Supplementary material

Store-Operated Calcium Entry in Breast Cancer Cells Is Insensitive to Orai1 and STIM1 N-linked Glycosylation

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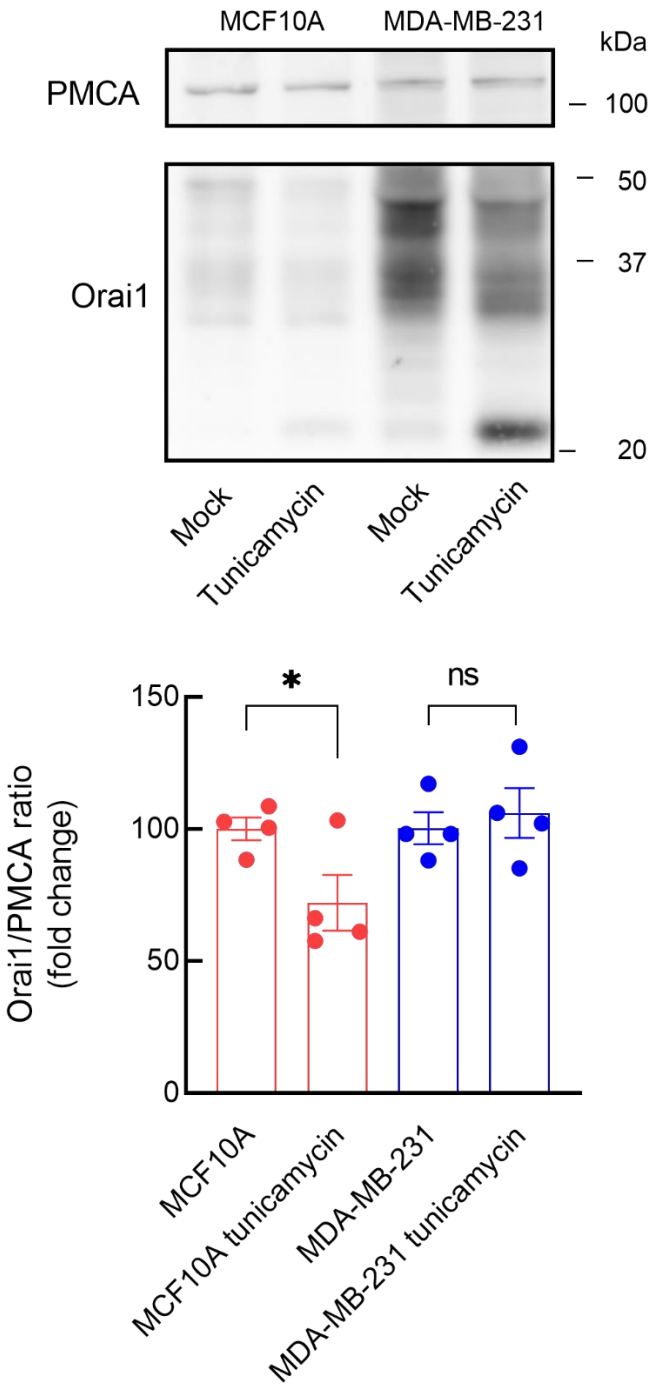

Figure S1. Effect of tunicamycin on Orai1 plasma membrane expression. MCF10A and MDA-MB-231 cells were incubated with 2 μM tunicamycin overnight or the vehicle (Mock), as indicated. Cells were mixed with biotinylation buffer containing EZ-Link sulfo-NHS-LC-biotin, and cell surface proteins were labeled by biotinylation, as described in "Material and methods". Labeled proteins were pulled down with streptavidin-coated agarose beads. The pellet (containing the plasma membrane fraction) was analyzed by SDS-PAGE and Western blotting using anti-Orai1 or anti-PMCA antibody, as indicated. Molecular masses indicated on the right were determined using molecular-mass markers run in the same gel. These results are representative of four separate experiments. Scatter plots represent the quantification of Orai1 plasma membrane expression under the different experimental conditions normalized to the PMCA expression and expresed as mean ± SEM. Data were statistically analyzed using Kruskal–Wallis test with multiple comparisons (Dunn's test). *$p < 0.05$ as compared to mock-treated MCF10A cells.

Table S1. Tunicamycin attenuates Orai1 and STIM1 protein expression in MCF10A and MDA-MB-231 cells.

		Orai1		
	control	tunicamycin	CHX	CHX+tunicamycin
MCF10A	1.00 ± 0.14	0.75 ± 0.12*	0.59 ± 0.14**	0.58 ± 0.14**
MDA-MB-231	1.00 ± 0.14	0.96 ± 0.09	0.75 ± 0.03**	0.71 ± 0.04**
		STIM1		
	control	tunicamycin	CHX	CHX+tunicamycin
MCF10A	1.00 ± 0.25	0.55 ± 0.19**	0.66 ± 0.14*	0.56 ± 0.18**
MDA-MB-231	1.00 ± 0.14	0.90 ± 0.21	0.91 ± 0.18	0.84 ± 0.16

MCF10A and MDA-MB-231 cells were incubated with 2 μM tunicamycin overnight or the vehicle (Mock) and further treated for 6h with 100 μg/mL cycloheximide (CHX) or the vehicle, as indicated, and lysed. Cell lysates were analyzed by Western blotting using specific anti-Orai1 antibody or anti-STIM1 antibody. The membrane was reprobed with anti-β-actin antibody for protein loading control. Data represents Orai1 and STIM1 expression normalized with β-actin and presented as fold-increase (experimental/control). Data are mean ± SEM of 4 independent experiments. Data were statistically analyzed using Kruskal–Wallis test with multiple comparisons (Dunn's test). * $p < 0.05$, ** $p < 0.01$ compared to control.