

Supplementary information

Generation of human appetite-regulating neurons and tanycytes from stem cells

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Supplementary information

Gene symbol	Gene name	Forward primer sequence	Reverse primer sequence
<i>ACTB</i>	Actin beta	ATGTGGCCGAGGA CTTTGATTG	ATGGCAAGGGACTTC CTGTAAC
<i>AGRP</i>	Agouti related neuropeptide	TCCCTGTCCTGTG GAAATTTGT	TCTCCAATTTGGGGTG AGGTTT
<i>AQP4</i>	Aquaporin 4	TTGGACCTGCAGT TATCATGGG	CTATGATGGGCCCAAC CCAATA
<i>ARX</i>	Aristaless related homeobox	CCTGAGCACTTTC CTCGGAGCG	TGGAAAAGAGCCTGC CGAATGCC
<i>BRN2</i>	POU3F2, POU class 3 homeobox 2	ATGCGCGGCTCCT TTAACCGG	TTAGACGCTGCGGTC GCCATG
<i>BSX</i>	Brain specific homeobox	GAGAAGAGGTTTCG AGATCCAGC	GTTCTGGAACACGT TTTCACC
<i>CART</i>	CART prepropeptide	GCGTCCATTCTCC TCCATACAT	GTTGCTTAAGCCAAAC TCCAGG
<i>CRH</i>	Corticotropin releasing hormone	CTGTACCATAGCG CTGCTCTTA	TTGTGCATGCTAAGTA AGGGGT
<i>CITED1</i>	Cbp/p300 interacting transactivator with Glu/Asp rich carboxy-terminal domain 1	AGTGGATGAGGAA GTGCTGATG	AAGTCAAACCTCATTCT GCCCCA
<i>COL1A1</i>	Collagen type I alpha 1 chain	CTTATGAAACCCC AATGCTGCC	GGGAGACAGATTTGG GAAGGAG
<i>CRYM</i>	Crystallin mu	ACAGAGCCCATT TGTTTGGTG	CATCCAGTTCTCTCCA GTCAGG
<i>DBX1</i>	Developing brain homeobox 1	GAAGTTTGGAGTG AACGCCATC	GACCCTTCGAAGTAG GGAAAGG
<i>DIO2</i>	Iodothyronine Deiodinase 2	GTTATAAGGCAAC CCCCGGTAT	AACCTCAGCTAATGGG ACCAAG
<i>DLX2</i>	Distal-less homeobox 2	ACCAGACCTCGG GATCCGCC	CTGCGGGGTCTGAGT GGGGT
<i>EN1</i>	Engrailed homeobox 1	CGTGGCTTACTCC CCATTTA	TCTCGCTGTCTCTCCC TCTC
<i>FEZF1</i>	FEZ family zinc finger 1	GGTACATTCCACA TTCGTGAGC	TCACGTGCAATAATCA AAACCA
<i>FGF10</i>	Fibroblast growth factor 10	GTTGCTGTTCTTG GTGTCTTCC	TGACACCATGTCCTGA CCAAG
<i>FOXA1</i>	Forkhead box A1	GGGCAGGGTGGC TCCAGGAT	TGCTGACCGGGACGG AGGAG
<i>FOXA2</i>	Forkhead box A2	CCGTTCTCCATCA ACAACCT	GGGGTAGTGCATCAC CTGTT
<i>FOXB1</i>	Forkhead box B1	GTGGTCGGACTTA AGCACCTT	GTGGTCGGACTTAAG CACCTT
<i>FOXD1</i>	Forkhead box D1	CCTGTCCAGTGTC GAGAACTTT	AACCACCAAGACGAG AAAAGGA
<i>FOXG1</i>	Forkhead box G1	TCAACGGCATCTA CGAGTTCAT	AAGCACTTGTTGAGG GACAGAT
<i>GAPDH</i>	Glyceraldehyde-3- phosphate dehydrogenase	TTGAGGTCAATGA AGGGGTC	GAAGGTGAAGGTCGG AGTCA

<i>GBX2</i>	Gastrulation brain homeobox 2	GTTCCCGCCGTCG CTGATGAT	GCCGGTGTAGACGAA ATGGCCG
<i>GHRH</i>	Growth hormone releasing hormone	AGGAACTCCCAGG GATGAAGAT	CAGTTGCATTTTGGCT ACAGGT
<i>GRH</i>	Growth hormone receptor	ACTAGCAATGGTG GTACAGTGG	TCAGTAAAGTCCAGTT GAGGGC
<i>GPR149</i>	G protein-coupled receptor 149	GACTGGGAGTGG TGTAGGAGTA	GGCATAACCGGAACG CTGAC
<i>HCRT</i>	Hypocretin neuropeptide predursor	CCTCAAGGTTCTT GGCTTTTTG	GGAAGGAAGGTTTCT GGTGTCT
<i>ISL1</i>	ISL LIM homeobox 1	AAGCGCAGGAAG AGAGACTG	CCAAGAGACCCAGGA TTTCA
<i>KISS1</i>	KiSS-1 metastasis suppressor	TGAACTTCAGACC CCAAAGGAG	TCTTTTATTGCCTCGG GTTGGA
<i>LEPR</i>	Leprin receptor	TACTGTTACGGTT CTGGCCATC	TGCTCATAGGCCATGA AAAGGT
<i>LHX2</i>	LIM homeobox 2	GGGCGACCACTTC GGCATGAA	CGTCGGCATGGTTGA AGTGTGC
<i>LHX6</i>	LIM Homeobox 6	AGGCAAGAACATC TGCTCCAG	GCCAGATGAGGTTGT TGACCTT
<i>LHX8</i>	LIM Homeobox 6	AGGCAAGAACATC TGCTCCAG	GCCAGATGAGGTTGT TGACCTT
<i>LHX9</i>	LIM Homeobox 9	TGCCTGAAGTGCT GTGAATGTA	TTGCAGTAAATGCTAC CGTCCT
<i>MASH1</i>	ASCL1, achaete-scute family bHLH transcription factor 1	CTAAAGATGCAGG TTGTGCG	GGAGCTTCTCGACTT CACCA
<i>MC4R</i>	Melanocortin 4 receptor	CTTTTTTCATCTGCA GCTTGGCT	TGTGAAACTCTGTGCA TCCGTA
<i>MCH</i>	Melanin concentrating hormone	TACATTCAGGTTG GGGAAAGGC	CCAGGGAAGGAGCAA TAACTGA
<i>MPZ</i>	Myelin protein zero	CTCAGGTCACGCT GTATGTCTT	GAACCACGTAGAAAA GCAGCAG
<i>NCAM1</i>	Neural cell adhesion molecule 1	GTCAGAGGCCAC CGTCAACGTG	CTTCCCCCTCCCGGA ACTCCTG
<i>NGN3</i>	Neurogenin 3	CTGAACTTGGCGA CCAGAAGC	TTGAGGCGTCATCCTT TCTACC
<i>NHLH2</i>	Nescient helix-loop-helix 2	ACCCACTGGAGAC TTTGAGTTC	GCATACTCTGAACTTC TGCCCT
<i>NKX2.1</i>	NK2 homeobox 1	AGGGCGGGGCAC AGATTGGA	GCTGGCAGAGTGTGC CCAGA
<i>NPY</i>	Neuropeptide Y	GAAAATGTTCCCA GAACTCGGC	TAGGAAAAGGCCAGA GAGCAAG
<i>NR5A1</i>	Nuclear receptor subfamily 5 group A member 1	GGAACAAGTTTGG GCCGATGTA	GTGCCTTCTTCTGCTG TTTCAG
<i>NROB1</i>	Nuclear receptor subfamily 0 group B member 1	GCCATCAAGTGCT TTCTTTCCA	TAGGCGTACTCCTTGG TACTGA
<i>NTS</i>	Homo sapiens neurotensin	GCTCAAAGTACTA CAGCAAAGCC	GGCGCTATTACTTTGT TTTGGGT
<i>ONECUT2</i>	One cut homeobox 2	AAGGGGTAGAGCT GGTGTATCT	TGTTGTTTCAGGGGT GACTTGA
<i>OTP</i>	Orthopedia homeobox	TAGAAGGGAAGGC TTCTCAGGA	GTCAGATCACCTCTTC CTCGTC

<i>OTX2</i>	Orthodenticle homeobox 2	ACAAGTGGCCAAT TCACTCC	GAGGTGGACAAGGGA TCTGA
<i>PAX6</i>	Paired box 6	TGGTATTCTCTCC CCCTCCT	TAAGGATGTTGAACGG GCAG
<i>PCSK1</i>	Proprotein convertase subtilisin/kexin type 1	GCAATGCCCCGTAA TGCTTAGAG	TTCCAAGGACAGAGT GATTCCG
<i>PCSK2</i>	Proprotein convertase subtilisin/kexin type 2	GATTGACTATCTCC ACCCGGAC	TCTGTGTACCGAGGG TAAGGAT
<i>PDYN</i>	Prodynorphin	CTCATTCCCAGGC ACTCTCTTT	TTTCCTCTCCTATCCA GCCTCA
<i>PITX2</i>	Paired like homeodomain 2	AACTCTATGAACG TCAACCCCC	CGACATGCTCATGGA CGAGATA
<i>POMC</i>	Proopiomelanocortin	TTTCATGACCTCC GAGAAGAGC	GATGATGGCGTTTTTG AACAGC
<i>PRDM12</i>	PR/SET domain 12	GTGGGAGGTGTTC AATGAGGAT	GTTCTGTACGTGCACA CTTGAT
<i>PVALB</i>	Parvalbumin	GGACAAGGACAAA AGTGGCTTC	CAGCCATCAGCATCTT GGTTTC
<i>RAX</i>	Retina and anterior neural fold homeobox	CCTCTCAGTTCAC CAAGCAGAT	TGATCAACCTTGGGT GTTAGGG
<i>RFX4</i>	Regulatory factor X4	ACCTTGCCATCTG TCTTGTCAT	ATAGGGATGGTACCAC CAGGAA
<i>SHH</i>	Sonic hedgehog	CCAATTACAACCC CGACATC	AGTTTCACTCCTGGC CACTG
<i>SIM1</i>	Single-minded family bHLH transcription factor 1	AAAGGGGGCCAA ATCCCGGC	TCCGCCCACTGGCT GTCAT
<i>SIM2</i>	SIM bHLH transcription factor 2	GGCTACTTGAAGA TCAGGCAGT	ATCTGGTAGCAGGAG TCGTACA
<i>SIX3</i>	SIX homeobox 3	ACCGGCCTCACTC CCACACA	CGCTCGGTCCAATGG CCTGG
<i>SIX6</i>	SIX homeobox 6	CTCAACAAGAATG AGTCGGTGC	ACTCCTTGGTGAACCT GTGGTT
<i>SOX14</i>	SRY-box 14	CATACATCGATGAA GCCAAGCG	CTGTCCTTCTTGAGCA GGTTCT
<i>SP8</i>	Sp8 transcription factor	CCTGTCTGTCCGG ACTTCAA	AGGGGCAGAAACAGA AAGAGAC
<i>SP9</i>	Sp9 transcription factor	TCTGGCCCCAACG ACTCTTAG	CTCGTTCGTTCTCGGT GTCTC
<i>SST</i>	Somatostatin	GGAACCTGAAGAT CTGTCCCAG	ATAGCCGGGTTTGAGT TAGCAG
<i>TBX3</i>	T-box transcription factor 3	GGGGGTAGGAGTT CCAACATTT	GCACTGAGGGAGATG TCTTTGA
<i>TH</i>	Tyrosine hydroxylase	CGGGCTTCTCGGA CCAGGTGTA	CTCCTCGGCGGTGTA CTCCACA
<i>TRH</i>	Thyrotropin releasing hormone	TCCTGGATGACCT GAGTAGGAG	TCAGGGAAAAGTGGG TTCACTC
<i>VSX2</i>	Unc-5 netrin receptor D	CCTCTGCCCTCTG TAAATGTGT	AGAGACCTCTGCGAG AACTTTG

Supplementary Table 1: List of qRT-PCR primers.

Antigen	Host species	Dilution	Manufacturer (#Cat)
NKX2-1	Rabbit	1:100-200	Abcam (#ab1333737)
PAX6	Mouse	1:1000	Sigma-Merck (#AMAb91372)
AGRP	Rabbit	1:1000	Phoenix Pharma (#H-003-53) – discontinued
AGRP	Goat	1:100-200	R&D Systems AF634
POMC (aMSH)	Sheep	1:1000	Millipore (#AB5087)
MAP2	Mouse	1:1000	Sigma (#M1406)
TRH	Rabbit	1:1000	Thermo Fisher (PA5-57331)
TH	Mouse	1:1000	Immunostar (173-22941)
GHRH	Rabbit	1:200	Abcam (ab18751)
OTP	Rabbit	1:500	GeneTex (GTX119601)
FOXG1	Rabbit	1:500	Abcam (#ab18259)
CRH	Rabbit	1:1000	Proteintech (10944-1-AP)
AQP4	Rabbit	1:1000	Sigma-Merck (#HPA014784)
hNCAM1	Mouse	1:1000	Santa Cruz Biotechnology #SC-106
HuNu	Mouse	IF: 1:1000 ISH: 1:200	Millipore #AB1281
NPY	Sheep	1:500	Millipore #AB1583
S100b	Mouse	1:1000	Sigma (#S2532)
Somatostatin	Mouse coupled- Alexa Fluor 488	1:500	BD Biosciences (#566032)
NFIA	Rabbit	1:200	Abcam (#ab228897)
Vimentin	Chicken	1:500	Millipore (#AB5733)
NR5A1	Mouse	1:1000	Thermo Fisher (#434200)

Supplementary Table 2: List of primary antibodies.

ANTIGEN	FLUOROPHORE	DILUTION	MANUFACTURER (#CAT)
RABBIT	Alexa488	1:200-500	Jackson Imm. Res. (#711-545-152)
MOUSE	Alexa647	1:200-500	Jackson Imm. Res. (#711-605-151)
SHEEP	Alexa Cy3	1:200-500	Jackson Imm. Res. (#713-165-147)
MOUSE	Alexa Cy3	1:200	Jackson Imm. Res. (#715-165-151)
MOUSE	Alexa 488	1:200-500	Jackson Imm. Res. (#715-545-150)
RABBIT	Alexa CY3	1:200	Jackson Imm. Res. (#711-165-152)
GOAT	Alexa488	1:200-500	Jackson Imm. Res. (#705-545-147)
SHEEP	Alexa647	1:200-500	Jackson Imm. Res. (#713-605-147)
GOAT	Alexa647	1:200	Jackson Imm. Res. (#705-605-147)
MOUSE	Biotinylated	1:500	Vector Laboratories #BA9200
DAPI	-	1:500	Thermo (#D3571)

Supplementary table 3. List of secondary antibodies.

Probe	Host species	Dilution	Manufacturer (#Cat)
Hs-RAX-C1	Homo sapiens	Ready-to-use	ACD Bio-Techne (#579951)
Hs-TBX3-C2	Homo sapiens	1:50	ACD Bio-Techne (#557441)
Hs-SIM1-C3	Homo sapiens	1:50	ACD Bio-Techne (#544281)
Hs-POU3F2-C2	Homo sapiens	1:50	ACD Bio-Techne (#543011)
Hs-PNOC-C2	Homo sapiens	1:50	ACD Bio-Techne (#1045241)
Fluorophore			
Opal 480		1:500	Akoya Bio FP1500001KT
Opal 570		1:500	Akoya Bio FP1488001KT
Opal 690		1:500	Akoya Bio FP1497001KT

Supplementary table 4. List of RNAscope probes and fluorophores.