Supplementary material

Supplementary Table S1 - Primer sequences and details of the cycling conditions and annealing temperatures

Gene	Forward primer (5' to 3')	Reverse primer (5' to 3')	Cycling conditions	Melting temperature (Tm)
VDR [1] Vitamin D receptor [1-3]	AGATGACCCTTCTGTGACCC	AGCTTCTTCAGTCCCACCTG	95°C for 15s 60°C for 15s 72°C for 20s	Forward: 59°C Reverse: 59°C
CEBPA [2] CCAAT/enhancer binding protein, alpha [2-3]	AGCCTTGTTTGTACTGTATG	AAAATGGTGGTTTAGCAGAG	95°C for 15s 60°C for 15s 72°C for 20s	Forward: 54.3°C Reverse: 58.3°C
CEBPB [2] CCAAT/enhancer binding protein, beta [2-3]	ATAAACTCTCTGCTTCTCCC	CCGTAGGAACATCTTTAAGC	95°C for 15s 60°C for 15s 72°C for 20s	Forward: 56.6°C Reverse: 57.9°C
CEBPD [2] CCAAT/enhancer binding protein, delta [2]	CAGACTTTTCAGACAAACCC	TTTCGATTTCAAATGCTGC	95°C for 15s 60°C for 15s 72°C for 20s	Forward: 58.8°C Reverse: 61.4°C
PPARG [1] Peroxisome proliferator-activated receptor gamma [1-3]	CGACCAAGTAACTCTCCTCA	GTTCCGTGACAATCTGTCTG	95°C for 15s 60°C for 15s 72°C for 20s	Forward: 55°C Reverse: 57°C
CAPN1[2] Calpain 1, large subunit [4-5]	AGACCATGTTCCGATTTTTC	TGCAACCACTTAAACAAGTC	95°C for 15s 60°C for 15s 72°C for 20s	Forward: 60.4°C Reverse: 57.8°C
BCL2[2] B-cell CLL/lymphoma 2 [6]	GATTGTGGCCTTCTTTGAG	GTTCCACAAAGGCATCC	95°C for 15s 60°C for 15s 72°C for 20s	Forward: 59.8°C Reverse: 59°C
CASP3 [3]	GTGCTACAATGCCCCTGGAT	GCCCATTCATTTATTGCTTTCC	95°C for 15s	Forward: 59.3°C

Caspase 3, apoptosis-related cysteine peptidase [5-7]			60°C for 15s 72°C for 20s	Reverse: 54.2°C
CASP9[2] Caspase 9, apoptosis-related cysteine peptidase [7]	CTCTACTTTCCCAGGTTT	TTTCACCGAAACAGCATT	95°C for 15s 60°C for 15s 72°C for 20s	Forward: 60.4°C Reverse: 57.8°C
CDKN1A[2] Cyclin-dependent kinase inhibitor 1A [8-9]	CAGCATGACAGATTTCTACC	CAGGGTATGTACATGAGGAG	95°C for 15s 60°C for 15s 72°C for 20s	Forward:57.3°C Reverse: 57°C
RPL13A[4] Ribosomal protein L13a [1]	CCTGGAGGAGAAGAGGAAAGGA	TTGAGGACCTCTGTGTATTTGTCAA	95°C for 15s 60°C for 15s 72°C for 20s	Forward: 62°C Reverse: 63°C

[1] Lahnalampi M, Heinäniemi M, Sinkkonen L, Wabitsch M, Carlberg C: Time-resolved expression profiling of the nuclear receptor superfamily in human adipogenesis. PLoS One 2010; 5(9): e12991.

[2] KiCqStart™ SYBR® Green Primers, Sigma-Aldrich Co. LLC, USA.

[3] Aquino I, Tsuboy MS, Marcarini JC, Mantovani MS, Perazzo FF, Maistro EL: Genotoxic evaluation of the antimalarial drugs artemisinin and artesunate in human HepG2 cells and effects on CASP3 and SOD1 gene expressions. Genetics and molecular research: GMR. 2013; 12: 2517-2527.

[4] Brandimarto, Jeffrey Alan: Molecular regulation of insulin-like growth factor binding protein-5 by signaling molecules downstream of the IGF-I receptor in mammary epithelial cells. Retrieved from http://dx.doi.org/doi:10.7282/T32J6C4V

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[1] Lahnalampi M, Heinäniemi M, Sinkkonen L, Wabitsch M, Carlberg C: Time-resolved expression profiling of the nuclear receptor superfamily in human adipogenesis. PLoS One 2010; 5(9): e12991.

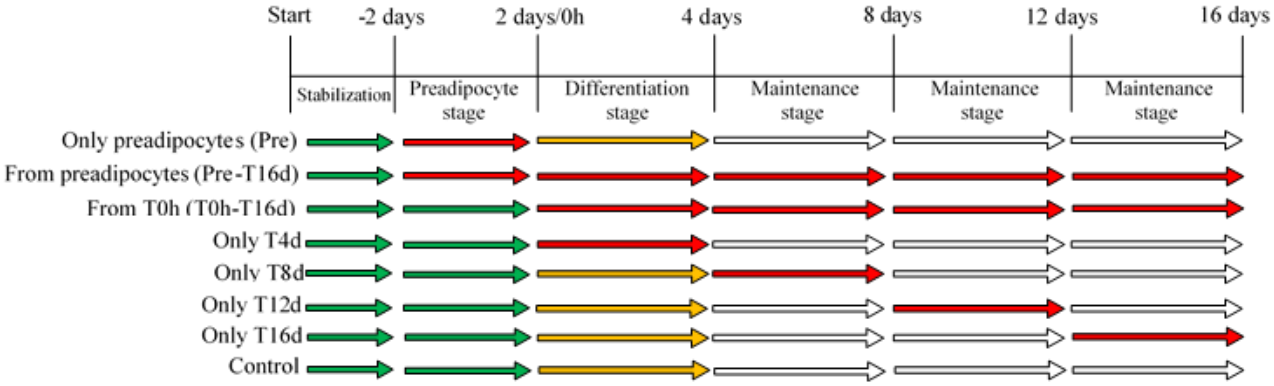
[2] Wood RJ: Vitamin D and adipogenesis: new molecular insights. Nutr Rev. 2008; 66:40-46.

[3] Nimitphong H, Holick MF, Fried SK, Lee MJ: 25-hydroxyvitamin D_3 and 1,25-dihydroxyvitamin D_3 promote the differentiation of human subcutaneous preadipocytes. PLoS One. 2012; 7:e52171

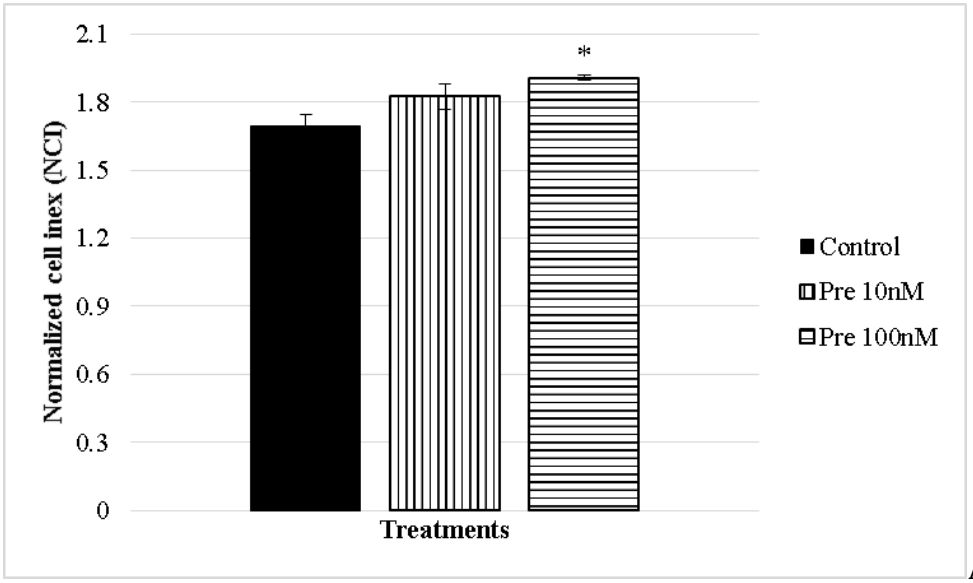
[4] Sergeev IN: 1,25-Dihydroxyvitamin D3 induces Ca2+-mediated apoptosis in adipocytes via activation of calpain and caspase-12. Biochem Biophys Res Commun. 2009; 384: 18-21.

[5] Sergeev IN: Vitamin D-mediated apoptosis in cancer and obesity. Horm Mol Biol Clin Invest. 2014; 20: 43-49.

[6] Nagel SA, Keuper M, Zagotta I, Enlund E, Ruperez AI, Debatin KM, Wabitsch M, Fischer-Posovszky P: Up-regulation of Bcl-2 during adipogenesis mediates apoptosis resistance in human adipocytes. Mol Cell Endocrinol. 2014; 382: 368-76.
[7] Yoon S, Park SJ, Han JH, Kang JH, Kim JH, Lee J, Park S, Shin HJ, Kim K, Yun M, Chwae YJ: Caspase-dependent cell death-associated release of nucleosome and damage-associated molecular patterns. Cell Death Dis. 2014; 5: e1494.
[8] Saramäki A, Diermeier S, Kellner R, Laitinen H, Vaïsänen S, Carlberg C: Cyclical chromatin looping and transcription factor association on the regulatory regions of the p21 (CDKN1A) gene in response to 1alpha,25-dihydroxyvitamin D3. J Biol Chem. 2009; 284: 8073-82.
[9] Rodríguez-Acebes S, Palacios N, Botella-Carretero JI, Olea N, Crespo L, Peromingo R, Gómez-Coronado D, Lasunción MA, Vázquez C, Martínez-Botas J: Gene expression profiling of subcutaneous adipose tissue in morbid obesity using a focused microarray: Distinct expression of cell-cycle- and differentiation-related genes. BMC Med Genomics. 2010; 3:61.



Supplementary Figure S1 - RTCA study design. The arrows indicate the treatment/stabilization stage. Green: only the growth medium; yellow: only the differentiation medium; white: only the maintenance medium; and red: $1,25(OH)_2D_3$ treatment (10 nM and 100 nM), with the respective medium for the stage.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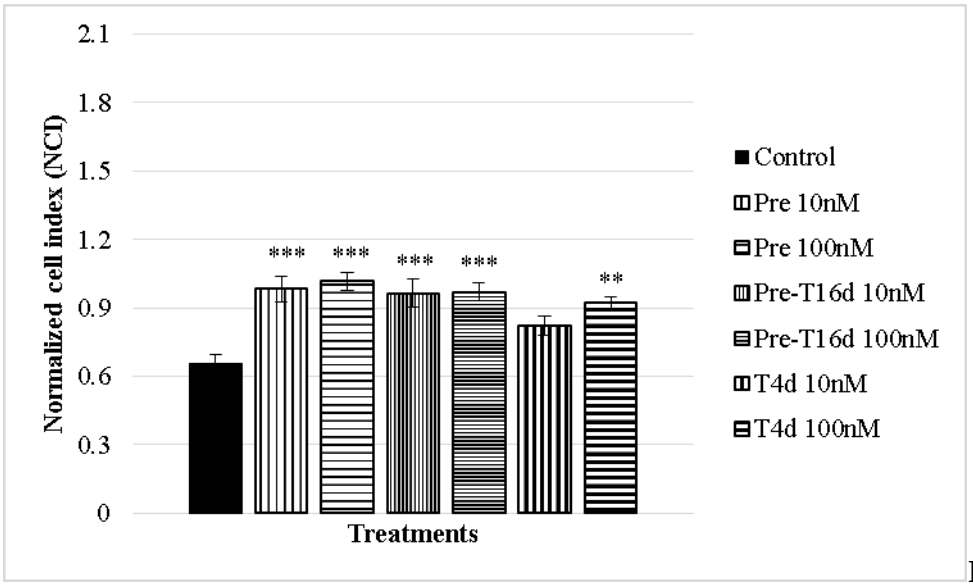
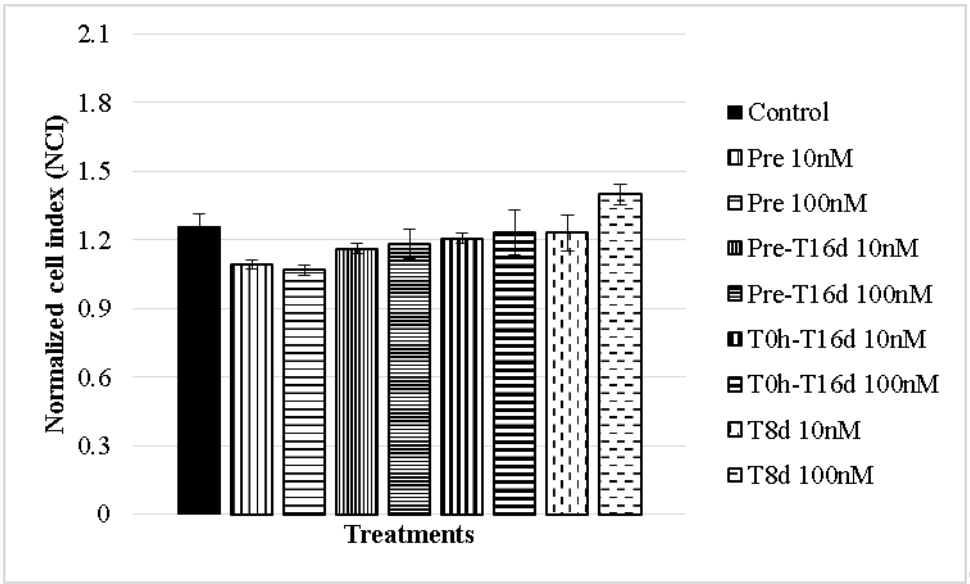
1.8
1.5
1.2
0.9
0.6
0.3
0
Normalized cell inex (NCI)
*
Treatments
Control
Pre 10nM
Pre 100nM

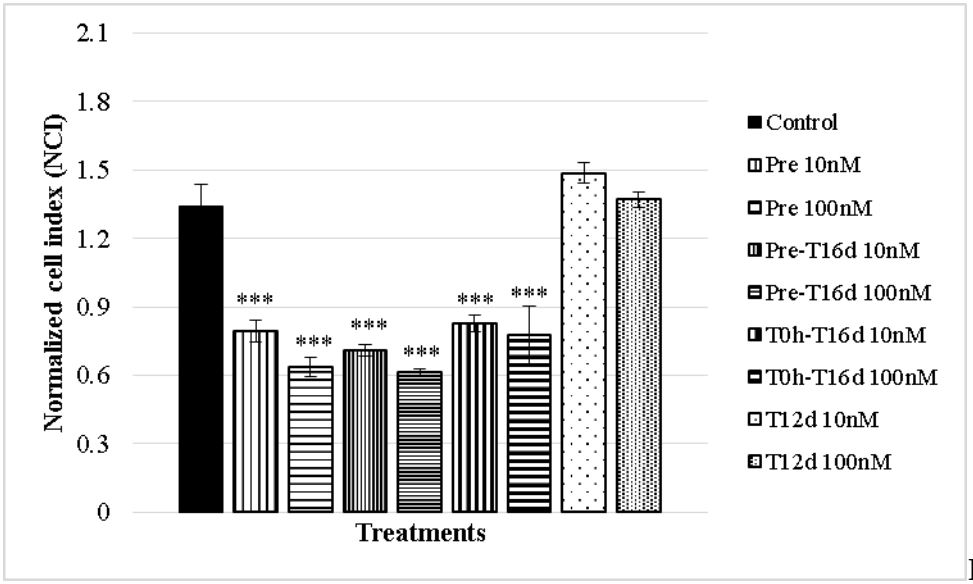
A


2.1
1.8
1.5
1.2
0.9
0.6
0.3
0
Normalized cell index (NCI)
***
***
***
***
**
Treatments
Control
Pre 10nM
Pre 100nM
Pre-T16d 10nM
Pre-T16d 100nM
T4d 10nM
T4d 100nM

B


2.1
1.8
1.5
1.2
0.9
0.6
0.3
0
Normalized cell index (NCI)
Treatments
Control
Pre 10nM
Pre 100nM
Pre-T16d 10nM
Pre-T16d 100nM
T0h-T16d 10nM
T0h-T16d 100nM
T8d 10nM
T8d 100nM

C



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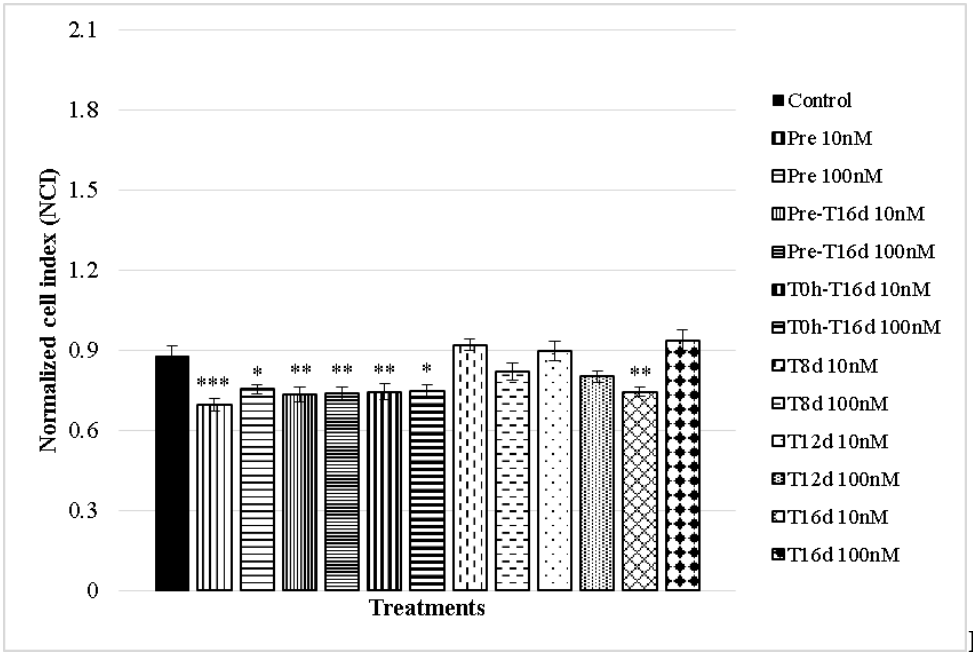
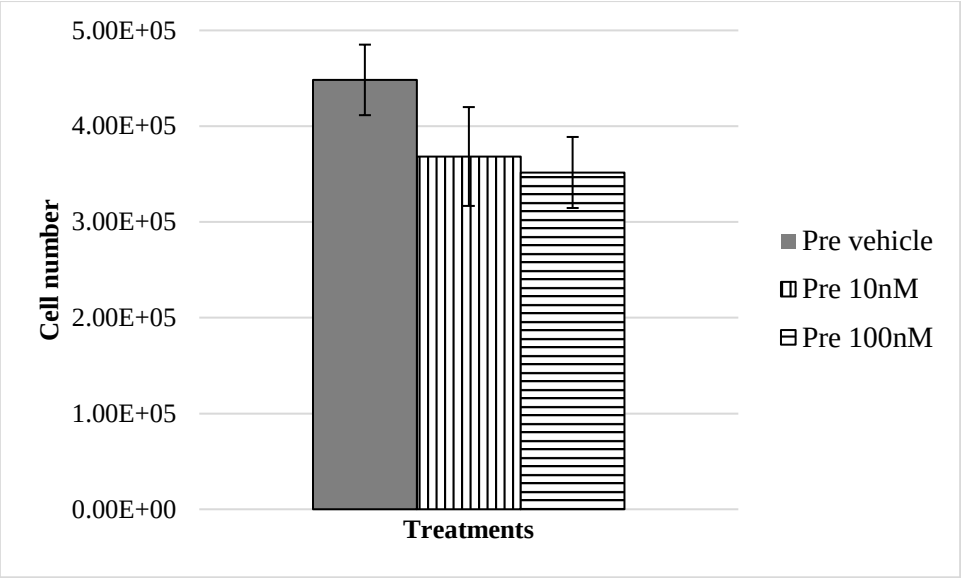
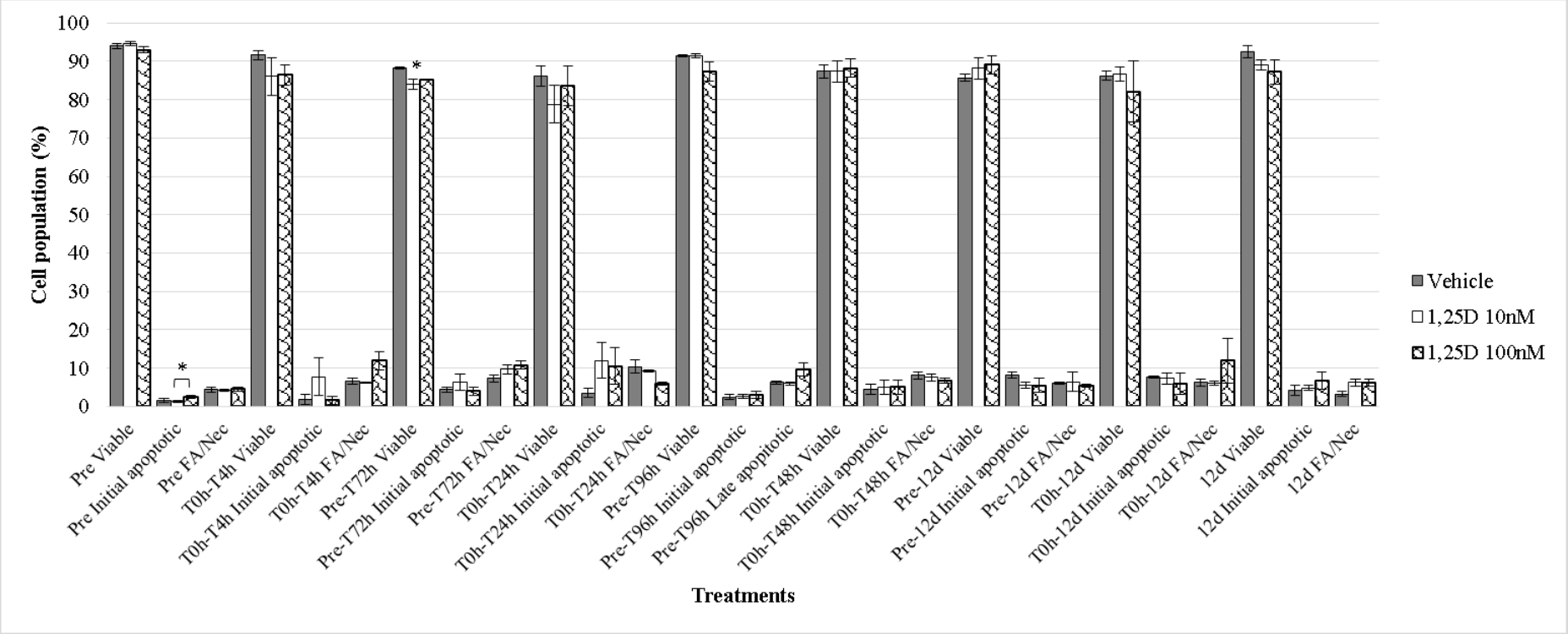


E

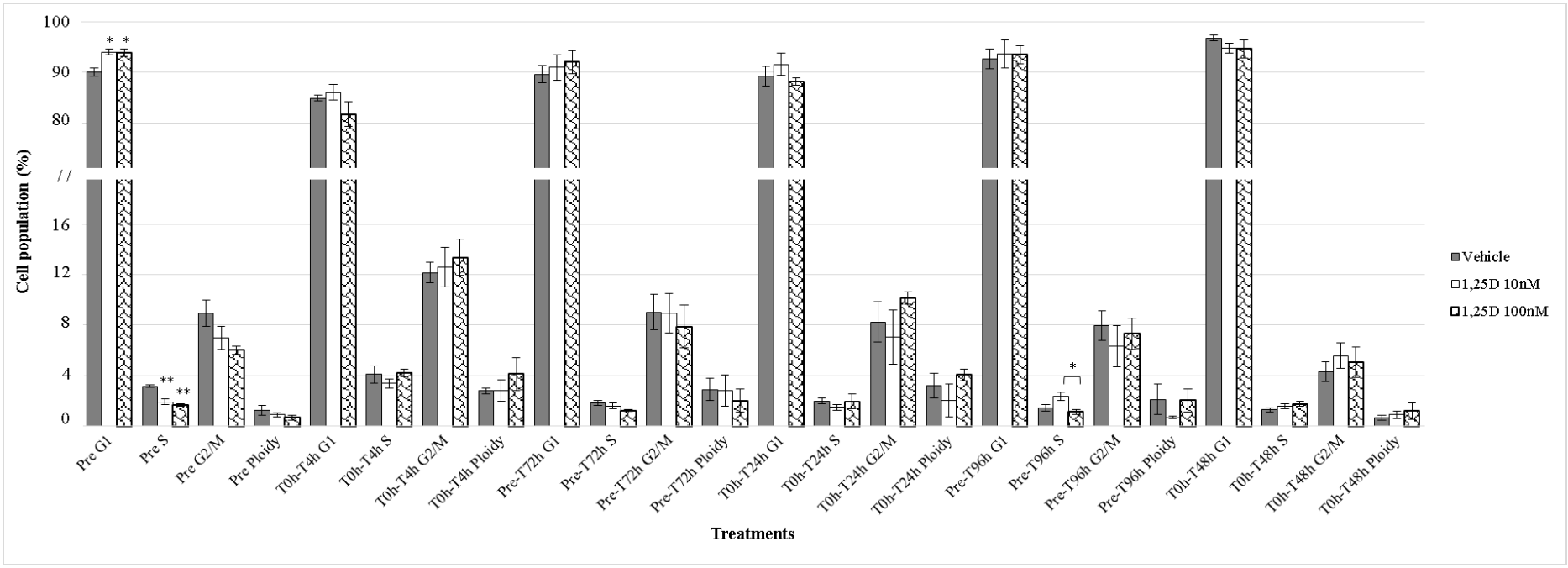
Supplementary Figure S2 - Real-time cell analysis (RTCA) of (A) human preadipocytes and adipocytes treated with $1,25(OH)_2D_3$ for (B) 4, (C) 8, (D) 12, and (E) 16 days after differentiation. The data are presented as the mean ± SEM. ANOVA was used to test significant differences between the treatments, and Dunnett's test was used to compare results with those of the control treatment. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$. Control: untreated cells; Pre: cells treated only during the preadipocyte stage; Pre-T16d: cells treated continuously from the preadipocyte stage; T0h-T16d: cells treated continuously from the differentiation stage (T0h); T4d: cells treated only 4 days after differentiation; T8d: cells treated only 8 days after differentiation; T12d: cells treated only 12 days after differentiation. All cells were differentiated at the same time, and differentiation proceeded equally among treatments for 16 days.



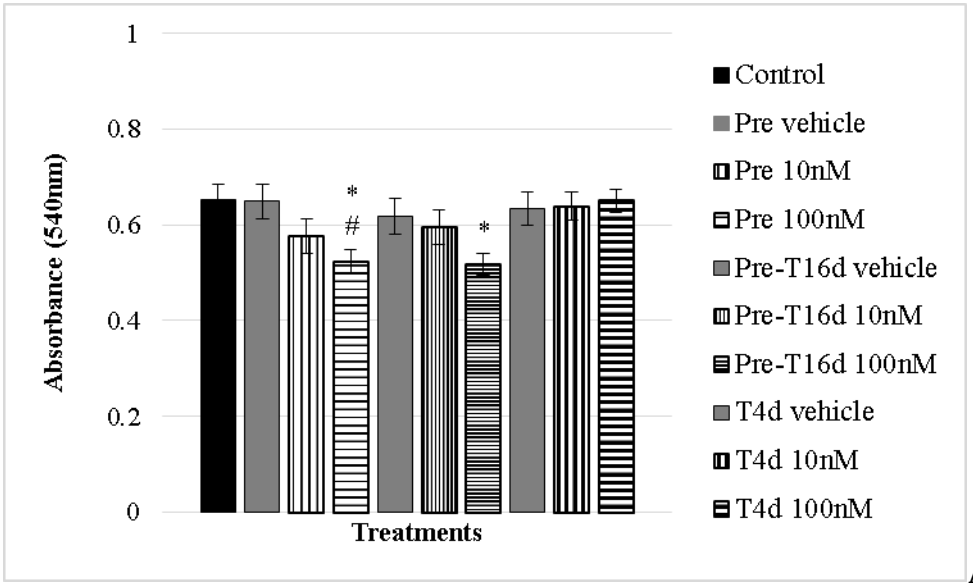
Supplementary Figure S3 - Proliferation assay in human preadipocytes treated for 48 h with 1,25$(OH)_2D_3$. ANOVA was used to test significant differences between treatments, and Dunnett's test was used to compare results with those of the vehicle treatment. The data are presented as the mean ± SEM.



Supplementary Figure S4 - Flow cytometry analysis (cell apoptosis analysis). The data are presented as the mean ± SEM. ANOVA was used to test significant differences between the treatments, and Dunnett's test was used to compare results with those of the vehicle treatment. Student's *t*-test was used to test significant differences between the $1,25(OH)_2D_3$ (10 nM and 100 nM) treatments. *$p < 0.05$. FA: Final apoptotic; Nec: Necrotic; 1,25D: $1,25(OH)_2D_3$.



Supplementary Figure S5 - Flow cytometry analysis results (cell cycle analysis). The data are presented as the mean ± SEM. ANOVA was used to test significant differences between the treatments, and Dunnett's test was used to compare results with those of the vehicle treatment. Student's *t*-test was used to test significant differences between the 1,25$(OH)_2D_3$ (10 nM and 100 nM) treatments. *$p < 0.05$ and **$p < 0.01$. 1,25D: 1,25$(OH)_2D_3$.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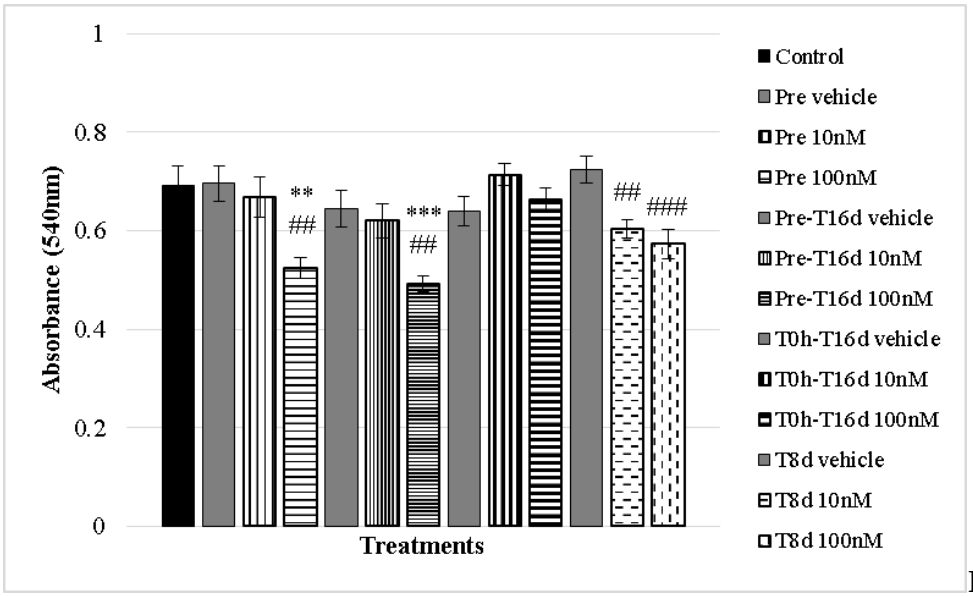
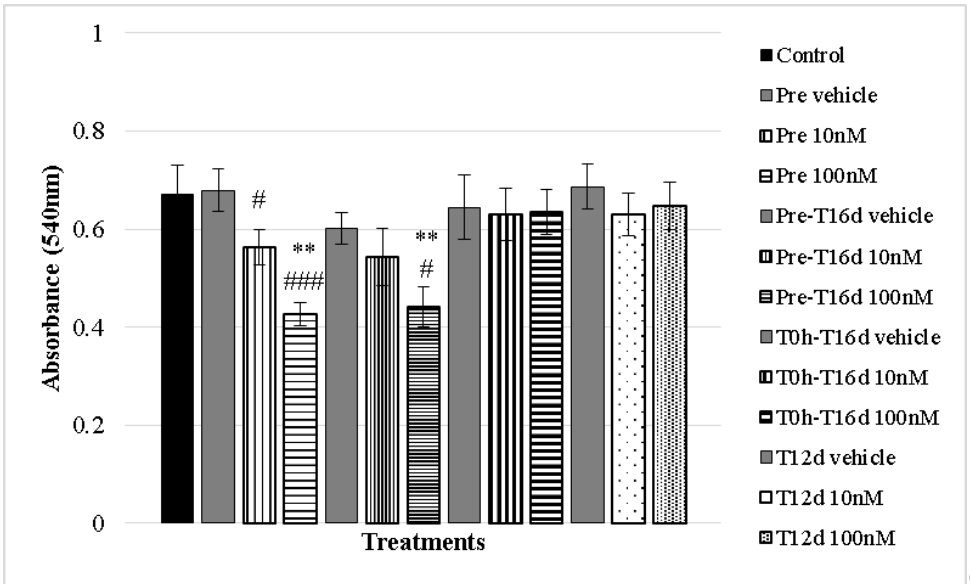
0.8
0.6
0.4
0.2
0
Absorbance (540nm)
Treatments
*
#
*
Control
Pre vehicle
Pre 10nM
Pre 100nM
Pre-T16d vehicle
Pre-T16d 10nM
Pre-T16d 100nM
T4d vehicle
T4d 10nM
T4d 100nM

A


1
0.8
0.6
0.4
0.2
0
Absorbance (540nm)
Treatments
**
##
***
##
##
####
Control
Pre vehicle
Pre 10nM
Pre 100nM
Pre-T16d vehicle
Pre-T16d 10nM
Pre-T16d 100nM
T0h-T16d vehicle
T0h-T16d 10nM
T0h-T16d 100nM
T8d vehicle
T8d 10nM
T8d 100nM

B


1
0.8
0.6
0.4
0.2
0
Absorbance (540nm)
Treatments
#
**
####
**
#
Control
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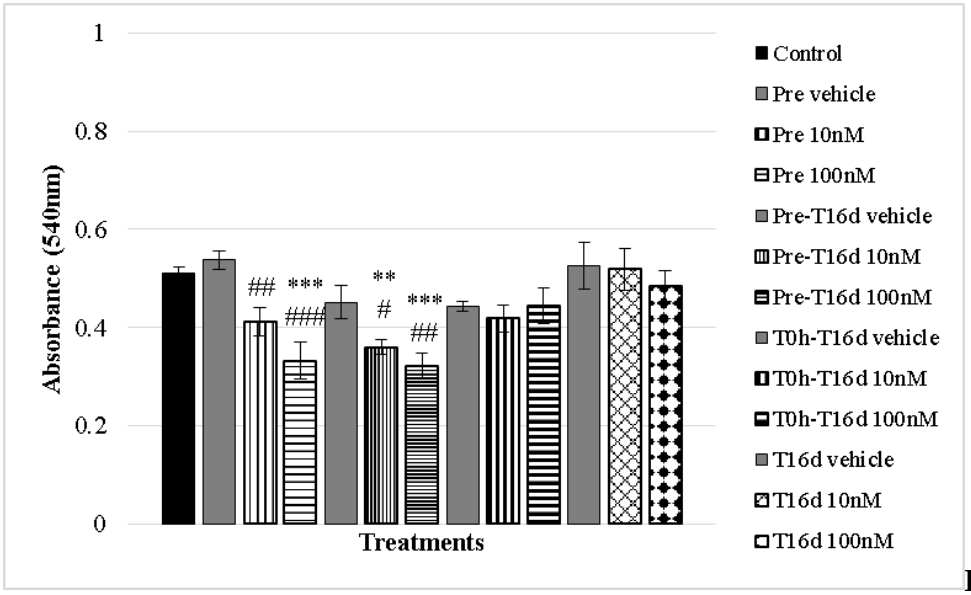
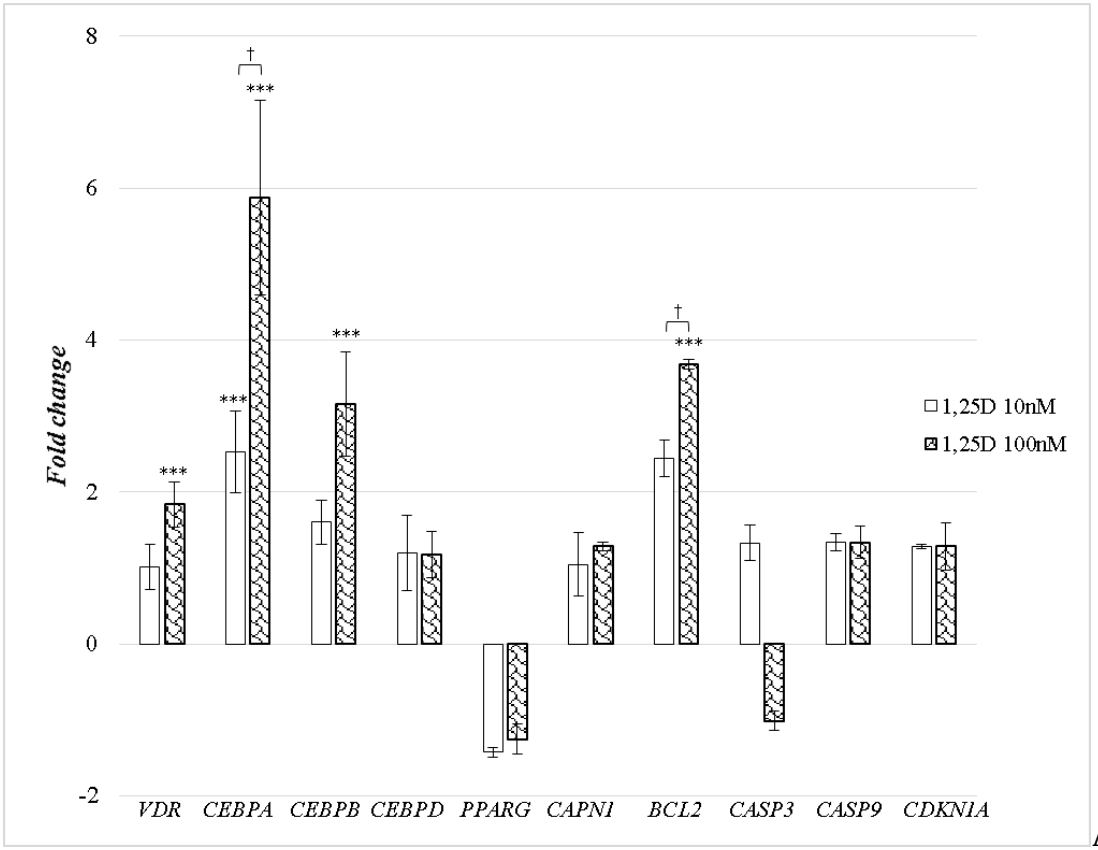
Pre 10nM
Pre 100nM
Pre-T16d vehicle
Pre-T16d 10nM
Pre-T16d 100nM
T0h-T16d vehicle
T0h-T16d 10nM
T0h-T16d 100nM
T12d vehicle
T12d 10nM
T12d 100nM

C

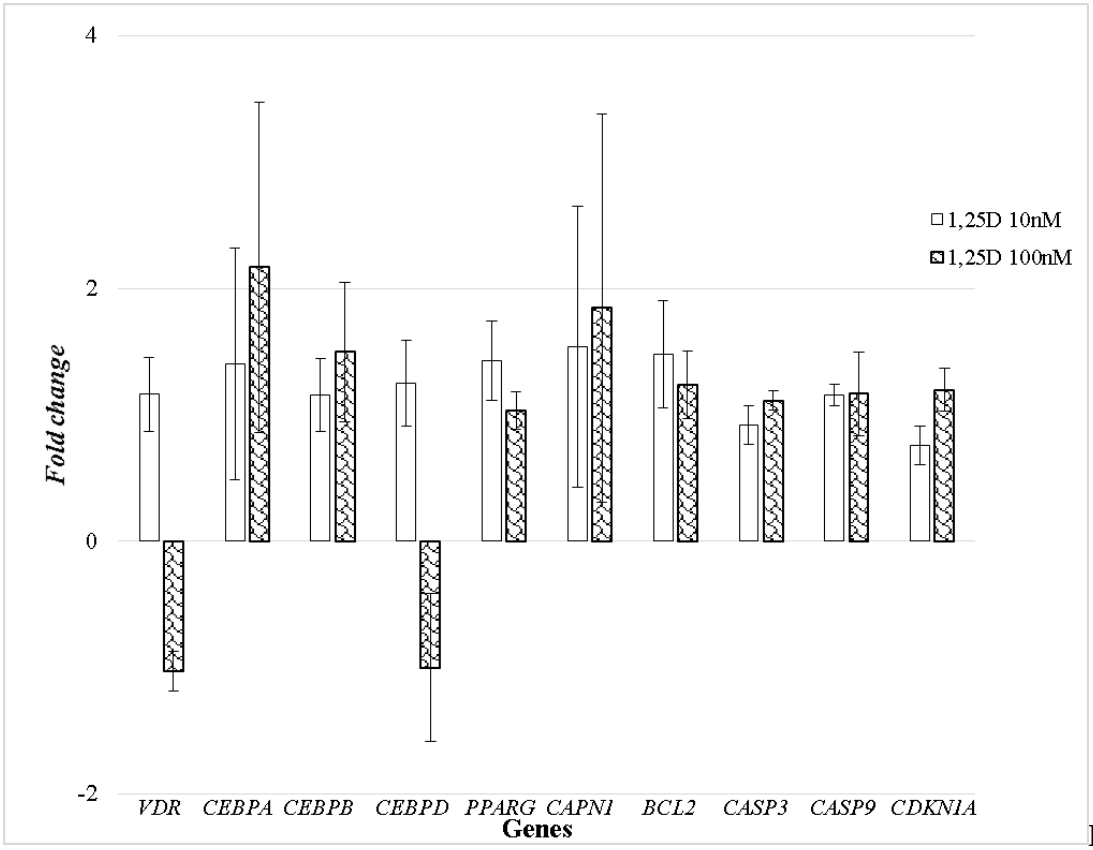


D

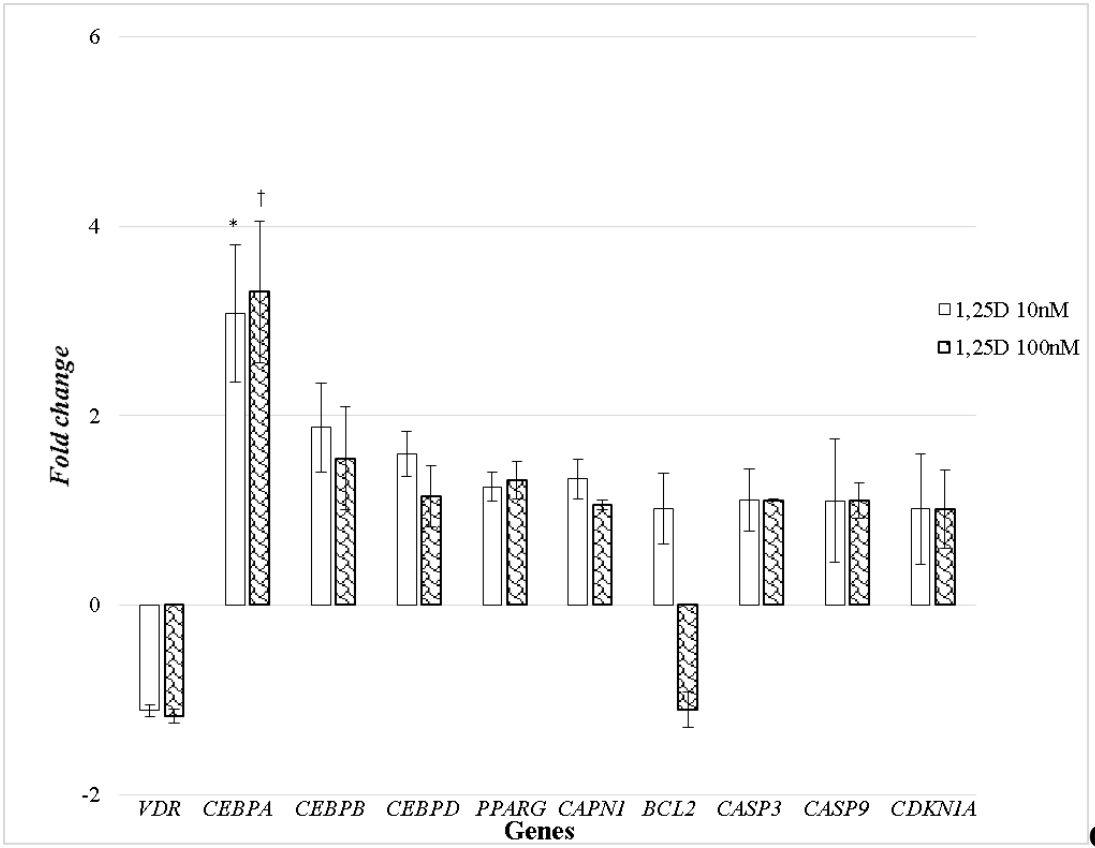
Supplementary Figure S6 - MTT assay results of SGBS adipocytes treated with $1,25(OH)_2D_3$ (A) 4, (B) 8, (C) 12, and (D) 16 days after differentiation. The data are presented as the mean ± SEM. ANOVA was used to test significant differences between the treatments, and Dunnett's test was used to compare the results with those of the control (*) and vehicle (#) treatments. */#$p < 0.05$, **/##$p < 0.01$, and ***/###$p < 0.001$. Control: untreated cells; vehicle: cells treated with ethanol; Pre: cells treated only during the preadipocyte stage; Pre-T16d: cells treated continuously from the preadipocyte stage; T0 h-T16d: cells treated continuously from the differentiation stage (T0 h); T4d: cells treated only 4 days after differentiation; T8d: cells treated after 8 days after differentiation; T12d: cells treated only 12 days after differentiation. All cells were differentiated at the same time, and differentiation proceeded equally among treatments for 16 days.


8
6
4
2
0
-2
Fold change
***
***
***
***
†
***
†
***
1,25D 10nM
1,25D 100nM
VDR
CEBPA
CEBPB
CEBPD
PPARG
CAPN1
BCL2
CASP3
CASP9
CDKN1A

A


4
2
0
-2
Fold change
1,25D 10nM
1,25D 100nM
VDR
CEBPA
CEBPB
CEBPD
PPARG
CAPN1
BCL2
CASP3
CASP9
CDKN1A
Genes

B



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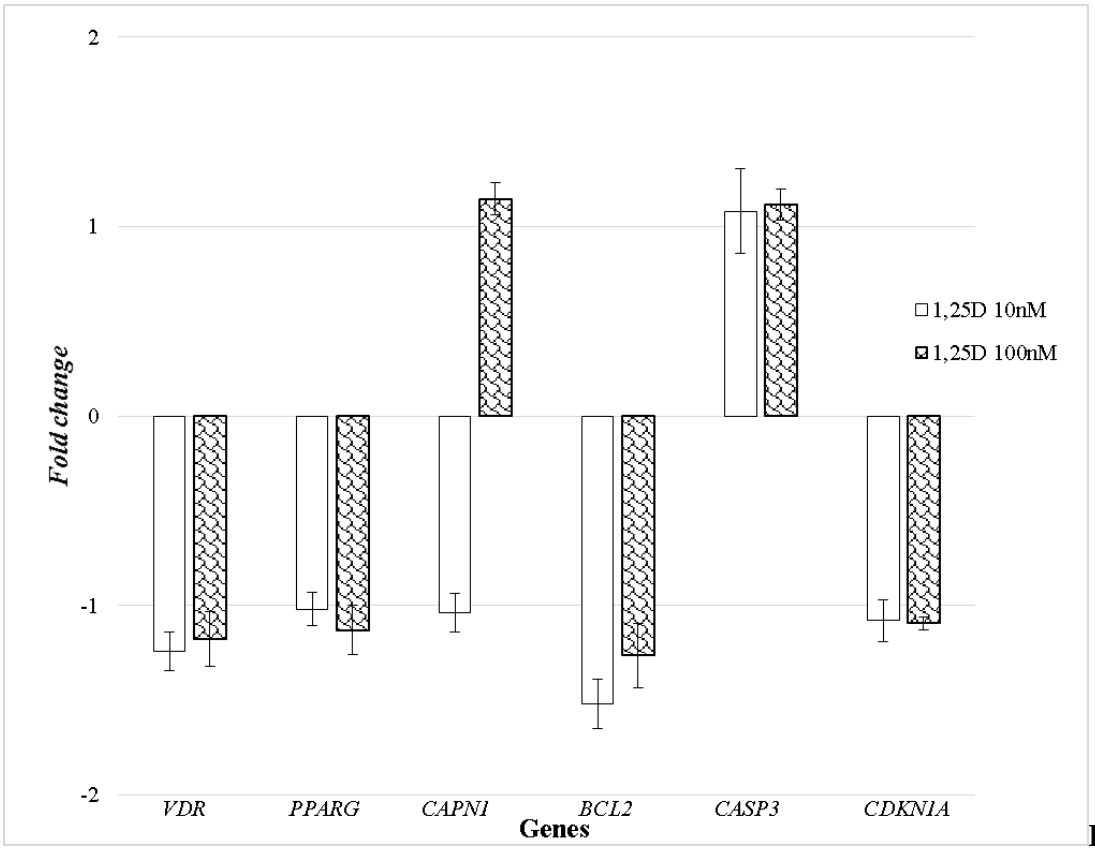

D

Supplementary Figure S7 - RT-qPCR gene expression results. Gene expression of human preadipocytes treated with 10 nM or 100 nM $1,25(OH)_2D_3$ after (A) 48 h in the preadipocyte stage and after (B) 4 h, (C) 24 h and (D) 12 days of induction of

differentiation. Columns represent the means of at least three independent experiments, and bars indicate SEM. REST software was used to calculate significant differences between normalized fold changes. Student's *t*-test was used to test significant differences between $1,25(OH)_2D_3$ concentrations. 1,25D: $1,25(OH)_2D_3$. $*p < 0.05$, $***p < 0.001$, and $\dagger p \leq 0.065$.