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Molecular Pharmacology

Supplemental Information

CRISPR/Cas9 Genome Editing of the Human Topoisomerase IIα Intron-19 5′ Splice Site Circumvents Etoposide Resistance in Human Leukemia K562 Cells

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Supplemental Table 1: Sequences of primers, single strand oligonucleotides, and qPCR hybridization probes utilized in this study

Primer/Oligonucleotide Name	Primer Orientation	Primer/Oligonucleotide	Annealing Region/ Function
Infusion Cloning TOP2α E19 For	Sense	5′-CCCAAGCTGGCTAGCGTCAG AGAAAGGTTTTGTTTACT-3′	Top2α E19
Infusion Cloning TOP2α E20 Rev	Antisense	5′-CCCTCTAGACTCGAGCTGA GCATTGTAAAGATGTATCG-3′	Top2α E20
TOP2α E19/I19 Consensus 5′ SS Mutation	Sense	5'-CAGGTAAGTACACAATCCATGTTTCC-3′	TOP2α minigene E19/I19 5′ SS
TOP2α E19 Inverse PCR Rev	Antisense	5'-ACCATGATGATAAGAAGACATTTCAGC-3′	TOP2α minigene E19
T7 For	Sense	5'-CGAAATTAATACGACTACTATAGG-3′	TOP2α I19
Minigene TOP2α I19 Rev	Antisense	5'-CAGACTTATGAATATCCCTGCAGG-3′	TOP2α I19
Minigene TOP2α Ex 20 Rev	Antisense	5'-GCAAGAGGTTTAGATTATTGCTACC-3′	TOP2α E20
GCD HPRT1 For	Sense	5'-AGAGGAGGGCCTTACTAATTAC-3′	HPRT1 I2
GCD HPRT1 Rev	Antisense	5'-CATGCATAGCCAGTGCTTGAG-3'	HPRT1 I2
GCD TOP2α E18 For	Sense	5'-GATCTATCCCTTCTATGGTGG-3	TOP2α E18
GCD TOP2α I19 Rev	Antisense	5'-CAGAAATCAAAGGGCAAGCAG-3'	TOP2α I19
HPRT1 sgRNA	Sense	5'-GCAUUUCUCAGUCCUAAACA-3' + Scaffold	gRNA/Cas9 mediated DSB in HPRT1 I2
TOP2α crRNA #1 (gRNA-1)	Sense	5'-GTCTTCTTATCATCATGGTG-3' + Scaffold	gRNA/Cas9 mediated DSB in TOP2α E19
TOP2α crRNA #2 (gRNA-2)	Sense	5'-GAAATGTCTTCTTATCATCA-3' + Scaffold	gRNA/Cas9 mediated DSB in TOP2α E19
TOP2α crRNA #3 (gRNA-3)	Antisense	5'-TATAATGCTTTCTGGAAACA-3' + Scaffold	gRNA/Cas9 mediated DSB in TOP2α I19
Enhanced E19/I19 5′ SS/No PAM-2	Antisense	5'-GAGCTTATACTTTCACCAAATCTGTTTTGAGA ATGACTCTGCAGGGATTTCTGATATAATGCTTT CTGGAAACATGGATTGTGTACTTACCTCAGCA TGATGATAGAAGACATTTCAGCCACTGATCCA GCTAATTGGGCAACCTTTACTTCTCGCTTGTCA TTCCGTTTGAAGCAAGT-3'	HDR repair template of the TOP2α E19/I19 boundary
Custom Wild type TOP2α E19/I19 boundary Taqman qPCR probe	Sense	5'-TCATGGTGAGGTAAACACACAATCC-3'	Wild type TOP2α E19/I19 boundary
Wild type TOP2α E19/E20 boundary Taqman qPCR probe (Assay ID Hs01032135_m1)	Sense	5'-TCATGGTGAGATGTCACTAATGATG-3'	Wildtype TOP2α E19/E20 boundary
Custom TOP2α E19/I19 5′ SS edited (-PAM and optimized 5′ SS) qPCR Taqman probe	Sense	5'-TCATGCTGAGGTAAGTACACAATCC-3'	CRISPR edited TOP2α E19/I19 boundary
Custom TOP2α E19/E20 edited boundary qPCR Taqman probe	Sense	5'-TCATGCTGAGATGTCACTAATGATG-3'	CRISPR edited TOP2α E19/E20 boundary

Supplemental Table 2: Cell line growth characteristics and etoposide-induced growth inhibition

Cell Line	Doubling Time (hours)[a]	Etoposide IC_{50} (μM)[b]	Relative Resistance[d]
K562	18.7 ± 0.5 (7)[c]	0.19 ± 0.04 (8)[c]	---
K/VP.5	19.9 ± 0.7 (7)	2.65 ± 0.48 (16)	13.9
K/VP.5/edit-1	20.1 ± 0.8 (7)	1.60 ± 0.24 (7)	8.4
K/VP.5/edit-2	20.0 ± 0.8 (7)	0.59 ± 0.09 (6)	3.1
K/VP.5/edit-3	19.9 ± 0.9 (7)	0.08 ± 0.01 (14)	0.4

[a]Calculated from log-linear regression plots over 3-4 days of growth

[b]Fifty percent inhibitory concentration (IC_{50}) in a 48 hour growth inhibition assay.

[c]Mean ± S.D.; numbers in parentheses, number of independent experiments performed on different days.

[d]IC_{50} of K/VP.5 cells and gene-edited clones divided by that of the parental K562 cell line.

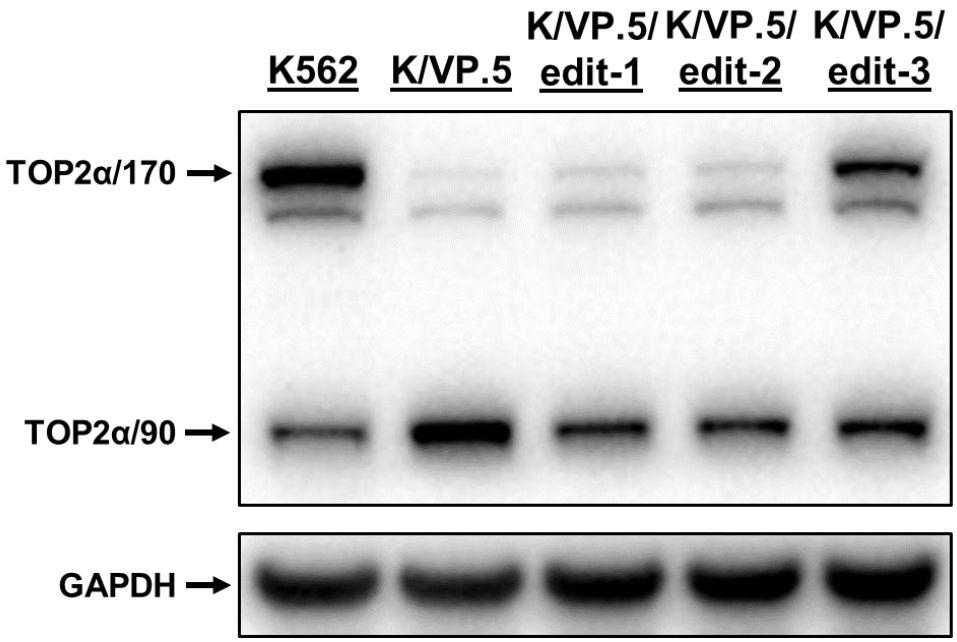
Supplemental Figure Legends

Supplemental Fig. 1. TOP2α/170 and TOP2α/90 protein levels in parental K562 cells, drug resistant K/VP.5 cells, and gene-edited clonal cell lines K/VP.5/edit-1, K/VP.5/edit-2, and K/VP.5/edit-3.

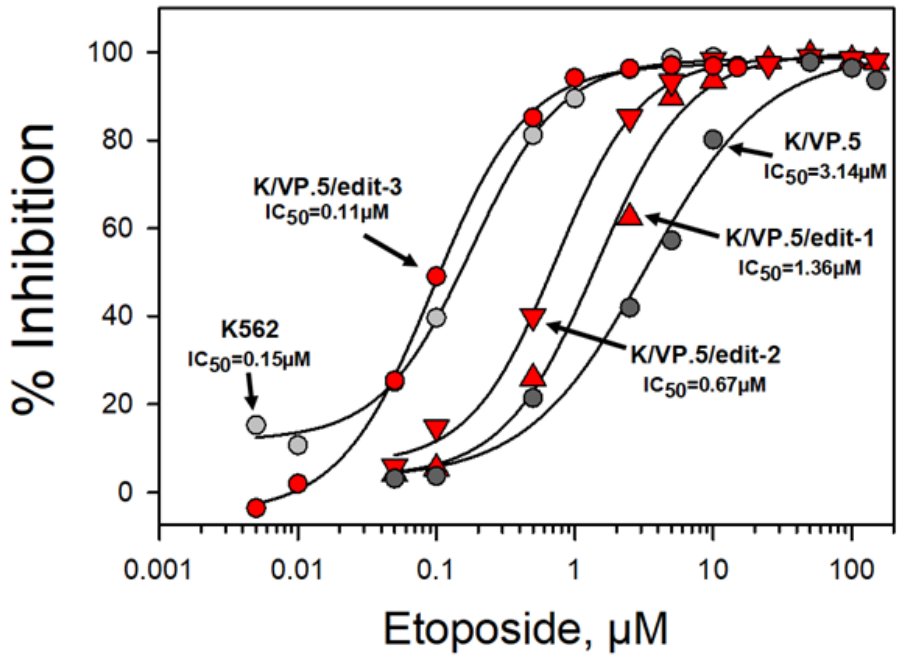
Supplemental Fig. 2. Growth inhibitory effects of etoposide in K562, K/VP.5 and gene-edited clonal cell lines. Log-phase cells were incubated for 48 hours with various concentrations of etoposide after which cells were counted on an electronic particle counter (model Z1 Coulter counter). The extent of growth beyond the starting concentration in drug-treated versus DMSO controls was expressed ultimately as percent inhibition. Shown are representative concentration-response (inhibitory) curves for each cell line tested with 50% inhibitory concentrations (IC-50-values) indicated. Compilation of replicate experiments performed on different days is shown in Supplemental Table 2.

Supplemental Fig. 3. Scatter plot of gene expression between gene-edited K/VP.5/edit-3 and K/VP.5 cells for 625 essential genes (denoted in blue) (Wang et al., 2019a) and the top 20 putative Cas9/g-RNA-2 off-target genes predicted by the CCTop algorithm (https://cctop.cos.uni-heidelberg.de/) (Stemmer et al., 2015) and expressed in K/VP.5/edit-3 and K/VP.5 cells. Dotted lines denote 2 fold-change in gene expression.

Supplemental Fig. 1



Supplemental Fig. 2



Supplemental Fig. 3