Supplemental Method Tables

Supplemental Method Table 1. PCR amplification and sequencing primers used for *ONECUT1* sequencing and phase determination of variants

a. ONECUT1 gene exon sequencing

Amplified Region	Map Position (hg19) Chr	Start	End	PCR amplification and sequencing primers (5'-3') Forward	Reverse	Product size (bp)
exon 1(5')	15	53081496	53082200	CTTGCTGGGAGTTGTGGATG	GAGAGGAAGGAAGGCAACAG	705
exon 1(3')	15	53080900	53081609	TCCTACCCTTCCTCCTTTGG	TGGCCTCCATGAATAACCTC	710
exon 2	15	53049374	53050223	TACACCTTCGTGGCATGGTA	TCCCAATTCTCCATTCTGCT	850

Chr: chromosome; bp: base pairs.

b. Primers used to determine the phase of ONECUT1 variants P75A and P215A in one double-heterozygous subject

Primer orientation, allele specificity	Primer sequences 5'>3'	Map position (hg19)	Size (bp)	Allele determined
P215A-forward, G-specific (WT, c.643C, p.P215A-P)	GCTTCGAAGCCGTTGGg	53081423-53081439	466	P75A, conditioned to P215A allele
P215A-forward, C-specific (variant, c.643G, p.P215A-A)	GCTTCGAAGCCGTTGGc			
Reverse	GCGGAGATTACCACCACCAC	53081869-53081888		
Forward	GATGGTGCGGAGGAAGG	53081328-53081344	549	P215A, conditioned to P75A allele
P75A-reverse, G-specific (Ref, c.223C, p.P75A-P)	ACCACCACCACCGGGCCc	53081859-53081876		
P75A-reverse, C-specific (Alt, c.223G, p.P75A-A)	ACCACCACCACCGGGCCg			

Allele-specific primers were designed to amplify across variants (P75A-P215A). Bases shown in lowercase are specific to each allele, as indicated. The size of the PCR-amplified fragment is shown. The allele of the second variant was determined by Sanger sequencing of the allele-specific PCR-amplified fragment. Ref: reference allele; Alt: alternative allele (for the low frequency P75A variant); WT: wild type (for the mutated/rare variants position).

Supplemental Method Table 2. List of guide and zinc finger sequences for gene editing

Guide name	Sequence
ONECUT1 crRNA guide 1	5' CAAAGAGGATCCGCGCCGTT 3'
ONECUT1 crRNA guide 2	5' ACTTCAGCAGGGCGGCGACT 3'
ONECUT1 ZFN 1	5' CAGCAAGGGCTCCCCCACTATnCCCACCCGGGGGCCGCCATG 3'
ONECUT1 ZFN 2	5' CTTCGAAGCCCACCACCCGGnnATGCTCGGCCGCCACGGGGA 3'
sgRNA E231	5' GCGUGAGGUGCUGCUCCCCG 3'
ssDNA repair E231D	CCCCCAACGGCTTCGAAGCCCACCACCCGGCCATGCTCGGCCGACACGGGGACCAGCACCTCACGCCCACCTCGGCCGGCATGGTGCCCATCAACGGCCT

Supplemental Method Table 3. PCR amplification/primers used for screening after gene editing

Amplified Region	Map Position*			PCR amplification and sequencing primers (5'-3')		Product size (bp)
	Chr	Start	End	Forward	Reverse	
CRISPR KO External	15	53049137	53082451	AAAGAGAATGCACTGGTTT AGGTG	AGCCGGAGACCTCAGAATTTTA	508 (KO) /33.315 (WT)
CRISPR KO Internal	15	53077508	53078057	TGGGCGAGACTTTCTCATGG	CGGTCACGAAATAACAGCGG	550
Indel + E231D	15	53081020	53081587	CAGCCACTTCCACATCCTCC	TACCCCCTACCACAAGGACG	568

Supplemental Method Table 4. List of primary antibodies

Primary Antibodies	Host	Company	Catalogue Number	Dilution	Application
GATA6	rabbit	Santa Cruz	sc-9055	-	ChIP
HNF6 (H100)	rabbit	Santa Cruz	sc-13050	-	ChIP
Flag (M2)-agarose conjugated	mouse	Sigma	A2220	1:2, 25 µl	CoIP
Flag, M5	mouse	Sigma	F4042	1:10000	CoIP, WB, EMSA
c-KIT-APC conjugated	mouse	Invitrogen	CD11705	1:100	FC
CXCR4-PE conjugated	mouse	Life Technologies	MHCXCR404	1:33.33	FC
NKX6.1-647 conjugated	mouse	BD	563338	1:35	FC
PDX1-PE conjugated	mouse	BD	562161	1:35	FC
NKX6.1	mouse	DSHB	F55A12 concentrate	1:150	FC, IF
NKX6.1	mouse	DSHB	F55A10 concentrate	1:100	IF
PDX1	goat	R&D	AF2419	1:500	FC, IF
NANOG	rabbit	Cell Signaling	#3580	1:100	IF
Oct3/4	mouse	Santa Cruz	sc-5279	1:200	IF
SOX17	goat	R&D	AF1924	1:500	IF
GFP (7.1 and 13.1)	mouse	Sigma	11814460001	1:1000	WB
HNF6 (H100X)	rabbit	Santa Cruz	sc-13050	1:2000	WB
HNF6	rabbit	Abcam	Ab186743	1:1000	WB
ß-Actin	mouse	Sigma	A1978	1:5000	WB
ß-Actin	mouse	Sigma	A5316	1:2000	WB
C-peptide	rabbit	Cell Signaling	4593	1:100	FC, IF
Glucagon	mouse	Sigma	G2654	1:500	FC, IF

Supplemental Method Table 5. List of secondary antibodies

Secondary Antibodies	Host	Company	Catalogue Number	Dilution	Application
ECL anti-mouse-HRP linked	sheep	GE Healthcare	NA931V	1:5000	CoIP, WB
anti-goat AlexaFluor 488nm	donkey	Invitrogen	A11055	1:500	FC, IF
anti-mouse AlexaFluor 568nm	donkey	Invitrogen	A10037	1:500	FC, IF
anti-mouse AlexaFluor 647nm	donkey	Invitrogen	A31571	1:500	IF
anti-rabbit AlexaFluor 568nm	donkey	Invitrogen	A10042	1:500	IF
ECL anti-mouse-HRP linked	sheep	GE Healthcare	NA9310V	1:5000	WB
ECL anti-rabbit-HRP linked	donkey	GE Healthcare	NA9340V	1:5000	WB

Supplemental Method Table 6. Expression constructs for EMSA, Co-IP and Luciferase assays

cDNA	pcDNA3-F1	pcDNA3-GFP	Tag
ONECUT1 WT	x	x	N-terminus
ONECUT1 D26E	x	x	N-terminus
ONECUT1 P215R	x	x	N-terminus
ONECUT1 K412R	x	x	N-terminus
ONECUT1 V242A	x	x	N-terminus
ONECUT1 E231D	x	x	N-terminus
ONECUT1 E231X	x	x	N-terminus
ONECUT1 G30S	x	x	N-terminus
ONECUT1 H33Q	x	x	N-terminus
ONECUT1 G81D	x	x	N-terminus
NKX6.1	x	x	N-terminus
NKX2.2	x	x	N-terminus
GATA4		x	C-terminus
PDX1		x	N-terminus
GLIS3	x	x	N-terminus
NGN3	x	x	N-terminus
FOXA1		x	N-terminus
FOXA2		x	N-terminus
GATA6		x	N-terminus
PAX4		x	N-terminus

Supplemental Method Table 7. List of cell lines and respective modifications

Parental cell line	Cell type (source)	Genotype / genetic modification	Specification
HUES8	hESC (Harvard University, HSCI iPS Core)	ONECUT1 WT	CRISPR control
HUES8	hESC (Harvard University, HSCI iPS Core)	ONECUT1 KO	gene deletion
HUES8	hESC (Harvard University, HSCI iPS Core)	ONECUT1 trunc	frameshift after indel
HUES8	hESC (Harvard University, HSCI iPS Core)	ONECUT1 E231D	point mutation
Patient-derived	hiPSC	ONECUT1 E231X	point mutation
Patient-derived	hiPSC	ONECUT1 WT	wild type
CyT49	hESC (ViaCyte, Inc. Stem Cell Derivation)	E1-NKX6.1-GFP	enhancer reporter construct
CyT49	hESC (ViaCyte, Inc. Stem Cell Derivation)	E2-NKX6.1-GFP	enhancer reporter construct
CyT49	hESC (ViaCyte, Inc. Stem Cell Derivation)	E3-NKX6.1-GFP	enhancer reporter construct
HeLa	tumor cell line (ATCC CCL2)	GFP- and FLAG-tagged overexpression constructs	for reporter assays and cellular localization
HEK293	tumor cell line (ATCC CRL 1573)	GFP- and FLAG-tagged overexpression constructs	for IP assays