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SUPPLEMENTARY MATERIAL

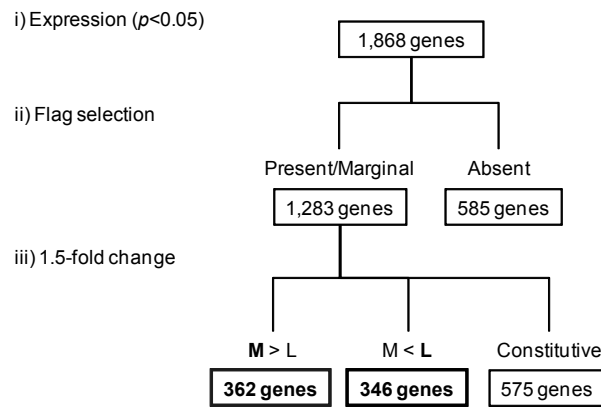
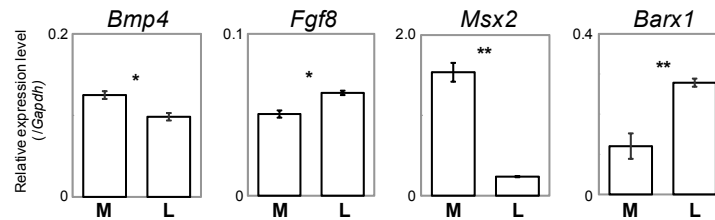
corresponding to:

**Molecular signaling at the fusion stage
of the mouse mandibular arch:
involvement of insulin-like growth factor family**

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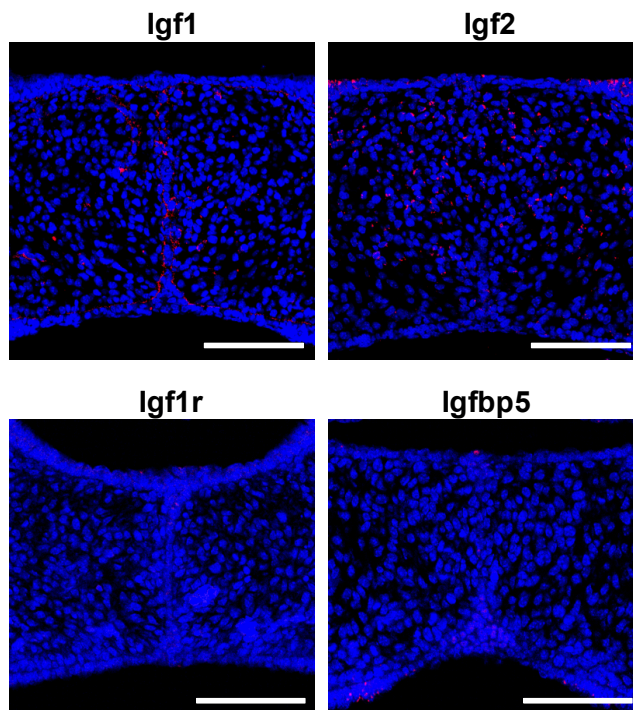
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A**Microarray dataset: Medial (M) and Lateral (L) regions of MA****B**

Supplementary Fig. S1. Threshold setting and qPCR-based evaluation for microarray data. (A) Screening of microarray dataset for region-specific genes. Probe signals were selected by i) p-values below 0.05, ii) present (or marginal) flag, and iii) a 1.5-fold-change between the M and L regions. In total, 362 and 346 differentially expressed genes were grouped as M region and L region-specific genes, respectively. **(B)** A cDNA pool from each M or L region sample was evaluated by qPCR for representative region-specific genes (*Bmp4*/*Fgf8* for medial/lateral epithelia, and *Msx2*/*Barx1* for medial/lateral mesenchyma, respectively). The epithelial genes (*Bmp4* and *Fgf8*) showed less difference between the M and L regions. Although *Fgf8* was omitted due to a p-value > 0.05 in the present microarray analysis, *Fgf8* showed significant expression in the L region consistent with a previous report (MGI:1335427). * $p < 0.05$; ** $p < 0.01$.

Secondary palatal shelves at E14.3



Supplementary Fig. S2. Localization of Igf family proteins in secondary palatal shelves. Frontal sections of secondary palatal shelves at E14.3, where the midline epithelial seam becomes disintegrated for the mesenchymal confluence, were immunolabeled for Igf1, Igf2, Igf1r, and Igfbp5. The corresponding signals and DAPI-stained cell nuclei are shown in red and blue, respectively. Scale bar, 100 μm.

SUPPLEMENTARY TABLE S1

PATTERN COLLATION OF THE REGION-SPECIFIC GENES WITH GENE EXPRESSION DATA PROVIDED BY
MOUSE GENOME INFORMATICS (MGI)

Gene symbol	The present microarray results		MGI Gene expression data				Reference: Acc. No.
	FC	P-value	M-region		L-region		
			epi	mes	epi	mes	
M>L							
<i>Bmp4</i>	3.65	6.27E-4	+				MGI:2450240
<i>Bambi</i>	3.54	7.18E-3	+	+			MGI:2450240
<i>Ptch1</i>	2.16	6.84E-3	+	+			MGI:3833903
<i>Wnt5a</i>	2.20	3.17E-2	+	+			MGI:3510682
<i>Zfp503</i>	2.96	2.62E-2	+	+			MGI:3785619
<i>Alx1</i>	44.69	9.40E-4		+			MGI:3039372
<i>Alx3</i>	11.87	8.71E-4		+			MGI:2152057
<i>Cux2</i>	5.82	1.00E-3		+			MGI:2687306
<i>Gata3</i>	14.71	2.37E-2		+			MGI:3510696
<i>Hand2</i>	2.98	3.68E-2		+			MGI:3510694
<i>Msx1</i>	1.78	3.77E-2		+			MGI:4948321
<i>Msx2</i>	2.25	3.84E-2		+			MGI:2687331
<i>Fgfr2</i>	1.71	2.28E-3	+	+	+		MGI:3042171
M<L							
<i>Pitx1</i>	0.27	9.12E-3	+		+	+	MGI:2151020
<i>Dlx2</i>	0.20	8.49E-5	+		+	+	MGI:1351463
<i>Dlx3</i>	0.39	3.36E-2	+		+	+	MGI:3703043
<i>Dlx1</i>	0.03	2.37E-2			+	+	MGI:3510683
<i>Dlx5</i>	0.30	1.26E-2			+	+	MGI:3510691
<i>Gpc4</i>	0.53	1.64E-2			+	+	MGI:3582047
<i>Barx1</i>	0.08	1.54E-2				+	MGI:2135647
<i>Cyp26a1</i>	0.12	8.12E-3				+	MGI:2429436
<i>Foxc2</i>	0.46	3.01E-2				+	MGI:1339703
<i>Shox2</i>	0.03	3.09E-2				+	MGI:3039375
<i>Spry1</i>	0.36	7.73E-3				+	MGI:1345456

Twenty-four region-specific genes ($p < 0.05$) which have the corresponding MGI reference (categorized as the embryonic stage TS17, mandibular component, and RNA in situ, in the database) are listed. The relative expression level in the M region over the L region is shown as fold change (FC). FC below 0.67 denotes M<L by 1.5-fold.

SUPPLEMENTARY TABLE S2 (EXCEL FILE)

LIST OF WHOLE GO CATEGORIES FOR REGION-SPECIFIC GENE SETS

Whole GO terms obtained from DAVID bioinformatics resources v6.7 (<http://david.abcc.ncifcrf.gov/>) are listed. All gene probe IDs are included in the list. "Count" denotes the number of genes within the GO category. Percentage of the gene among the total number of genes submitted is shown.

SUPPLEMENTARY TABLE S3

KEGG PATHWAYS IN THE MEDIAL (M) AND LATERAL (L) REGIONS

Term	P-value		KEGG-Id	Genes
	M	L		
Signaling pathway				
TGF-beta signaling pathway	3.99E-5	-	mmu04350	<i>Bmp4, Bmp5, Dcn, Fst, Id2, Id4, Pitx2, Smad6, Smad7, Tgfb2</i>
Hedgehog signaling pathway	2.00E-2	-	mmu04340	<i>Bmp4, Bmp5, Ptch1, Shh, Wnt5a</i>
Calcium signaling pathway	3.10E-2	-	mmu04020	<i>Adcy1, Cacna1d, Camk2d, Camk4, Cd38, Ednrb, Itpr2, Mylk, Ppp3ca</i>
p53 signaling pathway	4.39E-2	-	mmu04115	<i>Gadd45b, Gadd45g, Igf1, Perp, Pmaip1</i>
Cell function				
Gap junction	6.14E-3	-	mmu04540	<i>Adcy1, Gja1, Gucy1b3, Itpr2, Pdgfc, Prkg1, Tubb2a-ps2</i>
Long-term potentiation	1.10E-2	-	mmu04720	<i>Adcy1, Camk2d, Camk4, Itpr2, Ppp3ca, Rps6ka3</i>
Axon guidance	1.31E-2	5.00E-2	mmu04360	(M) <i>L1cam, Plxna2, Plxnc1, Ppp3ca, Sema3a, Sema6d, Unc5b, Unc5c</i> (L) <i>Sema3g, Nfatc1, Cxcl12, Gnai3, Sema3e</i>
Diseases				
Arrhythmogenic right ventricular cardiomyopathy (ARVC)	3.12E-3	-	mmu05412	<i>Cacna1d, Dsc2, Dsg2, Dsp, Gja1, Itga6, Pkp2</i>
Pathways in cancer	9.06E-3	-	mmu05200	<i>Bmp4, Egl3, Evi1, Fgfr2, Fzd7, Igf1, Itga6, Ptch1, Runx1t1, Shh, Tgfb2, Vegfc, Wnt5a</i>
Basal cell carcinoma	2.12E-2	-	mmu05217	<i>Bmp4, Fzd7, Ptch1, Shh, Wnt5a</i>
<GO>				
Enzyme linked receptor protein signaling pathway	2.89E-6	-	GO:0007167	<i>Angpt1, Axl, Bambi, Bmp4, Ddr1, Fgfr2, Fst, Fut8, Ghr, Msx1, Hpgd, Igf1, Smad6, Smad7, Ndn, Pdgfc, Tgfb2, Vegfc</i>

KEGG signaling pathways associated with differentially expressing genes between the M and L regions were analyzed using the DAVID database. Gene symbols involved in the pathways are shown. Note that "Axon guidance" was the only term obtained from the L region sample. Genes involved in the GO term "Enzyme-linked receptor protein signaling pathway" are also shown at the bottom of the list. Half of the genes (bold type) are not found in these KEGG pathways.