



Faculteit Geneeskunde en Farmacie
Vrije Universiteit Brussel
Laarbeeklaan 103
B-1090 Brussel

Exploring the Role of Macrophages in Pregnancy-associated Islet Adaptations

Bo Petroons

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Promotor: Prof. Dr. Nico De Leu
Copromotor: Prof. Dr. Willem Staels

Supervisor: Lien Willems

Genetics, Reproduction and Development Research Group (GRAD)
Beta Cell Neogenesis Unit (BENE)
Brussels Health Campus VUB
Laarbeeklaan 103, 1090 Brussels, Belgium



Abstract

Background/aims. Despite significant progress in identifying regulators of pancreatic beta cell proliferation, controlling beta cell mass expansion for diabetes therapy currently remains elusive. Pregnancy represents a physiological model of reversible beta cell mass plasticity, characterized by gestational beta cell mass expansion to meet increased insulin demands and subsequent regression postpartum. Islet-resident macrophages have emerged as potential regulators in this process, yet their role remains unclear with conflicting evidence regarding their contribution to gestational beta cell proliferation and postpartum regression. We aimed to elucidate the role of islet-resident macrophages in gestational beta cell mass remodeling.

Methods. Pancreata from female C57BL/6J mice were collected at defined gestational stages (G10.5, G14.5, G15.5, and G18.5) and postpartum day 5 (PP5) as well as in non-pregnant mice to quantify beta cell proliferation, apoptosis, and macrophage abundance by immunostaining. A macrophage-depleted mouse model (Fcgr1-Cre;R26-iDTR) was generated to assess the contribution of macrophages to beta cell adaptations. Metabolic phenotyping – including body weight, (non)-fasting glycemia, glucose and insulin tolerance test – was performed in (non-)pregnant and postpartum Fcgr1-Cre;R26-iDTR mice and single transgenic control mice to evaluate the functional consequences of macrophage depletion.

Results. Beta cell proliferation peaked at G14.5 and returned to baseline, non-pregnant rates by PP5, whereas apoptosis remained unchanged. Macrophage abundance remained unchanged during gestation yet increased at PP5. The Fcgr1-cre;R26-iDTR model achieved efficient macrophage depletion yet with persistence of a residual proliferating macrophage subset. Macrophage depletion did not alter beta cell proliferation or glucose homeostasis, but impaired beta cell mass expansion during pregnancy.

Conclusions. Mid-gestational macrophage depletion did not affect beta cell proliferation or glucose homeostasis, but it significantly precluded beta cell mass expansion, suggesting macrophages may contribute to the regulation of these adaptive changes. These findings highlight the complexity of immune-endocrine crosstalk regulating beta cell plasticity and provide relevant insights into physiological beta cell mass expansion.

Introduction

Diabetes mellitus is a widespread chronic disease characterized by hyperglycemia, resulting from insufficient insulin secretion, increased insulin resistance, or a combination of both. This metabolic disorder is a developing pandemic, with a prevalence of 589 million adults in 2025, which is projected to increase to approximately 853 million by 2050 (1). Diabetes is generally classified into two primary forms, type 1 (T1D) and type 2 diabetes (T2D). T1D is an autoimmune disorder in which the insulin producing beta cells are destroyed by autoreactive cytotoxic T cells (2, 3). In contrast, T2D arises from the progressive development of insulin resistance in peripheral tissues and/or a diminished ability of beta cells to produce insulin (2, 4). Importantly, both forms are characterized by a disruption of the tightly regulated balance between beta cell growth and beta cell apoptosis, ultimately resulting in an overall loss of beta cell mass (5, 6). Although treatment strategies have improved significantly, optimal glycemic control remains difficult to achieve (2, 7). This underscores the need to better understand the fundamental mechanisms that control beta cell mass maintenance (5).

Traditionally, beta cell mass has been considered largely static after early development, with replication rates declining sharply after childhood, suggesting a limited proliferative capacity during adulthood (8-11). However, adult beta cells retain the ability to re-enter the cell cycle in response to increased metabolic demand by transitioning from a near-quiescent to a replicative state (5, 12, 13). Importantly, this adaptive capacity is tightly regulated, resulting in a dynamically maintained beta cell mass through a balance between proliferation and apoptosis. This potential is particularly evident under physiological conditions associated with increased metabolic stress, such as pregnancy (6, 14). To ensure an adequate supply of glucose to the fetus, maternal peripheral insulin sensitivity decreases, resulting in elevated maternal blood glucose levels and increased insulin demand (15-17). In response, beta cell proliferation increases dramatically mid-gestation, leading to a significant expansion of the beta cell mass (5, 16, 18). After parturition, the beta cell mass again declines, returning to its pre-pregnancy level through increased apoptosis and reduced beta cell proliferation (6, 18, 19). This reversible physiological phase of beta cell mass expansion and regression therefore

provides a unique model for beta cell mass plasticity and an opportunity for investigating the mechanisms that control beta cell growth and decline in a physiological manner (5, 6, 14).

Despite systemic metabolic cues having a significant impact on beta cell mass adaptations, beta cell plasticity is significantly influenced by the islet microenvironment (7, 10, 20). Islet-resident macrophages represent the most abundant immune cell type within the islets of non-diabetic humans, mice, and even NOD mice, a model for T1D (20-22). Macrophages are highly heterogeneous cells that are present in all tissues and are well-known for their role in immune responses (3). In addition to their established function in phagocytosis and antigen presentation, they play essential roles in the maintenance of tissue homeostasis (3, 20). Traditionally, macrophages are categorized into a M1-like or M2-like phenotype. M1-like or “classically-activated” macrophages exhibit pro-inflammatory activity and mediate cytotoxic responses through the secretion of pro-inflammatory cytokines (3, 23). In contrast, M2-like or “alternatively-activated” macrophages are anti-inflammatory and contribute to resolving inflammation and promoting tissue repair and regeneration (3, 20, 23). Although this classification remains widely used, it is an oversimplification as macrophages are highly plastic and capable of phenotype shifting in response to environmental cues, thereby often displaying mixed characteristics (3, 24-26).

Within islets, resident macrophages are in close proximity to beta cells and are typically characterized as CD11b⁺, F4/80^{high}, and MHCII⁺ (3, 21, 25). The effect of islet-resident macrophages on beta cells is highly dependent on their activation state and the local microenvironment, determining whether they support survival or contribute to dysfunction. Pro-inflammatory macrophages are generally considered detrimental to beta cell function and survival, contributing to the progression of T1D and T2D (3, 23, 26). In contrast, anti-inflammatory macrophages play a protective role by promoting beta cell survival, replication, and function (23, 27).

Previous studies have already shown the importance of macrophages during pancreatic development since their depletion causes structural abnormalities and reduced beta cell mass, highlighting their importance in establishing and maintaining the beta cell mass (3, 26, 27). Early evidence linking macrophages to beta cell proliferation was provided by Banaei-Bouchareb et al., who showed that macrophage-deficient mice display abnormal islet

morphogenesis and impaired beta cell proliferation, both at weaning and during late pregnancy (28). Consistent with this concept, Riley et al. demonstrated that macrophages are necessary for CTGF-induced beta cell proliferation following injury (21, 29). Further evidence for the stimulatory role of macrophages in beta cell proliferation was obtained from studies using various models of metabolic stress and injury, where depletion of the macrophage population led to a significant reduction in beta cell proliferation, providing direct evidence for their key role in this response (24, 30-32). Collectively, these findings reinforce the concept that macrophages act as key regulators of beta cell regenerative capacity.

Recent studies suggest that macrophages may also play a key role in regulating gestational beta cell growth. For example, Yang et al. demonstrated that placental growth factor triggers gestational beta cell expansion by recruiting macrophages (17). In addition, Endo et al. showed that macrophages are involved in the postpartum reduction of the beta cell mass to its pre-pregnancy size (19). These findings indicate a potential link between macrophage activity and beta cell mass adaptation during pregnancy and postpartum. Nevertheless, the precise mechanisms underlying this relationship remains unclear.

This master thesis aims to elucidate the functional contribution of islet-resident macrophages to pregnancy-associated beta cell mass adaptation in mouse. Specifically, we will comprehensively characterize islet macrophage abundance and assess the role of macrophages in regulating adaptive changes (i.e. expansion and subsequent regression) in beta cell mass and glucose homeostasis during pregnancy and postpartum using a transgenic mouse model of macrophage depletion. Understanding how this dynamic process of beta cell mass expansion is regulated, and elucidating the role of macrophages within it, may provide novel insights into the mechanisms of beta cell mass plasticity and inform the development of physiologically safe strategies to expand the beta cell mass for diabetes treatment.

Results

Mouse beta cell proliferation peaks mid-gestation while intra-islet macrophage abundance only increases postpartum

To define the critical timepoint(s) of beta cell mass expansion and regression during pregnancy and postpartum in mouse and assess whether changes in intra-islet macrophage abundance are correlated, pancreata from pregnant and postpartum C57BL/6J mice were collected at multiple gestational time points (G10.5, G14.5, G15.5, and G18.5) and postpartum day 5 (PP5), along with non-pregnant (NP) controls at corresponding post-coital time points. Beta cell proliferation was assessed by co-immunostaining for insulin (INS), the beta cell marker NKX6.1, and the proliferation marker Ki67, followed by a quantitative analysis of the percentage of Ki67⁺NKX6.1⁺/NKX6.1⁺ cells (**Fig. 1A, B**). Beta cell proliferation was significantly increased during mid-gestation compared to both NP controls and PP mice (% beta cell proliferation: 7.79 ± 0.65 in G14.5 vs. 1.69 ± 0.11 in NP controls; $p < 0.0001$, vs. 2.30 ± 0.17 in PP5; $p < 0.0001$). G14.5 exhibited the highest beta cell proliferation rate compared to G10.5 (% beta cell proliferation: 6.74 ± 0.84 ; $p = 0.34$), G15.5 (5.96 ± 0.28 ; $p = 0.026$), and G18.5 (2.66 ± 0.37 ; $p < 0.0001$) (**Fig. 1B**). These findings indicate that beta cell proliferation peaks at G14.5 and subsequently declines toward the postpartum period.

To evaluate macrophage abundance, co-immunostaining for insulin and the pan-macrophage marker F4/80 was performed, and macrophages were quantified as the number of F4/80⁺ cells/islet (**Fig. 1C, D**). In contrast to the beta cell proliferation pattern, macrophage abundance remained similar throughout pregnancy but increased significantly at PP5 compared to controls (# macrophages/islet: 13.84 ± 1.12 in PP5 vs. 8.88 ± 0.83 in non-pregnant controls; $p = 0.0056$) (**Fig. 1D**).

Given the decline in beta cell proliferation after G14.5 and published reports indicating reduced beta cell mass postpartum, beta cell apoptosis was analyzed at G14.5 and PP5 using TUNEL staining and quantified as the percentage of TUNEL⁺INS⁺/INS⁺ cells. However, no significant differences in beta cell apoptosis were observed between G14.5, PP5, and NP controls (**Fig. 1E**).

Based on these findings, subsequent analyses focused on G14.5, representing peak beta cell proliferation, and PP5, when intra-islet macrophage abundance increases.

The Fcgr1-Cre;R26-iDTR model enables efficient macrophage depletion accompanied by compensatory macrophage proliferation

To elucidate a potential role for macrophages during beta cell mass remodeling, we used a transgenic macrophage depletion model (Fcgr1-Cre;R26-iDTR). Previous scRNA-sequencing data generated by the host laboratory demonstrated that Fcgr1 is specifically expressed by islet-resident macrophages, supporting its use for targeted macrophage depletion (**Supplementary Fig. S1**). By driving diphtheria toxin receptor (DTR) expression under the control of the Fcgr1 gene promotor, DTR expression is restricted to Fcgr1⁺ cells, resulting in the depletion of monocytes and macrophages upon diphtheria toxin (DT) administration. Control animals consisted of DT-treated R26-iDTR mice lacking Cre recombinase and therefore lacking DTR expression (**Fig. 2A**). To assess the contribution of macrophages to peak beta cell proliferation during pregnancy, DT was administered every two days starting one week before pancreas harvest at G14.5. To investigate the role of macrophages during postpartum beta cell mass remodeling, DT was likewise administered every two days starting one week before tissue collection at PP5, coinciding with peak intra-islet macrophage abundance.

Co-immunostaining for insulin and the pan-macrophage marker F4/80 was performed on pancreas sections from pregnant, postpartum, and non-pregnant Fcgr1-Cre;R26-iDTR mice with their respective R26-iDTR controls (**Fig. 2B**). Quantification of the number of F4/80⁺ cells per islet revealed a significant reduction in macrophage abundance in DT-injected experimental mice compared to control littermates, confirming effective, albeit incomplete, macrophage depletion (# macrophages/islet: 12.61 ± 0.73 in NP-CTRL vs. 1.89 ± 0.38 in NP-EXP; p<0.0001, 9.86 ± 1.43 in G14.5-CTRL vs. 2.61 ± 0.63 in G14.5-EXP; p<0.0001) (**Fig. 2C**). Notably, a residual intra-islet macrophage population persisted under all experimental conditions (**Fig. 2D**).

To assess the mechanism underlying incomplete macrophage depletion, co-immunostaining for F4/80 and the proliferation marker Ki67 was performed (**Fig. 2D**), revealing a marked

increase in macrophage proliferation (Ki67⁺F4/80⁺) in DT-injected pregnant, postpartum, and NP experimental mice compared to controls (% Ki67⁺ F4/80⁺: 4.59 ± 0.99 in NP-CTRL vs. 36.19 ± 6.10 in NP-EXP ; p=0.0026, 9.99 ± 1.03 in G14.5-CTRL vs. 41.85 ± 4.05 in G14.5-EXP; p=0.0069) (**Fig. 2E**), suggesting compensation to restore the depleted intra-islet macrophage niche.

Macrophage depletion does not alter glucose homeostasis during pregnancy

To assess the impact of macrophage depletion on pregnancy-associated beta cell mass remodeling and metabolic adaptation, we investigated whether macrophage depletion impairs the ability of experimental mice to maintain normoglycemia. Proper beta cell mass expansion during pregnancy is essential for maintaining maternal normoglycemia, and its disruption may predispose to gestational diabetes.

Longitudinal metabolic follow-up (**Fig. 3A**) of 10-week-old Fcgr1-Cre;R26-iDTR mice and their corresponding R26-iDTR controls revealed no significant changes in body weight following DT-mediated macrophage depletion. As expected, body weight progressively increased over time in pregnant mice and decreased postpartum following parturition (**Fig. 3B**). Non-fasting, 2-hour fasting, and 6-hour fasting blood glucose levels remained similar between macrophage-depleted and control mice across all groups (**Fig. 3C, D, and supplementary Fig. S2**).

To determine whether macrophage depletion affects beta cell function, intraperitoneal glucose tolerance tests (IPGTTs) were performed. No significant differences in glucose disposal were detected between macrophage-depleted and control mice in any physiological state, indicating preserved beta cell function and glucose clearance (**Fig. 3E, F**). Interestingly, postpartum mice exhibited lower glycemic excursions and a reduced area under the curve compared to both pregnant and non-pregnant mice in both experimental and control animals. However, these differences could not be statistically evaluated due to a limited sample size.

To evaluate the effect of macrophage depletion on peripheral insulin sensitivity, insulin tolerance tests (ITTs) were performed in experimental and control mice under non-pregnant, pregnant, and postpartum conditions. Insulin sensitivity remained largely unaffected by macrophage depletion across all conditions (**Fig. 3G, H and supplementary Fig. S3**). An

apparent difference was observed in the postpartum group between experimental and control animals (**Fig. 3G, H**). However, this observation should be interpreted with caution since the control group consisted of only a single animal.

Macrophage depletion does not affect peak beta cell proliferation at mid-gestation, despite an altered beta cell volume

To assess the effect of macrophage depletion on beta cell mass expansion during pregnancy, with a particular focus on beta cell proliferation, pancreas sections from pregnant, postpartum, and non-pregnant female *Fcgr1-Cre;R26-iDTR* mice and their respective controls were co-immunostained for insulin, NKX6.1, and Ki67 (**Fig. 4A**). Following depletion of *Fcgr1*⁺ cells, no significant differences were observed in the proportion of proliferating beta cells between experimental and control groups in either pregnant or non-pregnant females. As expected, pregnancy induced a robust increase in beta cell proliferation compared to non-pregnant littermates. Importantly, this increase was preserved upon macrophage depletion, indicating that mid-gestational beta-cell proliferation appears macrophage-independent. Although a modest difference was observed between postpartum experimental and control mice, statistical comparison was not feasible due to the limited sample size (**Fig. 4A, B**).

Beta cell area was calculated by quantifying the insulin-positive area relative to total pancreas area. In control animals, the insulin-positive fraction was significantly increased in both pregnant and postpartum mice compared to non-pregnant females, consistent with physiological beta cell mass expansion (% insulin volume/total pancreas volume: 0.59 ± 0.018 in NP-CTRL vs. 0.78 ± 0.040 in G14.5-CTRL; $p = 0.0021$) (**Fig. 4C**). Notably, this increase was attenuated in macrophage-depleted pregnant mice, resulting in a significant reduction compared to their control littermates (% insulin volume/total pancreas volume: 0.78 ± 0.040 in G14.5-CTRL vs. 0.63 ± 0.075 in G14.5-EXP; $p = 0.029$) (**Fig. 4C**). Together, these data indicate that, although macrophages are dispensable for mid-gestation peak beta cell proliferation, they appear to contribute to the regulation of beta cell mass expansion through alternative mechanism.

Discussion

Despite significant progress in identifying the pathways and factors regulating beta cell proliferation, the development of safe and effective strategies to restore the beta cell mass using mitogenic cues in the context of diabetes remains challenging (33). Understanding the mechanisms governing beta cell mass plasticity and the balance between proliferation and apoptosis remains a key priority in diabetes research (5, 34). Pregnancy offers a unique physiological model to study these processes, as beta cell mass dynamically adapts to increased insulin demands and subsequently returns to baseline postpartum levels (5, 6, 35). Understanding how this reversible process is regulated is highly relevant, as failure of these adaptive mechanisms contribute to the development of gestational diabetes (6, 33). Emerging evidence suggests that islet-resident macrophages may play a role in this remodeling process, but their precise contribution remains incompletely understood. This thesis therefore aimed to characterize pregnancy-induced beta cell adaptations and elucidate the contribution of macrophages to this dynamic process using a transgenic macrophage depletion model.

We first sought to define the temporal dynamics of beta cell mass adaptation during pregnancy and postpartum in mouse. Consistent with previous studies, beta cell proliferation peaked at mid-gestation (G14.5) and declined toward the postpartum period (5, 6, 15). Although postpartum regression of the beta cell mass is often attributed to increased apoptosis (6), we did not observe elevated beta cell apoptosis at PP5. These findings are in line with earlier reports and suggest that postpartum beta cell mass regression is primarily driven by reduced proliferation and, potentially, decreased cell size, rather than increased cell death (19, 34, 36).

The role of macrophages in these dynamics remains debated. While some studies report increased macrophage abundance in the pancreas during gestation (17), others reported a postpartum-specific increase (19). In agreement with the latter, we found that intra-islet macrophage numbers remained stable during pregnancy but increased significantly at PP5. These observations suggest that macrophage accumulation is not required for the gestational increase in beta cell proliferation but instead points toward a potential role for the specific

macrophage phenotype or functional state involved. Future studies focusing on macrophage polarization and secretory profiles may therefore provide important novel insights.

To directly assess macrophage contribution, we employed the Fcgr1-Cre;R26-iDTR macrophage depletion model and focused on two key timepoints: G14.5 (peak beta cell proliferation) and PP5 (peak macrophage abundance). Although DT administration resulted in a marked reduction in islet macrophage numbers, complete depletion was not achieved. A residual population of proliferating macrophages persisted across all experimental conditions, consistent with the well-described self-renewing capacity of tissue-resident macrophages (37). These cells are known to maintain their population through local proliferation and can rapidly repopulate depleted niches (37, 38). Additionally, monocyte recruitment may contribute to replenishment following depletion (24, 37). Together, these mechanisms likely explain the incomplete depletion observed in our study.

Notably, the capacity of islet-resident macrophages to rapidly repopulate the depleted macrophage niche likely underscores their importance in maintaining islet homeostasis. To further elucidate the origin of the remaining macrophage population after depletion, additional characterization using markers specific for monocyte-derived or tissue-resident macrophages will be required. However, distinguishing these populations remains challenging due to the substantial overlap in cell surface marker expression (39). Nevertheless, several studies have proposed candidate markers to discriminate monocyte-derived from tissue-resident macrophages across tissues, including CXCR3 and Ly6C as monocyte specific markers (19, 39, 40), and TIMD4 or FOLR2 as tissue-resident markers (41, 42). For pancreas specifically, Van Gassen et al. used CCR2-KO mice to distinguish the contribution of monocyte-derived versus tissue-resident macrophages during tissue injury responses (24, 25).

Given the increasing evidence that islet-resident macrophages contribute to beta cell proliferation and functional adaptations during metabolic stress, we hypothesized that their depletion would impair beta cell mass remodeling and thereby compromise glucose homeostasis. However, macrophage depletion did not alter body weight nor non-fasting, or fasting glycemia under non-pregnant, pregnant, or postpartum conditions. These findings indicate that, despite macrophage depletion, the remaining beta cell mass and functional

beta cell capacity were still sufficient to maintain normoglycemia in the absence of a metabolic challenge (15). Similarly, glucose tolerance remained unchanged during pregnancy and postpartum. These findings are in contrast with previous studies reporting an impaired glucose response after macrophage depletion (15, 17, 19). Such contrasting results may be explained by differences in depletion models or timing of macrophage depletion. In addition, the residual macrophage population may still have been sufficient to preserve normal beta cell function. To fully exclude the possibility of macrophage depletion affecting beta cell function, additional in vitro glucose-stimulated insulin secretion (GSIS) assays should be performed (15).

As the *Fcgr1-Cre;R26-iDTR* model does not selectively target islet-resident macrophages, DT administration results in systemic macrophage depletion. Given the known role of adipose tissue-resident macrophages in regulating insulin sensitivity, we additionally evaluated peripheral insulin responsiveness (43, 44). Following macrophage depletion, no significant differences in insulin sensitivity were observed between experimental and control groups, indicating that macrophage depletion did not markedly affect peripheral insulin responsiveness.

Although the metabolic readouts were largely unaffected, we further sought to elucidate the underlying mechanisms through which macrophages may contribute to pregnancy-associated beta cell mass adaptations. Several studies have reported that macrophage depletion impairs gestational beta cell proliferation, resulting in reduced beta cell mass expansion during pregnancy (15, 17). In contrast, our findings demonstrate that beta cell proliferation did not change following macrophage depletion, suggesting that islet-resident macrophages may not be essential regulators of beta cell proliferation during pregnancy. These discrepancies with published reports may be explained by the use of different depletion models and depletion regimens, including longer periods of diphtheria toxin administration. Additionally, the sample size of the pregnant experimental group should be increased before drawing definitive conclusion, as we observed considerable variability among the datapoints within this group.

Interestingly, despite unchanged beta cell proliferation, we observed a significant reduction in beta cell mass expansion during pregnancy compared to controls. These findings suggest

that macrophages may contribute to gestational beta cell mass expansion through mechanisms independent of beta cell proliferation, such as by affecting beta cell size (34). During the postpartum period, macrophage depletion inhibits beta cell mass regression, resulting in the persistence of an enlarged beta cell mass, comparable to that observed during mid-gestation (19). However, due to the limited sample size in our postpartum group, no definitive conclusions can currently be drawn regarding their role in beta cell mass regression. Additional experiments should be performed to establish a larger sample size and multiple postpartum timepoints should be included to further characterize these dynamics.

A limitation of our study is the persistence of a residual macrophage population following macrophage depletion. The presence of this remaining macrophage population limits the ability to draw definitive conclusions regarding the precise contribution of islet-resident macrophages to pregnancy- and postpartum-associated beta cell mass adaptations, as this residual population may still exert biologically relevant effects on beta cell remodeling. To achieve a more complete macrophage depletion, future studies could target CSF1R, as CSF1 is a critical survival factor for macrophages and its inhibition could efficiently deplete the residual population (21, 41, 45). In addition, further characterization of the islet-resident macrophage population is required to identify more selective markers that would allow a more specific targeting of the islet-resident macrophage population and prevent off-target effects associated with whole-body macrophage depletion.

Although pregnancy-associated beta cell mass plasticity is well described in rodents, its translational relevance to human remains unclear. In human, beta cell proliferation during pregnancy is relatively low and expansion of the beta cell mass appears to involve formation of small islets rather than enlargement of existing ones (6, 35, 46). This suggests a more pronounced role for de novo beta cell generation (neogenesis) rather than proliferation (6). Consequently, it remains unclear whether macrophages exert similar effects on human beta cells, which are generally considered less plastic and more resistant to proliferative stimuli than rodent beta cells (13, 35, 46).

In conclusion, our study demonstrates that beta cell proliferation peaks at mid-gestation and declines postpartum, whereas macrophage abundance increases predominantly after parturition. Although macrophage depletion did not impair glucose homeostasis nor beta cell proliferation, it attenuated beta cell mass expansion during pregnancy. Together, these results suggest that macrophages contribute to beta cell remodeling through mechanisms independent of direct regulation of proliferation, emphasizing the complexity of immune–endocrine interactions in metabolic adaptation. A deeper understanding of how beta cell mass can be induced to expand in a controlled and reversible manner may ultimately enable the development of novel strategies to safely increase the functional beta cell mass for therapeutic purposes.

Materials and methods

Animal procedures

All procedures were approved by the Ethical Committee of the Vrije Universiteit Brussel and were performed in accordance with institutional and national guidelines. Mice were housed under standard housing conditions in individually ventilated cages with ad libitum access to water and food and kept on a 12/12-hour light-dark cycle. C57BL/6JRj (Janvier, Saint Berthevin, France) and Fcgr1-Cre;R26-iDTR mice were used for experiments. Fcgr1-Cre;R26-iDTR mice were obtained by crossing Fcgr1-Cre with R26-iDTR mice, which were generously provided by Prof. Movahedi (BCIM, VUB). Pregnant C57BL/6JRj mice were purchased or pregnancy was induced by timed mating of 10-week-old nulliparous female Fcgr1-Cre;R26-iDTR mice with syngeneic males. To synchronize estrus, females were exposed for 72 hours to male bedding after which males were placed overnight into female cages. The following morning, males and females were separated and this timepoint was considered day post coitum 0.5. Age-matched non-pregnant female mice were used as controls. Throughout the experimental period, body weight and blood glucose levels (non-fasting between 8-10 AM and 2 hours fasting) were monitored using a glucometer (Glucomen Aero, Menarini, Machelen, Belgium). Measurements were performed at gestational days 7.5 and 13.5 to assess the effects of macrophage depletion during pregnancy, and at gestational day 17.5 and postpartum day 4 to evaluate its effect during postpartum. For the IPGTT, Fcgr1-Cre;R26-iDTR mice were fasted for 6 hours, with free access to water. Subsequently, all mice received an intraperitoneal injection of glucose (2 g D-glucose/kg body weight). Blood glucose levels were subsequently measured at 15, 30, 60, 90, and 120 minutes post-injection. For the ITT, Fcgr1-Cre;R26-iDTR mice were fasted for 6 hours. Thereafter, mice were intraperitoneally injected with insulin (0.75 IU insulin/kg body weight, Actrapid, NovoNordisk, Bagsvaerd, Denmark). Glycemia was measured at 0, 15, 30, and 60 minutes after insulin injection. To achieve macrophage depletion, Fcgr1-Cre;R26-iDTR mice received 4 intraperitoneal injections of diphtheria toxin (DT, 250 μ L per injection, 500 ng total per mouse, Sigma-Aldrich, St.Louis, MO, USA) dissolved in PBS every other day starting at gestational day 7.5 or 17.5. DT-injected single transgenic mice (R26-iDTR) were used as controls. Mice were sacrificed at gestational

day 14.5 or at postpartum day 5, after which the pancreas and spleen were collected for further analysis.

Genotyping

Genotyping was performed using the Phire Animal Tissue Direct PCR Kit (Thermo Fisher Scientific, Waltham, MA, USA). Ear tissue samples of 3-week-old mice were digested for 30 min at 56°C in 50 µL buffer L (Mouse Direct PCR kit (For Genotyping), Selleck Biotechnology, Cologne, Germany) supplemented with 2 µL protease (Mouse Direct PCR kit (For Genotyping)) and inactivated for 5 min at 95°C. Samples were vortexed and centrifuged for 30 sec at 13600 RPM. The polymerase chain reaction (PCR) mix consisted of 12 µL nuclease free water, 5 µL 2x Phire Animal Tissue PCR Buffer, 0.31 µL of 20 µM forward and reverse primer (**Supplementary Table 1**), 0.20 µL Phire Hot Start II DNA Polymerase, and 2 µL of the digested ear tissue sample. DNA was amplified using the GeneAmp PCR System 2700 (Applied Biosystem, Thermo Fisher Scientific, Waltham, MA, USA) with 1 cycle of initial denaturation (5 min 98°C), 35 cycles of amplification (denaturation: 5 sec 98°C, annealing: 5 sec 62°C, extension: 20 sec 72°C), and 1 cycle of final extension (1 min 72 °C). PCR samples were run for 50 min at 130V on a 1.5% agarose gel containing ethidium bromide in 1x TBE buffer.

Tissue processing

Animals were anaesthetized by injecting a mix of ketamine (100 mg/kg body weight) and xylazine (10 mg/kg body weight) intraperitoneally. To remove blood cells and minimize background signal in staining, systemic perfusion with phosphate-buffered saline (PBS; Gibco, Thermo Fisher Scientific, Waltham, MA, USA) was performed. Pancreas and spleen were harvested and fixed for 24h at room temperature under gentle orbital shaking in 4% neutral-buffered formaldehyde (VWR International, Radnor, PA, USA). Tissues were embedded in paraffin (Leica TP1020 Tissue Processor, Leica Biosystems, Nussloch, Germany), cut at 4 µm thickness with a rotary microtome (HM3553, Eppendorf, Breda, The Netherlands), and mounted on Superfrost Plus slides (VWR International, Radnor, PA, USA). Slides were dried overnight at 40°C and stored at 15°C.

Immunostaining

Slides were baked for 60 min at 60°C, deparaffinized in xylene (2 x 5 minutes), rehydrated in 95% ethanol (2 x 2 minutes) and rinsed for 2 min under running distilled water. Depending on the antigen, heat-induced epitope retrieval was performed using citrate pH 6.0 (ImTec Diagnostics, Antwerpen, Belgium) in a Retriever (Electron Microscopy Sciences, Hatfield, PA, USA) with cooling down of the slides for 30 min at room temperature. Slides were rinsed with distilled water (2 min) and transferred to a staining dish containing PBST (PBS + 0.1 % Tween-20 (VWR International)). Next, sections were covered for 30 min with Casein Blocking Buffer (Blocker Casein in PBS (Thermo Fisher Scientific, diluted 1:4 in PBST), followed by incubation overnight at 4°C with the primary antibody mix (**Supplementary Table 2**), diluted in PBST. The following day, slides were washed (3 x 10 min) in PBST, and incubated for 1 hour with the secondary antibody mix (**Supplementary Table 3**), diluted in PBST and supplemented with Hoechst (1/500). After washing (3 x 10 min) in PBST, slides were mounted with Fluoroshield Mounting Medium (Abcam, Cambridge, UK) and dried overnight.

For Immunohistochemical staining for beta cell area/mass measurements, sections were processed as above and incubated overnight at 4°C with guinea pig anti-insulin antibody (1:3000). The following day, slides were washed in PBST (3 x 10 min) and incubated for 30 min with biotin-SP donkey anti-guinea pig (1:500 in PBST). After washing in PBST (3 x 10 min), sections were incubated during 30 min with Vectastain ABC-AP (Vector Laboratories, Burlingame, CA, USA). After washing in PBST (3 x 10 min), color was developed for 5 min with ImmPACT Vector Red (Vector Laboratories). Slides were rinsed in distilled water (2 min), dehydrated through 99% ethanol (2 x 2 min), cleared in xylene (2 x 2 min) and mounted with Pertex (VWR International).

TUNEL staining

For TUNEL staining, slides were subjected to heat-induced epitope retrieval as described above using citrate pH 6.0 and subsequently incubated for 1 hour at 37°C under Hybrid-slips (Sigma-Aldrich) with TUNEL reaction mix (40 U/μL TdT (Roche, Basel, Switzerland), 3.33 μM AlexaFluor 488-11-dUTP (Jena Bioscience, Jena, Germany) in TUNEL buffer (25 mM Tris-HCl pH6.6, 0.2 M sodium cacodylate trihydrate (Sigma-Aldrich, St.Louis, MO, USA), 2.5 mM cobalt

chloride hexahydrate (Sigma-Aldrich), 0.25 mg/mL BSA (Sigma-Aldrich) in nuclease-free water).

Image acquisition and analysis

Fluorescent images were acquired using an Olympus BX61 microscope (Olympus, Tokyo, Japan). Brightfield images were captured using an Aperio GT 450 Digital Pathology Slide Scanner (Leica Biosystems). Image analysis was performed using Fiji image processing software.

Statistical analysis

GraphPad Prism 11.0 (GraphPad Software, San Diego, CA, USA) was used for statistical analysis. Normality was assessed with the Shapiro-Wilk normality test or by analyzing QQ plots. Different experimental groups were compared using One-Way ANOVA or Two-Way ANOVA. P-values were considered statistically significant if $p \leq 0.05$. *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$, ****: $p \leq 0.0001$, ns: not significant ($p > 0.05$). Data are represented as mean $\pm$ standard error of the mean (SEM) and single data points are represented as dots within each group.

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For basic language support, including grammar and spelling corrections, AI tools were used.

Figures

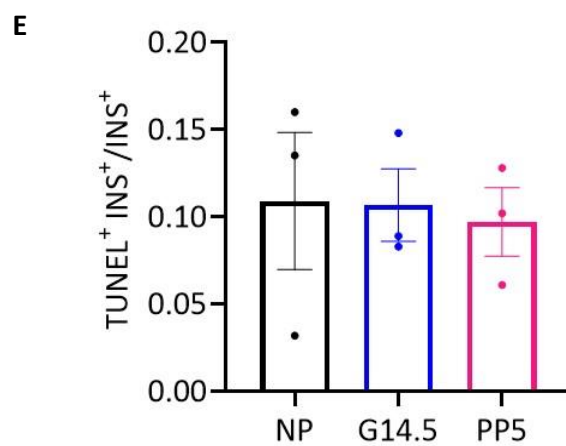
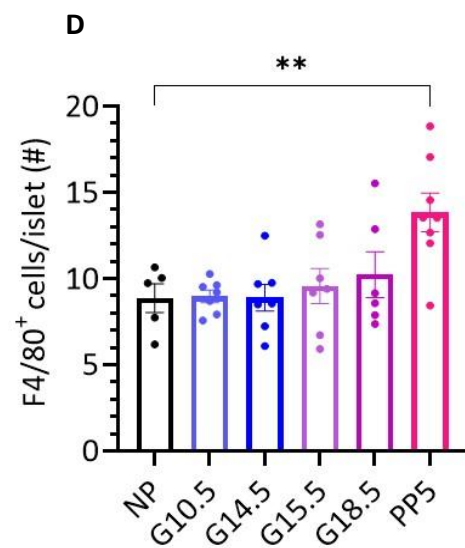
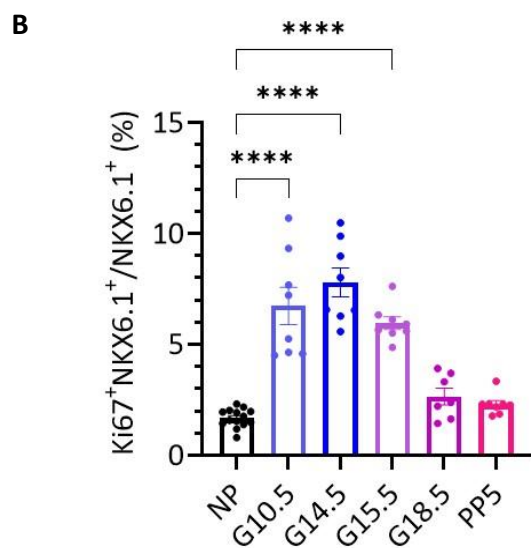
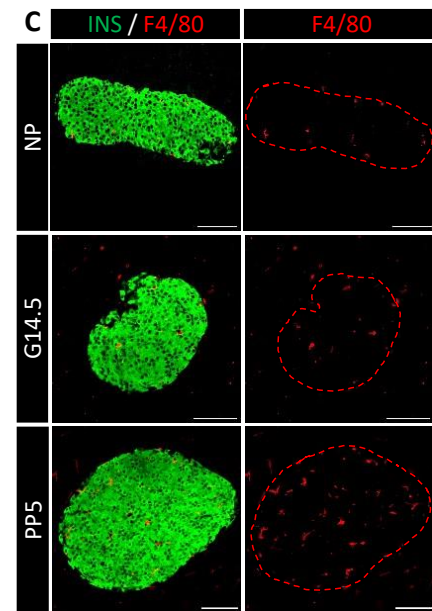
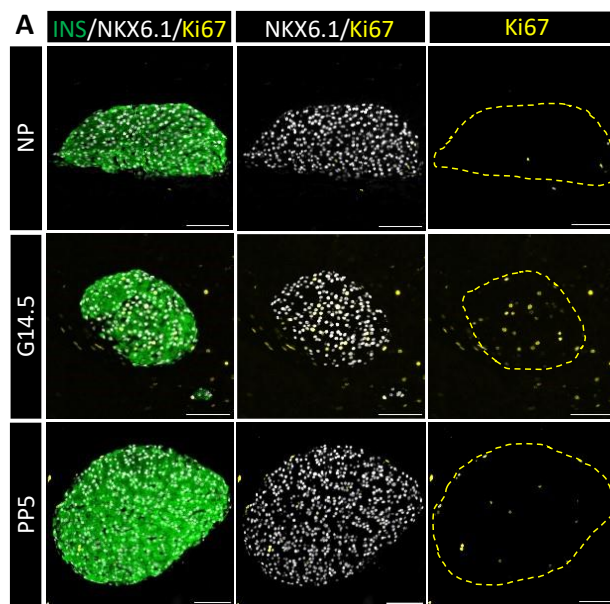


Figure 1: Beta cell proliferation peaks mid-gestation and declines postpartum with islet macrophage abundance only rising postpartum. (A) Immunostaining for insulin (INS, green), NKX6.1 (white), and proliferation marker Ki67 (yellow) on pancreas sections of 12-14 week-old C57BL/6J mice on gestational day (G)14.5, postpartum (PP) day 5, or non-pregnant (NP) female controls. **(B)** Quantification of beta cell proliferation at different gestational timepoints (G10.5, G14.5, G15.5, and G18.5), postpartum day 5, and non-pregnant controls, expressed as percentage of Ki67⁺NKX6.1⁺/NKX6.1⁺. Female vs. G10.5 ****: $p < 0.0001$; Female vs. G14.5 ****: $p < 0.0001$; Female vs. G15.5 ****: $p < 0.0001$. **(C)** Immunostaining for insulin (INS, green) and the pan-macrophage marker F4/80 (red) on pancreas sections of 12-14 week-old C57BL/6J mice on gestational day 14.5, postpartum day 5, or non-pregnant controls. **(D)** Quantification of macrophage abundance at different gestational timepoints (G10.5, G14.5, G15.5, and G18.), postpartum day 5, and non-pregnant controls expressed as the number of F4/80⁺ cells per islet. Female vs. PP5 ** $p = 0.0056$. **(E)** Quantification of beta cell death, expressed as the percentage of TUNEL⁺INS⁺/INS⁺ cells at mid-gestation (G14.5), postpartum (PP5), and from non-pregnant C57BL/6J controls. Scale bars: 100 μm . Data are represented as mean $\pm$ SEM and were analyzed with a One-Way ANOVA test. Three datapoints from each individual group were analyzed by the author and added to the existing datasets from B and D.

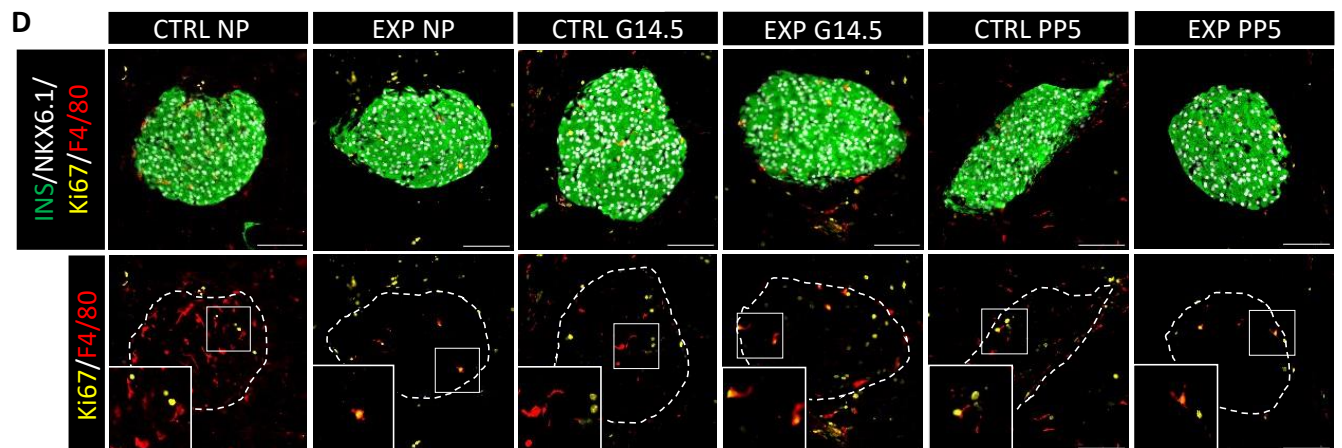
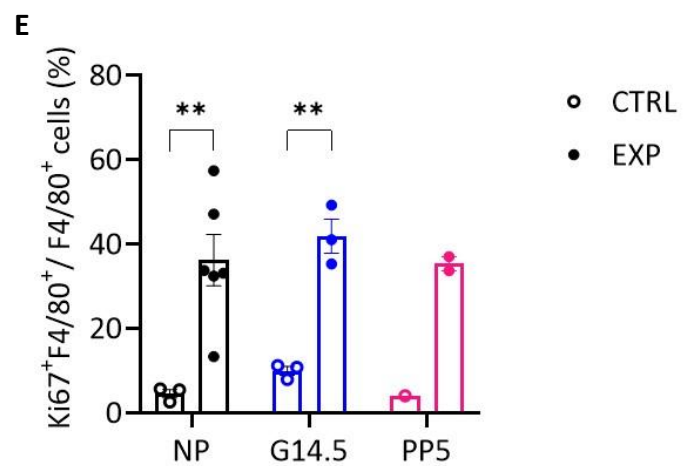
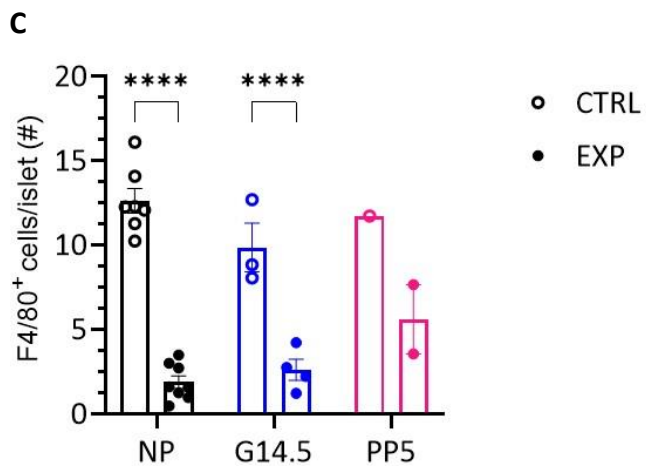
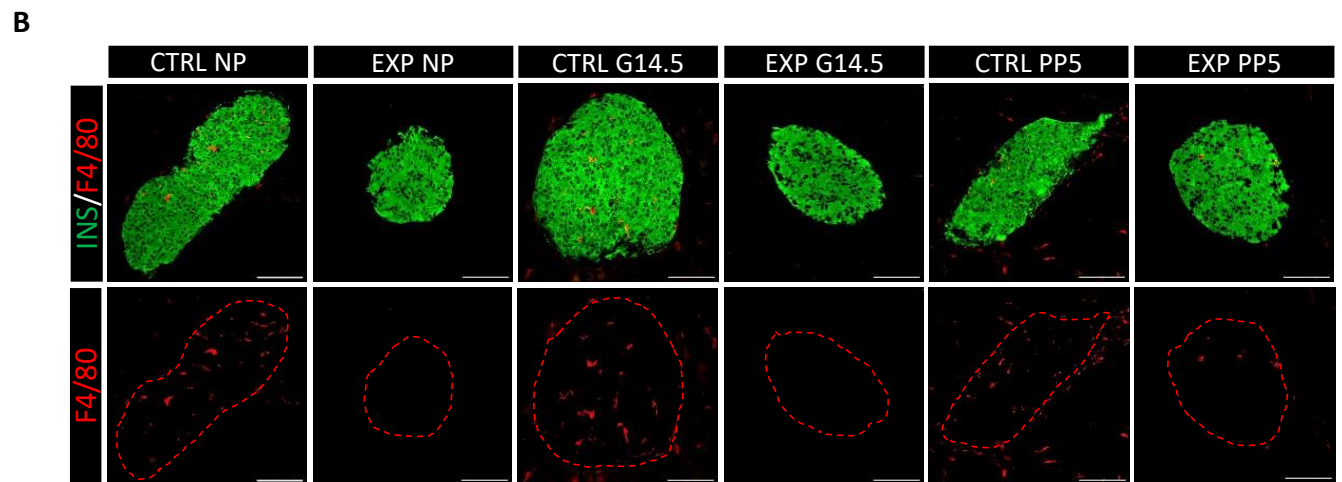
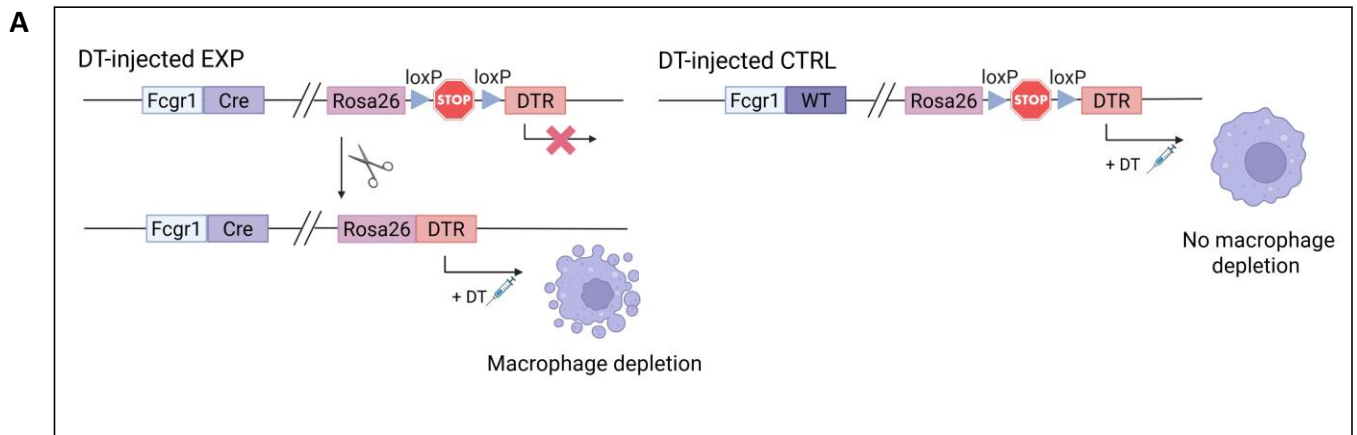


Figure 2. The Fcgr1-Cre;R26-iDTR model enables efficient macrophage depletion accompanied by compensatory macrophage proliferation. (A) Schematic overview of the Fcgr1-Cre;R26-iDTR and control construct. **(B)** Immunostaining for Insulin (INS) and the pan-macrophage marker F4/80 on pancreas sections from Fcgr1-cre;R26-iDTR mice (EXP) and R26-iDTR mice (CTRL), either non-pregnant, pregnant (G14.5), or postpartum (PP5). **(C)** Quantification of F4/80⁺ cells per islet for both experimental and control mice, either non-pregnant, pregnant, or postpartum. NP: CTRL vs. EXP ****: $p < 0.0001$; G14.5: CTRL vs. EXP ****: $p < 0.0001$. **(D)** Immunostaining for F4/80 and proliferation marker Ki67 on control and experimental pancreas sections harvested at G14.5 (pregnant) and PP5 (postpartum). Inset image corresponds to a 100 μm field of view. **(E)** Quantification of the number of proliferating islet macrophages expressed as islet Ki67⁺F4/80⁺ cells over the total islet F4/80⁺ population across all conditions. NP: CTRL vs. EXP **: $p = 0.0026$; G14.5: CTRL vs. EXP **: $p = 0.0069$. Scale bars: 100 μm . Data are represented as mean $\pm$ SEM and were analyzed with a Two-Way ANOVA test. In this figure, the macrophage proliferation data were analyzed by the author.

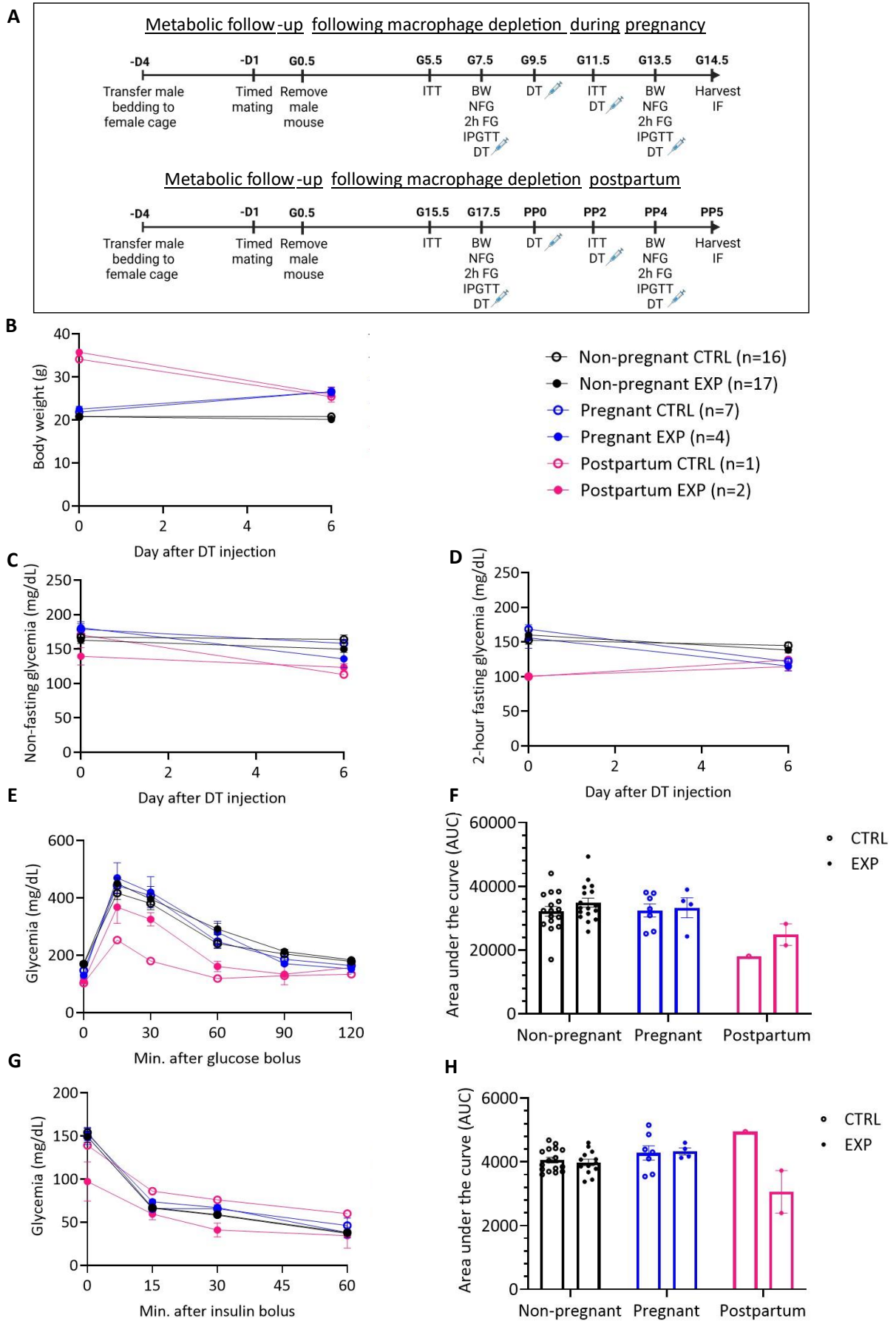


Figure 3. Macrophage depletion does not alter glucose homeostasis during pregnancy. (A)

Overview of the experimental set-up during pregnancy and postpartum with the non-pregnant control group undergoing identical experimental procedures as the pregnant/postpartum mice. **(B)** Body weight of Fcgr1-cre;R26-iDTR mice across non-pregnant, pregnant, and postpartum conditions before and after DT injections. **(C, D)** Non-fasting glycemia and 2-hour fasting glycemia across all conditions before and after DT administration. **(E, F)** IPGTTs performed after DT injections in experimental and control mice under pregnant, postpartum, and non-pregnant conditions. **(E)** Glycemia was measured at 0, 30, 60, 90, and 120 minutes after glucose bolus. **(F)** Area under the curve (AUC) was calculated to quantify glucose homeostasis after macrophage depletion. **(G, H)** ITTs performed after DT injections in experimental and control mice under pregnant (EXP; n=4, CTRL; n=7), postpartum (EXP; n= 2, CTRL; n= 1), and non-pregnant conditions (EXP; n= 13, CTRL; n=16). **(G)** Blood glucose levels were measured at 0, 15, 30, and 60 minutes after insulin bolus. **(H)** Area under the curve (AUC) was calculated to quantify insulin sensitivity in each condition. Data are represented as mean $\pm$ SEM and were analyzed with a Two-Way ANOVA test.

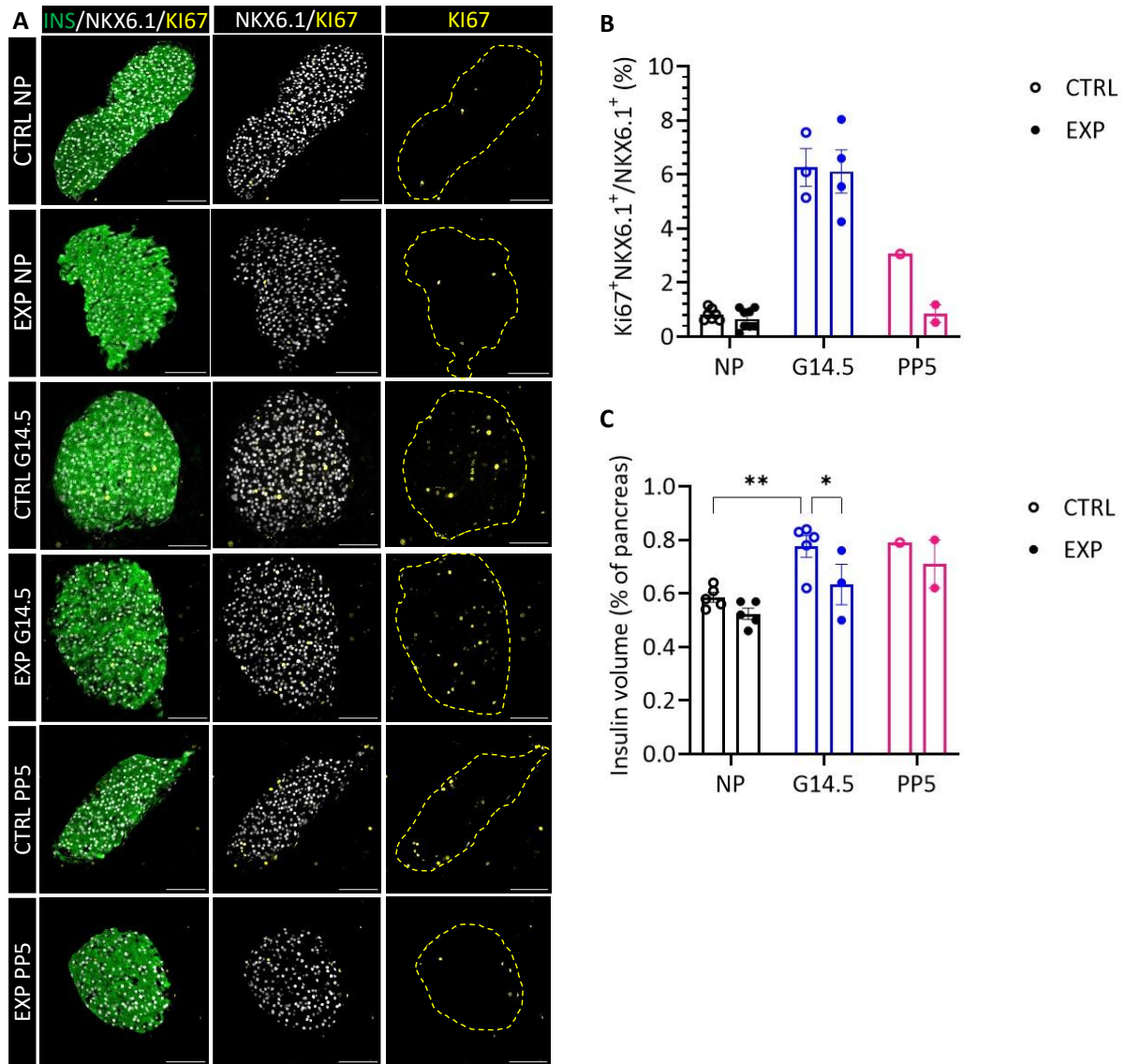
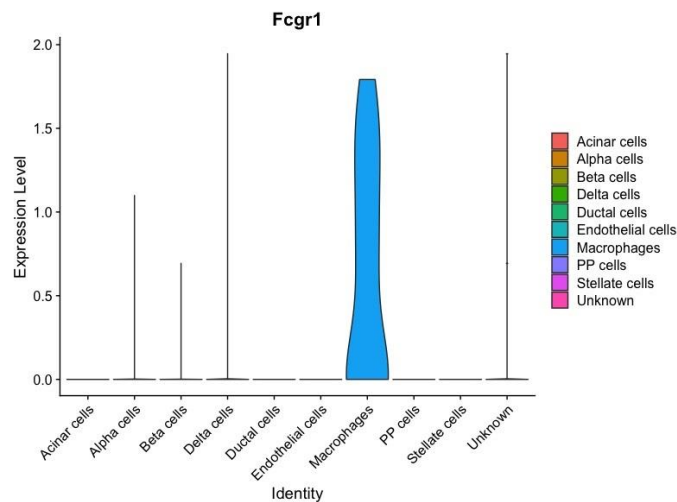


Figure 4. *In vivo* macrophage depletion does not alter mid-gestation peak beta cell proliferation despite a decreased beta cell volume. (A) Immunostaining for Insulin (INS), the beta cell marker NKX6.1, and the proliferation marker Ki67 on pancreas sections from non-pregnant, pregnant, and postpartum mice at gestational timepoint G14.5 and postpartum day 5 to evaluate beta cell proliferation after macrophage depletion. **(B)** Quantification of proliferating beta cells represented as Ki67⁺NKX6.1⁺ cells relative to the total NKX6.1⁺ population. **(C)** Quantification of insulin volume normalized to the total pancreas volume, expressed as the percentage of insulin positive area to total pancreas area for control and experimental mice across pregnant, postpartum, and non-pregnant conditions. G14.5: CTRL vs. EXP *: $p=0.0287$; CTRL: NP vs. G14.5 **: $p=0.0021$. Scale bars: 100 μ m. Data are represented as mean $\pm$ SEM and were analyzed with a Two-Way ANOVA test

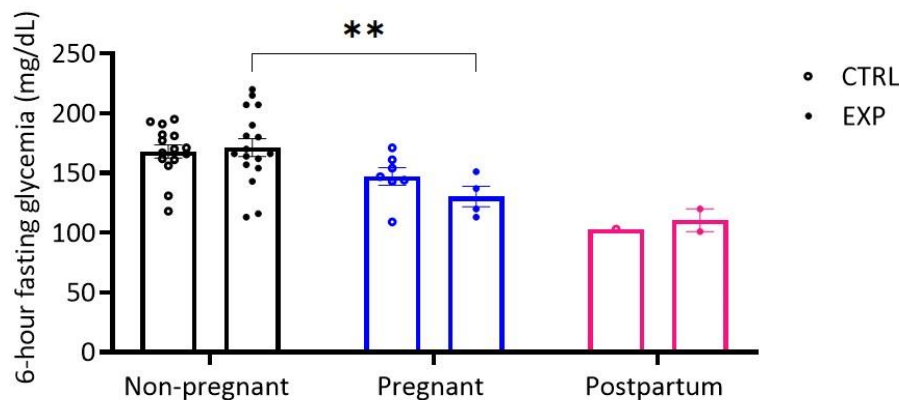
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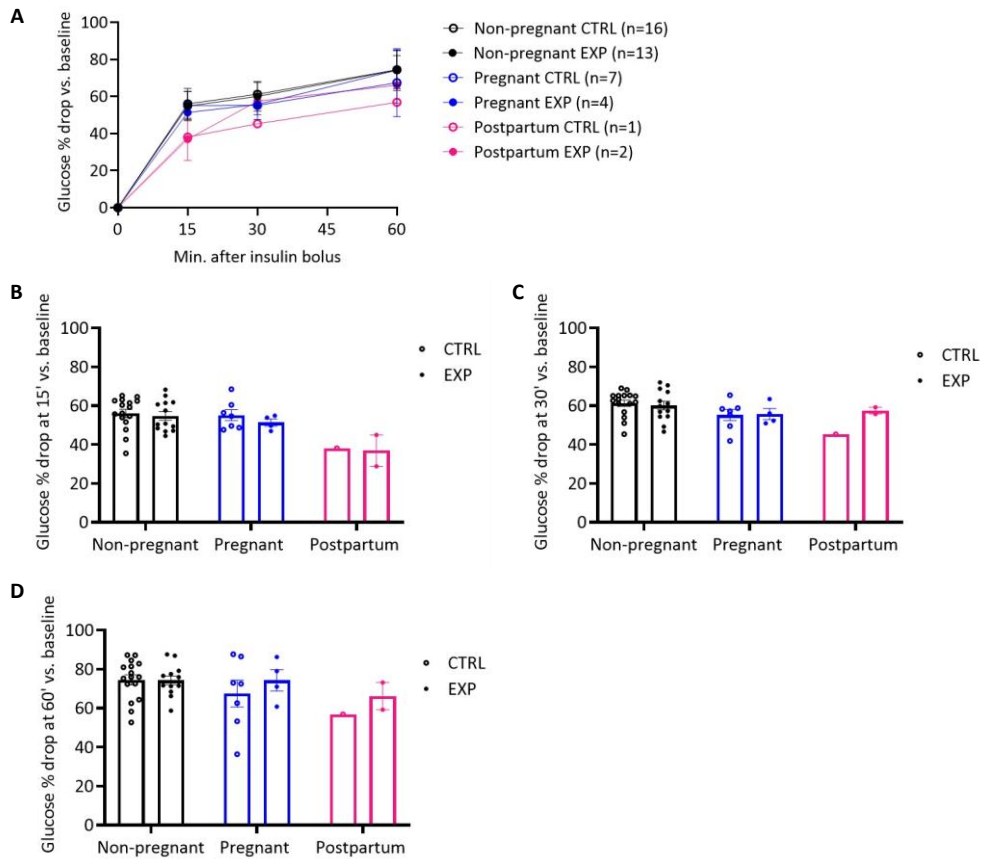
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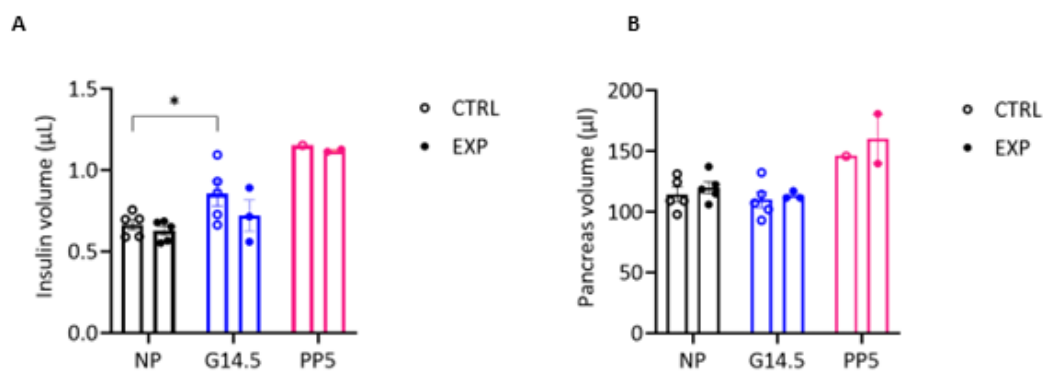
Supplementary Figure S1. Islet-specific Fcgr1 expression. Single-cell RNA sequencing of C57BL/6 islet cells shows Fcgr1 expression in islet macrophages (data provided by Annelore Van Mulders, BENE).



Supplementary Figure S2. Effect of macrophage depletion on 6-hour fasting glycemia. 6-hour fasting glycemia (mg/dL) measured during IPGTT for Fcgr1-cre;R26-iDTR mice and their respective control in pregnant, postpartum, and non-pregnant females.



Supplementary Figure S3. Effect of macrophage depletion on blood glucose decrease at 15, 30, and 60 minutes following insulin injection. (A) Blood glucose decrease at 15', 30', and 60' after insulin injection, expressed as percentage across all conditions. **(B-D)** Blood glucose drop at 15', 30', and 60' after insulin injection across all conditions.



Supplementary Figure S4. Effect of macrophage depletion on insulin volume and pancreas volume. Quantification of insulin volume (A) and pancreas volume (B) expressed in μL for Fcgr1-cre;R26-iDTR mice and their respective control in pregnant, postpartum, and non-pregnant females.

Supplementary Table S1: Primers used for genotyping. All primers were purchased from Invitrogen.

Name	Target	5'-3' sequence
Fcgr1-iCre	Wild type Forward	CCCTTCCTCCCAGTGACACTACTG
Fcgr1-iCre	Mutant Forward	GACGGCATGGACGAGCTGTACA
Fcgr1-iCre	Common Reverse	TGAACCCATCCACCCTGTGAG
DTR (IMR8052)		GCGAAGAGTTTGTCTCAACC
DTR (IMR8545)		AAAGTCGCTCTGAGTTGTTAT
DTR (IMR8546)		GGAGCGGGAGAAATGGATATG

Supplementary Table S2: Primary antibodies used for immunostaining.

Target	Host species	Heat-induced antigen retrieval	Dilution	Supplier
INS	Guinea Pig	/	1:5000	DRC VUB
F4/80	Rabbit	/	1:500	Cell Signaling
NKX6.1	Goat	10 mM Citrate pH 6	1:500	R&D Systems
Ki67	Rat	10 mM Citrate pH 6	1:5000	eBioscience

Supplementary Table S3: Secondary antibodies used for immunostaining.

All antibodies were purchased from Jackson ImmunoResearch.

Fluorophore	Target species	Host species	Dilution
Alexa Fluor 488	Guinea Pig	Donkey	1:500
Cyanine 3	Rabbit	Donkey	1:500
Alexa Fluor 647	Goat	Donkey	1:500
DyLight 755	Rat	Donkey	1:500

Supplementary Table S4. Statistical analysis of beta cell proliferation across pregnancy and postpartum in C57BL/6J mice.

Ki67 across pregnancy and postpartum in C57BL/6J mice						
Descriptive statistics						
	NP	G10.5	G14.5	G15.5	G18.5	PP5
Number of values	14	8	8	8	7	8
Mean	1.687	6.739	7.792	5.958	2.661	2.298
Std. Deviation	0.416	2.381	1.826	0.804	0.990	0.471
Std. Error of Mean	0.111	0.842	0.646	0.284	0.374	0.167
Test for normal distribution: Shapiro-Wilk test						
W	0.968	0.873	0.914	0.908	0.914	0.790
P value	0.848	0.162	0.380	0.337	0.424	0.0225
Passed normality test (alpha=0.05)?	Yes	Yes	Yes	Yes	Yes	No
P value summary	ns	ns	ns	ns	ns	*
Ordinary One-way ANOVA: multiple comparisons						
Dunnett's multiple comparisons test	Mean diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P value	
NP vs. G10.5	-5.051	-6.550 to -3.553	Yes	****	<0.0001	
NP vs. G14.5	-6.105	-7.604 to -4.606	Yes	****	<0.0001	
NP vs. G15.5	-4.271	-5.770 to -2.772	Yes	****	<0.0001	
NP vs. G18.5	-0.974	-2.540 to 0.591	No	ns	0.382	
NP vs. PP5	-0.611	-2.109 to 0.888	No	ns	0.766	
G14.5 vs. G10.5	1.054	-0.6105 to 2.718	No	ns	0.3397	
G14.5 vs. G15.5	1.834	0.1701 to 3.498	Yes	*	0.0260	
G14.5 vs. G18.5	5.131	3.408 to 6.853	Yes	****	<0.0001	

G14.5 vs. PP5	5.495	3.830 to 7.159	Yes	****	<0.0001
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Supplementary Table S5. Statistical analysis of macrophage abundance across pregnancy and postpartum in C57BL/6J mice.

F4/80⁺ cells/islet across pregnancy and postpartum in C57BL/6J mice						
Descriptive statistics						
	NP	G10.5	G14.5	G15.5	G18.5	PP5
Number of values	5	7	7	7	6	8
Mean	8.875	8.978	8.902	9.570	10.23	13.84
Std. Deviation	1.855	0.9680	2.047	2.696	3.244	3.158
Std. Error of Mean	0.830	0.366	0.774	1.019	1.324	1.116
Test for normal distribution: Shapiro-Wilk test						
W	0.902	0.955	0.961	0.939	0.852	0.965
P value	0.420	0.772	0.825	0.628	0.164	0.857
Passed normality test (alpha=0.05)?	Yes	Yes	Yes	Yes	Yes	Yes
P value summary	ns	ns	ns	ns	ns	ns
Ordinary One-way ANOVA: multiple comparisons						
Dunnett's multiple comparisons test	Mean diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P value	
NP vs. G10.5	-0.103	-3.905 to 3.699	No	ns	>0.9999	
NP vs. G14.5	-0.0274	-3.830 to 3.775	No	ns	>0.9999	
NP vs. G15.5	-0.695	-4.497 to 3.107	No	ns	0.981	
NP vs. G18.5	-1.359	-5.291 to 2.574	No	ns	0.808	
NP vs. PP5	-4.963	-8.665 to -1.261	Yes	**	0.0056	

Supplementary Table S6. Statistical analysis of TUNEL analysis comparing NP, G14.5, and PP5 in C57BL/6J mice.

TUNEL ⁺ INS ⁺ /INS ⁺ across pregnancy and postpartum in C57BL/6J mice					
Descriptive statistics					
	NP		G14.5		PP5
Number of values	3		3		3
Mean	0.109		0.107		0.0970
Std. Deviation	0.0679		0.0359		0.0338
Std. Error of Mean	0.0392		0.0207		0.0195
Test for normal distribution: Shapiro-Wilk test					
W	0.890		0.819		0.984
P value	0.354		0.160		0.755
Passed normality test (alpha=0.05)	Yes		Yes		Yes
P value summary	ns		ns		ns
Ordinary One-way ANOVA: multiple comparisons					
Dunnett's multiple comparisons test	Mean diff.	95.00 % CI of diff.	Significant?	Summary	Adjusted P value
NP vs. G14.5	0.00233	-0.111 to 0.116	No	ns	0.997
NP vs. PP5	0.0120	-0.101 to 0.125	No	ns	0.936

Supplementary Table S7. Statistical analysis of macrophage abundance comparing NP, G14.5, and PP5 after macrophage depletion.

F4/80+ cells/ islet after macrophage depletion						
Descriptive statistics						
	NP		G14.5		PP5	
	CTRL	EXP	CTRL	EXP	CTRL	EXP
Number of values	7	8	3	4	1	2
Mean	12.612	1.890	9.862	2.612	11.728	5.607
Std. Deviation	1.924	1.066	2.484	1.256	0.000	2.906
Std. Error of Mean	0.727	0.377	1.434	0.628	0.000	2.055
Test for normal distribution on residuals: Shapiro-Wilk test						
W	0.921	0.935	0.872	0.985	N too small	
P value	0.477	0.567	0.301	0.930	/	
Passed normality test (alpha=0.05)	Yes	Yes	Yes	Yes	/	
P value summary	ns	ns	ns	ns	/	
Ordinary Two-way ANOVA: multiple comparisons						
Sidak's multiple comparisons test	Mean diff.	95. 00 % CI of diff.	Significant?	Summary	Adjusted P value	
NP: CTRL vs. EXP	10.72	8.404 to 13.04	Yes	****	<0.0001	
G14.5: CTRL vs. EXP	7.250	3.829 to 10.67	Yes	****	<0.0001	

Supplementary Table S8. Statistical analysis of macrophage proliferation comparing NP, G14.5, and PP5 after macrophage depletion.

Ki67 ⁺ F4/80 ⁺ cells after macrophage depletion						
Descriptive statistics						
	NP		G14.5		PP5	
	CTRL	EXP	CTRL	EXP	CTRL	EXP
Number of values	3	6	3	3	1	2
Mean	4.590	36.19	9.993	41.85	4.080	35.35
Std. Deviation	1.709	14.95	1.780	7.007	0.000	2.355
Std. Error of Mean	0.987	6.102	1.028	4.045	0.000	1.665
Test for normal distribution on residuals: Shapiro-Wilk test						
W	0.7965	0.9426	0.8383	0.9889	N too small	
P value	0.1062	0.6799	0.2096	0.7985	/	
Passed normality test (alpha=0.05)	Yes	Yes	Yes	Yes	/	
P value summary	ns	ns	ns	ns	/	
Ordinary Two-way ANOVA: multiple comparisons						
Sidak's multiple comparisons test	Mean diff.	95. 00 % CI of diff.	Significant?	Summary	Adjusted P value	
NP: CTRL vs. EXP	- 31.60	-51.46 to -11.75	Yes	**	0.0026	
G14.5: CTRL vs. EXP	- 31.86	-54.79 to -8.931	Yes	**	0.0069	

Supplementary Table S9. Statistical analysis of body weight comparing NP, G14.5, and PP5.

Body weight across pregnant, postpartum, and non-pregnant conditions							
Descriptive statistics							
		NP		G14.5		PP5	
		CTRL	EXP	CTRL	EXP	CTRL	EXP
Number of values		16	17	7	4	1	2
D0	Mean	20.781	20.782	21.843	22.500	34.100	35.700
	Std. Deviation	1.478	1.528	0.854	1.311	0,000	0.141
	Std. Error of Mean	0.370	0.371	0.323	0.656	0,000	0.100
D6	Mean	20.769	20.100	26.543	26.550	25.400	25.900
	Std. Deviation	1.290	1.496	1.456	1.900	0.000	2.546
	Std. Error of Mean	0.323	0.363	0.550	0.950	0.000	1.800
Test for normal distribution on residuals: Shapiro-Wilk test							
W		0.9989					
P value		>0.9999					
Passed normality test (alpha=0.05)		Yes					
P value summary		ns					
Ordinary Two-way ANOVA: multiple comparisons							
Sidak's multiple comparisons test		Mean diff.	95. 00 % CI of diff.	Significant?	Summary	Adjusted P value	
D0 vs. D6	NP CTRL	0.01250	-0.3889 to 0.4139	No	ns	0.9502	
	NP EXP	0.6824	0.2930 to 1.072	Yes	**	0.0010	
	G14.5 CTRL	-4.700	-5.307 to -4.093	Yes	****	<0.0001	
D6	NP: CTRL vs. EXP	0.669	-0.846to 2.183	No	ns	0.9550	
D6	G14.5: CTRL vs. EXP	-0.00714	-2.733 to 2.719	No	ns	>0.9999	

Supplementary Table S10. Statistical analysis of non-fasting glycemia comparing NP, G14.5, and PP5.

Non-fasting glycemia across pregnant, postpartum, and non-pregnant conditions							
Descriptive statistics							
		NP		G14.5		PP5	
		CTRL	EXP	CTRL	EXP	CTRL	EXP
Number of values		16	17	7	4	1	2
D0	Mean	167.313	162.912	178.857	181.000	170.500	139.500
	Std. Deviation	15.832	20.546	21.583	17.645	0.000	17.678
	Std. Error of Mean	3.958	4.983	8.157	8.822	0.000	12.500
D6	Mean	164.063	149.824	158.143	135.750	113.000	123.500
	Std. Deviation	24.726	20.007	16.324	24.851	0.000	7.778
	Std. Error of Mean	6.182	4.852	6.170	12.426	0.000	5.500
Test for normal distribution on residuals: Shapiro-Wilk test							
W		0.9737					
P value		0.0546					
Passed normality test (alpha=0.05)		Yes					
P value summary		ns					
Ordinary Two-way ANOVA: multiple comparisons							
Turkey's multiple comparisons test		Mean diff.	95. 00 % CI of diff.	Significant?	Summary	Adjusted P value	
D0 vs. D6	NP CTRL	3.250	-11.00 to 17.50	No	ns	0.6512	
	NP EXP	13.09	-0.7326 to 26.91	No	ns	0.0631	
	G14.5 CTRL	20.71	-0.8240 to 42.25	No	ns	0.0592	
	G14.5 EXP	45.25	16.76 to 73.74	Yes	**	0.0022	
D6	NP: CTRL vs. EXP	14.24	-6.350 to 34.83	No	ns	0.341	

D6	G14.5: CTRL vs. EXP	22.39	-14.66 to 59.44	No	ns	0.495
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Supplementary Table S11. Statistical analysis of 2-hour fasting glycemia comparing NP, G14.5, and PP5.

2-hour fasting glycemia across pregnant, postpartum and non-pregnant conditions							
Descriptive statistics							
		NP		G14.5		PP5	
		CTRL	EXP	CTRL	EXP	CTRL	EXP
Number of values		16	17	7	4	1	2
D0	Mean	152.531	160.176	168.429	156.000	100.000	101.000
	Std. Deviation	23.876	26.761	17.038	30.452	0.000	4.243
	Std. Error of Mean	5.969	6.491	6.440	15.226	0.000	3.000
D6	Mean	144.938	138.382	121.857	115.250	124.000	114.500
	Std. Deviation	19.334	17.426	13.434	13.745	0.000	9.192
	Std. Error of Mean	4.833	4.226	5.078	6.872	0.000	6.500
Test for normal distribution on residuals: Shapiro-Wilk test							
W		0.9790					
P value		0.1352					
Passed normality test (alpha=0.05)		Yes					
P value summary		ns					
Ordinary Two-way ANOVA: multiple comparisons							
Turkey's multiple comparisons test		Mean diff.	95. 00 % CI of diff.	Significant?	Summary	Adjusted P value	
D0 vs. D6	NP CTRL	3.250	-11.00 to 17.50	No	ns	0.6512	
	NP EXP	13.09	-0.7326 to 26.91	No	ns	0.0631	
	G14.5 CTRL	20.71	-0.8240 to 42.25	No	ns	0.0592	

	G14.5 EXP	45.25	16.76 to 73.74	Yes	**	0.0022
D6	NP: CTRL vs. EXP	6.555	-14.96 to 28.07	No	ns	0.948
D6	G14.5: CTRL vs. EXP	6.607	-32.11 to 45.33	No	ns	0.996

Supplementary Table S12. Statistical analysis of AUC ITT comparing NP, G14.5 and PP5 after macrophage depletion.

AUC ITT across pregnant, postpartum and non-pregnant conditions after macrophage depletion						
Descriptive statistics						
	NP		G14.5		PP5	
	CTRL	EXP	CTRL	EXP	CTRL	EXP
Number of values	16	13	7	4	1	2
Mean	4057.313	3978.615	4279.571	4325.750	4943.000	3062.000
Std. Deviation	358.087	369.112	597.401	220.436	0.000	941.866
Std. Error of Mean	89.522	102.373	225.796	110.218	0.000	666.000
Test for normal distribution on residuals: Shapiro-Wilk test						
W	0.982					
P value	0.725					
Passed normality test (alpha=0.05)	Yes					
P value summary	ns					
Ordinary Two-way ANOVA: multiple comparisons						
Sidak’s multiple comparisons test	Mean diff.	95. 00 % CI of diff.	Significant?	Summary	Adjusted P value	
NP: CTRL vs. EXP	78.70	-319.6 to 477.0	No	ns	0.9469	
G14.5: CTRL vs. EXP	-46.18	-714.8 to 622.4	No	ns	0.9975	

Supplementary Table S13. Statistical analysis of AUC IPGTT comparing NP, G14.5, and PP5 after macrophage depletion.

AUC IPGTT across pregnant, postpartum, and non-pregnant conditions after macrophage depletion						
Descriptive statistics						
	NP		G14.5		PP5	
	CTRL	EXP	CTRL	EXP	CTRL	EXP
Number of values	16	17	7	4	1	2
Mean	32142.188	34867.765	32405.571	33268.500	18053.000	24859.000
Std. Deviation	6160.302	5777.460	5409.283	6297.138	0.000	4746.101
Std. Error of Mean	1540.075	1401.240	2044.517	3148.569	0.000	3356.000
Test for normal distribution on residuals: Shapiro-Wilk test						
W	0.986					
P value	0.851					
Passed normality test (alpha=0.05)	Yes					
P value summary	ns					
Ordinary Two-way ANOVA: multiple comparisons						
Sidak's multiple comparisons test	Mean diff.	95. 00 % CI of diff.	Significant?	Summary	Adjusted P value	
NP: CTRL vs. EXP	-2726	-7829 to 2378	No	ns	0.471	
G14.5: CTRL vs. EXP	-862.9	-10046 to 8320	No	ns	0.994	

Supplementary Table S14. Statistical analysis of the effect of macrophage depletion on beta cell proliferation comparing NP, G14.5, and PP5.

Ki67+ NKX6.1+ cells after macrophage depletion						
Descriptive statistics						
	NP		G14.5		PP5	
	CTRL	EXP	CTRL	EXP	CTRL	EXP
Number of values	7	8	3	4	1	2
Mean	0.825	0.661	6.262	6.110	3.066	0.858
Std. Deviation	0.215	0.376	1.213	1.601	0.000	0.467
Std. Error of Mean	0.0812	0.133	0.700	0.801	0.000	0.331
Test for normal distribution on residuals: Shapiro-Wilk test						
W	0.8952	0.8624	0.9855	0.9984	N too small	
P value	0.3031	0.1270	0.7694	0.9952	/	
Passed normality test (alpha=0.05)	Yes	Yes	Yes	Yes	/	
P value summary	ns	ns	ns	ns	/	
Ordinary Two-way ANOVA: multiple comparisons						
Sidak's multiple comparisons test	Mean diff.	95. 00 % CI of diff.	Significant?	Summary	Adjusted P value	
NP: CTRL vs. EXP	0.1640	-0.9177 to 1.246	No	ns	0.9719	
G14.5: CTRL vs. EXP	0.1514	-1.445 to 1.748	No	ns	0.9928	

Supplementary Table S15. Statistical analysis of the effect of macrophage depletion on insulin volume (% of pancreas volume) comparing NP, G14.5, and PP5.

Insulin volume (% of pancreas volume)						
Descriptive statistics						
	NP		G14.5		PP5	
	CTRL	EXP	CTRL	EXP	CTRL	EXP
Number of values	5	5	5	3	1	2
Mean	0.5860	0.5240	0.7760	0.6333	0.7900	0.7100
Std. Deviation	0.03975	0.04722	0.09017	0.1301	0.000	0.1273
Std. Error of Mean	0.01778	0.02112	0.04032	0.07513	0.000	0.09000
Test for normal distribution on residuals: Shapiro-Wilk test						
W	0.9758	0.9082	0.7705	0.9980	N too small	
P value	0.9112	0.4567	0.0455	0.9152	/	
Passed normality test (alpha=0.05)	Yes	Yes	No	Yes	/	
P value summary	ns	ns	*	ns	/	
Ordinary Two-way ANOVA: multiple comparisons						
Sidak's multiple comparisons test	Mean diff.	95. 00 % CI of diff.	Significant?	Summary	Adjusted P value	
NP: CTRL vs. EXP	0.06200	-0.04686 to 0.1709	No	ns	0.2436	
G14.5: CTRL vs. EXP	0.1427	0.01696 to 0.2684	Yes	*	0.0287	
CTRL: NP vs. G14.5	-0.1900	-0.2989 to -0.08114	Yes	**	0.0021	
EXP: NP vs. G14.5	-0.1093	-0.2350 to 0.01637	No	ns	0.0835	

Supplementary Table S16. Statistical analysis of the effect of macrophage depletion on insulin volume comparing NP, G14.5 and PP5.

Insulin volume						
Descriptive statistics						
	NP		G14.5		PP5	
	CTRL	EXP	CTRL	EXP	CTRL	EXP
Number of values	5	5	5	3	1	2
Mean	0.668	0.628	0.856	0.722	1.153	1.120
Std. Deviation	0.0732	0.0634	0.170	0.166	0.000	0.0103
Std. Error of Mean	0.0327	0.0284	0.0760	0.0958	0.000	0.00727
Test for normal distribution on residuals: Shapiro-Wilk test						
W	0.865	0.838	0.970	0.999	N too small	
P value	0.248	0.160	0.873	0.930	/	
Passed normality test (alpha=0.05)	Yes	Yes	Yes	Yes	/	
P value summary	ns	ns	ns	ns	/	
Ordinary Two-way ANOVA: multiple comparisons						
Sidak's multiple comparisons test	Mean diff.	95. 00 % CI of diff.	Significant?	Summary	Adjusted P value	
NP: CTRL vs. EXP	0.03966	-0.1191 to 0.1985	No	ns	0.6023	
G14.5: CTRL vs. EXP	0.1336	-0.04982 to 0.3169	No	ns	0.1414	
CTRL: NP vs. G14.5	-0.1884	-0.3472 to -0.02962	Yes	*	0.0231	
EXP: NP vs. G14.5	-0.09454	-0.2779 to 0.08883	No	ns	0.2891	

Supplementary Table S17. Statistical analysis of the effect of macrophage depletion on pancreas volume comparing NP, G14.5, and PP5.

Pancreas volume						
Descriptive statistics						
	NP		G14.5		PP5	
	CTRL	EXP	CTRL	EXP	CTRL	EXP
Number of values	5	5	5	3	1	2
Mean	114.4	119.8	109.9	113.5	145.9	160.3
Std. Deviation	13.34	11.42	14.85	3.296	0.000	28.97
Std. Error of Mean	5.965	5.107	6.640	1.903	0.000	20.49
Test for normal distribution on residuals: Shapiro-Wilk test						
W	0.941	0.967	0.966	0.759	N too small	
P value	0.675	0.853	0.847	0.0204	/	
Passed normality test (alpha=0.05)	Yes	Yes	Yes	No	/	
P value summary	ns	ns	ns	*	/	
Ordinary Two-way ANOVA: multiple comparisons						
Sidak's multiple comparisons test	Mean diff.	95. 00 % CI of diff.	Significant?	Summary	Adjusted P value	
NP: CTRL vs. EXP	-5.458	-24.45 to 13.53	No	ns	0.5493	
G14.5: CTRL vs. EXP	-3.665	-25.59 to 18.26	No	ns	0.7266	
CTRL: NP vs. G14.5	4.528	-14.46 to 23.52	No	ns	0.6187	
EXP: NP vs. G14.5	6.320	-15.61 to 28.25	No	ns	0.5481	

Supplementary Table S18. Statistical analysis of the effect of macrophage depletion on 6-hour fasting glycemia comparing NP, G14.5, and PP5.

6-hour fasting glycemia across pregnant, postpartum and non-pregnant conditions after macrophage depletion						
Descriptive statistics						
	NP		G14.5		PP5	
	CTRL	EXP	CTRL	EXP	CTRL	EXP
Number of values	15	17	7	4	1	2
Mean	168.067	171.235	147.000	130.250	103.000	110.500
Std. Deviation	21.456	30.932	19.536	17.115	0.000	13.435
Std. Error of Mean	5.540	7.502	7.384	8.557	0.000	9.500
Test for normal distribution on residuals: Shapiro-Wilk test						
W	0.9747					
P value	0.5850					
Passed normality test (alpha=0.05)	Yes					
P value summary	ns					
Ordinary Two-way ANOVA: multiple comparisons						
Turkey's multiple comparisons test	Mean diff.	95. 00 % CI of diff.	Significant?	Summary	Adjusted P value	
NP: CTRL vs. EXP	-6.373	-27.66 to 14.91	No	ns	0.545	
G14.5: CTRL vs. EXP	16.75	-13.78 to 47.28	No	ns	0.271	
CTRL: NP vs. G14.5	20.90	-8.087 to 49.89	No	ns	0.194	
EXP: NP vs. G14.5	44.02	9.679 to 78.37	Yes	**	0.0098	

Supplementary Table S19. Statistical analysis on % glucose drop at 15, 30 and 60 min following insulin injection comparing NP, G14.5 and PP5 after macrophage depletion.

% glucose drop at 15, 30, 60 min following insulin injection across pregnant, postpartum and non-pregnant conditions after macrophage depletion							
Descriptive statistics							
		NP		G14.5		PP5	
		CTRL	EXP	CTRL	EXP	CTRL	EXP
Number of values		16	13	7	4	1	2
15'	Mean	56.045	54.773	55.128	51.334	38.130	36.930
	Std. Deviation	8.351	7.955	7.495	3.697	0.000	11.413
	Std. Error of Mean	2.088	2.206	2.833	1.849	0.000	8.070
30'	Mean	61.237	60.044	55.208	55.700	45.324	57.436
	Std. Deviation	6.432	7.966	7.550	5.611	0.000	2.448
	Std. Error of Mean	1.608	2.209	2.853	2.805	0.000	1.731
60'	Mean	74.497	74.213	67.430	74.236	56.835	66.161
	Std. Deviation	10.308	7.880	18.321	10.929	0.000	9.891
	Std. Error of Mean	2.577	2.186	6.925	5.465	0.000	6.994
Test for normal distribution on residuals: Shapiro-Wilk test							
W		0.9974					
P value		0.9929					
Passed normality test (alpha=0.05)		Yes					
P value summary		ns					
Ordinary Two-way ANOVA: multiple comparisons							
Turkey's multiple comparisons test		Mean diff.	95. 00 % CI of diff.	Significant?	Summary	Adjusted P value	
15' tov 0'	NP: CTRL vs. EXP	1.271	-4.711 to 7.254	No	ns	0.669	
	G14.5: CTRL vs. EXP	3.794	-6.249 to 13.84	No	ns	0.449	

30' tov 0'	NP: CTRL vs. EXP	1.193	-4.120 to 6.506	No	ns	0.652
	G14.5: CTRL vs. EXP	-0.4917	-9.410 to 8.426	No	ns	0.912
60' tov 0'	NP: CTRL vs. EXP	0.2840	-8.341 to 8.909	No	ns	0.947
	G14.5: CTRL vs. EXP	-6.806	-21.29 to 7.672	No	ns	0.347