Fig. S1

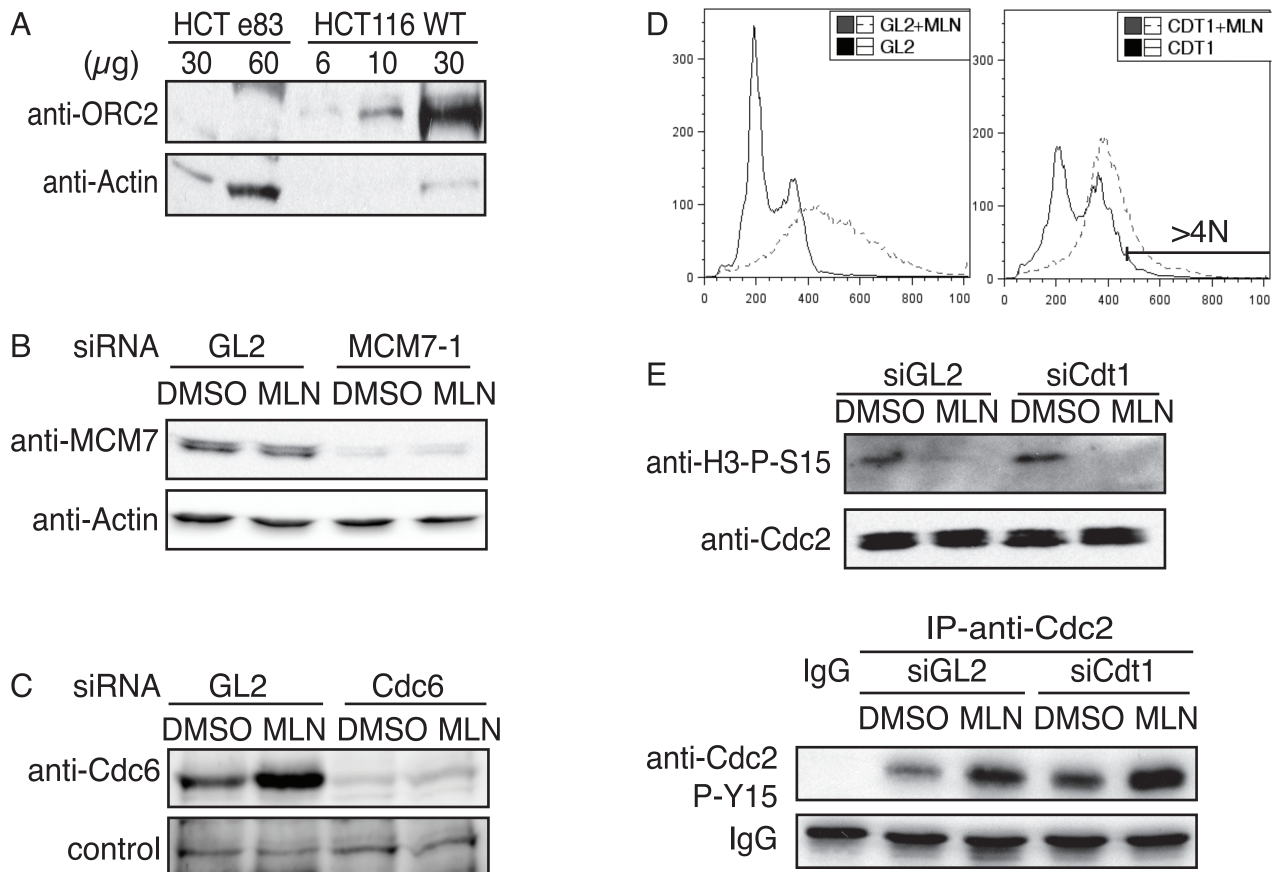

Figure S1. Cdt1 protein level is important for MLN4924 induced re-replication but not G2/M arrest in HCT116 cells
(A) Different amounts of cell lysates from Fig. 1C were immuno-blotted with indicated antibodies.
(B) HCT116 cells were treated as described in Fig. 1D. The levels of Mcm7 were shown. Actin was shown as a loading control. (C) Cell lysates from Fig. 1E were immuno-blotted with indicated antibodies.
(D) FACS profiles of samples described in Figure 1(A). (E) Total cell lysates of the above samples were blotted with indicated antibodies to show the G2/M arrest. Total cdc2 proteins were immuno-precipitated before blotting with cdc2-P-Y15 antibody to show Y15 phosphorylated cdc2.

Fig. S2

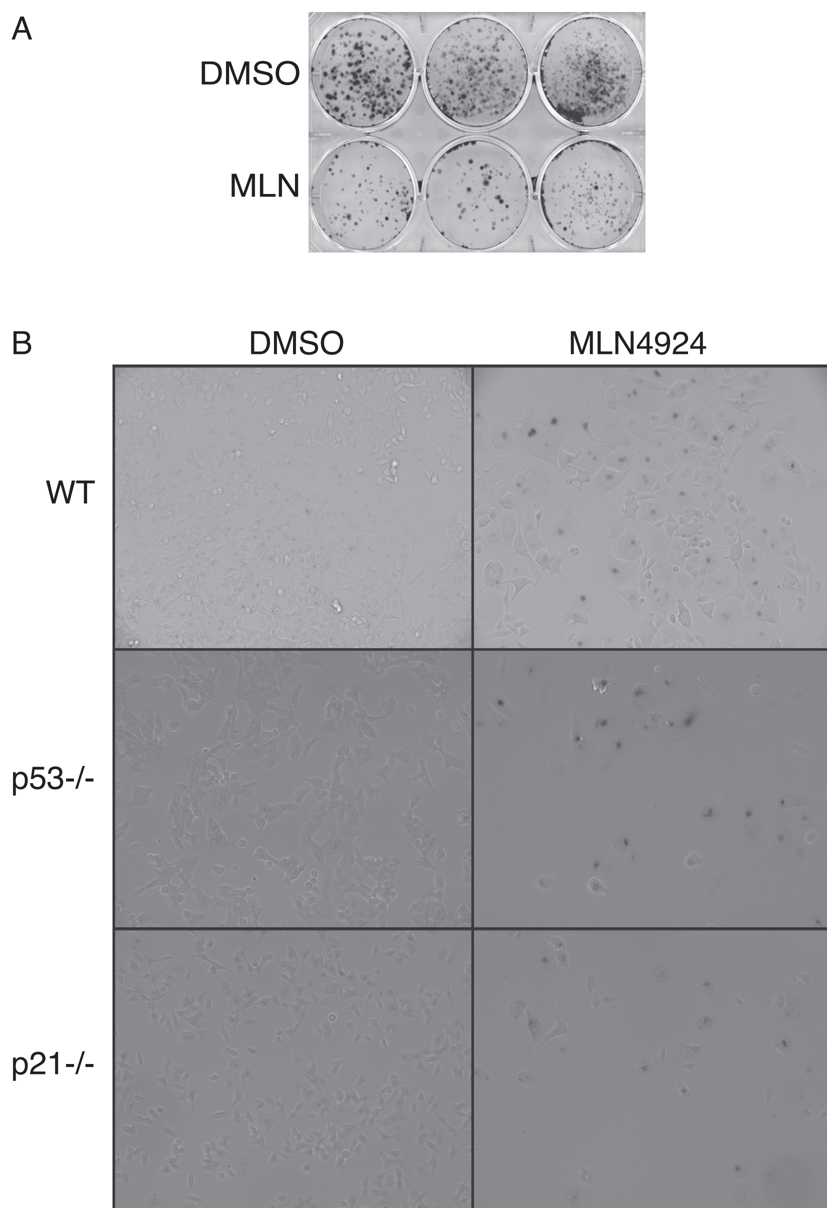

Figure S2. p21 plays an important role in causing cellular senescence following transient exposure to MLN4924
(A) HCT116 cells were treated as described in Fig. 6B. Colonies were stained with crystal voilet to show cell clonogenicity. (B) HCT116 WT, p53-/- or p21-/- cells were treated with DMSO or 1μM MLN4924 for 8 hours. SA-β-gal staining assay was performed 72 hours after the washout. Representative pictures were shown.

Fig. S3

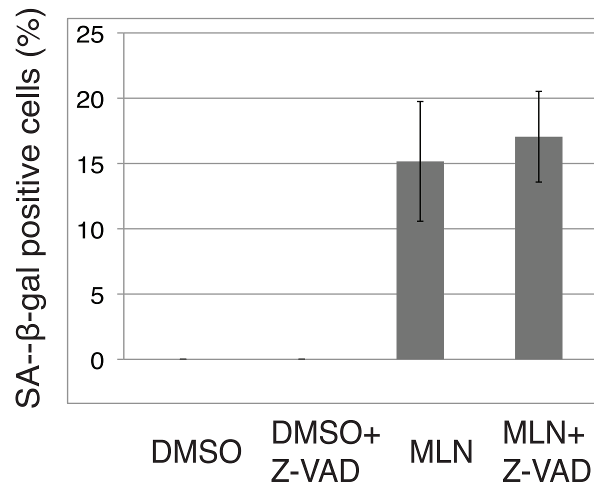

Figure S3. Apoptosis inhibitor Z-VAD-FMK cannot reduce MLN4924 induced senescence
HCT116 cells were treated with DMSO or 1μM MLN4924 for 8 hours and incubated in fresh medium for 24 hours before treated with 50μM Z-VAD for 48 hours. Fresh Z-VAD were added every 24 hours. Cells were then stained with SA-β-gal. SA-β-gal positive cells were counted and plotted as a percentage of total cells.

Fig. S4

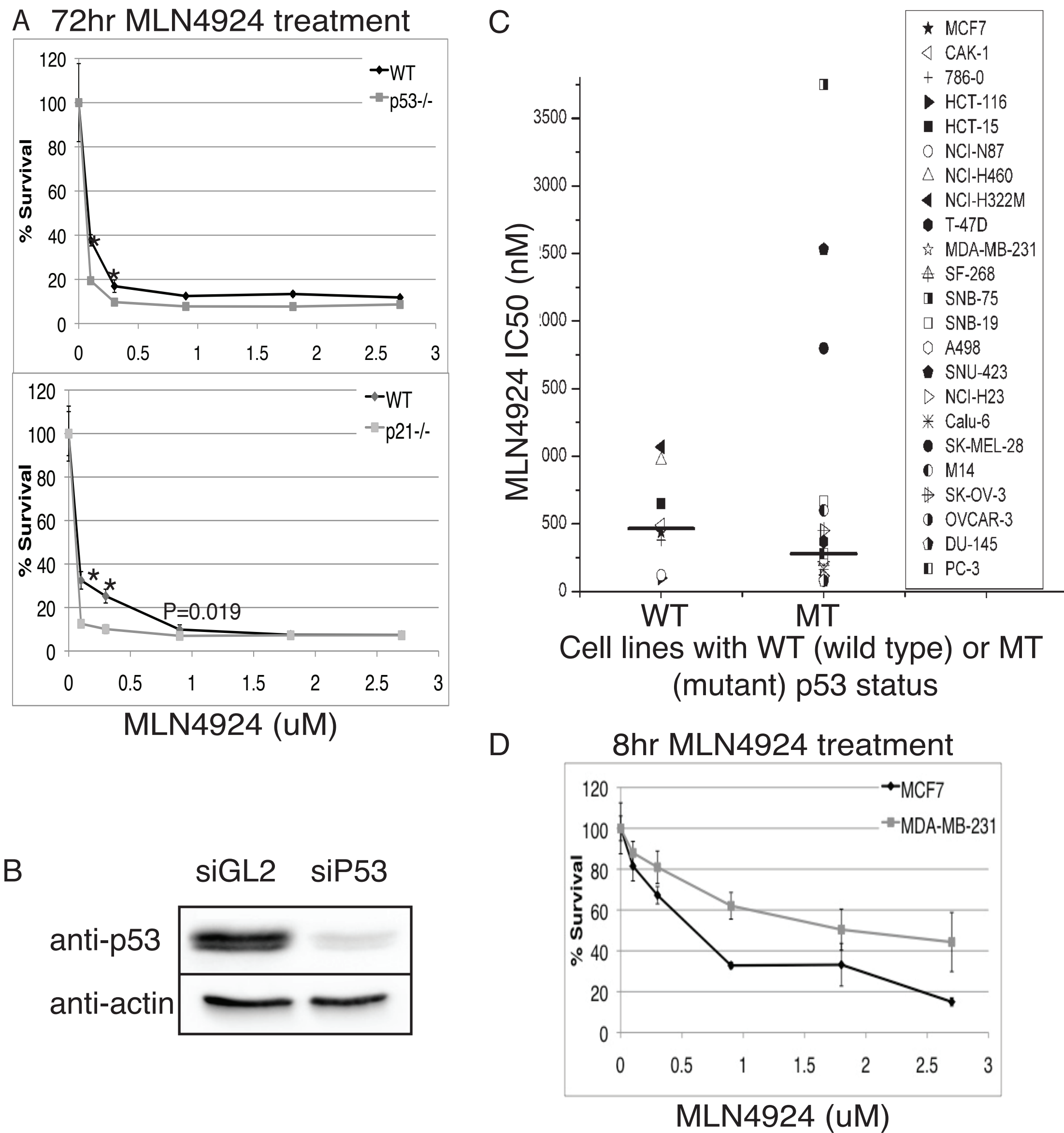

Fig S4. p53 status is one of the factors that affect MLN4924 induced cell death

(A) Viable HCT116 cells after 72hr MLN4924 treatment at different doses were measured using MTT assay. The points indicate mean and standard deviation of triplicates. * indicates statistically significant difference at various MLN4924 concentrations between the WT and the mutant cells ($p<0.01$). (B) MCF7 cells were transfected with siGL2 or siP53 24hr before MLN4924 exposure. Cell lysates were harvested before MLN4924 treatment and blotted with p53 and actin. These are the cells used in Fig. 7B. (C) 3,000–8,000 cells were seeded per well in 96-well culture plates and incubated overnight at 37ºC. MLN4924 was added to the cells and incubated for 72 hours. Cell viability was determined using the ATPlite assay. Median values were indicated with lines. The cells are classified based on their p53 status: wild type or mutant. (D) Viable MCF7 (p53 WT) and MDA-MB-231 cells (p53 mutant) after 8hr MLN4924 treatment at different doses were measured using MTT assay. The points indicate mean and standard deviation of triplicates.

Supplemental methods

Cell viability assays were performed by Southern Research (Birmingham, Alabama). Exponentially growing cell suspensions were seeded at 3,000–8,000 cells per well in 96-well culture plates and incubated overnight at 37ºC. MLN4924 was added to the cells in complete growth media and incubated for 72 hours at 37ºC. Cell viability was determined using the ATPlite assay (Perkin Elmer, Waltham, MA).

Supplemental figure legends

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