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Supplemental Data

Homozygosity Mapping and Candidate Prioritization Identify Mutations, Missed by Whole-Exome Sequencing, in *SMOC2*, Causing Major Dental Developmental Defects

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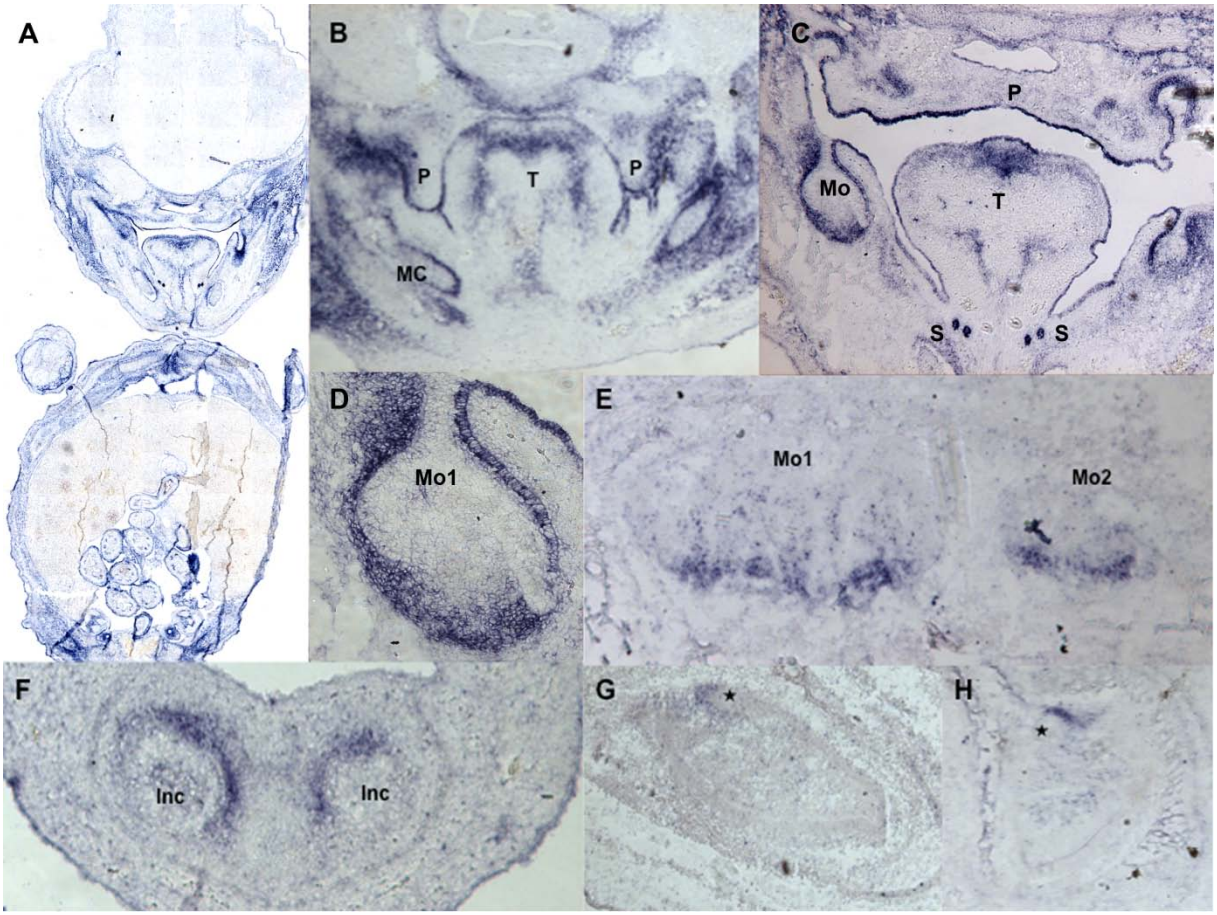
Figure S1. In situ hybridization of *Smoc2* during mouse development



(A) General view of an E14.5 dpc mouse embryo, *Smoc* 2 was expressed in various organs and systems like the digestive, urinary, respiratory (lung), nervous systems, vibrissae, olfactory nasal epithelium, axial skeleton.

(B) On this frontal view of the oral cavity, at E12.5 mouse embryo, *Smoc 2* transcripts were detected in the oral ectoderm especially in the maxilla and lining the palatal shelves (P) or the tongue (T), in the gingivolingual sulcus and in various mesenchymal area within the craniofacial region - preenchondral

condensation especially the skull basis - preossification area - around Meckel’s cartilage – and within specific region of tongue mesenchyme.

(C) On this view frontal of the oral cavity (E14.5), *Smoc 2* labeling was fainting but still visible in the oral ectoderm, the salivary glands excreting canals (S), the perichondrium of Meckel's cartilage (MC), tongue muscle bundles (T) and in the condensing mesenchymal cells of the maxillary/palatal bone rudiments.

The expression of *Smoc2* was present from day E14.5 onwards both during molar (Mo) and incisor (Inc) development.

(D) At E14.5 the signal was visible both in ectodermal (asymmetric labeling of the outer dental epithelium) and mesenchymal structures (cervical region of the dental papilla, follicular sac) of the cap stage first molar (Mo1).

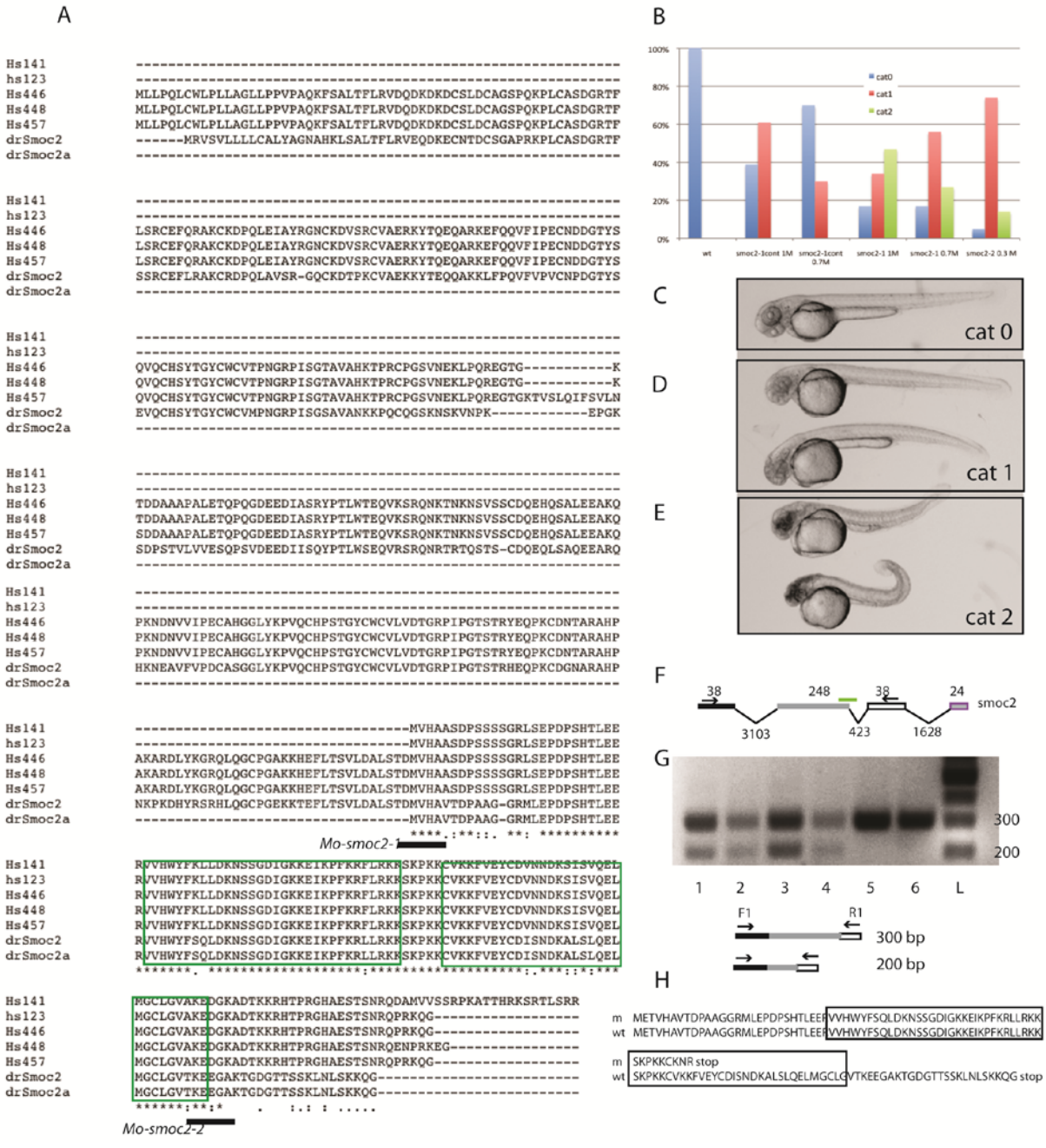
(E) On this sagittal view of the lower first (Mo1, bell stage with differentiated odontoblast) and second molar (Mo2) (early bell stage) at day E18.5 the labeling was restricted to the mesenchymal dental papilla and specifically to the area facing the epithelial loop (proliferative compartment) from which the root will further develop.

(F) Frontal view of lower incisors (Inc) at E14.5, the *Smoc 2* transcripts were located in the dental papilla especially on the lingual side.

(G) At E16.5 the labeling was restricted to the posterior part of the lower incisor (sagittal section) and stronger in the area facing the lingual epithelial loop (★).

(H) The asymmetrical lingual mesenchymal signal (★) was still visible on day E18.5 upper incisor (sagittal plane). Note that the lingual side of the continuously growing incisor is considered to be the analogue root side. Ameloblasts differentiation and enamel deposition occur only on the opposite labial side.

Figure S2. Zebrafish *smoc2* morpholino efficiency



(A) Alignment of human (Hs) and zebrafish (Dr) SMOC2 proteins. Human proteins correspond to 5 different transcripts (hs141, hs446, hs448, hs457, and hs123, amino acid numbers are indicated). The isolated full length zebrafish sequence (DrSmoc2, amplified by PCR with primers forward: gagcacagtctgcgacagca and reverse: caggttcagtttactagatg) and the short splice transcript Smoc2a (ENSDARP00000108925). Green blocks: calcium binding domain.

Black bars show the *smoc2-2* (CAGGAAACAGTGAGACTCATT) and *smoc2-1* (CTGGGTCAGTTACTGCATGGACCAT) morpholino positions. Morpholino control is:

CTGGcTCAcTTAgTGgATcGACCAT

(B) Chart showing the percentage of the different phenotypic categories obtained after morpholino injection.

(C–E) three different categories of phenotype after *smoc2-1* or *smoc2-2* morpholino injection. C: Cat 0 (wild-type); D: cat 1 (small head, slightly curved); E: cat 2 (necrotic head, strongly curved, reduced body size).

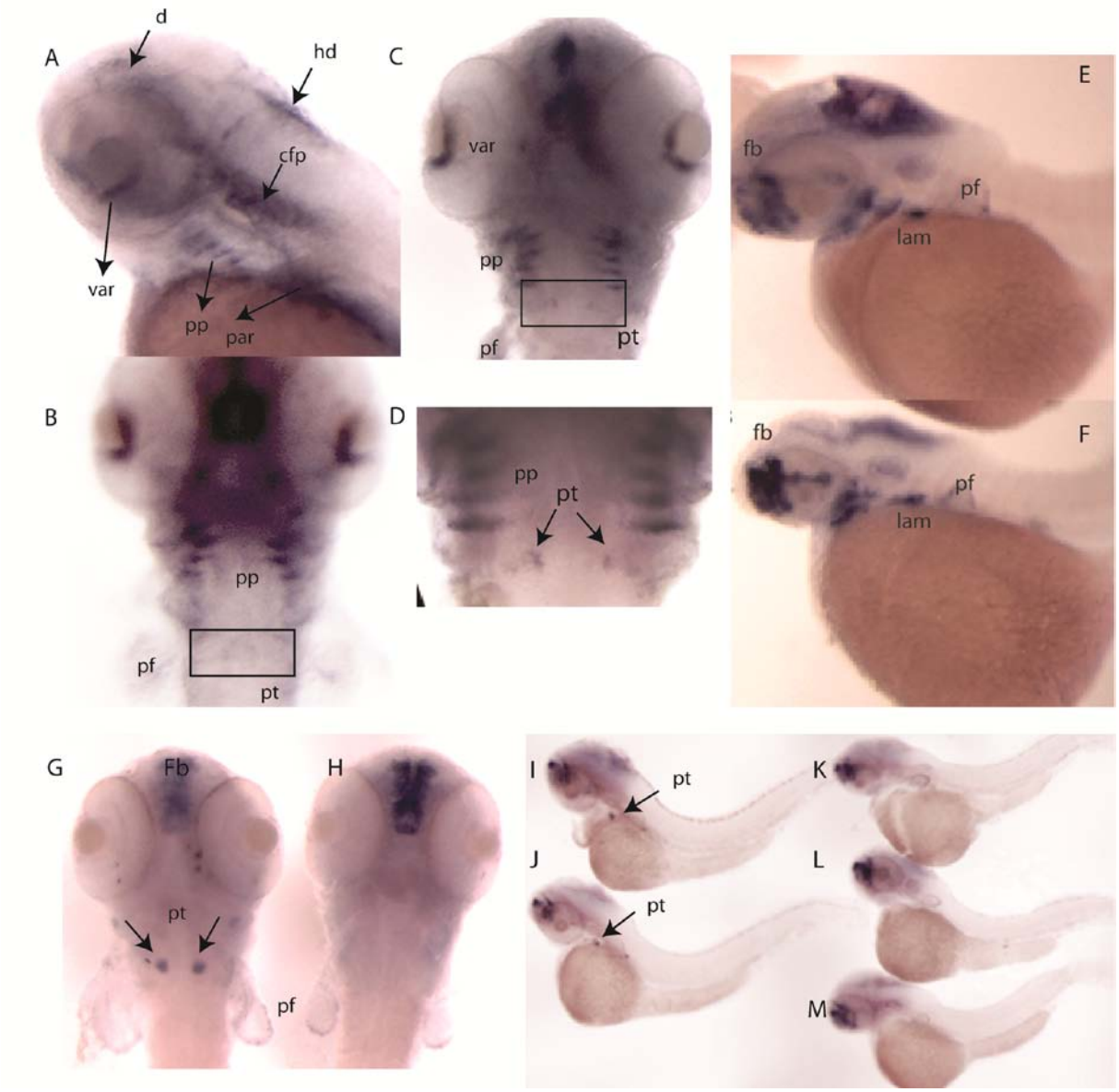
(F–H) Determination of *Mo-smoc2-2* efficiency

(F) *smoc2a* pre-mRNA. Bars represent the 4 exons flanking the 3 introns. Arrows show location of the primers (F1: 5'-GCAGTAACTGACCCAGCAGC-3' ; R2 : 5'- CTAGATGTTGTGCCATCACCTG-3'). Green line represents the morpholino target site. Numbers above indicate the size of exons, and below the size of introns (in bp).

(G) PCR amplification of *smoc2* cDNA prepared from single *smoc2-2* morphant (lane 1-4) shows 2 products: the 300 bp fragment indicates the correctly spliced transcript, and the 200 bp fragment represents the transcript with partial loss of exon 2. Wild-type siblings (lane 5-6) show only the correctly spliced variant (300 bp). All the products were sequenced. Scheme shows the corresponding amplified product. Lane L: DNA ladder.

(H) Tentative protein sequence of truncated Smoc2 in *smoc2-2* morphants. Sequencing of the shortest fragment (200 bp) revealed the formation of a premature stop codon removing the second calcium binding domain.

Figure S3. Zebrafish *smoc2* is expressed in future dental germ and inhibits specifically *dlx2b*



(A–D) *In situ* hybridization on wild type embryos with *smoc2* probe (900 bp). A: lateral view, C-D: ventral view of embryos without yolk. A, B: 56 hpf; C, D: 72 hpf. D: magnification of C. Black box: pharyngeal tooth area.

(E–F) *In situ* hybridization with *dlx2a* probe. Control (E) and *smoc2-2* morphant (F) embryos at 56 hpf did not reveal any obvious difference. Lateral view.

(G–M) *dlx2b* expression in 72 hpf embryos. G, I: control, H, K: 0.3 M *smoc2-2* morphant; J: 0.3 mM *p53* morphant; L: 0.2m M morphant; M: 0.3 mM *smoc2-2*/ 0.3 mM *p53* morphant. G-H: ventral view, I-M: lateral view.

var: ventral and anterior retina ; cfp: cephalic floor plate ; par : pharyngeal area ; pp: pharyngeal pouches ; d: diencephlon. pf: pectoral fin. pt: pharyngeal teeth area. Fb: forebrain; lam: lateral arch mesenchyme: pf: pectoral fin.

Table S1. Primer sequences of *SMOC2* gene used in this study

Primer name	Sequence (5' -> 3')
SMOC2-ex1F	GAAGCTCCGGGGTATTTGAC
SMOC2-ex1R	GCTGCGTCCTACCTGCTC
SMOC2-ex2F	TGAGAACCGGGTGGTGTTAT
SMOC2-ex2R	GAAATTACTCTTCTTCCTTACATGCTG
SMOC2-ex3F	GCTCCCTTTTCTGAGAGCTG
SMOC2-ex3R	GCTGATTTCCCCAGAGAACA
SMOC2-ex4F	GGGTCAAGACCTGCACACTT
SMOC2-ex4R	GCTTCACCCTTCCCCTCTTA
SMOC2-ex5F	AGCGCCAGAAGCAGAAACTA
SMOC2-ex5R	GAGCTGGTGATCGTGAGTGA
SMOC2-ex6F	TTCCTTCCTCATCTGCCCTA
SMOC2-ex6R	CGTGGTCTTTTCTGTCTGTGTC
SMOC2-ex7F	TGGATAGAGAGAAGAATGCCAAA
SMOC2-ex7R	GGCTGCCAGTCTACAAGAGG
SMOC2-ex8F	GAGGTTCTGCCTCTCCTGCT
SMOC2-ex8R	CCAAAGCCTCCCATGGTC
SMOC2-ex9F	CCACAAAGGTGACAGAAAGGA
SMOC2-ex9R	CCTTCCATACACAGCAGCAG
SMOC2-ex10F	AATGCCTGTGAATTTTAGTCTCA
SMOC2-ex10R	GCAACTTCCTGTGTTTGCAG
SMOC2-ex11F	TTGTTATATCTGATGACCAGTGCT
SMOC2-ex11R	CGCCAATTATCATTATTATTTCTGC
SMOC2-ex12F	GGAACCAATCCCTTCTGGAT
SMOC2-ex12R	GTAAGCTTGCCTCCCTTGAA
SMOC2-ex13F	CCAGCCCTCTTTGGTTCA
SMOC2-ex13R	TCTCCATTTTCACAGATTAAAACA