SUPPLEMENTAL

Supplemental Figure 1: Glucagon-Venus coexpression in control islets- Illustrative images corresponding to pancreatic sections of CTRL mice were performed using anti-Venus (green, panel C) and anti-glucagon (red, panel B) immunostaining as well as DAPI staining. Merge images represent DAPI, Venus and Glucagon signals (A). White arrows indicate Glucagon positive/Venus negative cells.

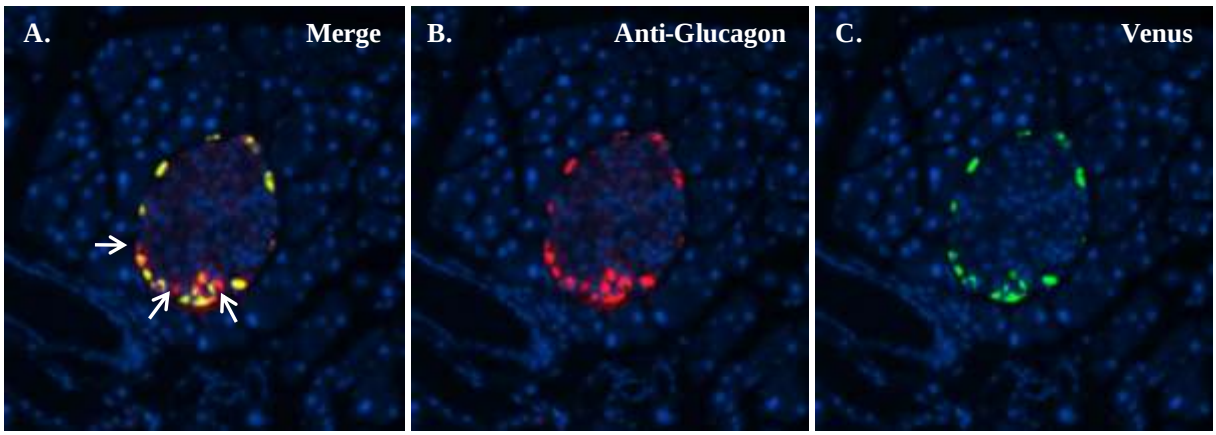
Proportion of Glucagon-positive cells which expressed Venus as well as proportion of Venus-positive cells which express Glucagon was evaluated by manual counting from 5 different islets of 3 different male control mice (D). Immunofluorescence from pancreatic sections of CTRL mice were also performed using anti-Venus (green) and anti-insulin (red) as well as DAPI staining (E) to confirm that no Venus+ cells express insulin.

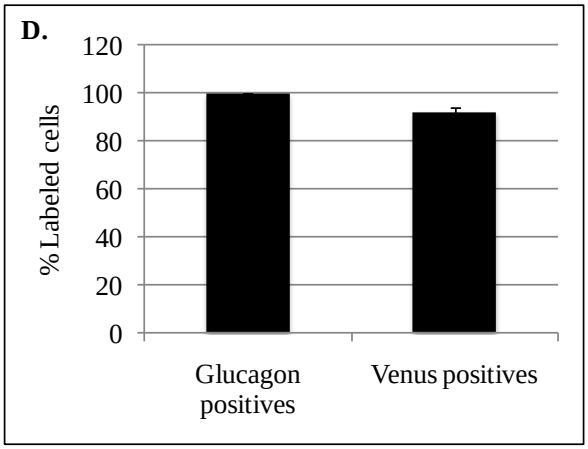
Supplemental Figure 2-4: Facs-profile of CTRL, STZ and STZ+Insulin implant mice- Images represent illustrative FACS-sorting profiles from Venus mice for CTRL (Fig.2), STZ (Fig.3) and STZ+Insulin implant (Fig.4) groups. Facs-sorting settings include usual parameters such as SSC and FSC Area (panel A) of living cells (panel B), fluorescence detection (panel E) and doublet exclusion (panel C and D). Collected Venus-positive α cells thus represent single living fluorescent cells (panel G). Pancreatic β cells were also gated without sorting just to monitor efficiency of the protocol (STZ-induced insulin-producing cell ablation, panel F and H). We collected an average of 13641 +/-1354 Venus+ α cells per mouse for CTRL, 5742+/-549 and 5315+/-806 for STZ and STZ+Insulin implant groups respectively.

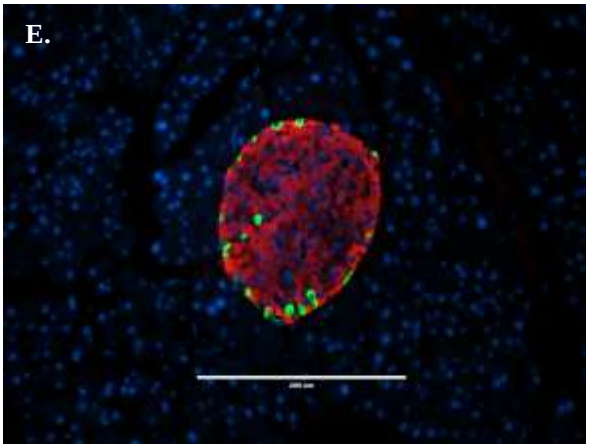
Supplemental Figure 5: Molecular characterization of FACS-sorted Venus+ alpha cells- After islet isolation, pancreatic endocrine cell dissociation and FACS-sorting (BioRad-S3), specific mRNA levels were evaluated from amplified cDNA (obtained from 10ng of total RNA) of 6 Venus Transgenic mice (Male and Female). The measurement of different mRNA levels of target genes were performed by real-time PCR (Light-cycler LC480) and obtained from Venus+ α cell fraction (A) and β cell fraction

(B). Data are expressed relative to Rps9, beta-actin and Tubulin Beta mRNA values using Roche software. Venus- and beta-cell fractions were collected after islets dissociation using typical Facs profile (C). Data were also expressed by specific ratios between glucagon and insulin 1 and 2 mRNA levels as well as Pcsk2 compared to Pcsk1 (D) to specify the high purity of Venus sorted α cells. Contamination of Venus+ α cell fraction by pancreatic β cells is estimated under 1‰.

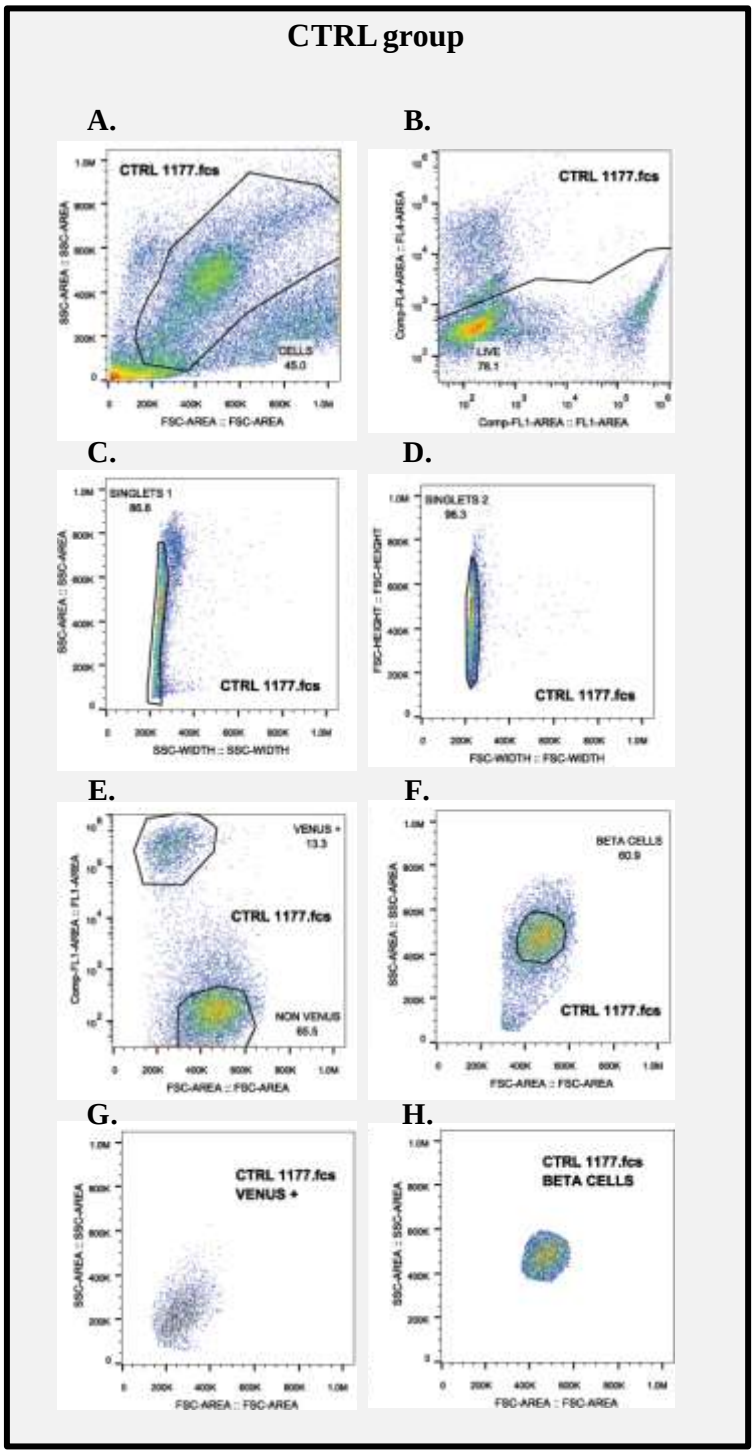
Supplemental Table 1-3: The table indicates the sequence of the different oligonucleotides used for real-time PCR experiments. Each target gene was designed in our laboratory and evaluated for specificity and amplification efficiency. Real-time PCR were performed for each gene in mouse primary Venus+ α cells using Light-cycler 480 SYBR Green I Master Mix and Light-Cycler 480 (Roche Diagnostics). For quantification, a standard curve was systematically generated with four different amounts of total amplified cDNA. Each assay was performed in duplicate and validation of the real time PCR runs was assessed by evaluation of the melting temperature of the products and by the slope and error obtained with the standard curve (slope between -3.1 and -3.3). The analyses were performed using the Light-Cycler software (Roche Diagnostics). PCR products were also analyzed on ethidium bromide-stained 3 % agarose gels for qualitative aspects (single PCR product and correct size) and sequenced for final validation.



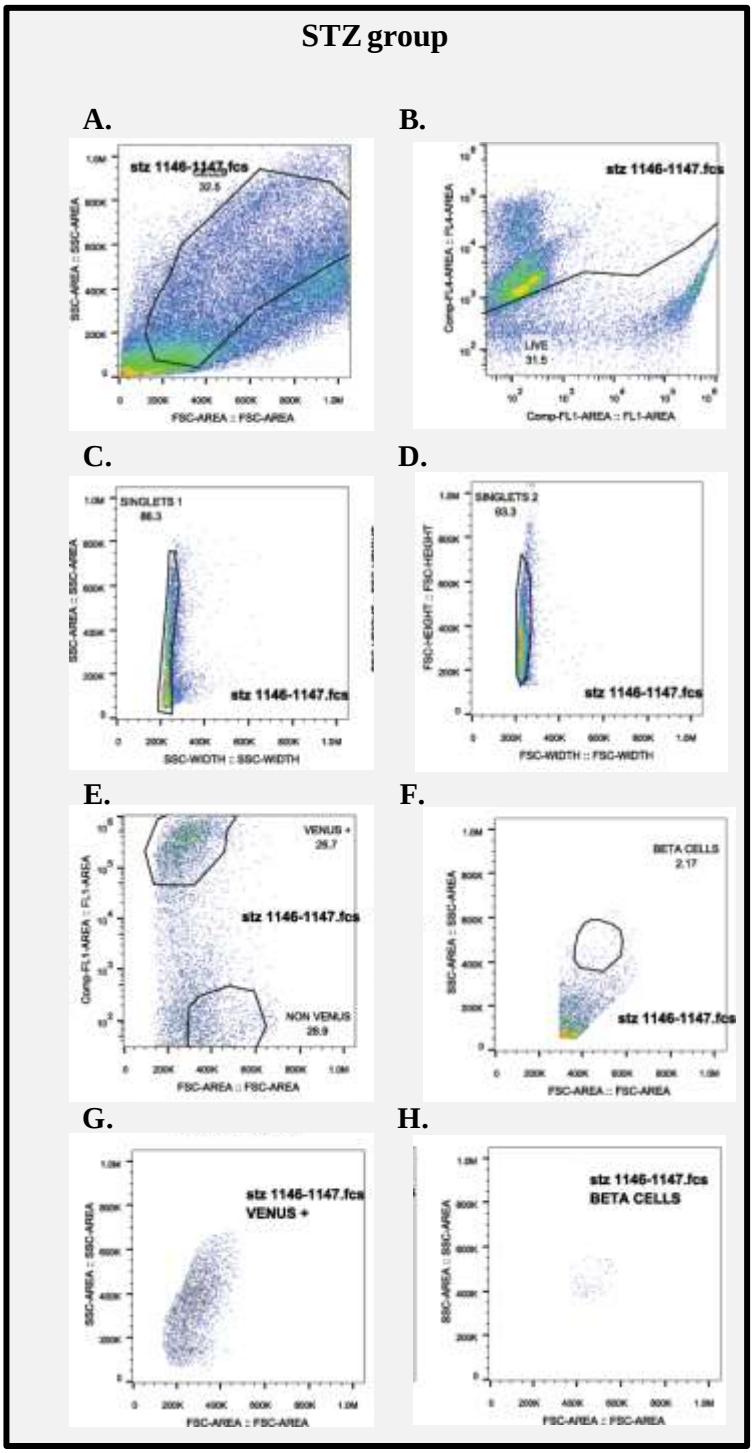




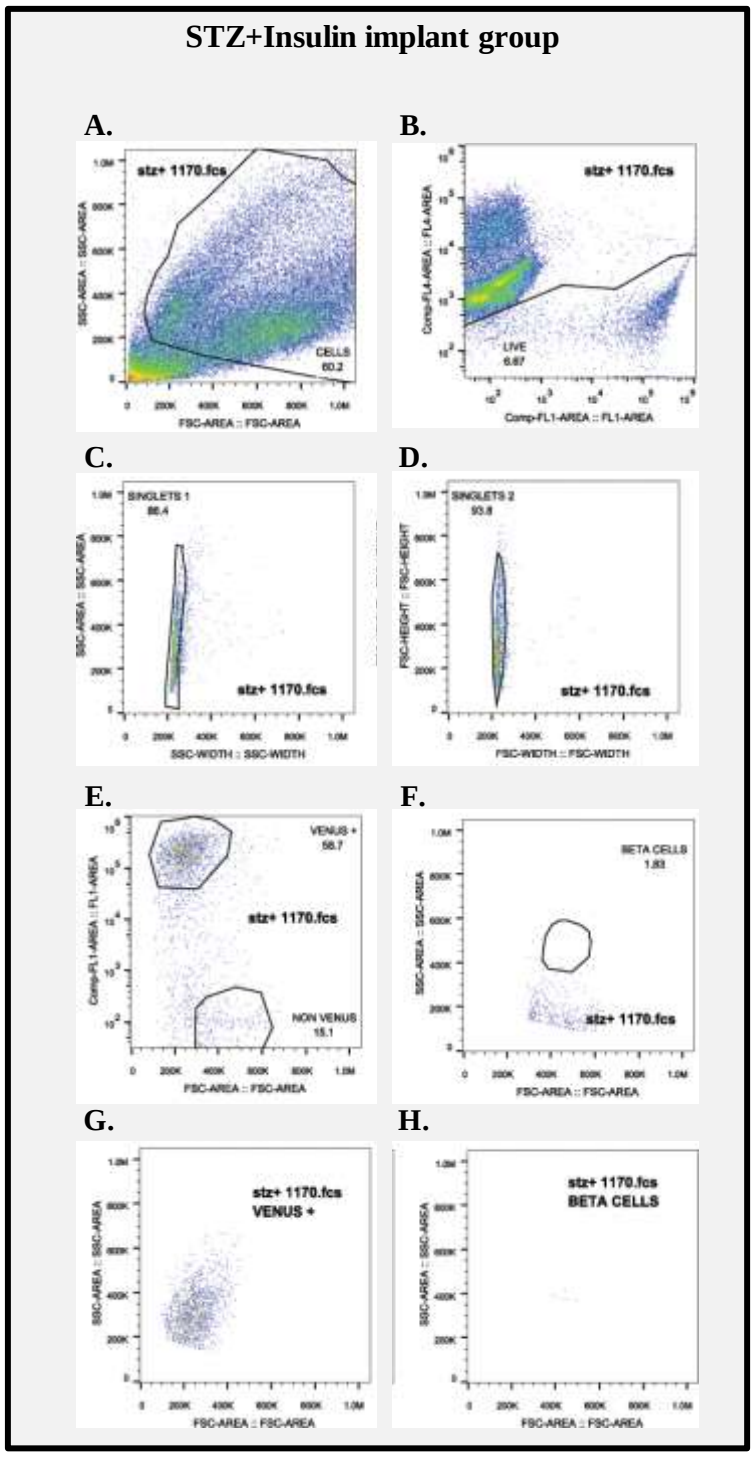
Supplemental Figure 1: Glucagon-Venus coexpression in control islets



Supplemental Figure 2: CTRL islets Facs Profile



Supplemental Figure 3: STZ islets Facs Profile



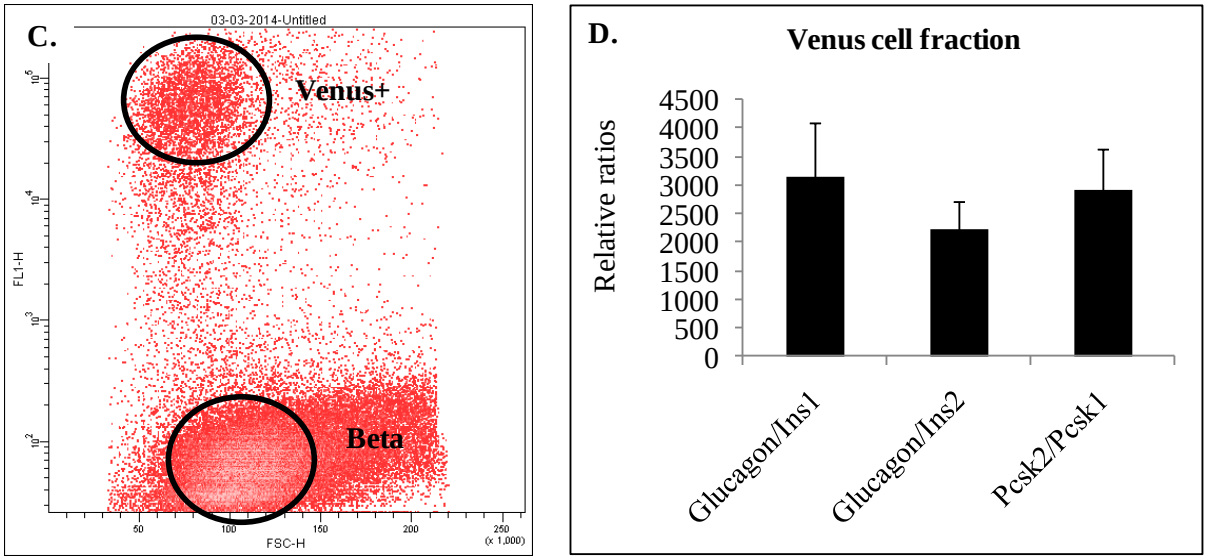
Supplemental Figure 4: STZ+ Insulin Implant islets Facs Profile

A. Venus + cell fraction

Ratio between gene targets and references	Glucagon	Insulin 1	Insulin 2	Pcsk1/3	Pcsk2
Mean	3762000	1280.1	1781.7	16.61	38460
SEM	523998	547.2	869.6	4.69	7738

B. Beta cell fraction

Ratio between gene targets and references	Glucagon	Insulin 1	Insulin 2	Pcsk1/3	Pcsk2
Mean	132.7	1184000	1723333	3482.3	12815
SEM	67.5	45011	35276	263.6	1224



Supplemental Figure 5: Molecular characterization of FACS-sorted Venus+ alpha cells

Name	sequence 5'-3'
Glucagon S/AS	gatcattcccagcttcccag and ctggtaaaggtcccttcagc
PC2 S/AS	gaattgactatctccacccagacc and agtcatctgtgtatcgagggtatgg
Brain4 S/AS	ttcctcaagtgtcccaagcc and gcttcctccagtcagagatc
Pax6 S/AS	ttacccaagagcagattgagg and caggtagatctattttggct
MafB S/AS	accaaggacgaggtgatcc and caggtgatgtttctgctgga
cMaf S/AS	atggcttcagaactggcaat and ccggttccttttttcacttca
Foxa1 S/AS	catgagagcaacgactggaa and ttggcgtaggacatgttgaa
Foxa2 S/AS	cagctactacgcggagcc and gctcattccagcgcccac
Foxa3 S/AS	tgctgggctcagtgaagatg and catgtaggagttgagagggg
HNF1α S/AS	atcccagcagatcctgttcc and cagttgtagacacgcacctc
HNF4α S/AS	tatgaaggagcagctgctgg and tcattgcctaggagcagcac
TCF7L2 S/AS	tcaatgaatcagagacgaatcaaaac and ttggccgcttcttccaaac
NeuroD1 S/AS	tccagggttatgagatcgtcac and gttcctcgtcctgagaactgag
Nkx2.2 S/AS	agcaccgagggcctccaata and gccctgggtctccttgtcat
Isl1 S/AS	tgagggtttctccggatttg and gcatcacgaagtcgttcttg
PC1 S/AS	gggctgaacaacagtatgaaaaag and catgaaggtccagcttgggc

Supplemental Table 1

Name	sequence 5'-3'
GCK S/AS	catccccgaggacgccatgac and ggagaaggtgaagcccagggg
Glut1 S/AS	gtgtcgctgtttgttgtagag and caaagccaaagatggccacga
Sglt1 S/AS	tggtgtacggatcaggtcattg and ttcagatagccacacagggtacag
Sglt2 S/AS	gctggatttgagtggaatgc and cggtcagatacactggcaca
IR S/AS	ggagagaccttggaaattggg and ttggtcttcagggcaatgtcg
IRS1 S/AS	cgctccagtgaggatttaagc and gagtctgggtacccatgagtt
IRS2 S/AS	tctcccaaagtggcctacaac and atcatctgccccccaggttac
PIk3cd S/AS	gcagtgcaccttcatggactc and gtccatgagctggatcatctgc
PIk3r1 S/AS	gagcagcaaccgaaacaaagc and atagccggtggcagtcttgtt
MapK8 S/AS	cacaccacagaaatccctagaag and gaaggtgcttgattccacacag
MapK14 S/AS	gattctggattttgggctggc and aacagctcagccatgatgcag
Ptpn1 S/AS	ctgatggacaagaggaaagaccc and agctccttccactgatcctgc
Pten S/AS	tcttccacaaacagaacaagatgc and ttttctgaggtttcctctggtcc

Supplemental Table 2

Name	sequence 5'-3'
GipR S/AS	gtgtcttgcccctggtatct and tctctccaagatccccactg
IL6R S/AS	cactgtgcgttgcaaacagtg and ccttccaggtatggctgatacc
Gp130 S/AS	gaagccatagtcgtgcctgtg and ggtgaccactgggcaatatgac
GPR40 S/AS	tgagccacaaacggaagctc and gttgagcaccacactccagg
GPR119 S/AS	ccactcggagtctccatattcc and accttgggtgaaacacagcaa
GPR120 S/AS	gggaccaggaaattccgatt and tcatgggatgtgtttttgtgac
Kir6.2 S/AS	gtgtccaagaaaggcaactg and gcacaggaaggacatggtg
Sur1 S/AS	aatgaccagcctcagggcc and cagcaggaacagcggtgtg
Cav1.2 S/AS	cctttccggaagacgactc and gaagagaagtccgtaggcaat
Cav2.1 S/AS	tcagtgaagacaacgtggtga and gccaggacgatgcagttag
Cav2.2 S/AS	atcgccaactgcattgttct and agcaaaagatcccgatgaagt
Cav2.3 S/AS	ccaatgcatcactatggaagg and cgatgatgatgagagggatga
Nav1.3 S/AS	ggagacaggtttcccaagtcg and aacagggagccacggatactc
Nav1.7 S/AS	tctagatttgaaggaatgaggg and actcatagaacttgccagcaaa
Stx1A S/AS	gaaaacgtggaggaggtgaa and ttcgctgtcttcttaatgtcc
Snap25 S/AS	atcagcggacagcatcctc and gtcttcggccatggtactg
Sumo1 S/AS	gacgtagaggaagtccctgcagcc and tttgcctcctggtcagacatggt
Sumo2 S/AS	gcggacccggtgcctctttt and tgcctcattgacaaaccctgcc
Sumo3 S/AS	gggagcgtccccacacccaa and atgctctcgccccgcagtgt
Rps9 S/AS	ctccggaacaaacgtgaggt and tccagcttcatcttgccctc
HPRT S/AS	gattttatcagactgaagagc and tccagttaaagttgagagatc
Actin Beta S/AS	ctaaggccaaccgtgaaaagat and cacagcctggatggctacgt
Tubulin Beta S/AS	gcagtgcggcaaccagat and agtgggatcaatgccatgct
Cyclophilin S/AS	acacgccataatggcactgg and ttgccattcctggacccaaa

Supplemental Table 3