THE MODEL OF *PPARγ* DOWNREGULATED SIGNALING IN PSORIASIS

Vladimir Sobolev[1, 2], Anna Soboleva[2], Oxana Svitich[1], Evgenia Dvoriankova[2], Anastas Piruzyan[2], Dzerassa Mildzikhova[2], Irina Korsunskaya[2] and Anastasia Nesterova[3]

[1]I. Mechnikov Research Institute for Vaccines and Sera RAMS, Russian Federation, 105064, Moscow, Malyy Kazennyy per., 5

[2]Centre of Theoretical Problems of Physico-Chemical Pharmacology, Russian Academy of Sciences, Russian Academy of Sciences, Russian Federation, 119334, Moscow, Kosygin str., 4

[3]Life Science Research and Development Department, Elsevier Inc., Rockville, USA

ABSTRACT

Interactions of genes in intersecting signaling pathways, as well as environmental influences, are required for the development of psoriasis. Peroxisome proliferator-activated receptor gamma (*PPARγ*) is a nuclear receptor and transcription factor which inhibits the expression of many proinflammatory genes. We tested the hypothesis that low levels of *PPARγ* expression promote the development of psoriatic lesions. We combined experimental results and network functional analysis to reconstruct the model of *PPARγ* downregulated signaling in psoriasis. We found that the expression of *PPARγ* maybe be slightly downregulated in human psoriatic skin and laser treatment may facilitate it. We tested the reconstructed model and found that at least on mRNA level the expression of *IL17*, *STAT3*, *FOXP3*, and *RORC* and *FOSL1* genes in psoriatic skin before and after laser treatment were correlated with the level of *PPARγ* mRNA expression suggesting that genes belong to the same signaling pathway that may regulate the development of psoriasis lesion.

INTRODUCTION

Psoriasis is an example of chronic inflammatory skin disorder with a complex multifactorial origin. Multiple genes cause heterogeneous heredity of psoriasis (Nickoloff and Nestle, 2004; Peters et al., 2000). Interactions of predisposing genes, as well as environmental influences, are required for the development of the disease.

Family genotyping supports the hypothesis that different phenotypes or manifestations of psoriasis are determined by different genetic loci (Samuelsson et al., 1999). These loci are associated with psoriasis and located at least on 13 different chromosomes and are named

PSORS (Psoriasis Susceptibility) PSORS1-PSORS13 (Hız Meliha Merve, 2017). Each PSORS contains a list with several revealed genes-candidates (Barker, 2001).

Peroxisome proliferator-activated receptors (PPARs) do not get on lists of gene-candidates for psoriasis, however, the important role of PPARs in anti-inflammatory and immunomodulatory cellular signaling pathways has been established. Recently association of proline12/alanine gene polymorphism (rs1801282) in peroxisome proliferator-activated receptor gamma (*PPARγ*, NCBI Gene ID: 5468) was found to be associated with psoriasis and obesity in Egyptian patients (Seleit et al., 2019).

PPARs perform function primarily as ligand-dependent transcription factors which activate genes with PPAR-responsive elements (PPREs) in their promoter. *PPARγ* is detected mostly in well-differentiated suprabasal keratinocytes within the human epidermis (Icre et al., 2006). Human hair follicle epithelial stem cells also express *PPARγ* which maintains their survival in normal conditions (Billoni, Bruno Buan, Brigitte Gauti, 2000). Skin adipocytes and sebocytes are the next large *PPARγ* depositions (Alestas et al., 2006; Inoue et al., 2014) and the protein is vital for their differentiation (Nehrenheim et al., 2013; Paus et al., 2007). The *PPARγ* expression was reported to be downregulated in the psoriatic skin of mice and DDH1 dose-dependently could restore the gene expression (Kitahata et al., 2018). In vitro experimental models of psoriasis showed the expression of other PPARs (PPARa) was also decreased in the skin, while PPARb and PPARd expression were increased (Friedmann et al., 2005). Mice model of inflammatory skin diseases revealed that the expression of *PPARγ* and PPARa was decreased in the skin due to the absence of the *Dlx3* gene (Hwang et al., 2011). The medical suppression of *PPARγ* improved the health of the mice model of atopic dermatitis (Jung et al., 2011). Wang X at all. reported that gene *PPARγ* had high level of expression in the skin of IMQ-induced psoriasis mice, and a *PPARγ* -selective antagonist GSK3787 was able to decrease the inflammation in the skin (Wang et al., 2016). Finally, another animal model research showed that mutant mice with deleted *PPARγ* did not have sebaceous glands and normal hair follicles (HF), and developed scarring alopecia and skin inflammation (Sardella et al., 2018). There is no experimental evidence about *PPARγ* activity level in human skin of patients with psoriasis to our knowledge.

PPARγ signaling in psoriasis has been studied at a good level, but conflicting experimental results do not allow describing a clear picture of protein-protein interactions and pathological changes in cell pathways leading to the development of psoriasis (read below, section “Pathway model of PPARγ signaling in psoriasis”).

In this work we tested a hypothesis that low levels of *PPARγ* may change the activity of cellular signalling pathways in the skin and facilitate the chronic inflammatory and immune response in psoriatic lesion in humans. Based on the literature-based protein-protein interactome (PPI) and pathway analysis we proposed that low *PPARγ* activity promotes the development of psoriatic lesions due to changes in the inflammatory signaling pathways regulated by STAT3, RORC, FOXP3, FOSL1 and IL17A. To check the hypothesis, we measured the expression of these genes altogether with *PPARγ* on the mRNA level in the skin of patients with psoriasis before and after low-intensity laser treatment.

MATERIALS AND METHODS

Protein-protein interactome (PPI) analysis and pathway model reconstruction

To reconstruct the *PPARγ*-psoriasis interactome we used the literature-based database PSD (Resnet - 2020 ®, Elsevier Pathway Studio database). PSD is a mammal - centered database where relationships between biological terms and molecules extracted from published papers with natural language processing technology (NLP). Data from public databases with experimental types of connections are also present in PSD. Resnet - 2020 contains over one million objects and more than 12 million relationships with more than 55 million supporting sentences ((Nesterova et al., 2019), www.pathwaystudio.com).

For PPI analysis we used SQL language and ran queries to filter PSD connections and found inhibited by *PPARγ* expression targets that simultaneously have positive relationships with psoriasis (see "*PPARγ* targets and regulators" file, list 1 in supplemental materials). To find *PPARγ* regulators we selected genes that negatively regulate expression of *PPARγ* and simultaneously negatively regulate *PPARγ* expression targets (see "*PPARγ* targets and regulators" file, list 2 in supplemental materials). To focus only on gene expression signaling and exclude other molecular types of interaction, we considered only two types of relationships in PSD that indexed sentences about the changing of mRNA or gene expression ("Expression" and "PromoterBinding). Queries and other parameters of network filtering are available by a request.

We used Pathway Studio software to reconstruct the model of *PPARγ* signaling. Models are interactive networks which describe connections between molecules and related phenotype or biological processes. Models are kept in RNEF format, connected with PSD and include different annotations of molecules and relationships (synonymes, identificators, references, sentences, effects, mechanism of actions and more). All files can be found in supplemental materials (see below).

Pathway functional analysis

List of proteins that we had identified in the PPI analysis was set up with Sub-Network Enrichment Analysis (SNEA, Pathways Studio), Fisher exact test, Enrichr tool (Chen et al., 2013), and KEGG mapping tool (Kanehisa and Sato, 2020). SNEA was used to find cell processes statistically enriched with genes from list 1 and 2. SNEA is the modification of gene set enrichment analysis that accounts for relationships between genes in the network (Kotelnikova et al., 2010). Fisher test was used to find associated Pathway Studio pathways and Gene Ontology (GO) functional gene groups (Ashburner et al., 2000). Associated KEGG pathways we found with the KEGG mapping tool and other associations we found with Enrichr tool.

Cell processes were selected if more than 5 genes from the list 3 (combined genes from list 1 and list 2) were overlapped with total genes associated with the pathway, and if more than 5% genes from the list 1 and 2 were overlapped with a sub-network or GO group. We selected top sub-networks and KEGG pathways filtered by rank, and top PS pathways and

GO groups filtered by Jaccard index. For the comparison of methods, we selected top 50 sub-networks, 50 pathways, and 50 GO groups after manual filtering off unrelated diseases (such as cancer), viral and bacterial KEGG pathways. See supplemental materials for results of pathway functional analysis ("PPARG network analysis" file and "PPARG Enrichr analysis" file).

Microarray in-silico analysis

Public microarray data (GEO, GSE13355) was used to verify the reconstructed model of *PPARγ* signaling in psoriasis. GSE13355 contains data about the expression of the human genome in skin samples of 58 patients with psoriasis (Ding et al., 2010). DE (differentially expressed genes) were identified with a two-class unpaired T-test between samples of lesional skin of each patient (PP samples) and non-lesional skin uninvolved samples (PN samples). Multiple probes were averaged by the best p-value or maximum magnitude. Pathway Studio software was used for calculation of DE and pathway analysis.

Skin samples

We analysed biopsies from 23 patients who were treated in the V G Korolenko Hospital, Moscow Scientific and Practical Centre of Dermatovenerology and Cosmetology. Patients were diagnosed with *Psoriasis vulgaris*. The diagnoses were confirmed by the pathomorphological examination of skin biopsies. The age of patients varied from 25 to 56 years. There were 10 men and 13 women Common PASI scale for all 23 patients was 22,1±6,25 (PASI evaluates the severity of lesions between 0 and 72 score). See scores for each patient in supplemental materials, "PPARG expression" file). Local anesthesia and dermatological punch (4 mm) were used for the collection of skin samples. Healthy skin samples were taken at a distance of 3 cm from a psoriatic lesion. The research was approved by the Local Ethical Committee at the Center for Theoretical Problems of Physical-Chemical Pharmacology, Russian Academy of Science, and complies with the principles of the Helsinki Declaration. The laser treatment was provided 2-3 times a week. Skin samples were collected before the treatment and one day after the 7th laser seance.

Genes Expression

Qiagen spin column and standard RNeasy Mini Kit® for the skin were used for the RNA isolation. Additional treatment of samples with the DNAase (Qiagen) was used to remove DNA traces. RNA concentration was measured with NanoDrop 1000 (Thermo Scientific, США).

Reverse transcription was done in 200 µl volume; the mixture included the buffer, dNTP, 100 units of reverse transcriptase (M_MLV, Promega), 20 units of RNAses inhibitor (RNasin, Promega), 500 ng of oligo(dT) primers (DNA-Synthes®), and RNA sample (no more than 100 ng/µl). The mixture was incubated at 37°C for 1 hour.

Real-time PCR was performed in 96-well optical plates using fluorescent dyes SYBR Green (Eurogen®) and custom primers (DNA-Synthesis®). Primer sequences: PPAR-γ F: 5′-TCTGGCCCACCAACTTTGGG-3′ R: 5′-CTTCACAAGCATGAACTCCA-3′; STAT3 F: 5′-ACCAGCAGTATAGCCGCTTC-3′ R: 5′-GCCACAATCCGGGCAATCT-3′; IL17A F: 5′-ACAACCGATCCACCTCACCTT-3′ R: 5′- CTTTGCCTCCCAGATCACAGA-3′ ; RORC F: 5′-

GTAGAACAGCTGCAGTACAATC-3′ R: 5′-CTTCCAGGTCACTTGGAC-3′ ; FOXP3 F: 5'-TCCCAGAGTTCCTCCACAAC-3' R: 5'-ATTGAGTGTCCGCTGCTTCT-3'. PCR amplifier (Bio-Rad, CFX96™) was used for the amplification with the following program: (1) denaturation at 95 ° C for 4 min, (2) denaturation at 94 ° C for 15 sec, (3) annealing at 55 ° C for 15 sec, (4) elongation at 72 ° C for 15 seconds, (5) steps 2-4 were repeated 50 times. Levels of the GAPDH gene were used as a control for the expression of targeted genes. Amplification of the GAPDH gene and the studied genes was performed in different test tubes.

Statistical analysis

To calculate the results, we used numbers from real-time PCR reactions with primer efficiency at least 95%, 0.99 correlation coefficient and the curve (slope) -3.4 ± 0.2. PCR results were analyzed using the 2-ΔΔCT method to compare the levels of expressions detected in affected and unaffected samples [18]. Each ΔCt was calculated as ΔCt = Ct (tested gene) - Ct (GAPDH). ΔΔCt was calculated as ΔΔCt = ΔCt (psoriatic skin sample) - ΔCt (health skin sample). The experiments were repeated three times for each sample. Intergroup differences were calculated using the Mann-Whitney U-test. See results for each genes in supplemental materials, "PPARG expression" file.

Supplemental materials

All supplemental materials are available to download from ResearchGate resource by the link https://www.researchgate.net/publication/340427568_Supplemental_Materials_The_role_of_PPARg_downregulated_signaling_in_psoriasis (Sobolev, 2020). All pathways models and their annotations are available for browsing and can be downloaded at http://www.transgene.ru/ppar-pathways.

RESULTS AND DISCUSSION

Reconstruction of downregulated *PPARγ* pathway model associated with psoriasis.

For testing the hypothesis that low levels of *PPARγ* trigger inflammatory signaling pathways in the skin, we analysed protein-protein interaction literature-based network (PSD, Elsevier Pathway Studio) and several public ontologies and databases (Gene Ontology, Human Protein Atlas, KEGG, Reactome).

First, in the PSD network, we identified *PPARγ* downstream expression targets and upstream regulators (inhibitors) of *PPARγ* expression. For researching the downstream targets, we looked for genes and proteins which were reported to be inhibited by *PPARγ* and simultaneously were positive biomarkers for psoriasis. 146 associated with psoriasis genes and gene families whose expressions are repressed by *PPARγ* had been found. For researching the upstream of *PPARγ* signaling we focused on the transcriptional factors which can inhibit both the expression of *PPARγ* and his direct targets. 99 associated with psoriasis unique negative regulators of *PPARγ* had been identified. Then we combine regulators with targets to obtain 182 names of unique genes forming the *PPARγ* down-regulated sub-network associated with psoriasis (see supplemental materials, "PPARG regulators and targets" file, list 3). (Figure 1).

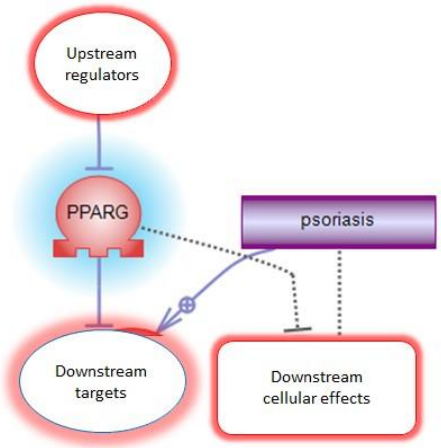

Figure 1. The logic of discovering the members of *PPARγ* down-regulated sub-network associated with psoriasis.

Comparative pathway analysis of PPARγ downregulated signaling associated with psoriasis

Several methods of pathway analysis were performed to explore the functional roles of 182 targets and regulators of *PPARγ* revealed in PPI analysis. Methods of pathway functional analysis are widely used for discovering cellular processes and signalings that are statistically associated with the list of genes or proteins (Nesterova et al., 2020).

We compared results from pathway functional analysis with three resources: Gene Ontology, Elsevier Pathways, and KEGG Pathways. Gene Ontology is the source of groups of proteins or genes manually assigned by their different functional roles. Elsevier Pathways and KEGG Pathways are manually reconstructed schemas or models of interactions between proteins describing molecular mechanisms of one or several biological processes. Gene Set Enrichment Analysis (GSEA) is a well-known method to analyse predefined and manually created collections of gene groups and pathways (Subramanian et al., 2005). Besides GSEA we used SNEA method which allows finding associated cellular processes based on literature - based PPI network. SNEA does not use predefined groups of genes or pathways and is considered less biased (Kotelnikova et al., 2010; Nesterova et al., 2020).

According to the results of comparative pathway analysis, *PPARγ* downregulated signaling is associated with adipogenesis, activation of myeloid pro-inflammatory cells (with a predominance of mast cells and dendritic cells), and activation of overall immune system response (with a predominance of Th17 cells). Also, fibrogenesis, cell-to-cell contacts, vascular-related processes and universal cell processes, such as cell proliferation or cell death, were identified (Figure 2). Cellular possesses directly associated with psoriasis were present in results from each source that we compared (Table 1).

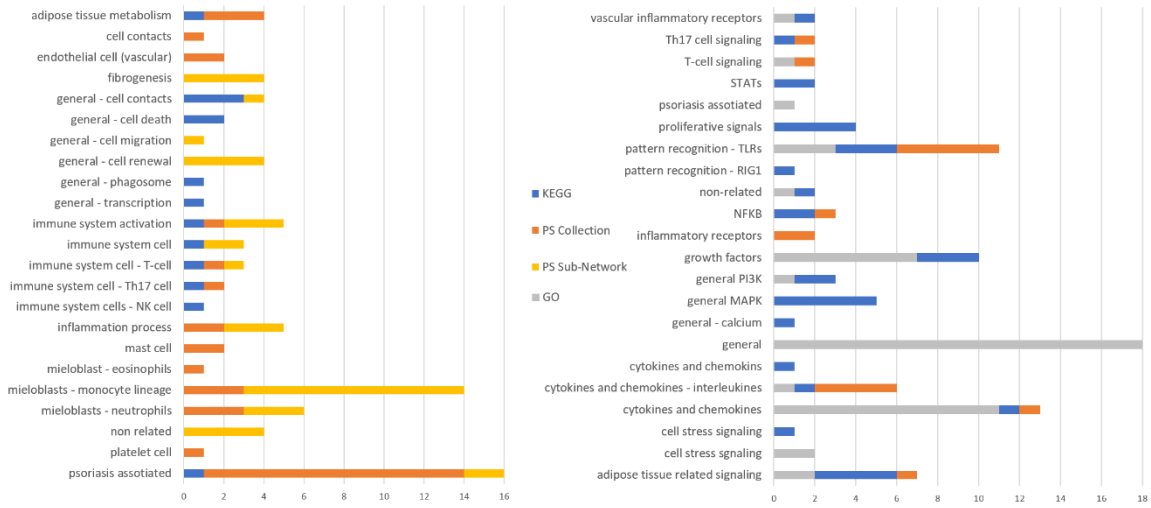

Figure 2. Cellular processes associated with members of PPARγ down-regulated signaling associated with psoriasis. Numbers are for the sum of pathways, subnetworks or GO groups in each category. Different sources are highlighted with blue (KEGG database), orange (Pathway Studio Pathway Collection), light orange (Resnet-2020 database), and grey (GO). For the complete list of results, see supplemental materials, "PPARG network analysis" file).

Table 1. Cell processes directly related to psoriasis and enriched with genes and proteins from the *PPARγ* downregulated signaling. See complete results with additional statistics in supplemental materials, "PPRAG network analysis" file.

Name of the process or signaling	Source	Rank or Jaccard similarity (the closer to 10%, the more similarity)	Category
Keratinocyte Activation in Psoriatic Arthritis	PS Collection	9.13%	Disease
lesion size	PS Sub-Network	46	Targets neighbors
keratinocyte proliferation	PS Sub-Network	100	Targets neighbors
Skin Fibrosis	PS Collection	9.09%	Disease
T-Cells Differentiation Block in Psoriasis	PS Collection	8.88%	Disease
Th17-Cell and Th1 Immune Response in Psoriatic Arthritis	PS Collection	8.82%	Disease
Dendritic Cell Dysfunction in	PS Collection	8.44%	Disease

Psoriatic Arthritis			
T-Cell Cytotoxic Response against Melanocytes in Vitiligo	PS Collection	6.20%	Disease
Synovial Fibroblast Activation in Psoriatic Arthritis	PS Collection	8.18%	Disease
Inflammatory Reaction in Acne Vulgaris	PS Collection	5.70%	Disease
Apoptotic Keratinocytes Clearance Recession in Systemic Lupus Erythematosus	PS Collection	5.69%	Disease
Atopic Dermatitis	PS Collection	5.62%	Disease
Hair Follicle Keratinocyte Apoptosis	PS Collection	5.56%	Disease
Vitiligo	PS Collection	5.07%	Disease
Melanogenesis - Homo sapiens	KEGG	n/a	Pathway
positive regulation of timing of anagen	GO	1.10%	GO: biological_process
glycosaminoglycan binding	GO	4.24%	GO: molecular_ function

Top sub-networks from SNEA were neighbours of adipogenesis and adipocyte differentiation, followed by the immune response, and T-development. The sub-networks "neighbours of monocyte recruitment or differentiation" and "macrophage differentiation" had the most percent (9%) of overlapped genes from *PPARγ* down-regulated signaling.

GSEA analysis of PS Pathway Collection and KEGG pathways resulted in many cancer-related processes. The disease taxonomy filtering with PS pathways about skin and immune system identified processes related to adipokines and IL17 signaling (Table 2). The signaling of aryl hydrocarbon receptor (AHR) in Th17 cells was the pathway with the biggest percent (48%) of overlapped genes from *PPARγ* down-regulated signaling.

Table 2. List of PS pathways associated with members of PPARγ down-regulated signaling associated with psoriasis. See complete results with additional statistics in supplemental materials, "PPRAG network analysis" file.

Pathway name	# of Entities	Overlap	Percent Overlap	p-value	Jaccard similarity	Pathway Taxonomy Top

						Category
EGFR -> Expression Targets in Skin	98	26	26	4.68E-11	10.28%	Biomarkers
GPCRs Family -> Expression Targets in Lymphoid System and Blood	89	24	26	1.97E-10	9.76%	Biomarkers
Adipokines Production by Adipocyte	58	20	34	4.8E-16	9.13%	Biological Process
Skin Fibrosis	83	22	26	3.29E-17	9.09%	Disease
Scavenger Receptor OLR1 in Inflammation Related Endothelial Dysfunction	73	21	28	2.8E-17	9.01%	Disease
T-Cells Differentiation Block in Psoriasis	52	19	36	5.87E-18	8.88%	Disease
Adipokines Production by Adipocyte Impaired in Obesity	56	19	33	2.99E-17	8.72%	Disease
CD40 -> Expression Targets in Thymus	58	19	32	4.67E-10	8.64%	Biomarkers
Lymphocyte Mediated Myocardial Injury in Myocarditis	84	21	25	6.67E-16	8.61%	Disease
IL17 Signaling in Psoriasis	49	18	36	4.08E-17	8.49%	Disease
Th17-Cell Differentiation	73	19	26	8.94E-13	8.09%	Biological Process

Among top KEGG pathways enriched with our gene list, we identified general MAPK and PI3K signaling and cancer-related pathways (for example, "hsa05200 Pathways in cancer - Homo sapiens"). TNF signaling pathway (hsa04668), as well as Th17-cell (hsa04659) and IL17 pathway (hsa04657), were also in the top 10 results. The cytokine-cytokine receptor interaction (hsa05200) and PI3K-Akt signaling (hsa04151) had the highest number of overlapped entities (48 and 34).

The list of revealed in pathway analysis molecular cascades complete the lists of cell processes.

There was no surprise that activation of general cellular flows like ERK/MAPK, RAS/ACT1, and adipose cells related AMPK, mTOR, and cAMP cascades were associated with the list of *PPARγ* targets and regulators. Also, among the top of associated molecular signalings there were well predictable inflammatory cascades like Toll-like receptors, interleukins and

interleukins receptors signaling (IL17, IL1B, IL6, and IL1R1) altogether with all-purpose cytokines and cytokines receptors signaling (CXCR3, CCR1, TNF). Signalings related to transcription factors NFKB and STATs also were significantly associated with the analysed list. GO functional group "GO: glycosaminoglycan binding"; "IL1R1 signaling in Pneumocytes'' from PS Pathway Collection; and "ErbB signaling pathway" (hsa04012) from KEGG had the maximum rank (See complete results with additional statistics in supplemental materials, "PPRAG network analysis" file). Glycosaminoglycans are essential for skin functioning. IL1R1 is a receptor commonly activated in any non-specific inflammatory processes. Finally, the ERbB/EGFR family is involved in cell proliferation and tumor development.

Additional comparison of pathway analysis results with other pathways databases (WikiPathways, Reactome, Biocarta analysed with Enrichr tool) confirmed results obtained with PS Pathway Collection (Figure 3). Pathways from all sources revealed skin inflammatory processes, TLRs and interleukins related cascades. However, the list of molecules was different compared with PS and KEGG results presenting IL10 and IL22R and no IL17 associations. In addition, analysis with DisGeNET (Piñero et al., 2017) confirmed that the *PPARγ* regulators and targets are connected with psoriasis since top diseases associated with the list 3 were: psoriasis, epithelial hyperplasia of skin, and inflammatory dermatosis. Allergic reaction, neutrophilia, and vascular diseases were also in the top 10 results (see supplemental materials, file "PPARG Enrichr analysis" file).

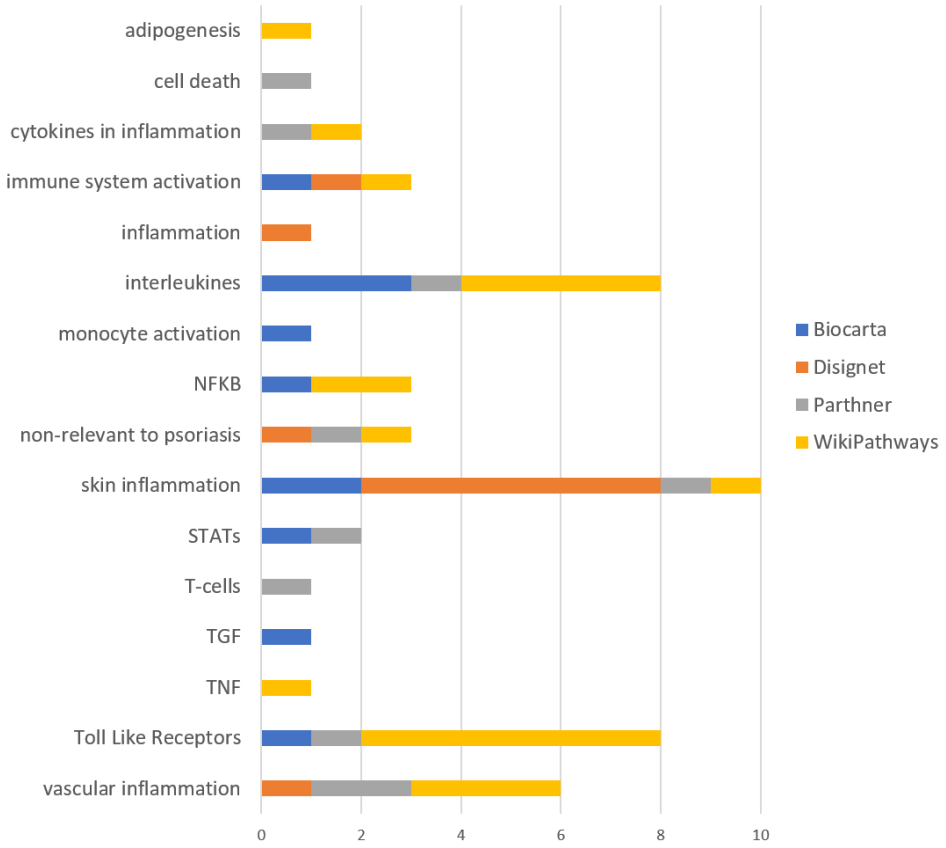

Figure 3. Comparison of results of pathway analysis (GSEA) with different sources for PPARγ down-regulated signaling associated with psoriasis. Results were calculated with the Enrichr tool. Numbers are for the sum of pathways in each category. Different sources are highlighted with blue (Biocarta database), grey (Partner database), orange (DisGeNET) and yellow (WikiPathways).

Pathway model of PPARγ signaling in psoriasis

Considering results of PPI network and functional pathway analysis we build a hypothetical model that describes cellular molecular mechanisms of involvement of *PPARγ* in the maintenance of chronic inflammatory and immune response in human psoriatic skin. Literature - based network (PSD) were used to build the model. Figure 4 described the adopted for the publication simplified version of the downregulated PPARγ pathway model. See supplemental materials for the completed version of the pathway model.

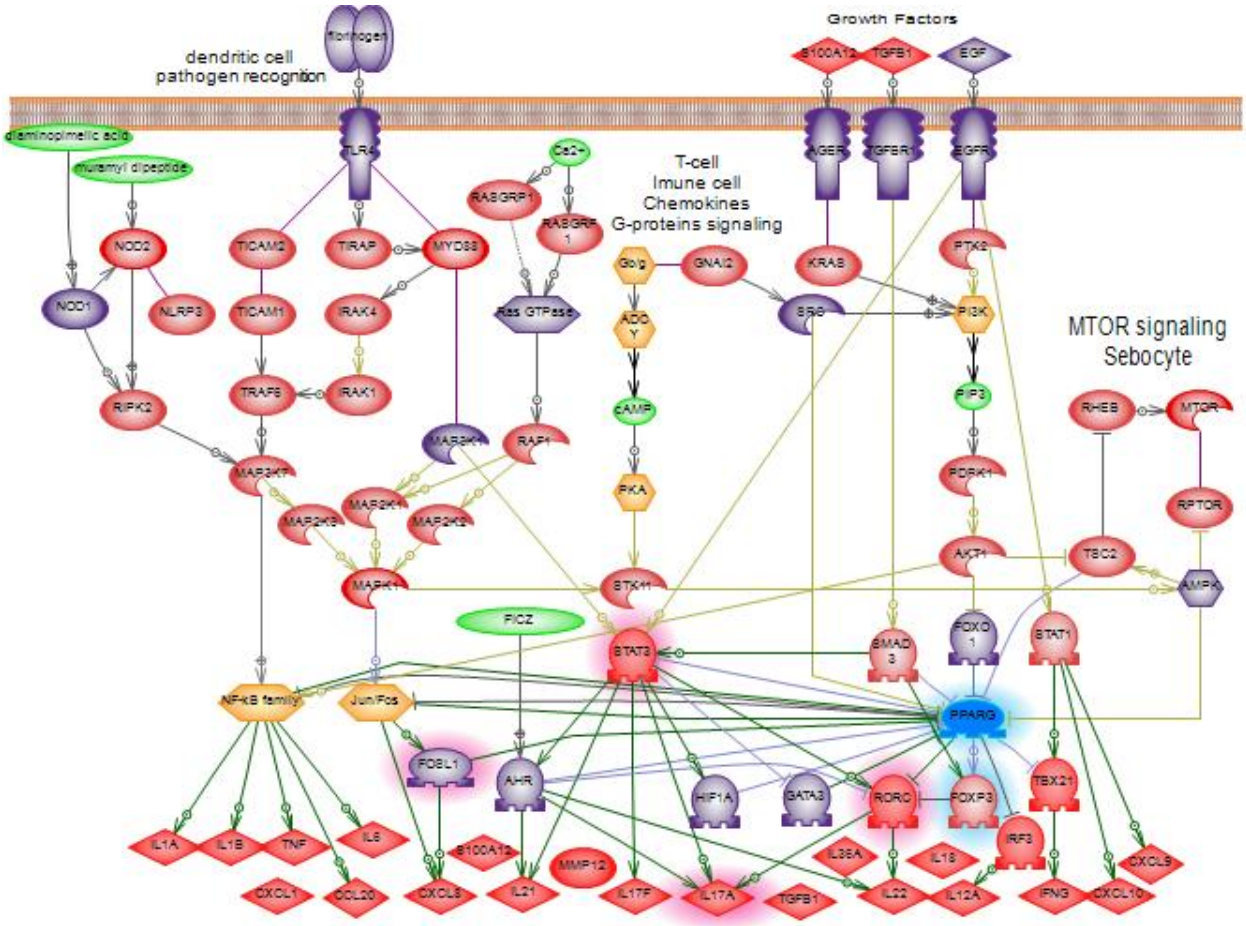

Figure 4. Model of downregulated *PPARγ* signaling in psoriasis. *PPARγ* which expression is downregulated in psoriasis is colored blue. Regulators that may inhibit the expression of *PPARγ* (data based on PPI and pathway functional analysis) are colored in violet. Targets which may be over-expressed in psoriasis due to the decrease of the negative impact of *PPARγ* are colored in bright red. *FOXP3*, *STAT3, IL17A, RORC* and *FOSL1* are highlighted according to own experiments (read section "Preliminary analysis of *PPARγ* signaling in human psoriatic skin"). Down-expressed genes (*PPARγ, FOXP3*) are highlighted in blue, overexpressed are highlighted in red.

Based on the model, reducing the level of the *PPARγ* gene expression may be a result of the over-regulation of several cascades. Pattern recognition receptors (TLRs, NOD1, NOD2, CLEC7A) that sensor pathogens and highly expressed in keratinocytes and monocytes during the infection may be one of such cascades. All-purpose cellular cascades like growth factors signaling, G-proteins and MTOR signaling also were reported to be inhibitors of *PPARγ* expression in literature and revealed in our analysis. Moreover, transcription factors including NF-kBs, JUN-FOS, AHR, GATA3, HIF1A, FOXO1 and FOSL1 can directly inhibit *PPARγ* expression. All these transcription factors are over-stimulated in the inflammatory and immune response. For example, it is reported that NF-kBs are stimulated in systemic

inflammatory processes in general, and in psoriasis as well (Tang et al., 2010; Xu et al., 2015).

In healthy skin *PPARγ* inhibits mentioned transcriptional factors in a feedback regulation loop. *PPARγ* may directly bind and suppress transcriptional factors STAT3 and RORC, by thus blocking the synthesis of pro-inflammatory cytokines including IL17. Less quantity of expressed cytokines decreases the Th17 cell proliferation, minimises chemotaxis of neutrophils and monocytes and results in the reduction of inflammation in psoriatic lesions.

IL17 which is produced mostly by TH17 cells plays the central role in the development of psoriasis because it stimulates keratinocytes to secrete pro-inflammatory cytokines and anti-bacterial peptides (Srivastava et al., 2017). IL17 pathway and Th17 cells had a strong association with *PPARγ* downregulated signaling confirmed by our network and functional analysis.

Th17 cells need robust activity of STAT3 gene for their function and differentiation. Also, STAT3 is described as an important linkage between keratinocytes and immune cells (Chowdhari and Saini, 2014). Previously the expression of *STAT3* was shown to be repressed due to *PPARγ* activation (Hsu et al., 2016). *STAT3* may also act as a regulator of *PPARγ* expression however it is not clear whether with positive or negative effect (Tuna et al., 2014).

As a transcription factor, STAT3 is reported to be a strong activator of RORC (ROR*γ*) and, probably, IL17 gene expression. From the other side, gene RORC is the major inductor of the expression of IL17 cytokines family (Takaishi et al., 2017). *PPARγ* was shown to bind the *RORC* promoter and suppress its expression altogether with *RORC*-mediated Th17 cell differentiation (Hermann-Kleiter et al., 2012).

Transcription factor FOXP3 is closely associated with psoriasis and the diminishing of Treg-cell number (Jorn Bovenschen et al., 2011; Shu et al., 2017). It was shown that activated *PPARγ* induces the stable *FOXP3* expression by strong inhibiting effect on DNA-methyltransferases. The activating effect of *PPARγ* on FOXP3 results in the proliferation of iTreg-cells (Lei et al., 2010).

FOSL1 is the transcriptional factor which plays important role in many processes related to cell differentiation and tissue remodeling (Sobolev et al., 2011, 2010; Young and Colburn, 2006). *FOSL1* (FOS-like antigen 1) is expressed in low level in healthy tissues, however its expression rises due to presence of mitogens or toxins. The accumulation of the FOSL1 protein in the skin depends on the stage of the keratinocyte differentiation (Mehic et al., 2005). Markers of stratum corneum differentiation like gene IVL are the main expression targets of FOSL1 (Adhikary et al., 2004).

The degree of the pathogenicity of downregulated *PPARγ* in psoriatic lesion depends on the cell type. It is known that *PPARγ* is expressed in Th17 cells as well as in keratinocytes, sebocytes and other cells of the psoriatic lesion (Billoni, Bruno Buan, Brigitte Gauti, 2000; Icre et al., 2006; Inoue et al., 2014; Nehrenheim et al., 2013; Paus et al., 2007). Functional

and network analysis supported the association of *PPARγ* down-regulated signaling with keratinocytes, vascular endothelium, vascular smooth muscle cells, macrophages, fibroblasts and adipocytes, and monocytes lineage (particular with CD33+, CD14+ monocytes) (Figure 5). However, we did not attempt to separate the *PPARγ* pathway model by appropriate cell types which is a disadvantage of this work. There is no reliable way to take in account cell specificity in our modeling paradigm. Moreover, we expect that most of the revealed from the literature network analysis cascades will be equal for different human cells due to insufficient experimental studies.

#	Gene Set Seed	Percent Overlap	p-value
1	epithelial cell	5	2.50E-62
2	keratinocyte	8	1.91E-58
3	vascular smooth muscle cell	10	5.08E-57
4	umbilical vein endothelial cell	12	1.39E-56
5	monocyte	5	2.55E-51
6	endometrial stromal cell	24	1.58E-50
7	alveolar macrophage	10	6.13E-50
8	synoviocyte	21	2.17E-49
9	macrophage	3	3.44E-49
10	adipocyte	7	2.61E-48
11	activated monocyte	18	8.78E-48
12	fibroblast	4	8.18E-47
13	airway epithelial cell	12	2.55E-46
14	PBMC	6	1.66E-45
15	stromal cell	7	5.12E-45
16	airway smooth muscle cell	23	6.18E-44
17	osteoblast	7	7.00E-44
18	pneumocyte	10	7.14E-44
19	endometrium cell	23	3.11E-43
20	microglia	6	1.15E-42

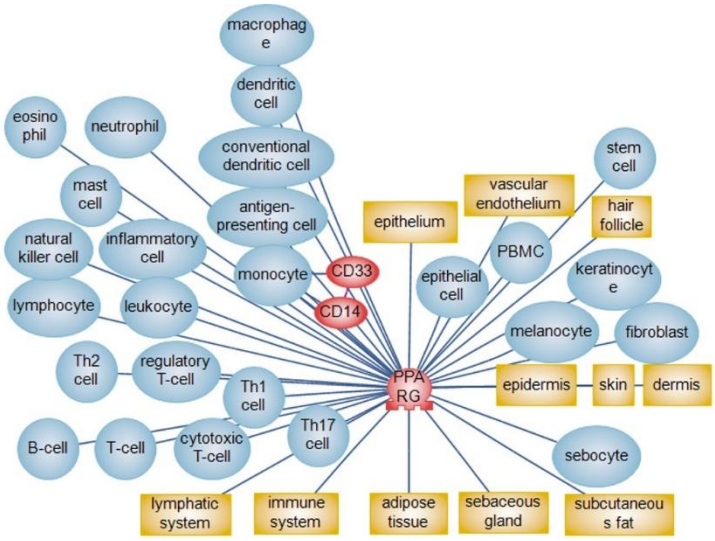

Figure 5. Cells associated with downregulated *PPARγ* signaling. SNEA method and Pathway Studio were used to calculate the results. See the complete list with statistics in supplemental materials, "PPARG and cell" file.

For additional evaluation of the reconstructed model, we analysed the public microarray data (GEO:GSE13355). In that experiment biopsies from 58 psoriatic patients were run on Affymetrix microarrays containing more 50 000 gene probes (Nair et al., 2009). We uploaded raw data from GEO and calculated differentially expressed genes (DE) between samples of psoriatic skin and unaltered samples for all patients. Then we used pathway analysis to explore the difference in the expression for genes of the *PPARγ* model we build (Figure 6).

PPARγ gene was slightly down regulated in psoriatic lesions comparing to non-altered lesions in GSE13355 microarray data (Figure 6, *PPARγ* expression diagram).

We assumed that regulators of *PPARγ* signaling should have higher expression in psoriatic lesion than in normal skin. Only S100A12 (S100 calcium binding protein A12) had a significantly higher level of expression in analyzed microarray data comparing with all regulators of *PPARγ* that we selected for the model (Figure 6, 7). S100A12 binds to the

AGER receptor which belongs to the immunoglobulin superfamily and is involved in many processes of inflammation and immune response. S100A12 is thought to be the most prominent biomarker of psoriasis (Wilsmann-Theis et al., 2016). Also, polymorphisms in AGER receptor were found to be associated with psoriasis (Puig and López-Ferrer, 2017).

The EGFR signaling almost completely was down-expressed in this microarray data including the *FOXO1* expression which is one of the direct inhibitors of *PPARγ*. Therefore, EGFR / FOXO1 signaling probably does not play an important role in the regulation of *PPARγ* in psoriasis (Figure 7).

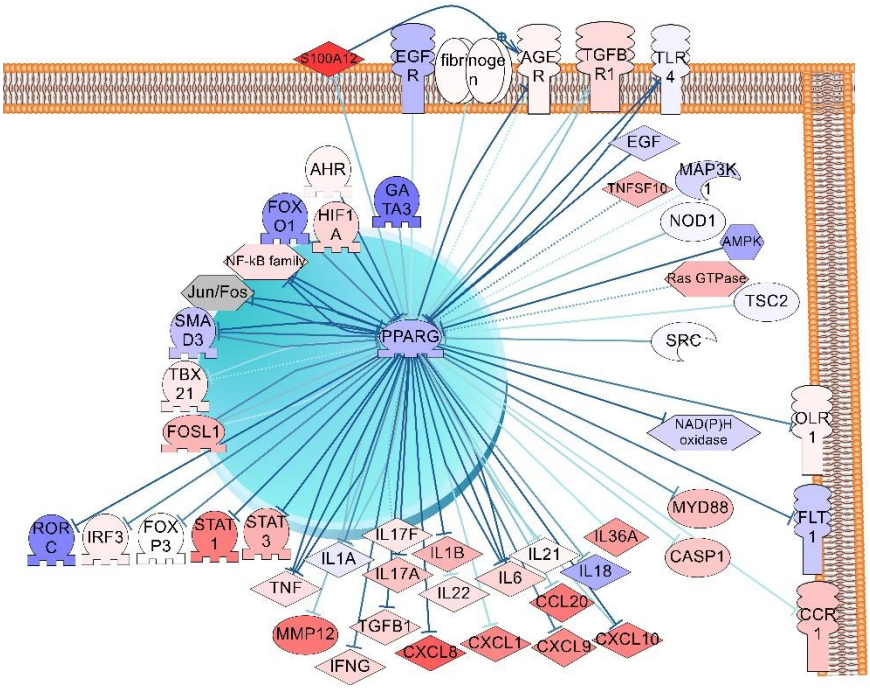

Figure 6. Evaluation of *PPARγ* downregulated sub-network (selected *PPARγ* regulators and targets) associated with psoriasis using microarray data analysis (results of differential expression analysis of psoriatic lesions vs unaltered lesions). The saturation in blue indicates the degree of genes down-expression in psoriatic samples in comparison with unaltered samples. The saturation in red indicates the degree of genes over-expression. The list of targets and regulators see in supplemental materials, "PPARG regulators and targets", list 3.

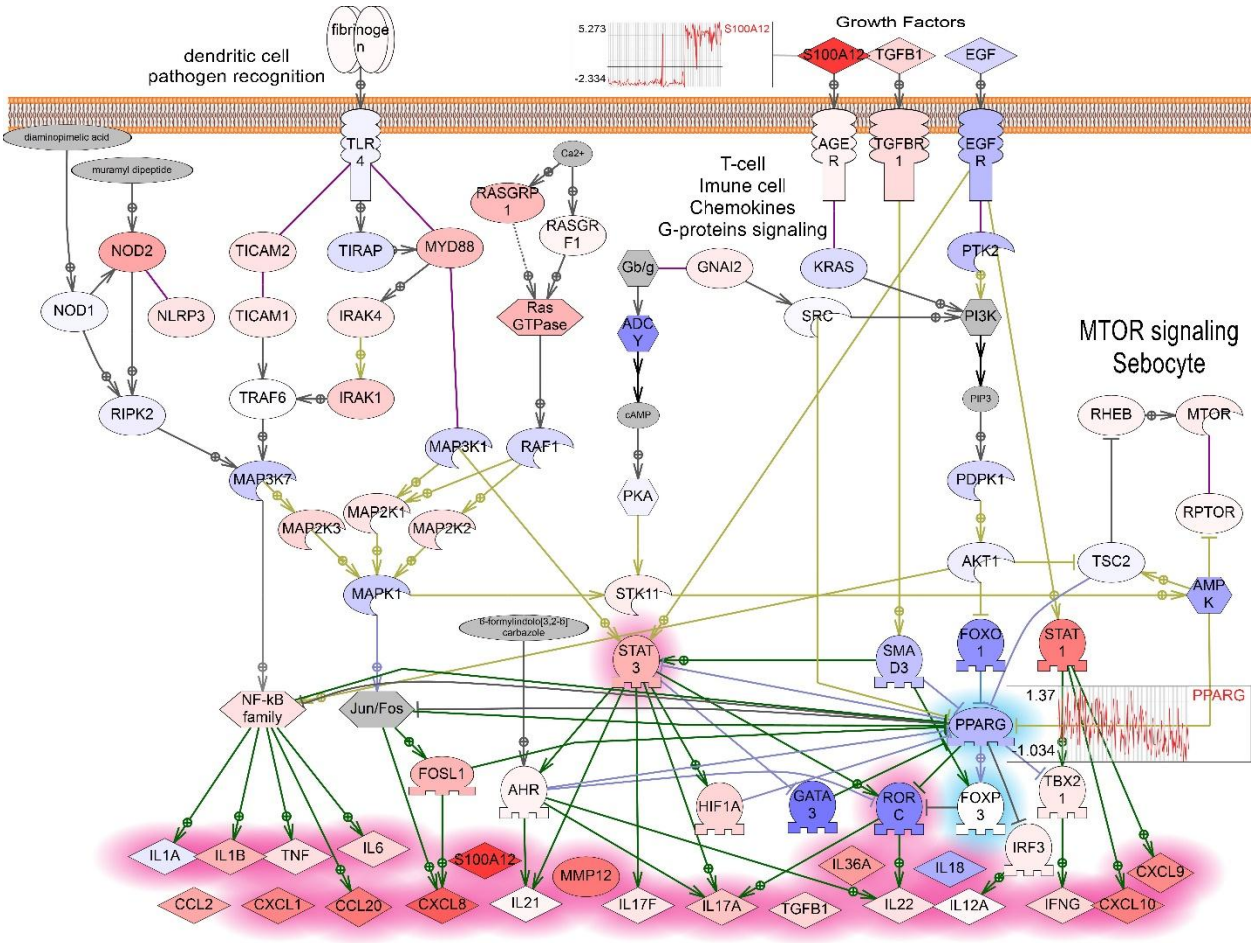

Figure 7. Evaluation of *PPARγ* downregulated model associated with psoriasis using microarray data analysis (results of differential expression analysis of psoriatic lesions vs unaltered lesions from GEO:GSE13355). The saturation in blue indicates the degree of genes down-expression in psoriatic samples in comparison with unaltered samples. The saturation in red indicates the degree of genes over-expression. The plots of expression pattern in psoriatic lesions compared with healthy skins samples are shown for *PPARγ* and *S100A12* genes.

Preliminary analysis of PPARγ signaling activity in human psoriatic skin

PPARγ expression is slightly downregulated in psoriatic skin

To test the hypothesis of downregulated *PPARγ* signaling in psoriasis, we measured gene expression of *PPARγ* and several key members of the reconstructed model in skin samples from patients with psoriasis.

Several key players in the *PPARγ* signaling were selected for experimental validation of the hypothesis that low levels of *PPARγ* may contribute to the development of psoriatic lesions. There were IL17A gene (interleukin 17A), *STAT3* gene (signal transducer and activator of

transcription 3), *RORC* gene (retinoid-related orphan receptor-gamma), FOXP3 gene (forkhead box P3) and *FOSL1* (FOS-like antigen 1) gene.

For each of 23 patients, we compared levels of expression of *PPARγ* in the psoriatic skin samples and the skins without visually noticed lesions at the distance of 3 cm from the psoriatic surface. Such comparison helps to exclude the influence of unrelated psoriasis factors on the molecular profile (Yao et al., 2008). Results of real-time PCR showed that in the psoriatic samples the *PPARγ* gene was expressed below the level of its expression in unaffected skin. The average level of the *PPARγ* expression in the skin of patients was slightly reduced in 1.3±0.27 times in psoriatic skin compared with the skin without visually noticed lesions. We found a significant increase in the level of the gene IL17 expression in 42.39±16.68 times, gene STAT3 in 4.42±0.90 times, gene RORC in 7.68±1.62, and FOSL1 in 9.72 ±4.98 times. The level of expression of gene FOXP3 was decreased in 1.72±0.14 times, and of gene PPARγ in 1.3±0.18times (Figure 8).

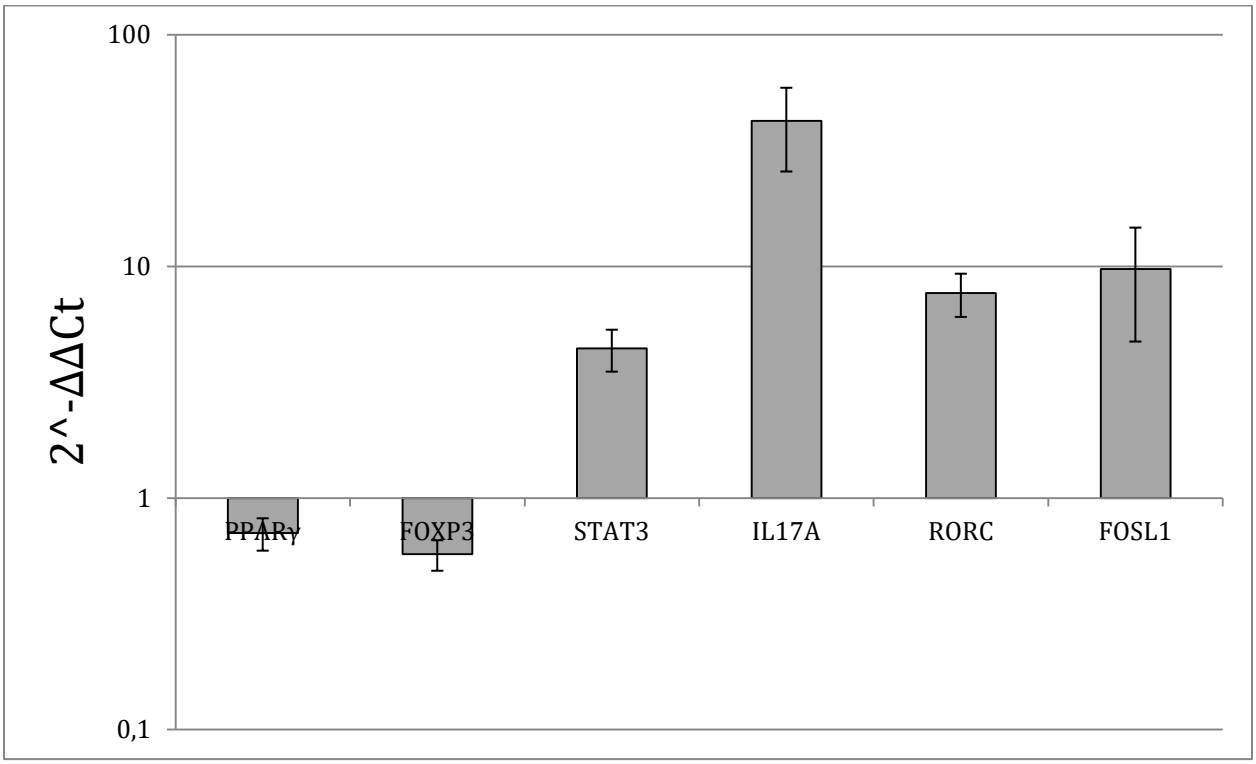

Figure 8. Comparison of *PPARγ*, *STAT3, IL17A, RORC, FOXP3* and *FOSL1* gene expression in the skin affected by psoriasis in 23 patients. The level of gene expression in visually unaffected skin is taken equal to conditional 1 ($p < 0.05$). See supplemental materials for detailed statistics ("PPARG expression file).

Low laser treatment stabilises PPARγ related signalings in psoriatic skin

For the next step of validation, we studied the expression of *PPARγ*, *STAT3, IL17A, RORC, FOXP3*, and *FOSL1* in human psoriatic skin samples and visually healthy skin samples before and after laser treatment.

Patients received low-intensity laser treatment with 1.27 microns wavelength (infrared short waves). The molecular mechanism of laser treatment is not well understood. Low-intensity laser waves are absorbed by oxygen, CO2, water molecules switching them into an activated state. Proteins with activated molecules participate in interactions more intensively. There was shown that low laser treatment stimulates Ca2-related signaling pathways including general membrane reparation and cell proliferation. There are expectations that low-level laser treatment will result in the replacement of "old" cells with new ones thus reducing the inflammation in the psoriatic lesion (Avci et al., 2013).

We detected a reliable reduction in the expression of studied *PPARγ*, *STAT3, IL17A, RORC, FOXP3* and *FOSL1* genes after low level (1.27 microns) laser treatment. The level of *STAT3* expression was decreased in 2.08±0.33 times (Figure 9 D), *IL17A* in 10.48±3.36 times, *RORC* in 3.20±0.68 times and *FOSL1* in 0.57±0.17 (Figure 9 C). The level of the expression of *PPARγ* was increased 2.13±0.47 times (Figure 9 A). The level of *FOXP3* was also increased in 2.62±0.39 times (Figure 9 B).

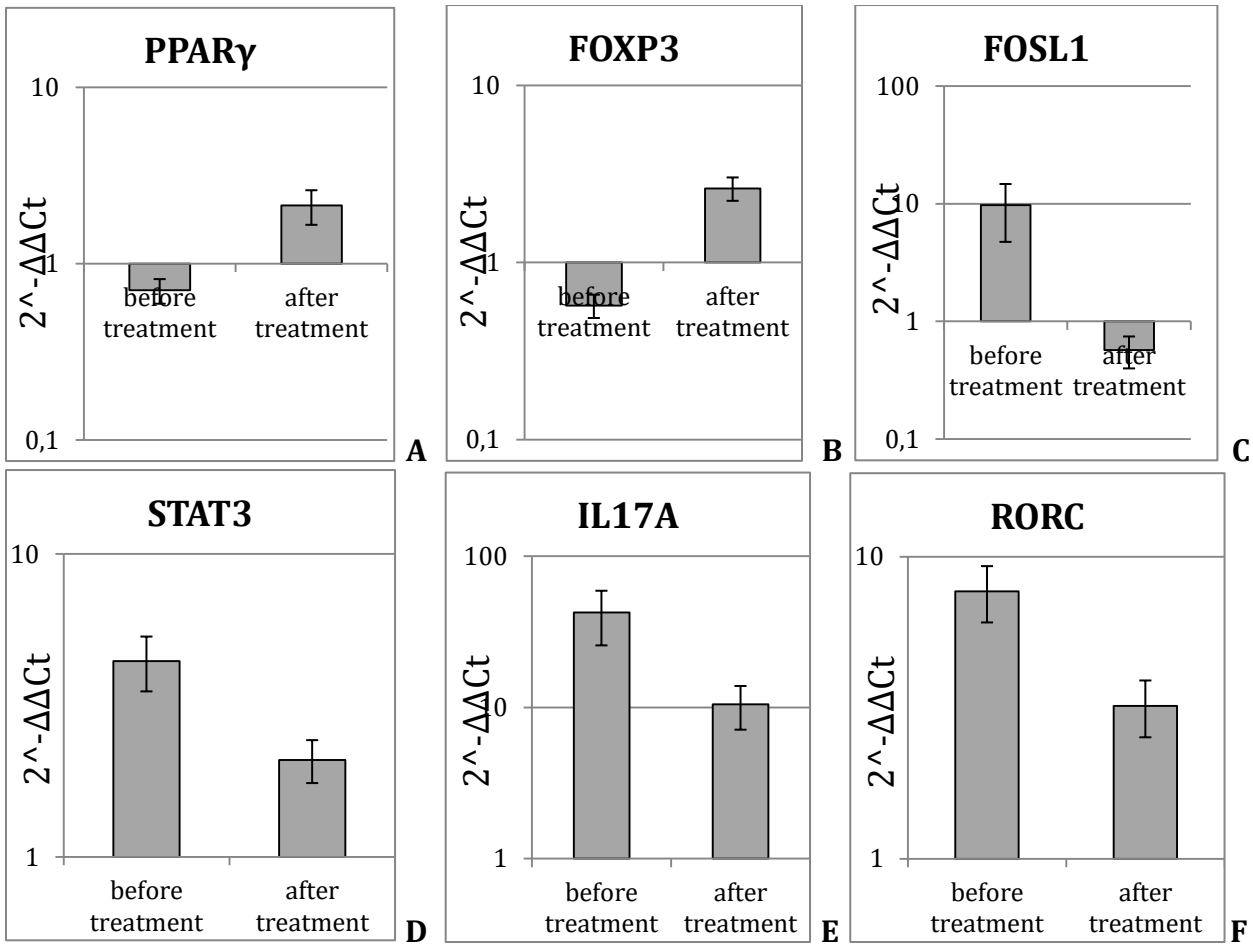

Figure 9. Comparison of *PPARγ (A)*, *FOXP3 (B), FOSL1* (C), *STAT3 (D), IL17A (E)* and *RORC (F)* genes expression in the skin of 23 patients with psoriasis before and after low-level laser therapy. The levels of mRNA concentration for genes in psoriatic skin samples was calculated in relation to the level of the same genes in unaffected skin samples (which was taken as conditional 1, $p<0.05$). See supplemental materials for detailed statistics ("PPARG expression file).

Therefore, low laser treatment caused significant growth of the *PPARγ* and *FOXP3* expression while reducing the expression of *STAT3, IL17A*, and *RORC*.

Similar to previously published results by different groups of medical researchers, the low-laser treatment had a positive effect on the health of observed patients and reduction of psoriatic skin inflammation was achieved.

CONCLUSION

In our previous work, we reviewed the recent progress in psoriasis pathways and published two pathway models. The first pathway model described the shift to TH17 cell production during the differentiation of psoriatic T cells. The primary cause of the shift of the T-cell differentiation is supposed to be genetic mutations, for example in IL23R receptors. The second model showed how elevated levels of IL17 and IL22 may activate keratinocytes to release different cytokines and chemokines for attracting neutrophils and other inflammatory cells in the psoriatic lesion (Nesterova et al., 2019).

In this work, we tested the hypothesis that *PPARγ* signaling when downregulated may promote psoriasis. We built the model of *PPARγ* dependent pathways involved in the development of the psoriatic lesions. However, we used a different approach for reconstructing the pathway model and selected key members with bioinformatic analysis. We included in the pathway model top statistically significant regulators of *PPARγ* gene expression and *PPARγ*-depended expression targets. Then we included significant molecular cascades and cell processes from results of the functional analysis (IL17 signaling, TLRs signaling, activation of STAT3 or NFKB transcription factors and others). We tested the model with analysis of published microarray data. Finally, we included in the model data from own experimental analysis of genes expression in human psoriatic skin.

We detected down-regulation of *PPARγ* gene expression in human psoriatic skin from 23 patients with real-time PCR method. Our results are similar to data from microarray on 58 patients where average *PPARγ* gene expression also is slightly down-regulated in psoriatic lesions (Nair et al., 2009). Our results do not confirm the work of Westergaard M et al. which described the slightly higher level of the *PPARγ* expression in human psoriatic skin compared to normal skin. However, the level of *PPARγ* mRNA was close to the detection limit in their research (Lei et al., 2010). This difference may be due detection of different isoforms of *PPARγ* which all have different patterns of the expression (Meirhaeghe and Amouyel, 2004). More research on protein level is needed to answer the question about the level of the *PPARγ* expression in human psoriatic skin. The limitation of current study is that we analysed gene expression only on the mRNA level using only one method of RT-

PCR. Additional analysis on protein levels, more samples, and analysis with specific cell types rather than with whole skin are needed to conclude whether *PPARγ* gene expression is downregulated in psoriatic lesions.

Within the framework of the model validation we supposed that signalling related to repressed *PPARγ* activity is correlated with the development of psoriasis. IL17A, STATS3, and RORC (RORg) are statistically significant *PPARγ* negative targets and they have expected higher levels of expression in psoriatic lesion which decrease after laser treatment. Since *PPARγ* may act as a suppressor of the *IL17* gene transcription by inhibiting his direct regulator gene RORC, our preliminary experimental results showing the high levels of expression for *IL17* and *RORC* are aligned well with low activity of *PPARγ* in psoriatic skin.

While the prominent role of *RORC* in psoriasis as the major controller of Th17 cell differentiation is well described, however, the evidence of *RORC* expression in psoriasis is controversial and supported by work where mice T-cells and dendritic cells had increased STAT3/RORC expression (Nadeem et al., 2017) still patients with psoriasis had elevated level of *RORC* (RORG-t isoform) (Mendoza et al., 1989). In analysed published microarray data, the level of expression of *RORC* was downregulated in most of 58 patients. We detected the high level of *RORC* mRNA in the psoriatic skin of patients and this level was reduced after laser treatment. We report the downregulation of *FOXP3* which is a direct inhibitor of *RORC* and positive target of *PPARγ*. Though, low level of *PPARγ* as well as high level of *RORC* is supported by down-regulated *FOXP3* expression and validates reconstructed model.

FOSL1 and *STAT3* were overexpressed in psoriatic lesion on mRNA level in our experiment and may directly inhibit *PPARγ* expression. The expressions of *FOSL1* and *STAT3* were reduced significantly after laser treatment that may play role in the stabilization of psoriatic inflammation as well as *PPARγ* related pathways. The limitation of current work when only one methodology was used to study gene expression, does not allow us to assert the high reliability of biological conclusions. However, the alignment of our results with microarray data and PPI network analysis shows that the model of *PPARγ* downregulated signaling in psoriasis reconstructed in this work can be useful for further research.

ACKNOWLEDGEMENTS AND FUNDING

We thank the “University Diagnostic Laboratory” LLC for funding along with Elsevier Inc. and Pathway Studio® for software and the database.

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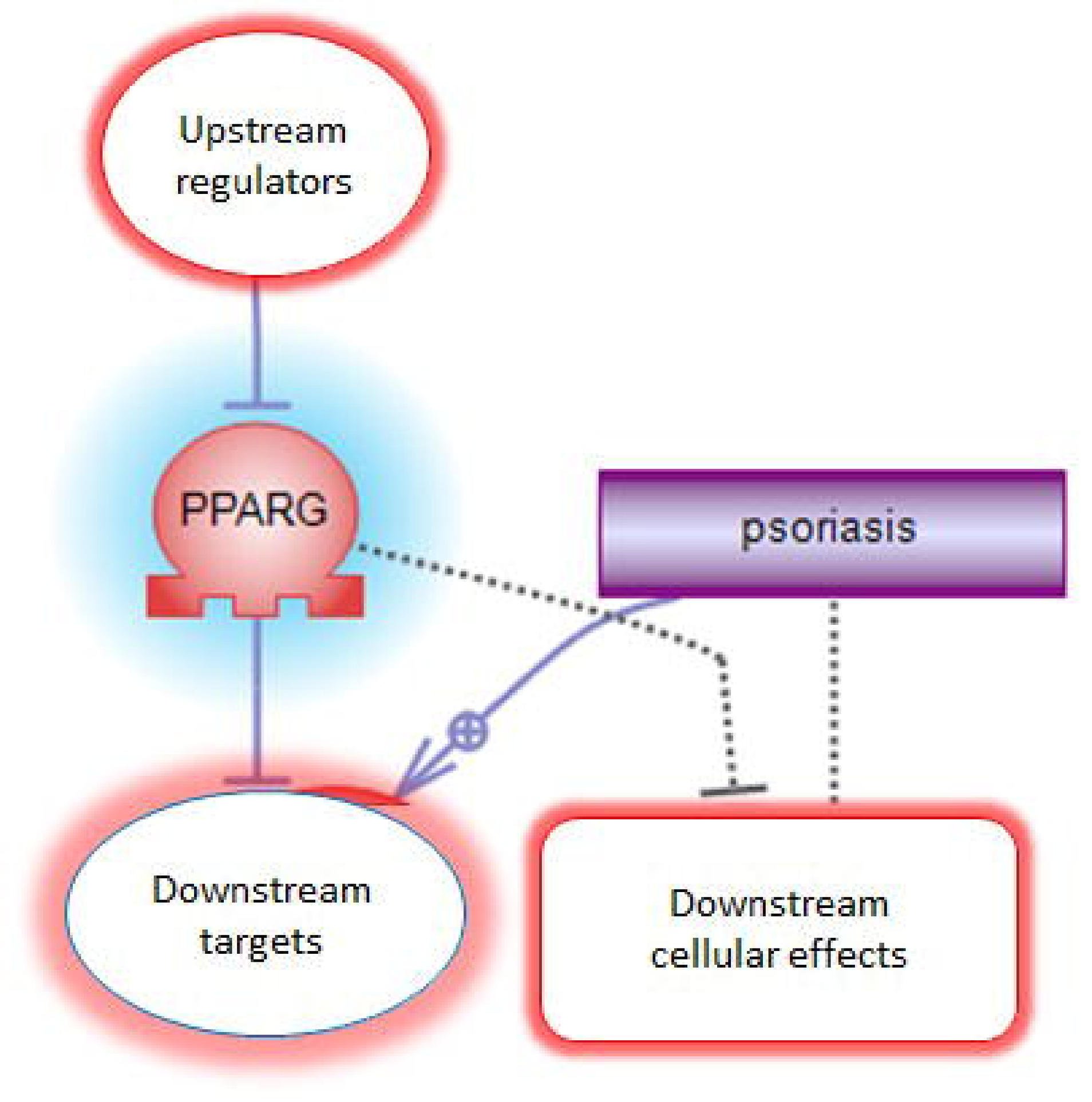
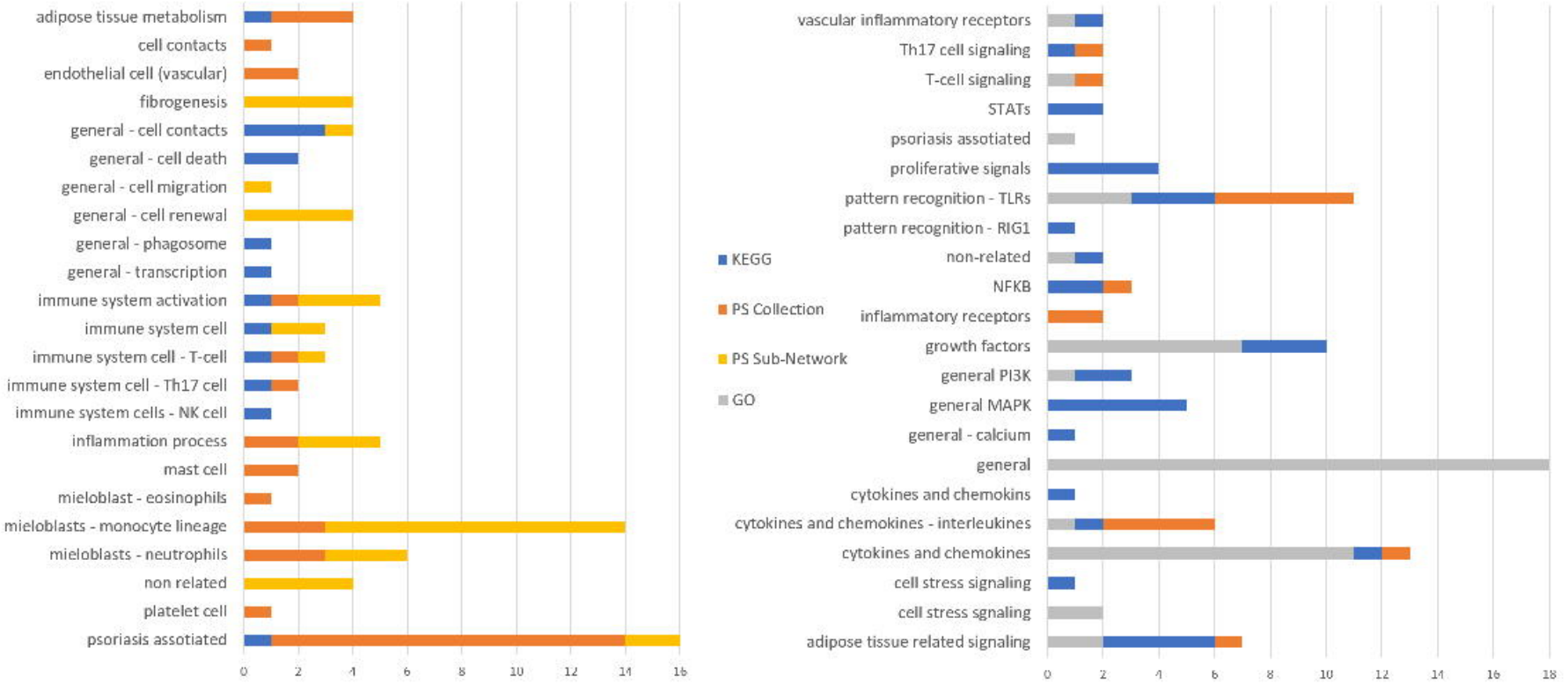
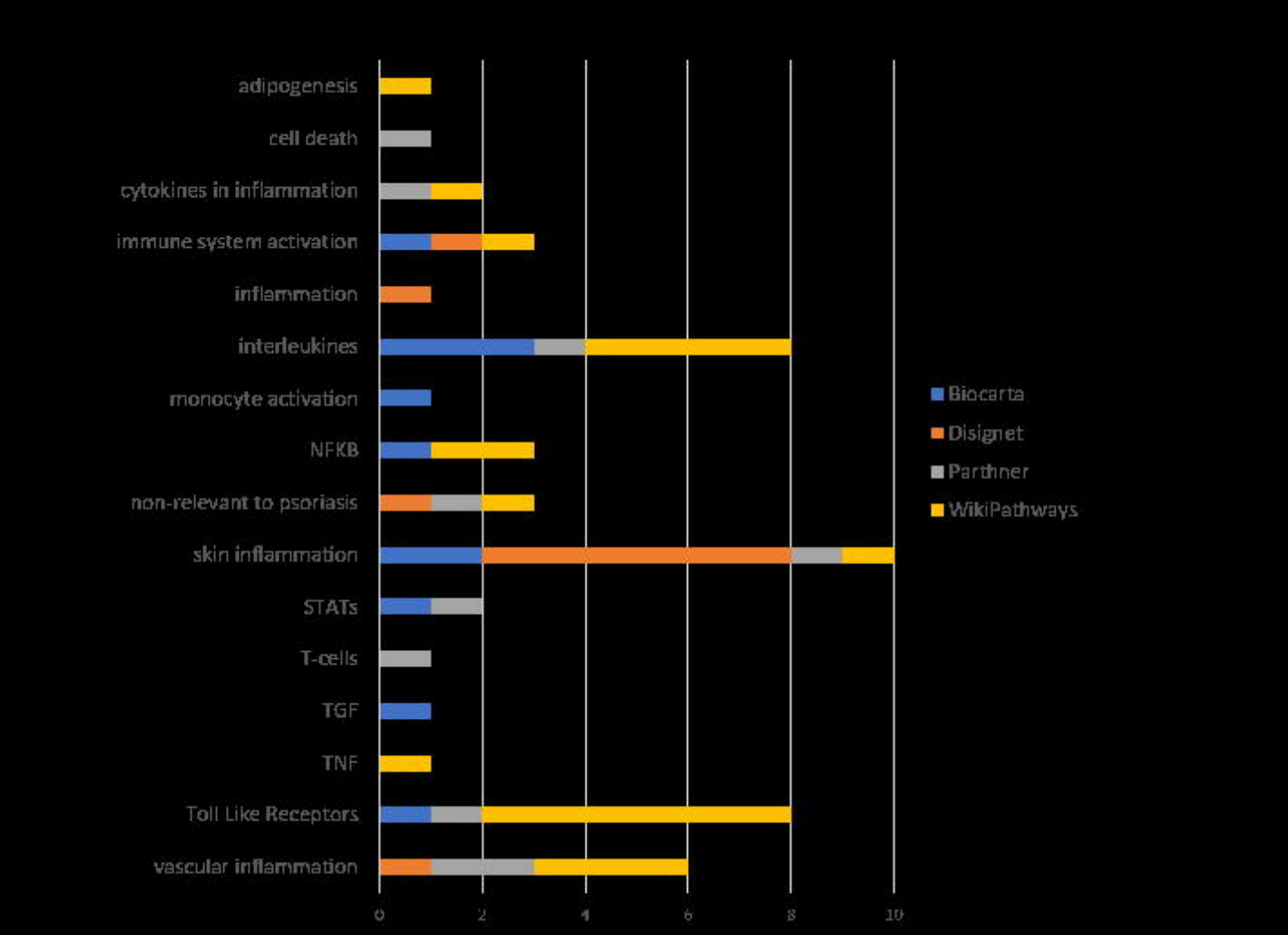
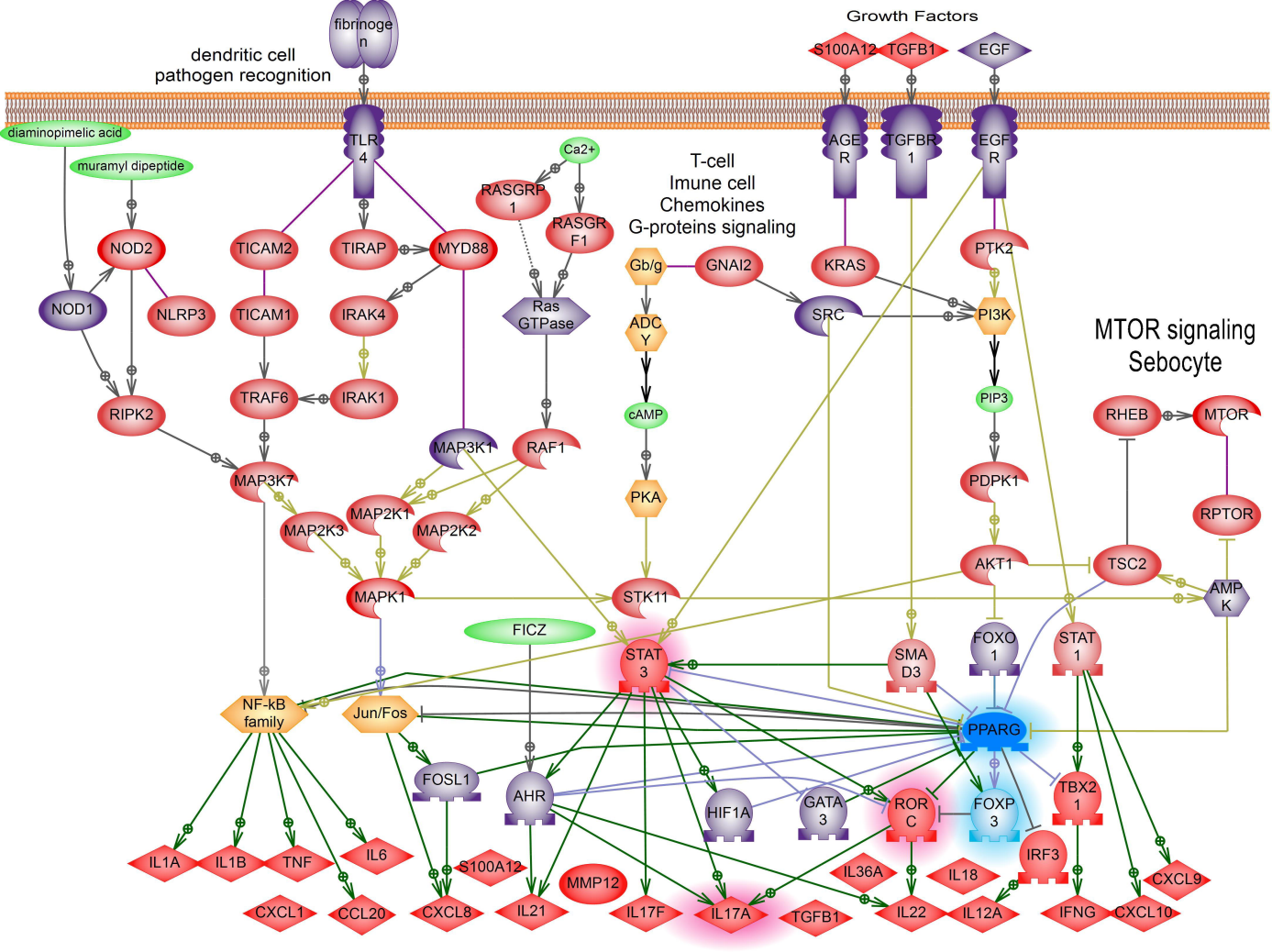
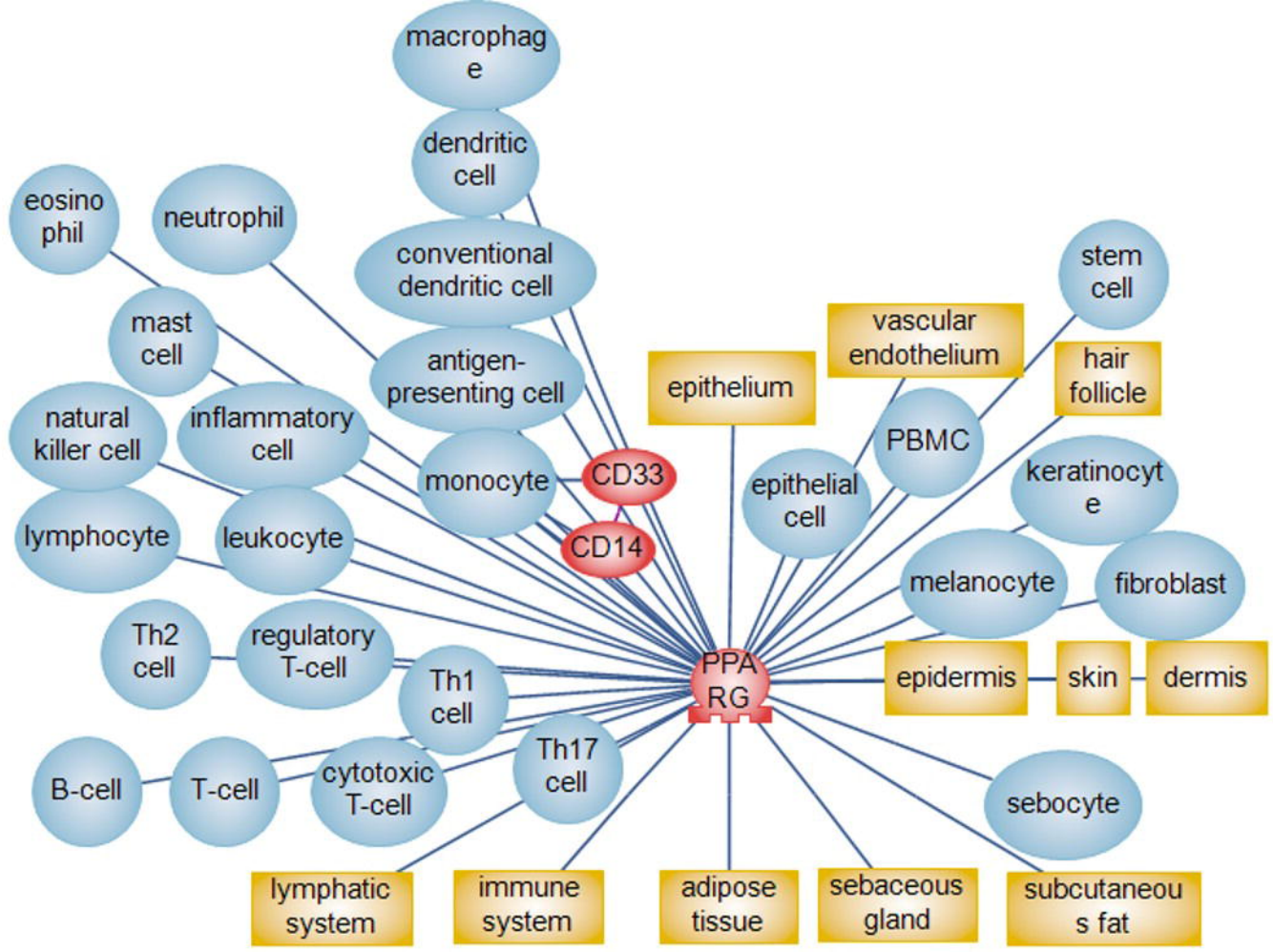

Upstream regulators
PPARG
psoriasis
Downstream targets
Downstream cellular effects

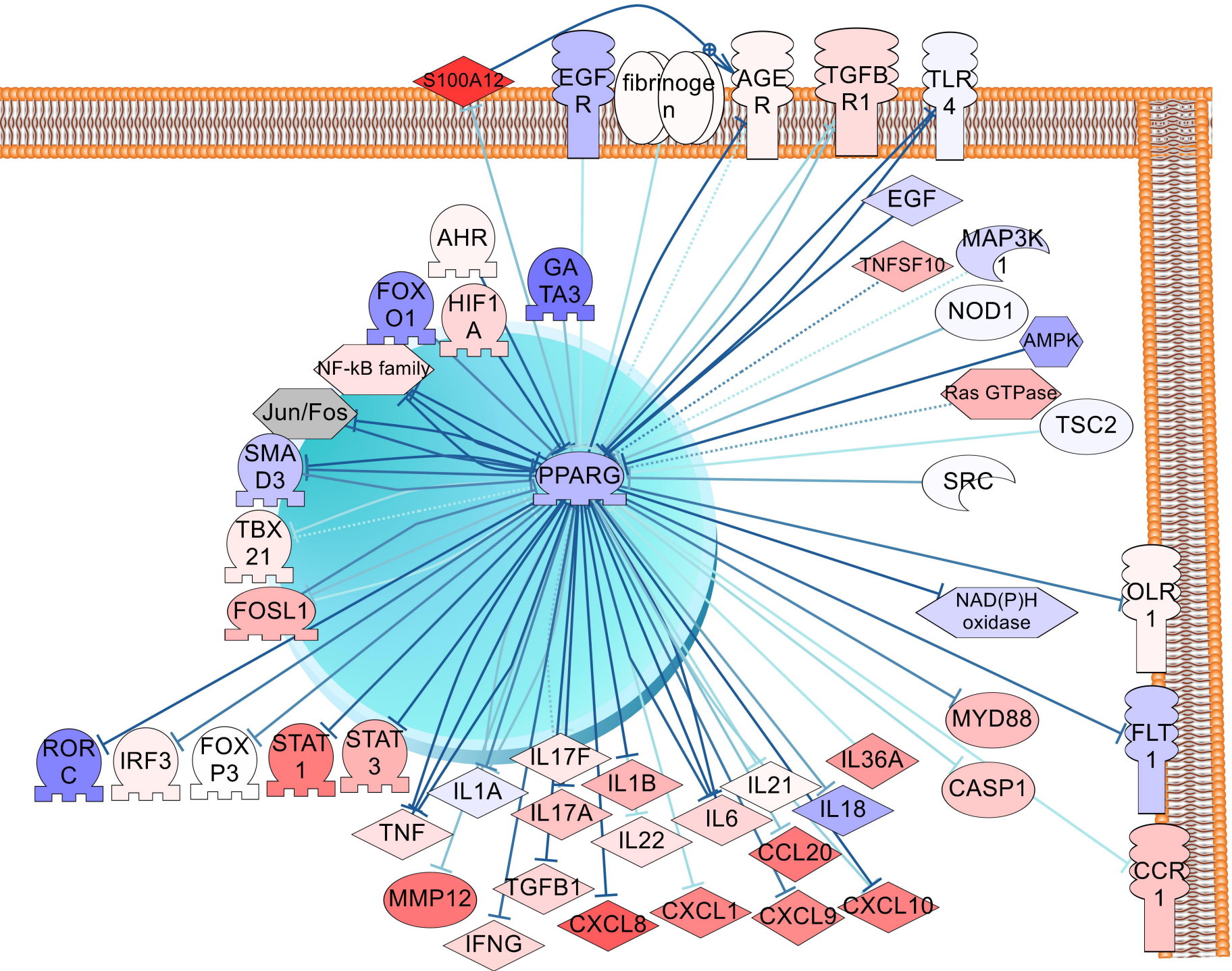

adipose tissue metabolism
cell contacts
endothelial cell (vascular)
fibrogenesis
general - cell contacts
general - cell death
general - cell migration
general - cell renewal
general - phagosome
general - transcription
immune system activation
immune system cell
immune system cell - T-cell
immune system cell - Th17 cell
immune system cells - NK cell
inflammation process
mast cell
mieloblast - eosinophils
mieloblasts - monocyte lineage
mieloblasts - neutrophils
non related
platelet cell
psoriasis assotiated
0
2
4
6
8
10
12
14
16
KEGG
PS Collection
PS Sub-Network
GO
vascular inflammatory receptors
Th17 cell signaling
T-cell signaling
STATs
psoriasis assotiated
proliferative signals
pattern recognition - TLRs
pattern recognition - RIG1
non-related
NFKB
inflammatory receptors
growth factors
general PI3K
general MAPK
general - calcium
general
cytokines and chemokins
cytokines and chemokines - interleukines
cytokines and chemokines
cell stress signaling
cell stress sgnaling
adipose tissue related signaling
0
2
4
6
8
10
12
14
16
18

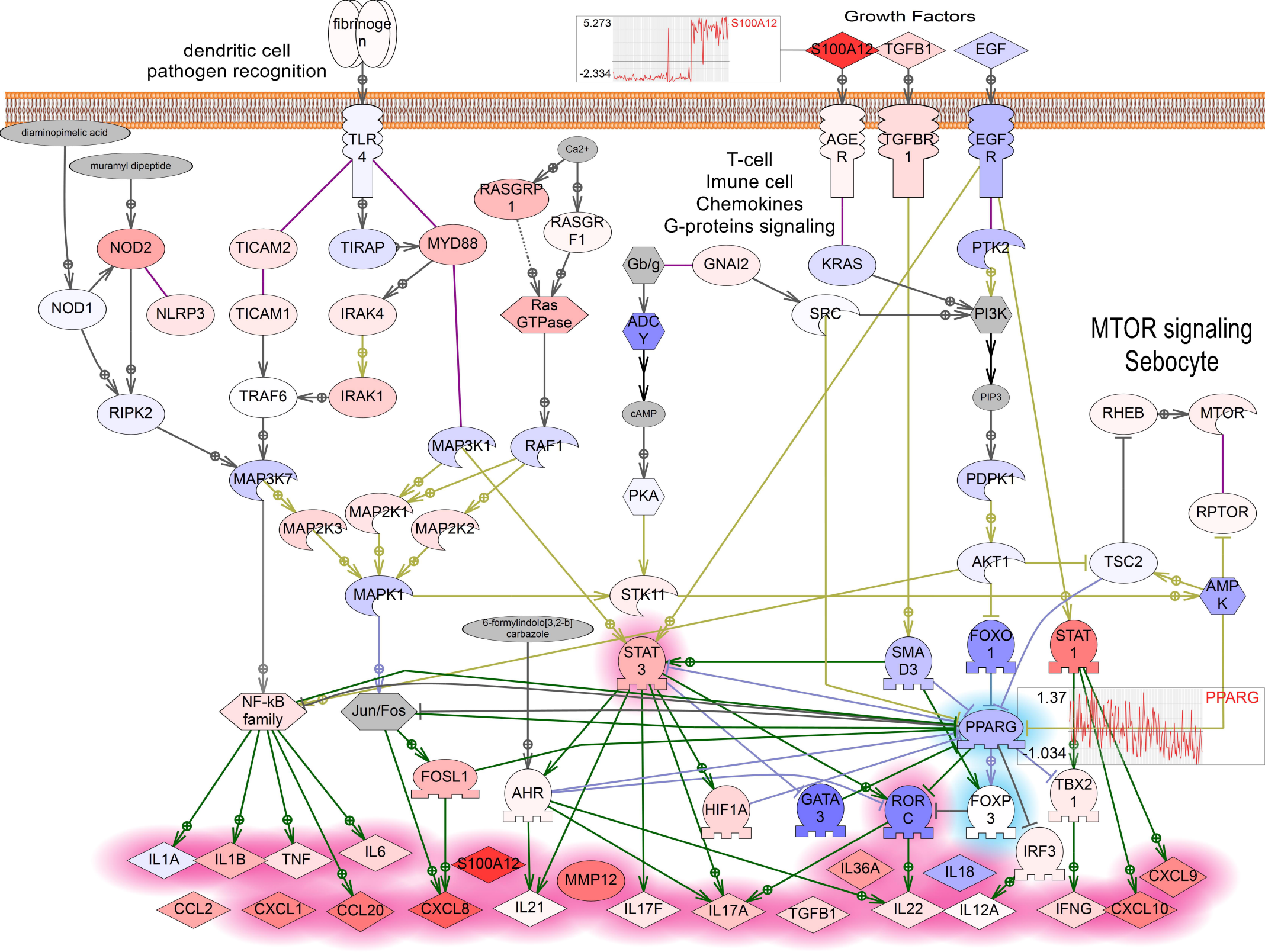

adipogenesis
cell death
cytokines in inflammation
immune system activation
inflammation
interleukines
monocyte activation
NFKB
non-relevant to psoriasis
skin inflammation
STATs
T-cells
TGF
TNF
Toll Like Receptors
vascular inflammation
0
2
4
6
8
10
Biocarta
Disignet
Parthner
WikiPathways


fibrinogen
dendritic cell
pathogen recognition
Growth Factors
S100A12
TGFB1
EGF
diaminopimelic acid
muramyl dipeptide
TLR4
AGER
TGFBR1
EGFR
Ca2+
RASGRP1
RASGRF1
T-cell
Imune cell
Chemokines
G-proteins signaling
NOD2
NOD1
NLRP3
TICAM2
TICAM1
TIRAP
IRAK4
MYD88
Gb/g
GNAI2
KRAS
PTK2
SRC
PI3K
ADCY
cAMP
PKA
Ras GTPase
MTOR signaling
Sebocyte
RIPK2
TRAF6
IRAK1
MAP3K1
RAF1
PIP3
PDPK1
RHEB
MTOR
RPTOR
MAP3K7
MAP2K1
MAP2K3
MAP2K2
AKT1
TSC2
AMPK
MAPK1
STK11
FICZ
STAT3
SMAD3
FOXO1
STAT1
NF-kB family
Jun/Fos
PPARG
FOSL1
AHR
HIF1A
GATA3
RORC
FOXP3
TBX21
IRF3
IL1A
IL1B
TNF
IL6
S100A12
MMP12
IL36A
IL18
CXCL9
CXCL1
CCL20
CXCL8
IL21
IL17F
IL17A
TGFB1
IL22
IL12A
IFNG
CXCL10

#	Gene Set Seed	Percent Overlap	p-value
1	epithelial cell	5	2.50E-62
2	keratinocyte	8	1.91E-58
3	vascular smooth muscle cell	10	5.08E-57
4	umbilical vein endothelial cell	12	1.39E-56
5	monocyte	5	2.55E-51
6	endometrial stromal cell	24	1.58E-50
7	alveolar macrophage	10	6.13E-50
8	synoviocyte	21	2.17E-49
9	macrophage	3	3.44E-49
10	adipocyte	7	2.61E-48
11	activated monocyte	18	8.78E-48
12	fibroblast	4	8.18E-47
13	airway epithelial cell	12	2.55E-46
14	PBMC	6	1.66E-45
15	stromal cell	7	5.12E-45
16	airway smooth muscle cell	23	6.18E-44
17	osteoblast	7	7.00E-44
18	pneumocyte	10	7.14E-44
19	endometrium cell	23	3.11E-43
20	microglia	6	1.15E-42

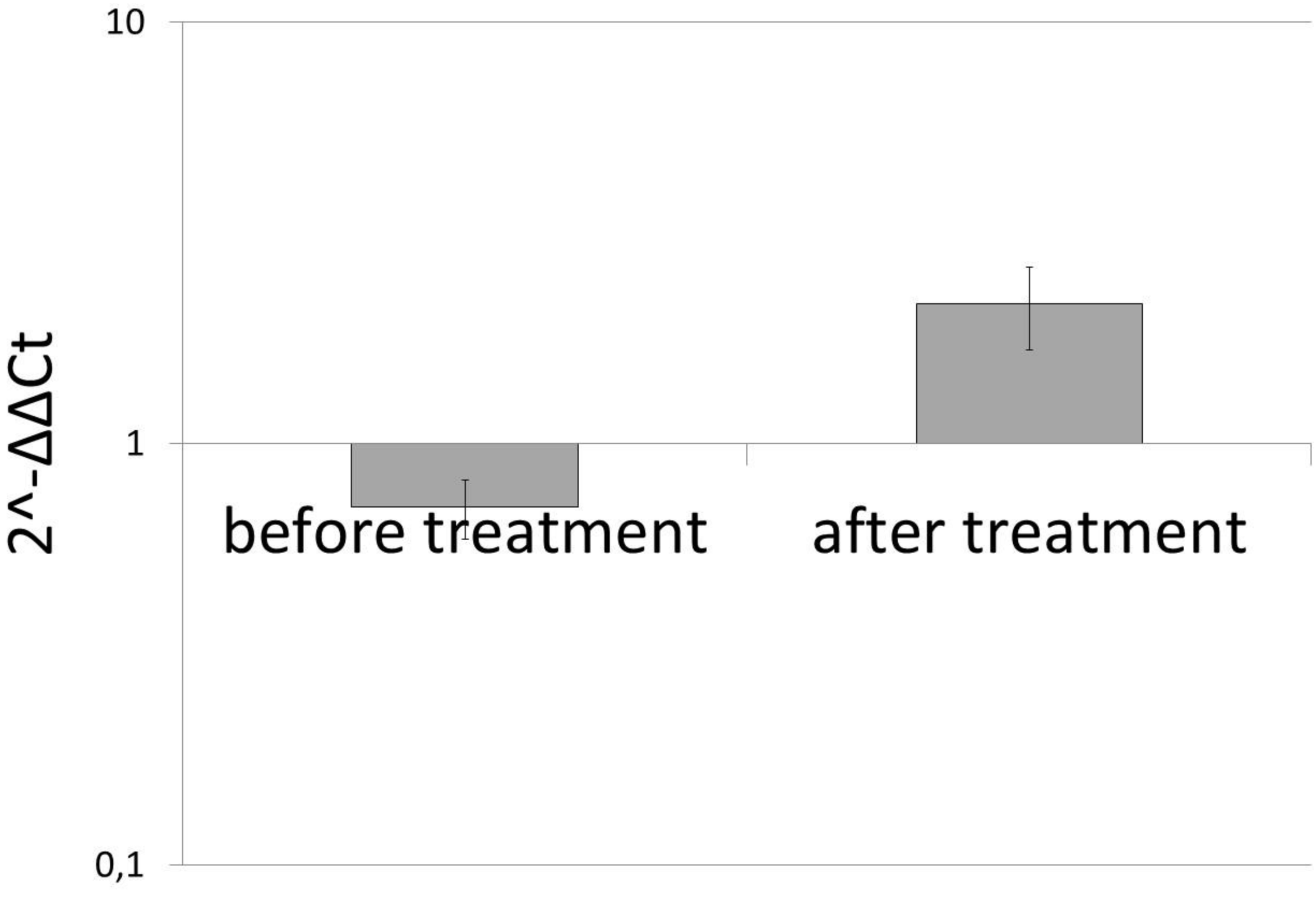



S100A12
EGFR
fibrinogen
AGER
TGFBR1
TLR4
EGF
AHR
FOXO1
HIF1A
GATA3
TNFSF10
MAP3K1
NOD1
AMPK
NF-kB family
Ras GTPase
Jun/Fos
TSC2
SMAD3
PPARG
SRC
TBX21
FOSL1
NAD(P)H oxidase
OLR1
MYD88
FLT1
RORC
IRF3
FOXP3
STAT1
STAT3
IL17F
IL1B
IL21
IL36A
IL1A
IL17A
IL6
IL18
CASP1
TNF
IL22
CCL20
CCR1
MMP12
TGFB1
CXCL1
CXCL9
CXCL10
CXCL8
IFNG

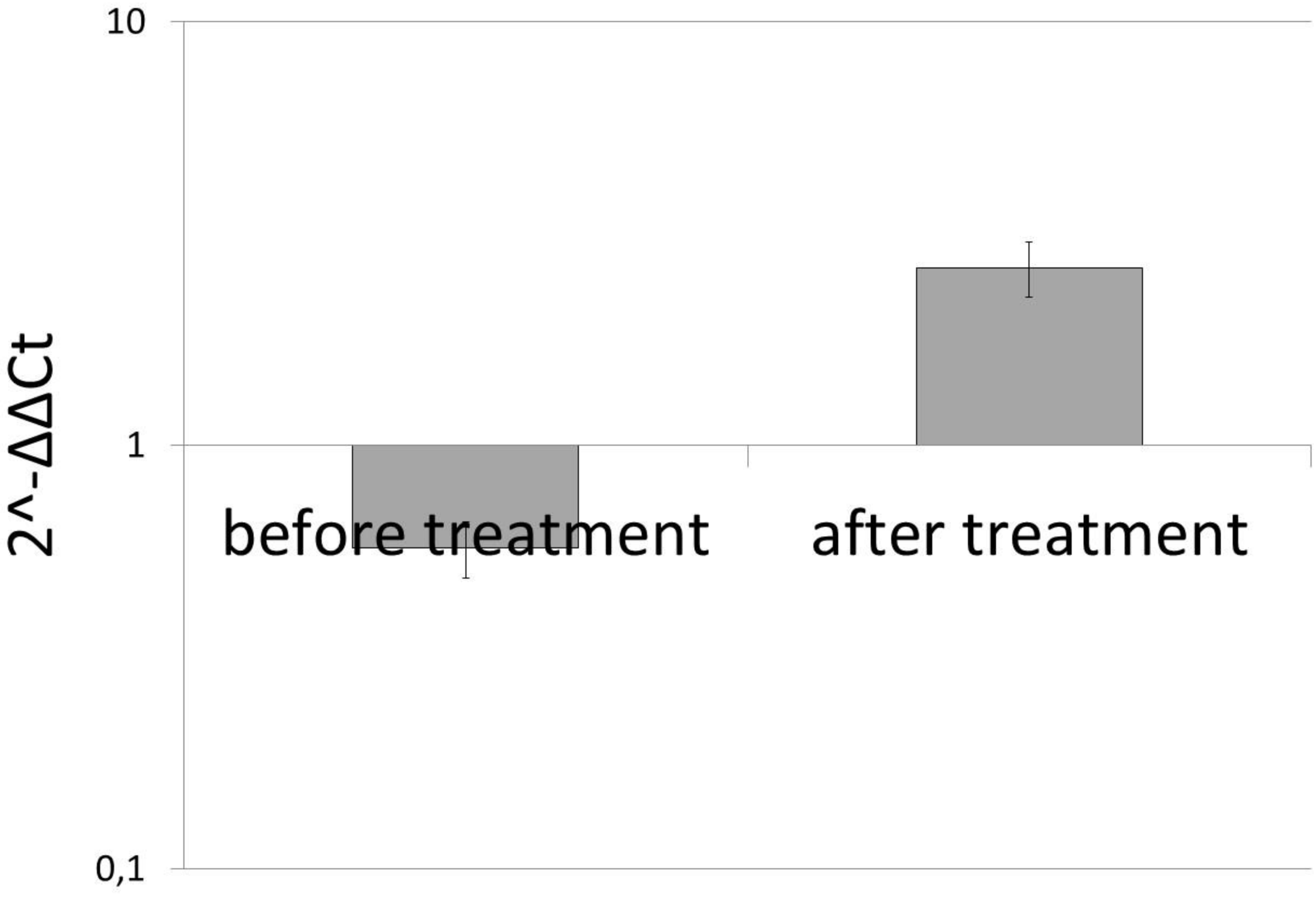

dendritic cell
pathogen recognition
fibrinogen
5.273
S100A12
-2.334
Growth Factors
S100A12
TGFB1
EGF
diaminopimelic acid
muramyl dipeptide
TLR 4
AGE R
TGFBR 1
EGF R
Ca2+
RASGRP 1
RASGR F1
T-cell
Imune cell
Chemokines
G-proteins signaling
NOD2
NOD1
NLRP3
TICAM2
TICAM1
TIRAP
MYD88
IRAK4
IRAK1
TRAF6
RIPK2
Gb/g
GNAI2
ADCY
cAMP
PKA
KRAS
SRC
PTK2
PI3K
PIP3
PDPK1
AKT1
MTOR signaling
Sebocyte
RHEB
MTOR
RPTOR
TSC2
AMPK
Ras GTPase
RAF1
MAP3K1
MAP3K7
MAP2K1
MAP2K3
MAP2K2
MAPK1
STK11
6-formylindolo[3,2-b] carbazole
STAT 3
SMAD3
FOXO 1
STAT 1
NF-kB family
Jun/Fos
PPARG
1.37
-1.034
PPARG
FOSL1
AHR
HIF1A
GATA 3
RORC
FOXP 3
TBX21
IRF3
IL1A
IL1B
TNF
IL6
S100A12
MMP12
IL36A
IL18
CXCL9
CCL2
CXCL1
CCL20
CXCL8
IL21
IL17F
IL17A
TGFB1
IL22
IL12A
IFNG
CXCL10

100
10
1
0,1
2^-ΔΔCt
PPARγ
FOXP3
STAT3
IL17A
RORC
FOSL1

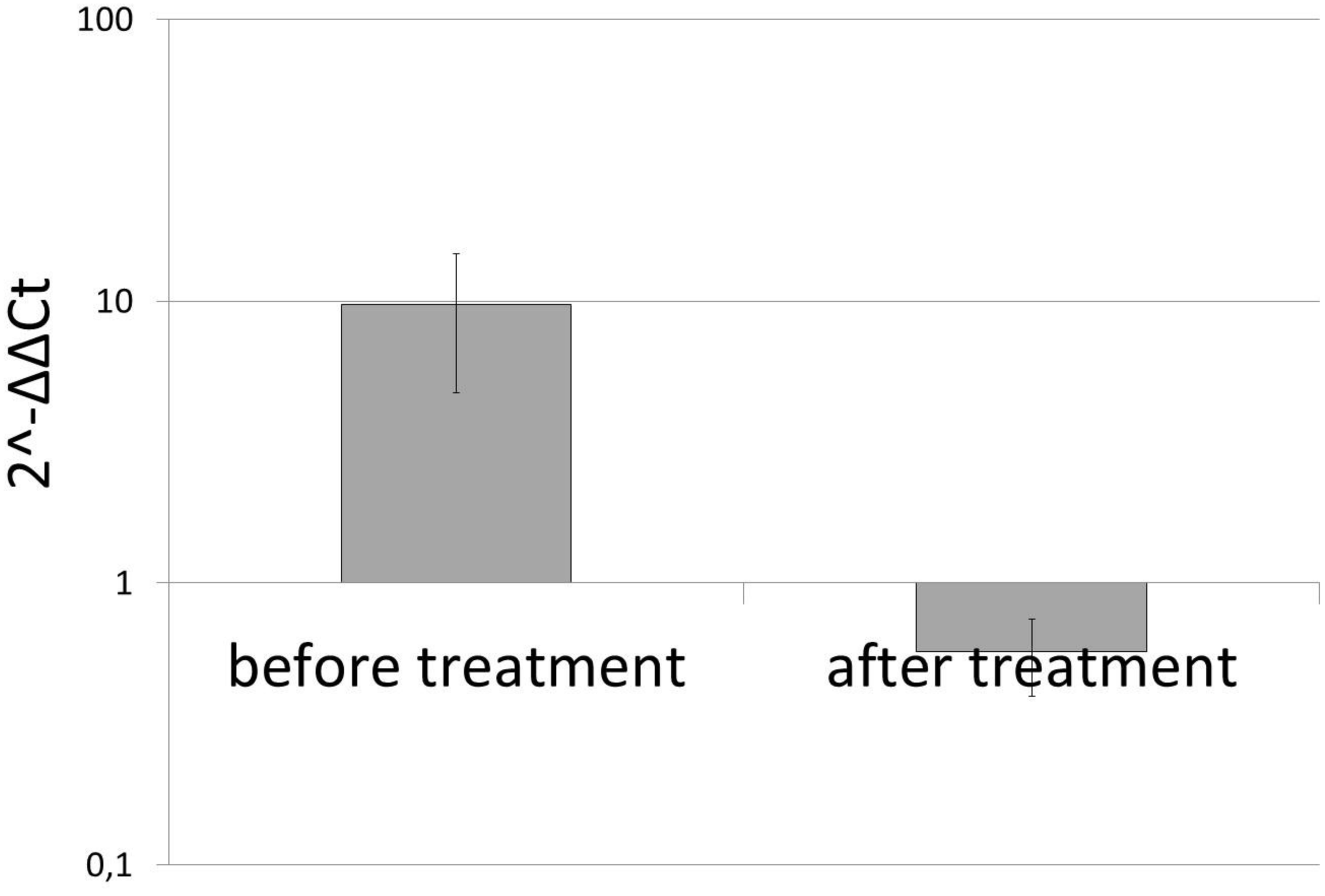
PPARγ



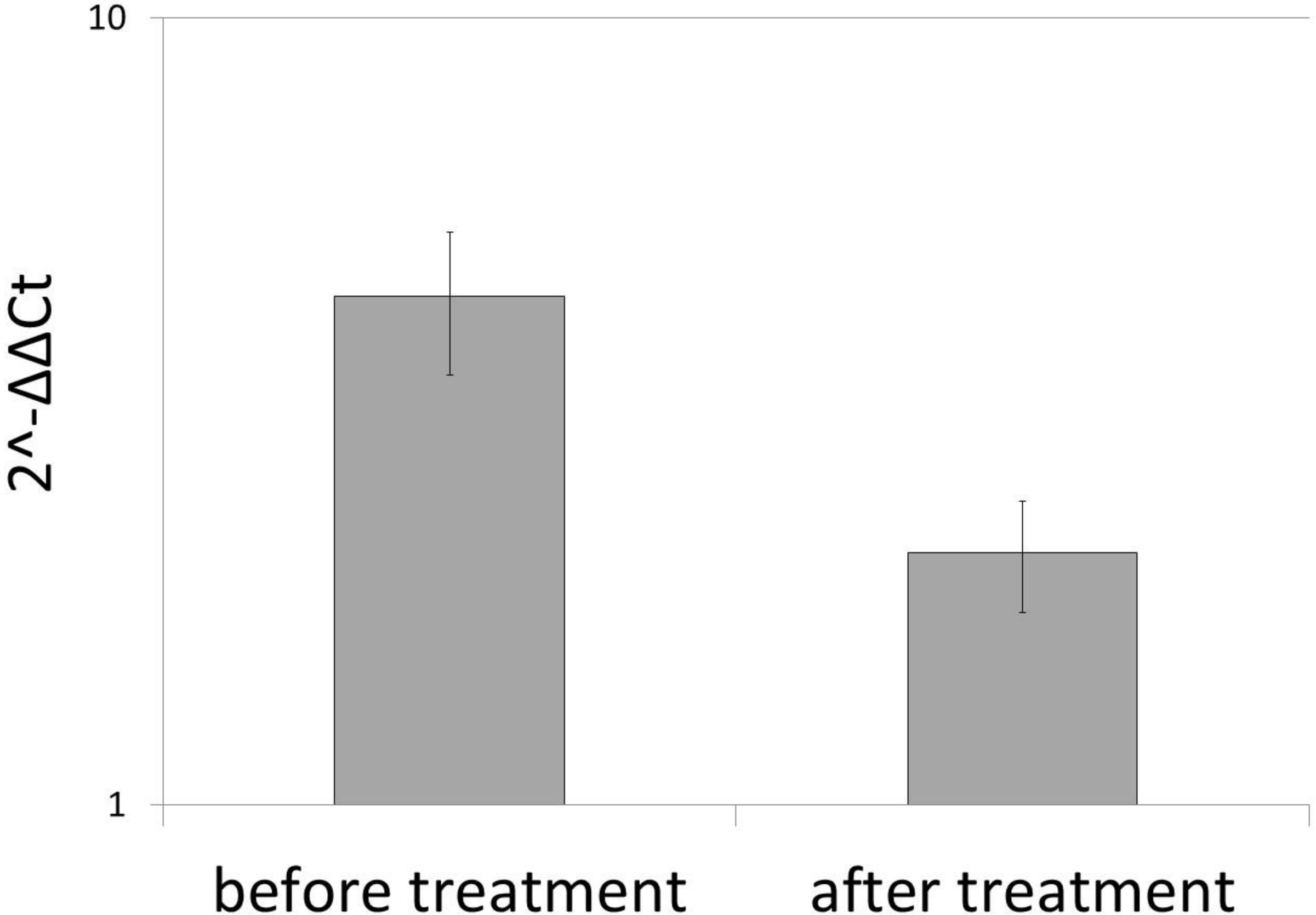
FOXP3



FOSL1



STAT3



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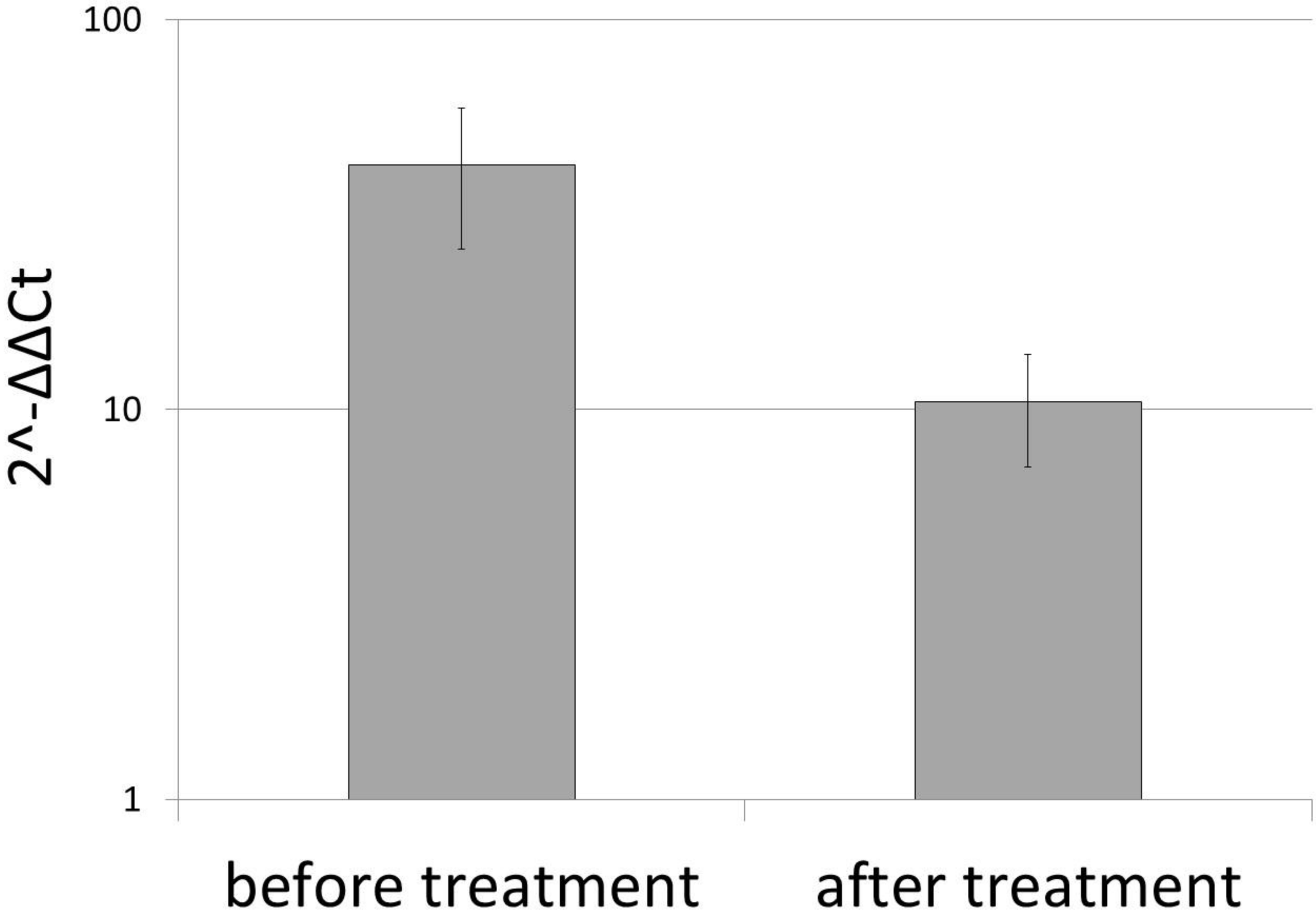

RORC