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Supplemental Information

KLF3 Mediates Epidermal Differentiation through the Epigenomic Writer CBP

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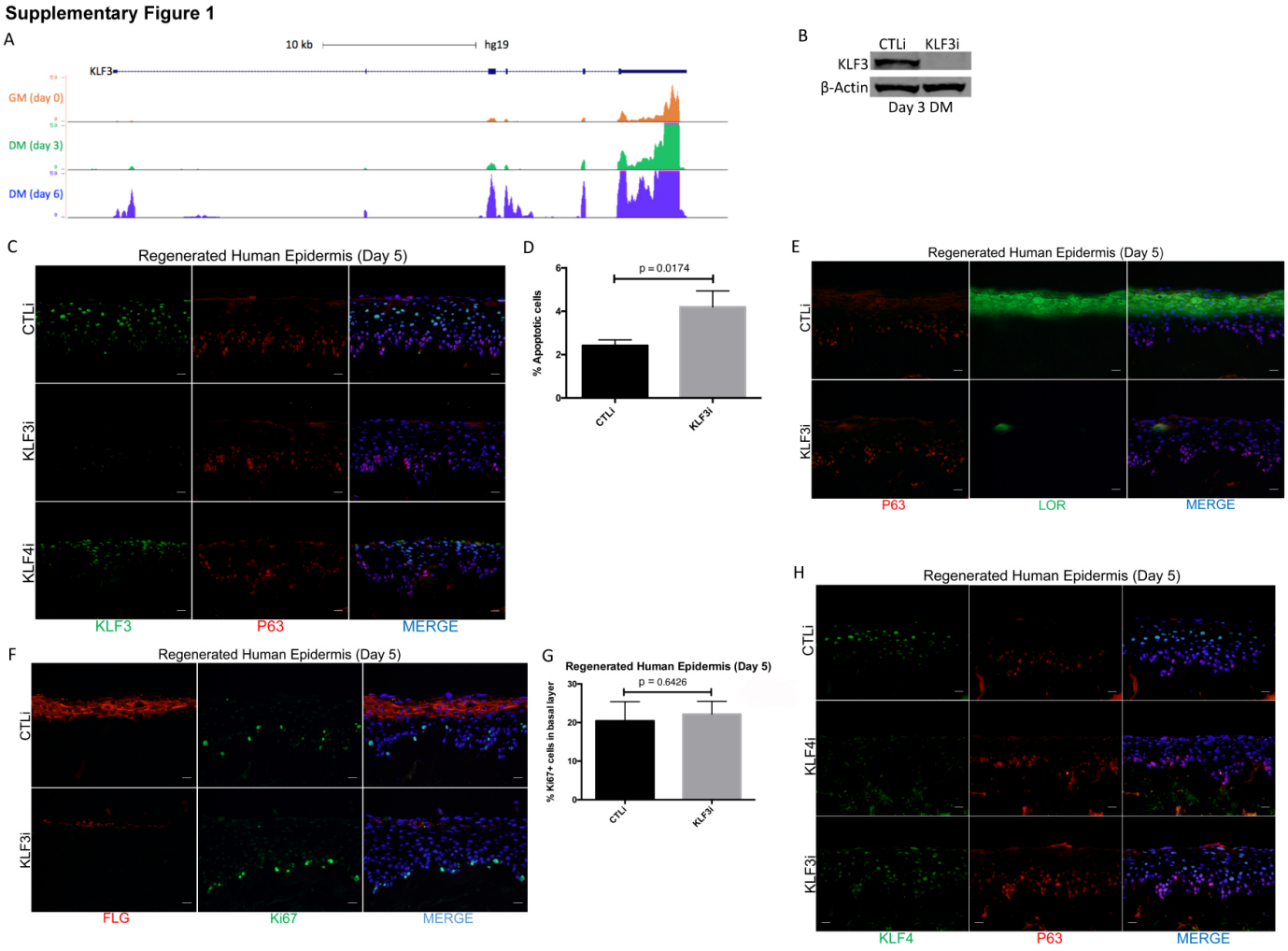


Figure S1. Increase in KLF3 expression is necessary for differentiation. Related to Figure 1
(A) UCSC Genome Browser tracks displaying representative RNA-sequencing data of KLF3 expression in growth media (GM, orange track) and differentiation media (DM, day 3:green track, day 6:blue track). **(B)** Western blot of KLF3 protein levels in day 3 differentiated keratinocytes treated with control (CTLi) or KLF3 targeting (KLF3i) siRNA. Representative image is shown, n=3. **(C)** Immunofluorescent staining of KLF3 (green) and P63 (red, a marker of the basal layer) in day 5 regenerated human epidermis treated with control (CTLi), KLF3 targeting (KLF3i), or KLF4 targeting (KLF4i) siRNAs. Merged image includes Hoechst staining of nuclei. n=3. Scale bar =20μm. **(D)** Annexin V staining was used to determine the percentage of apoptotic cells in CTLi and KLF3i cells cultured for 4 days. n=3, t-test. Data are graphed as the mean ± SD. **(E)** Immunofluorescent staining of late differentiation marker LOR (green) and basal layer marker P63 (red) in day 5 regenerated human epidermis treated with control (CTLi) or KLF3 targeting (KLF3i) siRNA. Merged image includes Hoechst staining of nuclei. n=3. Scale bar = 20μm. **(F)** Immunofluorescent staining of late differentiation marker FLG (red) and proliferation marker ki67 (green) in day 5 regenerated human epidermis treated with control (CTLi) or KLF3 targeting (KLF3i) siRNA. Merged image includes Hoechst staining of nuclei. n=3. Scale bar = 20μm. **(G)** Quantitation of the %KI67 positive cells in the basal layer. n=3, t-test. Mean values are shown with error bars=SD. **(H)** Immunofluorescent staining of KLF4 (green) and P63 (red, a marker of the basal layer) in day 5 regenerated human epidermis treated with control (CTLi), KLF4 targeting (KLF4i), or KLF3 targeting (KLF3i) siRNAs. Merged image includes Hoechst staining of nuclei. n=3. Scale bar =20μm.

Supplementary Figure 2

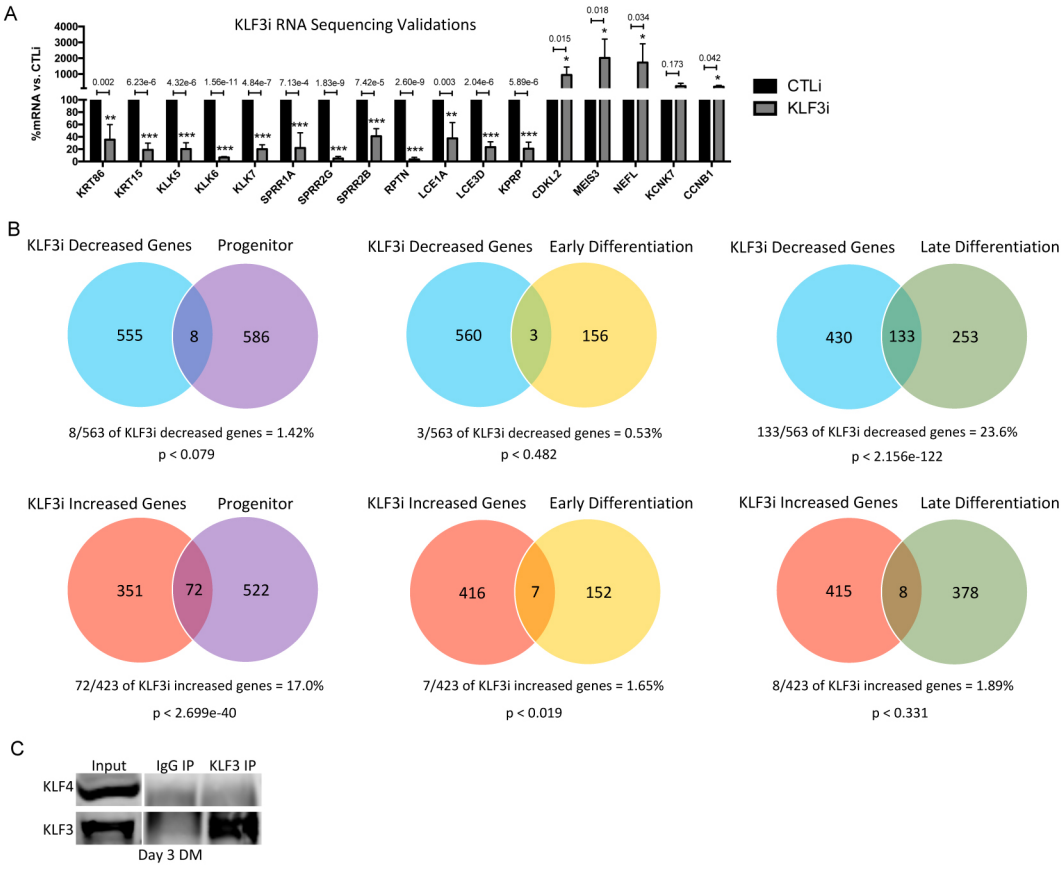


Figure S2. KLF3 is necessary to induce late differentiation gene expression and suppress progenitor genes. Related to Figure 2.

(A) RT-qPCR validating the RNA-Seq data from CTLi and KLF3i keratinocytes after three days of differentiation. Both increased and decreased genes upon KLF3 depletion identified from the RNA-Seq data were validated. N=4, statistics: t-test, *p < 0.05, ** p < 0.01, ***p < 0.001. Mean values are shown with error bars=SD. **(B)** Overlap of the 563 genes decreased upon KLF3 depletion with a previously published timecourse of epidermal differentiation which defined progenitor, early, and late differentiation genes (top panel). Overlap of the 423 genes increased upon KLF3 depletion with a previously published timecourse of epidermal differentiation which defined progenitor, early, and late differentiation genes (bottom panel). **(C)** A KLF3 antibody was used to immunoprecipitate (IP) KLF3 from day 3 differentiated primary human keratinocyte lysates. IgG pulldowns were used as a negative control. KLF3 or IgG immunoprecipitates were Western blotted for KLF4 or KLF3, n=3.

Supplementary Figure 3

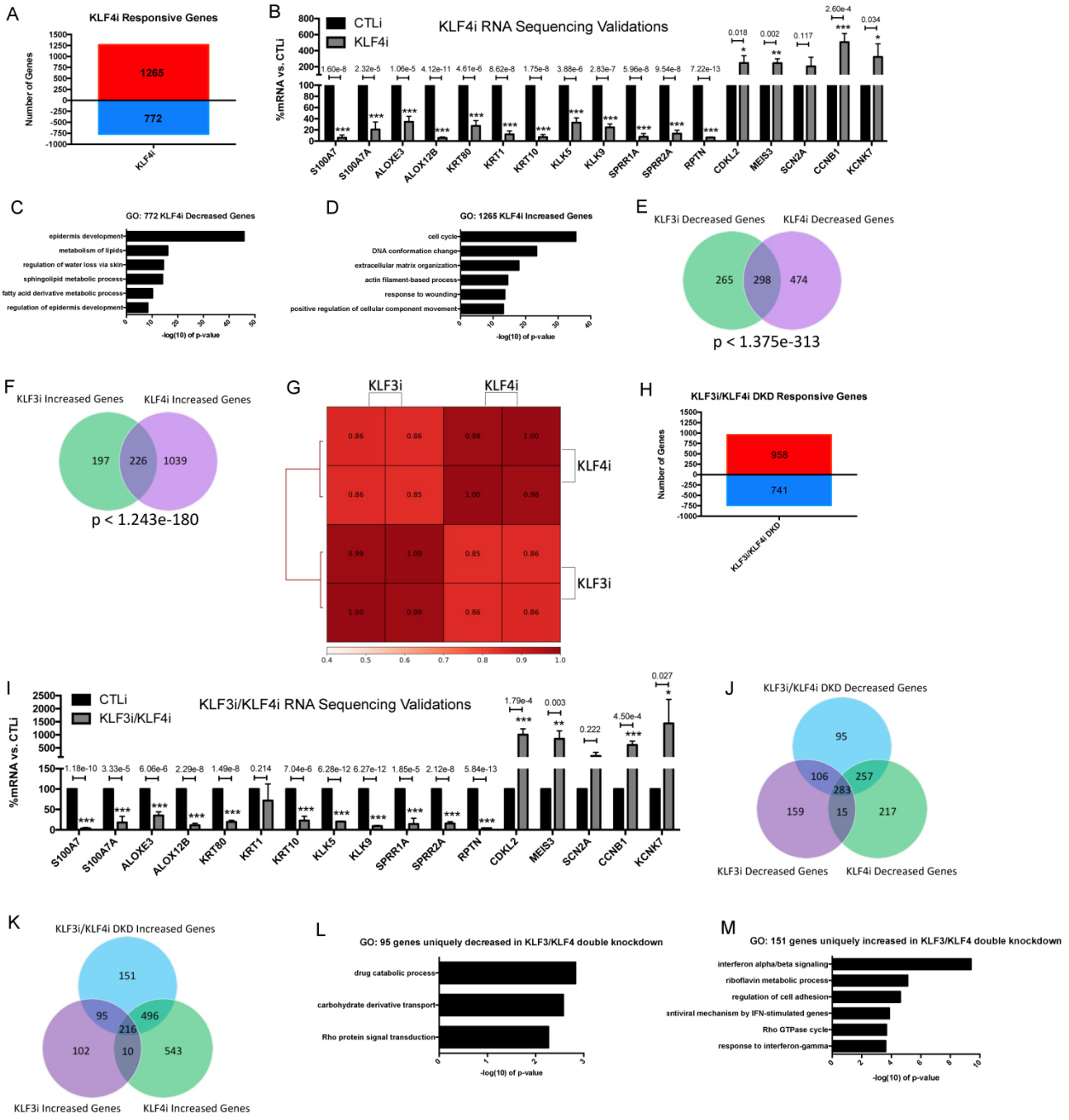


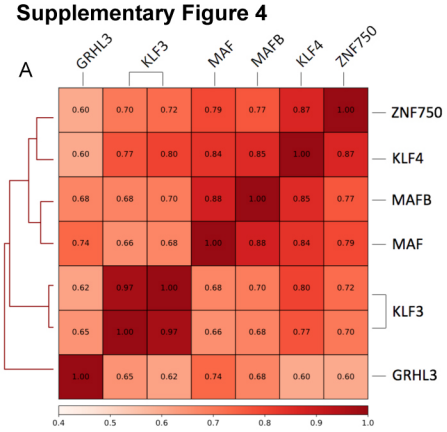
Figure S3. KLF3 and KLF4 are not redundant in the regulation of the epidermal differentiation gene expression program. Related to Figure 2.

(A) Histogram summarizing the gene expression changes identified by RNA Seq in KLF4 knockdown (KLF4i) keratinocytes when compared to controls (CTLi) after three days of differentiation. Number of increased genes is displayed in red, while decreased genes are shown in blue. Differentially expressed genes were selected by a FDR ≤ 0.05 and fold change ≥ 2 vs. CTLi, n=2. **(B)** RT-qPCR validating the RNA-Seq data from CTLi and KLF4i keratinocytes after three days of differentiation. Both increased and decreased genes upon KLF4 depletion identified from the RNA-Seq data were validated. N=4, statistics: t-test, *p < 0.05, ** p < 0.01, ***p < 0.001. Mean values are shown with error bars=SD. **(C)** Gene ontology (GO) term enrichment for the 772 genes significantly decreased in expression upon KLF4 knockdown. **(D)** Gene ontology term enrichment for the 1265 genes significantly increased in expression upon KLF4 knockdown. **(E)** Venn diagram showing the number of shared and uniquely decreased genes

in the KLF3i and KLF4i gene signatures. Overlap significance in the Venn diagram was calculated using hypergeometric distribution p-values. **(F)** Venn diagram showing the number of shared and uniquely increased genes in the KLF3i and KLF4i gene signatures. **(G)** Heatmap plot of Pearson correlation coefficients between replicate KLF3i and KLF4i RNA sequencing data sets (RPKM normalized). **(H)** Histogram summarizing the gene expression changes identified by RNA Seq in KLF3/KLF4 double knockdown (DKD) keratinocytes when compared to controls (CTLi) after three days of differentiation. Increased gene counts are displayed in red, while decreased genes are shown in blue. n=2. **(I)** RT-qPCR validating the RNA-Seq data from CTLi and KLF3/KLF4 double knockdown keratinocytes after three days of differentiation. Both increased and decreased genes upon KLF3/KLF4 depletion identified from the RNA-Seq data were validated. N=4, statistics: t-test, *$p < 0.05$, ** $p < 0.01$, ***$p < 0.001$. Mean values are shown with error bars=SD. **(J)** Venn diagram showing the number of unique and shared decreased genes in the KLF3i/KLF4i double knockdown versus the KLF3i and KLF4i gene signatures. **(K)** Venn diagram showing the number of unique and shared increased genes in the KLF3i/KLF4i double knockdown versus the KLF3i and KLF4i gene signatures. **(L)** Gene ontology term enrichment for the 95 genes uniquely decreased in the KLF3i/KLF4i double knockdown condition. **(M)** Gene ontology term enrichment for the 151 genes uniquely increased in the KLF3i/KLF4i double knockdown condition.

Supplementary Figure 4

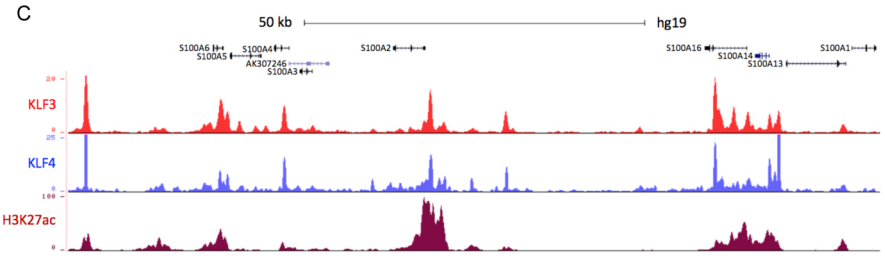
A



B

ChIP	Total Peaks	Shared Peaks with KLF3	% of peaks
KLF4	43408	15203	35.0
ZNF750	53922	10552	19.6
MAF	3521	856	24.3
MAFB	6489	2946	45.4
GRHL3	4383	357	8.2

C



D

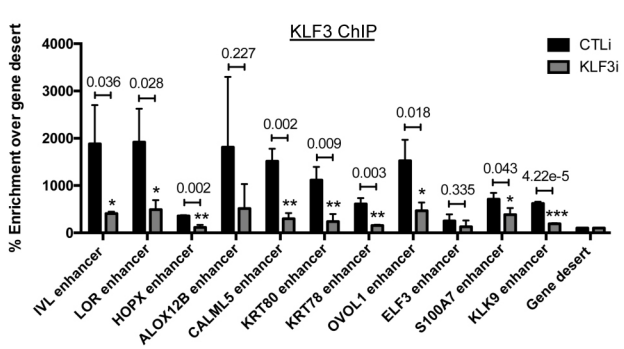


Figure S4. KLF3 and epidermal lineage determining transcription factors bind to similar regions in the genome. Related to Figure 3.

(A) Heatmap plot of Pearson correlation coefficients between KLF3 ChIP Seq data and ChIP Seq data for the epidermal LDTFs KLF4, ZNF750, MAF, MAFB, and GRHL3 (RPKM normalized). **(B)** Table displaying the peak overlap between KLF3 and each epidermal LDTFs. Each LDTFs total bound peak numbers identified by HOMER are shown with the number and percentage of overlap with KLF3 peaks. **(C)** UCSC genome browser track displaying KLF3 (red) and KLF4 (blue) ChIP Seq profiles near a cluster of differentiation associated S100 genes. H3K27ac (maroon) is included to represent open chromatin. **(D)** ChIP-qPCR of KLF3 pulldown in CTLi and KLF3i keratinocytes (day 3 differentiation). Each pulldown was normalized by calculating pulldown efficiency as a percent enrichment over its gene desert value. n=3, t-test, *p < 0.05, ***p < 0.001. Mean values are shown with error bars=SD.

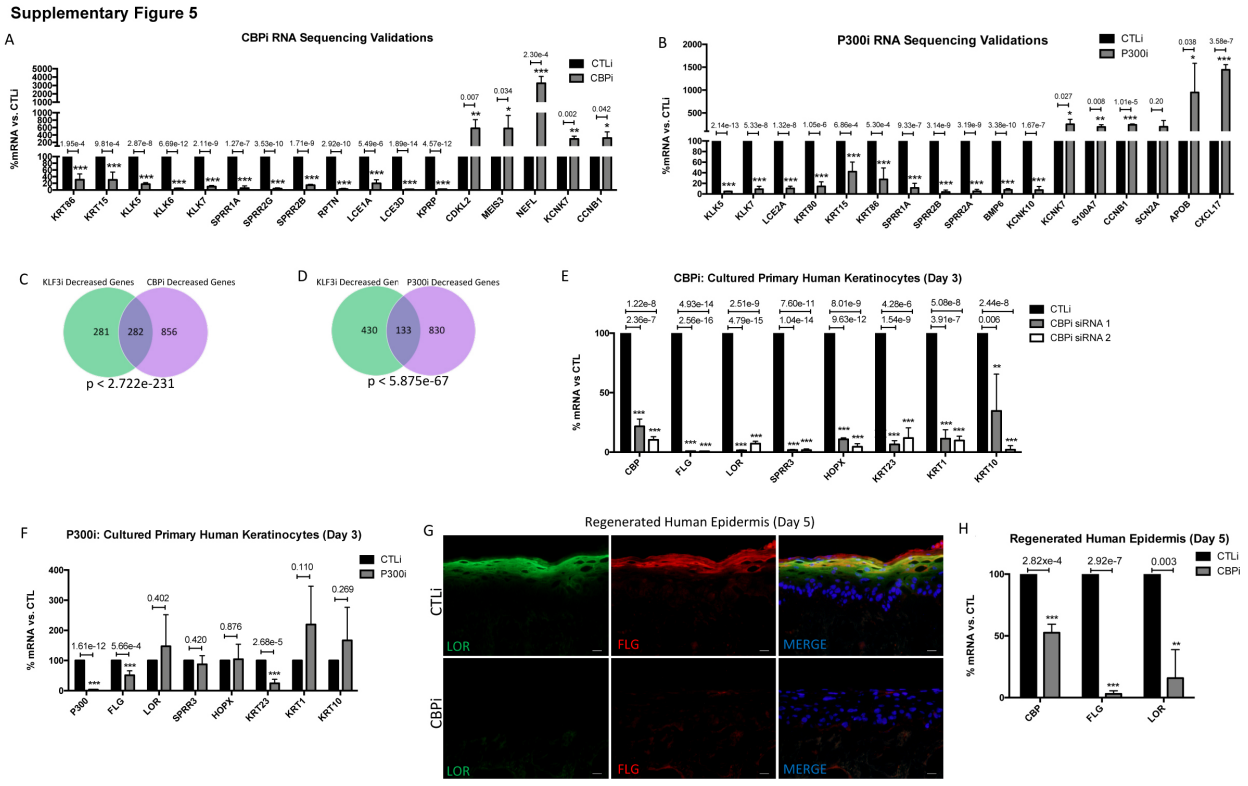
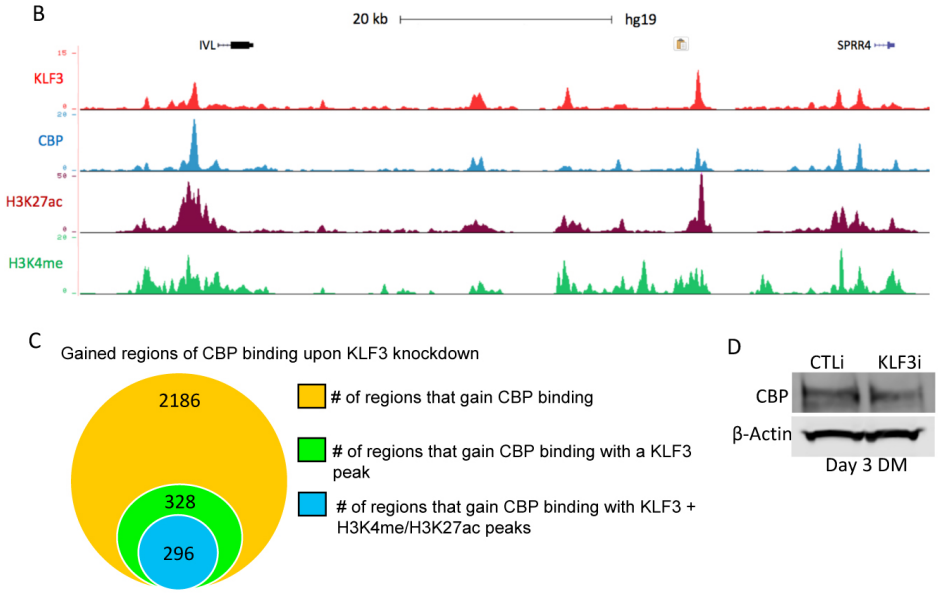


Figure S5. CBP is necessary for human epidermal differentiation. Related to Figure 4.
(A) RT-qPCR validating the RNA-Seq data from CTLi and CBPi keratinocytes after three days of differentiation. Both increased and decreased genes upon CBP depletion identified from the RNA-Seq data were validated. N=4, statistics: t-test, *p < 0.05, ** p < 0.01, ***p < 0.001. Mean values are shown with error bars=SD. **(B)** RT-qPCR validating the RNA-Seq data from CTLi and P300i keratinocytes after three days of differentiation. Both increased and decreased genes upon P300 depletion identified from the RNA-Seq data were validated. N=4, statistics: t-test, *p < 0.05, ** p < 0.01, ***p < 0.001. Mean values are shown with error bars=SD. **(C)** Venn diagram showing the number of shared and uniquely decreased genes in the KLF3i and CBPi gene signatures. Overlap significance in the Venn diagram was calculated using hypergeometric distribution p-values. **(D)** Venn diagram showing the number of common and uniquely decreased genes in the KLF3i and P300i gene signatures. **(E)** RT-qPCR quantifying the relative mRNA levels of a panel of epidermal differentiation genes in CTLi and CBPi keratinocytes after three days of differentiation. Two separate siRNAs (siRNA 1 and siRNA 2) targeting different regions of CBP were used, n=4. Data are graphed as the mean ± SD. Statistics: t-test, *p < 0.05, ** p < 0.01, ***p < 0.001. **(F)** RT-qPCR quantifying the relative mRNA levels of a panel of epidermal differentiation genes in CTLi and P300i keratinocytes after three days of differentiation, n=4. Data are graphed as the mean ± SD. Statistics: t-test, *p < 0.05, ** p < 0.01, ***p < 0.001. **(G)** Immunofluorescent staining of late differentiation markers LOR (green) and FLG (red) in day 5 regenerated human epidermis treated with control (CTLi) or CBP targeting (CBPi) siRNAs. Merged image includes Hoechst staining of nuclei, n=3. Scale bar = 20μm**. (H)** RT-qPCR quantifying the relative mRNA expression levels of LOR and FLG in CTLi and CBPi day 5 regenerated human epidermis, n=3. Data are graphed as the mean ± SD. Statistics: t-test, *p < 0.05, ** p < 0.01, ***p < 0.001.

Supplementary Figure 6

A

ChIP	Total Peaks	Shared Peaks with KLF3	% of peaks
CBP	24357	8461	34.74



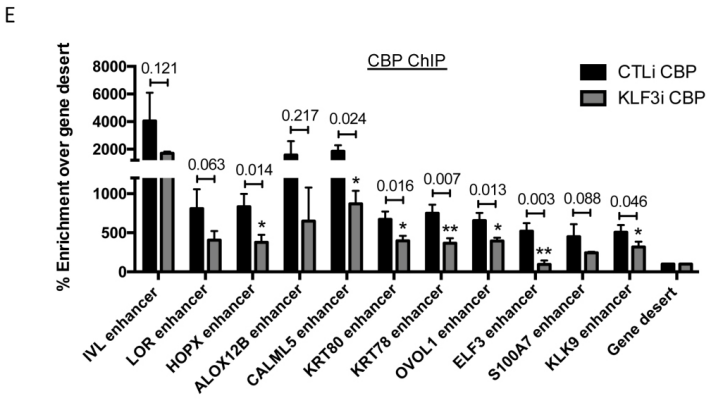


Figure S6. CBP and KLF3 directly overlap at ~35% of their binding sites in the genome. Related to Figure 5. **(A)** Table displaying the peak overlap between KLF3 and CBP. The total CBP bound peak numbers identified by HOMER are shown with the number and percentage of overlap with KLF3 peaks. **(B)** UCSC genome browser tracks displaying KLF3 (red) and CBP (blue) ChIP Seq profiles near the differentiation genes IVL and SPRR4. H3K27ac (maroon) and H3K4me (green) are included to represent open and active chromatin. **(C)** Summary of significant CBP ChIP signal enrichment events in day 3 differentiated keratinocytes knocked down for KLF3. The orange circle represents the total number of regions in the genome that gain CBP binding upon KLF3 knockdown. The green circle represents the number of regions that gain CBP binding that occur at a KLF3 peak upon KLF3 depletion. The blue circle represents the regions that gain CBP binding which also contain KLF3, H3K4me, and H3K27ac binding upon KLF3 knockdown. CBP ChIP Seq was performed in replicates in CTLi and KLF3i cells. Significant signal changes were identified by Diffreps. **(D)** Western blot of CBP protein levels in CTLi and KLF3i keratinocytes (day 3 differentiation) with B-Actin as a loading control. n=3 **(E)** ChIP-qPCR of CBP pulldown in CTLi and KLF3i keratinocytes (day 3 differentiation). Each pulldown was normalized by calculating pulldown efficiency as a percent enrichment over its gene desert value. n=3. Data are graphed as the mean ± SD. Statistics: t-test, *p < 0.05, ** p < 0.01, ***p < 0.001.

Supplementary Figure 7

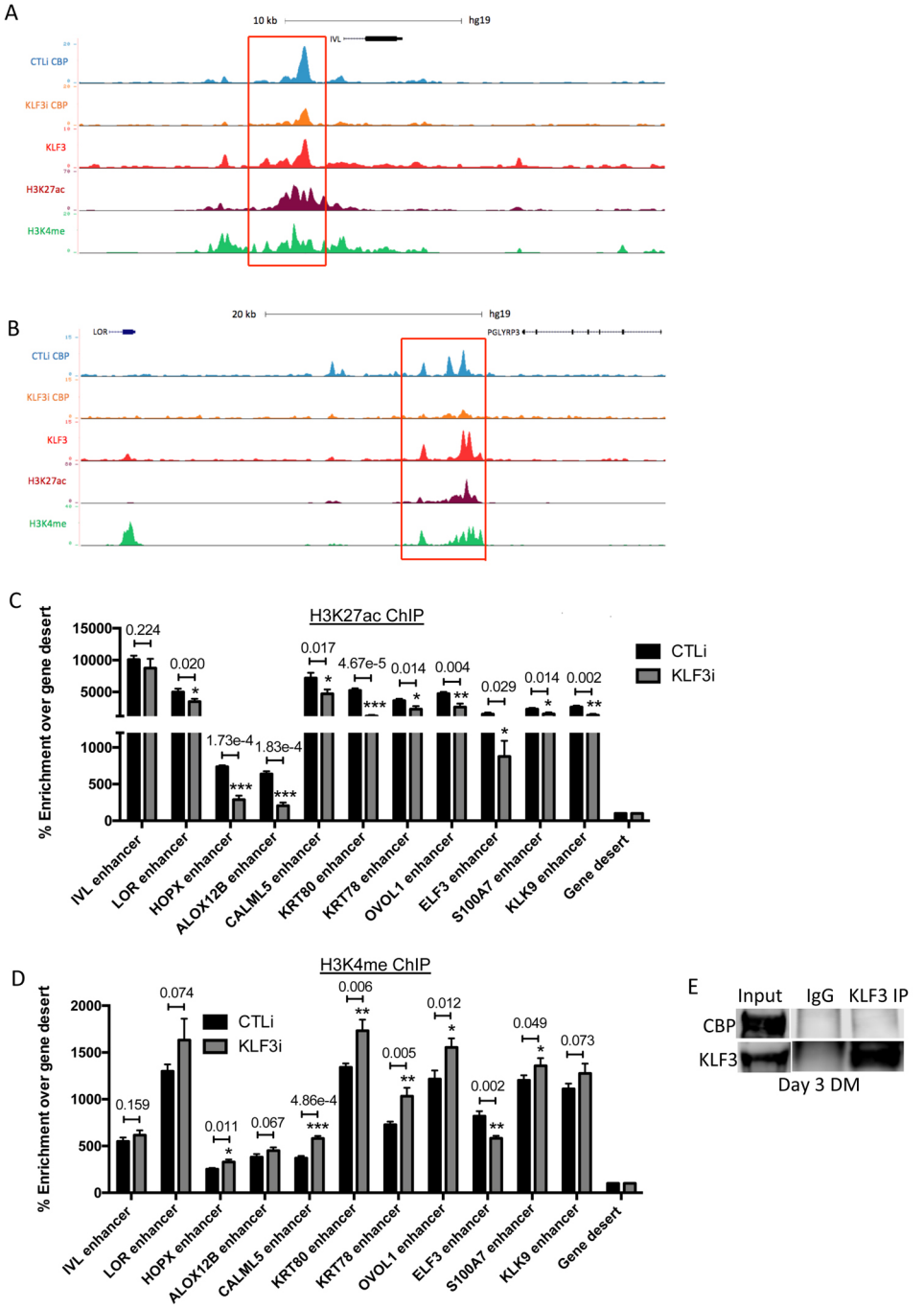


Figure S7. KLF3 is necessary for CBP localization to genomic sites. Related to Figure 6.
(A) UCSC genome browser tracks displaying CBP binding (CBP ChIP Seq profiles) from CTLi (blue) and KLF3i (orange) cells, near differentiation gene *IVL*. KLF3 (red), H3K27ac (maroon), and H3K4me (green) binding are also shown. **(B)** UCSC genome browser tracks displaying CBP binding (CBP ChIP Seq profiles) from CTLi (blue) and KLF3i (orange) cells, near differentiation genes *PGLYRP3* and *LOR*. KLF3 (red), H3K27ac (maroon), and H3K4me (green) binding are also shown. **(C)** ChIP-qPCR of H3K27ac pulldown in CTLi and KLF3i keratinocytes (day 3 differentiation). Each pulldown was normalized by calculating pulldown efficiency as a percent enrichment over its gene desert value. n=3. Data are graphed as the mean ± SD. Statistics: t-test, *p < 0.05, ** p < 0.01, ***p < 0.001. **(D)** ChIP-qPCR of H3K4me pulldown in CTLi and KLF3i keratinocytes (day 3 differentiation). Each pulldown was normalized by calculating pulldown efficiency as a percent enrichment over its gene desert value. n=3. Data are

graphed as the mean ± SD. Statistics: t-test, *p < 0.05, ** p < 0.01, ***p < 0.001. **(E)** A KLF3 antibody was used to immunoprecipitate (IP) KLF3 from day 3 differentiated primary human keratinocyte lysates. IgG pulldowns were used as a negative control. KLF3 or IgG immunoprecipitates were Western blotted for CBP or KLF3, n=3.

TRANSPARENT METHODS

Cell Culture

Primary human epidermal keratinocytes derived from human neonatal foreskin were used for all cell culture studies. These cells were primarily cultured in EpiLife medium (ThermoFisher MEPI500CA), which was treated with penicillin and streptomycin (HyClone SV30010), as well as human keratinocyte growth supplement (HKGS, ThermoFisher S0015). The differentiation of primary keratinocytes was carried out by plating the cells to full confluence and adding 1.2 mM calcium (Sen et al., 2012).

siRNA mediated knockdown

Lipofectamine RNAiMAX (ThermoFisher 13778) was used to transfect siRNAs into keratinocytes. 25ul of RNAiMAX was used for each 10cm plate transfection, and 50ul was used for each 15cm plate transfection. The siRNAs were mixed with RNAiMAX in Epilife media for 5 minutes and then added to plates of subconfluent keratinocytes at a final concentration of 10nM. Transfection was carried out for 18 hours at 37 degrees Celsius. The siRNAs used are as follows: Control siRNA (Ambion Silencer Select negative control 4390844), KLF3 siRNA1 (Ambion Silencer Select s229899), KLF3 siRNA 2 (Ambion Silencer Select s229898), KLF4 siRNA (Dharmacon:custom sequence), CBP siRNA 1 (Dharmacon D-003477-21), CBP siRNA 2 (Dharmacon D-003477-18), P300 siRNA (Dharmacon D-003486-02). The sequences of these siRNAs can be found in the supplementary materials.

Regenerated human epidermis

Human dermis for regeneration experiments was acquired from the New York Firefighters skin bank. Upon arrival, the dermis was separated from the epidermis. In order to create the three-dimensional constructs, the dermis was sized, and placed in a cassette. The bottom of the dermis was then coated in Matrigel. One million keratinocytes were seeded onto the dermis for each construct, and this construct was placed in KGM media with the keratinocytes above the liquid interface. They were then cultured for five days to allow full stratification. The constructs were then subjected to RNA extraction or placed in OCT for sectioning and staining (Li and Sen, 2015; Mistry et al., 2012; Noutsou et al., 2017).

Immunofluorescent staining

Tissue sections were fixed with 10% formalin solution (Sigma HT5012) for 12 minutes and then blocked for 30 minutes with PBS containing 2.5% normal goat serum, 2% bovine serum albumin, and 0.3% triton X-100. Sections were then stained with primary antibodies in the same blocking buffer for 1 hour. The following antibodies were for IF staining: KLF3 at 1:300 (Sigma HPA049512), KRT10 at 1:400 (ThermoFisher MS-611-P0), FLG at 1:200 (Abcam ab3137), LOR at 1:400 (Abcam ab198994), KLF4 at 1:1000 (Cell Signaling 4038P), Ki67 at 1:300 (Abcam ab15580), P63 at 1:500 (mouse, Biolegend 687203), P63 at 1:600 (rabbit, Abcam ab124762). The secondary antibodies Alexa Fluor 555 goat anti-mouse IgG (ThermoFisher: A21424) and Alexa Fluor 488 donkey anti-rabbit IgG (ThermoFisher: A21206) were then added to blocking buffer at 1:500 and applied for 30 minutes . Hoechst 33342 (ThermoFisher H3570) was used at 1:1000 to label cell nuclei.

KLF3 quantification in human skin sections

KLF3 immunofluorescent intensity in human skin sections was quantified using ImageJ. 15 cells/layer per sample were analyzed, resulting in the analysis of 45 total cells per layer across n=3 samples. KLF3 intensity was plotted relative to the basal layer signal.

Ki67 quantification in regenerated human epidermis

Ki67 signal in the basal layer of regenerated human epidermis samples was quantified by counting the number of Ki67 positive cells relative to the total amount of cells in the basal layer. The basal layer was considered to be the bottom two rows of cells from each section. This quantification was performed for n=3 control (CTLi) and KLF3 knockdown (KLF3i) regenerated human epidermis samples. 3 different sections from each sample were used for quantification. Cell counting was performed with ImageJ.

H&E staining
Regenerated human epidermis was sectioned and fixed with 10% formalin solution (Sigma HT5012) for 12 minutes. These sections were then treated with 0.25% Triton-X-100 in PBS for 5 minutes. Sections were then stained with Hematoxylin (Vector H-3401) for 8 minutes, followed by a water dip, and then an acid alcohol dip (1% HCL in 70% ethanol). The sections were then rinsed, put into 0.2% ammonia water for 1 minute. and then rinsed with water once more. This was followed by dipping in 95% ethanol. Eosin (Richard-Allan Scientific 71304) staining was then done for 30 seconds, with a subsequent 95% ethanol dip for 1 minute. Sections were then dipped in 100% ethanol for 4 minutes and Xylene for 2 minutes.

RNA extraction and RT-qPCR
RNA was extracted from keratinocytes with the GeneJET RNA purification kit (Thermo Scientific K0732). Nanodrop was used to measure RNA concentration. The Maxima cDNA synthesis kit (Thermo Fisher: K1642) was used to generate cDNA for each RNA sample. The Roche 480 Light Cycler was used to run qPCR on the generated cDNA samples. L32 or GAPDH (housekeeping genes) were used for normalization of qPCR data. Primer sequences for all reported genes can be found in the supplementary materials.

Western blotting
IP samples or 20-80ug of cell lysates were loaded onto 4-12% Bis-Tris (ThermoFisher NW04122BOX) or 3-8% Tris-acetate (ThermoFisher EA03752BOX) gels and transferred to PVDF membranes. Membranes were blocked in 5% BSA in TBS. Membranes were exposed to primary antibodies in blocking buffer overnight at 4 degrees. The following primary antibodies were used: KLF3 at 1:1000 (Sigma HPA049512), CBP at 1:1000 (Cell Signaling D6C5 #7389), KLF4 at 1:1000 (Cell Signaling 4038P). The loading controls Beta-Actin (Santa Cruz sc-47778) and Beta-Tubulin (Santa Cruz sc-9104) were used at 1:5000. The secondary antibodies used were donkey anti-rabbit IRDye 680RD (Li-Cor 926-68073) and donkey anti-mouse IRDye 800CW (Li-Cor 926-32212) at 1:5000.

RNA sequencing and analysis
Keratinocytes knocked down by siRNA were differentiated for 3 days and used for RNA extraction and sequencing. Sequencing was performed on the Illumina Hi Seq 4000, carried out by the Institute of Genomic Medicine core facility at UC San Diego. Sequenced reads were aligned with STAR (default settings, hg19). Partek Genomic Suite (Partek Incorporated, http://www. partek.com/partek-genomics-suite) was used for downstream differential gene expression analysis, and genes with differential expression were identified by ANOVA. In order to remove genes with minimal expression across all samples from the analysis, those genes with fewer than ten reads in all samples were filtered out. The statistical threshold for identifying differentially expressed genes was a $\geq$ 2-fold change (+/-) compared to control samples, and a significant p-value with FDR (< 0.05). Partek Genomic Suite was also used to generate heatmaps representing the relative expression of genes between samples. Metascape was used to generate gene ontology (GO) terms for lists of differentially expressed genes (Zhou et al., 2019). RPKM normalization and Pearson Correlation coefficients (and the associated heatmaps for these values) were generated using DeepTools (Ramirez et al., 2014; Ramirez et al., 2016). Nemates.org was used to calculate the p-value of gene list overlaps: (http://nemates.org/MA/progs/overlap_stats.html). The number of reference genes used was 25000.

ChIP sequencing and analysis
20 million differentiated (day 3) keratinocytes were used for each individual immunoprecipitation. The cells were crosslinked with 1% formaldehyde (ThermoFisher 28908) and 2mM DSG (disuccinimidyl glutarate, Thermo Fisher 20593). The cells were treated with Farnham lysis buffer (5 mM PIPES pH 8.0, 85 mM KCl, 0.5% IGEPAL CA-630) and sheared with a syringe to assist lysis. After shearing, the cells were spun down and then resuspended in SDS-Lysis Buffer (1% SDS, 10 mM EDTA, 50 mM Tris, pH 8.0). Once resuspended, the cells were sonicated with a water bath sonicator. Once the appropriate fragment size was achieved, the lysate was centrifuged, and the supernatant was then diluted 1:10 in low ionic strength ChIP dilution buffer (50mM NaCl, 10mM HEPES, pH 7.4, 1% IGEPAL CA-630, 10% Glycerol) to reduce SDS concentration. Antibody was then added to this diluted lysate and incubated overnight at 4 degrees Celsius. 5ug of KLF3 antibody (Sigma HPA049512), 20ul of CBP antibody (Cell Signaling D6C5 #7389), 3ug of H3K27ac antibody (Active Motif 39133), and 2ug of H3K4me antibody (Abcam ab8895) were used for each respective pulldown. 50ul of Protein-G dynabeads were added to each sample and rotated for an additional 4 hours at 4 degrees Celsius. Washes were then carried out to reduced non-specific binding, including two

washes with low ionic strength buffer, one wash with high salt buffer (500 mM NaCl, 0.1% SDS, 1% IGEPAL CA-630, 2 mM EDTA, 20 mM Tris, pH 8.0), one LiCl wash (0.25 M LiCl, 1% IGEPAL CA-630, 1% Sodium Deoxycholate, 1 mM EDTA 10 mM Tris-Hcl, pH 8.0), and two washes with TE (10 mM Tris-Cl, pH 7.5, 1 mM EDTA). The beads were then placed in elution buffer (0.09 M $NaHCO_3$, 1% SDS, 0.1 M $NaHCO_3$) for 1 hour at 65 degrees, and the supernatant was isolated. This was treated with RNAse A for 30 minutes at 37 degrees to prevent RNA contamination during sequencing (Mistry et al., 2014; Noutsou et al., 2017). Finally, de-crosslinking mixture (0.2 M NaCl, 0.1M EDTA,0.4 M Tris-HCl, pH6.8, 0.4 mg/ml proteinase K) was added and samples were incubated at 65 degrees overnight, followed by DNA purification. These samples were sequenced using the Illumina Hi Seq 4000 by the Institute of Genomic Medicine core facility at UC San Diego. Reads were trimmed and aligned to hg19 using BowTie 2 (Langmead and Salzberg, 2012). Duplicate and low-quality reads were filtered out. HOMER's findPeaks was used with default settings (p-value < 0.0001, FDR < 0.001, 4x enrichment vs. input) and the following options to identify significant peaks: for KLF3, CBP, KLF4, ZNF750, MAF, MAFB, and GRHL3: style=factor, minDist 200; for H3K27ac and H3K4me: style=region, size=1000, minDist=2500)(Heinz et al., 2010). Sequenced Input sample was used as background to identify significant enrichment. Peaks were mapped to nearby genes using HOMER's annotatePeaks (Refseq hg19 transcription start sites)(Heinz et al., 2010). Motif enrichment for each ChIP was analyzed using HOMER's findMotifsGenome command. UCSC genome browser tracks for each sample were generated using HOMER's makeUCSCfile command. Other analyses by HOMER included normalized mean density plots (annotatePeaks), and the identification of directly overlapping (d=given) peaks between samples using mergePeaks. RPKM normalization and Pearson Correlation coefficients (as well as the associated heatmaps for these values) were generated using DeepTools (Ramirez et al., 2014; Ramirez et al., 2016). Diffreps 1.55.4 was used to identify differential enrichment between control and knockdown sequencing samples (negative binomial test, scanning window size of 1000 bp, step size of 100 bp, and a cutoff p-value of 0.0001)(Shen et al., 2013). Metascape was used to generate gene ontology (GO) terms for sets of genes (Zhou et al., 2019). Nemates.org was used to calculate the p-value of gene list overlaps: (http://nemates.org/MA/progs/overlap_stats.html). The number of reference genes used was 25000.

Co-immunoprecipitation

Differentiated keratinocytes were harvested in IP lysis buffer (25 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% NP-40 and 5% glycerol) and sheared with a syringe. 5ug of KLF3 antibody (Sigma HPA049512) or control rabbit IgG (Millipore 12-370) was conjugated to 50ul of Protein G dynabeads (Life Technologies 10004D) for 30 minutes at room temperature. Lysis buffer was diluted to 1ml per sample and was added to the antibody conjugated beads and incubated overnight at 4 degrees. The following day the beads were washed with IP lysis buffer and boiled in RIPA buffer (25 mM Tris-HCl (pH 7.6), 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS) supplemented with NuPAGE LDS Sample Buffer (Life Technologies: NP0008) to elute. Samples were then loaded for western blotting.

Apoptosis Assay

Control and KLF3 knockdown cells were stained with Annexin V conjugated to Alexa Fluor 488 (Life Technologies: A13201) and analyzed using the Guava flow cytometer (Millipore) according to manufacturers instructions (Li et al., 2020).

Statistics

Statistics were generated using GraphPad Prism. Histogram data are graphed as the mean ± SD and the significance of data was determined by student's t tests.

Accession numbers: RNA-Seq and ChIP-Seq has been deposited with GEO accession number: GSE142225

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