Supplemental Materials and Methods

Quantitative PCR
Total RNA from whole dorsal back skin was purified with TRIzol Reagent (Life Technologies), cDNA was synthesized with iScript cDNA Synthesis Kit (Bio-Rad), and qPCR was performed with SsoFast EvaGreen/Probes Supermix (Bio-Rad). The Taqman probes for Dbp (Mm00497539_m1), Arntl (Mm00500223_m1) and Irf7 (Mm00516793_g1) were purchased from Applied Biosystems. Target genes are normalized to Mouse GAPDH Endogenous Control (Thermo Fisher Scientific).

Animals for 1% IMQ experiments
Mice were housed under 12:12 LD cycles with food and water ad libidum. Lights were switched on at 6 a.m. and off at 6 p.m. Zeitgeber time (ZT) refers to lights on = ZT0 and lights off = ZT12. All mice used in microarray experiments were male C57BL/6J from Jackson Laboratories. Mice were housed under 12:12 LD cycles for at least 7 days prior to the beginning of experiments. Animal handling after lights off was done by red light only. *Bmal1* germline KO (*Bmal1* KO) mice were generated as described in (1). Mice were maintained according National Institutes of Health guidelines and approved by the Institutional Animal Care and Use Committee of the University of California, Irvine (protocol number 2001-2239).

EdU+ cell quantification and epidermal width measurement
2 hours prior to collection, mice were injected I.P. with EdU at 5 mg/kg of body weight. The dorsal skin of the mice was collected, fixed in 10% formalin for 48 hours, ethanol dehydrated and embedded in paraffin. After sectioning, dewaxing and rehydrating, staining with the EdU click-it kit was performed. Twenty-fold magnified mosaic images were acquired on a Keyence microscope, with at least 1,000 μm skin length per image. EdU+ cell quantification per length of epidermis was performed by a blinded researcher using ImageJ software. Epidermal width was also measured. At least 2 images per mouse were analyzed and 15 measurements for each skin compartment per image were averaged.

Whole skin RNA isolation in IMQ experiments
RNA extraction was performed on whole skin with TRIzol™ reagent (Invitrogen) following manufacturers' recommendations; briefly, skin was suspended in 1-2ml of trizol and homogenized using Precellys tubes (Burtin), cellular debris was then removed by centrifugation at 12,000g for 10 minutes, total RNA was then extracted using chloroform followed by purification using Zymogen's Direct-zol RNA Isolation kit. RNA was resuspended in RNase-free water (Ambion) and the quality was checked on an Agilent Bioanalyzer 2100 (Agilent Technologies).

Gene Set Enrichment Analysis (GSEA)
GSEA was performed on samples after both 1 dose or 5 consecutive doses of 1% IMQ, compared to control. To distinguish genes of interest, False-Discovery Rate (FDR) with Q-value of < 0.25 and nominal (NOM) P-value was ≤ 0.05 was utilized to decrease the number of false positive "discovered" genes.

GSEA 2D Heatmaps
GSEA 2D heatmaps were created using the ggplot2 package in R. The circle color of each point corresponds to the normalized enrichment score for that gene ontology category and sample as reported by GSEA. The circle size of each point is a stepwise transformation of the false discovery rate for that gene ontology category and sample as reported by GSEA, with the cutoff

values for each step being described in the legend for the figure. Gene ontologies for each sample are only reported when the nominal p-value is less than or equal to 0.05.

Single cell sequencing of adult mouse epidermal cells
Adult male mice were treated as described in Methods and epidermal cells were isolated after 6h of 1% IMQ treatment. Live (double negative for DAPI and BV510 Viability dye (Tonbo Biosciences)), single epidermal cells were sorted and resuspended at 1 x 10^6 cells/mL in 0.04% UltraPure BSA and loaded into the Chromium™ Single Cell platform at a target yield of 6,000 cells. Sequencing was performed multiple times, if necessary, to obtain a depth of approximately 2,000 median genes per cell. Transcripts were mapped to the mm10 reference genome using Cell Ranger Version 2.1.0. Alignment and data filtering was done using Cell Ranger (10x Genomics).

Single-cell RNA-seq analysis: Dimension reduction and clustering analysis
To begin clustering cell populations from the Control and IMQ samples in Seurat R package (2), digital gene expression matrices were column-normalized and log-transformed with pseudocount 1. Principle component analysis (PCA) was first performed on a list of highly variable genes (parameters for FindVariableGenes function: x.low.cutoff = 0.01, x.high.cutoff = 3.5, y.cutoff = 0.5). Significant PCs were determined based on the Jackstraw method. The top PCs (for Control, 20 PCs; for IMQ, 21 PCs) were used for the first round of clustering with the Louvain modularity-based community detection algorithm (3) to generate cell clusters (FindClusters function; for Control, 29 clusters with resolution = 3; for IMQ, 20 clusters with resolution = 3.5). Cell clusters were annotated based on the canonical makers (4). Clusters with similar expressions of these canonical markers were merged together, leading to 14 and 10 clusters for Control and IMQ, respectively. To present high dimensional data in two-dimensional space, we performed t-distributed stochastic neighbor embedding (t-SNE) (5) using the significant PCs as input.

Canonical Correlation Analysis (CCA) was performed using the union of the top 2,000 genes with the highest dispersion from both samples. By examining the heatmaps of the top genes driving each component, we selected 18 significant components for downstream analyses such as dimension reduction and cell clustering (FindClusters function, 22 clusters with resolution = 1.2). To distinguish fibroblast subpopulation from melanocyte subpopulation, we performed sub-clustering analysis of the cluster with co-expression of both markers. The top 6 PCs were used for principal components analysis and Louvain clustering (resolution = 0.6) in this sub-clustering analysis. After merging clusters with similar expressions of the canonical markers, we obtained 14 clusters. Cells were visualized by performing t-SNE using the significant components from CCA.

Single-cell RNA-seq analysis: Comparison of cluster-specific Irf7 expression between Control and IMQ
Based on the integrative analysis using CCA, we compared the percentage and the distribution of Irf7 expression in each cell cluster between Control and IMQ samples. For a cell cluster (such as KC B, KC D, TC, LC, Mono) with total n1 cells, the number of cells positively expressing Irf7 (log-transformed data > 0.5) is denoted by n2. Then the percentage of Irf7+ cells in this cell cluster is given by n2/n1*100%. For one cell cluster, the expression levels of Irf7 are divided into different bins (the bin size is set to be 0.25). Then the proportion of cells in each bin is counted and the histogram plot is created for each cell cluster.

Single cell RNA-seq analysis: Comparison of the proportion of each cell cluster between Control and IMQ samples

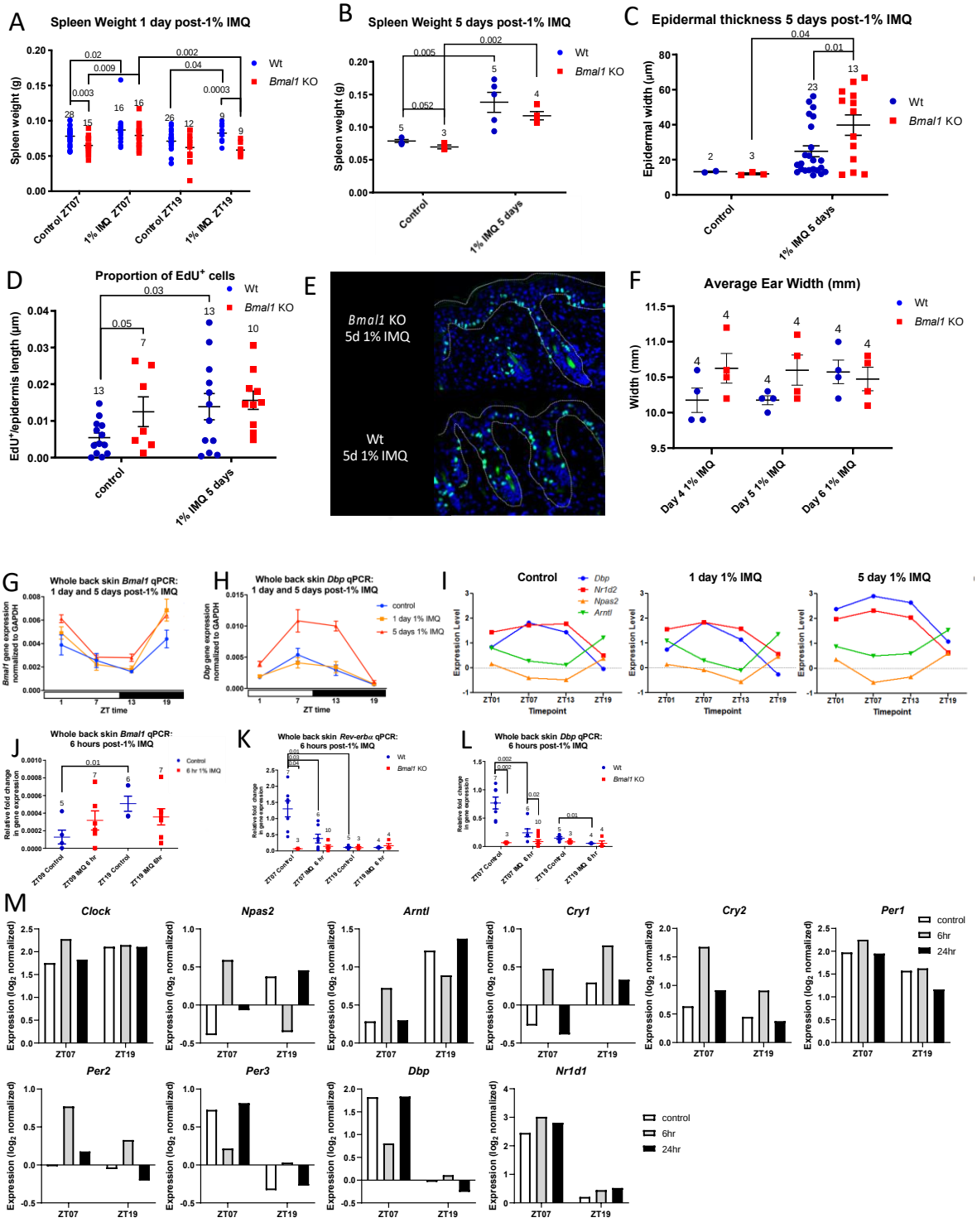
The relative change of the proportion of each cell cluster between Control and IMQ was quantified by (PIMQ - PCtrl)/PCtrl*100%, where PCtrl and PIMQ are the percentage of the cells in a cell cluster in Control and IMQ samples, respectively. Positive percentage values reflect more cells of a cell cluster were found in the IMQ treated sample over the control and negative percentage values indicate more cells of a cell cluster were present in the control sample over the IMQ treated sample.

IFN-β Serum ELISA

Two or 6h after 1% IMQ treatment, mice were sacrificed by CO_2 inhalation and peripheral blood was collected by cardiac puncture. Blood was left to clot and then centrifuged at 5,000g for 10 minutes. The serum was collected and stored at -80°C until ready for further use. The serum was later run on ELISA plates: IFNα/IFNβ 2Plex Mouse ProcartaPlex Panel (eBioscience) (for 2 and 6h post-1% IMQ samples), or Mouse IFN Beta ELISA kit (TCM, Serum) (PBL assay science) (for 1 day 1% IMQ samples). A standard curve was run to determine the concentrations of IFNα/β.

Staining of Epidermal cells for Imagestream and qPCR analysis

Mouse back skin was excised and dermal fat was scraped off with forceps. Skin was cut into 10cm^2 pieces and floated epidermal side upwards in 0.25% trypsin (Gibco) for 2h at 37°C. Epidermis was then scraped into Accutase® Cell Detachment Solution and then disrupted using 3 mL syringes (BD). Epidermal cells were filtered through 70 µm filters, centrifuged and resuspended in 50 µL of 1:200 Fc block (anti-CD16/32 from Tonbo Biosciences) in FACS buffer containing PBS, 2mM EDTA, 5% FBS, and Penicillin/Streptomycin. After resuspending in FACS buffer, cells were blocked on ice for 15 minutes, then 1 µL of each antibody (combinations of: APC Rat Anti-Mouse CD45 (BD Biosciences), Anti-Mouse CD11c PE-Cy7 (Tonbo Biosciences), PE/Dazzle™ 594 Anti-human/mouse CD49f (BioLegend), Anti-Mouse CD3e PerCP-Cy5.5 (Tonbo Biosciences)) were added and cells were incubated 25 minutes on ice in the dark. During the last 5 minutes of incubation, 0.5 µL of Brilliant Violet 510 viability dye (Tonbo Biosciences) was added. For qPCR analysis, cell types were sorted with a FACSaria Fusion flow cytometer, RNA was isolated with the Qiagen RNeasy Micro kit and cDNA was amplified with the Bio-Rad iScript cDNA Synthesis Kit. For Imagestream analysis, stained cells were fixed with 2% paraformaldehyde and permeabilized using the Foxp3 permeabilization kit (eBiosciences) followed by staining with Anti-IRF-7 (pS477/pS479)-PE, human and mouse (Miltenyi Biotec) and AlexaFluor® 488 anti-IRF7 (BioLegend).


A
Spleen Weight 1 day post-1% IMQ
Spleen weight (g)
Wt
Bmal1 KO
Control ZT07
1% IMQ ZT07
Control ZT19
1% IMQ ZT19
B
Spleen Weight 5 days post-1% IMQ
Control
1% IMQ 5 days
C
Epidermal thickness 5 days post-1% IMQ
Epidermal width (μm)
D
Proportion of EdU+ cells
EdU+/epidermis length (μm)
E
Bmal1 KO
5d 1% IMQ
Wt
5d 1% IMQ
F
Average Ear Width (mm)
Width (mm)
Day 4 1% IMQ
Day 5 1% IMQ
Day 6 1% IMQ
G
Whole back skin Bmal1 qPCR:
1 day and 5 days post-1% IMQ
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H
Whole back skin Dbp qPCR:
1 day and 5 days post-1% IMQ
control
1 day 1% IMQ
5 days 1% IMQ
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Control
1 day 1% IMQ
5 day 1% IMQ
Dbp
Nr1d2
Npas2
Arntl
Expression Level
Timepoint
J
Whole back skin Bmal1 qPCR:
6 hours post-1% IMQ
K
Whole back skin Rev-erbα qPCR:
6 hours post-1% IMQ
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Whole back skin Dbp qPCR:
6 hours post-1% IMQ
M
Clock
Npas2
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24hr

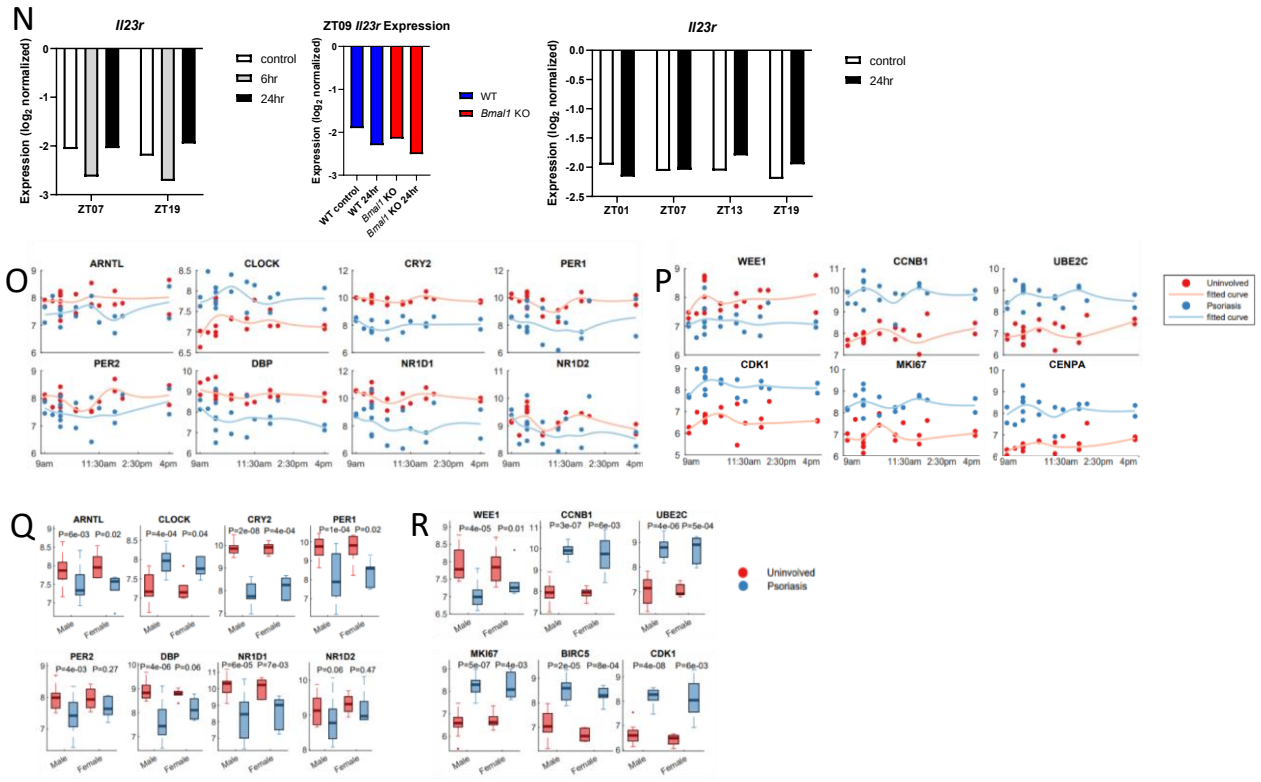

Figure S1. Clock-controlled gene expression is affected by IMQ and *Bmal1* systemic deletion and Psoriasis. (A) Spleen weight 1 day post-1% IMQ. Spleens from wildtype (blue) or *Bmal1* KO (red) mice were collected at ZT07 and ZT19 **(B)** Spleen weight after 5 days of IMQ. Mice were topically treated daily with 1% IMQ for 5 days and Wt (blue) and *Bmal1* KO mice (red) spleens were collected and weighed. **(C)** Epidermal thickness. Wt (blue) and *Bmal1* KO (red) mice were untreated or treated with 1% IMQ for 5 days and epidermal thickness was measured by microscopy. **(D)** Epidermal cell proliferation (EdU+ cells). Wt (blue) and *Bmal1* KO (red) mice were treated with 1% IMQ for 5 days. Two hours prior to collection, mice were i.p. injected with EdU+. **(E)** Representative images of back skin sections stained for EdU in green. **(F)** Average ear width after 4, 5, and 6 days post-1% IMQ treatment. Wt and *Bmal1* KO mice were treated with 1% IMQ and ear width was measured with calipers. **(A-F)** Each data point represents 1 mouse and Mean ± SEM is indicated. The numbers above each group indicate the number of samples analyzed. **(G)** Mice were treated with vehicle or 1% IMQ for 1 day or 5 days as described in Figure 1A and collected at ZT13. Following treatment, whole skin was extracted and RNA was isolated. One-way ANOVA for control, 1 day 1% IMQ, and 5 days 1% IMQ across ZT times shows significance (p=0.03, 0.0001, 0.0001, respectively). Two-way ANOVA shows significance between time points (p<0.0001) and treatments (p=0.003). Tukey's post-hoc test for control vs. 5 days 1% IMQ (p=0.0059). **(H)** *Dbp* expression in mouse back skin. One-way ANOVA for control, 1 day 1% IMQ, and 5 days 1% IMQ across ZT times shows significance (p=0.0009, 0.0035, 0.0001, respectively). Two-way ANOVA shows significance between time points and treatments (p<0.0001). Tukey's post-hoc test for control vs. 5 days 1% IMQ 5 and 1 day 1% IMQ vs. 5 days 1% IMQ (p<0.0001). **(G-H)** Data represents mean ± SEM of N= 5-7 mice per group. **(I)** Expression of core clock genes *Dbp*, *Nr1d1*, *Npas2*, and *Arntl* (*Bmal1*) in pooled RNA samples determined by microarray. Gene expression of *Bmal1* **(J)** *Rev-erba* **(K)**, and *Dbp* **(L)** determined by qPCR on whole back skin RNA from Wt (blue) and *Bmal1* KO (red) mice after 6 hours of 1% IMQ treatment during the day (ZT07) or night (ZT19). Each data point

represents 1 mouse and Mean ± SEM is indicated. **(J-L)** Statistical significance was determined by Student's paired t-test and significant or near-significant P-values are shown. **(M)** Microarray data from pooled (N=5-7) mouse back skin samples collected 6h or 24h post-1% IMQ treatment at ZT07 and ZT19. **(N)** *Il23r* expression from the same microarray experiment presented in *G*. Gene expression of core clock genes **(O)** and cell cycle genes **(P)** from the human skin biopsy RNAseq data (6). Uninvolved and psoriatic biopsies were collected from the same patient (N=19) at the same time (between 9 a.m. – 4 p.m). (Each point represents a single biopsy. The solid lines represent average expression of each gene that is estimated by fitting cubic regression splines. Human skin RNAseq data separated based on gender, core clock genes **(Q)** and cell cycle genes **(R)**. **(Q-R)** N=13 males and N=6 females. Median ± interquartile range is indicated.

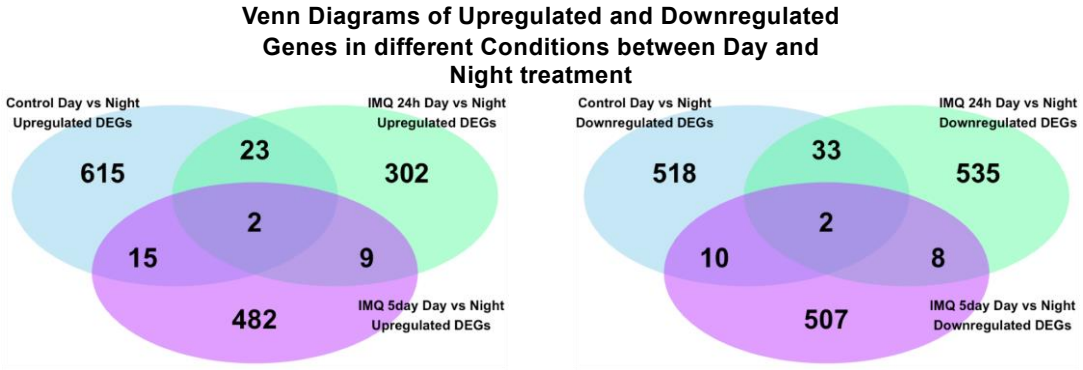

Figure S2. Comparison of differential gene expression during the day and night timepoints, both with and without IMQ. Venn diagrams of upregulated and downregulated genes between daytime treatment and nighttime treatment for control, 1 day 1% IMQ, and 5 days 1% IMQ. Differentially expressed genes were identified using Tukey's post hoc test with a $P<0.05$.

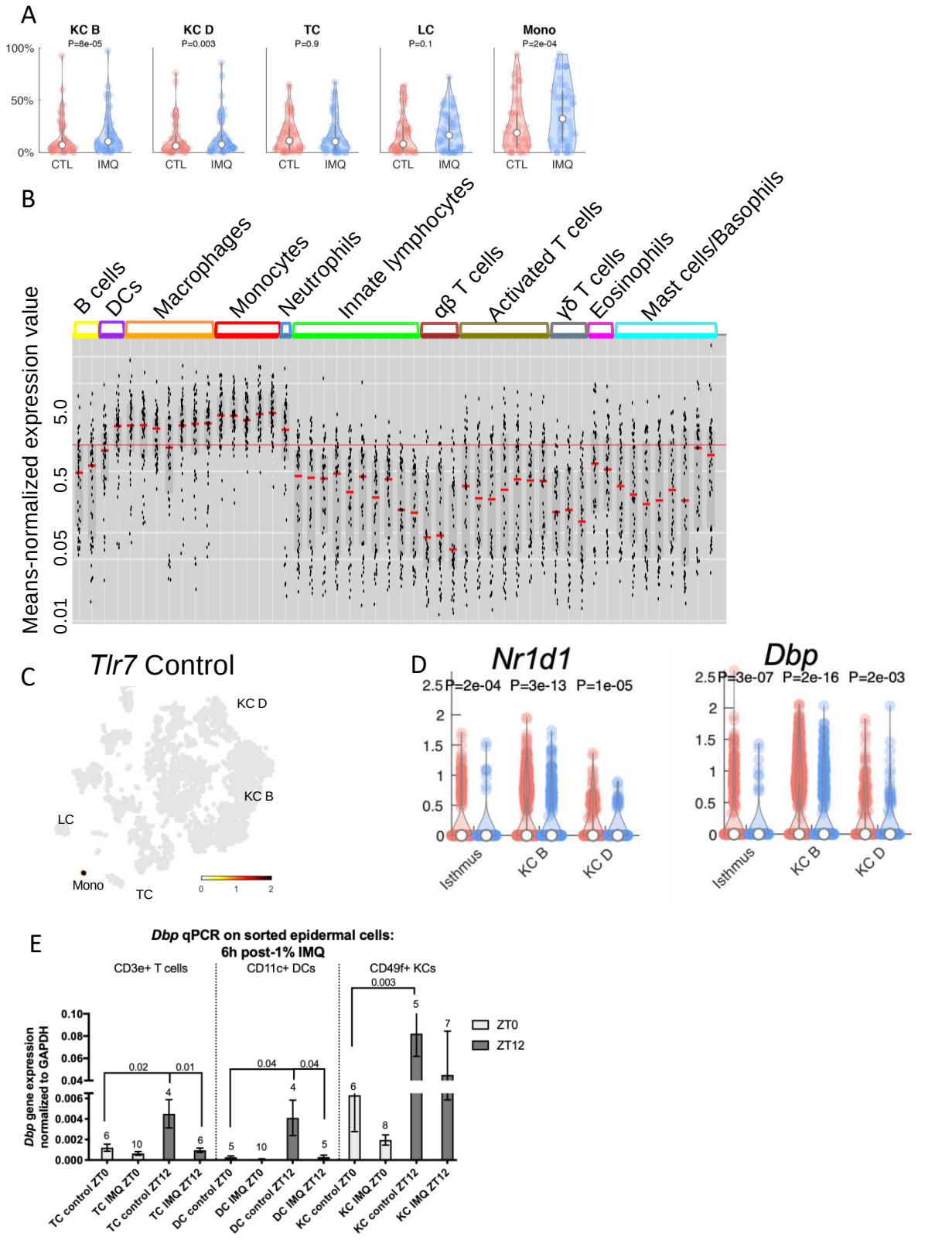
A
KC B
P=8e-05
KC D
P=0.003
TC
P=0.9
LC
P=0.1
Mono
P=2e-04
100%
50%
0%
CTL
IMQ
B
B cells
DCs
Macrophages
Monocytes
Neutrophils
Innate lymphocytes
αβ T cells
Activated T cells
γδ T cells
Eosinophils
Mast cells/Basophils
Means-normalized expression value
5.0
0.5
0.05
0.01
C
Tlr7 Control
KC D
KC B
LC
Mono
TC
0
1
2
D
Nr1d1
P=2e-04
P=3e-13
P=1e-05
Dbp
P=3e-07
P=2e-16
P=2e-03
Isthmus
KC B
KC D
E
Dbp qPCR on sorted epidermal cells:
6h post-1% IMQ
CD3e+ T cells
CD11c+ DCs
CD49f+ KCs
Dbp gene expression normalized to GAPDH
ZT0
ZT12
0.02
0.01
0.04
0.04
0.003
TC control ZT0
TC IMQ ZT0
TC control ZT12
TC IMQ ZT12
DC control ZT0
DC IMQ ZT0
DC control ZT12
DC IMQ ZT12
KC control ZT0
KC IMQ ZT0
KC control ZT12
KC IMQ ZT12

Figure S3. Expression of top genes expressed by monocytes within the epidermis across different immune cell types (A) Violin plots showing the distribution of the percentage of positive *Irf7*-related genes (in total 50 genes). Significance was determined by the Student's paired t-test shown in each cell type. Pink = control, blue = 6h 1% IMQ **(B)** Output from the Immgen database (7), which was used to identify the monocyte cell population within the epidermis. W-plot showing gene expression of the top 50 genes differentially expressed in the monocyte population in various immune cell populations from the Immgen database. Cell types are on the x-axis and means-normalized gene expression is on the y-axis. **(C)** Tlr7 expression in single cell data. TLR7 mRNA expression shown in the t-SNE plot of the control sample. Grey = no expression, red = intensity of expression. **(D)** Violin plot showing expression of the *Nr1d1* and *Dbp* in isthmus, basal keratinocytes (KC B), and differentiated keratinocytes (KC D). Pink = control, blue = 6h 1% IMQ. Significance was determined by the Mann-Whitney U-test. **(E)** *Dbp* expression on sorted epidermal cells (T cells, dendritic cells (DCs), and keratinocytes (KCs)) as determined by qPCR. Significance was determined by the Student's paired t-test.

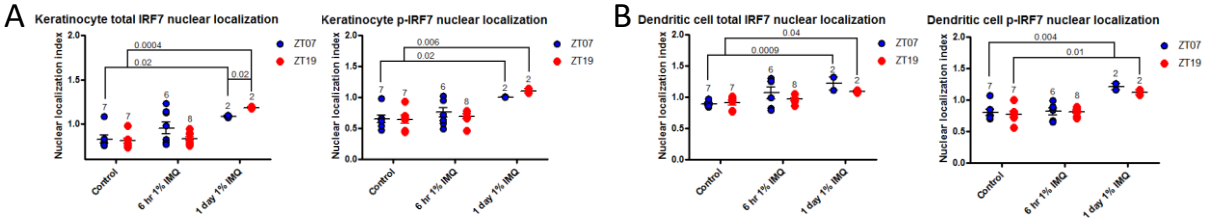

Figure S4. IMQ induced nuclear localization of total and phosphorylated IRF7 in epidermal keratinocytes and dendritic cells. Male Wt mice were treated with 1% IMQ for 6h or 1 day during the day (ZT07) or night (ZT19) and back epidermis was dissociated, stained for cell surface markers and intracellular IRF7 and p-IRF7, and analyzed on the Imagestream Flow Cytometer. Nuclear localization index was calculated using IDEAS software. **(A)** Average nuclear translocation index for total IRF7 (left) and p-IRF7 (right) in KCs ($CD45^-$). **(B)** Average nuclear translocation index for total IRF7 (left) and p-IRF7 (right) in DCs ($CD45^+CD11c^+CD3e^-$). **(A-B)** Each data point represents 1 mouse and Mean ± SEM is indicated. Statistical significance was determined by Student's paired t-test and significant or near-significant P-values are shown.

References

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