SUPPLEMENTARY DATA

Supplementary Methods:

Chemicals and reagents

Cisplatin, oxaliplatin and Myricetin were purchased from Sigma Aldrich. All other chemicals and reagents were purchased from standard suppliers.

Cell lines

Wild-type (92TAg) and Polβ null (88TAg) primary mouse embryonic fibroblasts were cultured in high glucose DMEM supplemented with 10% FBS plus antibiotics at 37ºC in a humidified atmosphere under 10% CO_2

Western blot analysis

Cells were harvested at 96,120,144 and168 hrs after the infection, washed with PBS and lysed in lysis buffer (10 mM Tris pH 8.0, 120 mM NaCl, 0.5% NP-40, 1mM EDTA) containing protease inhibitors (0.5 M phenyl methyl sulphonyl fluoride PMSF, 1mg/`ml Leupeptin and 1 mg/ml pepstatin A). The proteins were separated on 8% SDS-polyacrylamide gels and transferred onto Immobilon-P transfer membranes (Millipore). After blocking (2% non-fat dry milk), the membranes were probed with primary antibodies recognizing human MSH2, MSH3, MSH6, XPF and ERCC1 with α-tubulin as loading control. The membranes were incubated with appropriate secondary antibodies and the signal was detected by using Enhanced Chemiluminescence detection system. The quantification for the relative protein levels was done using imageJ software.

Real time PCR for the measurement of transcript levels

At indicated post-transfection time points, cells were harvested and pelleted. RNA was isolated using TRIzol reagent (Invitrogen) by standard procedures. The total RNA was reverse transcribed using MMLV reverse transcriptase enzyme (Invitrogen) as per the manufacturer's protocol. The transcript levels were quantified using iQ SYBR green

supermix (Bio-Rad) in iCycler iQ System, with β-actin or GAPDH as an endogenous control. The percent transcript knockdown was determined from $2^{\wedge-\Delta\Delta CT}$ values.

MTS assays

The CellTiter 96 Aq_{ueous} Non-Radioactive Cell Proliferation Assay (Promega) was used to evaluate cisplatin or oxaliplatin cytotoxicity. Cells were treated with increasing concentration of cisplatin for 2 hr. After 72 hr, 20 µL of combined MTS/PMS solution [(3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium and phenazine methosulfate] was added to each well and incubated for 2-4 hr at 37°C. The absorbance was measured at 490 nm in Spectramax M5 plate reader (Molecular Devices). The percentage of cell survival was calculated from the average OD of treated cells/average OD of control cells x 100.

Drug treatment

The cells were treated with Myricetin alone to assess its effect on cell viability (Data not shown). From these curves, a concentration of 10 µM (IC20) was chosen, so as to have minimum influence on cell viability with Myricetin alone. The cells were pretreated with this concentration for 2 hrs followed by treatment with increasing concentration of cisplatin for 2 more hrs. MTS assay was then performed as described.

Supplementary Figure Legends

Supplementary Figure S1. shRNA mediated downregulation of MSH3 in wildtype (A) and Polβ KD (B) MDA-MB-231 cells and MSH6 KD in wildtype (C) and Polβ KD (D) MDA-MB-231 cells . Cells were transfected with shRNA directed against MSH3 or MSH6 as described in Materials and Methods. Proteins were extracted at indicated time points and probed with MSH3 and MSH6 antibody with α-tubulin as loading control. Percent transcript knockdown in wildtype and Polβ KD MDA-MB-231 cells: MSH3 KD (E) (G) MSH6 KD (F) (H) respectively. Cells were transiently transfected with siRNA directed against MSH3. At 96 and 120 hr post transfection, total RNA was extracted from cells and the transcript levels were quantified as described in Materials and Methods with GAPDH as an endogenous control. The percent transcript knockdown was determined from $2^{\wedge-\Delta\Delta CT}$ values with non-targeting shRNA as controls.

Supplementary Figure S2. Western blot analysis for the expression of MSH2, MSH3 and MSH6 in MSH3 KD in WT and Polβ MDA-MB-231 cells (A)(D), MSH6 KD in WT and Polβ MDA-MB-231 cells (B)(E), MSH3 + MSH6 KD in WT and Polβ MDA-MB-231 cells (C)(F)

Supplementary Figure S3. DLD-1 and DLD-1 + chr 2 cells were tested for expression of MSH2, MSH3 and MSH6 using western blot analysis.

Supplementary Figure S4. (A) The colony survival assay was performed in WT and Polβ -/- MEF using an MSH3 specific siRNA. The cells were treated with increasing concentration of cisplatin to assess cytotoxicity. (B) (C) Percent transcript levels show the knockdown efficiency of MSH3 in WT and Polβ null MEFs respectively.

Supplementary Figure S5. (A) Oxaliplatin cytotoxicity was measured by using MTS assay for MSH6 KD in WT and pol beta deficient MDA-MB-231 cells, (B) MSH3 KD in WT and polβ deficient MDA-MB-231 cells AND (C) HCT116 MLH1 ATPase mutant cells. The cells were treated with increasing concentration of oxaliplatin for 2 hrs.

Supplementary Figure S6. MDA MB 231 and DLD-1 and DLD-1 + chr 2 Cells were transfected using siRNA directed against MLH1. The knockdown efficiency was checked at protein level using western blot analysis (A) (B) (C)

Supplementary Figure S7: MTS assay was used to assess cisplatin cytotoxicity after myricetin pretreatment. The cells were pretreated with myricetin for 2 hrs followed by treatment with increasing concentration of cisplatin.

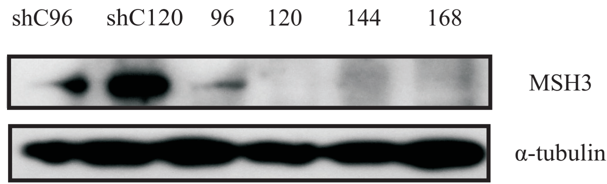
Supplementary Figure S8: (A) Expression of PMS2 in HCT116 cells. (B) Expression of XPF and ERCC1 was checked in HCT116 cells after cisplatin treatment. The cells were treated with cisplatin for 2 hrs and were harvested over a period of 0 – 72 hr time points.

The western blot analysis was performed as described previously. Quntification of the relative levels of XPF and ERCC1 protein levels was done using imageJ software (C-F).

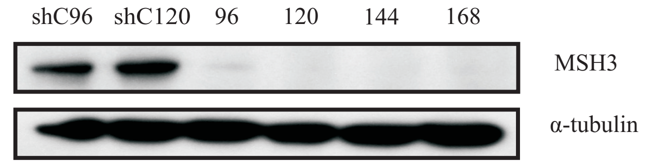
Supplementary Figure S9: MDA-MB-231 cells were transfected with shRNA directed against MSH3. Expression of XPF was tested using western blot analysis.

Supplementary Figure S1

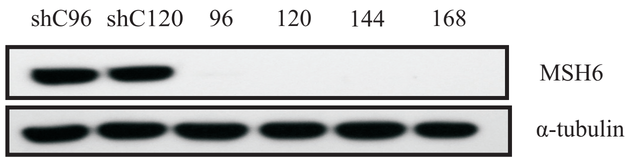
A MSH3 Knockdown in wt MDA-MB-231



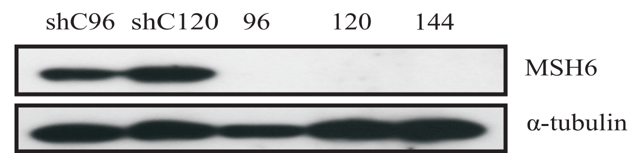
B MSH3 Knockdown in Polβ KD MDA-MB-231



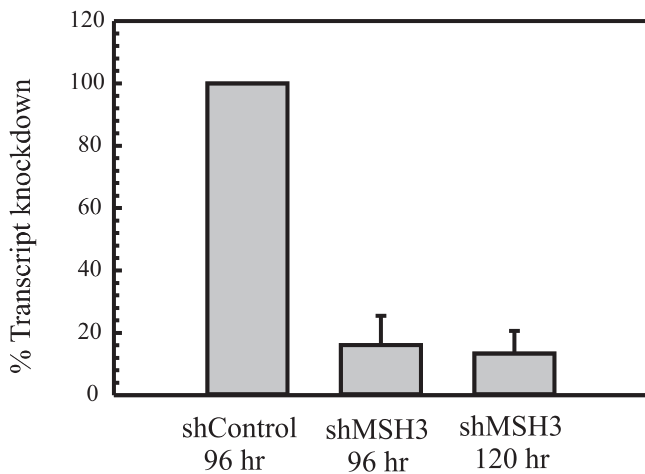
C MSH6 Knockdown in wt MDA-MB-231



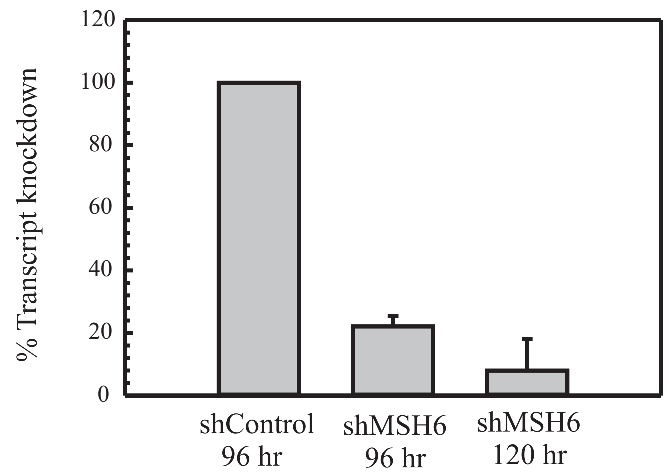
D MSH6 Knockdown in Polβ KD MDA-MB-231



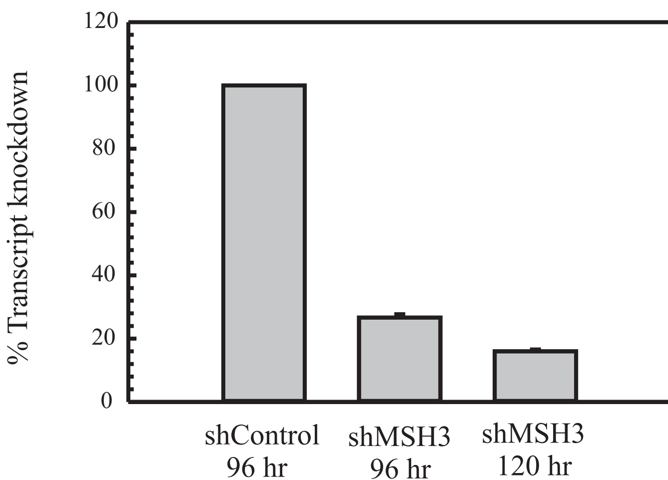
E MSH3 Transcript knockdown in WT MDA MB 231 cells



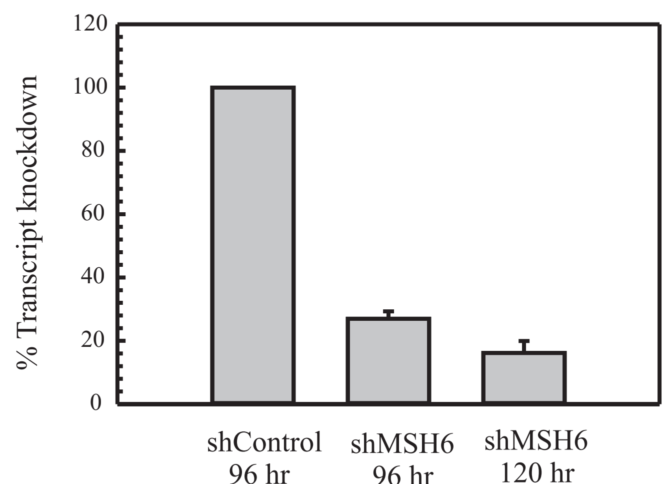
F MSH6 Transcript knockdown in WT MDA MB 231 cells



G MSH3 Transcript knockdown in Polβ MDA MB 231 cells

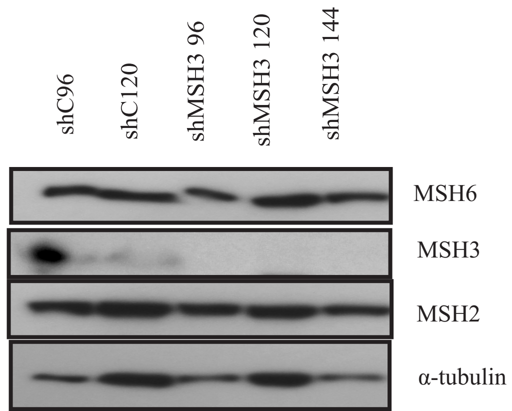


H MSH6 Transcript knockdown in Polβ MDA MB 231 cells

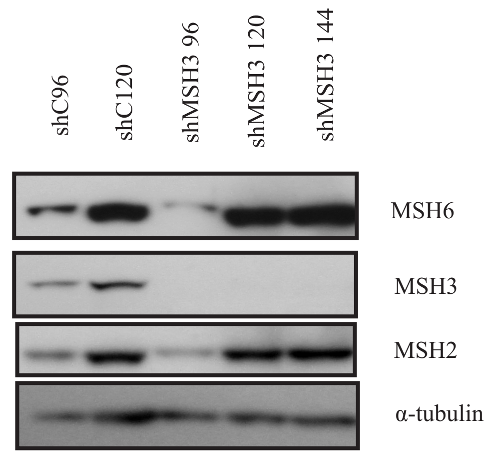


Supplementary Figure S2

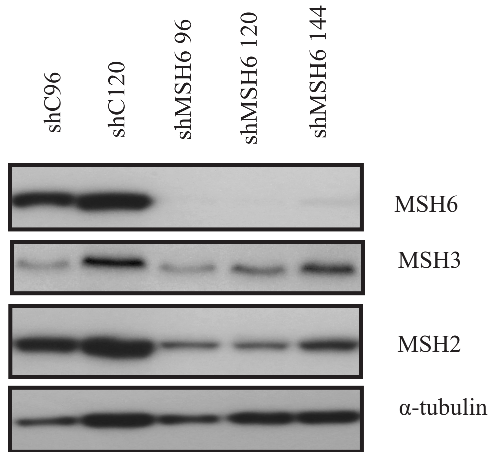
A MSH3 Knockdown in wt MDA-MB-231



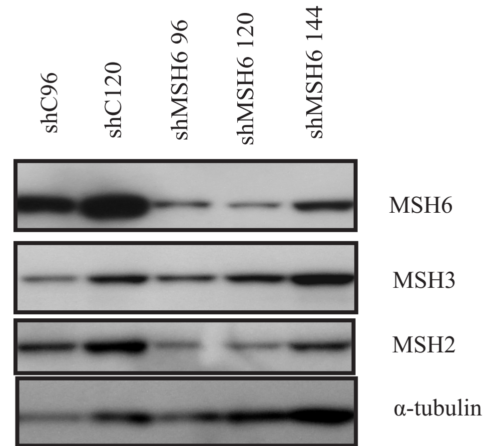
D MSH3 Knockdown in Polβ KD MDA-MB-231



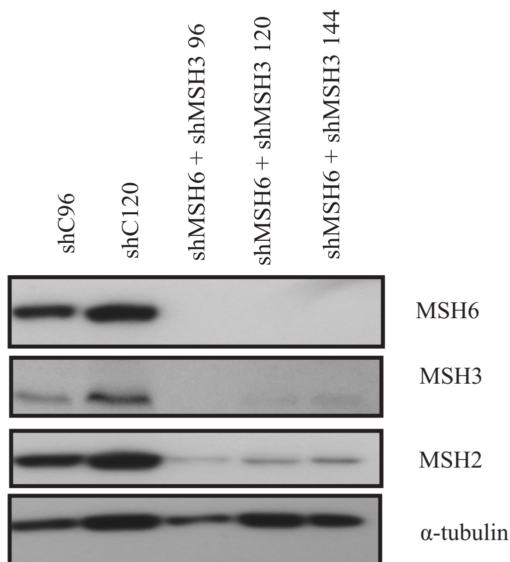
B MSH6 Knockdown in wt MDA-MB-231



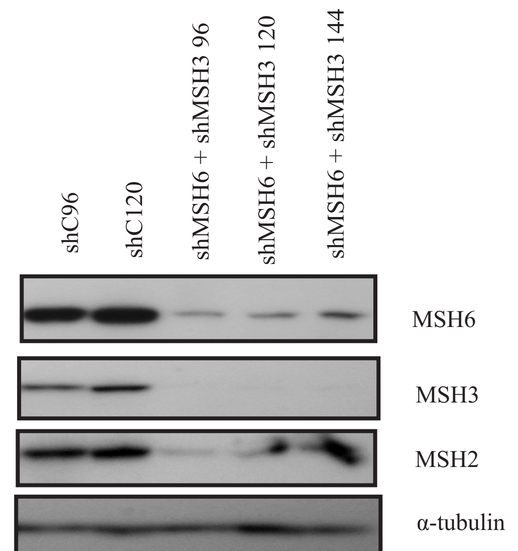
E MSH6 Knockdown in Polβ KD MDA-MB-231



C MSH3+MSH6 Knockdown in wt MDA-MB-231

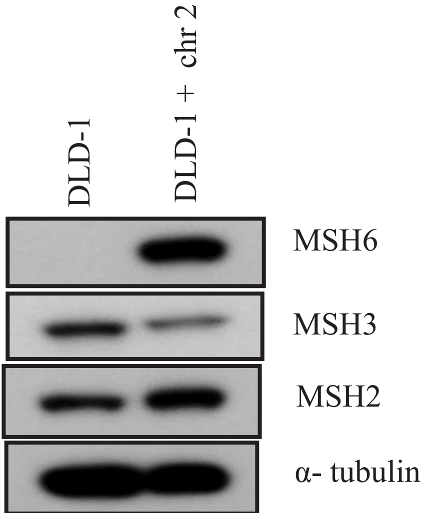


F MSH3 + MSH6 Knockdown in Polβ KD MDA-MB-231



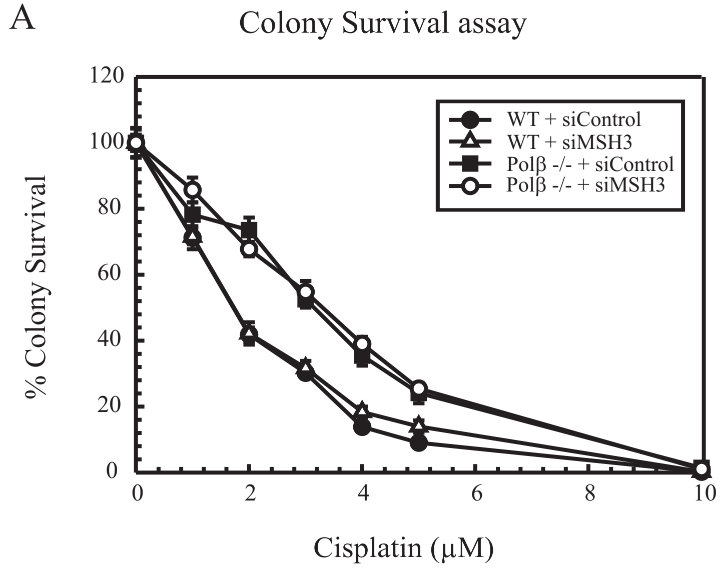
Supplementary Figure S3

Expression of MMR proteins in DLD-1 cells



Supplementary Figure S4

A



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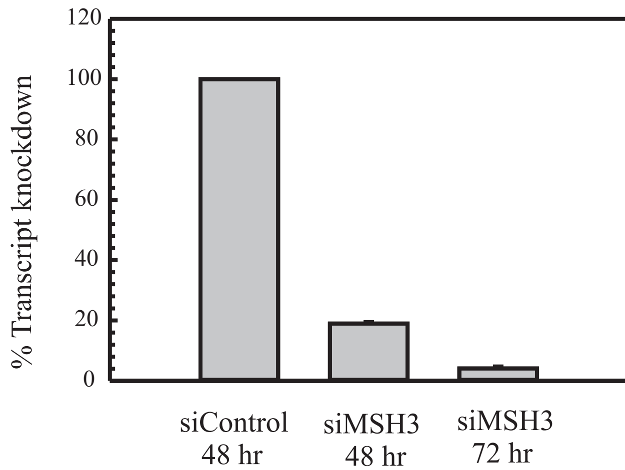

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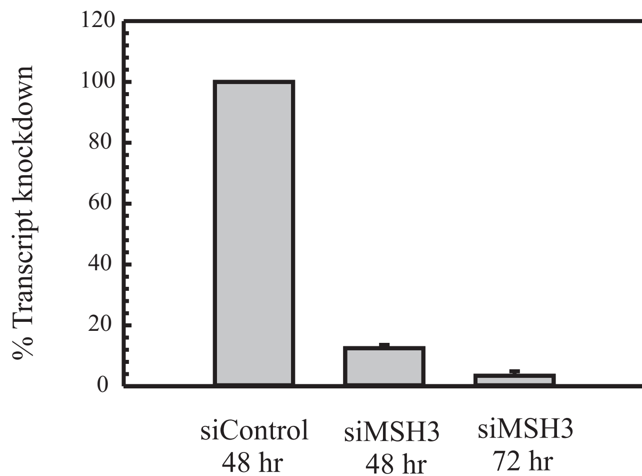
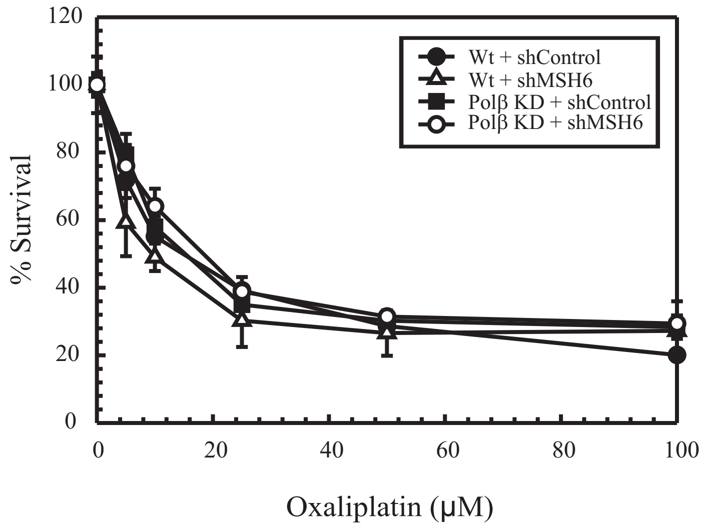
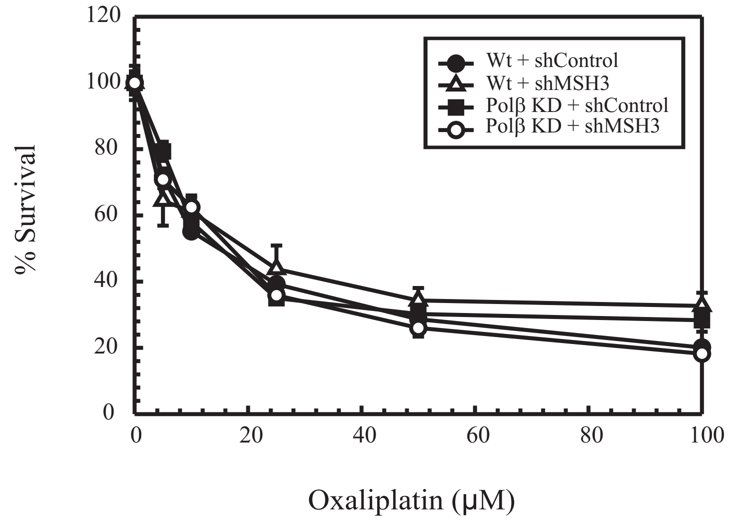
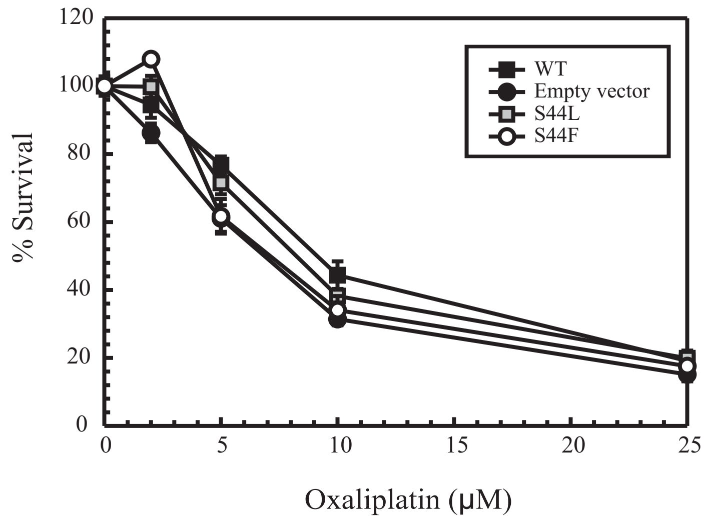


Supplementary Figure S5

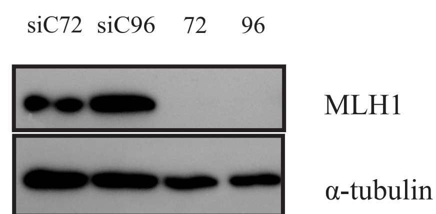




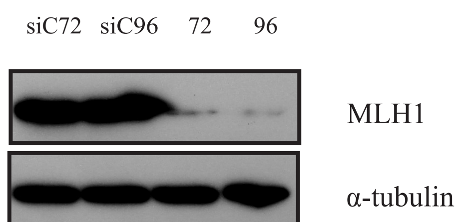


Supplementary Figure S6

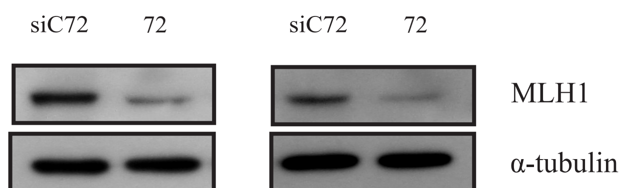
A MLH1 Knockdown in wt MDA-MB-231



B MLH1 Knockdown in Polβ KD MDA-MB-231

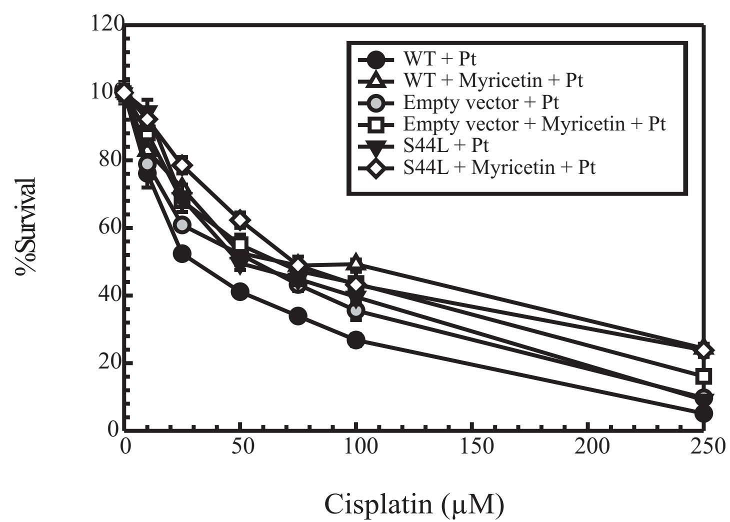


C MLH1 Knockdown in DLD-1 and DLD-1 + chr 2 cells



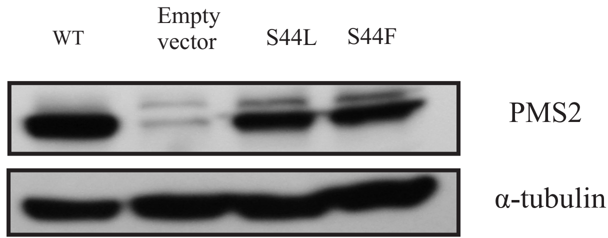
Supplementary Figure S7

A Myricetin pretreatment in HCT116 cells

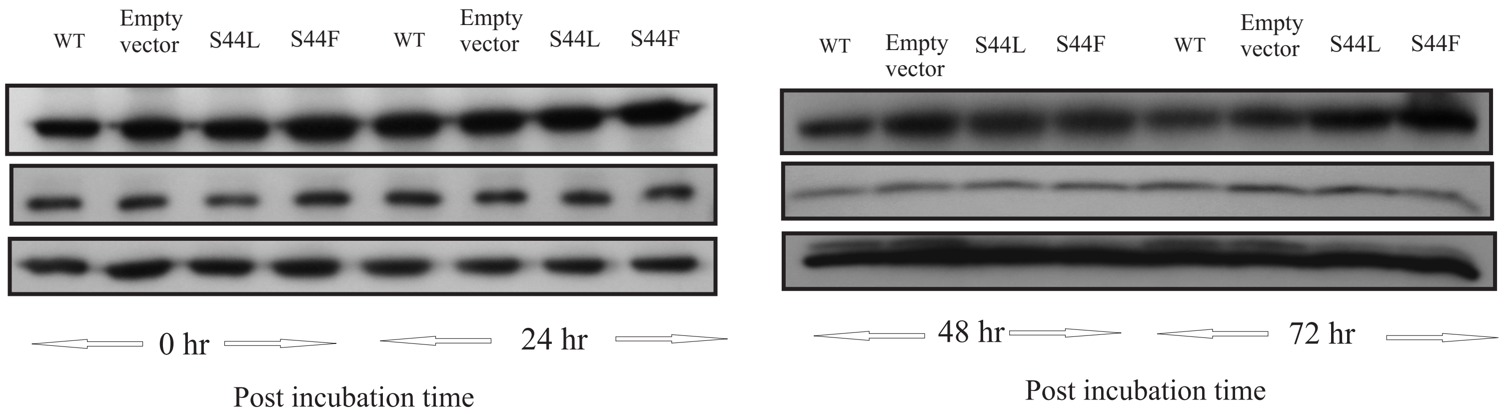


Supplementary Figure S8

A PMS2 expression in HCT116 cells



B XPF-ERCC1 expression in HCT116 cells after cisplatin treatment



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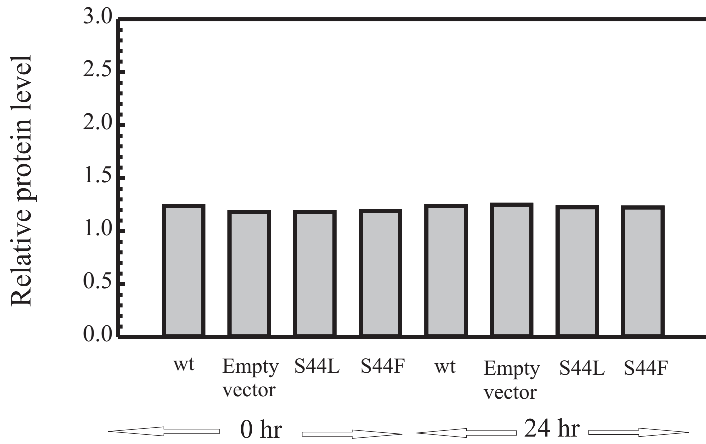

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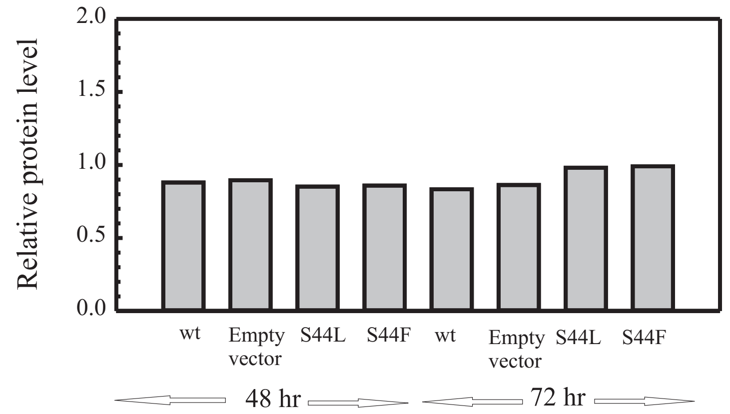

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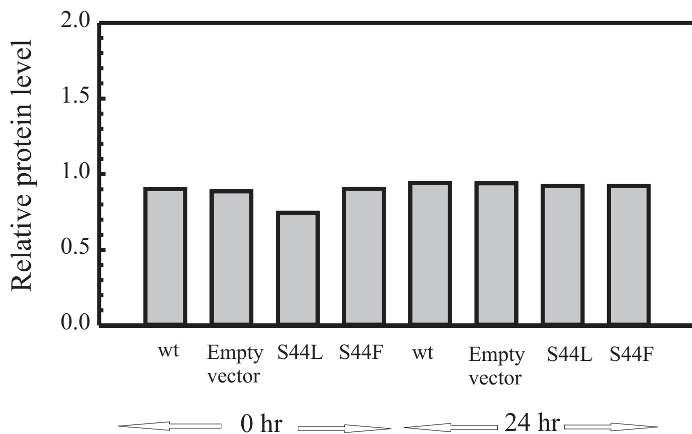

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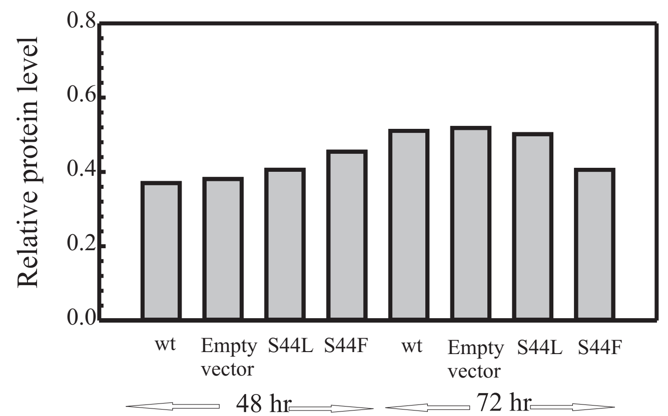

Supplementary Figure S9