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Supplementary Materials and Methods

Reagents and antibodies

TRIzol reagent for RNA extraction was from Invitrogen (Carlsbad, CA). The SYBR Green Master Mix for qRT-PCR was from Bio-Rad (Hercules, CA). Antibodies against SOX9 (ab185966) and Lhx2/LH2 (ab184337) were from Abcam (Cambridge, MA, USA). Biotinylated secondary antibodies for immunohistochemistry staining were from Beyotime Biotechnology (A0208). The DAB kit was from Cell Signaling Technology Inc. (#8059, Danvers, MA, USA). All other molecular-grade reagents or chemicals were from Sigma (St. Louis, MO) or Fisher (Pittsburgh, PA) unless otherwise mentioned.

Mice

The Paxbp1$^{fl/fl}$ mice were generously provided by Professor Wu from Hong Kong University of Science and Technology (Zhou et al., 2021). The B6N.Cg-Tg(KRT14-cre)1Amc/J (K14-Cre) mice were from the Jackson Laboratory. A conditional deletion of Paxbp1 in skins was generated by cross-breeding Paxbp1$^{fl/fl}$ mice with K14-Cre mice as previously described (Huang et al., 2025). Animals were maintained under specific pathogen-free conditions. All animal experiments were approved by the Committee for the Ethics of Animal Experiments, Shenzhen Peking University-The Hong Kong University of Science and Technology Medical Center.

Isolation of mouse epidermis

Mouse epidermal tissues were isolated as previously described (Huang et al., 2025).

H&E and immunohistochemistry staining

Mouse skins were isolated and formalin-fixed, followed by being embedded in paraffin. Hematoxylin and eosin (H&E) staining was performed as previously described (Huang et al., 2025). For immunohistochemistry (IHC) staining, SOX9 (Abcam, ab185966, 1:500) and Lhx2/LH2 (Abcam, ab184337, 1:500) antibodies were used to detect indicated proteins as described previously (Huang et al., 2025). The images were scanned and captured using a PANNORAMIC™ Digital Slide Scanner (3DHISTECH Ltd., Hungary). The relative signaling intensities were analyzed using the Image-Pro Plus 6.0 software (Media Cybemetics, USA).

RNA isolation and quantitative real time-PCR (qRT-PCR)

Total RNA preparation, reverse transcription, and qRT-PCR was performed as described previously (Huang et al., 2021). The primers used in the present study were designed using PrimerBank (https://pga.mgh.harvard.edu/primerbank/index.html).

RNA sequencing and data analysis

Total RNA extraction and RNA sequencing was performed as previously described (Huang et al., 2025). The GO term, heatmap, and gene set enrichment analysis (GSEA) were visualized using the R software. All sequencing data have been submitted to the NCBI Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo/) under accession numbers GSE272102 and GSE272402.

Statistics

All experiments were repeated at least three times unless otherwise mentioned. The data are presented as Mean ± SEM. Statistical analysis was conducted using GraphPad Prism software (GraphPad Software Inc., La Jolla, CA). The data were analyzed for significance using one-way ANOVA with Tukey's post-hoc tests. A value of $P < 0.05$ was considered statistically significant.

Supplementary References

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