Supplemental Figures

S1. NSC697923 has no inhibitory effect on PMA-induced ERK activation. 293T cells were treated with NSC697923 as described in Figure 1. The levels of phosphorylated (activated) ERK and total ERK protein at the indicated time points were analyzed by western blotting.

S2. Effect of partial Ubc13 knockdown on the growth of GCB-DLBCL cells. (A) Analysis of Ubc13 expression. OCI-Ly7 cells were infected with lentiviruses that express either shControl or shUbc13. The levels of Ubc13 protein in the infected cells were analyzed 7 days after infection by western blotting with a Ubc13-specific antibody. (B) The infected OCI-Ly7 cells were replated at 2.5 x 10^5 cells/ml 7 days after infection, and the live cells were counted by trypan blue exclusion assay at the indicated times after replating.

S3. (A) NSC697923 increases nuclear accumulation of p53 and p21 expression. OCI-Ly10 cells were treated with the indicated concentrations of NSC697923 for 3.5 hours. The level of p53 in the nuclear extracts and the level of p21 in the whole cell lysates were analyzed by western blotting. (B) **Ubc13 knockdown induces expression of p21 and GADD45, two of the known p53 targets.** The levels of p21 and GADD45 proteins in OCI-Ly10 cells expressing shControl and shUbc13, respectively, were analyzed by western blotting as described in Figure 8.

S4. NSC697923 inhibits DNA double-strand break (DSB) induced K63-linked ubiquitination and the recruitment of essential repair proteins to the sites of DSBs. (A)

Inhibition of FK2 focus formation by NSC697923. Osteosarcoma U2OS cells were pretreated with 1 μM NSC697923 for 1 hour before γ-irradiated (10 Gy). 30 minutes after irradiation, the cells were fixed and stained with the monoclonal antibody FK2, which recognizes protein-conjugated, but not free, ubiquitin and marks the foci formed by K63-linked ubiquitinated proteins at sites of DSBs.[1,2] (B) NSC697923 inhibits recruitment of DNA repair proteins 53BP1 and BRCA1 to the sites of DSBs after ionizing radiation. U2OS cells were treated as described in (A), and the focus formation of the indicated proteins was detected with their respective antibodies.

S5. Effect of the combination of NSC697923 and CHOP agents on the growth of DLBCL cells. OCI-Ly10 cells, seeded at 3 x 10^5 cells/ml, were treated with NSC697923 (1 μM) alone, CHOP agents (dissolved in DMSO), or a combination of NSC697923 and CHOP agents (the CHOP agents used here consist of cyclophosphamide monophosphate, doxorubicin, vincristine, and prednisone at concentrations of 5.84pM, 1.5pM, 260pM, and 1.0μM, respectively[3,4]). Shown are the live cells (mean +/- SD; n=3) measured with the trypan blue exclusion assay.

S6. Effect of NSC697923 on osteosarcoma U2OS and breast cancer MDA-MB231 cells. 1 x10^6 cells were plated in 10-cm plates and cultured overnight. The indicated concentrations of NSC697923 were then added into culture medium for 24 hour before the cell viability was analyzed by trypan blue exclusion assay. Shown are the averages from three independent experiments.

References

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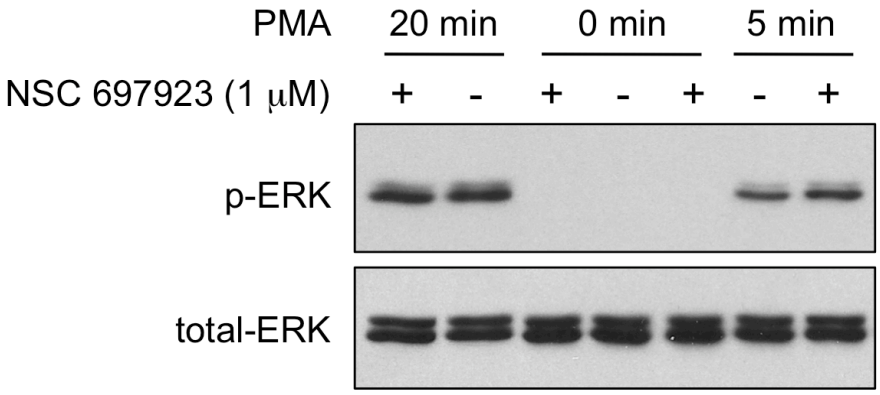
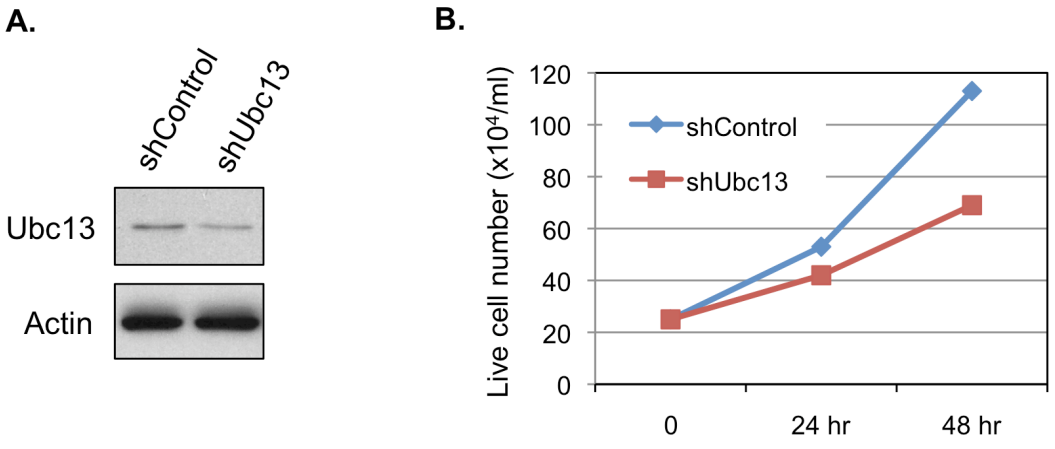
3. Kim SW, Oleksyn DW, Rossi RM, et al. Protein kinase C-associated kinase is required for NF-kappaB signaling and survival in diffuse large B-cell lymphoma cells. *Blood*. 2008;111(3):1644-1653.

4. Mohammad RM, Al-Katib A, Aboukameel A, Doerge DR, Sarkar F, Kucuk O. Genistein sensitizes diffuse large cell lymphoma to CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) chemotherapy. *Mol Cancer Ther*. 2003;2(12):1361-1368.

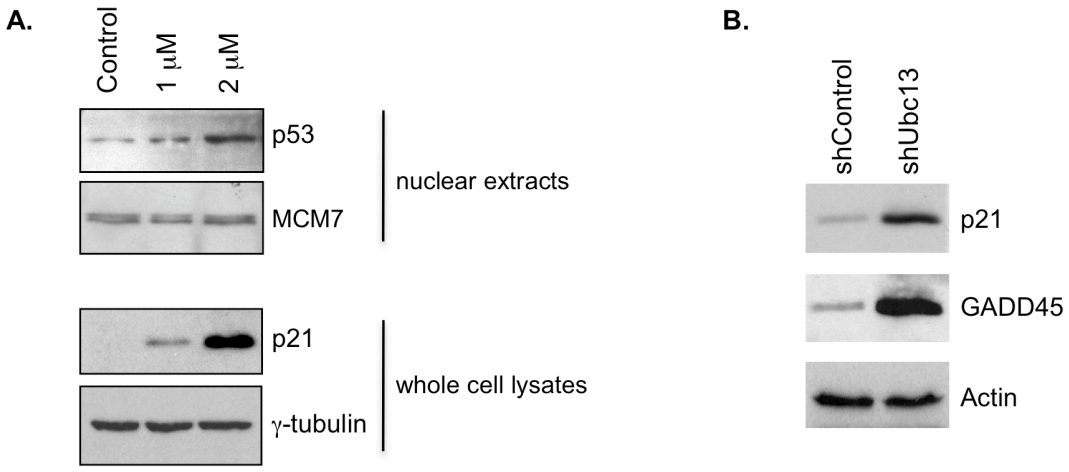
Supplemental Figure S1



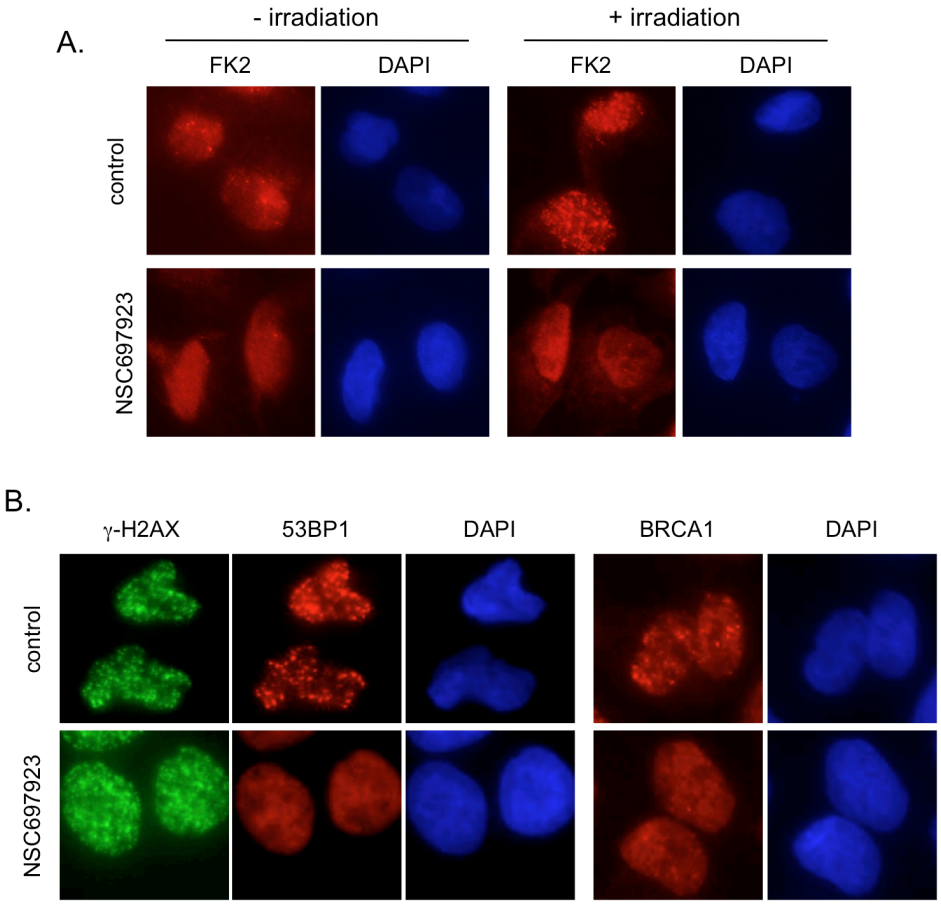
Supplemental Figure S2



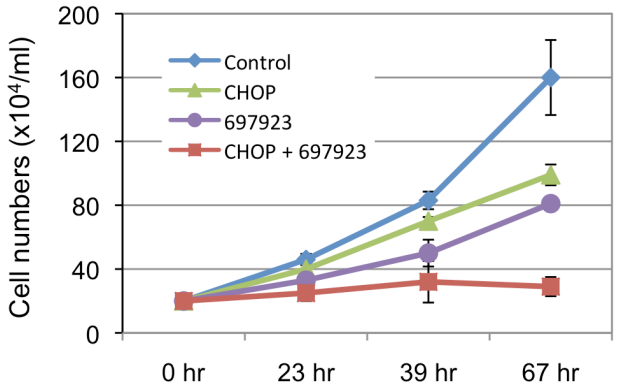
Supplemental Figure S3



Supplemental Figure S4



Supplemental Figure S5



Supplemental Figure S6