

Supplementary Materials for
**Mettl3-catalyzed m⁶A regulates histone modifier and modification expression
in self-renewing somatic tissue**

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The PDF file includes:

Supplementary Materials and Methods
Figs. S1 to S6

Other Supplementary Material for this manuscript includes the following:

Data S1 and S2

Figure S1

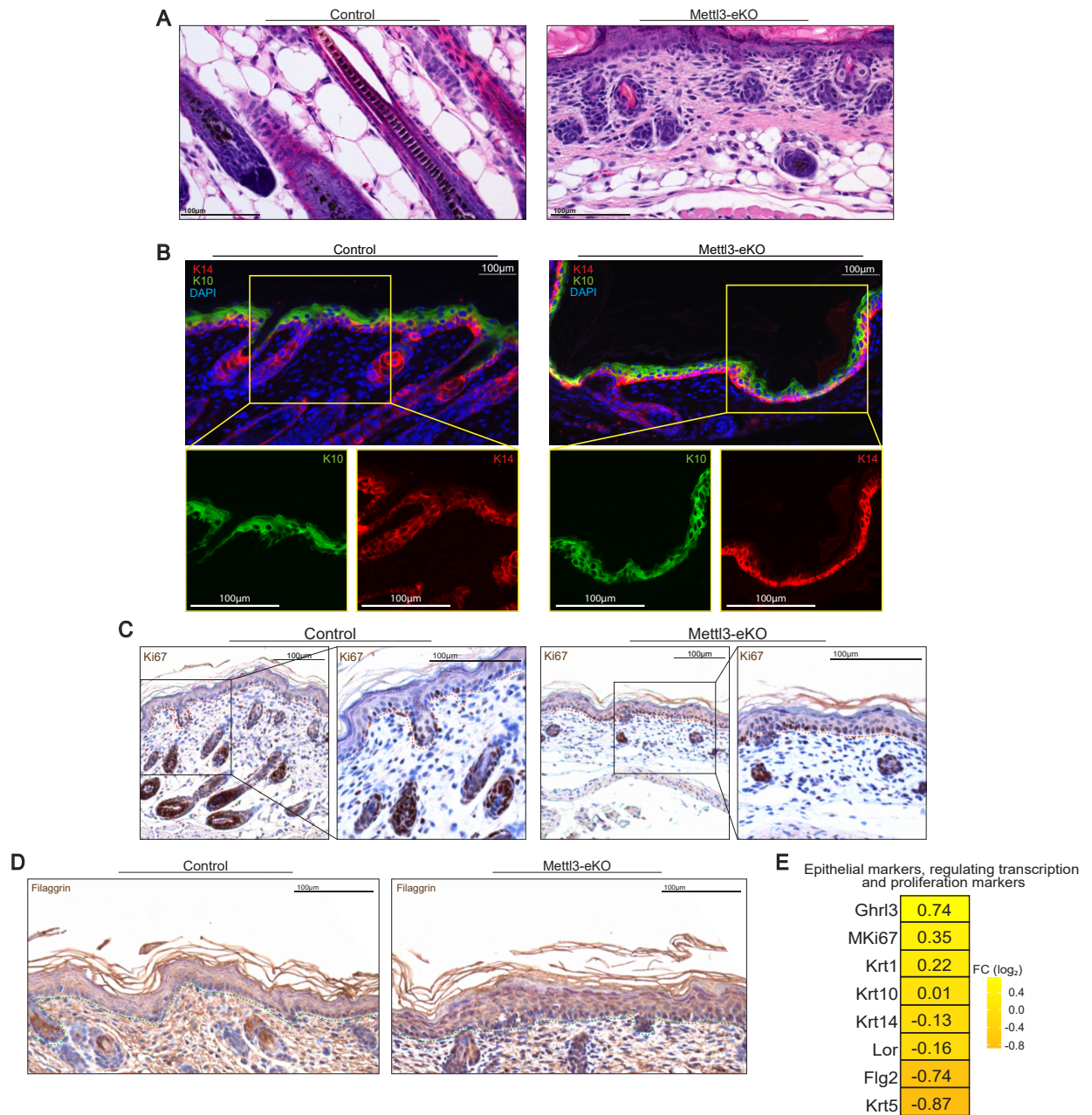


Fig. S1. Histological analysis of *Mettl3*-eKO murine skin. (A) *Mettl3*-eKO skin displays underdeveloped, malformed hair follicles. (B) IF of murine dorsal skin of cytokeratin 14 (K14) in the basal layer and cytokeratin 10 (K10) at the suprabasal layer. Controls have distinct basal and suprabasal layers, while there is overlapping staining of both K14 and K10 in the *Mettl3*-eKO epidermis. (C) Ki67 IHC of murine dorsal skin, the dotted orange line demarcates the epidermis-dermis junction. *Mettl3*-eKO epidermis have more positive Ki67 cell staining in the basal layer in comparison to control epidermis. (D) IHC of murine dorsal skin, the dotted green line demarcates the epidermis-dermis junction. Filaggrin is present normally in control and in *Mettl3*-eKO epidermis. (E) Representative markers of epithelial stemness and differentiation and their changes in gene expression by RNA-seq in *Mettl3*-eKO mice epidermis in comparison to WT mice.

Figure S2

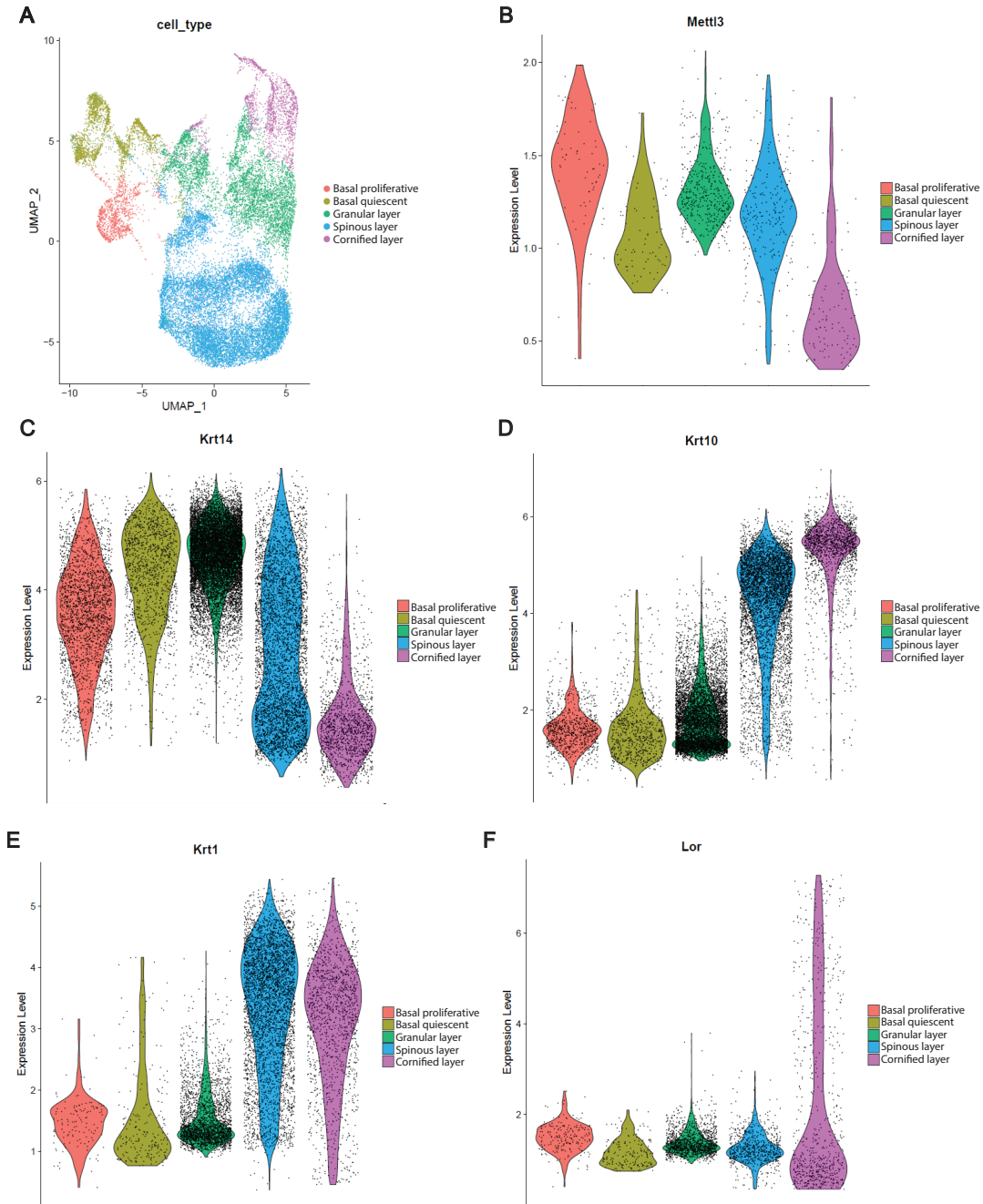


Fig. S2. Single-cell expression analysis of murine epidermal keratinocytes. (A) Epidermal cell types identified in mice in a UMAP projection of GSE154679. Violin plots show gene expression in cells on the single-cell level. Each dot represents a single mouse keratinocyte. Statistically significant gene expression is observed only if a violin-shaped fitting area can be calculated. (B) *Mettl3* expression is reduced in the more differentiated cornified layer in comparison to the basal and suprabasal layers of the epidermis similar to what is observed with *Krt14* (C). In contrast, *Krt10* (D), *Krt1* (E), and *Lor* (F) all display increased expression with differentiation.

Figure S3

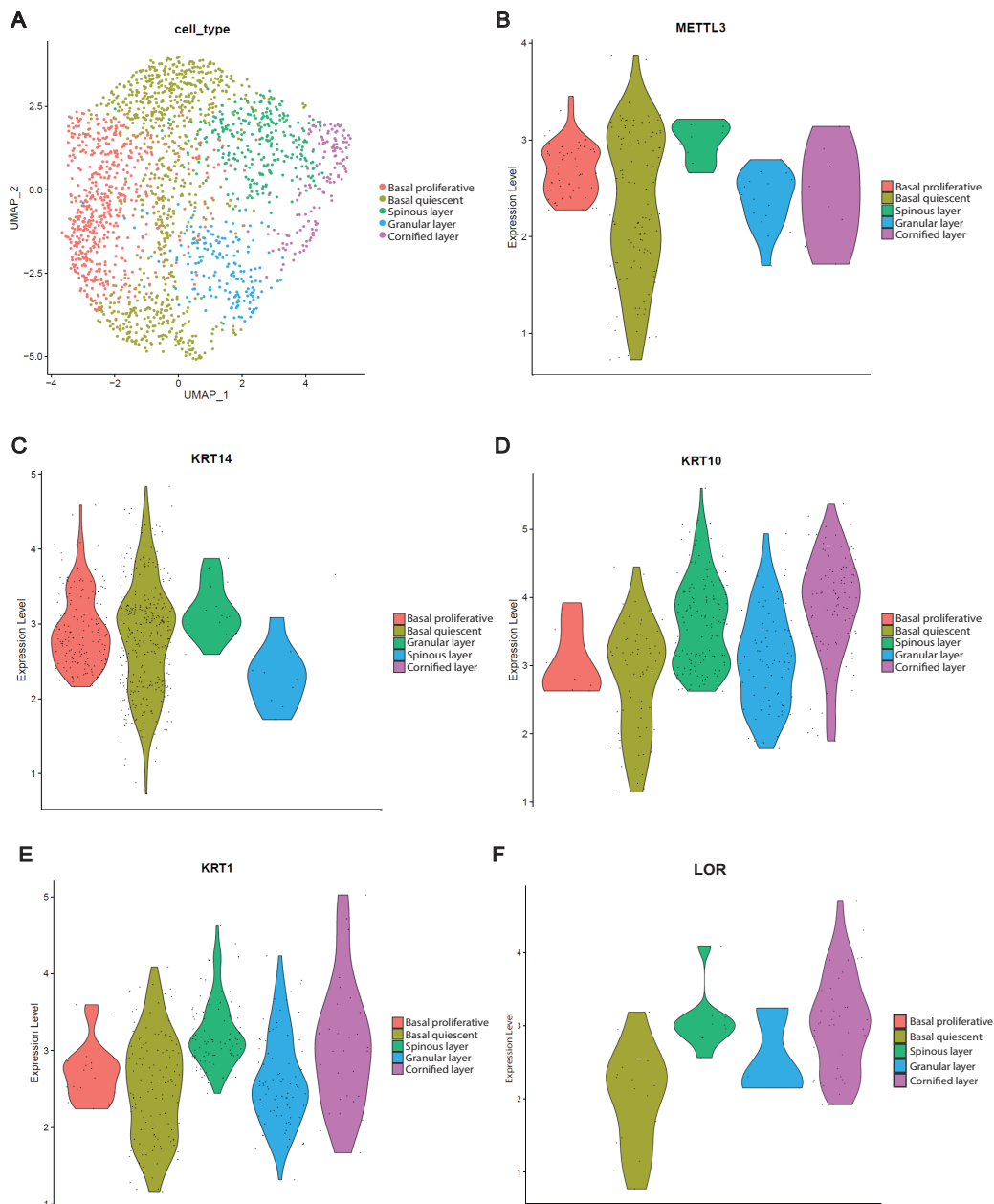


Fig. S3. Single-cell expression analysis of human epidermal keratinocytes. (A) Epidermal cell types identified in mice in a UMAP projection of scRNA-seq data (GTEx Portal dbGaP accession number phs000424.v9). Violin plots show gene expression in cells on the single-cell level. Each dot represents a single human keratinocyte. Statistically significant gene expression is observed only if a violin-shaped fitting area can be calculated. (B) *METTL3* expression is reduced in the more differentiated cornified layer in comparison to the basal and suprabasal layers of the epidermis similar to what is observed with *KRT14* (C). In contrast, *KRT10* (D), *KRT1* (E), and *LOR* (F) all display increased expression with differentiation.

Figure S4

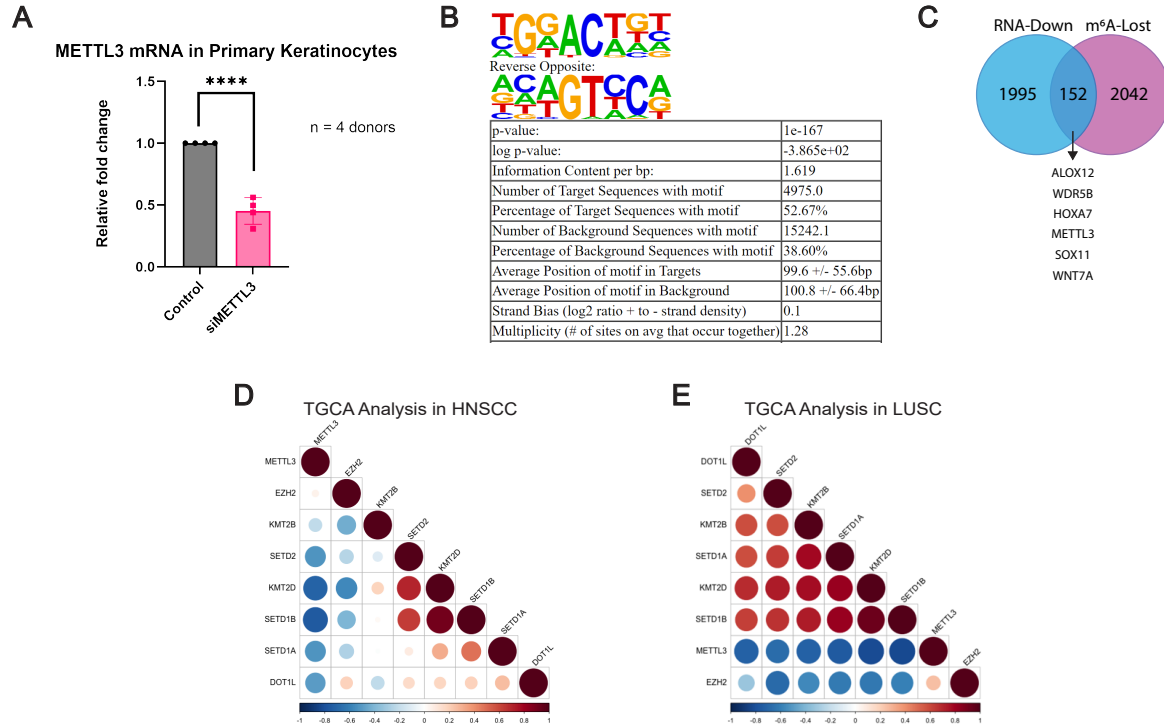


Fig. S4. METTL3-m⁶A dynamics in epithelial tissues. (A) RT-qPCR for METTL3 demonstrating knockdown with siMETTL3 treatment. (B) HOMER-identified enriched motifs for m⁶A peaks in proliferating epidermal progenitors (normal human epidermal keratinocytes, or NHEKs). (C) Overlap of transcripts that demonstrate a concomitant loss of mRNA expression by RNA-seq (blue) as well as a significant reduction in m⁶A enrichment by m⁶A-seq (purple) with depletion of Mettl3. TCGA analysis of Head and Neck Squamous Cell Carcinoma (HNSCC) (D) and Lung Squamous Cell Carcinoma (LUSC) (E) showing the inverse correlation of METTL3 expression and multiple histone methyltransferases.

Figure S5

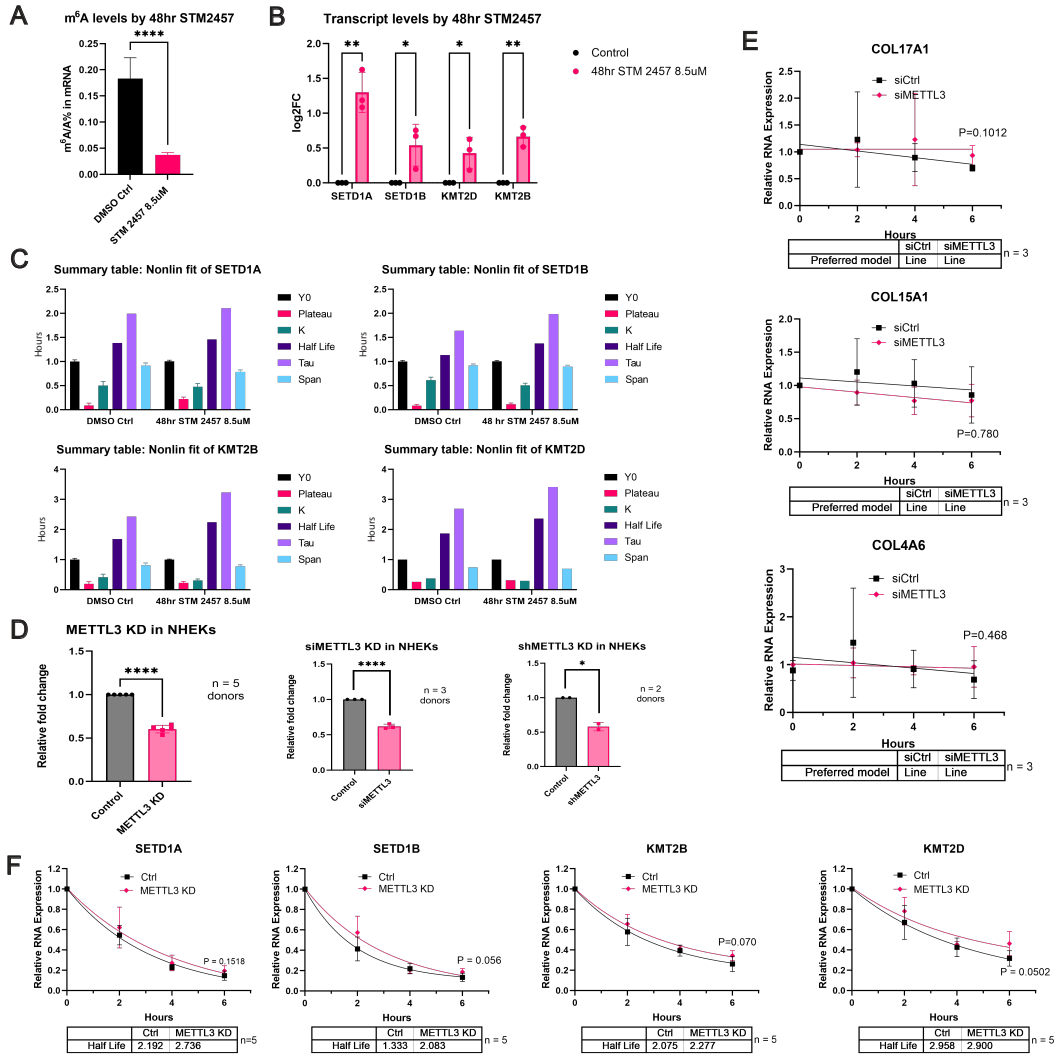


Fig. S5. mRNA half-life is dysregulated with Mettl3 loss. (A) Quantitative analysis of the m⁶A level by LC-MS/MS done in NHEKs demonstrates reduced global m⁶A with METTL3 catalytic inhibition by STM2457 treatment at 6 hr timepoint. (B) RT-qPCR was done in STM2457-treated NHEKs vs. DMSO vehicle control. A log₂ Fold Change of ± 0.5 and an adjusted p-value of <0.01 was done to visualize the significant changes that recapitulate what is seen in the bulk RNA-seq done in *Mettl3*-eKO epidermis. Transcripts were normalized by *GAPDH*. (C) Non-linear, one phase decay fit summary that was used to calculate mRNA half-life ($t_{1/2}$) of *SETD1A*, *SETD1B*, *KMT2B* and *KMT2D*. (D) RT-qPCR for METTL3 shows METTL3 knockdown when treated with siRNA or shRNA treatment (siMETTL3 KD and shMETTL3 KD were added to make METTL3-KD). (E) mRNA turnover assay to measure the mRNA half-life transcripts of *COL17A1*, *COL15A1* and *COL4A6* in NHEKs with METTL3 depletion show that the transcripts follow a linear model instead of a one phase decay model, meaning that the transcripts are stable and not undergoing mRNA degradation. Transcripts were normalized by *GAPDH*. (F) mRNA turnover assay to measure the mRNA half-life transcripts in epidermal progenitors demonstrates a trend in prolonged half-life of *SETD1A*, *SETD1B*, *KMT2B* and *KMT2D* transcripts with METTL3 depletion. Transcripts were normalized by *GAPDH*.

Figure S6

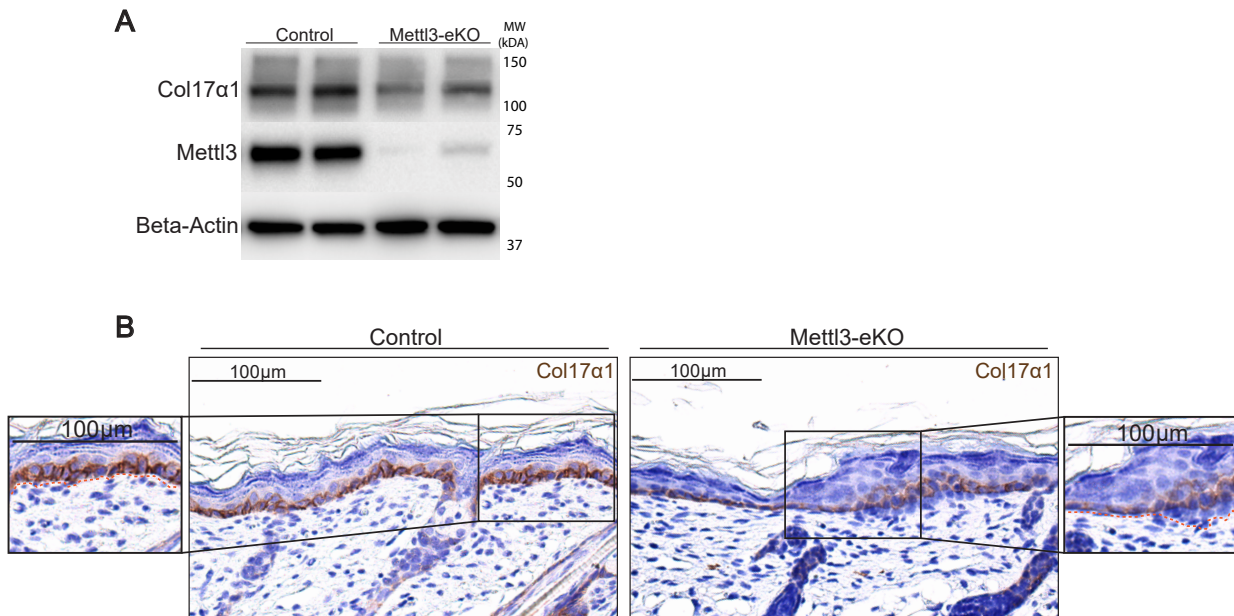


Fig. S6. Collagen 17 alpha 1 is decreased with deletion of *Mettl3*. (A) Western blot for Col17 α 1 in P6 mice epidermis. (B) IHC for Col17 α 1 in P6 mice epidermis.

Supplementary Tables

Antibodies

Antibody	Company	Catalog Number	Application
Mettl3	Abcam	ab195352	Immunoblot, IHC
Setd1a	Invitrogen	PA5-78298	Immunoblot, IHC
H3K4me2	Abcam	ab7766	IHC
H3K4me3	Abcam	ab8580	IHC
Cytokeratin 14	Abcam	ab7800	ICC, IF
Cytokeratin 10	Abcam	ab76318	IF
Ki67	Abcam	ab15580	IHC
Filaggrin	Abcam	ab81468	IHC
Beta-Actin	Cell Signaling	4970S	Immunoblot
Collagen XVII	Novus Biologicals	NBP2-67316	Immunoblot, IHC
m ⁶ A	Synaptic Systems	202003	ICC
Alexa Fluor 555	Invitrogen	A31570	ICC, IF
Alexa Fluor 488	Invitrogen	A21206	ICC, IF
Rb IgG HRP Linked	Cell Signaling	7074S	Immunoblot

siRNAs and shRNAs

Accell Human <i>METTL3</i> siRNA - SMARTpool	Catalog ID: E-005170-00-0020
Accell Non-targeting siRNA #1	Catalog ID: D-001910-01-20
Sigma <i>METTL3</i> shRNA	SKU: TRCN0000034717
Sigma pLKO.1-puro Non-Mammalian shRNA Control Plasmid DNA	SKU: SHC002
STM2457 - Selleck Chem	Catalog No: S9870

Primers (Genotyping and RT-PCR)

<i>Mettl3</i> (mouse)	F' 5-AAGTGCTGCCATGTGAATGA-3 R' 5-TAAAGTGGAAAGGGTCAGTC-3
<i>Keratin 14-Cre</i> (mouse)	F' 5-GAACCTGATGGACATGG-3 R' 5-AGTGCGTTCGAACGCTAGAGCCTGT-3
<i>METTL3</i> (human)	F' 5-CAAGCTGCACTTCAGACGAA-3 R' 5-GCTTGCGGTGTGGTCTTT-3
<i>SETD1A</i> (human)	F' 5-TTGCCATGTCAGGTCCAAAAA-3 R' 5-CGTACTIONTACGGCACATATCCTTC-3
<i>SETD1B</i> (human)	F' 5-AGCGAGCTCCAGAACATGAC-3 R' 5-ATGGGTGTGAGGCATCTGTG-3
<i>KMT2D</i> (human)	F' 5-ATCCTGGAGACACCCATCAG-3 R' 5-GACAGGCTCAGGGTCAGTG-3
<i>KMT2B</i> (human)	F' 5-GGAGGAAGCAGCAAGCAGTA-3 R' 5-GCTCAGGTTTGGGGATTGT-3
<i>COL17A1</i> (human)	F' 5-GCAGAGCTGAGTAGTCGCAT-3 R' 5-AATTCAGACCCTCGCAGCAA-3
<i>COL15A1</i> (human)	F' 5-CTGGGAGTCCAGAGCTCATC-3

	R' 5-ATCAAGTGGAGGACCTGGTG-3
<i>COL4A6</i> (human)	F' 5-AAAGGAGCCAGAGGAGATCG-3 R' 5-GAGGCCTCGAGACCCTTTAG-3
<i>Beta-Actin</i> (human)	F' 5-TGAAGTGTGACGTGGACATC-3 R' 5-GCAGGAGCAATGATCTTGAT-3
<i>GAPDH</i> (human)	F' 5-ATCATCCCTGCCTCTACTGG-3 R' 5-GTCAGGTCCACCACTGACAC-3

Data S1. (separate file)

Supplemental Data Table S1: RNA-seq data

Data S2. (separate file)

Supplemental Data Table S2: m⁶A-seq data