

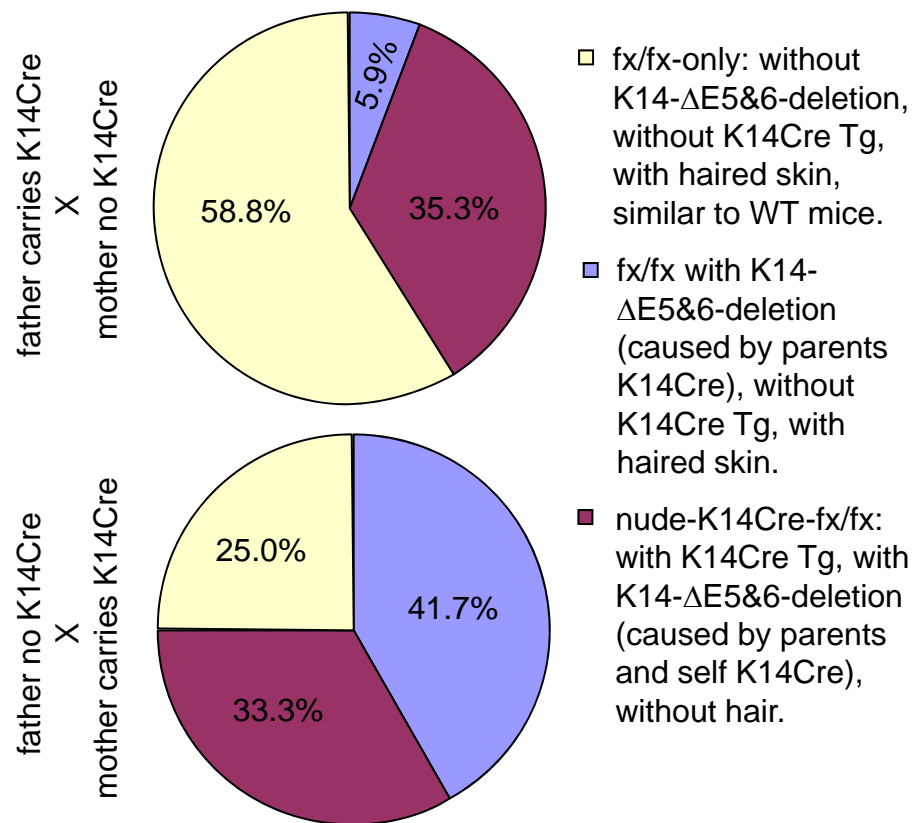
Morphogenesis and maintenance of the 3D-thymic medulla and prevention of nude skin phenotype require FoxN1 in pre- and post-natal K14 epithelium

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Supplemental Data (FiguresS1 – S4 and Table S1 – S2)

% fx/fx offspring with/without K14Cre and phenotype



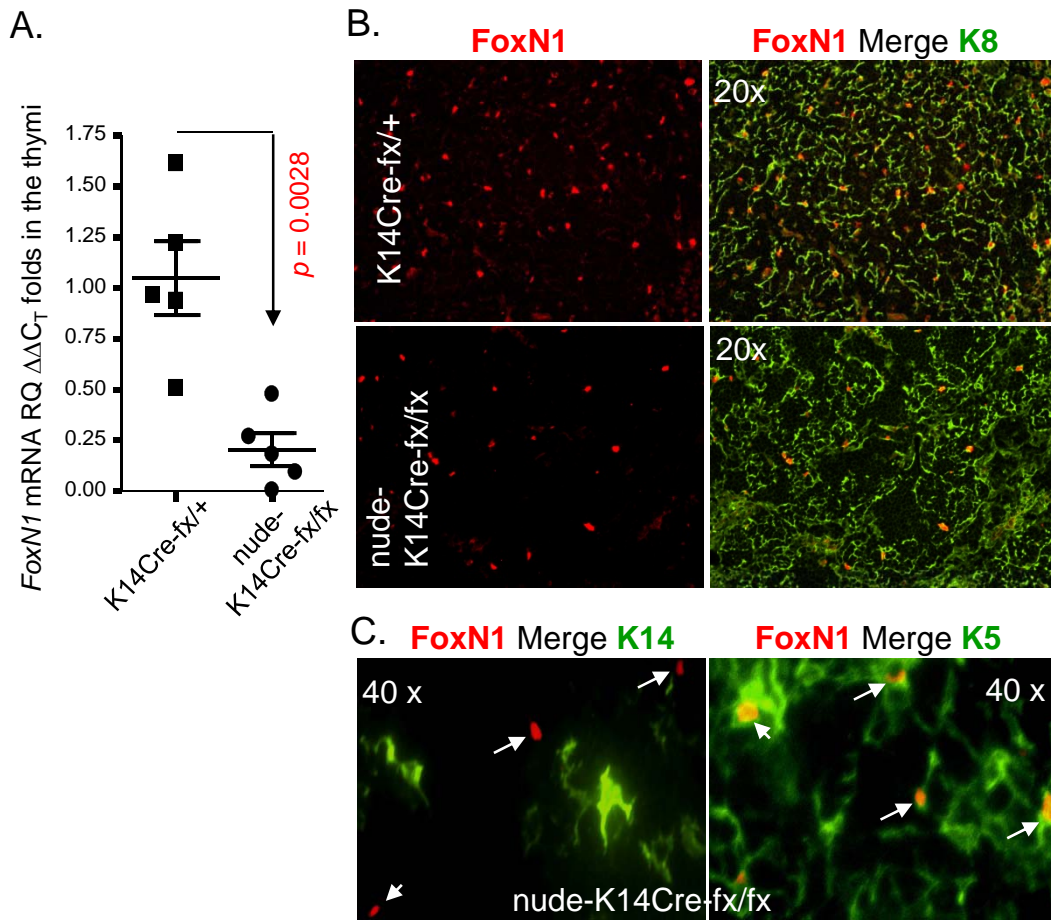
Supplemental Figure S1. Haired/nude K14Cre-fx/fx mice derived from alternate mating strategies.

Top panel shows fx/fx homozygous offspring from K14Cre-carrying male crossing with non-K14Cre-carrying female mice. Bottom panel shows fx/fx homozygous offspring from non-K14Cre-carrying male crossing with K14Cre-carrying female mice.



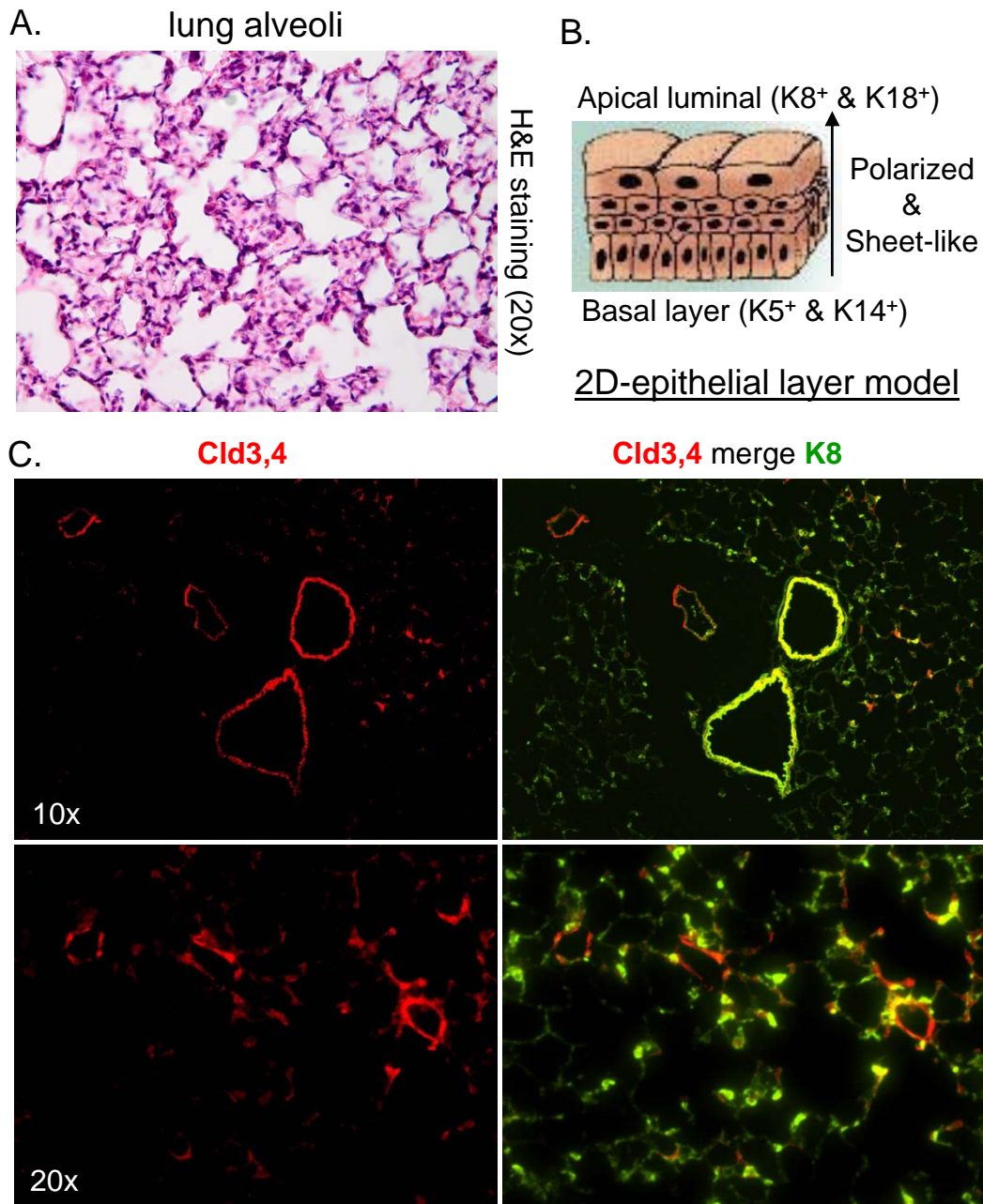
Supplemental Figure S2. An un-cropped image of the mice in Fig. 2A.

Left mouse shows the K14Cre-fx/+ thymus (arrow) and haired skin; right mouse shows the nude-K14Cre-fx/fx thymus (arrow) and nude skin.



Supplemental Figure S3. FoxN1 expression in the thymi of heterozygous (K14Cre-fx/+) and nude homozygous (K14Cre-fx/fx) mice.

(A) A summarized real-time RT-PCR result of FoxN1 mRNA levels in the thymi of K14Cre-fx/+ (squares) and nude-K14Cre-fx/fx (circles) mice. Each square and circle represents data from one animal. **(B)** Immunofluorescent staining image of the thymi from K14Cre-fx/+ (top panels) and nude-K14Cre-fx/fx (bottom panels) mice using antibodies against keratin-8 (green) and FoxN1 (red, left panels – antibody from Dr. Amagai). A representative result is shown of two experiments, with essentially identical results. The right panels show merged images. **(C)** Immunofluorescent staining image of the thymi from nude-K14Cre-fx/fx mice using antibodies against FoxN1 (red – antibody from Santa Cruz, clone G20) vs. k14 (green, left panels) or vs. K5 (green, right panel). Arrows show FoxN1 without co-localization to K14⁺ epithelial cells, but with co-localization to K5⁺ epithelial cells.



Supplemental Figure S4. Micro-structure of lung tissue.

- (A) H&E staining of the mouse lung showing normal lung alveoli.
- (B) Model of 2D-epithelial layer in the skin and lung airway, but not in the normal thymus (adopted from Wikipedia encyclopedia).
- (C) Immunofluorescent staining showing Cld3,4 expression in 2D airway epithelium: top panels show Cld3,4 expression in large bronchiole, bottom panels show Cld3,4 expression in small alveolar ducts and/or alveoli.

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Supplemental Table S1. PCR primers for mouse genotyping and semi-quantitative RT-PCR

PCR for	Primer Sequence:
loxP-floxed <i>FoxN1</i> genotyping (fx PCR)	Primer-A: 5'- cca acc tcc tgg gga cat ga -3'
	Primer-B: 5'- tag gag gag ggg agc gcc ta -3'
	Primer-C: 5'- gtg ggc ttt tca cca tcc ta -3'
K14Cre transgene and Internal Control	Transgene 5'- GCG GTC TGG CAG TAA AAA CTA TC-3'
	Transgene 5'- GTG AAA CAG CAT TGC TGT CAC TT -3'
	Internal Ctr 5'-CTA GGC CAC AGA ATT GAA AGA TCT-3'
	Internal Ctr 5'-GTA GGT GGA AAT TCT AGC ATC ATC C-3'
Semi-quantitative RT- PCR for Aire mRNA	5'- GGG ACT GGT TTA GGT CCA CA-3'
	5'- AGG TGG GGA TGG AAT GCT AC-3'
FoxN1 real-time RT- PCR primers and probe (TaqMan)	5'-primer: 5'-GGC CAA CGC CGA AGG-3'
	3'-primer: 5'-TGA AGA TGA GGA TGC TGT AAG AGT AGA-3'
	Probe: 6FAM-CAC CAG CCA CTC TTC CCA AAG CCC-TAMRA

Note: fx PCR was also used for measuring the excision of FoxN1 $\Delta E5&6$ mediated by K14Cre Tg: The % excision was calculated as the band density ratio of the signal from the excised band / (excised band + fx band) in K14Cre-fx/fx homozygous mice, or the excised band /1/2 (excised band + fx and WT band) in K14Cre-fx/+ heterozygous mice (ref. Fig. 1C top panel).

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Supplemental Table S2. Similarities and differences in the roles of FoxN1 in the skin and thymus

Functions	In the skin	In the thymus	References
Epithelial differentiation	Yes	Yes	(1,2)
Primary role in fetal organogenesis	Mainly in hair follicle cortex	In both cortex and medulla	(3-5)
Primary role in postnatal life	Mainly in hair follicle cortex	Mainly in the medulla	(3,4,6)
Formation and maintenance of epithelial 3D structure	N/A	Yes	Novel finding in this manuscript
Determination of pigmentation pattern	Yes	N/A	(4)
Cell types affected by knockout of FoxN1 in K14 epithelial cells	Skin stem cells (causes nude phenotype, similar to FoxN1-null mice)	A subset of TEC stem cells and postnatal mTECs (hypomorphic mutation, different from FoxN1-null mice)	Novel finding in this manuscript

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