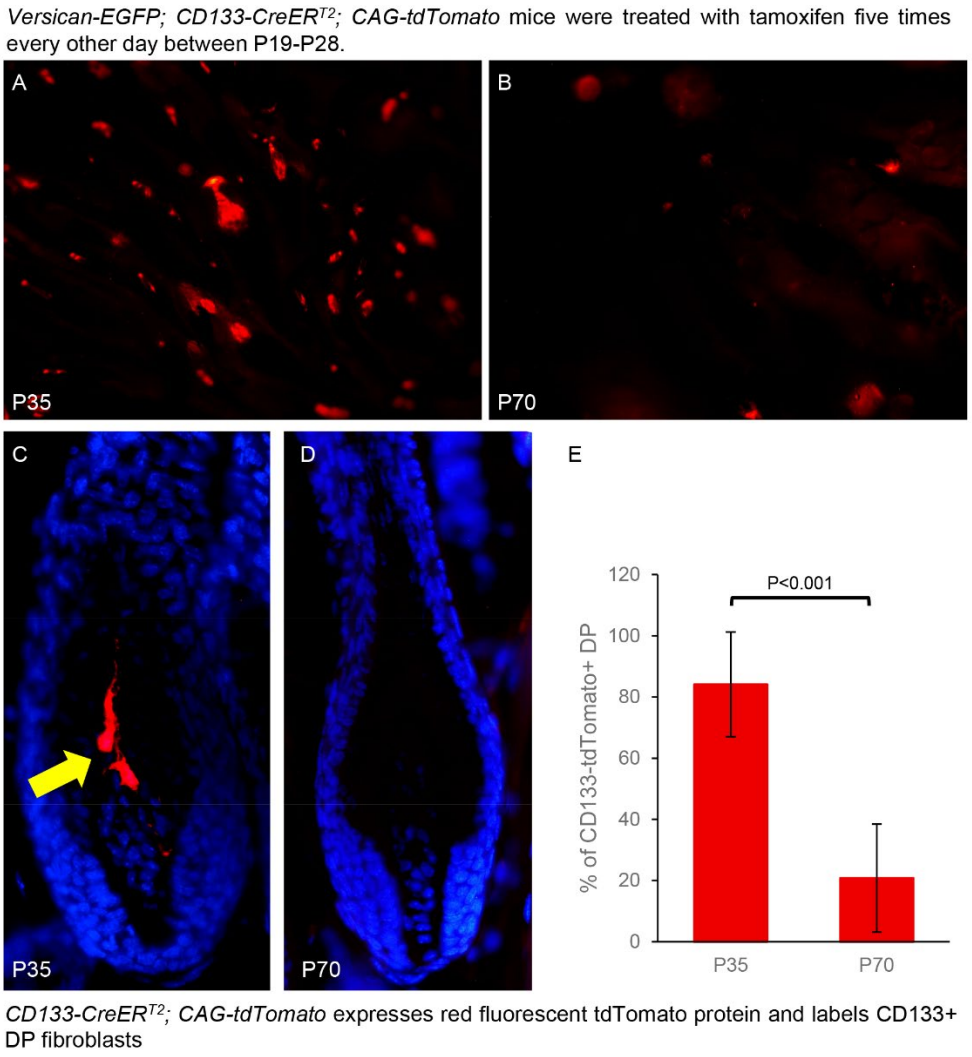

Figure S1 CD133+ DP fibroblasts are not maintained through the hair follicle telogen phase. *Versican-EGFP; CD133-CreER^{T2}; CAG-tdTomato* mice were treated with tamoxifen to induce tdTomato expression in CD133+ cells five times every other day between P19-P28. No tamoxifen was administered afterwards. **A, C**. At P35, red fluorescence can be seen in the DP region of each hair follicle (**C**) from the dermal side of the skin (**A**). **B, D**. At P70, red fluorescence can be seen in their DP regions as shown from the dermal side (**B**) and in individual hair follicle (**D**). **E**. Percentages of hair follicles harboring red fluorescence in P35 and P70 mouse skin. A minimum of 30 hair follicles in three mice at P35 and P70 respectively were counted. *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; ns, not significant.

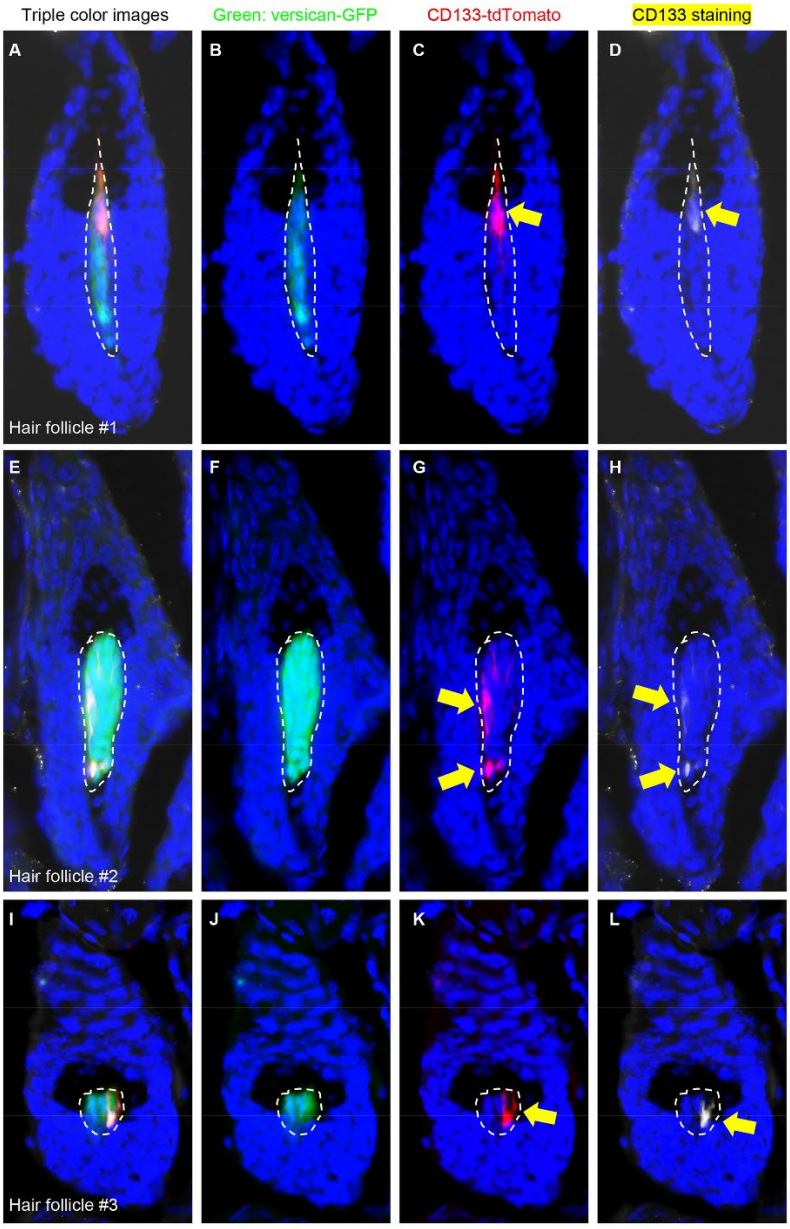

Figure S2 Red fluorescent tdTomato+ cells overlap with APC+ cells in the DP region of *Versican-EGFP; CD133-CreER^{T2}; CAG-tdTomato* hair follicles. Frozen section of three PP8 hair follicles collected from *Versican-EGFP; CD133-CreER^{T2}; CAG-tdTomato* mice were stained using an anti-CD133 primary antibody and a APC-conjugated secondary antibody and showed red fluorescent DP cells were the same population that was stained as APC+ for CD133 expression. **A-D**, hair follicle #1; **E-H**, hair follicle #2; **I-L**, hair follicle #3.

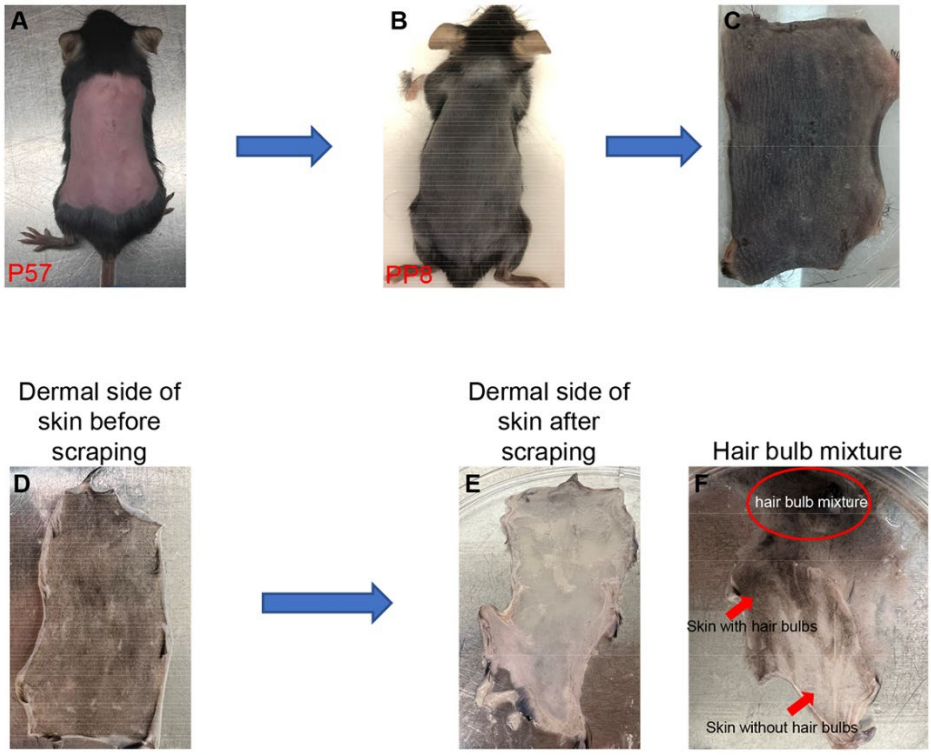

Figure S3 Isolation of versican+; CD133+ and versican+; CD133- DP cells from *versican-GFP; CD133-CreER^{T2}; CAG-tdTomato* transgenic mice. A. Hairs on the back of the mouse were plucked at P57. **B**. Mouse back skin was collected eight days after hair plucking. **C.** Collected skin was floated in a solution of collagenase I, collagenase IV, and hyaluronidase with the dermal side facing down for 30 minutes. **D**. The dermal side of the skin. HF bulbs could be seen. **E-F**. The HF bulbs were scraped off using a razor blade. No black HFs could be seen on the dermal side of skin. The HF bulb mixture in the dish is circled with a red circle.

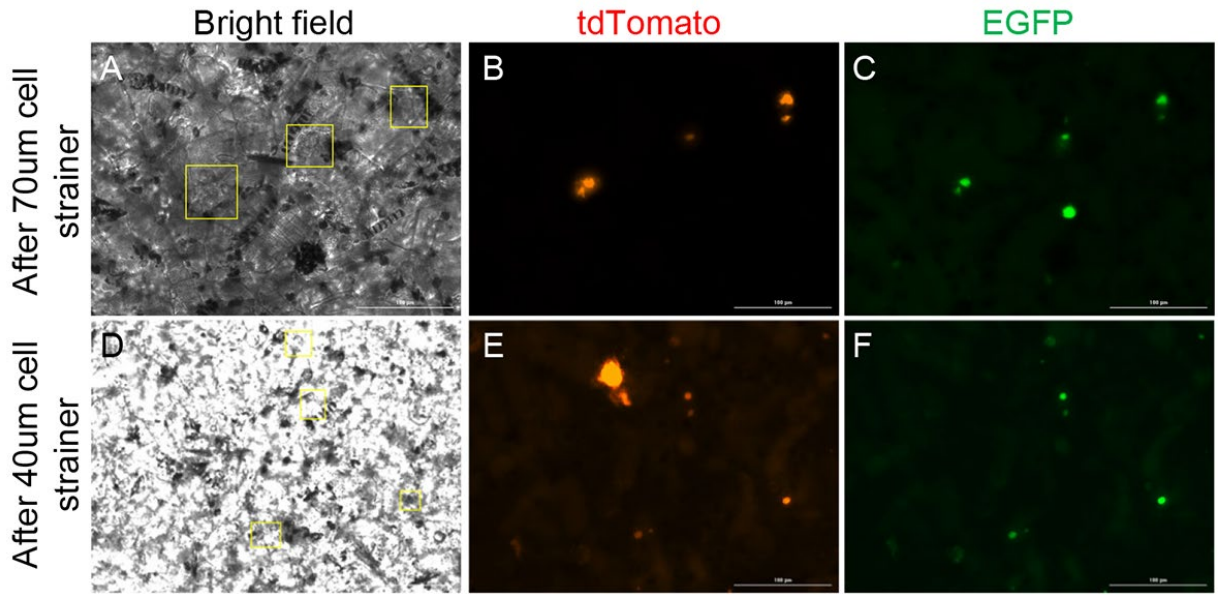

Figure S4 Preparation of versican+; CD133+ and versican+; CD133- single DP cell mixtures from anagen hair follicles. The HF bulb mixture was digested to generate a single-cell mixture. Cell debris and tissue aggregates were removed by filtering the cell mixture through a 70-μm cell strainer (**A-C**) and a 40-μm cell strainer (**D-F**). Red and green DP fibroblasts and green-only DP fibroblasts can be seen in the filtered mixture using a cytation imager. Scale bar: 100 μm.

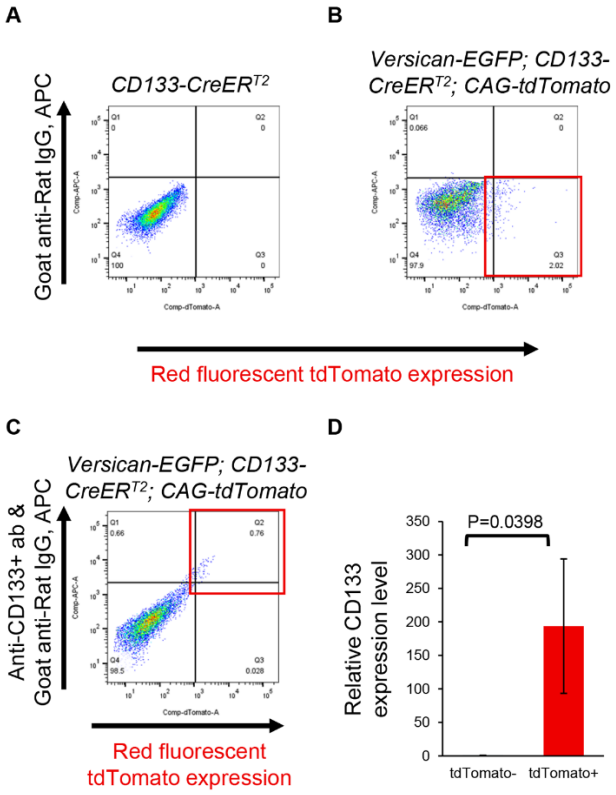

Figure S5 Red fluorescent tdTomato- cells in the DP region of *Versican-EGFP; CD133-CreER^{T2}; CAG-tdTomato* hair follicles express CD133. Flow cytometry analysis of isolated red tdTomato-tagged DP fibroblasts express CD133. In **A**, isolated Green-; red- DP cells from *CD133-CreER^{T2}* mouse were stained with APC-conjugated goat anti-Rat IgG secondary antibody and used as APC negative control. In **B**, isolated DP cells from *versican-EGFP*; *CD133-CreER^{T2}; CAG-tdTomato* mouse were stained with APC-conjugated secondary antibody and showed a population of red+; APC- cells in quadrant 3 (Q3 in red box). In **C**, isolated DP cells from *versican-EGFP*; *CD133-CreER^{T2}; CAG-tdTomato* mouse were stained with an anti-CD133 primary antibody and a APC-conjugated secondary antibody and showed red+ DP cells to be APC+ in Q2 (in red box). **D**. Relative CD133 expression levels in isolated red fluorescent (tdTomato+) and tdTomato- DP cells by qPCR. The expression level of CD133 in tdTomato-DP cells was set as 1 to normalize the expression level of CD133 in tdTomato+ DP cells. n=3. The data are presented as the mean ± SD.

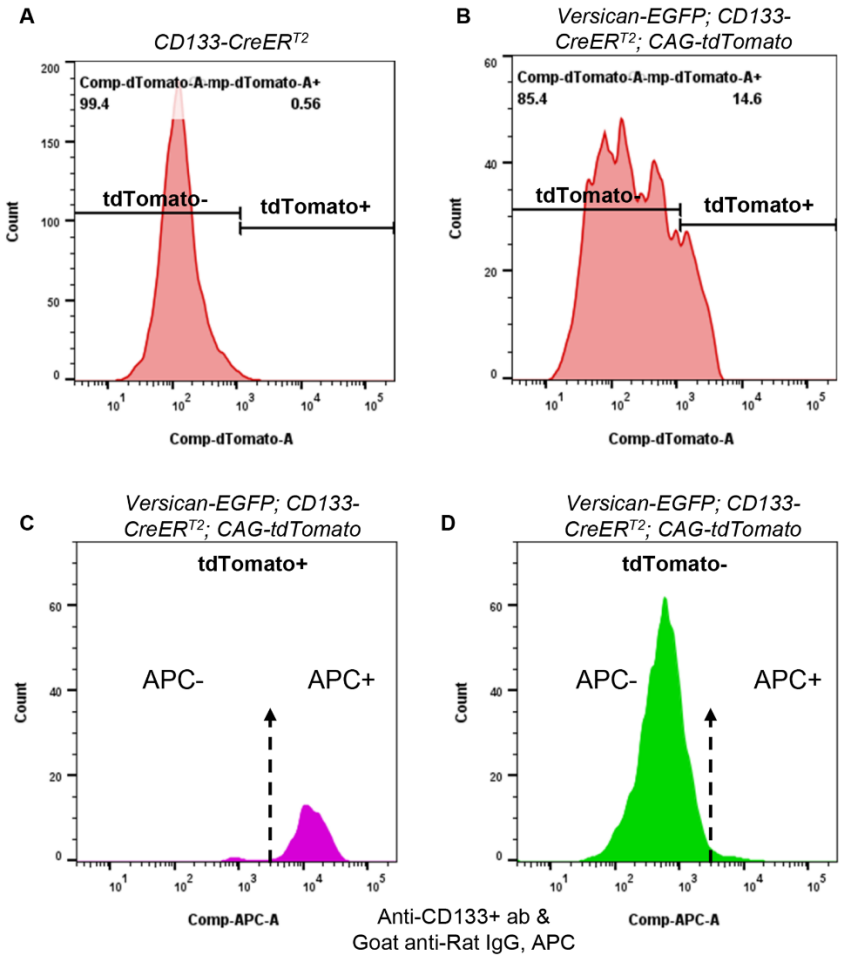

Figure S6 Non-red fluorescent tdTomato- cells in the DP region of *Versican-EGFP; CD133-CreER^{T2}; CAG-tdTomato* hair follicles do not express CD133. Flow cytometry analysis of isolated DP cells from *Versican-EGFP; CD133-CreER^{T2}; CAG-tdTomato* PP8 hair follicles stained with an anti-CD133 primary antibody and a APC-conjugated secondary antibody. **A**. Isolated DP cells from *CD133-CreER^{T2}* hair follicles were used as a negative control for red fluorescent tdTomato expression. **B**. Isolated DP cells from *Versican-EGFP; CD133-CreER^{T2}; CAG-tdTomato* hair follicles showed two subpopulations with and without red fluorescent tdTomato expression. **C**. tdTomato+ DP cells gated in B showed CD133 expression indicated by positive APC staining. **D**. tdTomato- DP cells gated in B showed no CD133 expression indicated by negative APC staining. Flow analysis was repeated three times using isolated DP cells from three different mice.

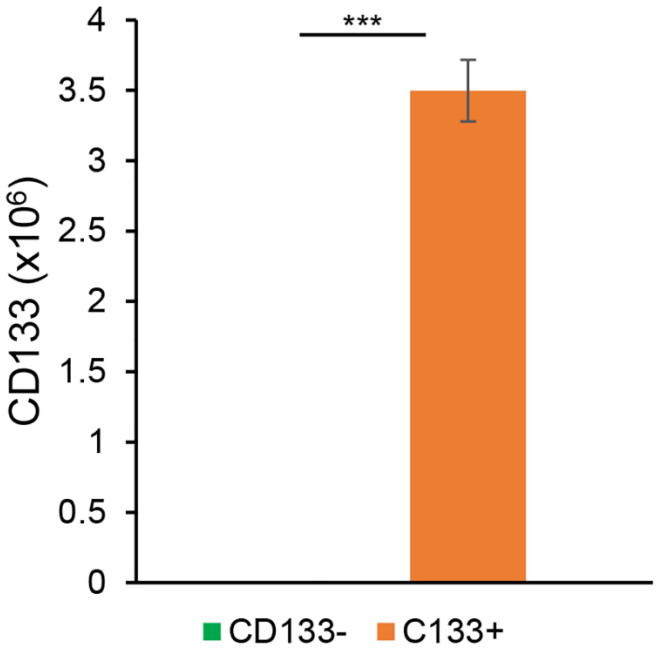

Figure S7 CD133+ DP fibroblasts but not CD133- DP cells retain CD133 expression in 3D spheroid culture. CD133+ and CD133- spheroids cultured for three days were collected for the evaluation of CD133 expression by qPCR. The Y-axis represents the relative CD133 expression levels with the level in versican+; CD133- DP cells set to 1. n=3. The data are presented as the mean ± SD. *P ⩽ 0.05; **P ⩽ 0.01; ***P ⩽ 0.001; ns, not significant.