

SHH signaling directed by two oral epithelium-specific enhancers controls tooth and oral development

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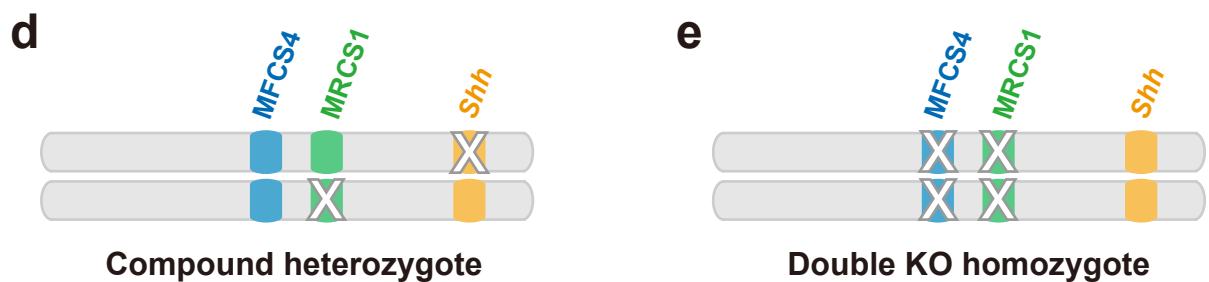
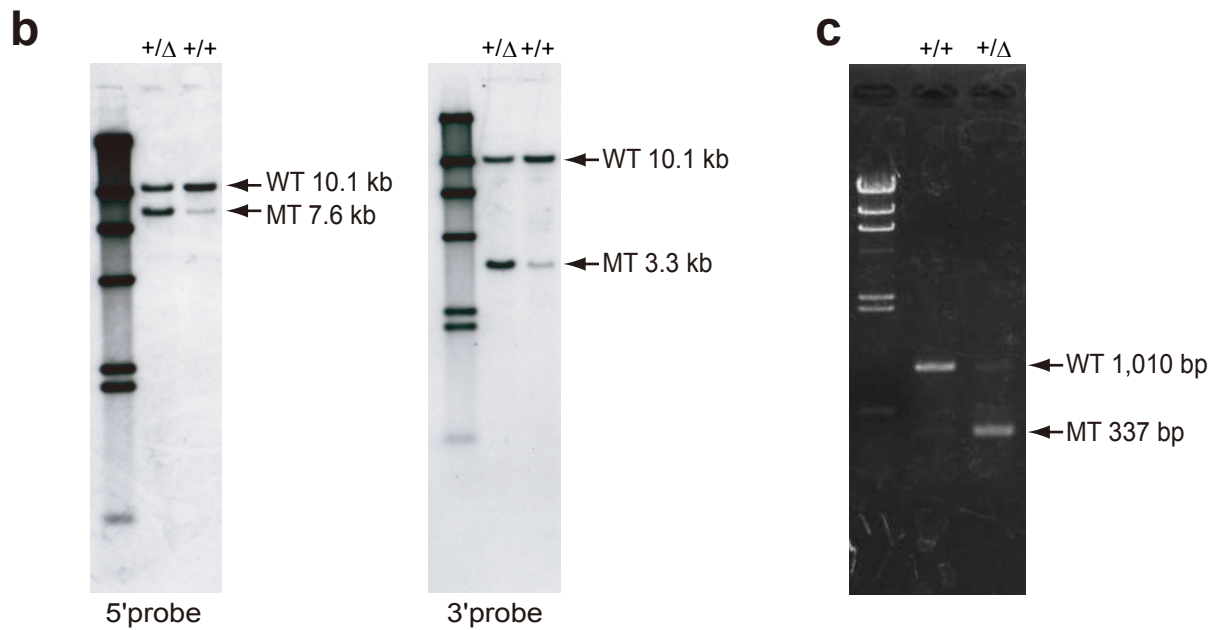
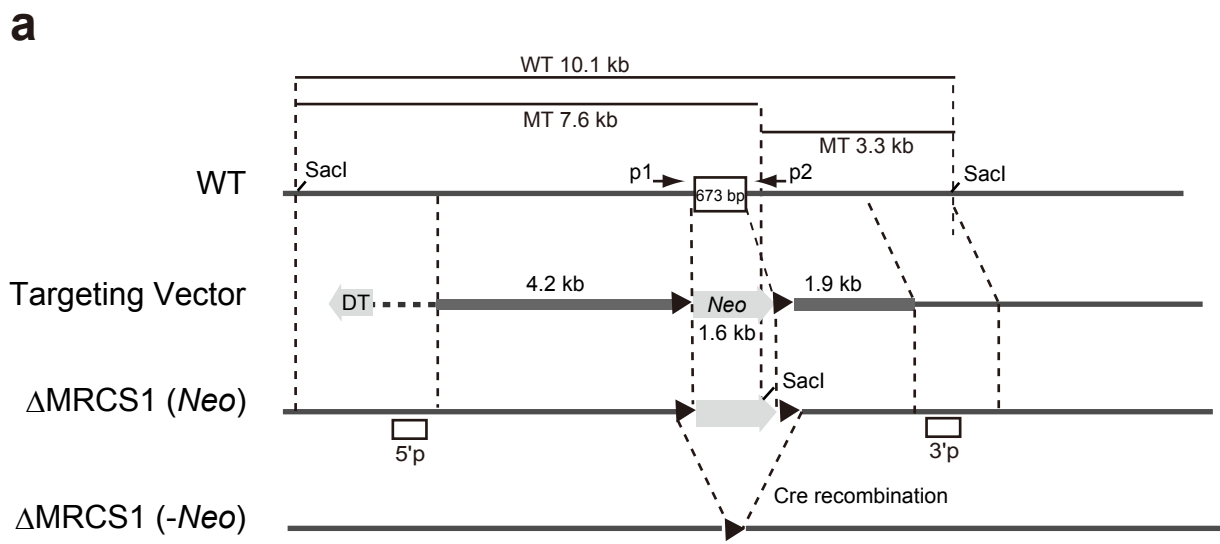
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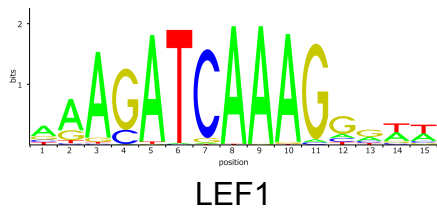
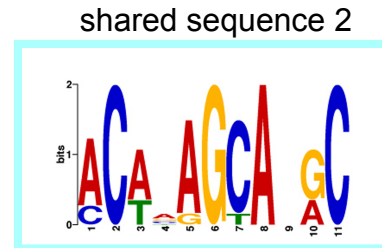
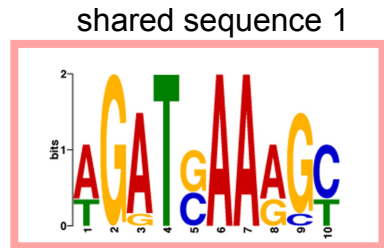
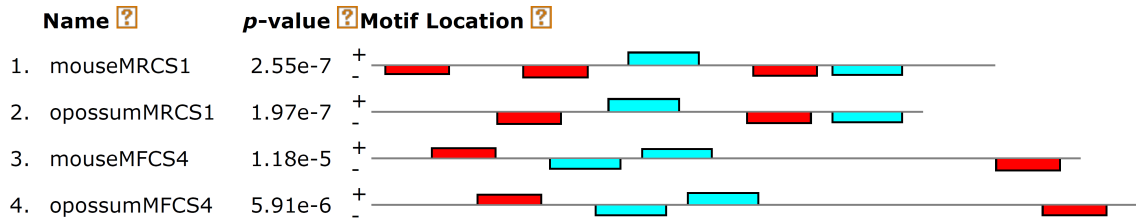
#These two authors contributed equally to this study.

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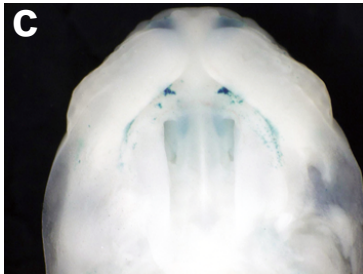


Supplementary Fig. 1. Generation of mutant mice in this study. (a) Schematic diagram of the ES cell targeting of MRCS1. (b) Southern blot analysis was carried out for Sacl-digested genomic DNAs. The 5' probe (5' p in (a)) detected fragments of 10.1 kb in the wild type allele (WT) and 7.6 kb in the mutant allele (MT). The 3' probe (3' p in (a)) detected fragments of 10.1 kb in WT and 3.3 kb in MT. (c) PCR amplification of the WT allele (1,010 bp) and the MT allele (337 bp) with a primer pairs p1-p2. The left lanes are λ /HindIII marker. Schematic diagram of the mouse chromosome 5 illustrating composition of mutant alleles in the compound heterozygote of the MRCS1 KO and the Shh coding sequence KO alleles (d), and the homozygote of the double KO mouse of MRCS1 and MFCS4 (e).

a



b



d

KB021656:129180776-129180914 (xenTro7)

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CATGGGATTCTTTCCGTGGGCTGATGAAAGTCATTTCTTGTTTGCCG
motif1
TGTCAAACAAAGCATGCTTATTTGTCACATTGTGGTTTGTGTATCTT
motif2
TTTAGAAGCTTTCAGCTTTGATCTCGATCAAGATCCAGCGAGTAA
motif3

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Supplementary Fig. 2. Sequences shared between MRCS1 and MFCS4. (a) Shared short sequences in MRCS1 and MFCS4 of mouse and opossum were identified by MEME (red and blue boxes). Position weight matrix of the two shared sequences and LEF1 binding sites are shown. (b) The *LacZ* reporter transgene construct with three tandem repeats. (c) The maxillary occlusal view of the E13.5 mouse embryo. The *LacZ* expression driven by the three tandem copies of the core sequence of mouse MRCS1 was observed in the incisor and molar rudiments but not in the palatal rugae. (d) The three shared motifs in the core sequence of *Xenopus* MFCS4.

Supplementary Table1 Incidence of the supernumerary tooth

Genotype	Mice with Maxillary sn		Mice with Mandibular sn		Incidence
	Right	Left	Right	Left	
wild type	0	0	0	0	0/20 (0%)
Δ MRCS1/+	0	0	0	0	0/12 (0%)
<i>Shh</i> KO/+	0	0	0	0	0/12 (0%)
Δ MRCS1/ Δ MRCS1	0	1	1	1	1/21 (4.7%)
Δ MRCS1/ <i>Shh</i> KO	8	6	10	12	15/19 (78.9%)
Δ MFCS4/ Δ MFCS4	0	0	0	0	0/15 (0%)
DKO/DKO	4	3	6	5	8/12 (66.7%)

sn, supernumerary tooth. Incidence is indicated as affected mice/observed mice.

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DKO/DKO	4	3	6	5	8/12 (66.7%)

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Supplementary Table 2 Primers and oligonucleotides used in this study

For ESC targeting		
Long arm	F	AGGAACTTTCAGTCTCACTGGG
	R	AATCTCGAGCTTGTGGGTTTGTCTCCTTG
Short arm	F	TAAGGATCCCAGAGAGTGACTGCCAAATG
	R	TTACCCGGGTATGCTCTCTTGAGCCTAGG
Probes for Southern blotting		
5' probe	F	TGCCTTATTAGATCTTGATC
	R	CTATGCTCTCCTGCACTGTT
3' probe	F	AGGCCAGAGCCCAGAACCTA
	R	GATGAGGTCAGAGGATGACT
For genotyping		
P1	F	CATTGCCACAGAGCAGAAGT
P2	R	TGTGCACCTCTATGTGTGCC
For cloning enhancers		
Mouse whole MRCS1	F	AGGAGACAAACCCACAAGAG
	R	CATTTGGCAGTCACTCTCTG
Mouse core MRCS1	F	AAGGCTTCACCTGCTTTTGC
	R	CCTTTAATACCGGTCCTGAG
<i>Xenopus</i> whole MFCS4	F	TGTGTATCAGGAAGCAGAGC
	R	AAGACAGAACATGGGCATGG
<i>Xenopus</i> core MFCS4	F	CCTGCAGACAACACAATTAG
	R	GTGACAATATACATCCCTGG
Mouse whole MFCS4	F	AGCAGGTTAAACCGCAGTTC
	R	TCCATCCCAGCTTCTAATGC
Mouse core MFCS4	F	TAATCAGGCTGATGAAAGTC
	R	TTTAGATCAAAGCGCAAAC
For deletion constructs		
del1	F	CTGCCAAACAACAAAGCTT
	R	CATTTGGCAGTCACTCTCTG
del2	F	AGGAGACAAACCCACAAGAG

	R	CCTCTTGGTTTTCTTTGCT
del3	F	AGGAGACAAACCCACAAGAG
	R	AAGGCTTCACCTGCTTTTGC
del4	F	AGGAGACAAACCCACAAGAG
	R	CTCAGGACCGGTATTAAGG
Δ mo1 (inverse PCR)	F	CTTTGCAGACAAAGCAGGCT
	R	CCGGGCAAAGCAGGTGAAG
Δ mo2 (inverse PCR)	F	GGCTGGGTTGGGCCTTGATC
	R	TGCAAAGATGAAAGCCGGGC
Δ mo3 (inverse PCR)	F	AGGGCGTGCTCAGGACCGGT
	R	GCCCAACCCAGCCTGCTTTG
mut mo1 (inverse PCR)	F	TGCATCTTTGCAGACAAAGCAGGCTGGGTTG
	R	CGCCGGGCAAAGCAGGTGAAGCCTTTTTTG
Oligonucleotides for EMSA		
MRCS1-motif1-WT	S	CCTGCTTTTGCCCGGCTTTCATCTTTGCAGACAAAGC
	A	GCCTGCTTTGTCTGCAAAGATGAAAGCCGGGCAAAG
MRCS1-motif1-mut	S	CCTGCTTTTGCCCGGcTgCATCTTTGCAGACAAAGC
	A	GCCTGCTTTGTCTGCAAAGATGcAcGCCGGGCAAAG
MFCS4-motif1-WT	S	ATTCTTAATCAGGCTGATGAAAGTCATTTATTGTTTG
	A	ACAGCAAACAATAAATGACTTTCATCAGCCTGATTAA
MFCS4-motif1-mut	S	ATTCTTAATCAGGCTGATGcAcGTCATTTATTGTTTG
	A	ACAGCAAACAATAAATGACgTgCATCAGCCTGATTAA

* F, forward primer; R, reverse primer; S, sense oligonucleotide; A, antisense oligonucleotide.