HHS Public Access

Author manuscript

J Invest Dermatol. Author manuscript; available in PMC 2022 May 01.

Published in final edited form as:

J Invest Dermatol. 2021 May ; 141(5): 1352–1356.e3. doi:10.1016/j.jid.2020.09.024.

ELL facilitates RNA Polymerase II mediated transcription of human epidermal proliferation genes

Jingting Li[1], **Varun Bansal**[1], **Manisha Tiwari**[1], **Yifang Chen**[1], **George L. Sen**[1,+]

[1]Department of Dermatology, Department of Cellular and Molecular Medicine, UCSD Stem Cell Program, University of California, San Diego, La Jolla, CA 92093-0869

Keywords

Stem Cell; Proliferation; Epidermis; Skin Differentiation; ELL; SMC4; CCNB2; PBK; CDC20; IGFL3; Skin; Transcription Elongation; RNA Polymerase II; Super Elongation Complex; Pre-initiation Complex; Keratinocytes

TO THE EDITOR

The first step of messenger RNA synthesis begins with the recruitment and stabilization of RNA Polymerase II (Pol II) and other components of the transcription apparatus to the promoter and formation of a pre-initiation complex. This step of transcription initiation has been considered the rate-limiting step and controls expression of a significant number of genes. Recent evidence has also demonstrated that transcriptional control at the post-initiation steps, including promoter proximal pausing and elongation are central for regulating gene expression(Adelman and Lis, 2012). Shortly after transcription initiation, Pol II is paused at the promoter-proximal region by negative elongation factors NELF and DSIF. Positive elongation factor b (P-TEFb) formed by CDK9 and cyclin T comes and phosphorylates the negative elongation factors to overcome the pausing of Pol II and initiate early elongation. P-TEFb also phosphorylates the C-terminal domain of Pol II and enables elongation-coupled mRNA processing. To prevent Pol II pervasive pausing and backtracking, the formation of super elongation complexes (SECs) is required which enhances processivity of Pol II and promotes elongation(Smith et al., 2011).

[+]Correspondence: George L. Sen, 9500 Gilman Drive, La Jolla, CA 92093-0869, Phone:(858) 246-0268, gsen@ucsd.edu.

AUTHOR CONTRIBUTIONS

Conceptualization: GLS, JL; Data curation: JL, VB, MT, YC; Formal Analysis: JL, GLS; Funding Acquisition: GLS; Investigation: JL, VB, MT, YC.

DATA AVAILABILITY

The ELL ChIP-Seq and RNA Pol II ChIP-Seq data has been deposited in GEO with the following accession number: GSE155667 with reviewer access token: alwlgeqklvevjyn at the following site: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE155667

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

Patient consent for experiments was not required because primary human keratinocytes were purchased from Life Technologies (C0015C).

The eleven-ninteen lysine-rich leukaemia (ELL) protein was identified as part of the SEC which promotes transcription elongation with other SEC components such as AFF1, P-TEFb, ENL, AF9, and AFF4(Chen et al., 2018). It was found in rat liver nuclear extracts and purified by its ability to stimulate the elongation rate by reducing Pol II stalling during active transcription(Shilatifard et al., 1996). More recently, ELL was shown to be important for transcription initiation by promoting the stabilization of RNA Pol II recruitment. This function was shown to be independent of the SEC in order to turn on rapidly induced genes in HEK293 cells(Byun et al., 2012). In mammals, ELL knockout mice resulted in an embryonic lethal phenotype, indicating that ELL is required for early embryogenesis(Mitani et al., 2000). However, it is unknown whether ELL plays any role in the context of adult stem and progenitor cells, let alone human epidermis. It is also unclear whether ELL acts through stabilization of Pol II recruitment to the pre-initiation complex or with the SEC to promote elongation in primary cells.

For the past decade, our lab has been focused on characterizing critical players in the maintenance of self-renewal and prevention of premature differentiation of human epidermal stem and progenitor cells, including transcription factors (ZNF750, SNAI2, TEAD1/3, KLF3) (Jones et al., 2020b, Li et al., 2020, Mistry et al., 2014, Sen et al., 2012) and epigenetic factors (DNMT1, JMJD3,cohesin complex, BRD4)(Jones et al., 2020a, Noutsou et al., 2017, Sen et al., 2010, Sen et al., 2008). Mutations or dysfunction of these regulators of epidermal homeostasis could result in various disorders of the skin, such as psoriasis, cancer, eczema, and ichthyosis(Lopez-Pajares et al., 2013). Thus, the elucidation of transcriptional regulatory mechanisms in human epidermis would provide more insights into potential therapeutics of skin diseases.

To determine whether ELL plays a role in human epidermal function, we first transfected human keratinocytes with control siRNA (CTLi) as well as two individual ELL siRNAs (ELL-A/Bi) targeting different regions of the ELL gene and observed consistent loss of cell number due to depletion of ELL (Figure 1a,1e). Part of the decreased cell number was due to apoptosis as ELL knockdown cells had higher levels of apoptotic cells (Figure 1b). To recapitulate the in vivo physiological conditions of human epidermis, we used our three-dimensional organotypic culture system(Li et al., 2019, Li and Sen, 2015), which regenerates human skin by seeding primary human keratinocytes on devitalized human dermis. This allows the cells to establish cell-cell and cell-basement membrane contact to allow proper growth, differentiation, and stratification all in a 3D context. CTLi or ELL knockdown cells (ELL-A/Bi) were seeded onto the dermis to regenerate human skin. The proliferative capacity of ELL knockdown basal layer cells were abolished 4 days after seeding on dermis, which can be seen from the dramatic decrease in Ki67 staining (Figure 1c and 1d). This correlated with decreased gene expressions of *MKI67* and cell cycle gene *CDK1*, in the ELL knockdown tissue (Figure 1e). However, depletion of ELL did not impact levels of early differentiation markers such as K1 and K10 (Figure 1c,1e,1f). Late differentiation factors (*IVL*, *SPRR1A*, *ZNF750*) were mostly unchanged except for FLG which was diminished in ELL knockdown epidermis (Figure 1e–1f). Epidermis regenerated by ELL knockdown cells still formed a stratum corneum but were hypoplastic, with its thickness less than half of control tissue ($p < 0.05$) (Figure 1g and h). These results suggest

that ELL is required for maintaining the proliferative capacity of the basal layer of the epidermis.

To determine the gene expression program changes upon ELL loss, we performed RNA sequencing (RNA-Seq) on CTLi and ELL knockdown cells. 661 genes were downregulated in ELLi cells, which were enriched in gene ontology (GO) terms including DNA replication, DNA metabolic process, mitotic cell cycle phase transition, mitotic sister chromatid segregation, and G1/S transition of mitotic cell cycle (Figure 2a and 2b, Supplementary Table 1), confirming that ELL is necessary for the proliferative capacity of epidermal cells. 1280 genes were upregulated with GO terms including extracellular matrix organization, positive regulation of endothelial cell migration, and regulation of smooth muscle cell migration (Figure 2a and 2c, Supplementary Table 1).

To gain insight into ELL's genomic binding in primary human keratinocytes, we performed chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-Seq) with an ELL specific antibody. We identified a total of 6,184 peaks enriched with ELL binding using the encode ChIP-Seq pipeline based on MACS2 (Figure 2d). High reproducibility was observed between the duplicates with a Pearson Correlation coefficient of 0.99 (Supplementary Figure 1a). The vast majority (~68%) of ELL peaks were mapped to genic regions which included promoter, exon, intron, 5'UTR, 3'UTR, and transcription termination site (TTS) (Figure 2d). A de novo motif analysis of ELL bound peaks showed enrichment for HINFP, ETV2, RUNX2, Ahr:Arnt, NFIC, ZNF652, Gfi1, E2F, E-box(bHLH) and ZNF263 (Supplementary Figure 1b). Among these, HINFP, RUNX2 and E2F are critical transcription factors involved in cell cycle regulation(Glotzer et al., 2008, Xie et al., 2009, Zheng et al., 1999), suggesting ELL may regulate epidermal self-renewal downstream of these transcription factors. The 6,184 ELL bound regions were mapped back to 4,135 genes, which were enriched in GO terms including positive regulation of cell proliferation, negative regulation of cell differentiation, positive regulation of transcription from RNA Polymerase II promoter, hemidesmosome assembly, and regulation of cell migration (Figure 2e, Supplementary Table 2). Since ELL can promote Pol II mediated transcriptional elongation and/or facilitate stabilization of Pol II recruitment, we performed Pol II ChIP-Seq to compare it to the ELL binding profile (Supplementary Table 3). Pol II and ELL had similar binding at ELL bound genes (Figure 2f, Supplementary Figure 1c). However, slight differences were observed where ELL binding peaked at the transcription start site (TSS) and waned at the gene body and transcription end site (TES) (Supplementary Figure 1c). Pol II also peaked at the TSS but had a second peak near the TES (Supplementary Figure 1c). This suggests that ELL may function with Pol II in transcription initiation rather than elongation in epidermal stem and progenitor cells.

To identify genes that are directly regulated by ELL binding, we intersected the differentially expressed genes due to ELL loss (RNA-Seq) with genes bound by ELL (Figure 2g). Interestingly, 220 of the 1,280 genes upregulated upon ELL depletion were also bound by ELL and were enriched in GO terms such as positive regulation of apoptotic process and regulation of cell migration (Figure 2g–h). The increase in expression of positive regulators of apoptosis such as *BTG1*, *GADD45A*, *GADD45B*, *RHOB*, and *PMAIP1* may cause the increased apoptosis seen in ELL depleted cells (Figure 2h,1b, Supplementary Table 1).

These results suggest that ELL may be acting as a transcriptional repressor on a subset of genes that it binds. 120 of the 661 downregulated genes were bound by ELL and were enriched for genes involved in CENP-A containing nucleosome assembly, centromere complex assembly, positive regulation of cell cycle G2/M phase transition, DNA replication-independent nucleosome assembly and mitotic sister chromatid segregation (Figure 2g,2i). Among these 120 genes, we confirmed the bindings of ELL to the TSS of a group of proliferation related genes (*PBK*, *SMC4*, *CDK1*, *FGFBP1*, *IGFL3*) by ChIP-QPCR as demonstrated by the significant enrichment of ELL pulldown over the IgG control pulldown (Figure 2j–k, Supplementary Figure 1d–1g). Next, we wanted to determine how ELL loss impacts Pol II localization. If ELL is promoting transcription elongation as part of the SEC, then its knockdown should result in accumulation/stalling of Pol II at the TSS since it can't properly elongate. However, if ELL is necessary for Pol II stabilization at the TSS in the pre-initiation complex, then its loss should result in decreased Pol II localization to the TSS. Importantly, depletion of ELL caused a dramatic reduction of Pol II from the TSS of proliferation genes (*PBK*, *SMC4*, *CDK1*, *FGFBP1*, *IGFL3*) that ELL binds (Figure 2l). Loss of Pol II from the TSS of these genes in turn led to the downregulation of these proliferation genes on the mRNA level (Figure 2m,1e). Interestingly, loss of CDK1 expression may not only lead to decreased proliferation but also an increase in apoptosis as others have previously shown(Castedo et al., 2002). Our data suggests that ELL is necessary for Pol II stabilization at its bound proliferation genes. ELL may also act as a transcriptional repressor on genes involved in promoting apoptosis, however it is unclear the mechanism of how ELL does this as previous studies have focused on its role in promoting transcription(Byun et al., 2012).

Through profiling ELL's gene expression program and genomic binding, we characterized the regulatory role of ELL on the transcription of proliferation genes in the context of primary human keratinocytes. Our findings suggest that ELL plays an essential role in human epidermal proliferation by binding to proliferation genes and facilitating the stabilization of RNA Polymerase II at the TSS to sustain their gene expression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

This work was supported by grants from the National Institutes of Health (NIH R01AR066530 and R01CA225463) to G.L. Sen.

REFERENCES

Adelman K, Lis JT. Promoter-proximal pausing of RNA polymerase II: emerging roles in metazoans. Nat Rev Genet 2012;13(10):720–31. [PubMed: 22986266]

Byun JS, Fufa TD, Wakano C, Fernandez A, Haggerty CM, Sung MH, et al. ELL facilitates RNA polymerase II pause site entry and release. Nature communications 2012;3:633.

Castedo M, Perfettini JL, Roumier T, Kroemer G. Cyclin-dependent kinase-1: linking apoptosis to cell cycle and mitotic catastrophe. Cell Death Differ 2002;9(12):1287–93. [PubMed: 12478465]

Chen FX, Smith ER, Shilatifard A. Born to run: control of transcription elongation by RNA polymerase II. Nat Rev Mol Cell Biol 2018;19(7):464–78. [PubMed: 29740129]

Glotzer DJ, Zelzer E, Olsen BR. Impaired skin and hair follicle development in Runx2 deficient mice. Dev Biol 2008;315(2):459–73. [PubMed: 18262513]

Jones J, Chen Y, Tiwari M, Li J, Ling J, Sen GL. BRD4 Is Necessary for Differentiation Downstream of Epidermal Lineage-Determining Transcription Factors. J Invest Dermatol 2020a.

Jones J, Chen Y, Tiwari M, Li J, Ling J, Sen GL. KLF3 Mediates Epidermal Differentiation through the Epigenomic Writer CBP. iScience 2020b;23(7):101320. [PubMed: 32659720]

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).

Li J, Tiwari M, Xu X, Chen Y, Tamayo P, Sen GL. TEAD1 and TEAD3 Play Redundant Roles in the Regulation of Human Epidermal Proliferation. J Invest Dermatol 2020.

Lopez-Pajares V, Yan K, Zarnegar BJ, Jameson KL, Khavari PA. Genetic pathways in disorders of epidermal differentiation. Trends Genet 2013;29(1):31–40. [PubMed: 23141808]

Mistry DS, Chen Y, Wang Y, Zhang K, Sen GL. SNAI2 controls the undifferentiated state of human epidermal progenitor cells. Stem Cells 2014;32(12):3209–18. [PubMed: 25100569]

Mitani K, Yamagata T, Iida C, Oda H, Maki K, Ichikawa M, et al. Nonredundant roles of the elongation factor MEN in postimplantation development. Biochem Biophys Res Commun 2000;279(2):563–7. [PubMed: 11118326]

Noutsou M, Li J, Ling J, Jones J, Wang Y, Chen Y, et al. The Cohesin Complex Is Necessary for Epidermal Progenitor Cell Function through Maintenance of Self-Renewal Genes. Cell Rep 2017;20(13):3005–13. [PubMed: 28954219]

Sen GL, Boxer LD, Webster DE, Bussat RT, Qu K, Zarnegar BJ, et al. ZNF750 is a p63 target gene that induces KLF4 to drive terminal epidermal differentiation. Dev Cell 2012;22(3):669–77. [PubMed: 22364861]

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. [PubMed: 20081831]

Sen GL, Webster DE, Barragan DI, Chang HY, Khavari PA. Control of differentiation in a self-renewing mammalian tissue by the histone demethylase JMJD3. Genes Dev 2008;22(14):1865–70. [PubMed: 18628393]

Shilatifard A, Lane WS, Jackson KW, Conaway RC, Conaway JW. An RNA polymerase II elongation factor encoded by the human ELL gene. Science 1996;271(5257):1873–6. [PubMed: 8596958]

Smith E, Lin C, Shilatifard A. The super elongation complex (SEC) and MLL in development and disease. Genes Dev 2011;25(7):661–72. [PubMed: 21460034]

Xie R, Medina R, Zhang Y, Hussain S, Colby J, Ghule P, et al. The histone gene activator HINFP is a nonredundant cyclin E/CDK2 effector during early embryonic cell cycles. Proc Natl Acad Sci U S A 2009;106(30):12359–64. [PubMed: 19590016]

Zheng N, Fraenkel E, Pabo CO, Pavletich NP. Structural basis of DNA recognition by the heterodimeric cell cycle transcription factor E2F-DP. Genes Dev 1999;13(6):666–74. [PubMed: 10090723]

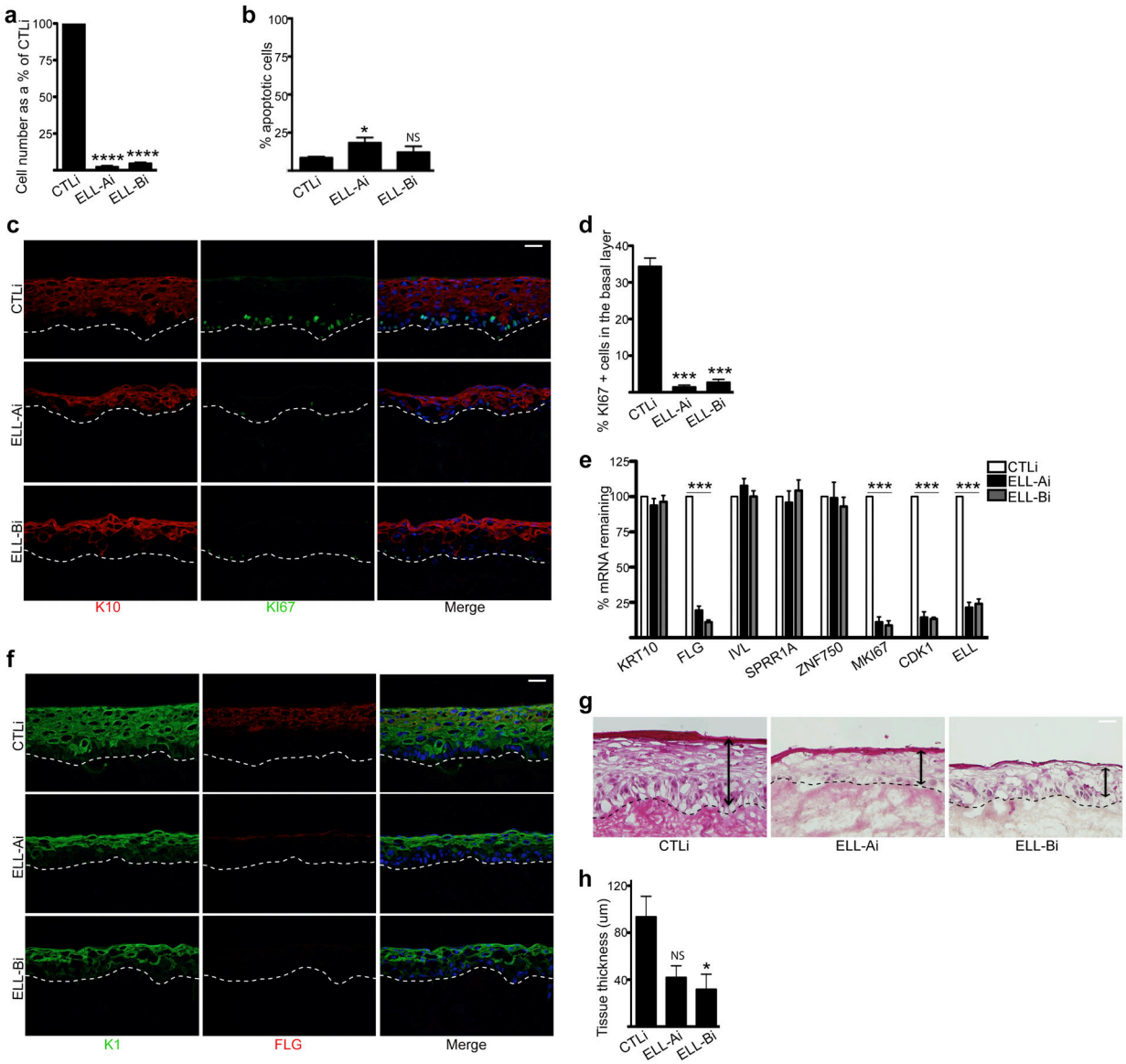

Figure 1. ELL is required for epidermal proliferation.

(**a**) Primary human epidermal keratinocytes were transfected using control (CTLi) and two distinct ELL siRNAs (ELL-Ai and ELL-Bi). The next day, CTLi and ELL-A/Bi cells were seeded at 300,000 and counted 4 days later, (**b**) Annexin V staining was used to determine the percentage of apoptotic cells in CTLi and ELL-A/Bi cells after 4 days of culture, (**c**) Regenerated human skin using three-dimensional organotypic cultures made from CTLi or ELL-A/Bi cells were harvested after 4 days of culture. Expression of K10 (red) and proliferation marker Ki67 (green) were characterized by immunostaining. Nuclei was stained using Hoechst (blue), (**d**) Quantification of the percentage of Ki67-positive cells in the basal layer, (**e**) RT-QPCR for epidermal differentiation and proliferation genes in CTLi and ELL-A/Bi tissue, (**f**) Expression of K1 (green) and FLG (red) were characterized by immunostaining in the regenerated human skin after culture for 4 days. Nuclei was stained using Hoechst (blue), (**g**) H&E staining of regenerated human epidermis at day 4. Bar = 20

μm. (**h**) Quantification of the tissue thickness of CTLi and ELL-A/Bi regenerated epidermis. Mean values are shown with error bars = SD, n = 3 for all panels. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$ using one-way ANOVA with post-hoc Tukey. CTLi, control small interfering RNA; ELL-Ai, ELL small interfering RNA A; ELL-Bi, ELL small interfering RNA B; K, keratin; NS, not significant; siRNA, small interfering RNA.

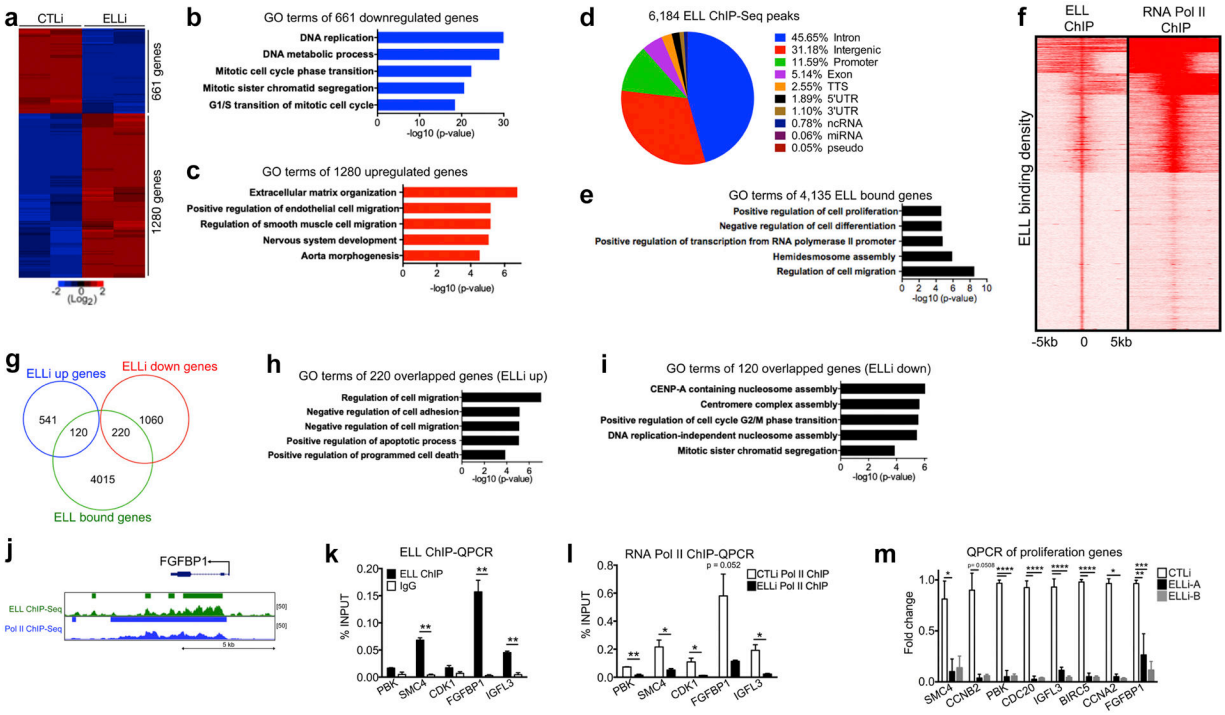

Figure 2. ELL binds to epidermal proliferation genes and promotes Pol II stabilization at their transcriptional start site.

(**a**) RNA-seq analysis of CTLi and ELLi cells harvested 4 days after transfection. A total of 1,280 genes were upregulated (red) and 661 genes were downregulated (blue). Heatmaps are presented in Log_2 scale, n = 2. (**b**) GO terms for the 661 downregulated genes on ELLi knockdown, (**c**) GO terms for the 1,280 genes upregulated in ELLi cells, (**d**) Genomic localization of the 6,184 ELL-bound peaks, (**e**) GO terms of the 4,135 genes to which the 6,184 ELL-bound peaks mapped back, (**f**) Heatmap of ELL ChIP-seq and Pol II ChIP-seq at ELL binding regions. X-axis shows genic regions −5 kb to +5 kb of the TSS. (**g**) Venn diagram of the overlap between ELLi differentially regulated genes and ELL-bound genes, (**h**) GO terms for the 220 overlapped genes from ELLi upregulated and ELL-bound genes, (**i**) GO terms for the 120 overlapped genes from ELLi downregulated and ELL-bound genes, (**j**) Gene track of *FGFBP1*. ELL ChIP-seq (green) and Pol II ChIP-seq (blue) tracks are shown in proliferation conditions. Green or blue bars over peaks represent significant peaks. Y-axis denotes signal strength, which is shown as reads per million, (**k**) ChIP-QPCR of proliferation genes in primary human keratinocytes with ELL pulldown. IgG pulldowns were used as a negative control. All primers were targeted toward the TSS of each proliferation gene. Significant binding was determined using *t*-test. (**l**) ChIP-QPCR of Pol II pulldown in both CTLi and ELLi cells. Each pulldown was normalized to its respective input and enrichment shown as a percent of input. All primers were targeted toward the TSS of each proliferation gene. Significant differences were determined using *t*-test. (**m**) RT-QPCR of epidermal proliferation genes in CTLi and ELLi cells. Kruskal-Wallis (Dunn post-hoc) for *SMC4, CCNB2*, and *CCNA2* and one-way ANOVA (Tukey post-hoc) were used to determine significant differences for the rest of the genes, n = 3. Mean values are shown with error bars = SD. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$. ChIP-QPCR,

chromatin immunoprecipitation and QPCR; ChIP-seq, chromatin immunoprecipitation followed by high-throughput sequencing; CTLi, control small interfering RNA; ELLi, ELL small interfering RNA; GO, Gene Ontology; kb, kilobase; Pol II, RNA polymerase II; RNA-seq, RNA sequencing; TSS, transcription start site.