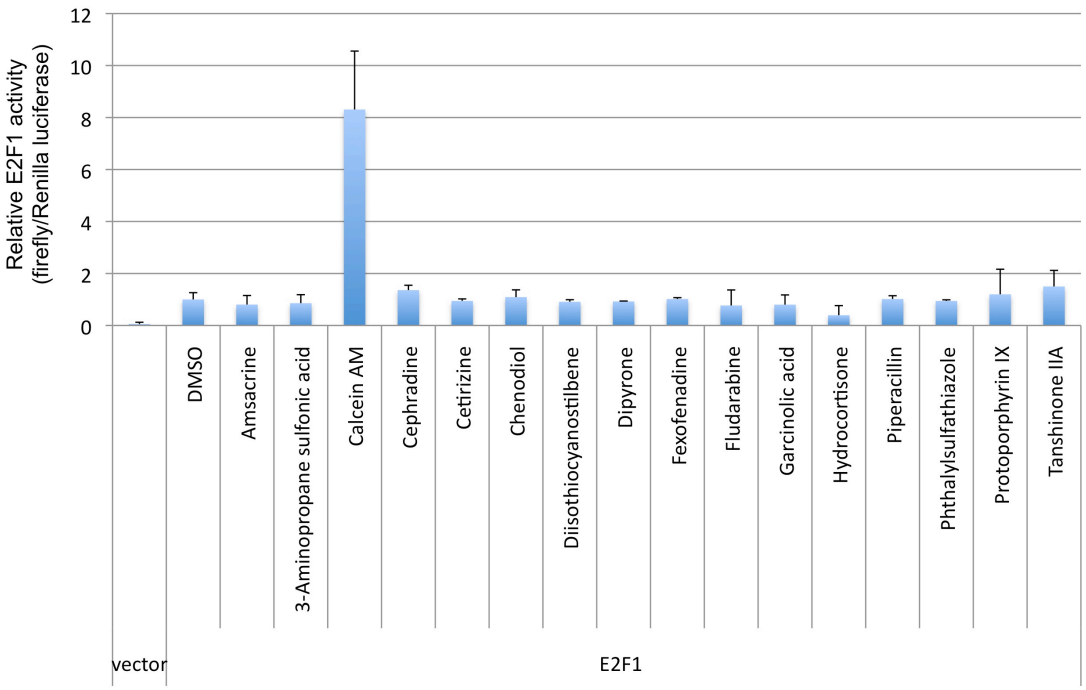
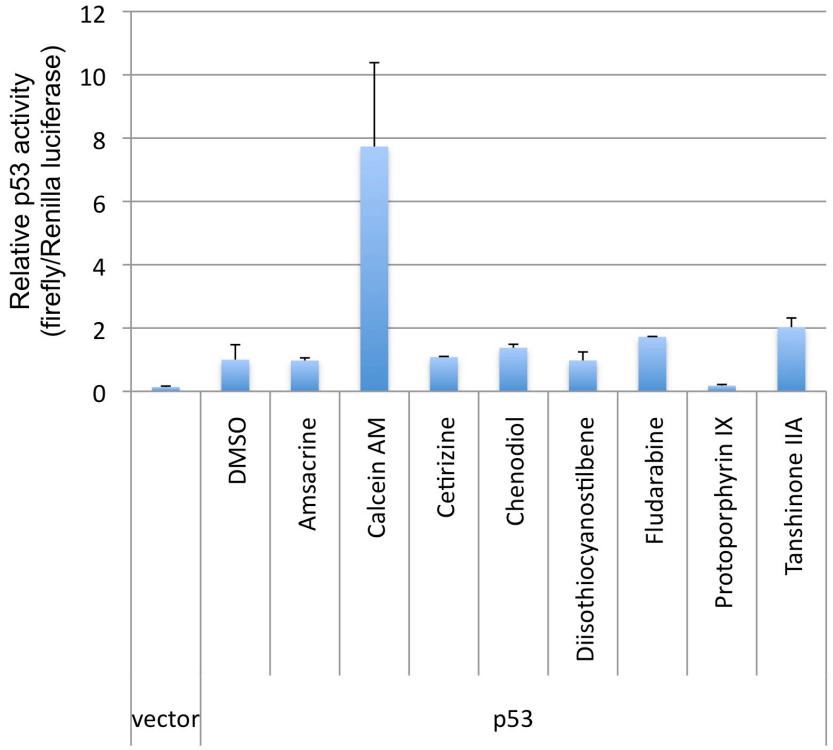


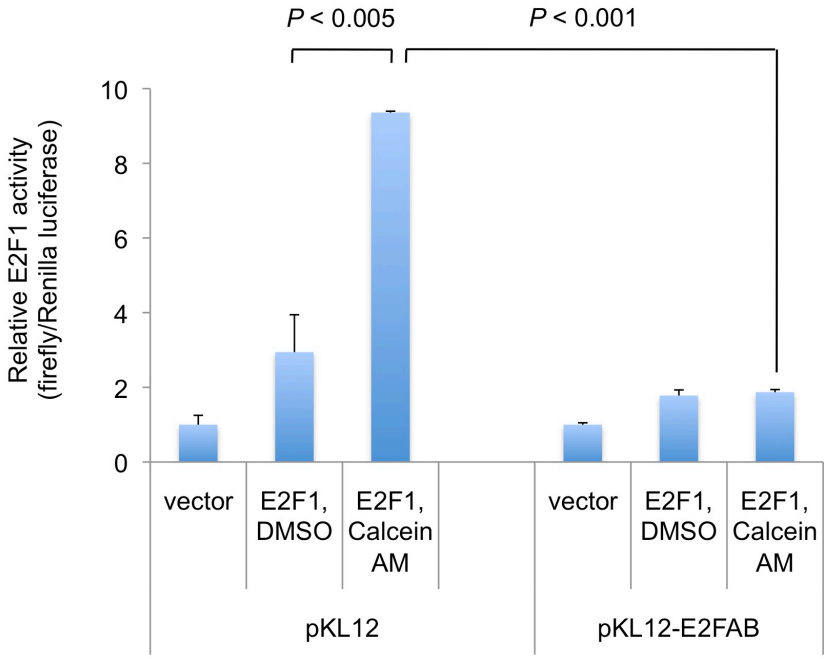
Supplementary Figure 1. Compound screening by an E2F1 activity reporter assay.

E2F1 transcriptional activity was measured by a $p14^{Arf}$ promoter-firefly Luc (along with control Renilla luciferase pRL-TK) dual luciferase assay in HEK293T cells after treatment of various compounds. The normalized activities of E2F1 were shown as fold of change relative to the DMSO control. The compounds were tested at 20 μM for overnight. The results derived from six independent experiments. Each compound was tested in triplicate in each experiment, and most of them were examined independently in two to five experiments. Shown are means ± S.D.



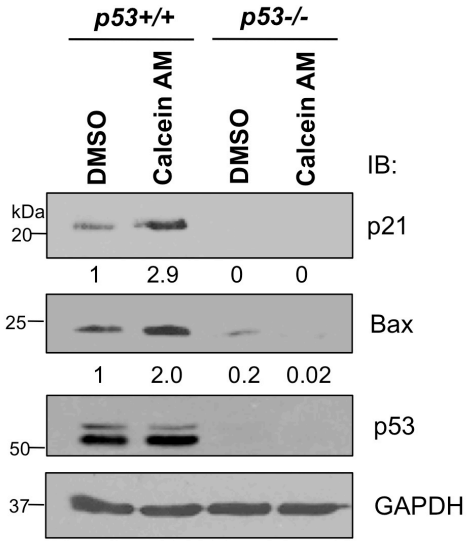
Supplementary Figure 2. Compound screening by a p53 activity reporter assay.

Selected compounds were further screened by p53 transcriptional reporter assay (by p21 promoter-firefly Luc and pRL-TK dual luciferase assay) in H1299 at 20 μM for 5 h. The normalized activities of p53 (means ± S.D.) were shown as fold of change relative to that of the DMSO control.



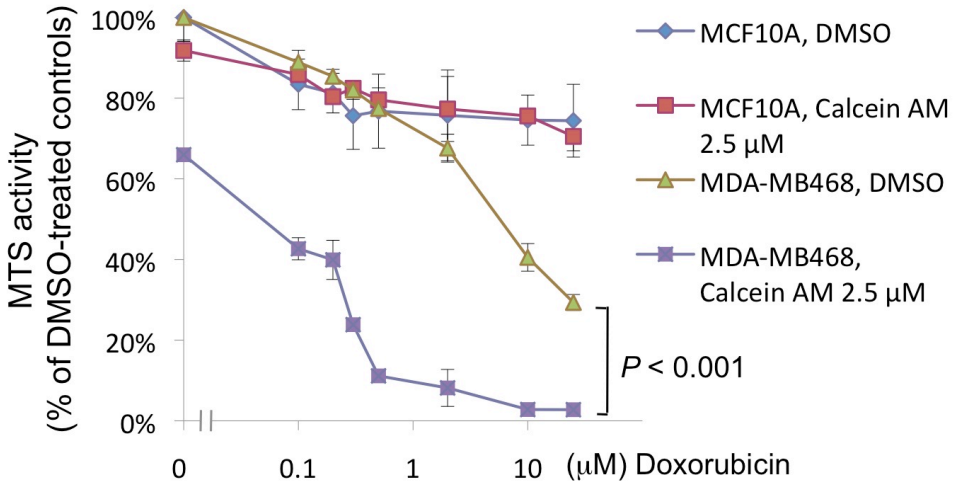
Supplementary Figure 3. Calcein AM activates E2F1 transcriptional activity in an E2F site-dependent manner.

E2F1 transcriptional activity was measured by a DNA polymerase α promoter-firefly Luc construct pKL12 (along with control Renilla luciferase pRL-TK) dual luciferase assay in HEK293T cells after treatment of Calcein AM (5 μM for 3 h). pKL12-E2FAB contains mutations on two E2F sites in the promoter of polymerase α. The normalized activities of E2F1 were shown as fold of change relative to the DMSO control. Results shown are the means ± S.D. (n = 3 biological replicates; *P* values are based on two-tailed *t* test).



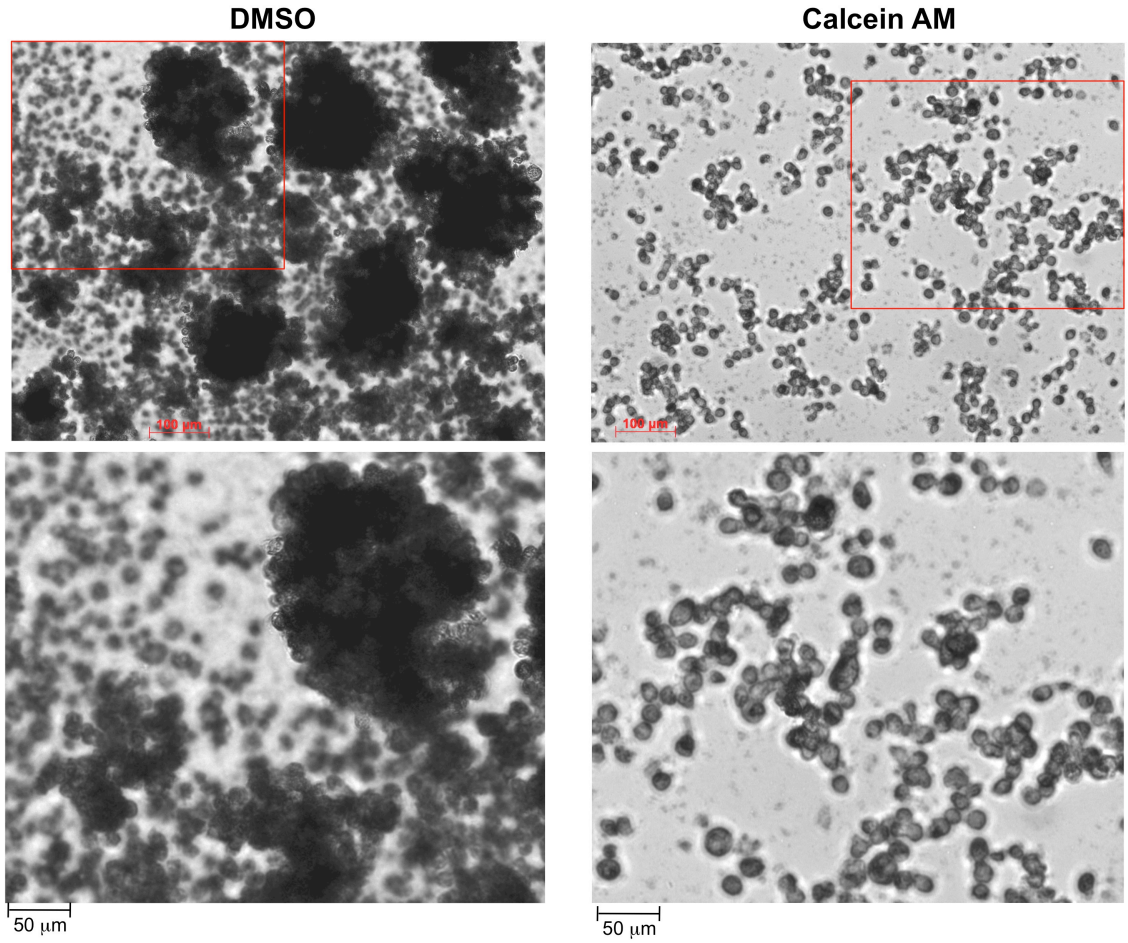
Supplementary Figure 4. Calcein AM induces the expression of p21 and Bax in a p53-dependent manner.

HCT116 cells with either $p53^{+/+}$ or $p53^{-/-}$ status were treated with Calcein AM (2.5 μM for 3 h) or DMSO vehicle. Cells were harvested and cell lysates were then subjected to SDS-PAGE and immunoblottings. The p21 and Bax signals were quantified by ImageJ. The relative values compared to the $p53^{+/+}$ DMSO control are shown below each panel.



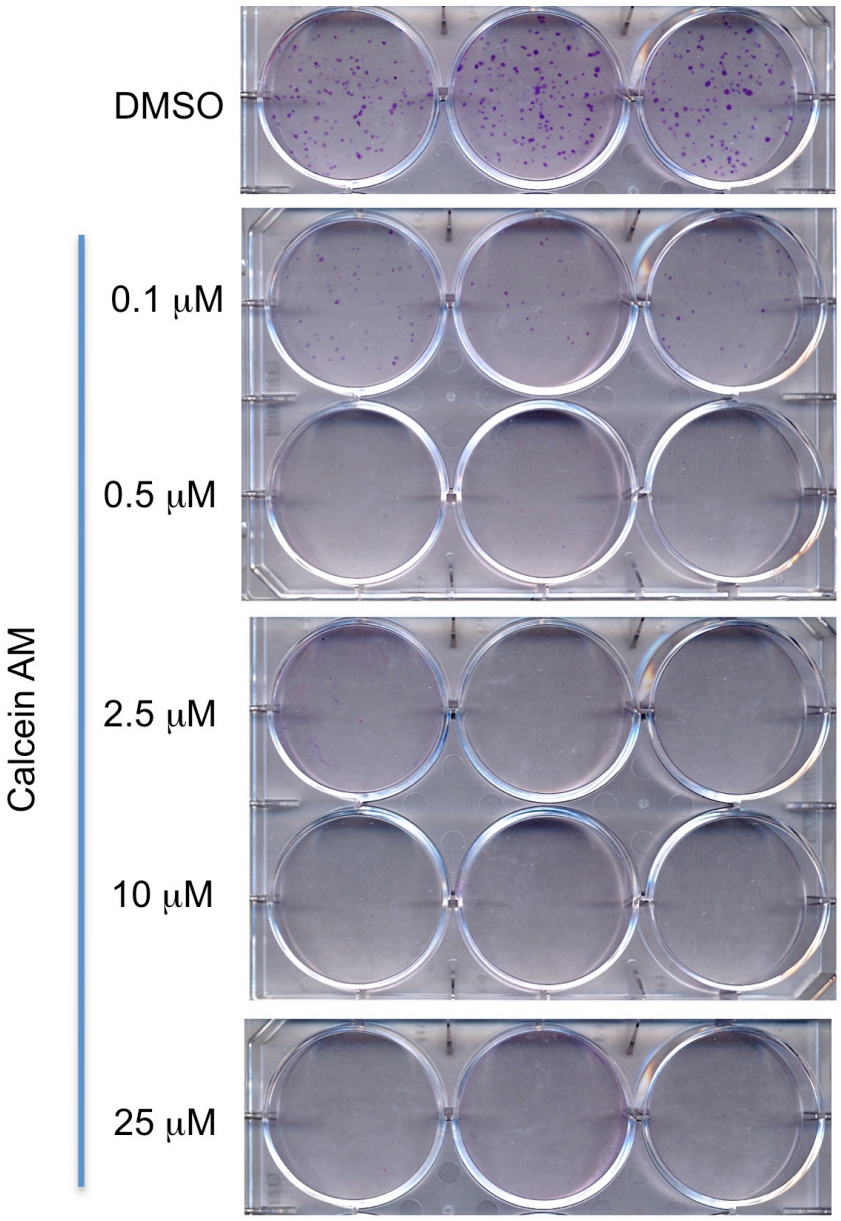
Supplementary Figure 5. Fig. 4c data normalized to DMSO controls.

The 490nm absorbance readings in "MCF10A, Calcein AM 2.5 μM" and "MDA-MB468, Calcein AM 2.5 μM" groups in Fig. 4c were normalized to that of "DMSO, 0 μM-Doxorubicin" MCF10A and MDA-MB468 control cells, respectively.

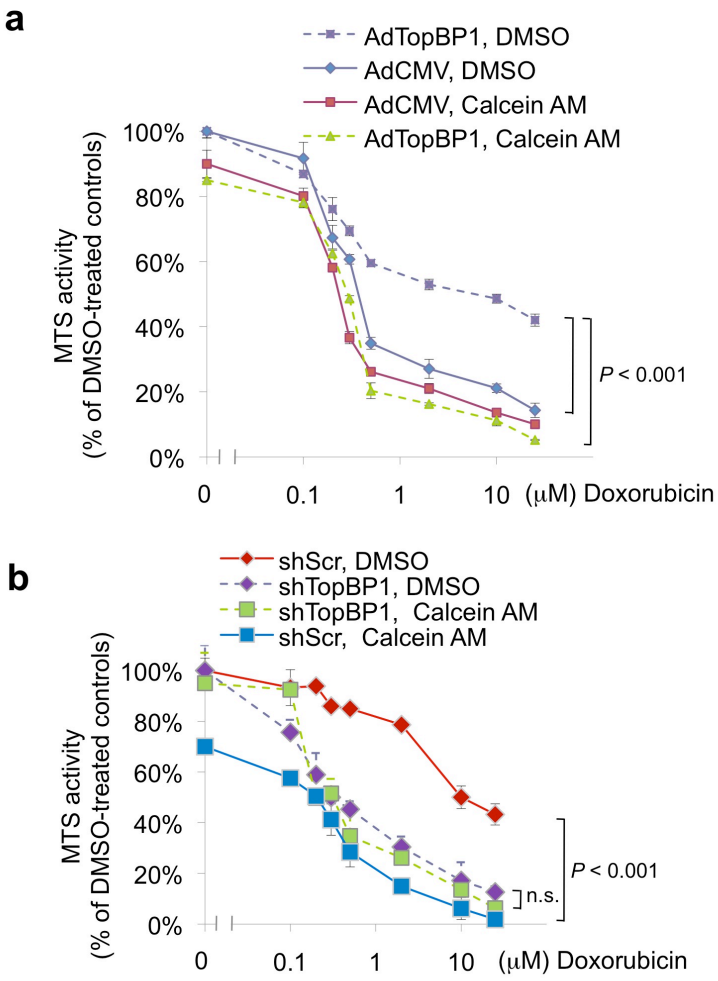


Supplementary Figure 6. High-magnification images of Fig. 4d.

High-magnification images of Fig. 4d show sphere formation in DMSO-treated MDA-MB468 cells, whereas in Caclein AM treated wells, presence of morphologically intact cells without forming mammospheres as well as many dead cell debris.

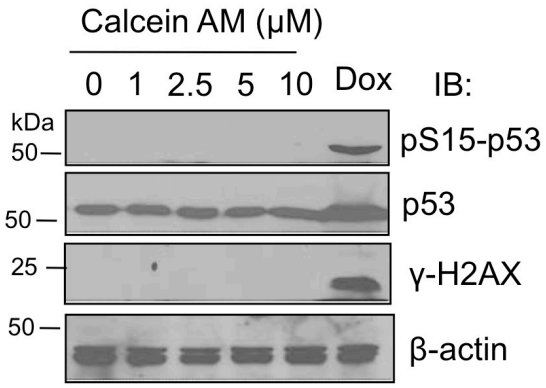


Supplementary Figure 7. A complete set of colony formation pictures for Fig. 4e.



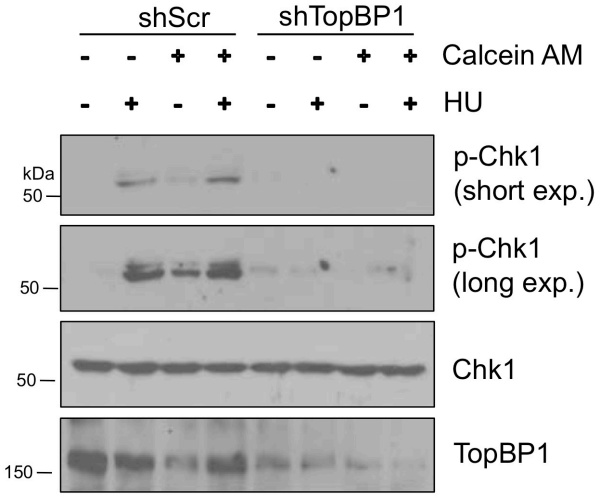
Supplementary Figure 8. Fig. 8a&b data normalized to DMSO controls.

The 490nm absorbance readings of the MTS assay in Fig. 8a and b were normalized to those of "DMSO, 0 μM-Doxorubicin" cells.



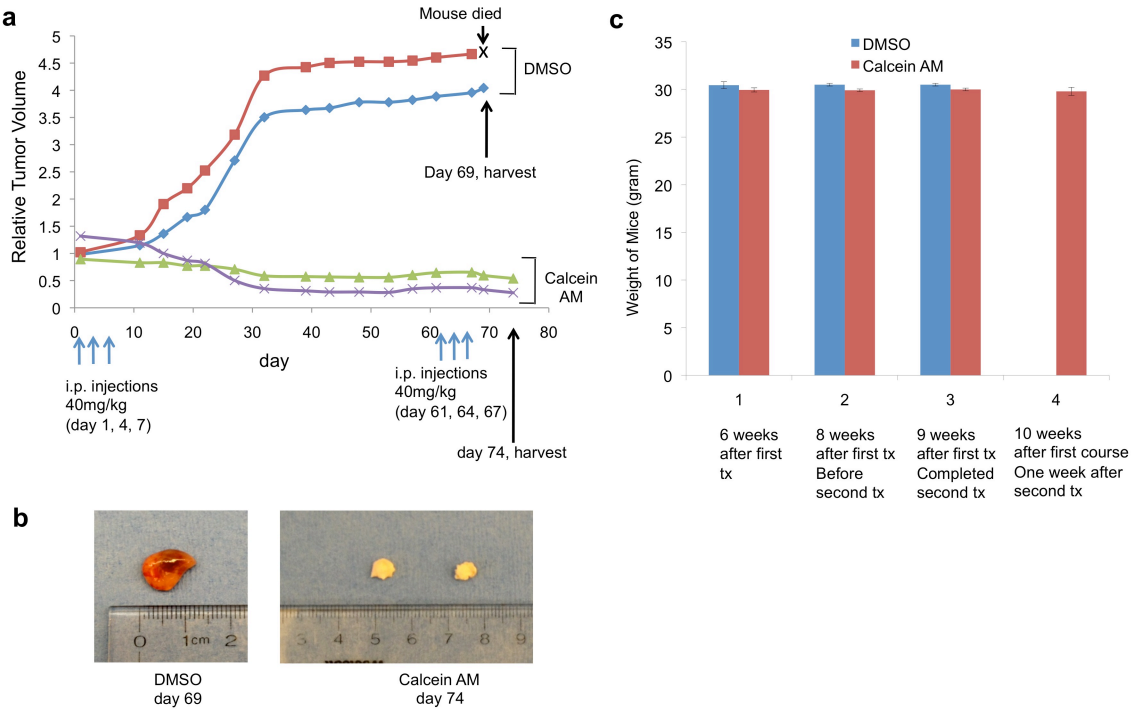
Supplementary Figure 9. Calcein AM treatment does not induce DNA damage response.

MDA-MB468 cells were treated with Calcein AM at the indicated concentrations or doxorubicin (Dox, 5 μM) for 5 h. Cell lysates were subjected to Western blot analysis. Doxorubicin treatment serves as a positive control for DNA damage.



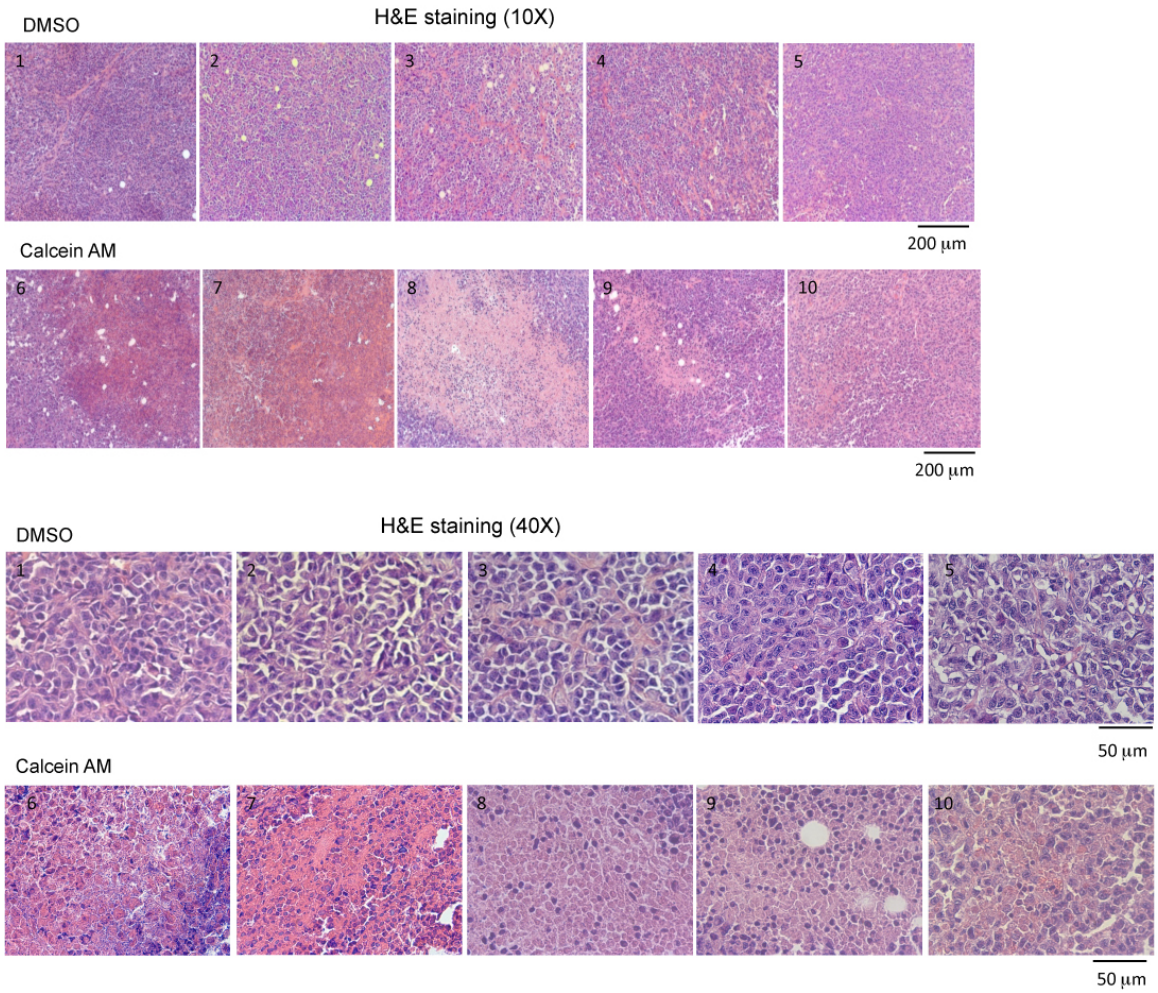
Supplementary Figure 10. Calcein AM does not block HU-induced Chk1 activation.

MDA-MB468 stable cell lines expressing either a control siScr or siTopBP1 were treated with hydroxyurea or (2 μM) or Calcein AM (2.5 μM) or combination, or vehicle control for 12 h. The cells were harvested and the lysates were analyzed by Western blot as indicated.



Supplementary Figure 11. The activity of Calcein AM in MDA-MB468 xenografts.

(a) Nude mice bearing MDA-MB468 xenografts were administered with Calcein AM or vehicle DMSO via i.p. injection. **(b)** Photographs of the MDA-MB468 xenograft tumors. **(c)** Mouse body weights during two courses of treatment.

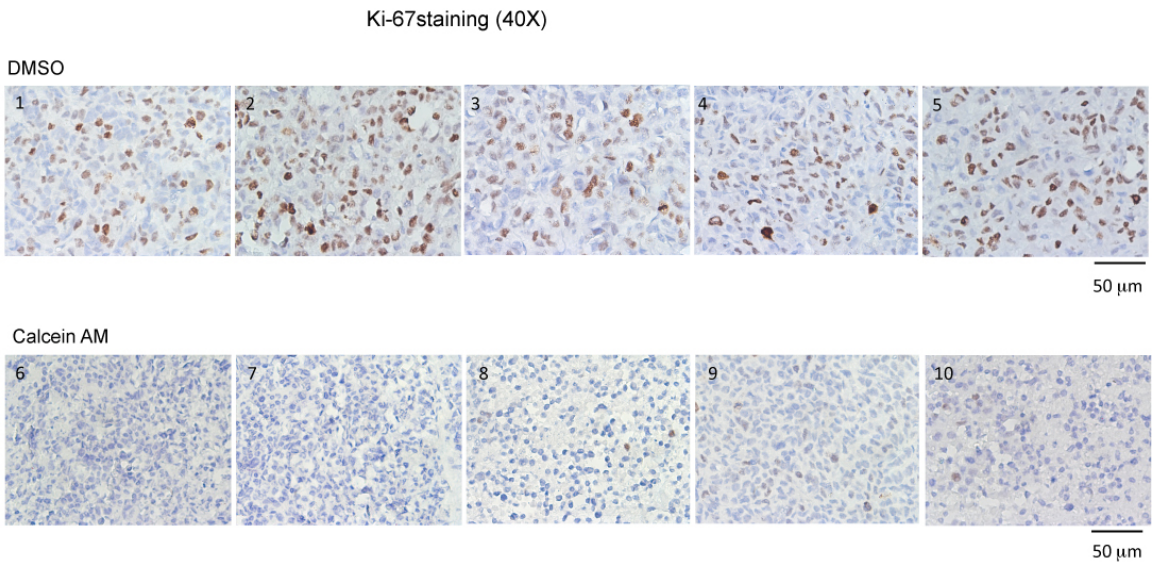


Supplementary Figure 12. H&E staining of MDA-MB468 xenografts in Figure 9b.

Representative images of H&E of xenografts at 10X and 40X magnifications.

Xenografts 1-5: DMSO control

Xenografts 6-10: Calcein AM

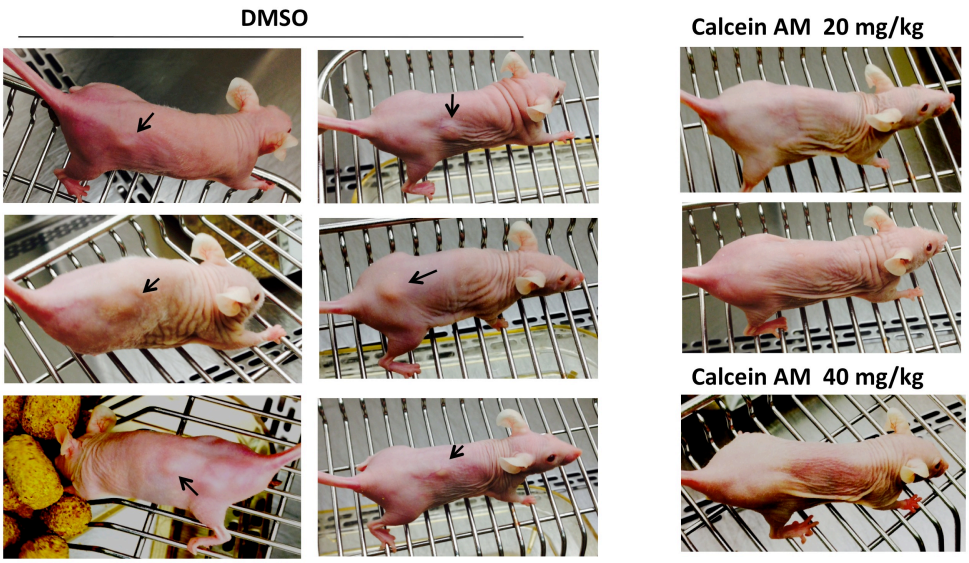


Supplementary Figure 13. Ki-67 staining of MDA-MB468 xenografts in Figure 9b.

Representative images of Ki-67 immunohistochemical staining of xenografts at 40X magnification.

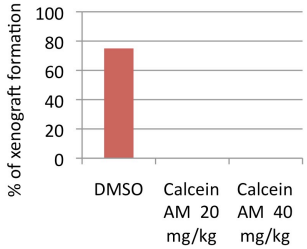
Xenografts 1-5: DMSO control

Xenografts 6-10: Calcein AM



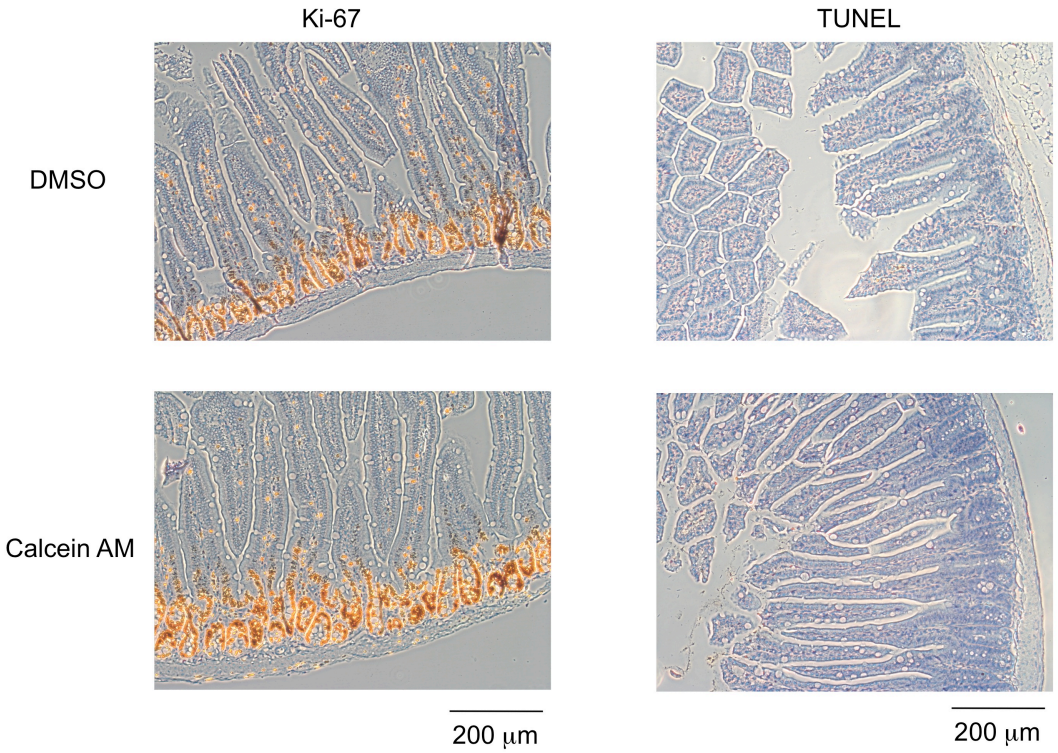
DMSO	Calcein AM 20 mg/kg	Calcein AM 40 mg/kg
6/8	0/8 **	0/4 *

* p = 0.0143 (Chi-square, compared with DMSO group)
** p = 0.0019 (Chi-square, compared with DMSO group)



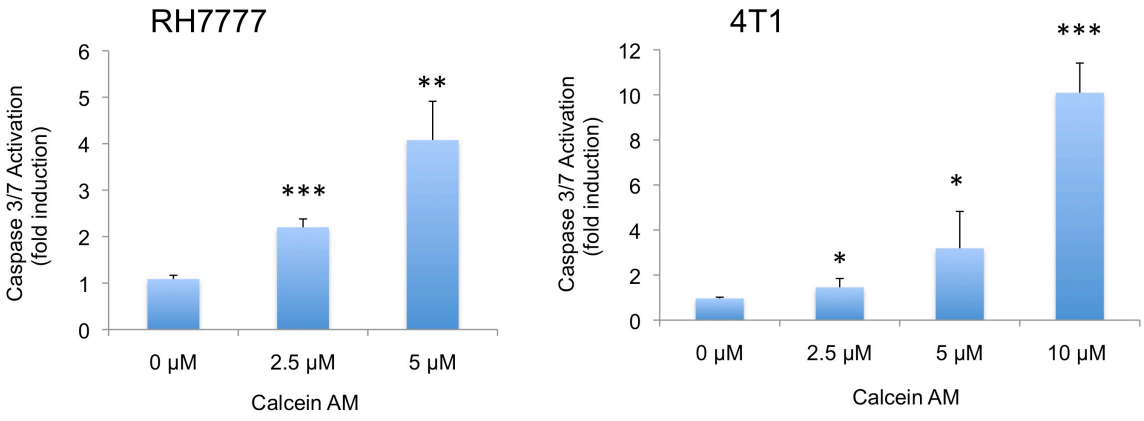
Supplementary Figure 14. Calcein AM inhibits BT549 xenograft formation.

Twenty 5 week-old nude mice were injected with BT549 cells (2.5 million cells, s.c. in the right flank). Four days later, the mice were randomized to three groups and injected i.p. with either DMSO control (8 mice), Calcein AM 20 mg/kg (8 mice) or Calcein AM 40 mg/kg (4 mice) every three days for three injection. Six out of 8 DMSO-injected mice developed tumors later, but none of the Calcein AM-treated mice developed xenografts. Shown are six DMSO-treated mice with xenografts and representative pictures of the mice treated with Calcein AM.



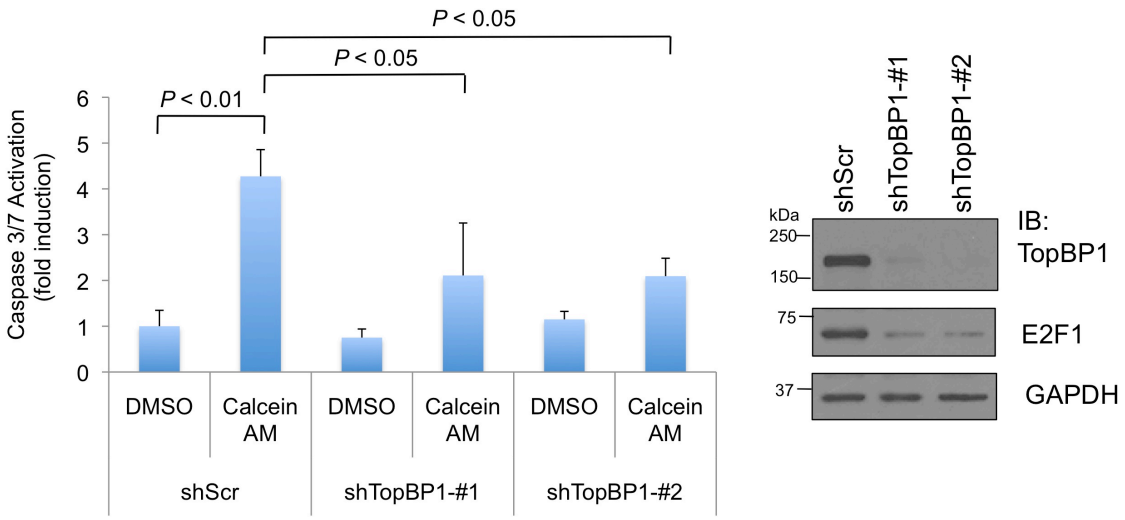
Supplementary Figure 15. Calcein AM injection does not inhibit proliferation, nor induce apoptosis of intestinal tissues.

Four week-old C57BL/6 mice were injected with Calcein AM i.p. at 40 mg/kg. 48 h later, mice were euthanized and intestinal tissues were harvested for Ki-67 staining and TUNEL staining. Shown are representative images at 10X magnification.



Supplementary Figure 16. Calcein AM induces apoptosis in a rat hepatoma cell line McA-RH7777 and mouse breast tumor cell line 4T1.

RH7777 and 4T1 cells were treated with Calcein AM at indicated doses overnight. Apoptosis was then analyzed by Caspase-Glo 3/7 activity assay. Shown are means ± S.D. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, compared with DMSO control (two-tailed *t* test, n = 3 biological replicates for each treatment).



Supplementary Figure 17. TopBP1 depletion in MDA-MB468 affects Calcein AM response.

MDA-MB468 cells were transfected with either scrambled (Scr) shRNA or two different shRNAs against TopBP1. Two days after transfection, cells were treated with Calcein AM (2.5 μM) overnight and then subjected to Caspase-Glo 3/7 activity assay. The data represent means ± S.D. *P* values were based on two-tailed *t* test, n = 3 biological replicates. Depletion of TopBP1 was verified by Western blot analysis (right panel). E2F1 expression was also examined by Western blot analysis.

Primers used for Real-time RT-PCR

p73: forward, 5'-TTTAACAGGATTGGGGTGTC-3'
reverse, 5'-ACAGGGCCTTGAGCACCAGTTTGC-3'

Bax: forward, 5'-AGGGTTTCATCCAGGATCGAGC-3'
reverse, 5'-CGTGAACTCCTCCTTGATGG-3'

Apaf-1: forward, 5'-AATGGACACCTTCTTGGACG-3'
reverse, 5'-GCACTTCATCCTCATGAGCC-3'

Cyclin A2: forward, 5'-AGCAGCCTGCAAACTGCAAAGTTG-3'
reverse, 5'-TGGTGGGTTGAGGGAGAGAAACAC-3'

Caspase 3: forward, 5'-TCGGTCTGGTACAGATGTCG-3'
reverse, 5'-CATACAAGAAGTCGGCCTCC-3'

Cdc25c: forward, 5'-GTATCTGGGAGGACACATCCAGGG-3'
reverse, 5'-CAAGTTGGTAGCCTGTTGGTTTG-3'

MDM2: forward, 5'-ATCTTGGCCAGTATATTATG-3'
reverse, 5'-GTTCCTGTAGATCATGGTAT-3'

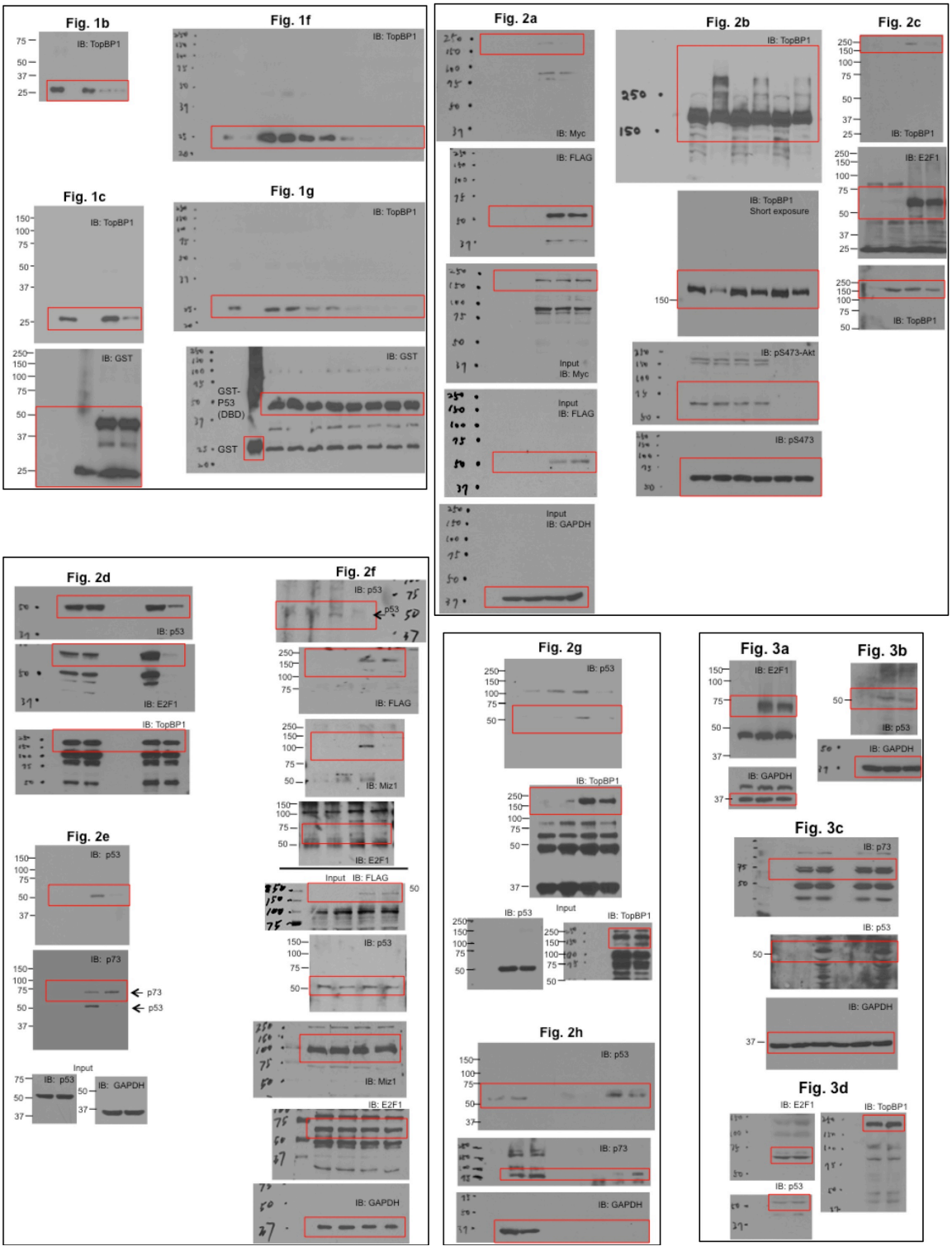
Cdk1: forward, 5'-CCTTGCCAGAGCTTTTGGAATACC-3'
reverse, 5'-GACATGGGATGCTAGGCTTCCTGG-3'

NOXA: forward, 5'-ACTGTTCGTGTTCAGCTC-3'
reverse, 5'-GTAGCACACTCGACTTCC-3'

GAPDH: forward, 5'-TGAAGGTCGGAGTCAACGGATTTGGT-3'
reverse, 5'-AAATGAGCCCCAGCCTTCTCCATG-3'

Supplementary Figure 18. Primer sequences used for real-time RT-PCR

Supplementary Figure 19. Full blots.



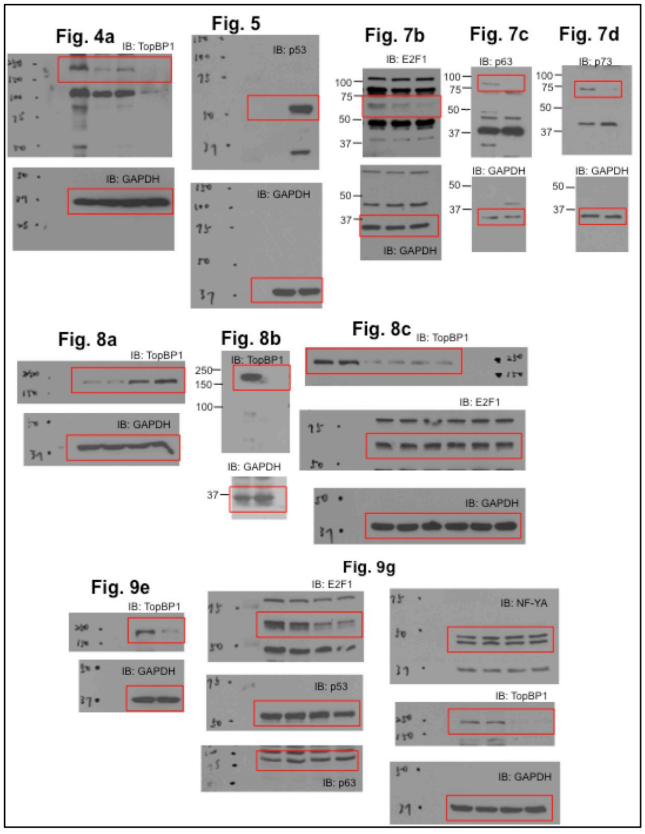


Fig. 4a
IB: TopBP1
IB: GAPDH
Fig. 5
IB: p53
IB: GAPDH
Fig. 7b
IB: E2F1
IB: GAPDH
Fig. 7c
IB: p63
IB: GAPDH
Fig. 7d
IB: p73
IB: GAPDH
Fig. 8a
IB: TopBP1
IB: GAPDH
Fig. 8b
IB: TopBP1
250
150
100
IB: GAPDH
37
Fig. 8c
IB: TopBP1
IB: E2F1
IB: GAPDH
Fig. 9e
IB: TopBP1
IB: GAPDH
Fig. 9g
IB: E2F1
IB: NF-YA
IB: p53
IB: TopBP1
IB: p63
IB: GAPDH

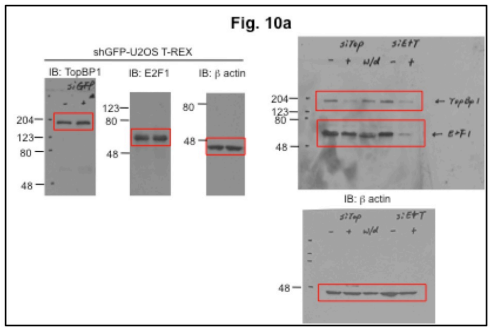
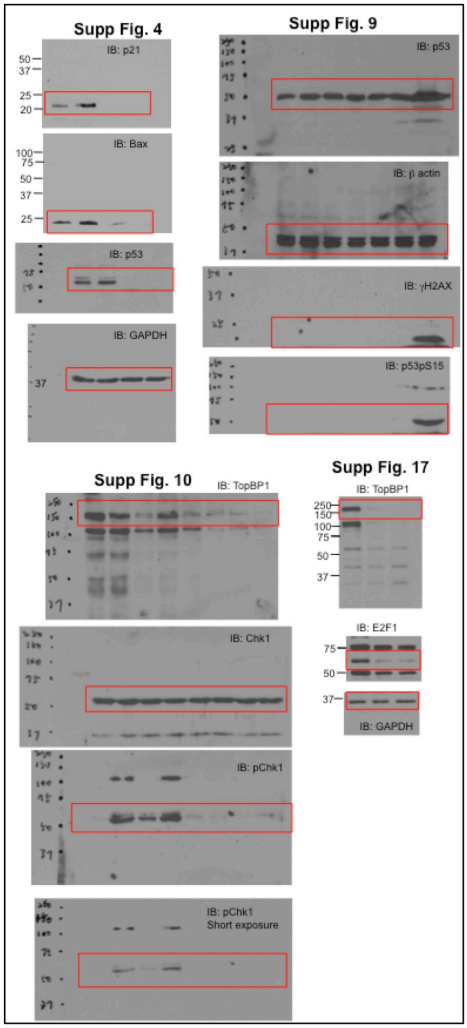


Fig. 10a
shGFP-U2OS T-REX
IB: TopBP1
IB: E2F1
IB: β actin
si:GFP
204
123
80
48
123
80
48
80
48
si:Top
si:E2F
- + w/d - +
204
123
80
48
← TopBp1
← E2F1
IB: β actin
si:Top
si:E2F
- + w/d - +
48


Supp Fig. 4
IB: p21
50
37
25
20
IB: Bax
100
75
50
37
25
IB: p53
IB: GAPDH
37
Supp Fig. 9
IB: p53
IB: β actin
IB: γH2AX
IB: p53pS15
Supp Fig. 10
IB: TopBP1
IB: Chk1
IB: pChk1
IB: pChk1
Short exposure
Supp Fig. 17
IB: TopBP1
250
150
100
75
50
37
IB: E2F1
75
50
37
IB: GAPDH