

Genetic deficiency and pharmacological stabilization of mast cells reduce diet-induced obesity and diabetes in mice

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Although mast cell functions have classically been related to allergic responses¹⁻³, recent studies indicate that these cells contribute to other common diseases such as multiple sclerosis, rheumatoid arthritis, atherosclerosis, aortic aneurysm and cancer⁴⁻⁸. This study presents evidence that mast cells also contribute to diet-induced obesity and diabetes. For example, white adipose tissue (WAT) from obese humans and mice contain more mast cells than WAT from their lean counterparts. Furthermore, in the context of mice on a Western diet, genetically induced deficiency of mast cells, or their pharmacological stabilization, reduces body weight gain and levels of inflammatory cytokines, chemokines and proteases in serum and WAT, in concert with improved glucose homeostasis and energy expenditure. Mechanistic studies reveal that mast cells contribute to WAT and muscle angiogenesis and associated cell apoptosis and cathepsin activity. Adoptive transfer experiments of cytokine-deficient mast cells show that these cells, by producing interleukin-6 (IL-6) and interferon- γ (IFN- γ), contribute to mouse adipose tissue cysteine protease cathepsin expression, apoptosis and angiogenesis, thereby promoting diet-induced obesity and glucose intolerance. Our results showing reduced obesity and diabetes in mice treated with clinically available mast cell-stabilizing agents suggest the potential of developing new therapies for these common human metabolic disorders.

In addition to adipocytes, it has been shown that WAT in obese humans and mice contains macrophages and lymphocytes⁹⁻¹¹. As expected from these previous studies, we found that WAT from obese subjects displayed more staining by the macrophage-specific monoclonal antibody HAM56 (cell numbers per mm²) than those from lean donors (Fig. 1a), with some of these stained macrophages bordering microvessels (Fig. 1b). These inflammatory cells furnish cytokines,

growth factors, chemokines and proteases in WAT^{11,12}. However, the role of these cells in the pathogenesis of obesity and its associated metabolic complications remains uncertain. Other heretofore unrecognized cells may also be crucial contributors. Like macrophages or lymphocytes, mast cells are also inflammatory cells. Staining WAT sections with a mast cell tryptase monoclonal antibody revealed higher numbers of mast cells in WAT from obese subjects compared with that from lean donors (Fig. 1c-e). Like macrophages, many of these mast cells adjoin microvessels (Fig. 1c,d), although WAT had significantly fewer mast cells than macrophages (Fig. 1e). This increased mast cell content of WAT from obese subjects suggests increased systemic mast cell protease levels. Obese subjects (body mass index ≥ 32 kg mm⁻²) had significantly higher serum tryptase concentrations than lean individuals (body mass index < 26 kg per mm²) before ($P = 0.01$) and after adjusting for gender ($P = 0.01$, multivariate analysis) (Fig. 1f). These observations suggest an association between mast cells and obesity.

To assess direct participation of mast cells in obesity, we studied diet-induced obesity in mast cell-deficient *Kit^{W-sh/W-sh}* mice, which lack mature mast cells owing to an inversion mutation of the promoter region of the gene encoding c-Kit¹³, a growth factor receptor required for mast cell differentiation and survival. Six-week-old male (Fig. 2a) and female (Supplementary Fig. 1a) *Kit^{W-sh/W-sh}* mice fed a Western diet for 12 weeks gained significantly less body weight than wild-type (WT) congenic controls. Similarly, WT mice receiving a daily intraperitoneal (i.p.) injection of the mast cell stabilizer disodium cromoglycate (DSCG)¹⁴⁻¹⁶ also had attenuated body weight gain (Fig. 2a). DSCG treatment did not further affect the body weight of the *Kit^{W-sh/W-sh}* mice, suggesting it acted through mast cells. Consistent with their lower body weight, both male and female *Kit^{W-sh/W-sh}* mice or those receiving DSCG had significantly less total subcutaneous and visceral fat than untreated WT controls (Fig. 2a and Supplementary Fig. 1a).

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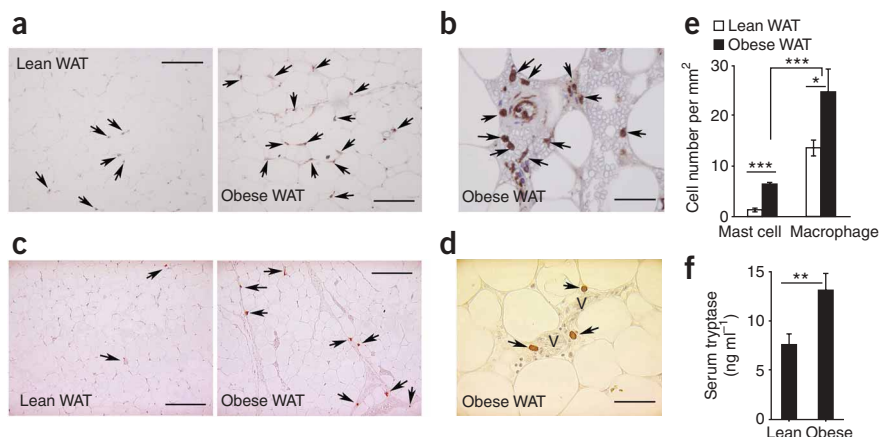


Figure 1 Macrophages and mast cells in human WAT. (a) Human HAM56 immunostaining of WAT from obese and lean subjects. (b) HAM56⁺ macrophages near the microvessels in obese WAT. (c) Human mast cell tryptase immunostaining of WAT from obese and lean subjects. (d) Tryptase⁺ mast cells close to microvessels in obese WAT. Arrows indicate HAM56⁺ macrophages or tryptase⁺ mast cells; V, microvessel; scale bars: 100 μ m (a,c) and 25 μ m (b,d). (e) Mast cell and macrophage contents in WAT from obese ($n = 12$) and lean ($n = 10$) subjects. Nonparametric Mann-Whitney test. (f) ELISA analysis of mast cell tryptase in human serum from obese ($n = 80$) and lean ($n = 32$) subjects. Unpaired t -test. * $P < 0.02$, ** $P = 0.01$ and *** $P < 0.01$. All data in e and f are means $\pm$ s.e.m.

Earlier studies revealed normal numbers and activities of monocytes or macrophages and neutrophils in WT and *Kit^{W-sh/W-sh}* mice^{6,7,17}. Using T or B lymphocytes from *Kit^{W-sh/W-sh}* mice, we detected no significant activity differences in a mixed lymphocyte reaction assay compared to WT mice (data not shown). Whereas Western diet-fed WT mouse livers were steatotic with abundant c-Kit CD117⁺ mast cells, those from *Kit^{W-sh/W-sh}* or WT mice treated with DSCG under the same dietary conditions did not contain mast cells and had a similar histological appearance to chow diet-fed WT mice (Supplementary Fig. 1b). Histological analysis of the intestine, colon, stomach and pancreas also did not reveal obvious phenotypic differences between chow diet-fed WT mice and *Kit^{W-sh/W-sh}* or DSCG-treated WT mice fed any of the dietary regimens (data not shown). Body composition analysis revealed a lower fat mass but a higher lean mass in *Kit^{W-sh/W-sh}* mice or both WT and *Kit^{W-sh/W-sh}* mice that received DSCG as compared to WT mice that did not receive DSCG after 12 weeks of a Western diet (Supplementary Fig. 1c). From the data presented so far, it seems that the reduced obesity in *Kit^{W-sh/W-sh}* or DSCG-treated mice resulted from a loss of mast cell function rather than from a confounding general health issue.

Similar to the results in human WAT (Fig. 1c–e), immunostaining with a monoclonal antibody to CD117 detected significantly more mast cells in WAT from obese mice fed a Western diet than in WAT from lean mice fed a chow diet, with many of these mast cells located near microvessels (Fig. 2b,c). Notably, DSCG treatment did not significantly reduce WAT mast cell content in Western diet-fed WT mice (Fig. 2b,c), but these mice responded similarly to *Kit^{W-sh/W-sh}* mice in body weight gain, suggesting that DSCG inactivated mast cells but did not affect their recruitment to the WAT. In contrast, immunostaining with a Mac-2-specific monoclonal antibody detected significantly fewer macrophages in *Kit^{W-sh/W-sh}* and DSCG-treated mice than in untreated Western diet-fed WT mice (Fig. 2d). High mast cell numbers but low macrophage numbers in WAT from DSCG-treated, Western diet-fed WT mice suggest that mast cells appear in WAT before macrophages, a hypothesis that merits further examination.

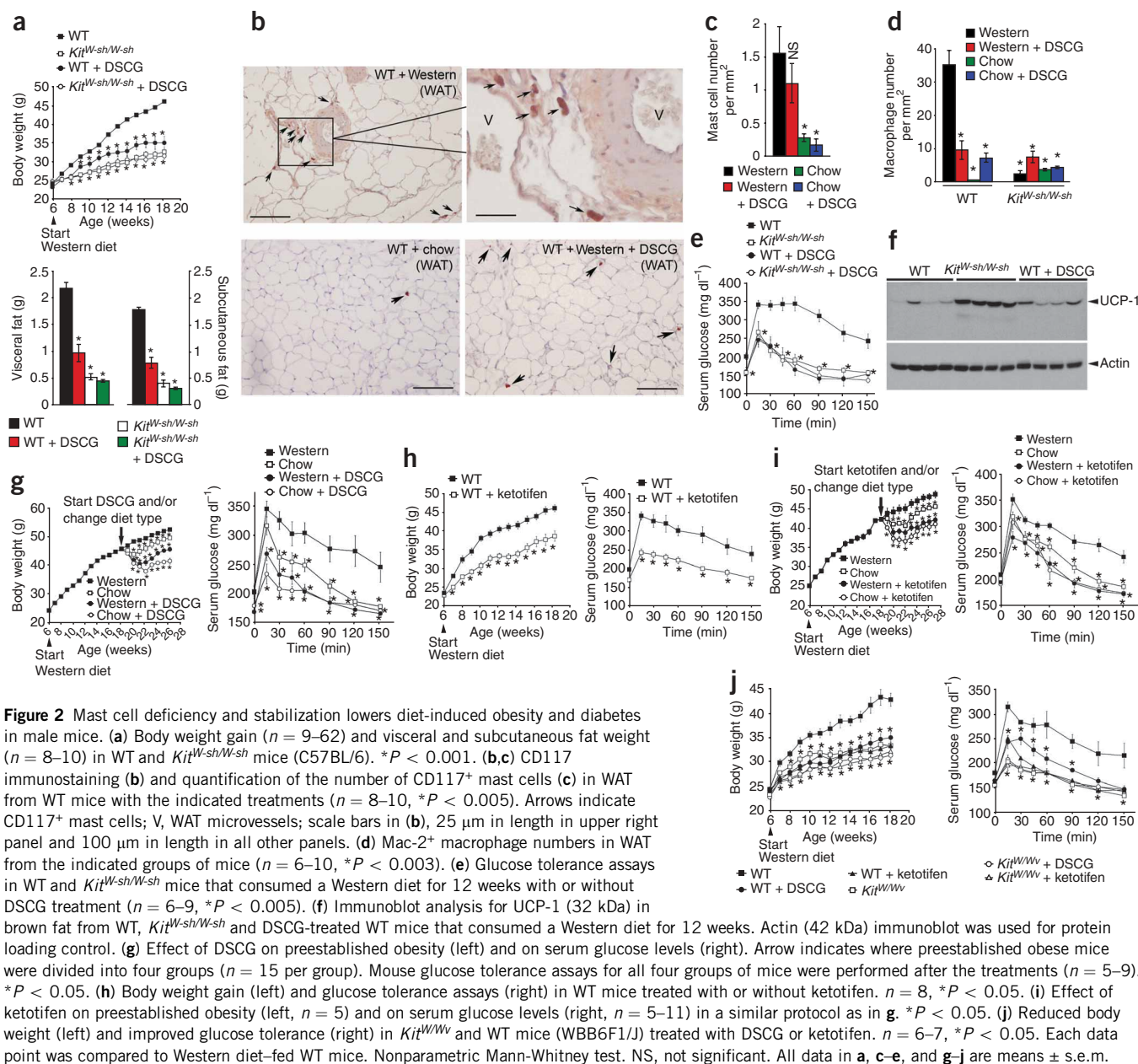
Along with lower body weight, male and female *Kit^{W-sh/W-sh}* mice or those receiving DSCG also had significantly lower concentrations of serum leptin than WT controls (Supplementary Fig. 1d). Glucose tolerance assays (Fig. 2e) and insulin sensitivity assays (Supplementary Fig. 1e) in Western diet-fed mice revealed significantly higher glucose tolerance and insulin sensitivity of *Kit^{W-sh/W-sh}* mice or WT mice receiving DSCG than WT control mice. These mice also had lower serum insulin concentrations than the WT control mice (Supplementary Fig. 1f).

To understand the mechanisms of these salutary effects of mast cell deficiency or inactivation, we performed energy expenditure assays and measured brown fat uncoupled protein-1 (UCP-1), a marker of energy expenditure^{18,19}. When we measured food and water intake, fecal and urine production and O₂ consumption and CO₂ production, we found that *Kit^{W-sh/W-sh}* mice and DSCG-treated WT mice had a higher resting metabolic rate, illustrated by significantly more O₂ consumption and CO₂ production than untreated WT mice (Supplementary Table 1). These mice had higher expression of UCP-1 in their brown fat, as compared to WT control mice (Fig. 2f).

To test whether DSCG also reverses preestablished obesity and diabetes, we fed WT mice a Western diet for 12 weeks to produce obesity and diabetes and grouped these obese and diabetic mice into four treatment groups: group 1, continued on a Western diet; group 2, switched to a chow diet; group 3, continued on a Western diet but with a daily i.p. injection of DSCG; and group 4, switched to a chow diet and treated with DSCG. Although changing the diet (group 2) also reduced body weight (8%) and improved glucose tolerance as expected, combined diet change and DSCG administration (group 4) yielded the greatest improvement of both body weight and glucose tolerance (Fig. 2g). Eight weeks after DSCG treatment, the body weight of group 3 mice decreased by 12%, but that of group 4 mice decreased by 19% and stabilized around 40–41 g, with these mice showing the highest glucose tolerance among the four groups (Fig. 2g), suggesting the possibility of managing preexisting obesity and diabetes by stabilizing mast cells. Use of a second mast cell stabilizer, ketotifen^{20,21}, confirmed this hypothesis. Like DSCG treatment, daily i.p. injection of ketotifen lowered diet-induced body weight gain and enhanced glucose tolerance (Fig. 2h). As we anticipated, ketotifen also improved preestablished obesity and diabetes in mice that had consumed a Western diet for 12 weeks (Fig. 2i).

To affirm that the phenotypes we observed from the *Kit^{W-sh/W-sh}* mice or those receiving DSCG or ketotifen stemmed from the loss of mast cell functions, we used *Kit^{W/W^v}* mice as an additional model of genetically induced mast cell deficiency²². *Kit^{W/W^v}* mice carry a compound mutation (one impaired allele and the other null) of the *Kit* gene. Whereas WT mice (WBB6F1/J background) continued gaining body weight and developed glucose intolerance on a Western diet, *Kit^{W/W^v}* mice and those receiving DSCG or ketotifen showed a smaller body weight gain and higher glucose tolerance (Fig. 2j). These observations provided further support for the direct participation of mast cells in both obesity and diabetes.

The development of obesity involves extracellular matrix remodeling and angiogenesis²³. Inhibition of angiogenesis blocks adipose tissue



development in mice²⁴. Besides supplying the WAT with nutrients, microvessels provide a path for leukocyte infiltration followed by adipokine release^{25,26}. The appearance of macrophages and mast cells next to the microvessels in WAT (Fig. 1b,d) supported this hypothesis. Furthermore, we found substantial immunostaining of CD31 along endothelial cells in WAT and muscle tissue from obese WT mice (Fig. 3a,b). In contrast, Western diet-fed *Kit^{W-sh/W-sh}* mice or those receiving DSCG had CD31⁺ areas similar to those from chow diet-fed lean mice (Fig. 3a,b). Reduced angiogenesis should limit nutrient supply, impairing cell viability^{24,27}. Thus, we performed TUNEL staining and found higher numbers of apoptotic cells in WAT from *Kit^{W-sh/W-sh}* mice or those receiving DSCG compared with WT controls (Fig. 3c). Reduced angiogenesis may also impair leukocyte infiltration (Fig. 2d) and therefore reduce WAT production of inflammatory mediators²⁸. Consistent with this notion, *Kit^{W-sh/W-sh}* mice or those receiving DSCG had lower concentrations of IL-6, tumor necrosis

factor- α (TNF- α), IFN- γ , monocyte chemoattractant protein-1, matrix metalloproteinase-9 and cathepsin S in serum and WAT, although some adipokines and proteases (for example, adiponectin and cathepsin L) did not change significantly (Supplementary Table 2).

Extracellular matrix proteolysis contributes to angiogenesis by releasing proangiogenic peptides²⁹. We have previously shown that cathepsin S has a crucial role in angiogenesis by degrading antiangiogenic peptides and generating proangiogenic lamin-5 fragment $\gamma 2$ (ref. 30). The lower angiogenesis in *Kit^{W-sh/W-sh}* and DSCG-treated mice (Fig. 3a,b) accompanied low cathepsin S amounts in WAT and serum (Fig. 3d-f). After incubating WAT protein extracts with [¹²⁵I]-JPM, a cysteine protease inhibitor E64 derivative that selectively labels active cathepsins³¹, we found smaller active cathepsin B, cathepsin S, and cathepsin L amounts in *Kit^{W-sh/W-sh}* mice than in WT mice (Fig. 3d). Notably, WAT from DSCG-treated mice had levels of active cathepsins similar to WT controls, consistent with

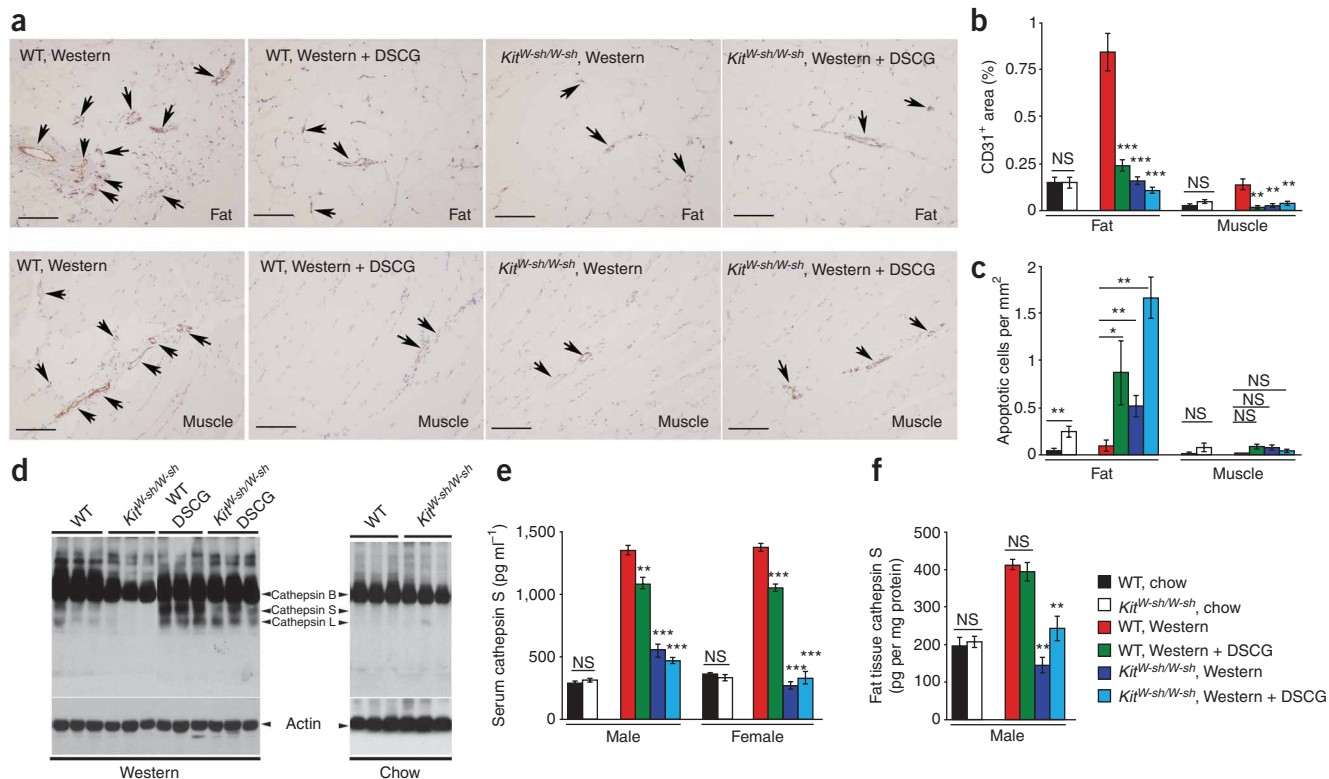


Figure 3 Mast cell functions in angiogenesis, apoptosis and protease expression. **(a)** CD31 immunostaining of WAT and muscle from WT and *Kit^{W-sh/W-sh}* mice that consumed a Western diet for 12 weeks with or without receiving DSCG. Arrows indicate CD31⁺ microvessels in the WAT or muscle. Scale bars, 100 μ m. **(b)** Quantification of CD31⁺ areas in WAT and muscle. **(c)** Quantification of apoptotic cells in WAT and muscle. **(d)** Cysteine protease cathepsin active site JPM labeling using WAT extracts from different groups of mice that consumed a Western or chow diet for 12 weeks. Arrowheads indicate active cathepsins. Actin immunoblot was used as a protein loading control. **(e)** Serum cathepsin S ELISA from the indicated groups of mice. **(f)** WAT extract cathepsin S ELISA from the indicated groups of mice. Statistical significance is shown compared to WT mice consuming a Western diet or as otherwise indicated using a nonparametric Mann-Whitney test. $n = 6-10$. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. All data in **b**, **c**, **e** and **f** are means $\pm$ s.e.m.

our observation of large numbers of mast cells in these tissues (Figs. 2c and 3d). Cathepsin S ELISA allowed us to quantify local and systemic levels among different mice. Similar to our observations from the JPM labeling experiment, both male and female *Kit^{W-sh/W-sh}* mice showed lower serum and WAT cathepsin S concentrations than the WT controls (Fig. 3e). In contrast, DSCG treatment significantly reduced the cathepsin S amounts in serum but not in WAT extracts (Fig. 3f), suggesting that DSCG effectively stabilized mast cells *in vivo*.

We wanted to further substantiate our hypothesis that the improvement in *Kit^{W-sh/W-sh}* mice of experimentally induced metabolic disorders result from the loss of mast cells, rather than from a generalized illness that may cause them to eat less or alter their glucose homeostasis. Thus, we reconstituted *Kit^{W-sh/W-sh}* mice with bone marrow-derived mast cells (BMMCs) prepared *in vitro* from WT mice and mice lacking the three common mast cell cytokines IL-6, TNF- α and IFN- γ , which are known stimulators of vascular cell cathepsin activity^{6,7}. After 13 weeks on a Western diet, mice reconstituted with WT and *Tnf^{-/-}* BMMCs but not *Il6^{-/-}* and *Ifng^{-/-}* BMMCs gained significantly more body weight than nonreconstituted mice, although they remained leaner than WT controls (Fig. 4a). Consistent with the body weight differences, those receiving WT and *Tnf^{-/-}* BMMCs had significantly higher serum leptin, insulin and glucose concentrations than nonreconstituted mice or those receiving *Il6^{-/-}* and *Ifng^{-/-}* BMMCs (Fig. 4b-d). *Kit^{W-sh/W-sh}* mice receiving *Il6^{-/-}* and *Ifng^{-/-}* BMMCs but not WT or *Tnf^{-/-}* BMMC also showed improved glucose tolerance (Fig. 4e), suggesting that

mast cell-derived IL-6 and IFN- γ contribute to these metabolic derangements. Although we found smaller WAT adipocytes in *Kit^{W-sh/W-sh}* mice compared to WT controls, WAT adipocyte size did not vary significantly between the various BMMC-reconstituted mice (data not shown). Notably, WAT from the various BMMC-reconstituted *Kit^{W-sh/W-sh}* mice had similar numbers of mast cells between the groups but fewer than those in WT mice (Fig. 4f), which could explain the incomplete restoration of body weight gain and serum levels of leptin, insulin and glucose (Fig. 4a-d). Along with increased body weight gain, reconstitution of WT BMMCs also lowered brown fat UCP-1 levels in *Kit^{W-sh/W-sh}* mice (Fig. 4g), affirming a key role of mast cells in energy expenditure.

As discussed, mast cells may contribute to obesity by stimulating WAT protease expression, thereby promoting microvessel growth. The body weight recovery in *Kit^{W-sh/W-sh}* mice after engraftment of WT BMMCs and *Tnf^{-/-}* BMMCs but not *Il6^{-/-}* or *Ifng^{-/-}* BMMCs suggests a role for mast cell-derived IL-6 and IFN- γ in WAT protease expression and the associated angiogenesis. To test this hypothesis, we incubated mouse 3T3-L1 preadipocytes with degranulated BMMC extract (Fig. 4h) or live BMMCs (data not shown) and found that both WT and *Tnf^{-/-}* BMMCs but not *Il6^{-/-}* or *Ifng^{-/-}* BMMCs increased adipocyte cathepsin activities in a JPM-labeling assay. Indeed, reconstitution of WT and *Tnf^{-/-}* BMMCs but not *Il6^{-/-}* or *Ifng^{-/-}* BMMCs in *Kit^{W-sh/W-sh}* mice resulted in higher WAT cathepsin activities (Fig. 4i). Consistent with our hypothesis, both WAT and muscle from *Kit^{W-sh/W-sh}* mice receiving WT and

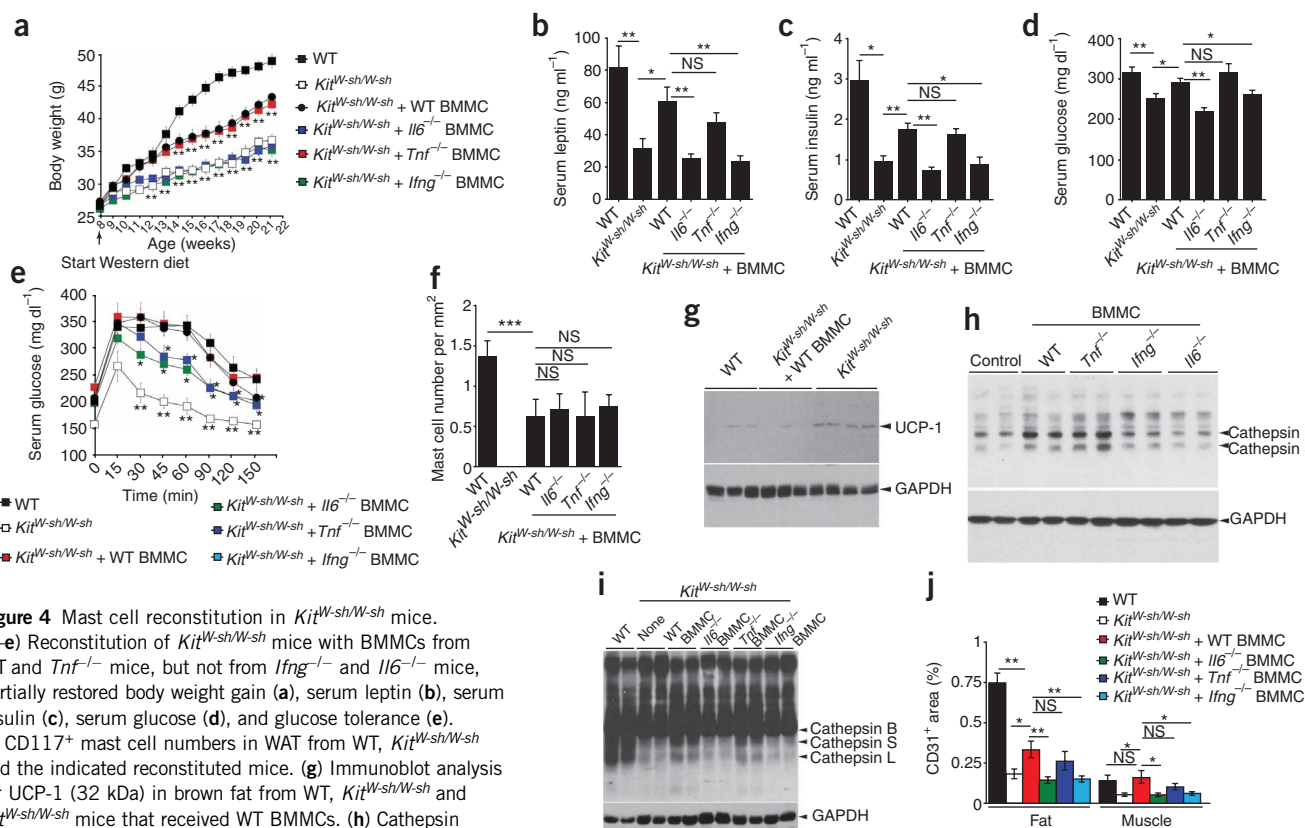


Figure 4 Mast cell reconstitution in *Kit^{W-sh/W-sh}* mice. (a–e) Reconstitution of *Kit^{W-sh/W-sh}* mice with BMMCs from WT and *Tnf^{-/-}* mice, but not from *Ifng^{-/-}* and *Il6^{-/-}* mice, partially restored body weight gain (a), serum leptin (b), serum insulin (c), serum glucose (d), and glucose tolerance (e). (f) CD117⁺ mast cell numbers in WAT from WT, *Kit^{W-sh/W-sh}* and the indicated reconstituted mice. (g) Immunoblot analysis for UCP-1 (32 kDa) in brown fat from WT, *Kit^{W-sh/W-sh}* and *Kit^{W-sh/W-sh}* mice that received WT BMMCs. (h) Cathepsin active site JPM labeling of cell lysate from 3T3-L1 cells treated with the indicated BMMCs. Active cathepsin B and cathepsin S are indicated. (i) WAT extract JPM labeling to detect active cathepsins as indicated by arrowheads. Immunoblot analysis for glyceraldehyde 3-phosphate dehydrogenase (GAPDH; 37 kDa) was used as a protein loading control. (j) Quantification of CD31⁺ areas in WAT and muscle from the indicated groups of mice. All data in a–f and j are means ± s.e.m. *n* = 4–10. Nonparametric Mann-Whitney test. **P* < 0.05, ***P* < 0.01 and ****P* = 0.06.

Tnf^{-/-} BMMCs but not *Il6^{-/-}* or *Ifng^{-/-}* BMMCs had partially preserved CD31⁺ areas (Fig. 4j). Although other mechanisms may be at play in this process, mast cell-mediated protease expression and associated angiogenesis may favor WAT growth.

This study establishes a previously undescribed role of mast cells in mouse obesity and diabetes and suggests a potential new therapeutic approach for these common human metabolic diseases.

METHODS

Methods and any associated references are available in the online version of the paper at <http://www.nature.com/naturemedicine/>.

Note: Supplementary information is available on the Nature Medicine website.

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AUTHOR CONTRIBUTIONS

J.L. induced mouse obesity and diabetes, monitored body weight, performed insulin tolerance and glucose tolerance assays and completed all immunoblot

analyses. A. Divoux performed human adipose tissue immunohistology. J.S. cultured mouse mast cells and performed mast cell reconstitution. J.Z. performed human serum trypsin ELISA. K.C. collected and provided all human serum samples and assisted with manuscript writing. J.N.G. helped with the mouse gastrointestinal tract tissue histology analysis. G.K.S. performed mouse tissue immunohistology. P.J.W. helped with data explanation and manuscript writing. J.D. helped with mouse tissue immunohistology. C.Z.G. performed mouse body tissue composition analysis. A. Doria performed human serum trypsin statistic analysis. P.L. assisted with the data interpretation and manuscript writing. R.S.B. helped with the gastrointestinal tract tissue histology analysis and assisted with the manuscript writing. B.B.K. assisted with the data interpretation and manuscript writing. G.S.H. helped with the body tissue composition data analysis. G.-P.S. designed the experiments, participated in all data analyses and prepared the manuscript.

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- Galli, S.J., Nakae, S. & Tsai, M. Mast cells in the development of adaptive immune responses. *Nat. Immunol.* **6**, 135–142 (2005).
- Bingham, C.O. III & Austen, K.F. Mast-cell responses in the development of asthma. *J. Allergy Clin. Immunol.* **105**, S527–S534 (2000).
- Robbie-Ryan, M. & Brown, M. The role of mast cells in allergy and autoimmunity. *Curr. Opin. Immunol.* **14**, 728–733 (2002).
- Secor, V.H., Secor, W.E., Gutekunst, C.A. & Brown, M.A. Mast cells are essential for early onset and severe disease in a murine model of multiple sclerosis. *J. Exp. Med.* **191**, 813–822 (2000).
- Lee, D.M. *et al.* Mast cells: a cellular link between autoantibodies and inflammatory arthritis. *Science* **297**, 1689–1692 (2002).
- Sun, J. *et al.* Mast cells promote atherosclerosis by releasing proinflammatory cytokines. *Nat. Med.* **13**, 719–724 (2007).
- Sun, J. *et al.* Mast cells modulate the pathogenesis of elastase-induced abdominal aortic aneurysms in mice. *J. Clin. Invest.* **117**, 3359–3368 (2007).

8. Coussens, L.M. *et al.* Inflammatory mast cells up-regulate angiogenesis during squamous epithelial carcinogenesis. *Genes Dev.* **13**, 1382–1397 (1999).
9. Weisberg, S.P. *et al.* Obesity is associated with macrophage accumulation in adipose tissue. *J. Clin. Invest.* **112**, 1796–1808 (2003).
10. Rocha, V.Z. *et al.* Interferon- γ , a T_H1 cytokine, regulates fat inflammation: a role for adaptive immunity in obesity. *Circ. Res.* **103**, 467–476 (2008).
11. Wu, H. *et al.* T-cell accumulation and regulated on activation, normal T cell expressed and secreted upregulation in adipose tissue in obesity. *Circulation* **115**, 1029–1038 (2007).
12. Fantuzzi, G. Adipose tissue, adipokines, and inflammation. *J. Allergy Clin. Immunol.* **115**, 911–919 (2005).
13. Duttlinger, R. *et al.* The Wsh and Ph mutations affect the c-kit expression profile: c-kit misexpression in embryogenesis impairs melanogenesis in Wsh and Ph mutant mice. *Proc. Natl. Acad. Sci. USA* **92**, 3754–3758 (1995).
14. Eigen, H. *et al.* Evaluation of the addition of cromolyn sodium to bronchodilator maintenance therapy in the long-term management of asthma. *J. Allergy Clin. Immunol.* **80**, 612–621 (1987).
15. Paterson, W.G. Role of mast cell-derived mediators in acid-induced shortening of the esophagus. *Am. J. Physiol.* **274**, G385–G388 (1998).
16. Shin, H.-Y., Kim, J.S., An, N.H., Park, R.K. & Kim, H.M. Effect of disodium cromoglycate on mast cell-mediated immediate-type allergic reactions. *Life Sci.* **74**, 2877–2887 (2004).
17. Grimaldeston, M.A. *et al.* Mast cell-deficient W-sash c-kit mutant *Kit^{W-sh/W-sh}* mice as a model for investigating mast cell biology *in vivo*. *Am. J. Pathol.* **167**, 835–848 (2005).
18. Cannon, B. & Nedergaard, J. Brown adipose tissue: function and physiological significance. *Physiol. Rev.* **84**, 277–359 (2004).
19. Koban, M. & Swinson, K.L. Chronic REM-sleep deprivation of rats elevates metabolic rate and increases UCP1 gene expression in brown adipose tissue. *Am. J. Physiol. Endocrinol. Metab.* **289**, E68–E74 (2005).
20. Santone, D.J., Shahani, R., Rubin, B.B., Romaschin, A.D. & Lindsay, T.F. Mast cell stabilization improves cardiac contractile function following hemorrhagic shock and resuscitation. *Am. J. Physiol. Heart Circ. Physiol.* **294**, H2456–H2464 (2008).
21. Serna, H., Porras, M. & Vergara, P. Mast cell stabilizer ketotifen [4-(1-methyl-4-piperidylidene)-4h-benzol[4,5]cyclohepta[1,2-b]thiophen-10(9H)-one fumarate] prevents mucosal mast cell hyperplasia and intestinal dysmotility in experimental *Trichinella spiralis* inflammation in the rat. *J. Pharmacol. Exp. Ther.* **319**, 1104–1111 (2006).
22. Galli, S.J. & Kitamura, Y. Genetically mast-cell-deficient W/W^v and Sl/Sl^d mice. Their value for the analysis of the roles of mast cells in biologic responses *in vivo*. *Am. J. Pathol.* **127**, 191–198 (1987).
23. Crandall, D.L., Hausman, G.J. & Kral, J.G. A review of the microcirculation of adipose tissue: anatomic, metabolic and angiogenic perspectives. *Microcirculation* **4**, 211–232 (1997).
24. Rupnick, M.A. *et al.* Adipose tissue mass can be regulated through the vasculature. *Proc. Natl. Acad. Sci. USA* **99**, 10730–10735 (2002).
25. Pang, C. *et al.* Macrophage infiltration into adipose tissue may promote angiogenesis for adipose tissue remodeling in obesity. *Am. J. Physiol. Endocrinol. Metab.* **295**, E313–E322 (2008).
26. Kintscher, U. *et al.* T-lymphocyte infiltration in visceral adipose tissue: a primary event in adipose tissue inflammation and the development of obesity-mediated insulin resistance. *Arterioscler. Thromb. Vasc. Biol.* **28**, 1304–1310 (2008).
27. Sato, K. *et al.* Autophagy is activated in colorectal cancer cells and contributes to the tolerance to nutrient deprivation. *Cancer Res.* **67**, 9677–9684 (2007).
28. Skoura, A. *et al.* Essential role of sphingosine 1-phosphate receptor 2 in pathological angiogenesis of the mouse retina. *J. Clin. Invest.* **117**, 2506–2516 (2007).
29. Xu, J. *et al.* Proteolytic exposure of a cryptic site within collagen type IV is required for angiogenesis and tumor growth *in vivo*. *J. Cell Biol.* **154**, 1069–1079 (2001).
30. Wang, B. *et al.* Cathepsin S controls angiogenesis and tumor growth via matrix-derived angiogenic factors. *J. Biol. Chem.* **281**, 6020–6029 (2006).
31. Shi, G.P., Munger, J.S., Meara, J.P., Rich, D.H. & Chapman, H.A. Molecular cloning and expression of human alveolar macrophage cathepsin S, an elastolytic cysteine protease. *J. Biol. Chem.* **267**, 7258–7262 (1992).

ONLINE METHODS

Mice. We purchased WT (C57BL/6 and WBB6F1/J), *Il6*^{-/-} (C57BL/6, N11), *Ifng*^{-/-} (C57BL/6, N10) and *Kit*^{W^{sh}/W^{sh} (WBB6F1/J) mice from the Jackson Laboratories. We generated congenic *Tnf*^{-/-} (C56BL/6, N10) and *Kit*^{W^{sh}/W^{sh} (C57BL/6, N10) mice as previously described^{6,32}. The Harvard Medical School Standing Committee on Animals approved all mouse protocols. To induce obesity, we fed 6-week-old mice (females and males) a Western diet containing 22.6% protein, 45.2% carbohydrate, 20.1% fat, and 1.25% cholesterol (Research Diet) for 12–13 weeks. We monitored body weight weekly. At the end of each course of Western diet consumption, we performed glucose tolerance, insulin tolerance and energy expenditure assays, and we determined body lean and fat mass as previously described^{33,34}. We collected mouse blood samples for serum adipokine measurement. We collected subcutaneous, visceral, and brown fat and skeletal muscle for protein extraction and paraffin section preparation. We used tissue protein extracts for ELISA, immunoblot analysis and cathepsin active site JPM labeling. For immunoblots, we used 30 µg of proteins to detect UCP-1 (1 in 3,000 antibody dilution, Abcam), actin (1 in 500 antibody dilution, Abcam) or glyceraldehyde-3-phosphate dehydrogenase (1 in 1,000 antibody dilution, Santa Cruz). We labeled the cathepsin active site as previously described^{31,33}. We collected the liver and gastrointestinal tract for histological analysis.}}

Human subject selection. In this study, we enrolled 80 obese subjects that had been prospectively recruited between 2003 and 2007 at the Department of Nutrition of Hôtel-Dieu Hospital, Paris, France. Detailed clinical and biological parameters are listed in **Supplementary Table 3**. These subjects were candidates for either a dietary intervention or gastric surgery in a clinical investigation program. We excluded those with evidence of inflammatory or infectious diseases, cancer, alcohol abuse or kidney disease. At the time of gastric surgery, we obtained subcutaneous adipose tissue from the periumbilical area in a subset of the obese subjects ($n = 12$, **Supplementary Table 4**). We recruited healthy, nonobese, age-matched ($P = 0.11$) individuals ($n = 32$) living in the same geographic area as controls (**Supplementary Table 3**). In ten lean controls (**Supplementary Table 4**), we obtained subcutaneous adipose tissue in the periumbilical area by needle biopsy. We determined serum insulin concentrations with an IRMA kit (CisBio International). We evaluated insulin sensitivity by the quantitative insulin sensitivity check index (QUICKI) = $1 / [\log \text{fasting insulin } (\mu\text{M}) + \log \text{fasting glycemia } (\text{mg dl}^{-1})]$. We processed all tissue samples under the same conditions. The Ethics Committee of the Hôtel-Dieu Hospital approved the clinical investigations, and all subjects gave written informed consent.

Immunohistology. We used antibodies to human HAM56 (1 in 100, Dako), human tryptase (1 in 100, Dako), mouse Mac-2 (1 in 25,000, Cedarlane), mouse CD117 (1 in 10, eBiosciences) and mouse CD31 (1 in 400, Pharmingen) to immunostain for macrophages, mast cells and microvessels on WAT, muscle and liver paraffin sections. Researchers blinded to the origin of tissue counted the total human HAM56⁺ macrophages, human tryptase⁺ and mouse CD117⁺ mast cells on each section, and data are presented as cell numbers per mm². We determined CD31⁺ areas in WAT and muscle with Image-Pro Plus

software as described^{30,35}. H&E staining assisted histological assessment of the gastrointestinal tract.

Enzyme-linked immunosorbent assay. We pulverized frozen mouse WAT and lysed it in RIPA buffer (Pierce). We subjected both WAT and serum samples to ELISA analysis for IL-6 (BD Biosciences), TNF- α , IFN- γ , monocyte chemo-attractant protein-1 (PeproTech), adiponectin, matrix metalloprotease-9, cathepsin S (R&D Systems) and cathepsin L (Bender MedSystems), according to the manufacturers' instructions.

To measure human serum tryptase abundance, we precoated a 96-well plate with a polyclonal antibody to human tryptase (1 in 1,000, Calbiochem). We added human serum samples (1:1 diluted in 1× PBS) along with recombinant human tryptase as a standard to the antibody-coated plate. After a 2-h incubation at 25 °C, we washed the plate and incubated it for 1 h with a monoclonal antibody to human tryptase (1 in 2,000, AbD Serotec). We used horseradish peroxidase-conjugated antibody to mouse IgG (1 in 1,000, Thermo Scientific) as the detecting antibody.

Cell culture. We prepared BMMCs and performed reconstitution experiments in 6-week-old male *Kit*^{W^{sh}/W^{sh} mice as we reported previously^{6,7}. Two weeks after BMMC reconstitution, we gave the mice a Western diet. We recorded mouse body weight weekly and performed the glucose tolerance assay before harvesting the WAT.}

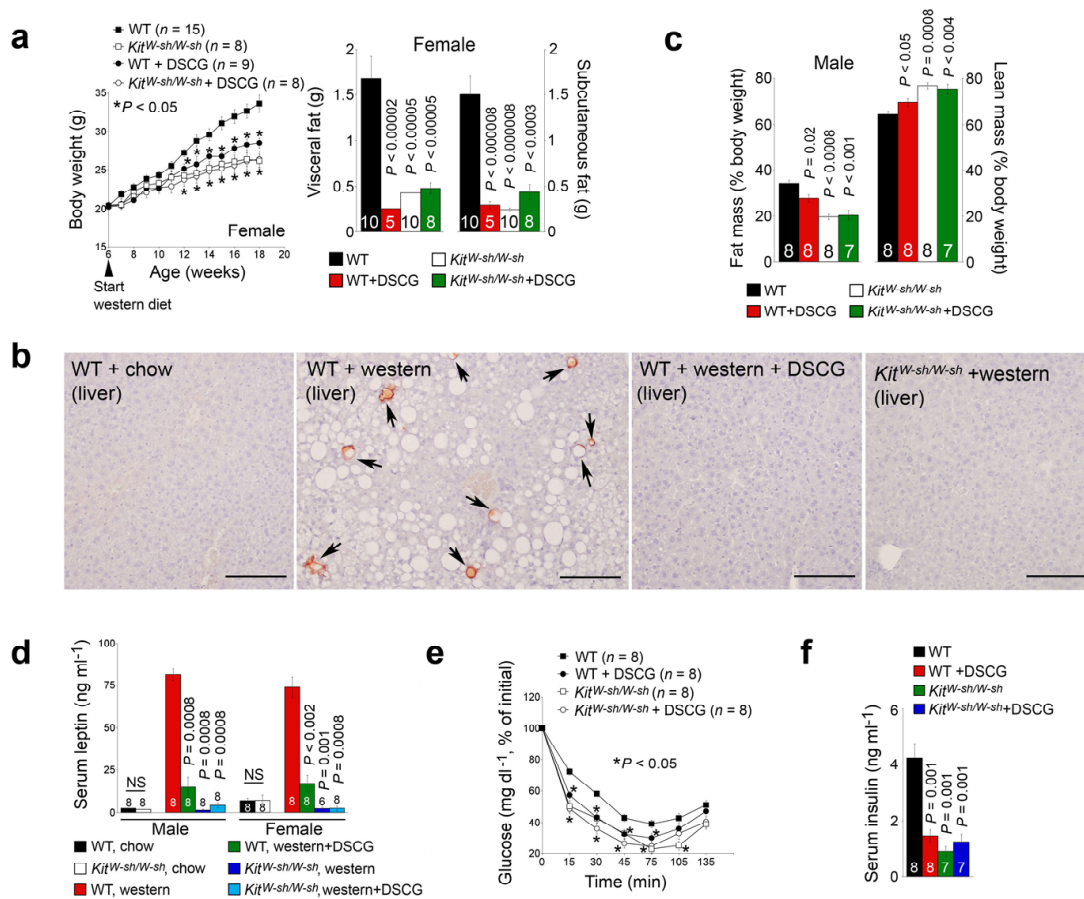
To assess the role of mast cells in 3T3-L1 cell cathepsin expression, we added either live BMMCs (2,000,000 cells per well for six-well plate) or their degranulated protein extract (equivalent to the live cell numbers) into 100% confluent 3T3-L1 cells. We maintained the co-cultures for 7–9 d, and we replaced culture medium with live BMMCs or BMMC protein extracts every 2 d. We then used 3T3-L1 cells for cathepsin active site labeling as previously described³¹.

Statistical analyses. All data from mice are expressed as means $\pm$ s.e.m., and we determined statistical significance with a nonparametric Mann-Whitney test, owing to our small data size and abnormal data distribution. Human serum tryptase ELISA data are expressed as means $\pm$ s.e.m. We used Student's *t* test, analysis of variance and Chi-square test for noncontinuous values for comparisons between groups. We used standard least squares regression method for multivariate analysis. We considered $P < 0.05$ statistically significant.

32. Wolters, P.J. *et al.* Tissue-selective mast cell reconstitution and differential lung gene expression in mast cell-deficient *Kit*^{W^{sh}/W^{sh} mice. *Clin. Exp. Allergy* **35**, 82–88 (2005).}
33. Yang, M. *et al.* Cathepsin L activity controls adipogenesis and glucose tolerance. *Nat. Cell Biol.* **9**, 970–977 (2007).
34. Hadigan, C. *et al.* Fasting hyperinsulinemia and changes in regional body composition in human immunodeficiency virus-infected women. *J. Clin. Endocrinol. Metab.* **84**, 1932–1937 (1999).
35. Wild, R., Ramakrishnan, S., Sedgewick, J. & Griffioen, A.W. Quantitative assessment of angiogenesis and tumor vessel architecture by computer-assisted digital image analysis: effects of VEGF-toxin conjugate on tumor microvessel density. *Microvasc. Res.* **59**, 368–376 (2000).

Deficiency and pharmacological stabilization of mast cells reduce diet-induced obesity and diabetes in mice

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Supplementary Figure 1. Mast cell deficiency and stabilization reduced diet-induced obesity and diabetes in mice. **a**, Body weight gain (left) and visceral and subcutaneous fat weight (right) of female WT and *Kit^{W-sh/W-sh}* mice and those receiving DSCG (all in C57BL/6 background). **b**, CD117 immunostaining for mast cells in liver paraffin sections from different mice as indicated. Arrows indicate CD117+ mast cells. Scale bar: 100 μ m. Only Western diet-fed WT mice developed steatotic livers with abundant mast cell accumulation. Western diet-fed *Kit^{W-sh/W-sh}* mice or WT mice receiving DSCG had normal liver histology similar to that in chow diet-fed WT mice. **c**, Fat and lean mass (% of body weight) in male WT and *Kit^{W-sh/W-sh}* mice treated with or without DSCG. **d**, Serum leptin levels in male and female mice with indicated treatments. **e**, Insulin tolerance test (e) and serum insulin levels (f) in male WT and *Kit^{W-sh/W-sh}* mice that consumed a Western diet for 12 weeks with and without DSCG treatments. $P < 0.05$ was considered statistically significant; non-parametric Mann-Whitney test. NS: no significant difference.

Supplementary Table 1: Energy expenditure analysis in *Kit^{W-sh/W-sh}* and DSCG-treated mice.

Parameters	WT (<i>n</i> = 6) (mean ± SEM)	<i>Kit^{W-sh/W-sh}</i> (<i>n</i> = 8) (mean ± SEM)	P Value*	WT + DGSC (<i>n</i> = 8) (mean ± SEM)	P Value*
Food consumption (gram per 24 hours)	3.17 ± 0.08	3.83 ± 0.26	0.03	3.25 ± 0.28	0.87
Water consumption (ml per 24 hours)	7.03 ± 0.28	6.38 ± 0.57	0.30	10.75 ± 0.62	0.02
Fecal output (gram per 24 hours)	1.53 ± 0.16	1.45 ± 0.14	0.77	1.56 ± 0.23	0.89
Urine volume (ml per 24 hours)	1.40 ± 0.10	1.65 ± 0.16	0.75	1.40 ± 0.17	0.52
O ₂ consumption (ml per kg per h)	2620.31 ± 27.65	2994.16 ± 67.79	0.002	2867.29 ± 57.65	0.005
CO ₂ production (ml per kg per h)	2129.04 ± 54.48	2619.41 ± 100.18	0.01	2534.96 ± 94.70	0.01

*Compared with WT mice, non-parametric Mann Whitney test. *P* < 0.05 was considered significant.

Supplementary Table 2. Serum and adipose tissue cytokine, chemokine, adipokine, and protease levels (mean $\pm$ SEM).

Serum	IL-6 (pg mL ⁻¹)	TNF- α (pg mL ⁻¹)	IFN- γ (pg mL ⁻¹)	MCP-1 (pg mL ⁻¹)	Eotaxin (pg mL ⁻¹)	RANTES (pg mL ⁻¹)	Adiponectin (ng mL ⁻¹)	MMP-9 (pg mL ⁻¹)	Cathepsin L (ng mL ⁻¹)
Males									
Wt + Chow	32.5 $\pm$ 7.5	54.1 $\pm$ 5.8	1328.4 $\pm$ 205.2	164.5 $\pm$ 18.4	419.0 $\pm$ 30.9	7.2 $\pm$ 2.6	6.7 $\pm$ 0.7	70.5 $\pm$ 0.3	4.6 $\pm$ 0.6
<i>Kit</i> ^{W-sh/W-sh} + Chow	30.9 $\pm$ 3.8	51.9 $\pm$ 6.9	1572.1 $\pm$ 87.2	161.6 $\pm$ 19.8	680.7 $\pm$ 63.4***	25.1 $\pm$ 6.1**	6.3 $\pm$ 0.5	62.6 $\pm$ 1.1***	5.7 $\pm$ 0.7
Wt + Western	74.4 $\pm$ 9.4	146.3 $\pm$ 15.7	1714.5 $\pm$ 123.7	459.2 $\pm$ 24.3	477.6 $\pm$ 26.0	8.9 $\pm$ 1.9	8.2 $\pm$ 0.9	71.4 $\pm$ 0.2	5.4 $\pm$ 0.7
<i>Kit</i> ^{W-sh/W-sh} + Western	41.6 $\pm$ 8.6	43.4 $\pm$ 24.9**	1467.2 $\pm$ 33.4*	115.2 $\pm$ 14.8***	630.5 $\pm$ 61.0	6.1 $\pm$ 1.3	6.3 $\pm$ 0.5	64.5 $\pm$ 1.4***	5.6 $\pm$ 0.9
Wt + DSCG + Western	26.7 $\pm$ 7.7**	129.4 $\pm$ 20.9	1403.9 $\pm$ 180.9**	285.5 $\pm$ 39.6**	571.7 $\pm$ 38.2	6.1 $\pm$ 1.6	9.0 $\pm$ 0.6	70.8 $\pm$ 0.2*	4.4 $\pm$ 0.7
Females									
Wt + Chow	18.9 $\pm$ 4.0	44.0 $\pm$ 8.0	900.2 $\pm$ 68.0	170.4 $\pm$ 11.9	560.3 $\pm$ 47.0	21.9 $\pm$ 9.3	9.1 $\pm$ 0.7	69.1 $\pm$ 3.9	8.0 $\pm$ 0.4
<i>Kit</i> ^{W-sh/W-sh} + Chow	30.4 $\pm$ 9.1	41.5 $\pm$ 9.3	950.1 $\pm$ 73.9	184.2 $\pm$ 16.8	531.2 $\pm$ 36.0	23.4 $\pm$ 6.3	7.4 $\pm$ 0.8	70.0 $\pm$ 4.5	8.3 $\pm$ 0.5
Wt + Western	28.6 $\pm$ 8.3	91.9 $\pm$ 16.8	1502.9 $\pm$ 213.	648.6 $\pm$ 73.9	549.1 $\pm$ 33.0	16.7 $\pm$ 6.5	9.0 $\pm$ 0.6	71.5 $\pm$ 0.2	9.1 $\pm$ 0.5
<i>Kit</i> ^{W-sh/W-sh} + Western	27.9 $\pm$ 7.3	30.6 $\pm$ 7.1***	994.3 $\pm$ 120.7	343.7 $\pm$ 44.4***	491.3 $\pm$ 27.0	17.1 $\pm$ 4.6	6.3 $\pm$ 0.5***	65.8 $\pm$ 4.9	8.2 $\pm$ 0.5
Wt + DSCG + Western	31.7 $\pm$ 4.9	91.5 $\pm$ 16.0	1408.9 $\pm$ 131.5	299.6 $\pm$ 38.7***	564.5 $\pm$ 47.0	33.3 $\pm$ 3.4	8.5 $\pm$ 0.7	71.0 $\pm$ 0.2	9.5 $\pm$ 0.6
WAT	IL-6 (pg mg ⁻¹)	TNF- α (pg mg ⁻¹)	IFN- γ (pg mg ⁻¹)	MCP-1 (pg mg ⁻¹)	Eotaxin (pg mg ⁻¹)	RANTES (pg mg ⁻¹)	Adiponectin (ng mg ⁻¹)	MMP-9 (pg mg ⁻¹)	Cathepsin L (ng mg ⁻¹)
Males									
Wt + Chow	21.0 $\pm$ 2.7	198.6 $\pm$ 12.1	196.0 $\pm$ 32.7	22.0 $\pm$ 6.8	9.0 $\pm$ 4.0	22.1 $\pm$ 2.7	379.2 $\pm$ 37.0	42.4 $\pm$ 4.1	9.0 $\pm$ 0.5
<i>Kit</i> ^{W-sh/W-sh} + Chow	22.5 $\pm$ 1.5	225.1 $\pm$ 18.8	204.5 $\pm$ 25.3	32.4 $\pm$ 6.1	10.4 $\pm$ 3.5	8.6 $\pm$ 3.1**	357.7 $\pm$ 31.0	40.1 $\pm$ 2.9	9.7 $\pm$ 0.7
Wt + Western	28.7 $\pm$ 2.8	187.6 $\pm$ 12.5	212.5 $\pm$ 29.7	115.4 $\pm$ 18.2	5.8 $\pm$ 2.2	15.7 $\pm$ 4.9	373.1 $\pm$ 23.0	35.4 $\pm$ 2.2	8.8 $\pm$ 0.4
<i>Kit</i> ^{W-sh/W-sh} + Western	20.9 $\pm$ 1.8*	124.5 $\pm$ 14.4**	192.5 $\pm$ 18.2	41.7 $\pm$ 12.2**	6.0 $\pm$ 3.4	20.2 $\pm$ 3.7	426.5 $\pm$ 46.0	41.4 $\pm$ 5.1	9.1 $\pm$ 0.5
Wt + DSCG + Western	30.8 $\pm$ 5.2	231.2 $\pm$ 13.3	245.0 $\pm$ 9.5	57.4 $\pm$ 7.7**	16.4 $\pm$ 3.2**	26.4 $\pm$ 3.2	266.1 $\pm$ 27.0*	41.4 $\pm$ 3.4	8.8 $\pm$ 0.5

* $P < 0.04$; ** $P < 0.02$; *** $P < 0.005$. Non-parametric Mann-Whitney test to compare the corresponding WT groups. $P < 0.05$ was considered significant.

Supplementary Table 3. Clinical and biological parameters of patients used for serum tryptase ELISA.

Parameter	Obese subject (average [range])	Lean subject (average [range])	<i>P</i> value [†]
Patient number	80	32	N/A
Age (year)	37.3 [20.0~66.0]	41.4 [20.0~62.0]	0.11
Body mass index (BMI, kg per mm ²)	48.5 [32.0~85.3]	22.9 [19.9~25.6]	<0.01
Sex (female to male)	69/11	22/10	0.45
Fasting glucose (mM)	5.1 [3.7~7.4]	4.5 [3.4~5.3]	<0.01
Fasting insulin (μU mL ⁻¹)	15.4 [3.9~68.3]	3.6 [0.4~7.9]	<0.01
Homeostasis Model Assessment (HOMA)	3.5 [0.8~15.3]	0.7 [0.1~0.8]	<0.01
Quantitative insulin-sensitivity check index (QUICKI)	0.3 [0.3~0.4]	0.4 [0.4~0.7]	<0.01
Total cholesterol (mmol)	4.8 [2.8~7.1]	4.9 [3.1~7.8]	0.67
High density lipoprotein (mmol)	1.4 [0.6~2.2]	1.6 [0.8~2.6]	0.03
Triglyceride (mmol)	1.2 [0.4~3.7]	1.0 [0.5~2.2]	0.06
Tryptase (ng mL ⁻¹)	13.1 [1.0~73.0]	7.7 [0.0~23.3]	0.01

[†]Comparison between obese and lean subjects. *Chi* square test was used for qualitative traits and unpaired *t*-test for quantitative traits. *P* < 0.05 was considered statistically significant.

Supplementary Table 4. Clinical and biological parameters for patients used for immunohistology analysis.

Parameter	Obese subject (average [range])	Lean subject (average [range])	<i>P</i> value [†]
General measurement			
Patient number	12	10	N/A
Sex (female to male)	6/6	10/0	0.002
Age (year)	47.8 [20~63]	35.2 [30~42]	0.004
Body mass index (BMI, kg per mm ²)	49.9 [36.0~62.1]	23.7 [21.9~26.2]	<0.0001
Glucose homeostasis			
Glucose (mmol L ⁻¹)	6.4 [4.2~10.3]	4.8 [3.7~6.3]	0.11
Insulin (μU mL ⁻¹)	22.0 [4.7~84.2]	7.2 [4.0~13.0]	<0.03
Homeostasis Model Assessment (HOMA)	7.3 [1.3~29.3]	1.7 [0.7~3.6]	0.38
Quantitative insulin-sensitivity check index (QUICKI)	0.3 [0.2~0.4]	0.4 [0.3~0.4]	<0.03
Type 2 diabetes			
Glycemia (>7 mmol L ⁻¹ or treatment)	<i>n</i> = 5	<i>n</i> = 0	0.007

[†]Comparison between obese and lean subjects. *Chi* square test was used for qualitative traits and Mann-Whitney *U* test for quantitative traits. *P* < 0.05 was considered statistically significant.