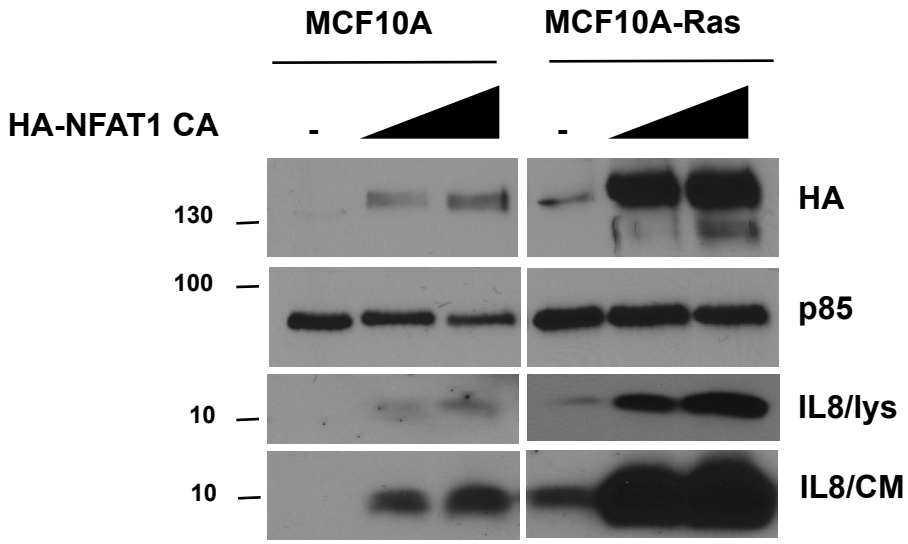
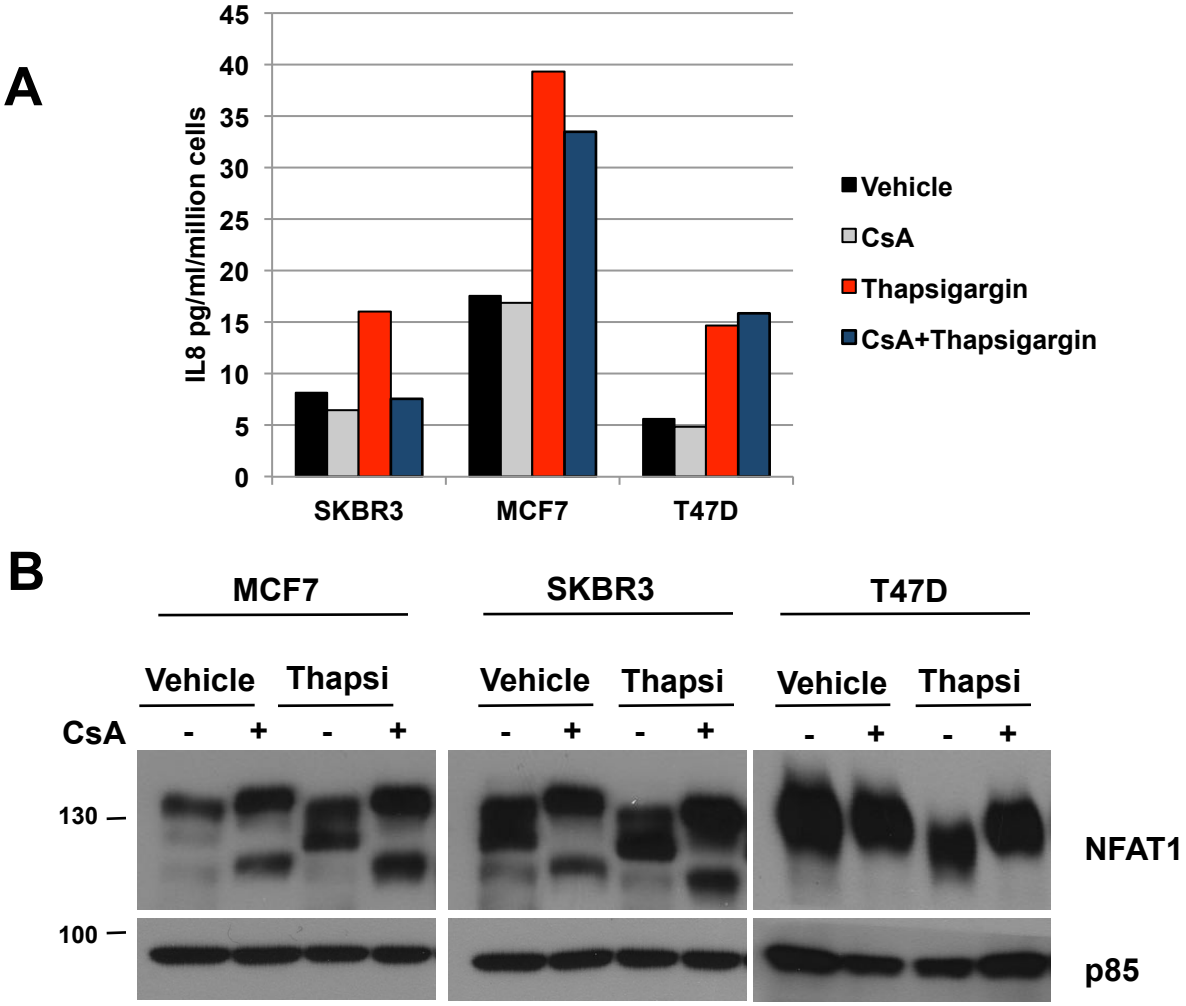
Supplementary table 1. mRNA copy # fold changes upon knock-down of NFAT1 in MDA-MB-231 cells.

Gene	Copy # per cell	Fold change upon NFAT1 knock-down
NFAT1	3	1
CCL2	1	0.7
PGF	4	1.4
IL6	4	1.8
CLIP1	5	1.2
TGFA	6	1.4
PDGFA	9	1.2
VEGFC	10	1.6
THBS1	10	2
MMP9	15	0.75
HB-EGF	20	1.2
CXCL1-2	30	0.5
VEGFA	40	1.3
CXCL1-3	50	0.5
IL8	60	0.8

Supplementary table 1. Table 1 shows the number of mRNA copy per cell in MDA-MB-231 cells and the relative change upon NFAT1 silencing. We infected MDA-MB-231 breast cancer cells with a tet-inducible lentiviral shRNA construct, and collected RNA from cells either untreated or treated with doxycycline (100ng/ml) for 72h. We used cDNA generated from these samples to determine the mean mRNA copy numbers by quantitative real-time RT-PCR by the ΔCt method normalized to the 18S housekeeping gene.

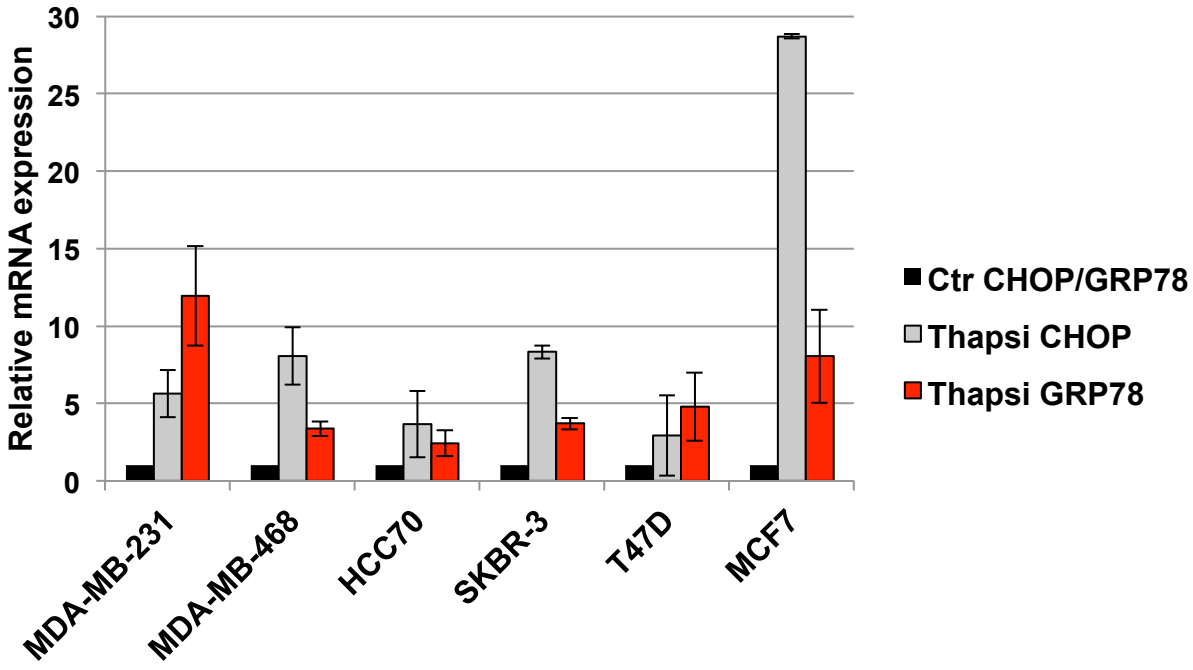


Supplementary Figure S1. Induction of IL8 by active NFAT1 in MCF10A and MCF10-Ras cells. The levels of IL8 are higher in MCF10A-Ras cells than in the naïve MCF10A cells, and the overexpression of active NFAT1 strongly elevates the protein levels of IL8 in both MCF10A and MCF10A-Ras cells (doxycycline 100 and 300ng/ml, 24h; lys: lysates; CM: conditioned media).



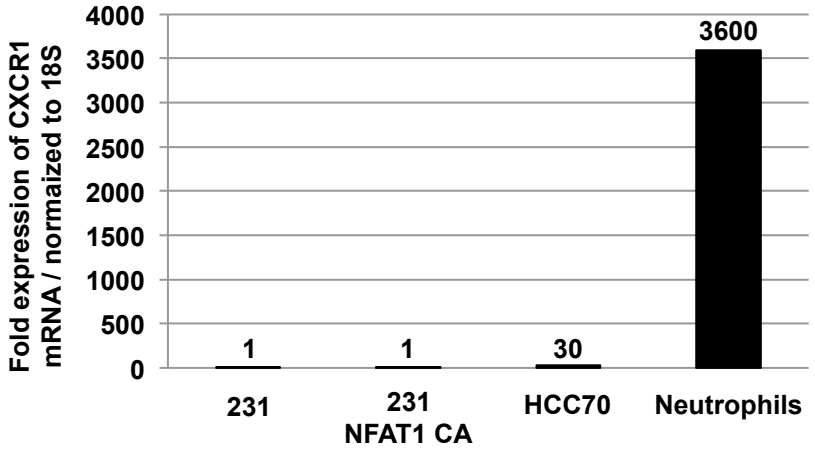
Supplementary Figure S2. Thapsigargin activates NFAT1, but fails to significantly promote IL8 production in non-triple negative breast cancer cell lines. A, To measure IL8 levels from the media, MCF7, SKBR3 and T47D were vehicle-treated or pretreated with cyclosporine A (CsA; 1μM, 1h) and then treated with thapsigargin (thapsi; 50nm) with or without cyclosporine A for 24h. Media was collected and filtered through 0.45-μm filters. **B,** For Western blot analyses, non-triple-negative breast cancer cell lines MCF7, SKBR3 and T47D were vehicle-treated or pretreated with cyclosporine A (1μM, 1h) and then treated with thapsigargin (200nm) with or without cyclosporine A for 20h.

C



Supplementary Figure S2. Thapsigargin activates NFAT1, but fails to significantly promote IL8 production in non-triple negative breast cancer cell lines. C, Expression of CHOP and GRP78 is induced in both triple negative and non-triple negative breast cancer cell lines in response to thapsigargin treatment (50nm, 16h). Figure shows mean fold induction +/-SD (n=2).

A



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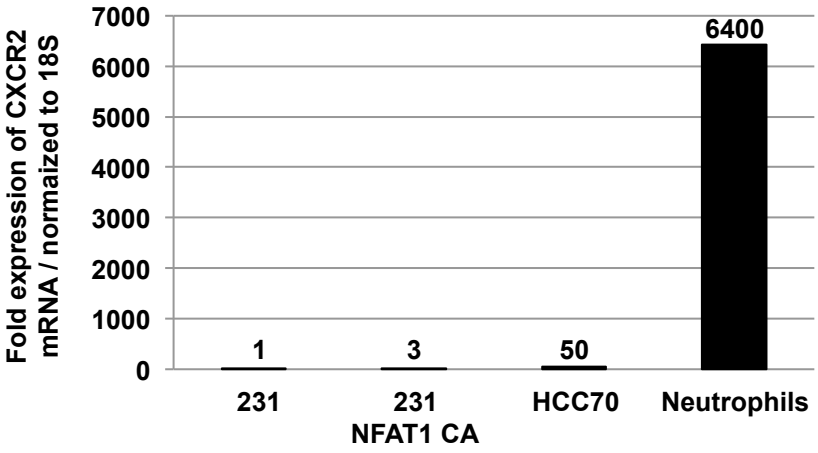
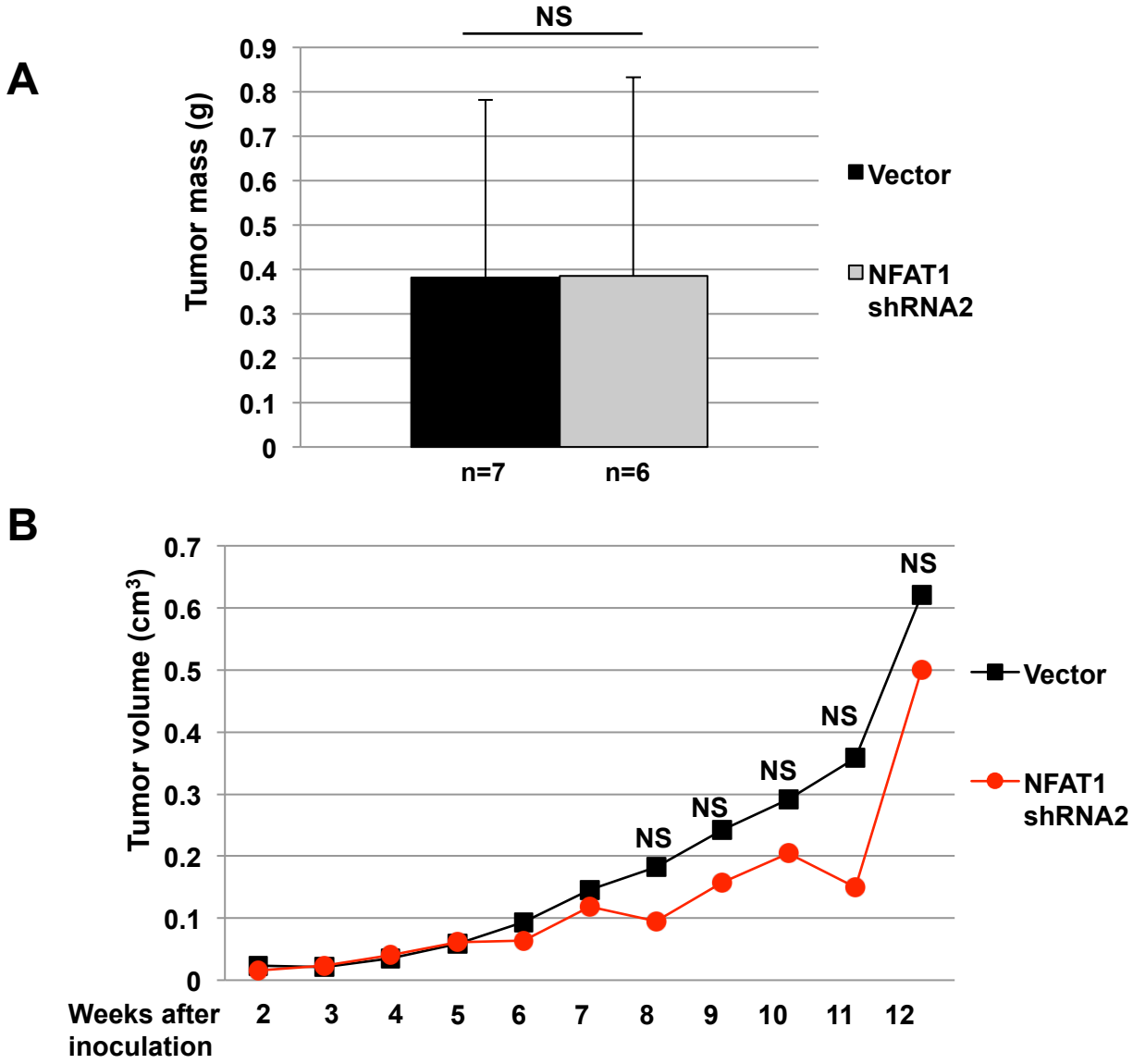
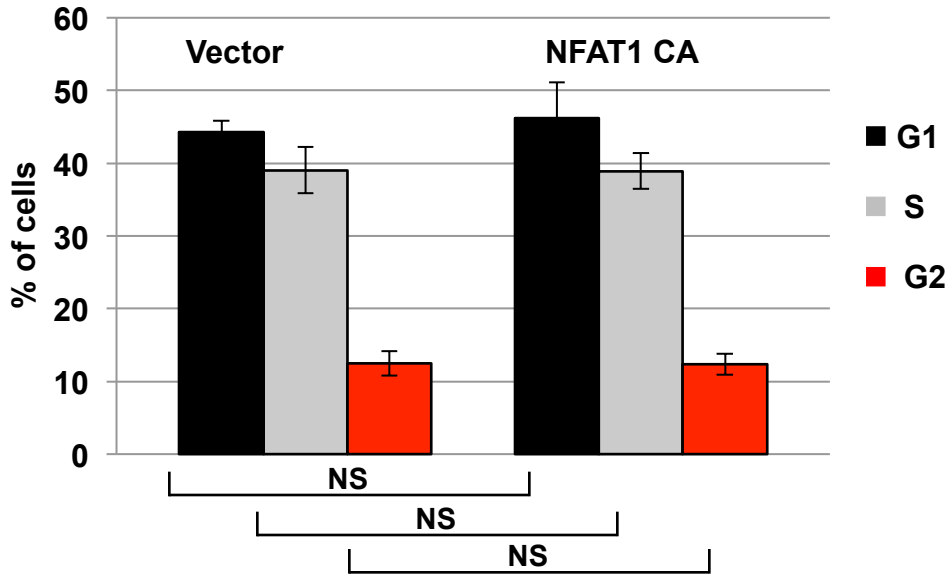


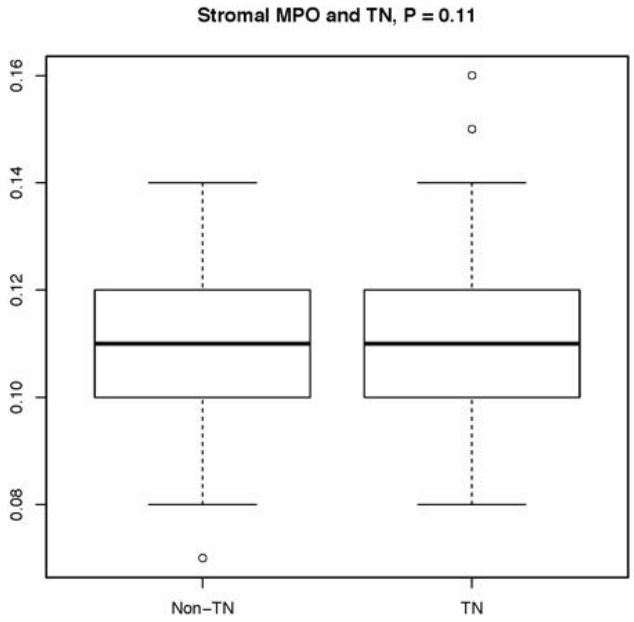
Supplementary Figure S3. Neutrophils express high levels of IL8-binding CXCR1 and CXCR2 receptors, whereas the breast cancer cell lines do not. A-B, We measured the relative mRNA expression levels of CXCR1 (A) and CXCR2 (B) by real-time RT-qPCR in MDA-MB-231 and HCC70 breast cancer cell lines and primary human neutrophils. Compared to MDA-MB-231 cells, primary human neutrophils expressed dramatically higher CXCR1 and CXCR2 mRNA levels. We had previously determined the mRNA copy number per cell for CXCR1 and CXCR2 to be less than one in MDA-MB-231 cells, suggesting these receptors are not expressed (data not shown).

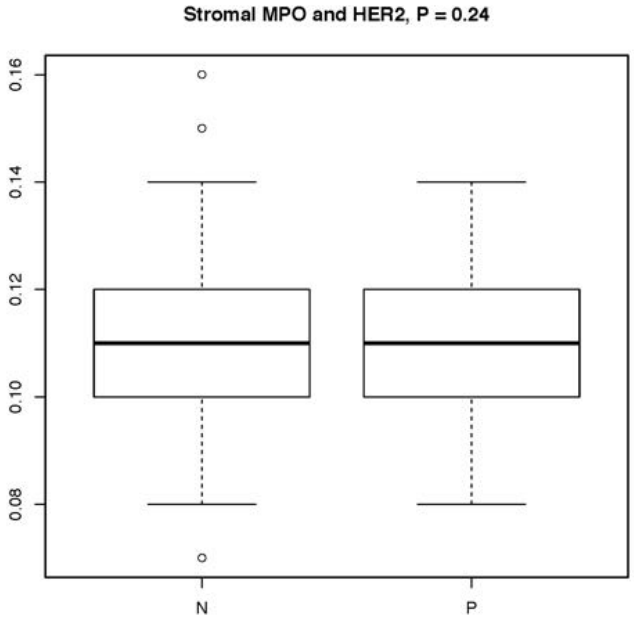


Supplementary Figure S4. Silencing of NFAT1 does not affect tumor growth. A-B, We determined the effect of knocking down NFAT1 on tumor growth by inoculating $0.3x10^6$ MDA-MB-231 cells containing the doxycycline-inducible NFAT1-targeting shRNA construct into the mammary fat pads of female nude mice. Doxycycline was added to the drinking water of the NFAT1 shRNA group (Tet-NFAT1 shRNA2 Dox), while the control group (Tet-NFAT1 shRNA Ctr) received fresh water. Tumor growth was measured weekly for 12 weeks after inoculation. Knockdown of NFAT1 does not significantly affect MDA-MB-231 xenograft growth. Statistical significance was determined by Student's unpaired t-test. NS, not statistically significant. **C,** Immunohistochemical analysis shows efficient knock-down of NFAT1.



Supplementary Figure S5. Overexpression of active NFAT1 does not affect the cell cycle profile of MDA-MB-231 cells. Doxycycline-inducible MDA-MB-231 cells expressing active NFAT1 were treated with doxycycline (200ng/ml, 48h), stained with propidium iodide, and the cell cycle profile was analyzed by flow cytometry using the FlowJo software (version 8.8.6). Samples were analyzed in duplicate, and mean values calculated from two independent experiments. Error bars indicate -/+SD, and statistical significance was analyzed using t-test.





Supplementary Figure S6. Stromal levels of myeloperoxidase (MPO) do not correlate with triple-negative status or Her2 status in breast cancer patient samples. Average MPO marker intensity per cell was computationally quantified. Statistical significance was analyzed using t-test. Non-TN: Non-triple negative; TN: triple-negative; N: Her2 negative; P: Her2 positive.