

## RESEARCH ARTICLE

# LC-MS/MS analysis of lesional and normally looking psoriatic skin reveals significant changes in protein metabolism and RNA processing

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## Abstract

### Background

Plaque psoriasis is a chronic autoimmune disorder characterized by the development of red scaly plaques. To date psoriasis lesional skin transcriptome has been extensively studied, whereas only few proteomic studies of psoriatic skin are available.

### Aim

The aim of this study was to compare protein expression patterns of lesional and normally looking skin of psoriasis patients with skin of the healthy volunteers, reveal differentially expressed proteins and identify changes in cell metabolism caused by the disease.

### Methods

Skin samples of normally looking and lesional skin donated by psoriasis patients (n = 5) and samples of healthy skin donated by volunteers (n = 5) were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). After protein identification and data processing, the set of differentially expressed proteins was subjected to protein ontology analysis to characterize changes in biological processes, cell components and molecular functions in the patients' skin compared to skin of the healthy volunteers. The expression of selected differentially expressed proteins was validated by ELISA and immunohistochemistry.

### Results

The performed analysis identified 405 and 59 differentially expressed proteins in lesional and normally looking psoriatic skin compared to healthy control. In normally looking skin of the patients, we discovered decreased expression of KNG1, APOE, HRG, THBS1 and PLG. Presumably, these changes were needed to protect the epidermis from spontaneous

activation of kallikrein-kinin system and delay the following development of inflammatory response. In lesional skin, we identified several large groups of proteins with coordinated expression. Mainly, these proteins were involved in different aspects of protein and RNA metabolism, namely ATP synthesis and consumption; intracellular trafficking of membrane-bound vesicles, pre-RNA processing, translation, chaperoning and degradation in proteasomes/immunoproteasomes.

## Conclusion

Our findings explain the molecular basis of metabolic changes caused by disease in skin lesions, such as faster cell turnover and higher metabolic rate. They also indicate on down-regulation of kallikrein-kinin system in normally looking skin of the patients that would be needed to delay exacerbation of the disease. Data are available via ProteomeXchange with identifier PXD021673.

## Introduction

Psoriasis is a chronic inflammatory skin disorder that affects ~2%–3% of human population, primarily in the industrialized countries. The most common form of psoriasis is *psoriasis vulgaris* or plaque psoriasis, which is characterized by scaly red skin lesions often located on the scalp, trunk and the extensor surfaces of the elbows and knees. Histology of psoriasis is characterized by incomplete degradation of cell nuclei and intracellular organelles in upper layers of the epidermis, thickening the epidermis due to hyperproliferation of the epidermal keratinocytes, hypogranulosis and massive infiltration of the skin by immune cells, as well as epidermal and vascular remodeling. Although the etiology and pathogenesis of psoriasis remain unclear, nowadays, psoriasis is regarded as an autoimmune disorder, of which the precise mechanism has to be clarified [1].

The inflammatory response in psoriasis is driven by pro-inflammatory cytokines, primarily, interleukin-17, interleukin-23, tumor necrosis factor (TNF) and interferon- $\gamma$  [2]. For a better understanding of the molecular mechanism underlying this process, mRNA profiles from the skin affected by the disease and skin of the healthy volunteers have been compared to each other using microarray and RNA-seq techniques [3, 4]. These and other studies have discovered several thousand genes differentially expressed in lesional skin and highlighted the cellular pathways involved in the pathogenesis of psoriasis. They also suggested new treatment options and drug targets [5, 6]. In the same time, the changes in mRNA expression often did not correlated with the changes at the posttranscriptional level [7] because the abundance of a protein in proteome, its location and functionality depends on the series of posttranslational events, such as post-translational modification, interactions with binding partners, refolding, proteolytic activation and degradation.

Compared with few thousands of differentially expressed genes (DEGs) revealed in the genomic studies [3–6], the authors of the first proteomic papers that had to rely on two-dimensional electrophoresis achieved very modest results and identified only several dozens of differentially expressed proteins (DEPs) [8–10]. Primarily, it happened due to limited detection range of the used staining method, low reproducibility of the experiments and difficulties in separation of hydrophobic proteins. In contrast, the more advanced LC-MS/MS technique, which is rapidly developing in our time, allowed a detection of more than 1000 DEPs in the

same kind of samples [11]. Using this technique in our study we compared the global protein expression in the skin of psoriasis patients and healthy volunteers. Based on the results of performed analysis, we revealed coordinated changes in intracellular signaling and protein and RNA metabolism caused by the disease.

## Experimental

### Ethics statement

All samples were obtained with informed written consent from healthy volunteers and psoriasis patients in accordance with Declaration of Helsinki principles. All protocols were approved by an institutional review board (I.I. Mechnikov Institute of Vaccines and Sera, Moscow, Russia).

### Collection of the skin samples

Skin biopsies for LC-MS/MS study were obtained from 5 psoriasis patients: 3 males and 2 females between the ages of 30 and 68 years (mean age 49.2 years) and equal number of healthy volunteers: 3 males and 2 females between the ages of 39 and 79 years (mean age 61.6 years). Skin biopsies for ELISA assays were obtained from 15 psoriasis patients: 8 males and 7 females between the ages of 27 and 55 years (mean age 43 years) and 10 healthy volunteers: 5 males and 5 females between the ages of 22 and 54 years (mean age 35.7 years).

The additional details on participants of this study can be found in Tables 1 and 2, respectively. The analysis of data variability in LC-MS/MS study is given in the comment to S1 Fig. The patients participated in our study discontinued systemic therapies for 2 weeks prior to biopsy collection (1 week in case of topical treatment). Each patient donated two 4 mm punch biopsies of lesional and normally looking (asymptomatic) skin following a local anesthesia. Biopsies of normally looking patients' skin were taken at least 6 cm away from the nearest skin lesion. The collected biopsies were flash frozen in liquid nitrogen and stored at  $-85^{\circ}\text{C}$  until processing.

### Preparation of the samples for analysis

Each sample was washed twice with 0.5 mL phosphate-buffered saline. The samples were homogenized by mechanical disruption in liquid nitrogen. Reduction, alkylation and digestion of the proteins were performed as previously described [12] with minor changes. Briefly, sodium deoxycholate (SDS) lysis, reduction and alkylation buffer pH 8.5, which contained 100 mM TRIS, 1% (w/v) SDS, 10 mM TCEP and 40 mM 2-chloroacetamide was added to the homogenized samples. The samples were sonicated and boiled for 10 min. Then, protein concentration was determined by Bradford assay and the equal volumes of 1% trypsin solution (w/v) prepared in 100 mM TRIS pH 8.5 were added.

After overnight digestion at  $37^{\circ}\text{C}$ , peptides were acidified with 1% trifluoroacetic acid (TFA). The samples (2 x 20  $\mu\text{g}$ ) were loaded on 14-gauge StageTips containing 2 layers of SDB-RPS discs. Respectively, 2 tips per a sample were used. The tips were consequently washed with equal volumes of ethyl acetate, 100  $\mu\text{l}$  of 1% TFA prepared in ethyl acetate and 100  $\mu\text{l}$  of 0.2% TFA. After each washing, the excess of liquid was removed by centrifugation (300 g; 1.5 min.) Then, the peptides were eluted with 60  $\mu\text{l}$  of 5% ammonium hydroxide prepared in 80% acetonitrile. The eluates were vacuum-dried and stored at  $-80^{\circ}\text{C}$ . Prior the experiment, the vacuum-dried samples were dissolved in 2% acetonitrile/0.1% TFA buffer) and sonicated for 2 min.

**Table 1. Clinical characteristics of psoriasis patients (n = 5) and volunteers (n = 5) participated in the LC-MS/MS study.**

ID	Sex	Age	Primary diagnosis	Comorbidities	PASI	Medical history
<i>Patients</i>						
1	male	30	plaque psoriasis		35	
2	male	40	plaque psoriasis		32	stage 1 arterial hypertension, hyperuricemia, obesity
3	female	48	plaque psoriasis		25	stage 2 hypertension
4	female	60	plaque psoriasis		20	stage 2 hypertension
5	male	68	plaque psoriasis	psoriatic	20	stage 2 hypertension, chronic arthritis, bronchitis, hyperuricemia, obesity 1st degree
<i>Volunteers</i>						
6	female	39				white line hernia
7	male	53				phlebeurysm
8	female	60				incisional ventral hernia
9	male	77				arthrosclerosis
10	female	79				arthrosclerosis, abdominal aortic aneurysm, hypertension, dyslipidemia, chronic pyelonephritis

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## LC/MS-MS analysis

The reverse-phase chromatography was performed on Ultimate 3000 Nano LC System (Thermo Fisher Scientific) coupled to the Q Exactive Plus benchtop Orbitrap mass spectrometer (Thermo Fisher Scientific) using a chip-based nanoelectrospray source (Thermo Fisher Scientific). The columns were packed in the lab as previously described [13]. Samples prepared in the loading buffer (0.1% TFA and 2% acetonitrile in water) were loaded on Inertsil ODS3 (GLSciences, USA) trap column (0.1 x 20 mm, 3  $\mu$ m), at 10  $\mu$ l/min and separated on Reprosil PUR C18AQ (Dr. Maisch, Germany) fused-silica column (0.1 x 500 mm, 1.9  $\mu$ m) with linear gradient of 3–35% buffer B (0.1% formic acid, 80% acetonitrile in water) for 55 min; 35–55% B for 5 min, and 55–100% B for 1 min at flow rate of 440 nl/min. Prior to injection of the next sample, the column was washed with buffer B for 5 min and reequilibrated with buffer A (0.1% formic acid, and 3% acetonitrile in water) for 5 min.

Peptides were analyzed on the mass spectrometer with one full scan (350–2000  $m/z$ ,  $R = 70,000$  at 200  $m/z$ ) at a target of  $3 \times 10^6$  ions and max ion fill time 50 ms, followed by up to 10 data-dependent MS/MS scans with higher-energy collisional dissociation (HCD) (target  $1 \times 10^5$  ions, max ion fill time 45 ms, isolation window 1.4  $m/z$ , normalized collision energy (NCE) 27%), detected in the Orbitrap ( $R = 17,500$  at fixed first mass 100  $m/z$ ). Other settings: charge exclusion—unassigned, 1, more than 6; peptide match—preferred; exclude isotopes—on; dynamic exclusion 40 s was enabled.

## Analysis of LC-MS data

Label-free protein quantification was performed using MaxQuant software version 1.5.6.5 [14], and a common contaminants database by the Andromeda search engine [15] with

**Table 2. Clinical characteristics of psoriasis patients (n = 15) and volunteers (n = 10) participated in the ELISA assays.**

ID	Sex	Age	Primary diagnosis	PASI	Disease history, years	Medical history
<i>Patients</i>						
1	male	27	plaque psoriasis	20	13	
2	male	43	plaque psoriasis	25	20	chronic bronchitis
3	male	50	plaque psoriasis	22	15	stage 2 hypertension
4	female	40	plaque psoriasis	25	20	
5	female	41	plaque psoriasis	35	25	
6.	male	52	plaque psoriasis	20	12	obesity, hypertension
7.	male	33	plaque psoriasis	26	16	
8.	female	51	plaque psoriasis	25	12	stage 2 hypertension
9.	female	44	plaque psoriasis	25	23	
10.	male	39	plaque psoriasis	18	15	
11.	female	42	plaque psoriasis	16	22	
12.	male	37	plaque psoriasis	26	16	
13.	female	50	plaque psoriasis	32	15	pyelonephritis
14.	male	59	plaque psoriasis	9	20	stage 2 hypertension
15.	female	41	plaque psoriasis	20	21	
<i>Volunteers</i>						
16.	female	41				
17.	female	43				
18.	female	41				pyelonephritis
19.	female	33				
20.	female	25				
21.	male	22				
22.	male	25				
23.	male	54				stage 2 hypertension
24.	male	41				
25.	male	32				

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cysteine carbamidomethylation as a fixed modification. Oxidation of methionine and protein N-terminal acetylation were used as variable modifications. Peak lists were searched against the human protein sequences extracted from the Uniprot (28.06.19) database. The false discovery rate (FDR\_ was set to 0.01 for both proteins and peptides with a minimum length of seven amino acids. Peptide identification was performed with an allowed initial precursor mass deviation up to 20 ppm and an allowed fragment mass deviation of 20 ppm. Downstream bioinformatics analysis was performed using Perseus [16] (versions 1.5.5.1). Protein groups only identified by site, only from peptides identified also in the reverse database, or belonging to the common contaminants database were excluded from the analyses. For Student's t-test, missing values were imputed with a width of 0.3 and a downshift of 1.8 over the total matrix. Two sample tests were performed in Perseus with s0 set to 0. Label free quantification was performed using a minimum ratio count of 1 [17]. The protein levels were assessed by the iBAQ (intensity-based absolute quantification) method using MaxQuant software [18]. To determine the relative abundance of identified proteins in the samples (riBAQ), we divided the obtained iBAQ values by the sum of all iBAQ values, and expressed this ratio as a percentage [19]. The proteins were considered as differentially expressed if they exhibited a fold-change of at least 1.5 and FDR was less than 0.05. The results were analyzed using Venn diagrams and heat maps.

Protein ontology analysis of the differentially expressed proteins (PO) was performed on gene ontology terms to catalog the molecular functions, cellular components and biological processes using DAVID Bioinformatics resources, 6.7 (Frederick National Laboratory for Cancer Research, USA).

## ELISA

The protein expression of NAMPT, FABP5, S100A2, AKR1B10, GLUL, TYMP in lesional and healthy skin was evaluated using ELISA kits (Wuhan Fine Biotech, China) according to the manufacturer's protocol. Briefly, standards and tested samples prepared in assay buffer were loaded on 96-well plate and incubated with immobilized specific antibody for 1h at 37°C. After washing with provided solution, the specific antibody conjugated with HRP-streptavidin was added and incubation continued for another 30 min. Then, the presence of antigen was visualized with the chromogenic substrate TMB and assayed using a microplate reader (Bio-Rad, USA) at wavelength 450 nm. The antigen was quantified with a standard curve generated with standards of known concentration.

## Statistical analysis

Data were analyzed with a two-sided, unpaired Student's t-test and were shown as mean + SE. Differences were considered statistically significant when  $p < 0.05$ . The mass spectrometry proteomics data were deposited to the ProteomeXchange Consortium via the PRIDE [20] partner repository with the dataset identifier PXD021673.

## Results and discussion

### Proteomes of normally looking and lesional skin of psoriasis patients are different from the skin proteome of healthy volunteers

LC/MS-MS analysis of the skin samples taken from psoriasis patients ( $n = 5$ ) and healthy volunteers ( $n = 5$ ) identified 899 proteins (S1 Table in [S1 File](#)) and 520 proteins were differentially expressed (S2 Table in [S1 File](#)). The numbers of identified proteins and DEPs were comparable with other proteomic studies [7, 11]. The distribution of these proteins between the groups of samples is shown on a Venn diagram ([Fig 1](#)). Sixteen proteins located in the center of the diagram were differentially expressed regardless, which two groups of samples were compared. Since the expression of 8 DEPs, namely S100A7, RPL39, NACA, RAB1A, RAB11B, CKMT1A, AHCY and TGM3, increased in normally looking skin of the patients compared to healthy skin and in lesional skin of the patients compared to their normally looking skin, they could be considered as potential disease biomarkers. Presumably, the expression levels of the named DEPs could be used to identify the individuals predisposed to psoriasis and monitor the disease progression in the patients. In contrast, the three peripheral sets of 12, 59 and 82 proteins were differentially expressed when two specific groups of samples were compared. Respectively, any of these sets could be used to describe differences between these groups. For instance, the set of 82 DEPs ([Fig 1](#)) could help to characterize the transition from normally looking to lesional skin. In the other words, it could be used to track the development of psoriatic plaque. In turn, the remaining three sets of 14, 25 and 314 proteins distinguished a specific group of samples from the others. For instance, 314 proteins mentioned above ([Fig 1](#)) were differentially expressed in lesional skin when the samples obtained from lesional skin were compared to the samples obtained from either normally looking skin or skin of the healthy volunteers. However, these proteins were not differentially expressed when samples obtained from normally looking skin of the patients were compared to the samples obtained from skin



**Fig 1. Venn diagram comparing DEPs in skin samples obtained from lesional and normally looking skin of the same psoriasis patients (n = 5) and skin of the healthy volunteers (n = 5) as assessed by LC/MS-MS analysis.** The numbers indicated in the diagram are the numbers of DEPs in compared groups of samples (FDR < 0.05, fold change > 1.5).

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of the healthy volunteers. Thus, the mentioned 314 DEPs could be used to characterize lesional skin.

### Proteins differentially expressed in uninvolved skin were overrepresented in extracellular compartments and secretory granules

When normally looking skin of psoriasis patients was compared to the skin of healthy volunteers, 59 proteins were differentially expressed. PO analysis of biological processes (S3 Table in S1 File) revealed their enrichment in platelet degranulation ( $P = 0.0076$ ). In turn, PO analysis of cellular components identified 10 terms as significantly enriched (S4 Table in S1 File). The highest enrichment was for extracellular exosomes ( $P = 5.10 \times 10^{-16}$ ), blood microparticles ( $P = 1.60 \times 10^{-14}$ ) and platelet  $\alpha$  granule lumen ( $P = 1.60 \times 10^{-4}$ ). The largest mentioned group (platelet degranulation) included two others, except HBD, which was associated only with "platelet  $\alpha$  granule lumen". This result suggested us that major differences of normally looking skin from the skin of healthy volunteers were presumably caused by differential expression of proteins involved in intercellular signaling, and particularly ones that were associated with specialized granules, such as extracellular exosomes. In turn, when the DEPs were analyzed for overrepresentation in terms of molecular functions, all identified terms had  $FDR > 0.05$  (S5 Table in S1 File).

A separate analysis of 26 DEPs with increased expression revealed no significant enrichment in terms of biological processes or molecular functions. In terms of cellular components (S4a Table in [S1 File](#)), we discovered enrichment for extracellular exosomes ( $P = 6.10 \times 10^{-4}$ ). In contrast, PO analysis of 33 DEPs with decreased expression (S3a Table in [S1 File](#)) revealed enrichment for two biological processes, namely regulation of blood coagulation ( $P = 4.70 \times 10^{-4}$ ) and regulation of coagulation ( $P = 4.00 \times 10^{-4}$ ). In both processes, DEPs were the same (KNG1, APOE, HRG, THBS1 and PLG). Their suppression could slow down the activation of kallikrein-kinin system and the recruitment of the immune cells to the sites of inflammation. Analyzing the cellular components (S4b Table in [S1 File](#)), we discovered that DEPs were enriched in extracellular region ( $P = 6.20 \times 10^{-3}$ ; FDR = 0.055). In turn, the analysis of molecular functions (S4a Table in [S1 File](#)) revealed that DEPs were enriched in heparin ( $P = 2.00 \times 10^{-4}$ ) and glycosaminoglycan ( $P = 4.50 \times 10^{-4}$ ) binding. This finding was not surprising because immunocytes and proinflammatory cytokines (e.g. CXCL8/IL8) directly interact with glycoproteins located on the surface of vascular endothelial cells. Thus, the threshold of the inflammatory response in normally looking skin of the patients supposed to be higher than in the skin of healthy volunteers. This phenomenon could be a part of an adaptive mechanism that prevents the disease from spreading over the body.

### **Proteins differentially expressed in uninvolved skin contributed to the acute phase of inflammatory response, metabolism of the traced metals and endocytosis**

The further analysis of 33 DEPs those levels were decreased in uninvolved skin compared to healthy skin also revealed several groups of proteins that contributed to the inflammatory response, metabolism of the traced metals and endocytosis. Although these biological processes were not identified by standard PO analysis, their characterization could reveal other differences between uninvolved and healthy skin.

Particularly, we discovered seven DEPs, namely KNG1, C9, ITIH4, C1S, FN1, HP and CP that participate in the acute phase of inflammatory response. Their decreased expression in uninvolved skin compared to healthy skin supports the existence of a protective mechanism that prevents the appearance of new lesions. Based on the previous findings, we proposed that low expression of C9 and C1S in uninvolved skin was needed to avoid an accidental activation of the complement pathways (classic and alternative). Moreover, the downregulation of KNG1 and PLG indicated that bradykinin signalling was not activated despite the cells expressing bradykinin receptors had to be present in uninvolved and lesional skin [21]. In addition, the decreased level of ITIH4, which is an inhibitor of elastase [22], was consistent with the absence of activated immune cells, primarily, macrophages and neutrophils. The mentioned immune cells infiltrate the skin in response to awakening the resident immune cells that secrete proinflammatory cytokines causing a dilation of dermal microcapillaries [23].

The absence of activated immune cells is also consistent with our previous statement that the complement pathways would be more difficult to activate in uninvolved skin. Otherwise, the expression of ITIH4 would be increased due to the invasion of immune cells from circulation. Moreover, a relatively low FN1 level in normally looking skin is consistent with the remodeling of blood vessels that normally occurs in lesional skin and follows the appearance of skin lesions [24]. However, it should not occur in uninvolved skin where we detected a relatively low FN1 level (S2 Table in [S1 File](#)). In addition, it is also consistent with two our previous statements that the complement pathways and bradykinin signalling were not activated since the dermal blood vessels were not dilated and there were no infiltrated immune cells in normally looking skin.



In addition, we found the decreased expression of ceruloplasmin (CP) in uninvolved skin ( $0.52 \pm 0.09$ ;  $p = 0.02$ ). According to the others, CP level was increased in the blood of psoriasis patients. The Cu/CP ratio correlated with the severity of the disease [25]. Moreover, CP is often considered as an early marker of the acute inflammatory response. In our best knowledge, this is the first study that reports of significantly lower CP level in normally looking skin compared to healthy skin. As the others discovered, the decreased CP level if it was accompanied by APOE deficiency, resulted in downregulation of VCAM-1 and ICAM-1 and delayed inflammatory response [26]. The latter is important because both VCAM-1 and ICAM-1 contribute to the activation of kallikrein-kinin system by interacting with infiltrating immune cells. In addition, the decreased expression of CP and APOE shall decrease the levels of proinflammatory cytokines, such as TNF, IL1 and -6, in the bloodstream [26, 27]. The latter could be important for two reasons. First, the named cytokines guide the immune cells to the sites where they suppose to invade the skin. Second, TNF and IL1 are also secreted by the resident immune cells that supposed to be dormant in uninvolved skin and be active during the acute phase of the inflammatory response.

In addition, we showed that haptoglobin (HP), which is a circulating biomarker of inflammation, was decreased in uninvolved skin compared to healthy skin (S2 Table in S1 File). Taking in account that HP is the primary scavenger of hemoglobin, we would like to mention that the low expression level of HP in lesional and uninvolved psoriatic skin is consistent with accumulation of heme metabolites, such as the heme precursor, protoporphyrin IX in skin lesions [28] and 10–24-fold higher losses of iron through the skin by psoriasis patients [29]. Despite HP is one of the most abundant plasma proteins, its concentration rapidly drops in the acute phase of the inflammatory response due to a receptor-dependent absorption of iron containing HP complexes by macrophages and hepatocytes [29].

Among 59 proteins differentially expressed in uninvolved skin, compared to healthy skin, we discovered six proteins that directly participate in endocytosis, namely HP, GC/VDBP, APOE, THBS1, NME1, and CP. Three mentioned DEPs (HP, CP and NME1) are directly involved in the iron metabolism. Particularly, NME1 and CP regulate the iron trafficking through the cellular membrane. NME1 promotes utilization of TF/transferrin that transfers iron from the extracellular space to the cytoplasm [30] whereas CP interacts with SLC40A1/ferroportin, the only known protein that exports iron out of the cell, and oxidizes  $\text{Fe}^{2+}$  into  $\text{Fe}^{3+}$ . Their interaction increases efflux of iron and stabilizes ferroportin. In contrast, ferroportin rapidly internalizes and degrades if CP is absent or decreased [31]. The changes in the expression of NME1 and CP in normally looking skin looked coordinated because both of them were needed to lessen the secretion of iron from the cells.

In normally looking skin, the expression of NME1, HP and CP was 2-fold less compared to healthy skin (S2 Table in S1 File). In lesional skin, the expression levels of HP and CP that were mainly synthesized in the liver did not change significantly whereas the expression of NME1 that was synthesized in the skin increased by a factor 2.5 exceeding its expression level in healthy skin in 1.3 fold. In this regard, we find the splash in NME1 expression to be consistent with higher loss of iron by lesional skin discovered by the others (e.g. [28]). In contrast, as we mentioned above, the decreased expression of NME1 in uninvolved skin could be needed to preserve iron in skin cells.

The protein of extracellular matrix THBS1 is known as an important modulator of the immune response. Its binding to the transmembrane receptor CD47 stimulates the migration of neutrophils and monocytes, activates neutrophils, stimulates phagocytosis and inhibits angiogenesis (reviewed in [32]). In addition, the deficiency of THBS1 causes a delay in differentiation of  $\text{T}_{\text{H}17}$  cells attenuating the response to proinflammatory cytokines IL17 and IFN- $\gamma$  [33].

According to our data, THBS1 and NME1 had similar expression profiles (S2 Table in [S1 File](#)). In uninvolved skin, the expression of THBS1 was ~2-fold less compared to healthy skin. In lesional skin, its expression increased by a factor 1.7 approaching its expression level in healthy skin ( $0.71 \pm 0.16$ ;  $p = 0.15$ ). As we believe, the ability of THBS1 to inhibit IL17- and IFN- $\gamma$ -dependent signaling as well as the differentiation of T<sub>h17</sub> cells should not significantly influence the inflammatory response in lesional skin. However, the low expression level of THBS1 in uninvolved skin should make it more susceptible to the activated Th<sub>1</sub> and Th<sub>17</sub> cells.

The major carrier of vitamin D (VD), vitamin D binding protein/GC globulin (VDBP/GC), transports VD precursors between skin, liver and kidney, as well as the other peripheral tissues (reviewed in [34]). The role of VDBP ligands, primarily calcitriol (1,25[OH]<sub>2</sub>D<sub>3</sub>) in psoriasis is well-documented (reviewed in [35]). Particularly, calcitriol increases the influx of calcium to the cells, increasing the level of calcium in the cytoplasm. Acting jointly, VD and calcium mediated signalling limit cell proliferation to the stratum basale and normalize the terminal differentiation of epidermal keratinocytes. Controlling the production of glucosylceramides, they also improve the skin barrier. As a modulator of innate immunity, VD induces the toll-like receptor TLR2, and its co-receptor CD14. In turn, their activation induces CYP27B1, which increases the production of calcitriol [36]. In addition, VD increases the chemotactic activity of lymphocytes (reviewed in [35]). In this regard, a partial deficiency of VDBP that we observed in uninvolved and lesional skin should contribute to well-documented VD-deficiency in psoriasis patients.

In previous reports, Jensen T et al. and Sølvssten H et al. did not find a serious impairment of VD-dependent signaling in lesional and uninvolved skin [37, 38]. They came to conclusion that the expression levels of VD receptor (VDR) and retinoic acid receptor RXR $\alpha$  were decreased in lesional skin of psoriasis patients, compared to uninvolved skin. However, they found these changes to be statistically insignificant.

Moreover, Safadi F.F, et al, did not see any visible changes in the skin of VDBP null mice although they reported that the levels of VD metabolites in the blood were low and their excretion with urine was substantially higher compared to control [39]. The former could be explained by the fact that, unlike humans, genetically unaltered mice do not suffer from psoriasis. Thus, additional studies are still needed to clarify the role of VDBP in the disease.

VDBP is also an important part of extracellular actin scavenger system (EASS). Acting in concert with gelsolin (GSN), VDBP transports actin monomers to the liver preventing their polymerization and clotting in the blood vessels. Tissue injuries and diseases are often accompanied by cell damages that cause a release of cytoplasmic actin into the circulation. In psoriasis, the levels of actin and other cytoplasmic proteins (calgranulin, calprotectin etc.) in the blood is significantly increased compared to the same parameters in control group (reviewed in [40]). Taking in account a short half-life of actin-VDBP complexes compared the half-life of free VDBR (30 min and 60 hours, respectively) we would like to propose that low VDBR levels detected by us in normally looking and lesional skin of psoriasis patients (S2 Table in [S1 File](#)) could be explained in part by its higher utilization rate in the liver followed tissue damages in the skin.

APOE is a lipid transport protein associated with lipoproteins. Being a part of a lipoprotein particle, APOE interacts with specific receptors (LDLR) located in the cellular membrane (reviewed in [41]). Their interaction initiates endocytosis that leads to the degradation of the lipoprotein particle in a lysosome. Although ~70% APOE is synthesized by the liver, the epidermal keratinocytes are also capable of expressing and secreting APOE. This locally synthesized protein enters the circulation to participate in reverse cholesterol transport and redistribution of lipids between the peripheral tissues.

Psoriasis is associated with a higher prevalence and incidence of dyslipidemia. The blood levels of total cholesterol and cholesterol associated with low-density lipoproteins ("bad cholesterol") are higher in patients, whereas high-density lipoprotein ("good cholesterol") is lower,

compared to healthy control [42]. In turn, higher levels of free and total cholesterol that were found in normally looking and lesional skin [43] could be a part of a mechanism that compensates the skin cells for lower APOE levels (S2 Table in S1 File).

The decreased expression of APOE in uninvolved skin could be the tip of the iceberg covering more significant changes in lipid metabolism and beyond. These changes might potentially impair the barrier permeability in psoriatic skin, stimulate proliferation and alter the terminal differentiation of the epidermal keratinocytes. To the notice, the previous studies discovered lower levels of esterified cholesterol, and significant changes in the composition of free fatty acids in normally looking and lesional skin of psoriasis patients [44]. Being the ligands of the nuclear receptors, known as LXRs (liver X receptors), free fatty acids and their derivatives influence a wide range of LXR controlled biological processes including ones that we mentioned above. For instance, the activation of LXRs in cultured epidermal keratinocytes by APOE-enriched lipoproteins increases the cell proliferation rate [45].

Recently, Cardoso et al. [46], reported that APOE deficiency in mice resulted in conformational changes of the lipids constituting the skin barrier and substantial increase of the barrier permeability for water (+18%) and the organic compound, E-PABA (+285%). They also discovered there higher levels of free fatty acids and altered expression of numerous genes involved in the metabolism of cholesterol. Although the skin phenotype described by Cardoso et al. in mice was different from one that we normally see in human patients (e.g. skin xanthomas would look very unusual in lesional psoriatic skin), their data could be used for an assessment of how lower APOE level might affect the barrier permeability in the skin of psoriasis patients. Primarily, their results proved that the maintenance of APOE at normal level was needed for the proper functioning of the skin barrier. Moreover, as they and the others noticed, a partial APOE deficiency could be responsible for higher losses of cholesterol in normally looking and lesional skin [43, 46].

In addition to its traditional role as lipid carrier and LDLR ligand, APOE was proven to be an immunosuppressant (reviewed in [41]). Interacting with the specific membrane receptor LILRB4, APOE activates the NF $\kappa$ B signalling pathway that inhibits the proliferation of T-cells and their infiltration into the skin [47]. In contrast, blocking LILRB4 with specific antibodies reverses the mentioned APOE suppressive effect and restores the mobility of T-cells [48]. Although Deng M, et al. and Li Z. et al. [47, 48] performed their research to evaluate LILRB4 on the role of molecular target for cancer therapy; their data could be also used to assess the therapeutic potential of APOE in psoriasis. Indeed, increased expression and secretion of APOE by the epidermal keratinocytes could interfere with the developing inflammatory response. In contrast, the decreased expression of APOE that we and the others observed [43, 49] could increase the proliferation rate of the epidermal keratinocytes and the formation of psoriatic plaques. The authors of the cited papers considered a partial APOE deficiency in normally looking skin to be an early sign of the exacerbating disease and believed that a normalization of APOE expression could cause a significant clinical improvement of psoriasis [49].

### **The proteins differentially expressed in lesional skin are responsible for higher metabolic rate accelerating pre-RNA processing, translation, protein biosynthesis and degradation**

A comparative analysis of the skin samples obtained from lesional skin of the psoriasis patients and the skin of the healthy volunteers revealed 405 differentially expressed proteins. The expression levels of 331 proteins were significantly increased whereas the expression levels of 74 proteins were significantly decreased. The DEPs with highest expression in lesional skin were S100A7, SERPINB3 and NAMPT (Table 3).

**Table 3. The most abundant proteins of lesional skin.**

Protein	Uniprot ID	LC-MS/MS Fold increase*	ELISA P-value	Fold increase*	P-value
S100A7	P31151	80.10	1.33E-05	N.D.	N.D.
SERPINB3	P29508	67.86	4.48E-03	N.D.	N.D.
NAMPT	A0A024R718	45.08	6.39E-05	3.49 $\pm$ 0.81	5.0E-04
SCCA1-SCCA2	Q5K684	35.60	2.16E-03	N.D.	N.D.
TYMP	P19971	29.56	1.25E-03	2.62 $\pm$ 1.15	4.9E-02
A2ML1	A8K2U0	26.10	5.96E-05	N.D.	N.D.
S100A8	P05109	25.80	1.12E-05	N.D.	N.D.
KRT16	P08779	25.47	3.49E-02	N.D.	N.D.
TGM3	Q08188	23.36	1.44E-03	N.D.	N.D.
FABP5	Q01469	22.17	8.79E-06	7.18 $\pm$ 1.94	3.0E-04
IDE	A0A3B3ISG5	21.35	6.99E-06	N.D.	N.D.
RPL39	P62891	18.49	2.15E-06	N.D.	N.D.
S100A2	P29034	17.63	3.96E-03	1.85 $\pm$ 0.58	4.63E-02
IVL	P07476	14.81	1.01E-03	N.D.	N.D.
AKR1B10	O60218	12.67	1.31E-05	2.86 $\pm$ 0.81	7.1E-03
GLUL	A8YXX4	12.38	2.01E-02	2.53 $\pm$ 0.78	1.3E-02
LCN2	P80188-2	12.03	9.64E-03	N.D.	N.D.
S100A9	P06702	11.78	8.77E-05	N.D.	N.D.
GSDMA	Q96QA5	11.21	3.08E-03	N.D.	N.D.
STAT1	P42224	10.82	2.69E-05	N.D.	N.D.

\* The expression level in skin of the healthy volunteers was equal to 1; N.D.—not determined.

<https://doi.org/10.1371/journal.pone.0240956.t003>

Based on the results LC-MS/MS experiments we verified the expression of six proteins, namely AKR1B10, GLUL, FABP5, NAMPT, S100A2, and TYMP, differentially expressed in lesional skin using ELISA. In ELISA experiments, we confirmed that the expression levels of all six proteins were elevated in the plaques compared to the skin of healthy volunteers (Table 3). On the other hand, the fold changes of their expression that we observed were significantly less than those detected by LC-MS/MS. This was not surprising because LC-MS/MS is more sensitive than ELISA [50]. Moreover, the protein concentrations measured by the LC-MS/MS method are often consistently higher than those measured by ELISA in the same samples [51, 52]. In addition, the differential expression of three proteins, namely NAMPT, S100A2 and TYMP was confirmed using immunohistochemistry analysis (S2 Fig).

Previously, three different groups of researchers compared the skin samples obtained from psoriasis patients and healthy volunteers using 2D gel electrophoresis followed by MS. Piruzian et al. [9] reported of the 10 most abundant proteins in skin lesions. All these proteins were also identified in our study (S2 Table in S1 File). Particularly, we confirmed the elevated expression of KRT16, SERPINB4, SERPINB3, S100A9, S100A7, KRT17 and ENO1. Moreover, the first five of them exhibited the highest expression levels in lesional skin of our patients (Table 3). On the other hand, the expression of KRT14, ENO1 and LGALS7B did not exceed 2 or 3 fold. In this regard, we would like to mention that differences between their and our data could be caused by technical problems with scanner and densitometer. Moreover, the band intensities did not suppose to be proportional to the protein amounts in the samples if they tended to approach the upper detection limit.

Carlén et al. [8] identified 11 DEPs in lesional psoriatic skin. In their paper, the authors reported of 9 proteins those expression was increased and 2 proteins those expression was decreased, compared to the skin of the healthy volunteers. Unlike us, they did not detect

KRT10 in lesional skin that had to be abundant in stratified epithelia (S2 Table in [S1 File](#)). In turn, we could not identify KRT15 that was detected in their study. Moreover, we had a problem with interpretation of the terms "SCCA2, low pI" and "SCCA2, high pI" that Carlén et al. used as "protein names". This problem could be avoided if they provided valid Uniprot IDs. After all, we confirmed the expression of SERPINB5/maspin, KRT17, GSTP1/GST- $\pi$ , HSPB1/HSP27, ARHGDI1/RhoGDI-1, CALR/calreticulin and SFN/14-3-3  $\sigma$ , although according to our data, only KRT17 ( $P = 0.040$ ), GSTP1 ( $P = 0.018$ ) and CALR ( $P = 0.004$ ) could be differentially expressed.

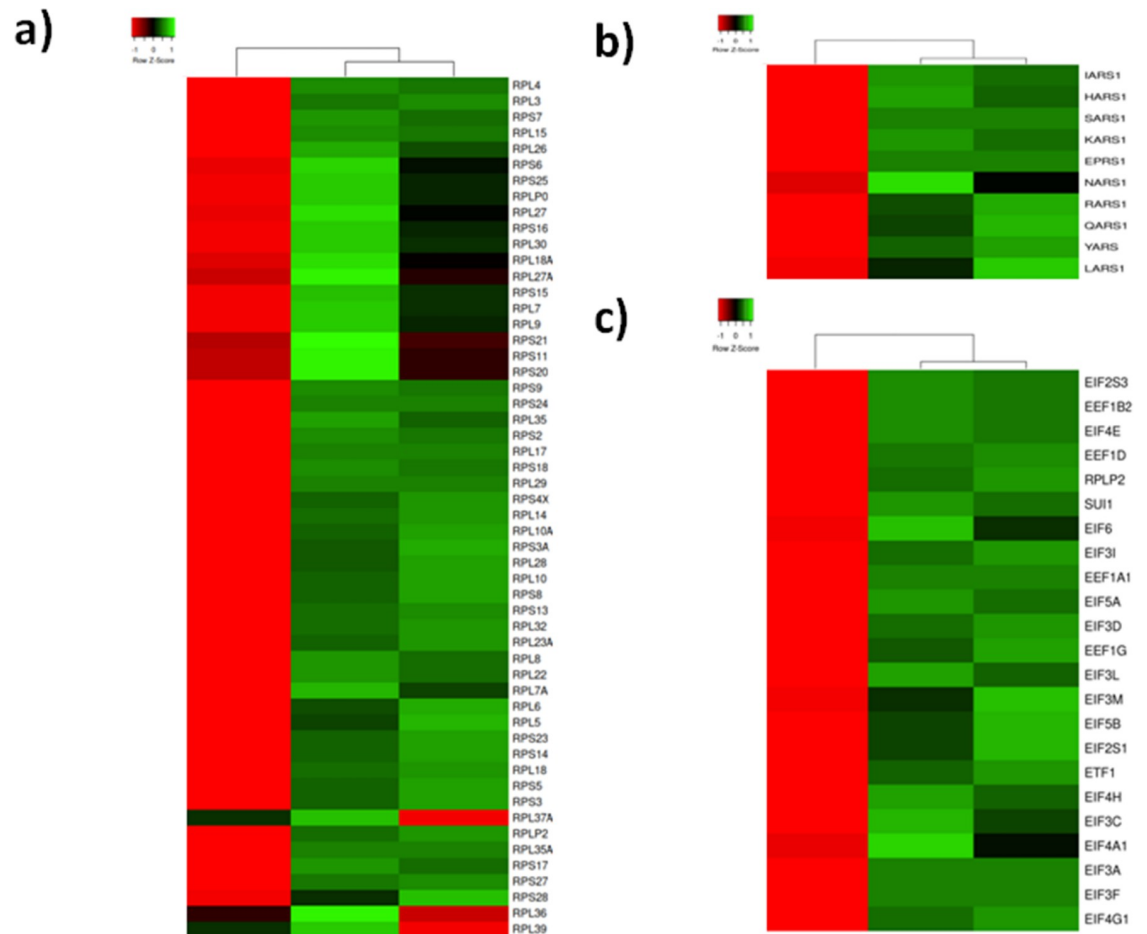
In turn, Ryu et al. [10] identified 30 DEPs in skin lesions of psoriasis patients, compared to their normally looking skin. Comparing their data with ours, we could not confirm the differential expression of TF, ATP5B, HSP90B1, PARK7, PDIA3, APCS and SERPINF1. In turn, four other proteins, namely DPYSL2, HBB, PRDX2 and SERPINA1 that Ryu et al. reported as proteins exhibiting an increased expression were suppressed in the skin of our patients. On the other hand, we confirmed the elevated expression of 17 other proteins, including GSTP1 ( $P = 0.018$ ), which was reported by them as DEP for the first time. In addition, the expression levels of the five remained proteins reported by them (HSPB1, MDH1, SFN, TUBB2C and YWHAZ), changed less than 1.5-fold in our study. In addition, we were not sure whether we and their group used the same criteria to identify DEPs because they did not provide any quantitative estimate of protein expression levels in their paper.

Comparing lesional skin and healthy control, we identified several DEPs controlling the terminal differentiation of epidermal keratinocytes. Particularly, we found that the expression level of the early differentiation marker IVL was increased ( $14.81 \pm 5.36$ ,  $P = 0.001$ ), whereas the expression level of the late differentiation marker FLG was decreased ( $0.50 \pm 0.16$ ,  $P = 0.016$ ). We also discovered the bidirectional changes in the expression of cytokeratins. The expression levels of KRT1 and KRT10 were decreased ( $0.58 \pm 0.11$ ,  $P = 0.011$  and  $0.56 \pm 0.09$ ,  $P = 0.005$ , respectively). In this regard, we would like to mention that KRT1 and KRT10 are the binding partners that interact in 1:1 ratio. In the cells, their expression had to be coordinated and changes in their expression levels be comparable to each other. The expression levels of two other cytokeratins KRT16 ( $25.47 \pm 18.69$ ,  $P = 0.035$ ) and KRT17 ( $2.85 \pm 1.38$ ,  $P = 0.090$ ) were increased.

Moreover, we identified three other cytokeratins, which were not abundant in healthy skin. In lesional skin, the expression of KRT23, the paralog of KRT14 in the human genome, was increased ( $2.79 \pm 1.01$ ,  $P = 0.002$ ). In contrast, the expression levels of KRT72 and KRT77 were decreased ( $0.49 \pm 0.21$ ,  $P = 0.05$  and  $0.04 \pm 0.03$ ,  $P = 0.003$ ). Although the role of KRT72 and KRT77 in the epidermis remains uncharacterized, their lower expression levels in lesional skin compared to healthy control were already confirmed by the others on both protein and mRNA levels [53, 54].

In addition, we showed an increased expression of psoriasin/S100A7 ( $80.10 \pm 25.67$ ,  $P = 10^{-5}$ ), S100A8 ( $25.80 \pm 8.45$ ,  $P = 10^{-05}$ ) and S100A9 ( $11.78 \pm 8.47$ ,  $P = 8E-05$ ). According to the others [11, 53, 54], the named proteins were overexpressed in lesional skin. Unfortunately, we did not identify the differential expression of the proliferation marker MKI67 that supposed to be significantly increased and the late differentiation marker LOR that had to be decreased in lesional skin.

The PO analysis showed enrichment of 46 biological processes (S6 Table in [S1 File](#)). The highest enrichment was observed in translation elongation and translation ( $P = 1.60 \times 10^{-62}$  and  $1.80 \times 10^{-48}$  respectively), positive regulation of ubiquitin-protein ligase activity, regulation of ubiquitin-protein ligase activity during mitotic cell cycle ( $P = 3.10 \times 10^{-10}$  and  $4.00 \times 10^{-10}$  respectively) and positive regulation of ligase activity ( $P = 5.50 \times 10^{-10}$ ). When PO analysis was performed separately on DEPs that exhibited an increased expression (S6a Table in [S1 File](#)), the most enriched biological processes kept the same order.



**Fig 2. Heat map representing three clusters of DEPs involved in mRNA translation, according to the average quantitative expression levels in two groups of samples.** a) Ribosome proteins; b) Aminoacyl-tRNA synthetases; c) Translation initiation elongation and termination factors; From left to right column—the results of comparative analysis for normally looking skin of the patients vs. skin of the healthy volunteers, lesional vs. normally looking skin of the patients, and lesional skin of the patients vs. skin of the healthy volunteers. Red color indicates low expression levels whereas green color indicates high expression levels. The DEPs uniprot ID, official gene symbols of the encoding genes, fold changes and Benjamini corrected P-values are shown in S2 Table in [S1 File](#).

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It is well-known that the development of psoriatic plaques is accompanied by a shift in the expression of several thousand genes [7] and changes in the mechanisms that regulate the translation of mRNAs into the proteins. Since the proteins involved in the translation machinery have different roles, we split them in several subgroups. Primarily, we recognized 23 and 31 proteins of the small and large ribosomal subunits. Most of them were underexpressed in the patients' normally looking skin compared to the skin of healthy volunteers (Fig 2A). In contrast, their expression levels were significantly increased in lesional skin of the same patients. Similarly, 10 differentially expressed aminoacyl-tRNA synthetases (Fig 2B) and 21 factors that control initiation, elongation and termination of translation (Fig 2C) were suppressed in healthy looking skin and elevated in skin lesions.

These findings were supported by the other researches. A long time ago, Freedberg reported a 7-fold increase of translation in lesional skin [55] and explained it by a delayed degradation of ribosomal proteins. Recently, Swindell et al. discovered [7] an elevated expression of translation-related proteins, such as the ribosomal proteins, in lesional skin. In turn, we noticed that

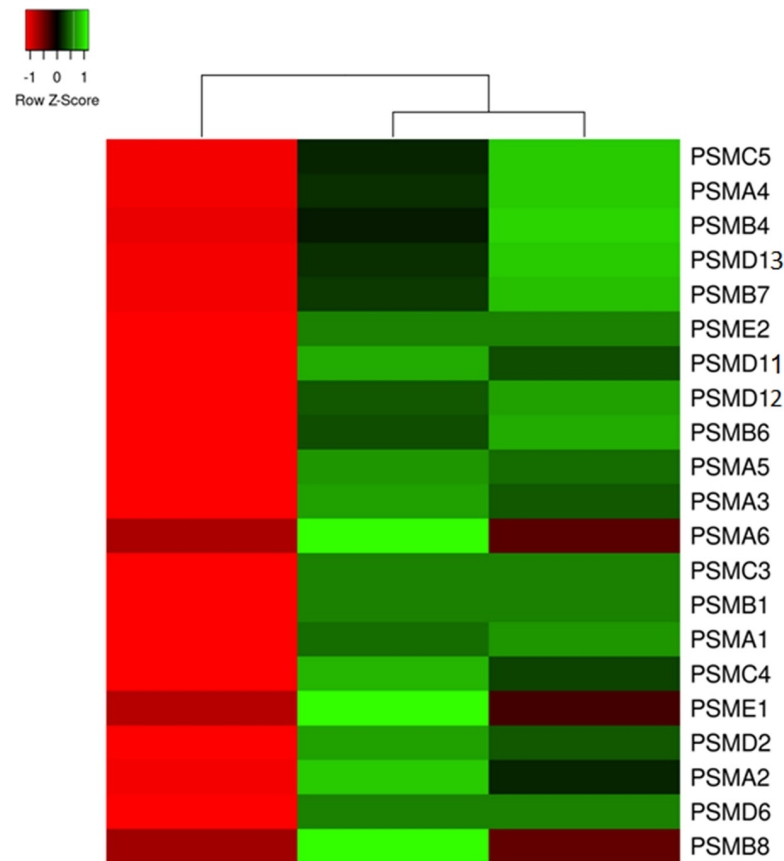
about a half of aminoacyl-tRNA synthetases and multiple translation factors were also differentially expressed. Based on these findings, we would like to hypothesize that major changes in psoriatic proteome were needed to maintain a higher metabolic rate in lesional skin.

Similarly to the other methods, ontology analysis has own limitations and some of its results could be controversial. For instance, about a half of biological processes that we presented in S6 Table in [S1 File](#) were enriched by the same group of DEPs, which was mainly composed of proteasome and immunoproteasome proteins. These biological processes were associated with different aspects of ubiquitin-protein ligase activity and its regulation. Some of these biological processes, like negative and positive regulation of protein ubiquitination ( $P = 3.10 \times 10^{-10}$  and  $8.10 \times 10^{-10}$  respectively) supposed to have the opposite outcomes. To our surprise, the same DEPs, except SKP1, which was associated only with the first one, were enriched in both of them. Taking in account that SKP1 catalyzed the ubiquitination of proteins designated for proteasomal degradation whereas the other DEPs (proteasome and immunoproteasome subunits) directly participated in the 26S proteasomal degradation pathway, we would consider a positive regulation of protein ubiquitination as the only true outcome and, respectively, disregard negative regulation of protein ubiquitination.

To the notice, the expression pattern of proteasome/immunoproteasome subunits ([Fig 3](#)) looked similar to the pattern of DEPs involved in translation ([Fig 2](#)). Most of them exhibited decreased expression in normally looking skin and increased expression in lesional skin of the patients, compared to the control group. The mentioned group of DEPs consisted 6 of 7  $\alpha$ -subunits (PSMA1-6), two  $\beta$ -subunits (PSMB1 and PSMB7) shared by proteasome and immunoproteasome, one  $\beta$ -subunit of immunoproteasome (PSMB8, a substitute of the non-mentioned  $\beta$ -subunit of proteasome PSMB5), 3 subunits exhibiting ATPase activity (PSMC3, PSMC4 and PSMC5), 4 subunits (PSMD2, PSMD6, PSMD11 and PSMD12) that did not exhibit ATPase activity and belonged to the regulatory module of proteasome (19S) and two subunits of E-type (PSME1 and PSME2) that composed the regulatory module of immunoproteasome (11S). Since all of them exhibited increased expression in lesional skin, we suggested a balance shift between 11S and 19S in favor of 11S because both its subunits (PSME1 and PSME2) were increased in skin lesions. Moreover, our data suggested that both proteasome and immunoproteasome had to be more active in lesional skin. The former is consistent with sustained inflammation in skin lesions. The latter is consistent with faster metabolism in the skin cells affected by the disease.

Enrichment analysis applied to the GO cellular component terms (S7 Table in [S1 File](#)) showed the highest representation for cytosol ( $P = 6.32 \times 10^{-56}$ ), cytosolic ribosome ( $P = 3.93 \times 10^{-54}$ ) and cytosolic parts ( $P = 3.98 \times 10^{-50}$ ). The terms proteasome complex ( $P = 3.12 \times 10^{-12}$ ), membrane-bound vesicle ( $P = 1.80 \times 10^{-4}$ ) and melanosome ( $P = 2.11 \times 10^{-10}$ ) were also enriched. In turn, the DOPs exhibiting an increased expression were enriched the same terms (S6a Table in [S1 File](#)), except eukaryotic translation initiation factor 3 complex ( $P = 1.67 \times 10^{-4}$ ), which was not enriched when all DEPs regardless their expression level were analyzed.

Similarly to the DEPs enriched in translation and proteasomal degradation (see above), most of the DEPs enriched in membrane-bound vesicles exhibited decreased expression in normally looking skin and increased expression in lesional skin of the patients ([Fig 4](#)). Primarily, we would like to mention two adaptin proteins (AP2B1 and AP2M1) that contributed to the AP-2 complex located on the surface of clathrin-coated vesicles. We would also mention CLTC, which is the heavy chain of clathrin. Moreover, we would acknowledge differential expression of several small GTPase that regulate the functioning of the clathrin-coated vesicles. One of them, RAB14 was found in vesicles that carried newly synthesized proteins from the Golgi apparatus to early endosomes. In turn, RAB11B controlled the performance of exo- and endocytosis. RAB5C secured docking and fusion of the vesicles to a target membrane. RAB7A



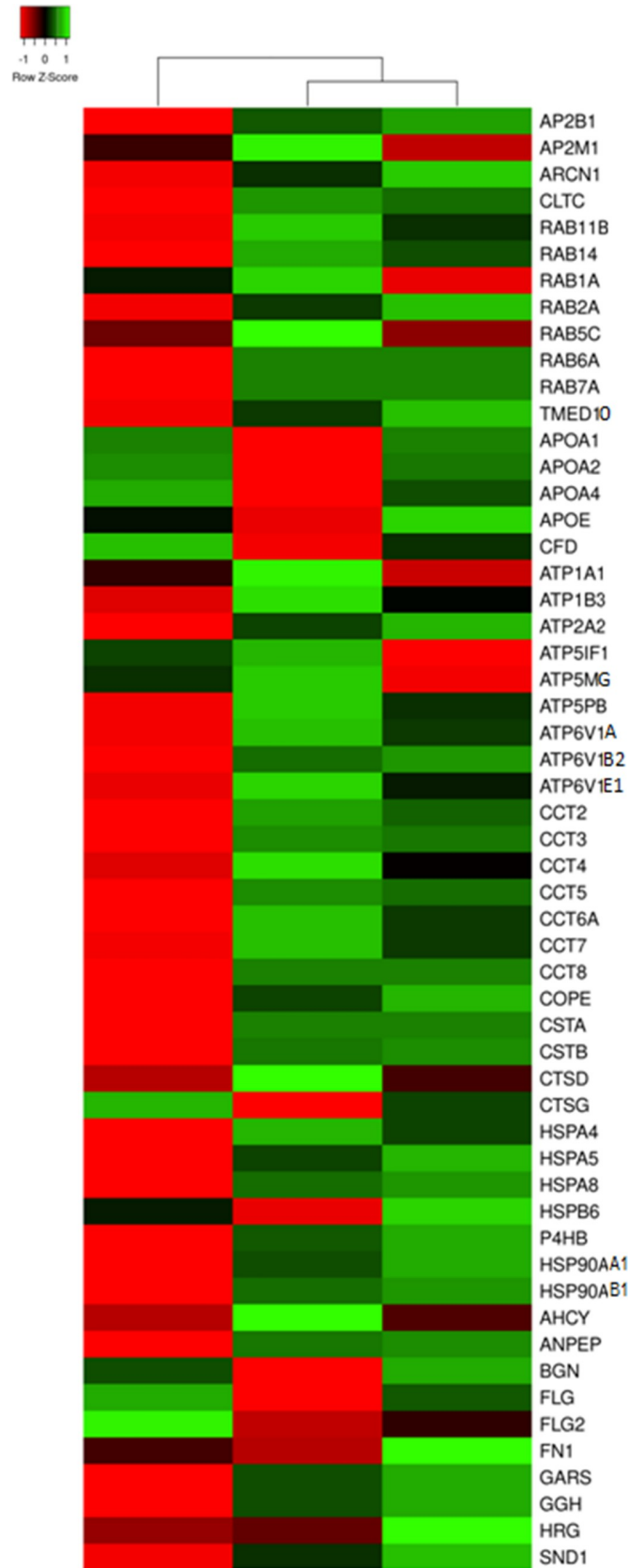
**Fig 3. Heat map representation of the differentially expressed subunits of proteasome and immunoproteasome, according to the average quantitative expression levels in two groups of samples.** From left to right column—the results of comparative analysis for normally looking skin of the patients vs. skin of the healthy volunteers, lesional vs. normally looking skin of the patients, and lesional skin of the patients vs. skin of the healthy volunteers. Red color indicates low expression levels whereas green color indicates high expression levels. The DEPs uniprot ID, official gene symbols of the encoding genes, fold changes and Benjamini corrected P-values are shown in S2 Table in [S1 File](#).

<https://doi.org/10.1371/journal.pone.0240956.g003>

regulated the formation of late endosomes as well as their transition to lysosomes. Since all these enzymes were strongly expressed in lesional skin, it would be reasonable to propose an activation of the protein trafficking toward the primary endosomes and lysosomes. This assumption is in a good agreement with our hypothesis that changes in psoriatic proteome are primarily needed to support a faster metabolism in the disease-affected skin because it links together protein biosynthesis and degradation.

Expectedly, we observed significant changes in expression of lysosomal proteins. Most of them exhibited decreased expression in normally looking skin and increased expression in lesional skin of the patients. Three of these proteins ATP6V1A, ATP6V1B2 and ATP6V1E1 were the subunits of vacuolar ATPase (v-ATPase). ATP6V1A was shown to be a catalytic subunit of the enzyme that hydrolyzed ATP. The other subunits played a role in interaction with cytoskeletal proteins and aldolases (like overexpressed ENO1 that we already mentioned in connection with Piruzian's paper), which were the major suppliers of ATP for v-ATPase. In the lysosomes, v-ATPases were needed to maintain the proper pH in their lumen. Four other identified lysosomal proteins were cathepsins (CTSD and CTSG) and endogenous inhibitors of cathepsins (CSTA and CSTB). The observed changes in their expression suggested us a balance shift between the lysosomal hydrolases due to a decreased expression of CTSG, inhibition





**Fig 4. Heat map showing mean expression profiles of the DEPs associated with membrane-bound vesicles, according to the average quantitative expression levels in two groups of samples.** From left to right column—the results of comparative analysis for normally looking skin of the patients vs. skin of the healthy volunteers, lesional vs. normally looking skin of the patients, and lesional skin of the patients vs. skin of the healthy volunteers. Red color indicates low expression levels whereas green color indicates high expression levels. The DEPs uniprot ID, official gene symbols of the encoding genes, fold changes and Benjamini corrected P-values are shown in S2 Table in [S1 File](#).

<https://doi.org/10.1371/journal.pone.0240956.g004>

of CTSB, CTSH and CTSL by CSTA and CSTB and increased expression of CTSD. The suggested imbalance of lysosomal proteins according to the others [56] could be linked to an activation of mTORC pathway (see below) and be partially responsible for incomplete degradation of the nucleus and other intracellular organelles in lesional epidermal keratinocytes.

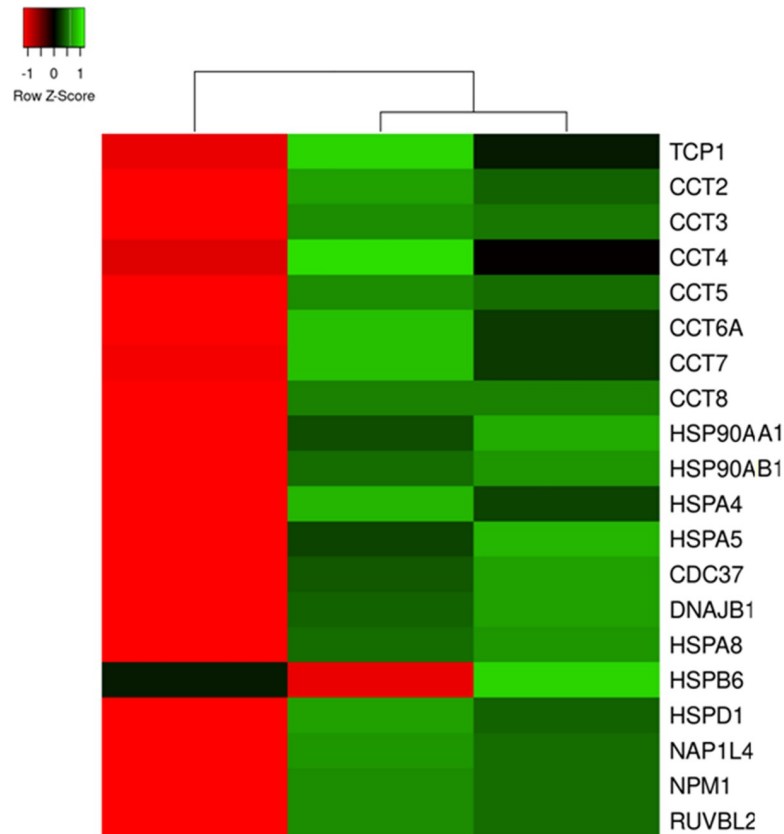
Unfortunately, some results of the PO analysis could be easily misinterpreted. For instance, we reported above that melanosome was strongly enriched for DEPs exhibited increased expression in lesional skin. However, a closer look on these proteins revealed that they were neither restricted to melanosome nor involved in the metabolism of melanin. Moreover, membrane-bound vesicles that we discussed before were also enriched for these DEPs.

In contrast to normally looking skin (S4 Table in [S1 File](#)), the extracellular components (exosomes, microparticles extracellular matrix etc.) were not enriched in lesional skin. However, similar terms, such as extracellular region ( $P = 1.12 \times 10^{-16}$ ), extracellular matrix ( $P = 3.16 \times 10^{-10}$ ) and extracellular space ( $P = 5.33 \times 10^{-8}$ ) were enriched when we performed a separate analysis of DEPs that exhibited decreased expression (S6b Table in [S1 File](#)). This observation suggested us that two different scenarios could be implemented simultaneously to prepare the epidermis for remodeling: one—to modulate the developing inflammatory response in the extracellular space and another—to accelerate the protein metabolism in the cells affected by the disease.

Enrichment analysis applied to the GO molecular function terms (S8 Table in [S1 File](#)) showed the strongest enrichment for structural constituent of ribosome ( $P = 9.37 \times 10^{-40}$ ), structural molecule activity ( $P = 9.29 \times 10^{-33}$ ) and RNA binding ( $P = 5.4 \times 10^{-23}$ ). This result did not surprise us, since proteins linked to translation, such as ribosome proteins were increased in lesional skin as we already showed above (Fig 2). We also found enrichment for unfolded protein binding ( $P = 4.25 \times 10^{-7}$ ), ATPase ( $P = 7.85 \times 10^{-5}$ ) and RNA helicase activities. The DEPs that exhibited an increased expression in lesional skin were enriched in the same terms (S8a Table in [S1 File](#)). The only new molecular function that we identified for this set of proteins was GTPase activity ( $P = 0.039$ ). In contrast, the DEPs that exhibited decreased expression in lesional skin (S8b Table in [S1 File](#)) were mainly enriched for glycosaminoglycan binding ( $P = 3.82 \times 10^{-7}$ ), phosphatidylcholine-sterol O-acyltransferase activator activity ( $P = 1.15 \times 10^{-5}$ ) and extracellular matrix structural constituent binding ( $P = 1.74 \times 10^{-5}$ ) highlighting the link between decreased expression of proteins involved in binding to proteoglycans in lesional skin and the progression of the inflammatory response.

The DEPs that were involved in unfolded protein binding were represented by four groups of chaperones, TRiC, HSP70, HSP90 proteins and nuclear chaperones. In normally looking skin (Fig 5), the expression levels of HSP70s (HSPA4, HSPA5 and HSPA8), HSP90s (HSP90AA1 and HSP90AB1), nuclear chaperones (NAP1L4, NPM1 and RUVBL2), co-chaperones (CDC37 and DNAJB1) and mitochondrial chaperone HSPD1 were decreased. In contrast, their expression levels were elevated in lesional skin regardless their location and role in the cell. The latter could be done to support accelerated mRNA translation and minimize losses due to protein misfolding in the cells affected by the disease.

Particularly, we discovered that all TRiC subunits (TCP1 and CCT1-8) exhibited elevated expression levels. Taking in account that the primary TRiC function in the cell is to fold



**Fig 5. Heat map for the expression levels of the DEPs that function as chaperones in the cell.** From left to right column—the results of comparative analysis for normally looking skin of the patients vs. skin of the healthy volunteers, lesional vs. normally looking skin of the patients, and lesional skin of the patients vs. skin of the healthy volunteers. Red color indicates low expression levels whereas green color indicates high expression levels. The DEPs uniprot ID, official gene symbols of the encoding genes, fold changes and Benjamini corrected P-values are shown in S2 Table in S1 File.

<https://doi.org/10.1371/journal.pone.0240956.g005>

misfolded cytoskeletal proteins, such as actin and tubulin as well as the components of mTORC complexes [57], we proposed that lesional skin experienced an increased demand for protein folding. Earlier Swindell et al. [7] suggested that mTOR, the key component of mTORC, was governing the hypertranslation in psoriasis. In this regard, their hypothesis is supported by our findings that there was more TRiC complexes in lesional skin. In turn, mTORC complexes were needed to organize the microfilaments and other cytoskeletal proteins in the cells and mTORCs activity supposed to be higher due to the ability of TRiC to activate mTORCs.

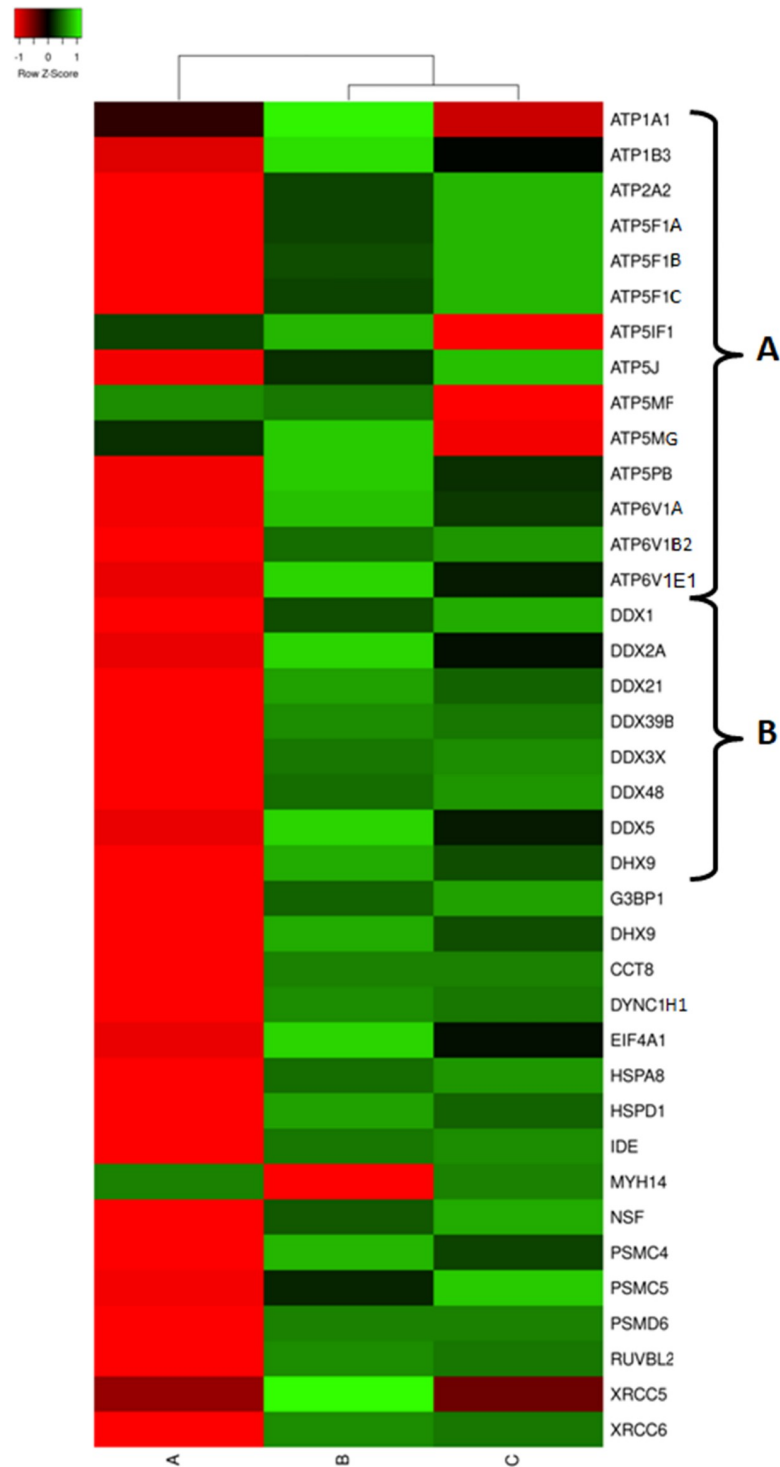
Notably, the expression profile of small chaperone HSPB6 was different from the others. Particularly, the expression level of HSPB6 was decreased in lesional skin and increased—in normally looking skin of the patients (Fig 5). To date, it is still not much known of the role that HSPB6 may play in the lesional cell. Previously, it was reported that HSPB6, a cytoplasmic co-chaperone of HSP70, was activated by oxidative stress [58] and had the ability to sequester cytochrome C, which was leaked by the mitochondria to the cytoplasm and, respectively, prevent the apoptosis [59]. In our opinion, the downregulation of HSPB6 in lesional skin could be driven by the necessity to shift the balance between apoptosis and autophagy of intracellular organelles toward autophagy and increase the cell survival rate in the epidermis.

The rapidly proliferating psoriatic epidermis is on a high demand of energy. In turn, the cellular ATPases and ATP synthase are primary consumers and suppliers of energy in the cell.

In this regard, we would like to mention a large group of DEPs, constituents of these enzymes, namely ATP1A1 and ATP1B3 ( $\alpha_1\beta_3$  Na<sup>+</sup>/K<sup>+</sup> ATPase), ATP2A2 (the Ca<sup>2+</sup>-ATPase SERCA2), ATP6V1A, ATP6V1B2 and ATP6V1E1 (the subunits of vacuolar ATPases) and ATP5F1A/ $\alpha$ -F<sub>1</sub>, ATP5F1B/ $\beta$ -F<sub>1</sub>, ATP5F1C/ $\gamma$ -F<sub>1</sub>, ATP5J/F6-F<sub>0</sub>, ATP5MF/f-F<sub>0</sub>, ATP5MG/g-F<sub>0</sub> and ATP5PB/b-F<sub>0</sub> (the subunits of ATP-synthase). All named proteins exhibited higher expression in lesional skin and lower expression in normally looking skin of the patients, compared to control (Fig 6A) suggesting higher rates for both-ATP consumption and production. Particularly, it could be needed to achieve a faster turnover of epidermal keratinocytes during their shortened life cycle. Moreover, this finding is in a good agreement with our hypothesis that changes in psoriatic proteome were aimed to maintain a higher metabolic rate in lesional skin.

In addition, we discovered a group of RNA helicases that were also upregulated in lesional skin and downregulated in normally looking skin of our patients (Fig 6B). Due to the term "RNA helicases" seems to be very restrictive we have to mention that RNA helicases are associated with many aspects of RNA metabolism and functions ranging from transcription and translation, to processing and decay (reviewed in [60]). Since RNA metabolism as we showed above was deeply affected in lesional skin (Fig 2), it was not surprising that RNA helicases exhibited a similar expression pattern. Expectedly, the differentially expressed RNA helicases identified in our study were deeply involved in any part of RNA metabolism that we named above. DDX1 was found to regulate the translation of insulin mRNA. DDX3 was shown to regulate mRNA transcription, splicing, participate in translocation of spliced mRNA to the cytoplasm and influence the translation. Moreover, DDX3 was implicated in Drosha-mediated processing of pri-miRNAs and regulation of cell cycle. DDX5 was discovered to unwind miRNA precursor duplex to facilitate the loading of guide RNA on to miRISCs. DDX6 was previously demonstrated to participate in the repression of translation, mRNA storage and decay. DHX9 was shown to influence DNA replication, mRNA transcription, processing, exporting and translation. Moreover, it was reported that DHX9 was capable of binding and resolving RNA G4 structures. DDX39B was shown to have an active role in exporting the spliced mRNA from the nucleus to the cytoplasm. Finally, DDX48/EIF4A3 was found to participate in pre-RNA splicing. In addition, DDX48 was also demonstrated to unwind the 5' untranslated region in the cytoplasm (reviewed in [60, 61]). Similarly to the other groups of DEPs that we reviewed above the differential expression of the named RNA helicases was needed to enforce rapid changes in the proteome necessary to maintain a higher metabolic rate in lesional skin.

In conclusion, performing LC-MS/MS analysis of skin samples obtained from psoriasis patients and healthy volunteers, we identified 520 DEPs that exhibited higher and lower expression levels in lesional and normally looking skin of psoriasis patients compared to the skin of healthy volunteers. The following PO analysis revealed an activation of opposite metabolic processes, such as proteins biosynthesis and protein degradation. Analyzing the proteins differentially expressed in lesional skin, we discovered 54 ribosome proteins, 10 aminoacyl-tRNA synthetases, 10 RNA helicases and 21 protein factors controlling initiation elongation and termination of translation those expression was increased. On the other hand, we detected ~20 molecular chaperones and about the same number proteasome subunits those expression levels were also elevated. We also found an increased expression of the proteins that constitute ATP-synthase and ATPases, the major suppliers and consumers of ATP in the cell. In addition, we reported of an increased expression of proteins that maintain the normal work of protein degrading cell organelles—endosomes and lysosomes. Collectively, our results explain the molecular basis of a higher metabolic rate in psoriatic skin lesions and provide new insights to the metabolic processes affected by the disease.



**Fig 6. Heat map for the expression levels of DEPs capable of ATP binding.** A—proteins constituting ATP synthase and ATPases. B.—RNA helicases. From left to right column—the results of comparative analysis for normally looking skin of the patients vs. skin of the healthy volunteers, lesional vs. normally looking skin of the patients, and lesional skin of the patients vs. skin of the healthy volunteers. Red color indicates low expression levels whereas green color indicates high expression levels. The DEPs uniprot ID, official gene symbols of the encoding genes, fold changes and Benjamini corrected P-values are shown in S2 Table in [S1 File](#).

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## Supporting information

**S1 Fig. PDF file that contains the statistical analysis of data variability in the samples of healthy, uninvolved and lesional skin.** In the figure, rows and columns refer to the individuals participated in LC-MS/MS study (Table 1). The entry in the *i*-th row and *j*-th column is the Pearson correlation  $r_{ij}$  of protein expression profiles of skin donors' *i* and *j*. The color scale at the bottom indicates the correlation strength.

(PDF)

**S2 Fig. PDF file that contains the results of immunohistochemistry analysis of the selected proteins differentially expressed in lesional skin of psoriasis patients and healthy skin of the volunteers.**

(PDF)

**S1 File. Excel-file with the raw data from all experiments and the results of PO analysis.**

(XLSX)

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