Supplemental Figure 1. *Stable RB knockdown in several MCF7 clones.* Several stable RB-deficient MCF7 clones (siRb17, siRb21, siRb28, siRb44) were generated through transfection of an shRNA plasmid and tested for RB expression by immunoblot and compared to the RB-proficient control clone (MCF7 donor1, Lane 1). Cdk4 served as a loading control.

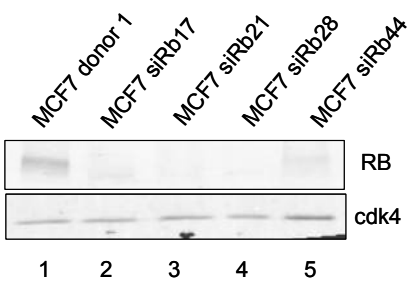
Supplemental Figure 2. *RB-deficiency enables accelerated cell cycle progression.* RB-deficient clones (siRb17, siRb21, siRb28, siRb44) and donor control cells were labeled with BrdU in culture and BrdU immunofluorescence was performed and scored.

Supplemental Figure 3. *Bypass of anti-estrogen checkpoint is evident in several RB-deficient MCF7 clones.* Clones from supplemental figure 1 were cultured in media containing FBS, CDT/Tam, or CDT/ICI for 3 days while BrdU labeling for the final 10h. Cells were then fixed and BrdU immunofluorescence was performed and scored.

Supplemental Figure 4. *Effect of RB- verses p53- deficiency on cell growth, DNA damage checkpoint, and response to hormone ablation.* (A.) Lysates from MCF7 donor1 and MCF7 siRb28 cells infected with retroviruses encoding LXSN or LXSN-p53dd were immunoblotted for expression levels of p53 and p21. Lamin B served as a loading control. Lysates from the same cells treated with 16μM CDDP for 18 hours were immunoblotted for induction of p21 expression. Lamin B served as a loading control. (B.) MCF7 donor1 and MCF7 siRb28 cells infected with retroviruses encoding LXSN or LXSN-p53dd were labeled with BrdU for 5 hours in culture. BrdU immunofluorescence was performed and scored as previously described. (C.) Cells from B were treated with IR and CDDP as before and labeled with BrdU for 5 hours. Cells were then fixed and immunofluorescence was performed and counted. (D.) Cells from B were seeded at $3x10^5$ and treated with 5 Gy IR. Cell growth assays were performed for 12 days while cells were counted every 3 days. Data is represented as relative cell growth with respect to MCF7 donor 1 LXSN controls at day 12. (E.) Cells from B were cultured in media containing FBS or CDT/Tam for 3 days while BrdU labeling for the final 5h. BrdU staining was performed and counted as before.

Supplemental Figures:

1.



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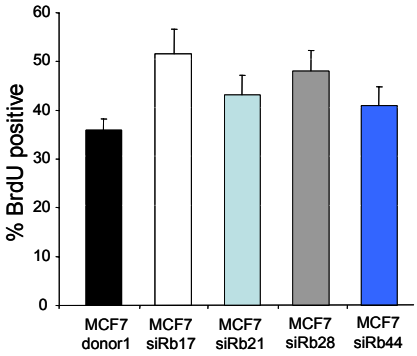

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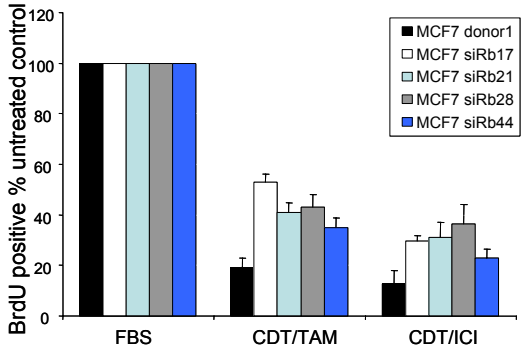

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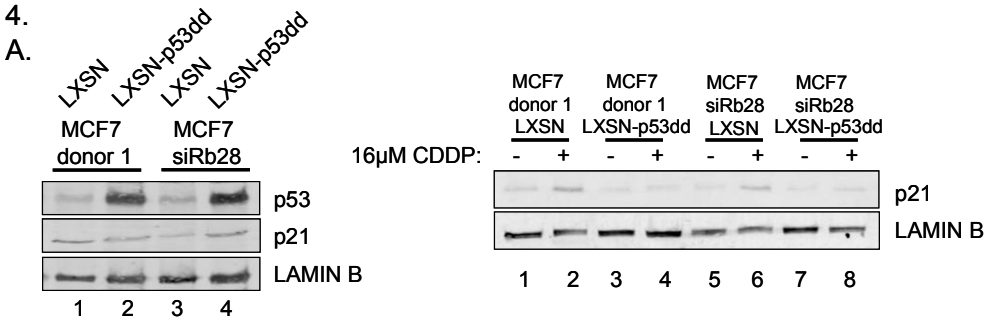
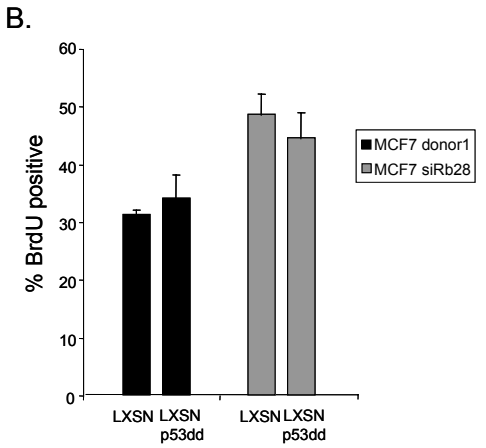
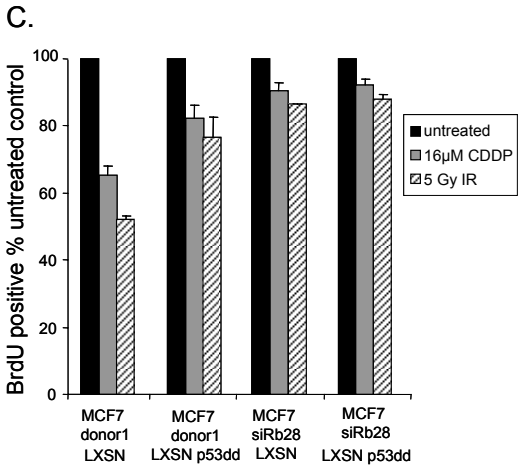
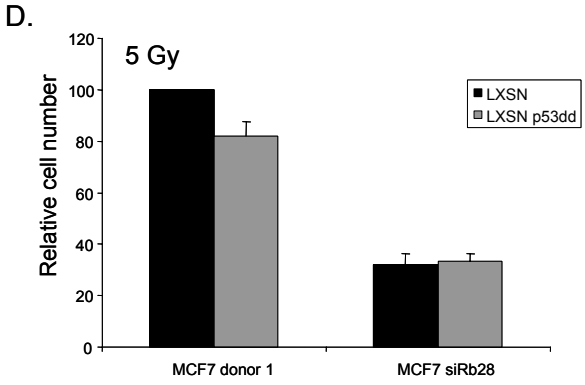
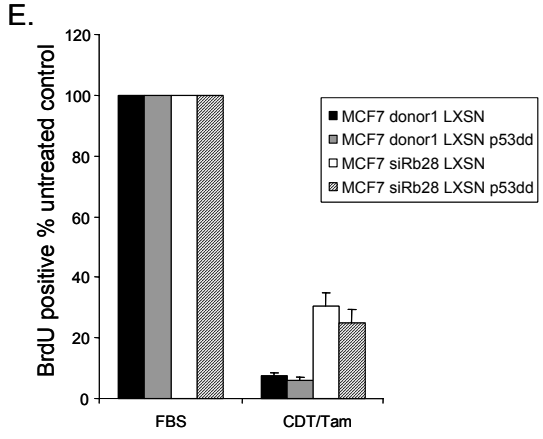

A.
LXSN
LXSN-p53dd
LXSN
LXSN-p53dd
MCF7 donor 1
MCF7 siRb28
p53
p21
LAMIN B
1 2 3 4
16μM CDDP:
MCF7 donor 1 LXSN
MCF7 donor 1 LXSN-p53dd
MCF7 siRb28 LXSN
MCF7 siRb28 LXSN-p53dd
- + - + - + - +
p21
LAMIN B
1 2 3 4 5 6 7 8


B.
% BrdU positive
MCF7 donor1
MCF7 siRb28
LXSN
LXSN p53dd
LXSN
LXSN p53dd


C.
BrdU positive % untreated control
untreated
16μM CDDP
5 Gy IR
MCF7 donor1 LXSN
MCF7 donor1 LXSN p53dd
MCF7 siRb28 LXSN
MCF7 siRb28 LXSN p53dd


D.
5 Gy
Relative cell number
LXSN
LXSN p53dd
MCF7 donor 1
MCF7 siRb28


E.
BrdU positive % untreated control
MCF7 donor1 LXSN
MCF7 donor1 LXSN p53dd
MCF7 siRb28 LXSN
MCF7 siRb28 LXSN p53dd
FBS
CDT/Tam