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Disease tolerance mediated by commensal *E. coli* via inflammasome and IGF-1 signaling

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Abstract

Infections and inflammation can lead to cachexia and wasting of skeletal muscle and fat tissue by as yet poorly understood mechanisms. We observed that gut colonization of mice by a strain of *Escherichia coli* prevents wasting triggered by infections or physical damage to the intestine. During intestinal infection with the pathogen *Salmonella* Typhimurium or pneumonic infection with *Burkholderia thailandensis*, the presence of this *E. coli* did not alter changes in host metabolism, caloric uptake or inflammation, but instead sustained signaling of the IGF-1/PI3K/AKT pathway in skeletal muscle, required for prevention of muscle wasting. This effect was dependent on engagement of the NLRC4 inflammasome. Therefore, this commensal promotes tolerance to diverse diseases.

Infections and inflammation lead to profound metabolic alterations that are primarily driven by responses of muscle, fat and the liver (1, 2). Coupled with loss of appetite dysregulated metabolism can lead to the severe metabolic pathology called wasting syndrome (cachexia). Such wasting constitutes loss of skeletal muscle (with and without adipose tissue depletion) resulting in weight loss (2). Tuberculosis, sepsis and HIV, as well as inflammatory diseases including colitis (1, 3, 4), can trigger wasting. Wasting can also interfere with therapeutic interventions and causes untreatable morbidity and mortality (5).

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Data described in this paper can be found in the supplementary materials.

The authors declare no conflict of interest.

Supplementary Material:

Materials and Methods

Supplementary Figures S1–S19

Tables S1–S2

Mammals have coevolved with a complex gut microbial community, the gut microbiome, and depend on the metabolic benefits that it confers on the host (6, 7). Here, we have investigated whether constituents of the microbiome have any protective effect during metabolic dysregulation caused by gut trauma and/or infection.

Intestinal injury and inflammation can cause muscle wasting and inflammatory bowel disease (IBD) and Crohn's disease (CD) patients suffer from muscle wasting (8). Antibiotics cause ecological perturbations in the microbiota, (9) and coupling antibiotics with disease models can reveal how specific constituents of the microbiota impact disease. The dextran sulfate sodium (DSS) intestinal injury model is one of the best-studied models of IBD/CD-associated pathologies, including intestinal inflammation, mucus erosion and microbiota decompartmentalization (10). C57Bl/6 mice treated with DSS, exhibited muscle and fat wasting that was associated with anorexia, colonic inflammation and bloody, diarrhea (Fig 1A–B, S1). Administration of the broad-spectrum antibiotic cocktail Ampicillin, Vancomycin, Neomycin and Metronidazole (AVNM) had no significant impact on the severity of DSS-induced wasting in C57Bl/6 mice obtained from Jackson Laboratories (Jax mice) (Fig. S2). Surprisingly, we found that C57Bl/6 from the UC Berkeley colony (CB mice) showed significantly less wasting when DSS was coupled with AVNM treatment (Fig. 1C, S2).

We hypothesized that differences in the microbiota composition between Jax and CB mice may account for the differences we observed in wasting pathogenesis in response to AVNM plus DSS. We cultured from the ceca of CB mice an AVNM resistant (AVNM^R) population of bacteria that was not present in Jax mice (Fig. S2). Upon co-housing with AVNM-treated CB mice, AVNM-treated Jax mice became colonized with AVNM^R bacteria and were now protected from DSS-induced wasting, demonstrating that protection from wasting and CB associated AVNM^R bacteria can be horizontally transferred to otherwise wasting-susceptible animals (Fig S2).

We performed culture-independent analysis of amplicons generated by primers specific to the V3 and V4 variable regions of bacterial 16S ribosomal RNA genes of cecal content samples from CB and Jax mice treated with AVNM for 5-days (11). The resulting bacterial communities detected were compared phylogenetically (11). We found that AVNM-treated CB mice hosted large numbers of *Escherichia* spp. compared to AVNM-treated Jax mice (Fig. S2). Using culturing techniques, serotyping, genetic and molecular characterization (11), we isolated a single AVNM^R *Escherichia* from the ceca of CB mice that we classified as an *E. coli* O21:H+ strain that was absent from Jax mice (Fig. S2 and (11)). We performed multilocus sequence typing (MLST) by analyzing seven loci of *E. coli* O21:H+ ((11) Table S1, Table S2) and found this *E. coli* to be a perfect match at these loci for *E. coli* of the strain ST101.

Oral administration of *E. coli* O21:H+ resulted in colonization of the intestine of Jax mice, but had no significant effect on weight, muscle mass, fat mass or food consumption under homeostatic conditions (Fig. S3). However, upon DSS treatment, *E. coli* O21:H+ Jax mice showed significantly less wasting than DSS-treated vehicle mice (Fig. 1D, S4). Jax mice administered heat-killed *E. coli* O21:H+ or live doses of the commensal strain *E. coli*

MG1655 exhibited similar wasting as vehicle mice (Fig. S4). In accordance with our animal protocol we used >15% weight loss as a clinical endpoint. We terminated all experiments on the day post challenge in which one or more animals exhibited >15% weight loss and harvested tissues for postmortem analyses. This time point is consistent for a particular disease model but varies among models due to different disease kinetics. In all experiments, the number of animals and replication numbers were chosen as necessary to provide significance or lack thereof and are reported in the figure legends (11).

We monocolonized germ free Swiss Webster (SW) mice by oral gavage with *E. coli* O21:H+ or *E. coli* MG1655, treated with DSS and monitored wasting. These strains colonized the intestines of SW mice equivalently (Fig. S4). Compared to *E. coli* MG1655-colonized Jax mice, SW *E. coli* MG1655-monocolonized mice were more susceptible to DSS with animals of the later beginning to exhibit >15% weight loss 5-days post treatment due to muscle and fat loss (Fig. 1E–F, S4). By contrast, *E. coli* O21:H+-monocolonized mice did not lose weight (Fig. 1E–F, S4). Thus, *E. coli* O21:H+ mediated protection does not require other microbiota constituents.

E. coli O21:H+ and *E. coli* MG1655 LPS were equivalent in their ability to stimulate the innate immune mediator Toll-like receptor 4 (TLR4) (Fig. S4). *E. coli* O21:H+-monocolonized mice were more susceptible to an i.p. challenge of *E. coli* O21:H+ compared to *E. coli* MG1655-monocolonized mice that received an i.p. challenge of *E. coli* MG1655 (Fig. S4). The canonical proinflammatory cytokines associated with wasting, TNF α , IL-1 β and IL-6, were not downregulated in *E. coli* O21:H+ mice nor was there any difference in intestinal inflammation between DSS-treated vehicle and *E. coli* O21:H+ mice (Fig. S5). Thus differences in LPS levels, stimulatory capacity or responsiveness to *E. coli* O21:H+ were not responsible for the reduced wasting observed in *E. coli* O21:H+ mice upon DSS challenge. Likewise, we found no difference in intestinal tissue damage in wasting susceptible and wasting protected animals as indicated by no differences in epithelial cell loss, hyperplasia, edema or fibrosis (Fig. S5).

Metabolic changes observed during wasting are distinct from those observed under conditions of food deprivation, and nutritional interventions cannot reverse wasting (12–14). The DSS-induced anorexic response was equivalent in vehicle and *E. coli* O21:H+ animals (Fig. S4). The comprehensive lab animal monitoring system (CLAMS), revealed no differences in the rates of oxygen consumption or metabolic rate, carbon dioxide production, respiratory exchange ratio, activity or heat production of DSS-treated animals with and without *E. coli* O21:H+ (Fig. S6).

In untreated mice, *E. coli* O21:H+ had no effect on host survival (carried out to one year). Interestingly, upon DSS treatment, 70% of Jax mice given *E. coli* O21:H+, survived, whereas 100% of control Jax mice that were wasting susceptible died within twelve days of treatment initiation (Fig. 1G).

We previously identified an *E. coli* O21:H+ strain in a sepsis model caused by intestinal insult (15). Muscle wasting is a consequence of sepsis. Mice in this model and a model of sepsis induced by i.v. injection with this microbe, were protected from wasting (15). We

therefore asked if *E. coli* O21:H+ ST101 identified in the current study prevents wasting induced by other microbes as follows.

The oral pathogen *Salmonella* Typhimurium, induces wasting of muscle and adipose tissue in Jax mice (Fig. 2A–B, S7). *E. coli* O21:H+ Jax mice did not show weight loss, skeletal muscle or adipose tissue wasting when infected orally with *S. Typhimurium* compared to vehicle-treated infected Jax mice (Fig. 2C–D, Fig. S7).

We tested whether *E. coli* O21:H+ could antagonize wasting in the *Burkholderia thailandensis* pneumonia model in which there is no compromise of the gut barrier. Consistent with critically ill patients with pulmonary dysfunction (16), intranasally infected Jax mice exhibited rapid wasting (~15% weight loss by 2-day postinfection) characterized by a depletion of both muscle and fat (Fig. 2B, E, S7). Histological analysis showed that the intestinal barrier remained intact during infection in contrast to DSS-treated animals that exhibited leaky gut (Fig. S5, S7). To assess gut barrier function, *B. thailandensis* infected mice were gavaged with FITC-dextran, but serum FITC remained negative, in contrast to serum from DSS-treated mice, confirming the gut barrier was not breached (Fig. S7). *B. thailandensis*-infected/*E. coli* O21:H+-colonized Jax mice showed a ~2% decrease in body weight with significantly less muscle and fat wasting than infected mice given vehicle, which exhibited a ~15% decrease in weight (Fig. 2D, F, S7). Consistent with our findings with DSS, *E. coli* O21:H+ mitigation of *S. Typhimurium*- and *B. thailandensis*-induced wasting was independent of changes in the infection-induced anorexic response, caloric absorption by the intestine or alterations in oxygen consumption, carbon dioxide production, activity levels or heat production (Fig. S7, S8). *E. coli* O21:H+ colonization did not result in down-regulation of systemic TNF α , IL-1 β or IL-6, nor with a reduction in tissue damage (*S. Typhimurium*-liver, spleen; *B. thailandensis*-lung, liver, spleen) (Fig. S9–S11). Consistent with previous reports (17), intestinal pathology in *S. Typhimurium* infected animals was minimal and indistinguishable in animals with and without *E. coli* O21:H+, remaining at background levels (Fig. S10). The pathogen burdens in *E. coli* O21:H+ animals were not reduced compared to infected animals treated with vehicle (Fig. 2G, *B. thailandensis* did not disseminate to the spleen and liver at our doses). Thus, the presence of *E. coli* O21:H+ in the intestinal microbiota appears to reduce/inhibit lean body wasting induced by pathogens or intestinal injury. It is this protective effect, rather than pathogen killing, that significantly promoted survival of *S. Typhimurium*-infected animals given *E. coli* O21:H+ (Fig. 2H, S7).

Transcriptional induction of the E3 ubiquitin ligases Atrogin-1 and Murf1 is crucial for muscle atrophy (18–20) and are induced upon challenge with *B. thailandensis*, *S. Typhimurium* or DSS (Fig. 3A, S12). Induction of *Atrogin-1* and *Murf1* did not occur in the muscle of *E. coli* O21:H+ Jax mice challenged with pathogen or DSS and were at equivalent levels found in untreated mice with and without *E. coli* O21:H+ (Fig. 3A, S12).

RNAseq analysis of leg muscles from *S. Typhimurium*-infected mice revealed that components of the insulin/insulin like growth factor-1 (IGF-1) signaling pathway, muscle physiology and metabolism, were upregulated in *E. coli* O21:H+ mice compared to infected vehicle mice (Fig. S13). The IGF-1/PI3K/AKT pathway is a master regulator of muscle size

that activates factors for protein synthesis and regeneration as well as the down-regulation of *Atrogin-1* and *Murfl* expression in atrophying muscle (21–23). Untreated Jax mice with or without *E. coli* O21:H+ had comparable levels of serum IGF-1 as measured by ELISA ((11) and Fig. S13) and showed no muscle hypertrophy (Fig. S3); likewise when infected with *S. Typhimurium*, *B. thailandensis* or given DSS, serum levels of IGF-1 and downstream signaling components in muscle (IGFR and AKT) in *E. coli* O21:H+ mice were sustained activation levels comparable to untreated animals, while activation levels in challenged animals given vehicle decreased significantly (Fig. 3B, S13, S14).

IGF-1 is produced primarily by the liver in response to growth hormone (GH) (24). We found that serum levels of GH, liver *Igf-1* expression and IGF-1 protein levels in the liver, muscle and intestine were unchanged in challenged *E. coli* O21:H+ animals compared to challenged animals treated with vehicle or unchallenged animals with and without *E. coli* O21:H+ (Fig. S13). Instead, during challenge with pathogens or DSS, white adipose tissue (WAT) from challenged *E. coli* O21:H+ mice showed higher levels of IGF-1 compared to WAT from challenged vehicle animals and unchallenged animals with and without *E. coli* O21:H+ (Fig. 3C, S15). This was associated with *E. coli* O21:H+ colonization of the WAT, but not the muscle or WAT associated lymph nodes (Fig. 3D, S15). *B. thailandensis* and *S. Typhimurium* did not infect the WAT. Thus sustained systemic levels of IGF-1 by *E. coli* O21:H+ is associated with increased WAT IGF-1 levels and colonization of the WAT by this commensal. A role for WAT-derived IGF eliciting systemic effects is supported by previous studies (25).

Systemic administration of an IGF-1 neutralizing antibody to *E. coli* O21:H+ mice in the absence of pathogen infection had no effect on mouse weight (Fig. S15). When given the neutralizing antibody i.p., *E. coli* O21:H+ mice infected with *B. thailandensis* were no longer protected from wasting (Fig. 3E–F, S15). These animals showed significant weight loss, muscle depletion (but not fat loss), a decrease in muscle IGF-1 signaling and an increase in muscle expression of *Atrogin-1* and *Murfl* compared to infected *E. coli* O21:H+ Jax mice given an IgG isotype control antibody (Fig. 3E–H, S15–16). There was no significant differences in the ceca levels of *E. coli* O21:H+ or *B. thailandensis* lung infection levels in *E. coli* O21:H+ mice that were treated with anti-IGF-1 antibody or the IgG isotype control (Fig S15).

The inflammasome – a cytoplasmic multi-protein complex required for activation of the Caspase-1 (CASP1) protease (26), has recently emerged as a critical regulator of host-microbiota interactions and metabolism (26, 27). We therefore examined *Casp1*^{−/−} mice, which are also deficient for CASP11 (28). In contrast to wild type mice, oral administration of *E. coli* O21:H+ to *B. thailandensis*-infected *Casp1*^{−/−} *Il1*^{−/−} mice did not protect against muscle wasting nor prevented the upregulation of *Murfl* and *Atrogin-1* in muscle nor prevented an infection-induced drop in systemic IGF-1 (Fig. 4A–C, S17). Similarly, IGF-1 levels in the WAT were less in *B. thailandensis*-infected *Casp1*^{−/−} *Il1*^{−/−} *E. coli* O21:H+ mice than in infected wild type *E. coli* O21:H+ mice (Fig. S17). Lung infection levels of *B. thailandensis* and intestinal levels of *E. coli* O21:H+ were not significantly different between infected *Casp1*^{−/−} *Il1*^{−/−} and wild type mice with or without *E. coli* O21:H+ (Fig S17).

Several distinct inflammasomes have been described, each of which contains a protein of the nucleotide-binding domain-containing (NLR) protein superfamily. The NLRC4 inflammasome is activated by the inner rod protein of type three secretion systems (TTSS) and by flagellin of Gram-negative bacteria (26), both of which are encoded by *E. coli* O21:H+ (Table S2). Similarly to *Casp1*^{-/-}*Il1*^{-/-} mice, the presence of *E. coli* O21:H+ in *B. thailandensis*-infected *Nlrc4*^{-/-} mice did not antagonize wasting nor prevented an infection-induced drop in systemic IGF-1 levels (Fig. 4D, S18).

Colonization levels of *E. coli* O21:H+ in the WAT deposits of *B. thailandensis*-infected *Casp1*^{-/-}*Il1*^{-/-} were equivalent to those in the WAT of infected-wild type mice (Fig. S17). Therefore the inflammasome is not necessary for translocation of *E. coli* O21:H+ from the intestine to the WAT.

Activation of any inflammasome leads to maturation of the pro-inflammatory cytokines interleukin-1 β (IL-1 β) and IL-18. Systemic levels of IL-18 in *B. thailandensis*-infected *E. coli* O21:H+ wild type mice were increased compared to infected vehicle mice, with no differences in IL-1 β levels (Fig. 4E, S5). The protective effect of *E. coli* O21:H+ functioned in *Il1 β* ^{-/-} mice but not in *Il1 β* ^{-/-}*Il18*^{-/-} mice in which *E. coli* O21:H+ did not protect from *B. thailandensis*-induced muscle and fat wasting (Fig. S18). *B. thailandensis*-infection levels in the lung and *E. coli* O21:H+ levels in the intestine and WAT were similar between infected wild type and *Il1 β* ^{-/-}*Il18*^{-/-} mice given *E. coli* O21:H+ (Fig. S18). When *Il1 β* ^{-/-}*Il18*^{-/-} mice were challenged with *B. thailandensis* IGF-1 loss was not prevented by *E. coli* O21:H+ (Fig. 4F). We conclude that the ability of *E. coli* O21:H+ to maintain levels of IGF-1 during challenge is mediated by the *Nlrc4* inflammasome via IL-18, (with a possible synergistic role for IL-1 β) and thereby prevents muscle wasting and promotes disease tolerance.

The intestinal microbiota performs essential functions for its host to achieve metabolic homeostasis. Our results strongly suggest that a specific constituent of the murine intestinal microbiota, *E. coli* O21:H+, antagonizes wasting triggered by infections and intestinal injury. During homeostasis, *E. coli* O21:H+ remains in the intestine, has no effect on weight, body composition or IGF-1 levels. During challenge, *E. coli* O21:H+ sustains systemic IGF-1 and signaling of IGF-1 in muscle to prevent wasting and promote disease tolerance (29–31) independent of metabolic changes, food consumption, caloric uptake, inflammation or tissue damage. This protection is associated with challenge-induced translocation of *E. coli* O21:H+ to WAT and activation of the NLRC4 inflammasome that correlates with increased production of IGF-1 by WAT. The ability to translocate to and colonize the WAT distinguishes *E. coli* O21:H+ from other microbes (including *B. thailandensis* and *S. Typhimurium*) that can activate inflammasomes where its presence antagonizes wasting in an inflammasome-dependent manner possibly induced by its flagellin or the inner rod protein of the its TTSS, eprJ. (Fig. S19).

The microbiota is crucial for the development and maintenance of a robust immune system and resistance defenses (32, 33). Discovery of a molecular mechanism by which the microbiota can promote tolerance to infection provides a perspective into the evolutionary forces that have driven the co-evolution of host-microbiota interactions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Protective Effects of an *E. coli* strain

Infection and intestinal damage can trigger cachexia, culminating in severe muscle wasting and loss of fat. How this happens is poorly understood. Palaferri-Schieber *et al.* have discovered a protective *Escherichia coli* strain in their mouse colony. If mice were intestinally colonized with the *E. coli* and infected with *Salmonella* or with *Burkholderia*, which only infects lungs, they did not waste away. Without the *E. coli* the mice became fatally sick. The protective effect of the *E. coli* was mediated via an innate immune mechanism that ensured muscle-signalling pathways were not damaged by infection. Thus, the *E. coli* allowed its host to tolerate and survive the pathogens.

Fig. 1. Effects of *E. coli* O21:H+ on DSS-induced skeletal muscle atrophy

(A–B) Jax mice were treated with 5% DSS. (A) Percent original weight. (B) Leg muscle mass at 7-days. Representative experiment ($N = 4–6$) using 5 animals per group, mean $\pm$ S.D. Unpaired t -test. (C) Percent original weight of colony born C57Bl/6 mice (UC Berkeley) treated with ampicillin, vancomycin, neomycin, metronidazole and 5% DSS (methods). Representative experiment ($N = 3$) using 4 animals per group, mean $\pm$ S.D. Unpaired t -test. (D) Percent original weight of 5% DSS-treated Jax mice gavaged with 5×10^8 CFU *E. coli* O21:H+ or vehicle (methods). Representative experiment ($N = 6$) using 4 animals per group, mean $\pm$ S.D. Unpaired t -test. Fig. S3 shows untreated control mice $\pm$ *E. coli* O21:H+. (E–F) Germ free Swiss Webster (SW) mice gavaged with 5×10^8 *E. coli* O21:H+ or *E. coli* MG1655 (methods) and treated with 5% DSS. (E) Percent original

weight. **(F)** Leg muscle mass at 5-days from a subset of mice in **(E)** that were weight matched at Day 0. Representative experiment ($N = 4$) using 5–7 animals per group, mean $\pm$ S.D. Unpaired t -test. Fig. S3 shows monocolonized SW controls. **(G)** Percent survival of Jax mice administered *E. coli* O21:H+ or heat-killed *E. coli* O21:H+ (methods) and treated with 5% DSS for 7-days, after which DSS was no longer administered. Results are from two independent experiments using 4–7 mice per group per experiment. Log rank analysis. Survival analyses of untreated control $\pm$ *E. coli* O21:H+ mice have been carried out for one year with no deaths from either group. Vehicle/DSS treated mice exhibit similar death kinetics as heat-killed/DSS treated mice. **** $p < 0.0001$, *** $p < 0.0005$, ** $p < 0.005$, * $p < 0.05$. EDL = extensor digitorum longus, TA = tibialis anterior. Experiments terminated for postmortem analyses at indicated day when one or more mice lost **(A, B, D, E, F)** >15% body weight in accordance with our Salk animal protocol or **(C)** >20% weight loss in accordance with our animal protocol (Berkeley).

Fig. 2. Effects of *E. coli* O21:H+ colonization on muscle wasting induced by infections

(A) Percent original weight of Jax mice orally infected with 8.5×10^6 *S. Typhimurium*. Representative experiment ($N = 3$) using 5 animals per group, mean $\pm$ S.D. Unpaired *t*-test. (B) Leg muscle mass from *S. Typhimurium*-infected mice in (A) at Day 5 (ST) or *B. thailandensis*-infected mice in (E) at Day 2 (Bt). Representative experiment ($N = 3$) using 5 animals per group, shown as mean $\pm$ S.D. Unpaired *t*-test. (C) Percent original weight of Jax mice gavaged with 5×10^8 CFU *E. coli* O21:H+ or vehicle and orally infected with 2×10^7 *S. Typhimurium*. Representative experiment ($N = 7$) using 4–5 animals per group, mean $\pm$ S.D. Unpaired *t*-test. (D) Tissue weight (mg) of leg muscles from *S. Typhimurium*-infected mice in (A) at Day 5 (ST) or *B. thailandensis*-infected mice in (E) at Day 2 (Bt). Representative experiment ($N = 3$) using 5 animals per group, shown as mean $\pm$ S.D. Unpaired *t*-test. (F) Percent original weight of Jax mice gavaged with 5×10^8 CFU *E. coli* O21:H+ or vehicle and orally infected with 2×10^7 *S. Typhimurium*. Representative experiment ($N = 7$) using 4–5 animals per group, mean $\pm$ S.D. Unpaired *t*-test. (G) Pathogen CFU/organ of Jax mice orally infected with 8.5×10^6 *S. Typhimurium* or 5×10^8 CFU *E. coli* O21:H+ or vehicle and orally infected with 2×10^7 *S. Typhimurium*. Representative experiment ($N = 7$) using 4–5 animals per group, mean $\pm$ S.D. Unpaired *t*-test. (H) Percent survival of Jax mice orally infected with 8.5×10^6 *S. Typhimurium* or 5×10^8 CFU *E. coli* O21:H+ or vehicle and orally infected with 2×10^7 *S. Typhimurium*. Representative experiment ($N = 7$) using 4–5 animals per group, mean $\pm$ S.D. Unpaired *t*-test.

S.D. Unpaired *t*-test. Fig. S3 shows untreated control mice +/- *E. coli* O21:H+. **(D)** Leg muscle mass from infected *E. coli* O21:H+ or vehicle mice from **(C)** at Day 3 (*ST*) and from **(F)** at Day 2 (*Bt*). Representative experiment (*N* = 3–7) using 4–5 animals per group, mean +/- S.D. Unpaired *t*-test. Fig. S3 shows control mice +/- *E. coli* O21:H+. **(E–F)** Jax mice were **(E)** left untreated or **(F)** gavaged with 5×10^8 CFU *E. coli* O21:H+ or vehicle (methods) and intranasally infected with 2500 CFU *B. thailandensis*. Representative experiment (*N* = 3–7) using 4–5 animals per group, shown as mean +/- S.D. Unpaired *t*-test. **(G)** *S. Typhimurium* (2×10^7 CFU inoculum) or *B. thailandensis* (2500 CFU inoculum) burdens in target tissues from infected mice +/- *E. coli* O21:H+ at 3-day (*ST*) or 2-day (*Bt*). *B. thailandensis* was not detected in the liver and spleen at this dose. Representative experiment (*N* = 3–7) using 3–7 animals per group, mean +/- S.D. Unpaired *t*-test. Dotted line indicates limit of detection. **(H)** Percent survival of Jax mice treated with 5×10^8 CFU *E. coli* O21:H+ or vehicle orally infected with 2×10^7 *S. Typhimurium*. Results are from two independent experiments with 10–12 animals per group per experiment. Log rank analysis. *****p* < 0.0001 *** *p* < 0.0005, ** *p* < 0.005, * *p* < 0.05. Postmortem analyses at day when one or more mice lost >15% body weight. EDL = extensor digitorum longus, TA = tibialis anterior.

Fig. 3. Gut colonisation by *E. coli* O21:H+ associated with down-regulation of muscle atrophy programs and maintenance of IGF-1 signaling during infection

(A–B) Jax mice gavaged with 5×10^8 CFU *E. coli* O21:H+ or vehicle and intranasally infected with 1000 CFU *B. thailandensis*. (A) *Murf1* and *Atrogin-1* expression in leg muscle at 2-day postinfection normalized to ribosomal protein S17 (RPS17) expression. Representative experiment ($N = 3$) using 4–9 animals per group, mean $\pm$ S.D. ANOVA.

(B) Serum IGF-1 at 2-days. Representative experiment ($N = 3$) using 7–9 animals per group, shown as mean $\pm$ S.D. ANOVA. See Fig. S14 for additional controls. (C–D) Jax mice

were given 5×10^8 CFU *E. coli* O21:H+ or vehicle and intranasally infected with 2500 CFU *B. thailandensis*. (C) IGF-1 levels in WAT and (D) *E. coli* O21:H+ levels in the WAT at Day 2. Representative experiment ($N = 4$) using 6–10 animals per group, mean $\pm$ S.D. ANOVA. Red line = limit of detection. (E–H) Mice given 5×10^8 CFU *E. coli* O21:H+ or vehicle were injected i.p. with anti-IGF-1 or IgG isotype control antibody and intranasally infected with 1000 CFU *B. thailandensis*. (E) Percent original weight. Results are from two independent experiments using 6–9 mice per group per experiment (12–17 mice total), mean $\pm$ S.D. ANOVA. Fig. S14 for additional controls. (F) Leg muscle mass and (G) Adipose tissue mass at 2-days. EDL = extensor digitorum longus, TA = tibialis anterior. GWAT = gonadal white adipose tissue, RPWAT = retroperitoneal WAT, IWAT = inguinal WAT, BAT = brown adipose tissue. Results are from the two independent experiments described in (E) using 5–9 mice weight matched at day 0 (~18g) for each group for each experiment (10–14 mice total), mean $\pm$ S.D. ANOVA. (H) *Murfl* and *Atrogin-1* expression in leg muscle at 2-day and normalized to RPS17. Representative experiment ($N = 2$) using 5–9 animals per group, mean $\pm$ S.D. ANOVA. **** $p < 0.0001$, *** $p = 0.0006$, ** $p < 0.005$, * $p < 0.05$. Postmortem analyses at day when one or more mice lost >15% body weight.

Fig. 4. The Nlr4 inflammasome is required for sustaining IGF-1 levels and mediates the *E. coli* O21:H+ associated protection against muscle wasting promoted by pathogenic infection (A–C) Wild-type and *Casp1^{-/-}11^{-/-}* mice were administered 5×10^8 CFU *E. coli* O21:H+ or vehicle and then intranasally infected with 2500 CFU *B. thailandensis* as described in methods. (A) Percent original weight at day 2. See Fig. S3 for additional controls. (B) Expression of *Murf1* and *Atrogin-1* were determined in leg muscle at 2-day postinfection normalized to expression levels of RPS17. (C) Serum IGF-1 quantified at day 2. Representative experiment ($N = 3$) using 10 animals per wildtype group and 5–7 mice per

Casp1^{-/-}Il1^{-/-} group, mean $\pm$ S.D. ANOVA. **(D)** Wild-type and *Nlrc4^{-/-}* mice were given 5×10^8 CFU *E. coli* O21:H+ or vehicle and infected intranasally with 2500 CFU *B. thailandensis*. Serum levels of IGF-1 at 2-days postinfection were measured. Representative experiment ($N = 2$) using 10 animals per wildtype group and 5 mice per *Nlrc4^{-/-}* group, shown as mean $\pm$ S.D. ANOVA. **(E)** Jax mice were given with 5×10^8 CFU *E. coli* O21:H+ or vehicle and then intranasally infected with 2500 CFU *B. thailandensis* as described in Methods and serum IL-18 levels were measured at 2-days postinfection. Representative experiment ($N = 2$) using 10 animals per group, mean $\pm$ S.D. ANOVA. **(F)** Wild-type and *Il1 β ^{-/-}Il18^{-/-}* mice were administered 5×10^8 CFU *E. coli* O21:H+ or vehicle and then intranasally infected with 2500 CFU *B. thailandensis*. Serum levels of IGF-1 at 2-days postinfection were measured. Representative experiment ($N = 3$) using 10 animals per wildtype group and 8 mice per *Il1 β ^{-/-}Il18^{-/-}* group, mean $\pm$ S.D. ANOVA. *** $p < 0.0003$, ** $p < 0.006$, * $p < 0.05$. Experiment was terminated at 2-days for postmortem analyses when one or more mice lost $>15\%$ body weight in accordance with our Salk animal protocol. **(C, D, F)** see Fig. S14 for additional controls.