Aus der Diabetes Research Group, Abteilung Biomedizin

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betreut durch Dr. Daniel Zeman-Meier

Metabolic Consequences of Adipocyte-specific Deletion of the IL-1 Receptor in Mice

Inauguraldissertation zur Erlangung der
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Fakultät der Universität Basel

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Summary

With the increasing abundance of food and the deleterious consequences of overnutrition, metabolic stress may act on the immune system and trigger local and systemic inflammation. My host laboratory previously found that adipose tissue macrophages are the first immune cells responding to feeding after fasting by upregulating the interleukin-1 (IL-1) pathway. Therefore, we generated mice that lack the receptor for IL-1specifically in adipocytes (*Il1r1*$^{fl/fl}$ *Adipoq*-Cre$^{tg/0}$) and assessed metabolic consequences.

Despite unaltered body weight development under regular chow- and high-fat diet (HFD) feeding, we found *Il1r1*$^{fl/fl}$ *Adipoq*-Cre$^{tg/0}$ mice on chow diet to have metabolic changes indicative of pre-diabetes at 1 year of age. With HFD feeding, glucose intolerance was already apparent after 10 weeks of HFD feeding. Further, we found the gene encoding for IL-6 to be lower in adipocytes of knock out mice fed chow diet. Fluorescence-activated cell sorting (FACS) analysis revealed that the immune cell composition of the stromal vascular fraction of *Il1r1*$^{fl/fl}$ *Adipoq*-Cre$^{tg/0}$ mice are comparable to controls. Further, *Il1r1*$^{fl/fl}$ *Adipoq*-Cre$^{tg/0}$ mice pre-injected with IL-1β in a glucose tolerance test showed a blunted insulin response compared to control mice, suggesting that a part of the insulin secretagogue action of IL-1β signals via adipocytes. Interestingly, *Il1r1*$^{fl/fl}$ *Adipoq*-Cre$^{tg/0}$ mice on HFD showed an increased pancreas weight, which might point to a novel crosstalk of IL-1 signaling in fat tissue and the pancreas. Overall, our adipocyte-specific *Il1r1* knock out mice show similar body weight development as control mice but show signs of impaired glucose metabolism when aging or fed a HFD.

Contents

Introduction

The link between metabolism and inflammation has been observed for more than 100 years, with the use of high doses of NSAIDs (Non-Steroid Anti-Inflammatory Drugs) observed to reduce diabetes in patients with presumed type 2 diabetes[1]. The function of metabolism is to convert food to energy for cellular processes and store nutrition, while the function of the immune system is a host defense system that protects our body against disease. Interestingly, there is evidence of a crosstalk between metabolism and inflammation. This crosstalk is considered as a necessary response to cope with stressors from our body to restore homeostasis[2,3]. Indeed, pathogens and metabolic changes are regarded as two different type of stress to the organism. For metabolism, with the current excess of food intake, obesity and overnutrition can be considered a burden to the human body. Similar to the body's immune response to pathogens, excess nutrition can also trigger an inflammatory response [4]. Due to prolonged overnutrition, this activation of the immune system may become harmful. This crosstalk between immunity and metabolism has generated a new research field called "immunometabolism"[5].

My host laboratory has already identified a pathologic role of chronic inflammation in metabolism[6]. We know that IL-1β and the IL-1-dependent IL-6 have an important role in insulin secretion and glucose disposal[7,8]. HFD stimulates a systemic low-grade upregulation of the pro-inflammatory IL-1β. Acutely, postprandial hyperglycemia also induces IL-1β secretion from macrophages, which stimulates insulin secretion[8,9].

In a preliminary rodent study from my host laboratory (Sophia Wiedemann, personal communication), acute feeding activated the IL-1 system specifically in adipose tissue macrophages but not in resident immune macrophages from other organs (Fig 1). Pathway analysis of differentially regulated genes revealed that adipose tissue macrophages show a distinct upregulation of a pro-inflammatory gene cluster following acute feeding (Fig 2). In

mice, activation of pro-inflammatory pathways has a physiological role and is required for adipose tissue expansion and remodeling[10]. Further, human preadipocytes and adipocytes express the IL-1 receptor 1 (*Il1r1*), and IL-1β prominently regulates metabolic genes in adipocyte cultures[11]. These observations point to a role for the IL-1 system not only in the regulation of immune cells in fat tissue but also to a prominent role in the regulation of adipocyte biology. Therefore, we hypothesize that IL-1β signaling in adipocytes may be the first response to nutrition and may relay inflammation to other organs of the body, triggering a pro-inflammatory cascade. To test this hypothesis we made adipocyte-specific IL-1 receptor knock out mice and evaluated their metabolic phenotype.

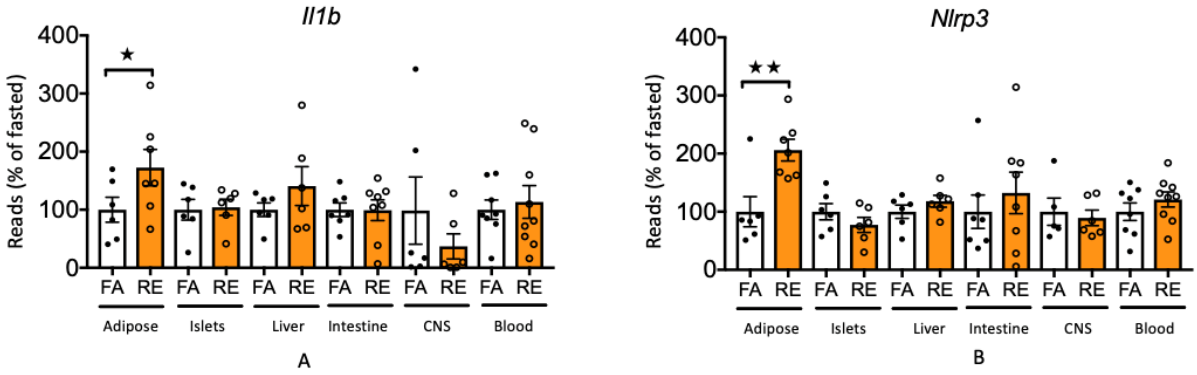


Fig. 1. Refeeding increases IL-1β signaling specifically in fat-derived macrophages. RNA expression levels of (A) *Il1b* and (B) *Nlrp3* following continous fasting (FA) or fasting followed by 2h feeding (RE) in FACS-sorted macrophages derived from various organs, analyzed using bulk RNA sequencing. Central nervous system (cns).

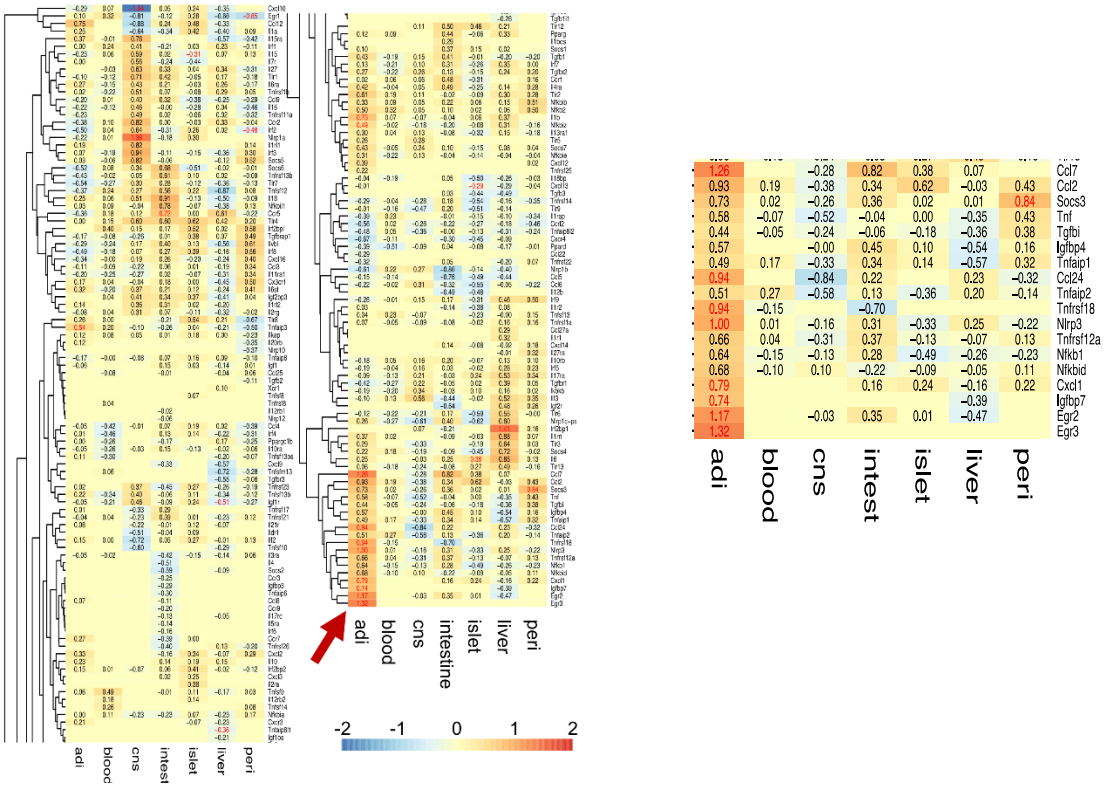


Fig. 2. Heat map of changes in genes from macrophages of adipose tissue (adi), blood, central nervous system (cns), intestine, islet, liver and the peritoneum (peri) following 2h refeeding. Note a cluster of highly upregulated pro-inflammatory genes in adipocytes (red arrow).

Materials and Method

Animal experiments

All animal experiments were performed in mice on a C57BL/6J background. B6.129(Cg)-Il1r1$^{tm1.1Rbl}$/J (Jax stock No:028398, *Il1r1*$^{fl/fl}$) mice[12] were obtained from Jackson laboratories (Ellsworth, USA). B6.-Tg (*Adipoq*-Cre)1Evdr/J (Jax stock No:010803, *Adipoq*-Cre)[13] were provided by Stephan Wüest (University of Zürich). These two mouse strains were intercrossed to generate adipocyte-specific deletion of IL-1 receptor 1 (*Il1r1*$^{fl/fl}$ *Adipoq*-Cre$^{tg/0}$). As control mice, we used Cre-recombinase-negative littermates (*Il1r1*$^{fl/fl}$). Only male mice were used for experiments. All animal experiments were conducted according to the Swiss Veterinary Law and Institutional Guidelines and were approved by the cantonal authorities. All animals were housed in a temperature-controlled room with a 12 h light–12 h dark cycle and had free access to food and water. For the high-fat diet experiments, 10-week-old mice were fed a HFD for 10–40 weeks (D12492, Ssniff, Soest, Germany; containing 60 %, 20 % and 20 % calories from fat (lard & soybean oil), carbohydrate, and protein, respectively). Experimental mice were between 12 and 54 weeks of age. Body weights were measured once per week starting at 6 weeks of age.

Genotyping

Toe biopsies were incubated in 200 ul 50 mM NaOH for 1 hour at 98 °C and the extract was subsequently neutralized by adding 50 ul Tris (1 M, pH 8) and spun down for 3 mins at 4000 rpm. Then 1.25 ul were used for polymerase chain reaction (PCR).

The primer sequences are as follows:

Cre Forward: GCA CTG ATT TCG ACC AGG TT, *Cre* Reverse: CCC GGC AAA ACA, *Actb* Forward: TGT TAC CAA CTG GGA CGA CA, *Actb* Reverse: GAC ATG CAA GGA

GTG CAA GA, *Il1r* flox Forward GAA AAG TGC TAG AAC ATC CTT TGA G, *Il1r* flox Reverse: GTA CCA ATG GAG GCC AGA AG.

PCR Reaction Mix (Cre-Actin)

	(uL)
5x Green reaction buffer	2.5
PCR nucleotide mix	0.25
Cre fwd (10 uM)	0.375
Cre rev (10 uM)	0.375
Actb fwd (10 uM)	0.75
Actb rev (10 uM)	0.75
GoTaq G2 polymerase	0.0625
Nuclease-free water	7.4375
Template DNA	1.25
Total	13.75

PCR Program (Cre-Actin)

	Time	Temp.°C	
Initial hot start	3'	94°C	
Denaturation	30"	94°C	**35 cycles**
Annealing	30"	60°C	
Elongation	1'	72°C	
Final elongation	10'	72°C	
Hold	Pause	10°C	

PCR Reaction Mix (Il1r1 flox)

	(uL)
5x Green reaction buffer	2.5
PCR nucleotide mix	0.25
Il1r1 flox fwd (10 uM)	0.5
Il1r1 flox rev (10 uM)	0.5
GoTaq G2 polymerase	0.0625
nuclease-free water	7.4375
template DNA	1.25
Total	12.5

PCR Program (Il1r1 flox)

	Time	Temp.°C	
Initial hot start	2'	94°C	
Denaturation	20"	94°C	**10 cycles**
Annealing	15"	65°C	
Elongation	10"	68°C	
Denaturation	15"	94°C	**28 cycles**
Annealing	15"	60°C	
Elongation	10"	72°C	
Final elongation	2'	72°C	
Hold	Pause	10°C	

The primers above were all purchased from Microsynth (Balgach, Switzerland). Following PCR, 10 ul of the reaction products were separated on a 1.5 % agarose gel (V3125 Analytical Grade, Madison, USA). The gel was imaged on a FUSION FX6 (Witec AG, Switzerland).

Glucose tolerance testing

For glucose tolerance testing (GTT), mice were fasted for 6 hours starting in the morning (8:00). All mice received an intraperitoneal (i.p.) injection of 2 g glucose per kg of body weight. Blood glucose was measured twice per time point prior to, and 15, 30, 60, 90 and 120 mins after glucose application, each with a drop of blood from the tail vein, using FreeStyle Lite glucose meters (Abbott AG, Baar, Switzerland). At the first three-time points, additional 25 ul of blood were collected from the tail vein into tubes containing EDTA for later measurement of plasma insulin with the MSD Mouse/Rat Insulin Kit (K152BZC-3, Meso Scale Discovery, Rockville MD, USA) according to the manufacturer's instructions.

Insulin tolerance testing

For insulin tolerance testing (ITT), mice were fasted for 4 hours starting in the morning (8:00). Fasted mice received an i.p. injection of 1 U per kg of body weight human insulin (Actrapid HM Penfill, Novo Nordisk Pharma, Switzerland). Blood glucose was measured twice per time point prior to, and 15, 30, 60 and 90 mins after insulin application, each with a drop of blood from the tail vein, using FreeStyle Lite glucose meters.

Isolation of adipocyte and stromal vascular fraction

Mice were euthanized using CO_2 and gonadal adipose tissue was excised. All tissues were minced and transferred to a 50 ml tube (Sarstedt, Nümbrecht, Germany) with 4 ml HBSS (#24020091, ThermoFisher, Massachusetts, USA). 4 ml 2x digestion mix (4 ml HBSS, 0.04 ml 1 M HEPES, 6 mg collagenase IV (LS004189, Worthington Cylinders, USA), 1.32 ul

DNAse I was added and then the mix was digested at 37 °C on a ThermoMixer (#5382000015, Eppendorf, Lustadt, Germany) shaking at 400 rpm for 20-30 mins. 20 ml STOP buffer (445 ml dH_20, 50 ml 10xD-PBS, 2.5 g BSA, 5 ml 0.5M EDTA) was added to stop the digestion and the solution was filtered through medical gaze into a new tube. Following centrifugation at 300 g for 2 mins, the tube was incubated at room temperature for 5 mins which floats the adipocytes on top of the liquid. For stromal vascular fraction, the infranatant was collected by poking through the layer of adipocytes using a syringe and the infranatant was transferred to the new tube. The adipocytes were then resuspended in 20 ml STOP buffer, vortexed and then spun down 5 mins at 450 g and the infranatant was again collected as described above. This washing procedure was repeated a third time using 10 ml STOP buffer.

RNA extraction and quantitative PCR (qPCR)

Pieces of liver, muscle, stromal vascular fraction, hypothalamus, and pituitary gland (20–30 ug) were homogenized with 5 mm stainless steel beads (69989; Qiagen, Maryland, USA) in a Tissue Lyser (85300; Qiagen, Maryland, USA) in 350 ul lysis buffer of the RNA extraction kit and total RNA was isolated using the NucleoSpin RNA II Kit (#740955 Macherey Nagel, Düren, Germany) according to the manufacturer's instructions.

For adipose tissue, cells were incubated in 500 ul NucleoZOL buffer of the RNA extraction kit and total RNA was isolated using the NucleoSpin RNA Set for NucleoZOL (#740406.50 Macherey Nagel, Düren, Germany) according to the manufacturer's instructions.

Complementary deoxyribonucleic acid was prepared using the GoScript™ Reverse Transcriptase (A5003, Promega, Catalys, Switzerland). For Sybr Green qPCR we used GoTaq qPCR Master Mix (A6002, Promega, Catalys, Switzerland) with Applied Biosystems™ 7500 Fast Real-Time PCR System (ThermoFisher, Massachusetts, USA). We used a 96well plate setup and the program as follows:

	Time	Temp.°C	
Holding stage	20"	95°C	
Denaturation	3"	100°C	**40 cycles**
Annealing	30"	60°C	
Hold	Pause	10°C	

Relative change in gene expression was normalized to the endogenous reference gene (*18 S* rRNA). The primer pairs used to quantify the expression of different genes are presented in Table 1.

Gene	Forward sequence	Reverse sequence
18S	GGGAGCCTGAGAAACGGC	GGGTCGGGAGTGGGTAATTT
Il1r1	GCACGCCCAGGAGAATATGA	AGAGGACACTTGCGAATATCAA
Tnf	ACTGAACTTCGGGGTGATCG	TGAGGGTCTGGGCCATAGAA
Il6	GCCTTCTTGGGACTGATGCT	TGCCATTGCACAACTCTTTTC
Adgre1	GCCCAGGAGTGGAATGTCAA	CAGACACTCATCAACATCTGCG
Adipoq	TGACGACACCAAAAGGGCTC	CACAAGTTCCCTTGGGTGGA
Slc2A4	GCTCTGACGATGGGGAACC	TCAATCACCTTCTGTGGGGC
Irs1	TTAGGCAGCAATGAGGGCAA	TCTTCATTCTGCTGTGATGTCCA
Pparg	GCCTATGAGCACTTCACAAGAAAT	GGAATGCGAGTGGTCTTCCA
Il1b	GCAACTGTTCCTGAACTCAACT	ATCTTTTGGGGTCCGTCAACT

Table 1. Primer pairs used for Sybr green real-time quantitiative PCR

GTT with IL-1β preinjection

For assessing glucose tolerance after IL-1β pre-injection, mice were fasted for 6 hours starting in the morning (8:00). Fasted mice received an i.p. injection of 1ug per kg of body weight recombinant IL-1β (R&D Systems) in saline or saline alone and 20 mins later an i.p. injection of 2 g glucose per kg of body weight or saline alone. Blood glucose was measured twice per time point prior to IL-1β and glucose injection, as well as 15, 30, 60, 90 and 120 mins after

glucose application, each with a drop of blood from the tail vein, using Free Style Lite glucose meters. At the first 4 time points, additional blood samples were collected from the tail vein into tubes containing EDTA for later measurements of plasma insulin with the MSD Mouse/Rat Insulin Kit according to manufacturer's instructions.

Fluorescence-activated cell sorting (FACS)

All the isolated cells from stromal vascular fractions (SVF) were kept in 300 ul FACS buffer (445 ml dH_20, 50 ml 10xD-PBS, 2.5 g BSA, 5 ml 0.5 M EDTA). 200 ul of every sample were added into each well of a 96-well plate (92096, TPP, AG, Switzerland) and plates were spun down at 1500 rpm for 5 mins. The supernatant was removed and the cells were washed twice with 200 ul FACS buffer per well, followed by resuspension in 50 ul antibody master mix (see below) and incubation on ice for 30 mins.

Panel 1

marker	Fluorochrome	Cat/Ref	Company	Volume
CD45	APC	17-0451-82	eBioscience San Diego, CA, USA	7 ul
CD11b	PE-Cy7	25-0112-81	eBioscience San Diego, CA, USA	7 ul
F4/80	PE	12-4801-80	eBioscience San Diego, CA, USA	7 ul
CD19	BV605	563148	BD Biosciences Eysins Switzerland	7 ul
CD4	FITC	11-0041-81	eBioscience San Diego, CA, USA	7 ul
CD8a	PreCp-Cy5.5	561109	BD Biosciences Eysins Switzerland	7 ul
CD11c	BV711	563048	BD Biosciences Eysins Switzerland	7 ul

Panel 2

marker	Fluorochrome	Cat/Ref	Company	Volume
CD45	APC	17-0451-82	eBioscience San Diego, CA, USA	6 ul
CD11b	PE	12-0112-81	eBioscience San Diego, CA, USA	6 ul
F4/80	PerCp Cy5.5	123127	Biolegend San Diego, CA, USA	6 ul
MHC II	PE-Cy7	107630	Biolegend San Diego, CA, USA	6 ul
CD206	FITC	141703	Biolegend San Diego, CA, USA	6 ul
CD11c	BV711	563048	BD Biosciences Eysins Switzerland	6 ul

After incubation, plates were centrifuged and cells were washed with 150 ul FACS buffer. 0.2 ul DAPI (20 ug/ul, BioLegend) were added per well before the plate was run on a CytoFLEX Flow Cytometer (Beckman Coulter, Kalifornien, USA) according to manufacturer's instructions.

For the analysis, we divided dead and live cells using DAPI as a marker for dead cells and we used CD45 to distinguish immune cells from non-immune cells. Immune cells were further separated into macrophages that are F4/80 and CD11b positive and subdivided using CD206 and MHC II to distinguish M I macrophages and M II macrophages. Also, B cells were marked by CD19 and T cells were marked by CD4 and CD8.

Statistics

Data are expressed as means +/- standard error of the mean (SEM). Tests, as stated in the figure legends, were used for comparison of groups and $p<0.05$ was considered significant. Data analysis was performed using GraphPad Prism v8.0d Software (San Diego, USA).

Results

Knock out efficiency and specificity of adipocyte-specific *Il-1r1* knock out mice

To test the validity of our newly generated mouse model, we first checked whether *Il1r1* gene expression is ablated only in adipocytes and what the degree of reduction is. For this, various IL-1 receptor1-expressing tissues were isolated from *Il1r1*$^{fl/fl}$ *Adipoq*-Cre$^{tg/0}$ and control mice and gene expression analyzed. As expected, *Il1r1* expression was strongly reduced (95 %) in adipocytes isolated from gonadal fat (Fig 3a). In liver, muscle and SVF of gonadal fat pad, *Il1r1* was not different from control mice (Fig 3b-d).

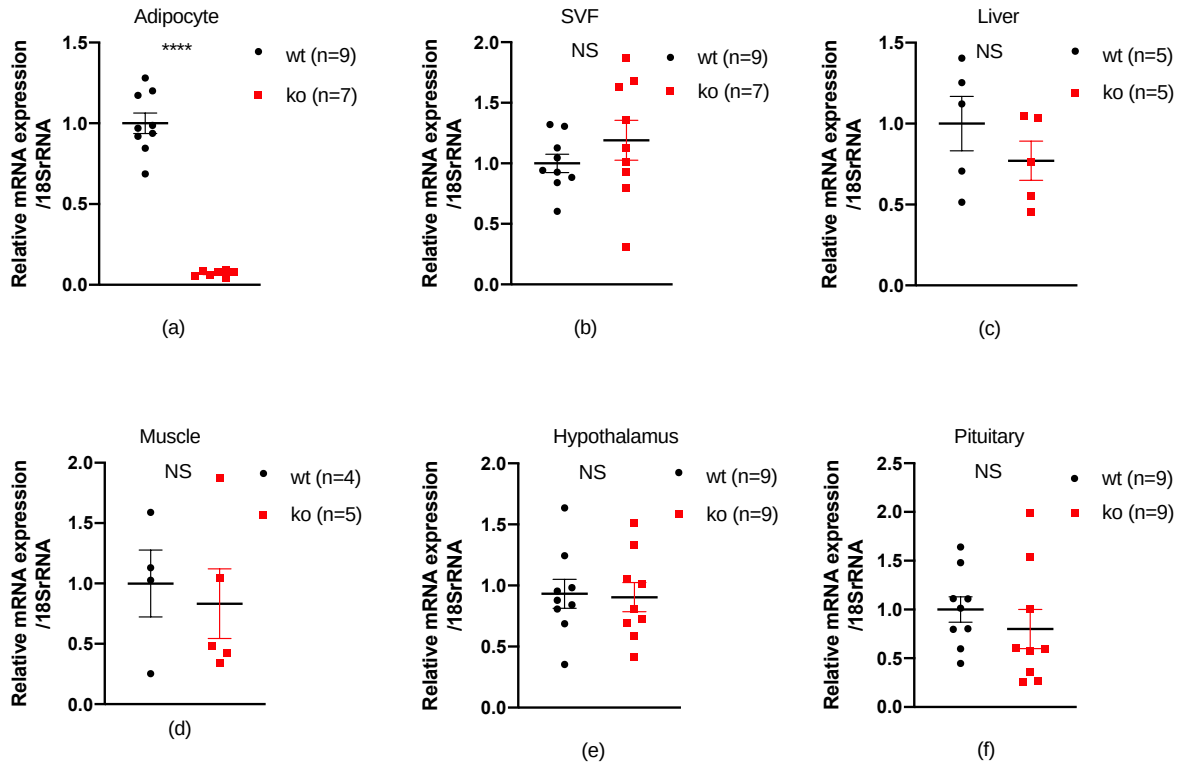


Fig 3 Knock out efficiency of *Il1r1*$^{fl/fl}$ *Adipoq*-Cre$^{tg/0}$ mice (a) – (f) representing adipocyte, SVF, liver, muscle, hypothalamus, and pituitary, respectively. Data were analyzed by student unpaired t-test. *** represents $p < 0.001$. NS represents non-significant.

Wilkison et al reported expression of adiponectin in the hypothalamus and especially in the pituitary. Further, they found that Cre is expressed in the hypothalamus and the pituitary of A*dipoq*-Cre driver mice[14]. We therefore also measured *Il1r1* expression in these tissues in our

Il1r1$^{fl/fl}$ *Adipoq*-Cre$^{tg/0}$ mouse model. In the hypothalamus (Fig 3e) and pituitary (Fig 3f) *Il1r1* expression did not differ between *Il1r1*$^{fl/fl}$ *Adipoq*-Cre$^{tg/0}$ and controls.

Body weight development in adipocyte-specific *Il-1r1* knock out mice

We followed the mice from 6 to 54 weeks of age and found no significant differences in body weight development in the mice on chow diet (Fig 4a). To metabolically challenge our mice, we then fed them a HFD starting at 10 weeks of age. Body weight development was still equal between the groups (Fig 4b).

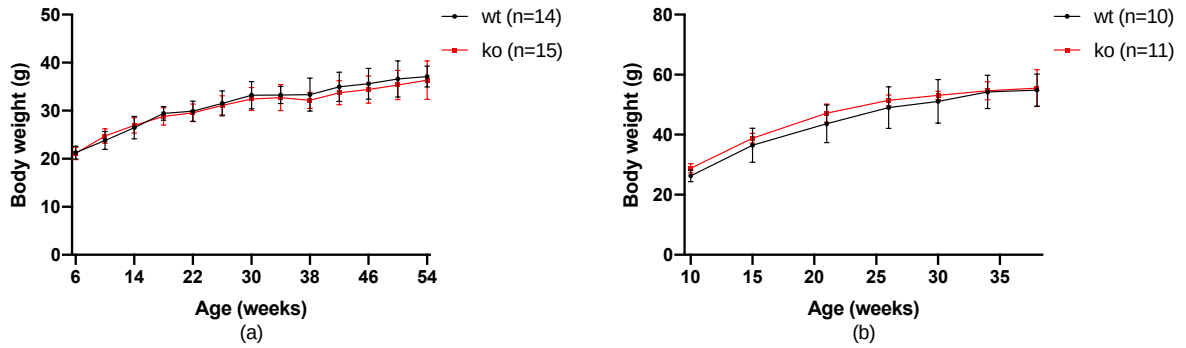


Fig 4 Body weight development. Body weight of (a) chow-fed and (b) HFD-fed male *Il1r1*$^{fl/fl}$ *Adipoq*-Cre$^{tg/0}$ and control mice. HFD was started at 10 weeks of age.

Aged adipocyte-specific *Il-1r1* knock out mice show impaired glucose tolerance

To evaluate the effect of adipocyte-specific *Il-1r1* knock out on glucose homeostasis, we performed GTT and one week later ITT. In chow-fed mice, insulin tolerance at 12, 20, 32 and 52 weeks of age was similar between *Il1r1*$^{fl/fl}$ *Adipoq*-Cre$^{tg/0}$ and control mice (Fig 5a-d). Glucose tolerance was also comparable at 13, 21 and 33 weeks of age (Fig 5e-g). However, glucose tolerance at 53 weeks of age resembled a pattern that is observed in pre-diabetes, with an early peak in glucose followed by a rapid clearance (Fig 5h). The corresponding insulin secretion also showed abnormalities as observed in prediabetes, with increased fasting insulin,

impaired stimulation and prolonged increase, although these insulin changes did not reach statistical significance (Fig 5i).

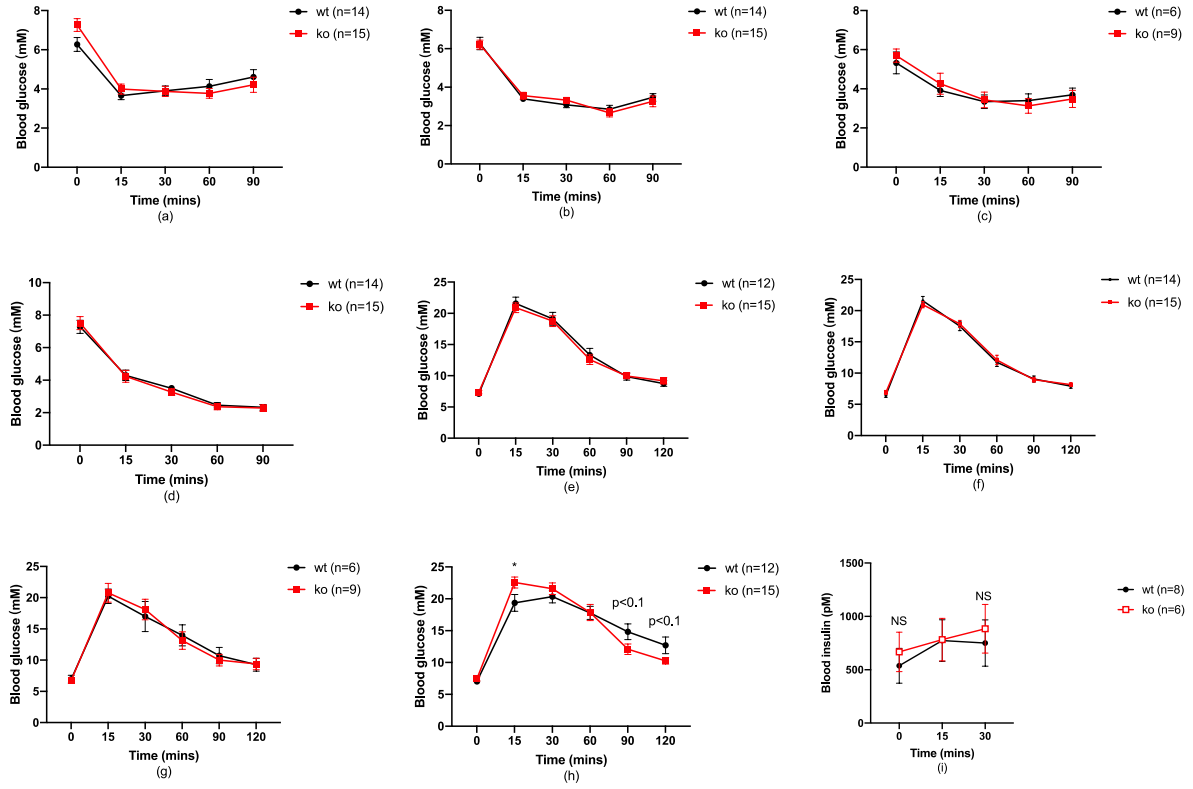


Fig 5 GTT and ITT over time in chow-fed mice. Blood glucose levels during an ITT at (a) 12, (b) 20, (c) 32 and (d) 52 weeks of age. GTT at (e) 13, (f) 21, (g) 33 and (h) 53 weeks of age. (i) Circulating insulin levels during the GTT at 53 weeks of age. Data were analyzed by student unpaired t-test. * represents $p < 0.05$. NS represents non-significant.

On HFD, insulin tolerance at 20 and 35 weeks of age (10 and 25 weeks of HFD-feeding, respectively) did not differ between knock out and control mice (Fig 6a-b). While glucose excursion during GTT at 21 and 36 weeks of age (11 and 26 weeks of HFD-feeding, respectively) was comparable between the groups (Fig 6c-d), peripheral insulin concentrations during the GTT were significantly increased in *Il1r1*$^{fl/fl}$ *Adipoq*-Cre$^{tg/0}$ mice compared to control mice (Fig 6e-f).

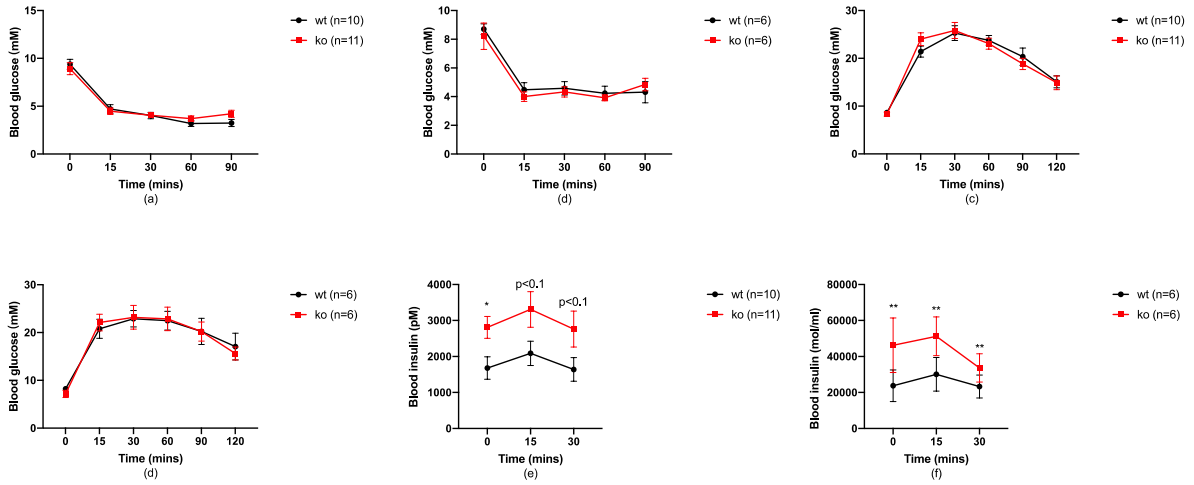


Fig 6 GTT and ITT over time in mice fed a HFD from 10 weeks of age onwards. ITT at (a) 20 and (b) 35 weeks of age. Glucose tolerance at (c) 21 and (d) 36 weeks of age. Circulating insulin levels during the GTT at (e) 21 and (f) 36 weeks of age, respectively. Data were analyzed by student unpaired t-test. ** represents $p < 0.001$. * represents $p < 0.05$.

IL-1β-mediated insulin secretion is reduced in adipocyte-specific *Il-1r1* knock out mice

Preinjection of IL-1β 20 mins before a GTT strongly enhances glucose-stimulated insulin secretion and therefore reduces glucose excursion[8]. To test whether the *Il-1r1* on adipocytes plays a role in this effect, 22-week-old *Il1r1*$^{fl/fl}$ *Adipoq*-Cre$^{tg/0}$ mice were injected with 1 ug/kg IL-1β or saline 20 mins before a GTT. Mice were divided into 4 groups, control mice ± IL-1β and knock out mice ± IL-1β. As expected, in control mice IL-1β-preinjection significantly boosted insulin secretion and thereby reduced glucose excursion during the GTT (Fig 7a-b). Knock out and control mice did not differ in insulin levels or glucose excursion during GTT upon saline preinjection. However, compared to control mice preinjected with IL-1β, *Il1r1*$^{fl/fl}$ *Adipoq*-Cre$^{tg/0}$ mice preinjected with IL-1β showed a blunted insulin response and thus only a partial improvement in glucose excursion during GTT.

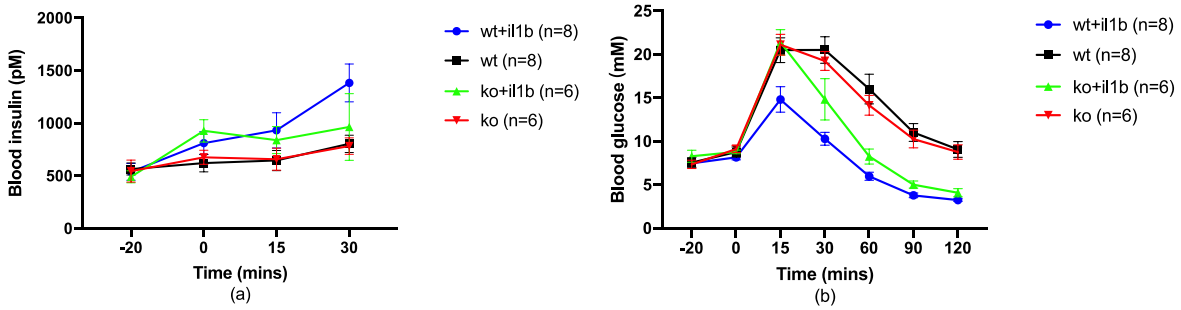


Fig 7 IL-1β preinjected GTT test. (a) insulin and (b) glucose excursion during a glucose tolerance test in *Il1r1*$^{fl/fl}$ *Adipoq*-Cre$^{tg/0}$ and control mice preinjected with IL-1β or saline.

Inflammatory gene changes in adipocyte-specific *Il-1r1* knock out mice on chow diet (preliminary data)

Next, we assessed whether knocking out the IL-1 receptor1 on adipocytes locally changes immune- and inflammatory parameters. For this, we isolated adipocytes from gonadal fat from 40-week-old chow-fed mice and performed RNA analysis using qPCR. While immune cell markers *Adgre1* (encoding for F4/80), *Il1b* and *Tnf* were similar between *Il1r1*$^{fl/fl}$ *Adipoq*-Cre$^{tg/0}$ mice and *Il1r1*$^{fl/fl}$ control mice, *Il6* was significantly lower in knock out mice (Fig 8a,b,c,d). Insulin signaling genes *Irs1* and *Slc2a4* (encoding for Glut4) did not differ between knock out and controls (Fig 8e,f). Two genes important for adipocyte metabolism, *Pparg* and *Adipoq* (encoding for adiponectin) were found to be similar between *Il1r1*$^{fl/fl}$ *Adipoq*-Cre$^{tg/0}$ and control mice although *Adipoq* showed a trend towards reduction in knock out mice (Fig 8g,h).

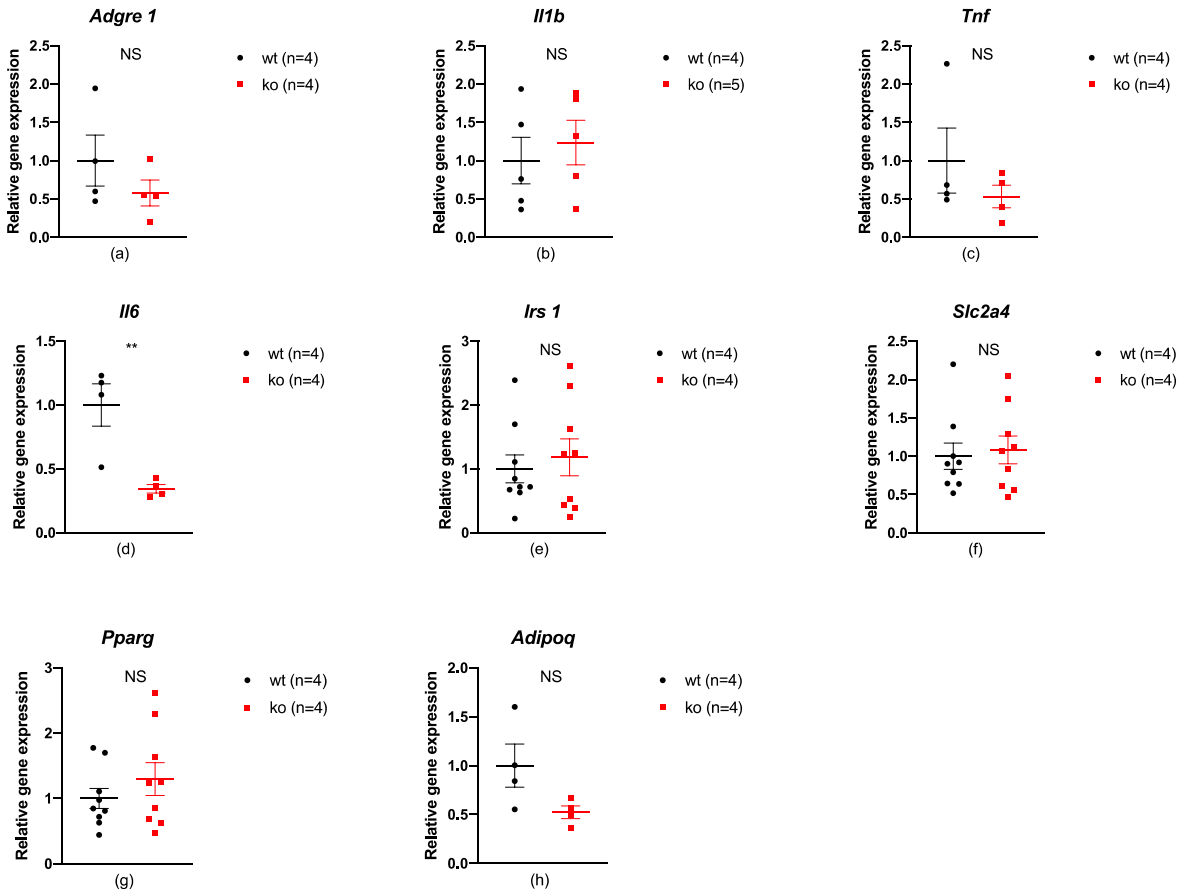


Fig 8 RNA expression profile in adipocytes of 40-week-old chow-fed *Il1r1*$^{fl/fl}$ *Adipoq*-Cre$^{tg/0}$ and control mice. Expression of (a) *Adgre1*, (b) *Il1b*, (c) *Tnf*, (d) *Il6*, (e) *Irs1*, (f) *Slc2a4*, (g) *Pparg* and (h) *Adipoq*. Data were analyzed by student unpaired t-test. * *represents $p < 0.001$. NS represents non-significant.

Physiological changes in adipocyte-specific *Il-1r1* knock out mice on HFD (preliminary data)

Next, we investigated a cohort of animals that had been fed HFD for 35 weeks (diet started at 10 weeks of age). For this, the mice were sacrificed and insulin-sensitive tissues isolated and RNA expression analyzed using qPCR. Body weight was comparable between knock out and control mice (Fig 9a). Surprisingly, pancreas weight in knock out mice was increased by 55 % (Fig 9b), and the there was still a trend towards an increased pancreas weight when normalized to body weight (Fig 9c).

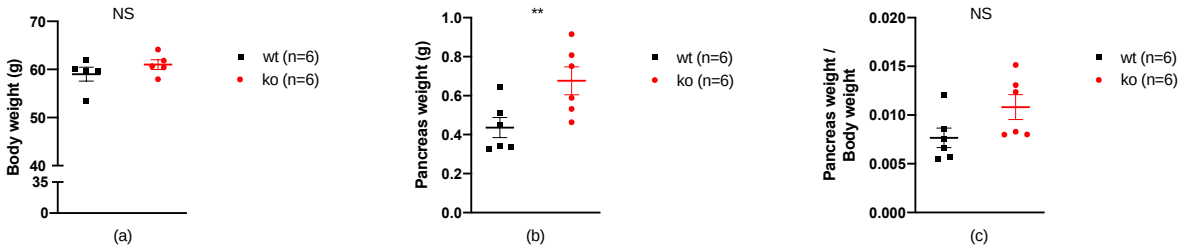
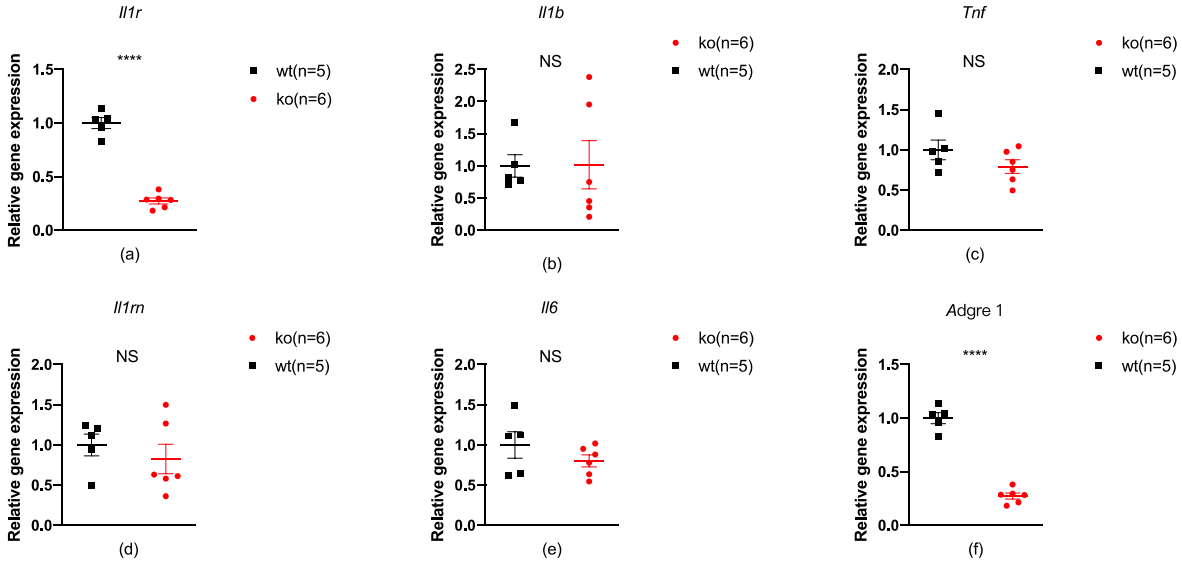


Fig 9 Sacrifice of 45-week-old mice on HFD for 35 weeks. (a) body weight and (b) pancreas weight upon sacrifice (c) pancreas weight to body weight proportion. Data were analyzed by student unpaired t-test. ** represents $p < 0.001$. NS represents non-significant.

As expected, *Il1r1* gene expression in adiopocytes was significantly reduced in *Il1r1*$^{fl/fl}$ *Adipoq*-Cre$^{tg/0}$ mice compared to control mice, suggesting that also under HFD-feeding the model works well (Fig 10a). While cytokines *Il1b*, *Tnf*, *Il1rn* (encoding for IL-1 receptor antagonist) and *Il6* were similar between genotypes (Fig 10b-e). *Adgre1* (encoding for F4/80) was significantly reduced in knock out adipocytes (Fig 10f). Markers of adipokines *Adipoq* and *Lep* were similar between genotypes (Fig 10g-h).



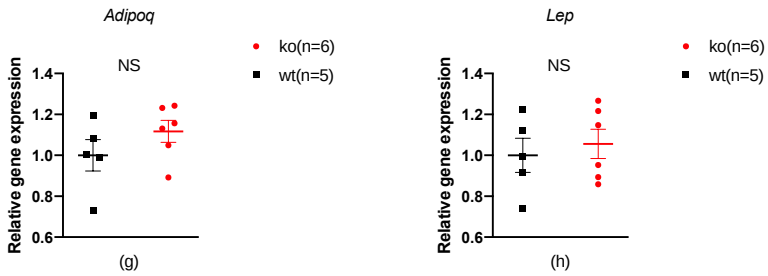


Fig 10 RNA expression profile in adipocytes of 45-week-old HFD-fed (for 35 weeks) *Il1r1*$^{fl/fl}$ *Adipoq*-Cre$^{tg/0}$ and control mice. Expression of (a) *Il1r1*, (b) *Il1b*, (c) *Tnf*, (d) *Il1rn*, (e) *Il6*, (f) *Adgre1*, (g) *Adipoq* and (h) *Lep*. Data were analyzed by student unpaired t-test. ****represents $p < 0.0001$. NS represents non-significant.

Further, from the same HFD-fed mice, we also isolated SVF from the gonadal fat pad. We used FACS analysis to characterize these immune cells on the level of protein expression. The distribution of CD45+ cells (as a marker of broad immune cells) was comparable between genotypes (Fig 11a, Fig 12). Distribution of total macrophages (F4/80+ and CD11b+) was also not different between genotypes (Fig 11b, Fig 13). Macrophage subtypes CD 206 and MHC II did also not differ (Fig 11c-d). Type I macrophages (top left of image) accounted for the vast majority of cells compare to type II macrophages (bottom right of image) in the mice on HFD (Fig 14). B cells (CD19+) showed a tendency towards an increase in *Il1r1*$^{fl/fl}$ *Adipoq*-Cre$^{tg/0}$ mice, although this was statistically not significant (Fig 11e, Fig 15). Finally, we tested the distribution of CD 4 and CD 8 T cells and found CD 4 T cells to be similar between genotypes but CD8 T cells to show a statistically non-significant trend towards a reduction in knock out mice (Fig 11f-g, Fig 16).

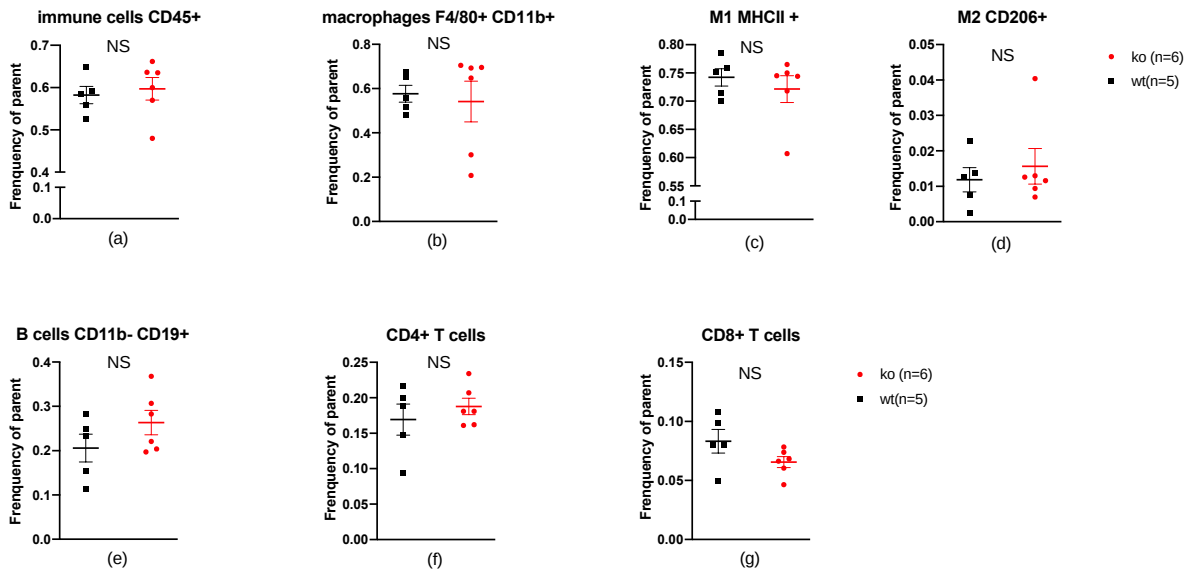
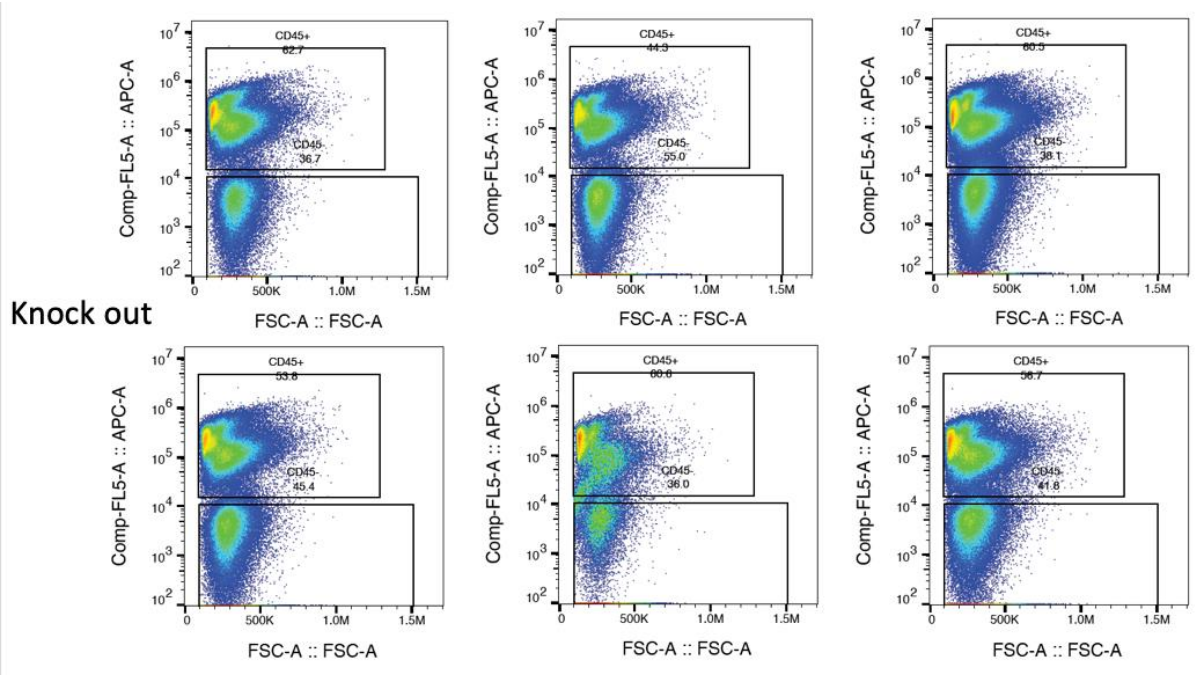


Fig 11 Immune cell distribution in SVF of gonadal fat pads isolated from *Il1r1*$^{fl/fl}$ *Adipoq*-Cre$^{tg/0}$ and control mice. (a) general immune cell distribution, (b) broad macrophages distribution, (c) type I macrophages distribution, (d) type II macrophages distribution, (e) B cells distribution, (f) CD4+ T cell distribution and (g) CD8+ T cell distribution. Data were analyzed by student unpaired t-test. NS represents non-significant.



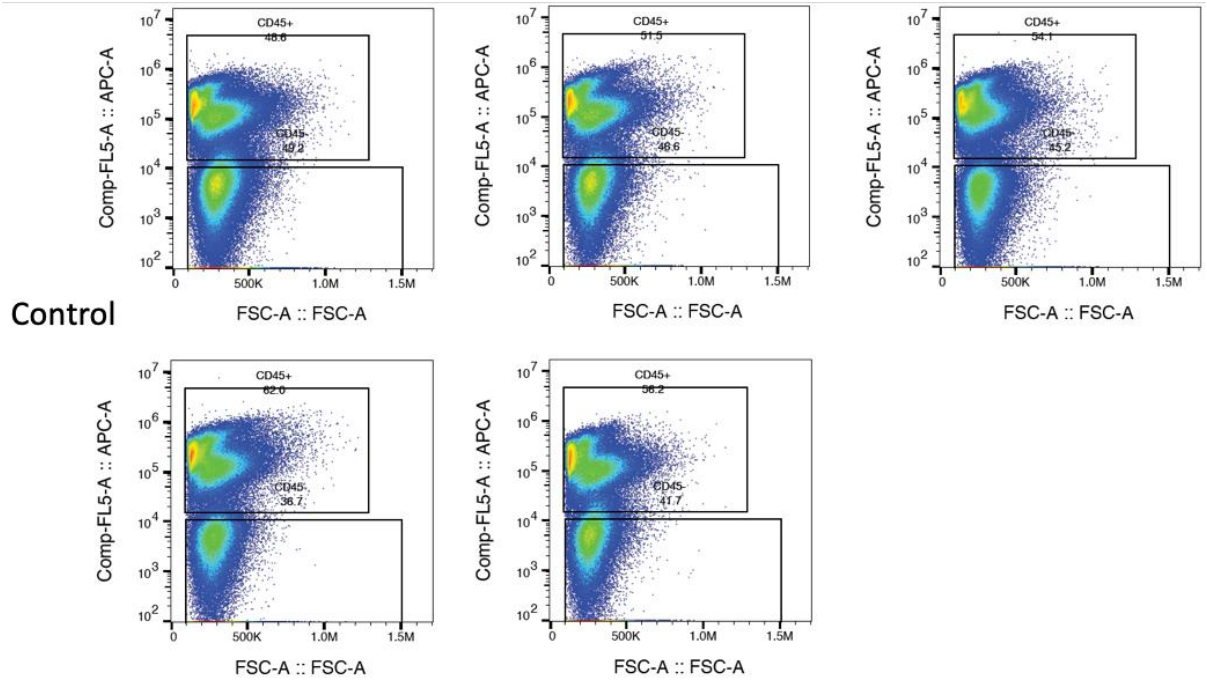
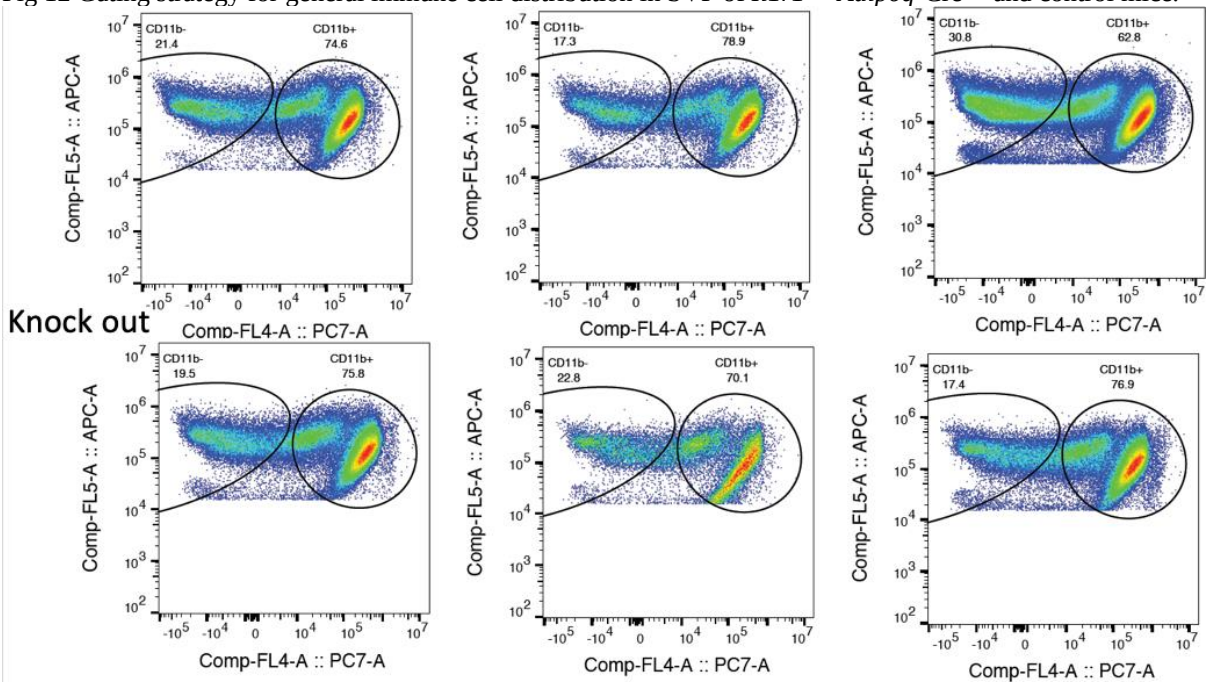


Fig 12 Gating strategy for general immune cell distribution in SVF of *Il1r1*$^{fl/fl}$ *Adipoq*-Cre$^{tg/0}$ and control mice.



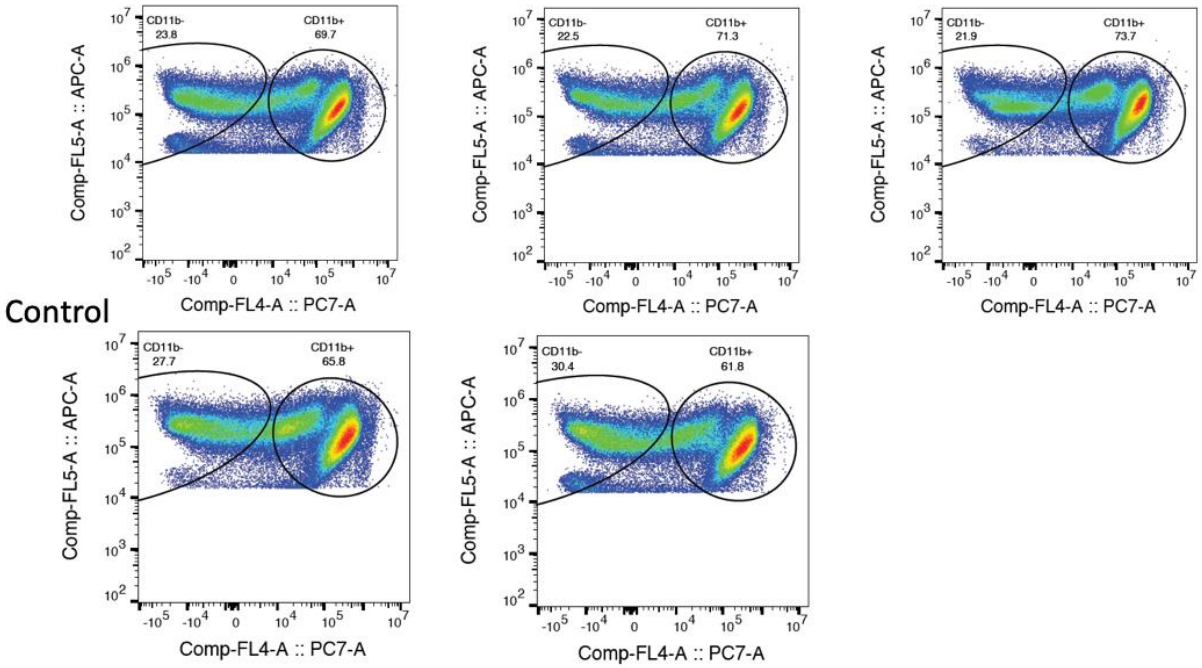
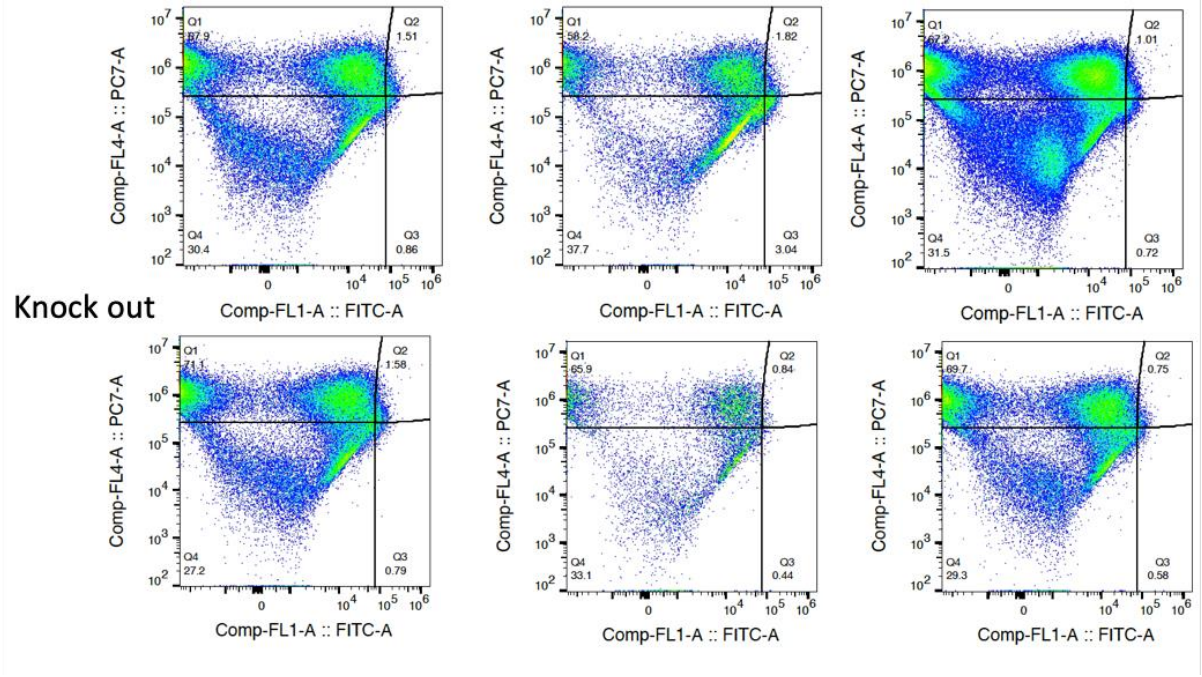


Fig 13 Gating strategy for macrophage distribution in SVF of *Il1r1*$^{fl/fl}$ *Adipoq*-Cre$^{tg/0}$ and control mice.



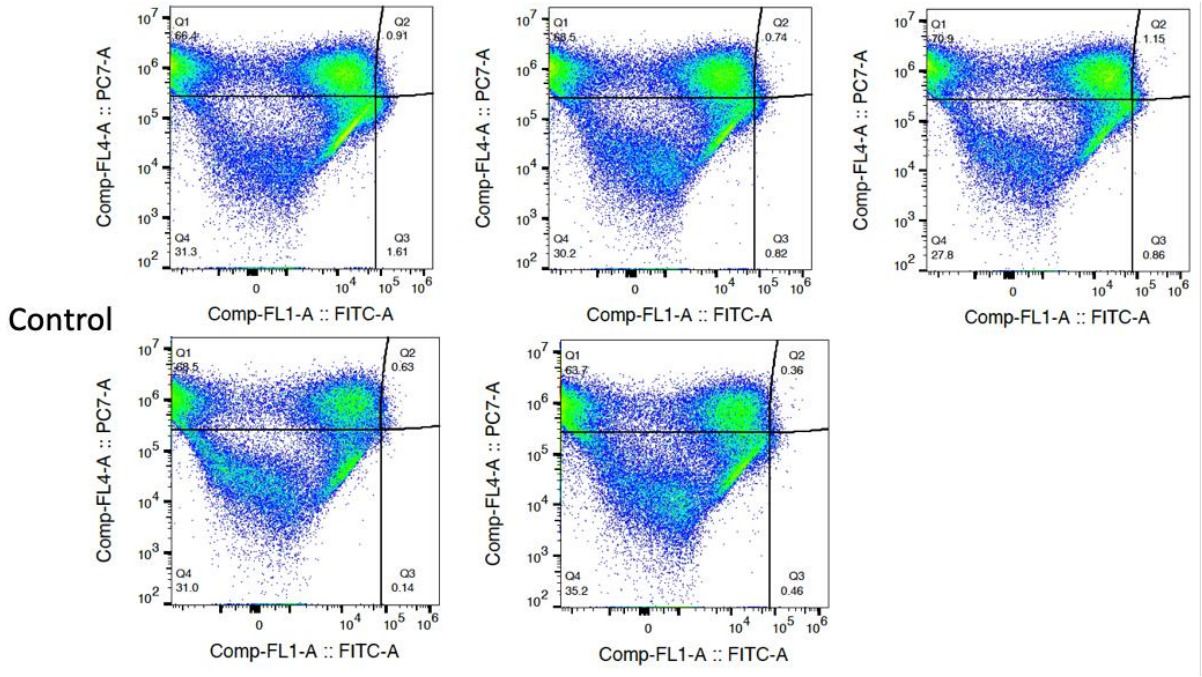
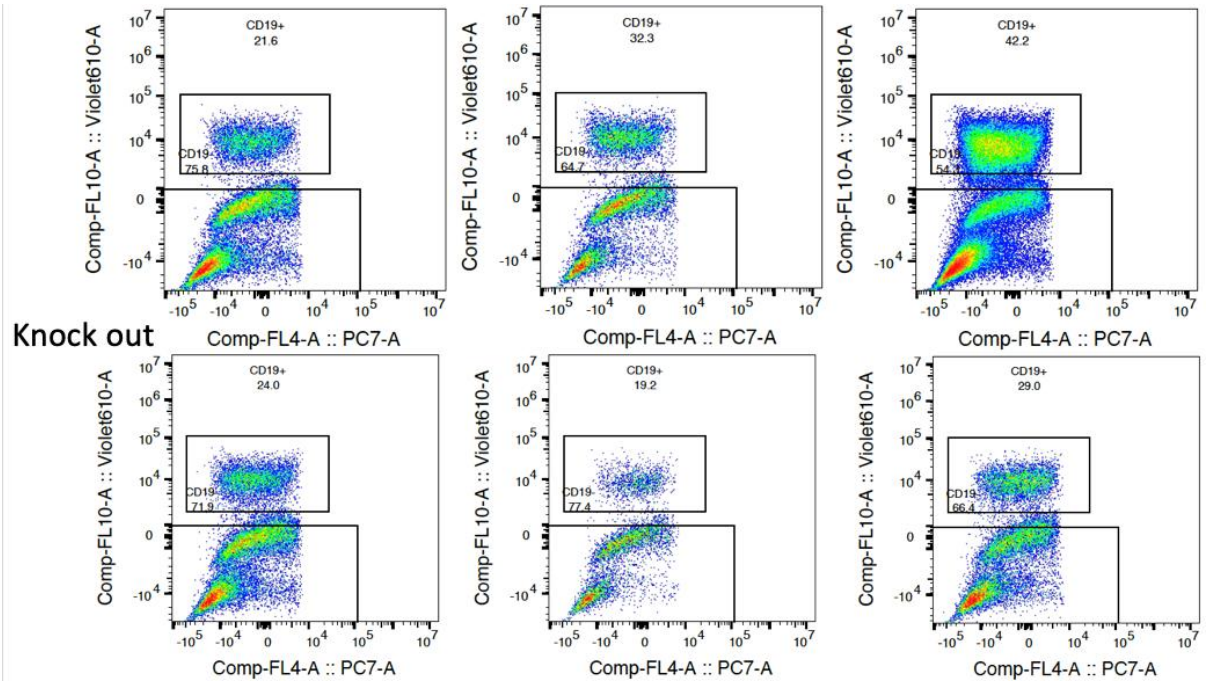


Fig 14 Gating strategy for M I and M II macrophage distribution in SVF of *Il1r1*$^{fl/fl}$ *Adipoq*-Cre$^{tg/0}$ and control mice.



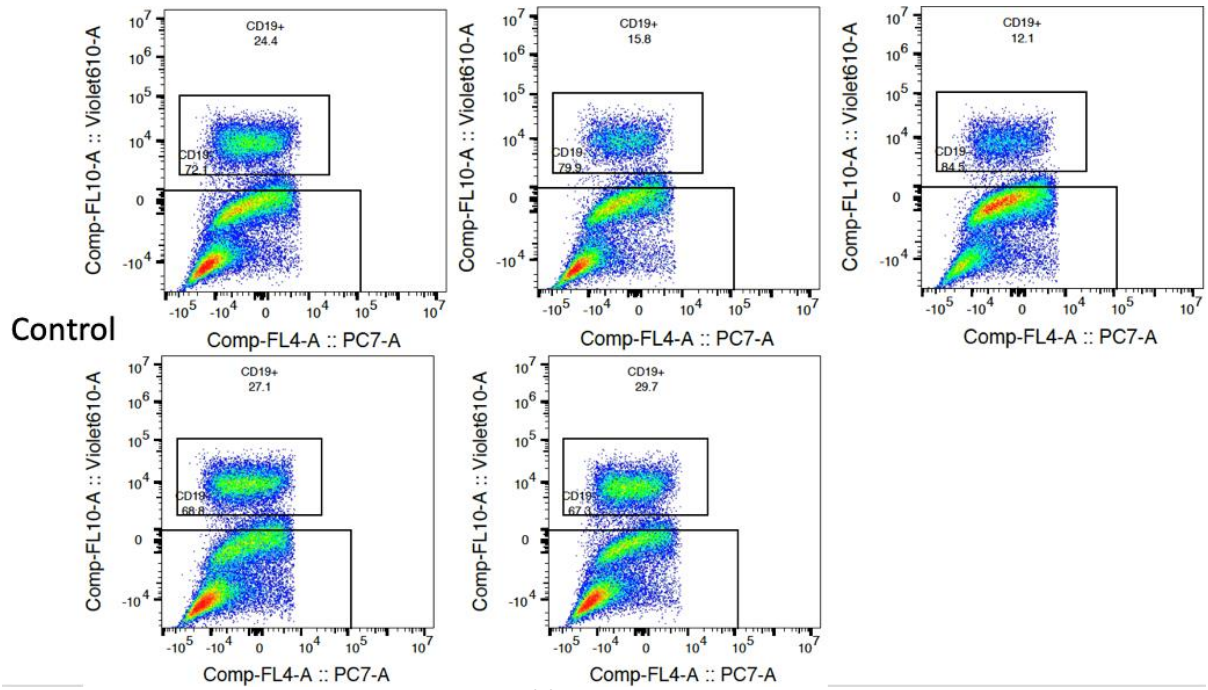
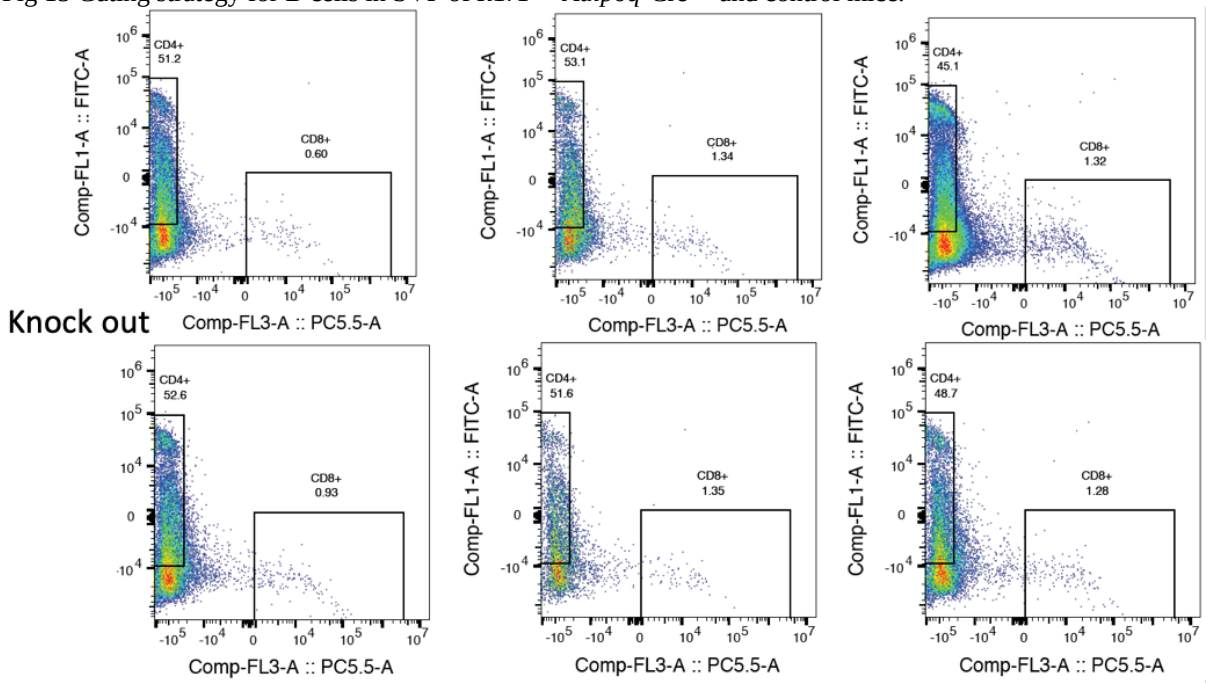


Fig 15 Gating strategy for B cells in SVF of *Il1r1*$^{fl/fl}$ *Adipoq*-Cre$^{tg/0}$ and control mice.



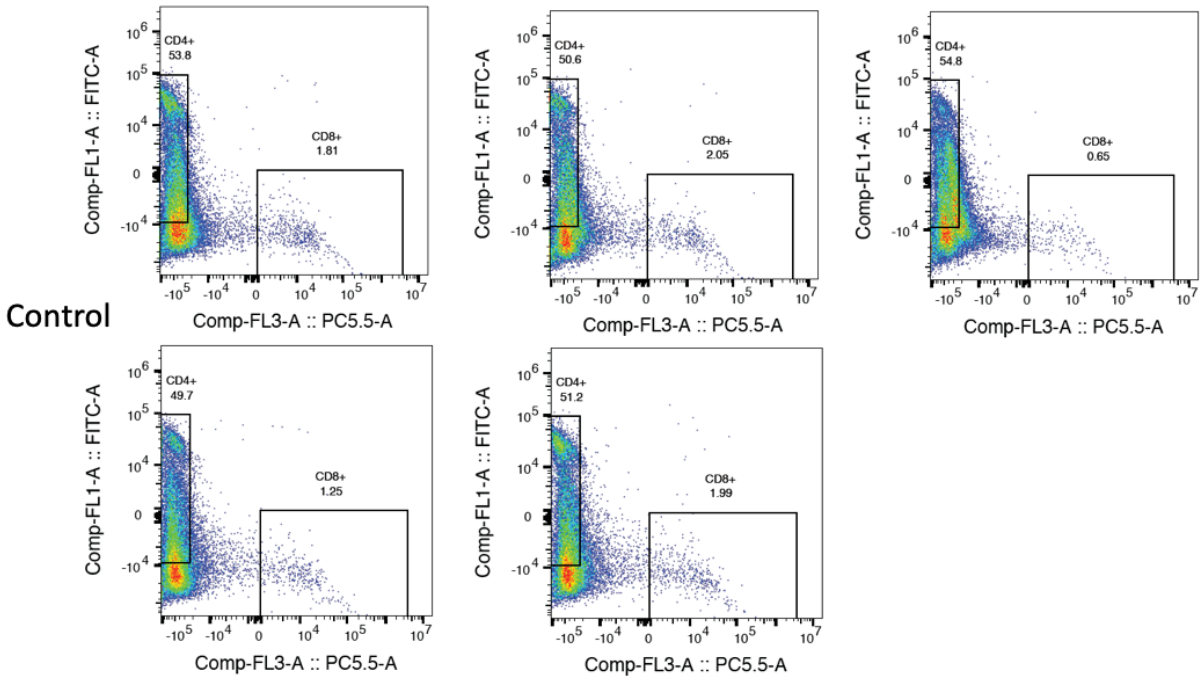


Fig 15 Gating strategy for T cells in SVF of *Il1r1*$^{fl/fl}$ *Adipoq*-Cre$^{tg/0}$ and control mice.

Discussion

Rather unexpectedly, our pilot study showed that following refeeding the most prominent early changes in inflammation gene expression happen in the IL-1 pathway of fat-derived immune cells. In the present project, we therefore knocked out the receptor of the major cytokine IL-1 specifically on adipocytes and assessed the metabolic consequences.

Whole-body IL-1 receptor1 knock out mice show increased accumulation of fat mass and subsequently increased body weight gain starting at 24 weeks of age[15]. Our tissue-restricted *Il1r1*$^{fl/fl}$ *Adipoq*-Cre$^{tg/0}$ mice did not show differences in body weight gain up to one year of age, neither when fed a chow-diet nor a HFD. This suggests that lack of the IL-1 receptor1 on adipocytes does not mediate the obesity phenotype observed in whole-body IL-1 receptor1 knock out mice.

Fasting glycemia and glucose tolerance were unchanged upon chow feeding, until 52 weeks of age, where blood glucose upon a glucose challenge rose higher and fell faster in knock out mice compared to controls, as seen in patients with prediabetes. However, peripheral insulin concentrations during the GTT did not significantly differ between the two groups, although the insulin pattern was also reminiscent to prediabetes. Insulin sensitivity, assessed by ITT, was not changed at the same timepoint. This suggests that aged *Il1r1*$^{fl/fl}$ *Adipoq*-Cre$^{tg/0}$ mice might develop a prediabetic phenotype on chow diet. These changes might become more prominent when the mice are followed even longer. When the mice were metabolically challenged with HFD-feeding, fasting glycemia did not differ between groups. However, starting at 10 weeks of HFD-feeding (20 weeks of age), *Il1r1*$^{fl/fl}$ *Adipoq*-Cre$^{tg/0}$ mice showed increased insulin secretion following a glucose challenge, although the glucose excursion remained comparable between groups. This suggests that on HFD, these mice become insulin resistant compared to control mice. However, ITT was similar between the two groups. This is in line with our observation, that ITT is not a very sensitive method to assess insulin sensitivity.

To conclusively assess whether *Il1r1*$^{fl/fl}$ *Adipoq*-Cre$^{tg/0}$ mice have reduced insulin sensitivity, one would need to perform hyperinsulinemic-euglycemic clamp studies.

Injection of IL-1β before a glucose challenge greatly enhances glucose-stimulated insulin secretion and thus reduces glucose excursion[8]. This effect was apparent in our control mice but was partly lost in *Il1r1*$^{fl/fl}$ *Adipoq*-Cre$^{tg/0}$ mice. This suggests that part of the insulin-stimulating-effect of IL-1β is mediated by the IL-1 receptor1 on adipocytes. There are two potential scenarios how this could happen. First, IL-1β could induce glucose uptake in adipocytes and thereby create a "glucose sink". This would then lead to partially reduced circulating glucose levels as observed following IL-1β injection. This glucose-lowering effect could be absent in *Il1r1*$^{fl/fl}$ *Adipoq*-Cre$^{tg/0}$ mice, leading to increased circulating glucose concentrations compared to control mice injected with IL-1β. Supporting this hypothesis is that IL-1β injection indeed induces glucose uptake into fat (Figure 7 on the paper[8]). A second hypothesis is that IL-1β induces the secretion of adipokines which then stimulate the β cell to secrete insulin in concert with IL-1β. Indeed, RNA expression of the adipokine adiponectin is reduced in adipocytes of *Il1r1*$^{fl/fl}$ *Adipoq*-Cre$^{tg/0}$ mice on chow-diet and not changed on HFD. Expression of leptin however is not changed between genotypes. Further, pancreata of *Il1r1*$^{fl/fl}$ *Adipoq*-Cre$^{tg/0}$ mice were found to be 55 % bigger after HFD-feeding, suggesting that there might be a β cell trophic signal arising from the adipocytes of adipocyte-specific IL-1 receptor1 knock out mice. Adipsin is another potential candidate adipokine that was shown to potentiate insulin secretion from β cells[16]. To shed light on the role of adipokines in our mouse model, circulating protein levels of leptin, adiponectin and adipsin could be assessed. However, the partly ablated IL-1β effect in our knock out mice could also simply be a consequence of an insulin secretion defect. In this scenario, *Il1r1*$^{fl/fl}$ *Adipoq*-Cre$^{tg/0}$ mice would have an inherent insulin-secretion defect and would therefore be unable to mount the same insulin response as the control mice, which then manifests in only a partial reduction of glucose excursion compared to control mice

injected with IL-1β. To investigate this in more detail *in vivo*, other insulin boosting agents would need to be tested and compared in control and *Il1r1*$^{fl/fl}$ *Adipoq*-Cre$^{tg/0}$ mice. Further, to test whether these islets are dysfunctional, islets could be isolated and tested *ex vivo* with a glucose-stimulated insulin secretion assay.

RNA markers of the insulin signaling pathways *Irs1* and *Slc2a4* (encoding for Glut4) were similar between genotypes, suggesting that insulin signaling in adipocytes is not changed. However, since insulin receptor signaling is highly regulated by protein phosphorylation, RNA analysis should be complemented by protein analysis by Western blotting before firm conclusions are drawn.

RNA markers of inflammation were not changed dramatically in adipocytes of *Il1r1*$^{fl/fl}$ *Adipoq*-Cre$^{tg/0}$ mice. On chow diet, *Il1b* and *Tnf* were similar between genotypes, while *Il6* was reduced. On HFD, all markers (*Il1b*, *Tnf*, *Il1rn*, *Il6*) were comparable between groups. In agreement with these results, the immune cell composition of the SVF isolated from aged HFD-fed mice was similar between *Il1r1*$^{fl/fl}$ *Adipoq*-Cre$^{tg/0}$ and control mice. Noteworthy, these results are preliminary and derived from only one cohort and need to be validated in other cohorts.

Overall, our adipocyte-specific *Il-1r1* knock out mice show similar body weight development as control mice but show signs of impaired glucose metabolism at one year of age on chow diet or at younger ages following 10 weeks of HFD-feeding. Interestingly, these adipocyte-specific knock out mice seem to have a pancreas and/or insulin phenotype which might point out a novel crosstalk of IL-1 signaling in fat tissue and the pancreas.

Acknowledgement

I would like to express my gratitude to all those who have helped me during the last 18 months of M.D. work: My mentor, my supervisor and my colleagues.

Specifically, I would like to express my sincere gratitude to Prof. Dr. Marc Donath and PD Dr. Marianne Boeni, as well of those who provided me for the M.D. position and supported my life in Switzerland. My gratitude goes to Dr. Daniel Zeman-Meier, who taught me basic experimental skills and rigorous scientific thinking. As my supervisor, Daniel always gave me great help and input, not only in study but also in personal life. I am also thankful to other members of the Donath lab for continous help: Leila Rachid, Sophia Wiedemann, Josua Wehner, Axel De Baat and Hélène Mereau.

At last, I want to show my gratitude to my beloved parents and my wife. They are the most thankful people in my life and always supported my studies and career.

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Deklaration

My thesis is focused on studying the metabolic alterations in transgenic mice that lack the interleukin 1 receptor specifically on adipocytes. I therefore spent 18 months phenotyping this mouse model.

At the beginning of my education, I did the LTK1 (Labor Tier Kunde Kurs), the license that allowed me to perform animal experiments. I then started in vivo and ex vivo animal experiments.

For the in vivo part of this thesis, I did the mouse colony management and husbandry, including taking care of mice, checking their health condition, measuring body weight, genotyping and glucose and insulin tolerance testing. This included learning and using Pyrats software, the animal managing software of the Univesity of Basel.

For the ex vivo experiments, I dissected various metabolic tissues from mice including brain regions, pancreas, adipose tissues, liver and muscle. Further, I isolated adipocytes and stromal-vascular fraction from primary fat depots. From these tissues and organs I isolated RNA, reverse transcribed it to cDNA and analyzed it using quantitative real-time PCR (Taqman and Sybr green). In addition, I also did fluorescence-activated cell sorting (FACS) from stromal-vascular fractions.

For data processing, I used FlowJo software, Fiji and Graphpad Prism. Finally, I wrote and revised my thesis.