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Supplemental Information

LSD1 Inhibition Promotes Epithelial Differentiation through Derepression of Fate-Determining Transcription Factors

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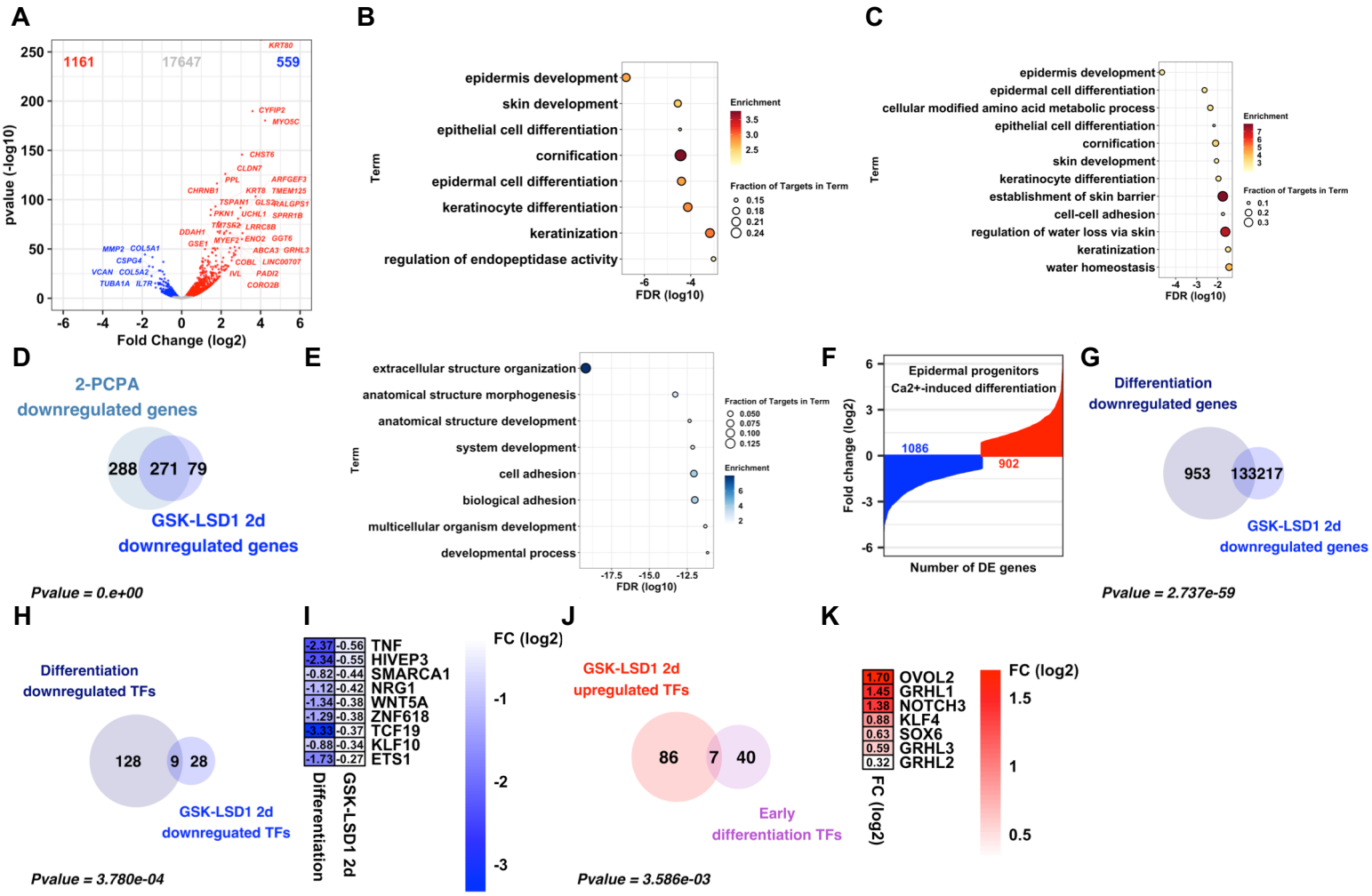

Figure S1. Treatment of epidermal progenitors with LSD1 inhibitors unleashes a pro-differentiation transcriptional program. Related to Figure 1. A. Volcano plot representation of differentially expressed genes upon LSD1 inhibition by 2-PCPA for 2 days, as determined by RNA-seq (n=3). **B.** Biological processes associated with 2-PCPA upregulated genes, as determined by PANTHER gene ontology analysis. **C.** Biological processes associated with genes upregulated by both GSK-LSD1 and 2-PCPA treatments for 2 days, as determined by PANTHER gene ontology analysis. **D.** Venn diagram representing the overlap between genes downregulated by 2 days of treatment with GSK-LSD1 (350 genes) and genes downregulated by 2 days of 2-PCPA treatment (559 genes). **E.** Biological processes associated with genes commonly downregulated by 2 days of treatment with GSK-LSD1 or 2-PCPA, as determined by PANTHER gene ontology analysis. **F.** Bargraph representation of differentially expressed genes upon *in vitro* Ca^{2+}-induced epidermal differentiation, as determined by RNA-seq (n =2). **G.** Venn diagram representing the overlap between genes downregulated by 2 days of GSK-LSD1 treatment (350 genes) and genes downregulated during *in vitro* Ca2+-induced keratinocyte differentiation (1,086 genes). **H.** Venn diagram representing the overlap between transcription factor-encoding genes downregulated by 2 days of GSK-LSD1 treatment (37 genes) and transcription factor-encoding genes downregulated during *in vitro* Ca2+-induced keratinocyte differentiation (137 genes). **I.** Heatmap representation of differential gene expression for transcription factor-encoding genes commonly downregulated by 2 days of GSK-LSD1 treatment and during *in vitro* Ca^{2+}-induced keratinocyte differentiation. **J.** Venn diagram representing the overlap between transcription factor-encoding genes upregulated by 2 days of GSK-LSD1 treatment (93 genes) and transcription factor-encoding genes associated with early keratinocyte differentiation (47 genes). **K.** Heatmap representation of differential gene expression for transcription factor-encoding genes commonly upregulated by 2 days of GSK-LSD1 treatment (93 genes) and during early keratinocyte differentiation (47 genes).

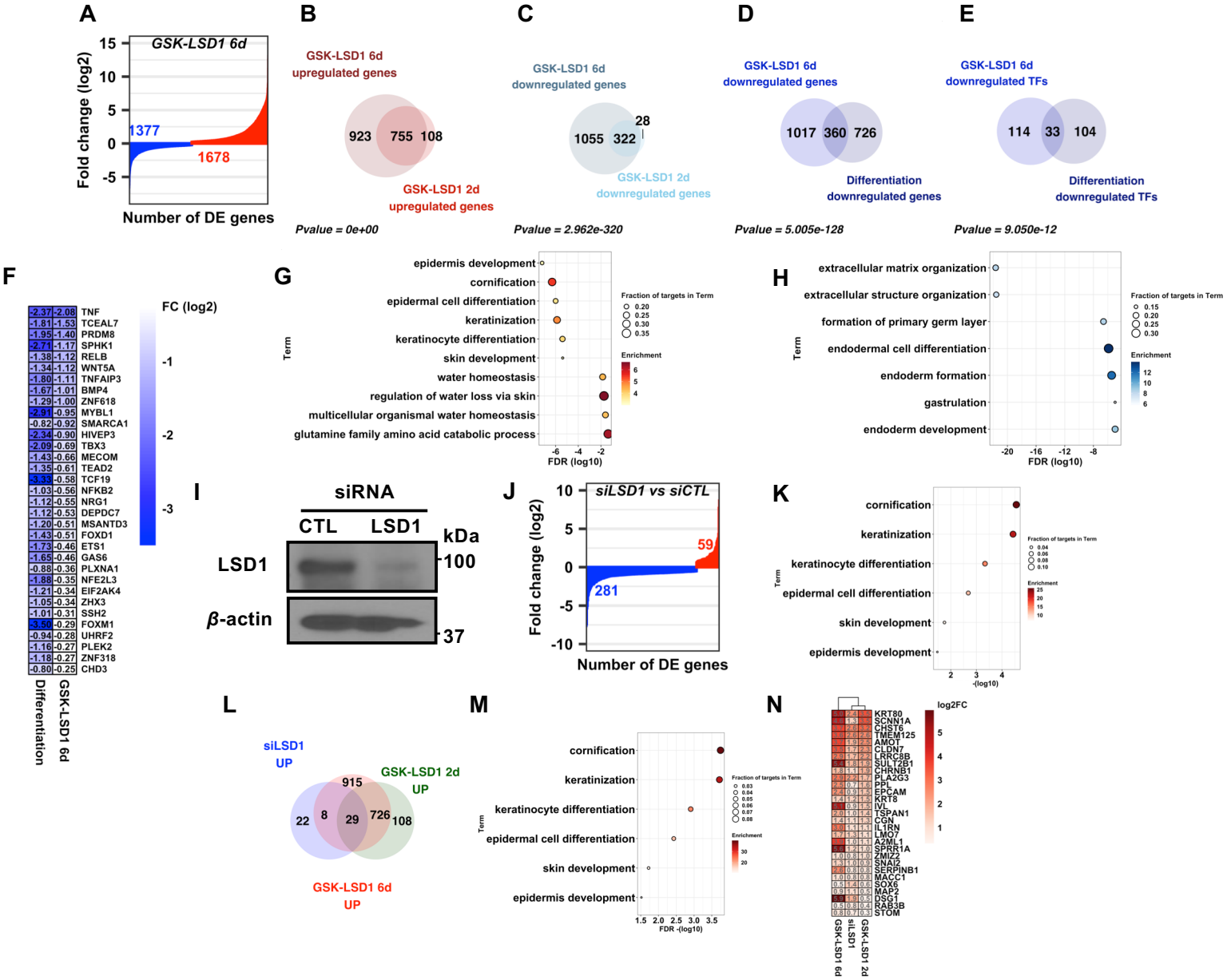

Figure S2. Treatment of epidermal progenitors with GSK-LSD1 inhibitor for 6 days further promotes epidermal cell differentiation compared to 2 days of treatment with GSK-LSD1 inhibitor while LSD1 genetic knockdown partially recapitulates many transcriptional features of pharmacological LSD1 inhibition. Related to Figure 1. A. Number of differentially expressed genes (DEG) in proliferating epidermal progenitor (PEPs) treated with GSK-LSD1 during 6 days compared to vehicle-treated PEPs, as determined by RNAseq (n = 3). **B.** Overlap between genes upregulated by 2 and by 6 days of GSK-LSD1 treatment (863 and 1,678 genes, respectively). **C.** Overlap between genes downregulated by 2 and by 6 days of GSK-LSD1 treatment (350 genes and 1,377 genes, respectively). **D.** Overlap between genes downregulated by 6 days of GSK-LSD1 treatment or by *in vitro* Ca^{2+}-induced keratinocyte differentiation (1,377 and 1,086 genes, respectively). **E.** Overlap between transcription factor-encoding genes downregulated by either 6 days of GSK-LSD1 treatment or *in vitro* Ca^{2+}-induced keratinocyte differentiation (147 and 137 genes, respectively). **F.** Heatmap representation of differential gene expression for transcription factor-encoding genes commonly downregulated by 6 days of GSK-LSD1 treatment and *in vitro* Ca^{2+}-induced keratinocyte differentiation. **G.** Biological processes associated with genes commonly upregulated by 2 and 6 days of GSK-LSD1 treatment, as determined by PANTHER gene ontology analysis. Terms with a FDR <=0.05 and a fold-enrichment >= 3 were removed. **H.** Biological processes associated with genes commonly downregulated by 2 and 6 days of GSK-LSD1 treatment, as determined by PANTHER gene ontology analysis. Terms with –log10(FDR)<=4 and a fold-enrichment <= 5 were removed. **I.** Western blot demonstrating LSD1 protein expression level in human epidermal progenitors treated with either scrambled siRNA (CTL) or with LSD1-specific siRNA (LSD1). **J.** Number of differentially expressed genes upon LSD1 genetic knockdown by siRNA, as determined by RNA-seq (n =2). **K.** Biological processes overrepresented in genes significantly upregulated in human epidermal progenitors upon LSD1 knockdown (siLSD1) compared to scrambled control (siCTL) (59 genes, n=2), as determined by PANTHER gene ontology analysis. **L.** Overlap between genes upregulated upon LSD1 knockdown by siRNA (59 genes, n=2), genes upregulated upon 2d GSK-LSD1 (863 genes, n=3) and genes upregulated upon 6d GSK-LSD1 (1,678 genes, n=3). **M.** Biological processes overrepresented in genes commonly upregulated in human epidermal progenitors by LSD1 siRNA knockdown (siLSD1) and by 2d or 6d GSK-LSD1 (29 genes), as determined by PANTHER gene ontology analysis. **N.** Heatmap representation of differential gene expression level (log2 fold changes) for the set of genes commonly upregulated by siRNA LSD1 knockdown (siLSD1) and by 2d or 6d GSK-LSD1 (29 genes).

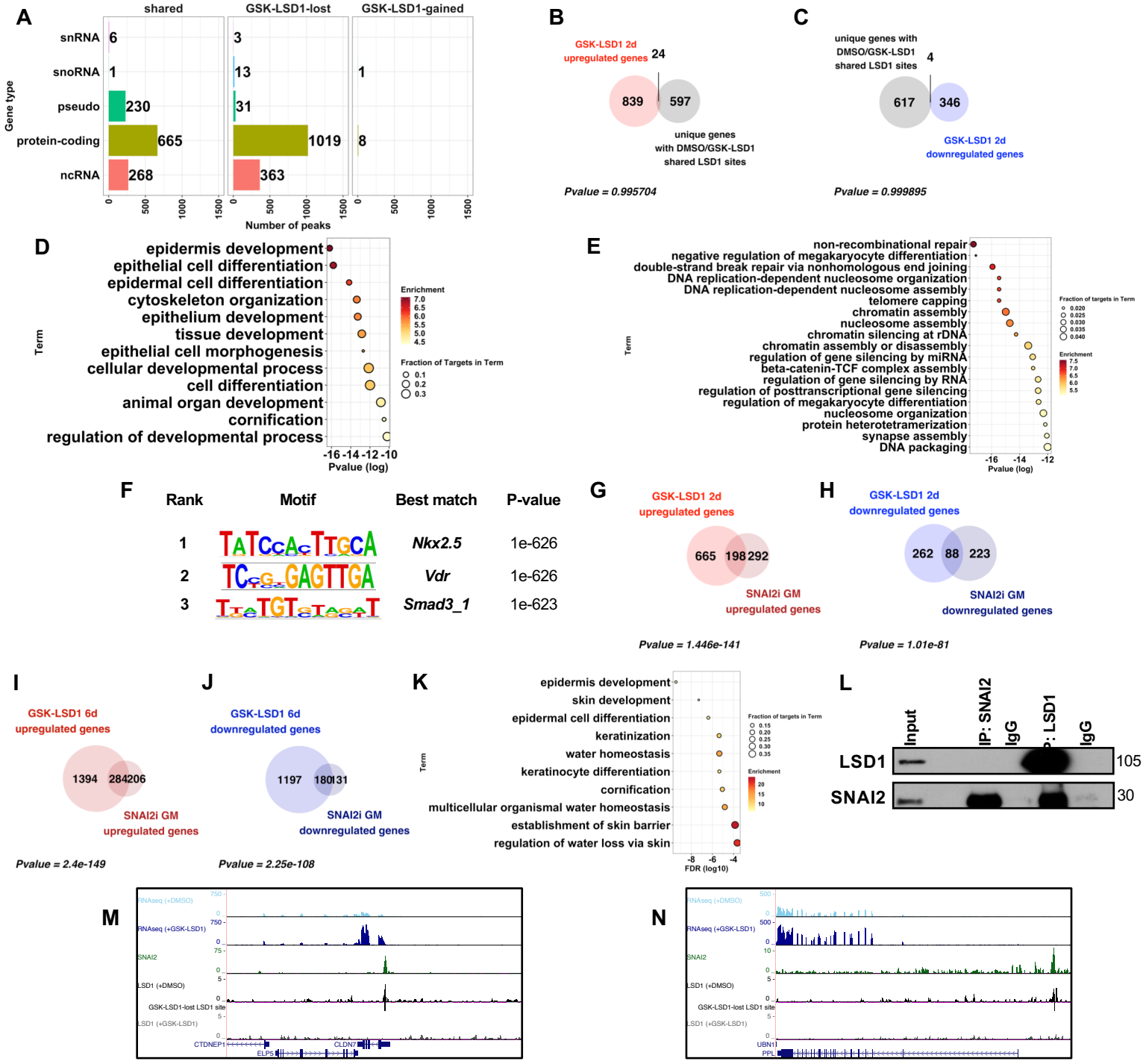

Figure S3. LSD1 binding sites that are maintained following GSK-LSD1 treatment show no association with SNAI2 binding sites, nor with GSK-LSD1 or epidermal differentiation upregulated genes. Related to Figure 2. A. Gene-type annotation of LSD1 binding sites at shared, GSK-LSD1-lost and GSK-LSD1-gained LSD1 sites. **B-C.** Overlap between DMSO / GSK-LSD1-shared LSD1 binding sites and genes up- **(B)** or down-regulated by 2 days of GSK-LSD1 treatment (863 and 350 genes, respectively). **D.** Biological processes associated with genes being concomitantly upregulated by 2 days of GSK-LSD1 treatment and loosing LSD1 binding sites upon LSD1 inhibition by 2 days of GSK-LSD1 treatment. **E.** Biological processes associated with genes upregulated by 2 days of GSK-LSD1 treatment and presenting similar LSD1 binding intensity in DMSO- and GSK-LSD1 treated samples. **F.** Top *de novo* motifs associated with shared LSD1 sites between DMSO- and GSK-LSD1-treated epidermal progenitors. **G.** Overlap between genes upregulated by 2 days of GSK-LSD1 treatment (863 genes) and genes upregulated upon SNAI2 (SNAI2i GM, 490 genes). **H.** Overlap between genes downregulated by 2 days of GSK-LSD1 treatment (350 genes) and genes downregulated upon SNAI2 knock-down (SNAI2i GM, 311 genes). **I.** Overlap between genes upregulated by 6 days of GSK-LSD1 treatment (1,678 genes) and genes upregulated upon SNAI2 knock-down (SNAI2i GM, 490 genes). **J.** Overlap between genes downregulated by 6 days of GSK-LSD1 treatment (1,377 genes) and genes downregulated upon SNAI2 knock-down (SNAI2i GM, 311 genes). **K.** Biological processes associated with genes found commonly upregulated by 2 days of GSK-LSD1-treatment or after siRNA-mediated SNAI2 knockdown. **L.** Representative result of co-immunoprecipitation experiments from whole human epidermal progenitor cells using anti-LSD1 or anti-SNAI2 antibodies. **M-N.** Representative UCSC tracks illustrating the common binding of LSD1 and SNAI2 at two keratinocyte differentiation genes, *CLDN7* **(M)** and *PPL* **(N)**, and how GSK-LSD1 treatment affects both LSD1 binding and transcription of LSD1 bound genes as determined by RNA-seq (top tracks) and ChIP-seq (bottom tracks).

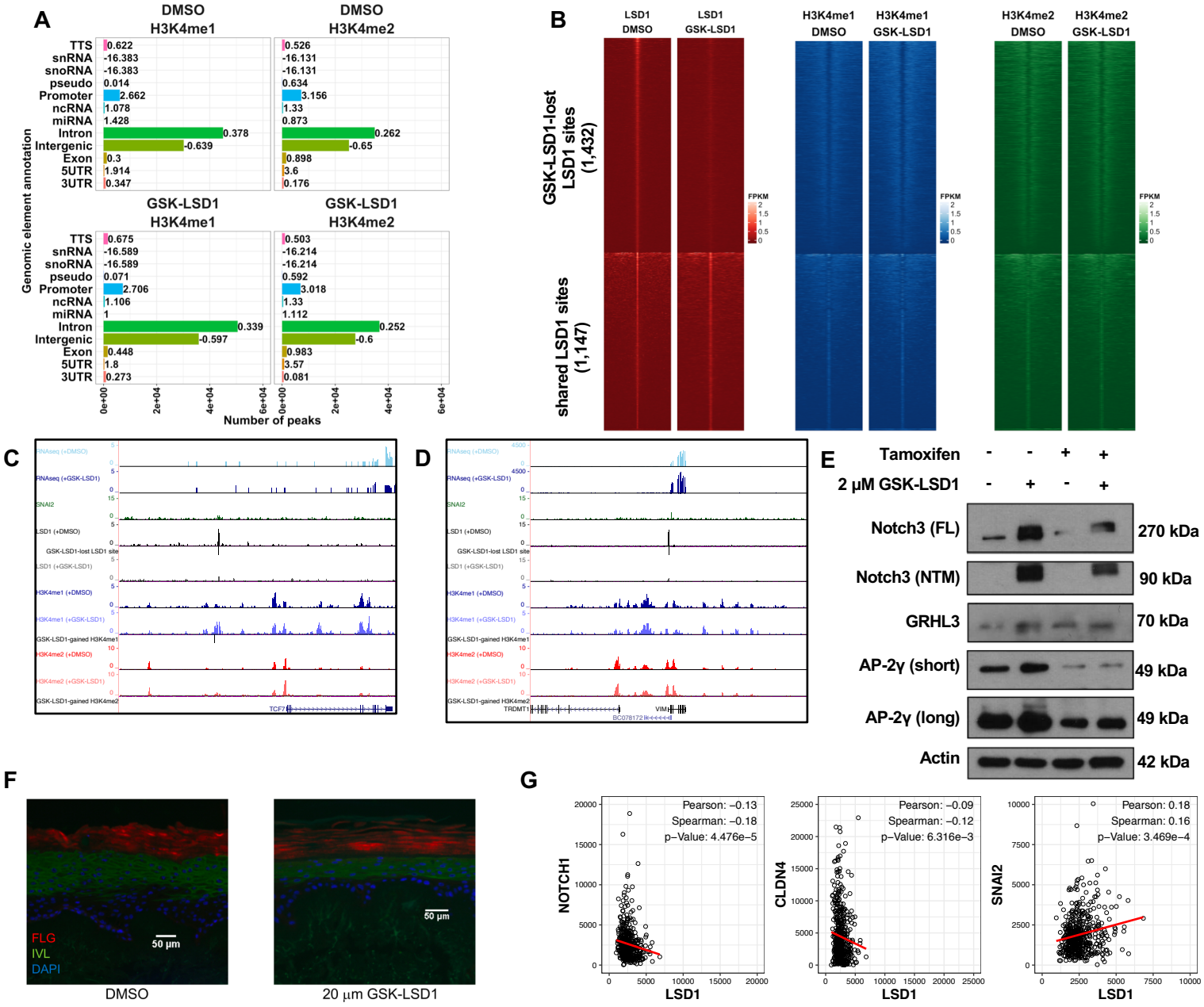

Figure S4. H3K4 methylation marks are enriched at gene promoters and show increased deposition near sites where LSD1 binding is lost while inhibition of LSD1 activates epidermal differentiation genes and inhibits squamous cell carcinoma invasion in a 3D organotypic model of epidermal neoplasia. LSD1 expression also inversely correlates with the expression of epithelial differentiation genes in head and neck squamous cell carcinoma (HNSCC). Related to Figures 3 and 4. A. Genomic distribution of H3K4me1/me2 marks in DMSO- or GSK-LSD1-treated epidermal progenitors, numbers at the top of each bar represent the log2 enrichment for a particular class of genomic annotation as determined by HOMER. **B.** Heatmaps representing LSD1, H3K4me1 and H3K4me2 binding enrichment +/- 2kb apart center of GSK-LSD1 differential LSD1 peaks (eg, GSK-LSD1-lost LSD1 sites, DMSO/GSK-LSD1-shared LSD1 sites and GSK-LSD1-gained LSD1 sites). **C.** UCSC browser track example of a locus with increased H3K4me1 and a GSK-LSD1-lost LSD1 site, but no increase in H3K4me2 (*TCF7*). **D.** UCSC browser track example of a locus with GSK-LSD1-lost LSD1 site without any concomitant GSK-LSD1-gained H3K4me1 or H3K4me2 (*VIM*). **E.** NOTCH3, GRHL3, and AP2-γ (TFAP2C) are upregulated in GSK-LSD1-treated (2 μM) oncogenic keratinocytes transfected with constitutively activated mutant CDK4 (R24C) and tamoxifen-inducible mutant H-RAS (G12V) with either inactivated (tamoxifen -) or activated H-RAS (tamoxifen +) as compared to corresponding DMSO controls determined by immunoblotting. **F.** Immunofluorescence staining of oncogenic keratinocytes transfected with constitutively activated mutant CDK4 (R24C) and activated H-RAS mutation treated with GSK-LSD1 (20μM) or vehicle (DMSO) for the epidermal differentiation markers Involucrin (red) and Filaggrin (green) or DAPI nuclear stain (blue). Magnification: 20X, scale bar =50μM **G.** Transcriptional correlation plots for human HNSCC data from TCGA show that LSD1 expression negatively correlates with expression of canonical epidermal differentiation transcription factors while being positively correlated with SNAI2 expression. P-values retrieved from TCGA.