





Fig. S2. Principal component analysis (PCA) of the anterior body and ramus portion of hemimandibles of three mouse models of craniosynostosis. Scatter plots of individual scores based on PCA from left hemimandibles of mutant and unaffected littermates of  $Fgfr2^{+/S252W}$  (A) and  $Fgfr2^{+/P253R}$  (B) Apert syndrome mouse models; and  $Fgfr2c^{C342Y/+}$  (C) Crouzon/Pfeiffer syndrome mouse model along first and second Principal Components axes (PC1 and PC2). Separate PCAs were estimated for the anterior portion (anterior body, shown in blue in inset image) and posterior portion (ramus, shown in red in inset image) of the hemimandibles. PCA of the anterior body of the left hemimandible is shown in the top panel of each column while PCA of the left ramus portion is shown in the bottom panel.



**Fig. S3. Bone mineral density mapping.** Bone mineral density (BMD) was mapped for each of the genotypes of interest: (A, B) buccal view of left hemimandibles of mice carrying *Fgfr2* mutations (B) and their respective unaffected littermates (A); (C, D) lingual view of left hemimandibles of mice carrying *Fgfr2* mutations (D) and their respective unaffected littermates (C). Though there are differences across the models, the contrast between mutant and respective unaffected littermates is greatest in the *Fgfr2*+/S252W mice.



**Fig. S4. Cortical bone thickness mapping.** Cortical bone thickness was mapped for each of the genotypes of interest: (A, B) buccal view of left hemimandibles of mice carrying *Fgfr2* mutations (B) and their respective unaffected littermates (A); (C, D) lingual view of left hemimandibles of mice carrying *Fgfr2* mutations (D) and their respective unaffected littermates (C).



Fig. S5. Histological analysis of the mandibles of  $Fgfr2^{+/P253R}$  and  $Fgfr2c^{C342Y/+}$  embryos at E16.5. (A-D) ALP and alcian blue staining showed osteogenic tissues (red) and Meckel's cartilage (blue) in the mandible of  $Fgfr2^{+/P253R}$  (B),  $Fgfr2c^{C342Y/+}$  (D) and their  $Fgfr2^{+/+}$  littermate embryos (A, C, respectively). MB, mandibular bone. MC, Meckel's cartilage. (E-H) Alizarin red S staining and (I-L) von Kossa staining showed ossification in the mandible of  $Fgfr2^{+/P253R}$  (F, J) and  $Fgfr2^{+/+}$  littermates (E, I), and  $Fgfr2c^{C342Y/+}$  (H, L) and  $Fgfr2^{+/+}$  littermates (G, K). Scale bar=100 µm. Boxes in G, H, K and L indicate the small area that exhibits impaired microarchitecture at a higher magnification in  $Fgfr2c^{C342Y/+}$  embryos compared with  $Fgfr2^{+/+}$  littermates.



## ltgb3 Dcstamp, Oscar I 2 Car2, Tnfrsf11a | 2 Csf1r Rac2 Acp5 Ctsk Vcam1 Thbs3 Aspn C1qb Aldh1a3, Cryab I 2 Bpgm, Hba-a1, Hba-a2, Hbb-bs I 4 Pdpn Fbn1 Negr1 Cd24a Tnn lgf1, Ptn I 2 Ackr3, Egfl7 I 2 Chac1 Fmod, Matn2, Ogn I 3 Sgcg Cdkn1c, D3Bwg0562e, Gpr126, Nrk I 4 Sfrp2 Chrdl1 Mgp Acta2, Mcc, Penk, Slc26a7, Srl I 5 Fabp4 Abi3bp, Mfap4, Smoc2 I 3 Ckb, Nav2 I 2 Akr1c18, Atp6v0d2, Atp6v1a, Dgki, Slc4a2, Unc13c, Xpr1 I 7 4930506M07Rik, Abl2, Dock5, Egr1, Fbln1, ltgb2, Plxna4, Sema5a I 8 Mmp9 Chd7 Enpp1 Stard9 Cyfip2, Etv1, Ext1 I 3 Gfra1, Srgap3 | 2 Arsb, II1r1, Megf10 | 3 Anpep, Atp1b1, Pls1, Pros1 I 4 Calcr Ocstamp Agap1, Emb, Gnptab, Rapgef6, Slc37a2 I 5 Ank





**Fig. S6. Gene ontology analysis of DEGs in mandibular bone of** *Fgfr2*<sup>+/S252W</sup> **embryos at E16.5.** (A) Biological process. (B) Cellular component. (C) Molecular function.



Fig. S7. Osteoclast activity in the mandibles of three FGFR2-related craniosynostosis mouse models at E16.5. (A-E) TRAP staining (purple) in mandibular bone of  $Fgfr2^{+/+}$  (A),  $Fgfr2^{+/S252W}$  (B) and  $Fgfr2^{+/P253R}$  (C) embryos on a C57BL6/J (B6) background; and  $Fgfr2c^{+/+}$  (D) and  $Fgfr2c^{C342Y/+}$ (E) on a CD1 background. Scale bar=100 µm. The boxes in D and E indicate the area that shows increased signal in  $Fgfr2c^{C342Y/+}$  embryos compared with unaffected littermates.



Fig. S8. Apoptosis in the mandible of  $Fgfr2^{+/s252W}$  embryos at E16.5. (A) TUNEL assay in the mandibular area. TUNEL signal was detected in the mandibular bone (MB) and the perichondrium of Meckel's cartilage (MC) of both  $Fgfr2^{+/+}$  and  $Fgfr2^{+/s252W}$  embryos. Scale bar=100 µm. (B-C) Quantification shows the number of TUNEL-positive cells in the mandibular bone (B) and the perichondrium (C) of  $Fgfr2^{+/+}$  (n=3) and  $Fgfr2^{+/s252W}$  (n=3) embryos. The experimental data were analyzed by two-tailed Welch's t-test and expressed as the mean±s.e.m.

Landmark (Left, Right)	Anatomical Definition	Region
1, 2	Inferior-most point on incisor alveolar rim at midline of the incisor at bone-tooth junction	AB
3, 4	Junction of the rim of the alveolar process with incisor, most lateral and centered along the cranial-caudal axis (taken on bone, not tooth)	AB
5, 6	Superior-most point on incisor alveolar rim at midline of bone- tooth junction	AB
7, 8	Anterior lip of the mental foramen	AB
9, 10	Antero-lateral edge of the molar alveolar process	AB
11, 12	Intersection of molar alveolar rim and base of coronoid process (posterior molar alveolus)	R, AB
13, 14	Most dorsal point of the coronoid process	R
15, 16	Most cranio-ventral point between the coronoid and condyloid processes	R
17, 18	Most caudal point on the cranial angle of the condyloid process	R
19, 20	Most caudal point on the ventral angel of the condyloid process	R
21, 22	Most anterior point between the angle that separates the condyloid and angular processes	R
23, 24	Midpoint on the cranial caudal axis of the most posterior aspect of the angular process	R
25, 26	Cranial-most point of the angular process along the ventral surface of the mandible	R, AB
27, 28	Dorsal mandibular foramen	R
29, 30	Most ventral edge of the molar alveolar process, toward the midline	R
31, 32	Ventral mandibular foramen	AB

Table S1. Anatomical definitions of landmarks displayed in Fig. 1.

Landmarks are classified as located on Anterior Body (AB; *n*=8) or Ramus (R; *n*=10) to create subsets for analysis. Additional landmarks defined on the embryonic mouse skull can be found at: <u>http://www.getahead.la.psu.edu/landmarks</u>.

Table S2. Results (*P* values) of nonparametric null hypothesis tests for form differences (EDMA) between mice (P0) carrying a mutation for a specific craniosynostosis syndrome and their littermates without the mutation.

Craniosynostosis Model	Left and Right Hemimandibles (32 landmarks)	<b>Left Hemimandible</b> (16 landmarks)	<b>Right Hemimandible</b> (16 landmarks)
Fgfr2 <sup>+/S252W</sup>	0.001	0.001	0.001
Fgfr2 <sup>+/P253R</sup>	0.001	0.001	0.001
Fgfr2c <sup>C342Y/+</sup>	0.001	0.001	0.001

## Table S3. Differentially expressed genes in the mandibular bone of Fgfr2<sup>+/S252W</sup> embryos at

E16.5 compared to their  $Fgfr2^{+/+}$  littermates.

Ensembl ID	Gene	log <sub>2</sub> FC	Average	t	P Value	Adjusted P
	Symbol		Expression			Value
ENSMUSG0000030257	Srgap3	1.27673267	6.610930486	9.640369417	5.20142E-07	0.003057916
ENSMUSG0000037370	Enpp1	1.831773279	8.59074871	10.20061207	2.82417E-07	0.003057916
ENSMUSG0000045573	Penk	-1.928167528	4.158086959	-9.22055957	8.37469E-07	0.003188606
ENSMUSG0000034573	Ptpn13	1.133258416	6.830424831	8.998531675	1.08474E-06	0.003188606
ENSMUSG0000069917	Hba-a2	-1.622438006	4.106158794	-8.558032456	1.83915E-06	0.003902154
ENSMUSG0000022265	Ank	1.326616334	7.721340364	8.493163202	1.99123E-06	0.003902154
ENSMUSG0000073940	Hbb-bt	-1.628190337	8.121203499	-8.263608921	2.64729E-06	0.004446698
ENSMUSG0000042436	Mfap4	-1.15168023	6.836719917	-8.135654118	3.11049E-06	0.004571637
ENSMUSG0000024593	Megf10	0.927309625	6.425005115	7.528605255	6.85637E-06	0.008957467
ENSMUSG0000036905	C1qb	-2.263114164	2.013981763	-6.999425722	1.41583E-05	0.011193739
ENSMUSG0000052305	Hbb-bs	-1.57470114	9.839937637	-7.157779	1.13549E-05	0.011193739
ENSMUSG0000035783	Acta2	-1.163348891	5.560032653	-6.993321688	1.42802E-05	0.011193739
ENSMUSG0000006369	Fbln1	0.877748065	5.903300431	7.109264313	1.21449E-05	0.011193739
ENSMUSG0000054594	Oscar	1.217936999	5.141501095	7.270388572	9.72461E-06	0.011193739
ENSMUSG0000040703	Cyp2s1	1.331780479	3.815520063	6.998767749	1.41714E-05	0.011193739
ENSMUSG0000031351	Zfp185	-2.478906784	3.159340528	-6.742466291	2.03933E-05	0.011887187
ENSMUSG0000033491	Prss35	-1.351275459	5.889560643	-6.721057207	2.1031E-05	0.011887187
ENSMUSG0000040569	Slc26a7	-1.106708548	6.18519432	-6.763093111	1.97983E-05	0.011887187
ENSMUSG0000037664	Cdkn1c	-0.880989857	8.705576379	-6.714493501	2.12307E-05	0.011887187
ENSMUSG0000024621	Csf1r	0.932343075	7.271710056	6.790660552	1.90318E-05	0.011887187
ENSMUSG0000020340	Cyfip2	1.222456601	6.830403391	6.838067414	1.77866E-05	0.011887187
ENSMUSG0000028047	Thbs3	-1.146915292	4.350320931	-6.66151792	2.29185E-05	0.012248919
ENSMUSG0000052512	Nav2	0.954836312	6.560031841	6.618351607	2.43993E-05	0.012473328
ENSMUSG0000014846	Тррр3	-2.657022312	2.498697296	-6.399689044	3.36333E-05	0.015732053
ENSMUSG0000055013	Agap1	0.824170057	6.949305293	6.376950077	3.47877E-05	0.015732053
ENSMUSG0000023964	Calcr	1.39086817	4.385746777	6.386112042	3.43176E-05	0.015732053
ENSMUSG0000044667	Plppr4	-1.64880105	3.281452634	-6.337299974	3.69024E-05	0.016070294
ENSMUSG0000021388	Aspn	-1.565468808	7.851433763	-6.235000826	4.30138E-05	0.017532802
ENSMUSG0000026321	Tnfrsf11a	0.878575936	6.328271017	6.231469435	4.3243E-05	0.017532802

ENSMUSG0000069516	Lvz2	-1 48397449	5 657315666	-5 984140315	6 30167E-05	0.021283798
ENSMUSG0000030218	Man	-1 221549215	4 443285577	-6.029622637	5.87632E-05	0.021283798
ENSMUSC0000041559	Emod	-0.935657006	6.937668196	-6.047249988	5.07032E 05	0.021283798
ENSMUSC0000026460	Vnr1	0.756419695	0. <i>737</i> 008170	5.065/20507	5.71775E-05	0.021283778
ENSMUSC0000020409	Cantah	0.730416063	0.300400909	6.049247217	0.46399E-03	0.021203790
ENSMUSG00000033311	Giptab	0.877333017	7.078081271	0.048247317	5./1102E-03	0.021283798
ENSMUSG00000022303	Destamp	1.01951/844	5.581854423	5.945315992	6.69054E-05	0.021283798
ENSMUSG0000040061	Plcb2	1.240168118	3.840512082	5.944636192	6.69/57E-05	0.021283798
ENSMUSG0000021214	Akr1c18	1.299377329	4.04982874	5.9744297	6.39663E-05	0.021283798
ENSMUSG0000020053	Igfl	-1.273626277	6.98869413	-5.913249668	7.03087E-05	0.021754992
ENSMUSG0000028238	Atp6v0d2	0.857306076	8.426331011	5.891337145	7.27394E-05	0.021929993
ENSMUSG0000031283	Chrdl1	-0.834377044	5.949540909	-5.813408207	8.21296E-05	0.021947271
ENSMUSG0000001270	Ckb	0.899333325	8.11410426	5.841953781	7.85492E-05	0.021947271
ENSMUSG0000050390	C77080	1.100606755	4.008047025	5.824856679	8.06733E-05	0.021947271
ENSMUSG0000058897	Col25a1	1.145484143	6.457814805	5.817728948	8.15768E-05	0.021947271
ENSMUSG0000025089	Gfra1	1.467929152	4.267875815	5.853457969	7.71533E-05	0.021947271
ENSMUSG0000012405	Rpl15	-0.906856775	6.677200075	-5.773069762	8.74862E-05	0.022362233
ENSMUSG0000021728	Emb	0.914199758	5.859278388	5.781838677	8.62911E-05	0.022362233
ENSMUSG0000032948	Lipi	-0.936729854	5.444723773	-5.663403914	0.000104002	0.025438301
ENSMUSG0000022324	Matn2	-0.694566276	5.740143219	-5.640097671	0.000107918	0.025438301
ENSMUSG0000052459	Atp6v1a	0.718305056	8.851933228	5.638604413	0.000108174	0.025438301
ENSMUSG0000044447	Dock5	1 048477353	6 571337781	5 639942804	0.000107945	0.025438301
ENSMUSG0000017737	Mmn9	1 19764316	9 755281083	5 569229207	0.000120814	0.027853587
ENSMUSC0000035296	Saca	-1.17704510	-0.922779598	-5 517657881	0.000120014	0.027033507
ENSMUSC0000033230	Grobp1	-4.411/10/92	2 115220671	5 407116925	0.000131213	0.028010594
ENSMUSC00000052251	Nuls	-2.133204427	5.115259071	-5.49/110625	0.000135018	0.028010594
ENSMUSC0000001248	INIK Apr	-1.48/002892	0.023819730	-3.34/231304	0.000123130	0.028010394
ENSMUSG0000001348	Acp3	0.793398373	6.02/342407	5.510155705	0.000131333	0.028010394
ENSMUSG0000021306	Gpr137b	0.861604003	6.046324047	5.519626396	0.000130802	0.028010594
ENSMUSG0000032122	SIc3/a2	1.1910/3369	7.077239901	5.496333193	0.000135789	0.028010594
ENSMUSG0000069919	Hba-al	-1.33/990689	7.815219545	-5.422264917	0.000153024	0.030909916
ENSMUSG0000020689	Itgb3	1.110567359	7.880369008	5.41393388	0.000155102	0.030909916
ENSMUSG0000027562	Car2	0.745533042	6.189698692	5.394819185	0.000159983	0.031351269
ENSMUSG0000022519	Srl	-0.874711972	4.426953711	-5.369870117	0.000166598	0.03211249
ENSMUSG0000040037	Negr1	-2.06759258	3.207329165	-5.347098779	0.000172888	0.032266944
ENSMUSG0000041362	Shtn1	0.856606818	5.623009126	5.348913307	0.000172378	0.032266944
ENSMUSG0000029765	Plxna4	1.049654389	5.846000814	5.319457787	0.000180861	0.033227474
ENSMUSG0000035258	Abi3bp	-1.564181951	4.695670152	-5.29502116	0.000188231	0.033533688
ENSMUSG0000027962	Vcam1	-1.033135854	4.531254283	-5.295700595	0.000188022	0.033533688
ENSMUSG0000021390	Ogn	-1.35118064	7.552490868	-5.285796718	0.000191095	0.033535785
ENSMUSG0000047139	Cd24a	-0.988039433	5.207433327	-5.274863718	0.000194549	0.033639831
ENSMUSG0000026072	Il1r1	0.782735636	4.957639421	5.248885822	0.000203022	0.034101898
ENSMUSG0000028581	Laptm5	0.879244011	6.642567459	5.25154349	0.000202138	0.034101898
ENSMUSG0000032060	Crvab	-1.110150001	3.610742127	-5.220757626	0.000212635	0.035213634
ENSMUSG0000024236	Svil	0.624813843	7.133248611	5.188180982	0.000224371	0.036640971
ENSMUSG0000026576	Atp1b1	1.17396213	4,596809676	5,172977602	0.000230079	0.037058409
ENSMUSG0000042082	Arsh	0.97021764	7 506223253	5 160310942	0.000234951	0.037331753
ENSMUSG0000004151	Ftv1	0.730282583	4 988104973	5 132028881	0.000236224	0.038601426
ENSMUSG0000002014	Ssr4	-0.810564086	5 903376453	-5.097609777	0.000240224	0.039301265
ENSMUSC0000034707	Gns	0.72700/1370	7 459110088	5 10/733751	0.000257645	0.039301265
ENSMUSC0000034707	Ocstamp	1.010536701	5 872442547	5 107174041	0.000257645	0.030301265
ENSMUSC0000027070	Evt1	0.662550112	7 426421905	5.060295912	0.000230001	0.039301203
ENSMUSG0000001751		0.005550112	7.430421893	5.009383813	0.000273207	0.0400/181
	Puzrn3	-0.//5//0383	5.110505724	-3.04/300/03	0.000283499	0.04100/193
	itgb2	0.939153536	5.118505724	5.024342179	0.000294628	0.042/68391
ENSMUSG0000033220	Rac2	0.//4/666	6.46652/16/	5.001754487	0.000305993	0.043876437
ENSMUSG0000023886	Smoc2	-1.0/6440655	5.01/46/359	-4.98683623	0.000313751	0.043917621
ENSMUSG0000039116	Gpr126	-0.981742058	4.952859094	-4.988178407	0.000313044	0.043917621
ENSMUSG00000015134	Aldh1a3	-1.172030326	5.661165159	-4.96080253	0.000327786	0.044910263
ENSMUSG0000026921	Egfl7	-0.844921515	4.284607323	-4.957154752	0.000329805	0.044910263
ENSMUSG0000018567	Gabarap	-0.694331619	7.756610472	-4.952676418	0.000332301	0.044910263
ENSMUSG0000037533	Rapgef6	0.614649699	7.303891696	4.92217191	0.000349838	0.046743173

ENSM	USG0000026596	Abl2	0.608326336	6.50724323	4.910707644	0.000356678	0.047121542
ENSM	USG00000044468	Fam46c	-0.684580972	6.669624759	-4.897416701	0.000364783	0.047656834
ENSM	USG0000004730	Emr1	-2.239807676	1.348603199	-4.832587645	0.000407175	0.049783334
ENSM	USG0000027313	Chac1	-2.213689517	4.887600415	-4.764393172	0.000457375	0.049783334
ENSM	USG0000027996	Sfrp2	-1.171324394	4.710267004	-4.788885324	0.000438639	0.049783334
ENSM	USG0000062515	Fabp4	-1.05622216	3.509023446	-4.704234021	0.000507036	0.049783334
ENSM	USG0000028583	Pdpn	-1.045235352	3.565172539	-4.802701535	0.000428426	0.049783334
ENSM	USG00000044337	Ackr3	-1.019213136	4.030412869	-4.708330855	0.000503482	0.049783334
ENSM	USG0000029838	Ptn	-0.936047162	8.138301746	-4.752584264	0.000466706	0.049783334
ENSM	USG0000027204	Fbn1	-0.801254068	6.408244189	-4.808538301	0.000424186	0.049783334
ENSM	USG0000026421	Csrp1	-0.783880408	6.759861112	-4.787747037	0.000439492	0.049783334
ENSM	USG0000026725	Tnn	-0.777064263	7.437115497	-4.847170068	0.000397209	0.049783334
ENSM	USG0000071856	Mcc	-0.73828315	5.345518229	-4.714396914	0.000498266	0.049783334
ENSM	USG0000024534	Sncaip	-0.723674183	4.868083805	-4.694867838	0.000515261	0.049783334
ENSM	USG0000038871	Bpgm	-0.682666203	5.249711873	-4.7094474	0.000502517	0.049783334
ENSM	USG0000022912	Pros1	0.663131966	6.459136494	4.749861565	0.000468885	0.049783334
ENSM	USG0000033705	Stard9	0.706268577	6.374308981	4.698304549	0.000512227	0.049783334
ENSM	USG0000061175	Fnip2	0.710174275	5.074804605	4.84910423	0.000395907	0.049783334
ENSM	USG0000028111	Ctsk	0.713395398	9.9623244	4.816083029	0.000418771	0.049783334
ENSM	USG0000075324	Fign	0.737656804	5.609855492	4.780463665	0.000444989	0.049783334
ENSM	USG0000028962	Slc4a2	0.773555819	6.05593102	4.795937482	0.000433394	0.049783334
ENSM	USG00000041235	Chd7	0.793445297	5.723571848	4.736175534	0.000480003	0.049783334
ENSM	USG0000039062	Anpep	0.813927796	8.056912398	4.76404628	0.000457646	0.049783334
ENSM	USG0000068854	Hist2h2be	0.830691553	4.786180283	4.735427416	0.000480619	0.049783334
ENSM	USG0000038665	Dgki	0.831832322	4.196277449	4.707501683	0.000504199	0.049783334
ENSM	USG0000079057	Cyp4v3	0.83836864	4.764351948	4.838702637	0.000402965	0.049783334
ENSM	USG0000022231	Sema5a	0.839094343	9.365098271	4.837629137	0.0004037	0.049783334
ENSM	USG0000026437	Cdk18	0.88555389	3.867251827	4.693416906	0.000516548	0.049783334
ENSM	USG0000022012	Enox1	0.892698352	3.686678832	4.709026013	0.000502881	0.049783334
ENSM	USG0000018774	Cd68	0.959283668	7.245963357	4.703581561	0.000507605	0.049783334
ENSM	USG0000079625	Tm4sf19	1.030522587	3.924772294	4.762574467	0.000458799	0.049783334
ENSM	USG0000038418	Egr1	1.120357726	6.447009919	4.772930692	0.000450751	0.049783334
ENSM	USG00000049493	Pls1	1.373575186	2.360582503	4.733077184	0.000482558	0.049783334
ENSM	USG0000062151	Unc13c	1.540649612	3.574785275	4.723859106	0.000490244	0.049783334

## Table S4. Primers used to generate riboprobe templates for RNA *in situ* hybridization.

Gene	Primers	References
Ank	5'-GAGTAATACGACTCACTATAGGGTGGGATGTGCCTCAATCTCA-3'	Uzuki et al., 2014
	5'-GAGATTAACCCTCACTAAAGGGACACAGAGTTCTGCAAAGGCAA-3'	
Csf1r	5'-GAGTAATACGACTCACTATAGGGAGGAGGTGTCTGTGGGTGAC-3'	Designed using
	5'-GAGATTAACCCTCACTAAAGGGATGGTACTTCGGCTTCTGCTT-3'	Primer3
Enpp1	5'-GAGTAATACGACTCACTATAGGGGGCTGTCTGAGACTCCCTTGG-3'	Designed using
	5'-GAGATTAACCCTCACTAAAGGGAGTCCCCAGACCACGTACACT-3'	
ltgb3	5'-GAGTAATACGACTCACTATAGGGGAAAATGTCGTCAGCCTTTACC-3'	Diez-Roux et al.,
	5'-GAGATTAACCCTCACTAAAGGGAGCAGGAGAAGTCATCGCACTC-3'	2011