HHS Public Access

Author manuscript

Science. Author manuscript; available in PMC 2024 February 14.

Published in final edited form as:

Science. 2023 September 08; 381(6662): 1092–1098. doi:10.1126/science.add5649.

A type 2 immune circuit in the stomach controls mammalian adaptation to dietary chitin

Do-Hyun Kim[1], **Yilin Wang**[1], **Haerin Jung**[1], **Rachael L. Field**[1], **Xinya Zhang**[1], **Ta-Chiang Liu**[1], **Changqing Ma**[1], **James S. Fraser**[2], **Jonathan R. Brestoff**[1], **Steven J. Van Dyken**[1,*]

[1]Department of Pathology & Immunology, Washington University School of Medicine, St. Louis, MO, USA

[2]Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco, San Francisco, CA, USA

Abstract

Dietary fiber improves metabolic health, but host-encoded mechanisms for digesting fibrous polysaccharides are unclear. Here, we describe a mammalian adaptation to dietary chitin that is coordinated by gastric innate immune activation and acidic mammalian chitinase (AMCase). Chitin consumption causes gastric distension and cytokine production by stomach tuft cells and group 2 innate lymphoid cells (ILC2s), driving expansion of AMCase-expressing zymogenic chief cells that facilitate chitin digestion. Although chitin influences gut microbial composition, ILC2-mediated tissue adaptation and gastrointestinal responses are preserved in germ-free mice. In the absence of AMCase, sustained chitin intake leads to heightened basal type 2 immunity, reduced adiposity, and resistance to obesity. These data define an endogenous metabolic circuit that enables nutrient extraction from an insoluble dietary constituent by enhancing digestive function.

One-Sentence Summary:

Gastric innate immune cells coordinate digestion and metabolic responses to an abundant insoluble dietary polysaccharide.

Dietary fiber intake is associated with lower risk of metabolic disorders such as obesity (1, 2) and type 2 immune activation has been implicated in metabolic homeostasis (3–5), but little is known about how degradation of specific fibers influences host immunity and metabolism. In mammals, digestion is initiated in the upper gastrointestinal (GI) tract and is facilitated by mechanical forces, neural feedback, and enzymatic activities that coordinate chemical and physical disruption of the food bolus prior to passage into the highly absorptive small intestine. Digestion is essential for nutrient extraction, evident in bariatric surgical approaches that counteract overnutrition by reducing or bypassing gastric

*Corresponding author. svandyken@wustl.edu.

Author contributions: D-H.K. and S.J.V.D. designed the study and performed experiments. Y.W. and H.J. performed experiments and provided advice. R.F., X.Z., and J.R.B. performed metabolic cage experiments, analyzed data, and provided expertise with metabolic studies. C.M. and T-C.L. provided human stomach specimens and expertise, and J.S.F. provided biochemical reagents and expertise. All authors edited the final manuscript. S.J.V.D. directed the studies and wrote the manuscript with D-H.K.

Competing interests: J.R.B. is on the Scientific Advisory Board of LUCA Science, Inc. The other authors declare that they have no competing financial interests.

digestion (6). Nutrient availability is also limited by dietary fiber enrichment, as most bulky insoluble polysaccharides are resistant to digestion by mammalian enzymes and undergo only limited degradation by distal gut microbes (7). A notable exception is chitin (β-1,4-poly-N-acetylglucosamine). One of the most abundant natural polysaccharides on earth, chitin is a component of arthropods and fungi and is an initiator of type 2 immune responses. We hypothesized that chitin is digested in a distinct manner, as widely conserved chitinases are encoded by both commensal microbes and mammals, particularly those that consume chitin (8–13).

Dietary chitin induces gastric distension and type 2 immune triggering

Chitin activates lung ILC2s via interleukin (IL)-25, IL-33, and thymic stromal lymphopoietin (TSLP) (14). To test GI responses to dietary chitin, “YRS” mice expressing reporter alleles for ILC2 signature genes arginase-1 (Yarg; *Arg1*^{YFP}), IL-5 (Red5; *Il5*^{tdTomato}), and IL-13 (Smart13; *Il13*^{hCD4}) on wild-type (WT) and IL-25-, IL-33R-, and TSLPR-triple-knockout (TKO) (15) backgrounds were fed with standard chow containing either cellulose (control) or chitin as fiber (Fig. 1A and table S1). We tested 5 to 20% chitin, approximating the dietary composition of insectivorous mammals (11, 12). Food intake was similar and GI transit time was unaffected by diet. However, we observed marked gastric distention and greater stomach contents in chitin- versus control-fed mice (Fig. 1B and fig. S1, A and B), indicating that dietary fiber influences stomach retention and stretch. Gastric epithelium rapidly responded to chitin, inducing expression of the ILC2-activating cytokines *Il25* and *Il33* in stomach tuft cells and non-tuft epithelial cells, respectively (Fig. 1C).

Dietary chitin increased stomach IL-5- and IL-13-producing ILC2s (Fig. 1D), alternatively activated Arg1$^+$ macrophages, serum IL-5, and blood eosinophils, whose numbers expanded over time and in proportion with chitin content (fig. S1, C to E). Few IL-5- or IL-13-expressing stomach CD4$^+$ T cells were detected, however, and chitin responses were intact in *Rag1*-KO mice, which lack B and T cells (fig. S1, F to I), consistent with predominantly innate immune activation. Chitin ingestion increased stomach expression of the ILC2-activating neuropeptide neuromedin U (*Nmu*) (16, 17) along with its receptor, *Nmur1*, among stomach-resident ILC2s (fig. S1, J and K). Expression of calcitonin gene-related peptide (CGRP), another ILC2-activating neuropeptide (18), was unaltered by dietary chitin (fig. S1L). Thus, gastric ILC2s may be synergistically activated by IL-25 and NMU, similar to intestinal ILC2s (16, 17).

Dietary chitin also increased gastrin and glucagon-like peptide-1 (GLP-1), gastrointestinal hormones induced by mechanical stretch following food ingestion (19). By contrast, serotonin, which responds to IL-33 (20), was unaffected by chitin (fig. S1, M and N). In addition, although IL-33 can be released by mechanical perturbation (21) and drives ILC2 responses to *Helicobacter pylori* infection and chemical injury (22, 23), chitin-induced ILC2 accumulation and eosinophilia were unaffected in *Il1rl1*-KO mice (fig. S2, A and B). By contrast, ILC2 activation and cytokine production was abolished in TKO mice (Fig. 1, C and D) and eosinophilia was abrogated in both tuft cell–deficient *Pou2f3*-KO and TKO mice (fig. S2, C and D), while chitin-induced stomach distension was maintained. Thus,

tuft cell–derived IL-25 appears to be the primary signal in gastric ILC2 responses to dietary chitin-induced stretch.

We inflated the stomach with air to recapitulate chitin-induced distension (fig. S2E). Within 2 hours, we observed increased expression of *Il25*, *Nmu*, and *Edn1*, a target of the mechanosensitive ion channel Piezo1 (24, 25). Conversely, in vivo administration of GsMTx-4, a stretch-activated channel inhibitor, blocked this response (Fig. 1E and fig. S2F) and abrogated ILC2 cytokine induction after chitin ingestion (Fig. 1F). Administration of the Piezo1 agonist Yoda1 induced ILC2 IL-5 production in WT but not TKO mice (Fig. 1G), suggesting that Piezo1 signaling activates gastric ILC2s via tissue cytokines including IL-25. Accordingly, Yoda1 did not enhance gastric ILC2 responses prior to tuft cell development (fig. S2, G and H). *Nmur1* expression was reduced in TKO compared to WT ILC2s (fig. S2I), consistent with prior reports (16) and suggesting that gastric ILC2s acquire basal IL-5 expression and receptivity to multiple stretch signals during development. Combined Yoda1 and NMU administration also increased ILC2 IL-13 and KLRG1 expression compared to Yoda1 alone (Fig. 1H and fig. S2J). Thus, dietary chitin and mechanical stretch initiates type 2 immune responses that sensitize ILC2s to additional synergistic neuroimmune activating signals.

Sustained chitin intake promotes gastrointestinal remodeling and adipose ILC2 responses

Dietary chitin remodeled GI tissues, inducing gastric epithelial proliferation, epithelial and submucosal thickening, and increased tuft cell abundance (Fig. 2, A to C). Proliferation was reduced in TKO compared to WT mice (Fig. 2B), indicating a requirement for IL-25, IL-33, and TSLP signaling in remodeling. Chitin lengthened the small intestine (SI), which was enriched with tuft cells, activated ILC2s, and eosinophils in WT, but not TKO mice (Fig. 2D and fig. S3, A and B). These SI effects closely resembled those induced by helminths, protozoa (*Tritrichomonas spp.*), and increased luminal succinate (26–29). However, control and chitin-fed mice were *Tritrichomonas*-free and cecal succinate levels were unaffected by chitin (fig. S3C). Thus, dietary chitin appears to initiate a distinct type 2 immune circuit within the GI tract.

Chitin intake also stimulated type 2 immune responses in metabolically active tissues. Although lung ILC2s and eosinophils were unaffected by diet (fig. S3, D and E), visceral adipose from chitin-fed mice contained elevated eosinophils and IL-5-producing ILC2s (Fig. 2E). Inhibiting tissue lymphocyte egress using FTY720 reduced circulating ILCs (fig. S3F), but did not alter dietary chitin-induced eosinophil and ILC2 responses (fig. S3, G and H), suggesting that interorgan ILC2 migration did not mediate adipose effects (30). By contrast, adipose ILC2 activation and eosinophilia were abrogated in TKO mice (Fig. 2E), indicating that tissue-derived cytokines coordinate local ILC2 responses to chitin. Moreover, mice lacking the shared signaling receptor for IL-4 and IL-13, IL4Rα, failed to induce stomach ILC2s, tuft cells, SI lengthening, adipose ILC2s, and eosinophils in response to chitin (fig. S3, I to M). ILC depletion in *Rag1*-KO mice impaired chitin-induced gastric ILC2 and tuft cell expansion (fig. S3, N and O), whereas tuft cells expanded normally in both

Il4-KO and *Il5*-KO mice (fig. S3P), supporting a role for IL-13–producing ILC2s in chitin responses. Finally, GI tissue resilience, which relies on ILC2s and IL-13 in the absence of adaptive immunity (31), was enhanced in chitin-fed *Rag1*-KO mice infected with the helminth *Nippostrongylus brasiliensis* compared to controls, as marked by increased ILC2s, serum IL-5, eosinophils, and improved worm expulsion (fig. S4, A to D).

Since dietary polysaccharides can be degraded by intestinal bacteria (7, 8, 32), we profiled the fecal microbiota from chitin- and control diet-fed mice. Mice maintained similar body weights regardless of diet (fig. S5A), indicating equivalent nutrient extraction. However, chitin intake significantly enriched Bacteroidetes phyla, whereas Firmicutes were proportionally decreased compared to controls (fig. S5B and table S2). Thus, chitin alters GI bacterial composition consistent with prior work on dietary fiber enrichment (8).

We then tested whether type 2 immune responses to dietary chitin were dependent on commensal microbiota using germ-free (GF) and specific-pathogen-free (SPF) mice. GF and SPF mice maintained similar body weights regardless of diet and dietary chitin induced gastric distension, SI lengthening, eosinophilia, ILC2 expansion, and tuft cell hyperplasia in both GF and SPF conditions (fig. S5, C to G). These results indicated that dietary chitin induces innate type 2 immune responses independent of commensal microbes. To address possible developmental alterations in GF mice, we also depleted bacteria by administering antibiotics to adult SPF mice prior to dietary chitin intake. Consistent with GF results, stomach, SI, and adipose tissue chitin responses were unaffected by antibiotics (fig. S5, H to K). Thus, although chitin alters GI microbial composition and commensal microorganisms influence tuft cell succinate responses (27, 28), dietary chitin initiates type 2 immune responses in the absence of commensal microbiota.

AMCase is required for dietary chitin digestion in mammals

Chitinases (EC 3.2.1.14) are widely conserved enzymes that cleave soluble chitooligomers and crystalline chitin substrates, liberating *N*-acetylglucosamine (GlcNAc). In contrast to insoluble dietary chitin fibers, GlcNAc did not induce gastric distension or gastrointestinal type 2 immune responses (fig. S6, A to D), suggesting that undigested, insoluble chitin causes mechanical stretch and type 2 immune triggering. Chitinases can be expressed by microbes, including gut-resident bacteria (8). However, the lack of microbial involvement in chitin responses led us to consider AMCase, a mammalian chitinase secreted by respiratory epithelium, salivary glands, and stomach that has been evolutionarily linked to chitin consumption (10–12, 33).

AMCase protein was expressed at the base of gastric glands in tissues from human-sleeve-gastrectomy patients and 8-week-old mice (Fig. 3A), consistent with RNA-seq data (fig. S7A) and prior reports (10, 33–35), which supported chief cells as the main source of AMCase in the mammalian stomach. We further investigated AMCase-expressing cells using ChiaRed reporter mice, in which tdTomato and Cre recombinase are knocked into *Chia1* (encoding AMCase). Homozygous ChiaRed (CC) mice lack AMCase (36). We lineage-traced AMCase-expressing cells by crossing ChiaRed with Rosa26-flox-stop-zsGreen (R26(LSL)-zsGreen) mice. Consistent with antibody staining, AMCase-expressing

ChiaRed^{+}zsGreen^{+} cells were localized in gastric glands, and concordantly coexpressed ChiaRed and zsGreen with age (fig. S7B), indicating that AMCase expression is sustained in terminally differentiated chief cells. ChiaRed^{+} cells were also enriched for mature chief cell markers gastric intrinsic factor (*Gif*), colipase (*Clps*), and pepsinogen C (*Pgc*) (Fig. 3, B and C), supporting AMCase as a marker of zymogenic digestive cells.

We tested the contribution of AMCase to chitin digestion using gastrointestinal luminal secretions from WT and CC (AMCase-deficient) mice. Chitinase activity was assessed on soluble chitooligomers and crystalline chitin substrates (37) and chitin-binding proteins were isolated with magnetized chitin (Fig. 3D). WT stomach lavage contained AMCase that bound insoluble chitin (Fig. 3E) and exhibited robust chitinase activity that was absent in CC lavage and could be depleted by preincubation with chitin (Fig. 3F), indicating that AMCase binds chitin and nonredundantly mediates stomach chitinase activity. Dietary chitin intake also reduced stomach pH (Fig. 3G), which enhances AMCase enzymatic activity (10, 33), suggesting a coordinated physiological digestion response involving acid-secreting parietal cells. Crystalline dietary chitin fibers were visibly digested and converted to soluble GlcNAc by WT stomach lavage, reflecting highly efficient chitinase digestive activity. However, this activity was absent in CC lavage, which retained undigested crystalline chitin particles and failed to produce soluble GlcNAc reaction products (Fig. 3, H and I). Neither control nor chitin chow elicited detectable epithelial ChiaRed or *Chia1* expression in lower GI tissues, including SI and cecum (fig. S8, A to C). However, WT SI lavage still contained chitinase activity that was absent in CC SI lavage, suggesting that AMCase produced in the stomach transits into lower GI tract, where it also comprises the major source of chitinase activity (fig. S8D). Thus, although most insoluble dietary polysaccharides consumed by mammals resist digestion or undergo only limited degradation in the lower GI tract by commensal microbiota-derived glycosyl hydrolases (7, 8), dietary chitin is primarily digested by host-encoded AMCase.

Stomach adaptation to dietary chitin is controlled by a type 2 immune circuit

Because lung AMCase expression is promoted by type 2 cytokines (36, 38), we tested whether stomach AMCase is similarly regulated. Indeed, sustained dietary chitin intake increased *Chia1* expression in WT, but not *Il4ra*-KO, *Pou2f3*-KO, ILC2-deleter (15), or TKO mice (Fig. 4, A and B). Tuft cell-derived IL-25, ILC2s, and IL-4–IL-13 signaling were therefore implicated as major drivers of gastric AMCase expression. Type 2 triggering was recapitulated by IL-25 administration, which stimulated IL-13 production by ILC2s and stomach *Chia1* expression in WT, but not ILC2-deficient mice (Fig. 4C and S8E). Thus, ILC2-derived IL-13 is implicated in the expansion of AMCase-expressing chief cells.

To test this further, we crossed ChiaRed with *Stat6*-KO (CR-*Stat6*-KO) and *Il4ra*$^{fl/fl}$ mice (39) (CR-*Il4ra*$^{fl/fl}$), enabling specific deletion of IL4Rα from AMCase-expressing cells (Fig. 4D). *Il4ra* was reduced in ChiaRed^{+} stomach epithelial cells from CR-*Il4ra*$^{fl/fl}$ mice compared to ChiaRed controls, indicating successful Cre-mediated excision (Fig. 4E). Dietary chitin increased AMCase-expressing chief cells in WT ChiaRed mice, but failed

to occur in both CR-*Stat6*-KO and CR-*Il4ra*$^{fl/fl}$ mice (Fig. 4F), indicating that dietary chitin promotes chief cell AMCase expression via cell-intrinsic IL4Rα signaling. We also examined acute gastric injury and transient chief cell depletion by high-dose tamoxifen treatment (HDT), which models aspects of human atrophic corpus gastritis and AMCase loss due to *H. pylori* infection (40, 41). Following HDT, WT mice activated gastric ILC2s coincident with chief cell recovery, whereas TKO mice failed to recover *Chia1* (fig. S9, A and B). Thus, restoration of gastric homeostasis and chitin digestive capacity after epithelial injury depends on type 2 circuit activation.

These data suggested that mammals adapt to dietary chitin by inducing endogenous stomach type 2 immune responses to boost AMCase production. Accordingly, CC mice failed to reduce gastric distension compared to WT mice after 2 weeks of chitin intake (Fig. 4G). Stomach ILC2 activation and tuft cell hyperplasia were also sustained in CC versus WT mice over several weeks (Fig. 4H), reflecting unresolved circuit activation without AMCase-catalyzed chitin digestion. Consistent with improved digestion, WT mice attenuated distal type 2 immune triggering in the SI and adipose tissues. CC mice, by contrast, exhibited enhanced and prolonged type 2 triggering, characterized by greater SI tuft cell abundance, increased eosinophils, and IL-13–producing ILC2s in SI and adipose tissues after 4 weeks of chitin intake (Fig. 4I). Adipose tissue weight was also reduced in proportion to body weight in CC compared to WT mice whereas body weights were similar (Fig. 4, J and K). Thus, both GI and adipose tissue homeostasis are regulated by the AMCase-mediated adaptation to dietary chitin.

Dietary chitin improves metabolic health in obesity

We next tested the impact of dietary chitin on obesity, which is influenced by neuronal–ILC2 interactions and type 2 cytokines (3–5, 42). We fed WT and CC mice isocaloric high-fat diets containing either cellulose (control; HFD) or chitin (CHFD) fiber (Fig. 5A). Food intake was similar among all groups, and HFD-fed mice showed comparable body weight gain. However, CHFD-fed CC mice gained significantly less weight, with reduced adiposity and fat mass compared to WT (Fig. 5, B to D, and fig. S10A). Resistance to obesity in CHFD-fed CC mice was accompanied by adipose ILC2 and eosinophil accumulation (Fig. 5E), cells previously linked with metabolic homeostasis (3–5) and suggesting that altered dietary chitin digestion could modulate metabolism. Indeed, ILC2s and tuft cells were elevated in the stomach and SI tissues of CHFD-fed CC compared to WT mice (Fig. 5F), reflecting sustained type 2 triggering and suggesting that AMCase-mediated dietary chitin digestion contributes to metabolic homeostasis.

Both dietary chitin and AMCase influenced metabolism in the context of high-fat diet, as CHFD-fed WT and CC mice exhibited significantly improved insulin sensitivity compared to HFD-fed WT mice (Fig. 5, G and H). CHFD-fed CC mice exhibited lower fasting glucose compared to WT (fig. S10B), consistent with differences in body weight and suggesting that AMCase activity influences glucose homeostasis after chitin ingestion. Additionally, light-phase energy expenditure was increased in CC mice and glucose utilization (RER) was increased after CHFD feeding compared to HFD in both WT and CC mice, despite no differences in core body temperature or activity (Fig. 5I and fig. S10, C to E), consistent

with sustained type 2 immune triggering. Indeed, tuft cell-deficient *Pou2f3*-KO mice failed to exhibit CHFD-induced effects on insulin sensitivity (fig. S11, A to G), suggesting that type 2 circuit initiation is required for some metabolic aspects of chitin in a high-fat diet. Thus, disruption of the mammalian stomach's adaptation to dietary chitin alters nutrient uptake and metabolic homeostasis manifesting in obesity.

Discussion

Chitin consumption has been linked to *CHIA* gene selection throughout primate evolution (11, 12), and chitin-rich fungi and arthropods are constituents of the diets of both modern and ancient human populations (13). Mammalian glycosyl hydrolase gene selection has also been linked with starch consumption (43, 44), but adaptations to specific dietary fibers after ingestion are mainly ascribed to shifts in gut microbial composition. As shown here, mammals encode an endogenous circuit that enables chitin catabolism and nutrient extraction through AMCase production. This pathway is triggered by gastric distension, neuropeptide release, and type 2 cytokine production caused by insoluble chitin fibers, which result in gastrointestinal remodeling and chief cell AMCase induction over time. This in turn enables enhanced chitin digestion. In humans, *CHIA* isoforms resulting in lower chitinase activity are linked with asthma (45, 46), supporting dual roles for AMCase in mucosal defense and nutrient extraction, as proposed initially (33). AMCase-producing chief cells produce additional digestive enzymes such as pepsinogen and lipase, suggesting that gastric ILC2s may coordinate a response that improves overall digestion of recalcitrant insect or fungal foods, perhaps representing a strategy for omnivores to adapt to varied diets. Although microbial composition is altered in response to chitin and other polysaccharides, we show that the mammalian adaptation does not rely on commensal microbiota and that AMCase is the primary source of chitinase activity in the GI tract, thereby distinguishing chitin digestion from other abundant insoluble polysaccharides such as cellulose. Our results further elucidate a mechanism for how chitin initiates type 2 immune initiation via mechanical stretch, thus connecting physical tissue perturbation with a branch of immunity increasingly recognized to maintain homeostasis in response to a wide variety of environmental disruptions as well as neural and dietary fluctuations. Intriguingly, the mammalian adaptation to chitin influences innate resistance to helminth infection and metabolic homeostasis, suggesting that chitin digestive pathways coevolved with intestinal helminths and may represent a therapeutic target in metabolic diseases such as obesity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments:

We thank K. Ravichandran for comments on the manuscript, J. Mills, R. Locksley, H.-E. Liang, J. Kipnis, M. Baldridge, C. Wilen, and C. Hsieh for advice and reagents, L. Mosby for expert technical assistance, and J. M. White at the Department of Pathology and Immunology Gnotobiotic Facility at Washington University in St. Louis for assistance with germ-free experiments.

Funding:

This work was supported in part by the Bursky Center for Human Immunology and Immunotherapy Programs, Center for Cellular Imaging, and Rheumatic Diseases Research Resource-based Center (P30 AR073752; National Institutes of Health, NIH) at Washington University in St. Louis, NIH DP5 OD028125 (J.R.B.), Burroughs Wellcome Fund CAMS #1019648 (J.R.B.), NIH R01HL148033, R01AI176660, R21AI163640 (S.J.V.D.), and the National Research Foundation of Korea Award NRF-2020R1A6A3A03037855 (D-H.K.).

Data and materials availability:

All data are available in the main text or supplementary materials. 16S sequencing data are available in Dryad (47).

References and Notes

1. Slavin JL, Dietary fiber and body weight. Nutrition. 21, 411–418 (2005). [PubMed: 15797686]
2. Gill SK, Rossi M, Bajka B, Whelan K, Dietary fibre in gastrointestinal health and disease. Nat. Rev. Gastroenterol. Hepatol 18, 101–116 (2021). [PubMed: 33208922]
3. Wu D, Molofsky AB, Liang H-E, Ricardo-Gonzalez RR, Jouihan HA, Bando JK, Chawla A, Locksley RM, Eosinophils sustain adipose alternatively activated macrophages associated with glucose homeostasis. Science. 332, 243–247 (2011). [PubMed: 21436399]
4. Molofsky AB, Nussbaum JC, Liang H-E, Van Dyken SJ, Cheng LE, Mohapatra A, Chawla A, Locksley RM, Innate lymphoid type 2 cells sustain visceral adipose tissue eosinophils and alternatively activated macrophages. J. Exp. Med 210, 535–549 (2013). [PubMed: 23420878]
5. Brestoff JR, Kim BS, Saenz SA, Stine RR, Monticelli LA, Sonnenberg GF, Thome JJ, Farber DL, Lutfy K, Seale P, Artis D, Group 2 innate lymphoid cells promote beiging of white adipose tissue and limit obesity. Nature. 519, 242–246 (2015). [PubMed: 25533952]
6. Nguyen NT, Varela JE, Bariatric surgery for obesity and metabolic disorders: state of the art. Nat. Rev. Gastroenterol. Hepatol 14, 160–169 (2017). [PubMed: 27899816]
7. Flint HJ, Scott KP, Duncan SH, Louis P, Forano E, Microbial degradation of complex carbohydrates in the gut. Gut Microbes. 3, 289–306 (2012). [PubMed: 22572875]
8. Sonnenburg JL, Xu J, Leip DD, Chen C-H, Westover BP, Weatherford J, Buhler JD, Gordon JI, Glycan foraging in vivo by an intestine-adapted bacterial symbiont. Science. 307, 1955–1959 (2005). [PubMed: 15790854]
9. Paoletti MG, Norberto L, Damini R, Musumeci S, Human Gastric Juice Contains Chitinase That Can Degrade Chitin. Ann. Nutr. Metab 51, 244–251 (2007). [PubMed: 17587796]
10. Ohno M, Kimura M, Miyazaki H, Okawa K, Onuki R, Nemoto C, Tabata E, Wakita S, Kashimura A, Sakaguchi M, Sugahara Y, Nukina N, Bauer PO, Oyama F, Acidic mammalian chitinase is a proteases-resistant glycosidase in mouse digestive system. Sci. Rep 6, 37756 (2016). [PubMed: 27883045]
11. Emerling CA, Delsuc F, Nachman MW, Chitinase genes (CHIAs) provide genomic footprints of a post-Cretaceous dietary radiation in placental mammals. Sci Adv. 4, eaar6478 (2018). [PubMed: 29774238]
12. Janiak MC, Chaney ME, Tosi AJ, Evolution of Acidic Mammalian Chitinase Genes (CHIA) Is Related to Body Mass and Insectivory in Primates. Mol. Biol. Evol 35, 607–622 (2018). [PubMed: 29216399]
13. Wibowo MC, Yang Z, Borry M, Hübner A, Huang KD, Tierney BT, Zimmerman S, Barajas-Olmos F, Contreras-Cubas C, García-Ortiz H, Martínez-Hernández A, Luber JM, Kirstahler P, Blohm T, Smiley FE, Arnold R, Ballal SA, Pamp SJ, Russ J, Maixner F, Rota-Stabelli O, Segata N, Reinhard K, Orozco L, Warinner C, Snow M, LeBlanc S, Kostic AD, Reconstruction of ancient microbial genomes from the human gut. Nature. 594, 234–239 (2021). [PubMed: 33981035]
14. Van Dyken SJ, Mohapatra A, Nussbaum JC, Molofsky AB, Thornton EE, Ziegler SF, McKenzie ANJ, Krummel MF, Liang H-E, Locksley RM, Chitin activates parallel immune modules that direct distinct inflammatory responses via innate lymphoid type 2 and γδ T cells. Immunity. 40, 414–424 (2014). [PubMed: 24631157]

15. Van Dyken SJ, Nussbaum JC, Lee J, Molofsky AB, Liang H-E, Pollack JL, Gate RE, Haliburton GE, Ye CJ, Marson A, Erle DJ, Locksley RM, A tissue checkpoint regulates type 2 immunity. Nat. Immunol 17, 1381–1387 (2016). [PubMed: 27749840]
16. Cardoso V, Chesné J, Ribeiro H, García-Cassani B, Carvalho T, Bouchery T, Shah K, Barbosa-Morais NL, Harris N, Veiga-Fernandes H, Neuronal regulation of type 2 innate lymphoid cells via neuromedin U. Nature. 549, 277–281 (2017). [PubMed: 28869974]
17. Wallrapp A, Riesenfeld SJ, Burkett PR, Abdulnour R-EE, Nyman J, Dionne D, Hofree M, Cuoco MS, Rodman C, Farouq D, Haas BJ, Tickle TL, Trombetta JJ, Baral P, Klose CSN, Mahlakõiv T, Artis D, Rozenblatt-Rosen O, Chiu IM, Levy BD, Kowalczyk MS, Regev A, Kuchroo VK, The neuropeptide NMU amplifies ILC2-driven allergic lung inflammation. Nature. 549, 351–356 (2017). [PubMed: 28902842]
18. Sui P, Wiesner DL, Xu J, Zhang Y, Lee J, Van Dyken S, Lashua A, Yu C, Klein BS, Locksley RM, Deutsch G, Sun X, Pulmonary neuroendocrine cells amplify allergic asthma responses. Science. 360 (2018), doi:10.1126/science.aan8546.
19. Zhang T, Perkins MH, Chang H, Han W, de Araujo IE, An inter-organ neural circuit for appetite suppression. Cell. 0 (2022), doi:10.1016/j.cell.2022.05.007.
20. Chen Z, Luo J, Li J, Kim G, Stewart A, Urban JF Jr, Huang Y, Chen S, Wu L-G, Chesler A, Trinchieri G, Li W, Wu C, Interleukin-33 Promotes Serotonin Release from Enterochromaffin Cells for Intestinal Homeostasis. Immunity. 54, 151–163.e6 (2021). [PubMed: 33220232]
21. Kakkar R, Hei H, Dobner S, Lee RT, Interleukin 33 as a mechanically responsive cytokine secreted by living cells. J. Biol. Chem 287, 6941–6948 (2012). [PubMed: 22215666]
22. Satoh-Takayama N, Kato T, Motomura Y, Kageyama T, Taguchi-Atarashi N, Kinoshita-Daitoku R, Kuroda E, Di Santo JP, Mimuro H, Moro K, Ohno H, Bacteria-Induced Group 2 Innate Lymphoid Cells in the Stomach Provide Immune Protection through Induction of IgA. Immunity. 52, 635–649.e4 (2020). [PubMed: 32240600]
23. Meyer AR, Engevik AC, Madorsky T, Belmont E, Stier MT, Norlander AE, Pilkinton MA, McDonnell WJ, Weis JA, Jang B, Mallal SA, Peebles RS Jr, Goldenring JR, Group 2 Innate Lymphoid Cells Coordinate Damage Response in the Stomach. Gastroenterology. 159, 2077–2091.e8 (2020). [PubMed: 32891625]
24. Solis AG, Bielecki P, Steach HR, Sharma L, Harman CCD, Yun S, de Zoete MR, Warnock JN, To SDF, York AG, Mack M, Schwartz MA, Dela Cruz CS, Palm NW, Jackson R, Flavell RA, Mechanosensation of cyclical force by PIEZO1 is essential for innate immunity. Nature. 573, 69–74 (2019). [PubMed: 31435009]
25. Oh Y, Lai JS-Y, Min S, Huang H-W, Liberles SD, Ryoo HD, Suh GSB, Periphery signals generated by Piezo-mediated stomach stretch and Neuromedin-mediated glucose load regulate the Drosophila brain nutrient sensor. Neuron. 109, 1979–1995.e6 (2021). [PubMed: 34015253]
26. von Moltke J, Ji M, Liang H-E, Locksley RM, Tuft-cell-derived IL-25 regulates an intestinal ILC2-epithelial response circuit. Nature. 529, 221–225 (2016). [PubMed: 26675736]
27. Schneider C, O'Leary CE, von Moltke J, Liang H-E, Ang QY, Turnbaugh PJ, Radhakrishnan S, Pellizzon M, Ma A, Locksley RM, A Metabolite-Triggered Tuft Cell-ILC2 Circuit Drives Small Intestinal Remodeling. Cell. 174, 271–284.e14 (2018). [PubMed: 29887373]
28. Lei W, Ren W, Ohmoto M, Urban JF Jr, Matsumoto I, Margolskee RF, Jiang P, Activation of intestinal tuft cell-expressed Sucnr1 triggers type 2 immunity in the mouse small intestine. Proc. Natl. Acad. Sci. U. S. A 115, 5552–5557 (2018). [PubMed: 29735652]
29. Nadjsombati MS, McGinty JW, Lyons-Cohen MR, Jaffe JB, DiPeso L, Schneider C, Miller CN, Pollack JL, Nagana Gowda GA, Fontana MF, Erle DJ, Anderson MS, Locksley RM, Raftery D, von Moltke J, Detection of Succinate by Intestinal Tuft Cells Triggers a Type 2 Innate Immune Circuit. Immunity. 49, 33–41.e7 (2018). [PubMed: 30021144]
30. Huang Y, Mao K, Chen X, Sun M-A, Kawabe T, Li W, Usher N, Zhu J, Urban JF Jr, Paul WE, Germain RN, S1P-dependent interorgan trafficking of group 2 innate lymphoid cells supports host defense. Science. 359, 114–119 (2018). [PubMed: 29302015]
31. Price AE, Liang H-E, Sullivan BM, Reinhardt RL, Eisley CJ, Erle DJ, Locksley RM, Systemically dispersed innate IL-13-expressing cells in type 2 immunity. Proc. Natl. Acad. Sci. U. S. A 107, 11489–11494 (2010). [PubMed: 20534524]

32. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI, An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 444, 1027–1031 (2006). [PubMed: 17183312]
33. Boot RG, Blommaart EF, Swart E, Ghauharali-van der Vlugt K, Bijl N, Moe C, Place A, Aerts JM, Identification of a novel acidic mammalian chitinase distinct from chitotriosidase. J. Biol. Chem 276, 6770–6778 (2001). [PubMed: 11085997]
34. Suzuki M, Fujimoto W, Goto M, Morimatsu M, Syuto B, Iwanaga T, Cellular expression of gut chitinase mRNA in the gastrointestinal tract of mice and chickens. J. Histochem. Cytochem 50, 1081–1089 (2002). [PubMed: 12133911]
35. Han X, Wang R, Zhou Y, Fei L, Sun H, Lai S, Saadatpour A, Zhou Z, Chen H, Ye F, Huang D, Xu Y, Huang W, Jiang M, Jiang X, Mao J, Chen Y, Lu C, Xie J, Fang Q, Wang Y, Yue R, Li T, Huang H, Orkin SH, Yuan G-C, Chen M, Guo G, Mapping the Mouse Cell Atlas by Microwell-Seq. Cell. 172, 1091–1107.e17 (2018). [PubMed: 29474909]
36. Van Dyken SJ, Liang H-E, Naikawadi RP, Woodruff PG, Wolters PJ, Erle DJ, Locksley RM, Spontaneous Chitin Accumulation in Airways and Age-Related Fibrotic Lung Disease. Cell. 169, 497–509.e13 (2017). [PubMed: 28431248]
37. Barad BA, Liu L, Diaz RE, Basilio R, Van Dyken SJ, Locksley RM, Fraser JS, Differences in the chitinolytic activity of mammalian chitinases on soluble and insoluble substrates. Protein Sci. 29, 966–977 (2020). [PubMed: 31930591]
38. Reese TA, Liang H-E, Tager AM, Luster AD, Van Rooijen N, Voehringer D, Locksley RM, Chitin induces accumulation in tissue of innate immune cells associated with allergy. Nature. 447, 92–96 (2007). [PubMed: 17450126]
39. Herbert DR, Hölscher C, Mohrs M, Arendse B, Schwegmann A, Radwanska M, Leeto M, Kirsch R, Hall P, Mossmann H, Claussen B, Förster I, Brombacher F, Alternative macrophage activation is essential for survival during schistosomiasis and downmodulates T helper 1 responses and immunopathology. Immunity. 20, 623–635 (2004). [PubMed: 15142530]
40. Huh WJ, Khurana SS, Geahlen JH, Kohli K, Waller RA, Mills JC, Tamoxifen induces rapid, reversible atrophy, and metaplasia in mouse stomach. Gastroenterology. 142, 21–24.e7 (2012). [PubMed: 22001866]
41. Nookaew I, Thorell K, Worah K, Wang S, Hibberd ML, Sjövall H, Pettersson S, Nielsen J, Lundin SB, Transcriptome signatures in Helicobacter pylori-infected mucosa identifies acidic mammalian chitinase loss as a corpus atrophy marker. BMC Med. Genomics 6, 41 (2013). [PubMed: 24119614]
42. Cardoso F, Klein Wolterink RGJ, Godinho-Silva C, Domingues RG, Ribeiro H, da Silva JA, Mahú I, Domingos AI, Veiga-Fernandes H, Neuro-mesenchymal units control ILC2 and obesity via a brain-adipose circuit. Nature. 597, 410–414 (2021). [PubMed: 34408322]
43. Perry GH, Dominy NJ, Claw KG, Lee AS, Fiegler H, Redon R, Werner J, Villanea FA, Mountain JL, Misra R, Carter NP, Lee C, Stone AC, Diet and the evolution of human amylase gene copy number variation. Nat. Genet 39, 1256–1260 (2007). [PubMed: 17828263]
44. Axelsson E, Ratnakumar A, Arendt M-L, Maqbool K, Webster MT, Perloski M, Liberg O, Arnemo JM, Hedhammar A, Lindblad-Toh K, The genomic signature of dog domestication reveals adaptation to a starch-rich diet. Nature. 495, 360–364 (2013). [PubMed: 23354050]
45. Seibold MA, Reese TA, Choudhry S, Salam MT, Beckman K, Eng C, Atakilit A, Meade K, Lenoir M, Watson HG, Thyne S, Kumar R, Weiss KB, Grammer LC, Avila P, Schleimer RP, Fahy JV, Rodriguez-Santana J, Rodriguez-Cintron W, Boot RG, Sheppard D, Gilliland FD, Locksley RM, Burchard EG, Differential enzymatic activity of common haplotypic versions of the human acidic Mammalian chitinase protein. J. Biol. Chem 284, 19650–19658 (2009). [PubMed: 19435888]
46. Okawa K, Ohno M, Kashimura A, Kimura M, Kobayashi Y, Sakaguchi M, Sugahara Y, Kamaya M, Kino Y, Bauer PO, Oyama F, Loss and Gain of Human Acidic Mammalian Chitinase Activity by Nonsynonymous SNPs. Mol. Biol. Evol 33, 3183–3193 (2016). [PubMed: 27702777]
47. Kim DH, Wang Y, Jung H, Field RL, Zhang X, Liu TC, Ma C, Fraser JS, Brestoff JR, Van Dyken SJ, A type 2 immune circuit in the stomach controls mammalian adaptation to dietary chitin, version 1, Dryad (2023); 10.5061/dryad.12jm63z3m.

48. Walker FC, Hassan E, Peterson ST, Rodgers R, Schriefer LA, Thompson CE, Li Y, Kalugotla G, Blum-Johnston C, Lawrence D, McCune BT, Graziano VR, Lushniak L, Lee S, Roth AN, Karst SM, Nice TJ, Miner JJ, Wilen CB, Baldridge MT, Norovirus evolution in immunodeficient mice reveals potentiated pathogenicity via a single nucleotide change in the viral capsid. PLoS Pathog. 17, e1009402 (2021). [PubMed: 33705489]

49. QuPATH, version 0.3.2; https://qupath.github.io.

50. Brestoff JR, Wilen CB, Moley JR, Li Y, Zou W, Malvin NP, Rowen MN, Saunders BT, Ma H, Mack MR, Hykes BL Jr, Balce DR, Orvedahl A, Williams JW, Rohatgi N, Wang X, McAllaster MR, Handley SA, Kim BS, Doench JG, Zinselmeyer BH, Diamond MS, Virgin HW, Gelman AE, Teitelbaum SL, Intercellular Mitochondria Transfer to Macrophages Regulates White Adipose Tissue Homeostasis and Is Impaired in Obesity. Cell Metab. 33, 270–282.e8 (2021). [PubMed: 33278339]

51. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP, DADA2: High-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583 (2016). [PubMed: 27214047]

52. Tissue expression of CHIA, version 8, GTEx (2023); https://gtexportal.org.

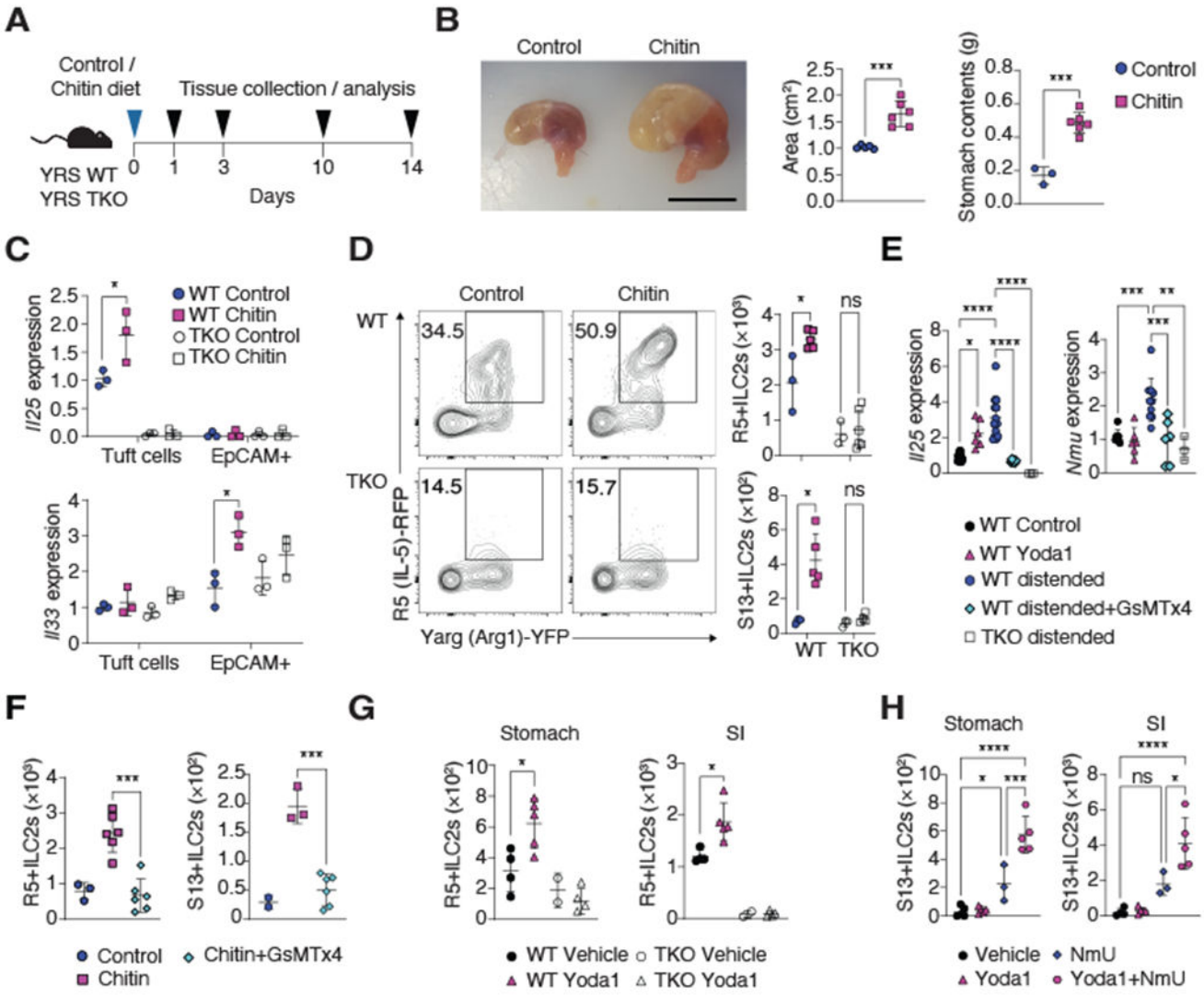

Fig. 1. Innate type 2 immune responses are triggered by gastric distension and dietary chitin.

(**A**) Dietary responses in wild-type (WT) or TKO mice on triple-reporter (YRS) backgrounds. (**B**) Representative stomach image (scale bar: 1 cm), stomach size, and luminal content, (**C**) relative *Il25* and *Il33* expression in stomach tuft ($CD45^{-}EpCAM^{+}SiglecF^{+}$) and epithelial cells ($CD45^{-}EpCAM^{+}$), and (**D**) expression of R5 (*Il5*) and S13 (*Il13*) reporter alleles among stomach ILC2s ($Yarg^{+}$, pre-gated on $CD45^{+}Lin^{-}Thy1.2^{+}$) in WT and TKO mice on indicated diet for 24 hours. (**E**) Relative stomach gene expression in WT or TKO mice after Yoda1 administration or gastric distension. (**F**) R5 and S13 expression in stomach ILC2 after GsMTx4 administration on indicated diet for 12 hours. (**G**) R5 or (**H**) S13 expression in stomach and small intestine ILC2s after vehicle, Yoda1, and/or NmU administration. Data represent individual biological replicates except in (C), which are pooled from 2-3 mice and are presented as means±SD comprising two or more independent experiments (n≥3 mice per group). *P*-values were calculated by unpaired *t* test (B, F, and G), one-way ANOVA (E and H), or two-way ANOVA with Tukey's multiple comparisons test (C and D). **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001, NS: not significant.

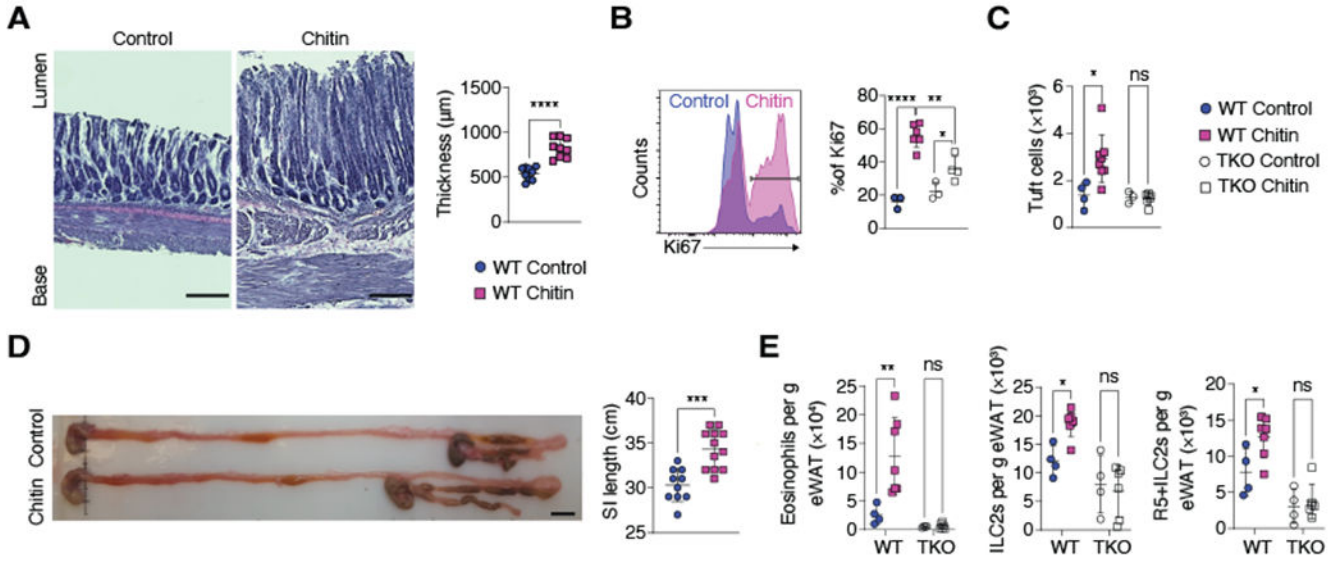

Fig. 2. Sustained chitin intake promotes gastrointestinal remodeling and adipose ILC2 responses.

(**A**) Representative stomach histology (H&E) (scale bar: 100 μm), (**B**) Ki67-expressing stomach epithelial cells, (**C**) stomach tuft cells, and (**D**) representative image of small intestine and small intestinal length in indicated mice on diet for 2 weeks. Scale bar: 1 cm. (**E**) Eosinophils, total ILC2s, and R5-expressing ILC2s per gram of epididymal white adipose tissue (eWAT) in WT and TKO mice on diet for 2 weeks. Data represent individual biological replicates and are presented as means±SD comprising two or more independent experiments (n≥3 mice per group). *P*-values were calculated by unpaired *t* test (A, C, and D), one-way ANOVA (B), or two-way ANOVA with Tukey's multiple comparisons test (E). **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001, NS: not significant.

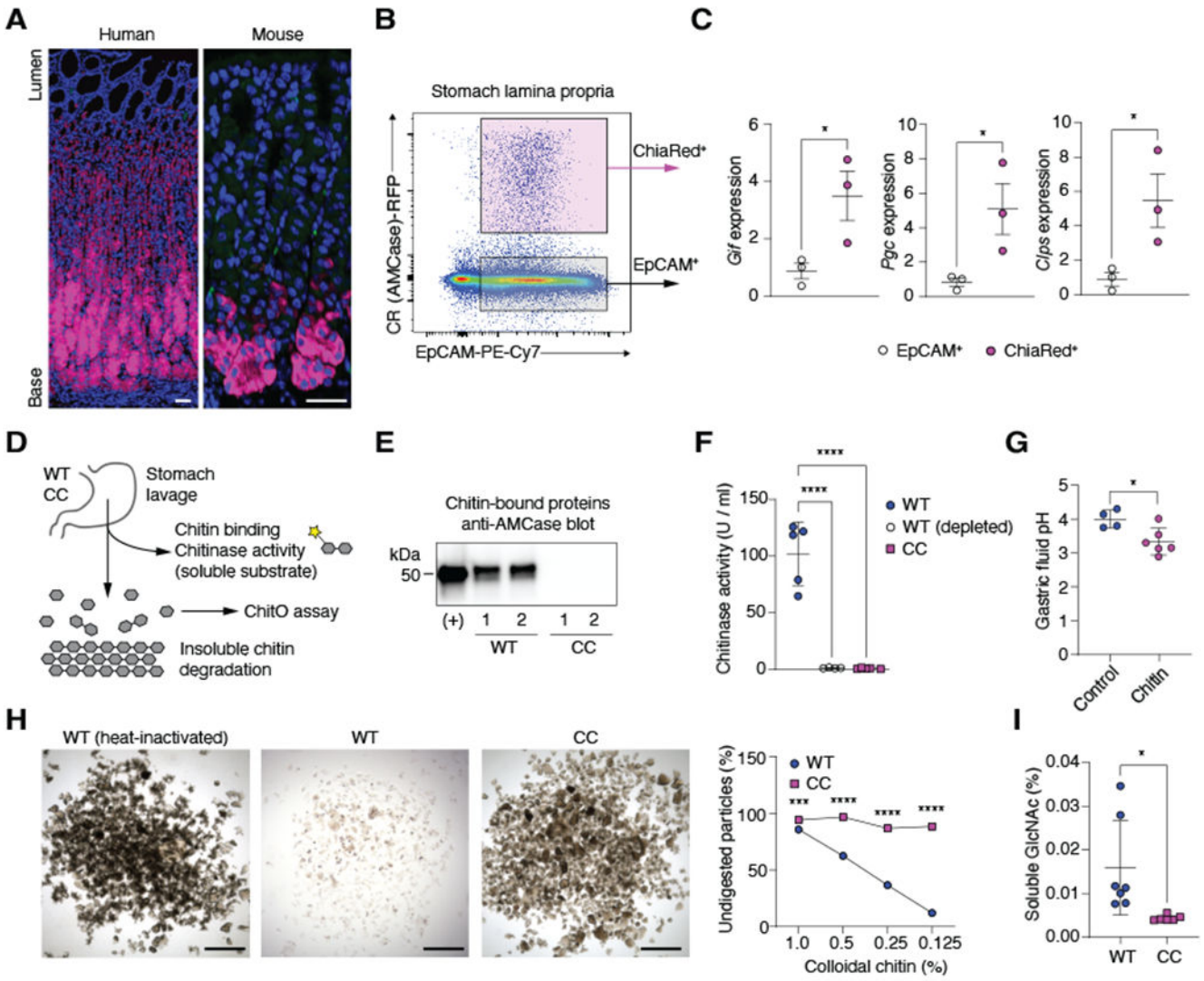

Fig. 3. AMCase is required for dietary chitin digestion.

(**A**) Immunostaining of AMCase-expressing chief cells in glandular stomach. Magenta: AMCase; blue: DAPI; green: autofluorescence. Scale bar: 50 μm. (**B**) Stomach ChiaRed$^+$ (CR: AMCase reporter; CD45$^-$EpCAM$^+$CR$^+$) or total (CD45$^-$EpCAM$^+$CR$^-$) epithelial cells were sorted and analyzed for (**C**) relative expression of *Gif*, *Clps*, and *Pgc* by qPCR. (**D**) Analysis of chitin binding, digestion of soluble chitooligomer and insoluble crystalline chitin substrates, and production of GlcNAc reaction products by chitooligomer oxidase (ChitO) assay in stomach lavage. (**E**) Immunoblot of chitin-bound AMCase proteins. (+) indicates recombinant AMCase postive control. (**F**) Chitinase activity with soluble substrate in stomach lavage samples, with and without predepletion of AMCase using insoluble chitin. (**G**) Gastric fluid pH in mice fasted overnight after 2 weeks on indicated diet. (**H**) Digestion of insoluble colloidal chitin after 96-hour incubation with inactivated (heat-treated) or fresh stomach lavage from WT or CC mice. Scale bar: 500 μm. (**I**) ChitO assay for soluble GlcNAc reaction products in supernatants from insoluble particle digestion in (H). Data points represent individual biological replicates except in (C), which represent samples

pooled from 3-4 mice. Data represent two or more independent experiments (n≥3 mice per group) and are presented as means±SD. *P<0.05, ***P<0.001, ****P<0.0001 (unpaired t test).

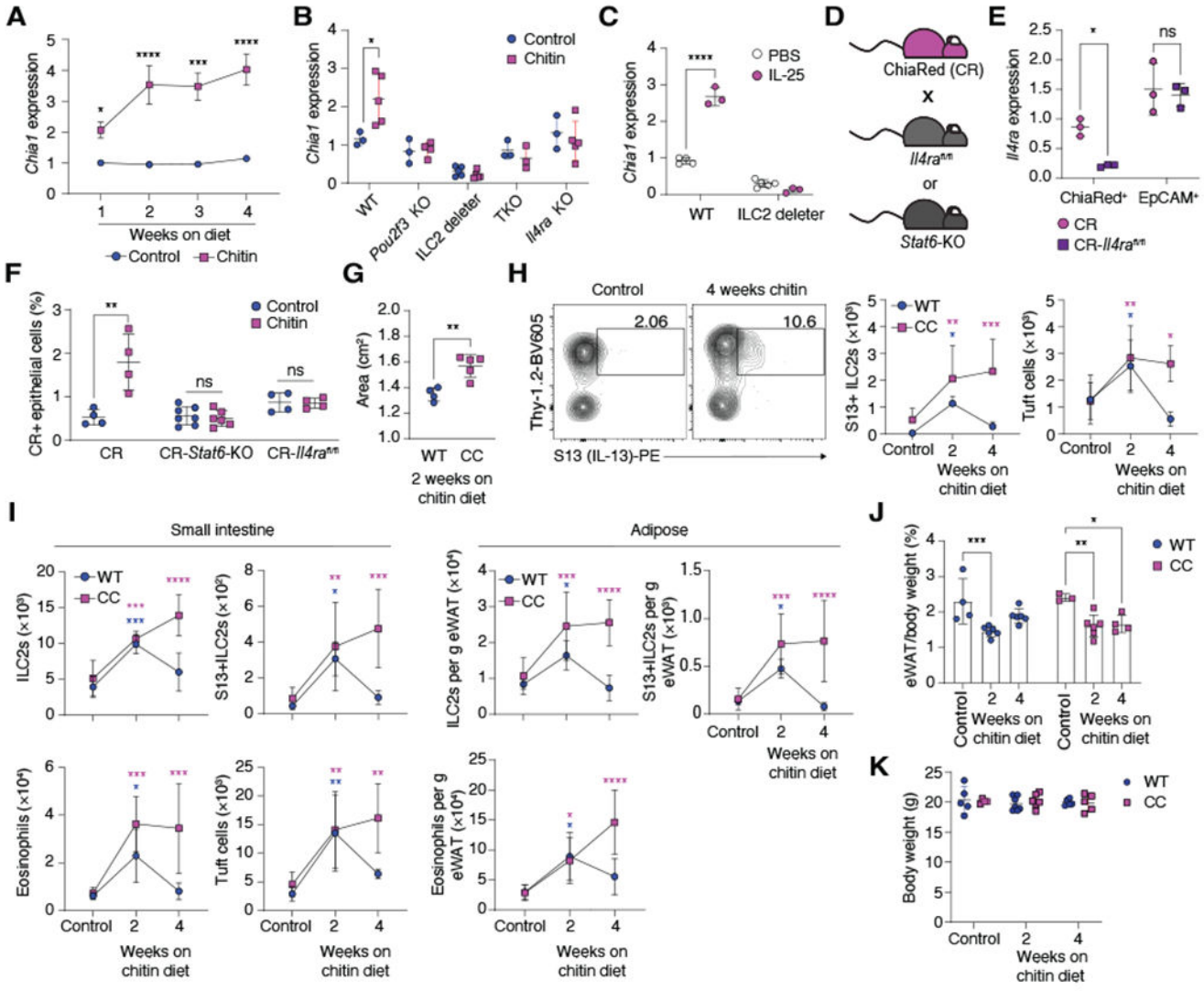

Fig. 4. Stomach adaptation to dietary chitin is controlled by a type 2 immune circuit.

(**A**) Relative *Chia1* stomach expression in WT and (**B**) indicated mouse strains (2 weeks on diet). (**C**) *Chia1* expression in stomach tissue from WT and ILC2 deleter mice after IL-25 administration. (**D**) Breeding scheme and (**E**) relative *Il4ra* expression in ChiaRed^{+} or total stomach epithelial cells (EpCAM^{+}) from ChiaRed and CR-*Il4ra*$^{fl/fl}$ mice. (**F**) Percentage of ChiaReD^{+} (CR) chief cells out of total stomach epithelial cells in ChiaRed, CR-*Il4ra*$^{fl/fl}$, and CR-*Stat6*-KO mice on diet for 2 weeks. (**G**) WT and CC stomach size on diet for 2 weeks. (**H**) S13 (IL-13)-expressing ILC2s and tuft cells in stomach, (**I**) SI tuft cells, SI and adipose eosinophils, ILC2s, and S13^{+} ILC2s, (**J**) eWAT to body weight ratio, and (**K**) body weights of WT and CC mice on control or chitin diets as indicated. Data represent individual biological replicates except in (A, E, H, and I), which are pooled from 3-8 mice and are presented as means±SD comprising two or more independent experiments (n≥3 mice per group). *P*-values were calculated by unpaired *t* test (A to C and E to G) or two-way ANOVA with Tukey's multiple comparisons test (H to K). **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001, NS: not significant.

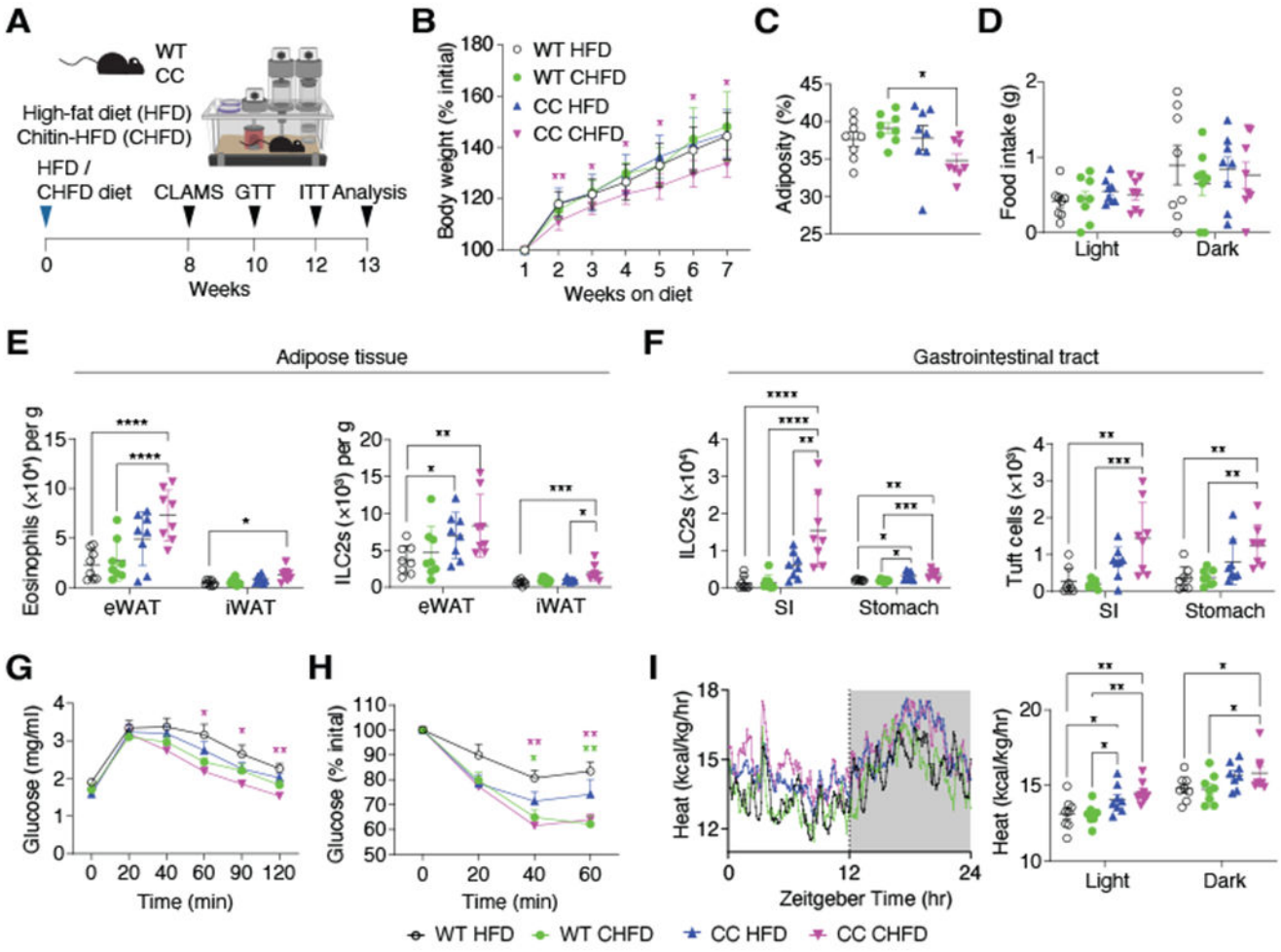

Fig. 5. Dietary chitin improves metabolic health in high fat diet-induced obesity.

(**A**) Experimental design. WT or CC mice were fed a high-fat diet (HFD) or chitin-containing HFD (CHFD) followed by metabolic cage analyses (CLAMS), glucose tolerance (GTT) and insulin tolerance tests (ITT). Part of the illustration was created with Biorender.com. (**B**) Body weights, (**C**) adiposity, and (**D**) food intake (24-hour average) at 8 weeks on diet. (**E**) Eosinophils and ILC2s in adipose tissue, and (**F**) tuft cells and ILC2s in SI and stomach tissues at 13 weeks on diet. (**G**) GTT and (**H**) ITT curves at 10 and 12 weeks, respectively. (**I**) Heat curve and averages over 24 hours in CLAMS cages at 8 weeks. Data represent individual biological replicates except in (B, E, and F), where each data point represents 8 pooled mice and are presented as means±SD (E, F) or means±SEM (B to D and G to I), comprising two or more independent experiments (n=8 mice per group). *P*-values were calculated by one-way ANOVA (C), two-way ANOVA (E, F), or two-way ANOVA with repeated measures (B, D, and G to I) with Tukey's multiple comparisons test. **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001.