Single-Dose GLP-1-Based Pancreatic Gene Therapy Durably Maintains Body Composition and Glycemia after Semaglutide Withdrawal in a Murine Model of Obesity

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Disclosure Statement

Authors
Harith Rajagopalan, Alice Liou Fitzpatrick, Suya Wang, Rebecca Reese, Nicole Picard, Emily Cozzi, Timothy Kieffer, and Jay Caplan are employees and shareholders of Fractyl Health, Inc. Harith Rajagopalan is a board member of Fractyl Health, Inc.

The Pancreatic Gene Therapy (PGTx) platform is in early development and has not been assessed by any regulatory body for investigational or commercial use.
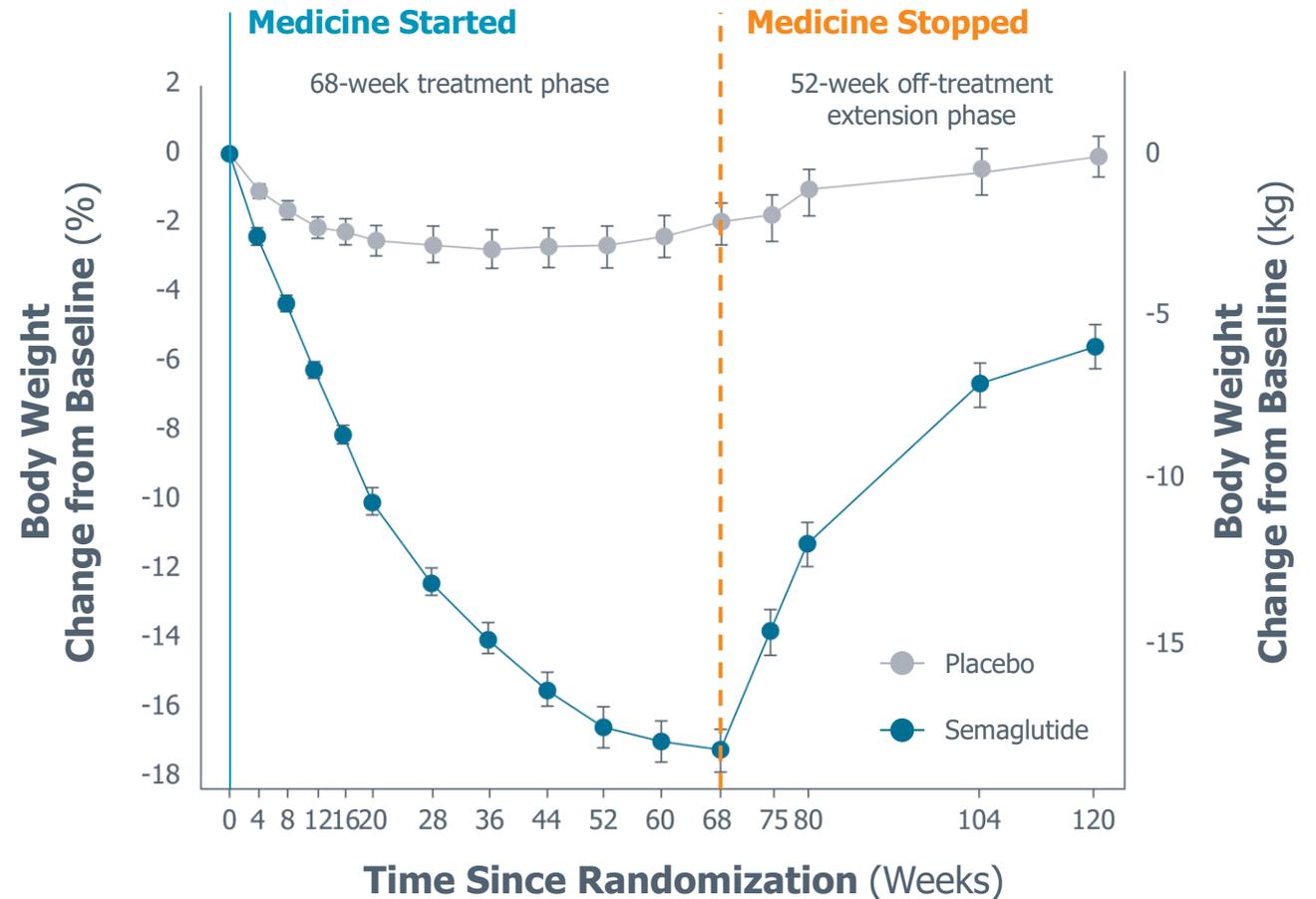
GLP-1 Drugs: Weight Rebound is a Significant Problem
Current GLP-1 drugs do not durably alter metabolic setpoint

Discontinuation of therapy leads to **near total loss of metabolic benefit**[1]

GLP-1 therapies support weight loss and glucose control, **but weight maintenance has become a critical challenge**

STEP-1 Trial Extension – Semaglutide 2.4 mg

Figure adapted from Wilding et al. Diabetes Obes Metab. 2022 24:1553-1564. 1. Wilding et al. Diabetes Obes Metab. 2022 24:1553-1564. GLP-1=glucagon-like peptide 1

ADA 2024
84th Scientific Sessions

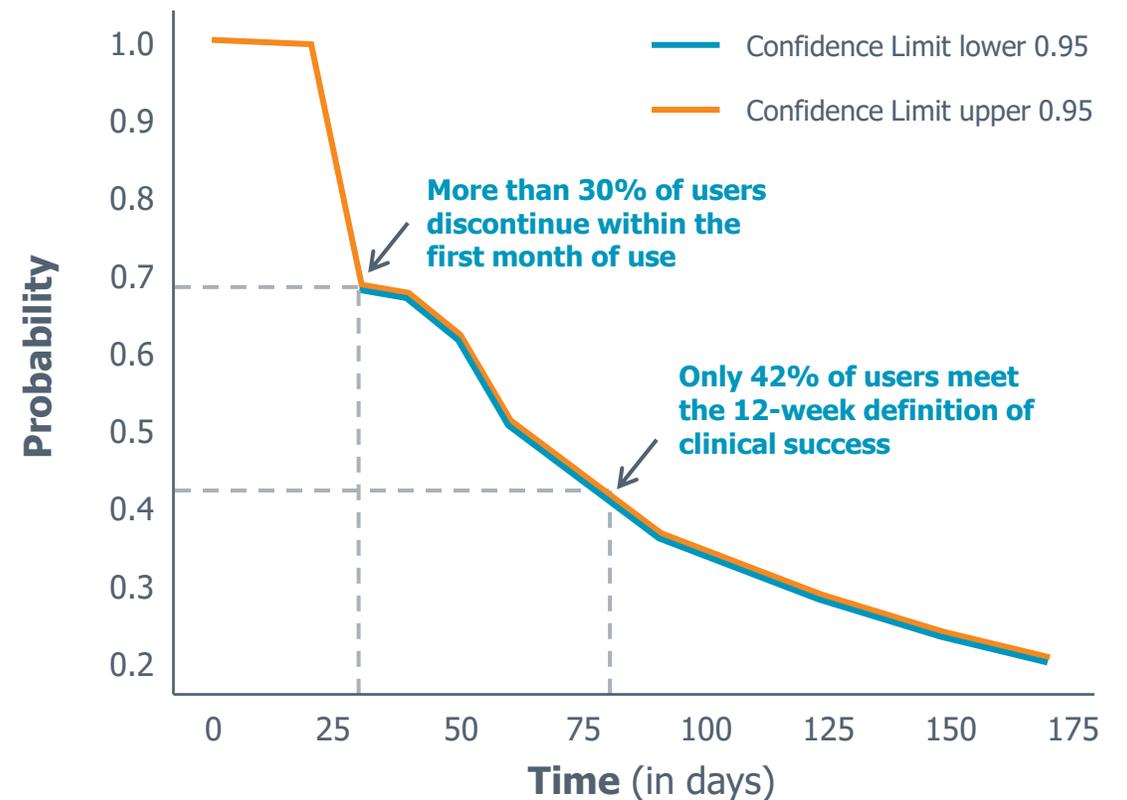# Real-World Evidence Shows High Discontinuation Rates
BCBS data show 50% GLP-1 drug discontinuation within 3 months

Private insurer **survey of ~170K unique GLP-1 drug users** for weight loss from January 2014 to December 2023[1]

Early discontinuation is often due to tolerability, but medium-term discontinuation rates still remain high across a range of tested variables:

- Cost (out of pocket expense)
- Type of prescriber (PCP vs endocrinologist)
- Age of patient
- Comorbidity index

Overall time to treatment discontinuation in GLP-1 drug users for weight management

Confidence Limit lower 0.95
Confidence Limit upper 0.95

More than 30% of users discontinue within the first month of use

Only 42% of users meet the 12-week definition of clinical success

Probability

Time (in days)

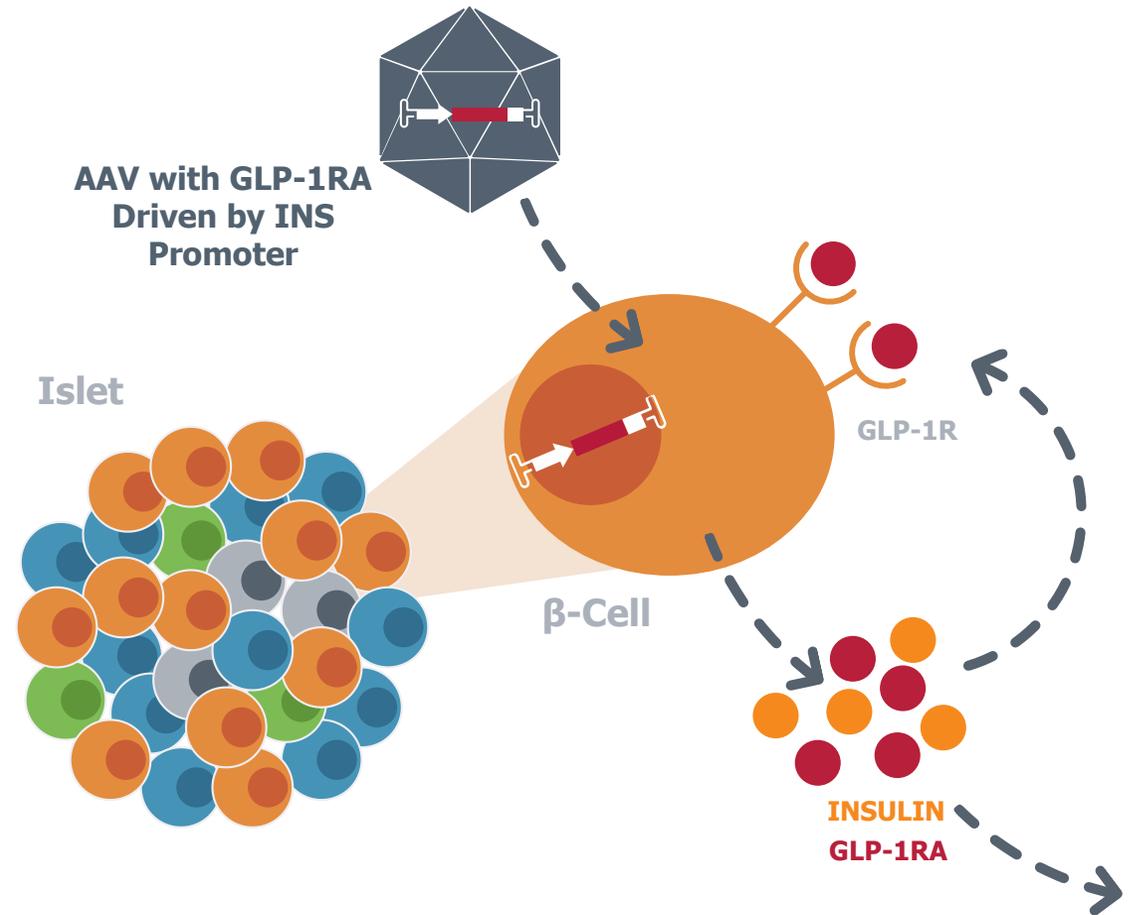# Pancreatic Gene Therapy (PGTx) to Modify Islet Function
Potential for durable improvement in metabolic health

GLP-1 gene therapy, targeted to pancreatic islets, may offer differentiated benefit

β-cell machinery can be leveraged to produce nutrient-stimulated hormones[1,2]

Islet cells are terminally differentiated,[3] making adeno-associated virus (AAV) suitable for durable effect

Opportunity to amplify islet GLP-1 signaling to improve β-cell health

AAV with GLP-1RA Driven by INS Promoter

Islet

β-Cell

GLP-1R

INSULIN

GLP-1RA

Figure adapted from Saikia et al. JCI Insight. 2021 6:e1418511. 1. Lubaczeuski et al. Keystone 2023 oral presentation. Poster no. 1025. 2. Rajagopalan et al. ADA 2023 oral presentation. Abstract no. 181-OR. 3. Perl et al. J Clin Endocrinol Metab. 2010 95: E234–E239. AAV=adeno-associated virus, GLP-1=glucagon-like peptide 1, GLP-1R=GLP-1 receptor, GLP-1RA=GLP-1R agonist, INS=insulin, PGTx=pancreatic gene therapy
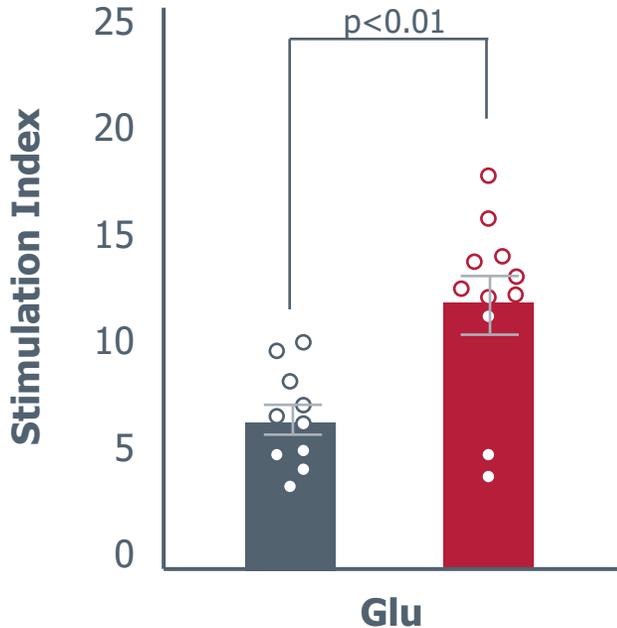
GLP-1RA PGTx in Human Islets and Human β-Cell Line
Improves glucose-stimulated insulin secretion via GLP-1R

A) Human Islet Transduction
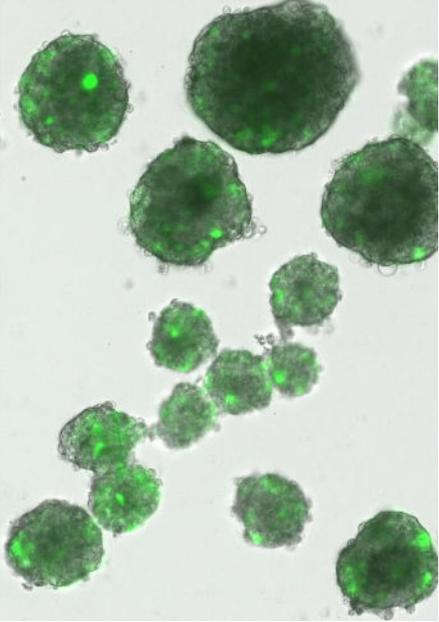
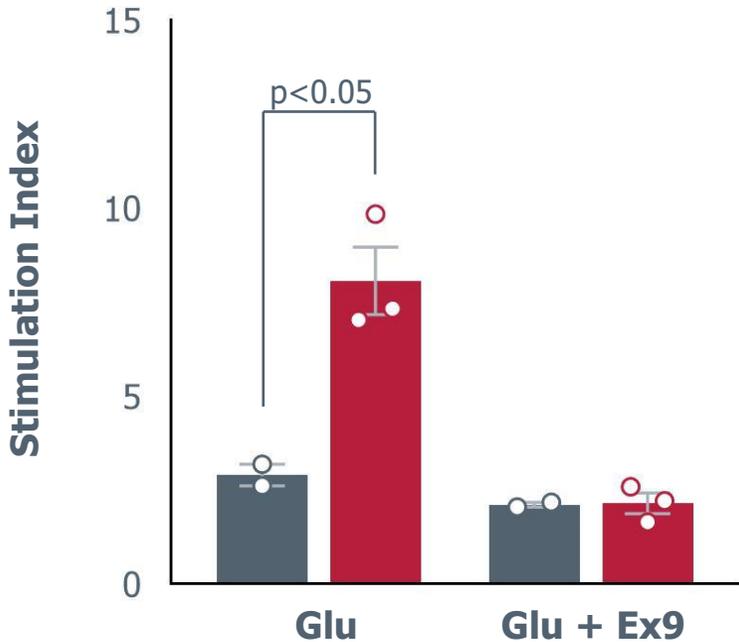
GFP Expression

B) Human Islet GSIS

p<0.01

C) Human β-cell Line GSIS ± Ex9
(GLP-1R Antagonist)
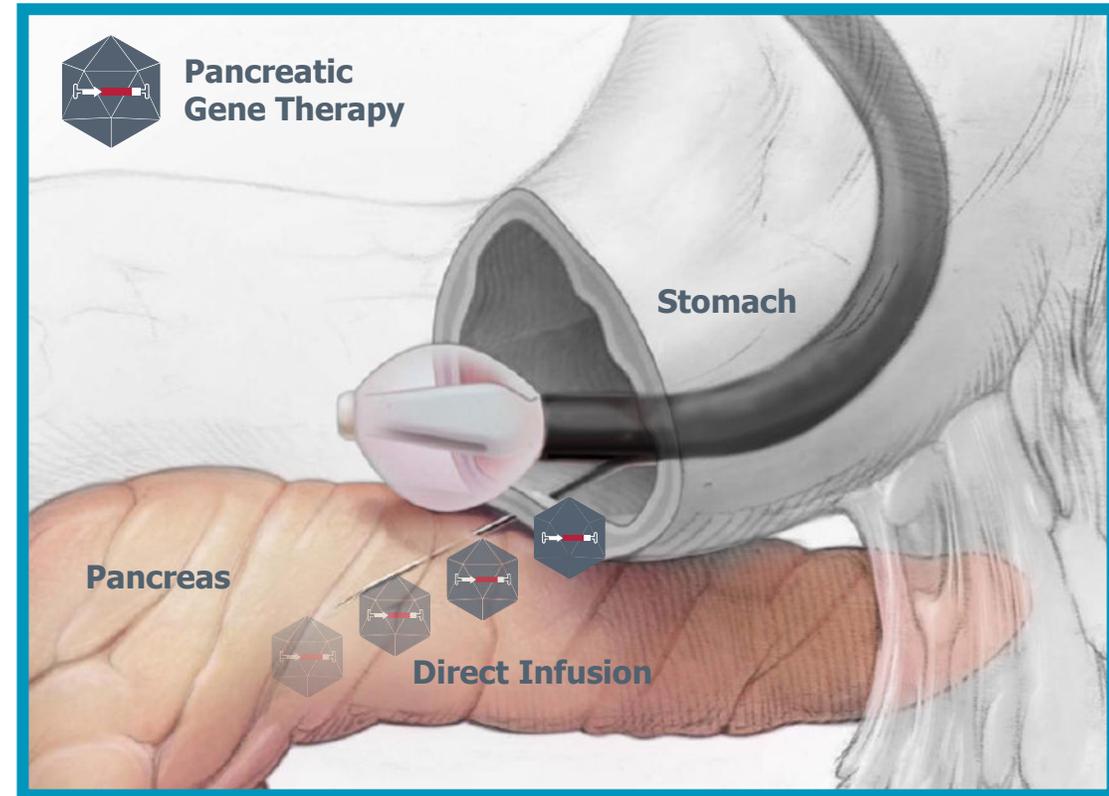

p<0.05

Legend: ■ Untransduced   ■ GLP-1RA PGTx

Gene Therapy Route of Administration to Pancreas

Proprietary, automated, endoscopic delivery device

Local delivery enables low viral genome dosing with limited systemic virus exposure[1]

Islets are readily accessible[2,3] via already established, routine, upper endoscopic ultrasound procedures,[4] performed in ~300K patients per year in US[5]

Procedural risk is further mitigated with device design (e.g., needle size, volume, controlled infusion rate)

Endoscopic Procedure & PGTx Delivery

1. Rajagopalan et al. ASGCT 2023 oral presentation. Abstract no. 191. 2. Docherty & Russ. Encyclopedia of Tissue Engineering and Regenerative Medicine 2019, pg. 367-374. 3. Ravi et al. Medicine (Baltimore). 2021 Apr 30;100(17):e25642. 4. Hasan & Hawes. Gastrointest Endosc Clin N Am. 2012 Apr;22(2):155-67. 5. Peery et al. Gastroenterology 2022 Feb: 162(2):621-644. PGTx=pancreatic gene therapy

Dose-Dependent Viral Transduction in Yucatan Pig Model
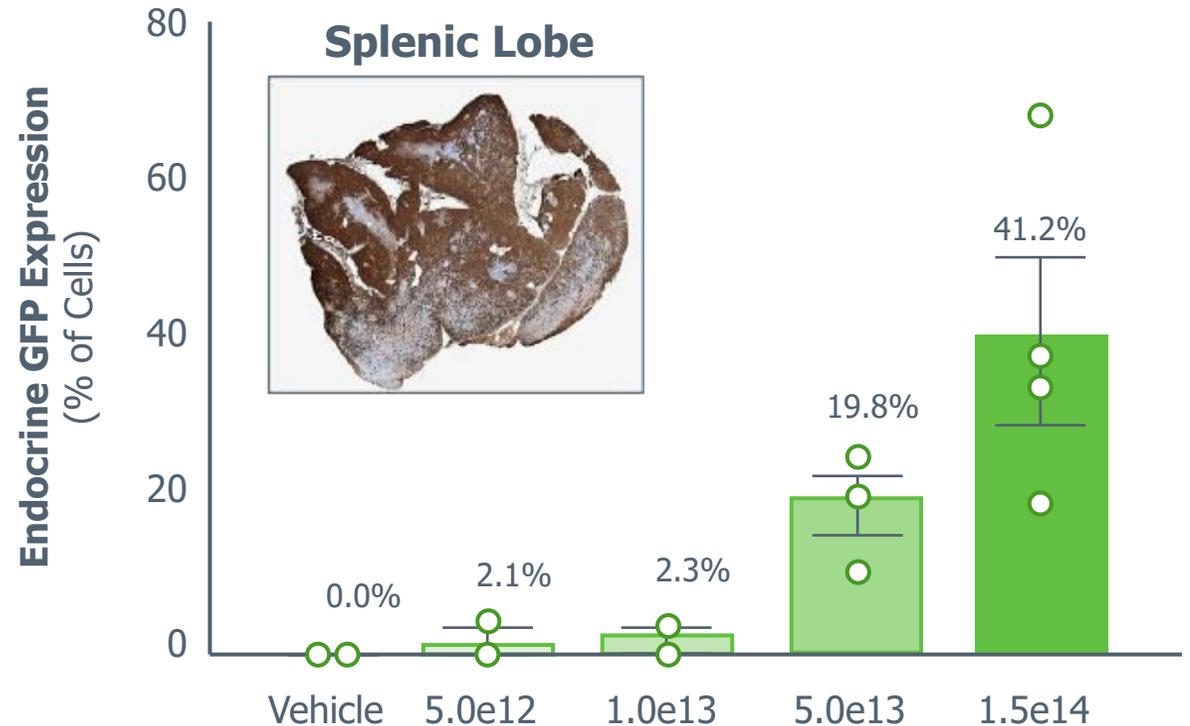## Local delivery effectively and reliably targets islets

Yucatan pig model **anatomy similar to humans**[1]

Dose-dependent AAV-GFP expression in targeted pancreatic lobe[2,3]

>60 animals treated with 100% technical success

No adverse safety signals to date (e.g., pancreatitis)

Yucatan Pig Islet Transduction

Splenic Lobe

Endocrine GFP Expression (% of Cells)

0.0% — Vehicle
2.1% — 5.0e12
2.3% — 1.0e13
19.8% — 5.0e13
41.2% — 1.5e14

GLP-1RA PGTx T2D Efficacy Study: Head-to-Head vs. Semaglutide

db/db murine model is *de facto* standard for T2D development

Single I.P. Injection
(5e12 VG GLP-1-based PGTx or Vehicle)

8-week-old Mice

Days 1 14 28 42 56 62
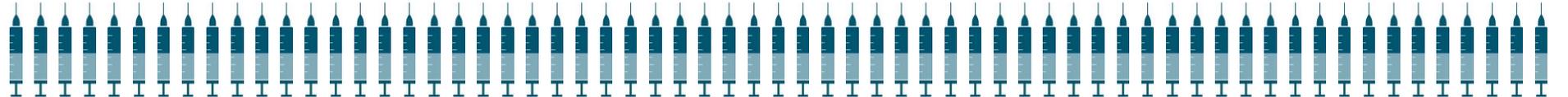
Daily S.C. Injections
Semaglutide (10 nmol/kg/d) or Vehicle

GLP-1=glucagon-like peptide 1, GLP-1RA=glucagon-like peptide 1 receptor agonist, I.P.=intraperitoneal, PGTx=pancreatic gene therapy, S.C.=subcutaneous, T2D=type 2 diabetes, VG=vector genomes

Glucose-Lowering Efficacy in *db/db* Murine Model
GLP-1RA PGTx improves glucose, insulin, and weight vs. daily semaglutide
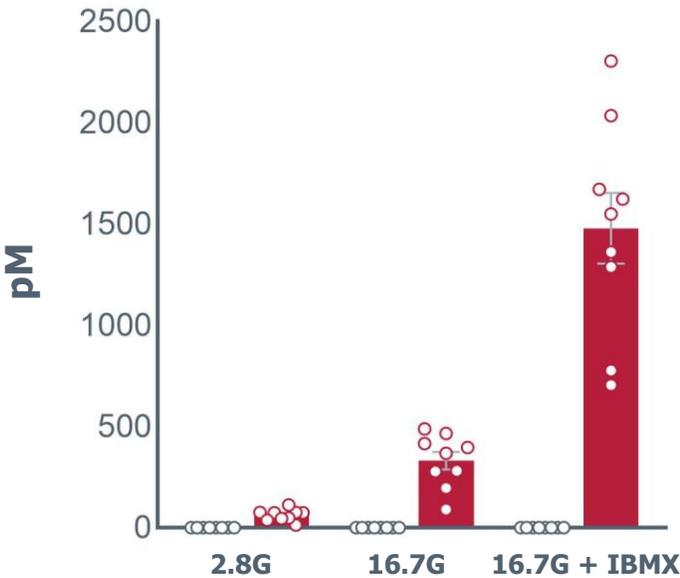

A) Fasting Blood Glucose
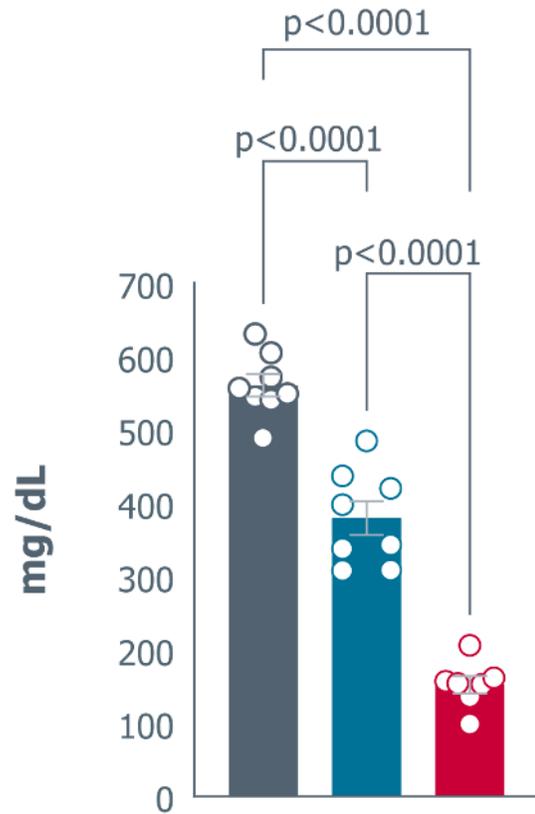
B) Fasting Plasma Insulin
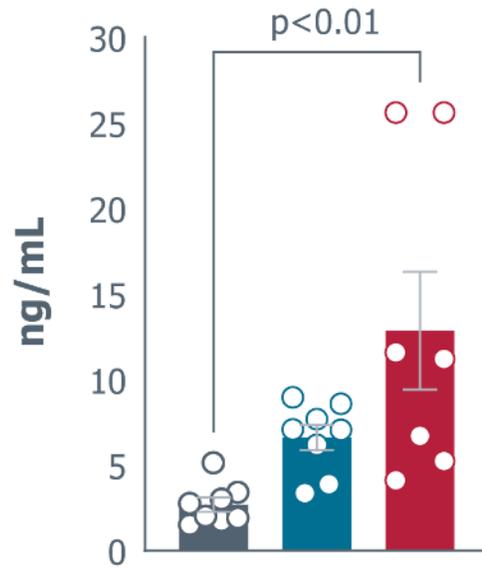
C) Body Weight Change from Baseline

Legend:
- Vehicle Control
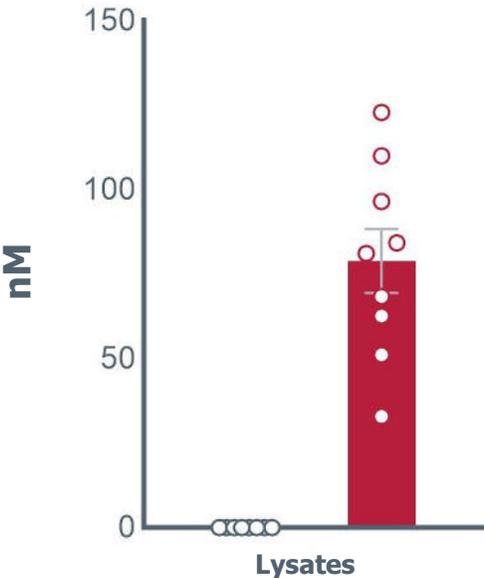- Semaglutide (10 nmol/kg/d)
- GLP-1RA PGTx (5e12)
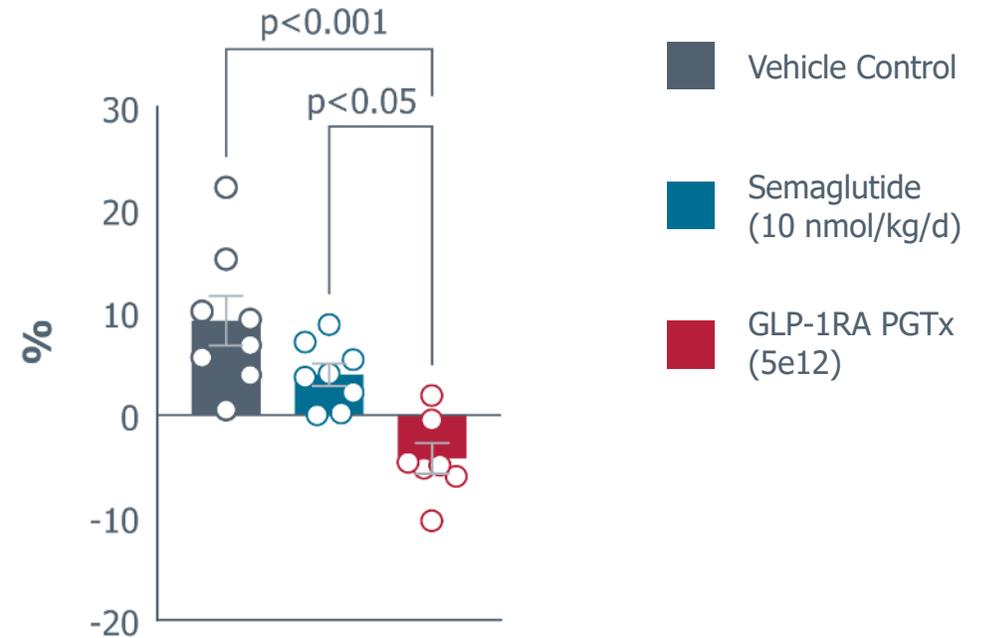
GLP-1RA PGTx Secretion in Isolated *db/db* Islets
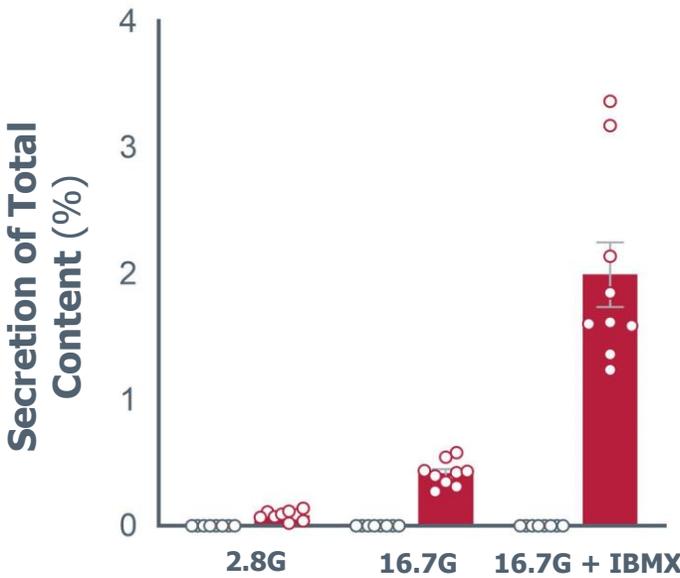## Glucose-dependent GLP-1RA release with ample secretory reserve

A) GLP-1RA Released

B) GLP-1RA Total Content

C) GLP-1RA Secretion
(% of Total Content)
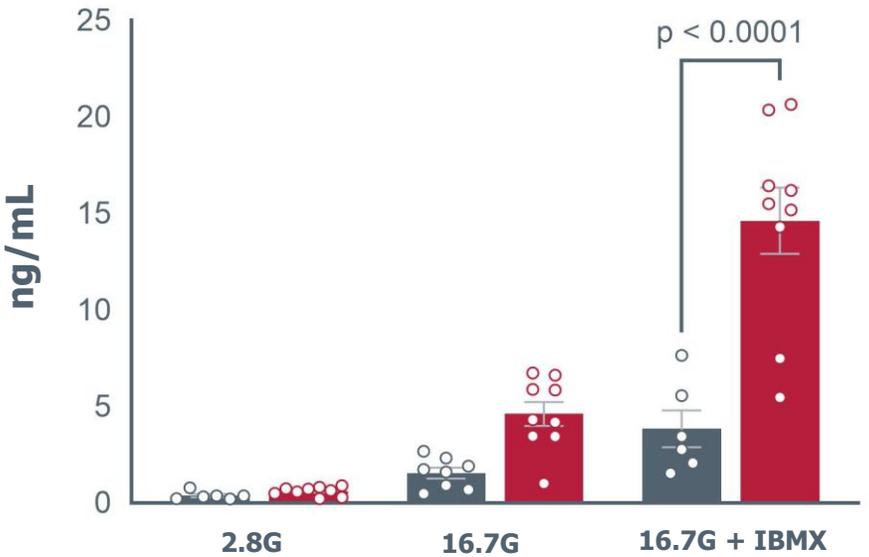

Vehicle Control   GLP-1RA PGTx

Islets were isolated 9 weeks post-GLP-1RA PGTx treatment, N=3 mice/group, 15 islets/well, performed in triplicate. 2.8G=2.8mM glucose, 16.7G=16.7mM glucose, GLP-1RA=glucagon-like peptide 1 receptor agonist, IBMX=3-isobutyl-1-methylxanthine, PGTx=pancreatic gene therapy
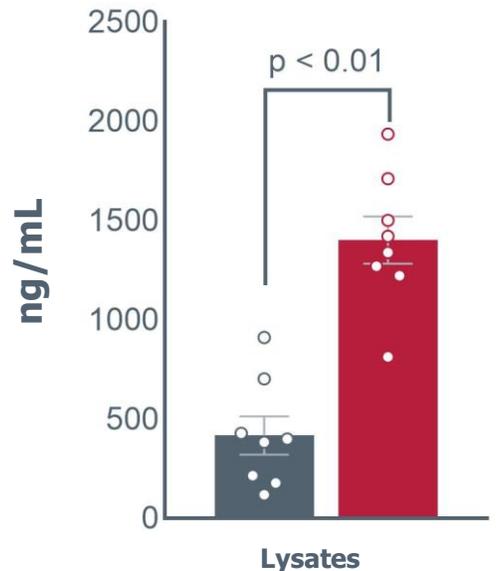
GLP-1RA PGTx-Induced Insulin Secretion in Isolated *db/db* Islets

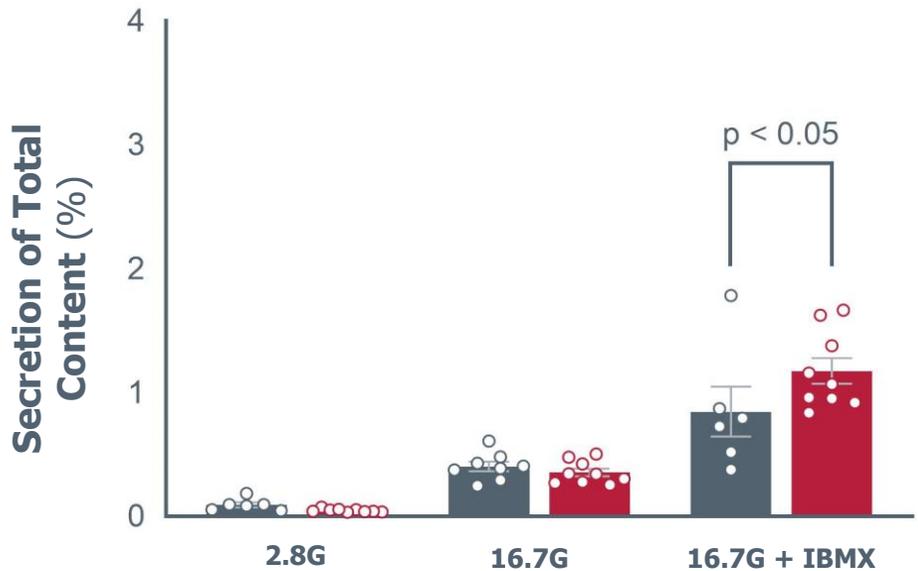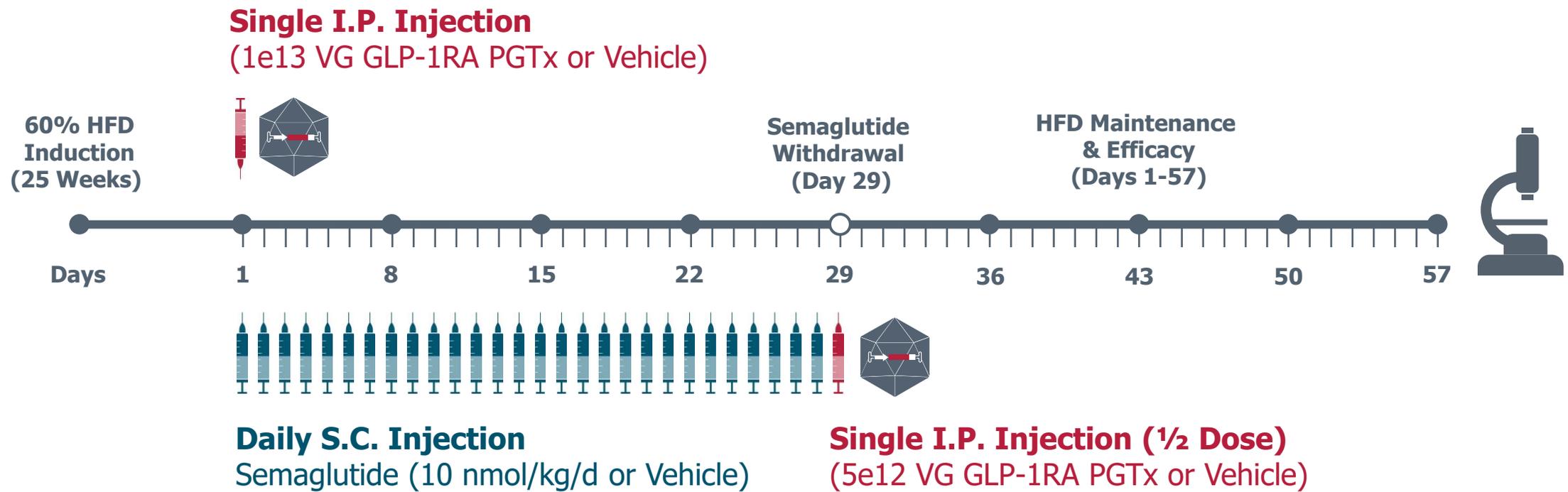## Increases insulin content and secretion with ample secretory reserve

A) Insulin Released

B) Insulin Total Content

C) Insulin Secretion (% of Total Content)

Legend: Vehicle Control — GLP-1RA PGTx

GLP-1RA PGTx Obesity Efficacy Study: Head-to-Head vs. Semaglutide
DIO murine model is *de facto* standard for obesity development

Single I.P. Injection
(1e13 VG GLP-1RA PGTx or Vehicle)

60% HFD Induction (25 Weeks)

Semaglutide Withdrawal (Day 29)

HFD Maintenance & Efficacy (Days 1-57)

Days   1   8   15   22   29   36   43   50   57

Daily S.C. Injection
Semaglutide (10 nmol/kg/d or Vehicle)

Single I.P. Injection (½ Dose)
(5e12 VG GLP-1RA PGTx or Vehicle)

DIO=diet-induced obesity, GLP-1RA=glucagon-like peptide 1 receptor agonist, HFD=high fat diet, I.P.=intraperitoneal, PGTx=pancreatic gene therapy, S.C.=subcutaneous, VG=vector genomes

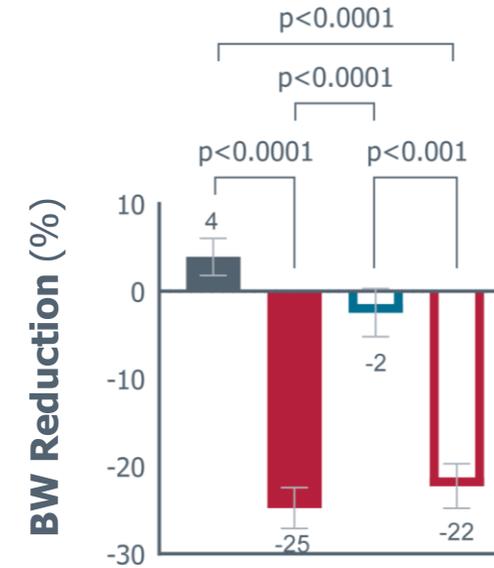# Body Weight Change in DIO Murine Model
Single-dose GLP-1RA PGTx sustains weight loss after semaglutide withdrawal
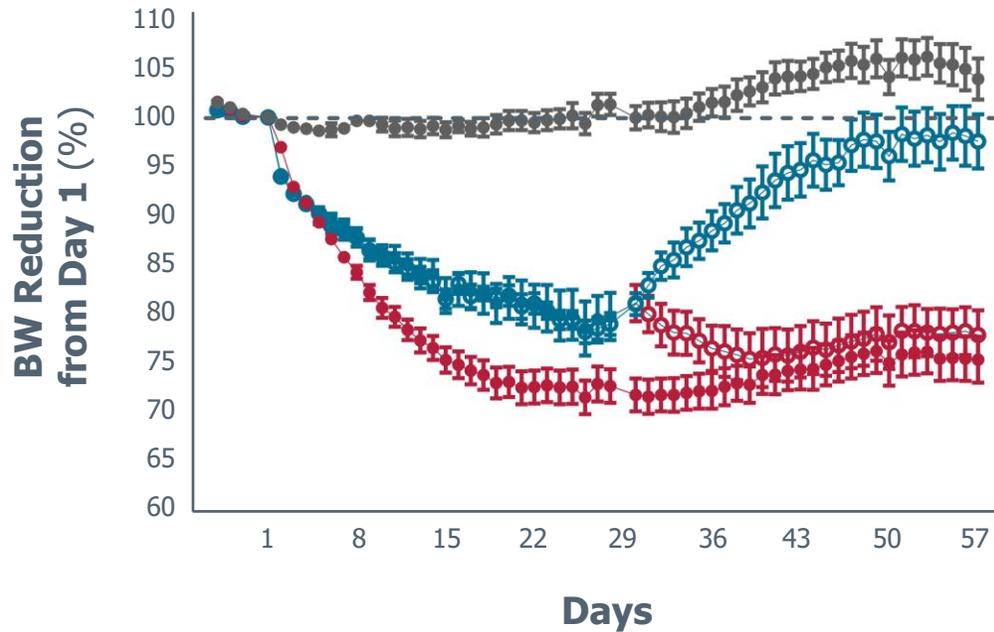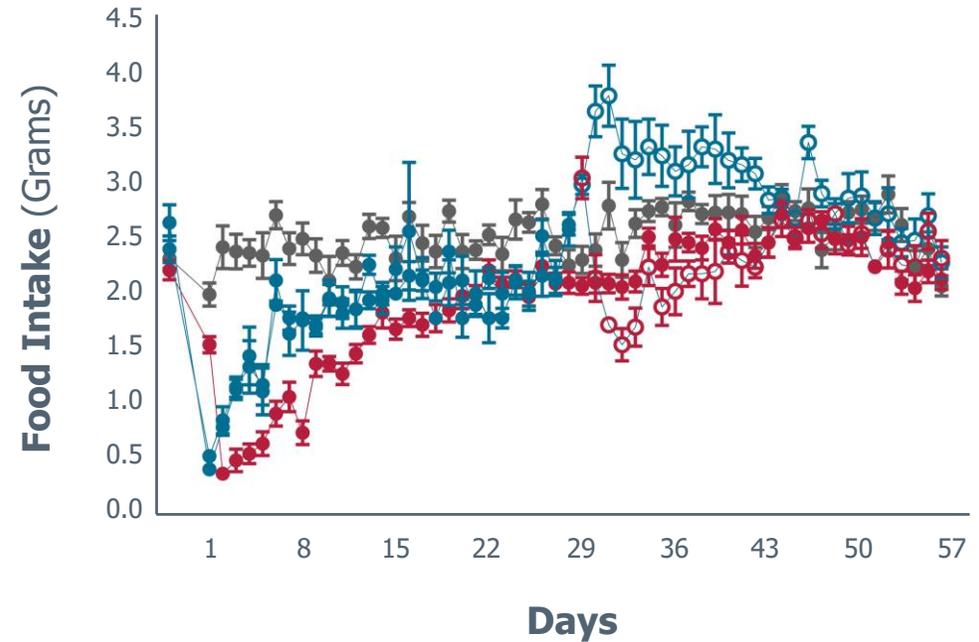
A) Change in BW Over Time

B) End of Study BW Change

Legend:
- Vehicle Control
- Sema (10 nmol/kg/d)
- GLP-1RA PGTx (1e13 VG)
- Sema Withdrawal + Vehicle
- Sema Withdrawal + GLP-1RA PGTx (5e12 VG)
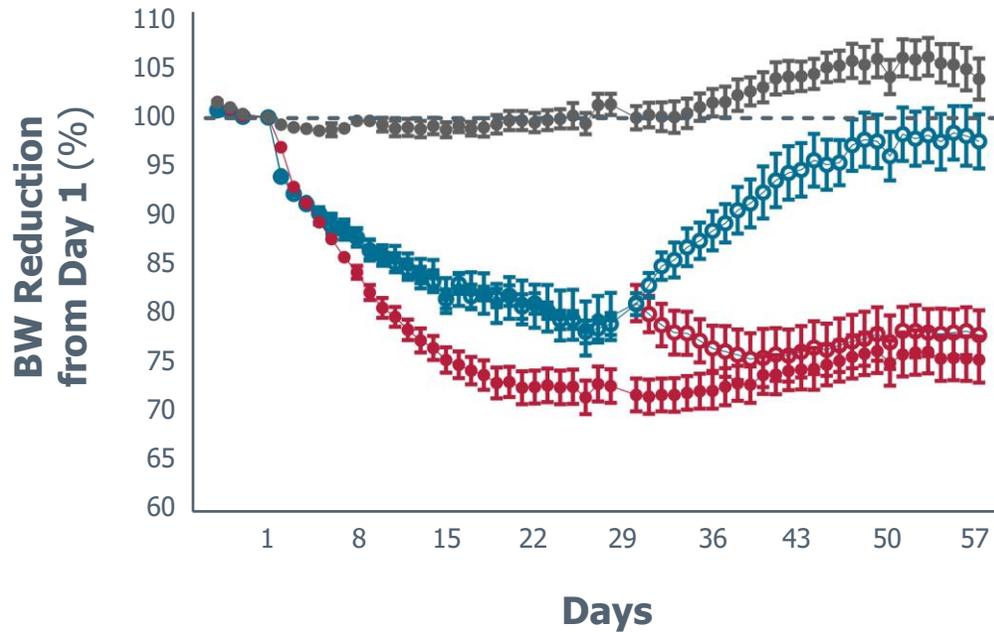
Food Intake Change in DIO Murine Model
Body weight changes are reflected by alterations in food intake

A) Change in BW Over Time

B) Food Intake Over Time

Legend:
- Vehicle Control
- Sema (10 nmol/kg/d)
- GLP-1RA PGTx (1e13 VG)
- Sema Withdrawal + Vehicle
- Sema Withdrawal + GLP-1RA PGTx (5e12 VG)
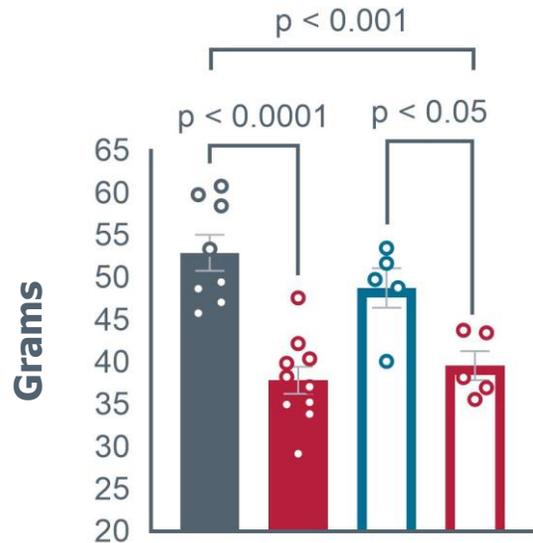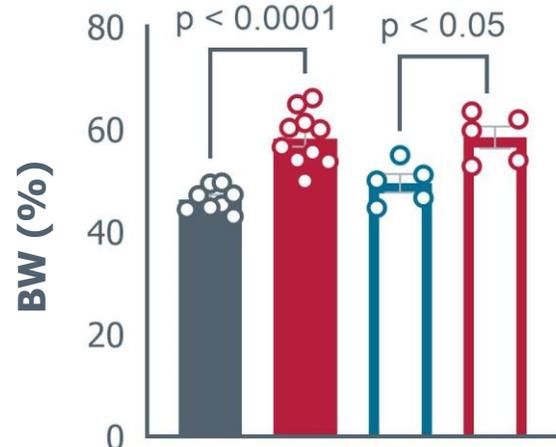
Body Composition Change in DIO Murine Model
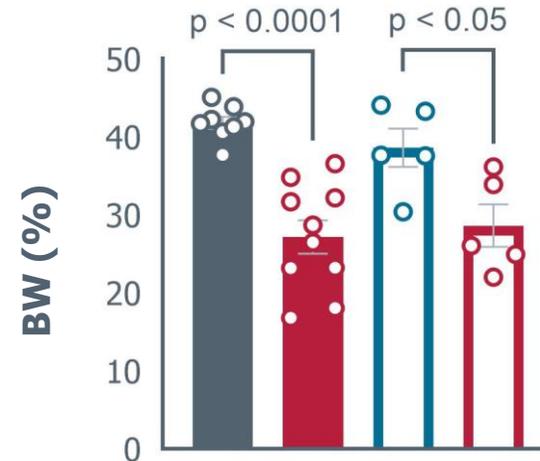## Preservation of lean mass: body weight loss primarily from fat mass
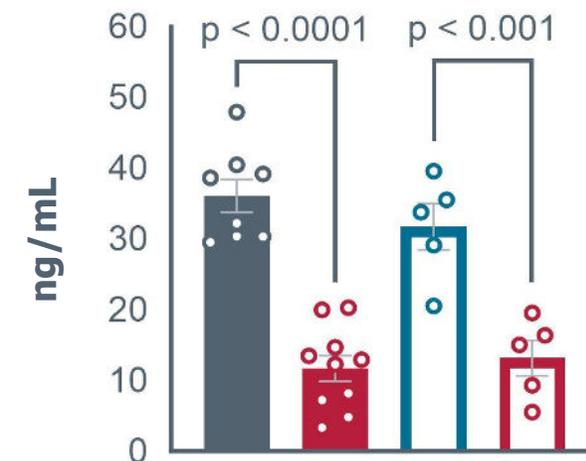

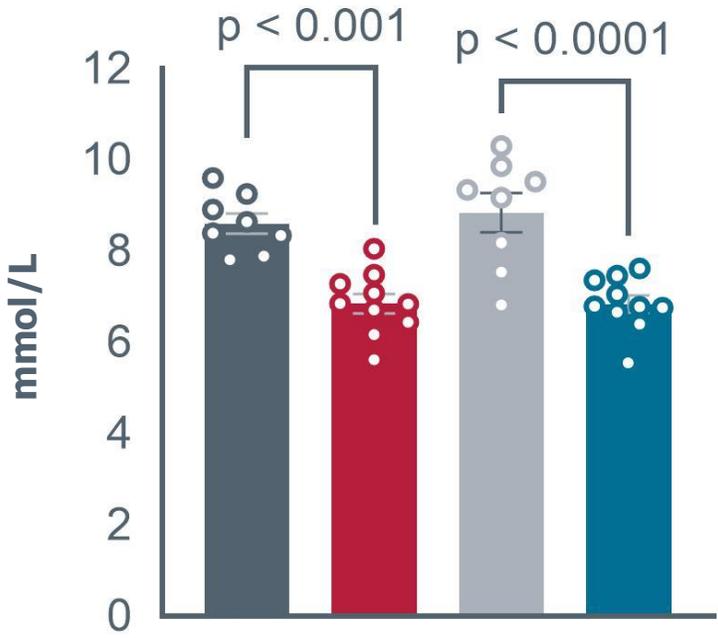A) Body Weight   B) Lean Mass   C) Fat Mass   D) Plasma Leptin

Legend:
- Vehicle Control
- GLP-1RA PGTx (1e13 VG)
- Sema Withdrawal + Vehicle
- Sema Withdrawal + GLP-1RA PGTx (5e12 VG)

Mean ± SEM shown; n=5-10 per group, 8 weeks post-treatment. BW=body weight, GLP-1RA=glucagon-like peptide 1 receptor agonist, PGTx=pancreatic gene therapy, Sema=semaglutide, VG=vector genomes
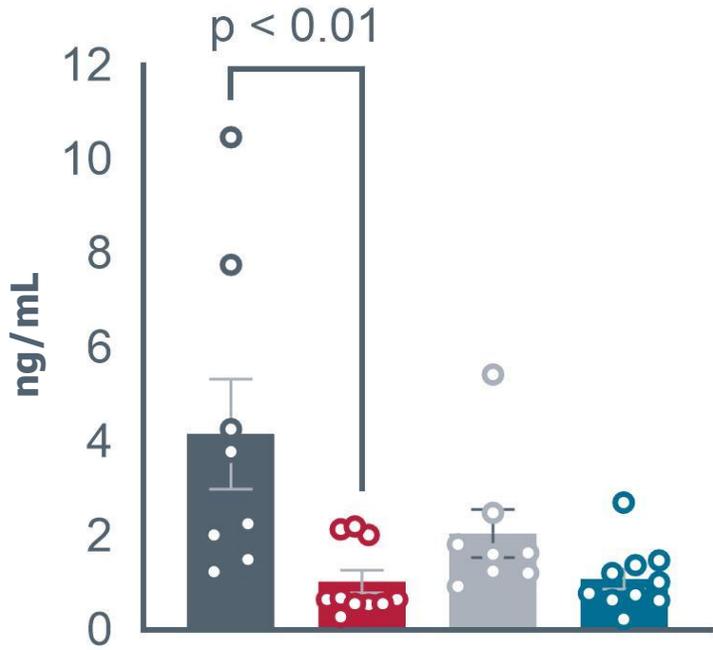
Fasting Blood Glucose and Insulin Changes in DIO Murine Model
Single-dose GLP-1RA PGTx improves FBG and insulin at 4 weeks

A) Fasting Blood Glucose

B) Fasting Plasma Insulin

Legend: AAV Vehicle Control | GLP-1RA PGTx (1e13 VG) | Sema Vehicle | Sema (10 nmol/kg/d)

Mean ± SEM shown; n=5-10 per group. AAV=adeno-associated virus, DIO=diet-induced obesity, FBG=fasting blood glucose, GLP-1RA=glucagon-like peptide 1 receptor agonist, PGTx=pancreatic gene therapy, Sema=semaglutide, VG=vector genomes

Fasting Blood Glucose and Insulin Changes in DIO Murine Model
Single-dose GLP-1RA PGTx improves FBG and insulin at 8 weeks

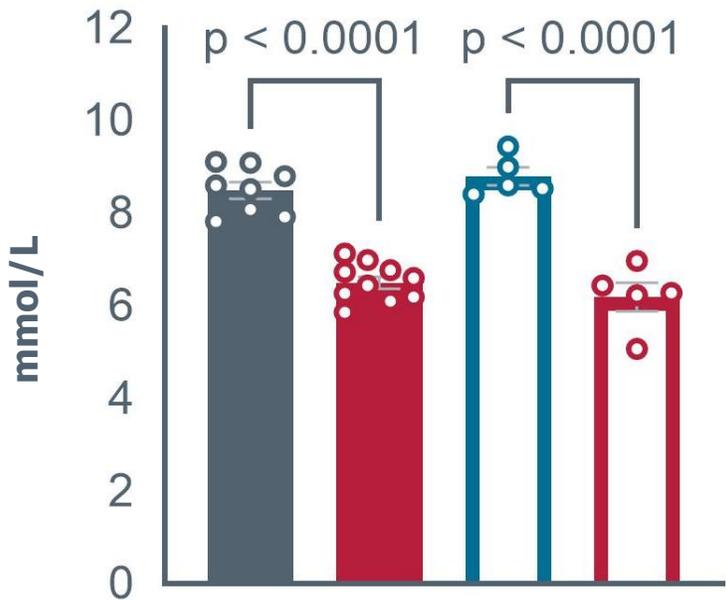**A)** Fasting Blood Glucose
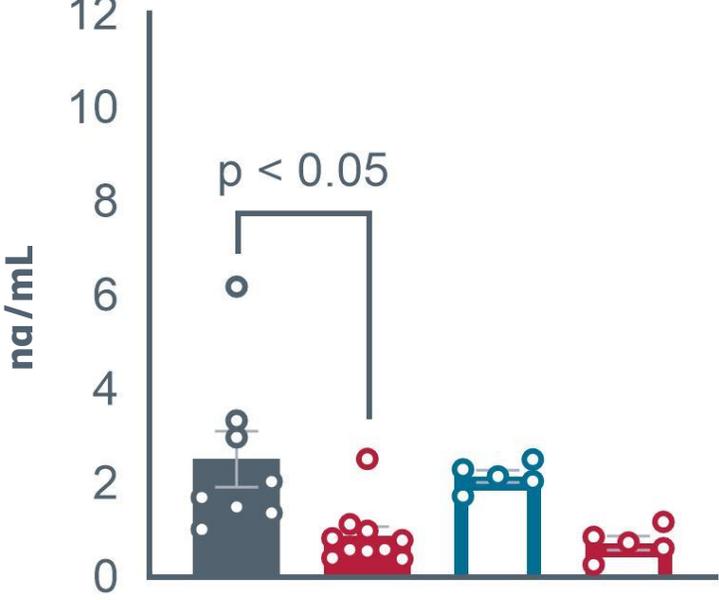
B) Fasting Plasma Insulin

Legend: AAV Vehicle Control | GLP-1RA PGTx (1e13 VG) | Sema Withdrawal + Vehicle | Sema Withdrawal + GLP-1RA PGTx (5e12 VG)

Mean ± SEM shown; n=5-10 per group. AAV=adeno-associated virus, DIO=diet-induced obesity, FBG=fasting blood glucose, GLP-1RA=glucagon-like peptide 1 receptor agonist, PGTx=pancreatic gene therapy, Sema=semaglutide, VG=vector genomes

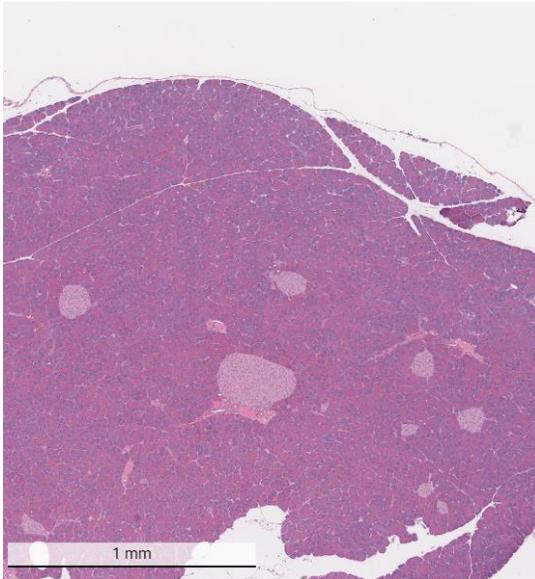# Pancreatic Islet Histopathology in DIO Murine Model
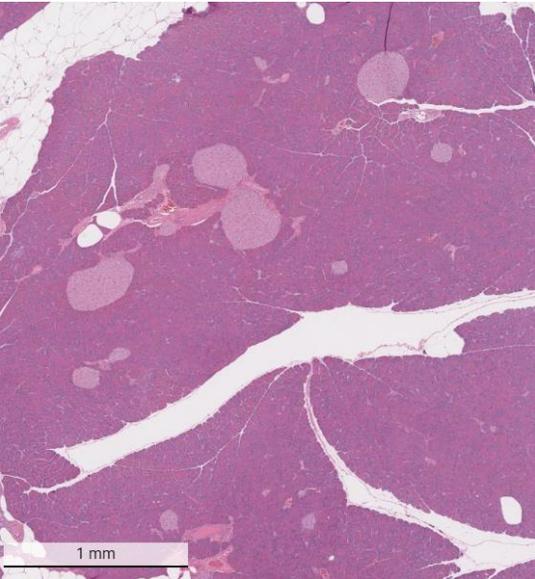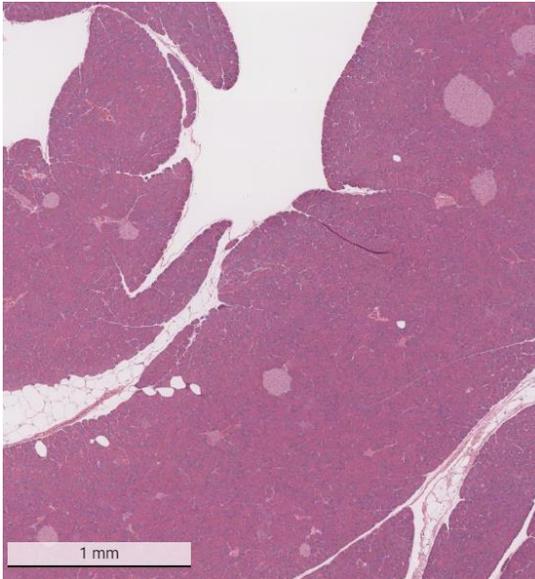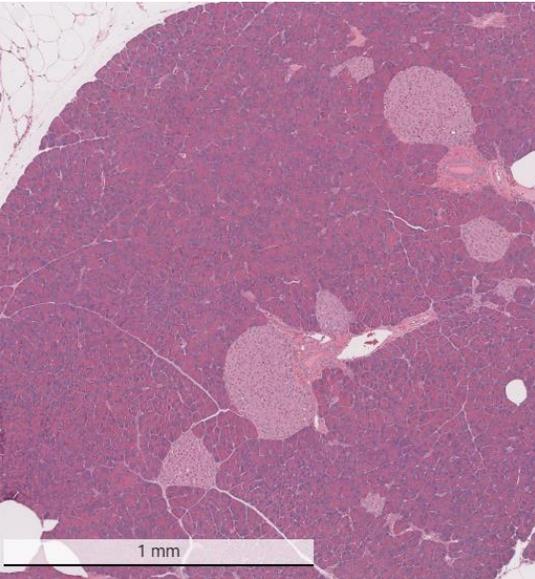## GLP-1RA PGTx was not associated with inflammation

Vehicle Control	GLP-1RA PGTx	Sema Withdrawal	Sema Withdrawal + PGTx

Score: 0.25 ± 0.16	0.30 ± 0.15	0.20 ± 0.20	0.20 ± 0.20

Histopathology Scorecard[1]
0 – No cellular infiltrates
1 – Infiltrates in small foci at islet periphery
2 – Infiltrates surround islets [peri-insulitis]
3 – Intra-islet infiltration <50% islet

4 – Extensive infiltrations = 50% islet, cellular, destruction, prominent cytoarchitectural derangement
5 – Islet atrophy because of beta cell loss

There were no significant differences between groups

GLP-1RA PGTx Safety and Feasibility Studies in Model Systems
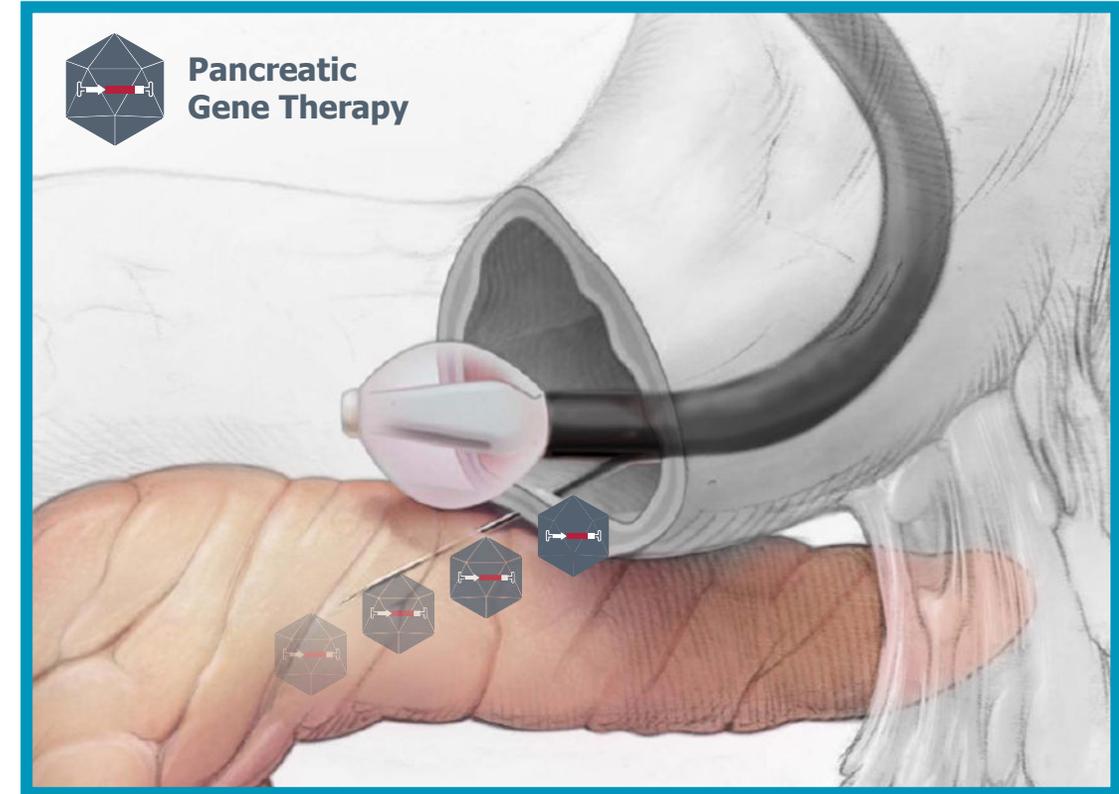
Conclusions to date and next steps

Early safety and feasibility observations in *db/db* and DIO mice and Yucatan pigs are encouraging

First demonstration of **nutrient-responsive GLP-1RA secretion in islets**

PGTx **improves fasting glucose and insulin** in *db/db* and DIO models of T2D and obesity

PGTx leads to **durable weight loss** and **weight and body composition maintenance after semaglutide withdrawal** in DIO mice

RJVA-001 IND-enabling studies are underway

Pancreatic Gene Therapy

DIO=diet-induced obesity, GLP-1RA=glucagon-like peptide 1 receptor agonist, IND=investigational new drug, PGTx=pancreatic gene therapy, T2D=type 2 diabetes

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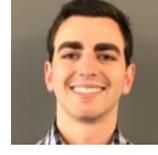**Rebecca Reese, Assoc.**
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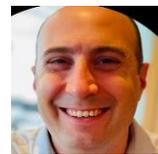Assoc. Scientist

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