Supplemental Material

Supplemental Data include Supplemental Figures S1-S6, Supplemental Table S1, the corresponding figure legends and Supplemental Materials & Methods.

Supplemental Materials and Methods

Quantitative RT-PCR Analysis

Expression of PARP1, PARP2, MLH1, MSH2, and MSH6 mRNA after shRNA knockdown of the gene was measured by quantitative RT-PCR using an Applied Biosystems StepOnePlus system as described previously (21). Applied Biosystems TaqMan® Gene Expression Assays used are as follows: human PARP1: Hs00811369-G1; human PARP2: Hs00193931-M1; human MLH1: Hs00179866-M1; human MSH2: Hs00953523, and human MSH6: Hs00264721. Each are normalized to the expression of human ß-actin (part #4333762T).

Cell extract preparation and immunoblot

Nuclear extracts were prepared and protein concentration was determined as previously described (15). Immunoblots for PAR formation utilized whole cell extracts prepared for the quantitative PAR ELISA. 30 microgram of protein was loaded on a pre-cast 4-20% Tris-Glycine gel for all immunoblots (InVitrogen).

The following primary antibodies were used in immunoblot assays: anti-human MPG (mAb; clone 506-3D) (15); anti-Pol ß (mAb clone 61; Thermo Fisher Scientific); anti-APE1 (EMD Biosciences); anti-PCNA (Santa Cruz); anti-poly(ADP-ribse) (PAR) (mAb Clone 10H, kindly provided by M. Ziegler); anti-poly(ADP-ribose) polymerase-1 (PARP1) (BD

Pharmingen); anti-XRCC1 (Bethyl laboratories), anti-MGMT (Novus biologicals) and anti-NAMPT (Bethyl laboratories).

Figure Legends

Supplemental Figure S1. Protein expression of BER proteins in LN428 and LN428/MPG cells.

Immunoblot analysis of BER proteins and NAMPT in Lane 1) LN428 cells and Lane 2) LN428/MPG cells. The proteins are identified on the left.

Supplemental Figure S2. Characterization of PARP1 and PARP2 knockdown cell lines.

A, RT-PCR analysis of LN428/MPG/PARP1-KD and LN428/MPG/PARP2-KD isogenic cell lines. Results reported as % mRNA remaining compared to LN428/MPG control.

B, ATP levels after 0.5 mM MMS (striped bars) or 1.5 mM MMS (black bars) in LN428/MPG, LN428/MPG/PARP1-KD and LN428/MPG/PARP2-KD cell lines.

C, Immunoblot analysis of PAR generation after 0, 15 and 30 minutes of MMS exposure at a dose of 0.5 mM in LN428/MPG, LN428/MPG/PARP1-KD and LN428/MPG/PARP2-KD cell lines. All samples were the same as utilized in the quantitative PAR ELISA. The blot shown is a representative blot (N=3).

Supplemental Figure S3. Prolonged exposure to FK866 is cytotoxic in a BER independent manner.

Cell survival assayed by MTS assay after prolonged exposure to FK866 in the LN428 (open circles) and LN428/MPG (closed circles) cell lines. Cells were treated with 10nM FK866 for 72 hours and cell survival was assayed after 48 hours of recovery.

Supplemental Figure S4. Characterization of TMZ resistant cell lines.

A, Cell survival determined by a long-term survival assay after increasing doses of TMZ in the LN428/MPG cell line (open circles) compared to the LN428/MPG/MGMT cell line (closed circles).

B, qRT-PCR mRNA analysis of LN428/MPG/MLH1-KD, LN428/MPG/MSH2-KD and LN428/MPG/MSH6-KD isogenic cell lines. Results reported as % mRNA remaining compared to the LN428/MPG control.

Supplemental Figure S5. The 3-drug combination of FK866, MX and TMZ enhances cell killing in TMZ resistant glioblastoma model cell lines.

A, Cell survival determined by a long-term survival assay in cells modified to over-express MGMT. LN428/MPG/MGMT cells were treated with either TMZ alone for 12 days, 24 hours of 10nM FK866 followed by 12 days of TMZ, a 30 minute pre-treatment of 10mM MX followed by MX (5mM) and TMZ co-treatment for 12 days, or the three-drug combination (a 24 hour pre-treatment of FK866, followed by a 30 minute pre-treatment with MX and a 12 day co-treatment with MX and TMZ. TMZ was used at a range of doses 0-75μm.

B-D, Cell survival determined by a long-term survival assay in cells stably expressing shRNA to MLH1 (B), MSH2 (C) or MSH6 (D). LN428/MPG MMR knockdown cells were treated with either TMZ alone for 12 days, 24 hours of 10nM FK866 followed by 12 days of TMZ, a 30 minute pre-treatment of 10mM MX followed by MX (5mM) and TMZ co-treatment for 12 days, or the three-drug combination (a 24 hour pre-treatment of FK866, followed by a 30 minute pre-treatment with MX and a 12 day co-treatment with MX and TMZ. TMZ was used at a range of doses 0-75μm.

Supplemental Figure S6. Characterization of NAD^+ content and FK866 cytotoxicty in T98G cells.

A, Basal NAD^+ content of the T98G cell line compared to basal NAD^+ content of the LN428/MPG cell line.

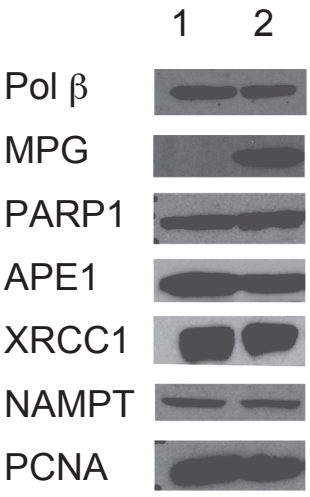
B, Cell survival determined by a long-term survival assay in T98G cells after 24 hours of varying doses of FK866.

Supplemental Table 1.

SER_{50} calculations for the LN428/MPG/MGMT cell line. Sensitization enhancement in long-term survival assay

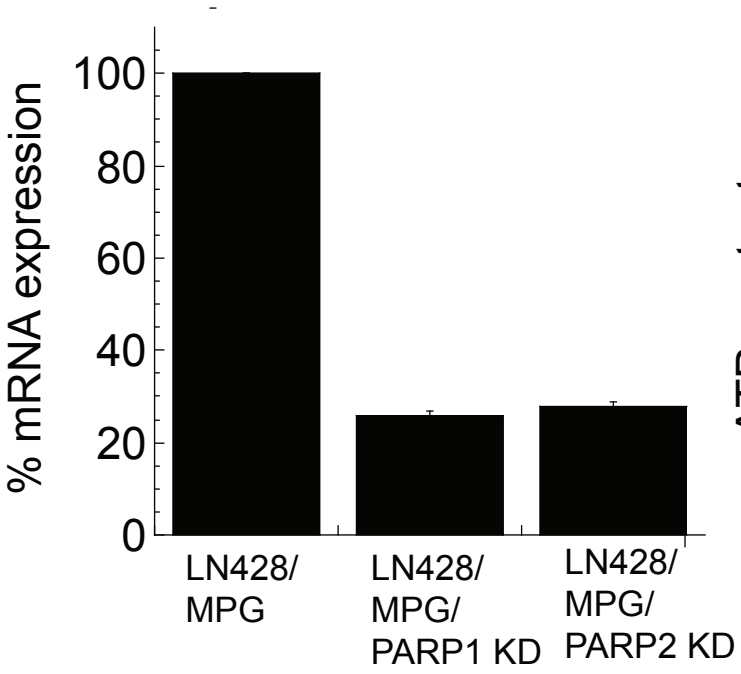
Treatment	**IC_{50} (μM)**	**SER_{50}**
TMZ	270	1
FK866 + TMZ	175	1.54
MX + TMZ	160	1.68
3-Drug combination	17	15.88

Goellner et al - Supplemental Figure S1



Goellner et al - Supplemental Figure S2

A.



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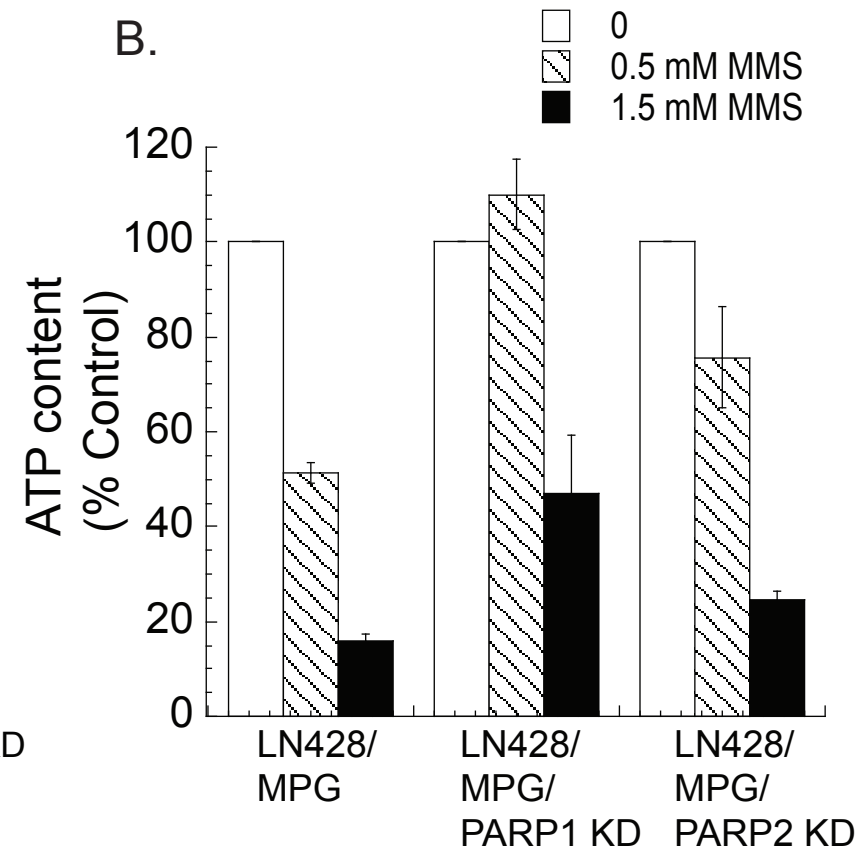

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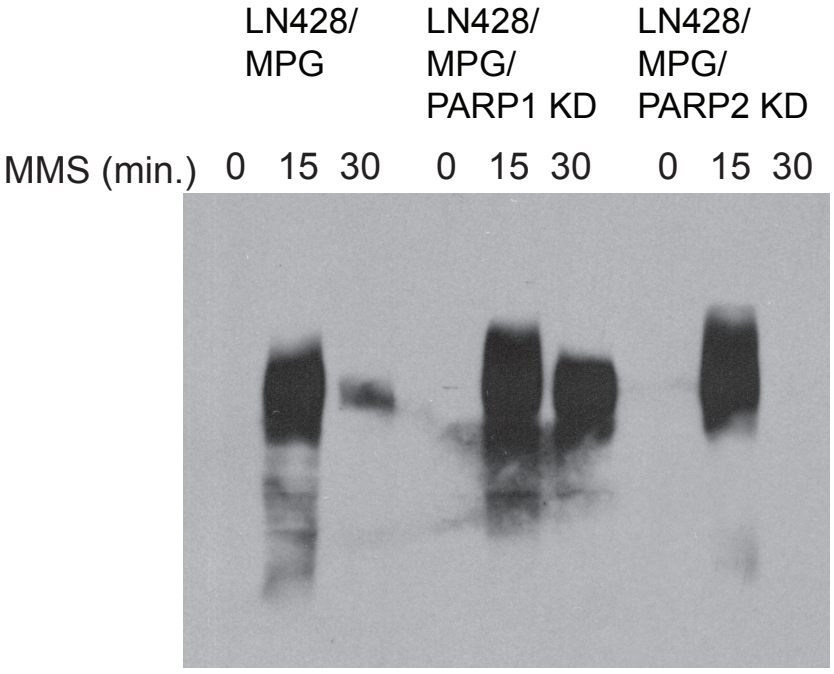
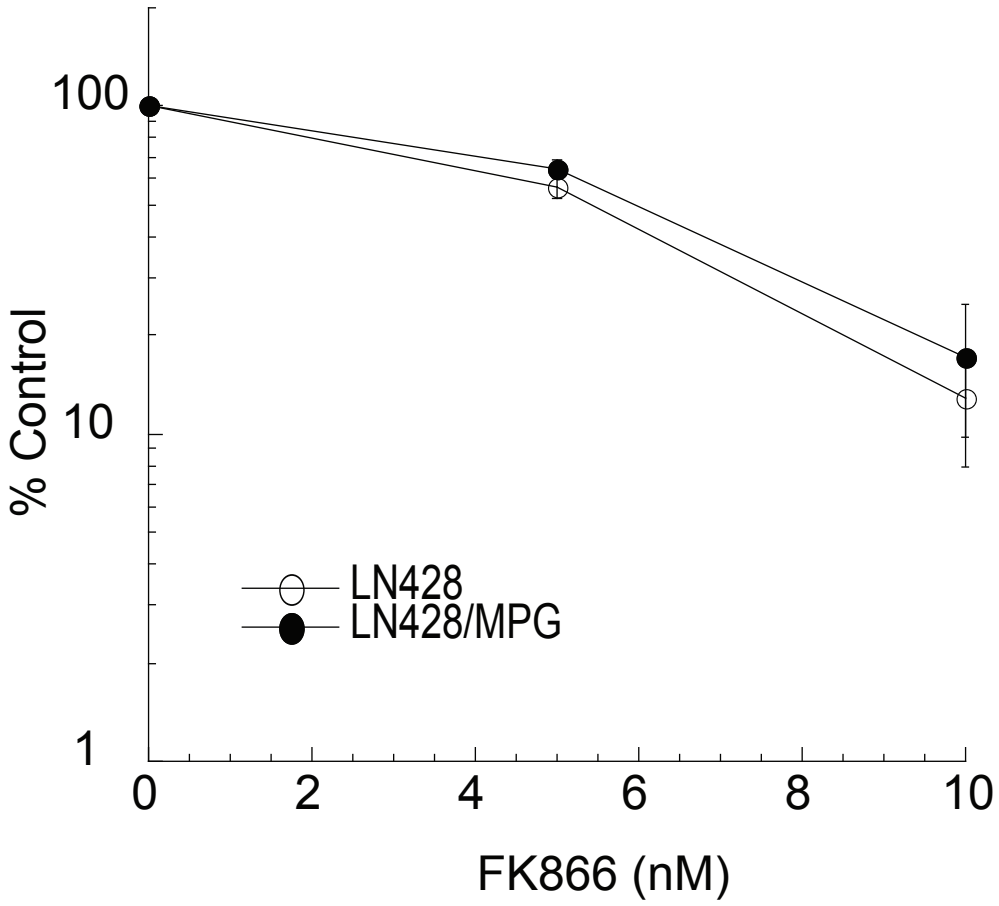
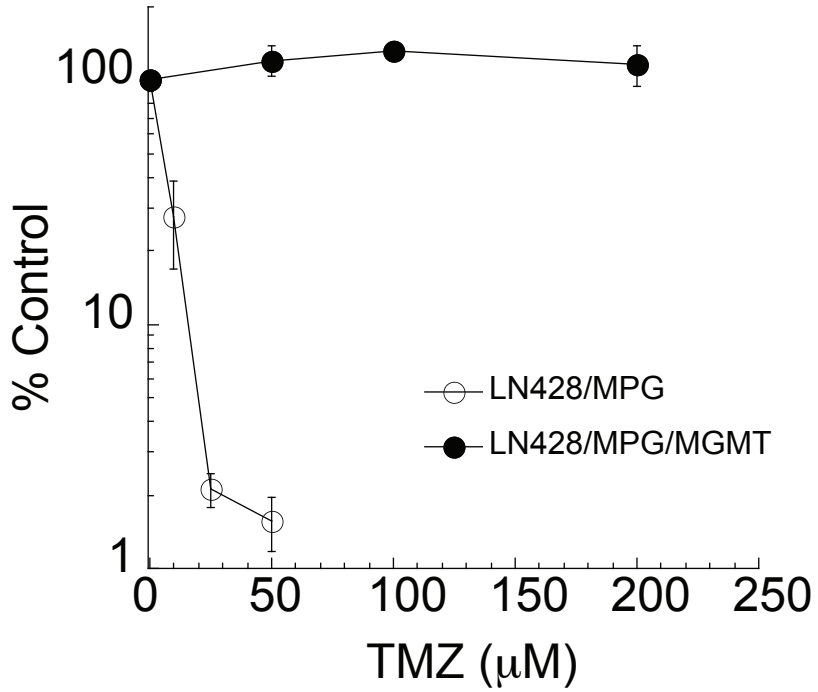


Goellner et al - Supplemental Figure S3



Goellner et al - Supplemental Figure S4

A.



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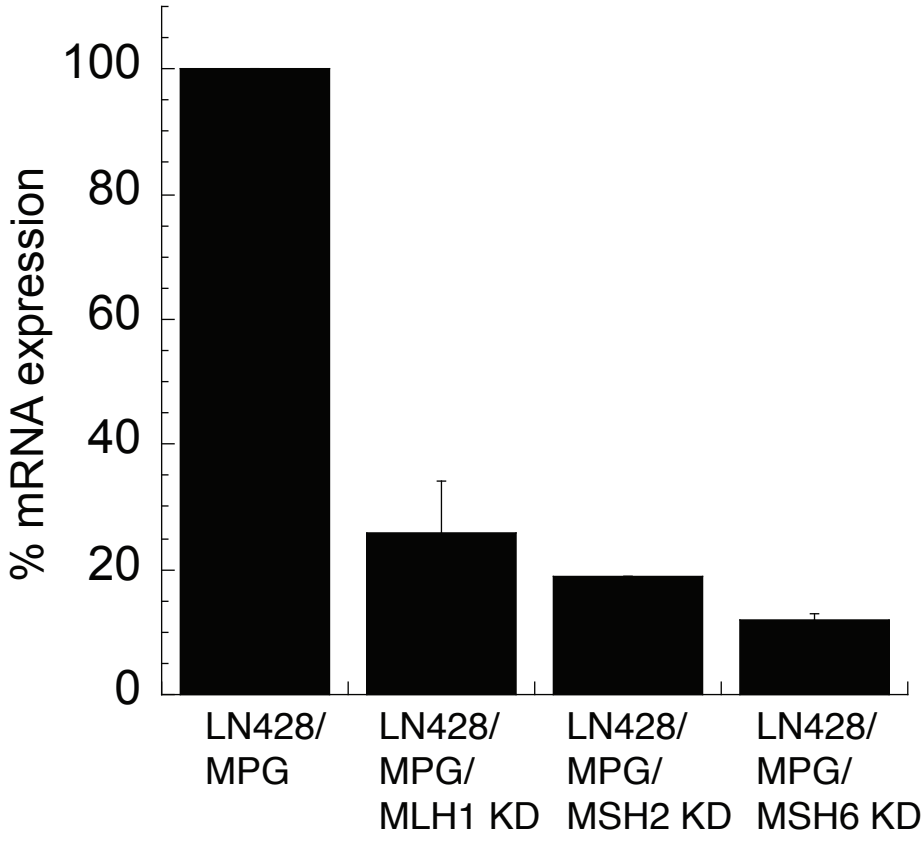
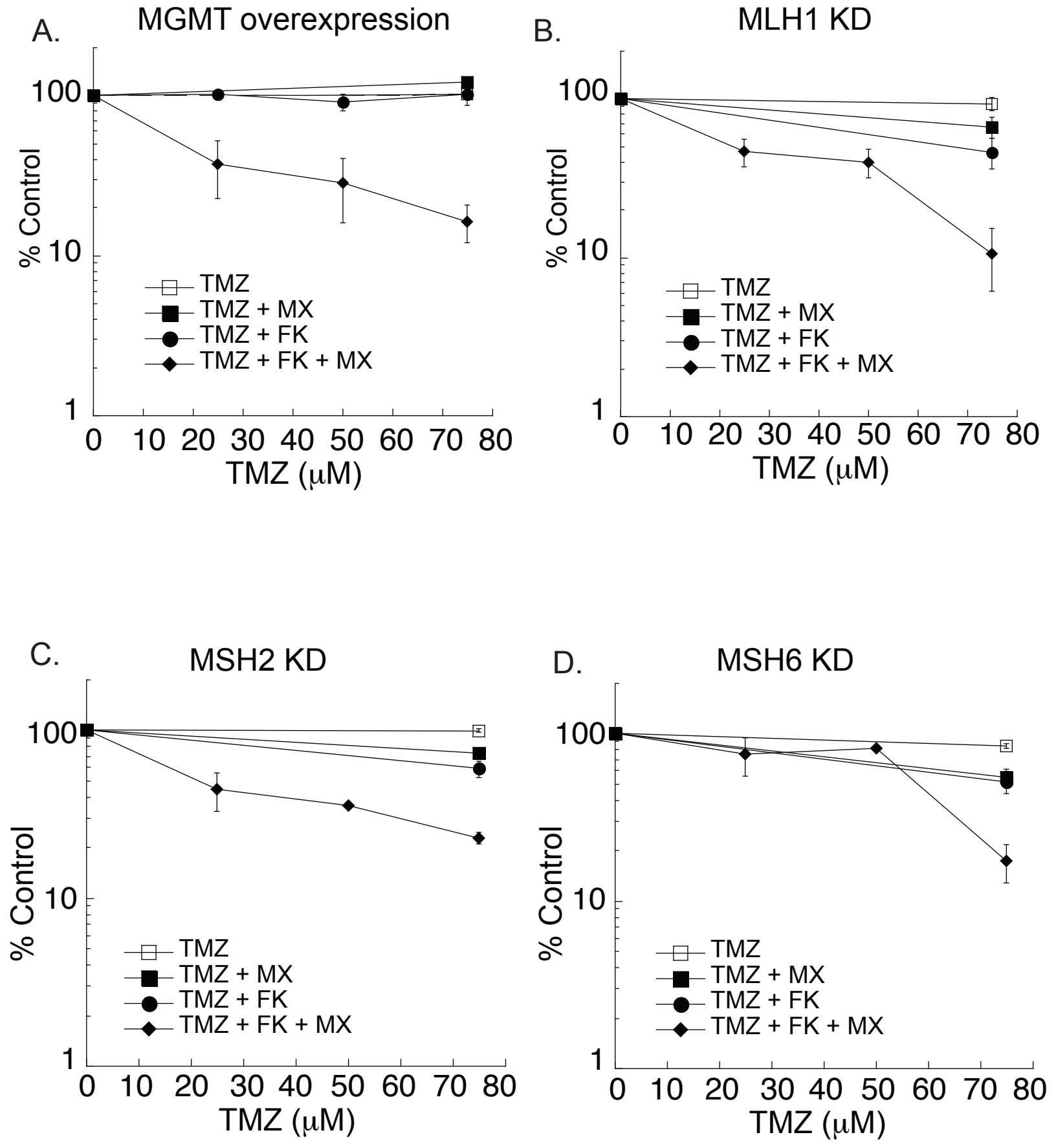


Goellner et al - Supplemental Figure S5



Goellner et al - Supplemental Figure S6