Supplemental Data

Combining single-strand oligodeoxynucleotide and CRISPR/CAS9 to correct the gene mutation in β-thalassemia induced Pluripotent Stem Cell

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Supplementary Figure1. The whole exome sequencing to detect the top 10 predicted off-target sites of gRNA1 in the exome.

Through the whole exome sequencing, the figures showed sequence results of the 10 off-target sites of gRNA1 in the exome.

Supplementary Table1. The top 10 predicted off-target sites in the exome.

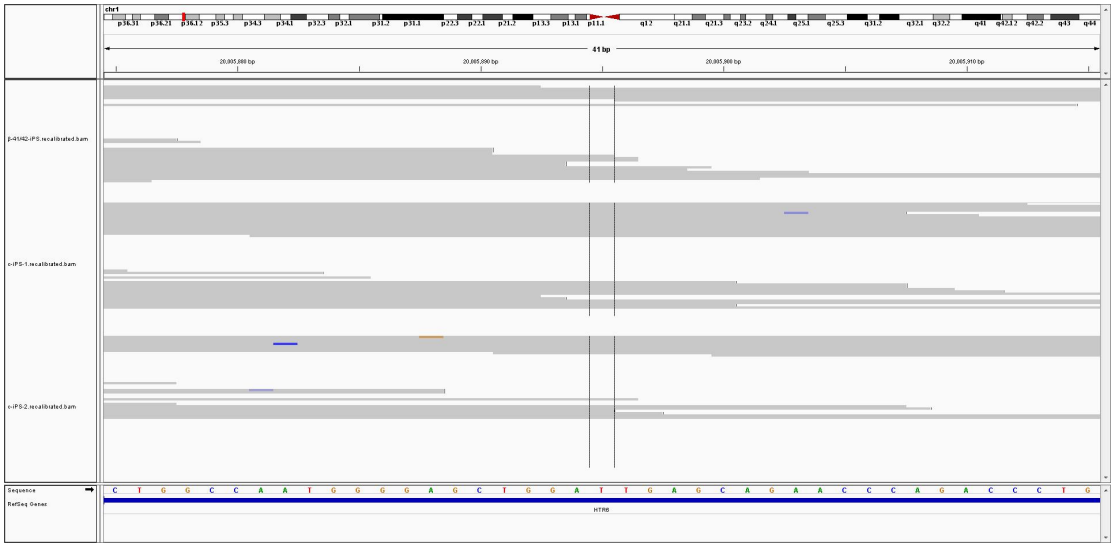
Supplementary Table2. The involved genes of each variants detected by microarray and exome sequencing.

Supplementary Table3. The primers used in the manuscript.

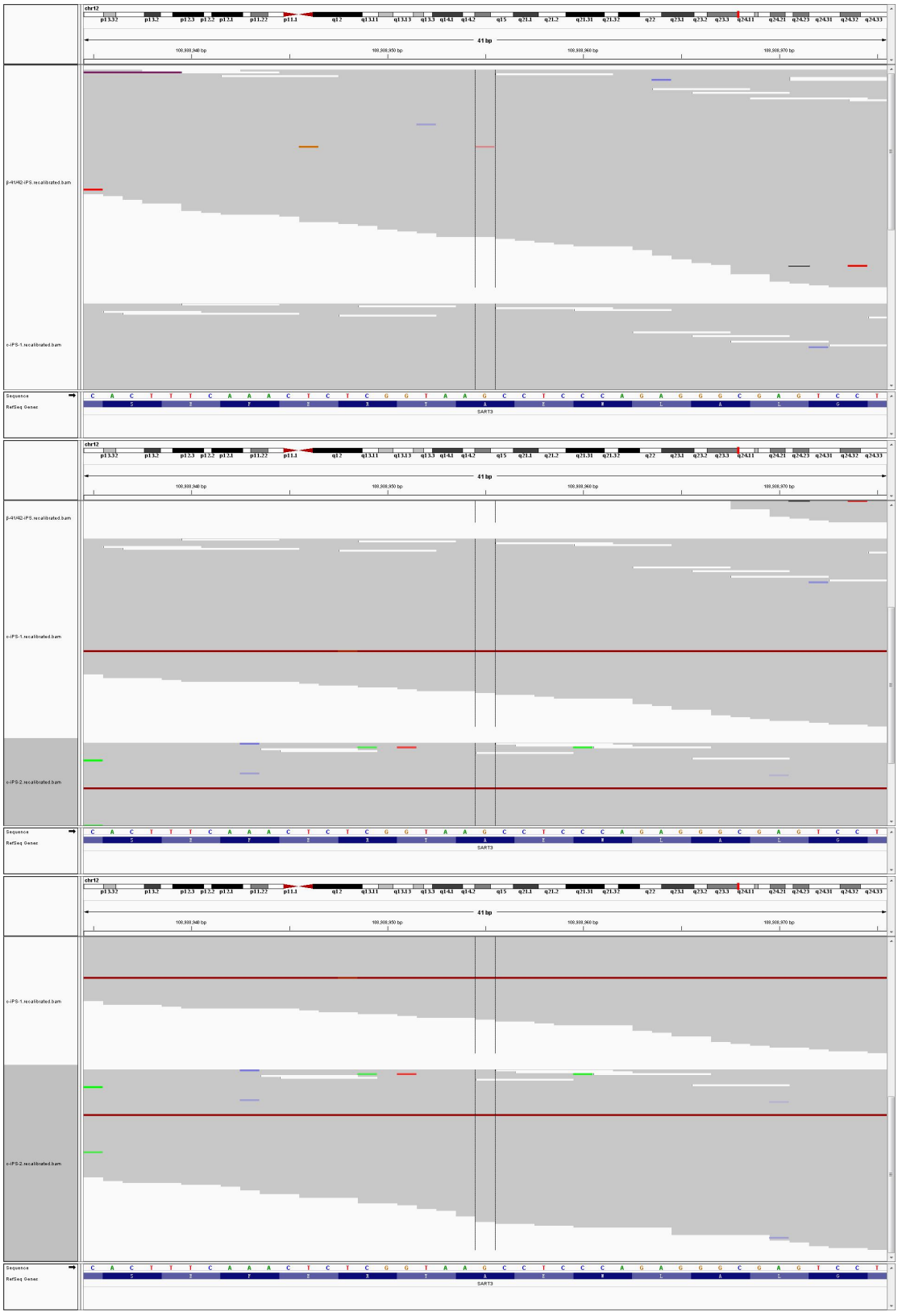
Supplementary Figure1. The whole exome sequencing to detect the top 10 predicted off-target sites of gRNA1 in the exome.

Offtarget site 1

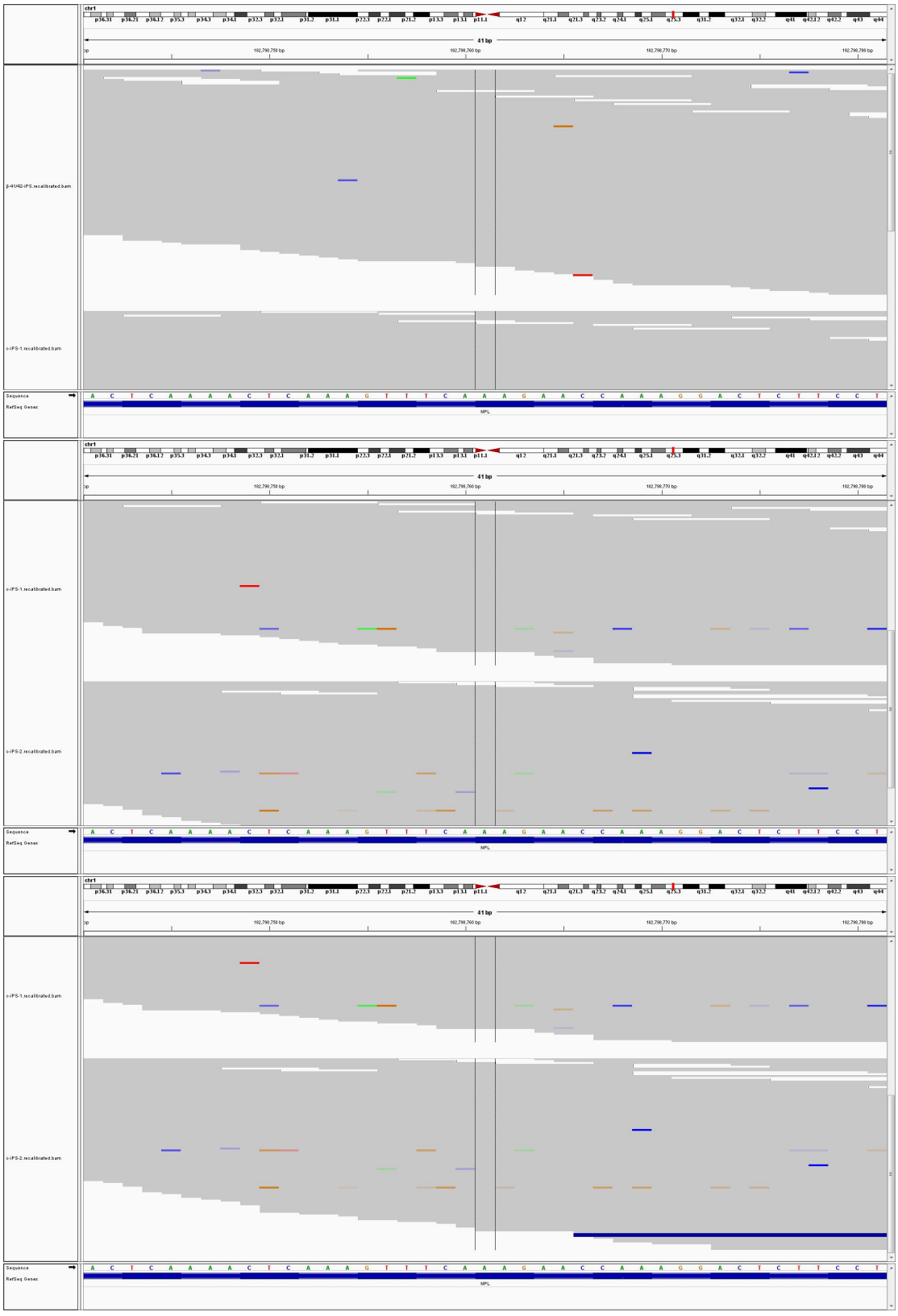
Offtarget site2



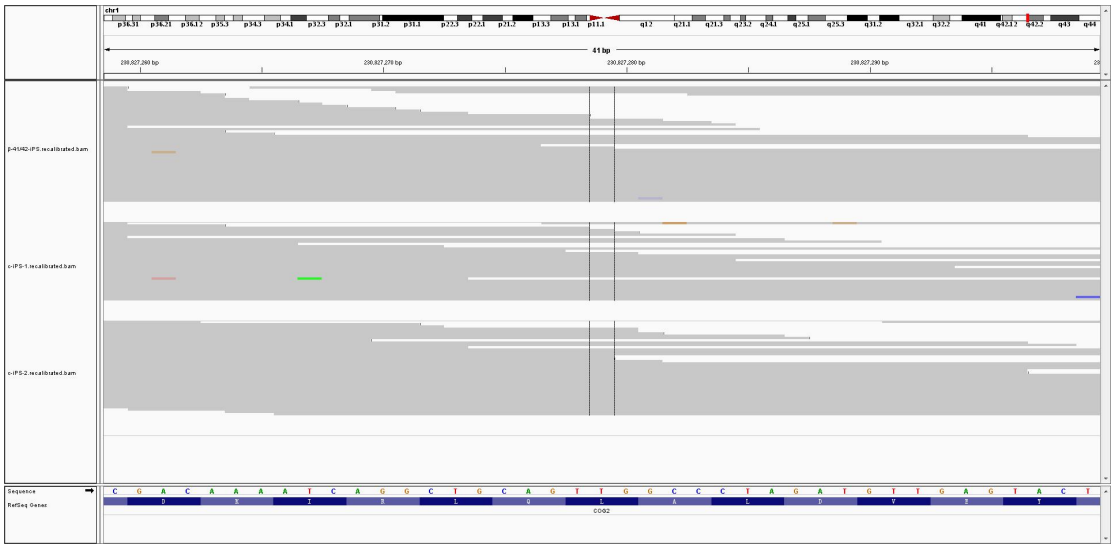
Offtarget site3



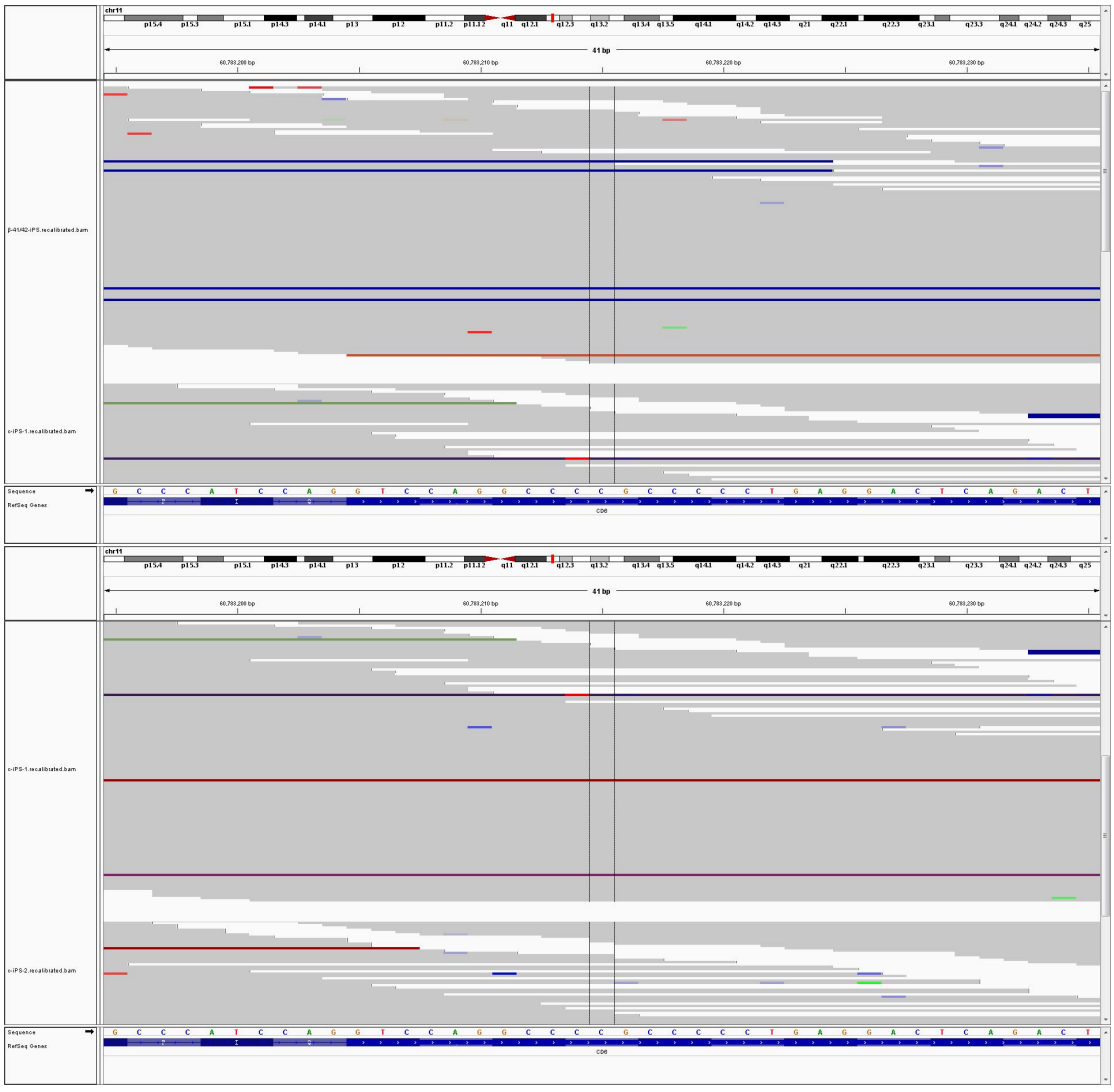
Offtarget site 4

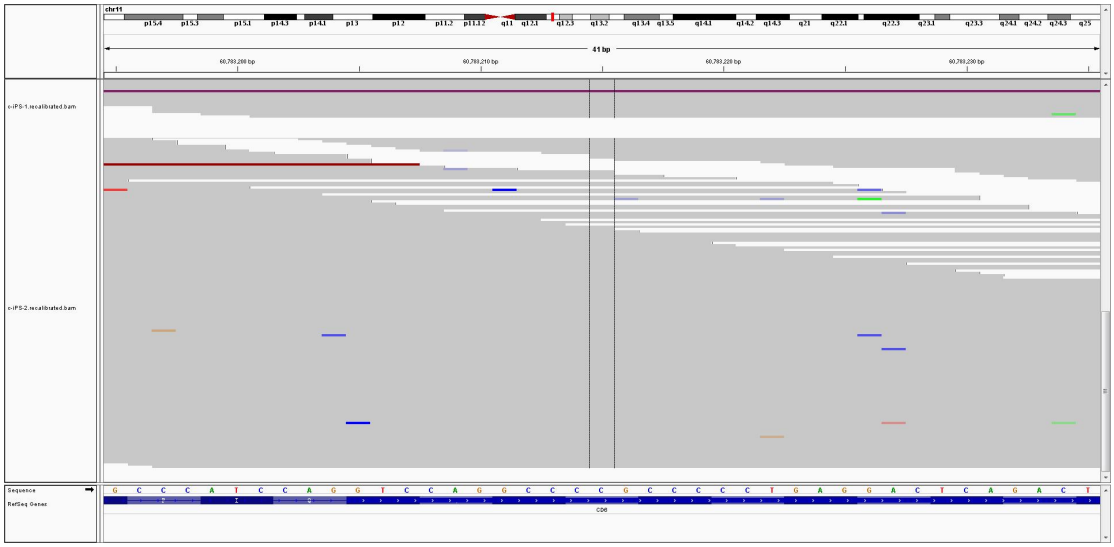


Offtarget 5

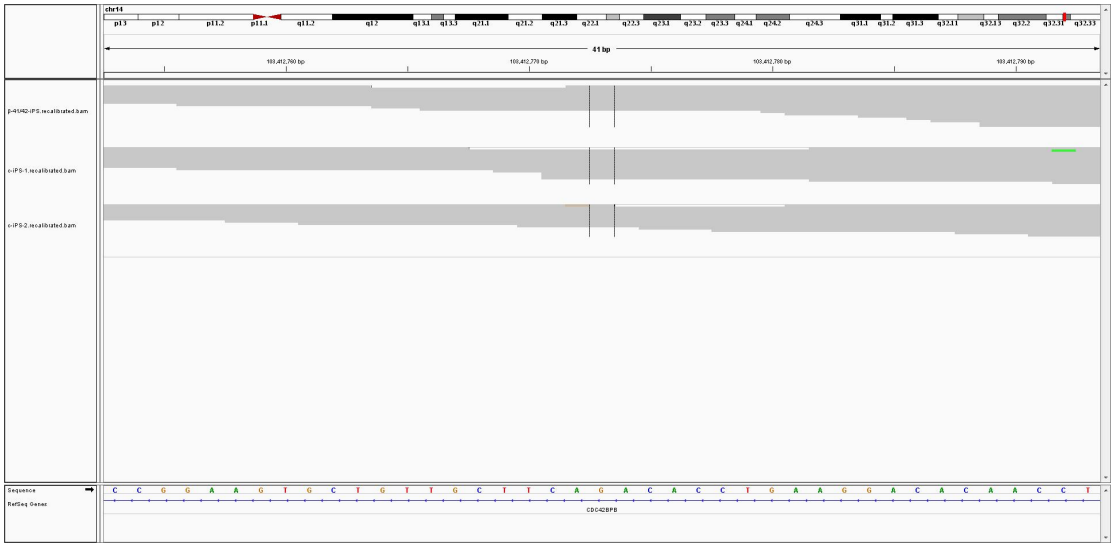


Offtarget 6



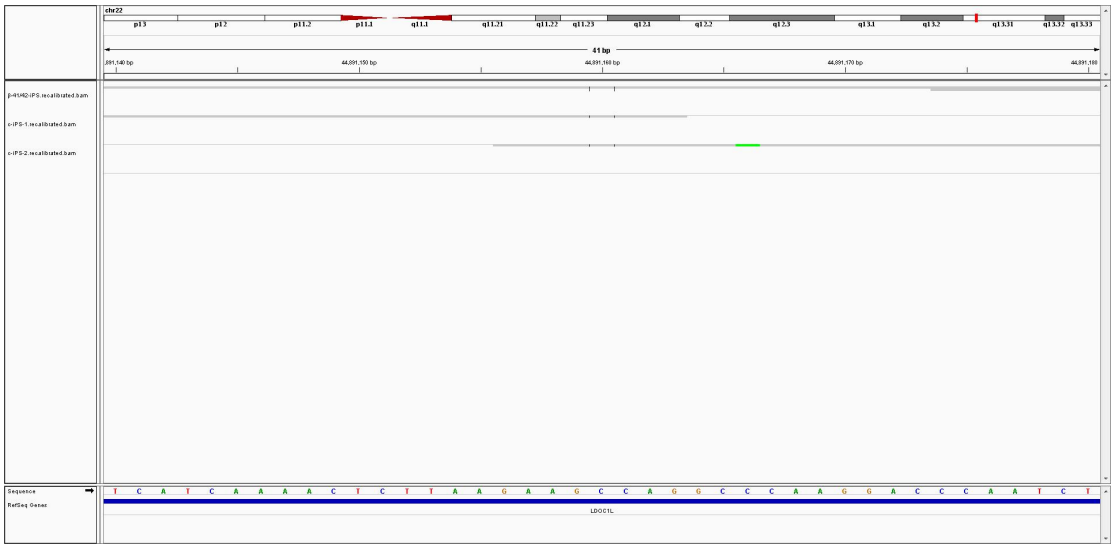


Offtarget 7

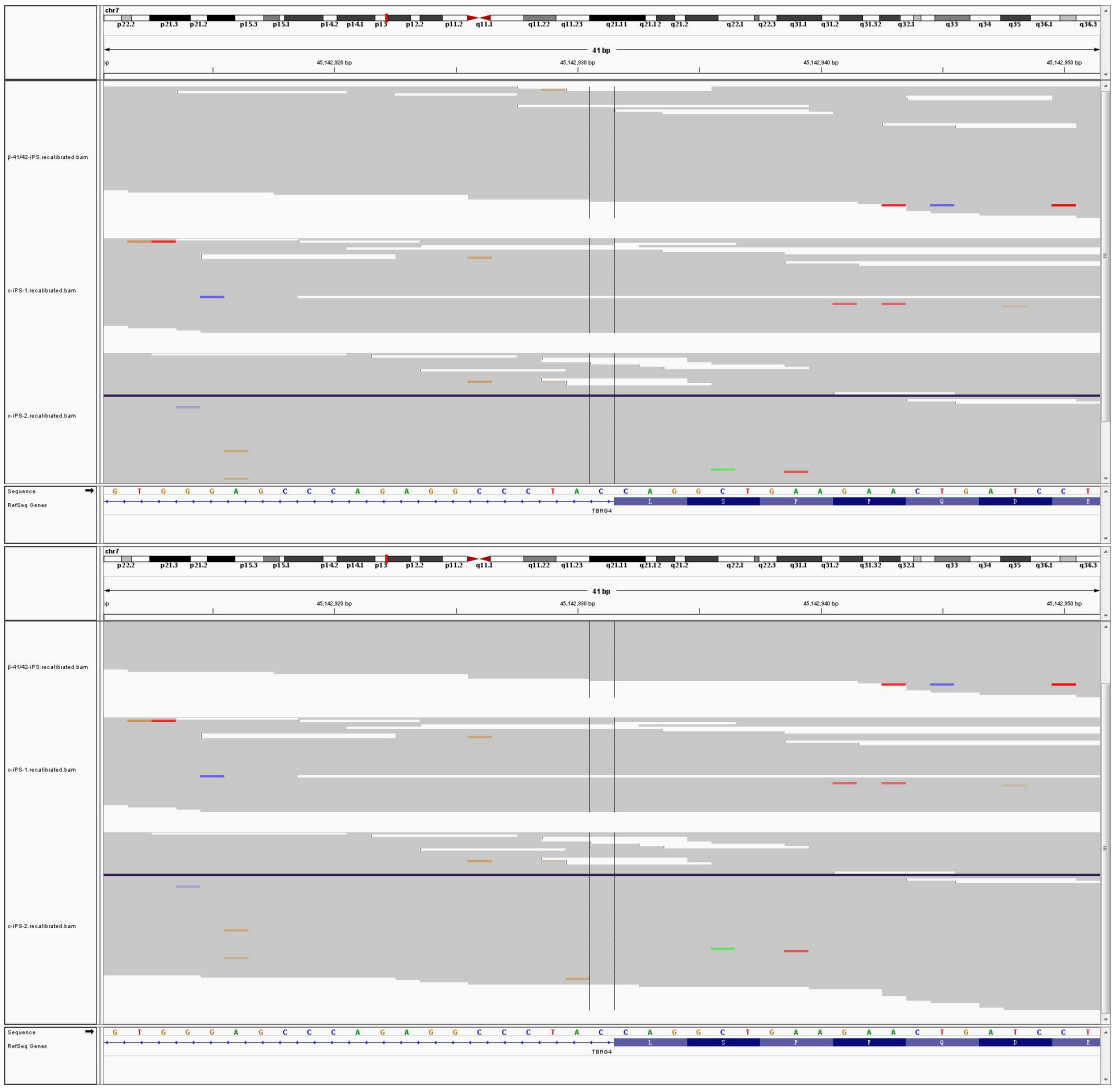


Offtarget 8

Offtarget 9



Offtarget 10



Supplementary Table1.The top 10 predicted off-target sites in the exome.

HBB gRNA1	GACCCAGAGGTTGAGTCCTT		
OFFTARGET1	CTCCCAGGGGTGGAGTCCTT	NM_006634	chr2:-85820593
OFFTARGET2	AACCCAGACCCTGAGTCCTT	NM_000871	chr1:+20005901
OFFTARGET3	CTCCCAGAGGGCGAGTCCTT	NM_014706	chr12:+108938955
OFFTARGET4	TGCCCAGAGGAAGAGTCCTT	NM_001200051	chr1:-182798761
OFFTARGET5	GCCCTAGATGTTGAGTACTT	NM_001145036	chr1:+230827279
OFFTARGET6	GAGCCAGAGTCTGAGTCCTC	NR_045638	chr11:-60783215
OFFTARGET7	GTCCCAAAGGTTGTGTCCTT	NM_006035	chr14:-103412773
OFFTARGET8	GAGCCAGAGACTGAGTGCTT	NM_006807	chr17:-46178379
OFFTARGET9	ATCCCAGAGATTGGGTCCTT	NM_032287	chr22:-44891160
OFFTARGET10	GACCAAGAGGATCAGTTCTT	NM_001261834	chr7:-45142931

Supplementary Table2. The involved genes of each variants detected by microarray and exome sequencing.

Method	Varients (to β-41/42-iPS)	Reffered Gene	Gene ID	PANTHER Family/Subfamily
Microarray	Duplication	DEFB115	HUMAN|HGNC=18096|UniProtKB=Q30KQ5	BETA-DEFENSIN 115 (PTHR15001:SF20)
		DEFB116	HUMAN|HGNC=18097|UniProtKB=Q30KQ4	BETA-DEFENSIN 116 (PTHR15001:SF17)
		DEFB118[#]	HUMAN|HGNC=16196|UniProtKB=Q96PH6	BETA-DEFENSIN 118 (PTHR15001:SF24)
		DEFB119	HUMAN|HGNC=18099|UniProtKB=Q8N690	BETA-DEFENSIN 119 (PTHR15001:SF6)
		DEFB121	HUMAN|HGNC=18101|UniProtKB=Q5J5C9	BETA-DEFENSIN 121 (PTHR15001:SF30)
		DEFB122	/	/
		DEFB123	HUMAN|HGNC=18103|UniProtKB=Q8N688	BETA-DEFENSIN 123 (PTHR15001:SF3)
		DEFB124	HUMAN|HGNC=18104|UniProtKB=Q8NES8	BETA-DEFENSIN 124 (PTHR15001:SF1)
		REM1	HUMAN|HGNC=15922|UniProtKB=O75628	GTP-BINDING PROTEIN REM 1 (PTHR24070:SF260)
		LINC00028	/	/
		HM13	HUMAN|HGNC=16435|UniProtKB=Q8TCT9	MINOR HISTOCOMPATIBILITY ANTIGEN H13 (PTHR12174:SF23)
		PSIMCT-1	/	/
		HM13-AS1	/	/
		ID1	HUMAN|HGNC=5360|UniProtKB=P41134	DNA-BINDING PROTEIN INHIBITOR ID-1 (PTHR11723:SF4)
		MIR3193	/	
		COX4I2	HUMAN|HGNC=16232|UniProtKB=Q96KJ9	CYTOCHROME C OXIDASE SUBUNIT 4 ISOFORM 2, MITOCHONDRIAL (PTHR10707:SF11)
		BCL2L1	HUMAN|HGNC=992|UniProtKB=Q07817	BCL-2-LIKE PROTEIN 1 (PTHR11256:SF12)
		TPX2	HUMAN|HGNC=1249|UniProtKB=Q9ULW0	TARGETING PROTEIN FOR XKLP2 (PTHR14326:SF9)
		MYLK2	HUMAN|HGNC=16243|UniProtKB=Q9H1R3	MYOSIN LIGHT CHAIN KINASE 2, SKELETAL/CARDIAC MUSCLE (PTHR24347:SF118)
		FOXS1	HUMAN|HGNC=3735|UniProtKB=O43638	FORKHEAD BOX PROTEIN S1 (PTHR11829:SF68)
		DUSP15	HUMAN|HGNC=16190|UniProtKB=Q9H1R2	DUAL SPECIFICITY PROTEIN PHOSPHATASE 15 (PTHR10159:SF30)
		TTLL9	HUMAN|HGNC=16118|UniProtKB=Q3SXZ7	TUBULIN POLYGLUTAMYLASE TTLL9-RELATED (PTHR12241:SF39)
		PDRG1	HUMAN|HGNC=16119|UniProtKB=Q9NUG6	P53 AND DNA DAMAGE-REGULATED PROTEIN 1 (PTHR21162:SF0)
		XKR7	HUMAN|HGNC=23062|UniProtKB=Q5GH72	XK-RELATED PROTEIN 7 (PTHR32129:SF9)
		C20orf160	/	/
		HCK	HUMAN|HGNC=4840|UniProtKB=P08631	TYROSINE-PROTEIN KINASE HCK (PTHR24418:SF245)
		TM9SF4	HUMAN|HGNC=30797|UniProtKB=Q92544	TRANSMEMBRANE 9 SUPERFAMILY MEMBER 4 (PTHR10766:SF55)
		TSPY26P	HUMAN|HGNC=16256|UniProtKB=Q9H489	TESTIS-SPECIFIC Y-ENCODED-LIKE PROTEIN 3-RELATED (PTHR11875:SF51)
		PLAGL2	HUMAN|HGNC=9047|UniProtKB=Q9UPG8	ZINC FINGER PROTEIN PLAGL2 (PTHR10032:SF124)
		POFUT1	HUMAN|HGNC=14988|UniProtKB=Q9H488	GDP-FUCOSE PROTEIN O-FUCOSYLTRANSFERASE 1 (PTHR21420:SF3)
Exon sequencing	INDEL	MTMR11	HUMAN|HGNC=24307|UniProtKB=A4FU01	MYOTUBULARIN-RELATED PROTEIN 11 (PTHR10807:SF51)
		MTR	HUMAN|HGNC=7468|UniProtKB=Q99707	METHIONINE SYNTHASE (PTHR21091:SF112)
		HGC63	/	/
		FAM90A1	HUMAN|HGNC=25526|UniProtKB=Q86YD7	PROTEIN FAM90A1 (PTHR16035:SF9)
		CCT6B	HUMAN|HGNC=1621|UniProtKB=Q92526	T-COMPLEX PROTEIN 1 SUBUNIT ZETA-2 (PTHR11353:SF58)
		OR7E24	HUMAN|HGNC=8396|UniProtKB=Q6IFN5	OLFACTORY RECEPTOR 7E24 (PTHR26451:SF173)
		MCM8	HUMAN|HGNC=16147|UniProtKB=Q9UJA3	DNA HELICASE MCM8 (PTHR11630:SF47)
		TMEM191B	HUMAN|HGNC=33600|UniProtKB=P0C7N4	/
		BEX5	HUMAN|HGNC=27990|UniProtKB=Q5H9J7	PROTEIN BEX5 (PTHR19430:SF0)
		ZFHX4	HUMAN|HGNC=30939|UniProtKB=Q86UP3	ZINC FINGER HOMEOBOX PROTEIN 4 (PTHR24208:SF40)
		HTT	HUMAN|HGNC=4851|UniProtKB=P42858	HUNTINGTIN (PTHR10170:SF10)
		FBXO46	HUMAN|HGNC=25069|UniProtKB=Q6PJ61	F-BOX ONLY PROTEIN 46 (PTHR16271:SF10)
		GSE1	HUMAN|HGNC=28979|UniProtKB=Q14687	GENETIC SUPPRESSOR ELEMENT 1 (PTHR17608:SF4)
	SNV	GABRG2	HUMAN|HGNC=4087|UniProtKB=P18507	GAMMA-AMINOBUTYRIC ACID RECEPTOR SUBUNIT GAMMA-2 (PTHR18945:SF498)
		SPRN	HUMAN|HGNC=16871|UniProtKB=Q5BIV9	SHADOW OF PRION PROTEIN (PTHR28552:SF1)
		BUD13	HUMAN|HGNC=28199|UniProtKB=Q9BRD0	BUD13 HOMOLOG (PTHR31809:SF0)
		MMP17	HUMAN|HGNC=7163|UniProtKB=Q9ULZ9	MATRIX METALLOPROTEINASE-17 (PTHR10201:SF21)
		A4GALT	HUMAN|HGNC=18149|UniProtKB=Q9NPC4	LACTOSYLCERAMIDE 4-ALPHA-GALACTOSYLTRANSFERASE (PTHR12042:SF17)
		GRID21P	/	/
		PIK3R2	HUMAN|HGNC=8980|UniProtKB=O00459	PHOSPHATIDYLINOSITOL 3-KINASE REGULATORY SUBUNIT BETA (PTHR10155:SF1)

Red text indicated pathogenic genes in OMIM, while yellow text indicated likely pathogenic genes

Supplementary Table 3. The primers used in the manuscript.

The Primers Used To Assambly The gRNA Vector		
	FROWARD	REVERSE
gRNA1	CACCGACCCAGAGGTTGAGTCCTT	AAACAAGGACTCAACCTCTGGGTC
gRNA2	CACCGCCCAGAGGTTGAGTCCTTTG	AAACCAAAGGACTCAACCTCTGGGC
gRNA3	CACCGCCCCAAAGGACTCAACCTC	AAACTTGGACCCAGAGGTTGAGTCC
gRNA4	CACCGGACTCAACCTCTGGGTCCA	AAACTGGACCCAGAGGTTGAGTCC
gRNA5	CACCGACTCAACCTCTGGGTCCAA	AAACTTGGACCCAGAGGTTGAGTC
The Primers For PCR Assay To Selection The Corrected Clones		
P1	CAAGACAGGTTTAAGGAGAC	
P2	CTTATCCCCTTCCTATGACA	
P3	AGATCCCCAAAGGACTCAAAGA	
P4	TTGGACCCAGAGGTTCTTTGAG	
The Primers Used For T7E1 Assay		
	FORWARD	REVERSE
HBB	GAAAACATCAAGCGTCCCA	GGCAGGTTGGTATCAAGGTT
The Primers Used For Gene Expression Assay		
	FORWARD	REVESE
OCT3/4	GAAGGTATTCAGCCAAACGA	GGCCGCAGCTTACACAT
NANOG	AGAATAGCAATGGTGTGCGC	GGTTGCTCCAGGTTGAATTGT
SOX2	ACCAGCTCGCAGACCTACAT	ACTTGACCACCGAACCCAT
ACTIN	ACCTTCTACAATGAGCTGCG	CCTGGATAGCAACGTACATGG
HBB	CTCGGTGCCTTTAGTGATGG	ACACAGACCAGCACGTTG
HBG1	AATGTGGAAGATGCTGGAGG	GCCAAAGCTGTCAAAGAACC
CD71	ACTTGCCCAGATGTTCTCAG	GTATCCCTCTAGCCATTCAGTG
The Primers Used For SSA System Construct		
	FORWARD	REVERSE
INSERT	GCATGGTACCTTCTCAGGGATACACGTGTG	CAGAAGATCTGTGCAAAGAGGCATGATAC
5'GFP ARM	TTAAGCTTATGGCCACAACCATGGTGAGCAA	CAGAAGATCTTTAGGGCGGACTGGGTGCTCAGGTAG
3'GFP ARM	GCATGGTACCCATCGAGCTGAAGGGCATCGACT	GCTCTAGATTACTTGTACAGCTCGTCCATGC
HBB DONOR SSODN	CCACCCTTAGGCTGCTGGTGGTCTACCCTTGGACCCAGAGGTTCTTTGAGTCCTTTGGGGATCTGTCCACTCCTGATGCTGTTATGG	