

Spatial proteomics identifies JAKi as treatment for a lethal skin disease

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Thierry M. Nordmann^{1,2,3}✉, Holly Anderton^{4,5}, Akito Hasegawa⁶, Lisa Schweizer¹, Peng Zhang⁷, Pia-Charlotte Stadler^{1,3}, Ankit Sinha¹, Andreas Metousis¹, Florian A. Rosenberger¹, Maximilian Zwiebel¹, Takashi K. Satoh³, Florian Anzengruber^{2,8}, Maximilian T. Strauss⁹, Maria C. Tanzer^{4,5,10}, Yuki Saito⁶, Ting Gong⁷, Marvin Thielert¹, Haruna Kimura⁶, Natasha Silke^{4,5}, Edwin H. Rodriguez¹, Gaetana Restivo², Hong Ha Nguyen⁶, Annette Gross¹¹, Laurence Feldmeyer¹², Lukas Joerg¹³, Mitchell P. Levesque², Peter J. Murray¹¹, Saskia Ingen-Housz-Oro¹⁴, Andreas Mund⁹, Riichiro Abe⁶, John Silke^{4,5}, Chao Ji^{7,15}✉, Lars E. French^{3,16}✉ & Matthias Mann^{1,9}✉

Toxic epidermal necrolysis (TEN) is a fatal drug-induced skin reaction triggered by common medications and is an emerging public health issue^{1–3}. Patients with TEN undergo severe and sudden epidermal detachment caused by keratinocyte cell death. Although molecular mechanisms that drive keratinocyte cell death have been proposed, the main drivers remain unknown, and there is no effective therapy for TEN^{4–6}. Here, to systematically map molecular changes that are associated with TEN and identify potential druggable targets, we utilized deep visual proteomics, which provides single-cell-based, cell-type-resolution proteomics^{7,8}. We analysed formalin-fixed, paraffin-embedded archived skin tissue biopsies of three types of cutaneous drug reactions with varying severity and quantified more than 5,000 proteins in keratinocytes and skin-infiltrating immune cells. This revealed a marked enrichment of type I and type II interferon signatures in the immune cell and keratinocyte compartment of patients with TEN, as well as phosphorylated STAT1 activation. Targeted inhibition with the pan-JAK inhibitor tofacitinib *in vitro* reduced keratinocyte-directed cytotoxicity. *In vivo* oral administration of tofacitinib, baricitinib or the JAK1-specific inhibitors abrocitinib or upadacitinib ameliorated clinical and histological disease severity in two distinct mouse models of TEN. Crucially, treatment with JAK inhibitors (JAKi) was safe and associated with rapid cutaneous re-epithelialization and recovery in seven patients with TEN. This study uncovers the JAK/STAT and interferon signalling pathways as key pathogenic drivers of TEN and demonstrates the potential of targeted JAKi as a curative therapy.

The skin is the organ that is most frequently affected by adverse drug reactions, and nearly 2% of such reactions are severe and life-threatening⁹. The spectrum of cutaneous adverse drug reactions (CADRs) ranges from self-resolving maculopapular rash (MPR) to rare and life-threatening conditions, such as drug reaction with eosinophilia and systemic symptoms (DRESS), Stevens–Johnson syndrome (SJS) and TEN⁵. TEN is characterized by fulminant epidermal detachment exceeding 30% of the body surface area, whereas

its milder form SJS–TEN overlap disease affects 10–30% of skin surface area. TEN is fatal in one-third of all cases. Despite diverse proposed pathogenic mechanisms^{10–24}, the main drivers of cytotoxicity remain unknown and consensus therapy of TEN remains primarily supportive care²⁵.

Spatial omics has recently emerged as a powerful strategy to obtain a global, molecular view of intact tissue samples^{26,27}. When applied at single-cell resolution, these methods can profile different cell

¹Department of Proteomics and Signal Transduction; Max Planck Institute of Biochemistry, Martinsried, Germany. ²Department of Dermatology, University Hospital Zurich, University of Zurich, Zurich, Switzerland. ³Department of Dermatology and Allergy, University Hospital, Ludwig Maximilian University (LMU) Munich, Munich, Germany. ⁴Inflammation division, Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria, Australia. ⁵Department of Medical Biology, University of Melbourne, Parkville, Victoria, Australia. ⁶Division of Dermatology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan. ⁷Department of Dermatology, The First Affiliated Hospital of Fujian Medical University, Fuzhou, China. ⁸Department of Internal Medicine, Division of Dermatology, Cantonal Hospital Graubünden, Chur, Switzerland. ⁹Proteomics Program, Novo Nordisk Foundation Center for Protein Research, University of Copenhagen, Faculty of Health and Medical Sciences, Copenhagen, Denmark. ¹⁰Advanced Technology and Biology division, Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria, Australia. ¹¹Immunoregulation Research Group, Max Planck Institute of Biochemistry, Martinsried, Germany. ¹²Department of Dermatology, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland. ¹³Division of Allergy and Clinical Immunology, Department of Pneumology, Allergy and Clinical Immunology, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland. ¹⁴Dermatology Department, AP-HP, Henri Mondor Hospital, Créteil, France. ¹⁵Key Laboratory of Skin Cancer of Fujian Higher Education Institutions, Fujian Medical University, Fuzhou, China. ¹⁶Dr. Philip Frost Department of Dermatology and Cutaneous Surgery, University of Miami Miller School of Medicine, Miami, FL, USA. ✉e-mail: nordmann@biochem.mpg.de; jichaofy@fjmu.edu.cn; Lars.French@med.uni-muenchen.de; mmann@biochem.mpg.de

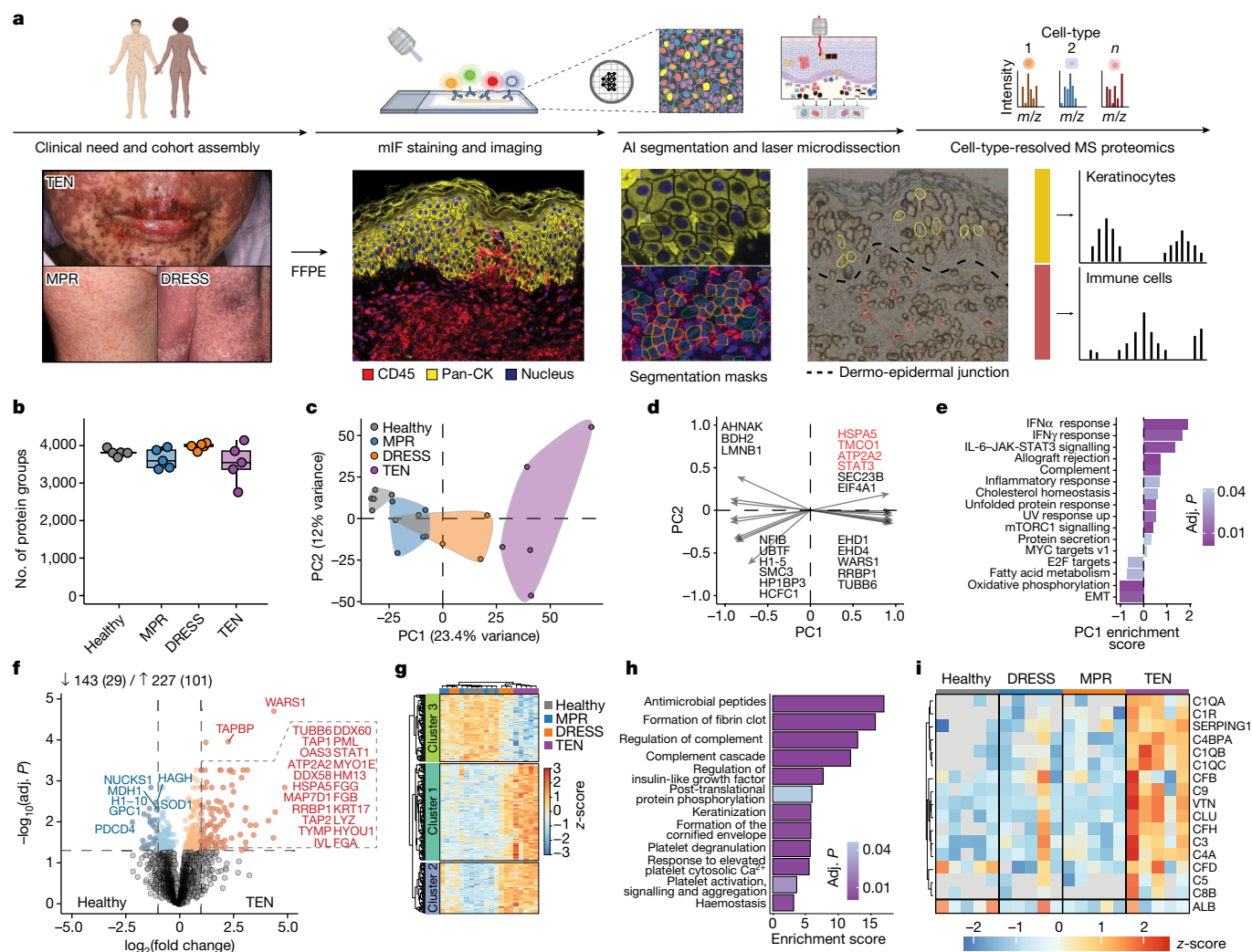


Fig. 1 | DVP workflow and cell-type-specific proteome of keratinocytes in cutaneous drug reactions. **a**, Top, schematic of the DVP workflow for cell-type-resolved proteomics in CADR (n = 21; 5 each for DRESS, MPR and healthy, and 6 for TEN). Bottom, representative images of each step, including excised keratinocytes (outlined in yellow in the image on the far right) and immune cells (outlined in red in the far right image) from FFPE tissue sections. Partially created with Biorender.com. AI, artificial intelligence; mIF, multicolour immunofluorescence; MS, mass spectrometry; pan-CK, pan-cytokeratin. **b**, Number of proteins identified in keratinocytes across cohorts. n = 5 individuals per cohort. **c–e**, Principal components analysis (**c**), main drivers (loadings) of PC1–PC2 separation (**d**) and gene set enrichment analysis (**e**) (GSEA) (MSigDB Hallmark) of PC1. Adj. P, adjusted P value; EMT, epithelial–mesenchymal transition. **f**, Differential expression of proteins in TEN and healthy keratinocytes. Coloured dots indicate significant DEPs, the dashed

vertical line indicates $\log_2$ -transformed fold change of ± 1 . Numbers indicate the total number of DEPs in each direction. n = 5 individuals per cohort. **g**, Hierarchical clustering of significant proteins by ANOVA. Colour indicates normalized intensity (z-score). n = 5 individuals per cohort. **h**, Overrepresentation analysis (Reactome) of ANOVA cluster 1, ordered by enrichment score. Colour indicates degree of significance. **i**, Semi-supervised heat map of complement factors (and albumin to exclude blood contamination) in the indicated conditions. Colour indicates normalized values (z-score). n = 5 individuals per cohort. **f**, Unpaired two-sided t-test. **g**, One-way ANOVA with post hoc Tukey's Honest significant difference (HSD). **e–h**, Benjamini–Hochberg correction for multiple comparisons (false discovery rate (FDR) < 0.05). In box plots the centre is the media, box edges delineate the 25th to 75th centiles and whiskers extend to furthest points within 1.5× the interquartile range.

types within their native environment. Although a variety of such technologies exist, translation into direct clinical benefit remains largely aspirational. Deep visual proteomics (DVP) provides spatial proteomics data of individual cell types from formalin-fixed paraffin-embedded (FFPE) archived biopsies. DVP combines high content imaging, artificial intelligence-guided cell segmentation and classification and laser microdissection of individual target cells coupled with ultra-sensitive mass spectrometry-based proteomics⁷. Here, we utilized DVP to study mechanisms of cutaneous drug reactions, investigate molecular features in the different CADR, and functionally validate targeted small-molecule inhibitors for rapid therapeutic intervention in TEN, the most severe CADR.

DVP workflow

We performed DVP on a retrospective cohort of lesional FFPE skin biopsies from patients with mild (MPR) and severe (TEN or DRESS) CADR, as well as healthy individuals (Fig. 1a). We stained FFPE tissue sections for CD45⁺ immune cells and pan-cytokeratin⁺ keratinocytes, followed by machine learning-based cell segmentation (Extended Data Fig. 1a,b). For each individual, contours per cell type were laser microdissected and combined separately to maintain patient and cell-type specificity, prior to peptide extraction and acquisition of mass spectrometry data (Fig. 1a). This identified around 5,000 unique proteins in each cell type across all participants, with distinct profiles for keratinocytes and immune cells (Extended Data Fig. 1c–f).

Proteome of lesional keratinocytes

To identify disease- and cell-type-specific molecular mechanisms, we first analysed the protein expression profiles of lesional keratinocytes from the CADR_s (Fig. 1b and Extended Data Fig. 2a–c). Keratinocyte proteomes clustered according to the type of drug reaction, with principal component 1 (PC1) separating TEN from other CADR_s and healthy individuals (Fig. 1c). PC1 was strongly associated with proteins involved in calcium homeostasis and cellular stress response (HSPA5, TMCO1 and ATP2A2; Fig. 1d). Of note, STAT3 was a main driver of separation, along with a dominant interferon signature (Fig. 1d,e). The number of differentially expressed proteins (DEPs) compared with cells from healthy individuals increased with the severity of the CADR_s (Extended Data Fig. 2d). DRESS keratinocytes expressed more than double the amount of the major histocompatibility complex class I proteins TAP1, TAP2, TAPBP and HLA-A, suggesting that keratinocytes participate actively in culprit-drug-associated antigen presentation (Extended Data Fig. 2e). Among significantly regulated proteins in keratinocytes from patients with TEN, many were involved in antimicrobial response, such as lysozyme and clusterin (Fig. 1f). WARS1, one of the most potently upregulated proteins, is strongly induced by inflammatory signals and especially IFN γ ²⁸, and has not been associated with TEN. Superoxide dismutase 1 (SOD1) levels were halved in the TEN keratinocytes, potentially exacerbating the oxidative stress cascade that is known to upregulate keratinocyte expression of Fas ligand, a key cytolytic molecule that is involved in keratinocyte apoptosis and epidermal detachment in TEN^{20,29}.

To identify protein signatures of keratinocytes that are uniquely associated with specific CADR_s, we examined hierarchical clusters of DEPs, which showed a clear segregation for TEN among three distinct protein clusters (Fig. 1g). The TEN cluster 1 was enriched for terms involved in the regulation of complement activation and blood clotting (Fig. 1h and Extended Data Fig. 2f,g). In accordance, nearly all complement factors were uniquely upregulated in keratinocytes of TEN and their presence may reflect the degree of dying keratinocytes in TEN (Fig. 1i and Extended Data Fig. 2h). Thus, proteomes of keratinocytes derived from DRESS and TEN reveal distinct disease-associated protein expression signatures.

Proteome of lesional immune cells

Cutaneous immune cell infiltration patterns differ markedly among CADR_s³⁰. To understand underlying molecular differences in immune cells, we assessed their proteomes (Extended Data Fig. 2i–l). They clustered by disease phenotype, but in contrast to keratinocytes, PC1 separated DRESS from other CADR_s (Fig. 2a and Extended Data Fig. 2m). It was prominently associated with targets of E2F and MYC, indicating a highly proliferative state (Extended Data Fig. 2o). PC1 and PC3 together revealed unique features of DRESS and TEN. In DRESS, multiple viral pathway terms were enriched, consistent with viral reactivation in its pathogenesis (Fig. 2b). By contrast, TEN had a dominant interferon signature with STAT1 as a main driver (Fig. 2b and Extended Data Fig. 2n). DRESS resulted in the largest number of DEPs compared with healthy immune cells, followed by TEN and MPR (Fig. 2c–e and Extended Data Fig. 2p). Few proteins were unique to specific CADR subsets (Extended Data Fig. 2q). One such protein was histone methylase EZH2, which was detectable only in immune cells from patients with DRESS (Fig. 2f). This proteomic observation was confirmed by immunofluorescence and by a report of almost exclusive transcription in a patient with therapy-resistant DRESS³¹ (Extended Data Fig. 2r,s). Among proteins previously associated with specific CADR_s, granzyme B has been described as a key mediator of cytotoxicity in TEN²⁴, however, it was present in similar amounts in non-cytotoxic DRESS (Fig. 2f).

Unsupervised hierarchical clustering analysis on DEPs in immune cells across all cohorts revealed three visually distinct protein clusters

with perfect disease alignment (Fig. 2g). Cluster 2, which predominantly represents DRESS, was enriched for DNA replication processes, highlighting the proliferative nature of infiltrating immune cells in this disease (Extended Data Fig. 2t). Its most prominent term (activation of ATR in response to replication stress) was dominated by the DNA replication factors RFC and MCM, establishing a potential link to the viral pathogenesis of DRESS³² (Extended Data Fig. 2u). Notably, cluster 1 contained proteins that are predominantly associated with type I and II interferon signalling (Fig. 2h). These proteins were markedly overexpressed in TEN, but showed only slightly increased expression in MPR and DRESS (Fig. 2g).

Prominent macrophage involvement in TEN

Although they indicated a role for the interferon pathway in TEN, our experiments did not identify the specific immune cell subtypes that contributed to this response. To this end, we harnessed a multiplexed data-independent acquisition (mDIA) workflow with a reference channel and the novel Astral mass analyser for the DVP workflow^{33,34}. This enabled proteomic investigation of CD163⁺ macrophages, CD4⁺ T helper cells and CD8⁺ cytotoxic T cells infiltrating the skin of patients with TEN and SJS–TEN overlap, despite their extreme sparsity (Fig. 3a). From only 20 cell equivalents per immune cell type and participant, we covered a median of 2,104 and a total of 5,302 proteins (Fig. 3b,c). Macrophages had the highest expression of proteins related to the interferon pathway, potentially reflecting the higher expression of IFN γ receptor (Fig. 3d). In particular, the amount of the mediator of interferon signalling STAT1 was consistently higher in macrophages than in CD4⁺ and CD8⁺ T cells from the same individuals (Fig. 3e). Compared with cytotoxic T cells, macrophages showed significant enrichment for myeloid-specific lineage markers (MND4 and MRC1) and interferon-driven proteins (STAT1 and GBP5; Fig. 3f).

Spatial proteome of epidermal detachment

We next investigated spatial differences between detached (blister roof) and attached (blister side) keratinocytes from within the same biopsy from only 20 cells per type and patient (Fig. 3g,h). Among three distinct clusters, the largest comprised the complement system and inflammation (Fig. 3i). These proteins were upregulated in both attached and detached keratinocytes of TEN, indicating that the inflammatory pathways driving TEN are already active in keratinocytes adjacent to blistered skin. Several proteins followed a linear relationship from healthy to attached to detached keratinocytes (Extended Data Fig. 3a). Proteomic differences between attached and detached keratinocytes were subtle, but sufficient to separate them according to spatial location and away from healthy keratinocytes (Fig. 3j). This separation was again driven by proteins of the complement and inflammation cascade (including MX1, C3, KRT6 and KRT16, among others; Fig. 3k–m). Furthermore, five proteins were associated with attached versus detached cells and may represent early proteomic changes before detachment (Fig. 3k–m).

The JAK/STAT pathway is highly active in TEN

Severe inflammatory disorders are characterized by altered cellular cross-talk and feedback loops that exacerbate the immune response³⁵; we investigated this using the cell-type-resolved proteome (Extended Data Fig. 3b). To further unravel such disease-specific feedback loops, we hypothesized that proteins that were strongly regulated in both keratinocytes and immune cells would identify therapeutically relevant pathways. Six such proteins (WARS1, STAT1, S100A9, LYZ, GBP1 and APOL2) were upregulated at least fourfold in keratinocytes and immune cells of TEN compared with healthy controls (Fig. 4a). We confirmed the

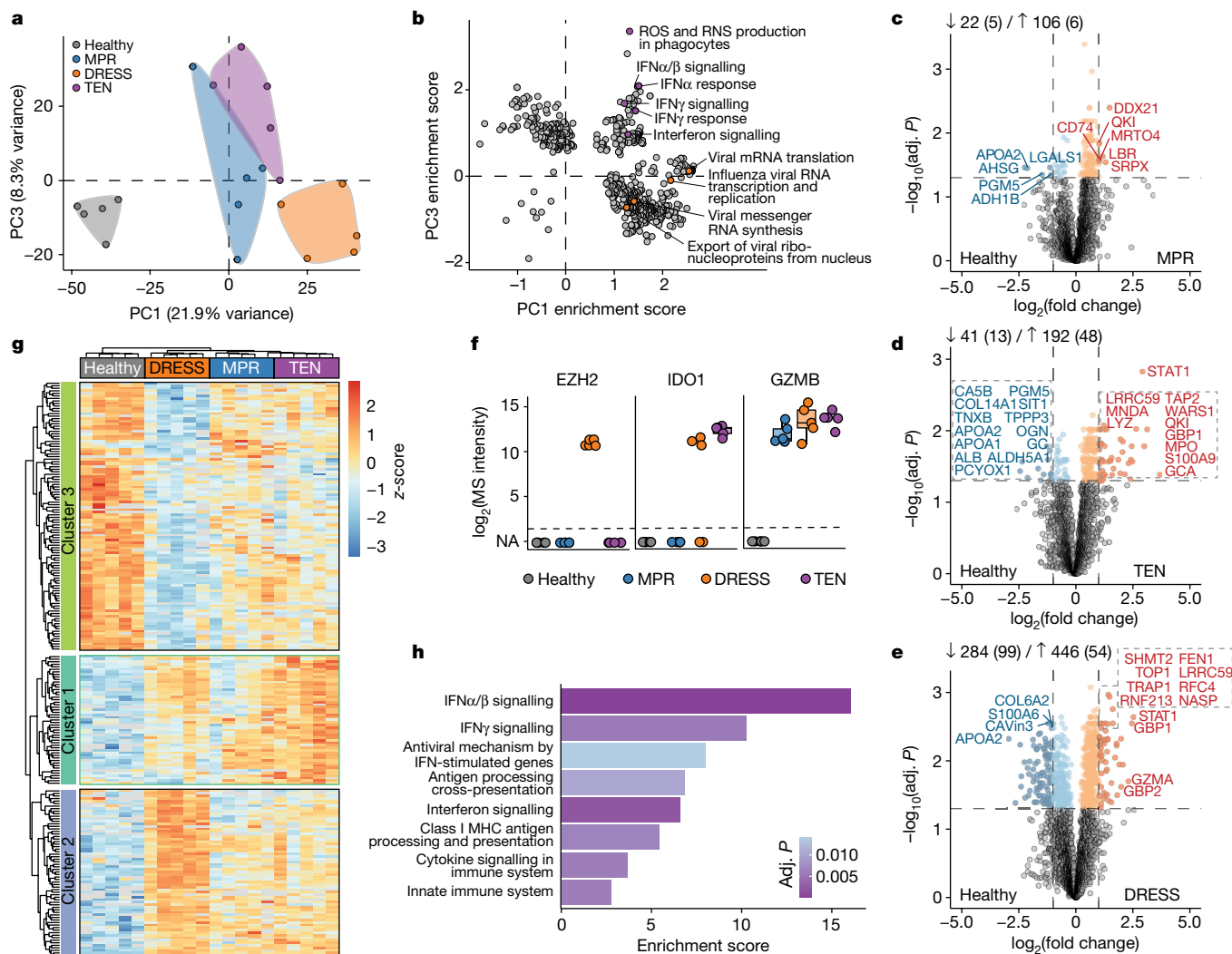


Fig. 2 | Cell-type-specific proteome of lesional immune cells. a, Principal component analysis. $n = 5$ individuals per cohort. **b**, Scatter plot of gene set enrichment scores (MsigDB Hallmark, Gene Ontology Biological Processes) present in both PC1 and PC3. Terms that include 'viral' or 'interferon' are highlighted. RNS, reactive nitrogen species; ROS, reactive oxygen species. **c–e**, DEPs between healthy and MPR (**c**), TEN (**d**) or DRESS (**e**) immune cells. Coloured dots indicate significant DEPs, dashed vertical lines indicate $\log_2$ -transformed fold change of ± 1 . Numbers indicate the total number of DEPs

in each direction. $n = 5$ individuals per cohort. **f**, Selected proteins with distinct intensities across cohorts. **g**, Hierarchical clustering of significant proteins by ANOVA. Colour indicates normalized intensities (z-score). $n = 5$ individuals per cohort. **h**, Overrepresentation analysis (Reactome) of ANOVA cluster 1, ordered by enrichment score. MHC, major histocompatibility complex. Colour indicates degree of significance. **c–e**, Unpaired two-sided t -test. **g**, One-way ANOVA with post hoc Tukey's HSD. **b–e, g, h**, Benjamini–Hochberg correction for multiple comparisons (FDR < 0.05).

proteomic findings of WARS1 using immunohistochemistry (Extended Data Fig. 4a–c). By contrast, DRESS had only one (STAT1) and MPR had no such overlapping proteins (Extended Data Fig. 3c). Notably, all six of these proteins are in the interferon pathway, responsible for signal transduction (via STAT1) or triggered by it^{36–40}. Interferons signal through the JAK/STAT pathway; we therefore examined molecules in this pathway (Fig. 4b and Extended Data Fig. 4d,e). STAT1 and STAT3 were upregulated in both immune cells and keratinocytes, whereas STAT2 was upregulated only in keratinocytes, and STAT5 was upregulated in immune cells. Most interferon-stimulated gene (ISG) products were either potentially upregulated, illustrated by a ninefold increase of GBP1, or only detectable in TEN—for example, GBP5.

Cytokines activate the JAK/STAT pathway; to study the activation of this pathway, we utilized targeted transcriptomics in an expanded cohort of patients with CADR (Extended Data Fig. 5a–c). Among the genes encoding cytokines that are known to signal through the JAK/STAT pathway, *IFNG* (encoding IFN γ) expression was the most potentially upregulated in TEN, exceeding the upregulation of genes such as *TNF*,

which is already known to be involved in TEN⁴¹ (Fig. 4c). Whereas targeted transcriptomics was not able to separate between the different CADRs, specific interrogation of JAKs and STATs confirmed broad upregulation of these molecules in TEN and SJS–TEN overlap (Fig. 4d and Extended Data Fig. 5d–i). Antibody-based visualization of STAT1 in FFPE tissue sections further confirmed that it was significantly upregulated and localized to the nucleus in keratinocytes and other cell types in TEN (Fig. 4e and Extended Data Fig. 3d). Notably, the degree of single-cell nuclear STAT1 upregulation in immune cells of TEN was similar to that in DRESS, in which therapeutic intervention with JAKi has proved successful, despite their differing histopathology, clinical presentation and disease course³¹. This upregulation of STAT1 suggests that severe CADRs share a common inflammatory pathway, potentially driven by T cell activation. To further validate STAT1 activation in TEN at the signalling level, we assessed the phosphorylation status of STAT1. Histological analysis revealed a profound degree of STAT1 phosphorylation in skin-infiltrating immune cells and keratinocytes of patients with TEN (Fig. 4f). We extended this

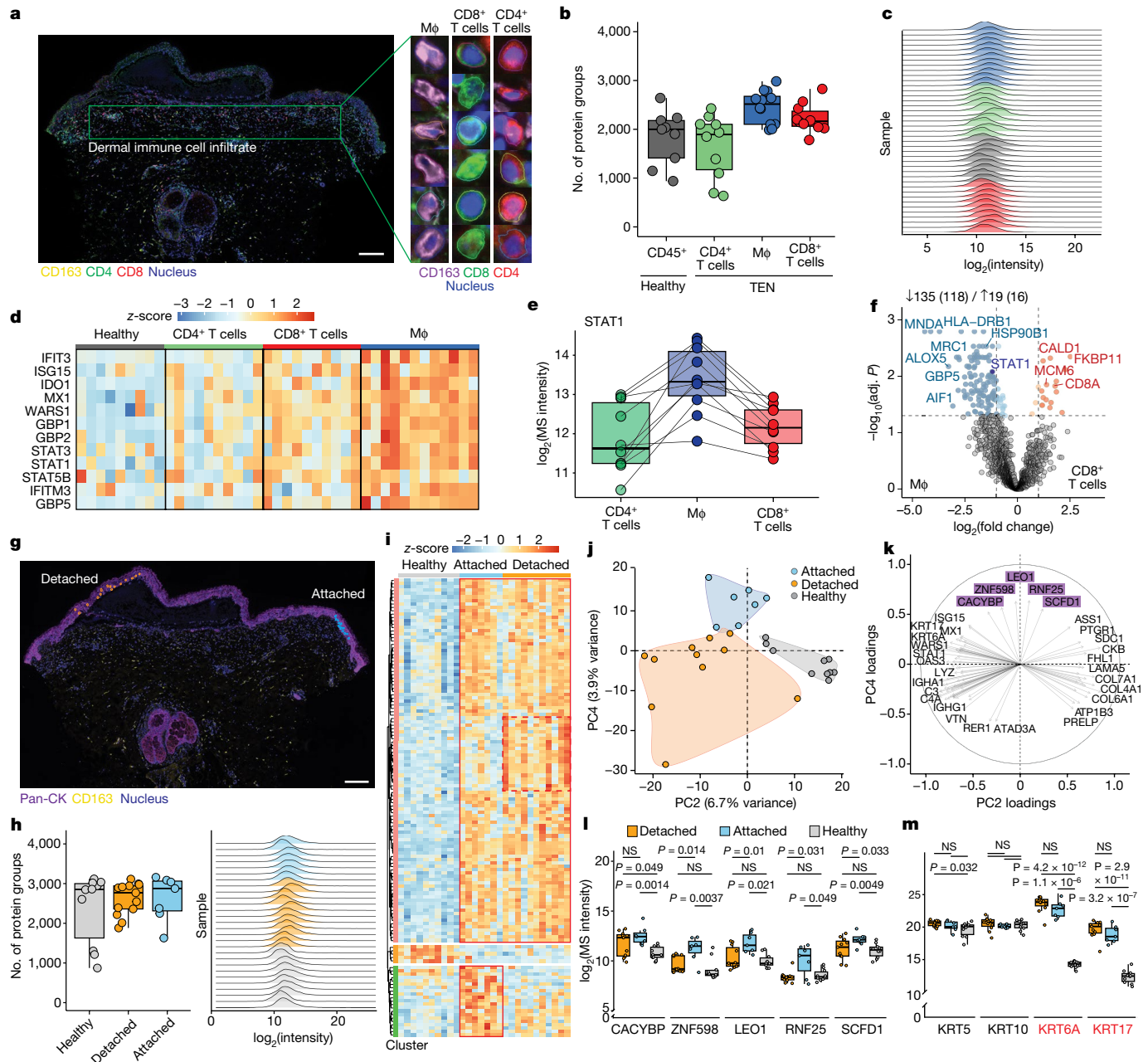


Fig. 3 | Spatial proteomic profiling of immune cell subtypes in TEN.

a, Left, immunofluorescence image of TEN with segmented CD163⁺ macrophages, CD4⁺ and CD8⁺ T cells. Right, representative single-cell images and segmentation contours. Mφ, macrophage. Scale bar, 200 μm. **b,c**, Number of identified proteins (**b**) and their intensity (**c**) across all samples, colour-coded by cell type. **d**, Heat map of interferon pathway proteins across samples grouped by cell type. Colour indicates normalized intensities (z-score). **e**, STAT1 intensity values across immune cell subtypes. Lines connect measurements from the same patient. **f**, DEPs between indicated cell types. Coloured dots represent significant DEPs, dashed lines indicate log₂ transformed fold change of ±1. Numbers indicate the total number of DEPs in each direction. **g**, Immunofluorescence of a TEN sample with segmented keratinocytes from detached (orange; blister roof) and attached (blue; adjacent to blister) regions. Scale bar, 200 μm. **h**, Number of proteins identified in keratinocytes (left) and

intensity distribution (right) across samples. **i**, Semi-supervised hierarchical clustering of significant proteins by ANOVA with fold change greater than 1. Colour indicates normalized intensities (z-score). Borders of visually distinct subclusters are outlined in red. **j,k**, Principal component analysis (**j**) and main drivers of PC2 and PC4 (**k**). **l,m**, Proteins of interest that vary between detached versus attached cells (**l**; highlighted in **k**) and across cohorts (**m**). Alarmins are labelled in red. **b–d**, $n = 12$ macrophage, 10 CD4⁺ cell, 10 CD8⁺ cell and 9 healthy samples from $n = 12$ disease-affected and 7 healthy individuals. **e,f**, paired samples for each cell type in $n = 10$ individuals. **h–m**, $n = 7$ attached, 11 detached and 9 healthy samples from $n = 11$ disease-affected and 7 healthy individuals. **a,g**, Immunofluorescence staining was performed on all samples, representative images are shown. **f**, Paired two-sided t -test. **i**, One-way ANOVA with post hoc Tukey's HSD. **f,i**, Benjamini–Hochberg correction for multiple comparisons (FDR < 0.05). NS, not significant.

observation using global phosphoproteome analysis, measuring more than 16,000 class I phosphosites in an independent cohort of TEN, which further confirmed our findings (Extended Data Fig. 6). Collectively, these results demonstrate that activation of the JAK/STAT pathway is a key driver of TEN.

Targeting JAK/STAT in models of TEN

To investigate the clinical significance of our experimental findings, we developed a novel in vitro model to authentically replicate key aspects of cutaneous drug reactions⁴² (Fig. 5a). In this autologous co-culture

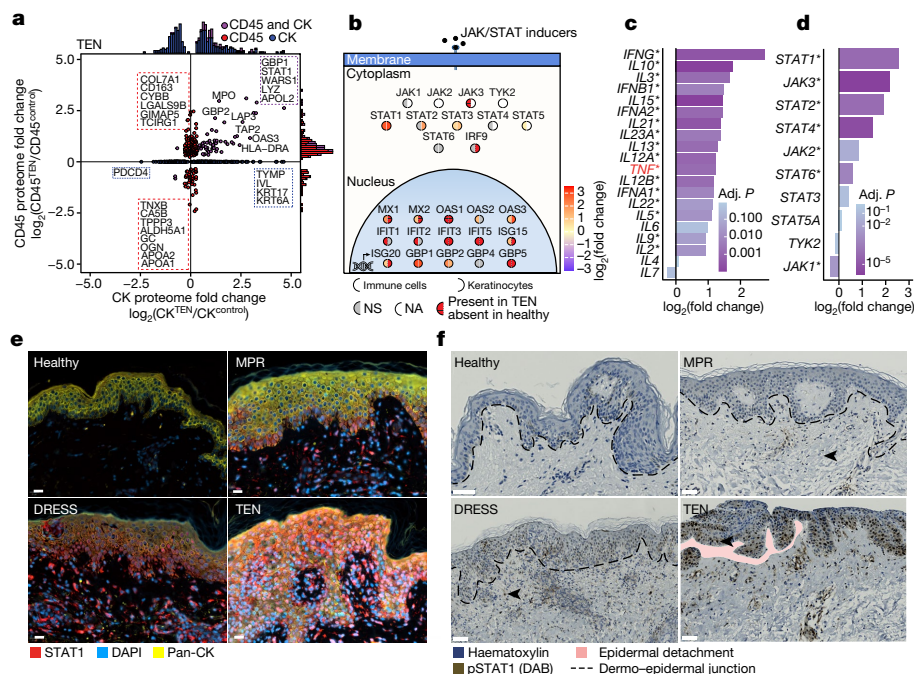


Fig. 4 | The JAK/STAT pathway is potentially activated in TEN. **a**, DEPs in keratinocytes (CK) and immune (CD45) cells in patients with TEN compared with healthy participants. Each dot represents a protein and colour indicates cell type. Histograms above and to the right display the distribution of fold changes for the indicated cell type. *n* = 5 individuals per cohort and cell type. **b**, JAK/STAT pathway proteins in immune cells (left semicircle) and keratinocytes (right semicircle). Colour represents fold change in TEN compared with healthy donors; red with horizontal black lines indicates proteins identified in TEN only. *n* = 5 individuals per cohort and cell type. NA, data not available.

c, d, Differentially expressed mRNA transcripts in TEN compared with healthy tissue for selected cytokines (**c**) and components of the JAK/STAT pathway (**d**). Asterisks indicate significant differences between groups. *n* = 10 individuals per cohort. TNF is highlighted. **e, f**, Representative images of STAT1 and pan-cytokeratin (**e**) and phosphorylated STAT1 (pSTAT1) (**f**) staining in tissue sections across cohorts. *n* = 5 individuals per cohort, representative images shown. Scale bars: 20 μm (**e**), 50 μm (**f**). **a–d**, Unpaired two-sided *t*-test. **a, c, d**, Benjamini–Hochberg correction for multiple comparisons (FDR < 0.05).

model, activated peripheral blood mononuclear cells (PBMCs) efficiently killed keratinocytes within 72 h, and this effect was inhibited in a dose-dependent manner by the pan-JAK inhibitor tofacitinib (Fig. 5b).

Encouraged by these results, we next evaluated the efficacy of oral JAKi in established mouse models of TEN^{22,43–47}. Subcutaneous injection of a small-molecule antagonist of inhibitor of apoptosis (IAP) proteins (second mitochondria-derived activator of caspases (smac) mimetic) induced TEN-like cutaneous inflammation, epidermal detachment and histological features of TEN (Fig. 5c–f). Oral treatment with the pan-JAK inhibitor tofacitinib or the JAK1 and JAK2 inhibitor baricitinib successfully reduced macroscopic disease severity on days 1 and 3 of treatment (Fig. 5d and Extended Data Fig. 7a, b). Mirroring human TEN, a strong increase in cutaneous phosphorylated STAT1 signal was detectable, which was significantly reduced upon JAKi treatment (Fig. 5e and Extended Data Fig. 7c). Similarly, average dermal thickness was restored to normal levels (Fig. 5f and Extended Data Fig. 7d). Further, JAKi decreased keratinocyte cell death (TUNEL⁺ cleaved caspase-3⁺ cells; Fig. 5e and Extended Data Fig. 7e) and reduced immune cell infiltration (CD45⁺ cells; Extended Data Fig. 7c). Of note, epidermal recovery was accelerated in JAKi-treated mice, as demonstrated by the rapid clinical recovery and upregulated expression of the proliferation marker Ki67 in keratinocytes of the basal layer (Extended Data Fig. 7c, f). This argues against detrimental effects of JAKi treatment on wound healing in the context of TEN.

As JAK1 is the primary mediator of interferon signalling, we tested whether inhibition of JAK1 alone would be sufficient to treat the disease. Indeed, treatment with the JAK1 inhibitors abrocitinib or upadacitinib significantly reduced disease severity (Fig. 5g and Extended Data Fig. 7g, h). We also explored a therapeutic model in which JAKi was administered after disease induction (Fig. 5h). This similarly

significantly reduced clinical severity, lesion size and time of recovery (Fig. 5h–j).

We further tested the efficacy of baricitinib in an established humanized mouse model of TEN, in which we injected PBMCs from a TEN survivor intravenously into immunodeficient mice (Fig. 5k). Severe ocular conjunctivitis and cell death of conjunctival epithelium reminiscent of TEN occurred at day 14 following daily oral administration of the causative drug²² (Fig. 5l–n and Extended Data Fig. 8a–c). Baricitinib treatment significantly reduced ocular conjunctivitis and cell death of conjunctival epithelium compared with vehicle treatment (Fig. 5l–n and Extended Data Fig. 8a–d). Together, the in vitro and in vivo data clearly demonstrate the efficacy of JAKi in preclinical models of TEN.

JAKi treatment resolves TEN

On the basis of our compelling preclinical data and the urgent clinical need in this devastating disease, we treated seven patients with TEN or SJS–TEN overlap with JAKi off-label. One such individual was a 59-year-old man who was receiving carboplatin, etoposide and the PD1 antagonist serplulimab for small cell lung cancer (Fig. 6). After the third cycle of cancer treatment, he developed TEN with epidermal detachment affecting 35% of his body surface and a severity-of-illness score (SCORTEN⁴⁸) of 4, indicating a predicted mortality rate of 58.3%. Epidermal detachment had progressed despite high-dose intravenous methylprednisolone, prompting us to initiate JAK1 inhibitor rescue therapy with abrocitinib, which was recently approved for treatment of atopic dermatitis⁴⁹. The disease stopped progressing within 2 days and re-epithelialization was visible within four days, reaching near-completeness after 16 days (95%), after which the patient was discharged following full recovery from TEN. All seven patients

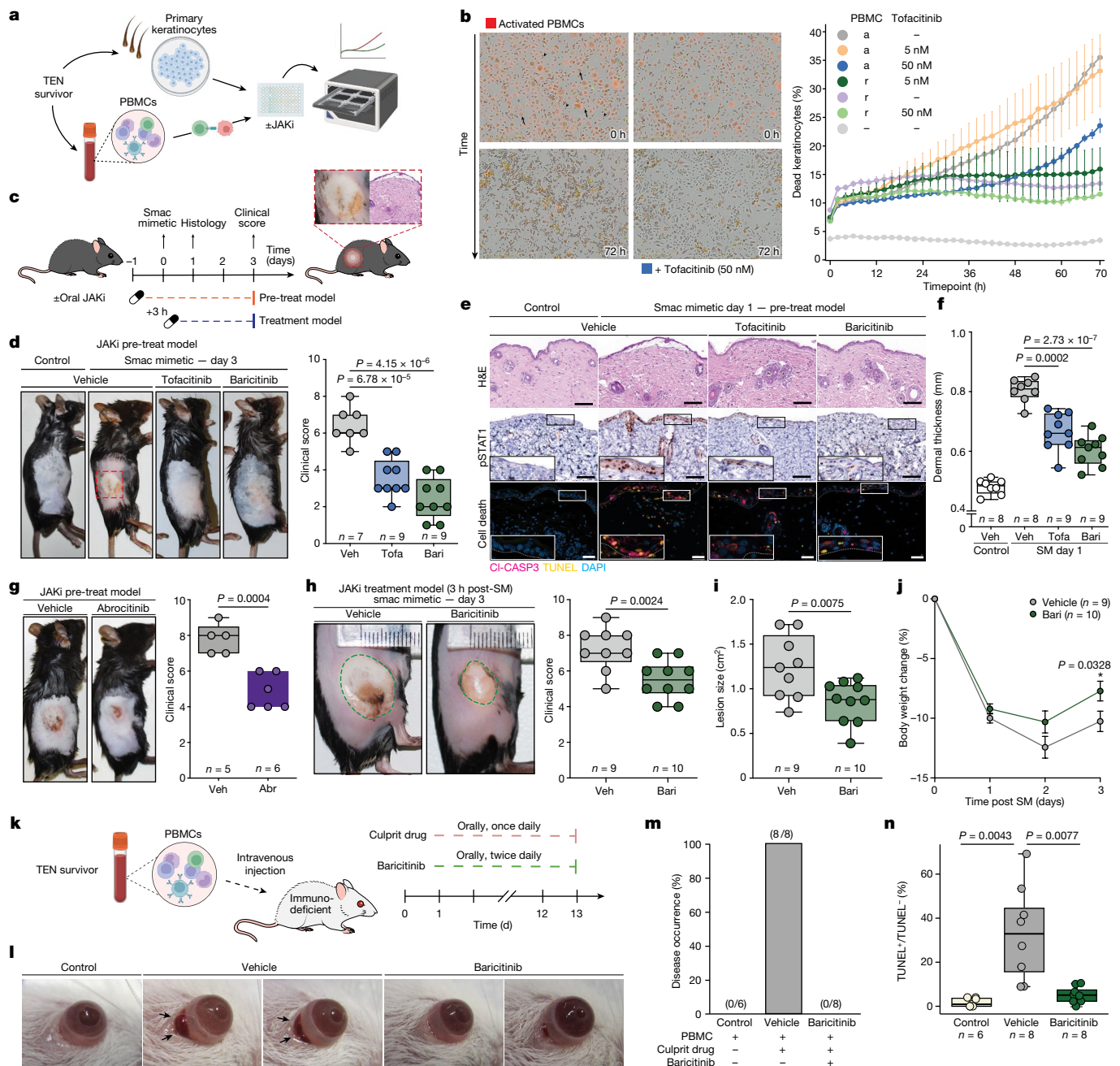


Fig. 5 | JAK/STAT inhibition reduces severity of TEN in vitro and in vivo. **a, b**, Schematic of live-cell imaging assay (**a**) with representative images of labelled keratinocytes (arrows) and unlabelled PBMCs (arrowhead) in co-culture for the indicated duration, with or without 50 nM tofacitinib to measure cytotoxicity over time (**b**). r, resting; a, activated. *n* = 6 biological replicates per condition, 4 field of views per well. Data are mean ± s.e.m. **c**, Schematic of oral JAKi treatment in the smac-mimetic-induced model of TEN. Oral JAKi (tofacitinib (tofa), 30 mg kg⁻¹; baricitinib (bari), 10 mg kg⁻¹; abrocitinib (abr), 20 mg kg⁻¹) was started one day before ('pre-treat model') or 3 h after ('treatment model') subcutaneous injection of smac mimetic. Outcome assessment was performed on day 1 (histology) and day 3 (clinical score). **d–j**, Clinical assessment (**d, g, h**), histology (**e**), dermal thickness (**f**; 15 measurements per mouse),

lesion size (**i**) and change in body weight (**j**) in the pre-treat (**d–g**) and treatment models (**h–j**). **e**, Insets show magnification of indicated area. Scale bars, 100 μm. Number of mice per cohort as indicated; each data point represents 1 mouse; 2 independent experiments; representative images are shown. **j**, Weight change data are mean ± s.e.m. CI-CASP3, cleaved caspase-3; H&E, haematoxylin and eosin; SM, smac mimetic; veh, vehicle. **k**, Schematic of oral JAKi treatment in the humanized mouse model of TEN. **l–n**, Ocular reaction (**l**, black arrows), disease occurrence (**m**) and percentage of subepithelial cell death (**n**) in the corresponding cohort following daily culprit-drug administration with or without oral baricitinib (10 mg kg⁻¹). Number of mice as indicated. **d, f–j**, One-tailed unpaired Welch's *t*-test. **n**, Unpaired two-sided *t*-test. **a, c, k**, Partially created with Biorender.com.

treated with JAKi survived at day 30 with no side effects (Extended Data Figs. 9a–d and 10a–c). Notably, cutaneous levels of phosphorylated STAT1 were markedly reduced in all seven patients following JAKi treatment (Extended Data Figs. 9e and 10d). These clinical data show the potential of JAKi or pan-JAK inhibition for the treatment of TEN.

Discussion

Here we used DVP to characterize the spatial proteome of keratinocytes and immune cells in FFPE tissue sections from patients with drug-induced skin reactions, quantifying up to around 5,000 proteins

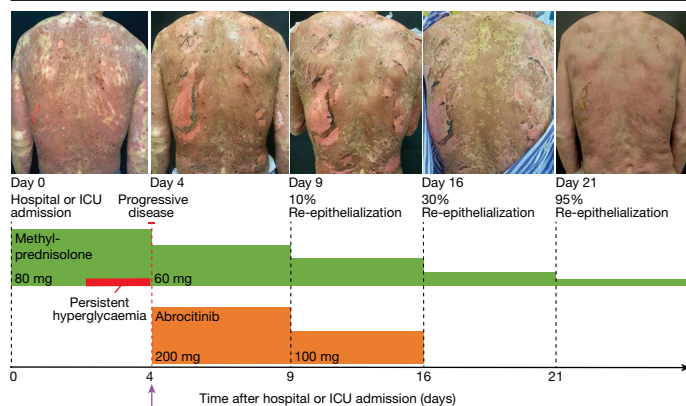


Fig. 6 | Beneficial effect of JAK/STAT inhibition in patients with TEN.

Disease course in a patient with TEN (SCORTEN 4) associated with cancer treatment. Disease progression was observed during high-dose intravenous methylprednisolone treatment and the patient developed persistent hyperglycaemia. JAKi rescue therapy with abrocitinib was initiated on day 4, resulting in visible cessation of progression within 48 h and initial re-epithelialization within 4 days. Top, photographs of the back of the patient, showing the degree of re-epithelialization at the indicated timepoints after hospital admission. Bottom, Treatment schedule. Arrow marks start of abrocitinib treatment.

per cell type. These proteomic analyses across all major types of cutaneous drug reactions with cell-type resolution provided insights into the molecular mechanism of each CADR that we leveraged for therapeutic interventions.

Proteomics analysis revealed an in-depth view of the biological molecules involved in CADR. For instance, the histone methyltransferase EZH2 was detected exclusively in skin-infiltrating immune cells in DRESS. EZH2 has a key role in epigenetic gene regulation by modifying histone H3 at lysine 27 (H3K27me3). In addition, E2F pathway enrichment detected in our proteomic dataset of immune cells in DRESS correlates with overexpression of EZH2, which functions as a downstream effector in this signalling cascade⁵⁰. These findings suggest a role of EZH2, and by extension epigenetic modifications, in the pathogenesis of DRESS that would be of interest for future studies.

The key finding of our study is the marked upregulation of the JAK/STAT pathway in TEN, which is not observed in less severe CADR. Our results pinpoint this signalling pathway as the main driver of cutaneous inflammation, keratinocyte cytotoxicity and epidermal detachment, with macrophages showing the greatest inflammatory response among tissue immune cells. Whereas activation of STAT1 and STAT2 specifically indicates interferon signalling, STAT3 can be activated by cytokines other than interferons. Our spatial analysis revealed pronounced inflammation in keratinocytes, both at the site of epidermal detachment and immediately adjacent to it. This observation aligns closely with clinical findings showing that light pressure near an existing blister induces further blistering ('indirect Nikolsky sign'). It also suggests an active role for keratinocytes in contributing to the skin-directed cytotoxic immune response. Upregulation of JAK/STAT signalling in both immune cells and keratinocytes may explain the extensive tissue damage in TEN through a potentially detrimental self-amplifying inflammatory cascade⁵¹. Further study of macrophages in this context could clarify the role of the innate immune system in TEN. Finally, we comprehensively investigated the effect of JAKi in vitro in a novel cell culture model and in vivo in two independent mouse models, all of which demonstrated consistent therapeutic benefits.

Having identified the JAK/STAT pathway as an actionable therapeutic target in TEN using spatial proteomics, and given our extensive preclinical evidence, we treated seven patients with TEN or SJS–TEN overlap syndrome, including three who were previously unresponsive

to high-dose systemic corticosteroids. All seven responded well to JAKi and were discharged in good health after treatment. These data pave the way for a clinical trial of early JAKi therapy in TEN with the potential to change the outcome of this lethal adverse reaction to common drugs.

Owing to the immediate risk of mortality associated with TEN and the short duration of specific therapy required, the rapid onset of action and benefits of short-term JAKi therapy would be substantial, if they are confirmed in larger clinical trials. Given the strong association of TEN with immune checkpoint inhibitor therapy⁵² (odds ratio = 4.33), JAKi may also prove useful in such cases by improving outcome and enabling rapid reintroduction of oncological treatment^{53,54}.

Our study is a successful example of directly linking emerging cell-type-resolved spatial omics technologies, especially spatial proteomics, with a new treatment modality that is of benefit to patients. We envision that similar approaches could be transformative in a broad set of inflammatory or oncological conditions by helping to identify key druggable targets and select treatments more precisely.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41586-024-08061-0>.

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Methods

Patient biopsies

Skin tissue biopsies were obtained during routine histopathological diagnostic procedures. In the standard DVP proteomic cohort, patients with TEN had a minimal affected skin area of 30%. In the other cohorts, patients with SJS–TEN overlap were included (minimal affected skin >10%). All patients with DRESS had a RegiSCORE of 6–7 (definite diagnosis). In addition to the typical clinical features, the lymphocyte transformation test or skin tests were positive in all patients with MPR. The number of samples per condition is indicated in the relevant figures and corresponding legends. Each sample represents one individual. Exceptions are the phosphoproteomic cohort where two distinct biopsies from each of the four healthy individuals yielded eight samples, and the mDIA–DVP cohort, where two distinct biopsies from three out of seven patients resulted in a total of ten samples. Histopathological diagnosis was re-validated in all cases by a board-certified dermatopathologist using a fresh H&E-stained tissue section. Baseline characteristics of all cohorts are described in Supplementary Tables 1–5 and were statistically evaluated using ANOVA for numeric variables (age) and a Chi-squared test of independence for categorical variables (sex). Retrospective analysis was performed with informed consent and ethical approval in place (Munich: 22-0342, 22-0343; Zurich: BASEC: Req-2021-00226 and 2017-00494; Fujian: MRCTA, ECFAH of FMU[2023]400). All experiments were performed in accordance with the Declaration of Helsinki.

Treatment of patients with JAKi

Regular assessment and vigilant surveillance of the vital signs, haematological parameters and coagulation markers was performed throughout treatment. Patients with active infections were excluded. All patients were additionally treated according to current best supportive care guidelines²⁵. Oral abrocitinib was administered at a regimen of 200 mg daily for 5–7 days, and tapered to 100 mg daily for 5–7 days. Similarly, oral tofacitinib was administered at a dosage of 10 mg daily for 5–7 days, and then tapered to 5 mg daily for 5–7 days. In cancer patients with high SCORTEN, a reduction in JAKi dosage was initiated after observing a certain degree of re-epithelialization. Treatment was approved by the local ethics committee and the institutional review board of the First Affiliated Hospital of Fujian Medical University (Fujian: MRCTA, ECFAH of FMU[2023]400), and the patient provided written informed consent.

Mice

Smac-mimetic induced TEN mouse model. As previously published⁴³, C57BL/6 mice were injected subcutaneously with 100 µl of 1 mg ml⁻¹ smac mimetic (CompA) (TetraLogic Pharmaceuticals) or vehicle (12% Captisol). Weight dependent doses of JAKi (10 mg kg⁻¹ baricitinib, 30 mg kg⁻¹ tofacitinib, 20 mg kg⁻¹ abrocitinib or 10 mg kg⁻¹ upadacitinib; Selleckchem) or vehicle (20% Captisol; 50 µl; Selleckchem) was administered by oral gavage twice daily starting one day before (pre-treat model) or 3 h after (treatment model) subcutaneous injection of smac mimetic. Mice were euthanized on day one or day three after smac-mimetic injection, the site was photographed, scored, and samples collected for ex-vivo analysis. We used a four-point ordinal scale (0–4) to assess the three clinical associated macroscopic criteria of TEN (Oedema, rubor and epidermal disruption (Nikolsky sign/lesion severity)) at day one or three after smac-mimetic injection. They were assessed and scored as 0 (not present), 1 (mild), 2 (moderate) or 3 (severe) and the scores were combined for an overall clinical score. *P* values were calculated using the unpaired *t*-test with Welch's correction. The corresponding data can be found in Supplementary Table 13. All pre-treatment experiments were performed using male mice only, and the treatment model was performed using female mice. Mice were maintained at the appropriate biosafety level under constant

temperature and humidity conditions with a 12 h light cycle. Animals were allowed food and water ad libitum. Experiments were approved by the local ethics committee (WEHI AEC 2022.009).

Humanized mouse model of TEN. Immunocompromised NOD/Shi-scid, *Il2rg*^{null} (NOG) mice at 6 weeks of age were included. PBMCs (2×10^6) from an individual who had recovered from SJS–TEN 1 year earlier were injected intravenously into the NOG mice, followed by oral administration of the causative drugs (acetaminophen, 1.5 mg per 100 µl) at day one and once daily thereafter. The dosage used in this model was based on mg per kg body weight, converted from the normal adult human dose. Baricitinib (10 mg kg⁻¹) or vehicle (20% Captisol; 50 µl) was administered by oral gavage twice daily from day one. Body weight, ocular reactions or cutaneous changes were assessed daily. On day 14, the degree of conjunctivitis was evaluated after ocular dislocation under general anaesthesia and scored (0, no conjunctivitis; 1, mild conjunctivitis; 2, severe conjunctivitis). The ratio of the number of TUNEL-positive cells to the total conjunctival cell count in a 400× magnified field was calculated for all mice. The corresponding data can be found in Supplementary Table 14. The patients received no systemic glucocorticoids at the time of PBMCs collection. Mice were maintained at the appropriate biosafety level under constant temperature and humidity conditions with a 12 h light cycle. Animals were allowed food and water ad libitum. Experiments were approved by the local ethics committee and the institutional review board of Niigata University, and the patient provided written informed consent.

Membrane slide tissue mounting, immunofluorescence staining, imaging and laser microdissection

Two-micrometre PEN membrane slides (MicroDissect GmbH) were exposed to UV light (254 nm) for one hour and then coated with Vectabond (Vector laboratories; SP-1800-7) according to the manufacturers protocol. Three-micrometre-thick tissue sections of each FFPE block were mounted on the pretreated membrane slides and dried overnight at 37 °C. Immunofluorescence was performed according to our previously optimized protocol for membrane slides⁵⁵. In brief, slides were heated to 56 °C for 20 min and immediately deparaffinized and rehydrated (Xylo 2× 2 min, 100% ethanol 2× 1 min, 90% ethanol 2× 1 min, 75% ethanol 2× 1 min, 30% ethanol 2× 1 min, ddH₂O 2× 1 min). Slides were then transferred to prewarmed glycerol-supplemented Antigen Retrieval buffer⁵⁵ (DAKO pH9 S2367 + 10% Glycerol) at 88 °C for 20 min, followed by a 20 min cooldown at room temperature. Slides were then washed in water and blocked with 5% BSA/PBS for 30 min, followed by overnight primary antibody incubation at 4 °C (CD45 or cytokeratin) or 90 min at room temperature (CD4, CD8 and CD163; cytokeratin and CD163) in a humid staining chamber. After washing in PBS, secondary antibodies were incubated for 1 h at room temperature. All primary and secondary antibodies used reported in the Supplementary Table 6. After washing in PBS, sections were counterstained with SYTOX Green (1:400, Invitrogen S7020) for 10 min, washed again and coverslipped using Slowfade Diamond Antifade Mountant (Invitrogen, S36963). Sections were imaged on a Zeiss AxioScan at 20× magnification with a tile overlap of 10%. At the corresponding excitation wavelength at 100% laser intensity, except for 50% for the 493 nm channel. Illumination time was adapted to the optimal spectral properties. Up to 5 z-stacks at an interval of 1.25 µm were acquired. Multi-scene images were then split into single scenes, z-stacks were combined to a single plane by extended depth of focus where applicable (variance method, standard settings) and stitched, using the proprietary Zeiss Zen Imaging software. Images were imported as.czi files into the Biological Image Analysis Software (BIAS) with the packaged import tool⁷. Single-cell segmentation for Figs. 1 and 2 was performed using deep neural network on the basis of pan-cytokeratin for keratinocytes and CD45 for immune cells at 1.0 input spatial scaling, 50% detection confidence and 30% contour confidence. Segmentation of images for Fig. 3 was performed using a

custom-trained Cellpose model⁵⁶. Only contours between 30 μm^2 and 200 μm^2 were taken into consideration. After removal of duplicates at tile-overlapping regions, a supervised machine learning approach was used to remove false identifications and overlapping cell types. Contours were exported together with three calibration points that were chosen at characteristic tissue positions. Contour outlines were simplified by removing 99% of data points. For keratinocytes, only maximally every second shape was (randomly) chosen in order to prevent membrane instability while cutting multiple adjacent cells. Contour outlines were imported after reference point alignment, and shapes were cut by laser microdissection with the LMD7 (Leica) with a 63 $\times$ objective in a semi-automated manner at the following settings: power 57, aperture 1–2, speed 23, middle pulse count 1, final pulse –3, head current 46–53%, pulse frequency 2,600, offset 210.

Sample preparation, LC–MS/MS (TimsTOF) and raw data analysis for standard DVP

For proteomic analysis of CD45⁺ immune cells and pan-CK⁺ keratinocytes, we collected 700 contours for each keratinocyte sample and 1,000 for each immune cell sample. For each participant, biological duplicates were collected when possible, from the same slide at different regions. Dissected contours for each participant and cell type were collected directly into the same underlying low-binding 384-well plate (Eppendorf 0030129547), with immune cells in the top half of the plate and keratinocytes in the bottom half, leaving an empty well between each sample.

Sample preparation. Semi-automated sample preparation and digestion was performed in the collection plate using a Bravo pipetting robot (Agilent) as previously described⁷. For this, wells were washed with 28 μl of 100% acetonitrile and dried in a SpeedVac for 20 min at 45 °C. The contours were then resuspended in 4 μl of 60 mM triethylammonium bicarbonate (TEAB, Sigma) in mass spectrometry-grade H₂O, sealed with two adhesive foils and heated at 95 °C for 60 min in a 384-well thermal cycler (Eppendorf). 1 μl of 60% acetonitrile in 60 mM TEAB (12.5% final acetonitrile concentration) was added, followed by heating at 75 °C for 60 min in a 384-well thermal cycler. Samples were then predigested with 4 ng of trypsin for 4 h followed by overnight digestion with 6 ng LysC in a 384-well thermal cycler at 37 °C. After 18 h the reaction was quenched with 1.5 μl 6% trifluoroacetic acid (1% final concentration). Samples were then manually transferred to single PCR tubes, dried in a SpeedVac for approx. 60 min at 45 °C and stored at –20 °C.

LC–MS/MS analysis. Peptides were resuspended in 4.1 μl MS loading buffer (2% acetonitrile (v/v) + 0.1% trifluoroacetic acid (v/v) in mass spectrometry-grade H₂O) immediately prior to measurement. All samples were stratified into cell type and replicates, and further randomized using the rand function in Excel. An EASY nanoLC 1200 (Thermo Fisher Scientific) was coupled to a timsTOF SCP mass spectrometer (Bruker) via a nanoelectrospray ion source (Captive spray source, Bruker). Peptides were separated on a 50 cm in-house packed HPLC column (75 μm inner diameter, 1.9 μm ReproSil-Pur C18-AQ silica beads (Dr. Maisch)), which was heated to 60 °C by an in-house manufactured oven. A linear gradient of 120 min was ramped at a constant flow rate of 300 nl min^{–1} from 3 to 30% buffer B in 95 min, followed by an increase to 60% for 5 min, washed at 95% buffer B for 10 min and re-equilibration for 10 min at 5% buffer B (buffer A: 0.1% formic acid and 99.9% ddH₂O; buffer B: 0.1% formic acid, 80% acetonitrile and 19.9% ddH₂O). The timsTOF SCP mass spectrometer was operated in dia-PASEF mode using the standard 16 dia-PASEF scan acquisition scheme⁵⁷ with 4 IM steps per dia-PASEF scan, covering a m/z range from 400 to 1,200 and ion mobility of 0.6 to 1.6 V cm^{–2}. All other settings were standard as described in ref. 58.

Raw data analysis with DIA-NN. A library-free search was performed, using a DL predicted spectral library in DIA-NN (v1.8.0)⁵⁹. Uniprot human

databases UP000005640_9606 and UP000005640_9606_additional were used. Mass spectrometry raw files from immune cells and keratinocytes were searched separately in DIA-NN, apart from data shown in Extended Data Fig. 1d–f and Supplementary Fig. 1 (ligand–receptor interaction). Methionine oxidation was defined as variable modification and missed cleavages were limited to one. The precursor charge ranged from 2 to 4, precursor mass range was set to 300 to 1,800, and peptide length from 7 to 35. Mass and MS1 accuracy were set to 15, based on prior estimation. Isotopologues, and MBR were enabled and neural network classifier was set to single-pass mod. The ‘–relaxed-prot-inf’ function was activated using the command line for more conservative protein grouping. Proteins were inferred from FASTA. Library generation was set as ‘Smart profiling’, ‘RT-dependent’ as cross-run normalization and ‘Robust LC (high precision)’ as quantification strategy.

Sample preparation, LC–MS/MS (Orbitrap Astral) and raw data analysis for mDIA–DVP

For proteomic analysis of different immune cell subsets (CD163⁺ macrophages, CD8⁺ and CD4⁺ T cells) and spatially resolved keratinocytes, we collected ~35 contours for each keratinocyte sample and ~100 for each immune cell sample (equivalent to 5,000 μm^2) into an underlying 384-well plate and further processed.

Sample preparation. Dimethyl labelling of reference channel ($\Delta 0$) and samples (laser microdissected macrophages, CD8⁺ T cells, CD4⁺ T cells or keratinocytes; $\Delta 8$) was performed as previously described³³. For the reference channel, an equal amount (measured prior using the tryptophan assay) of digested bulk TEN tissue (5 μm FFPE section), tonsil tissue (5 μm FFPE section) and primary keratinocytes (cell culture) was combined and diluted to 1 ng μl^{-1} . Each sample was loaded together with 10 ng of the reference channel on an Evotip Pure (Evosep).

LC–MS/MS analysis. Peptides were separated by the Evosep One liquid chromatography system and the standardized whisper 40SPD connected to the Orbitrap Astral (Thermo) using the EASY-Spray source (Thermo). Spray voltage was set to 1,900 V. The 15 cm column with an inner diameter of 75 μm containing 1.7 μm C18 beads (Aurora Elite TS, IonOpticks) was heated to 50 °C. All samples on the Orbitrap Astral were acquired in data-independent acquisition (DIA) mode at an MS1 resolution of 240,000 and scan range of 380–980 m/z . Normalized AGC target was 500%. Isolation windows of 8 Th scanning with a maximum injection time of 16 ms were recorded. Isolated ions were fragmented at an HCD collision energy of 25%. All samples were acquired with field asymmetric ion mobility spectrometry (FAIMS) enabled at a compensation voltage of –40 V. Gas flow was reduced to 2.5 l min^{–1}.

Raw data analysis. The DIA-NN search was performed using a custom spectral library and postprocessed with Refquant as described previously in detail³³. The ‘Channel.Q.Value’ cut-off was likewise set to 0.15. The spectral library was created by gas-fractionation of the bulk samples used for the reference channel (10 fractions, 50 ng each, same mass spectrometry method as above). The output of Refquant was then used for further bioinformatic analysis³³.

Phosphoproteomic workflow, LC–MS/MS (Orbitrap Astral) and data analysis

Frozen tissue samples stored in RNA Later at –80 °C were transferred with as little RNA Later as possible to single tissueTUBE TT1 (Covaris) and immediately pulverized using a chilled hammer. Five-hundred microlitres of PreOmics Lysis Buffer was then added, resuspended and transferred to a 1.5 ml Eppendorf tube, followed by boiling at 95 °C for 60 min and protein concentration measurement (Tryptophan

assay). To remove any potential remnants of RNA Later that could inhibit tryptic digestion, proteins were transferred to a new 1.5 ml Eppendorf tube using Resyn magnetic beads (Hydroxyl modified, 20 $\mu\text{g } \mu\text{l}^{-1}$ stock) at a protein to bead ratio of 1:5 and according to the manufacturers protocol. Samples and beads were resuspended in 200 μl 60 mM TEAB followed by overnight tryptic digestion (LysC and Trypsin at a protein to enzyme ratio of 1:100) at 37 °C and 1,200 rpm. Next day, enzyme activity was quenched using trifluoroacetic acid (1% final concentration) and peptide concentration was again measured. Twenty micrograms from each sample was transferred to a low-bind 96-well Eppendorf plate followed by fully automated phospho enrichment on the Agilent AssayMAP Bravo using High-Capacity Fe(III)-NTA cartridges and elution in 500 mM ammonium hydrogen phosphate (pH 4). Enriched peptides were then loaded on Evotip Pure (Evosep). Peptides were eluted into the mass spectrometer using the Evosep One liquid chromatography system and the standardized whisper 40SPD. A 15 cm column with an inner diameter of 75 μm containing 1.7 μm C18 beads (IonOpticks), heated at 50 °C and connected to the Orbitrap Astral (Thermo) using the EASY-Spray source (Thermo). Spray voltage was set to 1,900 V. All samples on the Orbitrap Astral were acquired in DIA mode at an MS1 Orbitrap resolution of 120,000 and scan range of 380–1,180 m/z . Normalized AGC target was 500%. Isolation windows of 3 Th scanning with a maximum injection time of 5 ms were recorded. Isolated ions were fragmented at an HCD collision energy of 25%. All samples were acquired with field asymmetric ion mobility spectrometry (FAIMS) enabled at a compensation voltage of –40 V. Gas flow was reduced to 2.5 l min^{-1} . MS raw files were processed by the Spectronaut software version 17 in directDIA mode (Biognosys) against the human FASTA database (21,039 entries, 2019). Peptides between 7–52 with a FDR less than 1% at the peptide and protein levels were taken into account. Variable modification were defined as serine/threonine/tyrosine phosphorylation, N-terminal acetylation and methionine oxidations. Cysteine carbamidomethylation was defined as a fixed modification. Enzyme specificity was set as Trypsin/P with a maximum of two missed cleavages. The resulting report was then exported and analysed in R. Class I phosphosites were defined as phosphosites with a localization probability > 75% or otherwise defined as non-class I. Functional analysis was performed on the imputed dataset. Redundancies in data, caused by multiplicity, were removed by retaining the ones that have higher fold change and higher –log (p.value), as previously described⁶⁰. *t*-test was performed using the base R function, and the resulting *P* values were adjusted for multiple testing (Benjamini–Hochberg, FDR < 0.05). Gene-centric enrichment analysis was performed using the clusterProfiler package, accessing the MsigDB C5 database. Specifically, overrepresentation analysis was performed on proteins of the corresponding phosphosites proteins upregulated by fold change >0.5, while gene set enrichment analysis was performed on all proteins, including the corresponding fold change. The GOplot package was implemented to generate the circosplot⁶¹. Phosphosite-specific analysis was performed using the ssGSEA2 package accessing the PTMSigDB and with the default settings⁶².

Preparation and subsequent transcriptomic analysis of FFPE tissue sections

Ten-micrometre FFPE human skin sections were cut from each sample and deparaffinized using ROTICLEAR (Carl Roth). RNA was isolated using RecoverAll Total Nucleic Acid Isolation Kit (AM1975, Thermo Fisher Scientific) and concentrated using the Savant SpeedVac DNA 130 Integrated Vacuum Concentrator (Thermo Fisher Scientific). Concentration was measured using Nanodrop One/OneC (Thermo Fisher Scientific). One-hundred and fifty micrograms of RNA from each sample was hybridized overnight with unique probe pairs for 624 genes, including 594 genes from the nCounter Immunology Panel, 15 internal reference genes, and 30 user defined genes using the Panel

Plus option (NanoString Technologies). Data were collected using the nCounter SPRINT Profiler (NanoString Technologies). Normalization was performed using nanostringr v0.4.2.

Bioinformatics data analysis

Bioinformatics data analysis was carried out using the R statistical computing environment version 4.0.2. For subsequent statistical analysis, protein or gene intensities were $\log_2$ -transformed and average intensities of the biological replicates were computed for each participant and cell type where applicable. Box plots show the median (centre line) with interquartile range of 25% to 75%, whiskers extend to furthest data points within 1.5 $\times$ the interquartile range from the box boundaries. For the ANOVA, the R stats package was used; variables were zero-centred and scaled prior to analysis. Subsequent multiple testing correction (adj. *P*) was conducted using the Benjamini–Hochberg method with a FDR cut-off of 5%; post hoc Tukey's HSDs were calculated at a confidence level of 0.95. For differential expression, the non-paired *t*-test was performed using the rstatix package with standard settings, followed by multiple comparison correction (Benjamini–Hochberg method, FDR 5%) from the stats package. Overrepresentation analyses (ORA) was performed with WebGestalt 2019 (ref. 63) and GSEA with the clusterProfiler package (MsigDB H, C2 or C5 database) within the R environment. The heat maps were generated using the pheatmap package, with the data being zero-centred and scaled before display. Only proteins that exhibited an identification rate of 70% or higher in at least one cohort for each cell type independently were included for functional analysis of the proteomic data. Imputation was performed per individual based on a normal distribution (width of 0.3, downshift of 1.8) for principal component analyses, functional analysis of the phosphoproteomic dataset and the mDIA–DVP data. All other statistical analyses were performed on non-imputed data. For the comparison between TEN/DRESS/MPR and healthy for selected proteins in both cell types (shown in Fig. 4b and Extended Data Fig. 3c), a non-paired *t*-test was performed and the $\log_2$ fold change value of TEN over healthy was visualized in the figure using a consistent range and colour-coding for both cell types. The fill colour of the (semi-circles) was manually adjusted using the 'eyedropper' tool in Adobe illustrator.

Cell–cell interaction analysis

Cell–cell interaction analysis was performed in Python. CK and CD45 files were processed together and loaded via Pandas. Data was filtered to have fewer than 30% missing values on protein level. Missing data points were imputed using scikit-learn's IterativeImputer with a RandomForestRegressor. Significant genes were identified by performing one-way-anova using Scipy's stats package and a *P* value cut-off of 0.01. Significant genes were identified by performing a one-way ANOVA using the stats package from SciPy, applying a *P* value cut-off of 0.01. Results were further adjusted by applying a pairwise Tukey test from the Pingouin package, requiring a minimum of five significant comparisons at a Tukey cut-off of 0.05. Protein expression data were averaged by condition. Potential interactions were retrieved from OmniPath (version 0.16.4) and matched by gene name. Interaction plots were generated using CircosPlots. For each potential interaction, the intensity difference was calculated in each direction by subtracting the target intensity from the source intensity. To identify disease-specific interactions, the difference in healthy conditions was subtracted from the difference in disease conditions. The plots were created using the D3Blocks package, and the resulting HTML files were automatically converted to figures using Selenium, CairoSVG, and pdf2image. Only interactions with a delta greater than zero were displayed in the plots. Bubble plots were created using Matplotlib. For each interaction, a matrix was constructed to compare all differences across disease conditions and cell types and plotted as scatterplot. Colour indicates the difference according to a colormap and bubble size reflects the magnitude. Bubble plots for the selected

protein interactions across different cell types and conditions are provided in the Supplementary Fig. 1.

Generation of hair follicle-derived keratinocytes for co-culture assay

Hair follicles were placed on thin-Matrigel (Corning, 354234) coated T25 flasks and covered with 10 μ l of 1.8 mg ml⁻¹ Matrigel to prevent hair follicle detachment. Following 1 h incubation at 37 °C/5% CO₂, 1 ml of conditioned mouse embryonic fibroblast medium, from irradiated J2-3T3 cells, supplemented with 10 μ M Y-27632 (CST 13624) and 10 ng ml⁻¹ FGF2 (Cell Guidance GFH146-50), was carefully added. Medium was changed every 48 h. When outgrowth of keratinocytes became visible, medium was aspirated and replaced with 2 ml Keratinocyte Expansion Medium DermaCult (Stemcell 100-0500, with supplement), and changed every 48 h. When confluent, keratinocytes were detached using TrypLE Express Enzyme (Gibco 12604039) and defined trypsin inhibitor (Gibco R007100) and replated on collagen I (Corning, 354236) coated plates for further expansion.

Isolation of PBMCs for co-culture assay

PBMCs were isolated using Ficol according to standard protocol. Cells were frozen in liquid nitrogen until further usage. Keratinocyte expansion and PBMC isolation were approved by the local ethics committee with written informed consent (Munich: 23-0544).

Live-cell imaging co-culture assay

Human PBMCs (2×10^5 per well per 200 μ l) were stimulated in U-shape 96-well plates in the presence or absence of CD3/28 (Gibco Dynabeads, 11161D) at a ratio of 1:1, in X-VIVO 15 (Lonza, 881026) for 5 days at 37 °C/5% CO₂. On day 4 of PBMC stimulation, hair follicle-derived keratinocytes from the same person were plated on collagen I-coated 96-well plates at a density of 8×10^3 per well. On day 5 of PBMC stimulation, keratinocytes were fluorescently labelled (Celltracker Red; Invitrogen C34552) according to the manufacturer's recommendation. Activated or non-activated ('resting') PBMCs were then added to the keratinocytes at a ratio of 1:1, after magnetic removal of CD3/28 beads where necessary. Tofacitinib (CST, 14703) or control (DMSO) was added in the indicated concentration as well as Celltox Green (Promega, G8731, 1:8,000 final dilution) to reach a final volume of 200 μ l per well. Every 2 h images were acquired using a live-cell analysis system (Incucyte Sx3, Sartorius) for 72 h at 37 °C/5% CO₂. Quantification of dead keratinocytes (Celltox*Celltracker*/total Celltracker*) was performed using the proprietary analysis software (Incucyte, Sartorius).

Immunostaining on regular glass slides

Three-micrometre FFPE tissue sections were mounted on regular Superfrost-Plus glass slides and exposed to high temperatures for improved tissue adherence. Subsequently, sections were deparaffinized and subjected to heat-induced antigen retrieval and blocking solution. The primary antibody was applied to the tissue section for one hour at room temperature or overnight at 4 °C (pSTAT1 and cleaved caspase-3). For immunofluorescence staining, a species-specific secondary antibody coupled to a fluorophore was added for an additional hour, followed by a nuclear counterstain. Alternatively, immunohistochemical staining was conducted using horseradish peroxidase (HRP) coupled secondary antibodies, or signal amplified using biotinylated secondary antibodies followed by labelling with and Avidin-Biotin Conjugate (ABC) HRP Kit. HRP-labelled samples were then colour-developed with 3,3'-diaminobenzidine (DAB) and haematoxylin counterstain. TUNEL staining was performed according to the manufacturer's instruction prior to application of the anti-cleaved caspase-3 primary antibody for co-staining. H&E staining was performed according to standard protocol. Images were acquired on a Zeiss Axioscan 7 or a DP72 microscope and cellSens imaging software (Olympus), or entire slides were scanned with a 3D Histech Virtual Slide Microscope (Olympus), viewed

using CaseCenter (3D Histech) and image captured on a MacBook Pro 13-inch retina display (Ki67, CD45 and H&E). Reagents used are reported in Supplementary Table 6.

Quantification of STAT1 expression in immunofluorescence images

Three-micrometre-thick FFPE tissue sections of all participants from the proteomic cohort were subjected to multiplex immunofluorescence staining for STAT1, pan-cytokeratin and Hoechst. All stainings were then uniformly acquired as.czi files using the Zeiss Axioscan 7. Single-cell segmentation was performed within QuPath (v0.4.1) using the standard nuclear segmentation algorithm. After segmentation, all available features were extracted, including staining intensities for all channels and nuclei. Segmentation and feature extraction was automated with QuPath using the script editor, to ensure equality. In R (v4.0.2) 5000 nuclei and their corresponding measurements were then randomly chosen per individual and merged into a single file, excluding samples with fewer than 5,000 cells ($n = 1$). To account for staining variation between slides, mean STAT1 fluorescence intensity was divided by its own mean Hoechst intensity on a per cell basis. Statistical significance between cohorts was determined by a unpaired two-sided *t*-test on mean normalized STAT1 values. Data are visualized using ggplot2.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Mass spectrometry and transcriptomic data of this study have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD044477. Data tables of all proteomic and transcriptomic measurements performed throughout this manuscript are provided in Supplementary Tables 7–12. Clinical scores, average dermal thickness, lesion size and percentage weight change measurements performed in the smac-mimetic mouse model are provided in Supplementary Table 13. Quantification of subepithelial cell death in the humanized mouse model is provided in Supplementary Table 14. Immunofluorescence images of skin sections from DRESS, TEN, MPR and healthy cohorts stained for CD45 and pan-cytokeratin have been uploaded to BioImage Archive (<https://www.ebi.ac.uk/bioimage-archive/>; accession number S-BSST1430).

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Article

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Author contributions T.M.N., L.E.F. and M.M. conceived the project. T.M.N., H.A., A.H., R.A., J.S., L.E.F. and M.M. designed the experimental framework. T.M.N. performed the proteomic experiments with help from L.S., A. Metousis, M.Z., P.-C.S. and M.T. T.M.N. performed the statistical analysis of the proteomic data with help from M.T.S. and M.Z. H.A., M.C.T., N.S. and J.S. performed and analysed the smac-mimetic mouse model experiments. A.H., Y.S., H.K., H.H.N. and R.A. performed and analysed the humanized mouse model experiments. P.Z., T.G. and C.J. performed and analysed JAKi in patients. T.M.N., H.A., J.S., L.E.F. and M.M. designed the figures with help from F.A.R., P.-C.S., M.Z., M.T. and L.S. T.M.N. performed the live-cell imaging experiments and analyses, with substantial experimental guidance from A.G. and P.J.M. Transcriptomics experiments and analyses were performed by P.-C.S. and T.K.S., and L.S., A.S., A. Metousis, F.A.R., M.Z., M.T., E.H.R. and A. Mund provided intellectual input and helped interpret data. F.A., G.R., M.P.L., L.F., L.J. and S.I.-H.-O. provided intellectual input and

helped construct the cohorts. T.M.N., L.E.F. and M.M. wrote the manuscript. All authors read, revised and approved the manuscript.

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Competing interests A patent for treatment of TEN, SJS–TEN and SJS with JAKi has been filed (US patent: provisional application no. 63/531,677; European patent: provisional application no. 23 190 686.8, with T.M.N., L.E.F. and M.M. as inventors). The patent application belongs to the Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V. M.M. is an indirect investor in Evosep Biosystems. The authors declare no other competing interests.

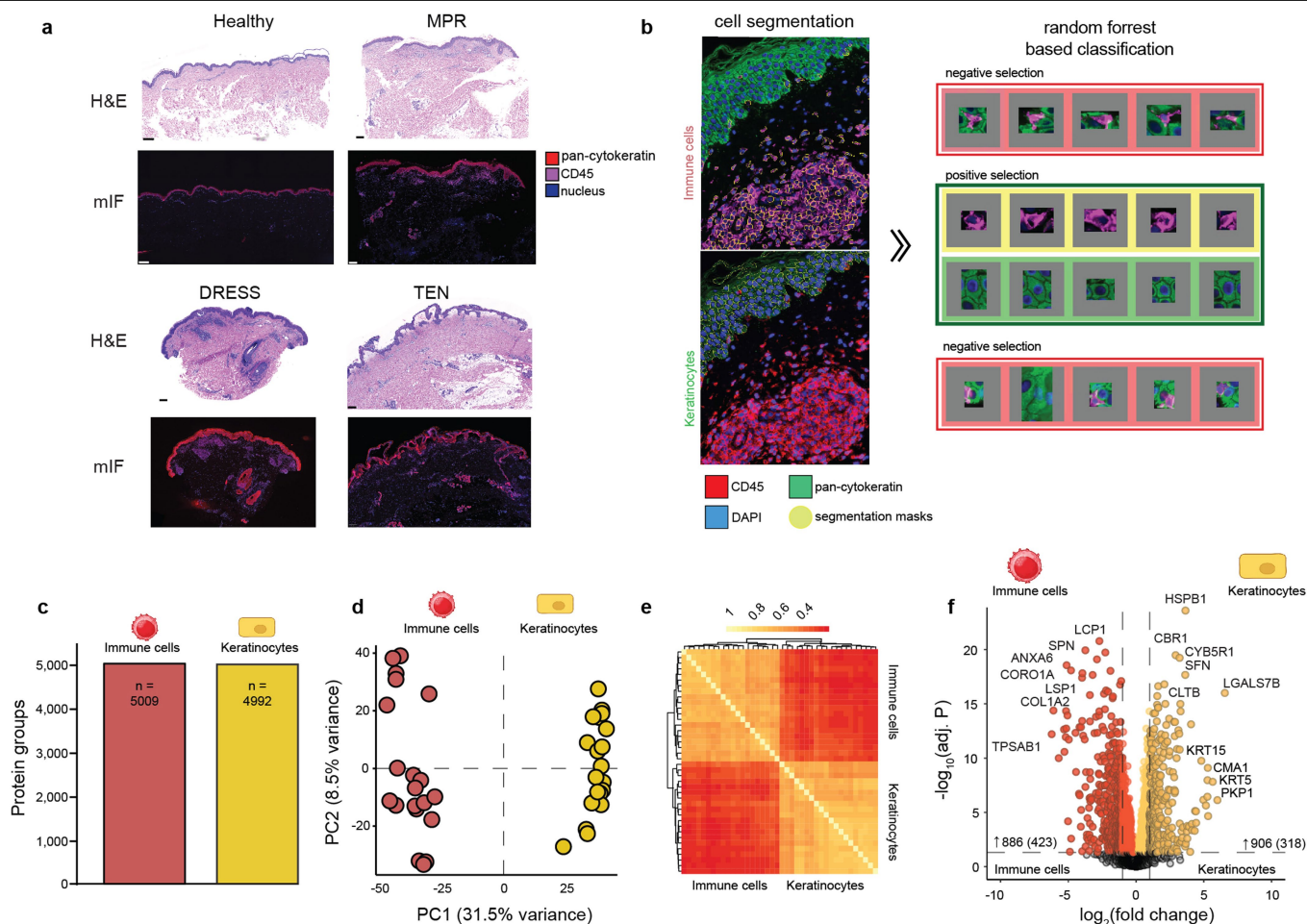
Additional information

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41586-024-08061-0>.

Correspondence and requests for materials should be addressed to Thierry M. Nordmann, Chao Ji, Lars E. French or Matthias Mann.

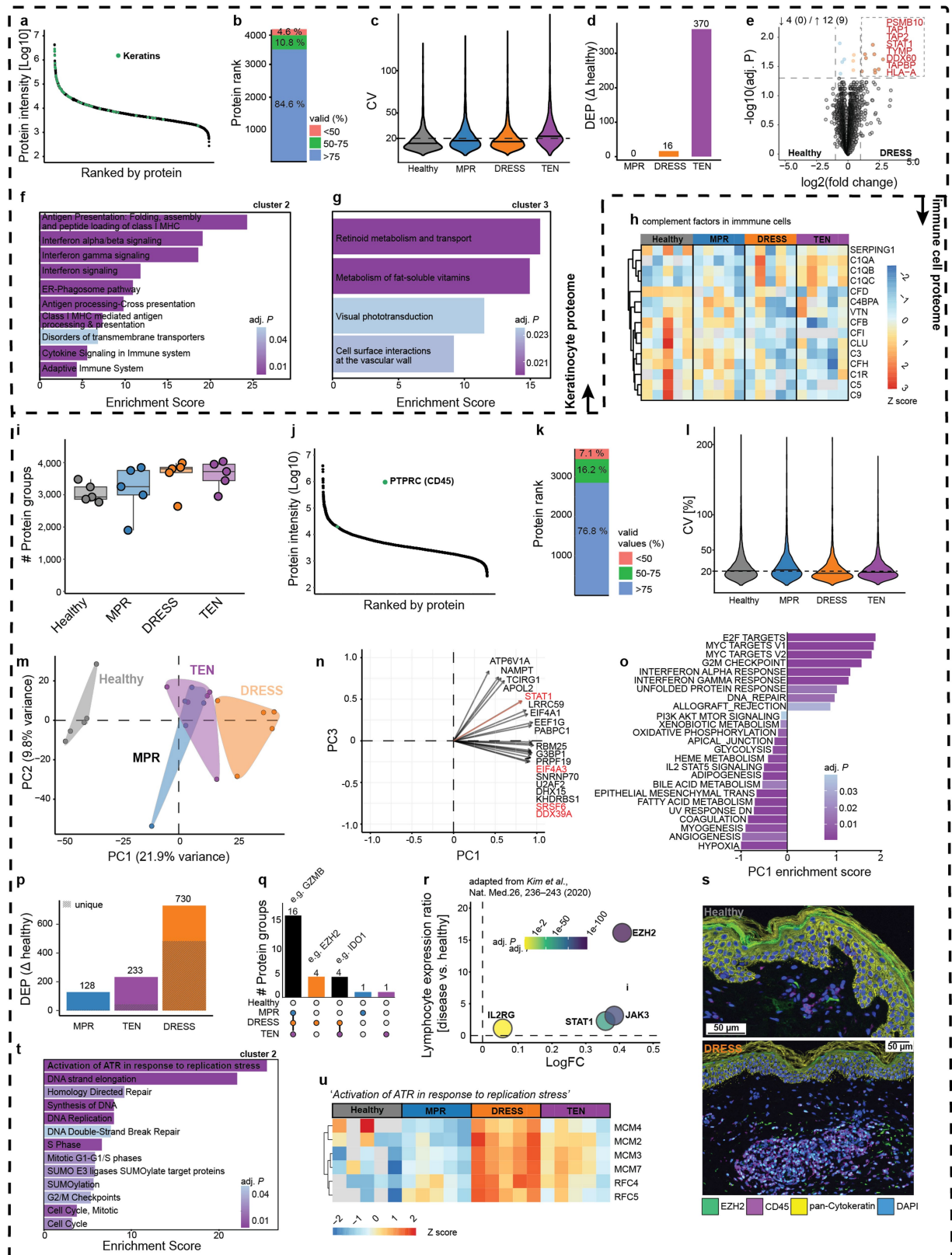
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Extended Data Fig. 1 | Deep Visual Proteomics workflow. **a**, Side-by-side H&E and multicolor immunofluorescence (mIF) of 3 μm FFPE tissue sections across all cohorts, representative images shown. Scale bar, 200 μm . **b**, Machine learning based image segmentation was followed by random-forest based classification to ensure no overlapping contours ('negative selection') were microdissected, yielding pure and cell-type specific contours ('positive selection'). **c,d**, Identification of unique protein groups (**c**) and principal component analysis (**d**) across all patients in the indicated cell type. Each dot

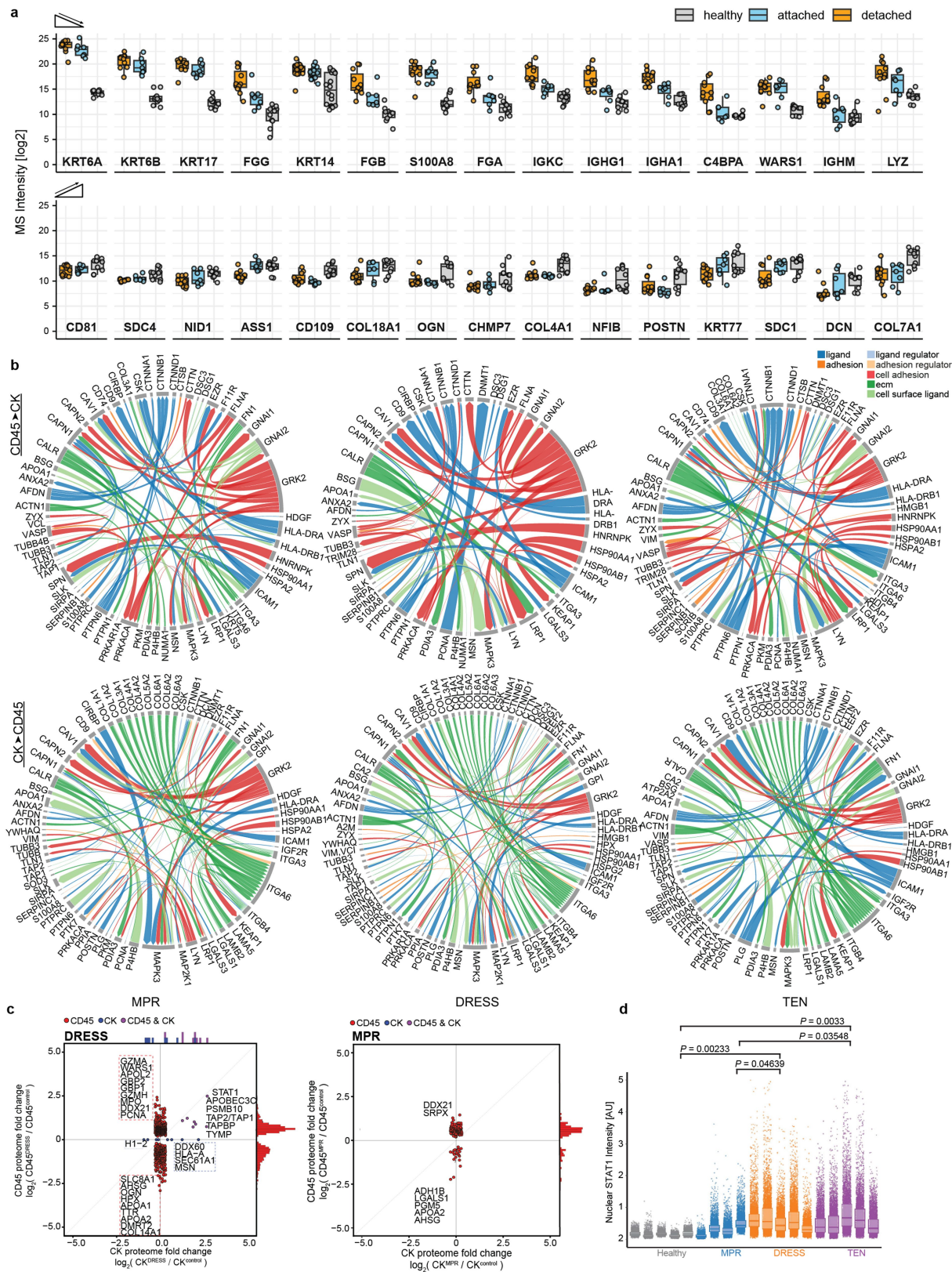
represents a patient, color represents the cell type. **e,f**, Pearson correlation (**e**) and differentially expressed proteins (DEP, **f**) quantified across all patients in the indicated cell type. Colored dots are significant DEPs, dashed vertical line indicate $\log_2$ fold change of ≤ 1 or ≥ 1 . Unpaired two-sided t-test with Benjamini-Hochberg correction for multiple comparisons ($\text{FDR} < 0.05$). **a-f**, $n = 21$ individuals (5 each DRESS/MPR/Healthy, 6 for TEN). TEN, Toxic Epidermal Necrolysis; MPR, Maculopapular Rash; DRESS, Drug Reaction with Eosinophilia and Systemic Symptoms.



Extended Data Fig. 2 | See next page for caption.

Extended Data Fig. 2 | Keratinocyte and immune cell proteome. a-c, Rank plot of median protein intensity (a), data completeness per protein (b) and coefficient of variation (CV) per cohort (c) of the keratinocyte proteome. Keratins are highlighted in green (a). **d,** Number of differentially expressed proteins (DEP) in keratinocytes of MPR/DRESS/TEN compared to healthy. **e,** DEPs in keratinocytes of DRESS versus healthy. Colored dots are significant DEPs, dashed vertical line indicate log2 fold change of ≤ 1 or ≥ 1 . Numbers indicate DEPs in each direction. **f, g,** Overrepresentation analysis (reactome) of ANOVA cluster 2 and 3 (shown in Fig. 1g), ordered by enrichment score. Color indicates degree of significance. **h,** Semisupervised heatmap of complement factors in immune cells of the indicated conditions. Color indicates normalized intensity levels (z score). **i,** Number of proteins identified in immune cells across cohorts. **j-l,** Rank plot of median protein intensity (j), data completeness per protein (k) and coefficient of variation (CV) per cohort (l) of the immune cell proteome. Ptpcr is highlighted in green (j). **m-o,** Principal component analysis (m), top 20 proteins (loadings) contributing to PC1/ 3 (n) and PC1 gene set enrichment analysis (MSigDB Hallmark) of immune cells, ordered according to enrichment score (o). **p,** DEPs in the indicated conditions compared to healthy. Shaded area within columns indicates the proportion of non-overlapping differentially regulated proteins. **q,** Upset plot of uniquely

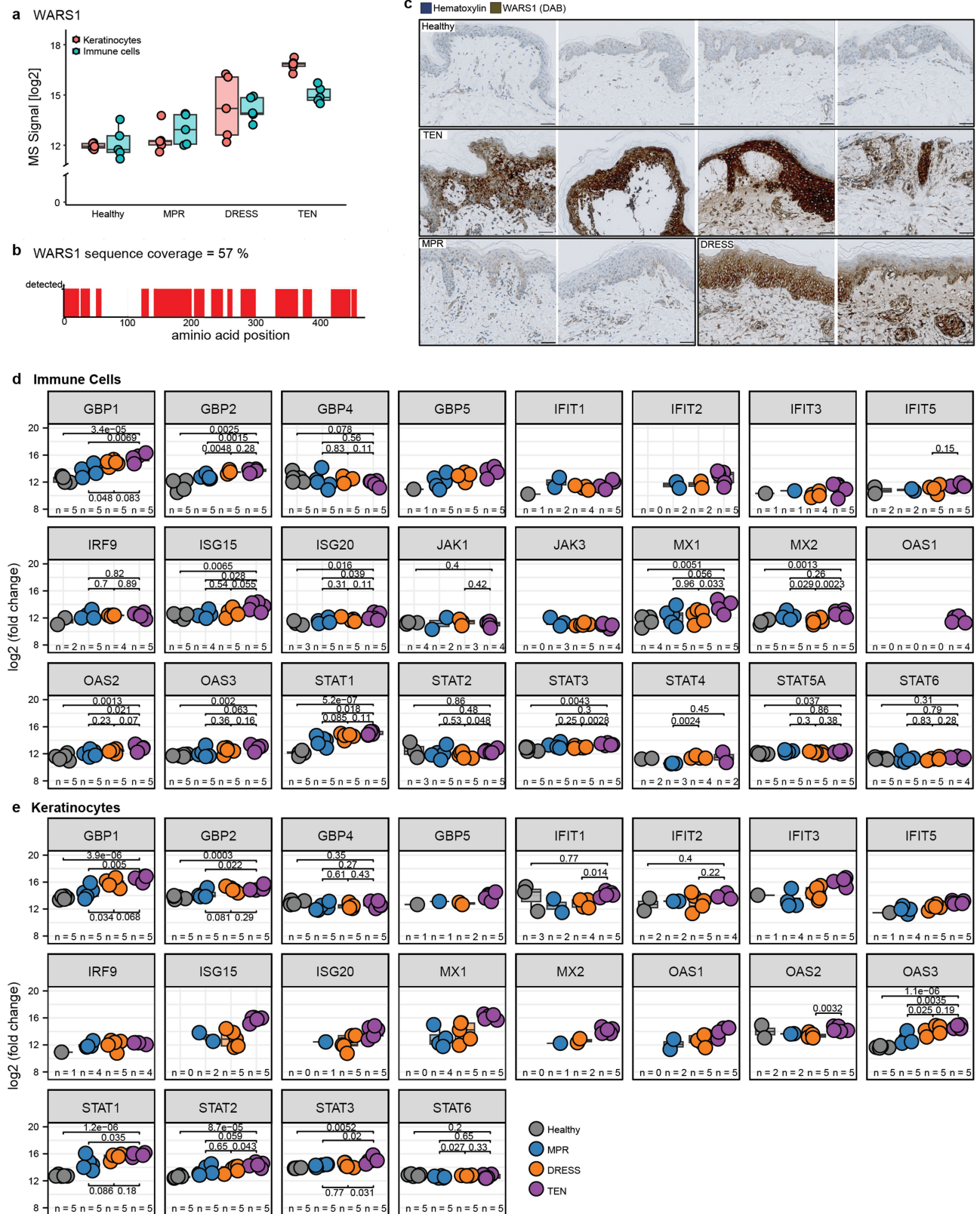
identified proteins specific to each of the indicated conditions. **r,** Source Data from Kim, D., Kobayashi, T., Voisin, B. et al. Targeted therapy guided by single-cell transcriptomic analysis in drug-induced hypersensitivity syndrome: a case report. *Nat Med* 26, 236–243 (2020). Scatter plot of EZH2 and previously identified markers (STAT1, JAK3, IL2RG) in DRESS showing fold change (x-axis) and the ratio of cell expression in diseased versus healthy samples (y-axis) in PBMC lymphocyte clusters of DRESS. **s,** Representative histology stained for EZH2, CD45 and pan-Cytokeratin in DRESS (n = 5) and healthy (n = 5) skin tissue sections. Representative images shown. **t,u,** Overrepresentation analysis (“Reactome”) of ANOVA cluster 2 (shown in Fig. 2g), ordered by enrichment score (t) and semisupervised heatmap of enriched proteins of “activation of ATR in response to replication stress” (u). Color indicates degree of statistical significance (t) and normalized intensities (u). **d,e,p** unpaired two-sided t-test. **d-g,p,t,** Benjamini-Hochberg correction for multiple comparisons (FDR < 0.05). **a-q,t-u,** n = 5 individuals per cohort (MPR, DRESS, TEN, healthy). Box plots: median (center), interquartile range (box, 25th-75th percentiles), whiskers (furthest points within 1.5 * interquartile range). TEN, Toxic Epidermal Necrolysis; MPR, Maculopapular Rash; DRESS, Drug Reaction with Eosinophilia and Systemic Symptoms.



Extended Data Fig. 3 | See next page for caption.

Extended Data Fig. 3 | a. Top 15 proteins that significantly follow a linear relationship from healthy to attached to detached keratinocytes. The proteins are displayed in order of highest MS intensity values in detached keratinocytes (top panel) and healthy keratinocytes (bottom panel). Colors denote sample origin. Box plots: median (center), interquartile range (box, 25th-75th percentiles), whiskers (furthest points within 1.5 * interquartile range). **b.** Circos plots to visualize ligand-receptor interactions between keratinocytes (CK) and immune cells (CD45) across the cutaneous drug reactions (MPR, DRESS, TEN), normalized to healthy. In the bottom panels (CK > CD45) immune cells are the recipient, while in the top panels (CD45 > CK) keratinocytes are the recipient. Arrows indicate interaction direction, colors the different functional categories as indicated, and width of arrow the difference in average

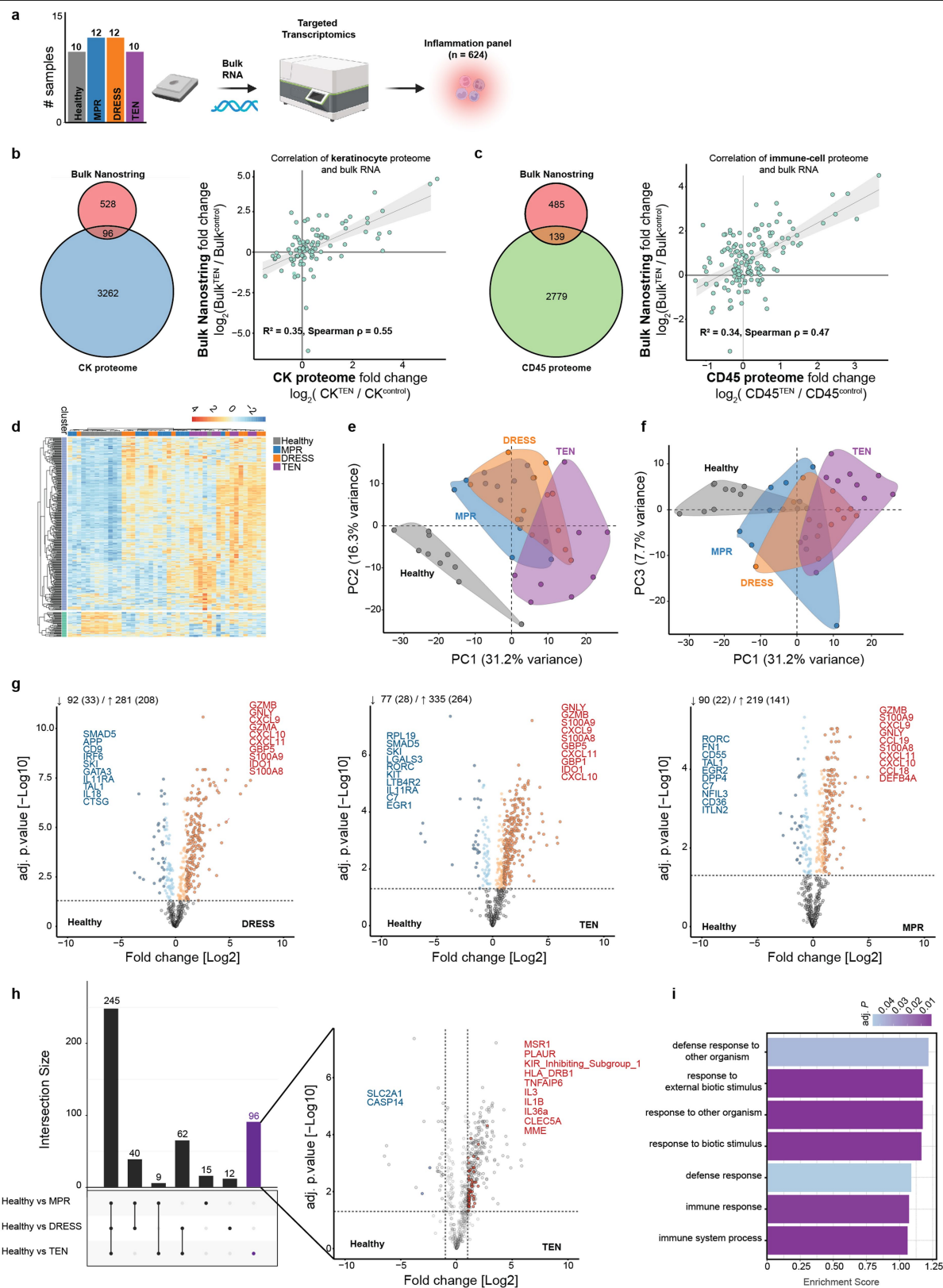
expression levels. **c.** Differentially expressed proteins in keratinocytes (x-axis) and immune cells (y-axis) of DRESS (left) or MPR (right) compared to healthy. Each dot represents a protein, color indicates cell type. n = 5 individuals per cohort and cell type. Histograms above and to the right display the distribution of fold changes along the respective cell type. **d.** Nuclear Stat1 intensity per cell (shown in Fig. 4e), normalized to its nuclear stain intensity. Each dot represents a single cell measurement. n = 5000 cells per individual, n = 5 individuals each for TEN, DRESS, Healthy, 4 for MPR. Box plots: median (center), interquartile range (box, 25th-75th percentiles). Unpaired two-sided t-test on mean normalized Stat1 values. TEN, Toxic Epidermal Necrolysis; MPR, Maculopapular Rash; DRESS, Drug Reaction with Eosinophilia and Systemic Symptoms.



Extended Data Fig. 4 | WARS1 expression in cutaneous drug reactions.

a. Protein expression level of WARS1 across all cohorts and cell types as indicated.
b. Identified amino acid sequence and sequence coverage of WARS1 using mass-spectrometry across all samples. **c.** Histology of WARS1 across cohorts as indicated. Scale bar = 50 μ m. **d,e.** Differential expression analysis of proteins depicted in Fig. 5b for immune cells (**c**) and keratinocytes (**d**). Two-sided t-test.

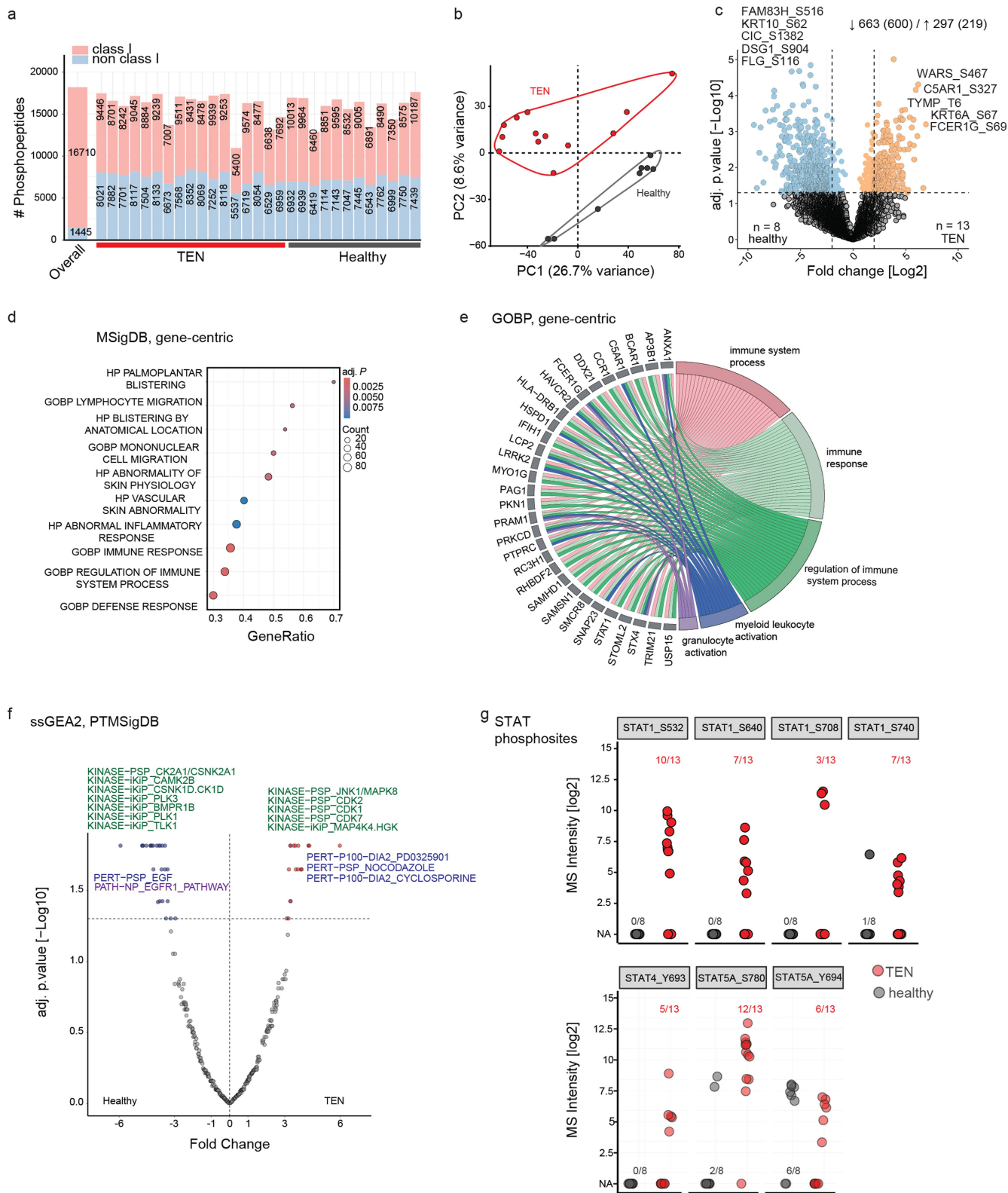
Valid values per protein and cohort as depicted. Box plots: median (center), interquartile range (box, 25th-75th percentiles), whiskers (furthest points within $1.5 \times$ interquartile range). TEN, Toxic Epidermal Necrolysis; MPR, Maculopapular Rash; DRESS, Drug Reaction with Eosinophilia and Systemic Symptoms.



Extended Data Fig. 5 | See next page for caption.

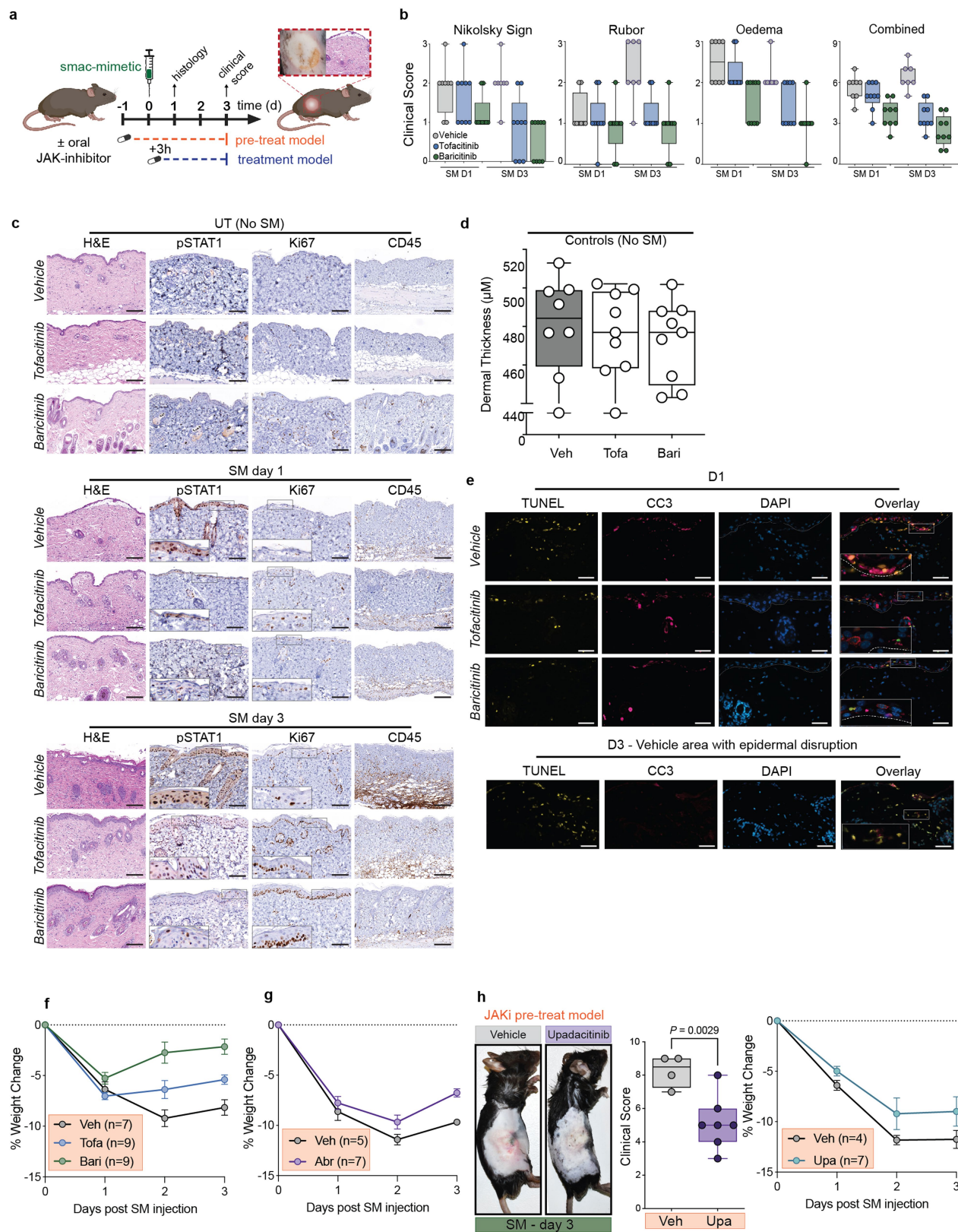
Extended Data Fig. 5 | Targeted transcriptomics of bulk tissue sections.
a, Schematic representation of the targeted transcriptomic analysis performed on bulk FFPE scrolls using the nCounter Inflammation panel. Number of measured transcripts as indicated (n = 624). **b-c**, Venn diagram of identified transcripts detected by targeted transcriptomics of bulk tissue (top) and proteins identified using DVP (bottom) of keratinocytes (b) or immune cells (c). Correlation analysis metrics for overlapping proteins / transcripts as indicated. **d**, Hierarchical clustering of ANOVA significant proteins. Color indicates normalized intensities (z score). **e,f**, Principal Component analysis (PCA) of transcriptome from bulk tissue sections across all cohorts as indicated. **g**, Differentially expressed transcripts between healthy and DRESS (left),

TEN (middle) or MPR (right). Colored dots are significant transcripts, and the top ten in each direction are shown. **h,i**, Upset plot of differentially regulated transcripts across all conditions compared to healthy (h). Those uniquely regulated in TEN are color-marked in the original volcano plot (right), followed by overrepresentation analysis (GOBP, i). **a-i**, n = 44 individuals (10 each for Healthy, TEN and 12 for DRESS and MPR). **d**, One-way ANOVA with post-hoc Tukey HSD. **g,h**, unpaired two-sided t-test. **d, g-i**, Benjamini-Hochberg correction for multiple comparisons (FDR < 0.05). TEN, Toxic Epidermal Necrolysis; MPR, Maculopapular Rash; DRESS, Drug Reaction with Eosinophilia and Systemic Symptoms.



Extended Data Fig. 6 | Phosphoproteomic analysis of TEN. **a**, Number of Class I phosphosites (localization probability > 75%; otherwise, non-class I) identified in TEN and Healthy per sample and overall. **b**, Principal Component Analysis (PCA). **c**, Differentially regulated phosphosites between TEN and healthy. Colored dots are significant, dashed vertical line indicate log2 fold change of ≤ 1 or ≥ 1 . **d-e**, Gene-centric enrichment analysis shown as dotplot (MSigDB, d) or chord diagram of top five terms and related proteins (GOBP, e) of upregulated phosphosites in TEN. Size of dots correlate to the number of mapped proteins per term and color represents degree of statistical

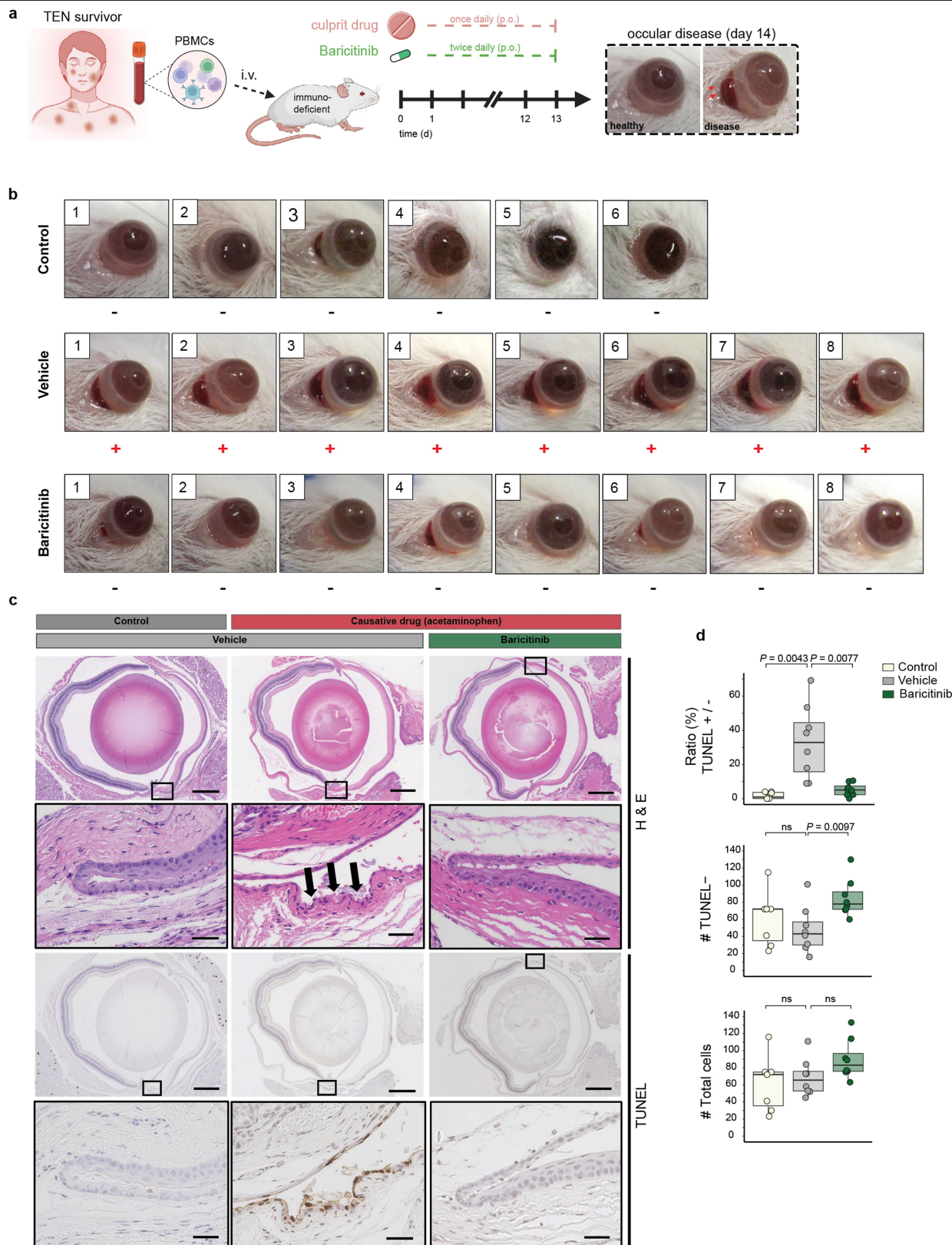
significance (d). **f**, Site-specific enrichment analysis (ssGEA2, PTMSigDB) of upregulated phosphosites in TEN. Points are color-coded according to the origin of the signature set (kinase-substrate interaction = green, perturbation = blue, molecular signaling pathway = purple). **g**, Number of identified STAT1/4/5 phosphosites in healthy and control samples, with numerical annotations indicating the identified phosphosites per group. **c-f**, Benjamini-Hochberg correction for multiple comparisons (FDR < 0.05). **a-g**, $n = 8$ (healthy) and 13 (TEN). TEN, Toxic Epidermal Necrolysis.



Extended Data Fig. 7 | See next page for caption.

Extended Data Fig. 7 | JAK inhibition in smac-mimetic mouse model of TEN.
a, Schematic experimental protocol for assessing JAK inhibitor efficacy in the smac-mimetic (SM) induced TEN model. Evaluation on day 1 (D1, histology) and day 3 (D3, clinical score). JAK inhibitors (10 mg/kg Baricitinib, 30 mg/kg Tofacitinib, 20 mg/kg Abrocitinib or 10 mg/kg upadacitinib) or vehicle (20% Captisol, 50 μ l) were given orally twice daily. Partially created with Biorender. com. **b**, Individual and combined clinical scores for the three assessment criteria (Epidermal disruption, Rubor, Oedema) evaluating clinical severity on D1 and D3 after SM-injection. **c**, Representative histology (H&E, pSTAT1, Ki67 and CD45) of the corresponding cohorts without SM (UT, untreated) and on D1 and D3 post-SM. Insets magnify indicated area of interest. Scale bars = 100 μ m. **d**, Dermal thickness of the control cohorts, without SM. Each data point

represents 1 mouse, n = 15 measurements/mouse. **e**, Immunofluorescence staining for cell death in the indicated cohorts following SM injection. Dotted lines indicate the dermo-epidermal junction. **b-e**, n = 7 (Vehicle D3), 8 (Vehicle D1) or 9 (tofacitinib and baricitinib) mice examined over 4 independent experiments (2 each D1 & D3 cohorts). **f,g**, Percentage weight change over three days post SM injection in the corresponding treatment groups. **h**, Representative macroscopic image, clinical assessment score and relative weight change following SM injection and pretreatment with upadacitinib (n = 7) or vehicle (n = 4). Weight change data points (**f, g, h**) show mean $\pm$ SEM. **h**, one-tailed Welch t-test. All box plots: median (center), interquartile range (box, 25th-75th percentiles), whiskers (furthest points within 1.5 * interquartile range); Veh, vehicle; Tofa = tofacitinib; Bari = baricitinib; Abr = abrocitinib.

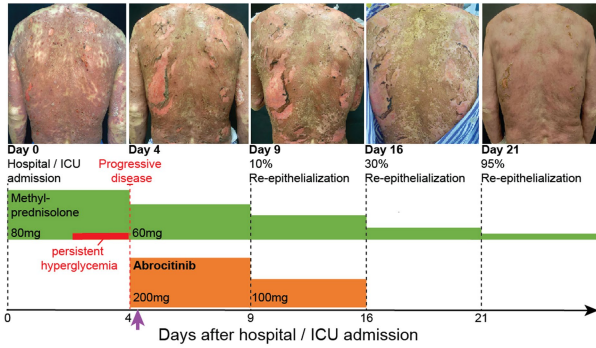
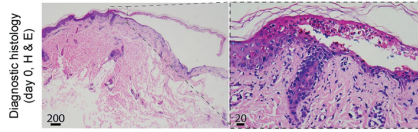


Extended Data Fig. 8 | JAK inhibition in humanized mouse model of TEN.

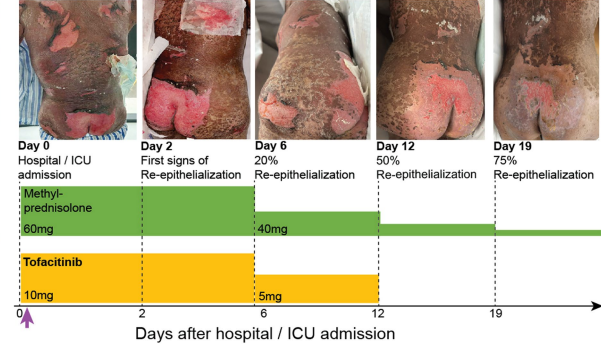
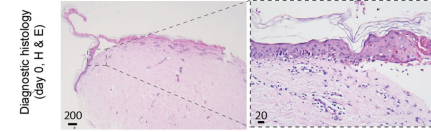
a, Schematic of oral JAK inhibitor treatment in the humanized mouse model of TEN. Partially created with Biorender.com. **b**, Ocular reaction following daily culprit drug administration ± oral baricitinib (10 mg/kg) with a plus or minus under each picture depending on the occurrence of an immune-reaction. **c**, Representative H&E and TUNEL immunohistochemistry images of the murine eye (scale bars = 500 μm) across the cohorts. Insets magnify the

conjunctival subepithelial area of interest (scale bars = 50 μm). Arrows indicate epithelial cell death (TUNEL +). **d**, Quantification of TUNEL positive, TUNEL negative, their ratio and total count of subepithelial cells of the corresponding cohort. Each data point is a mouse. Box plots: median (center), interquartile range (box, 25th-75th percentiles), whiskers (furthest points within 1.5* interquartile range). Unpaired, two-sided t-test. **b-d**, n = 6 control, 8 vehicle and 8 baricitinib treated mice.

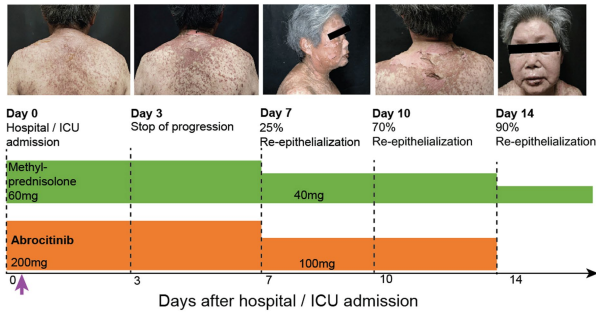
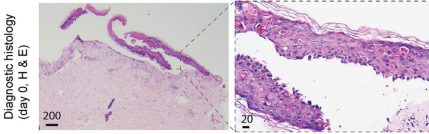
a Patient 1 (TEN)



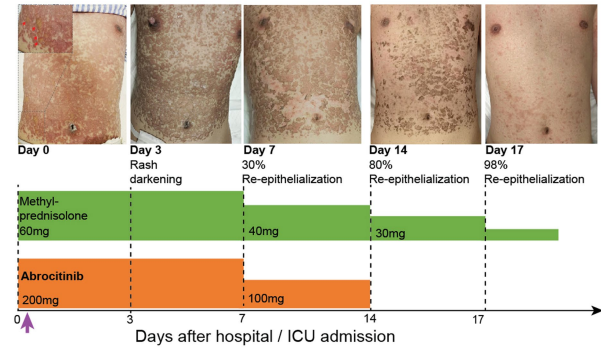
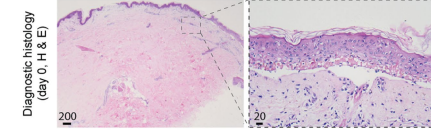
b Patient 2 (TEN)



c Patient 3 (SJS/TEN)

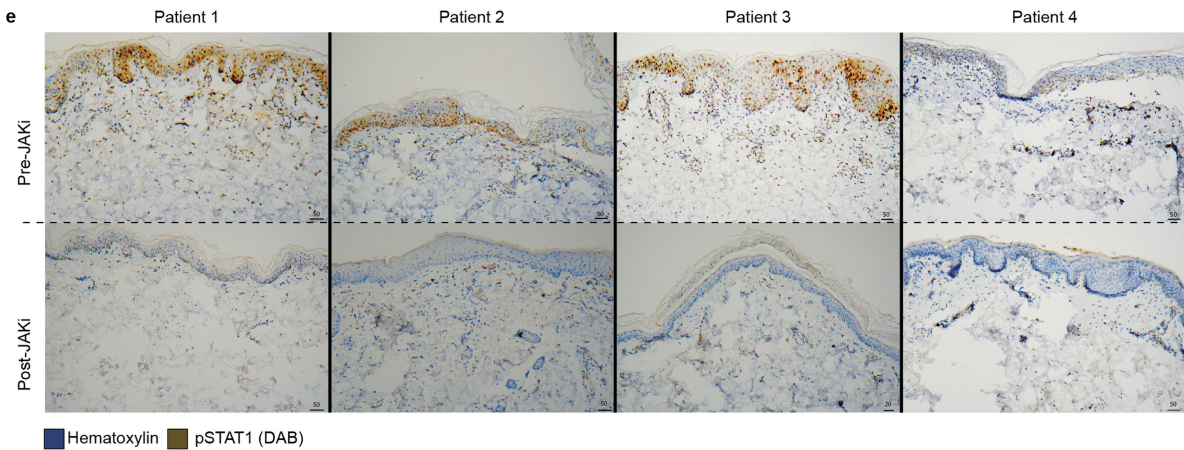


d Patient 4 (SJS/TEN)



↑ = initiation of JAK inhibitor therapy ↗ = epidermal detachment

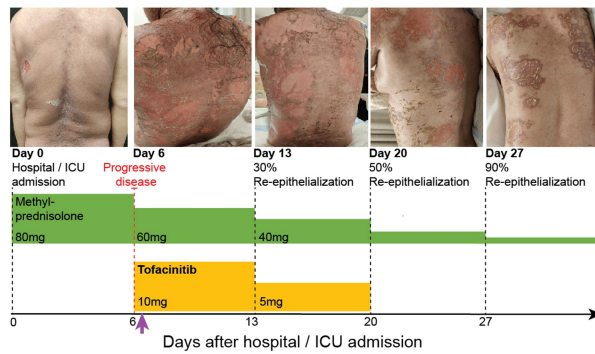
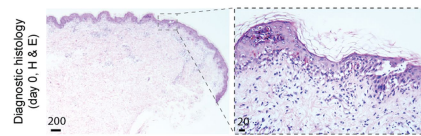
e



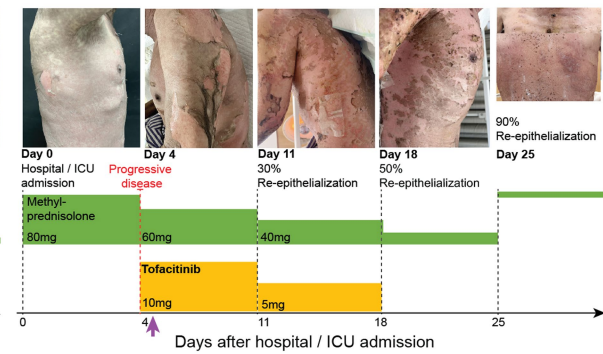
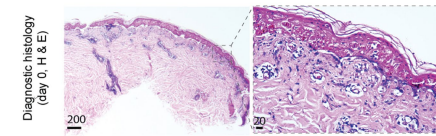
Extended Data Fig. 9 | See next page for caption.

Extended Data Fig. 9 | JAK inhibition in 4 patients with TEN and SJS/TEN.
a, 45-year-old male as described in Fig. 6. **b**, 67-year-old male with hepatocellular carcinoma under checkpoint inhibitor (Tislelizumab) therapy with clinically and histologically confirmed toxic epidermal necrolysis (TBSA 35%, SCORTEN 3, predicted mortality 35.3%). Methylprednisolone (1 mg/kg/day) and tofacitinib (10 mg/day for six days, 5 mg/day for six days) was given. Rapid cessation of disease progression was observed followed by 20% re-epithelialization of detached skin areas six days later. **c**, 72-year-old female with clinically and histologically confirmed Stevens-Johnson-Syndrome / TEN overlap syndrome (TBSA 10%, SCORTEN 2, predicted mortality 12.1%) associated with allopurinol intake. Upon admission she was immediately treated with methylprednisolone (1 mg/kg/day) and abrocitinib (200 mg/day for seven days, 100 mg/day for seven days) resulting in cessation of progression with regression of erythema within three days and 25% re-epithelialization of detached skin areas within seven days. **d**, 18-year-old male with clinically and histologically confirmed Stevens-Johnson-Syndrome/TEN overlap syndrome (TBSA 12%, SCORTEN 1, predicted mortality 3.2%) associated with allopurinol intake. Upon admission, the patient was treated with methylprednisolone (1 mg/kg/day) and abrocitinib (200 mg/day for seven days, 100 mg/day for seven days), resulting in cessation of progression with regression of erythema within three days and 30% re-epithelialization of detached skin areas by day seven. All H&E stained FFPE lesional skin biopsies were taken on day 0 (timepoint of hospital admission). **e**, pSTAT1 staining before and after JAK-inhibitor treatment, per patient. TBSA, total body surface area. Scale bars as indicated [μ m].

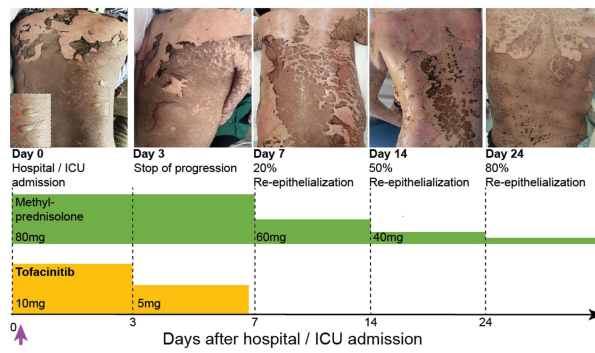
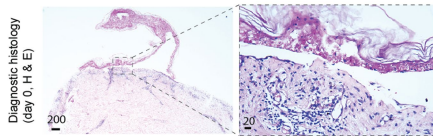
a Patient 5 (TEN)



b Patient 6 (SJS/TEN)

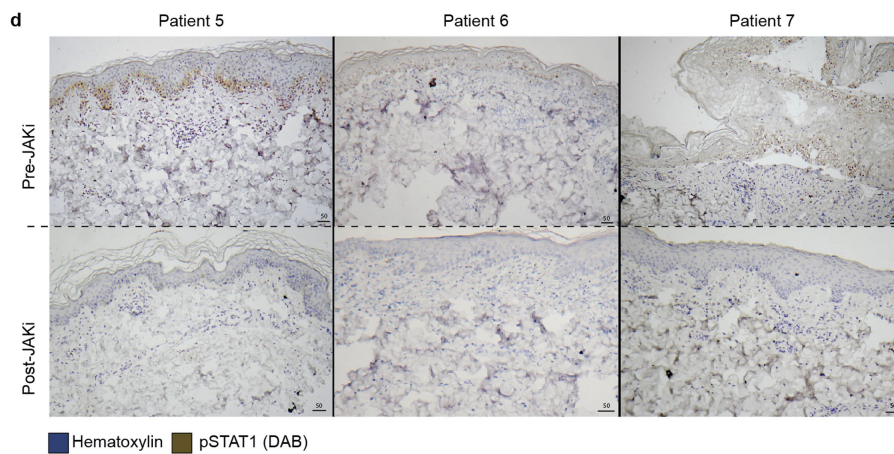


c Patient 7 (TEN)



↑ = initiation of JAK inhibitor therapy

↗ = epidermal detachment



Extended Data Fig. 10 | See next page for caption.

Extended Data Fig. 10 | JAK inhibition in 3 patients with TEN and SJS/TEN.
a, 67-year-old female with clinically and histologically confirmed toxic epidermal necrolysis (TBSA 35%, SCORTEN 2, predicted mortality 12.1%) associated with metronidazole intake, unresponsive to methylprednisolone (1 mg/kg/day) monotherapy was given tofacitinib (10 mg/day for seven days, 5 mg/day for seven days). Rapid cessation of disease progression was observed followed by 30% re-epithelialization of detached skin areas seven days later.
b, 45-year-old male with clinically and histologically confirmed Stevens-Johnson-Syndrome/TEN overlap syndrome (TBSA 25%, SCORTEN 5, predicted mortality >90%) associated with levofloxacin, cefixime intake, unresponsive to methylprednisolone (1 mg/kg/day) monotherapy was given tofacitinib (10 mg/day for seven days, 5 mg/day for seven days). Rapid cessation of disease

progression was observed followed by 30% re-epithelialization of detached skin areas seven days later. **c**, 64-year-old male with laryngeal cancer under checkpoint inhibitor (Zimberelimab) therapy clinically and histologically confirmed toxic epidermal necrolysis (TBSA 30%, SCORTEN 3, predicted mortality 35.3%) associated with carboplatin. Upon admission, he was given methylprednisolone (1 mg/kg/day) and tofacitinib (10 mg/day for seven days, 5 mg/day for seven days). Rapid cessation of disease progression was observed three days later and followed by 20% re-epithelialization of detached skin areas seven days later. All H&E stained FFPE lesional skin biopsies were taken on day 0 (timepoint of hospital admission). **d**, pSTAT1 staining before and after JAK-inhibitor treatment, per patient. TBSA, total body surface area. Scale bars as indicated [μm].

Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☐	☒ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	Zeiss Zen blue (v3.7.97.07000), Incucyte Sx1 (2022B Rev2), Leica LMD (v8.3), Bruker HyStar (v6.0), timsControl (v3.0.20), Evosep One Software (RCNet Driver 2.2.74.0), Orbitrap Astral Tune Application (1.0.100.40), Thermo Scientific Xcalibur (v4.7.69.37), nCounter® SPRINT Profiler (NanoString Technhologies)
Data analysis	DIA-NN (v1.8.0), R(4.0.2), QuPath 0.4.1, Biological Image Analysis Software (BIAS v1.3.0), Python (v3.11), Spectronaut (v17), Cellpose (v2.0), Zeiss Zen Imaging software (ZEN blue v3.7.97.07000), Incucyte analysis software (2022B Rev2), R-packages: ggplot2(3.4.3), pheatmap(v1.0.12), rstatix (v0.7.2), clusterProfiler (v4.6.0), WebGestaltR (v0.4.6), GOplot (v1.0.2), ssGSEA2 (v1.4), nanostringr(v0.4.2); Python-packages: SciPy (v1.11.1), Matplotlib (v3.7.2), Pandas (v2.0.3), Pingouin (v0.5.3), D3Blocks (v1.2.3), OmniPath (v0.16.4)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

UniProt human proteome (UP000005640_9606 and UP000005640_9606_additional, <https://www.uniprot.org/proteomes/UP000005640>), MsigDB C5 and Hallmark database (<https://www.gsea-msigdb.org/gsea/msigdb/collections.jsp>), PTMSigDB (<https://proteomics.broadapps.org/ptmsigdb/>), WebGestalt 2019 (<http://www.webgestalt.org/>), Reactome pathway database (<https://reactome.org/>), Gene Ontology (GO) database (<http://geneontology.org/>). Mass spectrometry and transcriptomic data of this study have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD044477 (username: reviewer_pxd044477@ebi.ac.uk, password: 6Lhi7MGM). Data tables of all proteomic and transcriptomic measurements performed throughout this manuscript are provided in Supplementary Tables 7 - 12. Clinical scores, average dermal thickness, lesion size and % weight change measurements performed in the smac-mimetic mouse model are provided in Supplementary Table 13. Quantification of subepithelial cell death in the humanized mouse model is provided in Supplementary Table 14.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender

To study molecular mechanisms of cutaneous drug reactions, we assembled an age and sex matched, retrospective cohort of lesional FFPE skin biopsies for A) proteomics: i) from patients with mild (MPR) or severe (TEN, DRESS) CADRs alongside healthy controls (Fig. 1a and Extended Data Figure 1 / Supplemental Table 1, n = 21), ii) from patients with SJS/TEN and TEN alongside healthy controls (Fig. 3 / Supplemental Table 2, n = 24) and B) transcriptomics: from patients with mild (MPR) or severe (TEN, DRESS) CADRs alongside healthy controls (Fig. 4c-d and Extended Data Fig. 5 / Supplemental Table 3, n = 44). For phosphoproteomics frozen tissue biopsies from patients with SJS/TEN and TEN alongside healthy controls were used (Extended Data Figure 6 / Supplemental Table 4, n = 21).

Reporting on race, ethnicity, or other socially relevant groupings

No socially constructed or socially relevant categorization variables were used in this study.

Population characteristics

Baseline characteristics were statistically evaluated using Analysis of Variance (ANOVA) for numeric variables (age) and a Chi-squared test of independence for categorical variables (sex), a summary of which can be found in the corresponding Extended Data Tables.

Recruitment

Proteomic and transcriptomic analyses were performed on a retrospective cohort. Participants were identified through a review of medical records at the Departments of Dermatology and Allergology of the University Hospital Zurich and Ludwig Maximilian University of Munich. Eligible patients were those who had undergone routine histopathological diagnostic procedures for suspected cutaneous adverse drug reactions (CADRs). Inclusion criteria for the CADR groups were i) TEN patients: a minimal affected skin area of 30% (standard DVP proteomic cohort) or >10% (other cohorts, including SJS/TEN overlap). ii) DRESS patients: a RegiSCORE of 6-7 (definite diagnosis). iii) MPR patients: typical clinical features and a positive lymphocyte transformation test (LTT) or skin test. Healthy control samples were obtained from individuals undergoing routine dermatological procedures unrelated to drug reactions during the same period. We do not assume any self-selection bias in this study due to the comprehensive and systematic approach used in patient identification and sample collection.

Ethics oversight

Ethical permission and informed consent was conducted for all involved patients and samples. Treatment with JAK inhibitors was approved by the local ethics committee and the institutional review board of the First Affiliated Hospital of Fujian Medical University (Fujian: MRCTA, ECFAH of FMU[2023]400), and the patient was provided written informed consent. Skin biopsies were performed in the context of routine clinical workup for retrospective analysis with informed consent and ethical approval in place (Munich: 22-0342, 22-0343; Zurich: BASEC: Req-2021-00226 and 2017--00494; Fujian: MRCTA, ECFAH of FMU[2023]400, all experiments were performed in accordance with the Declaration of Helsinki.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Proteomics cohorts n = 45 patients. Phosphoproteomics n = 21 patients. Transcriptomics cohort n = 44 patients. JAK inhibitor treatment n = 7 patients. Mouse cohorts n = 78. For the proteomics, phosphoproteomics, and transcriptomics cohorts, sample size was primarily determined by the availability of rare TEN cases, which naturally limited the cohort size. We first identified available TEN samples that met our inclusion criteria, then added samples from other CADR types and healthy controls to create balanced cohorts, keeping in mind to achieve a number of samples in total that remain processable within the technological aspects. No formal statistical methods were used to predetermine sample sizes. Considering the rarity of the disease and in comparison to existing literature we achieved a high number of analyzed TEN samples that allow the statements made within the manuscript, further supported by the biological follow up experiments. For the JAK inhibitor treatment group, we included all consecutive eligible patients with this rare condition who received the treatment during the study period.
Data exclusions	None.
Replication	We used independent biological samples of different patients per disease (TEN, DRESS, MPR) and across different omic entities. All experiments confirmed the experimental findings. In the phosphoproteomic cohort and mDIA-DVP cohort, 2 different biopsy sites were used in the healthy cohort from a subset of healthy patients, as specified in the methods section.
Randomization	Samples were allocated into the experimental groups based on the type of drug reaction and thus based on the clinical-histological diagnosis. Histopathological diagnosis was re-validated in all cases by a board-certified dermatopathologist using a fresh H&E-stained tissue section. Mass-spectrometry sample injection was randomized using the rand function in Excel.
Blinding	Investigators were not blinded to group allocation during data collection and analysis, as blinding was not feasible due to the nature of the study. Given the distinct clinical and histological features of each type of drug reaction, blinding would not have been practical or meaningful in this context.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☐	☒ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	see Supplemental Table 6 for all used antibodies and their usage details.
Validation	Only commercially available antibodies that have been validated for immunohistochemistry as documented on the manufacturer's website were used. Dilutions used are mentioned in Extended Data Table 8. abcam 228724 / abcam 156769: validated by manufacturer for Western blotting and immunohistochemistry using recombinant human WARS1 protein fragment as immunogen. CST #9167S / CST #5246T / CST #14994 / CST #9661 / CST #98941 / CST #98941 / : validated by manufacturer with Western blotting. DAKO #M0701 / DAKO, #Z0622: validated by manufacturer for immunohistochemistry on formalin-fixed, paraffin-embedded tissues and acetone-fixed frozen sections. LSBio #LS-B16812: validated by manufacturer for immunohistochemistry on formalin-fixed, paraffin-embedded tissues. Thermo Fisher #MA514520 / Invitrogen #13-2444-82 / BD Pharmingen, # BD553076: validated by manufacturer for Western blot, immunohistochemistry (paraffin and frozen), immunocytochemistry, and flow cytometry.

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	Male and female BL6 mice at 6 weeks of age; male immunocompromised NOD/Shi-scid, IL-2R γ null (NOG) mice at 6 weeks of age. Mice were maintained at the appropriate biosafety level under constant temperature and humidity conditions with a 12h light cycle. Animals were allowed food and water ad libitum.
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Wild animals	No wild animals were used in this study.
Reporting on sex	Male and female mice were used according to previously published models.
Field-collected samples	No field-collected samples were used in this study.
Ethics oversight	Smac mimetic mouse model experiments were approved by the local ethics committee (WEHI AEC# 2022.009). Humanized mouse model experiments were approved by the local ethics committee and the institutional review board of Niigata University.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about [clinical studies](#)
 All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration	n/a
Study protocol	n/a
Data collection	Tissue biopsies represent the original biopsies used for diagnostic purposes during clinical routine.
Outcomes	n/a