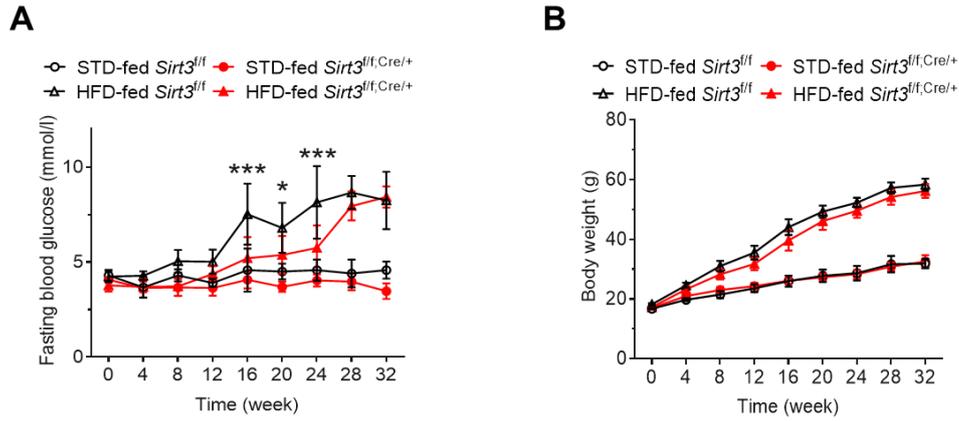


Supplementary Fig. 1 Pancreatic beta cell selective deletion of *Sirt3*. A:

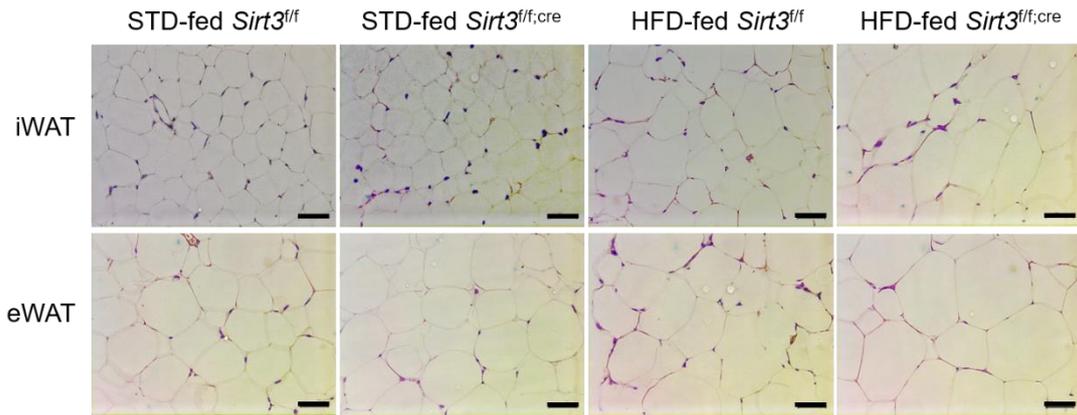
Immunohistochemistry staining of SIRT3 in islets from *Sirt3^{fl/fl;Cre/+}* and *Sirt3^{fl/fl}* mice (scale

bar, 100 μ m). B: Representative western blot data of SIRT3 protein expressions in several

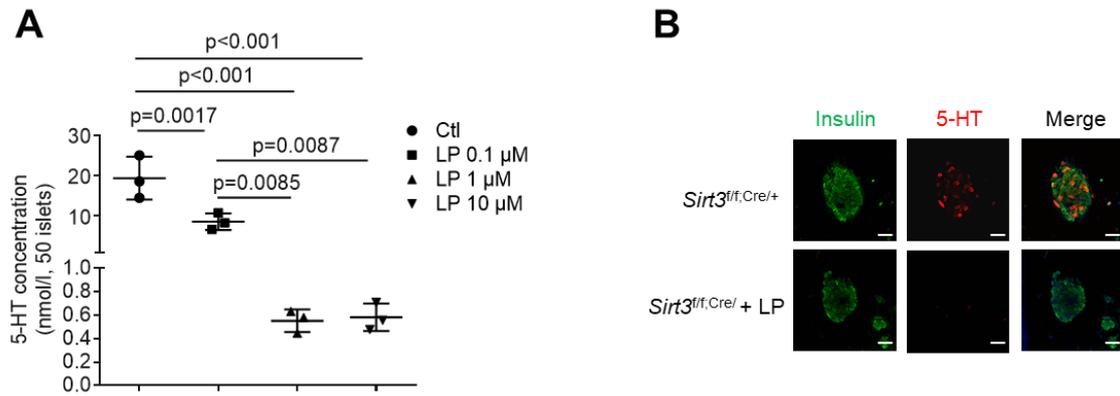
organs and tissues from *Sirt3^{fl/fl;Cre/+}* and *Sirt3^{fl/fl}* mice, n=3.



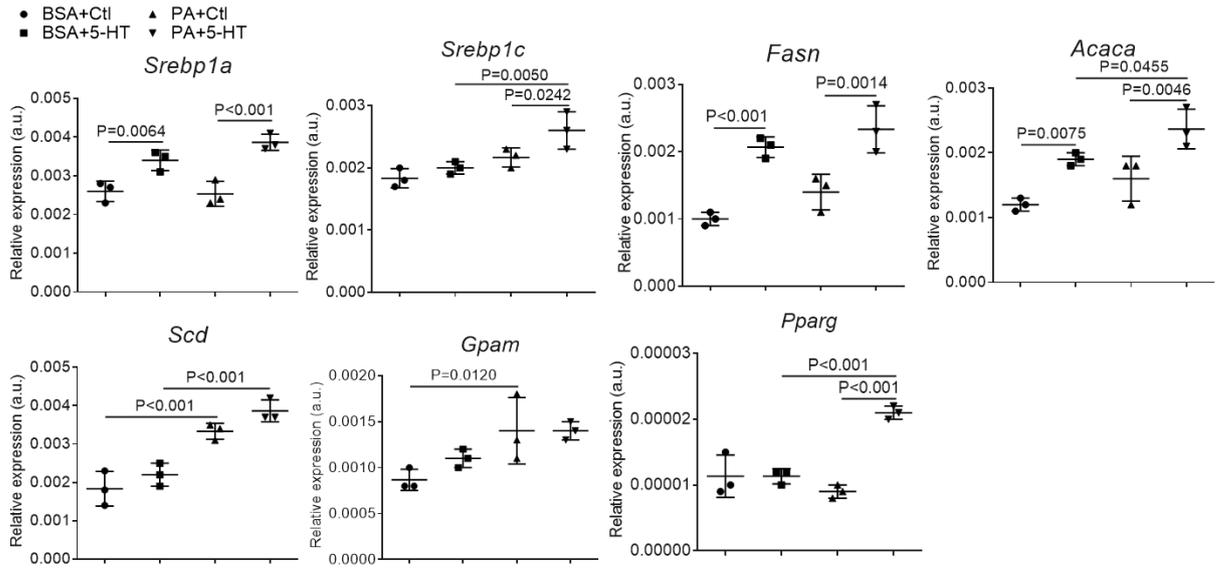
Supplementary Fig. 2 HFD-fed *Sirt3^{fl/fl};Cre/+* mice have lower fasting blood glucose than HFD-fed *Sirt3^{fl/fl}*, despite no significant difference in body weight. *A*: The fasting blood glucose was measured every 4 weeks around 9:00–10:00 am after overnight fasting. *B*: The body weight was monitored every 4 weeks. Data are presented as mean-SD. *HFD-fed *Sirt3^{fl/fl};Cre/+* vs. HFD-fed *Sirt3^{fl/fl}*; * $p < 0.05$, * * $p < 0.01$, * * * $p < 0.001$; HFD, high fat diet; STD, standard diet, n=5.



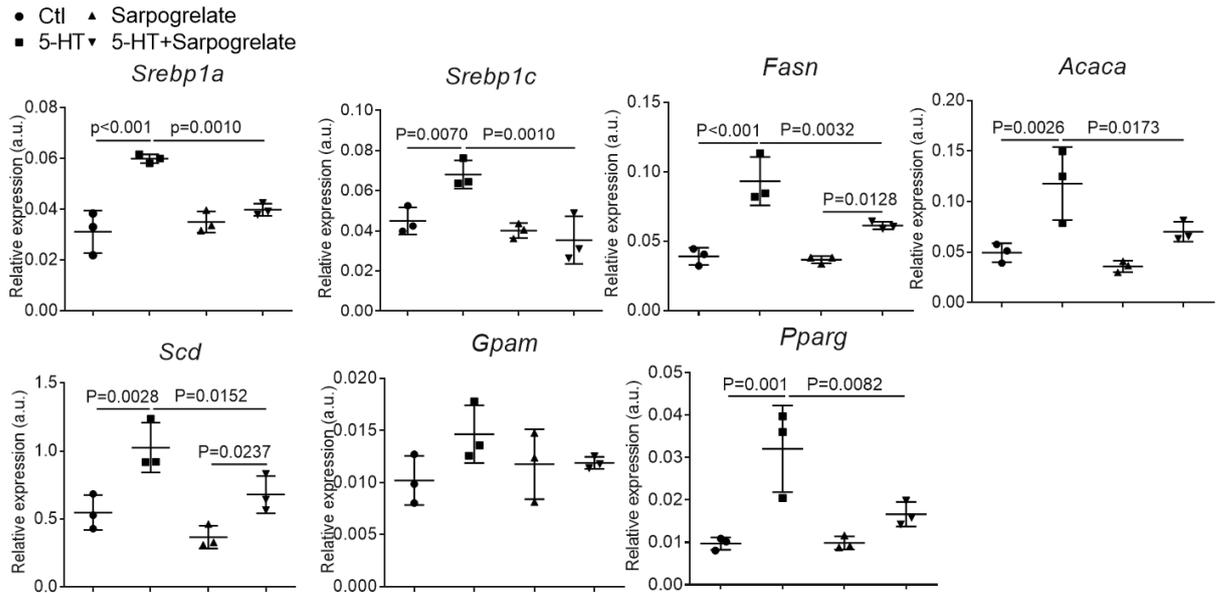
Supplementary Fig. 3 Adipose cell size was increased in HFD-fed *Sirt3^{fl/fl};Cre/+* mice compared to HFD-fed *Sirt3^{fl/fl}*. H&E staining was conducted on iWAT and eWAT from four groups of mice. eWAT: epididymal adipose tissue; high fat diet; iWAT: inguinal adipose tissue; STD, standard diet, n=3.



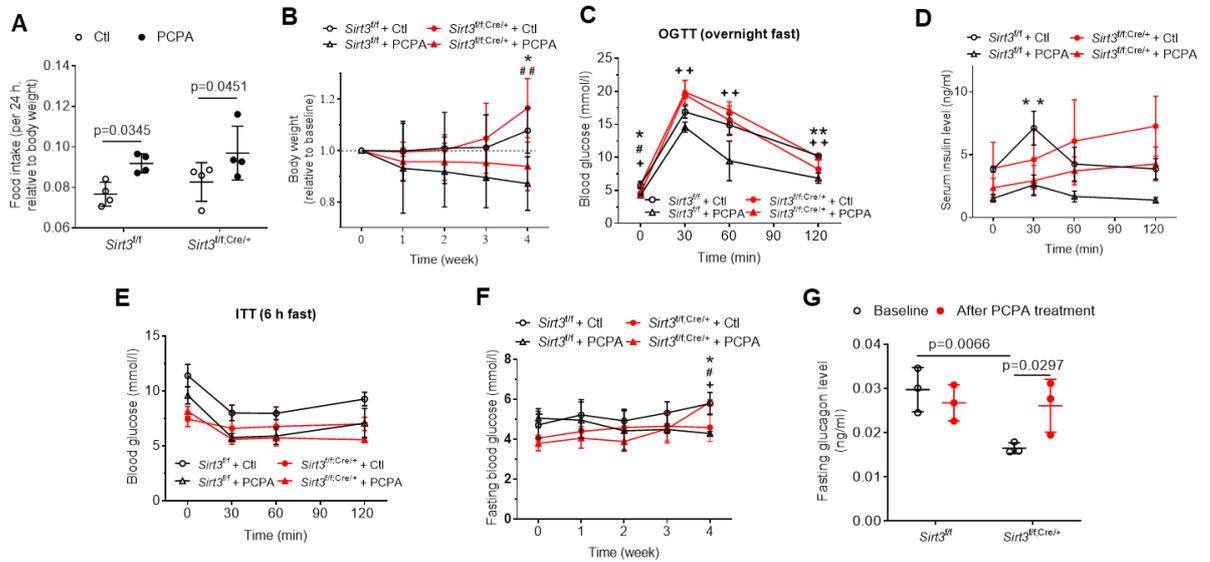
Supplementary Fig. 4 TPH1 inhibitor dose dependently inhibit 5-HT synthesis. *A*: 5-HT concentration in 50 pancreatic islets from *Sirt3^{fl/f; Cre/+}* mice after different concentration of LP treatment for 48 h. *B*: Immunofluorescence staining of insulin and 5-HT in pancreatic islets from *Sirt3^{fl/f; Cre/+}* mice with or without treatment of 10 μ M LP for 48 h. (scale bar, 100 μ m, green: Insulin, red: 5-HT, blue: DAPI). Data are presented as Mean-SD. LP, LP533401, tryptophan hydroxylase 1 (TPH1) inhibitor, n=3.



Supplementary Fig. 5 Lipogenic genes were elevated by 5-HT in HepG2 cells. Data are presented as Mean-SD. *Acaca*, acetyl-CoA carboxylase; BSA, bovine serum albumin, which is used to dissolve PA; *Fasn*, fatty acid synthase; *Gpam*, Glycerol-3-phosphate acyltransferase 1; PA, palmitic acid; 5-HT, 5-hydroxytryptamine; *Pparg*, peroxisome proliferator-activated receptor gamma; *Scd*, steroyl CoA desaturase-1; *Srebp1*, sterol regulatory element-binding protein 1, n=3.



Supplementary Fig. 6 Lipogenic effect of 5-HT was counteracted by HTR2A antagonist in HepG2 cells. Data are presented as Mean-SD. *Acaca*, acetyl-CoA carboxylase; Anti-HTR2A, 5-HT receptor 2A antagonist; BSA, bovine serum albumin, which is used to dissolve PA; *Fasn*, fatty acid synthase; *Gpam*, Glycerol-3-phosphate acyltransferase 1; PA, palmitic acid; 5-HT, 5-hydroxytryptamine; *Pparg*, peroxisome proliferator-activated receptor gamma; *Scd*, steroyl CoA desaturase-1; *Srebp1*, sterol regulatory element-binding protein 1, n=3.



Supplementary Fig. 7 Different role of PCPA in glucose homeostasis of *Sirt3^{fl/fl};Cre/+* and *Sirt3^{fl/fl}* mice. 20 weeks high fat diet fed *Sirt3^{fl/fl};Cre/+* and *Sirt3^{fl/fl}* mice were treated with TPH1 inhibitor, PCPA for four weeks and sacrificed. **A: 24h food intake was measured (n=4). **B:** Body weight relative to baseline was calculated every 4 weeks (n=3). **C:** OGTT was performed before sacrifice (n=3). **D:** During OGTT, blood samples were collected at each time point, serum was extracted from blood samples for insulin level measurement (n=3). **E:** ITT was performed 1 week before OGTT (n=3). **F:** The fasting blood glucose was measured every 4 weeks around 9:00–10:00 am after overnight fasting (n=3). **G:** The fasting glucagon level was measured at baseline and after 4 weeks of treatment (n=3). Data are presented as mean-SD. * *Sirt3^{fl/fl}* + Ctl vs. *Sirt3^{fl/fl}* + PCPA, # *Sirt3^{fl/fl};Cre/+* + Ctl vs. *Sirt3^{fl/fl};Cre/+* + PCPA, + *Sirt3^{fl/fl}* + PCPA vs. *Sirt3^{fl/fl};Cre/+* + PCPA; * p , # p or + p < 0.05, * p , # p or ++ p < 0.01, *** p , ### p or +++ p < 0.001. PCPA, p-chlorophenylalanine.**

Supplementary Table S1 QC and statistics of RNA-Seq

Sample name	Raw reads	Clean reads	raw bases	clean bases	Error rate(%)	Q20(%)	Q30(%)	GC content(%)
STD-fed <i>Sirt3^{flf1}</i>	51393012	48708370	7.7G	7.3G	0.01	97.49	93.54	52.72
STD-fed <i>Sirt3^{flf2}</i>	40496700	38410428	6.1G	5.8G	0.01	97.55	93.65	52.83
STD-fed <i>Sirt3^{flf3}</i>	45253246	42708532	6.8G	6.4G	0.01	97.53	93.58	53.25
STD-fed <i>Sirt3^{flf;Cre/+1}</i>	43710938	41260142	6.6G	6.2G	0.01	97.51	93.56	52.41
STD-fed <i>Sirt3^{flf;Cre/+2}</i>	42707530	40169312	6.4G	6G	0.01	97.67	93.88	53.15
STD-fed <i>Sirt3^{flf;Cre/+3}</i>	45398442	42798032	6.8G	6.4G	0.01	97.61	93.76	52.16
HFD-fed <i>Sirt3^{flf1}</i>	51047030	48150600	7.7G	7.2G	0.01	97.6	93.75	53.43
HFD-fed <i>Sirt3^{flf2}</i>	40672074	38520994	6.1G	5.8G	0.01	97.49	93.47	53.23
HFD-fed <i>Sirt3^{flf3}</i>	43137038	40666922	6.5G	6.1G	0.01	97.5	93.5	52.86
HFD-fed <i>Sirt3^{flf;Cre/+1}</i>	43735732	41027742	6.6G	6.2G	0.01	97.6	93.72	53.09
HFD-fed <i>Sirt3^{flf;Cre/+2}</i>	40388526	37808536	6.1G	5.7G	0.01	97.87	94.29	53.09
HFD-fed <i>Sirt3^{flf;Cre/+3}</i>	47990468	45104154	7.2G	6.8G	0.01	97.48	93.49	52.74

Supplementary Table S2 The ten most upregulated genes in HFD-fed *Sirt3^{fl/f};Cre/+* vs. HFD-fed *Sirt3^{fl/f}*

NO.	Gene name	Gene annotation	Log2 fold change	P-value
1	<i>Tph1</i>	tryptophan hydroxylase 1	9.725	4.62E-51
2	<i>Gbp11</i>	guanylate binding protein 11	8.901	1.58E-28
3	<i>Prok1</i>	prokineticin 1	8.793	1.40E-37
4	<i>Lrrc55</i>	leucine rich repeat containing 55	8.670	3.97E-51
5	<i>Syt10</i>	synaptotagmin X	8.433	1.37E-12
6	<i>Gad2</i>	glutamic acid decarboxylase 2	8.326	9.22E-04
7	<i>Kcnj5</i>	potassium inwardly-rectifying channel, subfamily J, member 5	7.798	8.23E-23
8	<i>Sox11</i>	SRY (sex determining region Y)-box 11	7.287	4.09E-13
9	<i>Popdc3</i>	popeye domain containing 3	7.234	4.74E-04
10	<i>Rph3a</i>	rabphilin 3A	7.033	3.98E-39

Supplementary Table S3 The ten most downregulated genes in HFD-fed *Sirt3^{fl/f};Cre/+* vs. HFD-fed *Sirt3^{fl/f}*

NO.	Gene name	Gene annotation	Log2 fold change	P-value
1	<i>Nrk</i>	Nik related kinase	-5.119	7.87E-05
2	<i>Dlgap2</i>	DLG associated protein 2	-4.049	7.00E-03
3	<i>Rprml</i>	reprimin-like	-3.432	5.81E-07
4	<i>Tmem179</i>	transmembrane protein 179	-3.342	1.21E-02
5	<i>Cplx3</i>	complexin 3	-3.250	4.57E-02
6	<i>B3gat1</i>	beta-1,3-glucuronyltransferase 1 (glucuronosyltransferase P)	-2.645	2.86E-03
7	<i>Lmx1b</i>	LIM homeobox transcription factor 1 beta	-2.609	1.03E-07
8	<i>Greb1l</i>	growth regulation by estrogen in breast cancer-like	-2.553	5.69E-03
9	<i>Sgcz</i>	sarcoglycan zeta	-2.509	2.96E-04
10	<i>Dok7</i>	docking protein 7	-2.401	2.21E-04

Supplementary Table S4 List of mouse primers used for RT-PCR

Gene name	Forward primer (5' to 3')	Reverse primer (5' to 3')
<i>Acaca</i>	CTCCCGATTGATAATTGGGTCTG	TCGACCTTGTTTTACTAGGTGC
<i>Actb</i>	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT
<i>Cd36</i>	GTTATTGGTGCAAGTCCTGGC	GGTGCCTGTTTTAACCCAGT
<i>Cpt1a</i>	GGCATAAACGCAGAGCATTCTG	CAGTGCCATCCTCTGAGTAGC
<i>Fasn</i>	GGAGGTGGTGATAGCCGGTAT	TGGGTAATCCATAGAGCCAG
<i>Gck</i>	CCAAGCACCAAGCGGTATCA	GTCAGTGGGTTGGACTTCTCT
<i>Glut1</i>	TGCAGTTCGGCTATAACT	GTAGCGGTGGTCCATGTTT
<i>Glut2</i>	TCAGAAGACAAGATCACCAGGA	GCTGGTGTGACTGTAAGTGGG
<i>Gpam</i>	ACCAGCGATCCTTCCTCAA	AATGTGCTCAGCCAGGTTTG
<i>G6p</i>	CGACTCGCTATCTCCAAGTGA	GTTGAACCAGTCTCCGACCA
<i>Pepck</i>	ATCTTTGGTGGCCGTAGACCT	CCGAAGTTGTAGCCGAAGAA
<i>Pfk</i>	AACCAGTCTGTGAGGCTACC	GTCAAACCTCTCCTCGTCCA
<i>Pk</i>	TGAAAGCTTTGCAACCTCCC	ATTTCCACCTCCGACTCTGG
<i>Ppara</i>	AAGGGCTTCTTTGGCGAAC	TGACCTTGTTTCATGTTGAAGTTCTTCA
<i>Pparg</i>	GGAAGACCACTCGCATTCTT	GTAATCAGCAACCATTGGGTCA
<i>Tph1</i>	ACGTTCTCTCTTGGCTGAA	AAGGGCTTGACTTTGGCATG
<i>Tph2</i>	GAATGAAGTTGGTGGGCTGG	TTCCTCCGTCCAAATGCTCT
<i>Tph1 promoter</i>	ACGTGCTACTGACATTC CCT	TGAGCTTCATCCTGGAAAAGGT
<i>Tph2 promoter</i>	ACGGCTGTTTTGTTGCAAGA	AGCTTGGGGTT ATCTGTGCT
<i>Scd</i>	CTGTACGGGATCATACTGGTTC	GCCGTGCCTTGTAAAGTTCTG
<i>Srebp1c</i>	CGGAAGCTGTCTGGGGTAG	GTTGTTGATGAGCTGGAGCA
<i>18S rRNA</i>	GCAATTATCCCATGAACG	GGCCTCACTAAACCATCCAA

Supplementary Table S5 List of human primers used for RT-PCR

Gene name	Forward primer (5' to 3')	Reverse primer (5' to 3')
<i>Acaca</i>	TCACACCTGAAGACCTTAAAGCC	AGCCACACTGCTTGACTG
<i>Actb</i>	TGGCACCACACCTTCTACAA	CCAGAGGCGTACAGGGATAG
<i>Fasn</i>	CCGAGACACTCGTGGGCTA	CTTCAGCAGGACATTGATGCC
<i>Gpam</i>	GACAGTGACTTTGGGGAGGA	AACAAAGATGGCAGCAGAGC
<i>Pparg</i>	ACAGATCCAGTGGTTGCAGA	ATGAGGGAGTTGGAAGGCTC
<i>Scd</i>	CCCCTACGGCTCTTTCTGAT	CTGAAAACCTGTGGTGGGCA
<i>Srebp1a</i>	TCAGCGAGGCGGCTTTGGAGCAG	CATGTCTTCGATGTCGGTCAG
<i>Srebp1c</i>	CGGAGCCATGGATTGCA	GGAAGTCACTGTCTTGGTTGTTGA

Supplementary Table S6 List of mouse primers for ChIP

Input	Gene name	Forward primer (5' to 3')	Reverse primer (5' to 3')
H4K16Ac	<i>Tph1</i> <i>promoter</i>	ACGTGCTACTGACATTC CCT	TGAGCTTCATCCTGGAAAGGT
H4K16Ac	<i>Tph2</i> <i>promoter</i>	ACGGCTGTTTTGTTGCAAGA	AGCTTGGGGTT ATCTGTGCT
CREB	<i>Tph1</i> <i>promoter</i>	CCCCTCTCTCTGTTCACA	CTCTCAAGTAGCTGAAACCACA
CREB	<i>Tph2</i> <i>promoter</i>	ACGTCATGCCAGCAGAAAAG	CAACAAAACAGCCGTCGTCT
STAT5	<i>Tph1</i> <i>promoter</i>	ACGTGCTACTGACATTC CCT	TGAGCTTCATCCTGGAAAGGT
STAT5	<i>Tph2</i> <i>promoter</i>	ATTGCTGGGTCTGATCAGGT	GTGTGTGTGTGACGGCTTT