Supplemental information for Materials and Methods

Exposure to YTR107: Cells were exposed to YTR107 for 30 min prior to, during and for 90 min after irradiation. The YTR107 treatment was at 37°C. This incubation time based on the time required for repair of DNA DSBs, as measured by neutral comets assays. We found that 90 min after irradiation is sufficient for 90% or more rejoining of DSBs.

Immunofluorescence: γH2AX foci were visualized following 1 Gy and Rad51 foci visualized after 4 Gy. These doses were used because they yielded clear countable foci.

Legends for Supplemental Figures

Fig S1 Radiation-induced Repair Foci. A) Representative confocal microscopy images for irradiated cells immunostained for γ H2AX and counterstained with DAPI. B & C) NPM1 null MEFS were transiently transfected with Myc-tagged wild type NPM1 or Myc-tagged T199A NPM1 expression plasmid, a gracious gift from Prof T Ohta, Department of Translational Oncology, St. Marianna University Graduate School of Medicine, Kawasaki, Japan. Twenty four hrs after transfection some cells were immunoblotted for NPM1 (B) while others were administered 1 Gy. One hr after irradiation, cells were fixed, immunostained for γH2AX, and imaged by confocal microscopy (C). Panel B lane 1 illustrates an immunoblot of cell lysate from NPM1 null MEFs transfected with wild type NPM1, lane 2 illustrates lysate from cells transfected with Myc-tagged T199A NPM1, lane 3 represents lysate from untransfected NPM1 wild type MEFs, and lane 4 illustrates lysate from cells transfected with insertless expression vector.

Fig S2 Representative images for NPM1 wild type and null fixed cells immunostained for Rad51 foci (detected using Alexa 488 secondary antibody). Additionally, cells were imaged using Differential Interference Contrast (DIC) and displayed as inverted black and white images. NPM1 wt and null MEFs were administered 0 or 4 Gy and imaged 4 hrs after irradiation. Images were acquired using scanning confocal microscopy – 63x oil objective.

Fig S3 YTR107 binds the oligomerization domain of NPM1. A) Chemical structure of YTR107. B) Recombinant (full length or truncated (aa 1-122) His-tagged NPM1 was expressed from a pET28a vector, a gracious gift from Dr, Jason Weber, Washington University. Protein was purified using Ni-NTA agarose gel [1]. 10 ug of NPM1 was added to biotinylated-YTR107 bound to streptavidin magnetic beads and incubated for 30 min while mixing at 4°C. The magnetic beads were washed 5 times to remove unbound protein, boiled with 5x Laemmle buffer and immunoblotted for NPM1. His-NPM1-FL – Full length NPM1; His-NPM1 (1-222) – truncated NPM1, aa 1-122. C) Migration of NPM1 on a 4-16% NativePage Bis Tris gel (Life Technologies). Recombinant human NPM1 (2.4 μM) was exposed to solvent control (lane 1), YTR107 (24μM, lane 2), or positive control NSC348884 (24μM, lane 3), resolved by native gel electrophoresis according to the manufacturer's instructions and stained with Coomassie blue. Arrows identify NPM1.

Fig S4 pNPM1 is a nuclear protein and forms IRIF following irradiation. HeLa cells, used for their excellent optical properties, were grown on cover slips, exposed to DMSO or 25 μM YTR107 in DMSO for 30 min, administered 0 or 3 Gy, and then incubated at 37°C for 1.5 hrs. Paraformaldehyde-fixed cells were incubated with pT199 NPM1 rabbit monocolonal (Abcam). Detection of primary antibody was accomplished using Rhodamine Red. Nuclei were stained with DAPI. Immunostaining was visualized by scanning confocal microscopy using a 63x oil objective. Zen2009 Light Edition software was used to create images. Z section = 0.576 μM

thickness, image has been enlarged 160%. A) Representative nuclei from cells exposed to DMSO for 90 min at 37°C. B) Representative nuclei from cells exposed to 25 μM YTR107 for 90 min at 37°C. C) Representative nuclei of cell administered 3 Gy and incubated at 37°C for 90 min prior to fixation. D) Representative nuclei from cells exposed to 25 μM YTR107 for 30 min prior, during and for 90 min/37°C after administration of 3 Gy.

Fig S5 YTR107 inhibits pNPM1 IRIF co-localization with γH2AX foci. HeLa cells were grown on cover slips, exposed to DMSO or 25 μM YTR107 in DMSO for 30 min, administered 0 or 3 Gy, and then incubated at 37°C for 1.5 hrs. Paraformaldehyde-fixed cells were incubated with pT199 NPM1 rabbit monocolonal (Abcam). Detection of primary antibody was accomplished using Rhodamine Red. γH2AX foci were immunostained using pS139 γH2AX antibody from Millipore. Detection of primary antibody was accomplished using Alexa 488. Nuclei were stained with DAPI. Immunostaining was visualized by scanning confocal microscopy using a 63x objective. Zen2009 Light Edition software was used to create images. Z section = 0.576 μM thickness, image has been enlarged 160%. Representative images showing co-localization of pNPM1(yellow dots denoted by white arrow) and γH2AX IRIF in cells treated with DMSO. Large numbers of γH2AX foci are observed in cells treated with YTR107 that lack pNPM1 IRIF.

Fig S6 YTR107 inhibits formation of pNPM1 IRIF. HeLa cells, were administered 0 or 3 Gy in growth medium supplemented with DMSO or 25 μM YTR107, incubated at 37°C for 4 hrs, fixed and stained for pT199NPM1 (Rhodamine, red) and NPM1 (Alexa 488, green). Staining for NPM1 (green) is a well characterized marker for nucleoli. Administering 3 Gy results in formation of pNPM1 IRIF (panel C, white arrow), consistent with the work of [2] who observed such foci in MCF7, MCF10A, 293T, and T47D cells. Exposure to YTR107 during and after irradiation blocked formation of pNPM1 IRIF (panels D & E, $P < 0.038$, n = 8 random fields per point). Immunostaining was visualized by scanning confocal microscopy using a 63x objective. Z section = 0.91 μM thickness.

Fig S7 Analysis of NPM1 expression in NSCLC using the Oncomine database. Data derived from Hou et al PLOS ONE 2010, 5(4):e10312 PMID: 20421987.

Fig S8 YTR107 increases radiation sensitivity of NSCLC. A) A549 and B) H226 NSCLC cells were exposed to DMSO solvent control or 25 μM YTR107 for 30 min prior to, during and for 90 min after irradiation. Survival (+/-SD) was determined from colony formation assays. C) Calu1, HCC827, PC9/BRC1 and PC9/BRC4 cells were exposed to 25 μM YTR107 for 30 min prior to irradiation, during irradiation (6 Gy), and for 90 min afterward. Survival (+/- SD) was determined from colony formation assays. Fold change relative to control represents the ratio of (cell survival of cells irradiated in the presence of DMSO divided by the cell survival of cells irradiated in the presence of YTR107) x -1. D) A549 cells growing sc in the legs of athymic nu/nu mice were injected intraperitoneally with DMSO or 20 mg/kg of YTR107 and 30 minutes later the tumors were administered 0 or 2.4 Gy. This protocol was administered for 7 consecutive days. Fold change in tumor volume +/- SE is shown. N= 5 mice per point.

Reference

1. Sekhar KR, Reddy YT, Reddy PN, Crooks PA, Venkateswaran A, McDonald WH, Geng L, Sasi S, Van Der Waal RP, Roti JL, Salleng KJ, Rachakonda G, Freeman ML. The novel chemical entity ytr107 inhibits recruitment of nucleophosmin to sites of DNA damage, suppressing repair of DNA double-strand breaks and enhancing radiosensitization. *Clin Cancer Res* 2011;17:6490-6499.

2. Koike A, Nishikawa H, Wu W, Okada Y, Venkitaraman AR, Ohta T. Recruitment of phosphorylated npm1 to sites of DNA damage through rnf8-dependent ubiquitin conjugates. *Cancer Res* 2010.

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