

Supplementary information, Tables S1 and S2

Table S1 The off-target analysis and mosaic analysis of mutant offspring

A. Potential off-targets of site 1

NCBI Seq. ID	Potential Off-target Seq. (mismatch in red)	Primers	Product size (bp) and code
NC_022277.1 chr6	GGCAGCACTGACacT CAcTGTGG	TGTCAGCATCAGAATTCCCCC	355 T1 s1
		CCAGGCAGACCCATGAAGAAT	
NC_022273.1 chr2	aGcTgCACTGACGGTg AGTGTGG	GGCACATAATGCAGGGACCA	794 T1 s2
		AACGGGTGAGTTGAGGCCAT	
NC_022284.1 chr13	GGCAGtACTGAgGGT CAcTGAGG	CCACTTGGGTGTACCTGGTT	462 T1 s3
		ATGTTcAGGAGTCAGGTTGGG	
NC_022276.1 chr5	GGCAGCACTGAgtGT CtGTGTGG	AGGTGTGGATGGTTCACTCA	177 T1 s4
		CCCCAGCCAGTGTTAATAGGG	
NC_022285.1 chr14	GGCAGCACTGAatGcC AGTGTGG	CTAGAGGCCTTTGGGGTGT	605 T1 s5
		CACCAGATGCCTCTTAGGGTC	
NC_022281.1 chr10	GGCAGCACTGctGGg CAGTGAGG	TACAGTGTCTGCATTGGCGG	517 T1 s6
		TTGACCCTGGTGTGGTTGAG	
NC_022281.1 chr10	GGgAGCAgTGAaGGT CAGTGAGG	GGGCACCATAGTAAGACCTC	404 T1 s7
		GAGCCACTGAAACTCCCTT	
NC_022290.1 chr19	GGCAGCcCTGACTGTg AGTGAGG	TGCCACTGCTAAGCTGACAT	284 T1 s8
		GGATGCTCGCTGCTGGTTAT	
NC_022280.1 chr9	GGCAGtgCTGAaGGTC AGTGAGG	TTCCTGCCTAAACACCCCAG	378 T1 s9
		GTCGAAGTCCTAACGAGGCA	
NC_022292.1 chrX	GtCAGCAaTGgCGGTC AGTGAGG	TGGAGGTAGTGTGGCTTG	456 T1 s10
		CTTACAGGGAAGTCTCAGGC	
NC_022276.1 chr5	GGCAGCAgTGAaaGT CAGTGGGG	ACTGACACACAAGGAATGGTAG	263 T1 s11
		TGTAAGAGAAGGCTGAGTAGGA	
NC_022280.1 chr9	GGCAGCAaTGcCGGc CAGTGTGG	CGCTGTACAATGTGGCATGG	316 T1 s12
		GATGTGCCAGGGCCTTTAGT	

B. Potential off-targets of site 2

NCBI Seq. ID	Potential Off-target Seq. (mismatch in red)	Primers	Product size (bp) and code
NC_022277.1 chr6	GGCCACTGATGTgGta CTCCAGG	CATCCCCTCTTCTCTAGCCC	434 T2 s1
		CCTAAGTAGCTCCGCCTGAAG	
NC_022279.1 chr8	GGCCAgtcATgaTGCT CTCCGGG	TCAGTGAGAGGGCTGTTAGC	398 T2 s2
		CTTTAGAGGACACCGTGGA	
NC_022273.1 chr2	GGCCcCTGATGcTGC TCTCaAGG	CTTGTGCCCTACGTTGCTTG	479 T2 s3
		CTTGTGCCCTACGTTGCTTG	
NC_022281.1 chr10	GGCCACTGcTGTTGgc CTCCTGG	GGACTTGCCCTTCACTCACC	787 T2 s4
		TCTGTGTGCTCCAGATGGGT	
NC_022290.1 chr10	GGCCACTGcaGTTGT CTCCTGG	TCCTTTGACTTGGGCTAACCA	399 T2 s5
		AGAAGTTGGAAGTCGGCAGAG	
NC_022288.1 chr17	caCCACTGATGcTGCT CTCCTGG	GACCATAGGTGTGCCTCTACA	323 T2 s6
		TGAAAGACGAGCCCAAGTT	
NC_022286.1 chr15	aGCCACTGATGTTGC TCctCAGG	CAATAAAGCAATCAGCCAGC	399 T2 s7
		CTAACACCCAAGCACCTCTT	
NC_022286.1 chr15	GaCCtCTGATGTTGCT CTgCAGG	TCTGGATGTGTAGTCTGGAAAA GAG	304 T2 s8
		TCCACCCTGTCCCCTACCAA	

C. Primers used in mosaic analysis

	sgRNA Targeted Sequence	Primers	Product size (bp)
Target site 1	GGCAGCACTGAC GGTCAGTG	GCTGCATAGCACGGACAAGGT	546 (305+241)
		GGATCTCCTGCCAGCCATTCTC	
Target site 2	GGCCACTGATGTT GCTCTCC	ACTCCTTTGGTTTGCCTGGAAG	547 (361+186)
		GAAGCCCTAGCCCATCACTACA	
Large deletion		GCTGCATAGCACGGACAAGGT	
		GAAGCCCTAGCCCATCACTACA	
Deep amplicon sequencing at site 2	GGCCACTGATGTT GCTCTCC	CTCCTCCTGGCTCAGGTCCA	222
		AGCCAGCAGCTTCTCCTGGACC	

D. Barcodes used in deep amplicon sequencing

Tissues	Forward barcode	Reverse barcode	Offspring
PFC	TTAGGC	TGACCA	M1 and M2
striatum	ATCACG	CGATGT	M1 and M2
liver	CAGATC	ACTTGA	M1 and M2
kidney	GATCAG	TAGCTT	M1 and M2
gonad	ATGTCA	CCGTCC	M1 and M2
blood	ATCACG	CGATGT	M3
hair	TTAGGC	TGACCA	M3
placenta	CAGATC	ACTTGA	M3

Table S2 Antibodies used for western and immunostaining analyses

Antibody	Source	Cat. No.	Company	WB/IHC Dilution
Shank3	Rabbit		This study	WB 1:5000; IHC 1:5000
α -tubulin	mouse	T5168	Sigma	WB 1:25000
GluN1	Rabbit	5704S	Cell Signaling	WB 1:1000
GluN2B	Rabbit	4212S	Cell Signaling	WB 1:2000
GluA2	Rabbit	ab133477	Abcam	WB 1:2000
mGluR5	Rabbit	ab5675	Millipore	WB 1:2000
Homer1b/c	Rabbit	sc-20807	Santa Cruz	WB 1:1000; IHC 1:400
Homer	Mouse	sc-17842	Santa Cruz	WB 1:2000
PSD95	Mouse	CP35	Cal Biochem	WB1:2000; IHC 1:200
NeuN	Mouse	Ab104224	Abcam	WB 1:5000; IHC 1:1000
GFAP	Rabbit	Z0334	Dako	WB 1:20000; IHC 1:5000
Doublecortin	Rabbit	4604	Cell Signaling	WB 1:2000