

Inhibiting HuR-Mediated mRNA Stabilization Attenuates Psoriasis-Like Inflammation in Preclinical Models

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Purpose: Psoriasis is a chronic inflammatory skin disease characterized by hyperproliferative keratinocytes and activated Th17/Th1 lymphocytes, which sustain a self-perpetuating cycle by releasing pro-inflammatory cytokines. The RNA-binding protein (RBP) Human Antigen R (HuR) stabilizes many of these cytokine mRNAs, contributing to disease progression. This study aims to investigate the role of the HuR protein in psoriasis and modulate its activity to develop novel therapeutic strategies.

Patients and Methods: We evaluated the effects of Human Antigen R (HuR) silencing on psoriatic markers in both patient-derived keratinocytes and lymphocytes. Furthermore, we identified novel HuR targets within a psoriatic context using enhanced Cross-Linking and ImmunoPrecipitation sequencing (eCLIP-seq) in psoriasis-like HaCaT cells. Finally, we investigated the pharmacological effects of the TM11, a Tanshinone-mimic (TM) molecule, in an in vivo mouse model and an ex vivo 3D human skin model.

Results: Studies on HuR function in HaCaT cells and in patient-derived primary keratinocytes and lymphocytes, demonstrated that keratinocyte proliferation and lymphocyte activation and differentiation are both dependent on HuR. In psoriatic-like HaCaT cells, eCLIP-seq data identified novel psoriatic-related HuR targets, and its mRNA-stabilizing activity was confirmed. Treatment with TM11, that competes with HuR-mRNA binding, reduced keratinocyte proliferation and psoriasis proteins (Cyclin B, Survivin, S100A7, eFABP), and almost suppressed T-cell activation (CD25⁺CD69⁺) and Th17/Th1 cytokine production. In vivo, TM11 reduced psoriasis-like symptoms in Imiquimod-treated mice, reducing Psoriasis Area and Severity Index (PASI) scores, systemic inflammation and histological alterations. In ex vivo InflammSkin[®] models, TM11 restored epidermal morphology, decreased Ki-67 positive cells and lowered IL17AF and IFN- γ secretion.

Conclusion: Overall, these findings highlight HuR as a key driver of psoriasis pathogenesis and provide preclinical evidence that pharmacological modulation of HuR activity, by TM11 treatment, could provide a conceptual therapeutic rationale for psoriasis, potentially contributing to the reduction of keratinocyte hyperproliferation and immune activation while favouring the restoration of skin homeostasis. However, further preclinical and clinical validation will be required to establish its translational feasibility.

Keywords: RBP, TMs, primary cells, in-vivo, ex-vivo

Introduction

Psoriasis is a chronic inflammatory skin disease characterized by keratinocyte hyperproliferation and impaired differentiation, leading to erythematous scaly plaques.¹ It affects 2–4% of the population, with higher prevalence in individuals of European ancestry.² Disease arises in genetically predisposed individuals, exposed to environmental stimuli, such as drugs, stress and smoking.^{3–5} Crosstalk between skin and immune cells plays a key role in disease progression.⁶ In recent years, many pathways have been involved in the pathogenesis of psoriasis. Among the pathways involved, the *IL23/IL17 axis* represents a central link between keratinocyte hyperproliferation and T-cell activity. Upon skin injury, dendritic cells produce IL23 and IL12, activating Th17 and CD8+ cells to secrete IL17.^{7,8} This cytokine stimulates keratinocytes to produce inflammatory mediators (IL1, IL8, IL20), promoting immune responses and neutrophil recruitment.⁹ The resulting cytokine network recruits additional Th17 and Th1 cells, amplifying inflammation and sustaining a self-perpetuating cycle of keratinocyte proliferation.^{10,11} Inflammatory mediators are regulated at the post-transcriptional level through AU-rich elements (AREs) in the 3'-UTR, which are recognized by RNA-binding proteins (RBPs) controlling mRNA stability.^{12,13} Targeting these mechanisms represents a promising strategy to limit persistent cytokine production.

Human antigen R (HuR) is one of the most studied RBPs. Under physiological conditions, it is mainly nuclear, where it regulates pre-mRNA processing. Upon stimulation, HuR translocates to the cytoplasm, stabilizing target mRNAs and modulating gene expression.¹⁴ It binds primarily to AREs via its RNA recognition motifs (RRM1 and RRM2), while RRM3 contributes to complex stability.^{15–18} HuR regulates both pro- and anti-inflammatory responses and has been implicated in several inflammatory diseases, where it promotes T-cell activation and stabilizes cytokine mRNAs such as TNFA, IL6, and IL1B.^{19–22} In the hypothesis that post-transcriptional mechanisms drive the excessive production of the cytokine IL-20 in psoriatic skin, a recent study conducted in psoriatic keratinocytes, demonstrated that HuR translocates to the cytoplasm upon AMPK activation, where it stabilizes cytokine mRNAs driving immune responses.²³ In addition, inflammatory networks associated with skin diseases can also impact T-cell activation pathways,²⁴ in which the deletion of HuR associates with loss of peripheral T-cells.²⁵

However, the extent of HuR activity in psoriatic keratinocytes remains poorly defined, given the large number of inflammatory transcripts that may be stabilized by HuR protein during disease progression. Consistent with the hypothesis that inhibition of HuR function may promote the destabilization and degradation of pro-inflammatory transcripts, several anti-psoriatic approaches have been proposed. HuR-inhibiting peptides modified with polyethylene glycol were developed to form nanoparticles capable of reducing levels of IL-23 and VEGF in mice.²⁶ The treatment successfully eased physical symptoms such as skin thickness and scaling without showing signs of toxic side effects in major organs like the liver, corroborating the idea that post-transcriptional regulation can be targeted for developing new therapies. On the other hand, Chinese herbal medicines have gained attention.^{27,28} Compounds derived from *Salvia miltiorrhiza*, such as Tanshinone IIA (Tan IIA), induce apoptosis and cell cycle arrest in psoriatic keratinocytes by targeting the *IL17/IL23 axis*.^{29,30} Another derivative, 15,16-dihydrotanshinone-I (DHTS), competes with mRNA for HuR binding, reducing its stabilizing activity.³¹ New-generation derivatives (TM7nox, TM7nred, and TM11), known as Tanshinone mimics (TMs), further modulate inflammatory responses.^{32,33} TM7nox/TM7nred exist in redox equilibrium, while TM11 shows improved solubility and preferentially reduces proliferation over apoptosis.^{32,33}

We hypothesized that HuR acts as a central post-transcriptional regulator of psoriasis by stabilizing inflammatory and proliferation-associated transcripts, and that its pharmacological modulation could attenuate disease-associated pathways. To test this hypothesis, and to collect the first proof-of-principle of the efficacy of HuR targeting, we combined patient-derived keratinocytes, cytokine-stimulated HaCaT cells, ex vivo 3D skin models, and the imiquimod-induced murine model. Although the HaCaT and imiquimod models have inherent limitations and cannot fully recapitulate the complexity of human psoriasis, the use of complementary experimental systems provides converging preclinical evidence that supports HuR as a promising therapeutic target and justifies further pharmacokinetic, safety, and clinical evaluation of HuR-targeting compounds.

Patients and Methods

Patient Recruitment

A total of 32 subjects were enrolled across IRCCS Fondazione Ca' Granda Ospedale Maggiore Policlinico di Milano (approval no. 485_2021) and Azienda Ospedaliero-Universitaria di Modena (approval no. 1343/2020). Among the 32 enrolled

donors, 14 (9 male + 5 female) are non-psoriatic, and 18 (14 male + 4 female) are newly diagnosed treatment naive psoriatic donors, with the average age of 73.3 and 56.2, respectively. PASI average is 15.6 in psoriatic patients compared to 0 in healthy donors. Written informed consent was obtained from all participants prior to inclusion. Enrolled subjects underwent blood sampling to purify T cells from total PBMCs and a biopsy of lesional skin for psoriasis keratinocyte isolation. Healthy skin was collected from unaffected surgical resection margins during other dermatological surgery interventions. All donor samples were de-identified.

Primary Cells and Cell Line

Primary Keratinocytes and T-Cells

Human Keratinocytes were isolated from skin biopsies and the isolated cells were cultured on the 3T3 feeder layer, as previously indicated.³⁴ Passage 1 or 2 keratinocytes were plated and maintained in Keratinocyte Growth Medium (KGM) (Lonza, Clonetics, San Diego, CA, USA) and subjected to further experiments: TMs treatment or siRNA transfection.

Peripheral blood mononuclear cells (PBMCs) were isolated from fresh human blood samples obtained from healthy donors or psoriasis patients. See [Supplementary Materials and Methods](#) for T-cell isolation and culturing.

HaCaT Cell Line

HaCaT human keratinocytes were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, ThermoFisher Scientific, Massachusetts, USA) supplemented with 10% FBS (Gibco, ThermoFisher Scientific) and 1% penicillin-streptomycin (10,000 U/mL) (Gibco, ThermoFisher Scientific).

HaCaT cells are stimulated with TNFA (10 ng/mL), IL17 (10 ng/mL), and IL22 (20 ng/mL) (reconstituted in 0.1% BSA) to induce an inflammatory response.³⁵ For cell stimulation procedure see [Supplementary Materials and Methods](#).

Statistical Analysis

Statistical analyses were performed using the number of independent biological replicates indicated in the Results section and in the corresponding figure legends. Biological replicates consisted of independent donor-derived samples for experiments involving primary human cells and individual animals for in vivo experiments. Technical replicates represented repeated measurements of the same biological sample and were averaged before statistical analysis. Technical replicates were not considered independent biological replicates. Unless otherwise indicated, each dot in the graphs represents one biological replicate. Values in the figures are expressed as mean $\pm$ Standard Error of the Mean (SEM). Analysis was performed using GraphPad Prism 10.0. For all datasets, distributional assumptions were evaluated by normality testing, and homogeneity of variance was assessed where appropriate. Donor-matched comparisons (eg, siCtrl versus siHuR conditions in cells derived from the same patient) were analysed using paired tests. Unpaired t-tests with Welch's correction were applied for two-group comparisons when variances were unequal. For experiments involving more than two conditions, one-way or 2-way ANOVA followed by Dunnett's multiple comparisons test or Sydák's procedure was used, as specified in the figure legends. Statistical significance is indicated by * symbols (*: $0.01 < P < 0.05$; **: $0.001 < P < 0.01$; *** $0.0001 < P < 0.001$; **** $P < 0.0001$), ns indicates not significant.

Ethical Study Approval

This study was performed in accordance with the Declaration of Helsinki. Collection of human tissue samples for this study was approved as part of the study protocol. The human study was approved by the Ethical Committee of Area Vasta Emilia Nord for the Azienda Ospedaliero-Universitaria di Modena unit (Approval Prot. N. 1343/2020) and IRCCS Fondazione Ca' Granda-Ospedale Maggiore Policlinico di Milano unit (Approval Prot. N. 485_2021). All adult participants provided written informed consent to participate in this study. The animal study was reviewed by OPBA (Organismo Preposto al Benessere degli Animali, the Institutional Animal Welfare Body) of the University of Modena and Reggio Emilia and approved by the Italian Ministry of Health - approval: N. 261/2025-PR, according to the 3 R (replacement, reduction, and refinement) principles and guidelines established by the EU legislation on animals in science.

Data Availability

Data supporting the findings of this study are available in the article and [Supplementary Material](#). Values for all data points in graphs are reported in the [Supporting Data Value file](#). The accession number for the eCLIP-sequencing data reported in this paper is GEO GSE314160.

Additional materials and methods are listed and described in the [Supplementary Materials and Methods](#) and [Supplementary Tables S1](#) and [S2](#), reporting Primers Sequences and Reagents.

Results

Protein Levels of Psoriatic Markers are Dependent on HuR Expression in Primary Keratinocytes and T-Cells

We collected primary keratinocytes and T-cell tissue samples from psoriatic and non-psoriatic donors. We first characterized the cohort of donors to determine whether psoriatic donors exhibit a distinct molecular profile compared to healthy individuals, including HuR expression and localization. To this end, we measured the HuR localization in immunofluorescence images (Protocol in [Supplementary Materials and Methods](#)). In non-psoriatic keratinocytes, HuR is mainly nuclear (higher mean), whereas in psoriatic cells it localizes mainly to the cytoplasm, suggesting a marked cytoplasmic function which is unexpected in resting cells.²³ Notably, psoriatic keratinocytes were also found to be smaller in size compared with non-psoriatic cells ([Figure 1a](#)). Then, we silenced HuR in primary keratinocytes by siHuR pools and analysed HuR mRNA and protein levels after 24 and 48 hours. After verifying that HuR mRNA was efficiently silenced 24 hours post-transfection and that HuR protein levels were reduced at both time points ([Figure 1b](#)), we measured the expression patterns of psoriatic markers in keratinocytes from healthy and psoriatic group, focusing on pathways previously implicated in psoriasis pathogenesis and correlating them to HuR expression.

Indeed, silencing HuR for 24 and 48 hours led to marked alterations in the expression of psoriasis-associated markers in psoriatic, but not in normal primary keratinocytes. IL1B protein levels were reduced at 48 hours, as was the keratinocyte-related marker S100A7. Epidermal Fatty Acid-Binding Protein (eFABP) protein levels did not decrease after treatment, but its expression increased in psoriatic cells compared to healthy cells. Interestingly, STAT3, a key mediator of psoriatic signaling, displayed a reduced activation in psoriatic cells treated with siHuR for 24 hours, as shown by the decreased ratio of phosphorylated STAT3 over the total STAT3 ([Figure 1c](#)). Moreover, through an ELISA assay, we observed that the production and release of IL17AF (a key modulator of pathogenesis) is reduced in psoriatic cells after 24 and 48 hours of HuR silencing ([Figure 1d](#)).

We next examined HuR expression in primary T cells derived from psoriatic and non-psoriatic donors. Cells were nucleofected with either siHuR (100 nM) or siSCR pools and subsequently activated using anti-CD3/CD28 beads. HuR protein was overexpressed in psoriatic T cells compared to controls and effectively silenced 48 and 96 hours after siHuR nucleofection, as shown by Western blot ([Figure 2a](#)). HuR silencing significantly impaired psoriatic T lymphocytes activation, reducing the CD25/CD69 double-positive population at 48 hours ([Figure 2b](#)). Furthermore, the decrease in CD8+ T cells suggests an impaired differentiation program ([Figure 2c](#)). Finally, HuR silencing significantly altered Th1/Th17 polarization evaluated after 72 hours, resulting in a reduced percentage of CD4+/IL17A+ (Th17) cells ([Figure 2d](#)). This modulation of polarization was further supported by ELISA analyses, which showed significantly decreased production levels of IL-17AF and IFN- γ after 48 and 96 hours ([Figure 2e](#) and [2f](#)). These data suggest that HuR can mediate the activation and differentiation of keratinocytes and lymphocytes involved in the pathogenesis of psoriasis.

eCLIP-Seq Identifies HuR-Bound Psoriasis-Associated Transcripts and Reveals their Redistribution Toward 3'UTR Binding Following Inflammatory Stimulation in HaCaT Cells

Primary cells present technical challenges as the extensive amplification required to obtain sufficient numbers often leads to the loss of the psoriatic phenotype. Consequently, we employed stimulated HaCaT cells to investigate HuR activity under psoriatic-like conditions (stimulated HaCaT model is described in [Supplementary data](#) and [Supplementary Figure S1](#)). To determine the relevance of HuR in mediating psoriatic progression, we performed a genome-wide investigation using an

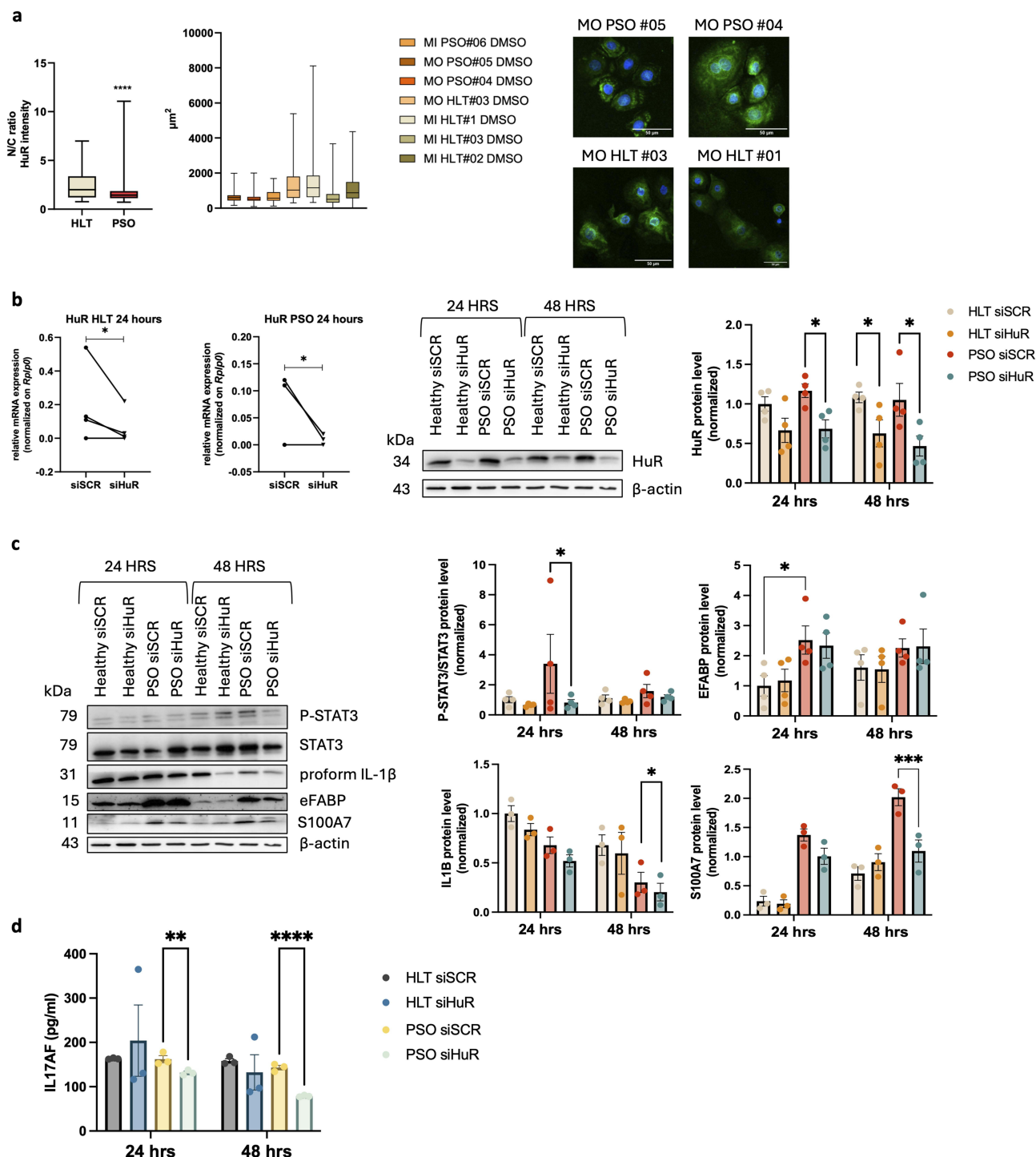


Figure 1 HuR nuclear-cytoplasmic distribution and functional silencing in primary keratinocytes. (a) The nuclear/cytoplasmic (N/C) fluorescence intensity ratio of HuR localization in primary keratinocytes isolated from patients with psoriasis (PSO) and non-psoriatic control donors (healthy: HLT). Data are presented as box-and-whisker plots indicating Min/Max values of three psoriatic and four healthy patients. Statistical analysis was performed using a two-tailed nonparametric Mann-Whitney test for independent groups (healthy $n=4$; $psn=3$) (**** $P < 0.0001$). The adjacent plot shows the mean keratinocyte area calculated for each donor from immunofluorescence images. Representative micrographs of HuR distribution in PSO and HLT samples. Scale Bar = 50 μm . (b) Primary keratinocytes from psoriatic and non-psoriatic donors were treated with human siHuR or scrambled siRNA (siSCR), used as a negative control, for 24 or 48 hours. HuR mRNA and protein silencing were assessed by qRT-PCR and Western blotting, respectively. *RPLP0* mRNA and β -actin protein were used as housekeeping and loading controls, respectively. Data of three psoriatic and four healthy patients were normalized on the control mean of siSCR and presented as paired dot plots. qRT-PCR statistical analysis was performed using paired parametric Student's *t*-test while Dunnett's multiple comparisons test was used for WB analysis (*: $0.01 < P < 0.05$). (c) Effects of HuR silencing on IL-1 β , S100A7, p-STAT3, and eFABP protein levels in primary keratinocytes. IL1 β and S100A7 proteins level reduction after 48 hours of siHuR treatment. P-STAT3 protein expression reduced after 24 hours of treatment in psoriatic cells. eFABP protein increases in psoriatic cells. Quantification of different WB in graphs (Data plotted as mean $\pm$ SEM; n of biological replicates presented by dots). 2way-ANOVA followed by Dunnett's multiple comparisons test (*: $0.01 < P < 0.05$; *** $0.0001 < P < 0.001$). (d) ELISA-based quantification of IL17AF heterodimer in psoriatic primary keratinocytes treated with siHuR for 24 and 48 hours. Data plotted as mean $\pm$ SEM of three healthy and three psoriatic donors. 2way-ANOVA followed by Sydak's multiple comparisons test (*: $0.001 < P < 0.01$; **** $P < 0.0001$).

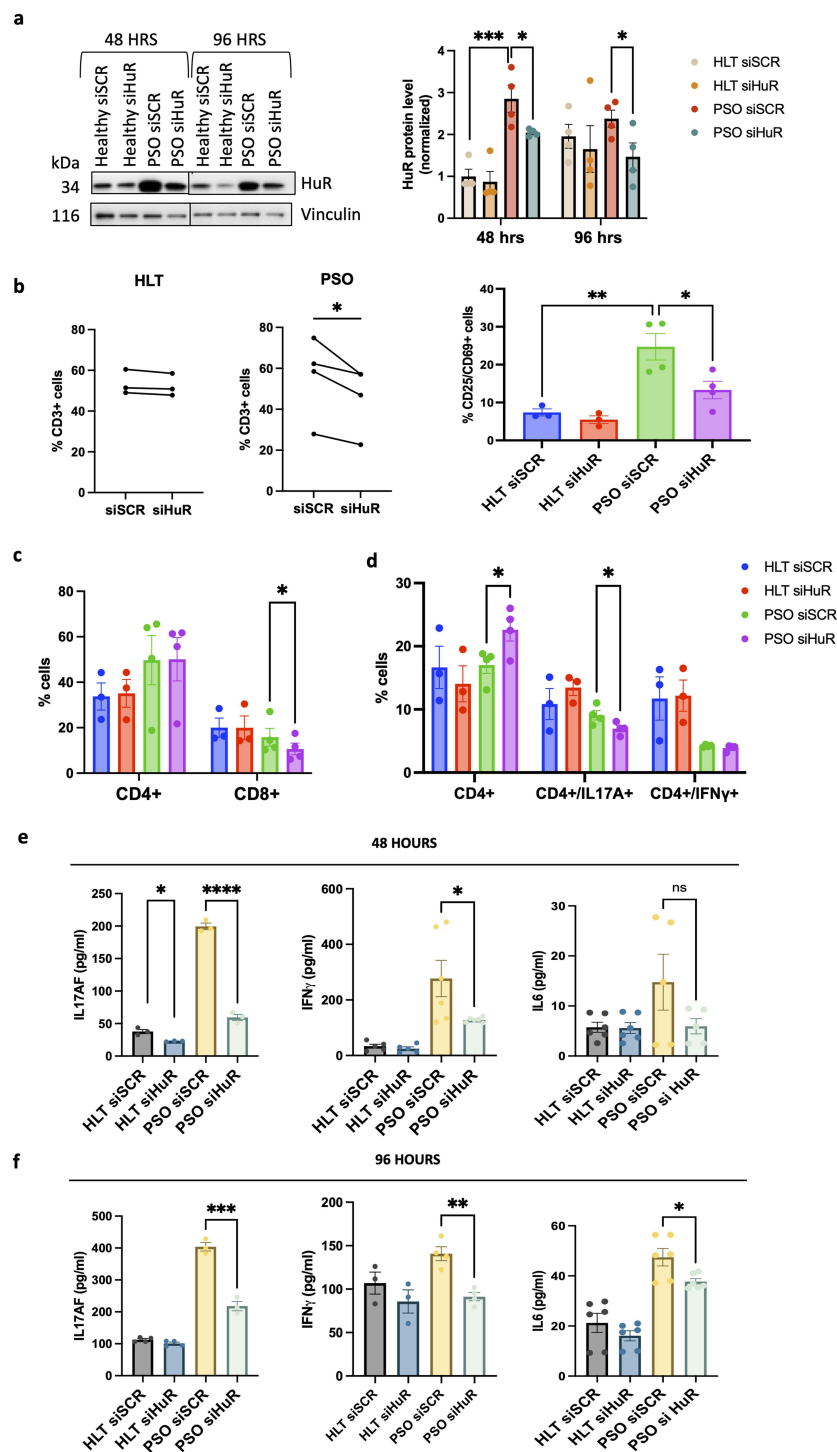


Figure 2 Effects of HuR silencing on gene expression and T-cell activation in primary lymphocytes. **(a)** Primary lymphocytes from psoriatic and non-psoriatic donors were nucleofected with 100 nM siHuR or 100 nM scrambled siRNA (siSCR), used as a negative control. HuR protein levels were evaluated by Western blotting 48 and 96 hours after nucleofection, using Vinculin as a loading control. Data were normalized to the mean value of the non-psoriatic siSCR control and are presented as mean $\pm$ SEM. Statistical analysis was performed using 2way-ANOVA followed by Sydak's multiple comparisons test (*: 0.01 < P < 0.05; ****0.0001 < P < 0.001). **(b)** Paired dot plots show the percentages of CD3-positive cells (paired t-test analysis is shown) while the bar plot represents cells expressing activation markers (double-positive CD25/CD69) within the CD3+ population. Data are presented as mean $\pm$ SEM and analysed by one-way ANOVA followed by Dunnett's multiple comparisons test (*: 0.01 < P < 0.05; **0.001 < P < 0.01). **(c)** Flow-cytometric analysis of CD4⁺ T-helper and CD8⁺ cytotoxic T-cell populations gated on CD3⁺ cells 96 hours after HuR silencing. Data are presented as mean $\pm$ SEM and analysed by paired t-test (*: 0.01 < P < 0.05). **(d)** Flow cytometry analysis of Th1 and Th17 subsets in healthy and psoriatic T lymphocytes 72 hours after silencing. The plot reports the percentages of CD4-positive cells and cells positive for intracellular IL17A or IFN- γ , analysed within the CD4⁺ population. Data are presented as mean $\pm$ SEM and analysed by paired Student's t-test (*: 0.01 < P < 0.05). **(e)** ELISA-based quantification of IL17AF heterodimer, IFN- γ , and IL6 in the supernatant of healthy and psoriatic T lymphocytes 48 hours after siRNA treatment. Data are presented as mean $\pm$ SEM and were analyzed using an unpaired Welch's t-test (*: 0.01 < P < 0.05; ****P < 0.0001; ns, not significant). **(f)** ELISA-based quantification of IL17AF heterodimer, IFN- γ and IL6 in the supernatant of healthy and psoriatic T lymphocytes 96 hours after siRNA treatment. Data are presented as mean $\pm$ SEM and were analyzed using an unpaired Welch's t-test (*: 0.01 < P < 0.05; **: 0.001 < P < 0.01; ***P < 0.0001).

Enhanced Cross-Linked ImmunoPrecipitation sequencing (eCLIP-seq) experiment. Experimental details are provided in [Supplementary Materials and Methods](#), while quality control and binding landscape analyses are shown in [Supplementary Figures S2](#) and [S3](#). Briefly, following stimulation, HaCaT cells were cross-linked and lysed. HuR-bound RNA was immunoprecipitated using an anti-HuR antibody ([Supplementary Material](#) and [Methods](#)), isolated, reverse-transcribed into cDNA, used for library preparation, and subsequently sequenced to identify HuR-bound transcripts. We compared immunoprecipitated material after stimulation (STIM, 2 replicates) and not stimulation (NS, 3 replicates), and peak classification was performed according to the comparison between IP and Input samples, as well as between experimental conditions. Matched Input controls were processed in parallel to account for the background signal,³⁶ and replicate consistency was assessed by principal component analysis (PCA) based on normalized Fragments Per Kilobase of transcript per Million mapped reads (FPKM) values calculated across identified binding regions/peaks ([Supplementary Table S3](#)). The different peak categories and their interpretation are summarized in [Supplementary Table S4](#). The number of increased (+) and decreased (−) altered crosslinked events obtained from the different comparisons is summarized in [Supplementary Table S5](#). To assess the sequence specificity of HuR binding, motif enrichment analysis was performed on identified HuR-bound regions as described in [Supplementary Materials and Methods](#) and [Supplementary Table S6](#).

We next examined the transcriptomic distribution of HuR binding changes in the stimulated condition. Stimulation induced a redistribution of HuR binding: decreased binding was observed in intronic and intergenic regions, while enrichment in 3'-UTRs suggests a functional shift toward post-transcriptional regulation of mature transcripts ([Figure 3a](#)).

To investigate the functional significance of HuR binding changes after stimulation, RBP-bound peaks (IP versus input) significantly enriched in at least one condition (either NS or STIM) were further selected for downstream analysis. We then carried out enrichment analyses on the set of genes associated with RBP-driven changes in both stimulated and non-stimulated conditions. Enrichment analysis of MSigDB Hallmarks in STIM and NS conditions revealed that stimulation redirects HuR binding toward stress, inflammation and cell fate regulatory genes. ([Supplementary Figure S4](#)).

To determine if binding patterns varied by transcriptomic region, we separately analysed increased and decreased HuR binding events in 3'-UTRs and introns. Functional enrichment (Biological Process, Molecular Function and Reactome) was then performed on transcripts showing region-specific binding changes. For transcripts with decreased HuR binding, enrichment in the 3'-UTR was observed for processes related to cell division, chromosome organization, the mitotic prometaphase and cell cycle checkpoints ([Figure 3b](#)). In intronic regions, enriched terms included DNA repair, chromosome organization and catalytic activity ([Supplementary Figure S5a](#)). For transcripts with increased HuR binding ([Figure 3c](#)), enrichment in the 3'-UTR included negative regulation of transcription, response to growth factor, cell proliferation and differentiation. Molecular function analysis also highlighted transcription factor binding, while Reactome terms indicated TGFB signalling and NGF-stimulated transcription. In introns, enriched categories involved actin filament organization, cell–matrix adhesion, kinase activity and the RHO GTPase cycle ([Supplementary Figure S5b](#)).

Taken together eCLIP results suggest that psoriasis-like stimulation redistributes HuR binding from intronic and intergenic regions toward 3'-UTRs of transcripts involved in inflammatory and growth factor signalling. This pattern is consistent with stimulus-dependent cytoplasmic relocalization and target switching of HuR, potentially promoting stabilization and/or translation of response genes while releasing control of cell-cycle transcripts.^{37,38}

According to sequencing results, we investigated HuR effects in NS and STIM HaCaT cells at the single-gene level. After 24h of cytokine stimulation, we performed HuR RNA immunoprecipitation (RIP) using an anti-HuR antibody (see [Supplementary Materials and Methods](#)), with an anti-IgG antibody as a negative control. The enrichment of target mRNAs was quantified by qRT-PCR and expressed as the fold change relative to the mRNA levels detected in the IgG immunoprecipitate. We revealed significant enrichment of psoriasis-related mRNAs, including *RAB27A*, *ATF2*, *H3C6*, *IL6*, *IL8* and *IL1B* (all linked to inflammatory pathways and cytokine release in psoriatic inflammation), compared to IgG controls; while 1% of lysate was used as Input ([Figure 3d](#)). We further confirmed that HuR regulates *IL6* and *IL1B* mRNAs by human siHuR pools transfection. qRT-PCR revealed more than 50% of HuR mRNA silencing, along with *IL6* and *IL1B* mRNA decrease ([Figure 3e](#)). Additional experiments were conducted on *IL1B* and *IL6* mRNAs as HuR targets, considering HuR stabilization activity.³⁹ We treated STIM and NS HaCaT cells with Actinomycin D (ActD) for 30, 60 and 90 minutes. No ActD treatment (0 min) was used as the control condition. In STIM cells, target mRNAs remain stable over time, while in NS mRNA levels

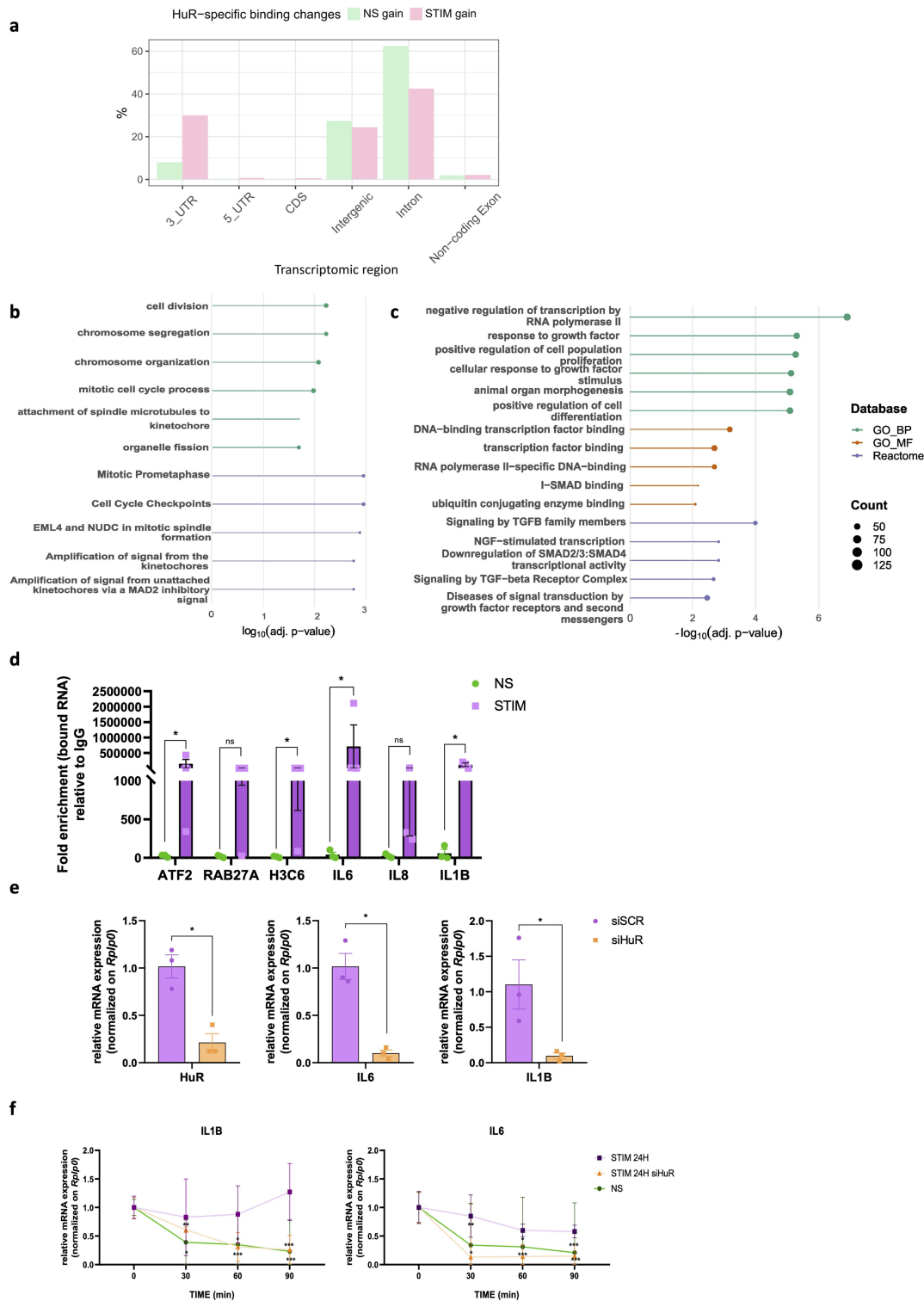


Figure 3 HuR binds inflammation-related 3'-UTR transcripts associated with psoriasis in stimulated HaCaT cells. **(a)** Distribution of RBP-driven peak change gained (green) and lost (pink) upon stimulation. Data are shown as percentages of the total of RBP-driven changes in stimulated (STIM) and non-stimulated (NS) conditions, independently. **(b and c)** Dot plot showing significantly enriched terms in the Biological Processes (BP) and Reactome for genes with, respectively, decreased and increased HuR binding upon stimulation in the 3'UTR. The size of the dots represents the number of genes associated with each term, while the color denotes the category. The significance ($-\log_{10}$ P-value) is reported on the x-axis. **(d)** *IL6*, *IL8*, *IL1B*, *RAB27A*, *ATF2* and *H3C6* mRNAs confirmed as HuR target in STIM cells by qRT-PCR, following RIP assay. Fold enrichment indicates the values of mRNA bound with HuR, normalized to IgG-bound RNA, used as negative control. Data plotted as mean $\pm$ SEM; n=3; statistical analysis was performed using Welch's t-test (*: 0.01 < P < 0.05; ns, not significant). **(e)** *HuR*, *IL6* and *IL1B* mRNA levels in STIM cells, transfected with siHuR or siSCR used as control. Bar graphs show mean $\pm$ SEM; n=3; Welch's t-test (*: 0.01 < P < 0.05). **(f)** qRT-PCR of *IL6* and *IL1B* mRNA levels in HaCaT cells in three conditions: NS, STIM and STIM after transfection of siHuR treated with actinomycin D (ActD; 2 μ M) for 30, 60 and 90 minutes after 24 hours of stimulation. Data were normalized to no-ActD treatment (0 minutes). Data plotted as mean $\pm$ SEM; n=3; Welch's t-test (*: 0.01 < P < 0.05; **: 0.001 < P < 0.01; ***0.0001 < P < 0.001).

decrease. Silencing HuR in HaCaT cells before stimulation and ActD treatment led to reduced *IL6* and *IL1B* mRNAs stability over time (Figure 3f).

Tanshinone Mimic (TM) Molecules Regulate HuR Activity in Psoriatic Keratinocytes and Lymphocytes

Taking advantage of previously characterized HuR–mRNA competitors, TMs bind HuR and induce conformational changes that impair mRNA interaction, reducing its activity.^{32,33} We examined the impact of pharmacological modulation of HuR on psoriasis-associated markers. Human primary healthy and psoriatic keratinocytes were treated with TM11, TM7nox and TM7nred at two concentrations, 1.56 μ M and 12.5 μ M. As shown in Figure 4a, HuR protein was unaffected by these treatments in both non- and psoriatic donors. However, we observed reduced levels of proliferative psoriatic markers,^{40–44} particularly Cyclin B after TM7red and TM7ox treatment at 12.5 μ M. Survivin showed a broader decrease following TM7red, TM7ox, and TM11 at the same concentration. FABP5 was reduced after TM7ox at both concentrations and TM11 at the highest dose, while S100A7 decreased following TM7red and TM11 at 12.5 μ M, as well as TM7ox at 1.56 μ M (Figure 4b). The P-STAT3/STAT3 ratio was also reduced after TM11 treatment at 1.56 μ M (Figure 4c), indicating that these markers are regulated through HuR-dependent post-transcriptional mechanisms.

Given the similar effects of TMs and the favourable properties of TM11, we further investigated its mechanism. HaCaT cells were treated with TM11 (20 μ M) for 2 hours. qRT-PCR showed no change in target expression (Figure 4d). TM11 treatment restored basal HuR–mRNA binding for all tested targets (*RAB27A*, *ATF2*, *H3C6*, *IL6*, *IL8*, *IL1B*) following stimulation (Figure 4e), indicating that its inhibitory effect occurs through the disruption of post-transcriptional complexes.

Moreover, we assessed whether TMs affected the activity of psoriatic lymphocytes. During the early phases of activation, primary T cells derived from healthy and psoriatic donors were treated with three concentrations of TM7red and TM11 (1.56, 5, and 12.5 μ M). Cells were stimulated using anti-CD3/CD28–coated immunomagnetic beads and analysed by flow cytometry after 3 and 6 days of treatment. The profile of the CD25/CD69 double-positive subset within CD3+ cells, particularly affected in psoriatic T cells, is shown in Figure 4f. In psoriatic donors, the percentage of CD3/CD25/CD69 positive cells decreased following treatment with both compounds. This reduction in activated cells persisted after 6 days, although it remained significant only at the highest concentration (Figure 4g). Both compounds exhibited clear immunomodulatory properties. ELISA confirmed a dose-dependent decrease in IL17AF and IFN- γ secretion after 6 days of treatment with TM7red and TM11 in psoriatic T lymphocytes (Figure 4h).

Overall, results from HaCaT cells, keratinocytes and lymphocytes demonstrate the functional efficacy of TM11 in the psoriatic context, supporting its use in subsequent ex vivo and in vivo experiments.

TM11 Treatment Rescues in vivo Murine Model of Psoriasis-Like Dermatitis

To assess the in vivo effects of TM11, an acute psoriasis-like dermatitis model was established in C57BL/6 mice using topical Imiquimod (IMQ). Five groups were treated as follows: negative control (Vaseline, CTRL), IMQ alone, IMQ and Dexamethasone (positive control), and IMQ with topically administered TM11 at 10 μ M or 20 μ M. IMQ was applied daily from day 1 to 7, while treatments were administered from day 2 to 7, a few hours prior to IMQ (Figure 5a). Daily assessment of skin inflammation using a murine-adapted PASI showed a significant reduction in TM11-treated groups, with TM11 10 μ M effective from day 3 and TM11 20 μ M from day 4, compared to IMQ alone (Figure 5b). This improvement persisted throughout the study, as confirmed by cumulative PASI AUC analysis (Figure 5c). We evaluated the single characteristics that contribute to the total PASI score, such as redness/erythema, thickness and scaling, demonstrating that TM11 treatment dramatically decreases all these aspects over time (Supplementary Figure S6a–c). Body weight monitoring indicated minimal loss and recovery in TM11 groups, suggesting good tolerability and low systemic toxicity (Figure 5d). At day 7, spleen and blood analyses revealed that TM11 10 μ M significantly reduced the Spleen Index and circulating levels of Total white blood Cell (WBC) and Neutrophils, indicating systemic immunomodulatory effects (Figure 5e and f). Histological analysis showed that TM11 restored epidermal structure, reducing IMQ-induced thickening, spongiosis, and dermal infiltrate to near-normal levels (Figure 5g). Quantitative epidermal thickness

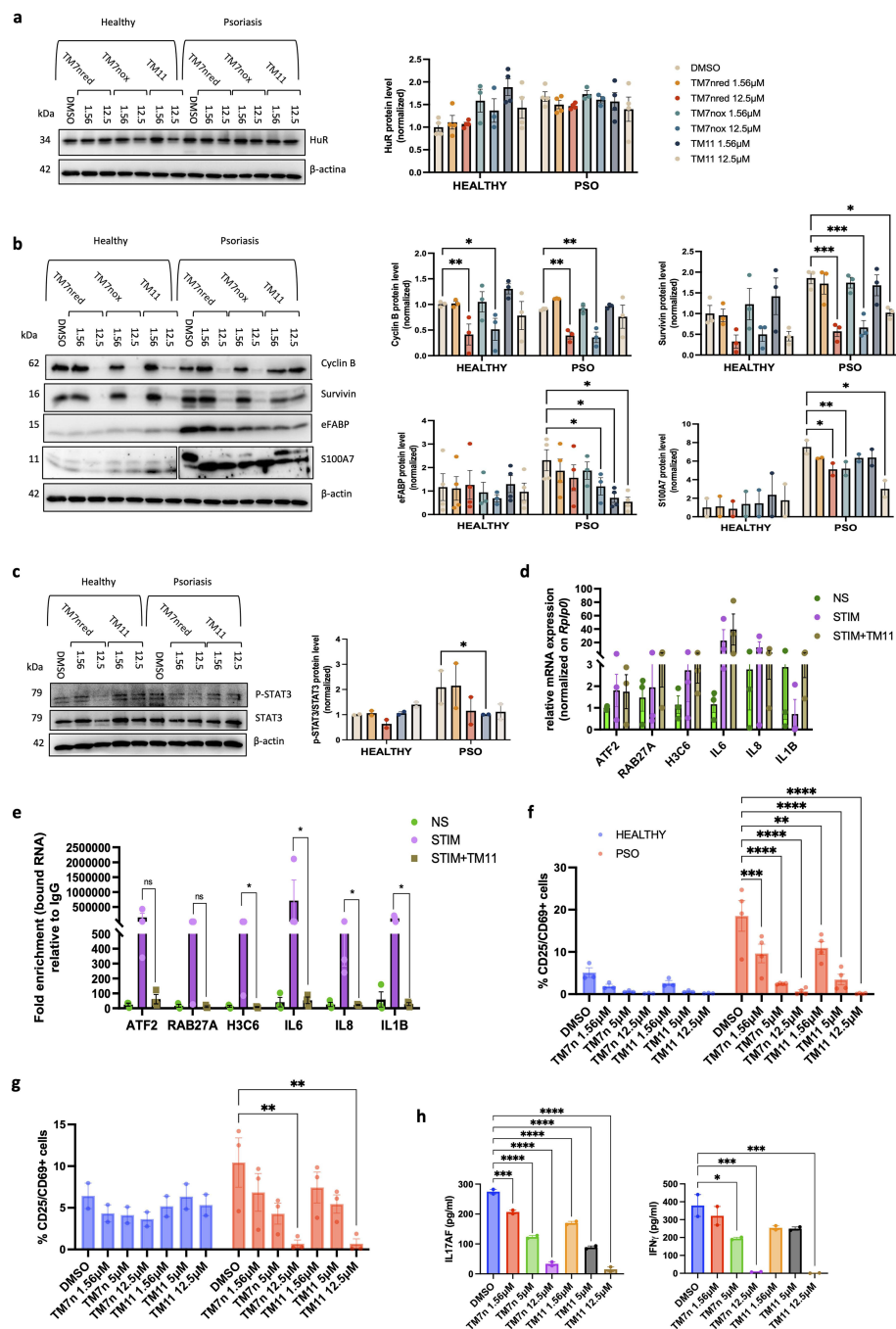


Figure 4 TM compounds modulate psoriasis-associated markers and inflammatory signalling in keratinocytes and lymphocytes. **(a)** Healthy and psoriatic keratinocytes treated with either TM7nox or TM7nred or TM11 at 1.56 μ M and 12.5 μ M. HuR protein abundance was measured by Western blotting. Protein levels were normalized to β -actin and expressed relative to the mean value of the healthy DMSO-treated control. **(b)** Representative images of Cyclin B, Survivin, eFABP and S100A7 WB. Quantification plots show data normalized to β -actin and expressed relative to the mean value of the healthy DMSO-treated control. Data are presented as mean $\pm$ SEM and analysed by 2-way ANOVA followed by Dunnett's multiple comparisons test (*: 0.01 < P < 0.05; **: 0.001 < P < 0.01; ***: 0.0001 < P < 0.001). **(c)** Representative Western blot images and quantification of the p-STAT3/STAT3 ratio. Data were expressed relative to the mean value of the healthy DMSO-treated control and are presented as mean $\pm$ SEM. Statistical analysis was performed using two-way ANOVA followed by Dunnett's multiple-comparisons test. (*: 0.01 < P < 0.05). **(d)** qRT-PCR was performed on HaCaT cells under three conditions: unstimulated (NS), stimulated for 24 hours (STIM) and stimulated for 24-hours followed by a two-hour treatment with TM11 (20 μ M) (STIM+TM11). Data represent mRNA levels of data normalized on NS conditions, using *RPLP0* as housekeeping. Plots are represented as mean $\pm$ SEM (n=3; Welch's t-test). **(e)** Plots are represented as Fold Enrichment of mRNA level bound on HuR normalized to the mRNA normalized to the mRNA bound with IgG, used as negative control. Data plotted as mean $\pm$ SEM; n=3; Welch's t-test (*: 0.01 < P < 0.05; ns, not significant). **(f)** Primary lymphocytes from healthy and psoriatic donors were treated with TM11 or TM7n at concentrations of 1.56, 5, or 12.5 μ M for 3 days during T-cell activation. Flow cytometry was used to determine the percentage of CD25⁺CD69⁺ double-positive cells within the CD3⁺ population. Data are presented as mean $\pm$ SEM and were analyzed using two-way ANOVA followed by Sidak's multiple-comparisons test. (*: 0.001 < P < 0.01; ***: 0.0001 < P < 0.0001). **(g)** T-cell activation markers were analyzed after 6 days of treatment with TM11 or TM7n at concentrations of 1.56, 5, or 12.5 μ M. The percentage of CD25⁺CD69⁺ double-positive cells was determined within the CD3⁺ population. Data are presented as mean $\pm$ SEM and were analyzed using two-way ANOVA followed by Sidak's multiple-comparisons test. (*: 0.001 < P < 0.01). **(h)** ELISA-based quantification of the IL-17A^F heterodimer and IFN- γ in culture supernatants from psoriatic primary T cells treated with TM11 or TM7n for 6 days. Data are shown as mean $\pm$ SEM and analysed by 2-way ANOVA followed by Dunnett's multiple comparisons test (*: 0.01 < P < 0.05; ***: 0.0001 < P < 0.001; ****: P < 0.0001).

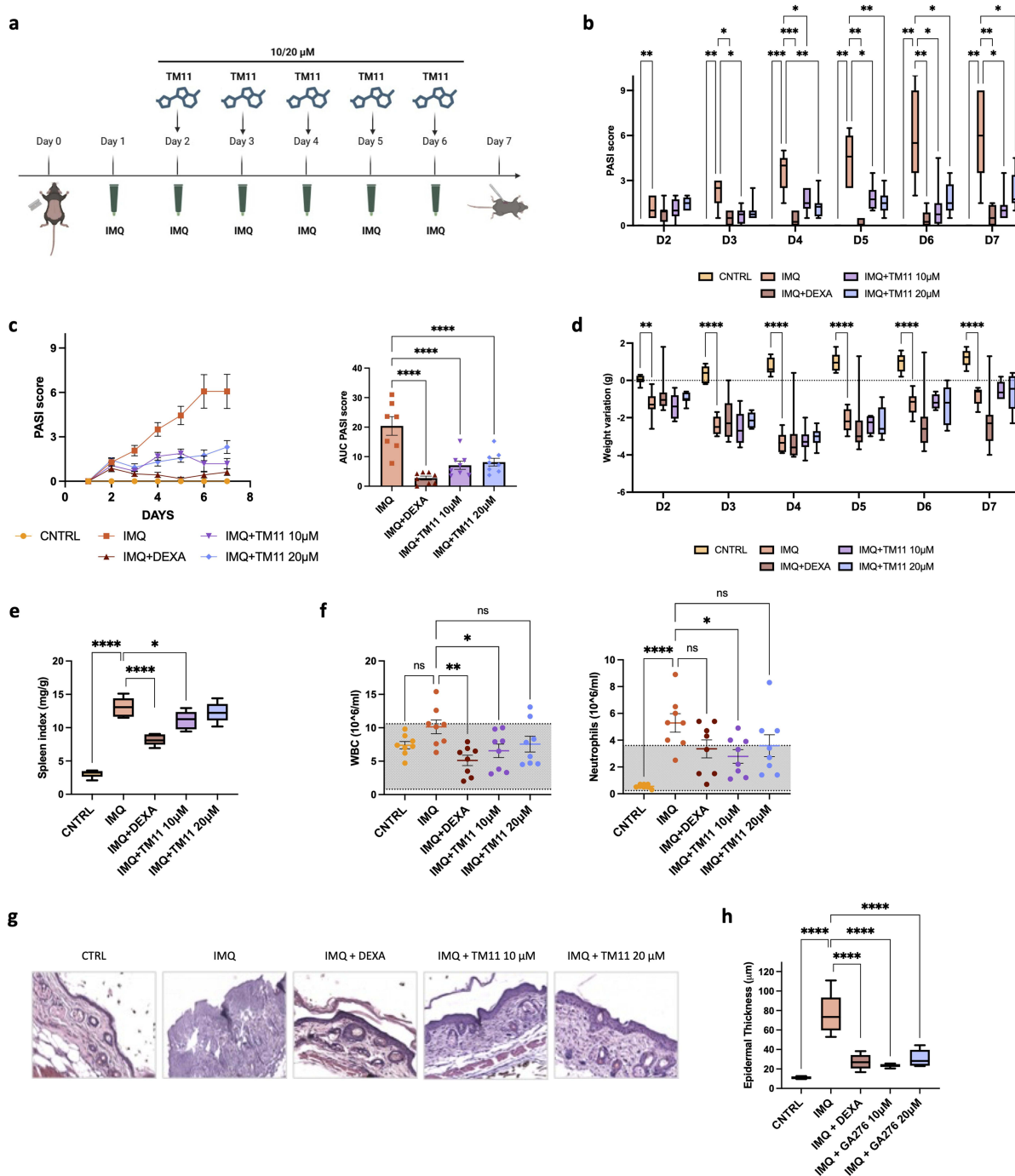


Figure 5 TM11 compound attenuates psoriasis-like inflammation in an in vivo murine model. **(a)** Experimental timeline of the in vivo murine study comprising five experimental groups ($n=8$ mice per group) divided as: Vaseline group (CNTRL); Imiquimod (IMQ); Imiquimod + Dexamethasone (IMQ+DEXA); Imiquimod + TM11 10 μ M (IMQ + TM11 10 μ M); Imiquimod + TM11 20 μ M (IMQ + TM11 20 μ M). On day 0, the dorsal fur was shaved over a 2x2 cm area. Mice were then treated with either IMQ or Vaseline, which served as the control treatment, once daily for 7 days. Dexamethasone or TM11 treatment was administered daily from day 2 through day 7. Mice were euthanized on day 7. **(b)** Daily evaluation of the murine PASI score. Data are presented as box-and-whisker plots showing the minimum and maximum values and were analyzed using two-way repeated-measures ANOVA followed by Dunnett's multiple-comparisons test. (*: $0.01 < P < 0.05$; **: $0.001 < P < 0.01$; ***: $0.0001 < P < 0.001$). **(c)** PASI score overtime and corresponding Area Under Curve (AUC). Data are expressed as mean $\pm$ SEM and analysed by ordinary one-way ANOVA followed by Sydak's multiple comparisons test (**** $P < 0.0001$). **(d)** Daily body-weight measurements, normalized to the day 0 value for each mouse. Data are presented as box-and-whisker plots showing the minimum and maximum values and were analyzed using two-way repeated-measures ANOVA followed by Dunnett's multiple-comparisons test. (**: $0.01 < P < 0.05$; **** $P < 0.0001$). **(e)** Spleen index, expressed as spleen weight normalized to body weight (mg/g), measured after euthanasia on day 7. Data are presented as box-and-whisker plots showing the minimum and maximum values and were analyzed using one-way ANOVA followed by Dunnett's multiple-comparisons test. (*: $0.01 < P < 0.05$; **** $P < 0.0001$). **(f)** Analysis of peripheral blood cellular parameters on day 7, immediately before euthanasia, using an automated hematology analyzer. White blood cell (WBC) and neutrophil counts are presented as mean $\pm$ SEM and analysed by ordinary one-way ANOVA followed by Dunnett's multiple-comparisons test. The gray dashed line indicates the normal physiological range. (*: $0.01 < P < 0.05$; **: $0.001 < P < 0.01$; **** $P < 0.0001$ ns, not significant). **(g)** Representative hematoxylin-and-eosin-stained skin sections from the indicated treatment groups. Scale Bar = 100 μ m. **(h)** Quantification of epidermal thickness. Data are presented as box-and-whisker plots showing the minimum and maximum values and were analyzed using one-way ANOVA followed by Dunnett's multiple-comparisons test. (**** $P < 0.0001$).

measurements confirmed a 60–65% reduction in TM11-treated groups, compared to the IMQ group, and similar to the steroid treatment (Figure 5h). Overall, TM11 demonstrated rapid onset of action, sustained efficacy, and good tolerability in this murine model of psoriasis-like dermatitis.

TM11 Compound Restores Physiological Phenotype in ex vivo 3D Human Psoriatic Skin Model

Given the in vivo evidence, we further investigated HuR modulation by TM11 in an ex vivo human-like psoriatic model. We used the InflammSkin® 3D skin system, developed by Genoskin, to replicate Th17/Th1-mediated inflammation.⁴⁵ The model is based on healthy human skin biopsies, where resident T cells are activated with anti-CD3 and anti-CD28 antibodies plus rhIL2, and polarization toward a Th17/Th1 phenotype is induced using IL1B, IL23 and TGFβ. A key advantage of this system is its ability to be maintained in culture for additional 7 days, providing a validated human-like platform to assess the efficacy and mechanism of psoriasis treatments. In our study, six experimental groups were established: NativeSkin (untreated, non-activated skin); InflammSkin; InflammSkin and Betamethasone (BETA; positive control); InflammSkin and DMSO; InflammSkin and TM11 10 μM; InflammSkin and TM11 20 μM. Histological analysis showed that untreated NativeSkin maintained normal tissue integrity, with only few pyknotic/eosinophilic cells in the epidermis, confirming model stability. In contrast, untreated InflammSkin exhibited marked alterations, including keratinocyte pyknosis, cytoplasmic vacuolization, epidermal detachment, and increased dermal inflammatory infiltrate, consistent with a strong inflamed phenotype. Treatment with either steroid or TM11 at both concentrations restored tissue morphology to near-normal levels. (Figure 6a). Quantitative analysis of epidermal thickness showed a significant reduction in the TM11-treated groups at both doses, comparable to that observed with betamethasone (Figure 6b). The proliferation marker Ki-67, elevated in the InflammSkin model, returned to physiological levels following TM11 treatment at both concentrations (Figure 6c). We also demonstrate a reduction of the amount of infiltrating cells (CD45+) and in particular of CD3+ cells in InflammSkin models treated with both doses of TM11 (Figure 6c). ELISA quantification of IL17AF and IFN-γ in the supernatant at days 1, 4, and 7 revealed a significant dose-dependent reduction with TM11 treatments at 10 and 20 μM overtime (Figure 6d). TM11, at both doses, modulated inflammatory responses and proliferation of human keratinocytes and lymphocytes in co-culture, recapitulating key features of the effect in a psoriatic patient's dermal environment.

Discussion

Upon cytoplasmic relocalization, the RNA-binding protein HuR (also known as ELAVL1) stabilizes the mRNAs of numerous pro-inflammatory mediators, thereby amplifying both innate and adaptive immune responses.^{14,46} Supporting the hypothesis that HuR contributes to keratinocyte hyperactivation and chronic cutaneous inflammation, recent evidence has shown that HuR post-transcriptionally stabilizes cytokines involved in psoriasis pathogenesis, including IL-20.²³ Interestingly, a proof-of-concept study also indicated that pharmacological inhibition of HuR using PEGylated peptides could attenuate inflammatory responses by reducing VEGF and IL-23 expression, highlighting HuR as a potential therapeutic target for psoriasis. Despite these findings, mechanistic evidence linking ELAVL1 (HuR) to psoriasis pathogenesis remains limited. In this work, we characterized the HuR-bound transcript profiles in stimulated HaCaT cells and primary psoriatic keratinocytes, confirming the exerted stabilizing activity on a subset of interleukin transcripts.

Furthermore, using complementary mouse models and an ex vivo human skin model, we explored the restorative properties of TM11, a small-molecule inhibitor of HuR–RNA interactions that we recently characterized,³³ reinforcing the HuR-mediated post-transcriptional regulatory layer as a promising strategy for the development of novel anti-psoriatic therapies. In particular, we investigated the role of HuR in sustaining the proliferative and inflammatory phenotype of primary human psoriatic keratinocytes and lymphocytes, as well as the efficacy of HuR modulators in reverting this phenotype in vitro, ex vivo and in vivo. Consistent with Garcin et al.,²³ HuR was predominantly cytoplasmic in psoriatic keratinocytes, suggesting enhanced mRNA stabilization. HuR knockdown reduced IL1B and S100A7 protein levels and destabilized *IL1B* and *IL6* mRNAs, leading to decreased IL17AF secretion. These findings confirm that HuR sustains inflammation, as IL1B promotes T-cell proliferation⁴⁷ and IL8 drives neutrophil infiltration and microabscess formation.^{21,48} HuR also stabilizes S100A7,

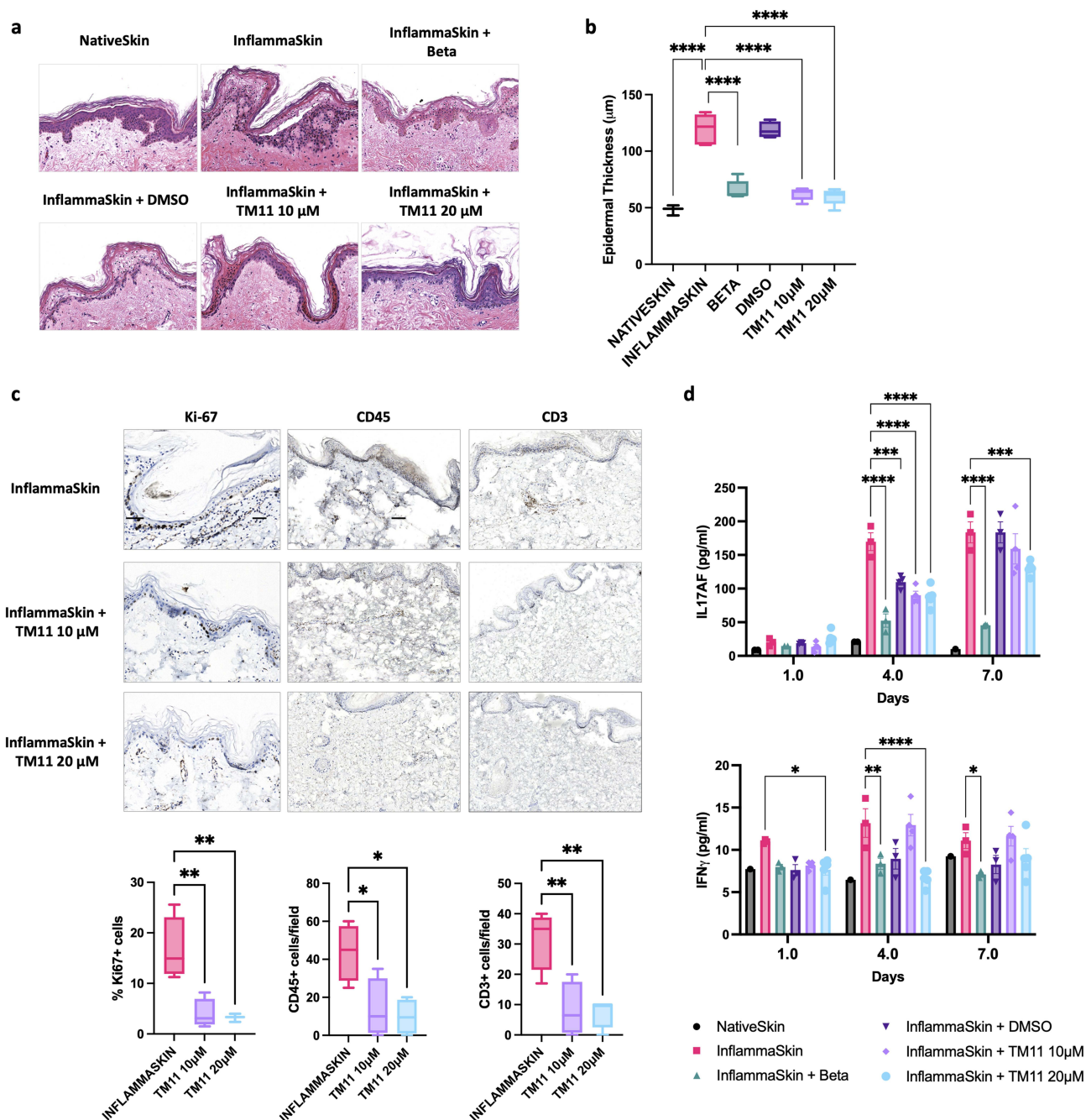


Figure 6 TM11 improves tissue morphology and attenuates inflammation in a human-derived 3D InflammaSkin model. The following groups were analyzed: NativeSkin (untreated, n=3); InflammaSkin (n=5); InflammaSkin + Betamethasone (n=5) (positive control); InflammaSkin + DMSO (n=5); InflammaSkin + TM11 10 μ M (n=5); InflammaSkin + TM11 20 μ M (n=5). (a) Representative hematoxylin-and-eosin-stained sections from the indicated InflammaSkin treatment groups. Scale Bar = 100 μ m. (b) Quantification of epidermal thickness. Data are presented as box-and-whisker plots showing the minimum and maximum values and were analyzed using one-way ANOVA followed by Dunnett's multiple-comparisons test (****P < 0.0001). (c) Representative immunohistochemical images of InflammaSkin, InflammaSkin treated with TM11 at 10 μ M, and InflammaSkin treated with TM11 at 20 μ M. Separate immunohistochemical stainings were performed for Ki-67, CD45, and CD3. Positive staining was visualized using 3,3'-diaminobenzidine (DAB; brown staining), whereas hematoxylin was used as a counterstain to visualize cell nuclei. Scale Bar = 100 μ m. Quantitative data are presented as box-and-whisker plots showing the minimum and maximum values. Ki-67-positive staining was analyzed using one-way ANOVA followed by Dunnett's multiple-comparisons test, whereas CD45- and CD3-positive staining was analyzed using one-way ANOVA followed by Sidak's multiple-comparisons test (*: 0.01 < P < 0.05; **: 0.001 < P < 0.01). (d) ELISA-based quantification of IL17AF, IFN- γ in the culture medium of the 3D model after 1, 4, and 7 days of culture. Data are presented as mean $\pm$ SEM. Statistical analysis was performed using a mixed-effects model (REML), with matched values entered in stacked subcolumns, followed by Dunnett's multiple-comparisons test (*: 0.01 < P < 0.05; **: 0.001 < P < 0.01; ****0.0001 < P < 0.001; *****P < 0.0001).

amplifying inflammation via IL1B and IL17 signaling.^{49–51} Moreover, HuR interacts with STAT3 mRNA, supporting its translation by limiting miRNA repression.⁵² STAT3 regulates transcription upon phosphorylation,⁵³ and its activation was reduced following HuR depletion, as indicated by decreased phosphorylation. Together with previous findings indicating that

STAT3 promotes HuR nuclear retention,⁵⁴ our data suggest a context-dependent reciprocal regulation between HuR and STAT3.

HuR also regulates patient-derived lymphocytes. Its silencing reduced CD25⁺, CD69⁺ and CD25⁺CD69⁺ T-cell populations within CD3⁺ cells, which are associated with psoriasis progression.^{55–57} Th17 and Th1 subsets and their cytokines (IL17AF, IL6, IFN- γ) were also reduced, indicating impaired T-cell activation, proliferation, and differentiation. Taken together, these results suggest that HuR is required not only for keratinocyte-driven inflammation but also plays a key role in activating, proliferating and differentiating T cells in the psoriatic context.

To overcome limitations related to sample heterogeneity and difficulties in collecting high-content material from primary samples, we performed eCLIP-seq in stimulated HaCaT cells. Although these cells and stimulation protocols provide only a partial representation of inflammatory targets with no heterologous interactions, we adopted this system to investigate HuR-dependent keratinocyte-intrinsic inflammatory responses. Indeed, we identified novel HuR-bound mRNAs at single-nucleotide resolution. Compared to previous RIP-seq studies,²³ this approach revealed dynamic, condition-specific HuR-RNA interactions. We identified several psoriasis-related HuR targets, including RAB27A,⁵⁸ H3C6,⁵⁹ ATF2,⁶⁰ IL6, IL8 and IL1B. These transcripts showed preferential HuR binding and were reduced upon HuR silencing, confirming post-transcriptional regulation. ActD assays demonstrated that the stability of these transcripts depends on HuR, particularly under stimulated conditions, supporting its central role in psoriatic keratinocyte development.

Current psoriasis treatments depend on disease severity, ranging from topical therapies for mild forms,^{61–63} to systemic drugs and biologics for moderate-to-severe disease.^{64,65} However, important limitations remain, highlighting the need for new therapeutic strategies. Here, we identified small molecules targeting HuR that restore a healthy phenotype.^{32,33}

These compounds reduced HuR-mediated mRNA-stabilizing activity without affecting HuR protein levels, likely by acting in the cytoplasm. Treatment decreased pro-inflammatory gene expression and modulated keratinocyte proliferation and differentiation, as indicated by reduced Cyclin B and Survivin levels, key regulators of cell cycle and apoptosis.^{40–43} In our treated keratinocytes, Cyclin B and Survivin downregulation suggested a partial restoration of the differentiation program and a return toward physiological proliferation and differentiation, in agreement with previous studies.²⁹ S100A7 and eFABP were also downregulated, indicating partial recovery of normal function.^{44,50} Similarly, treated lymphocytes showed reduced activation, as indicated by the lower frequency of CD3⁺CD25⁺CD69⁺ cells, and decreased IL17AF and IFN- γ production, supporting anti-inflammatory effects of TM11 *in vitro* and *in vivo*.

TM11 exhibited selective cytotoxicity toward psoriatic cells together with potent anti-inflammatory activity *in vitro*, thus, we prioritized topical TM11 in the IMQ-induced psoriasis-like mouse model for *in vivo* testing. In C57BL/6 mice with induced skin inflammation, topical TM11 treatment exerted a robust anti-psoriatic effect, as shown by the marked reduction in the PASI score, including erythema/redness, epidermal thickening/induration, and scaling/desquamation. In parallel, TM11 also attenuated systemic inflammation, as indicated by significantly lower circulating WBC and neutrophil counts, as well as a spleen index. Importantly, mice receiving TM11 regained body weight after day 4, similarly to control animals, supporting a favourable tolerability profile. Together with the observed selective cytotoxicity toward psoriatic cells and the favourable tolerability profile *in vivo*, these data provide a preliminary rationale for further safety evaluation. However, pharmacokinetic, toxicological and tissue-selectivity studies will be necessary before clinical translation. Histological analysis further supported the therapeutic effect of TM11, which markedly reduced epidermal thickening and partially restored skin architecture toward a near-normal morphology. Nevertheless, some limitations of the IMQ-induced psoriasis-like mouse model should be considered when interpreting these *in vivo* findings. IMQ application elicits an acute, TLR7-driven inflammatory response that only partially recapitulates the complex, chronic and relapsing nature of human psoriasis. Moreover, its transcriptomic and cellular features may vary depending on the mouse strain, sex and induction protocol. Moreover, IMQ-induced dermatitis activates a broad spectrum of cutaneous inflammatory pathways that are not unique to psoriasis and predominantly reflects IL-23/IL-17 axis-dependent mechanisms.⁶⁶ Although the small number of primary patient samples represents a limitation of this study and independent cohorts will be necessary to confirm the broader applicability of our findings, the complementary models used here highlight the promising pre-clinical potential of TM11 as anti-psoriatic therapy. Together, these models demonstrated both local and systemic anti-inflammatory activity and support further investigation for TM11 applicability to therapeutic regimens. Although TM11 was previously characterized as an inhibitor of HuR–RNA interactions, we cannot

exclude that some effects may reflect additional context-dependent mechanisms; thus, direct *in vivo* target-engagement studies will be required to confirm HuR dependence across all models.

Further validation of TM11 was performed in an *ex vivo* human skin system, the InflammSkin model, that recapitulates key features of psoriatic lesions, including inflammation, keratinocyte hyperproliferation and immune infiltrates. In this context, topical TM11 showed selective cytotoxicity toward psoriatic cells and potent anti-inflammatory activity. These findings highlight TM11 as a promising pre-clinical anti-psoriatic candidate. *Ex vivo* validation in the InflammSkin model confirmed dose-dependent effects of TM11 on inflammation and keratinocyte/immune cell activity, with improved tissue morphology. TM11 reduced Ki-67, IL17AF and IFN- γ levels, supporting its ability to counteract psoriasis-associated inflammatory pathways. Although the InflammSkin *ex vivo* model reproduces several key features of psoriatic inflammation, it does not fully recapitulate the complexity of patient lesions, including their chronic inflammatory environment, systemic influences, and interactions among immune, stromal, vascular, and neural components. Therefore, the findings obtained in this model should be considered supportive and require further validation in independent clinical studies.

However, beyond the intrinsic partial selective profile of the small molecule, the applicability of TM11 or its analogues depends on the biology of the target protein. HuR controls a wide and context-dependent RNA network in multiple cell types and numerous transcripts involved in inflammation, cell proliferation, survival, stress responses, and tissue homeostasis. Therefore, the systemic inhibition of the protein may produce unintended effects on immune function, epithelial integrity, vascular responses, or normal tissue repair. Since HuR inhibition may attenuate psoriasis-associated inflammatory mediators, its therapeutic targeting could also affect pathways beyond the markers here evaluated. Overall, our study demonstrates that HuR is a key regulator of the psoriatic phenotype and that its pharmacological modulation restores normal cellular function. Limitations include the small number of primary samples and the need for further studies to assess long-term effects and mechanisms in humans. Nonetheless, our findings support HuR as a promising therapeutic target for psoriasis.

Clinically, these findings provide a preclinical rationale for investigating topical HuR inhibition as a skin-directed therapeutic strategy. However, TM11 cannot yet be considered suitable for clinical use, and further studies are required to assess its formulation, skin penetration, pharmacokinetic and pharmacodynamic properties, target engagement, long-term safety, and efficacy in independent patient-derived lesions.

Conclusions

The data support the contribution of HuR to psoriasis-relevant inflammatory and proliferative pathways and that small molecules affecting the post-transcriptional function of the protein can reduce psoriasis-like phenotypes in preclinical models. Because our experiments were limited to short-term treatment, the persistence of the therapeutic response after treatment withdrawal remains unknown and should be addressed in longitudinal studies. In the context presented here, TM11 represents a promising preclinical candidate that warrants further investigation to support future therapeutic development. We acknowledge that additional target-engagement studies *in vivo* will be required to definitively demonstrate that all biological effects are, at least partially, mediated through HuR, and that the durability of the therapeutic response remains to be investigated in long-term preclinical studies including pharmacokinetic, toxicological, and selectivity profiles.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work. All authors have read and agreed to the published version of the manuscript.

Disclosure

Roberta Lotti reports patents from WO2024200287 - ANTI-FAS LIGAND (FASL) ANTIBODIES IN THE TREATMENT OF SJS/TEN DISEASES, WO2025196289 - NEW TARGET AFFINITY AND SPECIFICITY OF ANTI SFAS-L ANTIBODY, PC111, outside the submitted work. The authors report no other conflicts of interest in this work.

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