SUPPLEMENTARY MATERIALS AND METHODS

Lentivirus used for knockdown experiments

Four shRNAs with IDs TRCN0000057393, TRCN0000057394, TRCN0000057395, and TRCN0000057396 (abbreviated as sh-1, sh-2, sh-3, and sh-4, respectively) were used to generate individual stable galectin-7 knockdown HaCaT pooled cell lines.

Lentivirus-encoded shRNAs against human JNK1 and ΔNP63 were obtained from the National RNAi Core Facility (clone IDs TRCN0000352648, TRCN0000342576, TRCN0000342626, and TRCN0000352709 for JNK1; TRCN0000006503, TRCN0000006504, TRCN0000006505, and TRCN0000006506 for ΔNP63).

MicroRNA microarray and small-RNA deep-sequencing analysis of keratinocytes

RNAs for microarray and deep-sequencing analyses were extracted using the TRIzol reagent (Invitrogen, CA, USA). Human miRNA microarray (Agilent, CA, USA), array type 31181V16.0 with 1205 human and 144 human viral miRNAs represented) was used to profile the miRNA expression pattern in HaCaT cells. The data were analyzed using the Agilent Genespring software.

Small-RNA deep sequencing was performed using pooled RNAs from two samples. We pooled equal amounts of RNA from HaCaT parental and empty-vector-transfected cells for use as

controls (abbreviated as P/V). This procedure was utilized for galectin-7 knockdown cells, and RNAs from the clones sh-1 and sh-3 were pooled for analysis (abbreviated as sh-1/sh-3). Small RNAs from the pooled samples of P/V and sh-1/sh-3 were sequenced using the Illumina Solexa Sequencing platform. After removal of low-quality reads and adapters, the remaining clean reads were subjected to bioinformatic analysis. A total of 10,929,993 and 13,404,249 small RNAs were sequenced in P/V and sh-1/sh-3, respectively. After mapping back to the genome and miRbase, we identified 631 miRNAs in P/V and 632 miRNAs in sh-1/sh-3 out of 1539 annotated miRNAs (mirBase V. 16.0).

RNA extraction, mRNA, and miRNA real-time PCR analysis

RNAs from each HaCaT cell clone were extracted using the TRIzol reagent (Invitrogen). To generate cDNA for real-time PCR, we used the iScript cDNA synthesis kit (Bio-Rad; Hercules, CA, USA). Real-time PCRs were performed using TaqMan buffer (Roche; Indianapolis, IN, USA); the primers and probes were designed based on the Roche Universal Library (UPL).

We used the following forward primers, reverse primers, and probes: galectin-7 (NM_002307): forward primer 5′-cagacgacggcttcaagg-3′, reverse primer 5′-aagatcctcacggagtccag-3′, and probe #10 (cat. # 04685091001); *JNK-1* (NM_139049.1): forward primer 5′-gggcagccctctccttta-3′, reverse primer 5′-cattgacagacgacgatgatg-3′, and probe #89 (cat.

04689143001); *JNK2* (NM_139068.2): forward primer 5′-tgatattccaaggcactgacc-3′, reverse primer 5′-tcttcatgaactctgctgatgg-3′, and probe #33 (cat. # 04687663001); *GAPDH* (NM_002046): forward primer 5′-agccacatcgctcagacac-3′, reverse primer 5′-gcccaatacgaccaaatcc-3′, and probe #33 (cat. # 04687663001); *KRT1* (ENSG00000167768.3): forward primer 5′-tctgggggtgtcaagtcct-3′, reverse primer 5′-tccggaataagtggtagaaacaa-3′, and probe #87; and *KRT10* (ENSG00000186395.5): forward primer 5′-ggtggctatggaggattagga-3′, reverse primer 5′-cccaccaaagctgctacttc-3′, and probe #22. Real-time PCR was performed on an ABI 7500 cycler, and the data were analyzed using the SDS software and ΔΔCt method with normalization to *GAPDH* as the housekeeping gene.

For miRNA real-time PCR analysis, we used Mir-X miRNA First-Strand Synthesis Kit (Clontech; Mountain View, CA, USA) and SYBR qRT-PCR (Applied Biosystems; Foster City, CA, USA). Reverse transcription and PCR were performed according to the manufacturer's instructions. The primers for miR-146a and miR-203 were based on their mature sequences in miRbase (V16.0). Real-time PCR was performed on the ABI 7500, and relative quantification was performed by means of the ΔΔCt method with normalization to the *U6* endogenous control in the SDS 2.1 software.

Immunoblot, immunofluorescence staining, and immunoprecipitation analyses

Lysates from different HaCaT cell clones were extracted, and aliquots containing 30 µg of protein were analyzed using either 10% or 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The polyvinyl difluoride immobilin H-bond membrane (EMD Millipore, Armstadt, Germany) was used for immunoblot analysis. The primary antibody (against galectin-7) from Novagen (Madison, WI, USA) was used as described previously (Madsen *et al.*, 1995). The anti-ΔNp63 antibody was from Cell Signaling Technology, MA, USA), the anti-KRT1 antibody was purchased from Covance, CA, USA, and the anti-KRT-10 (Mab-LH2) antibody was purchased from Santa Cruz Biotechnology, USA. A rabbit monoclonal anti-JNK1/2 (EPR140) antibody was purchased from Epitomics, CA, USA. The JNK1-tGFP and pCI-HisHbi vectors were purchased from Origene (clone Id: RG218407) and Addgene (ID: #31815). To immunoprecipitate JNK1-tGFP, transfected cells were lysed in RIPA buffer (50 mM Tris-HCl, pH 7.4, 15 mM NaCl, 0.25% deoxycholic acid, 1% NP-40, 1 mM EDTA) with 1 mM PMSF, 1 mM sodium orthovanadate, 1 mM sodium fluoride, and protease inhibitor cocktail. Mouse anti-tGFP 2H8 (TA183081) and anti-His-tag (27E8) antibodies from Origene and Cell Signaling, USA, were used according to the previously described protocol (Young *et al.*, 2011).

To perform calcium-induced HaCaT cells differentiation, we followed the protocol described in (Deyrieux and Wilson, 2007; Pleguezuelos and Kapas, 2006) with slight modifications. Briefly, we cultured cells at a sub-confluent level in medium with the calcium concentration of 0.02 mM

or 1.8 mM for 7 days. Immunoblot analyses were done using antibodies against keratin-1, loricrin and filaggrin from Covance, USA; involucrin from Sigma, USA; and anti-KRT-10 from Santa Crus Biotechnology, USA.

The Epithelial-Mesenchymal Transition (EMT) Antibody Sampler Kit #9782 was purchased from Cell Signaling Technology, USA, and that included anti-E-cadherin, N-cadherin, claudin-1, β-catenin, SNAIL and SLUG antibodies.

Reconstitute human epidermis using HaCaT cells

To perform organotypic culture, HaCaT-v and HaCaT-sh1 clones were prepared as described in (Boelsma *et al.*, 1999; Schoop *et al.*, 1999) with slight modifications. Briefly, collagen gel with primary fibroblast Detroit 551 (a generous gift from Prof. Jin-Jer Lin, National Taiwan University, Taiwan) were used as a feeder layer of organotypic culture in a 24-well transwell. After 24 hour for solidification of collagen, HaCaT cells were seeded on the collagen well and incubated for another 24 hours. The transwells with HaCaT cells were lifted to the air and the medium on top of the collagen was removed. Cells in the transwells were cultured in submerged conditions for 7 days before 4 % Paraformaldehyde fixation, sucrose dehydration and cryosection. The organotypic section was then stained with 4,6-diamidino-2-phenylindole (DAPI) staining and L-PHA-FITC (Vector Lab, USA).

HEKn culture and siRNA knockdown of galectin-7

HEKn cells (C-001-5C) were purchased from Invitrogen, USA and cultured in serum free keratinocyte culture medium with supplement as described in (Koster et al., 2004). Silencer select negative control #1 siRNA and pre-designed siRNA targeting human galectin-7 (s230574-75-76) were purchased from Invitrogen. 30 pmole siRNA each were used per-well in 6 well plate setup. Lipofectamine 2000 (Invitrogen, USA) was used for transfection and the procedure we used is as described in manufacture's protocol.

Confocal imaging and high-content image analysis

The PerkinElmer UltraVIEW microscope system was used to acquire Z-stack images of HaCaT cells after immunofluorescent staining. After scanning with a 100× objective lens (Zeiss), the Z-stack images were analyzed by the Volocity software (PerkinElmer). To quantify the colocalization of JNK1 and ubiquitination signals, we used the Molecular Devices ImageXpress system with a 40× objective lens. The images were analyzed in the MetaXpress software.

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. The expression of differentiation and EMT markers, and organotypic culture of galectin-7 knockdown HaCaT cells. a) Immunoblot analysis of keratin-1, keratin-10, loricrin, filaggrin and involucrin expression in galectin-7 knockdown cells in response of different calcium concentrations in culture. (b) The expression of EMT markers (E-cadherin, N-cadherin, claudin-1, β-catenin, SNAIL and SLUG in galectin-7 knockdown HaCaT cells was examined by immunoblot analysis. (c) The IFA staining of reconstitute human epidermis of HaCaT cells using vector and sh-1 clone was conducted by using DAPI and L-PHA-FITC. Scale bar = 50μm.

Supplementary Figure 2. Galectin-7 knockdown HEKn cells keratinocytes exhibit reduced expression of differentiation markers keratin-1 and keratin-10 and a hyperproliferative phenotype. HEKn cells were treated with siRNA control (siNC) and three siRNA against galectin-7 (si-1, si-2 and si-3). (a) The mRNA levels of galectin-7 were measured by real-time PCR analysis. (b) The protein levels of galectin-7, and JNK1, keratin-1 and keratin-10 were analyzed by immunoblot analysis. (c) Time-lapse images. (d) The numbers of cells from individual colonies in each group. Scale bar = 50μm. All statistical analyses were performed by individually comparing with the siRNA control (siNC), ns: not significant, *P < 0.05, **P < 0.01, ***P < 0.001. (e and f) MiR-203 and miR-146a were quantified using real-time PCR.

Supplementary Figure 3. The expression levels of miR-203 in transient expression and

knockdown HaCaT cells. (a and b) HaCaT cells were transiently transfected with indicated plasmids. All experiments were performed at least in triplicate, and the statistical analyses were performed by individually comparing with HaCaT mock treatment cells, ns: not significant, **P < 0.01, ***P < 0.001.

Supplementary Figure 4. Increase of JNK1 and ubiquitination localization in galectin-7 knockdown cells. High-content image analysis was performed to quantify colocalization of JNK1 with anti-ub staining. Immunofluorescence for galectin-7, JNK1, and ubiquitin was processed using Molecular Devices High-content ImageXpress, and the data were analyzed in the MetaXpress software. Quantification of the percentage of colocalization was performed using 100 cells from galectin-7 knockdown stable cell clones and parental and empty-vector control cell lines (a). Images of staining for galectin-7, ubiquitin, JNK1, or nuclei (4, 6-diamidino-2-phenylindole; DAPI) in galectin-7 knockdown cell clones (sh-1 and sh-4) are presented in panel (b). ***P < 0.001. Scale bar = 50μm.

REFERENCES

Boelsma E, Verhoeven MC, Ponec M (1999) Reconstruction of a human skin equivalent using a spontaneously transformed keratinocyte cell line (HaCaT). *J Invest Dermatol* 112:489-98.

Deyrieux AF, Wilson VG (2007) In vitro culture conditions to study keratinocyte differentiation using the HaCaT cell line. *Cytotechnology* 54:77-83.

Madsen P, Rasmussen HH, Flint T*, et al.* (1995) Cloning, expression, and chromosome mapping of human galectin-7. *The Journal of biological chemistry* 270:5823-9.

Pleguezuelos O, Kapas S (2006) Differentiation of the HaCaT keratinocyte cell line: modulation by adrenomedullin. *Br J Dermatol* 154:602-8.

Schoop VM, Mirancea N, Fusenig NE (1999) Epidermal organization and differentiation of HaCaT Keratinocytes in organotypic coculture with human dermal fibroblasts. *J Invest Dermatol* 112:343-53.

Young JA, Sermwittayawong D, Kim HJ*, et al.* (2011) Fas-associated death domain (FADD) and the E3 ubiquitin-protein ligase TRIM21 interact to negatively regulate virus-induced interferon production. *The Journal of biological chemistry* 286:6521-31.

Table S1. Differentially expressed miRNAs in microarray analysis

	Fold Change		
miRs	**sh-1**	**Sh-3**	**Probe sequence of Mature miRs**
hsa-miR-146a	21.30	6.28	AACCCATGGAATTCAGTTC
hsa-miR-4270	5.80	2.88	GCCCTCCCCTGAC
hsa-miR-10b	4.62	1.90	CACAAATTCGGTTCTACAGGG
hsa-miR-203	-4.32	-2.80	CTAGTGGTCCTAAACATT

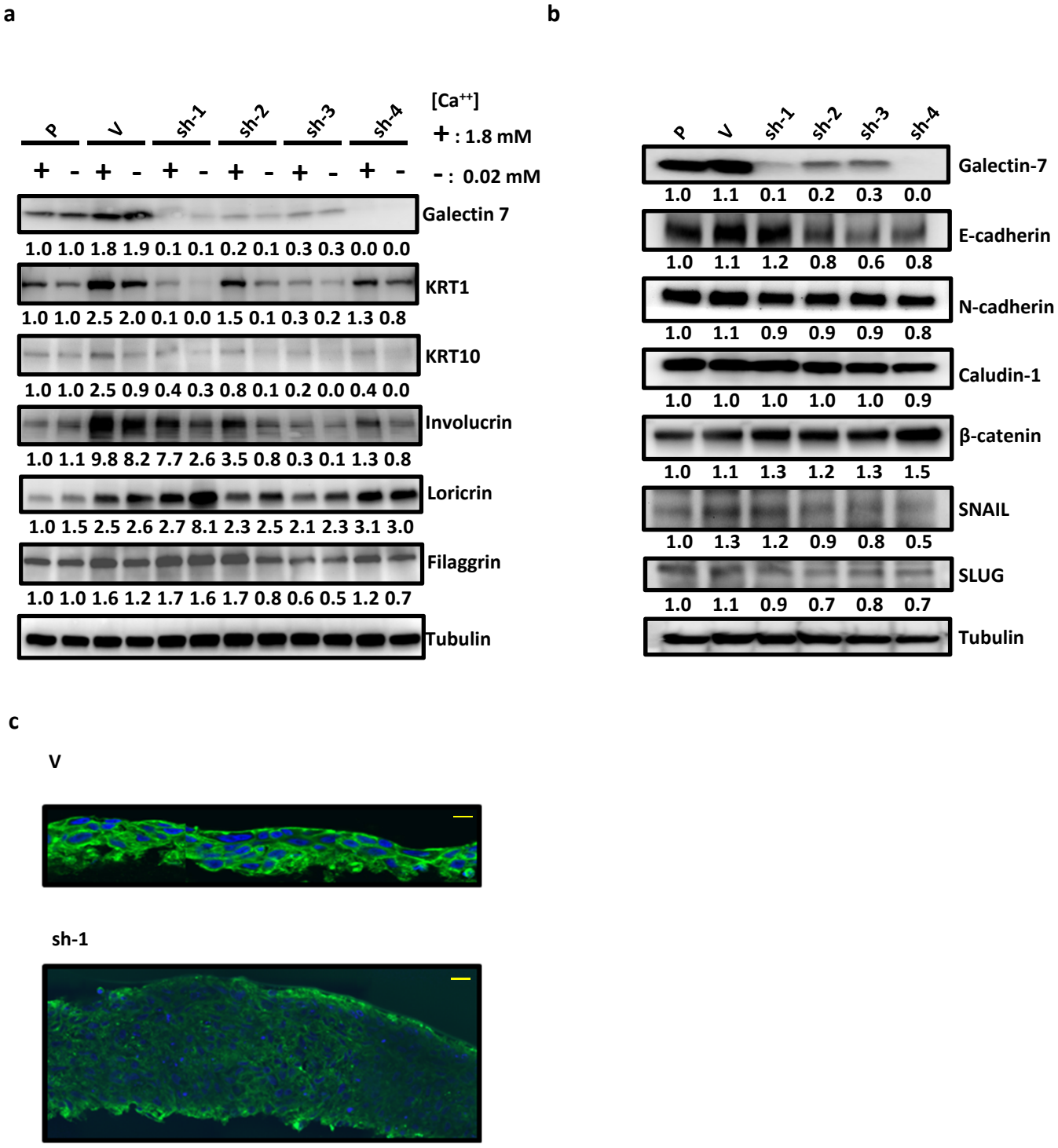
The differentially expressed miRNAs were identified by miRNA microarray in galectin-7 knockdown HaCaT cells (sh-1 and sh-3) compared with vector control cells. MicroRNAs with fold change in one of the galectin-7 knockdown cells greater than 4 are listed in the table. 221 miRNAs were detected in array for differential expression analysis out of 1368 entities (using Agilent array 31181 based on mirbase V16.0).

Table S2. Differentially expressed miRNAs in deep-sequencing analysis

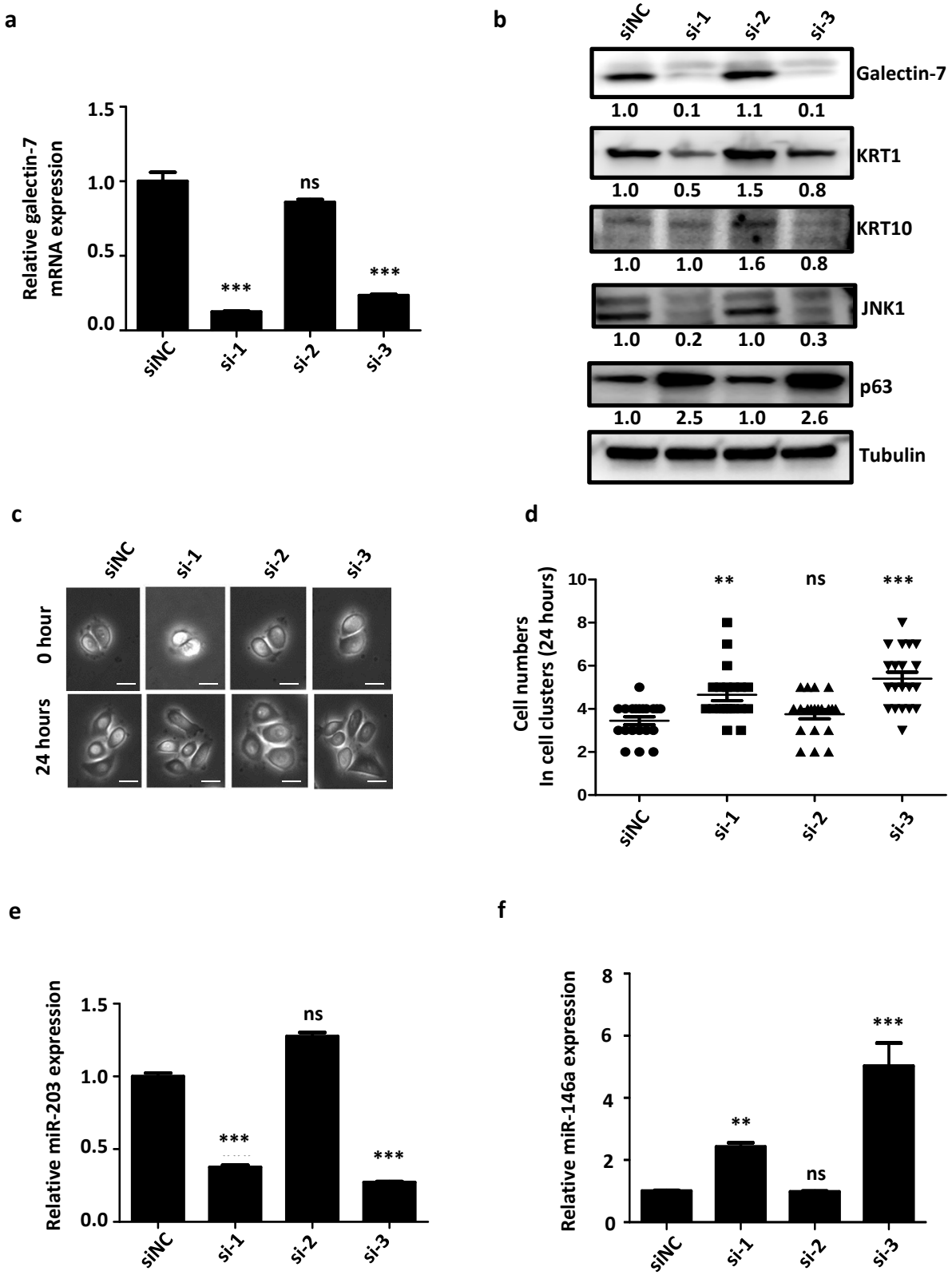
miRNA	Reads in HaCaT-P/V pooled cells	Reads in sh-1 and sh-3 pooled cells	Ratio of G7KD/control
hsa-miR-146a	2224	41950	18.86
hsa-miR-155	2278	8428	3.7
hsa-miR-10a	4117	9007	2.19
hsa-miR-9	639	1371	2.15
hsa-miR-125b	5581	11990	2.15
hsa-miR-222*	503	1008	2
hsa-miR-224	15101	6883	0.46
hsa-let-7c	28773	12305	0.43
hsa-miR-203	37485	15747	0.42
hsa-miR-452	23936	10066	0.42

Solexa illumina small RNA deep-sequencing analysis was used to detect miRNAs expression in HaCaT cell lines. Pooled samples of HaCaT-P and –V were used as control to compare with galectin-7 knockdown cells (sh-1 and sh-3 pooled cells). 258 miRNAs were identified mapped to mirbase (V16.0) and have reads over 100. The ratio of reads was calculated by dividing reads in G7KD by control. MicroRNAs with raw reads over 1000 and ratio over 2 or less than 0.5 are listed in this table.

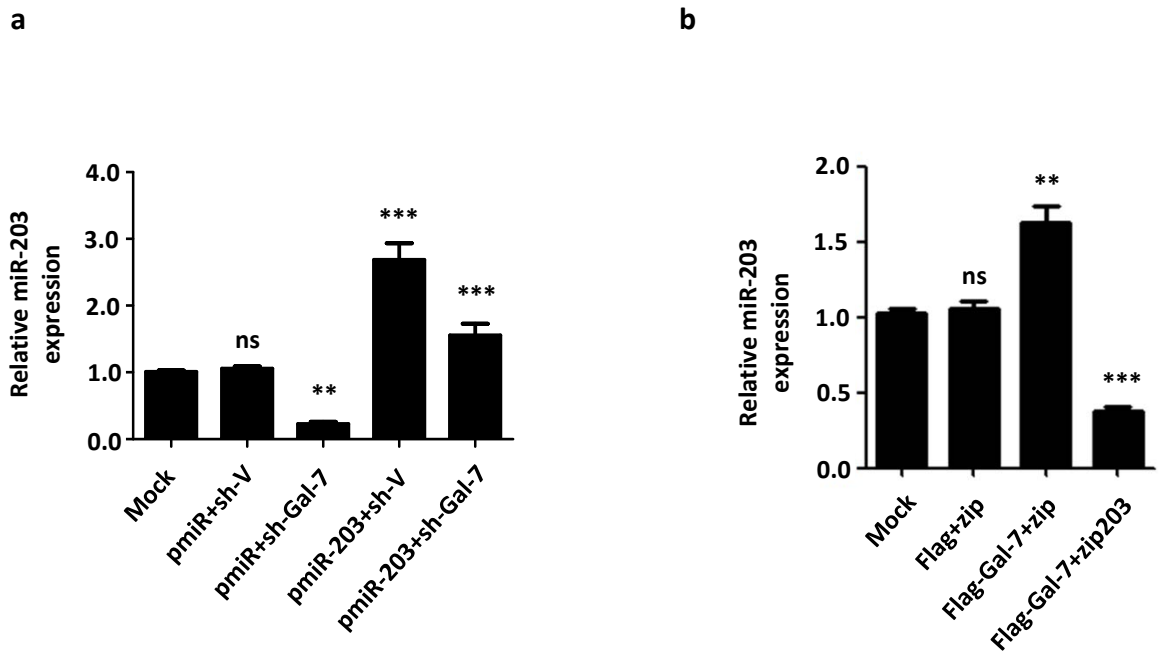
Supplementary Figure S1



Supplementary Figure S2



Supplementary Figure S3



Supplementary Figure S4