Supplemental Figures

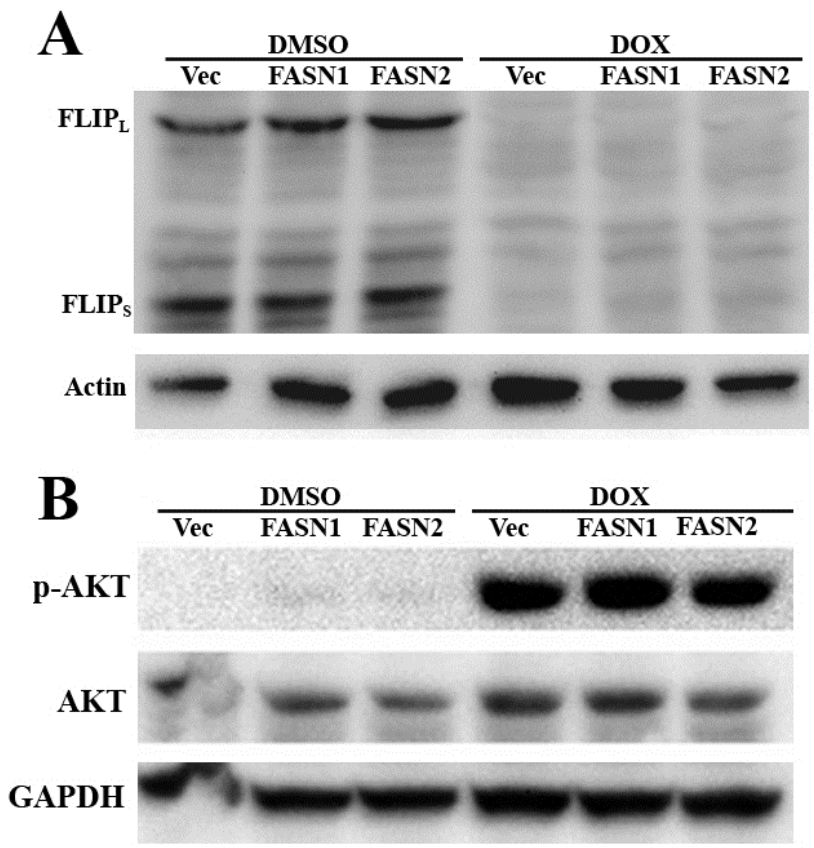

Figure S1. Effect of FASN on c-FILP and AKT. Stable MCF7 cells with FASN over-expression (FASN1 and FASN2) and vector-transfected control (Vec) cells were treated with 1 μM doxorubicin (DOX) or DMSO vehicle control for 24 hrs followed by analysis of c-FLIP$_L$, c-FLIP$_S$, total AKT, and activated AKT (p-AKT) using Western blot. Actin and GAPDH were used as loading controls.

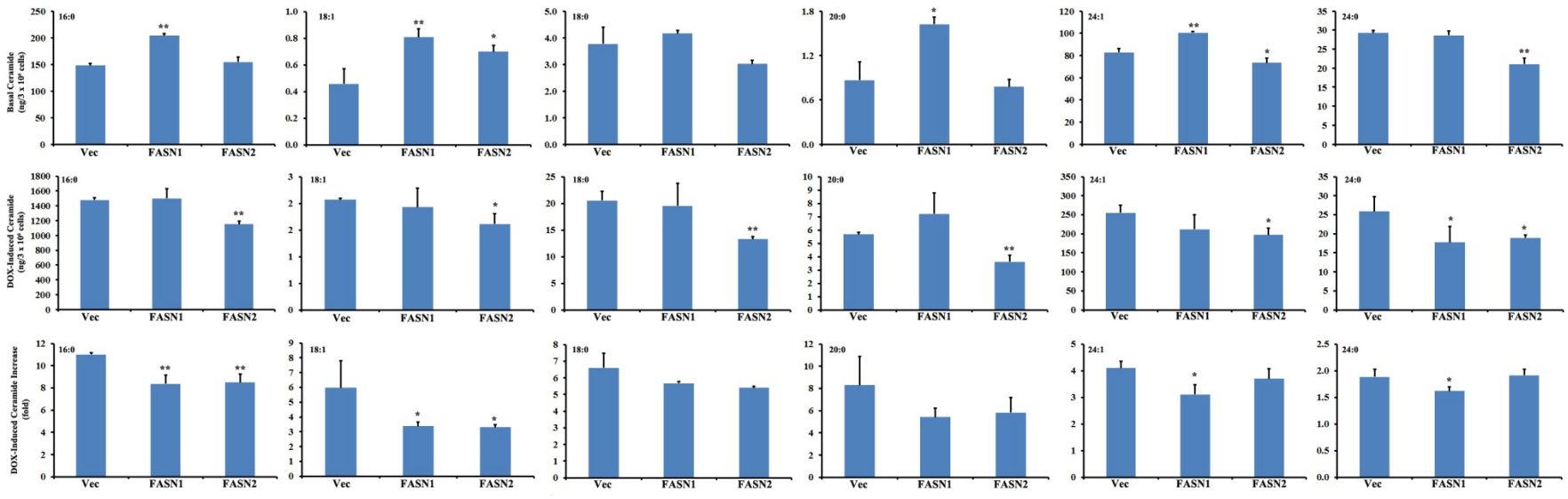

Figure S2. Effect of FASN over-expression on basal and doxorubicin-induced individual ceramide generation. A. Basal level of ceramide C16:0, C18:0, C18:1, C20:0, C24:0, and C24:1. Basal level of individual ceramide in stable MCF7 cells with FASN over-expression (FASN1 and FASN2) and vector-transfected control (Vec) cells were determined using mass spectrometry as described in Materials and Methods. B and C. Doxorubicin-induced increase of ceramide ceramide C16:0, C18:0, C18:1, C20:0, C24:0, and C24:1. Individual ceramides were determined in stable MCF7 cells with FASN over-expression (FASN1 and FASN2) and vector-transfected control cells (Vec) following treatment with 1 μM doxorubicin for 48 hrs. DOX-induced ceramide increase (fold) =ceramide in cells treated with doxorubicin/ceramide in cells treated without doxorubicin. (* $p<0.05$; ** $p<0.01$)

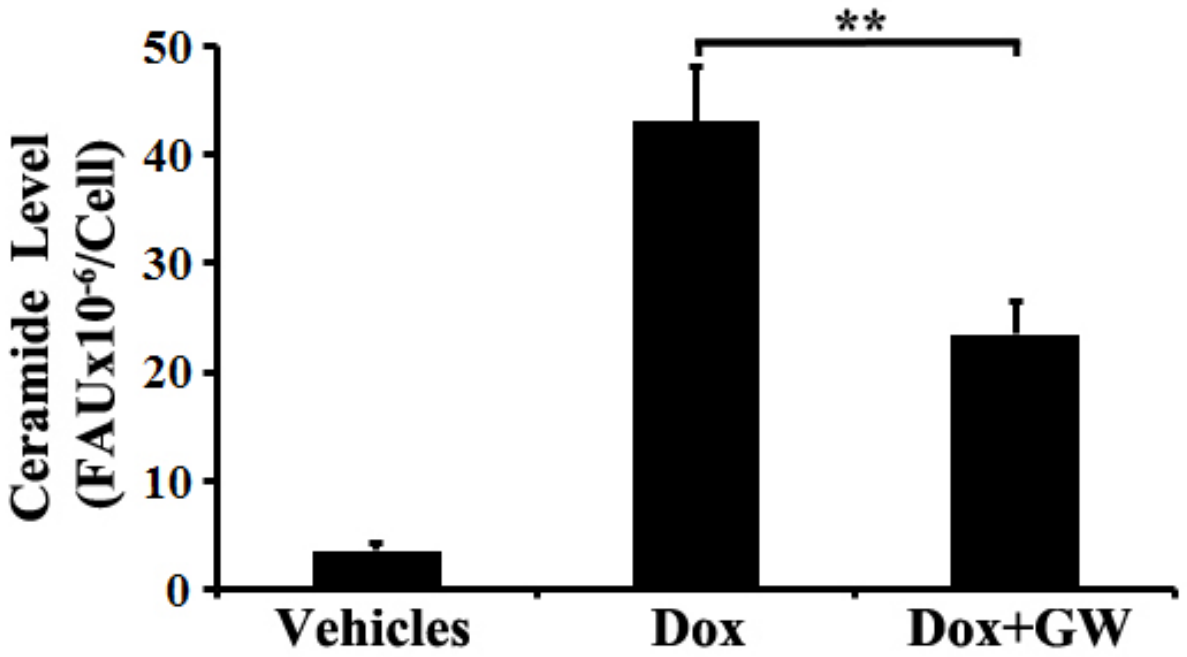

Figure S3. Inhibition of doxorubicin-induced ceramide production by GW4869. MCF7 cells were treated with vehicle (5% methane sulfonic acid) or 10 µM GW4869 (GW) for 30 min, followed by addition and incubation with 1 µM doxorubicin (Dox) or DMSO vehicle for 24 hrs. Cells were then harvested for determination of ceramide level using immunofluorescence staining as described in Materials and Methods. (**p<0.01; FAU=fluorescence arbitrary units).

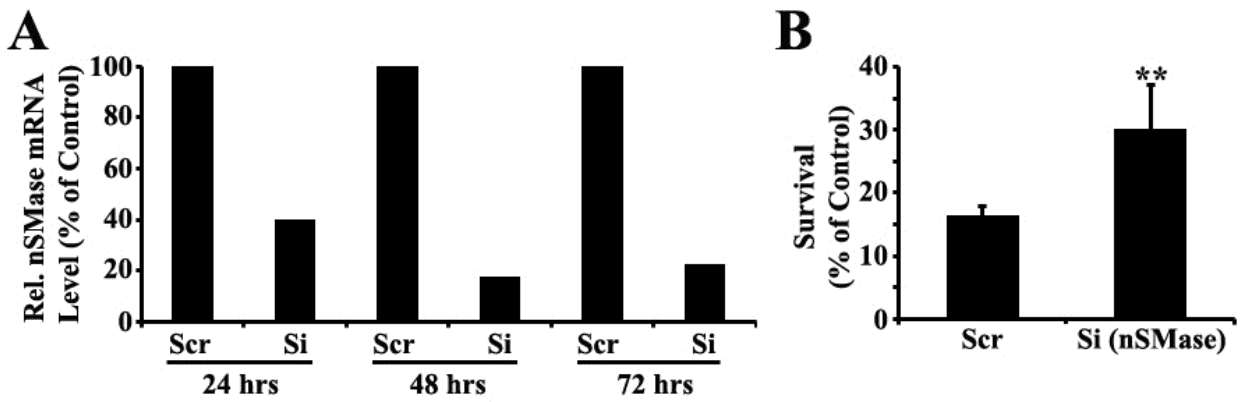

Figure S4. Effect of nSMase knockdown on survival. MCF7 cells were transiently transfected with siRNAs against nSMase (Si) or scrambled control siRNAs (Scr) followed by isolation of RNAs at different times for real time RT-PCR analysis of nSMase expression (A) or by treatment with 1 μM doxorubicin for 48 hrs and SRB assay for survival (B) as described in Materials and Methods. (**$p<0.01$)