

Supplementary Information

Title

CRISPR/Cas9 mediated genome editing in ES cells and its application for chimeric analysis in mice

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Supplementary Figure Legends

Supplementary Figure S1. Generation of *Cetn1* mutant ESCs

(a) *Cetn1* was targeted in ESCs with sgRNA #22 (see **Supplementary Table S1**). The black box, scissors, and arrows indicate the coding region, DSB site, and primer set a/b (see **Supplementary Table S1**), respectively.

(b) Biallelic deletions were identified by sequencing. The 330 bp and 268 bp were deleted between black and red character in *em51* and *em52*, respectively. Green arrow indicates the sgRNA target.

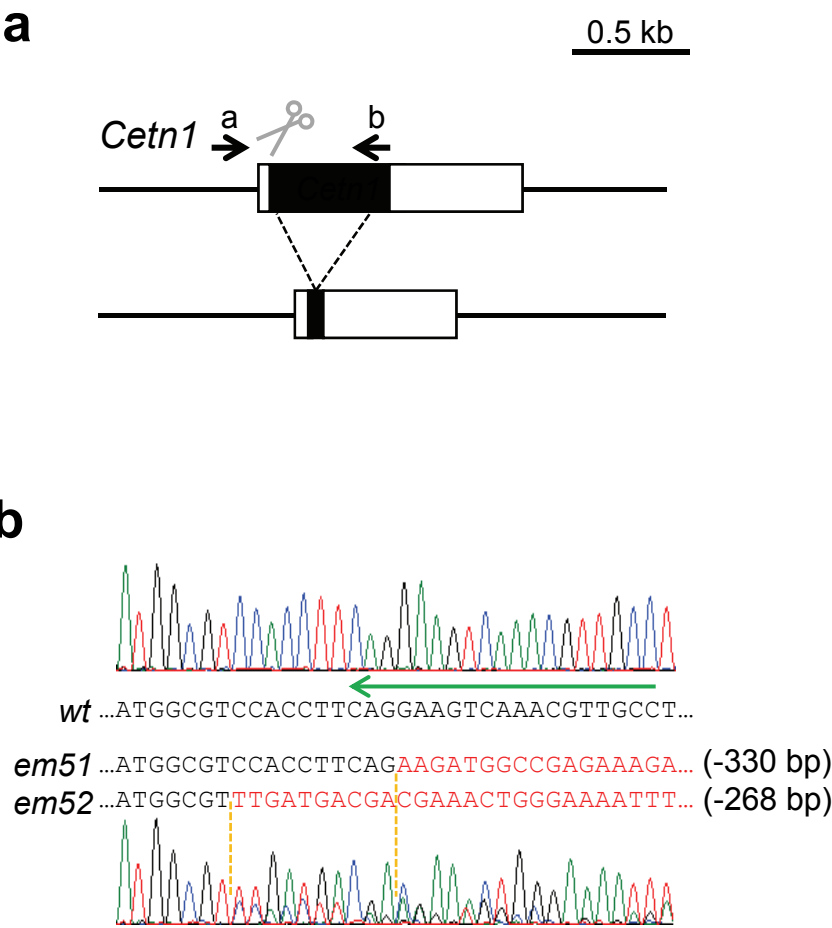
Supplementary Figure S2. Suppression of hydrocephalus and lethality in *Dnajb13* chimeric KO mice

(a) *Dnajb13* exon 8 was targeted in ESCs with sgRNA #43 (see **Supplementary Table S1**). The black boxes, scissors, and arrows indicate the coding region, DSB site, and primer set g/h (see **Supplementary Table S1**), respectively.

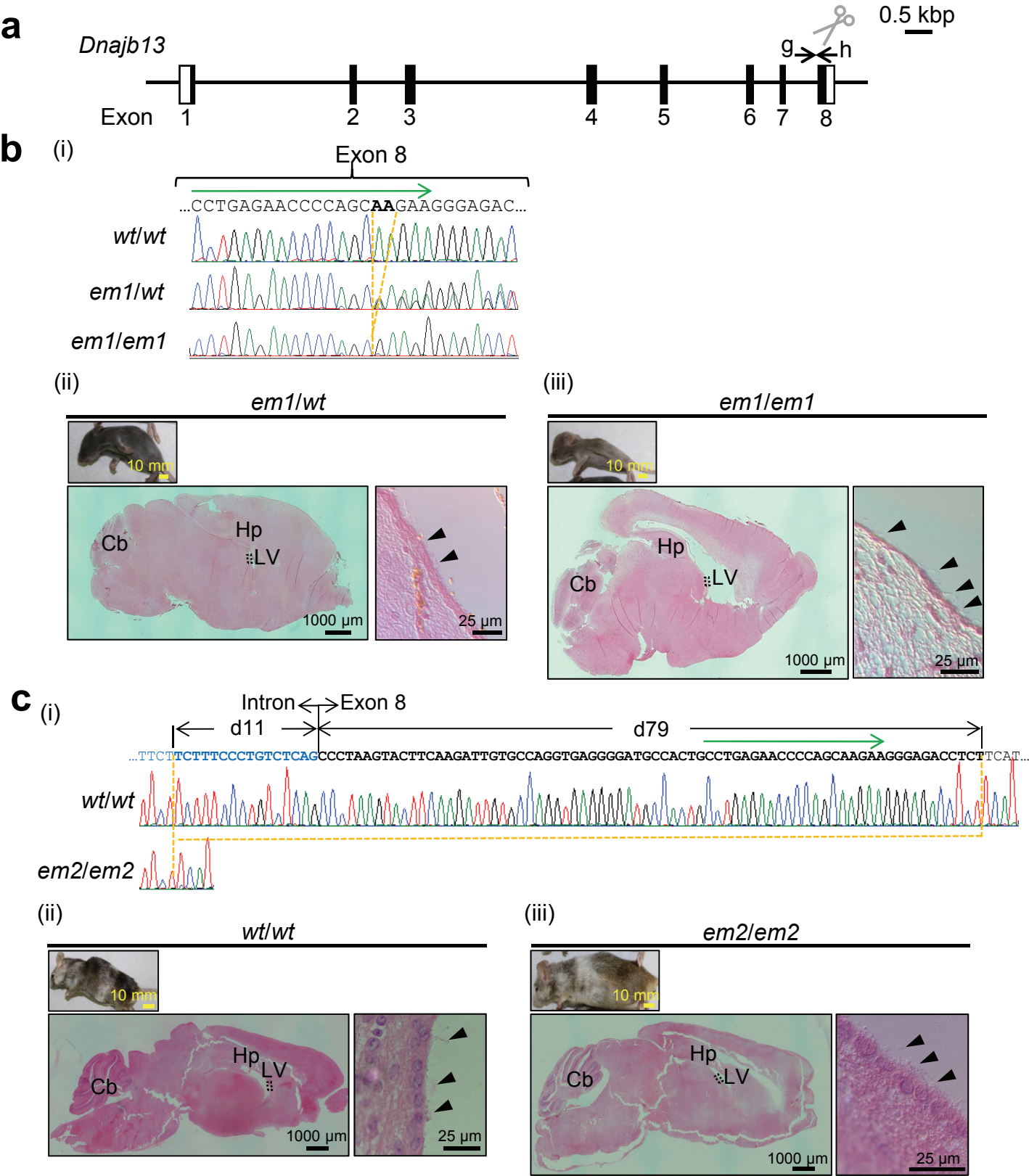
(b) *Dnajb13* mutant mice (*em1/em1*) generated by pronuclear injection. The deletion of 2 bp was identified by PCR and sequencing (i). The brain sections were prepared from 1 week old mice (upper panels) and observed after HE staining (lower panels) (ii).

(c) Chimeric mutant mice generated with WT and KO ESCs (*wt/wt* and *em2/em2*, respectively). The deletion of 90 bp was identified by PCR and sequencing (i). The brain sections were prepared from adult mice (upper panels) and observed after HE staining (lower panels) (ii). (b and c) Cb, Hp and LV indicate the cerebellum, hippocampus and lateral ventricle, respectively. A region of the LV shown as dashed-lined squares was magnified and then cilia were observed (arrowheads).

Supplementary Figure S1



Supplementary Figure S2



Supplementary Table S1. Target sequence of sgRNAs and oligonucleotides used for genotyping

sgRNA	target sequence (5' to 3')	PAM		PCR primer sequence (5' to 3')
#01	GAGGTTCTTGCTTGTGGCGC	TGG	F R	CCCTGTGTTGGTCAGTTTGCTTTCTACATTC GGAATGTTCTCTCTCCACAGCC
#02	GGCCAAGGTTGGGCTACTCC	AGG	F R	GGGTATGTCTTTCTCTCAGGGTCCTAGAG GCCTTGGCTTCATCAGTCTCTAGGT
#03	TTCCAGCAAGATGTAAGCTT	TGG	F R	GGTGGAAGCTGAGACAGCCC ATAAACACGGACTAACCTGC
#04	CCCCAGTGAGGGTCCGCACC	AGG	F R	GGGCAGGGTGGGAACAATCAAT GCAGCCTGTAGAAAGGAAAGTCGAGG
#05	GAGCAACAGGAGGTGTTTCA	TGG	F R	GGCCCTCAGCTGGGGGTCC CCAGCATTAGAAGGTAGAGGCAGGAGG
#06	TCTGATAGTGAGTGCAAGCT	TGG	F R	CCTTCTGTCTGGCAATGGAAT TGACAACTGTAGGTAGCCCCA
#07	GTTAGAGTGATGCACAAGGTT	TGG	F R	CACGTGTGATGCCACAGGTGCC GCAAGTAAGCCTCTGGGCTGTCC
#08	CAAAAGATGGCGACGCCCT	TGG	F R	ATTGAACCACAAGCCCGGCTCT TGAGGTCTCTACCGCTAAACGTGGA
#09	GGGTGGTCGCAGGGGGGCTC	TGG	F R	ATTGAACCACAAGCCCGGCTCT TGAGGTCTCTACCGCTAAACGTGGA
#10	CAGAACACACAAATGGCGGA	AGG	F R	GGGGAGAACAACCAATTGTTCAGCCC CTTGAAGCCATTGGGCCAGTTTGTGTTG
#11	CCACAAGGAAGTGAACGACC	TGG	F	AGGCCAGAAGTGCCTGCCTC
#12	ACAACAGAACAGCTCGGGAA	TGG	R	CATGGGAAACAGTCCGGTGG
#13	CAGGAAATATGGCACGAGCT	AGG	F	TACTTAGTCCACTCTGTACC
#14	GGTGATCGAGAGATAATACG	CGG	R	GGTCAGCGTTACAGTGAAGG
#15	CATCACAGTAAACAGTCCAA	AGG	F	GACCACATCTTTCATGTCC
#16	CTTCTGCGAGCTCATGAAGT	TGG	R	AGCAACTGAGAATGCAACCC
#17	CATCTATCTCATCACCATTG	TGG	F R	GTTTCATTTCAGGGACACCC CCACACACACAGACCATTGG
#18	ATCATGACAATCACAACTCG	TGG	F	AAATTAGAGATTTTAGGGCCTGGCATATGG
#19	TGAGGAAAAGAAAATGGCGG	CGG	R	TTTCAGAGTAGGGAGAGGCTCCAG
#20	AGGTATTTTCCAATTGCAGT	GGG	F	CCCTTGAACCTGGGATTGTTT
#21	TTGTACAAGTCTCATTCAAT	GGG	R	AGCCAGCCTCTAGTGGAGACA
#22	GGCAACGTTTGACTTCCTGA	AGG	F (a) R (b)	TGCAGGCGCATAGGGGCTGG AGTCACTTAATAAAGGTTGG
#23	AGCCACACATTTGATCTTCA	GGG	F R	CTTTGAAGACCCAGGAAAGG AAATGTCCTTAAGGAGTCCC
#24	CATCACAGTAAACAGTCCAA	AGG	F R	TAAGACAGAGGGGAAAGATTGG AGGGGCTAATTGCTCTCTTGG
#25	CACTTGATCAAACATTACCC	AGG	F R	TGGAGTCTGAGCAGATCCTG ACAGCGATATCCCTAACCCC
#26	GTGTTAGAGGATGAGCTAAC	AGG	F R	ATAAACCTTGGACATGGTAG TGGGACTTTACAAAGCAAG
#27	GCTGTCTGGAAATTGACTGC	TGG	F R	ATGACTAGGGAGGAGCAGAGAC ATTCCCATGACCACTCACTACC
#28	TACTCTGGTGATGAATGCCG	CGG	F R	GAAAGATGCTGCGCCAGAGG TAGCATCAAGGAGAATCTGG
#29	GAGAGATACTCAGAAGCTGCC	CGG	F R	CTACGTCAGTGCTGCCAAGTAGC CCATCATCACTAATGACCTCATGGCTGG
#30	GTCACATCCAGGACCCTGACA	CGG	F R	GGAGAACAGCCTGTGTAATGGTGGG CAAGACAGGGTTTCTCTGTGCTCCC
#31	GTTCCCTAAAGTGAAGTACCCT	GGG	F R	GGATGGATGGAAAGGAAACG GGAGCCAGAAGTCAGAACCA
#32	GAGAAAGGAAGGCCAGATCA	CGG	F R	ACTTCGTCTATTCTGCTCCGG ATGAGTCAGAGTGTGCACAGC
#33	AACAAGCGTCCAAGATGCTG	AGG	F R	AGGTCGATTGTCTGTGTGG CTCATTCCCAAGATGCTAGG

#34	GTTCGAGGAAATCATGAATGC	AGG	F R	GGTAGCTTTGCCTAGAGAGTCC ACCTTGTCAAATGGAGACTACC
#35	CGGCCCAGATCATGAAATCA	TGG	F R	CATTGTGATGAAAGACTTGAGGG ATTCTCTCCCCGACCTGACC
#36	TTTTGTTGATGACTTTCTGT	TGG	F R	TCCATCACCACCACCAGGTG TCTGTGCCTCAGGCCTTGGC
#37	GTCCCCGGTTCCTGGAGCAAC	TGG	F R	AGAGAGAGATGGCTTAGCCG GCAAAACACCCAAACACATC
#38	GTCATGAGCAAAGTCATCAGT	AGG	F R	TGTTCTAGTTAGTGCGTTGG CACCCACGTCATGAGAGAAG
#39	GAGACAGGGTTAGTCCAAAA	TGG	F R	CAGTGAGTGCCTAGGTGAAC CTCAGCTGCTGCTCCAGTAG
#40	GAATTCTGTAGTCTAAGATG	TGG	F R	CTAGACCGACTATTCTCAGG TGTACAAGCAGACAACGTCAGG
#41	AGGGATAGACGGTGATCTGA	TGG	F R	CTAGACCGACTATTCTCAGG TGTACAAGCAGACAACGTCAGG
#42	GTTGCAGATCACGAGGGAAG	AGG	F R	GCTGCGGGGCGGGTGC CTGCTTACATAGTCTAACTCGCGACACTG
#43	CCTGAGAACCCAGCAAGAA	GGG	F (g) R (h)	GCTCTGGTGTGTGAGACCTG TAGAGATAGTCTGTTGTGAG

Supplementary Table S2. HDR-mediated small mutations using ssODN as a reference

pronuclear-injection				ESC-transfection		
sgRNA	analyzed pups	GMO	HDR (%)	sgRNA	analyzed clones	HDR (%)
#22	13	5	1 (7.7)	#22	56	7 (12.5)
#23	29	17	0 (0)	#23	24	0 (0)
#24	5	0	0 (0)	#24	16	2 (12.5)
#25	8	5	2 (25.0)	-	-	- -
#26	4	1	1 (25.0)	-	-	- -
#27	6	2	0 (0)	-	-	- -
#28	26	0	0 (0)	-	-	- -
#29	35	1	0 (0)	#29	96	0 (0)
#30	34	2	1 (2.9)	#30	96	0 (0)
#31	13	1	0 (0)	-	-	- -
#32	27	9	1 (3.7)	-	-	- -
#33	14	7	2 (14.3)	-	-	- -
#34	18	8	1 (5.6)	-	-	- -
-	-	-	- -	#35	16	3 (18.8)
-	-	-	- -	#36	144	0 (0)
total (13) ^a	232	58	9 (3.9)	total (7) ^a	448	12 (2.7)

GMO: Genetically Modified Organism, HDR: Homology-Dependent Repair.

^aThe numbers in parentheses indicate the number of targets.

Supplementary Table S3. HDR-mediated small mutations using dsDNA as a reference

pronuclear-injection				ESC-transfection			
sgRNA	analyzed pups	GMO	HDR (%)		sgRNA	analyzed clones	HDR (%)
#22	25	2	0	(0)	#22	55	13 (23.6)
#23	15	3	0	(0)	#23	56	24 (42.9)
#24	2	0	0	(0)	#24	16	6 (37.5)
-	-	-	-	-	#35	16	13 (81.3)
-	-	-	-	-	#36	16	7 (43.8)
#37	6	3	0	(0)	#37	16	6 (37.5)
-	-	-	-	-	#38	16	5 (31.3)
-	-	-	-	-	#39	24	6 (25.0)
-	-	-	-	-	#40	16	7 (43.8)
-	-	-	-	-	#41	16	15 (93.8)
total (4) ^a	48	8	0	(0)	total (10) ^a	247	102 (41.3)

GMO: Genetically Modified Organism, HDR: Homology-Dependent Repair.

^aThe numbers in parentheses indicate the number of targets.

Supplementary Table S4. Oligonucleotides used for HDR-mediated point mutations or tag insertions

sgRNA-mutation	sequence (5' to 3')
#22-EcoRI	CAGGCTTAGGACCCACCTTTCTCTTGTAGCTGGTAGAGGCAACGTTTGAA TTCCTGAAGGTGGACGCCATCCTCCACAACAGTTGGGATCTTAACGGTGC
#23-FLAG	ACGTTTGGGATTTTTTCCTTTATATAATTAGCCACACATTTGATCTTCAG TGT TTCGTCGTCATCCTTATAATCCATGGCGGCAACGGGGCAGGAAC AGCTGAAGGAAGGGCATGGGACCTGCCATCCATTTT
#24-FLAG	TGCGAACCTTGAACCTTCCTCACTGTTTATTTATGTTTGGTACTTCAGAG AG GATTACAAGGATGACGATGACAAGTGACAGTCCAAAGGAATGTTGGTA CATCTTTTATTAACCATAAAAATAAAAGACT
#25-PM	GAACGCGGCTCAGGTGATGGTAACACAACAGTCCCTCTTGGAGCACTTGA T TAAACATCATCCAGGTAAAGCAATTCCGCCCTTGGTGTTTCATCCGAATG TGTTTGGTTTCC
#26-PM	CTTCACGTGGTCGTTTGGAAACACCGTAGACTTCTATGATCACTACTATGT CAGCGGTGTTAGACA AAGCTTGAACAGGCAACTGGATCCCCTGATGATC TGTGCAAACACACATGACCAGAAGAACTTA
#27-PM	TATGACACTATTTTGCCTCCCTTTTGCTAGTGCTCTTGTGAAAGGCTGCC GCTGTCTGGAAAT ACGTTGCTGGGATGGATCCCAAAATGAACCCATTGTG TACCATGGTTACACATTCACCAGCAAGCTT
#28-PM	TGACTACAAATGTTTCAAACATTTTCATTTCTTTTTCTCTTTGTAGTCC ATGAGTTTATTTTCC CACAGCTGTCATTCATCACCAGAGTATACCCCAAA ATGATGAGAGCAGACTCTTCTAACTTTAAC
#29-PM	AAACCATCTTCGTCAGGCAGCCGTGTTTAGATGTCTGGAGAGATACTCAG AG GCCGCCCCAGTATGTTTGTACAACTTTCTGAAGATTATTTAAAGTTA AATTAATTTT
#30-FLAG	ACTGGCTCCAGAGTGACGTGTCACATCCAGGATCCTGACACG CCACCATG GACTACAAGGATGACGATGACAAGATACTGCAGGCCTGGAGATCTCTGCA GCTGCTGTACCTCTTAGAAGCCAT
#31-PM	TGATCTGACTTGGATCCTGACGCGGTGGGTTTACTTCGTCATTGCATT CTTCAATTTAGCGCT AGACTTTTCTAAAGTGAAGTACCCTGGGGCAGGAC ATAGGTGCACAGAACCACATTAGGTACAG
#32-FLAG	GGCACAGGGTACTTGGCAGGTGGGAGAAAGGAAGGCCAGATCACGGAGCC TGATCA TTTATCGTCGTCATCCTTATAATCTGGAGCTGGAGCCTCTGGGA AGACATGGCTTGCGTGATATGTTGAGCCCT
#33-FLAG	GGAGCAAGAGGTGGCCCTGTGCAGGGAAGGAGGAGCGGGAACAAGCGGCC GCCATG GATTATAAGGATGACGACGATAAACTGAGGGTTGTTGTGGAGTC CGCTTCGATTAACCTCCCTCAGCACCAC
#34-PM	GTGCTGTATCTATGGAGCTTAAAGATTAAACCATCCTAAACATTGTTTCT GCTTCGAGGAAAT GCTGAATGCAGGCATCTTACAGAGTACTTCACCTTCA AACAGGAATGTGAGTATACATCTCTCCAGA
#36-PM	TCTGTGAGACCTCTTGATGTGTCTTTATTTAGGTTGCTTTTACGTTTTG T GGCCGCTTCTCTGCTGGTGACGCCTCACTTGGACCAAGCAAAAACCTTC CTCAGGTAAGGACCATCAT

PM: point mutation.

Red-colored letters indicate point mutations and/or FLAG sequences.

Supplementary Table S5. Oligonucleotides used for cloning of homology arms

sgRNA	homology arm (kb)		sequence (5' to 3')
#22	5' (0.5)	F	CTGCCAGGAAAGTTTACTAAGG
		R	TTgaattcCTGAAGGTGGACGCCATCC
	3' (0.5)	F	AGgaattcAAACGTTGCCTCTACC
		R	AActcgagCAGTCACTTAATAAAGGTTGG
#23	5' (0.5)	F	TTggatccGGGTCAGGAAACTGTAAAGG
		R	TTgaattcCCATGGCGGCAACGGGGCAGGAACAGCTGAAGG
	3' (0.5)	F	CGCCATG GATTATAAGGATGACGACGATAAA AACTGAAG
		R	ATCAAATGTGTGG
#24	5' (0.5)	F	TTtctagaTGGATCATTTTCGGGGCTGG
		R	TTggatccTCA CTTGTCATCGTCATCCTTGTAATC CTCTC
	3' (0.5)	F	TGAAGTACCAAACAT
		R	TTggatccAGGAATGTTGGTACATCTT
#35	5' (0.5)	F	TTtctagaTGAGAAAGAGTATGGTTCCC
		R	TTggatcc CTTGTCATCGTCATCCTTGTAATC GATCATGA
	3' (0.5)	F	TTTCATGATCTGGGCCG
		R	TTggatccGAAAGCCAACTTCATGAGCT
#36	5' (0.5)	F	TTtctagaTACAGAAGTCAGAGCACTGG
		R	TCTGTGAGACCTCTTGATGTGTCTTTTATTTAGGTTGCTT
	3' (0.5)	F	TTACGTTTTGT ggccGCCTTCTGCT GGTGACGCCTCACT
		R	TGGACCAAGCAAAAACCTTCCTCAGGTAAGGACCATCAT
#37	5' (0.5)	F	AAgaattcGGCAAATAGCCCTTCTGTGG
		R	AAgaattcACCACATGGTGGCTCACAGC
	3' (0.5)	F	AAgaattcAATCCATGGCGGCCGTGCTGCCTTGTGGCTAGG
		R	CGccatg ATTATAAGGATGACGACGATAAA GGCAAGACC
#38	5' (0.5)	F	ATTCCCCGGTTCC
		R	AActcgagACTCTGGGCAAAAGGTGTCTC
	3' (0.5)	F	AAgctagcGTGTCAGGAGACTAAAAGAGC
		R	AAgaattcATCCATGGCGGCTGGAGAAAGGAGAACGAC
#39	5' (0.5)	F	CGccatg ATTATAAGGATGACGACGATAAA GGTACTAGG
		R	AAAAAGGTTCAAGCG
	3' (0.5)	F	AAactcgagCTGCAAGCTGCTCTCTGACTTCC
		R	ATtctagaCCATAGCTTTCCTTCTCCTCC
#40	5' (0.5)	F	TGgatatcTTAG GGTGCTGTCCAGGCCCAGCAGGGGGTTGG
		R	GGATGGGCTTGCC GTCCAAAATGGTCTGAAAGTCTGTGC
	3' (0.5)	F	AAgatatcCATTGTAGCCTCAGCACTCC
		R	AAactcgagAAAATCCTGCTTTTATAAGTTGG
#42	5' (0.5)	F	AAgctagcACCCAAGACTGAAATGAAGG
		R	AAgaattcTCAGTACAGTTGTTAGTGG
	3' (0.5)	F	GAgaatcTGTAAGT CGACTACAAAGACGATGACGACAAGT
		R	AAgctagcATCTCCAGGGAGGGTGGGAGG
#42	5' (1.0)	F	AAgagctcGGTCAGCGAAAGTAGCTCGC
		R	AAaccgcggAAAGGTATTGCAACACTCCC
	3' (1.0)	F	AAactcgagGTGGGCGTTGTCCTGCAGGG
		R	TTggtaccATGCCAATGCTCTGTCTAGG

Red-colored letters indicate point mutations and/or FLAG sequences.

Small letters indicate the restriction enzyme sites.

Supplementary Table S6. Oligonucleotides used for genotyping PCR

gene		sequence (5' to 3')	
#22-EGFP	5'	F (c)	ATGTAACAATACAGTGGATTCTCC
		R (d)	TCACCTTGATGCCGTTCTTCT
	3'	F (e)	GCCACCATGGTGAGCAAGGGCGAG
		R (f)	AGATGACCTGGAGTTATGTAAGCC
#42-zeocin	5'	F	CTCAGAGAGCCTCGGCTAGG
		R	AACGGCACTGGTCAACTTGG
	3'	F	CAACTGCGTGCACTTCGTGG
		R	AGAATGCCATGAGTCAAGCC
Gapdh (control)		F	AGTGGAGATTGTTGCCATCAACGAC
		R	GGGAGTTGCTGTTGAAGTCGCAGGA

Supplementary Movies S1 and S2. Motility of tailless *Dnajib13* KO spermatozoa

The DIC image and GFP fluorescence of the *Dnajib13* KO spermatozoa were shown in Movies S1 and S2, respectively.

Supplementary Movies S3 and S4. Motility of short-tailed *Dnajib13* KO spermatozoa

The DIC image and GFP fluorescence of the *Dnajib13* KO spermatozoa were shown in Movies S3 and S4, respectively.