

Architectural Niche Organization by LHX2 is Linked to Hair Follicle Stem Cell Function

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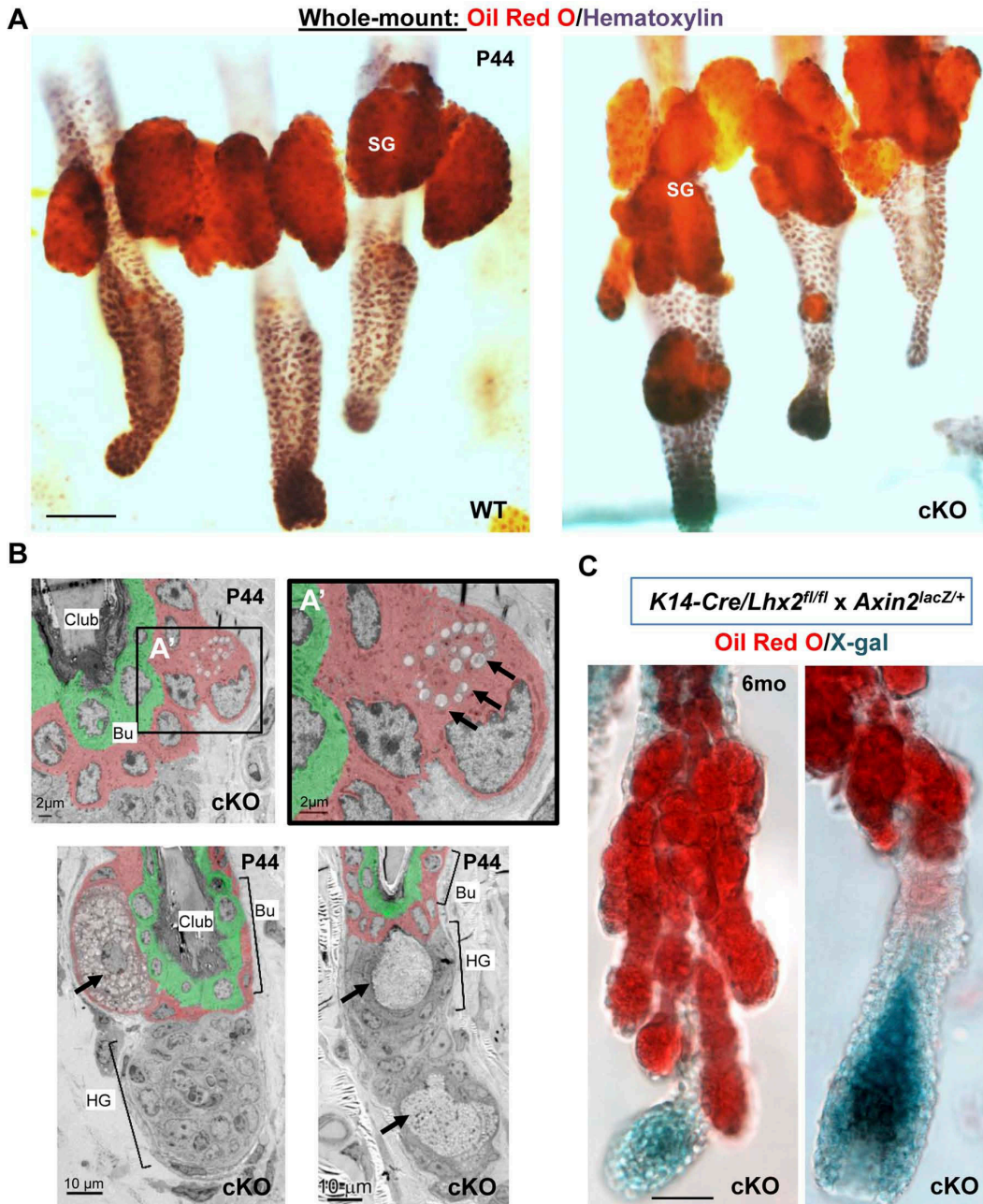
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In adult skin, self-renewing, undifferentiated hair follicle stem cells (HF-SCs) reside within a specialized niche, where they spend prolonged times as a single-layer of polarized, quiescent epithelial cells. When sufficient activating signals accumulate, HF-SCs become mobilized to fuel tissue regeneration and hair growth. The mechanisms underlying the intricate balance between long-term self-renewal of HF-SCs and their commitment into differentiated lineages are still poorly understood. The niche provides a rich milieu of activating and inhibitory signals to control SC dynamics. While recent studies begin to suggest how stemness is influenced by external signaling pathways, less is known about the impact of cytoarchitecture on HF-SC behavior.

In this study, we use HFs as our model system to explore whether and how LHX2 functions in adult SC biology and normal homeostasis. We first perform *in vivo* anti-LHX2 chromatin immunoprecipitation and deep sequencing (ChIP-seq) on purified HF-SCs taken directly from their native niche. Second, we perform skin-specific conditional ablation of *Lhx2* in mice and couple our newfound knowledge of LHX2-bound genes in HF-SCs with transcriptional profiling of messenger RNAs (mRNAs) from wild-type and *KJ4-Cre/Lhx2^{fl/fl}* conditional knockout (*Lhx2*-cKO) HF-SC-enriched populations. Our studies show that LHX2 regulates a significant number of HF-SC signature genes and cytoskeletal and adhesion molecules within the niche. Delving into the physiological relevance of these findings, we unearth a surprising reason for why LHX2 loss leads to baldness. Rather than exhausting HF-SC proliferative activity or blocking Wnt signaling at the niche base, LHX2 deficiency profoundly perturbs HF-SC polarization and niche architecture. Without LHX2, the niche can no longer anchor its hair and maintain the proper behavior of its SCs. These findings suggest that niche organization underlies the requirement for LHX2 in hair follicle structure and function.

We conclude that architectural organization of the HF-SC niche by transcription factor LHX2 plays a critical role in HF-SC behavior. Conditional ablation of LHX2 results in gross cellular disorganization and HF-SC polarization within the niche. LHX2 loss leads to a failure to maintain HF-SC quiescence and hair anchoring, and progressive transformation of the niche into a sebaceous gland.

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LHX2-Deficient HF-SCs Spontaneously Differentiate Into Sebocytes Within Their Niche

(A) Oil Red O staining of whole-mount tail skin samples at P44 shows ectopic sebocyte formation in the bulge of tail *Lhx2*-cKO HF. Scale bar, 40 μ m.

(B) Ultrastructure analysis of *Lhx2*-cKO bulge at P44 shows a developing sebocyte located among HF-SCs (left), never seen in WT. Box area is magnified in A'. Arrows indicate lipid droplets. Scale bar, 2 μ m. Enlarged sebocytes (arrows) within bulge and HG (right). Scale bar, 10 μ m.

(C) Oil Red O Staining of 6 month old WT and *Lhx2*-cKO whole-mount back skin samples, in the AxinLacZ background. Sample are counterstained with X-gal. Note that *Lhx2*-cKO HF. still cycle, and that TACs are still sensitive to Wnt signaling (right). Scale bar, 40 μ m.

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