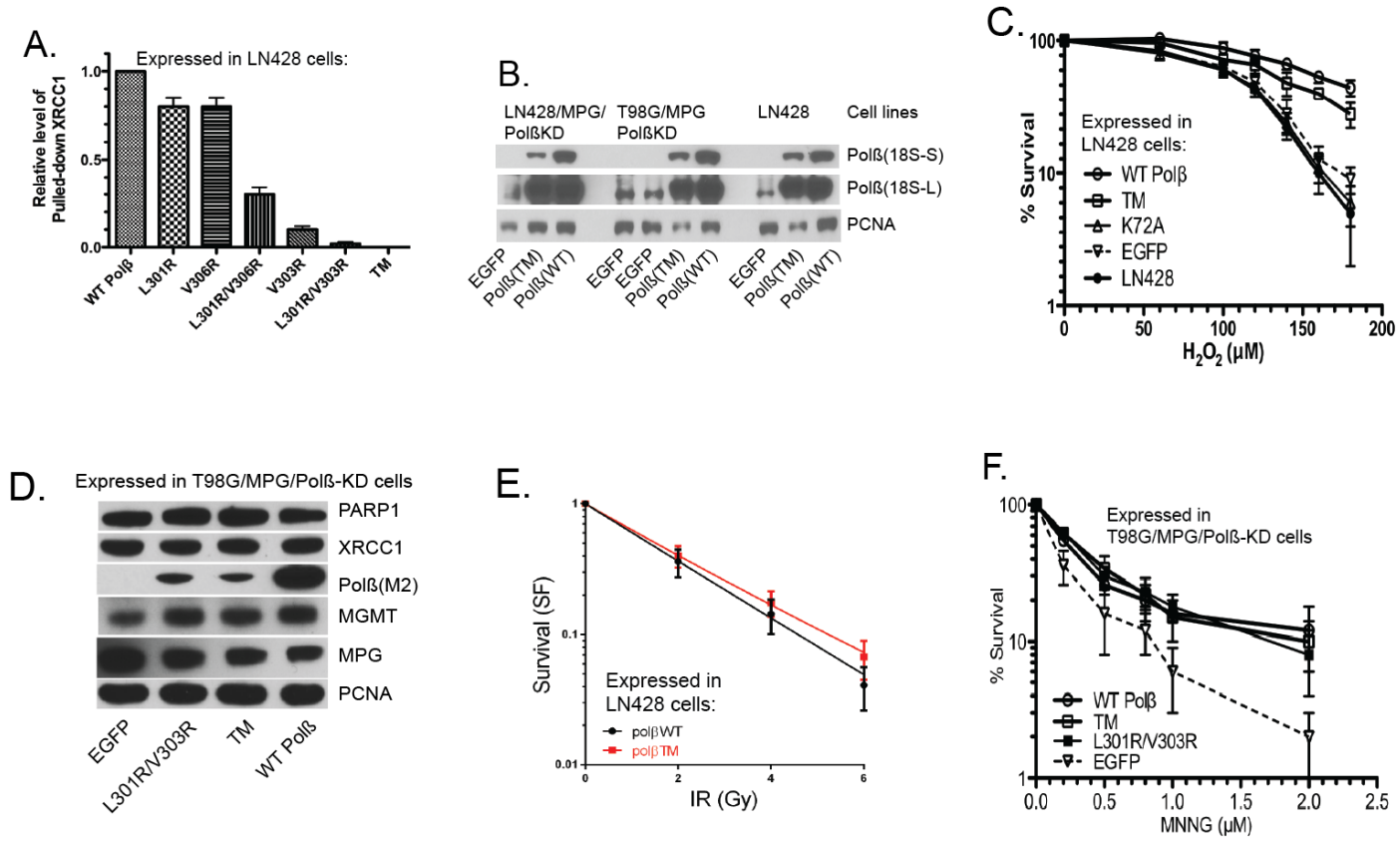
Fang et al. - Supplementary Figure 1



Supplementary Figure 1

The Polß/XRCC1 interaction does not play a key role in the cellular survival response to DNA damage

(A) Polß, mutated at residues L301, V303 and V306, can disrupt the Polß/XRCC1 interaction (shown in **Figure 1A,B**). The band densitometry of Polß and mutants was quantified and the relative ratio of XRCC1 bound to indicated Flag-Polß(mutants) or Flag-Polß(WT) was calculated and is shown in the bar graph. The result indicates mean ± SD of three independent experiments.

(B) Immunoblot analysis of LN428 and T98G cells to determine the relative level of endogenous Polß as compared to the expression of the transgenic Flag-Polß. The relative level of Polß in LN428/MPG/PolßKD, T98G/MPG/PolßKD and LN428 cells expressing either endogenous Polß, Flag-Polß(WT) or Flag-Polß(TM) was examined by immunoblot as shown. "S": short exposure time; "L": long exposure time. Levels of PCNA are shown as a loading control. The cells (LN428/MPG/PolßKD, T98G/MPG/PolßKD or LN428) were modified to express either EGFP, Flag-Polß(WT) or Flag-Polß(TM) by lentiviral transduction, as indicated in the figure.

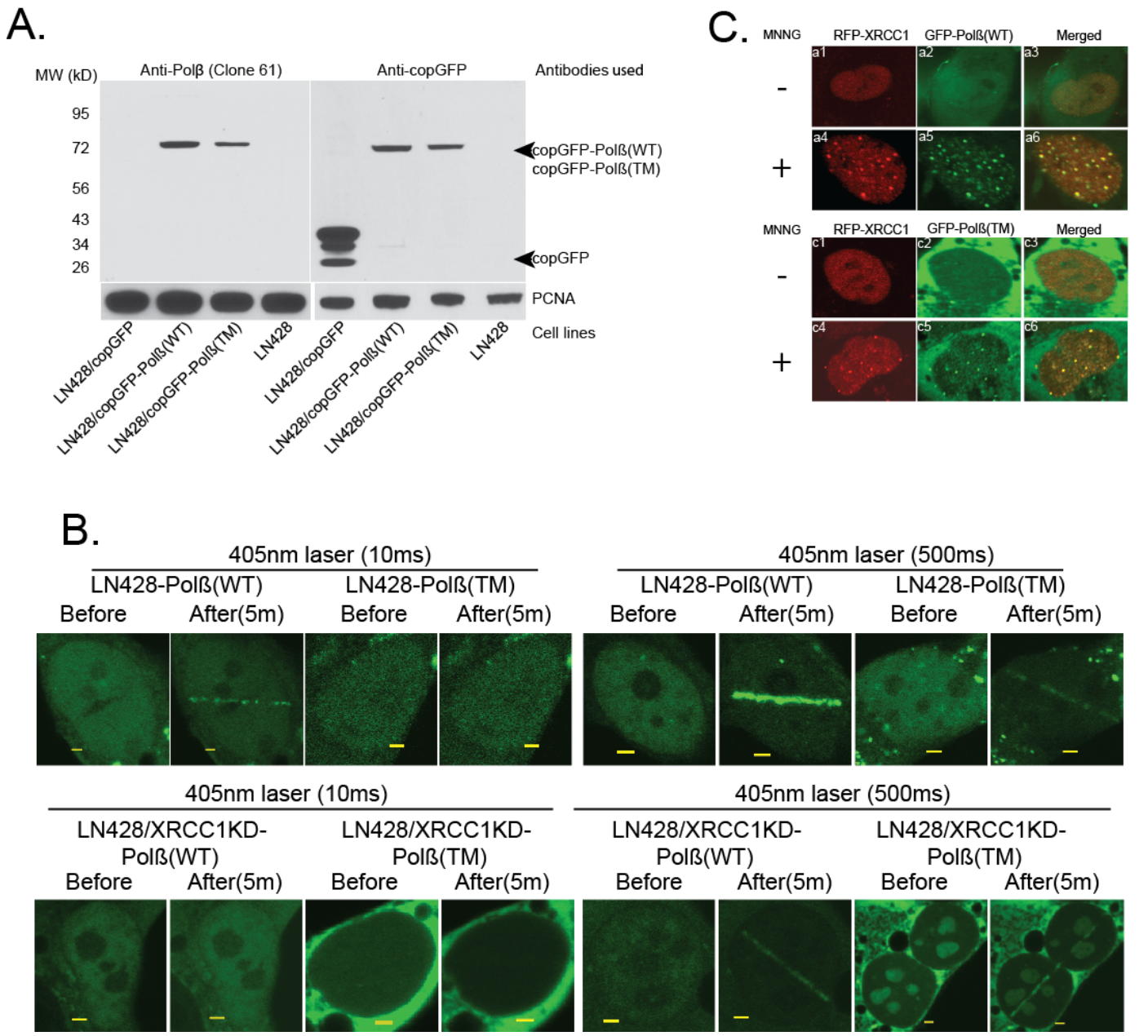
(C) The disruption of the Polß/XRCC1 interaction does not significantly enhance H_2O_2-induced short-term cytotoxicity measured by an MTS assay. The plot shows the survival of cells expressing the indicated Polß proteins (or EGFP control) after hydrogen peroxide exposure, as described in the materials and methods. LN428 cells or those modified to express Flag-Polß(WT), Flag-Polß(TM), Flag-Polß(K72A) or EGFP (control) were treated with H_2O_2 and evaluated for cell viability. The plot shows the % viable cells as compared to untreated (control) cells (MTS assay). Results indicate the mean ± SD of three independent experiments.

(D) The level of Polß and mutants [Flag-Polß(L301R/V303R), Flag-Polß(TM)] or EGFP in nuclear extracts and the expression of Polß(WT) and mutants do not affect the protein level of some key BER repair proteins in stable T98G/MPG/Polß-KD cells, as determined by immunoblot.

(E) Lack of XRCC1 binding by Polß does not alter the cellular radiation response. Expression of Flag-Polß(TM) radiosensitizes cells to the same extent as expression of Flag-Polß(WT). Clonogenic survival of LN428 cells expressing Flag-Polß(TM) (squares) compared to LN428 cells expressing Flag-Polß(WT) (circles). Data points represent means and SD of seven independent experiments.

(F) The disruption of the Polß/XRCC1 interaction does not significantly increase MNNG-induced long-term cytotoxicity in modified T98G cells as determined by the CyQuant assay. T98G cells or those modified to express Flag-Polß(WT), Flag-Polß(TM), Flag-Polß(L301R/V303R) or EGFP (control) were treated with MNNG and evaluated for cell viability. The plot shows the % viable cells as compared to untreated (control) cells (CyQuant assay). Results indicate the mean ± SD of three independent experiments.

Fang et al. - Supplementary Figure 2



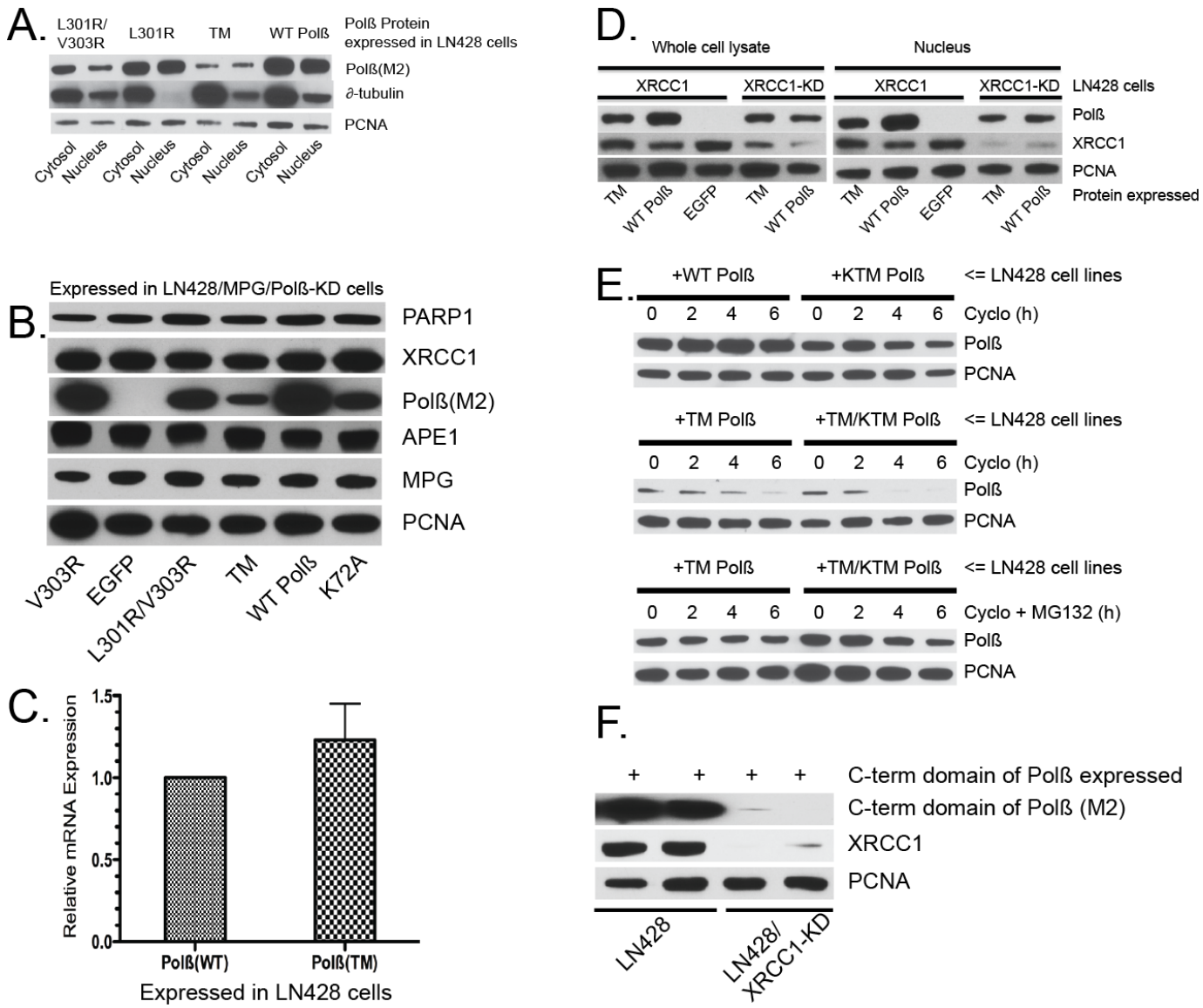
Supplementary Figure 2
Recruitment of copGFP-Polß(WT) or copGFP-Polß(TM) to the site of damage in LN428 or LN428/XRCC1-KD cells induced by laser treatment

(A) Immunoblot assay confirms the expression of copGFP-Polß(WT) and copGFP-Polß(TM). LN428 cells were transduced with lentiviral vectors to express copGFP, copGFP-Polß(WT) or copGFP-Polß(TM). Whole cell lysates were prepared and probed by anti-Polß (Clone 61) and anti-copGFP antibody respectively, as shown. PCNA is shown as a loading control.

(B) The fluorescence images of cells treated with a 405nm laser (10ms or 500ms) after 5 min. The quantified data was plotted and shown in **Figure 2E**. The scale bar in the image indicates 2 μm.

(C) Fluorescent images depicting DNA damage-induced foci of RFP-XRCC1 and copGFP-Polß(WT) (panels a1-a6) or copGFP-Polß(TM) (panels c1-c6) expressed in LN428/MPG cells before or after MNNG treatment (5 μM, 5 min), as indicated.

Fang et al. - Supplementary Figure 3



Supplementary Figure 3

The expression level of some DNA repair proteins and their distribution in the cytosol and nucleus and no evidence to suggest that Mule is involved in the ubiquitin proteasome pathway-mediated degradation of Polß when separated from XRCC1

(A) The expression and distribution of Flag-Polß(WT) and mutants in established stable LN428 cells, as determined by immunoblot.

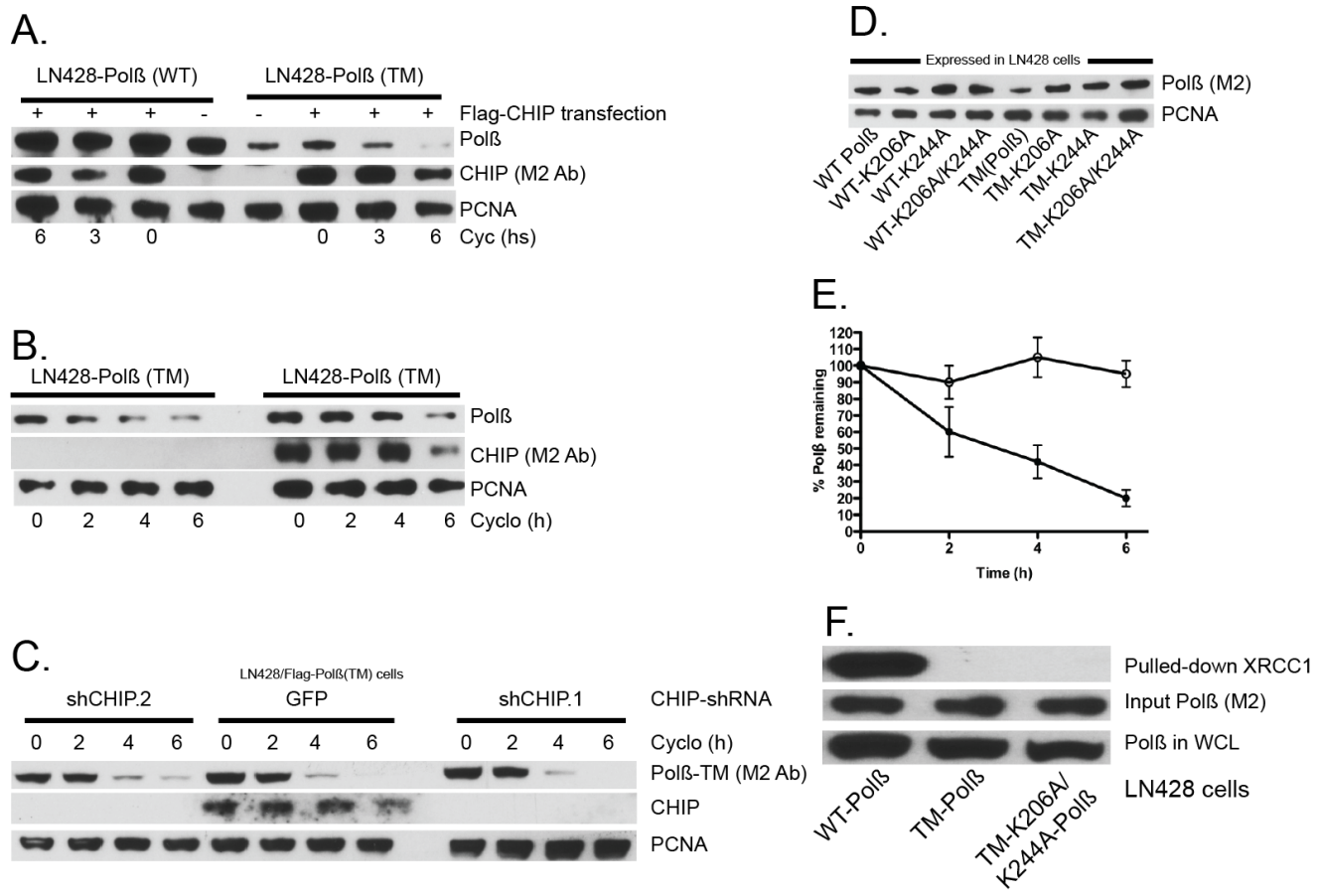
(B) The level of Flag-Polß(WT) and mutants [Flag-Polß(V303R), Flag-Polß(L301R/V303R), Flag-Polß(TM), Flag-Polß(K72)] or EGFP in nuclear extracts and related BER repair proteins in stable LN428/MPG/Polß-KD cells, as determined by immunoblot.

(C) The relative expression of Polß in LN428 cells expressing Flag-Polß(WT) or Flag-Polß(TM) was examined by qRT-PCR, normalized to ß-actin.

(D) The level of Flag-Polß(WT) and Flag-Polß(TM) in nuclear extracts or whole cell lysates from LN428 cells with or without XRCC1 knockdown was determined by immunoblot. The quantified result is shown in **Figure 3B**.

(E) LN428 cells expressing Flag-Polß(WT), Flag-Polß(TM) or Flag-Polß(KTM) were analyzed for protein stability by immunoblot after Cyclo treatment for the times indicated (KTM = MULE-mediated mono-ubiquitylation sites as reported[1]; Polß(K41R/K61R/K81R); TM = Polß(L301R/V303R/V306R); TM/KTM = Polß(K41R/K61R/K81R/L301R/V303R/V306R).

(F) The C-terminal domain of Polß (C-term) was expressed in LN428 and LN428/XRCC1-KD cells (transient transfection). The steady-state level of C-term was determined by immunoblot. The blot also shows the reduced level of XRCC1 in the LN428/XRCC1-KD cells and the level of PCNA as a loading control.



Supplementary Figure 4

No evidence to suggest that CHIP is involved in the ubiquitin proteasome pathway-mediated degradation of Polß when separated from XRCC1. Amino acid residues K206 and K244 are the ubiquitylation sites on Polß when separated from XRCC1

(A) LN428 cells expressing Flag-Polß(WT) and Flag-Polß(TM) were analyzed for protein stability by immunoblot after Cyclo treatment for the times indicated and after CHIP over-expression.

(B) LN428 cells expressing Flag-Polß(TM) were analyzed for protein stability by immunoblot after Cyclo treatment for the times indicated with and without CHIP over-expression.

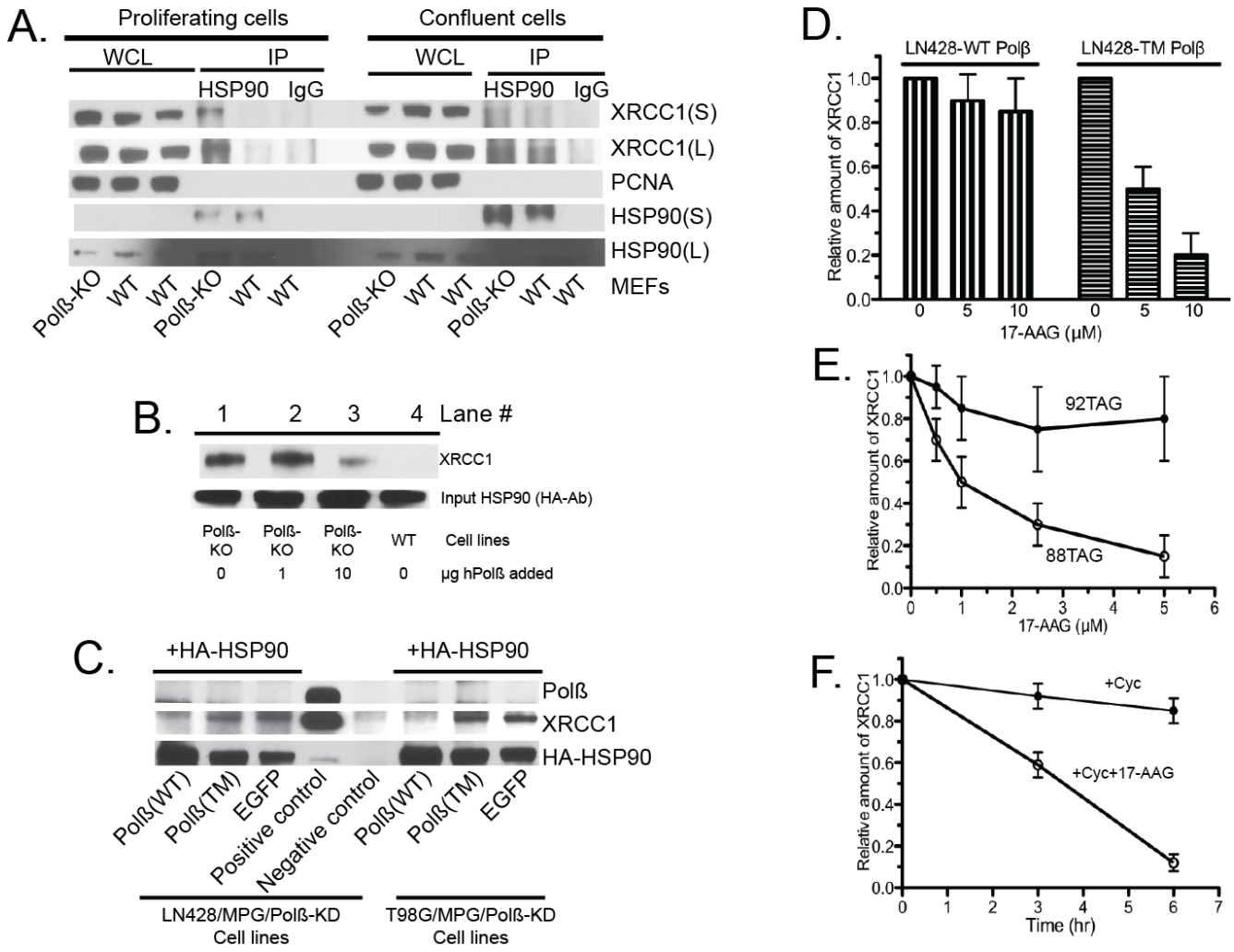
(C) LN428 cells expressing Flag-Polß(TM) were analyzed for protein stability by immunoblot after Cyclo treatment for the times indicated after transduction with GFP-expressing lentivirus or two separate CHIP shRNA-expressing lentiviruses.

(D) Alanine mutation of the predicted ubiquitylation sites K206 and K244 increases the steady-state level of Flag-Polß(TM) in established stable LN428 cell lines. Also refer to **Figure 4B**. Shown is an immunoblot from the cell lines as listed in the figure.

(E) The band intensity (densitometry) of Flag-Polß(TM) and Flag-Polß(TM/K206A/K244A) treated with 0.2mM Cyclo for different times was quantified and plotted as a function of time after the start of treatment. Also refer to **Figure 4C**.

(F) The Polß K206A/K244A mutation does not affect the interaction of Polß/XRCC1. Flag-Polß(WT), Flag-Polß(TM) and Flag-Polß(TM/K206A/K244A) in LN428 cells were immunoprecipitated with M2 antibody. The pulled-down XRCC1 was probed as shown.

Fang et al. - Supplementary Figure 5



Supplementary Figure 5

HSP90 inhibitor (17-AAG) treatment induces the degradation of free XRCC1 and Polß competes with HSP90 to bind to XRCC1

(A) XRCC1 is efficiently pulled-down by antibodies specific to endogenous HSP90 in proliferating 88TAg cells but weakly or not at all in 92TAg cells (as shown in **Figure 5B**). Proliferating or confluent 88TAg and 92TAg cells were collected and IP was performed using an antibody specific to endogenous HSP90. The pulled-down XRCC1 was examined by immunoblot as shown. ”S”: short exposure time; “L”: long exposure time.

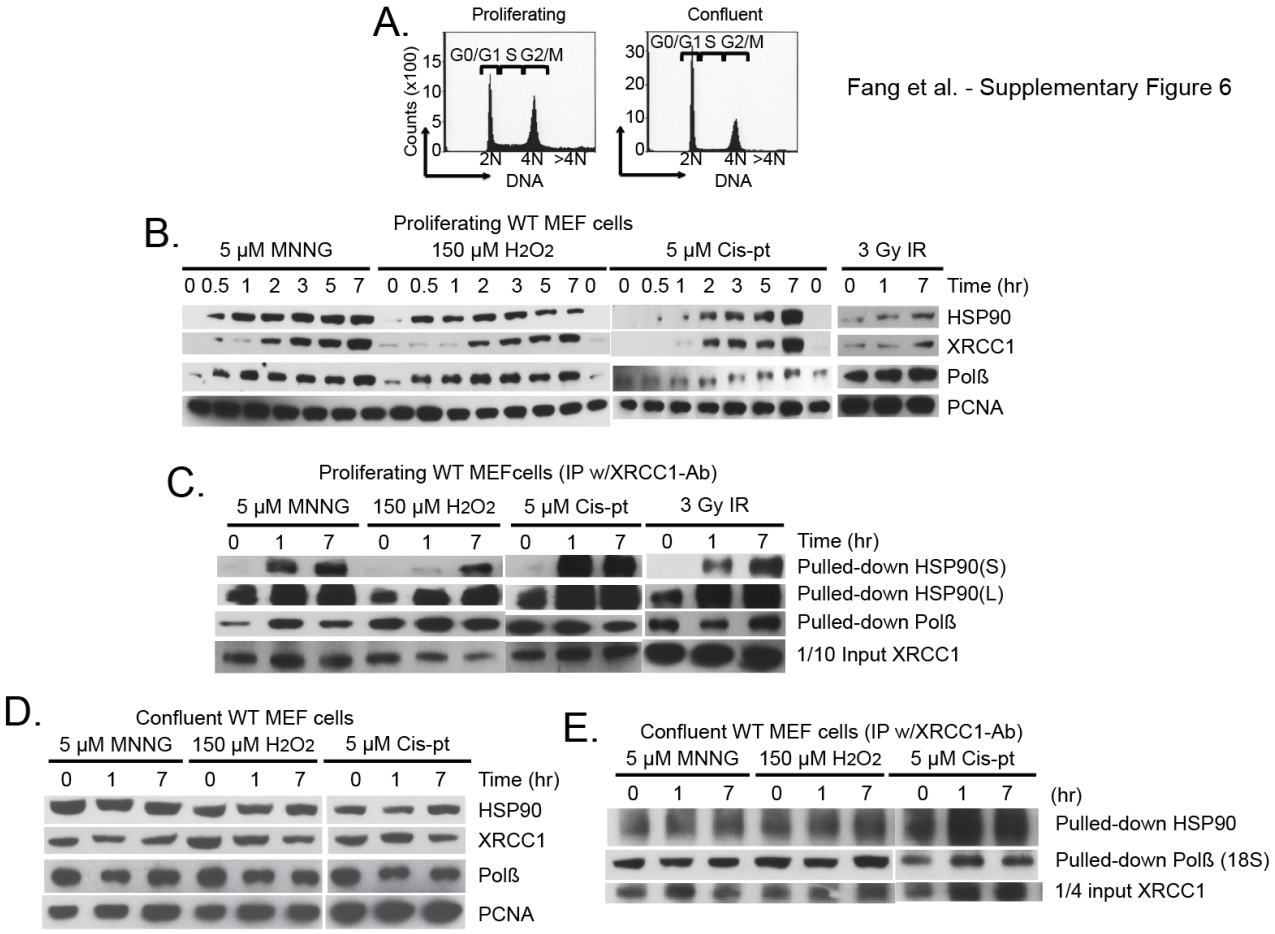
(B) Polß competes with HSP90 to bind to XRCC1. Whole cell lysates (WCLs) were prepared from 88TAg and 92TAg cells. 1 or 10μg of purified human Polß was added to the WCLs of 88TAg and rotated for 1 hr at 4°C. IP was performed with anti-HA antibody to detect the HA-HSP90/XRCC1 complex. The pulled-down XRCC1 was determined by immunoblot, as shown.

(C) HA-HSP90 interacts with XRCC1 in LN428/MPG/Polß-KD and T98G/MPG/Polß-KD cells expressing Flag-Polß(TM) or EGFP: Stable LN428 and T98G cell lines expressing Flag-Polß(WT), Flag-Polß(TM) or EGFP, after expression of HA-HSP90, were probed for HSP90 interacting proteins by IP of the expressed HA-HSP90 via the N-terminal HA epitope tag and probing for XRCC1 and Polß by immunoblot. Positive control is a whole cell lysate from LN428/Flag-Polß(TM) cells after transfection with pcDNA-HA-HSP90; Negative control is the immunoprecipitate of anti-HA agarose gel from the T98G/MPG/Polß-KD/Flag-Polß(TM) cell lysate (w/o expression of HA-HSP90).

(D) XRCC1 band densitometry in LN428/Flag-Polß(WT) cells or LN428/Flag-Polß(TM) cells (as shown in **Figure 5C**) treated with 17-AAG for the indicated doses was quantified. The relative amount of free XRCC1 is shown.

(E) XRCC1 band densitometry in Polß in WT MEFs (92TAg) or Polß knockout MEFs (88TAg) treated with 17-AAG (shown in **Figure 5C**) was quantified and the relative amount of free XRCC1 was plotted against the dose of 17-AAG as shown.

(F) XRCC1 band densitometry LN428/Flag-Polß(TM) cells treated with Cyclo or Cyclo+17-AAG (as shown in **Figure 5D**) was quantified. The relative amount of XRCC1 is plotted against the time course for Cyclo treatment as shown.



Supplementary Figure 6
Heterodimer complexes Polß/XRCC1 and XRCC1/HSP90 in 92TAg cells in response to cell cycle and DNA damage

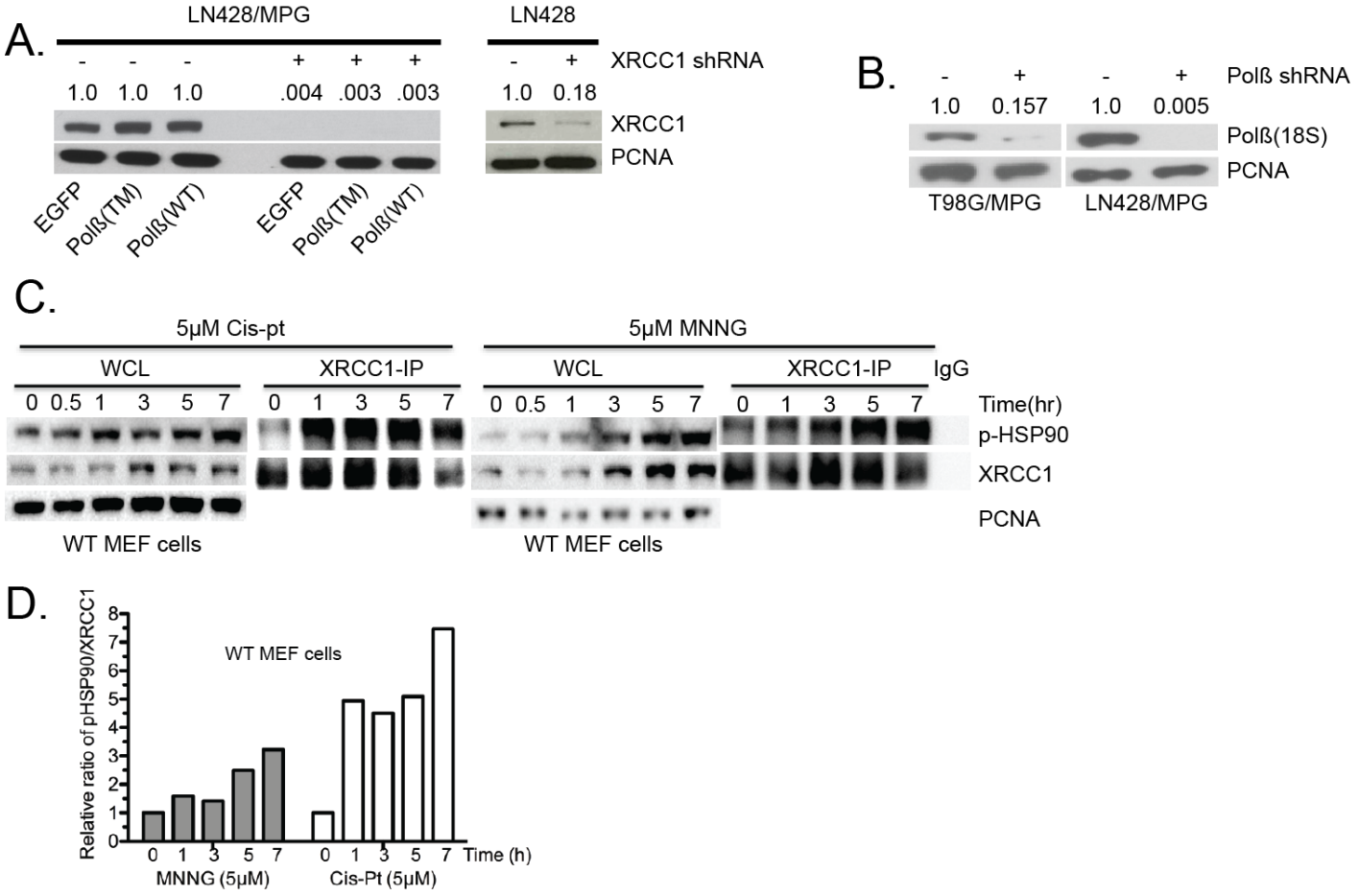
(A) Cell cycle distribution of proliferating and confluent 92TAg MEF cells determined by flow cytometric analysis of propidium iodide-stained cells.

(B) Proliferating 92TAg MEFs were treated with MNNG, H_2O_2, Cis-pt and IR for the times indicated. The expression level of HSP90, XRCC1 and Polß at different time course was determined by immunoblot, as shown. PCNA level is shown as a loading control.

(C) Proliferating 92TAg MEFs were treated with MNNG, H_2O_2, Cis-pt and IR for 0, 1 and 7 h(s). Whole cell lysates were prepared and an IP was performed with the XRCC1-Ab. The level of HSP90 or Polß bound to XRCC1 was evaluated by immunoblot, as shown.

(D) Confluent 92TAg MEFs were treated with MNNG, H_2O_2, Cis-pt and IR for the times indicated. The expression level of HSP90, XRCC1 and Polß at different time course was determined by immunoblot, as shown. PCNA level is shown as a loading control.

(E) Confluent 92TAg MEFs were treated with MNNG, H_2O_2, Cis-pt and IR for 0, 1 and 7 h(s). Whole cell lysates were prepared and an IP was performed with the XRCC1-Ab. The level of HSP90 or Polß bound to XRCC1 was evaluated by immunoblot, as shown.



Supplementary Figure 7
shRNA expression can efficiently knockdown XRCC1 and Polß and phosphorylated HSP90 (T7-P) is induced by DNA damage agents promoting an interaction with XRCC1 in 92TAg cells

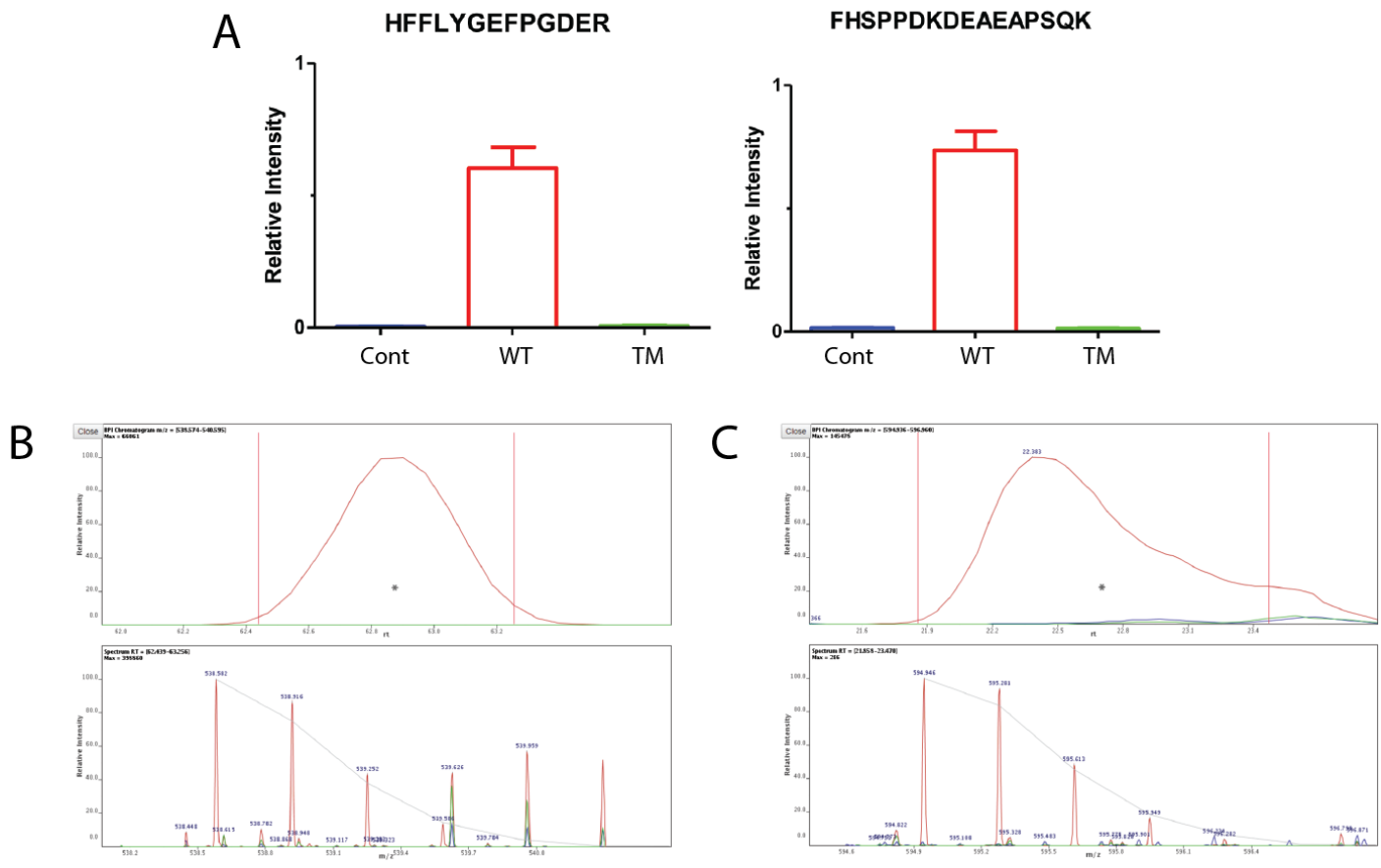
(A) Stable LN428, LN428/XRCC1-KD, LN428/MPG or LN428/MPG/XRCC1-KD cells expressing EGFP, Polß(WT) or Polß(TM) were established and the level of XRCC1 was determined by immunoblot, as shown.

(B) The level of Polß in T98G/MPG and LN428/MPG cells with or without expressing Polß shRNA was examined with immunoblot and shown.

(C) pHSP90 in 92TAg cells can be induced by cis-pt and MNNG and interacts with XRCC1. Proliferating 92TAg cells were treated with 5μM cis-pt or MNNG for different time course. Whole cell lysates (WCL) were were prepared and a fraction of the WCL was used for IP. IP was performed with the XRCC1 antibody and protein G. The level of pHSP90 bound to XRCC1 was evaluated by immunoblot, as shown.

(D) The relative ratio of pHSP90/XRCC1 was calculated by quantifying the band intensity of immunoblots following immunoprecipitation of XRCC1 and analysis for HSP90 (shown in **Figure S7C**) from proliferating cells (92TAg MEFs) treated with 5μM MNNG or Cis-pt.

Fang et al. - Supplementary Figure 8



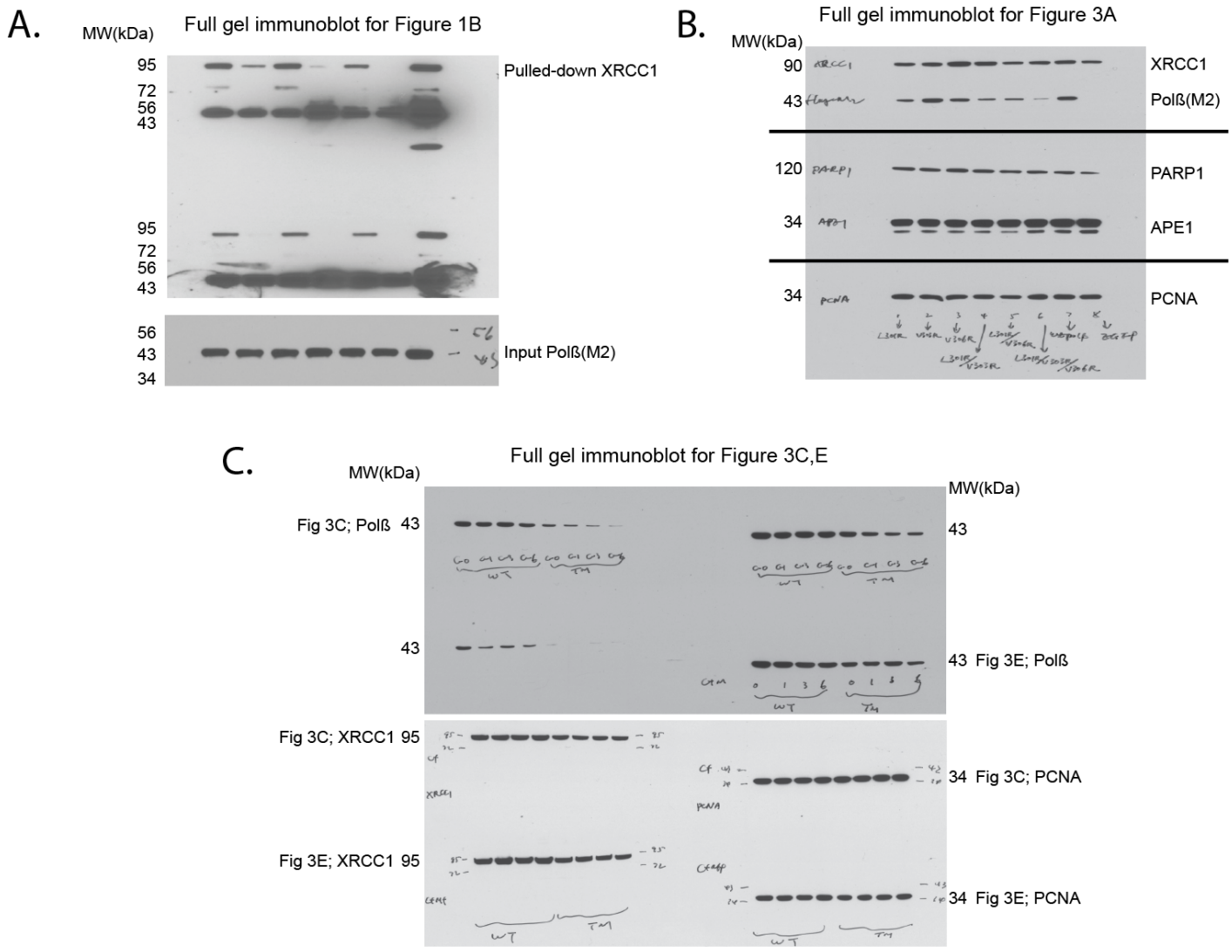
Supplementary Figure 8
(LC-MS/MS) analysis of immunoprecipitated samples

(A) Quantification results for two proteotypic XRCC1 peptides, HFFLYGEFPGDER and FHSPPDKDEAEAPSQK. Bar graphs of mean and SEM following affinity purification and high resolution LC-MS analysis of LN428 cells expressing an empty vector (Cont), WT Flag-Polß (WT) and the TM mutant Flag-Polß (TM). In total, twenty proteotypic peptides with amino acid sequences matching XRCC1 were quantified in independent affinity purification samples (9 samples per condition). The vast majority of XRCC1 peptides have relative intensities at or below background levels in the Cont and TM groups.

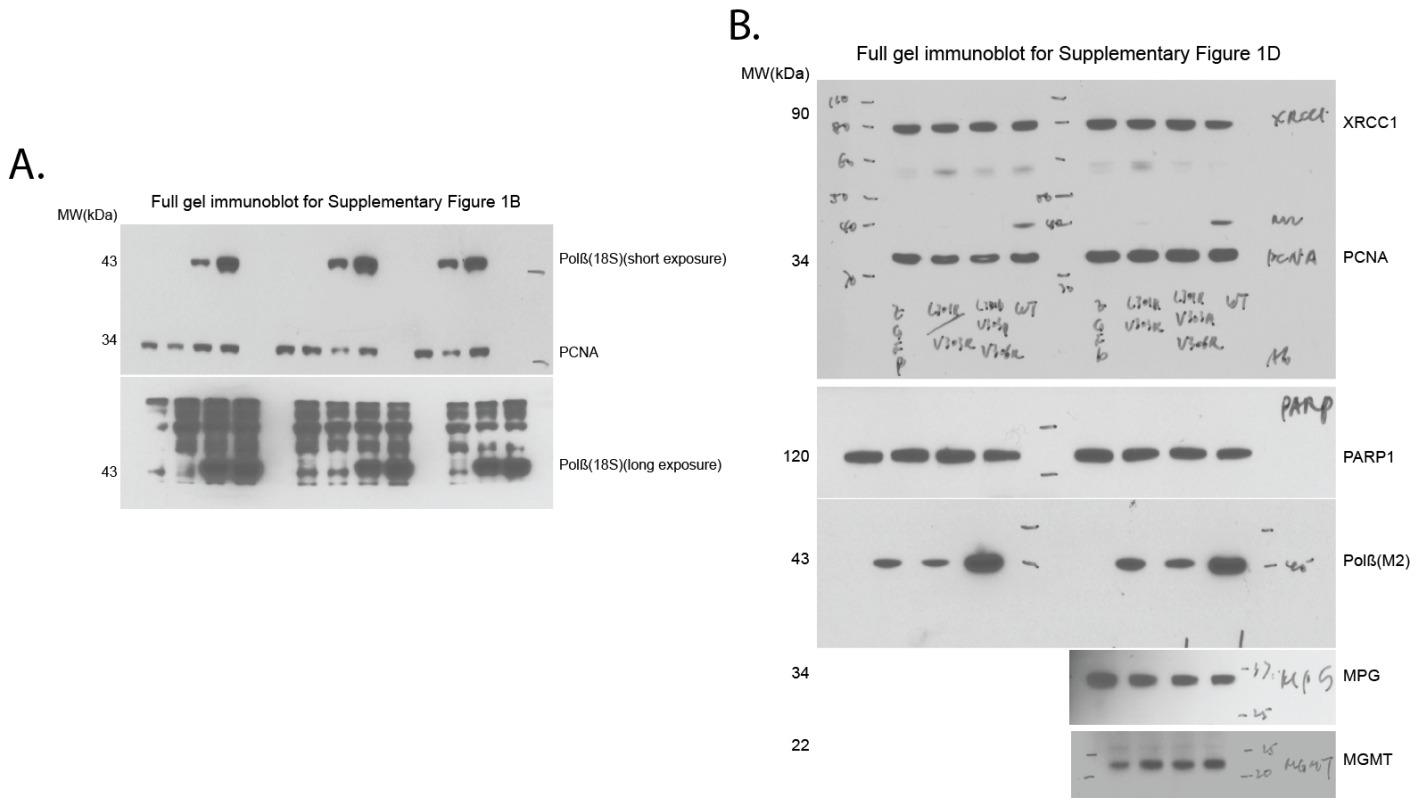
(B) High resolution LC-MS data for a proteotypic peptide with m/z = 538.583 and retention time 62.8 minutes , and amino acid sequence = HFFLYGEFPGDER, from DNA repair protein XRCC1. Overlay selected ion chromatograms (top) and full scan high resolution mass spectra (bottom) for 9 Cont (blue), WT (red) and TM (green) affinity purification samples.

(C) High resolution LC-MS data for a proteotypic peptide with m/z = 594.946 and retention time 22.4 minutes , and amino acid sequence = FFLYGEFPGDER, from DNA repair protein XRCC1. Overlay selected ion chromatograms (top) and full scan high resolution mass spectra (bottom) for 9 Cont (blue), WT (red) and TM (green) affinity purification samples.

Fang et al. - Supplementary Figure 9



Supplementary Figure 9
Full gel immunoblots for main text figures
(A) Full gel immunoblot for Figure 1B.
(B) Full gel immunoblot for Figure 3A.
(C) Full gel immunoblot for Figure 3C and Figure 3E.



Supplementary Figure 10
Full gel immunoblots for supplementary text figures
(A) Full gel immunoblot for Supplementary Figure 1B.
(B) Full gel immunoblot for Supplementary Figure 1D.

Supplementary Table 1. Oligodeoxynucleotides used

Oligo name	Sequence (5' to 3')*
PolBK72A-F	GGAACAAAAATTGCTGAA**GC**GATTGATGAGTTTTTAGCA ACTGG
PolBK72A-R	CCAGTTGCTAAAAACTCATCAATCGCTTCAGCAATTTTTGTTCC
PolBL301R-F	CAATCAATGAGTACACCATCCGTCCC**CG**GGGAGTCACTGGAGTTGCAGGAGAACCCC
PolBL301R-R	GGGGTTCTCCTGCAACTCCAGTGACTCCC**CG**GGGACGGATGGTGTACTCATTGATTG
PolBV303R-F	CACAATCAATGAGTACACCATCCGTCCCTTGGGA**CG**CACTGGAGTTGCAGGAGAACCCC TGCCAGTGG
PolBV303R-R	CCACTGGCAGGGGTTCTCCTGCAACTCCAGTG**CG**TCCCAAGGGACGGATGGTGTACTC ATTGATTGTG
PolBV306R-F	CCATCCGTCCCTTGGGAGTCACTGGA**CG**TGCAGGAGAACCCCTGCCAGTGG
PolBV306R-R	CCACTGGCAGGGGTTCTCCTGCA**CG**TCCAGTGACTCCCAAGGGACGGATGG
PolBL301R-F-3	CAATCAATGAGTACACCATCCGTCCC**CG**GGGA**CG**CACTGGAGTTGCAGGAGAACCCC
PolBL301R-R-3	GGGGTTCTCCTGCAACTCCAGTG**CG**TCCC**CG**GGGACGGATGGTGTACTCATTGATTG
PolBL301R-F-6	CAATGAGTACACCATCCGTCCC**CG**GGGAGTCACTGGA**CG**TGCAGGAGAACCCC
PolBL301R-R-6	GGGGTTCTCCTGCA**CG**TCCAGTGACTCCC**CG**GGGACGGATGGTGTACTCATTG
PolBV303R-F-6	CACAATCAATGAGTACACCATCCGTCCCTTGGGA**CG**CACTGGA**CG**TGCAGGAGAACCCC TGCCAGTGG
PolBV303R-R-6	CCACTGGCAGGGGTTCTCCTGCA**CG**TCCAGTG**CG**TCCCAAGGGACGGATGGTGTACTC ATTGATTGTG
PolBL301R-F-3-6	GAGTACACCATCCGTCCC**CG**GGGA**CG**CACTGGA**CG**TGCAGGAGAACCCCTGCC
PolBL301R-R-3-6	GGCAGGGGTTCTCCTGCA**CG**TCCAGTG**CG**TCCC**CG**GGGACGGATGGTGTACTC
PolBT304I-F	ATCCGTCCCTTGGGAGTCA**T**TGGAGTTGCAGGAGAACC
PolBT304I-R	GGTTCTCCTGCAACTCCA**A**TGACTCCCAAGGGACGGAT
PolBK41R-F	ATGCTTACAGAA**G**AGCAGCATCTGTTA
PolBK41R-R	GCTATAACAGATGCTGCT**C**TTCTGTAA
PolBK61R-F	AGCTGAAGCTAAGA**G**ATTGCCTGGAG
PolBK61R-R	CTACTCCAGGCAAT**C**TCTTAGCTTCAGCT
PolBK81R-F	TTAGCAACTGGAA**G**ATTACGTAAACTG
PolBK206A-F	CTTCACTTCAGAATCAACC**GC**ACAGCCAAAACTGTTACAT
PolBK206A-R	GATGTAACAGTTTTGGCTGT**GC**GGTTGATTCTGAAGTGAAG
PolBK244A-F	GGTGTTTGCCAGCTTCCCAGT**GC**AAATGATGAAAAAGAATATCC
PolBK244A-R	GGATATTCTTTTTCATCATTT**GC**ACTGGGAAGCTGGCAAACACC
PolBGFPC24F	CTGCCAGGGTCTAGAATGGACTACAAAGACGATGAC
PolBGFPC24R	CGCAGAGCCGGATCCTCATTCGCTCCGGTCCTTGG
shRNA1/HSP90	GTTATCCTACACCTGAAAGAA
shRNA2/HSP90	CCAGAATGAAGGAGAACCAGA
shRNA3/HSP90	TCATCAATACTTTCTACTCGA
shRNA4/HSP90	GAAGGATGGTGACAAGAAGAA
shRNA5/HSP90	CCCTCTAAACCATATCCCGTGA
shRNA-1/CHIP	CCCAAGTTCTGCTGTTGGACT
shRNA-2/CHIP	GAAGAGGAAGAAGCGAGACAT
shRNA-3/CHIP	GACGCATTCATCTCTGAGAAT
shRNA-4/CHIP	GCAGTCTGTGAAGGCGCACTT
shRNA-5/CHIP	CGCGAAGAAGAAGCGCTGGAA

*The bold bases indicate the mutated bases.

Supplementary Table 2. LN428 cell lines developed and used in this study

Cell line name	Cell line description	Growth media*
LN428	Human glioblastoma tumor cell line	Media #1
LN428/Flag-Polß(WT)	LN428 cells expressing Flag-Polß(WT)	Media #2
LN428/Flag-Polß(L301R)	LN428 cells expressing Flag-Polß(L301R)	Media #2
LN428/Flag-Polß(V303R)	LN428 cells expressing Flag-Polß(V303R)	Media #2
LN428/Flag-Polß(V306R)	LN428 cells expressing Flag-Polß(V306R)	Media #2
LN428/Flag-Polß(L301R/V303R)	LN428 cells expressing Flag-Polß(L301R/V303R)	Media #2
LN428/Flag-Polß(L301R/V306R)	LN428 cells expressing Flag-Polß(L301R/V306R)	Media #2
LN428/Flag-Polß(L301R/V303R/V306R)	LN428 cells expressing Flag-Polß(L301R/V303R/V306R)	Media #2
LN428/Flag-Polß(K72A)	LN428 cells expressing Flag-Polß(K72A)	Media #2
LN428/EGFP	LN428 cells expressing EGFP	Media #2
LN428/Flag-Polß(K41R/K61R/K81R)	LN428 cells expressing Flag-Polß(K41R/K61R/K81R)	Media #2
LN428/Flag-Polß(L301R/V303R/V306R-K41R/K61R/K81R)	LN428 cells expressing Flag-Polß(L301R/V303R/V306R/K41R/K61R/K81R)	Media #2
LN428/MPG/Polß-KD	LN428/Polß-KD cells expressing MPG	Media #3
LN428/MPG/Polß-KD/Flag-Polß(WT)	LN428/Polß-KD cells expressing MPG and Flag-Polß(WT)	Media #4
LN428/MPG/Polß-KD/Flag-Polß(L301R/V303R/V306R)	LN428/Polß-KD cells expressing MPG and Flag-Polß(L301R/V303R/V306R)	Media #4
LN428/MPG/Polß-KD/Flag-Polß(K72A)	LN428/Polß-KD cells expressing MPG and Flag-Polß(K72A)	Media #4
LN428/MPG/Polß-KD/EGFP	LN428/Polß-KD cells expressing MPG and EGFP	Media #4
LN428/XRCC1-KD	LN428/XRCC1-KD cells	Media #3
LN428/XRCC1-KD/ Flag-Polß(WT)	LN428/XRCC1-KD cells expressing Flag-Polß(WT)	Media #4
LN428/XRCC1-KD/Flag-Polß(L301R/V303R/V306R)	LN428/XRCC1-KD cells expressing Flag-Polß(L301R/V303R/V306R)	Media #4
LN428/XRCC1-KD/EGFP	LN428/XRCC1-KD cells expressing EGFP	Media #4
LN428/MPG	LN428 cells expressing MPG	Media#3
LN428/MPG-EGFP	LN428/MPG cells expressing EGFP	Media#4
LN428/MPG-Flag-Polß(WT)	LN428/MPG cells expressing Flag-Polß(WT)	Media#4
LN428/MPG-Flag-Polß(L301R/V303R/V306R)	LN428/MPG cells expressing Flag-Polß(L301R/V303R/V306R)	Media#4
LN428/MPG/XRCC1-KD	LN428/MPG cells with XRCC1 knockdown	Media#4
LN428/MPG/XRCC1-KD/EGFP	LN428/MPG/XRCC1-KD cells expressing EGFP	Media#5
LN428/MPG/XRCC1-KD/Flag-Polß(WT)	LN428/MPG/XRCC1-KD cells expressing Flag-Polß(WT)	Media#5
LN428/MPG/XRCC1-KD/Flag-Polß(L301R/V303R/V306R)	LN428/MPG/XRCC1-KD cells expressing Flag-Polß(L301R/V303R/V306R)	Media#5
LN428/Flag-Polß(K206A)	LN428 cells expressing Flag-Polß(K206A)	Media#2
LN428/Flag-Polß(K244A)	LN428 cells expressing Flag-Polß(K244A)	Media#2
LN428/Flag-Polß(K206A/K244A)	LN428 cells expressing Flag-Polß(K206A/K244A)	Media#2
LN428/Flag-Polß(L301R/V303R/V306R/K206A)	LN428 cells expressing Flag-Polß(L301R/V303R/V306R/K206A)	Media #2
LN428/Flag-Polß(L301R/V303R/V306R/K244A)	LN428 cells expressing Flag-Polß(L301R/V303R/V306R/K244A)	Media #2
LN428/Flag-Polß(L301R/V303R/V306R-K206A/K244A)	LN428 cells expressing Flag-Polß(L301R/V303R/V306R-K206A/K244A)	Media #2

*Media #1: α-MEM with 10% heat inactivated FBS, 5μg/ml Gentamycin, 80u Penicillin/80μg Streptomycin/0.32μg Amphotericin per ml, 2mM L-Glutamine.

*Media #2: Media #1 supplemented with Puromycin (1.0 μg/ml)

*Media #3: Media #1 supplemented with Geneticin (0.6 mg/ml)

*Media #4: Media #1 supplemented with Puromycin (1.0 μg/ml) and Geneticin (0.6 mg/ml)

*Media #5: Media #1 supplemented with Puromycin (1.0 μg/ml), Geneticin (0.6 mg/ml) and Hygromycin B (50mg/ml)

Supplementary Table 3. T98G cell lines developed and used in this study

Cell line name	Cell line description	Growth media*
T98G	Human glioblastoma tumor cell line	Media #5
T98G/Flag-Polß(WT)	T98G cells expressing Flag-Polß(WT)	Media #6
T98G/Flag-Polß(L301R/V303R/V306R)	T98G cells expressing Flag-Polß(L301R/V303R/V306R)	Media #6
T98G/EGFP	T98G cells expressing EGFP	Media #6
T98G/MPG/Polß-KD	T98G/Polß-KD cells expressing MPG	Media #7
T98G/MPG/Polß-KD/Flag-Polß(WT)	T98G/Polß-KD cells expressing MPG and Flag-Polß(WT)	Media #8
T98G/MPG/Polß-KD/Flag-Polß(L301R/V303R)	T98G/Polß-KD cells expressing MPG and Flag-Polß(L301R/V303R)	Media #8
T98G/MPG/Polß-KD/Flag-Polß(301R/V303R/V306R)	T98G/Polß-KD cells expressing MPG and Flag-Polß(L301R/V303R/V306R)	Media #8
T98G/MPG/Polß-KD/EGFP	T98G/Polß-KD cells expressing MPG and EGFP	Media #8

*Media #5: MEM minimum essential medium with 10% FBS, 5μg/ml Gentamycin, 80u Penicillin//80μg Streptomycin/0.32μg Amphotericin per ml, 1mM Sodium Pyruvate, 0.1mM MEM non-essential amino acids.

*Media #6: Media #5 supplemented with Puromycin (1.0 μg/ml)

*Media #7: Media #5 supplemented with Geneticin (0.4 mg/ml)

*Media #8: Media #5 supplemented with Puromycin (1.0 μg/ml) and Geneticin (0.4 mg/ml)

Supplementary Table 4. Vectors developed for and used in this study

Lab Stock #	Plasmid name	Insert description	Parental vector
136	pRS1427	Flag-Polß(31K N-terminal deletion mutant)	pIRES-Neo
727	pLVX-EGFP-IRES-puro	EGFP	pLVX-GW-IRES-puro
728	pLVX-Flag-Polß(WT)-IRES-puro	Flag-Polß(WT)	pLVX-GW-IRES-puro
729	pLVX-Flag-Polß(L301R)-IRES-puro	Flag-Polß(L301R)	pLVX-GW-IRES-puro
730	pLVX-Flag-Polß(V306R)-IRES-puro	Flag-Polß(V306R)	pLVX-GW-IRES-puro
731	pLVX-Flag-Polß(L301R/V306R)-IRES-puro	Flag-Polß(L301R/V306R)	pLVX-GW-IRES-puro
732	pLVX-Flag-Polß(TM)-IRES-puro	Flag-Polß(L301R/V303R/V306R)	pLVX-GW-IRES-puro
755	pLVX-Flag-Polß(L301R/V303R)-IRES-puro	Flag-Polß(L301R/V303R)	pLVX-GW-IRES-puro
756	pLVX-Flag-Polß(V303R)-IRES-puro	Flag-Polß(V303R)	pLVX-GW-IRES-puro
767	pLVX-EGFP-IRES-Neo	EGFP	pLVX-GW-IRES-Neo
791	pLVX-Flag-Polß(K72A)-IRES-puro	Flag-Polß(K72A)	pLVX-GW-IRES-puro
792	pLVX-Flag-Polß(WT)-IRES-Neo	Flag-Polß(WT)	pLVX-GW-IRES-Neo
793	pLVX-Flag-Polß(L301R/V303R)-IRES-Neo	Flag-Polß(L301R/V303R)	pLVX-GW-IRES-Neo
814	pCT-CMV-copGFP/Polß(WT)-Puro	copGFP-Polß(WT)	pCYTO-CMV-copGFP-MCS-EF1-puro
815	pCT-CMV-copGFP/Polß(TM)-Puro	copGFP-Polß(L301R/V303R/V306R)	pCYTO-CMV-copGFP-MCS-EF1-puro
834	pLVX-Flag-Polß(WT)-IRES-Hyg	Flag-Polß(WT)	pLVX-GW-IRES-Hyg
835	pLVX-Flag-Polß(TM)-IRES-Hyg	Flag-Polß(L301R/V303R/V306R)	pLVX-GW-IRES-Hyg
837	pLVX-Flag-Polß(TM)-IRES-Neo	Flag-Polß(L301R/V303R/V306R)	pLVX-GW-IRES-Neo
885	pLVX-Flag-Polß(KTM)-IRES-Neo	Flag-Polß(K41R/K61R/K81R)	pLVX-GW-IRES-Neo
886	pLVX-Flag-Polß(KTM)-IRES-Puro	Flag-Polß(K41R/K61R/K81R)	pLVX-GW-IRES-puro
887	pLVX-Flag-Polß(KTM)-IRES-GFP	Flag-Polß(K41R/K61R/K81R)	pLVX-GW-IRES-ZsGreen1
888	pLVX-Flag-Polß(TM/KTM)-IRES-Neo	Flag-Polß(L301R/V303R/V306R/(K41R/K61R/K81R)	pLVX-GW-IRES-Neo

889	pLVX-Flag-Polß(TM/KTM)-IRES-Puro	Flag-Polß(L301R/V303R/V306R/(K41R/K61R/K81R)	pLVX-GW-IRES-puro
890	pLVX-Flag-Polß(TM/KTM)-IRES-GFP	Flag-Polß(L301R/V303R/V306R/K41R/K61R/K81R)	pLVX-GW-IRES-ZsGreen1
893	pLVX-Flag-Polß(D256A)-IRES-Puro	Flag-Polß(D256A)	pLVX-GW-IRES-puro
900	pLVX-Flag-Polß(K72A)-IRES-Neo	Flag-Polß(K72A)	pLVX-GW-IRES-Neo
901	pLVX-Flag-Polß(D256A)-IRES-Neo	Flag-Polß(D256A)	pLVX-GW-IRES-Neo
937	pLVX-Flag-Polß(K206A)-IRES-Puro	Flag-Polß(K206A)	pLVX-GW-IRES-puro
938	pLVX-Flag-Polß(TM/K206A)-IRES-Puro	Flag-Polß(L301R/V303R/V306R/K206A)	pLVX-GW-IRES-puro
939	pLVX-Flag-Polß(TM/K244A)-IRES-Puro	Flag-Polß(L301R/V303R/V306R/K244A)	pLVX-GW-IRES-puro
941	pLVX-Flag-Polß(K244A)-IRES-Puro	Flag-Polß(K244A)	pLVX-GW-IRES-puro
947	pLVX-Flag-Polß(K206A/K244A)-IRES-Puro	Flag-Polß(K206A/K244A)	pLVX-GW-IRES-puro
949	pLVX-Flag-Polß(TM/K206A/K244A)-IRES-Puro	Flag-Polß(L301R/V303R/V306R/K206A/K244A)	pLVX-GW-IRES-puro

Supplementary Reference

1. Parsons, J.L. *et al.* Ubiquitin ligase ARF-BP1/Mule modulates base excision repair. *EMBO J* **28**, 3207-3215 (2009).