Supplemental Information

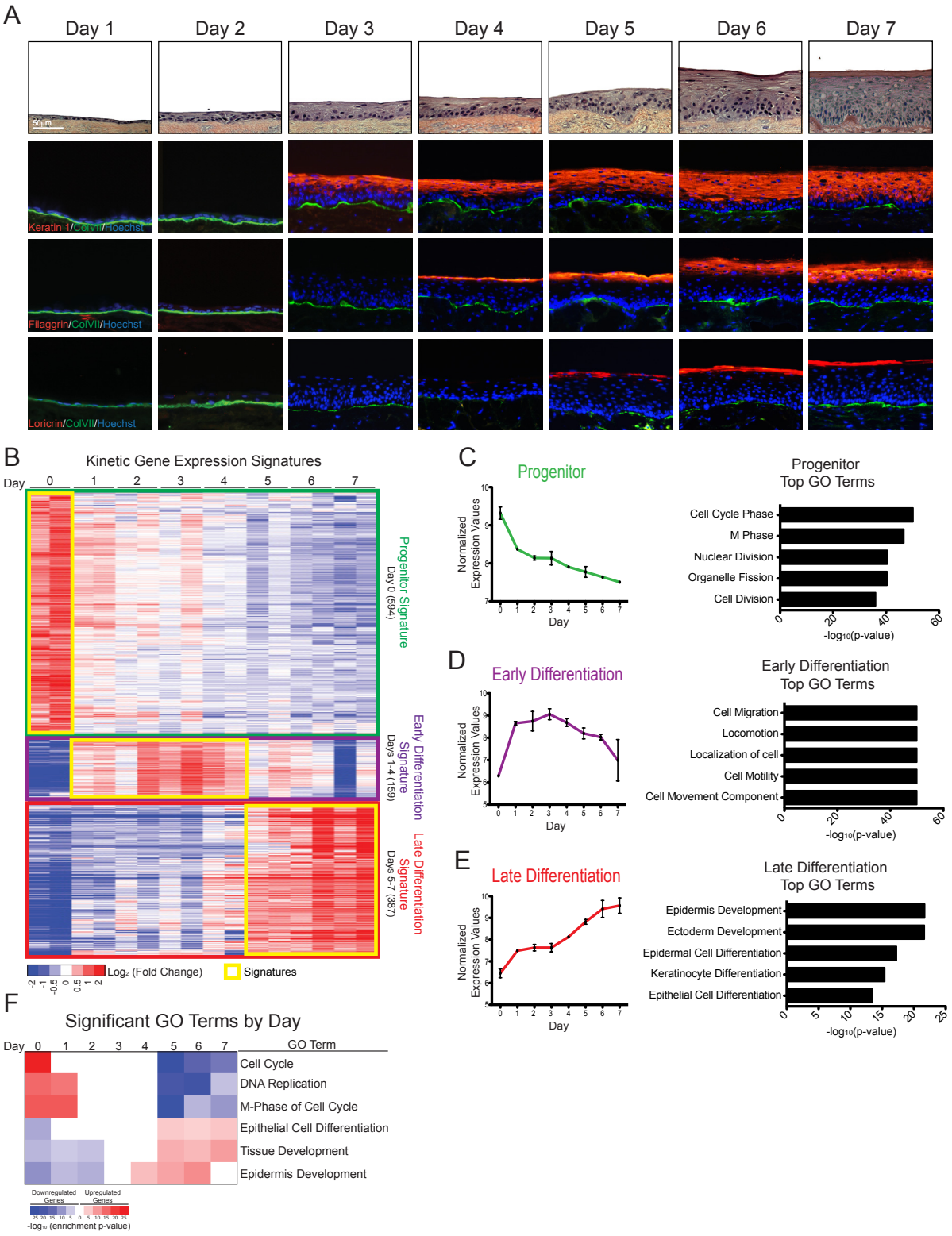

Figure S1. Dynamically expressed gene sets during regeneration of differentiated epidermis (Related to Figure 1). (A) Time course of regeneration of differentiated epidermal tissue. Epidermis regenerated in organotypic tissue using primary human epidermal keratinocytes seeded onto devitalized human dermis pre-embedded with primary human fibroblasts over a 7-day time course. Histology and differentiation markers keratin 1, filaggrin and loricrin (orange) are shown with epidermal basement membrane marked with collagen VII (green); scale bar=50μm. (B) Epidermal differentiation gene sets. Heatmap of duplicate, mean-centered microarray analysis of RNAs with ≥2 fold change over the time course, FDR≤0.05. Signature of gene sets with increased expression at day 0 (progenitor, 594 genes), days 1-4 (early differentiation, 159 genes) and days 5-7 (late differentiation, 387 genes); each gene set is boxed in yellow. Average normalized gene set expression values and associate GO terms for progenitor-enriched genes (C), early differentiation-enriched genes (D), and late differentiation-enriched genes (E). (F) Representation of overall gene expression pattern changes based on GO term analysis over the time-course.

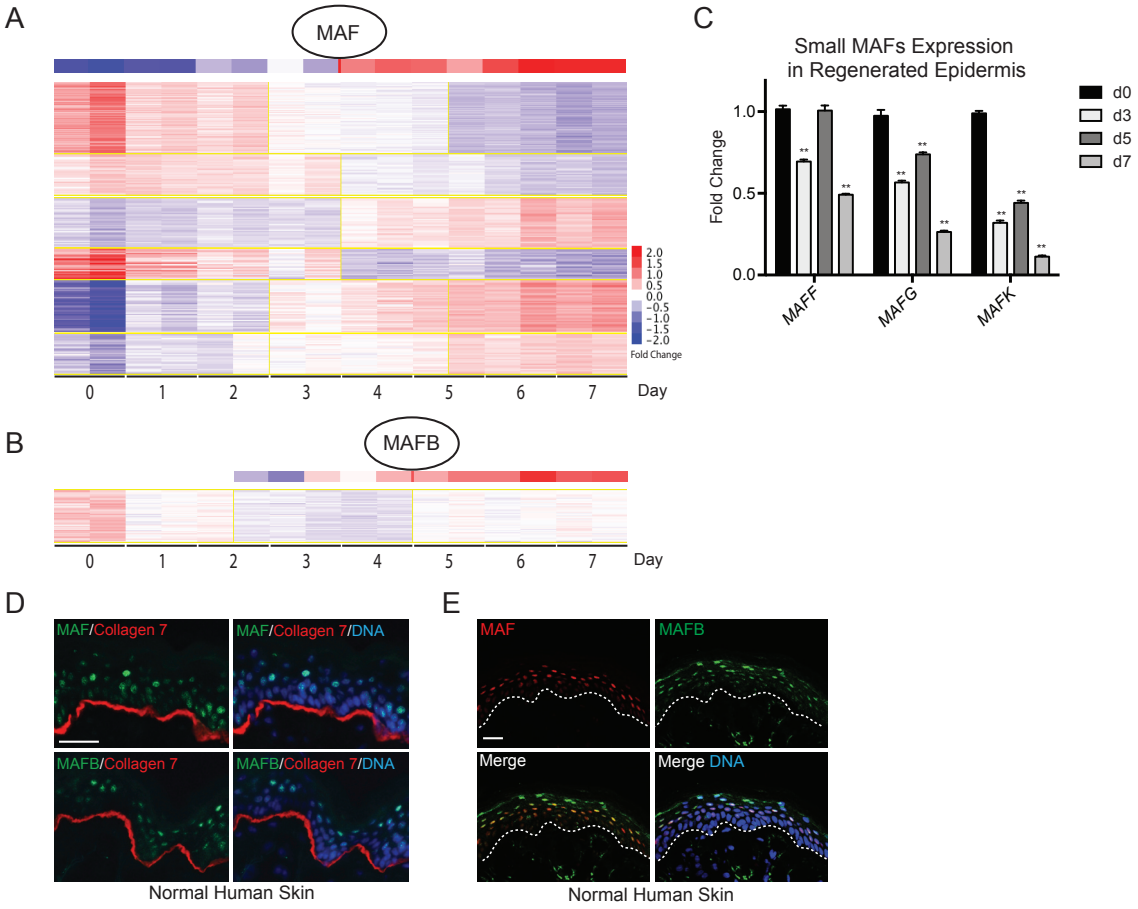

Figure S2. Module maps of MAF and MAFB-correlated genes and small MAFs expression in regenerated epidermis (Related to Figure 1). (A) Expression modules correlated with MAF over the time-course. (B) Expression modules correlated with MAFB over the time-course. (C) Quantification of mRNA expression levels of the small MAF family members. Mean ± SEM; n=2, **p<0.01. (D) Immunostaining of normal human skin for MAF or MAFB (green) and collagen VII (orange) showing MAF and MAFB expression in differentiated layers. (E) Co-immunostaining of MAF (orange) and MAFB (green) showing co-localization in differentiated layers of normal human skin [white dotted line demarcates epidermal basement membrane]; scale bar=50μm.

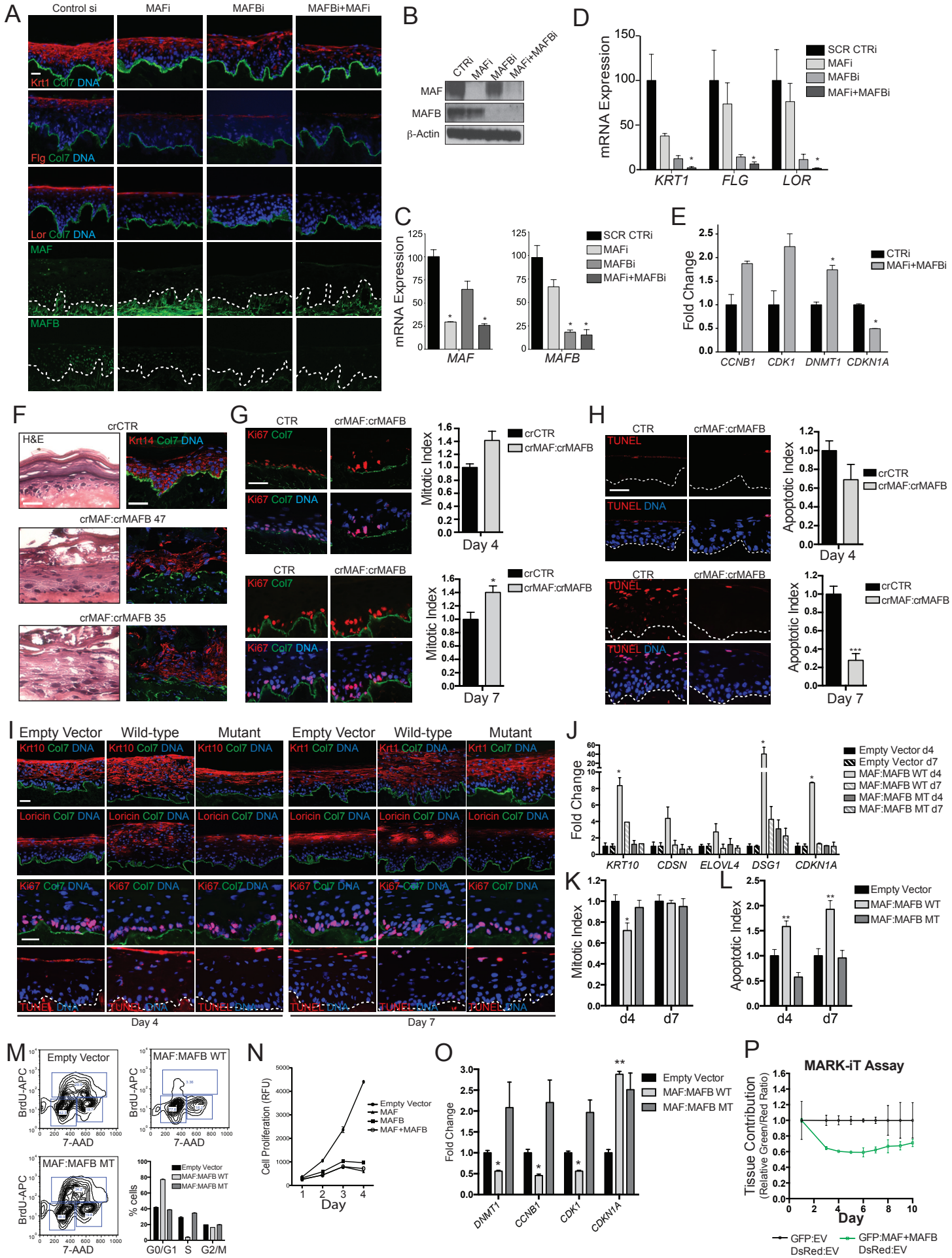
A
Control si
MAFi
MAFBi
MAFBi+MAFi
Krt1 Col7 DNA
Flg Col7 DNA
Lor Col7 DNA
MAF
MAFB
B
CTRi
MAFi
MAFBi
MAFi+MAFBi
MAF
MAFB
β-Actin
C
mRNA Expression
SCR CTRi
MAFi
MAFBi
MAFi+MAFBi
MAF
MAFB
D
mRNA Expression
KRT1
FLG
LOR
E
Fold Change
CTRi
MAFi+MAFBi
CCNB1
CDK1
DNMT1
CDKN1A
F
crCTR
H&E
Krt14 Col7 DNA
crMAF:crMAFB 47
crMAF:crMAFB 35
G
CTR
crMAF:crMAFB
Ki67 Col7
Ki67 Col7 DNA
Mitotic Index
crCTR
crMAF:crMAFB
Day 4
Day 7
H
CTR
crMAF:crMAFB
TUNEL
TUNEL DNA
Apoptotic Index
crCTR
crMAF:crMAFB
Day 4
Day 7
I
Empty Vector
Wild-type
Mutant
Empty Vector
Wild-type
Mutant
Krt10 Col7 DNA
Krt1 Col7 DNA
Loricin Col7 DNA
Ki67 Col7 DNA
TUNEL DNA
Day 4
Day 7
J
Fold Change
Empty Vector d4
Empty Vector d7
MAF:MAFB WT d4
MAF:MAFB WT d7
MAF:MAFB MT d4
MAF:MAFB MT d7
KRT10
CDSN
ELOVL4
DSG1
CDKN1A
K
Mitotic Index
d4
d7
L
Apoptotic Index
Empty Vector
MAF:MAFB WT
MAF:MAFB MT
d4
d7
M
Empty Vector
MAF:MAFB WT
MAF:MAFB MT
BrdU-APC
7-AAD
% cells
G0/G1
S
G2/M
N
Cell Proliferation (RFU)
Empty Vector
MAF
MAFB
MAF+MAFB
Day
O
Fold Change
Empty Vector
MAF:MAFB WT
MAF:MAFB MT
DNMT1
CCNB1
CDK1
CDKN1A
P
MARK-iT Assay
Tissue Contribution
(Relative Green/Red Ratio)
Day
GFP:EV
DsRed:EV
GFP:MAF+MAFB
DsRed:EV

Figure S3. Effects of MAF and MAFB depletion or overexpression in keratinocytes (Related to Figure 2). (A) Depletion of MAF and MAFB in organotypic epidermis. Individual or double siRNA-mediated knockdown of MAF (MAFi) and/or MAFB (MAFBi) with staining for differentiation markers keratin 1, filaggrin and loricrin (orange), collagen VII (green) and nuclei (blue). Note loss of differentiation proteins upon MAF:MAFB loss. (B) Western blots for MAF and MAFB depletion by siRNA. Quantification of mRNA expression levels of MAF and MAFB (C), differentiation markers (D), or basal markers (E) in MAFi:MAFBi tissue. Mean ± SEM, n=2 biological replicates, *p<0.05. (F) Hematoxylin and eosin (left panel) or keratin 14 (orange), collagen 7 (green) and nuclei (blue) staining (right panel) of xenograft tissue generated from CRISPR/Cas9 targeted control or MAF:MAFB primary keratinocytes from two independent combinations of sgRNAs (crMAF:crMAFB 47 and crMAF:crMAFB 35) at day 21. (G) Ki67 staining of control or crMAF:crMAFB tissue at day 4 and day 7 of differentiation in organotypic culture with quantitation. Mean ± SEM; n=5, *p<0.05. (H) TUNEL staining of control or crMAF:crMAFB tissue at day 4 and day 7 of differentiation in organotypic culture with quantitation. Scale bar=50μm. Mean ± SEM; n=5, ***p<0.001. (I) Immunostaining for differentiation markers keratin 1 and loricrin (orange), collagen VII (green) and nuclei (blue) of tissue overexpressing empty vector, wild-type or DNA-binding deficient mutants of MAF and MAFB at day 4 and day 7 of differentiation in organotypic culture (top 2 rows) and Ki67 and TUNEL staining (bottom 2 rows). (J) Quantitation of differentiation gene induction in MAF:MAFB overexpressing tissue comparing empty vector, wild-type and DNA-binding deficient mutants. Mean ± SEM, n=2 biological replicates, *p<0.05. (K) Quantitation of Ki67 staining in MAF:MAFB overexpressing tissue. Mean ± SEM; n=5, *p<0.05. (L) Quantitation of TUNEL staining in MAF:MAFB overexpressing tissue. Mean ± SEM; n=5, **p<0.01. (M) Cell cycle profiling of empty vector, wild-type or mutant MAF:MAFB overexpressing progenitor keratinocytes assessed by BrdU flow cytometry and corresponding quantitation of cell cycle phase. Mean ± SEM; n=2. (N) Cell proliferation of MAF and MAFB overexpressing progenitor keratinocytes. (O) Quantitation of basal marker gene expression in MAF:MAFB overexpressing progenitor keratinocytes. Mean ± SEM; n=2, *p<0.05, **p<0.01. (P) MARK-iT cell competition assay showing relative green/red ratio for GFP:MAF+MAFB/DsRed:empty vector mixed samples. This is the inverse experiment of that shown in Figure 2I in which GFP and DsRed marker genes are switched. For all images, scale bar=50μm.

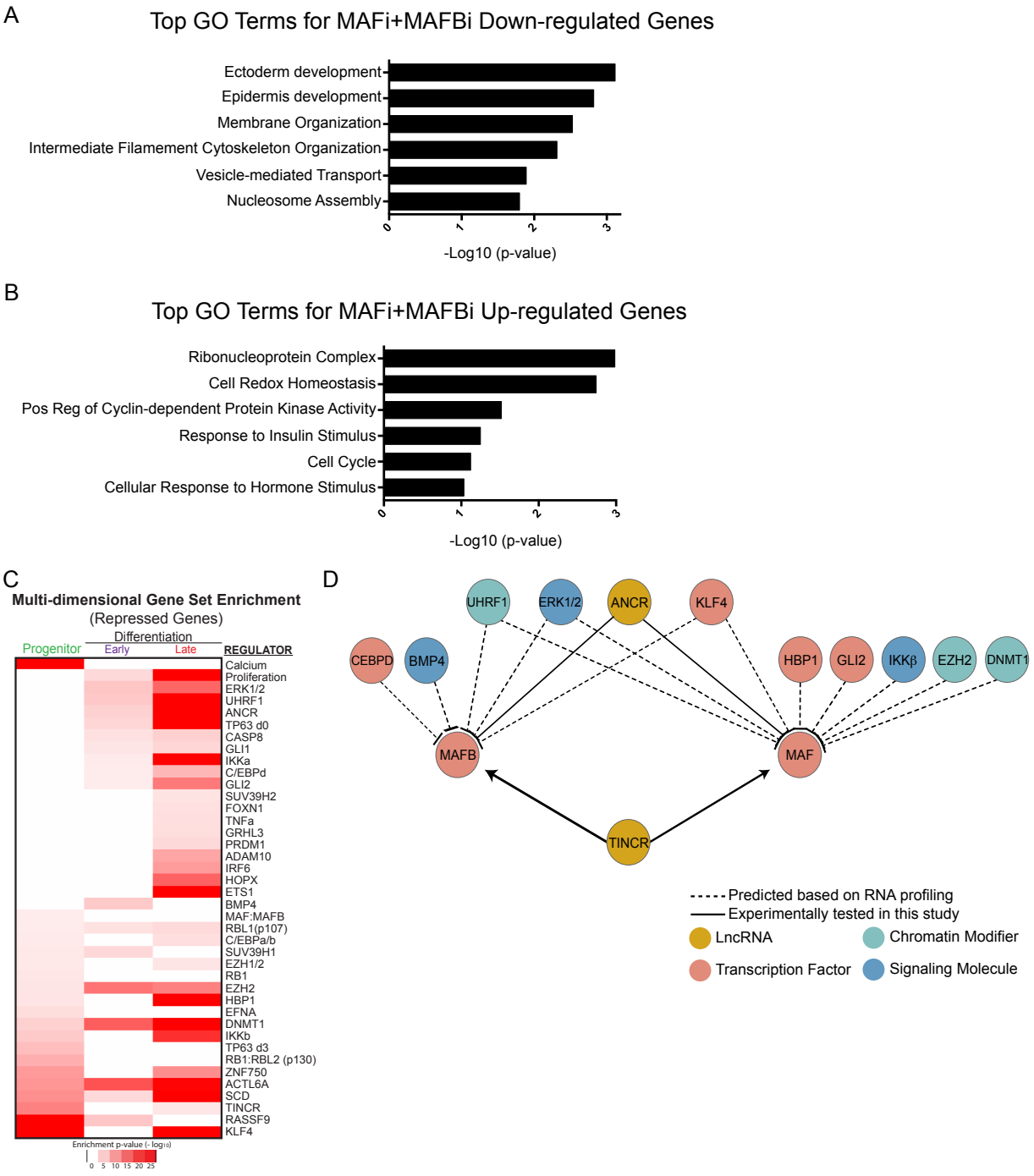

Figure S4. Enrichment terms for MAF:MAFB regulated genes and incorporation of published gene sets into epidermal regeneration signatures with predicted MAF and MAFB regulator network (Related to Figure 3). (A) Top GO terms for MAFi+MAFBi down regulated genes. (B) Top GO terms for MAFi+MAFBi up regulated genes. (C) Multi-dimensional gene set enrichment analysis performed on “repressed” genes (up regulated genes in knockdown or knockout of listed factor or down

regulated genes in overexpression of listed factor) against the differentiation signatures. (D) Network of predicted MAF and MAFB upstream regulators based on presence of MAF or MAFB in a regulator gene set. These interactions may be indirect and may involve feedback (for example KLF4).

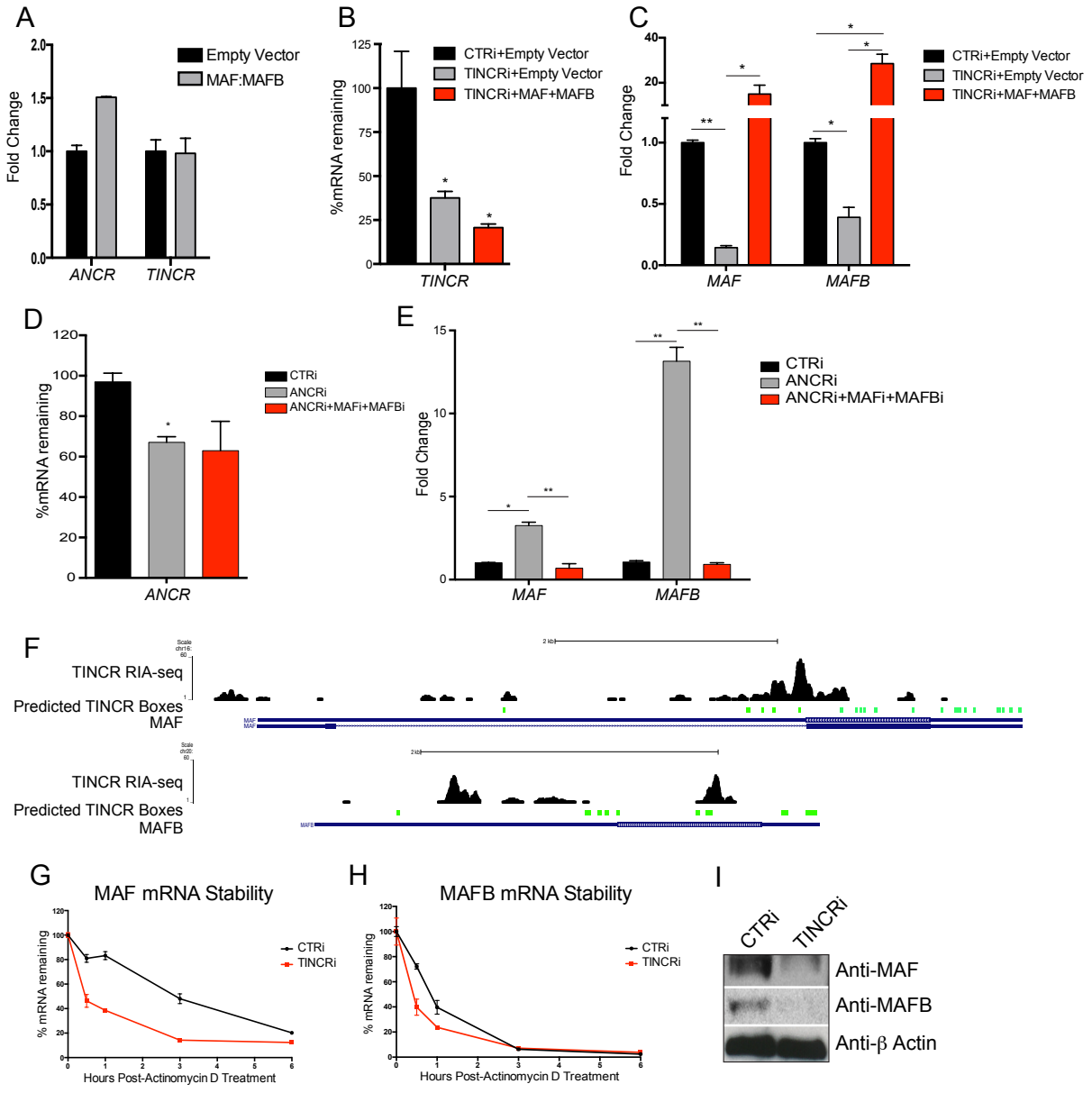

Figure S5. Effects of lncRNAs on MAF and MAFB (Related to Figure 4). (A) mRNA expression levels of ANCR and TINCR in keratinocytes overexpressing MAF:MAFB. Mean ± SEM, n=2. (B,C) mRNA expression levels of TINCR, MAF and MAFB in TINCR-depleted tissue with or without MAF:MAFB overexpression. Mean ± SEM, n=2 biological replicates, *p<0.05, **p<0.01. (D,E) mRNA expression levels of ANCR, MAF and MAFB in ANCR-depleted progenitor keratinocytes with or without MAF:MAFB depletion. Mean ± SEM, n=2 biological replicates, *p<0.05, **p<0.01. (F) RIA-seq tracks showing TINCR binding to MAF (top) and MAFB (bottom) mRNA with predicted TINCR boxes in green. Stability of MAF (G) and MAFB (H) mRNA in control versus TINCR-depleted, actinomycin D treated differentiated keratinocytes. (I) Western blot of MAF and MAFB in TINCR depleted keratinocytes.

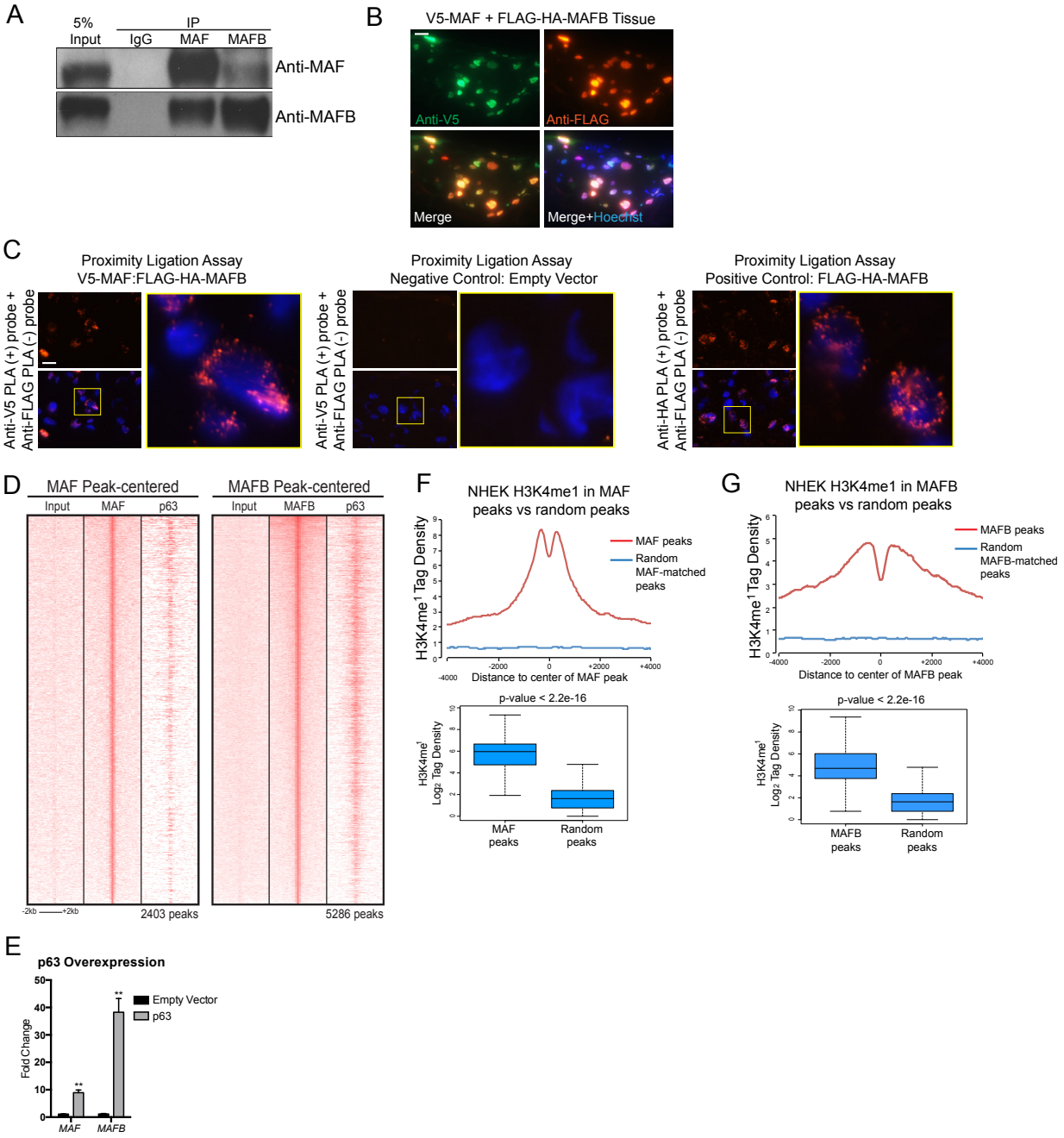

Figure S6. MAF and MAFB are physically proximal to each other and correlate with p63 genomic occupancy and enhancer marks (Related to Figure 5). (A) Physical interaction of MAF:MAFB determined by endogenous co-immunoprecipitation of MAF and MAFB in differentiated keratinocytes. (B) Immunostaining of organotypic human epidermal tissue expressing V5-MAF and FLAG-HA-MAFB. Scale bar=50μm. (C) Proximity ligation assay (PLA) using V5 and FLAG antibodies to detect MAF and MAFB proximal association *in situ* (left panel); PLA of empty vector control cells using V5 and FLAG antibodies (middle panel); PLA of MAFB using FLAG and HA antibodies (right panel). Scale bar=50μm.

(D) Peak-centered heatmaps of ChIP-seq peak alignment of MAF (left) and MAFB (right) with p63 ChIP-seq peaks. (E) mRNA expression levels of MAF and MAFB in keratinocytes overexpressing p63. Mean ± SEM, n=2, **p<0.01. Detection of enhancer elements by analyzing enrichment of normalized H3K4me^{1} tag density, acquired from NHEK ENCODE ChIP-seq data, across (F) MAF or MAF-matched random peaks; statistically represented by boxplot (left) and (G) MAFB or MAFB-matched random peaks, statistically represented by boxplot (left).

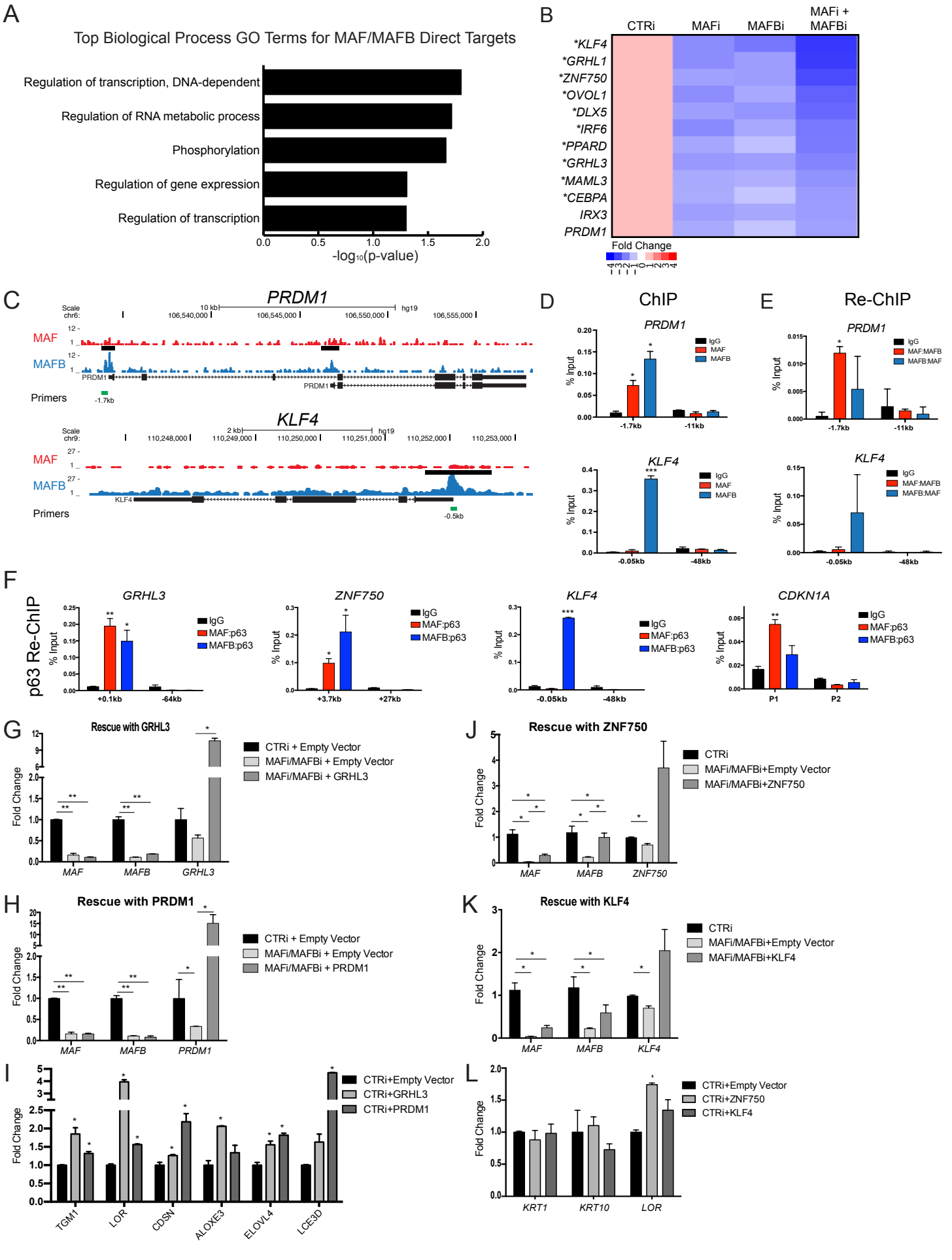
A
Top Biological Process GO Terms for MAF/MAFB Direct Targets
Regulation of transcription, DNA-dependent
Regulation of RNA metabolic process
Phosphorylation
Regulation of gene expression
Regulation of transcription
0.0
0.5
1.0
1.5
2.0
-log10(p-value)
B
CTRi
MAFi
MAFBi
MAFi + MAFBi
*KLF4
*GRHL1
*ZNF750
*OVOL1
*DLX5
*IRF6
*PPARD
*GRHL3
*MAML3
*CEBPA
IRX3
PRDM1
Fold Change
C
PRDM1
Scale
chr6:
10 kb
hg19
106,540,000
106,545,000
106,550,000
106,555,000
MAF
MAFB
PRDM1
Primers
-1.7kb
KLF4
chr9:
2 kb
110,248,000
110,249,000
110,250,000
110,251,000
110,252,000
110,253,000
KLF4
-0.5kb
D
ChIP
PRDM1
% Input
IgG
MAF
MAFB
-1.7kb
-11kb
KLF4
-0.05kb
-48kb
E
Re-ChIP
PRDM1
IgG
MAF:MAFB
MAFB:MAF
-1.7kb
-11kb
KLF4
-0.05kb
-48kb
F
p63 Re-ChIP
GRHL3
% Input
IgG
MAF:p63
MAFB:p63
+0.1kb
-64kb
ZNF750
+3.7kb
+27kb
KLF4
-0.05kb
-48kb
CDKN1A
P1
P2
G
Rescue with GRHL3
Fold Change
CTRi + Empty Vector
MAFi/MAFBi + Empty Vector
MAFi/MAFBi + GRHL3
MAF
MAFB
GRHL3
J
Rescue with ZNF750
CTRi
MAFi/MAFBi+Empty Vector
MAFi/MAFBi+ZNF750
MAF
MAFB
ZNF750
H
Rescue with PRDM1
CTRi + Empty Vector
MAFi/MAFBi + Empty Vector
MAFi/MAFBi + PRDM1
MAF
MAFB
PRDM1
K
Rescue with KLF4
CTRi
MAFi/MAFBi+Empty Vector
MAFi/MAFBi+KLF4
MAF
MAFB
KLF4
I
CTRi+Empty Vector
CTRi+GRHL3
CTRi+PRDM1
TGM1
LOR
CDSN
ALOXE3
ELOVL4
LCE3D
L
CTRi+Empty Vector
CTRi+ZNF750
CTRi+KLF4
KRT1
KRT10
LOR

Figure S7. MAF and MAFB regulate epidermal transcription factors (Related to Figure 6). (A) Top biological process GO terms enriched in MAF:MAFB direct target gene list. (B) Effect of MAF and MAFB depletion on mRNA expression levels of TFs induced during differentiation in organotypic epidermal culture (qPCR, T-test p-value<0.05 comparing CTRi to MAFi+MAFBi). (C) MAF [top, red] and MAFB [bottom, blue] ChIP-seq tracks at epidermal transcription factor genes, *PRDM1* and *KLF4*; black bars denote ChIP-seq peaks called by MACS and green bars denote sequences for ChIP-qPCR. (D) ChIP-qPCR of MAF and MAFB at *PRDM1* and *KLF4*. Mean ± SEM, n=2. (E) Re-ChIP-qPCR of MAF and MAFB at *PRDM1* and *KLF4*. Mean ± SEM, n=2. (F) Re-ChIP of MAF:p63 or MAFB:p63 at differentiation genes. Mean ± SEM, n=2. mRNA expression levels of GRHL3 (G), PRDM1 (H), ZNF750 (J) and KLF4 (K) during MAFi+MAFBi rescue in tissue. Mean ± SEM, n=2 biological replicates. mRNA expression of differentiation genes in GRHL3 and PRMD1 (I) or ZNF750 and KLF4 (L) overexpressed in control tissue. Mean ± SEM, n=2 biological replicates. For all data shown: *p<0.05, **p<0.01, ***p<0.001.

Table S1. List of epidermal differentiation gene signatures (Related to Figure 1). Gene expression profiling for epidermal differentiation time-course showing three distinct signatues: progenitor, early differentiation and late differentiation.

Table S2. Transcription factors predicted to regulate epidermal differentiation modules based on Genomica analysis (Related to Figure 1). List of transcription factors correlated with modules built from time course gene expression data with corresponding number of modules regulated by each transcription factor over 100 permutations.

Table S3. MAFi:MAFBi expression profiles (Related to Figure 3). Gene expression profiles of MAFi:MAFBi regenerated tissue.

Table S4. FOCIS motif enrichment analysis (Related to Figure 6). List of factors with enriched DNA binding sequences that correlate with MAF and MAFB ChIP-seq binding sites.

Extended Experimental Procedures

RNAi and CRISPR/Cas9 knockout

For MAF knockdown, ON-TARGETplus siRNA (J000-007) was used (Dharmacon); for MAFB, Life Technologies siRNA (s19279) was used. For ANCR and TINCR custom siRNA were used as described (Kretz et al., 2012, 2013). For CRISPR/Cas9 gene ablation sgRNA sequences were designed using a CRISPR design tool (Shalem et al., 2014) and chosen for their high quality score, predictive of their low off-target effects, as well as effective deletion of MAF or MAFB expression based on western blot screens. The following sgRNA sequences were used: Control (GACCGGAACGATCTCGCGTA); AAVS1 (GGGGCCACTAGGGACAGGAT); MAF sg3 (GCAGCTGAACCCCGAGGCGC); MAF sg4 (GATCACGGCGGACACCACGG); MAFB sg5 (GACGCAGCTCATTCAGCAGG); MAFB sg7 (ATGGCCGCGGAGCTGAGCAT).

Plasmids

Overexpression was performed by lentiviral-mediated gene transfer using the pLEX vector (Thermo). Clones for MAF, MAFB, KLF4, ZNF750, GRHL3 and PRDM1 were purchased from OpenBioSystems and epitope tagged with V5, HA or FLAG. MAF and MAFB DNA binding deficient mutants were generated as previously described (Kataoka et al., 1994). Briefly, the MAF and MAFB L2PL4P mutants were generated using PCR mutagenesis by mutating two conserved leucine residues in the leucine zipper motif to proline residues to eliminate DNA binding activity of MAF and MAFB. These mutant genes were subcloned into the pLEX vector (Thermo).

Protein Expression, Tissue Analysis and Histology

For immunoblot analysis, 20-30 mg of cell lysates were loaded per lane for SDS-PAGE and transferred to PVDF membranes. For immunofluorescence staining, tissue sections (7 μm thick) were fixed using either 50% acetone and 50% methanol, or 4% formaldehyde. Primary antibodies were incubated at 4°C overnight and secondary antibodies were incubated at room temperature for 1 hour. MAF (M153) and MAFB (P20) antibodies were purchase from Santa Cruz, while a separate MAFB antibody (Sigma) was used for IF. Other antibodies used in this study include: anti-Krt14 (Covance), anti-Krt1 (Covance), anti-Krt10 (Neomarkers), anti-Loricrin (Covance), anti-Filaggrin (Covance), ms-anti-CollagenVII (Millipore), pAb-anti-CollagenVII (Calbiochem), anti-HA (Cell Signaling), anti-V5 (Abcam), anti-FLAG (Sigma), anti-Ki67 (Thermo), anti-actin (Sigma). Proximity ligation assay was performed with the Duolink system using plus PLA probe anti-mouse and minus PLA probe anti-rabbit (Sigma). TUNEL assay was conducted using the In Situ Cell Death Detection Kit-TMR Red (Roche). For histology, 7 μm frozen sections were stained with hematoxylin and eosin according to standard methods.

Quantitative RT-PCR Gene Expression Analysis

For qRT-PCR, total RNA was extracted using the RNeasy Plus kit (Qiagen) and subsequently subjected to reverse transcription using iSCRIPT cDNA synthesis kit (Biorad). qRT-PCR analysis was performed using the Roche480 Lightcycler instrument with Maxima SYBR Green Master Mix (Fermentas). Samples were run in duplicate and normalized to levels of L32 ribosomal RNA. Statistical analysis was performed using Prism5 software (GraphPad).

Primer Sequences for quantitative RT-PCR:

NAME	Forward Primer	Reverse Primer
ABCA12	AACAGTCCAAAGCCATCCAG	GAGCAGCAGCAATTTCACG
ALOXE3	ACTCCCCCACACTCGATACA	GAGGTAGATGAGGCCTTGCC
ANCR	GCCACTATGTAGCGGGTTTC	ACCTGCGCTAAGAACTGAGG
ATP2C2	TCGGCTTTCTCAGGAACCAC	GGGGATGTAAATGACCGCCA
CASP14	GGCCTGTCGAGGAGAACAAA	ACGTGCAAGGCATCTGTGTA
CCNB1	AACCTGAGCCAGAACCTGAG	TGGGCTTGGAGAGGCAGTAT
CDK1	TGCTTATGCAGGATTCCAGGTT	CATGTACTGACCAGGAGGGA
CDKN1A	GGGTCGAAAACGGCGGCAGA	CCTCGCGCTTCCAGGACTGC
CDSN	CCTTGAGCTGCCATCAGTCAG	GTCTGAGAAGGTGCCAATGCT
CEBPA	GAGGAGGGGAGAATTCTTGG	GAGGCAGGAAACCTCCAAAT
CRNN	GGGTCACACACCGAGACT	TCTCCCTTGAGTGGTCATC
DNMT1	AAGCCCGTAGAGTGGGAATG	GCTAGGTGAAGGTTCAGGCTT
DSG1	AGCCTGTCGTGAAGGTGAAG	TCCTACTCCAGAGATGCGGT
ELOVL4	TAAACGTAGTGTCCACGGCA	GAGACTGCATCAGAGGCCAA
FLG	AAAGAGCTGAAGGAACTTCTGG	AACCATATCTGGGTCATCTGG
GRHL1	GCCTACCCACTCCATCAAGA	GAGTCTGGAGTTCGCCTTTG
GRHL3	GGTGTTCATCGGCGTAAACT	CCCAAGCCACAGTCATAGGT
IVL	TGCCTGAGCAAGAATGTGAG	TGCTCTGGGTTTTCTGCTTT
K1	GAAGTCTCGAGAAAGGGAGCA	ATGGGTTCTAGTGGAGGTATCTA
K10	GCAAATTGAGAGCCTGACTG	CAGTGGACACATTTCGAAGG
KLF4	GCCTCCTCTTCGTCGTC	GGCTCACGTCGTTGATGT

L32	AGGCATTGACAACAGGGTTC	GTTGCACATCAGCAGCACTT
LCE1E	CCTCCAGTCTCTTCCTGCTG	AGAGCTCTGGGTCTGTGAC
LCE3D	GCTGCTTCCTGAACCAC	GGGAACTCATGCATCAAG
LOR	CTCTGTCTGCGGCTACTCTG	CACGAGGTCTGAGTGACCTG
MAF	TATGCCCAGTCCTGCCGCTT	CGCTGCTCGAGCCGTTTTCT
MAFB	GACGCAGCTCATTCAGCAG	CCGGAGTTGGCGAGTTTCT
MAFF	TCGTGGGCCCTGTCTTCCTC	ACCTCTGCCCCATCCCCAAC
MAFG	TCCCAGCCCTGCCCTTCTTC	CAGCGCCAGGAGAGCAACAG
MAFK	AACCAGCACCTGCGGGGTCTC	CAGCTCCTCCTTCTGCGTCACC
OVOL1	GCTCCCGGCTTCAGTTA	GGGCTGTGGTGGGCAGAA
PPARD	GTGTGGAAGCAGTTGGTGAA	TGCACGCCATACTTGAGAAG
PRDM1	CGGGACTCCTACGCTTAC	TGCCATTCATGCTGCTCA
S100A8	ATTTCCATGCCGTCTACAGG	CCAACTCTTTGAACCAGACG
S100A9	CGCAGCTGGAACGCAACAT	GCCCTCGTCACCCTCGTG
SPINK5	TTAGCAAGAGCTCCCAAGGC	CCATCTGTGCCACAAACAGC
SPRR1A	GCCACTGGATACTGAACA	AGGAAGACTAGGGATGGT
SPRR3	CCAGGCTACACAAAGCTAC	GCTTAATTCAGGGGCTTAC
TGM1	CTTCAAGAACCCCCTTCCCG	TGAGGATCTTGGGCCTCTGT
TINCR	TGTGGCCCAAACTCAGGGATACAT	AGATGACAGTGGCTGGAGTTGTCA
ZNF750	GCACAGAATGCCTACCTGCC	CCGTTCACAACATTGAGGCTTACT

mRNA Expression Profiling and Analysis

Amplification and labeling of cDNA probes and hybridization to the HG-U133 plus 2.0 microarray chip (Affymetrix) was performed by the Stanford PAN Facility. Data analysis was performed using the Bioconductor suite in R or Genespring GX11 (Agilent). Raw data was RMA normalized, filtered for probes with expression value ≥100 in at least 1 of the samples, and probes were averaged over genes. For time-course analysis, gene signatures were obtained by performing SAM analysis on each signature against all other days with FDR ≤ 0.05 and ≥ 2-fold expression change. Pair-wise comparisons between the RNAi-treated samples and the control samples were performed to find probes that showed ≥ 1.5-fold expression change with p- value ≤ 0.05 based on T-test. Gene ontology

of differential expressed genes was performed through DAVID (Dennis et al., 2003). For multi-dimensional gene set enrichment, the following datasets were used: progenitor keratinocytes, calcium-differentiated keratinocytes and DNMT1, UHRF1, SUV39H1/2 (GSE18590), p63 (GSE33495), KLF4 and ZNF750 (GSE32685), TINCR (GSE40122), NOTCH1 (GSE1569), ERK1/2 (GSE15417), IKKβ (GSE17511), CEBPD (Borrelli et al., 2010a), SCD (GSE24243), IRF6 (GSE5800), ETS1 (Nagarajan et al., 2010), CEBPα/β (E-MEXP-1719), RB, RBL1, and RBL2 (GSE9562), GLI1/GL12 (GSE1434), TNFα (GSE2489), GRHL3 (GSE7381), RASSF9 (GSE24190), ANCR (GSE34528), ADAM10 (GSE25480), EFNA (GSE26521), BLIMP1/PRDM1 (Magnúsdóttir et al., 2007), GR (GSE23724), CASP8 (GSE17345), FOXN1 (GSE16937), BMP4 (GSE16111), EZH2 (GSE26616), SRF (GSE25548), HOPX (Yang et al., 2010), HBP1 (Borrelli et al., 2010b), TCFAP2g (GSE10175), IL-1 (GSE9120).

Colony Formation Assay

Mouse fibroblast 3T3 cells were treated with 15 mg/mL mitomycin C (Sigma) in DMEM for 2 h, then trypsinized and plated at $8x10^5$ cells per well in a 6-well plate. The media was changed to keratinocyte growth media 24 h after plating. One hundred keratinocytes were seeded onto the feeder layer 24 h after the media change. Media was changed every two days for 14 days. At the end of 14 days, the cells were washed with PBS to remove the 3T3 cells and fixed in 1:1 acetone/methanol for 5 minutes. The plate was allowed to air dry for 3-5 minutes and then colonies were stained with cresyl violet.

MARK-iT (Mosaic Analysis of Regeneration Kinetics-in Tissue) Assay

500,000 keratinocytes (250,000 each of GFP- and DsRed-labeled cells) were seeded onto a devitalized human dermis and cultured in at the air-liquid interface for 10 days. Each fluorescent population was transfected with MAF, MAFB, MAF+MAFB, or an empty vector control. Total fluorescent reading underwent spectral un-mixing to quantify each cell population using the CR-Maestro imaging system and software (Perkin-Elmer) and this value was plotted relative to the matched control sample.

Cell Proliferation and Flow Cytometry

Cells were seeded at low density in duplicate in 24-well plates. Twenty-four hours after seeding the medium was removed and replaced with 500 µl of a 5:1 mixture of media and cell titer blue reagent (Promega). Cells were incubated for 2h in the dark at 37°C and fluorescence at 560nm/590nm was read for 100 µl triplicate aliquots for each sample. Readings were repeated every day for 4 days to measure proliferation. For flow cytometry, the BrdU-APC kit (BD biosciences) was used following the manufacturer's protocol. Cells were interrogated on a BD FACScalibur and analysis was done with

FlowJo software (Treestar). All graphs and statistical analysis were performed using Prism5 software (GraphPad).

Chromatin Immunoprecipitation and ChIP-qPCR

Human keratinocytes was cross-linked with 1% formaldehyde. The chromatin was sonicated to achieve fragments with an average length between 200-500 bp for ChIP-qPCR and 150-250 bp for ChIP-seq. The sonicated chromatin was immunoprecipitated overnight at 4°C. Following reversal of cross-links, the samples were treated with RNaseA and the DNA was purified using the Qiagen PCR Purification Kit. For ChIP-qPCR, 1µl of DNA was used as template. Antibodies for EZH2 and H3K27Me^3 ChIP were purchased from Active Motif. The following primers were used for EZH2 and H3K27Me^3 ChIP-qPCR: MAF (For: CTGCTTGAGGTGGTCGACTT Rev: TTCAAGAGGGTGCAGCAGAG); MAFB (For: CCCTCTCCTTTCCTCGTTGC Rev: GCTTTCTGAACTTTGCGCGT); OVOL1 (For: AGCGACGAGGGTTCGAAAAT Rev: CTCGCTCCAGTTCCTCTTGC). For MAF and MAFB ChIP or re-ChIP qPCR the following primers were used: ZNF750 +3.7kb (For: GGCAGGGATCTGAAAGCTGA Rev: GGACGCAGCTCTCACTTGAT); ZNF750 +27kb (For: GTCACACTTTAACACCAAACGC Rev: TCATCAAGAGCAAGGGGAACA); KLF4 -0.5kb (For: GCGCGTTCCTTACTTATAACTTCC Rev: TAGCAACGATGGAAGGGAGC); KLF4 -48kb (For: CATTGGTGTGGGAGCCAGAT Rev: CCCCCATTACCTACCCTCCA); PRDM1 -1.7kb (For: AGGCCGCCTCCAGTATGAC Rev: AGAAATCCAGCCTGCTCAAGAG); PRDM1 -11kb (For: TCCACTCCCGGCATAGGTAA Rev: TTGGAGCTGGACACAGAACC); GRHL3 +0.1kb (For: CTGGGAACCTACCTGTCAGC Rev: ACACAGACATTCTCTGCCCG); GRHL3 -64kb (For: TGGTGCTTAGGTTACGGCAG Rev: TGGTGGAAATTACCCGCTCC). For ChIP-seq, MAF (M153) and MAFB (P20) antibodies were used (Santa Cruz). Libraries were prepared using the NEB Next ChIP-seq library prep master mix set for Illumina (NEB) with minor modifications. Barcoded libraries were submitted for Illumina HiSeq2500 massive parallel sequencing with read length 50bp. Heatmaps of ChIP-seq signals show occupancy profiles around peak summits (+/- 2kb region) calculated using a 50bp sliding window using the following formula:

$$signal = \log_2\left(\sum_{i=1}^{50} \frac{\text{coverage\_of\_base}_i}{number\_of\_unique\_mappable\_reads} \times 10{,}000{,}000\right)$$

Peak classification was defined as: Promoter: -2kb to transcriptional start site (TSS); enhancer: 2-10kb upstream of TSS; TSS: 100bp downstream of TSS; Stop: -200bp-200bp around transcriptional end site (TES), Gene tail: 2kb downstream of TES; Intergenic: none of the above.

Supplemental References:

Borrelli, S., Fanoni, D., Dolfini, D., Alotto, D., Ravo, M., Grober, O., Weisz, A., Castagnoli, C., Berti, E., Vigano, M., et al. (2010a). C/EBPδ Gene Targets in Human Keratinocytes. PloS One *5*, e13789.

Borrelli, S., Candi, E., Hu, B., Dolfini, D., Ravo, M., Grober, O., Weisz, A., Dotto, G., Melino, G., ViganÒ, M., et al. (2010b). The p63 target HBP1 is required for skin differentiation and stratification. Cell Death Differ. *17*, 1896–1907.

Dennis, G., Jr, Sherman, B.T., Hosack, D.A., Yang, J., Gao, W., Lane, H.C., and Lempicki, R.A. (2003). DAVID: Database for Annotation, Visualization, and Integrated Discovery. Genome Biol. *4*, P3.

Kataoka, K., Fujiwara, K., Noda, M., and Nishizawa, M. (1994). MafB, a new Maf family transcription activator that can associate with Maf and Fos but not with Jun. Mol. Cell. Biol. *14*, 7581–7591.

Kretz, M., Webster, D.E., Flockhart, R.J., Lee, C.S., Zehnder, A., Lopez-Pajares, V., Qu, K., Zheng, G.X.Y., Chow, J., Kim, G.E., et al. (2012). Suppression of progenitor differentiation requires the long noncoding RNA ANCR. Genes Dev. *26*, 338–343.

Kretz, M., Siprashvili, Z., Chu, C., Webster, D.E., Zehnder, A., Qu, K., Lee, C.S., Flockhart, R.J., Groff, A.F., Chow, J., et al. (2013). Control of somatic tissue differentiation by the long non-coding RNA TINCR. Nature *493*, 231–235.

Magnúsdóttir, E., Kalachikov, S., Mizukoshi, K., Savitsky, D., Ishida-Yamamoto, A., Panteleyev, A.A., and Calame, K. (2007). Epidermal terminal differentiation depends on B lymphocyte-induced maturation protein-1. Proc. Natl. Acad. Sci. *104*, 14988–14993.

Nagarajan, P., Chin, S.S., Wang, D., Liu, S., Sinha, S., and Garrett-Sinha, L.A. (2010). Ets1 blocks terminal differentiation of keratinocytes and induces expression of matrix metalloproteases and innate immune mediators. J. Cell Sci. *123*, 3566–3575.

Shalem, O., Sanjana, N.E., Hartenian, E., Shi, X., Scott, D.A., Mikkelsen, T.S., Heckl, D., Ebert, B.L., Root, D.E., Doench, J.G., et al. (2014). Genome-scale CRISPR-Cas9 knockout screening in human cells. Science *343*, 84–87.

Yang, J., Sim, S., Kim, H., and Park, G. (2010). Expression of the homeobox gene, HOPX, is modulated by cell differentiation in human keratinocytes and is involved in the expression of differentiation markers. Eur. J. Cell Biol. *89*, 537–546.