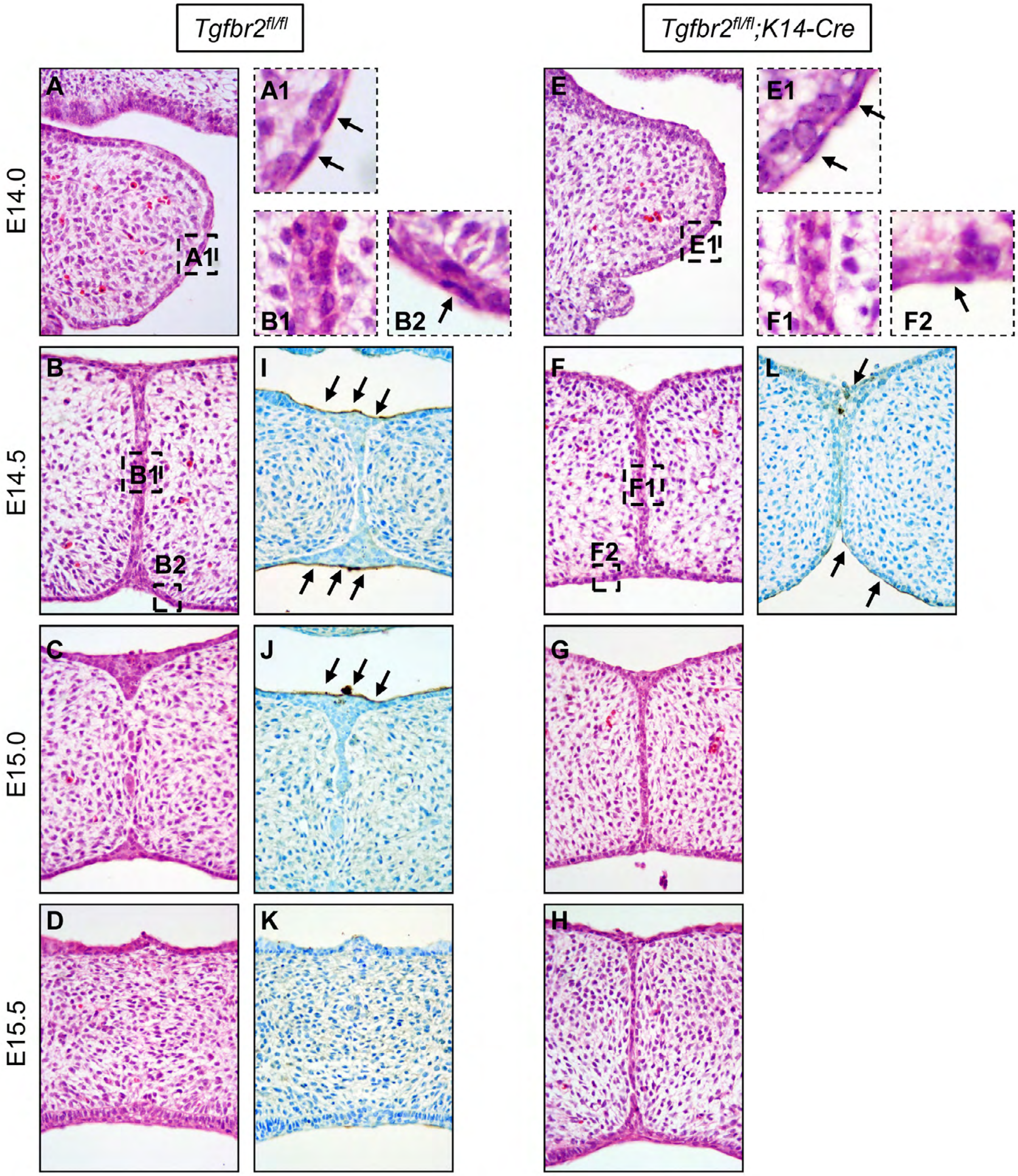

Fig. S1. Periderm disappearance is unaffected in *Tgfbr2$^{fl/fl}$;K14-Cre* mice. (**A-H**) H&E staining of *Tgfbr2$^{fl/fl}$* control (A-D) and *Tgfbr2$^{fl/fl}$;K14-Cre* (E-H) mice at the indicated developmental stages. Boxed areas are shown magnified. Arrows indicate peridermal cells. (**I-L**) Staining of the periderm with anti-Lex/SSEA1 antibody in *Tgfbr2$^{fl/fl}$* control (I-K) and *Tgfbr2$^{fl/fl}$;K14-Cre* (L) mice. Arrows indicate positive signal.

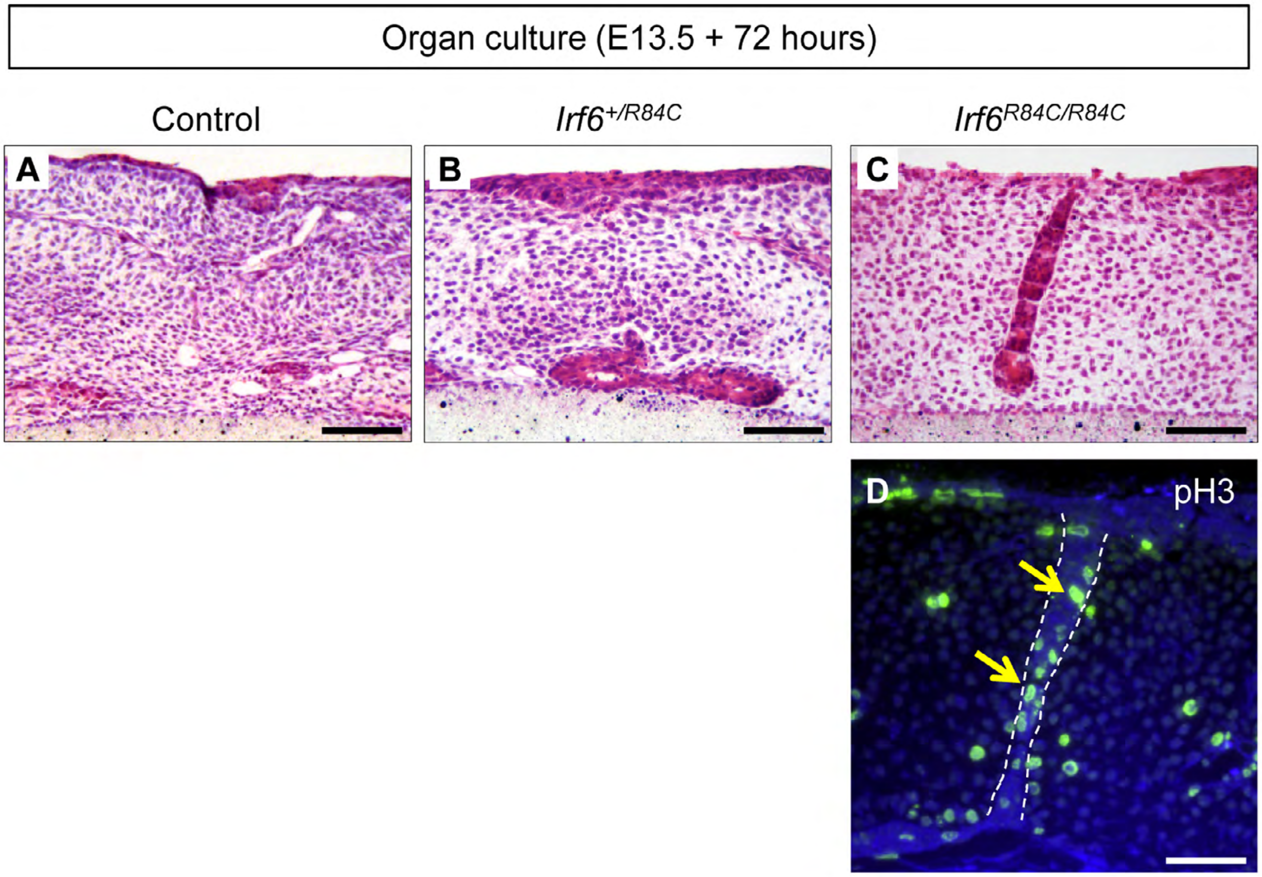

Fig. S2. Loss of IRF6 activity causes MEE persistence. (**A-C**) H&E staining in palatal explants of control (A), *Irf6*$^{+/R84C}$ (B) and *Irf6*$^{R84C/R84C}$ (C) mice after 3 days culture. (**D**) Immunostaining of phosphorylated histone H3 (pH3) in *Irf6*$^{R84C/R84C}$ palatal shelf explants after 3 days culture. Dashed lines indicate the MEE. Arrows indicate positive signal (green). Nuclei were counterstained with DAPI (blue). Scale bars: 100 µm.

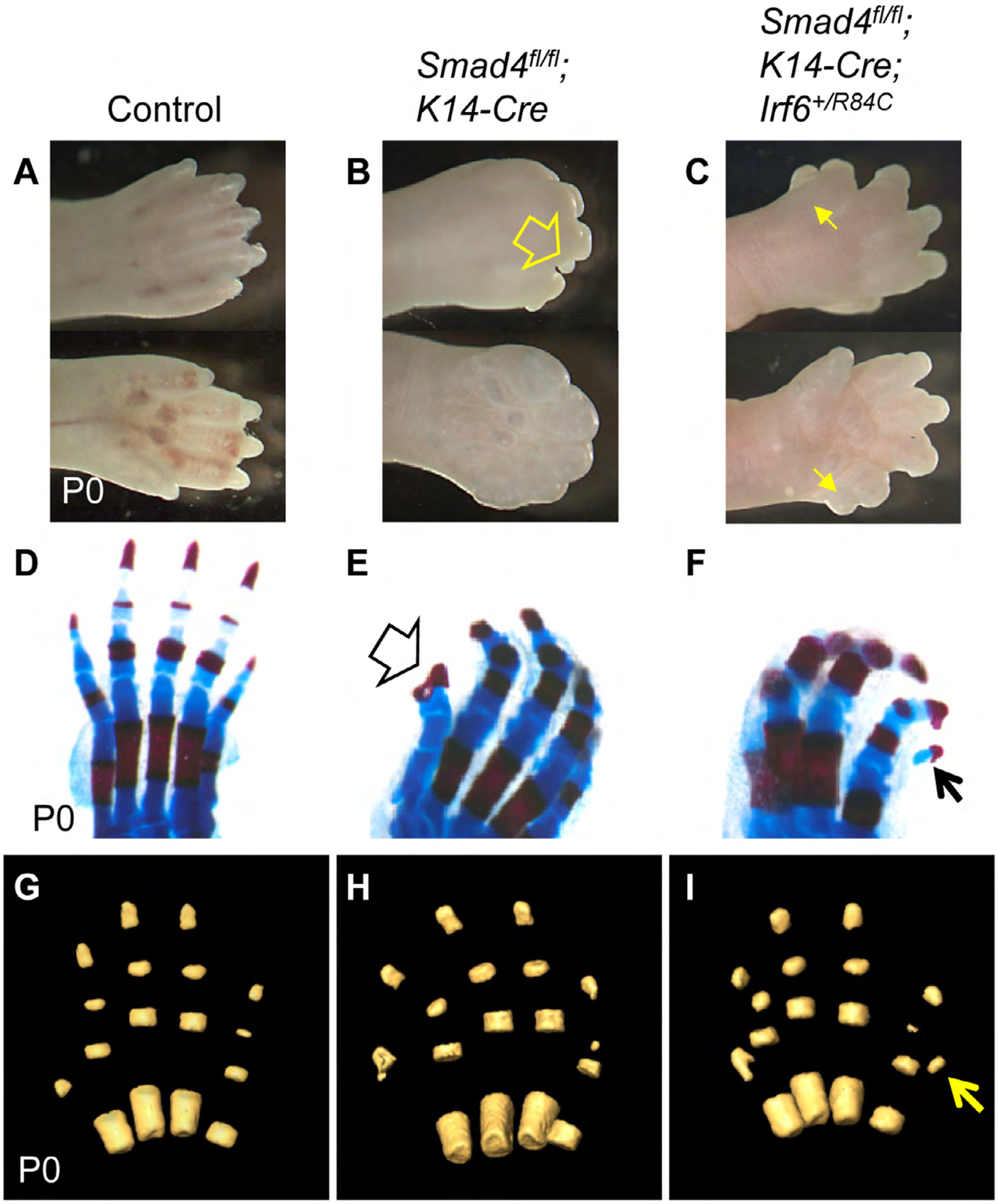

Fig. S3. Haploinsufficiency of *Irf6* in *Smad4*$^{fl/fl}$*;K14-Cre* mice causes syndactyly. (**A-C**) Limb morphologies of newborn control (A), *Smad4*$^{fl/fl}$*;K14-Cre* (B) and *Smad4*$^{fl/fl}$*;K14-Cre;Irf6*$^{+/R84C}$ (C) mice. Open arrow indicates abnormal nails in *Smad4*$^{fl/fl}$*;K14-Cre* mice. Arrows indicate extra digits in *Smad4*$^{fl/fl}$*;K14-Cre;Irf6*$^{+/R84C}$ mice. (**D-F**) Whole-mount Alcian Blue-Alizarin Red skeletal staining of newborn control (D), *Smad4*$^{fl/fl}$*;K14-Cre* (E) and *Smad4*$^{fl/fl}$*;K14-Cre;Irf6*$^{+/R84C}$ (F) mice. Open arrow indicates abnormal nails. Arrow indicates extra digit. (**G-I**) microCT images of the hind-limb in newborn control (G), *Smad4*$^{fl/fl}$*;K14-Cre* (H) and *Smad4*$^{fl/fl}$*;K14-Cre;Irf6*$^{+/R84C}$ (I) mice. Arrow indicates extra digit.

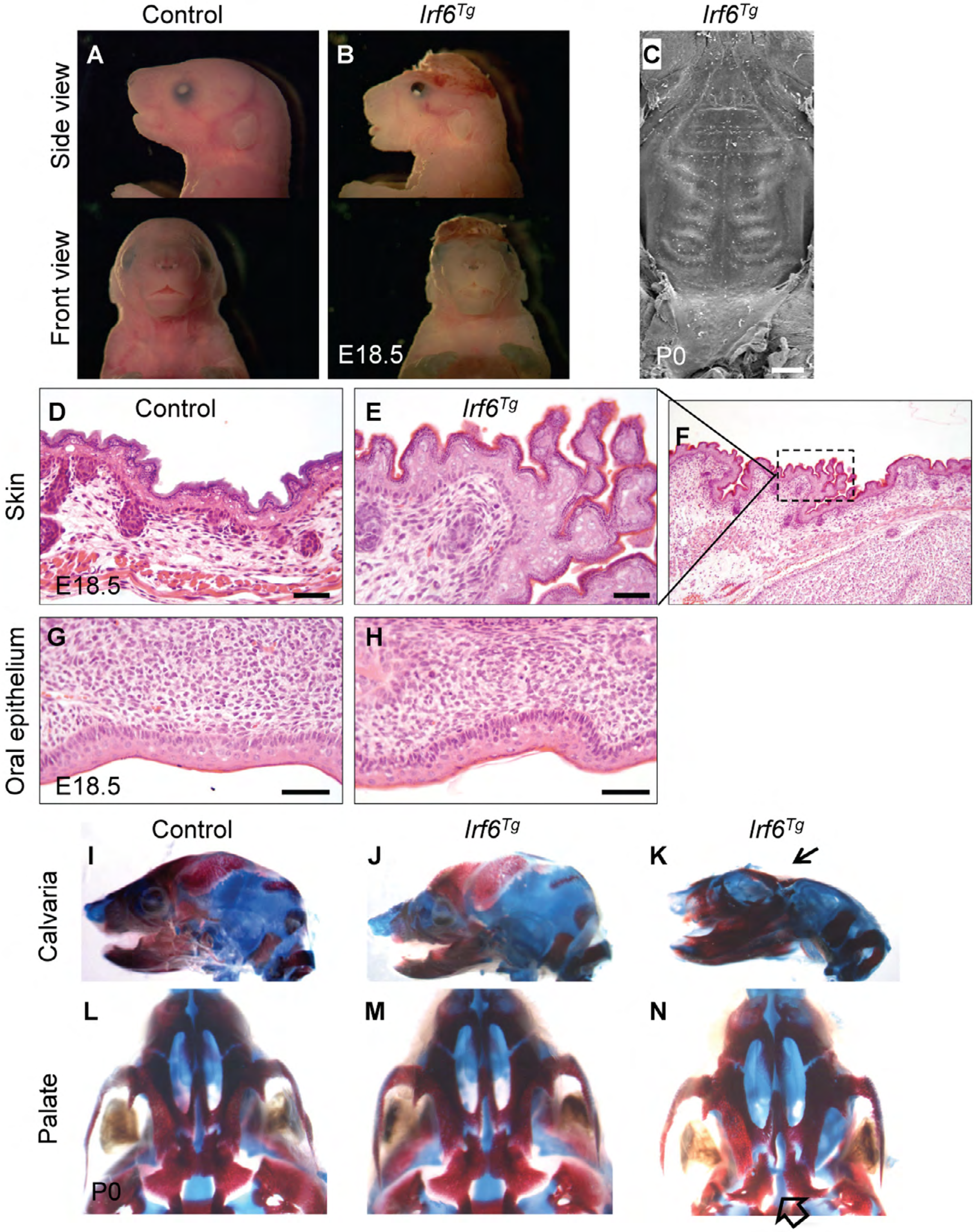

Fig. S4. Morphologies of *Irf6* transgenic mice. (**A**,**B**) Top panels show side views of wild-type (A) and *Irf6*^{Tg} (B) mice, bottom panels show frontal views. Approximately 22% of *Irf6* transgenic embryos exhibit a defect in calvaria formation and open eye at E18.5. (**C**) SEM image of the palate of newborn *Irf6*^{Tg} mice. Scale bar: 500 µm. (**D-H**) H&E staining of the skin (D-F) and oral epithelium (G,H) of wild-type control (D,G) and *Irf6*^{Tg} (E,F,H) mice at E18.5. Boxed area in F is shown magnified in E. Scale bars: 50 µm. (**I-N**) Whole-mount Alcian Blue-Alizarin Red skeletal staining of wild-type control (I,L) and *Irf6*^{Tg} (J,K,M,N) newborn mice. Arrow indicates a defect in the calvaria. Open arrow indicates a defect in the palatine bone. Eighty percent of *Irf6*^{Tg} mice show normal craniofacial development (middle panels; J,M), but 20% of *Irf6*^{Tg} mice exhibit defects in the calvaria and the palatine bone (right panels; K,N).

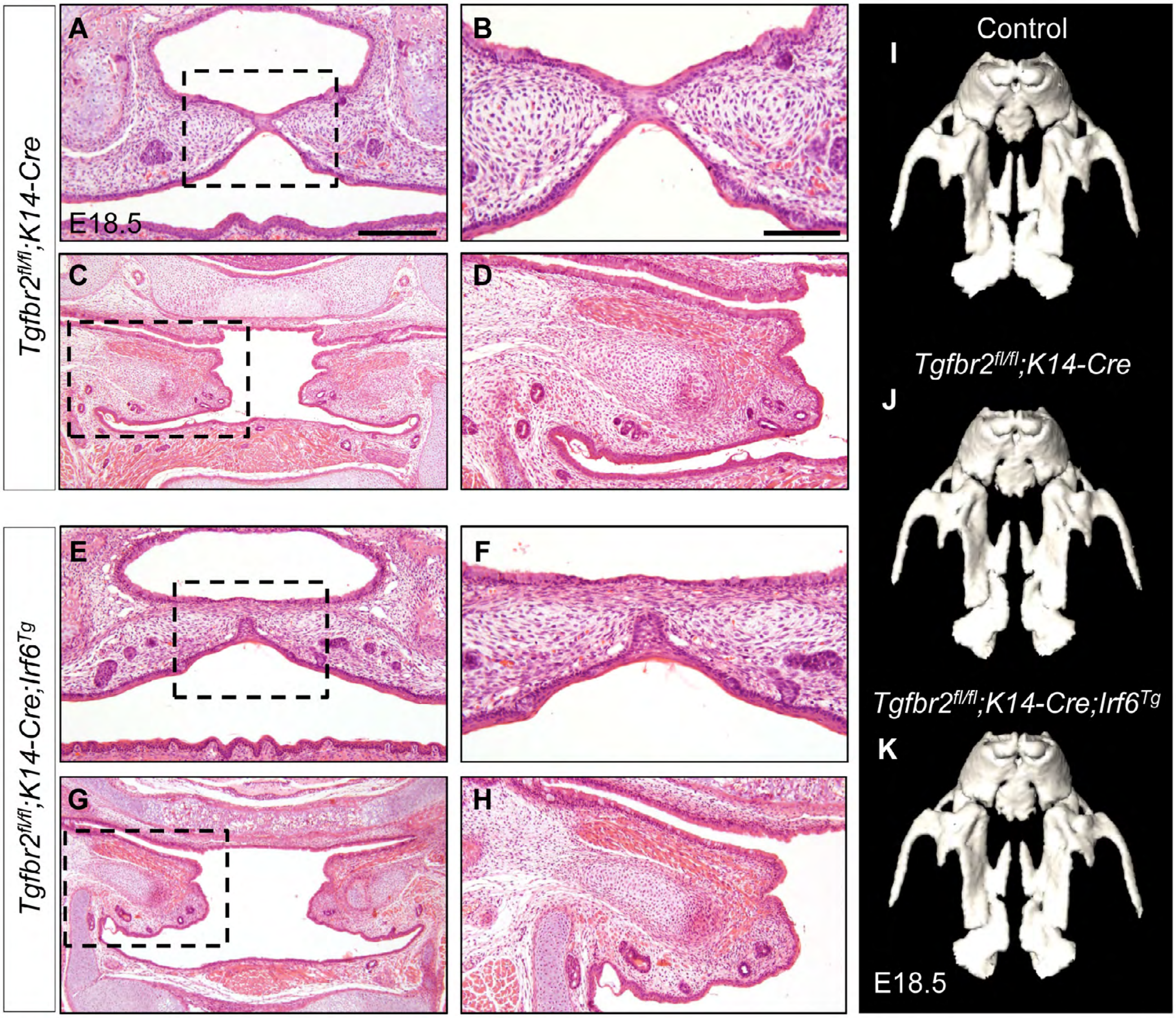

Fig. S5. Cleft soft palate in *Tgfbr2$^{fl/fl}$;K14-Cre* and *Tgfbr2$^{fl/fl}$;K14-Cre;Irf6^{Tg}* mice. (**A-H**) H&E staining of *Tgfbr2$^{fl/fl}$;K14-Cre* (A-D) and *Tgfbr2$^{fl/fl}$;K14-Cre;Irf6^{Tg}* (E-H) mice at E18.5. Boxed areas are enlarged in panels to the right. Scale bars: 250 µm in left-hand panels; 100 µm in right-hand panels. (**I-K**) MicroCT analysis of the hard tissues of E18.5 control (I), *Tgfbr2$^{fl/fl}$;K14-Cre* (J) and *Tgfbr2$^{fl/fl}$;K14-Cre;Irf6^{Tg}* (K) mice.

Table S1. Conserved SMAD recognition sequences in the *Irf6* gene

Binding site position relative to TSS	Genomic context	Binding site sequence context*	Conservation of 4-bp site
–2375 to –2372	Upstream	TGT**GTCT**TGA	All but rat
–1695 to –1692	Upstream	GAC**GTCT**TCT	All but dog/horse
–274 to –271	Upstream	CCA**GTCT**CAG	All eight species

*Binding site motif is underlined in all cases. It is noted in red if conserved in all eight species, and in blue if conserved in all but the noted species in the rightmost column. The eight species include: human (Build 19), chimpanzee (Build 2.1.3), orangutan (Build 2.0.2), rhesus macaque (Build 1.0), mouse (Build 38), rat (Build 3.4), dog (Build 2) and horse (Build equCab2).

Table S2. Penetrance of abnormalities in *Irf6* transgenic mice

Abnormality	Wild-type mice	*Irf6* transgenic mice
Skull defect	1.28% (2/156)	21.92% (32/146)
Open eye	0% (0/156)	21.92% (32/146)
Cleft lip	0% (0/156)	2.74% (4/146)

Phenotype of wild-type littermate control and *Irf6* transgenic mice was scored at E18.5.