Human ex vivo 3D bone model recapitulates osteocyte response to metastatic prostate cancer

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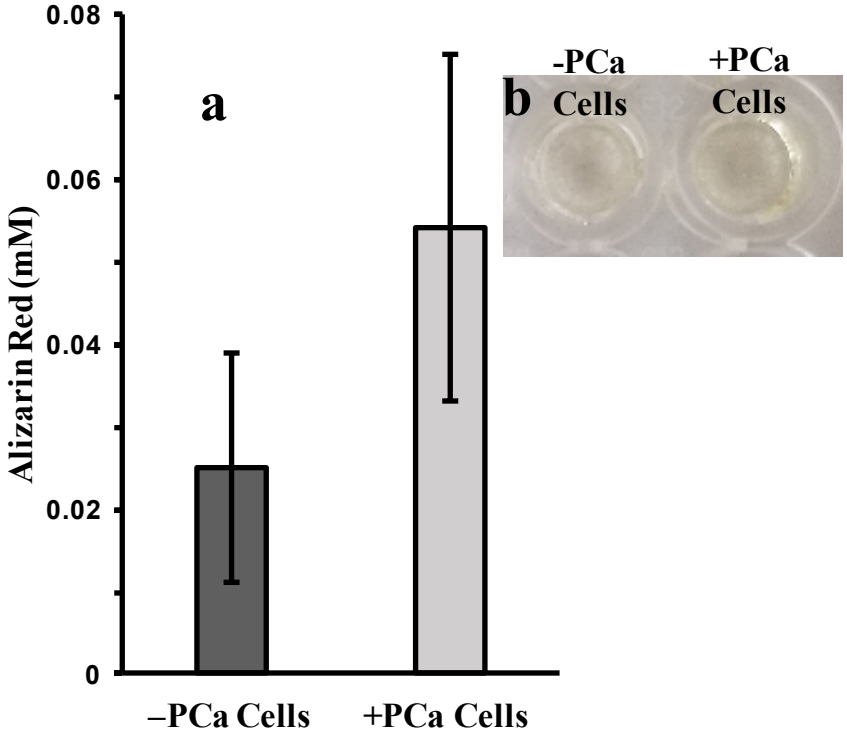
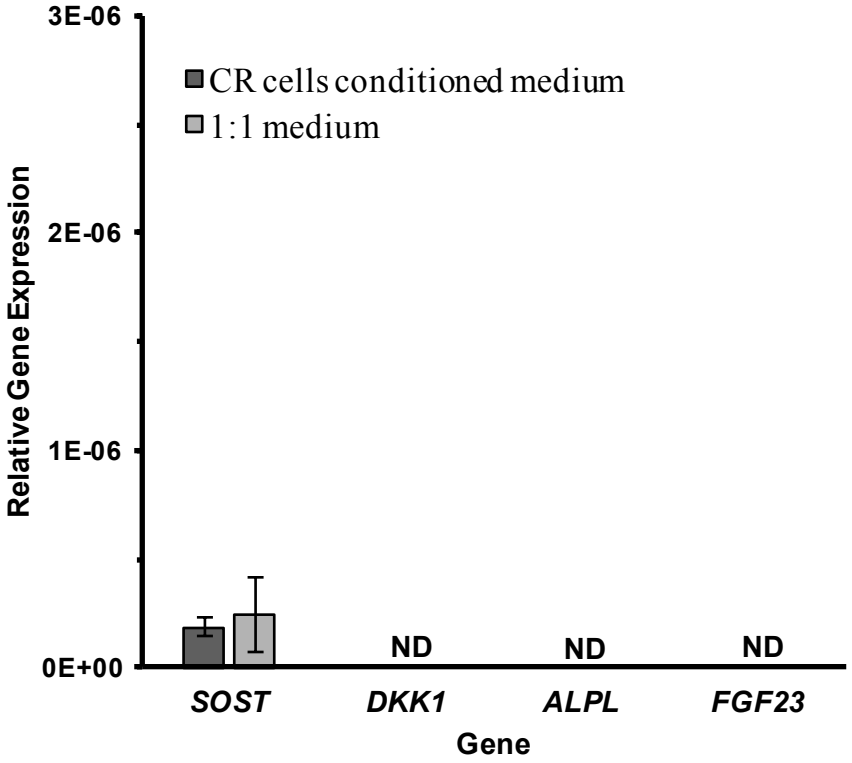
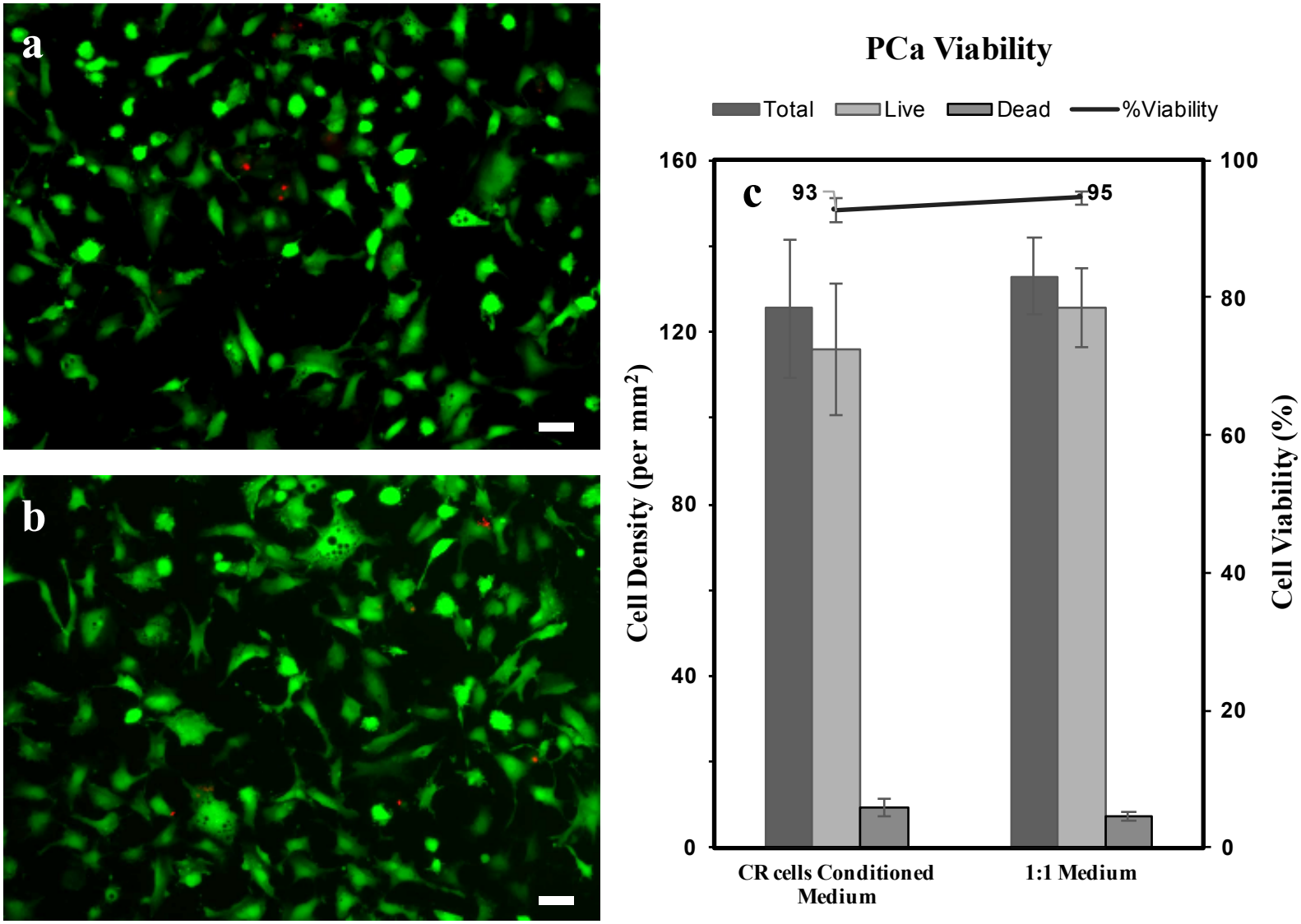


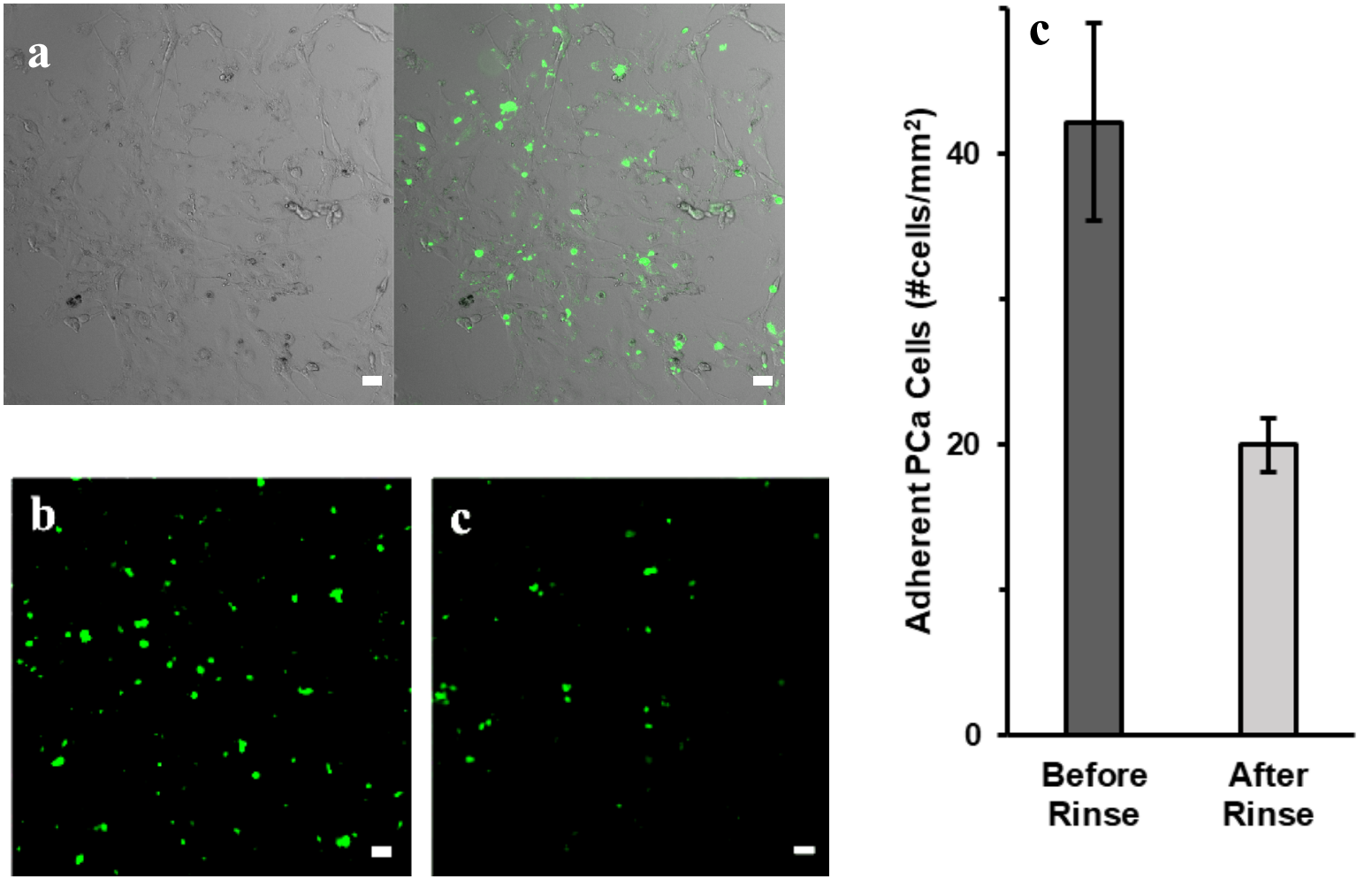
Supplementary Figure 1: Mineralization of primary human osteocytic cells in 2D culture. (a) Quantification of mineralization in 2D cultures of osteocytes with no PCa cells and with PCa cells. (b) Representative image of extracted Alizarin Red S from 2D cultures of osteocytes with no PCa cells (left) and with PCa cells (right).



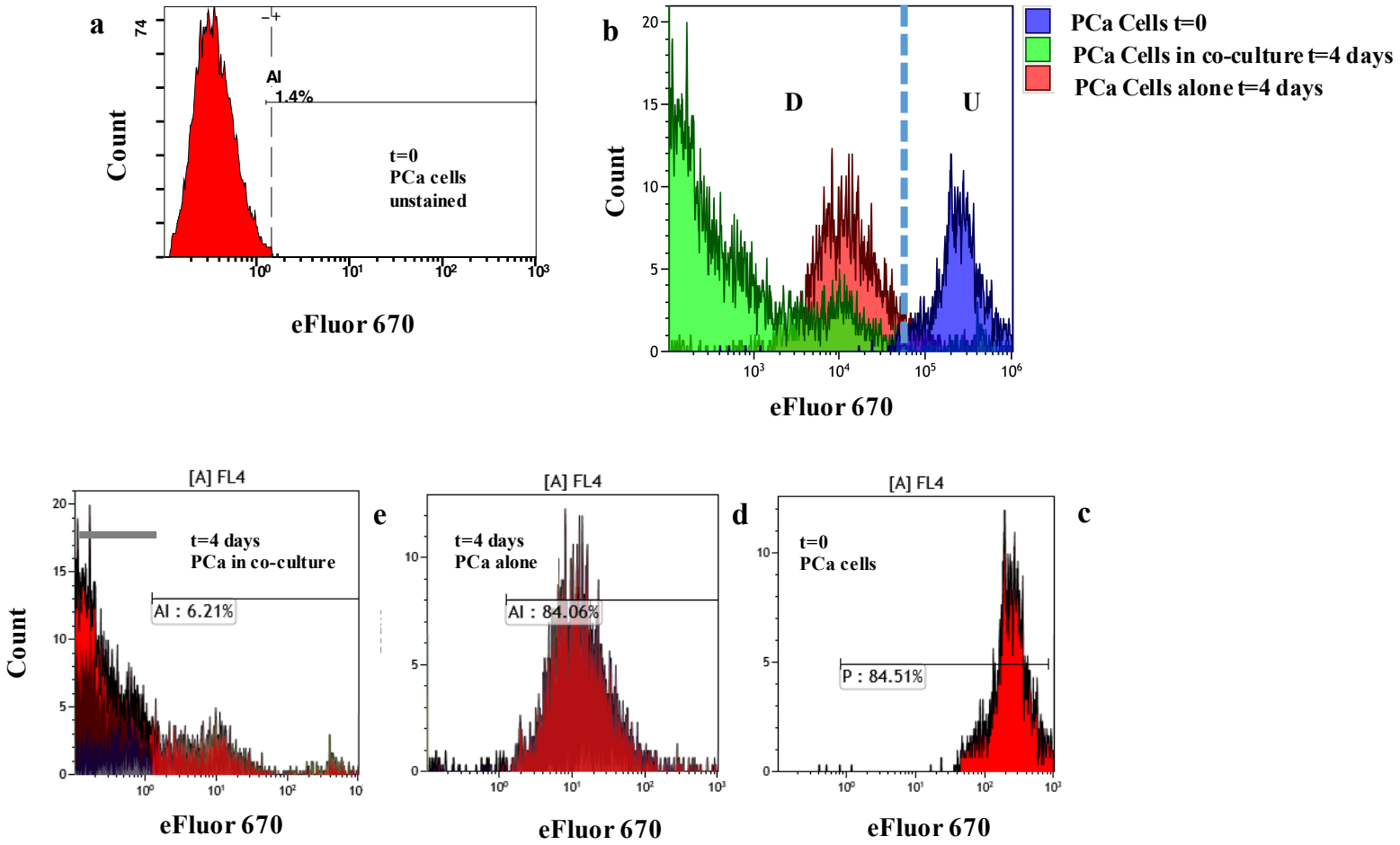
Supplementary Figure 2: Relative gene expression of PCa cells cultured in CR cells conditioned medium or in a mixture of 1:1 medium containing conditioned osteogenic medium collected as the effluent from 3D osteocyte cultures.



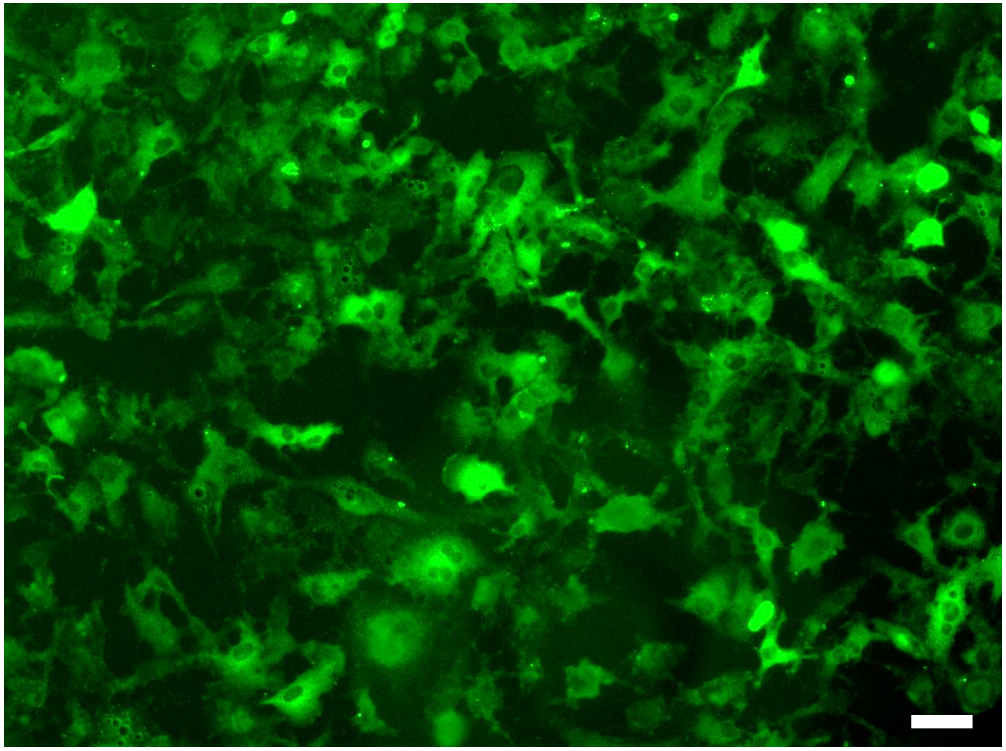
Supplementary Figure 3: PCa viability under osteogenic co-culture environment. Representative images of live (green) and dead (red) PCa cells cultured in (a) CR cells conditioned medium and (b) 1:1 mixture of CR cells conditioned medium and osteogenic differentiation medium. (c) Quantification of total, live, dead cells and % viability of PCa cells under different culture conditions. Scale bars = 50 μm.



Supplementary Figure 4: Adhesion of CFSE-labeled PCa cells to human primary osteocytic cells. Representative images of PCa cells (green) at (a) 24 h, and after 4 days (b) before rinsing, and (c) after rinsing. (c) Quantification of PCa cell density before and after rinsing. Scale bars = 50 μm.



Supplementary Figure 5: eFluor 670-labeled PCa cells proliferation in co-culture with 3D bone tissues. (a) Unstained PCa cells. (b) Composite histogram plot. (c,d,e) Individual histogram plots. U= undivided (at time = 0) and (D) divided cells (4 days post-culture). Gray bar on (d) indicates non-eFluor 670+ cells (i.e., osteocytic cells).



Supplementary Figure 6: Pan-cytokeratin staining of CR PCa cells. Scale bars = 50 μm.