Supporting Information

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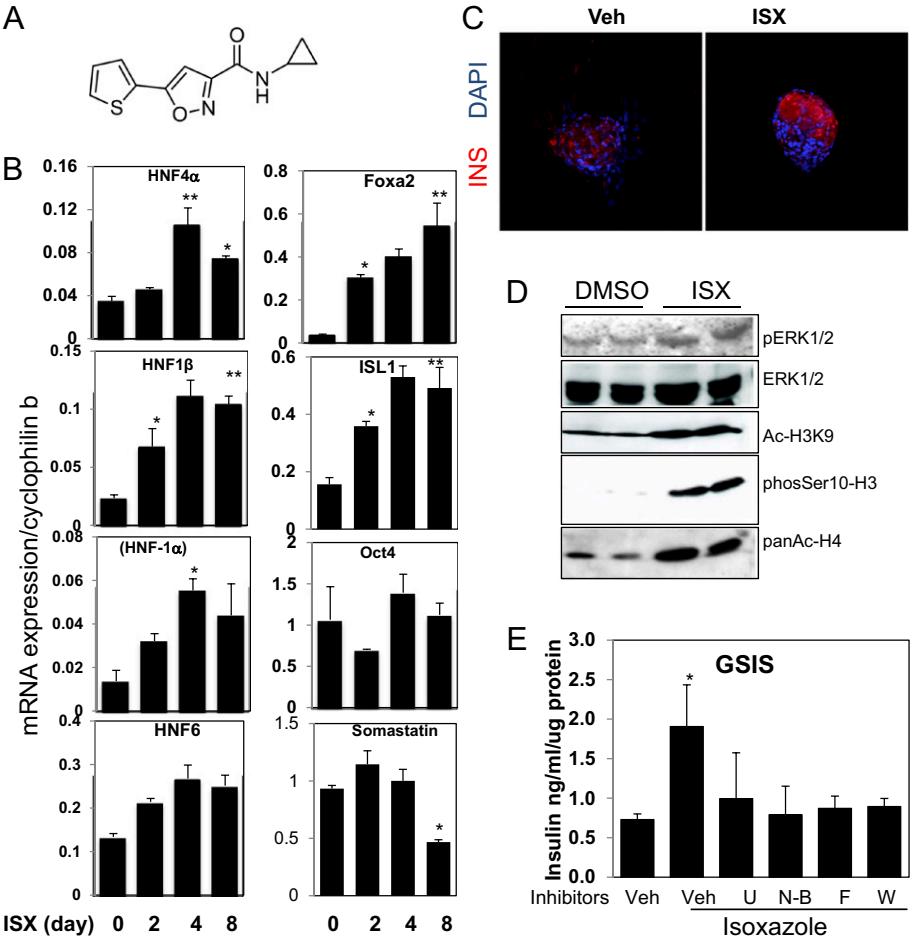

Fig. S1. Isoxazoles (Isx) are small molecules that induce insulin expression in human pancreatic islets maintained in culture for 2–12 mo. Gene expression induced by Isx in human islet cultures. (*A*) Structure of the lead compound, *N*-cyclopropyl-5-(thiophen-2-yl)isoxazole-3-carboxamide (Isx), used in this study. (*B*) Expression profiles of relevant islet factors by Sybr-Green quantitative PCR analyses from human islets cultured for 1 y as in Fig. 1*D*. Foxa2, forkhead box A2; Hnf, hepatic nuclear factor; Oct4, octamer-binding transcription factor 4. (*C*) Insulin immunohistochemical staining (INS, red) in human islets cultured for 2 mo. Nuclei are stained with DAPI (blue). (*D*) Immunoblotting with antibodies against acetylated histone 3 lysine 9 (AcH3K9) and pan-acetylated histone 4 (panAc-H4) of whole-cell extracts from human islets cultured for 6 mo and treated with vehicle (DMSO) or Isx for 24 h. (*E*) Human islets cultured for 6 mo were treated with DMSO (Veh) or Isx for 48 h. To implicate signaling pathways that are required for the isoxazole-driven increase in β-cell function, measured as glucose-stimulated insulin secretion, the Isx-treated islets also were treated with vehicle (Veh), 10 μM U0126 (U), 10 μM nifedipine1,2-bis(o-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA) (N-B), 0.5 μM FK506 (F), or 0.1 μM wortmannin (W) for the duration of the Isx treatment. GSIS, glucose-stimulated insulin secretion.

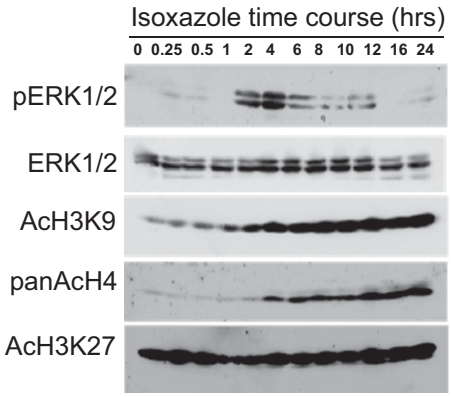

Fig. S2. Time course of Isx-induced activation of ERK1/2 and histone acetylation. Time course of Isx treatment in MIN6 cells. Immunoblotting of whole-cell extracts from MIN6 cells grown for 24 h in complete DMEM containing 4.5 mM glucose and treated with 20 μM Isx for 15 min to 24 h. The control (0 min) was treated with DMSO for 24 h.

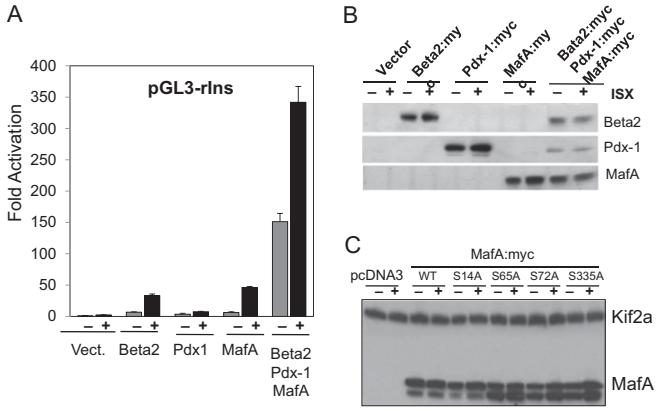

Fig. S3. Isx augments the transactivation capacities of islet transcription factors BETA2 and v-maf musculoaponeurotic fibrosarcoma oncogene homolog A (MafA) in transient transfection assays in HEK-293 cells. Transient transfections and insulin reporter assays in HEK-293 cells. (*A*) BETA2:myc, pancreatic and duodenal homeobox 1 (PDX-1):myc and MafA:myc were transfected singly or in combination in HEK-293 cells together with the rat Insulin 1 promoter (−410, +1) luciferase reporter (pGL3-rIns). The basal activity of the promoter was assessed by transfection of the empty pcDNA vector alone. Transfected cells were treated with vehicle or 20 μM Isx for 24 h. sv40-Renilla luciferase was used as an internal control. (*B*) Expression of recombinant factors Beta2:myc, Pdx-1:myc, and MafA:myc, transfected in HEK-293 cells as in *A*, was determined by immunoblot using NeuroD1, PDX-1, and cMaf antibodies. (*C*) Expression profile of transfected WT and mutant MafA:myc in HEK -293 cells treated with vehicle or Isx for 24 h.

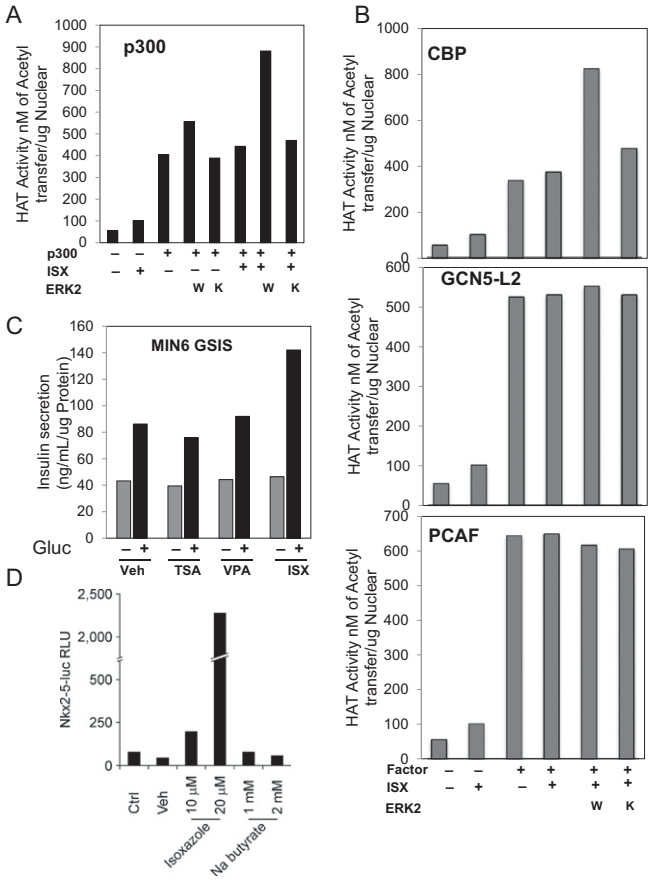

Fig. S4. Isx induces histone acetyltransferase (HAT) activity in p300 and cAMP response element-binding (CREB)-binding protein (CBP) and has a differential effect on β-cell function compared with histone deacetylase (HDAC) inhibitors. Isx stimulated HAT activity. (*A* and *B*) Combined effect of Isx treatment for 24 h and WT ERK2 (W) or kinase dead (K52R) ERK2 (R) on the HAT activities of p300 (*A*) and other HATs [CBP, p300/CBP-associated factor (PCAF), and GCN5-L2] (*B*) transfected in HEK-293 cells. (*C* and *D*) Specific effects of Isx on GSIS in MIN6 cells (*C*) and Nkx2.5 promoter activity in P19 progenitor cells (*D*) compared with HDAC inhibitors such as trichostatin A (TSA) or valproic acid (VPA) after pretreatment for 48 h.

Table S1. Human quantitative PCR primer sets

Primer name	Forward	Reverse
Cyclophilin	5′-CATGTCAGTGGGACTTGTGC-3′	5′- CTGGCCCAATTTCAAAGAAG-3′
Beta2	5′-ATGACCAAATCGTACAGCGAG-3′	5′-GTTCATGGCTTCGAGGTCGT-3′
Neurogenin3	5′-ACTGTCCAAGTGACCCGTGAG-3′	5′- CTCAGTGCCAACTCGCTCTTAG-3′
MafA	5′-CTTCAGCAAGGAGGAGGTCATC	5′-CTCGTATTTCTCCTTGTACAGGTCC-3′
Pdx1	5′-ACCAAAGCTCACGCGTGGAAA-3′	5′-TGATGTGTCTCTCGGTCAAGTT-3′
Glut2	5′-GCTACCGACAGCCTATTCTA-3′	5′-CAAGTCCCACTGACATGAAG-3′
Isl-1	ATTTCCCTATGTGTTGGTTGCG	CGTTCTTGCTGAAGCCGATG-3′
NKX2.2	5′-ATGTCGCTGACCAACACAAAG-3′	5′-GATGTCCTTGACCGAAAACCC-3′
Oct4	5′-CCGAAAGAGAAAGCGAACCAG-3′	5′-ATGTGGCTGATCTGCTGCAGT-3′
Foxa2	5′-CTGAGCGAGATCTACCAGTGGA-3′	5′-CAGTCGTTGAAGGAGAGCGAGT-3′
Hnf4a	5′-ACTACATCAACGACCGCCAGT-3′	5′-ATCTGCTCGATCATCTGCCAG-3′
Hnf1b	5′-GCACCTCTCCCAGCATCTCA-3′	5′-GTCGGAGGATCTCTCGTTGC-3′
Hnf6	5′-TGTGGAAGTGGCTGCAGGA-3′	5′-TGTGAAGACCAACCTGGGCT-3′
Hnf1a	5′-TGATGAGCTACCAACCAAGAAGG-3′	5′-CTATTGCACTCCTCCACTAGCGT-3′
Pax4	5′-TCTCCTCCATCAACCGAGTC-3′	5′-GAGCCACTATGGGGAGTGAG-3′
Pax6	5′-CGAATTCTGCAGGTGTCCAA-3′	5′-ACAGACCCCCTCGGACAGTAAT-3′
Nkx6.1	5′-AGACCCACTTTTTCCGGACA-3′	5′-CCAACGAATAGGCCAAACGA-3′
Somatostatin	5′-CCCAGACTCCGTCAGTTTCT-3′	5′-ATCATTCTCCGTCTGGTTGG-3′
Insulin	5′-GCAGCCTTTGTGAACCAACAC-3′	5′-CCCCGCACACTAGGTAGAGA-3′

Table S2. Mouse quantitative PCR primer set

Primer name	Forward	Reverse
Cyclophilin	5′-ATGTGGTTTTCGGCAAAGTTCTA-3	5′-GGCTTGTCCCGGCTGTCT-3′
Insulin 1	5′-GACCAGCTATAATCAGAGACC-3′	5′-AGTTGCAGTAGTTCTCCAGCTG-3′
Insulin 2	5′-AGCCCTAAGTGATCCGCTACAA-3′	5′-AGTTGCAGTAGTTCTCCAGCTG-3′