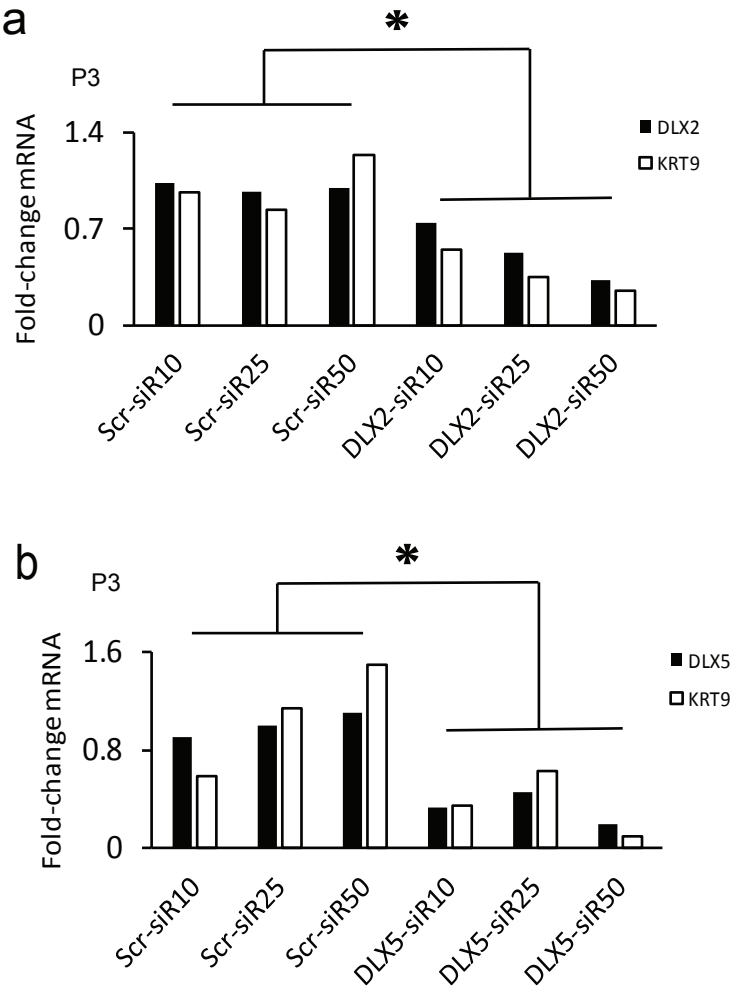
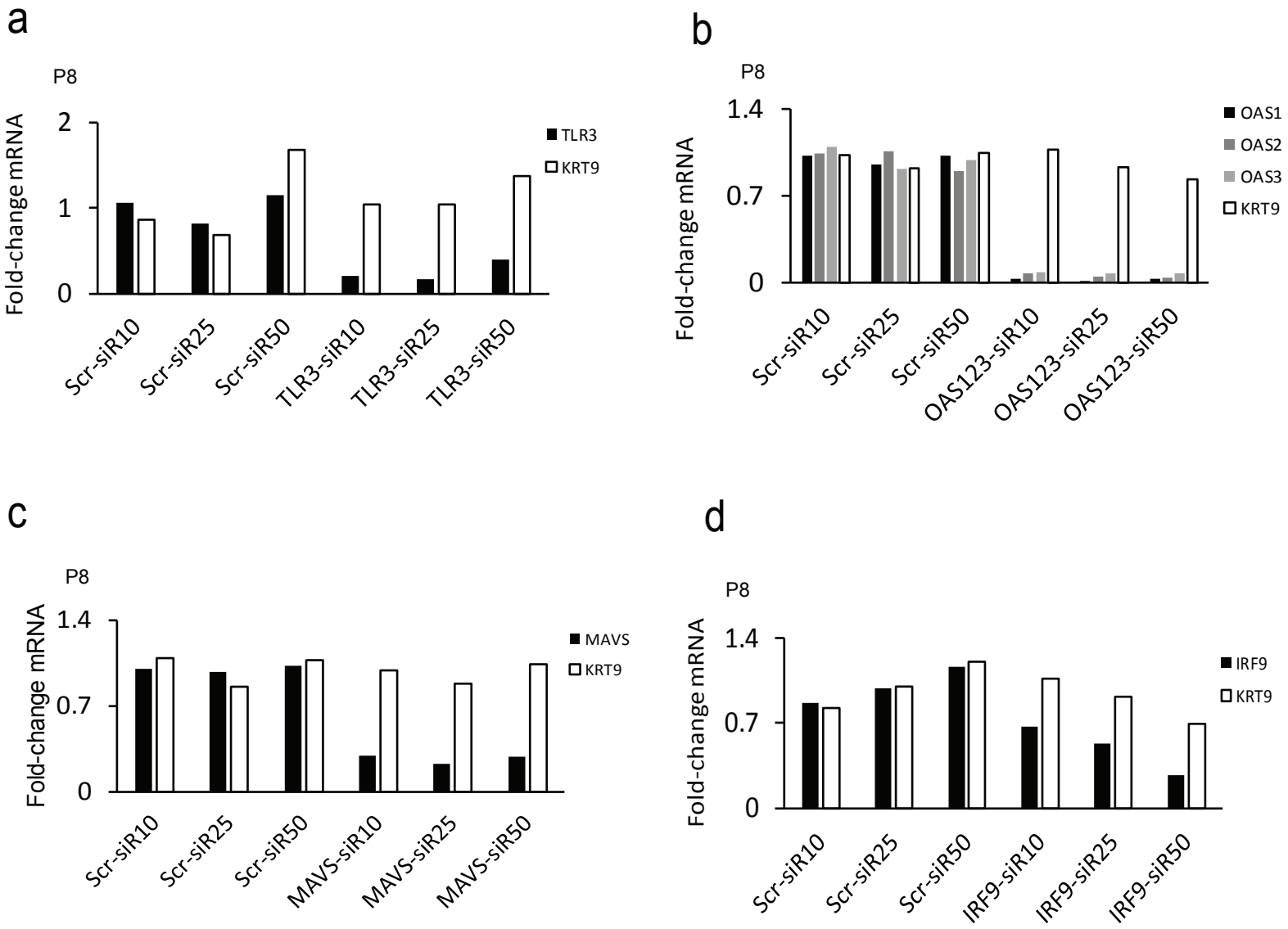
Supplement Figure 1



Homeobox DLX2 and 5 are necessary for volar keratinocyte expression of KRT9

(a-b) siRNA to DLX5(b) or DLX2(a) decreases KRT9 expression in P3 early passage volar keratinocytes

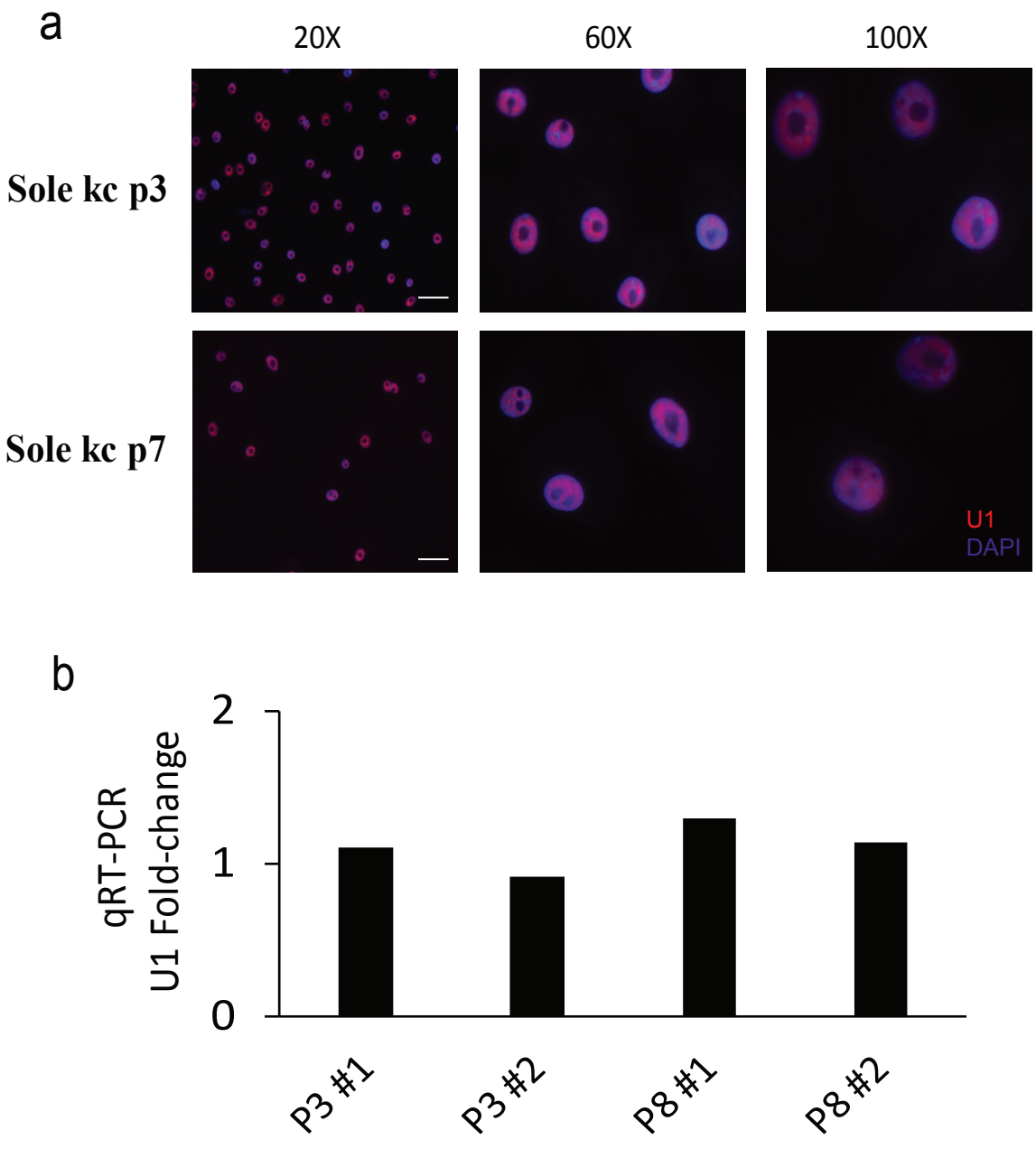
Supplement Figure 2



Multiple innate immune genes cannot rescue KRT9 expression.

(a) siRNA to the dsRNA receptor TLR3(a) does not rescues KRT9 expression in P8 late passage keratinocytes.
(b) siRNA to OAS1,2 and 3 does not rescue KRT9 expression in P8 late passage keratinocytes.
(c-d) siRNA to MAVS (c) or IRF9 (d) do not rescue KRT9 expression in P8 late passage keratinocytes.

Supplement Figure 3



The endogenous U1 dsRNA/snRNA is not increased in late passage keratinocytes

(a) U1 expression (red) is not elevated with no significant difference in volar keratinocytes of late passage versus early passage as stained by FISH (blue nuclear DAPI stain; up). Scale bar = 100 μm.
(b) U1 expression does not increase significantly in late passage compare to early passage as detected by qRT-PCR.

Supplementary Materials and Methods

Methylation. DNA of either human palm, hand, sole, foot epidermis or early and late passages cultured sole and foreskin keratinocytes was isolated and submitted for pyrosequencing methylation analysis (Johns Hopkins Genetic Resources Core Facility). DNA was bisulfite converted using EpiTect Bisulfite Kit (Qiagen) following the manufacturer's protocol. 20 ng of converted DNA was PCR amplified using a PyroMark PCR Kit (Qiagen) following manufacturer's protocol for bisulfite converted DNA. The final concentration of primers was 0.2 μM and no additional $MgCl_2$ or Q-Solution was added. Amplification was carried out in a Veriti thermocycler (Applied Biosystems).

PCR reactions were carried out following the procedures in the Qiagen pyromark PCR manual. For the pyrosequencing, we have the following assays: KRT9 Promotor

Qiagen part number: PMC0085878

Sequence to Analyze:

ATTTTATGTTGTTTTYGTTGTTTAGGTYGTTTTYGTTATTTTYGTYGTTGYGG TTTAAGTAGGAYGAGGAGAATTGTTTGTAGTTTATGATATAGTTGGTAGTTT AYGGGTTGAGAAGTAGTGATAGGAGTGTTATYGGTTTTT

Dispensation Order:

TATTATGCTAGTTCTGTGTATGTCTAGTTCTGTGATTCTAGTCTGTAGTCGTA GTAGTATCGAGAGATGTGTAGTATGATATAGTGTAGTGATCGTGAGAGTAGT GATAGAGTGTGATCTG

Forward primer: AAGAGGTTGTAGGAAGATTTTATGTTG

Reverse primer: AAACAACTCTATACCCTACACT

Sequencing primer: AAGAGGTTGTAGGAAG

These primer sets were ordered from IDT Inc (Coralville, IA).

All assays were designed using the Pyromark Assay design software v2. The assay with the FF initials was optimized in software by Frank Fischinger at Qiagen. Data was generated using either a Pyromark Q24 with Advanced chemistry or a Pyromark Q48 (anything since June 2016), following standard Qiagen protocols.

Small interfering RNA Transfection. Keratinocytes in 12-well plates were transfected with 10 nmol/L of siRNA specific (see table below) or scrambled sequences using Lipofectamine RNAiMAX (Thermo Fisher Scientific) for 48 hours. Then, protein and RNA was isolated from cells for Western blot and qRT-PCR, respectively.

Western Blot. Protein was isolated from the epidermis of human skin specimen and fibroblasts and keratinocytes using M-PER buffer (Thermo Scientific) consisting of protease inhibitors (Thermo Scientific). The protein concentration was measured by BCA protein assay (Thermo Scientific). All procedures were followed with the manual of the NuPAGE® Bis-Tris Electrophoresis System (Thermo Fisher Scientific). After gel electrophoresis and membrane transfer process, PVDF membrane was blocked in 5% nonfat dry milk for 3 hours. Then the membrane was incubated with rabbit anti-human KRT9 (ab85283; Abcam, Cambridge, MA) overnight at 4℃, followed by incubation with anti-rabbit HRP (7074S; Cell Signaling Technology, Danvers, MA) for 1 hour at room temperature. Finally, protein samples were developed with a Chemiluminescence substrate kit (SuperSignal ^{TM} West Pico PLUS and SuperSignal^{TM} West Femto Maximum Sensitivity Substrate;Thermo Scientific). Grey mean value of Western bolt bands was quantified by Image J .

Poly(I:C) Treatment: 3 x 10^5 keratinocytes at passages 3-5 per well were plated into 12-well plates and treated with 1 μg/ml Poly(I:C) (InvivoGen, San Diego, CA)twice every other day (Day1 and Day3). mRNA and protein was isolated as above at Day 5 and analyzed by qRT-PCR and Western blot, respectively.

Immunofluorescence. For cells, keratinocytes were cultured on coverslip for 4 days in KGM media and fixed with 4% paraformaldehyde for 20 minutes at room temperature. After three washes with PBS, cells were incubated in PBS-T (PBS with 0.1% Triton X-100) solution for 15 min at room temperature to enhance the permeability of antibodies. After blocking with 10% goat serum for 1 hour at room

temperature, cells were incubated with KRT9 antibody overnight (ab85283; Abcam), at 4°C. Then, cells were stained with goat anti-rabbit antibody labeled with Alexa Fluor 488 for 1 hour at room temperature. Cell nuclei were stained by DAPI contained in mounting media (Vector Lab, Burlingame, CA). For tissues, mouse fore- and hind-paw were fixed in 4% paraformaldehyde for 48 hours at room temperature prior to paraffin embedding protocol. Sections of 4 μm thickness were stained with Krt9 antibody overnight (ab85283; Abcam) at 4°C and then subjected to immunofluorescence analysis as previously described.

Fluorescence in situ hybridization. Stellaris® FISH Probes were designed against U1 by utilizing the Stellaris® RNA FISH Probe Designer (Biosearch Technologies, Inc., Petaluma, CA) available online at www.biosearchtech.com/stellarisdesigner. Human sole keratinocytes were hybridized with the U1 Stellaris RNA FISH Probe set labeled with Quasar 570 (Biosearch Technologies, Inc.), following the manufacturer's instructions available online at www.biosearchtech.com/stellarisprotocols.

Mouse Experiments. All animal protocols are approved by Johns Hopkins Medical Institution Animal Care and Use Committee. C57BL/6J mice (Jackson laboratory) were used as wild-type control. Ddx58 null mice in 129Sv/C57BL/6 background were provided from the Adolfo Garcia-Sastre laboratory. Poly(I:C) or PBS were injected subcutaneously into paws. 20 μl 1 μg/ml Poly(I:C) or PBS was injected into forelimbs, while 40 μl 1 μg/ml Poly(I:C) or PBS was injected into hindlimbs of four to five-week-old male and female mice. Mice were injected 3 times every other day. 24 hours after the last injection, paw skin was used for qRT-PCR and immunostaining.

Taqman probes

KRT9	Applied Biosystems	Hs00413861_m1

DDX58	Applied Biosystems	Hs01061436_m1
STAT1	Applied Biosystems	Hs01013996_m1
RNASEL1	Applied Biosystems	Hs00221692_m1
KRT7	Applied Biosystems	Hs00559840_m1
OAS1	Applied Biosystems	Hs00973635_m1
OAS2	Applied Biosystems	Hs00942643_m1
OAS3	Applied Biosystems	Hs00196324_m1
OASL	Applied Biosystems	Hs00984387_m1
DLX2	Applied Biosystems	Hs00269993_m1
DLX5	Applied Biosystems	Hs01573641_mH
TLR3	Applied Biosystems	Hs01551078_m1
MDA5	Applied Biosystems	Hs00223420_m1
MAVS	Applied Biosystems	Hs00920075-m1
IRF9	Applied Biosystems	Hs00920075-m1
RPLP0	Applied Biosystems	Hs00420895_gH
Krt9	Applied Biosystems	Mm01701803_m1
Ddx58	Applied Biosystems	Mm01216853_m1
Oas1a	Applied Biosystems	Mm00836412_m1
Actb	Applied Biosystems	Mm02619580_g1

siRNA probes

STAT1 siRNA	Santa Cruz Biotechnology	Sc-44123
DDX58 siRNA	Santa Cruz Biotechnology	Sc-61480
DLX2 siRNA	Santa Cruz Biotechnology	Sc-38651
DLX5 siRNA	Santa Cruz Biotechnology	Sc-38657
TLR3 siRNA	Dharmacon	L-007745-00-0005
MDA5 siRNA	Santa Cruz Biotechnology	Sc-61010
OAS1 siRNA	Santa Cruz Biotechnology	Sc-61241
OAS2 siRNA	Santa Cruz Biotechnology	Sc-61243
OAS3 siRNA	Santa Cruz Biotechnology	Sc-61245
MAVS siRNA	Santa Cruz Biotechnology	Sc-75755
IRF9 siRNA	Santa Cruz Biotechnology	Sc-38013
Scramble siRNA	Sigma-Aldrich	SIC001