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Supplementary Materials for

Cis-regulatory chromatin loops analysis identifies GRHL3 as a master regulator of surface epithelium commitment

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

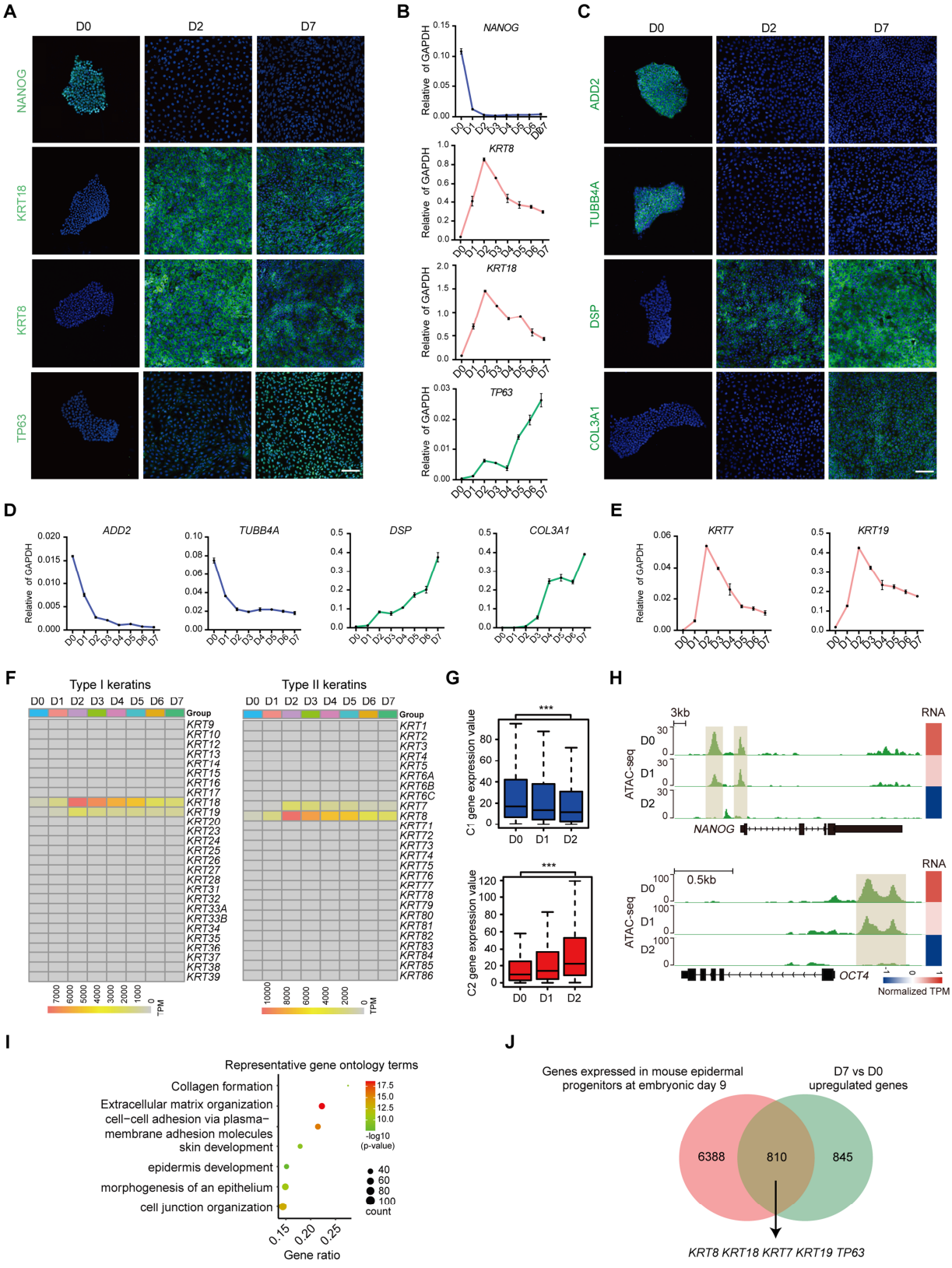
A
D0
D2
D7
NANOG
KRT18
KRT8
TP63
B
NANOG
KRT8
KRT18
TP63
Relative of GAPDH
C
ADD2
TUBB4A
DSP
COL3A1
D
ADD2
TUBB4A
DSP
COL3A1
E
KRT7
KRT19
F
Type I keratins
Type II keratins
Group
TPM
G
C1 gene expression value
C2 gene expression value
***
H
3kb
0.5kb
RNA
ATAC-seq
NANOG
OCT4
Normalized TPM
I
Representative gene ontology terms
Collagen formation
Extracellular matrix organization
cell−cell adhesion via plasma−membrane adhesion molecules
skin development
epidermis development
morphogenesis of an epithelium
cell junction organization
-log10 (p-value)
count
Gene ratio
J
Genes expressed in mouse epidermal progenitors at embryonic day 9
D7 vs D0 upregulated genes
6388
810
845
KRT8 KRT18 KRT7 KRT19 TP63

Fig. S1.

Gene expression profile during SE differentiation (A) Immunofluorescence staining of NANOG, KRT8, KRT18, and TP63 in the differentiated hESCs on D0, D2 and D7. Scale bar, 100um. (B) qRT-PCR validation of the expression levels of NANOG, KRT8, KRT18, and TP63 during SE differentiation. Values are shown as means ± SD (n = 3 biological replicates). (C) Immunofluorescence staining of ADD2, TUBB4A, DSP and COL3A1 in the differentiated hESCs on D0, D2 and D7. Scale bar, 100um. (D) qRT-PCR validation of the expression levels of ADD2, TUBB4A, DSP and COL3A1 during SE differentiation. Values are shown as means ± SD (n = 3 biological replicates). (E) qRT-PCR validation of the expression levels of KRT7 and KRT19 during SE differentiation. Values are shown as means ± SD (n = 3 biological replicates). (F) Heatmap of type I and type II keratin expression changes during SE differentiation. The color bar shows the expression value (TPM) from the RNA-seq. (G) Box plots of the expression levels of genes closest to the C1 and C2 peaks in the differentiated hESCs on D0, D1, and D2. (H) Snapshots of genome browser showing chromatin accessibility at NANOG and OCT4 loci. Gene expression is also displayed in heatmaps (log_2 TPM). The genome browser view scales were adjusted on the basis of the global data range. (I) Representative gene ontology terms identified from the genes highly expressed in D7-differentiated cells (compared to D2-differentiated cells). (J) Venn diagram showing overlap between genes expressed (TPM [transcripts per kilobase of exon model per million mapped reads] >10) in mouse epidermal progenitors at embryonic day 9 and genes (TPM > 10) highly expressed in SE cells (compared to hESCs).

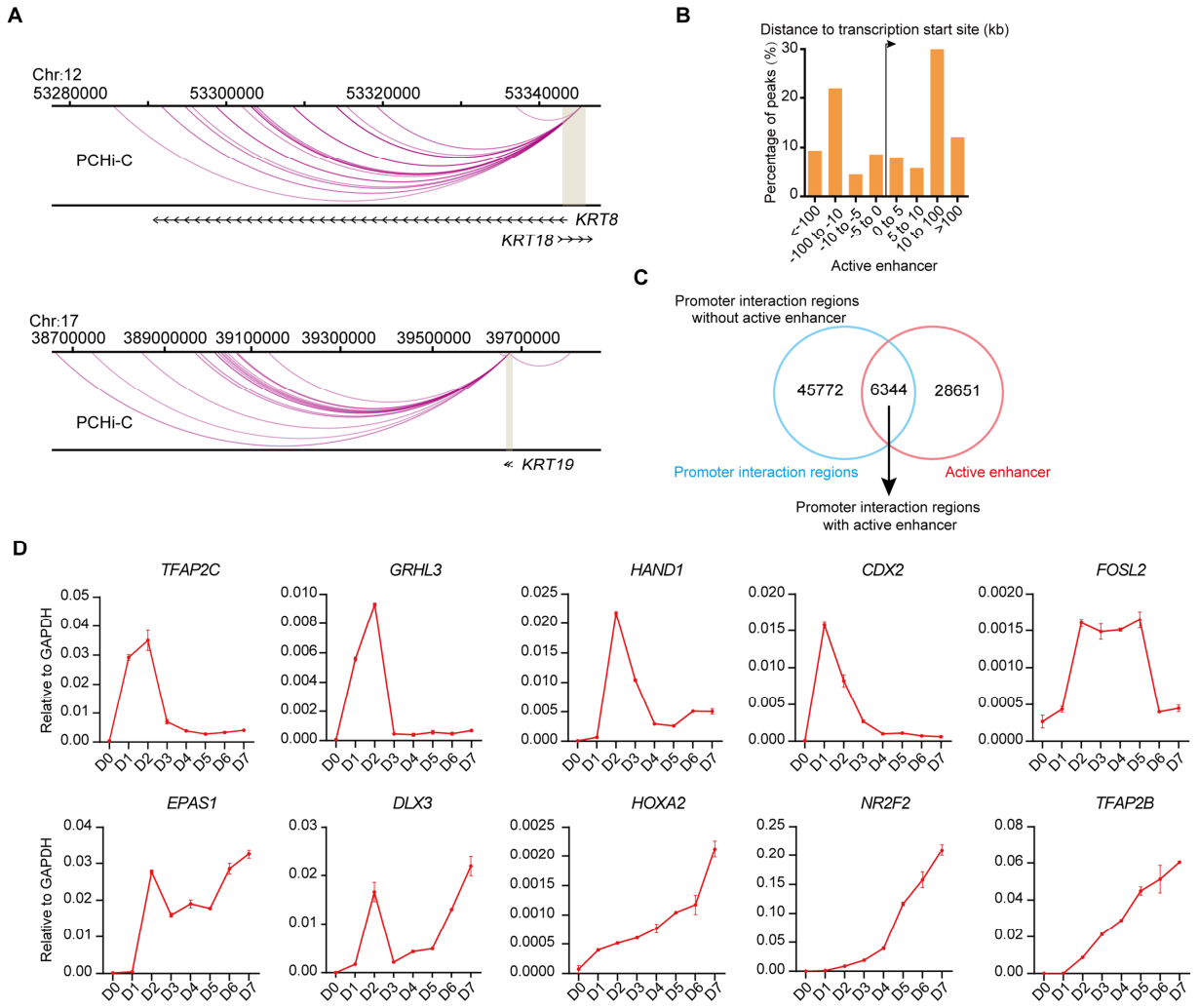

Fig. S2.

Promoter interactions and potential regulators of SE-initiating cells (A) Examples of promoter-anchored chromatin loops at *KRT8*/*18* and KRT19 loci in SE-initiating cells. (B) Histogram showing the distribution of the distance between active enhancer and TSSs in SE-initiating cells. (C) Venn diagram showing the identification of promoter interaction regions with active enhancers in SE-initiating cells. (D) qRT-PCR analysis of top 10 candidate transcription factors during SE differentiation. Values are shown as means ± SD (n = 3 biological replicates).

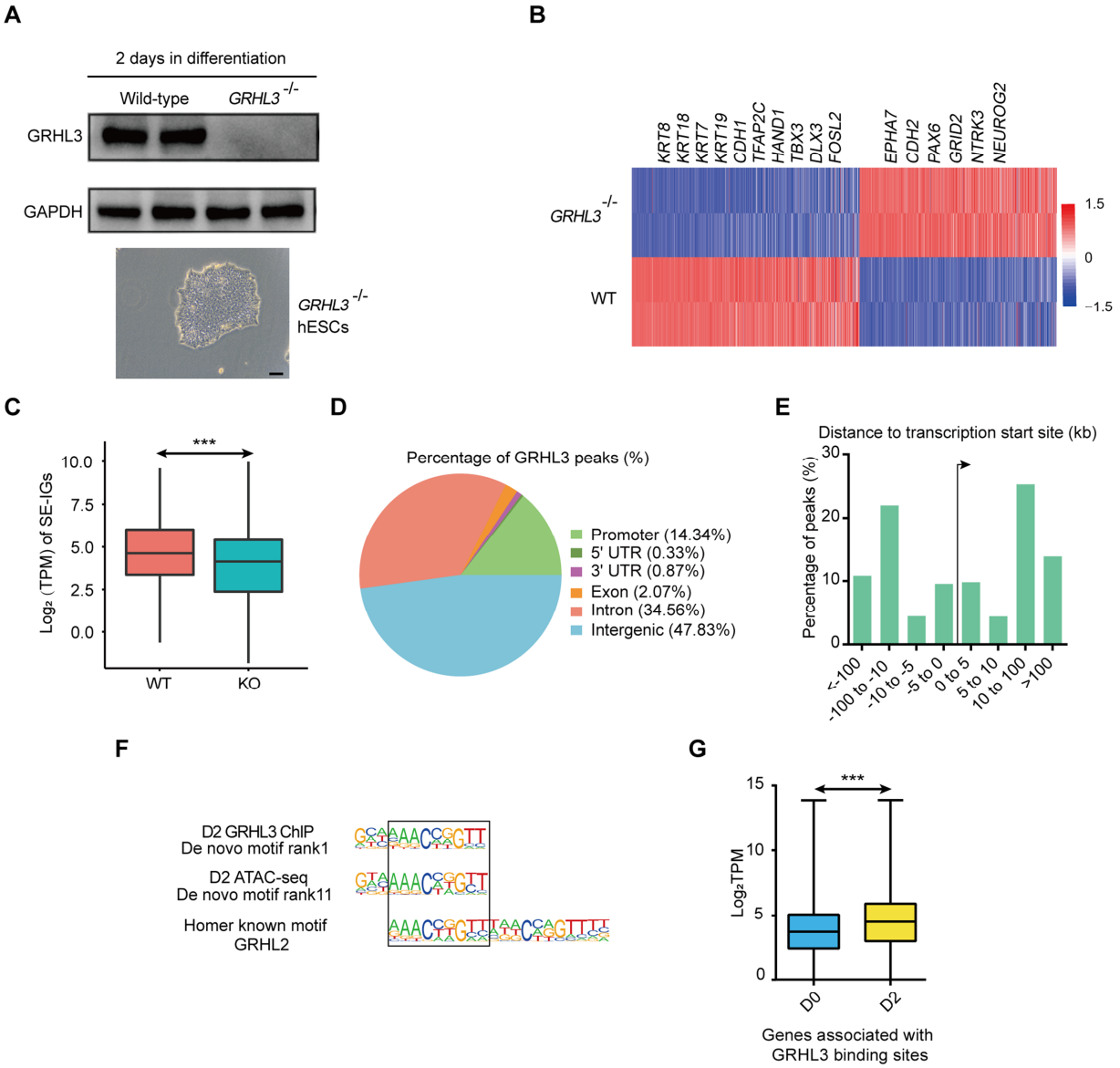

Fig. S3.

Loss of GRHL3 decreases chromatin accessibility in surface ectodermal genes (A) Top panel, western blot analysis of GRHL3 in wild-type and *GRHL3*-knockouthESCs after 2 days differentiation; Bottom panel, phase contrast image of GRHL3-knockout hESCs. Scale bar, 100um. (B) Heatmap of differentially expressed genes in wild-type and *GRHL3*-knockout hESCs after 2 days differentiation. (C) Box plot representing the expression level of SE identity genes in wild-type and *GRHL3*-knockout hESCs after 2 days differentiation. (D) Pie chart showing the distribution patterns of GRHL3 peaks of SE-initiating cells. (E) Histogram showing the distribution of the distance between GRHL3 peaks and transcription start site. (F) *De novo* motif-enrichment analysis of GRHL3-binding sites (top). *De novo* motif-enrichment analysis of ATAC-seq peaks in SE-initiating cells (middle). The GRHL2 known motif identified by HOMER (bottom). (G) Box plot representing the expression level of genes associated with GRHL3 peaks in hESCs and SE-initiating cells.

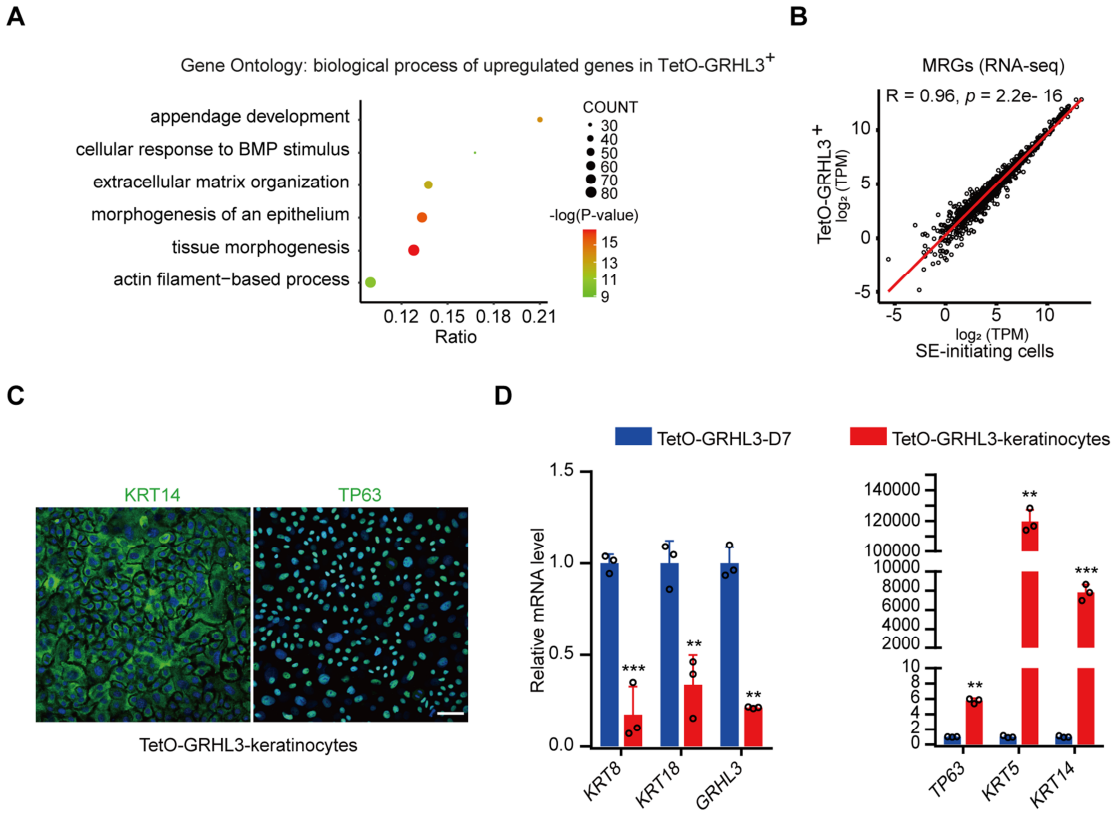

Fig. S4.

Differentiation of GRHL3-induced SE cells into keratinocytes (A) Gene ontology (biological process) analysis of the upregulated genes in TetO-GRHL3^{+} cells. (B) Scatterplot of gene expression from RNA-seq of MRGs in TetO-GRHL3^{+} cells and SE-initiating cells with Pearson correlation value. (C) Immunofluorescence staining of KRT14 and TP63 in keratinocytes derived from TetO-GRHL3^{+} cells (TetO-GRHL3-keratinocytes). Scale bar, 50um. (D) Comparison of gene expression level in TetO-GRHL3^{+} and TetO-GRHL3-keratinocytes. qRT-PCR values were normalized to the values in TetO-GRHL3^{+} cells. Values are shown as means ± SD (n = 3 biological replicates; $^{**}P < 0.01$; $^{***}P < 0.001$ *t* test).

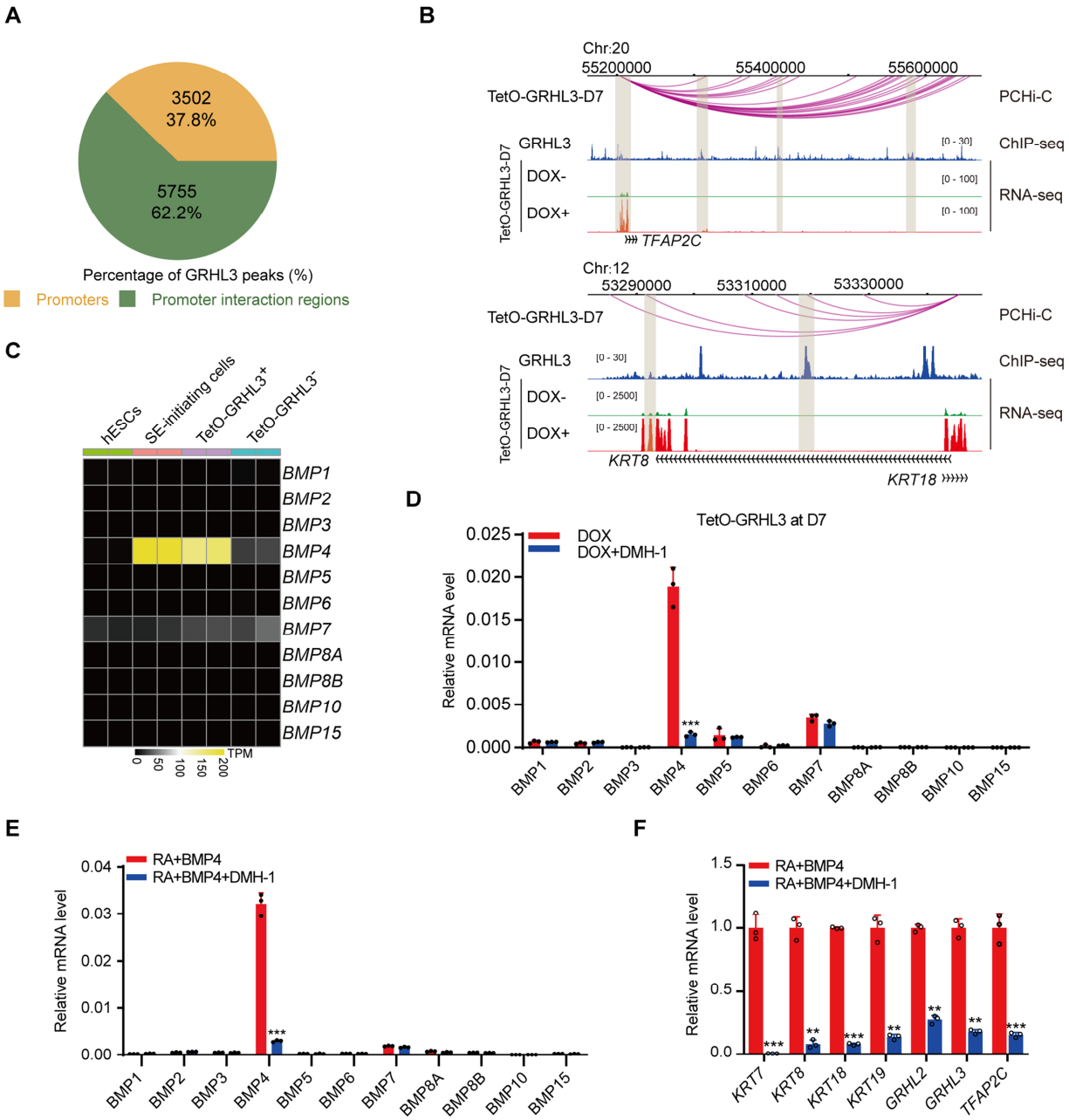

Fig. S5.

Characteristics of GRHL3-mediated promoter interactions (A) Pie chart showing the percentage of GRHL3 peaks in promoter interaction regions or promoters. (B) Genome browser view of promoter interactions, GRHL3, and RNA-seq signals at *TFAP2C* and *KRT8*/*18* loci. (C) Heatmap of the expression level of BMP family members in hESCs, SE-initiating cells, TetO-GRHL3^{+} and TetO-GRHL3^{-} cells. (D) qRT-PCR analysis of BMP family members in hESCs treated with or without DMH-1 upon SE differentiation for 2 days. qRT-PCR values were normalized to the values in RA/BMP4 group. Values are presented as means ± SD (n = 3 biological replicates; ***P < 0.001 t test). (E) qRT-PCR analysis of BMP family members in TetO-GRHL3^{+} cells with or without DMH-1 for 7 days. qRT-PCR values were normalized to the

values in control cells. Values are shown as means ± SD (n = 3 biological replicates; ***P < 0.001 t test). (F) Comparison of gene expression level in hESCs treated with or without DMH-1 upon SE differentiation for 2 days. qRT-PCR values were normalized to the values in SE-initiating cells. Values are shown as means ± SD (n = 3 biological replicates; ***P* < 0.01; ****P* < 0.001 *t* test).

table S1

List of primers used in this study.

Primers for RT-qPCR		
Gene Name	**Forward**	**Reverse**
ADD2	ATGAGCGAAGAGACGGTCC	TTGCGAAGGCGCATGTACT
CDX2	GACGTGAGCATGTACCCTAGC	GCGTAGCCATTCCAGTCCT
BMP4	ATGATTCCTGGTAACCGAATGC	CCCCGTCTCAGGTATCAAACT
COL3A1	GGAGCTGGCTACTTCTCGC	GGGAACATCCTCCTTCAACAG
DLX3	CTCGCCCAAGTCGGAATATAC	CTGGTAGCTGGAGTAGATCGT
DSP	GCAGGATGTACTATTCTCGGC	CCTGGATGGTGTTCTGGTTCT
EPAS1	CGGAGGTGTTCTATGAGCTGG	AGCTTGTGTGTTCGCAGGAA
FOSL2	CAGAAATTCCGGGTAGATATGCC	GGTATGGGTTGGACATGGAGG
GAPDH	CTGAGAACGGGAAGCTTGT	GGGTGCTAAGCAGTTGGT
GATA3	GCGGGCTCTATCACAAAATGA	GCCTTCGCTTGGGCTTAAT
GRHL2	TCAATACCCGAAGAGCCTACA	CTTGGCTGTCACTTGCTTTGC
GRHL3	GCCAGTTCTACCCCGTCA	GTCAATGACCCGCTGCTT
HAND1	CCATGCTCCACGAACCCTTC	CCTGGCGTCAGGACCATAG
KRT5	ATCTCTGAGATGAACCGGATGATC	CAGATTGGCGCACTGTTTCTT
KRT7	TCCGCGAGGTCACCATTAAC	GCTCTGTCAACTCCGTCTCAT
KRT8	GATCGCCACCTACAGGAAGCT	ACTCATGTTCTGCATCCCAGACT
KRT14	TGCCGAGGAATGGTTCTTCACC	GCAGCTCAATCTCCAGGTTCTG
KRT18	CCGTCTTGCTGCTGATGACT	GGCCTTTTACTTCCTCTTCGTG
KRT19	AACGGCGAGCTAGAGGTGA	GGATGGTCGTGTAGTAGTGGC
NANOG	AAGGTCCCGGTCAAGAAACAG	CTTCTGCGTCACACCATTGC
NR2F2	TCATGGGTATCGAGAACATTTGC	TTCAACACAAACAGCTCGCTC
TFAP2A	CAGATATGCAAAGAGTTCACCGAC	TCAAGCAGCTCTGGATGCC
TFAP2B	CCATCCCGGAATGGAAGACG	TCACCGATTTGGGAGGAACTG
TFAP2C	AGATTGGGTTGAATCTTCCG	GGCTTCACAGACATAGGCAA
TP63	TTTCCCACCCCGAGATGA	TGCGGCGAGCATCCAT
TUBB4A	CCGGACAACTTCGTGTTTGG	TCGCGGATCTTACTGATGAGC
BMP1	GGGTCATCCCCTTTGTCATTG	GCAAGGTCGATAGGTGAACACA
BMP2	ACCCGCTGTCTTCTAGCGT	TTTCAGGCCGAACATGCTGAG
BMP3	GCAGGGAGAGAGACCGAAG	TGGACCGTGCTGTACCTGT
BMP5	GCTGCTGGGTTCTAGTGGG	TTCGTGGTTCCGTAGTCTTCTA
BMP6	AGCGACACCACAAAGAGTTCA	GCTGATGCTCCTGTAAGACTTGA
BMP7	GGAACGCTTCGACAATGAGAC	GCAGGAAGAGATCCGATTCCC
BMP8A	CACCCTTCTCATCTGGATCG	CAGGAAGTAGGCACCGAGAG
BMP8B	AGGTGGCTTCCTTATCTGCG	ATGTGCCAACTCTGCTTCGT
BMP10	CCTCTGCCAACATCATTAGGAG	TTTTCGGAGCCCATTAAAACTGA
BMP15	TGTGAACTCGTGCTTTTCATG	CTCAATCAGGGGCAAAGTAGG

Supplemental Tables:

Table S2: Gene list of MRGs.
Table S3: Gene list of SE identity genes.
Table S4: The annotation of GRHL3 peaks.
Table S5: Gene list of TetO-GRHL3^{+} upregulated genes.