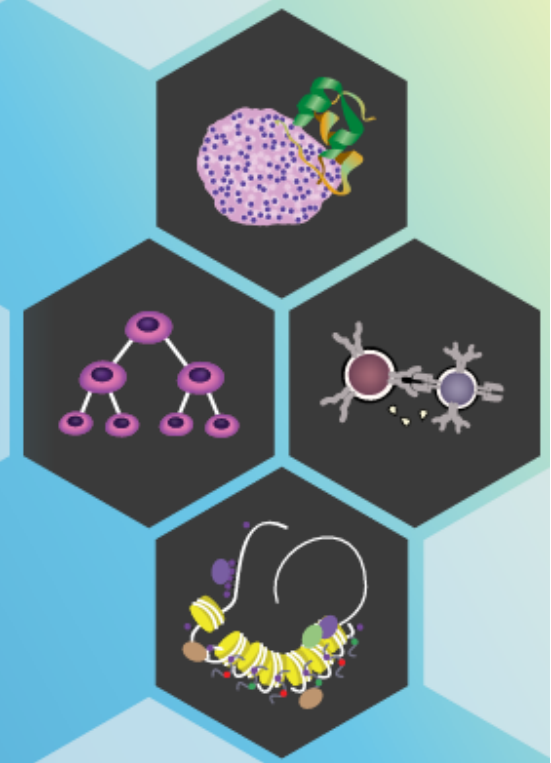
2024 RACHMIEL LEVINE-ARTHUR RIGGS

Diabetes Research Symposium

TXNIP



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Nancy R. and Eugene C. Gwaltney Family Endowed Chair in Juvenile Diabetes Research

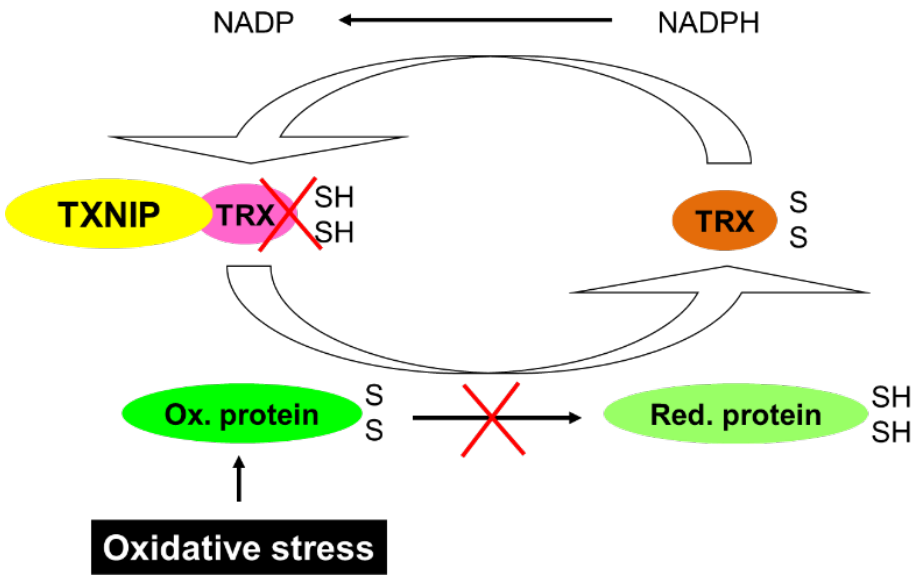
University of Alabama at Birmingham

Disclaimer

This is a Non-CME Accredited Presentation.

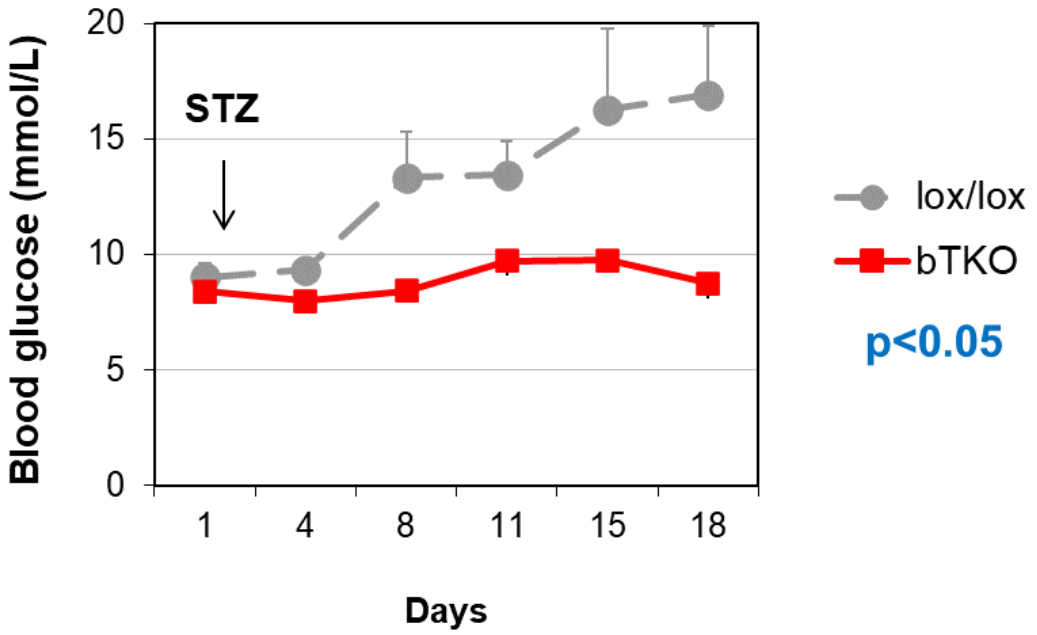
Therapeutic target: Thioredoxin-interacting protein (TXNIP)

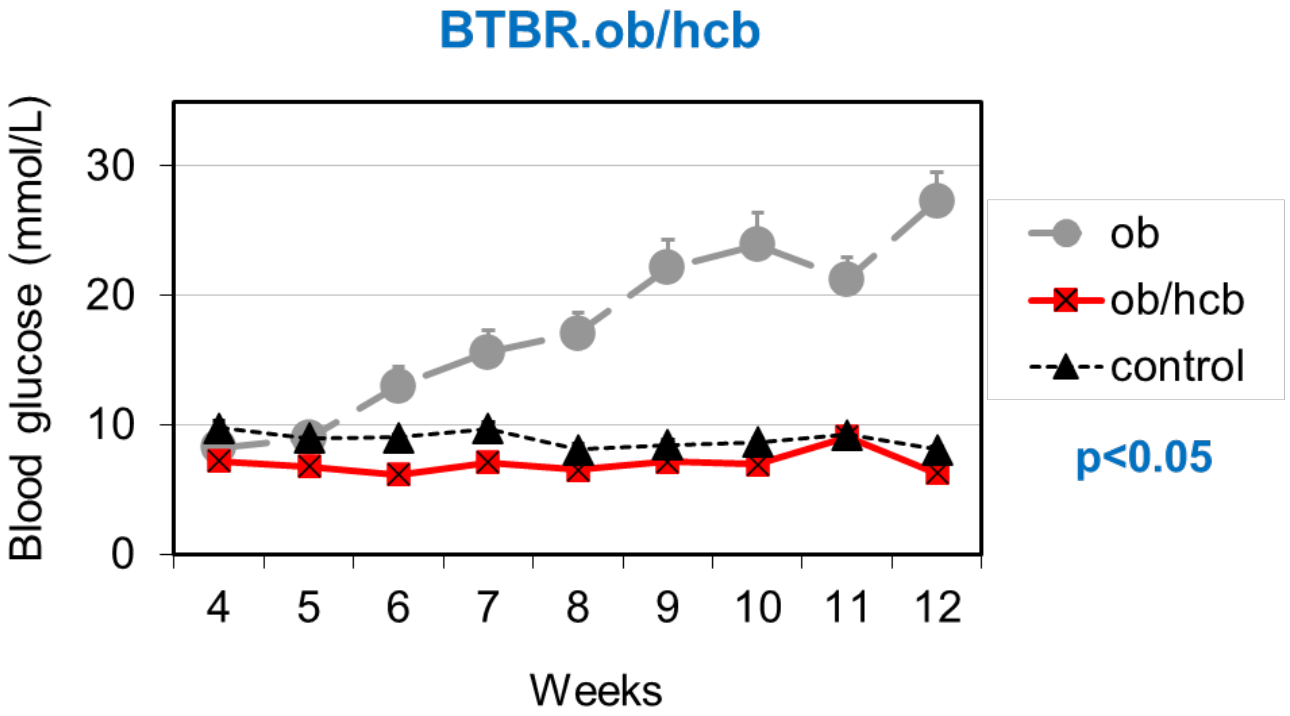
- Identified in human islet microarray as top glucose-induced gene, increased in human T1D and T2D islets and diabetic mouse islets.
- 50 kD protein, ubiquitously expressed and highly conserved.
- Functions by binding and inhibiting thioredoxin and induces ox. stress and beta cell apoptosis.
- Increases inflammasome activation.
- Inhibits glucose uptake in fat and muscle and promotes hepatic glucose production.
- Elevation has detrimental effects on heart, kidney and retina.



→ ”Inhibition has generalized beneficial effects”

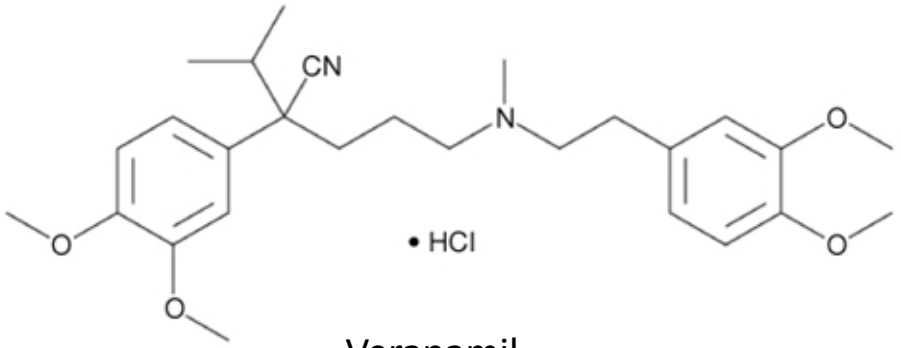
Deletion of TXNIP protects against streptozotocin (STZ) and obesity-induced diabetes



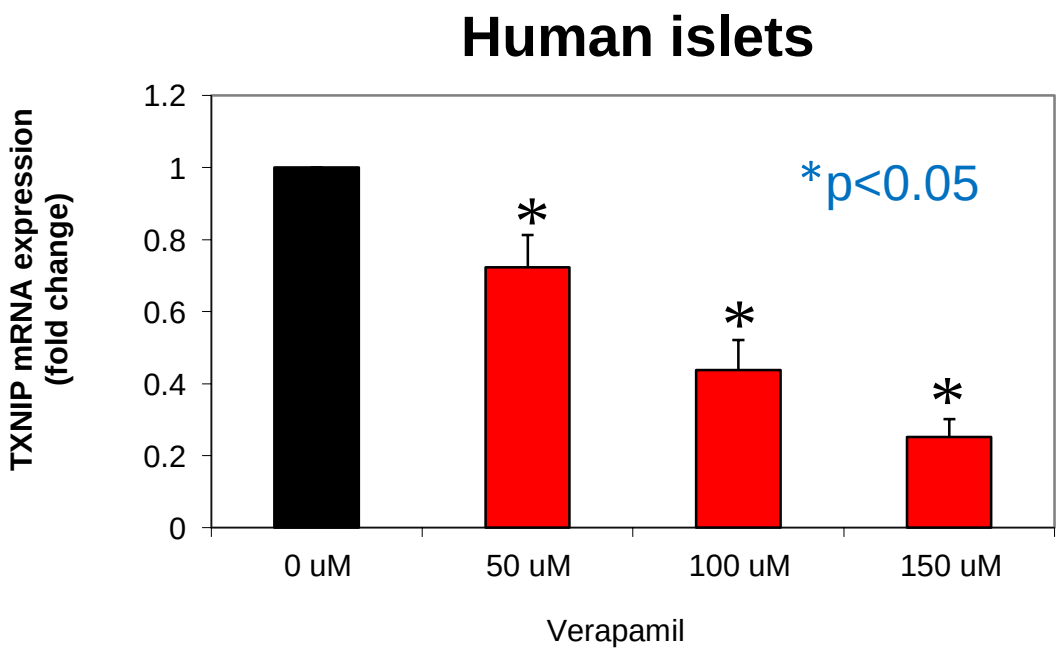


Chen…Shalev: FASEB (2008)

Verapamil Ca^{2+}-channel blocker anti-hypertensive inhibits expression of thioredoxin-interacting protein (TXNIP)

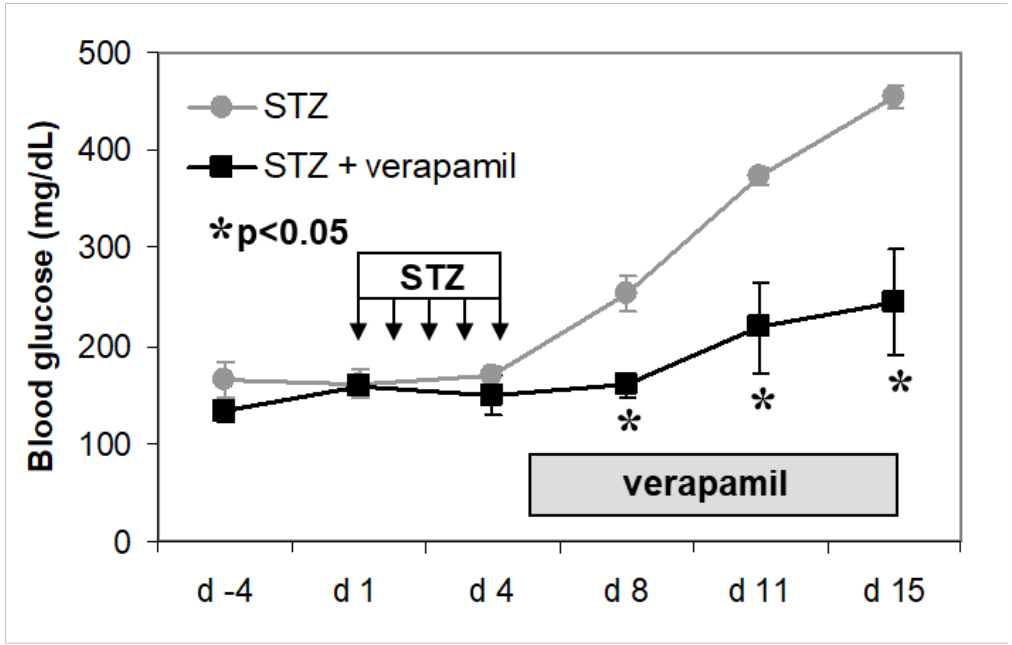


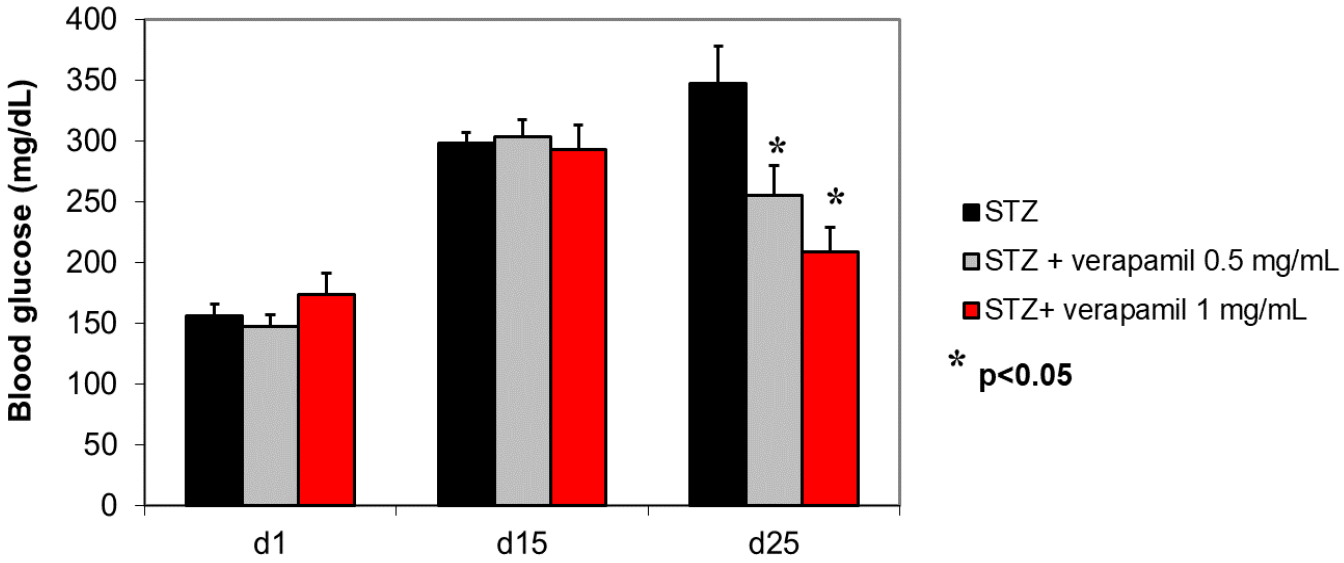
Verapamil



Xu…Shalev: Diabetes (2012)

Oral verapamil (100mg/kg/d) protects against and reverses STZ-induced diabetes





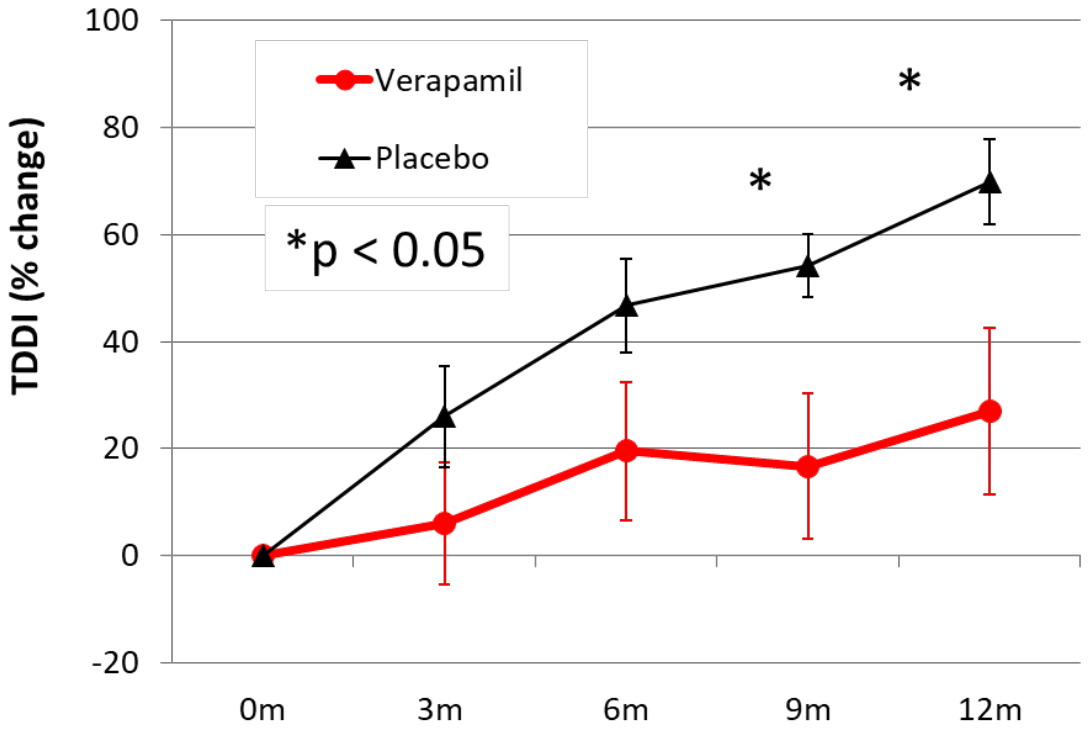
Consistent with findings in response to genetic TXNIP deletion

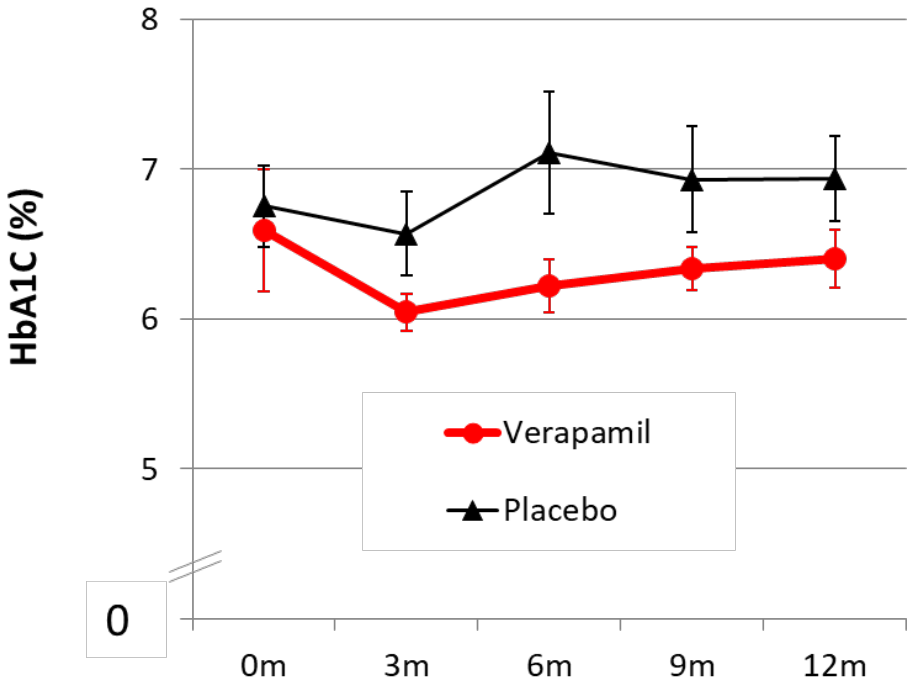
Xu…Shalev: Diabetes (2012)

Clinical Trial (JDRF-funded)
Repurposing of verapamil as a beta cell survival therapy in T1D

- Double-blind, placebo-controlled study
- New-onset T1D (within 3 months), age 18-45
- Participants randomized to receive oral verapamil (360mg/d) or placebo once a day for 1 year in addition to standard insulin therapy
- Primary endpoint: Functional beta cell mass (mixed meal-stimulated C-peptide)
- Secondary endpoints: Insulin requirements and glucose control (CGMS)

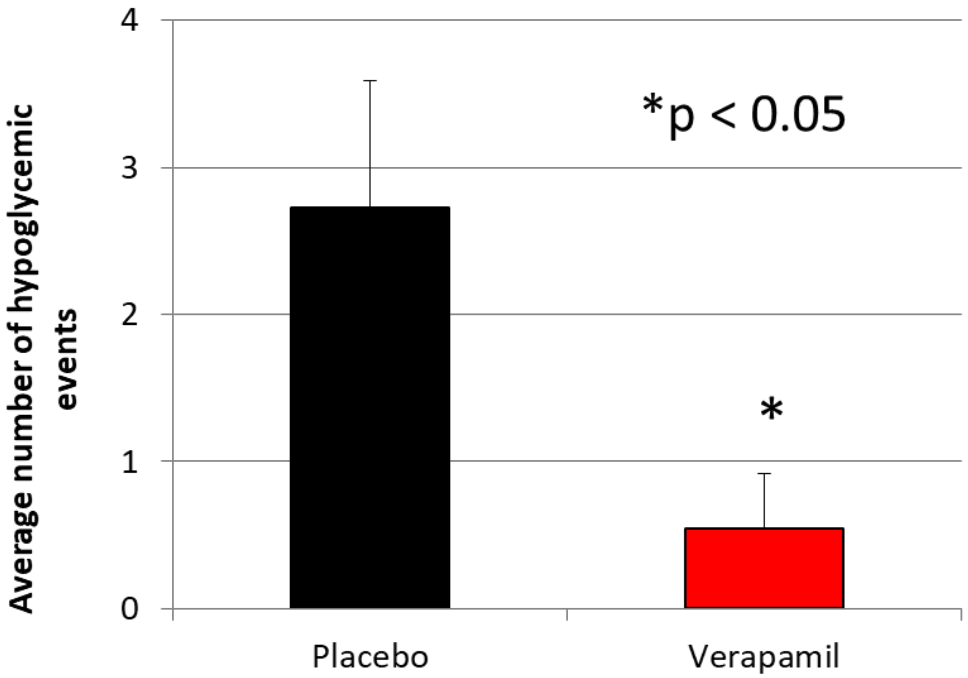
Verapamil limits the increase in total daily dose of insulin (TDDI) required to maintain glycemic control

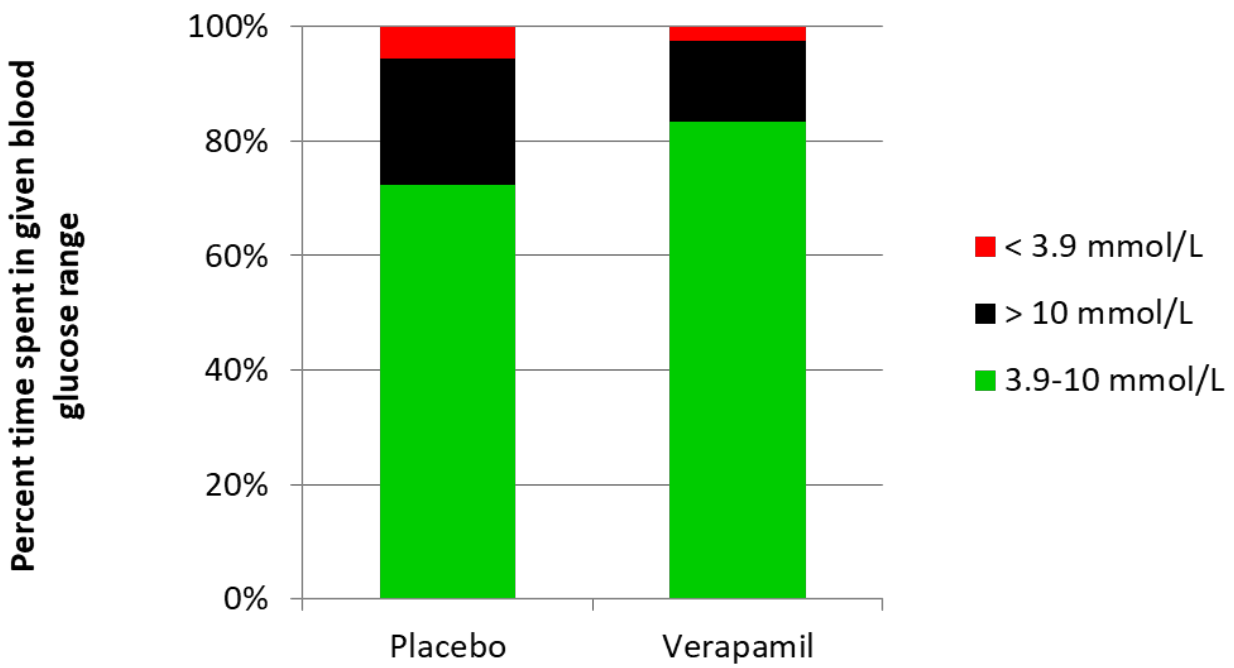




Ovalle…Shalev: Nat Med (2018)

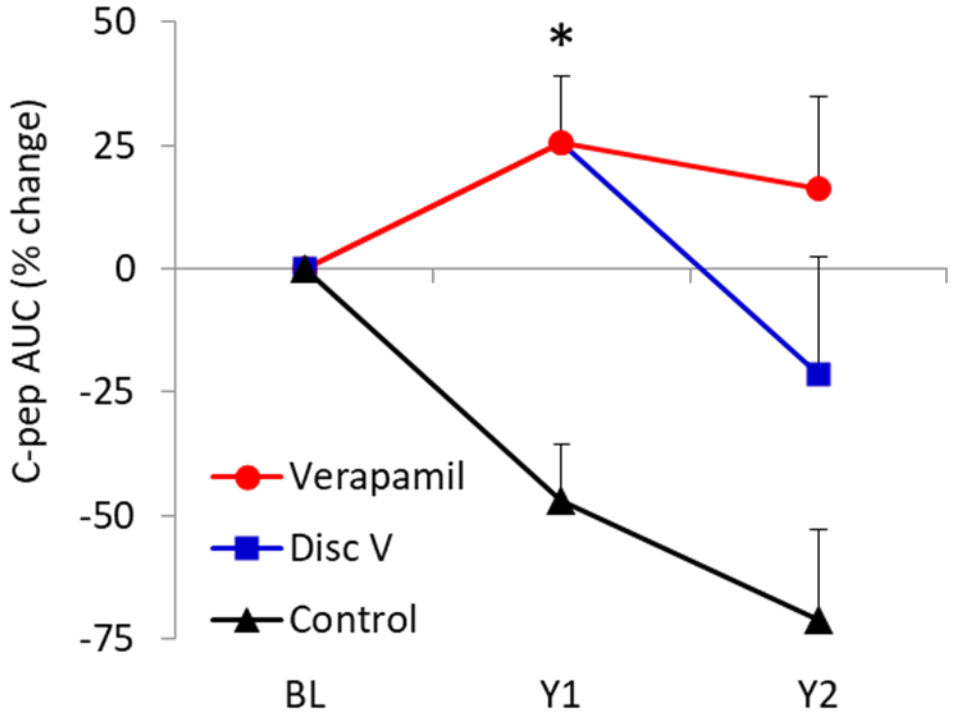
Verapamil decreases the number of hypoglycemic events





Ovalle…Shalev: Nat Med (2018)

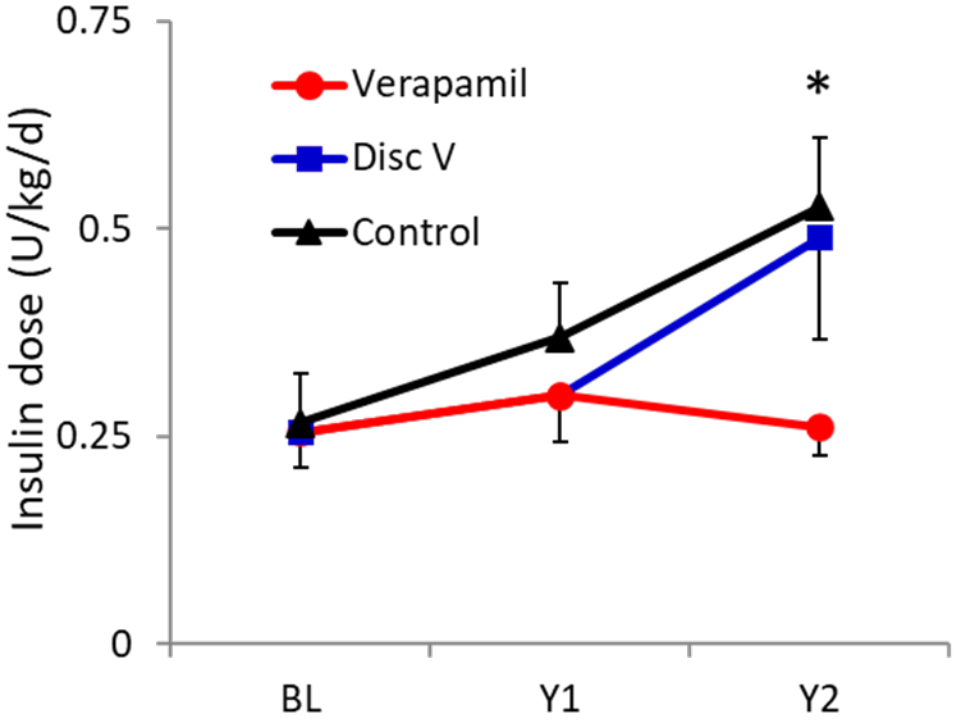
Beta cell function – Beneficial effects of verapamil persist for at least 2 years with continuous use

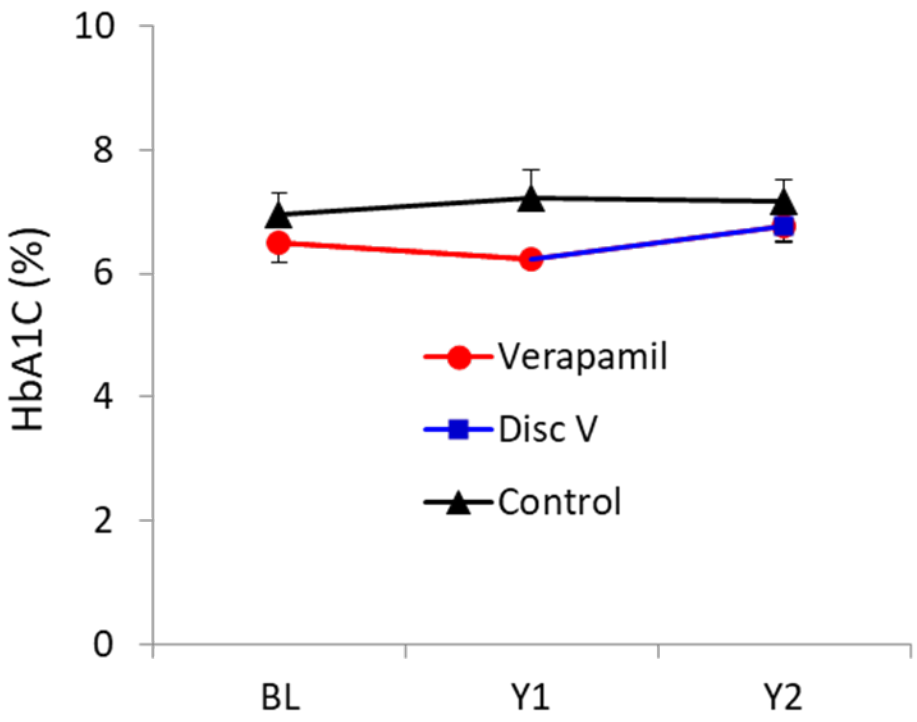


→ *Further evidence of causality*

Xu…Shalev: Nat Commun (2022)

Insulin requirements remain low and blood glucose control stable for at least 2 years with continuous verapamil use





Xu, et. al. Nat Commun (2022)

Independent validation of verapamil findings in children: CLVer Trial

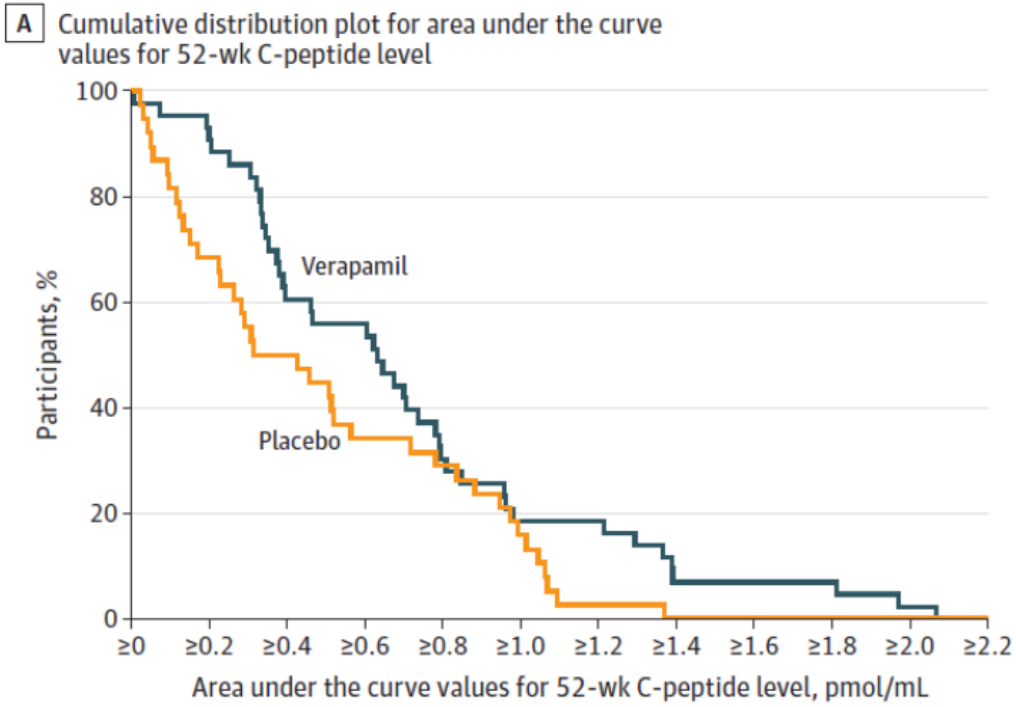
JAMA | Original Investigation

Effect of Verapamil on Pancreatic Beta Cell Function in Newly Diagnosed Pediatric Type 1 Diabetes A Randomized Clinical Trial

Gregory P. Forlenza, MD; Jennifer McVean, MD; RoyW. Beck, MD, PhD; Colleen Bauza, PhD, MPH; Ryan Bailey, MS; Bruce Buckingham, MD; Linda A. DiMeglio,MD, MPH; Jennifer L. Sherr,MD, PhD; Mark Clements, MD, PhD; Anna Neyman, MD; Carmella Evans-Molina, MD, PhD; Emily K. Sims, MD; Laurel H. Messer, PhD, RN; Laya Ekhlaspour, MD; Ryan McDonough, DO; Michelle Van Name, MD; Diana Rojas, BS; Shannon Beasley, APRN, CPNP; Stephanie DuBose, MPH; Craig Kollman, PhD; Antoinette Moran, MD; for the CLVer Study Group

→ …verapamil partially preserved stimulated C-peptide secretion (30% higher) at 52 weeks from diagnosis compared with placebo.

→ FACTORIAL DESIGN:…automated insulin delivery, achieved excellent glucose control but did not affect the decline in pancreatic C-peptide secretion at 52 weeks



In A and B, the area under the curve values for the C-peptide levels were obtained from a mixed-meal tolerance test and computed using the trapezoidal rule as a weighted sum of the measurements for C-peptide level at time 0 and after 15, 30, 60, 90, and 120 minutes. In A, for any given C-peptide area under the curve level, the percentage of participants in each treatment group with a value at that level or higher can be determined from the Figure.

Implications

- We cannot make general recommendations for the use of verapamil for diabetes as not approved for this indication. Ongoing efforts: Vera-T1D trial in Europe etc.
- Verapamil is approved and used for hypertension for ~40 years and physicians can prescribe it to their patients on a one-by-one basis.

→ BUT verapamil is not a specific TXNIP inhibitor

→ As a Ca+ channel blocker, verapamil can cause arrhythmias, heart block and hypotension limiting its use

High throughput screening & optimization

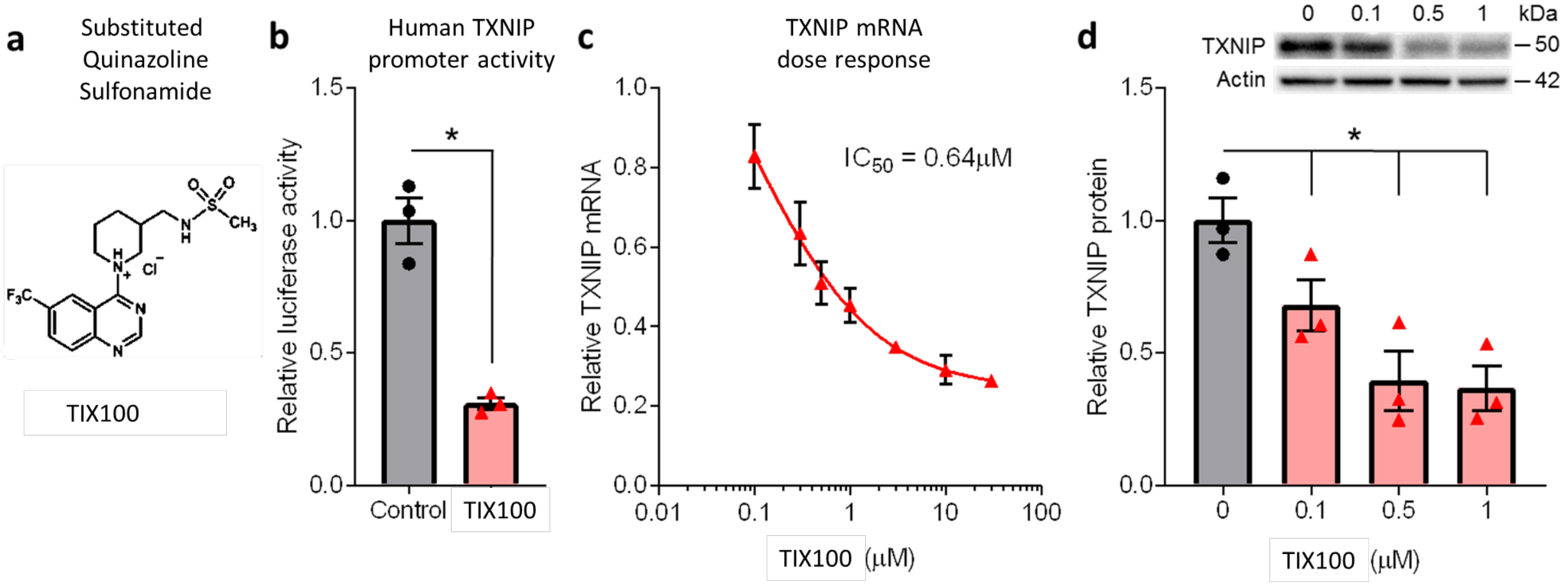
Goal

- **Identify first-in-class compounds that inhibit TXNIP** transcription at high glucose concentrations

Results

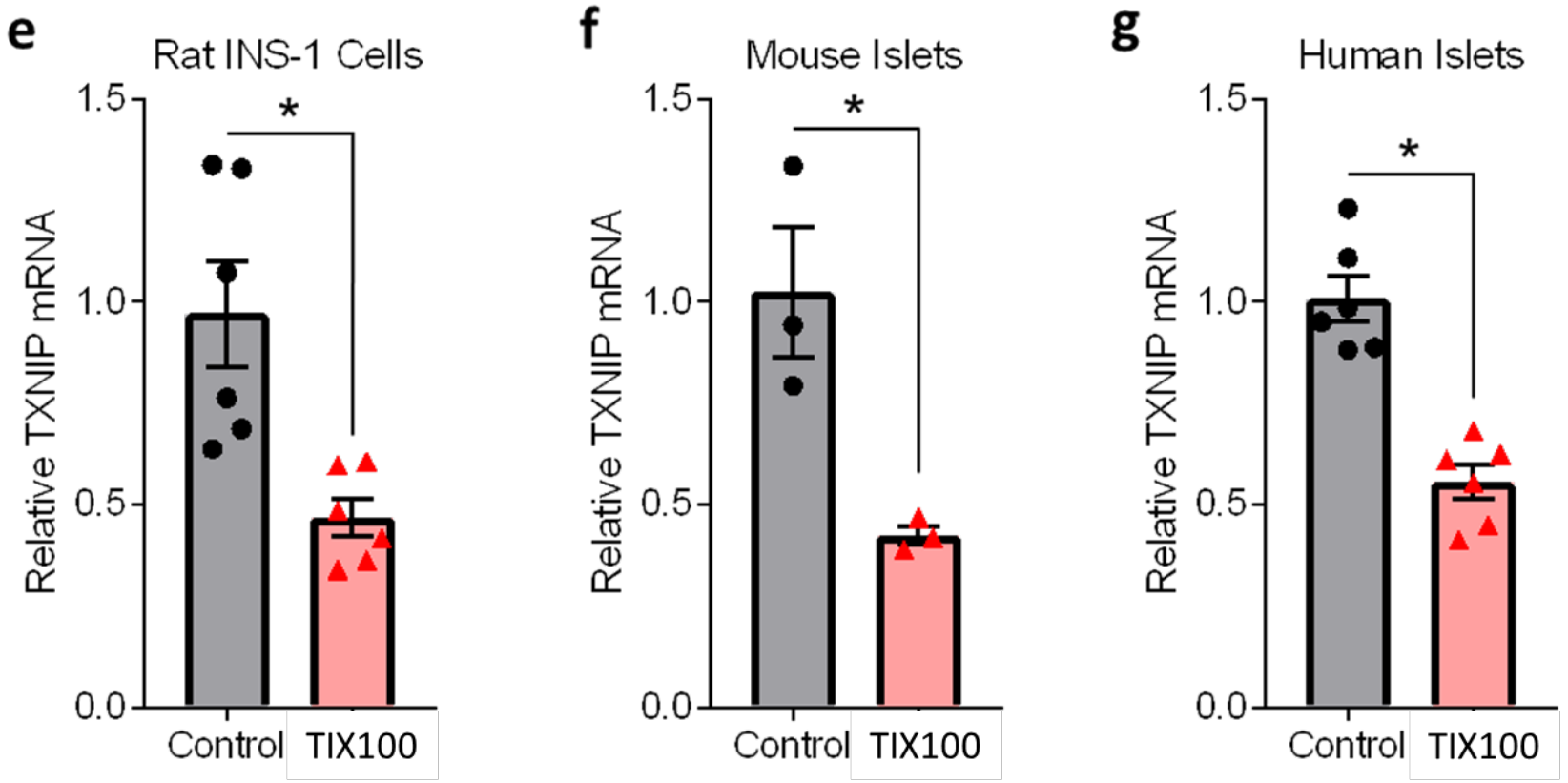
- 300,000-compound primary high-throughput screen
- Extensive Medicinal Chemistry
- Hit-to-Lead Studies → lead compound **TIX100** (aka SRI-37330)
- Obtained Patent for composition-of-matter (new chemical entity) and methods-of-use

TIX100 inhibits TXNIP promoter activity, mRNA and protein expression



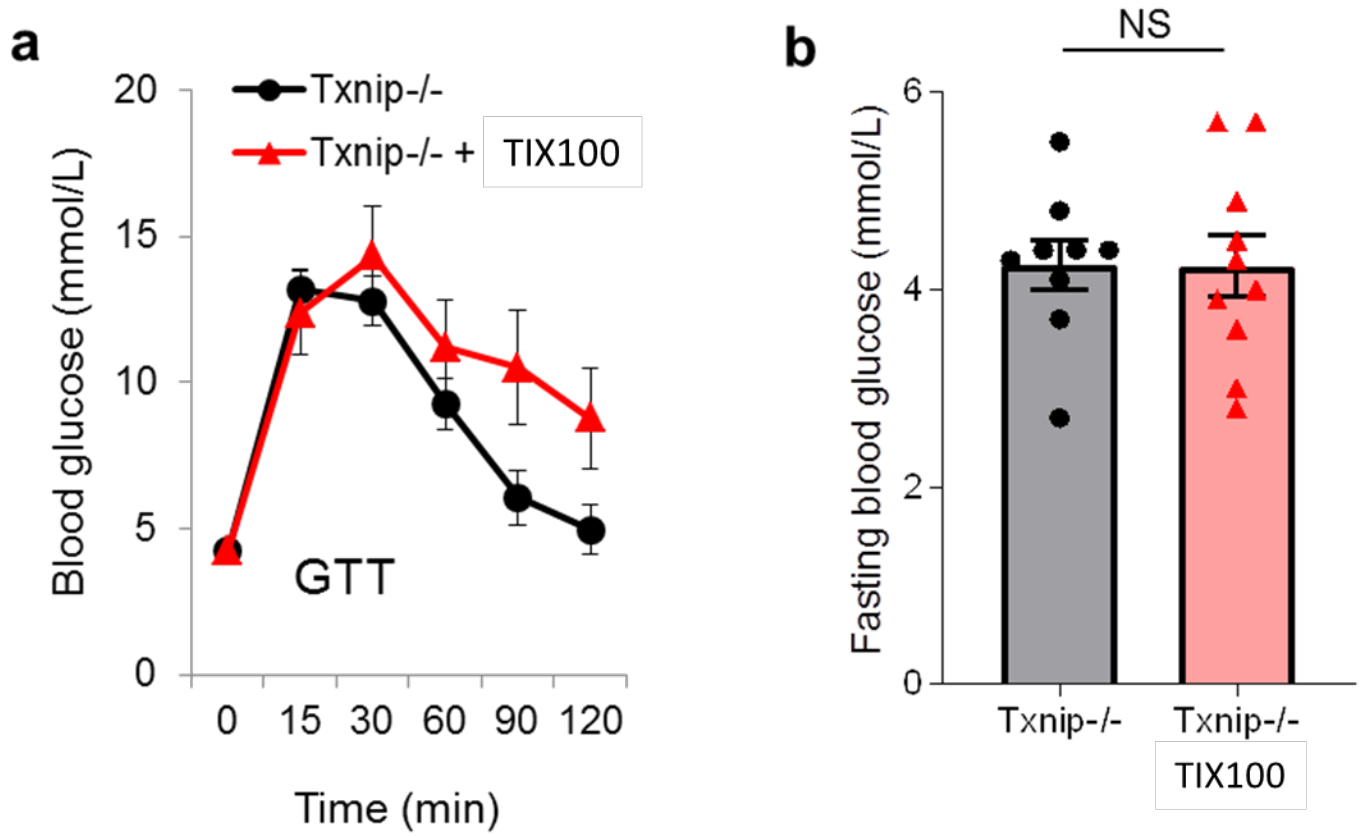
Thielen…Shalev: Cell Metabolism 32, 353–365, 2020

TIX100 inhibits TXNIP expression in mouse and human islets in the context of high glucose



Thielen…Shalev: Cell Metabolism 32, 353–365, 2020

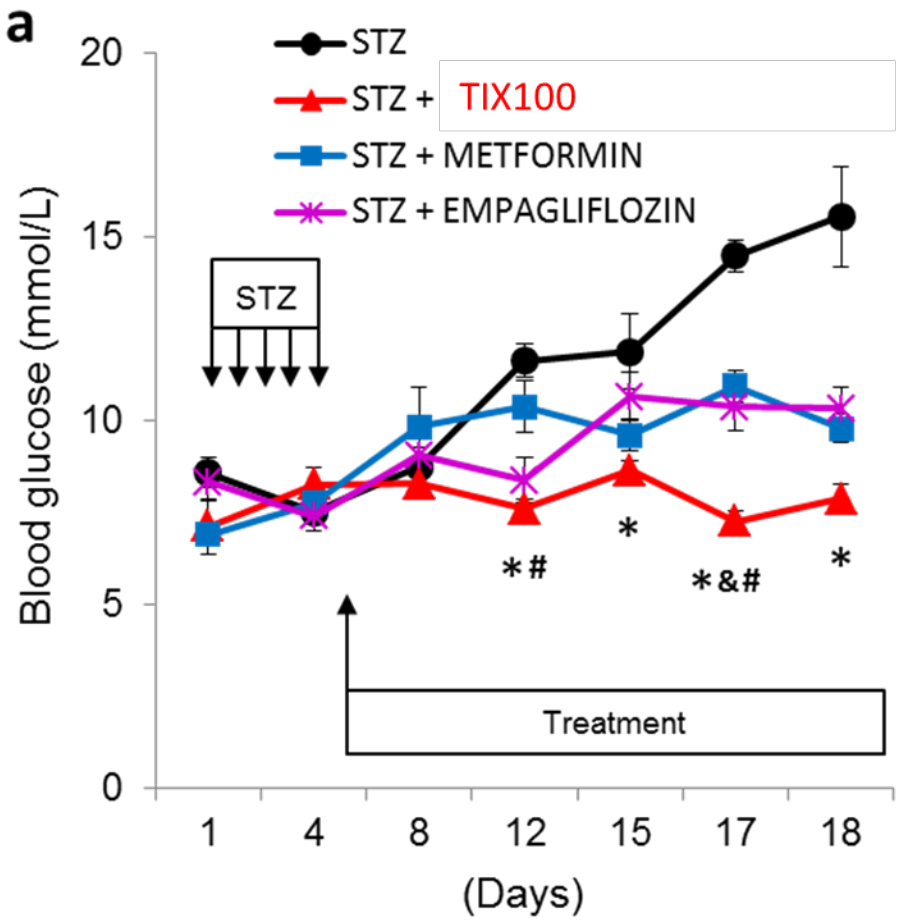
TIX100 does not improve glucose homeostasis in the absence of TXNIP



TXNIP deficient Txnip-/- mice

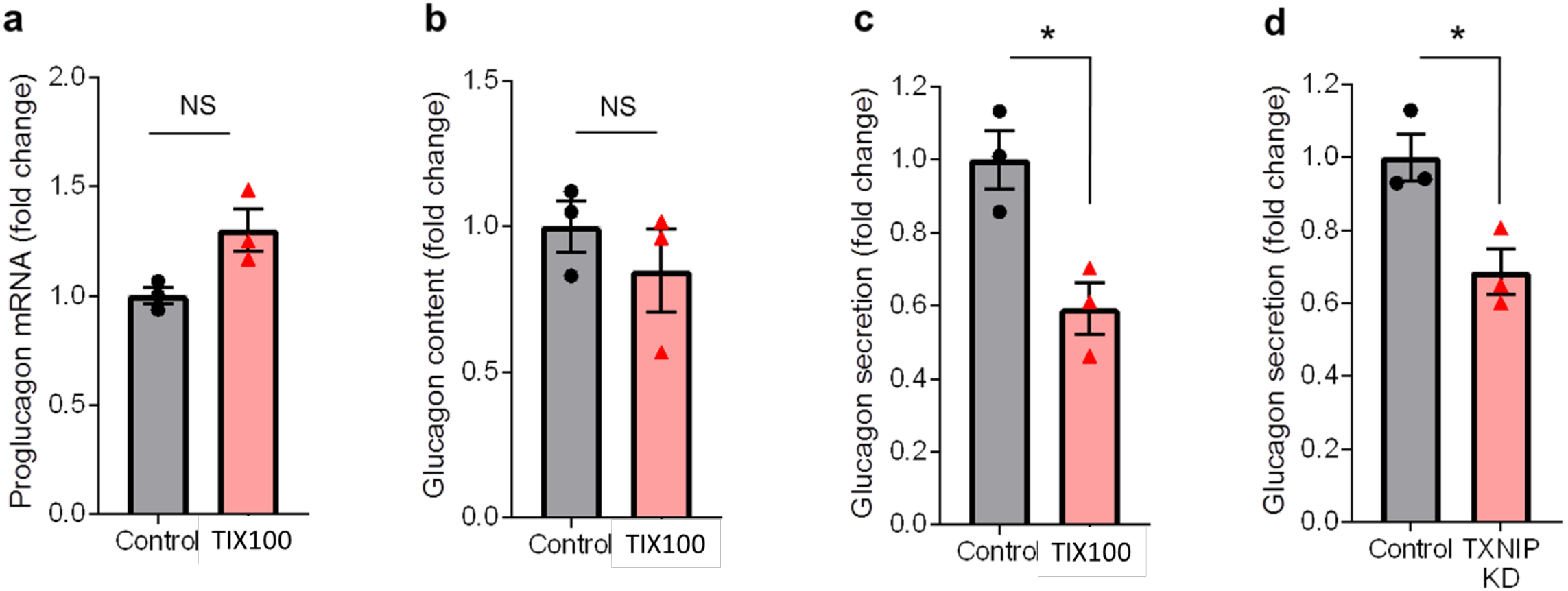
Thielen…Shalev: Cell Metabolism 32, 353–365, 2020

Oral TIX100 is highly effective in improving glucose homeostasis in the context of diabetes



Thielen…Shalev: Cell Metabolism 32, 353–365, 2020

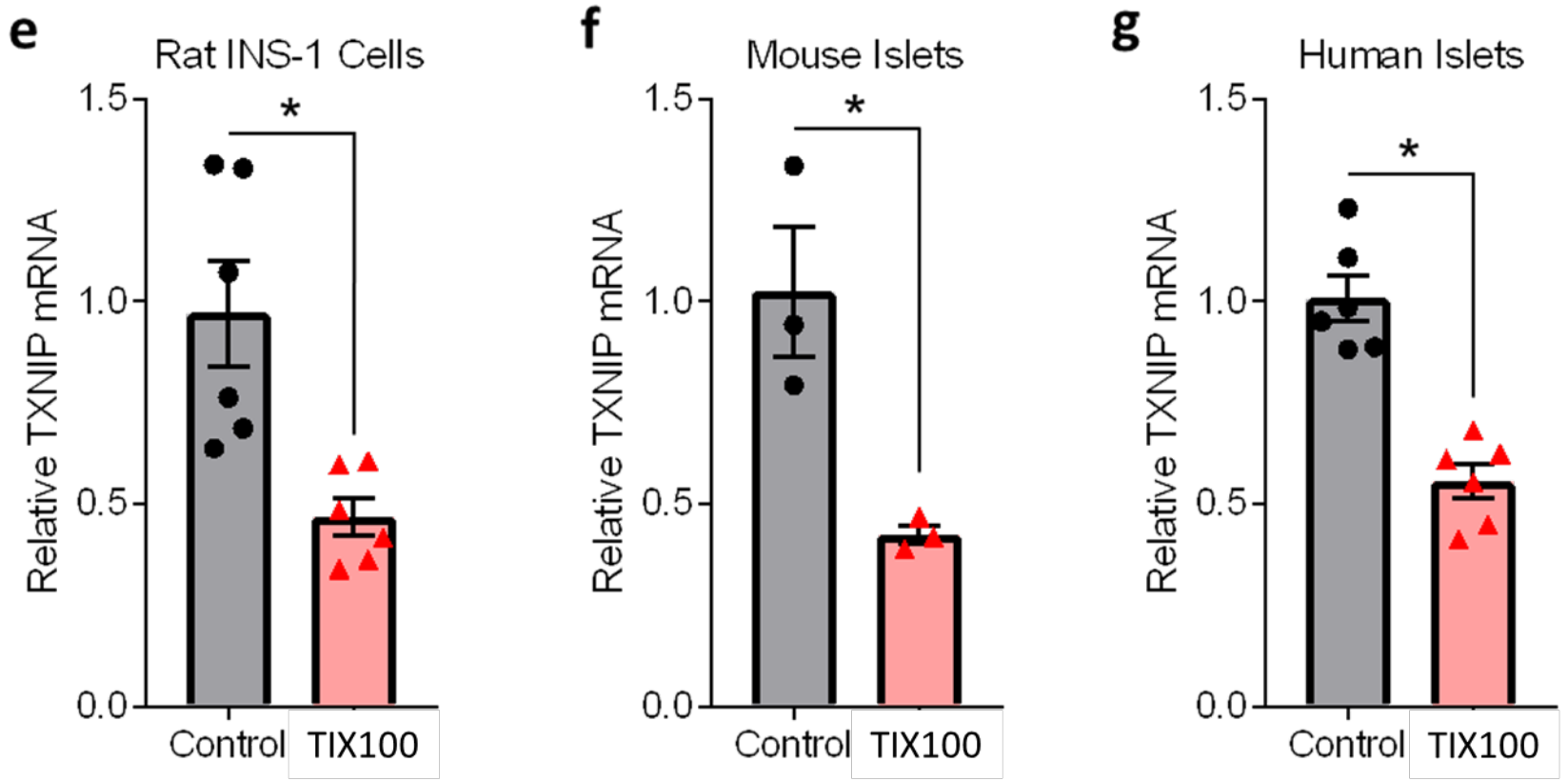
TIX100 controls alpha cell glucagon secretion



alphaTC1-6 cells

Thielen…Shalev: Cell Metabolism 32, 353–365, 2020

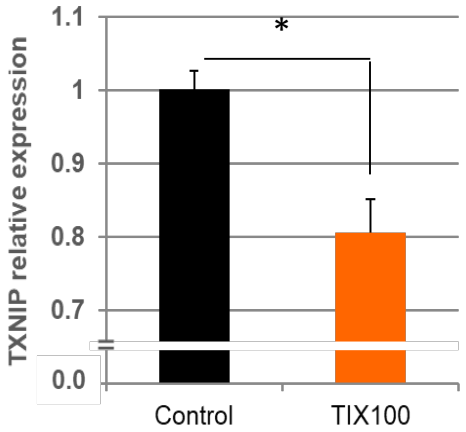
TIX100 does not impair glucagon secretion in response to stress or hypoglycemia

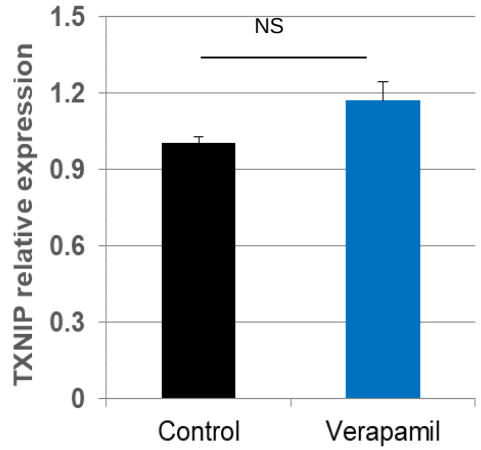


→ *Safety feature, minimizing risk of low blood sugar*

Thielen…Shalev: Cell Metabolism (2020)

TIX100 (but not verapamil) inhibits alpha cell TXNIP expression



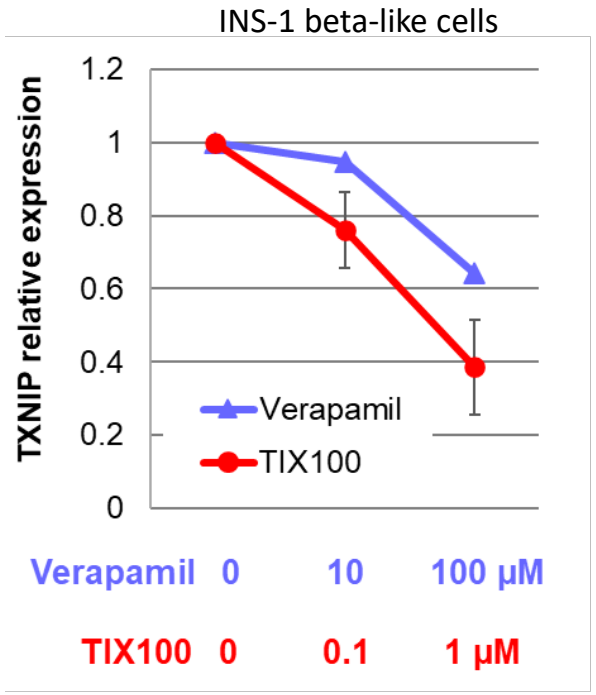


Safety - TIX100 has obtained FDA clearance to proceed to human trials

Pharmacokinetic or Safety Property	Target Value	TIX100
IC_{50} Compound concentration that inhibits TXNIP expression by 50%	< 1 µM	0.64 µM
CC_{50}/72h Compound concentration that reduces cell viability by 50%	> 50 µM	> 50 µM
Maximum tolerated dose in vivo – mouse		> 800 mg/kg/d for 6 days
Ames mutagenicity assay	Negative	Negative
CYP450 inhibition	Negative	Negative
Safety Screen for off-target interactions including L-type Calcium Channels (Eurofins Cerep-Panlabs)	Negative	Negative
hERG inhibition IC_{50} Risk for long QT	> 10 µM (Negative)	26 µM (Negative)
Log D Distribution coefficient/compound lipophilicity at pH 7.4	2-4	2.6
$t_{1/2}$ Mouse liver microsomes	> 60 min	46 min
$t_{1/2}$ Dog liver microsomes		113 min
$t_{1/2}$ Human liver microsomes		116 min
Mouse hepatocyte metabolic stability (% compound remaining after 2h)	> 20 %	22 %
$t_{1/2}$ Mouse in vivo $PO_{(5mg/kg)}$	> 1 h	1.5 h
Bioavailability (%)	> 30 %	95 %
Fraction unbound to human plasma proteins	> 10%	22 %

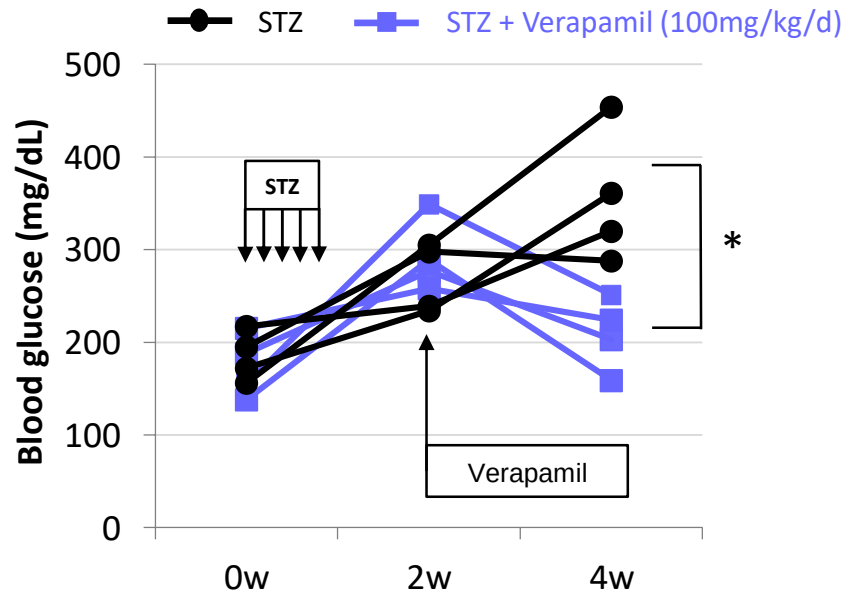
- All IND-enabling toxicology & pharmacokinetics studies completed → TIX100 has a favorable safety profile → FDA clearance to proceed to human trials
- Unlike verapamil, TIX100 is NOT a calcium channel blocker and as such does not have the associated cardiovascular side effect risks.
- TIX100 caused NO hypoglycemia, NO fatty liver, NO hyperlipidemia, and NO weight gain.
- TXNIP deletion in animal models and TXNIP inhibition with verapamil in humans has so far proven to be safe (including in adults and children with T1D).
- Therapeutic goal is normalization of pathologically elevated TXNIP expression, back to non-diabetic values.

TIX100 is more potent than verapamil in inhibiting beta cell TXNIP expression



maximal effect

Oral TIX100 is also more potent *in vivo* and even and reverses established diabetes

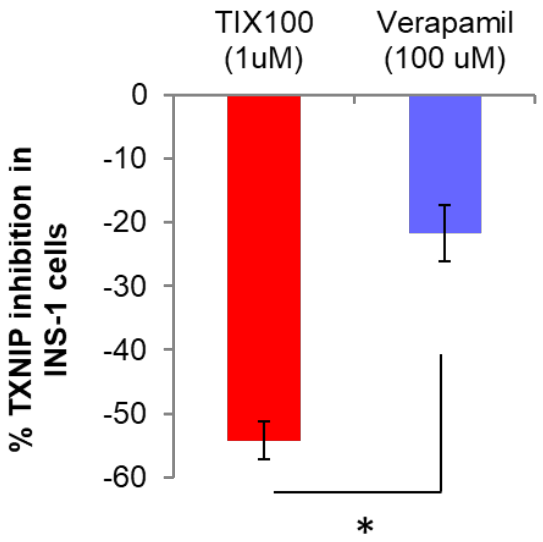


** p < 0.05; ** p < 0.005*

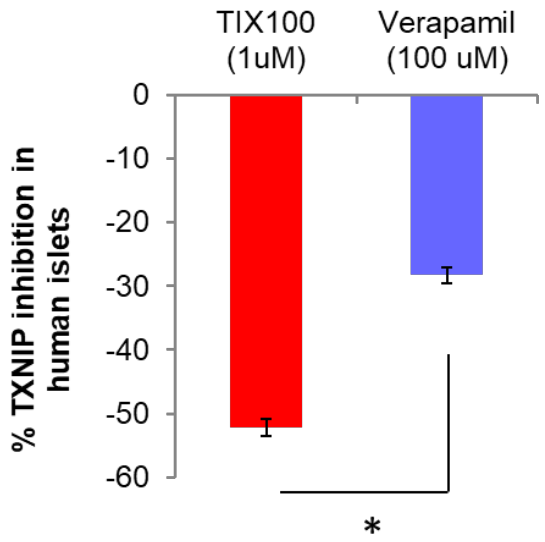
Unpublished

TIX100 is more effective than verapamil in lowering TXNIP and normalizing blood glucose

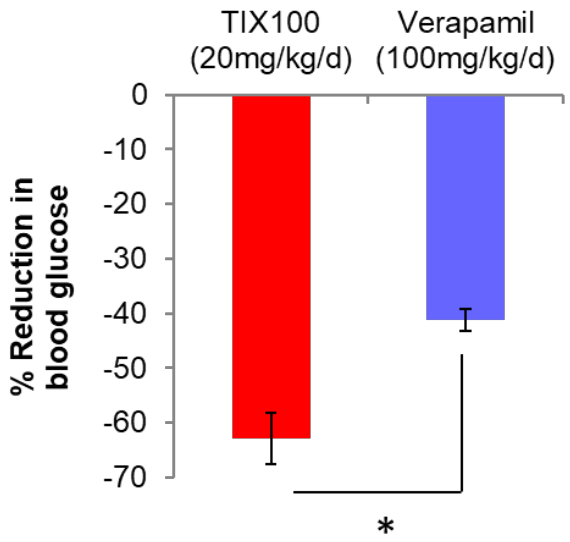
INS-1 cells



Human islets



Diabetic mice



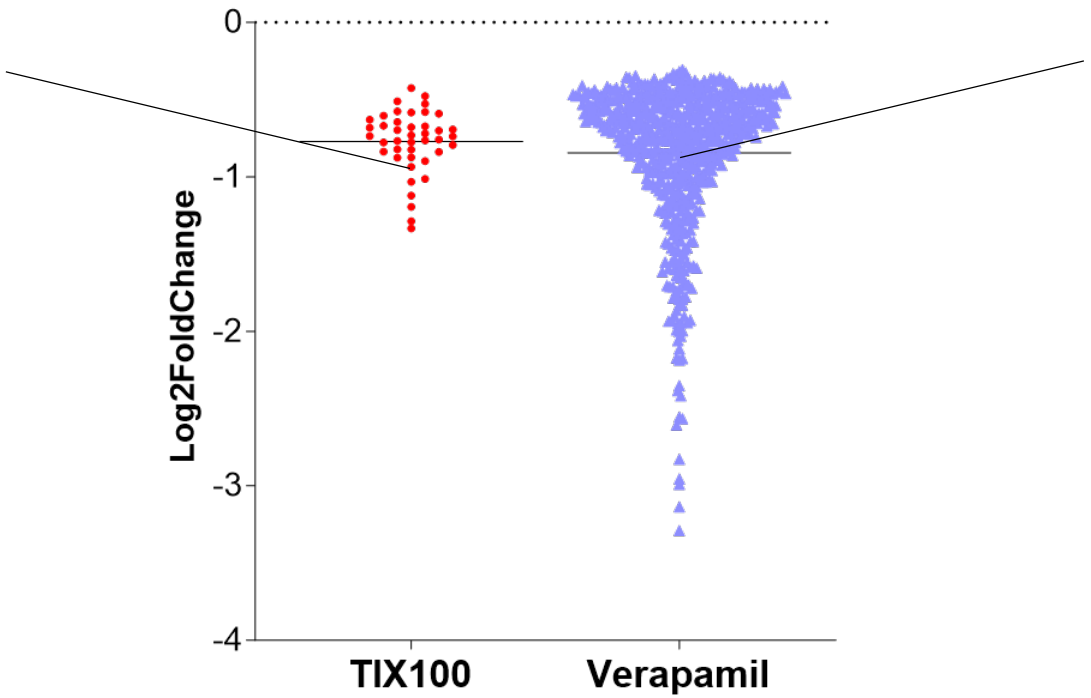
** p < 0.05*

Unpublished

TIX100 is more specific than verapamil in inhibiting human islet TXNIP expression

TXNIP = #7th of 42 genes downregulated by **TIX100** in human islets

→ TIX100 15-times more selective for TXNIP than verapamil
→ Less off-target effects anticipated



TXNIP = #192nd of 619 genes downregulated by **verapamil** in human islets

n = 3 individual islet donors, each serving as their own control (islets from same donor used in parallel for TIX100 and verapamil treatment)

Unpublished

Comparison of key features of TIX100 and verapamil

	TIX100	Verapamil
▪ Controls beta cell TXNIP & improves beta cell health	↑↑	↑
▪ Controls alpha cell TXNIP & protects against hyperglucagonemia	↑	=
▪ Controls excessive glucose production by liver	↑	=
▪ Provides increased potency, effectiveness & specificity	↑	↓
▪ Maintains cellular calcium & avoids arrhythmia, heart block, hypotension risks	↑	↓↓

Conclusions

TXNIP is an attractive target for the treatment of T1D (in vitro, ex vivo, genetic deletion studies, pharmacological studies in mice and humans)

Verapamil - repurposed → not specific for TXNIP, not FDA approved for T1D, but in the interim available for off-label use

-POC for targeting TXNIP as a translatable, therapeutic approach for T1D

-Limitations due to potential cardiovascular side effects (Ca+ channel blocker)

TIX100 New Chemical Entity (NCE) → IND approved by FDA, ready to enter clinical trials

-More potent, effective & specific than verapamil and not a Ca+ channel blocker

-Additional beneficial effects, e.g., on hyperglucagonemia

Acknowledgments

Shalev Lab:

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Clinical Trial Team

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UAB School of Nursing

Li Peng

UMass MMPC

Jason Kim

Southern Research Institute

UAB DRC Physiology Core

UAB Small Animal and Glycemic Clamp Core

IIDP and islet donors

Study partners & participants

TIX100 generous gift of:

TIXiMED

JDRF IMPROVING LIVES. CURING TYPE 1 DIABETES.

NIDDK NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

HiRN HUMAN ISLET RESEARCH NETWORK

UAB COMPREHENSIVE DIABETES CENTER The University of Alabama at Birmingham

Questions?