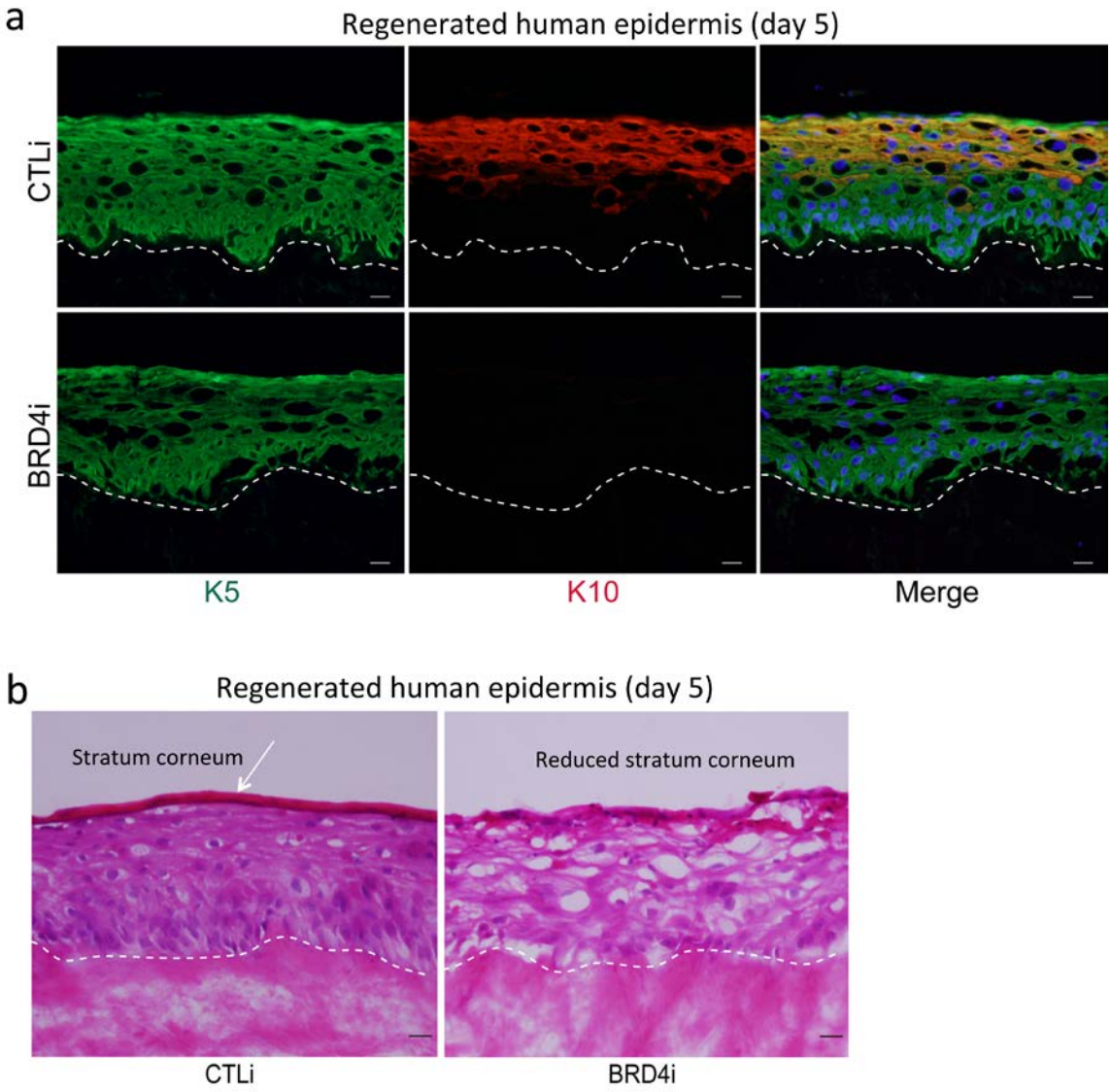
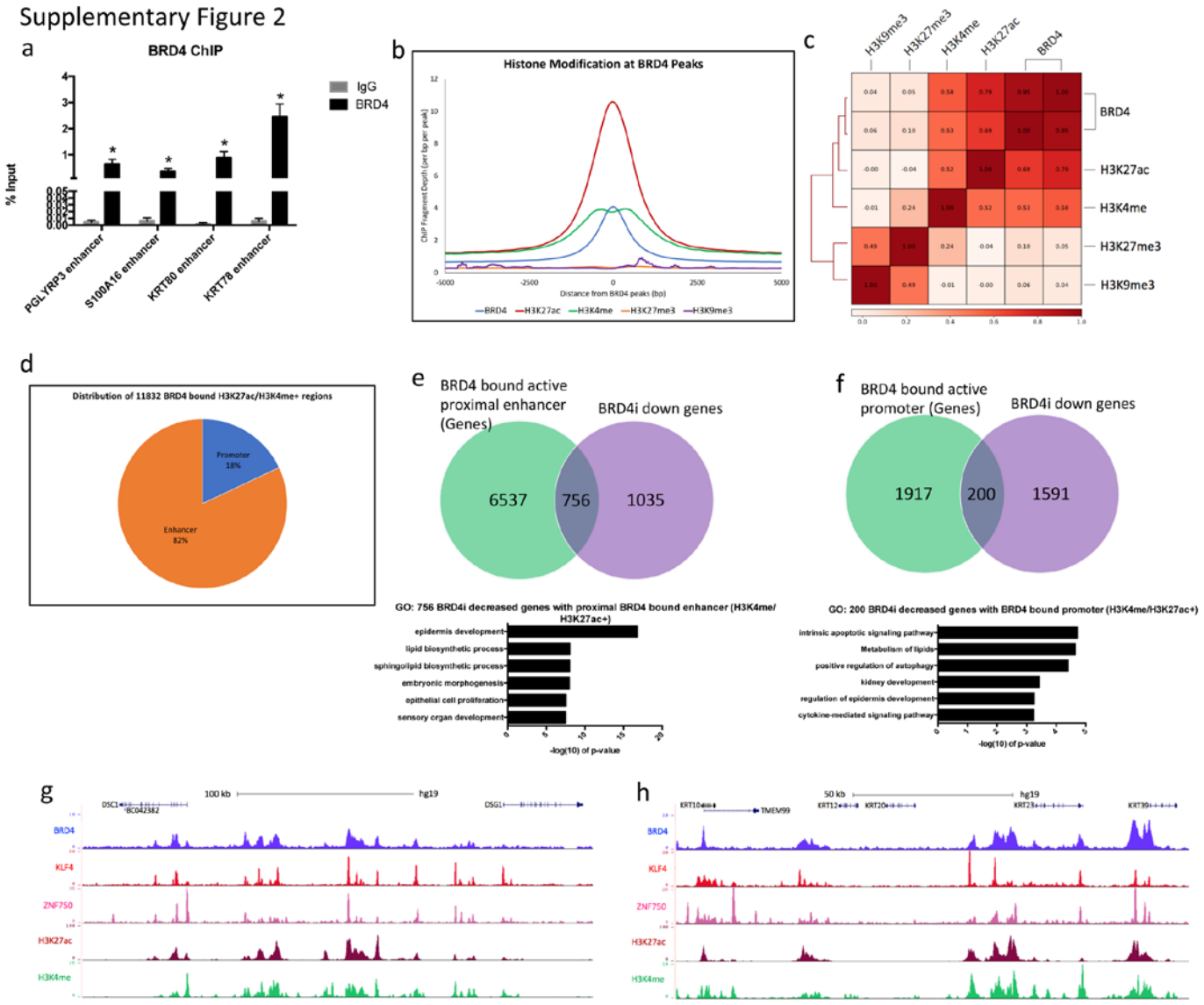
# Supplementary Figure 1

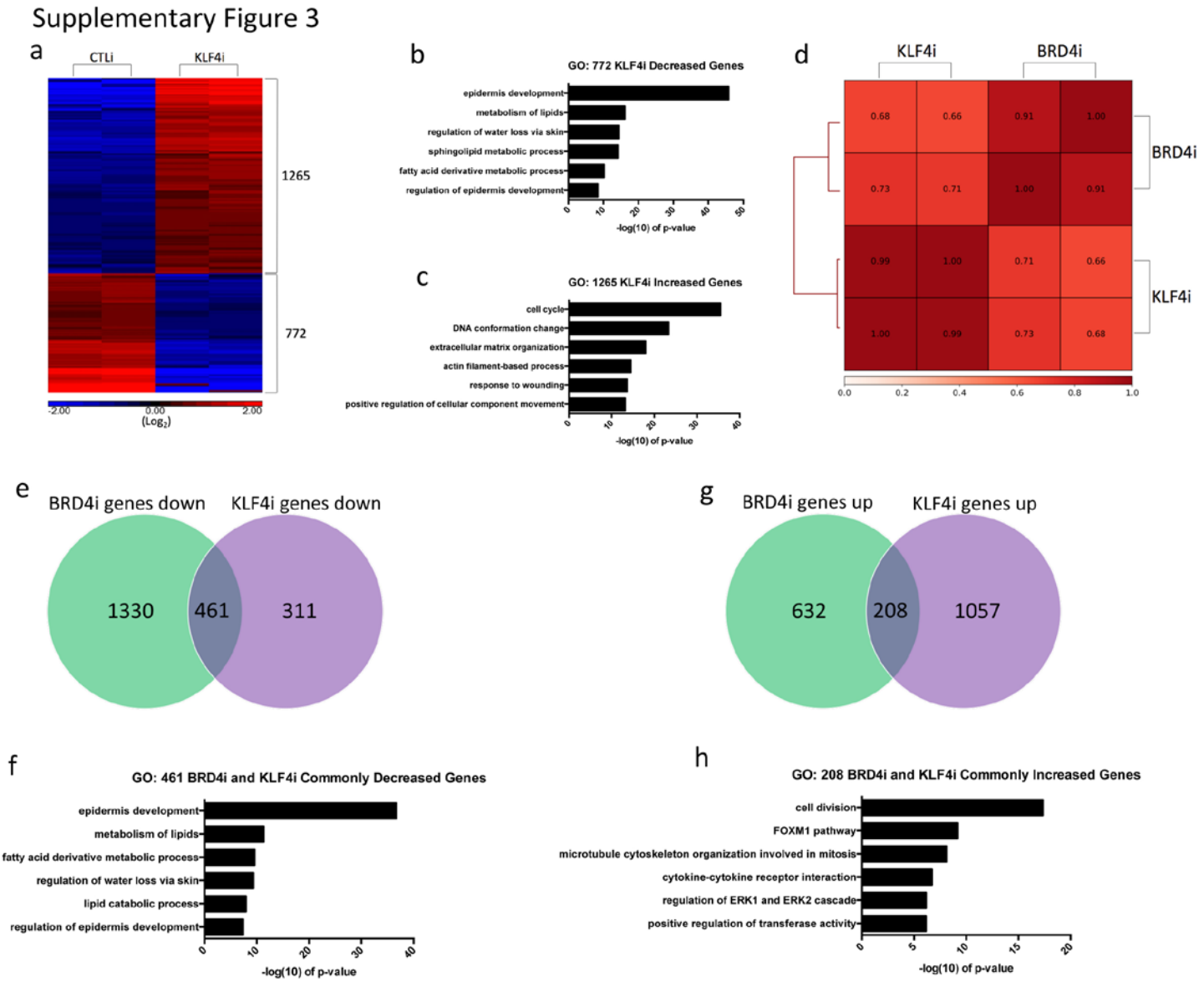



**Supplementary Figure 1. BRD4 is necessary to promote epidermal differentiation** **(a)** Immunofluorescent staining of K5 (green) and K10 (red) in CTLi and BRD4i day 5 regenerated human epidermis. Merged image includes Hoechst staining of nuclei. n=3. White scale bar = 20μm. **(b)** Hematoxylin and eosin staining of CTLi and BRD4i day 5 regenerated human epidermis. n=3, white scale bar = 20μm.




**Supplementary Figure 2. BRD4 is bound to active enhancers and promoters**
**(a)** ChIP qPCR showing BRD4 pulldown and an IgG negative control at differentiation gene proximal sites in keratinocytes differentiated for 3 days. Enrichment is represented as a percent of input. n=2. Statistics: t-test, *$p < 0.05$. **(b)** Mean density profile displaying histone mark ChIP-Seq profiles (H3K27ac: red, H3K4me: green, H3K27me3: orange, H3K9me3: purple) centered around BRD4 peaks (blue). The 17,569 BRD4 peaks were used as the reference coordinates and the profiles are displayed +/- 5kb from the BRD4 peak centers. **(c)** Heatmap plot of Pearson correlation coefficients between replicate BRD4 ChIP-Seq data sets and histone ChIP-Seq data sets (RPKM normalized). **(d)** Percent distribution of the 11,832 regions where BRD4 peaks overlap with both H3K27ac and H3K4me. These 11,832 regions with colocalized BRD4/H3K27ac/H3K4me were annotated using HOMER and all regions not mapped to promoters were considered enhancers. **(e)** Venn diagram showing the number of genes (756) decreased in the BRD4 knockdown that also have a proximal BRD4 bound active (H3K27ac/H3K4me+) enhancer, and the associated GO term enrichments for the 756

genes. **(f)** Venn diagram showing the number of genes (200) decreased in the BRD4 knockdown that also have a BRD4 bound active (H3K27ac/H3K4me+) promoter, and the associated GO term enrichments for the 200 genes. **(g)** UCSC genome browser track displaying BRD4 (blue), KLF4 (red), ZNF750 (pink), H3K27ac (maroon), and H3K4me (green) ChIP-Seq profiles near *DSC1* and *DSG1*. **(h)** UCSC genome browser track displaying BRD4 (blue), KLF4 (red), ZNF750 (pink), H3K27ac (maroon), and H3K4me (green) ChIP-Seq profiles near a cluster of *KRT* genes.




**Supplementary Figure 3. BRD4 and KLF4 regulate a similar transcriptional program (a)** Heatmap generated for replicate (n=2) RPKM normalized RNA-Seq data from CTLi and KLF4i keratinocytes differentiated for 3 days. The expression of genes significantly increased (red) or decreased (blue) is shown. Differential expression was determined with FDR ≤ 0.05 and fold change ≥ 2 vs. CTLi. Graphs are displayed in log2 scale. **(b)** Gene ontology (GO) term enrichment for the 772 genes significantly decreased in KLF4 knockdown cells. **(c)** Gene ontology (GO) term enrichment for the 1,265 genes significantly increased in expression in KLF4 knockdown cells. **(d)** Heatmap plot of Pearson correlation coefficients between replicate BRD4i RNA-Seq data sets and replicate KLF4i RNA-Seq data sets (RPKM normalized). **(e-f)** Venn diagram showing the number of genes (461) decreased in both the BRD4 and KLF4 knockdowns and the associated GO term enrichments for the 461 genes. **(g-h)** Venn diagram showing the number of genes (208) increased in both the BRD4 and KLF4 knockdowns and the associated GO term enrichments for the 208 genes.

## METHODS

### Cell Culture

Primary human epidermal keratinocytes (derived from neonatal foreskin) were cultured in EpiLife medium (ThermoFisher MEPI500CA). Patient consent for experiments was not required because primary keratinocytes were purchased from Life Technologies (C0015C). The media was supplemented with Penicillin/streptomycin (HyClone SV30010) and human keratinocyte growth supplement (HKGS, ThermoFisher S0015). Keratinocytes were differentiated by seeding in full confluence and subsequently adding 1.2 mM calcium for 3 days as previously described (Sen et al., 2012).

### Gene knockdown

siRNAs were placed into EpiLife media with the transfection reagent Lipofectamine RNAiMAX (25ul for 10cm plate transfection, 50 ul for 15cm plate transfection) (ThermoFisher 13778) and incubated for 5 minutes. This siRNA media was then diluted 1:10 and added to sub-confluent keratinocytes, with the siRNA at a final concentration of 10nM. The keratinocytes were then incubated in this media for a minimum of 18 hours to carry out siRNA knockdown. The siRNAs used in this study are as follows: Control siRNA (Ambion Silencer Select negative control 4390844), BRD4 siRNA1 (Dharmacon D-004937-05), BRD4 siRNA 2 (Dharmacon D-004937-04), KLF4 siRNA (Dharmacon:custom sequence). siRNA sequences can be found in the supplemental materials.

### Regenerated human epidermis

Human dermis obtained from the New York Firefighters skin bank was devitalized, cut, and placed upon a cassette. The bottom of the dermis was coated in matrigel and the cassette was placed in KGM media, with the bottom of the dermis contacting the media and the top at the air interface. One million keratinocytes were then seeded on the top of the dermis and allowed to regenerate and stratify for five days. The constructs were then harvested for RNA or embedded in OCT for sectioning and staining. Additional details for regenerating human epidermis have been previously described (Li and Sen, 2015, Mistry et al., 2012, Noutsou et al., 2017).

### Immunofluorescent staining

Sectioned tissue derived from regenerated human epidermis was fixed with 10% formalin solution (Sigma HT5012) for 12 minutes. Sections were blocked (PBS, 2% bovine serum albumin, 2.5% normal goat serum, and 0.3% triton X-100) for 30 minutes. Primary antibodies were then added to blocking buffer and put onto sections for 1 hour. The following antibodies were used at the following concentrations: FLG at 1:200 (Abcam ab3137), LOR at 1:400 (Abcam ab198994), KRT10 at 1:400 (ThermoFisher MS-611-P0), and KRT5 at 1:500 (Biolegend PRB-160P). Secondary antibodies were used at 1:500 for 30 minutes and included Alexa Fluor 555 goat anti-mouse IgG (ThermoFisher: A21424) and Alexa Fluor 488 donkey anti-rabbit IgG (ThermoFisher: A21206). Hoechst 33342 (ThermoFisher H3570) was used at 1:1000 to stain nuclei.

### H&E staining

Sectioned tissue derived from regenerated human epidermis was fixed with 10% formalin solution (Sigma HT5012) for 12 minutes. Sections were then dipped in 0.25% Triton-X-100 in PBS for 5 minutes. Haemotoxylin (Vector H-3401) staining was performed for 8 minutes, rinsed in water, and then dipped in acid alcohol (1% HCL in 70% ethanol). After subsequent rinsing, the sections were dipped in 0.2% ammonia water for

1 minute, rinsed again, and then dipped in 95% ethanol. Eosin (Richard-Allan Scientific 71304) staining was performed for 30 seconds followed by 95% ethanol rinsing for 1 minute. Sections were then put into 100% ethanol for 4 minutes followed by 2 minutes in Xylene.

**RNA extraction and analysis by RT-qPCR**

The GeneJET RNA purification kit (Thermo Scientific K0732) was used to extract RNA from cultured keratinocytes. RNA concentration for each sample was measured by nanodrop and 1µg of RNA was used for generating cDNA. This cDNA was generated using the Maxima cDNA synthesis kit (Thermo Fisher: K1642). qPCR was performed using this cDNA on the Roche 480 Light Cycler. The house-keeping genes L32 or GAPDH were used for normalization of signal. Primer sequences for all genes tested are listed in the supplementary materials.

**RNA sequencing and bioinformatic analysis**

RNA isolated from day 3 differentiated keratinocytes was used for sequencing. Samples were sequenced by the Institute of Genomic Medicine core facility at UCSD on the Illumina Hi Seq 4000. Reads were aligned to the hg19 genome build using STAR with default settings. Identification of differential gene expression and downstream analysis was carried out using Partek Genomic Suite (Partek Incorporated, http://www.partek.com/partek-genomics-suite). Differential expression between control and knockdown samples was analyzed using ANOVA. Genes with fewer than ten reads among all samples were filtered out before analysis to avoid genes with low expression. Differentially expressed genes were selected by $\geq$ 2-fold change (+/-) compared to controls and a significant p-value with FDR ($\leq$0.05). Heatmaps representing RNA sequencing data were generated in Partek Genomic Suite. GO terms for selected gene lists were generated using Metascape (Zhou et al., 2019). DeepTools was used to RPKM normalize sequencing files and generate Pearson correlations and the representative heatmaps of those values (Ramirez et al., 2014, Ramirez et al., 2016).

**ChIP sequencing and bioinformatic analysis**

For each pulldown, 20 million keratinocytes were differentiated for 3 days and subsequently crosslinked. These cells were crosslinked using 2mM DSG (disuccinimidyl glutarate, Thermo Fisher 20593) and 1% formaldehyde (ThermoFisher 28908). Cells were placed in Farnham lysis buffer (5 mM PIPES pH 8.0, 85 mM KCl, 0.5% IGEPAL CA-630) and sheared with a syringe. The cells were then pelleted and resuspended in SDS-Lysis Buffer (1% SDS, 10 mM EDTA, 50 mM Tris, pH 8.0) and sonicated in a water bath sonicator. Sonicated lysate was then centrifuged, and the supernatant was diluted 1:10 in low ionic strength ChIP dilution buffer (50mM NaCl, 10mM HEPES, pH 7.4, 1% IGEPAL CA-630, 10% Glycerol) and used for ChIP. 5ug of BRD4 antibody (Bethyl A301-985A) or control rabbit IgG (Millipore 12-370) was used for each pulldown. Antibody/lysate incubation was carried out at 4 degrees overnight. 50ul of Protein-G dynabeads were added to each solution of antibody/lysate the following day and incubated for 4 hours at 4 degrees. The beads were then washed twice with low ionic strength buffer, once with high salt wash buffer (500 mM NaCl, 0.1% SDS, 1% IGEPAL CA-630, 2 mM EDTA, 20 mM Tris, pH 8.0), once with LiCl wash (0.25 M LiCl, 1% IGEPAL CA-630, 1% Sodium Deoxycholate, 1 mM EDTA 10 mM Tris-Hcl, pH 8.0), and twice with TE (10 mM Tris-Cl, pH 7.5, 1 mM EDTA). Samples were then eluted at 65 degrees in elution buffer (0.09 M $NaHCO_3$, 1% SDS, 0.1 M $NaHCO_3$) and de-crosslinking mixture (0.2 M NaCl, 0.1M EDTA,0.4 M Tris-HCl, pH6.8, 0.4 mg/ml

proteinase K) and treated with RNAse A to prevent RNA contamination during sequencing (Mistry et al., 2014, Noutsou et al., 2017). Samples were sequenced by the Institute of Genomic Medicine core facility at UCSD on the Illumina Hi Seq 4000. Sequenced reads were trimmed and aligned to the genome build hg19 using BowTie 2 (Langmead and Salzberg, 2012). Duplicate and low-quality reads were removed. To identify significant peaks, HOMER's findPeaks was used with default statistical settings (p-value < 0.0001, FDR < 0.001, 4x enrichment vs. input) and the following options: for BRD4: style=region, size=150, minDist=500; for KLF4, ZNF750, MAF, MAFB, and GRHL3: style=factor, minDist 200; for H3K27ac and H3K4me: style=region, size=1000, minDist=2500(Heinz et al., 2010). Input sample was used as background in peak calling. Genomic localization was annotated using HOMER's annotatePeaks (Refseq hg19 transcription start sites)(Heinz et al., 2010). HOMER was also used to identify motifs (findMotifsGenome), create UCSC tracks (makeUCSCfile) to be visualized on the UCSC genome browser, and generate normalized mean density plots (annotatePeaks). mergePeaks was used to identify directly overlapping (d=given) peaks between samples. DeepTools was used to RPKM normalize sequencing files and generate Pearson correlations and the representative heatmaps of these values(Ramirez et al., 2014, Ramirez et al., 2016). GO terms for selected gene lists were generated using Metascape (Zhou et al., 2019).

**Statistics**

Statistical analyses were performed using GraphPad Prism. Histogram data are presented as the mean ± SD and the significance of differences between samples was determined by student's t tests.

**Accession numbers:** ChIP-Seq and RNA-Seq data was deposited in GEO with accession number: GSE140992 with reviewer access token: mlilywusnnqdvmh
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**REFERENCES FOR METHODS**


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