

Supplemental Table S1a. Related to Figure 1: Pharmacokinetic, metabolic and safety properties of SRI-37330

Pharmacokinetic or Safety Property	Target Value	SRI-37330
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		46 min
t _{1/2} Dog liver microsomes	> 60 min	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 %

Supplemental Table S1b. Related to Figure 1: Human islet genes significantly changed by SRI-37330

GENE	adjusted p-value	GENE	adjusted p-value	GENE	adjusted p-value	GENE	adjusted p-value
DNAAF4-CCPG1	3.56E-06	NR0B1	0.0016	COL4A1	0.0120	STK39	0.0306
LRFN2	3.23E-11	THBD	0.0017	MLLT10	0.0123	TIPARP	0.0306
LOXL4	3.23E-11	FAM181B	0.0017	AC096636.1	0.0125	ZNF469	0.0312
STUM	1.18E-10	SEMA6D	0.0017	CLDN2	0.0125	ITGB8	0.0312
SIPA1L2	1.38E-10	AP001793.1	0.0017	MMP2	0.0125	ZNF415	0.0312
SULF2	3.72E-10	CCDC191	0.0019	THBS2	0.0127	TMEM132D	0.0317
SLC2A2	1.32E-09	IGFN1	0.0020	IL24	0.0136	CALB1	0.0318
KRT6B	8.26E-09	KIF26B	0.0021	IL17RB	0.0138	LDLRAD3	0.0323
CXCL12	1.73E-07	TXNIP	0.0021	NMI	0.0139	CNTN2	0.0323
WSCD2	2.64E-07	PTPRU	0.0021	PTPRCAP	0.0139	LRRC17	0.0323
PCDH7	4.95E-07	PKP1	0.0022	GNG4	0.0139	TAGLN	0.0323
GCNT4	8.06E-07	GBP3	0.0022	IGF1R	0.0140	GLIS3	0.0323
AC000095.2	2.33E-06	AC053481.2	0.0022	PTPN3	0.0141	PROS1	0.0323
ENTPD3	4.35E-06	LHFPL2	0.0022	HDHD2	0.0144	CHRFAM7A	0.0323
PTPRC	9.06E-06	MYC	0.0025	TNC	0.0146	ST13P6	0.0325
SLC14A1	1.10E-05	TMEM87B	0.0025	CYP27B1	0.0146	MIRLET7DHG	0.0325
COL24A1	1.12E-05	AL139241.1	0.0025	FAM84A	0.0152	AL135925.1	0.0326
HSPB6	1.25E-05	KIRREL3	0.0026	WWTR1	0.0152	ABO	0.0341
POSTN	1.25E-05	HIST1H2AC	0.0026	LINC02447	0.0152	MIR7-3HG	0.0344
NPTX2	1.25E-05	MPO	0.0026	CHODL	0.0153	FMRD6	0.0346
NEFM	1.74E-05	MICAL2	0.0027	TRPM3	0.0153	TNFAIP3	0.0346
SFRP2	1.77E-05	ALK	0.0027	H2AFZ	0.0153	AKR1C2	0.0346
ISL1	1.92E-05	KRT17	0.0027	CPA1	0.0153	MICAL2	0.0346
TWNK	5.01E-05	SLC7A2	0.0031	ATP2B2	0.0153	AC007368.1	0.0346
CTGF	6.42E-05	OR211P	0.0035	NBL1	0.0154	AL354733.3	0.0350
SYNDIG1L	6.70E-05	KIAA1549L	0.0036	DUSP5	0.0165	CCDC103	0.0350
FSTL4	7.05E-05	KCNMA1	0.0037	AL356234.1	0.0170	CTRB2	0.0357
TRIM6	7.05E-05	RGN	0.0038	PYCR1	0.0170	PLEKHG4	0.0358
SPARCL1	7.15E-05	SMG1P3	0.0043	IBSP	0.0174	SRP68	0.0361
AMIGO3	7.37E-05	TGFB11	0.0043	FYN	0.0174	F11	0.0366
CPNE4	8.70E-05	SCD	0.0043	BCL2L1	0.0174	RPS6KA1	0.0368
EDN1	9.34E-05	SLC6A6	0.0045	NAV2	0.0182	UBR4	0.0369
MDGA1	1.17E-04	MXD3	0.0045	TJP3	0.0182	GOS2	0.0372
APCDD1L	1.17E-04	SUGCT	0.0050	HIST1H2BD	0.0192	SHISAL1	0.0385
KRT75	1.65E-04	MDM1	0.0050	ZDHHC14	0.0194	THBS1	0.0387
SLC26A9	1.65E-04	HIST2H2BE	0.0053	EDN3	0.0198	CCND1	0.0396
JAG1	2.20E-04	CUZD1	0.0061	FUT4	0.0202	PRDM6	0.0406
C2CD4A	2.29E-04	LUZP2	0.0064	CENPBD1P1	0.0212	TPD52L2	0.0406
PAPPA2	2.29E-04	TNFRSF21	0.0064	NELL2	0.0215	SLC30A8	0.0422
VLDLR	2.29E-04	MUC1	0.0064	CFI	0.0217	SLC31A1	0.0422
AC092647.5	2.29E-04	RCOR2	0.0064	CCDC136	0.0222	MAFA	0.0423
TAL1	2.29E-04	PUS7	0.0065	ANXA10	0.0225	SUSD4	0.0423
LRRC4	2.35E-04	SYNM	0.0065	APPL1	0.0236	CDH1	0.0424
RAB31L1	2.75E-04	HMGN1P4	0.0068	CDC25A	0.0237	PNMA8B	0.0428
GALNT14	3.34E-04	EGR1	0.0068	SLC26A6	0.0238	PTPRT	0.0430
IGF2-AS	3.84E-04	CALD1	0.0074	GRIA3	0.0240	EPHA1-AS1	0.0434
TNFSF14	4.04E-04	LDHA	0.0074	IGDCC4	0.0244	COL3A1	0.0446
OTOGL	4.89E-04	OLMALINC	0.0076	TUBB2B	0.0265	PDZK1	0.0456
ALCAM	4.98E-04	NCOA3	0.0078	TMEM178B	0.0265	RF01957	0.0467
CALCB	5.26E-04	ENC1	0.0078	SLC12A2	0.0267	YIF1A	0.0472
PDE3B	5.43E-04	KCTD18	0.0078	FUT2	0.0267	EEF2K	0.0473
ROBO2	6.33E-04	AC084756.1	0.0080	TOB1	0.0277	SGPP2	0.0483
PAPPA	6.71E-04	ARMC9	0.0083	MCM3AP	0.0279	PALLD	0.0483
TMOD1	6.71E-04	CDH6	0.0097	PNLIPRP2	0.0281	FP236383.3	0.0483
ST6GALNAC5	7.98E-04	STXBP5	0.0097	CXCL8	0.0285	TGFB2	0.0483
ELMO1	9.62E-04	SYNDIG1	0.0097	AC068888.1	0.0286	FP236383.2	0.0483
SORL1	9.62E-04	BTBD11	0.0098	NQO1	0.0292	EPB41L2	0.0490
TGFB3	0.0010	SURF4	0.0098	NLRP1	0.0293	MS4A6A	0.0497
KRT23	0.0010	ATP7B	0.0104	AC005785.1	0.0299	CCZ1	0.0497
MMP12	0.0011	MLXIPL	0.0104	RNU1-70P	0.0299	CDR2	0.0497
GREM2	0.0011	RAD51-AS1	0.0105	FAT3	0.0299	MALAT1	0.0497
OSTC	0.0011	IL32	0.0107	CMKLR1	0.0299	DRAM2	0.0497
RPL36A-HNRNPH	0.0012	EXTL3	0.0107	MGP	0.0299	TMEM130	0.0497
SHISA9	0.0012	FAM20A	0.0109	TMEM108	0.0299	GOLGA8N	0.0497
H1FO	0.0012	SEC61A1	0.0109	NA	0.0299	LRRC2	0.0497
GCNT1	0.0013	ACSM3	0.0110	RGS2	0.0299	SULT1C2	0.0497
SCGN	0.0014	KRT5	0.0115	ANGPTL3	0.0305	AC008403.1	0.0497

Supplemental Table S1c. Related to Figure 1: Gene ontology analysis of SRI-37330-mediated changes in human islet gene expression

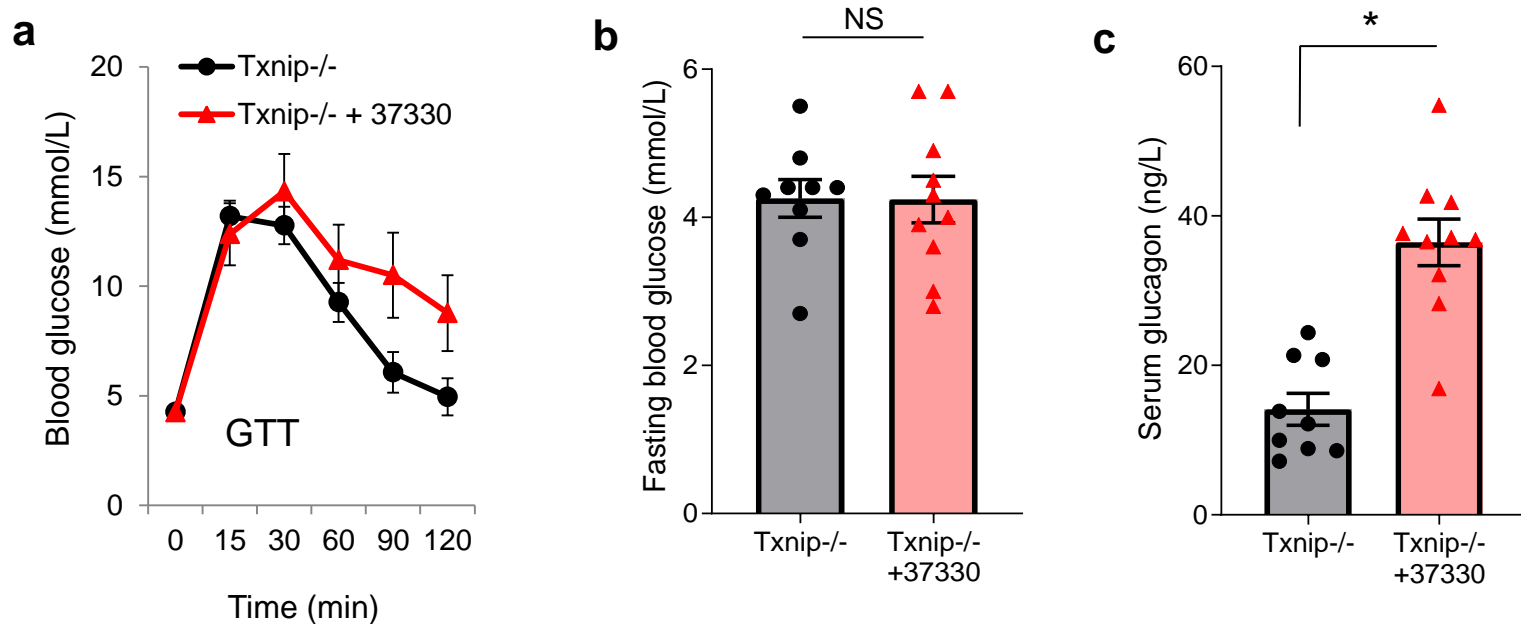
	Biological Process*	Gene of Interest*	P-value
Downregulated Genes	Negative regulation of cell division (GO:0051785)	TXNIP	0.02611
	Neuron apoptotic process (GO:0051402)	NLRP1	0.06403
	Regulation of energy homeostasis (GO:2000505)	MLXIPL	0.04315
Upregulated Genes	Negative regulation of intrinsic apoptotic signaling pathway in response to DNA damage (GO:1902230)	BCL2L1	0.00015
	Positive regulation of cell proliferation (GO:0008284)	IGF1R	0.00285
	Response to glucose (GO:0009749)	MAFA	0.00073
	Positive regulation of cell differentiation (GO:0045597)	CTGF	0.00007

**Enrichr Gene Ontology (GO) Biological Process 2017 was used and TXNIP-associated processes and genes are shown; P-values refer to the significance of the over-representation of the pathway according to Enrichr.*

Supplemental Table S2: Related to STAR Method Details: Primers used in this study

Description	Sequence (5' – 3')
18S 5' qPCR primer	AGTCCCTGCCCTTTGTACACA
18S 3' qPCR primer	GATCCGAGGGCCTCACTAAAC
Rat	
TXNIP 5' qPCR primer	CGAGTCAAAGCCGTCAGGAT
TXNIP 3' qPCR primer	TTCATAGCGCAAGTAGTCCAAGGT
TXNIP E-box 5' ChIP primer	AAGGACCAAGTAGCCAATGGG
TXNIP E-box 3' ChIP primer	GTGCTGGCCCGGAGG
TXNIP Upstream 5' ChIP primer	TACCCTGGATGAGGTTTCAGG
TXNIP Upstream 3' ChIP primer	CGTGGACACAGTGTGCTCTT
IRS2 5' ChIP primer	TCTCCTATTACATCCAGAACAGG
IRS2 3' ChIP primer	TCATCAGAGCCATTCACTTGTC
Mouse	
TXNIP 5' qPCR primer	CGAGTCAAAGCCGTCAGGAT
TXNIP 3' qPCR primer	TTCATAGCGCAAGTAGTCCAAGGT
IGF1R 5' qPCR primer	CGGCACAACACTACTGCTCCA
IGF1R 3' qPCR primer	CAGTTTTAGGGCAAGCGCAG
CTGF 5' qPCR primer	CCACTCTGCCAGTGGAGTTC
CTGF 3' qPCR primer	GTAATGGCAGGCACAGGTCT
BCL2L1 5' qPCR primer	TGACCACCTAGAGCCTTGGA
BCL2L1 3' qPCR primer	CTGGCCTTTCCGGCTCTC
MLXIPL 5' qPCR primer	CACTCAGGGAATACACGCCTAC
MLXIPL 3' qPCR primer	ATCTTGGTCTTAGGGTCTTCAG
NLRP1 5' qPCR primer	CACTCCAGTGAAGAGTGGG
NLRP1 3' qPCR primer	TTGTTGCAAGCAGGAGACCA
GCG 5' qPCR primer	ATCTTGCCACCAGGGACTTC
GCG 3' qPCR primer	AAGTGACTGGCACGAGATGT
PCK1 5' qPCR primer	CCCAAGGCAACTTAAGGGCTAT
PCK1 3' qPCR primer	CTGAGGTGCCAGGAGCAACT
G6PC 5' qPCR primer	TCTGCAAGAGCGCAACAGTT
G6PC 3' qPCR primer	CGGGCTAGGCAGTATGGGATA
Human	
TXNIP 5' qPCR primer	ACTCGTGTCAAAGCCGTTAGG
TXNIP 3' qPCR primer	TCCCTGCATCCAAAGCACTT
IGF1R 5' qPCR primer	TCGTGGGAGGGTTGGTGAT
IGF1R 3' qPCR primer	CCCCAGCCTGCTGTTATTTC
MAFA 5' qPCR primer	CCGCTGGCCATCGAGTAC
MAFA 3' qPCR primer	GGCTCCTTCTTCACCTCGAA
CTGF 5' qPCR primer	TGCATCCGTAATCCCAAATC
CTGF 3' qPCR primer	ATGCTGGTGCAGCCAGAAAG
BCL2L1 5' qPCR primer	GAGCTGGTGGTTGACTTTCTC

BCL2L1 3' qPCR primer	TCCATCTCCGATTCAGTCCCT
MLXIPL 5' qPCR primer	GGCGGCGACAGCCAT
MLXIPL 3' qPCR primer	CCGCGGGACCTGCAA
NLRP1 5' qPCR primer	GGCTGCAAGTGAAAGACAAGAA
NLRP1 3' qPCR primer	CAGGCATGAGATCTCCTGGTTT
ARRB1 5' qPCR primer	CTGCGCGGAGAATTTGGA
ARRB1 3' qPCR primer	GGATGACCAGACGCACAGAA
ARRDC3 5' qPCR primer	AGAGGAACAAAGGCGGAACA
ARRDC3 3' qPCR primer	GGGCTCTCTCAAAGTCATCACAA
TXN 5' qPCR primer	CATGCCAACATTCCAGTTTTTTAA
TXN 3' qPCR primer	GCTTTTCCTTATTGGCTCCAGAA

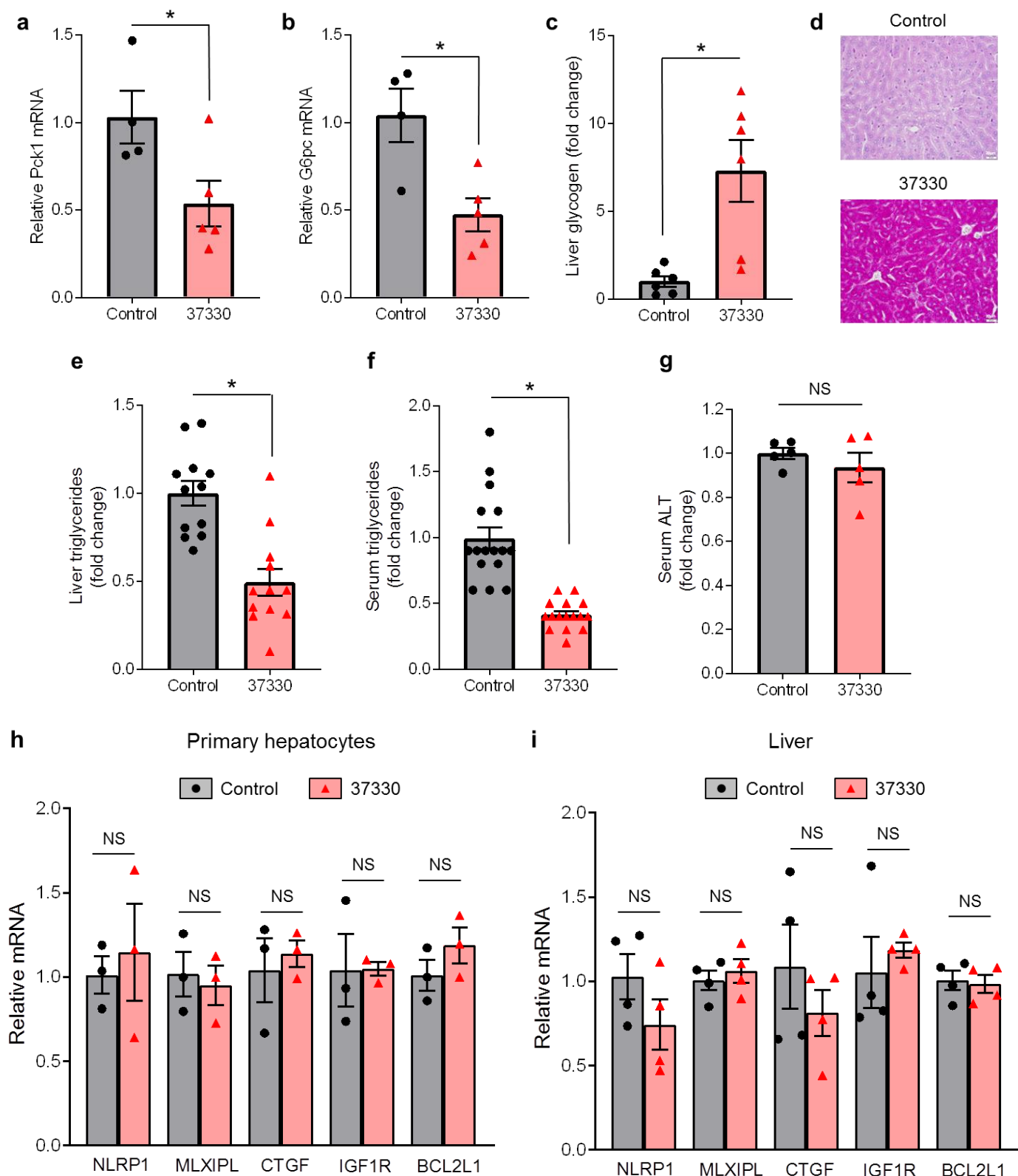


Supplemental Figure S1. Related to Figure 3: Oral administration of SRI-37330 does not improve glucose homeostasis in the absence of TXNIP.

(a) I.p. glucose tolerance tests (GTT) in TXNIP-deficient (Txnip^{-/-}) mice treated with or without SRI-37330, ($F_{5,85} = 2.76$, $P = 0.0231$), $n = 9-10$ mice per group.

(b) Fasting blood glucose in Txnip^{-/-} mice treated with or without SRI-37330, (N.S.), $n = 9-10$ mice per group.

(c) Fasting serum glucagon levels in Txnip^{-/-} mice treated with or without SRI-37330, ($t_{17} = 5.78$, $*P < 0.0001$), $n = 9-10$ mice per group.



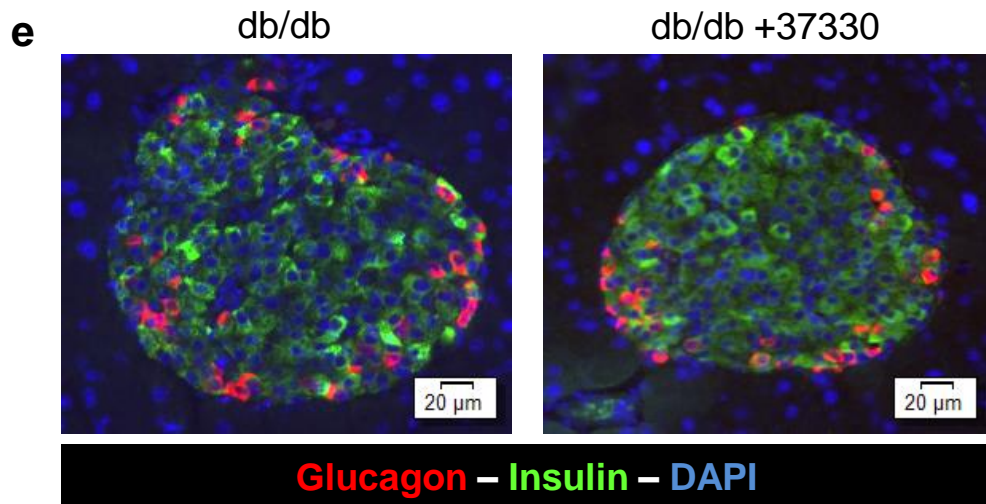
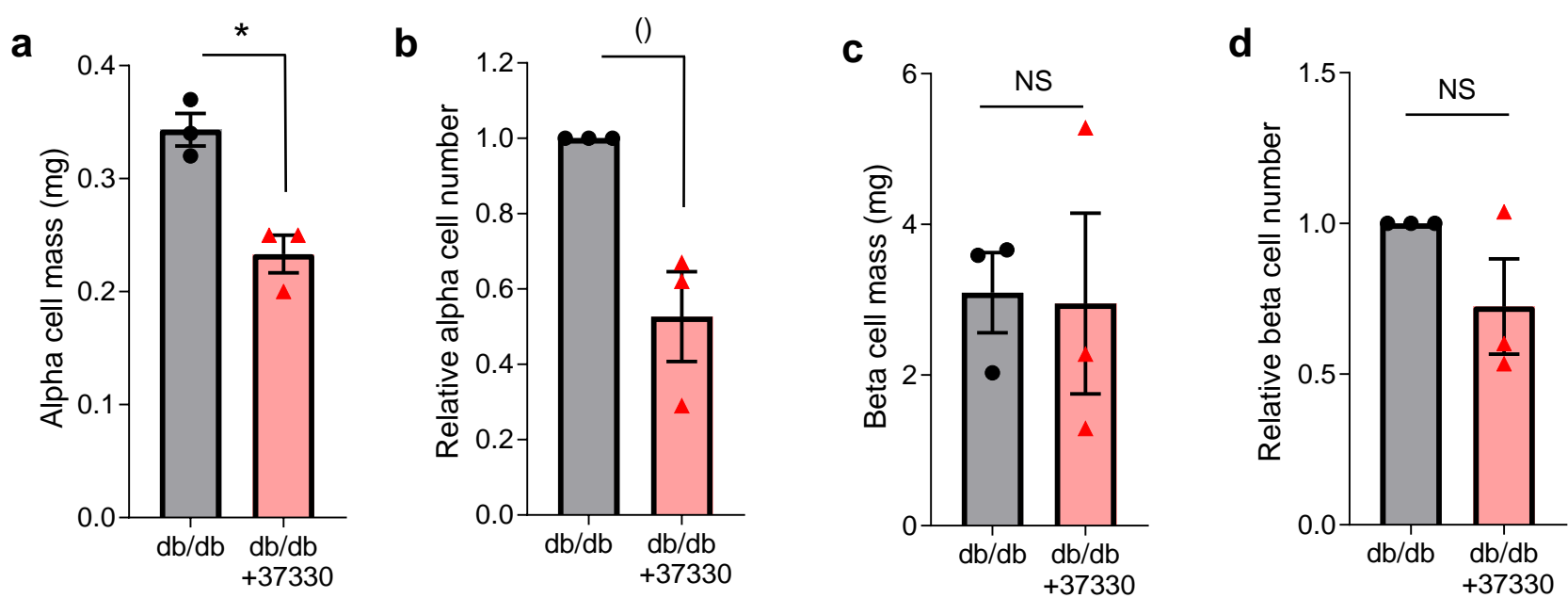
Supplemental Figure S2. Related to Figure 3 and 5: SRI-37330 effects on the liver.

(a) Expression of phosphoenolpyruvate carboxykinase (Pck1) as assessed by qPCR in livers of C57BL/6J mice treated with or without SRI-37330 ($t_7 = 2.46$, $*P = 0.0436$), $n = 4-5$ mice per group.

(b) Expression of glucose-6-phosphatase (G6pc) in livers of SRI-37330 treated and untreated mice ($t_7 = 3.30$, $*P = 0.0131$), $n = 4-5$ mice per group.

Supplemental Figure S2. (continued)

- (c)** Hepatic glycogen content in response to treatment with or without SRI-37330 as assessed by colorimetric assay ($t_{10} = 3.82$, $*P = 0.0019$) $n = 6$ mice per group.
- (d)** Representative, PAS stained, histological liver sections ($n = 12$ total sections analyzed) of SRI-37330 treated and untreated mice, (glycogen = magenta), scale bar = $20\mu\text{m}$
- (e)** Liver triglycerides in SRI-37330 treated and untreated mice ($t_{22} = 4.88$, $*P < 0.0001$) $n = 12$ mice per group.
- (f)** Serum triglycerides in SRI-37330 treated and untreated mice ($t_{18} = 6.49$, $*P < 0.0001$), $n = 16$ mice per group.
- (g)** Serum levels of alanine transaminase (ALT) in SRI-37330 treated and untreated mice (N.S., not significant), $n = 5$ mice per group.
- (h)** Expression of TXNIP-associated genes in response to SRI-37330 as assessed by qPCR in primary hepatocytes (N.S.), $n = 3$ independent experiments.
- (i)** Liver expression of TXNIP-associated genes in response to treatment with or without SRI-37330 (N.S.), $n = 3$ independent experiments.



Supplemental Figure S3. Related to Figure 6: Effects of SRI-37330 treatment on alpha and beta cell morphometry.

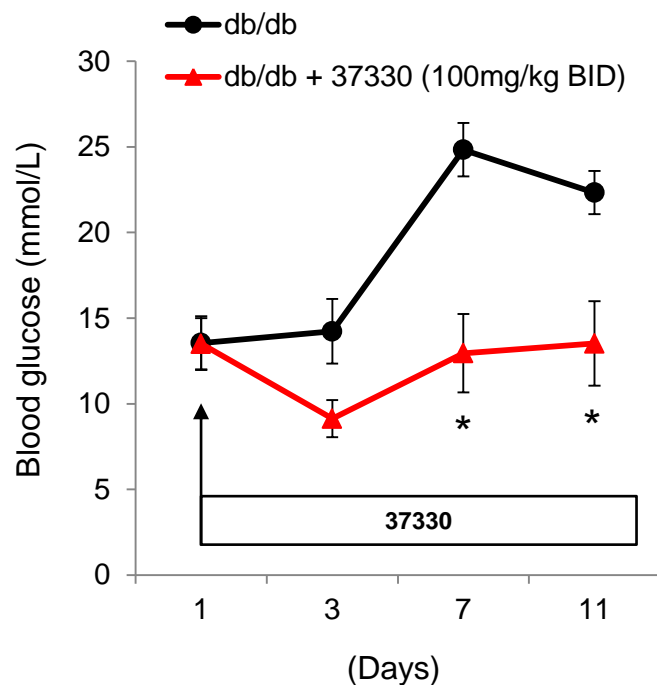
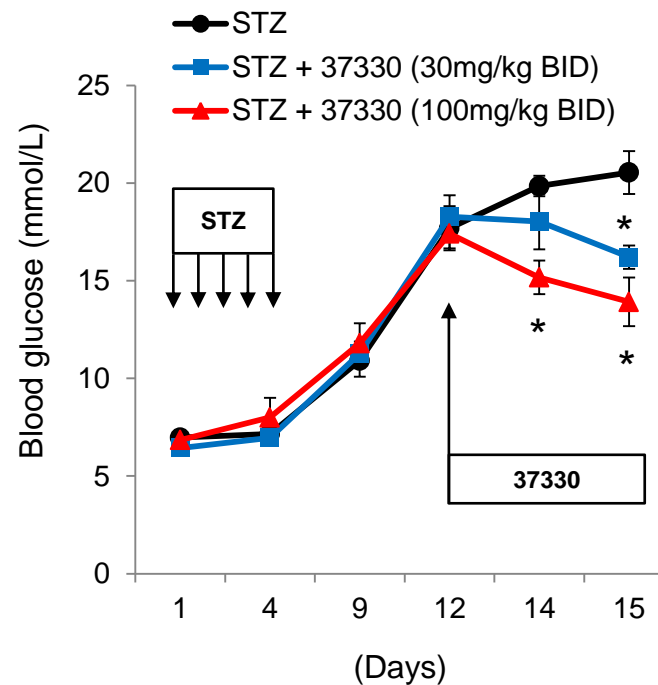
(a) Pancreatic alpha cell mass of SRI-37330 treated and untreated *db/db* mice ($t_4 = 4.97$, $*P = 0.0076$), $n = 3$ mice per group.

(b) Pancreatic alpha cell number of SRI-37330 treated and untreated *db/db* mice ($t_2 = 4.00$; ${}^{\circ}P = 0.0572$), $n = 3$ mice per group.

(c) Pancreatic beta cell mass of SRI-37330 treated and untreated *db/db* mice, (N.S., not significant), $n = 3$ mice per group.

(d) Pancreatic beta cell number of SRI-37330 treated and untreated *db/db* mice, (N.S.), $n = 3$ mice per group.

(e) Representative islet images ($n = 14$ sections total analyzed) of *db/db* mice treated with or without SRI-37330, scale bar = 20 μ m

a**b**

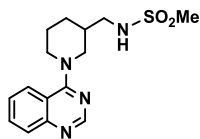
Supplemental Figure S4. Related to Figure 7: Effects of oral gavage SRI-37330 on obesity and STZ-induced diabetes.

(a) Obese, insulin resistant and diabetic male db/db mice were treated twice a day (BID) with SRI-37330 or vehicle and their non-fasting blood glucose was assessed at the designated time points ($F_{1,44} = 17.04$, $*P = 0.0002$), $n = 8$ mice per group.

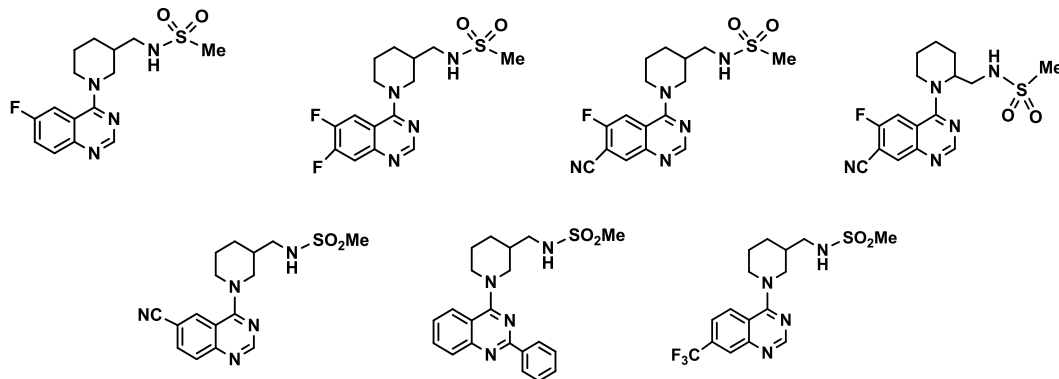
(b) C57BL/6J mice were rendered diabetic by multiple low-dose STZ injections and started receiving the designated doses of SRI-37330 by oral gavage after they had developed overt diabetes; their non-fasting blood glucose levels are shown ($F_{10,95} = 4.92$, $*P < 0.0001$) $n = 7-8$ mice per group.

a

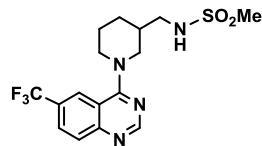
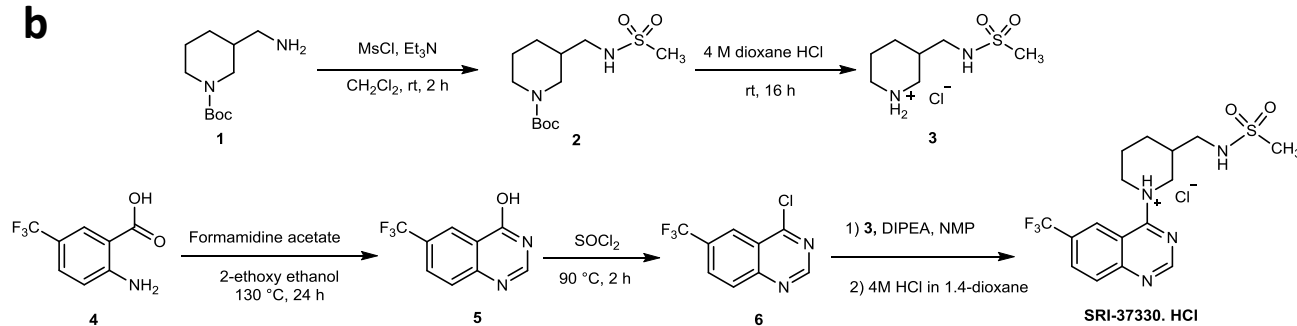
Hit

*N*-(1-(quinazolin-4-yl)piperidin-3-yl)methylmethanesulfonamide

Analog



Lead

*N*-((1-(6-(trifluoromethyl)quinazolin-4-yl)piperidin-3-yl)methyl) methanesulfonamide**b**

Supplemental Figure S5.
**Related to high-throughput
 screening and compound
 synthesis sections of the
 STAR Methods:**

Development of SRI-37330.

(a) Optimization from original hit to lead compound with a few representative analogs shown.

(b) Chemical synthesis pathway of SRI-37330.HCl.