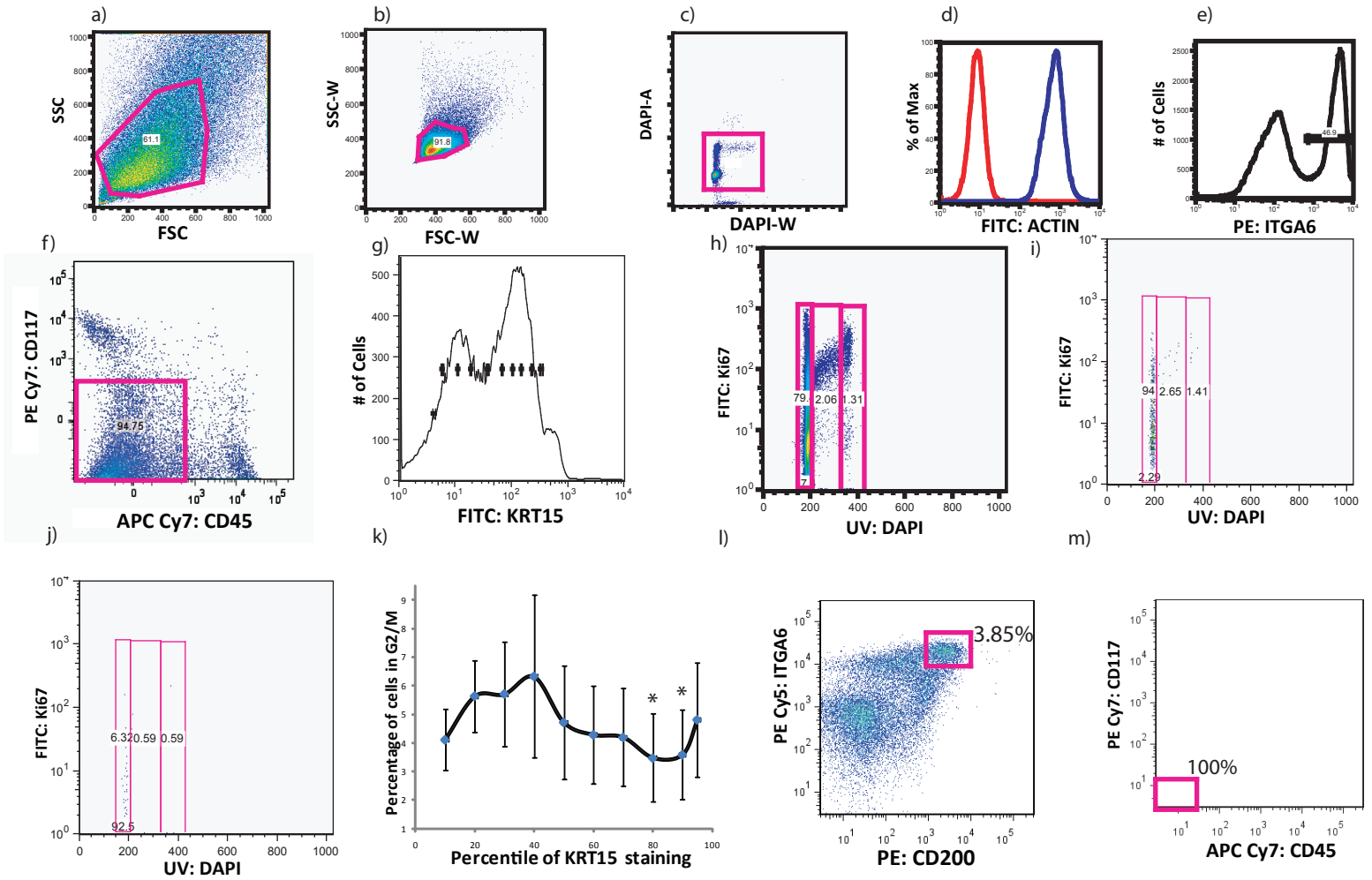
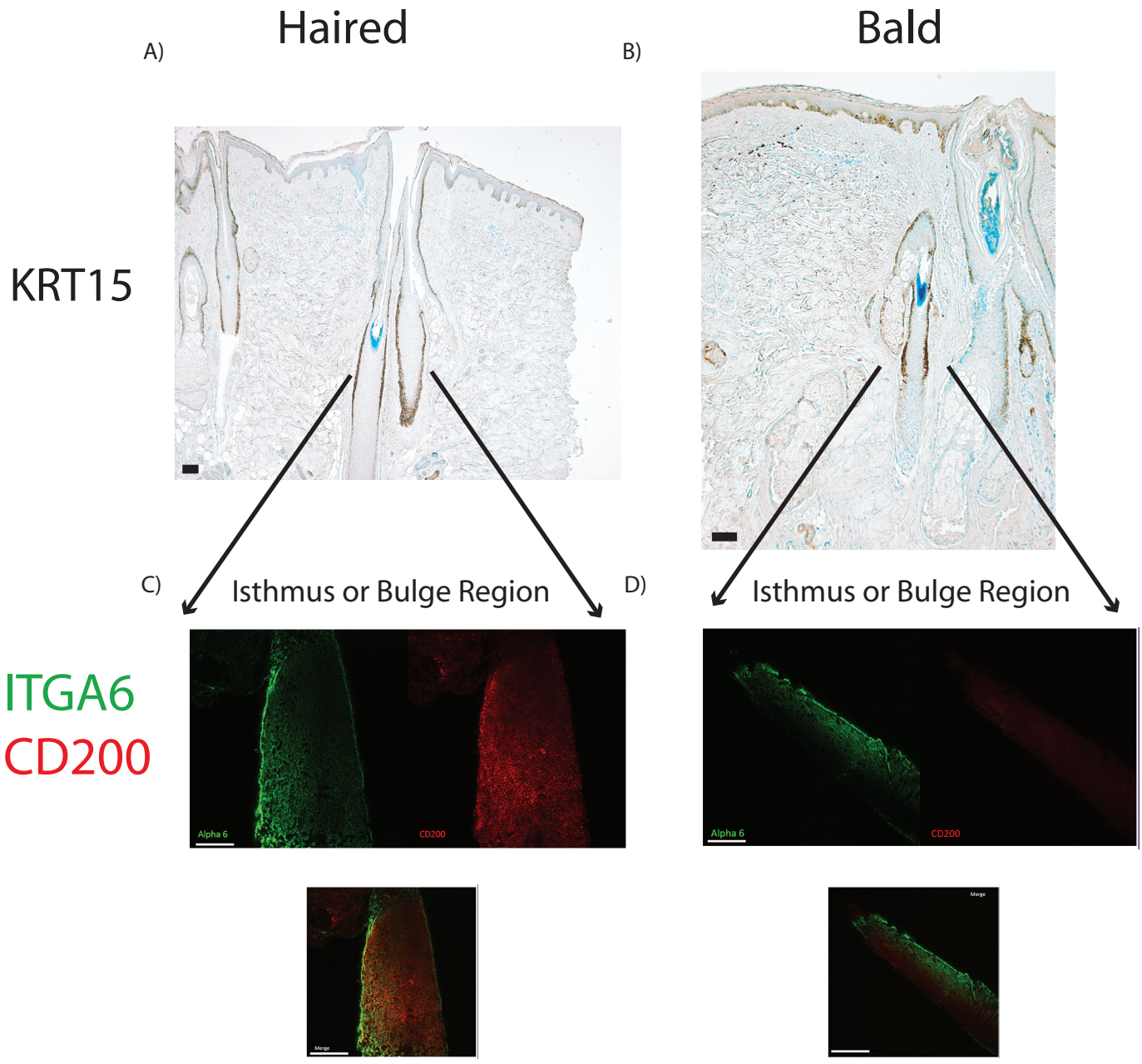
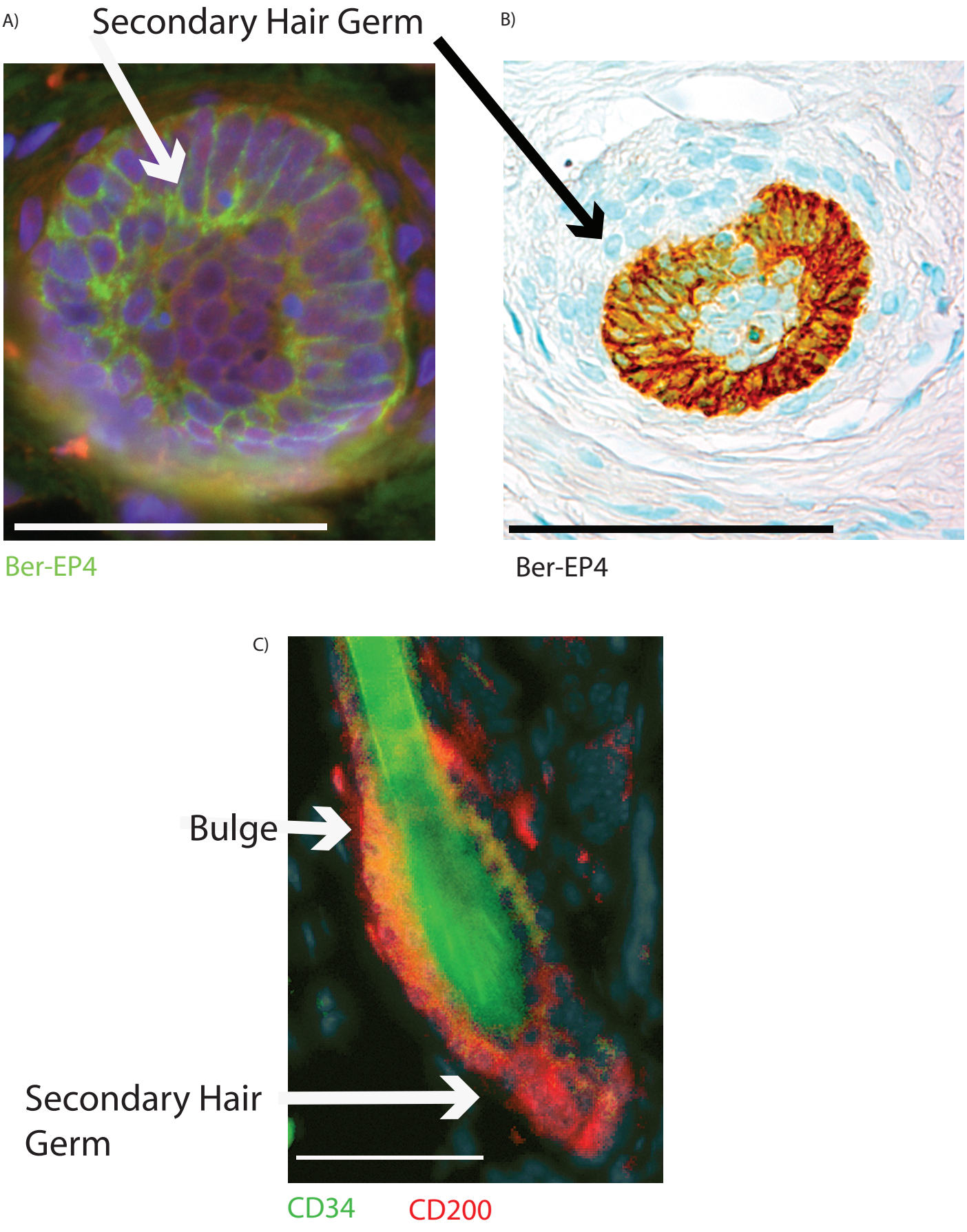


S. Figure #1: Gating strategy for human flow cytometry

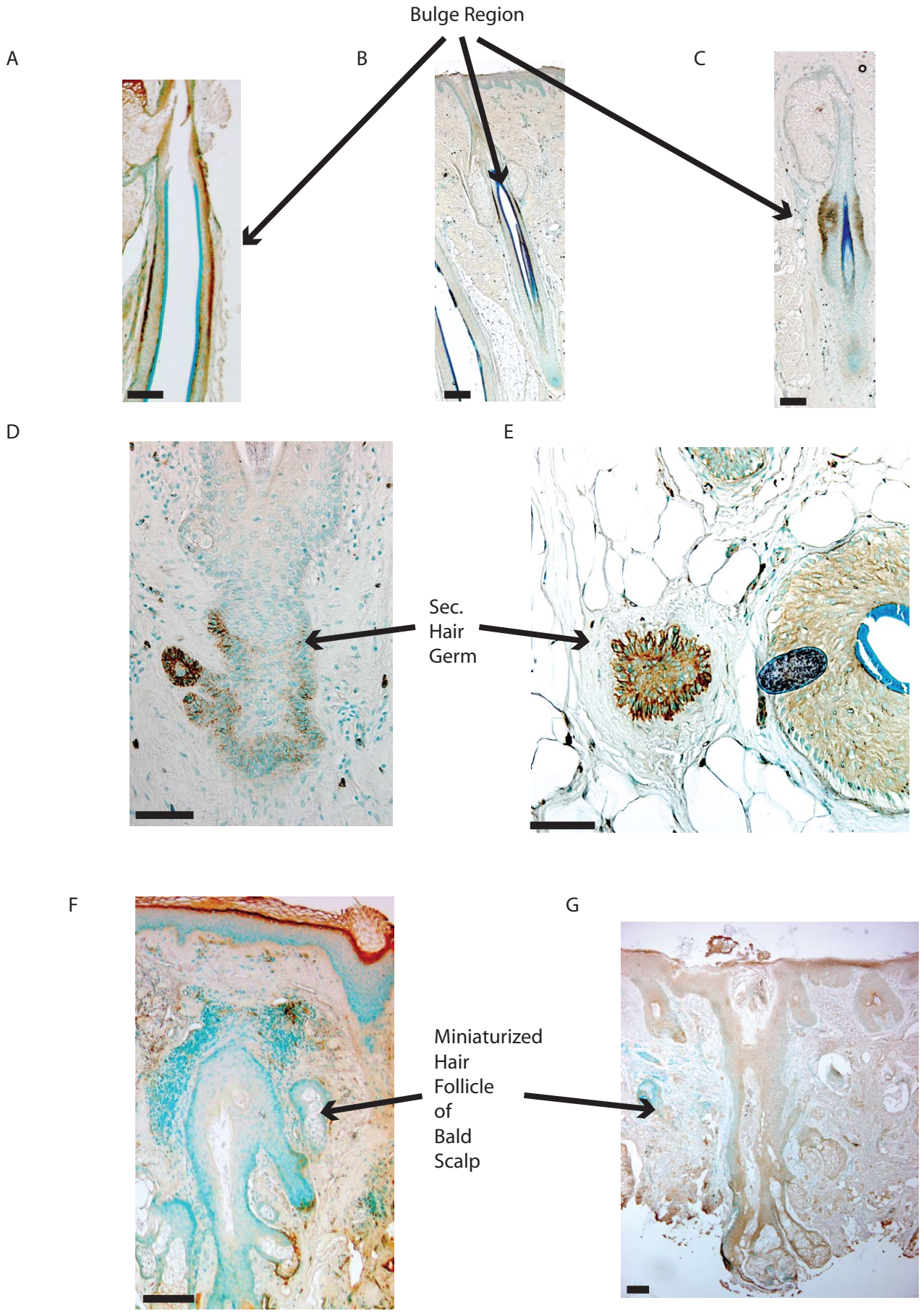
A-C) Pseudocolor plots of: A)Forward Scatter (FSC) versus Side Scatter (SSC) with gating of dominant population of human epidermal keratinocytes from haired scalp; B) FSC-width versus SSC-width for elimination of doublets; C) DAPI-area versus DAPI-width for inclusion of only cells containing diploid through tetraploid quantities of DNA as a further method to remove cell aggregates or debris. If fixation was not employed, a live-dead discrimination was used to gate on DAPI-excluding cells. D) Histogram demonstrating complete permeabilization of cells as detected by anti-actin (blue) with no overlap of IgG nonspecific staining (red). E) Histogram of ITGA6 with gating for ITGA6 positive cells. F) Confirmation was made that all analyzed populations were CD45 (hematopoietic marker) and CD117 (melanocyte marker) negative. G) Histogram of KRT15 percentiles used for gating. H-J) Cell cycle analysis for keratinocytes can be divided operationally into G0, G1, S, and G2&M based on Ki67 and DAPI staining. G0 is defined as undetectable levels of Ki67. Cells of the 10th percentile of KRT15 expression demonstrate abundant G1, S and G2&M percentages (I), while cells in the 95th percentile of KRT15 staining demonstrate enrichment of G0 (J). K) In analyzing these dot blots, the levels of G2/M trended lower in cells expressing more KRT15 (n=5, $p=0.037$ at 80th and $p=0.049$ at 90th versus 20th percentile). L&M) An example of gated CD200highITGA6high human population (L), without previous exclusion, where CD45 and CD117 levels were undetectable(M).



S. Figure #2: By immunohistochemistry, KRT15 and ITGA6 are maintained, but CD200 hair follicle expression is lost. A-D) Haired (A,C) and bald (B,D) scalp stained with antibodies to KRT15 (A,B) or double stained with ITGA6 (Green) and CD200 (Red) (C,D). Anagen terminal follicles extend towards subcutaneous fat (A) and miniaturized follicles extend just inferiorly to the sebaceous gland (B). Note that sections of terminal hair follicle and miniaturized hair follicle both stain for the bulge stem cell marker KRT15 indicating presence of stem cell compartment in bald scalp (A,B). However, while ITGA6 is maintained CD200 expression is lost in the bald scalp bulge region (D). Scale bars= 100μm.



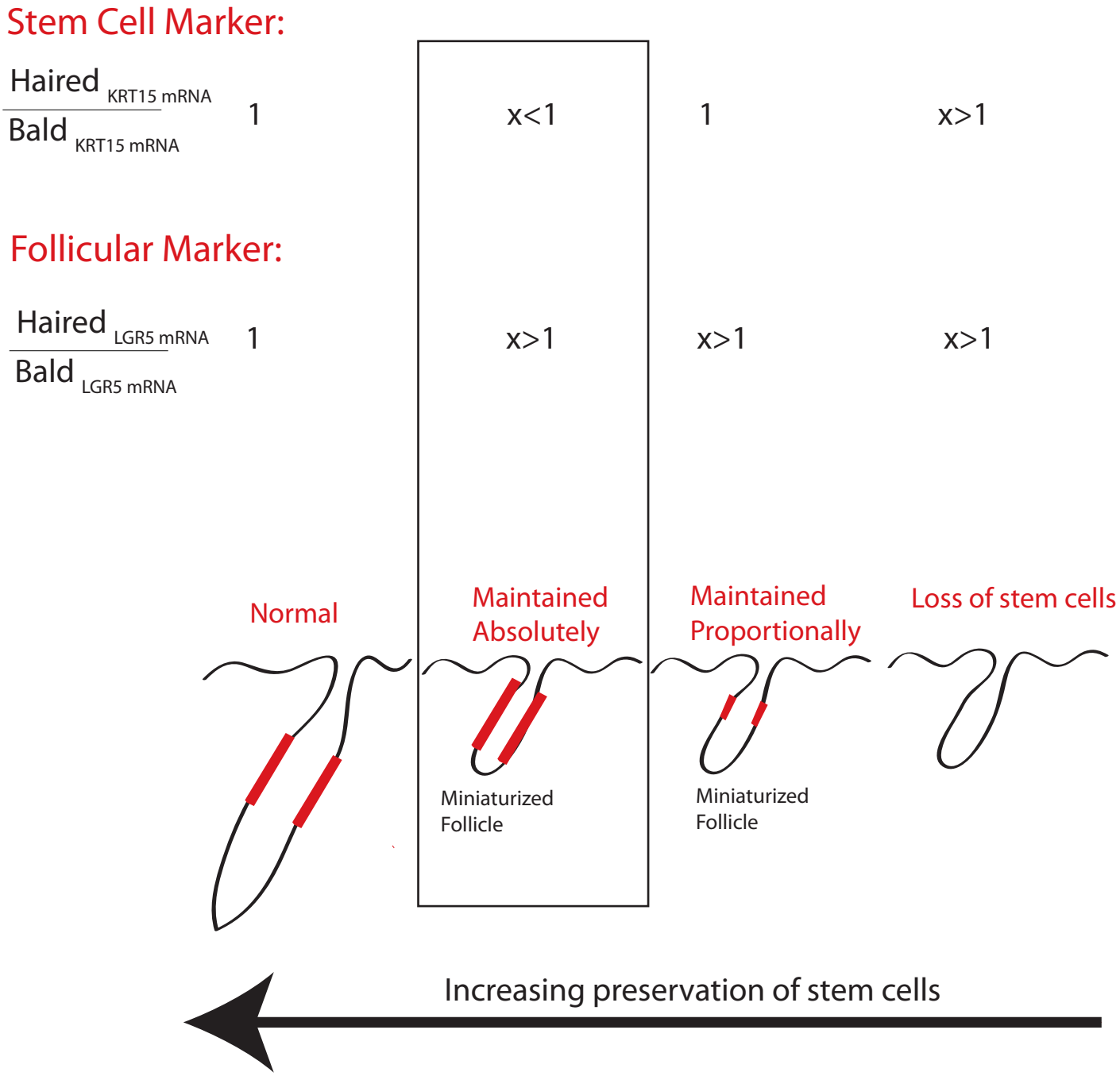
S. Figure #3: Bulge and non-bulge marker immunohistochemical staining.
A&B) Human Ber-EP4 is expressed strongly in secondary hair germs at the level of the dermal papillae and can be detected by both immunofluorescence (A) and immunohistochemistry (B). C) Mouse telogen hair follicles express both CD200 (red) and CD34 (green) in the bulge area (yellow), but only CD200 in the secondary hair germ (red). Note green autofluorescence of the hair shaft. Scale bars= 100μm.



S. Figure #4: Human CD200 immunohistochemistry.
Immunohistochemistry of human haired scalp (A-E), bald scalp (F-G). A) CD200 is expressed in the bulge region of hair follicles. B&C) Often the brightest staining of CD200 was noted in small follicles possibly in early anagen. D&E) CD200 staining was found in upper secondary hair germs. F&G) In bald scalp, CD200 expression was limited to small areas near enlarged sebaceous glands. Scale bars= 100μm.

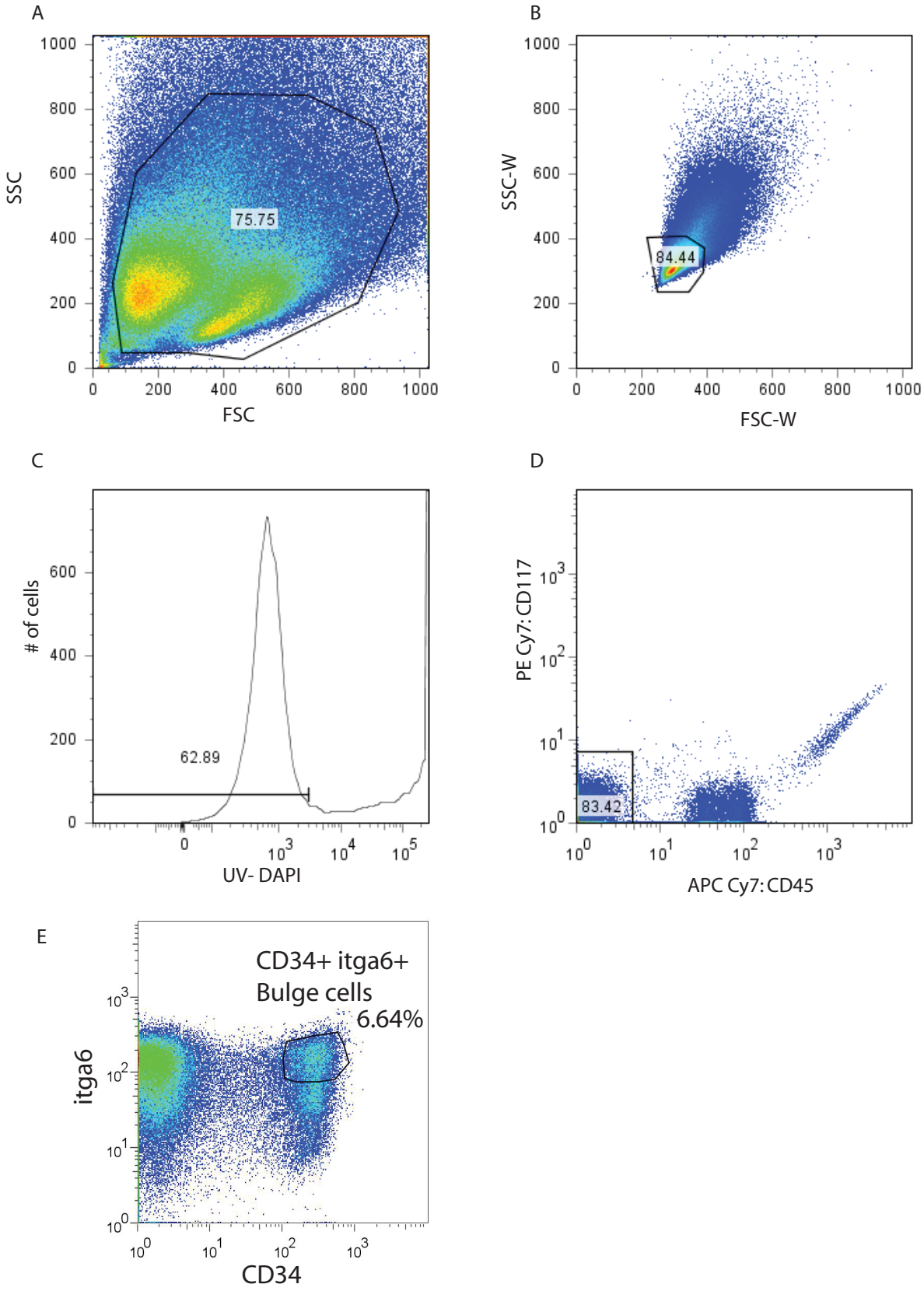
Status of Stem Cell Compartment

$\Delta\Delta_{CT}$ method with actin



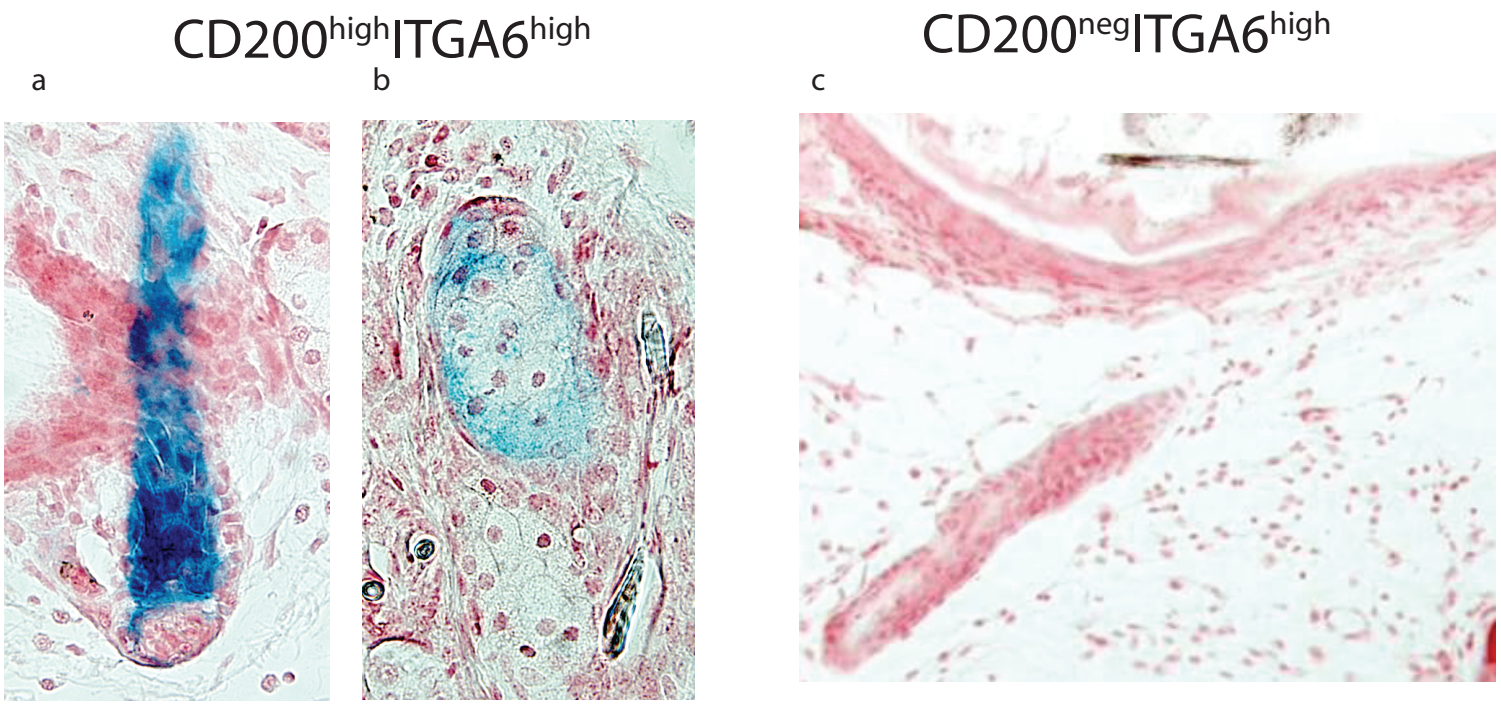
S Figure #5: Stem cell models in disease.

A) Schematic depicting models for how hair follicle stem cells might be affected during hair follicle miniaturization. Stem cells may be maintained in absolute number, maintained proportionally, or decreased in the miniaturized hair follicle. To discriminate between these models, we measured both a stem cell marker (KRT15) and a distinct follicular marker (LGR5) for mRNA abundance relative to actin using qPCR. For each, we made a ratio between haired and bald scalp, so that in the event of loss of the marker in bald scalp the ratio should be x>1 and in the event of a gain of the marker in the bald scalp the ratio should be x<1. While we noticed this ratio was greater than 1 for LGR5, it was less than 1 for KRT15. For LGR5, this suggests that loss of this follicle specific marker is happening in the miniaturization of the hair follicle. For KRT15, this suggests that given the loss of hair follicle volume, the KRT15 message is proportionally more abundant and therefore more likely absolutely maintained.



S. Figure #6: Mouse flow cytometry gating strategy
A) Mouse keratinocytes were first gated out for the dominant population by FSC-A vs. SSC-A selection. B) Doublets were gated out using FSC-W vs. SSC-W. C) Live cells excluding DAPI were gated. D) CD45 negative (Common leukocyte antigen present on hematopoietic cells such as Langerhans cells), CD117 negative (c-kit present on melanocytes) cells were chosen for further analysis. E) Bulge cells were gated as CD34+Itga6+ cells.

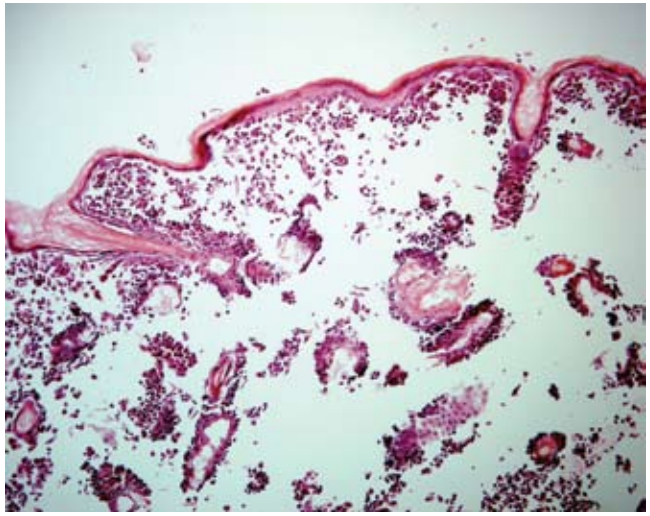
Supp. Figure #7



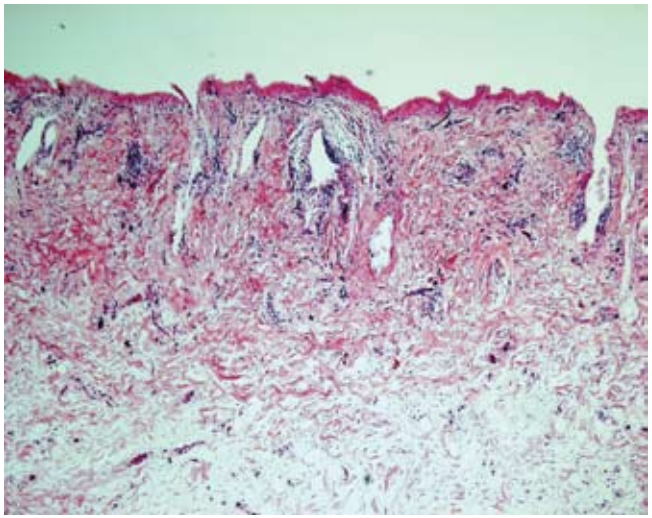
S. Figure #7: Reconstitution Histology:
A-C) Nuclear fast stained histologic sections demonstrating contribution of CD200 high Itga6 high cells (from ROSA26 mice expressing β-galactosidase) to all cells of the hair follicle (A-B), but not dermal papillae (A). CD200 high Itga6 high keratinocytes contribute to sebaceous glands (B). However, CD200 neg Itga6 high cells did not contribute to reconstituted follicles (C).

i

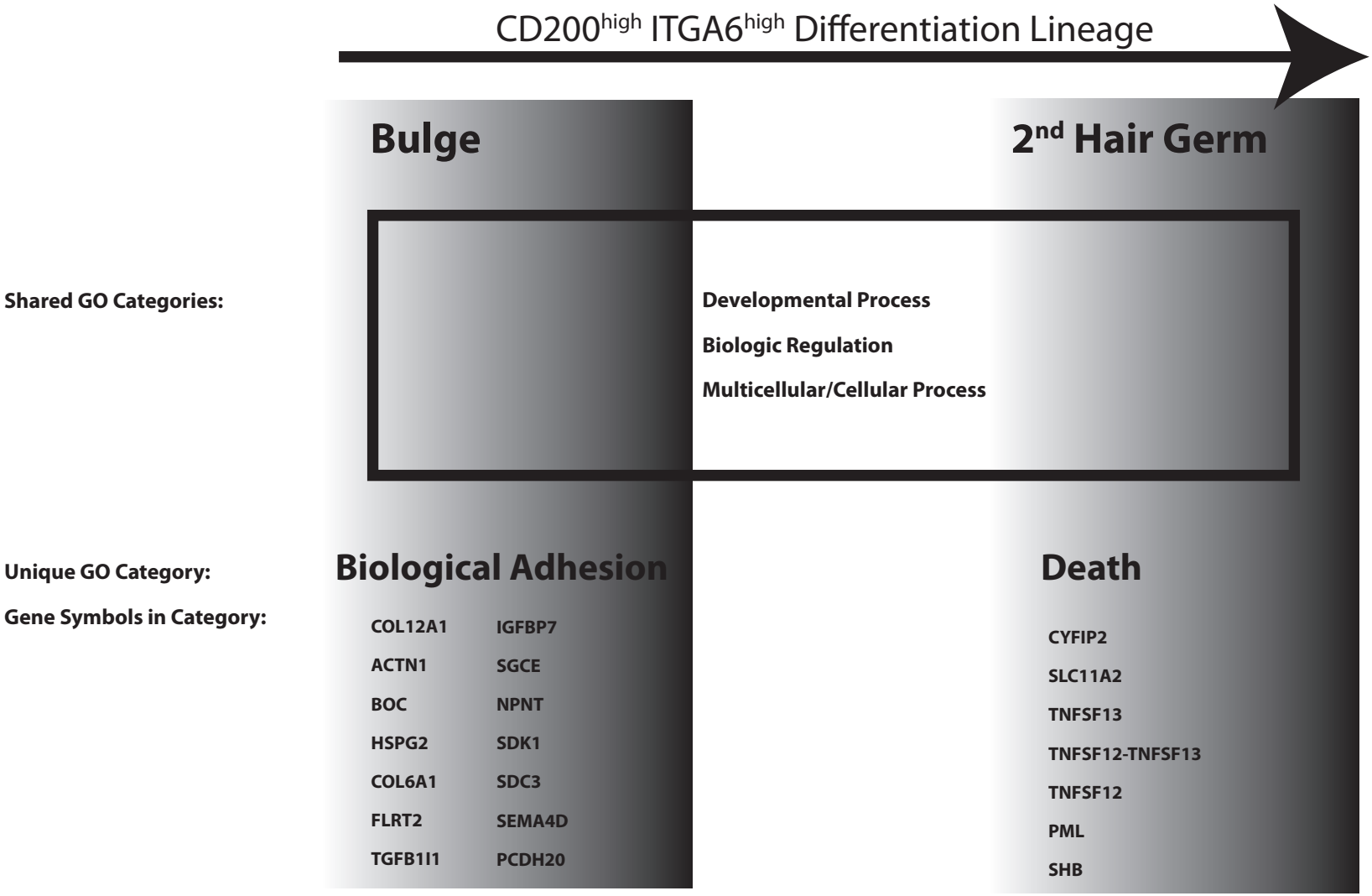
A



B



S. Figure #8: Verification of adequate tissue separation of epidermis and dermis prior to flow cytometry. A-B) By hematoxylin and eosin staining of samples post separation of epidermis and dermis, we verified that the complete epidermis was present in epidermal samples (A) and that few, if any, keratinocytes were present in the dermal sample (B).



S. Figure #9: Array Analysis

To better understand the biological significance of the unique gene lists from human CD200high ITGA6high keratinocytes and either mouse bulge and mouse secondary hair germ (Figure 5H), we performed Gene Ontology (GO) DAVID analysis of common and unique categories within each gene list. We sought to identify unique categories of genes present during differentiation of bulge cells into secondary hair germ cells. We show that shared human and mouse genes present in the bulge are uniquely enriched in biologic adhesion proteins, while transcripts of the secondary hair germ are uniquely enriched in genes regulating death and apoptosis. These results are consistent with the notion of substrate attachment for maintenance of the quiescent phenotype of the bulge, and tha t the loss of these adhesions is associated with differentiation to secondary hair germ cells. The upregulation of transcripts relating to death and apoptosis in the secondary hair germ is consistent with its susceptibility to loss during disease as we find in human androgenetic alopecia where CD200 high ITGA6 high cells are decreased. This analysis also allows insights into the shared biology of hair follicle stem cell differentiation in mice and humans.