is a recipient of the Research Fellowship award from the Crohn's and Colitis Foundation of America (CCFA). We thank B. Zhang, L. Etienne-Mesmin, H. Q. Tran and E. Viennois for technical assistance.

**Author Contributions** B.C. and A.T.G. conceived the project, designed the experiments, interpreted the results, and wrote the manuscript. B.C. performed all experiments and analysis with advice and guidance from O.K., J.K.G., and A.C.P. S.S. and R.E.L. guided experimental design and data interpretation.

**Author Information** Sequencing data are deposited in the European Nucleotide Archive under accession number PRJEB8035. Reprints and permissions information is available at [www.nature.com/reprints](http://www.nature.com/reprints). The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to A.T.G. (agewirtz@gsu.edu).

**METHODS**

**Mice.** Wild-type C57BL/6 and *Il10<sup>-/-</sup>* mice were purchased from Jackson Laboratories. Swiss Webster mice were purchased from Taconic, Inc. *Tlr5<sup>-/-</sup>* mice were originally generated by S. Akira (Osaka University, Japan)<sup>31</sup> and backcrossed/maintained as previously described<sup>27</sup>. All mice were then bred and housed at Georgia State University, Atlanta, Georgia, USA under institutionally-approved protocols (Institutional Animal Care and Use Committee no. A14033). For wild-type mice, experiments were also performed on mice purchased directly from the aforementioned providers and used within 3 days of receipt. Mice were fed Purina rodent chow no. 5001, which is commonly used in many vivaria. Mice were weaned at 3 weeks of age and put into new cages (randomized to littermates) such that each experimental group contained mice from at least two different litters, and that each litter were used for at least three experimental groups. Experiments used either male or female mice (comparisons within a gender) except for Figs 1m-o and 2 which used both male and female mice. For littermate control experiments (Extended Data Fig 3), all the mice from 10 (Extended Data Fig. 3a-g) and 8 (Extended Data Fig. 3h-n) different litters (from distinct mothers) were used and placed into cages such that each litter was split equally amongst groups that were to receive water, CMC or P80 (three (Extended Data Fig. 3a-g) or two (Extended Data Fig. 3h-n) cages per sex per condition). For experiments using older mice (Extended Data Fig. 5a-p), mice were weaned at 3 weeks of age, put into new cages, and subjected to emulsifier treatment starting 3 months later.

**Materials.** Sodium carboxymethylcellulose (CMC, average  $M_w \sim 250,000$ ), polysorbate-80 (P80) and sodium sulfite were purchased from Sigma (Sigma, St. Louis, Missouri).

**Emulsifier agent treatment.** Mice were exposed to CMC, P80 or sodium sulfite diluted in drinking water (1.0% or indicated concentration) (not blinded). The same water (reverse-osmosis treated Atlanta city water) was used for the water-treated (control) group. These solutions were changed every week. Emulsifier solutions were autoclaved for experiments presented in Fig. 4a-h. When required, emulsifier solutions were replaced by water after 6 weeks of treatment for an additional 6 weeks. Body weights were measured every week and are expressed as a percentage compared to the initial body weight (day 0) defined as 100%. Mice developing colitis (LCN2 level  $\geq 500$  ng per g of faeces) were excluded for metabolic syndrome parameters analysis. Fresh faeces were collected every week for downstream analysis. After 3 months of emulsifier treatment, mice were fasted for 5 h, at which time blood was collected by retrobulbar intraorbital capillary plexus. Haemolysis-free serum was generated by centrifugation of blood using serum separator tubes (Becton Dickinson, Franklin Lakes, New Jersey). Mice were then euthanized and colon length, colon weight, spleen weight and adipose weight were measured. Organs were collected for downstream analysis. Incorporation of CMC and P80 into the diet was performed by Research Diets Inc. (New Brunswick, New Jersey) using Purina Rodent Chow diet no. 5001. These diets are now referenced by Research Diets as C13050701 and C13050702, respectively.

**Food intake measurement.** Groups of mice were placed in a clean cage with a known amount of food. Twenty-four hours later, the amount of remaining food was measured with the difference viewed as food intake per 24 h. Error bars represent s.e.m. of three measurements made one week apart.

**Overnight fasting blood glucose measurement.** Mice were placed in a clean cage and fasted for 15 h. Blood glucose concentration was then determined using a Nova Max Plus Glucose Meter and expressed in mg dl<sup>-1</sup>.

**Glucose and insulin tolerance test.** Wild-type and *Tlr5<sup>-/-</sup>* mice were treated with CMC or P80 diluted to 1% drinking water for 8 weeks. Following a 5-h fasting, baseline blood glucose levels were measured using a Nova Max Plus Glucose Meter and expressed in mg dl<sup>-1</sup>. Mice were then injected intraperitoneally with 2 g glucose per kg body weight in sterile PBS or with 0.5 U insulin per kg body weight (Sigma, St. Louis, Missouri), and blood glucose levels were measured 30, 60, 90 and 120 min after injection, as previously described<sup>27</sup>.

**In vivo epithelial barrier permeability.** *In vivo* assay of intestinal barrier function was performed using an FITC-labelled dextran method, as previously described<sup>32</sup>. Mice were deprived of food and water for 4 h, and were then gavaged with 15 mg of permeability tracer FITC-labelled dextran 4 kDa (FD4, Sigma, St. Louis, Missouri). Blood was collected retro-orbitally after 3 h, and fluorescence intensity was measured in the serum (excitation, 490 nm; emission, 520 nm; BIOTEK Fluorescence Spectrophotometer). FITC-dextran concentrations were determined using a standard curve generated by serial dilution of FITC-dextran in mice serum.

**Germ-free experiments.** Germ-free Swiss Webster mice were kept under germ-free conditions in a Park Bioservices isolator in our germ-free facility. CMC and P80 were diluted to 1% in water and then autoclaved for germ-free purpose. The same water was used for the water-treated (control) group. After 3 months of emulsifier agent treatment, mice were fasted for 5 h and then removed from the isolator to be euthanized. Samples were collected as previously described. For the analysis of mucus layer integrity, germ-free C57BL/6 mice were kept under germ-free conditions and

treated with CMC or P80 as described above. After 2 months of emulsifier agent treatment, mice were removed from the isolator, and inoculated with 0.5  $\mu\text{m}$  green fluorescent beads (Polysciences, Warrington, PA). Seven hours post-inoculation, mice were euthanized and colonic tissue was collected and placed in methanol-Carnoy's fixative solution (60% methanol, 30% chloroform, 10% glacial acetic acid), followed by immunostaining of mucins, as described below.

**Microbiota transplantation.** Caecal contents from detergent treated mice were suspended in 30% glycerol diluted in PBS (1.0 ml) and stocked at  $-80^{\circ}\text{C}$  until analysis. Germ-free Swiss Webster mice (4 weeks old) were removed from the isolator and were orally administered 200  $\mu\text{l}$  of faecal suspension made using glycerol stocks. Transplanted mice were then monitored as previously described.

**Colonic myeloperoxidase assay.** Neutrophil influx in tissue was analysed by assaying the enzymatic activity of myeloperoxidase, a marker for neutrophils. In brief, tissue ( $50 \text{ mg ml}^{-1}$ ) was thoroughly washed in PBS and homogenized in 0.5% hexadecyltrimethylammonium bromide (Sigma, St. Louis, Missouri) in 50 mM PBS (pH 6.0), freeze-thawed three times, sonicated and centrifuged. Myeloperoxidase was assayed in the clear supernatant by adding 1  $\text{mg ml}^{-1}$  of dianisidine dihydrochloride (Sigma, St. Louis, Missouri) and  $5 \times 10^{-4}\%$  H<sub>2</sub>O<sub>2</sub> and the change in optical density measured at 450 nm. Human neutrophil myeloperoxidase (Sigma, St. Louis, Missouri) was used as a standard. One unit of myeloperoxidase activity was defined as the amount that degraded 1.0  $\mu\text{mol}$  of peroxide per min at  $25^{\circ}\text{C}$ <sup>33</sup>.

**Haematoxylin and eosin staining and histopathologic analysis.** Following euthanasia, mouse colons and small intestines were fixed in 10% buffered formalin for 24 h at room temperature and then embedded in paraffin. Tissues were sectioned at 5  $\mu\text{m}$  thickness and stained with haematoxylin and eosin (H&E) using standard protocols. H&E-stained slides were scored as follows. Each colon was assigned four scores based on the degree of epithelial damage and inflammatory infiltrate in the mucosa, submucosa and muscularis/serosa, as previously described<sup>34</sup>. A slight modification was made to this scoring system<sup>23</sup>; each of the four scores was multiplied by 1 if the change was focal, 2 if it was patchy and 3 if it was diffuse. The four individual scores per colon were added, resulting in a total scoring range of 0–36 per mouse.

**Periodic acid-Schiff staining.** Following euthanasia, mouse colons were fixed in 10% buffered formalin for 24 h at room temperature and then embedded in paraffin. Tissues were sectioned at 5  $\mu\text{m}$  thickness, deparaffinized and oxidized for 5 min in 0.5% periodic acid solution (Sigma, St. Louis, Missouri). After rinsing, tissues were placed in Schiff reagent (Sigma, St. Louis, Missouri) for 15 min, washed in warm water, and counterstained using haematoxylin (Sigma, St. Louis, Missouri) for one minute.

**Immunostaining of mucins and localization of bacteria by fluorescent *in situ* hybridization.** Mucus immunostaining was paired with fluorescent *in situ* hybridization (FISH), as previously described<sup>11</sup>, in order to analyse bacteria localization at the surface of the intestinal mucosa. In brief, colonic tissues (proximal colon, second cm from the caecum) containing faecal material were placed in methanol-Carnoy's fixative solution (60% methanol, 30% chloroform, 10% glacial acetic acid) for a minimum of 3 h at room temperature. Tissue were then washed in methanol 2  $\times$  30 min, ethanol 2  $\times$  15 min, ethanol/xylene (1:1) 15 min and xylene 2  $\times$  15 min, followed by embedding in paraffin with a vertical orientation. Five- $\mu\text{m}$  sections were cut and dewaxed by preheating at  $60^{\circ}\text{C}$  for 10 min, followed by bathing in xylene at  $60^{\circ}\text{C}$  for 10 min, xylene at room temperature for 10 min and 99.5% ethanol for 10 min. The hybridization step was performed at  $50^{\circ}\text{C}$  overnight with an EUB338 probe (5'-GCTGCCTCCCGTAGGAGT-3', with a 5' Alexa 647 label) diluted to a final concentration of 10  $\mu\text{g ml}^{-1}$  in hybridization buffer (20 mM Tris-HCl, pH 7.4, 0.9 M NaCl, 0.1% SDS, 20% formamide). After washing for 10 min in wash buffer (20 mM Tris-HCl, pH 7.4, 0.9 M NaCl) and 3  $\times$  10 min in PBS, a PAP pen (Sigma, St. Louis, Missouri) was used to mark around the section and block solution (5% FBS in PBS) was added for 30 min at  $4^{\circ}\text{C}$ . Mucin 2 primary antibody (rabbit H-300, Santa Cruz Biotechnology) was diluted to 1:1500 in block solution and applied overnight at  $4^{\circ}\text{C}$ . After washing 3  $\times$  10 min in PBS, block solution containing anti-rabbit Alexa 488 secondary antibody diluted to 1:1500, Phalloidin-Tetramethylrhodamine B isothiocyanate (Sigma, St. Louis, Missouri) at 1  $\mu\text{g ml}^{-1}$  and Hoechst 33258 (Sigma, St. Louis, Missouri) at 10  $\mu\text{g ml}^{-1}$  was applied to the section for 2 h. After washing 3  $\times$  10 min in PBS slides were mounted using Prolong anti-fade mounting media (Life Technologies). Observations were performed with a Zeiss LSM 700 confocal microscope with software Zen 2011 version 7.1. This software was used to determine the distance between bacteria and the epithelial cell monolayer, as well as the mucus thickness.

**Quantification of faecal LCN2 by ELISA and determination of colitis incidence.** For quantification of faecal LCN2 by ELISA, frozen faecal samples were reconstituted in PBS containing 0.1% Tween 20 to a final concentration of 100  $\text{mg ml}^{-1}$  and vortexed for 20 min to produce a homogenous faecal suspension<sup>23</sup>. These samples were then centrifuged for 10 min at 14,000g and  $4^{\circ}\text{C}$ . Clear supernatants were collected and stored at  $-20^{\circ}\text{C}$  until analysis. LCN2 levels were estimated in the

supernatants using Duoset murine LCN2 ELISA kit (R&D Systems, Minneapolis, Minnesota) using the colourimetric peroxidase substrate tetramethylbenzidine, and optical density was read at 450 nm (Versamax microplate reader). For determination of colitis incidence, a faecal LCN2 level  $\geq 500$  ng per g of faeces was used to determine colitic mice.

**Faecal flagellin and LPS load quantification.** We quantified flagellin and LPS as previously described<sup>21</sup> using human embryonic kidney (HEK)-Blue-mTLR5 and HEK-Blue-mTLR4 cells, respectively (Invivogen, San Diego, California). We resuspended faecal material in PBS to a final concentration of 100 mg ml<sup>-1</sup> and homogenized for 10 s using a Mini-Beadbeater-24 without the addition of beads to avoid bacteria disruption. We then centrifuged the samples at 8000g for 2 min and serially diluted the resulting supernatant and applied to mammalian cells. Purified *Escherichia coli* flagellin and LPS (Sigma, St Louis, Missouri) were used for standard curve determination using HEK-Blue-mTLR5 and HEK-Blue-mTLR4 cells, respectively. After 24 h of stimulation, we applied cell culture supernatant to QUANTI-Blue medium (Invivogen, San Diego, California) and measured alkaline phosphatase activity at 620 nm after 30 min.

**Serum flagellin- and LPS-specific immunoglobulins.** Flagellin- and LPS-specific IgG levels were quantified by ELISA, as previously described<sup>22,35</sup>. Microtitre plates were coated overnight with purified *E. coli* flagellin (100 ng per well) or LPS (1  $\mu$ g per well). Serum samples diluted 1:500 were then applied. After incubation and washing, wells were incubated with anti-mouse IgG. Quantification was performed using the colourimetric peroxidase substrate tetramethylbenzidine, as described above. Data are reported as optical density corrected by subtracting background (determined by readings in samples lacking serum).

**Gene expression analysis by quantitative reverse-transcription PCR (qRT-PCR).** Total RNAs were isolated from colonic tissues using TRIzol (Invitrogen, Carlsbad, California) according to the manufacturer's instructions. Quantitative RT-PCR was performed using the iScriptTM One-Step RT-PCR Kit with SYBR Green (Bio-Rad, Hercules, California) in a CFX96 apparatus (Bio-Rad, Hercules, California) with specific mouse oligonucleotides. The oligonucleotides used were: 36B4 (sense) 5'-TCCAGGCTTGGGCATCA-3' and (antisense) 5'-CTTTATTCTAGCTGCA CATCACTCAGA-3', Tff3 (sense) 5'-CCTGGTTGCTGGGTCTCTGG-3' and (antisense) 5'-GTCTCCTGCAGAGGTTGAAGC-3', Klf4F (sense) 5'-TGTGA CTATGCAGGCTGTGG-3' and (antisense) 5'-AGTCGCTGGTCAGTTCATC G-3'. Results were normalized to the housekeeping 36B4 gene.

**Bacterial quantification by qPCR.** For quantification of total faecal bacterial load, total bacterial DNA was isolated from weighted faeces using QIAamp DNA Stool Mini Kit (Qiagen). DNA was then subjected to quantitative PCR using QuantiFast SYBR Green PCR kit (Biorad) with universal 16S rRNA primers 8F: 5'-AGAGTTT GATCCTGGCTCAG-3' and 338R: 5'-CTGCTGCCCTCCGTAGGAGT-3' to measure total bacteria<sup>36</sup>. Results are expressed as bacteria number per mg of stool, using a standard curve. For quantification of mucosa-associated bacteria, total DNA was isolated from PBS-washed and weighted colonic tissue using DNeasy Blood & Tissue Kit (Qiagen). DNA was then subjected to quantitative PCR as described above, and results are expressed as bacteria number per mg of colonic tissue, using a standard curve.

**Faecal microbiota analysis by 16S rRNA gene sequencing using Illumina technology.** 16S rRNA gene amplification and sequencing were done using the Illumina MiSeq technology following the protocol of Earth Microbiome Project with their modifications to the MO BIO PowerSoil DNA Isolation Kit procedure for extracting DNA (<http://www.earthmicrobiome.org/emp-standard-protocols>)<sup>37,38</sup>. Bulk DNA were extracted from frozen extruded faeces using a PowerSoil-htp kit from MO BIO Laboratories (Carlsbad, California) with mechanical disruption (bead beating). The 16S rRNA genes, region V4, were PCR amplified from each sample using a composite forward primer and a reverse primer containing a unique 12-base barcode, designed using the Golay error-correcting scheme, which was used to tag PCR products from respective samples<sup>38</sup>. We used the forward primer 515F 5'-A ATGATACGGCGACCACGGATCTACACTATGTTAATTGTGTGCCAGCM GCCCGGGTAA-3'; the italicized sequence is the 5' Illumina adaptor B, the bold sequence is the primer pad, the italicized and bold sequence is the primer linker and the underlined sequence is the conserved bacterial primer 515F. The reverse primer 806R used was 5'-CAAGCAGAACGGCATACGAGAT XXXXXXXX XXXX AGTCAGTCAG CC GGACTACHVGGGTWTCTAAT-3'; the italicized sequence is the 3' reverse complement sequence of Illumina adaptor, the 12 X sequence is the Golay barcode, the bold sequence is the primer pad, the italicized and bold sequence is the primer linker and the underlined sequence is the conserved bacterial primer 806R. PCR reactions consisted of Hot Master PCR mix (Five Prime), 0.2  $\mu$ M of each primer, 10–100 ng template, and reaction conditions were 3 min at 95 °C, followed by 30 cycles of 45 s at 95 °C, 60 s at 50 °C and 90 s at 72 °C on a Biorad thermocycler. Four independent PCRs were performed for each sample, combined, purified with Ampure magnetic purification beads (Agencourt), and products were visualized by gel electrophoresis. Products were then quantified

(BIOTEK Fluorescence Spectrophotometer) using a Quant-iT PicoGreen dsDNA assay. A master DNA pool was generated from the purified products in equimolar ratios. The pooled products were quantified using a Quant-iT PicoGreen dsDNA assay and then sequenced using an Illumina MiSeq sequencer (paired-end reads, 2  $\times$  250 base pairs) at Cornell University, Ithaca.

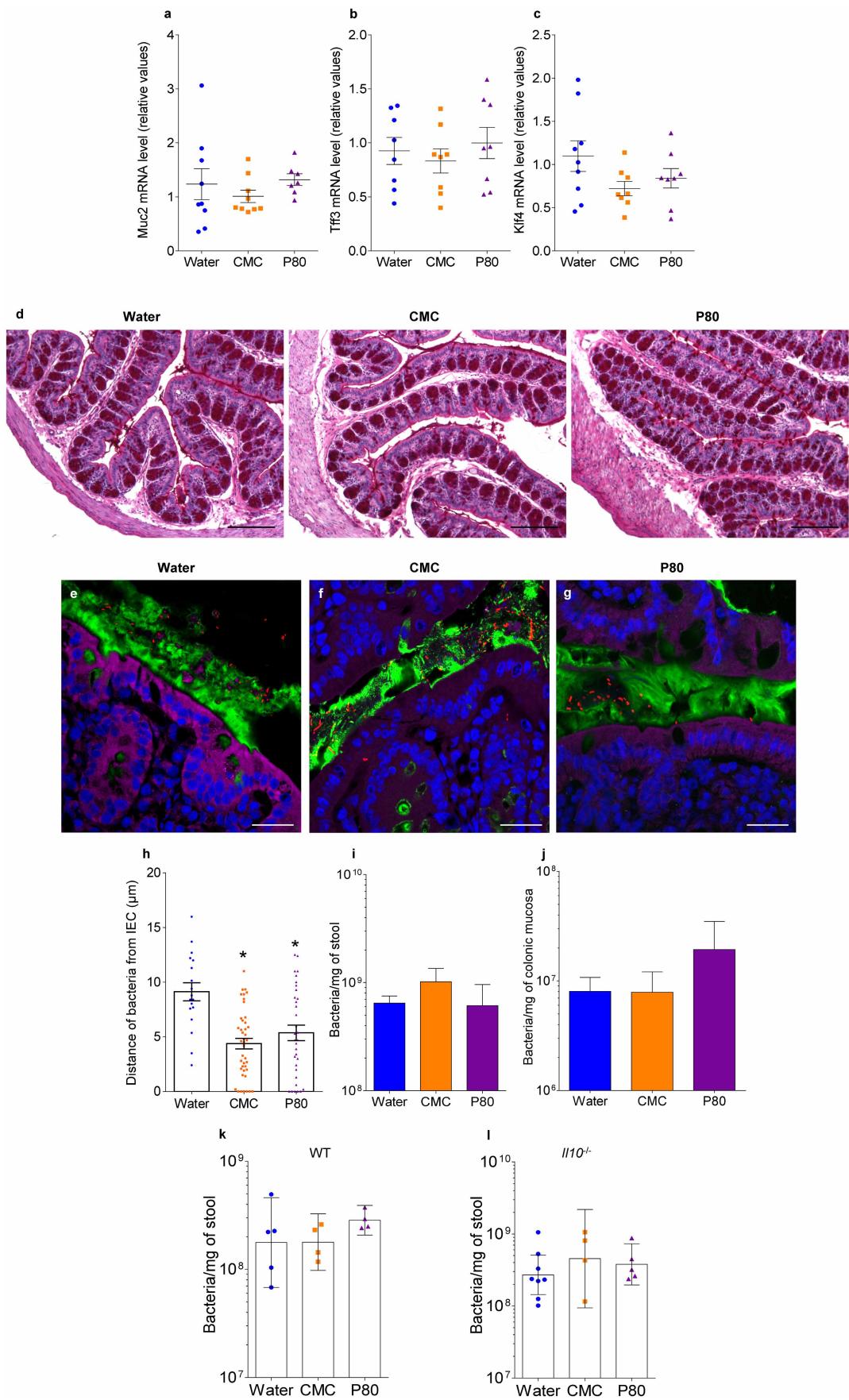
**16S rRNA gene sequence analysis.** The sequences were demultiplexed, quality filtered using the Quantitative Insights Into Microbial Ecology (QIIME, version 1.8.0) software package<sup>39</sup>, and forward and reverse Illumina reads were joined using the fastq-join method (<http://code.google.com/p/ea-utils>)<sup>40</sup>. We used QIIME default parameters for quality filtering (reads truncated at first low-quality base and excluded if: (1) there were more than three consecutive low quality base calls; (2) less than 75% of read length was consecutive high quality base calls; (3) at least one uncalled base was present; (4) more than 1.5 errors were present in the barcode; (5) any Phred qualities were below 20; or (6) the length was less than 75 bases). Sequences were assigned to OTUs using the UCLUST algorithm<sup>41</sup> with a 97% threshold of pairwise identity (without the creation of new clusters with sequences that do not match the reference sequences), and classified taxonomically using the Greengenes reference database<sup>42</sup>. A single representative sequence for each OTU was aligned and a phylogenetic tree was built using FastTree<sup>43</sup>. The phylogenetic tree was used for computing the unweighted UniFrac distances between samples<sup>12,44</sup>, rarefaction were performed (3,500–20,000 sequences per sample) and used to compare abundances of OTUs across samples. Principal coordinates analysis (PCoA) plots were used to assess the variation between experimental group (beta diversity) and jackknifed beta diversity was used to estimate the uncertainty in PCoA plots. In addition to using PCoA, samples were clustered using UPGMA (unweighted pair group method with arithmetic mean). Alpha diversity curves were determined for all samples using the determination of the number of observed species, and the Shannon diversity index was used to characterize species diversity in a community. Sequencing data are deposited in the European Nucleotide Archive under accession number PRJEB8035.

**Short-chain fatty acids and bile acids composition analysis.** Faecal samples were used to analyse short-chain fatty acids and bile acid composition at the Metabolomics Core of the University of Michigan (supported by grant U24 DK097153 of NIH Common Funds Project to the University of Michigan).

**Statistical analysis.** All replicates in this study were biological; that is, repeat experiments with additional mice. The D'Agostino-Pearson omnibus test was used to verify that all data were normally distributed. Significance was determined using t-tests, one-way ANOVA corrected for multiple comparisons with a Sidak test, two-way ANOVA corrected for multiple comparisons with a Bonferroni test, or two-way group ANOVA (GraphPad Prism software, version 6.01). Differences were noted as significant at  $P \leq 0.05$ . A 'nearest-shrunken centroid' classification approach was performed to detect the OTUs that were particularly representative of each experimental group<sup>45</sup>. The amount of shrinkage was set to minimize the misclassification error. When OTUs were used to classify samples, accuracy of the classification was perfect (overall error rate = 0). These analyses allowed the identification of the 15 OTUs whose abundances were the most significantly different between experimental groups, which were then used for heat map generation and sample clustering. These analyses were performed using the prediction analysis for micro-arrays (PAM) package within R software<sup>45</sup>. No statistical methods were used to predetermine sample size.

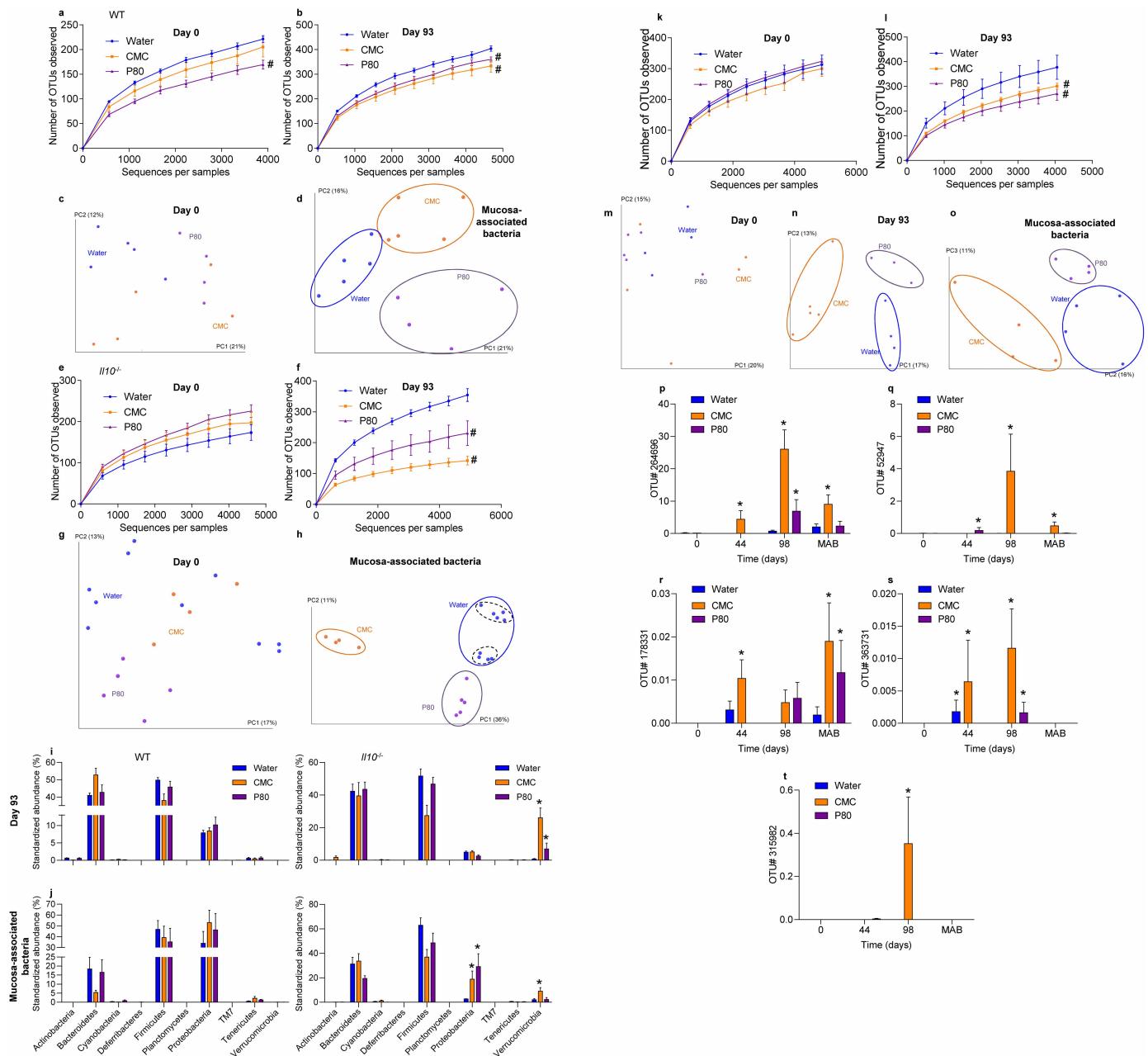
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**Extended Data Figure 1 | Effects of emulsifiers on mucus-microbiota interaction in wild-type, *Il10*<sup>-/-</sup> and *Tlr5*<sup>-/-</sup> mice.** **a–d**, Dietary emulsifiers did not affect mucus and mucus-related genes expression in wild-type mice. Wild-type (WT) mice were exposed to drinking water containing CMC or P80 (1.0%) for 12 weeks. **a–c**, mRNA expression analysis by qRT-PCR of *Muc2* (**a**), *Tff3* (**b**) and *Klf4* (**c**) genes in the colonic mucosa. Points are from individual mice, bar represent the mean  $\pm$  s.e.m., ( $n = 9$ ). **d**, Colons were stained using periodic acid-Schiff stains. Scale bar, 200  $\mu$ m. Pictures are representative of 10 biological replicates. **e–j**, Dietary emulsifiers alter microbiota localization, composition and pro-inflammatory potential in *Tlr5*<sup>-/-</sup> mice. *Tlr5*<sup>-/-</sup> mice were exposed to drinking water containing CMC or P80 (1.0%) for 12 weeks. **e–g**, Confocal microscopy analysis of microbiota localization: MUC2, green; actin, purple; bacteria, red; and DNA, blue.

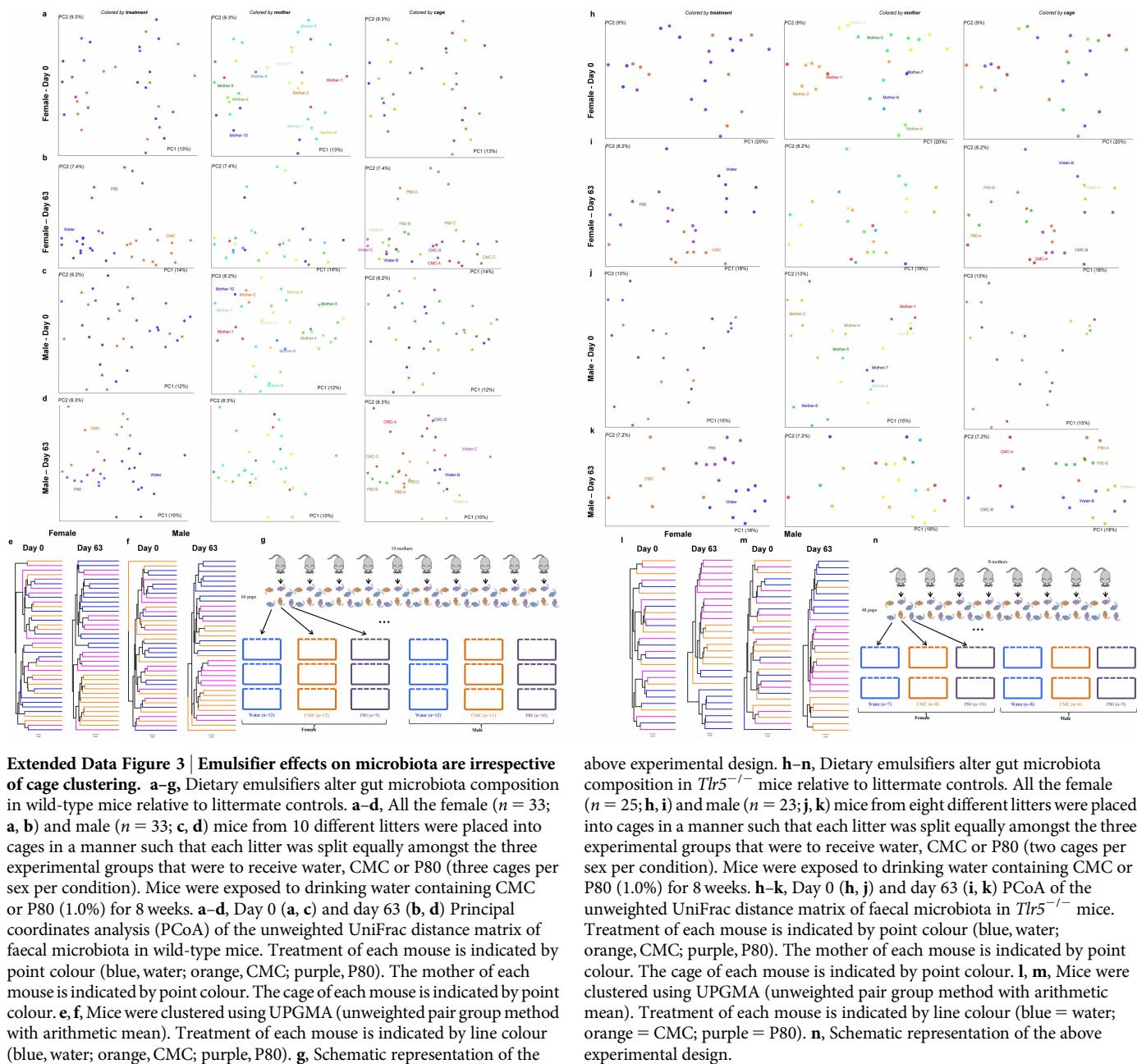
Scale bar, 20  $\mu$ m. Pictures are representative of five biological replicates. **h**, Distances of closest bacteria to intestinal epithelial cells (IEC) per condition over five high-powered fields per mouse. **i, j**, PCR-based quantification of total bacterial load (**i**) and bacterial load adhered to colonic mucosa (**j**). Data are the means  $\pm$  s.e.m.,  $n = 5$ . Significance was determined using one-way ANOVA corrected for multiple comparisons with a Sidak test; \* $P < 0.05$  compared to water-treated group. **k, l**, Dietary emulsifiers do not modify total bacterial load in wild-type and *Il10*<sup>-/-</sup> mice. Wild-type and *Il10*<sup>-/-</sup> mice were exposed to drinking water containing CMC or P80 (1.0%) for 12 weeks. Total bacterial load in stool of wild-type (**k**) and *Il10*<sup>-/-</sup> (**l**) mice. Points are from individual mice. Data are geometric means with 95% confidence interval ( $n = 5$  for **k** except  $n = 4$  for CMC- and P80-treated groups; for **l**,  $n = 8, 4$  and 6 for water-, CMC- and P80-treated groups, respectively).



### Extended Data Figure 2 | Emulsifiers alter microbiota composition.

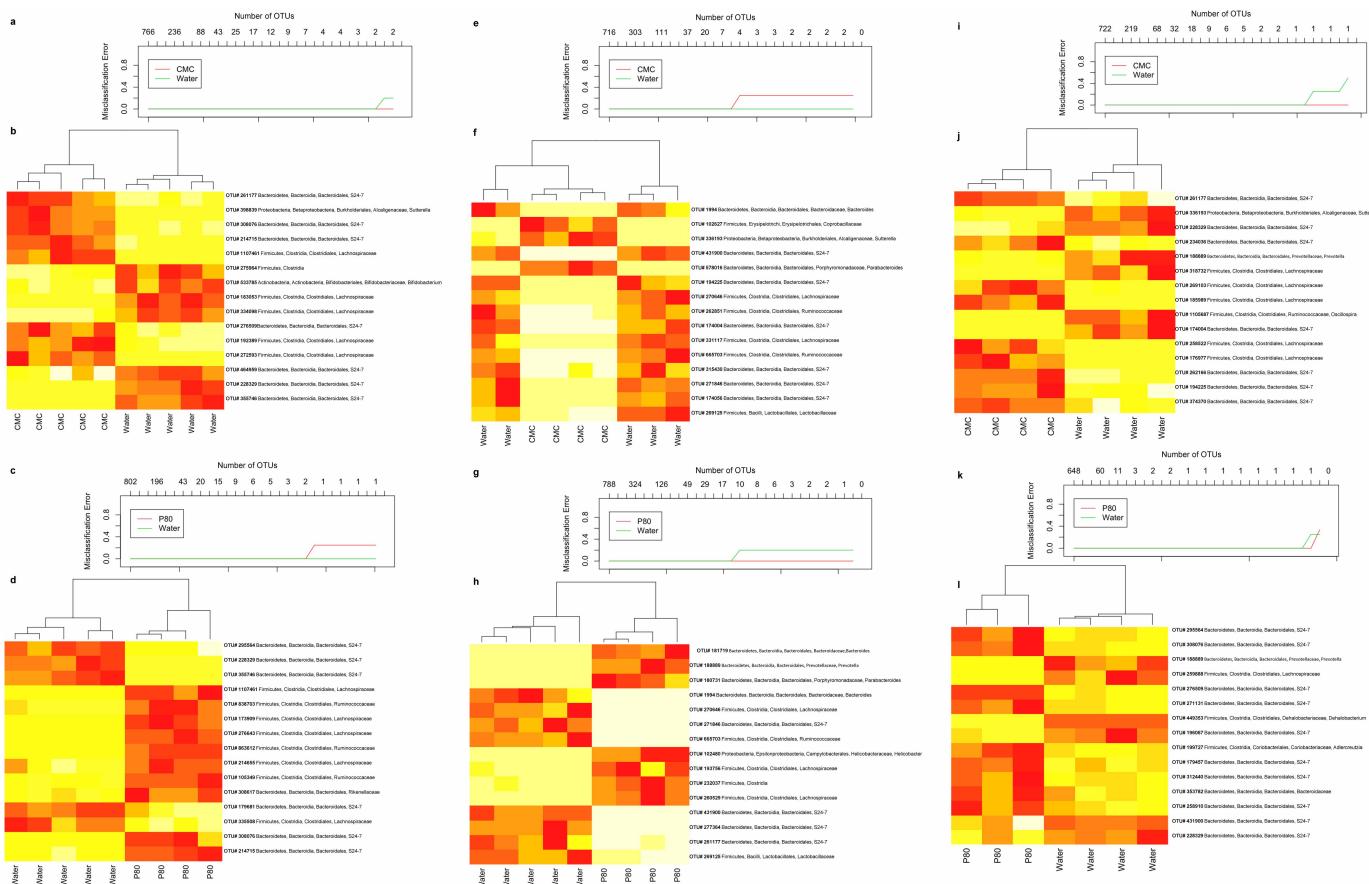
**a-h.** Dietary emulsifiers induce profound alterations in gut microbiota composition in wild-type and *IL10<sup>-/-</sup>* mice. Wild-type and *IL10<sup>-/-</sup>* mice were exposed to drinking water containing CMC or P80 (1.0%) for 12 weeks. **a, b, e, f.** Day 0 (**a, b**) and day 93 (**e, f**) microbiota richness and diversity in wild-type (**a, b**) and *IL10<sup>-/-</sup>* (**e, f**). **c, d, g, h.** Principal coordinates analysis (PCoA) of the unweighted UniFrac distance matrix of faecal microbiota (**c, g**) and mucosa-associated bacteria (**d, h**) in wild-type (**c, d**) and *IL10<sup>-/-</sup>* (**g, h**) mice. Treatment of each mouse is indicated by point colour and matching coloured circles represent clustering by treatment (blue, water; orange, CMC; purple, P80). Black dashed circles represent mice sharing a cage. Data are the means  $\pm$  s.e.m.;  $n = 5$ , except  $n = 4$  for P80-treated wild-type mice;  $n = 4$  for CMC-treated *IL10<sup>-/-</sup>* mice; and  $n = 9$  for water-treated *IL10<sup>-/-</sup>* mice. Significance was determined using two-way group ANOVA ( $\#P < 0.05$ ) compared to the water-treated group. **i, j.** Phylum characterization of emulsifier-induced alteration of gut microbiota composition in wild-type and *IL10<sup>-/-</sup>* mice. Wild-type and *IL10<sup>-/-</sup>* mice were exposed to drinking water containing CMC or P80 (1.0%) for 12 weeks. Relative abundance of phyla are represented for faecal microbiota at day 93 (**i**) and for colonic mucosa-associated bacteria (**j**). Data are the means  $\pm$  s.e.m.,  $n = 5$ . Significance was determined using two-way ANOVA corrected for multiple comparisons with a

Bonferroni test,  $*P < 0.05$  compared to water-treated group. **k-o.** Dietary emulsifiers induce profound alterations in gut microbiota composition in *Thrb<sup>-/-</sup>* mice. *Thrb<sup>-/-</sup>* mice were exposed to drinking water containing CMC or P80 (1.0%) for 12 weeks. **k, l.** Day 0 (**k**) and day 93 (**l**) microbiota richness and diversity ( $n = 5$ ). **m-o.** PCoA of the unweighted UniFrac distance matrix of faecal microbiota at day 0 (**m**), day 93 (**n**) and of mucosa-associated bacteria (**o**). Data are the means  $\pm$  s.e.m. (for **m**,  $n = 4, 5$  and 5 for water-, CMC- and P80-treated groups, respectively; for **n**,  $n = 4, 5$  and 3 for water-, CMC- and P80-treated groups, respectively; for **o**,  $n = 4$ ). Significance was determined using two-way group ANOVA ( $\#P < 0.05$ ) compared to the water-treated group. **p-t.** Prevalence analysis of OTUs related to mucolytic bacteria in *IL10<sup>-/-</sup>* mice treated with dietary emulsifier. *IL10<sup>-/-</sup>* mice were exposed to drinking water containing CMC or P80 (1.0%) for 12 weeks. OTUs Prok\_MSA # 52947 (**p**; related to *Clostridium perfringens*), 264696 (**q**; related to *Akkermansia muciniphila*), 315982 (**r**; related to *Clostridium perfringens*), 363731 (**s**; related to *Akkermansia muciniphila*), and 178331 (**t**; related to *Akkermansia muciniphila*) were analysed. Data are expressed as a percentage of the total sequences analysed and are the means  $\pm$  s.e.m. ( $n = 6$ , except  $n = 9$  for water-treated *IL10<sup>-/-</sup>* mice). Significance was determined using two-way ANOVA corrected for multiple comparisons with a Bonferroni test,  $*P < 0.05$  compared to water-treated group.

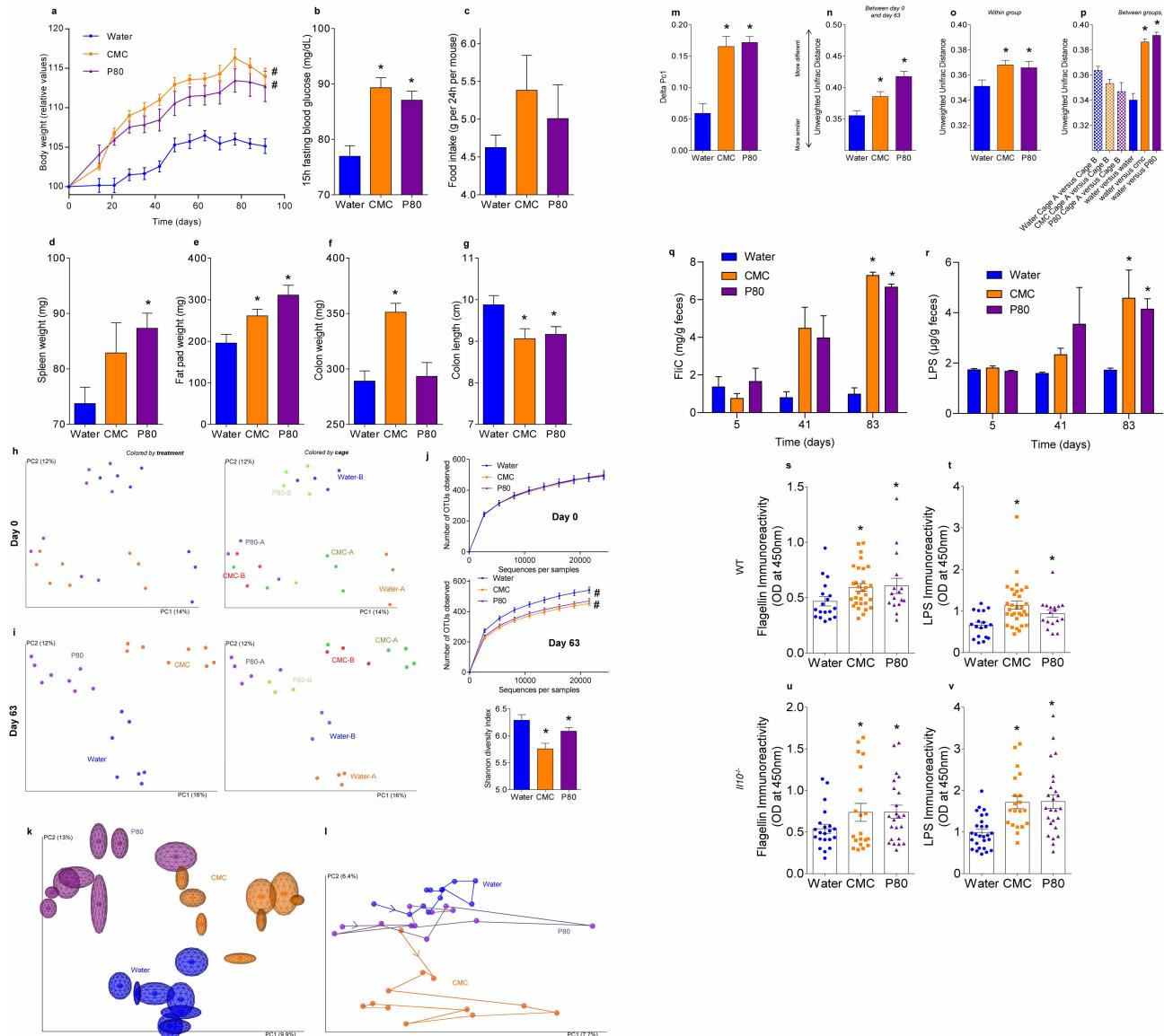


**Extended Data Figure 3 | Emulsifier effects on microbiota are irrespective of cage clustering.** **a–g.** Dietary emulsifiers alter gut microbiota composition in wild-type mice relative to littermate controls. **a–d.** All the female ( $n = 33$ ; **a, b**) and male ( $n = 33$ ; **c, d**) mice from 10 different litters were placed into cages in a manner such that each litter was split equally amongst the three experimental groups that were to receive water, CMC or P80 (three cages per sex per condition). Mice were exposed to drinking water containing CMC or P80 (1.0%) for 8 weeks. **a–d.** Day 0 (**a, c**) and day 63 (**b, d**) Principal coordinates analysis (PCoA) of the unweighted UniFrac distance matrix of faecal microbiota in wild-type mice. Treatment of each mouse is indicated by point colour (blue, water; orange, CMC; purple, P80). The mother of each mouse is indicated by point colour. The cage of each mouse is indicated by point colour. **e, f.** Mice were clustered using UPGMA (unweighted pair group method with arithmetic mean). Treatment of each mouse is indicated by line colour (blue, water; orange, CMC; purple, P80). **g.** Schematic representation of the

above experimental design. **h–n.** Dietary emulsifiers alter gut microbiota composition in *Tlr5*<sup>-/-</sup> mice relative to littermate controls. All the female ( $n = 25$ ; **h, i**) and male ( $n = 23$ ; **j, k**) mice from eight different litters were placed into cages in a manner such that each litter was split equally amongst the three experimental groups that were to receive water, CMC or P80 (two cages per sex per condition). Mice were exposed to drinking water containing CMC or P80 (1.0%) for 8 weeks. **h–k.** Day 0 (**h, j**) and day 63 (**i, k**) PCoA of the unweighted UniFrac distance matrix of faecal microbiota in *Tlr5*<sup>-/-</sup> mice. Treatment of each mouse is indicated by point colour (blue, water; orange, CMC; purple, P80). The mother of each mouse is indicated by point colour. The cage of each mouse is indicated by point colour. **l, m.** Mice were clustered using UPGMA (unweighted pair group method with arithmetic mean). Treatment of each mouse is indicated by line colour (blue = water; orange = CMC; purple = P80). **n.** Schematic representation of the above experimental design.

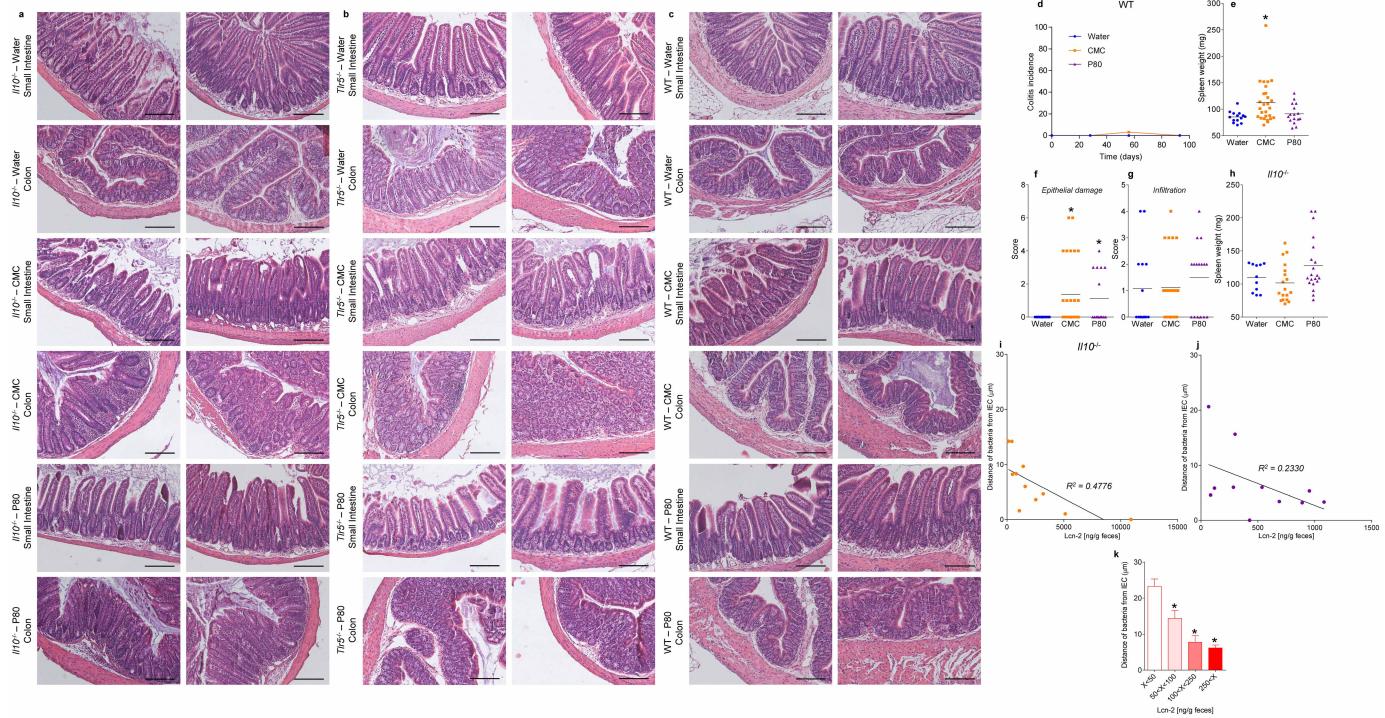


overrepresented, respectively). The 15 OTUs are listed on the right using their Greengenes Prok\_MSA identities, and assigned taxonomy are labelled starting phylum, then class, order, family and genus. Dendrogram on the upper part represents sample clustering. **e-h**, As for **a-d** with *Il10*<sup>-/-</sup> mice. **i-l**, As for **a-d** with *Tlr5*<sup>-/-</sup> mice. For **a, b**, *n* = 5; for **c, d**, *n* = 5 and 4 for water- and P80-treated groups, respectively; for **e, f**, *n* = 5 and 4 for water- and CMC-treated groups, respectively; for **g, h**, *n* = 5 and 4 for water- and P80-treated groups, respectively; for **i, j**, *n* = 4; for **k, l**, *n* = 4 and 3 for water- and P80-treated groups, respectively.



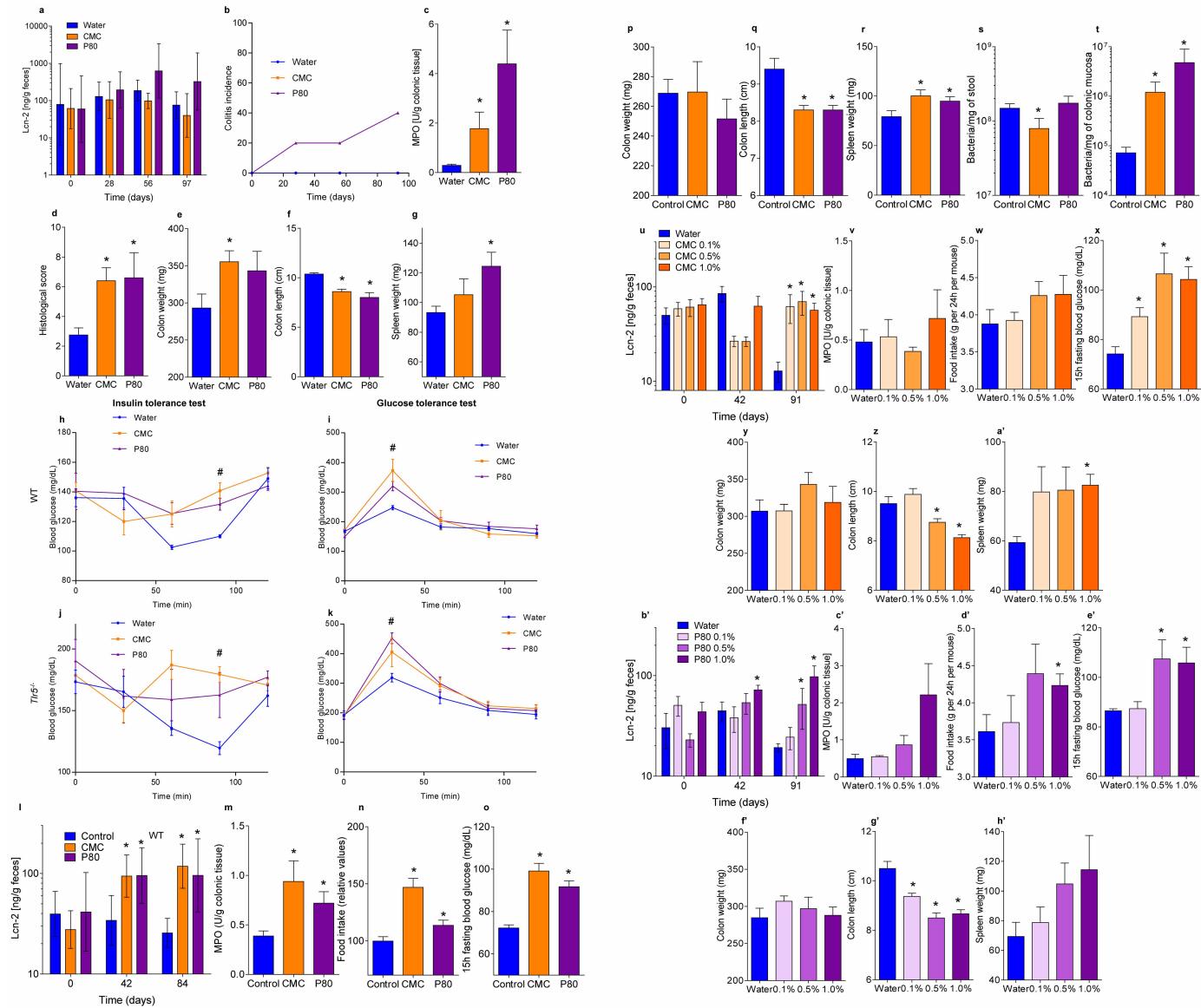
**Extended Data Figure 5 | Emulsifier-induced changes in adult mouse microbiota.** **a–g**, Dietary emulsifiers promote metabolic syndrome in adult wild-type mice. **a–g**, Four-month-old male wild-type (WT) mice were exposed to drinking water containing CMC or P80 (1.0%) for 8 weeks (two cages per condition). **a**, Body weight over time; **b**, 15 h fasting blood glucose concentration; **c**, food intake measurement; **d**, spleen weights; **e**, fat-pad weights; **f**, colon weights; and **g**, colon lengths. Data are the means  $\pm$  s.e.m.,  $n = 10$ . Significance was determined using one-way ANOVA corrected for multiple comparisons with a Sidak test (\* $P < 0.05$  compared to water-treated group) or two-way group ANOVA (# $P < 0.05$  compared to water-treated group). **h–p**, Dietary emulsifiers alter gut microbiota composition in adult wild-type mice. Four-month-old male wild-type mice were exposed to drinking water containing CMC or P80 (1.0%) for 8 weeks (two cages per condition). **h, i**, Day 0 (**h**) and day 63 (**i**) Principal coordinates analysis (PCoA) of the unweighted UniFrac distance matrix of faecal microbiota in wild-type mice. Treatment of each mouse is indicated by point colour (blue, water; orange, CMC; purple, P80). The cage of each mouse is indicated by point colour (for **h**,  $n = 7$ , 8 and 8 for water-, CMC- and P80-treated groups, respectively). **j**, Day 0 and day 63 microbiota richness and diversity. **k**, Day 63 jackknifed PCoA of the unweighted UniFrac distance matrix of faecal microbiota in wild-type mice. Treatment of each mouse is indicated by point colour (blue, water; orange, CMC; purple, P80) ( $n = 8$ ). **l**, After clustering of mouse faecal microbiota using PCoA of the unweighted UniFrac distance matrix, a representative mouse has been used to illustrate the time point evolution of the microbiota ( $n = 1$ ). **m**, After clustering of mouse faecal microbiota using PCoA of the unweighted UniFrac distance matrix, evolution of the principal coordinate 1 between day 0 and day 63 has been calculated for

each mouse ( $n = 10$ ). **n**, Average of the UniFrac unweighted distance for each group (water, CMC and P80) between day 0 and day 63 has been calculated ( $n = 10$ ). **o**, Average of the UniFrac unweighted distance within group (water, CMC and P80) has been calculated ( $n = 10$ ). **p**, Average of the UniFrac unweighted distance between group (cages, water, CMC and P80) or within group (water) at day 63 has been calculated ( $n = 10$ ). Significance was determined using one-way ANOVA corrected for multiple comparisons with a Sidak test (\* $P < 0.05$  compared to water-treated group) or two-way group ANOVA (# $P < 0.05$  compared to water-treated group). **q, r**, Dietary emulsifiers increase pro-inflammatory potential of intestinal microbiota in  $Tlr5^{-/-}$  mice.  $Tlr5^{-/-}$  mice were exposed to drinking water containing CMC or P80 (1.0%) for 12 weeks. Bioactive levels of faecal flagellin (**q**) and LPS (**r**) assayed with TLR5 and TLR4 reporter cells. Data are the means  $\pm$  s.e.m.,  $n = 10$ . Significance was determined using two-way ANOVA corrected for multiple comparisons with a Bonferroni test; \* $P < 0.05$  compared to water-treated group. **s–v**, Dietary emulsifiers increase serum immune reactivity. Wild-type (**s, t**) and  $Il10^{-/-}$  (**u, v**) mice were exposed to drinking water containing CMC or P80 (1.0%) for 12 weeks. Serum immune reactivity (IgG) to flagellin (**s, u**) and LPS (**t, v**) in wild-type (**s, t**) and  $Il10^{-/-}$  (**u, v**) mice. Points are from individual mice. Data are the means  $\pm$  s.e.m. (for **s**,  $n = 18$ , 30 and 16 for water-, CMC- and P80-treated groups, respectively; for **t**,  $n = 18$ , 31 and 17 for water-, CMC- and P80-treated groups, respectively; for **u**,  $n = 21$ , 20 and 23 for water-, CMC- and P80-treated groups, respectively; for **v**,  $n = 27$ , 20 and 25 for water-, CMC- and P80-treated groups, respectively). Significance was determined using one-way ANOVA corrected for multiple comparisons with a Sidak test; \* $P < 0.05$  compared to water-treated group.



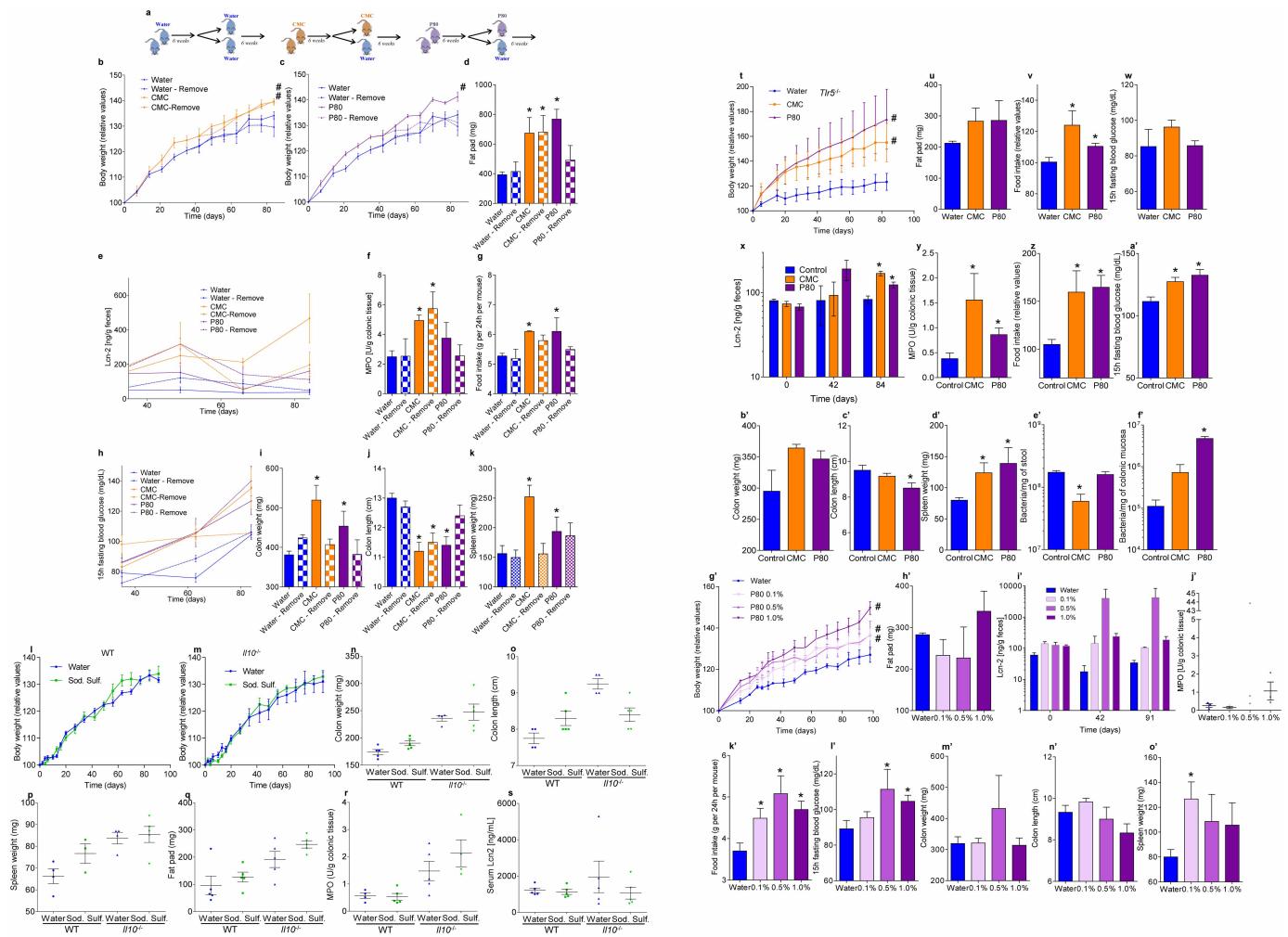
**Extended Data Figure 6 | Histopathologic changes in emulsifier-treated wild-type and *II10*<sup>-/-</sup> mice.** **a**, Dietary emulsifiers induce histopathologically robust inflammation in *II10*<sup>-/-</sup> mice. **a**, *II10*<sup>-/-</sup> mice were exposed to drinking water containing CMC or P80 (1.0%) for 12 weeks. Colon and small intestine were haematoxylin and eosin (H&E) stained. Scale bar, 200 μm. Pictures are representatives of 15 biological replicates. **b**, Dietary emulsifiers induce histopathologically robust inflammation in *Tlr5*<sup>-/-</sup> mice. *Tlr5*<sup>-/-</sup> mice were exposed to drinking water containing CMC or P80 (1.0%) for 12 weeks. Colon and small intestine were H&E stained. Scale bar, 200 μm. Pictures are representative of five biological replicates. **c**, Histopathology of emulsifier-treated wild-type (WT) mice. Wild-type mice were exposed to drinking water containing CMC or P80 (1.0%) for 12 weeks. Colon and small intestine were H&E stained. Scale bar, 200 μm. Pictures are representative of 15 biological replicates. **d–h**, Dietary emulsifiers elicit low-grade intestinal inflammation in WT and splenomegaly in *II10*<sup>-/-</sup> mice. Wild-type (**d–g**) and *II10*<sup>-/-</sup> (**h**) mice were exposed to drinking water containing CMC or P80 (1.0%) for 12 weeks. **d**, Colitis incidence over time; **e**, **h**, spleen weights; **f**, epithelial damage; and **g**, infiltration scores. Points are from individual mice, bars represent the mean. For **e–g**,  $n = 14, 27$  and  $16$  for water-, CMC- and P80-treated groups,

respectively; for **h**,  $n = 11, 18$  and  $20$  for water-, CMC- and P80-treated groups, respectively. Significance was determined using one-way ANOVA corrected for multiple comparisons with a Sidak test; \* $P < 0.05$  compared to water-treated group. **i–k**, Extent of intestinal inflammation correlates with perturbation in microbiota localization in wild-type and *II10*<sup>-/-</sup> mice. *II10*<sup>-/-</sup> mice were exposed to drinking water containing CMC (**i**) or P80 (**j**) (1.0%) for 12 weeks. Faecal levels of the inflammatory marker LCN2 as well as confocal microscopy analysis of microbiota localization and estimation of the distances of the closest bacteria to intestinal epithelial cells (IEC) were determined, and plotted in the  $x$  and  $y$  axis, respectively. Linear regression line was calculated and  $R^2$  was determined;  $n = 11$ . **k**, Analysis of bacterial–epithelial distance upon stratification of levels of gut inflammatory marker faecal LCN2, using both wild-type and *II10*<sup>-/-</sup> mice exposed to drinking water containing CMC or P80 (1.0%) for 12 weeks. Mice were grouped according to their faecal LCN2 levels and bacterial–epithelial distances were then plotted (mean  $\pm$  s.e.m.). Significance was determined using one-way ANOVA corrected for multiple comparisons with a Sidak test; \* $P < 0.05$  compared to  $X < 50$  ng per g group.



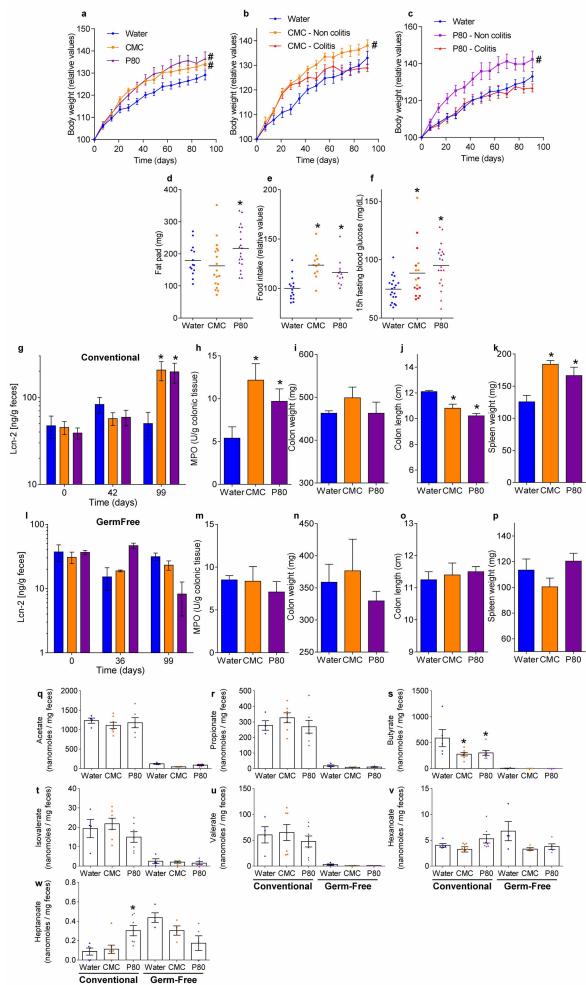
**Extended Data Figure 7 | Inflammatory and metabolic parameters in emulsifier-treated wild-type and *Tlr5<sup>-/-</sup>* mice.** **a-g**, Dietary emulsifiers promote intestinal inflammation in *Tlr5<sup>-/-</sup>* mice. **a-g**, *Tlr5<sup>-/-</sup>* mice were exposed to drinking water containing CMC or P80 (1.0%) for 12 weeks. **a**, Faecal levels of the inflammatory marker LCN2 over time; **b**, colitis incidence over time; **c**, myeloperoxidase levels; **d**, histological score; **e**, colon weights; **f**, colon lengths; and **g**, spleen weights. Data are the means  $\pm$  s.e.m. or geometric means with 95% confidence interval (the latter for a),  $n = 5$ . Significance was determined using one-way ANOVA corrected for multiple comparisons with a Sidak test or using two-way ANOVA corrected for multiple comparisons with a Bonferroni test; \* $P < 0.05$  compared to water-treated group. **h-k**, Dietary emulsifiers induce metabolic syndrome in wild-type (WT) and *Tlr5<sup>-/-</sup>* mice. Wild-type (**h-i**) and *Tlr5<sup>-/-</sup>* (**j-k**) mice were exposed to drinking water containing CMC or P80 (1.0%) for 12 weeks. Glucose tolerance (**h-j**) and insulin sensitivity (**i-k**) were analysed. Data are the means  $\pm$  s.e.m.,  $n = 5$ . Significance was determined using two-way group ANOVA (# $P < 0.05$ ) compared to water-treated group. **l-t**, Emulsifier-supplemented chow elicits low-grade intestinal inflammation in wild-type mice. Wild-type mice were given mouse chow containing CMC or P80 (1.0%) for 12 weeks. **l**, Faecal levels of the inflammatory marker LCN2 over time; **m**, myeloperoxidase levels; **n**, food intake measurement; **o**, 15 h fasting blood glucose concentration; **p**, colon weights; **q**, colon lengths; **r**, spleen weights; and **s, t**, PCR-based quantification of total bacterial load (**s**) and bacterial load adhered to colonic mucosa (**t**). Data are the means  $\pm$  s.e.m. or geometric means with 95% confidence interval (the latter for l),  $n = 5$ . Significance was determined using one-way ANOVA corrected for multiple comparisons with a Sidak test or using two-way ANOVA corrected for multiple comparisons with a Bonferroni test; \* $P < 0.05$  compared to control group. **u-h'**, Dose-response characterization of dietary emulsifiers on intestinal inflammation. Wild-type mice were exposed to drinking water containing 0.1–1.0% CMC (**u-a'**) or P80 (**b'-h'**) for 12 weeks. **u, b'**, Faecal levels of the inflammatory marker LCN2 over time; **v, c'**, myeloperoxidase levels; **w, d'**, food intake measurement; **x, e'**, 15 h fasting blood glucose concentration; **y, f'**, colon weights; **z, g'**, colon lengths; and **a', h'**, spleen weights. Data are the means  $\pm$  s.e.m.,  $n = 5$ . Significance was determined using one-way ANOVA corrected for multiple comparisons with a Sidak test or using two-way ANOVA corrected for multiple comparisons with a Bonferroni test; \* $P < 0.05$  compared to water-treated group.

of the inflammatory marker LCN2 over time; **m**, myeloperoxidase levels; **n**, food intake measurement; **o**, 15 h fasting blood glucose concentration; **p**, colon weights; **q**, colon lengths; **r**, spleen weights; and **s, t**, PCR-based quantification of total bacterial load (**s**) and bacterial load adhered to colonic mucosa (**t**). Data are the means  $\pm$  s.e.m. or geometric means with 95% confidence interval (the latter for l),  $n = 5$ . Significance was determined using one-way ANOVA corrected for multiple comparisons with a Sidak test or using two-way ANOVA corrected for multiple comparisons with a Bonferroni test; \* $P < 0.05$  compared to control group. **u-h'**, Dose-response characterization of dietary emulsifiers on intestinal inflammation. Wild-type mice were exposed to drinking water containing 0.1–1.0% CMC (**u-a'**) or P80 (**b'-h'**) for 12 weeks. **u, b'**, Faecal levels of the inflammatory marker LCN2 over time; **v, c'**, myeloperoxidase levels; **w, d'**, food intake measurement; **x, e'**, 15 h fasting blood glucose concentration; **y, f'**, colon weights; **z, g'**, colon lengths; and **a', h'**, spleen weights. Data are the means  $\pm$  s.e.m.,  $n = 5$ . Significance was determined using one-way ANOVA corrected for multiple comparisons with a Sidak test or using two-way ANOVA corrected for multiple comparisons with a Bonferroni test; \* $P < 0.05$  compared to water-treated group.

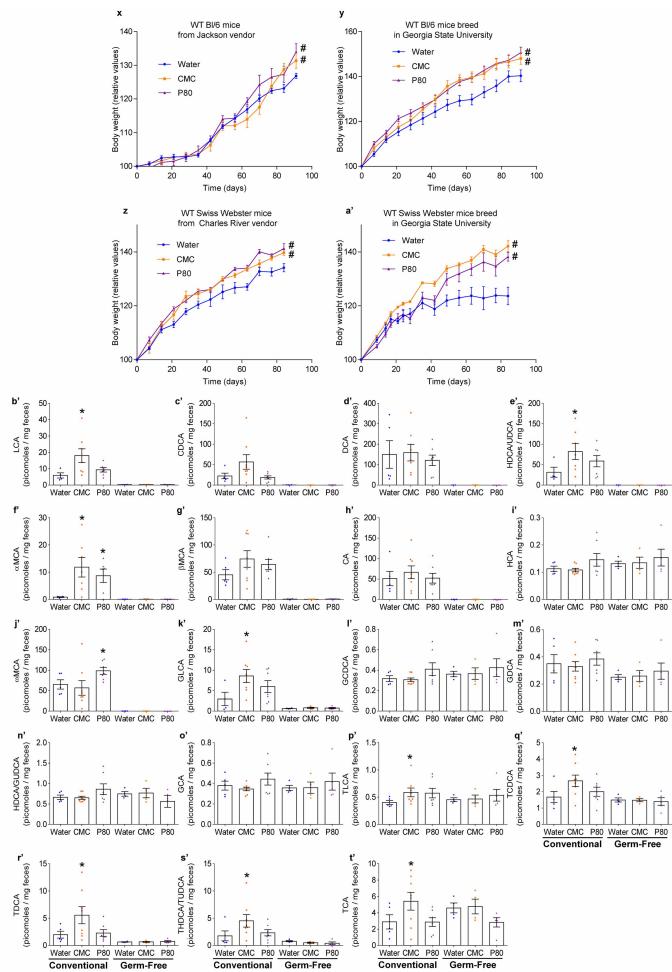


**Extended Data Figure 8 | Reversibility and dose dependence of emulsifier-induced effects on inflammation and metabolism.** **a–k**, Emulsifier-induced metabolic syndrome in Swiss Webster mice is partially reversible by 6 weeks after emulsifier treatment. **a**, Schematic representation of the experiment. **b, c**, Body weight over time; **d**, fat-pad weight; **e**, faecal levels of the inflammatory marker LCN2 over time; **f**, myeloperoxidase levels; **g**, food intake measurement; **h**, 15 h fasting blood glucose concentration; **i**, colon weights; **j**, colon lengths; and **k**, spleen weights. Data are the means  $\pm$  s.e.m.,  $n = 5$ . Significance was determined using one-way ANOVA corrected for multiple comparisons with a Sidak test (\* $P < 0.05$  compared to water-treated group) or two-way group ANOVA (# $P < 0.05$  compared to water-treated group). **l–s**, Sodium sulfite did not induce robust or low-grade intestinal inflammation. Wild-type and *Il10*<sup>-/-</sup> mice were exposed to drinking water containing sodium sulfite (1.0%) for 12 weeks. **l, m**, Body weight over time; **n**, colon weights; **o**, colon lengths; **p**, spleen weights; **q**, fat-pad weight; **r**, myeloperoxidase levels; and **s**, serum levels of the inflammatory marker LCN2. Data are the means  $\pm$  s.e.m.,  $n = 5$ . Points are from individual mice. Significance was determined using one-way ANOVA corrected for multiple comparisons with a Sidak test. **t–w**, Dietary emulsifiers promote metabolic syndrome in *Tlr5*<sup>-/-</sup> mice. *Tlr5*<sup>-/-</sup> mice were exposed to drinking water containing CMC or P80 (1.0%) for 12 weeks. **t**, Body weight over time; **u**, fat-pad weight; **v**, food intake measurement; and **w**, 15 h fasting blood glucose concentration. Data are the means  $\pm$  s.e.m.,  $n = 5$ . Significance was determined using one-way

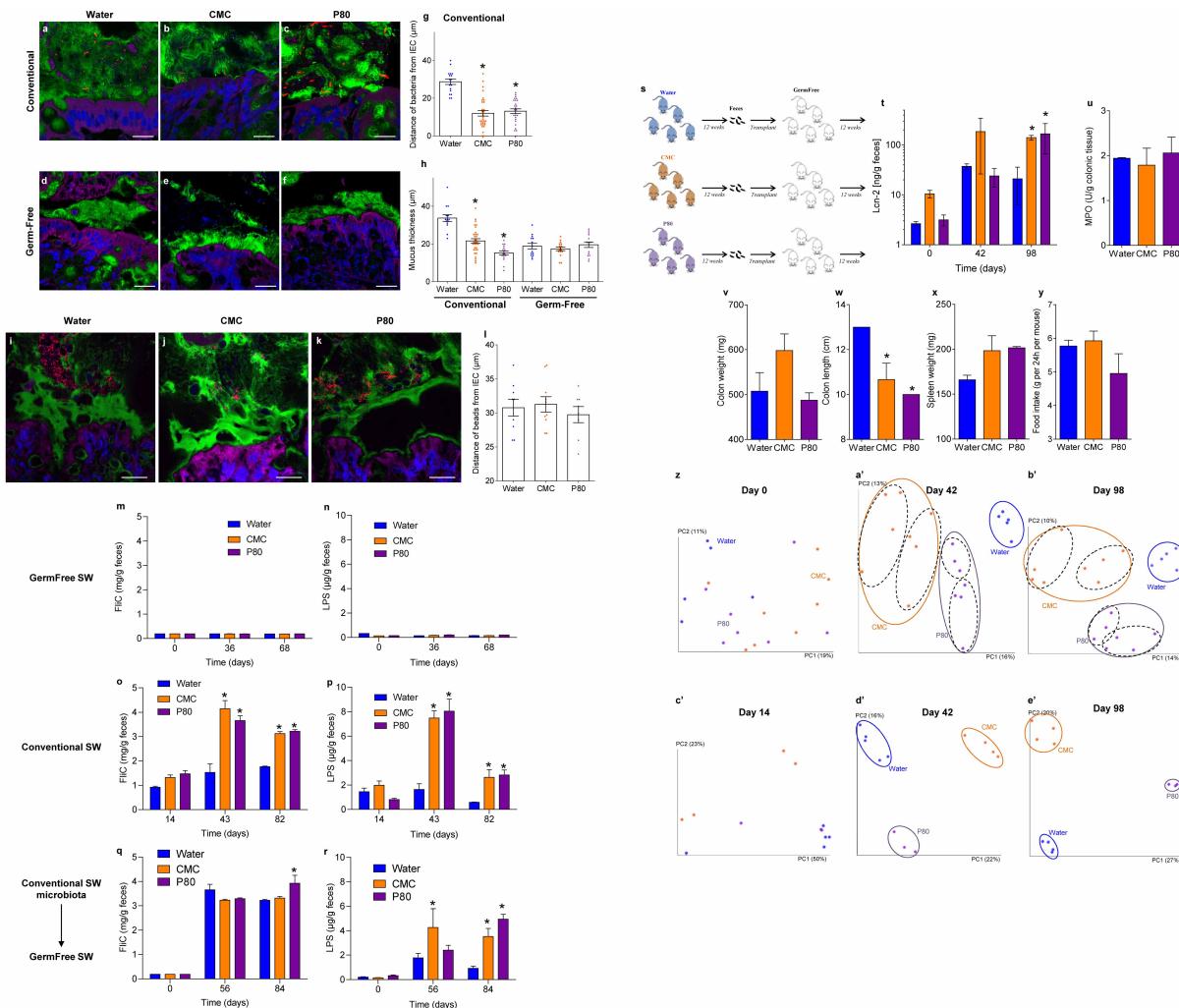
ANOVA corrected for multiple comparisons with a Sidak test (\* $P < 0.05$  compared to water-treated group) or two-way group ANOVA (# $P < 0.05$  compared to water-treated group). **x–f'**, Emulsifier-supplemented chow promotes intestinal inflammation in *Tlr5*<sup>-/-</sup> mice. *Tlr5*<sup>-/-</sup> mice were given mouse chow containing CMC or P80 (1.0%) for 12 weeks. **x**, Faecal levels of the inflammatory marker LCN2 over time; **y**, myeloperoxidase levels; **z**, food intake measurement; **a'**, 15 h fasting blood glucose concentration; **b'**, colon weights; **c'**, colon lengths; **d'**, spleen weights; and **e', f'**, PCR-based quantification of total bacterial load (**e'**) and bacterial load adhered to colonic mucosa (**f'**). Data are the means  $\pm$  s.e.m.,  $n = 5$ . Significance was determined using one-way ANOVA corrected for multiple comparisons with a Sidak test or using two-way ANOVA corrected for multiple comparisons with a Bonferroni test; \* $P < 0.05$  compared to control group. **g'–o'**, Dose-response characterization of dietary emulsifiers on intestinal inflammation in *Tlr5*<sup>-/-</sup> mice. *Tlr5*<sup>-/-</sup> mice were exposed to drinking water containing 0.1–1.0% P80 for 12 weeks. **g'**, Body weight over time; **h'**, fat-pad weight; **i'**, faecal levels of the inflammatory marker LCN2 over time; **j'**, myeloperoxidase levels; **k'**, food intake measurement; **l'**, 15 h fasting blood glucose concentration; **m'**, colon weights; **n'**, colon lengths; and **o'**, spleen weights. Data are the means  $\pm$  s.e.m.,  $n = 3$ . Points in **d** are from individual mice. Significance was determined using one-way ANOVA corrected for multiple comparisons with a Sidak test (\* $P < 0.05$  compared to water-treated group) or two-way group ANOVA (# $P < 0.05$  compared to water-treated group).



**Extended Data Figure 9 | Effects of emulsifiers on metabolic parameters and bile acids in conventional and germ-free mice.** **a–f**, Dietary emulsifiers promotes metabolic syndrome in *Il10<sup>-/-</sup>* mice. *Il10<sup>-/-</sup>* mice were exposed to drinking water containing CMC or P80 (1.0%) for 12 weeks. **a–c**, Body weight over time; **d**, fat-pad weight; **e**, food intake measurement; and **f**, 15 h fasting blood glucose concentration. Data are the means  $\pm$  s.e.m. (for **a**,  $n = 24, 18$  and  $21$  for water-, CMC- and P80-treated groups, respectively; for **b**,  $n = 14, 11$  and  $8$  for water-, CMC- and P80-treated groups, respectively; for **c**,  $n = 14, 9$  and  $9$  for water-, CMC- and P80-treated groups, respectively; for **d**,  $n = 14, 18$  and  $20$  for water-, CMC- and P80-treated groups, respectively; for **e**,  $n = 15, 11$  and  $12$  for water-, CMC- and P80-treated groups, respectively; for **f**,  $n = 21, 17$  and  $20$  for water-, CMC- and P80-treated groups, respectively). Significance was determined using one-way ANOVA corrected for multiple comparisons with a Sidak test ( $*P < 0.05$  compared to water-treated group) or two-way group ANOVA (# $P < 0.05$  compared to water-treated group). Points are from individual mice and red points in **f** represent mice with overt colitis. **g–p**, Emulsifier-induced low-grade intestinal inflammation was abolished under germ-free conditions. Conventionally housed (**g–k**) and germ-free (**l–p**) Swiss Webster mice were exposed to drinking water containing CMC or P80 (1.0%) for 12 weeks. **g, l**, Faecal levels of the inflammatory marker LCN2 over time; **h, m**, myeloperoxidase levels; **i, n**, colon weights; **j, o**, colon lengths; and **k, p**, spleen weights. Data are the means  $\pm$  s.e.m.;  $n = 8$  for conventionally housed mice and  $n = 4$  for germ-free mice. Significance was determined using one-way ANOVA corrected for multiple comparisons with a Sidak test or using two-way ANOVA corrected for multiple comparisons with a Bonferroni test,  $*P < 0.05$  compared to control group. **q–w**, Dietary emulsifiers induce perturbations in faecal short-chain fatty acid composition. Faecal short-chain fatty acids composition was analysed at the Metabolomics Core of the University of Michigan. Acetate (**q**), propionate (**r**), butyrate (**s**), isovalerate (**t**), valerate (**u**), hexanoate (**v**) and heptanoate (**w**) were analysed. Data are the means  $\pm$  s.e.m. ( $n = 5, 8$  and  $7$  for water-, CMC- and P80-treated conventional mice groups, respectively;  $n = 4, 4$  and  $5$  for water-, CMC- and P80-treated germ-free mice groups, respectively). Significance was



determined using one-way ANOVA corrected for multiple comparisons with a Sidak test; \* $P < 0.05$  compared to water-treated group. x-a', Dietary emulsifiers promote metabolic syndrome in mice from different vendors. Wild-type mice were exposed to drinking water containing CMC or P80 (1.0%) for 12 weeks. x, y, Body weight over time of Bl/6 mice used upon receipt from Jackson Laboratories (x) or bred at Georgia State University (y). z, a', Body weight over time of Swiss Webster mice used upon receipt from Charles River company (z) or bred at Georgia State University (a'). Data in x are not used elsewhere in report, while data in y, z, and a' are from Figs 3a and 4a and Extended Data Fig 8a-k. Data are the means  $\pm$  s.e.m. n = 8 for Bl/6 mice used upon receipt from Jackson Laboratories, n = 16 for Bl/6 mice bred at Georgia State University, n = 10 for Swiss Webster mice used upon receipt from Charles River company, n = 8 for Swiss Webster mice bred at Georgia State University. Significance was determined using two-way group ANOVA (# $P < 0.05$  compared to water-treated group). b'-t', Dietary emulsifiers induce perturbations in faecal bile acids composition. Faecal bile acids composition was analysed at the Metabolomics Core of the University of Michigan. Lithocholic acid (LCA; b'), chenodeoxycholic acid (CDCA; c'), deoxycholic acid (DCA; d'), hyodeoxycholic acid/ursodeoxycholic acid (HDCA/UDCA; e'),  $\alpha$ -muricholic acid ( $\alpha$ -MCA; f'),  $\beta$ -muricholic acid ( $\beta$ -MCA; g'), cholic acid (CA; h'), hyocholic acid (HCA; i'),  $\omega$ -muricholic acid ( $\omega$ -MCA; j'), glycolithocholic acid (GLCA; k'), glycochenodeoxycholic acid (GCDCA; l'), glycdeoxycholic acid (GDCA; m'), hyodeoxycholic acid/glycoursodeoxycholic acid (HDCA/GUDCA; n'), glycocholic acid (GCA; o'), taurolithocholic acid (TLCA; p'), taurine-conjugated chenodeoxycholic acid (TCDCA; q'), taurodeoxycholic acid/taurusodeoxycholic acid (TDCA/TUDCA; r'), taurohyodeoxycholic acid (s'), and taurocholic acid (TCA; t') were analysed. Data are the means  $\pm$  s.e.m. (n = 5, 8 and 7 for water-, CMC- and P80-treated conventional mice groups, respectively; n = 4, 4 and 5 for water-, CMC- and P80-treated germ-free mice groups, respectively). Significance was determined using one-way ANOVA corrected for multiple comparisons with a Sidak test; \* $P < 0.05$  compared to water-treated group.



**Extended Data Figure 10 | Faecal transplants transfer some effects of emulsifiers.** **a–h**, Dietary emulsifiers do not alter mucus thickness under germ-free conditions. **a–h**, Conventionally-housed (**a–c**, **g–h**) and germ-free Swiss Webster (SW; **d–f**, **h**) mice were exposed to drinking water containing CMC or P80 (1.0%) for 12 weeks. **a–f**, Confocal microscopy analysis of microbiota localization: MUC2, green; actin, purple; bacteria, red; and DNA, blue. Scale bar, 20  $\mu$ m. **g**, Distances of closest bacteria to intestinal epithelial cells (IEC) per condition over five high-powered fields per mouse. Pictures are representative of five biological replicates. **h**, Mucus thickness over five high-powered fields per mouse. Data are the means  $\pm$  s.e.m.,  $n = 5$ . Significance was determined using one-way ANOVA corrected for multiple comparisons with a Sidak test; \* $P < 0.05$  compared to water-treated group. **i–l**, Dietary emulsifiers do not induce drastic perturbations of mucus layer integrity under germ-free conditions. Germ-free Swiss Webster mice were exposed to drinking water containing CMC or P80 (1.0%) for 8 weeks. Mice were removed from the isolator, inoculated with 0.5  $\mu$ m green fluorescent beads (Polysciences, Warrington, Pennsylvania), and euthanized 7 h post-inoculation. **i–k**, Confocal microscopy analysis of fluorescent beads localization: MUC2, green; actin, purple; fluorescent beads, red; and DNA, blue. Scale bar, 20  $\mu$ m. **l**, Distances of closest fluorescent beads to intestinal epithelial cells per condition over five high-powered fields per mouse. Pictures are representatives of five biological replicates. Data are the means  $\pm$  s.e.m.,  $n = 4$ . Significance was determined using one-way ANOVA corrected for multiple comparisons with a Sidak test. **m–r**, Dietary emulsifiers increase pro-inflammatory potential of intestinal microbiota in Swiss Webster mice, transferable to germ-free mice recipients. **m, n**, Germ-free Swiss Webster mice were exposed to drinking water containing CMC or P80 (1.0%) for 12 weeks. Bioactive levels of faecal flagellin (**m**) and LPS (**n**) were assayed with TLR5 and TLR4 reporter cells. **o, p**, Swiss Webster mice were exposed to drinking water containing CMC or P80 (1.0%) for 12 weeks. Bioactive levels of faecal flagellin (**o**) and LPS (**p**) were assayed with TLR5 and TLR4 reporter cells. **q, r**, Germ-free Swiss Webster mice were

conventionalized via microbiota transplant from the Swiss Webster mice treated with emulsifiers described above. Bioactive levels of faecal flagellin (**q**) and LPS (**r**) were assayed with TLR5 and TLR4 reporter cells. Data are the means  $\pm$  s.e.m.  $n = 4$  for germ-free mice,  $n = 8$  for conventionally housed mice and  $n = 5$  for conventionalized mice. Significance was determined using two-way ANOVA corrected for multiple comparisons with a Bonferroni test; \* $P < 0.05$  compared to control group. **s–y**, Microbiota transplant transfers emulsifier-induced low-grade intestinal inflammation. Germ-free Swiss Webster mice were conventionalized via microbiota transplant from mice that received standard drinking water or drinking water containing CMC or P80 (1.0%). **s**, Schematic representation of the experiment. **t**, Faecal levels of the inflammatory marker LCN2 over time; **u**, myeloperoxidase levels; **v**, colon weights; **w**, colon lengths; **x**, spleen weights; and **y**, food intake measurement. Data are the means  $\pm$  s.e.m.,  $n = 4$ . Significance was determined using one-way ANOVA corrected for multiple comparisons with a Sidak test or using two-way ANOVA corrected for multiple comparisons with a Bonferroni test; \* $P < 0.05$  compared to control group. **z–e'**, Dietary emulsifiers induce profound alterations in gut microbiota composition in Swiss Webster mice, transferable to germ-free mice recipients. **z–b'**, Swiss Webster mice were exposed to drinking water containing CMC or P80 (1.0%) for 12 weeks. Principal coordinates analysis (PCoA) of the unweighted UniFrac distance matrix of faecal microbiota at day 0 (**z**), day 42 (**a'**) and day 98 (**b'**). **c'–e'**, Germ-free Swiss Webster mice were conventionalized via microbiota transplant from the Swiss Webster mice treated with emulsifiers described above. PCoA of the unweighted UniFrac distances of faecal microbiota at day 14 (**c'**), day 42 (**d'**) and day 98 (**e'**) post-transplant. For **z–b'**,  $n = 5, 8$  and 7 for water-, CMC- and P80-treated groups, respectively; for **c'–e'**,  $n = 5, 4$  and 3 for water-, CMC- and P80-treated groups, respectively. Treatment of each mouse is indicated by point colour and matching coloured circles indicate mice receiving the same treatment (blue, water; orange, CMC; purple, P80). Black dashed circles represent mice sharing a cage.