Tricyclic Compounds increase IRS2 in Human Islets and promote β-Cell Growth and Function in Mice

Alexandra Kuznetsova[1], Yue Yu[2], Jennifer Hollister-Lock[¶,3], Lynn Opare-Addo[4],Aldo Rozzo[5], Marianna Sadagurski[6], Lisa Norquay[7], Jessica Reed[†8], Ilham El Khattabi[¶,9], Susan Bonner-Weir[¶,10], Gordon C. Weir[¶,11], Arun Sharma[¶,12], Morris F. White

Division of Endocrinology
Department of Medicine
Boston Children's Hospital
Harvard Medical School
Boston, Massachusetts 02115

[¶]Section of Islet Cell and Regenerative Biology
Department of Medicine
Joslin Diabetes Center
Harvard Medical School
Boston, Massachusetts

[†]Housey Pharmaceutical Research Laboratories
Southfield, Michigan 48076

Corresponding author:
Morris F. White
Division of Endocrinology
Boston Children's Hospital
Harvard Medical School
CLS, Rm 16020
3 Blackfan Circle
Boston MA 02115
TEL: (617) 919 2846
FAX: (617) 730-0244
300 Longwood Avenue
Email: morris.white@childrens.harvard.edu

TABLE S1

All compounds that increased IRS2 mRNA expression more than the 95%ile in isolated human islets.

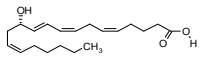
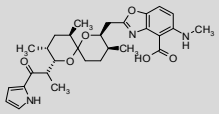
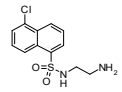
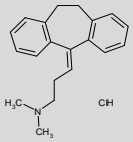
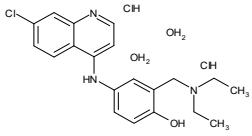
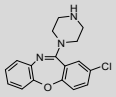
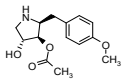
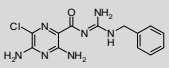
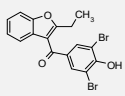
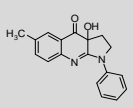
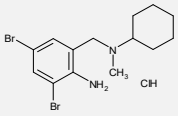
Structure	name	Conc	Δ >95%ile	SE	Sig	Action
	12(S)-HETE	0.1mM	2.55	1.02	0.01	
	A-23187	5mg/mL	8.83	3.45	0.01	Calcium ionophore
	A-3	5mg/mL	2.41	0.29	0.00	kinase inhibitor
	Amitriptyline • HCl	10 mM	4.13	1.49	0.01	antidepressant
	Amodiaquin •2HCl	2 mg/ml 10 mM	3.47 3.94	0.47 1.88	0.00 0.04	Bcl-2/Bcl-XL ligand induces apoptosis
	Amoxapine	2 mg/ml	5.10	0.60	0.00	reuptake inhibitor of serotonin and norepinephrine, respectively, and binds to 5-HT2, 3, 6 and 5-HT7, D2, α1-
	Anisomycin	2 mg/ml 5mg/mL 10 mM	6.92 6.86 3.90	0.25 1.05 1.17	0.00 0.00 0.00	protein synthesis inhibitor
	Benzamil	5mg/mL	3.36	0.43	0.00	Calcium channels
	Benzbromarone	2 mg/ml	5.29	0.25	0.01	a uricosuric agent and non-competitive inhibitor of Xanthine Oxidase
	Blebbistatin	5mg/mL	2.86	0.79	0.00	Myosin II inhibitor
	Bromhexine • HCl	10 mM	3.56	1.08	0.00	expectorant

TABLE S1 cont.

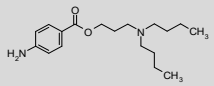
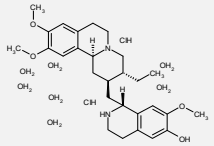
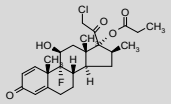
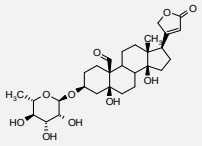
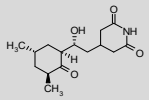
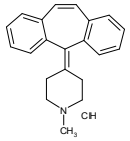
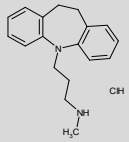
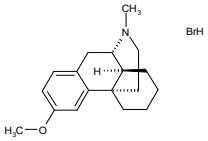
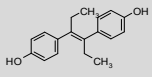
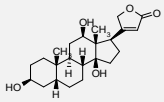
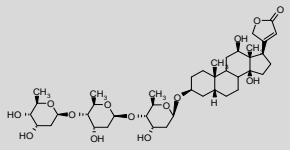
	Butacaine	2 mg/ml	2.49	1.20	0.04	a local anesthetic.
	Cephaeline •2HCl •7H2O	2 mg/ml	7.41	0.25	0.00	an alkaloid chemical closely related to emetine
	Clobetasol propionate	2 mg/ml	3.25	1.26	0.01	a corticosteroid
	Convallatoxin	10 mM	12.05	3.90	0.00	cardiotonic
	Cycloheximide	2 mg/ml 5mg/mL	8.18 5.71	0.59 1.38	0.00 0.00	protein synthesis inhibitor
	Cyproheptadine • HCl	2 mg/ml	6.54	1.11	0.00	H1-receptor antagonist
	Desipramine • HCl	10 mM	3.25	1.55	0.04	antidepressant
	Dextromethorphan • HBr	10 mM	3.86	1.13	0.00	antitussive
	Diethylstilbestrol	2 mg/ml	3.19	1.11	0.00	
	Digoxigenin	2 mg/ml	4.77	2.11	0.02	
	Digoxin	10 mM	6.04	1.38	0.00	cardiac stimulant

TABLE S1 cont.

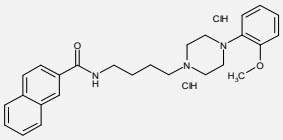
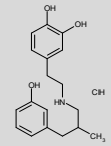
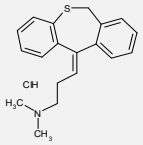
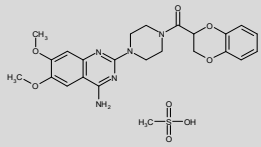
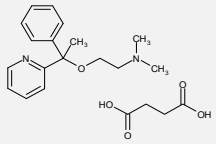
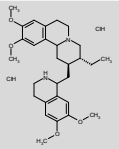
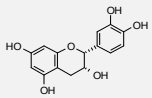
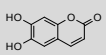
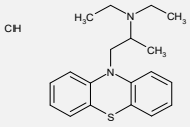
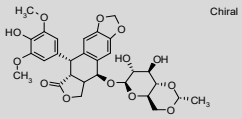
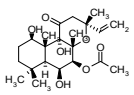
	DO 897/99	2 mg/ml	4.53	0.25	0.02	
	Dobutamine • HCl	10 mM	3.67	1.56	0.02	cardiotonic
	Dosulepin •HCl	2 mg/ml	4.94	0.25	0.01	
	Doxazosin mesylate	2 mg/ml	3.36	1.14	0.00	
	Doxylamine succinate	2 mg/ml	2.43	0.95	0.01	
	Emetine	10 mM 2 mg/ml	9.26 5.70	3.32 0.25	0.01 0.00	inhibits RNA, DNA and protein synthesis
	Epicatechin	10 mM	2.48	0.93	0.01	antioxidant
	Esculetin	2 mg/ml	5.83	0.82	0.00	
	Ethopropazine •HCl	2 mg/ml	5.56	1.49	0.00	anticholinergic, antihistamine, and antiadrenergic actions.
	Etoposide	2 mg/ml	7.47	0.25	0.00	
	Forskolin	5mg/mL	9.26	2.63	0.00	Adenylate cyclase activator

TABLE S1 cont.

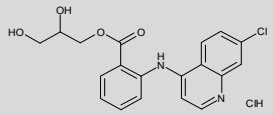
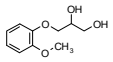
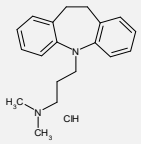
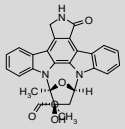
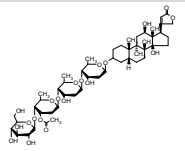
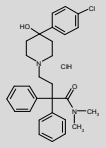
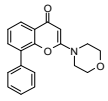
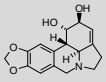
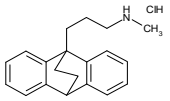
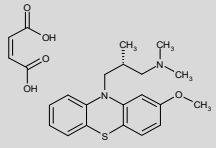
	Glafenine • HCl	2 mg/ml	3.80	0.45	0.01	a non-steroidal anti-inflammatory drug
	Guaifenesin	2 mg/ml	4.10	0.26	0.00	Expectorant
	IBMX	5mg/mL	3.40	1.25	0.01	PDE inhibitor (broad spec), adenosineR agonist
	Imipramine • HCl	2 mg/ml	5.06	2.47	0.04	serotonin transporter inhibitor; noradrenalin transporter inhibitor
	K252a	0.5mg/mL	8.76	3.19	0.01	Kinase inhibitor (Broad spectrum)
	Lanatoside C	2 mg/ml 10 mM	4.27 6.90	0.69 2.76	0.00 0.01	cardiotonic
	Loperamide • HCl	2 mg/ml	2.75	1.25	0.03	
	LY294002	5mg/mL	3.46	0.27	0.01	PI-3-Kinase inhibitor
	Lycorine	5mg/mL	3.24	0.53	0.00	inhibits TNFa production
	Maprotiline • HCl	2 mg/ml 10 mM	7.63 4.99	0.25 0.98	0.00 0.00	antidepressant
	Methotrimeprazine maleat salt	2 mg/ml	3.17	0.29	0.02	

TABLE S1 cont.

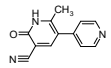
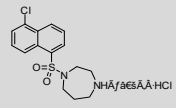
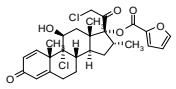
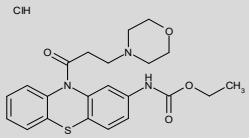
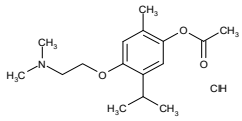
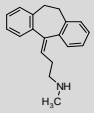
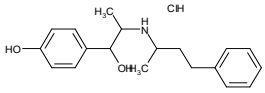
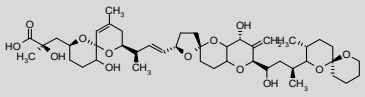
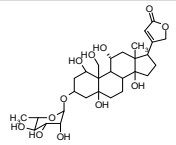
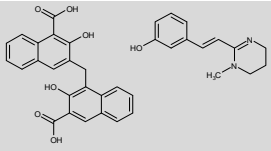
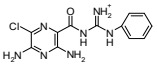
	Milrinone	2 mg/ml	2.92	0.97	0.00	
	ML9	5mg/mL	6.86	3.05	0.02	kinase inhibitor
	Mometasone furoate	2 mg/ml	5.22	0.25	0.01	
	Moricizine • HCl	2 mg/ml	3.27	0.53	0.00	voltage-gated sodium channel blocker
	Moxisylyte • HCl	2 mg/ml	3.91	0.25	0.01	
	Nortriptyline	10 mM 2 mg/ml	3.88 6.72	1.49 2.94	0.01 0.02	antidepressant; serotonin transporter inhibitor and noradrenalin transporter inhibitor
	Nylidrin • HCl	10 mM	3.52	0.73	0.00	vasodilator (peripheral)
	Okadaic acid	0.5mg/mL	10.39	4.07	0.01	PP1 PP2A inhibitor
	Ouabain	5mg/mL	6.78	1.11	0.00	Na+K+ATPase inhibitor
	Oxantel pamoate	2 mg/ml	7.85	0.25	0.00	Anthelmintic
	Phenamil	5mg/mL	4.73	2.40	0.05	Sodium channels

TABLE S1 cont.

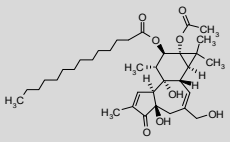
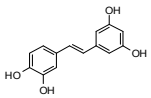
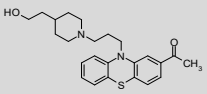
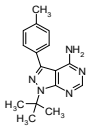
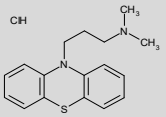
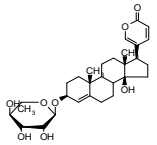
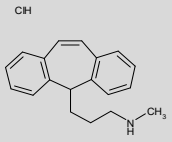
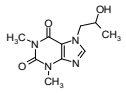
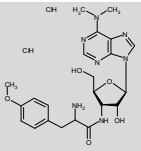
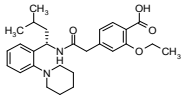
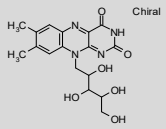
	Phorbol 12 myristate 13 acetate	5mg/mL 5mg/mL	4.95 2.97	1.11 0.69	0.00 0.00	PKC activator
	Piceatannol	5mg/mL	6.90	3.21	0.03	Syk inhibitor
	Piperacetazine	2 mg/ml	3.10	1.07	0.00	
	PP1	5mg/mL	3.19	0.25	0.02	src family trosine kinase inhibitor
	Promazine • HCl	2 mg/ml	3.45	0.88	0.00	
	Proscillaridin A	2 mg/ml	6.00	3.01	0.05	
	Protriptyline • HCl	2 mg/ml	6.85	2.37	0.00	
	Proxyphylline	2 mg/ml	8.34	4.12	0.04	
	Puromycin • 2HCl	2 mg/ml	4.62	0.25	0.02	
	Repaglinide	2 mg/ml	5.70	1.23	0.00	sulfonylurea receptor 1 agonist
	Riboflavine	2 mg/ml	8.66	0.25	0.00	enzyme co-factor

TABLE S1 cont.

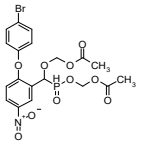
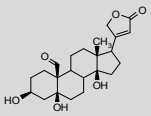
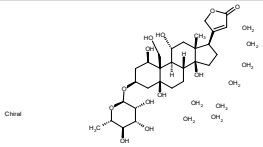
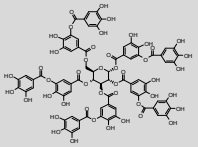
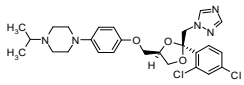
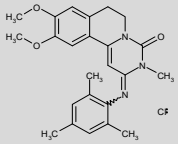
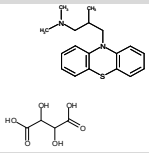
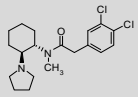
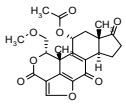
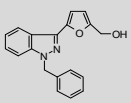
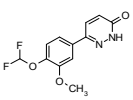
	RWJ-60475-(AM)3	5mg/mL	5.06	1.33	0.00	CD45 phosphatase inhibitor
	Strophanthidin	10 mM	4.61	1.66	0.01	cardiotonic
	Strophantine • 8H2O	2 mg/ml	10.41	0.25	0.00	
	Tannic acid	10 mM	7.30	2.84	0.01	nonspecific enzyme/receptor blocker
	Terconazole	2 mg/ml	4.59	0.25	0.02	sterol 14alpha-demethylase ihibitor
	Trequinsin	5mg/mL	6.40	3.24	0.05	phosphodiesterase (PDE3) inhibitor
	Trimeprazine tartrate	2 mg/ml	5.85	1.89	0.00	H1-receptor antagonist
	U-50488	5mg/mL	3.92	0.58	0.00	Calcium channels
	Wortmannin	5mg/mL	2.48	1.25	0.05	PI-3Kinase, other kinases inhibitor
	YC-1	5mg/mL	3.83	0.49	0.00	guanylyl cyclase stimulator
	Zardaverine	2 mg/ml	4.50	2.12	0.03	Selective, dual inhibitor of PDE III and PDE IV

TABLE S2

Analysis of Nuclear and Cytoplasmic Pdx1 Distribution

Islet	Geno	Treat	$Area^{Ins}$	$Pdx1^{Cyto}$	$\#Nuc^{Tot}$	$\#Nuc^{Pdx1}$	$Pdx1^{>MED}$
1	WT	Saline	4283	649	16	6	0.375
2	WT	Saline	8097	869	28	13	0.464
3	WT	Saline	1503	350	4	0	0.000
4	WT	Saline	2302	500	7	7	1.000
5	WT	Saline	45010	856	154	64	0.416
6	WT	Saline	15986	485	67	41	0.612
7	WT	Saline	1360	518	11	6	0.545
8	WT	Saline	2756	674	9	3	0.333
9	WT	Saline	2666	472	9	4	0.444
10	WT	Saline	78389	705	200	167	0.835
11	WT	Saline	11919	802	46	6	0.130
12	WT	Saline	16636	808	61	39	0.639
13	WT	Saline	20310	817	67	21	0.313
14	WT	Saline	18014	670	56	6	0.107
15	WT	Saline	7864	782	26	5	0.192
16	WT	Saline	17937	769	77	44	0.571
1	WT	Trimep	85880	388	200	135	0.675
2	WT	Trimep	26410	461	110	50	0.455
3	WT	Trimep	3461	499	16	13	0.813
4	WT	Trimep	11963	885	44	19	0.432
6	WT	Trimep	3047	759	13	12	0.923
7	WT	Trimep	3874	745	20	17	0.850
8	WT	Trimep	10181	637	49	39	0.796
9	WT	Trimep	14732	344	66	52	0.788
10	WT	Trimep	3358	583	11	7	0.636
11	WT	Trimep	24440	407	87	63	0.724
12	WT	Trimep	7446	684	24	22	0.917
13	WT	Trimep	20658	844	59	24	0.407
14	WT	Trimep	6147	530	24	21	0.875
15	WT	Trimep	51694	690	200	147	0.735
16	WT	Trimep	10709	527	31	18	0.581
1	IRS2KO	Saline	0	813	15	4	0.267
2	IRS2KO	Saline	10698	800	44	12	0.273
3	IRS2KO	Saline	11784	679	45	11	0.244
4	IRS2KO	Saline	4767	618	14	6	0.429
5	IRS2KO	Saline	19439	572	76	39	0.513
6	IRS2KO	Saline	12558	518	43	20	0.465
7	IRS2KO	Saline	10346	586	33	17	0.515
8	IRS2KO	Saline	12137	665	35	15	0.429
9	IRS2KO	Saline	6302	542	26	16	0.615
10	IRS2KO	Saline	28555	542	113	30	0.265
11	IRS2KO	Saline	4460	510	17	6	0.353
12	IRS2KO	Saline	3497	484	12	1	0.083
13	IRS2KO	Saline	12334	541	42	23	0.548
1	IRS2KO	Trimep	2769	385	25	15	0.600
2	IRS2KO	Trimep	3496	578	25	13	0.520
3	IRS2KO	Trimep	691	404	9	7	0.778
4	IRS2KO	Trimep	6939	516	27	10	0.370
5	IRS2KO	Trimep	32314	78	121	60	0.496
6	IRS2KO	Trimep	8022	269	27	10	0.370
7	IRS2KO	Trimep	19521	192	90	39	0.433
8	IRS2KO	Trimep	16368	690	67	27	0.403
9	IRS2KO	Trimep	5704	383	21	17	0.810
10	IRS2KO	Trimep	7774	568	24	10	0.417
11	IRS2KO	Trimep	31411	699	109	71	0.651
12	IRS2KO	Trimep	3426	481	15	9	0.600
13	IRS2KO	Trimep	19641	310	88	17	0.193
14	IRS2KO	Trimep	30773	506	139	68	0.489

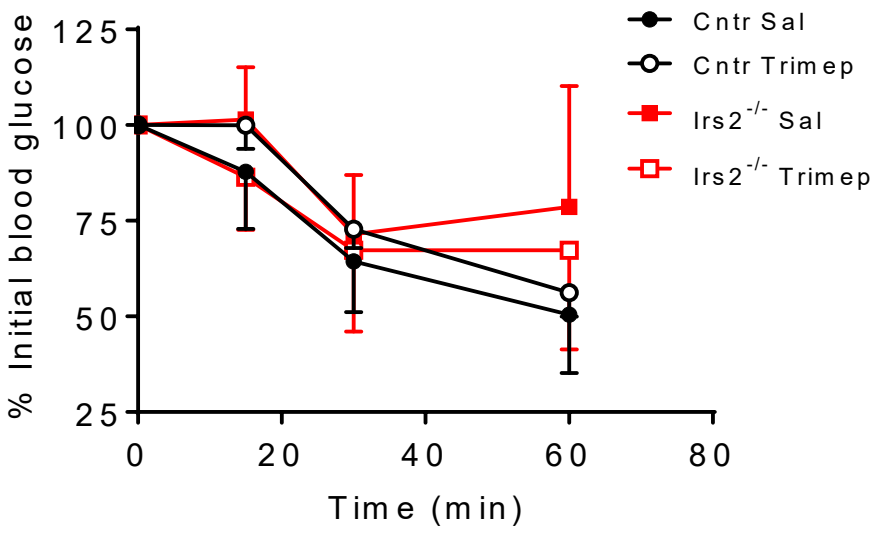

% Initial blood glucose
125
100
75
50
25
0
20
40
60
80
Time (min)
Cntr Sal
Cntr Trimep
Irs2-/- Sal
Irs2-/- Trimep

Figure S1. Insulin tolerance test in 9 week-old chow-fed Cntr or Irs2-/- mice treated between 6-9 weeks of age with saline injections or 10 mg/kg trimeprazine in saline. For ITT, blood was drawn beginning 3 hr after the end of dark phase, after which mice were fasted 4 hr, then injected intraperitoneally with 1 U/kg body weight humulin R (Lilly), and blood glucose levels were measured at indicated time points.

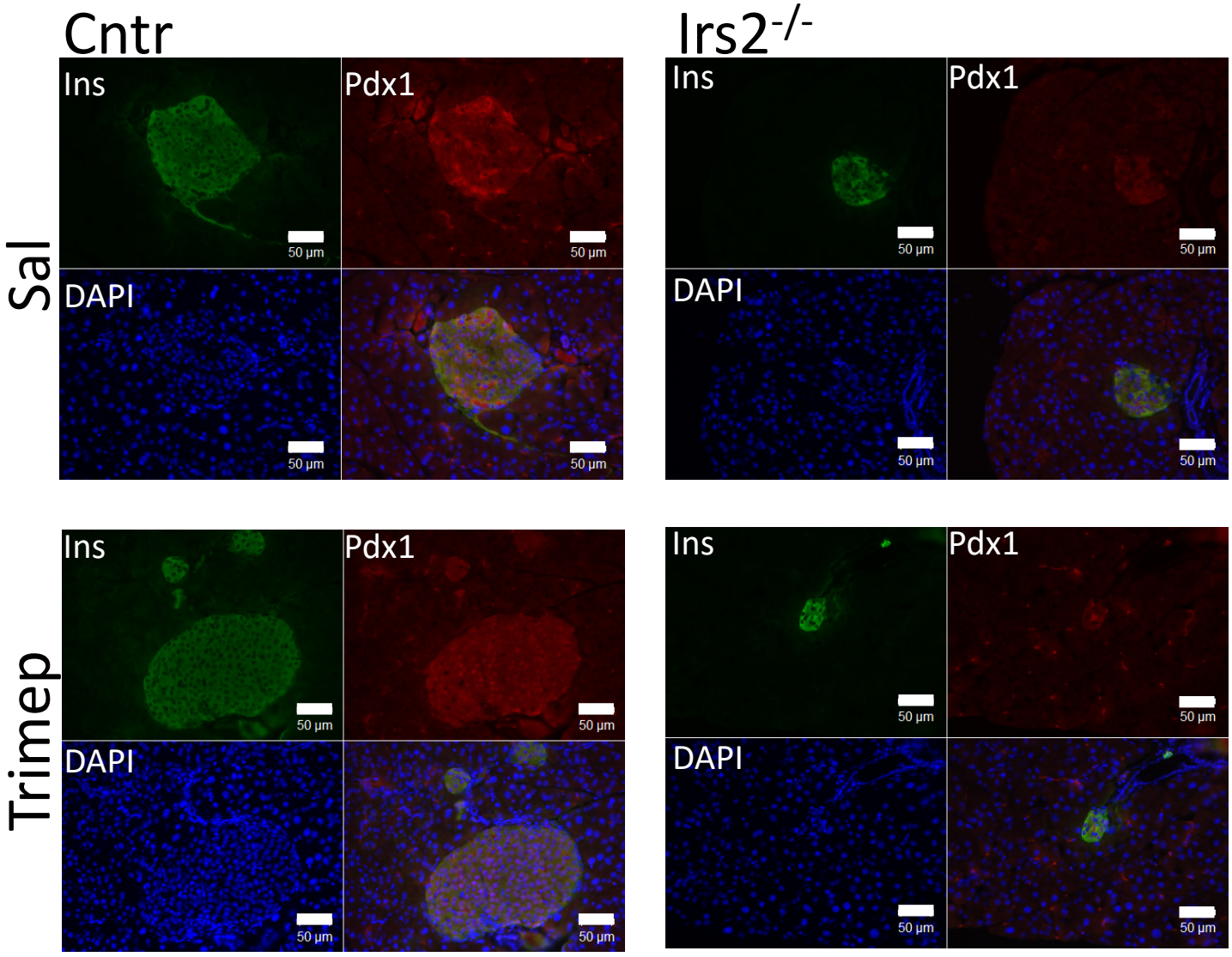

Cntr
Irs2-/-
Sal
Trimep
Ins
Pdx1
DAPI
50 µm
green = insulin; red = Pdx1; blue = DAPI

Figure S2 Representative pancreas sections from control (Cntr) or $Irs2^{-/-}$ mice treated without or with 10 mg/kg daily trimeprazine for 3 weeks. The 10μ sections were stained with DAPI and immunostained with antibodies against insulin (Ins) and Pdx1. The color channels were separated using Imaris software. The merged image is shown in the lower right quarter of each panel.

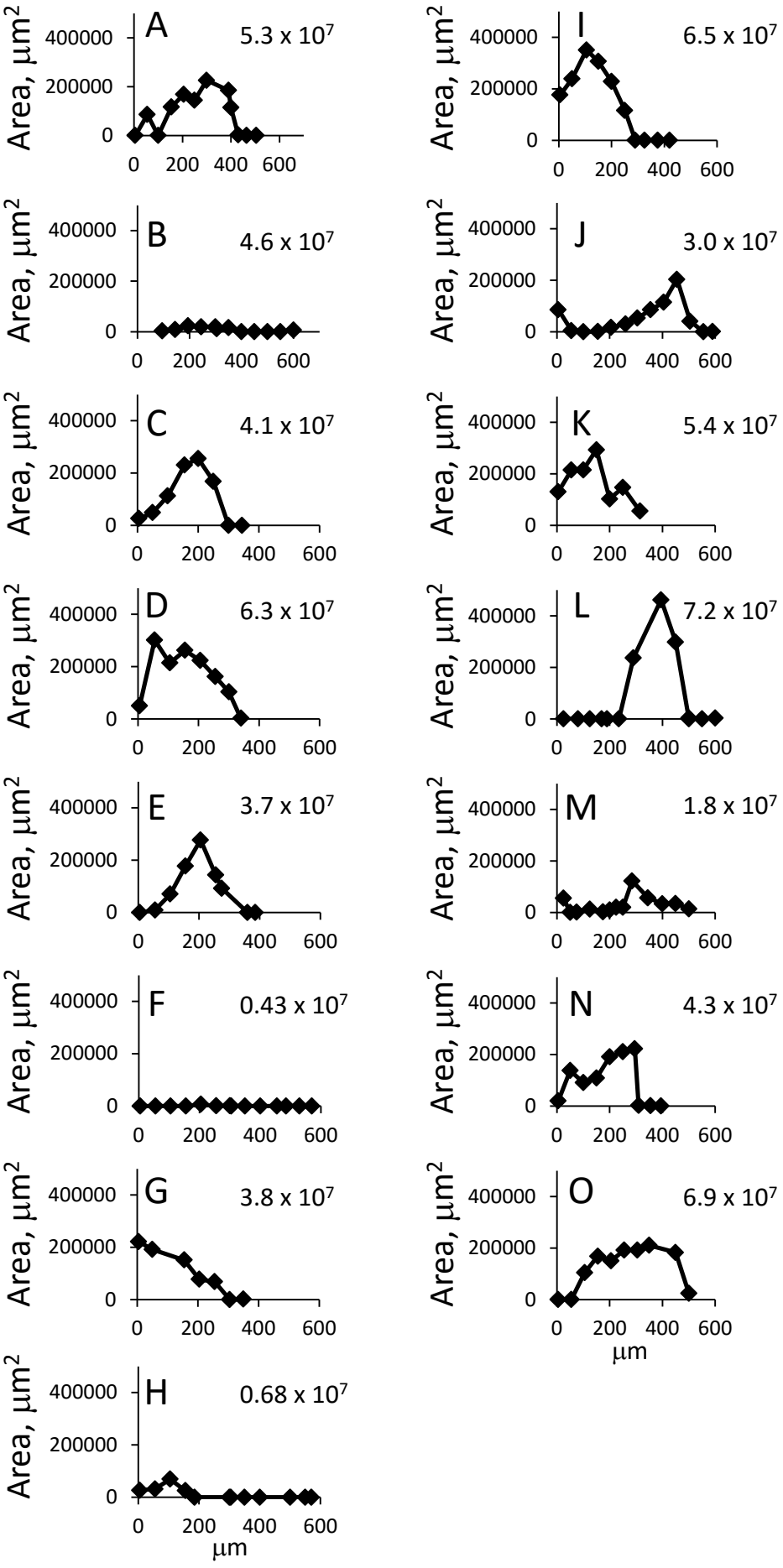

A
5.3 x 10^7
B
4.6 x 10^7
C
4.1 x 10^7
D
6.3 x 10^7
E
3.7 x 10^7
F
0.43 x 10^7
G
3.8 x 10^7
H
0.68 x 10^7
I
6.5 x 10^7
J
3.0 x 10^7
K
5.4 x 10^7
L
7.2 x 10^7
M
1.8 x 10^7
N
4.3 x 10^7
O
6.9 x 10^7
Area, µm2
400000
200000
0
0 200 400 600
µm

Figure S3. Human islet graft area determined 75 days after transplantation of 800 islets under the kidney capsule of streptozotocin diabetic Beige Scid Fox Chase' mice treated without (A-H) or with trimeprazine (I-O). The graft was excised and sectioned at 5μ intervals and selected sections were stained with antibodies against insulin and visualized by a peroxidase conjugated secondary antibody. The insulin positive area (μm^2) was determined using Imagej software and plotted against the position of the section in the graft. The volume (μm^3) of each graft was estimated by determining the area under the curves (shown in the upper right corner of each panel).

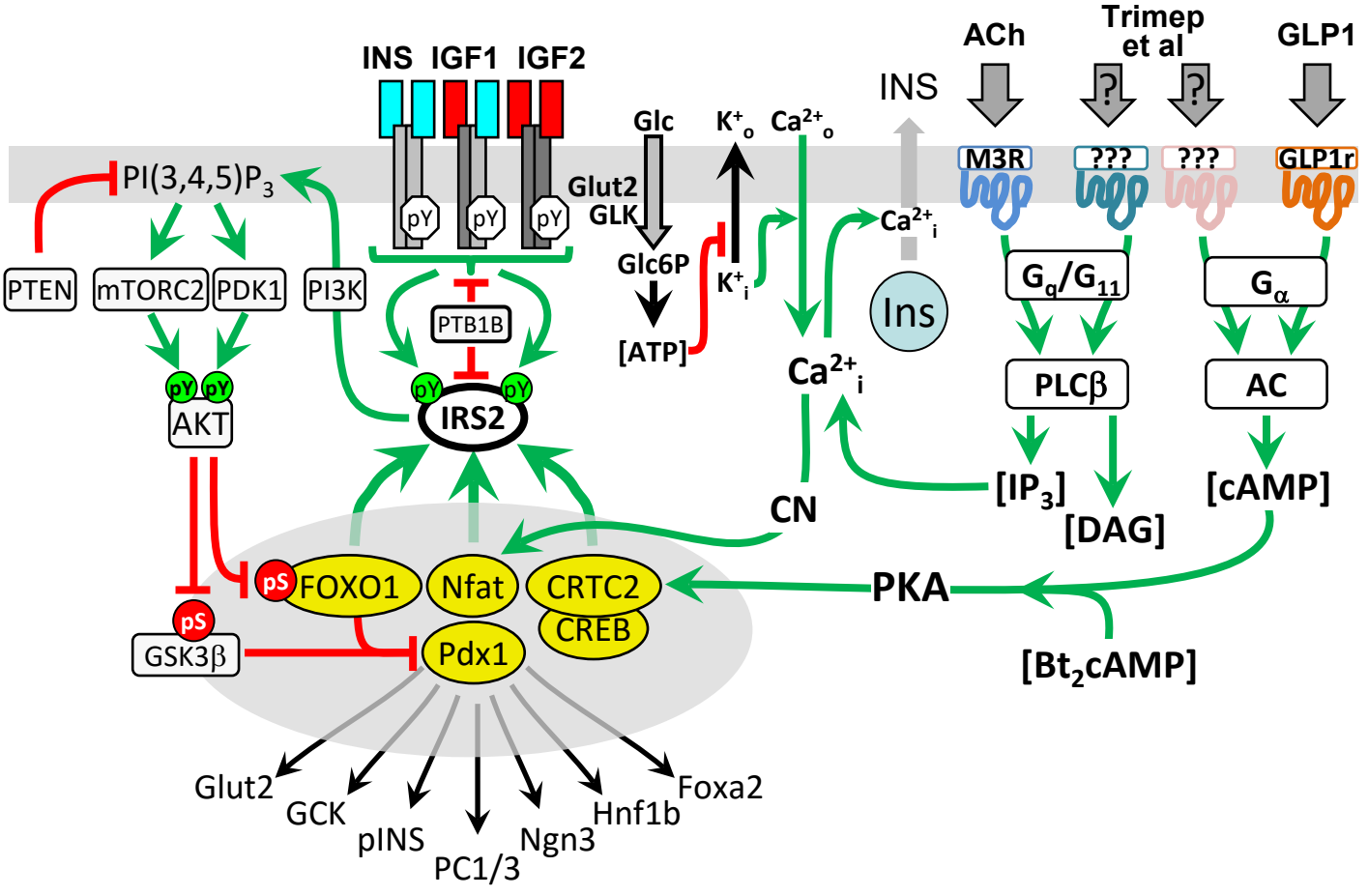

INS IGF1 IGF2
Glc
K+o
Ca2+o
INS
ACh
Trimep et al
GLP1
M3R
???
???
GLP1r
PI(3,4,5)P3
Glut2
GLK
Glc6P
[ATP]
K+i
Ca2+i
Ins
Gq/G11
Gα
PTEN
mTORC2
PDK1
PI3K
PTB1B
AKT
IRS2
Ca2+i
PLCβ
AC
CN
[IP3]
[DAG]
[cAMP]
FOXO1
Nfat
CRTC2
CREB
PKA
GSK3β
Pdx1
[Bt2cAMP]
Glut2
GCK
pINS
PC1/3
Ngn3
Hnf1b
Foxa2

Figure S4. An integrative model of IRS2 signaling in pancreatic β-cell function. The diagram shows the relation between the IRS2-branch of the insulin signaling pathway and upstream and downstream mechanisms regulating β-cell growth and function. The production of PI3,4,5P3 by the PI3K recruits the Ser/Thr-kinases PDK1 and AKT to the plasma membrane where AKT is activated by PDK1 and mTORC2-mediated phosphorylation. AKT phosphorylates many proteins that play important physiological roles—GSK3β (glycogen synthesis), the BAD•BCL2 heterodimer (apoptosis inhibition), TSC1•TSC2 (protein synthesis and nutrient sensing), and FOXO (transcriptional regulation). Activation of GLP1→cAMP→PKA→CREB, glucose→Ca^{2+}→CRTC2 and calcineurin→NFAT induce IRS2 expression in β-cells revealing a mechanism that places β-cell growth, function and survival under the control of glucose and incretins. GPCR engaged by trimeprazine and its tricyclic analogs can also stimulate these pathways. Since insulin and IGF1R are constitutively active, IRS2 expression can act as the regulatory gateway for these signaling cascades: IRS2→PI3K→AKT signaling promotes mTOR signaling and inhibits Foxo1 and $p27^{kip}$; Irs2⊣Gsk3β might couple into the β-catenin cascade regulated by Wnt. PTEN and PTP1B modulate IRS2 signaling. Together this integrated pathway shows how signals known to promote β-cell/islet growth and function can be integrated by the IRS2 signaling cascade into common pathways regulated by heterologous factors.