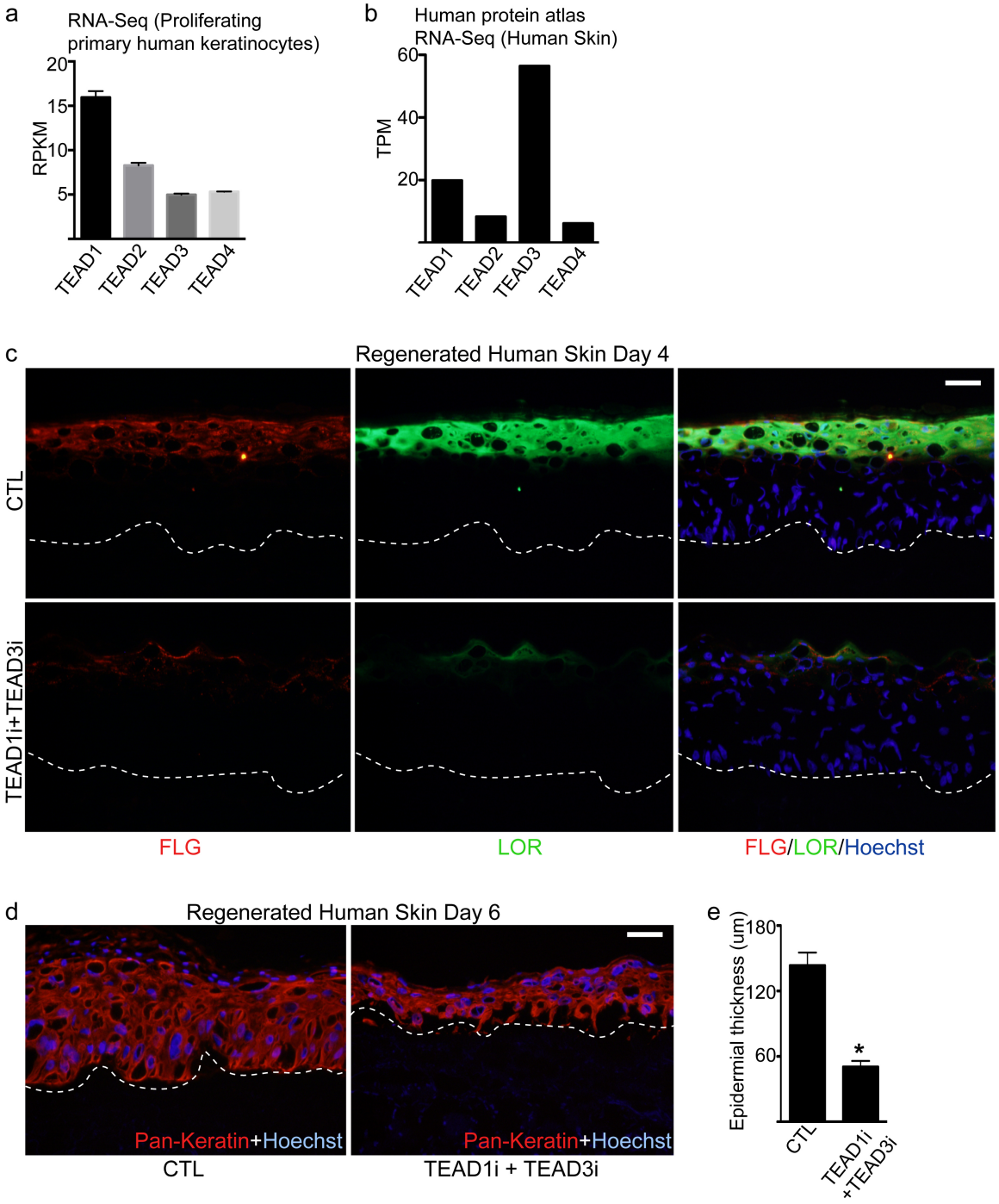
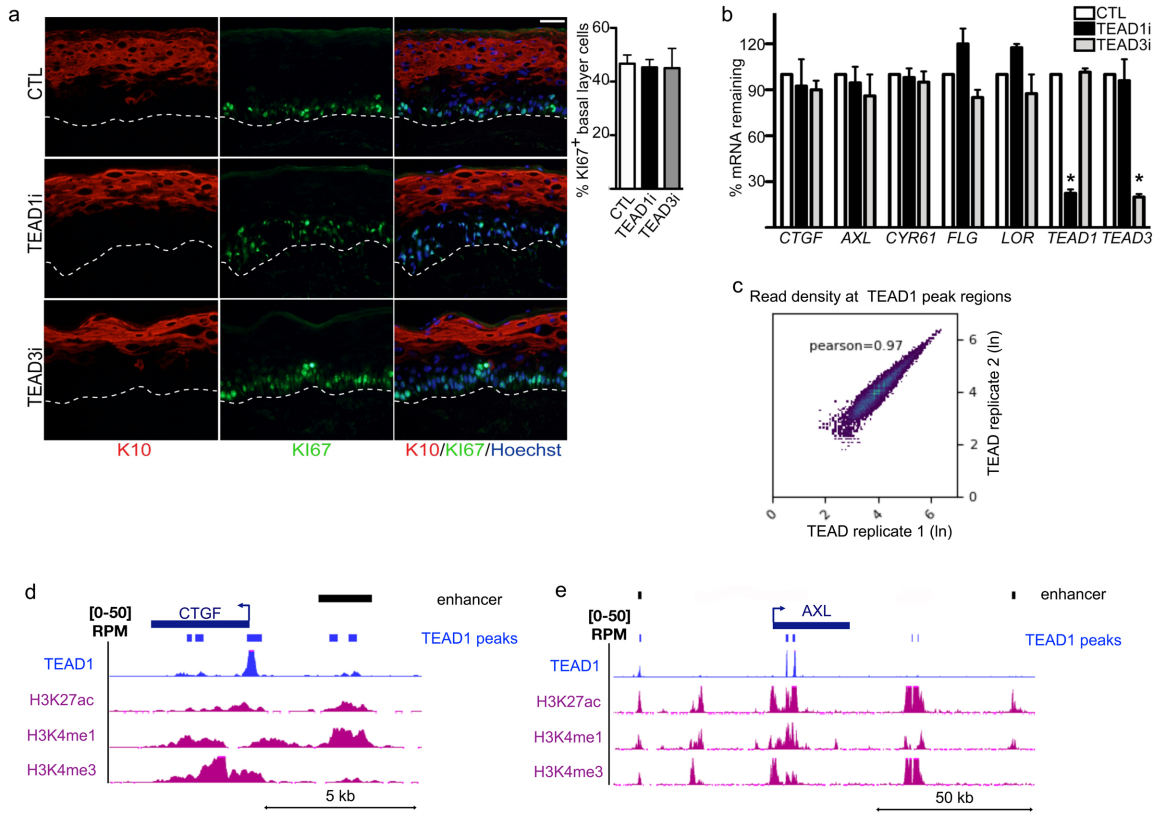
Supplementary Figure 1



Supplementary Figure 1. TEAD1 and TEAD3 are highly expressed in the skin and are necessary for epidermal growth

(a) RNA-seq expression levels of TEAD1-4 in primary human keratinocytes cultured in growth conditions. Expression levels are represented in RPKM. **(b)** RNA-seq expression levels of TEAD1-4 from human skin biopsies (RNA-seq data derived from the Human protein atlas). Expression levels are represented in TPM. **(c)** Human epidermis was regenerated using three-dimensional organotypic cultures with CTLi or TEAD1i+TEAD3i cells. Expression of terminal differentiation proteins filaggrin (FLG:red) and loricrin (LOR:green) were characterized by immunostaining at day 4. White scale bar= 40μm. **(d)** Human epidermis was regenerated using three-dimensional organotypic cultures with CTLi or TEAD1i+TEAD3i cells. Tissue was harvested 6 days after cell seeding. Tissue sections were stained with a Pan-Keratin (red) antibody to determine tissue thickness. White scale bar= 40μm. n=3 for each group. **(e)** Quantitation of epidermal thickness represented in μm. Mean values are shown with error bars=SD. $^{*}p$ <0.05 (t-test).

Supplementary Figure 2



Supplementary Figure 2. Single knockdown of TEAD1 or TEAD3 has no impact on epidermal growth and binding of TEAD1 to the genome

(a) Left Panel: Human epidermis was regenerated using three-dimensional organotypic cultures with CTLi, TEAD1i, or TEAD3i cells. Expression of Keratin 10 (K10:red) and proliferation marker (Ki67:green) were characterized by immunostaining at day 4. White scale bar= 40μm. **(a) Right Panel:** Quantitation of the %KI67 positive cells in the basal layer. **(b)** RT-QPCR for *CTGF*, *AXL*, *CYR61*, *FLG*, *LOR*, *TEAD1*, and *TEAD3* expression levels in tissue knocked down for control (CTL), TEAD1, or TEAD3. Tissue was harvested 4 days after seeding of cells. Mean values are shown with error bars=SD. *p <0.05 (t-test). **(c)** Pearson correlation between the replicate TEAD1 ChIP-Seq data. **(d-e)** Gene tracks showing TEAD1 (blue), H3K27ac (purple), H3K4me1 (purple), and H3K4me3 (purple) binding. Active enhancer (shown as black bars) regions are shown as regions that have H3K27ac and H3K4me1 peaks without the presence of H3K4me3. The presence of H3K4me3 is used to rule out gene promoters and gene bodies.

MATERIALS AND METHODS

Cell culture

Primary human epidermal keratinocytes derived from neonatal foreskin were cultured in EpiLife medium (ThermoFisher: MEPI500CA) mixed with human keratinocyte growth supplement (HKGS, ThermoFisher:S0015) and pen/strep. Patient consent for experiments was not required because primary human keratinocytes were purchased from Life Technologies (C0015C).

Knockdown of genes

siRNAs targeting TEAD1, TEAD2, TEAD3, TEAD4 and control siRNAs were purchased from Dharmacon and Life Technology. Cells were transfected using Lipofectamine RNAiMax (ThermoFisher: 13778100) to knockdown genes. The following siRNA sequences were used to target the following genes: TEAD1: GGACATTCGTCAGATTTAT, TEAD3: GATTGCACGCTATATTAAA, TEAD2: GCAGTTGATTCTTACCAGA, TEAD4: GGACACTACTCTTACCGCA.

RNA isolation and RT-QPCR

Total RNA from cells or tissue was extracted using the GeneJET RNA purification kit (Thermo Scientific: K0732) and quantified using a Nanodrop. One µg of total RNA was reversed transcribed using the Maxima cDNA synthesis kit (Thermo Fisher: K1642). Quantitative PCR was performed using the Bio-Rad CFX96 Touch Real-Time PCR Detection System. L32 was used as an internal control for normalization. Primer sequences for *L32*, *CTGF*, *AXL*, *TEAD1*, *TEAD2*, *TEAD3*, *TEAD4*, *BIRC5*, *FGFBP1*, *CCNA2*, *MYC*, *CDK1*, *CYR61* are as follows:
L32 forward: AGGCATTGACAACAGGGTTC, L32 reverse: GTTGCACATCAGCAGCACTT;
CTGF forward: GCGAAGCTGACCTGGAAG, CTGF reverse: GCAGGGTGGTGGTTCTGT;
AXL forward: CGGTGTCAGCTCCAGGTT, AXL reverse: ACTGTCCCGTGTCGGAAA;
TEAD1 forward: GGACAGGCAAGACGAGGA, TEAD1 reverse: AGTGGCCGAGACGATCTG;
TEAD2 forward: CCTTCTCTGCAGGCCAAA, TEAD2 reverse: GCTCGTACCCTGGGAGGT;
TEAD3 forward: GCCACTGTTCTGCGCTTT, TEAD3 reverse: GTGGGTGGGGGTACAGTG;
TEAD4 forward: TGGACATCCGCCAAATCT, TEAD4 reverse: TCGGGGCTCTCATACTGG;
BIRC5 forward: TTCGCTGGAAACCTCTGG, BIRC5 reverse: CTCTGCCAGGACGCTCAT;
CDK1 forward: GCTGGGGTCAGCTCGTTA, CDK1 reverse: TTCCACTTCTGGCCACACT;
CCNA2 forward: AGACGAGACGGGTTGCAC, CCNA2 reverse: AAAGCCAGGGCATCTTCA;
FGFBP1 forward: TCTGGGCAACACCCAGAT, FGFBP1 reverse: GGCATGAGGTTGGATTGC;
CYR61 forward: GGTTTCCAGGGCACACCT, CYR61 reverse: AGTGTCCATCCGCACCAG;
MYC forward: CGGACACCGAGGAGAATG, MYC reverse: GCTTGGACGGACAGGATG.

Regenerated human skin and immunofluorescence

For the organotypic skin cultures, one million control or TEAD1 and TEAD3 knockdown cells were seeded onto devitalized human dermis to regenerate human skin as previously described (Li and Sen, 2015, Mistry et al., 2012, Sen et al., 2010). Tissue was harvested 4 days after initial seeding. Tissue was sectioned and fixed in 4% paraformaldehyde for 11 minutes followed by blocking in PBS with 2.5% normal goat serum, 0.3% triton X-100, and 2% bovine serum albumin for 30 minutes. Primary antibodies used were Keratin 10 (Abcam: ab9025) at 1:500, Filaggrin (Abcam: ab3137) at 1:200, MKi67 (Abcam: ab16667) at 1:300, loricrin (Abcam: ab198994) at 1:1000 for 1 hour. The secondary antibodies used were Alexa 555 conjugated goat anti-mouse IgG (ThermoFisher: A11029) or Alexa 488 conjugated donkey anti-rabbit IgG (ThermoFisher: A21206) both at 1:500. Nuclear dye, Hoechst 33342 was used at 1:1000 (ThermoFisher: H3570).

ChIP-Seq and bioinformatic analysis

Ten million cells and 5 µg of TEAD1 antibody (Abcam:133533) or rabbit IgG (Millipore:12-370) were used for pulldown experiment for ChIP. Cells were fixed in both 1% formaldehyde and 2 mM disuccinimidyl glutarate (Thermo Fisher 20593) for ChIP-Seq as described previously (Li et al., 2019). The ChIP DNA library was prepared using the TrueSeq DNA sample prep kit (Illumina). Sequencing was performed on HiSeq 4000 System using single 1×75 reads at the Institute for Genomic Medicine core, UCSD. TEAD1 ChIP-Seq was performed in duplicates. As described previously (Li et al., 2019), the ChIP-Seq data were processed by the ENCODE Transcription Factor and Histone ChIP-Seq processing pipeline (https://github.com/ENCODE-DCC/chip-seq-pipeline2). The reads were aligned to the hg19 reference genome using bwa 0.7.13 and then deduplicated using picard 2.10.6. MACS2.1.1 was then used to call the peaks by applying a cutoff q-value of

0.05 (Zhang et al., 2008). Track hub hosting all the relevant tracks of peaks or signal was generated to enable UCSC genome browser visulization and track annotation. To identify common enhancers, we used mergePeaks in HOMER (Heinz et al., 2010) to generate H3K27ac and H3K4me1 overlapped peaks and the VennDiagram R package to generate the Venn Diagrams (Chen and Boutros, 2011). Genomic Regions Enrichment of Annotations Tool (GREAT) analysis was used to identify associated genes and distance to TSS (McLean et al., 2010). Pearson correlation density plot was generated using deeptools plot correlation (Ramirez et al., 2014). De novo motif analysis was performed using HOMER (Heinz et al., 2010).

Statistical analysis

Graph data are presented as mean ±SD. Statistical analyses were performed using GraphPad prism. Student's t tests a were used to compared between groups and significant changes were defined as p <0.05.

Data availability

The TEAD1 ChIP-Seq data has been deposited in GEO with the following accession number: GSE138727 at the following site: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE138727

REFERENCES FOR METHODS

Chen H, Boutros PC. VennDiagram: a package for the generation of highly-customizable Venn and Euler diagrams in R. BMC Bioinformatics 2011;12:35.

Heinz S, Benner C, Spann N, Bertolino E, Lin YC, Laslo P, et al. Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. Mol Cell 2010;38(4):576-89.

Li J, Chen Y, Xu X, Jones J, Tiwari M, Ling J, et al. HNRNPK maintains epidermal progenitor function through transcription of proliferation genes and degrading differentiation promoting mRNAs. Nature communications 2019;10(1):4198.

Li J, Sen GL. Generation of Genetically Modified Organotypic Skin Cultures Using Devitalized Human Dermis. J Vis Exp 2015(106):e53280.

McLean CY, Bristor D, Hiller M, Clarke SL, Schaar BT, Lowe CB, et al. GREAT improves functional interpretation of cis-regulatory regions. Nat Biotechnol 2010;28(5):495-501.

Mistry DS, Chen Y, Sen GL. Progenitor function in self-renewing human epidermis is maintained by the exosome. Cell Stem Cell 2012;11(1):127-35.

Ramirez F, Dundar F, Diehl S, Gruning BA, Manke T. deepTools: a flexible platform for exploring deep-sequencing data. Nucleic Acids Res 2014;42(Web Server issue):W187-91.

Sen GL, Reuter JA, Webster DE, Zhu L, Khavari PA. DNMT1 maintains progenitor function in self-renewing somatic tissue. Nature 2010;463(7280):563-7.

Zhang Y, Liu T, Meyer CA, Eeckhoute J, Johnson DS, Bernstein BE, et al. Model-based analysis of ChIP-Seq (MACS). Genome biology 2008;9(9):R137.