

Supplementary information

To accompany Mitxelena et al., “An E2F7-dependent transcriptional program modulates DNA damage repair and genomic stability”

Figure Legends

Supplementary Figure 1. Cell synchronization in U2OS cells. **A.** U2OS cells were treated with 4 mM HU and 12 h later were transfected with NT siRNA and E2F7 siRNA. Cells were washed after 24 h of HU treatment, and harvested for Western blot and FACS analyses at 0h, 3h and 12h. For flow cytometric analysis, cells were stained with of propidium iodide (PI). The percentage of cells in G1 (green), S (red) and G2/M (blue) of a representative experiment of three independent replicates is shown. **B.** E2F7 and CYCLIN E1 (CCNE1) expression levels were evaluated by Western blot analysis. α -TUBULIN (TUBA1A) served as loading control.

Supplementary Figure 2. Cellular responses after genotoxic damage in E2F7-depleted cells. **A.** E2F7 knockdown does not interfere with checkpoint activation caused by DNA-damaging agents. U2OS cells were treated with 4mM HU for 24 hours and transfected with siRNAs. Cells released from HU-induced block were treated with 250 nM MMC, 8 μ M CSP or 20ng/ml NCS. Cells were harvested for subsequent analyses 15 hours after treatments. Cell-cycle profiles as determined by PI staining and FACS analysis. This experiment is representative of 3 independent experiments. **B.** Asynchronously growing U2OS cells were transfected with siNT and siE2F7 and subsequently treated with 250 nM MMC, 8 μ M CSP or 1,5 μ M OLA for 24h. Nocodazole was present in the culture for the last 14h of culture. The percentage of mitotic pH3-positive cells was scored by FACS analysis. Results are expressed as fold-change over siNT values (mean \pm SD) of pH3-positive cells from three independent experiments. **C.** U2OS cells were HU-synchronized and transfected with siNT and siE2F7. Subsequently, cells were released into the cell cycle and treated for 24h with 1,5 μ M OLA. Nocodazole was present in the culture for the last 14h of culture. The percentage of mitotic pH3-positive cells is shown in a representative figure obtained by FACS analysis.

Supplementary Figure 3. Validation of U2OS E2F7 CRISPR knockout cell line. **A.** Schematic overview of E2F7 gene, including exons (grey boxes) and introns (grey line). CRISPR-targeted exon 2 is highlighted in purple, and its sequence is indicated below. Guide RNA (gRNA) sequence is indicated in italics, and PAM sequence is underlined. Sequences corresponding to the wild type (WT) and knockout (KO) E2F7 cell lines are represented. Regarding to the KO cell line, each allele carries a different mutation after the PAM sequence: allele 1 has a single base pair insertion, and allele 2 has the P2A-Puromycin-cassette insertion. **B.** Western blot analysis confirmed efficient E2F7 depletion in the KO cell line at the protein level. β -ACTIN (ACTB) was used as a loading control.

Supplementary Figure 4. E2F7 controls cell cycle reentry upon MMC, CSP or OLA treatments. U2OS cells were transfected with NT and E2F7 siRNAs. After HU treatment for 24h, cells were released into the cell cycle and treated with 250nM MMC, 8 μ M CSP, 1,5 μ M OLA and 20ng/ml NCS for 32 hours. Cell-cycle profiles were determined by PI staining. Shown is the percentage of cells in G1 (green), S (red) and G2/M (blue) of a representative experiment.

Supplementary Figure 5. Overexpression of cell cycle genes in E2F7-depleted cells after CSP treatment. Asynchronously growing U2OS cells were transfected with siNT and siE2F7 and subsequently treated with 8 μ M CSP for 24h. RT-Q-PCR analyses of indicated genes are shown. Expression values are normalized to the expression of *EIF2C2*, used as standard control. Data are represented as fold-change (mean \pm SEM) relative to siNT-transfected samples from three independent experiments (*, $P < 0.01$). UT, untreated.

Supplementary Figure 6. Depletion of E2F7 results in reduced γ -H2AX levels. E2F7-knockout and wild-type U2OS cells were treated with 250nM MMC, 4 μ M CSP and 2 μ M OLA, and fixed after 7h or 24h. Cells were stained for γ -H2AX with a FITC-conjugated specific antibody. Fluorescence intensity was analyzed by flow cytometry. Data are represented as the ratio of γ -H2AX-positive cells in E2F7 knockout samples relative to control samples from three independent analyses (*, $P < 0.05$, one-sample t-test).

Supplementary Figure 7. Increased RAD51 foci upon E2F7 loss. siNT and siE2F7 transfected U2OS cells were treated with various doses of MMC for 24h. Cells were fixed and stained for RAD51. Cells with more than 10 RAD51 foci were scored as positive. A representative experiment of two independent experiments is presented.

Supplementary Figure 8. Knockdown of RAD51 and E2F7 expression with siRNAs. U2OS-DR-GFP cells were transfected with siE2F7, RAD51 or both, and RT-QPCR expression analyses were performed after 24h. Expression values are normalized to the expression of *EIF2C2*, used as standard control. Data are represented as fold-change (mean \pm SEM) relative to siNT-transfected samples from two independent experiments (*, $P < 0.05$).

Supplementary Figure 9. Efficient knockdown of BRCA2 and E2F7 expression with siRNAs. U2OS-DR-GFP cells were transfected with siE2F7, siBRCA2 or both, and RT-QPCR expression analyses were performed after 24h. Expression values are normalized to the expression of *EIF2C2*, used as standard control. Data are represented as fold-change (mean \pm SEM) relative to siNT-transfected samples from two independent experiments (*, $P < 0.05$).

Supplementary Figure 10. CAPAN-1 cell sensitivity to PARP-1 inhibitor Olaparib (10 μ M) is alleviated by E2F7 knockdown, as measured by trypan blue exclusion. Data are represented as fold-change (mean \pm SEM) relative to siRNA-transfected control samples from two independent experiments (*, $P < 0.05$).

Supplementary Table S1: Nucleotide sequences of siRNAs used for gene knockdown

Gene	Sense (5'>3')	Antisense (5'>3')
<i>BRCA2</i>	GGAUUUAUACAUUUUCGCAtt	UGCGAAAUUUGUAUAAUCCag
<i>E2F7</i>	CCAAGAUUGUCACUCUGGAtt	UCCAGAGUGACAAUCUUGGtt
<i>RAD51</i>	GGUAGAAUCUAGGUAUGCAtt	UGCAUACCUAGAUUCUACCat
<i>TP53</i>	GGGUUAGUUUACAAUCAGCtt	GCUGAUUGUAAACUAACCCtt

Supplementary Table S2: Nucleotide sequences of primers used for RT-QPCR experiments

Gene	Forward	Reverse	Conc. (nM)
<i>E2F7</i>	GGAAAGGCAACAGCAAACCTCT	TGGGAGAGCACCAAGAGTAGAAGA	50/50
<i>EIF2C2</i>	GTCCCTTTTGAGACGATCCAG	AGCCAAACCACACTTCTCG	50/50
<i>RAD51</i>	TGCTTATTGTAGACAGTGCCACC	CACCAAACCTCATCAGCGAGTC	50/50
<i>BARD1</i>	GCTCGCGTTGTAACATTC	TTCAAGTCTTGTATCCAGGCC	50/50
<i>CTIP</i>	ACAGCTGAGGGAACAGCAGAAA	TCTGCTGGAGTTGTTCAAAAAGC	300/300
<i>FANCE</i>	TCTGGATGATGCTAAAGGTCTGG	TGAAGAAGCTGTAGCTCAACTG	900/900
<i>FANCI</i>	CACCACACTTACAGCCCTTG	ATTCCTCCGAGCTCTGAC	300/300
<i>BRIP1</i>	GCGTATCACCCTGCTACTTT	ACTTCTTGCCTCCTCTTTAC	50/300
<i>MCM2</i>	CTCATTGGAGATGGCATGCA	AGCTCCTCTACGTCCTCATC	300/300
<i>CDC6</i>	CGGATTCTGCAAGACCTCAA	CTGAGCAATAGCTGGGAATACA	300/300
<i>E2F1</i>	TGACATCACCAACGTCCTTGA	CTGTGCGAGGTCTGGGTC	300/300
<i>E2F2</i>	ACGTGCTGGAAGGCATCC	GCTCCGTGTTTCATCAGCTCC	300/50
<i>BRCA2</i>	CCAAAGTTTGTGAAGGGTCG	GTAGAACTAAGGGTGGGTGGTG	50/50

Supplementary Table S3: Nucleotide sequences of primers used for ChIP-QPCR experiments

Gene	Forward	Reverse	Conc. (nM)
<i>ACTB</i>	GTGTGGTCTGCGACTTCTAAGT	CCTGGGCTTGAGAGGTAGAGTGT	300/300
<i>RAD51</i>	GCTCAGACGATACTCTCGCCTC	CGCTAACCAAGACGGGAG	50/50
<i>FANCE</i>	ACACCGCCACAACTAAC	AAGTCCAGCCGGAGTAGG	300/300
<i>FANCI</i>	CCCTTCAGTCTTCATGGTACAC	TCCGTTACCCGTAACAACAAG	300/900
<i>CTIP</i>	TGTGTCTGATGAAATAGGCCG	AGACCCAGAAGTAGTACTGAGG	300/300
<i>BARD1</i>	TCCCCTAAGTCCCACACG	CCGCAATGCTCCAAAATAG	900/900
<i>BRIP1</i>	AGGATTTGGCACTCTGGTG	TTCTGAGGCTGTGAAAGGAC	300/300

Supplementary Table S4: E2F binding motifs in promoter regions of genes significantly upregulated after knockdown of E2F7 in at least 2 time-points, identified by TOUCAN within a -1000/+500 bp genomic region.

Gene	E2F motif		
	% Similarity ^a	Location	Sequence
<i>BARD1</i>	80	672,679	TTTCGACC
	80	1045,1052	TTTCGAGT
	80	1077,1084	TTCCCGA
	80	1352,1359	TCTGCCGC
	85	415,422	TTCCCGG
	85	1198,1205	TTCCGCGC
	85	1390,1397	GTTCCCGC
<i>BRCA1</i>	80	1196,1203	TTTGACGG
	85	232,239	TTCGGCGC
	85	1112,1119	TTCCCGG
<i>BRIP1</i>	80	155,162	TTGCCTC
	80	571,578	ATTCCCGC
	80	728,735	TTTGCTGC
	80	967,974	TTGGGCGC
	80	996,1003	ATTCCCGC
	80	1224,1231	TTCCCTC
	85	484,491	TTCCCGG
	85	551,558	TTGGCTC
<i>CCNA2</i>	80	1015,1022	TATCCCGC
	85	731,738	TTGGCGA
	85	1081,1088	TTGGCTC
	85	1261,1268	CTTGGCGC
<i>CCNE1</i>	80	571,578	TGTCCCGC
	85	773,78	TCTGGCGC
	85	1050,1057	TTCCGCGC
	90	711,718	TTGCCCGC
<i>CDC6</i>	80	983,99	TTTGCGGC
	80	1276,1283	TTTGCTGC
	90	1219,1226	TTGGCGG
	95	1254,1261	TTGGCGC
<i>CDC25A</i>	80	656,663	TTTGCCC
	80	1252,1259	TTGCCCGC
	85	900,907	GTTGGCGC
	90	18,25	TTCGGCGC
	90	955,962	TTTCGCGG
	95	896,903	TTGGCGC
<i>CHEK1</i>	80	1028,1035	TTGGCTC
	80	1136,1143	TTGGAGC
	80	1331,1338	TTTCTCGG
	85	1212,1219	TTCCCGG

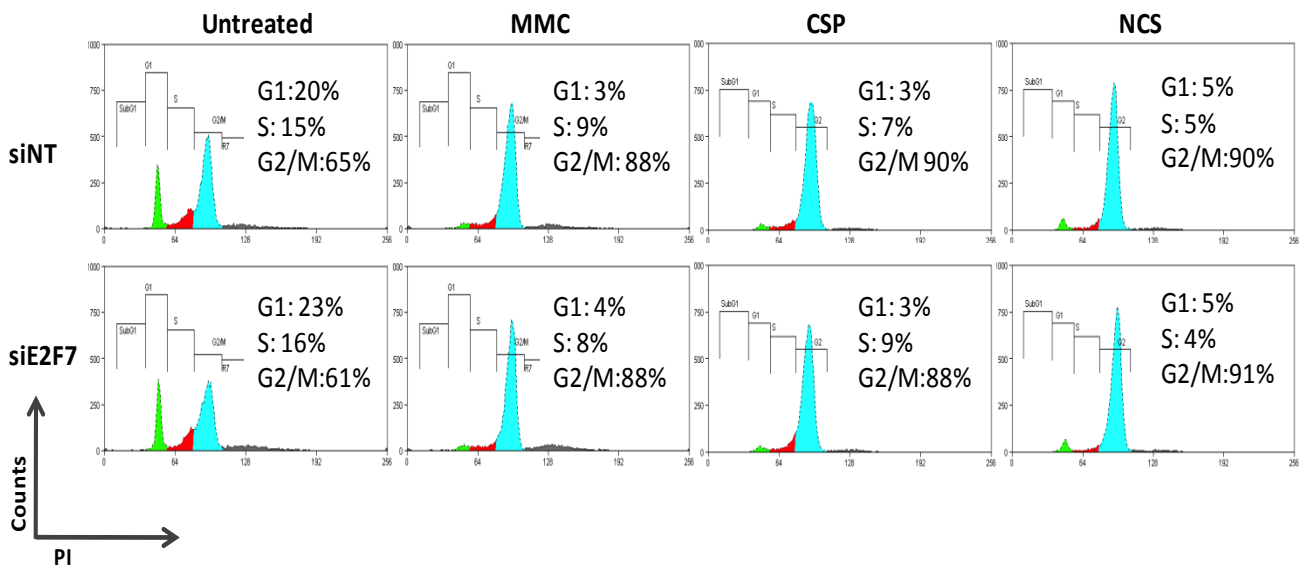
CTIP	80	711,718	ATTCGCGA
	80	975,982	TTTCGCCG
	80	1116,1123	TTTGCCCC
	80	1454,1461	TTTTGCGA
	85	618,625	TTTGGCAC
	85	641,648	TTTCGCCC
	90	709,716	TTTCGCGA
FANCA	80	127,134	TCTGCCGC
	80	1162,1169	TTTCGCCG
	80	1339,1346	TTTCCCGA
	85	798,805	TCTCGCGC
	85	1021,1028	CTTGCGGC
	95	828,835	TTTGCCGC
FANCC	80	818,825	TTCCGCGC
	80	1082,1089	TTTGCTC
	80	1333,134	GTTCCCGC
	85	948,955	TTTCCCGC
	85	1046,1053	TTTCCCGC
	85	1468,1475	TTCCGCGC
	95	822,829	TTTCGCGC
FANCE	80	996,1003	GTTCCCGC
FANCI	85	563,57	TTTTGCGC
	90	1243,125	TTTCGCGG
	90	1465,1472	TTTCCCGC
MCM2	80	90,97	TTTGCTGC
	80	1244,1251	TATGGCGG
	85	1383,1391	TTTCTCGC
	95	1191,1198	TTTCGCGC
	95	1195,1202	TTTCGCGC
MCM7	80	759,766	TTTCGAAC
	80	1254,1261	TTTCGCCG
	85	857,864	TTTGGCGG
	90	1357,1364	TTTCCCGC
PCNA	80	19,26	TTTGCTC
	80	313,32	CTTCGCGG
	85	339,346	CTTCGCGC
	90	374,381	TTTGTCGC
	90	946,953	TTTCCCGC
RAD51	80	1159,1166	TTACGCTC
	85	469,476	TTTGGCAC
	85	1430,1437	TTTCGCCC
	90	1006,1013	TTTGGCGG
RFC2	80	721,728	TTTGCCCG
	85	1054,1061	TCTCGCGC
RFC3	80	1002,1009	TTACGCGG
	85	827,834	TTTGGCGT

	85	1036,1043	TTTGGAGC
	85	1046,1053	TTTCCCGC
	95	1271,1278	TTTCGCGC
RFC4	80	804,811	TTCGTTCGC
	80	1175,1182	TTACGTGC
	80	1203,121	ATTCCCGC
	80	1345,1352	TATCGCGG
	85	381,388	TTTGGCAC
	85	1217,1224	TTTCGCC
RMI2	80	254,261	TTGGCCGC
	80	255,262	TTTGGCCG
	80	295,302	TTTTCCGC
	80	713,72	GTTTCGCGG
	85	357,364	TCTCGCGC
	90	819,826	GTTTCGCGC
	95	976,983	TTTGGCCGC
RPA1	85	852,859	CTTGGCGC
	85	1011,1018	TTGCGCGC
RPA2	80	763,770	ATTCCCGC
	80	1044,1051	TTTGGAGC
	80	1169,1176	ATTCGCGG
	80	1240,1247	TTTTCCGC
	80	1434,1441	TTTGGGGG
	85	853,860	GTTTCGCGC
	95	849,856	TTTGGCGC
TIPIN	80	1013,1020	TATCGCGA
	80	1429,1436	TTCCCGC
	90	1177,1184	TTTCCCGC
	95	966,973	TTTGGCGC
	95	970,977	TTTGGCGC
TOPBP1	80	190,197	TTTTCCGC
	80	434,441	TTAGGCGG
	80	627,633	CGGGAAA
	80	1176,1183	GTTTCGCGA
	85	942,949	GTTGGCGC
	90	1253,126	TTTGACGC
	95	946,953	TTTCGCGC
USP1	80	330,337	GTTCCCGC
	80	628,635	GTACGCGC
	80	636,643	GTTTCGTGC
	80	747,754	TTTCGCTG
	80	1453,146	TTCGGCGA
	85	721,728	TTTGGCGT
	85	767,774	ATTGGCGC
	85	1401,1408	CTTGGCGC

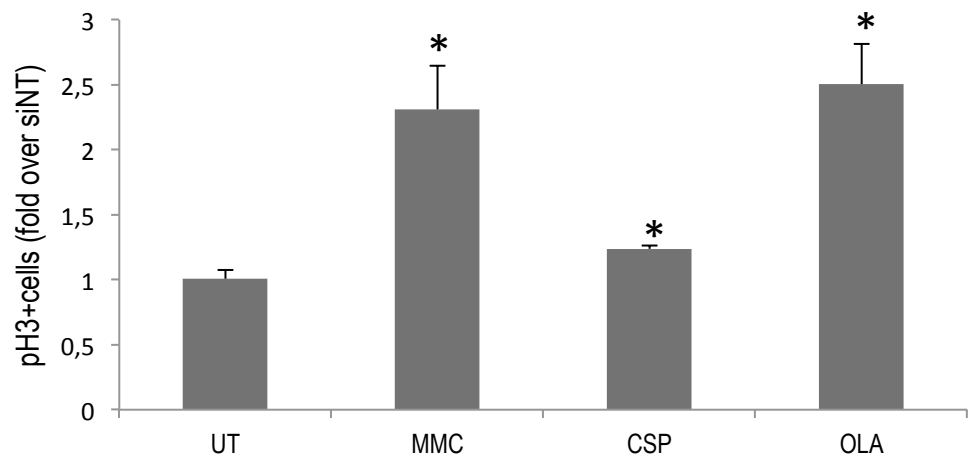
^a Threshold level of for similarity with the canonical E2F motif recorded in the TRANSFAC database (TTTSSCGC)

Supplementary figure S2A

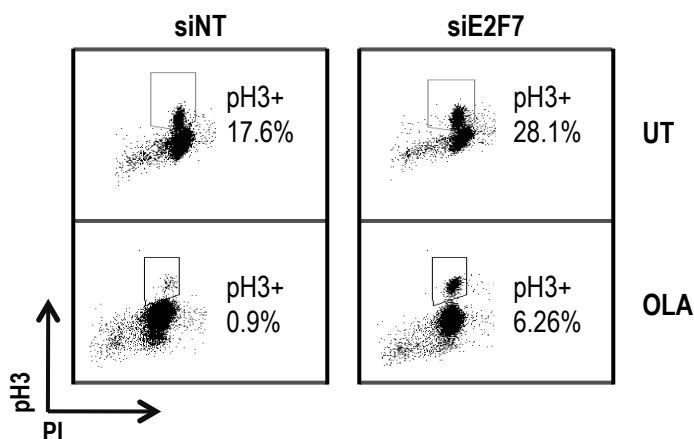
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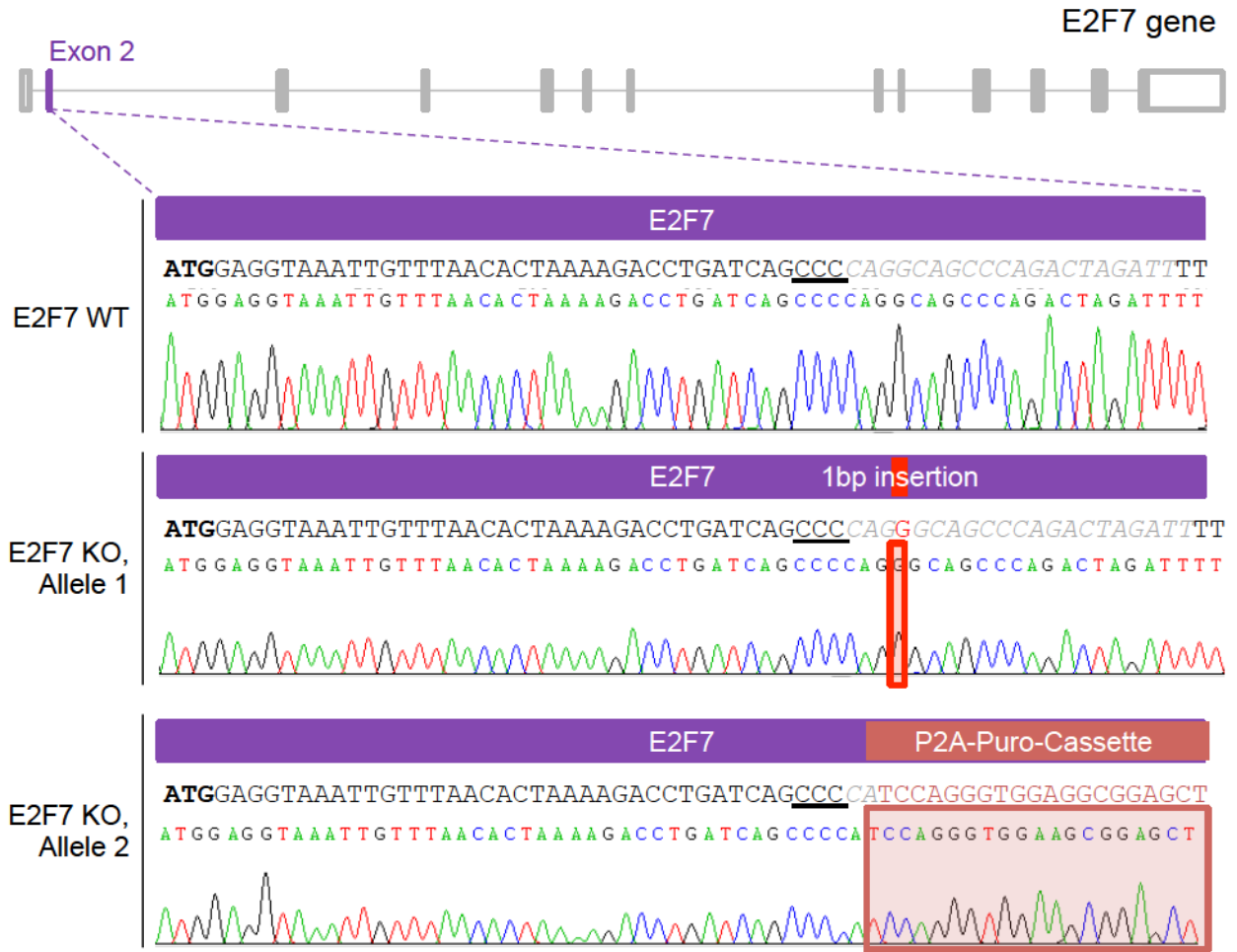
Supplementary Figure S2B



Supplementary Figure S2C

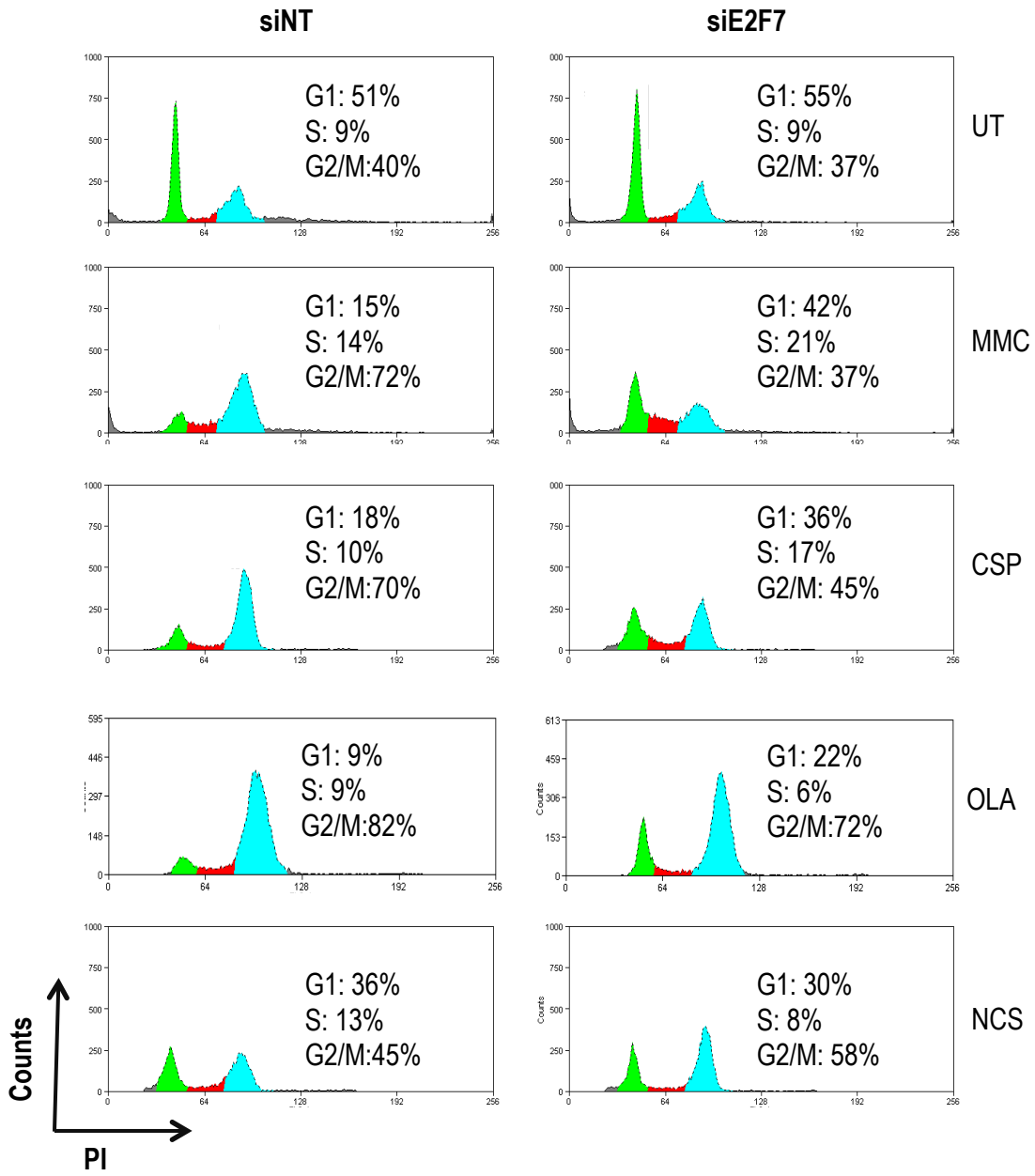


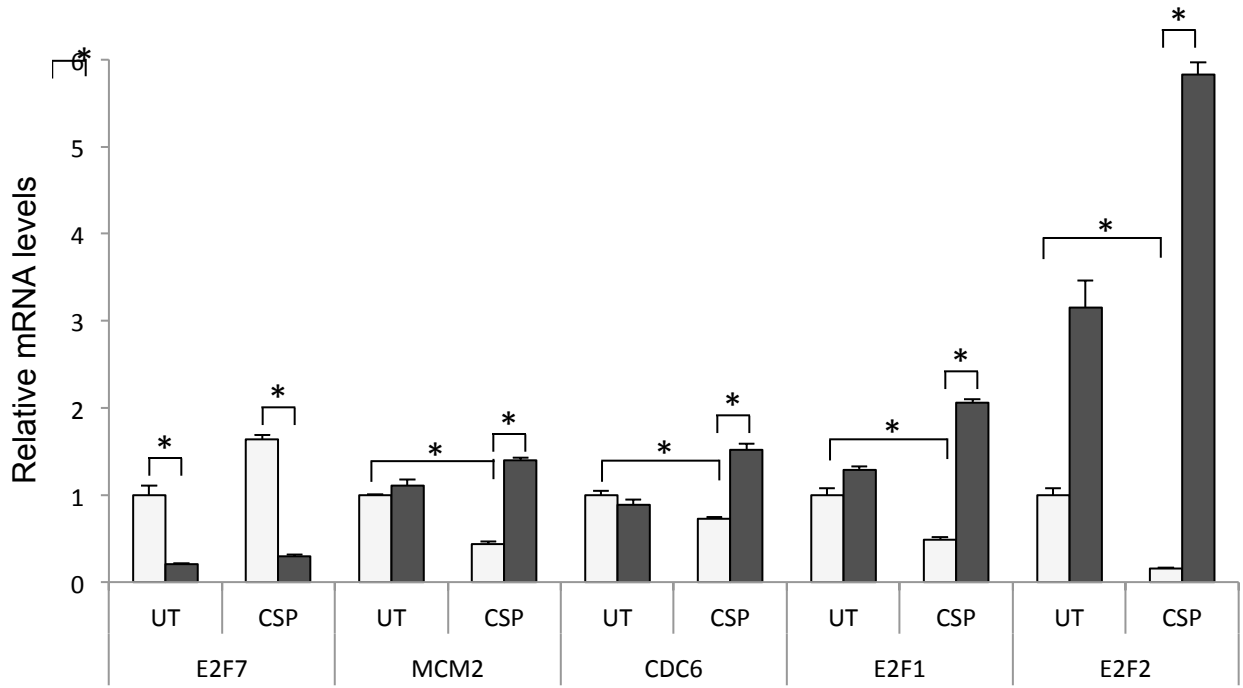
Supplementary figure S3A

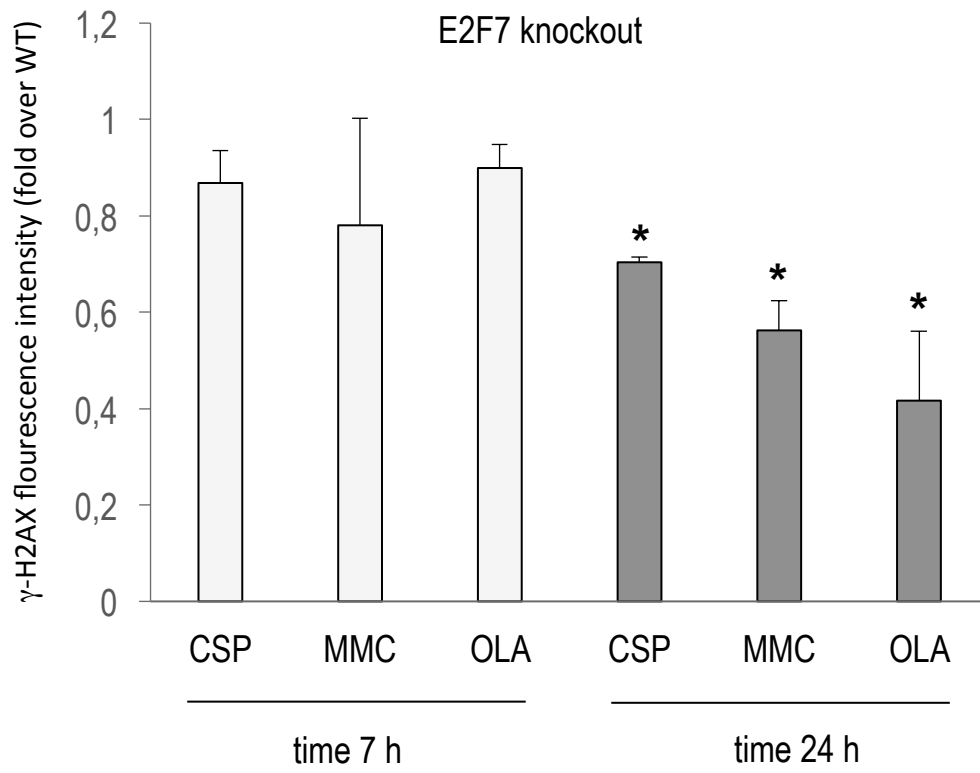


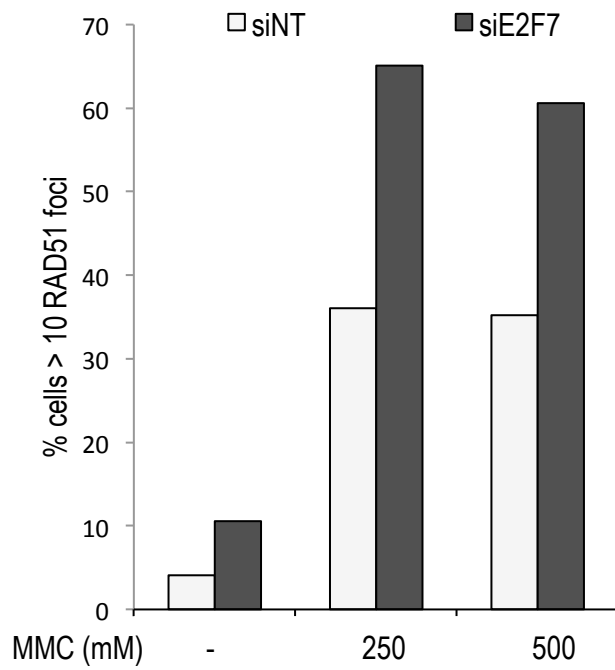
Supplementary figure S3B

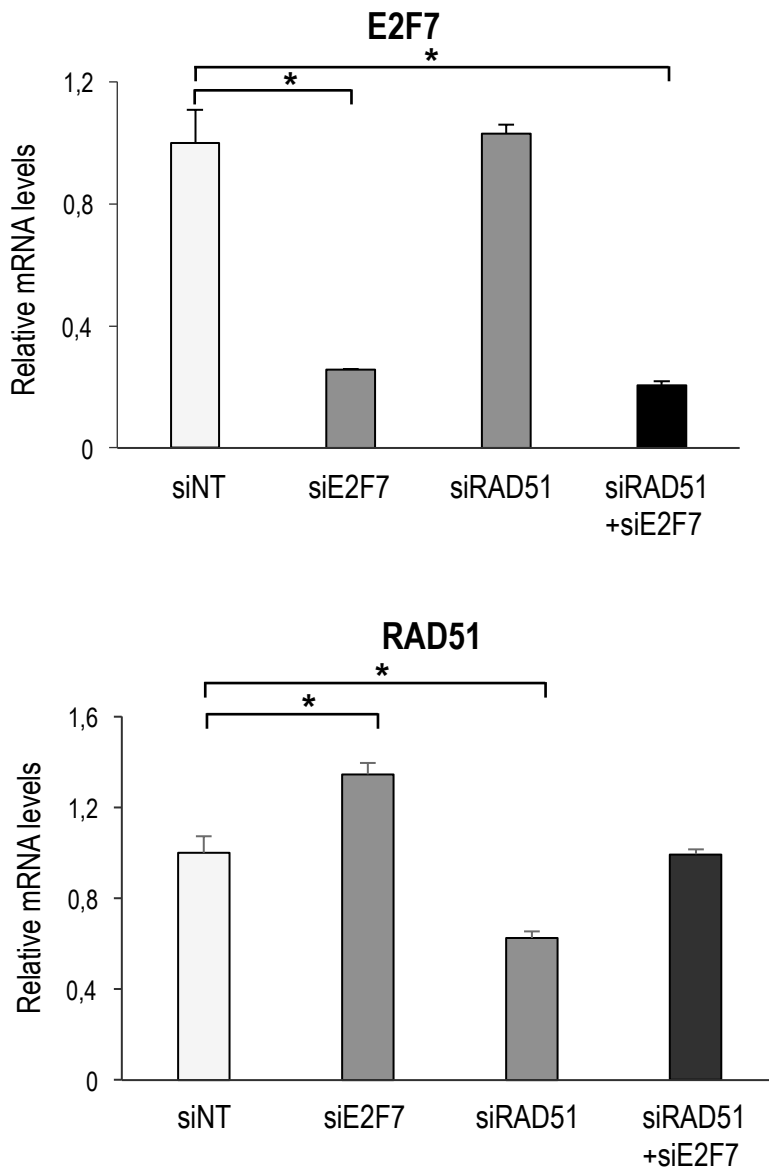


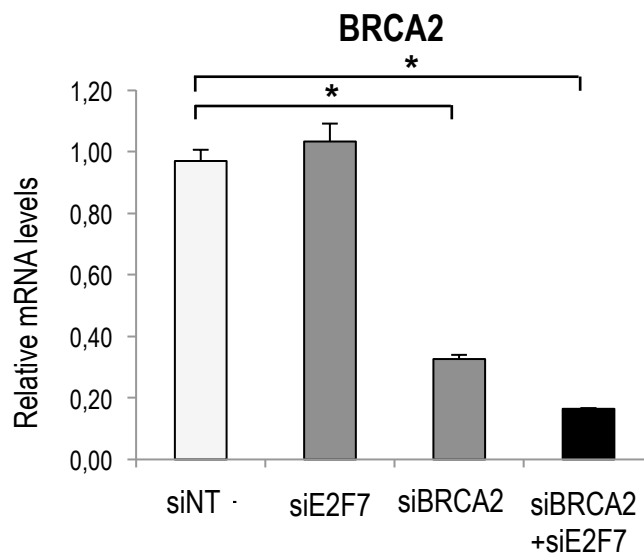
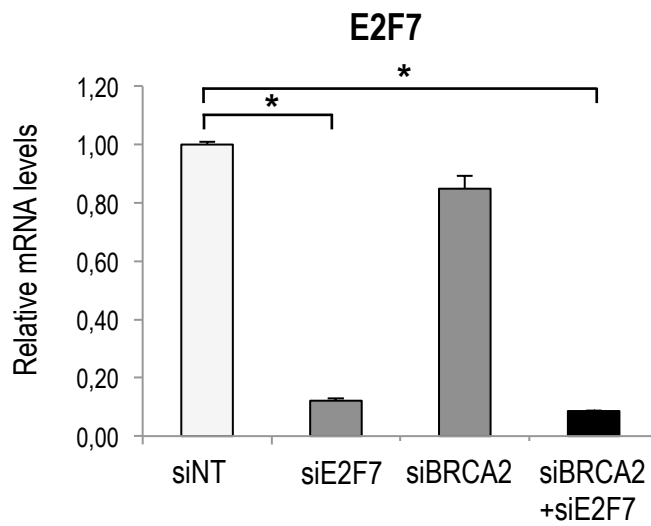












Supplementary Figure S10

