

Supplemental Data

FGF20 governs formation of primary and secondary dermal condensations in developing hair follicles

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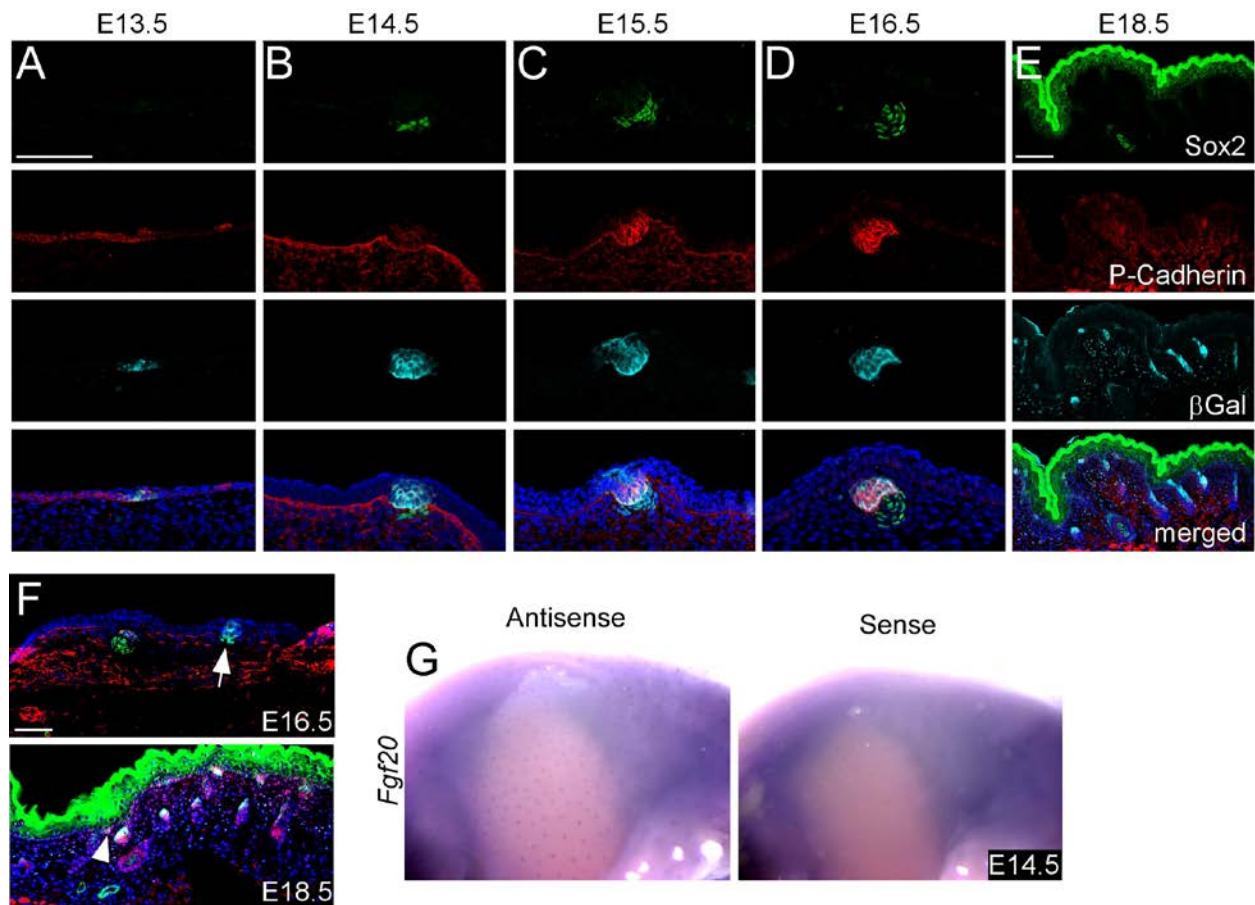


Figure S1. Expression of $FGF20^{\beta Gal}$ during hair follicle development. (A-F) Co-immunostaining for Sox2, P-Cadherin, and βGal in $Fgf20^{\beta Gal/+}$ embryos showing restricted epithelial expression of $FGF20^{\beta Gal}$ in pre-placode epidermis at E13.5 (A), hair placode at E14.5 (B), hair germ at E15.5 (C), hair peg at E16.5 (D), and bulbous peg at E18.5 (E). $FGF20^{\beta Gal}$ is expressed in secondary (arrow in F at E16.5) and tertiary (arrowhead in F at E18.5) hair placodes. (G) *In situ* hybridization for $Fgf20$ at E14.5 showing expression in primary hair placodes. Scale bar 100 μm .

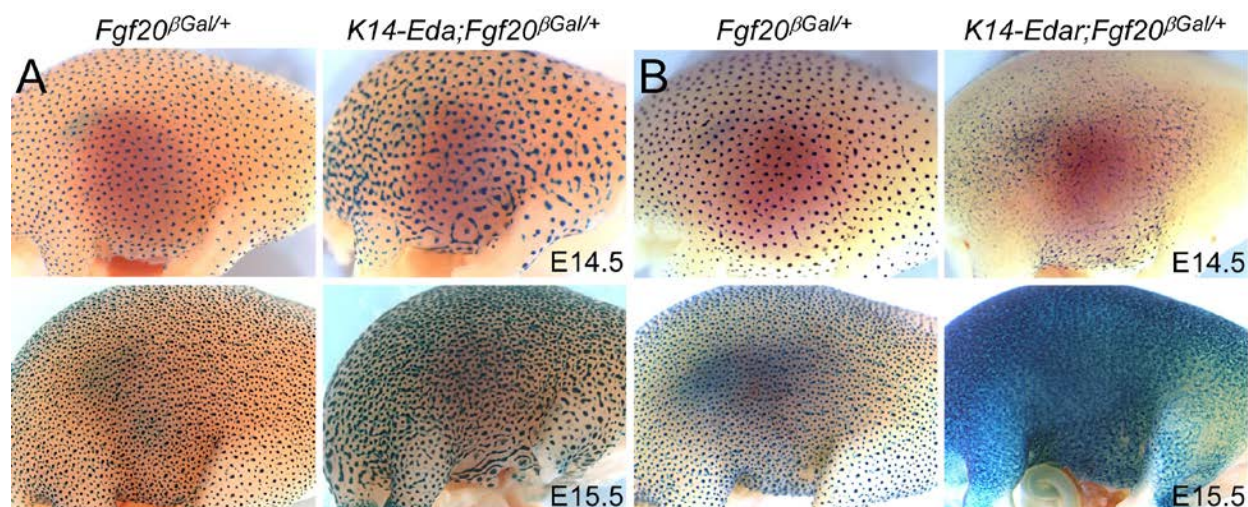


Figure S2. *Fgf20* is induced by Eda/Edar signaling *in vivo* in skin. (A) Increased βGal staining observed in *K14-Eda;Fgf20*^{βGal/+} compared to *Fgf20*^{βGal/+} embryos at E14.5 (upper) and E15.5 (lower). (B) Increased βGal staining observed in *K14-Edar;Fgf20*^{βGal/+} embryos throughout the epithelium compared to focal staining in *Fgf20*^{βGal/+} embryos at E14.5 (upper) and E15.5 (lower).

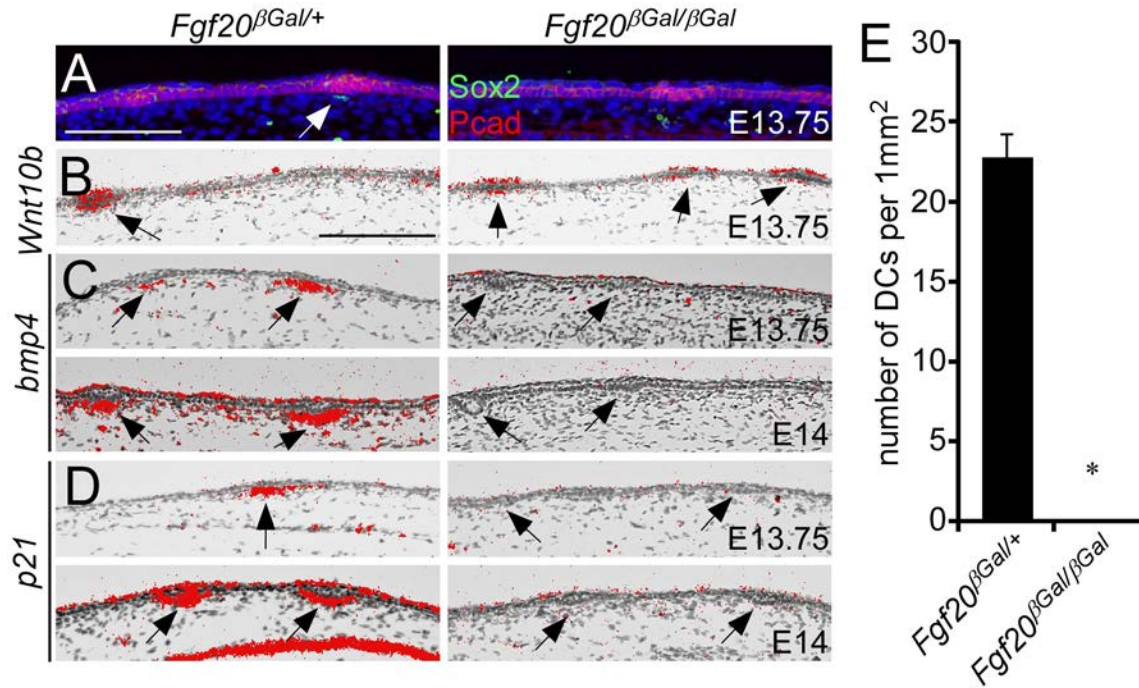


Figure S3. Primary dermal condensations fail to form in *Fgf20*^{βGal/βGal} embryos. (A) Co-immunostaining for Sox2 and P-Cadherin(PCad) showing that PCad+ placodes were observed at E13.75 in both *Fgf20*^{βGal/+} and *Fgf20*^{βGal/βGal} embryos. PCad+ foci were occasionally associated with Sox2+ cells in *Fgf20*^{βGal/+} but not in *Fgf20*^{βGal/βGal} embryos. (B-D) *In situ* hybridization for *Wnt10b* (B), *Bmp4* (C) and *p21* (D) showing presence of nascent placodes at E13.75 and E14 in both *Fgf20*^{βGal/+} and *Fgf20*^{βGal/βGal} embryos induced by Wnt10b staining (B). The dermal condensation markers, *Bmp4* and *p21*, were detected in *Fgf20*^{βGal/+} control embryos (C, D) but not in *Fgf20*^{βGal/βGal} embryos. (E) Counting number of dermal condensates showing there is no dermal condensates in *Fgf20*^{βGal/βGal} embryos. Scale bar 100 μm. Data are shown as mean ± S.D.

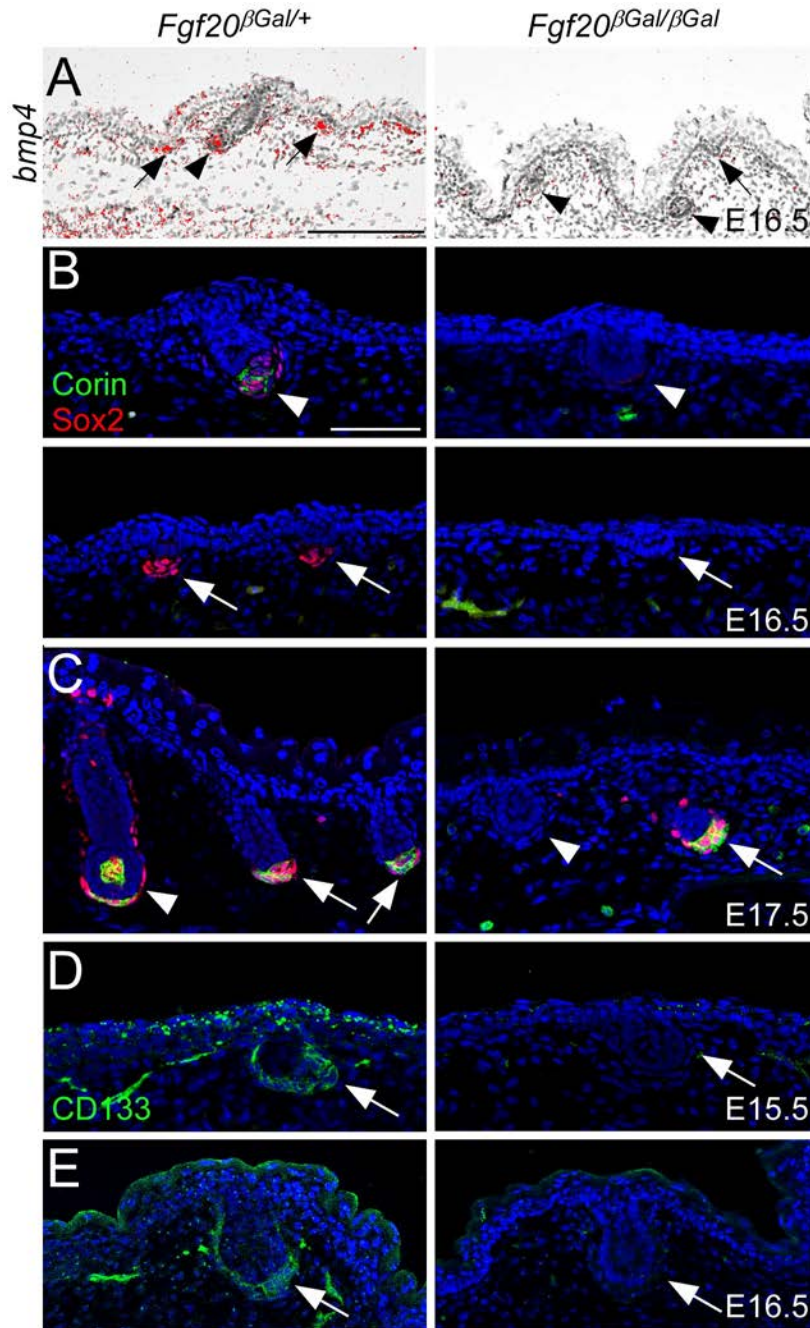


Figure S4. Delayed formation of secondary dermal condensations in *Fgf20*^{βGal/βGal} embryos. (A) At E16.5, primary and secondary hair follicles were associated with *Bmp4* positive dermal condensation in control (left), but rarely in *Fgf20*^{βGal/βGal} embryos (right). (B-C), At E16.5 (B), co-immunostaining for Sox2 and Corin showed mature dermal condensation formation

(Sox2+,Corin+) in primary hair follicles (A, upper left, arrowhead) and initiation of dermal condensations (Sox2+,Corin-) in secondary hair follicles (B, lower left, arrows) in *Fgf20* ^{β Gal/+} embryos. Corin and Sox2 expression were not observed in *Fgf20* ^{β Gal/ β Gal} embryos at E16.5. At E17.5 (C), primary hair follicle dermal papilla (arrowhead, left) and maturing condensations of secondary follicles (arrow, left) were Sox2+ and Corin+ in *Fgf20* ^{β Gal/+} embryos. In *Fgf20* ^{β Gal/ β Gal} embryos, bud stage follicles did not stain with either marker (arrowhead, right) but more advanced follicles (arrow, right) were Sox2+ and Corin+. (D-E) CD133 staining showing dermal condensates are positive of CD133 at E15.5 (left in D) and E16.5 (left in E) in *Fgf20* ^{β Gal/+} embryos. No CD133 staining in *Fgf20* ^{β Gal/ β Gal} embryos at E15.5 (right in D) and E16.5 (right in E). Scale bar 100 μ m.

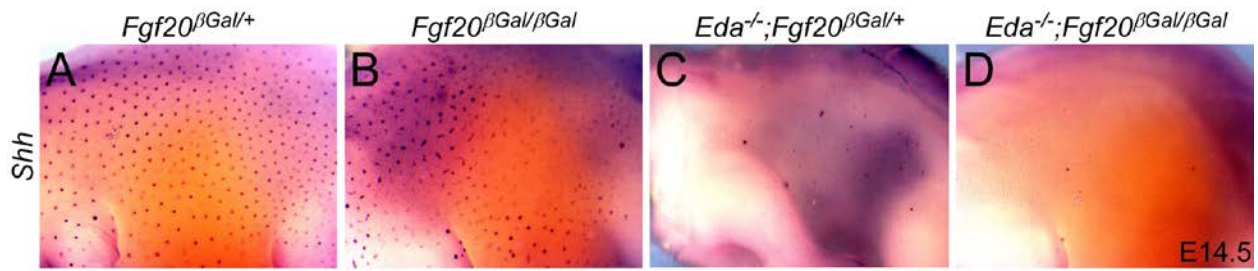


Figure S5. *Eda* is required for *Shh* expression in *Fgf20* ^{β Gal/ β Gal} embryos. *In situ* hybridization for *Shh* showing focal expression in *Fgf20* ^{β Gal/+} (A) and *Fgf20* ^{β Gal/ β Gal} (B) embryos, but not in *Eda*^{-/-}; *Fgf20* ^{β Gal/+} (C), or *Eda*^{-/-}; *Fgf20* ^{β Gal/ β Gal} (D) embryos at E14.5.

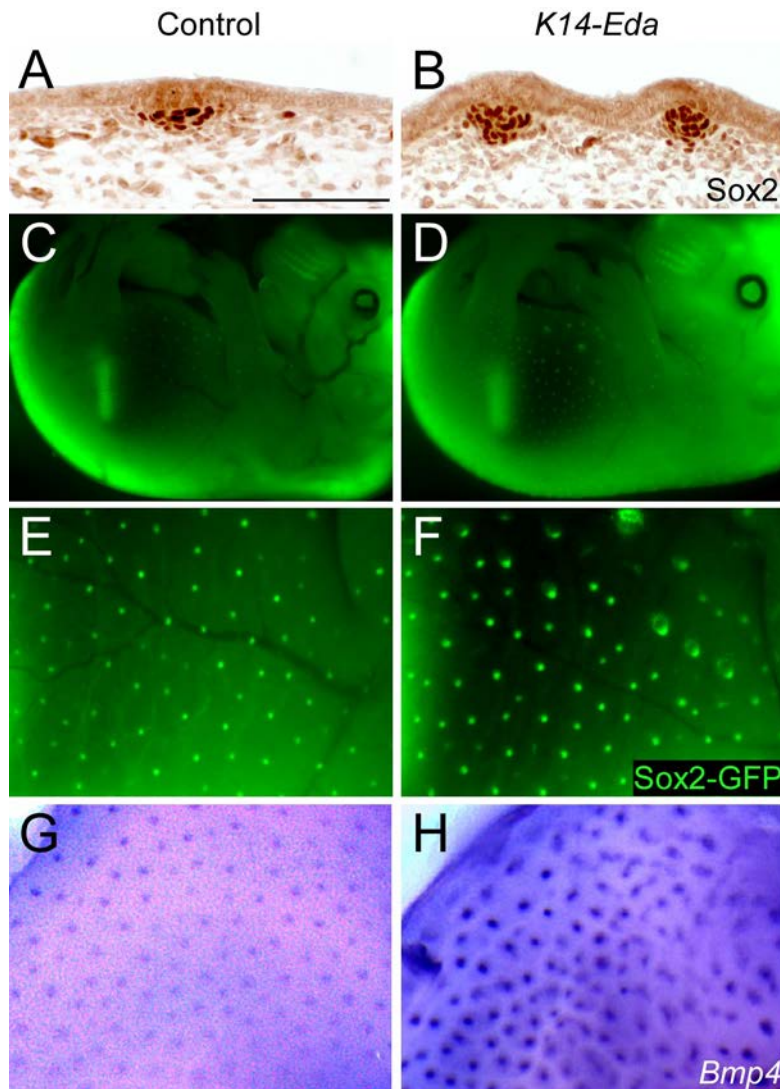


Figure S6. Primary dermal condensations are larger in K14-Eda embryos. (A, B)

Immunostaining for Sox2 revealing more Sox2+ cells in K14-Eda embryos compared to controls at E14.0. (C-F) Transgenic Sox2-GFP expression in control and K14-Eda embryos showing enlarged dermal condensations in K14-Eda embryos at E14.5. (G, H) In situ hybridization for Bmp4 showing more intense expression in enlarged areas in K14-Eda embryos compared to controls at E14.5. Scale bar 100 μ m (A, B).