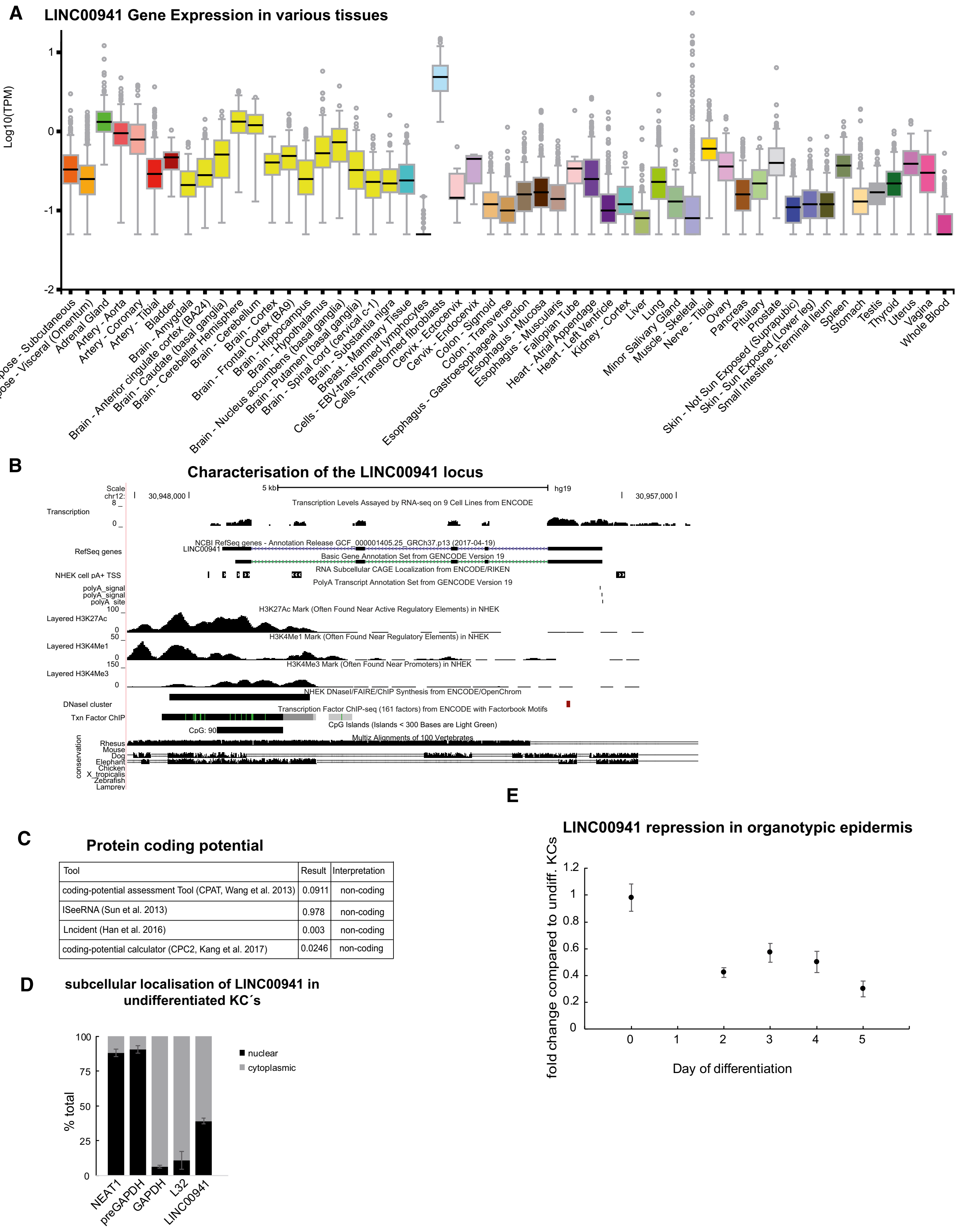
Expanded View Figures

Figure EV1. Coding potential of LINC00941 and validation of RNA-Seq results.

- A Utilization of the GTEx Portal to access the expression of LINC00941 in various tissues. Boxplot displaying the distribution of log10 transformed TPM. Mid-line represents median. Whiskers display the upper and lower extreme.
- B UCSC view showing the genomic locus of LINC00941 with two different Isoforms. Transcription start sites and poly-A sites as well as chromatin marks are included.
- C Usage of different bioinformatical algorithms to investigate the protein-coding potential of LINC00941.
- D qRT–PCR analysis for LINC00941 subcellular localization in undifferentiated keratinocytes (D0) (n = 3 biological replicates). Data are presented as mean ± SD.
- E qRT–PCR analysis of LINC00941 repression during keratinocyte differentiation organotypic epidermal tissue compared to undifferentiated keratinocytes (D0) (n = 3 biological replicates). Data are presented as mean ± SD.



Tool	Result	Interpretation
coding-potential assessment Tool (CPAT, Wang et al. 2013)	0.0911	non-coding
ISeeRNA (Sun et al. 2013)	0.978	non-coding
Lncident (Han et al. 2016)	0.003	non-coding
coding-potential calculator (CPC2, Kang et al. 2017)	0.0246	non-coding

Figure EV1.

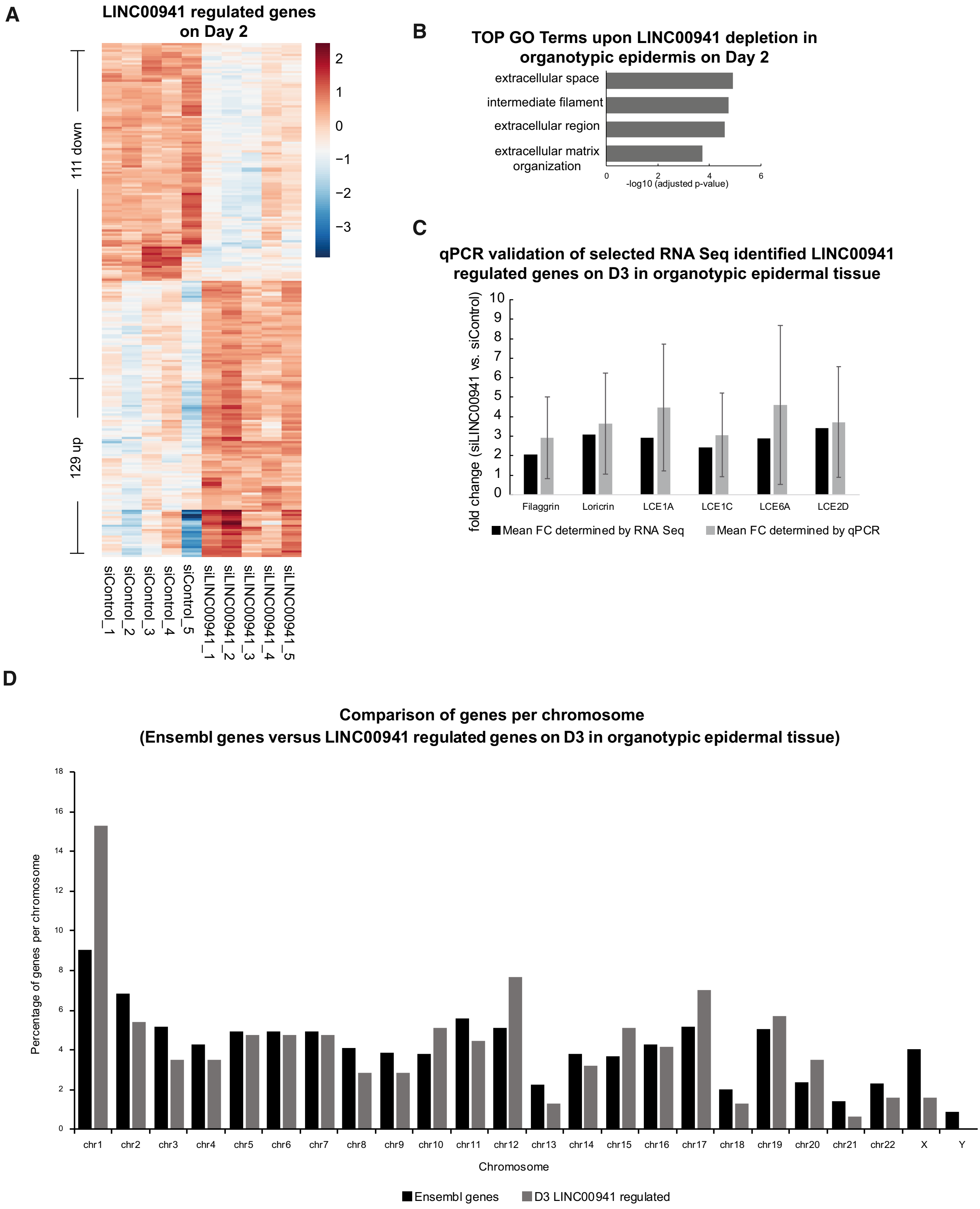

A
LINC00941 regulated genes on Day 2
111 down
129 up
2
1
0
−1
−2
−3
siControl_1
siControl_2
siControl_3
siControl_4
siControl_5
siLINC00941_1
siLINC00941_2
siLINC00941_3
siLINC00941_4
siLINC00941_5
B
TOP GO Terms upon LINC00941 depletion in organotypic epidermis on Day 2
extracellular space
intermediate filament
extracellular region
extracellular matrix organization
0
2
4
6
-log10 (adjusted p-value)
C
qPCR validation of selected RNA Seq identified LINC00941 regulated genes on D3 in organotypic epidermal tissue
fold change (siLINC00941 vs. siControl)
10
9
8
7
6
5
4
3
2
1
0
Filaggrin
Loricrin
LCE1A
LCE1C
LCE6A
LCE2D
Mean FC determined by RNA Seq
Mean FC determined by qPCR
D
Comparison of genes per chromosome
(Ensembl genes versus LINC00941 regulated genes on D3 in organotypic epidermal tissue)
Percentage of genes per chromosome
18
16
14
12
10
8
6
4
2
0
chr1
chr2
chr3
chr4
chr5
chr6
chr7
chr8
chr9
chr10
chr11
chr12
chr13
chr14
chr15
chr16
chr17
chr18
chr19
chr20
chr21
chr22
X
Y
Chromosome
Ensembl genes
D3 LINC00941 regulated

Figure EV2.

◀

Figure EV2. RNA-Seq validation of LINC00941 depletion.

A Heatmap of differentially expressed genes ($P_{adj} < 0.05$ and $-1 > \log_2FC > 1$) upon LINC00941 depletion on day 2 in organotypic epidermal tissue with marked keratinocyte differentiation genes (n = 5 epidermal tissue cultures/knockdown group).

B GO term analysis of downregulated ($P_{adj} < 0.05$ and $-1 > \log_2FC < 1$) genes in LINC00941-deficient organotypic epidermal tissue on day 2 (n = 5 epidermal tissue cultures/knockdown group).

C qRT–PCR validation of the obtained RNA-Seq results for the expression of selected genes on day 3 in LINC00941-depleted organotypic epidermal tissue (n = 4–5 epidermal tissue cultures/knockdown group). Data are presented as mean ± SD.

D Comparison of the genomic localization of LINC00941-regulated genes ($P_{adj} < 0.05$ and $-1 > \log_2FC > 1$) on day 3 (D3) and distribution of Ensembl genes per chromosome (n = 4–5 epidermal tissue cultures/knockdown group).

Figure EV3. SPRR5 transcript characterization.

▶

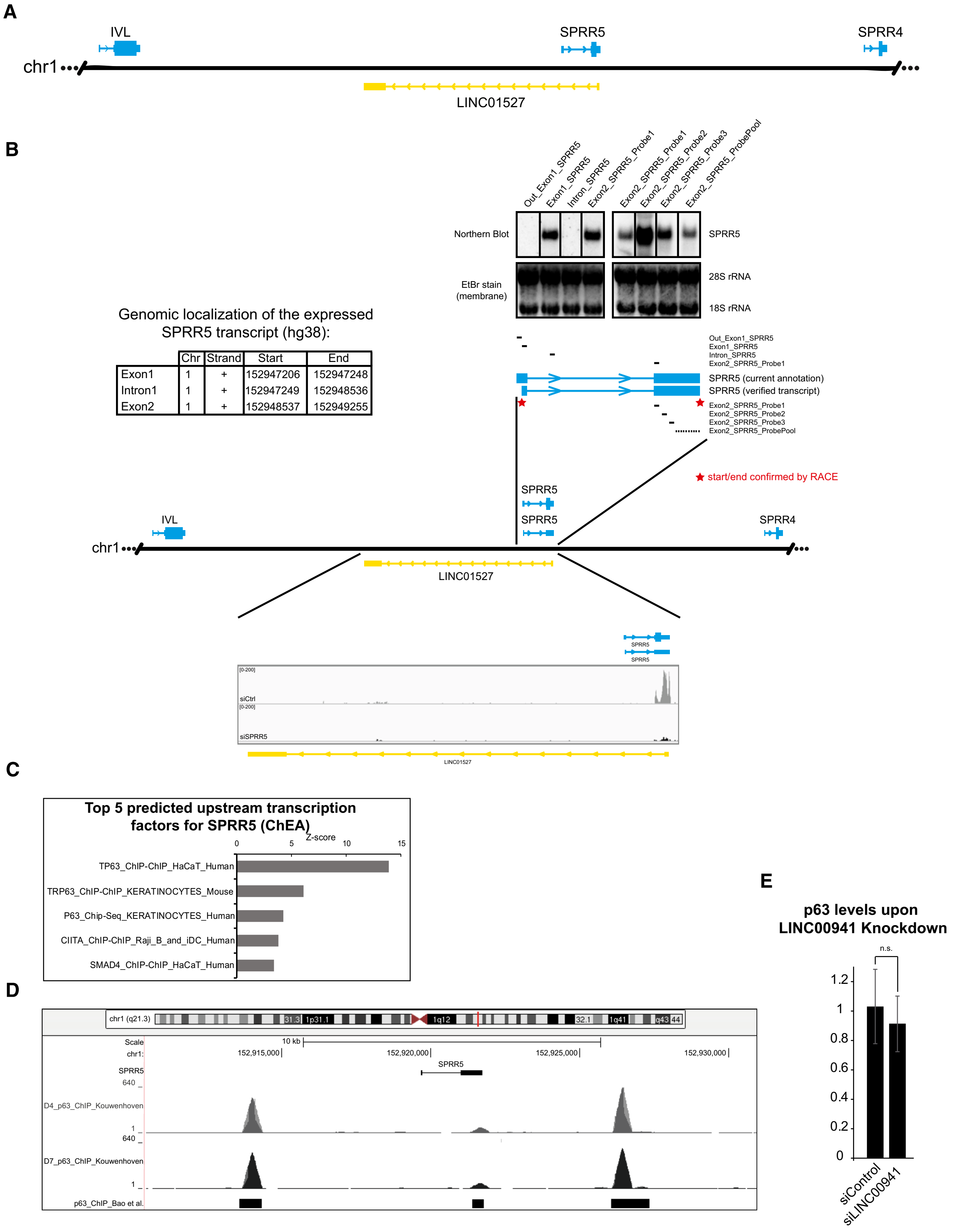
A Schematic overview of the genomic localization of *SPRR5*.

B Exact genomic coordinates for the verified SPRR5 transcript (genome release: hg38) and exemplary pictures of aligned and mapped RNA-Seq reads for control (siCtrl) and SPRR5 knockdown (siSPRR5) with respect to *LINC01527* (lower part). The top part indicates the localization of the utilized probes for northern blot mapping as well as the results from hybridization of membranes with RNA from differentiated keratinocytes. Furthermore, the verified transcript and the Ensembl annotated version are shown and red stars indicate the RACE (rapid amplification of cDNA ends) verification of start and end points of the transcript.

C Upstream transcription factor predictions for SPRR5 obtained by ARCHS[4].

D UCSC view showing the genomic locus of SPRR5 as well as the p63 ChIP-Seq peaks from studies by Kouwenhoven *et al* and Bao *et al*.

E Knockdown of LINC00941 does not affect pan-p63 levels in human epidermal tissue (n = 3–4 epidermal tissue cultures/knockdown group). Data are presented as mean ± SD.



	Chr	Strand	Start	End
Exon1	1	+	152947206	152947248
Intron1	1	+	152947249	152948536
Exon2	1	+	152948537	152949255

Figure EV3.

A

Verified SPRR5 Sequence (hg38):

Exon Intron

5‘AAACCCCTCCACACCTATCTGCTCCAGTCGCTGGTGAACCTTGGTAAGTCTGGAAAGGTAGTACCTCTTATAG
CAGCCTTGCCTTGCCTGCATCCCTAACCAACTATGGACTGGGCTGATTCTCTGGGTGGTGGTCAGAAAGGATG
GGTCTTTTGGGGTACCTCTTTTTTCCATATCATTTGTGACAAACCTATCTTGGGTCCAGCAAGTTATCCAGCAGA
GTAGTGTGGGAGGCTGGGCTGAGCAGGGAGAAGGAGGGGGGTATCTACAAGAGGGTCAGTATCTCAGCGAT
GCTGCTGGCATGGTGTGGGTGTTGCATCTTGTCAGGTGCTAAGAGGGAATTAGTAGGAGAGGAGAGACCAGT
TCTGGGCAGAGCAACTGGAAGGACTGTCCTTTCAAAGAAAAGAGAAGGCAGGAAGGAAAGAGAACAGACTC
CCTTGGGACAGCAGAGTTGTGGATGTCCATGGAGCAAAGGGCTGCAGGGACAAGACGATGTGGCACCATGG
AAGGCTCTGCTCTGGGAATCAGGAGACCTCTTTTCCCACCATCTATGCCGAGTGGCTTTGAAGCCATTCCTTCCC
TCTGAGGTTTGCTTTCCTCAACTGTAATTGAGAGAGGCAAGCTAGATAGTCCATTTCATCTATCTCTAGAGTTTT
CTCTTTATAAACTCCTTTAGAGAGTGGTTAAGATGCGCCTTTTAAATTGTTTATGAATTTACTTAACAAATTACA
ACTCTTATATAGTGTTCATTTTGTGCTACTACTCTGCATGTGTGTGTCGGTACATACATACCTACGTATGCATGC
ATATTTTAAGTATGTATTTTATATACTCATTTAATTTTTGCACCAACTCTAAGAGAGGTAAGTATGGAAAACCTT
AGTTGCTCAAAATCATATAGTTAATAAATGATTTAGCCAGAACTCAGATCCCAGGCAATTTATTCCAGATGCCA
CAGGTTTTGCTATAATAGGATACAACAGTCTGCTCTTTCATTCTTCCTCTTTTTTTTCCTCTCTCTCCCTTTTCTTTT
TTTATGTTTTTCTTTCTTTTCAACCCCTCCTAACATTTCTGTTTTCCCTCCCTTCCTCCTTTCCTATCTTTCCTTCCTT
CCATGTTTCTTTGTCTTGAAAAGAGTTGAAATAACTTGTAAACACTTAAAGTTTACACATGCGCGCGCGCACAC
ACACACACACACTCCTCTTAAATCTGTGAAGGGATCAGGGCAAGGTAAAAGCAGAGCAGGACAGGAATGTGG
CAGGGGGTTCACCTGGGCTTAGGGTCTCTCTATGGCTCAAGTGTTCCTCATTTCTGTGTCTTTCTGTTTCCAGTT
GCAAGTCCAGCCCAGAATGTCTCAGCAGAAGCAGAAGCAGTGTGCTCCCCCGCAGCAGTGCTGCCCCCCACCC
CAGCAGCGCTGCCCCCCACCCCAGCAGTGCTGCCCCCCGCCTCAACAGTGCTGCCCCCCACCCCAGCAGTGCTG
CCCGCCGCCTCAACAATGCTGCCCCCCTCCCCAGCAGTGCTGTCCCCCACCCCAGCAGTGCTGCCCTCCACCTCA
GCAGTACTGCCCCCCACCCCAACAGACCAAGCAGCCTTGCCAGCCCCCACCCAAGTGCCAGGAGCCCTGTGCC
CCCAAGTGCCCACCCCCTCAGCAGTGCCAGACGTCCAAGCAGAAGTAAGCCCTGGGCATGTGATCAGAGGCG
AACCCCCAGGAAAAACAGAGCATGAAGTTCCTTCTGGGCCAGCATCTTACACTCCACAGGCCAAGCAAAGCCC
TGCTCACCAGTGCTTCTGGATGCAGCCACACTTCACACTTGCAGGAGAGGCAGCACCAGGCTGGCAGCTGGCG
GCTTCCTCGAGCTCTACTCTTCTTAATTAGCAGCTGCAGTTTCCATCTCAGACAAGGATCTGGTTCTAAACTATC
TAATCATCTTTCCTGACATGCATCTACATTGCCCTGCTGGAAACATGAGCGGCATGCACAGGCATCTTCTCCTAC
CACGCTTCCCCTCAGCTCCTGTTTTCAAACAATTAAAACAGAATTCCTCTGGCA 3‘

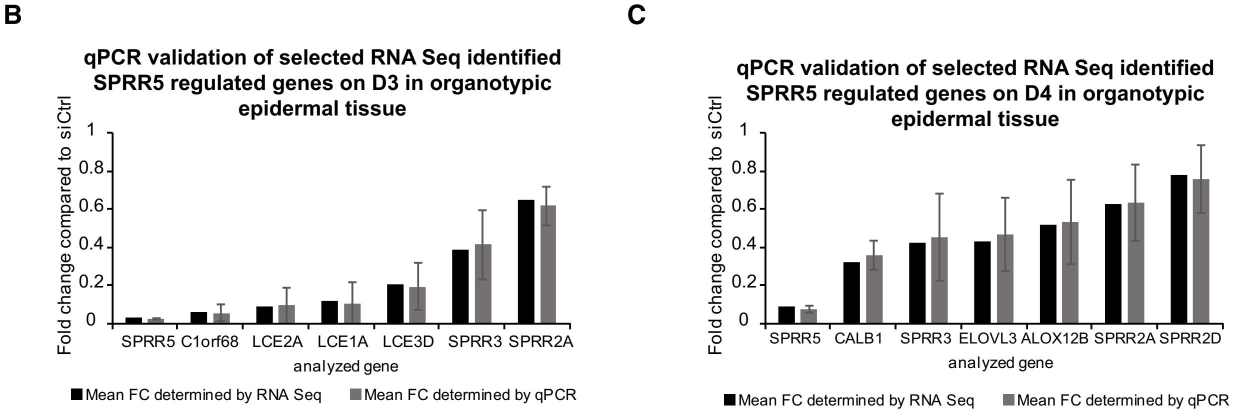


Figure EV4. Sequence of the human SPRR5 transcript and validation of RNA-Seq results.

A The SPRR5 sequence as well as the exon (black)/intron (red) structure of the human SPRR5 transcript are shown.

B, C qRT–PCR validation of the obtained RNA-Seq results for selected genes on day 3 (D3; B) or day 4 (D4; C) in SPRR5-depleted organotypic epidermal tissue (*n* = 3–5 epidermal tissue cultures/knockdown group). Data are presented as mean ± SD.

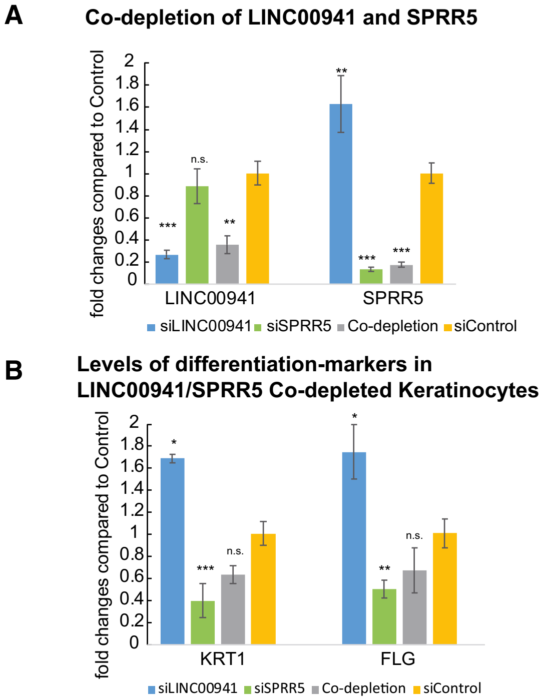


Figure EV5. Co-depletion of LINC00941 and SPRR5.

A, B siPool-mediated LINC00941, SPRR5 knockdown, and co-depletion of LINC00941 and SPRR5 on day 3 (D3) of calcium-induced keratinocyte differentiation (n = 3 biological replicates/knockdown group). Data are presented as mean ± SD. Statistical significance was tested by an unpaired t-test and corrected for multiple testing after Bonferroni (*adj. P-value < 0.05, **adj. P-value < 0.01, ***adj. P-value < 0.001, and n.s. = not significant).