FRACTYL® health

20TH WORLD CONGRESS
on INSULIN RESISTANCE, DIABETES & CARDIOVASCULAR DISEASE
Exploring New Frontiers in Metabolism – Tomorrow's Clinical Science Today

AAV-Mediated Pancreatic Gene Therapy for Type 2 Diabetes

Alice Liou Fitzpatrick, Nidhi Khanna, Jacob Wainer, Rebecca Reese, Jason West, Jay Caplan, and Harith Rajagopalan

December 2, 2022

Disclosure Statement

Author(s): Alice Liou Fitzpatrick, Nidhi Khanna, Jacob Wainer, Rebecca Reese, Jason West, Jay Caplan, and Harith Rajagopalan are employees and shareholders of Fractyl Health, Inc.
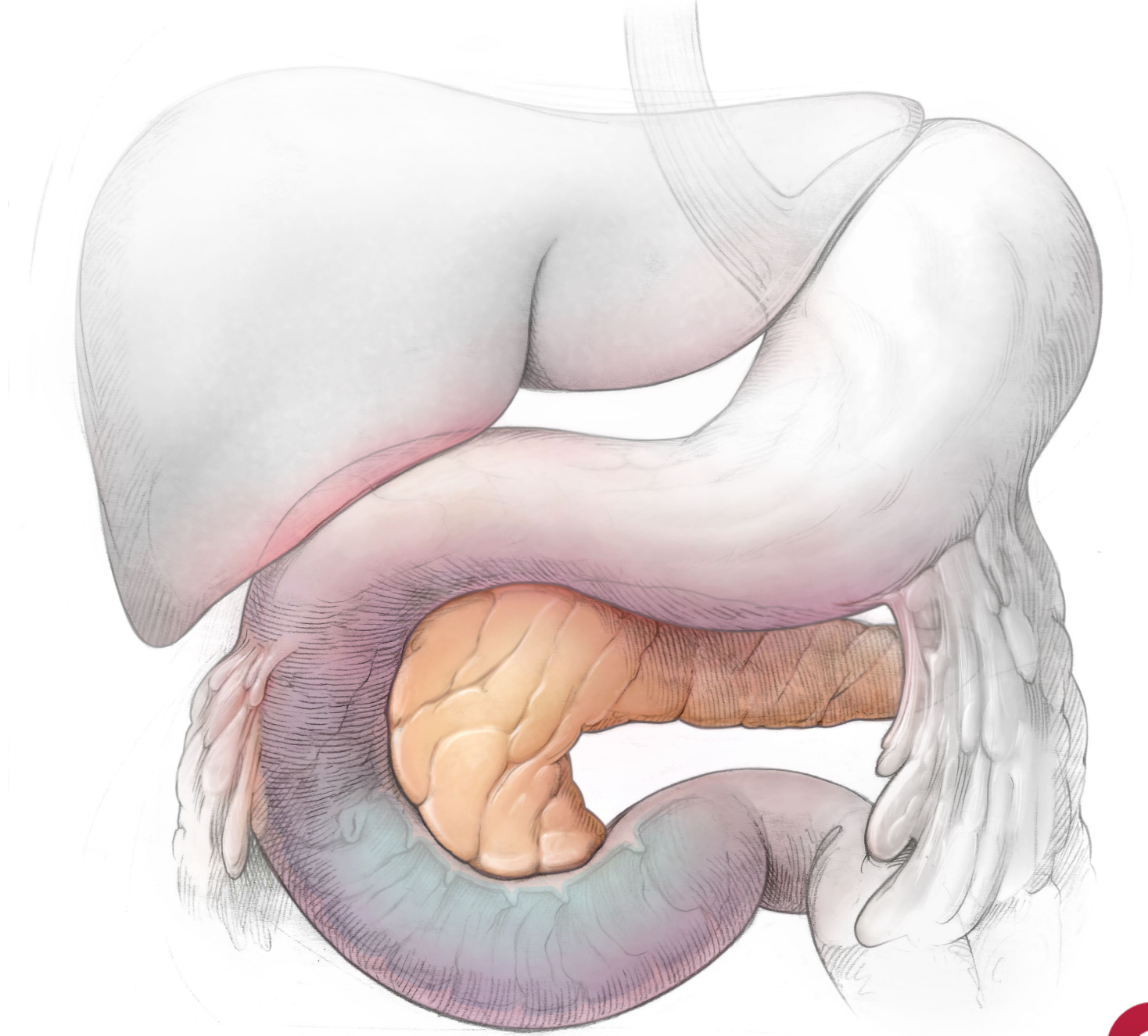
Fractyl Health's Mission

Target organ-level root causes of metabolic disease

Revita System™ for Duodenal Mucosal Resurfacing (DMR)

Restore morphology and metabolic function to the duodenum

Rejuva® Platform for Pancreatic Gene Therapy

Restore metabolic regulation of the pancreas with locally delivered gene therapy

The Revita™ system is for investigational use only in the United States. The Rejuva® platform is in early development and not approved by any regulatory body for investigational or commercial use.

GLP-1 therapies have proven benefits for pancreatic health in T2D but are limited by adherence and tolerability of systemic delivery

Real world persistence with systemic GLP-1 therapy remains low[1]
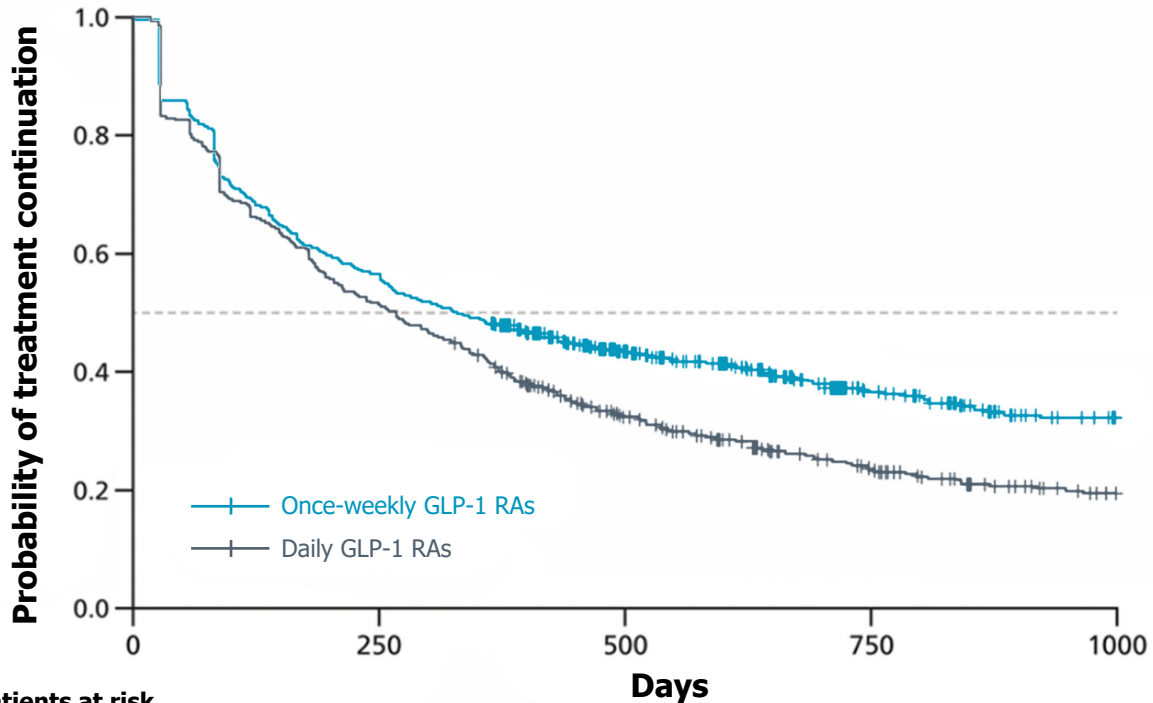
Patients at risk

Once-weekly GLP-1 Ras	784	443	258	139	83
Daily GLP-1 RAs	784	403	222	133	78

GLP-1 RA dosing	Median stay time
Once-weekly	333 days
Daily	269 days

- GLP-1 stimulates glucose-dependent insulin secretion and improves overall beta-cell health.[1]

- In the US, 50% of patients discontinue therapy within 330 days after initiating weekly GLP-1 therapy.[2]

- Side effects are primary reason for discontinuation.[3]

- Discontinuation of GLP-1RA therapy is associated with total loss of metabolic benefit. Ongoing exposure is needed for lasting patient benefit.[4]

1 Müller 2019 Mol Metab 30:72-130
2 Weiss 2020 Patient Pref Adherence 14:2337-2345
3 Polonsky 2021 Diabetes spectr 34(2):175-183
4 RISE Consortium Diabetes Care. 2019;42(9):1742-1751

Gene therapy approach for improvements in beta-cell health
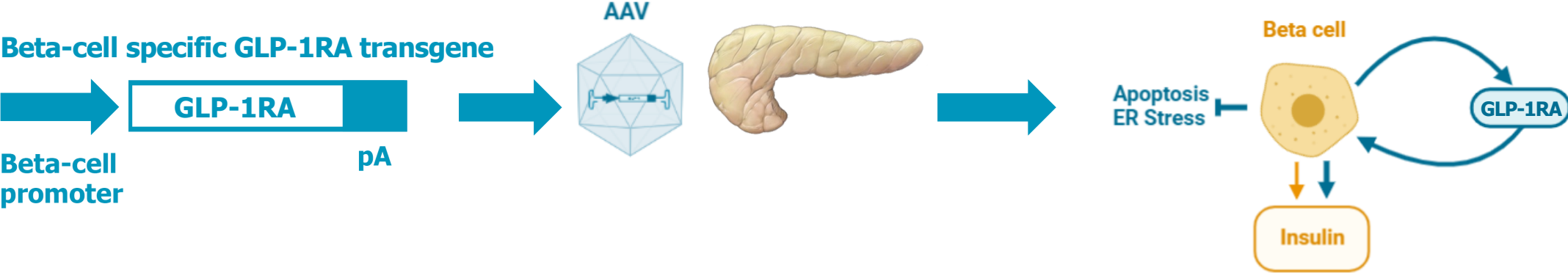
pA = Polyadenylation
AAV = Adeno-associated virus
ER = Endoplasmic reticulum

Screening a DNA construct library to identify top functional GLP-1RA producers in a beta-cell line
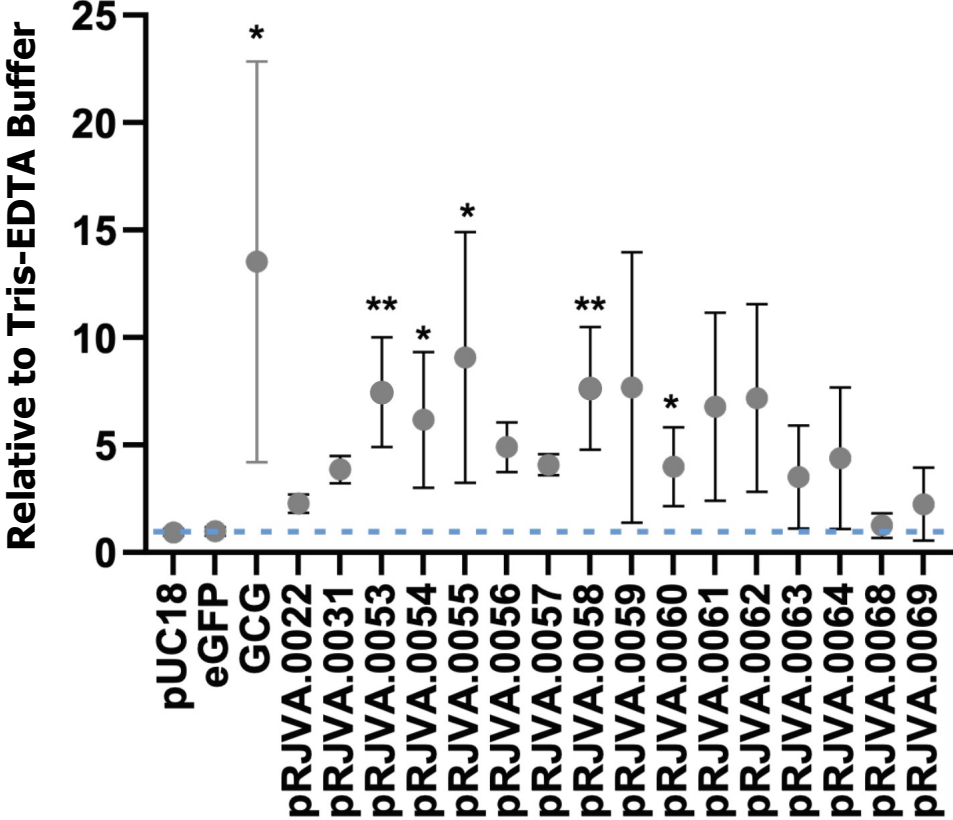

DNA construct template

**Beta-cell specific GLP-1RA transgene**

GLP-1RA

Plasmid Library

Transfection
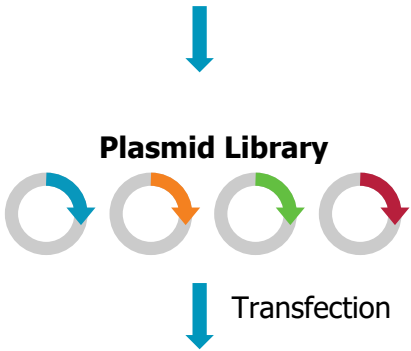
MIN6 Cells

GLP-1RA secretion

25 mM Glucose stimulation

Means ± Std Dev. One sample t-test *P<0.05, **P<0.01

MIN6 = Mouse insulinoma cell line 6
eGFP = enhanced green fluorescent protein
GCG = Preproglucagon gene

Screening a DNA construct library to identify top functional GLP-1RA producers in a beta-cell line

DNA construct template

Beta-cell specific GLP-1RA transgene

GLP-1RA

Plasmid Library

Transfection
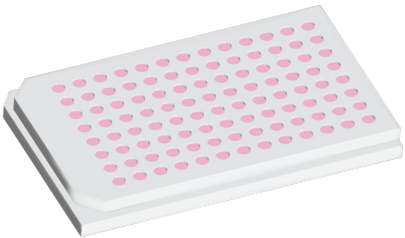
MIN6 Cells

cAMP signaling
CHO-K1 hGLP-1R Gs cell line

MIN6 = Mouse insulinoma cell line 6
eGFP = enhanced green fluorescent protein
GCG = Preproglucagon gene
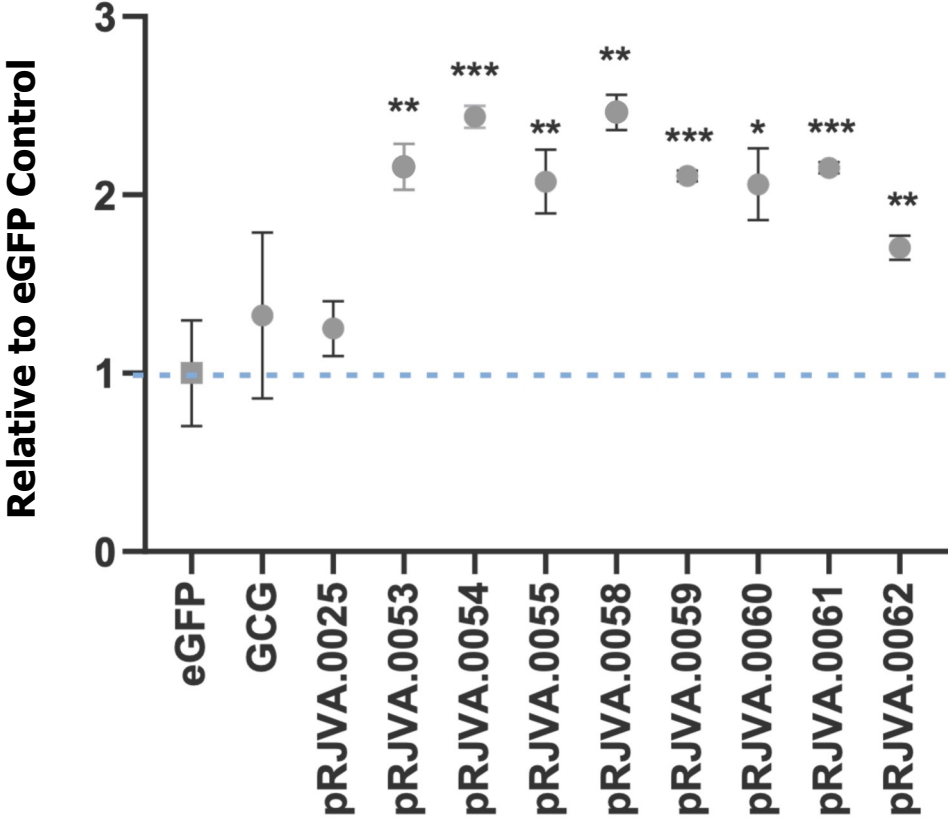
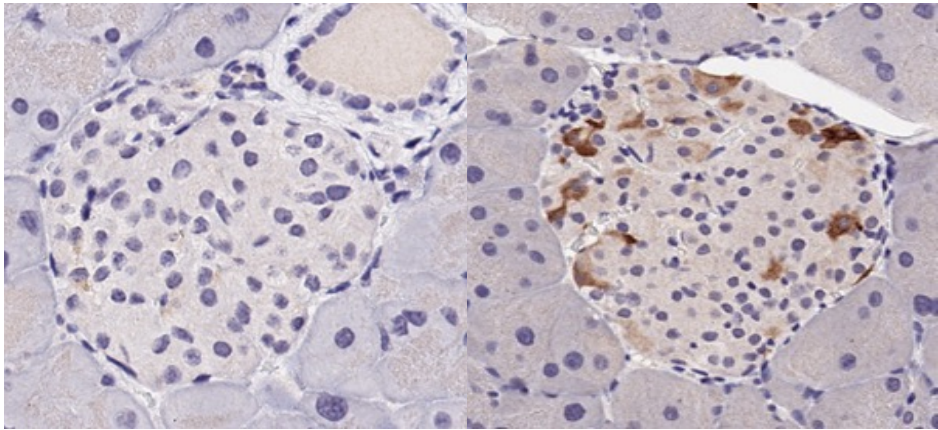Means ± Std Dev. One sample t-test *P<0.05, **P<0.01, ***P<0.001

Confirmed dose-dependent islet-restricted expression of GLP-1RA via AAV-mediated delivery in the BKS db/db mouse
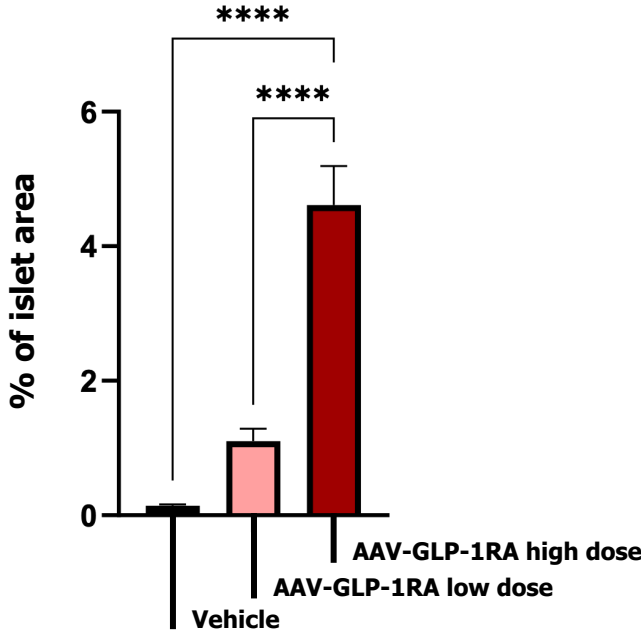
Single IP injection
5 weeks old
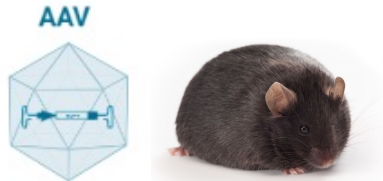
- AAV-GLP-1RA Low
- AAV-GLP-1RA High
- Vehicle Control

Week 10 post-injection
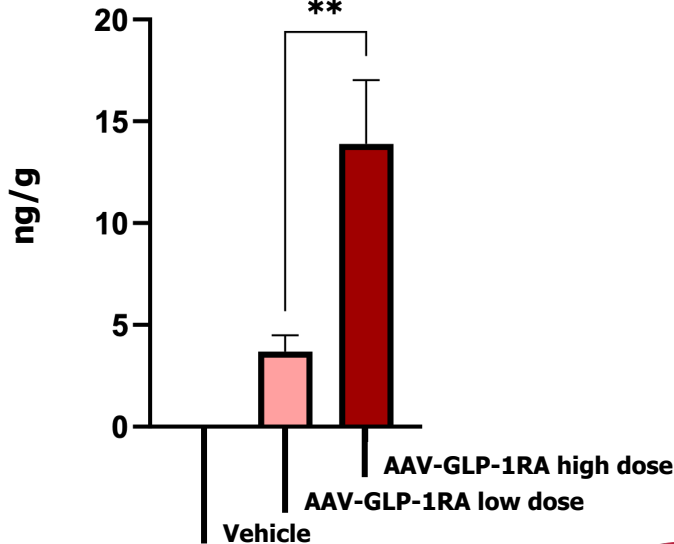**GLP-1RA transgene expression in mouse islets**

Vehicle

AAV-GLP1RA high dose

Pancreatic GLP-1RA protein % islet expression by IHC

Whole pancreas GLP-1RA protein expression

IP = Intraperitoneal
AAV = Adeno-associated virus
IHC = Immunohistochemistry

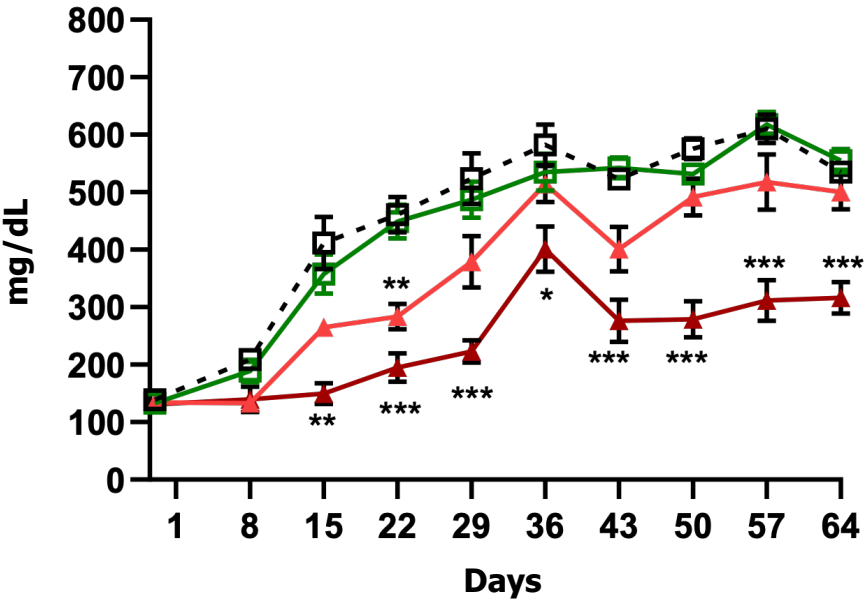Means ± SEM. One-Way ANOVA, post-hoc Tukey Test
**P<0.01, ****P<0.0001

Dose-dependent reduced glycemia with elevated insulinemia with AAV-GLP-1RA gene delivery in the BKS db/db mouse
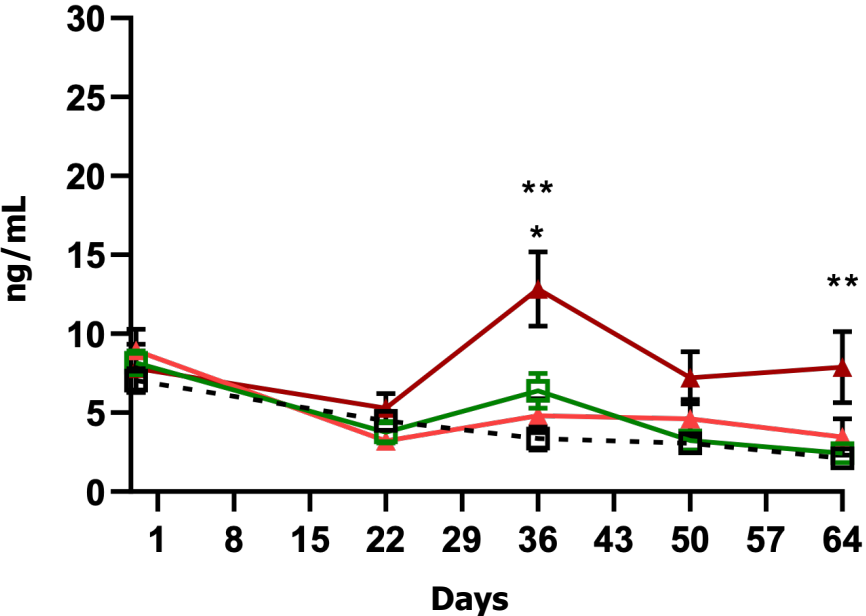
Single IP injection
5 weeks old
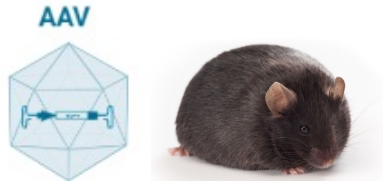
- AAV-GLP-1RA Low
- AAV-GLP-1RA High
- Vehicle Control
- AAV-eGFP Control

Weekly FBGs
4–6 hour fasted

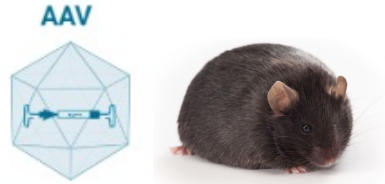**Biweekly insulin**
4–6 hour fasted

IP = Intraperitoneal
AAV = Adeno-associated virus
eGFP = Enhanced green fluorescent protein

Means ± SEM. Two-way ANOVA, post-hoc Tukey Test
**P<0.01, ****P<0.0001

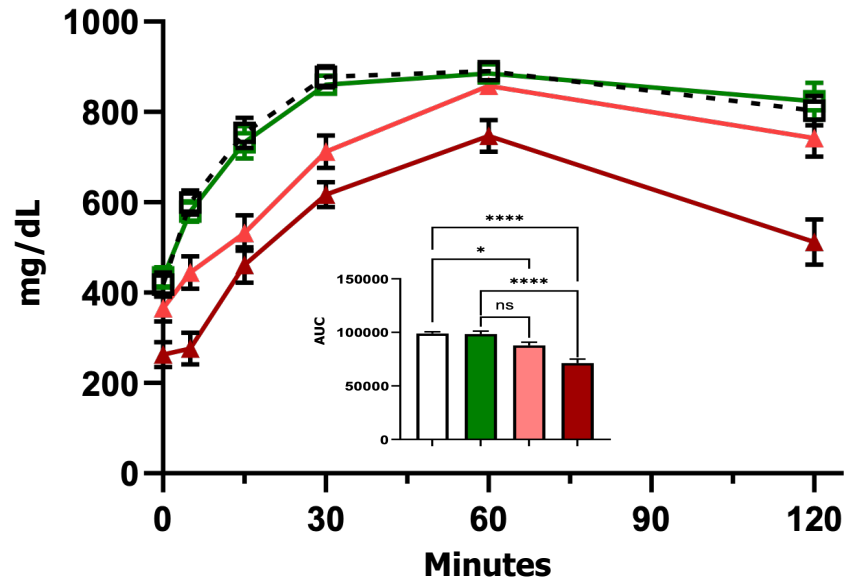# Improved glucose tolerance and 1st phase insulin secretion with AAV-GLP-1RA gene delivery in the BKS db/db mouse
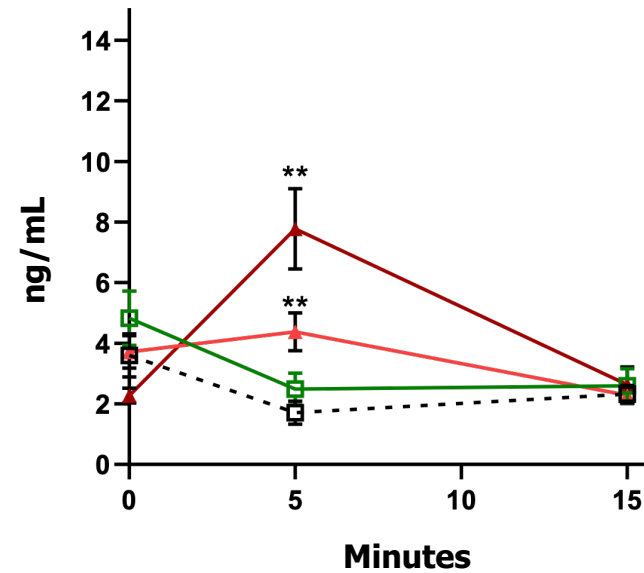
Single IP injection
5 weeks old

- AAV-GLP-1RA Low
- AAV-GLP-1RA High
- Vehicle Control
- AAV-eGFP Control

Week 6 post-injection

Glucose tolerance test

Glucose-stimulated insulin secretion

IP = Intraperitoneal
AAV = Adeno-associated virus
eGFP = Enhanced green fluorescent protein

Means ± SEM. One-Way ANOVA, post-hoc Tukey Test
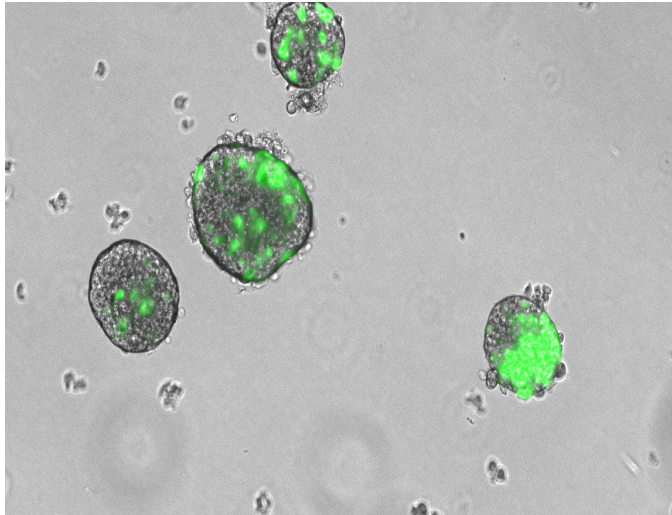**P<0.01, ****P<0.0001

GLP-1RA transgene expression and improved insulin secretion from primary BKS db/db islets *ex vivo*
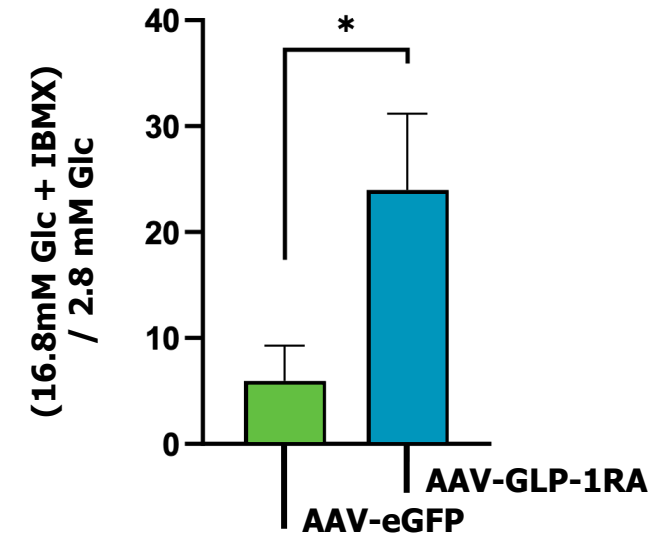
AAV

- AAV-eGFP Control
- AAV-GLP-1RA

Day 4 eGFP expression

Total GLP-1RA content
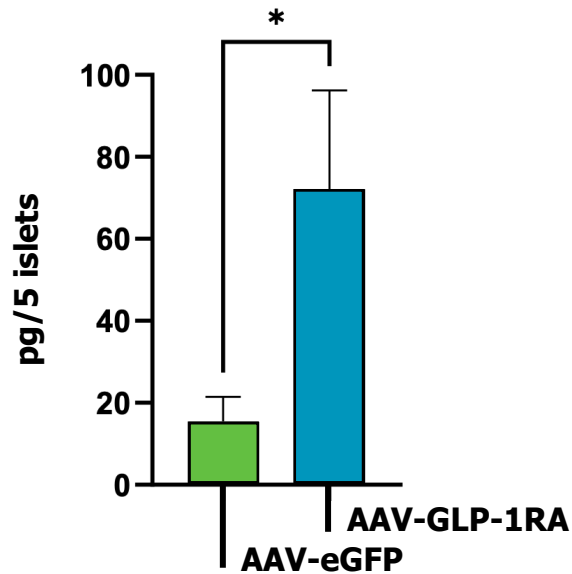
y-axis: pg/5 islets (0–100)
x-axis: AAV-eGFP, AAV-GLP-1RA

*

Glucose-stimulated insulin secretion

y-axis: (16.8mM Glc + IBMX) / 2.8 mM Glc (0–40)
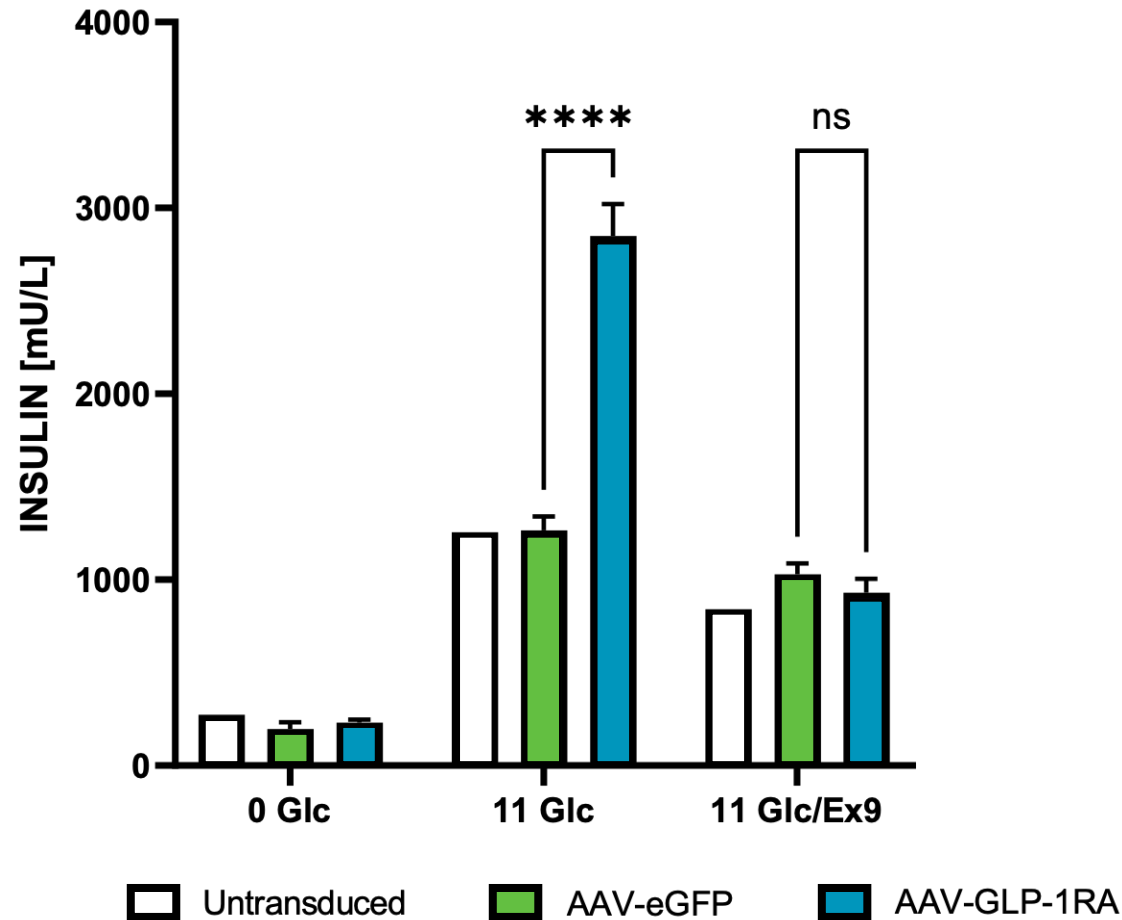x-axis: AAV-eGFP, AAV-GLP-1RA

*

AAV = Adeno-associated virus
eGFP = Enhanced green fluorescent protein
Glc = Glucose
IBMX = 3-isobutyl-1-methylxanthine

Means ± Std Dev. Unpaired t-test, *P<0.05

AAV-mediated delivery of GLP-1RA enhances insulin secretion in a GLP-1R dependent manner in the human beta-cell line EndoC-BH5

AAV = Adeno-associated virus
EX9 = Exendin-9, GLP-1R antagonist
Glc = Glucose

Means ± Std Dev. Two-Way ANOVA, post-hoc Tukey Test ****P<0.0001

Summary

1. Fractyl Health is developing an AAV-mediated gene therapy approach to locally deliver a GLP-1RA to the pancreas to improve beta-cell health and function for T2D.

2. We identified top plasmid constructs yielding functional GLP-1RA production via *in vitro* screening in a beta-cell line.

3. We tested the metabolic effect of an AAV-delivered GLP-1RA candidate in the db/db mouse model, demonstrating delayed disease progression, improved glycemia and glucose tolerance, and sustained insulin secretion.

4. AAV-GLP-1RA directly improves glucose-stimulated insulin secretion in primary db/db mouse islets and in a human beta-cell line.

Conclusion

- These studies provide proof of concept that a single dose islet-targeted gene therapy durably improves beta-cell function in a diabetic mouse model.

- Targeted gene therapy has the potential to improve glycemic control, modify or reverse disease progression, and reduce therapeutic burden in patients with T2D.

- This approach may offer a durable way to address patient need that is still unresolved with current therapeutics.

ER = Endoplasmic reticulum

Fractyl Health 2022