**Supplementary materials to:**

# Divergent proliferation patterns of distinct human hair follicle epithelial progenitor niches *in situ* and their differential responsiveness to prostaglandin D2

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| Antibody/ assay | Host | Localisation | Dilution/ Concentration | Catalogue | Vendor |
|---|---|---|---|---|---|
| **Alexa Fluor 488/594** | Goat anti mouse/rabbit | - | 1:200 | A11001, A11005, A11008, A11037 | Thermo Fisher Scientific |
| **CD200** | Mouse | Bulge | 1:100 | MCA1960GA | AbD Serotec / Bio-Rad |
| **CD34 (My10)** | Mouse | Sub-bulge | 1:10 | 347660 | BD Biosciences |
| **EdU incorporation** | - | - | 20 µM | C10339 | Thermo Fisher Scientific |
| **Keratin 15 (LHK15)** | Mouse | Bulge & pbORS | 1:200 | AB1385 | Abcam |
| **Keratin 19** | Mouse | Bulge & pbORS | Pre-diluted | AB87000 | Abcam |
| **Ki-67** | Rabbit | - | 1:50 | AB16667 | Abcam |

**Table S1 Antibodies used for immunofluorescence**

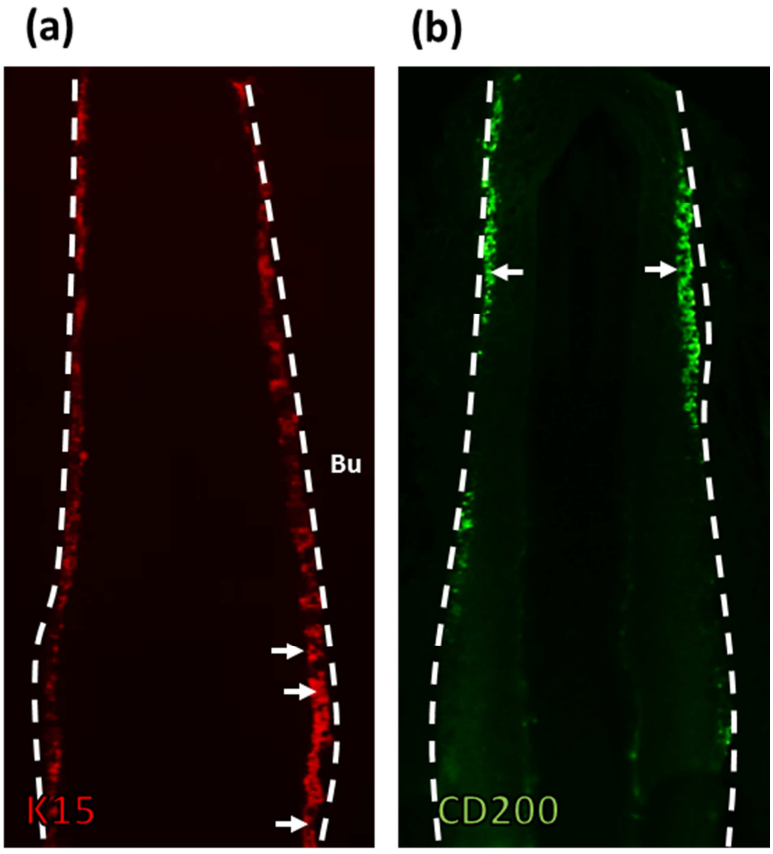


**Figure S1** Co-localisation of CD200 and K15 expression within the human HF bulge

Serial sections highlighting that CD200 and K15 signal co-localise to the bulge (Bu), where the most prominent expression of these markers localises to the upper and lower bulge respectively (arrows). Note that CD200 labels a more restricted subset of cells. Images taken at 200x and subsequently merged using Keyence image analysis software.

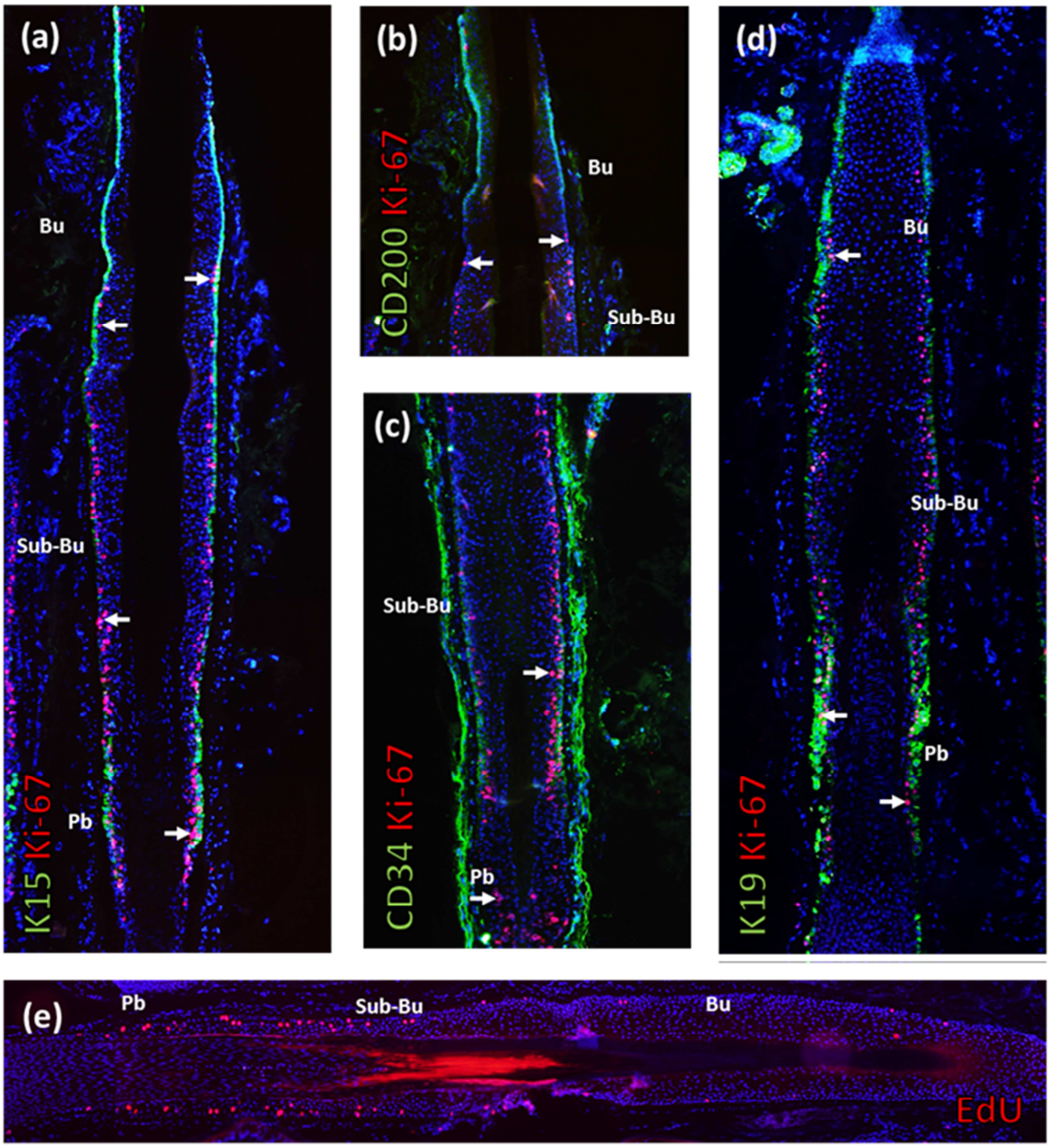


**Figure S2** Localisation of stem/progenitor markers and proliferation in the human HF ORS

**(a-d)** Ki-67 double immunofluourescence with K15 (a), CD200 (b), CD34 (c) and K19 (d). CD200 marks the bulge (Bu), a zone of minimal Ki-67 expression, whereas K15 marks this zone too, but extends beyond it, coinciding with an increase in the total number of Ki-67+ cells. K15 (and K19, where expression is intermittent) is then downregulated in the sub-

bulge (Sub-Bu), within a region where CD34 expression increases, and vice versa again in the proximal bulb (Pb) ORS (pbORS) compartment. Arrows indicate proliferating cells in the HF epithelium. Note that expression of K15/K19 co-localise but show distinct expression patterns, as described previously [7,10,11] suggesting that they represent functionally distinct progenitor cell populations. Images taken at 200x and merged using Keyence image analysis software.

**(e)** Localisation of S-phase+ cells in the ORS resembles the distribution of Ki-67 cells, with limited DNA synthesis within the bulge compared to the sub-bulge and pbORS under normal conditions. Arrows indicate proliferating cells in the HF epithelium. Regions are approximate. Pb – pb(proximal bulb)ORS.

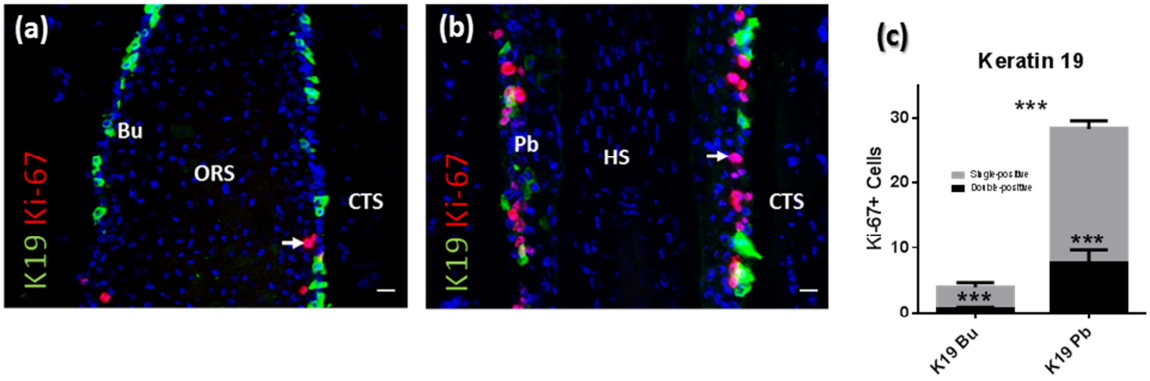


**Figure S3** Patterns of proliferation in K19+ stem/progenitor populations

**a-c)** Total number of Ki-67+ cells is significantly greater within the K19+ pbORS region compared to K19+ bulge region (Mann Whitney U test). Both the bulge and pbORS show significantly fewer double positive cells (K19+Ki-67+) compared to single positive cells (K19-Ki-67+) (paired t-tests). Patient N=6 (total 12 HFs analysed per region). P=<0.001 (***).

Significance asterisks *within* bars denote double vs. single comparisons. Significance asterisks *between* bars in chart denote comparisons of total Ki-67+ counts. Arrows indicate proliferating cells in the HF epithelium. 20 µm scale bars. Error bars are standard error. Bu – Bulge; CTS – connective tissue sheath; HS - hair shaft ORS; outer root sheath; Pb – pb(proximal bulb)ORS

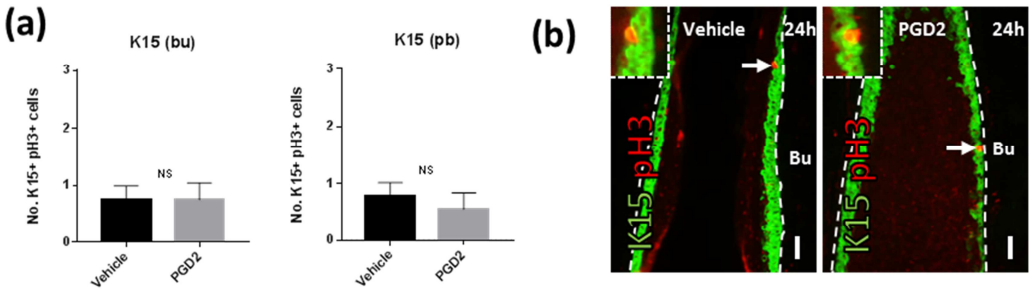


**Figure S4** The number of K15+ pH3+ cells remains constant following PGD2 treatment

**(a)** Number of K15+/phospho histone H3 (pH3)+ double-positive cells in bulge and pbORS is unaffected by 24h PGD2 treatment (Mann Whitney U tests) **(b)** Representative images of pH3 bulge expression K15+ cells in control and treated HFs . Patient N=3; Bu - 10 HFs per group, Pb - 7-8 HFs per group. Scale bars are 10 µm. Arrows point to positive signal in the HF epithelium.

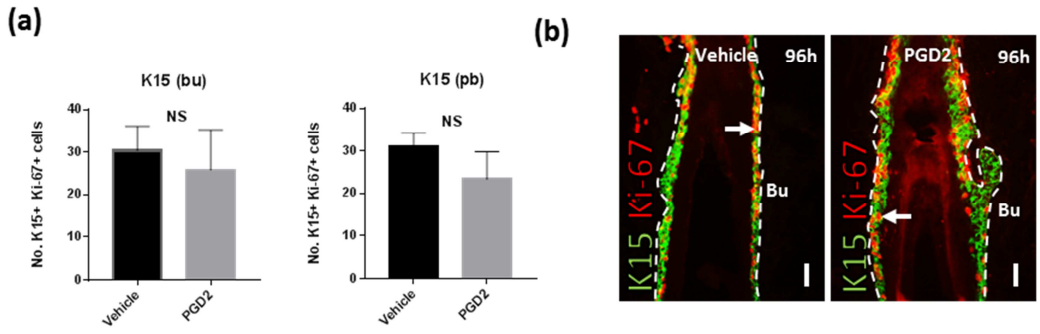


**Figure S5** Effects of 96 h PGD2 culture on K15+ cell proliferation

**(a)** 96h HF organ culture in the presence of 10µM PGD2 (administered daily) did not significantly alter the number of bulge or pbORS K15+ Ki-67+ double positive cells compared to vehicle. Instead, extended organ culture resulted in the hyper proliferation of this otherwise quiescent compartment. Patient N=2; Bu - 6-8 HFs per group; pbORS - 5-10 HFs per group (Mann Whitney U tests). Scale bars are 10 µm. Positive cell number analyses conducted using mean values per HF analysed. Arrows point to positive signal in the HF epithelium.