Supplementary Information for

ΔNp63-Senataxin circuit controls keratinocyte differentiation by promoting the transcriptional termination of epidermal genes
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Supplementary Material and Methods

Yeast-two hybrid screening
Ultimate Yeast two-hybrid assay (Y2H) was performed by the HYBRIGENICS services (http://www.hybrigenics-services.com). ULTImate Y2H™ is an optimized version of the yeast two-hybrid (Y2H) screening technique, which, unlike classical Y2H, takes advantage of a patented cell-to-cell mating process to test on average 83 million interactions per screen. The p63 fragment containing aminoacids from 444 to 680 was PCR-amplified and sent to HYBRIGENICS, which cloned it into pB27 vector containing the DNA binding domain of LexA. As cDNA library we utilized the Human Breast Tumor Epithelial Cells cDNA library (prepared by HYBRIGENIC), based on the pivotal role of p63 in regulating breast epithelial homeostasis. A total of 70 millions of interaction has been analyzed and a confidence score (PBS, for Predicted Biological Score, automatically computed through algorithms) was attributed to each interaction. The list of 27 hits with their relative PBS score has been included as supplementary material (Dataset 1).

Growth Curves, Clonogenic, Wound Healing and Transwell migration assays.
A253, A431 and FaDu cells were transfected with SCR, sip63 or siSETX and 48hours after transfection were subjected to growth curves, clonogenic assays, wound healing or transwell migration assays. For clonogenic assay 200 cells were seeded on 6-well plates and incubated at 37°C for 14 days changing the medium every three days. Cells were fixed and stained with a solution of 20% methanol and 0.5% crystal violet. Crystal violet was eluted with 33% acetic acid and the absorbance at 590 nm of the eluent was measured. Cell growth and wound healing ability of transfected cells were measured by Incucyte Live-Cell Analysis System (Essen Biosciences) and
plotted as percentage of confluence or percentage of wound confluence respectively. For transwell migration assay, transfected cells were seeded in the upper chamber of transwell cell culture inserts (8.0 µm, Falcon) in serum-free medium. Culture medium with 10% FBS was used as chemoattractant and added to the lower chambers. After overnight incubation cells were fixed, stained with propidium iodide and fluorescence was measured by Incucyte Live-Cell Analysis System.

**Immunoblotting, Immunoprecipitation and Chromatin Immunoprecipitation (ChIP)**

Immunoblot analysis was performed using total cell extracts obtained by lysing cell pellets with 0.1% Triton buffer (50 mM Tris-HCl pH 7.5, 250 mM NaCl, 50 mM NaF, 1 mM EDTA pH 8, 0.5% Triton), supplemented with protease inhibitors (Roche), PMSF, and sodium orthovanadate. Proteins were separated by SDS/PAGE, transferred onto PVDF membranes, and blocked with PBS-T (phosphate-buffered saline and 0.1% Tween-20) containing 5% non-fat dry milk for 1 h at room temperature (RT). The incubation with primary antibodies was performed for 2 h at RT, followed by incubation with the appropriate horseradish peroxidase-conjugated secondary antibody. Detection was performed with ECL Western Blot Reagent (Perkin-Elmer). Immunoprecipitation was performed by preclearing total cell lysates with protein A or protein G-Sepharose beads (GE Healthcare) for 2 h at 4°C followed by incubation overnight at 4°C with the appropriate amount of antibody. Finally, the immunocomplexes were collected by incubation with protein A or G-Sepharose beads, washed six times in Triton lysis buffer and then eluted by boiling in SDS Laemmli sample buffer. Chromatin Immunoprecipitations were performed using MAGnify Chromatin Immunoprecipitation system (Thermo Fisher) following the manufacturer’s recommendations. Briefly, cells were cross-linked for 10 min at room temperature with 1% formaldehyde (Sigma). The cross-linking reaction was stopped by addition of 125 mM glycine for 5 min. Pellet was lysed and chromatin was sheared using sonicator (Bioruptor UCD-200; Diagenode). The chromatin extract was incubated with Dynabeads Protein A/G coupled to 5 µg of rabbit anti-Senataxin (Novus Biologicals) or rabbit IgG (negative control) overnight at 4 °C. The immune complexes were washed and treated with proteinase K (20 mg/mL) at 55 °C for 15 min to reverse the cross-linking. DNA was isolated with DNA purification magnetic beads and used for qPCR analysis.

**DNA-RNA Immunoprecipitation (DRIP)**

DRIP was performed as described (1). Briefly Ker-Ct were harvested and lysed overnight with SDS and proteinase K. DNA was extracted with phenol/ chloroform/isoamyl alcohol (Sigma), precipitated and then digested overnight using a cocktail of restriction enzymes (BsrGI, EcoRI, HindIII, SspI and
XbaI. New England BioLabs) RNase H specificity control was performed treating 10 μg of digested DNA with 4 μL of RNase H (New England BioLabs) overnight. Digested and RNase H-pretreated DNA were immunoprecipitated with 15 μg of S9.6 antibody (Millipore) for 14–17 h at 4 °C while gently inverting on rotator. Immunocomplexes were collected by incubation with Dynabeads™ Protein G magnetic beads (Thermo Fisher). Immunoprecipitated DNA was eluted incubating dynabeads with proteinase K (20 mg/mL) at 55 °C for 45 min, purified with phenol/ chloroform/isoamyl alcohol and analyzed by qPCR.

**R-loop immunofluorescence and Dot Blot**

Differentiated Ker-CT, transfected with SCR, sip63 or siSETX were cultured on coverslips, fixed with 100% ice-cold methanol for 7 min at -20°C and then blocked with 5 % BSA in PBS overnight at 4°C. Incubation with S9.6 primary antibody was performed overnight at 4°C and was followed by incubation with Alexa Fluor 568 goat anti-mouse secondary antibody (Thermo Fisher) and DAPI 1 hour at RT. Coverslips are placed on a microscope slide with ProLong Gold Antifade Mountant (Thermo Fisher). Fluorescence intensity was measured with ImageJ software (https://imagej.net).

For dot blot, genomic DNA, extracted and treated as described for DRIP, was spotted on a positively charged nylon membrane (Amersham) and then cross-linked with UV (0.12J/m2). Membranes were blocked with PBS-T for 1 h at RT and incubated with S9.6 or anti-adenosine antibodies overnight at 4°C. After incubation with horseradish peroxidase-conjugated anti mouse secondary antibody, detection was performed with ECL Western Blot Reagent (Perkin-Elmer). Densitometric analysis was performed by using ImageJ software. Two biological replicates were analyzed.

**Skin immunofluorescence and haematoxylin/eosin staining**

Paraffin-embedded slides were incubated at 58°C for 1 hr, dewaxed by Bio-Clear washing (Bio-Optica) and rehydrated by serial dilution of ethanol (100, 90, 80, 70 and 50% ethanol). Antigen retrieval was performed by boiling in 0.01 M Sodium Citrate Buffer pH 6.0 and was necessary for co-detecting p63 with differentiation markers. IF staining was performed as follows: 1-h blocking in 5% goat serum (Gibco) in PBS at RT; 3-h primary antibody incubation at RT; and 1-h secondary antibody and DAPI (4',6-diamidino-2-phenylindole) incubation at RT. IF images were acquired by Nikon A1 confocal laser microscope. The following antibodies were used: anti-KRT10 (Covance PRB159P); anti-Loricrin (Covance PRB145P); anti-KRT1 (BioLegend, PRB-149P); anti-p63 (Abcam, ab735); anti ZNF750 (Sigma-Aldrich HPA023012); goat anti-rabbit 488 (Life Technologies, A11034); goat anti-mouse 568 (Life Technologies, A11019).
For haematoxylin/eosin staining, sections were dewaxed and rehydrated as described before, then incubated 5 min in Mayer's haematoxylin (Bio-Optica) solution, extensively washed in distilled water, incubated 5 min in Eosin Y alcoholic solution (Bio-Optica), extensively washed in distilled water and finally dehydrated by 70, 90, 100% ethanol solution incubation.Slides were mounted using Bio Mount HM (Bio-Optica).

**Immunohistochemistry and Tissue Microarray (TMA)**

Cutaneous Squamous Cell Carcinoma TMA was purchased by US Biomax, Inc (SK802c). TMA slide includes 76 cases of squamous cell carcinoma, 2 each of adjacent normal skin tissue, plus 2 normal skin tissues. TMA slides were deparaffinized and antigen retrieval has been performed (in HIER solution at pH 6; 98 °C for 30 min). Sections were incubated for 1 h with anti-SETX Ab #1 (Rabbit polyclonal, Novus Biologicals NBP1-94712, dilution 1:1000), anti-SETX Ab#2 (kind gift from Prof. Delia, dilution 1:1500) and p63 (Mouse Monoclonal clone (4A4) - pre-diluted; Ventana, Tucson, AZ, USA). The UltraTek HRP Anti-Polyvalent Staining System (Seytek) was used for detection. As shown in table S1, for each investigated marker, immunohistochemical analyses has been evaluated by assigning a score (0-3+).

**RNA Isolation and RT-qPCR.**

Total mRNA was isolated from cell lines using the RNeasy mini kit (Qiagen), quantified using a NanoDrop Spectrophotometer (Thermo Scientific) and retrotranscribed by GoScript Reverse Transcription System (Promega) according to the manufacturer's protocols. Real-time PCR was performed using GoTaq qPCR Master Mix (Promega) with the QuantStudio 5 Real-Time PCR Systems (Applied Biosystems). The expression of each gene was defined from threshold cycle (Ct), and the relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method. The primers utilized for qRT-PCR are listed in Table S2.

**RNA-seq and bioinformatic analyses.**

Total RNA was extracted using the RNeasy mini kit (Qiagen) and purified from DNA contamination through a DNase I (Qiagen, IT) digestion step. Quantity and integrity of the extracted RNA were assessed by NanoDrop Spectrophotometer (NanoDrop Technologies, DE) and by Agilent 2100 Bioanalyzer (Agilent Technologies, CA), respectively. RNA libraries for sequencing were generated in triplicate using the same amount of RNA for each sample according to the Illumina TruSeq Stranded Total RNA kit with an initial ribosomal depletion step using Ribo Zero Gold (Illumina, CA). The libraries were quantified by qPCR and sequenced in paired-end mode (2x100 bp) with NovaSeq.
For each sample generated by the Illumina platform, a pre-process step for quality control was performed to assess sequence data quality and to discard low-quality reads. RNA-seq data were analyzed with “rnaseq” version 3.3 pipeline of nf-core community (https://nf-co.re/rnaseq). The alignment and quantification were performed with STAR+salmon method. The pipeline was run with default parameters (2). Enrichment analysis and geneset interpretation were performed with the oncoEnrichR software (3). Differential expression analysis was carried out using DESeq2 package (4). Normalized counts were expressed as the variance stabilizing transformation (VST function). Data were further scaled applying the difference between the expression value of each gene in each sample and the relative mean of expression associated to the gene among the dataset. Unsupervised hierarchical clustering was performed with the ComplexHeatmap R package (5). TCGA and GTEx expression data were downloaded from UCSC Xena platform (6). Mutation and clinical data of squamous cell cancer were downloaded from cBioportal (7-8). Survival curves were estimated and compared by the Kaplan-Meier product-limit method and log-rank test, respectively. Median values of expression were compared by the Wilcoxon test. The level of significance was defined as p<0.05. Statistical analyses were carried out using R software. RNA-seq data have been deposited in the NCBI’s Gene expression Omnibus (GEO: GSE193205).

**Primary non-melanoma skin tumors samples, ethics approval and consent to participate.**

Total RNA was isolated from clinical specimens using RNeasy Lipid Tissue Mini Kit (Qiagen, Hilden, Germany) and retrotranscribed by a SensiFAST cDNA Synthesis Kit (Bioline, Memphis, TN, USA) according to the manufacturer’s protocol. RT-qPCR was performed as described above. Clinicopathological features and patient details have been already described (9). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Bioethics Committee of IDI-IRCCS (Protocol No. 552/1, December 14, 2018). Informed consent was obtained from all individual participants included in the study.

**Organotypic epidermis culture**

Organotypic human epidermis were generated using 3D full thickness starter kit from CELLnTECH following the manufacturer's protocol. Briefly HEKn were transfected with SCR or siSETX siRNA oligos and 24 hours after transfection were seeded on top of the established dermal fibroblast layers, previously arranged into PET inserts starting from normal human fibroblast (Cascade Biologics, Thermo Fisher). After three days, the inserts were transferred into new plates containing 2.5 mm-
thick spacers and the models were grown to the air-liquid interface for 12 days before being fixed with 4% paraformaldehyde over night at 4°C.

**Statistical information**

For qRT-PCR studies, the expression of each gene was defined from threshold cycle (Ct), and the relative expression levels were calculated using the 2-ΔΔCt method. The relative expression levels of indicated genes were displayed as bars representing the mean of three technical replicates (n=3, PCR runs) ± SD. The number of biological replicates per experiment varied and it is mentioned in the figure legends.

For R-loop quantification, we analyzed 114 nuclei and plotted S9.6 fluorescence intensity as the mean ± SD. p value was calculated by one way ANOVA. For quantification of whole skin or stratum corneum, n=30 measurements in control (SCR) and SETX depleted skin were performed by ImageJ. Data were plotted as mean ± SD and p value was calculated by Welch’s t-test. Differences were considered statistically significant at p < 0.05.

**Supplementary Figures**
**Figure S1.** (A) Whole-cell extract from MCF7 cell line was subjected to immunoprecipitation with an anti-SETX antibody followed by immunoblotting with the indicated antibodies. (B) Human immortalized keratinocytes (Ker-CT) were transfected with scramble (SCR) or siSETX siRNA oligos. 48 hours after transfection, transfected cells were fixed and subjected to immunofluorescence staining for SETX (green). DAPI (blue) were used to visualize nuclei.
Figure S2. (A) Immortalized keratinocytes (Ker-CT) were transfected with siRNA targeting p63 (sip63), SETX (siSETX) or a nonrelevant mRNA (scramble, SCR). Transfected cells were fixed and stained with S9.6 (red) and DAPI (blue) to visualize R-loops and nuclei, respectively. S9.6 fluorescence intensity per nucleus (n=114) was quantified and plotted (central panel). Data are shown as the mean ± SD. p value was calculated by t-test. ns= not significant. In parallel, total protein lysates were analyzed by immunoblotting using antibodies to the indicated proteins (right panel).

(B) RNA/DNA hybrid dot blot. Immortalized keratinocytes were transfected as in (A), genomic DNA was extracted, digested with a restriction enzyme cocktail and either treated (+) or not (-) with RNase H. Genomic extracts were spotted on a membrane in a two-fold dilution (1,000 to 125 ng per spot from left to right) and probed with anti-DNA-RNA Hybrid (clone S9.6) antibody. S9.6 signal was quantified and normalized to the adenosine signal, used as loading control (left panel). Densitometric analysis (central panel) was performed by Image J and is shown as the mean ± SD of two biological replicates (n=2). ns=not significant. In parallel, total protein lysates were analyzed by immunoblotting using antibodies to the indicated proteins (right panel).
Figure S3. A253 cells were transfected with siRNA targeting p63 (sip63), Senataxin (siSETX) or a nonrelevant mRNA (scramble, SCR). Transfected cells were subjected to protein extraction and immunoblotting using the indicated antibodies.
Figure S4. SETX regulates the expression of epithelial genes in immortalized human keratinocytes. (A) Heatmap showing the unsupervised hierarchical clustering of the most variables (Deseq2 VSD) genes (n=200) in the SCR (orange), sip63 (green), siSETX (blue) transfected immortalized human keratinocytes (Ker-CT) treated for three days with CaCl2. Color scheme: Violet (highest) Yellow (lowest) VSD score. (B) Venn diagram showing the shared downregulated (top panel) and upregulated genes (bottom panel) in sip63 (yellow) and siSETX (violet) transfected cells. (C) Barplot showing the top 10 gene ontology (GO) terms for Biological Process, Molecular Function and Cell Compartment ordered by FDR. (D) Heatmap showing the unsupervised hierarchical clustering of genes associated to the Epidermis development GO term in SCR (orange), sip63 (green), siSETX (blue) transfected cells. Color scheme: Violet (highest) Yellow (lowest) VSD score.
Figure S5. (A) Quantification by qPCR of DNA-RNA immunoprecipitation (DRIP) signal in A253 cells treated with SCR or SETX siRNAs. Values at 3’ends were normalized to ZNF750 intron 1 signal. Bars represent the mean of three replicates (n=3, PCR runs) ± SD and are representative of two independent experiments (n=2 biological replicates). p value was calculated by t-test. (B) Genomic DNA extracted from differentiated human immortalized keratinocytes (Ker-CT) was treated or not with RNase H1 and subjected to DRIP followed by qPCR quantification. Bars represent the mean of three replicates (n=3, PCR runs) ± SD and are representative of two independent experiments (n=2 biological replicates). p value was calculated by t-test. (C) Genomic DNA of differentiated normal human keratinocytes (HEKn) was treated or not with RNase H1 and subjected to DRIP followed by qPCR quantification of the indicated loci. Bars represent the mean of three replicates (n=3) ± SD. (D) Quantification by qPCR of ChIP analysis of endogenous SETX occupancy at p63 binding site (BS#2) in proliferating keratinocytes. Bars represent the mean of three replicates (n=3, PCR runs) ± SD and are representative of two independent experiments (n=2 biological replicates). p value was calculated by t-test.
Figure S6. SETX loss does not affect R-loop signals and the production of read-through transcripts at the 3’ ends of HAS3 gene. (A) Quantification by qPCR of DNA-RNA immunoprecipitation (DRIP) signal in differentiated keratinocytes treated with SCR or siSETX siRNAs (left panel). Values at 3’ends were normalized to HAS3 intron signal. Bars represent the mean of two independent experiments (n=2 biological replicates) ± SD. p value was calculated by t-test. In parallel, genomic DNA of differentiated keratinocytes was treated or not with RNase H1 and subjected to DRIP followed by qPCR quantification. Bars represent the mean of three replicates (n=3, PCR runs) ± SD and are representative of two independent experiments (n=2 biological replicates). p value was calculated by t-test. * P < 0.05, ns= not significant. (B) qRT-PCR analysis of read-through transcripts at HAS3 3’ region in SCR or siSETX differentiated keratinocytes. Values are normalized to ZNF750 intron 1 and showed as fold enrichment of SCR over siSETX. Bars represent the mean of two biological replicates (n=2 biological replicates) ± SD.
Figure S7. SETX regulates R-loop formation on the 3’ end of the p63 target gene KRT1. (A) Schematic representation of KRT1 genetic locus (modified from genome browser, https://genome.ucsc.edu) showing from top to bottom p63 ChIP-seq peaks, GC skew signal and gene structure with exons (boxes) and introns (lines). (B) Quantification by qPCR of DNA-RNA immunoprecipitation (DRIP) signal in differentiated keratinocytes treated with SCR or SETX siRNAs. Values at 3’ends were normalized to KRT1 intron signal. Bars represent the mean of three replicates (n=3, PCR runs) ± SD and are representative of two independent experiments (n=2 biological replicates) (C) Genomic DNA of differentiated keratinocytes was treated or not with RNaseH1 and subjected to DRIP followed by qPCR quantification. Bars represent the mean of three replicates (n=3, PCR runs) ± SD and are representative of two independent experiments (n=2 biological replicates) (D) RT-qPCR analysis of read-through transcripts at KRT1 3’ region in SCR or siSETX differentiated keratinocytes. Values are normalized to KRT1 intron1 and showed as fold enrichment of siSETX over SCR. Bars represent the mean of three replicates (n=3, PCR runs) ± SD and are representative of two independent experiments (n=2 biological replicates). (E,F) Quantification by qPCR of ChIP analysis of endogenous SETX occupancy at p63 binding site (p63BS) in proliferating (E) and differentiated (F) keratinocytes. Bars represent the mean of three replicates (n=3, PCR runs) ± SD and are representative of two independent experiments (n=2 biological replicates).
**Figure S8.** (A) qRT-PCR analysis of p63 and SETX mRNA levels from normal human keratinocytes (HEKn) at different time points (0, 3, 6 and 9 days) upon CaCl₂ treatment. The quantification is relative to mRNA expression in proliferating cells (time 0). The data shown represent the mean ± SD of three biological replicates (n=3). P value was calculated by t-test. ns= not significant. (B) Immunofluorescence staining of senataxin (green) and p63 (red) in normal human epidermis. DAPI (blue) were used to visualize nuclei. Dotted lines underline the keratinocyte-fibroblast border. (C) Immunofluorescence staining of the differentiation marker ZNF750 (green) in senataxin-depleted (siSETX) compared to scramble control (SCR) organotypic epidermis. p63 staining (red) and DAPI (blue) were used to visualize the basal layer and nuclei, respectively. Dotted lines underline the keratinocyte-fibroblast border.
Figure S9. SETX expression is downmodulated in psoriatic lesions. Violin plot showing SETX mRNA expression in skin samples of normal healthy controls (n=64) and psoriatic patients (N=58) (GEO dataset GSE13355). **** p value < 0.0001.
Figure S10. SETX depletion does not affect the proliferation and migration of SCC cells. (A) Total protein lysates from the indicated cell lines were analyzed by immunoblotting using anti-SETX or anti-p63 antibodies. Vinculin was used as a loading control. (B–D) A431, Fadu and A253 cells were transfected with scramble (SCR) or SETX siRNA oligos (siSETX#1 and siSETX#2). 48 hours after transfection, transfected cells were utilized for monitoring cell growth by Incucyte Live-Cell Analysis System (upper panels) or plated for clonogenic assays (lower panels). A representative of n = 3 experiments is shown; Colony forming cells were quantified by measuring the absorbance of eluted
crystal violet. Data are averages from three (A431) or two (Fadu and A253) independent experiments ±SD. Representative images of colonies were shown in the bottom left panel. In parallel, total protein lysates were immunoblotted for the indicated proteins. (E-F) A431 and A253 cells, transfected as in A and D respectively, were utilized for wound healing (left panels) or transwell migration assays (right panels). Percentage of wound confluence was measured by the Incucyte Live-Cell Analysis System. A representative of n = 3 experiments is shown (left). Transwell inserts were fixed and stained with propidium iodide. Histograms in the right panels show migration quantification plotted as ratio of migrated to total cells. Data are shown as average of three independent experiments ±SD (n=3 biological replicates, A431 cells) or as a representative of two independent experiments (n=2 biological replicates, A253). p value was calculated by t test. ns= not significant.
Figure S11. Uncropped immunoblotting images.
Table S1. Immunohistochemical score based on both number of positive cells and intensity of staining.

| Score | Meaning               |
|-------|-----------------------|
| 0     | No/rare positive cells|
| 1+    | Weak staining         |
| 2+    | Moderate staining     |
| 3+    | Strong staining       |

Table S2. qPCR primer list

| Primer name | Sequence 5→3          |
|-------------|-----------------------|
| hZNF750 for | AGCTCGCCTGAGTGTTGAC   |
| hZNF750 rev | TGCAGACTCTGGCTGTGA    |
| hKRT1 for  | GGTGAAGTCTCGAGAAAGGA  |
| hKRT1 rev  | TGGTCCACTCTCCCTCGGA   |
| hGRHL3 for | GTGGAACGTGAATGAAGAGGC |
| hGRHL3 rev | GACAGACGGGTGTACACAGG  |
| hKRT75 for | TACGAGGACATTGCGAACCG  |
| hKRT75 rev | TTTGCAAGCGGAACTCTGC   |
| hDNp63 for | GAAGAAAGGACACAGCAGT   |
| hDNp63 rev | GGGACTGGTAGGACAGGAG   |
| hSETX for  | TGAGTTGTCCGCGGACTTT   |
| hSETX rev  | CGGGTATCAAATCCTCAGAT  |
| hKRT10 for | AGGAGGAGTGTCACTCCCTAAG|
| hKRT10 rev | AAGCTGCGCTTCTAAAACCTC |
| hZNF750 intr1 for | GGAAGTAGAGAAGCGCGGT |
| hZNF750 intr1 rev | TCTCAGCCTTCAAGACAG |
| hZNF750 3’ end for | ATACCCACCCCAACAATC |
| hZNF750 3’ end rev | TGTGTGCAGTCCCTCATC |
| hZNF750 BS#1 for | GGAGGGAGCTCTTCAAGAG |
| hZNF750 BS#1 rev | CCTCCGATTAAGCAGCAAG |
| hZNF750 BS#2 for | GGAAGTAGAGAAGCGGGTG |
| hZNF750 BS#2 rev | TCTCAGCCTTCAAGACAG |
| Primer          | Sequence                        |
|----------------|---------------------------------|
| hRPL13A for    | AGGTGCCTTGCTCACAGAGT            |
| hRPL13A rev    | GGGTGCATTGCCCTCATATTAC          |
| hCALM3 for     | GAGGAATTGTGGCGTTGACT            |
| hCALM3 rev     | AGAGTGCCAAATGAGCAGT             |
| hTFTP for      | TCTGGGAGTCCAAGCAGACT            |
| hTFTP rev      | AAGGAGCCACTGAAGGGTTT            |
| hKRT1 intr1 for| AGTCCCAATTCCGCTCAAGA            |
| hKRT1 intr1 rev| GTCGACCTAAAGGTTGGTCAG           |
| hKRT1 3’end for| ACGTGGAAACAGCCAGTAAGG           |
| hKRT1 3’end rev| TGAGCTATGTGTCAACAAGGGA          |
| hKRT1 ChIP for | AGTAGCGTCCCTGGGAAGA             |
| hKRT1 ChIP rev | AGAGCTGGCTTGATCTGTGG            |
| hGAPDH for     | AGCCACATCGCTCAGACA              |
| hGAPDH rev     | GCCCAATACGACCACAAATC            |
| hTBP for       | CTGACAGCTAAGGAGGACGC            |
| hTBP rev       | AGTTACCTGACCTCTCCCCC            |
| hHAS3 for      | TGTGCATTGCGCGCATTACC            |
| hHAS3 rev      | CCGAGCGGCAGGCCACTT              |
| hHAS3 intr for | CTCAGGACAGGGTGTTGGGG            |
| hHAS3 intr rev | GTCAAAGCCACCTCGGTCT             |
| hHAS3 3’end for| TGGGCAATATGTCCATCTGCTGG         |
| hHAS3 3’end rev| GGCCCATTCTCATCGCCAGTTA          |
| hHK2 for       | GGGCATCCTTGAACAAAAG             |
| hHK2 rev       | GGTCCTCAAGCCCTAAAG              |

SUPPLEMENTARY REFERENCES
1. Sanz LA, Chédin F (2019) High-resolution, strand-specific R-loop mapping via S9.6-based DNA-RNA immunoprecipitation and high-throughput sequencing. Nat Protoc. 14(6):1734-1755.
2. Ewels PA, et al. (2020) The nf-core framework for community-curated bioinformatics pipelines. Nat Biotechnol. 38(3):276-278
3. Nakken, S., et al. (2021). OncoEnrichR: cancer-dedicated gene set interpretation (preprint).
4. Love MI, Huber W, Anders S. (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 15:550.
5. Gu Z, Eils R, Schlesner M. (2016) Complex heatmaps reveal patterns and correlations in multidimensional genomic data. Bioinformatics 32:2847-2849
6. Goldman, M.J., et al. (2020) Visualizing and interpreting cancer genomics data via the Xena platform. Nat Biotechnol. 38(6):675-678
7. Cerami E, et al. (2012) The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2(5):401-4.
8. Gao J, et al. (2013) Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal. 6(269):pl1
9. Mancini M, et al. (2021) Involvement of transcribed lncRNA uc.291 and SWI/SNF complex in cutaneous squamous cell carcinoma. Discov Onc 12,14.

Additional materials for this manuscript include the following
Dataset 1: list of p63 interacting proteins identified by yeast two-hybrid (Y2H) screen