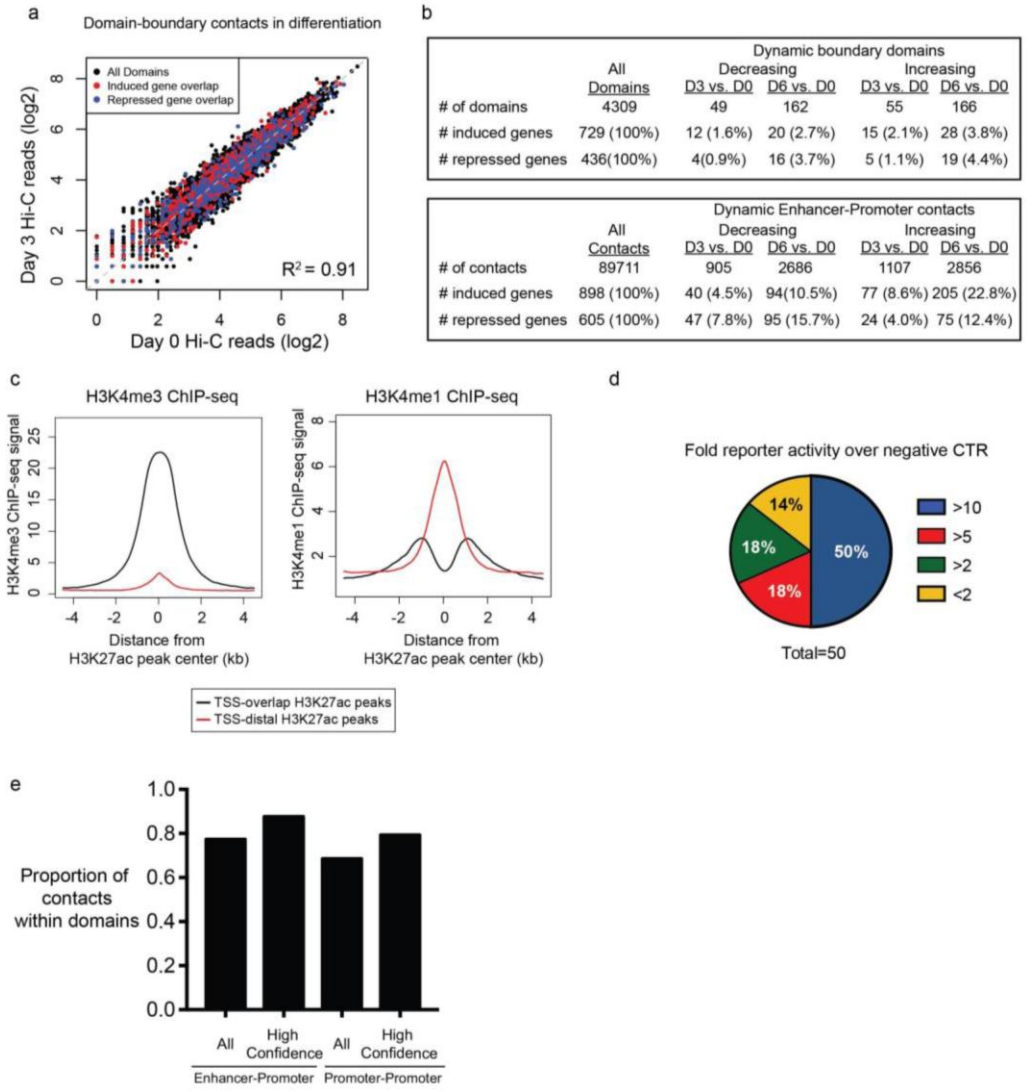
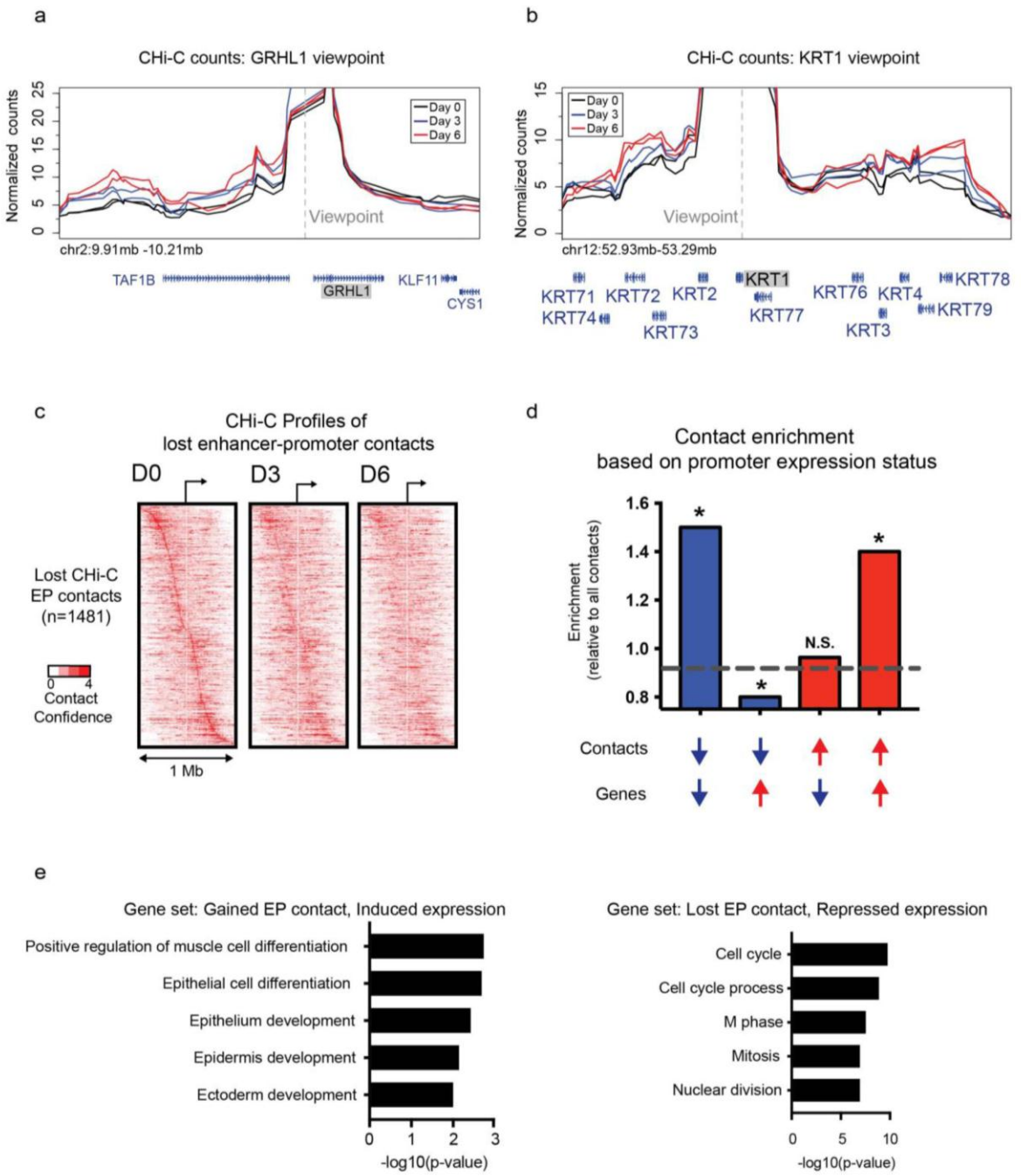


Supplementary Figure 1

Characterization of Hi-C/CHi-C dynamics and enhancer identification.

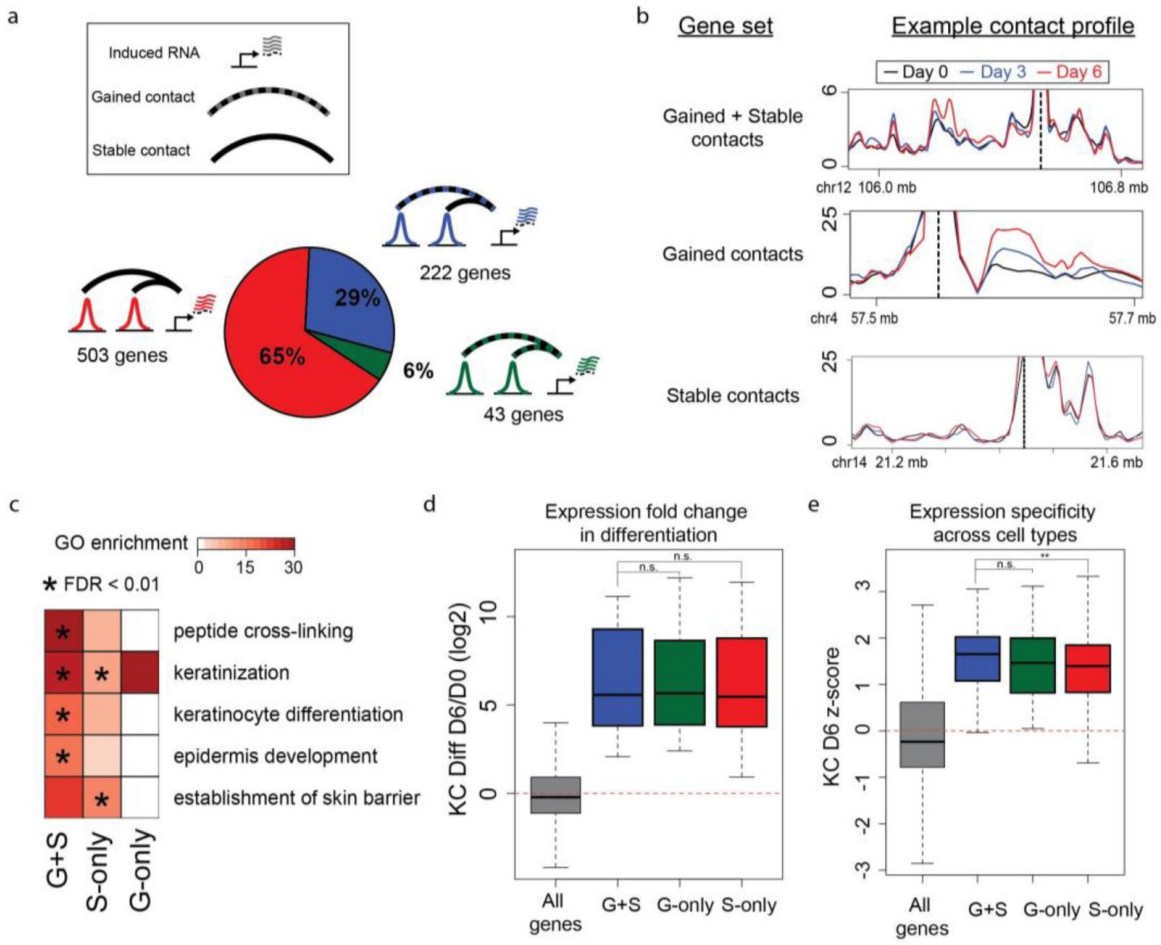
(**a**) Scatterplot of Hi-C read counts supporting contacts between domain boundaries. Contacts enclosing domains containing differentially expressed genes are highlighted. (**b**) Prevalence of differentially expressed genes associated with dynamic domain contacts and dynamic enhancer–promoter contacts. Increasing and decreasing contact sets refer to contacts significantly gained and lost, respectively, on day 3 or day 6 versus day 0 (dynamic contact calls by edgeR, FDR < 0.1, minimum of 15 reads supporting contact in at least one condition). (**c**) Metaplots of ChIP–seq signal from the ENCODE Project, derived from normal human epidermal keratinocyte (NHEK) cells. H3K27ac peaks identified in this study. TSS distal indicates peaks greater than 5 kb away from a TSS. TSS-proximal peaks exhibit the expected promoter-associated H3K4me1-low, H3K4me3-high signature, while TSS-distal peaks exhibit the expected enhancer-associated H3K4me1-high, H3K4me3-low signature. (**d**) Distribution of enhancer reporter activity from H3K27ac-marked regions. Measurements of 50 putative H3K27ac-marked enhancer elements were made in progenitor keratinocytes. The proportion of elements showing different fold inductions during differentiation over empty control vector is shown. (**e**) Proportion of enhancer–promoter and promoter–promoter contacts contained within domains. The high-confidence set corresponds to contacts supported by greater than 15 reads in any replicate of CHi-C (this set was used for edgeR differential contact analysis).



Supplementary Figure 2

Characterization of CHi-C dynamics and relationship with dynamic gene expression.

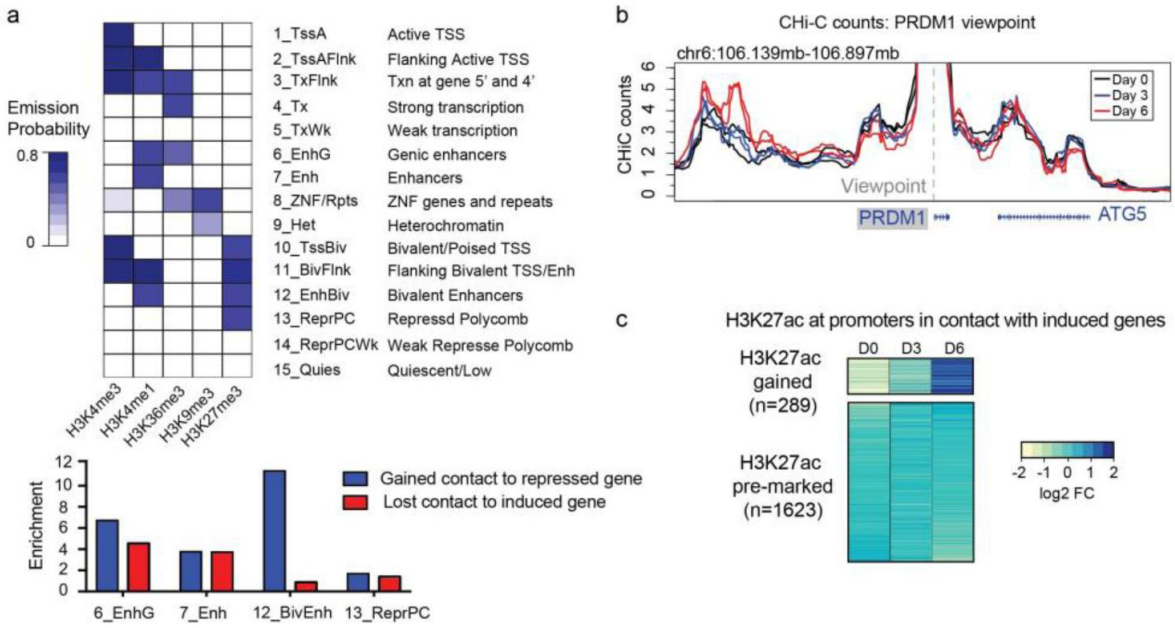
(**a**) Normalized CHi-C read count profile for contacts from the *GRHL1* promoter (normalized to total reads associated with the viewpoint fragment in each condition, days 0–6 of differentiation). The gray dotted line marks the position of the viewpoint fragment. (**b**) As in **a**, for contacts from the *KRT1* promoter. (**c**) Heat map of CHi-C *q* scores for 1,481 distal genomic region contacts displaying decreasing contact with target promoters during epidermal cell differentiation. Contacts are sorted in order of enhancer–promoter distance (edgeR, FDR < 0.1, fold change > 2). (**d**) Enrichment of overlap between dynamic contacts and gene subsets by dynamic expression. Enrichment >1.0 represents the scenario in which the contact and gene subsets co-occurred more frequently than expected by chance (empirical FDR, N.S., $P > 0.05$, $*P < 0.05$). (**e**) Enriched GO terms for induced or repressed gene sets displaying either gained (left) or lost (right) enhancer–promoter (EP) contacts.



Supplementary Figure 3

Identification and characterization of gene sets based on contact dynamics.

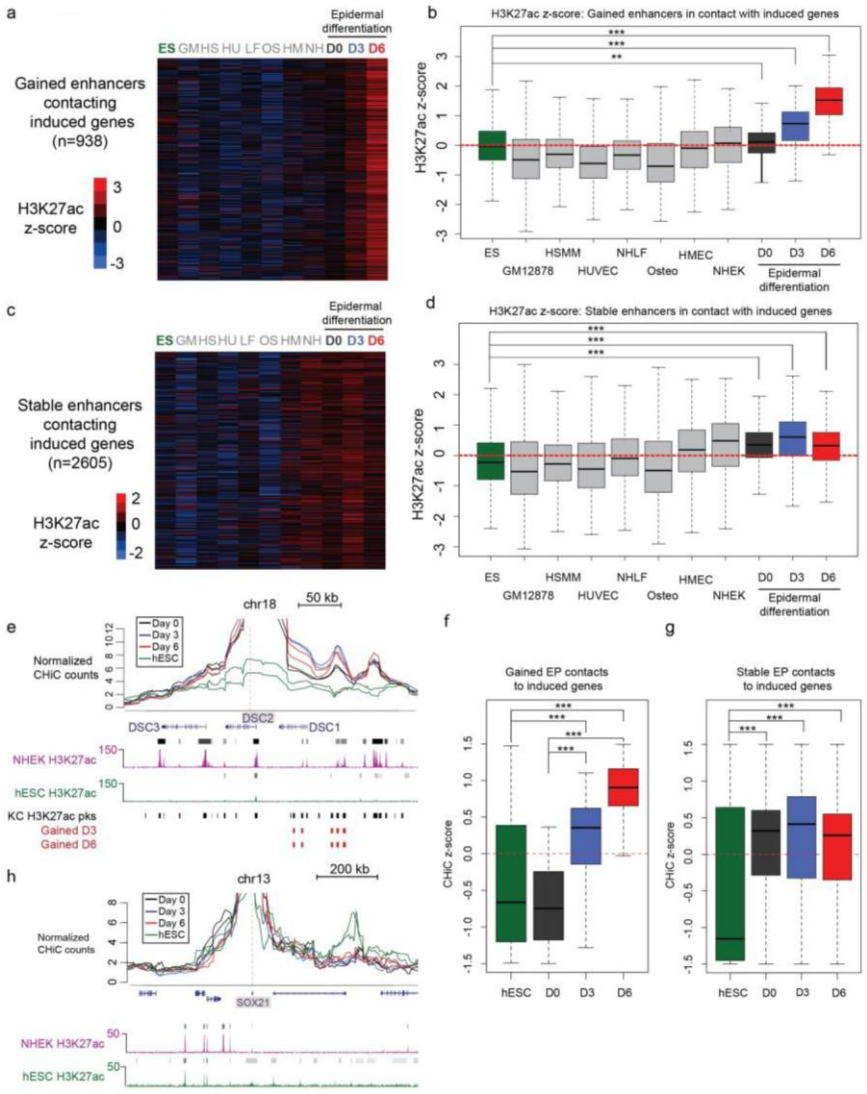
(**a**) Pie chart representation of three classes of genes induced at day 6 versus day 0 of differentiation. Genes are classified by association with stable or gained contacts in differentiation. (**b**) Example promoter viewpoint CHi-C read count profiles for genes of each of three classes of induced genes. (**c**) Enrichment values for GO terms enriched at FDR < 0.01 in at least one of three gene classes. An asterisk indicates that the term was significantly enriched in that specific gene set. (**d**) Box-and-whisker plots representing expression fold change on day 6 versus day 0 of differentiation for all genes or classes of induced genes (two-sided KS test, GS versus G $P = 0.9503$, GS versus S $P = 0.4915$). (**e**) Box-and-whisker plots representing the z score of expression on day 6 versus other differentiation days and collection of ENCODE cell types (Online Methods) for all genes or each class of induced genes (two-sided KS test, GS versus G $P = 0.7501$, GS versus S $P = 0.2.536 \times 10^{-3}$). KS test, n.s., $P > 0.05$, *$P < 0.05$, **$P < 0.01$.



Supplementary Figure 4

Chromatin state and dynamics of induced gene-linked enhancers and promoters.

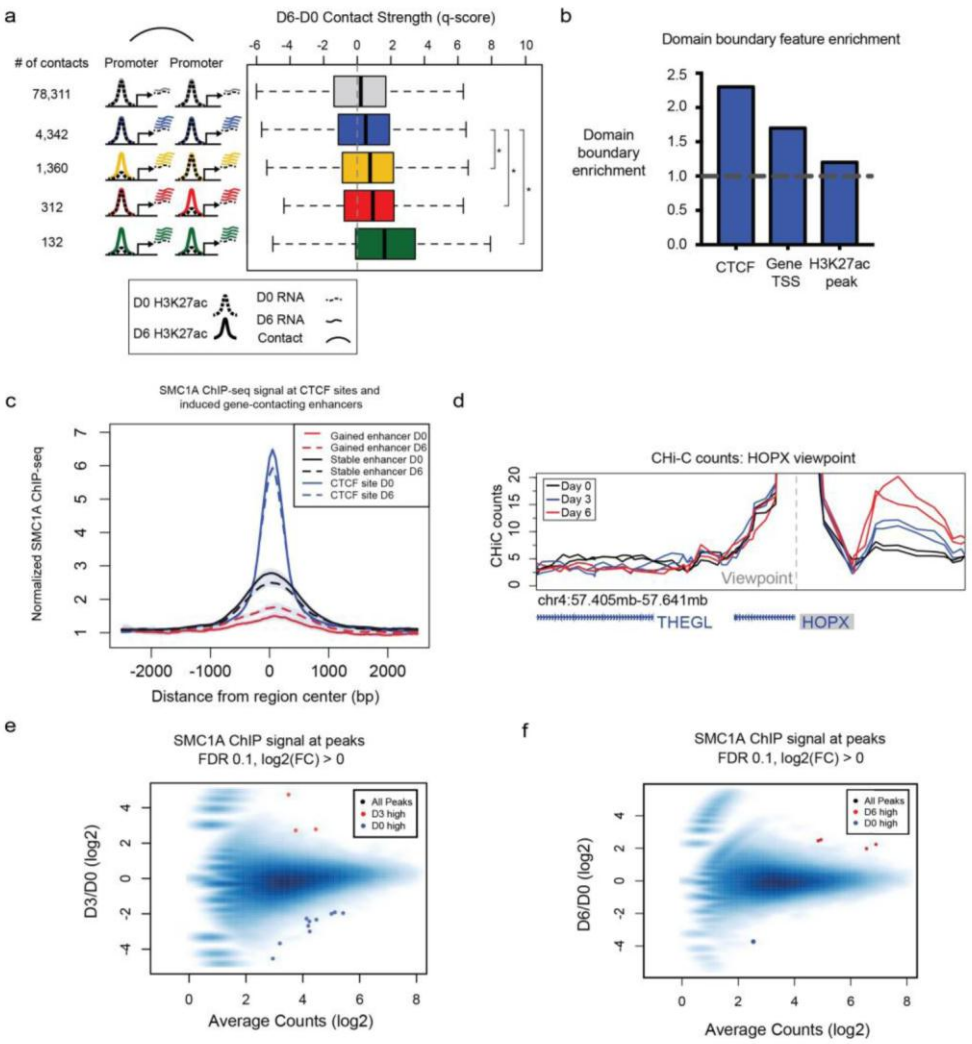
(**a**) Top, heat map of chromHMM chromatin state segmentation emission probabilities for NHEKs from the Roadmap Epigenomics Project. State codes are accompanied by descriptions. Bottom, enrichment fold changes of putative repressive distal regions in select enhancer (genic enhancer and enhancer) and repressive (bivalent enhancer and repressed Polycomb) chromHMM states. (**b**) Normalized CHi-C read count profile for contacts from the *PRDM1* promoter (normalized to total reads associated with the viewpoint fragment in each condition). The gray dotted line marks the position of the viewpoint fragment. (**c**) H3K27ac at genomic regions as in **Figure 2c** showing H3K27ac at promoter elements in contact with the promoters of induced differentiation genes.



Supplementary Figure 5

Developmental and lineage specificity of induced gene-linked enhancers.

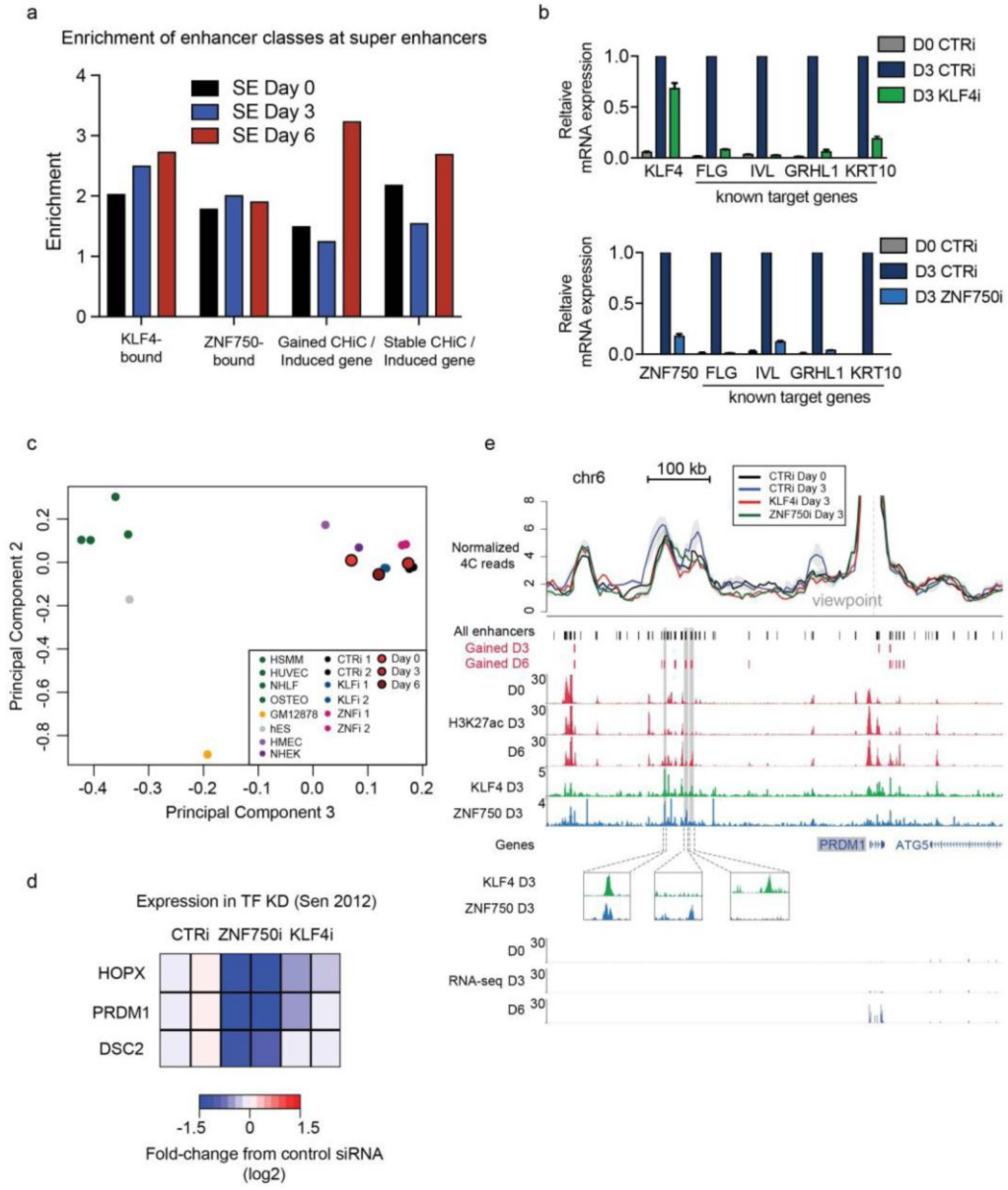
(**a**) Heat map of H3K27ac ChIP–seq signal at enhancers contacting induced genes and exhibiting gained H3K27ac in differentiation. *z* score is calculated for ChIP–seq signal across rows (cell types). Column labels correspond to the following: ES, H1 hESC human embryonic stem cells; GM, GM12878 immortalized B lymphocytes; HS, human skeletal muscle cells and myoblasts; HU, HUVEC human umbilical vein endothelial cells; LF, NHLF normal human lung fibroblasts; OS, OSTEO human primary osteoblasts; HM, HMEC human mammary epithelial cells; NH, NHEK normal human epidermal keratinocytes; D0, day 0; D3, day 3; D6, day 6. (**b**) Box-and-whisker plots representing the heat map in **d** (two-sided KS test, D0 versus hES $P = 7.292 \times 10^{-9}$, D3 versus hES $P < 2.2 \times 10^{-16}$, D6 versus hES $P < 2.2 \times 10^{-16}$). (**c**) Similar to **d**, including enhancers contacting induced genes and exhibiting no significant dynamics of H3K27ac in differentiation. (**d**) Box-and-whisker plots representing the heat map in **f** (two-sided KS test, D0 versus hES $P < 2.2 \times 10^{-16}$, D3 versus hES $P < 2.2 \times 10^{-16}$, D6 versus hES $P < 2.2 \times 10^{-16}$). (**e**) Normalized CHi-C read count profile for contacts from the *DSC2* promoter (normalized to total reads associated with the viewpoint fragment in each condition). The gray dotted line marks the position of the viewpoint fragment. NHEK and hESC H3K27ac signal tracks and peak calls are taken directly from the ENCODE portal of the UCSC Genome Browser. (**f**) Box-and-whisker plots representing CHi-C read count *z* scores for induced gene-associated contacts gained in epidermal differentiation (two-sided KS test, D6 versus hES $P < 2.2 \times 10^{-16}$, D3 versus hES $P < 2.2 \times 10^{-16}$). (**g**) Similar to **f** for induced gene-associated contacts that are stable in epidermal differentiation (two-sided KS test, D0 versus hES $P < 2.2 \times 10^{-16}$, D3 versus hES $P < 2.2 \times 10^{-16}$, D6 versus hES $P < 2.2 \times 10^{-16}$). (**h**) Similar to **e**, for profiles of the *SOX21* promoter, which is expressed specifically in hESCs. KS test, $^{**}P < 1 \times 10^{-5}$, $^{***}P < 1 \times 10^{-15}$.



Supplementary Figure 6

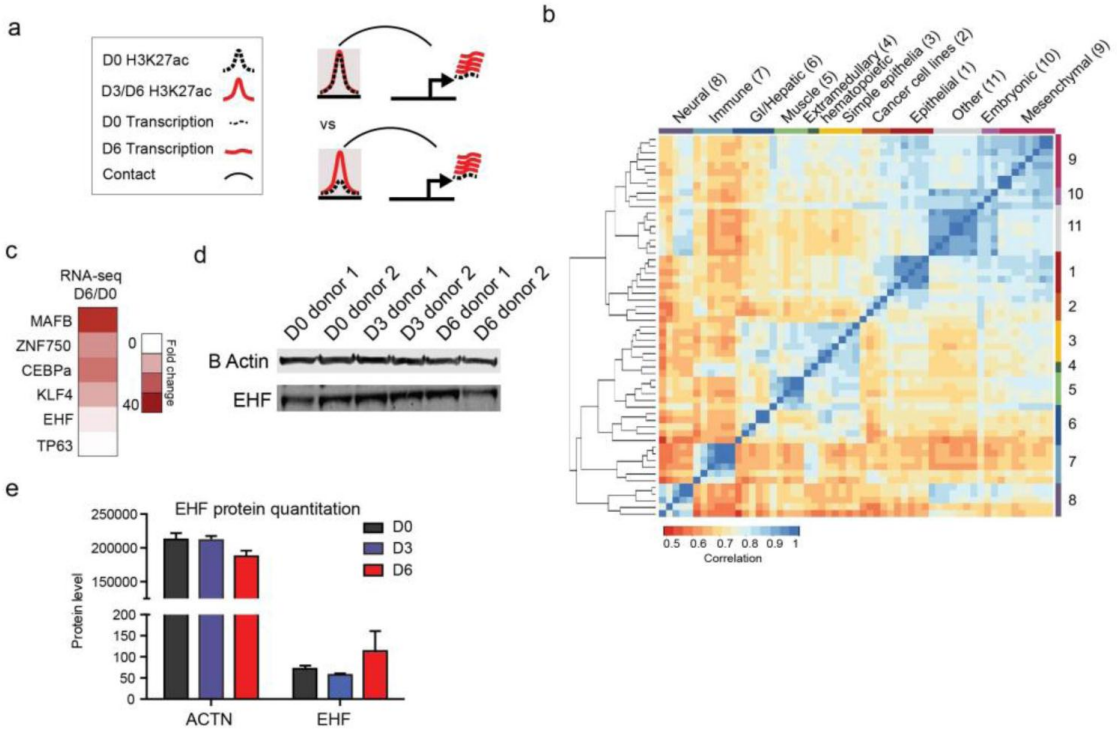
Characterization of contact dynamics and cohesin association of induced gene-linked enhancers.

(**a**) Contact strength between promoter elements in contact with the promoters of induced differentiation genes as in **Figure 2d**. Box-and-whisker plots represent the median and interquartile range of the change in contact *q* score between day 6 and day 0. Contact sets are defined by H3K27ac dynamics at the promoter locus (bait HindIII fragment) or distal promoter. Dotted lines denote day 0 for either H3K27ac signal or differentiation gene mRNA expression; solid lines denote day 6. (**b**) Enrichment of genomic features in 10-kb regions surrounding domain boundaries relative to 10-kb regions in domain centers. (**c**) SMC1A ChIP–seq metaplot signal at H3K27 premarked and H3K27ac gained putative enhancers that contact induced genes as well as CTCF peaks. Error bands represent 98% boostrapped confidence intervals. (**d**) Normalized CHi-C read count profile for contacts from the *HOPX* promoter (normalized to total reads associated with the viewpoint fragment in each condition). The gray dotted line marks the position of the viewpoint fragment. (**e**) MA plot (Online Methods) of SMC1A ChIP–seq signal at enhancer loci on day 3 versus day 0. Loci identified as significantly dynamic are highlighted (edgeR, FDR < 0.1, fold change > 0). (**f**) MA plot of SMC1A ChIP–seq signal at enhancer loci on day 6 versus day 0. Loci identified as significantly dynamic are highlighted (edgeR, FDR < 0.1, fold change > 0).



Supplementary Figure 7

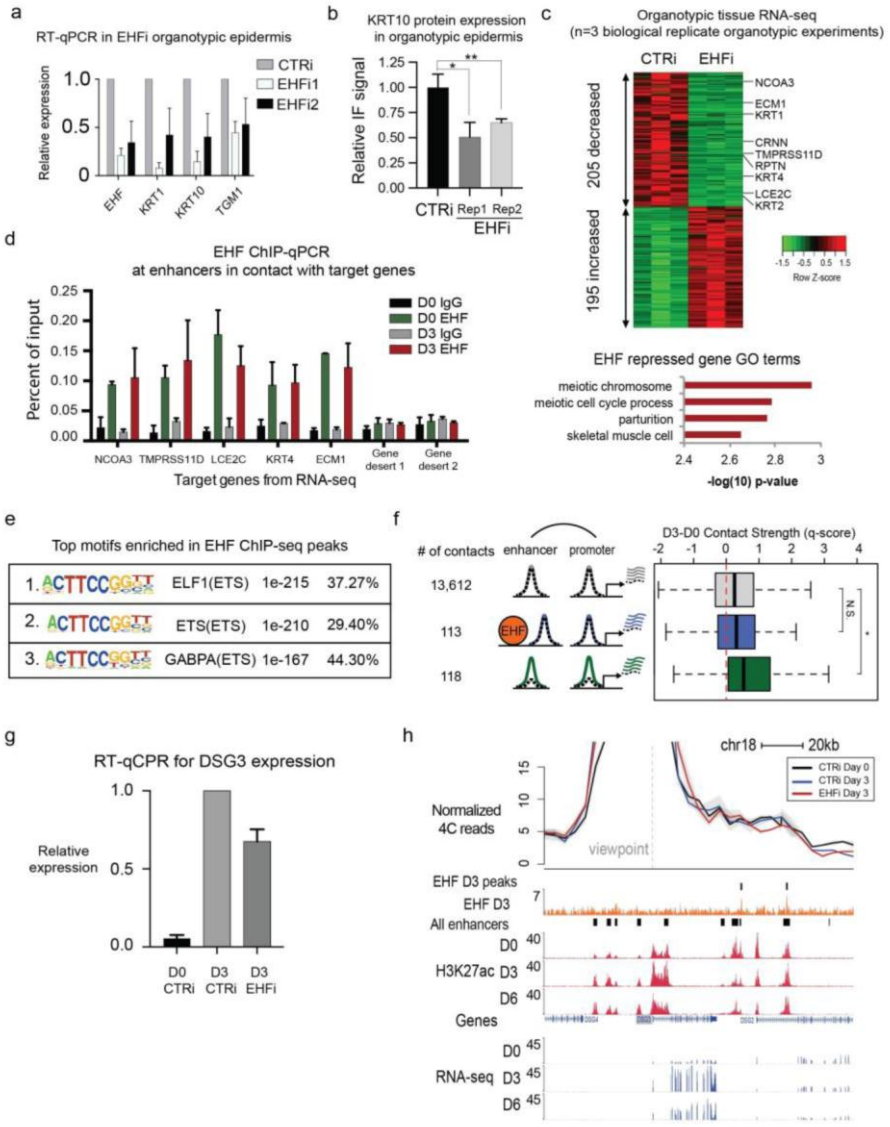
Developmental context of transcription factor depletion and further characterization of effect on chromatin conformation. (**a**) Enrichment of enhancer subsets versus all enhancers in super-enhancers identified on day 0, day 3, or day 6. (**b**) Relative expression by RT–qPCR for *KLF4*, *ZNF750*, and several known target genes in siRNA-treated cells. Error bars represent s.d. across two technical replicates (independent samples from a pool of siRNA-treated cells). (**c**) Principal-component analysis of H3K27ac ChIP–seq signal in a panel of ENCODE cell types, normal epidermal differentiation time points, and differentiating epidermal cells treated with control or transcription factor–targeting siRNA. CTRi, control siRNA; KLFi, KLF4 siRNA; ZNFi, ZNF750 siRNA. Epidermal cells, including transcription factor–depleted cells, cluster closely. (**d**) Relative expression by published data (*Dev. Cell* **22**, 669–677, 2012) for genes analyzed by UMI-4C in siRNA-treated cells. (**e**) UMI-4C profile of interactions anchored by the *PRDM1* promoter in control and KLF4- or ZNF750-knockdown conditions. Error bands represent s.e.m. between replicates (independent samples of siRNA-treated cells).



Supplementary Figure 8

Discovery and characterization of EHF as a novel regulator of epidermal differentiation

(**a**) Comparative approach to identify transcription factor motifs enriched in H3K27ac premarked enhancers relative to H3K27ac gained enhancers in contact with induced differentiation genes. (**b**) Heat map of Pearson correlations for mRNA expression values derived from RNA-seq (Roadmap Epigenomics). Hierarchical clustering was used to identify sets of cell types labeled by names (columns) and group IDs (rows). (**c**) Gene expression fold change of *EHF* and other transcription factors during differentiation by RNA-seq. Day 6 was compared with day 0. (**d**) Western blot of EHF at days 0, 3, and 6 of differentiation for primary human keratinocytes derived from two independent donors. (**e**) Quantification of the western blot in **d**, with normalization to actin loading control.



Supplementary Figure 9

Characterization of EHF and functional effects of EHF depletion on chromatin.
(**a**) RT–qPCR of *EHF* and differentiation-specific genes in organotypic epidermis depleted of EHF using two independent siRNAs targeting EHF versus scrambled controls. Error bars represent s.d. across two biological replicates (independent organotypic tissues). (**b**) Quantification of differentiation protein immunofluorescence signal area within the epidermis across technical replicates for control and two biological replicate organotypic epidermal tissues depleted of EHF (Student's *t* test, one-sided, Rep 1 versus Control $P = 3.54 \times 10^{-2}$, Rep 2 versus Control $P = 9.3 \times 10^{-3}$; $^{*}P < 0.05$, $^{**}P < 0.01$). (**c**) Heat map of RNA-seq *z* scores for all differentially expressed genes in triplicate biological replicate EHF-depleted (EHFi) versus control (CTRi) organotypic epidermal tissue. GO enrichments correspond to genes exhibiting increased expression with EHF depletion. (**d**) ChIP–qPCR of EHF, day 0 and day 3, demonstrating enrichment at a panel of enhancers in contact with promoters of genes induced in differentiation. Genes are noted in the heat map in **b**. Error bars represent s.d. across biological replicates (independent samples of cells from a single culture). (**e**) Top motifs identified by HOMER in EHF ChIP–seq peaks relative to a random background. (**f**) Box-and-whisker plots representing the median and interquartile range of the change in contact *q* score between day 3 and day 0. Contact sets are defined by EHF binding and H3K27ac dynamics at the promoter locus (bait HindIII fragment) or enhancer (empirical FDR, $^{*}P < 0.01$). (**g**) RT–qPCR of *DSG3* in control and EHF-depleted cells. Error bars represent s.d. across technical replicates. (**h**) UMI-4C profile of interactions anchored by the *DSG3* promoter in control and EHF-knockdown conditions. Error bands represent s.e.m. between biological replicates.