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Excess calorie intake early in life increases susceptibility to colitis in adulthood

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Epidemiological data reveal an association between obesity and inflammatory bowel disease (IBD). Furthermore, animal models demonstrate that maternal high-fat diet (HFD) and maternal obesity increase susceptibility to IBD in offspring. Here we report that excess calorie intake by neonatal mice, as a consequence of maternal HFD, forced feeding of neonates or low litter competition, leads to an increase during weaning in intestinal permeability, expression of pro-inflammatory cytokines and hydrogen sulfide production by the microbiota. These intestinal changes engage in mutual positive feedback that imprints increased susceptibility to colitis in adults. The pathological imprinting is prevented by the neutralization of IFN-γ and TNF-α or the production of hydrogen sulfide, or by normalization of intestinal permeability during weaning. We propose that excess calorie intake by neonates leads to multiple causally linked perturbations in the intestine that imprint the individual with long-term susceptibility to IBD.

Obesity, in particular childhood obesity, is a risk factor for the development of metabolic disorders and associated pathologies, such as atherosclerosis, hypertension, type 2 diabetes and fatty liver disease[1,2]. Obesity is also associated with IBD[3–6], and animal models demonstrate that maternal HFD and maternal obesity increase susceptibility to IBD in the offspring[7–11]. Given the increase in the prevalence of obesity worldwide, the identification of the mechanisms by which mother or child obesity favours the development of inflammatory pathologies may offer new targets and preventive measures against IBD.

Potential mechanisms that translate maternal obesity or excess calorie intake early in life into increased susceptibility to obesity, metabolic disorders and inflammatory pathologies later in life include transfer of feeding behaviour[12], transfer and development of an altered (dysbiotic) microbiota[13–16] and epigenetic modifications of genes involved in the control of inflammation[17,18]. Mothers with obesity or IBD transfer a dysbiotic microbiota to offspring and thereby increase offspring susceptibility to similar pathologies[14,15,19]. Furthermore, perturbations in the composition of early-life gut microbiota favours subsequent development of paediatric obesity and IBD[13,20,21]. In contrast, exposure to complex microbiota before weaning decreases the susceptibility to allergic inflammation later in life[18,22–24]. Finally, in a murine model of intestinal allergy mediated by invariant natural killer T (iNKT) cells, the gut-colonizing microbiota modifies the epigenetic code of *Cxcl16*, a chemokine that recruits iNKT cells[18].

We have recently reported that during weaning, the expanding microbiota induces a vigorous immune reaction termed the weaning reaction[25]. The weaning reaction is required for the development of a 'healthy' immune system, as inhibition of this reaction leads to increased susceptibility to inflammatory pathology, such as colitis, later in life. However, the impact of early-life diet on the immune system at weaning, and the consequences for later susceptibility to inflammatory pathology, remain to be assessed.

Here, we use three distinct murine models of excess calorie intake early in life to decipher how it increases the severity of IBD in adulthood. Offspring that were exposed, until weaning, to maternal HFD, increased access to maternal feeding or direct feeding with coconut oil developed higher intestinal permeability, expression of pro-inflammatory cytokines and bacterial H_2S production during weaning. Such intestinal perturbations promoted long-term susceptibility to chemically induced colitis, a susceptibility that could be normalized through inhibition of each perturbation independently. Our results demonstrate that an intestinal pro-inflammatory feedback loop triggered during weaning by excess calorie intake and microbiota induces a long-lasting pathological imprinting. These data suggest that there are early-life preventive measures against inflammatory pathologies later in life, which are based on diet and microbiota control.

Results

Excess calorie intake early in life increases susceptibility to colitis in adulthood. In order to assess the impact of excess calorie intake early in life on the susceptibility to colitis later in life, we used three distinct models of neonatal overfeeding that lead to overweight during weaning. Mothers feeding the pups were given a HFD (Fig. 1a), neonates were given a gavage of a preparation of coconut oil two times per day (Fig. 1b) or litters were reduced to three pups (control litters had seven pups; thus, pups in the smaller litters had more access to maternal milk) (Fig. 1c). All three conditions led to overweight of the male pups (Fig. 1d–f), characterized by increased fat depots (Extended Data. Fig. 1a–c). The early overweight was transient, and all groups of mice returned to normal body weight as early as 6 weeks of age and were undistinguishable before dextran sodium sulfate (DSS) treatment (Extended Data 1d–f). However, mice that had experienced neonatal overweight showed increased susceptibility (or pathological imprinting) to DSS-induced colitis in adulthood, characterized by increased weight loss, higher disease-activity index, increased intestinal permeability and inflammation,

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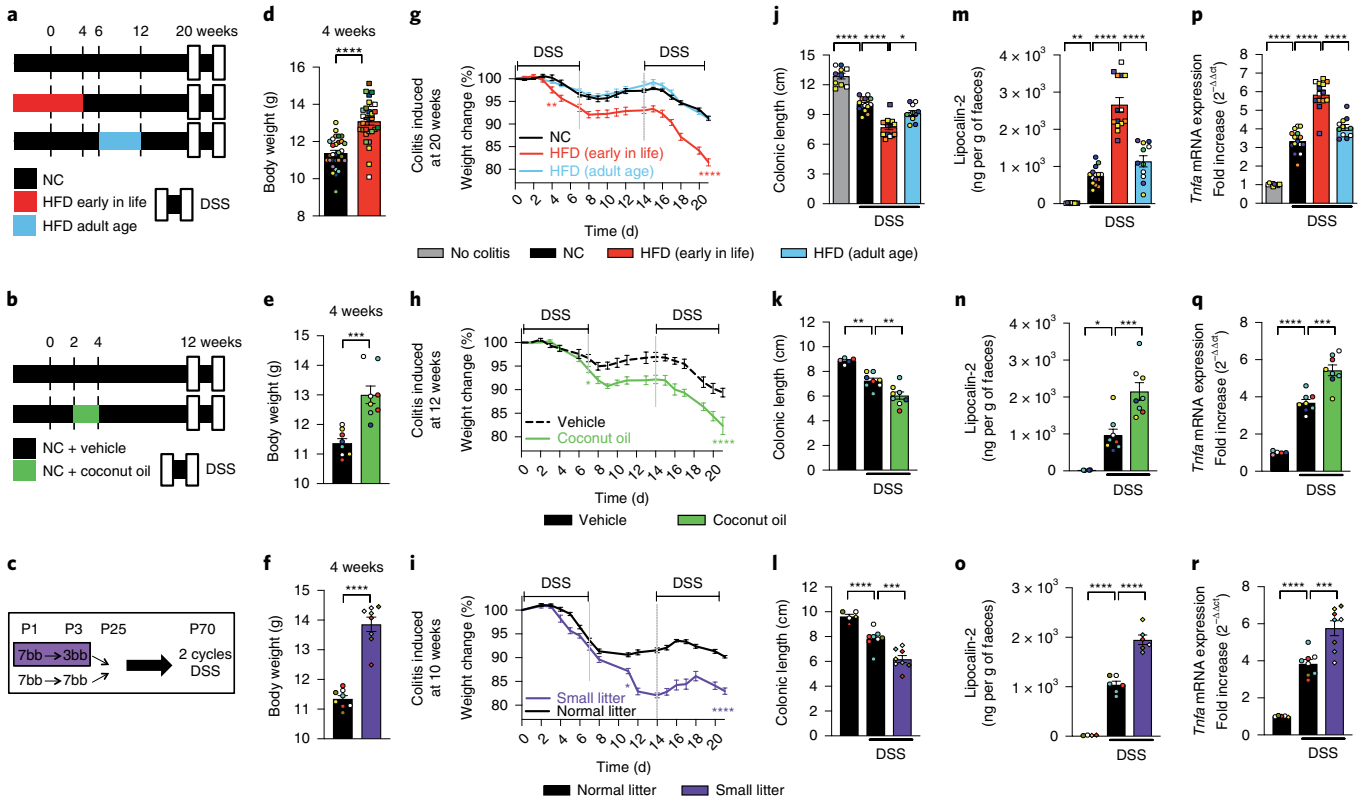

Fig. 1 | Excessive calorie intake early in life increases susceptibility to colitis in adulthood. a, Mice exposed to a HFD early in life were weaned to normal chow (NC) at 4 weeks old, or adult mice fed a HFD (between 6–12 weeks of age) were returned to NC. Colitis was induced when mice were 20 weeks old. **b**, Mice fed NC or NC supplemented coconut oil between 2 to 4 weeks after birth. Colitis was induced when mice were 12 weeks old. **c**, The number of mice fed NC was reduced at day 3 following birth to 3 pups (SL, small litter) or maintained at 7 pups per litter (normal litter, NL) and colitis was induced when mice were 10 weeks old. **d**–**f**, Body weight was measured in 4-week-old mice fed NC or a HFD (**d**; $n=30$ per group; litters = 11), fed NC supplemented or not with coconut oil (**e**; $n=8$ per group; litters = 5) and raised in a NL or SL (**f**; $n=8$ per group; litters = 5). **g**–**i**, Percentage of body weight loss in mice fed NC ($n=9$; litters = 6), a HFD early in life ($n=8$; litters = 6) or a HFD at adult age ($n=10$; litters = 6) (**g**), fed NC supplemented with coconut oil ($n=9$; litters = 6) or not (n = 8; litters = 6) (**h**), or grown in NL or SL ($n=8$ per group; litters = 5) (**i**). **j**–**r**, Colonic length (**j**–**l**), lipocalin-2 level (**m**–**o**) and *Tnfa* mRNA colonic expression (**p**–**r**) measured at day 21, in mice fed NC ($n=15$; litters = 7), a HFD early in life ($n=14$; litters = 5) or a HFD at adult age (n = 11; litters = 5) (**j**,**m**,**p**); NC supplemented with coconut oil or vehicle ($n=8$; litters = 5 per group) (**k**,**n**,**q**); or grown in NL (**l**,**r** ($n=8$; litters = 4), **o** ($n=6$; litters = 4)) or SL (**l**,**r** ($n=8$; litters = 5), **o** ($n=6$; litters = 5)). No colitis (**j**,**m**,**p** ($n=10$; litters = 7), **k**,**n**,**q** ($n=5$; litters = 5), **l** ($n=5$; litters = 4), **o** ($n=4$; litters = 4), **r** ($n=5$; litters = 4)). Post hoc Tukey-adjusted tests following significant ($P<0.05$, after correction) analyses of deviance (mixed-effect models); all ****$P<0.0001$; **e** (***$P=0.0002$); **g** (**$P=0.003$); **h** (*$P=0.03$); **i** (*$P=0.01$); **j** (*$P=0.02$); **k** (**$P=0.001$; **$P=0.007$); **l** (***$P=0.0007$); **m** (**$P=0.001$); **n** (*$P=0.02$; ***$P=0.0008$); **q** (***$P=0.0004$); **r** (***$P=0.0002$). Data were pooled from at least two independent experiments. Each dot represents one offspring mouse. Dots of the same colour and symbol represent mice from same litter. Data are shown as mean ± s.e.m.

colon shortening and, eventually, decreased survival (Fig. 1g–r and Extended Data Fig. 1g–s).

Excess calorie intake early in life perturbs intestinal homeostasis at weaning. We next examined the effect of the three regimens on the inflammatory status and permeability of the intestine. All three regimens led to an increased expression, at weaning but not at later time points, of transcripts coding for the pro-inflammatory cytokines tumour necrosis factor (TNF)-α, interferon (IFN)-γ, interleukin (IL)-1β, IL-12, IL-6 and IL-22 (not strictly a pro-inflammatory cytokine), as well as a decrease in transcripts for the anti-inflammatory cytokine IL-10 (Fig. 2a–c and Extended Data Fig. 2). This was paralleled by an increase in lipocalin 2 levels in the faeces (Fig. 2d–f), increased intestinal permeability, as measured by serum levels of orally fed FITC–dextran (Fig. 2g–i), higher expression of *Mylk*, which encodes the myosin light-chain kinase (MLCK) that regulates the gut epithelial tight junctions, and lower expression of *Tjp1* and *Tjp2*, which encode the tight junction proteins zona occludens 1 (ZO1) and ZO2 (Fig. 2a–c). The expression of other genes, involved in epithelial repair and mucus, such as trefoil factor 3 (*Tff3*) and mucin 2 (*Muc2*), were also affected (Extended Data Fig. 2). Abnormalities observed early in life were dependent on intestinal microbiota because most effects on the inflammatory reaction and increased permeability induced by neonatal overweight were prevented by concomitant treatment of the feeding mothers with a cocktail of antibiotics (Fig. 2 and Extended Data Figs. 2 and 3).

Prevention of pathological imprinting through gut normalization at weaning. We next addressed whether the increased inflammation and intestinal permeability in overweight weaning mice induced increased susceptibility to colitis (or pathological imprinting) in adults. Offspring of mothers fed a HFD after birth were treated with neutralizing antibodies against IFN-γ and TNF-α from 2 to 4 weeks after birth. Inhibition of these two pro-inflammatory cytokines was sufficient to prevent overweight in weaning mice (Fig. 3a), to prevent the increase in expression of other pro-inflammatory cytokines (Fig. 3b) and to normalize intestinal permeability (Fig. 3c and Extended Data Fig. 4a,b). Most importantly, neutralization of IFN-γ and TNF-α during weaning also prevented increased susceptibility to DSS-induced colitis in adulthood (Fig. 3d–g and

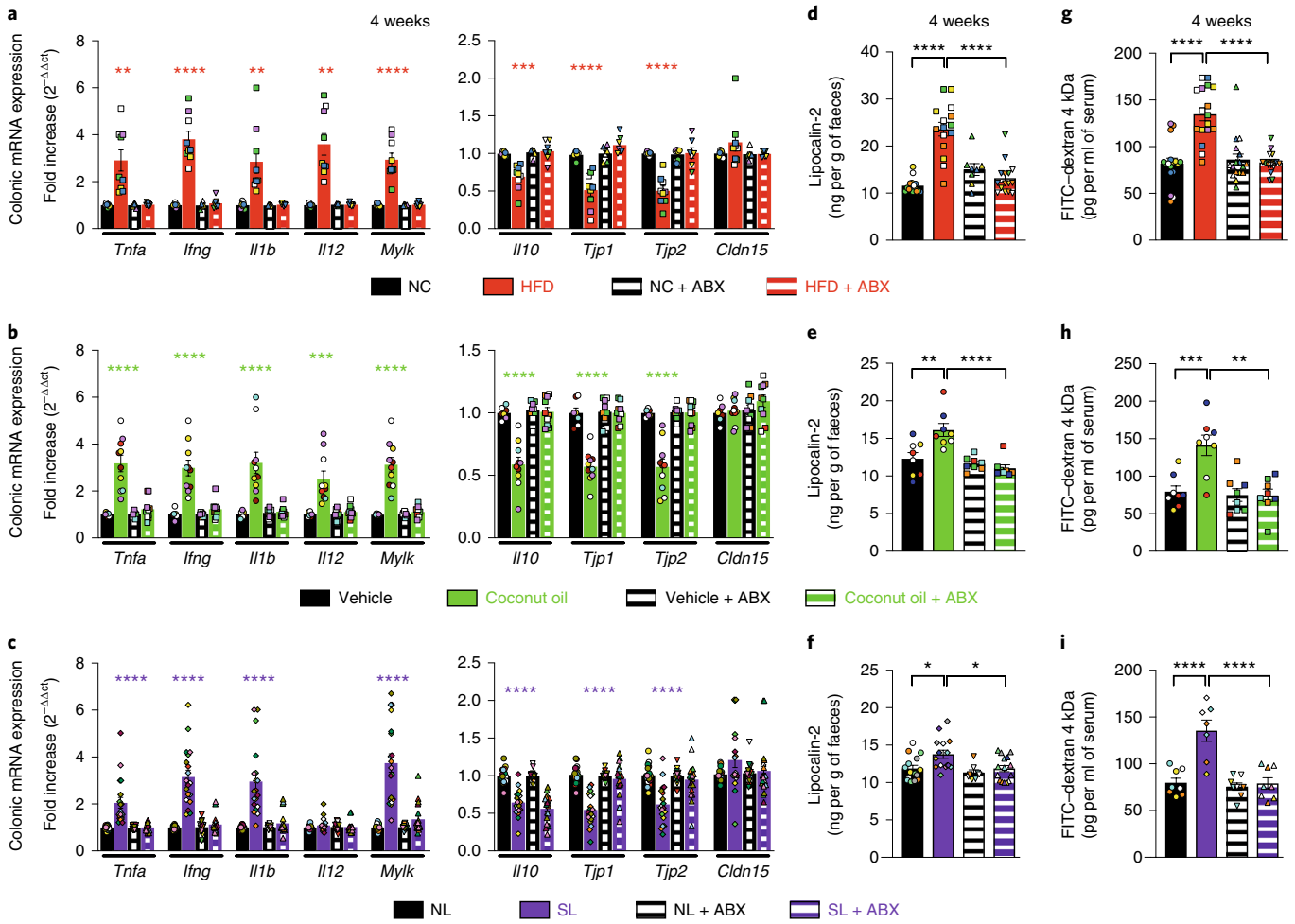

Fig. 2 | Excessive calorie intake early in life increases intestinal permeability and inflammation at weaning. a–c, Colonic mRNA expression of the indicated genes. **d–f**, Lipocalin-2 level. **g–i**, FITC-dextran 4-kDa level. Data in **a–i** were measured at 4 weeks of age in mice fed NC or HFD (**a**,**d**,**g**), supplemented with coconut oil or vehicle (**b**,**e**,**h**) and grown in NL or SL (**c**,**f**,**i**). For NC (**a** ($n=7$; litters = 5), **d** ($n=15$; litters = 5), **g** ($n=17$; litters = 6)); HFD (**a** ($n=9$; litters = 5), **d** ($n=15$; litters = 5), **g** ($n=17$; litters = 6)); NC + ABX (**a** ($n=6$; litters = 5), **d** ($n=8$; litters = 3), **g** ($n=17$; litters = 6)); HFD + ABX (**a** ($n=7$; litters = 5), **d** ($n=15$; litters = 5), **g** ($n=17$; litters = 6)); vehicle (**b** ($n=7$; litters = 5), **e** ($n=8$; litters = 4), **h** ($n=8$; litters = 6)); coconut oil (**b** ($n=10$; litters = 6), **e** ($n=8$; litters = 4), **h** ($n=8$; litters = 6)); vehicle + ABX (**b** ($n=9$; litters = 5), **e**,**h** ($n=8$; litters = 5)); coconut oil + ABX (**b** ($n=10$; litters = 6), **e**,**h** ($n=8$; litters = 5)); NL (**c** ($n=18$; litters = 10), **f** ($n=15$; litters = 5), **i** ($n=8$; litters = 4)); SL (**c** ($n=19$; litters = 10), **f** ($n=15$; litters = 9), **i** ($n=8$; litters = 4)); NL + ABX (**c** ($n=18$; litters = 10), **f**,**i** ($n=8$; litters = 4)); SL + ABX (**c** ($n=19$; litters = 10), **f** ($n=15$; litters = 9), **i** ($n=8$; litters = 5)). Post hoc Tukey-adjusted tests following significant ($P<0.05$, after correction) analyses of deviance (mixed-effect models); all ****$P<0.0001$; **a** (**$P=0.003$, ***$P=0.0009$); **b** (***$P=0.0009$); **e** (**$P=0.0015$); **f** (*$P=0.03$, *$P=0.02$); **h** (***$P=0.0006$, **$P=0.001$). The data were pooled from at least two independent experiments. Each dot represents one offspring mouse. Dots of the same colour and symbol represent mice from the same litter. Data are shown as mean ± s.e.m.

Extended Data Fig. 4c–f). Normalization was also achieved by the induction of RORγt$^+$ regulatory T (T_{reg}) cells[26] during this period of time, through the administration of short-chain fatty acids (SCFA) (Extended Data Fig. 5). Finally, similar results were obtained when MLCK was inhibited from 2–4 weeks after birth by the synthetic naphthalenesulfonyl derivative ML-7 (Fig. 4 and Extended Data Fig. 4g–l). Blocking MLCK normalized intestinal permeability as expected, but also prevented the increase in the expression of pro-inflammatory cytokines, and, as a consequence, prevented pathological imprinting. In contrast, neutralization of IFN-γ and TNF-α, or blocking MLCK, in adult mice fed a HFD did not impact body weight, pro-inflammatory responses or gut permeability (Extended Data Fig. 4m–p).

Bacterial H_2S in pathological imprinting by excessive calorie intake early in life. We next explored the mechanisms by which excessive calorie intake in neonates affected the expression of cytokines and gut permeability. Previous work shows that HFD favours the expansion of sulfide-producing bacteria[27,28]. Hydrogen sulfide (H_2S) leads to destabilization of the mucus layer by reducing S–S bonds in the protein network formed by essential mucus components, such as Muc2 (refs. [29,30]), and is toxic to colonic epithelial cells[31]. In offspring of mothers fed a HFD, in neonates given a coconut-oil gavage and in mice raised in small litters, the expression of bacterial *dsrA* coding for the dissimilatory sulfite reductase, a bacterial enzyme essential for the production of sulfide[32], was significantly increased during weaning, but not later, as was the concentration in faecal H_2S (Fig. 5a–c and Extended Data Fig. 6a–h). This increase in faecal H_2S was prevented by the concomitant treatment of feeding mothers with a cocktail of antibiotics (Extended Data Fig. 6i,j). Blocking the production of H_2S by bacteria with 5-aminosalicylic acid (5-ASA)[33] (Fig. 5d,e) prevented overweight in weaning mice,

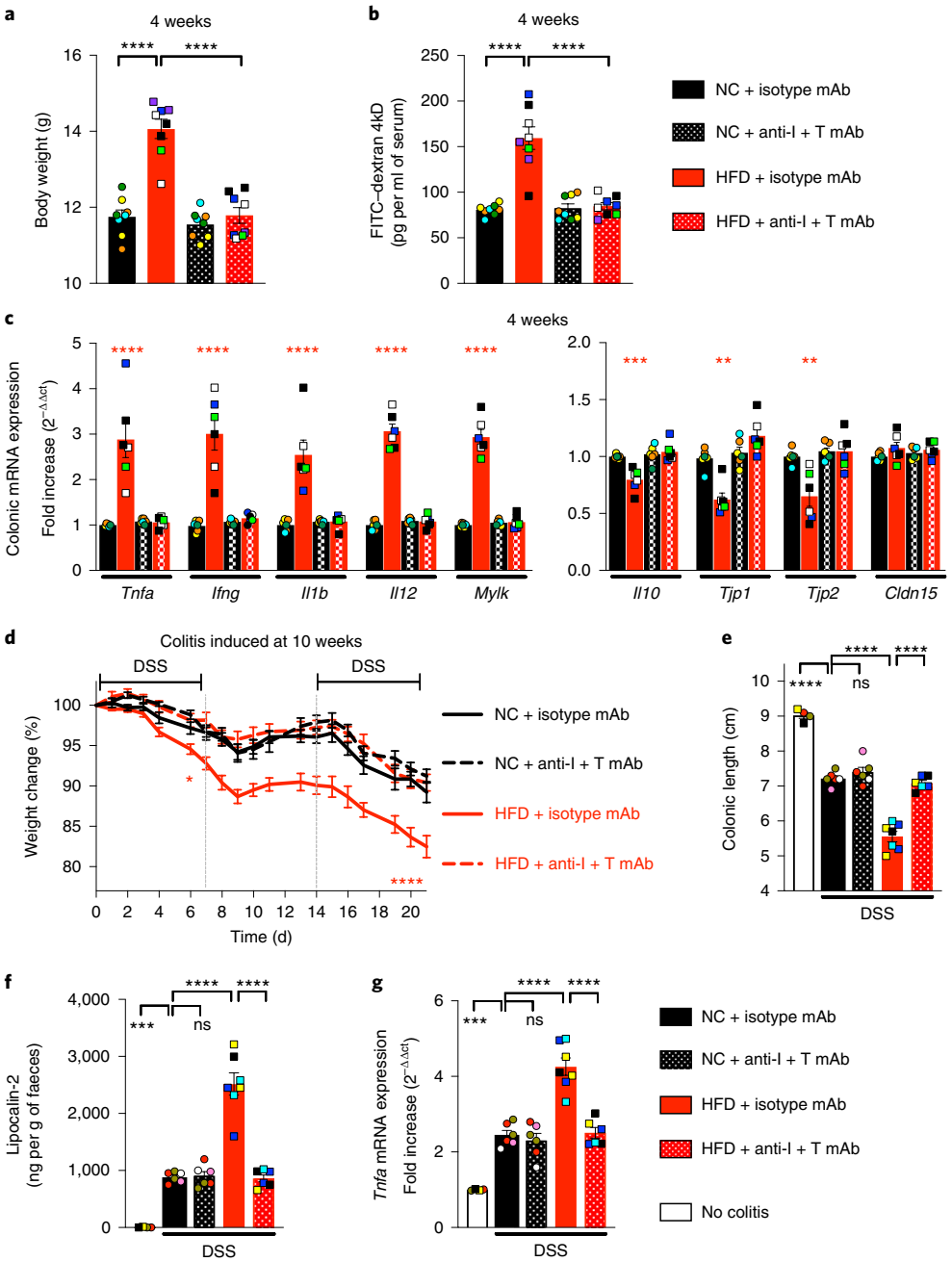

Fig. 3 | Prevention of increased adult susceptibility through normalization of pro-inflammatory responses at weaning. a–g, Mice fed NC or a HFD early in life were treated with anti-TNF-α and anti-IFN-γ (anti-I + T) neutralizing antibodies from 2 to 4 weeks of age and then were challenged with 2 cycles of DSS at 12 weeks of age. Body weight (**a**), intestinal permeability (**b**) and transcript profile (**c**) were measured at 4 weeks of age. We quantified percentage of weight loss during the 3 weeks after initiation of DSS challenge (**d**). Colonic length (**e**), lipocalin-2 level (**f**) and colonic expression (**g**) of *Tnfa* mRNA measured at day 21 after initiation of DSS challenge were quantified. For NC + isotype monoclonal antibody (mAb) (**a**,**b** ($n = 8$; litters = 4), **c**,**e**–**g** ($n = 6$; litters = 4), **d** ($n = 9$; litters = 6)); NC + anti-I + T mAb (**a**,**b** (n = 8; litters = 4), **c**,**e**–**g** ($n = 6$; litters = 4), **d** ($n = 9$; litters = 6)); HFD + isotype mAb (**a**,**b** ($n = 8$; litters = 5), **c** ($n = 6$; litters = 4), **d** ($n = 9$; litters = 7), **e**–**g** (n = 7; litters = 5)); HFD + anti-I + T mAb (**a**,**b** (n = 8; litters = 5), **c** (n = 7; litters = 4), **d** (n = 9; litters = 7), **e**–**g** ($n = 6$; litters = 4)); no colitis (**e**–**g** ($n = 4$; litters = 4)). Post hoc Tukey-adjusted tests following ($P < 0.05$, after correction) analyses of deviance (mixed-effect models); all ****$P < 0.0001$; **c** (*Il10* ***$P = 0.0008$; *Tjp1* **$P = 0.0014$, *Tjp2* **$P = 0.0038$); **d** (*$P = 0.02$); **f** (***$P = 0.0006$); **g** (***$P = 0.0001$). ns, not significant. Data were pooled from at least two independent experiments. Each dot represents one offspring. Dots of the same colour and symbol represent mice from the same litter. Data are shown as mean ± s.e.m.

reduced the expression of several pro-inflammatory cytokines and normalized intestinal permeability (Fig. 5f–h and Extended Data Fig. 6k,l). As a consequence, pathological imprinting was prevented, and the susceptibility of mice to DSS-induced colitis was reduced in adulthood (Fig. 5f–i and Extended Data Fig. 6m–q).

Furthermore, the gavage of germ-free (GF) mice, raised in small litters, from 2 to 4 weeks after birth with sodium hydrogen sulfide (NaHS) as an acute donor of H_2S, in the presence of microbial immunogens (administered as heat-killed microbiota), was sufficient to induce overweight, as well as an increase in the expression of

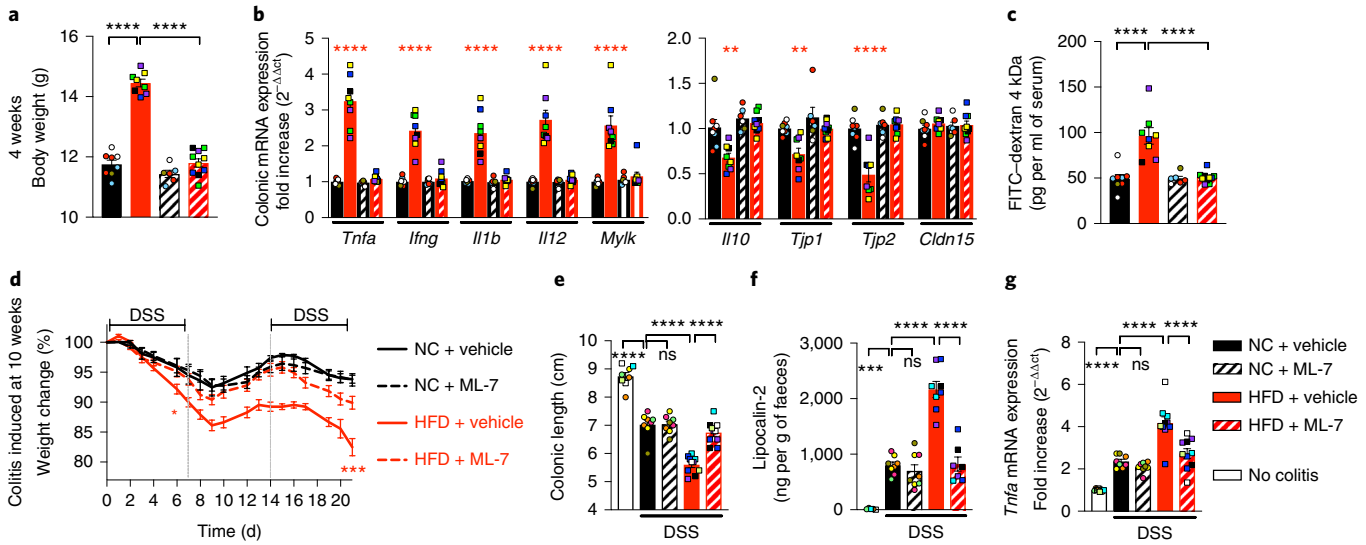

Fig. 4 | Prevention of increased adult susceptibility through gut permeability normalization at weaning. Mice fed NC or HFD early in life were treated with ML-7 (an inhibitor of MLCK) from 2 to 4 weeks of age and then were challenged with 2 cycles of DSS at 12 weeks of age. **a**–**c**, Body weight (**a**), transcript profile (**b**) and intestinal permeability (**c**) were measured at 4 weeks of age. **d**, Percentage of weight loss over the 3 weeks after initiation of DSS challenge. **e**–**g**, Colonic length (**e**), lipocalin-2 level (**f**) and colonic expression of *Tnfa* mRNA (**g**) measured at day 21 after initiation of DSS challenge. For NC + vehicle (**a**–**c** ($n = 8$; litters = 4), **d**–**g** ($n = 8$; litters = 5)); NC + ML-7 (**a**–**c** ($n = 6$; litters = 4), **d**–**g** ($n = 8$; litters = 5)); HFD + vehicle (**a**–**c** ($n = 8$; litters = 5), **d**,**e**,**g** ($n = 10$; litters = 6), **f** ($n = 8$; litters = 4)); HFD + ML-7 (**a**–**c** ($n = 10$; litters = 5), **d**,**e**,**g** ($n = 10$; litters = 6), **f** ($n = 7$; litters = 4)); no colitis (**d**,**e**,**g** ($n = 8$; litters = 7), **f** ($n = 5$; litters = 4)). Post hoc Tukey-adjusted tests following significant ($P < 0.05$, after correction) analyses of deviance (mixed-effect models); all $^{****}P < 0.0001$; **b** (*Il10* $^{**}P = 0.0018$; *Tjp1* $^{**}P = 0.0078$); **d** ($^{*}P = 0.04$, $^{****}P = 0.0004$); **f** ($^{***}P = 0.0006$). ns = not significant. Data were pooled from at least two independent experiments. Each dot represents one offspring. Dots of the same colour and symbol represent mice from the same litter. Data are shown as mean ± s.e.m.

pro-inflammatory cytokines and an increase in intestinal permeability (Fig. 6a–c and Extended Data 7a–e). Such mice developed pathological imprinting and increased susceptibility to DSS-induced colitis in adulthood (Fig. 6d–h and Extended Data Fig. 7f–g). Similar results were obtained using the slow hydrogen-sulfide-releasing compound GYY4137 (ref. [34]) (Extended Data Fig. 8). In contrast, gavage of 8-week-old adult GF mice, raised in small litters, until 10 weeks with NaHS in the presence of microbial immunogens did not impact body weight, the expression of pro-inflammatory cytokines or intestinal permeability, and also did not impact colitis severity (Extended Data Fig. 7h–m). Finally, pathological imprinting induced by NaHS gavage at weaning was prevented by the concomitant neutralization of TNF-α and IFN-γ, or by inhibition of MLCK (Extended Data Fig. 7n–r).

Discussion

This study shows that neonatal overweight induces long-lasting pathological imprinting, assessed here as increased susceptibility to colitis in adulthood. Such overweight induced by HFD in feeding mothers, direct gavage of neonates with lipid-rich food or low littermate competition (and thus increased access to maternal milk) led to increased production of hydrogen sulfide by the microbiota, increased intestinal permeability and increased expression of pro-inflammatory cytokines. These perturbations were causally linked, and even if limited in time to the weaning period, induced pathological imprinting into adulthood. Thus, the weaning period appears to be a critical time period for pathological imprinting, as exposure to a HFD after weaning did not lead to increased susceptibility to colitis (Fig. 1g,j,m,p and Extended Data Fig. 1g,h,m,n,o). Previous reports point to the notion that the time period between birth and weaning is a unique time window of opportunity for the normal development of the immune system, with pathological consequences later in life if perturbed[18,22–24,35,36].

The intestinal and lung microbiota have an important role during this period, as mice not exposed to microbiota before weaning develop increased susceptibility to allergies and colitis in adulthood[18,22–24,35]. We have recently reported that the expanding microbiota at weaning induces a vigorous immune weaning reaction that is required to prevent such pathological imprinting of the immune system[25]. A similar phenomenon is observed in humans, as neonates treated with antibiotics develop increased susceptibility to allergy and paediatric colitis[20,37], a phenomenon framed by the hygiene hypothesis (which states that reduced exposure to microbes increases susceptibility to inflammatory pathologies)[38].

In the context of neonatal overfeeding, we show that microbiota are a necessary element in the perturbation of intestinal permeability and inflammation, involving the production of hydrogen sulfide. Several bacterial taxa produce hydrogen sulfide in mice fed a HFD[27,28] or in patients with IBD[33]. It remains to be understood how excessive calorie intake before and during weaning promotes the expansion of H_2S-producing bacteria, and, for preventive measures, how the expansion of such bacteria can be blocked. Thus, even though our recent findings show that the expanding microbiota at weaning is required to prevent pathological imprinting[25], it now appears that not all bacteria are beneficial during weaning.

The mechanisms by which neonatal perturbations translate into pathological imprinting remain poorly understood. In a model of intestinal allergic reaction induced by invariant NKT cells, microbiota induces modification, in dendritic cells, of the epigenetic code of *Cxcl16*, involved in the recruitment of NKT cells[18]. In a transgenic T cell receptor model of colitis, the induction, during weaning, of T_{reg} cells carrying the transgenic TCR is required to prevent severe colitis in the adult[36]. Finally, maternal microbiota shaped by antibiotic treatment can transfer increased susceptibility to colitis by vertical transmission to offspring[19]. In the context of neonatal overweight reported here, epigenetic modifications in genes involved in immune reactivity later in life, decreased generation of specific T_{reg} cells and dysbiosis of the microbiota are all credible mechanisms of pathological imprinting that remain to be assessed.

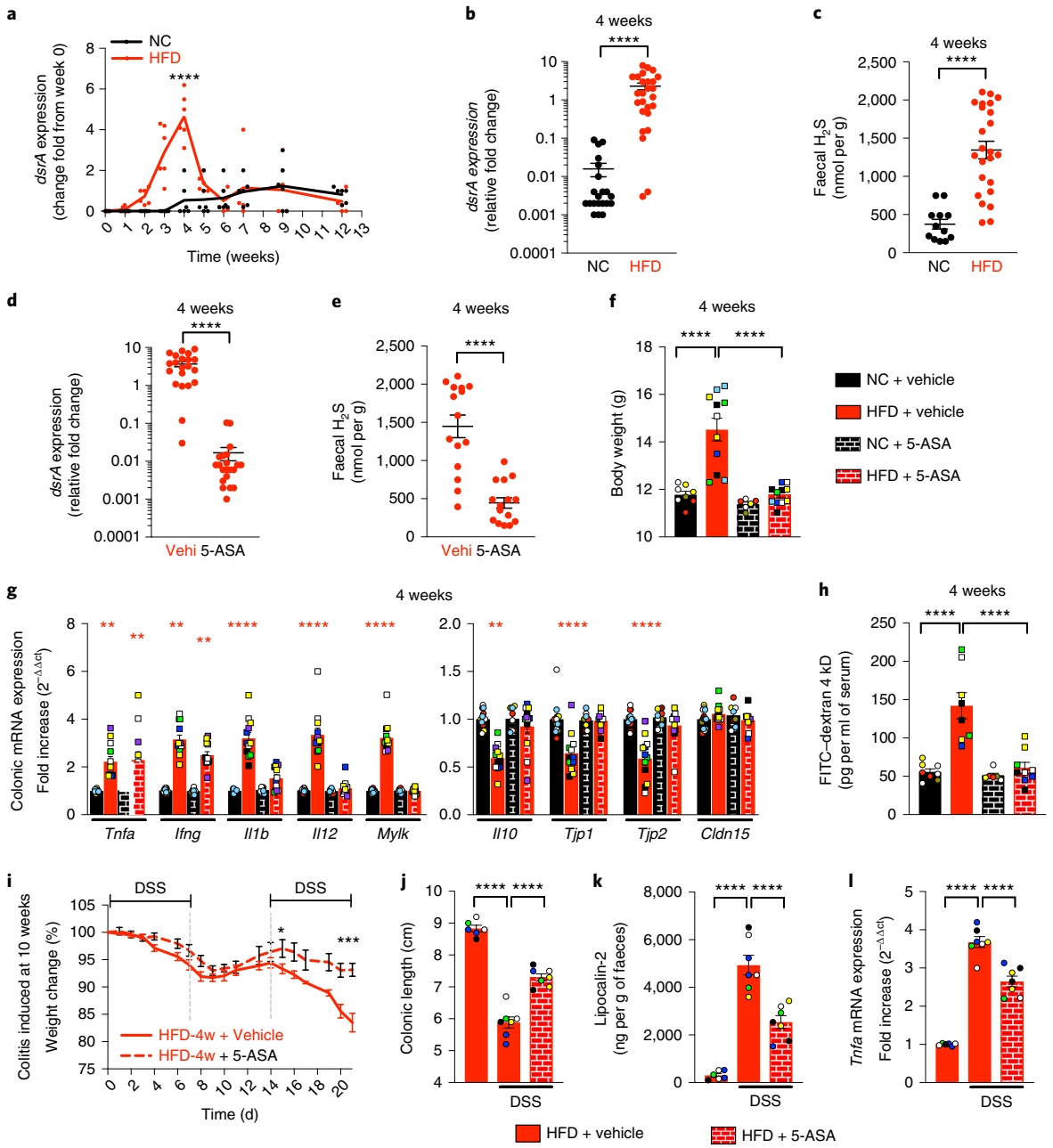

Fig. 5 | Involvement of H_2S in pathological imprinting by excessive calorie intake early in life. Mice fed NC or a HFD early in life were treated or not with 5-aminosalicylic acid (5-ASA) between 2 and 4 weeks after birth. **a–e**, The expression of bacterial *dsrA*, which encodes the bacterial dissimilatory sulfite reductase (**a**), and *dsrA* expression measured at 4 weeks after birth in mice fed NC or a HFD and treated or not with 5-ASA (**b**,**d**). Faecal H_2S levels in mice fed HFD or NC at 4 weeks of age treated or not with 5-ASA (**c**,**e**). **f–h**, Body weight (**f**), transcript profile (**g**) and intestinal permeability (**h**) measured at 4 weeks after birth. **i–l**, Mice fed a HFD early in life were treated with 5-ASA between 2 and 4 weeks after birth and challenged with 2 cycles of DSS at 10 weeks of age. **i**, Percentage of weight loss during the 3 weeks after initiation of DSS challenge. **j**, Colonic expression of *Tnfa* mRNA measured at day 21 after initiation of DSS challenge. For NC (**a** ($n=6$; litters = 4), **b** ($n=21$; litters = 9), **c** ($n=12$; litters = 5), **d** ($n=21$; litters = 9), **e** ($n=15$; litters = 6), **f** ($n=8$; litters = 4), **g** ($n=14$; litters = 6), **h** ($n=6$; litters = 4)); HFD (**a** ($n=6$; litters = 4), **b** ($n=26$; litters = 11), **c** ($n=24$; litters = 9), **d** ($n=21$; litters = 8), **e** ($n=15$; litters = 6), **f** ($n=11$; litters = 6), **g** ($n=12$; litters = 6), **h** ($n=8$; litters = 4), **i–l** ($n=7$; litters = 4)); NC + 5-ASA (**f** ($n=6$; litters = 4), **g** ($n=8$; litters = 6), **h** ($n=8$; litters = 5)); HFD + 5-ASA (**f** ($n=11$; litters = 6), **g** ($n=12$; litters = 6), **h** ($n=9$; litters = 5), **i–l** ($n=7$; litters = 4)); no colitis (**j–l** ($n=6$; litters = 4)). For **a–e** and **i**, a Mann–Whitney test was used for statistical analyses. For **f–l**, post hoc Tukey-adjusted tests following significant ($P<0.05$, after correction) analyses of deviance (mixed-effect models), all ****$P<0.0001$; **g** (*Tnfa* **$P=0.007$, **$P=0.004$; *Ifng* **$P=0.005$, **$P=0.003$; *Il10* **$P=0.007$); **i** (*$P=0.01$; ***$P=0.0003$). Data were pooled from at least two independent experiments. Each dot represents one offspring. Dots of the same colour and symbol represent mice from the same litter. Data are shown as mean ± s.e.m.

Our study demonstrates that neonatal overweight is a risk factor for the development of colitis later in life. In this context, it remains to be assessed whether any type of excess calorie intake, through lipids, carbohydrates or proteins, induces pathological imprinting. The multiple intestinal perturbations induced by excess calorie intake early in life, and their causal role in pathological imprinting, offer

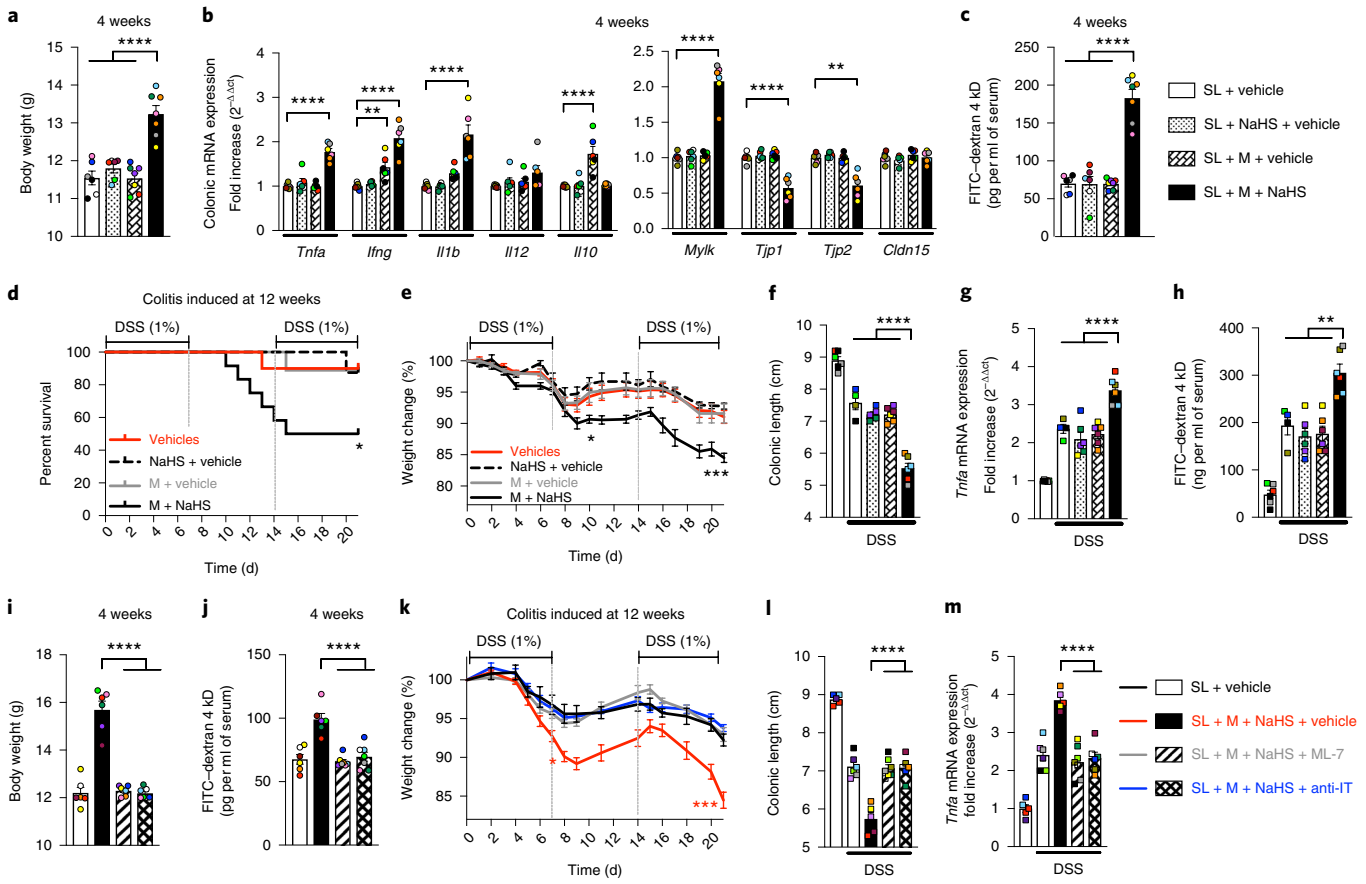

Fig. 6 | Involvement of H_2S in pathological imprinting by excessive calorie intake early in life. a–h, A small litter (SL) of germ-free mice was exposed to heat-killed microbiota (M) and/or to sodium hydrogen sulfide (NaHS) between 2 and 4 weeks after birth, and then colitis was induced at 12 weeks of age. Body weight (**a**), transcript profile (**b**) and intestinal permeability (**c**) were measured at 4 weeks after birth. **d–h**, Survival curves (**d**), percentage of weight loss during 3 weeks after initiation of DSS challenge (**e**), colonic length (**f**), lipocalin 2 level (**g**) and *Tnfa* mRNA colonic expression (**h**) measured at day 21 after DSS introduction. For SL + vehicle (**a**,**c** ($n=6$; litters = 5), **b** ($n=7$; litters = 6), **d**,**e** ($n=9$; litters = 6), **f–h** ($n=4$; litters = 4)); SL + NaHS (**a–c** ($n=6$; litters = 5), **d–e** ($n=7$; litters = 4), **f–h** ($n=6$; litters = 4)); SL + M (**a**,**c** ($n=7$; litters = 6), **b**,**f** ($n=6$; litters = 5), **d**,**e** ($n=8$; litters = 4)); SL + M + NaHS (**a**,**c** ($n=7$; litters = 6), **b**,**f** ($n=6$; litters = 5), **d**,**e** ($n=6$; litters = 4)); no colitis (**f–h** ($n=6$; litters = 4)). **i–m**, Total body weight (**i**) and intestinal permeability at 4 weeks of age (**j**) in GF mice grown in SL and exposed to sodium hydrogen sulfide (NaHS) and heat-killed microbiota (M), and treated with ML-7, with anti-IFNγ and anti-TNFα (anti-IT) neutralizing antibodies (mAb), or with vehicle control from 2 to 4 weeks of age. **k–m**, Percentage of body loss (**k**), colonic length (**l**) and colonic transcript profile (**m**) after colitis induction. For SL (**i**,**j** ($n=6$; litters = 5), **k** ($n=6$; litters = 4); **l**,**m** ($n=6$; litters = 6)); SL + M + NaHS (**i**,**j** ($n=6$; litters = 6), **k** ($n=7$; litters = 5), **l**,**m** ($n=5$; litters = 5)); SL + M + NaHS + ML-7 (**i–m** ($n=7$; litters = 7)); SL + M + NaHS + anti-IT (**i**,**j** ($n=7$; litters = 7), **k–m** ($n=6$; litters = 6)); no colitis (**l**,**m** ($n=5$; litters = 4)). For **a–c** and **e–m**, post hoc Tukey-adjusted tests following significant ($P<0.05$, after correction) analyses of deviance (mixed-effect models), all $^{****}P<0.0001$; **b** (*Ifng* $^{**}P=0.008$; *Tjp2* $^{**}P=0.005$); **e** ($^{*}P=0.03$,$^{***}P=0.0002$); **h** ($^{**}P=0.0012$); **k** ($^{*}P=0.02$, $^{***}P=0.0003$). For **d**, log-rank test ($P=0.04$). Data were pooled from at least two independent experiments. Each dot represents one offspring. Dots of the same colour and symbol represent mice from same litter. Data are shown as mean ± s.e.m.

preventive measures that must be applied before and during weaning. It is also desirable to identify measures to reverse pathological imprinting after weaning, once the offspring has been recognized as having experienced such risk factors. The identification of the nature of pathological imprinting will offer potential avenues that lead to the reversal to healthy imprinting.

Methods

Mice. All animal experiments were approved by the committee on animal experimentation of the Institut Pasteur and by the French Ministry of Research. SPF females and males C57BL/6 mice were purchased from Charles River and exposed to the environment of the local animal facility at Institut Pasteur at least for 2 weeks before mating. Germ-free C57BL/6 mice were generated at the Gnotobiology Platform of the Institut Pasteur. *Foxp3*^{Cre} × *Rorc(gt)*^{Fl} mice were maintained on a C57BL/6 background. All mice were weaned by 28 d after birth (D28) and were co-housed for all experiments in order to avoid cage-specific divergence in the composition of the gut microbiota[39], and experimental groups were both age- and sex-matched. The age of mice at the beginning of each experiment is indicated in the legend to the figures.

High-fat diet and coconut oil gavage. Mice were fed a HFD containing 60% of fat dominantly coconut oil (U8954 version 0140, SAFE, France) or normal chow diet (801954, SDS Diets) irradiated with 10 kGys. Mice were supplemented with coconut oil (5 ml kg^{-1} day^{-1}; 46949; Sigma; France) or a vehicle (distilled water) via oral gavage at 14 d until 28 d after birth. Only male pups, and a normal litter size, were used for these studies.

Control of litter size. Eight-week-old adult female C57BL/6 mice (Charles River) were individually housed. After 2 weeks of adaptation to the surroundings, they were mated overnight with males, at a ratio of 2 females to 1 male. The females were then housed in individual cages 4 d before delivery and during lactation. Mice were kept on a standard pellet diet (801954, SDS Diets) under a 12-h light–dark cycle in a room maintained at a controlled temperature of 22 °C and constant humidity. Litters were normalized to 7 pups per litter on postnatal day 1 (P1), with 4 male and 3 female pups per litter (NL, normal litter). On P3, some litters were culled to 3 pups per litter (2 male + 1 female pups; SL, small litter) to induce postnatal overfeeding. Animals were fed standard chow following weaning, unless otherwise specified. Only male pups were used for these studies. Each experimental group in all experiments consisted of offspring from at least four litters.

Mouse treatment with antibiotics, chemicals and antibodies. Mice were treated during the indicated periods of time with a cocktail of antibiotics in their drinking water, containing 2.5 mg ml^{-1} streptomycin, 1 mg ml^{-1} ampicillin, 0.5 mg ml^{-1} vancomycin, 1 mg ml^{-1} ciprofloxacin and 0.5 mg ml^{-1} metronidazole. The antibiotic-containing drinking water was changed twice a week. Once the treatment was terminated, treated and untreated mice were co-housed. For treatment with SCFAs, 50 µl of cocktail containing acetate (2 mg ml^{-1}), butyrate (1.65 mg ml^{-1}) and priopionate (1.45 mg ml^{-1}) was administered intraperitoneally 3 times per week between 2 and 4 weeks after birth. Where indicated, mice were given a gavage 3 times per week with 100 µl of 5-ASA (5-aminosalicylic acid; 200 µg ml^{-1}) to reduce H_2S and sulfide-reducing bacteria levels, or with 50 mg kg^{-1} of GYY4137 (morpholin-4-ium (4-methoxyphenyl)morpholin-4-ylphosphinodithioate) or 10 µmol kg^{-1} NaHS. For MLCK inhibition, mice were treated by intraperitoneal injection of ML-7 (2 mg kg^{-1}) 3 times per week between 2 and 4 weeks after birth[40]. For TNF-α and IFN-γ neutralization, mice were treated simultaneously with a combination of 25 µg of anti-TNF-α monoclonal antibody (clone MP6-XT22) and 25 µg of anti-IFN-γ monoclonal antibody (XMG1.2) via intraperitoneal injection 3 times per week between 2 and 4 weeks after birth.

Models of colitis. To test susceptibility to DSS-induced colitis, adult mice with similar body weights were exposed to two cycles of 2.5% DSS (approximately 40,000 g mol^{-1}) in their drinking water (except GF mice that received 1% of DSS), for 7 d, which was interrupted by 7 d of normal water. Control mice received drinking water without DSS. Weight and survival were monitored daily to determine progression of disease. Colitis severity was scored by daily observation of the following parameters: weight loss (0 point, no weight loss or weight gain; 1 point, 5–10% weight loss; 2 points, 11–15% weight loss; 3 points, 16–20% weight loss; 4 points, >21% weight loss); stool consistency (0 point, normal and well formed; 2 points, very soft and unformed; 4 points, watery stool); and blood in stool (0 point, normal colour stool; 2 points, reddish colour stool; 4 points, bloody stool). The disease activity index (DAI) was calculated as the combined scores of weight loss, bleeding and stool consistency, with a maximum score of 12. After 21 d, mice were euthanized, the lengths of the colons were measured and organs and blood were collected for biochemical analysis, and a small piece (0.2 cm) of distal colon was taken for the analysis of gene expression.

Gut permeability. In vivo permeability assays were performed using fluorescein isothiocyanate (FITC)–dextran 4 kDa as a paracellular permeability tracer. FITC–dextran was given by gavage to mice 6 d after initiation of the second cycle of DSS in the DSS-induced colitis model. Mice were gavaged with FITC–dextran (5 mg per 200 µl per mouse) 4 h prior to euthanization. The same dose was given to weaned mice (4 weeks old). Whole-blood FITC–dextran concentration was determined by spectrometry. FITC–dextran concentrations in serum were calculated from standard curves generated by serial dilution of FITC–dextran.

ELISA. Myeloperoxidase (MPO) and lipocalin-2 were measured using enzyme-linked immunosorbent assays (ELISAs) and following the manufacturers' recommendations (from R&D Systems).

Measure of H_2S. Faecal and colonic H_2S levels were quantified using the methylene blue method[41]. Colonic contents were collected after animals were euthanized. Material (0.1 g) was homogenized with 1% zinc acetate trapping solution, 20 mM *N*,*N*-dimethyl-p-phenylenediamine sulfate prepared in 7.2 N HCl and 30 mM $FeCL_3$ prepared in 1.2 N HCl, and incubated for 30 min. Samples were centrifuged at 12,000*g* for 3 min, and the clear upper phase was analysed at 670 nm in comparison with a calibration curve of standard H_2S solutions[42]. H_2S levels were expressed in nM g^{-1} of weight.

Quantification of sulfite-reducing bacteria. Faecal genomic DNA was extracted from the weighed stool samples as previously described[43]. Sulfite-reducing bacteria were quantified using specific primers for the *dsrA* gene (encoding a dissimilatory sulfite reductase alpha subunit) as described by Devkota[28]: F: 5′-CCAACATGCACGGYTCCA-3′, R: 5′-CGTCGAACTTGAACTTGAACTTGTAGG-3′.

Quantitative PCR. Frozen tissue samples were dissociated in lysing/binding buffer of Multi-MACS cDNA kit (Miltenyi) with 0.5% antifoam using the gentleMACS Octo Dissociator (Miltenyi). RNA isolation, cDNA synthesis and cDNA purification were performed using the MultiMACS M96thermo Separation Unit, following the manufacturer's instructions for the MultiMACS cDNA Synthesis Kit (Miltenyi). Real-time quantitative PCR on cDNA was performed using SybrGreen (BioRad) and Qiagen primers. Ct values were normalized to the mean Ct obtained with the three housekeeping genes, *Gapdh*, *Hsp90* and *Hprt*, in each sample.

Quantification and statistical analysis. Statistical analysis was performed with the R software 3.5.1. Quantitative data were analysed using linear mixed-effects models (R package lme4), in order to account for statistical dependence among individuals originating from a same family (each progeny being reared in a single cage). Depending on the experiment, data could be recorded at several time points; two statistical designs were therefore considered. In simple designs (non-repeated measures) comparisons were made between experimental treatments (fixed effects) while setting the variable cage as a random term. In designs involving repeated measures (that is, when variables were repeatedly recorded over 21 d), 2 fixed effects were included, the experimental treatment as well as the date of record (analysed as a categorical variable). The latter models included the mouse ID and the cage label as random factors. The overall statistical significance of experimental treatments was assessed based on an analysis of deviance (ANODE, R package car). Given the large number of statistical tests involved in this study, all *P* values returned by ANODE (including data displayed in Supplementary Figures) were adjusted using the Benjamini–Yekutieli (BY) procedure, in order to minimize the false discovery rate. Any ANODE results that remained significant after BY-adjustment (*P* adjusted < 0.05) were followed by post hoc tests. Pairwise testing among treatments was carried out using a Tukey-adjusted comparison (R package lsmeans). In the case of disease activity index, data were averaged across three consecutive days (for example, across days 19, 20 and 21) within individuals so that the distribution of the averaged index was suited for analysis with mixed-effect models.

Survival data were analysed by fitting survival curves with the Kaplan–Meier method and comparison among experimental groups was done using log-rank tests (R package survival).

All figures were performed in GraphPad Prism statistical software (version 8).

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data Availability

The data that support the plots within this paper and other findings of this study are available from the corresponding authors upon reasonable request.

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Author contributions

Z.A.N. and G.E. planned the project and the experiments; Z.A.N., S.D., E.L., B.P. and M.B. performed the experiments; Z.A.N. and P.C. analysed the data; M.B. provided germ-free resources; Z.A.N. and G.E. wrote the manuscript, acquired the funding and conceived the project.

Competing interests

The authors declare no competing interests.

Additional information

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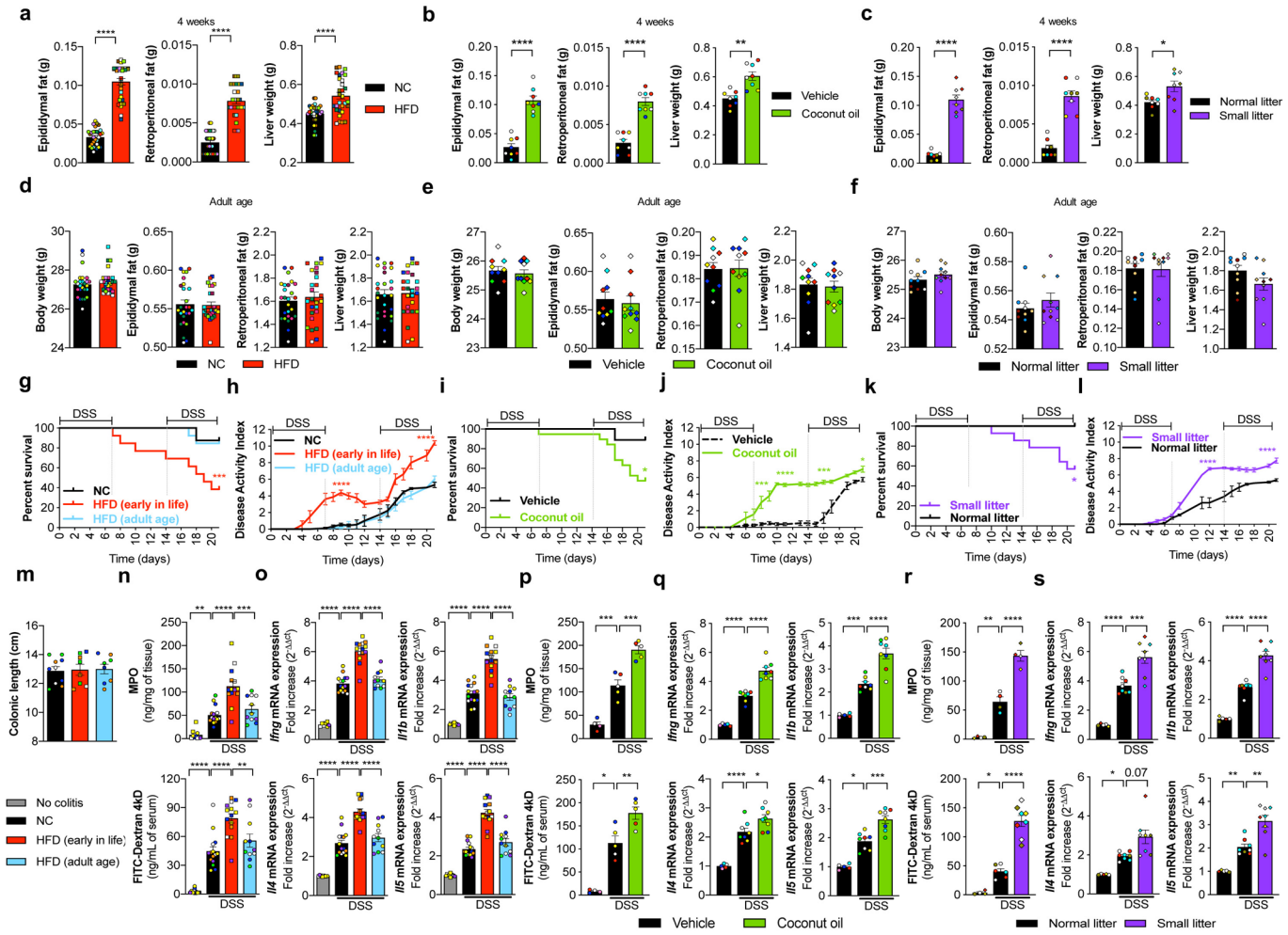
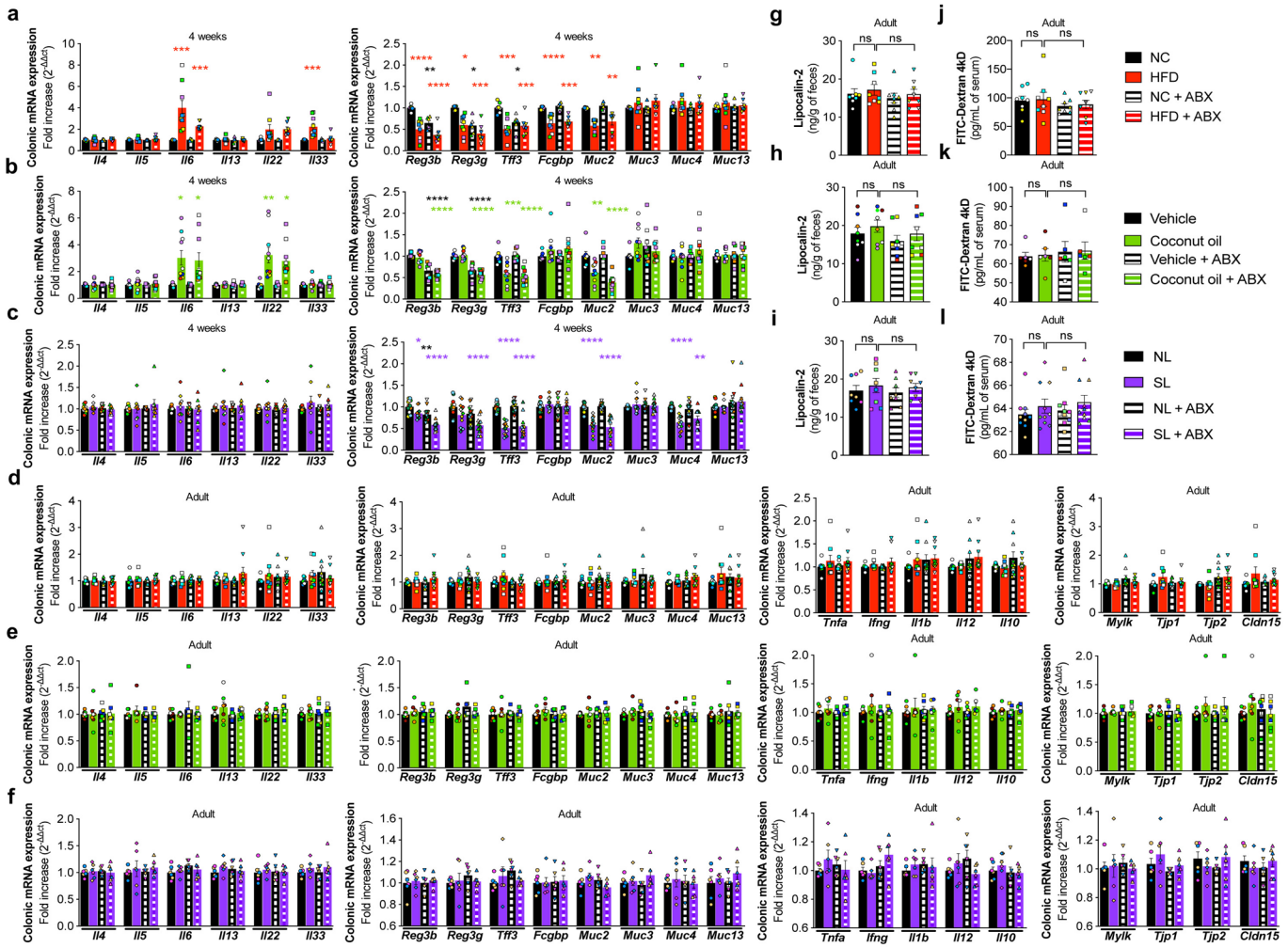
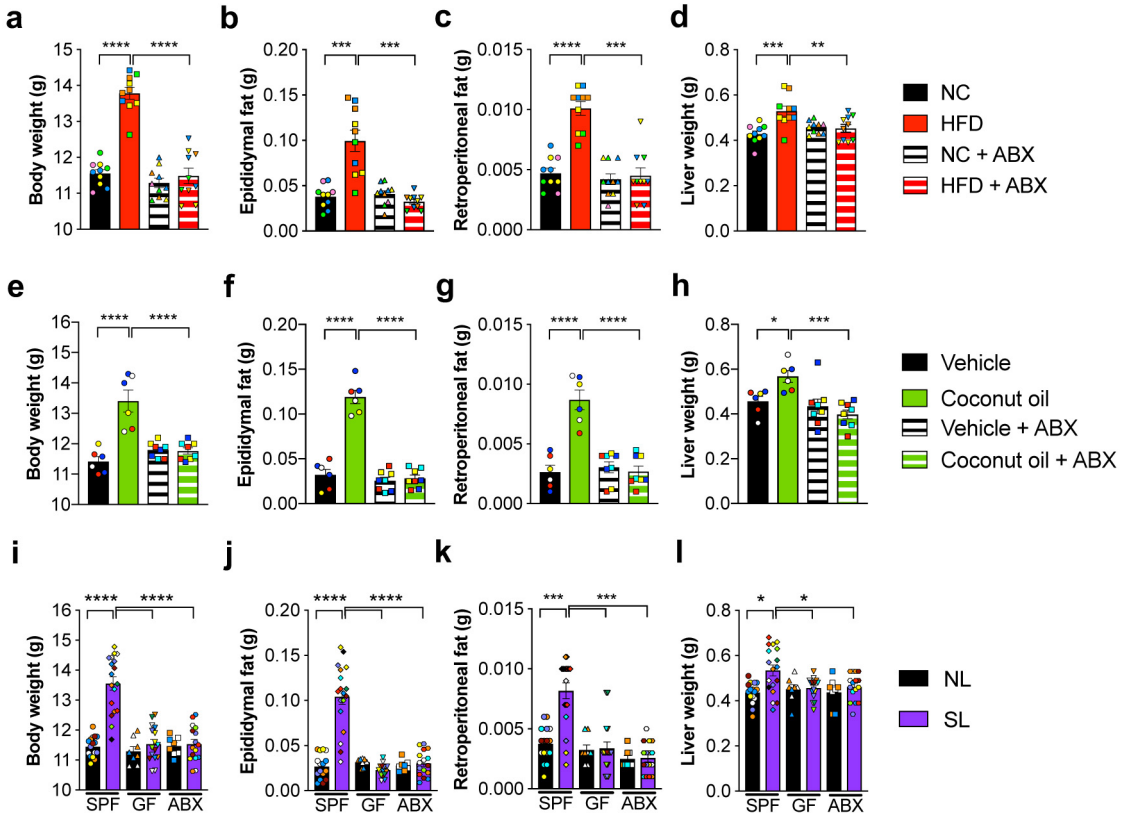


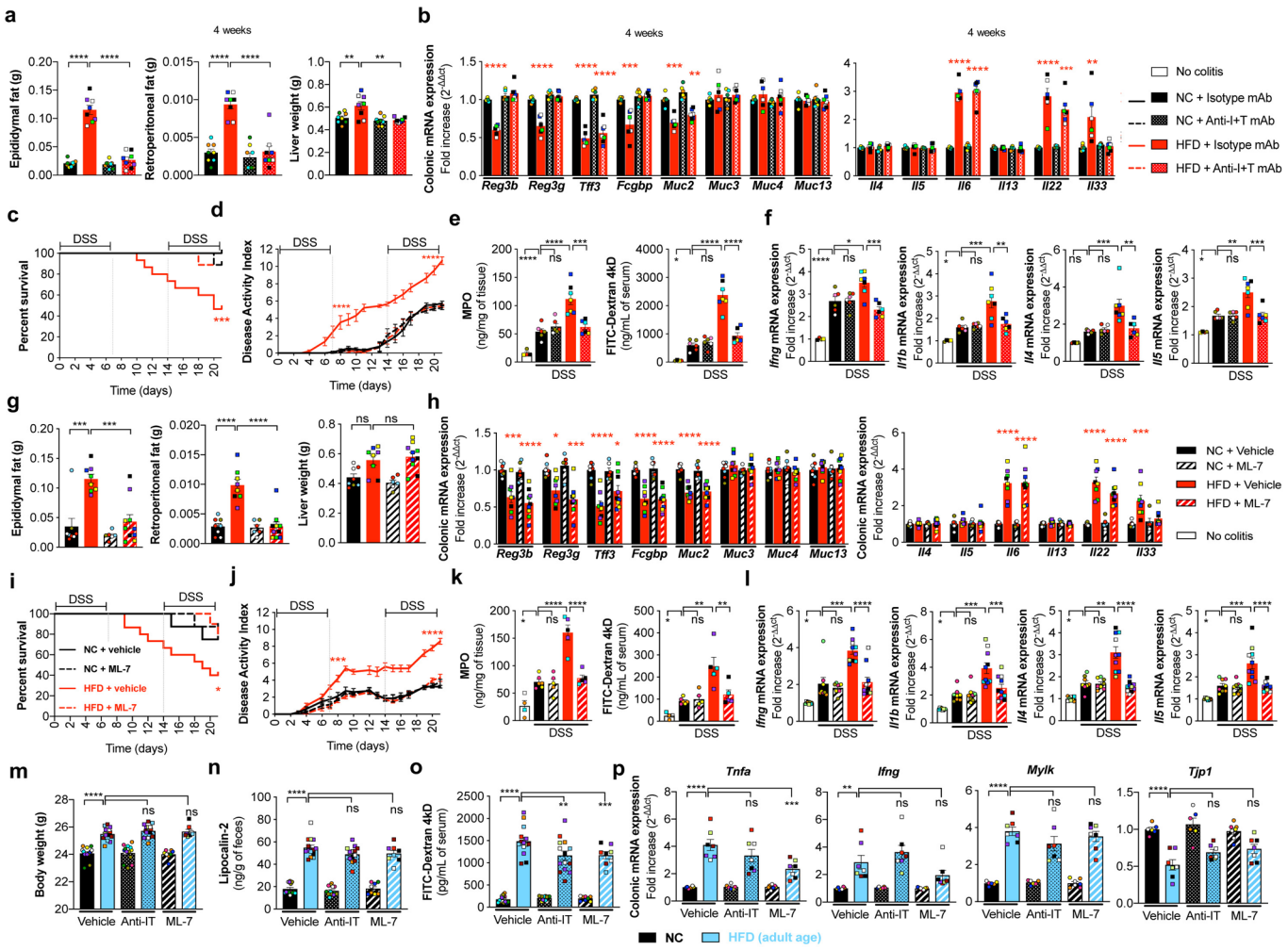
Extended Data Fig. 1 | Excess calorie early in life increased colitis severity in adult mice. (**a**,**d**,**g**,**h**,**m**,**n**,**o**) Mice exposed to a HFD early in life were weaned to NC. (**b**,**e**,**i**,**j**,**p**,**q**) Mice fed NC were supplemented or not with coconut oil between 2 to 4 weeks of age. (**c**,**f**,**k**,**l**,**r**,**s**) The number of mice fed NC was reduced at day 3 post-birth to 3 pups (SL) or maintained to 7 pups per litter as NL. Colitis induced at adult age. (**a**-**c**) Weight of total body, epididymal fat, retroperitoneal fat and liver in 4-week-old mice or (**d**-**f**) before DSS induction in adulthood. (**g**,**i**,**k**) Percentage of survival, (**h**,**j**,**l**) disease-activity index, (**m**) colonic length before DSS exposure, (**n**,**p**,**r**) myeloperoxidase and intestinal permeability levels and **(o,q,s)** colonic transcript profile after colitis induction. For **a** (n=30/group; litters=11), **b** (n=8/group; litters=5), **c** (n=8/group; litters/group=5), **d** (n=26/group; litters/group=9), **e** (n=10/group; litters=6), **f** (n=10; NL: 4 litters; SL: litters=6). NC (**m** (n=10; litters=4), **g** (n=8; litters=5), **h** (n=9; litters=6), **n-o** (n=15; litters=7)); HFD early in life (**m** (n=8; litters=4), **g** (n=15; litters=8), **h** (n=8; litters=6), **n-o** (n=14; litters=5)); HFD at adult age (**m** (n=8; litters=3), **g** (n=13; litters=7), **h** (n=10; litters=6), **n-o** (n=11; litters=5)); Vehicle (**i** (n=9; litters=9), **j** (n=8; litters=6), **p** (n=5; litters=4), **e** (n=8; litters=6)); Coconut oil (**i** (n=19; litters=9), **j** (n=9; litters=6), **p** (n=5; litters=4), **e** (n=8; litters=6)); NL (**k,l** (n=8; litters=5), **r** (n=4-6; litters=4), **s** (n=8; litters=4)); SL (**k** (n=14; litters=8), **l,s** (n=8; litters=5), **r** (n=4-8; litters=4-5)); No colitis (**n-o** (n=10; litters=7), **p,r** (n=4; litters=4), **q** (n=5; litters=5), **s** (n=5; litters=4)). For **g,i,k** Log-rank test (**g** ***P=0.0009, **i** *P=0.04; **k** *P=0.03). For **a-f,h,j,l-s** *post hoc* Tukey-adjusted tests following significant (P<0.05, after correction) Analyses of Deviance (mixed-effect models), all ****P<0.0001; **b** **P=0.001; **c** *P=0.03; **j** (***P=0.0005; *P=0.04); **n** (MPO **P=0.003; ***P=0.0002; Permeability **P=0.009); **p** (MPO ***P=0.0003, ***P=0.0002; Permeability *P=0.02, **P=0.004); **q** (*Il1b* ***P=0.0001; *Il4* *P=0.01; *Il5* **P=0.002); **r** (MPO **P=0.003; Permeability *P=0.01); **s** (*Ifng* ***P=0.002; *Il4* *P=0.04; *Il5* **P=0.002; **P=0.003). Data were pooled from at least two independent experiments. Each dot represents 1 offspring mouse. Dots of same color and symbol represent mouse from same litter. Data are shown as mean±s.e.m.



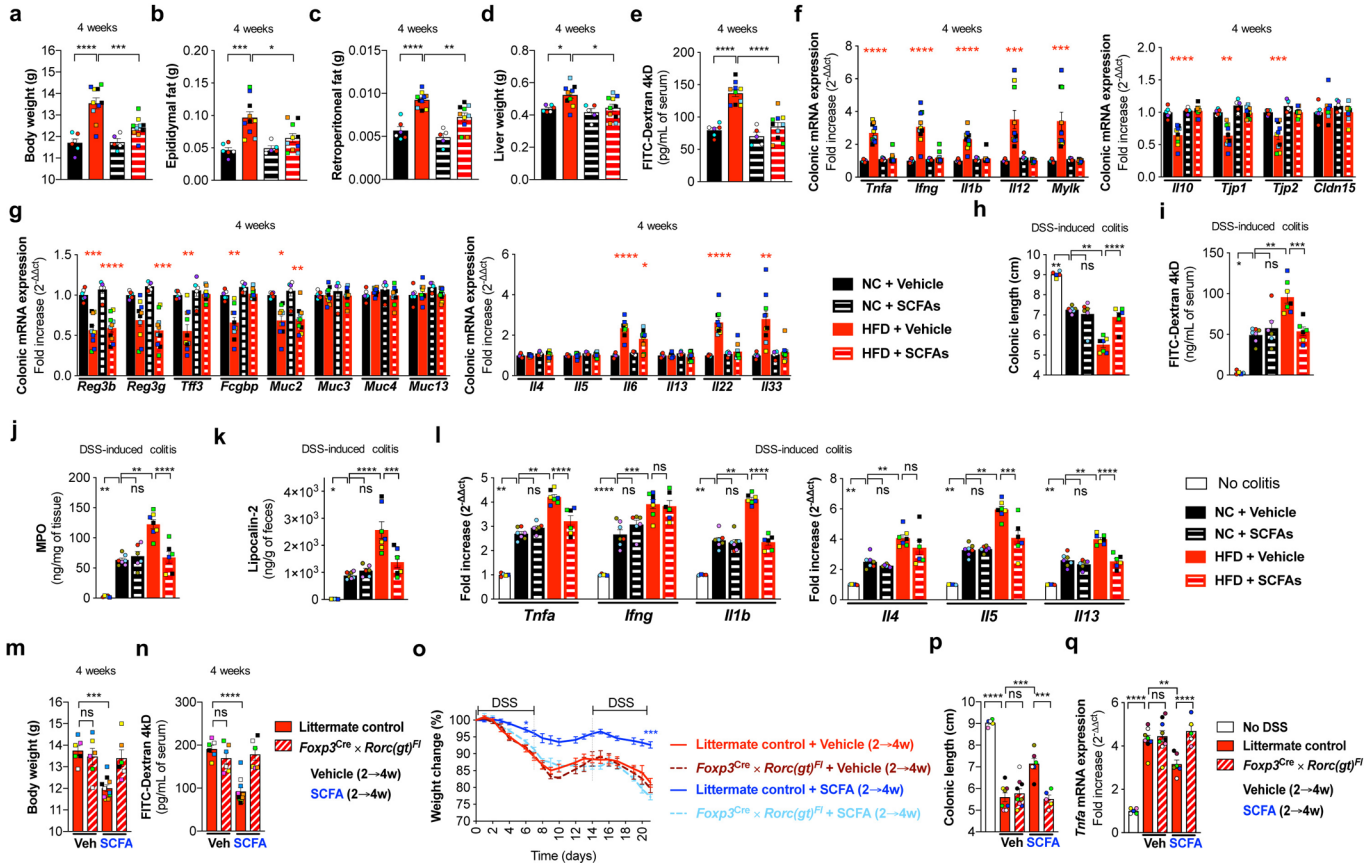
Extended Data Fig. 2 | Excessive calorie intake early in life induces gut dysfunctionality that does not persist later in life. (a,d,g,j) Mice were fed NC or a HFD early in life, and some were exposed to antibiotics (ABX). **(b,e,h,k)** Mice fed NC supplemented with coconut oil or vehicle for 2 to 4 weeks after birth. **(c,f,i,l)** Mice were overfed by reducing litter size (SL) or not (NL). **(a,d)** Colonic mRNA expression measured at **(a-c)** 4 weeks or **(d-f)** 20 weeks of age. **(g-i)** Levels of lipocalin 2 and **(j-l)** intestinal permeability measured before colitis induction in adult mice. For **a** (NC (n=7; litters=5), HFD (n=9; litters=5); NC+ABX (n=6; litters=5); HFD+ABX (n=7; litters=5)); **d** (NC (n=9; litters=5), HFD (n=9; litters=5), NC+ABX (n=10; litters=4), HFD+ABX (n=10; litters=4)); **g,j** (n=8/group; litters/group=4). For Vehicle (**b** (n=7; litters=5), **e** (n=7; litters=4), **h** (n=8; litters=6), **k** (n=6; litters=4)); Coconut oil (**b** (n=10; litters=6), **e** (n=7; litters=4), **h** (n=8; litters=5), **k** (n=6; litters=4)); Vehicle+ABX (**b** (n=9; litters=5), **e** (n=7; litters=4), **h** (n=8; 6 litters), **k** (n=6; litters=4)); Coconut oil+ABX (**b** (n=10; litters=6), **e** (n=7; litters=4), **h** (n=8; 5 litters), **k** (n=6; litters=4)). For NL (**c** (n=10-14; litters=5-7), **d** (n=6; litters=3), **i** (n=8; litters=3), **l** (n=10; litters=4)); SL (**c** (n=9-14; litters=5-7), **d** (n=6; litters=3), **i** (n=8; litters=4), **l** (n=10; litters=6)); NL+ABX (**c** (n=10-14; litters=5-7), **d** (n=6; litters=3), **i** (n=8; litters=3), **l** (n=10; litters=5)); SL+ABX (**c** (n=9-13; litters=5-8), **d** (n=6; litters=3), **i** (n=8; litters=4), **l** (n=10; litters=6)). **(a,g)** *Post hoc* Tukey-adjusted tests following significant ($P<0.05$, after correction) Analyses of Deviance (mixed-effect models), all $^{****}P<0.0001$; **a** (*Il6* $^{***}P=0.0002$, $^{***}P=0.0003$; *Il33* $^{***}P=0.0009$; *Reg3b* $^{**}P=0.005$; *Reg3g* $^{*}P=0.01$; *Tff3* $^{***}P=0.0006$, $^{*}P=0.01$; *Fcgbp* $^{***}P=0.0001$; *Muc2* $^{**}P=0.0018$, $^{**}P=0.0025$); **b** (*Il6* $^{*}P=0.011$, $^{*}P=0.015$; *Il22* $^{**}P=0.0018$, $^{*}P=0.036$; *Tff3* $^{***}P=0.0005$; *Muc2* $^{**}P=0.002$); **c** (*Reg3b* $^{*}P=0.025$, $^{**}P=0.005$; *Muc4* $^{**}P=0.006$). ns = not significant. Data were pooled from two independent experiments. Each dot represents 1 offspring mouse. Dots of same color and symbol represent mouse from same litter. Data are shown as mean±s.e.m.



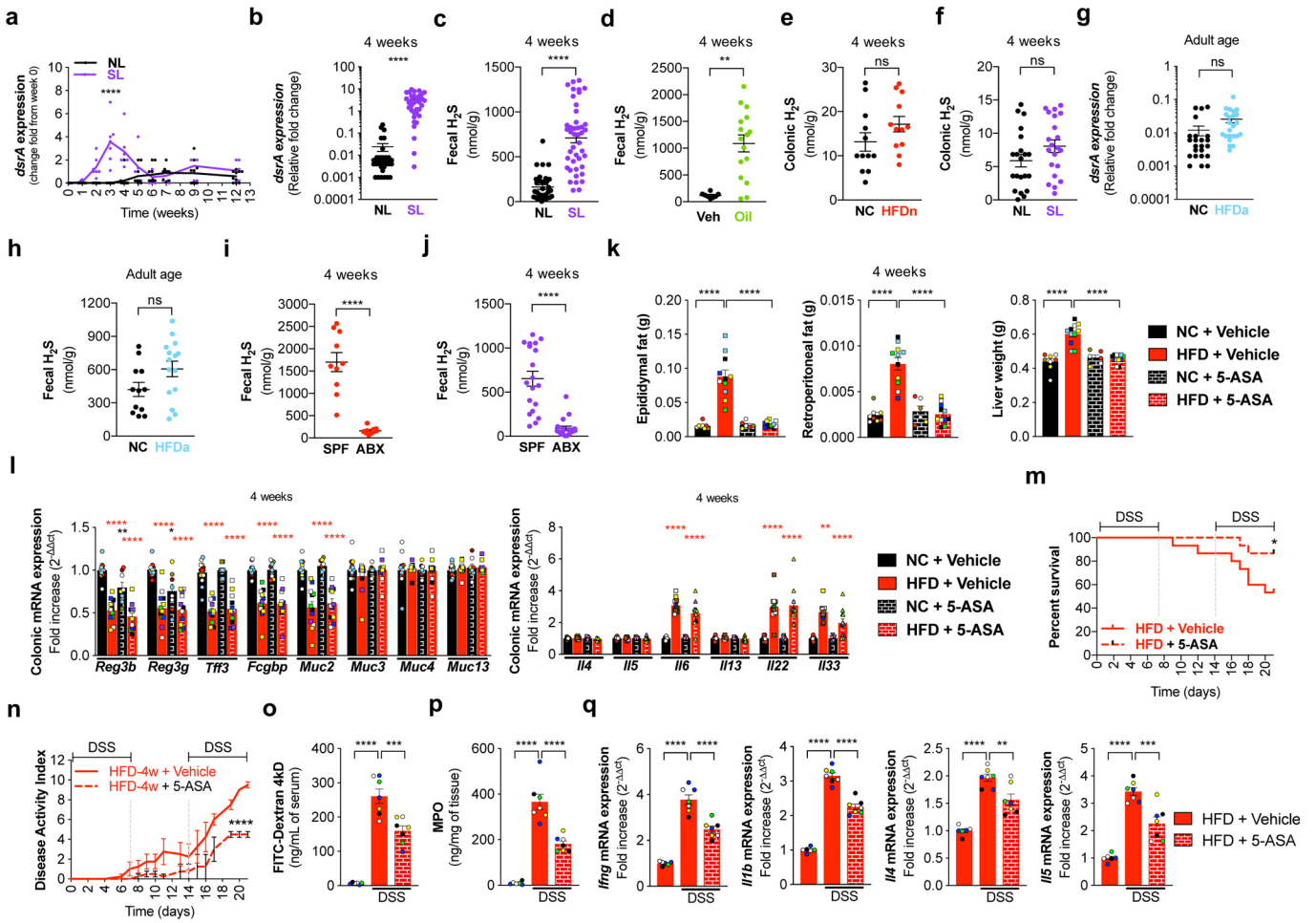
Extended Data Fig. 3 | Excessive calorie intake increases body weight through a microbiota-dependent mechanism. Weight of total body **(a,e,i)**, epididymal fat **(b,f,j)**, retroperitoneal fat **(c,g,k)** and liver **(d,h,l)** at 4 weeks after birth of mice exposed or not to antibiotics (ABX). Mice were **(a-d)** fed NC or a HFD early in life, **(e-h)** supplemented with coconut oil or vehicle during 2 to 4 weeks after birth, **(i-l)** overfeed by reducing litter size (SL) or not (NL) at neonatal age in SPF or in GF conditions. Post hoc Tukey-adjusted tests following significant ($P<0.05$, after correction) analyses of deviance (mixed-effect models), all $^{****}P<0.0001$; **b** ($^{***}P=0.0004$, $^{***}P=0.0001$); **c** ($^{***}P=0.0002$); **d** ($^{**}P=0.009$, $^{***}P=0.0004$); **h** ($^{*}P=0.049$, $^{***}P=0.0008$); **k** ($^{***}P=0.0001$, $^{***}P=0.0007$); **l** ($^{*}P=0.01$, $^{***}P=0.03$). For **a-d** (n=10/group; litters/group=4), **e-h** (Vehicle (n=6; litters=4), Coconut oil (n=6; litters=4), Vehicle+ABX (n=8, litters=4), Coconut oil+ABX (n=8, litters=4)), **i-l** (SPF+NL (n=16, litters=6), SPF+SL (n=18, litters=11), GF + NL (n=8, litters=3), GF+SL (n=16, litters=9), ABX+NL (n=8, litters=3), ABX+SL (n=16, litters=9)). Data were pooled from at least two independent experiments. Each dot represents 1 offspring mouse. Dots of same color and symbol represent mouse from same litter. Data are shown as mean±s.e.m.



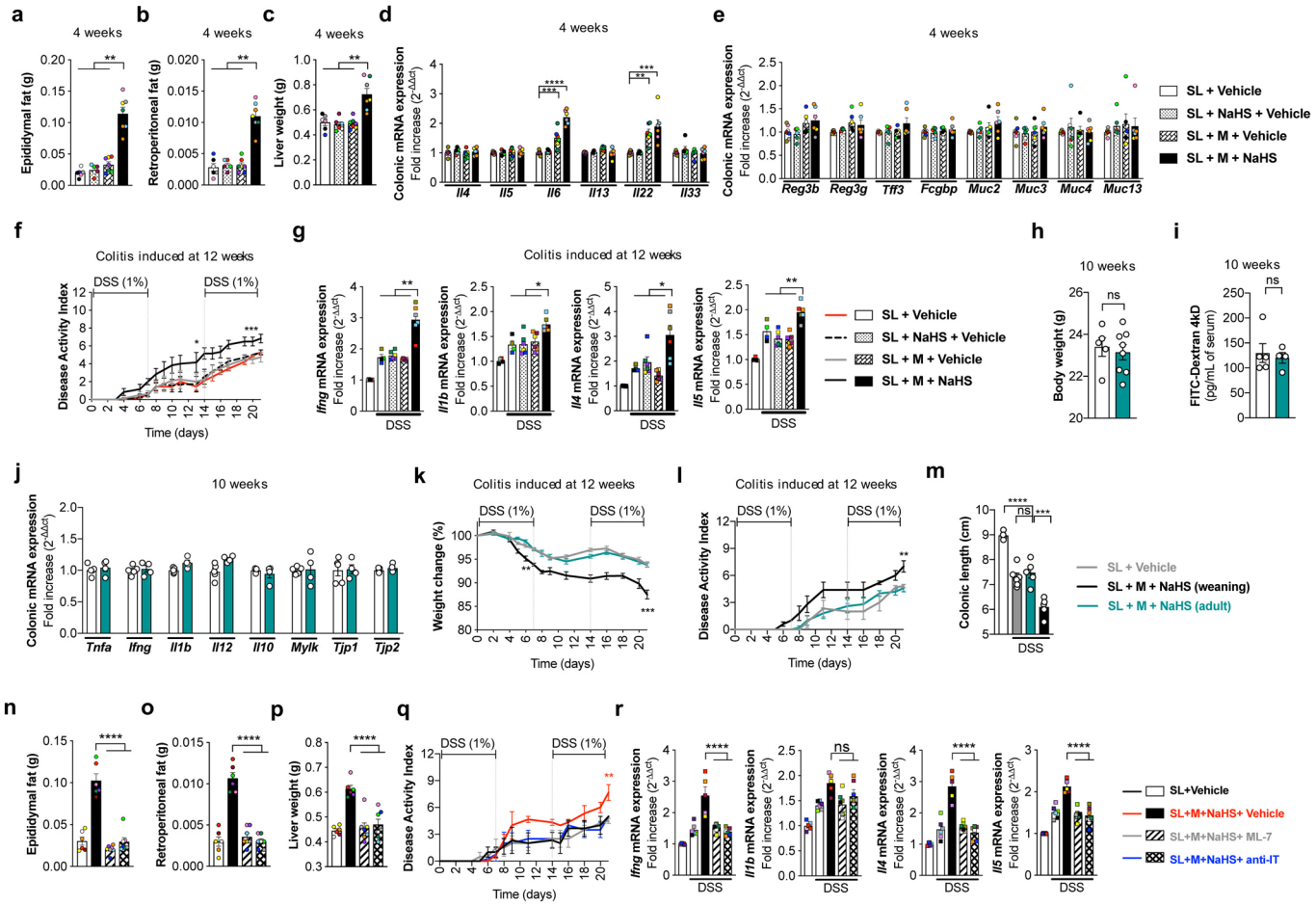
Extended Data Fig. 4 | Prevention of pathological imprinting early in life. Mice fed NC or a HFD early in life treated **(a-f)** with anti-IFN-γ and anti-TNF-α (anti-I+T) neutralizing antibodies (mAb) or **(g-l)** ML-7 versus controls from ages 2 to 4 weeks old, and colitis was induced in adulthood. **(a,g)** Weight of epididymal fat, retroperitoneal fat, liver and **(b,h)** colonic mRNA expression measured at 4-week-old. **(c,i)** Percentage of survival, **(d,j)** disease activity index, **(e,k)** levels of myeloperoxidase and intestinal permeability and **(f,l)** mRNA colonic transcript profile after colitis initiation. **(m-p)** Adult mice fed NC or HFD during 6- to 9-week-old are simultaneously treated or not with anti-I+T antibodies or with ML-7 *vs* controls. **(m)** Body weight, **(n)** lipocalin 2, **(o)** gut permeability and **(p)** colonic mRNA expression measured 9-week-old. For NC+Isotype and NC+Anti-I+TmAb (**a** (n=8; litters=4), **b**,**e-f** (n=6; litters=4), **c-d** (n=9; litters=6)); HFD+Isotype (**a** (n=8; litters=5), **b** (n=6; litters=4), **c** (n=15; litters=11), **d** (n=9; litters=7), **e-f** (n=7; litters=5)); HFD+Anti-I+TmAb (**a** (n=8; litters=5), **b** (n=7; litters=4), **c-d** (n=9; litters=7), **c-e** (n=6; litters=4)). For NC+Vehicle (**g-h** (n=8; litters=4), **i,j,l** (n=8; litters=5), **k** (n=6; litters=3)); NC+ML-7 (**g-h** (n=6; litters=4), **i,j,l** (n=8; litters=5), **k** (n=5; litters=3)); HFD+Vehicle (**g-h** (n=8; litters=5), **i** (n=15; litters=12), **j,l** (n=10; litters=6), **k** (n=5; litters=3)); HFD+ML-7 (**g** (n=10; litters=5), **h** (n=9; litters=5), **i,j,l** (n=10; litters=6), **k** (n=6; litters=3)). No colitis (**e-f** (n=4; litters=4), **k** (n=4; litters=3), **l** (n=8; litters=7)). For **c,i** Log-rank (Mantel–Cox) test (**c** (***P=0.0005); **i** (*P=0.02)). For **a,b,d-f,g,h,j-p**, *Post hoc* Tukey-adjusted tests following significant (P<0.05, after correction) Analyses of Deviance (mixed-effect models), all ****P<0.0001; **a** (**P=0.008, **P=0.008); **b** (*Fcgbp* ***P=0.0002; *Muc2* ***P=0.0002, **P=0.006; *Il22* ***P=0.0008; *Il33* **P=0.002); **e** (MPO ***P=0.0001; Permeability *P=0.01); **f** (*Ifng* *P=0.01, ***P=0.0001; *Il1b* *P=0.01, **P=0.002, ***P=0.0005; *Il4* **P=0.002, ***P=0.0005; *Il5* *P=0.03, **P=0.001, ***P=0.0003); **g** (***P=0.0003, ***P=0.0001), **h** (*Reg3b* ***P=0.0005; *Reg3g* *P=0.04, ***P=0.0004; *Tff3* *P=0.02; *Il33* ***P=0.0008). **k** (MPO *P=0.017; Permeability *P=0.012, **P=0.001, **P=0.004); **l** (*Ifng* *P=0.018, ***P=0.0001; *Il1b* *P=0.049, ***P=0.0001, ***P=0.0006; *Il4* *P=0.023, **P=0.003; *Il4* *P=0.039, ***P=0.0002); **o** (**P=0.001, ***P=0.0004); **p** (*Tnfa* ***P=0.0008; *Ifng* **P=0.005). ns=not significant. Data were pooled from at least two independent experiments. Each dot represents 1 offspring mouse. Dots of same color and symbol represent mouse from same litter. Data are shown as mean±s.e.m.



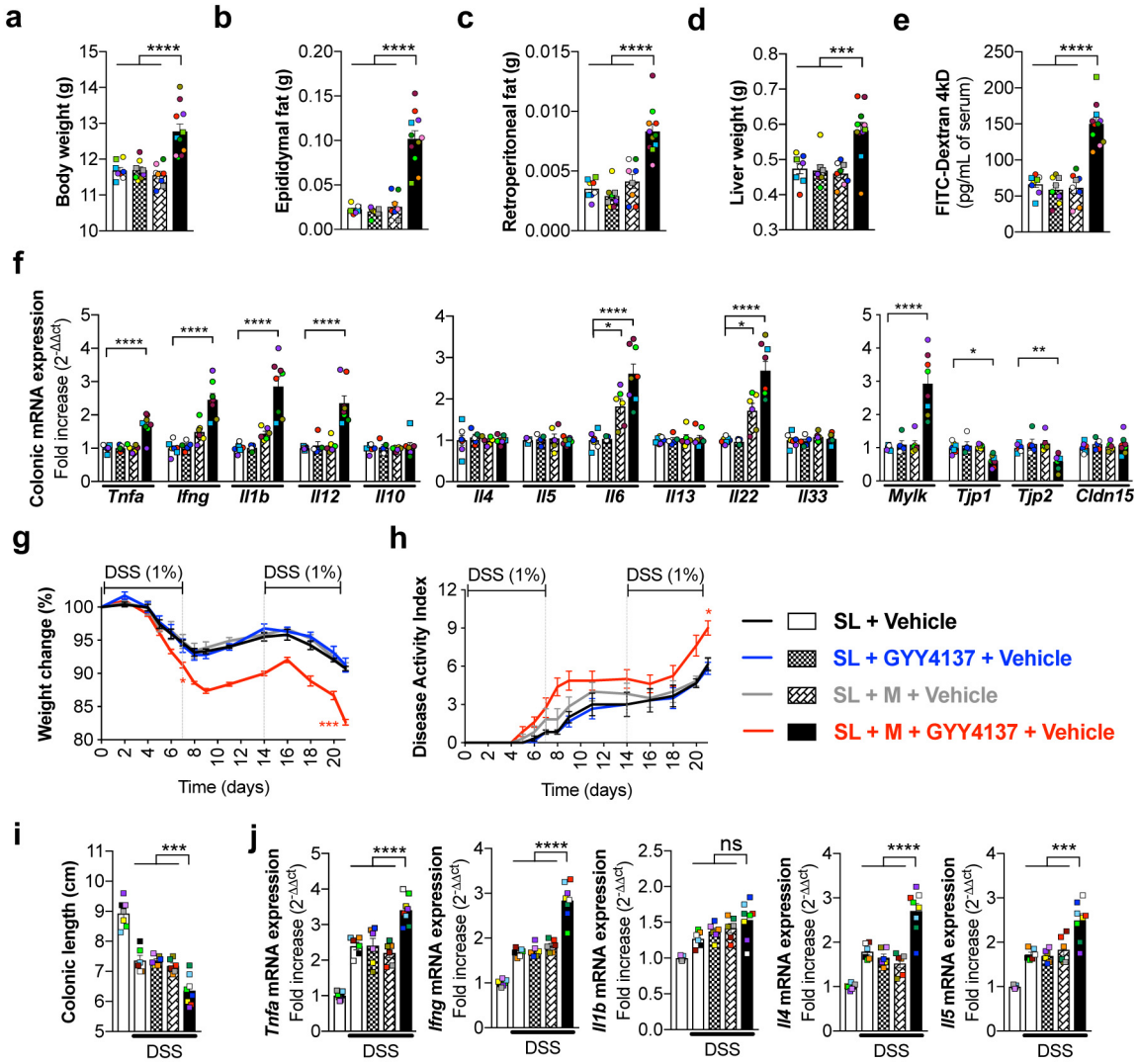
Extended Data Fig. 5 | Exposure to SCFAs in mice fed HFD early in life reduced the severity of colitis in adulthood by mechanism dependent on RORγt-expressing T_{reg}s. (a-i) Mice were exposed to NC or to a HFD early in life and treated with SCFAs or vehicle controls during 2 to 4 weeks of age. **(m-q)** *Foxp3*^{Cre} × *Rorc(gt)*^{Fl} mice and littermate control were fed HFD early in life and exposed to SCFAs or vehicle during weaning. **(a,m)** Weight of total body, **(b)** epididymal fat, **(c)** retroperitoneal fat, **(d) and** liver, **(e,n)** level of intestinal permeability, and **(f,g)** colonic mRNA expression measured post-weaning. **(h,p)** Colonic length, **(i)** intestinal permeability, **(j)** level of myeloperoxidase, **(k)** lipocalin-2, **(l,q)** colonic transcript profile and **(o)** loss of body weight measured after initiation of colitis at 9-10 weeks of age. For NC+Vehicle (**a-g** (n=6); **h-l** (n=7); litters=4); HFD+Vehicle (**a-g** (n=10; litters=6), **h-l** (n=7; litters=4)); NC+SCAFs (**a-g** (n=5), **h-l** (n=6); litters=4); HFD+SCFAs (**a-g** (n=10; litters=6), **h-l** (n=6; litters=4)); Littermate+Vehicle (**m-n** (n=6; litters=6), **o** (n=5; litters=5), **p-q** (n=8; litters=8)); Littermate+SCFAs (**m-n** (n=10; litters=7), **o** (n=10; litters=6), **p-q** (n=6; litters=6)); Foxp3^{Cre}RORγt+Vehicle (**m-n** (n=6; litters=6), **o** (n=5; litters=5), **p-q** (n=9; litters=8)); Foxp3^{Cre}RORγt+SCFAs (**m-n** (n=6; litters=6), **o** (n=6; litters=5), **p-q** (n=5; litters=5)); No colitis (n=5; litters=5). *Post hoc* Tukey-adjusted tests following significant ($P<0.05$, after correction) Analyses of Deviance (mixed-effect models), all ****$P<0.0001$; **a** (***$P=0.0006$); **b** (***$P=0.0004$, *$P=0.012$); **c** (**$P=0.003$); **d** (*$P=0.04$, *$P=0.02$); **f** (*Il12* ***$P=0.0002$; *Mylk* ***$P=0.0004$; *Tjp1* **$P=0.004$; *Tjp2* ***$P=0.0003$); **g** (*Reg3g* ***$P=0.0005$; *Reg3b* ***$P=0.0004$; *Tff3* **$P=0.004$; *Fcgbp* **$P=0.006$; Muc2 *$P=0.02$, **$P=0.008$; *Il6* *$P=0.03$; *Il33* **$P=0.007$); **h** (**$P=0.002$, **$P=0.008$); **i** (*$P=0.02$, **$P=0.007$, ***$P=0.0003$); **j** (**$P=0.005$, **$P=0.003$); **k** (*$P=0.04$, ***$P=0.0006$); **l** (*Tnfa* **$P=0.004$, **$P=0.005$; *Ifng* ***$P=0.0003$; *Il1b* **$P=0.003$, **$P=0.008$; Il4 **$P=0.008$, **$P=0.004$; Il5 **$P=0.003$, **$P=0.007$; Il13 **$P=0.006$, **$P=0.008$); **m** (***$P=0.0003$); **o** (*$P=0.01$, ***$P=0.0008$); **p** (***$P=0.0003$, ***$P=0.0009$); **q** (**$P=0.007$); ns = not significant. Data were pooled from at least two independent experiments. Each dot represents 1 offspring mouse. Dots of same color and symbol represent mouse from same litter. Data are shown as mean±s.e.m.



Extended Data Fig. 6 | The expression of bacterial *dsrA*, H_2S levels and impact of 5-ASA on colitis susceptibility. (a-b) The expression of bacterial *dsrA* coding for the dissimulatory sulfite reductase **(a)** by weeks and **(b)** at 4 weeks after birth in mice grown in reduced litters (SL=Small Litter) or normal litters (NL). For NL (**a** (n=10; litters=4), **b** (n=34; litters=15)); SL (**a** (n=7; litters=5), **b** (n=34; litters=20)). **(c-f)** Faecal H_2S levels **(c)** from mice grown in NL (n=34; litters=15) and SL (n=43; litters=25), **(d)** from mice gavaged with coconut oil (n=16; litters=10) or Vehicle (n=8; litters=6), or in the colon of **(e)** mice fed NC (n=12; litters=5) or HFD early in life (HFDn; n=12; litters=5), or **(f)** mice grown in NL (n=21; litters=9) or SL (n=21; litters=12). **(g)** The expression of *dsrA* at 12-week-old from mice fed HFD between 6- to 12-week-old (n=23; litters=15) or NC (n=23; litters=10). **(h-j)** Fecal H_2S levels **(h)** measured at 12-week-old mice that fed HFD in adulthood (n=15; litters=7) or NC (n=12; litters=5), **(i)** measured at 4-week-old mice that fed HFDn and treated with antibiotics (n=10; litters=4) or not (n=10; litters=5), and **(j)** mice grown in SL and treated or not with antibiotics (n=19/group; litters/group=13). **(k-q)** Mice fed NC or HFD early in life treated during weaning with 5-ASA or controls. **(k)** Weight of epididymal fat, retroperitoneal fat and liver, **(l)** colonic mRNA expression at 4-week-old. **(m)** Percentage of survival, **(n)** disease activity index, **(o)** levels of gut permeability, **(p)** myeloperoxidase and **(q)** colonic mRNA expression after colitis initiation in adulthood. HFD and HFD+5-ASA (**k** (n=11), **l** (n=12), litters=6); NC+5-ASA (**k** (n=6; litters=4), **l** (n=8; litters=6)). HFD (**i** (n=15; litters=12), **j-l** (n=7; litters=4)); HFD+5-ASA (**i** (n=15; litters=12), **j-l** (n=7; litters=4)); No colitis (**e-f** (n=4), **k-l** (n=6), litters=4). For **m** ($^{*}P$=0.03) Log-rank test; **a-l;n-q**, *Post hoc* Tukey-adjusted tests following significant (P<0.05, after correction) Analyses of Deviance (mixed-effect models), all $^{****}P$<0.0001; **d** ($^{**}P$=0.002); **l** (*Reg3b* $^{**}P$=0.009; *Reg3g* $^{*}P$=0.02; *Il33* $^{**}P$=0.003); **o** ($^{***}P$=0.0001); **q** (*Il4* $^{**}P$=0.001, *Il5* $^{***}P$=0.0001). Data were pooled from at least two independent experiments. Each dot represents 1 offspring mouse. Dots of same color and symbol represent mouse from same litter. Data are shown as mean±s.e.m.



Extended Data Fig. 7 | Pathological imprinting through H_2S and microbial immunogens early in life. (a-g) Germ-free mice grown in SL are exposed to sodium hydrogen sulfide (NaHS) and/or heat-killed microbiota (M), or to vehicle during 2- to 4-week-old. Weight of **(a)** epididymal fat, **(b)** retroperitoneal fat, **(c)** liver and colonic mRNA expression for **(d)** cytokines and **(e)** mucins and antimicrobial peptides measured at 4-week-old. **(f)** Disease activity index and **(g)** colonic transcript profile after colitis induction in adulthood. **(h-n)** Adult germ-free mice grown in SL are exposed to NaHS and/or M, or to vehicle during 8- to 10-week-old. **(h)** Body weight, **(i)** colonic mRNA expression and **(j)** gut permeability measured at 10-week-old. **(k)** Loss of body weight, **(l)** disease activity index and **(m)** colonic length after colitis induction at 12 week-old. **(n-r)** Germ-free mice grown in SL are exposed to NaHS and M, and treated with ML-7, or with anti-IFNγ and anti-TNFα (anti-IT) antibodies (mAb) or with vehicle, during 2- to 4-week-old. **(n)** epididymal fat, **(o)** retroperitoneal fat and **(p)** liver at 4-week-old. **(q)** Diseases activity index and **(r)** colonic transcript profile after colitis induction in adulthood. For SL+Vehicle (**a-c,k-m** (n=6; litters=5), **d-e** (n=7; litters=6), **f** (n=9; litters=6), **g** (n=4; litters=4), **h-j** (n=5; litters=5)); SL+NaHS (**a-c**,**d-e** (n=6; litters=5), **f** (n=7; litters=4), **g** (n=6; litters=4)); SL+M (**a-c** (n=7; litters=6), **d-e,g** (n=6; litters=5), **f** (n=8; litters=4)); SL+M+NaHS (**a-c** (n=7; litters=6), **d-e,g** (n=6; litters=5), **f** (n=6; litters=4), **k-m** (n=5; litters=5), **n-r** (n=6; litters=6)); SL+M+NaHS adult (**h** (n=8; litters=7), **i-j** (n=4; litters=4), **k-m** (n=5; litters=5)); SL (**n-r** (n=6; litters=5)); SL+M+NaHS+ML-7 (**n-r** (n=7; litters=7)); SL+M+NaHS+anti-IT (**n-r** (n=7; litters=7)); No colitis group (**g** (n=6), **m** (n=4), **r** (n=5), litters=4); *Post hoc* Tukey-adjusted tests following significant ($P<0.05$, after correction) Analyses of Deviance (mixed-effect models), all $^{****}P<0.0001$; **a** ($^{**}P=0.009$); **b** ($^{**}P=0.006$); **c** ($^{**}P=0.005$); **d** (*Il6* $^{***}P=0.0004$); *Il22* $^{**}P=0.002$, $^{***}P=0.0005$); **f** ($^{*}P=0.01$, $^{***}P=0.0002$); **g** (*Ifng* $^{**}P=0.009$, *Il1b* $^{*}P=0.02$, *Il4* $^{*}P=0.03$, *Il5* $^{**}P=0.007$); **k** ($^{**}P=0.002$, $^{***}P=0.0004$); **l** ($^{**}P=0.003$); **m** ($^{***}P=0.0005$); **q** ($^{**}P=0.005$). ns=not significant. Data were pooled from at least two independent experiments. Each dot represents 1 offspring mouse. Each color represent mouse from same litter. Data are shown as mean±s.e.m.



Extended Data Fig. 8 | Effect of hydrogen sulfide early in life on colitis susceptibility. Weight of **(a)** total body, **(b)** epididymal fat, **(c)** retroperitoneal fat, **(d)** liver, **(e)** intestinal permeability and (**f**) colonic expression of mRNA for cytokines and MLCK and epithelial tight junction proteins at 4 weeks of age in germfree mice grown in small litter (SL) and exposed to slow release of H_2S (GYY4137) and/or heat-killed microbiota (M), or to vehicle control during 2 to 4 weeks of age. **(g)** Percentage of body loss, **(h)** disease activity index, **(i)** colonic length and **(j)** colonic transcript profile after colitis induction in adult germfree mice exposed to a slow-release H_2S donor (GYY4137) and/or heat-killed microbiota (M) or to vehicle control, and grown in SL, during 2 to 4 weeks of age. *Post hoc* Tukey-adjusted tests following significant ($P<0.05$, after correction) Analyses of Deviance (mixed-effect models), all $^{****}P<0.0001$; **d** ($^{***}P=0.0005$); **f** (*Il6* $^{*}P=0.02$; *Il22* $^{*}P=0.03$; *Tjp1* $^{*}P=0.04$; *Tjp2* $^{**}P=0.004$); **g** ($^{*}P=0.03$, $^{***}P=0.0005$); **h** ($^{*}P=0.04$); **i** ($^{***}P=0.0005$); **j** ($^{***}P=0.0008$). ns= not significant. For SL+Vehicle (**a-e** (n=8; litters=7), **f** (n=7; litters=6), **g-j** (n=6; litters=6)); SL+GYY4137 (**a-e** (n=8; litters=6), **f** (n=5; litters=4), **g-j** (n=6; litters=6)); SL+M (**a-e** (n=8; litters=7), **f** (n=6; litters=5), **g-j** (n=6; litters=6)); SL+M+GYY4137 (**a-e** (n=10; litters=9), **f** (n=8; litters=7), **g-j** (n=8; litters=8)); No colitis (**g-j** (n=7; litters=7)). Data were pooled from at least two independent experiments. Each dot represents 1 offspring mouse. Dots of same color and symbol represent mouse from same litter. Data are shown as mean±s.e.m.

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For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

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☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☐	☒ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☐	☒ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Graphpad Prism software (version 7.0e) was used for statistical analyses of survival data, disease activity index, weights data, gene expression data, colonic lenght, and mesurement of lipocalin-2, MPO and FITC-dextran 4kD level. Prism software (Graphpad) was used to plot Kaplan-Meier survival curves, bar graphs, and scatter dot plots presented throughout the manuscript. Figure of Graphpad Prism was used in PPT to generate figures in the manuscript.
Data analysis	Statistical analysis was performed with the R software 3.5.1. Quantitative data were analysed using linear mixed-effects models (R package lme4), in order to account for statistical dependence among individuals originating from a same family (each progeny being reared in a single cage). Depending on the experiment, data could be recorded at several time points; two statistical designs were therefore considered. In simple designs (non-repeated measures) comparisons were made between experimental treatments (fixed effects) while setting the variable cage as a random term. In designs involving repeated measures (i.e., when variables were repeatedly recorded over 21 days), two fixed effects were included, the experimental treatment as well as the date of record (analysed as a categorical variable). The latter models included the mouse ID and the cage label as random factors. The overall statistical significance of experimental treatments was assessed based on an Analysis of Deviance (ANODE, R package car). Given the large number of statistical tests involved in this study, all p-values returned by ANODE (including data displayed in Supplementary Figures) were adjusted using the Benjamini–Yekutieli (BY) procedure, in order to minimize the False Discovery Rate. Any ANODE results that remained significant after BY-adjustement (P-adjusted < 0.05) were followed by post hoc tests. Pairwise testing among treatments was carried out using a Tukey-adjusted comparison (R package lsmeans). In the case of Disease Activity Index, data were averaged across three consecutive days (e.g., across days 19, 20 and 21) within individuals so that the distribution of the averaged index was suited for analysis with mixed-effect models. Survival data were analysed by fitting survival curves with the Kaplan-Meier method and comparison among experimental groups was done using log-rank tests (R package survival).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

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- A list of figures that have associated raw data
- A description of any restrictions on data availability

Provide your data availability statement here.

Field-specific reporting

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	For bacterial load, total protein, cytokine, lipocalin-2 and MPO levels measurements and survival studies, we used animal numbers that are precised in Figure legends.
Data exclusions	No data were excluded.
Replication	All experiments were performed at least twice, and data from individual mice were pooled from all experiments except post-weaning qPCR data where one of twice experiments was shown. The numbers of mice were used in each experiment are indicated in Figure legends. Differences between control and treatment groups showed consistent and reproducible results across our study. The exact number of independent experiments conducted for each experiment are described in Figure legends.
Randomization	All experiments involving mice were carried as described in material and methods. All group were randomly put together after weaning. All experiments involving treatments (e.g. NaHS vs. vehicle) were performed on equal groups of cagemates or littermates.
Blinding	For all experiments in mice, the experimenter measuring body weight, and other parameters was blinded to mouse treatment groups during weaning and at adult procedures until being unblinded at the analysis stage.

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Materials & experimental systems

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☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Specific pathogen free (SPF) females and males C57BL/6 mice were purchased from Charles River and offspring were bred and housed in the environment of the local animal facility at Institut Pasteur. Germ-free C57BL/6 mice were generated at the Gnotobiology Platform of the Institut Pasteur. All mice were weaned 28 days after birth (D28) and co-housing used in all experiments, experimental group were both age- and sex-matched. The age of mice at the beginning of each experiment is indicated in the Figure legends.
Wild animals	*Provide details on animals observed in or captured in the field; report species, sex and age where possible. Describe how animals*

Wild animals	*were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.*
Field-collected samples	*For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.*
Ethics oversight	All animal experiments were approved by the committee on animal experimentation of the Institut Pasteur and by the French Ministry of Research.

Note that full information on the approval of the study protocol must also be provided in the manuscript.