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**Background:** One of the dentinal hypersensitivity theories proposes that odontoblasts (ODs) could act as sensory receptor cells by the TRP receptors activity. The TRPV1 subtype in mouse ODs is activated by vanilloid agonists such as capsaicin. The structure-activity relationship analysis indicates that Eugenol, have a vanilloid structure and could also activate it.

**Objectives:** This work was aimed to evidence the TRPV1 expression and establish its possible chemical activation by Eugenol in human odontoblast-like cells (hOLCs).

**Methods:**

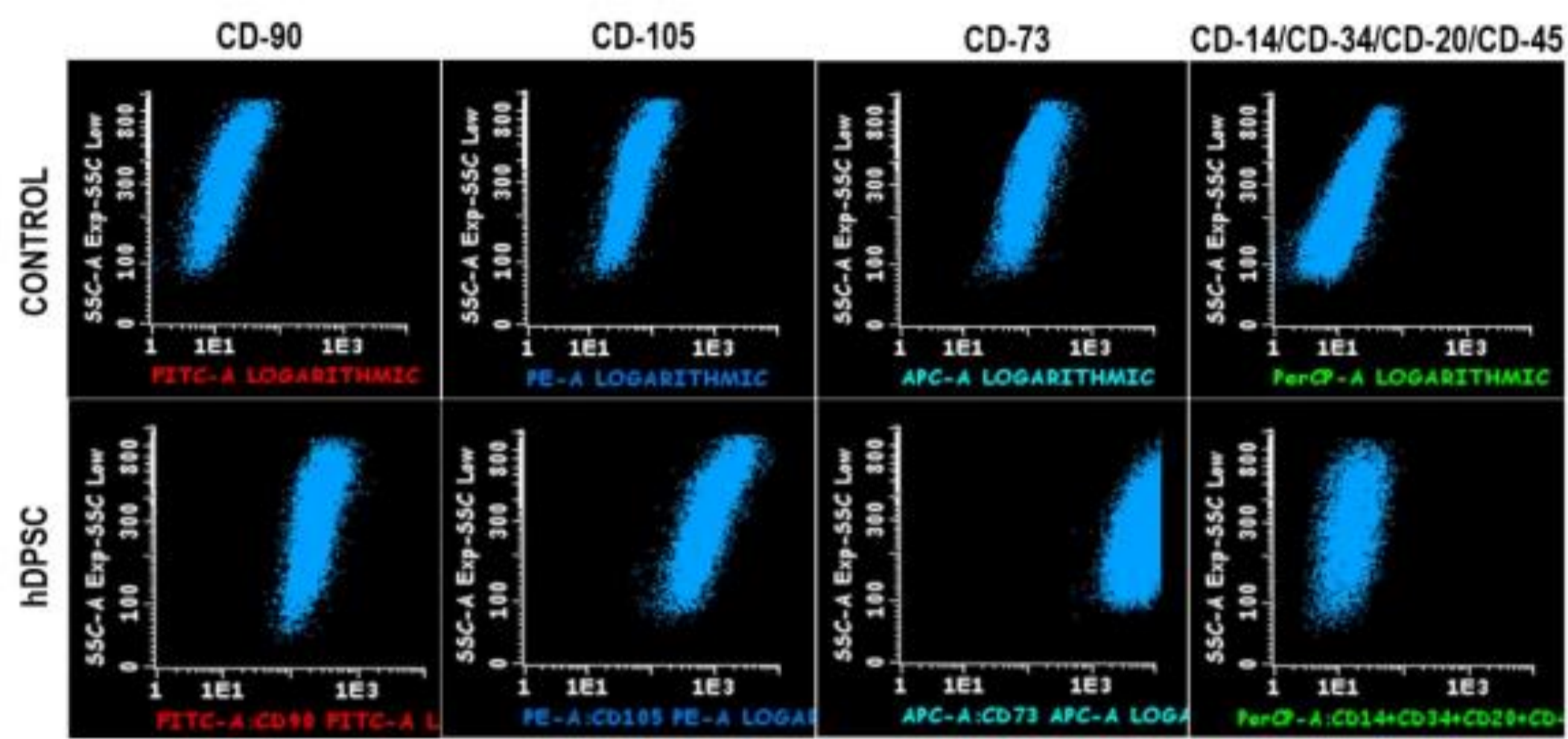
Isolation of hDPSCs and characterization (flow cytometry)

Differentiation process of hOLCs: 7, 14 and 21 days in odontogenic induction medium with TGFβ-1

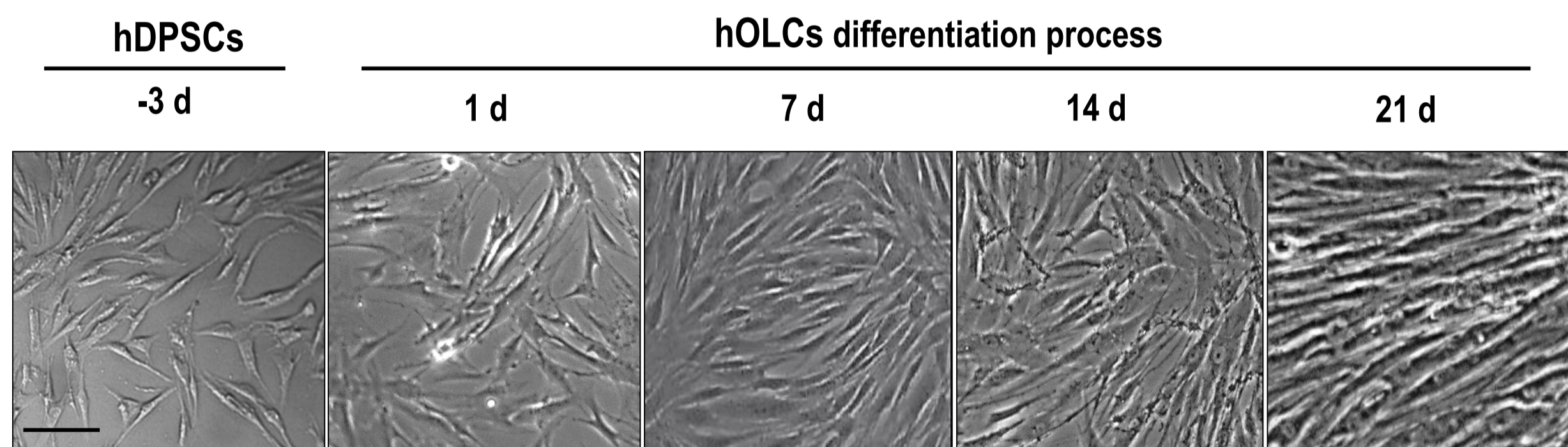
Evaluation of TRPV1 gene expression in the hOLCs (RTqPCR and immunofluorescence)

Determination of cell viability and Eugenol EC50 (resazurin assay) and the evaluation of TRPV1 activation (Fluo-4AM probe)

**Results**

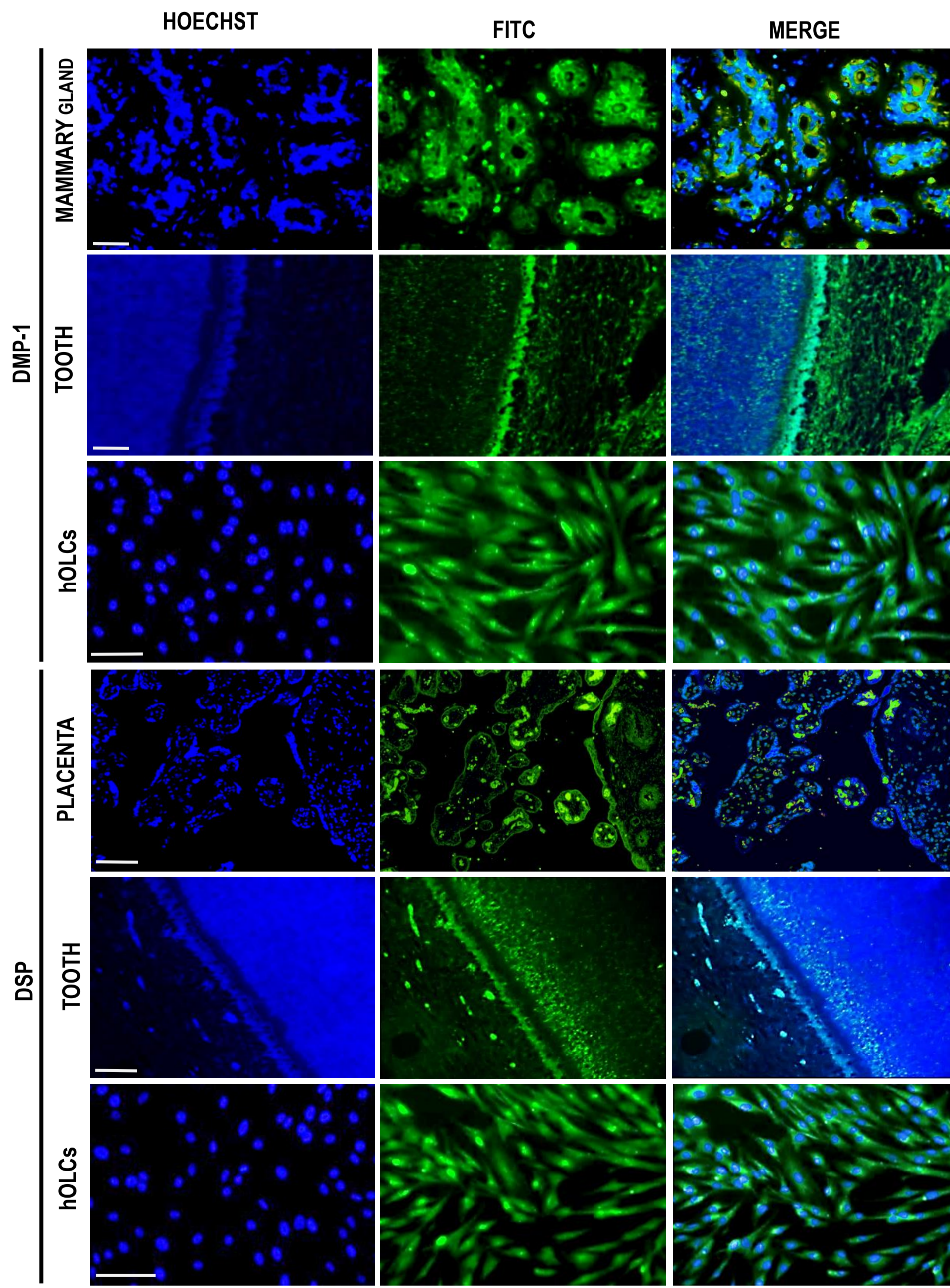


**Figure 1: Phenotypic characterization of hDPSCs.** Demonstrated positivity for mesenchymal markers CD90, CD105, and CD73 and negativity for the early hematopoietic markers CD14, CD20, CD34, and CD45.

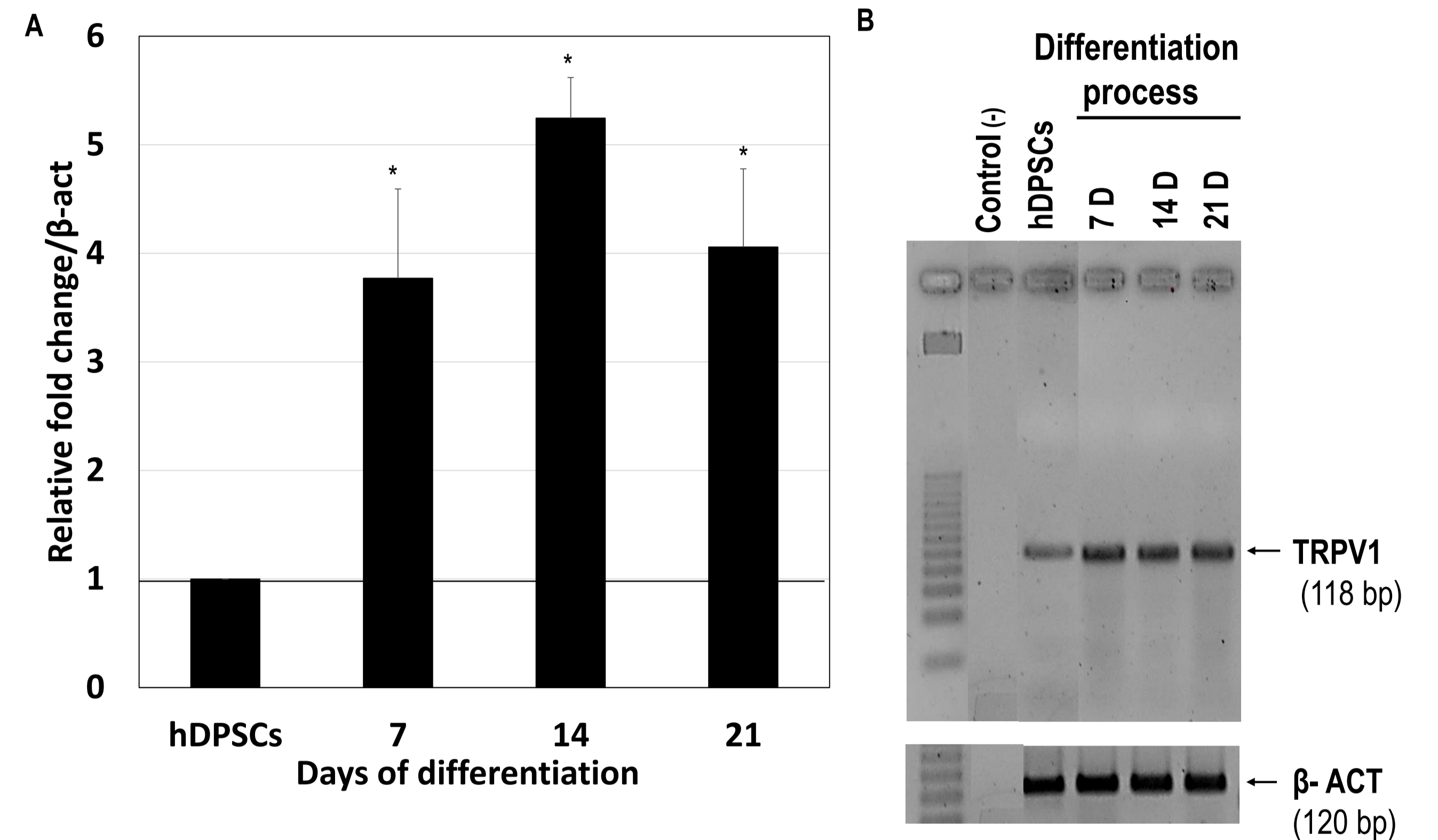
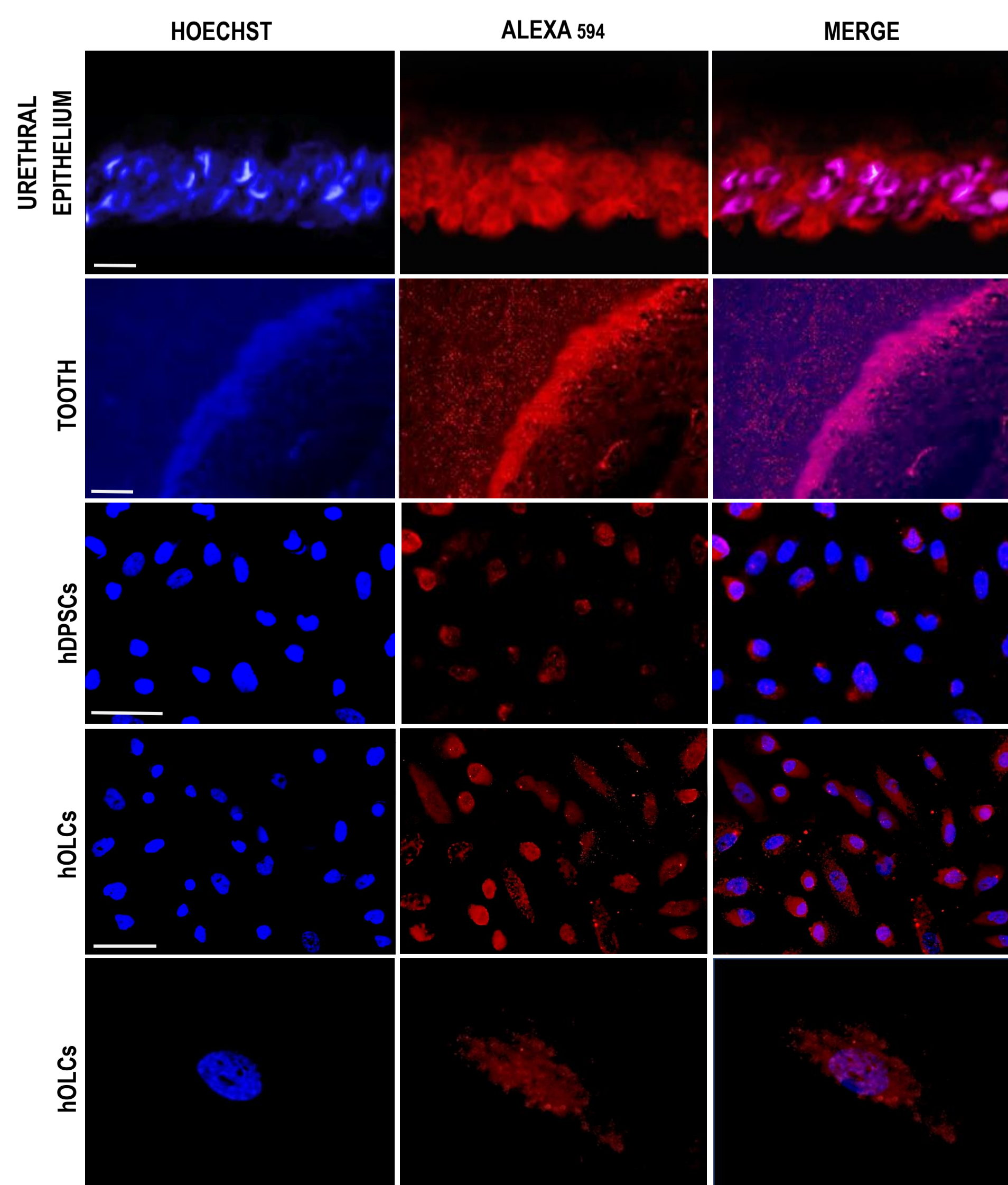


**Figure 2: Morphological cell appearance during the cell differentiation process.** hDPSCs with tapered, fibroblastic morphology, as well as hOLCs cells during the differentiation process at 7, 14, and 21 days. Scale bar: 100 μm.

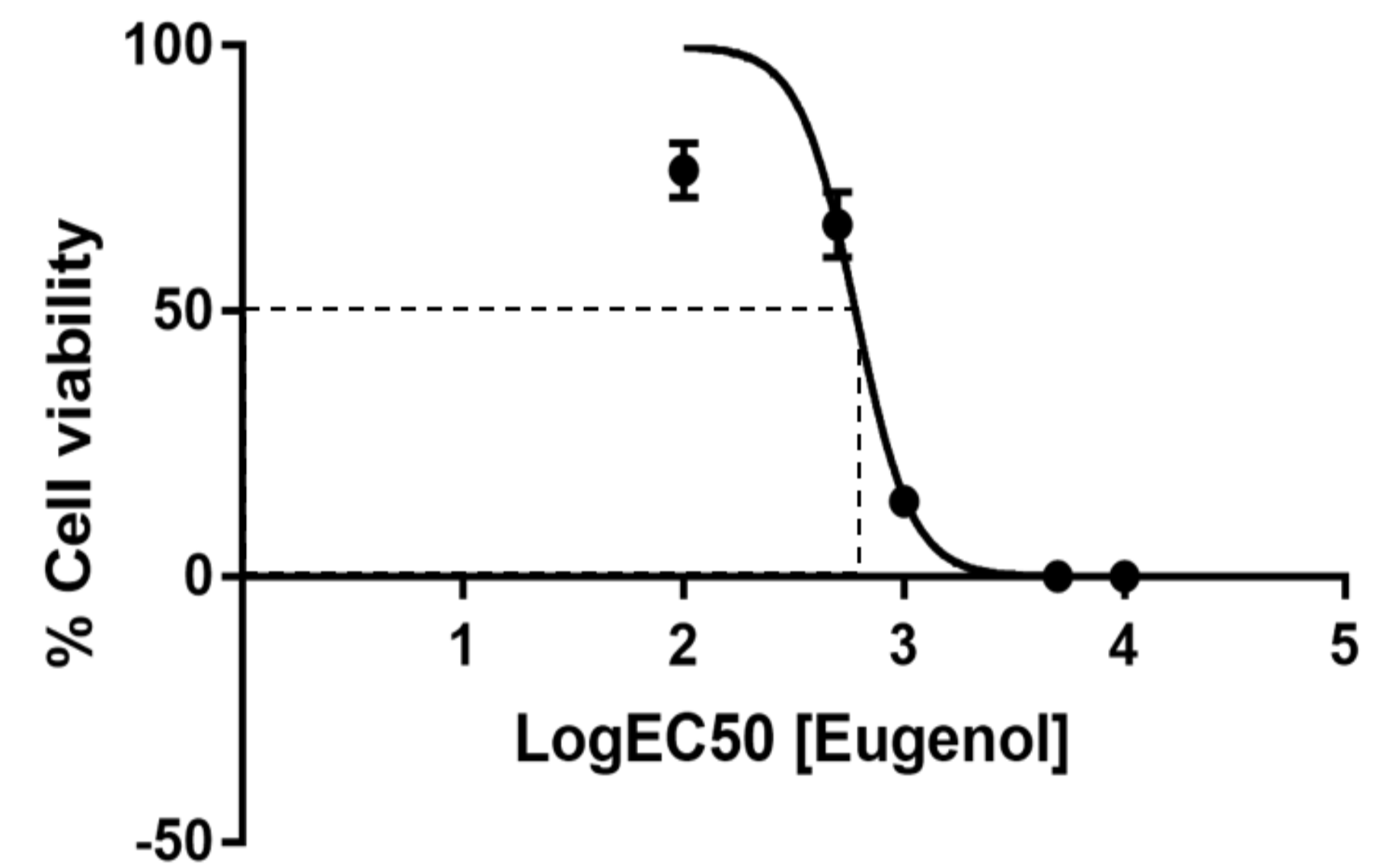
**Figure 3: Immunocytochemical detection of odontogenic markers DMP-1 and DSP.** The upper panel correspond to dentin matrix protein-1 (DMP-1), and lower panel to dentin sialoprotein (DSP). Note the positivity of these markers in hOLCs. Scale bar: 50 μm



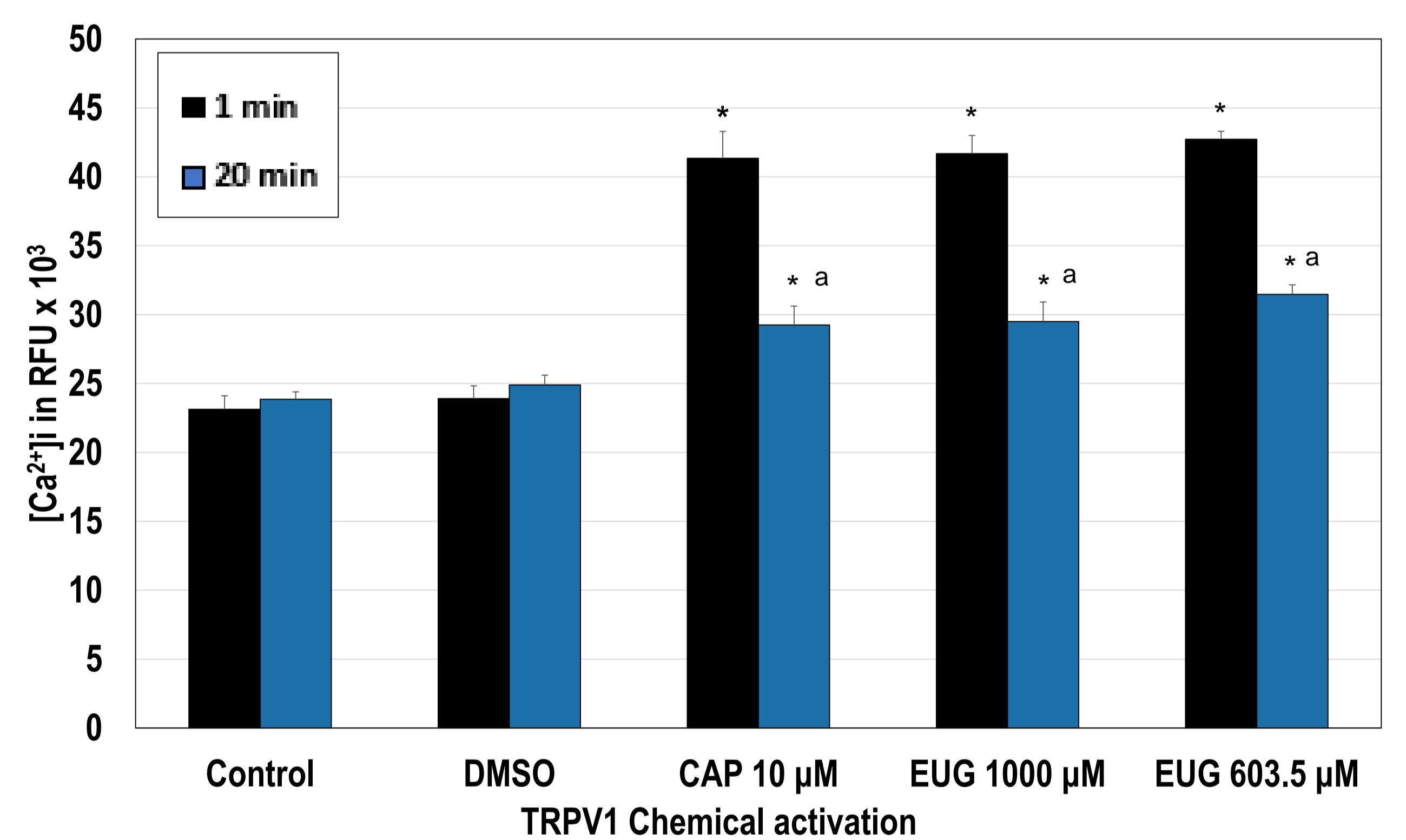
**Figure 4: Immunocytochemical detection of TRPV1.** Show positive staining to TRPV1 protein in the control of urethral epithelial tissue, in dental tissue, in hDPSCs and in hOLCs. Showed an increase in this immunostaining localized in the membrane is evident, compared to hDPSCs. Scale bar: 50 μm



**Figure 5: Relative quantification of TRPV1 at different differentiation times using RT-qPCR.** In hOLCs cells at 7, 14, and 21 days of differentiation, compared to hDPSCs (considered as 1). An overexpression of transcripts was observed for all the differentiation times. Mean ± SD (n = 9) (\*p < 0.05).



**Figure 6: Eugenol EC50 in hOLCs.** LogEC50: 2.781, equivalent to 603.5 μM. GraphPad Prism 7.00 software was used (n = 18) (p < 0.05).



**Figure 7: Chemical stimulation of the TRPV1 in hOLCs.** Stimulation assay were evaluated using the Fluo 4 AM probe. Statistically significant differences were found between the control and groups EUG and CAP at 1 min (\*p < 0.05) and TRPV1 receptor desensitization was confirmed at 20 min with both vanilloid substances (a = p < 0.05). Mean ± SD (n = 9).

**Conclusion**

hOLCs express active TRPV1 receptor and could be involved as transducer of dental painful sensation. This activation with Eugenol could induce desensitization and analgesia as capsaicin does. This finding provides information to understand the dental hypersensitivity and contribute to develop therapeutic alternative.