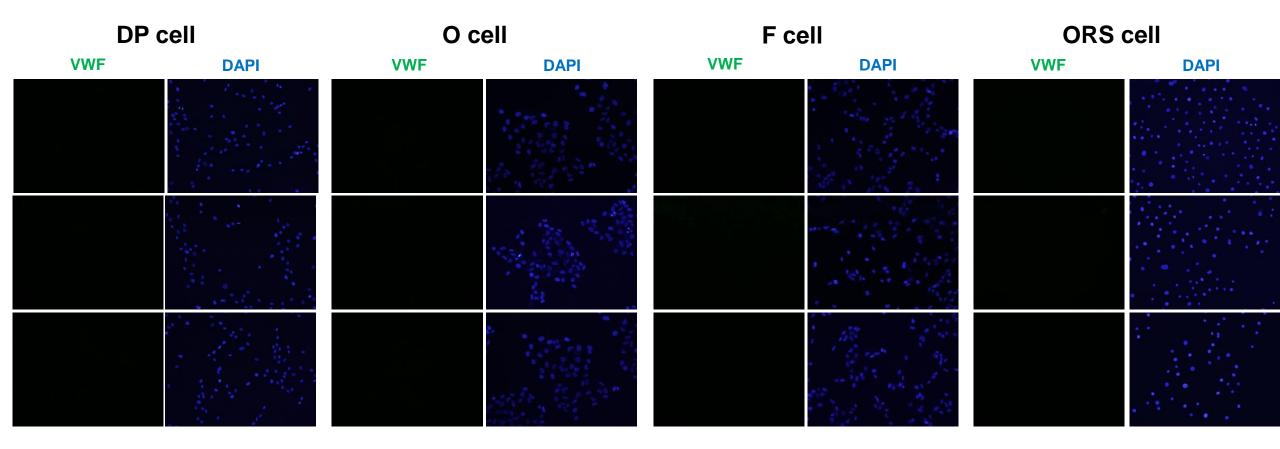
ALP activity

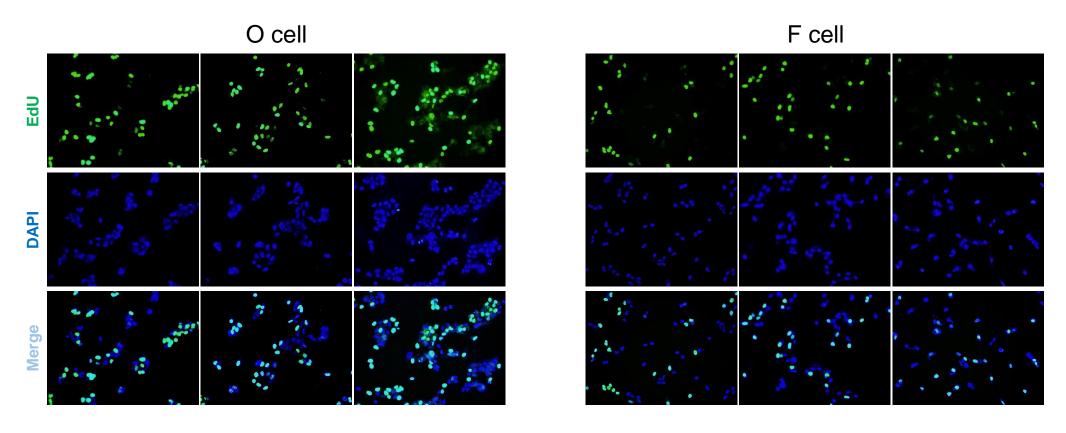


Primary DP cell (passage 2), O DP cell line (passage 45), F DP cell line (passage 45), primary ORS cell (passage 2) were assessed alkaline phosphatase (ALP) activity. ALP activity is detected in cultured primary DP cells and is weakly detected O DP cell line. However ALP activity is not detected in F DP cell line and ORS keratinocyte (ALP negative control).

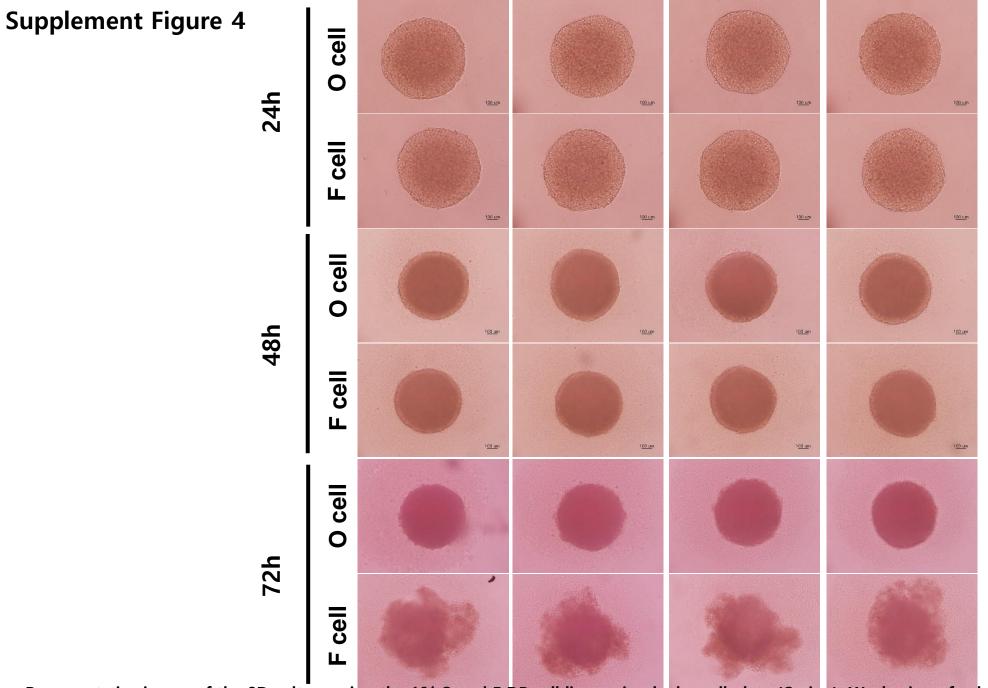
Supplement Figure 2 vWF



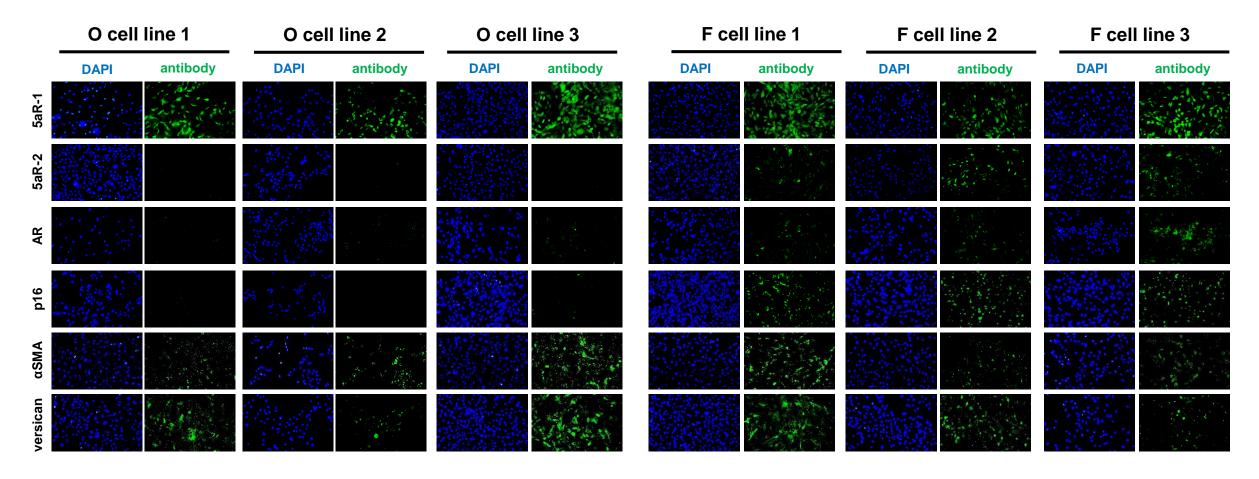
Primary DP cells (passage 2), O DP cell line (passage 45), F DP cell line (passage 45), primary ORS cells (passage 2) were assessed von Willebrand factor (vWF; endothelial cell marker). vWF is not expressed in primary DP cells, O and F DP cell lines and ORS keratinocyte.



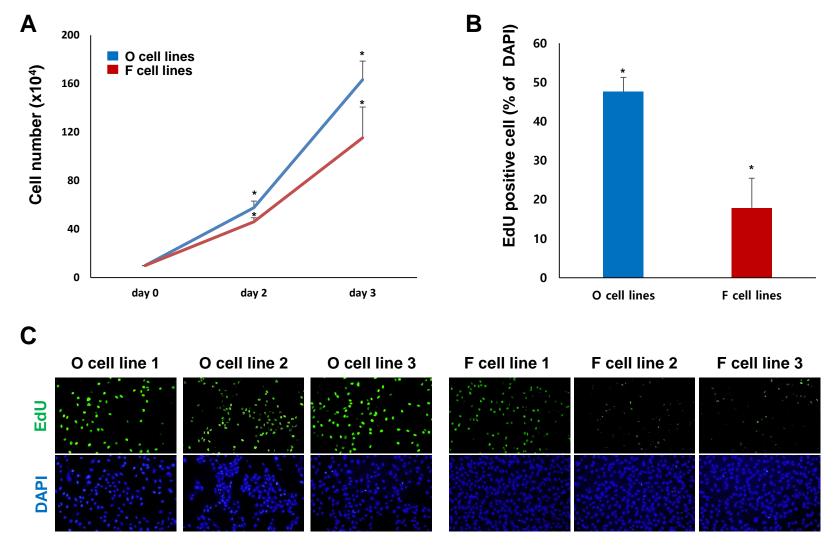
After 10⁵ cells O and F DP cell lines seeding for 24h, cells were incorporated Edu. After incorporating EdU into the cells, proliferating cells were observed (upper panel). Corresponding DAPI nuclear staining is also shown (middle panel).



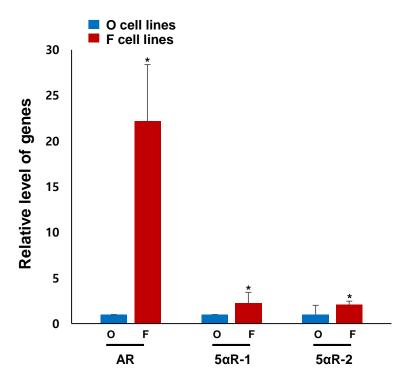
Representative image of the 3D culture using the 10⁴ O and F DP cell lines using hydro cell plate (Coring). Weakening of sphere aggregation is observed in the 3D culture of the F DP cell line after 72 hours.



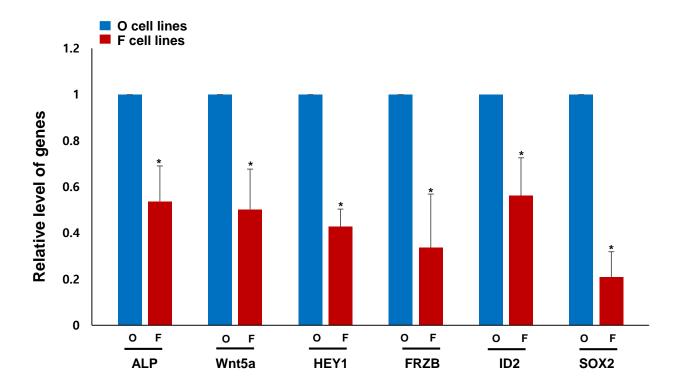
After 10⁵ cells three types of O and F DP cell lines seeding for 24h, cells were assessed AR related proteins (5aR-1, 5aR-2, AR and p16) and cultured DP cells markers (aSMA, versican). 5aR-2, AR and p16 are not expressed O DP cell lines.



(A) After seeding 10^5 cells, three types of O DP cell lines and three types of F DP cell lines were counted. Data are the mean \pm SD from three clones(*P < 0.05). (B) EdU-positive proliferative cells were counted. Data are the mean \pm SD from clones (*P < 0.05). (C) After incorporating EdU into the cells, proliferating cells were observed (left panel). Corresponding DAPI nuclear staining is also shown (middle panel).



Relative AR, 5aR-1 and 5aR-2 levels were analyzed by real-time PCR. Data are the mean \pm SD from three clones (*P < 0.05).



Relative levels of representative DP signature genes were analyzed by real-time PCR in 3D-cultured F and O DP cell lines (three clones) 48 hours after sphere formation. Data are expressed as means \pm SD from three clones (*P < 0.05).