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Eosinophils and type 2 cytokine signaling in macrophages orchestrate development of functional beige fat

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SUMMARY

Beige fat, which expresses the thermogenic protein UCP1, provides a defense against cold and obesity. Although a cold environment is the physiologic stimulus for inducing beige fat in mice and humans, the events that lead from the sensing of cold to the development of beige fat remain poorly understood. Here, we identify the efferent beige fat thermogenic circuit, consisting of eosinophils, type 2 cytokines interleukin (IL)-4/13 and alternatively activated macrophages. Genetic loss of eosinophils or IL-4/13 signaling impairs cold-induced biogenesis of beige fat. Mechanistically, macrophages recruited to cold-stressed subcutaneous white adipose tissue (scWAT) undergo alternative activation to induce tyrosine hydroxylase expression and catecholamine production, factors required for browning of scWAT. Conversely, administration of IL-4 to thermoneutral mice increases beige fat mass and thermogenic capacity to ameliorate pre-established obesity. Together, our findings have uncovered the efferent circuit controlling biogenesis of beige fat and provide support for its targeting to treat obesity.

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AUTHOR CONTRIBUTION

Y.Q., K.D.N., J.I.O., and X.T. designed and performed the main experiments, and X.C. provided technical assistance for the main experiments. R.M.L. and R.D.P. provided essential mouse lines for the completion of the studies. Y.Q., K.D.N., J.I.O., and A.C. discussed and interpreted the results from the study. Y.Q. and A.C. conceived, supervised, and wrote the paper. All animal care and procedures were performed in accordance with UCSF's IACUC guidelines.

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INTRODUCTION

Obesity, which affects 1.4 billion adults globally, represents the greatest current threat to human health (Finucane et al., 2011). Chronic imbalance between energy intake and energy expenditure causes obesity for which there is no effective therapy (Harms and Seale, 2013; Lowell and Spiegelman, 2000). Thus, a major challenge for biomedical sciences is to identify targetable pathways that can decrease energy intake or increase energy expenditure. One of the most promising targets for treatment of human obesity is brown adipose tissue (BAT) (Enerback, 2010; Harms and Seale, 2013), but adult humans lack this thermogenic interscapular organ (Lidell et al., 2013). However, recent studies have demonstrated that adult humans harbor a separate depot of brown adipocytes that are cold inducible and interspersed amongst white adipocytes in the supraclavicular, para-aortic, and suprarenal regions (Cypess et al., 2009; Saito et al., 2009; van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009). Since these human brown adipocytes share some molecular, histologic, and functional characteristics with cold-inducible beige adipocytes found in the subcutaneous white adipose tissue (scWAT) of mice (Cypess et al., 2013; Liu et al., 2013; Sharp et al., 2012; Wu et al., 2012; Wu et al., 2013), there is great clinical interest in the therapeutic targeting of beige fat for the treatment of obesity (Enerback, 2010; Harms and Seale, 2013). However, our lack of understanding of how cold triggers the development of functional beige fat is a major barrier for its therapeutic translation.

Uncoupling protein-1 (UCP1), which dissipates the mitochondrial electrochemical gradient to stimulate cellular respiration, mediates the thermogenic activity of both brown and beige adipocytes (Cannon and Nedergaard, 2010, 2011; Feldmann et al., 2009). Despite this similarity in thermogenesis, multiple lines of evidence indicate that brown and beige adipocytes have unique expression profiles that likely contribute to their tissue-specific functions (Harms and Seale, 2013). First, unlike interscapular brown adipocytes that arise from $Myf5^{+}/Pax7^{+}$ myogenic precursors (Lepper and Fan, 2010; Seale et al., 2008; Timmons et al., 2007), beige adipocytes residing in the scWAT of mice do not have a history of $Myf5^{+}$ expression (Seale et al., 2011). Second, brown adipocytes constitutively express Ucp1 after differentiation, whereas beige adipocytes specifically increase expression of thermogenic genes, such as Ucp1, in response to environmental cold, and agonists of the β-adrenergic receptor or peroxisome proliferator-activated receptor-γ (Ppar-γ) (Liu et al., 2013; Ohno et al., 2012; Wu et al., 2012). Third, a number of genes, such as Klhl13, Ear2, Tbx1, Tmem26, and CD137, are preferentially expressed in beige adipocyte precursors (Liu et al., 2013; Sharp et al., 2012; Wu et al., 2012). Together, these findings suggest that beige and brown adipocytes are likely to have complementary functions in the maintenance of energy balance and thermogenesis; however, rigorous proof for the therapeutic efficacy of beige fat in the treatment of obesity is lacking.

In the textbook view of thermogenesis, the sensing of cold by the neuronal system triggers the sympathetic efferents that promote the biogenesis and activation of beige fat (Cannon and Nedergaard, 2011; Lowell and Spiegelman, 2000). While this model works well for tissues that are densely innervated by the sympathetic nerves (Morrison and Nakamura, 2011), such as the interscapular BAT, it does not explain how cold exposure results in the rapid remodeling of the poorly innervated scWAT (Daniel and Derry, 1969; Slavin and

Ballard, 1978; Trayhurn and Ashwell, 1987). In these classic studies, adrenergic nerves only innervated 2-3% of all adipocytes in WAT, leading these authors to conclude that the sympathetic nerves primarily innervate blood vessels of the WAT (Daniel and Derry, 1969; Slavin and Ballard, 1978). These older observations thus suggest that the adrenergic tone of WAT must somehow be amplified during cold stress to stimulate biogenesis of beige fat. In this regard, we recently reported that acute cold stress results in type 2 (alternative) activation of macrophages in WAT, which locally secrete catecholamines, to sustain the metabolic adaptations to environmental cold (Nguyen et al., 2011). These findings, which provide a potential explanation for this remodeling conundrum, led us to investigate the functions of type 2 cytokines, IL-4 producing cells, and alternatively activated macrophages in the biogenesis of beige fat.

Here, we report that the cold-induced remodeling of scWAT into thermogenic beige fat is dependent on eosinophils, type 2 cytokines, macrophages, and myeloid cell-derived catecholamines. Genetic disruption of IL-4/13 signaling or tyrosine hydroxylase (*Th*), the rate limiting step in the synthesis of catecholamines, in myeloid cells prevents cold-induced biogenesis of beige fat, whereas treatment of thermoneutral obese mice with IL-4 enhances the growth of beige fat to mitigate obesity and its attendant sequelae. These data provide fundamental insights into how the sensing of cold is translated into the biological program of beige fat differentiation and thermogenesis in mice, findings that have potential implications for increasing beige fat mass and function in humans.

RESULTS

Type 2 cytokines are required for development of functional beige fat

To investigate the role of type 2 immunity in biogenesis of cold-induced beige fat, we quantified the expression of thermogenic genes in scWAT of WT and *Il4/13*$^{-/-}$ mice housed at thermoneutrality (30°C), 22°C, or 5°C for 48 hours. Quantitative RT-PCR analysis revealed that prolonged exposure to environmental cold (5°C) induced the expression of the core set of thermogenic genes, including *Ucp1*, *Ppargc1a*, *Cox8b*, *Cidea*, *Elovl3*, and *Cpt1b*, in the scWAT, whose induction was largely absent in eWAT of WT mice (Figure 1A and S1A). This cold-induced increase in expression of thermogenic genes was reduced by ~4-9-fold in the scWAT of *Il4/13*$^{-/-}$ mice (Figure 1A). Congruent with these observations, expression of UCP1 protein, which is required for uncoupled respiration in brown and beige adipocytes (Cannon and Nedergaard, 2011; Wu et al., 2012), was increased (~4-fold) after cold exposure in the scWAT of WT but not *Il4/13*$^{-/-}$ mice (reduced by ~80%, Figure 1B). This difference in scWAT UCP1 expression was also evident at 22°C, a housing temperature that is known to impose thermal stress in young mice (Figure 1B) (Cannon and Nedergaard, 2011). In contrast to the changes in scWAT, prolonged exposure to 5°C did not alter the expression or content of UCP1 in BAT of WT and *Il4/13*$^{-/-}$ mice (Figure 1B and Table S1A). Histologic analysis further affirmed that cold-induced remodeling of scWAT, but not eWAT, into beige fat was dependent on the type 2 cytokines IL-4 and-13, as evidenced by the paucity of multilocular, UCP1^{+} adipocytes in the scWAT of *Il4/13*$^{-/-}$ mice (Figure 1C, D and Figure S1B,C). Furthermore, measurement of oxygen consumption in scWAT and BAT of cold exposed mice confirmed that type 2 cytokine signaling was

preferentially required for the browning of scWAT, as evidenced by ~51% and ~70% reduction in the rate of oxygen of scWAT of *Il4/13*$^{-/-}$ mice housed at 22°C and 5°C, respectively (Figure 1E, F).

Since brown/beige fat thermogenesis is stimulated by environmental cold in humans (van der Lans et al., 2013; Yoneshiro et al., 2013), we next investigated the thermogenic capacity of WT and *Il4/13*$^{-/-}$ mice under different environmental temperatures. Consistent with the human studies, total oxygen consumption was not different between WT and *Il4/13*$^{-/-}$ mice when they were housed at the thermoneutral temperature of 30°C (Figure 1G). However, exposure of these animals to progressively colder temperatures increased oxygen consumption in WT mice, a response that was blunted in *Il4/13*$^{-/-}$ mice, especially at 4°C (Figure 1H, I). This difference in energy expenditure (~11-12%) between WT and *Il4/13*$^{-/-}$ mice, which was independent of body mass (Table S1B and Figure 1I), likely reflects the thermogenic activity of beige fat because interscapular BAT UCP1 content, respiratory exchange ratio (RER), muscle thermogenic gene expression, total activity, and food intake were not different amongst the genotypes (Tables S1A, C, S2A-B, and Figure S1D, E). These data thus demonstrate that cold-induced remodeling of scWAT into functional beige fat requires the type 2 cytokines IL-4 and 13.

Signaling via the IL4Rα and STAT6 regulates biogenesis of beige fat

We next investigated the downstream signaling pathways by which IL-4/13 mediate their browning effects in the scWAT. Since IL-4Rα is the shared subunit between the type I (IL-4Rα/γc) and II (IL-4Rα/IL-13Rα1) receptors (Kelly-Welch et al., 2003), which mediate the known biologic effects of IL-4/13, we first examined the requirement of IL-4Rα in the biogenesis of beige fat. Similar to *Il4/13*$^{-/-}$ mice, cold induced increases in thermogenic gene and UCP1 protein expression were reduced (~3-16- and 10-fold, respectively) in the scWAT of *Il4ra*$^{-/-}$ mice (Figure S2A and 2A). Histological examination confirmed that the scWAT of *Il4ra*$^{-/-}$ mice housed at 5°C had fewer multilocular, UCP1^{+} beige adipocytes (Figure S2B and 2B). Accordingly, cold-induced increase in energy expenditure (VO_2 consumption) was reduced by ~14-24% in *Il4ra*$^{-/-}$ mice (Figure 2C, D), whereas oxygen consumption was not different between WT and *Il4ra*$^{-/-}$ mice at thermoneutrality (Figure S2C). Moreover, deletion of Il4ra did not alter BAT UCP1 content, body mass, RER, muscle thermogenic gene expression, food intake, and total activity in these animals (Table S1A-C, Table S2A, B, and Figure S2D, E). These results thus demonstrate that cold-induced activation of canonical IL-4/13 signaling via IL-4Rα mediates the remodeling of scWAT into thermogenic beige fat.

Although stimulation with IL-4 or IL-13 activates PI3K-AKT and MAPK/ERK signaling pathways (Heller et al., 2008), the reprogramming of cellular networks requires the transcription factor STAT6 in most cells (Goenka and Kaplan, 2011; Maier et al., 2012). This observation prompted us to investigate whether STAT6 might also be necessary for cold-induced remodeling of scWAT into beige fat. Indeed, the molecular, histologic, and metabolic phenotypes of cold-challenged *Stat6*$^{-/-}$ mice were similar to those of *Il4/13*$^{-/-}$ and *Il4ra*$^{-/-}$ mice (Figures 2E-G and S2F-J). For instance, cold-induced expression of UCP1 protein in the scWAT was decreased by ~12-fold in *Stat6*$^{-/-}$ mice (Figure 2E, F), as was the

concomitant increase in oxygen consumption (Figure 2G, H). Accordingly, compared to WT mice, *Il4/13*$^{-/-}$, *Il4ra*$^{-/-}$ and *Stat6*$^{-/-}$ mice maintained thermal homeostasis at a lower body temperature (Figure 2I). In aggregate, these findings provide strong evidence to support the hypothesis that canonical type 2 immunity, specifically the IL4/13-IL4Rα-STAT6 pathway, is required for biogenesis of functional beige fat, and raise four important questions. First, what cells secrete IL-4 to support the remodeling of scWAT into thermogenic beige fat? Second, how does activation of IL4/13 signaling promote development of beige fat? Third, is administration of IL-4 sufficient to increase beige fat mass in thermoneutral mice? And fourth, can IL-4 induced increase in beige fat mass ameliorate metabolic disease in an established model of obesity?

IL-4 producing eosinophils are required for biogenesis of beige fat

To identify cells competent for IL-4 production in scWAT, we housed 4get mice, which express green fluorescent protein (GFP) from the endogenous IL-4 locus (Mohrs et al., 2001), at 30°C and 5°C. Flow cytometric analysis revealed that the majority (~90%) of GFP^{+} cells in the scWAT of 4get mice housed at 5°C were eosinophils, as they expressed Siglec F and had high side scatter (Figure S3A) (Heredia et al., 2013; Wu et al., 2011). Exposure to environmental cold increased numbers of GFP^{+} cells in the scWAT of 4get mice (Figure 3A), a response that was reduced by ~85-95% in 4get-ΔdblGATA mice (Figure 3B-D), which lack eosinophils (Yu et al., 2002).

Using 4get and 4get-ΔdblGATA mice, we next investigated the requirement of eosinophils in cold-induced browning of scWAT. In a manner similar to *Il4/13*$^{-/-}$, *Il4ra*$^{-/-}$ and *Stat6*$^{-/-}$ mice, exposure to 5°C for 48 hours failed to increase the expression of core set of thermogenic genes, including *Ucp1*, *Ppargc1a*, *Prdm16*, *Cidea*, *Elovl3*, *Cpt1b*, and *Nrf1*, in 4get-ΔdblGATA mice (Figure 3E). This was accompanied by attenuated induction of thermogenic protein UCP1 (reduced by ~70%) and a marked reduction in morphological transformation of scWAT into beige fat (Figure 3F-H). Furthermore, similar to mice impaired in type 2 cytokine signaling, cold-induced increase in energy expenditure was reduced by ~14-17% in 4get-ΔdblGATA mice (Figure 3I, J). This decrease in energy expenditure was independent of BAT UCP1 content, body mass, RER, muscle thermogenic gene expression, food intake, and total activity because these indices were similar between 4get and 4get-ΔdblGATA mice (Table S1A-C, Table S2A, B, and Figure S3B, C). Together, these results suggest that eosinophils, which are the primary IL-4 producing cells in the scWAT, are required for the cold-induced transformation of scWAT into beige fat.

Cold-induced biogenesis of beige fat requires CCR2^{+} macrophages

Since macrophages are an important target of IL-4/13 signaling in adipose tissue (Odegaard and Chawla, 2013), we investigated whether macrophage content of scWAT was altered by prolonged cold exposure. Exposure to 5°C progressively increased macrophage content of scWAT, with maximal numbers observed at 48 hours after initiation of cold exposure (Figure S3D). This increase in scWAT macrophage content likely resulted from recruitment of Ly6C^{hi} monocytes because majority of the recruited macrophages continued to express Ly6C (Figure 4A) (Geissmann et al., 2010). Congruent with this idea, exposure to environmental cold failed to increase the numbers of total and Ly6C^{hi} macrophages in

Ccr2$^{-/-}$ mice (Figure 4A, S3D), which lack the chemokine receptor required for the recruitment of Ly6C^{hi} monocytes to sites of tissue stress and injury (Charo and Ransohoff, 2006; Shi and Pamer, 2011).

Using *Ccr2*$^{-/-}$ mice (Charo and Ransohoff, 2006; Shi and Pamer, 2011), we next investigated whether recruitment of macrophages is necessary for the remodeling of scWAT from a site of energy storage to a site of thermogenesis. Cold-induced expression of core set of thermogenic genes, including the uncoupling protein UCP1, was reduced in the scWAT of *Ccr2*$^{-/-}$ mice (Figure S3E, 4B). This impairment in biogenesis of beige fat was also evident histologically, as the scWAT of *Ccr2*$^{-/-}$ mice had fewer multilocular, UCP1^{+} beige adipocytes (Figure S3F). Accordingly, *Ccr2*$^{-/-}$ mice had ~8-9% lower oxygen consumption (Figure 4C, D) without any significant differences in UCP1 BAT content, body mass, RER, muscle thermogenic gene expression, food intake, or total activity (Table S1A-C, Table S2A, B, and Figure S3G, H). This defect in beige fat thermogenesis was unlikely secondary to a developmental defect in *Ccr2*$^{-/-}$ mice because clodronate-mediated macrophage depletion in adult WT mice yielded similar results (Figures S4A-E). Together, these data support a model in which factors elaborated by recruited macrophages stimulate the growth of functional beige fat.

Signaling via IL-4Rα in macrophages is required for biogenesis of beige fat

Type 2 cytokines IL-4 and IL-13 promote the alternative activation of macrophages (Martinez et al., 2009; Odegaard and Chawla, 2011), prompting us to ask whether prolonged exposure to 5°C induces this program of macrophage activation in the scWAT. Compared to thermoneutral mice, macrophages residing in the scWAT of mice housed at 5°C had higher expression of alternative activation markers Arginase 1 and CD301 (Figures 4E, S4F). Moreover, the requirement of IL-4/13 and IL-4Rα for the induction of CD301 and Arginase 1 suggested that these were bona fide alternatively activated macrophages (Figures 4E, S4F). Together, these findings suggest that type 2 cytokine signaling via the IL-4Rα in macrophages might be required for development of functional beige fat in animals habituated to 5°C.

To test this postulate, we utilized *Il4ra*$^{f/f}$ and *Il4ra*$^{f/f}$*Lyz2*^{Cre}, the latter genetically engineered to lack *Il4ra* in myeloid cells (Herbert et al., 2004). The cold-induced increases in UCP1 protein and thermogenic gene expression was reduced by ~3- and 4-10-fold, respectively, in *Il4ra*$^{f/f}$*Lyz2*^{Cre} (Figure 4F, S4G), indicating that myeloid cells are an important target for the browning effects of IL-4/13. Consistent with this notion, scWAT of *Il4ra*$^{f/f}$*Lyz2*^{Cre} mice had fewer UCP1^{+} beige adipocytes (Figure S4H) and lower rate of oxygen consumption at 22°C and 5°C (reduced by ~53% and 62%, respectively) (Figure 4G). In contrast, oxygen consumption rates were not different in the BAT of *Il4ra*$^{f/f}$ and *Il4ra*$^{f/f}$*Lyz2*^{Cre} mice (Figure 4H), results that are similar to what was observed in *Il4/13*$^{-/-}$ mice (Figure 1E, F). These observations prompted us to investigate whether cold-induced increases in energy expenditure might be impaired in *Il4ra*$^{f/f}$*Lyz2*^{Cre} mice. Indeed, rate of oxygen consumption was ~11-13% lower in *Il4ra*$^{f/f}$*Lyz2*^{Cre} mice at colder temperatures but not at thermoneutrality (Figure 4I-K). Consequently, scWAT and eWAT mass was higher in *Il4ra*$^{f/f}$*Lyz2*^{Cre} mice (Figure 4L), and these animals maintained thermal homeostasis at a

lower core body temperature than *Il4ra*$^{f/f}$ mice (Figure 4M). These cold-induced changes in energy expenditure occurred without significant alterations in BAT UCP1 content, body mass, RER, muscle thermogenic gene expression, food intake, or total activity (Table S1A-C, Table S2A, B, and Figure S4I, J). These findings demonstrate for the first time that type 2 cytokines and alternatively activated macrophages mediate the long-term physiologic adaptations to environmental cold by stimulating the growth of functional beige fat. Moreover, our results suggest that factors secreted by alternatively activated macrophages likely work in *trans* to regulate the development and activity of beige fat.

Myeloid cell Th is required for development of beige fat

In the prevailing model of cold-induced thermogenesis, release of norepinephrine by the sympathetic nerves stimulates the development of beige fat (Cannon and Nedergaard, 2011; Lowell and Spiegelman, 2000). However, we have previously demonstrated that alternatively activated macrophages, which express all enzymes necessary for catecholamine synthesis, comprise an important accessory circuit for catecholamine production during acute cold stress (Nguyen et al., 2011). These observations led us to postulate that a similar mechanism might underlie the biogenic effects of macrophages on cold-induced browning of scWAT. To investigate this hypothesis, we first examined effects of cold-exposure on expression of tyrosine hydroxylase (TH), the rate-limiting step in biosynthesis of norepinephrine (Thomas and Palmiter, 1997; Zhou et al., 1995), in various adipose depots. As shown in Figure S5A, expression of TH protein was temperature responsive and inducible in all three adipose depots (BAT, scWAT, and eWAT). However, unlike BAT, basal expression of TH was nearly absent in scWAT and eWAT of mice housed at 22°C, but dramatically induced upon cold exposure (Figure S5A), findings that are consistent with published reports demonstrating the dense innervation of BAT but not scWAT and eWAT by the sympathetic nerves (Daniel and Derry, 1969; Morrison and Nakamura, 2011; Slavin and Ballard, 1978; Trayhurn and Ashwell, 1987). Since this change in TH expression correlated with alternative activation of scWAT macrophages (Figure S5A, S4E and 4E), we utilized intracellular flow cytometry to quantify TH protein content of scWAT macrophages. In an IL-4/13- and IL-4Rα-dependent manner, environmental cold induced expression of TH protein in the scWAT macrophages (Figure 5A and S5B), findings that implicate macrophage derived catecholamines in the biogenesis of beige fat.

To definitively address the contribution of myeloid cell-derived catecholamines to cold-induced browning, we generated mice in which the *Th* gene, which is required for synthesis of all catecholamines (Zhou et al., 1995), was deleted in myeloid cells (designated *Th*$^{f/f}$*Lyz2*^{Cre}) (Jackson et al., 2012). Immunoblot analysis revealed robust expression of TH protein in the peritoneal macrophages of control (*Th*$^{f/f}$) mice, which was reduced by ~80-85% in peritoneal macrophages isolated from *Th*$^{f/f}$*Lyz2*^{Cre} mice (Figure 5B). Since catecholamines mediate the browning of scWAT (Lee et al., 2012), this novel mouse model provided us a unique opportunity to dissect the contribution of myeloid cell-versus sympathetic nerve-derived catecholamines to cold-induced biogenesis of beige fat. In line with our hypothesis, steady state norepinephrine content was reduced by ~70% in the scWAT, but not BAT, of *Th*$^{f/f}$*Lyz2*^{Cre} mice housed at 5°C (Figure 5C, D). Consequently, cold-induced browning was impaired in *Th*$^{f/f}$*Lyz2*^{Cre} mice, as evaluated by the histological

appearance of multilocular, UCP1^{+} beige adipocytes (Figure 5E, S5C). Immunoblot analysis of UCP1 protein in scWAT (reduced by ~3-fold) provided independent confirmation that biogenesis of beige fat but not BAT was defective in *Th*$^{f/f}$*Lyz2*^{Cre} mice (Figure 5F). TH protein expression, which was induced by cold exposure in the scWAT and eWAT of *Th*$^{f/f}$ mice, was decreased (~4.5- and ~5.5-fold, respectively) in *Th*$^{f/f}$*Lyz2*^{Cre} mice (Figure 5F), findings that suggest that myeloid cells rather than sympathetic nerves are the primary source of catecholamines in these cold stressed WAT depots. In contrast, TH protein expression was similar in BAT of *Th*$^{f/f}$ and *Th*$^{f/f}$*Lyz2*^{Cre} mice at 22°C and 5°C, confirming that adrenergic tone of this richly innervated tissue is dependent on the sympathetic nerves. The importance of myeloid cell-derived catecholamines in the regulation of WAT function was further affirmed by the ~66-68% reduction in serum levels of free fatty acids and higher weights of WATs of *Th*$^{f/f}$*Lyz2*^{Cre} mice after cold challenge (Figure 5G, H). These findings collectively suggest that cold-induced lipolysis and mobilization of stored triglycerides is dependent on myeloid cell-derived catecholamines.

Based on these results, we next tested whether myeloid cell-derived catecholamines are required for the increase in energy expenditure that is necessary to maintain thermal homeostasis. While oxygen consumption rates were similar between *Th*$^{f/f}$ and *Th*$^{f/f}$*Lyz2*^{Cre} mice at thermoneutrality (30°C), indirect calorimetry revealed that oxygen consumption was reduced by ~7-8% in *Th*$^{f/f}$*Lyz2*^{Cre} at different ambient temperatures (Figure 5H, I). These differences in energy expenditure between *Th*$^{f/f}$ and *Th*$^{f/f}$*Lyz2*^{Cre} mice could not be accounted for by differences in BAT content of UCP1, body mass, RER, muscle thermogenic gene expression, food intake, or total activity (Table S1A-C, Table S2A, B, and Figure S5D, E). Congruent with these observations, *Th*$^{f/f}$*Lyz2*^{Cre} mice maintained thermal homeostasis at a lower body temperature during prolonged cold exposure (Figure 5L). Taken together, these data support our hypothesis that myeloid cell-derived catecholamines, in particular those secreted by alternatively activated macrophages, are necessary for the molecular, histologic, and metabolic remodeling of scWAT into thermogenic beige fat.

Administration of IL-4 increases beige fat mass in thermoneutral mice

Having established a requirement for type 2 cytokines in the cold-induced remodeling of scWAT, we next investigated whether pharmacological activation of this signaling pathway was sufficient to increase the total thermogenic capacity in thermoneutral mice. To test this postulate, we administered IL-4 to WT BALB/cJ mice housed at 30°C (Figure 6A), a temperature at which mice lack the thermal drive to activate BAT or beige fat (Cannon and Nedergaard, 2011). In a dose-dependent manner, treatment with IL-4 increased UCP1 protein expression (~3-fold) in the scWAT but not interscapular BAT (Figure S6A). Of note, at the higher dose of IL-4 (2μg), even eWAT, which was resistant to cold-induced browning, developed molecular and histologic features of beige fat (Figure S6A, D, E). Based on these data, we decided to use the 2μg dose of IL-4 complexed to anti-IL-4 antibody (10 μg) for all subsequent studies (Ricardo-Gonzalez et al., 2010).

We next investigated the requirement of IL-4Rα in mediating the browning effects of IL-4 in thermoneutral mice. Administration of IL-4 complex increased the expression of UCP1 (~15-fold), alternative activation markers Arginase 1 and CD206, and TH (~2-3 fold) in the

scWAT and eWAT of WT mice (Figures 6B and S5F-H), augmenting the steady state catecholamine content of these WAT depots by ~69-83% (Figure S5I). This increase in adrenergic tone of scWAT and eWAT was sufficient to cause these fat depots to acquire histological characteristics of beige fat (Figure S6B-E). Since IL-4-mediated browning of scWAT and eWAT was nearly absent in *Il4ra*$^{-/-}$ mice (Figure 6B and S6B-E), it suggests that the on-target effects of this type 2 cytokine control the growth of beige fat. In contrast to IL-4-mediated remodeling of scWAT and eWAT, BAT's expression of UCP1 protein or its catecholamine content were unaffected by IL-4 administration in WT or *Il4ra*$^{-/-}$ mice (Figure 6B, Table S1A, and Figure S5J).

A hallmark of human beige/brown fat is the cold-induced increase in its thermogenic activity (van der Lans et al., 2013; Yoneshiro et al., 2013), prompting us to ask whether administration of IL-4 increases total thermogenic capacity in mice. To address this question, we first measured oxygen consumption in vehicle or IL-4 treated WT mice under different environmental temperatures. Although whole-body energy expenditure was similar between the two groups at thermoneutrality, the IL-4 treated animals had higher energy expenditure when housed at progressively cooler temperatures (Figure 6C, S6F). For instance, the total thermogenic capacity of IL-4 treated mice was 8-11% higher at the cooler environmental temperatures (Figure 6C, S6F), likely reflecting the increased beige fat mass in these animals. This is an important point because the UCP1 content of interscapular BAT was not significantly different between vehicle and IL-4 treated WT mice (Table S1A). Furthermore, IL-4-mediated increase in total thermogenic capacity was absent in *Il4ra*$^{-/-}$ mice (Figure 6D, S6G), which lacked molecular and histologic evidence of WAT browning but had similar amounts of interscapular BAT (Figures 6B, S6B-E and Table S1A). In both genotypes, chronic treatment with IL-4 did not significantly alter body mass, RER, muscle thermogenic gene expression, total activity, or food intake (Table S1B, C, Table S2A, B, and Figure S6H-K).

To definitively quantify the contribution of recruited beige fat to the total thermogenic capacity of mice, we administered norepinephrine (NE) to thermoneutral mice to maximally activate all adrenoreceptors and thermogenesis. As reported previously, injection of NE into conscious, thermoneutral mice transiently increases energy expenditure (Golozoubova et al., 2006). This observed increase in metabolic rate was greatly augmented in BALB/cJ mice that were pretreated with IL-4 (Figure 6E). Again, the thermogenic effects of IL-4 were on target because IL-4 failed to increase the rate of oxygen consumption in *Il4ra*$^{-/-}$ mice (Figure 6F). Based on the results, we next sought to determine whether this observed increase in metabolic rate was dependent on UCP1. While treatment with IL-4 augmented energy expenditure in C57BL/6J mice, this increase was completely absent in congenic *Ucp1*$^{-/-}$ mice (Figure 6G, H), indicating that IL-4 therapy results in the recruitment of beige fat to specifically increase the UCP1-dependent component of metabolic rate. Together, these findings demonstrate for the first time that IL-4-mediated increase in beige fat mass significantly contributes to the total thermogenic capacity of mice, findings that are likely to be relevant for the cold-induced recruitment of beige fat in humans.

Beige fat ameliorates metabolic dysfunction in established models of obesity

Although recruitment of beige fat has been postulated to exert negative energy balance and mitigate the metabolic complications of obesity, most studies have focused on prevention rather than treatment of diet-induced obesity (Harms and Seale, 2013). Since administration of IL-4 selectively increased beige fat mass in thermoneutral mice, we investigated whether this newly recruited beige fat can ameliorate metabolic dysfunction in the setting of pre-established obesity. For these studies, thermoneutral C57BL/6J mice were fed normal chow (NC) or high fat diet (HFD) for 10 weeks (Figure 7A). After matching for adiposity (Figure S7A), mice on HFD were treated with vehicle or IL-4 complexes over a period of 14 days (Figure 7A). Remarkably, dual-energy X-ray absorptiometry (DXA) revealed that, compared to vehicle treated animals, treatment with IL-4 decreased total body mass (~5.7g) and fat mass (~13.5%) without significantly affecting lean body mass (Figures 7B, C and S7B). This decrease in adiposity was also reflected in the smaller mass of scWAT and eWAT (Figures S7C-D). Immunoblotting analysis of adipose tissues revealed that HFD feeding decreased expression of TH (~70%) and UCP1 (~85%) proteins in the scWAT, which were restored after IL-4 therapy (Figure 7D). This was not limited to UCP1 because expression of the entire set of core thermogenic genes was restored by IL-4 therapy in the scWAT (Figure S7E). Similar increases in expression of TH and UCP1 proteins were observed in eWAT, but not BAT, of mice treated with IL-4 (Figure 7D, S7F), findings that were confirmed by the histologic examination of scWAT and eWAT of treated animals (Figure 7E and S7G).

We next investigated whether IL-4-induced increase in beige fat mass can restore insulin sensitivity in obese mice. Glucose and insulin tolerance tests demonstrated improvement in glucose disposal and insulin sensitivity, respectively, in IL-4 treated obese animals (Figure 7F, G). This was further affirmed by provocative insulin signaling studies, which revealed that IL-4 therapy improved insulin action, as quantified by the phosphorylation of AKT, in eWAT, liver, and quadriceps (Figure 7H). This improvement in insulin action was also associated with a decrease in liver triglyceride content, and in the circulating levels of triglycerides and cholesterol (Figure S7H-J). In aggregate, these results demonstrate for the first time that restoration of beige fat mass and activity can mitigate pre-established obesity and insulin resistance in mice.

DISCUSSION

Adult human BAT, which is dispersed in the supraclavicular, para-aortic, and suprarenal regions, is cold inducible, both in terms of its activity and mass (Enerback, 2010; Harms and Seale, 2013; Wu et al., 2013). However, unlike the classical brown adipocytes found in the interscapular regions of rodents and human infants, adult human BAT shares many molecular, histologic, and functional characteristics with the cold-inducible beige fat found in the scWAT of rodents (Harms and Seale, 2013; Liu et al., 2013; Sharp et al., 2012; Wu et al., 2012). For instance, rodent beige fat is derived from a group of non-myogenic progenitors that express CD137 and Tmem26 and induce their thermogenic activity upon cold exposure (Harms and Seale, 2013). Since these characteristics are in part shared by adult human BAT, cold-induced recruitment and activation of human BAT is thought to

primarily involve beige adipocytes. These observations led us to ask the fundamental question of how exposure to environmental cold triggers the growth of functional beige fat, a question that previously had not been addressed.

In the classical paradigm of cold-induced thermogenesis, the central sensing of cold by the dorsomedial hypothalamus results in the activation of sympathetic outflow to stimulate BAT thermogenesis (Morrison et al., 2012). While this efferent thermogenic circuit works well for the richly innervated interscapular BAT, it fails to explain how cold exposure induces browning of the poorly innervated scWAT (Daniel and Derry, 1969; Slavin and Ballard, 1978; Trayhurn and Ashwell, 1987). Since we previously implicated alternatively activated macrophages in local production of catecholamines (Nguyen et al., 2011), we investigated here the requirement and sufficiency of type 2 immunity in supporting the browning of scWAT. Using mice impaired in type 2 immune responses, including Δ*dbl-GATA*, *Il4/13*$^{-/-}$, *Il4ra*$^{-/-}$, and *Stat6*$^{-/-}$ mice (Martinez et al., 2009), we demonstrate for the first time that eosinophils and type 2 cytokines comprise the efferent arm of the thermogenic circuit that regulates beige fat development (Figure 7I). Macrophages, which are recruited to the cold-stressed scWAT via the chemokine receptor CCR2, are the core integrators of these thermogenic signals. Accordingly, mice lacking CCR2 or IL-4Rα in myeloid cells are unable to remodel their scWAT into beige fat. The induction of tyrosine hydroxylase (Th), the rate limiting enzyme in the biosynthesis of catecholamines, provides a unifying mechanism by which type 2 cytokines and alternatively activated macrophages support the browning of scWAT (Figure 7I). For instance, cold stress selectively induces the expression of TH in alternatively activated scWAT macrophages, whereas myeloid specific deletion of *Th* impairs catecholamine production necessary for biogenesis of beige fat. Thus, in contrast to the neuronal thermogenic circuit that regulates interscapular BAT thermogenesis (Morrison and Nakamura, 2011), the development and function of beige fat requires catecholamine production by the hematopoietic circuit consisting of eosinophils, type 2 cytokines and alternatively activated macrophages, findings that reinforce another fundamental difference between beige and brown fat. Thus, in the future, it will be important to determine how neuronal sensing of cold results in the activation of the hematopoietic circuit described here to stimulate beige fat biogenesis.

A hallmark of human beige fat is its cold-induced recruitment and activation. For instance, chronic exposure to mild cold results in recruitment of new BAT in healthy young subjects, resulting in increased energy expenditure and weight loss (van der Lans et al., 2013; Yoneshiro et al., 2013). Interestingly, the contribution of this new beige/brown fat to total body energy expenditure can only be detected after mild cold exposure but not in thermoneutral subjects (Yoneshiro et al., 2013). We observe a similar dependence on environmental temperature for the thermogenic activation of beige fat. For instance, at thermoneutrality, oxygen consumption rates are similar between control and various knockout mice, including *Il4/13*$^{-/-}$,*Il4ra*$^{-/-}$, *Stat6*$^{-/-}$, *Il4ra*$^{f/f}$*Lyz2*^{Cre}, and *Th*$^{f/f}$*Lyz2*^{Cre} mice. In contrast, upon cold exposure, one observes a significant difference in the rates of oxygen consumption between control and knockout animals. For example, under colder environmental conditions, energy expenditure is reduced by ~10% in the various knockout animals, an amount that likely reflects the thermogenic activity of beige fat because UCP1

content of BAT remains unchanged. However, since myeloid cell-derived catecholamines are also required for maximal lipolysis of stored triglycerides, we cannot formally exclude the possibility that decreased delivery of fatty acids to BAT might also contribute to the reduced energy expenditure in these knockout mice.

Conversely, treatment of thermoneutral WT mice with IL-4 increases beige fat mass but not oxygen consumption. However, upon exposure to progressively colder temperatures, these IL-4 treated animals have ~8-12% higher energy expenditure, reflecting their higher thermogenic capacity. It is important to note that this IL-4-induced increase in thermogenesis is completely dependent on UCP1 and independent of BAT UCP1 content, suggesting that the recruitment of beige fat is primarily responsible for the observed increase in oxygen consumption. Finally, although pharmacological activation of all adrenoreceptors by NE results in marked increase in oxygen consumption in IL-4 treated animals, it likely represents an overestimate of beige fat thermogenic capacity because interscapular BAT mediated thermogenesis is reduced to ~20-25% when mice are housed at thermoneutrality for prolonged periods of time (Golozoubova et al., 2006). However, if one takes this into account, then the UCP1-dependent and IL-4-induced beige fat mass could account for ~15-20% of total thermogenic capacity in mice, findings that are in agreement with the recent report that suggested beige fat respiration can account for ~10-37% of interscapular BAT thermogenic capacity (Shabalina et al., 2013)

Cold and diet are the two physiological triggers known to activate the thermogenic activity of brown fat (Cannon and Nedergaard, 2011). Although the importance of cold in stimulating beige fat thermogenesis has previously been investigated (Harms and Seale, 2013), its role in diet-induced thermogenesis has not been systematically explored. By housing mice at thermoneutrality, which inactivates cold-induced thermogenesis, we found that high fat diet activates the thermogenic activity of recruited beige fat to promote negative energy balance. For example, treatment of thermoneutral obese animals with IL-4 increased beige fat mass, whose activation by high fat diet decreased adiposity and weight gain. In this experimental setting, BAT UCP1 content remained unchanged, indicating that weight loss was primarily driven by dietary activation of recruited beige fat. Not only do these findings provide strong experimental support for the therapeutic potential of beige fat in the setting of obesity, but they also outline a rigorous experimental strategy to systematically evaluate the potency and activity of other browning factors, such as irisin, Fgf21 and natriuretic peptides. Together, our results demonstrate that recruitment of new beige fat can ameliorate the established metabolic dysfunction resulting from diet-induced obesity, thereby providing strong support for the therapeutic targeting of beige fat for the treatment of human obesity and obesity-associated insulin resistance.

In summary, the studies presented here have elucidated the efferent thermogenic circuit consisting of eosinophils, type 2 cytokines, and alternatively activated macrophages, which regulates the development of cold-induced beige fat. Surprisingly, this thermogenic circuit requires local production of catecholamines by alternatively activated macrophages rather than sympathetic nerves to stimulate the conversion of scWAT into a thermogenic organ. Moreover, since this hematopoietic circuit is activated in response to the physiologic stimulus of cold, it is plausible that other factors promoting the browning of scWAT might

induce or activate components of this thermogenic circuit to increase beige fat mass. If so, it will suggest that the efferent beige fat thermogenic circuit identified here is a central, common pathway by which mammals increase their thermogenic capacity to meet their physiologic needs in response to a diverse set of stimuli.

EXPERIMENTAL PROCEDURES

Animals and *in vivo* studies

All mice were maintained in the vivarium under a 12 hour light:dark cycle and used at the designated environmental temperature. Male mice, 8-12 week old, were used for the cold exposure and thermoneutrality experiments. The following strains were on the BALB/cJ background: WT, *Il4/13*$^{-/-}$, *Il4ra*$^{-/-}$, *Stat6*$^{-/-}$, 4get, 4get-ΔdblGATA, *Il4ra*$^{f/f}$, and *Il4ra*$^{f/f}$*Lyz2*^{Cre}, whereas WT, *Ccr2*$^{-/-}$, *Ucp1*$^{-/-}$, *Th*$^{f/f}$, and *Th*$^{f/f}$*Lyz2*^{Cre} were on the C57BL6/J background. In addition, C57BL6/J mice were used for the obesity studies performed at thermoneutrality with IL-4. Animals were maintained at 30°C for 4 weeks prior to molecular and histologic quantification of brown or beige fat, or measurement of energy expenditure. For the cold-induced changes in energy expenditure, mice were chronically housed at 22°C and acutely shifted to 4-5°C environment for 48 hours. During the cold challenge experiments, mice were fed *ad lib* and housed in 5°C chamber (Powers Scientific) for 48 hours in groups of 2 mice per cage. Cages were pre-chilled overnight at 5°C and contained Enviro-dri as enrichment to allow animals to make large nests. At the end of experiments, tissues were harvested and snap frozen in liquid nitrogen for RNA and protein analysis, or fixed in 10% formalin for histology. Mice were acclimated to 30°C in laboratory incubator (Darwin Chambers) for 2-4 weeks prior to initiation of experiments at thermoneutrality. For IL-4-induced browning, 4-5 week old male mice were housed at 30°C for 3 weeks prior to intraperitoneal injection with PBS (Vehicle) or IL-4 (2 μg, Peprotech) that was complexed with anti-IL4 mAb (10 μg of clone 11B11). To deplete monocytes and tissue macrophages, mice were given 5 intraperitoneal injections of clodronate-containing or empty liposomes (100 μl) starting 4 days prior to through initiation of cold challenge. Depletion of circulating monocytes and resident macrophages was confirmed by flow cytometric analysis of blood, adipose tissues, and spleen. Cohorts of ≥ 4 mice per genotype or treatment were assembled for all *in vivo* studies, which were repeated 2-3 independent times.

Statistical analysis

All data are presented as mean ± s.e.m and analyzed using Prism (Graphpad). Statistical significance was determined using the unpaired two-tailed Student's t-test for single variables and two-way ANOVA followed by Bonferroni post-tests for multiple variables. A p-value of < 0.05 was considered to be statistically significant, and is presented as * ($p < 0.05$), ** ($p < 0.01$), or *** ($p < 0.001$).

Extended Experimental Procedures are included in the Supplemental Information.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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HIGHLIGHTS

- Eosinophils and type 2 cytokines are required for biogenesis of beige fat
- Alternatively activated macrophages support the development of functional beige fat
- Myeloid cell-derived catecholamines mediate the growth of cold-induced beige fat
- IL-4 therapy increases beige fat mass to ameliorate obesity in thermoneutral mice

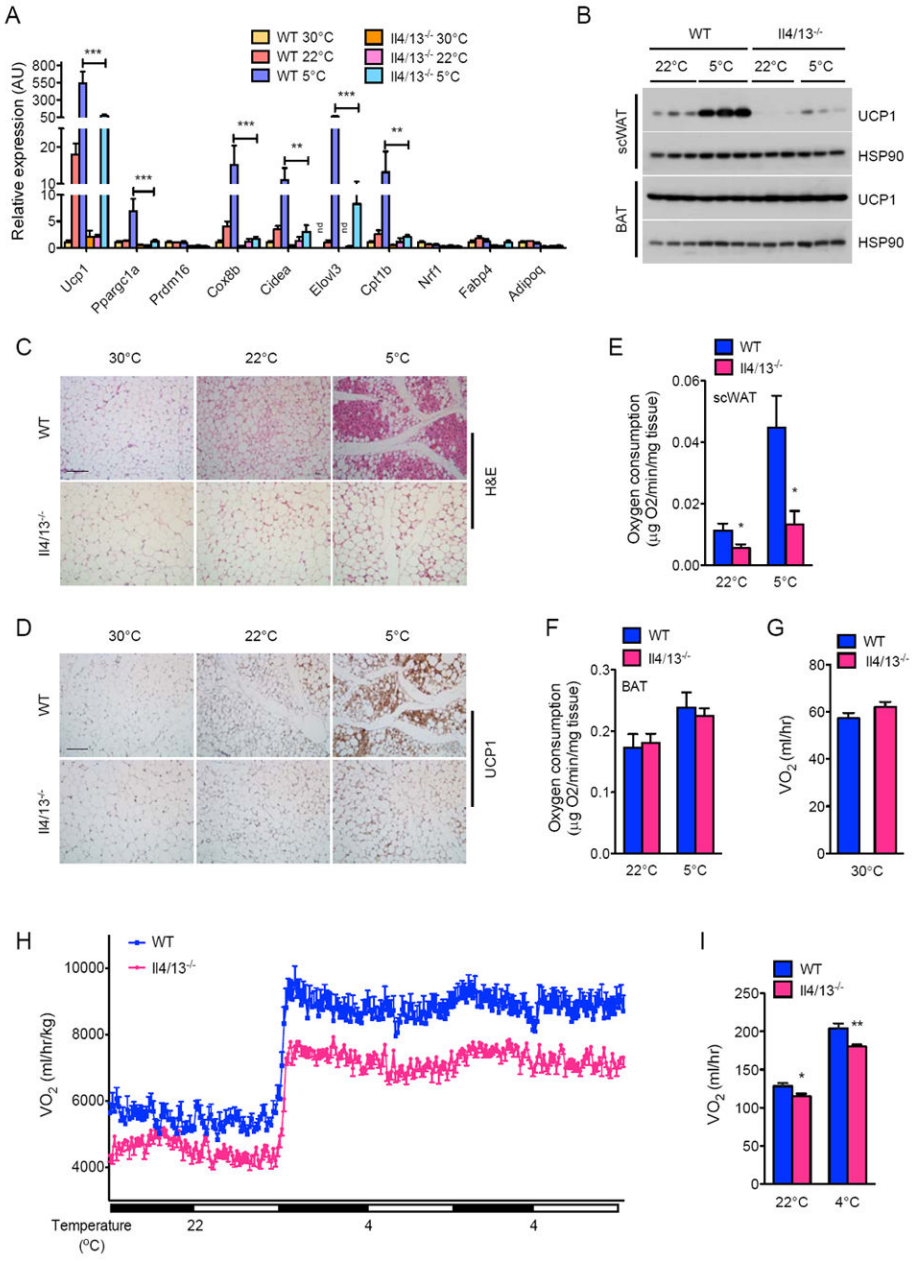

Figure 1. Type 2 cytokines IL-4/13 are required for biogenesis of cold-induced beige fat

(A) Quantitative RT-PCR analysis of thermogenic genes in scWAT of WT and *Il4/13*$^{-/-}$ mice housed at 30°C, 22°C, or 5°C for 48 hours (n=5 per genotype and temperature). (B) Immunoblot analysis of UCP1 in scWAT and BAT of WT and *Il4/13*$^{-/-}$ mice housed at 22°C or 5°C for 48 hours (n=3 per genotype and temperature). (C, D) Representative scWAT sections of WT and *Il4/13*$^{-/-}$ mice housed at 30°C, 22°C, or 5°C for 48 hours were stained with hematoxylin and eosin (C) or for UCP1 (D), scale bar 100 μm. (E, F) Oxygen consumption in scWAT (E) and BAT (F) from WT and *Il4/13*$^{-/-}$ mice housed at 22°C or 5°C for 48 hours (n=7-8 per genotype and temperature). (G-I) Oxygen consumption (VO_2) in WT and *Il4/13*$^{-/-}$ mice at different environmental temperatures: at thermoneutrality, 30°C

(G) or at different ambient temperatures (H, I), n=5-8 per genotype. Data are represented as mean ± SEM. See also Figure S1.

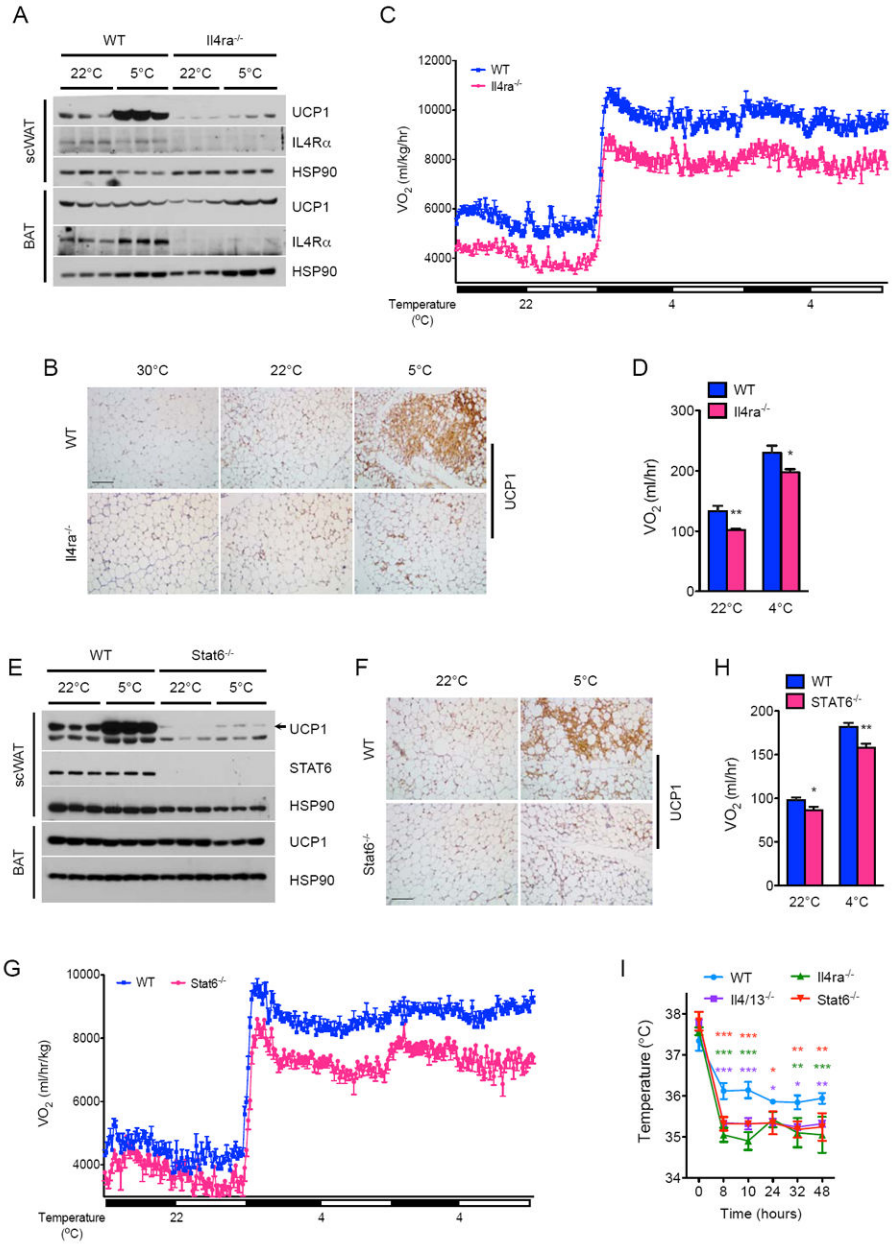

Figure 2. Signaling via IL-4Rα and STAT6 controls growth of functional beige fat

(A, E) Immunoblot analysis for UCP1 protein in the scWAT and BAT of WT, *Il4/13*$^{-/-}$ (A) and *Stat6*$^{-/-}$(E) mice housed at 22°C or 5°C for 48 hours (n=3 per genotype and temperature). (B, F) Representative sections of scWAT from WT, *Il4ra*$^{-/-}$ (B) and *Stat6*$^{-/-}$ (F) mice housed at various temperatures were stained for UCP1, scale bar 100 μm. (C-D, G-H) Oxygen consumption (VO_2) in WT, *Il4ra*$^{-/-}$ (C) and *Stat6*$^{-/-}$ (F) mice at 22°C and 4°C (n=8-10 per genotype). (I) Core body temperature of WT, *Il4/13*$^{-/-}$, *Il4ra*$^{-/-}$, and *Stat6*$^{-/-}$ mice during a 48 hour cold challenge (n=4-5 per genotype). Data are represented as mean ± SEM. See also Figure S2.

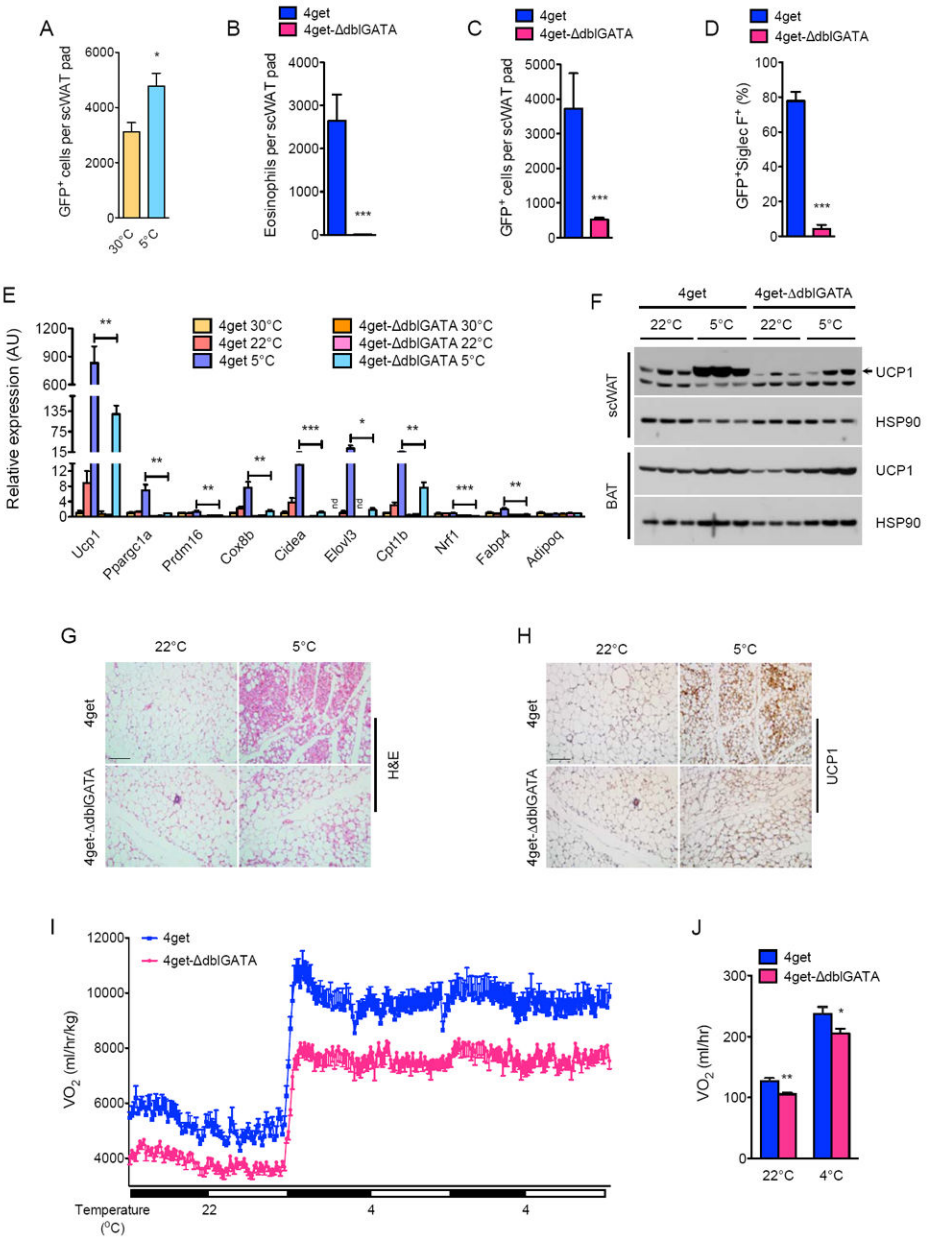

Figure 3. IL-4 producing eosinophils are required for development of cold-induced functional beige fat

(A) Quantification of IL-4 producing cells in the scWAT of 4get mice housed at 30°C and 5°C. GFP expression marks cells competent for production of IL-4 (n=6 per temperature). (B-D) Quantification of eosinophils (B), GFP^+ cells (C) and GFP^+ $SiglecF^+$ cells (eosinophils, D) in the scWAT of 4get and 4get-ΔdblGATA mice housed at 5°C (n=5-9 per genotype). (E) Quantitative RT-PCR analysis of thermogenic genes in scWAT of 4get and ΔdblGATA mice housed at 30°C, 22°C, or 5°C for 48 hours (n=5 per genotype and temperature). (F) Immunoblot analysis of UCP1 in scWAT and BAT of 4get and ΔdblGATA mice housed at 22°C or 5°C for 48 hours (n=3 per genotype and temperature). (G, H) Representative scWAT sections of 4get and ΔdblGATA mice housed at 22°C, or 5°C

for 48 hours were stained with hematoxylin and eosin (G) or for UCP1 (H), scale bar 100 μm. (I, J) Oxygen consumption (VO_2) in 4get and ΔdblGATA mice at different environmental temperatures (n=7-8 per genotype). Data are represented as mean ± SEM. See also Figure S3.

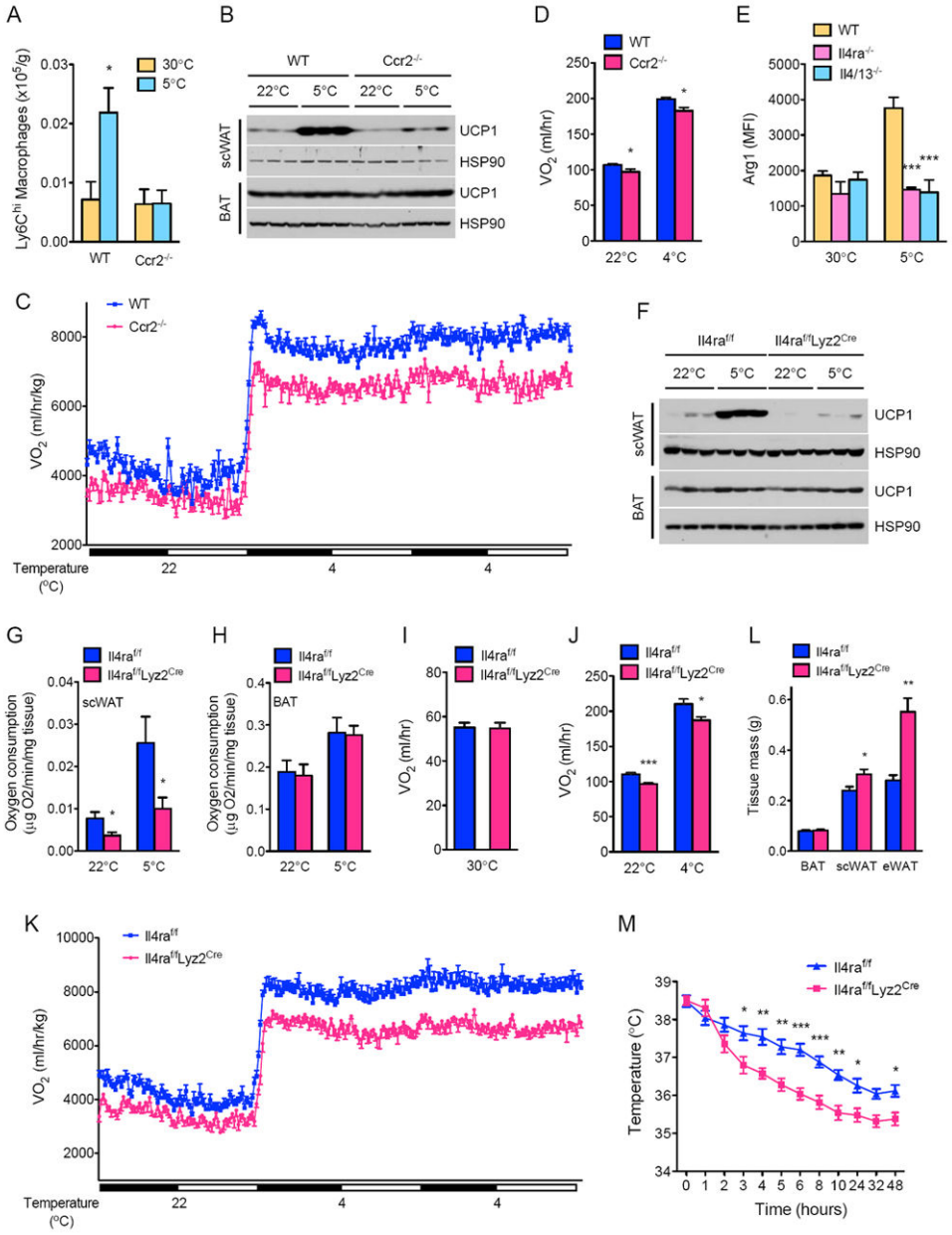

Figure 4. Macrophage recruitment via CCR2 and alternative activation via IL-4Rα is required for biogenesis of beige fat

(A) Ly6C^{hi} macrophage content of scWAT in WT and *Ccr2*$^{-/-}$ mice housed at thermoneutrality (30°C) or at 5°C for 48 hours (n=5 per genotype and temperature). (B, F) UCP1 protein expression in WT and *Ccr2*$^{-/-}$ mice (B) or *Il4ra*$^{f/f}$ and *Il4ra*$^{f/f}$*Lyz2*^{Cre} (F) mice at 22°C or 5°C (n=3 per genotype and temperature). (C, D) Cold-induced changes in oxygen consumption (VO_2) in WT and *Ccr2*$^{-/-}$ mice (n=8 per genotype). Data are represented as mean ± SEM. (E) Expression of alternative activation marker Arginase 1 in scWAT macrophages of WT, *Il4/13*$^{-/-}$, and *Il4ra*$^{-/-}$ mice housed at 30°C or 5°C (n=4-5 per genotype and temperature). (G, H) Oxygen consumption in scWAT (G) and BAT (H) from *Il4ra*$^{f/f}$ and *Il4ra*$^{f/f}$*Lyz2*^{Cre} mice housed at 22°C or 5°C for 48 hours (n=6 per genotype and

temperature). (I-K) Oxygen consumption (VO_2) in *Il4ra*$^{f/f}$ and *Il4ra*$^{f/f}$*Lyz2*^{Cre} mice at various environmental temperatures: thermoneutrality, 30°C (n=5 per genotype) or 22°C and 4°C (n=7-9 per genotype). (L) Adipose tissue weights of *Il4ra*$^{f/f}$ and *Il4ra*$^{f/f}$*Lyz2*^{Cre} mice after cold challenge at 5°C for 48 hours (n=5 per genotype). (M) Core body temperature of *Il4ra*$^{f/f}$ and *Il4ra*$^{f/f}$*Lyz2*^{Cre} mice during a 48 hour cold challenge (n=5 per genotype). Data are represented as mean ± SEM. See also Figures S3 and S4.

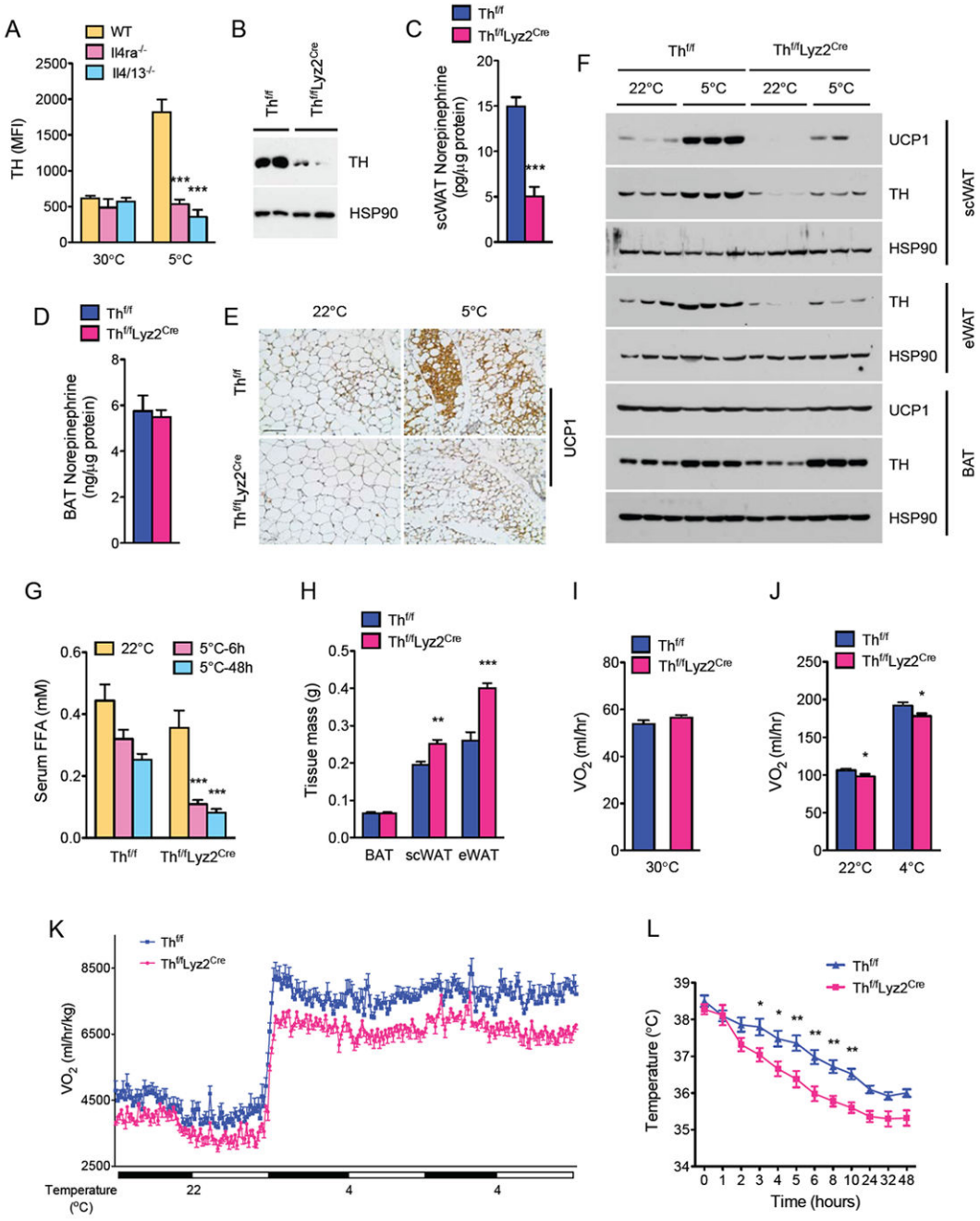

Figure 5. Myeloid cell tyrosine hydroxylase is required for biogenesis of functional beige fat

(A) Intracellular staining for TH expression in scWAT macrophages of WT, *Il4/13*$^{-/-}$ and *Il4ra*$^{-/-}$ mice housed at 30°C or 5°C. (B) Immunoblotting for TH protein in resident peritoneal macrophages of *Th*$^{f/f}$ and *Th*$^{f/f}$*Lyz2*^{Cre} mice. (C, D) Norepinephrine content of scWAT (C) and BAT (D) of *Th*$^{f/f}$ and *Th*$^{f/f}$*Lyz2*^{Cre} mice housed at 5°C (n=6-7 per genotype). (E) Representative sections of scWAT from *Th*$^{f/f}$ and *Th*$^{f/f}$*Lyz2*^{Cre} mice stained for UCP1, scale bar 100 μm. (F) Immunoblot analysis for TH and UCP1 in scWAT, eWAT, and BAT of *Th*$^{f/f}$ and *Th*$^{f/f}$*Lyz2*^{Cre} mice housed at 22°C or challenged with 5°C for 48 hours (n=3

genotype and temperature). (G) Serum concentration of free fatty acids in $Th^{f/f}$ and $Th^{f/f}Lyz2^{Cre}$ mice at various temperatures (n=6 per genotype and temperature). (H) Adipose tissue weights of $Th^{f/f}$ and $Th^{f/f}Lyz2^{Cre}$ mice after cold challenge at 5°C for 48 hours (n=5 per genotype). (I-K) Oxygen consumption (VO_2) in $Th^{f/f}$ and $Th^{f/f}Lyz2^{Cre}$ mice at different environmental temperatures: at thermoneutrality, 30°C (I, n=5 per genotype) or during at different ambient temperatures (J, K), n=8 per genotype. (L) Core body temperature of $Th^{f/f}$ and $Th^{f/f}Lyz2^{Cre}$ mice during a 48 hour cold challenge (n=5 per genotype). Data are represented as mean ± SEM. See also Figure S5.

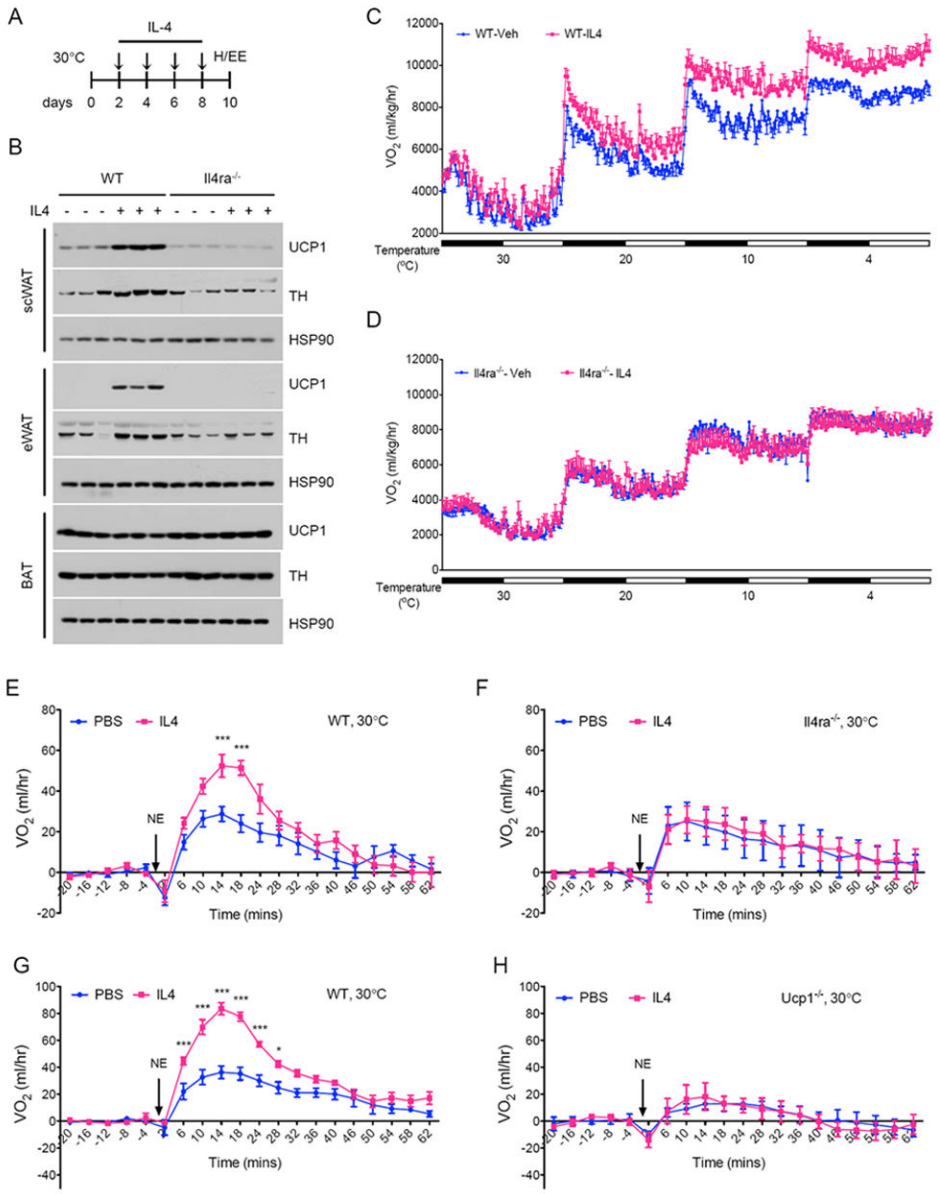

Figure 6. IL-4 induces beige fat in thermoneutral mice

(A) Schematic for IL-4 dosing and metabolic analysis in thermoneutral mice. (B) Immunoblotting for UCP1 and TH in scWAT, eWAT, and BAT of WT and *Il4ra*$^{-/-}$ thermoneutral mice administered IL-4 complex over 8 days (n=3 per genotype and treatment). (C, D) Cold-induced changes in oxygen consumption in WT (C) and *Il4ra*$^{-/-}$ (D) thermoneutral mice administered IL-4 complex over 8 days (n=4-5 per genotype and treatment). (E-H) Norepinephrine stimulated changes in oxygen consumption (VO_2) in conscious, thermoneutral mice that were pretreated with vehicle (Veh) or IL-4 complexes (IL-4): BALB/cJ (E), *Il4ra*$^{-/-}$ (F), C57BL/6J (G) and *Ucp1*$^{-/-}$ (H). All metabolic and histological analyses were performed 2 days after the last dose of IL-4 complex. Data are represented as mean ± SEM. See also Figure S6.

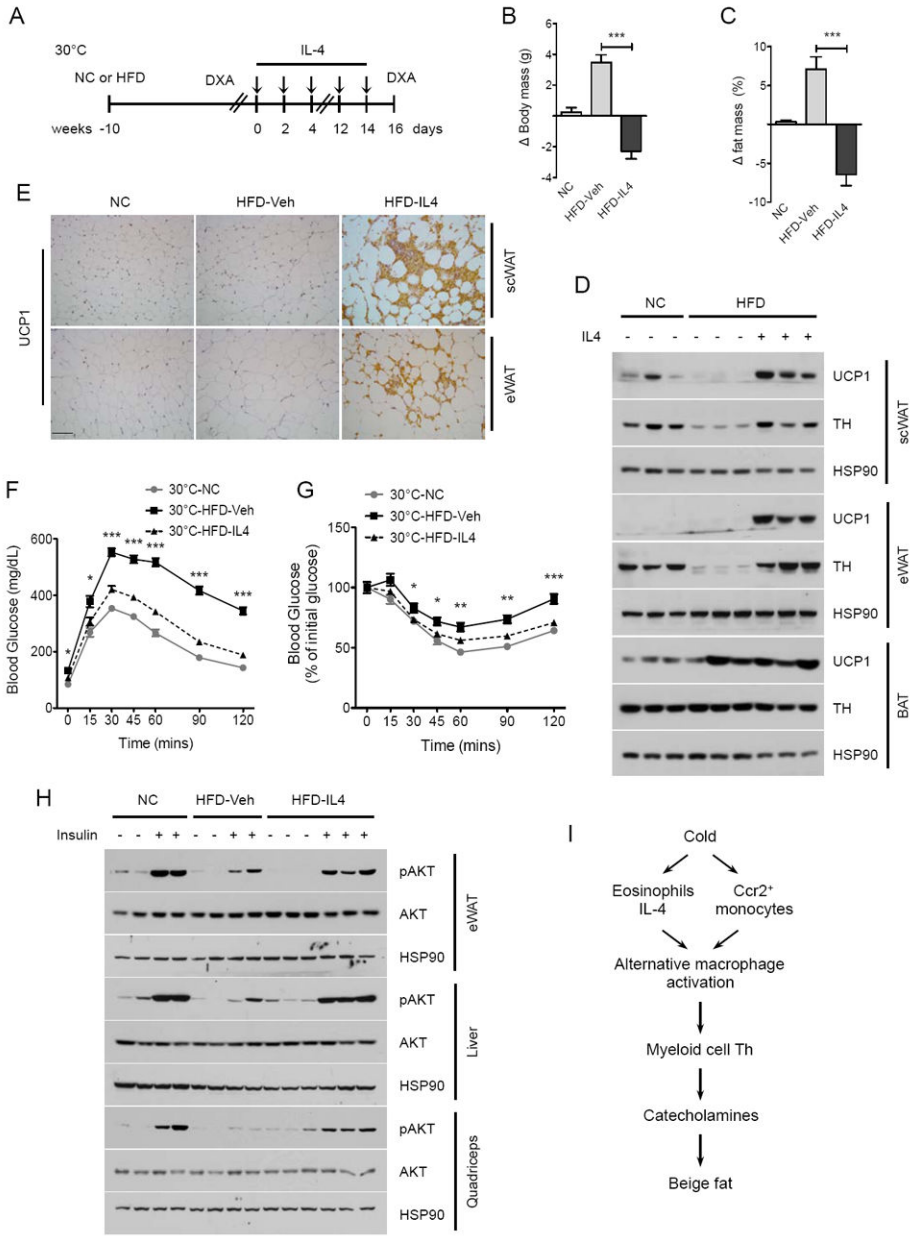

Figure 7. Administration of IL-4 to obese thermoneutral mice reverses high fat diet-induced metabolic dysfunction

(A) Schematic for treatment of pre-established obesity with IL-4 complex in thermoneutral mice. (B, C) Changes in body mass (C) and fat mass (C) in obese C57BL/6J thermoneutral mice treated with vehicle (Veh) or IL-4 complex over 14 days (n=5 per treatment). (D) Immunoblot analysis for UCP1 and TH in scWAT, eWAT, and BAT of obese C57BL/6J thermoneutral mice administered Veh or IL-4 (n=3 per treatment). (E) Representative sections of scWAT and eWAT from thermoneutral mice treated with vehicle or IL-4 complex for 14 days were stained for UCP1, scale bar 100 μm. (F, G) Glucose (F) and insulin (G) tolerance tests in obese thermoneutral mice treated with Veh or IL-4 (n=5-7 per treatment). (H) Assessment of insulin signaling, as quantified by the phosphorylation of

AKT, in obese thermoneutral mice treated with Veh or IL-4 (n=2-3 per treatment group). (I) Model for biogenesis and function of cold-induced beige fat. All metabolic and provocative testing was performed 2 days after the last dose of IL-4 complex. Data are represented as mean ± SEM. See also Figure S7.