Supplementary data

miR-146b probably assists miRNA-146a in the suppression of keratinocyte proliferation and inflammatory responses in psoriasis

Helen Hermann,[1,*] Toomas Runnel,[1,2,*] Alar Aab,[1] Hansjörg Baurecht,[3] Elke Rodriguez,[3] Nathaniel Magilnick,[4] Egon Urgard,[1] Liisi Šahmatova,[5,6] Ele Prans,[5,1] Julia Maslovskaja,[1] Kristi Abram,[6] Maire Karelson,[6] Bret Kaldvee,[5,6] Paula Reemann,[1] Uku Haljasorg,[1] Beate Rückert,[7] Paulina Wawrzyniak,[7] Michael Weichenthal,[3] Ulrich Mrowietz,[3] Andre Franke,[8] Christian Gieger,[9] Jonathan Barker,[10] Richard Trembath,[11] Lam C. Tsoi,[12] James T. Elder,[13] Eric R. Tkaczyk,[1,14] Kai Kisand,[1] Pärt Peterson,[1] Külli Kingo,[5,6] Mark Boldin,[4] Stephan Weidinger,[3,#] Cezmi A. Akdis[7,#] and Ana Rebane[1,#]

[1]Institute of Biomedicine and Translational Medicine, University of Tartu, Tartu, Estonia.

[2]Institute of Molecular and Cellular Biology, University of Tartu, Tartu, Estonia.

[3]Department of Dermatology, University Hospital Schleswig-Holstein, Christian-Albrechts-University of Kiel, Kiel, Germany.

[4]Department of Molecular and Cellular Biology, Beckman Research Institute of City of Hope National Medical Center, Duarte, CA, USA

[5]Department of Dermatology and Venereology, University of Tartu, Tartu, Estonia.

[6]Dermatology Clinic, Tartu University Hospital, Tartu, Estonia.

[7]Swiss Institute of Allergy and Asthma Research (SIAF), University of Zürich, Davos, Switzerland.

[8]Institute of Clinical Molecular Biology, Christian-Albrechts-University of Kiel, Kiel, Germany.

[9]Institute of Genetic Epidemiology, Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg, Germany.

[10]Division of Genetics and Molecular Medicine, King's College London, London, UK and St John's Institute of Dermatology, King's College London, London, UK.

[11]Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London, UK.

[12]Department of Dermatology, University of Michigan, Ann Arbor, MI, USA and Ann Arbor Veterans Affairs Hospital, Ann Arbor, MI, USA.

[13]Department of Biostatistics, Center for Statistical Genetics, University of Michigan, Ann Arbor, MI, USA

[14]Department of Medicine, Vanderbilt University Medical Center, Nashville, Tennessee, USA

*These authors contributed equally to this work

[#]These senior authors contributed equally to this work

Supplementary

MATERIALS AND METHODS

Patients

Altogether, 30 patients with plaque psoriasis and 30 control subjects were included in the study, of which 22 patient samples and 22 control samples were used for RNA purification, RT-qPCR and correlation analyses (see Supplemental Table S1). 8 patient samples and 8 control samples were used for ISH and IF, of which two representative ISHs and one IF are shown. Skin samples from patients with psoriasis were collected from the Dermatology Clinic of Tartu University Hospital or from University Hospital Kiel (Germany). All of the participants were unrelated Caucasians living either Estonia or Germany. The Psoriasis Area and Severity Index (PASI) score of the patients ranged from 2 to 27.6. Control subjects were recruited from among health care personnel, medical students and patients who turned to dermatological outpatient clinic for surgical excision of nevi. None of control subjects had any history of chronic skin disease or family history of psoriasis. Two punch biopsy skin samples (3-4 mm in diameter) were taken from each patient with psoriasis, one from the marginal zone of lesional skin and another from non-sun-exposed non-lesional skin. One punch biopsy skin sample (3-4 mm in diameter) from non-sun-exposed skin was taken from each control subject. The skin samples were immediately frozen using dry ice or liquid nitrogen and stored at -80˚C until RNA extraction. For *in situ* hybridization and immunofluorescence, skin biopsy specimens were embedded into the Tissue-Tek (Thermo Scientific, Waltham, Mass) before freezing.

Human cell culture

Pooled, normal human epidermal keratinocytes (Promocell, Heidelberg, Germany) were cultured and stimulated as described previously (Rebane *et al.*, 2014). Human primary fibroblasts were cultured in Dulbecco's Modified Eagle Medium (Life Technologies, Grand Island, NY) as described in (Reemann *et*

al., 2014). Human PBMCs were isolated from peripheral blood by using density gradient centrifugation on Ficoll (Biochrom, Berlin, Germany) as described previously (Tserel *et al.*, 2011). The following concentrations of cytokines were used: TNF-α 25 ng/ml, IL-1β 10 ng/ml, IFN-γ 10 ng/ml, IL-17A 10 ng/ml, IL-22 10 ng/ml (PeproTech, London, United Kingdom). For 3D keratinocyte culture in air-liquid interface (ALI), $5x10^5$ cells were seeded on ThinCert Cell Culture Inserts (0,4 µm pore, 0.33 cm^2) on 24-well plates (Greiner Bio-One, Kremsmünster, Austria) using Keratinocyte-SFM medium with supplements and Dulbecco's Modified Eagle Medium (both from Life Technologies, Grand Island, NY) containing High Glucose, GlutaMAX™ and Pyruvate in 1:1 ratio. To monitor the quality of 3D culture, transepithelial resistance (TER) was measured in every 24 h using Millicell ERS-2 Voltohmmeter (Merk Millipore, Darmstadt, Germany). $2x10^4$ keratinocytes per one well of 12-well or $5x10^3$ cells per one well of 96-well (Figure 3a, b and 5b) plate was seeded 24 hours before the transfection in 1.0 ml or 100 µl of Keratinocyte-SFM with supplements (Life Technologies), repectively. The transfection with siPORT NeoFX (Life Technologies, Grand Island, NY) was performed according to the manufacturer's instructions. 3 µl or 0.3 µl of siPORT NeoFX (Life Technologies, Grand Island, NY) per one well of 12-well or 96-well plate was used, respectively. Transfection with PepFect14 was used for Figure 5b and was performed as in (Urgard *et al.*, 2016). Briefly, miRNA mimics at 2:1 PepFect14-miRNA charge ratio (molar ratio 17:1) were incubated in 60 µl of water at room temperature for 1 h, then mixed with 0.6 ml of the growth media and shared to the cells in six welle of 96-well plate after removing the old growth media. After 24 h, keratinocytes were stimulated with cytokines as indicated for 48 h. Transfections in keratinocytes were performed at 60 nM of Pre-miR Precursors Molecule hsa-miR-146a, and Pre-miR Negative Control #1 or at 30 nM of miRNA mimic 2.0, miR-146b-5p and mirVana miRNA Mimic, Negative Control #1 (all from Life Technologies) and miRCURY LNA™ inhibitors (microRNA Power inhibitor hsa-miR-146a, has-miR-146 and Negative Control A, Exiqon, Vedbaek, Denmark). Silencer Select siRNAs for

IRAK1 (s323), CARD10 (s26577), FERMT1 (s31076) and negative control #1 (Life Technologies) were used at 50 nM concentration.

***In situ* hybridization (ISH)**

ISH was performed on 10 µm frozen sections using microRNA ISH Buffer, Controls Kit and 5`-DIG and 3`-DIG labeled hsa-miR-146a-5p (619856-360) and hsa-miR-146b (615935-360) miRCURY LNA™ Detection probes according to the manufacturer's protocol (Exiqon). Prehybridization, hybridization and washings were performed at 50 °C for miR-146a and at 48 °C for miR-146b. For detection, slides were incubated with alkaline phosphatase-conjugated sheep anti-DIG-AP (1:1500, Roche, Basel, Switzerland) for 1h at room temperature. The staining was visualized by adding BM purple AP substrate (Roche).

Array, pathway and target analysis

To study miR-146a impact in unstimulated keratinocytes, a previously published dataset, E-MTAB-1739 (Rebane *et al.*, 2014), was reanalyzed. The array data were analyzed with GenomeStudio 2011.1 Gene Expression Module using Average Normalization for miRNA data and Illumina's custom rank invariant method for mRNA arrays. Genes were considered differentially expressed at adjusted $P < 0.05$. Further analyses and visualizations were performed using Microsoft Excel and Multi Experiment Viewer 4.8.1. Pathway analysis was performed with g:Profiler (http://biit.cs.ut.ee/gprofiler), which estimates significance of the overlap between the functional groups and the list of studied genes by calculating enrichment P-value using Fisher's one-tailed test. Putative direct targets for miR-146a were selected using Targetscan search choosing only highly conserved and those with better context score (<-0.15) (Friedman *et al.*, 2009; Garcia *et al.*, 2011).

Isolation of RNA, cDNA synthesis and RT-qPCR

Total RNA was extracted using miRNAeasy Mini Kit (Qiagen) or Direct-zol RNA MiniPrep kit (Zymo Research (Irvine, CA). cDNA was synthesized from 100-900 ng of total RNA using oligo-dT and reagents from Thermo Scientific. RNA concentration was assessed with NanoDrop ND-1000. 5x HOT FIREPol EvaGreen qPCR Supermix (Solis BioDyne, Tartu, Estonia) and ABI Prism 7900 or Via7 were used for qPCR. The relative gene expression levels were normalized to the level of human EEF1A1 or mouse Hprt and calculated using the comparative Ct (ΔΔCt) method (Life Technologies). PCR primers were designed with assistance of Primer 3 and were ordered from Microsynth, Balgach, Switzerland or TAG Copenhagen (Copenhagen, Denmark). miRNA qPCR was carried out using TaqMan microRNA Assays (Life Technologies) and using 5× HOT FIREPol® Probe qPCR Mix Plus (ROX) (Solis BioDyne). miRNA RT-qPCR results were normalized to let-7a. Relative expression is shown compared to the mean of control experiments or control group (=1) both in mRNA and miRNA RT-qPCR.

Proliferation assays

KCs were transfected on 96-well plate as described above. Cytokines were added after 24 hours and ^{3}H thymidine (Hartmann Analytic) at end-concentration of 1 µCi/ml after 48 hours. During cell-division, ^{3}H thymidine was incorporated into the replicating DNA. After 14 hours, cells were harvested onto a glass-fiber filter, mounted with a cocktail containing scintillation liquid and the counts were monitored. β-counts of the control cells were in the range of 2.7×10^{4} and 1.2×10^{6} counts per minute (cpm). Alternatively, after 24h of the transfection, cell proliferation was analyzed using CellTiter-Glo® Luminescent Cell Viability Assay (Promega, Madison, Wis) in which the measured Luciferase activity is proportional with the amount of cellular ATP.

qPCR primers

The following primers were used to detect human mRNAs: FERMT1 for: 5'-TGATGCAGCCACCGGGATTCCA-3', FERMT1 rev: 5'-CGA TGACCACCTGCCGGGTTTC-3', NUMB for: 5'-AATGCCTTCAGCACACCTGA-3', NUMB rev 5'-AGTC AGTGCCATTAGCTTGGAA-3', Hprt for 5'-CTCTCGAAGTGTTGGATACAG-3', Hprt rev 5'-ACAAACGTGATTCAAATCCCC-3'. Sequences for human and CCL5, IRAK1, EEF1A1 (EF1A), human IL-8 and mouse Ccl5, Cxcl1, Irak and Card10 primers have been provided before (Rebane *et al.*, 2014).

Cloning and Luciferase assay

3'UTR fragments containing binding sites for miR-146a and the mutant binding sites of FERMT1 were cloned into the pmirGLO Dual-Luciferase miRNA Target Expression Vector (Promega) using the PCR primers containing NheI and SalI sites. The following primers were used: FERMT1-1 3'UTR for: 5'-ATTGCTAGCACCCACTGCTGGCACATCCCT-3', FERMT1-1 3'UTR rev: 5'-ATTGTCGACGCAGTTCC ATGAAGGACAGGCG-3', FERMT1-2 3'UTR for: 5'-ATAGCTAGCATTAACAGCTGCCTGAATT-3', FERMT1-2 3'UTR rev: 5'-ATAGTCGACAAGTCTGTCTCTATGTGGCT-3', FERMT1-1 3'UTR for mut: TGGTTACAGTAGTCTGACTTGC, FERMT1-1 3'UTR rev mut: GCA AGTCAGACTACTGTAACCA, FERMT1-2 3'UTR for mut: ATTTTTCAGTAGTCCTAGGATG, FERMT1-2 3'UTR rev mut: CATCCTAGGACTACTGAAAAAT. Transfections were carried out in 24-well plates using 0.8 µl of siPORT NeoFX (Life technologies), 30 nM of pre-miRNAs (Pre-miR™ Precursors Molecules hsa-miR-146a or Negative Control #1, Life Technologies), 50 ng of the reporter plasmid and $2x10^4$ human embryonic kidney epithelial cells HEK293 in 0.6 ml medium for 24 h. Firefly and renilla luciferase activities were measured using Dual-Luciferase® Reporter Assay System (Promega). Firefly luciferase activities were normalized to the values of the renilla luciferase.

Immunofluorescence

10 µm cryosections of skin biopsy specimens were fixed for 10 min with 4% formaldehyde in PBS followed by permeabilization with 0.1% Triton-X-100 in PBS for 10 min and blocking in 10% goat serum and 0.5% BSA (Sigma) in PBS for 1 hour. Specimens were further incubated for 1 hour either with rabbit polyclonal anti-FERMT1 or KRT10 antibodies (Atlas antibodies, Stockholm, Sweden). Alexa-488 labeled anti-rabbit-IgG (Life Technologies) with dilutions of 1:2000 was used as secondary antibody. Before and after antibody incubations, specimens were washed 3x5 min with PBS. As the last 10 min washing step, 1 µg/ml of 4',6-diamidino-2-phenylindol (DAPI) (Roche, Switzerland) in PBS was added. Slides were mounted with StayBrite Hardset Mounting Medium, (Biotium USA) and analyzed with Leica DM5500 B microscope (Leica Microsystems, Germany).

Generation of mice with a targeted deletion of miRNA-146b, mouse strains and maintenance

The miR-146b-/- mice were generated by deleting approximately 938 bp of genomic sequence that encompasses the miR-146b precursor on mouse chromosome 19. The linearized targeting cassette, containing two LoxP sites flanking the miR-146b genomic locus and a neomycin resistance gene flanked by FRT sites, was electroporated into C57BL/6N embryonic stem (ES) cells. Three correctly targeted ES cell lines were used for blastocyst injection and the generation of chimeric mice. The resulting chimeric mice were bred with C57BL6/J mice to obtain offspring with the targeted miR-146b locus in the germline (F1 generation). F1 mice were crossed with Cre-deleter mice (C57BL/6J-Tg(EIIA-Cre)) to remove the miR-146b locus and the neomycin selection cassette in the germline. The heterozygous miR-146b+/- mice were then interbred to homozygosity. MiR-146b-/- mice were generated at the Department of Molecular and Cellular Biology, Beckman Research Institute of City of Hope National Medical Center, Duarte, CA. The related animal experiments were approved by the Institutional Animal Care and Use Committee of City of Hope. miR-146a-/- mice in C57BL/6J background and C57BL/6J (B6)WT mice were

purchased from the Jackson Laboratory (Bar Harbor, Me). Before the isolation of keratinocytes, strains were maintained and bred either in the animal facility at the Laboratory Animal Centre at the Institute of Biomedicine and Translational Medicine, University of Tartu in accordance with the institute's regulations.

Isolation and culture of mouse skin keratinocytes and fibroblasts

For isolation of skin keratinocytes and fibroblasts, six-to seven-week-old and five- to six-week-old male mice were used, respectively. Isolation and short-term culture of keratinocytes and fibroblasts was perfomed according to (Lichti *et al.*, 2008). Separation of epidermis from the dermis of adult mouse skin was conducted with incubation with Dispase II (Life Technologies) at 4°C for overnight. For fibroblasts, the dermis was additionally incubated with 2 ml of 0.35% collagenase Type II (Gibco) at 37°C for 30 minutes in a shaking incubator at low speed and for 10 more minutes with added 12.5 µl of DNase (Zymo Research) per dermis. To achieve a single cell suspension, keratinocytes and fibroblasts were then incubated with 0.05% trypsin at 37°C for 15 minutes and collected separately using 70 µm cell strainers (BD Falcon). Keratinocytes were further cultured in Keratinocyte-SFM medium with supplements (Life Technologies) for 2 days and fibroblasts in Dulbecco's Modified Eagle Medium (Life Technologies, Grand Island, NY) for 4 days before stimulation with 25 ng/ml of TNF-α or 10 ng/ml of IL-17A (PeproTech, London, United Kingdom) for 48 hours.

Statistical analyses

Statistical analyses for RT-qPCR results, flow cytometry and proliferation assays were performed by ANOVA when more than 2 conditions were compared or Student's t-test, otherwise. *Post-hoc* analysis were Bonferroni corrected and the results were considered significant at $P<0.05$ (*) and highly significant at $P<0.01$ (**). Based on observations from the current study, miR-146a/b and 5 direct target

genes regulated by miR146a/b were selected. Association of SNPs in miR146a/b and these target genes (± 50 kb) was analyzed *in silico* using data from a meta-analysis of genome-wide association studies (GWAS) with a total of 4,489 psoriasis cases and 8,240 controls. Details of the meta-analysis have been previously reported (Baurecht *et al.*, 2015). SNPTEST was used to associate the imputed dosage for each SNP with psoriasis status separately in each study sample with adjustment for the first three principal components from a multidimensional scaling (MDS) analysis of population stratification. The association test results of those SNPs with relatively high confidence (PROPER_Info>0.4) were then meta-analyzed with METAL using the inverse-variance method based on a fixed-effect model. To address multiple testing, P-values were Bonferroni-corrected by the number of selected target regions resulting in a significance threshold of 0.05/7=0.00714. Association analysis of rs2910164 genotypes and stratification of patients based on the presence of HLA-Cw*0602 allele was performed using logistic regression models. The models were adjusted for gender and the first 4 principal components to account for ethnicic differences.

Supplementary

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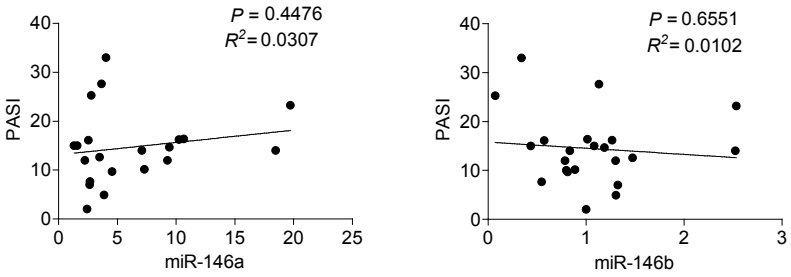

Figure S1. Correlation analysis of miR-146a/b expression and psoriasis severity. The relative expression levels of miR-146a/b in lesional skin of poriasis patients and the Psoriasis Area and Severity Index (PASI) score were included in the correlation analysis.

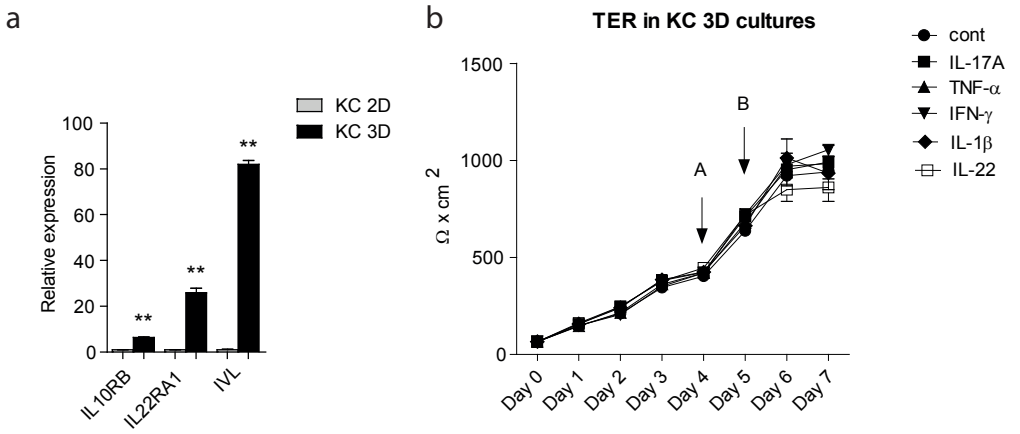

Figure S2. Relative expression of marker genes and transepithelial resistance measurements of keratinocyte 3D cultures. (**a**) Keratinocytes were grown either in proliferating conditions (KC 2D) or in KC 3D culture, data represent the mean ± SEM of three different experiments. Relative expression of indicated genes in KC 3D was measured compared to the value in KC 2D (=1), Student's t-test, **P<0.01. (**b**) 50,000 KCs was seeded per each well on day 0. The apical media was removed on day 4 (A), after 24h (B), KC 3D cultures were stimulated with indicated cytokines for 48h or left unstimulated (us). High expression of involucrin (IVL) and enhancement of transepithelial resistance confirmed the establishment of epidermis.

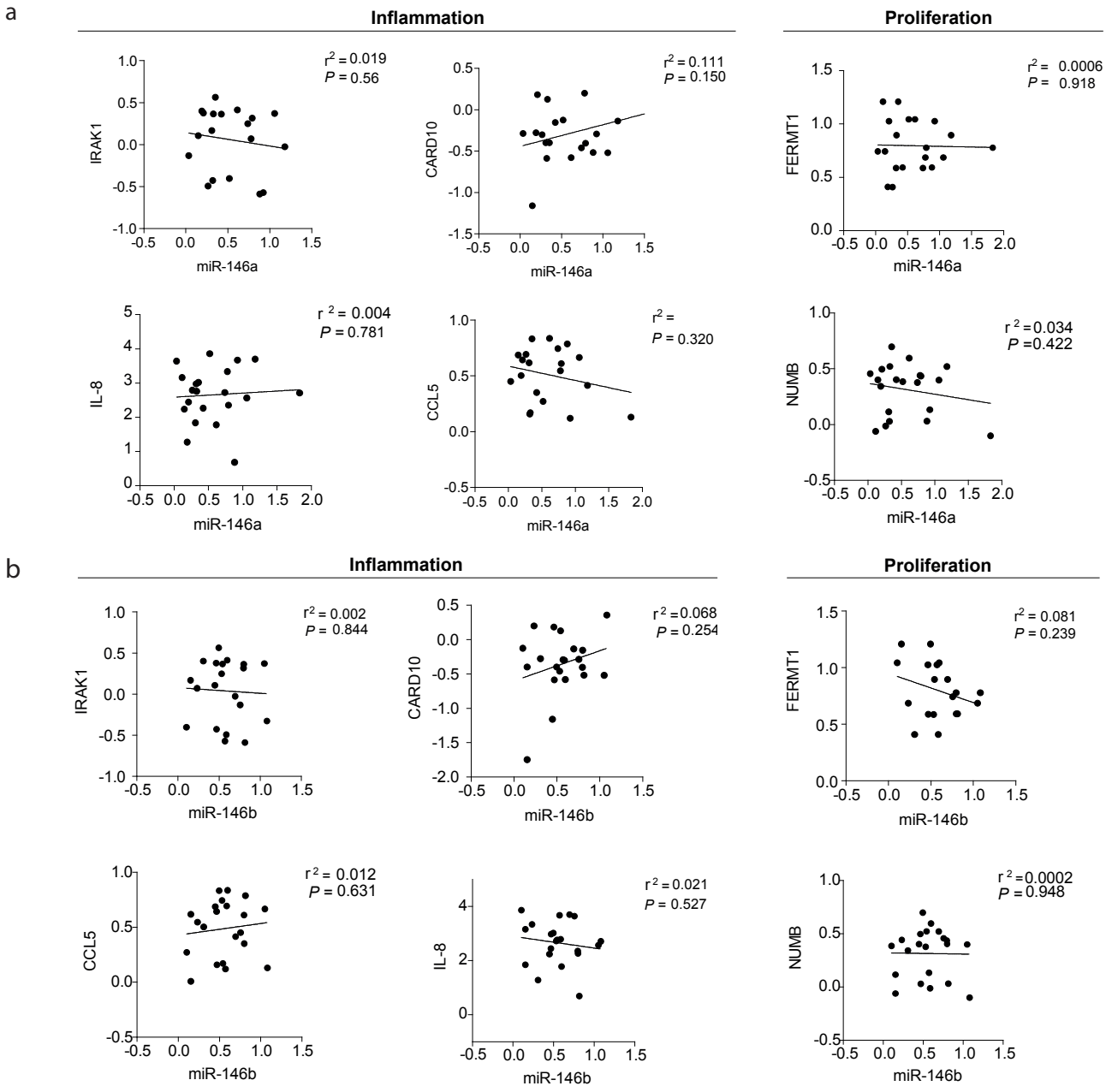

Figure S3. Correlation analysis miR-146a/b and target genes in the skin of psoriasis patients. Log10 values of relative expression of indicated mRNAs and miR-146a (**a**) and miR-146b (**b**) in lesional skin of psoriasis patients are included in the analysis.

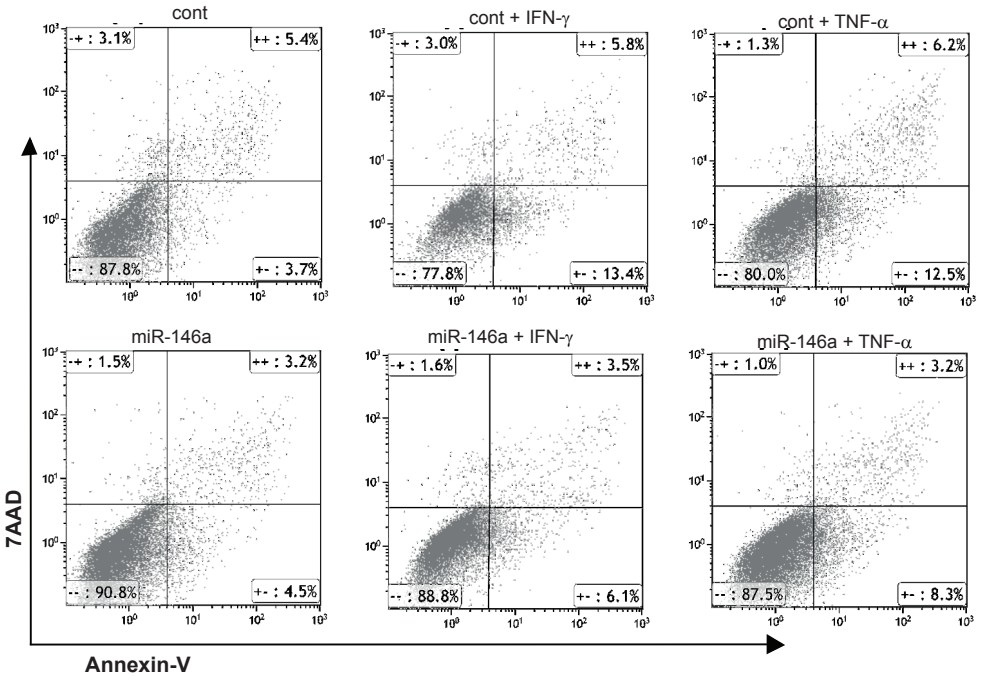

Figure S4. miR-146a has capacity to inhibit activation-induced apoptosis of human primary keratinocytes. Keratinocytes were transfected either with control (cont) or pre-miR-146a (miR-146a) for 24 h and then stimulated with IFN-γ or TNF-α 72 h or left unstimulated (us). The percentage of Annexin-V positive cells unstimulated (us) or stimulated primary KCs is presented. One representative experiment is shown.

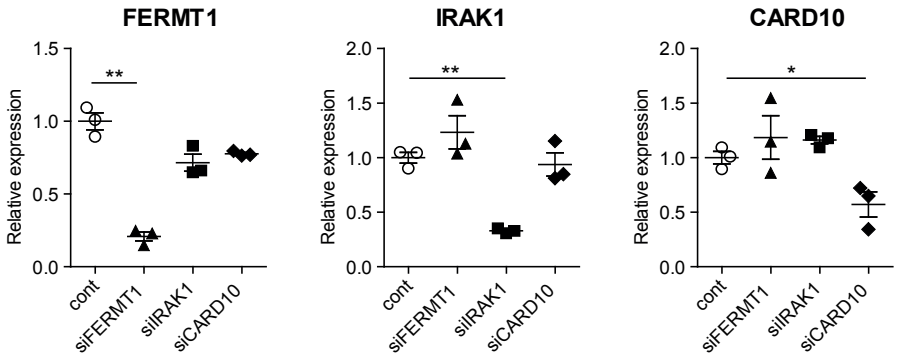

Figure S5. The efficiency of FERMT1, IRAK1 and CARD10 siRNAs. Keratinocytes transfected either with scrambled (cont) or indicated specific siRNAs for 24 h. Data represent the mean ± SEM Student's t-test, *P<0.05, **P<0.01.

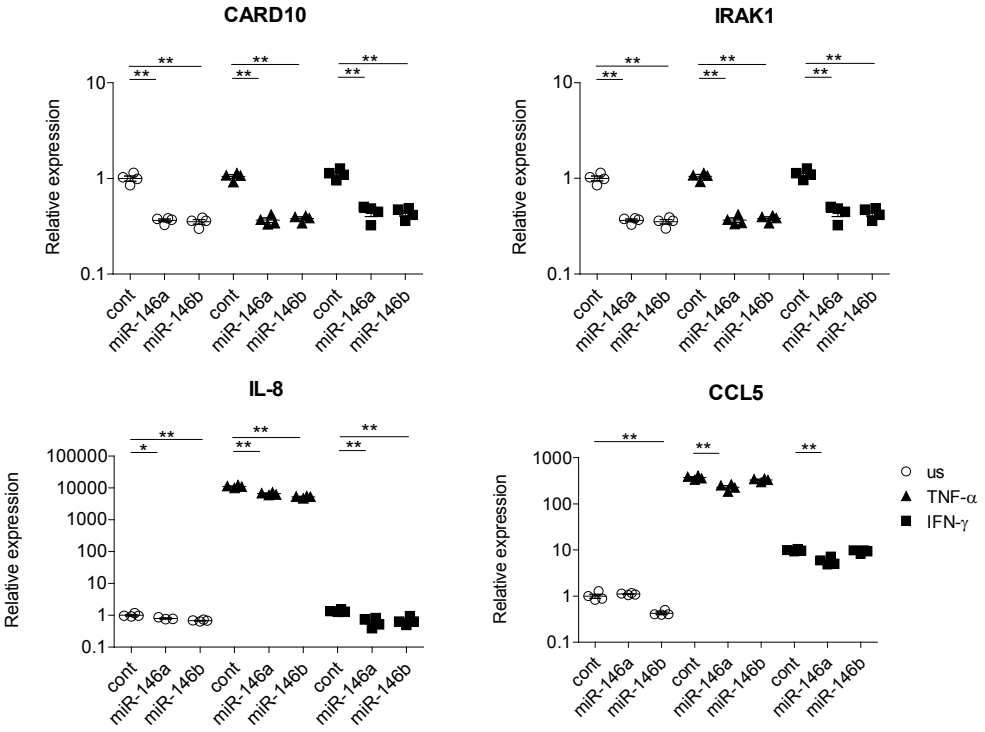

Figure S6. miR-146a/b inhibit the target genes in a similar extent in fibroblasts. Human primary skin fibroblasts were transfected either with control (cont) or miR-146a or miR-146b mimics. Relative expression compared to unstimulated cells (=1) is shown. Where indicated, the cells were stimulated with IFN-γ or TNF-α for 48 h or left unstimulated (us). Data represent mean ± SEM. Student's t-test, * $P < 0.05$, ** $P < 0.01$. Note that FERMT1 and NUMB were not analyzed in fibroblasts as their expression was not detectable.

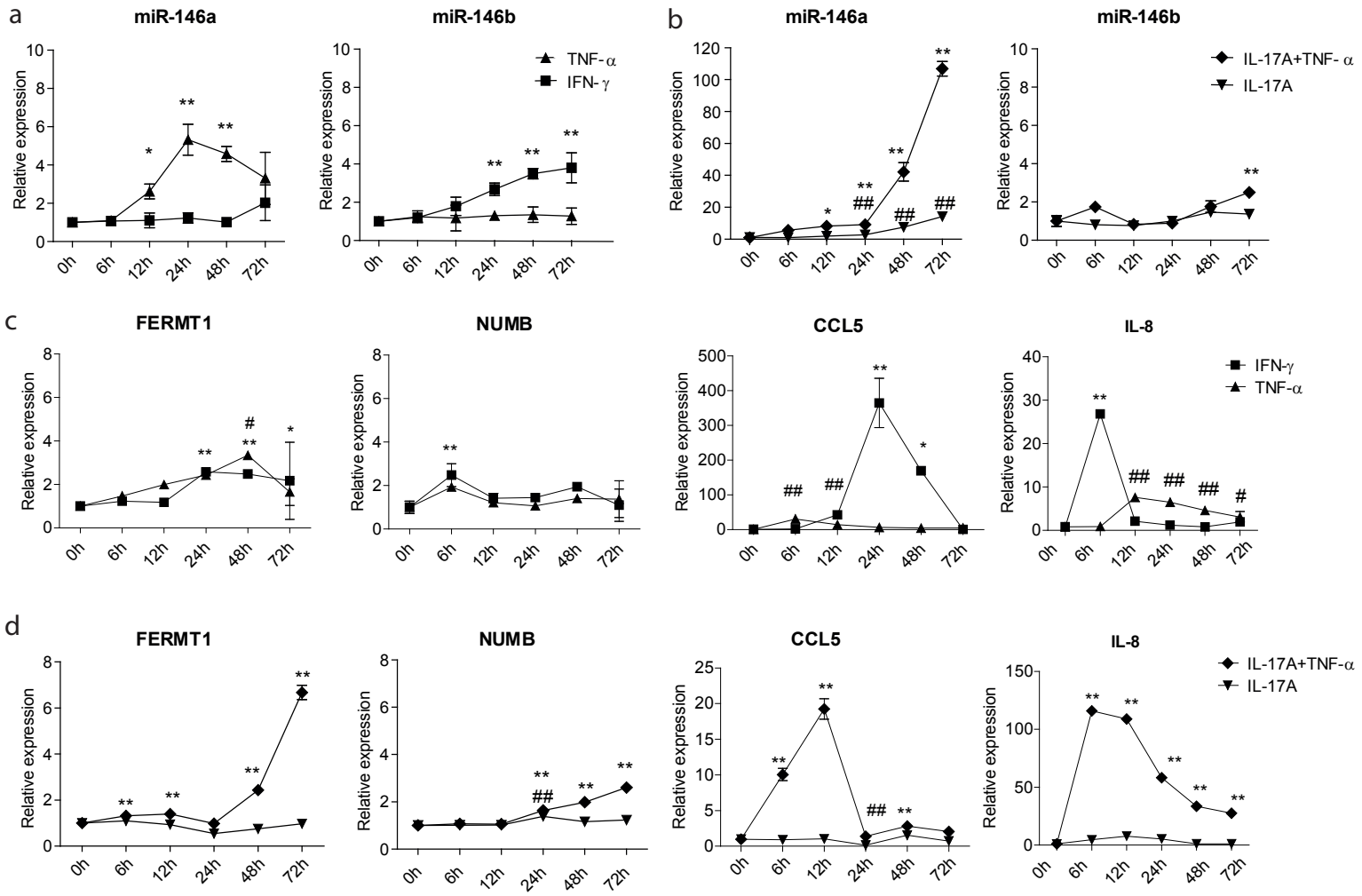

Figure S7. Relative expression of miR-146a/b and targets in keratinocytes. Keratinocytes were stimulated with indicated cytokines for 6, 12, 24, 48 or 72h or left unstimulated. Relative expression compared to unstimulated cells in each timepoint is shown. Data represent mean ± SEM of three different stimulations. Two-way ANOVA Bonferroni post-test,* P < 0.05, **P < 0.01; # P < 0.05 ## P < 0.01 when stimulated with TNF-α or IL-17A.

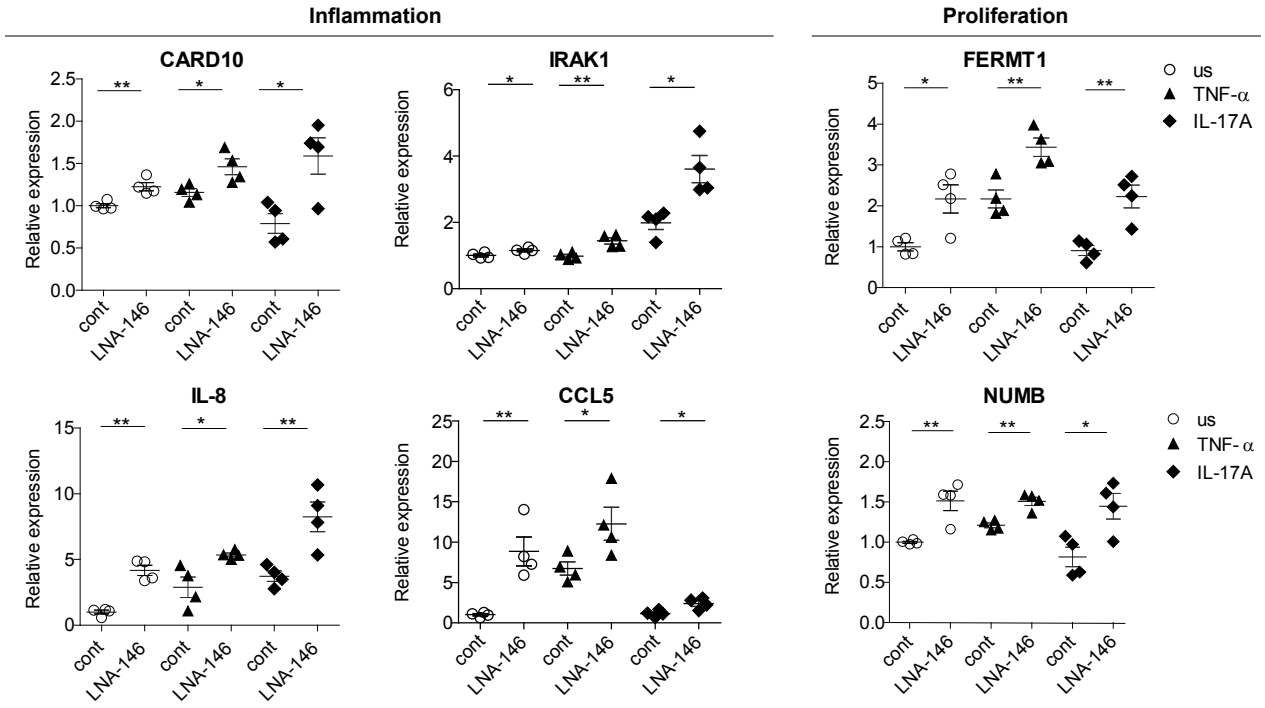

Figure S8. LNA inhibition of miR-146a/b leadst to the increased expression of the target genes. Keratinocytes were transfected either with the control LNA (cont) or LNA inhibitor for miR-146a/b (LNA-146) and stimulated with indicated cytokines for 48 h or left unstimulated (us). Data represent mean ± SEM of eight independent stimulations of cells from two mice in each group. Student's t-test, * $P < 0.05$, ** $P < 0.01$.

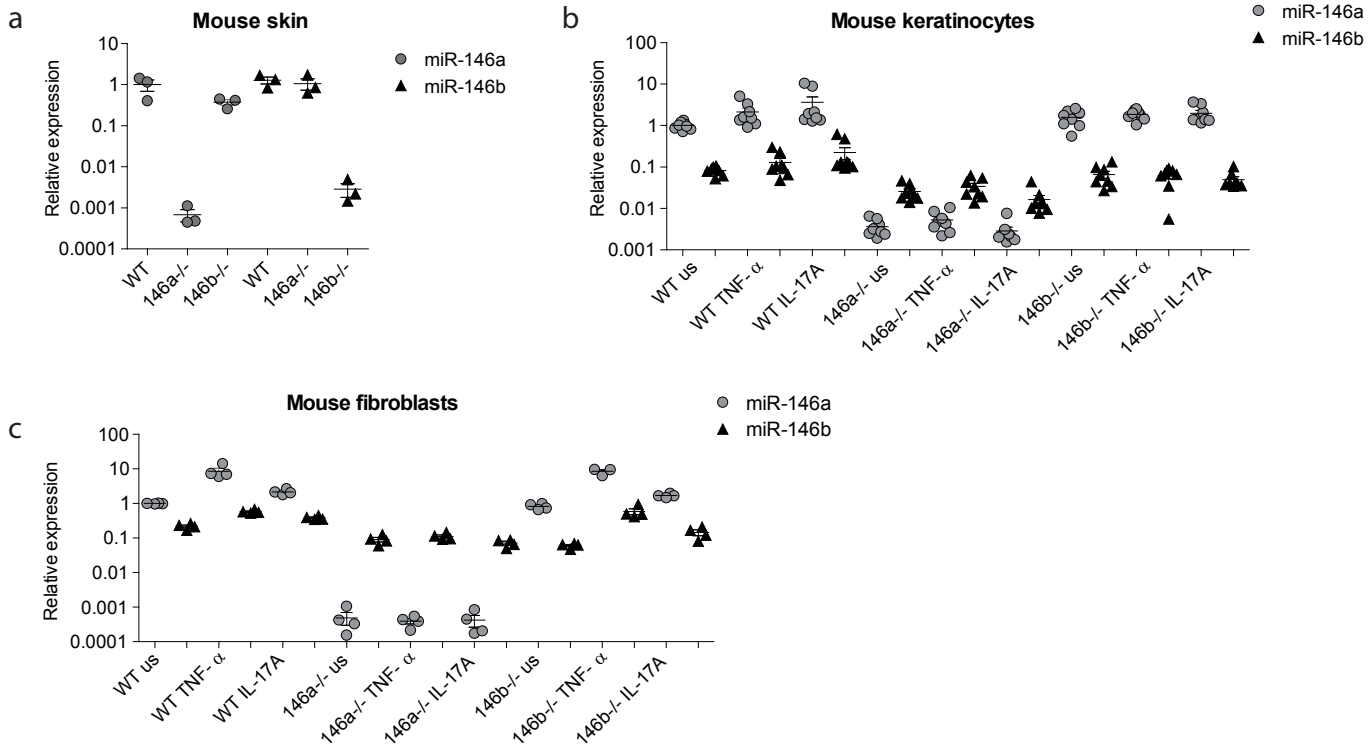

Figure S9. Relative expression of miR-146a/b and selected target genes in the skin, kertinocytes and fibroblasts from wild type and miR-146a-/- and miR-146b-/- mice (**a**) Relative expression of miR-146a/b is shown compared to the average levels of miR-146a in the skin of wild type (WT) mice (=1). Data represent mean ± SEM of three different mouse in each group. (**b** and **c**) Relative expression of miR-146a/b and is shown compared to the level of miR-146a in unstimulated (us) keratinocytes (**b**) or fibroblasts (**c**) from WT mice (=1). Note that due to apparently lower levels of miR-146b as compared to miR-146a (at least12-fold difference in keratinocytes and 4-fold difference in fibroblast) there is a strong crossreactivity of the miR-146b probe with miR-146a. (**b** and **c**) Data represent mean ± SEM of eight (**b**) or four (**c**) independent stimulations of cells from two mouse in each group. (**a** - **c**) Student's t-test, * $P < 0.05$, ** $P < 0.01$.

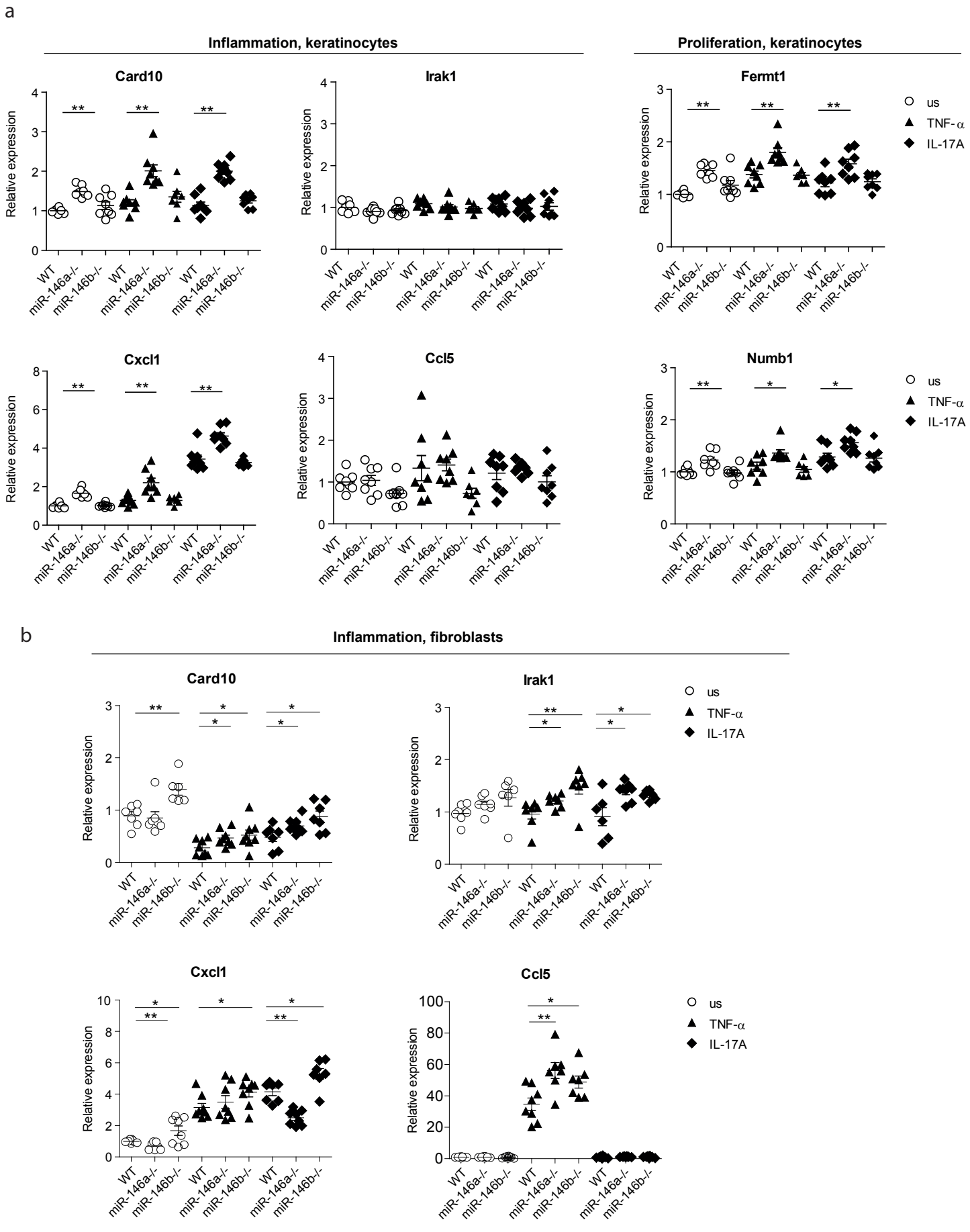

Figure S10. Deficiency of miR-146a or miR-146b results in the increased expression of the target genes. Keratinocytes (**a**) and skin fibroblast (**b**) from wild type (WT), miR-146a-/- or miR-146b-/- mice were stimulated with indicated cytokines for 24 h or left unstimulated (us). Data represent mean ± SEM of eight independent stimulations of cells from two mice in each group. Relative expression compared to the value of us cells is shown. Student's t-test, * $P < 0.05$, ** $P < 0.01$.

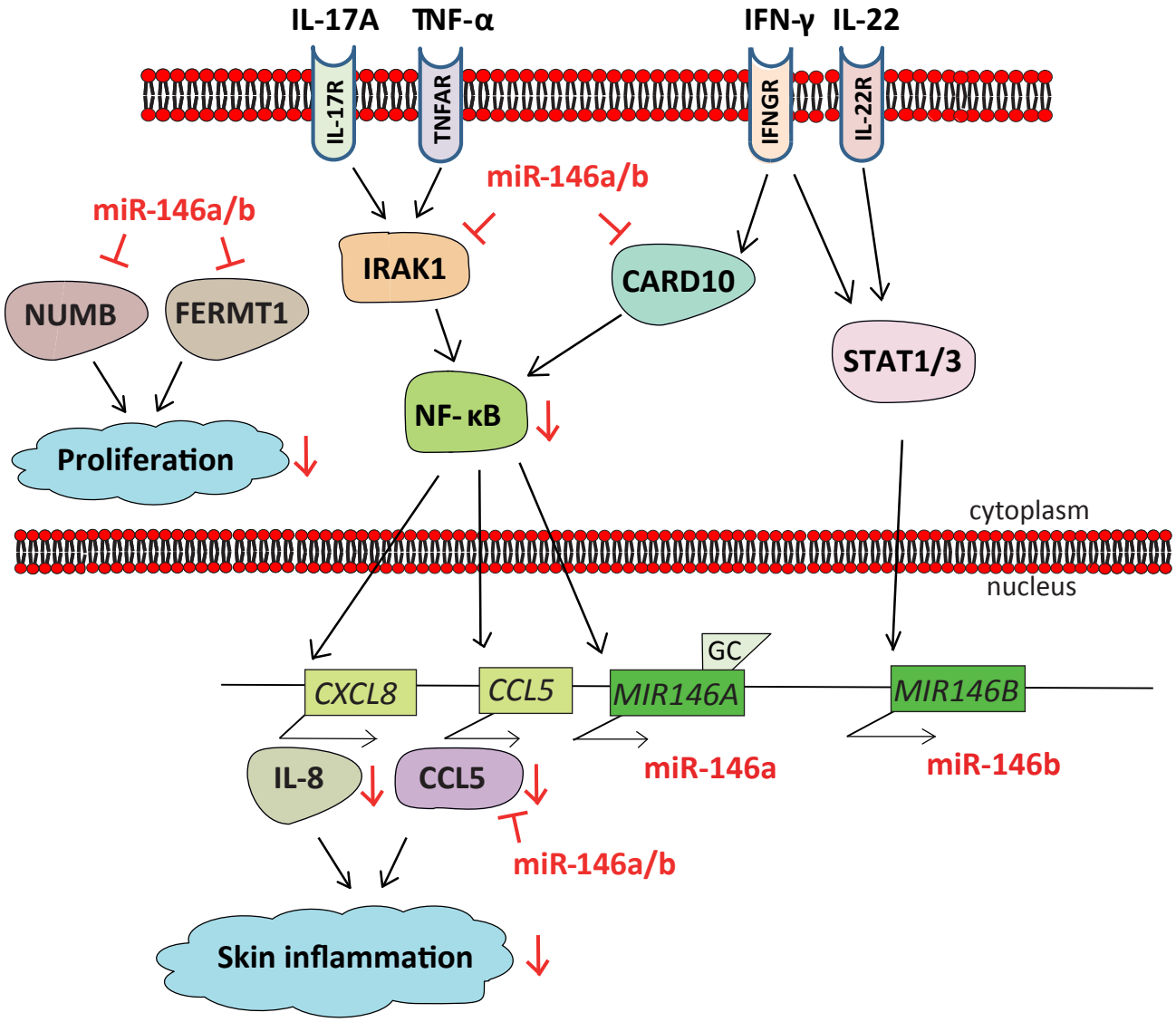

Figure S11. The function of miR-146a/b in keratinocytes and psoriasis. In psoriatic skin keratinocytes, the expression of miR-146a and miR-146b is increased in response to the activation of NF-κB and STAT1 (and/or STAT3), respectively. The elevated expression of miR-146a/b in turn contributes to suppression of pro-inflammatory factors, including IL-8 and CCL5, which can be activated by stimulation of TNF-α, IL-17A or IFN-γ. Previously, it has been demonstrated that the suppression of IL-8 and CCL5 by miR-146a/b occurs via targeting NF-κB signaling transducers IRAK1 or CARD10 and that CCL5 is a direct target of miR-146a. In addition to the supression of inflammatory responses, miR-146a/b inhibit the proliferation of keratinocytes through targeting of multiple factors, including FERMT1 and NUMB. The influence of miR-146a/b to the development of psoriasis is also supported by the moderate association of polymorfisms within the miR-146a-encoding region, of which rs2910164-GC has a protective effect. It should be noted that although miR-146a/b have capacity to inhibit inflammatory responses and the proliferation of keratinocytes, the increased expression of miR-146a/b in psoriatic skin is not sufficient to fully suppress disease-related changes in the skin.

Supplementary Table S1. The list of psoriasis patients included in the expression analysis with RT-qPCR

ID	Gender	Age (years)	PASI[1]	Sample collection place
P024	M	62	14	Dermatology Clinic, Tartu University Hospital (Estonia)
P025	M	27	7,7	Dermatology Clinic, Tartu University Hospital (Estonia)
P026	M	41	16,4	Dermatology Clinic, Tartu University Hospital (Estonia)
P027	M	58	9,7	Dermatology Clinic, Tartu University Hospital (Estonia)
P028	F	56	7	Dermatology Clinic, Tartu University Hospital (Estonia)
P029	F	63	27,6	Dermatology Clinic, Tartu University Hospital (Estonia)
P030	M	60	14,7	Dermatology Clinic, Tartu University Hospital (Estonia)
P031	M	51	25,3	Dermatology Clinic, Tartu University Hospital (Estonia)
P032	F	61	23,2	Dermatology Clinic, Tartu University Hospital (Estonia)
P033	M	27	10,1	Dermatology Clinic, Tartu University Hospital (Estonia)
P034	M	57	11,6	Dermatology Clinic, Tartu University Hospital (Estonia)
P035	M	56	25,6	Dermatology Clinic, Tartu University Hospital (Estonia)
01_01	M	42	15	University Hospital Kiel (Germany)
01_06	M	30	15	University Hospital Kiel (Germany)
01_07	F	60	33	University Hospital Kiel (Germany)
01_08	F	56	12	University Hospital Kiel (Germany)
01_09	F	37	10	University Hospital Kiel (Germany)
01_10	F	54	12	University Hospital Kiel (Germany)
01_11	F	34	14	University Hospital Kiel (Germany)
01_17	M	53	16,1	University Hospital Kiel (Germany)
01_18	F	21	16,2	University Hospital Kiel (Germany)
01_21	M	52	10,2	University Hospital Kiel (Germany)

[1] PASI = Psoriasis Area Severity Index

Supplementary Table S2. The list of genes suppressed by miR-146a in unstimulated human primary keratinocytes[1]

	us		TNF-α		IFN-γ		
SYMBOL	cont	miR-146a	cont	miR-146a	cont	miR-146a	Differential Score
CARD10	2451.6	891.4	2664.9	996.5	2273	589.1	-329.363
MT1G	5345.7	1795.4	3089.8	1930.5	5774.6	1617.2	-329.363
MMP9	1076.8	358.1	10375	4446.5	2088.2	204.4	-304.821
MT1F	1440.2	609.9	1327.8	609.4	2100.8	539.2	-258.428
CSF2	425.8	111.6	1544.6	582.2	527.7	90.1	-177.494
MAOA	3147.9	1748.6	3054.4	2306.7	1980.2	1750.5	-157.449
MMP10	1540.2	845.1	3969.8	2512.9	1655.4	989.3	-144.554
IRAK1	2674.1	771.8	3088.2	1296.2	3404.5	952.3	-143.324
SLC2A3	1193.9	454.1	1206	432.8	3744.9	1798.4	-120.57
PTGS2	2137.2	734.5	2593	1368.3	4766.5	3369	-87.973
SERPINE1	2724.6	1457.6	2388.1	1226.1	712.9	411	-82.436
ERRFI1	1664.2	1092.3	1286.8	1115.8	1430	1430.5	-82.168
DDEF2	937	564.3	929.1	839.9	590.7	830.2	-72.341
TNFAIP3	684.9	366.3	1941.4	1139.5	1623.1	1072.5	-65.42
PLAU	7540.4	4935.2	15322.8	11012.3	9237.8	4360.3	-64.46
TGFA	1608.9	1001	2513.9	1714.3	3308.6	2116.1	-60.554
MT1H	470.8	123.3	243.2	52.8	603.5	141.8	-60.205
PTPRK	1096.2	581.6	1045.7	842.5	512.3	644.9	-57.747
SUMO3	4358.8	3133.3	4319.8	3427.3	4019.2	2761.4	-56.799
AIF1L	829.5	497.5	1534.2	894.6	2086.5	1586.3	-56.047
MED20	931.2	523.7	716	385.2	605.4	350.6	-55.072
IL1F9	938.8	625.6	3493	2497.6	2052.1	1338.3	-53.085
NUMB	1255.5	732.8	1305.7	870.9	1475.9	1053.4	-51.932
SPRR2F	4481.1	2895	12432.1	8996.4	15507.3	10305.7	-50.089
CXCL5	203.5	59.6	814.8	293.2	135.3	47.3	-49.489
LOC642489	7457.2	4829.8	9794.4	7096	11396.1	7182.5	-47.667
SCG5	771.5	486.9	626	570.8	121.3	152.4	-45.071
ANAPC13	1735.8	1244.7	1120.1	1063	834.3	934.9	-43.666
CHST15	1035.8	556.1	1416.6	629.4	1240.8	595.9	-43.666
DUSP22	1007	540.6	1209.8	577.9	1026.6	491.4	-43.627
LCE3D	1372.6	974	4911.3	2799.8	4564.9	2327.8	-43.373
CEACAM6	668.9	441.6	1320.2	985.5	2130.6	2144.1	-40.095
SNRNP27	546.2	276.6	549.6	283.6	698.8	378	-38.781
EFNA1	606.4	398.4	1614	1076.8	964.2	519	-36.4
SRPRB	816.1	492.1	739.4	424	488.4	247.7	-35.522
SIK3	377.1	215.4	347.3	239.8	387	294.7	-35.496

PNLIPRP3	706.9	480.1	755.8	594.4	686	784.1	-35.436
FHL1	1160.4	553.2	1496	992.1	1355.4	723.7	-34.699
LOC644743	419.5	263.9	764.4	404.1	1295.6	544.2	-34.404
UGDH	352.5	197.3	245.1	254.7	326	395.7	-34.288
HYOU1	496.1	248.7	612.9	214.3	331.5	150	-34.101
FOXQ1	616.8	408.2	671.3	304.4	983.1	897.4	-33.104
KRT19	1447.1	917.7	3388.2	2236	4781.2	2039.2	-32.213
HERPUD1	1142.9	804.8	1522.5	1050.7	1007.8	638.7	-32.093
TMEM200A	857.2	506.7	722.5	474.7	381.6	440.4	-31.788
DEFB103B	3552.3	2092.5	11070.3	7176.4	21723.8	13218.3	-31.638
TMX1	345.9	161.9	208.8	111.5	205	295.6	-30.584
PPP4R1	2285.4	1653.8	2418.4	2000.3	2873.9	2429.1	-30.082
MARCKSL1	962.5	694.5	1649.3	1234.1	5653.4	3976.6	-29.993
PHKB	113.8	36.5	64.5	-34.2	46.6	55.7	-29.719
TOR1A	554.5	339.8	644.7	312	483.4	223	-28.636
ZMPSTE24	1769.6	1008	1299	1071.5	564.9	1207.8	-28.441
FOLR3	994.8	642.8	1128.2	746.9	976.8	584.3	-27.378
RNASE7	1011.7	637.9	2509.9	1442.2	2983.2	1383	-26.269
FERMT1	3878.2	1884.8	6315.3	4680.6	3954.5	2168	-26.034
SLC10A3	341.9	135.1	408.7	114.3	422.8	127.1	-25.761
TSPAN14	462	228	625.2	170.2	713.2	291.6	-25.761
IL8	457.3	170	1161.4	657.2	434.1	135.5	-25.42
C18orf25	492.1	324.5	338.6	305.5	491.7	741.9	-24.935
TIMM17B	199.3	81.9	266.2	108.6	273.5	73.4	-24.417
TIPARP	1862.7	1427.7	1518.6	1618.3	1003.6	1369	-24.417
ST13	877.8	507.8	608.1	441.2	397.3	540.9	-24.28
LOC730820	828.2	605	786.7	841.7	944.2	1127.9	-23.188
HBEGF	1597.4	1043.8	4003.9	3018.8	4710.9	2075.1	-23.151
MTSS1	537.3	350.1	852.3	568.2	545.4	470.5	-23.1
LOC283267	702.9	498.6	536.3	498.1	244.4	264.4	-22.922
CYGB	1102.9	690.9	1094.9	1118.7	1028.9	500.2	-22.747
PLD5	559.1	377	644.6	451.3	414	446.4	-21.817
SHISA5	2339.7	1811.1	2537.3	1835	3088.1	2195	-21.143
ENC1	942.7	704.4	932.9	803.6	476.8	519	-20.99
SH3PXD2A	3872	2371.6	4185.1	2874.9	2692.4	1817.5	-20.722
ADAMTS1	1197.7	762.8	930.4	647.8	426.7	512.7	-20.622
GOLPH3L	187.3	77.5	127.5	62.1	100.9	70.6	-20.409
KRT8	978	571.8	1426.2	1137.6	2183.2	996.5	-20.182
RTTN	1311.3	982.2	1612.4	1469.3	985.3	907.3	-20.181
HIST2H2BE	531.7	342.5	821.3	505.5	1078.6	672.1	-20.017
COPS8	303.1	166.5	212	52.5	260.9	170.3	-18.483

SLC7A1	2465.9	1948.2	2165.3	2083.6	2452.7	1928.7	-18.279
SERPINB2	9621.4	5871.8	15360.2	12993.3	24724.1	14168.4	-18.218
HSP90B1	2386.2	1680.1	2257.8	2199.1	795.4	1534.6	-18.143
TXNRD1	853	553	923.2	616.2	559.6	466	-17.775
HNRNPD	5226	3897.1	4853.2	3869.8	4052.4	3036.1	-17.056
KYNU	270.1	132.9	318.8	209.1	200.5	154.3	-16.649
PTPN1	2069.4	1638.4	2556.1	1758.9	2621.7	1764.1	-16.513
ZNF512	358.7	226.6	228.8	137.8	281.1	263.5	-16.513
BNIP3L	437.5	216.5	421.6	282.2	252.4	466.5	-16.49
GFPT1	511.4	337.2	531.7	397.9	432.3	555.4	-16.29
LANCL1	1073.2	571.3	682.7	520.9	277.1	402.3	-16.108
SMAD3	412.4	270.2	433.2	262.6	132.3	114.8	-15.591
FAF2	1380.9	972.3	1369	1081.3	1208.4	1080.9	-15.521
LOC151579	1893	848.6	1480	869	1471.4	2532.2	-15.463
ODC1	6649.5	4692.5	9733.9	7144.3	1487.4	815.6	-15.434
CCDC6	1568.1	1212.3	1106.9	1199.9	659.9	1098.8	-15.362
HDAC3	1008.2	748.5	1045.7	784	1144.8	752.3	-15.269
ARMCX2	527.3	378	465.7	272.5	231.4	228	-15.075
LOC100216001	602.7	391.9	531.3	543.2	105.6	81.3	-14.385
NDEL1	1548.9	1057.9	2046.6	1472	2385	1618.1	-14.331
RHOA	7172.4	5109.9	7485.8	6039.2	9111.2	6967.7	-14.317
RHOB	458.3	283.4	640.2	223.4	937.7	524.2	-13.772
EIF4A2	1916.1	1427.9	1433.6	1646.2	926.4	1184.6	-13.61
TMEM41B	989.4	675.1	846.3	790.9	944.8	1152.5	-13.575

[1] Keratinocytes were transfected either with control (cont) or pre-miR-146a (miR-146a) for 24 h and then stimulated with IFN-γ or TNF-α for 48 h or left unstimulated (us). Average expression signals (n=3) of mRNA with differential $P < 0.05$ (Differential Score < -13.00 in unstimulated conditions) are shown.

Supplementary Table S3. Pathway analysis of genes suppressed by miR-146a in unstimulated keratinocytes[1]

p-value	Functional group	Overlapped genes	Direct targets
5.80e-03	cell proliferation (GO:0008283) 1809 genes	CSF2, PTGS2, SERPINE1, TNFAIP3, PLAU, TGFA, PTPRK, NUMB, CXCL5, DUSP22, TMX1, MARCKSL1, FERMT1, IL8, HBEGF, MTSS1, ADAMTS1, TXNRD1, SMAD3, ODC1, NDEL1, RHOA	NUMB, TMX1, RHOA, PTGS2, FERMT1

[1]The *P*-value form Fisher exact test showing the significance of the overlap between the target list and indicated functional group.

Supplementary Table S4. *In silico* association anlaysis of genetic markers of *MIR146A* and *MIR146B*.[1]

Target gene ± **50kb**	MarkerName	CHR	POS	EA/OA*	EAF	OR	95% CI	P-value	mapped gene
miR-146a	rs2961918	5	159901408	A>C	0,6341	1,111	1.041-1.186	0,001523	*DQ658414*
miR-146a	rs2961919	5	159903134	A>G	0,6778	1,099	1.033-1.169	0,002774	*DQ658414*
miR-146a	rs888656	5	159903870	C>G	0,6723	1,093	1.028-1.162	0,004724	*DQ658414*
miR-146a	rs3096020	5	159905679	T>C	0,673	1,092	1.027-1.161	0,005138	*DQ658414*
miR-146a	**rs2961920**	**5**	**159911506**	**A>C**	**0,7677**	**1,117**	**1.043-1.196**	**0,001521**	***DQ658414***
miR-146a	rs2910164	5	159912418	G>C	0,768	1,115	1.042-1.194	0,001761	*miR-146a*
miR-146a	chr5:159913283:D	5	159913283	CTTTAGA>C	0,7687	1,113	1.040-1.192	0,002143	*DQ658414*
miR-146a	rs4921290	5	159914240	A>T	0,768	1,115	1.041-1.194	0,001892	*DQ658414*
miR-146a	rs17057868	5	159914665	T>C	0,768	1,115	1.041-1.193	0,001918	near-DQ658414
miR-146a	rs62390337	5	159914884	C>T	0,768	1,115	1.041-1.194	0,001858	near-DQ658414
miR-146a	rs61665417	5	159918335	C>T	0,7195	1,097	1.028-1.170	0,005085	near-DQ658414
miR-146a	**rs184776122**	**5**	**159949791**	**A<C**	**0,0565**	**0,799**	**0.682-0.935**	**0,005075**	**near-DQ658414**
CARD10	rs760721	22	37905645	G<C	0,273	1,111	1.034-1.194	0,004052	*CARD10*
CARD10	**37905648:D**	**22**	**37905648**	**C<CG**	**0,2541**	**1,128**	**1.046-1.217**	**0,001842**	***CARD10***
CARD10	rs760722	22	37905649	C<G	0,2757	1,112	1.034-1.195	0,003984	*CARD10*
CARD10	**rs7293163**	**22**	**37937829**	**G<C**	**0,3004**	**1,140**	**1.065-1.220**	**0,0001667**	**near-CDC42EP1**
CARD10	rs9610795	22	37939510	T<G	0,3547	1,110	1.039-1.185	0,001893	near-CDC42EP1
CARD10	rs7286403	22	37948293	A<T	0,3007	1,121	1.050-1.196	0,0006073	near-CDC42EP1
CARD10	rs6519071	22	37949320	A<G	0,2949	1,104	1.037-1.176	0,002053	near-CDC42EP1
CARD10	rs11703240	22	37950124	A<G	0,2957	1,103	1.036-1.174	0,002234	near-CDC42EP1
CARD10	rs9610801	22	37950530	A<G	0,2862	1,106	1.036-1.180	0,002463	near-CDC42EP1
CARD10	rs9610803	22	37951026	G<A	0,2666	1,111	1.038-1.189	0,002363	near-CDC42EP1
CARD10	rs5995460	22	37951451	G<A	0,309	1,093	1.027-1.163	0,005152	near-CDC42EP1
CARD10	rs2064133	22	37952493	G<A	0,3039	1,100	1.033-1.170	0,002814	near-CDC42EP1
CARD10	rs5845350	22	37952547	TG<T	0,3091	1,092	1.026-1.162	0,005667	near-CDC42EP1
CARD10	rs1016106	22	37952731	C<G	0,3036	1,099	1.033-1.170	0,002894	*near-CDC42EP1*

CARD10	rs1016107	22	37952779	T<C	0,2957	1,102	1.035-1.173	0,002439	near-CDC42EP1
CARD10	rs8142783	22	37953050	A<G	0,3035	1,100	1.033-1.170	0,002841	near-CDC42EP1
CARD10	rs1016108	22	37953067	G<A	0,2952	1,102	1.034-1.173	0,002533	near-CDC42EP1
CARD10	rs2064134	22	37953424	A<G	0,3039	1,100	1.033-1.170	0,002845	near-CDC42EP1
CARD10	rs2267365	22	37955081	G<T	0,2969	1,100	1.033-1.171	0,003101	near-CDC42EP1
CARD10	rs7284657	22	37955197	G<A	0,3048	1,097	1.030-1.167	0,003835	near-CDC42EP1
CARD10	rs9610804	22	37956214	G<A	0,3034	1,104	1.037-1.175	0,001896	near-CDC42EP1
CARD10	rs8140986	22	37956840	C<A	0,3133	1,095	1.029-1.165	0,00437	*CDC42EP1*
CARD10	rs2235335	22	37958163	C<G	0,3035	1,105	1.038-1.177	0,001711	*CDC42EP1*
CARD10	rs2235336	22	37958268	G<A	0,3034	1,106	1.039-1.177	0,001594	*CDC42EP1*
CARD10	rs2235337	22	37958312	G<A	0,307	1,101	1.034-1.172	0,002585	*CDC42EP1*
CARD10	37958775:D	22	37958775	T<TC	0,2957	1,111	1.043-1.183	0,001137	*CDC42EP1*
CARD10	rs34912439	22	37958776	C<CA	0,2953	1,111	1.04-1.183	0,001099	*CDC42EP1*
CARD10	rs5995463	22	37959214	C<T	0,3421	1,098	1.032-1.168	0,003027	*CDC42EP1*
CARD10	rs2076088	22	37960725	G<T	0,2851	1,138	1.062-1.220	0,0002778	*CDC42EP1*
CARD10	rs5756728	22	37961266	C<T	0,4075	1,109	1.043-1.178	0,000897	*CDC42EP1*
CARD10	rs5756729	22	37961353	C<T	0,4077	1,105	1.040-1.175	0,001235	*CDC42EP1*
CARD10	rs5756732	22	37961847	G<A	0,3985	1,098	1.033-1.167	0,002666	*CDC42EP1*
CARD10	rs5756733	22	37961949	C<T	0,404	1,106	1.041-1.175	0,001167	*CDC42EP1*
CARD10	rs2281098	22	37964299	C<G	0,4197	1,096	1.033-1.162	0,002455	*CDC42EP1*
CARD10	rs66468174	22	37964408	CCAGCGCCTGCTGCAAACCCCT<C	0,4454	1,090	1.027-1.158	0,005022	*CDC42EP1*

[1]Listed are only associated variants after Bonferroni-correction. Variants marked in bold are the lead SNPs of independent LD-blocks, separated by solid lines.

[2]CHR, chromosome

[3]POS, position in the genome according to UCSC genome build 37, hg19 (2009)

[4]EA/OA, effect allele/other allele, all variants are mapped on the forward strand

[5]EAF, effect allele frequency

[6]OR, odds ratio

[7]CI, confidencial interval

Supplementary Table S5. Association analysis of rs2910164 in the *MIR146A* gene in psoriasis patients and controls

Allele/Genotype rs2910164	Cases, n (%) n=4167	Controls, n (%) n=7971	*P*-value	OR [95% CI]
G	6525 (0.783)	12148 (0.762)		1
C	1809 (0.217)	3794 (0.238)	6.79E-04	0.89 (0.84-0.95)
GG	2572 (0.617)	4615 (0.579)		1
GC	1381 (0.331)	2918 (0.366)	2.92E-04	0.86 (0.79-0.93)
CC	214 (0.055)	438 (0.055)	0.1361	0.88 (0.81-1.04)
GG/GC	3953 (0.949)	7533 (0.945)		1
CC	214 (0.051)	438 (0.055)	0.3803	0.93 (0.78-1.1)
Stratified by HLA-Cw*0602				
Allele/Genotype rs2910164	HLA-Cw*0602	Cases, n (%) n=4167	Controls, n (%) n=7971	*P*-value	OR [95% CI]
G	-	3240 (0.785)	10113 (0.764)		1
C	**-**	**890 (0.215)**	**3123 (0.236)**	**0.0092**	**0.89 (0.82-0.97)**
G	+	3285 (0.718)	2035 (0.752)		1
C	**+**	**919 (0.219)**	**671 (0.248)**	**0.0063**	**0.85 (0.76-0.96)**
GG	-	1286 (0.623)	3856 (0.583)		1
GC	**-**	**668 (0.323)**	**2401 (0.363)**	**0.0016**	**0.84 (0.75-0.94)**
CC	-	111 (0.054)	361 (0.055)	0.4550	0.92 (0.82-1.15)
GG	+	1286 (0.612)	759 (0.561)		1
GC	**+**	**713 (0.339)**	**517 (0.382)**	**0.0123**	**0.83 (0.72-0.96)**
CC	**+**	**103 (0.049)**	**77 (0.057)**	**0.0986**	**0.77 (0.66-1.05)**
GG/GC	-	1954 (0.946)	6257 (0.945)		1
CC	-	111 (0.054)	361 (0.055)	0.8393	0.98 (0.78-1.22)
GG/GC	+	1999 (0.951)	1276 (0.943)		1
CC	+	103 (0.049)	77 (0.057)	0.2204	0.82 (0.6-1.12)
Recessive association model analysis					
Allele/Genotype rs2910164	HLA-Cw*0602	Cases, n (%) n=4167	Controls, n (%) n=7971	*P*-value	OR [95% CI]
GG	-	1286 (0.309)	3856 (0.484)		1
GC	**-**	**668 (0.160)**	**2401 (0.301)**	**0.0018**	**0.84 (0.76-0.94)**
CC	-	111 (0.027)	361 (0.045)	0.4825	0.92 (0.83-1.16)
GG	+	1286 (0.309)	759 (0.095)	1.39E-182	5.19 (4.66-5.78)
GC	+	713 (0.171)	517 (0.065)	5.42E-103	4.28 (3.84-5.37)
CC	+	103 (0.025)	77 (0.010)	5.13E-19	4.06 (3.64-5.09)
GG/GC	-	1954 (0.469)	6257 (0.785)		1
CC	-	111 (0.027)	361 (0.045)	0.8694	0.98 (0.79-1.23)
GG/GC	+	1999 (0.480)	1276 (0.160)	2.46E-286	5.13 (4.11-5.61)
CC	+	103 (0.025)	77 (0.010)	6.87E-21	4.32 (3.46-5.86)