The increased incidence of inflammatory bowel disease (IBD) has become a global phenomenon that could be related to adoption of a Western life-style. Westernization of dietary habits is partly characterized by enrichment with the ω-6 polyunsaturated fatty acid (PUFA) arachidonic acid (AA), which entails risk for developing IBD. Glutathione peroxidase 4 (GPX4) protects against lipid peroxidation (LPO) and cell death termed ferroptosis. We report that small intestinal epithelial cells (IECs) in Crohn’s disease (CD) exhibit impaired GPX4 activity and signs of LPO. PUFAs and specifically AA trigger a cytokine response of IECs which is restricted by GPX4. While GPX4 does not control AA metabolism, cytokine production is governed by similar mechanisms as ferroptosis. A PUFA-enriched Western diet triggers focal granuloma-like neutrophilic enteritis in mice that lack one allele of Gpx4 in IECs. Our study identifies dietary PUFAs as a trigger of GPX4-restricted mucosal inflammation phenocopying aspects of human CD.
Oxidation of biolipids, referred to as lipid peroxidation (LPO), is controlled by enzymatic (e.g., lipoygenase-mediated) and non-enzymatic (e.g., fenton-type) reactions that particularly affect polyunsaturated fatty acids (PUFAs) within biological membranes. LPO impairs cellular functions partly by forming cytotoxic protein adducts or damage to cellular membranes. Compensation is provided by glutathione peroxidase 4 (GPX4), the only selenoprotein that catalyzes the reduction of oxidized biolipids. Deletion of both Gpx4 alleles in mice or pharmacologic GPX4 inhibition in cells induces a distinct regulated form of iron-dependent cell death termed ferroptosis. Ferroptosis requires acyl-CoA synthetase long-chain family member 4 (ACSL4)-mediated membrane enrichment of the ω-6 PUFAs arachidonic acid (AA), which is prone to oxidation. Deletion of both alleles of Gpx4 culminates in organ injury of the kidney, brain, and skin, which is conceivably elicited or modulated by immune responses. While studies have identified key regulators of GPX4-restricted LPO and cellular demise, mechanisms(s) of concurrent inflammatory responses remain elusive.

Inflammatory bowel diseases (IBDs) and specifically Crohn's disease (CD) are characterized by chronic remittent intestinal inflammation that arises from complex interactions between environmental factors (e.g., diet) in a genetically susceptible host. However, plausible examples to support this assumption remain scarce. Notably, the increase in incidence of IBD parallels the increase in dietary intake of ω-6 PUFAs such as AA, which is a major component of a Western diet and contained in meat and eggs. Although AA intake entails a risk for developing CD, which is a major component of a Western diet and contained in meat and eggs. Although AA intake entails a risk for developing CD, which is a major component of a Western diet and contained in meat and eggs.

Results
Impaired epithelial GPX4 activity features CD. To investigate a role of reduced GPX4 activity and LPO in human IBD, we analyzed biopsy-derived IEC-enriched specimens from the lesional and non-lesional mucosa of CD and ulcerative colitis (UC) patients with active disease. Non-IBD patients who underwent screening colonoscopy and lacked demonstrable intestinal disease by endoscopic and histologic means served as healthy controls (HC). Clinical characteristics of this cohort are summarized in Table 1. IECs derived from the lesional small intestinal mucosa of CD patients exhibited decreased expression of GPX4, which was paralleled by decreased enzymatic activity (Fig. 1a-e and Supplementary Fig. 1A). In contrast, colonic GPX4 expression and activity in UC patients was indistinguishable from that in healthy controls (Fig. 1f, g), similar to GPX4 expression in colonic CD (Fig. 1f). In line with this, IECs of the lesional small intestinal mucosa of CD patients exhibited signs of LPO indicated by 4-HNE adducts (Fig. 1h), which was similarly notable in small intestinal epithelial organoids retrieved from lesional mucosa of CD patients (Supplementary Fig. 1B).

PUFAs evoke an inflammatory response of Gpx4-deficient IECs. To analyze the role of GPX4 in IECs, we first generated Gpx4+/− small intestinal epithelial MODE-K cells by CRISPR Cas9 editing of exon 1, which induced IEC death and thus prevented further studies (Supplementary Fig. 1C, D). To assess the consequences of reduced (but not completely abrogated) GPX4 activity in IECs, we silenced MODE-K cells with Gpx4 small-interfering RNA (siGpx4). siGpx4 silencing impaired GPX4 expression and enzymatic activity by ~75% (Supplementary Fig. 1E-G). As oxidation of PUFAs and specifically AA is restricted by GPX4, but in a first step, tested the impact of reduced GPX4 activity and AA exposure on intestinal epithelial LPO. Indeed, the ω-6 PUFA AA deteriorated LPO in Gpx4-deficient IECs (Fig. 2a and Supplementary Fig. 1H). Importantly, AA induced the expression of IL-6 and CXCL1 in Gpx4−/− IECs, but not in control IECs (Fig. 2b–e). Similarly, ω-3 and ω-6 PUFAs, i.e. stearidonic acid (SDA), docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and docosapentaenoic acid (DPA), induced LPO in Fig. 2f and IL-6 and CXCL1 production (Fig. 2g, h) in Gpx4−/−, but not in siCtrl IECs. The saturated long-chain fatty acid palmitic acid (PA) induced LPO, IL-6 and CXCL1 responses to a similar extent in Gpx4−/− and in siCtrl IECs (Fig. 2f–h). Monounsaturated fatty acids such as palmitoleic acid (POA) and oleic acid (OA) did not impact LPO or cytokine production in Gpx4−/− IECs (Fig. 2f–h). Other pro-inflammatory stimuli such as TNFα or IL-1β evoked cytokine responses from siGpx4 IECs that were comparable to siCtrl IECs (Fig. 2i, j). As such, PUFAs particularly elicited LPO and a cytokine response in GPX4-deficient IECs.

A PUFA-enriched Western diet induces enteritis in Gpx4−/−−IECs. Next, we set out to study the impact of PUFAs on intestinal inflammation in GPX4-deficient mice. We were unable to retrieve homozygous Gpx4fl/fl;Villin-Cre+/− (Gpx4−/−−IEC) mice as the offspring died in utero. However, 11% Gpx4−/−−IEC pups were born when the diet of the mothers during gestation

| Table 1 Patient characteristics. | HC | CD | UC |
|--------------------------------|----|----|----|
| Total N                         | 21 | 16 | 12 |
| male/female (%)                 | 43/57 | 50/50 | 75/25 |
| Age (years)                     | 51.62 ± 12.1 | 39.06 ± 14.4 | 40.38 ± 13.7 |
| Body mass index (kg/m²)         | 28.56 ± 6.17 | 24.06 ± 5.81 | 23.70 ± 3.48 |
| MAYO Score                      | N/A | N/A | N/A |
| Harvey-Bradshaw Index           | N/A | 4.67 ± 0.52 | N/A |
| CRP (mg/dl)                     | N/A | 0.55 ± 0.04 | 0.59 ± 0.14 |

HC healthy control, CD: Crohn’s disease, UC: ulcerative colitis, N: patient numbers, CRP: C-reactive protein.
was supplemented with α-tocopherol (Supplementary Table 1, Supplementary Fig. 2A, B). Gpx4<sup>−/−</sup>-IEC pups from α-tocopherol treated mothers had lower weight at birth but regained weight at 7 weeks of age (Supplementary Fig. 2C–F), with a mucosa that was morphologically comparable to WT littermates (Supplementary Fig. 2G, H). For further studies, we utilised Gpx4<sup>fl<sup>ox/</sup>fl<sup>−</sup></sup>/<sup>−</sup> (Gpx4<sup>−/−</sup>-IEC) mice, which specifically deleted one Gpx4 allele in the intestinal epithelium and resulted in a ~50% reduction of mRNA and protein levels in small and large intestinal IECs (Supplementary Fig. 3A, B). Gpx4<sup>−/−</sup>-IEC mice were viable, born at Mendelian ratios and exhibited a mucosal appearance indistinguishable from that of WT littermates (Fig. 3a and Supplementary Fig. 3C). LPO, IEC death and proliferation were comparable between Gpx4<sup>−/−</sup>-IEC and WT mice (Supplementary Fig. 3D–H). Notably, Gpx4<sup>−/−</sup>-IEC mice were susceptible to colonic inflammation induced by dextran sodium sulfate (DSS) (Supplementary Fig. 3I–M), as previously observed in mice with myeloid-specific deletion of Gpx4<sup>−/−</sup>.29

In a next step, we orally challenged Gpx4<sup>−/−</sup>-IEC and WT mice with a Western-style diet (ssniff TD88137) enriched with or without 10% fish oil (containing ω-3 and ω-6 PUFAs, Supplementary Table 2) for 3 months. As expected, WT mice were unaffected by a low-fat control diet (LFD), a Western diet (WD) or a PUFA-enriched Western diet (PUFA WD) (Fig. 3b and Supplementary Fig. 4A). In contrast, a PUFA-enriched Western diet evoked patchy small intestinal inflammation in Gpx4<sup>−/−</sup>-IEC mice, while the colon was unaffected. Specifically, PUFA WD-fed Gpx4<sup>−/−</sup>-IEC mice displayed mucosal to submucosal infiltration of neutrophil granulocytes and mononuclear cells, crypt hyperplasia, epithelial injury and granuloma-like accumulation of inflammatory cells resembling some aspects of small intestinal CD (Fig. 3b–d and Supplementary Fig. 4B). Intestinal inflammation in PUFA WD-fed Gpx4<sup>−/−</sup>-IEC...
Fig. 2 PUFAs trigger epithelial LPO and an inflammatory response restricted by GPX4. **a** LPO quantification by flow cytometry of BODIPY581/591 C11⁺-labeled IECs stimulated with arachidonic acid (AA) for 24 h (n = 6 biologically independent experiments). *P = 0.0183. **b, c** Quantification of IL-6 expression from siGpx4 and siCtrl IECs over a course of AA stimulation determined by qPCR (n = 4 biologically independent experiments), ***P<0.001 (b), and after 24 h by ELISA (n = 12 for vehicle and n = 24 for AA biologically independent experiments). ***P<0.001 (c). **d, e** Quantification of CXCL1 expression from siGpx4 and siCtrl IECs over a course of AA stimulation determined by qPCR (n = 3 biologically independent experiments). **P = 0.001 (d) and after 24 h by ELISA (n = 12 for vehicle and n = 24 for AA), **P = 0.0031 (e). **f** LPO quantification by flow cytometry of BODIPY581/591 C11⁺-labeled IECs stimulated with the saturated fatty acid palmitic acid (PA), monounsaturated fatty acids palmitoleic acid (POA) and oleic acid (OA) and polyunsaturated fatty acids stearidonic acid (SDA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) for 24 h (n = 4–12 biologically independent experiments). For all: ***P<0.001 and **P<0.01 (g, h). Quantification of IL-6 and CXCL1 expression from siGpx4 and siCtrl IECs stimulated with PA, POA, OA, SDA, EPA, DPA, and DHA for 24 h by ELISA (n = 4–11 biologically independent experiments). ***P<0.001 and **P<0.01. **i, j** Quantification of IL-6 and CXCL1 production from siGpx4 and siCtrl IECs stimulated with TNFα or IL-1β for 24 h by ELISA (n = 4 biologically independent experiments). For panel (a), (c) and (e–j) data are presented as boxplot with median and interquartile range (25th and 75th). The whiskers represent minimal and maximal values. For panel (b) and (d) data presented as mean ± SEM. For panel (a–j) to (j) one-way ANOVA with Bonferroni multiple comparison test or a Kruskal–Wallis test with Dunn’s multiple comparison test was used with the exception of panel (b) and (d) for which a two-way ANOVA with Bonferroni post-hoc test was used and panel (f) and (g) for which an unpaired two-tailed Student’s t test was used. Source data are provided as a Source Data file.
mice was characterized by signs of epithelial LPO (Fig. 3e), expression of Cxcl1 (Fig. 3f) and infiltration of MPO+ and GR1+ neutrophils (Fig. 3g, h). Gpx4+/−IEC mice also exhibited higher levels of circulating leukocytes and specifically neutrophil granulocytes in the blood (Fig. 3i). We observed a similar enteritis severity in male and female Gpx4+/−IEC mice (Supplementary Fig. 4C). Notably, a Western diet (rich in saturated fatty acids) did not induce intestinal inflammation in Gpx4+/−IEC mice (Fig. 3b, d and Supplementary Fig. 4D). Although PUFA stimulation (but not cytokines, bile acids or lipopolysaccharide) impaired epithelial GPX4 activity likely because of proteasomal degradation of GPX4 (Supplementary Fig. 5A-H) and Gpx4 deficiency induced ferroptosis of MODE-K IECs to some extent (Supplementary Fig. 6A-I), we did not observe IEC death in Gpx4+/−IEC mice on a PUFA-enriched Western diet (Supplementary Fig. 6J-L). These data indicated that cell death was not a prerequisite of PUFA-induced and GPX4-restricted intestinal inflammation in Gpx4+/−IEC mice.

Cytokine production is driven by ferroptosis mechanisms. We focused on regulatory mechanisms that control AA-induced cytokine production because AA entails a risk for developing IBD20,21. As we noted that AA stimulation promoted LPO only in Gpx4-deficient IECs (Fig. 2a) and cellular iron availability and lipoxgenases (LOX) control LPO in Gpx4-deficient cells1,30, we...
analyzed the impact of iron and LPO on GPX4-restricted cytokine production. Ferric iron promoted LPO (Fig. 4a) and AA-induced cytokine production in siGpx4 IECs (Fig. 4b, c), despite a reduced iron uptake that could not be explained by differential regulation of iron transporters (Supplementary Fig. 7A–G). Vice versa, iron chelation with deferoxamine (DFO) reduced IL-6 and CXCL1 responses of siGpx4 IECs upon AA stimulation (Fig. 4d, e). Moreover, AA-induced cytokine responses were ameliorated by the LPO scavenger ferrostatin-1 and a-tocopherol (Fig. 4f–h). Similarly, inhibition of LOX15 ameliorated AA-induced LPO (Supplementary Fig. 7H) and cytokine production of siGpx4 IECs (Fig. 4i, j). Co-silencing of Alox15 (or Alox12) also ameliorated the cytokine response of siGpx4 IECs after AA stimulation (Fig. 4k and Supplementary Fig. 7I, J). The non-selective COX1/2 inhibitor piroxicam abolished neither CXCL1 production nor LPO (Supplementary Fig. 7K, L). Collectively, these data demonstrate that iron availability promoted PUF-induced LPO and cytokine production in IECs with reduced GPX4 activity, which was reversed by a-tocopherol.

AA and ferric maltol induce enteritis in Gpx4+/–IEC mice. These findings led us to study the direct impact of AA and ferric iron on intestinal inflammation. Indeed, intestinal epithelial organoids from Gpx4+/–IEC mice, but not WT controls, displayed signs of LPO and increased Cxcl1 expression on AA and ferric iron exposure (Fig. 5a, b and Supplementary Fig. 8a), while unstimulated Gpx4+/–IEC organoids were indistinguishable from WT organoids (Supplementary Fig. 8B-G). Next, we orally challenged 8-week old WT and Gpx4+/–IEC mice with AA once daily for five consecutive days complementary to the standard chow diet in addition to iron supplementation with ferric maltol (Fig. 5c), which is approved for treatment of iron-deficiency anemia in IBDS. We used this model as it may reflect a daily PUFA and iron challenge in humans on a meat-enriched Western diet19. WT mice were unaffected by oral AA and ferric maltol exposure (Fig. 5d, e). In contrast, Gpx4+/–IEC mice exhibited inflammation in the intestine when exposed to AA and ferric maltol (Fig. 5d, e). More specifically, acute inflammation in Gpx4+/–IEC mice was characterized by neutrophil infiltration in the proximal small intestine (Fig. 5d, e), the locale of PUFA and iron absorption23,23, as corroborated by flow cytometry of GR1+ neutrophils (Fig. 5f) and immuno-labeling of MPO+ cells (Supplementary Fig. 8H). Neutrophil infiltration was paralleled by signs of epithelial LPO (Fig. 5g) and increased Cxcl1 expression (Fig. 5h). Notably, ferric maltol or AA exposure alone did not evoke neutrophilic inflammation in Gpx4+/–IEC mice (Fig. 5e). The abundance of other mucosal innate and adaptive immune cells remained comparable between WT and Gpx4+/–IEC mice exposed to AA and ferric maltol (Supplementary Fig. 9A–H) and we did not note colonic inflammation (Supplementary Fig. 10A). These data demonstrated that intestinal epithelial GPX4 restrained neutrophilic small intestinal inflammation induced by AA and ferric maltol. In line with a critical role of LPO in inflammation, a-tocopherol treatment protected against PUF-induced LPO and cytokine production (Fig. 4f–h) and neutrophilic infiltration in Gpx4+/–IEC mice challenged with AA and ferric maltol (Fig. 5i). Similarly, a-tocopherol, as well as liproxstatin-1 treatment, protected against enteritis in Gpx4+/–IEC mice induced by a PUFA WD (Fig. 5j, k), which was associated with reduced signs of LPO and neutrophil infiltration (Supplementary Fig. 10B–D).

Cytokine production is governed by ACSL4. To further explore how AA may instigate cytokine production, we used liquid chromatography tandem mass spectrometry (LC-MS/MS) to investigate the AA metabolite profile of siGpx4 IECs as compared to that of controls. AA may be metabolized by cyclooxygenases (COX), lipoxynegenases (LOX), and cytochrome P450 enzymes to bioactive lipid mediators, which occurred to a similar extent in AA-stimulated siGpx4 and siCtrl IECs (Fig. 6a–c, Supplementary Table 3). In line with this, Lox and Cox expression was comparable between siCtrl and siGpx4 IECs (Supplementary Fig. 11A). As Acsl4 is required for IEC ferroptosis (Supplementary Fig. 6I)2,6, we next hypothesized that AA-induced inflammation in siGpx4 IECs required ACSL4. Indeed, Acsl4 deletion abolished AA-induced IL-6 and CXCL1 production in siGpx4 IECs (Fig. 6d, e).

Next, we sought to understand how ACSL4 controlled AA-induced cytokine responses. Notably, Acsl4 deletion in siGpx4 IECs did not protect against LPO after AA exposure (Supplementary Fig. 11B). These data indicated that ACSL4 controlled AA-induced cytokine production using a distinct mechanism that was independent of LPO. To explore a role of ACSL4 in AA metabolism, we analyzed the metabolite profile in Acsl4−/−IECs by means of LC-MS/MS (Fig. 6a–c). Indeed, Acsl4−/−IECs exhibited a decreased abundance of LOX and COX metabolites after AA stimulation in siCtrl and siGpx4 IECs (Fig. 6a, b).
However, none of the abundant LOX and COX lipid mediators (i.e. 5-HETE, a 15-HETE precursor or PGE2) was able to promote the production of IL-6 or CXCL1 in our model (Supplementary Fig. 11C–H). These data suggested that modulation of LOX and COX metabolism by ACSL4 did not affect the inflammatory tone. However, we noted that P450 metabolites of AA (i.e. epoxyeicosatrienoic acids or ‘EETs’) were increasingly accumulating in Acsl4−/− IECs after AA stimulation (Fig. 6c). A combination of EET’s ameliorated AA-induced IL-6 and CXCL1 production in siGpx4 IECs (Fig. 6f, g), likely due to their anti-inflammatory effect that suppressed NF-κB34. In line with this, we noted that AA induced activation of NF-κB p65 in siGpx4 IECs (Supplementary Fig. 11I, J), and NF-κB inhibition with BAY11-7082 or MG132 abolished IL-6 production in siGpx4 IECs independent of LPO.
Discussion

Westernization of dietary habits, partially characterized by enrichment with PUFAs35,36, paralleled the increased IBD incidence19. Previous observations associated PUFA uptake and mucosal AA accumulation with the risk of developing IBD20,21,37. Large prospective clinical trials in CD patients (and patients without IBD) indicated that PUFA supplementation may cause gastrointestinal side effects (e.g. diarrhea), indicative for disturbed intestinal homeostasis38,39. In contrast, dietary restriction (e.g. by an elemental diet) ameliorates the course of CD40,41. These reports and other studies15 indicate that dietary cues impact the risk of developing CD and affect the natural history of disease. However, a direct link between PUFA uptake and intestinal inflammation remained elusive. Our study establishes that dietary-derived PUFAs trigger neutrophil inflammation in the small intestine resembling some aspects of human CD.

IECs have the delicate task of maintaining a physical and immunological line of defense to protect the host against a potentially hostile environment. At the same time IECs must continue to allow uptake of essential nutrients such as long-chain fatty acids42. A hypoxic milieu and exposure to luminal noxae may specifically require GPX4 activity in IECs to protect against cellular LPO, a critical condition that determines cell fate1. While substantial advances have shaped our understanding of ferrophtosis1, GPX4-restricted immunologic responses remain poorly explored despite reports of inflammatory tissue injury in GPX4-deficient animals9,11,27. We report that IECs with reduced GPX4 activity respond to non-toxic dietary PUFA exposure with an inflammatory response involving IL-6 and CXCL1. PUFA-induced CXCL1 production involving siGpx4 IECs was comparable to CXCL1 production induced by TNFa stimulation. In contrast IL-6 production was far less pronounced in PUFA-stimulated siGpx4 IECs, when compared to TNFa stimulation. Future studies will delineate the relevance of both cytokines as driver of mucosal inflammation in our model. Notably, exposing ω-3 PUFAs such as DHA (which is thought to exert anti-inflammatory effects43) elicited cytokine production similar to AA (which is thought to exert inflammatory effects) only in Gpx4-deficient IECs. As such it appears that the availability (rather than the positioning) of double bonds within PUFAs define their propensity to fuel LPO and an inflammatory response. These findings led us to explore the consequences of PUFA exposure on IECs with impaired GPX4 activity in the intestine. We generated mice with IEC-specific deletion of one Gpx4 allele (Gpx4+/−/IEC), which is a valuable tool for modeling the effects of reduced, but not completely abrogated epithelial GPX4 activity as observed in patients with small intestinal CD. Indeed, a PUFA-enriched WD containing 10% fish oil (with ω-3 and ω-6 PUFAs) evoked focal neutrophilic enteritis in male and female Gpx4+/−/IEC mice that was characterized by granuloma-like mucosal to submucosal accumulation of inflammatory cells and expression of the IL-8 homologue Cxcl1. As such, our findings represent first evidence that non-toxic dietary lipids trigger focal enteritis in a genetically susceptible host resembling some aspects of human small intestinal CD. Future studies are warranted to explore the immune-phenotype in more detail. Notably, a WD rich in saturated fatty acids (without addition of fish oil) did not induce intestinal inflammation in Gpx4+/−/IEC mice, indicating that PUFA supplementation specifically elicited the inflammatory phenotype.

We did not note colonic intestinal inflammation in PUFA WD- or AA/FM-exposed Gpx4+/−/IEC mice, suggesting that environmental cues may determine disease localization. We speculate that PUFAs and iron are specifically absorbed in the small intestine32,33,44, which could be one explanation for small intestinal disease localization and a requirement for GPX4 activity in the small intestine. In line with a small intestinal phenotype in mice, we specifically noted reduced epithelial GPX4 activity in active small intestinal CD. In contrast, we did not note altered GPX4 activity in colonic CD or UC.

Of note, fish oil supplementation has been tested in the maintenance of remission in CD patients with mixed results. More specifically, fish oil supplementation had favorable effects on maintenance of remission in some patients35, while larger studies observed no beneficial effect but worsening of gastrointestinal symptoms (e.g. diarrhea)35–47. This observation indicates that dietary PUFA exposure impacts the course of CD. Similarly, PUFA supplementation induces gastrointestinal symptoms (diarrhea, abdominal pain and nausea) in patients without IBD39. Which factors define a beneficial or detrimental response on PUFA challenge (e.g. fish oil or other sources such as fish, meat and eggs) in CD (or healthy) patients is unknown. Our data indicate that PUFAs trigger GPX4-restricted mucosal inflammation resembling some aspects of human CD.

Ferrophtosis is fundamentally controlled by lipooxygenase-driven LPO, which is limited by GPX4 and driven by cellular iron availability1. Similarly, we found that PUFA exposure induced LPO and cytokine production of siGpx4 IECs, while pharmacologic iron chelation with DFO, inhibition of lipooxygenases and LPO scavenging with α-tocopherol ameliorated this phenotype. Genetic deletion of Acs4 (which is required for ferrophtosis24), abrogated PUFA-induced cytokine production in IECs with reduced GPX4 activity. While GPX4 did not control AA metabolism, we found that ACSL4 limited the generation of anti-inflammatory AA metabolites (epoxyeicosatrienoic acids or EET’s), which have been demonstrated to inhibit NF-κB

( Supplementary Fig. 11K, L). As such, Acs4 deletion may limit AA-induced cytokine responses by modulating AA metabolism34, while GPX4 controlled LPO.
Indeed, a combination of EETs, but not a single EET alone, reduced AA-induced cytokine production similar to pharmacologic inhibition of NF-κB. These data suggest that GPX4-restricted LPO and ACSL4-controlled AA metabolism converge on NF-κB-mediated transcription of inflammatory cytokines specifically in GPX4-deficient IECs. As such, the same mechanisms (i.e. iron availability, LOX-mediated LPO and ACSL4) that control ferroptosis also control PUFA-induced cytokine production in Gpx4−/−IECs. These findings lay the foundation for understanding dietary lipid-induced intestinal inflammation. Indeed, AA and ferric maltol (approved for the treatment of iron-deficiency anemia in IBD) triggered epithelial LPO, Cxcl1 expression and small intestinal neutrophilic infiltration in WT mice. Similarly, Gpx4−/−IECs exposed to a PUFA WD exhibited increased LPO and neutrophilic inflammation, and LPO scavenging with α-tocopherol ameliorated enteritis in both models. These findings indicate that GPX4 protects against epithelial LPO, which sets a threshold for intestinal inflammation triggered by dietary lipids. Although LPO is a key feature of ferroptosis, cell death was not a prerequisite for intestinal inflammation indicated by a lack of epithelial TUNEL labeling in Gpx4−/−IECs. One reason for this may be that one Gpx4 allele is sufficient to protect against ferroptotic IEC death, but insufficient to prevent PUFA-induced cytokine production. Future work may define a role for ferroptotic cell death in mucosal inflammation.

Collectively, our data support a model in which dietary PUFAs elicit neutrophilic inflammation, which emanates from IECs with reduced GPX4 activity (Supplementary Fig. 12). Our findings turn the spotlight onto GPX4-restricted oxidative processes, which determine the competence of the epithelium to cope with environmental cues that inevitably occur on mucosal surfaces. Impaired GPX4 activity observed in small intestinal CD may arise from dietary long-chain fatty acids (Supplementary Fig. 5), (yet unidentified) immune mediators, bile salts or microbial activity. Indeed, a combination of EETs, but not a single EET alone, reduced AA-induced cytokine production similar to pharmacologic inhibition of NF-κB. These data suggest that GPX4-restricted LPO and ACSL4-controlled AA metabolism converge on NF-κB-mediated transcription of inflammatory cytokines specifically in GPX4-deficient IECs. As such, the same mechanisms (i.e. iron availability, LOX-mediated LPO and ACSL4) that control ferroptosis also control PUFA-induced cytokine production in Gpx4−/−IECs. These findings lay the foundation for understanding dietary lipid-induced intestinal inflammation. Indeed, AA and ferric maltol (approved for the treatment of iron-deficiency anemia in IBD) triggered epithelial LPO, Cxcl1 expression and small intestinal neutrophilic infiltration in WT mice. Similarly, Gpx4−/−IECs exposed to a PUFA WD exhibited increased LPO and neutrophilic inflammation, and LPO scavenging with α-tocopherol ameliorated enteritis in both models. These findings indicate that GPX4 protects against epithelial LPO, which sets a threshold for intestinal inflammation triggered by dietary lipids. Although LPO is a key feature of ferroptosis, cell death was not a prerequisite for intestinal inflammation indicated by a lack of epithelial TUNEL labeling in Gpx4−/−IECs. One reason for this may be that one Gpx4 allele is sufficient to protect against ferroptotic IEC death, but insufficient to prevent PUFA-induced cytokine production. Future work may define a role for ferroptotic cell death in mucosal inflammation.

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metabolites\textsuperscript{50} that are absorbed in the small intestine. As we specifically observed impaired GPX4 activity in IECs from the inflamed (but not uninflamed) mucosa of CD patients, it appears plausible that GPX4 deficiency is no primary defect in CD but occurs secondary to insults (likely caused by yet unidentified cellular stressors) in the inflamed mucosa. We speculate that IBD-related genetic cues may impinge on GPX4 activity during inflammation\textsuperscript{51}, and that genetic and environmental mucosal insults (other than PUFAs) trigger an inflammatory response in GPX4-deficient hosts\textsuperscript{52}. Future studies will advocate a critical role of oxidative processes on cellular membranes of IECs and decipher the impact of oxidized lipid species in mucosal inflammation and IBD\textsuperscript{33-35}.

\textbf{Methods}

\textbf{Human studies.} IBD patients were recruited in the gastrointestinal outpatient clinic of the Medical University of Innsbruck and included when a definitive IBD diagnosis (by clinical, endoscopic, and histopathological means) was established and informed consent was obtained. Healthy controls undergoing screening for patients were included after informed consent was obtained. Patients were excluded if they lacked endoscopic and histological signs of intestinal disease—were included after informed consent was obtained. Patients were excluded if any of these criteria were not fulfilled or if the histology report and the endoscopic report conflicted. Ileal and colonic biopsies were collected from IBD patients and healthy controls, stored on ice in RPMI medium or formalin and processed the same day. One biopsy was used for mRNA isolation and one biopsy was formalin-fixed and paraffin-embedded. IEC suspensions were snap-frozen in liquid nitrogen and stored at \(-80 °C\) until GPX4 enzymatic activity testing (see section IEC isolation. IEC suspensions were snap-frozen in liquid nitrogen and stored at \(-80 °C\) until GPX4 enzymatic activity testing (see section IEC isolation).

\textbf{Reagents.} The following reagents were used for cell stimulation: deferoxamine (0.1-20 µM, Sigma, D9533), \(\beta\)-thujaplicin (Hinokitol, Sigma, 469521), deferasirox (Exadjaste*, Novartis), fer(III) sulfate (2-20 µM, Sigma, F6038), arachidonic acid (AA, 20 µM, Sigma, A3611), docosahexaenoic acid (DHA, 500 µM, Sigma, D2534), palmitic acid (PA, 250 µM, Sigma, P5585), oleic acid (OA, 250 µM, Sigma, O1008), palmitoleic acid (POA, 100 µM, Sigma P9417), stearidonic acid (SDA, 50 µM, Sigma SM00291), eicosapentaenoic acid (EPA, 100 µM, Sigma E2011), docosapentaenoic acid (DPA, 25 µM, Sigma D1797), ferrostatin-1 (1-4, 0.1-1 µM, Sigma, SM0586), \(\alpha\)-tocopherol (\(\alpha\)-toco, 0.1-1 µM, Sigma, T2351), Z-VAD-FMK (0.1-100 µM, BD Bioscience, 250337), TNFa (50 ng/ml, PeproTech), IFN\(\gamma\) (50 ng/ml, R&D), IL-1\(\beta\) (50 ng/ml, PeproTech), LPS (100 ng/ml, Sigma L4524), IFN\(\gamma\) (250µg/ml, PBL, 12401-1), IL-4 (10 ng/ml, PeproTech 214-14), IL-6 (20 ng/ml, PeproTech 216-16), Liproxstatin-1 (1% Biochrome, 0257 F), HEK293T cells (Biochrome, S0115), HEPES (10 mM, Biochrome, L1613), non-essential amino acids (500 µM, Sigma, N2840), D-glucose (1% Biochrome, 90257 F), high-glucose DMEM (Lonza, BE12-040), RPMI (Lonza, BE12-040), fetal bovine serum (FBS, Biochrome, F0540), L-glutamine (250 µM, Sigma, G8504), pyruvate (10 mM, Sigma, P8383), penicillin (100 U/ml, Sigma P0789), streptomycin (100 µg/ml, Sigma S1523), 4-HNE-labeled organoids (green) from indicated genotypes after stimulation with AA and ferric iron or vehicle for 24 h. Scale bars indicate 20 µm (n = 3 biologically independent samples). Data are presented as boxplot with median and interquartile range (25th and 75th). The whiskers represent minimal and maximal values. *\(P<0.05\), †\(P<0.01\), ‡\(P<0.001\). **\(P<0.001\) Neutrophilic infiltration of IBD\textsuperscript{+} tissues by flow cytometry of indicated genotypes from the experiment shown in (c). Each dot represents an experimental animal (n = 6 WT mice and n = 5 Gpx4\textsuperscript{+/-}IEC mice). *\(P = 0.0228\). g Representative images of 4-HNE immunoreactivity (brown), indicative for LPO (n = 5 mice per group). Scale bars indicate 100 µm. h Quantification of 4-HNE expression determined by qPCR in intestinal epithelial scrapings of indicated genotypes from the experiment shown in (c). Each dot represents one experimental animal (n = 10 mice per group). P = 0.0435. Mann-Whitney test. I Histology score of AA- and ferric maltol-exposed mice with or without \(\alpha\)-tocopherol supplementation [0.4 mg/ml] in drinking water over the course of the experiment. Each dot represents one experimental animal (n = 4 mice for WT + vehicle, n = 7 mice for WT + \(\alpha\)-toco, n = 9 mice for Gpx4\textsuperscript{+/-}IEC + vehicle and n = 8 mice for Gpx4\textsuperscript{+/-}IEC + + + vehicle). P = 0.0302. j Entorist histology score of WT and Gpx4\textsuperscript{+/-}IEC mice exposed to a PUFAn-enriched WD (PUNA WD) for 3 months with and without \(\alpha\)-tocopherol supplementation [0.4 mg/ml] in drinking water over the course of the experiment. Each dot represents one experimental animal. Median shown (n = 9 mice for WT PUNA WD + vehicle, n = 14 mice for Gpx4\textsuperscript{+/-}IEC PUNA WD + vehicle, n = 9 mice PUNA WD + a-toco). **\(P<0.01\). k Enteritis histology score of WT and Gpx4\textsuperscript{+/-}IEC mice exposed to a PUFAn-enriched WD (PUNA WD) for 3 months with and without liproxstatin-1 treatment intraperitoneally from 6 weeks (10 mg/kg) until the closure of the experiment. Each dot represents one experimental animal. Median shown (n = 5 mice for WT PUNA WD + vehicle, n = 8 mice for Gpx4\textsuperscript{+/-}IEC PUNA WD + vehicle, n = 9 mice PUNA WD + liproxstatin-1). ***\(P<0.001\). For panel (e), (f), (h), and (i) data are presented as mean±SEM. For panel (b), (f) and (h) unpaired two-tailed Student’s t test and for panel (e) and (i-k) one-way ANOVA with Bonferroni’s multiple comparison test was used. Source data are provided as a Source Data file.
IL-22 (10 ng/ml, PeproTech 210-22), cholic acid (1 µM, Sigma C9282), deoxycholic acid (1 µM, Sigma D2510), ursodeoxycholic acid (1 µM, Sigma US5127), (−/−)11(12)- and (−/−)11(12)- and (−/−)14(15)-EETs (0.25 µM Cayman Chemicals, 50511, 50651, 50351), ML351 (10 µM, Sigma, SML1353), PDI46176 (0.5 µM, Sigma, P4620), zileuton (10 µM, Sigma, Z4277), NCTC 956 (2 µM, Sigma, SML0499), peroxiscam (20 µM, Sigma, P5634), 5-HETE (200nM-1µM, Cayman Chemicals, 34210), 15-HPETE (200nM-1µM, Cayman Chemicals, 44720), PGE2 (50 ng/ml Sigma, P0409), BAY117082 (10 µM, Sigma, B5556), MG132 (125 nM, ApexBio, A2585).

siRNA silencing. MODE-K IECs were seeded on 6-well plates at ~70% confluence for siRNA silencing with either Gpx4 siRNA (siGpx4, Ambion, s122089), Alox12 (siAlox12, Ambion, s62261), Alox15 (siAlox15, Ambion, s62271) or scrambled control siRNA (siCtrl, Ambion 4390843) and RNAiMAX (Thermo Fisher Scientific, 13778100) transfection over 48 h unless otherwise indicated, according to the recommended protocol.

CRISPR Cas9 gene editing. Target genes were disrupted in MODE-K IECs using the CRISPR/Cas9 system. For each gene three guide RNAs (gRNA) targeting different exons of the target gene were designed. gRNAs with specific overhangs were annealed into a BsmBI-digested plentiCRISPRv2 plasmid (Addgene #52961, gift from Feng Zhang). Vectors were transfected into HEK293T cells (ATCC® CRL-1573™) to produce viral particles. Harvested supernatants were used for gene knockout. For transfection, MODE-K cells were seeded on 6-well plates and infected with viral particles containing the constructed vectors. The guide RNA sequence used for studies in Gpx4−/−IECs was CTTGTTGCATGTCACCAACGC, which targeted exon 1. The guide RNA sequence used for studies in Acsl4−/−IECs was CAATAGACCAGATCCTCGT, which targeted exon 6. To generate Gpx4−/− and Acsl4−/− clones by CRISPR Cas9 gene editing, transfection was performed for 48 h followed by puromycin (Gibco, A1113803) selection for 10 days and subsequent seeding onto 96-well plates with one cell per well for expansion.

Human intestinal epithelial organoids. Human organoids were cultured from 4- to 8-week-old Gpx4−/−IECs and littersate WT mice using IntestiCult Organoid Growth Medium (Stemcell Technologies) and a protocol adapted from manufacturer’s instructions. Briefly, biopsies were flushed with 10 ml of ice cold PBS and minced into the small pieces. Tissue was then transferred to 5 ml Gentle Cell Dissociation Reagent (Stemcell) and incubated at 4 °C on a rocking platform for 30 min. After centrifugation at 4 °C and 250g for 5 min supernatant was removed and crypts were transferred to 1 ml of ice cold 1% RNA/DMEM. Crypts were dissolved by gentle mixing and passed through a 70 µm cell strainer. Seeding was identical to that for mouse organoids (see below). Human organoids were passaged with a split ratio of 1:3 every 7–14 days.

Mouse intestinal epithelial organoids. Intestinal organoids were cultured from 4- to 8-week-old Gpx4−/−IECs and littersate WT mice using IntestiCult Organoid Growth Medium (Stemcell Technologies, 06065) and a protocol adapted from manufacturer’s instructions as initially described. Briefly, small intestines were flushed with ice cold PBS, minced to pieces of approximately 2–3 mm in size and washed up to five times with 10 ml ice cold PBS. Samples were transferred to...
2 mEq EDTA/PBS and incubated at 4 °C on a rocking platform for 30 min. After sedimentation supernatant was removed and crypts eluted in 10 ml PBS by shaking vigorously. The eluted and permeabilized crypts through a 70 μm cell strainer to obtain Fraction I. This process was repeated three times to obtain Fractions 2–4. Fractions were analyzed under a light microscope and the optimal fraction was chosen to obtain crypts for organoid culture by centrifugation at 290g for 5 min at 4 °C. Crypts (100,000 per well) were seeded in 50 μl Matrigel (BD, 356231) on a pre-warmed 24-well plate and allowed to solidify at 37 °C, after which 500 μl IntestiCult Growth Medium supplemented with 100 μl penicillin and 100 μg/ml streptomycin (Biochrome, 0257F) was added. Medium was exchanged three times per week and organoids passed with a split ratio of 1:6 every 7–14 days (Supplementary Fig. 8A-G).

Stimulation of mouse organoids. Before stimulation, organoids were allowed to establish for 6 days. Organoids were stimulated with arachidonic acid (AA, 100 μM), Sigma, A3611) and Fe(III) sulfate (5 μM, Sigma, F6038) or vehicle for indicated time periods depending on the experiment setting. Medium was replaced every 24 h. For RNA extraction TRIZol reagent (Invitrogen, 15596026) was used according to the manufacturer’s instructions.

Immunofluorescence Imaging. Organoids were cultured with 15 μl Matrigel (BD, 356231) on chamber slides (Falcon, 354108) covered with 200 μl IntestiCult Growth Medium supplemented with 100 μl penicillin and 100 μg/ml streptomycin (Biochrome, 0257 F). Medium was exchanged three times a week and organoids were allowed to establish for 6 days. Organoids were stimulated as described above for 24 h, washed with PBS and fixed with 4% PFA at room temperature for 20 min, washed again twice with PBS and permeabilized with PBS-Triton X 0.5% for 20 min at room temperature. Organoids were washed with IF buffer (PBS, Triton X 0.3%, Tween 0.05%), blocked with blocking solution (PBS-Triton X 0.3%, Tween 0.05%, BSA 1%), followed by another wash with IF buffer and primary antibody. Blocking solution was added over night at 4 °C. Organoids were washed three times with IF buffer and secondary antibody was added for 1 h, washed three times with IF buffer, mounted (Invitrogen, P36962) and analyzed with a Zeiss Axio Observer Z1 confocal microscope and Zen 2012 software. Triton X 0.3%, Tween 0.05%, BSA 1%, followed by another wash with IF buffer and primary antibody. Signal was visualized with a Zeiss Axio Z1 confocal microscope and Zen 2012 software.

Immunohistochemistry, TUNEL, BrdU, and PAS labeling. Formalin-fixed paraffin-embedded sections were dewaxed with xylene and treated with 3% H2O2 for 15 min to quench endogenous peroxidases. After washing in PBS, sections were immersed in 0.3% H2O2 and 1% hydrogen peroxide for 10 min at room temperature for 30 min. After washing in PBS, sections were incubated for 20 min in 3% H2O2. The primary antibody was incubated overnight at 4 °C. The secondary biotinylated antibody mediated horseradish peroxidase (HRP)-conjugated 3,3’-diaminobenzidine (DAB, DAKO, K3468) turnover, which resulted in brown labeling of immunoreactive cells. Stained sections were analyzed with a light microscope (Zeiss, Germany) and captured with a Zeiss AxioCam. TUNEL labeling was performed according to the manufacturer’s instructions (Roche, 11684817910). TUNEL-positive cells were quantified in 50 consecutive histological sections. Histological scores (0, absent; 1, mild; 2, moderate; 3, severe) were mononuclear cell infiltration, crypt hyperplasia, epithelial injury/erosion, polymorphonuclear cell infiltration and transmural inflammation. The sum of these scores was multiplied by a factor that reflected the extent of inflammation along the intestine (1, 10%; 2, 10–23%; 3, 25–50%; and 4, >50%).

Mouse IEC isolation. Mouse IEC isolation protocol was performed as previously described57. Briefly, PBS-flushed and longitudinally cut intestinal pieces were vortexed with ice cold PBS 5 min, transferred to 30 ml EDTA, and digested overnight using 500 μl of 10 mg/ml collagenase (128U/ml, Sigma, C1889) digested on a shaker for 60 min at 37 °C. Tissue was washed through cell strainers (100 μm) and washed twice in FACS buffer and transferred using 40 μm cell strainer for flow cytometry. Annexin V, PI or 7AAD were used for cell death analysis.

LPO and cell death labeling. Cells derived from cell culture or from IEC isolation procedures were incubated with BODIPY FL 581/591 CI1 or the surface labeling antibodies (see below) at 37 °C in the dark for ten to 30 min in flow cytometry buffer (2% FCS, 2 μM EDTA in PBS). Cells were subsequently washed with PBS, resuspended in FACS buffer and transferred through a 40 μm cell strainer for flow cytometry. Annexin V, PI or 7AAD were used for cell death analysis.

Flow cytometry analysis. For LPO analysis, BODIPY-positive cells among DAPI-negative cells were analyzed as compared to a control sample using BD LSR II flow cytometry measurement. For cell death analysis, debris was excluded using FSC/SSC characteristics; Annexin V and PI or 7AAD positivity was determined by flow cytometry.

FACS gating strategy. The gating strategy for analyzing the mucosal cellular infiltrate is depicted in Supplementary Fig. 9A, E. Briefly, cells were gated using FSC/SSC characteristics. Single cell suspensions were analyzed using BD FACSDIVA software. Neutrophils were identified as CD45+, Lin1− (Lin1=CD3, CD19, CD49b, DAPI) and Gr1+ cells. Macrophages were identified by CD45+, Lin1+, Gr1−, CD11b+, MerTK+. Monocytes were characterized by CD45+, Lin1−, Gr1−, Ly6C+ and Ly6C+ dendritic cells were characterized by CD45+, Lin1−, Gr1−, CD11c+, MHCII+ and CD163−. CD4-positive helper cells were defined by Lin2− (Lin2=CD11c, F4/80, Gr1, DAPI), CD3+, CD19−, CD4+). Cytotoxic T cells were defined by Lin2−, CD3+, CD19−. B cells were defined as Lin2−, CD23−, CD19+. Details of antibodies used are found in Supplementary Table 4.

RNA extraction and qRT-PCR. RNA was isolated from 6-well plates, epithelial scrapings or human intestinal biopsies using a RNeasy mini kit (Qiagen 74104). cDNA was synthesized using M-MLV reverse transcriptase (Invitrogen, 20205013). Quantitative real time PCR was performed with SYBR green mastermix (Eurogentec RT-SY2X-06-w-WOULR) on a MX3005 Stratagene cycler (Agilent). The primer used in this study can be found in Supplementary Table 4.

Immunoblotting. Western blot analysis was performed according to standard protocols (Bio-Rad Laboratories). Briefly, isolated cells (from culture plates or scrapings) were lysed in RIPA buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, and 0.1% SDS) or M-Per (Thermo Fisher Scientific, 78501) and supplemented with protease and phosphatase inhibitors (Thermo Fisher Scientific, 78443). Protein quantity was determined by Bradford assay (BioRad Laboratories, 5000006) and equal amounts of protein were denatured at 95 °C in Laemmli buffer, resolved on SDS-PAGE and transferred to a polyvinylidene fluoride membrane (Sigma, GE10600023). After blocking the membrane in 5% skim milk, primary antibody was incubated over night at 4 °C. Signal was visualized with HRP-conjugated secondary antibodies (Cell Signalling Technology, 7074) and ECL Select Western Blotting Detection Reagent (Amersham, RNA2323). Densitometry of immunoblots was performed with ImageJ. All uncropped and unedited blots are available in the Source Data. The following antibodies were used: anti-GPX4 (1:2000, Abcam, ab125066), anti-ACSL4 (1:1000, Abcam, ab155282), anti-TIR (1:1000, Invitrogen, H68.4), anti-FPN1 (1:1000, Eurogentec, Liege, Belgium) anti-ferritin (1:1000, Sigma, F5012), anti-phospho-NF-κB p65 (1:1000, Cell Signalling Technology, 3039), anti-MPO (1:2000, Fisher Scientific), anti-MHCII (1:1000, Santa Cruz, sc-6242), with anti-GAPDH (1:2000, Cell Signalling Technology, 3039), anti-NF-κB (1:1000, Cell Signalling Technology, 2118) or anti-β-actin (1:2000, Sigma, A2066 and Abcam, ab9990) as loading control.
Cytokine quantification. Cellular supernatants were collected, centrifuged at 300g for 5 min and stored at −20 °C. Cytokine quantification was performed by ELISA (IL-6, BD Biosciences, 552540; CXCL1/RC, R&D, DY453) according to the manufacturer’s protocol.

Cell viability assay. Cells were seeded on 96-well plates (2000 cells per well) and treated with siRNA for 48 h. Cell viability was assessed by AlamarBlue turnover recommendations.

Quantification of iron uptake and release. Iron uptake and release were performed as previously described. Briefly, MODE-K IECs were silenced for 48 h with siGpx4 or siCtrl and washed with high-glucose DMEM (1% FCS, 1% peni-cillin/streptomycin, 25 mM HEPEs). To determine non-transferrin-bound iron uptake and release, cells were incubated with 5 µM ferric chloride (Perkin Elmer, NEZ037) for 2 h. After washing, cells were transferred to high-glucose DMEM (1% FCS, 1% penicillin/streptomycin, 25 mM HEPEs) and incubated for 1 h. Iron uptake and release were measured with a γ counter (Perkin Elmer). Uptake and release were performed on CPM (counts per minute) were normalized to protein quantity as determined by BCA Assay (Thermo Fisher Scientific, 23225).

GPX4 enzymatic activity assay. Cells were collected in lysis buffer (100 mM Tris pH 7.6, 5 mM EDTA, 1 mM Na3VO4 and 0.1% peroxide-free Triton-X100). Lysates were complemented with 0.6 U/ml glutathione reductase (Sigma, G3664), 0.2 mM nicotinamide adenine dinucleotide phosphate hydroxide (NADPH, Sigma, N7505), 3 mM reduced glutathione (GSH, Sigma, G4251) and 200 µM of the substrate composition. The reaction was initiated by addition of 10 nM of reduced glutathione and incubated for 30 min in the presence of 0.05 mM of the substrate (i.e. pg/sample). Validation and extraction efficiency of GPX4 enzymatic activity were performed on a 6-plex assay (Agilent Technologies), equipped with electrospray ionization source, performed in triplicates using an Infinity 1290 UPLC/MS/MS (Agilent Technologies). The dataset generated in this study are deposited in a publicly available platform (https://data.mendeley.com, 10.17632/k9ynzckd3g.2; Data for: Dietary lipids fuel GPX4-restricted enteritis resembling Crohn’s disease). The source data for Figs. 1-6 and Supplementary Fig. S1-11 are provided in the Source Data File.

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