Target gene	Forward primer	Reverse primer
Runx2	5′-GTCTCACTGCCTCTCACT	5′-TACACACATCTCCTCCCTTC
hCOL-1	5′-GGAGGAGAGTCAGGAAGG	5′-TCAGCAACACAGTTACACAA
OSX	5′-TTGACATGTACCCCTTTCTG	5′-AATACCCCTGATGAAGAGG
DMP-1	5′-ACTCTCAAGAAGACAGCAA	5′-GACTCACTCACCACCTCT
BSP	5′-ACCGAGCCTATGAAGATGA	5′-CTTCCTGAGTTGAACTTCGA
DSPP	5′-CAGTACAGGATGAGTTAAATGCCAGTG	5′-CCATTCCCTTCTCCCTTGTGAC
GAPDH	5′-GTATGACAACAGCCTCAAGAT	5′-CCTTCCACGATACCAAAGTT

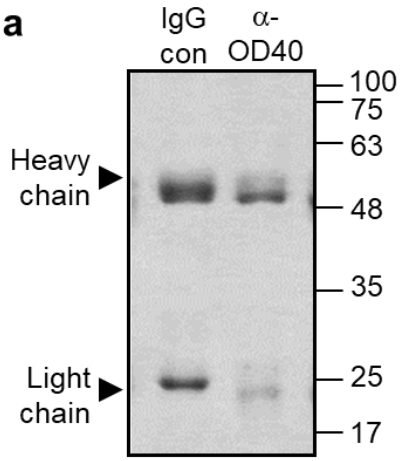
Supplemental Table 1. Oligonucleotide primers used for quantitative real-time PCR

Mouse heavy chain constant region primers, V_H primers	
IgG	5'-ATA GAC AGA TGG GGG TGT CGT TTT GGC-3'
5'MH1	5'-SAR CTN MAG CTG SAG SAG TC-3'
5'MH2	5'-SAR GTN MAG CTG SAG SAG TCW GG-3'

Mouse kappa chain constant region primers, V_L primers	
5'Mk	5'-GAY ATT GTG MTS ACM CAR WCT MCA-3'
Primer 6	5'-GAC ATT GTG CTG ACC CAA TCT CCA GCT TCT-3'
Primer 7	5'-GAC ATT CAG CTG ACC CAG TCT CCA-3'

Supplemental Table 2. DNA sequences of the primers used for reverse transcription PCR (RT-PCR) of mouse IgG VL and VH gene. Mixed base codes were indicated as followed; R=a/g, Y=c/t, M=a/c, K=g/t, S=c/g, W=a/t, V=a/c/g, and N=a/c/g/t.

a

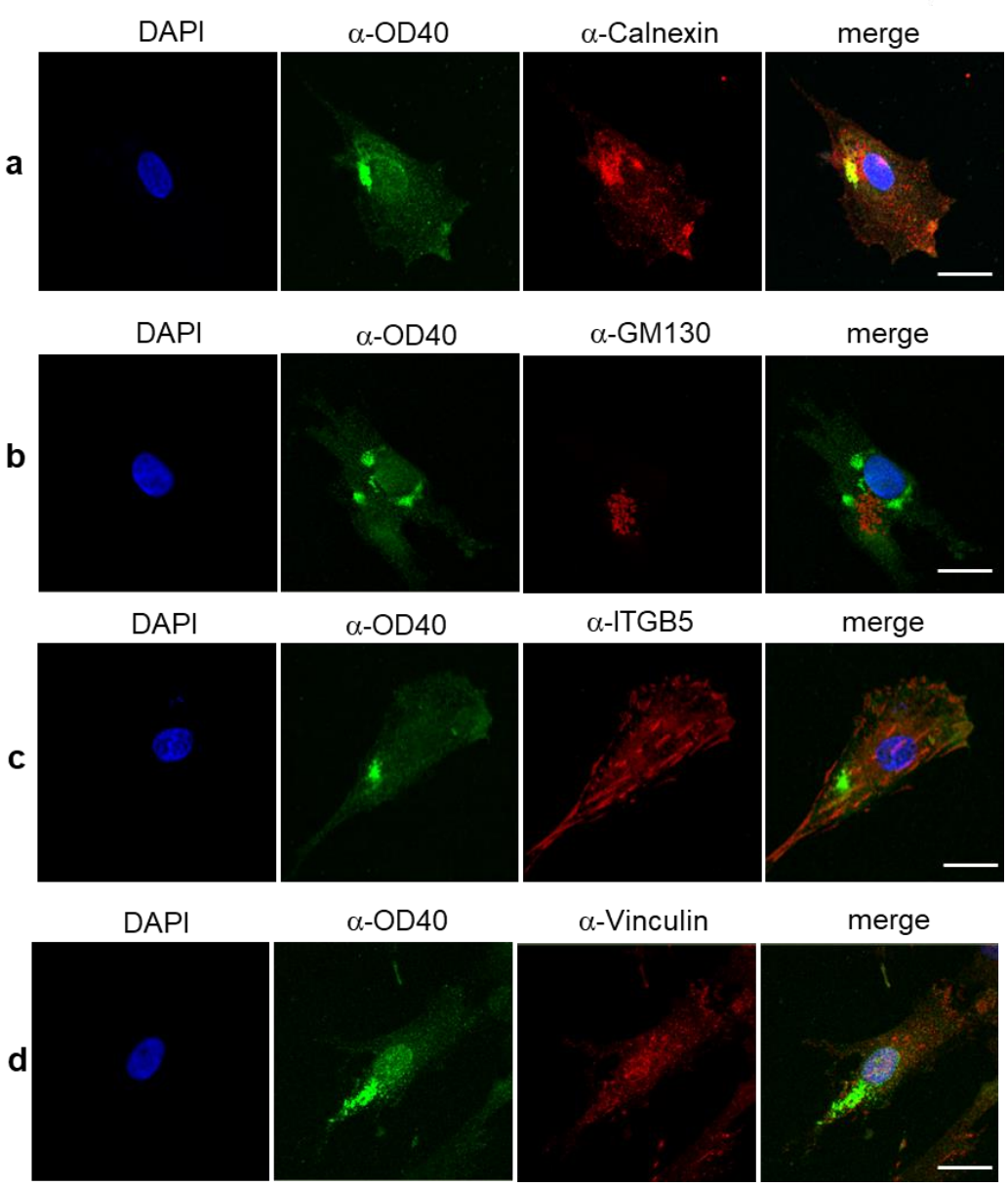


b

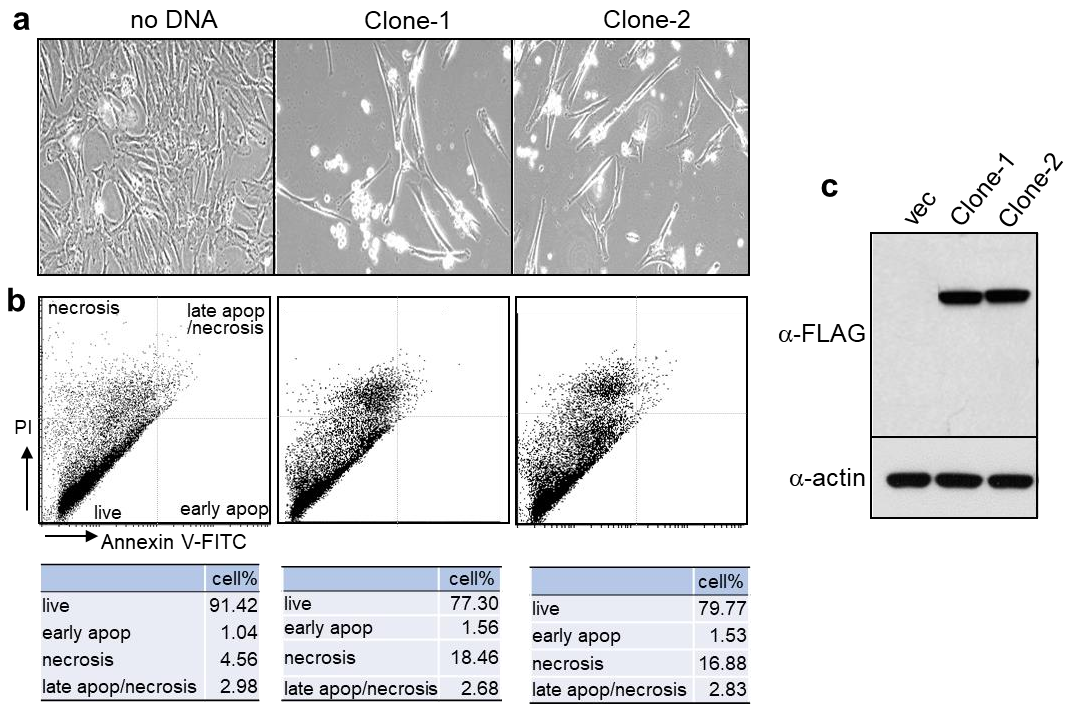
Segment of OD40 V_H	Gene and allele	Score (bit)	Identity, % (nt)
V	Musmus IGHV8-11*01F	413	identity = 95.92% (235/245 nt)
J	Musmus IGHJ4*01F	79.5	identity = 81.13% (43/53 nt)
D	Musmus IGHD2-2*01F	D-REGION is in reading frame 2	

Segment of OD40 V_L	Gene and allele	Score (bit)	Identity, % (nt)
V	Musmus IG_KV12-46*01F	424	identity = 98.28% (228/232 nt)
J	Musmus IG_KJ2*01	27.6	identity = 100% (14/14 nt)

Supplemental Figure 1. Purification and gene sequencing of anti-OD40 monoclonal antibody. (a) SDS-PAGE analysis of anti-OD40 antibody. Heavy and light chains of purified antibody were detected by Coomassie staining. (b) Detail results of the IMGT/V-QUEST analysis of V_H (heavy chain variable region) and V_L (light chain variable region) sequences of the anti-OD40 antibody. Scores were calculated by +5 for each nucleotide that is identical to the IMGT reference sequence, and -4 for each mismatch. Identity indicated percentage of nucleotides identical to the reference sequence. n indicated a ratio of nucleotides identical to reference sequence to total number of nucleotides. a, IMGT/V-QUEST analysis of V_H; b, IMGT/V-QUEST analysis of V_L.



Supplemental Figure 2. Subcellular localization of the antigenic molecule against anti-OD40 antibody in hDPCs. a, Determining the location of ER using calnexin; b, Determining the location of Golgi using GM130; c, Determining the location of plasma membrane using integrin β5; d, Determining the location of cytoskeletal network using vimentin. Nuclei are stained by DAPI. Green and red signals indicate OD40 and other markers, respectively. Bar indicates as 100 μm.

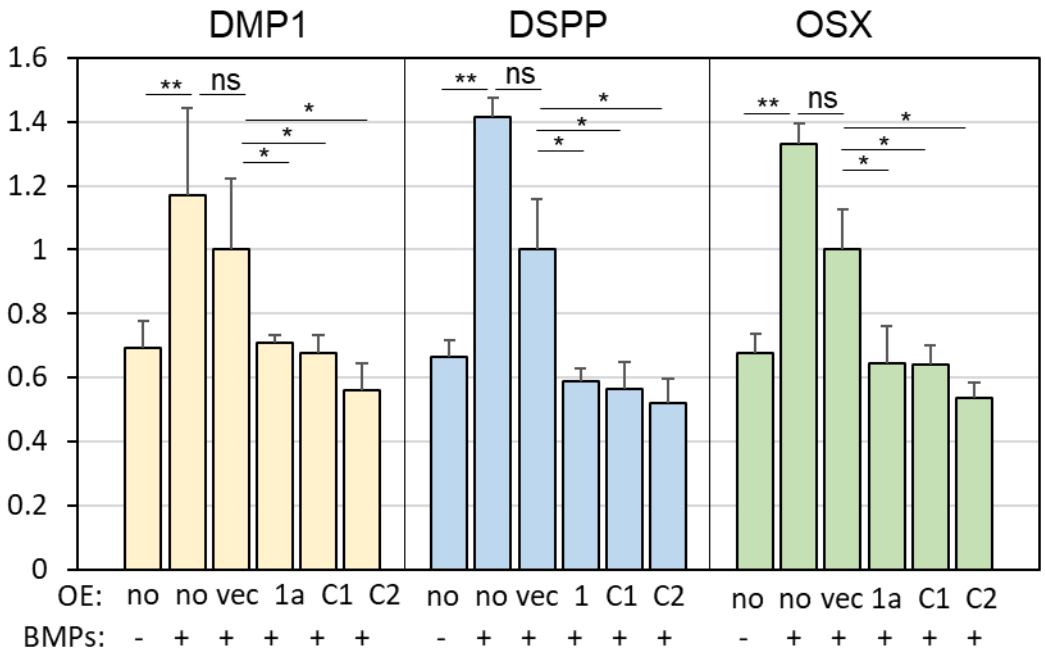


	cell%
live	91.42
early apop	1.04
necrosis	4.56
late apop/necrosis	2.98

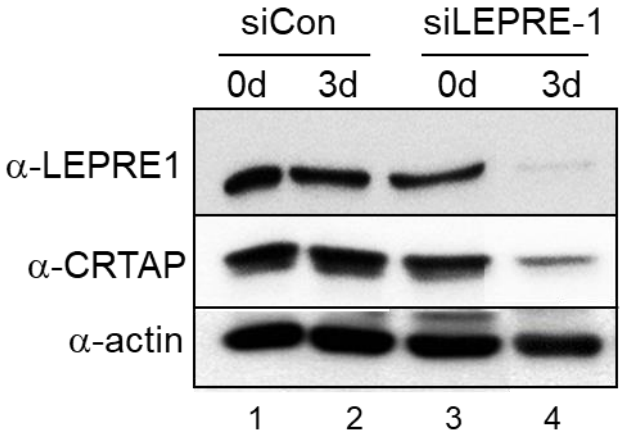
	cell%
live	77.30
early apop	1.56
necrosis	18.46
late apop/necrosis	2.68

	cell%
live	79.77
early apop	1.53
necrosis	16.88
late apop/necrosis	2.83

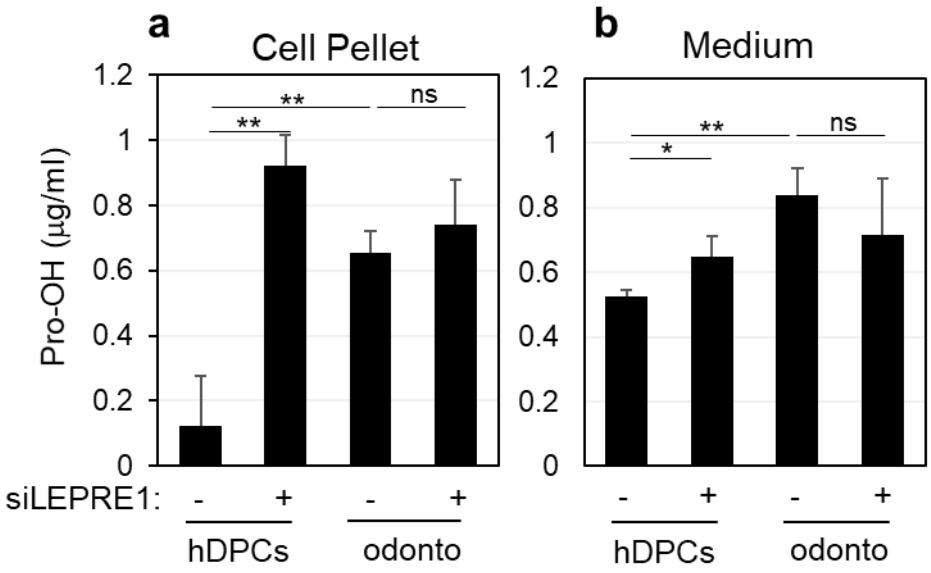
Supplemental Figure 3. Cellular defects are increased by Overexpression of amino acids 1-685 of LEPRE1a with or without ER-retention signal. a, morphological changes; b, FACS analysis by double staining of PI and annexin V; c, Expression of the indicated subclones. Clone-1, amino acids 1-685 of LEPRE1a without KDEL sequence; clone-2, amino acids 1-685 of LEPRE1a with KDEL sequence



Supplemental Figure 4. Overexpression of amino acids 1-685 of LEPRE1a inhibits odontogenic differentiation. mRNA expressions of the representative odontogenic markers were examined in hDPCs and odontoblasts. no, transfection with no DNA; vec, transfection with vector only; 1a, transfection with full length cDNA clone; C1, transfection with fragment 1-685 without KDEL; C2, transfection with fragment 1-685 with KDEL. Statistical analysis was performed using Student t-test (n=3). *, $P < 0.05$; **, $P < 0.01$.



Supplemental Figure 5. Depletion of endogenous LEPRE1 results in reduction of CRPAP.



Supplemental Figure 6. Depletion of endogenous LEPRE1 results in up-regulation of hydroxylation of intracellular collagen, not in medium. Statistical analysis was performed using Student t-test (n=3). *, $P < 0.05$; **, $P < 0.01$; ns, not significant.

Supplemental experimental procedures

Antibody purification

Anti-OD40 antibody was purified by column chromatography from the hybridoma culture media. Briefly, media were pooled and loaded on the column of Protein G agarose (Amicogen). After washing beads with PBS, the bound IgG was collected by elution with 100 mM glycine buffer (pH 2.8). The eluted fraction was quickly mixed with 1 M Tris-HCl (pH 9.0) for neutralization. After dialysis in PBS, antibody was quantified and analyzed in SDS-PAGE.

Antibody sequencing

The primers for antibody sequencing were shown in Supplementary Table 2 (Kang et al., 2017; Wang et al., 2000). Total RNA was extracted from hybridoma by using Easy-spin™ total RNA extraction kit (Intron), and then, cDNAs of the constant and variable regions of mouse heavy and light chains were direct synthesized by ONE-STEP™ RT-PCR premix (Intron) from total RNA according to the manual provided by the manufacturer. The cDNA products were cloned into pBluescript KS(+) vector, and sequencing was performed. Consensus domains of antibody were estimated by using the IMGT/V-QUEST program (version 3.2.21) (Ning et al., 2012).

Primary antibody information

The primary antibodies used in this study were as followed: anti-KDEL (Invitrogen, PA1-013), anti-LEPRE1 (Novus Biologicals, NBP2-45786), anti-GROS1 (Santa Cruz, sc-393003), anti-OSX (Santa Cruz, sc-393060), anti-DMP-1 (Santa Cruz, sc-73633), anti-DSPP (Santa Cruz, sc-73632), anti-actin (Santa Cruz, sc-1616), anti-FLAG (Sigma, F3165), anti-PARP (Cell Signaling Technology, 9532), anti-caspase-3 (Cell Signaling Technology, 9662), anti-CRTAP (Novus Biologicals, H00010491-M01), anti-GM130 (Cell Signaling Technology, 12480), anti-Calnexin (Santa Cruz, sc-11397), anti-Integrin-β5 (Invitrogen, PA5-17260), Anti-COL1A (Santa Cruz, sc-59772) antibodies.

Annexin V assay

The percentage of cells within a population that are actively undergoing apoptosis were quantitatively determined by using Annexin V-FITC Apoptosis Detection Kit (BD Pharmingen). Cells were detached and washed twice using ice-cold PBS. $1x10^5$ cells were resuspended in 10 mM HEPES (pH 7.4), 140 mM NaCl, 2.5 mM $CaCl_2$ containing propidium iodide and Annexin V-FITC. Cells were incubated for 15 min in the dark and signals were analyzed by flow cytometry.

Supplemental references

Kang, K.J., Ko, S.Y., Ryu, C.J., and Jang, Y.J. (2017). A monoclonal antibody recognizes undifferentiation-specific carbohydrate moieties expressed on cell surface of the human dental pulp cells. Stem Cell Res 21, 85-93. 10.1016/j.scr.2017.04.002.

Ning, B., Tang, Y., Song, H., Yang, S., and Shen, H. (2012). Cloning and sequencing of the light chain variable region from NS-1 myeloma. Oncol Lett 3, 1083-1086. 10.3892/ol.2012.601.

Wang, Z., Raifu, M., Howard, M., Smith, L., Hansen, D., Goldsby, R., and Ratner, D. (2000). Universal PCR amplification of mouse immunoglobulin gene variable regions: the design of degenerate primers and an assessment of the effect of DNA polymerase 3' to 5' exonuclease activity. J Immunol Methods 233, 167-177. 10.1016/s0022-1759(99)00184-2.

Gel or blot data before cropping

Figure 1

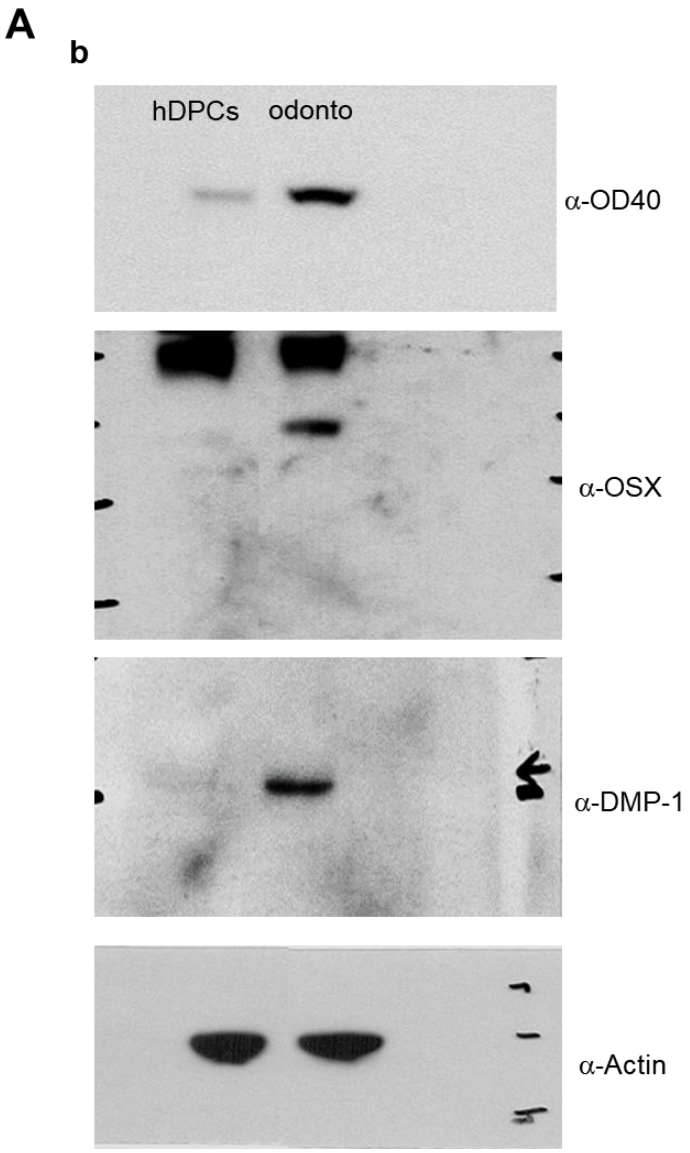


Figure 2

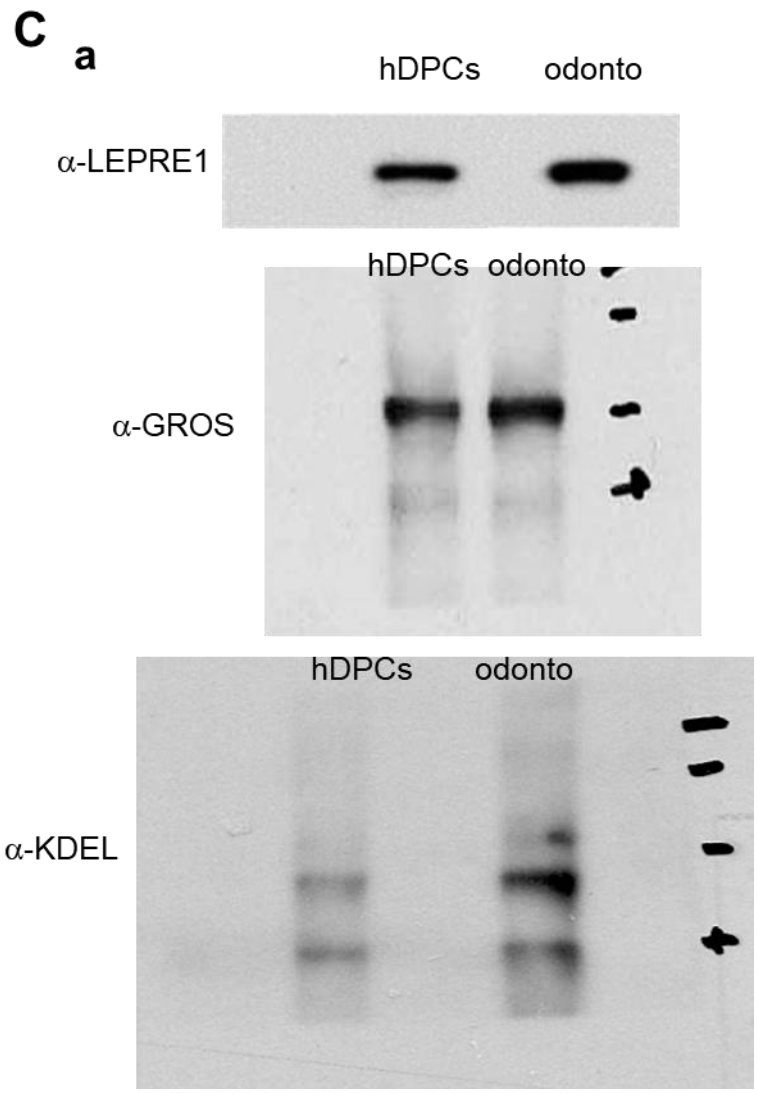

C a
hDPCs
odonto
α-LEPRE1
hDPCs
odonto
α-GROS
hDPCs
odonto
α-KDEL

Figure 3

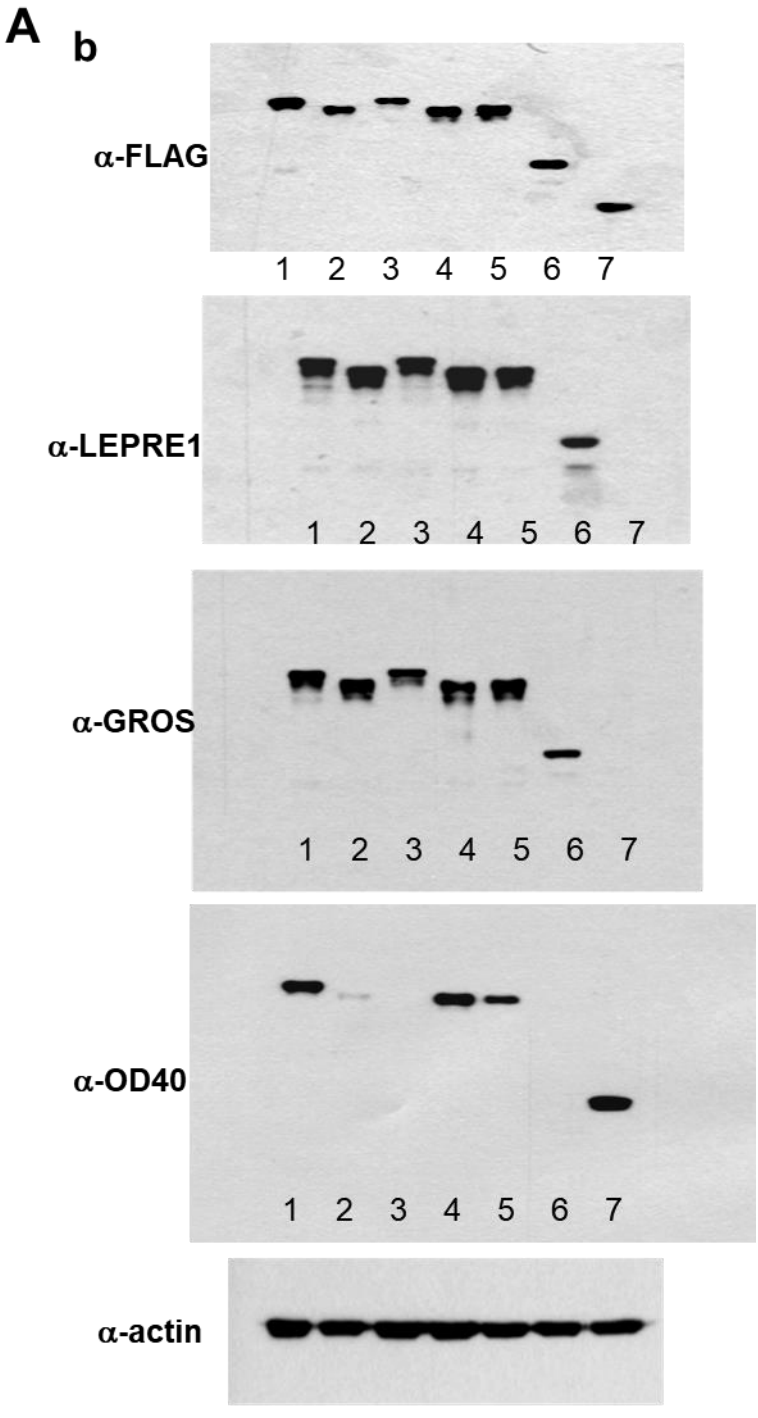

A
b
α-FLAG
1 2 3 4 5 6 7
α-LEPRE1
1 2 3 4 5 6 7
α-GROS
1 2 3 4 5 6 7
α-OD40
1 2 3 4 5 6 7
α-actin

Figure 4

A b

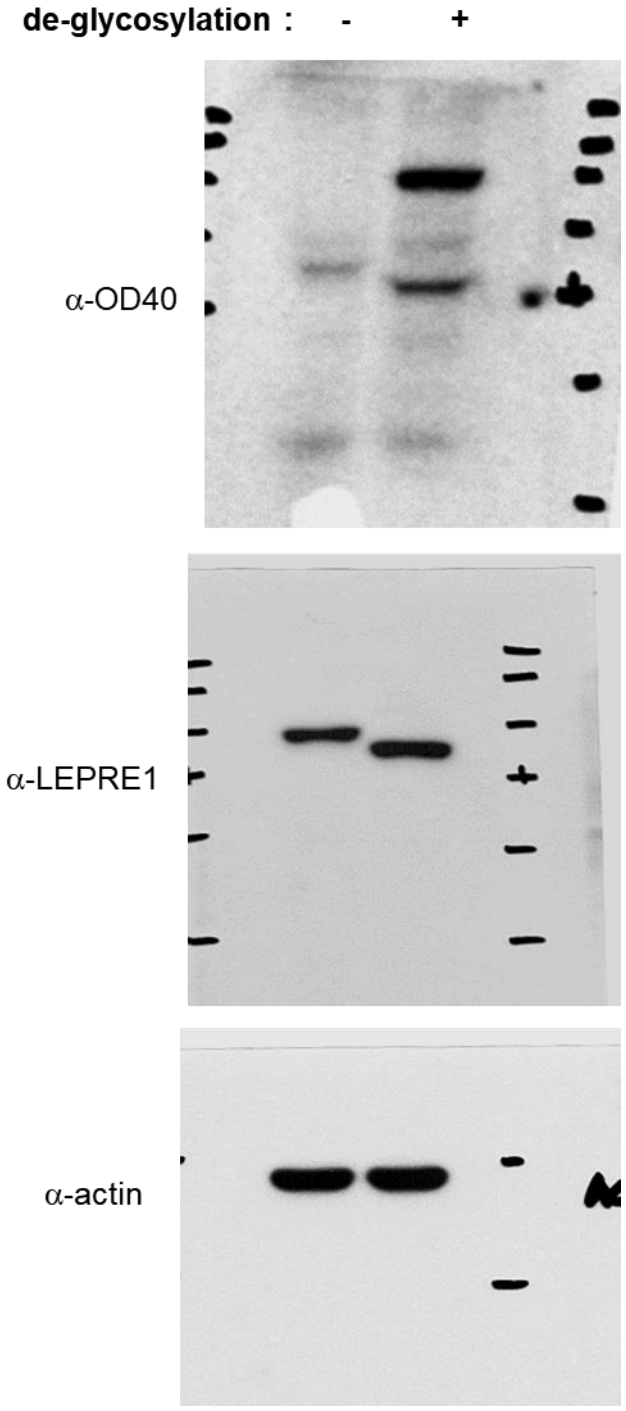


Figure 4

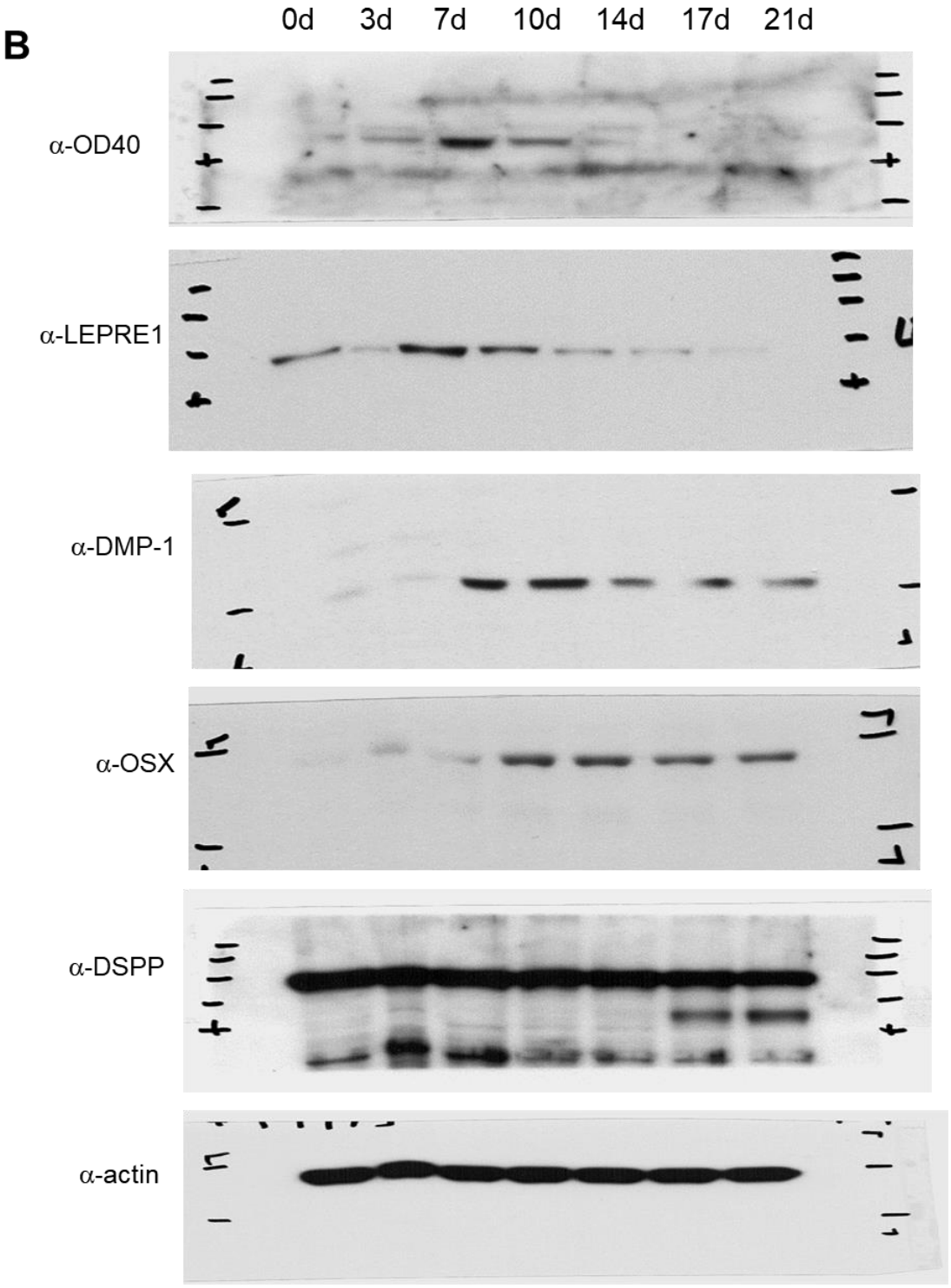

B
0d
3d
7d
10d
14d
17d
21d
α-OD40
α-LEPRE1
α-DMP-1
α-OSX
α-DSPP
α-actin

Figure 4

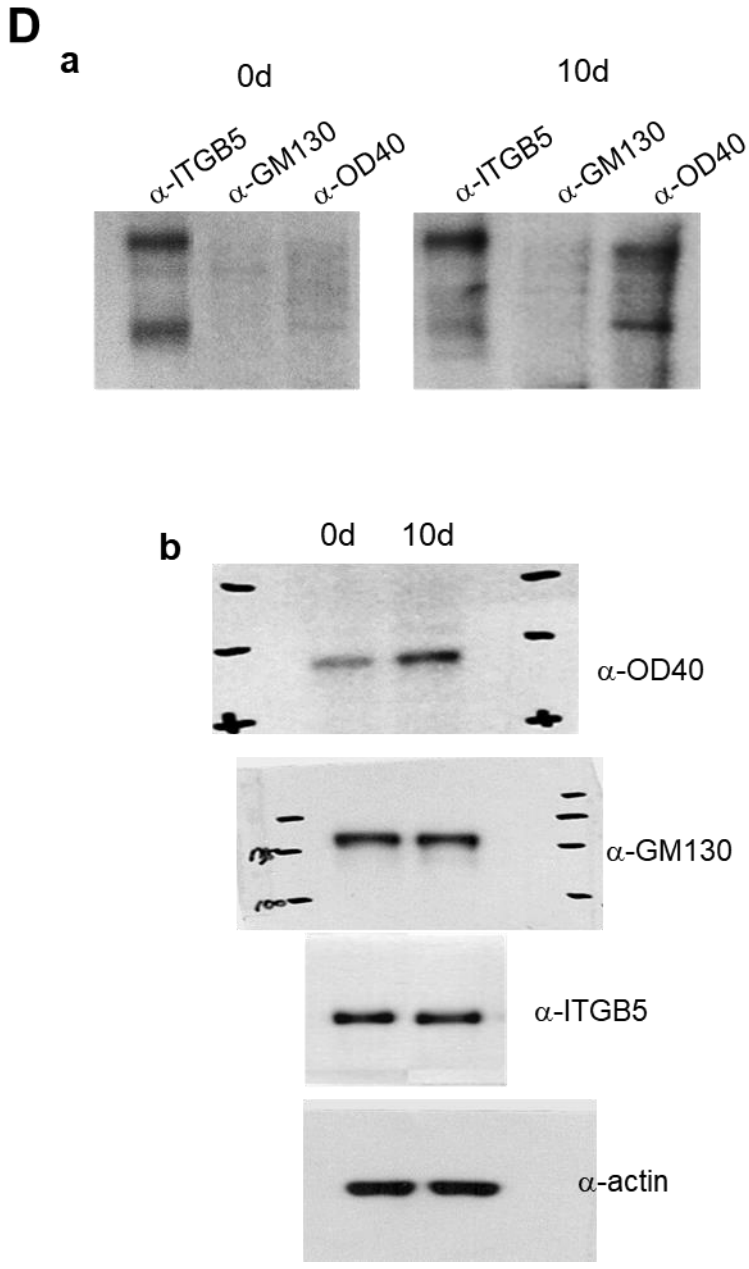

D
a
0d
10d
α-ITGB5
α-GM130
α-OD40
α-ITGB5
α-GM130
α-OD40
b
0d
10d
α-OD40
α-GM130
α-ITGB5
α-actin

Figure 5

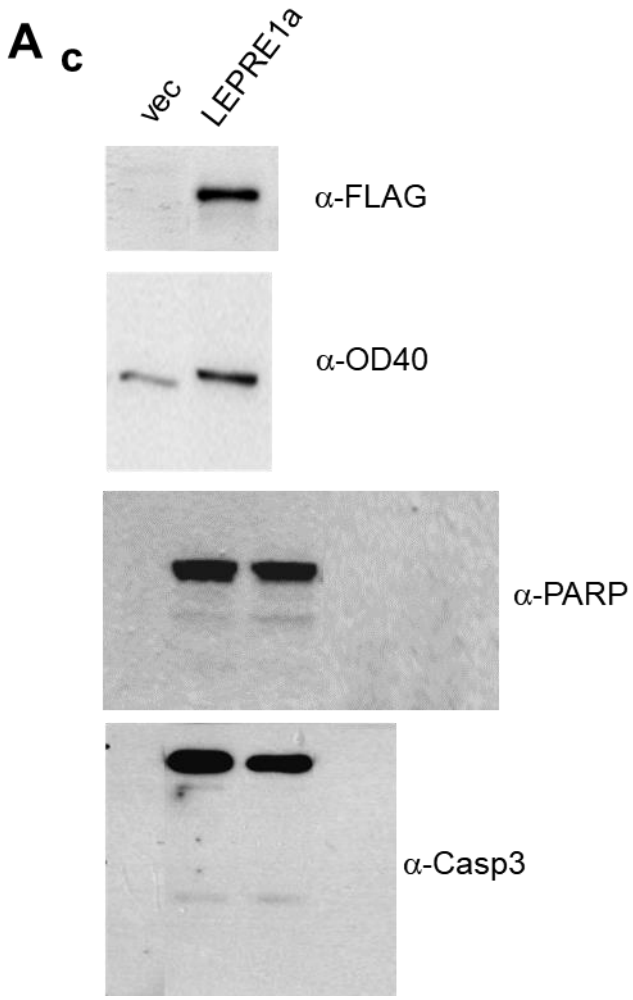


Figure 5

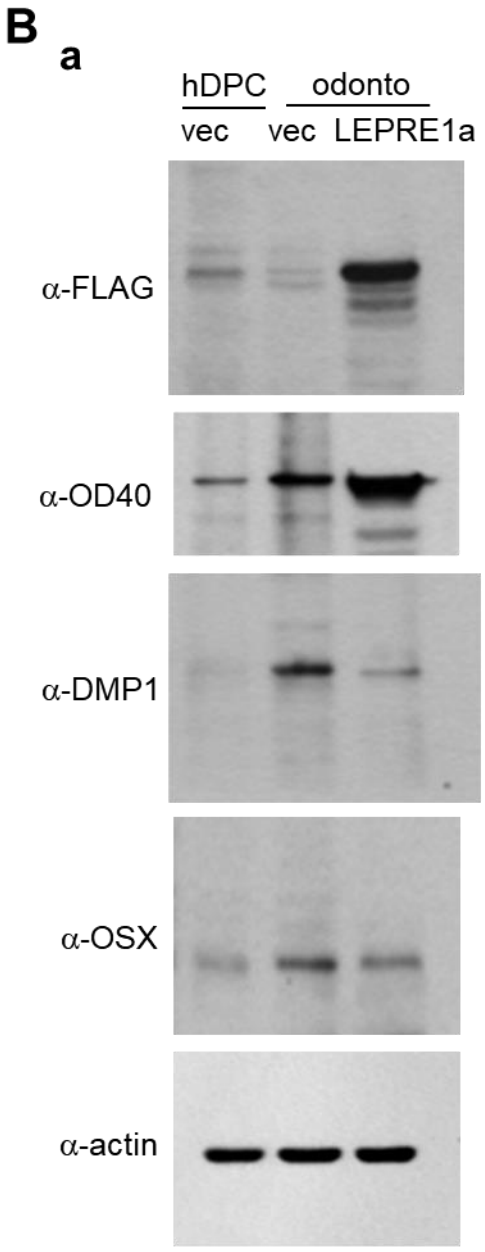

B
a
hDPC
odonto
vec
vec
LEPRE1a
α-FLAG
α-OD40
α-DMP1
α-OSX
α-actin

Figure 7