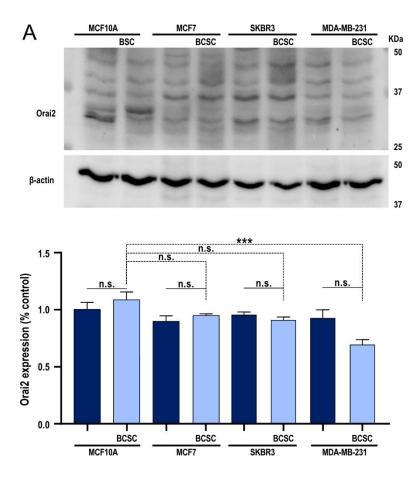
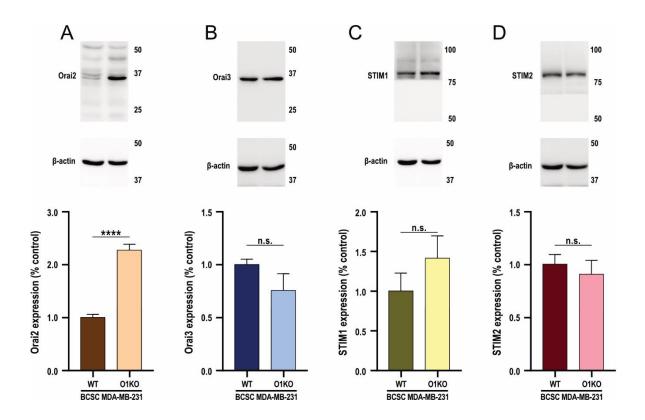
## Supplementary figures

## Orai1α and Orai1β support calcium entry and mammosphere formation in breast cancer stem cells

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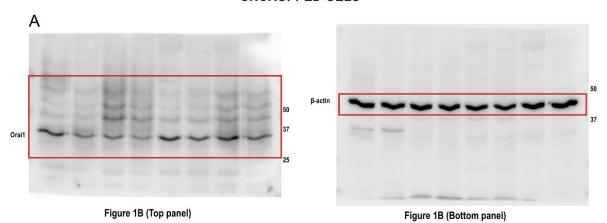
Supplementary Fig. 1. Expression of Orai2 protein in breast stem cells and breast cancer stem cells derived from the MCF10A, MCF7, SKBR3 and MDA-MB-231 cell lines. Whole cell lysates from non-stem cells and stem cells derived from MCF10A, MCF7, SKBR3 and MDA-MB-231 cell lines were subjected to 10% SDS-PAGE and Western blotting with specific anti-Orai2 antibody, as indicated. Blots were reprobed with anti- $\beta$ -actin antibody for protein loading control. Bar graph represents Orai2 protein expression presented as mean  $\pm$  SEM of 6 independent experiments. Data were statistically analyzed using Kruskal–Wallis test with multiple comparisons (Dunn's test). \*\*\*\*p < 0.001



Supplementary Fig. 2. Expression of Orai2, Orai3, STIM1 and STIM2 in breast cancer stem cells (BCSC) derived from WT MDA-MB-231 and Orai1-KO MDA-MB-231. Whole cell lysates from stem cells derived from WT and Orai1-KO (O1KO) MDA-MB-231 cells were subjected to 10% SDS-PAGE and Western blotting with specific anti-Orai2, anti-Orai3, anti-STIM1 and anti-STIM2 antibody, as indicated. Blots were reprobed with anti-β-actin antibody for protein loading control. Bar graph represents Orai2 (A), Orai3 (B), STIM1 (C) and STIM2 (D) protein expression presented as mean ± SEM of 4 separate experiments. Data were statistically analyzed using Mann-Whitney U-test. \*\*\*\*\* p < 0.0001

## Supplementary Fig. 3

## **UNCROPPED GELS**



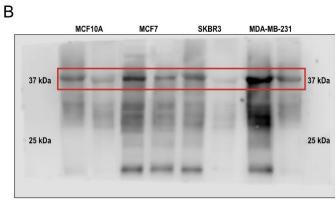


Figure 3A-D (Top panel)



Figure 3A-D (Bottom panel)

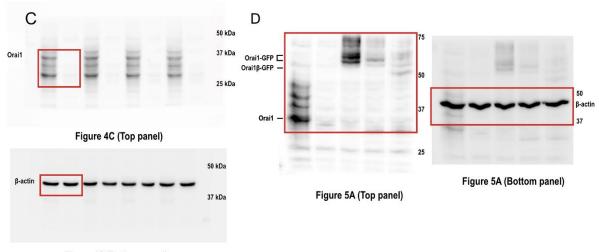
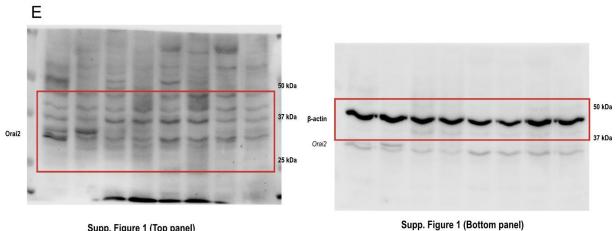
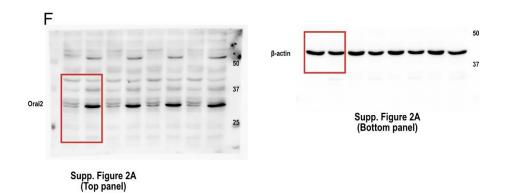


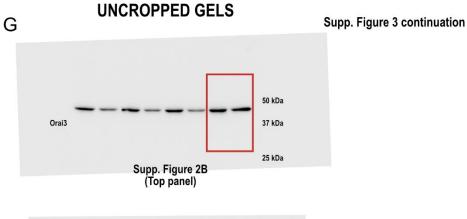
Figure 4C (Bottom panel)

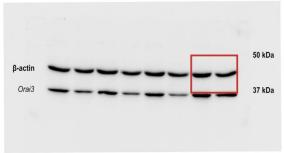


Supp. Figure 1 (Top panel)

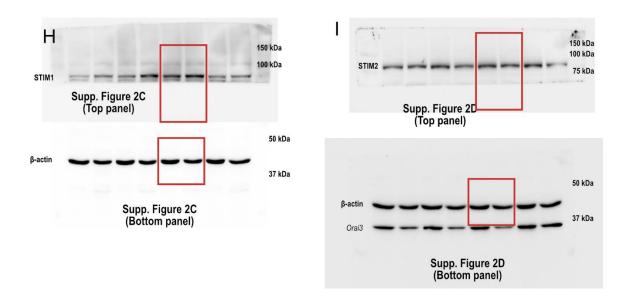


Supp. Figure 3 continue





Supp. Figure 2B (Bottom panel)



**Supplementary Fig. 3.** Original blots used in the manuscript. Uncropped and un-processed blots shown in figure 1B (A), Figure 3A-D (B), Figure 4C (C), Figure 5A (D), supplementary Figure 1 (E), supplementary Figure 2A (F), supplementary Figure 2B (G), supplementary Figure 2C (H) and supplementary Figure 2D (I). Routinely membranes were cut at approximately 65-70 kDa to analyze proteins in the upper and lower membranes simultaneously.