Gut intraepithelial T cells calibrate metabolism and accelerate cardiovascular disease

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The bioresponse to food intake must be precisely regulated. Because ingested sugars and fats can feed into many anabolic and catabolic pathways, how our bodies handle nutrients depends on strategically positioned metabolic sensors that link the intrinsic nutritional value of a meal with intermediary metabolism. Here we describe a subset of immune cells—integrin β7+ natural gut intraepithelial lymphocytes (IELs)—that is dispersed throughout the enterocyte layer of the small intestine and that modulates systemic metabolism. Integrin β7+ mice that lack natural IELs are metabolically hyperactive and, when fed a high-fat and high-sugar diet, are resistant to obesity, hypercholesterolemia, hypertension, diabetes and atherosclerosis. Furthermore, we show that protection from cardiovascular disease in the absence of natural IELs depends on the enteroendocrine-derived incretin GLP-12, which is normally controlled by IELs through expression of the GLP-1 receptor. In this metabolic control system, IELs modulate enteroendocrine activity by acting as gatekeepers that limit the bioavailability of GLP-1. Although the function of IELs may prove advantageous when food is scarce, present-day overabundance of diets high in fat and sugar renders this metabolic checkpoint detrimental to health.

Although integrin β7+ directs immune cells to the gut3,8, we know little about the influence of this integrin on metabolism, despite the strategic location of the gut as the site where dietary nutrients are absorbed. β7+ bone marrow (β7+/−) mice that were fed a chow diet gained weight in a similar way to control wild-type mice (Fig. 1a), but ate more food (Fig. 1b) despite being equally active (Extended Data Fig. 1a). This inconsistency prompted us to measure energy usage. We found that β7+/− mice expended more energy (Fig. 1c) and produced more heat (Fig. 1d), although their respiratory exchange rate was similar to wild-type mice (Extended Data Fig. 1b, c). The data suggest that these mice have a heightened basal metabolism. We therefore performed whole-body 18F-fluorodeoxyglucose ([18F] FDG) and non-invasive, high-resolution positron emission tomography/computed tomography (PET/CT) imaging to assess regional glucose uptake and found that β7+/− mice accrued more glucose in the brown fat compared to wild-type controls (Fig. 1e, f and Extended Data Fig. 1d). The β7+/− mice were more glucose tolerant (Fig. 1g), even at thermoneutrality (Extended Data Fig. 1e, f), and had higher levels of plasma insulin (Fig. 1f) without changes in insulin sensitivity (Fig. 1g). The microbiome did not appear to affect these differences (Extended Data Fig. 1f, g). Moreover, the phenomenon was neither restricted to glucose—because β7+/− mice had lower levels of fasting triglycerides (Fig. 1j) and better fat tolerance (Fig. 1k) without differences in hepatic secretion of triglycerides (Fig. 1i)—nor associated with fat absorption or gut permeability abnormalities (Extended Data Fig. 1g, h, i). We next tested whether the beneficial metabolic alterations in β7+/− mice were sustained in the context of the ‘metabolic syndrome’ component cluster10. β7+/− mice that were fed a diet high in fat, sugar and sodium (HFSSD) remained relatively lean, in contrast to wild-type controls, which became obese (Fig. 2a). Both inguinal white adipose tissue (IWAT) and perigonadal white adipose tissue (pWAT) were heavier in wild-type mice than in β7+/− mice, but other tissue weights remained similar (Fig. 2b). Furthermore, adipocytes in IWAT and pWAT were larger in wild-type mice than in β7+/− mice (Fig. 2c–e). Flow cytometry of both IWAT and pWAT showed that fewer Ly-6Chigh monocytes, neutrophils and macrophages had accumulated in β7+/− mice, indicating that β7+/− mice were protected from obesity-associated inflammation11,12 (Extended Data Fig. 2a, b). In contrast to wild-type control mice, β7+/− mice did not develop hypertension (Fig. 2f) and—similar to observations made in β7+/− mice that were on a chow diet—β7+/− mice that were fed a HFSSD remained more glucose-tolerant than wild-type mice (Fig. 2g and Extended Data Fig. 2c, d), indicating that β7+/− mice were protected against the adverse metabolic consequences of a high-fat diet.

Because β7−/− mice had a higher metabolism and exhibited fewer metabolic syndrome components, we tested whether they had lower rates of atherosclerosis, which is a chronic, lipid-driven inflammatory disease.13 We generated Ldlr−/−/− chimaeras reconstituted with bone marrow from either β7+/− (bmβ7+/−) or wild-type (bmβ7+/+) mice and found that Ldlr+/− mice with β7+/− bone marrow (bmβ7+/−Ldlr+/−) that were fed a diet high in cholesterol (HCD) had considerably lower levels of plasma total cholesterol than controls (bmβ7+/−Ldlr+/−) (Fig. 3a and Extended Data Fig. 2e). The bmβ7+/−Ldlr+/− mice had lower levels of very-low-density lipoprotein, intermediate-density lipoprotein and low-density lipoprotein, but similar levels of high-density lipoprotein (Fig. 3b). bmβ7+/−Ldlr+/− mice with Ldlr−/− bone marrow (bmβ7+/−Ldlr−/−) were more glucose-tolerant than controls (Extended Data Fig. 2f), yet not in bmβ7−/−Ldlr+/− mice fed a HCD (Fig. 3g), but not in bmβ7+/−Ldlr−/− mice fed a chow diet (Extended Data Fig. 3c). Similar to experiments obtained using β7+/− mice, we noted improved glucose tolerance in the bmβ7+/−Ldlr−/− chimaeras (Extended Data Fig. 3d). We also generated β7−/−Ldlr+/− mice. Following assessment of metabolic functions, which were similar to those in β7−/− mice.
we noted that $\beta^7\text{-}Ldlr^{-/-}$ mice had lower levels of plasma cholesterol, smaller aortic root lesions and fewer aortic leucocytes after HCD (Extended Data Fig. 4a–d). Finally, we injected anti-integrin $\beta^7$ antibodies into $Ldlr^{-/-}$ mice and found that these mice had improved glucose tolerance and attenuated atherosclerosis (Extended Data Fig. 4e–g). These data show that integrin $\beta^7$ deficiency protects against atherosclerosis.

Next, we investigated which cells account for our findings. Intraepithelial lymphocytes that reside in the small intestine had the highest integrin $\beta^7$ expression (Fig. 4a), in agreement with studies that have shown that integrin $\beta^7$ guides leucocytes to the gut.\(^{16,17}\) Although the intestinal intraepithelium had fewer CD3$^+$ cells in $\beta^7^{-/-}$ mice (Extended Data Fig. 5a), we nevertheless analysed the relative ability of CD$^+$ cells to enter tissues (Extended Data Fig. 5b). The blood contained $\beta^7^{-/-}$ and wild-type cells in similar proportions (Fig. 4b) and—although many tissues accumulated $\beta^7^{-/-}$ and wild-type cells in similar, albeit varied, proportions—considerably fewer $\beta^7^{-/-}$ cells accumulated in the gut and particularly in the small intestine intraepithelium (Fig. 4b and Extended Data Fig. 5b). These findings therefore confirm that integrin $\beta^7$ directs leucocytes to the gut.

We show that the leucocytes that rely on integrin $\beta^7$ influx to the gut are $\alpha\beta$ and $\gamma\delta$ T cells (Fig. 4c). B cells and myeloid cells (Extended Data Fig. 5c). Notably, $\beta^7^{-/-}$ mice had similar numbers of leucocytes as wild-type mice in metabolically important organs such as the liver and pancreas (Extended Data Fig. 5d, e). Although T cells were the most numerous integrin $\beta^7$-dependent population that was assessed in the gut, we nevertheless tested which of the three populations ($\alpha\beta$ and $\gamma\delta$ T cells, B cells or myeloid cells) mediated the metabolic effects. We therefore generated five different mixed chimeric groups of mice ($\beta^7^{-/-}$ wild-type, $\beta^7^{-/-}$ Tcrb$^{-/-}$ (hereafter $\beta^7^{-/-}$ TCR$^{-/-}$), $\beta^7^{-/-}$ Tcrd$^{-/-}$ (hereafter $\beta^7^{-/-}$ $\gamma\delta$ TCR$^{-/-}$), $\beta^7^{-/-}$ Ighm$^{-/-}$ (hereafter $\beta^7^{-/-}$ mIg) and $\beta^7^{-/-}$ Ccr2$^{-/-}$) on a wild-type background and performed a glucose-tolerance test to screen for the metabolic phenotype. We found that specific absence of integrin $\beta^7$ on either $\alpha\beta$ (hereafter $\beta^7^{-/-}$ TCR$^{-/-}$) or $\gamma\delta$ (hereafter $\beta^7^{-/-}$ $\gamma\delta$ TCR$^{-/-}$) T cells improved glucose tolerance (Fig. 4d), whereas no changes were found in the other mixed chimaeras (Extended Data Fig. 5f). Both Ig$\mu$$^{-/-}$ and Ccr9$^{-/-}$ mice\(^{9,19}\) showed a similar improvement in glucose tolerance (Fig. 4e). Moreover, given the importance of B cells for gut homeostasis,\(^{20,21}\) we analysed the contribution of this lymphocyte population in more detail, but found no differences in glucose tolerance, cholesterolaemia or atherosclerosis (Extended Data Fig. 6), indicating that $\beta^7$-dependent B cells do not contribute to the metabolic phenotype. These data show that intraepithelial $\alpha\beta$ and $\gamma\delta$ T cells regulate systemic metabolism.

In response to dietary nutrients, enteroendocrine L-cells in the gut produce the incretin hormone GLP-1, which induces postprandial glucose tolerance and attenuated atherosclerosis. \(^{16,17}\) GLP-1 mediates various other beneficial effects on metabolism, while its analogue improves cardiovascular outcomes in patients with diabetes.\(^{24,25}\) We found that bm$\beta^7^{-/-}$ Ldlr$^{-/-}$ mice that were fed a HCD had higher levels of fasting GLP-1 in the plasma (Fig. 4f), along with increased gut Gcg mRNA levels (Fig. 4g). In addition, $\beta^7^{-/-}$ mice had increased levels of GLP-1 compared to wild-type mice that were fed a HFDSS (Extended Data Fig. 7a, b). To test whether $\alpha\beta$ and $\gamma\delta$ T cells controlled the bioavailability of GLP-1, we measured the expression of the GLP-1 receptor in gut leucocytes and found that natural $\alpha\beta$ and
Integrin $\beta$7 deficiency protects against metabolic syndrome.

a. Body weights of wild-type and $\beta$7$^{-/-}$ mice that were fed a HFSSD for 5 months. $n = 9$ wild-type mice; $n = 8$ $\beta$7$^{-/-}$ mice. Representative images of wild-type and $\beta$7$^{-/-}$ mice are shown on the left. Black dots denote animals shown in the images. b. Tissue weights of wild-type and $\beta$7$^{-/-}$ mice after 5 months of a HFSSD. $n = 10$ mice per group, except for heart, $n = 5$. c. Representative haematoxylin and eosin images of iWAT and pWAT of wild-type ($n = 5$) and $\beta$7$^{-/-}$ ($n = 4$) mice fed a HFSSD for 5 months. d. e. Quantification of adipocytes at indicated size ranges in iWAT and pWAT of wild-type and $\beta$7$^{-/-}$ mice fed a HFSSD.

$\gamma$δ T cells in wild-type mice showed abundant expression of the GLP-1 receptor, consistent with data from Immgen (http://www.immgen.org/) and a previous study26 (Fig. 4h). By contrast, the guts of $\beta$7$^{-/-}$ mice were relatively deficient in expression of the GLP-1 receptor, containing fewer and mostly Glp1r$^{low}$ T cells (Extended Data Figs. 5a, 7c, d). These results indicate that loss of Glp1r$^{high}$ natural IELs is associated with increased plasma levels of the ligand (GLP-1), an observation that is consistent with previous studies that show increased levels of GLP-1 in Glp1r$^{-/-}$ mice27.

To determine whether the GLP-1 receptor on IELs protects against atherosclerosis through increased systemic levels of GLP-1, we generated mixed chimaeras (bmGlp1r$^{+//-}$; Fig. 4i) on a Ldlr$^{-/-}$ background. After performing quality-control experiments (Extended Data Figs. 7c, 8a–e), we found that bmGlp1r$^{+//-}$ Ldlr$^{-/-}$ mice had increased concentrations of GLP-1 (Fig. 4j), were more glucose tolerant (Fig. 4k), less hypercholesterolaemic (Fig. 4l) and developed smaller plaques (Fig. 4m) of oil-red O staining of aortic root sections from wild-type mice. Data are mean ± s.e.m., representing biological replicates. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$, two-tailed Mann–Whitney U-tests unless otherwise indicated.

Fig. 2 | Integrin $\beta$7 deficiency protects against metabolic syndrome.

Fig. 3 | Integrin $\beta$7 deficiency protects against atherosclerosis. Ldlr$^{-/-}$ mice were lethally irradiated and reconstituted with bone marrow cells from either wild-type (bm$^{+/+}$Ldlr$^{-/-}$) or $\beta$7$^{-/-}$ (bm$^{+/+}$Ldlr$^{-/-}$) mice. a. a. Plasma cholesterol in fed and overnight-fasted animals fed a HCD for 14 weeks. $n = 6$ bm$^{+/+}$Ldlr$^{-/-}$ mice; $n = 9$ bm$^{+/+}$Ldlr$^{-/-}$ fed mice; $n = 10$ bm$^{+/+}$Ldlr$^{-/-}$ mice; $n = 9$ bm$^{+/+}$Ldlr$^{-/-}$ fasted mice. b. Plasma lipoprotein distribution measured by fasted liquid chromatography in bm$^{+/+}$Ldlr$^{-/-}$ and bm$^{+/+}$Ldlr$^{-/-}$ mice. c. d. Representative images (c) and quantification (d) of oil-red O staining of aortic root sections from bm$^{+/+}$Ldlr$^{-/-}$ and bm$^{+/+}$Ldlr$^{-/-}$ mice fed a HCD for 14 weeks. $n = 12$ bm$^{+/+}$Ldlr$^{-/-}$ mice; $n = 13$ bm$^{+/+}$Ldlr$^{-/-}$ mice. e. Plaque volumes were calculated by measuring the plaque size at increasing distances from the aortic root. $n = 5$ mice per group. *$P < 0.05$, two-tailed Student’s t-test. f. g. Leukocyte quantification in aortas (f) and blood (g) of bm$^{+/+}$Ldlr$^{-/-}$ and bm$^{+/+}$Ldlr$^{-/-}$ mice fed a HCD for 14 weeks. $n = 6$ bm$^{+/+}$Ldlr$^{-/-}$ and $n = 5$ bm$^{+/+}$Ldlr$^{-/-}$ in f; $n = 5$ mice per group in g. Data are mean ± s.e.m., representing biological replicates. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$, two-tailed Mann–Whitney U-tests unless otherwise indicated.
apparent between Glp1r−/− and wild-type chimaeras on a wild-type (that is, not Ldlr−/−) background, although we did see differences in the Glp1r−/−:Ldlr−/− mixed chimaeras (Extended Data Fig. 8f–i). By contrast, we found attenuated atherosclerosis in E7–/–Ldlr−/– mice; KO indicates knockout (either βTCR−/− or γTCR−/−) bone marrow. e, Glucose-tolerance test (intraperitoneal injection) in wild-type (n = 4 and 5 mice) and Ilgαe−/− (n = 4 mice) or Ccr9−/− (n = 3 mice). f, g, Fasting plasma total GLP-1 (tGLP-1) levels (f) and Gg mRNA expression (g) in the ileum of bmβ2/−/−Ldlr−/− (n = 5 mice) and bmβ2/−/−Ldlr−/− (n = 4 and 5 mice) mice fed a HCD for 14 weeks. h, Glp1r in sorted IELs from wild-type mice. n = 3 mice. Epi., epithelial cells; Mye., myeloid cells; Nat., natural; Ind., induced. i, Experimental set-up for generating mixed bone marrow chimaeras. J, Fasting plasma total GLP-1 levels. n = 6 bmGlp1r+/+/β7−/−Ldlr−/− mice; n = 4 bmGlp1r−/−/β7−/−Ldlr−/− mice. k, Glucose-tolerance test. n = 5 mice. l, Plasma cholesterol. n = 5 bmGlp1r+/+/β7−/−Ldlr−/− mice; n = 4 bmGlp1r−/−/β7−/−Ldlr−/− mice. *P < 0.05, two-tailed unpaired Student’s t-test. m, Representative images and quantification of oil-red O-stained aortic roots. n = 4 bmGlp1r+/+/β7−/−Ldlr−/− mice; n = 6 bmGlp1r−/−/β7−/−Ldlr−/− mice. n, Quantification of the number of leukocytes in aortas. n = 5 mice. k–n, Mice were fed a HCD. Data are mean ± s.e.m., representing biological replicates. *P < 0.05, **P < 0.01, two-tailed Mann–Whitney U-tests unless otherwise indicated.

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Competing interests The General Hospital Corporation has filed a US patent application 62/771,668 with the US Patent and Trademark Office entitled ‘Targeting intraepithelial leukocytes for treatment of cardiometabolic diseases’, which names F.K.S. and S.H. as inventors. D.J.D. has served as an advisor or consultant to Intarcia, Forkhead Biopharmaceuticals Inc., Kallyope Inc., Merck Research Laboratories, Pfizer Inc., Novo Nordisk Inc. and Zafgen Inc. Mount Sinai receives funding from GSK, Merck and Novo Nordisk for GLP-1-related studies in the Drucker laboratory.

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METHODS

Mice. C57BL/6 (wild-type mice), B6.SJL-Ptprc<Pe>BoyJ (CD45.1−), Ilyg<γc>−/− (γδT−), LdGR<red>−/− (Ldr−), Tcrβm<all>−/− (TCRβ−), Tcrγδ<all>−/− (TCRγδ−), Ccr2<all>−/− (Ccr2−), IgM<all>−/− (μMT), CCR1<all>−/− (Ccr1−), and IgG<all>−/− (IgG−) mice were purchased from The Jackson Laboratory. Glp1r<all>−/− mice on the C57BL/6 background were lethally irradiated with 300 Gy before bone marrow transplantation. Bone marrow transplantation. Naïve C57BL/6 or Ldr−/− mice were lethally irradiated (950 cGy) and reconstituted with indicated bone marrow cells to generate different chimaera groups: (i) bm<all>−/+ Ldr−/− and bm<all>−/+ Ldr−/− (irradiated Ldr−/− mice reconstituted with either wild-type or Ldr−/− bone marrow); (ii) bm<all>−/+ CD45.1− or bm<all>−/+ GFP−/− Ldr−/− (irradiated Ldr−/− mice reconstituted with a 1:1 bone marrow mixture of CD45.1− wild-type and GFP−/− bone marrow); (iii) bm<all>−/+ CD45.1− or bm<all>−/+ GFP−/− (irradiated wild-type mice reconstituted with a 1:1 bone marrow mixture of CD45.1− wild-type and GFP−/− bone marrow); (iv) Ldr−/− wild-type, Ldr−/−/TCRβ− and Ldr−/−/γTCR− (irradiated wild-type mice reconstituted with 1:1 bone marrow mixture of Ldr−/− wild-type and GFP−/− bone marrow); (v) Ldr−/− wild-type, Ldr−/−/TCRβ− and Ldr−/−/γTCR−, and Ldr−/−/TCRβ− and Ldr−/−/γTCR− (irradiated Ldr−/−/TCRβ− and Ldr−/−/γTCR− mice reconstituted with 1:1 bone marrow mixture of Ldr−/− wild-type and Ldr−/−/TCRβ− and Ldr−/−/γTCR−, respectively); (v) bm<all>−/+ Ldr−/− and bm<all>−/+ Ldr−/− (irradiated Ldr−/− mice reconstituted with 1:1 bone marrow mixture of wild-type and Ldr−/−, and Ldr−/− and Ldr−/−, respectively); (vi) bm<all>−/+ and bm<all>−/+ (irradiated wild-type mice reconstituted with either wild-type or Glp1r<all>−/− bone marrow); (vii) bm<all>−/+ Glp1r<all>−/− and bm<all>−/+ Glp1r<all>−/− (irradiated wild-type mice reconstituted with 1:1 bone marrow mixture of wild-type and Glp1r<all>−/−, and Glp1r<all>−/− and Glp1r<all>−/−, respectively); (viii) bm<all>−/+ Ccr2<all>−/−, bm<all>−/+ μMT, and bm<all>−/+ wild-type (irradiated wild-type mice reconstituted with 1:1 bone marrow mixture of Ccr2<all>−/−, μMT, and wild-type, and Ccr2<all>−/− and μMT, and wild-type, respectively); (ix) bm<all>−/+ wild-type Ldr−/− or bm<all>−/+ μMT Ldr−/− (irradiated Ldr−/− mice reconstituted with 1:1 bone marrow mixture of wild-type and Ldr−/−, and wild-type, and μMT, respectively). Anti-integrin β7 antibody treatment. Ldr−/− mice on a HCD were treated with anti-integrin β7 antibodies (P1B5, BioxCell) or IgG isotype control (2A3, BioxCell) by intraperitoneal injection for 14 weeks for quantification of atherosclerotic plaque size after euthanasia (500 μg per mouse per week). Glp1r<all>−/− agonist treatment. Ldr−/− mice were treated with the Glp1r<all>−/− agonist exendin-4 (Abcam) at a dose of 100 μg per kg per day via osmotic minipumps (Alzet). After 8 weeks on a HCD, mice were euthanized for atherosclerotic lesion quantification.

Metabolic measurements. CLAMS. A comprehensive laboratory animal monitoring system (CLAMS, The Columbus Instruments) was used at the Joslin Diabetes Center Animal Physiology Core to simultaneously measure a series of metabolic parameters including energy expenditure (heat production), oxygen consumption (VO2), carbon dioxide production (VCO2), respiratory exchange ratio, food consumption and locomotor activity levels.

Blood pressure measurements. Systolic and diastolic blood pressures were measured using a non-invasive tail-cuff system (Kent Scientific). Mice were initially acclimated to the instrument for the three consecutive days before the measurements.

[18F]FDG PET/CT. The uptake and distribution of glucose in vivo were determined by [18F]FDG and non-invasive, high-resolution PET/CT imaging and ex vivo biodistribution. In brief, mice were anaesthetized with isoflurane and injected intraperitoneally with [18F]FDG. Mice were scanned at the indicated time points after injection or sacrifice for analysis of glucose uptake and distribution. The uptake and distribution of glucose in vivo were determined using a modified Feldkamp cone beam reconstruction algorithm (COBRA, Eximm Computing Corporation). A 20-min PET image was acquired and reconstructed using the ordered subsets expectation maximization followed by maximum a posteriori. Regions of interest were manually drawn for standard uptake value calculations. After imaging, animals were euthanized and tissues were collected for biodistribution analysis using gamma well counting on a 20% window on the 511 keV photopeak (Wizard, PerkinElmer). [18F]FDG levels were normalized to the weight of resected tissue and expressed as the percentage injected dose per gram tissue weight.

Glucose- and insulin-tolerance tests. For glucose- and insulin-tolerance tests, overnight-fasted mice were injected intraperitoneally or by oral gavage with glucose (2 g per kg body weight) or injected intraperitoneally with insulin (0.75 U per kg body weight). Blood glucose levels were measured at the basal level and at 15, 30, 60, 90 and 120 min after glucose or insulin administration using One Touch Ultra2 Blood Glucose Meter (OneTouch, LifeScan).

Fat-tolerance test. Overnight-fasted mice were injected intraperitoneally with 200 μl 20% Intralipid (vol/vol) fat emulsion (Sigma, MA), and blood that was drawn from the retroorbital plexus at the indicated time points for triglyceride measurement using the L-Type Triglyceride M kit (Wako Diagnostics, VA). To measure hepatic lipid export, overnight-fasted mice were injected with 1 g per kg poloxamer 407 (Pluronic F-127, Sigma) and plasma was collected at the indicated time points for triglyceride analysis.

Fat absorption test. To measure fat absorption in the gut, overnight-fasted mice were injected intraperitoneally with 1 g per kg poloxamer 407. After 1 min, the mice were gavaged with 0.1 ml 11.1 kV photopaque (Wizard, PerkinElmer). [18F]FDG levels were normalized to the weight of resected tissue and expressed as the percentage injected dose per gram tissue weight.

Blood Glucose Meter (OneTouch, LifeScan).
Cell culture. For all experiments, cells or ex vivo ileum tissues were kept in a humidified 5% CO₂ incubator at 37°C. (i) For in vitro GLP-1 receptor agonist-binding experiments, small-intestinal IELs were isolated and incubated with 50 pM fluorescently labelled GLP-R agonist exendin-4 Cys40SeTaa474 for 1 h, and IEL subsets were gated as follows: natural, Glp1rhigh IELs (CD45+CD3+CD90.2+CD5+); induced, Glp1rlow IELs (CD45+CD3+CD90.2+CD5-), non-T cells (CD45+CD3-), and epithelial cells (CD45+CD326+CD3-). The binding capacity was analysed for exendin-4 Cys40SeTaa474 by flow cytometry. (ii) For in vitro co-culture experiments, GLP-1-producing L-cells (GLUTag cells that were provided by D.J.D., authenticated multiple times and tested for mycoplasma) were cultured together with sorted Glp1rhigh or Glp1rlow IELs in DMEM and GlutaMAX-I with glucose 1 g l⁻¹ (Invitrogen), supplemented with 10% FBS and 1% penicillin–streptomycin (10³ GLUTag cells and 10⁵ IELs in a 96-well plate in 200 μl medium per well). After 24 h, the concentration of GLP-1 in the supernatant was measured using a total GLP-1 enzyme-linked immunosorbent assay (ELISA) kit (Millipore). In some experiments, the GLP-1 receptor agonist exendin-4 (Abcam) was added to co-culture wells (100 nM). (iii) In the case of two-step ex vivo experiments, sorted Glp1rhigh IELs were incubated with exendin-4 (100 nM) or control. After 24 h, samples were centrifuged (3000 g, 5 min) and supernatants were transferred to ileum ex vivo sections of previously euthanized wild-type mice. GLP-1 levels were determined 24 h later from ex vivo supernatants. (iv) In an ex vivo GLP-1 receptor-inhibition experiment, whole ileum preparations of wild-type or β7⁻/⁻ mice were treated with the GLP-1 receptor antagonist exendin-9 (100 nM) or control. After 24 h the concentration of GLP-1 in the supernatant was measured using a total GLP-1 ELISA kit (Millipore).

Molecular biology. PCR. Total RNA was isolated using the RNeasy Mini Kit (Qiagen) according to the manufacturer’s instructions. cDNA was generated from 1 μg of total RNA per sample using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Quantitative real-time TaqMan PCR was performed using the following TaqMan primers (Applied Biosystems): Glp1r (Mm00445292_m1), Cgc (Mm00801714_m1) and housekeeping gene actin (Actb) (Mm02615980_g1). PCR was run on a 7500 thermal cycler (Applied Biosystems) and data were quantified with the 2 -ΔΔCt method.

ELISA. Total GLP-1 levels were measured in plasma of overnight-fasted mice or during oral glucose-tolerance tests using a commercial ELISA kit (Millipore) according to the manufacturer’s instructions. Gut IgA was retrieved by flushing the lumens of dissected guts with 5 ml PBS, and both gut flush IgA and plasma IgA was detected using mouse IgA ELISA kit (Bethyl Laboratories).

Histology. Adipose tissue. iWAT and pWAT were excised, fixed in 10% formalin solution and paraffin-embedded. Haematoxylin and eosin staining was performed to assess overall tissue morphology. The adipocyte size distribution was determined with the NIH ImageJ program.

Aortas. Aortic roots were dissected, embedded in Tissue-Tek OCT compound (Sakura Finetek) and frozen in 2-methylbutane (Fisher Scientific) cooled with dry ice. For comparisons of lesion sizes between the groups, sections with the maximum lesion area were used. To measure lesion volume, sections were collected at the first appearance of the aortic valves until lesions were no longer visible. Oil-red O staining (Sigma–Aldrich) was performed to visualize lipid content and the lesion size was measured. To quantify lesion macrophage and smooth muscle cell content, immunohistochemistry was performed with anti-CD68 (BioLegend) and anti-Mvh H1 (Millipore) antibodies. The positive cells were visualized using the Vectastain ABC kit (Vector Laboratories) and AEC substrate (DAKO/Agilent Technologies) and the slides were counterstained with Harris haematoxylin (Sigma–Aldrich). To quantify collagen content, Masson trichrome staining (Sigma–Aldrich) was performed.

ileum CD3 cell numbers. Small intestines were dissected and cut open. After rinsing away the lumens contents in PBS, ileum was rolled from proximal to distal parts and embedded for histological sectioning. Immunohistochemistry was performed using an anti-CD3 antibody (BioLegend) and CD3+ cells were quantified.

ileum L-cell numbers. Ileum sections of wild-type and β7⁻/⁻ mice were paraffin-embedded and GLP-1 staining (Abcam) was performed to quantify GLP-1-producing L-cells in the entire ileum of each mouse. A biotinylated secondary antibody and streptavidin DyLight 594 (Vector Laboratories) were applied and nuclei were identified using DAPI (Thermo Fisher Scientific). All histological slides were scanned using a digital slide scanner NanoZoomer 2.0RS (Hamamatsu).

Statistics. Results are shown as mean ± s.e.m. Unless indicated, statistical tests included unpaired, two-tailed Student’s t-tests and nonparametric Mann–Whitney U-tests (when a Gaussian distribution was not assumed). For multiple-comparisons tests, nonparametric tests that compare the mean rank of each group (when a Gaussian distribution was not assumed) were performed. P values of 0.05 or less were considered to denote significance.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability All relevant data are included in the paper. Source Data are available for Figs. 1–4 and Extended Data Figs. 1–9 in the online version of the paper.

30. Kim, M. et al. GLP-1 receptor activation and Epac2 link atrial natriuretic peptide secretion to control of blood pressure. Nat. Med. 19, 567–575 (2013).
Extended Data Fig. 1 | Effects of integrin β7 deficiency on metabolism.

a, Metabolic cage measurements of activity in wild-type (WT) and β7−/− mice. n = 4 mice per group. b, O2 consumption and CO2 production. n = 5 wild-type mice; n = 4 β7−/− mice. c, Respiratory exchange rate (RER) by CLAMS in wild-type and β7−/− mice that were fed a chow diet. n = 5 wild-type mice; n = 4 β7−/− mice; *P < 0.05, two-tailed Mann–Whitney U-test. d, Overnight-fasted wild-type and β7−/− mice were administered [18F]FDG. The radioactivity in indicated organs was measured as the percentage injected dose per gram tissue weight (%ID/g). n = 6 wild-type mice; n = 5 β7−/− mice; *P < 0.05, Student’s t-test. e, Wild-type and β7−/− mice were housed in thermoneutral (TN) incubators for three days and then subjected to the intraperitoneal (IP) glucose-tolerance test. n = 5 mice per group; **P < 0.01, two-tailed Mann–Whitney U-test. f, Wild-type and β7−/− mice were treated with antibiotic cocktails in drinking water for 4 weeks and then subjected to the glucose-tolerance test. n = 4 mice per group; *P < 0.05, two-tailed Mann–Whitney U-test. g, Eight-week-old wild-type and β7−/− mice were cohoused at a ratio of 1:1 for 4 weeks and then subjected to the glucose-tolerance test. n = 7 mice per group; *P < 0.05, two-tailed Mann–Whitney U-test. h, Fat absorption was analysed using a fat-tolerance test in the presence of P407. n = 9 wild-type mice; n = 6 β7−/− mice. i, For assessment of permeability, mice were gavaged with FITC–dextran and fluorescence was measured in the plasma 4 h later. A wild-type mouse subjected to a dextran sulfate sodium (DSS)-induced colitis model was used as a positive control for increased gut permeability. n = 8 wild-type mice; n = 7 β7−/− mice; P = 0.17, two-tailed Mann–Whitney U-test. Data are mean ± s.e.m.
**Extended Data Fig. 2** | See next page for caption.
Extended Data Fig. 2 | Effects of integrin β7 deficiency on obesity, cholesterololemia and atherosclerosis. a, Representative flow cytometry plots gated on CD45+ non-T and B (TB) leukocytes and quantification of Ly-6C<sup>high</sup> monocytes, neutrophils and macrophages (Mφ) in iWAT of wild-type and β7<sup>−/−</sup> mice fed a HFSSD for 5 months. n = 5 wild-type mice; n = 4 β7<sup>−/−</sup> mice; *P < 0.05, two-tailed Mann–Whitney U-test. b, Representative flow cytometry plots and quantification of Ly-6C<sup>high</sup> monocytes, neutrophils and macrophages in pWAT of wild-type and β7<sup>−/−</sup> mice fed a HFSSD for 5 months. n = 5 wild-type mice; n = 4 β7<sup>−/−</sup> mice; *P < 0.05, two-tailed Mann–Whitney U-test. c, Plasma glucose levels measured in overnight-fasted animals fed a HFSSD for 5 months. n = 10 wild-type mice; n = 7 β7<sup>−/−</sup> mice; ***P < 0.001, two-tailed Mann–Whitney U-test. d, Insulin levels measured in overnight-fasted animals fed a HFSSD for 5 months. n = 9 wild-type mice; n = 8 β7<sup>−/−</sup> mice; *P < 0.05, two-tailed Mann–Whitney U-test. e, Fasting plasma total cholesterol levels of mice fed a chow diet. n = 6 mice per group. f, Body weight changes during a 14-week HCD diet of bm;β7<sup>+/+</sup>Ldlr<sup>−/−</sup> and bm;β7<sup>−/−</sup>Ldlr<sup>−/−</sup> mice. n = 5 mice per group. g, Faecal cholesterol levels in bm;β7<sup>+/+</sup>Ldlr<sup>−/−</sup> and bm;β7<sup>−/−</sup>Ldlr<sup>−/−</sup> mice after a 14-week HCD diet. n = 5 mice per group; P = 0.09, two-tailed unpaired Student’s t-test. h–j, Representative images and histological quantification of macrophages (h), collagen content and necrotic core size (i) and smooth muscle cell content (j) of bm;β7<sup>+/+</sup>Ldlr<sup>−/−</sup> and bm;β7<sup>−/−</sup>Ldlr<sup>−/−</sup> mice after 14 weeks on a HCD. n = 5 mice per group; **P < 0.01, ***P < 0.001, two-tailed unpaired Student’s t-test. Data are mean ± s.e.m.
Extended Data Fig. 3 | Effects of integrin β7 deficiency on myeloid cells and glucose tolerance. a, Ldlr−− mice were lethally irradiated and reconstituted with bone marrow mixtures of wild-type and β7−− mice (1:1) and fed a chow diet or HCD for 14 weeks. b, The aortic leukocytes from different origins were analysed by flow cytometry. n = 4 mice for both HCD recipients and chow recipients. c, Ly-6C^hi and Ly-6C^lo monocyte numbers in blood (n = 3 wild-type mice; n = 5 β7−− mice), bone marrow (n = 3 wild-type mice; n = 5 β7−− mice) and spleen (n = 6 mice per group) of wild-type and β7−− mice fed a chow diet. d, bmβ7−−Ldlr−− and bmWT Ldlr−− mice fed a HCD were subjected to an intraperitoneal glucose-tolerance test. n = 5 mice per group; *P < 0.05, two-tailed Mann–Whitney U-test. Data are mean ± s.e.m.
Extended Data Fig. 4  | Effects of genetic deficiency and blocking of integrin β7 on atherosclerosis. 

**a**, Body weights, cumulative food intake and energy expenditure were measured in Ldlr−/− mice and β7−/−Ldlr−/− mice. n = 4 mice per group. 

**b**, Ldlr−/− mice and β7−/−Ldlr−/− mice were fed a HCD for 14 weeks. Plasma cholesterol levels were determined in overnight-fasted mice. n = 7 Ldlr−/− mice; n = 5 β7−/−Ldlr−/− mice; **P < 0.01, two-tailed Mann–Whitney U-test.**

**c**, Representative oil-red O images and quantification of plaque size in the aortic roots. n = 7 Ldlr−/− mice; n = 5 β7−/−Ldlr−/− mice; *P < 0.05, two-tailed Mann–Whitney U-test.

**d**, Quantification of Ly-6Chigh monocytes, neutrophils and macrophages in plaques. n = 7 mice per group. *P < 0.05. **P < 0.01, two-tailed Mann–Whitney U-test.**

**e**, Ldlr−/− mice that were fed a HCD were treated with anti-integrin β7 antibodies or IgG isotype control (500 μg per mouse per week) for 14 weeks. Mice were subjected to a glucose-tolerance test after 8 weeks on the HCD. n = 6 mice per group; **P < 0.01, two-tailed Mann–Whitney U-test.**

**f**, Representative images of oil-red O-stained aortic cross-sections and quantification of plaque size in the aortic roots after 14 weeks on a HCD. n = 5 mice treated with IgG; n = 6 mice treated with anti-integrin β7 antibody; *P < 0.05. **P < 0.01, two-tailed Mann–Whitney U-test.** Data are mean ± s.e.m.
Extended Data Fig. 5 | Integrin \( \beta 7 \) guides leukocytes to gut intraepithelium. a, Top, representative histology staining for CD3 in small intestines of wild-type and \( \beta 7^{-/-} \) mice. Bottom, quantification of CD3\(^+\) cells in each villus (more than 15 villi were counted for each mouse). ***\( P < 0.001 \), two-way ANOVA. b, Schematic of the competitive transfer experiments. Mice (CD45.2\(^+\)) were lethally irradiated and transplanted with a 1:1 ratio mix of GFP\(^+\)\( \beta 7^{-/-} \) bone marrow cells and CD45.1\(^+\) wild-type bone marrow cells. The chimerism in different tissues was normalized to the ratio in blood. \( n = 5 \) mice. c, Representative flow cytometry plots and quantification of B cells and myeloid cells in mice depicted in b. \( n = 5 \) biologically independent recipients. d, Quantification of \( \gamma \delta \)T cells in the liver. \( n = 3 \) wild-type mice; \( n = 4 \) \( \beta 7^{-/-} \) mice. WBC, white blood cells. e, Quantification of \( \gamma \delta \)T cells in the pancreas. \( n = 4 \) wild-type mice; \( n = 5 \) \( \beta 7^{-/-} \) mice. f, Wild-type mice were lethally irradiated and reconstituted with bone marrow cell mixtures of \( \beta 7^{-/-} \) and wild-type (\( \beta 7^{-/-}: \) wild-type cells, 1:1 ratio) or \( \beta 7^{-/-} \) and indicated knockout mice (\( \beta 7^{-/-}: \) knockout cells, 1:1 ratio). The indicated mixed chimaeras that specifically lack intestinal B cells (\( \beta 7^{-/-}: \)μMT) or myeloid cells (\( \beta 7^{-/-}: \)Ccr2\(^{-/-} \)) were subjected to oral glucose-tolerance tests and the AUCs are shown. \( n = 5 \) mice per group for \( \beta 7^{-/-} \) wild-type and \( \beta 7^{-/-}: \)μMT mice; \( n = 4 \) mice per group for \( \beta 7^{-/-} \) wild-type and \( \beta 7^{-/-}: \)Ccr2\(^{-/-} \) mice. Data are mean ± s.e.m.
Extended Data Fig. 6 | B cells are dispensable for the altered metabolic phenotypes in integrin β7-deficient mice. Ldlr−/− mice were lethally irradiated and reconstituted with bone marrow cell mixtures of β7−/− and wild-type (β7−/− WT, 1:1 ratio) or β7−/− and μMT (β7−/− μMT, 1:1 ratio). The reconstituted mixed chimaeras were fed a HCD for 14 weeks. a, IgA levels in the gut flush (n = 5 β7−/− wild-type mice; n = 4 β7−/− μMT mice) and plasma (n = 5 β7−/− wild-type mice; n = 3 β7−/− μMT mice). **P < 0.01, two-tailed Mann–Whitney U-test. b, Number of IgD+ B cells in Peyer’s patches (PP) and IgA+ B cells and IgD+ B cells in lamina propria (LP) as determined by flow cytometry. n = 5 β7−/− wild-type mice; n = 3 β7−/− μMT mice; *P < 0.05, two-tailed Mann–Whitney U-test. c, Glucose-tolerance test in HCD-fed mixed chimaeras. n = 5 β7−/− wild-type mice; n = 3 β7−/− μMT mice. d, Plasma cholesterol levels in overnight-fasted mice. n = 5 β7−/− wild-type mice; n = 4 β7−/− μMT mice. e, Representative images and quantification of oil-red O staining in aorta root sections of bmβ7−/− wild-type Ldlr−/− mice and bmβ7−/− μMT Ldlr−/− mice that were fed a HCD for 14 weeks. n = 5 bmβ7−/− wild-type Ldlr−/− mice; n = 4 bmβ7−/− μMT Ldlr−/− mice. Data are mean ± s.e.m.
Extended Data Fig. 7 | Integrin β7 deficiency and GLP-1. **a,** Plasma total GLP-1 levels after overnight fasting and 15 min after oral glucose load (2 g per kg body weight) in wild-type and β7−/− mice that were fed a chow diet. Total GLP-1 fasting: *n* = 7 mice per group; total GLP-1 after oral glucose-tolerance test (OGTT) 15 min: *n* = 7 wild-type mice; *n* = 6 β7−/− mice.

**b,** Plasma total GLP-1 levels after 5 months of a HFSSD. Total GLP-1 fasting: *n* = 7 mice per group; total GLP-1 oral glucose-tolerance test 15 min: 6 mice per group.

**c,** Representative flow cytometry plots of small-intestinal IELs from wild-type and β7−/− mice. *d,* Glp1r mRNA levels in sorted different IEL subsets from wild-type and β7−/− mice. *n* = 4 wild-type mice; *n* = 5 β7−/− mice. **e,** Wild-type mice were lethally irradiated and transplanted with a 1:1 bone marrow mixture of wild-type and GFP+ or Glp1r−/− and GFP+. The chimerism in different tissues was analysed by comparing the percentage of GFP+ leukocytes normalized to wild-type GFP+ blood leukocytes. *n* = 4 mice per group. Data are mean ± s.e.m. *P* < 0.05, **P** < 0.001. All *P* values from two-tailed unpaired Student’s *t*-tests.
Extended Data Fig. 8 | Effects of Glp1r deficiency on IELs and atherosclerosis. a, Quantification of small-intestinal IEL subpopulations in bmGlp1r+/+β7−/−Ldlr−/− and bmGlp1r−/−β7−/−Ldlr−/− mice. n = 5 mice per group, mean ± s.e.m. b, Glp1r mRNA expression of sorted IEL subpopulations. n = 4 biologically independent bmGlp1r+/+β7−/− and n = 5 biologically independent bmGlp1r−/−β7−/− mice; two-tailed unpaired Student's t-test. c, Glp1r mRNA expression in the liver (n = 5 bmGlp1r+/+β7−/− and n = 4 bmGlp1r−/−β7−/− mice), heart and lung tissue (n = 5 mice per group). d, Quantification of γδ T cells from the liver (n = 5 mice per group) and pancreas (n = 5 bmGlp1r+/+β7−/−Ldlr−/− mice; n = 4 bmGlp1r−/−β7−/−Ldlr−/− mice). e, Glp1r mRNA expression of sorted γδ T cells from pancreas, liver and small-intestinal IELs. n = 3 mice per group; two-tailed unpaired Student's t-test. f, Oral glucose-tolerance test. n = 4 bmGlp1r+/+ mice; n = 3 bmGlp1r−/− mice. g, Oral glucose-tolerance test in bmGlp1r+/+β7−/− and bmGlp1r−/−β7−/− mice. n = 5 mice per group; two-tailed Mann–Whitney U-test. h, GLP-1 levels after overnight fasting or oral glucose challenge in bmGlp1r+/+β7−/− and bmGlp1r−/−β7−/− mice. n = 5 mice per group; two-tailed unpaired Student's t-test. i, GLP-1 levels after overnight fasting or oral glucose challenge in bmGlp1r+/+β7−/− and bmGlp1r−/−β7−/− mice. n = 5 mice per group; two-tailed Mann–Whitney U-test. j, Ldlr−/− mice were treated with the GLP-1 receptor agonist exendin-4 (Ex-4) at a dose of 100 μg per kg per day via osmotic minipumps (PBS was used as control). After 8 weeks on a HCD, mice were euthanized to enable the quantification of atherosclerotic lesions. Representative images of oil-red O-stained aortas and quantification of plaque size. k, Quantification of blood Ly-6C+ monocytes, Ly-6C− monocytes and neutrophils. n = 8 Ldlr−/− mice treated with exendin-4; n = 6 Ldlr−/− mice treated with PBS; two-tailed unpaired Student's t-test. Data are mean ± s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001.
Extended Data Fig. 9 | Gut intraepithelial Glp1r<sup>high</sup> IELs regulate the bioavailability of GLP-1. **a**, Immunohistochemistry and quantification of GLP-1-producing L-cells in whole ileum preparations of 6 wild-type and 5 β<sup>7</sup>−/− mice. **b**, Small-intestinal IEL mixtures were incubated with the fluorescently (Cys40SeTau647) labelled GLP-1R agonist exendin-4 and the capacity of agonist binding by the different subsets—natural IELs (Glp1r<sup>high</sup>), induced IELs (Glp1r<sup>low</sup>) and non-T cells—was analysed by flow cytometry. Sorted Glp1r<sup>high</sup> and Glp1r<sup>low</sup> cells were also incubated with recombinant GLP-1 and the remaining supernatant GLP-1 was plotted against the relative Glp1r mRNA levels of the cells. **c**, GLP-1-producing GLUTag cells were co-cultured with sorted natural (Glp1r<sup>high</sup>) or induced (Glp1r<sup>low</sup>) IELs. After 24 h, the concentration of GLP-1 in the supernatant was measured. **d**, Left, GLUTag cells were co-cultured with sorted Glp1r<sup>high</sup> IELs in the presence of exendin-4 (100 nM) or control. n = 3 independent biologically samples per group. Right, GLUTag cells were stimulated with exendin-4 (100 nM) or control. n = 4 independent biological samples per group. After 24 h, the concentration of GLP-1 in the supernatant was measured. **e**, Sorted Glp1r<sup>high</sup> IELs were incubated with exendin-4 (100 nM) or control. After 24 h, samples were centrifuged and supernatants were transferred to ex vivo ileum fractions of wild-type mice. GLP-1 levels were determined 24 h later from ex vivo supernatants. n = 10 biologically independent mice per group. **f**, Whole gut preparations of wild-type or β<sup>7</sup>−/− mice were treated with or without the GLP-1 receptor antagonist exendin-9 (100 nM). After 24 h, the concentration of GLP-1 in the supernatant was measured. n = 5 biologically independent samples for wild-type or β<sup>7</sup>−/− mice without exendin-9; n = 4 biologically independent samples for wild-type mice with exendin-9. Data are mean ± s.e.m. *P < 0.05, **P < 0.01. All P values from two-tailed unpaired Student’s t-test.

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In this study, we propose that integrin β7-dependent Glp1r^high IELs that reside in the small intestine modulate dietary metabolism in mice by restricting the bioavailability of GLP-1.

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**Extended Data Fig. 10 | Model.** In this study, we propose that integrin β7-dependent Glp1r^high IELs that reside in the small intestine modulate dietary metabolism in mice by restricting the bioavailability of GLP-1.
Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
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- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
- Clearly defined error bars
- State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

| Data collection | BD FACSDiVa™ software was used to collect data from flow cytometry; NPDview2 software, NanoZoomer 2.0RS software and NIH ImageJ program were used to collect immunohistochemistry data; Columbus CLAX 2.2.10 was used to collect data for metabolic measurements; Thermo Scientific™ Amira™ Software 5.3.2 was used for 18-FDG-PET/CT imaging. |
| Data analysis | Flow cytometric analyses were performed with the FlowJo analysis software (FlowJo 8.7.2); Statistical analyses were performed with the GraphPad Prism 7.0 software (GraphPad) |

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Data

Policy information about availability of data
All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
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- A list of figures that have associated raw data
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The authors declare that the data supporting the findings of this study are available within the paper [and its supplementary information files].

Field-specific reporting

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Life sciences
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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size calculations were carried out using group percentages (controls expressing 90% expected value) and variables expressing 20-30% change, which was deemed to be detectable changes using our current imaging and ex vivo analytical methods. Confidence levels were set at 5% with Beta levels established as 50%.

Data exclusions
No data was excluded from the analysis

Replication
All attempts at replication were successful. Findings were replicated in at least three biologically independent samples each.

Randomization
Allocation of animals (mice) into different experimental groups was done in a random manner

Blinding
Investigators were blinded to group allocation during data collection and analysis

Reporting for specific materials, systems and methods

Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☑️ | Unique biological materials |
| ☑️ | Antibodies |
| ☑️ | Eukaryotic cell lines |
| ☑️ | Palaeontology |
| ☑️ | Animals and other organisms |
| ☑️ | Human research participants |

Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☑️ | ChIP-seq |
| ☑️ | Flow cytometry |
| ☑️ | MRI-based neuroimaging |

Antibodies

| Antibodies used |
|----------------|
| anti-integrin \(\beta7\) (BioLegend, clone F1B27, Cat\#121008, Lot\#B238485), anti-CD45 (BioLegend, Clone30-F11, Cat\#103147, Lot\#B243834), anti-CD45.1 (BioLegend, clone A20, Cat\#110708), anti-CD45.2 (BioLegend, clone 104, Cat\#109802), anti-CD3 (BioLegend, clone 17A2, Cat\#100206), anti-CD90.2 (BioLegend, clone 30-H12, Cat\#105308, Lot\#B237375), anti-CD19 (BioLegend, clone 6D5, Cat\#115508, Lot\#B226581), anti-CD3 (BioLegend, clone PK136, Cat\#108708), anti-Ly-6G (BioLegend, clone RA3-6B2, Cat\#553089, Lot\#B217859), anti-CD11b (BioLegend, clone 1A8, Cat\#1257342, Lot\#B238485), anti-CD5 (BioLegend, clone M1/70, Cat\#101226, Lot\#B238268), anti-CD5 (BioLegend, clone 53-7.3, Cat\#100618, Lot\#B208122), anti-CD115 (BioLegend, clone G13, Cat\#118116, Lot\#B228498), anti-CD326 (BioLegend, clone G8.8, Cat\#118214), anti-i\(\gamma\)A (BD Biosciences, clone C10-3, Cat\#559354, Lot\#5089919), anti-i\(\delta\)G (BioLegend, clone 11-26c.2a, Cat\#05725, Lot\#B219485), anti-CD11a (BioLegend, clone AF598, Cat\#135517, Lot\#B265220), anti-CX3CR1 (BioLegend, clone SAD11F11, Cat\#149027, Lot\#B247422), anti-CD90.2 (BD Pharmingen, clone 53-2.1, Cat\#561642, Lot\#8145588), anti-CD5 (BD Horizon, clone 53-7.3, Cat\#149027, Lot\#B247422) |
Validation

These antibodies are all used for flow cytometry for experiments with mice. Antibody validations were performed by antibody suppliers per quality assurance literature provided by each supplier.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s) GLUTag cells were kindly provided by Dr. Daniel J. Drucker (Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Joseph & Wolf Lebovic Health Complex, Toronto, ON, Canada.

Authentication The GLUTag cell line was isolated from a glucagon-producing enteroendocrine cell tumor that arose in glucagon gene-SV40 T antigen transgenic mice. The cell line was generated, first described and authenticated by Dr. Daniel J. Drucker (Drucker et al. Mol Endocrinol. 1994; Brubaker et al. Endocrinology. 1998). The GLUTag cell line has been repeatedly authenticated by many different international laboratories by validating secretion of enteroendocrine hormones (i.e. GLP-1, GLP-2) and the typical gene expression profile. The respective references can be found at http://glucagon.com/glutagcells.html.

Mycoplasma contamination The GLUTag cell line has been tested negative for mycoplasma contamination

Commonly misidentified lines (See ICLAC register) GLUTag cells are not listed in the database of commonly misidentified cell lines

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals C57BL/6J (wild type, WT), Itgb7tm1Cgn (β7–/–), Ldlrtm1Her (Ldlr–/–), Tcrbtm1Mom (βTCR–/–), Tcrdtm1Mom (γδ TCR–/–), Ccr2tm1Ifc (Ccr2–/–), Ighmtm1Cgn (μMt), Ccr9tm1Lov (Ccr9–/–), Itgaetm1Cmp (itgae–/–) mice either male or female were purchased from The Jackson Laboratory (Bar Harbor, ME, USA). Glp1r–/– male mice in the C57BL/6 background were bred in-house as described. Unless otherwise indicated age- and sex-matched animals were used at 8–12 weeks of age. Where appropriate, animals were randomly assigned to interventions.

Wild animals This study did not involve wild animals

Field-collected samples This study did not involve samples collected from the field

Flow Cytometry

Plots

Confirm that:

Box The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

Box The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).

Box All plots are contour plots with outliers or pseudocolor plots.

Box A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation Peripheral blood was collected by retro-orbital bleeding and red blood cells were lysed in RBC lysis buffer (BioLegend, San Diego, CA). Aortas were excised after PBS (Thermo Fisher Scientific, MA) perfusion, minced and digested with 450 U/ml collagenase I, 125 U/ml collagenase XI, 60 U/ml DNase I and 60 U/ml hyaluronidase (Sigma-Aldrich, St. Louis, MO) in PBS for 40 minutes at 37°C. Total viable cell numbers were counted using trypsin blue (Cellgro, Mediatech, Inc. VA). Small intestine intraepithelial leukocytes (IEL) were isolated as follows: After excision of the small intestine, the Peyer’s patches were removed under microscope and the gut was cut open longitudinally to wash off the lumen contents in HBSS buffer. The gut was then cut into 1-2 cm pieces and subjected to 3x dissociation in EDTA-containing buffer (7.5 mM HEPES, 2% FCS, 2 mM EDTA, 10.000 U/ml Penicillin-Streptomycin, 50 μg/ml Gentamycin in HBSS; all Thermo Fisher Scientific, MA) in a shaker at 37°C for 15 minutes. After dissociation the IELs were collected by filtering lamina propria through a mesh. For sorting, the IEL flow-through after dissociation was further subjected to Percoll (GE Healthcare Bio-Sciences, MA) gradient centrifugation to remove the mucus. Single cell suspension of IELs from indicated animals were then stained to identify indicated cell populations.

Instrument FACS LSRII, FACS Aria II

Software DIVA, FlowJo

Cell population abundance After sorting, a small fraction of the sorted cells were run through Aria II and the same gating strategy was applied to check the
Cell population abundance: A general purity of higher than 95% were achieved for all the sorted population.

Gating strategy:

For Fig S2a,b, the gating strategy is: FSC-A/SSC-A (cells) --- FSC-A/FSC-H (singlets) --- CD45-BV711/SSC-A (leukocytes) --- CD45-BV711/Zombie-BV510 (live leukocytes) --- CD19-BV605/CD3-FITC (Non TB leukocytes). For Fig 3, Fig S3a,b,c, S4, S8k and Fig 4, the gating strategy is: FSC-A/SSC-A (cells) --- FSC-A/FSC-H (singlets) --- CD45-BV711/SSC-A (leukocytes) --- CD45-BV711/Zombie-BV510 (live leukocytes) --- Lin-PE/CD11b-APCCy7 (myeloid cells) --- Ly6C-BV605 or FITC/F4/80-PECy7 (Ly-6Chi monocytes, Ly-6Clo monocytes, neutrophils, macrophages). For Fig S5, the gating strategy is: FSC-A/SSC-A (cells) --- FSC-A/FSC-H (singlets) --- CD45-BV711/SSC-A (leukocytes) --- CD45-BV711/Zombie-BV510 (live leukocytes). For Fig S6, the gating strategy is: FSC-A/SSC-A (cells) --- FSC-A/FSC-H (singlets) --- CD45-BV711/SSC-A (leukocytes) --- CD45-BV711/Zombie-BV510 (live leukocytes) --- CD3-PE/CD19-APCCy7 (B cells) --- CD3-PE/IgD-BV421 or IgA-FITC (IgD+ or IgA+ B cells). For Fig S7c, S8 a,d, the gating strategy is: FSC-A/SSC-A (cells) --- FSC-A/FSC-H (singlets) --- CD45-BV711/SSC-A (leukocytes) --- CD45-BV711/Zombie-BV510 (live leukocytes) --- CD3-PE/CD19-APCCy7 --- bTCR-FITC/gdTCR-APC (abT, gdT cells) --- CD90.2-PECy7/CD5-PerCPcy5.5 or BV605 (natural abT or nabT, induced abT or iabT).

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.