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Research Article

# Distinct Osteogenic Differentiation Patterns in Dental Pulp Stromal Cells from Cystic Fibrosis Patients

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## Abstract

**Background:** Cystic fibrosis (CF) is a genetic disease caused by mutations in the Cystic Fibrosis Transmembra Conductance Regulator (CFTR) gene. It affects multiple organs, and some patients develop bone-related issu such as osteopenia and osteoporosis.

**Methods:** DPSCs were characterized by immunophenotyping for mesenchymal stromal cell (MSC) marke Proliferative capacity was assessed by Ki67 immunofluorescence. Osteogenic differentiation was induced evaluate the mineralized matrix and the expression of genes including CFTR, RUNX2, and OPG by qPCR.

**Results:** Here, we describe the osteogenic differentiation of dental pulp stromal cells (DPSCs) derived from tv CF patients carrying distinct genotypes: one with homozygous F508del and another with compound heterozygo F508del/R347H. DPSCs were characterized by mesenchymal stromal cell markers and showed no differences immunophenotype or proliferative capacity compared to healthy donors. However, osteogenic differentiati outcomes varied according to genotype. DPSCs from the F508del/F508del patient exhibited enhanc mineralized matrix formation and increased RUNX2 expression, while DPSCs from the F508del/R347H patie displayed reduced osteogenesis with RUNX2 levels comparable to healthy cells. In both CF patien osteoprotegerin (OPG) expression decreased after osteogenic induction, in contrast to the increase observed healthy donors.

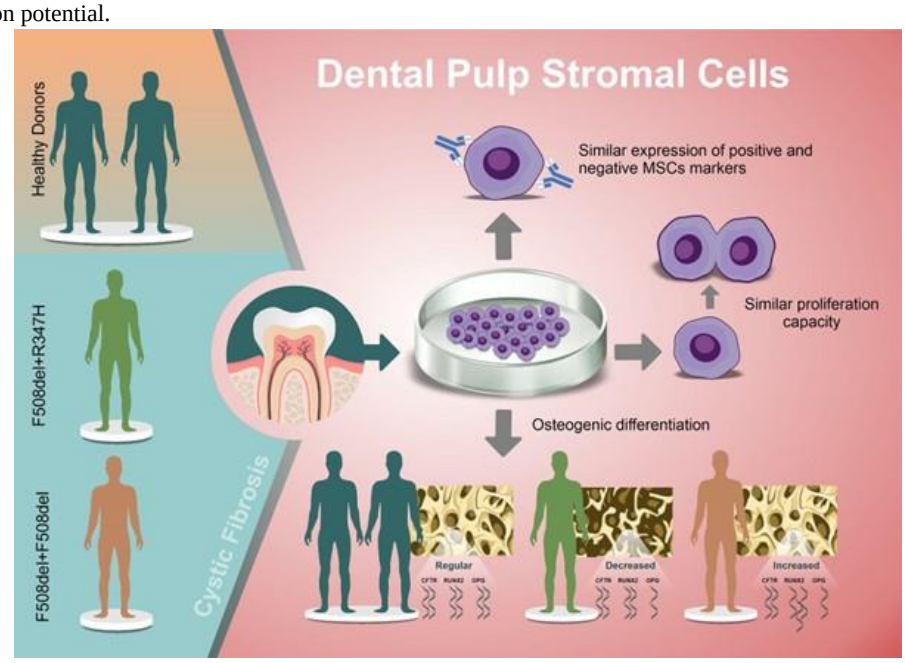
**Conclusion:** These findings demonstrate genotype-specific differences in the osteogenic behavior of DPS from CF patients, highlighting a potential contribution of CFTR mutations to bone fragility and underscoring t importance of considering molecular variability in CF-associated bone disease.

**Key Words:** cystic fibrosis; dental pulp stromal cells; osteogenesis; gene mutations; cell characterization

## Introduction

Dental pulp is a rich source of adult stromal cells, known as dental pulp stromal cells (DPSCs), which have shown promise in various regenerative medicine applications [1]. These cells have been extensively characterized by their properties such as multipotency and self-renewal [2], [3]. However, the behavior of DPSCs in individuals with chronic diseases, particularly cystic fibrosis (CF), remains largely unknown. CF is a life-threatening genetic disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene [4], which leads to defective chloride ion transport and results in the accumulation of thick, sticky mucus in the respiratory and digestive tracts [5]. The disease affects multiple organs, including the lung, pancreas, and liver, and patients with CF also suffer from a range of bone problems, such as osteopenia and osteoporosis [6]. This study aimed to assess whether DPSCs from cystic fibrosis patients preserve

their proliferative capacity and to determine how CFTR mutations influence their osteogenic differentiation potential.




## 2. Material and methods

### *2.1 Case Report Description*

Dental pulp stem cells (DPSCs) were obtained from four pediatric donors. The first donor CFTR(WT-1), a five-year-old boy, had no CFTR mutations, and the cells were isolated from a primary tooth. The second donor CFTR(WT-2), a girl, also carried no CFTR mutations, and DPSCs were collected from a primary tooth; however, clinical information such as age, weight, and height was not available. The third donor CFTR(F508del+R347H), a four-year-old girl, carried compound heterozygous mutations in the CFTR gene: F508del (deletion of phenylalanine at position 508) and R347H (arginine to histidine substitution at position 347). DPSCs were derived from primary tooth. The fourth donor CFTR(F508del+F508del), a two-year-old girl, carried the homozygous F508del / F508del mutation, and DPSCs were also isolated from a primary tooth. DPSCs derived from primary teeth are widely used as a source of mesenchymal stromal cells [7], [8], [9]10].

### *2.2 Study settings and ethical considerations*

This study was approved by the Ethics Committee of Fundação Oswaldo Cruz, Brazil (CAAE: 80641317.1.0000.5248), Hospital de Clínicas da Universidade Federal do Paraná (CAAE: 80641317.1.3003.0096), and Pontifícia Universidade Católica do Paraná - PUCPR (CAAE: 80641317.1.3001.0020). Written informed consent was obtained from all guardians or donors prior to sample collection, and all procedures were carried out in compliance with the guidelines set forth by the respective ethics committees. To ensure the privacy and confidentiality of the donors, all samples were coded and handled in accordance with the relevant ethical and legal regulations.

### *2.3 Tooth pulp collection, isolation and maintenance*

To isolate DPSCs, teeth were washed in phosphate-buffered saline solution (PBS) and the tooth pulp fragments were mechanically removed with a K file, as previously described by Fracaro et al., (2020). The pulp tissue was then dissociated using collagenase type II and centrifuged in PBS. The resulting cells were resuspended and plated in flasks with Iscove's Modified Dulbecco's Media IMDM supplemented with 1% antibiotic penicillin/streptomycin/amphotericin B, 8 µg/mL vancomycin and 15% fetal bovine serum FBS. All cell cultures were maintained in an incubator at 37°C with 5% CO2. DPSCs were maintained in DMEM medium supplemented with 10% FBS and antibiotics in a humidified chamber at 37°C with 5% CO2. All experiments were performed using cells in passages 3-5 to ensure consistency in culture conditions.

### *2.4 Sequencing by sanger*

The patients recruited for this study had a medical report and sequencing describing the mutations present in the gene CFTR, from this we confirmed the presence of the F508del and R347H mutation. The DNA was extracted from DPSCs samples from healthy donors and donors with cystic fibrosis using the QIAamp® DNA Mini Kit. The region of the CFTR gene corresponding to the mutation site F508del and R347H was amplified by PCR using the CFTR_DNA_F508del and CFTR_DNA_R347H primers (Table 1). The PCR products were sequencing by GoGenetics.

### *2.5 Immunophenotyping of DPSCs*

The cells were resuspended with trypsin and then treated with 1% PBS/BSA and incubated on ice for one hour. After incubation, the cells were centrifuged, and antibodies conjugated against CD90, CD105, CD73, CD34, CD11b, CD45, CD19, CD140b, CD31, and HLA-DR were added for labeling[11]. Following labeling, the cells were washed with PBS and fixed with 4% paraformaldehyde. Analysis of cell surface marker expression was performed using a FACSCanto II flow cytometer, and the data were analyzed using FlowJo™ version 10.6.3 software. The data were compared between healthy and cystic fibrosis donors to evaluate potential differences in DPSCs populations.

### *2.6 Proliferation assay*

DPSCs were plated at two different confluence levels, 40% (3000 cells/well) and 90% (8000 cells/well), in a 96-well plate. After 48 hours, the cells were fixed with 4% paraformaldehyde, washed, and permeabilized with Triton X-100 (0.5%) for 30 minutes. The cells were then washed and incubated with 1% PBS/BSA for one hour. Next, an anti-Ki67 primary antibody (anti-rabbit - Abcam, 1:300) diluted in 1% PBS/BSA was added and incubated for one hour under agitation. The cells were then washed and incubated with the

secondary antibody (anti-rabbit IgG 488 - Invitrogen, 1:800) diluted in 1% PBS/BSA for one hour under agitation. After incubation with DAPI for 10 minutes and washing, the samples were stored in PBS (1x) until the time of reading using the Operetta high content imaging system (Waltham, MA, USA) with 20x magnification, 25 photos each well, 4 wells each condition. To quantify the nuclei, images were acquired on the DAPI channel (355–385 nm excitation and 430–500 nm emission) and Ki67 positive cells by acquiring images on the Alexa 488 channel (460–490 nm excitation and 500–550 nm emission). Quantitative analysis was performed using the Harmony high-content analysis software 4.8 (PerkinElmer). The percentage of Ki67-positive cells was calculated and compared between DPSCs from healthy and CF donors[12].

### *2.7 Osteogenic differentiation*

DPSCs were plated at a concentration of 3.5 x 10^3 cells/well in a 96-well plate. After 24 hours, the medium was removed, and the induction medium for osteogenic differentiation (StemPro® Osteogenesis Differentiation Kit) was added. The medium was changed every three days for 28 days. Cells were labeled with OsteoImage™ Mineralization Assay and DAPI according to the manufacturer's instructions. The stained cells were examined under the Operetta CLS High-Content Imaging System, and images were acquired using Harmony 4.8 software (PerkinElmer). A total of 25 images were acquired per well at 20x magnification, from four wells and experimental triplicate. To quantify the nuclei, images were acquired on the DAPI channel (355–385 nm excitation and 430–500 nm emission), and nuclei with circularity > 0.9 were considered in the analysis to exclude cellular debris. The percentage of mineralization area was determined by acquiring images on the Alexa 488 channel (460–490 nm excitation and 500–550 nm emission). The data were analyzed by calculating the positive area for Alexa 488 (green) using high-content imaging analysis. The percentage of mineralization area was compared between DPSCs from healthy and cystic fibrosis donor[13].

### *2.8 RNA isolation, cDNA synthesis and quantitative PCR*

To assess gene expression, total RNA was extracted from DPSCs cultured under osteogenic conditions for 7 days and control conditions using the RNeasy Mini Kit , following the manufacturer's instructions. cDNA was synthesized using 1 µg of total RNA and the ImProm-II Reverse Transcription System . Quantitative PCR (qPCR) was performed using the Applied Biosystems QuantStudioTM5 Real-time PCR system and GoTaq Polymerase Mix with 5 µM forward and reverse primers (Table 1). Three technical replicates were performed for each reaction, and the expression ratio to POLR2A (Fold to POLR2A) was calculated using the Design and Analysis Software v2.6.[13].

### *2.9 Statistical analysis*

Statistical analysis was performed using GraphPad Prism version 7.0. The proliferation assay, osteogenic differentiation and quantitative PCR were evaluated by two-way repeated measures analysis of variance (ANOVA). The results are presented as mean ± standard deviation (SD), and statistical significance was $p < 0.05$.

## 3. Results

Isolating stromal cells from patients with CF presents several challenges, including the difficulty of obtaining viable cells from a rare sample and the presence of antibiotic-resistant microbiota, which leads to recurrent contamination in cell culture. Despite these obstacles, we successfully isolated stromal cells from the tooth pulp of two patients with CF, each with a specific genotype, and two health donors. Donors´ information, including confirmation of the R347H mutation and F508del mutation are shown in Figure 1. Immunophenotyping of DPSCs from healthy and cystic fibrosis donors present expression of mesenchymal stem cell markers CD90, CD105, CD73, and CD140b, and absence of negative markers CD34, CD11b, CD45, CD19, CD31, and HLA-DR (Figure 2 and Supplementary Figure S1, S2 and table S1). The effect of CFTR gene mutations on the proliferative activity of DPSCs, was analyzed by the rate at which cells divide and replicate using a Ki67 proliferation assay. DPSCs were plated at 40% and 90% confluence, and the expression of Ki67 was assessed using immunofluorescence. Images were captured using high-content imaging (Figure 3A), and the percentage of Ki67-positive cells was compared between cystic fibrosis and healthy donors (Figure 3B). Our results revealed no significant difference in the proliferative activity of DPSCs between cystic fibrosis and healthy donors, suggesting that CFTR mutations do not impact the proliferation of DPSCs.

Markers positive

| Markers | CFTR$^{(WT-1)}$ | CFTR$^{(WT-2)}$ | CFTR$^{(F508del+R347H)}$ | CFTR$^{(F508del+F508del)}$ |
|---|---|---|---|---|
| CD90 | 90,6 | 81,3 | 69 | 78,7 |
| CD73 | 90,08 | 82,5 | 84,2 | 98,8 |
| CD105 | 78,9 | 66,1 | 70,4 | 89,4 |
| CD140b | 57,2 | 91,5 | 63,8 | 85 |

Markers negative

| Markers | CFTR$^{(WT-1)}$ | CFTR$^{(WT-2)}$ | CFTR$^{(F508del+R347H)}$ | CFTR$^{(F508del+F508del)}$ |
|---|---|---|---|---|
| CD34 | 2,16 | 1,48 | 2,53 | 2,44 |
| CD11b | 2,47 | 1,19 | 2,49 | 3,62 |
| CD45 | 2,66 | 1,83 | 0,18 | 3,83 |
| CD19 | 1,98 | 1,25 | 1,89 | 2,44 |
| CD31 | 0,96 | 1,16 | 1,54 | 0,08 |
| HLA-DR | 3,11 | 2,66 | 0,69 | 3,62 |

**Table S1: Analysis of marker expression in MSCs.** Values referring to the percentage of positive cells after analysis using flow cytometry for positive and negative markers for MSCs.

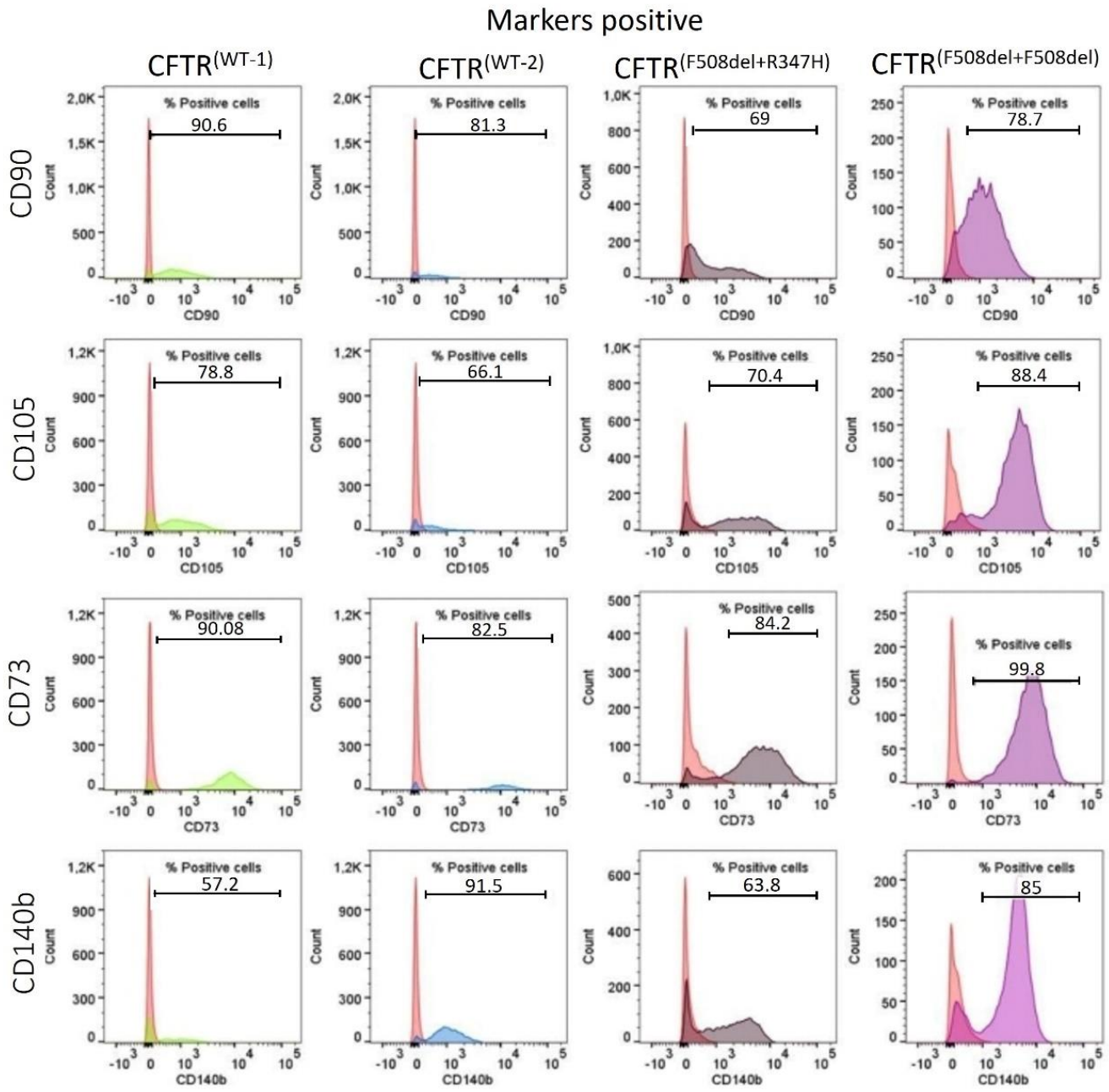



**Figure S1: Analysis of DPSCs marker expression.** Representative flow cytometry histograms showing positive markers for MSCs. The pink line represents the isotypic control, and the colors represent different donors: Green for CFTR$^{(WT-1)}$, Blue for CFTR$^{(WT-2)}$, Black for CFTR$^{(F508del+R347H)}$, and Purple for CFTR$^{(F508del+F508del)}$.

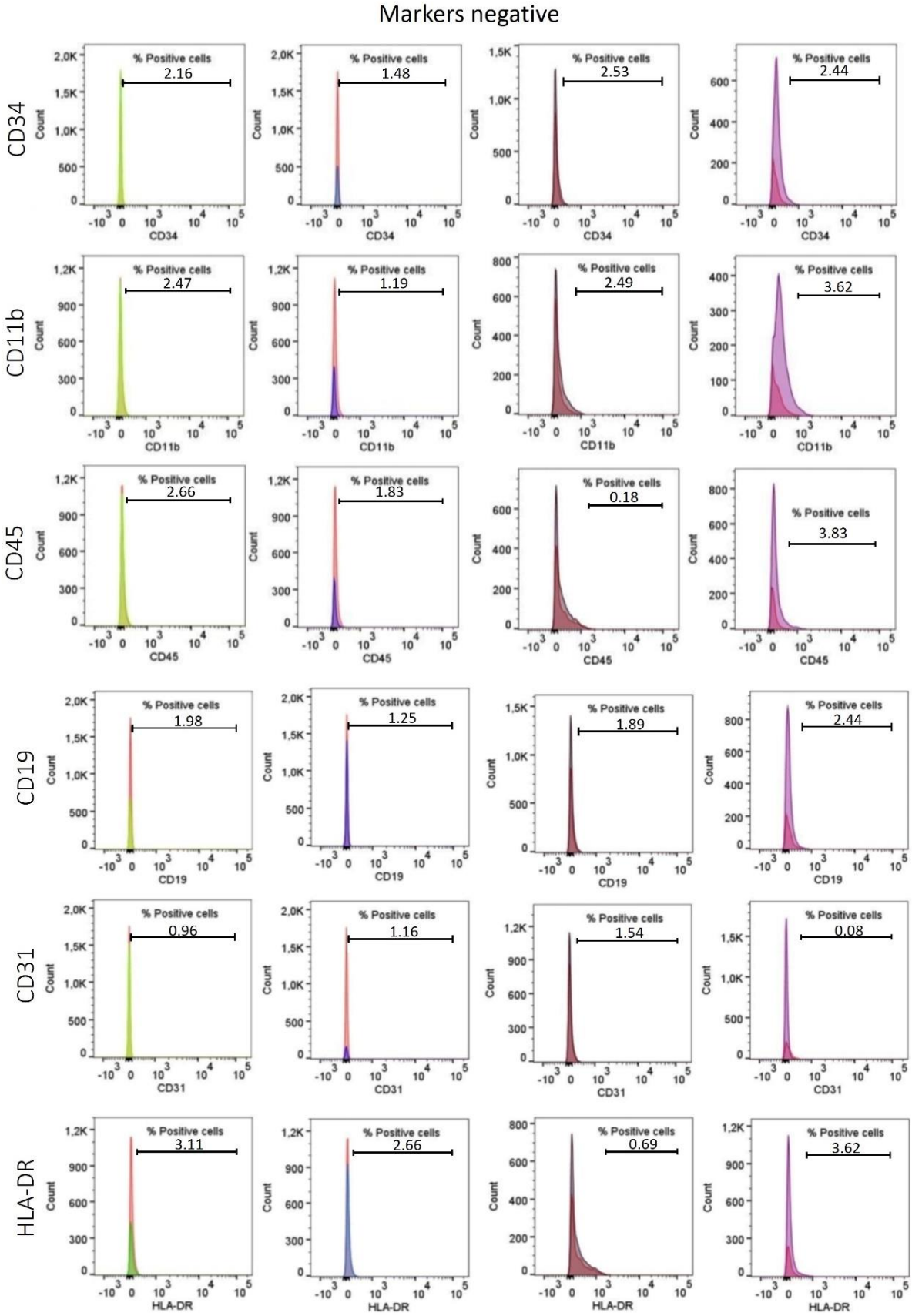



**Figure S2: Analysis of DPSCs marker expression.** Representative flow cytometry histograms showing negative markers for MSCs. The pink line represents the isotypic control, and the colors represent different donors: Green for $CFTR^{(WT-1)}$, Blue for $CFTR^{(WT-2)}$, Black for $CFTR^{(F508del+R347H)}$, and Purple for $CFTR^{(F508del+F508del)}$.

We hypothesized that CFTR gene mutations could affect osteogenesis in CF patients. To test this hypothesis, we induced osteogenic differentiation of DPSCs from healthy and cystic fibrosis donors and quantified the area of differentiation using the Osteoimage Mineralization Assay, which stains the mineralized matrix (Alexa 488) (Figure 4A). The quantification of osteogenic differentiation was evaluated using the positive area for Alexa 488 (Green) through high-content imaging (Figure 4B). Our results revealed that DPSCs with homozygous F508del mutations had a significantly higher ability to differentiate compared to cells with heterozygous F508del and R347H mutations. Conversely, cells with heterozygous mutations showed a reduction in osteogenic differentiation compared to cells with homozygous mutations and healthy (Figure 4B). These findings suggest that the osteogenic capacity of DPSCs may vary depending on the types of mutations present in the CFTR gene. However, further studies are needed to confirm these results and explore the potential mechanisms underlying these findings.

A

| ID Donor | Age | Weight (Kg) | Height (m) | BMI (Kg/m$^2$) | Sex | Cells Derived from | Mutation | Classe mutation |
|---|---|---|---|---|---|---|---|---|
| CFTR$^{(WT-1)}$ | 5 | 55 | 1.54 | 23.19 | M | Pulp of primary tooth | No | - |
| CFTR$^{(WT-2)}$ | n.i | n.i | n.i | - | M | Pulp of primary tooth | No | - |
| CFTR$^{(F508del+R347H)}$ | 4 | 24 | 1.19 | 24 | F | Pulp of primary tooth | F508del+R347H | II, IV |
| CFTR$^{(F508del+F508del)}$ | 2 | 22 | 1.07 | 22.07 | F | Pulp of primary tooth | F508del+F508del | II |

B

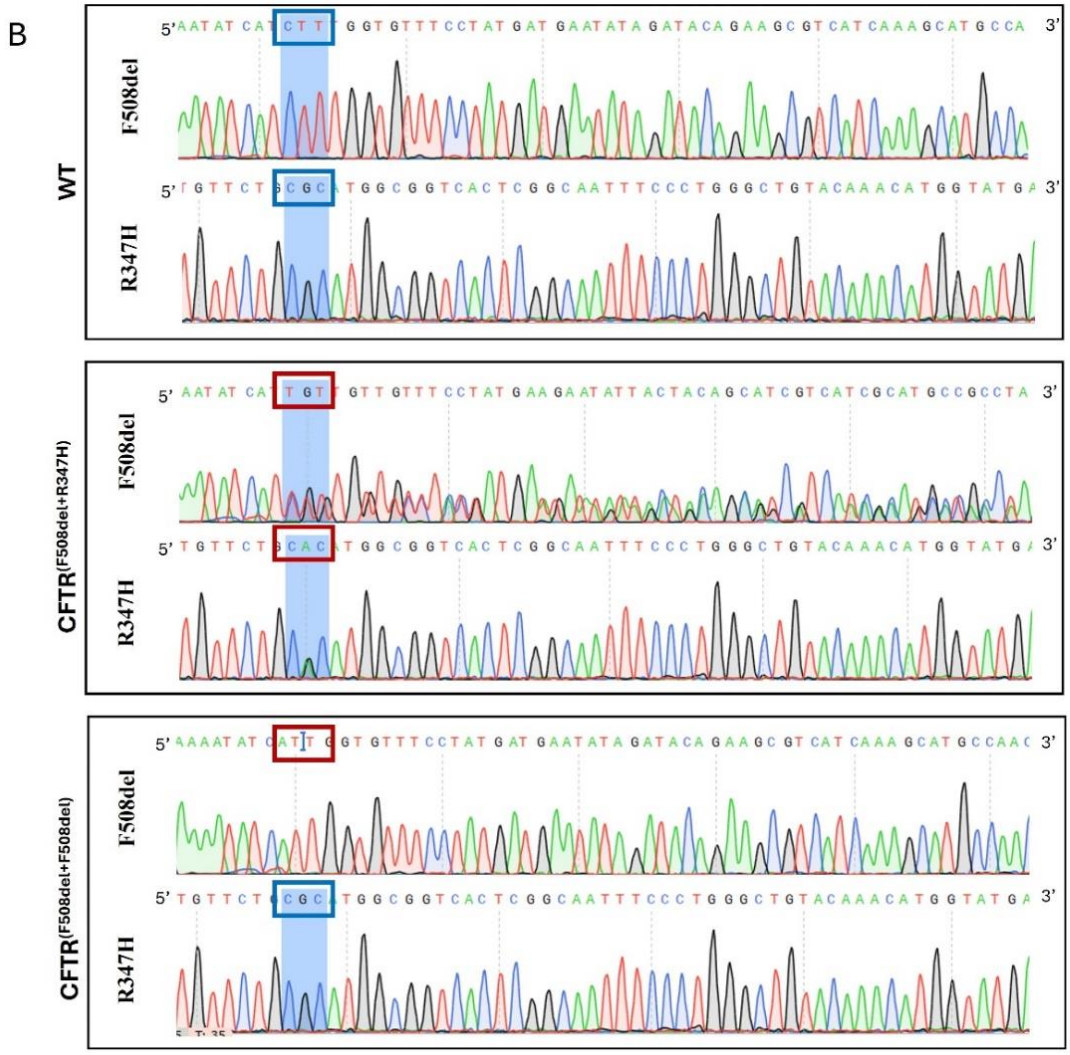



**Figure 1: Donor profiles and CFTR mutation status.** (A) Donor characteristics. Abbreviations: CFTR, cystic fibrosis transmembrane conductance regulator; WT, wild type; F, female; M, male; BMI, body mass index; n.i., not informed. (B) Sanger sequencing electropherograms of CFTR PCR products from wild-type (WT) and cystic fibrosis (CF) donors confirm the presence of the F508del deletion and the R347H substitution. The WT sample displays the expected CFTR sequence (blue), whereas the CF samples exhibit the respective mutations highlighted in red.

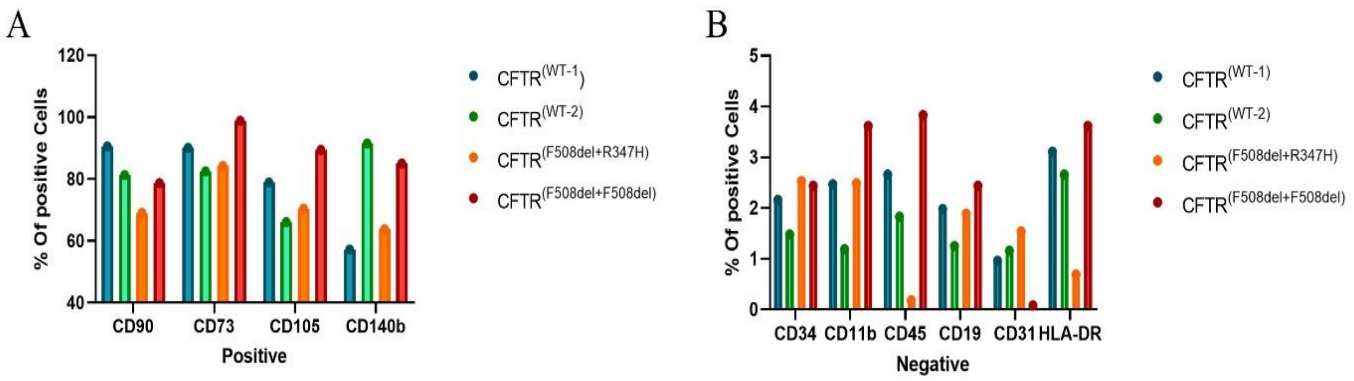



**Figure 2: Analysis of DPSC marker expression.** (A) Percentage of cells expressing positive mesenchymal markers. Each marker is shown on the X-axis, and donors are categorized as wild type CFTR (WT-1, WT-2) or cystic fibrosis CFTR (F508del/F508del, F508del/R347H). (B) Percentage of cells expressing negative markers. Each marker is shown on the X-axis, with the same donor categorization as in (A).

| Official Symbol | NCBI ID | Primer sequence (5'-3') | Amplicon (bp) |
|---|---|---|---|
| CFTR_RNA | NM_000492 | Forward: 5' - ATGGGAGAACTGGAGCCTTC - 3'<br>Reverse: 5' - CACCGAATGGTGCAGGCATACC - 3' | 92 |
| CFTR_DNA__F508del | NC_000007.14 | Forward: 5' - ATGGGAGAACTGGAGCCTTC - 3'<br>Reverse: 5' - AGTTTCTTACCTTCTCTAGTTG - 3' | 180 |
| CFTR_DNA__ R347H | NC_000007.14 | Forward: 5' - TATTTGAAAAATAAAATAAC - 3'<br>Reverse: 5' - GTATTAGCTGGCAACTTTTA - 3' | 443 |
| OPG | NM_002546.4 | Forward: 5' - GCTCACAAAGCACAGCATTTCCAG - 3'<br>Reverse: 5' - CTGTTTTCTACAGAGGCAAATATTTCT - 3' | 106 |
| POLR2A | NM_078569.3 | Forward: 5' - TACACGTCATCCTCTTTGATGGCT - 3'<br>Reverse: 5' - GTGCGGCTGCTTCCATAA - 3' | 186 |
| RUNX2 | NM_001015051.4 | Forward: 5' - ACTGGCGCTGCAACAAGAAC - 3'<br>Reverse: 5' - CCCGGCATGACAGTAACCA - 3' | 91 |

**Table 1:** Primer sets used for RT-qPCR analysis of gene expression. The primer sets were designed for CFTR (CF transmembrane conductance regulator), OPG (NF receptor superfamily member 11b), POLR2A (RNA polymerase II subunit A), and RUNX2 (RUNX family transcription factor 2) genes.

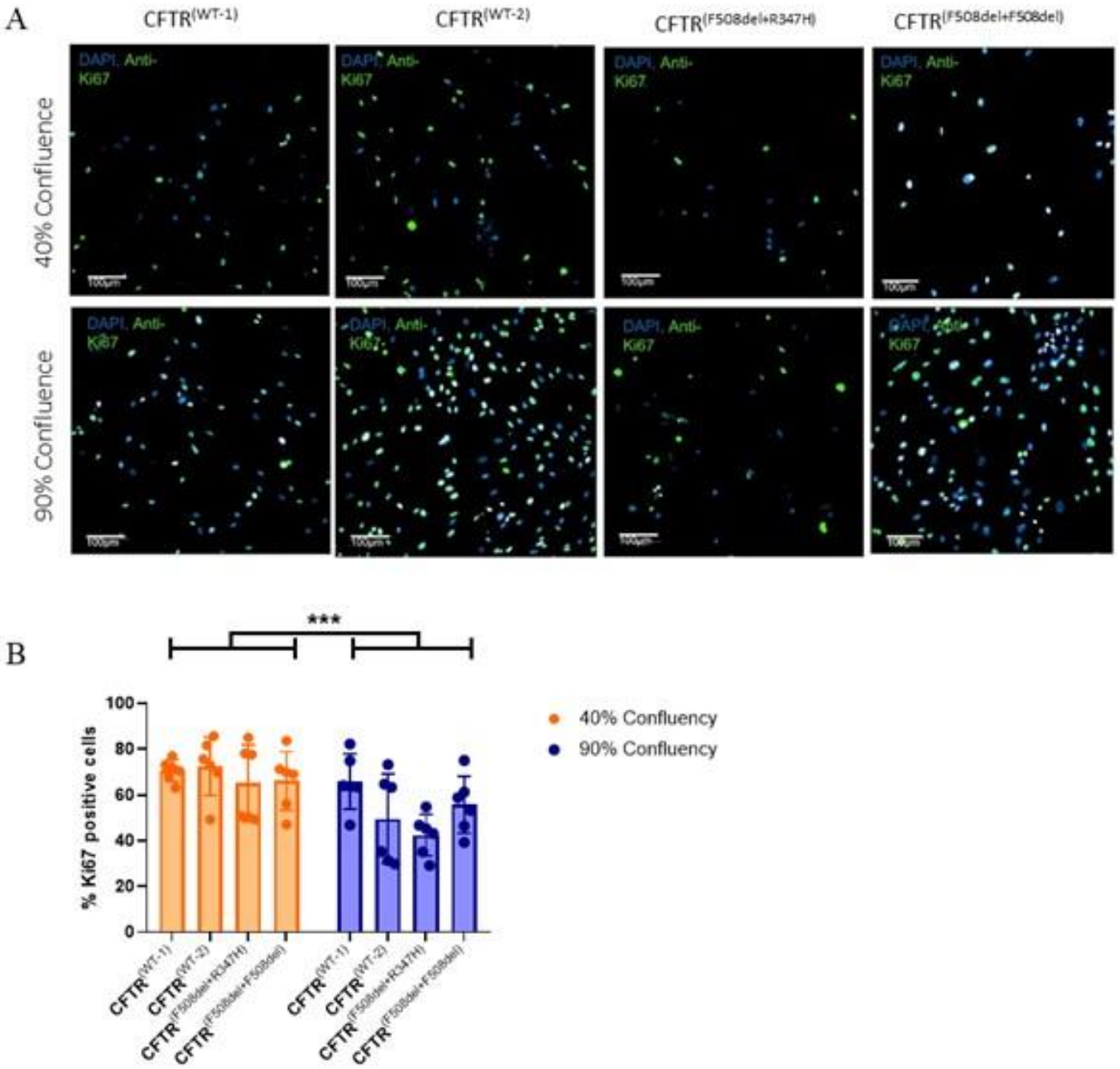



**Figure 3: Proliferation analysis of DPSCs.** (A) Immunofluorescence labeling of Ki67 in the nuclei of cells in the proliferative phase. Cells were plated at 40% and 90% confluence. Nuclei were stained with DAPI (blue) and Ki67 was detected with an anti-Ki67 antibody (green). Images were acquired using the Operetta system. Scale bar: 100 µm. (B) Quantification of Ki67-positive cells. Experimental replicates at 40% confluence are shown in orange and at 90% confluence in blue. Each donor is represented individually on the X-axis, with the percentage of Ki67-positive cells on the Y-axis. Statistical analysis: two-way ANOVA, ***$p < 0.001$.

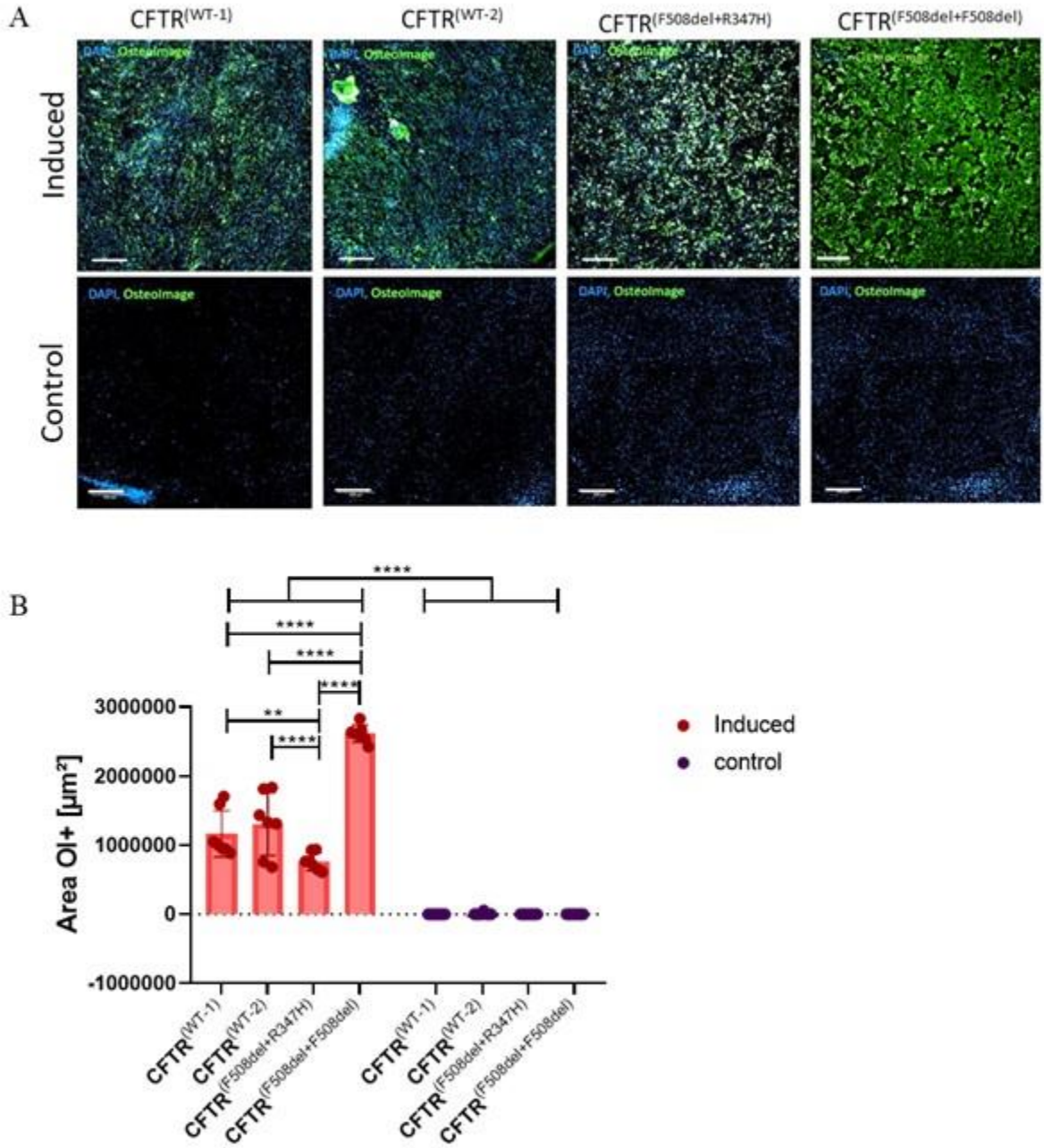



**Figure 4: Osteogenic differentiation of DPSCs**. (A) Representative images of cells induced for osteogenesis (Induced) and non-induced cells (Control) for 28 days. Mineralized matrix was stained with OsteoImage™ (green, Alexa 488), and nuclei were stained with DAPI (blue). Images acquired with Operetta. Scale bar: 500 µm. (B) Quantification of osteogenic differentiation based on the OsteoImage™-positive area (OI+, µm²). Induced samples are shown in red, and controls in purple. Statistical analysis: two-way ANOVA, **p < 0.01, ****p < 0.001.

The genes RUNX2 and OPG (also known as tumor necrosis factor receptor superfamily member 11b, TNFRSF11B) are key regulators of osteogenesis, where a tightly controlled balance is essential for maintaining bone tissue integrity. To investigate the molecular basis of the genotype-dependent differences observed in mineralized matrix formation, we performed quantitative PCR to assess the expression of CFTR, RUNX2, and OPG (Figure 5). CFTR expression remained unchanged after seven days of osteogenic induction. RUNX2 expression in DPSCs heterozygous for the F508del mutation was comparable to that of healthy cells, whereas DPSCs from homozygous F508del donors showed elevated RUNX2 levels. In parallel, OPG expression increased in healthy donors but decreased in CF donors after seven days of osteogenic induction, highlighting genotype-specific regulation of osteogenic pathways.

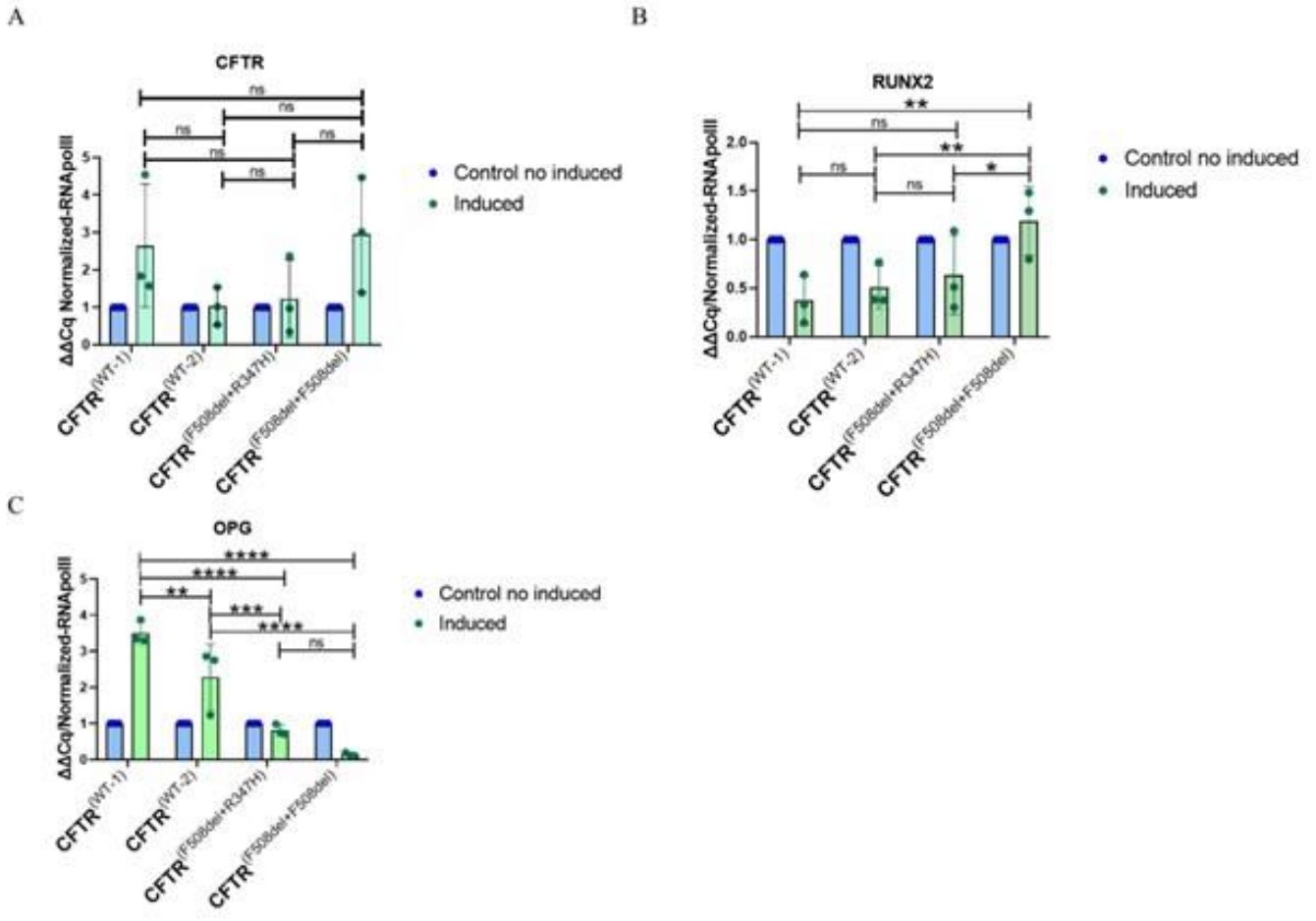



**Figure 5: Gene expression of *CFTR* (A), RUNX2 (B) and OPG (C) in DPSCs.** Evaluation of *CFTR*, RUNX2 and OPG gene expression in DPSCs from healthy donors and CF carriers, induced to osteogenic differentiation for 7 days. Samples normalized with POLR2A. OPG (osteoprogerin), CFTR (cystic fibrosis transmembrane conductance regulator), RUNX2 (Runt-related transcription factor 2), ns (no significative).

## 4. Discussion

One of the defining criteria for mesenchymal stem cells is the expression of CD73, CD90 and CD105 in ≥ 95% of the population, along with the absence (≤ 2% positive) of CD11b, CD19, CD34, CD45, and HLA-DR, [11].. Similarly, the DPSCs population maintained a consistent percentage of positive and negative markers [8]. However, due to the limited number of biological replicates in our study, we were unable to statistically determine whether the observed deviation from the expected marker percentages is significant. Further studies with a larger number of biological replicates are needed to elucidate this aspect.

Cystic fibrosis is a genetic disorder that affects many systems in the body, including the respiratory and digestive systems [14]. While CFTR expression has been shown to influence epithelial proliferation in lung development [15], the effects on proliferation seem to vary depending on the cell type and developmental stage. For instance, studies have demonstrated increased proliferation of intestinal stem cells and activation of the Wnt/β-catenin signaling pathway in the CF [16], while defective CFTR has been linked to inhibited endothelial cell proliferation and altered gene signatures related to migration and proliferation [17]. However, in our study, no differences in the proliferative activity of DPSCs from CF patients were detected. These findings suggest that the impact of CFTR mutations on proliferation is context-dependent, and further investigations are needed to fully understand the mechanisms involved.

Cystic fibrosis can also lead to osteoporosis and osteopenia [14]. These conditions are characterized by reduced bone density and increased risk of fractures [18], [19], and altered bone metabolism, including impaired turnover [20]. The imbalance between osteogenesis (formation of new bone) and osteoclastogenesis (resorption of existing bone) may contribute to the onset of these bone disorders. Osteoclastogenesis is a process by which bone-resorbing cells, called osteoclasts, are formed and mature. In healthy individuals, the balance between osteoclastogenesis and osteogenesis is carefully regulated to maintain bone homeostasis. However, in individuals with cystic fibrosis, this balance can be disrupted, leading to a reduction in bone density, but the exact mechanism is not well understood. Mice carrying the F508del CFTR mutation demonstrate reduced osteoblast differentiation and function, as reported by Le Henaff et al., (2015). These effects are associated with increased NF-κB activity and decreased Wnt/β-catenin signaling. In this study, we observed that the osteogenic capacity of DPSCs can vary depending on the specific mutations present in the CFTR gene. DPSCs with homozygous mutations in the CFTR gene (F508del) demonstrated enhanced osteogenic capacity, whereas DPSCs with heterozygous mutations (F508del and R347H) exhibited reduced osteogenic capacity compared to healthy DPSCs. The functional impact of CFTR

mutations, such as the βV348M mutation for example, may play a significant role in the pathophysiology of CF by dysregulating sodium and fluid absorption in the respiratory tract [22]. Furthermore, the combined effects of two mutations in cis can have a profound impact on CFTR function, contributing to the extensive phenotypic variability observed in CF. For instance, the coexistence of R347H and D979A mutations leads to a synergistic impairment of Cl (-) current, resulting in a significant decrease in CFTR function [23]. Certain genetic mutations can have a significant impact on the proper folding of a protein, leading to alterations in its three-dimensional structure or in post-translational modification sites crucial for protein function. These structural changes can affect protein stability, the ability to interact with other molecules, or even modify its enzymatic activity. Additionally, mutations may result in the exposure of new domains in the protein, allowing interactions with other signaling pathways that would not normally occur. These additional interactions can trigger altered signaling cascades, affecting various cellular processes such as proliferation, differentiation, and cell survival. Therefore, it is important to recognize that protein mutations can have effects beyond their direct function, potentially influencing the interaction with other signaling pathways and contributing to the complexity of genetic diseases.

The expression of osteogenesis markers, such as RUNX2 and OPG, undergoes modulation during the 7-day osteogenic differentiation period. Notably, at the early stages of osteogenesis, DPSCs homozygous for the F508del mutation exhibit higher expression levels of RUNX2 compared to other induced DPSCs. Robert et al., (2018) reported that RUNX2 was not detected in MSCs committed to osteoblast lineage after 24 hours of osteogenic induction. Furthermore, other studies have indicated that the expression of RUNX2 becomes evident at later stages of osteogenic differentiation [25]. Specifically, RUNX2 type I has been associated with early osteoblastogenesis, while RUNX2 type II is necessary for the terminal stages of osteoblastic maturation [26], [27]. In this study, the oligonucleotides used to detect RUNX2 expression can recognize all isoforms of RUNX2 mRNA, providing a general overview of gene expression. However, it should be noted that this approach cannot distinguish the specific isoform expressed at each stage. Interestingly, the expression of OPG shows an inverse relationship with RUNX2 expression. OPG is a member of the TNF receptor superfamily that acts as a dose-dependent inhibitor of osteoclast differentiation from precursor cells [28] corroborating these findings, Le Henaff et al. (2015) demonstrated a significant reduction in the mRNA level of OPG in osteoblasts derived from F508del-CFTR mice compared to osteoblasts from WT mice. In individuals homozygous for the F508del mutation in the CFTR gene, there appears to be an impact on osteogenesis, promoting osteoblast formation. Consequently, at a systemic level and over the long term, the imbalance between osteoclastogenesis and osteogenesis resulting from altered OPG expression by DPSCs could contribute to the development of osteoporosis and osteopenia in cystic fibrosis. These findings suggest that DPSCs from CF donors display distinct gene expression patterns related to osteogenesis compared to DPSCs from healthy donors and those with different genotypes. Further research is needed to fully understand the underlying mechanisms and to develop effective treatments for the bone complications associated with CF.

An important limitation of this study is the reduced number of donors included in each cystic fibrosis variant group. This limitation arises from the inherent difficulty in obtaining biological samples from patients with specific genotypes, since the frequency of certain mutations in the population is relatively low. In addition, the process of sample collection and cell isolation depends on donor availability and sample viability, which further restricts the final sample size. Nevertheless, the data obtained are consistent and allow the observation of relevant trends, although they should be interpreted with caution and validated in future studies with larger cohorts.

## 5. Conclusion

DPSCs from CF patients were successfully established and exhibited normal immunophenotype and proliferation, but their osteogenic potential varied according to CFTR genotype. Distinct RUNX2 and OPG expression profiles highlight genotype-specific modulation of osteogenesis, underscoring a potential role for CFTR mutations in CF-associated bone fragility.

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### Competing interests

The authors report that there are no potential conflicts of interest or financial interests.

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### Authors' contributions

EBS carried out cellular and molecular studies. FB and CLKR carried out DPSC isolation. JBL and LB carried out cell culture. CAR, MM, and NRF carried out patient recruitment. RGJ performed qPCR tests. PS carried out the cytometry experiments. EBS and PS drafted the manuscript. All authors read and approved the final manuscript.

### Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT to improve the clarity and understanding of the text. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

## References

1. M. Honda and H. Ohshima, (2022). 'Biological characteristics of dental pulp stem cells and their potential use in regenerative medicine', *Japanese Association for Oral Biology*.
2. F. Ferro, R. Spelat, and C. S. Baheney, (2014). 'Dental pulp stem cell (DPSC) isolation, characterization, and differentiation', Methods in Molecular Biology, vol. 1210, pp. 91–115,
3. Q. Dong et al., (2019). 'Dental pulp stem cells: Isolation, characterization, expansion, and odontoblast differentiation for tissue engineering', in Methods in Molecular Biology, vol. 1922, Humana Press Inc, pp. 91–101.
4. J. R. Riordan et al., (2023). 'Identification of the Cystic Fibrosis Gene: Cloning and Characterization of Complementary DNA', Science (1979), vol. 245, no. 4922.
5. K. M. Dickinson and J. M. Collaco, (2021). 'Cystic fibrosis', Pediatr Rev, vol. 42, no. 2, pp. 55–65.

6. F. Chedevergne and I. Sermet-Gaudelus,(2019). 'Prevention of osteoporosis in cystic fibrosis', *Curr Opin Pulm Med,* vol. 25, no. 6, pp. 660–665.
7. A. I. Tsai et al., (2017). 'Isolation of Mesenchymal Stem Cells from Human Deciduous Teeth Pulp', Biomed Res Int, vol. 2017.
8. L. Fracaro et al., (2020). 'The Expression Profile of Dental Pulp-Derived Stromal Cells Supports Their Limited Capacity to Di ff erentiate into Adipogenic Cells', *Int J Mol Sci,* vol. 21, no. 2753.
9. I. Kerkis and A. I. Caplan, (2012). 'Stem cells in dental pulp of deciduous teeth', Tissue Eng Part B Rev, vol. 18, no. 2, pp. 129–138.
10. G. Mori et al., (2011). 'Dental pulp stem cells: Osteogenic differentiation and gene expression', Ann N Y Acad Sci, vol. 1237, no. 1, pp. 47–52.
11. M. Dominici, T. J. Hofmann, and E. M. Horwitz, (2001). 'Bone marrow mesenchymal cells: Biological properties and clinical applications', *J Biol Regul Homeost Agents,* vol. 15, no. 1, pp. 28–37.
12. A. L. Ribeiro et al., (2021). 'Bismuth-based nanoparticles impair adipogenic differentiation of human adipose-derived mesenchymal stem cells', Toxicology in Vitro, vol. 77.
13. C. DS. Horinouchi, M. Julia. Barisón, A. W. Robert, Crisciele. Kuligovski, and Bruno. Aguiar, et al. (2020). 'Influence of donor age on the differentiation and division capacity of human adipose-derived stem cells', Stem Cells, vol. 12, no. 12, p. 11.
14. M.-C. M. R., H. A., Richard B. M. M. and L. K. B. M. Bhudhikanok Grace S., (1998). 'Bone acquisition and loss in children and adults with cystic fibrosis: A longitudinal study', *J Pediatr,* vol. 133.
15. J. E. Larson, J. B. Delcarpio, M. M. Farberman, S. L. Morrow, A. J. C. Cohen, et al. (2000). 'CFTR modulates lung secretory cell proliferation and differentiation'.
16. A. M. Strubberg et al., (2018). 'Cftr Modulates Wnt/b-Catenin Signaling and Stem Cell Proliferation in Murine Intestine Ashlee', Cell Mol Gastroenterol Hepatol, vol. 5, no. 3, pp. 253–271.
17. M. Declercq et al., (2021). 'Transcriptomic analysis of CFTR-impaired endothelial cells reveals a pro-inflammatory phenotype', *European Respiratory Journal,* vol. 57, no. 4.
18. R. M. Aris et al., (2005). 'Consensus statement: Guide to bone health and disease in cystic fibrosis', in *Journal of Clinical Endocrinology and Metabolism,* pp. 1888–1896.
19. A. B. Grey, R. W. Ames, R. D. Matthews, and I. R. Reid,(1993). 'Original articles Bone mineral density and body composition in adult patients with cystic fibrosis'.
20. F. D. L. G. M. T. A. C. S. C. C. S. B. & G. S. Giampiero I Baroncelli, (1997). 'pr1997127', *Pediatr Res,* vol. 41, pp. 397–403.
21. C. Le Henaff et al., (2015). 'Increased NF-κB activity and decreased Wnt/β-catenin signaling mediate reduced osteoblast differentiation and function in ΔF508 cystic fibrosis transmembrane conductance regulator (CFTR) mice', *Journal of Biological Chemistry,* vol. 290, no. 29, pp. 18009–18017.
22. R. Rauh et al., (2013). 'A mutation in the-subunit of ENaC identified in a patient with cystic fibrosis-like symptoms has a gain-of-function effect', *Am J Physiol Lung Cell Mol Physiol,* vol. 304, pp. 43–55.
23. J. Clain et al., (2001). 'Two Mild Cystic Fibrosis-associated Mutations Result in Severe Cystic Fibrosis When Combined in Cis and Reveal a Residue Important for Cystic Fibrosis Transmembrane Conductance Regulator Processing and Function', *Journal of Biological Chemistry,* vol. 276, no. 12, pp. 9045–9049.
24. A. W. Robert et al., (2018). 'Gene expression analysis of human adipose tissue-derived stem cells during the initial steps of in vitro osteogenesis', Sci Rep, vol. 8, no. 1, Dec.
25. N. A. Twine, L. Chen, C. N. Pang, M. R. Wilkins, and M. Kassem, (2014). 'Identification of differentiation-stage specific markers that define the ex vivo osteoblastic phenotype', Bone, vol. 67, pp. 23–32.
26. S. H. Lee et al., (2020). 'Haploinsufficiency of Cyfip2 Causes Lithium-Responsive Prefrontal Dysfunction', *Ann Neurol,* vol. 88, no. 3, pp. 526–543.
27. Z. S. Xiao, A. B. Hjelmeland, and L. D. Quarles, (2004). 'Selective Deficiency of the "Bone-related" Runx2-II Unexpectedly Preserves Osteoblast-mediated Skeletogenesis', Journal of Biological Chemistry, vol. 279, no. 19, pp. 20307–20313.
28. W. S. Simonet, D. L. Lacey, and C. R. Dunstan, (1997). 'Osteoprotegerin: A Novel Secreted Protein Involved in the Regulation of Bone Density'.

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