

1 **Classification:** Biological Sciences, Cell Biology

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3 **Intravital imaging of islet Ca^{2+} dynamics reveals enhanced β cell connectivity**
4 **after bariatric surgery in mice**

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23

24 **Author Contributions:**

25 E.A., K.S., V.S. and L.L.N. undertook the mouse studies. V.S. and G.A.R. designed and
26 supervised the study. E.A. undertook all data analyses. E.G. developed connectivity scripts
27 and P.C. contributed to connectivity analysis. I.L assisted with mouse studies. E.A. and G.A.R.
28 wrote the manuscript with contributions from all authors.

29

30 **This PDF file includes:**

31 Main text

32 Figures 1 to 4

33 Supplementary Figures 1 to 3

1 **Abstract:**

2 Bariatric surgery improves both insulin sensitivity and secretion in type 2 diabetes. However,
3 these changes are difficult to monitor directly and independently. In particular, the degree and
4 the time course over which surgery impacts β cell function, versus mass, have been difficult
5 to establish. In this study, we investigated the effect of bariatric surgery on β cell function *in*
6 *vivo* by imaging Ca^{2+} dynamics prospectively and at the single cell level in islets engrafted into
7 the anterior eye chamber. Islets expressing GCaMP6f selectively in the β cell were
8 transplanted into obese male hyperglycaemic mice that were then subjected to either vertical
9 sleeve gastrectomy (VSG) or sham surgery. Imaged *in vivo* in the eye, VSG improved
10 coordinated Ca^{2+} activity, with 90% of islets observed exhibiting enhanced Ca^{2+} wave activity
11 ten weeks post-surgery, while islet wave activity in sham animals fell to zero discernible
12 coordinated islet Ca^{2+} activity at the same time point. Correspondingly, VSG mice displayed
13 significantly improved glucose tolerance and insulin secretion. Circulating fasting levels of
14 GLP-1 were also increased after surgery, potentially contributing to improved β cell
15 performance. We thus demonstrate that bariatric surgery leads to time-dependent increases
16 in individual β cell function and intra-islet connectivity, together driving increased insulin
17 secretion and diabetes remission, in a weight-loss independent fashion.

18

19

20 **Significance Statement:**

21 Used widely to treat obesity, bariatric surgery also relieves the symptoms of type 2 diabetes.
22 The mechanisms involved in diabetes remission are still contested, with increased insulin
23 sensitivity and elevated insulin secretion from pancreatic β cells both implicated. Whilst the
24 speed of remission – usually within a few days – argues for improvements in β cell function
25 rather than increases in mass, a direct demonstration of changes at the level of individual β
26 cells or islets has been elusive. Here, we combine vertical sleeve gastrectomy with intravital
27 imaging of islets engrafted into the mouse anterior eye chamber to reveal that surgery causes
28 a time-dependent improvement in glucose-induced Ca^{2+} dynamics and β cell - β cell
29 connectivity, both of which likely underlie increased insulin release.

1 Introduction

2 An estimated 30 million individuals in the US (9.4 % of the population) have diabetes (1), with
3 ~90% of cases thought to be Type 2 Diabetes (T2D), while in the United Kingdom it is
4 predicted that by 2025 more than five million people will be diagnosed with the disease (2). In
5 response to this epidemic, an abundance of pharmacological, dietary, exercise and
6 behavioural interventions have been deployed but often focus on T2D management rather
7 than long-term disease resolution (3, 4). Several clinical trials have now reported that bariatric
8 surgery, a group of gastrointestinal procedures originally developed to aid weight loss,
9 improves long-term glycaemia more effectively than caloric restriction or medical intervention
10 (5-7).

11 Numerous studies (8-12) have attempted to unravel the mechanisms through which blood
12 glucose control is improved post-operatively. One hypothesis to explain post-bariatric T2D
13 remission is that it results from the increased release of incretins from the gut, such as the
14 gastrointestinal insulin-stimulating hormone Glucagon-like Peptide 1 (GLP-1), as upregulated
15 postprandial levels have been reported following bariatric surgery (13-15). Preclinical and
16 clinical data have shown that bariatric surgery improves both hepatic and peripheral insulin
17 sensitivity, as well as increases in insulin secretion (16-19). However, the exact mechanisms
18 through which surgery impacts the β cell, including the identity of all the extra-pancreatic
19 signals involved, and the relative importance of changes in β cell function and mass, have
20 remained elusive. Nonetheless, the rapid (hours-days) reversal of diabetes in human subjects
21 treated with bariatric surgery (20, 21) has provided powerful evidence that an improvement of
22 β cell function plays an important, and possibly the dominant, role in increasing pancreatic
23 insulin output.

24 A critical limitation in investigating β cell function in living humans or preclinical models is that,
25 in the absence of robust *in vivo* imaging technologies (22), function must rely mainly on indirect
26 measurements of circulating insulin or C-peptide. These approaches preclude any quantitation
27 of changes over time, a detailed examination of function at the level of single β cells, or the
28 connections between them. The latter has become an important issue since we (23) and
29 others (24) have reported that weaker intercellular connections, and the loss of highly
30 connected cells, that can often initiate Ca^{2+} waves (sometimes referred to as “hubs”), underlie
31 the loss of insulin secretion observed in response to challenges associated with diabetes
32 gluco(lipo)toxicity, low inflammation level, etc. (23, 25, 26). However, untangling these
33 functional changes from alterations in β cell mass *in vivo* is challenging, since the latter can
34 only reliably be determined post-mortem via pancreatic biopsies, and thus at a single time
35 point.

36 In an effort to overcome these limitations, the present study aimed to investigate the effect of
37 Vertical Sleeve Gastrectomy (VSG) on pancreatic β cell function in mice, by transplanting
38 “reporter” islets in the anterior chamber of the eye. This approach was established by Berggren
39 and colleagues (27) and has recently been developed by ourselves (26) to assess coordinated
40 islet behaviour *in vivo*. Importantly, this technique has allowed us to image Ca^{2+} dynamics
41 recursively, in the same islet, over time and with near single cell resolution, following surgery.
42 We show that VSG increases β cell Ca^{2+} dynamics within eight weeks post-surgery when
43 compared to pre-operative baseline and a sham operated group. Moreover, we demonstrate
44 that VSG increases the number and strength of β to β cell connections at ten weeks after
45 surgery. These changes were associated with increased fasting levels of GLP-1, suggesting

1 that enhanced incretin production may contribute to postoperative improvements in β cell
2 performance.

3

4 **Results**

5

6 *Vertical Sleeve Gastrectomy improves glucose tolerance*

7 Our experimental protocol is summarized in Figure 1A. In brief, mice were placed on a high
8 fat high sucrose diet (HFHSD), at eight weeks of age, eight weeks before sham or vertical
9 sleeve gastrectomy (VSG) surgery (week 0). This protocol led to fasting hyperglycaemia,
10 indicative of β cell decompensation and defective insulin secretion, as expected (28).
11 *Ins1Cre:GCaMP6f* islets were isolated from donor mice and transplanted at week (-4).
12 Baseline islet Ca^{2+} dynamics were imaged at week (-1).

13 VSG-treated mice experienced a larger decrease in body weight versus sham-operated
14 animals, that was statistically significant until week 8 (week 7 av. Sham $42.9 \pm 4.3\text{g}$, av. VSG
15 $34 \pm 2.4\text{g}$, $p > 0.05$), (Fig. 1B). Vertical sleeve gastrectomy significantly increased the glucose
16 clearance rate ($p < 0.01$ at 15, 30, 60 and 90 min.) as assessed by oral glucose tolerance test
17 (OGTT) at post-operative week 8 (Fig. 1C) and intraperitoneal glucose tolerance test (IPGTT)
18 four and ten weeks post operatively ($p < 0.01$ at min. 30, 60, 90 min, Fig. 1D, 1E respectively).
19 Strikingly, in all tolerance tests performed on VSG-treated mice, glucose peaked at 15 min.
20 post glucose injection (3g/kg) and dropped to baseline levels within 60 min. by week eight,
21 and near baseline levels at week ten. In contrast, in sham-operated mice, glucose peaked at
22 30 min. and did not fully recover within the first 2 h of measurement.

23

24 *Vertical Sleeve Gastrectomy improves insulin secretion and sensitivity but does not increase*
25 β cell mass

26 In order to understand the marked increase in the rate of glucose clearance in mice that had
27 undergone VSG, we measured insulin secretion *in vivo* as a response to an IP glucose load
28 (3g/kg). Insulin secretion was increased significantly in VSG versus sham mice as early as
29 four weeks post operatively (Fig. 1F, with the observed peak at 15 min. almost three-fold
30 higher compared to sham mice ($p < 0.05$). VSG mice were also significantly more insulin
31 sensitive when compared to sham mice, as assessed by intraperitoneal insulin tolerance test
32 (ITT, 1.5U/kg) ($p < 0.01$, Fig. 1 G). However, pancreatic β cell mass was not increased in the
33 VSG group relative to sham controls (Supp. Fig. 1A, 2). Notably, the ratio of α to β cell mass
34 was significantly higher in the VSG group, yet α cell mass was not significantly increased
35 (Supp Fig. 1B, C, 2).

36

37 *Vertical Sleeve Gastrectomy enhances GLP-1 secretion*

38 To assess whether enhanced incretin release may contribute to the euglycemic effect of VSG
39 we observed during IPGTT and OGTT, we measured plasma GLP-1 levels during fasting and

1 15 min. following an orally administered glucose load (3g/kg) (Fig. 1C). Fasting GLP-1 was
2 significantly higher in the VSG group, when compared to sham (Fig. 1H). Moreover, whilst
3 glucose failed to increase GLP-1 levels significantly in the sham group, a highly significant
4 increase in response to glucose gavage was observed in VSG-treated animals (Fig. 1H).
5 Significantly lower glucose levels were apparent in VSG-treated versus sham-treated mice,
6 both fasting and following glucose gavage (Fig. 1I).

7

8 *β cell Ca²⁺ dynamics are enhanced following Vertical Sleeve Gastrectomy*

9 In order to explore changes in β cell function after surgery, we monitored intracellular Ca²⁺
10 changes prospectively and in the same islets by confocal imaging of the anterior eye chamber
11 (26, 27). Ca²⁺ increases, measured at ambient blood glucose concentrations in the range
12 12.5±0.7 mmol/L for both VSG-treated and sham mice, which occurred at a single or multiple
13 site across the islet but did not advance across the islet, were defined as “oscillations”.
14 Increases that had a defined site of origin but did not spread across the full width of the imaged
15 plane, were defined as “partial” waves (Fig. 2Bi, Supp. Mov. 1D). Those increases spreading
16 across the whole islet were termed “waves” (Fig. 2Ai, 2Bii Supp. Mov. 1A, E). If the latter wave
17 type was recurrent, we defined the behaviour as a “super wave” (Fig 2Biii, Supp. Mov. 1F).
18 As illustrated in Fig. 2A, when imaged 0, 4 and 10 weeks after surgery, islets in sham-operated
19 animals displayed a progressive loss of Ca²⁺ dynamics, as defined by the frequency and type
20 of waves. Thus, when imaged at 0 weeks (Fig. 2Ai), wave behaviour (beginning at the bottom
21 right; red area) area moved rapidly across the areas identified in yellow and blue. Comparable
22 behaviour was seen at 4 weeks, with a similar site of origin of the wave (Fig. 2Aii) but was lost
23 at 10 weeks post sham surgery, even though there was no significant weight difference
24 between the two groups (Fig. 2Aiii, Supp. Mov. 1C).

25 In contrast, islets implanted into mice subject to VSG displayed sustained or gradually
26 improving Ca²⁺ dynamics following surgery. Thus, the islet shown in Fig. 2Bi initially showed
27 partial wave activity but progressed to full wave activity by week 4 (Fig. 2Bii, Supp. Mov. 1D)
28 and to super wave by week 10 (Fig. 2Biii, Supp. Mov. 1F). A similar progression was seen for
29 eight islets in three separate mice subjected to VSG (Fig. 3A, Supp. Fig. 3), whilst in six islets
30 in three sham-operated mice a decline in behaviour was apparent after surgery (Fig. 3A).
31 Remarkably, almost all islets transplanted into VSG animals displayed either wave or
32 superwave behaviour by week eight, even if VSG-treated animals did not display further
33 weight loss. This is significantly higher when compared to sham mice at the same timepoint
34 (p=0.02) (Fig. 3A). By week 10, the activity of all sham-transplanted islets dropped to almost
35 zero (p=0.004) (Fig. 3A). Mean wave front velocity, a measure of the speed of the wave
36 calculated by distance (μm) divided by time (sec), across the islet was not different between
37 groups at any time point explored. Similarly, no differences were apparent between wave
38 velocities for the different wave types in either VSG or sham operated (Fig. 3B).

39

40 *Vertical Sleeve Gastrectomy maintains the number and strength of β cell – β cell connections*

41 Coordinated activity of β cells is a feature of the healthy islet, and is likely to be important for
42 the regulation of pulsatile insulin secretion (29). As shown in Fig. 4A and B, Pearson
43 correlation analysis revealed no differences in apparent connectivity at week 0 (prior to

1 surgery), whereas a progressive decline in connectivity was observed in the sham group. The
2 number of connected cells (Fig. 4B), or the mean connectivity strength (R) (Fig. 4C, D)
3 remained relatively constant in the VSG group, such that by week 10 these islets displayed
4 significantly greater connectivity than the sham group (Fig. 4B). In summary, glucose-related
5 Ca^{2+} signalling in VSG mice was characterized by higher magnitude and higher sensitivity to
6 glucose when compared with sham mice, suggesting changes in glucose metabolism in the
7 islets following VSG.

8

9 **Discussion**

10 Using an intravital imaging approach developed in recent years to monitor islet function *in vivo*
11 (26, 27), we provide here evidence that VSG causes a dramatic improvement in β cell Ca^{2+}
12 dynamics, a useful assay of normal cellular function and proxy for insulin secretion (22, 30,
13 31). The use of such an approach addresses the challenges in dissecting the relative
14 importance of the actions of bariatric surgery in changes observed in: (a) pancreatic insulin
15 output versus peripheral insulin sensitivity, (b) β cell function versus mass, and (c) the time
16 courses of changes, post-surgery.

17 Critically, we demonstrate that at similar, stimulatory glucose concentrations, islet Ca^{2+}
18 dynamics and connectivity are dramatically increased in VSG versus sham-operated animals.
19 Our data provide the first evidence we are aware of that alterations in β cell function occur
20 both at the level of individual cells and across the islet ensemble after surgery, and are thus
21 likely to play a pivotal role in improving insulin output. Changes in both β cell identity, reflecting
22 altered gene expression (32-34), and in coordinated β cell activity across the islet, are
23 important features of T2D (25, 35). The normalization of either thus presents an attractive
24 therapeutic route towards improving insulin secretion in this disease. Importantly, whilst
25 several studies have demonstrated changes in islet gene expression in rodent models related
26 to hyperglycaemia and diabetes progression, such as obese diabetic (ZDF) rats and HFHSD
27 mice (36, 37), few have examined the potential for reversing these changes as a therapeutic
28 strategy (37, 38).

29 Central to the present study has been the use of VSG in obese mice as a model of human
30 bariatric surgery (39, 40). Roux-en-Y Gastric Bypass (RYGB) and VSG are routinely deployed
31 as an approach to treat human obesity, and both cause similar rates of T2D remission within
32 the first post-operative year in man (41). In mice, VSG leads to initial rapid weight loss followed
33 by a weight regain, unlike RYGB, but sustains improved glucose tolerance while offering a
34 more tractable approach, with lower mortality (42, 43). Importantly, our study had a ten-week
35 post-operative follow up and, by week eight, there was no significant weight difference
36 between sham and VSG group. This allowed us to separate marked improvements in insulin
37 secretion from significant weight loss without the need to pair-feed the sham group (43).
38 Moreover, it corresponds with our previous findings in lean VSG-treated mice that
39 demonstrated no weight difference when compared to sham mice at four weeks post-
40 operatively, yet displayed improved glucose tolerance and corresponding insulin secretion
41 curves during an IPGTT (44). Insulin tolerance tests at week eight demonstrated that VSG
42 mice had improved insulin sensitivity, an effect previously attributed in humans to rapid and
43 significant enhancement of post-operative hepatic insulin clearance (45). It has recently been

1 suggested that, in animal models of surgery, hepatic insulin clearance is related to peripheral,
2 rather than hepatic, insulin sensitivity (46).

3 Accelerated glucose clearance in VSG-treated animals was accompanied by increased insulin
4 secretion in response to glucose at 15 and 30 min., consistent with previous studies using
5 VSG models (39, 47, 48). The fact that the insulin response to IPGTT was equally robust
6 suggests that this effect is not solely due to an increased spike in blood glucose associated
7 with elevated gastric emptying rates and upregulated glucose absorption, as has been
8 previously postulated (9, 49). Increased insulin secretion in the face of lower plasma glucose
9 demonstrates enhanced β cell glucose sensitivity, consistent with cell-autonomous changes
10 in islet function, alterations in circulating levels of other regulators of secretion, or an increase
11 in β cell number. Analysis of the endogenous pancreatic β cell mass showed no increase in
12 VSG versus sham-operated mice, pointing to a functional change rather than a change in
13 endogenous β cell mass, as underlying increased insulin output. Moreover, and though this
14 could not be quantitated accurately due to the lack of focal distances stacking data, we saw
15 no evidence for a change in the β cell mass of islets engrafted into the eye. This is in line with
16 previous findings demonstrating that there is no islet hyperplasia or increased β cell turnover
17 following bariatric surgery in humans or rats with obesity (50, 51). These findings contrast
18 other studies that have reported -over similar times scales- increasing (52-54) or decreasing
19 (48, 55) β cell mass differences, which may reflect pre-operative metabolic state or other
20 factors.

21 Given many reports of increased GLP-1 release after bariatric surgery in both humans (56)
22 and rodents (42, 57), here we explored levels of both circulating fasting and post-glucose
23 gavage GLP-1 levels. Importantly, the peak in GLP-1 following oral gavage did not differ
24 between sham and VSG mice, indicating that an enhanced insulinotropic effect of the incretin
25 is unlikely to explain dramatic increase in insulin secretion observed. Furthermore, enhanced
26 insulin secretion was seen in mice treated with VSG even during IPGTT, where the stimulation
27 of GLP-1 secretion is negligible.

28 Interestingly, VSG-treated mice did display significantly higher circulating GLP-1 levels under
29 basal (fasting) conditions. Apart from increasing glucose-stimulated insulin secretion and
30 enhancing insulin gene transcription (58, 59), GLP-1 also inhibits β cell apoptosis in animal
31 models of diabetes (60, 61). Thus, although the underlying mechanisms remain unclear,
32 increased basal GLP-1 levels might provide a partial explanation for the enhanced responses
33 to glucose and the euglycemic effects observed after surgery. A number of studies have
34 shown that, in post-operative patients with T2D, GLP-1 receptor (GLP-1R) blockade with the
35 GLP-1R antagonist Exendin-(9-39) causes significant reduction of insulin secretion when
36 compared to a control group with lower GLP-1 levels, indicating an effect on β cell function
37 (62, 63). Nonetheless, other studies have demonstrated that blocking GLP-1R in bariatric
38 patients impairs glucose tolerance but not to a greater degree than before surgery, or when
39 compared to non-operated patients (64, 65). Furthermore, Ye and colleagues found that
40 pharmacological or genetic blockade or elimination of GLP-1R signaling in rats or mice,
41 respectively, had no impact on the ability of RYGB to lower body weight (66, 67). Taken
42 together, these earlier data suggest that GLP-1 signaling may not be the main mediator of
43 T2D remission but is likely to contribute (68). Changes in the levels of other circulating factors
44 are thus likely to be involved in the apparent increase in β cell function. Lowered levels of
45 circulating lipids (69), inflammatory cytokines (70), bile acids (71), glucocorticoids (72) or
46 microbiome-derived products (73) are potential candidates.

1 An important aspect of the present study has been to examine, at the cellular level, the
2 functional connectivity between β cells before and after VSG or sham surgery. The percentage
3 of significantly-connected cell pairs and correlation coefficient decreased substantially in the
4 sham group at week ten, while in the VSG group these parameters remained stable for the
5 duration of the study. We would note that hub/follower behaviour (i.e. the existence of a “power
6 law” in the degree of connectedness) (23) was not readily apparent in the present study. More
7 rapid acquisition rates are likely to be needed to reveal such a hierarchy. Furthermore, we
8 note that wave-like behaviour is more often apparent in the islet *in vivo* using GCaMP6f as the
9 Ca^{2+} sensor (26) than in some of our own and others’ earlier studies (23, 25) using entrapped,
10 synthetic Ca^{2+} probes. Nonetheless, clusters of apparent “leader” β cells, corresponding to
11 the point at which a rise in Ca^{2+} was first observed at the beginning of a wave, were easily
12 identified in many cases (e.g. Fig. 2A), and these have previously been reported (23) to
13 correspond to the hub cell population. Interestingly, the origin of the waves was similar within
14 a given islet assayed several weeks apart, indicating that the cells which initiate them (leaders)
15 represent a stable population, at least over the time frame (≤ 10 weeks) studied here.

16 Taken together, our results indicate that bariatric surgery improves glycaemic control at least
17 partially by maintaining: (a) functional β cell identity and (b) coordinated activity across the
18 islet. A possible explanation for our data may be that the improvement in islet function follows
19 the improvement in glycaemia. However, since insulin sensitivity was barely altered by VSG,
20 it is unclear whether extrapancreatic events could be the drivers for improved islet function
21 and insulin output.

22 Although the surgical model used provides us with novel information on β cell activity via
23 continuous monitoring, there are undoubtedly limitations in the use of islets engrafted into the
24 ACE. These include potential differences between the vascularization and innervation at this
25 site compared to pancreatic *in situ* islets (74). Our findings on ACE-engrafted islet reactivation
26 following VSG are however in line with previous results focusing on pancreatic islets isolated
27 postmortem. Thus, Douros et al (43) performed Ca^{2+} imaging *in vitro* in mouse islets
28 postmortem and showed that the percentage of islets displaying Ca^{2+} oscillations in response
29 to glucose was enhanced 2.2-fold in the VSG group, indicating increased islet glucose
30 sensitivity after surgery. In addition, VSG altered the islet transcriptome, affecting genes
31 involved in insulin secretion and Ca^{2+} signaling (43). However, these earlier studies were
32 cross-sectional in nature, and as such did not explore the apparent reactivation *in vivo* of
33 individual islets and β cells in the living animal, as described here.

34 In conclusion, our findings provide further evidence for the protective effect of bariatric surgery
35 in T2D, irrespective of weight loss, and demonstrate direct effects on β -cell function and
36 coordination in the living animal. Future challenges are to understand more fully the
37 mechanisms through which these changes are affected at the paracrine, endocrine and
38 cellular levels.

39

40

41 **Methods:**

42 *Animals* - All animal procedures undertaken were approved by the British Home Office under
43 the UK Animal (Scientific Procedures) Act 1986 (Project License PPL PA03F7F07 to I.L.) with

1 approval from the local ethical committee (Animal Welfare and Ethics Review Board, AWERB),
2 at the Central Biological Services (CBS) unit at the Hammersmith Campus of Imperial College
3 London.

4 Adult male C57BL/6J mice (Envigo, Huntingdon U.K.) were maintained under controlled
5 temperature (21-23°C) and light (12:12 hr light-dark schedule, lights on at 0700). From the
6 age of 8 weeks they were put on a 58 kcal% Fat and Sucrose diet (D12331, Research Diet,
7 New Brunswick, NJ) ad libitum to induce obesity and diabetes. Four weeks after the start of
8 this diet, the animals underwent islet transplantation into the anterior chamber of the eye of
9 genetically modified islets expressing GCaMP6f to allow for intravital measurements of
10 cytosolic Ca^{2+} . Four weeks after islet transplantation, the animals underwent either a vertical
11 sleeve gastrectomy or a sham surgery as described below.

12 *Ins1Cre:GCaMP6f^{f/f}* mice, used as donors for islet transplantation were generated by crossing
13 crossed *Ins1Cre* mice (provided by J Ferrer, this Department) to mice that express GCaMP6f
14 downstream of a LoxP-flanked STOP cassette (The Jackson Laboratory, stock no. 028865).
15 Islets donated from either sex were used for transplantation.

16 *Islet transplantation into the anterior chamber of the mouse eye (ACE)* - Pancreatic islets were
17 isolated and cultured as described previously (75). For transplantation, 10-20 islets were
18 aspirated with a 27-gauge blunt eye cannula (BeaverVisitec, UK) connected to a 100ul
19 Hamilton syringe (Hamilton) via 0.4-mm polyethylene tubing (Portex Limited). Prior to surgery,
20 mice were anaesthetised with 2-4% isoflurane (Zoetis) and placed in a stereotactic frame to
21 stabilise the head. The cornea was incised near the junction with the sclera, being careful not
22 to damage the iris. Then, the blunt cannula, pre-loaded with islets, was inserted into the ACE
23 and islets were expelled (average injection volume 20 μl for 10 islets). Carprofen (Bayer, UK)
24 and eye ointment were administered post-surgery.

25 *Vertical Sleeve Gastrectomy* - Three days before bariatric or sham surgery, animals were
26 exposed to liquid diet (20% dextrose) and remained on this diet for up to four days post
27 operatively. Following this, mice were returned to high fat/high sucrose diet until euthanasia
28 and tissues harvested ten weeks post bariatric surgery. Anaesthesia was induced and
29 maintained with isoflurane (1.5-2%). A laparotomy incision was made, and the stomach was
30 isolated outside the abdominal cavity. A simple continuous pattern of suture extending through
31 the gastric wall and along both gastric walls was placed to ensure the main blood vessels were
32 contained. Approximately 60% of the stomach was removed, leaving a tubular remnant. The
33 edges of the stomach were inverted and closed by placing two serosae only sutures, using
34 Lembert pattern. The initial full thickness suture was subsequently removed. Sham surgeries
35 were performed by isolating the stomach and performing a 1 mm gastrotomy on the gastric
36 wall of the fundus. All animals received a five-day course of SC antibiotic injections
37 (Ciprofloxacin 0.1mg/kg).

38 *In vivo Ca^{2+} imaging of *Ins1Cre:GCaMP6f^{f/f}* islets in the ACE* - A minimum of four weeks was
39 allowed for full implantation of islets before imaging. Imaging sessions were performed as
40 previously described (26) with the mouse held in a stereotactic frame and the eye gently
41 retracted, with the animal maintained under 2-4% isoflurane anaesthesia. All imaging
42 experiments were conducted using a spinning disk confocal microscope (Nikon Eclipse Ti,
43 Crest spinning disk, 20x water dipping 1.0 NA objective). The signal from GCaMP6f
44 fluorophore (ex. 488 nm, em. 525±25 nm) was monitored in time-series experiments for up to

1 20 min. at a rate of 3 frames/ sec. Ca^{2+} traces were recorded for three min, with a mean blood
2 glucose reading (across six islets in three separate animals per group) of 12.5 ± 0.7 mmol/L.
3 Islets were continuously monitored, and the focus was manually adjusted to counteract
4 movement. Animals were imaged 3 days prior to Vertical Sleeve Gastrectomy (baseline) and
5 then at four, eight and ten weeks post-operatively.

6 *Glucose Tolerance Tests* - Mice were fasted overnight (total 16 h) and given free access to
7 water. At 0900, glucose (3 g/kg body weight) was administered via intraperitoneal injection or
8 oral gavage. Blood was sampled from the tail vein at 0, 5, 15, 30, 60 and 90 min. after glucose
9 administration. Blood glucose was measured with an automatic glucometer (Accuchek;
10 Roche, Burgess Hill, UK).

11 *Insulin Tolerance Tests* - Mice were fasted for 8 h and given free access to water. At 1500,
12 human insulin (Actrapid, Novo Nordisk) (1.5U/kg body weight) was administered via
13 intraperitoneal injection. Blood was sampled from the tail vein at 0, 15, 30, 60 and 90 min after
14 insulin administration. Blood glucose was measured with an automatic glucometer (Accuchek;
15 Roche, Burgess Hill, UK).

16 *Plasma insulin and GLP-1 measurement* - To quantify circulating insulin and GLP-1(1-37)
17 levels, 100 μ l of blood was collected from the tail vein into heparin-coated tubes (Sarstedt,
18 Beaumont Leys, UK). Plasma was separated by sedimentation at 10,000g for 10 min. (4°C).
19 Plasma insulin levels were measured in 5 μ l aliquots and GLP-1(1-37) levels were measured
20 in 10 μ l aliquots by ELISA kits from Crystal Chem (USA).

21 *Immunohistochemistry of pancreas sections* - Isolated pancreata were fixed in 10% (vol/vol)
22 buffered formalin and embedded in paraffin wax within 24 h of removal. Slides (5 μ m) were
23 submerged sequentially in Histoclear (Sigma, UK) followed by washing in decreasing
24 concentrations of ethanol to remove paraffin wax. Permeabilised pancreatic slices were
25 blotted with ready-diluted anti-guinea pig insulin (Agilent Technologies, USA) and anti-mouse
26 glucagon (Sigma, UK) primary antibody (1:1000). Slides were visualised by subsequent
27 incubation with Alexa Fluor 488 and 568-labelled donkey anti-guinea pig and anti-mouse
28 antibody. Samples were mounted on glass slides using VectashieldTM (Vector Laboratories,
29 USA) containing DAPI. Images were captured on a Zeiss Axio Observer.Z1 motorised inverted
30 widefield microscope fitted with a Hamamatsu Flash 4.0 Camera using a Plan-Apochromat
31 206/0.8 M27 air objective with Colibri.2 LED illumination. Data acquisition was controlled with
32 Zeiss Zen Blue 2012 Software. Fluorescence quantification was achieved using Image J
33 (<https://imagej.nih.gov/ij/>). Whole pancreas was used to quantitate cell mass.

34 *Statistical Analysis* - Data were analysed using GraphPad PRISM 7.0 software. Significance
35 was tested using unpaired Student's two-tailed t-tests with Bonferroni post-tests for multiple
36 comparisons, or two-way ANOVA as indicated. $P < 0.05$ was considered significant and errors
37 signify \pm SEM.

38 *Pearson (R)-based connectivity and correlation analyses* - Correlation analyses between the
39 Ca^{2+} signal time series for all cell pairs in an imaged islet were performed in MATLAB using a
40 modified custom-made script (26). B cell intensity was measured in 100 consecutive frames
41 (30 sec.). Regions of Interest (ROI, 18-40 per islet, depending on size) were selected with
42 single or near single cell resolution (i.e. 10-20 μ m diameter) and captured approximately 95%
43 of the fluorescence of the image plane. Data were smoothed using a retrospective averaging

1 method (previous 10 values). The correlation function R between all possible (smoothed) cell
2 pair combinations (excluding the autocorrelation) was assessed using Pearson's correlation.
3 The Cartesian co-ordinates of the imaged cells were then incorporated in the construction of
4 connectivity line maps. Cell pairs were connected with a straight line, the colour of which
5 represented the correlation strength and was assigned to a colour-coded light-dark ramp
6 ($R=0.1-0.25$ [blue], $0.26-0.5$ [green], $R=0.51-0.75$ [yellow], $R=0.76-1.0$ [red]). Cells with the
7 highest number of possible cell pair combinations are shown in red. Data are also displayed
8 as heatmap matrices, indicating individual cell pair connections on each axis (min. = 0; max.
9 = 1). The positive R values (excluding the auto-correlated cells) and the percentage of cells
10 that were significantly connected to one another were averaged and compared between
11 groups.

12

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32

1 **Figure legends:**

2 Figure 1:

3 **VSG improves glucose and insulin tolerance in HFHSD mice.** (A) Timeline of procedures. (B) Body
4 weight monitoring following VSG (n=6 animals) or sham surgery (n=6). (C) Glucose was administered
5 via oral gavage (3 g/kg) after mice were fasted overnight and blood glucose levels measured at 0, 15,
6 30, 60 and 90 min. post gavage, six weeks after surgery, n=5-6 mice/group. (D) Glucose was
7 administered via intraperitoneal injection (3 g/kg) after mice were fasted overnight and blood glucose
8 levels measured at 0, 15, 30, 60 and 90 min. post injection, four weeks after surgery, n = 6 mice/group.
9 (E) Glucose was administered via intraperitoneal injection (3 g/kg) after mice were fasted overnight and
10 blood glucose levels measured at 0, 15, 30, 60 and 90 min. post injection, 10 weeks after surgery, n =
11 4-5 mice/group. (F) Corresponding insulin secretion levels measured on plasma samples obtained
12 during the IPGTT performed in D (n=6). (G) Insulin was administered via intraperitoneal injection
13 (1.5UI/kg) after mice were fasted for 5h and blood glucose levels measured at 0, 15, 30, 60 and 90 min.
14 post injection, 7-8 weeks after surgery, n = 6 mice/group. (H) Corresponding GLP-1 secretion levels
15 measured on plasma samples obtained during the OGTT performed in C (n=5). (I) Corresponding
16 glucose levels for 0, 15 min obtained during the OGTT performed in C (n=5). ## P<0.01 VSG 0 vs. VSG
17 15 min, *P<0.05, **P<0.01, ***P<0.001 VSG vs. Sham, following Student t-test or 2-way ANOVA. Data
18 are expressed as means \pm SEM.

19

20 Figure 2:

21 **Description of Ins1Cre:GCaMPf^{fl/fl} islet Ca²⁺ dynamics: super wave, full wave, partial wave and**

22 no activity. Ins1Cre:GCaMPf^{fl/fl} islets implanted in the anterior chamber of the eye and imaged for 400
23 frames (133 secs) using a spinning disk confocal microscope (see Materials and Methods) at baseline
24 week 0, postoperative week 4 and week 10. Each islet is separated in three Regions of Interest (ROIs)
25 in order to categorise its activity. Red represents area 1 (distal islet), yellow represents area 2 (middle
26 islet) and blue represents area 3 (proximal islet). Mean intensity is measured in each ROI for each
27 frame (3 frames/ sec) and presented as Ca²⁺ dynamics. (A). Ins1Cre:GCaMPf^{fl/fl} islets implanted in a
28 sham animal and imaged at (i) week 0 (full wave), (ii) 4 (full wave) and (iii) 10 (inactive). B (i)
29 Ins1Cre:GCaMPf^{fl/fl} islet implanted in a VSG-treated animal (i) at week 0 (partial wave), (ii) week 4 (full
30 wave) and (iii) week 10 (super wave). Scale: 100 μ m. Plasma glucose levels during imaging were
31 12.5 \pm 0.7 mmol/L.

32

33 Figure 3:

34 **Ca²⁺ dynamics of Ins1Cre:GCaMPf^{fl/fl} islets in Sham and VSG-treated animals** (A). Categorisation
35 of Ins1Cre:GCaMPf^{fl/fl} islets in sham (n=3 animals, n=6 islets) and VSG (n=3 animals, n=8 islets) mice
36 on weeks 0 (baseline), 4, 8 and 12. Categories: 1. No activity, 2. Oscillations, 3. Partial Wave, 4. Wave,
37 5. Super Wave (B). Velocity of waves and partial waves of Ins1Cre:GCaMPf islets in sham and VSG
38 animals calculated by d/ Δ t and measured as μ m/ sec. *P<0.05, **p<0.01, by Student t-test. Data are
39 expressed as means \pm SEM.

40

41 Figure 4:

42 **Cartesian functional connectivity and correlation coefficienty of islets before and after VSG or**
43 **Sham surgery.** (A) Cartesian functional connectivity maps displaying the correlation coefficients of β
44 cells within the x-y position of analysed cells (dots). Cells are connected with a line where the strength

1 of each cell pair correlation (the Pearson R statistic) is colour coded: red for R of 0.76 to 1.0, yellow for
2 R of 0.51 to 0.75, green for R of 0.26-0.5 and blue for R of 0.1 to 0.25. Red dots represent β cells with
3 the highest number of connected cell pairs. Ca^{2+} activity detected during the 30 sec (100 frames)
4 imaging period analysed is displayed at the top right of each connectivity map. (B) The percentage of
5 significantly connected cell pairs decreased significantly in the sham group at week 10. (C)
6 Representative heatmaps depicting connectivity strength (Pearson R correlation) of all β cell pairs (x- y
7 axis) presented in (A) (R values colour coded from 0 to 1, blue to yellow respectively). Yellow represents
8 β cell pairs with high connectivity strength. (D) The average of correlation coefficient (R) decreased
9 significantly in the sham group at week 10. N=3 animals (1 islet/ animal). Data are means \pm SEM and
10 *p<0.05, **p<0.01, ***p<0.001 following 1-way ANOVA.

11

12 Supplementary data:

13 Figure 1:

14 **Percentage of pancreatic area occupied by and ratio of α to β cells.** (A) Percentage of pancreatic
15 area occupied by β cells measured by immunofluorescent staining of pancreatic islets using anti-insulin
16 antibody against total pancreatic area. (B) Ratio of α to β cells in pancreatic islets of VSG (n=4) and
17 Sham (n=5) mice measured by immunofluorescent staining of pancreatic islets using anti-glucagon
18 (red) and anti-insulin (green) antibody. (C) Percentage of pancreatic area occupied by α cells measured
19 by immunofluorescent staining of pancreatic islets using anti-glucagon antibody against total pancreatic
20 area. Each point represents an average of all islets (10-30/ slide) present in three permeabilised
21 pancreatic slices, separated by 600 μ m. Total pancreatic area is measured in each slide and percentage
22 is calculated accordingly.*P<0.05, by unpaired Student's t-test. Data are expressed as means \pm SEM.

23

24 Figure 2:

25 **Immunofluorescence staining of pancreatic islets.** Anti-glucagon (red) and anti-insulin (green)
26 antibodies were used in VSG (n=4) and Sham (n=5) mice.

27

28 Figure 3:

29 **Ca^{2+} dynamics of Ins1Cre:GCaMPf^{fl/fl} islets in Sham and VSG-treated animals** Grouped
30 categorisation of Ins1Cre:GCaMPf islets in sham (n=3 animals, n=6 islets) and VSG (n=3 animals, n=8
31 islets) mice on weeks 0 (baseline), 4, 8 and 12.

32

33 Movie 1:

34 **Description of Ins1Cre:GCaMPf^{fl/fl} islet when in full wave, partial wave and no activity.** (A)
35 Ins1Cre:GCaMPf^{fl/fl} islet implanted in a sham animal, imaged using a spinning disk confocal microscope
36 at week 0 (full wave), (B) 4 (full wave) and (C) 12 (inactive). (D) Ins1Cre:GCaMPf^{fl/fl} islet implanted in
37 VSG-treated animal, imaged at week 0 (partial wave), (E) 4 (full wave) and (F) 12 (super wave). Plasma
38 glucose levels during imaging were 12.5 \pm 0.7 mmol/L measured at 5 min intervals.

39

40

Figure 1:

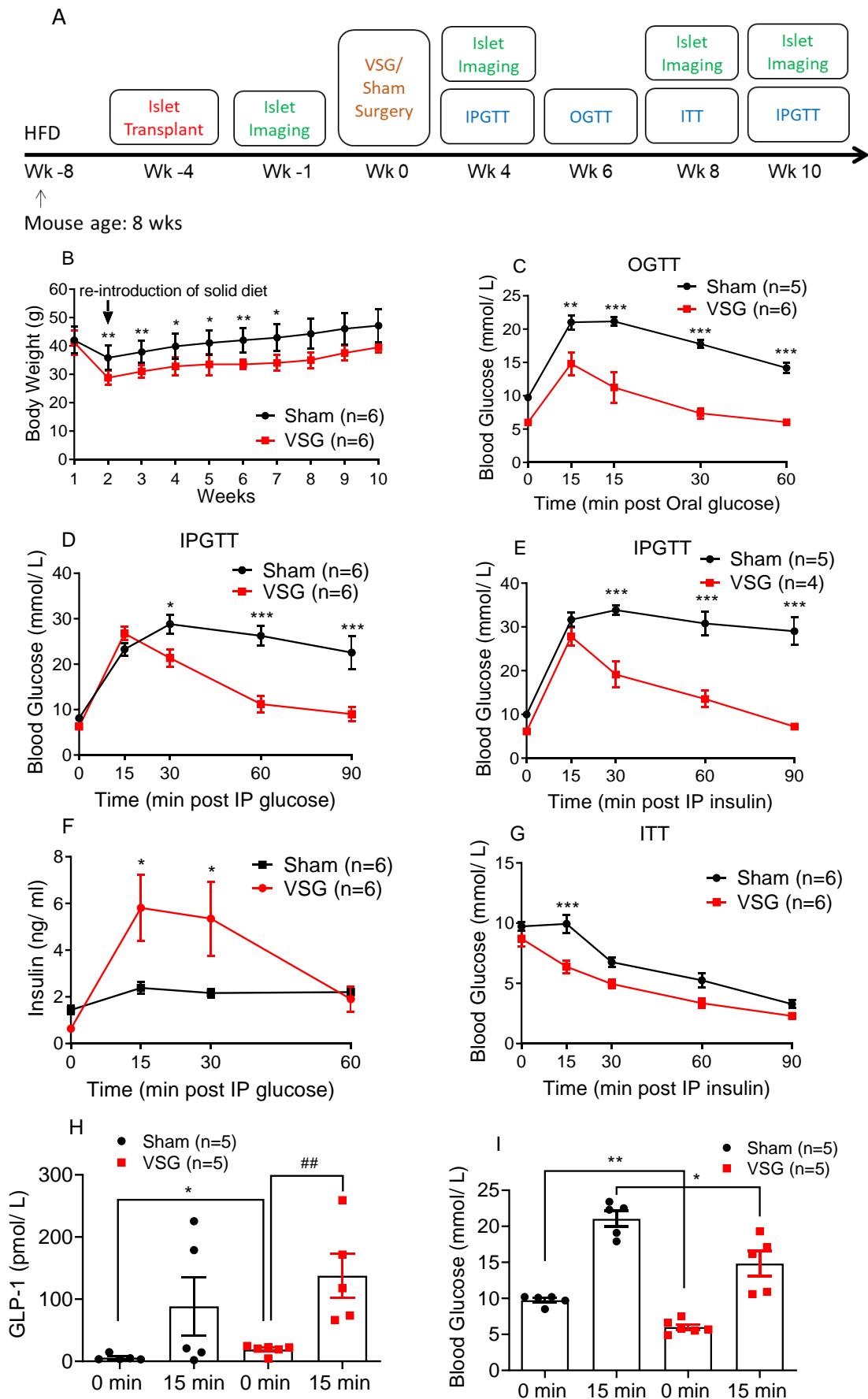


Figure 2:

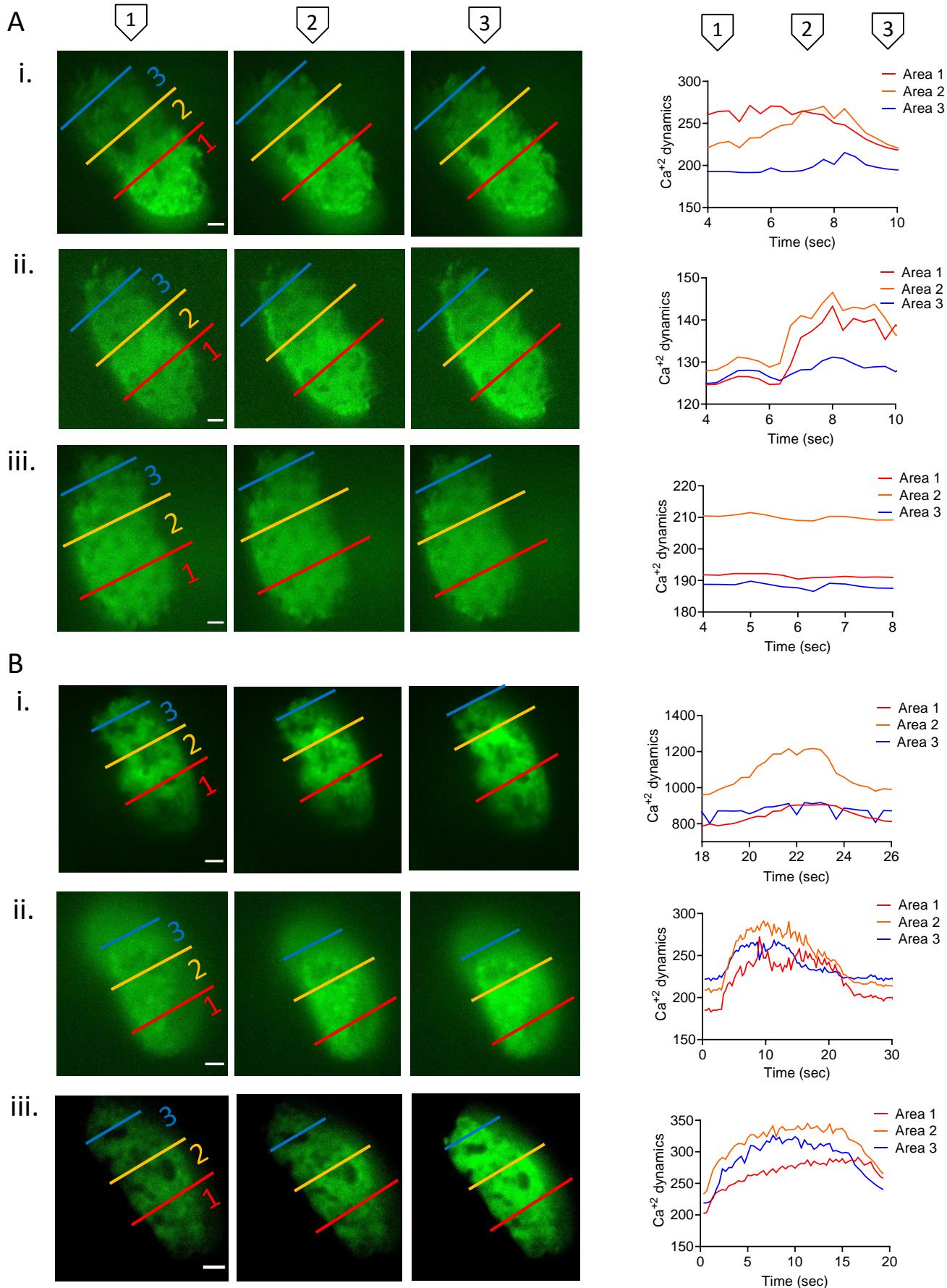


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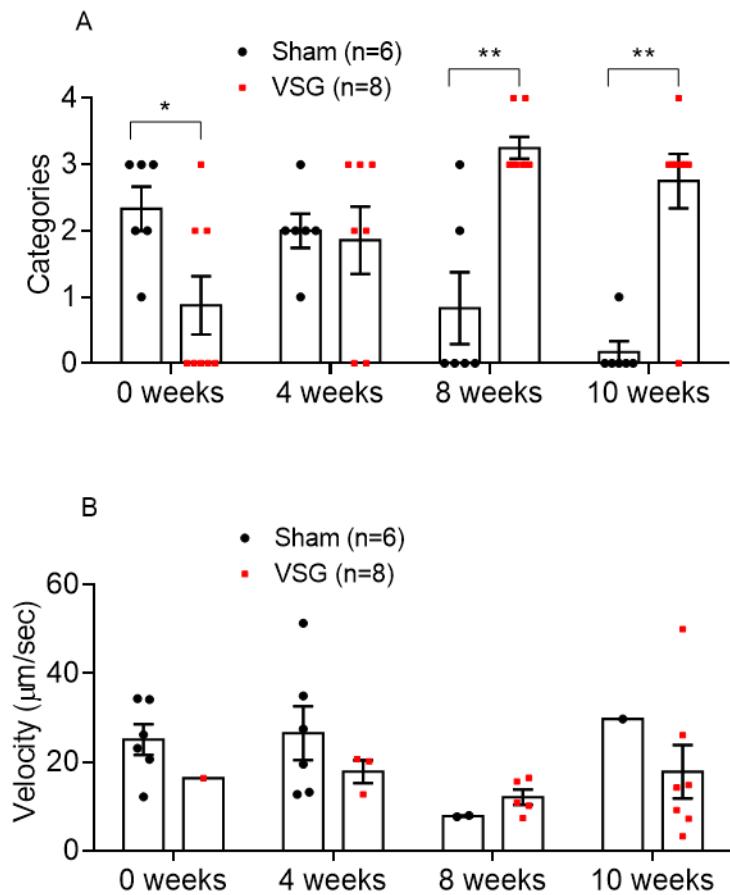
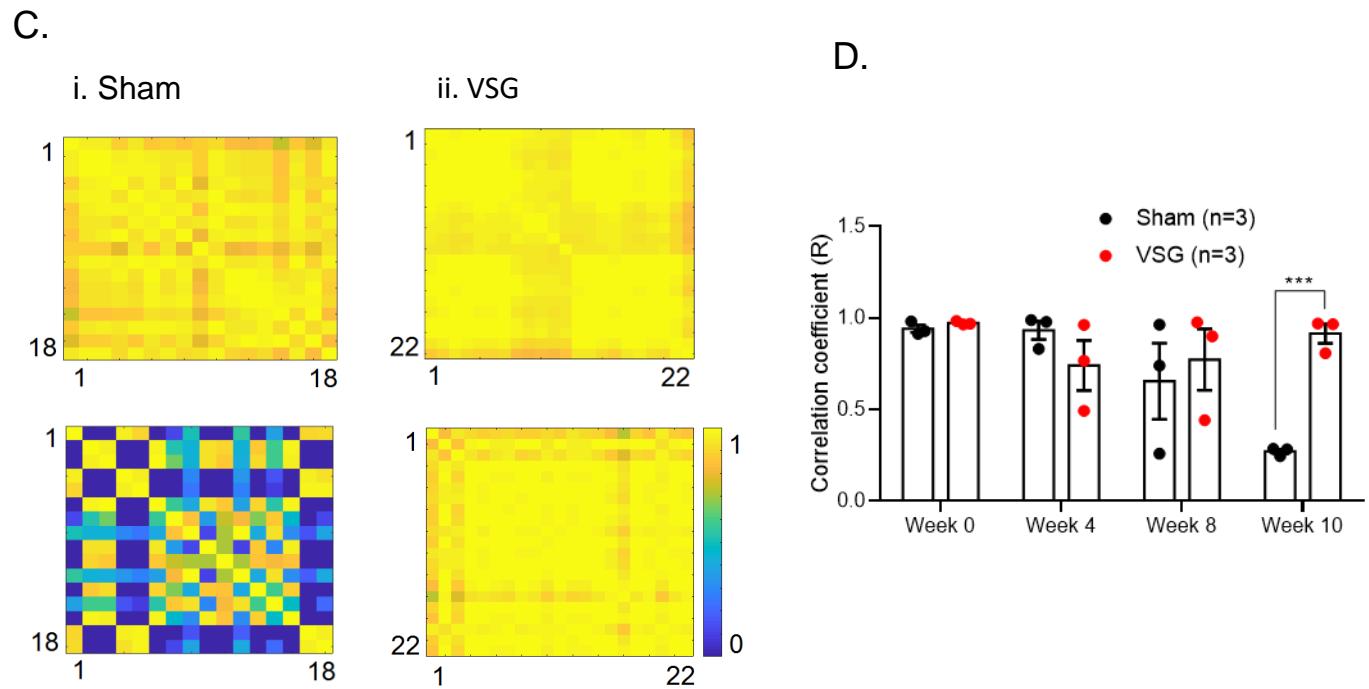
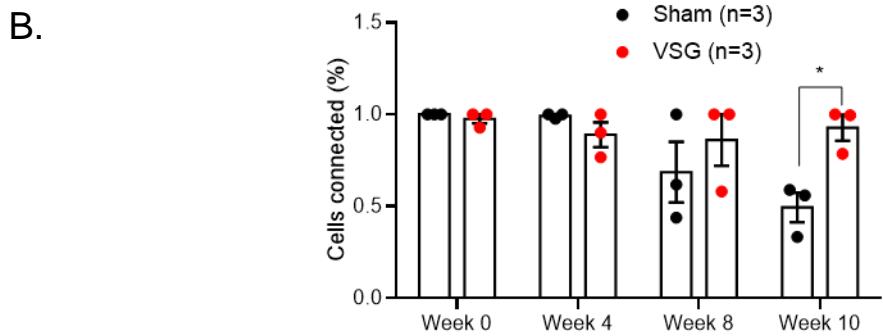
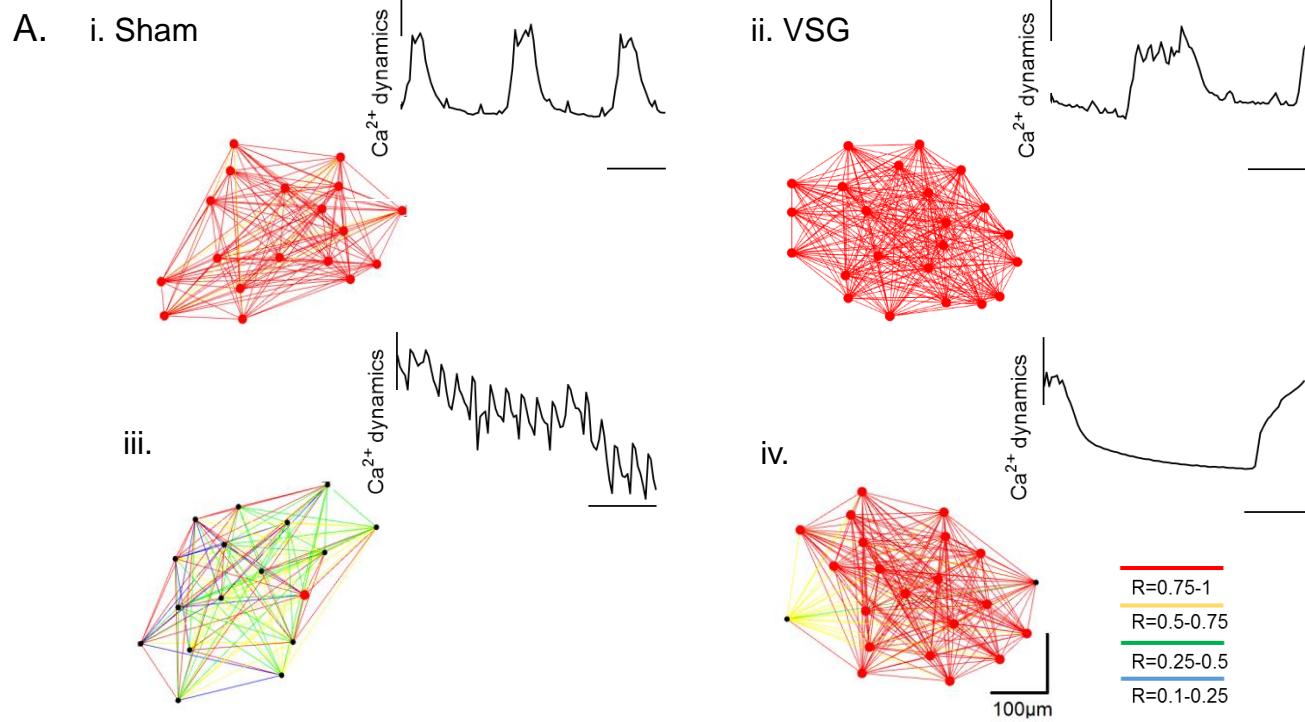
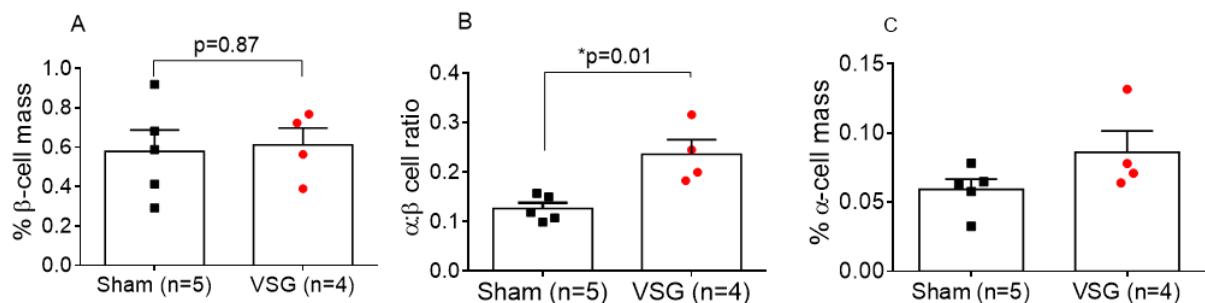


Figure 4:

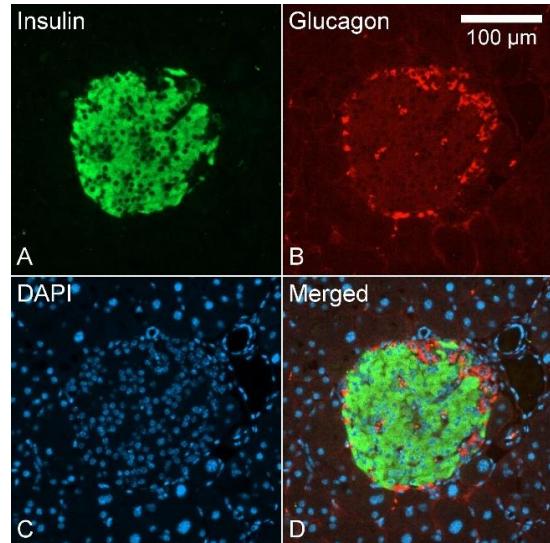


Supplementary Figure 1:

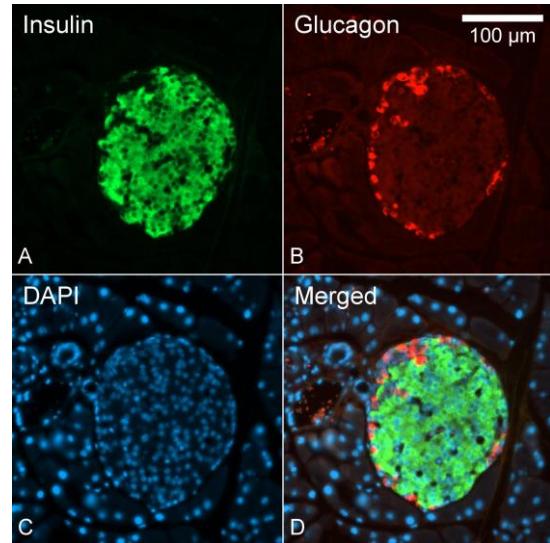


Supplementary Figure 2:

VSG



Sham



Supplementary Figure 3:

