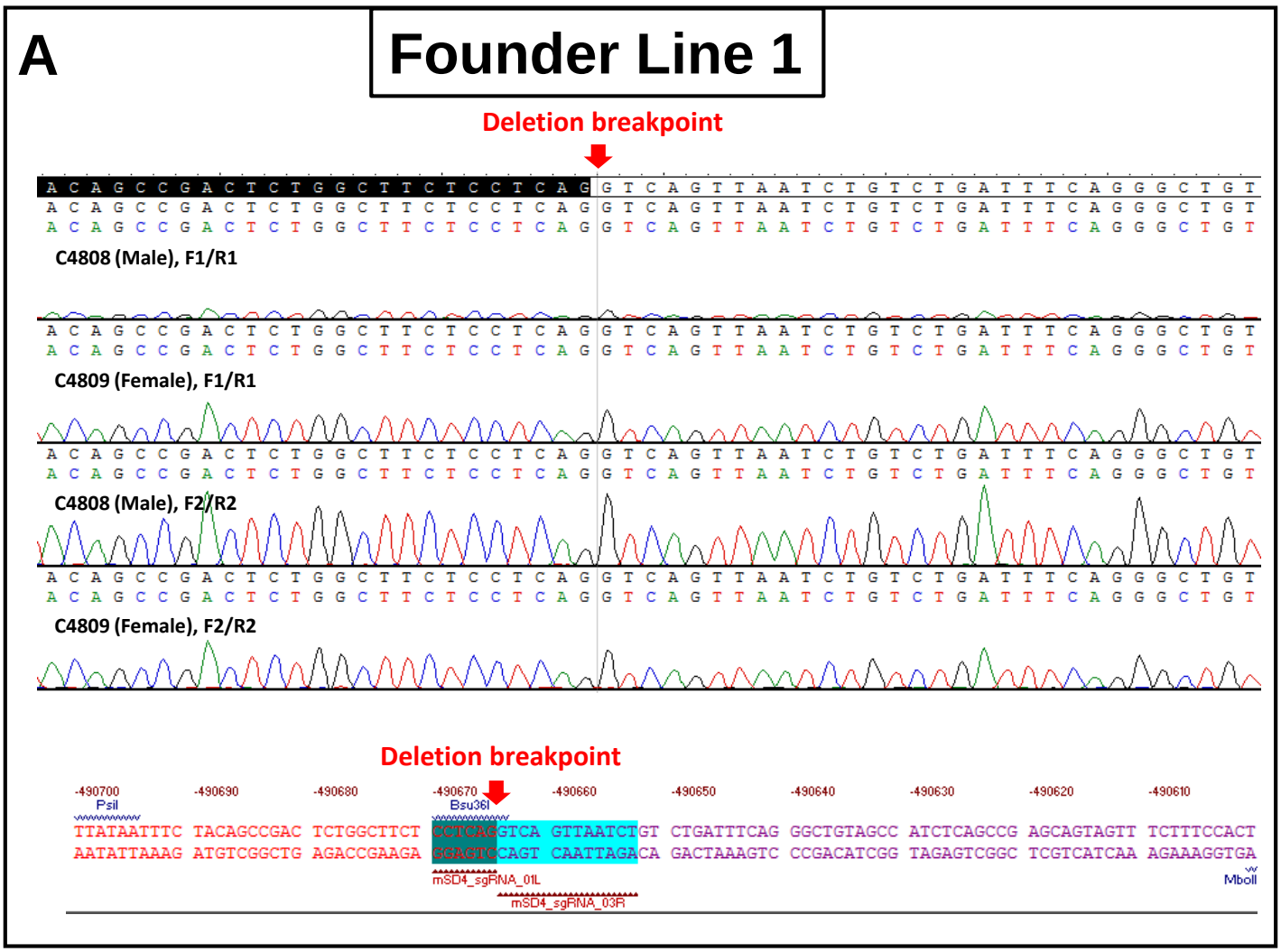
Table S1

Enhancer (rel. to TSS)	Forward primer seq (5' - 3')	Reverse primer seq (5' - 3')	Primer (genomic) co-ordinates	Amplicon size (kb)
-180kb	ACTCCAGGACCCTGTTTGTG	GCAAACAGCATCACAGAGGA	chr15:36,867,314 - 36,868,655	2,341
-97kb	AGGTCAGCCTCAGCACTGTT	AGAAGCTGCCAGGTGACTGT	chr15:37,064,191 - 37,066,506	2,316
-40kb	CCGCTCACTTCTGTGGTACA	AGCCAGTGCTGGGCTAGATA	chr15:37,121,875 - 37,122,942	1,068
-34kb	CAGAAACAGTCCCTCGCTTC	AGCAACAACTGTGTGCAAGG	chr15:37,127,220 - 37,129,961	2,742
-23kb	AAAAGGCGTTTCCTCTGGAT	TCCTTCTACCACGTGGGTTC	chr15:37,139,015 - 37,140,627	1,613
Prox. Prom.	GGAGAAGGGCCTTACCTGAG	TCGTACTCGGCCTCAAAATC	chr15:37,162,686 - 37,163,910	1,225

Table S2

sgRNA sequences	Founder line	Deletion co-ordinates (mm9)	Size of deletion (kb)	Genotyping primers used	Amplicon size (genotyping)
sgRNA_01L	C4379	chr15:37,126,673 - 37,130,417	3,745bp	F:5'-attttctgtgggcttgtctgtt-3'	761bp
sgRNA_03R	C4386	chr15:37,126,674 - 37,130,413	3,740bp	R:5'-gaggcagatcatacacaccaaa-3'	

Fig. S1


A
Founder Line 1
Deletion breakpoint
C4808 (Male), F1/R1
C4809 (Female), F1/R1
C4808 (Male), F2/R2
C4809 (Female), F2/R2
Deletion breakpoint
-490700
-490690
-490680
-490670
-490660
-490650
-490640
-490630
-490620
-490610
PsiI
Bsu36I
TTATAATTTC TACAGCCGAC TCTGGCTTCT CCTCAGGTCA GTTAATCTGT CTGATTTCAG GGCTGTAGCC ATCTCAGCCG AGCAGTAGTT TCTTTCCACT
AATATTAAAG ATGTCGGCTG AGACCGAAGA GGAGTCCAGT CAATTAGACA GACTAAAGTC CCGACATCGG TAGAGTCGGC TCGTCATCAA AGAAAGGTGA
mSD4_sgRNA_01L
mSD4_sgRNA_03R
MboII

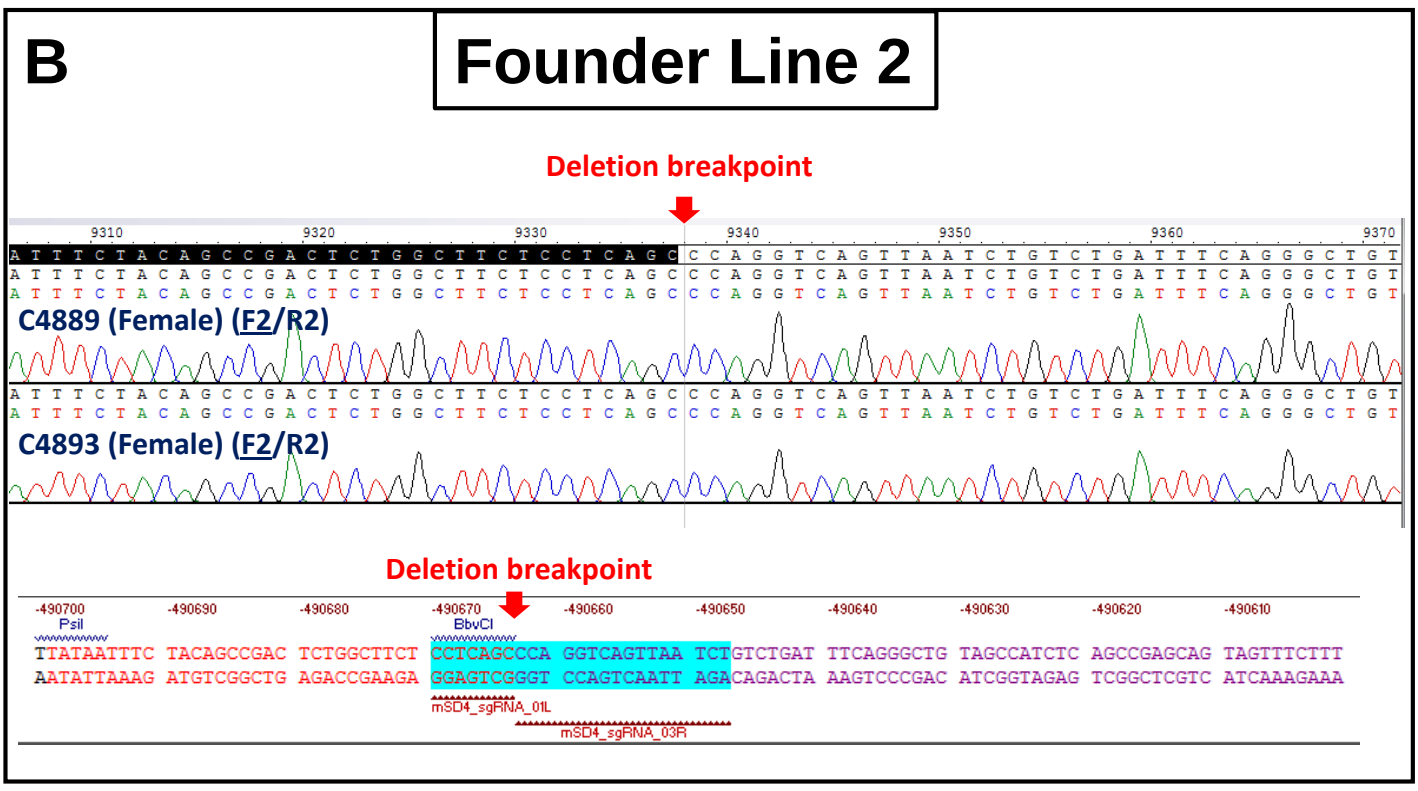
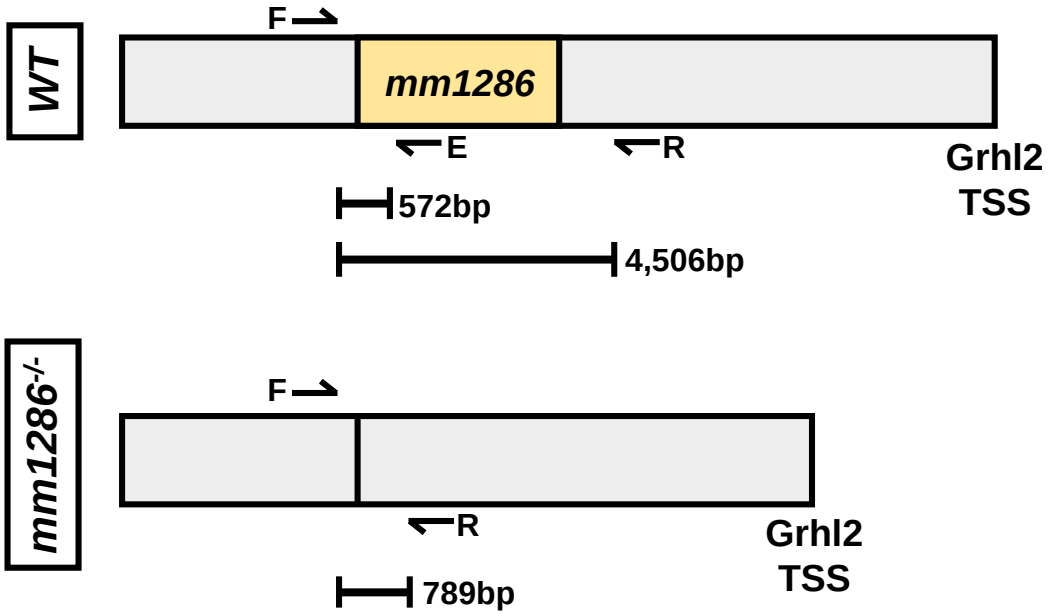

B
Founder Line 2
Deletion breakpoint
9310
9320
9330
9340
9350
9360
9370
C4889 (Female) (F2/R2)
C4893 (Female) (F2/R2)
Deletion breakpoint
-490700
-490690
-490680
-490670
-490660
-490650
-490640
-490630
-490620
-490610
PsiI
BbvCI
TTATAATTTC TACAGCCGAC TCTGGCTTCT CCTCAGCCCA GGTCAGTTAA TCTGTCTGAT TTCAGGGCTG TAGCCATCTC AGCCGAGCAG TAGTTTCTTT
AATATTAAAG ATGTCGGCTG AGACCGAAGA GGAGTCGGGT CCAGTCAATT AGACAGACTA AAGTCCCGAC ATCGGTAGAG TCGGCTCGTC ATCAAAGAAA
mSD4_sgRNA_01L
mSD4_sgRNA_03R

Fig. S2

A


WT
F
mm1286
E
R
Grhl2
TSS
572bp
4,506bp
mm1286-/-
F
R
Grhl2
TSS
789bp

B

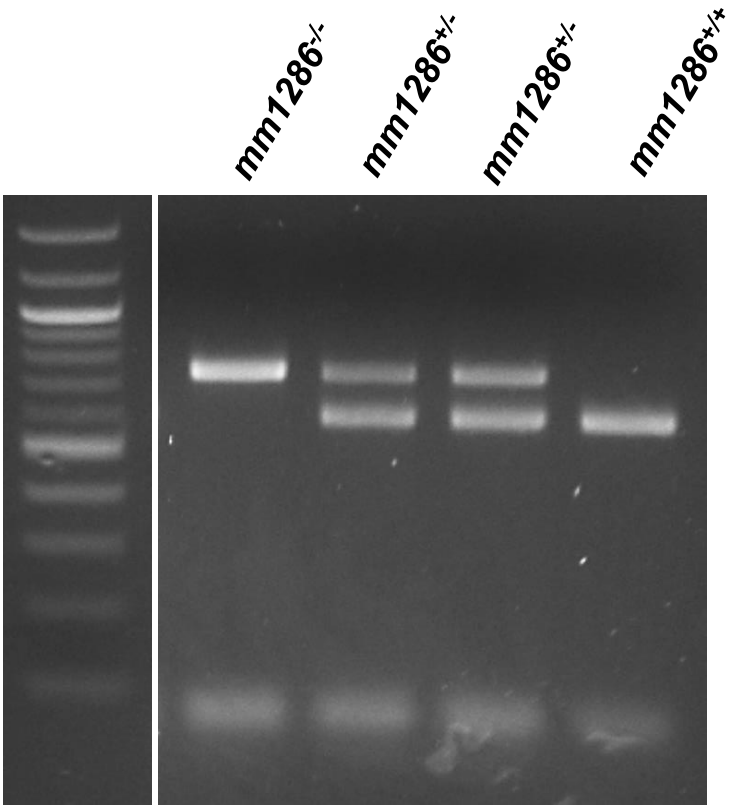

mm1286-/-
mm1286+/-
mm1286+/-
mm1286+/+

Fig. S3

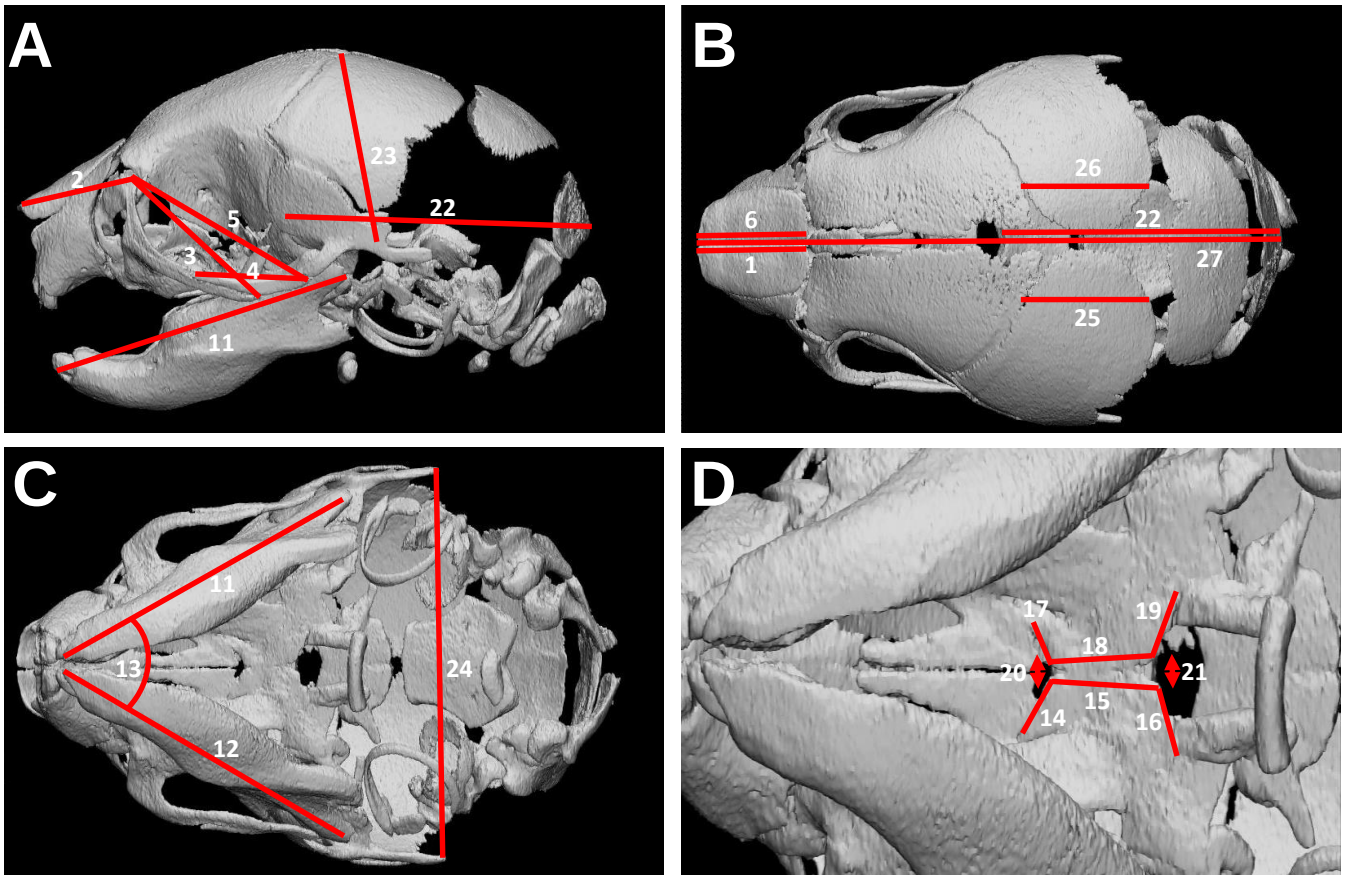


Table S3

Skull landmarks for μCT analysis

Mark	Location
N1	Most antero-medial point of the nasal bone, left side
N2	Most postero-medial point of the nasal bone, left side
N3	Most antero-medial point of the nasal bone, right side
N4	Most postero-medial point of the nasal bone, right side
M1	Intersection of frontal process of maxilla with frontal and lacrimal bones, left side
M2	Intersection of zygoma with zygomatic process of maxilla, taken on maxilla, left side
M3	Intersection of zygoma with zygomatic process of maxilla, taken on zygoma, left side
M4	Intersection of zygoma with zygomatic process of temporal, taken on zygoma, left side
M5	Intersection of frontal process of maxilla with frontal and lacrimal bones, right side
M6	Intersection of zygoma with zygomatic process of maxilla, taken on maxilla, right side
M7	Intersection of zygoma with zygomatic process of maxilla, taken on zygoma, right side
M8	Intersection of zygoma with zygomatic process of temporal, taken on zygoma, right side
J1	Inferior-most point on incisor alveolar rim at midline of the incisor at bone-tooth junction, left side
J2	Most caudal point on the ventral angle of the condyloid process, left side
J3	Inferior-most point on incisor alveolar rim at midline of the incisor at bone-tooth junction, right side
J4	Most caudal point on the ventral angle of the condyloid process, right side
Ja	Angle between J1-J2 and J3-J4 at the anterior point of the mandible
P1	Most anterolateral point on the posterior palatine plate, left side
P2	Most anteromedial point on the posterior palatine plate, left side
P3	Most posteromedial point on the posterior palatine plate, left side
P4	Most posterolateral point on the posterior palatine plate, left side
P5	Most anterolateral point on the posterior palatine plate, right side
P6	Most anteromedial point on the posterior palatine plate, right side
P7	Most posteromedial point on the posterior palatine plate, right side
P8	Most posterolateral point on the posterior palatine plate, right side
S1	Most antero-medial point on the body of the sphenoid
S2	Mid-point on the posterior margin of the foramen magnum, taken on squamous occipital
S3	Most medial intersection of the frontal and parietal bones, taken on the frontal, left side
S4	Most medial intersection of the frontal and parietal bones, taken on the frontal, right side
S5	Most postero-medial point on the parietal, left side
S6	Most postero-medial point on the parietal, right side
S7	Most antero-lateral point on corner of the basioccipital, left side
S8	Most antero-lateral point on corner of the basioccipital, right side
S9	Most posterior point on the posterior extension of the forming squamosal, left side
S10	Most posterior point on the posterior extension of the forming squamosal, right side

Fig. S4

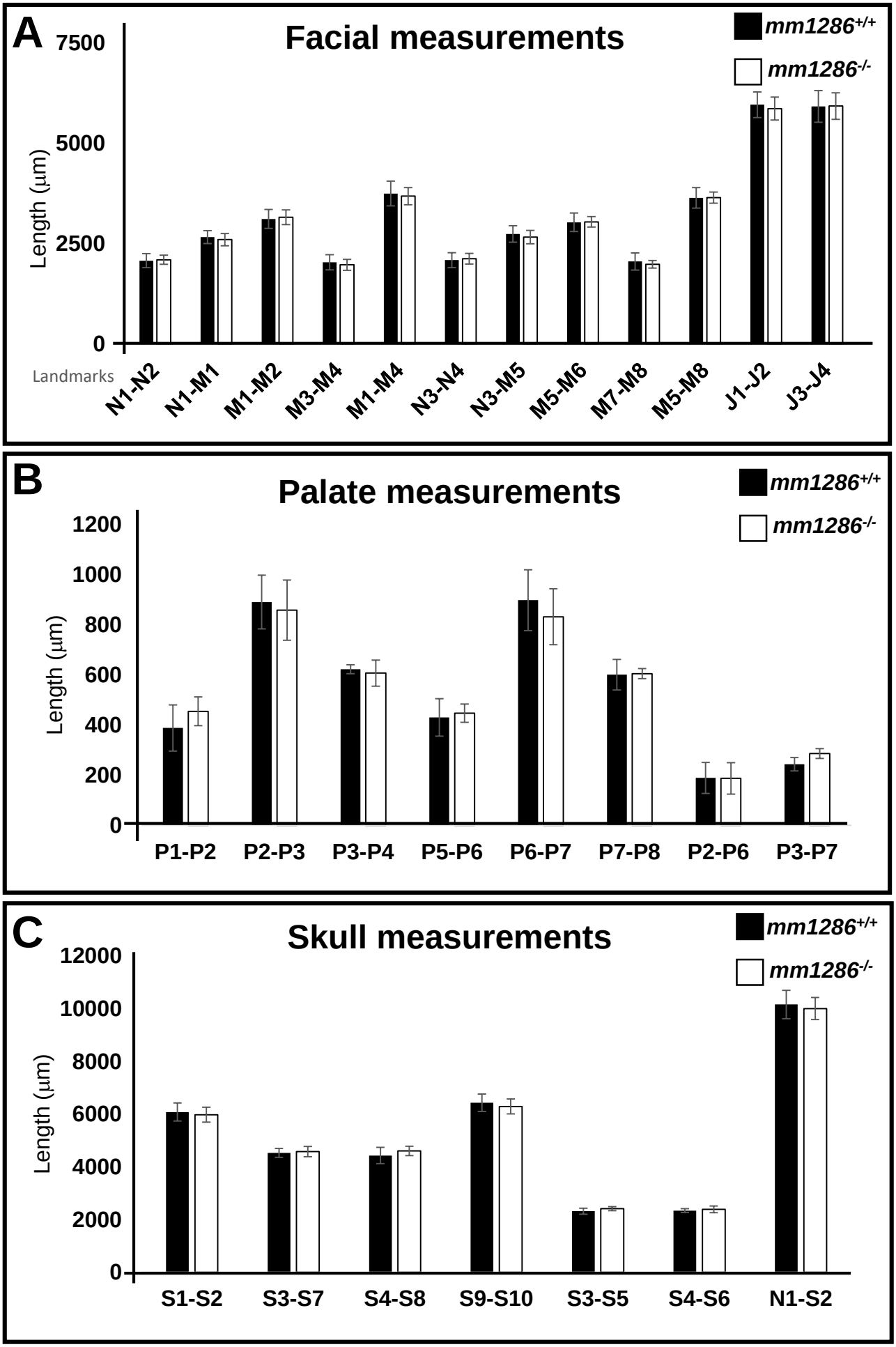

A
Facial measurements
mm1286+/+
mm1286-/-
Length (μm)
7500
5000
2500
0
Landmarks
N1-N2
N1-M1
M1-M2
M3-M4
M1-M4
N3-N4
N3-M5
M5-M6
M7-M8
M5-M8
J1-J2
J3-J4
B
Palate measurements
mm1286+/+
mm1286-/-
Length (μm)
1200
1000
800
600
400
200
0
P1-P2
P2-P3
P3-P4
P5-P6
P6-P7
P7-P8
P2-P6
P3-P7
C
Skull measurements
mm1286+/+
mm1286-/-
Length (μm)
12000
10000
8000
6000
4000
2000
0
S1-S2
S3-S7
S4-S8
S9-S10
S3-S5
S4-S6
N1-S2

Table S4

	μCT Measurements			
Point	**Marks**		**Description**	**Annotation**
	FACE			
1	N1	N2	Length of left nasal bone	N1-N2
2	N1	M1	Approximate premaxillary length, Left	N1-M1
3	M1	M2	Maxilla length, Left	M1-M2
4	M3	M4	Zygomatic length, Left	M3-M4
5	M1	M4	Maxilla zygomatic length, Left	M1-M4
6	N3	N4	Length of right nasal bone	N3-N4
7	N3	M5	Approximate premaxillary length, Right	N3-M5
8	M5	M6	Maxilla length, Right	M5-M6
9	M7	M8	Zygomatic length, Right	M7-M8
10	M5	M8	Maxilla zygomatic length, Right	M5-M8
11	J1	J2	Jaw length, Left	J1-J2
12	J3	J4	Jaw length, Right	J3-J4
13	J1-J2	J3-J4	Mandible angle	Ja
	PALATE			
14	P1	P2	Anterior palate shelf width,Left	P1-P2
15	P2	P3	Palate shelf length,Left	P2-P3
16	P3	P4	Posterior palate shelf width,Left	P3-P4
17	P5	P6	Anterior palate shelf width,Right	P5-P6
18	P6	P7	Palate shelf length,Right	P6-P7
19	P7	P8	Posterior palate shelf width,Right	P7-P8
20	P2	P6	Anterior space between shelves	P2-P6
21	P3	P7	Posterior space between shelves	P3-P7
	SKULL			
22	S1	S2	Length of skull vault	S1-S2
23	S3/S4	S7/S8	Height of skull vault	S3-S7/S4-S8
24	S9	S10	Width of skull vault	S9-S10
25	S3	S5/S6	Distance of parietal sutures	S3/S4
26	S4	S5/S7	Distance of parietal sutures	S4/S6
27	N1	S5/S8	Length of skull	N1-S2

Table S5

A

mm1286$^{+/-}$ x mm1286$^{+/-}$

E14.5	Observed	Expected	Observed %	Expected %	χ-square
mm1286 $^{+/+}$	4	5	20.0%	25%	0.861
mm1286 $^{+/-}$	11	10	55.0%	50%	
mm1286 $^{-/-}$	5	5	25.0%	25%	
TOTAL	20				

P0	Observed	Expected	Observed %	Expected %	χ-square
mm1286 $^{+/+}$	15	11	34.1%	25%	0.376
mm1286 $^{+/-}$	19	22	43.2%	50%	
mm1286 $^{-/-}$	10	11	22.7%	25%	
TOTAL	44				

B

Grhl2$^{+/-}$ x mm1286$^{+/-}$

P0	Observed	Expected	Observed %	Expected %	χ-square
Grhl2$^{+/+}$;mm1286$^{+/+}$	20	19.25	25.5%	25%	0.986
Grhl2$^{+/+}$;mm1286$^{+/-}$	19	19.25	23.2%	25%	
Grhl2$^{+/-}$;mm1286$^{+/+}$	20	19.25	25.5%	25%	
Grhl2$^{+/-}$;mm1286$^{+/-}$	18^	19.25	20.9%	25%	
TOTAL	77				

^3/18 embryos showed palatal clefting; p=0.011

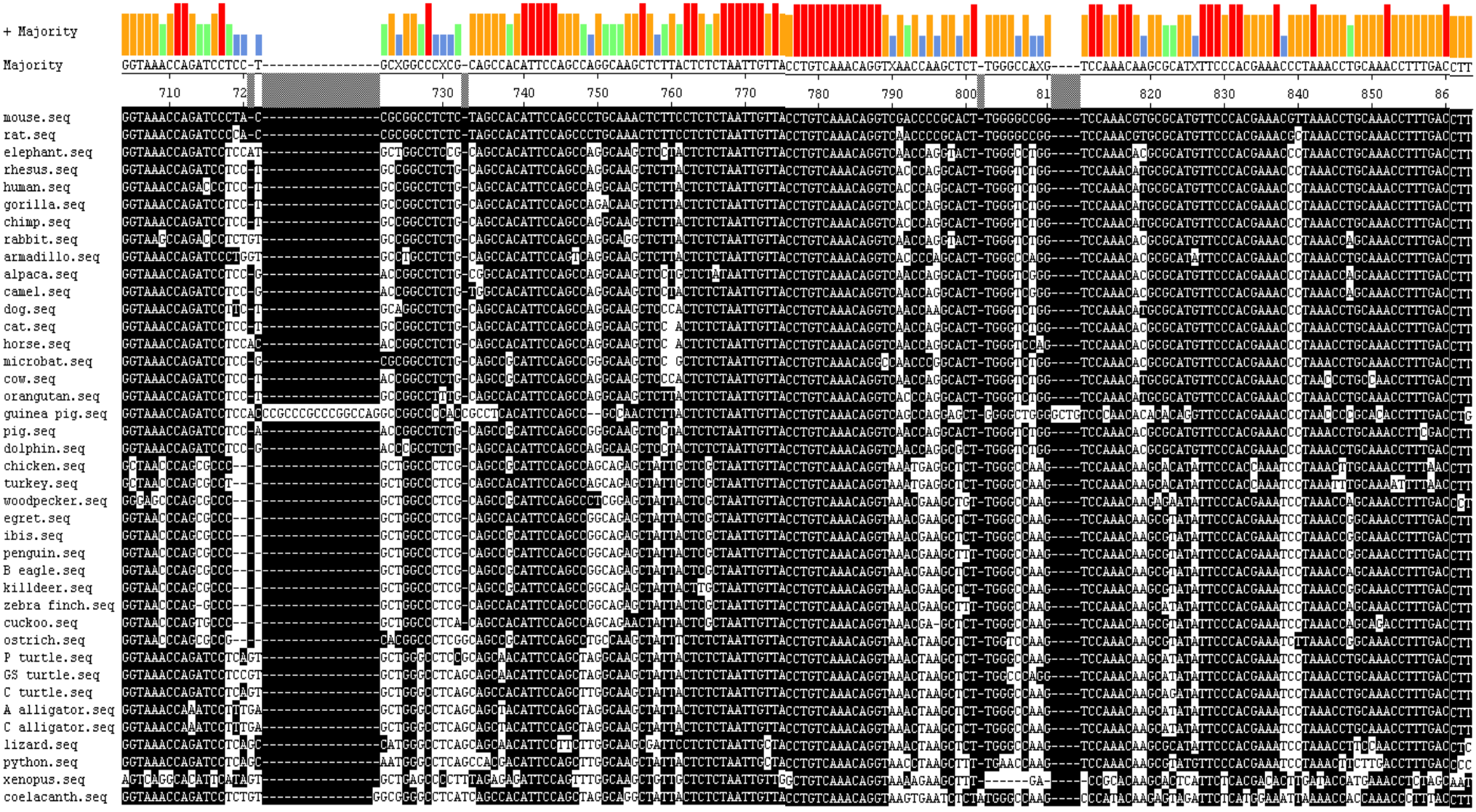

+ Majority
Majority
710
720
730
740
750
760
770
780
790
800
810
820
830
840
850
860
mouse.seq
rat.seq
elephant.seq
rhesus.seq
human.seq
gorilla.seq
chimp.seq
rabbit.seq
armadillo.seq
alpaca.seq
camel.seq
dog.seq
cat.seq
horse.seq
microbat.seq
cow.seq
orangutan.seq
guinea pig.seq
pig.seq
dolphin.seq
chicken.seq
turkey.seq
woodpecker.seq
egret.seq
ibis.seq
penguin.seq
B eagle.seq
killdeer.seq
zebra finch.seq
cuckoo.seq
ostrich.seq
P turtle.seq
GS turtle.seq
C turtle.seq
A alligator.seq
C alligator.seq
lizard.seq
python.seq
xenopus.seq
coelacanth.seq
Homeobox TF recognition sequence

Fig. S5