Supporting Information for
The developmental basis for scaling of mammalian tooth size

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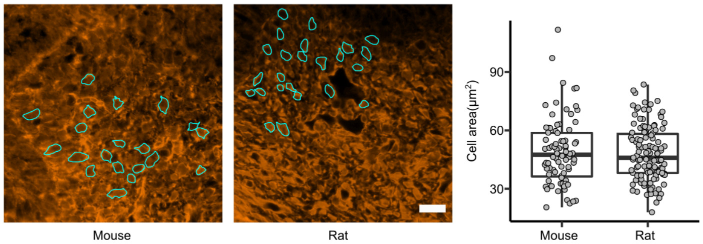

Fig. S1. Cell size does not differ between developing rat and mouse molars. Cell size (measured as the perimeter of cells stained with DiI on histological sections) does not differ between rat and mouse molars at bell stage of tooth development (E16 in mouse, n = 85, E19 in rat, n = 126, *P* = 0.665, randomization test). Scale bar 50 μm.

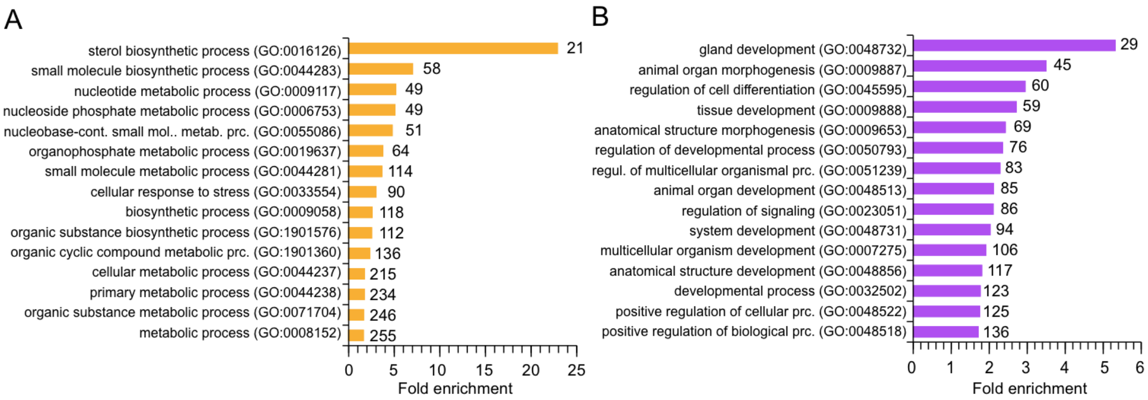

Fig. S2. IGF1 protein treatment up- and downregulates distinct gene groups. (*A*) In IGF1-treated mouse molars, fold enrichment of upregulated GO biological process categories is biased towards metabolic and biosynthesis related genes. (*B*) In contrast to (*A*), fold enrichment of downregulated GO biological process categories is biased towards developmental genes. Figures show the top 15 GO categories based on the *P*-value (all false discovery rate corrected values of shown categories are $P < 10^{-9}$). Number of observed genes in each category is shown next to the bars. For padj < 0.05 upregulated and downregulated genes, n = 439 and 268, respectively. See methods for details.

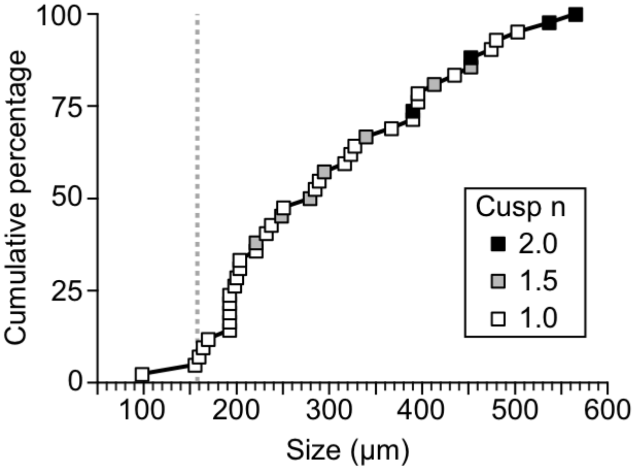

Fig. S3. Minimum sizes of cultured mouse molars with activated epithelial Wnt signaling are close to the predicted minimum. Size distribution of 42 teeth obtained from a single molar transplant experiment that was cultured under the kidney capsule [Fig. 3F in (1)]. Gray dashed line shows the predicted minimum tooth size (Fig. 7*B*, *SI Appendix*, Table S10). Teeth were classified into morphotypes that were clearly single or two cusped, and into an intermediate morphotype that showed an incipient second cusp (cusp n = 1.5). Measurements were made from (1).

Table S1. The areas, antero-posterior lengths and bucco-lingual widths of *Foxi3* and S*hh* expression areas of developing mouse and rat molars.

Species	Date	Sample	Structure	Gene	Area (mm^2)	Length (mm)	Width (mm)
Mouse	120818	NMRI_E12_t4_3	Placode	Foxi3	0.0376	0.242	0.223
Mouse	120818	NMRI_E12_t3_1	Placode	Foxi3	0.0410	0.317	0.186
Mouse	120818	NMRI E12 t4 1	Placode	Foxi3	0.0366	0.224	0.227
Mouse	120818	NMRI E12 t4 2	Placode	Foxi3	0.0347	0.253	0.221
Mouse	100718	NMRI_E12_2	Placode	Foxi3	0.0380	0.320	0.183
Mouse	230718	NMRI_E12_3	Placode	Foxi3	0.0200	0.214	0.137
Mouse	230718	NMRI_E12_1	Placode	Foxi3	0.0339	0.260	0.218
Mouse	230718	NMRI_E12_2	Placode	Foxi3	0.0346	0.290	0.192
Rat	100918	Wistar_E14_2	Placode	Foxi3	0.0419	0.279	0.217
Rat	100918	Wistar E14 4	Placode	Foxi3	0.0303	0.302	0.149
Rat	120818	Wistar_E14_t10_1	Placode	Foxi3	0.0290	0.273	0.149
Rat	120818	Wistar_E14_t10_2	Placode	Foxi3	0.0201	0.207	0.164
Rat	120818	Wistar_E14_t8_1	Placode	Foxi3	0.0257	0.295	0.138
Rat	120818	Wistar_E14_t8_2	Placode	Foxi3	0.0353	0.318	0.149
Rat	120818	Wistar_E14_t8_3	Placode	Foxi3	0.0335	0.326	0.131
Rat	230718	Wistar_E14_1	Placode	Foxi3	0.0482	0.273	0.250
Rat	240220	Wistar_E14_6	Placode	Foxi3	0.0299	0.239	0.175
Rat	240220	Wistar E14 1	Placode	Foxi3	0.0407	0.267	0.221
Rat	240220	Wistar E14 10	Placode	Foxi3	0.0248	0.220	0.181
Rat	240220	Wistar_E14_11	Placode	Foxi3	0.0361	0.290	0.214
Rat	240220	Wistar_E14_2	Placode	Foxi3	0.0331	0.295	0.203
Rat	240220	Wistar_E14_4	Placode	Foxi3	0.0333	0.271	0.166
Rat	240220	Wistar_E14_5	Placode	Foxi3	0.0361	0.239	0.241
Rat	240220	Wistar E14 7	Placode	Foxi3	0.0313	0.251	0.221
Rat	240220	Wistar_E14_9	Placode	Foxi3	0.0349	0.228	0.241
Mouse	130715	NMRI_E12_4	Initiation knot	Shh	0.0024	0.065	0.063
Mouse	120818	NMRI_E12_t2_1	Initiation knot	Shh	0.0030	0.061	0.069
Mouse	120818	NMRI_E12_t2_2	Initiation knot	Shh	0.0031	0.068	0.067
Mouse	290216	NMRI_E12_1	Initiation knot	Shh	0.0015	0.051	0.048
Mouse	290216	NMRI_E12_2	Initiation knot	Shh	0.0012	0.050	0.042
Mouse	230718	NMRI_E12_2	Initiation knot	Shh	0.0026	0.062	0.067
Mouse	230718	NMRI E12 3	Initiation knot	Shh	0.0026	0.058	0.067
Mouse	290216	NMRI E12 3	Initiation knot	Shh	0.0024	0.065	0.057
Mouse	290216	NMRI_E12_5	Initiation knot	Shh	0.0017	0.048	0.054
Rat	100918	Wistar_E14_5	Initiation knot	Shh	0.0064	0.102	0.087
Rat	100918	Wistar_E14_6	Initiation knot	Shh	0.0053	0.096	0.080
Rat	100918	Wistar_E14_7	Initiation knot	Shh	0.0060	0.104	0.085
Rat	100918	Wistar E14 8	Initiation knot	Shh	0.0072	0.107	0.095
Rat	100918	Wistar_E14_9	Initiation knot	Shh	0.0066	0.110	0.089
Rat	120818	Wistar_E14_t6_1	Initiation knot	Shh	0.0038	0.090	0.065

Species	Date	Sample	Structure	Gene	Area (mm^2)	Length (mm)	Width (mm)
Rat	120818	Wistar_E14_t6_3	Initiation knot	Shh	0.0072	0.119	0.089
Rat	120818	Wistar_E14_t7_1	Initiation knot	Shh	0.0021	0.067	0.052
Rat	120818	Wistar_E14_t7_3	Initiation knot	Shh	0.0043	0.092	0.070
Rat	140116	Wistar E14 4	Initiation knot	Shh	0.0030	0.074	0.064
Rat	230718	Wistar_E14_1	Initiation knot	Shh	0.0077	0.122	0.090
Mouse	140116	NMRI_E14_X	Primary enamel knot	Shh	0.0133	0.184	0.100
Mouse	140116	NMRI_E14_Y	Primary enamel knot	Shh	0.0146	0.167	0.121
Mouse	251115	NMRI_E14_1	Primary enamel knot	Shh	0.0120	0.201	0.089
Mouse	251115	NMRI_E14_9	Primary enamel knot	Shh	0.0227	0.205	0.158
Mouse	290216	NMRI_E14_1	Primary enamel knot	Shh	0.0210	0.230	0.130
Mouse	290216	NMRI_E14_3	Primary enamel knot	Shh	0.0118	0.197	0.084
Rat	290216	Wistar E16 1	Primary enamel knot	Shh	0.0273	0.335	0.116
Rat	290216	Wistar E16 5	Primary enamel knot	Shh	0.0410	0.442	0.124
Rat	290216	Wistar_E16_4	Primary enamel knot	Shh	0.0380	0.432	0.132

Table S2. Tooth germ growth and increase in complexity from cap stage onwards. Growth is tabulated as the occlusal area, antero-posterior length, and bucco-lingual width. Complexity is tabulated as OPCR values. The mouse E19 and rat E21 are marked as P0.

Species	Date	Sample	Days after placode	Tooth size (mm^2)	Length (mm)	Width (mm)	OPCR
Mouse	250119	NMRI_E14_3	2	0.0556	0.448	0.164	-
Mouse	250119	NMRI_E14_4	2	0.0524	0.458	0.158	15.8
Mouse	250119	NMRI_E14_5	2	0.0535	0.446	0.158	16.3
Mouse	271114	NMRI_E15_a	3	0.0570	0.482	0.164	14.8
Mouse	271114	NMRI_E15_b	3	0.0619	0.451	0.192	15.4
Mouse	271114	NMRI_E15_c	3	0.0552	0.447	0.164	12.5
Mouse	110618	NMRI_E16_a	4	0.1141	0.600	0.272	17.0
Mouse	110618	NMRI_E16_b	4	0.1058	0.581	0.274	16.3
Mouse	110618	NMRI_E16_c	4	0.1218	0.585	0.300	16.3
Mouse	270815	NMRI_E16_x	4	0.0899	0.505	0.254	13.8
Mouse	270815	NMRI_E16_y	4	0.0786	0.497	0.224	10.0
Mouse	210615	NMRI_E17_x	5	0.2001	0.708	0.417	32.9
Mouse	210615	NMRI_E17_y	5	0.2284	0.750	0.431	29.5
Mouse	210615	NMRI_E17_z	5	0.1807	0.683	0.381	29.3
Mouse	021020	NMRI_P0_1	7	0.5248	1.158	0.623	57.1
Mouse	021020	NMRI_P0_5	7	0.4776	1.104	0.585	57.0
Mouse	021020	NMRI_P0_3	7	0.5017	1.143	0.579	55.6
Mouse	240620	NMRI_P2_5	9	0.6204	1.221	0.642	51.1
Mouse	240620	NMRI_P2_1	9	0.6163	1.250	0.639	-
Mouse	240620	NMRI_P2_3	9	0.6118	1.227	0.636	52.4
Mouse	300620	NMRI_P4_5	11	0.8460	1.397	0.764	54.6
Mouse	300620	NMRI_P4_11	11	0.7875	1.410	0.711	55.9
Mouse	300620	NMRI_P4_1	11	0.7895	1.374	0.729	46.5
Mouse	060720	NMRI_P6_1	13	0.8180	1.377	0.747	57.8
Mouse	060720	NMRI_P6_3	13	0.8607	1.407	0.780	49.3
Mouse	060720	NMRI_P6_5	13	0.8984	1.391	0.810	49.9
Rat	051018	Wistar_E16_3	2	0.1069	0.566	0.266	9.1
Rat	181014	Wistar_E16_2	2	0.1116	0.635	0.261	10.9
Rat	181014	Wistar_E16_7	2	0.1105	0.633	0.294	9.4
Rat	210216	Wistar_E16_4	2	0.0869	0.566	0.233	14.0
Rat	270914	Wistar_E17_7	3	0.1094	0.627	0.279	9.1
Rat	270914	Wistar_E17_10	3	0.1065	0.633	0.294	10.6
Rat	270914	Wistar_E17_x	3	0.1084	0.624	0.234	12.3
Rat	190618	Wistar_E18_2	4	0.2411	0.891	0.405	12.8
Rat	190618	Wistar_E18_3	4	0.2041	0.825	0.369	11.5
Rat	190618	Wistar_E18_6	4	0.2322	0.867	0.426	11.1
Rat	221014	Wistar_E19_1	5	0.3913	1.068	0.567	28.3
Rat	221014	Wistar_E19_2	5	0.4591	1.122	0.633	31.6
Rat	160119	Wistar_E19_3	5	0.4398	1.107	0.606	32.6
Rat	220616	Wistar_E19_x	5	0.8180	1.412	0.804	37.3

Species	Date	Sample	Days after placode	Tooth size (mm^2)	Length (mm)	Width (mm)	OPCR
Rat	011215	Wistar_E20_3	6	0.9319	1.515	0.843	43.3
Rat	011215	Wistar_E20_x	6	0.9458	1.488	0.867	42.5
Rat	011215	Wistar_E20_y	6	1.0980	1.575	0.927	45.0
Rat	270421	Wistar P0 1	7	1.9773	2.148	1.224	56.3
Rat	280421	Wistar_P1_1	8	2.4448	2.352	1.388	59.8
Rat	280421	Wistar_P1_2	8	2.5100	2.488	1.308	63.1
Rat	290421	Wistar_P2_2	9	3.0075	2.632	1.472	64.6
Rat	290421	Wistar_P2_1	9	3.1956	2.648	1.584	65.9
Rat	290421	Wistar_P2_3	9	3.3538	2.672	1.601	64.1
Rat	300421	Wistar_P3_2	10	3.5497	2.836	1.632	69.8
Rat	300421	Wistar_P3_1	10	3.5770	2.764	1.712	67.1
Rat	300421	Wistar_P3_3	10	3.4811	2.715	1.655	65.6
Rat	060715	Wistar_P5_1	12	3.3949	2.776	1.600	68.0
Rat	060715	Wistar_P5_2	12	3.4157	2.700	1.648	62.9

Table S3. Tooth size and spacing of the secondary enamel knots detected using *Fgf4* expression (patterning area) in mouse and rat molars.

Species/ Genotype	Date	Sample	Tooth size (mm^2)	Patterning area (mm^2)
Mouse	110116	NMRI_E16_1	0.2587	0.1426
Mouse	110116	NMRI_E16_2	0.2176	0.1268
Mouse	110116	NMRI_E16_10	0.2809	0.1374
Mouse	130715	NMRI_E17_1	0.2636	0.1511
Mouse	130715	NMRI_E17_6	0.2555	0.1422
Mouse	130715	NMRI_E17_7	0.2235	0.1227
Mouse	130715	NMRI_E17_14	0.2294	0.1229
Mouse	130715	NMRI E17 X	0.2188	0.1341
Rat	190115	Wistar_E19_1	0.4553	0.2474
Rat	190115	Wistar_E19_2	0.4267	0.2371
Rat	190115	Wistar_E19_6	0.3810	0.2070
Rat	151214	Wistar_E19_3	-	0.2752
Rat	080822	Wistar E19 1	0.3402	0.1887
Rat	080822	Wistar_E19_2	0.3546	0.1958
Rat	080822	Wistar E19 5	0.3787	0.2103
Rat	080822	Wistar_E19_6	0.3608	0.1734
Rat	080822	Wistar_E19_8	0.3933	0.1891

Table S4. Expression of *Igf*-pathway related genes in rat and mouse molars. Genes with read counts below ten in both the species are not listed (e.g., *Igfbp1, Ins1, Ins2*). Bolded genes have a higher expression in the rat relative to the mouse molars at padj < 0.05, and bolded genes in brackets have a lower expression in the rat relative to the mouse molars at padj < 0.05.

Gene	Gene ID mouse (ENSMUSG 000000)	Gene ID rat (ENSRNOG 000000)	Stage	Mouse mean count	Rat mean count	log2 Fold Change	pvalue	padj
Igf1	20053	04517	Bud	1254.63	5900.52	2.234	1.16E-22	1.45E-21
Igf1			Cap	1671.01	6559.41	1.973	5.72E-17	4.84E-16
Igf1			Bell	1588.43	5678.52	1.838	7.69E-16	5.04E-15
Igf1r	05533	14187	Bud	5972.62	5981.88	0.002	0.978851	0.984267
Igf1r			Cap	5332.95	5544.75	0.056	0.501891	0.561991
Igf1r			Bell	5619.78	5807.20	0.046	0.565733	0.619579
Igf2	48583	20369	Bud	62517.93	69737.80	0.158	0.418821	0.486138
Igf2			Cap	45351.26	76597.68	0.756	0.000177	0.000389
Igf2			Bell	39471.46	73208.88	0.891	0.000005	0.000012
Igf2r	23830	14997	Bud	6197.70	9813.38	0.663	0.000003	0.000008
Igf2r			Cap	7006.37	12481.32	0.833	1.31E-08	4.87E-08
Igf2r			Bell	5796.93	12306.30	1.086	1.82E-14	1.07E-13
Igfbp2	39323	16957	Bud	1885.02	4697.42	1.318	2.76E-24	3.87E-23
Igfbp2			Cap	2571.93	6476.31	1.333	2.20E-23	2.94E-22
Igfbp2			Bell	2300.23	6638.37	1.528	3.10E-32	6.00E-31
Igfbp3	20427	61910	Bud	2211.35	8035.82	1.862	8.11E-27	1.34E-25
Igfbp3			Cap	3481.70	8819.48	1.341	7.74E-14	4.94E-13
Igfbp3			Bell	3084.31	7767.38	1.332	1.61E-14	9.54E-14
Igfbp4	17493	10635	Bud	8734.64	7888.97	-0.147	0.520044	0.585184
Igfbp4			Cap	8662.03	9265.02	0.097	0.680564	0.726755
Igfbp4			Bell	7878.39	12023.91	0.610	0.007524	0.012184
Igfbp5	26185	17206	Bud	27115.27	95790.36	1.821	4.70E-24	6.47E-23
Igfbp5			Cap	27072.84	121984.83	2.172	1.86E-31	4.25E-30
Igfbp5			Bell	33712.75	115067.75	1.771	7.55E-23	8.47E-22
Igfbp6	23046	10977	Bud	9.02	140.48	4.020	2.51E-27	4.28E-26
Igfbp6			Cap	6.02	166.83	4.947	4.61E-34	1.24E-32
Igfbp6			Bell	9.72	230.30	4.634	1.35E-37	3.58E-36
Igf2bp1	13415	06122	Bud	2824.98	3075.89	0.123	0.312116	0.376078
Igf2bp1			Cap	1304.36	2190.09	0.748	3.11E-09	1.24E-08
Igf2bp1			Bell	970.89	1728.21	0.830	1.29E-11	5.95E-11
Igf2bp2	33581	25946	Bud	5792.42	5142.98	-0.172	0.080936	0.114335
(Igf2bp2)			Cap	4906.97	3952.46	-0.312	0.002172	0.004004
(Igf2bp2)			Bell	4539.81	3445.87	-0.398	0.000054	0.000119
(Igf2bp3)	29814	09052	Bud	2513.81	1973.58	-0.351	0.000474	0.001014
(Igf2bp3)			Cap	1881.21	1459.65	-0.365	0.000467	0.000959
(Igf2bp3)			Bell	1622.97	1141.57	-0.507	6.18E-07	0.000002
Igfbpl1	35551	11320	Bud	3.46	29.50	3.638	1.53E-11	7.96E-11
Igfbpl1			Cap	39.46	28.03	-0.469	0.316667	0.376315

Gene	Gene ID mouse (ENSMUSG 000000)	Gene ID rat (ENSRNOG 000000)	Stage	Mouse mean count	Rat mean count	log2 Fold Change	pvalue	padj
(Igfbpl1)			Bell	186.31	24.84	-2.941	7.55E-11	3.24E-10
Igfals	46070	15061	Bud	4.39	4.78	0.170	0.885116	0.907325
Igfals			Cap	13.54	4.47	-1.663	0.165635	0.211764
(Igfals)			Bell	15.07	2.77	-2.681	0.024600	0.036497
(Insr)	05534	29986	Bud	2531.52	1219.10	-1.054	4.47E-23	5.75E-22
(Insr)			Cap	2780.62	1169.19	-1.250	8.98E-30	1.86E-28
(Insr)			Bell	2468.56	1114.99	-1.146	6.96E-27	9.93E-26

Table S5. Tooth size and spacing of the secondary enamel knots (patterning area) in Fucci-red mouse molars cultured in the presence and absence of IGF1 protein. Teeth were measured when at least five secondary enamel knots were visible.

Sample	Starting date	Treatment	Days *ex vivo*	Tooth size (mm^2)	Patterning area (mm^2)
1R	080221	Control	6	0.5159	0.1018
5R	080221	Control	6	0.6144	0.1536
2R	110321	Control	5	0.3642	0.1162
1L	110321	Control	5	0.4273	0.1243
6L	070421	Control	5	0.2478	0.0806
5R	070421	Control	5	0.3396	0.0948
1R	070421	Control	5	0.3450	0.1198
3R	070421	Control	5	0.3525	0.0974
2L	070421	Control	5	0.3673	0.1146
9L	110622	Control	5	0.3124	0.0800
7R	110622	Control	6	0.3800	0.1303
1L	080221	IGF1	6	0.6230	0.1897
6R	080221	IGF1	6	0.6815	0.1889
4R	080221	IGF1	6	0.8405	0.3074
2L	110321	IGF1	5	0.5230	0.1769
6R	110622	IGF1	5	0.3271	0.1285
5R	110622	IGF1	5	0.3612	0.1250
4R	110622	IGF1	5	0.4110	0.1343
8L	110622	IGF1	6	0.4582	0.1716
1L	110622	IGF1	5	0.4917	0.2335

Table S6. Tooth size and the spacing of the secondary enamel knots (patterning area) detected using *Fgf4*-expression (patterning area) in mouse and rat molars.

Species/ Genotype	Date	Sample	Tooth size (mm^2)	Patterning area (mm^2)
Mouse/IGF1R_KO	270121	IGF1R_E18_KO_1	0.1823	0.0932
Mouse/IGF1R_KO	270121	IGF1R_E18_KO_2	0.1938	0.1112
Mouse/IGF1R_KO	280622	IGF1R_E18_KO_3	0.1546	0.0841
Mouse/IGF1R_KO	280622	IGF1R_E18_KO_4	0.1272	0.0504
Mouse/IGF1R_KO	280622	IGF1R_E18_KO_7	0.1508	0.0703
Mouse/IGF1R_KO	280622	IGF1R_E18_KO_8	0.1524	0.0820
Mouse/IGF1R_WT	270121	IGF1R_E17_WT_6	0.2824	0.1655
Mouse/IGF1R_WT	030321	IGF1R_E17_WT_5	0.2563	0.1294
Mouse/IGF1R_WT	280622	IGF1R_E17_WT_8	0.2526	0.1324
Mouse/IGF1R WT	280622	IGF1R E17 WT 6	0.2645	0.1352

Table S7. The effect of IGF1 treatment on expression of genes known to be required for normal tooth development (2) of mouse molars. The genes in bold have expression level changes at padj < 0.05, all downregulation.

Gene	Gene ID	baseMean	log2FoldChange	pvalue	padj
Shh	ENSMUSG00000002633	158.008	-1.331553	1.86E-08	0.000002
Spry2	ENSMUSG00000022114	203.119	-0.899376	0.000003	0.000176
Lef1	ENSMUSG00000027985	651.643	-0.668914	0.000004	0.000240
Fgf3	ENSMUSG00000031074	78.791	-1.745747	0.000006	0.000349
Pdgfra	ENSMUSG00000029231	5187.024	-0.509024	0.000007	0.000409
Sostdc1	ENSMUSG00000036169	1056.792	-0.583204	0.000028	0.001276
Bmp4	ENSMUSG00000021835	276.550	-0.752379	0.001969	0.036465
Eda	ENSMUSG00000059327	125.645	-0.648440	0.002217	0.040236
Fst	ENSMUSG00000021765	393.073	-0.456395	0.004985	0.073447
Inhba	ENSMUSG00000041324	944.896	-0.371059	0.007682	0.097234
Yap1	ENSMUSG00000053110	2155.990	-0.260643	0.015872	0.157331
Spry4	ENSMUSG00000024427	312.648	-0.386442	0.016378	0.159866
Runx2	ENSMUSG00000039153	1442.066	-0.261197	0.017853	0.168808
Barx1	ENSMUSG00000021381	661.294	-0.269877	0.028907	0.228161
Pax9	ENSMUSG00000001497	1513.753	-0.231191	0.040367	0.279380
Edar	ENSMUSG00000003227	48.260	-0.740193	0.045058	0.294635
Bmp2	ENSMUSG00000027358	67.729	-0.539686	0.058984	0.339601
Jag2	ENSMUSG00000002799	402.313	0.280615	0.064589	0.355305
Fgfr2	ENSMUSG00000030849	1593.774	0.203518	0.073533	0.381244
Trp63	ENSMUSG00000022510	1239.197	-0.213861	0.077427	0.389638
Sp6	ENSMUSG00000038560	243.396	-0.455061	0.079825	0.394617
Acvr2a	ENSMUSG00000052155	392.015	-0.286330	0.079945	0.394774
Evc	ENSMUSG00000029122	512.253	0.220915	0.119441	0.482374
Chuk	ENSMUSG00000025199	391.518	0.241357	0.148289	0.535945
Msx1	ENSMUSG00000048450	1597.954	-0.116144	0.249078	0.668065
Bcl11b	ENSMUSG00000048251	490.290	-0.152262	0.281665	0.698493
Ctnna1	ENSMUSG00000037815	2230.010	-0.085910	0.343552	0.752505
Bmp7	ENSMUSG00000008999	185.512	0.175060	0.359283	0.762787
Foxi3	ENSMUSG00000055874	109.940	0.207778	0.403306	0.791898
Fgf10	ENSMUSG00000021732	197.830	-0.153683	0.422457	0.803655
Bmpr1a	ENSMUSG00000021796	1609.036	0.068240	0.483999	0.839166
Pitx2	ENSMUSG00000028023	717.020	-0.057882	0.658253	0.913127
Gas1	ENSMUSG00000052957	499.538	-0.056887	0.703741	0.925558
Wnt10a	ENSMUSG00000026167	42.444	0.152322	0.712022	0.928833
Apc	ENSMUSG00000005871	2008.212	0.028482	0.793381	0.948206
Dicer1	ENSMUSG00000041415	1643.814	-0.025564	0.798670	0.949471
Msx2	ENSMUSG00000021469	80.878	0.069767	0.808810	0.952988
Ctnnb1	ENSMUSG00000006932	8368.773	0.014435	0.874369	0.974468

Gene	Gene ID	baseMean	log2FoldChange	pvalue	padj
Smo	ENSMUSG00000001761	1119.973	-0.014494	0.884361	0.976782
Lrp4	ENSMUSG00000027253	1212.486	0.012883	0.913219	0.982982
Edaradd	ENSMUSG00000095105	176.398	0.006436	0.976009	0.993924
Rps6ka3	ENSMUSG00000031309	985.009	0.000284	0.998403	0.999149

Table S8. Simulations comparing the mouse molar pattern with those where growth (*Mgr*) has been increased or activation (*Act*) has been decreased. In rat molar simulation both the parameter values have been changed. Size and patterning area are relative to those of the mouse simulation.

Simulation	*Mgr*	*Act*	Relative size	Relative patterning area	Enamel knot *n*
Mouse	16000	1.6	1.0	1.0	5
Mouse + growth	160000	1.6	1.9	2.7	7
Mouse – activation	16000	0.8	1.0	0.8	4
Rat	160000	0.8	1.6	1.6	5

All the other parameters have identical values in all simulations: Iterations = 14000, Deg = 0.076, Set = 0.95, Sec = 0.14, Ds = 1.0, Da = 0.2, Lbi = 1.34, Bbi = 1.31, Ina = 0.0, Int = 0.19, Inh = 800.0, Di = 0.2, Egr = 0.013, Dgr = 10500.0, Dff = 0.0002, Adh = 0.005, Rep = 1.5, Ntr = 0.00013, Abi = 3.2, Pbi = 7.0, Bgr = 0.2, Boy = 0.17, Rad = 3.0, Swi = 1.51, Bwi =3.4. For parameters, see Methods and (3, 4). The model can be downloaded at https://github.com/jernvall-lab/ToothMaker.

Table S9. Measurements of tooth widths at the cap stage, at bell stage, and at the final mineralized size. The late bell stage data was measured when the last cusps have just become visible (only multicusped teeth included). When the required bell stage was between two stages in the data, an average was used. Data is from this study, literature, and from the Museum of Natural History Berlin, Embryological collections (ZMB EMB) and Finnish Museum of Natural History (MZH).

			Developmental width		Mineralized width		
Stage	Species	Tooth	µm	log10 µm	µm	log10 µm	Source
Cap	*Capreolus capreolus*	dp/3	177	2.249	5420	3.734	1
	Felis catus	dp4/	162	2.21	3100	3.491	2
	Homo sapiens	M1/	187	2.273	11500	4.061	3
	Loxodonta africana	M/3 (dp/4)	209	2.321	48000	4.681	4
	Macropus eugenii	M/1	154	2.188	4800	3.681	5
	Mus musculus	M/1	155	2.189	950	2.98	6
	Mustela erminea	P4/	174	2.24	2600	3.42	7
	Mustela putorius	dp/3	172	2.235	1220	3.087	8
	Ovis aries	dp4/	214	2.331	10550	4.023	9
		dp/4	185	2.266	7150	3.854	9
	Phocaena sp.	upper	148	2.171	2130	3.328	10
		lower	196	2.293	2410	3.382	10
	Rattus norvegicus	M/1	170	2.23	1970	3.294	11
	Sorex araneus	P/4	207	2.317	644	2.809	12
	Sus scrofa	M1/	193	2.286	14200	4.151	13
		dp3/	177	2.247	7900	3.898	13
		dp/3	154	2.188	4670	3.669	13
Bell	*Felis catus*	dp3/	1426	3.154	3420	3.534	14
		dp/4	946	2.976	1900	3.279	14
	Homo sapiens	M1/	3350	3.525	11500	4.061	3
	Loxodonta africana	M/2(dp/3)	4850	3.686	27400	4.438	4
		M2/(dp3/)	5530	3.743	31500	4.498	4
	Mesocricetus auratus	M/1	630	2.80	1410	3.149	15
	Macropus eugenii	M/1	1900	3.279	4800	3.681	5
	Mus musculus	M/1	596	2.775	950	2.98	6
	Rattus norvegicus	M/1	1225	3.088	1970	3.294	11

Sources: 1. Developmental: Lab collection, Mineralized: MZH_UN_3057; 2. Developmental: Section ZMB_EMB_A_Bolk_13-2-1, Mineralized: Fig. 3a in (5); 3. Developmental: for cap stage average of Fig. 3c in (6) and Fig. 4a in (7), for bell stage Fig. 7b in (8), Mineralized: average of right and left side measurements in table 1 in (9); 4. Developmental: This study, for cap stage file lox2011-10-1_headPTA_01, for bell stage file loxodonta_2013-7, Mineralized: MZH_UN_2845; 5. Developmental: Fig. S3, specimen 3935 and Fig. S4, specimen 4946, Mineralized: Fig. S5, specimen 7275, all in (10); 6. This study; 7. Developmental: section ZMB_EMB_B_Bolk_8-3-4, Mineralized: MZH_429; 8. Developmental: Lab collection, slide 0700_1_230609_MJ, Mineralized: MZH_KN_47857; 9. Developmental: ZMB_EMB_T_Bolk_4-4-11, ZMB_EMB_S_Bolk_5-3-12 , Mineralized: MZH_541_1960; 10. Developmental: ZMB_EMB_D_Bolk_7-2-6, ZMB_EMB_Bolk_9-1-5 , Mineralized: MZH_976_658_1960; 11. This study; 12. Developmental: Fig 2c, Mineralized: fig. 4b, both in (11); 13. Developmental: ZMB_EMB_U_Bolk_6-2-3, ZMB_EMB_V_Bolk_6-2-5, ZMB_EMB_V_Bolk_5-2-1, Mineralized: MZH reference collections; 14. Developmental: For dp/4 average of Fig 10 and 11, for dp/4 Fig 12, Mineralized: Figs 2a and 18a, all in (5); 15. Developmental: Average of Fig 10e and 10f, Mineralized: Fig. 7a, all in (12).

Table S10. Slopes using least-squares (LS) and major axis (MA) regression to estimate the minimum tooth size possible based on the cap-stage data, and the minimum size when the teeth can fully pattern based on the bell-stage data.

Data and regression	Slope	Intercept	r^2	P	Minimum tooth size (µm and 95% CI)	Minimum patterning size (µm and 95% CI)
Cap stage, LS	0.0427	2.095	0.1626	0.1113	154.3 (122.5-191.3)	
Cap stage, MA	0.0431	2.093	0.1626	0.1075	153.9 (122.0-191.8)	
Bell stage, LS	0.6398	0.885	0.9764	0.0001		286.4 (112.0-502.0)
Bell stage, MA	0.6443	0.869	0.9764	0.0001		277.4 (93.3-508.7)

Slopes and bootstrapped 95 percent confidence intervals were obtained using PAST (13). Major axis regression can be preferable when both variables have error and are in the same units of measurements (14).

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