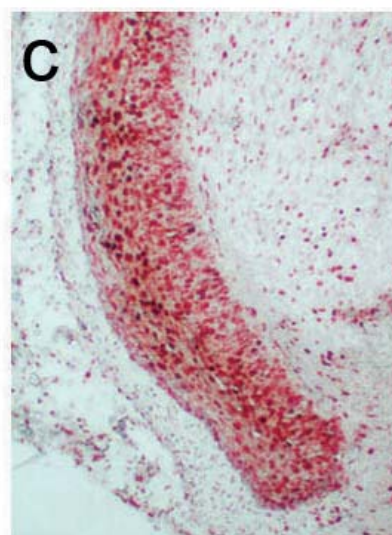
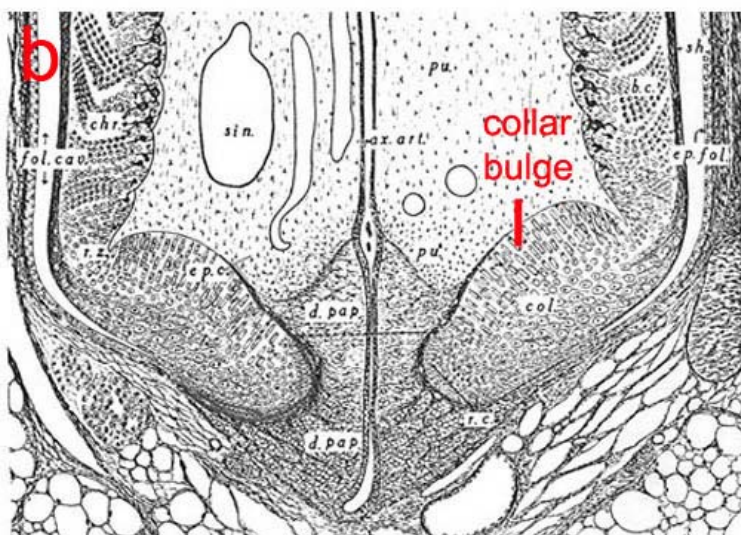
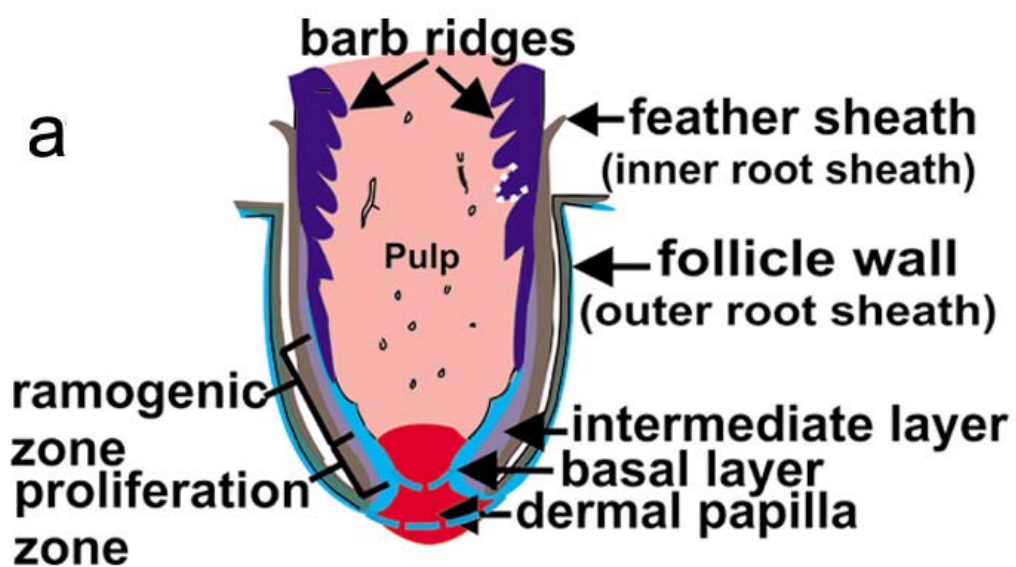
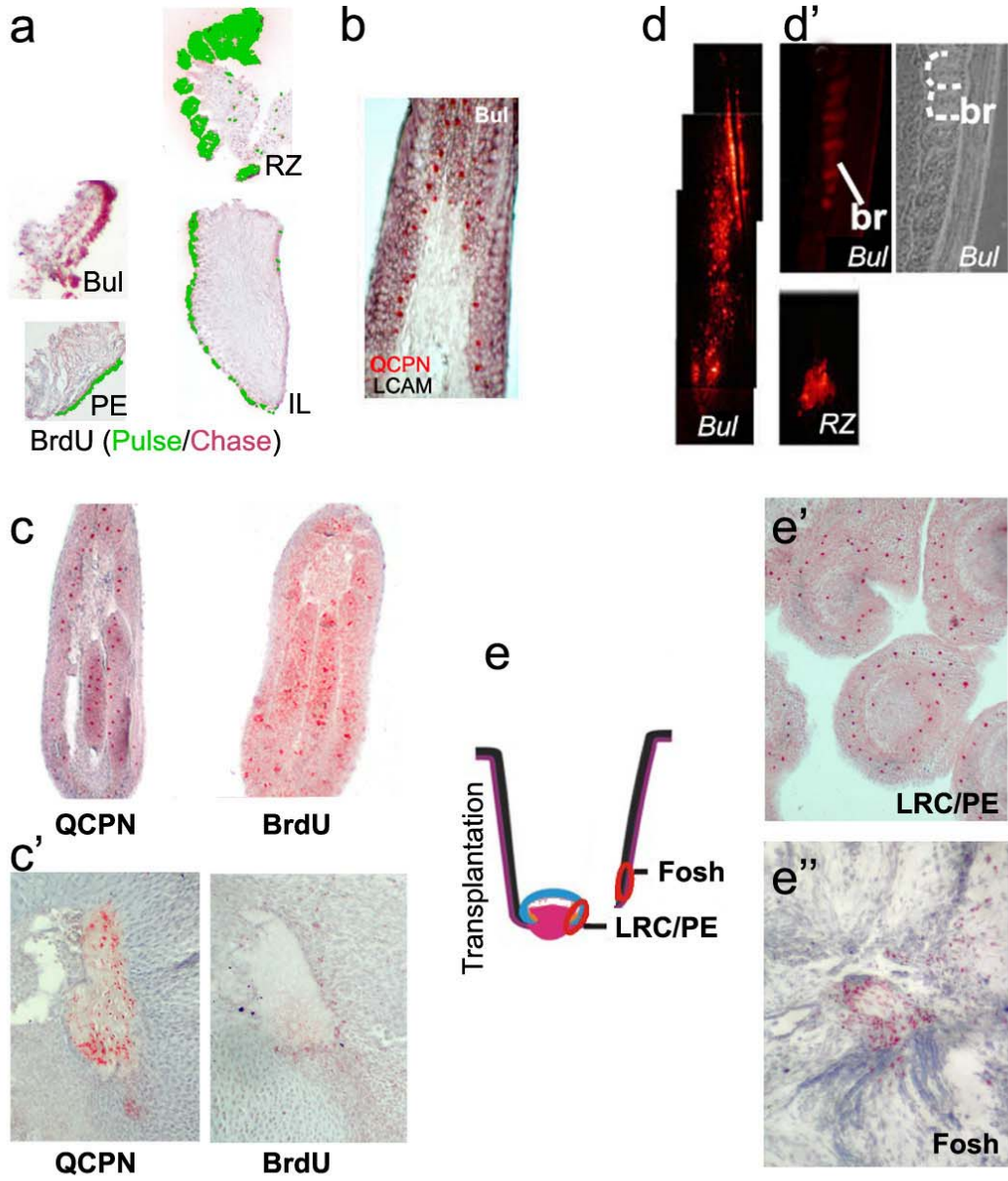


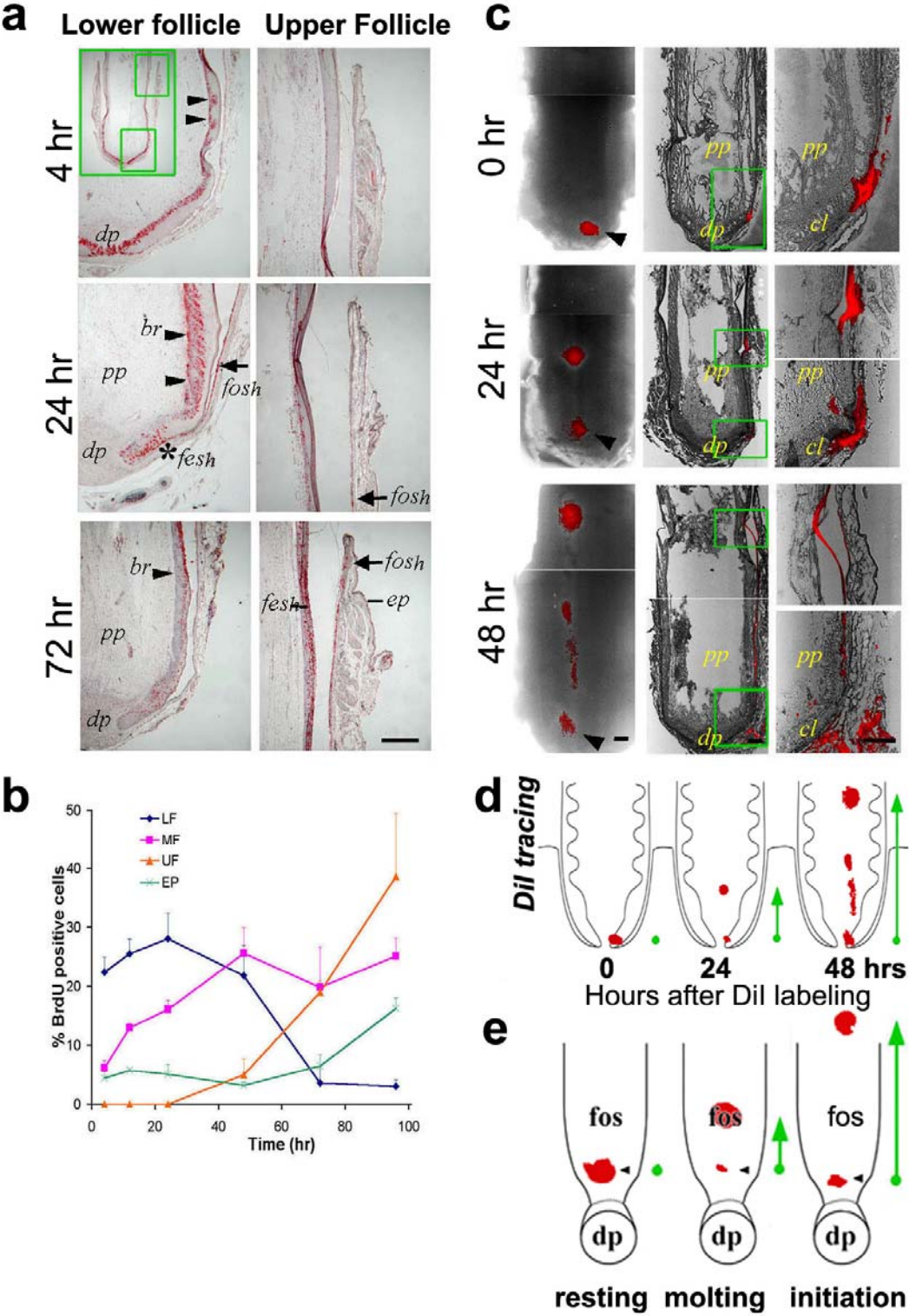
# Supplement Figure 1



# Supplement Figure 2

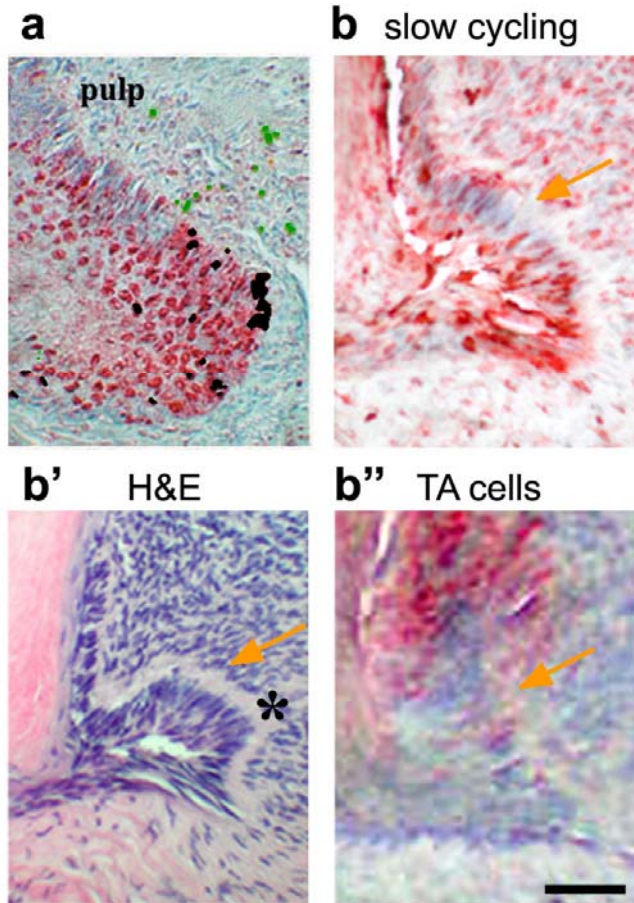


# Supplement Figure 3





# Supplement Fig 4



## Supplementary Figures

Supplementary figure 1. Follicle structure and cell labelling.

- a. Schematic drawings of a feather follicle with major zones marked. Adopted from Yu et al., 2002, The morphogenesis of feathers. *Nature* 420, 308.
- b, Drawing of the proximal feather follicle. Adopted from Lillie and Wang, 1941.
- c, BrdU labelling before 1 week chase, in the collar region. Enlargement of green boxed region of Figure 1b.

Supplementary figure 2. Transplantation to test multipotentiality.

- a. Pieces of feather follicle fragments dissected as shown in Fig. 1c. To verify regions we dissected follicles pre-labelled for LRC (red) or short term BrdU pulse (green). There is no mixing of cells between these epithelial cell populations. Bul, collar bulge; Fosh, follicle sheath; IL, collar intermediate layer; PE, papillar ectoderm; RZ, ramogenic zone.
- b, Collar bulge (Bul) transplant after 9 days (d') shows the incorporation of quail cells (red, stained with QCPN antibody) into barb ridges. This panel is double stained with antibody to L-CAM to show epithelia.
- c-c', Fate of LRC and TA cells in quail / chicken transplantation assay. After 5 days, the specimens were stained with QCPN for quail cells and anti-BrdU in

adjacent sections. c, longitudinal sections of feather filaments showing LRC cells are incorporated into different epithelial types including barbule plate, marginal plate, etc. c', TA cells remain in the original transplanted fragment without assimilation into the host skin.

d, Regions similarly dissected from adult chicken feather follicles were labelled with Dil and transplanted to another follicle of the same chicken. After 4 days, collar bulge fragments were incorporated into the feather filament, but the RZ fragment remained at the transplant site. d', enlargement with phase contrast to show the labelled bulge cells have become part of the barb ridges (br). e-e'', Testing the pluri-potentiality of components from late resting phase follicles with quail / chicken transplantation. LRC and papillar ectoderm (LRC/PE) cannot be separated at this time. They show good incorporation (e') while follicle sheath (Fosh) fragments cannot. Also see Table 1.

Supplementary figure 3. Pulse labelling and Dil tracing to follow cell lineages.

a, We injected a single dose of BrdU intraperitoneally and examined the distribution of labelled cells in feather follicles at 4, 12, 24, 48, 72 and 96 hr. We find TA cells migrate along the following routes:

*The basal layer.* Labelled cells are initially located in the collar region and are displaced upward. At the ramogenic zone, they start branching morphogenesis and form barb ridges.

*The barb ridges.* Along the feather filament cylinder, epidermal cells continue to proliferate at the top of the ridge (the pulp side) and contribute to the elongation of barb ridges. Labelled cells start to be stacked centrifugally (supra-basally) toward the periphery where differentiation begins (arrow head).

*The intermediate layer and feather sheath.* Some BrdU positive cells around the PE were displaced upward to form the intermediate layer and the feather sheath (asterisk).

*Follicle sheath and inter-follicular epidermis.* In the hair outer root sheath, cells are reported to move downward<sup>2</sup>. Here we observed cell flux in the opposite direction in the feather follicle sheath.

b, As time progresses, more and more labelled cells appeared in the middle follicle sheath, upper follicle sheath, and epidermis, while labelled cells decreased in the lower follicle sheath (arrow). Semi-quantitative measurement suggests a trend that TA cells are displaced upward.

c, Dil was injected into the lower part of a growing flight feather follicle *in vivo*. Dil labelled epithelial cells were displaced upward in both the feather filament and follicle sheath at 24 hr (n = 8) and 48 hr (n = 10) post labelling. In contrast,

labelled mesenchymal cells, in the dermal sheath and pulp, remained near the original injection sites.

d, e, Dil lineage tracing. d, Dil was injected into the lower part of the growing flight feather follicle in vivo and followed for 24 and 48 hours. Dil labelled epithelial cells were displaced upward in both the feather filament and follicle sheath at 24 hr (n = 8) and 48 hr (n = 10) post labelling. In contrast, labelled mesenchymal cells, in the dermal sheath and pulp, remained near the original injection sites. More data are in supplementary Fig. 2. e, Tracing cell movement in different phases of feather follicles. Dil was used to label the follicle sheath 48 hr earlier. The level of migration was indicated by the green arrow.

Bar: 100  $\mu$ m.

Supplementary figure 4. LRC and TA cells in molting stage follicles.

a, enlargement of LRCs in the molting phase boxed within Figure 2b. LRCs (orange arrow) located in the PE region have invaginated into the DP and are surrounded by a distinct translucent zone (\*), suggesting a unique microenvironment for special epithelial-mesenchymal interactions. These are shown labelled for slow cycling cells b and H&E staining, b'. On the other hand, TA cells, b'', do not form this zone.