

Localization-dependent effects of HSPA1A overexpression on skin inflammation and remodeling in a DNCB- induced mouse model of allergic dermatitis

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Abstract

Background

Allergic dermatitis (AD) is a chronic relapsing inflammatory skin disorder, and current therapies remain symptomatic. Heat shock protein 70 (HSP70) exerts pro- or anti-inflammatory effects depending on its localization, yet its role in AD remains unclear. This study aimed to determine how overexpression of human HSPA1A with predominantly intracellular or extracellular accumulation affects skin inflammation and remodeling.

Methods and Results

Dermatitis was induced by topical 2,4-dinitrochlorobenzene (DNCB) in 81 male mice: wild-type C57BL/6J (WT) and transgenic (Tg(HSP70[in])) (intracellular) and (Tg(HSP70[out])) (extracellular) lines (n = 27 per genotype). Skin was collected on Days 0, 11, and 17 (n = 9 per genotype per time point) and examined by histochemical staining and immunofluorescence for HSP70, α -SMA, and COL1A1. On Day 11, epidermal thickness was lower in both transgenic lines than in WT mice; by Day 17, it was lowest in (Tg(HSP70[out])) mice. On Day 11, HSP70out mice showed the smallest increase in dermal cellularity and fewer α -SMA-positive microvessels than WT mice, and elastic fibers were partially preserved. In (Tg(HSP70[in])) mice, mast cell numbers rose sharply by Day 17, exceeding those in the other groups, and focal COL1A1 accumulation was most pronounced.

Conclusions

The effects of HSPA1A overexpression on DNCB-induced dermatitis depend on protein localization: extracellular accumulation was associated with faster resolution of inflammation and attenuated epidermal hyperplasia, whereas intracellular accumulation was associated with more persistent inflammatory changes. These findings identify extracellular HSP70 as a candidate modulator of skin repair in AD, although the underlying mechanisms require direct validation.

Introduction

Allergic dermatitis (AD) comprises a group of chronic, relapsing inflammatory skin disorders characterized by dysfunctional Th2-mediated immune responses [1]. Clinical manifestations include eczematous lesions and intense pruritus, which disrupt normal skin architecture and the function of its derivatives, leading to a marked reduction in patients' quality of life [2]. From clinical and scientific perspectives, the study of the pathogenetic mechanisms of atopic dermatitis – as an example of allergic pathology – is of interest. As a polyetiological disease with high prevalence (particularly among children up to 3 years of age), atopic dermatitis carries a substantial socioeconomic burden. The relapsing nature of the pathology and the primarily symptomatic nature of current therapies, which alleviate acute

inflammatory signs without targeting disease pathogenesis, underscore the urgency of identifying novel molecular targets critical for disease induction and progression.

Numerous pharmacological modeling approaches have been developed to study the pathogenetic mechanisms of AD in experimental animals [3]. These models primarily target rodent skin through topical application of irritating agents. The most well-characterized models include hapten-induced models (2,4-dinitrochlorobenzene [DNCB], 1-fluoro-2,4-dinitrobenzene [DNFB]), ovalbumin (OVA) sensitization, and calcipotriol-induced (MC903) allergy. Additionally, transgenic lines carrying mutant or knockout alleles of genes have been developed, including filaggrin knockout mice (*Flg* ^{ft/ft} and *Flg* ^{-/-}), filaggrin-hornerin knockout (*FlgHnr* ^{-/-}), bleomycin hydrolase knockout (*Blmh* ^{-/-}), and SASPase knockout (*SASP* ^{-/-}) [4]. Furthermore, nontransgenic rodent lines with spontaneous AD-like phenotypes have been established through inbreeding. Notably, NC/Nga mice possess alleles that potentiate Th2-type immune responses. Despite this diversity of AD modeling approaches, the fundamental cellular and tissue mechanisms underlying pathology development and progression remain incompletely understood.

In the present study, we employed the DNCB-induced hIAD model, as it is one of the most characterized in the literature [5]. DNCB is an organic compound that acts as a sensitizing agent to activate type IV hypersensitivity reactions. Upon topical application, DNCB penetrates cells and induces protein haptenization [6]. The resulting cellular damage triggers Th2 activation, initiating immunological reactions in the skin.

AD progression is associated with diminished cellular regenerative potential [7]. Therefore, developing strategies to restore and maintain skin barrier function remains a pressing challenge [8]. Recent attention has focused on the effects and regulatory activity of cellular chaperones, heat shock proteins HSP70 [9] and HSP90 [10] as potential novel therapeutic targets for AD.

HSP70 proteins belong to a highly conserved family of molecules synthesized in response to cellular stress that regulate protein homeostasis [9]. They exhibit both intracellular and extracellular localization, and their role in proteostasis is localization-dependent. Intracellular HSP70 forms are involved in protein folding [11–12], refolding [13], and proteolysis [14]. Studies in oncology indicate that HSP70 regulates antiapoptotic [15] and mitophagy processes [16–17]. Inducible intracellular HSP70 forms (iHSP70s) are generally associated with anti-inflammatory effects [18].

Conversely, the extracellular form of HSP70 (eHSP70) functions as a damage-associated molecular pattern (DAMP) and interacts with innate immunity receptors, notably TLR2 [19] and TLR4 [20]. This interaction activates proinflammatory cytokine expression by immunocompetent cells such as monocytes [21]. However, several studies have indicated that eHSP70 can also exert anti-inflammatory effects by reducing proinflammatory cytokine levels [22]. This functional duality of eHSP70 has attracted interest in elucidating the mechanisms underlying its ambivalent properties [23]. Given the role of heat shock proteins in folding and refolding processes, investigating their contribution to reparative regeneration associated with allergic dermatitis holds promise for both scientific and clinical practice. This forms the hypothesis of our experiment.

It is known that allergic dermatitis is characterized by elevated basophil and eosinophil counts in the systemic circulation, as well as increased mast cell numbers in affected skin areas. Papakonstantinou et al. (2021) investigated bronchial asthma and assessed treatment effects by examining smooth muscle cell numbers, proliferating epithelial and mesenchymal cells, and HSP70 and HSP90 expression levels in these cells [24]. Given the limited understanding of HSP70 family proteins in AD pathogenesis and the dual pro- and anti-inflammatory effects of their extracellular forms, the aim of this study was to investigate the impact of overexpression of extracellular and intracellular forms of HSPA1A on structural changes in skin components during regeneration in a DNCB-induced mouse model of allergic dermatitis. Our study is exploratory in nature. The selection of criteria for analyzing the contribution of extracellular versus intracellular form overexpression to disease progression was based on key components of AD pathogenesis. Mast cell count and α -smooth muscle actin (α -SMA) reflect the severity of the pathological process, while collagen and elastic fibers – as products of fibroblast metabolism – indicate the rate of repair in damaged skin areas.

Materials and methods

Animals

The study was performed on 81 male mice weighing 26.0 ± 2.5 g. Animals were divided according to genotype into three groups ($n = 27$ in a group per genotype): 4-month-old C57BL/6J wild-type (WT) mice and age-matched transgenic HSP70in (overexpression of the cytoplasmic human *HSPA1A* via insertion of a transgenic cassette containing HSP70 nucleotide sequences (Tg(HSP70[in])) on a C57BL/6J background) and HSP70out mice (overexpression of the secreted form of HSP70 ((Tg(HSP70[out])))). Within each genotype, animals were assigned to three tissue-collection time points (Day 0, Day 11, and Day 17; $n = 9$ animals per genotype at each time point). The transgenic lines carried a cassette insertion of the human *HSPA1A* gene leading predominantly to intracellular or extracellular accumulation of HSP70 in the HSP70in and HSP70out lines, respectively (Fig. 1b). Only animals showing no signs of health abnormalities were included in the study. Experimental groups were formed according to genotype; only animals whose body weight deviated from the group mean by no more than 10% were included. First-generation animals were identified by PCR genotyping and subsequently bred to obtain homozygous offspring. Heterozygous and wild-type littermates were excluded from the transgenic groups to avoid potential effects of variable transgene copy number.

FIGURE 1

All animal procedures were performed in accordance with institutional ethical standards and the 3Rs principle and were approved by the Local Ethics Committee of Sechenov University (Protocol No. 04–23, 2 March 2023). The quarantine period before the experiment was 14 days. Mice were housed three per cage at 20–22°C and 55–60% relative humidity, with ad libitum access to clean water and pelleted feed. The animals were obtained from vivarium and assigned using stratified randomization.

Experimental design

For DNCB-exposed cohorts, 2,4-dinitrochlorobenzene (DNCB) was applied topically to a shaved 1 cm² interscapular skin area. DNCB solutions were prepared in acetone: ethanol (1:2, v/v) and applied using a brush saturated with the solution. Primary sensitization was performed on Day 1 with 5% DNCB. Skin reactions were monitored daily and scored using a 5-point scale: 0, no reaction; 1, mild erythema; 2, moderate hyperemia; 3, severe redness and edema; 4, erosions; and 5, crust formation and ulceration. Repeated 5% DNCB applications were performed from Day 4 through Day 8 inclusive. On Day 10, a 0.5% DNCB challenge was applied topically (Fig. 1a). Day 0 animals were collected without DNCB exposure.

Tissue collection was performed on Day 0, Day 11, and Day 17 (n = 9 animals in a group per genotype per time point; Fig. 1d). Mice were anesthetized with 5 mg/kg xylazine hydrochloride (Interchemie, Netherlands) and 40 mg/kg tiletamine/zolazepam (Virbac, France), euthanized by decapitation, and the experimental dorsal skin area was excised together with a 0.5-cm margin of macroscopically unaffected tissue.

Histological analysis

Skin biopsy material was fixed in 10% neutral buffered formalin (Biovitrum, Russia) for 48 h at 4°C. Tissue processing was performed through a graded series of isopropyl alcohol (Biovitrum, Russia), followed by paraffin embedding. Sections of 5 µm thickness were prepared using an HM325 microtome (Thermo Fisher Scientific, USA).

For histological evaluation, sections were stained with hematoxylin and eosin, Mallory's trichrome, and Weigert's resorcin-fuchsin with toluidine blue counterstaining.

Immunofluorescence staining was performed using antigen retrieval buffer (pH 9.0; Cat. No. E-IR-R220A; Elabscience, USA) at 98°C and 105 kPa for 20 min. After three washes in 10% PBS (Eco-Service, Russia), endogenous peroxidase activity was blocked with 3% H₂O₂ at 37°C for 30 min. Sections were washed in PBS and blocked with 1% bovine serum albumin (BSA; Cat. No. RM-T1725/1000; Biosera, France) for 1 h. Primary antibodies were incubated at 37°C for 3 h: rabbit monoclonal anti-α-SMA (1:200; Cat. No. ET1607-53; Lot H681762047; Huabio, China), rabbit polyclonal anti-HSP70 (1:200; Cat. No. E-AB-92322; Lot KL064ZP66846; Elabscience, USA), and rabbit polyclonal anti-COL1A1 (1:200; Cat. No. E-AB-66975; Lot UX194T2L3263; Elabscience, USA). Sections were then incubated for 2 h at 37°C with iFluor 488-conjugated goat anti-rabbit IgG (1:500; Cat. No. HA1121; Lot H680662011; Huabio, China). Nuclei were counterstained with propidium iodide (PI; Cat. No. G1021-10ML; Lot CR2212025; Servicebio, China) for 10 min. Sections were washed in PBS, dehydrated through graded alcohols, cleared in xylene, and mounted with VitroGel mounting medium (Cat. No. HM-VI-A500; Lot 0181; Biovitrum, Russia).

Positive and negative internal controls were used for each marker evaluated in the immunohistochemical analysis. Mouse liver fragments served as positive controls for anti-HSP70 and

anti- α -SMA, while ovarian tissue was used for COL1A1. Sections of the mouse cerebral cortex served as negative controls.

Morphometric and statistical analysis

Images of histological specimens were performed on Axio Imager.A1 microscope equipped with Axiocam 305 camera (Zeiss, Germany) and M8 histological scanner (PeciPoint, Germany) for light microscopy imaging. The intensity of immunohistochemical staining was assessed by optical density analysis using ImageJ software (version 1.52, National Institutes of Health, USA). Morphometric measurements of extended structures were performed after scanning the slides in ViewPoint software (PeciPoint, Germany) while preserving the scale. Morphometric analysis was conducted using ZEN 3.10 (Zeiss, Germany) and QuPath 0.7.0 [25]. One histological section per animal was analyzed. For each animal (served as statistical unit), 10 fields of view were evaluated at $\times 400$ for skin sebaceous-hair complex measurements, $\times 800$ for mast cell counts, and $\times 1260$ for total dermal cellularity. Mean fluorescence intensity of HSP70 immunofluorescence was quantified in QuPath from $\times 400$ images. Skin reactions and morphometric measurements were performed by an investigator blinded to the experimental group. The 10 fields from each animal were analyzed as individual field-level measurements, yielding 90 measurements per genotype at each time point. Statistical analysis was performed with GraphPad Prism 10.2.1 (Dotmatics, USA) and OriginPro 2024 (OriginLab, USA). Data distribution was assessed using the Shapiro–Wilk test. Differences among groups were assessed by one-way ANOVA followed by Tukey's post hoc test. Statistical significance was set at $p \leq 0.05$. The effect size was calculated based on pilot data on epidermal thickness using Hedges' g . For primary between-group comparison of HSP70in and WT on day 11 after DNCB application, the effect size was $g = 1.90$.

Sample size estimation

The sample size was determined based on pilot data on epidermal thickness. The effect size was derived from comparison of HSP70in group with WT on day 11 after DNCB application (Hedges' $g = 1.90$). With two-sided significance level of $\alpha = 0.05$, the minimum calculated sample size was 6 animals per group to achieve 80% statistical power and 7 animals per group for 90% power.

Results

Dermatitis development dynamics

Examination of the skin revealed signs of an acute inflammatory process with a typical course (Fig. 2a). By Day 3 after primary sensitization, the experimental skin area in all groups had mild to moderate hyperemia (scores of 1–2). Notably, animals of the HSP70out line exhibited a less pronounced reaction to the initial DNCB application (scores 0–1) compared to the other groups (scores 1–2) (Fig. 2b). By

Days 5–6, all animals had developed serous and/or hemorrhagic crusts, which had epithelialized by Days 11–12 (scores 4–5). Between Days 11 and 17, complete or partial skin restoration was observed.

Morphological validation of the hIAD model

In all DNCB-treated groups, the following pathomorphological features of an inflammatory allergic reaction were observed on Day 11: diffuse epidermal hyperplasia (acanthosis), spongiosis with focal vesiculation, hyperkeratosis and parakeratosis, scattered polymorphonuclear infiltrates, granulation tissue with nuclear debris, focal erythrocyte extravasation, mast cell infiltration, and a reduction of elastic fibers within the DNCB-treated area (Fig. 2c). The intensity of these changes decreased toward the periphery of the experimental skin region. By Day 17, partial epidermal recovery, reduced infiltration, and decreased mast cell numbers were observed, whereas restoration of elastic fibers was not evident.

FIGURE 2

Effect of the overexpression of extracellular and intracellular HSPA1A forms on epidermis and its appendages in the hIAD model

To analyze epidermal thickness, as well as the number and depth of sebaceous-hair complexes, hematoxylin and eosin (H&E) staining was performed (Fig. 3a). Skin from WT mice exhibited a progressive increase in epidermal thickness across all time points (Fig. 3b). In contrast, the epidermal thickness in HSP70ⁱⁿ and HSP70^{out} animals was significantly lower on Day 11 compared to WT. Notably, by Day 17, mice overexpressing extracellular HSPA1A displayed values significantly lower than those of the other two groups.

Changes in the depth of sebaceous-hair complexes were also assessed in H&E-stained sections (Fig. 3c). Wild-type animals showed a marked increase in the depth of these complexes from Day 0 to Day 17. Transgenic animals did not exhibit such an accelerated increase; only a slight increase was observed by Day 17 compared to WT for each transgenic line. Both lines demonstrated a similar dynamic pattern: an increase in the measured parameter by Day 11, followed by stabilization of the sebaceous-hair complex depth within a narrow range of values.

Analysis of hair follicle number was performed on H&E-stained sections (Fig. 3d). Skin from the HSP70^{out} group initially contained a greater number of sebaceous-hair complexes compared to the other groups. Subsequent changes in this parameter in transgenic mice were opposite to those in wild-type animals. By Day 11, transgenic lines showed a reduction in the number of sebaceous-hair complexes, most pronounced in HSP70ⁱⁿ animals, where the count dropped to 2.0 per field of view, with density increasing toward the periphery of the experimental area. On Day 11, wild-type skin presented a marked increase in sebaceous-hair complex numbers, with a relatively uniform distribution in the dermis, similar to that in the HSP70^{out} group. The HSP70^{out} group on Day 11 presented intermediate values compared to WT and HSP70ⁱⁿ groups; however, due to the initially higher number of complexes in this group, the values were closer to WT group. By Day 17, both transgenic and wild-type animals returned to

intact values: an increase in the number of sebaceous-hair complexes in the transgenic animals and a decrease in WT mice. Notably, despite the reduction in WT mice to 5.97 per field—similar to Day 0 values—the hair follicle components were hypertrophied, suggesting the presence of a chronic and fairly pronounced inflammatory process. By Day 17, the experimental skin areas of HSP70in and HSP70out animals exhibited an increased number of sebaceous-hair complexes per field of view, reflecting a similar pattern of changes across both lines.

FIGURE 3

Analysis of total dermal cellularity in the context of extracellular and intracellular HSPA1A form overexpression in the hIAD model

To visualize collagen fiber distribution, assess hair follicle number, and analyze cellularity per field of view, sections were stained with Mallory's trichrome (Fig. 4a).

On Day 11, all three groups presented polymorphonuclear inflammatory infiltrates in the superficial dermis, subepidermal and perivascular areas, as well as nuclear debris within granulation tissue, beneath which reepithelialization of the damaged skin area was observed. By Day 17, compared to Day 11, a reduction in the number of inflammatory infiltrates and the area of erythrocyte extravasation were noted. Skin from WT and HSP70in animals was characterized by more prolonged persistence of morphological signs of inflammation compared to the HSP70out line, which explains the later onset of the remodeling phase in the experimental skin area.

Total cellularity was analyzed on Mallory-stained sections (Fig. 4b). Cells from sebaceous-hair complexes, vessels, and adipose tissue were excluded from the count. Quantitative analysis revealed an increase in total cellularity on Day 11, followed by a decrease by Day 17 in all groups. While total cellularity does not directly indicate the severity of the inflammatory process as an increase may reflect not only alteration and exudation but also reparative regeneration in the present study, this parameter may indirectly reflect the degree of infiltrative changes and the rate of skin architecture restoration, taking into account the well-established timelines for each phase. On Day 11, all groups showed a general increase in cell number, attributed to heightened skin reactivity following DNCB application. HSP70in and WT displayed no statistically significant differences; however, the increase in this parameter was least pronounced in the HSP70out line and was significantly different from the other groups. By Day 17, all the groups exhibited a decrease in cell count, with no statistically significant differences between the animals.

FIGURE 4

Morphological assessment of elastic fiber distribution and dermal mast cell infiltration in the context of extracellular and intracellular HSPA1A form overexpression in the hIAD model

To evaluate the distribution of elastic fibers and the degree of dermal mast cell infiltration, sections were stained with Weigert's resorcin-fuchsin followed by toluidine blue counterstaining (Fig. 5a). The skin in

the affected area showed a complete absence of elastic fibers in the HSP70in and WT groups. A clearly discernible boundary between the experimental area and relatively preserved skin is shown in Fig. 5a (Day 11 column). The number of elastic fibers in the experimental area of all the groups remained virtually unchanged by the final day of the experiment, likely associated with enhanced neocollagenogenesis at the DNCB application site.

Morphometric analysis of mast cell counts revealed an overall trend toward an increase on Day 11 compared with Day 17 in all groups. Animals of the HSP70in line, despite an initially higher baseline mast cell level relative to WT, exhibited a lower mast cell count on Day 11. By the end of the experiment, the number of mast cells in this group increased sharply and substantially exceeded the values of the other groups. In the experimental skin areas of all animal groups, mast cells were distributed diffusely. The line overexpressing the extracellular form, after an increase in mast cell numbers by Day 11 compared to the HSP70in and WT groups, did not show further elevation by Day 17 relative to the other groups. Collectively, the results obtained on Day 17 suggest a higher intensity and prolonged persistence of allergic inflammatory signs in HSP70in and WT animals compared to the HSP70out transgenic line.

FIGURE 5

Immunofluorescence assessment of α -SMA-positive microvessels in the dermal microcirculatory bed

To analyze signs of neoangiogenesis as a component of tissue remodeling, mouse skin sections were stained by immunofluorescence using antibodies against alpha-smooth muscle actin (α -SMA) (Fig. 6a). This protein is expressed by smooth muscle and myoepithelial cells, enabling selective visualization of microvessel walls within the dermis of the examined samples. Comparative analysis of vessel counts in the experimental skin area across all groups is presented in Fig. 6b. An increase in the number of small-caliber vessels was observed in the dermis of all the animal lines. Statistical differences between groups within a given time point were minor; only on Day 11 did the HSP70out group exhibit a reduced extent of dermal vascularization compared with the WT group. By Day 17, vessel counts in the dermis showed no significant differences among groups, although all values were substantially elevated relative to their respective baseline levels.

FIGURE 6

Quantitative and qualitative assessment of HSP70 expression

Quantification of mean fluorescence intensity demonstrated an overall increase in HSP70 immunofluorescence from Day 0 to Day 17 in all three genotypes (Fig. 7b). Between Day 11 and Day 17, increased dermal staining was observed in individual cells and in areas of the extracellular matrix. Across the experimental period, HSP70-associated fluorescence was highest in HSP70out skin, lowest in WT skin, and intermediate in HSP70in skin.

Furthermore, on Day 11, in the skin of the HSP70ⁱⁿ line, the intracellular form of HSP70 was characterized by more pronounced intranuclear localization compared to the HSP70^{out} line, where cytoplasmic localization of the protein predominated. On Day 17, the skin of the HSP70ⁱⁿ line also predominantly contained cells with intranuclear HSP70 localization, whereas such cells were virtually absent in the skin of the HSP70^{out} line.

FIGURE 7

Qualitative assessment of COL1A1 expression

Qualitative assessment of collagen fiber distribution density was performed on sections stained with Mallory's trichrome (Fig. 4a) and using antibodies against the alpha-1 chain of type I collagen (COL1A1) (Fig. 8).

On sections immunostained for COL1A1, areas of locally increased fluorescence were observed on Day 17. This feature was most pronounced in HSP70ⁱⁿ skin and least pronounced in HSP70^{out} skin. These areas may correspond to zones of active neocollagenogenesis. A similar distribution was observed in Mallory-stained sections, where the corresponding regions contained increased numbers of cells with fibroblast-like morphology. By Day 11, collagen fiber staining appeared increased in all three genotypes and remained elevated during the remodeling period relative to baseline.

FIGURE 8

Discussion

In the present study, the hIAD model was selected to investigate the effects of the inducible HSPA1A form on skin regeneration. DNCB covalently modifies cell surface proteins and, in the early stages, induces a Th2 cellular response [4]. The choice of this model was based on the AD-like effects exerted by DNCB in *in vitro* cell line experiments. Exposure of human monocytic THP-1 cells to DNCB leads to their metabolic reprogramming [26–27] and increased IL-12 expression [28]. Treatment of NCTC 2544 epithelial-like cells with DNCB revealed elevated total IL-18 levels [29]. In turn, IL-12 and IL-18 are proinflammatory cytokines that stimulate IFN- γ production by Th1 cells [30]. Additionally, IL-18 mediates the differentiation of naive CD4 + T cells into Th2 cells and induces the secretion of the type 2 cytokines IL-4, IL-5, IL-13, and IFN- γ [31], which are involved in AD pathogenesis [32]. The immune response of NCTC 2544 cells to DNCB is partially mediated by the LMWHA/TLR4/NF- κ B signaling axis [33]. Thus, the DNCB-induced allergic dermatitis model is a classic and widely used model, allowing its application as a positive control in the development of novel therapeutic approaches [34].

Epidermal hyperplasia is a hallmark of allergic dermatitis [35]. By Day 17 of the experiment, skin samples from the HSP70^{out} group exhibited distinctly different changes in epidermal thickness compared to the other groups, characterized by a trend toward a decrease in this parameter by Day 17. Wang et al. (2011) demonstrated the ability of cultured keratinocytes to release HSP70 into the extracellular environment *in vitro*, as well as to selectively uptake this protein and its peptide complexes in the presence of HMGB-1,

IL-27, and TNF- α [36]. Their study also revealed a stimulatory effect of HSP70-peptide complexes on IFN- γ synthesis by CD3 + T cells. Elistratova et al. (2025) reported increased HSP70 expression levels in keratinocytes of allergic dermatitis patients, indicating involvement of these proteins in the stress response in AD [37]. Notably, HSPA1A has been identified among the proteins most susceptible to haptenization by DNCB [6]. Collectively, these data may explain the more pronounced and prolonged inflammatory response observed in the HSP70ⁱⁿ transgenic line upon DNCB exposure as a hapten. In our view, this response likely arises from the constitutive overexpression of the intracellular HSP70 form, its modification, and its translocation into the extracellular space following DNCB-induced damage. In turn, the anti-inflammatory effect of the extracellular HSP70 form, manifested as accelerated regeneration of the experimental skin area and partial restoration of the analyzed parameters toward baseline values, may be explained by studies on thymic stromal lymphopoietin (TSLP). HSPA1A released from keratinocytes reduces TSLP levels [38], a proinflammatory factor that drives Th2 responses in AD [39]. These findings support the notion of potential anti-inflammatory properties of the free extracellular form of HSP70, as previously demonstrated in several studies [9]. Furthermore, the etiopathogenesis and clinical features of atopic dermatitis bear considerable resemblance to autoimmune diseases [40], which may corroborate our results the increased severity and duration of inflammatory signs in the HSP70ⁱⁿ overexpression group and accelerated tissue remodeling in the HSP70^{out} transgenic group.

Morphological analysis of hair regeneration is not a classical parameter for describing AD-associated changes in the DNCB model. Nevertheless, several studies have addressed the comorbidity of atopic dermatitis and alopecia areata [41]. Moreover, cells located in the bulge region of hair follicles (HFBCs) are directly involved in skin and appendage regeneration. It is now known that HSP70 expression is higher during mid-anagen, exerting an anti-apoptotic effect on hair follicle keratinocytes and thereby preventing their regression [42]. Wild-type mice showed increased follicle depth on Day 11, unlike transgenic animals. We hypothesize that this may be related to earlier telogen phase activation in WT mice induced by the treatment.

Several studies have addressed the involvement of TSLP in AD pathogenesis but in the context of mast cells. Redhu et al. (2025) demonstrated that reduced TNF- α expression following repeated OVA exposure promotes mast cell hyperplasia and stimulates TSLP secretion by keratinocytes. The effect of eHSP70 on mast cell TNF- α expression remains ambiguous [43]. On the one hand, treatment of dendritic cells with extracellular HSP70 reduces the expression of the proinflammatory cytokines TNF- α , IFN- γ , and MCP-1 via the TLR-2/ERK/STAT3/IL-10 axis [22]. On the other hand, HSP70 release from mast cells stimulates TNF- α and IL-6 production through TLR4 [44]. Thus, the effects of HSP70 on TNF- α may be related to the form of extracellular HSP70 (free protein versus membrane-associated protein) that interacts with surface receptors. Mice of the (Tg(HSP70^{out})) line are characterized by overexpression of free protein into the extracellular space, whereas (Tg(HSP70ⁱⁿ)) animals can transport the intracellular form via multiple routes, including membrane-associated variants [45]. Under conditions of constitutive HSP70 gene overexpression, protein efflux from cells increases.

A key role in DNCB-induced allergic dermatitis pathogenesis is played by dermal infiltration by antigen-presenting cells, particularly macrophages [46]. It is known that M2 macrophages play a direct role in AD pathogenesis, their induction being mediated by type 2 cytokines [47]. Interaction of the membrane-bound extracellular HSP70 form stimulates M2 macrophages to produce TNF- α [48]. M2 macrophage-derived TNF- α closes a vicious cycle by stimulating TSLP secretion by keratinocytes, which enhances the Th2 immune response. Their activation leads to increased type 2 cytokine levels and exacerbation of AD.

Thus, in our study, the (Tg(HSP70[out])) group exhibited pronounced signs of reduced inflammation in DNCB-induced allergic dermatitis model mice. These findings underscore the importance of further investigating the morphological and microcirculatory effects of extracellular and intracellular HSP70 forms on skin regeneration, with an expanded panel of immunohistochemical markers. This approach may elucidate pathogenetic mechanisms not only of allergic dermatitis but also of other chronic inflammatory skin diseases. Given the described mechanisms, some researchers have proposed the use of synthetic and recombinant HSP70 forms for therapeutic purposes [49–50], which may be justified by the observed anti-inflammatory effects of extracellular HSP70.

Limitations of this study

1. The results obtained in mouse experiments should be adapted to humans with particular caution, as such studies may serve as a basis for subsequent pharmacological developments.
2. The immunohistochemical marker panel employed does not allow for a comprehensive characterization of all recorded effects.

Perspectives

1. Expanding the range of experimental models of inflammatory skin diseases is essential for a comprehensive, integrative assessment of effects of expression, aggregation, and regulation of proteostasis by HSP70 proteins.
2. Investigating inducers of HSP70 secretion (thymosin beta 4, ancoferd, or silver sulfadiazine), as well as studying factors that locally stimulate HSP70 expression at the lesion site, would be useful for comparative analysis of their effects versus those of HSP70.
3. A detailed analysis of molecular mechanisms (TNF- α - and TSLP-dependent pathways, profiling of the Th1/Th2 cytokine profile at the mRNA and protein levels) and use of extended panel of immunohistochemical markers (TLR2, TLR4, markers for macrophage (M1/M2) and T-cell phenotyping) are needed to study differences in skin remodeling between (Tg(HSP70[in])) and (Tg(HSP70[out])) mice.

Conclusion

The present findings indicate that the predominant cytoplasmic localization of overexpressed human HSPA1A is associated with distinct trajectories of DNCB-induced skin inflammation and remodeling. The

group of HSP70^{out} mice showed faster resolution of dermal infiltrates, lower mast cell numbers, reduced epidermal hyperplasia, and partial preservation of elastic fibers, whereas HSP70ⁱⁿ mice displayed more persistent inflammatory changes. These observations support a localization-dependent role of HSPA1A in skin repair; however, the molecular mechanisms underlying the divergent responses require direct experimental validation.

Abbreviations

AD

allergic dermatitis

BSA

bovine serum albumin

Blmh

bleomycin hydrolase

COL1A1

collagen type I alpha 1 chain

DAMP

damage-associated molecular pattern

DNCB

2,4-dinitrochlorobenzene

DNFB

1-fluoro-2,4-dinitrobenzene

eHSP70

extracellular heat shock protein 70

Flg

filaggrin

hIAD

human-like atopic dermatitis

H&E

Hematoxylin and Eosin

Hrnr

hornerin

HSP70

heat shock protein 70

HSP70ⁱⁿ

transgenic line with predominantly intracellular HSPA1A accumulation

HSP70^{out}

transgenic line with predominantly extracellular HSPA1A accumulation

HSPA1A

heat shock protein family A member 1A

iHSP70
intracellular heat shock protein 70
IFN
γ–Interferon–gamma
MC903
calcipotriol
OVA
ovalbumin
PBS
phosphate–buffered saline
SASPase
skin aspartic protease
Th
T helper cell
TLR
Toll–like receptor
TNF
α–Tumor Necrosis Factor alpha
TSLP
thymic stromal lymphopoietin
WT
wild type
α
SMA–α–smooth muscle actin

Declarations

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Ethics approval

All animal procedures were approved by the Local Ethics Committee of Sechenov University (Protocol No. 04–23, 2 March 2023) and were conducted in accordance with institutional ethical standards and the 3Rs principle.

Consent to participate

Not applicable.

Consent to publish

Not applicable.

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Author Contribution

The study was conceived and designed by G.P. and Kr. P. The first draft of the manuscript was written by N. M. The figures were prepared by N. M. and E. F. Statistical analyses were performed by E. F. and V. A. Histological samples were prepared by E. Kh., V. A. and S.D. The manuscript was reviewed and edited by Ks. P., E. K., G. P., and Kr. P. The final version of the manuscript was read and approved by all authors.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

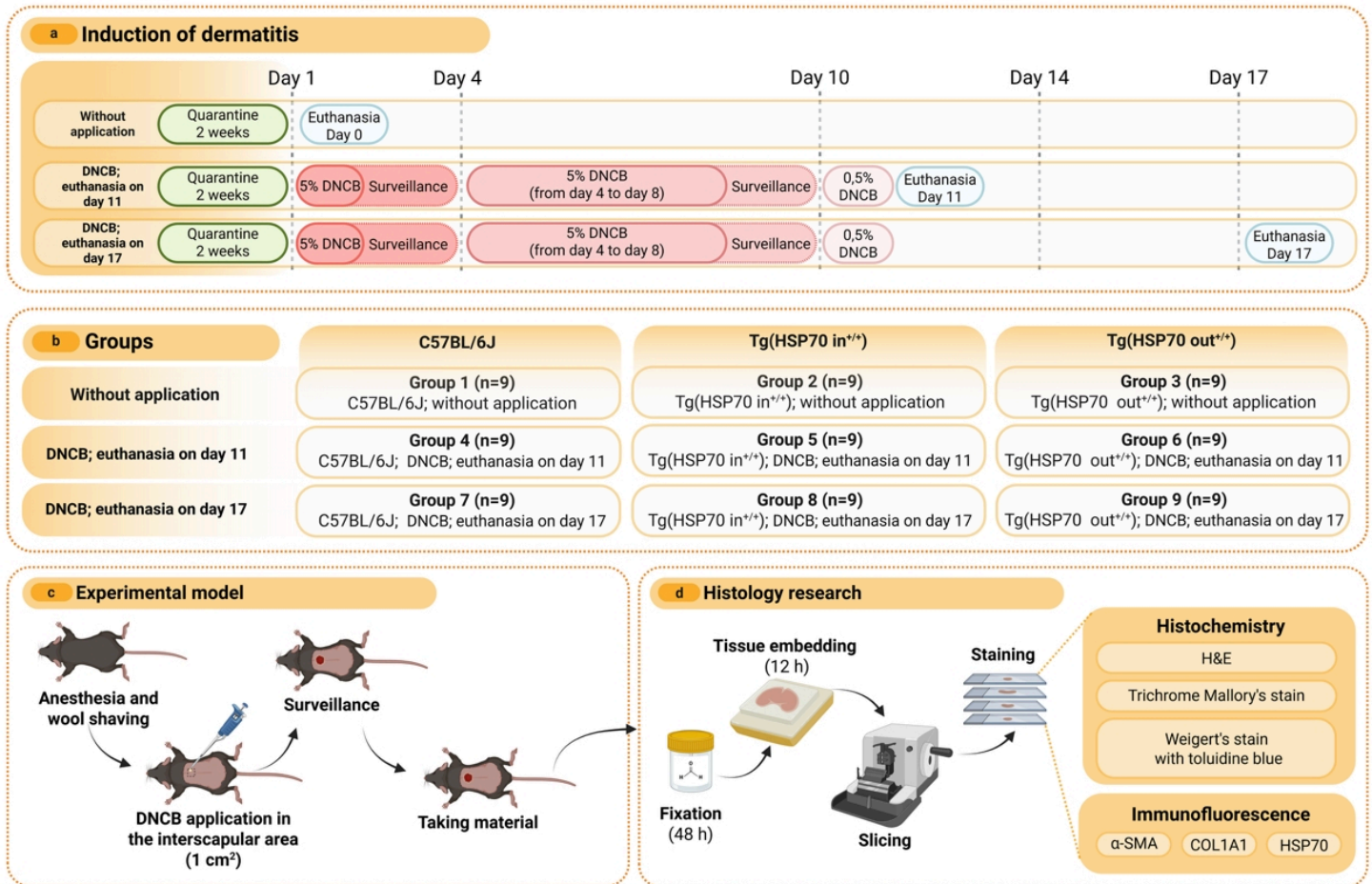


Figure 1

Overview of the experimental design. a Scheme of allergic dermatitis induction. Following a two-week quarantine, animals from the WT, HSP70ⁱⁿ, and HSP70^{out} groups received 5% DNCB on Day 1 and Days 4–8; a 0.5% DNCB challenge was applied on Day 10. Animals were euthanized at Day 0 (no DNCB exposure), Day 11, or Day 17. b Experimental groups and time-point allocation. c DNCB application to the interscapular skin before tissue collection. d Histological workflow including fixation, paraffin embedding, sectioning, histochemical staining, and immunofluorescence microscopy

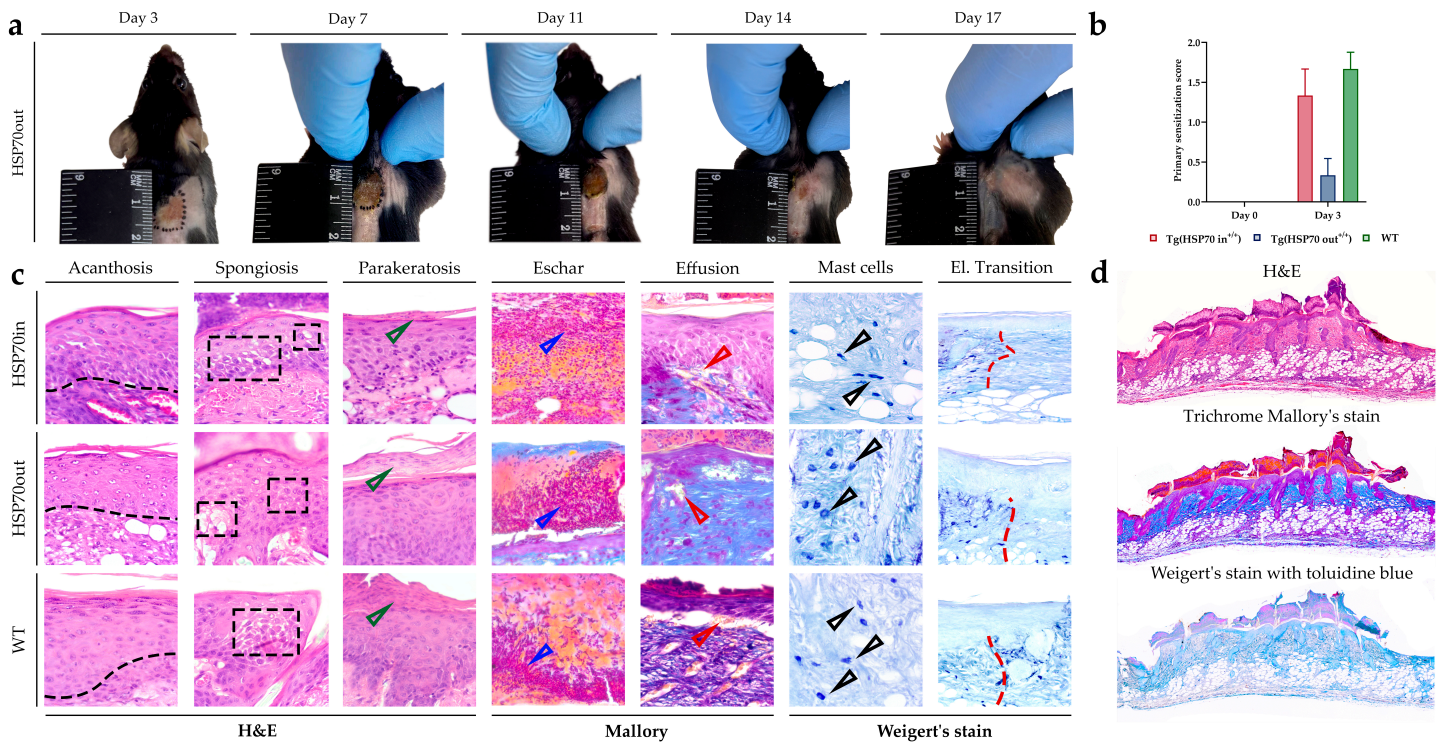


Figure 2

Macro- and microscopic changes in skin following 2,4-dinitrochlorobenzene exposure. **a** Macroscopic change in the experimental skin area illustrated by an HSP70out mouse. Moderate hyperemia was evident on Day 3, serous-hemorrhagic crusts appeared on Days 5–7, and partial restoration occurred between Days 14 and 17. **b** Assessment of primary skin sensitization following DNCB application. **c** Microscopic features of skin remodeling: acanthosis (dotted epidermal boundary), spongiosis with focal vesiculation (dotted rectangles), hyperkeratosis and parakeratosis (green arrows), granulation tissue with nuclear debris (blue arrows), focal erythrocyte extravasation (red arrows), mast cells (black arrows), and the transition between areas with reduced and relatively preserved elastic fibers (red dotted line). **d** Representative H&E, Mallory trichrome, and Weigert resorcin-fuchsin/toluidine blue staining

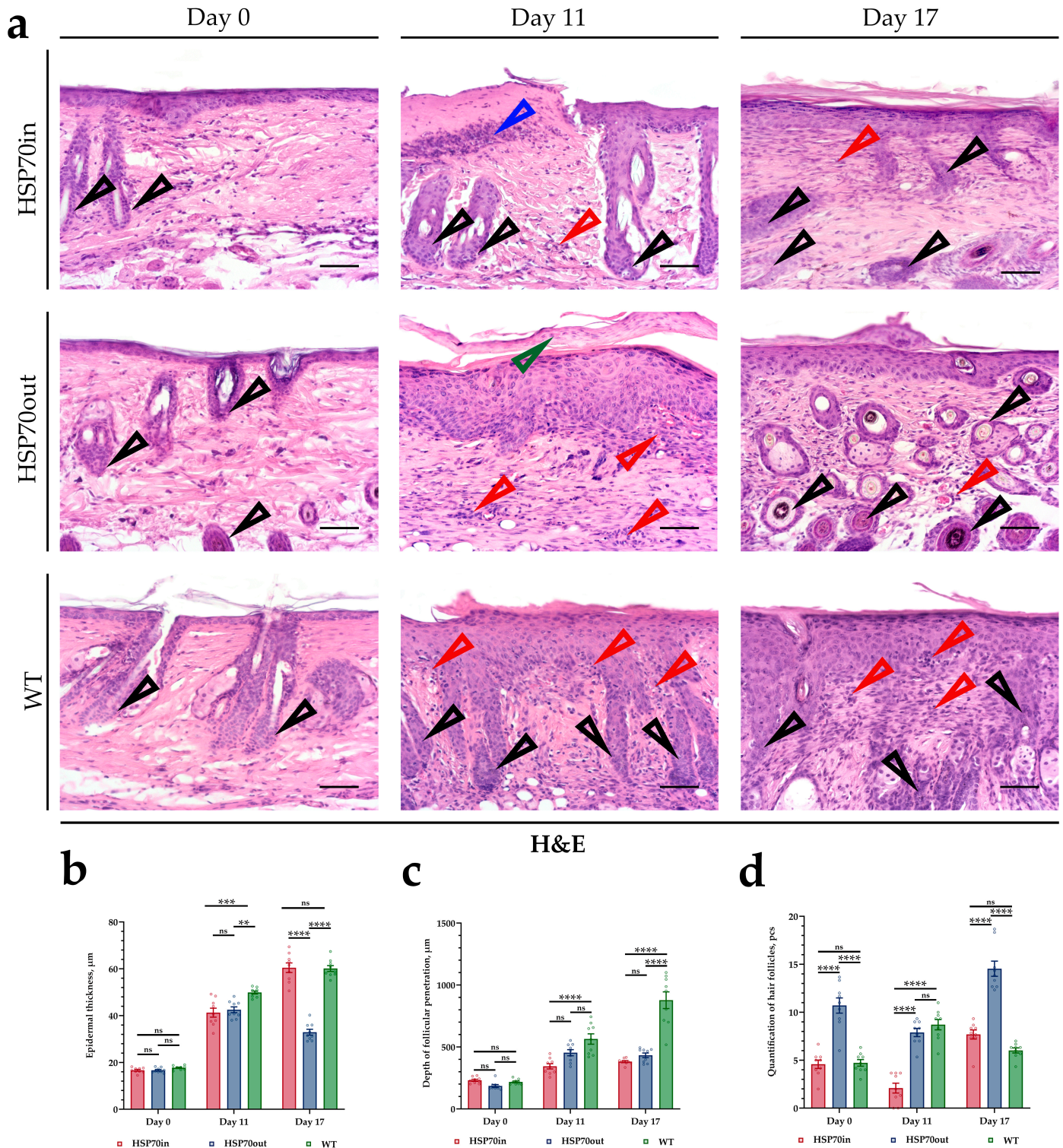


Figure 3

Skin morphology assessed by H&E staining. a Skin tissue sections in all experimental groups for Days 0, 11 and 17; scale bar = 100 μm. Green arrow, parakeratosis; black arrows, sebaceous-hair complexes; red arrows, superficial dermal infiltrates. b Epidermal thickness. c Sebaceous-hair complex depth. d Number of sebaceous-hair complexes. ns, not significant; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$

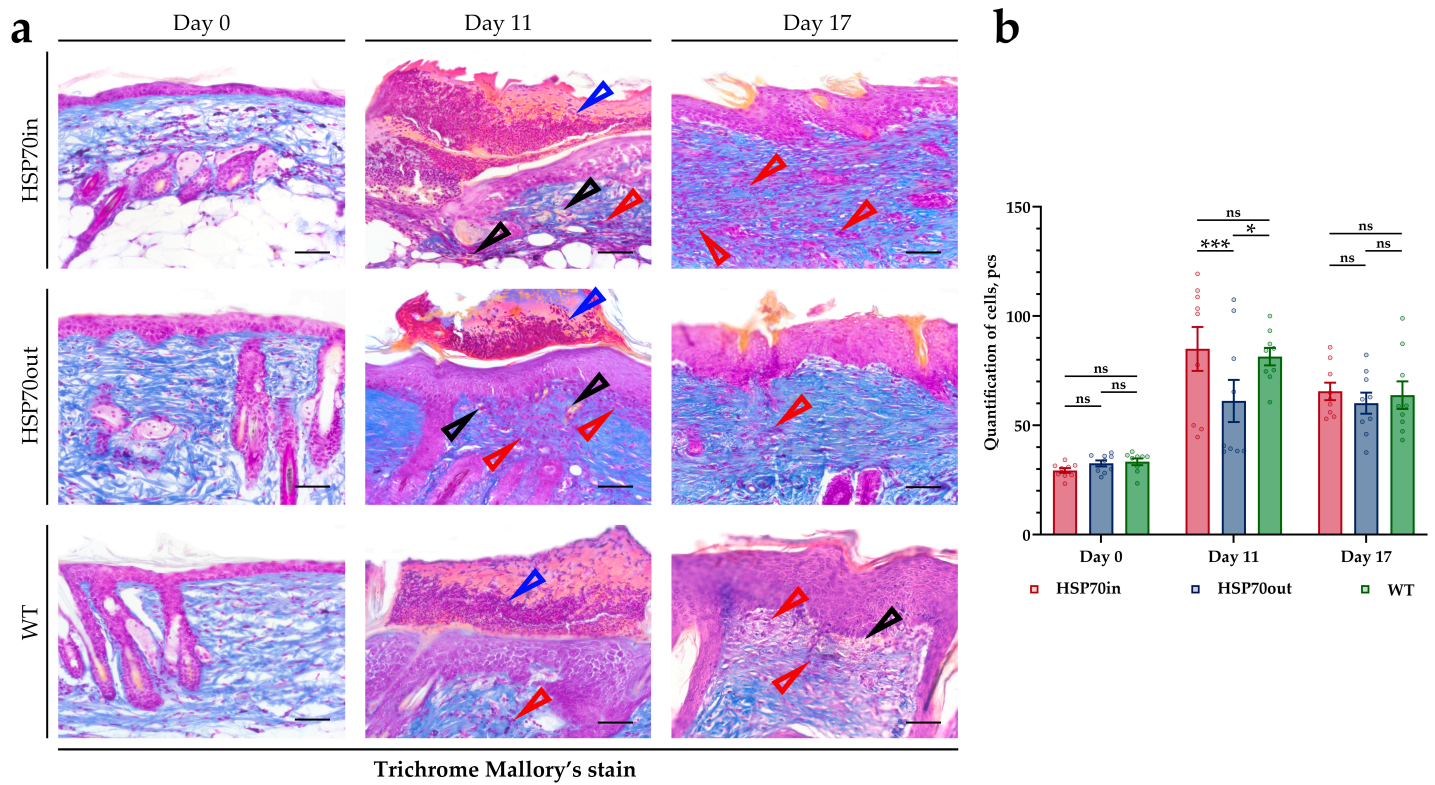


Figure 4

Skin morphology assessed by Mallory trichrome staining. a Skin with visible collagen fibers in all experimental groups for Days 0, 11 and 17; scale bar = 100 μ m. Black arrows, focal erythrocyte extravasation; blue arrows, hyperkeratosis/crust with necrotic tissue; red arrows, dermal and subepidermal infiltrates. b Total dermal cellularity. ns, not significant; * $p \leq 0.05$; *** $p \leq 0.001$; **** $p \leq 0.0001$

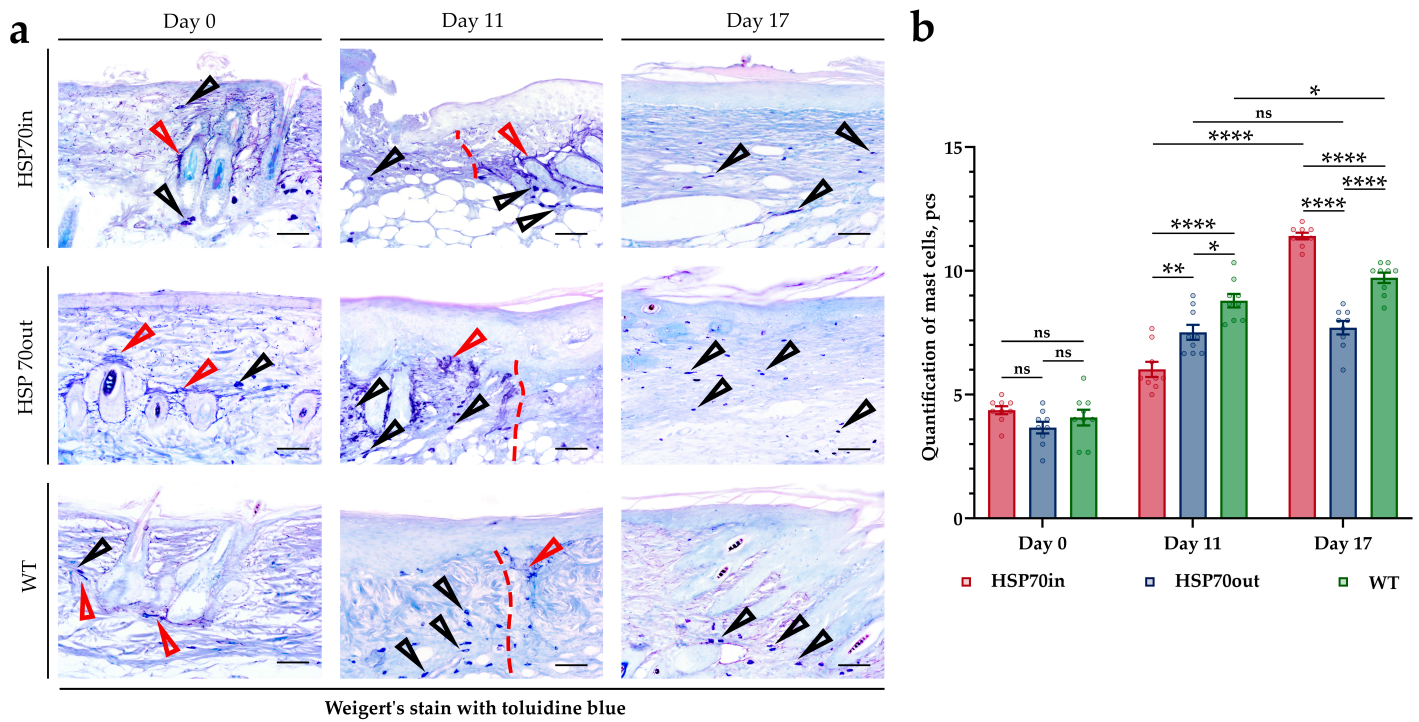


Figure 5

Elastic fibers and mast cells assessed by Weigert resorcin-fuchsin staining with toluidine blue counterstaining. a Skin tissue sections in all experimental groups for Days 0, 11 and 17; scale bar = 100 μ m. Red arrows, elastic fibers; red dashed line, boundary between experimental and relatively intact areas; black arrows, mast cells. b Mast cell counts. ns, not significant; * $p \leq 0.05$; ** $p \leq 0.01$; **** $p \leq 0.0001$

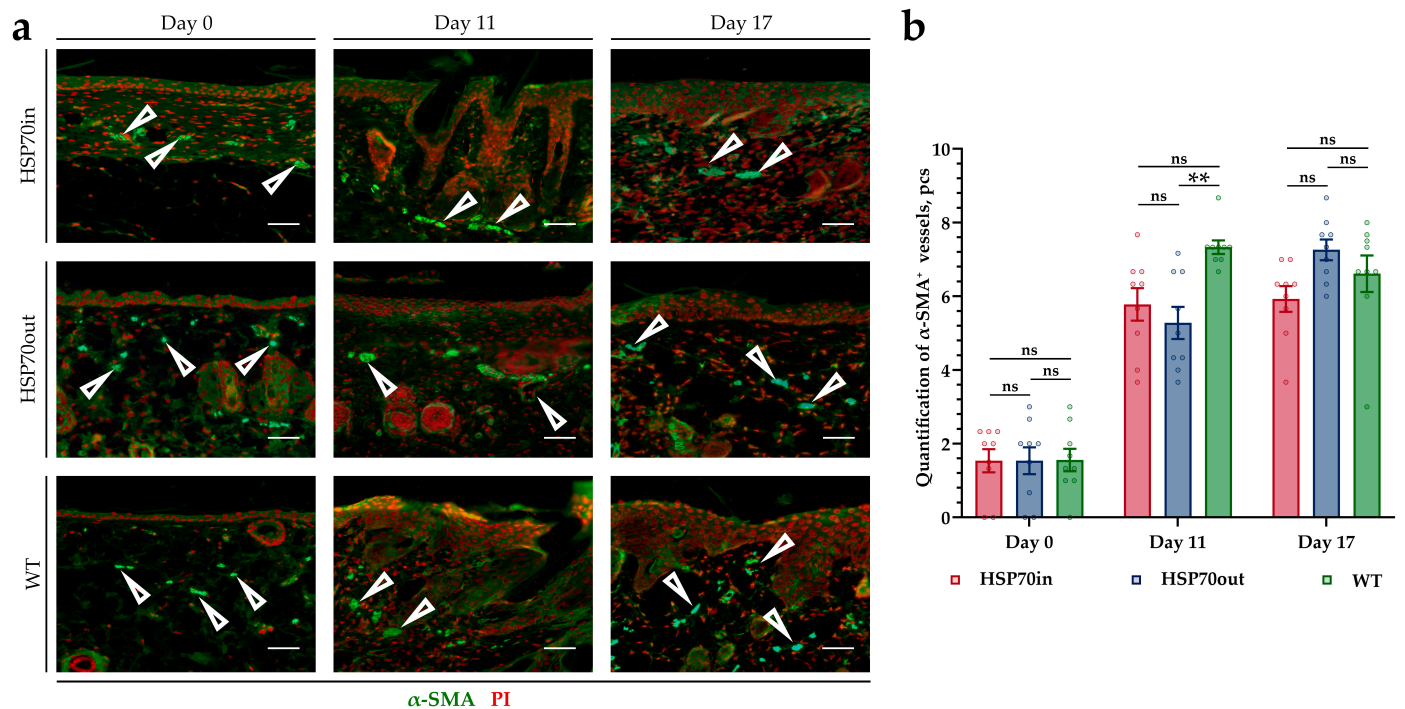


Figure 6

Dermal microvessels visualized by α -SMA immunofluorescence. a Skin sections with superficial dermal vessels (white arrows) in all experimental groups for Days 0, 11 and 17; scale bar = 100 μ m. b Number of α -SMA-positive dermal vessels. ns, not significant; ** $p \leq 0.01$

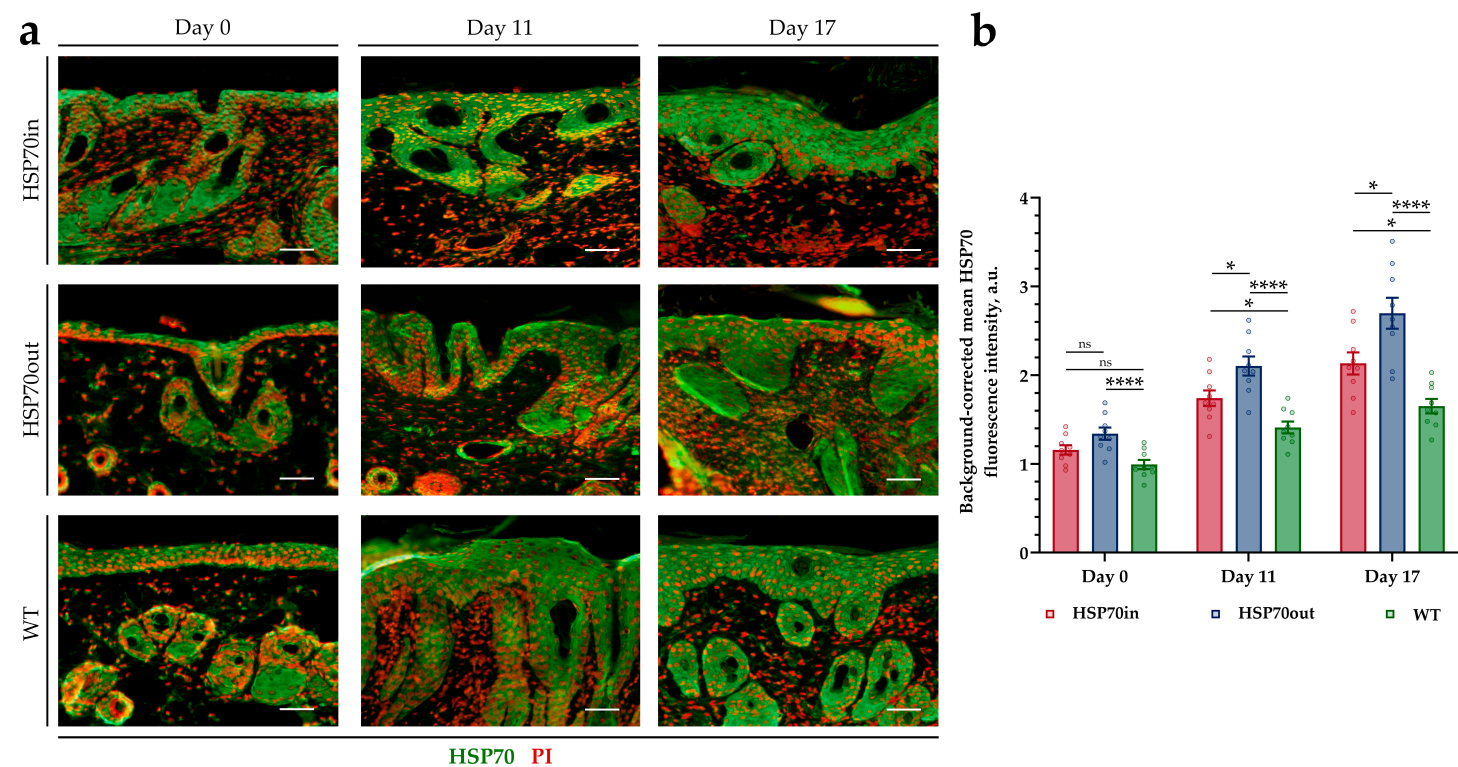


Figure 7

HSP70 immunofluorescence in skin. a Images of skin tissue in all experimental groups for Days 0, 11 and 17; scale bar = 100 μ m. b Quantification of mean fluorescence intensity. ns, not significant; * $p \leq 0.05$; **** $p \leq 0.0001$

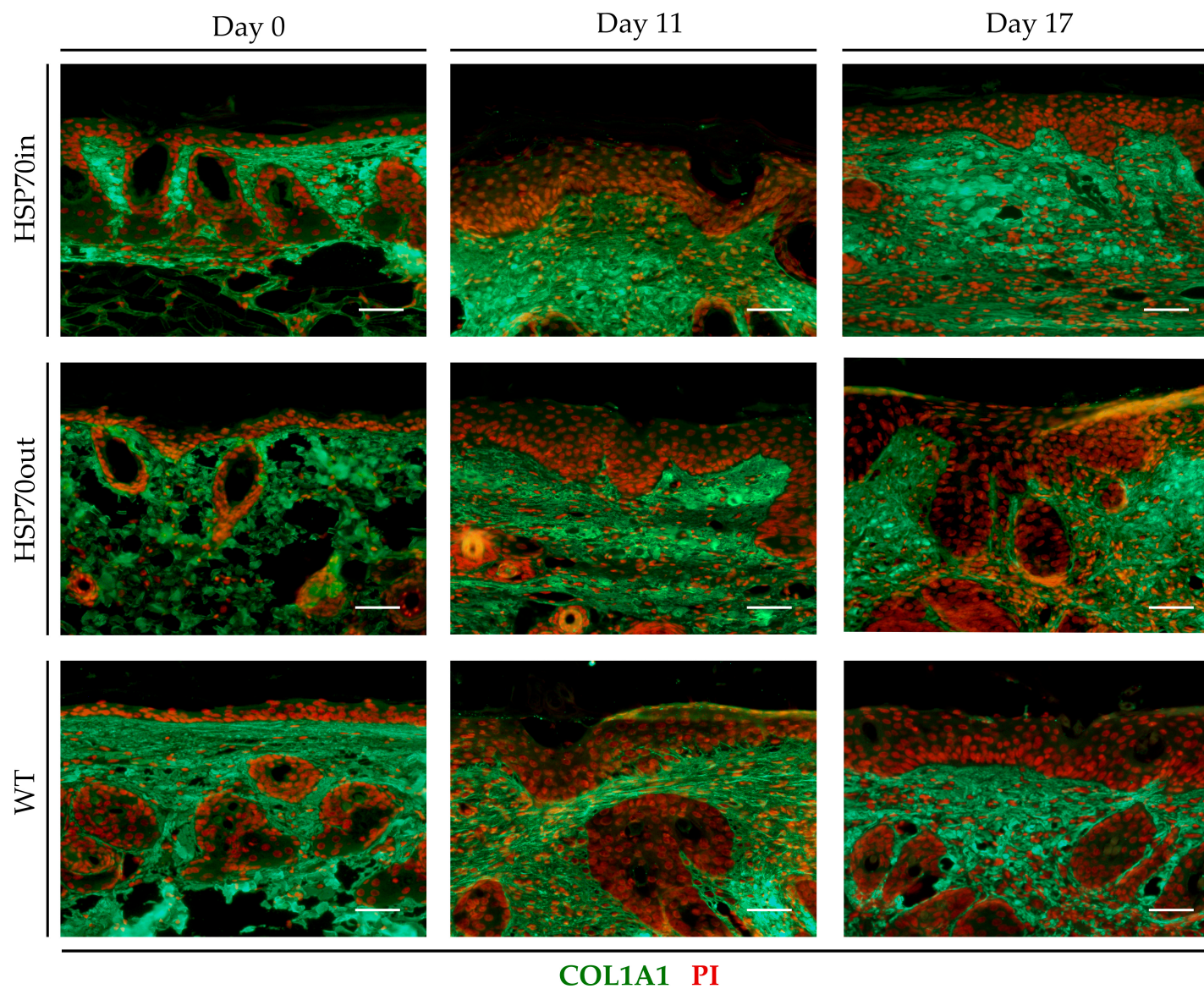


Figure 8

COL1A1 immunofluorescence in skin in all experimental groups for Days 0, 11 and 17; scale bar = 100 μ m