Supplementary Figure 11. The characterization data for reserpine .

Reserpine had high purity (>90%).

Reserpine was purchased as a dry powder from Calbiochem Novabiochem Novagen.

HPLC, LC-MS (ESI) and ^{1}H-NMR profiles are shown.

Supplementary Figure 12. The characterization data for Tetrabenazine.

Tetrabenazine had high purity (>90%).

Tetrabenazine was purchased as a dry powder from Tocris Bioscience.

HPLC, LC-MS (ESI) and ^{1}H-NMR profiles are shown.

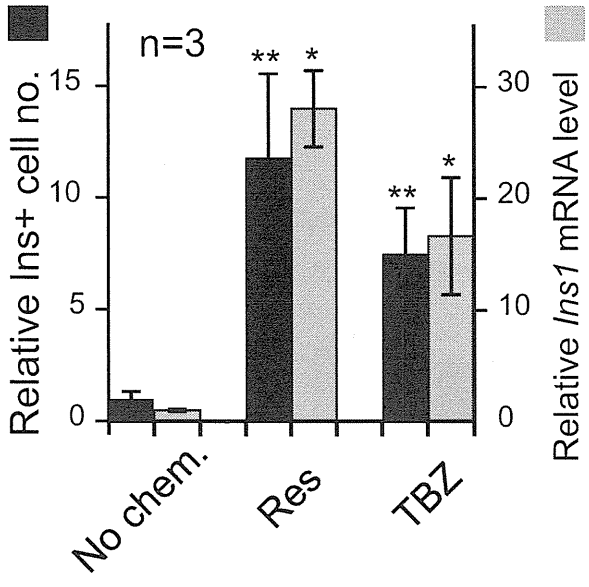
Supplementary Figure 13. The characterization data for dBu-cAMP.

dBu-cAMP had high purity (>90%).

dBu-cAMP was purchased as a dry powder from BIOMOL International. HPLC, LC-MS (ESI) and ^{1}H-NMR profiles are shown.

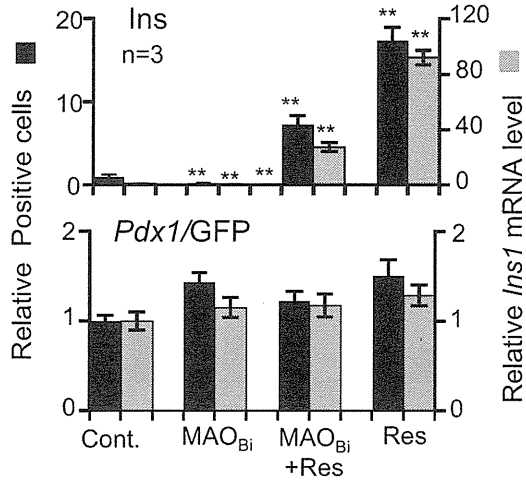
Nature Chemical Biology: doi:10.1038/nchembio.1410

Supplementary Figure 1



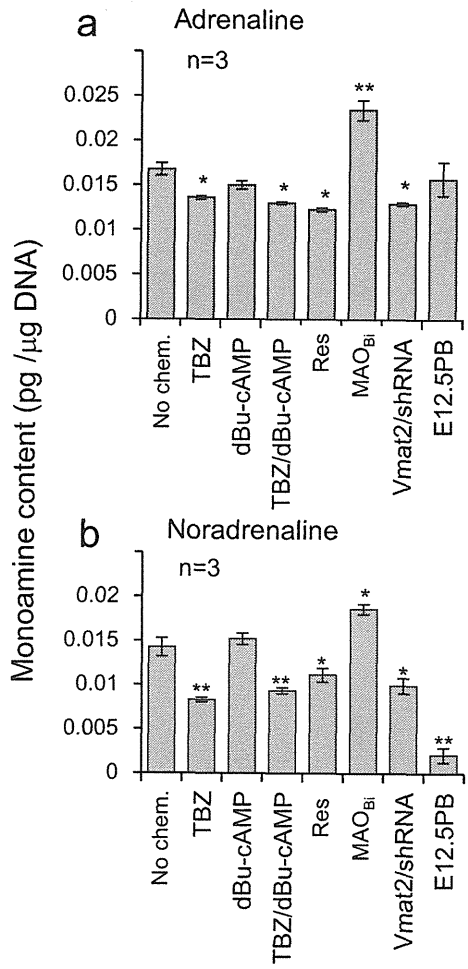
Nature Chemical Biology: doi:10.1038/nchembio.1410

Supplementary Figure 2



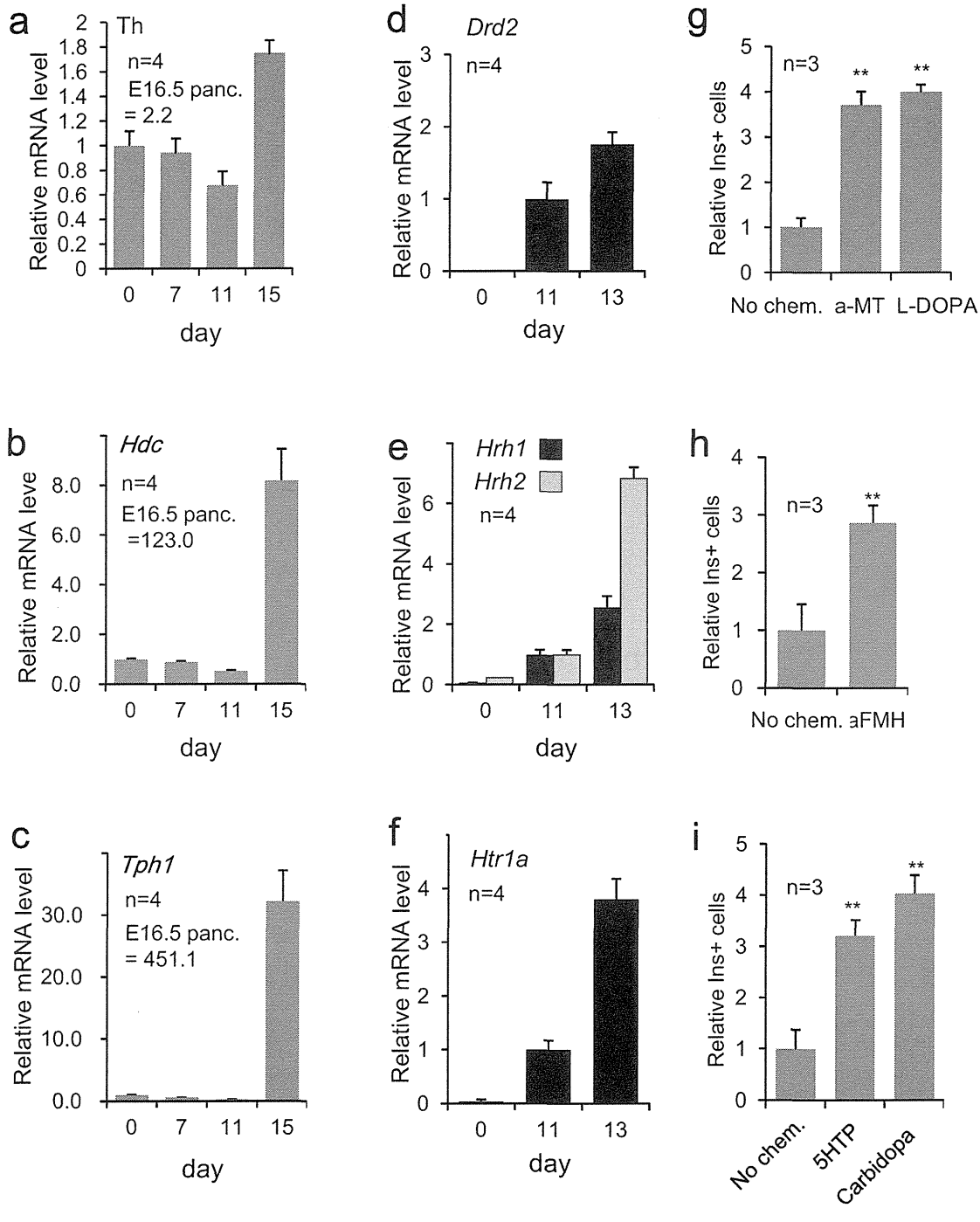
Nature Chemical Biology: doi:10.1038/nchembio.1410

Supplementary Figure 3



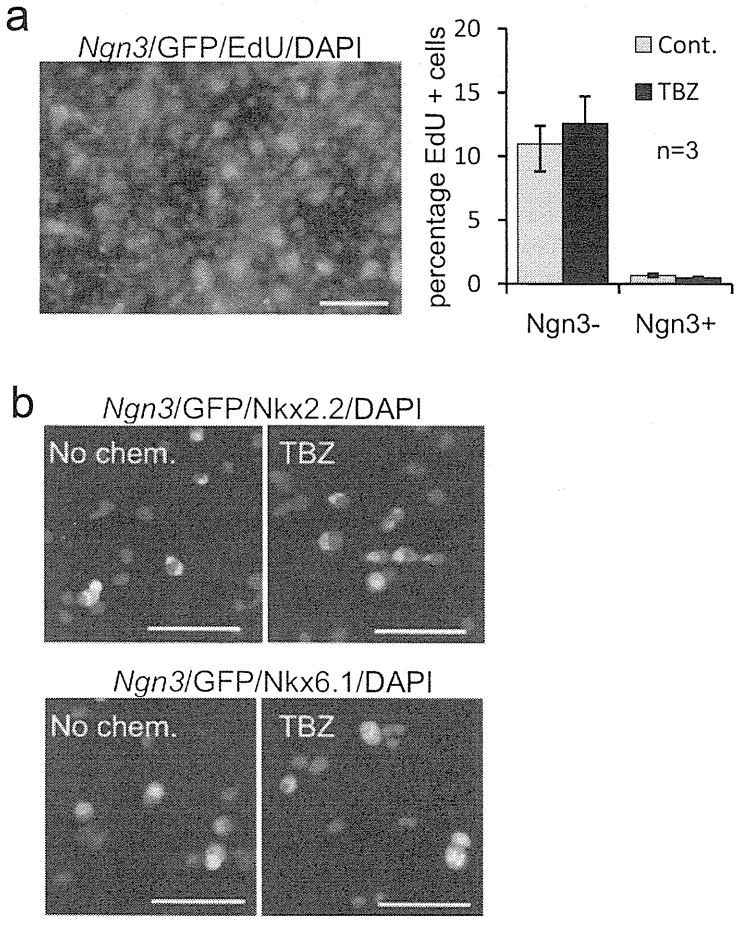
Nature Chemical Biology: doi:10.1038/nchembio.1410

Supplementary Figure 4

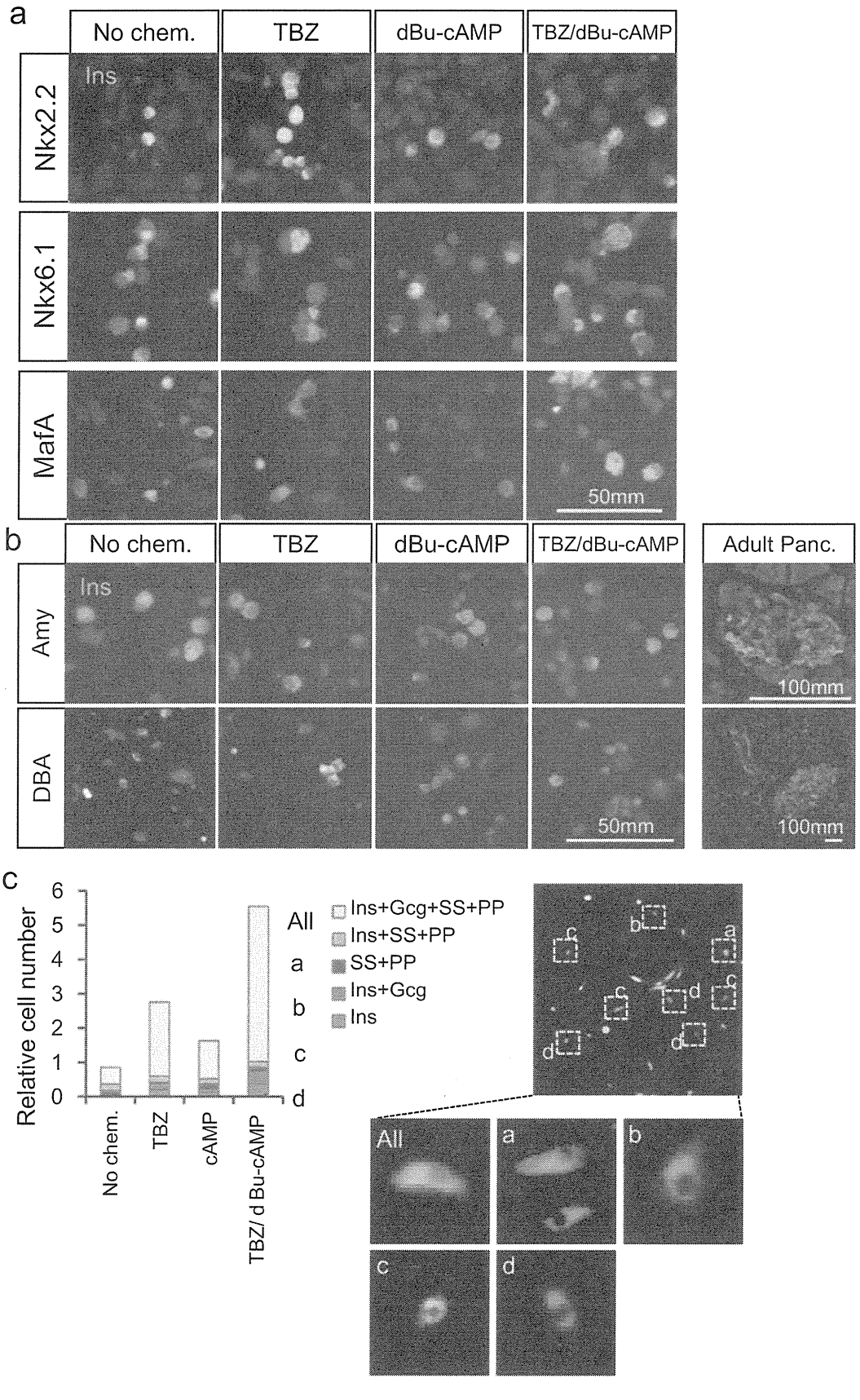


Nature Chemical Biology: doi:10.1038/nchembio.1410

Supplementary Figure 5



Supplementary Figure 6



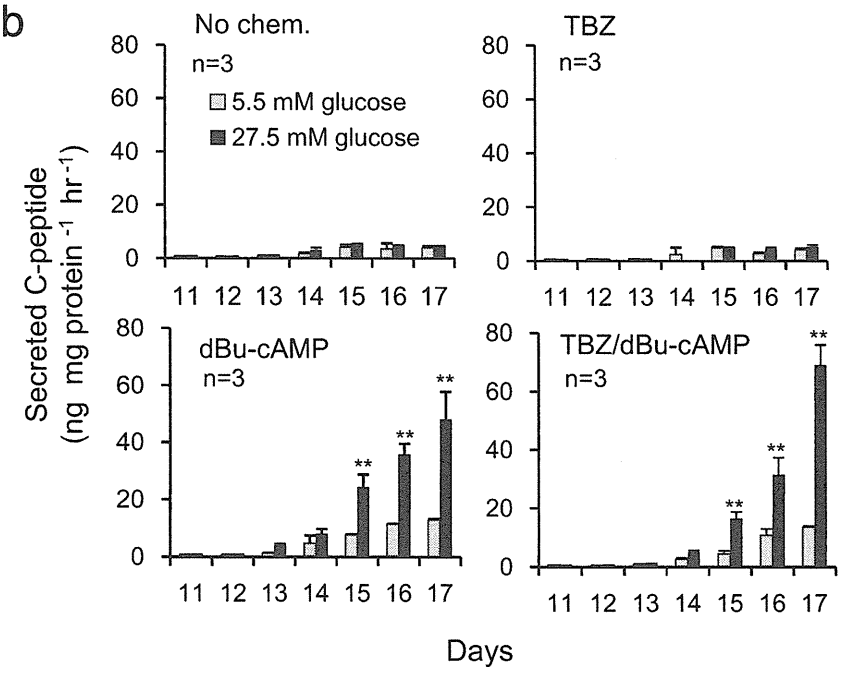
Nature Chemical Biology: doi:10.1038/nchembio.1410

Supplementary Figure 7

a

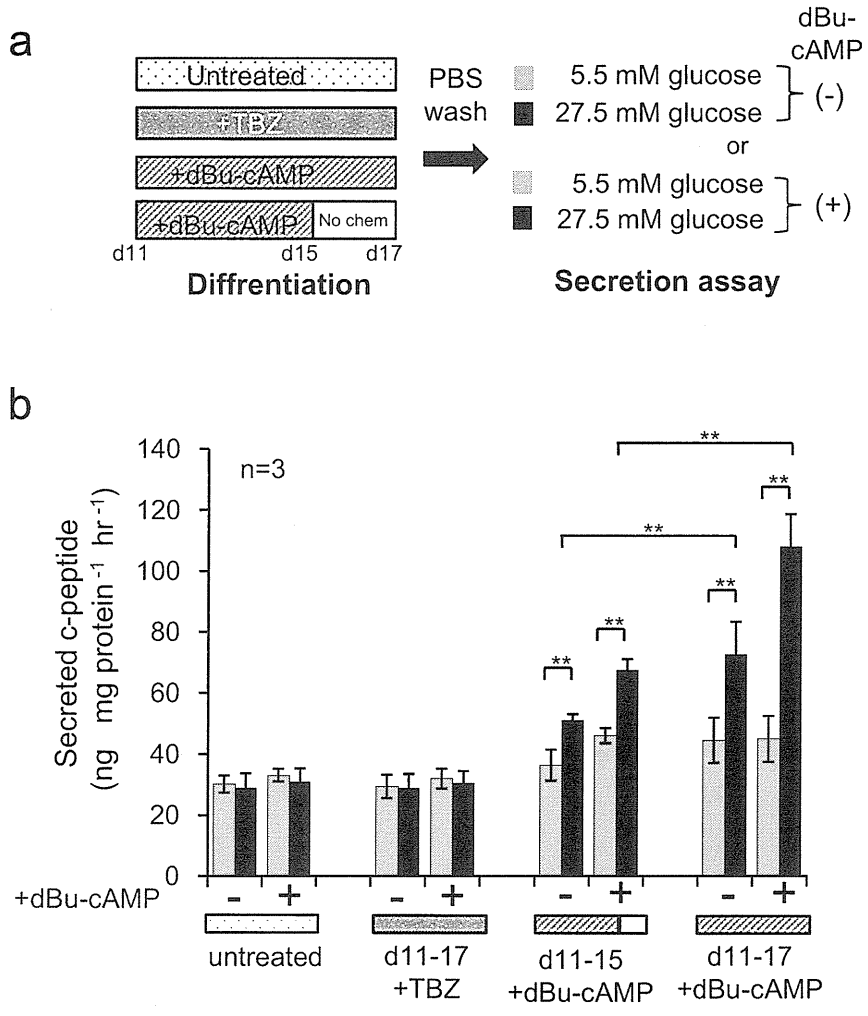
	C-peptide (ng/ well, ~1×10^7 ES cells)	C-peptide (ng/μg DNA)	Folds
No chem.	326±76	8.5±2.0	1
TBZ	1432±152	48.8±5.2	5.7
dBu-cAMP	604±11	22.9±0.4	2.7
TBZ/dBu-cAMP	1670±119	68.9±4.9	8.1
10 islets	16707±829 ng	118.7±5.9 ng	

b



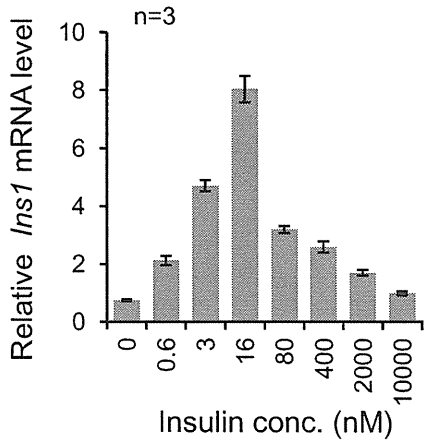
Nature Chemical Biology: doi:10.1038/nchembio.1410

Supplementary Figure 8



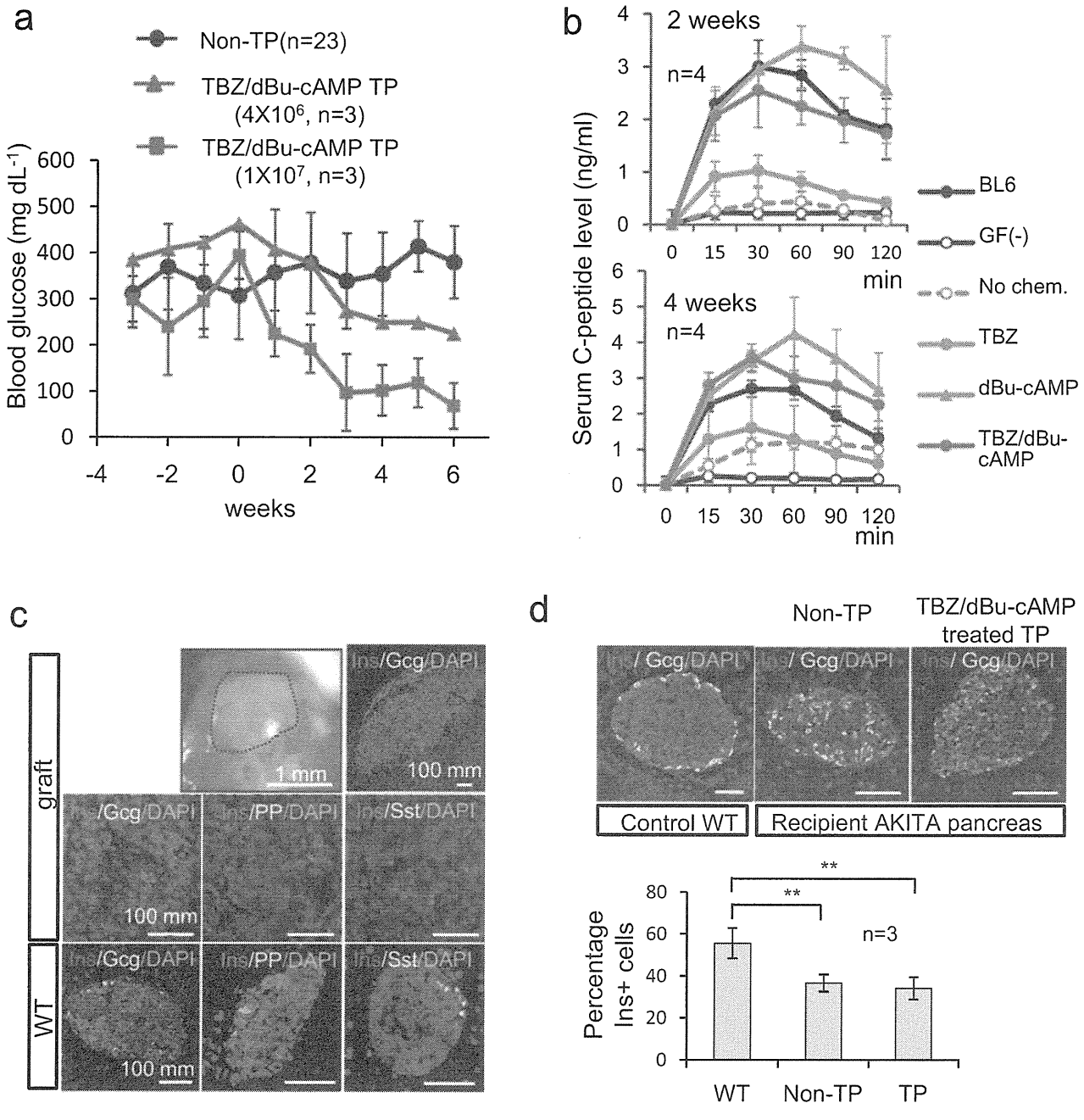
Nature Chemical Biology: doi:10.1038/nchembio.1410

Supplementary Figure 9



Nature Chemical Biology: doi:10.1038/nchembio.1410

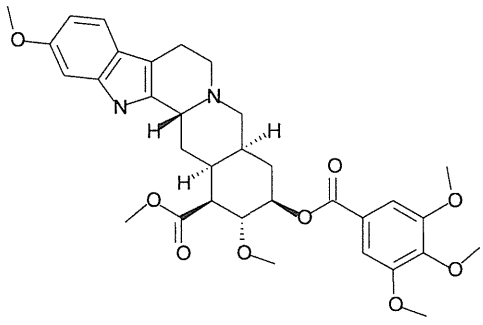
Supplementary Figure 10



Nature Chemical Biology: doi:10.1038/nchembio.1410

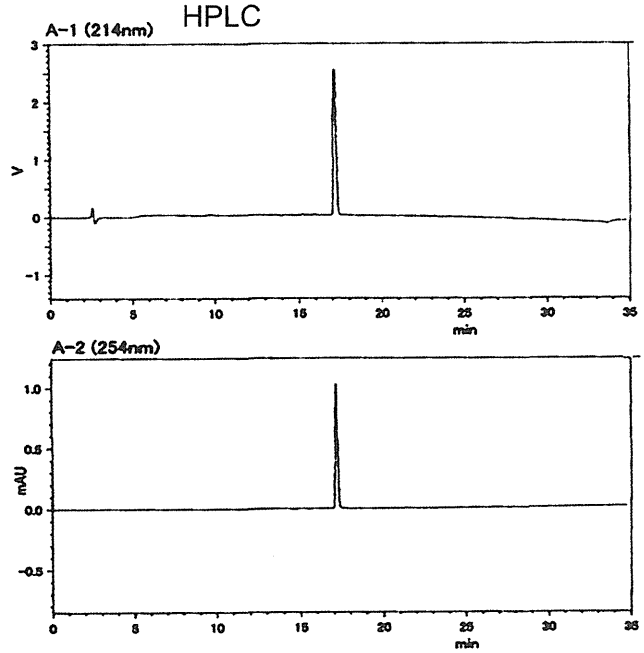
Supplementary Figure 11

Reserpine

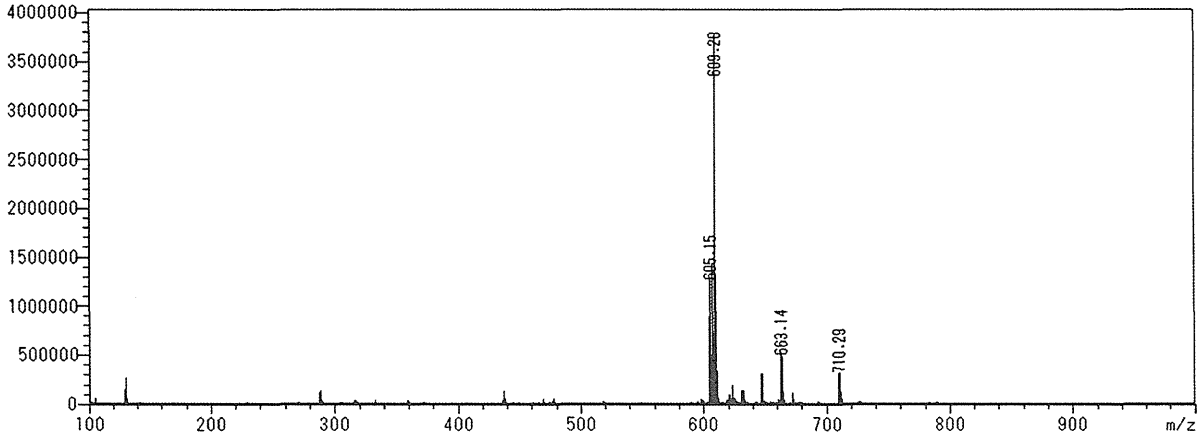


Chemical Formula: $C_{33}H_{40}N_2O_9$
Molecular Weight; 608.68

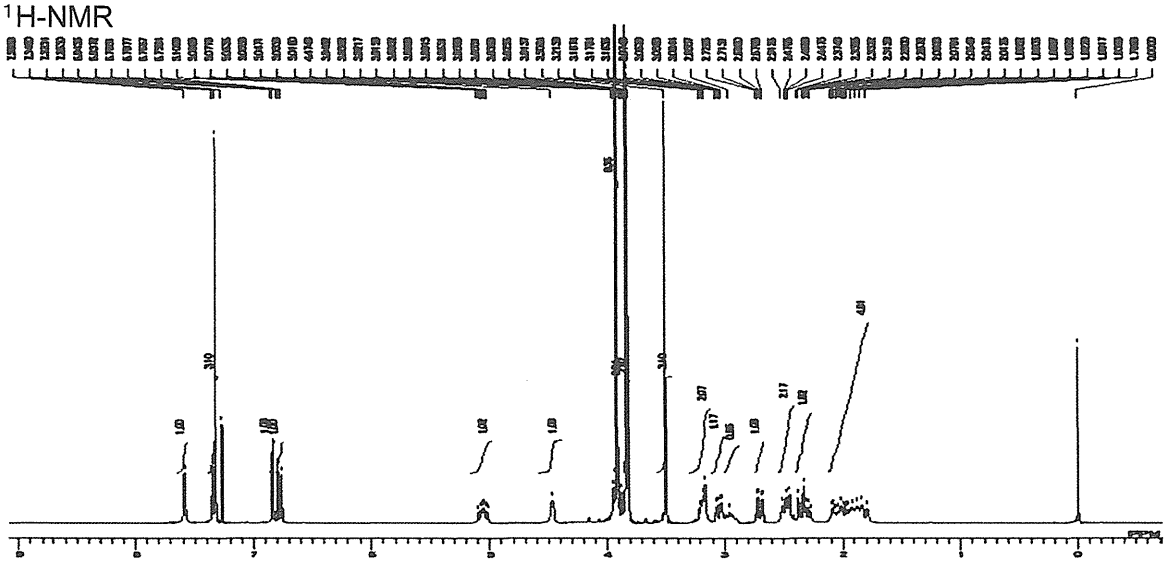
HPLC



LC/MS

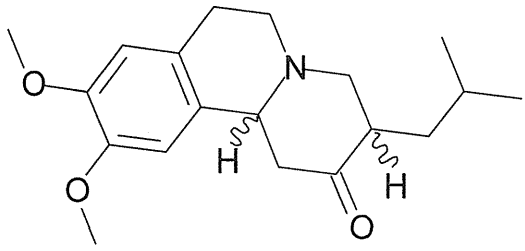


^{1}H-NMR



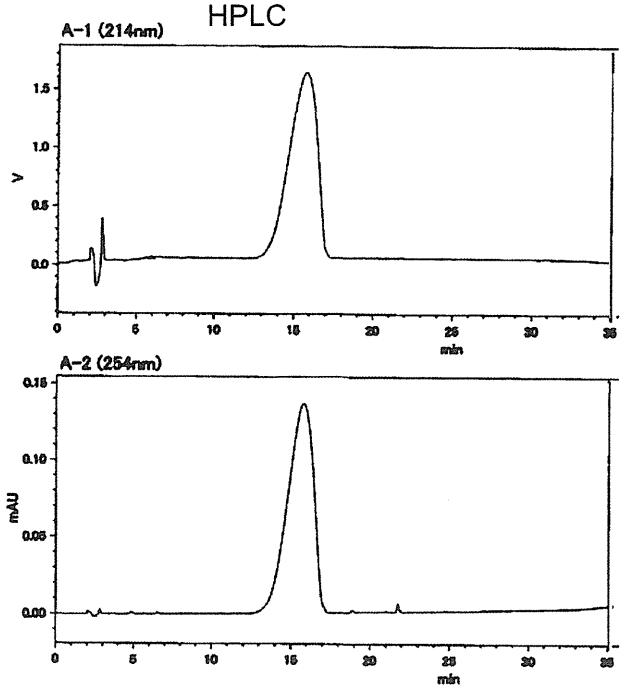
Supplementary Figure 12

(*RR,SS*)-Tetrabenazine

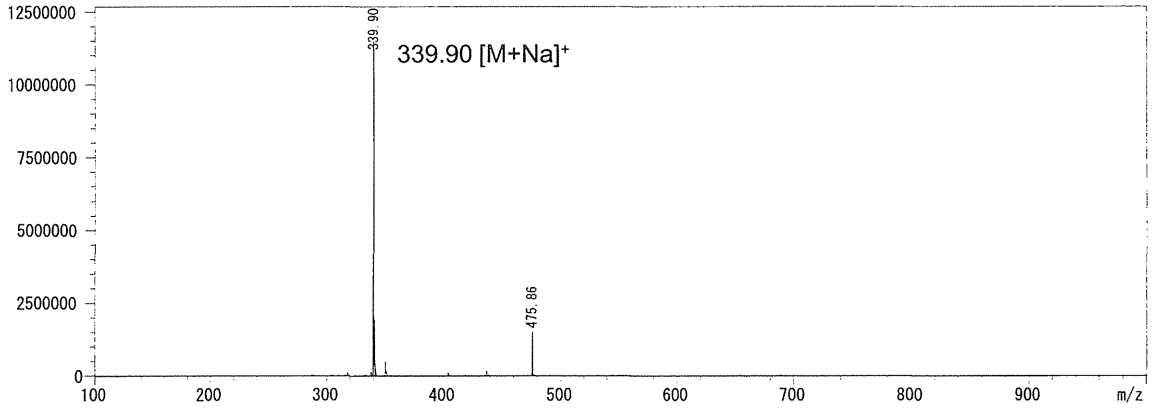


Chemical Formula: $C_{19}H_{27}NO_3$
Molecular Weight; 317.42

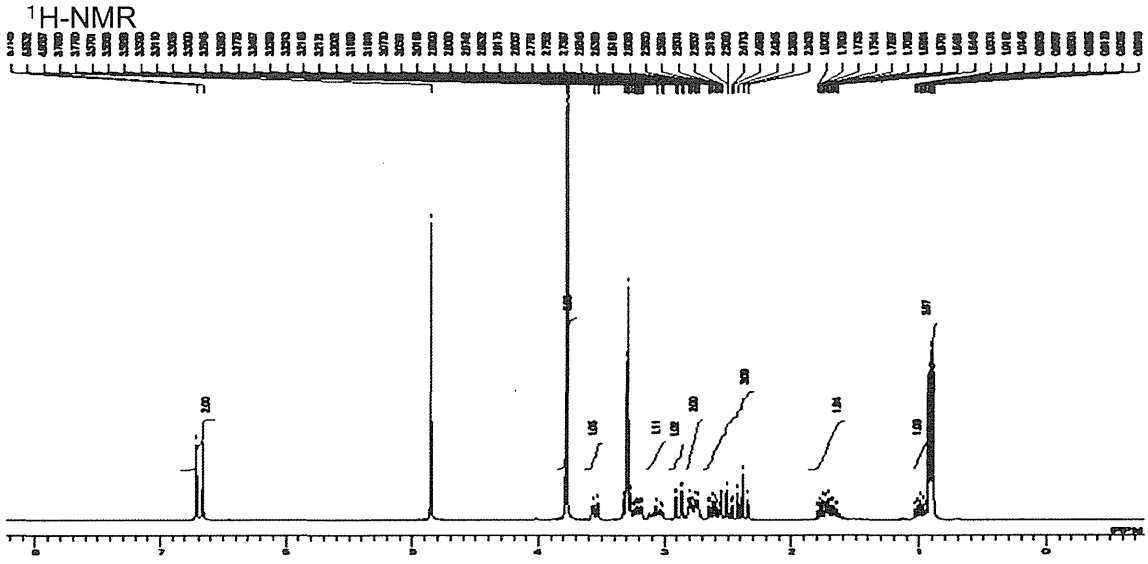
HPLC



LC/MS

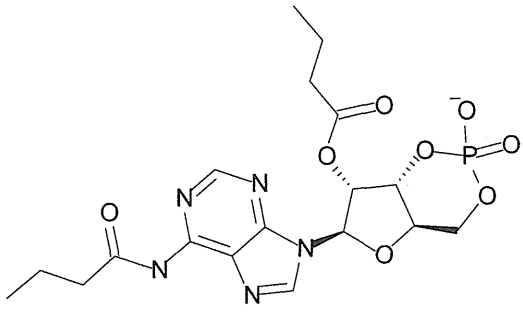


^{1}H-NMR



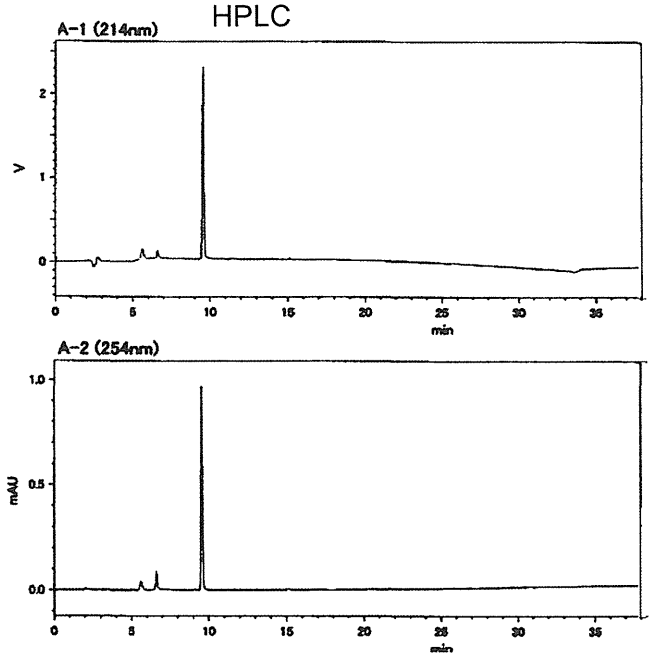
Supplementary Figure 13

N^6,2′-O-Dibutyryladenosine
3′,5′-cyclic monophosphate

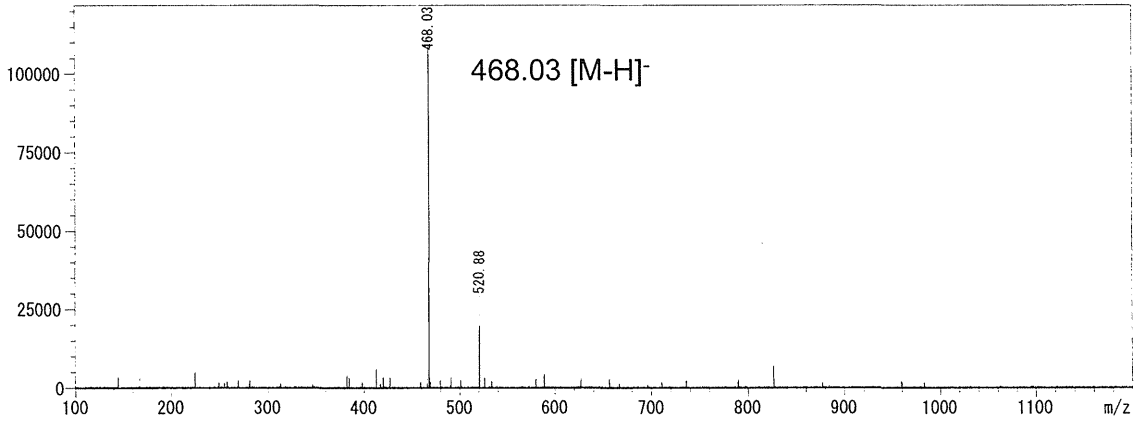


Chemical Formula: $C_{16}H_{24}N_5O_8P$
Molecular Weight; 469.39

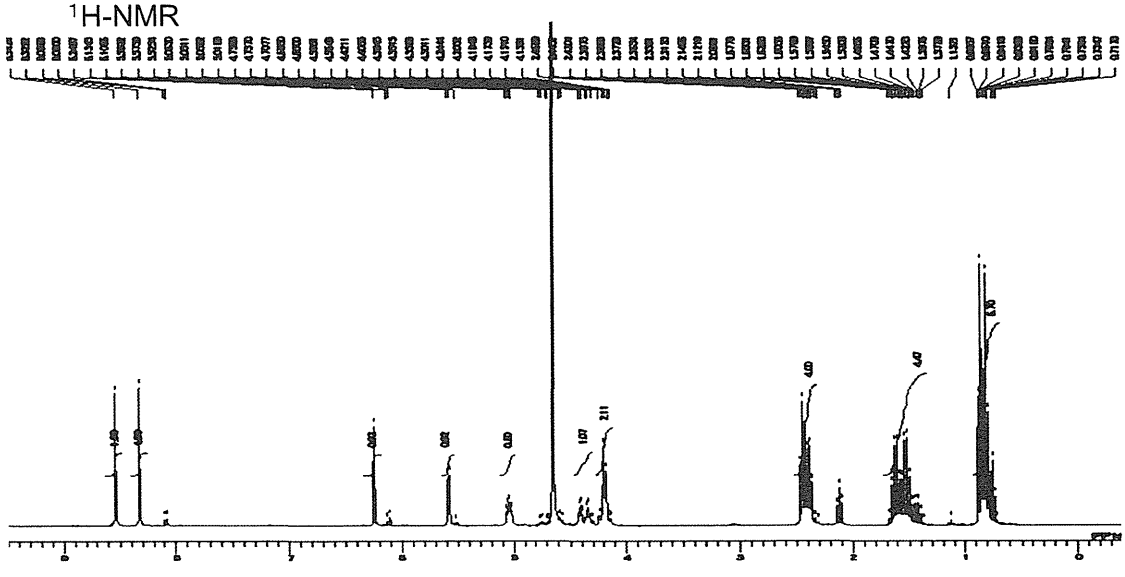
HPLC



LC-MS



^{1}H-NMR



Supplementary Table 1. Small molecule screening data

Category	Parameter	Description
Assay	Type of assay	cell-based (differentiating mouse ES cells)
	Target	Mouse Insulin 1 protein expression level
	Primary measurement	Counting the number of Insulin+ cells detected by immunocytochemistry analysis.
	Key reagents	Monoclonal anti-insulin Antibody K36AC10 (SIGMA)-
	Assay protocol	Described in this manuscript, partially referring to previous report report (doi: 10.1242/jcs.066886)
	Additional comments	
Library	Library size	1120
	Library composition	biologically active known compounds arrayed as single compounds in dimethyl sulfoxide (DMSO)
	Source	Prestwick Chemical Library
	Additional comments	
Screen	Format	96 well plates
	Concentration(s) tested	2 μM compounds and 1% DMSO
	Plate controls	10% pdx1/GFP positive cells should be appeared on d11 before chemical screening.
	Reagent/ compound dispensing system	manual
	Detection instrument and software	ImageXpress Micro scanning system and MetaXpress cellular image analysis software (Molecular Devices, Japan)
	Assay validation/QC	CV value = 0.36±0.0447
	Additional comments	Pdx1/GFP fluorescence detection as a criteria for continuing the assay
Post-HTS analysis	Hit criteria	>2-fold increase in the proportion of Insulin+ cells
	Hit rate	0.1% (10/1120)
	Confirmation of hit purity and structure	HPLC, LC-MS (ESI), and 1H-NMR profiles
	Additional comments	In 2nd screen, dose-dependencies were examined

Nature Chemical Biology: doi:10.1038/nchembio.1410

Supplementary Table 2 PCR primers used to detect mouse gene expressions

Gene	Forward primer	Reverse primer
Abcc8	CCTGCAGCCAGACATAGACA	GCTCTTGGTCTTGCCTGTTC
β-actin	GTGATGGTGGGAATGGGTCA	TTTGATGTCACGCACGATTTCC
Drd2	GAGAAGGCTTTGCAGACCAC	AGGACAGGACCCAGACAATG
Gk	TAT GAA GAC CGC CAA TGT GA	TTT CCG CCA ATG ATC TTT TC
Glut2	ACAGAGCTACAATGCAACGTGG	CAACCAGAATGCCAATGACGAT
Hdc	GATCAGATTTCTACCTGTGG	GTGTACCATCATCCACTTGG
Hrh1	AAGTGGTTTCTCCACGGTTG	CTTTTTGGAGAAGGCGAGTG
Hrh2	AAGCTTCAGGTGTGGAGTGG	ATTGCCACTGCCAGAGATTC
Htr1a	TTGGAACTACTTTGGGTTATGG	ATTGTCAATTTCTTTGGTGAGTG
Ins1	CAGCCCTTAGTGACCAGCTA	ATGCTGGTGCAGCACTGATC
MafA	TTTCCTCGGCAGCGTCCACTTGTA	GGGGGTTCCTCCGGGTTTTCTAAT
Ngn3	CTGCGCATAGCGGACCACAGCTTC	CTTCACAAGAAGTCTGAGAACACCAG
Nkx6.1	TACTTGGCAGGACCAGAGAG	CGCTGGATTTTGTGCTTTTTC
Pdx1	CCAAAACCGTCGCATGAAGTG	CTCTCGTGCCCTCAAGAATTTTC
Snap25	GAGAACCTGGAGCAGGTGAG	GGGGGTGACTGACTCTGTGT
Sox17	GAACAGTTGAGGGGCTACAC	GTTTAGGGTTTCTTAGATGC
Th	GGTATACGCCACGCTGAAGG	TAGCCACAGTACCGTTCCAGA
Tph1	CACAGTTCAGATCCCCTCTACA	GAACGTGGCCTAGGAGTTCA
Vmat2	TGTGAAGTCTGGTGTGTTTAAGG	CATCACATCACAAGGCATCC

Nature Chemical Biology: doi:10.1038/nchembio.1410