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miR-10a-5p is increased in atopic dermatitis and has capacity to inhibit keratinocyte proliferation

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Abbreviations

AD, atopic dermatitis; DAPI, 4',6-diamidino-2-phenylindole; HA, hyaluronic acid; HAS, hyaluronan synthase; IL, Interleukin; KC, keratinocyte; miRNA, microRNA; MAP3K7, mitogen-activated kinase kinase 7; NF, nuclear factor; RT-qPCR, real time quantitative PCR; Th, T helper; TGF- β , Transforming growth factor- β ; 3'UTR, 3' untranslated region.

Abstract

Background: miR-10a-5p has been shown to regulate cancer cell proliferation and invasiveness and endothelial cell inflammatory responses. The function of miR-10a-5p in the skin has not been previously studied. The aim of the current study was to examine miR-10a-5p expression, regulation and function in keratinocytes (KCs) in association with atopic dermatitis (AD).

Methods: The expression of miR-10a-5p and its target genes was analyzed using RT-qPCR, mRNA array analysis, *in situ* hybridization and immunofluorescence. The transfection of miRNA mimics, cell

cycle distribution analysis and luciferase assays were used to study miR-10a-5p functions in human primary KCs.

Results: miR-10a-5p was found to be upregulated in lesional skin from AD patients and in proliferating KCs. Array and pathway analysis of IL-1 β -stimulated KCs revealed that miR-10a-5p inhibited many genes that affect cell cycle progression and only a few inflammation-related genes. Accordingly, fewer cells in S-phase and reduced proliferation were detected as characteristics of miR-10a-5p-transfected KCs. The influence of miR-10a-5p on cell proliferation was also evident in KCs induced by AD-related cytokines, including IL-4, IL-17 and IL-1 β , as measured by the capacity to strongly suppress the expression of the proliferation marker Ki-67. Among AD-related putative direct target genes, we verified hyaluronan synthase 3, a damage-associated positive regulator of KC migration and proliferation, as a direct target of miR-10a-5p.

Conclusions: miR-10a-5p inhibits KC proliferation and directly targets hyaluronan synthase 3 and thereby may modulate AD-associated processes in the skin.

Introduction

The skin is an organism's first line of defense from the environment, including against pathogens and allergens. To be effective, coordinated proliferation and differentiation of keratinocytes (KCs) in the epidermis is needed. The most common inflammatory skin disease associated with defects in the skin barrier and KC differentiation is atopic dermatitis (AD), which affects up to 20% of children and up to 5% adults.^{1,2} It has been suggested that defects in the skin barrier, including low hydration, make the skin vulnerable to environmental factors, which may cause the activation of innate immune responses; expansion of T helper (Th)2 cells, including the Th22, Th17 and Th1 cell subsets; and increased IgE serum levels.³ Correspondingly, genetic studies focused on AD have identified candidate genes linked to the epidermal barrier, such as filaggrin (*FLG*), or to the regulation of innate immune responses and T cell functions.⁴⁻⁷ As AD is a heterogeneous disease, there are no treatments equally suitable for all patients.^{8,9} However, it is known that all patients benefit from continuous

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maintenance and epidermal barrier repair with emollients and the avoidance of individual trigger factors.³ Among others, hyaluronic acid (HA) has been characterized as a natural skin component needed for efficient barrier function. HA is a hydrophilic polysaccharide that is produced and exported to the extracellular space by hyaluronan synthases (HASs) 1, 2, and 3.¹⁰ HA confers strong hydrating properties and is widely used as an agent for intradermal injections to improve wrinkles.¹¹ Additionally, HA is important for proper KC proliferation and differentiation.^{12,13} As increased HA synthesis and HAS3 have been demonstrated in healing wounds and AD skin, the dysregulation of HAS3 may influence the development and severity of AD.^{14,15}

MicroRNAs (miRNAs) are small endogenous single-stranded RNAs approximately 21-24 nucleotides in length that control mRNA stability or inhibit the translation of target genes and thereby affect the expression of up to 50% of genes.^{16,17} Human miR-10a-5p is a member of the miR-10 family and is evolutionarily conserved in animals, including mouse, zebrafish, *Drosophila* and chicken.¹⁸ The human gene that encodes miR-10a-5p is located upstream of the *HOXB4* gene within the HOX gene cluster on chromosome 17q21.32.^{18,19} In line with its position in the genome, miR-10a-5p functions in development, but also has a role in the regulation of cell proliferation and migration in various cancers cells.²⁰⁻²³ In endothelial cells, miR-10a-5p downregulates pro-inflammatory cytokines and chemokines, including IL-6 and IL-8, which may occur through targeting of mitogen-activated kinase 3 kinase 7 (MAP3K7), indicating that miR-10a-5p may also have anti-inflammatory functions.²⁴ Our previous array analysis detected increased miR-10a-5p expression in keratinocytes from AD patients;²⁵ however, the function of miR-10a-5p in KCs and AD has not been previously studied.

In the current study, we report the increased expression of miR-10a-5p in the skin of AD patients and delineate its function in human primary KCs. We demonstrate that miR-10a-5p inhibits cell cycle progression and proliferation but only moderately affects genes associated with inflammation in KCs. Our data indicate that the effect of miR-10a-5p on KC proliferation is due its capacity to suppress multiple proliferation and cell cycle associated genes, among which we

detected AD-related hyaluronan synthase HAS3 as a direct target. Altogether, our data demonstrate that miR-10a-5p has capacity to inhibit KC proliferation and thereby may influence AD-associated processes in the skin.

Methods

Study participants

This study was approved by the Research Ethics Committee of the University of Tartu. All participants provided a written informed consent. One skin biopsy (diameter 4 mm) from non-lesional and one from lesional skin were collected from patients with AD with skin inflammation of 1-2-week (5 women and 5 men, age 22-58 years). For 9 out of 10 patients, elevated IgE or eosinophilia was detected. One patient was additionally diagnosed with bronchial asthma. One biopsy from each gender- and age-matched control individuals (7 men, 8 women, age 20-59 years) was collected. All control individuals were free from allergic disorders, had negative skin prick tests for common allergens and normal serum IgE at least for five years before inclusion into the study. None of the participants was under treatment with systemic medicines. Biopsies were snap-frozen and kept at -80°C until extraction of total RNA.

Cell culture, transfection and stimulation of KCs

Pooled normal primary human KCs (PromoCell, Heidelberg, Germany) were cultured as previously described²⁶ and transfected either with 30 nM of hsa-miR-10a-5p Precursor, mirVana™ miR-10a-5p Mimic or corresponding Negative Controls #1. Locked nucleic acid (LNA) inhibitor for hsa-miR-10a-5p and negative control A were used at 10 nM (Figure 4D) or 30 nM (Figure 4E) concentrations. Transfections were performed with siPORT™ NeoFX™ (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions or using cell penetrating peptide PepFect14 as described.²⁷ Stimulations were performed at final concentrations of 10 ng/ml for IL-1β, 25 ng/ml for

HB-EGF, 20 ng/ml for IL-22, 40 ng/ml for IL-4, 10 ng/ml for IL-17A (Peprotech, Rocky Hill, NJ, USA).

For additional information, please see Supporting Methods.

RNA purification, reverse transcription and real time quantitative PCR (RT-qPCR)

Total RNA was extracted with Direct-zol™ RNA MiniPrep Kit (Zymo Research, Irvine, CA, USA), miRNeasy Mini Kit (Qiagen, Hilden, Germany) or Total RNA Zol-Out Kit (A&A Biotechnology, Gdynia, Poland). Reverse transcription was performed with RevertAid First Strand cDNA Synthesis reagents (Thermo Fisher Scientific, Waltham, MA, USA) for mRNA analysis or TaqMan® MicroRNA Reverse Transcription Kit and TaqMan™ MicroRNA Assays (both from Thermo Fisher Scientific) for microRNA analysis. RT-qPCR was done using custom primers and 5x HOT FIREPol EvaGreen qPCR Supermix (Solis BioDyne, Tartu, Estonia) for mRNA analysis or TaqMan™ MicroRNA Assays and 5 x HOT FIREPol® Probe qPCR Mix Plus (ROX) (Solis BioDyne) for miRNA analysis. Primer sequences and additional information are provided in Supporting Methods.

Array, pathway and target analysis

mRNA profiling was done with Illumina HumanHT-12 Expression BeadChips version 4. The array data are available at ArrayExpress as E-MTAB-7245. For additional information, please see Supporting Methods.

Cell cycle analysis

Cell cycle was analysed using 1 µg/ml of 4',6-diamidino-2-phenylindole (DAPI) or Click-iT® EdU Alexa Fluor® 488 Flow Cytometry assay (Thermo Fisher Scientific). For additional information, please see Supporting Methods.

***In situ* hybridisation (ISH) and Immunofluorescence**

ISH and immunofluorescence analysis were performed on frozen skin sections. For ISH, miRCURY LNA™ 5'- and 3'-DIG labelled hsa-miR-10a-5p detection probe and 5'- and 3'-DIG labelled miRCURY scrambled ISH 49C (Qiagen) probes were used. Immunofluorescence was performed with the rabbit anti-HAS3 (Atlas Antibodies, Stockholm, Sweden). For additional information, please see Supporting Methods.

Cloning and luciferase assay

3' untranslated region (UTR) fragments containing miR-10a-5p binding sites of HAS3 and MAP3K7 were cloned into pmirGLO Dual-Luciferase miRNA Target Expression Vector (Promega, Madison, WI, USA) using NheI and Sall restriction sites. Luciferase (LUC) assay was performed in HEK293 cells. Cloning primers and additional information are provided in the Supporting Methods.

Statistics

Statistical analyses were performed with GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA). All comparisons were performed using unpaired Students *t*-test at 95% confidential interval. The results were considered significant at $P < 0.05$. Data are presented as mean with standard error of the mean (SEM).

Results

miR-10a-5p is upregulated in AD

A previously conducted miRNA profiling study demonstrated that miR-10a-5p is one of the most strongly upregulated miRNAs in KCs from AD patients compared to KCs from control individuals.²⁵ To test whether miR-10a-5p is also upregulated in skin from AD patients, we first performed RT-qPCR, which revealed an approximately 2-fold upregulation of miR-10a-5p in both non-lesional and lesional skin from AD patients compared to control skin (Figure 1A). Subsequent ISH showed that miR-10a-5p

staining was significantly increased in the epidermis from lesional AD samples, with the strongest signal in the basal epidermal layer, confirming that miR-10a-5p is more highly expressed in skin from AD patients (Figures 1B and C, Supplementary Figure S1).

miR-10a-5p expression is suppressed during KC differentiation

As the *in situ* hybridization results suggested that miR-10a-5p is more abundant in proliferating KCs, we next performed *in vitro* differentiation of KCs in monolayer culture by adding high concentrations of Ca^{2+} and air-liquid interphase. In both these cultures, a differentiation marker, keratin 10 (KRT10), was upregulated and the proliferation marker Ki-67 was downregulated compared to proliferating KCs (Supplementary Figure S1). To follow the dynamics of miR-10a-5p expression during KC differentiation, we measured miR-10a-5p and KRT10 relative expression (Figure 2A, B) and trans-epithelial electrical resistance (TER) (Figure 2B) at different time points. In line with the results from the AD patient skin samples, we observed an approximately 65% drop in miR-10a-5p expression over 4 days due to the addition of Ca^{2+} to the media, while KRT10 expression was strongly induced during the same time. Correspondingly, we measured a 63% reduction in miR-10a-5p expression over the 4 days from apical media removal, while the relative expression of KRT10 was increased by more than 15-fold over the same time period (Figure 2B). In line with the higher miR-10a-5p expression in the epidermis basal layer and AD, heparin-binding EGF-like growth factor (HB-EGF) induced the expression of miR-10a-5p in proliferating KCs at the 24 and 48 h time points (Figure 2C). In addition, during the reconstitution of the epidermis, miR-10a-5p expression was downregulated with a delay in the presence of HB-EGF as measured on days 3 and 6 from cell seeding (Figure 2D). The tested pro-inflammatory cytokines (IFN- γ , TNF- α , IL-1 β , IL-17A, IL-4 and IL-13) as well as TGF- β and retinoic acid, which have been previously shown to induce miR-10a-5p expression in T cells,²⁸ did not significantly alter miR-10a-5p expression in KCs (Supplementary Figures S2A, B). The expression of miR-146a, which was used as a positive control, increased in response to TNF- α , IL-1 β , IL-17A, and retinoic acid, as well as upon co-stimulations (Supplementary Figure S2C, D). These data indicate

that miR-10a-5p is increased in proliferating KCs where its expression is dependent on HB-EGF but not upon pro-inflammatory cytokines.

miR-10a-5p inhibits the expression of genes related to cell cycle regulation, cell adhesion and cytokine signaling in KCs

As a previous study verified MAP3K7 as a key miR-10a-5p target and suggested the NF- κ B-mediated inflammatory pathway to be affected by miR-10a-5p in human aortic endothelial cells,²⁴ we next studied whether this pathway is also affected by miR-10a-5p in KCs. We transfected miR-10a-5p mimics into KCs, stimulated the cells with IL-1 β or left nonstimulated and performed RT-qPCR analysis. IL-1 β was chosen for stimulation as it has been shown to be increased in the skin during filaggrin deficiency²⁹ and due to its capacity to activate the NF- κ B pathway in KCs. Indeed, the mRNA levels of the NF- κ B-dependent chemokines IL-8 and CCL5 were downregulated in cells transfected with miR-10a-5p mimics and stimulated with IL-1 β (Figure 3A-C) and MAP3K7 expression was inhibited in both conditions (Figure 3B).

To determine the other genes influenced by miR-10a-5p, we next performed Illumina array analysis from the same set of samples. Altogether, 317 and 522 genes were found to be upregulated and downregulated, respectively, in control-transfected KCs stimulated with IL-1 β (differential P -value < 0.05) compared to control-transfected and nonstimulated KCs. Of these genes, the top 200 are shown in Supplementary Table S1. We next selected the top 200 genes downregulated by miR-10a-5p based on fold change in KCs stimulated with IL-1 β (Supplementary Table S2). Because only 14 genes among the top 200 in IL-1 β -stimulated cells and 6 in nonstimulated cells were differentially expressed according to the selected P -value cut-off of 0.05, we included the full top 200 genes in the pathway analysis. Interestingly, cell cycle regulation was revealed as the most prominent gene ontology (GO) functional group with 63 downregulated genes (P -value of 7.0×10^{-18}) (Supplementary Table S3), of which 13 (Figure 3D, Supplementary Table S4) were found to be putative miR-10a-5p direct targets according to Targetscan 7.1.^{30,31} In addition, we found the miR-10a-5p-influenced

genes to be connected with cell adhesion, cytokine signaling in immune system (Figure 3D), epithelium development and abnormal skin morphology (Supplementary Table S3). Comparison of the top 200 list of downregulated genes with the list of 1335 genes differentially expressed in lesional skin from AD patients according to previously published array and RNA sequencing datasets³² revealed 21 overlapping genes (Supplementary Table S5), of which 14 were found to be associated with cell cycle regulation. In conclusion, the array and pathway analyses revealed that miR-10a-5p most strongly affects genes that regulate the cell cycle in KCs and among genes that are differentially expressed in AD skin.

miR-10a-5p inhibits the proliferation of human primary KCs

As the array analysis suggested that miR-10a-5p has the capacity to regulate the cell cycle, we next performed a set of experiments to analyze the effects of miR-10a-5p on cell cycle regulation and proliferation. We first performed a DAPI-based cell cycle distribution assay, which showed that approximately 50% of cells in control-transfected KCs were in S-phase, while the corresponding fraction was only approximately 38% in cells transfected with miR-10a-5p mimics. Changes were also detected in G1/G0-phase: in cells transfected with miR-10a-5p, approximately 48% were in G1/G0-phase compared with approximately 38% in the control (Figure 4A, Supplementary Figure S3A). To confirm these results and to study the effects on the cell proliferation rate, we next performed a 5-ethynyl-2'-deoxyuridine (EdU) incorporation assay. Figure 4B demonstrates that among the KCs transfected with miR-10a-5p mimics, approximately 18% of the cells were EdU-positive, while >22% of KCs transfected with control mimics were EdU-positive, indicating that miR-10a-5p inhibits KC proliferation. The cell cycle distribution based on the DAPI in this assay showed that approximately 18% of cells were in S-phase when KCs were transfected with miR-10a-5p mimics compared with the control with approximately 23% (Figure 4B, Supplementary Figure S3B). To test whether miR-10a-5p is able to inhibit KC proliferation under inflammatory conditions characteristic to AD, we transfected KCs with miR-10a-5p mimics and then stimulated the cells with IL-4, IL-17A or IL-1 β or left

nonstimulated and measured the expression of the proliferation marker Ki-67. In line with cell cycle distribution and proliferation assays (Figure 4A, B), we detected strongly reduced Ki-67 expression in the cells transfected with miR-10a-5p under all tested conditions (Figure 4C). Next, we assessed whether the endogenous levels of miR-10a-5p are sufficient to suppress KC proliferation. For that purpose, we transfected KCs with miR-10a-5p LNA inhibitors and miR-10a-5p mimics as a positive control and performed an ATP-based proliferation assay. Figure 4D shows increased luminescence values for the cells transfected with miR-10a-5p-specific inhibitors and reduced activity in cells transfected with miR-10a-5p mimics, indicating that inhibition of endogenous miR-10a-5p leads to enhanced KC proliferation. However, when we measured Ki-67 levels, LNA inhibition of miR-10a-5p (Supplementary Figure S4) only the cells stimulated with IL-17A showed increased expression of Ki-67 in the presence of miR-10a-5p inhibitor (Figure 4E). In line with thickening of the epidermis, increased expression of Ki-67 was detected in the skin from AD patients (Figure 4F). These data indicate that although miR-10a-5p has the capacity to inhibit KC proliferation, its increased expression in AD skin is regulatory, but not sufficient to eliminate disease-related enhanced KC proliferation.

HAS3 is a novel AD-related direct target of miR-10a-5p

Among the genes downregulated by miR-10a-5p and changed in AD,¹⁵ we detected HAS3 as the best scored putative direct target gene with two binding sites for miR-10a-5p (Supplementary Table S4). As dysregulated HAS3 and HA may influence multiple AD-related changes in the skin, including KC proliferation,^{14,15} we next performed a set of experiments to verify HAS3 increased expression in AD and to prove the influence of miR-10a-5p on HAS3 expression. First, we tested which AD-related cytokines impact HAS3 expression and found that stimulation with IL-4, IL-17A and IL-22 and combinations led to its enhanced mRNA (Figure 5A) and protein (Supplementary figure S5) expression in the reconstituted epidermis. Next, the increased expression of HAS3 in the lesional skin of AD patients was confirmed by RT-qPCR (Figure 5B) and immunofluorescence analysis, which

showed the strongest signal for the HAS3 protein in the epidermis of lesional skin from AD patients, while no signal was detected in the control and non-lesional skin (Figure 5C). To confirm that miR-10a-5p has the capacity to suppress HAS3 expression under inflammatory conditions, we measured HAS3 mRNA levels in KCs transfected with miR-10a-5p mimics and stimulated with the AD-related cytokines IL-4, IL-17, or IL-1 β or left nonstimulated. Note that IL-22 was not used in the transfection experiments as proliferating KCs are insensitive to IL-22 as they do not express IL22RA1.³³ Figure 5D demonstrates that HAS3 mRNA expression was reduced in miR-10a-5p-transfected cells under all the used conditions. To verify that HAS3 is a direct target for miR-10a-5p, we next cloned the 3'UTR fragments containing putative miR-10a-5p target sites and the mutated target sites (Figure 5E) into dual-luciferase reporter plasmids and performed a LUC assay in HEK293 cells. Figure 5F shows reduced LUC activity for the constructs with miR-10a-5p binding sites when miR-10a-5p was transfected, while no significant effect was observed in the case of the mutant constructs. The MAP3K7 3'UTR-containing construct was used as a positive control and was strongly inhibited by miR-10a-5p. The empty vector used as a negative control was unaffected. These data demonstrate that HAS3 is a novel direct target of miR-10a-5p. However, as both miR-10a-5p and HAS3 have increased expression in lesional skin from AD patients, miR-10a-5p's suppressive effect on HAS3 appears to be partial.

Discussion

Recent studies suggest that defects in the skin barrier play significant roles in the development of AD, highlighting the importance of KC functions.³ In this study, we identified miR-10a-5p as upregulated in the skin of AD patients, downregulated during KC differentiation and upregulated in response to the growth factor HB-EGF. The array analysis of IL-1 β -stimulated KCs revealed cell cycle regulation as the pathway most strongly affected by miR-10a-5p, while only a few inflammation-related genes were influenced. In line with the array analysis, miR-10a-5p-transfected KCs displayed

altered cell cycle and reduced proliferation. Among the putative miR-10a-5p targets, we determined damage-associated hyaluronan synthase HAS3 as a novel miR-10a-5p direct target. miR-10a-5p inhibited the mRNA expression of HAS3 and proliferation marker Ki-67, as well as in the presence of the AD-related pro-inflammatory cytokines IL-4, IL-17A and IL-1 β , indicating that miR-10a-5p can regulate its target genes in inflamed skin. Altogether, our results demonstrate that miR-10a-5p has the capacity to inhibit the proliferation of KCs, the expression of HAS3 as a regulator of cell adhesion as well as pro-inflammatory chemokines IL-8 and CCL5 and may thereby influence the development and severity of AD.

Several studies have demonstrated altered miR-10a-5p expression in cancerous cells and tissues.³⁴⁻³⁶ In the present study, we show that the growth factor HB-EGF, which is upregulated in lesional skin from AD patients,¹⁵ has the ability to enhance miR-10a-5p expression in KCs. It has been previously shown that miR-10a-5p expression is induced by other growth-inducing factors, such as retinoic acid^{22,37,38} and TGF- β ,²⁸ however, these factors did not affect miR-10a-5p expression in KCs. As we also did not detect the influence of AD-related pro-inflammatory cytokines on miR-10a-5p expression, our results suggest that miR-10a-5p expression is mainly affected by EGF-type growth factors in KCs.

In endothelial cells, miR-10a-5p inhibits the MAP3K7 and beta-transducin repeat-containing genes, thereby reducing NF- κ B activation and displaying anti-inflammatory effects in atherosusceptible endothelium.²⁴ Another study showed that the NF- κ B-inducible chemokine IL-8 is a direct target of miR-10a-5p.³⁹ The development of AD is associated with skin barrier abnormalities that cause the activation of the NF- κ B pathway in response to environmental and/or intrinsic factors in KCs, which among other cytokines start to produce IL-1 family cytokines.^{3,40} In this study, we showed that the transfection of miR-10a-5p leads to the inhibition of MAP3K7 and the NF- κ B-inducible chemokines IL-8 and CCL5 in IL-1 β -stimulated KCs. However, array analysis demonstrated that miR-10a-5p's effect on NF- κ B-related genes is not predominant compared to its effect on genes that regulate the cell cycle and proliferation.

miR-10a-5p has been previously shown to suppress cell proliferation in non-cancerous cells, including ovarian granulosa cells⁴¹ and airway smooth muscle cells.⁴² In contrast, in promyelocytic leukemia cells, miR-10a-5p expression was shown to promote cell proliferation, while suppressing leukemia cell granulocytic and monocytic differentiation.⁴³ In KCs, miR-10a-5p clearly inhibited proliferation, most likely by suppressing the expression of multiple cell cycle-regulating genes. As the expression of miR-10-5p was higher in proliferating KCs and decreased during differentiation, our data suggest that miR-10a-5p regulates proliferation rate in proliferating KCs, but does not influence KC differentiation.

The functions of several previously validated miR-10a-5p direct targets, including MAP3K7²⁴, Bcl-6²⁸ and tumor suppressor phosphatase and tensin homolog (PTEN), are associated with the regulation of cell proliferation and cancer development.³⁴ In the current study, we verified that HAS3, is a novel direct target of miR-10a-5p. HA and HAS proteins have been previously shown to modulate keratinocyte proliferation, differentiation, migration and skin hydration.^{44,45} Accordingly, increased HA synthesis has been implicated in AD, whereas HAS3 has been shown to be upregulated by HB-EGF, IL-13 and IFN- γ .^{14,15,46} We observed the strongest upregulation of HAS3 by the combination of IL-4 and IL-22 in the reconstituted epidermis, which did not have an effect on miR-10a-5p expression (data not shown). Nevertheless, the transfection of miR-10a-5p inhibited the HAS3 mRNA expression, as well as in KCs stimulated with the AD-related cytokines IL-4, IL-17A and IL-1 β . Interestingly, although we observed strong localized HAS3 protein staining with immunofluorescence in skin from AD patients, we could only detect very weak HAS3 levels with immunofluorescence and no signal in Western blot in nonstimulated and stimulated KCs (data not shown), which indicates that HAS3 protein level is tightly controlled. It is possible that HAS3 expression is required during inflammation and should be suppressed during the recovery phase when miR-10a-5p is induced by HB-EGF and/or any other factors.

Intriguingly, the increased expression of miR-10a-5p was accompanied by the enhanced expression of the proliferation marker Ki-67 and multiple cell cycle regulation- and AD-related targets, including HAS3, in skin from AD patients. This indicates that the effect of miR-10a-5p under diseased conditions is regulatory and not sufficient to suppress the increased proliferation of KCs in the AD epidermis. As inhibition of miR-10a-5p did not have as strong influence on proliferation and Ki-67 expression as the transfection of miR-10a-5p mimics, it is possible that other miR-10 family members or unknown small RNAs confer similar function as miR-10a-5p in KCs.

Interestingly, the expression level of miR-10a-5p was already increased in cultured KCs from AD patients²⁵ and in non-lesional skin of AD patients indicating to the epigenetic regulation of miR-10a-5p. However, as the analyzed miR-10a-5p-influenced genes, Ki-67 and HAS3 were not altered in non-lesional skin, the possible effect of miR-10a-5p is apparently balanced by other regulatory factors. Whether and how much epigenetic changes in non-lesional skin affect relapse and severity of AD, remains to be studied.

In conclusion, we have shown the increased expression of miR-10a-5p in skin from AD patients and its capacity to modulate cell cycle progression and inhibit KC proliferation. Additionally, we identified the AD-related hyaluronan synthase HAS3 as a novel direct target of miR-10a-5p. We suggest that miR-10a-5p has the capacity to suppress KC proliferation in AD skin through multiple target genes, including HAS3; however, its endogenous expression levels are not sufficient to prevent the enhanced proliferation of KCs in AD skin. Further studies using mice deficient or overexpressing miR-10a-5p and corresponding mouse disease model would help to clarify the importance of miR-10a-5p *in vivo* in AD. In addition, therapeutic potential of miR-10a-5p would be worth to assess using formulations enabling delivery of miRNA mimics into the skin cells in mice that naturally develop AD-like skin inflammation due the skin barrier defect.

Figure legends

Figure 1. MicroRNA hsa-miR-10a-5p is upregulated in the skin of AD patients. (A) Relative expression of miR-10a-5p in lesional (AD L) and non-lesional (AD NL) skin from AD patients was measured by RT-qPCR and compared to control skin. (B, C) ISH of miR-10a-5p in skin cryosections from the control, AD non-lesional (AD NL) and lesional (AD L) skin. (B) Blue color shows the expression of miR-10a-5p, the border of epidermis and dermis is designated with dashed line, bar = 75 μ m. (C) An integrated density value of the signal per area of interest was obtained for six areas from non-lesional and lesional skin from two patients and two controls. The relative intensity was calculated as percentage and was normalized to the average of control samples (100%). (A, C) Data represent the mean $\pm$ SEM. Student's t-test, * $P < 0.05$, ** $P < 0.01$.

Figure 2. miR-10a-5p is downregulated during KC differentiation and upregulated by HB-EGF. RT-qPCR analysis of miR-10a-5p was performed at indicated time-points from samples collected from KCs differentiated with high Ca^{2+} concentration (A), *in vitro* reconstructed epidermis (B) as well as from the proliferating KCs (C) and *in vitro* reconstructed epidermis (D) stimulated with HB-EGF or left nonstimulated (NS). Cells were seeded at day 0, grey arrow indicates the time when the apical media was removed. (A-D) Data represent mean $\pm$ SEM of four independent samples in each conditions, Student's t-test, * $P < 0.05$, ** $P < 0.01$.

Figure 3. miR-10a-5p inhibits the expression of genes associated with cell cycle regulation, cell adhesion and cytokine signalling in KCs. KCs were transfected either with control or miR-10a-5p mimic (miR-10a-5p) for 24 hours and then stimulated with IL-1 β or left nonstimulated (NS) for the next 24 hours. (A-C) Relative miRNA (A) and mRNA (B, C) levels are shown compared to nonstimulated control-transfected cells. Data represent mean $\pm$ SEM, Student's t-test, * $P < 0.05$, ** $P < 0.01$. (D) Heatmaps of miR-10a-5p influenced genes from the selected functional groups. Log2 values of mRNA expression signals are mean-centered for each gene separately. Color scale from

blue (lower) to yellow (higher) represents deviation from the mean (black). Predicted direct targets are designated with bold.

Figure 4. miR-10a-5p inhibits cell cycle progression and proliferation of primary KCs. (A-E) KC were transfected with miR-10a-5p mimic (miR-10a-5p), inhibitor (LNA-miR-10a) or corresponding controls for 48 h (A-C, E) or 72 h (D) and indicated assays were performed. (C, E) Indicated cytokines were added 24 h after transfection for additional 24 h. (A) Cell cycle distribution was measured based on DAPI signal. (B) Cells were labeled with EdU followed by measurement of DAPI and EdU signals by flow cytometry. (D) ATP-based proliferation assay, non-transfected (NT) cells were included as an additional control expected to proliferate faster compared to transfected cells. (C, E, F) RT-qPCR analysis of transfected KCs (C, E) or skin biopsies (F) from lesional (AD L) and non-lesional (AD NL) skin from AD patients and control individuals. Data represent the mean $\pm$ SEM. Student's t-test, *P < 0.05, **P < 0.01.

Figure 5. HAS3 is novel direct target of miR-10a-5p. (A) KCs in reconstituted epidermis were stimulated with indicated cytokines for 24 h or left nonstimulated (NS) and subjected to RT-qPCR analysis. Data is shown compared to the mean expression of HAS3 in NS cells (=1). (B) RT-qPCR analysis of skin biopsies from lesional (AD L) and non-lesional (AD NL) skin from AD patients and control individuals. (C) Immunofluorescence and DAPI staining of L and NL skin sections from AD patients and controls. Bars correspond to 50 μ m. (D) KC were transfected with miR-10a-5p mimic (miR-10a-5p) or control for 24 h before indicated cytokines were added for additional 24 h. (E) miR-10a-5p and the mutated binding sites (underlined). Positions indicate the distance from the beginning of HAS3 3'UTR. (F) The relative firefly luciferase (LUC) activity is normalized to the values of control-transfected cells (=1) (n=8). (A, B, D, F) Data represent the mean $\pm$ SEM. Student's t-test, *P < 0.05, **P < 0.01.

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Author contributions

H.V, T.R. and G.C.B performed the experiments, analyzed the data, contributed with design of the study and writing the manuscript. A.A., E.U., J.M. and T.A. and performed the experiments and analyzed the data, T.M., K. K., C.A.A., K.K. and E.R.T. contributed with design of the study and writing the manuscript, K.A., L.Š., B.K. and K.K. were responsible for the clinical part of the study. A.R. designed the study, supervised the project and writing the manuscript. All authors have commented and approved the final version of the manuscript.

Conflict of interest

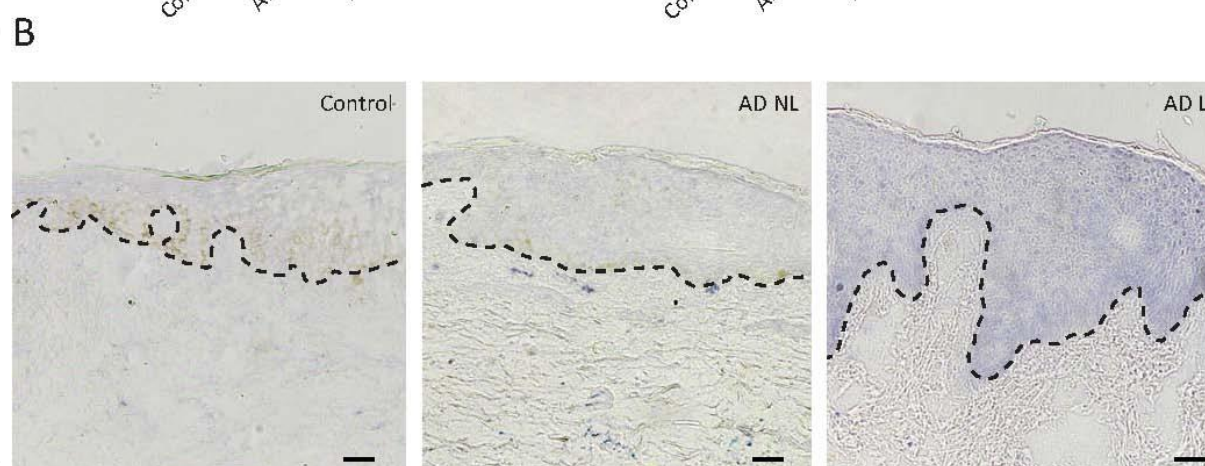
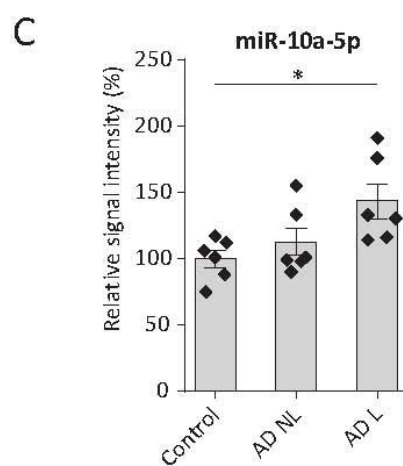
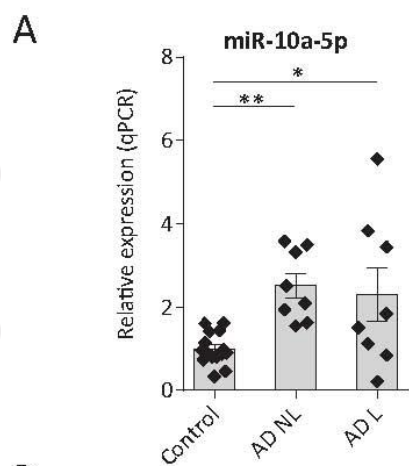
The authors have no conflict of interest in relation to this work.

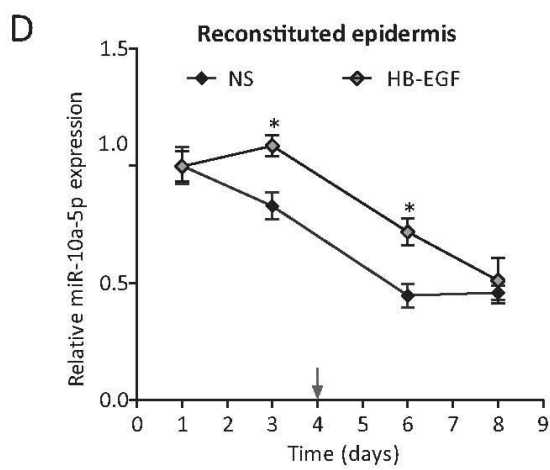
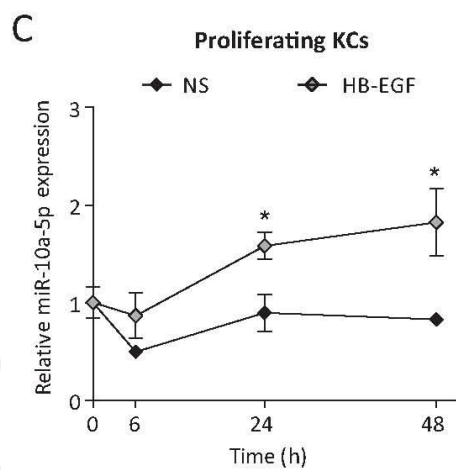
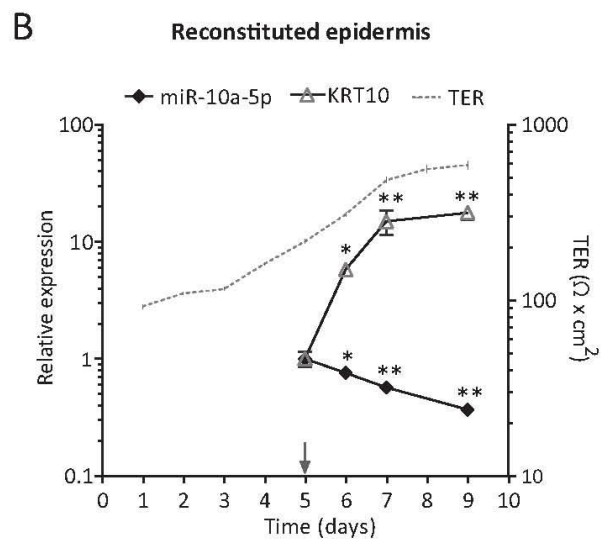
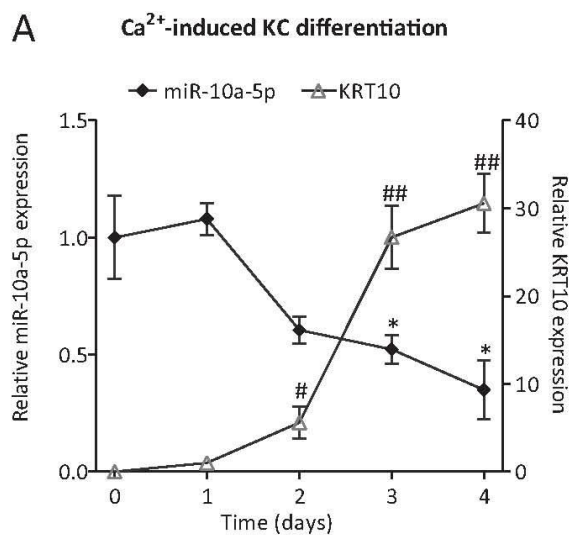
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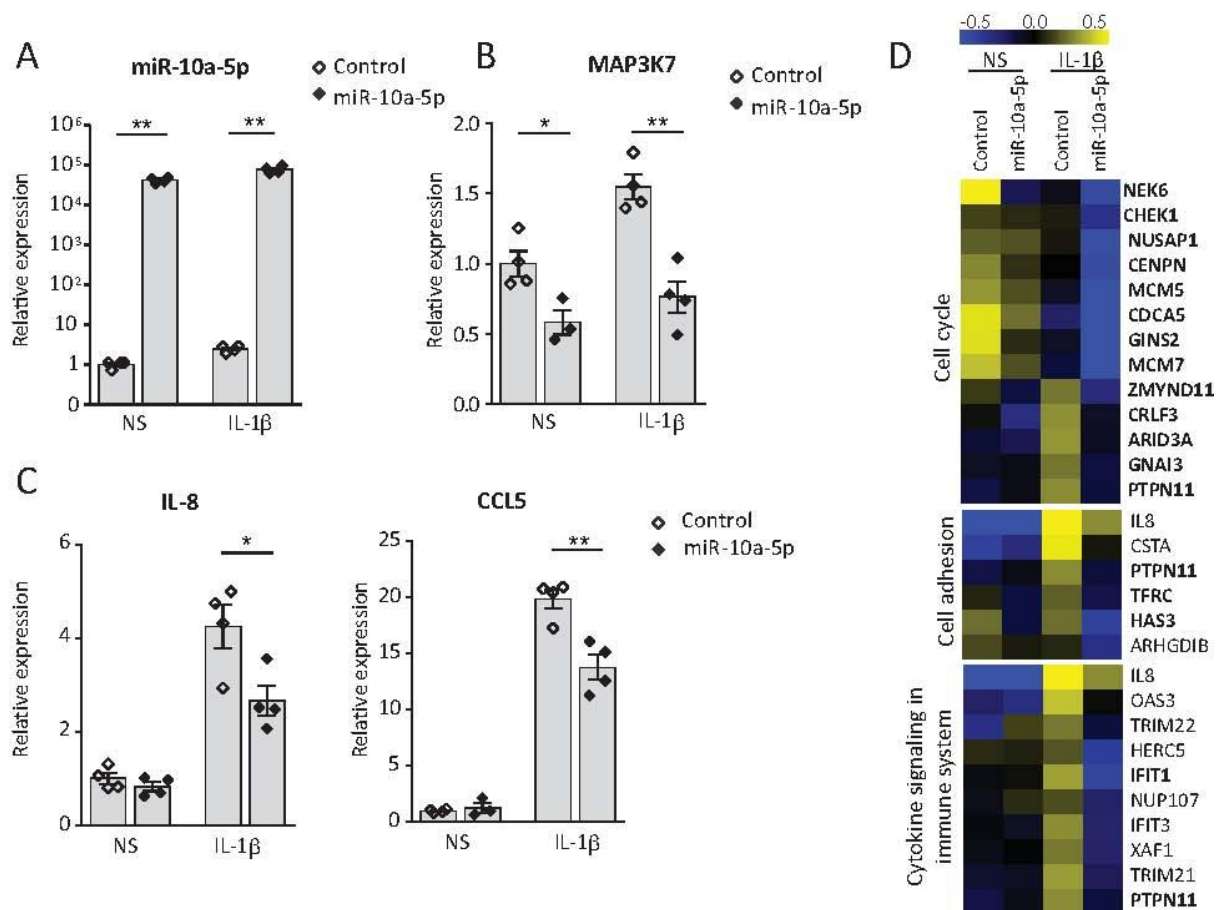
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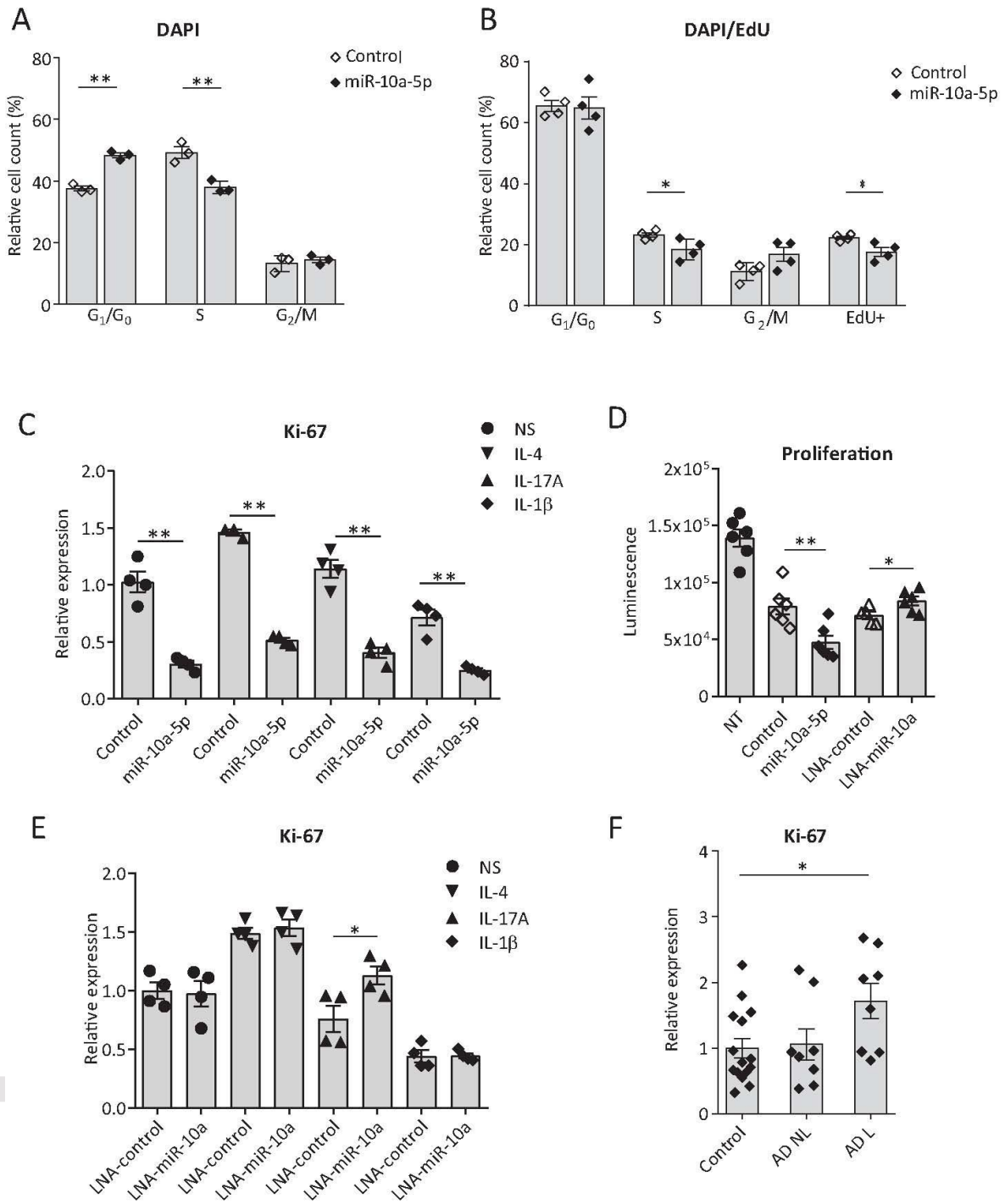
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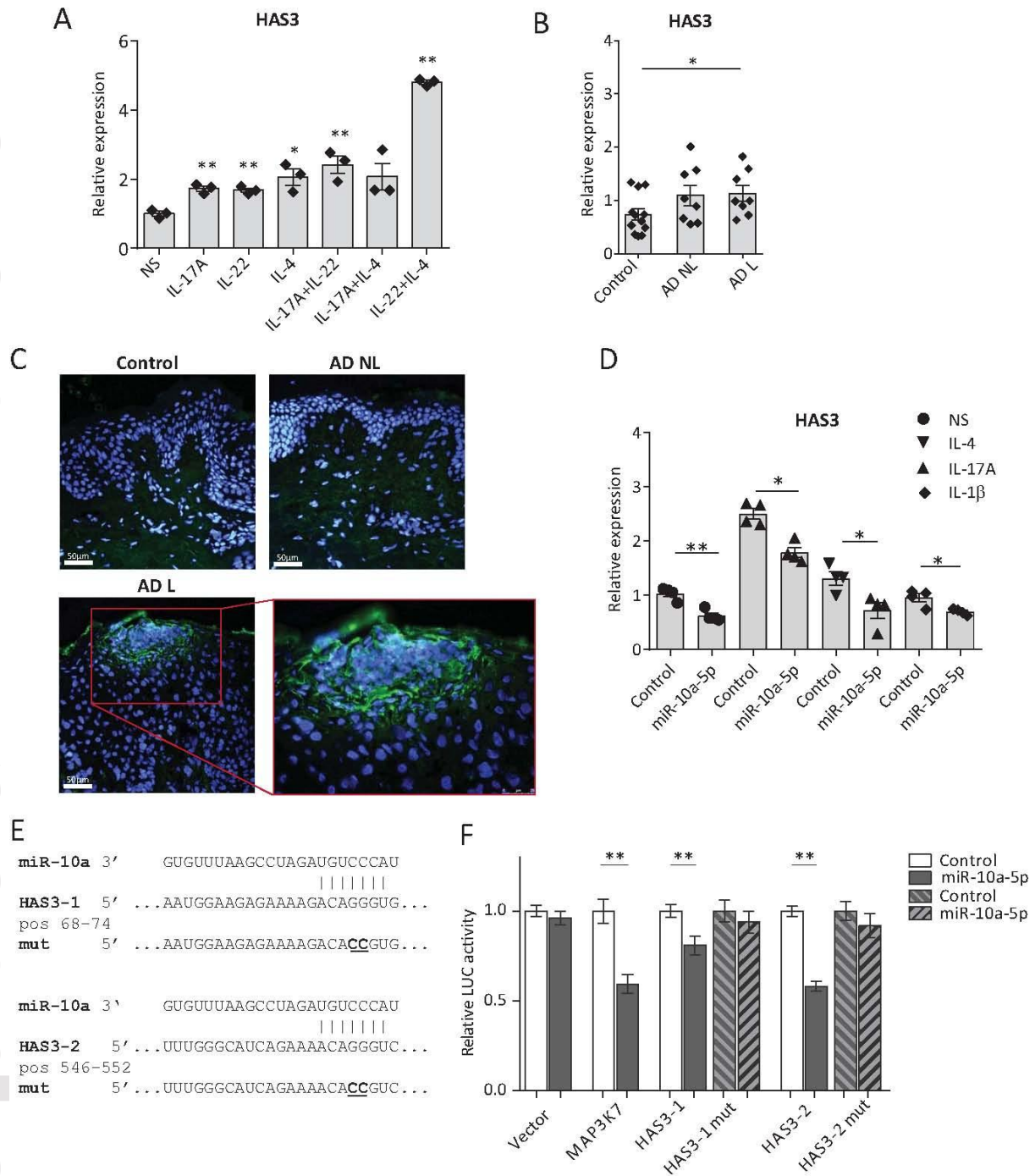
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Online Supporting Information

miR-10a-5p is increased in atopic dermatitis and has capacity to inhibit keratinocyte proliferation

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Online Supporting methods

Cell culture

Pooled normal primary human keratinocytes (KCs) (PromoCell, Heidelberg, Germany) between passages 5 to 7 were used in KC experiments. Monolayer cultures were grown in Keratinocyte-SFM (Thermo Fisher Scientific, Waltham, MA, USA) with supplements (2 $\mu\text{mol/L}$ L-glutamine, 50 $\mu\text{g/mL}$ bovine pituitary extract, 5 ng/mL human recombinant epidermal growth factor) and with low concentration of Penicillin and Streptomycin mixture (0.5 U/ml) (Lonza, Basel, Switzerland). Cells were split upon reaching >80% confluency and re-seeded at 20-30% confluency. KCs were differentiated in the presence of 1.8 mM calcium chloride. *In vitro* reconstruction of the epidermis was performed as described in.¹ Briefly, 5×10^5 cells were seeded per ThinCert™ Cell Culture Insert (0,4 μm pore, 0.33 cm^2) (Greiner Bio-One, Kremsmünster, Austria) using KC-SFM in equal volume of high glucose Dulbecco's Modified Eagle Medium (DMEM) supplemented with GlutaMAX™ (Thermo Fisher Scientific). HEK293 line cells were grown in DMEM high glucose with L-glutamine (Lonza, Basel, Switzerland), supplemented with 10% of Fetal Bovine Serum (FBS) (Capricorn Scientific, Ebsdorfergrund, Germany) and mixture of Penicillin and Streptomycin (5 U/ml) (Lonza, Basel, Switzerland). Cells were cultured in humidified atmosphere containing 5% CO₂ at 37°C and medium was replaced every 48 hours unless noted differently.

Transfection and stimulation of KCs

Transfections were performed 20-24 h hours after plating the cells with density of 10 000 cells/ cm^2 on 24- or 12-well plates. Either 30 nM of miRNA Precursor hsa-miR-10a-5p, Negative Control #1, mirVana™ miRNA Mimic miR-10a-5p or corresponding Negative Control #1 (Thermo Fisher Scientific) were used at 30 nM of concentration. Locked nucleic acid (LNA) inhibitor for hsa-miR-10a-5p and negative control A (Qiagen) were used at 10 nM (Figure 4D) or 30 nM (Figure 4E). Transfections were performed with siPORT™ NeoFX™ Transfection Agent (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions or using

cell penetrating peptide (CPP) PepFect14 (PF14), as described in.² Per one 12-well, PF14 and miRNA mimics were mixed at molar ratio 17:1 in final volume of 100 µl, incubated at room temperature for 1 hour, mixed with 900 µl of the growth media and applied to the cells. KC stimulations were started 24 hours after transfections, with final concentrations of 10 ng/ml for IL-1β, 25 ng/ml for HB-EGF, 20 ng/ml for IL-22, 40 ng/ml for IL-4, 10 ng/ml for IL-17A (Peprotech, Rocky Hill, NJ, USA). Stimulation of KCs in Supplementary Figure S2 was performed for 48 hours with the following concentrations of cytokines: TNF-α 25 ng/ml, IL-1β 10 ng/ml, IL-4 40 ng/ml, IFN-γ 10 ng/ml, IL-17A 10 ng/ml, TGF-β 20 ng/ml (R&D Systems, Minneapolis, MN, USA). Retinoic acid was used at 1 µM final concentration (Merck, Darmstadt, Germany).

RNA purification, reverse transcription and real time quantitative PCR.

Skin biopsies in Qiazol (Qiagen, Hilden, Germany) cell lysis reagent were homogenised with Precellys 24 (Precellys, Bertin Instruments, Montigny-le-Bretonneux, France) homogeniser. *In vitro* samples were lysed with TRI Reagent (Zymo Research, Irvine, CA, USA). Total RNA was extracted with Direct-zol™ RNA MiniPrep Kit (Zymo Research) or miRNeasy Mini Kit (Qiagen). In-column DNase digestion was performed with DNase (Zymo Research) or with RNase-Free DNase Set (Qiagen). RNA concentration and quality was measured with Nanodrop 2000c and Agilent 2100 Bioanalyzer, using Agilent RNA 6000 Nano Kit (Agilent Technologies, Santa Clara, CA, USA). Reverse transcription was performed with 0.1-1 µg of total RNA, oligo-dT primers and RevertAid First Strand cDNA Synthesis reagents (Thermo Fisher Scientific) for mRNA analysis or TaqMan® MicroRNA Reverse Transcription Kit and TaqMan™ MicroRNA Assays for microRNA analysis. Real-time quantitative PCR (RT-qPCR) was done using custom primers and 5x HOT FIREPol EvaGreen qPCR Supermix (Solis BioDyne, Tartu, Estonia) for mRNA analysis or TaqMan™ MicroRNA Assays and 5 x HOT FIREPol® Probe qPCR Mix Plus (ROX) (Solis BioDyne) for miRNA analysis. Machines used were ABI Prism 7900 or Viia7 (Life Technologies, Carlsbad, CA, USA). Gene expression analyses were done with Comparative Ct (ΔΔCt) method. Relative gene expression levels were normalised to human EEF1A1 for mRNAs or let-7a for miRNAs and

compared to mean of control experiments or control group (=1). The following primers were used: MAP3K7 fw: 5'-AGCCTGCTTGAATCCAGTGT-3', MAP3K7 rv: 5'-TTGCGTGGGCAGCAGTATAA-3', HAS3 fw: 5'-TGTCCAGATCCTCAACAAGTACG-3', HAS3 rv: 5'-CAGCCAAAGTAGGACTGGCA-3', Ki-67 fw: 5'-AAAGAGTGGCAACCTGCCTT-3', Ki-67 rv: 5'-TTTGCAACAATCAGATTTGCTTCCG-3'. All primers were designed with Primer 3 and synthesized by TAG Copenhagen, Frederiksberg, Denmark. Primers for human CCL5, IRAK1, EEF1A1 (EF1A) and IL-8 have been previously published.³

Cloning and luciferase assay

3'UTR (untranslated region) fragments of HAS3 and MAP3K7 were cloned into pmirGLO Dual-Luciferase miRNA Target Expression Vector (Promega, Madison, WI, USA) using NheI and SalI restriction sites. Each cloned 3'UTR fragment contained a single Targetscan-predicted (www.targetscan.org) miR-10a binding site or a miR-10a binding site with 2-nucleotide mutation. The following primers for fragment amplification were used: HAS3-1 3'UTR fw 5'-ATAGCTAGCGCAGAGCGGGTAAAGTG-3'; HAS3-1 3'UTR rv 5'-ATAGTCGACTGGTTTAGCACAACCTTCTGA-3'; HAS3-2 3'UTR fw 5'-ATAGCTAGCTCAGTGGCAACTTGTGATAG-3'; HAS3-2 3'UTR rv 5'-ATAGTCGACTGAACATGTTGGGGCTTAAT-3'; MAP3K7 3'UTR fw 5'-ATAGCTAGCCTAACAGAACAGGA-3'; MAP3K7 3'UTR rv 5'-ATAGTCGACATCCGGTAAACTGCTTTCA-3'. The mutations were introduced with following primers: HAS3-1 3'UTR 10a mut fw 5'-AGAAAAGACACCGTGGGAGGG-3'; HAS3-1 3'UTR 10a mut rv 5'-CCCTCCCACGGTGTCTTTTCT-3'; HAS3-2 3'UTR 10a mut fw 5'-TCAGAAAACACCGTCCAGGAAT-3'; HAS3-2 3'UTR 10a mut rv 5'-ATTCCTGGACGGTGTCTTTCTGA-3'; MAP3K7 3'UTR 10a mut fw 5'-CAAGTTAAAGACACCGTATTATAAATTT-3'; MAP3K7 3'UTR 10a mut rv 5'-AAATTTATAATACGGTGTCTTTAACTTG-3'. HEK293 cells were seeded on 24-well plates, 10⁴ cells per well, grown overnight and transfected with 50 ng of reporter plasmid and 30 nM of pre-miRNAs (Pre-miR Precursor Molecules hsa-miR-10a-5p or negative control #1, Thermo Fisher Scientific), using 1,5 µl siPORT NeoFX (Thermo Fisher Scientific). 24h after transfection, the firefly and renilla luciferase activity was measured with

Dual-Luciferase® Reporter Assay System (Promega). Firefly luciferase activities were normalised to internal renilla luciferase and miR-10a transfected samples were normalised to controls (=1).

Cell cycle analysis

Cells were trypsinised and collected 48 hours after transfection with miR-10a or control mimics, fixed 30 minutes with ice-cold 70% ethanol, permeabilized in 0,1 % Triton X-100 in phosphate-buffered saline 10 minutes at room temperature and stained with DAPI (1 µg/ml). Cell cycle phase distribution was determined with BD LSRFortessa™ cell analyzer (BD Biosciences Franklin Lakes, NJ, USA) and analysed using ModFit LT™ Software 2.5.1 (Verity Software House, Topsham, ME, USA). Alternatively, Click-iT® EdU Alexa Fluor® 488 Flow Cytometry assay (Thermo Fisher Scientific) was performed after the transfection for 48 hours according to the manufacturer's instructions and analysed on a BD LSRFortessa™ cell analyzer (BD Biosciences). EdU was added to the cells 2 hours before the harvesting the cells.

Proliferation assay

KCs were transfected on 96-well plate with 30 nM mirVana™ miR-10a-5p and Negative Control #1 miRNA Mimics (Thermo Fisher Scientific) or 10 nM miRCURY LNA™ inhibitor for hsa-miR-10a and Negative Control A (Exiqon, Vedbaek, Denmark). Transfections were performed with PF14 as described above. After 72h of the transfection, cell proliferation was analyzed using CellTiter-Glo® Luminescent Cell Viability Assay (Promega, Madison, WI, USA) in which the measured Luciferase activity is proportional with the amount of cellular ATP.

Array, pathway and target analysis

mRNA profiling was done with Illumina HumanHT-12 Expression BeadChips version 4 and data analysed with Illumina GenomeStudio V2009.1 Gene Expression Module using Quantile normalisation and background subtraction with Illumina custom errors model, and further with Microsoft Excel and Multi Experiment Viewer

4.8.1. Genes, with average expression values below 50 or average detection P values above 0.05 in all conditions, were discarded. The remaining genes were sorted based on gene expression fold change between control transfected and miR-10a transfected cells. 200 most upregulated genes were used in g:Profiler analysis (<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 ⁴. Putative direct targets for miR-146a were selected using Targetscan 7.1 (http://www.targetscan.org/vert_71/) choosing only highly conserved sites with context score <-0.15 ^{5,6}.

***In situ* hybridisation (ISH)**

ISH was performed on 6 µm frozen skin sections using microRNA ISH Buffer, Controls Kit and 5'-DIG and 3'-DIG labelled hsa-miR-10a-5p (612528-360) and 5'-DIG and 3'-DIG labelled miRCURY scrambled ISH 49C (Custom Detection Probe 339115) according to the manufacturer's protocol (Qiagen/Exiqon, Vedbaek, Denmark). Prehybridization, hybridization and washing were performed at 48°C. For detection, slides were incubated with alkaline phosphatase-conjugated sheep anti-DIG-AP (1:1500, Roche, Basel, Switzerland) for 1h at room temperature. Densitometry analysis of *in situ* hybridization images were performed with ImageJ software (NIH, USA). An integrated density value of the signal per area of interest was obtained for six areas from nonlesional and lesional skin from two patients and two controls. The relative intensity was calculated as percentage and was normalized to the average of control samples (100%).

Immunofluorescence

Immunofluorescence staining of HAS3 protein expression was analyzed from frozen human skin sections (5 µm) of healthy controls, nonlesional and lesional skin of atopic dermatitis patients or 10 µm cross-sections of *in vitro* reconstructed epidermis. Sections were fixed with 4% paraformaldehyde, permeabilized with 0.1% Triton X-100 and blocked with 10 % normal goat serum (Thermo Fisher Scientific). After that the slides were

incubated with the HAS3 (Rabbit anti-HAS3, 0.05 mg/ml, 1:100, Atlas Antibodies, Stockholm, Sweden or sc-365322 mouse monoclonal Anti-HAS3, Santa Cruz Biotechnology, Dallas, TX, USA) primary antibody for 1 hour. The omission of the primary antibody was used as a negative control. The sections were stained with Alexa Fluor 488-conjugated goat anti-rabbit IgG (2 mg/ml, 1:1000 or Goat anti-Mouse IgG1 Cross-Adsorbed Secondary Antibody, Alexa Fluor 488, Thermo Fisher Scientific) secondary antibody and after that incubated with DAPI (5 mg/ml, 1:5000) in PBS. Slides were mounted with StayBrite Hardset Mounting Medium (Biotium, Fremont, CA, USA) and visualized with a Leica DM5500 B microscope (Leica Microsystems, Wetzlar, Germany). Densitometry analysis of immunofluorescence images were performed with ImageJ software (NIH, USA). An integrated density value of the signal per area of interest was obtained for three microscopy fields from *in vitro* reconstructed epidermis. The relative intensity was normalized to the average intensity in nonstimulated conditions (=1).

Statistics

Statistical analyses were performed with GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA). All comparisons were performed using unpaired Students *t*-test at 95% confidential interval. The results were considered significant at $P < 0.05$. Data are presented as mean with standard error of the mean (SEM).

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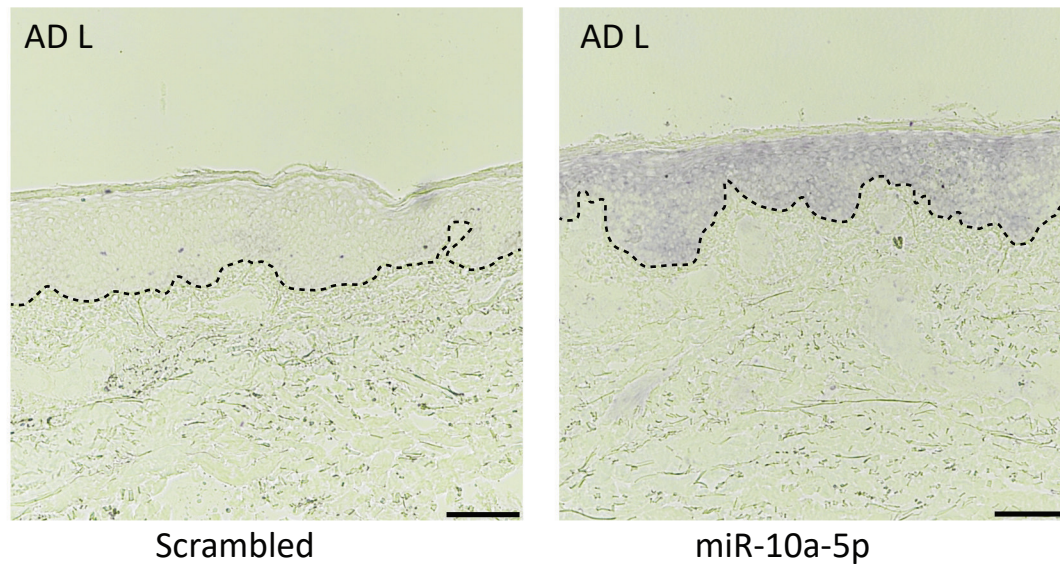


Figure S1. The expression of hsa-miR-10a-5p in the skin of AD patients. ISH of miR-10a-5p in skin cryosections from AD lesional (AD L) skin was performed with miR-10a-5p specific probe and scrambled ISH 49C as a control. Blue color shows the expression of miR-10a-5p, bar = 100 μ m.

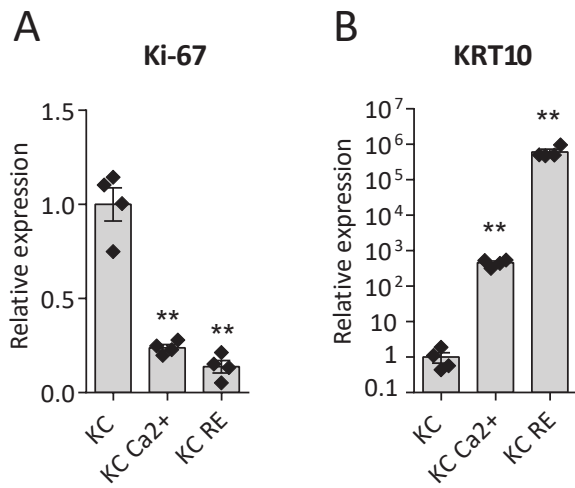


Figure S2 Relative expression levels of Ki-67 and KRT10 in proliferating and differentiated KCs. RT-qPCR analysis of Ki-67 and KRT10 was performed from proliferating KCs (KC), KCs differentiated with high Ca²⁺ concentration for 72h (KC Ca²⁺) and from *in vitro* reconstituted epidermis (KC RE) at day 0 before the apical media was removed. Data represent the mean ± SEM. Student's t-test as compared to KC, *P < 0.05, **P < 0.01.

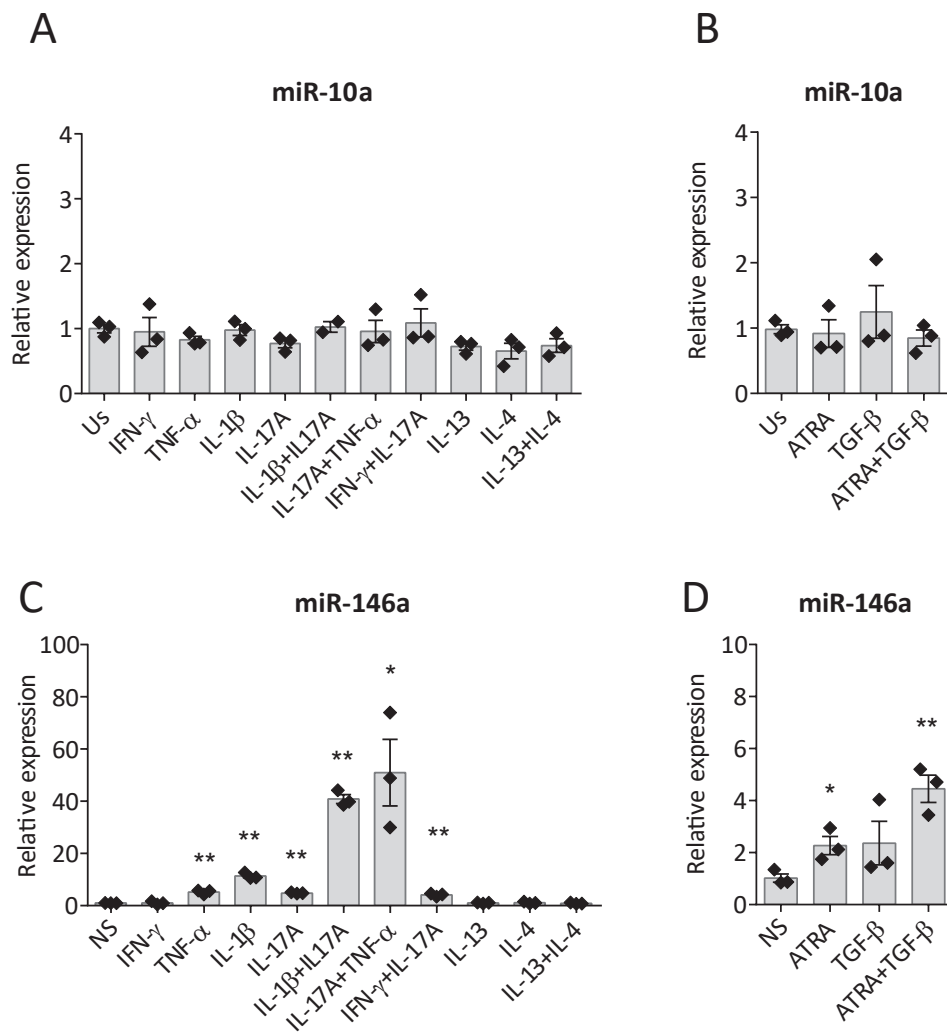


Figure S3 miR-10a-5p and miR-146a expression in stimulated KCs. KCs were stimulated or co-stimulated with indicated cytokines or retinoic acid (ATRA) for 48 h or left nonstimulated (NS). Data represent mean $\pm$ SEM, Student's t-test, *P < 0.05, **P < 0.05.

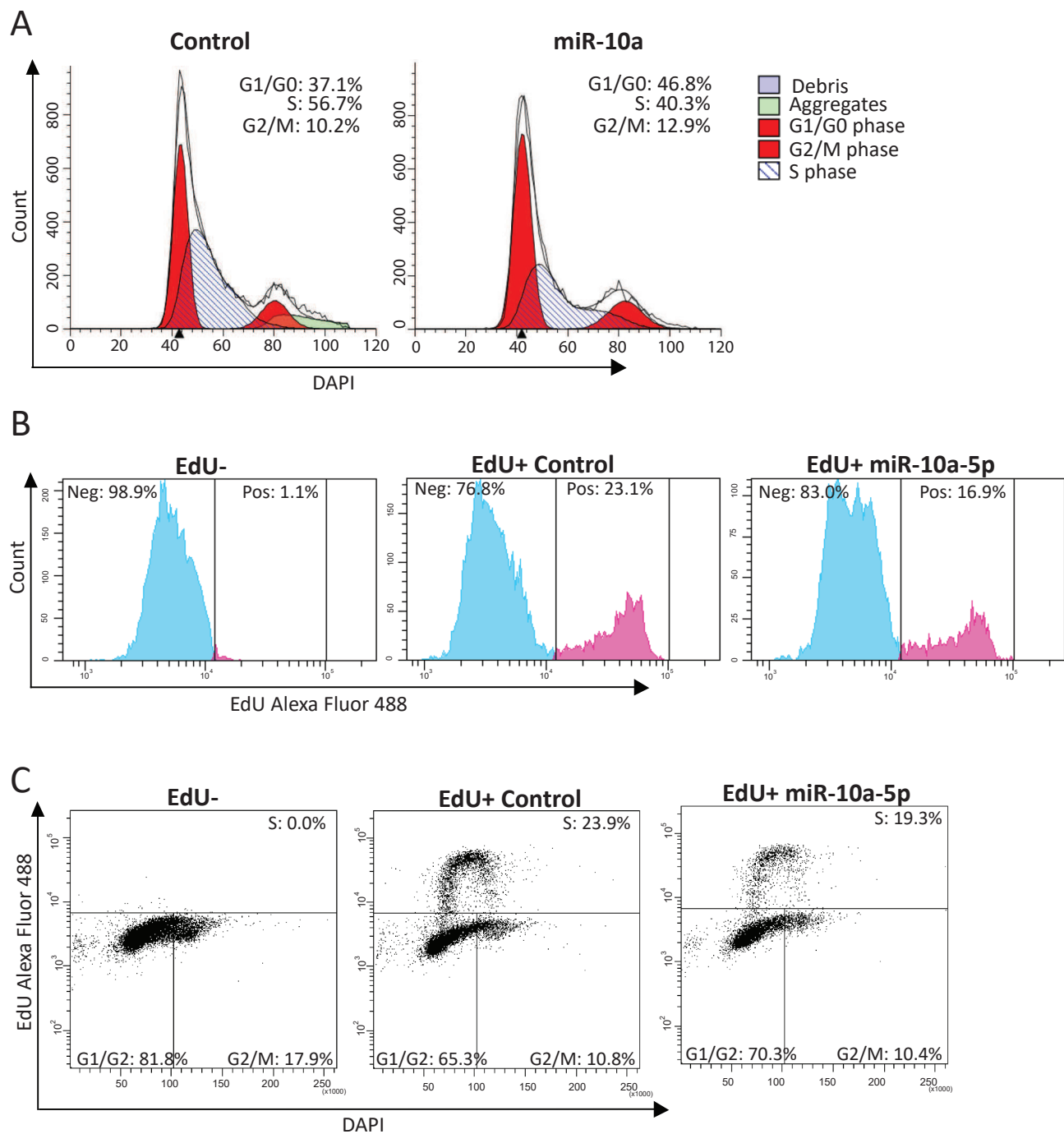
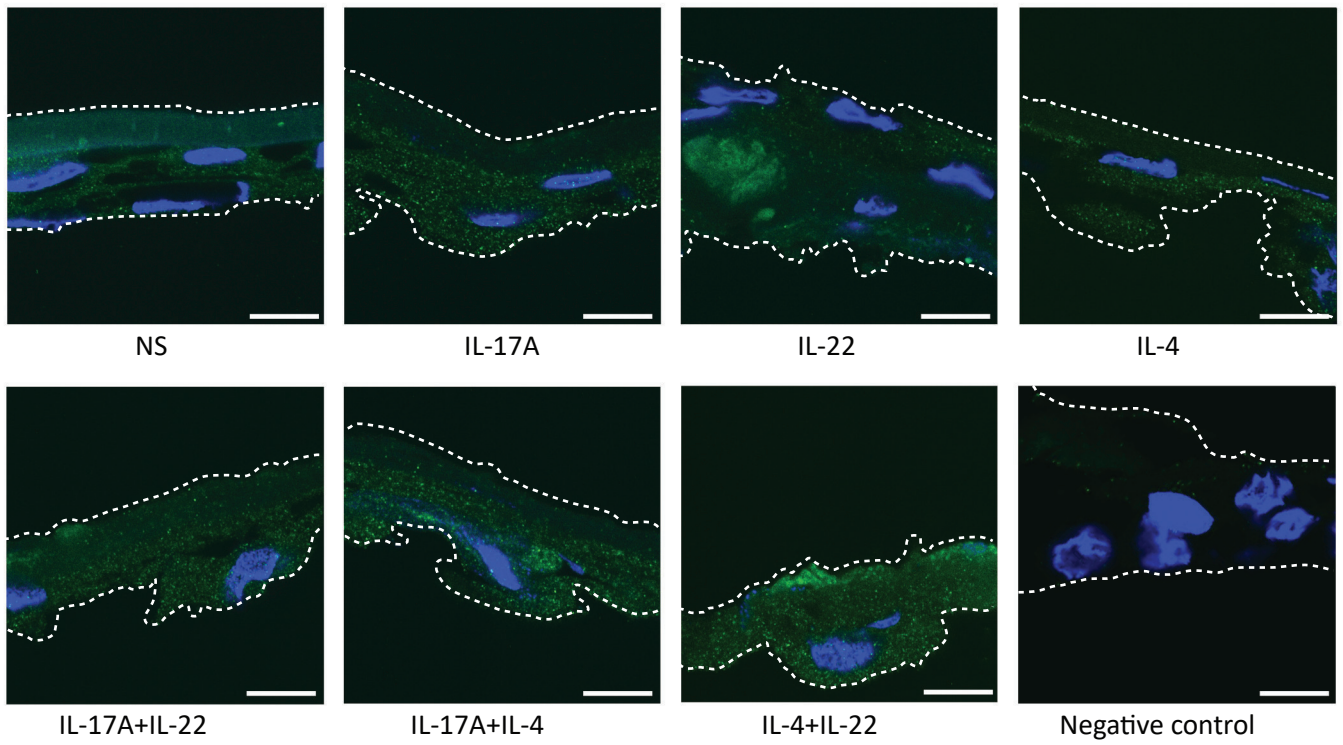


Figure S4 miR-10a-5p inhibits cell cycle progression and increases cell migration of primary KCs. (A-C) KC were transfected with miR-10a-5p mimic (miR-10a-5p) or control for 48 h and indicated assays were performed. One representative experiment is shown. (A) Cell cycle distribution was measured based on DAPI signal. (B, C) Cells were labeled with EdU followed by measurement of EdU (B) and DAPI and EdU(C) signal distribution by flow cytometry.

A



B

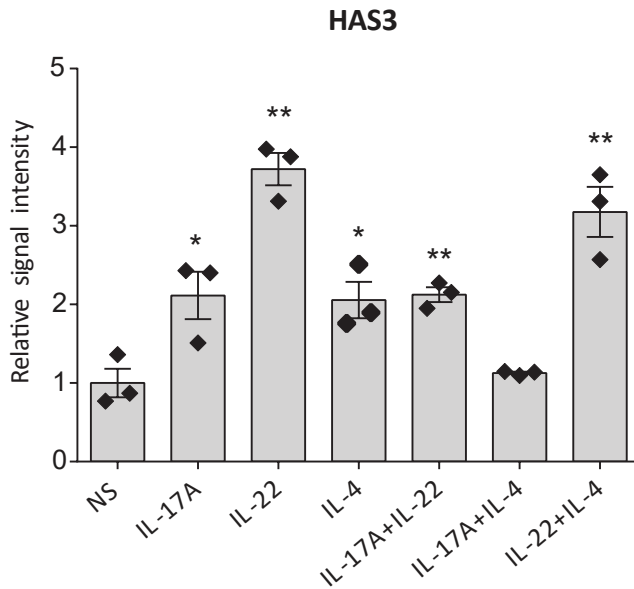


Figure S5 HAS3 expression in the presence of AD-realted cytokines in reconstituted epidermis. Transwells with reconstituted epidermis cultures were stimulated with indicated cytokines for 24 h or left nonstimulated (NS). Immunofluorescence with anti-HAS3 (green) and DAPI staining (blue) was performed on 10 μm frozen sections. (A) Reconstituted epidermis is designated with two dotted white lines. Negative control is performed without primary antibody. Bars correspond to 25 μm. (B) An integrated density value of immunofluorescence signal per area of interest was obtained for three areas in each condition. The relative intensity was normalized to the average of NS (=1). Data represent the mean ± SEM. Student's t-test, * P < 0.05, ** P < 0.01.

Table S1. Top 200 genes induced by IL-1 β ^A

SYMBOL	Average signal				control+IL-1 β /control	
	control	miR-10a-5p	control + IL-1 β	miR-10a-5p + IL-1 β	Differential <i>P</i> -value	FD
SPRR2A	202.8	260.4	1623.5	2176.1	0.000	8.0
SERPINA3	219.5	244.9	1286.8	1353	0.000	5.9
SPRR2F	264.3	334.8	1360.9	1807.9	0.000	5.1
SPRR2E	168.1	187.2	769.7	1018.7	0.000	4.6
S100A8	1326.3	1778.9	5215.4	5864.6	0.000	3.9
IL8	142	119.2	533.3	344.4	0.000	3.8
CXCL5	343.7	424.7	1059.3	1234.3	0.000	3.1
SPRR2G	230	231.6	600	803.4	0.000	2.6
S100A9	2586.8	3006.8	6512.8	8401.3	0.000	2.5
SPRR2D	1208.1	1498.4	2981.7	3232.4	0.000	2.5
CLDN7	195.1	250.8	440	500.8	0.000	2.3
MMP10	2435.2	3743.6	5197	7077.4	0.000	2.1
ZC3H12A	385.7	392.9	813.5	717.8	0.000	2.1
LCN2	1054.2	1176	2162.7	2798.3	0.000	2.1
CSF2	306.2	264.9	627.2	562.9	0.000	2.0
MMP9	4302.9	4981.8	8414.3	10253.5	0.000	2.0
SLPI	1092.8	1427.4	2106.4	1859.1	0.000	1.9
CRCT1	657.3	824.8	1246.8	1527.5	0.000	1.9
ABCB7	121.4	151.1	228.7	140.2	0.000	1.9
CXCL1	509.5	549.3	941.4	887.4	0.068	1.8
WFDC5	125.4	143.5	231.6	306.7	0.000	1.8
HLA-B	612.1	645.1	1108.2	959.3	0.000	1.8
CSTA	334.7	363.3	602.2	447.8	0.000	1.8
MED31	147.1	184.9	261.8	183.1	0.000	1.8
MYD88	287.4	360.2	510.9	418.9	0.000	1.8
C10orf10	158.5	184.7	278.4	341	0.000	1.8
TNFAIP3	562.7	602.2	966.6	908.3	0.000	1.7
IL13RA2	106.9	103.7	182.2	213.2	0.000	1.7
GNG12	101.9	145.6	172.4	167.4	0.018	1.7
ERO1L	369.6	428.1	623	611.5	0.000	1.7
KRT34	190.5	236.5	319.7	385	0.000	1.7
HLA-H	1096.6	1182.4	1836.1	1563.2	0.000	1.7
ITGAV	650.7	751	1085	1074.7	0.000	1.7
C9orf16	107.1	114.8	178	148.1	0.000	1.7
NFKBIZ	123.4	141.9	203.2	222.9	0.000	1.6
MAF1	131.1	145.7	214.8	150.1	0.000	1.6
NCCRP1	137	176.8	222.8	272.2	0.000	1.6
SC5DL	655.6	831.6	1059.7	1114.6	0.000	1.6
SRPK1	465	561.7	750.5	535.3	0.000	1.6
TNFSF10	154.3	168	248.9	214.5	0.000	1.6
CEACAM6	247.5	328.6	399.2	609.4	0.000	1.6
TJP1	208.5	286.2	336.1	323.4	0.000	1.6
PLAT	196.6	199.9	316.8	275	0.000	1.6
EHF	161.7	200.3	260.5	359.8	0.000	1.6
PAN3	191.6	231.2	306.8	240.1	0.000	1.6
RDX	224.5	346.1	358.2	361.5	0.000	1.6
IL1RN	329	358.5	524.9	650.6	0.001	1.6
CDSN	183.5	203.1	291.4	376.4	0.000	1.6
B4GALT1	199.1	194.3	316.1	279.5	0.000	1.6
NEU1	244.8	271.5	388.2	329.6	0.000	1.6
LYPLA2	245.5	268.4	385.9	309	0.000	1.6
ANAPC10	191.3	237.6	300.5	226.2	0.000	1.6

TSPAN13	213	246.9	333.6	352.1	0.004	1.6
ZFP36L2	333.4	477	520.6	543	0.000	1.6
ZNF212	118.4	150.1	184.5	156.3	0.000	1.6
CADM1	106.2	121.7	165.3	137.8	0.000	1.6
TNFSF9	112.3	136.5	174.7	185	0.000	1.6
YWHAЕ	413.5	482.2	642.8	484.1	0.024	1.6
GLIPR2	135.4	125.6	210.4	242	0.000	1.6
KCTD9	188.9	227.6	293.1	245.4	0.000	1.6
BMP2	224.1	248.1	347.5	336.9	0.000	1.6
TMEM167A	166.7	192.7	257.4	193.6	0.000	1.5
PSENE1	129.8	121.7	200.2	135.8	0.000	1.5
LIMA1	2205.5	2375	3395.8	2845.7	0.000	1.5
ARPC1B	230.7	289.5	354.9	317.9	0.378	1.5
SDC4	1672.1	1666.7	2572	2183.3	0.000	1.5
IGFBP4	116.6	138.5	178.8	165.3	0.010	1.5
ATP1B1	497.2	575.5	759.8	727.6	0.000	1.5
MTSS1	352.2	390.6	537.7	547.8	0.000	1.5
PDHX	119.6	158.4	182.5	146.8	0.000	1.5
MAMDC2	245	311.3	373.3	446.5	0.049	1.5
NRBP1	124.8	136.1	189.8	151.5	0.000	1.5
GRAMD3	195.9	270.4	297.5	315.7	0.000	1.5
ATF6	315.7	357.6	479	401.1	0.000	1.5
IFIH1	203	221.6	307.6	258.3	0.001	1.5
HN1L	223	244.1	337.8	269.6	0.000	1.5
OAS3	249.1	237.8	377.1	292	0.173	1.5
HLA-F	414	418.2	626	591.9	0.002	1.5
KLK10	459.9	540.4	695	650.5	0.000	1.5
SPRR1B	2355.9	3014.2	3557.4	3805.9	0.066	1.5
BLCAP	699.5	725.5	1056.1	755.4	0.000	1.5
SAMD4B	117.3	129.1	176.7	120.5	0.000	1.5
ATL2	152.4	197.7	229.4	241.4	0.000	1.5
SLC11A2	205.9	226.3	309.4	293.9	0.000	1.5
RAB6A	116	134.9	174.3	170	0.000	1.5
CMTM6	581.2	708	873.3	778.2	0.000	1.5
TSPYL1	452.2	594.3	678.1	631	0.000	1.5
UCHL1	135.8	144.5	203.6	157.7	0.000	1.5
CNOT2	398.8	497.2	597.8	512.7	0.000	1.5
CDC42	162.2	202.1	242.8	206.4	0.012	1.5
DOCK9	127.6	170.1	190.6	203.7	0.000	1.5
STRN3	212.9	276.9	317.9	267.7	0.000	1.5
TPM3	113.3	133	169.1	154.9	0.624	1.5
GLRX	361.8	411.1	539.3	531.4	0.000	1.5
TERF2IP	433.2	496.5	645.3	586.7	0.000	1.5
DEFB103B	468.8	444.2	698	907.5	0.000	1.5
L1CAM	117.1	115.8	174.3	157.7	0.001	1.5
TMEM62	132.2	122.4	196.6	175.4	0.000	1.5
NRIP1	581.8	780.9	865.2	878.4	0.004	1.5
MMP1	262.8	369.2	388.9	795.5	0.065	1.5
CHRNA1	300.9	317.9	444.6	457.7	0.000	1.5
JAK1	115.5	145.5	170.4	180.9	0.000	1.5
KPNA1	276.7	342.5	408.2	382.2	0.000	1.5
KYNU	300.8	297.9	443.5	488.6	0.000	1.5
EFNA1	398.8	392.9	585.8	594.6	0.000	1.5
POLD4	226.8	255	332.7	294.8	0.000	1.5
PGM3	201.2	234.8	295.1	319.5	0.000	1.5
PPP2CB	350.3	422.1	513	447.9	0.017	1.5

RAB1A	158.1	199.8	230.8	188.9	0.001	1.5
CLIC4	349	418.2	508.5	544.6	0.013	1.5
LCP1	533.4	538.9	776.4	766.1	0.000	1.5
PLAUR	365.5	439.5	531.6	643.6	0.010	1.5
MYO10	261.2	313.6	379.6	389.2	0.480	1.5
MFSD1	347.9	397	505.3	390.6	0.014	1.5
SDHD	182.9	239.8	265.4	205.1	0.000	1.5
EIF4EBP1	132.6	162.2	192.4	167.2	0.000	1.5
TRIM22	161.7	217.5	234.4	181.9	0.000	1.4
TAGLN2	2436.9	2620.9	3531.8	3108.7	0.000	1.4
PELI1	117.7	151.8	170.5	191.5	0.010	1.4
PNLIPRP3	760.8	860.9	1102	1037.6	0.001	1.4
IFNGR1	599.3	679	867.2	768.6	0.000	1.4
CRABP2	329.6	366.7	476.7	545	0.000	1.4
LMO4	556.6	653.4	805	862.6	0.014	1.4
SCOC	535.2	628.9	773.7	656	0.000	1.4
EIF1AY	142.5	165.6	206	173.3	0.001	1.4
NRAS	133.4	138.2	192.7	152	0.000	1.4
MAN2A1	131.4	151.9	189.7	150.3	0.739	1.4
HARS2	439.2	501.7	632.1	495.5	0.000	1.4
MOV10	164.8	196.7	236.8	187.9	0.001	1.4
RNF13	101.7	121.1	146.1	119.4	0.033	1.4
GFPT1	277.5	322.2	398.1	475.3	0.003	1.4
RIT1	151.7	180.4	217.5	214.8	0.070	1.4
CNFN	477.5	486.4	684.1	1022.3	0.000	1.4
KRT80	180.5	239.1	258.4	382.6	0.001	1.4
PIAS3	191.3	220	273.6	222.7	0.000	1.4
HERC6	459.9	439.9	656.4	517.1	0.001	1.4
IL1A	3741.7	4050.7	5334.3	4968.4	0.000	1.4
CCDC53	668	725.8	952.2	689	0.000	1.4
LYPD3	495.4	582.8	705.6	712.8	0.000	1.4
TAP1	993.6	1013.2	1414.8	1134.2	0.000	1.4
PPDPF	717.7	665	1021.9	746	0.135	1.4
FILIP1L	116.3	124.2	165.4	152.7	0.005	1.4
KIAA0391	364.7	433.1	518.1	416.4	0.000	1.4
DSC2	276.2	358.6	392.3	528.2	0.000	1.4
ZNF217	171.2	205.7	243	243.6	0.000	1.4
TUBB6	1666.5	1866.8	2360.3	1906.6	0.000	1.4
SDCBP	440.8	498	624.2	587.7	0.000	1.4
FGD6	152.3	187.4	215.1	225	0.000	1.4
CIB2	110.2	111.8	155.6	142.3	0.007	1.4
CMPK1	219.1	158.7	309.3	174.9	0.140	1.4
GTF2E1	103.5	117.8	146.1	113.6	0.001	1.4
NRD1	1126	1305.1	1588.8	1395.5	0.000	1.4
AXL	494.2	594.1	697	614.9	0.001	1.4
ELOVL4	210.6	274.6	297	326.1	0.001	1.4
APLP2	272.3	298.1	383.1	342.2	0.001	1.4
NFKBIA	1675.9	1620.5	2354.2	2107.3	0.000	1.4
MYO1B	582.4	687.7	817.6	832.4	0.000	1.4
AP2B1	156.6	164.2	219.8	167.6	0.006	1.4
PLA2G7	344.7	464.5	483.8	469.1	0.001	1.4
SPCS3	195.3	234.3	273.8	273.2	0.000	1.4
P4HA2	421	443.6	589.7	631.5	0.000	1.4
HERC4	114.3	132.4	160.1	155.6	0.034	1.4
KRT19	2113.6	2305.8	2960.5	3469.7	0.000	1.4
SMAD3	324.4	394.3	454.3	393.3	0.003	1.4

RAB11FIP1	209.6	275.6	293.5	392.3	0.098	1.4
BNIP3L	220.6	271.4	308.7	343.9	0.001	1.4
NCOA3	118.3	135.5	165.4	134.4	0.084	1.4
DPH3	222.7	249.5	311.3	248.3	0.000	1.4
SSFA2	120.7	157.5	168.7	172.5	0.009	1.4
DSC3	454.9	587.8	635.8	568.8	0.001	1.4
HYOU1	296	330.6	413.4	416.4	0.004	1.4
TPM4	584.8	517.5	816.4	588.7	0.000	1.4
SH3BGRL	228.6	290.4	319	278.7	0.002	1.4
MMD	292.2	357	407.7	476	0.000	1.4
SH3PXD2B	134.4	148.6	187.3	184.7	0.001	1.4
RBL2	175.8	218.1	244.9	235.5	0.011	1.4
FERMT1	3376.3	3607.6	4701.5	5148.3	0.000	1.4
MICB	186.3	185.8	259.3	234.7	0.000	1.4
KIF13B	131.7	161.8	183.3	230.4	0.003	1.4
GOS2	490.6	615.5	682.6	770.2	0.018	1.4
MSN	2839.8	3189	3950.1	3364.5	0.000	1.4
KRT8	346.6	341.8	482.1	459	0.039	1.4
SLC35F5	160.4	188.2	223.1	224.4	0.004	1.4
HMGB3	320.8	372.8	445.4	461.1	0.000	1.4
PRKAG1	168	201	233	188.2	0.000	1.4
ULBP2	436.6	464.2	605.2	587.5	0.001	1.4
ZFAND6	140.5	159.3	194.6	202	0.026	1.4
PICALM	259.3	313.5	359.1	394.1	0.050	1.4
PPP2R5A	128.9	156	178.5	188.1	0.010	1.4
KDELR2	732.2	905.3	1013.4	1123.9	0.000	1.4
UBE2A	409.6	447.1	566.7	499.6	0.002	1.4
CALR	1319.2	1407.7	1823.8	1575.7	0.015	1.4
SIAH2	177.6	205.9	245.5	231.5	0.002	1.4
RPL12P6	1003.7	1127.2	1386.4	1012.8	0.098	1.4
NDUFC1	496.9	504.9	685.7	507.2	0.000	1.4
TDRD7	114.8	123.5	158.4	144	0.580	1.4
FER	103.6	115.9	142.9	115	0.030	1.4
DDX60	176	191.7	242.7	228.8	0.019	1.4
UCHL3	1017.7	1110.1	1402.7	1134	0.000	1.4
DUSP7	158.1	165.9	217.8	180.7	0.547	1.4

^aAverage signals of top 200 differentially expressed genes in cells transfected with control mimics and stimulated with IL- β as compared to control-transfected cells that were left unstimulated. Top 200 gene list is generated based on fold difference (FD).

Table S2. Top 200 genes downregulated by miR-10a-5p^A

SYMBOL ^B	Average signal				miR-10a-5p+IL-1 β / cont+IL-1 β	
	control	miR-10a-5p	control+IL-1 β	miR-10a-5p + IL-1 β	Differential <i>P</i> -val	FD
MT1G	1090.3	674.7	964.1	558.3	0.1132	0.58
CMPK1*	219.1	158.7	309.3	174.9	0.1261	0.57
CDKN3	224.6	231.3	217.1	130.1	0.0612	0.60
TOP2A	295.8	334.4	277.3	166.3	0.0066	0.60
CEP55	261	266.8	273.3	164.8	0.0940	0.60
IFIT1*	375.8	387.7	477.2	290	0.6548	0.61
UBE2C	392.9	345.4	343.9	210.5	0.0659	0.61
ABCB7*	121.4	151.1	228.7	140.2	0.0667	0.61
UBE2T	248.2	253.1	272.2	172.1	0.23964	0.63
RAB7B	249.7	172	152.3	97.9	0.0053	0.64
UBIAD1*	145.8	120.6	136.1	87.6	0.0583	0.64
TPX2	137.3	137.8	151.8	97.8	0.31618	0.64
IL8	142	119.2	533.3	344.4	0.37597	0.65
TNS4*	347.6	366.4	458.8	296.7	0.21933	0.65
ST6GALNAC6*	249.4	192.9	272.2	178.4	0.0002	0.66
SLC29A1	117.1	93.2	93.8	61.6	0.36951	0.66
SLC38A5	231.9	247.2	314	206.4	0.0116	0.66
ANLN	127.3	135.8	143.6	94.5	0.22057	0.66
CKAP2L	130.2	114.7	87.5	57.6	0.0767	0.66
CDCA8	131.8	126.5	115.4	76.1	0.53852	0.66
HAS3*	566.3	445.8	566.6	374.3	0.0150	0.66
HIST1H4C	4419.8	4284.6	3919.8	2602.9	0.19049	0.66
CYP4V2*	119.3	97.9	115.6	76.9	0.22629	0.67
STARD5*	140.5	116.9	163.6	109.1	0.0527	0.67
CCNA2	217.4	226.7	199.4	133.3	0.38171	0.67
PCBD1*	235.1	193.5	277	186.1	0.13791	0.67
TACC3	139.2	130.5	90.9	61.2	0.0963	0.67
SPC24	105.6	92.4	70.5	47.6	0.99999	0.68
GINS2*	287.5	225.2	200	135.3	0.32445	0.68
PSENEN	129.8	121.7	200.2	135.8	0.33719	0.68
POLR3K	115.5	99.5	105.1	71.5	0.13862	0.68
SAMD4B*	117.3	129.1	176.7	120.5	0.0163	0.68
C3orf14*	243.2	235.8	279.1	191.2	0.16063	0.69
TK1	314.5	295	285.7	196.1	0.21479	0.69
IFFO2*	418.3	329.6	389.5	267.4	0.0037	0.69
MAD2L1	120.9	141.1	111.1	76.4	0.6554	0.69
CKS1B	871.9	888.4	882.4	607.6	0.21416	0.69
VRK1	151.3	146.5	133.4	91.9	0.41808	0.69
TRIP13	307.2	273.2	229.4	158.2	0.0091	0.69
HERC5	155.3	152.8	164.8	113.7	0.44621	0.69
HJURP	113.5	108.7	90.8	62.8	0.46208	0.69
ATP5G1	557.4	555.6	662.9	459	0.41582	0.69
MT1X	5490.9	4546.6	5369	3747.5	0.0230	0.70
MAF1	131.1	145.7	214.8	150.1	0.17078	0.70
MED31	147.1	184.9	261.8	183.1	0.77538	0.70
IPO4	229.8	212.1	201	141	0.19884	0.70
CSTF2	324.1	337.5	413.9	290.4	0.1257	0.70
NUSAP1*	142.8	140.2	128.8	90.5	0.0550	0.70
TMEM14A	337.3	328.2	392.5	276.8	0.90288	0.71
SS18L2*	1952.6	1625.3	2022.4	1426.4	0.0017	0.71

IFIT3	106	101.8	130.2	91.9	0.50752	0.71
ZMYND11*	203.1	170.8	220.5	155.8	0.66366	0.71
SNHG1	378.9	382.6	374.3	264.7	0.41452	0.71
SLC16A9	135.9	121.6	129.5	91.8	0.8656	0.71
SNORA10	249.8	254.4	326.6	232.1	0.2108	0.71
TRIM21	386.9	394	512.4	364.2	0.35589	0.71
PTTG1	800.2	761.8	764.4	544.1	0.15307	0.71
SRPK1	465	561.7	750.5	535.3	0.28639	0.71
SMARCD2*	145.8	156.3	188	134.2	0.0542	0.71
RPP40*	540.8	495.3	503.6	359.5	0.21937	0.71
CDC26	480.8	521.2	604.3	432.1	0.43029	0.72
BLCAP	699.5	725.5	1056.1	755.4	0.0727	0.72
SNORA18	172.1	180.4	211.2	151.3	0.73704	0.72
PCNA	125.1	110.2	100.5	72.1	0.93288	0.72
CDC45*	345.7	295.6	217.3	155.9	0.0675	0.72
SPR	192.8	186.9	220.4	158.4	0.59642	0.72
TPM4*	584.8	517.5	816.4	588.7	0.0013	0.72
LIG1	123.7	94.9	75.4	54.5	0.48576	0.72
CCDC53	668	725.8	952.2	689	0.27439	0.72
CKS2	757	751.6	721.1	521.9	0.62222	0.72
MRPS23*	335.8	367.5	417.9	302.5	0.63358	0.72
CBR1	164.7	167.5	191	138.4	0.31391	0.72
RPS26	1133.8	1174.7	1384.6	1003.4	0.14172	0.72
XAF1	138	141.8	168	121.8	0.1277	0.73
KHSRP	357.6	364.6	389.3	282.3	0.21028	0.73
NCAPG	210.8	209.7	148.2	107.5	0.1897	0.73
TMX1*	256.6	264.8	338.7	245.7	0.68122	0.73
BCYRN1	1387	1662.2	1721.4	1249.8	0.78512	0.73
POLR3B*	165.7	170.6	210.2	152.9	0.0896	0.73
MCM7*	351.8	302	248.1	181	0.20833	0.73
PPDPF*	717.7	665	1021.9	746	0.99999	0.73
RPL12P6	1003.7	1127.2	1386.4	1012.8	0.51684	0.73
CENPN*	201.1	178.9	167.1	122.4	0.16345	0.73
EXOSC2*	121.3	113.6	98.6	72.3	0.3528	0.73
PDE12*	108.2	104.6	128	93.9	0.99999	0.73
KIFC1	179	158.1	125.3	92	0.18211	0.73
PDCD2L	244.4	234.7	240.5	176.7	0.0311	0.73
CAD*	245.9	221.4	224.5	165.3	0.0419	0.74
MCM2	143.8	122.9	113.9	83.9	0.23694	0.74
PAK1IP1	310.7	300.7	299.6	220.8	0.32911	0.74
NDUFAF2	923	978.6	1095	807.3	0.34034	0.74
TMEM126A	593.8	626.7	744.9	549.2	0.64949	0.74
ZNF296	104.6	91.6	91.2	67.3	0.41866	0.74
RFC4	244.2	234.8	235.9	174.1	0.48661	0.74
C14orf2	347.7	368.8	400.3	296	0.24205	0.74
NDUFC1	496.9	504.9	685.7	507.2	0.41866	0.74
DDX21	1182.2	1281.7	1521.6	1126	0.27439	0.74
SNRPG	4362.2	4548	5128.9	3805.9	0.30688	0.74
CSTA	334.7	363.3	602.2	447.8	0.50781	0.74
MCM3	727.6	696.2	600.9	446.9	0.22937	0.74
GEMIN6*	341	349.7	324.1	241.2	0.28788	0.74
MTF1*	283	271.6	344.1	256.1	0.41866	0.74
TIMELESS	160.6	137.3	112.8	84	0.28813	0.74
TMEM106C	323.9	327.4	434.1	323.3	0.67432	0.74
MCM4	413.4	377.5	304.9	227.1	0.0182	0.74
TMEM138	171.1	179.1	222.6	165.9	0.57235	0.75

MRPS17*	650.4	675.9	676.6	504.7	0.72795	0.75
PNQ1*	176.2	171	172.3	128.6	0.51517	0.75
MCM5*	302.9	274.7	233.4	174.5	0.22125	0.75
SNORA67	273.1	233.4	186	139.2	0.56253	0.75
MTHFS	255.2	247.3	290.2	217.7	0.99999	0.75
ALDH18A1	231.6	264.7	280.4	210.8	0.56744	0.75
TMEM167A	166.7	192.7	257.4	193.6	0.87637	0.75
ANAPC10	191.3	237.6	300.5	226.2	0.99999	0.75
DLL3	135.6	103.8	109.3	82.3	0.62107	0.75
YWHAE	413.5	482.2	642.8	484.1	0.8522	0.75
C4orf27	146.4	162.8	194.7	146.7	0.1132	0.75
DKC1*	1062.9	1003.1	976.8	736.2	0.0767	0.75
NR2C2AP	146.8	145	117.5	88.7	0.4536	0.75
WDR77*	222.4	236	246.5	186.4	0.0963	0.76
BLOC1S1	390.3	411.4	372.7	282.2	0.99999	0.76
PLD1	323.7	333.6	427.6	323.8	0.35165	0.76
RPA3	1077.8	1036.4	1082.3	819.7	0.44108	0.76
PDSS1	148.5	147.6	132.3	100.2	0.96983	0.76
RRM1	1057.6	1133.6	1189.1	901	0.35589	0.76
PTPN11*	807	863.6	1078.7	817.4	0.1066	0.76
FAM36A	175.2	180.9	191.3	145	0.13549	0.76
CIRH1A	654.9	622.7	638.8	484.7	0.19189	0.76
SFRS3	114	123.5	129.1	98	0.71775	0.76
LSM3	1273.4	1343.6	1447.4	1098.9	0.40937	0.76
NEK6*	156.7	98.4	106.8	81.1	0.42954	0.76
DLGAP5	119.4	125	93.2	70.8	0.52983	0.76
FEN1	374.7	336.9	302.7	230.1	0.35456	0.76
CCDC58	113.7	109.9	74.7	56.8	0.99999	0.76
KNTC1	100.2	96.2	93.2	70.9	0.72795	0.76
NIP7	892.4	917.2	950.8	723.4	0.0563	0.76
KIF20A	139	146.5	99.7	75.9	0.50694	0.76
AP2B1	156.6	164.2	219.8	167.6	0.48118	0.76
SHKBP1	156.1	155.2	208.9	159.3	0.18357	0.76
PLP2	383.2	383.5	510.8	389.7	0.53852	0.76
APOBEC3C	176.2	191.9	217.4	165.9	0.1257	0.76
IFI44L	174.7	179.9	181.1	138.3	0.28788	0.76
PRC1	592.1	554.6	462.9	353.8	0.31024	0.76
POLR2L	256.5	242.7	220.6	169.3	0.23997	0.77
SEPW1	1352.5	1416.7	1742.9	1339	0.32763	0.77
ARHGDIB	1492.4	1393.5	1422.4	1094.1	0.0704	0.77
DRAM1*	215.8	196.1	245.7	189.1	0.13563	0.77
LSM5	664.8	682.2	708.5	545.5	0.84619	0.77
PRKRIR	413.9	469	486.5	374.6	0.50781	0.77
CINP	107.1	110.3	132.8	102.5	0.38468	0.77
NUP107	225.1	246.3	257.4	198.8	0.6296	0.77
MCM6	486.4	466.7	393.3	303.8	0.0767	0.77
UBL5	1051.9	1031.8	1203.3	929.6	0.73384	0.77
SDHD	182.9	239.8	265.4	205.1	0.99999	0.77
MFSD1	347.9	397	505.3	390.6	0.99848	0.77
PAK6	177.3	176	204	157.7	0.19884	0.77
SKP2	218.4	223.1	203.7	157.5	0.46789	0.77
SNHG9	487.1	396.7	328.1	253.9	0.78512	0.77
MT1F	507.1	353.8	534.3	413.5	0.0731	0.77
GNAI3*	130.6	133.3	162.1	125.5	0.47058	0.77
ARID3A*	218.4	209.4	288.9	223.7	0.17705	0.77
OAS3	249.1	237.8	377.1	292	0.95534	0.77

UCHL1	135.8	144.5	203.6	157.7	0.21028	0.77
MTHFD1	102.3	91.6	78.6	60.9	0.8228	0.77
FAM83D	115	111.1	78.2	60.6	0.99999	0.77
LYRM7*	144.4	132.2	130.2	100.9	0.99999	0.77
CRLF3*	111.5	90.7	133.8	103.7	0.7126	0.78
TMEM199*	112.1	100.4	120.1	93.1	0.96289	0.78
SNX12*	119.5	100.5	121.7	94.4	0.50743	0.78
TRIM22	161.7	217.5	234.4	181.9	0.37492	0.78
HSPA1A	346.6	277.8	351.3	272.7	0.99999	0.78
WDR55*	214.9	179.2	223.5	173.6	0.75279	0.78
GTF2E1	103.5	117.8	146.1	113.6	0.99999	0.78
CCDC34	165.1	143.3	121.9	94.8	0.41289	0.78
PTTG3P	457	435.3	394	306.5	0.72514	0.78
SRP19*	1087.4	1140.6	1447.8	1126.8	0.34034	0.78
C10orf2	150.1	143.2	119.3	92.9	0.65635	0.78
PRMT6	156.8	164.8	156.3	121.8	0.99999	0.78
DYNLL1	3541.8	3820.6	4504.6	3512.2	0.28639	0.78
CHEK1*	132.1	127.7	124.7	97.3	0.48326	0.78
WDR12*	349.6	364.5	345.5	269.7	0.54053	0.78
FKBP4	315.6	294.5	306.3	239.3	0.53852	0.78
C10orf35	160.1	129.8	176.8	138.2	0.33509	0.78
P15RS	734.5	791.7	890.7	696.7	0.0847	0.78
PAN3	191.6	231.2	306.8	240.1	0.6003	0.78
C9orf114*	191.2	166.3	181.1	141.9	0.99999	0.78
NUDT9*	102.5	107.3	129	101.1	0.65478	0.78
GPATCH4	166.4	158.9	147.5	115.6	0.7076	0.78
HARS2	439.2	501.7	632.1	495.5	0.64657	0.78
TSEN2	169.4	161.3	121.8	95.5	0.98712	0.78
ANKRD54	118.7	117.1	120	94.1	0.54519	0.78
ALKBH2	177	149.3	138.1	108.4	0.46208	0.78
BCS1L	196.5	195.9	175.5	137.8	0.99999	0.79
POLD2	356.4	359.8	335.3	263.4	0.8823	0.79
ORC5L	122.5	131.2	132	103.7	0.90968	0.79
PPIH	465.9	399.8	392.7	308.7	0.21028	0.79
HSPE1	904.9	894.6	794.5	625.1	0.6554	0.79
EXOC2	118	107.6	147.8	116.3	0.67834	0.79
TFRC*	1162.6	1013.5	1265.9	996.7	0.57465	0.79
C8orf59	822.6	826.5	840	661.5	0.99999	0.79

^AAverage signals of top 200 differentially expressed genes in cells transfected with miR-10a-5p mimics as compared to control-transfected cells in IL- β -stimulated condition. Top 200 gene list is generated based on fold difference (FD).

^BStar (*) designates genes predicted to be miR-10a-5p targets.

Table S3. miRNA-10a-5p downregulates genes associated with cell cycle regulation in KCs

P-value ^A	Nr of genes in functional group	Nr of genes in input list	Overlap	Functional Group ID	Functional group name	Downregulated genes
7E-18	1799	181	63	GO:0007049	Cell cycle	ANLN,TACC3,ZMYND11,GNAI3,TRIP13, MCM2, MCM6,TPX2,DYNLL1,MCM5, CDKN3,VRK1,CINP,FAM83D,MCM4,LIG1, RPA3,POLD2,YWHAE,NCAPG,NUP107, TIMELESS,MCM3,KIF20A,ARID3A,NEK6, HJURP,CKS2,PDCD2L,DLGAP5,GINS2, TOP2A, TRIM21,PCNA,CDCA8,NUSAP1,PAK6,CEP55, HERC5,CCNA2,SKP2,CDCA5,CHEK1,SPC24, RFC4,MAD2L1,ANAPC10,PTTG1,ORC5L, CENPN,MCM7,BLCAP,FEN1,IL8,CKS1B, UBE2C,CDC26,CRLF3,PTPN11,KNTC1,PRC1, HSPA1A,KIFC1
3.8E-10	293	133	23	REAC:69620	Cell cycle checkpoints	MCM2,MCM6,DYNLL1,MCM5,MCM4, RPA3,YWHAE,NUP107,MCM3,CDCA8, CCNA2,CHEK1,SPC24,RFC4,MAD2L1, ANAPC10,ORC5L,CENPN,MCM7, UBE2C,CDC26,KNTC1,HIST1H4C
1	1340	181	6	GO:0007155	Cell adhesion	TFRC,HAS3,ARHGDIB,CSTA,IL8,PTPN11
1	826	133	10	REAC:1280215	Cytokine signaling in Immune system	OAS3,NUP107,IFIT3,TRIM21,TRIM22, XAF1,HERC5,IL8,PTPN11,IFIT1
1	474	133	3	REAC:190236	Signaling by FGFR	POLR2L,PTPN11,MAF1
1	1346	42	12	HP:0011121	Abnormality of skin morphology	ALDH18A1,GNAI3,TRIP13,UBE2T, YWHAE,CSTA,DKC1,PCNA,PDSS1, CKAP2L,PTPN11,RPS26
1	137	181	3	GO:0051092	Positive regulation of NF-kappaB transcription factor activity	TRIM22,HSPA1A,RAB7B
1	1626	181	12	GO:0042127	Regulation of cell proliferation	TFRC,CDKN3,RPA3,MED31,WDR77, IFIT3,CCNA2,SKP2,CHEK1,IL8,PRC1, HSPA1A
1	640	181	8	GO:0071363	Cellular response to growth factor stimulus	CSTF2,MT1G,CCNA2,MCM7,IL8, POLR2L,PTPN11,HSPA1A
1	1253	181	8	GO:0060429	Epithelium development	AP2B1,DLL3,MTHFD1,TIMELESS, WDR77,CSTA,PCNA,CBR1
1	1495	181	8	GO:0048870	Cell motility	ANLN,PLD1,FAM83D,YWHAE, ARHGDIB,PAK6,IL8,PTPN11

^AThe *P*-value from Fisher exact test showing the significance of the overlap between the target list and indicated functional group.

Table S4. Selected genes predicted to be miR-10a-5p direct targets among top 200 genes downregulated by miR-10a-5p^A

SYMBOL	Site type ^B									Context score ^C
	conserved ^B				Unconserved					
	total	8mer	7mer-m8	7mer-A1	total	8mer	7mer-m8	7mer-A1	6mer	
CRLF3	1	1	0	0	1	0	1	0	1	-0.73
TFRC	1	1	0	0	1	0	0	1	0	-0.59
ZMYND11	1	0	1	0	0	0	0	0	1	-0.34
HAS3	1	0	1	0	1	0	1	0	0	-0.33
CMPK1	0	0	0	0	1	0	1	0	0	-0.23
NEK6	0	0	0	0	2	0	2	0	0	-0.22
GINS2	1	1	0	0	1	0	1	0	0	-0.16
CDCA5	0	0	0	0	1	0	0	1	1	-0.13
NUSAP1	0	0	0	0	1	0	1	0	0	-0.12
CENPN	0	0	0	0	2	0	2	0	0	-0.05
GNAI3	0	0	0	0	1	0	1	0	0	-0.05
ARID3A	0	0	0	0	1	0	0	1	5	-0.01
PTPN11	0	0	0	0	1	0	0	1	0	-0.01
CHEK1	0	0	0	0	1	0	1	0	0	0

^AOnly genes presented in Figure 3D heatmaps are shown. HAS3, a gene subjected to additional analysis is marked in bold.

^BConserved = conserved across most of the mammals, but usually not beyond placental.

^CThe context+ score for a specific site is the sum of the contribution of six features, including site-type, 3' pairing, local AU, position, target-site abundance and seed-pairing stability contributions

Table S5. Overlap between top 200 genes downregulated by miR-10a-5p^A and changed in the lesional skin of AD patients

SYMBOL ^B	Average signal				miR-10a+IL-1 β / cont+IL-1 β		Differentially expressed in AD ^C	
	control	miR-10a	control+ IL-1 β	miR-10a+ IL-1 β	Differential P-val	FD	RNASeq FD	Array FD
CCNA2	217.4	226.7	199.4	133.3	0.381	0.67	1.55	2.08
CDCA5*	345.7	295.6	217.3	155.9	0.067	0.72	2.46	2.57
CDKN3	224.6	231.3	217.1	130.1	0.061	0.6	2.15	3.15
CENPN*	201.1	178.9	167.1	122.4	0.163	0.73	1.96	2.36
CEP55	261	266.8	273.3	164.8	0.094	0.6	2.33	2.28
CKAP2L	130.2	114.7	87.5	57.6	0.076	0.66	1.84	2.27
DLGAP5	119.4	125	93.2	70.8	0.529	0.76	2.69	2.54
GIN52*	287.5	225.2	200	135.3	0.324	0.68	1.31	2.06
GPATCH4	166.4	158.9	147.5	115.6	0.707	0.78	1.59	2.06
HAS3*	566.3	445.8	566.6	374.3	0.015	0.66	1.73	4.76
HJURP	113.5	108.7	90.8	62.8	0.462	0.69	2.10	2.19
KIF20A	139	146.5	99.7	75.9	0.506	0.76	2.08	3.11
NCAPG	210.8	209.7	148.2	107.5	0.189	0.73	1.77	2.62
NUSAP1*	142.8	140.2	128.8	90.5	0.055	0.7	1.60	2.48
SLC38A5	231.9	247.2	314	206.4	0.011	0.66	2.01	1.29
SPC24	105.6	92.4	70.5	47.6	0.999	0.68	2.34	1.04
TACC3	139.2	130.5	90.9	61.2	0.096	0.67	1.88	2.22
TK1	314.5	295	285.7	196.1	0.214	0.69	1.56	2.20
TRIP13	307.2	273.2	229.4	158.2	0.009	0.69	2.39	2.20
UBE2C	392.9	345.4	343.9	210.5	0.065	0.61	1.73	2.11
XAF1	138	141.8	168	121.8	0.127	0.73	2.01	1.72

^AAverage signals of top 200 differentially expressed genes in cells transfected with miR-10a-5p mimics as compared to control-transfected cells in IL- β -stimulated condition. Top 200 gene list is generated based on fold difference (FD).

^BStar (*) designates genes predicted to be miR-10a targets. Genes associated with the regulation of cell cycle (GO:0007049) are designated with bold.

^CDifferentially expressed genes according to (Suárez-Fariñas et al, J Allergy and Clin Immunol 2015, Reference 34 in the main manuscript fail)