but the differences are not significant (n.s.) at 36 hpf. Symplectic cartilage was marked using *sox9a:GFP* and epithelial tissues were marked using anti-P63. Symplectic cartilage length and the endoderm-ectoderm gap were measured and recorded for each individual embryo. (B) Diagram of the *fras1* revertible allele experiment. Embryos trans-heterozygous for *fras1*^{mn0156Gt} and *fras1*^{te262d} were heat-shocked at different developmental stages to induce Cre recombinase expression in the half of the clutch that inherited the *hsp70l:Cre* transgene. Cre activity removes the insertion trap transgene (which, when present, encodes an mRFP tag and a premature stop codon after exon 15), and restores *fras1* function. Skeletal preparations of heat-shocked animals carrying the *Cre* transgene (Cre+, reverted) were compared to siblings that did not inherit the transgene (Cre-, control). (C-E) Larvae heat shocked at the indicated time were stained for cartilage and bone at 6 dpf, genotyped for *hsp70l:Cre*, and each fish was scored for the indicated skeletal trait. Graphs show the penetrance per fish, defined as the percent of fish with a given defect on at least one side of the embryo. Error bars in A are 95% confidence intervals: 1.95 times the standard error. Error bars in C-E are standard deviations; * indicates $P<0.05$, and ** denotes $P<0.001$.

Figure 8: Epithelial-mesenchymal Fras1-Itga8 interactions sculpt zebrafish facial development. (A) Proposed structure of a Fras1-Itga8 interacting complex. Fras1 protein (orange circle) is part of the FPC (gray filled circles), that interacts with Itga8, directly or indirectly (dotted lines), to attach mesenchymal cells to the lamina densa (orange bar), which is itself attached (orange arches) to epithelial cells. Fras1 may be able to participate in weak epithelial/mesenchymal interactions independently of Itga8 (narrow dotted lines), but most of their function occurs via one another (broad dotted lines). (B) Diagram modeling how epithelial-mesenchymal adhesion might narrow the space between endoderm and ectoderm, e.g. during endodermal pouching. (C) Illustration of late-p1 formation, with mesenchyme and skeletal elements shown. In *fras1* and *itga8* mutants, epithelial-mesenchymal interactions are lost and late-p1 fails to form. (D) Timeline of *fras1* and *itga8* functions during different stages of facial development.. Rescue experiments indicate that *fras1* is required (brown) during the cartilage morphogenesis phase of development (green), concurrent with late-p1 formation (orange). Fras1 is dispensable during early endodermal pouching (pink) and cartilage patterning (yellow). In both *fras1* and *itga8* mutants, phenotypes start to diverge from wild type by 36 hpf, and are severe by 72 hpf (red).

Figure S1: Expression of *fras1*-related and *itga8*-related genes in facial tissues. (A-N') RNA in situ hybridization on wild type tissue sections at (A-G') 60 hpf or (H-N') 72 hpf. Each image in A-N shows a lower magnification image of individual sections and A'-N' shows higher magnification images of these sections, focusing on the pharyngeal region. (A, H) *fras1* expression in facial endoderm and ectoderm is shown as a positive control. (B, I) *fbn2a* shows no detectable facial expression. (C, J) *fbn2b* is strongly expressed in arch mesenchyme. (D, K) The *fras1* processing gene *grip1* is expressed in brain and eyes, but not in the pharyngeal regions. (E, L) Similarly, *grip1* paralog *grip2b* is not visibly expressed in pharyngeal regions at 60 hpf (F), but is present by 72 hpf in the joint between the left and right Meckel's cartilage (M, arrow). This suggests that genes other than *grip1* and *grip2b* may process Fras1 in the face, or that earlier *grip* expression enables Fras1 processing. Two primer-sets failed to amplify *grip2a* cDNA at 3 dpf, so

we did not assay facial expression of this gene. (G, N) *npnt* expression is most intense along the lateral edge of facial endoderm (asterisk), where the proposed Itga8-FPC interactions occur. All scale bars are 100 µm, applicable to their respective columns.

Figure S2: *npnt* is expressed in sub-regions of arch epithelia, and *fbn2b* is expressed in arch mesenchyme. In situ hybridization of sections from the head of 60 hpf wild-type embryos. (A) *fras1* is expressed in epithelia. (B) *npnt* is expressed in a subset of these epithelia, while (C) *fbn2b* is expressed in a complementary pattern in arch mesenchyme. (D) Illustration of section level. Ectoderm is shown in blue; cartilages are green; anterior endoderm is orange; posterior endoderm is red. Anterior-posterior level for sections 1-5 are shown with lines in D. Scale bar is 50 µm, applicable to A-C.

Figure S3: Cartilage and late-p1 phenotypes largely correlate in critical window experiments. (A-B) Differential interference contrast images of alcian/alizarin stained tissues of 6 dpf WT (A) and *fras1*^{te262} mutant homozygotes (B). (A) In WT, skeleton is well formed, and a completely outpocketed late-p1 is apparent. (B) In *fras1*^{te262} homozygotes, cartilage defects are seen and late-p1 is absent, as evidenced by mesenchyme occupying the space between ceratohyal and more dorsal skeleton. (C-E) 6 dpf alcian/alizarin skeletal preparations of *fras1* reversion experiments, from crosses described in Fig. 7. Briefly, embryos trans-heterozygous for *fras1*^{mn0156Gt} and *fras1*^{te262d} were heat-shocked at different stages to induce Cre recombinase expression in the half of the clutch that inherited the *hsp70l:Cre* transgene; this Cre induction restores *fras1*^{mn0156Gt} function. (C) If Cre is activated before the *fras1* critical window (30 hpf), larvae form normal endodermal and skeletal morphology. (C') Inset is a different focal plane showing normal symplectic morphology. (D) At 30 hpf, however, heat shock of control Cre- siblings does not result in phenotypic rescue. (E) When heat-shock of Cre restores *fras1*^{mn0156Gt} function after the critical window for *fras1* function (72 hpf), both endodermal and skeletal phenotypes resemble *fras1*^{te262} homozygotes. (F) Plot of late-p1 defect penetrance as a function of time of development, determined by DIC imaging on one side of each embryo (n's range from 5 to 13 for each condition). In Cre+ fish, there is a significant correlation between heat shock time and late-p1 defect penetrance (R^2=0.92, P<0.05), however there is no significant correlation in Cre- fish (R^2=0.17, P=0.6). Scale bar is 100 µm, applicable to A-E.

Movie 1: Late-p1 is lost in *itga8*^{b1161} at 72 hpf, without affecting posterior pouch structures. Movie is a compilation of select transverse sections spanning the first three pharyngeal arches of (A) wild type and (B) *itga8*^{b1161} mutants, aligned using "StackReg" in ImageJ (Thevenaz et al., 1998). Expression of *sox9a:EGFP* reveals cartilage and anti-P63 labeling reveals epithelia. Late-p1 is highlighted in white. Section levels along the A-P axis are indicated by the black bar in (C). Cartilage abbreviations: Meckel's (Me), Retroarticular process (Ra), Palatoquadrate (Pq), Symplectic (Sy), Ceratohyal (Ch), Interhyal (Ih), Ethmoid plate (Ep), Trabecula (Tr), Ceratobranchial cartilage (Cb), basihyal cartilage (Bh). Endodermal abbreviations: Stomadeum

(St); an early forming portion of pouch-1 (early-p1); a late-forming portion of pouch-1 (late-p1); medial endoderm (Md). Scale bar: 100 μm.

References

Akimenko, M.-A., Ekker, M., Wegner, J., Lin, W., Westerfield, M., 1994. Combinatorial Expression of Three Zebrafish Genes Related to Distal-Less: Part of a Homeobox Gene Code for the Head. J. Neurosci. 14, 3475–3486.

Alazami, A.M., Shaheen, R., Alzahrani, F., Snape, K., Saggar, A., Brinkmann, B., Bavi, P., Al-Gazali, L.I., Alkuraya, F.S., 2009. FREM1 Mutations Cause Bifid Nose, Renal Agenesis, and Anorectal Malformations Syndrome. Am. J. Hum. Genet. 85, 414–418.

Alexander, C., Piloto, S., Le Pabic, P., Schilling, T.F., 2014. Wnt Signaling Interacts with Bmp and Edn1 to Regulate Dorsal-Ventral Patterning and Growth of the Craniofacial Skeleton. PLoS Genet. 10, e1004479.

Beattie, C.E., Raible, D.W., Henion, P.D., Eisen, J.S., 1999. Early pressure screens. Methods Cell Biol. 60, 71–86.

Benjamin, J.T., Gaston, D.C., Halloran, B.A., Schnapp, L.M., Zent, R., Prince, L.S., 2009. The role of integrin α8β1 in fetal lung morphogenesis and injury. Dev. Biol. 335, 407–417.

Bieritz, B., Spessotto, P., Colombatti, A., Jahn, A., Prols, F., Hartner, A., 2003. Role of alpha8 integrin in mesangial cell adhesion, migration, and proliferation. Kidney Int. 64, 119–127.

Bonkowsky, J.L., Chien, C.-B., 2005. Molecular cloning and developmental expression of foxP2 in zebrafish. Dev. Dyn. 234, 740–746.

Brandenberger, R., 2001. Identification and characterization of a novel extracellular matrix protein nephronectin that is associated with integrin alpha8beta1 in the embryonic kidney. J. Cell Biol. 154, 447–458.

Carney, T.J., Feitosa, N.M., Sonntag, C., Slanchev, K., Kluger, J., Kiyozumi, D., Gebauer, J.M., Talbot, J.C., Kimmel, C.B., Sekiguchi, K., others, 2010. Genetic analysis of fin development in zebrafish identifies furin and hemicentin1 as potential novel fraser syndrome disease genes. PLoS Genet. 6, e1000907.

Choe, C.P., Crump, J.G., 2015. Dynamic epithelia of the developing vertebrate face. Curr. Opin. Genet. Dev. 32, 66–72.

Clark, K.J., Balciunas, D., Pogoda, H.-M., Ding, Y., Westcot, S.E., Bedell, V.M., Greenwood, T.M., Urban, M.D., Skuster, K.J., Petzold, A.M., Ni, J., Nielsen, A.L., Patowary, A., Scaria, V.,