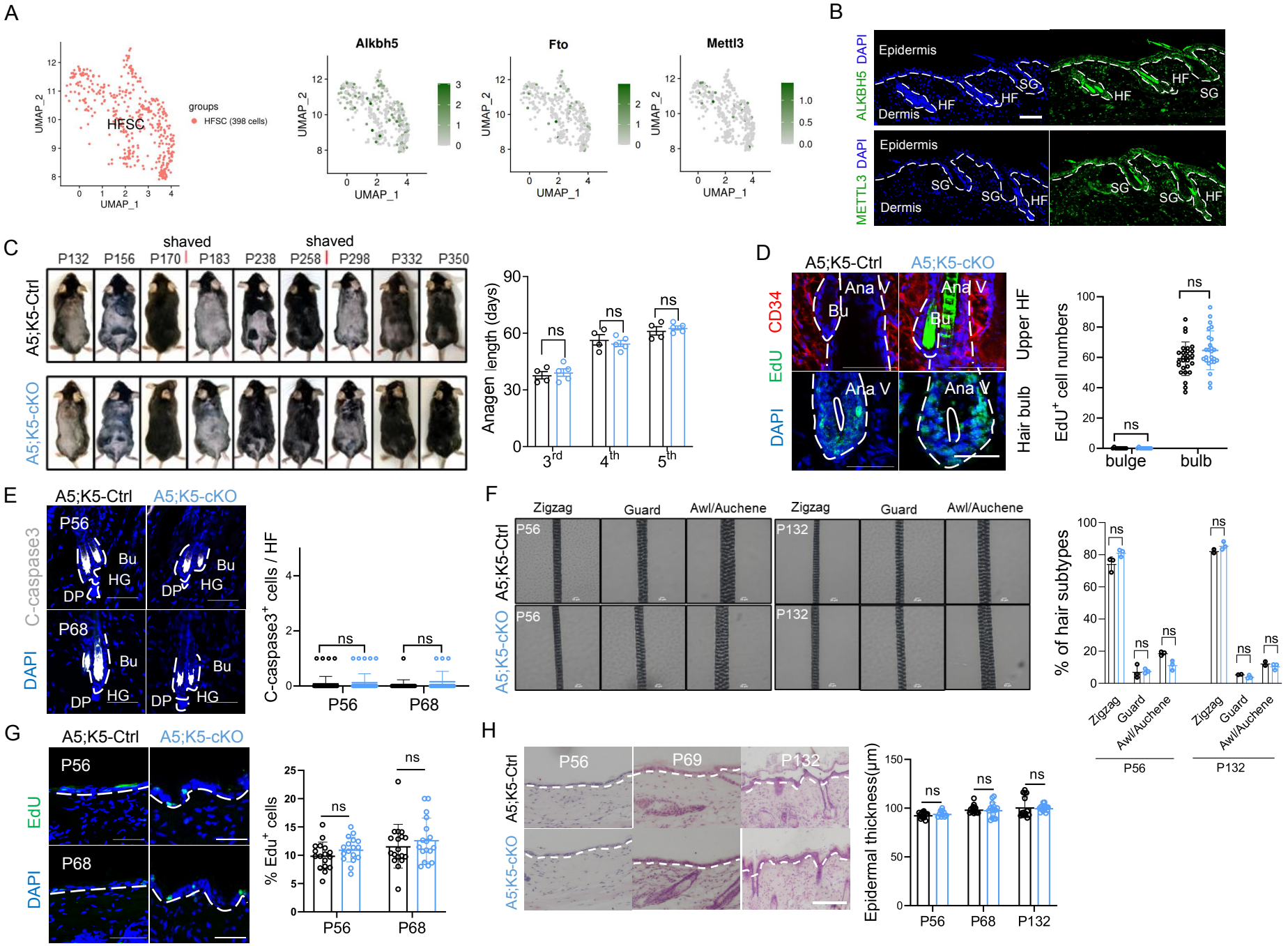
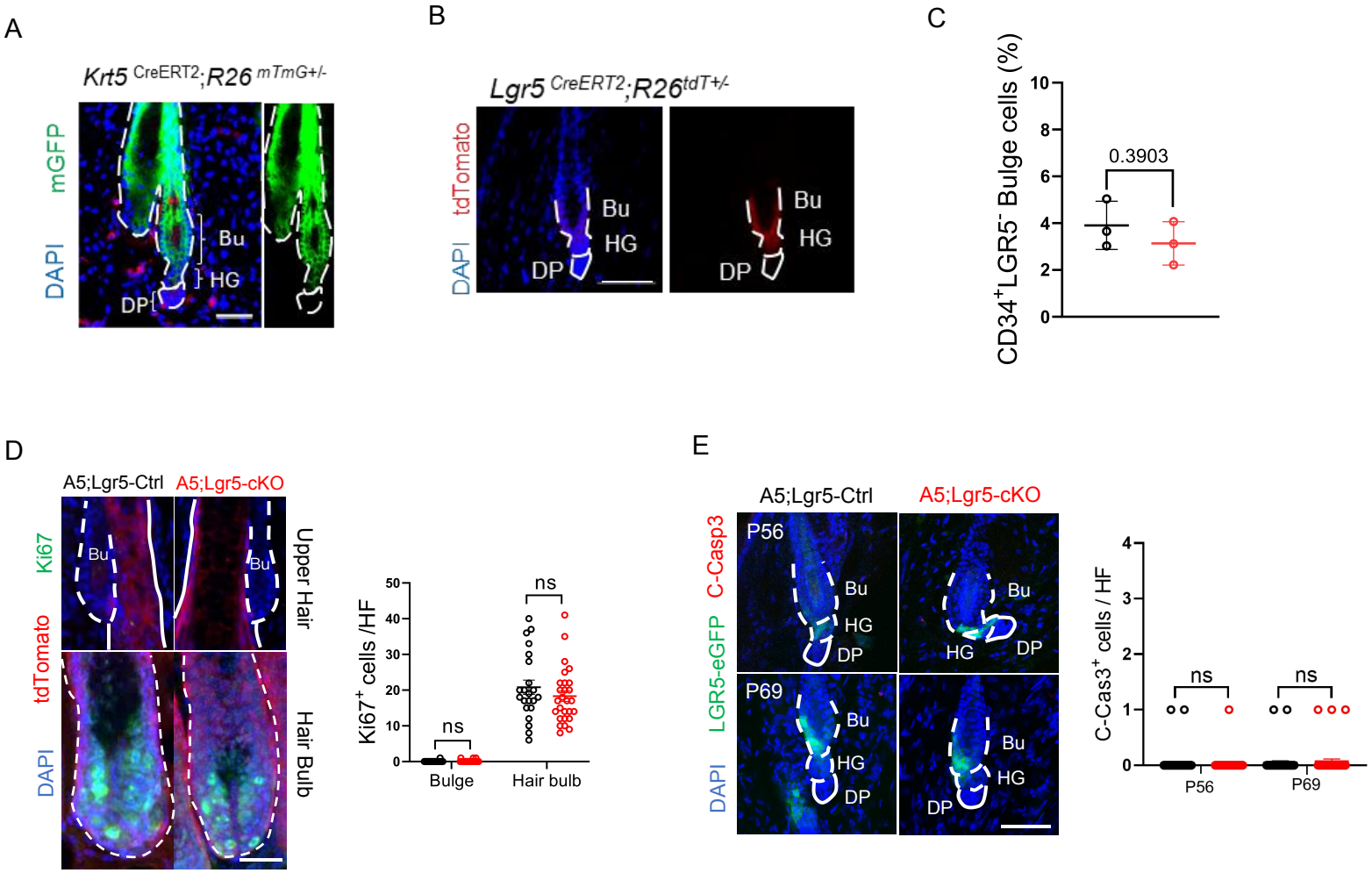

A
HFSC
groups
HFSC (398 cells)
UMAP_1
UMAP_2
Alkbh5
Fto
Mettl3
B
Epidermis
Dermis
DAPI
ALKBH5
METTL3
HF
SG
C
shaved
P132
P156
P170
P183
P238
P258
P298
P332
P350
A5;K5-Ctrl
A5;K5-cKO
Anagen length (days)
ns
3rd
4th
5th
D
A5;K5-Ctrl
A5;K5-cKO
CD34
EdU
DAPI
Ana V
Bu
Upper HF
Hair bulb
EdU+ cell numbers
bulge
bulb
E
C-caspase3
P56
P68
Bu
HG
DP
C-caspase3+ cells / HF
F
Zigzag
Guard
Awl/Auchene
P56
P132
% of hair subtypes
G
EdU
% Edu+ cells
H
P56
P69
P132
Epidermal thickness(μm)

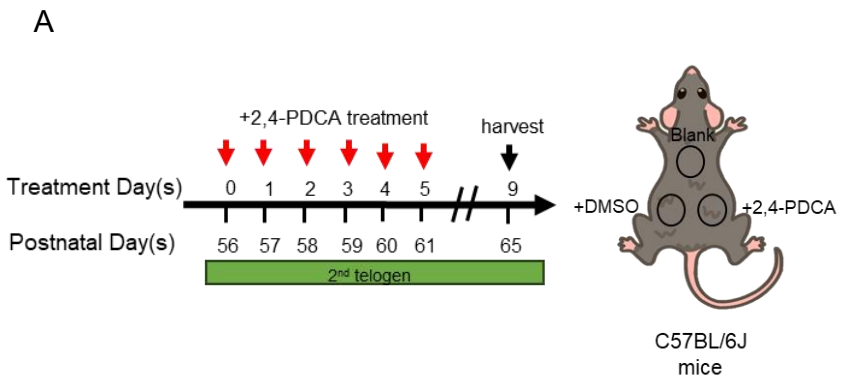
Supplementary Figure. 1 Hair cycle progression in *Alkbh5* knock out mice over time.

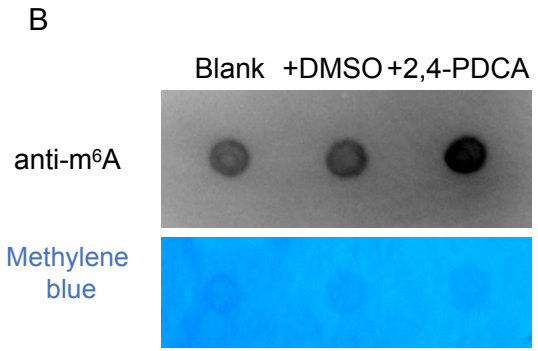
(A) Analysis of publicly available mouse single-cell RNA-seq datasets showing *Mettl3* and *Alkbh5* expression patterns in hair follicle stem cells (HFSCs). (B) Immunofluoresence staining of DAPI (Blue), ALKBH5 (Green) and METTL3 (Green) in mouse skin (n = 3) at P56. The white dashed line delineates the epidermis (Epidermis) from the hair follicle (HF). Scale bar, 50 μm. (C) (Left) Representative images showing dorsal hair regrowth in *Alkbh5*$^{fl/fl}$; *Krt5*$^{CreERT2+/-}$ (A5;K5-cKO, n = 5) and *Krt5*$^{CreERT2+/-}$ (A5;K5-Ctrl, n = 4) mice, starting from the third hair cycle. Red arrows indicate shaving of the dorsal skin. (Right) Quantification of the anagen phase duration (in days) across hair cycles. (D) (Left) Immunofluorescence staining for EdU (green), CD34 (red), and DAPI (blue) in bulge-region HFSCs and hair matrix cells from A5;K5-cKO and A5;K5-Ctrl mice (n = 3) during late anagen (Anagen V). Scale bar, 100 μm. (Right) Quantification of EdU$^+$ cell numbers in bulge HFSCs and hair bulb cells during late anagen. (E) (Left) Immunofluorescence staining for cleaved Caspase-3 (C-Caspase3, white) and DAPI (blue) in HFSCs from A5;K5-cKO and A5;K5-Ctrl mice (n = 3) at P56 and P68. Scale bar, 50 μm. (Right) Quantification of numbers of C-Caspase3$^+$ HFSCs. (F) Quantification of hair types—including guard, awl, auchene, and zigzag hairs—in A5;K5-cKO and A5;K5-Ctrl mice (n = 3) at P56 and P132. (G) (Left) Immunofluorescence staining for EdU (green) and DAPI (blue) in interfollicular epidermis (IFE) basal cells from A5 cKO and A5 Ctrl mice (n = 3) at P56 and P68. Scale bar, 50 μm. (Right) Quantification of numbers of EdU$^+$ IFE basal cells. (H) (Left) Hematoxylin and eosin (H&E) staining of skin sections from A5;K5-cKO and A5;K5-Ctrl mice (n = 3) at P56, P68, and P132. (Right) Quantification of IFE epidermal thickness from the corresponding images. Bars represent mean ± SD.

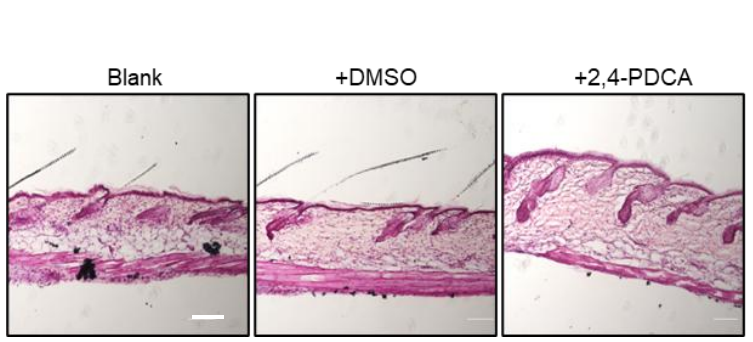

A
Krt5 CreERT2;R26 mTmG+/-
mGFP
DAPI
Bu
HG
DP
B
Lgr5 CreERT2;R26tdT+/-
tdTomato
DAPI
Bu
HG
DP
C
CD34+LGR5- Bulge cells (%)
0.3903
D
A5;Lgr5-Ctrl
A5;Lgr5-cKO
Ki67
tdTomato
DAPI
Upper Hair
Hair Bulb
Ki67+ cells /HF
ns
Bulge
Hair bulb
E
A5;Lgr5-Ctrl
A5;Lgr5-cKO
C-Casp3
LGR5-eGFP
DAPI
P56
P69
C-Cas3+ cells / HF
P56
P69

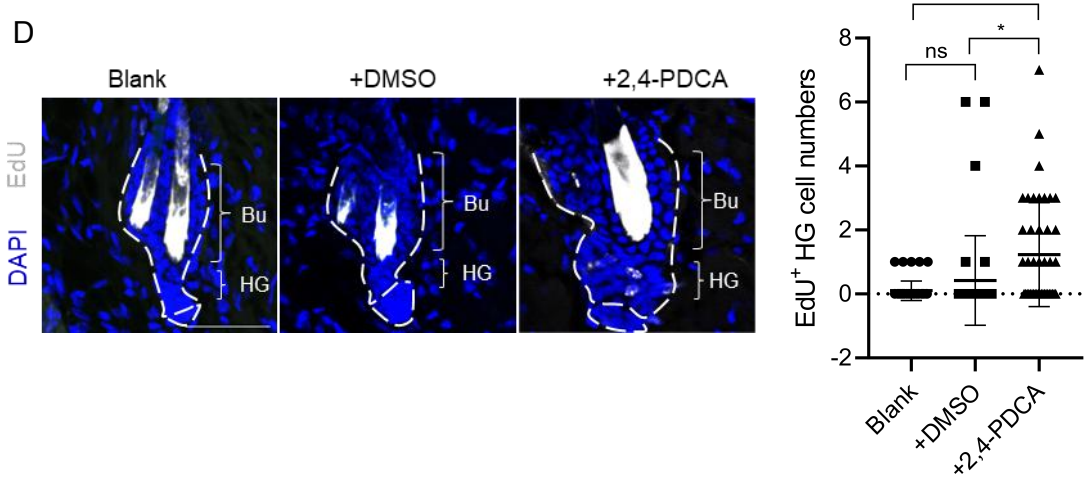
Supplementary Figure. 2 *Alkbh5* deficiency in hair follicle stem cells spares their differentiation.

(A) (Left) Fluorescence images of dorsal hair follicles from *Krt5*^{CreERT2}; *Rosa26*^{mTmG} mice at P46 showing membrane-tdTomato (mT, red), membrane-GFP (mG, green), and DAPI (blue). Scale bar, 50 μm. (Right) GFP channel only, representing KRT5^{+} cells within the hair follicle. White dashed lines outline individual hair follicles. (B) (Left) Fluorescence images of dorsal hair follicles from *Lgr5*$^{EGFP\text{-}IRES\text{-}CreERT2}$;*Rosa26*^{tdTomato} mice at P56 showing tdTomato (red) and DAPI (blue). Scale bar, 50 μm. (Right) tdTomato channel only, representing LGR5^{+} cells within the hair follicle. White dashed lines outline individual hair follicles. (C) Flow cytometric analysis of epidermal cells isolated from P70 A5; Lgr5-Ctrl (n = 3) and A5; Lgr5-cKO (n = 3) mice, the statistics of LGR5^{-} CD34^{+} bulge-region HFSCs. (D) (Left) Immunofluorescence staining for Ki67 (green), tdTomato (red), and DAPI (blue) in bulge-region HFSCs and hair matrix cells from A5;Lgr5-cKO and A5;Lgr5-Ctrl mice (n = 3) during late anagen (Anagen V). Scale bar, 200 μm. (Right) Quantification of Ki67^{+} cell numbers in bulge HFSCs and hair bulb cells during late anagen. (E) (Left) Immunofluorescence staining for cleaved- caspase-3 (C-caspase3, green) and DAPI (blue) in HFSCs from A5;Lgr5-cKO and A5;Lgr5-Ctrl mice (n = 3) at P56 and P68. Scale bar, 50 μm. (Right) Quantification of numbers of C-caspase3^{+} HFSCs. Bars represent mean ± SD.


A
+2,4-PDCA treatment
harvest
Treatment Day(s)
0 1 2 3 4 5 9
Postnatal Day(s)
56 57 58 59 60 61 65
2nd telogen
Blank
+DMSO
+2,4-PDCA
C57BL/6J
mice


B
Blank +DMSO +2,4-PDCA
anti-m6A
Methylene
blue


C
Blank
+DMSO
+2,4-PDCA

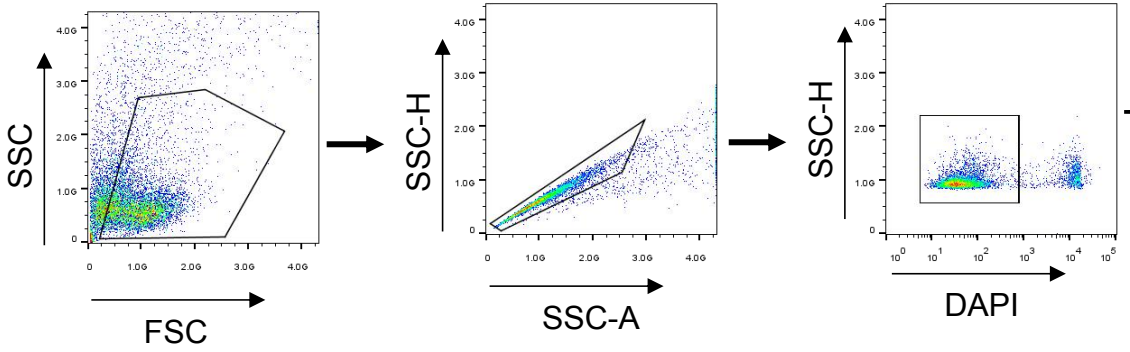

D
Blank
+DMSO
+2,4-PDCA
EdU
DAPI
Bu
HG
****
*
ns
EdU+ HG cell numbers
8
6
4
2
0
-2
Blank
+DMSO
+2,4-PDCA

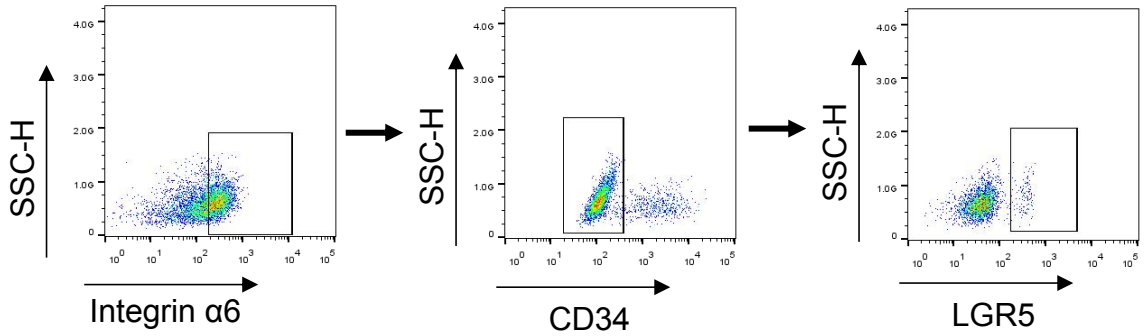
Supplementary Figure. 3 The ALKBH5 inhibitor 2,4-PDCA enhances proliferation of hair follicle stem cells.

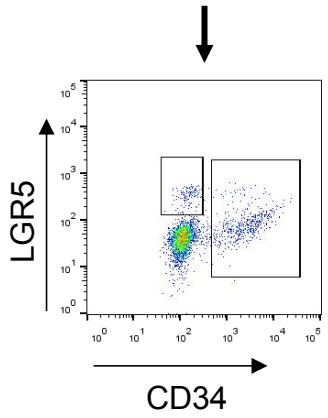
(A) C57BL/6J mice (n = 3) were shaved on the dorsal skin, and three separate areas were treated topically with 2,4-PDCA, 10% DMSO, or left untreated (Blank). Both 2,4-PDCA and 10% DMSO were applied once daily for 5 consecutive days, and skin tissues were collected on day 9 for analysis. (B) m^6 A dot blot assay (anti-m^6 A) and methylene blue staining of RNA extracted from Blank, 2,4-PDCA–treated and 10% DMSO–treated skin tissues (n = 3). (C) Hematoxylin and eosin (H&E) staining of skin tissues from the Blank (untreated), DMSO (10% DMSO-treated), and 2,4-PDCA (ALKBH5 inhibitor-treated) groups (n = 3). Scale bar, 200 μm. (D) (Left) Immunofluorescence staining for Ki67 (white) and DAPI (blue) in skin tissues from Blank, DMSO, and 2,4-PDCA groups (n = 3). Scale bar, 50 μm. (Right) Quantification of $Ki67^+$ cell numbers from the corresponding images. Bars represent mean ± SD.

The gating strategy of hair germ HFSC: Integrin α6⁺ CD34⁻ LGR5⁺

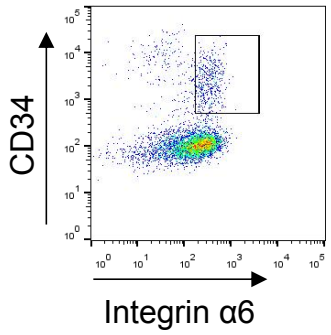
A







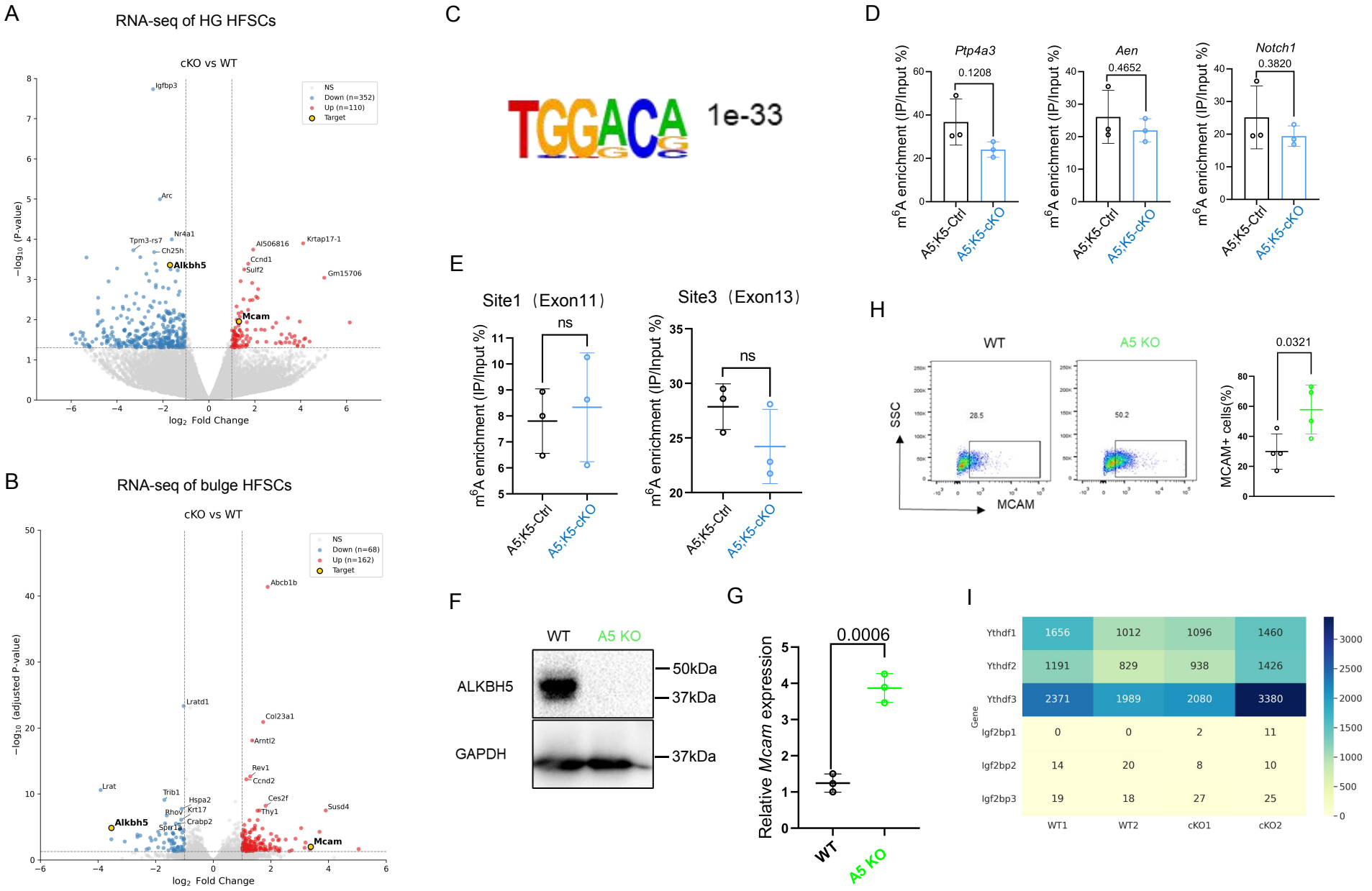
The gating strategy of hair germ HFSC and bulge HFSC



The gating strategy of bulge HFSC: Integrin α6⁺ CD34⁺

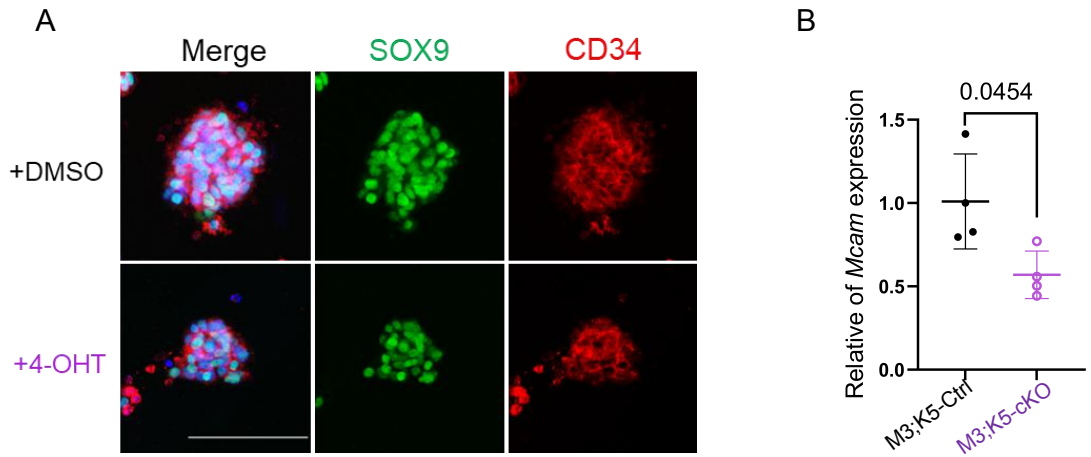
Supplementary Figure. 4 The gating strategies for flow cytometry.

(A) The gating strategy for identifying the hair germ HFSC, or the bulge HFSC, or both at the same time.


A
RNA-seq of HG HFSCs
cKO vs WT
NS
Down (n=352)
Up (n=110)
Target
Igfbp3
Arc
Nr4a1
Tpm3-rs7
Ch25h
Alkbh5
AI506816
Krtap17-1
Ccnd1
Sulf2
Gm15706
Mcam
−log10 (P-value)
log2 Fold Change
B
RNA-seq of bulge HFSCs
cKO vs WT
NS
Down (n=68)
Up (n=162)
Target
Abcb1b
Lratd1
Col23a1
Arntl2
Rev1
Ccnd2
Lrat
Trib1
Hspa2
Ces2f
Thy1
Susd4
Rhov
Krt17
Crabp2
Spry1
Alkbh5
Mcam
−log10 (adjusted P-value)
log2 Fold Change
C
TGGACA
1e-33
D
Ptp4a3
0.1208
Aen
0.4652
Notch1
0.3820
m6A enrichment (IP/Input %)
A5;K5-Ctrl
A5;K5-cKO
E
Site1 （Exon11）
Site3 （Exon13）
ns
ns
m6A enrichment (IP/Input %)
A5;K5-Ctrl
A5;K5-cKO
F
WT
A5 KO
ALKBH5
GAPDH
50kDa
37kDa
37kDa
G
0.0006
Relative Mcam expression
WT
A5 KO
H
WT
A5 KO
28.5
50.2
SSC
MCAM
0.0321
MCAM+ cells(%)
I
Gene
Ythdf1 1656 1012 1096 1460
Ythdf2 1191 829 938 1426
Ythdf3 2371 1989 2080 3380
Igf2bp1 0 0 2 11
Igf2bp2 14 20 8 10
Igf2bp3 19 18 27 25
WT1
WT2
cKO1
cKO2
3000
2500
2000
1500
1000
500
0

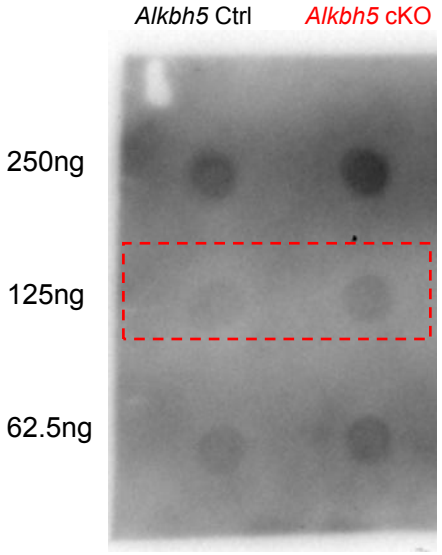
Supplementary Figure. 5 ALKBH5 regulates Mcam transcript stability and expression.

(A) Volcano plot showing differentially expressed genes between wild-type (WT, n = 3) and *Alkbh5*-deficient (n = 3) hair germ (HG) HFSCs. Red dots indicate upregulated genes, and blue dots indicate downregulated genes. (B) Volcano plot showing differentially expressed genes between WT (n = 4) and *Alkbh5*-deficient (n = 4) bulge-region HFSCs. Red dots represent upregulated genes, and blue dots represent downregulated genes. (C) Motif analysis of m^6A peaks identified by m^6A-seq in WT and *Alkbh5*-deficient HFSCs. (D) MeRIP–qPCR analysis of m^6A enrichment in *Notch1*, *Ptp4a3*, and *Aen1* transcripts from WT and *Alkbh5*-deficient HFSCs. (E) MeRIP–qPCR analysis of m^6A enrichment on exons 11 and 13 of the *Mcam* transcript. (F) Western blot analysis of ALKBH5 expression in WT and *Alkbh5*-knockout HaCaT cells (n = 3), with GAPDH as a loading control. (G) RT–qPCR analysis of *Mcam* transcript levels in WT and *Alkbh5*-knockout HaCaT cells (n = 3). (H) (Left) Flow cytometry analysis of MCAM protein expression in WT and *Alkbh5*-knockout HaCaT cells (n = 3). (Right) Quantification of the percentage of $MCAM^+$ cells. (I) RNA-seq analysis of bulge-region HFSCs showing expression levels of m^6A reader proteins. Bars represent mean ± SD.


A
Merge
SOX9
CD34
+DMSO
+4-OHT
B
0.0454
Relative of Mcam expression
1.5
1.0
0.5
0.0
M3;K5-Ctrl
M3;K5-cKO

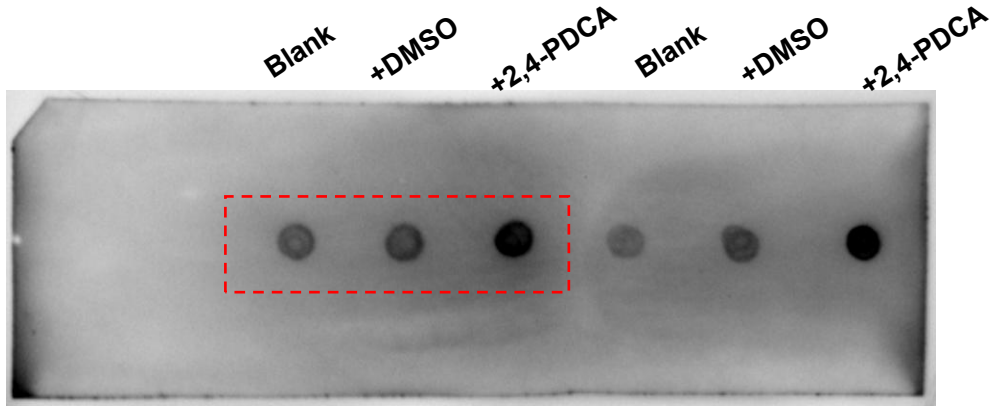
Supplemetary Figure. 6 *Mettl3* deletion does not affect the maintenance of HFSC， and downregulates *Mcam* expression. (A) Immunofluorescence staining for DAPI (blue), SOX9 (green), and CD34 (red) to evaluate the stemness of *in vitro*-cultured HFSCs. Scale bar: 50 μm. (B) RT–qPCR analysis of *Mcam* mRNA expression in HFSCs from M3;K5-Ctrl and M3;K5-cKO (n = 4) mice. Bars represent mean ± SD.

Uncropped blot images in Figure. 1B



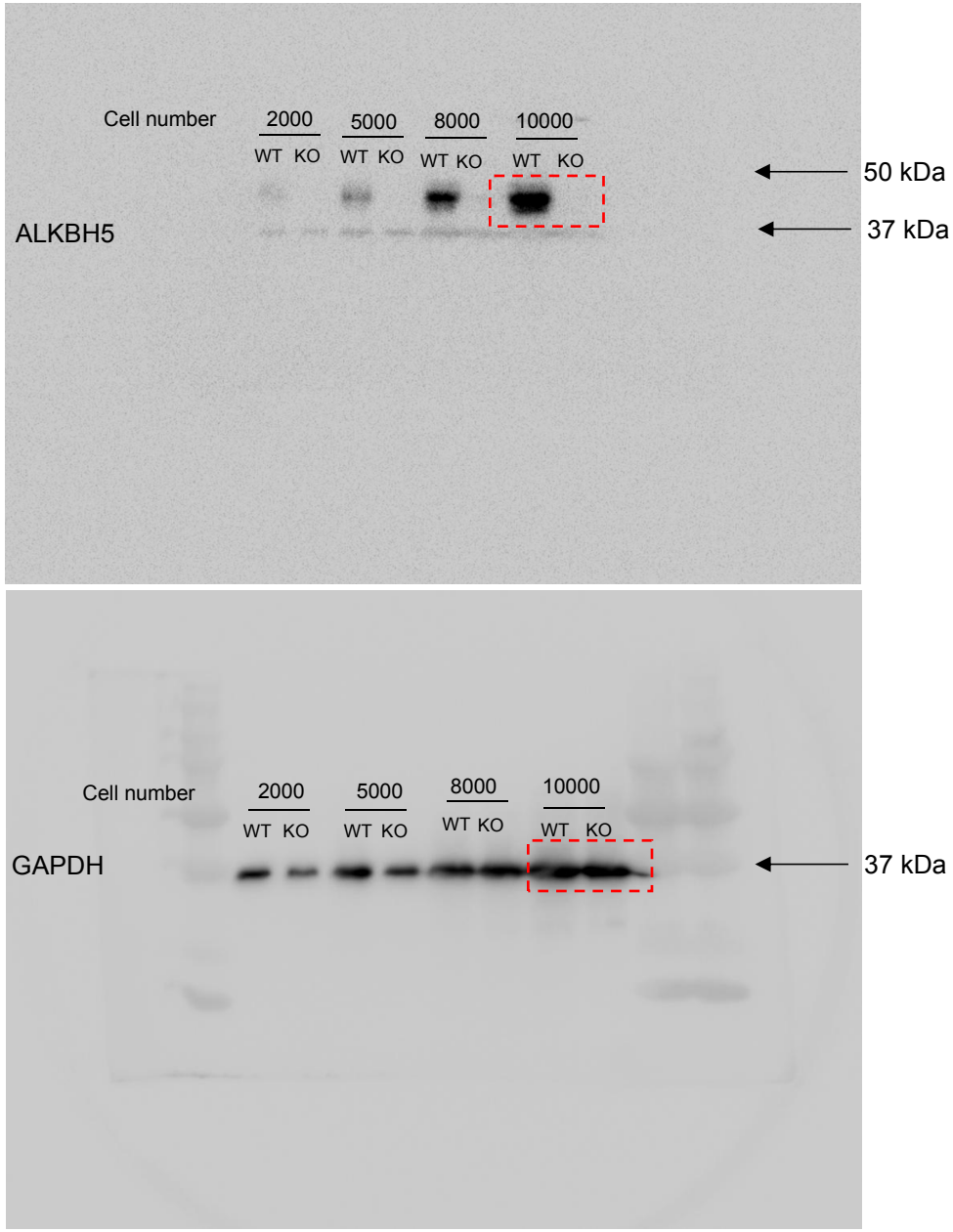
Uncropped m^{6}A dot blot images corresponding to Figure 1B. The red dashed box refers to the representative image displayed in the figure.

Uncropped blot images in Supplementary Figure. 3B



Uncropped m^{6}A dot blot images corresponding to Supplementary Figure 3B. The red dashed box refers to the representative image displayed in the figure.

Uncropped blot images in Supplementary Figure. 5F



Uncropped Western blot images corresponding to Supplementary Figure 5F. The red dashed box refers to the representative image displayed in the figure.