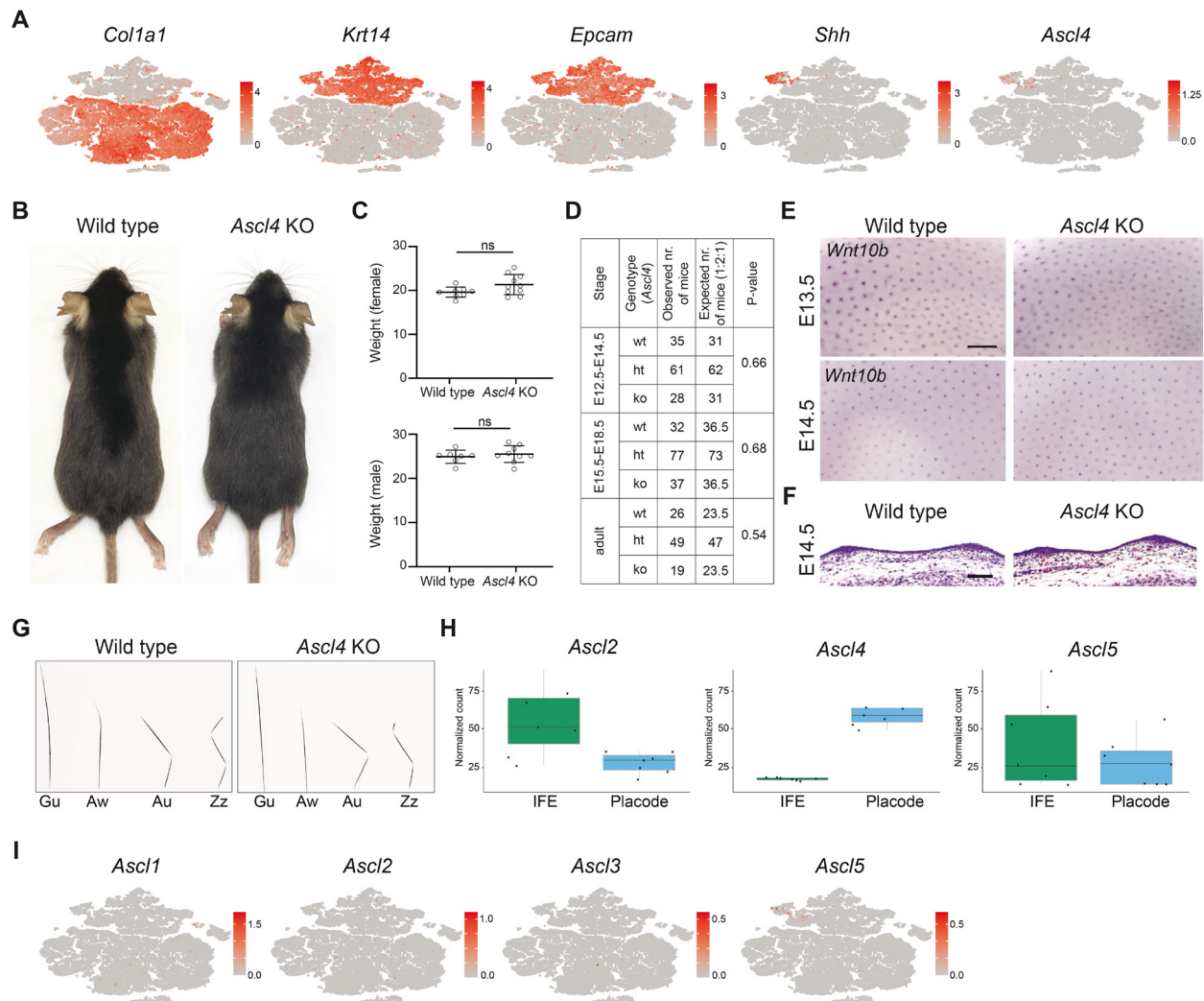


# Supplementary Material

corresponding to:

## **Wnt target gene *Ascl4* is dispensable for skin appendage development**

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**Supplementary Figure 1. Expression of *Ascl4* and phenotype analysis of *Ascl4* mice.** (A) tSNE plots showing expression of the mesenchymal marker *Col1a1*, the epithelial markers *Krt14* and *Epcam*, and the enamel knot markers *Shh*, as well as *Ascl4* in E14.5 molar tooth scRNA-seq dataset from Hallikas *et al.*, 2021. Colors span a gradient from red (high expression) to grey (low expression) for each gene. (B) Representative images of wild-type and *Ascl4* KO mice ( $n=8$  each) at 8 weeks of age. (C) The body weight of 8-week-old male and female *Ascl4* KO ( $n_{\text{male}}=9$ ;  $n_{\text{female}}=10$ ) and wild-type mice ( $n_{\text{male}}=7$ ;  $n_{\text{female}}=8$ ). Data are shown as mean  $\pm$  s.d. Statistical significance was assessed with two-tailed unpaired Student's t-test. ns, not significant. (D) The number of mice of various genotypes obtained from the *Ascl4* heterozygous matings is shown. The expected number was calculated according to the total number of mice born and based on the Mendelian 1:2:1 ratio. E12.5-E14.5 and E15.5-E18.5 embryos were pooled for statistical analysis. Statistical significance was assessed with Chi-square test. wt, *Ascl4*<sup>+/+</sup>; ht, *Ascl4*<sup>+/-</sup>; ko, *Ascl4*<sup>-/-</sup>. (E) Expression of *Wnt10b* was analyzed by whole mount *in situ* hybridization in E13.5 (top panel) and E14.5 (bottom panel) embryos ( $n=4$  each). Scale bar, 500  $\mu\text{m}$ . (F) Hematoxylin and eosin staining of wild-type and *Ascl4* KO E14.5 dorsal skin ( $n=6$  each). Scale bar, 100  $\mu\text{m}$ . (G) Hair types of wild-type and *Ascl4* KO mice ( $n=6$  each) at 8 weeks of age. Gu, guard; Aw, awl; Au, auchene; Zz, zigzag. (H) Box plots for *Ascl2*, *Ascl4* and *Ascl5* showing normalized count distributions between interfollicular epithelium and placode samples in E14.5 hair placode bulk RNA-seq dataset (7 biological replicates) from Sulic *et al.*, 2023. IFE, interfollicular epithelium. (I) tSNE plots showing expression of the transcription factors *Ascl1*, *Ascl2*, *Ascl3* and *Ascl5* in E14.5 molar tooth scRNA-seq dataset from Hallikas *et al.*, 2021. Colors span a gradient from red (high expression) to grey (low expression) for each gene.