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## Data Article

# Fibroblast and keratinocyte gene expression following exposure to extracts of neem plant (*Azadirachta indica*)



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## ABSTRACT

This data article provides gene expression profiles, determined by using real-time PCR, of fibroblasts and keratinocytes treated with 0.01% and 0.001% extracts of neem plant (*Azadirachta indica*), local name “Kohomba” in Sri Lanka, harvested in Sri Lanka. For fibroblasts, the dataset includes expression profiles for genes encoding hyaluronan synthase 1 (HAS1), hyaluronan synthase 2 (HAS2), hyaluronidase-1 (HYAL1), hyaluronidase-2 (HYAL2), versican, aggrecan, CD44, collagen, type I, alpha 1 (COL1A1), collagen, type III, alpha 1 (COL3A1), collagen, type VII, alpha 1 (COL7A1), matrix metalloproteinase 1 (MMP1), acid ceramidase, basic fibroblast growth factor (bFGF), fibroblast growth factor-7 (FGF7), vascular endothelial growth factor (VEGF), interleukin-1 alpha (IL-1 $\alpha$ ), cyclooxygenase-2 (cox2), transforming growth factor beta (TGF- $\beta$ ), and aquaporin 3 (AQP3). For keratinocytes, the expression profiles are for genes encoding HAS1, HAS2, HYAL1, HYAL2, versican, CD44, IL-1 $\alpha$ , cox2, TGF- $\beta$ , AQP3, Laminin5, collagen, type XVII, alpha 1 (COL17A1), integrin alpha-6 (ITGA6), ceramide synthase 3 (CERS3),

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elongation of very long chain fatty acids protein 1 (ELOVL1), elongation of very long chain fatty acids protein 4 (ELOVL4), filaggrin (FLG), transglutaminase 1 (TGM1), and keratin 1 (KRT1). The expression profiles are provided as bar graphs.

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## Specifications Table

Subject area	<i>Biology</i>
More specific subject area	<i>Cell biology</i>
Type of data	<i>Graph</i>
How data was acquired	<i>Quantitative RT-PCR (LightCycler 96 system, Roche)</i>
Data format	<i>Analyzed</i>
Experimental factors	<i>Isolation of total cellular RNA, cDNA amplification, PCR analysis</i>
Experimental features	<i>Analysis of gene expression by quantitative RT-PCR</i>
Data source location	<i>Negombo, Sri Lanka</i>
Data accessibility	<i>Data are available within this article</i>

## Value of the data

- Data showing changes in gene expression levels in response to neem extract exposure are valuable for estimating effects of the extract on fibroblasts and keratinocytes.
- The data presented in this article showing that neem extract up- or down-regulates the expression of genes involved in epidermal and dermal cells could be important for investigations in pharmacology and cosmetics.
- The present data can be referenced by investigations into chemicals and natural medicines for the epidermal and dermal tissues.

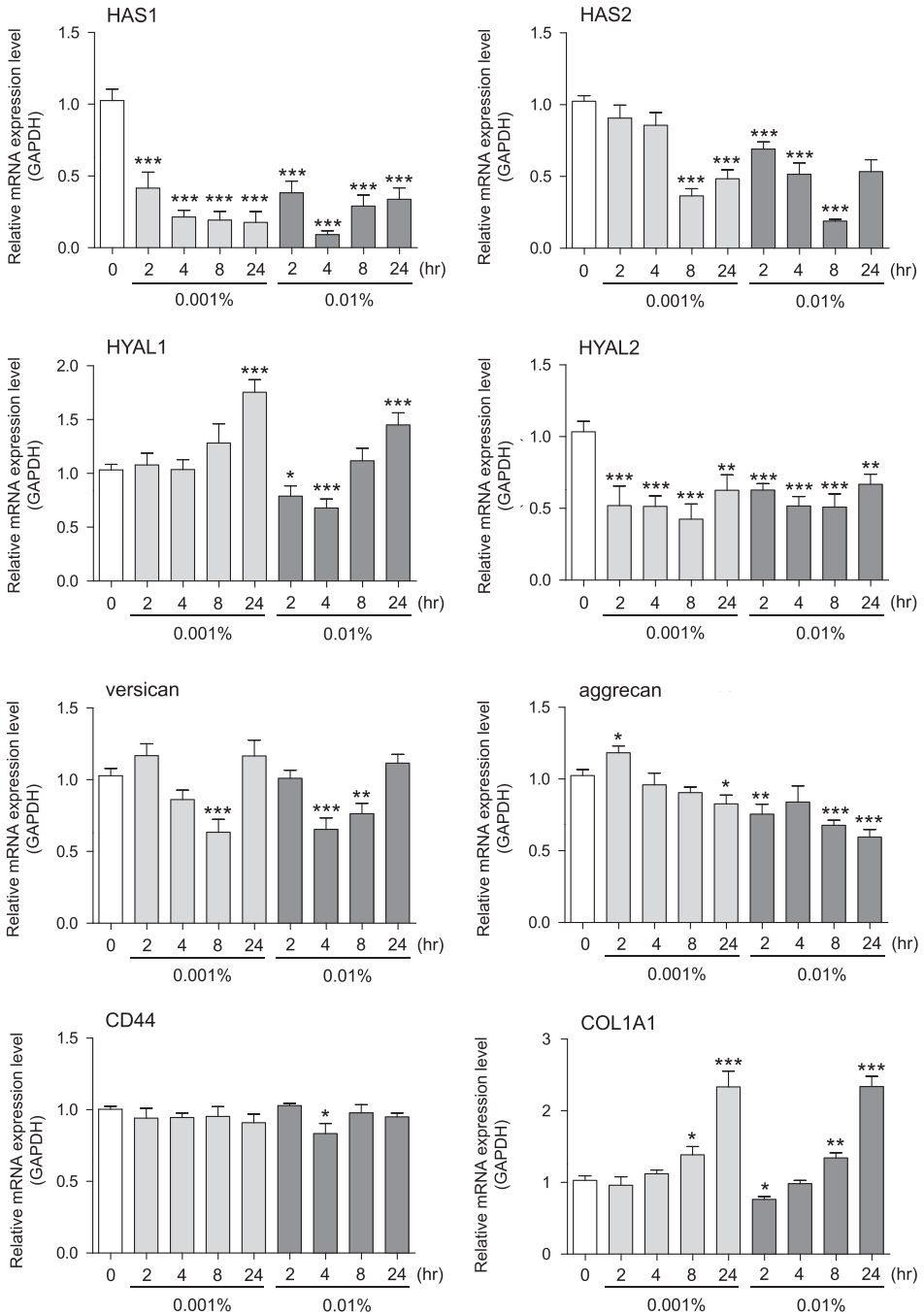
## 1. Data

This data article contains bar graphs showing gene expression levels in fibroblasts and keratinocytes in response to exposure to 0.01% and 0.001% neem plant (*Azadirachta indica*) extract, harvested in Negombo, Sri Lanka. For fibroblasts, the dataset includes expression profiles for genes encoding HAS1, HAS2, HYAL1, HYAL2, versican, aggrecan, CD44, COL1A1, COL3A1, COL7A1, MMP1, acid ceramidase, bFGF, FGF7, VEGF, IL-1 $\alpha$ , cox2, TGF- $\beta$ , and AQP3 (Fig. 1). For keratinocytes, the expression profiles are for genes encoding HAS1, HAS2, HYAL1, HYAL2, versican, aggrecan, CD44, IL-1 $\alpha$ , cox2, TGF- $\beta$ , AQP3, Laminin5, COL17A1, ITGA6, CERS3, ELOVL1, ELOVL4, FLG, TGM1, and KRT1 (Fig. 2). The data represent the mean  $\pm$  SE values from triplicate independent experiments (\* $P < 0.05$ , \*\* $P < 0.001$  and \*\*\* $P < 0.001$  vs. 0 time).

## 2. Experimental design, materials and methods

### 2.1. Materials

Neem plants (*Azadirachta indica*), local name “Kohomba” were harvested from a medicinal garden at the Institute of Traditional Plants in Sri Lanka (Negombo, Sri Lanka). The heat-treated plant leaves were extracted with 1,3-butylene glycol.



**Fig. 1.** Gene expression levels in fibroblast cells after exposure to neem extract. The mRNA expression levels were normalized to GAPDH expression, and the relative gene expression levels in the cells at 2, 4, 8, and 24 h after initiation of extract exposure were compared to the corresponding levels for unexposed cells, whose levels were defined as 1.0.

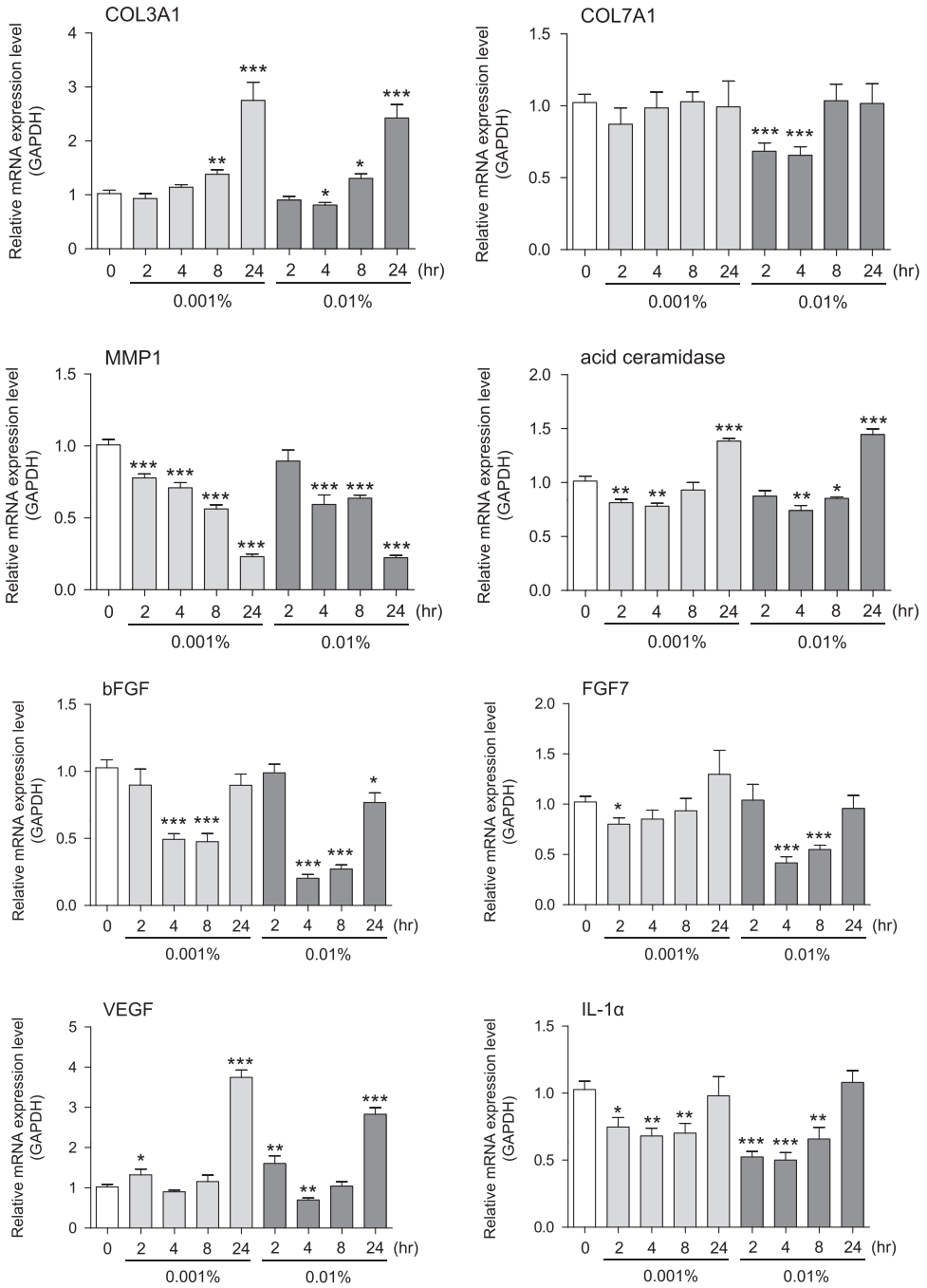


Fig. 1. (continued)

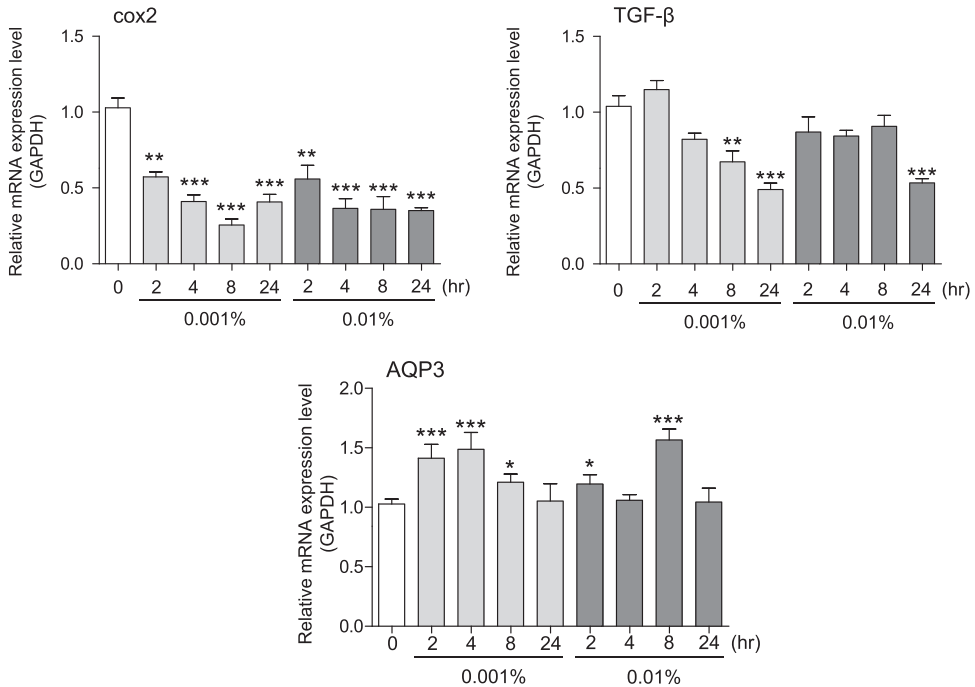


Fig. 1. (continued)

## 2.2. Fibroblast cell culture

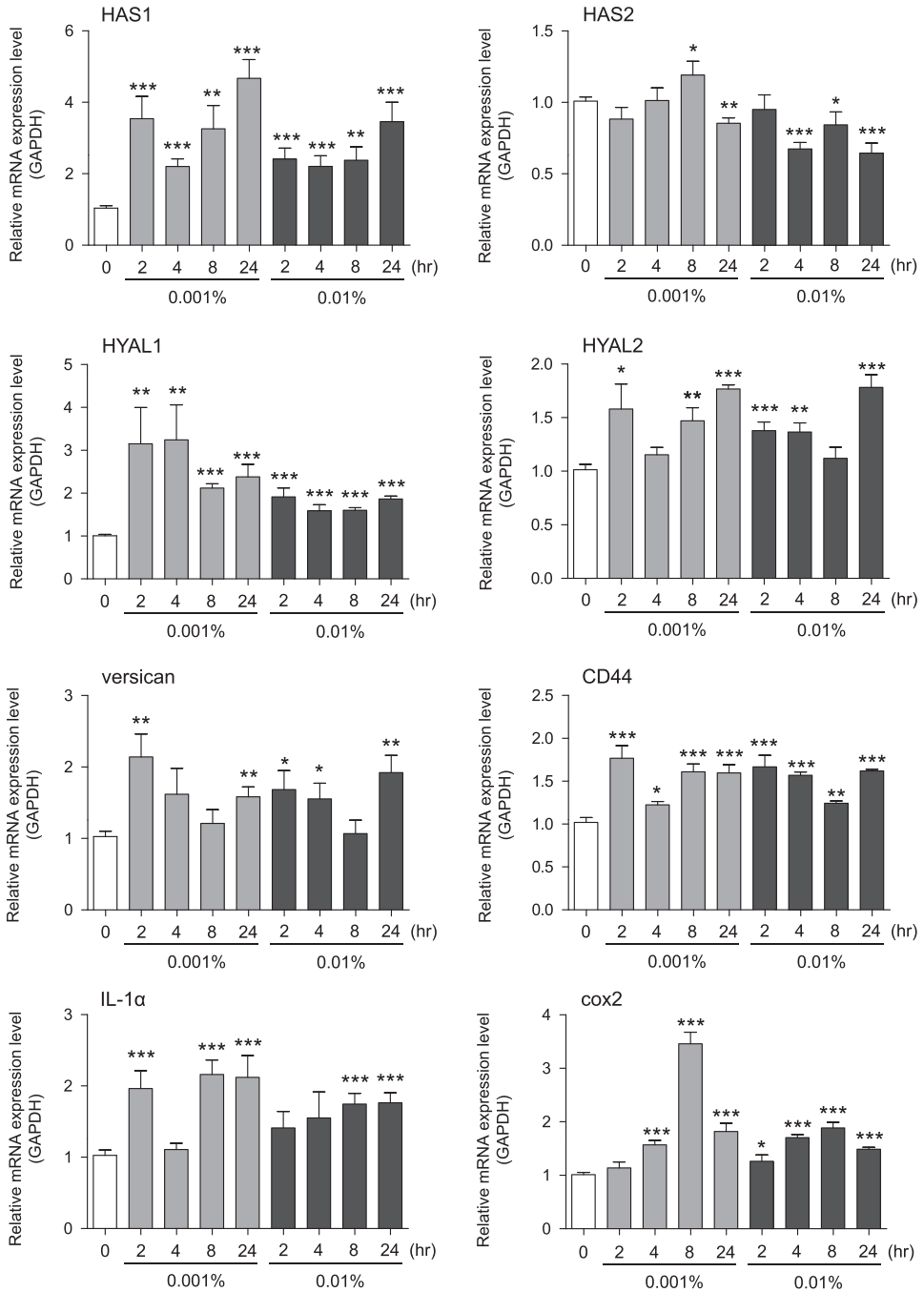
Normal human skin fibroblasts, RIKEN original (NB1RGB), were provided by the RIKEN BRC through the National Bio-Resource Project of the MEXT, Japan. The cells were cultured in Minimum Essential Media- $\alpha$  (MEM $\alpha$ ; Life Technologies Corp.) supplemented with 10% fetal bovine serum (FBS; Biowest) and 0.2% NaHCO<sub>3</sub>. Cells were grown at 37 °C in a humidified incubator containing 5% CO<sub>2</sub>, according to the manufacturer's instructions. For all of the experiments, human fibroblasts were seeded into a 60 mm dish ( $5 \times 10^4$  cells/dish) and incubated for 8 h with culture media containing 10% FBS. The cells were subsequently subjected to serum starvation for 16 h with serum-free MEM $\alpha$ .

## 2.3. Keratinocyte cell culture

Normal human epidermal keratinocytes (HEKn; GIBCO) were isolated from neonatal foreskin. The cells were cultured in Medium 154 (Invitrogen) supplemented with human keratinocyte growth factor (HKGS; Invitrogen), according to the manufacturer's instructions. Cells were grown at 37 °C in a humidified incubator containing 5% CO<sub>2</sub>. For all of the experiments, human keratinocytes were seeded into a collagen-coated 60 mm dish ( $5 \times 10^4$  cells/dish), and incubated for 8 h with culture media containing HKGS. The cells were next subjected to HKGS starvation for 16 h with Medium 154.

## 2.4. Exposure of the cells to plant extract, RNA isolation and quantitative real-time PCR

The cells were seeded into a 60 mm dish ( $5 \times 10^4$  cells/dish). Cells were exposed to 0.01% or 0.001% of plant extract, for 24 h at 37 °C. The cells were collected at 2, 4, 8, and 24 h after initiation of the exposure. Total RNA was extracted from the cells by using the TRI reagent (Merck). This RNA extract was used as a template for subsequent cDNA synthesis with oligo dT primers (Table 1), using the Primescript RT reagent Kit (Takara bio inc.). mRNA levels were quantified using a LightCycler 96 system (Roche) and SYBR Premix Ex Taq II (Takara Bio Inc.). The data were analyzed using the delta



**Fig. 2.** Gene expression levels in keratinocyte cells after exposure to neem extract. The mRNA expression levels were normalized to GAPDH expression, and the relative gene expression levels in the cells at 2, 4, 8, and 24 h after initiation of extract exposure were compared to the corresponding levels for unexposed cells, whose levels were defined as 1.0.

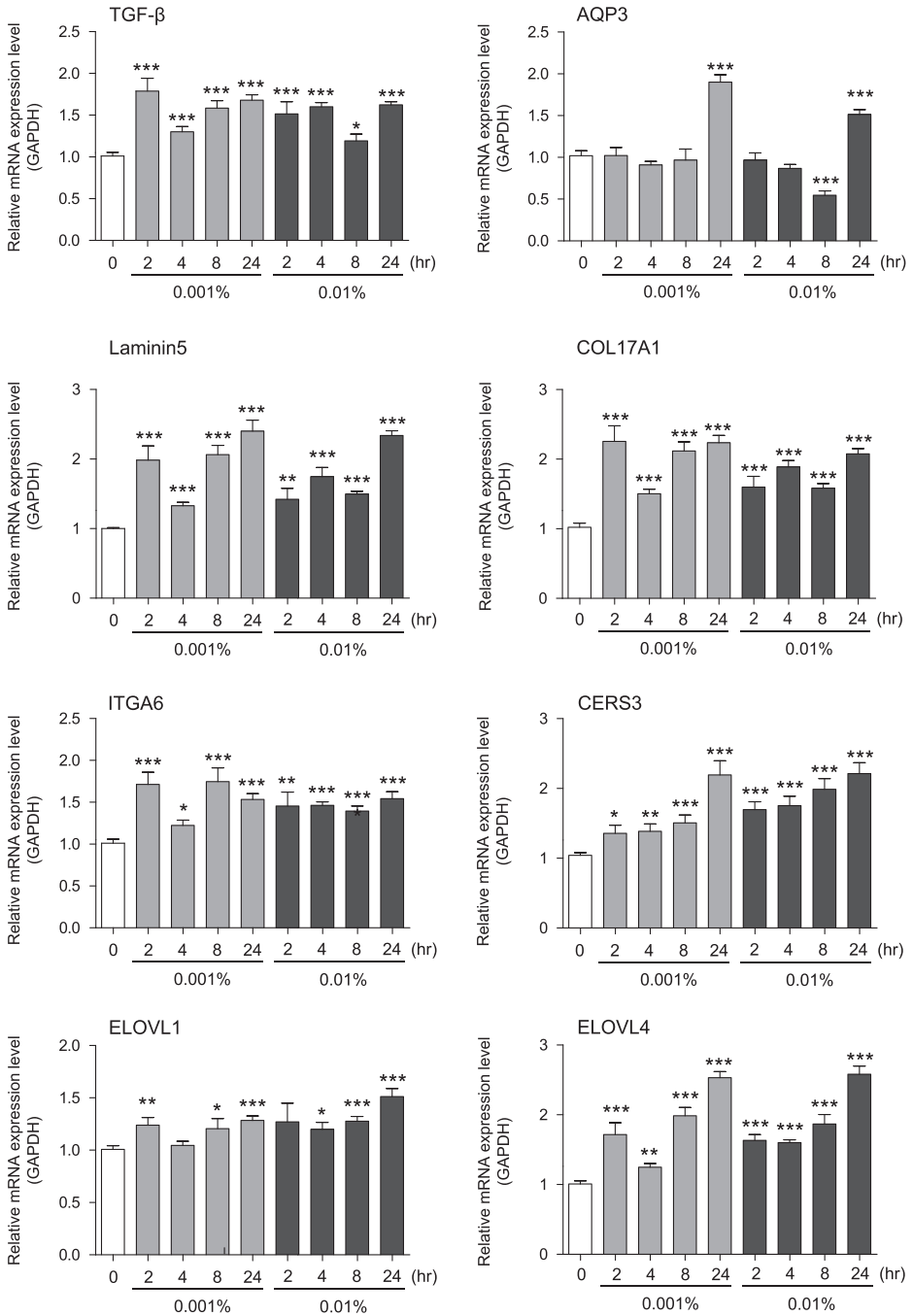


Fig. 2. (continued)

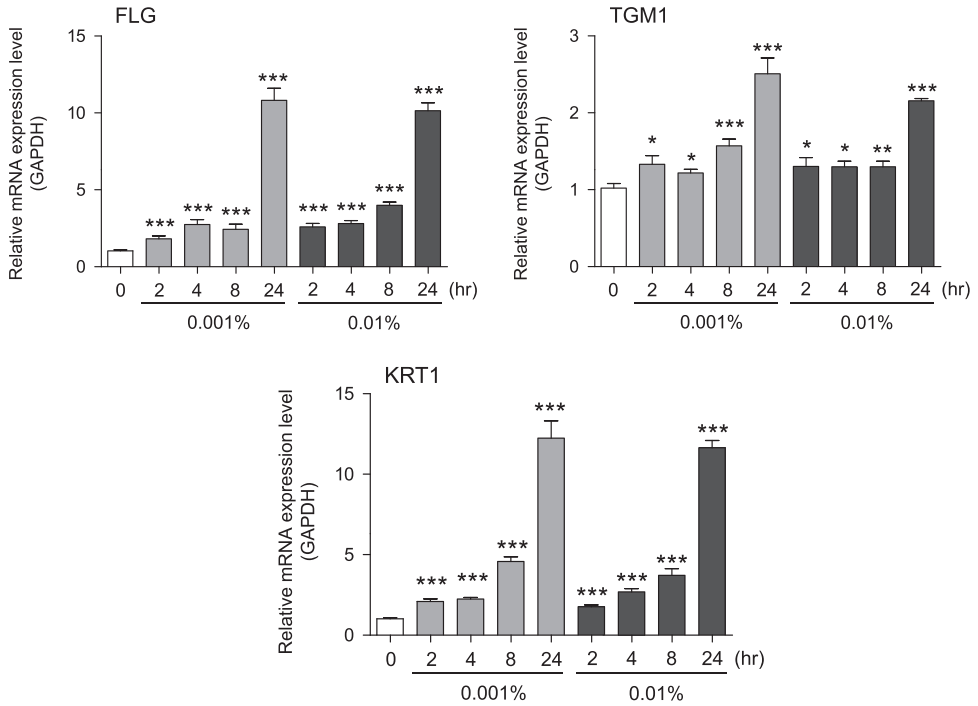


Fig. 2. (continued)

**Table 1**  
Nucleotide sequences of primers used in this study.

Primers	Sequences	Direction	References
<b>Quantitative real time-PCR</b>			
<i>HAS1</i>			
HAS1-F	3'-CGCTAACTACGTCCCTCTGC-5'	Sense	[1]
HAS1-R	3'-CCAGTACAGCGTCAACATGG-5'	Anti-sense	
<i>HAS2</i>			
HAS2-F	3'-GCCTCATCTGTGGAGATGGT-5'	Sense	[2]
HAS2-R	3'-ATGCACTGAACACACCCAAA-5'	Anti-sense	
<i>HYAL1</i>			
HYAL1-F	3'-CCAAGGAATCATGTACAGGCCATCAA-5'	Sense	[3]
HYAL1-R	3'-CCCCTGCTCACGTTCCAGG-5'	Anti-sense	
<i>HYAL2</i>			
HYAL2-F	3'-GGCTTAGTGAGATGGACCTC-5'	Sense	[3]
HYAL2-R	3'-CCGTGTCAGGTAATCTTTGAG-5'	Anti-sense	
<i>versican</i>			
VCAN 3-F	3'-TGAGAACCCTGTATCGTTTTGAGA-5'	Sense	[4]
VCAN 3-R	3'-CGTTAAGGCACGGGTTCAIT-5'	Anti-sense	
<i>aggrecan</i>			
ACAN-F	3'-TCGAGGACAGCGAGGCC-5'	Sense	[5]
ACAN-R	3'-TCGAGGGTGTAGCCTGTAGAGA-5'	Anti-sense	
<i>CD44</i>			
CD44-F	3'-GCTATTGAAAGCCTTGACAG-5'	Sense	[6]
CD44-R	3'-CGCAGATCGATTGTAATATAAC-5'	Anti-sense	
<i>COL1A1</i>			
COL1A1-F	3'-CACCAATCACCTGCGGTACAGAA-5'	Sense	[7]
COL1A1-R	3'-CAGATCACGTCATCGCACAAAC-5'	Anti-sense	



**Table 1** (continued)

Primers	Sequences	Direction	References
<i>COL3A1</i>			
COL3A1-F	3'-CCCCTATTATTTTGGCACAACAG-5'	Sense	[8]
COL3A1-R	3'-AACGGATCCTGAGTCACAGACA-5'	Anti-sense	
<i>COL7A1</i>			
COL7A1-F	3'-CTCAGCAGCTATCACCTGGAC-5'	Sense	[9]
COL7A1-R	3'-TGTCCACCACACGTAGTTCAA-5'	Anti-sense	
<i>MMP1</i>			
MMP1-F	3'-TGTGGTGTCTCACAGCTTCC-5'	Sense	[3]
MMP1-R	3'-CTTGCTCCCATCATTCTTC-5'	Anti-sense	
<i>acid ceramidase</i>			
acid ceramidase-F	3'-CGTACAGAGGTGCAGTTCCA-5'	Sense	original
acid ceramidase-R	3'-GTAGGCCAGGCAATTTTCA-5'	Anti-sense	
<i>bFGF</i>			
bFGF-F	3'-AGAGCGACCCTCACATCAAG-5'	Sense	[10]
bFGF-R	3'-ACTGCCAGTTCGTTTCAGT-5'	Anti-sense	
<i>FGF7</i>			
FGF7-F	3'-CATGAACACCCGGAGCACTAC-5'	Sense	[11]
FGF7-R	3'-CACTGTGTTCCAGACAAGAGTCTTC-5'	Anti-sense	
<i>VEGF</i>			
VEGF-F	3'-GGAGAGATGAGCTTCTACAG-5'	Sense	[12]
VEGF-R	3'-TCACCGCCTTGGCTTGCACA-5'	Anti-sense	
<i>IL-1<math>\alpha</math></i>			
IL-1 $\alpha$ -F	3'-TGGCTCATTTTCCCTCAAAGTTG-5'	Sense	[13]
IL-1 $\alpha$ -R	3'-AGAAATCGTGAATCCGAAGTCAAG-5'	Anti-sense	
<i>cox2</i>			
COX2-F	3'-TGAGCATCTACGGTTTGGCTG-5'	Sense	[14]
COX2-R	3'-TGCTTGTCTGGAACAACCTGC-5'	Anti-sense	
<i>TGF-<math>\beta</math></i>			
TGF- $\beta$ -F	3'-GCCCTGGACCAACTATTG-5'	Sense	[15]
TGF- $\beta$ -R	3'-GTCAGGCTCCAATGTAGG-5'	Anti-sense	
<i>AQP3</i>			
AQP3-F	3'-GTCACCTCTGGGCATCCTCAT-5'	Sense	[16]
AQP3-R	3'-TATTCAGCACCCAAGAAGG-5'	Anti-sense	
<i>Laminin5</i>			
Laminin5-F	3'-GCCTGGAGTACAACGAGGTC-5'	Sense	original
Laminin5-R	3'-AGTTGGCAAACCTGATGAGGAC-5'	Anti-sense	
<i>COL17A1</i>			
COL17A1-F	3'-CGAGACTTTCGACTACTCAGAGC-5'	Sense	original
COL17A1-R	3'-GAGGACGAGAACAAGCTGAC-5'	Anti-sense	
<i>ITGA6</i>			
ITGA6-F	3'-TCTCGCTGGGATCTTGATGC-5'	Sense	original
ITGA6-R	3'-CCTAGAGCGTTTAAAGAATCCAC-5'	Anti-sense	
<i>CERS3</i>			
CERS3-F	3'-TCTCTGCTGACTGCATCTATTG-5'	Sense	original
CERS3-R	3'-GAAGCCAGAATCTTCCAACC-5'	Anti-sense	
<i>ELOVL1</i>			
ELOVL1-F	3'-GGACTTCTCTGGCCCTG-5'	Sense	original
ELOVL1-R	3'-CGTGCTTCATCACCTCTTGG-5'	Anti-sense	
<i>ELOVL4</i>			
ELOVL4-F	3'-GATTCTCCCCTGTTACATC-5'	Sense	original
ELOVL4-R	3'-TTCAGACCCGAAGAATGAGTGAC-5'	Anti-sense	
<i>FLG</i>			
FLG-F	3'-GAAGGTGAAGGTCCGAGTC-5'	Sense	original
FLG-R	3'-GAAGATGGTGATGGGATTC-5'	Anti-sense	
<i>TGM1</i>			
TGM1-F	3'-CGAAGGCTCTGGGTACAGA-5'	Sense	original
TGM1-R	3'-TGTCACCTGTTTCATTCCTCC-5'	Anti-sense	
<i>KRT1</i>			
KRT1-F	3'-TGAGCTGAATCGTGTATCC-5'	Sense	original
KRT1-R	3'-CCAGGTCAITCAGCTTGTTCC-5'	Anti-sense	
<i>GAPDH</i>			
GAPDH-F	3'-GAAGGTGAAGGTCCGAGTC-5'	Sense	[17]
GAPDH-R	3'-GAAGATGGTGATGGGATTC-5'	Anti-sense	

cycle threshold method, and calculated based on the Cq values, and the expression of each gene was normalized to GAPDH. All values are reported as means  $\pm$  standard error, as previously described [18].

## Acknowledgements

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## Statistical analysis

All the values have been reported in terms of mean  $\pm$  SE values. The data were analyzed using the Student's *t*-test. A *P* value less than 0.05 was considered to be statistically significant.

## Transparency document. Supporting information

Transparency data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.dib.2017.12.035>.

## Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.dib.2017.12.035>.

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