Supplemental Materials

Molecular Biology of the Cell

Rajabi *et al*.

Supplementary Materials for

DNA damage-induced EMT controlled by the PARP dependent chromatin remodeler ALC1 promotes DNA repair efficiency through RAD51 in tumor cells

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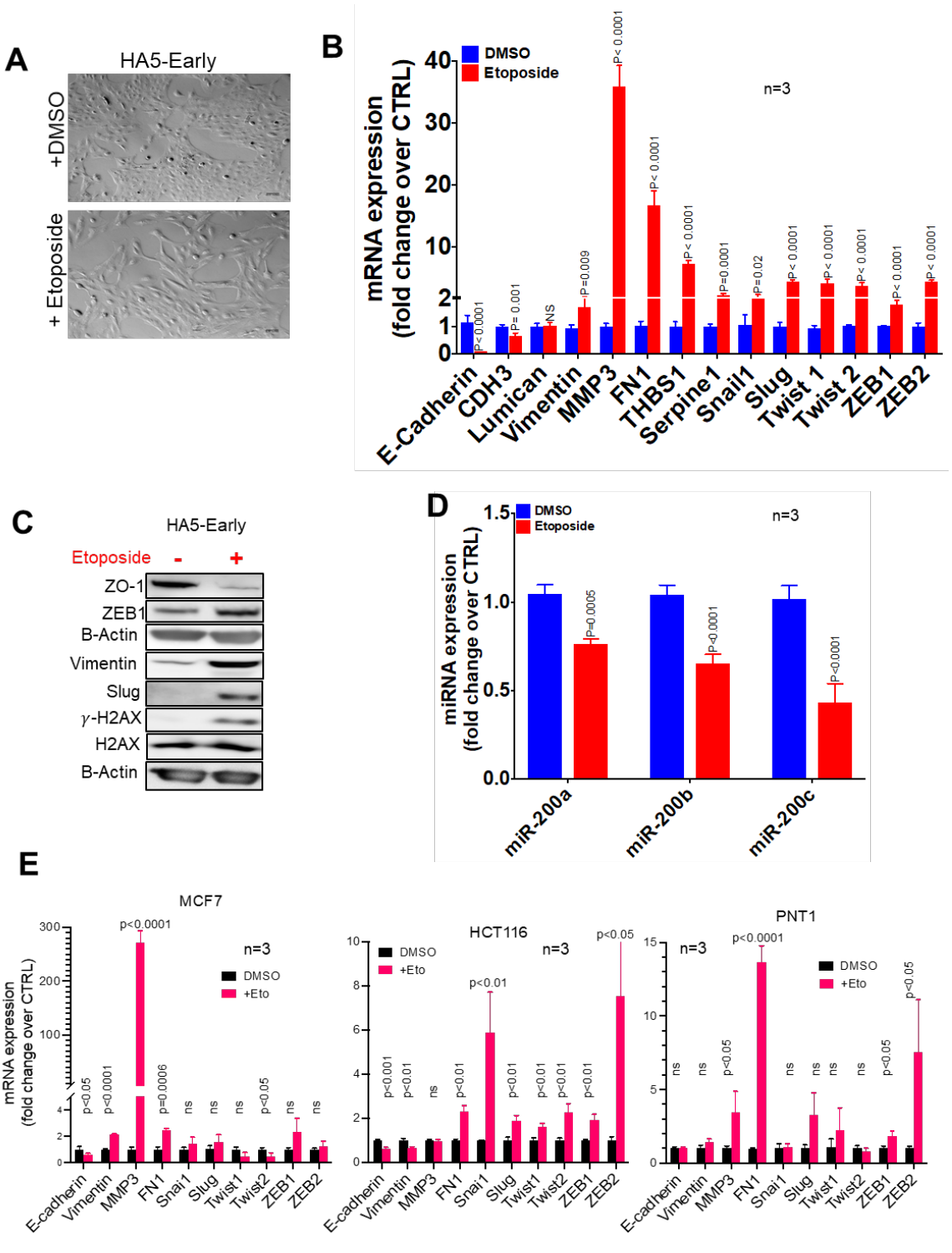
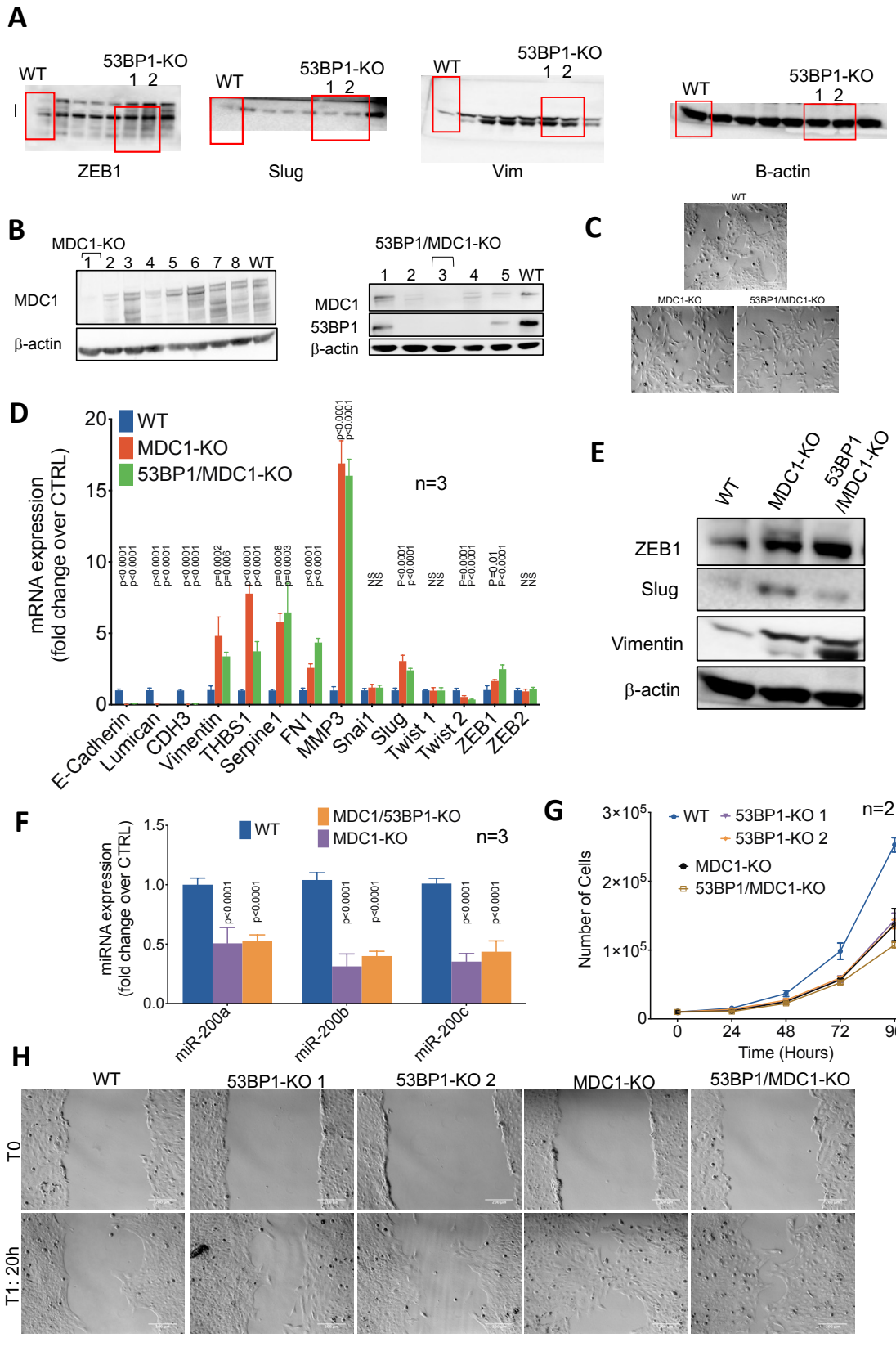
Supplementary figures and figure legends



Supp. Figure S1

Fig. S1.

DNA damage rapidly induces EMT in transformed epithelial cells. **A.** Cell morphology of HEK cells before (DMSO) and after 7 days treatment with etoposide. **B.** Relative mRNA level of expression of epithelial and EMT markers in HEK cells treated with etoposide and compared to the untreated cell line (DMSO). p values from paired comparisons are indicated. ns: non-significant. **C**. Detection by western blot of some epithelial and mesenchymal markers in HEK cells before and after treatment with etoposide. **D.** Relative expression level of three members of the miR-200 family after treatment with etoposide and compared to untreated cells (DMSO). p values from paired comparisons are indicated. **E.** Relative mRNA level of expression of epithelial and EMT markers in three different tumor-derived (MCF7 and HCT116) or transformed (PNT1A) cells treated with etoposide and compared to the untreated counterparts (DMSO). p values from paired comparisons are indicated. ns: non-significant.



Supp. Figure S2

Fig. S2.
Disabling the DNA Damage Response induces EMT.
A. Uncropped images for the western blots shown in Figure 1C. **B.** Identification by western blot of CRISPR-Cas9 transfected HEK-Early clones full KO for either DMC1 or both MDC1 and 53BP1. **C**. Cell morphology of KO clones compared with the parental cell line (WT). **D**. Relative mRNA level of expression of epithelial and EMT markers in MDC1- or 53BP1/MDC1-DKO clones compared to the parental cell line (WT). p values from paired comparisons are indicated. NS: non-significant. **E**. Western blot analyses for EMT-related markers in MDC1- and 53BP1/MDC1-DKO clones. **F**. Relative expression levels of three members of the miR-200 family upon deletion of MDC1 or of both 53BP1 and MDC1. p values from paired comparisons are indicated. **G**. Growth curves for WT and 53BP1-, MDC1- and 53BP1/MDC1-DKO clones. **H**. Representative images at 0h and 20h used for the wound healing assays carried out in 53BP1-, MDC1- and 53BP1/MDC1 double KO. Quantifications are presented in Figure 1J.

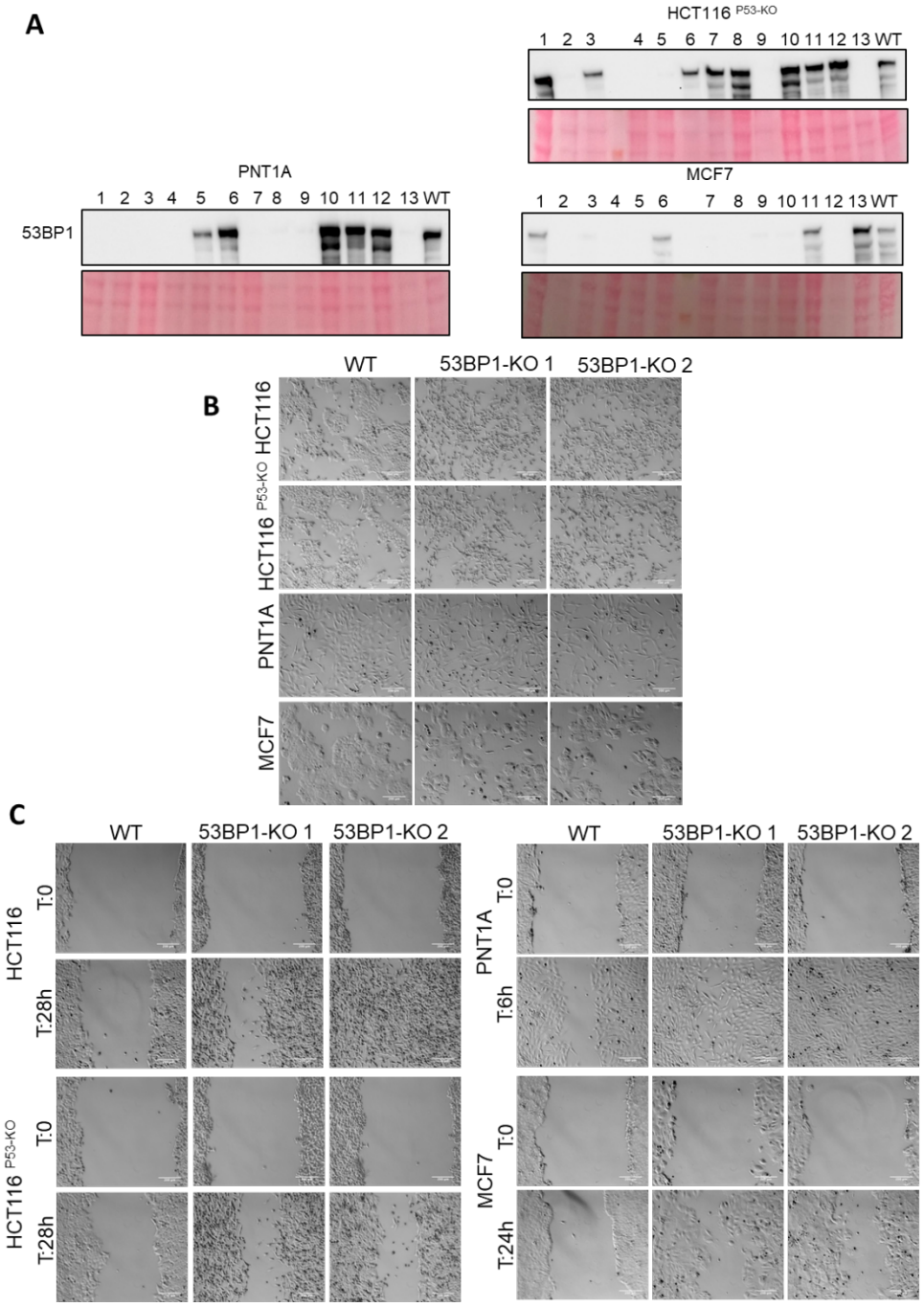


Fig. S3.
EMT induction following DDR attenuation is a widespread phenomenon in cancer cells.
A. Identification by western blot of CRISPR-Cas9 transfected HCT116, HCT116^{P53-KO}, MCF7 and PNT1A clones full KO for 53BP1. **B**. Cell morphology aspects of different 53BP1-KO clones compared with the parental cell line (WT). **C**. Representative images used for the wound healing assays carried out in 53BP1-KO clones obtained from HCT116, HCT116^{P53-KO}, MCF7 and PNT1A cells. Indicated readout times were determined depending on would healing capacities of parental (WT) cells. Quantifications are presented in Figure 2G.

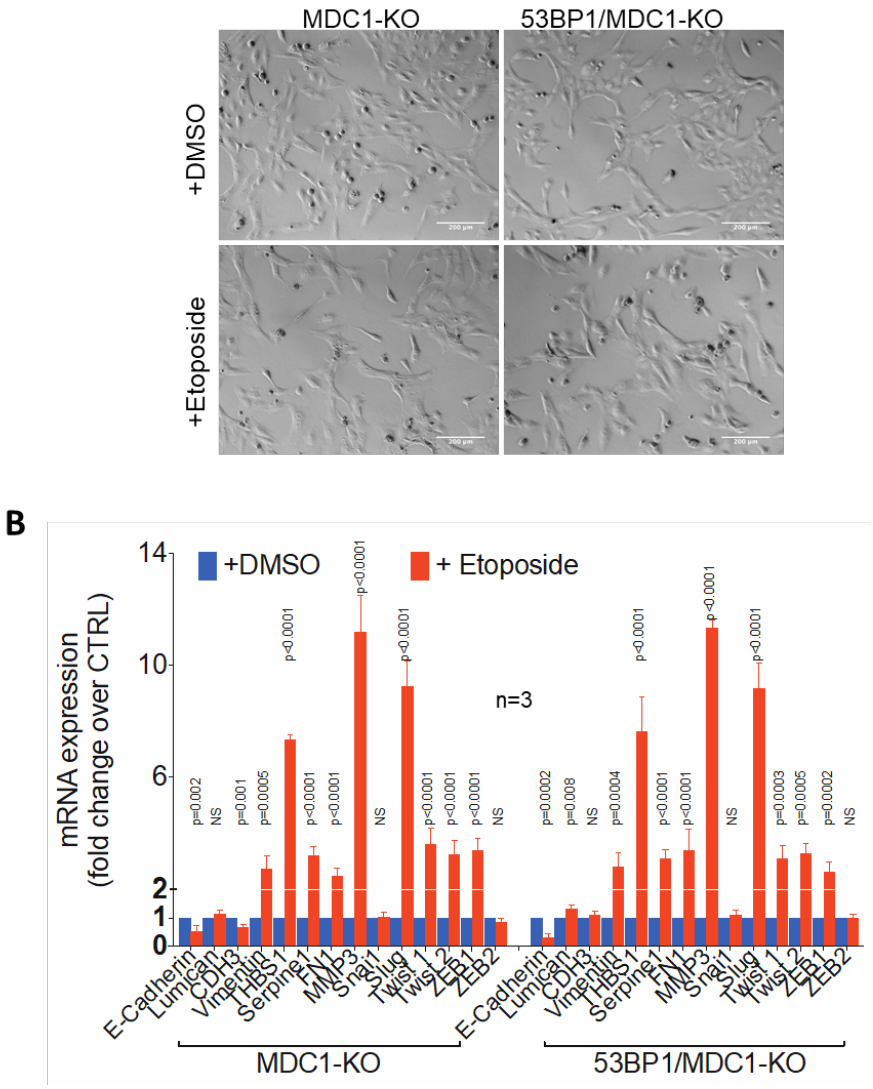


Fig. S4.

Exposure to exogenous DNA damage reinforces EMT hallmarks in a DDR deficient context.
A. Cell morphology aspect of WT parental cell line and 53BP1 KO clones after treatment with etoposide 0.1 □M for 7 days. **B**. Relative mRNA level of expression of epithelial and EMT markers in MDC1- and 53BP1/MDC1-DKO clones after exposure to etoposide (0.1 μM for 7 days). p values from paired comparisons are indicated. NS: non-significant.

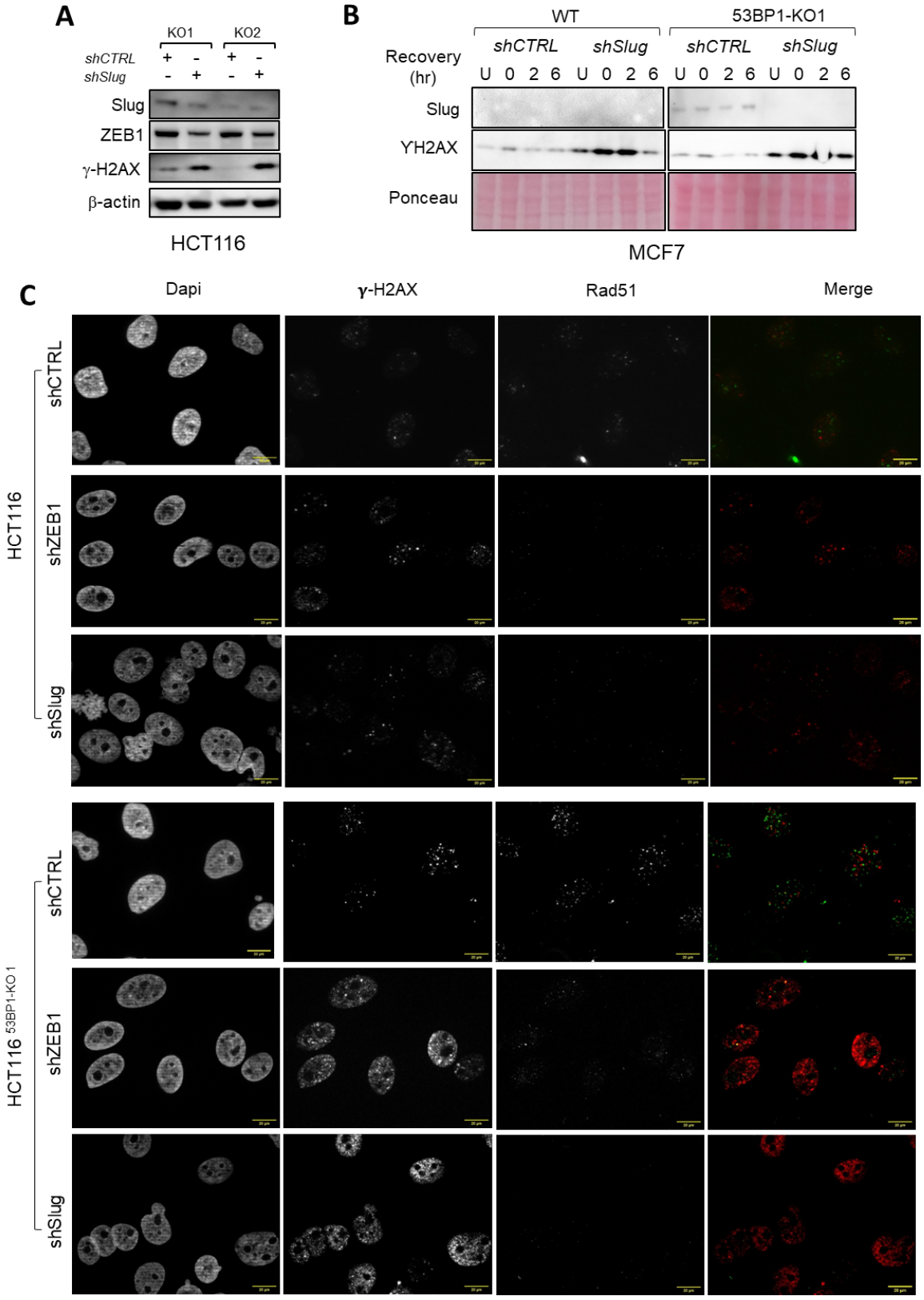
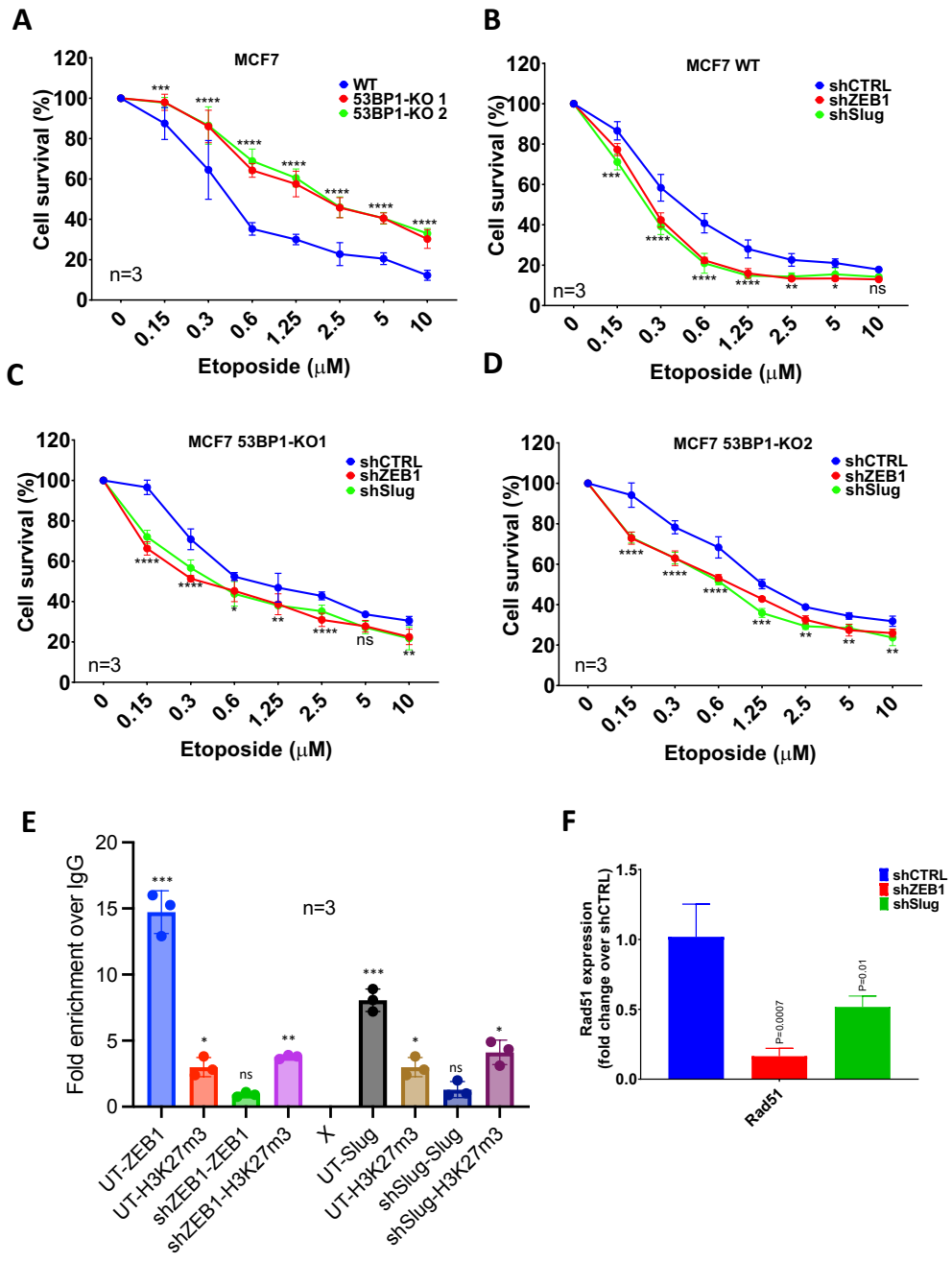

A
KO1
KO2
shCTRL
shSlug
Slug
ZEB1
γ-H2AX
β-actin
HCT116
B
WT
53BP1-KO1
shCTRL
shSlug
shCTRL
shSlug
Recovery (hr)
U 0 2 6 U 0 2 6 U 0 2 6 U 0 2 6
Slug
YH2AX
Ponceau
MCF7
C
Dapi
γ-H2AX
Rad51
Merge
HCT116
shCTRL
shZEB1
shSlug
HCT116 53BP1-KO 1
shCTRL
shZEB1
shSlug

Fig. S5.

EMT favors DNA repair efficiency.

A. Western blot analyses for Slug and ZEB1 after introduction of an shSlug expressing vector into HCT116 53BP1-KO clones. **B**. DNA damage recovery assay in MCF7 cells, WT and KO for 53BP1, and depleted or not for Slug. Cells were treated (or not, U) with etoposide (2μM for 24h) before medium change (0 hr) and let to recover for either 2 or 6 hours. Shown here are western blot analyses for Slug and the DNA damage marker γH2AX. **C**. Representative images immunofluorescence experiments aimed at detecting γH2AX and RAD51 foci in WT or 53BP1-KO HCT116 cells expressing either shZEB1 or shSlug. Quantitative analyses are presented in Figure 4G.

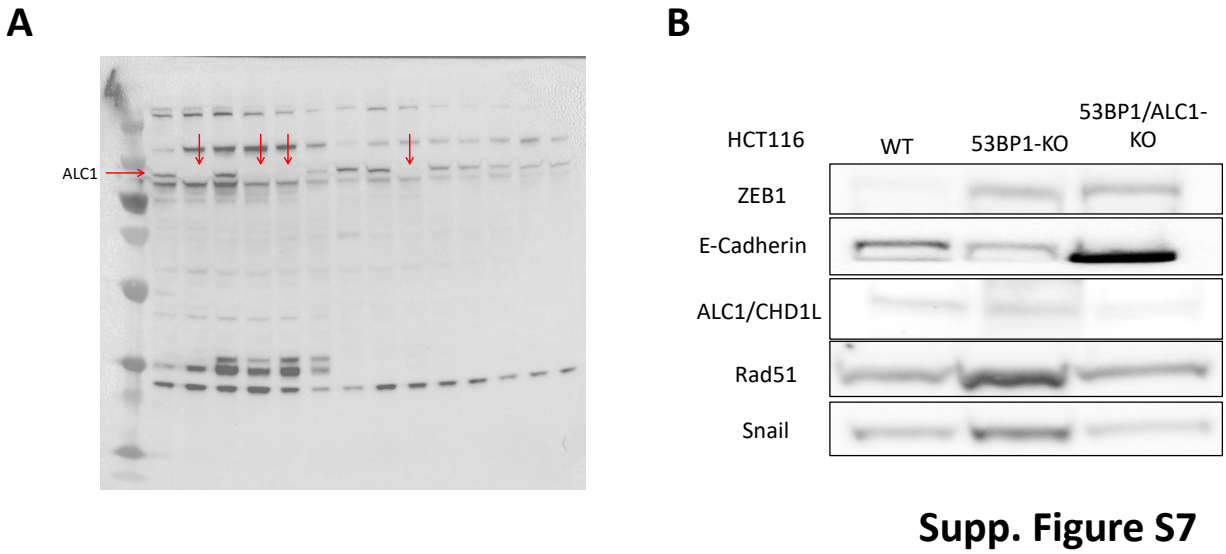


Supp. Figure S6

Fig. S6.

EMT promotes resistance to etoposide and stimulates RAD51 expression.

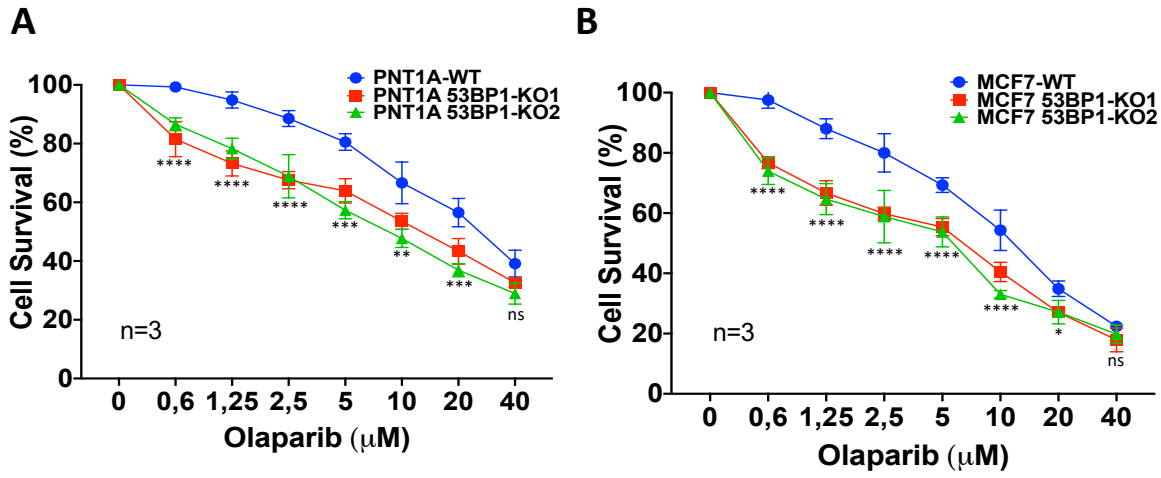
A to D. Cytotoxicity assays for WT and 53BP1-KO MCF7 cells, depleted or not for either ZEB1 or Slug using etoposide. Significant differences from multiple t-tests (adjusted) are shown for the following comparisons: KO vs WT (A) or shZEB1/shSlug vs shControl (B-D). **E.** Cut&Run analysis for EMT-TFs binding to the promoter of the Vimentin gene. **F.** Relative levels of expression (compared to untreated cells) after transfections of HCT116 WT cells with a random sh or sh targeting ZEB1 or Slug EMT-TFs.



Supp. Figure S7

Fig. S7.

A. Example of screening for ALC1 KO cells in HCT116 53BP1 KO cells after transfection with CRSPR constructs, sorting for strong GFP signal and clone isolation. The horizontal red arrow points to the ALC1 specific band detected by the antibody. Four different clones (indicated by the vertical arrows) are identified in this experiment. **B.** HCT116 cells (WT, 53BP1 KO and double 53NBP1/ALC1 KO) were tested in western blot for expression of EMT markers as well as RAD51 expression.



Supp. Figure S8

Fig. S8.

53BP1 KO cells are more sensitive to Olaparib than WT cells.

A-B. Cytotoxicity assays for WT and 53BP1-KO MCF7 cells as well as WT and 53BP1-KO PNT1A cells using Olaparib. Significant differences from multiple t-tests (adjusted) are shown for the comparisons KO vs WT.

Table S1.
Sequences of sgRNAs targeting sense and anti-sense strands in MDC1 and 53BP1 genes.

MDC1_S_F	accgCAGGATCAGAGCTGCTGAGA	Exon 9
MDC1_S_R	aaacTCTCAGCAGCTCTGATCCTG	Exon 9
MDC1_AS_F	accgCAGGTGGCATCTTGCAATTC	Exon 9
MDC1_AS_R	aaacGAATTGCAAGATGCCACCTG	Exon 9
53BP1_S_F	accgGCAGTCCCACAGAGCAAGAA	Exon 10
53BP1_S_R	aaacTTCTTGCTCTGTGGGACTGC	Exon 10
53BP1_AS_F	accgGAACGATAAAAGGAGTAGAT	Exon 10
53BP1_AS_R	aaacATCTACTCCTTTTATCGTTC	Exon 10

Table S2.

Sequences of forward and reverse primers bracketing CRISPR targeted sequences.

MDC1-F	CTGCTTGGAACTCAGCCACC	20	GENOTYPIC SCREENING
MDC1-R	GAAGATATAGAGATGACTTGTGGAATAGGAGG	32	GENOTYPIC SCREENING
53BP1-F	AAGGAATTCTTCAGATCTTGTTGC	24	GENOTYPIC SCREENING
53BP1-R	CAAGGCAGAAAAAGTGTTGCTC	22	GENOTYPIC SCREENING
MDC1-F	CCAAAGTGATCCTGGAGAGAGATAC	25	DNA SEQUENCING
MDC1-R	CTCTCCTCCATTAGACTGGGATCTA	25	DNA SEQUENCING
53BP1-F	TATTTCCTAGCACTGCTCATTTTGC	25	DNA SEQUENCING
53BP1-R	CTGAAGGGCTCCTCAAGTGC	20	DNA SEQUENCING

Table S3.
Antibodies used for immunoblots analyses.

Target	Host	Dilution ratio	Supplier	Reference number
γ-H2AX	Rabbit	1:1000	Cell Signaling	9718
H2AX	Rabbit	1:1000	abcam	ab11175
B-Actin	HRP	1:10000	Santa Cruz	sc-47778
53BP1	Rabbit	1:5000	novusbio	NB100-304
MDC1	Rabbit	1:5000	abcam	ab11171
Vimentin	Rabbit	1:1000	Cell signaling	9782
Slug	Rabbit	1:1000	Cell signaling	9782
Snai1	Rabbit	1:1000	Cell signaling	9782
ZEB1	Mouse	1:2000	Origene	TA802298
ZEB1	Rabbit	1:1000	Cell signaling	9782
ZO-1	Rabbit	1:1000	Cell signaling	9782
E-Cadherin	Rabbit	1:1000	Cell signaling	9782
Claudin-1	Rabbit	1:2000	Cell signaling	9782
Twist1	Mouse	1:1000	Santa Cruz	sc-81417
Rad51	Rabbit	1:1000	Sigma-Aldrich	PC130
P-Kap1 (S824)	Rabbit	1:1000	Bethyl	A300-767A-M
Kap1 (TIF1β)	Rabbit	1:1000	Cell signaling	4124
Phospho-ATM	Rabbit	1:10,000	abcam	ab81292
ATM	Rabbit	1:10,000	abcam	ab32420

Table S4.
Sequences of forward and reverse oligos used for RT-qPCR.

FN1-F	ACAACACCGAGGTGACTGAGAC
FN1-R	GGACACAACGATGCTTCCTGAG
THBS1-F	GCTGGAAATGTGGTGCTTGTCC
THBS1-R	CTCCATTGTGGTTGAAGCAGGC
Serpine1-F	CTCATCAGCCACTGGAAAGGCA
Serpine1-R	GACTCGTGAAGTCAGCCTGAAAC
B-Actin-F	CACCATTGGCAATGAGCGGTTC
B-Actin-R	AGGTCTTTGCGGATGTCCACGT
MMP3-F	CACTCACAGACCTGACTCGGTT
MMP3-R	AAGCAGGATCACAGTTGGCTGG
twist1-F	GCCAGGTACATCGACTTCCTCT
twist1-R	TCCATCCTCCAGACCGAGAAGG
twist2-F	GCAAGATCCAGACGCTCAAGCT
twist2-R	ACACGGAGAAGGCGTAGCTGAG
ZEB1-F	GGCATACACCTACTCAACTACGG
ZEB1-R	TGGGCGGTGTAGAATCAGAGTC
ZEB2-F	AATGCACAGAGTGTGGCAAGGC
ZEB2-R	CTGCTGATGTGCGAACTGTAGG
CDH3-F	CAGGTGCTGAACATCACGGACA
CDH3-R	CTTCAGGGACAAGACCACTGTG
Vimentin-F	CGAGGACGAGGAGAGCAGGATTTCTC
Vimentin-R	GGTATCAACCAGAGGGAGTGA
E-Cadherin-F	GCCTCCTGAAAAGAGAGTGGAAG
E-Cadherin-R	TGGCAGTGTCTCTCCAAATCCG
SNAIL1-F	ACCACTATGCCGCGCTCTT
SNAIL1-R	GGTCGTAGGGCTGCTGGAA
SNAIL2-F	TGTTGCAGTGAGGGCAAGAA

SNAIL2-R	GACCCTGGTTGCTTCAAGGA
Rad51-Promoter-1-F	AATGTCTTCCACTTCGCCC
Rad51-Promoter-1-R	TTCACGCCAGTAATCCCAG
Rad51-Promoter-2-F	CCATTTCCCACTTCTATCCATC
Rad51-Promoter-2-R	GTTGCCGTCTTCTGTTTACC
Alu-F	TACAAAAAATTAGCCGGGCG
Alu-R	GATCTCGGCTCACTGCAAG