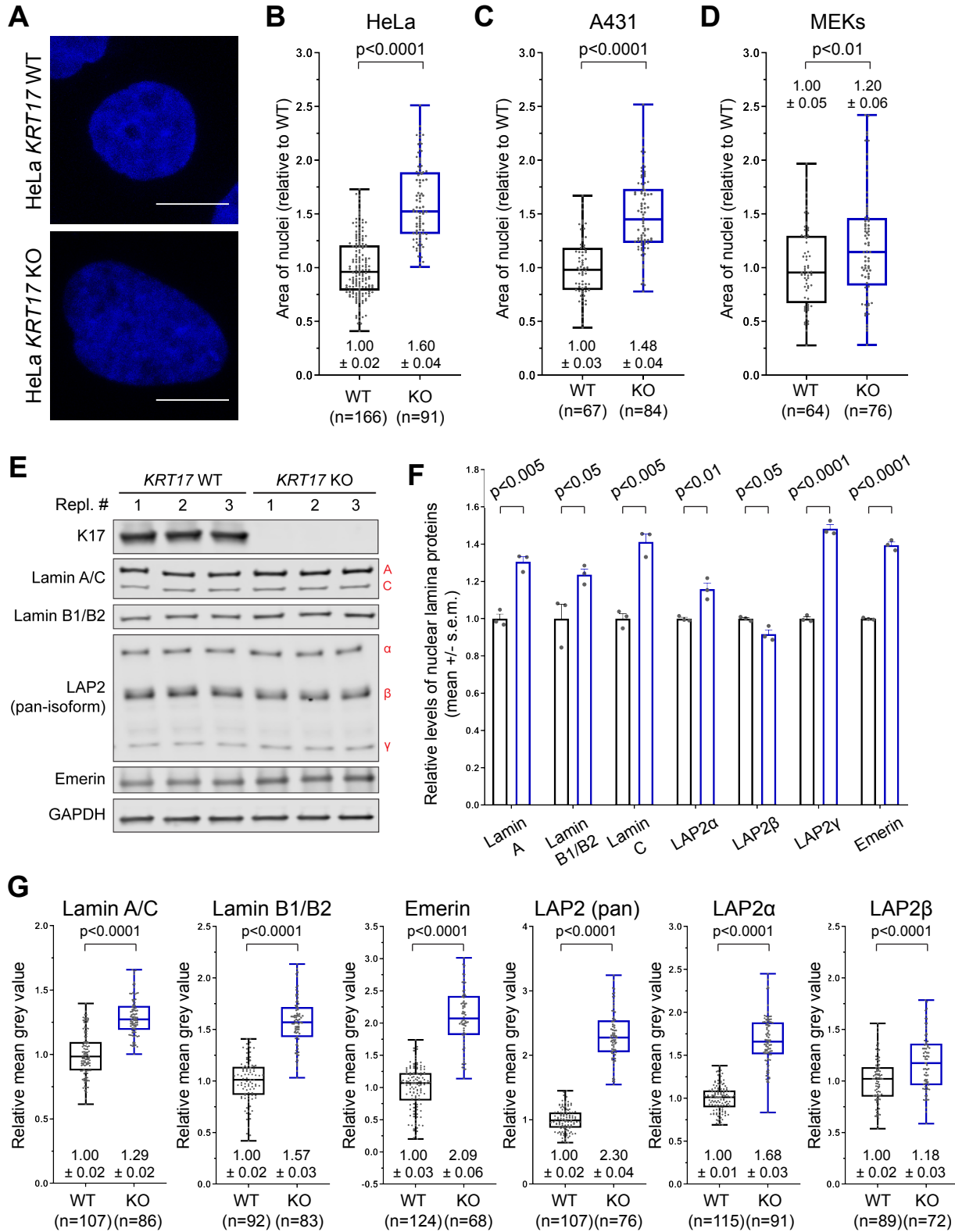Jacob et al. Figure S1
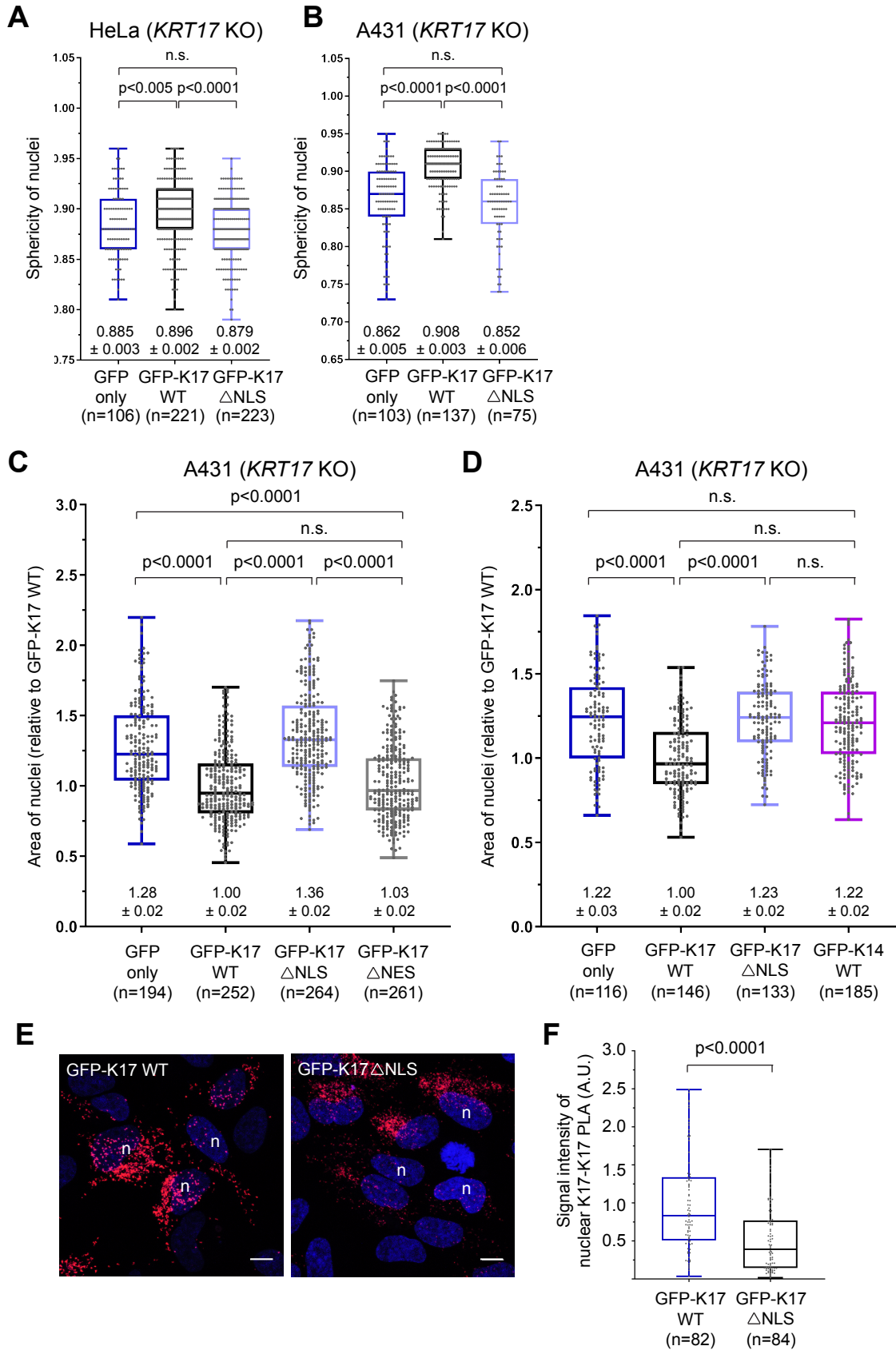
Figure S1
Loss of K17 alters nuclear morphology and nuclear lamina-associated proteins.
(A) Confocal micrograph maximum intensity projections (MIPs) of individual nuclei from *KRT17* WT vs. *KRT17* KO HeLa cells. Nuclei are stained with DAPI (blue). Scale bars, 10 µm. **(B, C, and D)** Quantified area measurements of nuclei from cultured *KRT17* WT vs. KO *KRT17* (B) HeLa and (C) A431 human epithelial tumor cells, and (D) primary mouse epidermal keratinocytes (MEKs). Numeric values above or below each box-and-whisker plot designate the mean +/- standard error of the mean (s.e.m.). **(E)** Western blots of nuclear lamina-associated proteins from whole-cell lysates of *KRT17* WT and *KRT17* KO HeLa cells (n=3 biological replicates per genotype). **(F)** Quantitation of Western blot band intensities in (E), each normalized to GAPDH loading control. **(G)** Quantitation of mean immunofluorescence signal intensity of confocal micrograph MIPs of nuclei immunostained with antibodies recognizing several nuclear lamina-associated proteins (refer to **Fig. 1E**).

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A HeLa (*KRT17* KO)

Sphericity of nuclei

n.s.

p<0.005 p<0.0001

0.885 ± 0.003   0.896 ± 0.002   0.879 ± 0.002

GFP only (n=106)   GFP-K17 WT (n=221)   GFP-K17 △NLS (n=223)

B A431 (*KRT17* KO)

Sphericity of nuclei

n.s.

p<0.0001 p<0.0001

0.862 ± 0.005   0.908 ± 0.003   0.852 ± 0.006

GFP only (n=103)   GFP-K17 WT (n=137)   GFP-K17 △NLS (n=75)

C A431 (*KRT17* KO)

Area of nuclei (relative to GFP-K17 WT)

p<0.0001

n.s.

p<0.0001   p<0.0001   p<0.0001

1.28 ± 0.02   1.00 ± 0.02   1.36 ± 0.02   1.03 ± 0.02

GFP only (n=194)   GFP-K17 WT (n=252)   GFP-K17 △NLS (n=264)   GFP-K17 △NES (n=261)

D A431 (*KRT17* KO)

Area of nuclei (relative to GFP-K17 WT)

n.s.

n.s.

p<0.0001   p<0.0001   n.s.

1.22 ± 0.03   1.00 ± 0.02   1.23 ± 0.02   1.22 ± 0.02

GFP only (n=116)   GFP-K17 WT (n=146)   GFP-K17 △NLS (n=133)   GFP-K14 WT (n=185)

E

GFP-K17 WT   GFP-K17△NLS

F

Signal intensity of nuclear K17-K17 PLA (A.U.)

p<0.0001

GFP-K17 WT (n=82)   GFP-K17 △NLS (n=84)

Figure S2
Nuclear-localized K17 rescues nuclear morphology.

(A and B) Quantified sphericity measurements of nuclei from (A) HeLa *KRT17* KO and (B) A431 *KRT17* KO cells transiently transfected (48-hour transfection) with GFP (only), GFP-K17 WT, or GFP-K17ΔNLS plasmid constructs. Numeric values below each box-and-whisker plot designate the mean +/- s.e.m. **(C and D)** Quantified area measurements of nuclei from A431 *KRT17* KO cells transiently transfected (48-hour transfection) with GFP (only), GFP-K17 WT, GFP-K17ΔNLS, and (C) GFP-K17ΔNES or (D) GFP-K14 WT plasmid constructs. Numeric values below each box-and-whisker plot designate the mean +/- s.e.m. **(E)** Confocal micrograph MIPs of proximity ligation assays (PLAs) of HeLa *KRT17* KO cells transfected (48-hour transfection) with GFP-K17 WT or GFP-K17ΔNLS. PLAs relied on the use of two different host-species antibodies recognizing K17. Scale bars, 10 μm. **(F)** Quantitation of nuclear PLA signal intensities in (E). A.U., arbirtrary units.

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A

Krt17 WT sequence

```
               ⊢— NLS WT —⊣
5'-gatgcccacctgactcagtacaagccaaaagaacctgtgaccacccgccaggtgcgcacc-3'
aa392 D  A  H  L  T  Q  Y  K  P  K  E  P  V  T  T  R  Q  V  R  T  aa411
3'-ctacgggtggactgagtcatgttcggttttcttggacactggtgggcggtccacgcgtgg-5'
                              aa        aa
                              399       401
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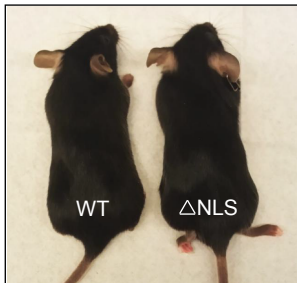
Krt17△NLS sequence

```
               ⊢— △NLS —⊣
5'-gatgcccacctgacacagtacgcgccagcagaacctgtgaccacccgccaggtgcgcacc-3'
aa392 D  A  H  L  T  Q  Y  A  P  A  E  P  V  T  T  R  Q  V  R  T  aa411
5'-ctacgggtggactgtgtcatgcgcggtcgtcttggacactggtgggcggtccacgcgtgg-3'
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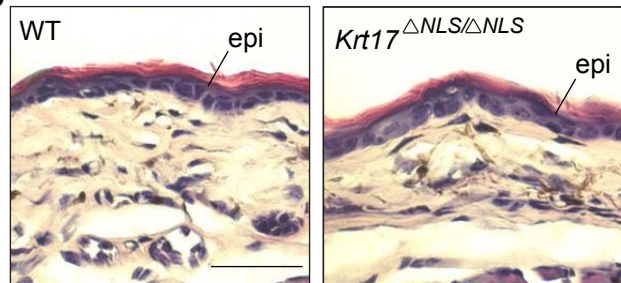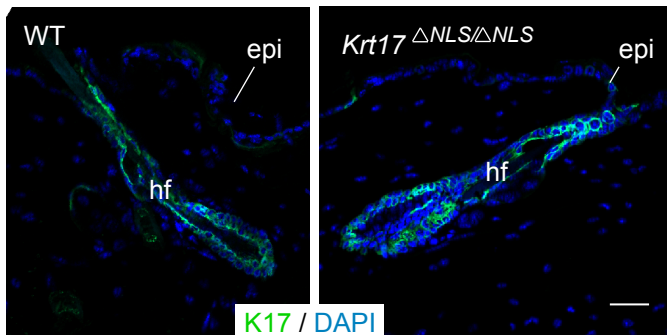
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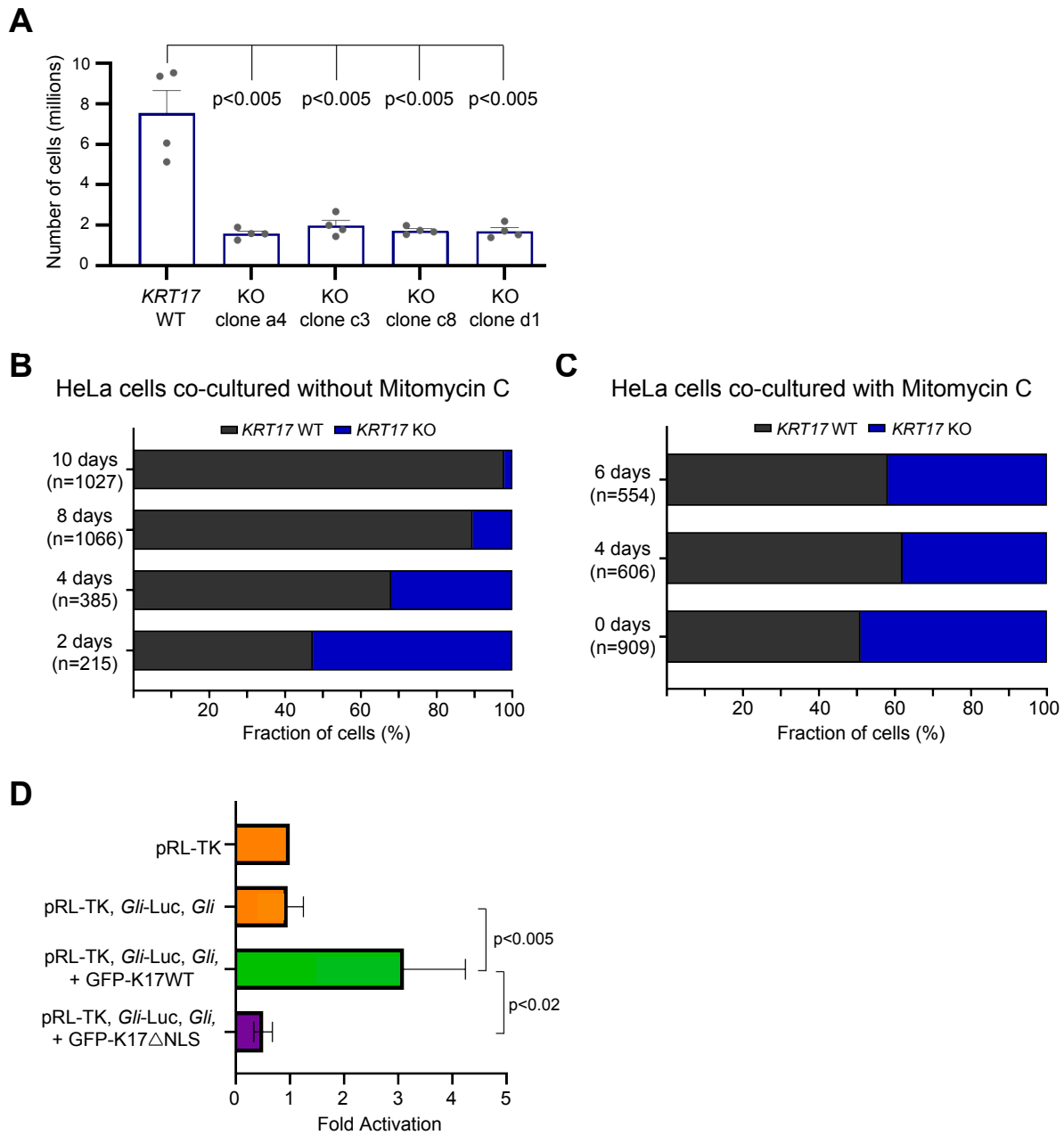
B
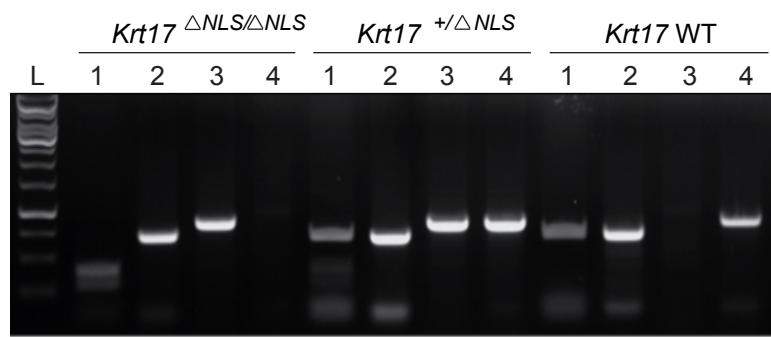
C

D

E

Figure S3
Genesis of a *Krt17^{ΔNLS/ΔNLS}* mouse strain.

(A) DNA and protein sequences for the *Krt17* WT and *Krt17^{ΔNLS}* alleles. Lysine-399 (K399) and Lysine-401 (K401) are both mutated to Alanine residues (K399A and K401A) in the *Krt17^{ΔNLS}* mutant allele. The *Krt17^{ΔNLS}* mutant allele contains a new BstUI restriction enzyme cleavage site (purple text and line). **(B)** RT-PCR genotyping results for *Krt17^{ΔNLS/ΔNLS}*, *Krt17^{+/ΔNLS}*, and *Krt17* WT mice. (lane 1) BstUI digest of the NLS flanking region; (lane 2) undigested control of the product digested in lane 1; (lane 3) PCR amplification using the *Krt17^{ΔNLS}* mutant-specific primer; and (lane 4) PCR amplification using the *Krt17* WT-specific primer. **(C)** Age-matched *Krt17* WT and *Krt17^{ΔNLS/ΔNLS}* mutant ("ΔNLS") mice demonstrating a comparable phenotype at baseline. **(D)** Hematoxylin and eosin staining of (P60) ear tissue sections from *Krt17* WT and *Krt17^{ΔNLS/ΔNLS}* mutant mice. Scale bars, 50 μm. **(E)** Confocal micrographs of hair follicles from *Krt17* WT and *Krt17^{ΔNLS/ΔNLS}* mice, immunostained for K17 (green). DAPI, blue. Scale bars, 50 μm.

Jacob et al., Figure S4

A

B

HeLa cells co-cultured without Mitomycin C

C

HeLa cells co-cultured with Mitomycin C

D

Figure S4
Impact of K17 loss on cell proliferation and GLI1 function.
(A) Cell counting assay using a hemocytometer to count the number of cells after seeding 500,000 cells per genotype (e.g. HeLa *KRT17* WT cells and four different HeLa *KRT17* KO clones) and culturing at 37ºC and 5% CO_2 for three days. **(B)** Quantitation of the percent of HeLa *KRT17* WT and *KRT17* KO cells after mixing and plating 1:1 and then culturing in normal growth media for different lengths of time. **(C)** Quantitation of the percent of HeLa *KRT17* WT and *KRT17* KO cells after mixing and plating 1:1 and then culturing in normal growth media (supplemented with Mitomycin C) for different lengths of time. **(D)** Luciferase assays in HeLa KO cells transfected with a *Gli1*-Luciferase reporter construct (see Methods). Data were normalized with regard to transfection efficiency and signal obtained with pRL-TK vector control. Data represent mean ± s.e.m. from 5 biological replicates each consisting of 6 technical replicates. Mann-Whitney tests were performed to compare each parameter using GraphPad Prism 8.

Table S1
Top entries from proteomics screen for K17-interacting proteins in nuclear extracts from A431 keratinocytes.
Data filtering (e.g., 127N/130N ratio >1.2; 127N/130N count >4; cf. Methods) yielded 77 proteins enriched by K17 IP in *KRT17* WT cells over *KRT17* KO cells.

TABLE S1 Source data file - Jacob et al. - Unbiased Proteomics Screen - K17 interacting proteins listed according to 127N / 130N count (>4)

Accession	Gene	Coverage	# Unique Peptides	# Peptides	PSMs	127N / 130N ratio	127N / 130N Count	MW [kDa]	calc. pI
4504309	HIST4H4	64.08%	13	14	648	1.472	216	11.4	11.4
530384720	HNRNPA2B1	71.67%	20	26	774	1.264	186	37.4	8.95
4885381	HIST1H1B	55.75%	17	27	644	1.361	143	22.6	10.9
5902076	SRSF1	42.34%	11	13	197	1.209	55	27.7	10.4
55956921	HNRNPAB	57.54%	12	18	169	1.292	54	30.6	7.91
40254924	LRRC59	41.04%	12	15	179	1.362	50	34.9	9.57
167234419	THRAP3	27.64%	18	28	170	1.273	47	108.6	10.2
4506901	SRSF3	38.41%	7	8	343	1.266	46	19.3	11.7
4885377	HIST1H1D	59.73%	7	26	739	1.25	44	22.3	11
238776833	ALYREF	46.21%	12	12	180	1.351	43	27.5	11.1
4506695	RPS19	59.31%	11	13	133	1.217	43	16.1	10.3
4885379	HIST1H1E	63.93%	7	27	793	1.347	41	21.9	11
7705373	LIMA1	37.42%	20	28	137	1.225	36	85.2	6.84
5031749	HMGN2	65.56%	3	10	224	1.895	33	9.4	9.99
4885375	HIST1H1C	61.50%	6	26	745	1.268	32	21.4	10.9
4557701	KRT17	52.31%	12	28	279	2.514	30	48.1	5.02
42734430	PTRF	32.82%	9	12	100	1.204	25	43.4	5.6
4506903	SRSF9	46.15%	9	11	95	1.251	24	25.5	8.65
106775678	HIST2H2AA3	68.46%	4	11	565	1.69	23	14.1	10.9
332801090	HNRNPDL	25.62%	8	10	80	1.289	19	40	9.96
578831350	UBTF	22.64%	10	18	71	1.227	19	89.4	5.81
4504253	H2AX	65.03%	4	11	409	1.638	18	15.1	10.7
530384472	CBX3	33.33%	5	7	82	1.32	17	20.8	5.33
321267473	SAFB	20.64%	6	17	97	1.454	16	95.1	6.46
190885499	COX5A	21.33%	4	4	47	1.304	15	16.8	6.79
4506715	RPS28	46.38%	3	3	41	1.203	15	7.8	10.7
4504297	HIST1H3A	51.47%	2	11	323	1.263	14	15.4	11.1
359279859	DPYSL5	1.24%	1	1	37	1.226	13	61.4	7.2
4506773	S100A9	51.75%	6	6	38	1.505	12	13.2	6.13
4504255	H2AFZ	53.91%	3	5	237	1.329	12	13.5	10.6
48255933	HMGN1	52.00%	6	7	37	1.375	11	10.7	9.6
4758302	ERH	28.85%	3	5	43	1.303	11	12.3	5.92
327478416	OCLN	35.06%	7	8	37	1.301	11	31.6	5.68
6912292	CBX5	31.94%	5	7	39	1.268	11	22.2	5.86
19557702	SURF6	20.50%	7	9	38	1.202	11	41.4	10.6
530364188	S100A16	39.81%	3	3	30	1.518	10	11.8	6.79
166795236	TACSTD2	23.53%	4	7	37	1.276	10	35.7	8.87
4502491	C1QBP	16.67%	2	4	40	1.251	10	31.3	4.84
6912676	SNW1	21.08%	7	8	32	1.206	10	61.5	9.52

Accession	Gene	Coverage	# Unique Peptides	# Peptides	PSMs	127N / 130N ratio	127N / 130N Count	MW [kDa]	calc. pI
54633315	DNTTIP2	12.04%	5	9	39	1.455	9	84.4	6.16
5454152	UQCRB	47.75%	4	6	38	1.235	9	13.5	8.78
255652953	ZC3H18	9.44%	6	9	30	1.228	9	106.3	8.32
386781463	LSR	17.01%	6	7	30	1.222	9	59.7	9.41
9624998	HNRNPH2	25.17%	2	8	129	2.092	8	49.2	6.3
21614544	S100A8	16.13%	2	2	13	1.59	8	10.8	7.03
5803137	RBM3	17.20%	2	3	24	1.347	8	17.2	8.91
20149675	EFHD2	25.83%	4	7	30	1.32	8	26.7	5.2
767948000	SRRT	8.78%	5	7	28	1.292	8	90.3	5.62
7661742	C19orf53	42.42%	4	6	28	1.264	8	10.6	11.6
767909532	PIGR	9.82%	5	6	24	2	7	83.2	5.74
27735127	SLC35F3	3.27%	1	2	9	1.255	7	54.7	9.51
768012048	TIMM50	15.25%	4	4	21	1.228	7	38.3	6.55
4506699	RPS21	20.48%	2	2	26	1.227	7	9.1	8.5
28195394	HIST2H2AB	70.00%	2	8	274	1.831	6	14	10.9
148664186	PKP2	7.77%	5	5	21	1.231	6	92.7	9.29
20270186	HMGN3	31.31%	3	5	45	1.421	5	10.7	9.66
14043026	VAMP8	27.00%	3	3	18	1.308	5	11.4	7.34
289577114	EIF4G2	5.64%	4	4	14	1.299	5	98.1	6.99
21361376	SF3A2	7.11%	2	3	21	1.267	5	49.2	9.64
61097912	NOM1	8.14%	5	6	16	1.238	5	96.2	8.1
7706326	SF3B6	34.40%	4	5	15	1.222	5	14.6	9.38
4885373	HIST1H1A	38.14%	4	13	363	1.211	5	21.8	11
530427503	HNRNPM	60.96%	1	44	715	1.203	5	75.6	8.57
767984396	CTDSPL2	18.28%	4	7	19	1.544	4	52.8	6.58
7705626	MRPS16	20.44%	3	3	15	1.506	4	15.3	9.5
187960037	CBX1	14.59%	1	2	39	1.483	4	21.4	4.93
260656008	SPINT2	13.33%	2	2	11	1.37	4	21.8	8.38
171543879	CD99	14.20%	2	2	11	1.358	4	17.1	4.56
47271443	SRSF2	13.57%	3	4	10	1.338	4	25.5	11.9
18765731	SNAP23	14.56%	2	2	12	1.282	4	17.8	4.69
10835240	HMGN4	25.56%	2	4	17	1.259	4	9.5	10.5
4506761	S100A10	17.53%	3	3	20	1.259	4	11.2	7.37
22208975	HMGA1	37.50%	1	6	93	1.25	4	10.7	10.3
7705704	GSTK1	28.76%	2	5	17	1.247	4	25.5	8.41
4507127	SNRPC	13.21%	2	2	12	1.236	4	17.4	9.67
27597059	DNAJC9	23.85%	3	5	19	1.221	4	29.9	5.73
262231786	MSH5	0.85%	1	1	4	1.215	4	91.8	6.2

n = 77