Supplemental Material

Implications of Inhibition of Rev1 Interaction with Y-family DNA Polymerases for Cisplatin Chemotherapy

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Table S1

Figures S1 to S7 and their legends

All the data and information in the Supplemental Material directly relates to the main manuscript.

Table S1. Effects of siRNA knockdown of TLS Pols on mutation frequencies and nucleotides inserted opposite a Pt-GG intrastrand crosslink carried on lagging strand DNA template in NER defective XPA HFs or in Polη defective XPV HFs

Cells	siRNA	# of *Kan*$^+$ blue colonies sequenced	Nucleotide inserted					Mutation frequency %
			A	G	C	T	Other[b]	
XPA HFs	NC	282 (6)	5 (3' G)[a]	-	276	-	1	2.1
	Polη	156 (6)	5 (3' G)	-	150	-	1	3.8
	Polι	72 (0)	-	-	72	-	-	0
	Polθ	64 (0)	-	-	64	-	-	0
	Polι + Polθ	64 (0)	-	-	64	-	-	0
XPV HFs	NC	96 (4)	4 (3' G)	-	92	-	-	4.2
	Polι	60 (0)	-	-	60	-	-	0
	Polθ	48 (0)	-	-	48	-	-	0
	Polι + Polθ	64 (0)	-	-	64	-	-	0

a. Insertion of an A occurred opposite the 3'G of the crosslink

b. Mutations at the flanking 3'-C of GG-cisplatin. 5'-TCGGCT-3' to 5'-TCGGGT-3'

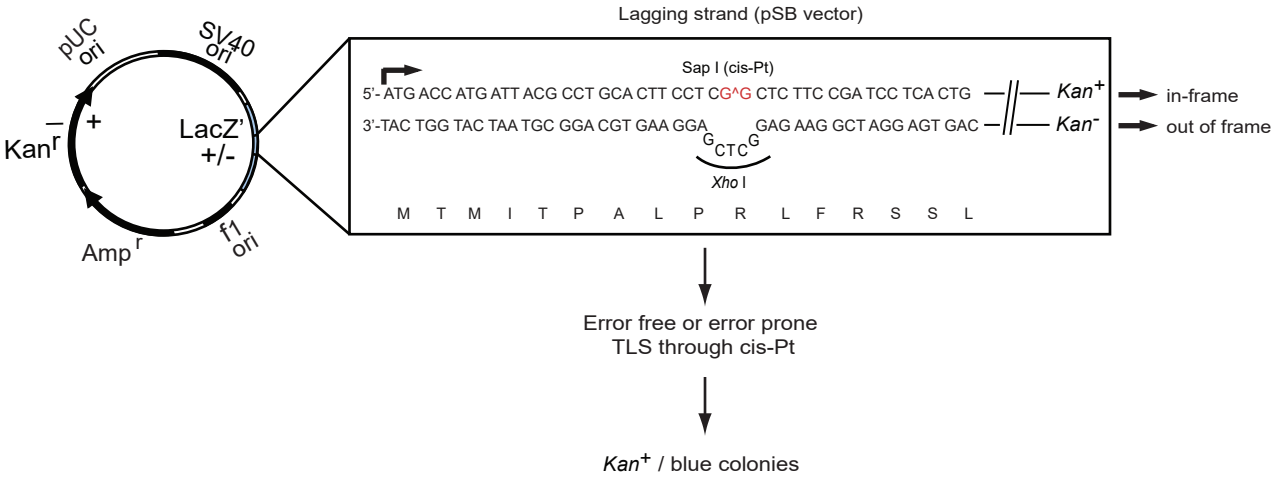

Figure S1. Assay for TLS through Pt-GG intrastrand crosslink. The lacZ sequence in the lagging strand template in the pSB vector containing the G^G crosslink is in-frame and it carries the *Kan*$^+$ gene. TLS through the crosslink generates Kan$^+$ blue colonies. The other DNA strand is out of frame and replication of this DNA strand generates white colonies.

Supplemental_Fig_S2.pdf

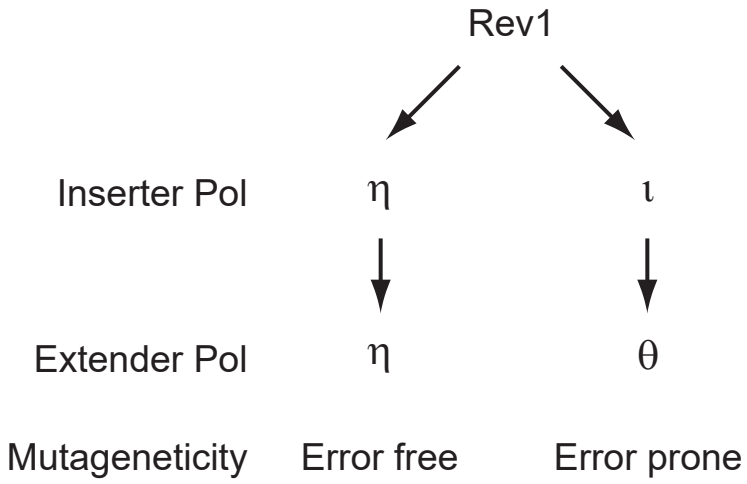

Figure S2. TLS pathways for replication through cisplatin induced intrastrand crosslinks. As determined from TLS analyses, cisplatin induced mutational analyses, and from analyses of RF progression through cisplatin induced DNA adducts, replication through cisplatin induced (GG and AG) intrastrand crosslinks occurs via a Polη dependent error free pathway and by Polι/Polθ dependent error prone pathway. As an essential scaffolding component of Polη and Polι, Rev1 is required for TLS by both the pathways.

Supplental_Fig_S3.pdf

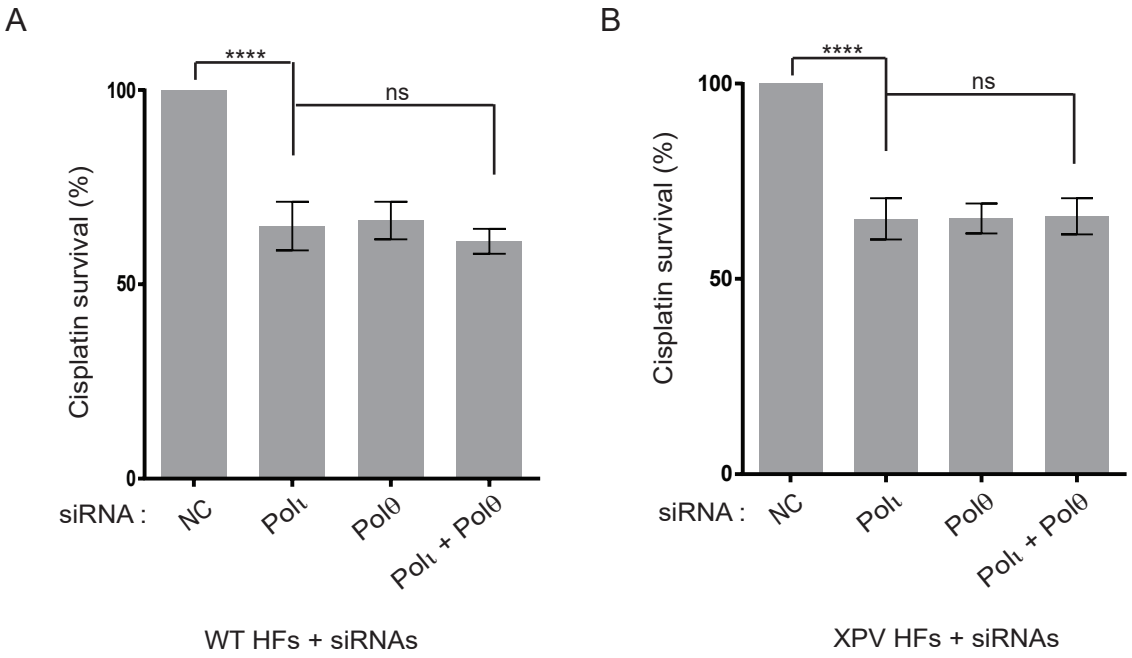

Figure S3. Epistatic effects of Polι and Polθ on survival in cisplatin treated human cells. **A.** Effects of Polι or Polθ depletion or of their co-depletion on cisplatin survival in WT HFs **B.** Effects of Polι or Polθ depletion or of their co-depletion on cisplatin survival in XPV HFs. In **A** and **B,** error bars indicate the standard deviation from four independent experiments. Student's two-tailed t-test p values, ns, not significant; ****, $p<0.0001$.

Supplemental_Fig_S4.pdf

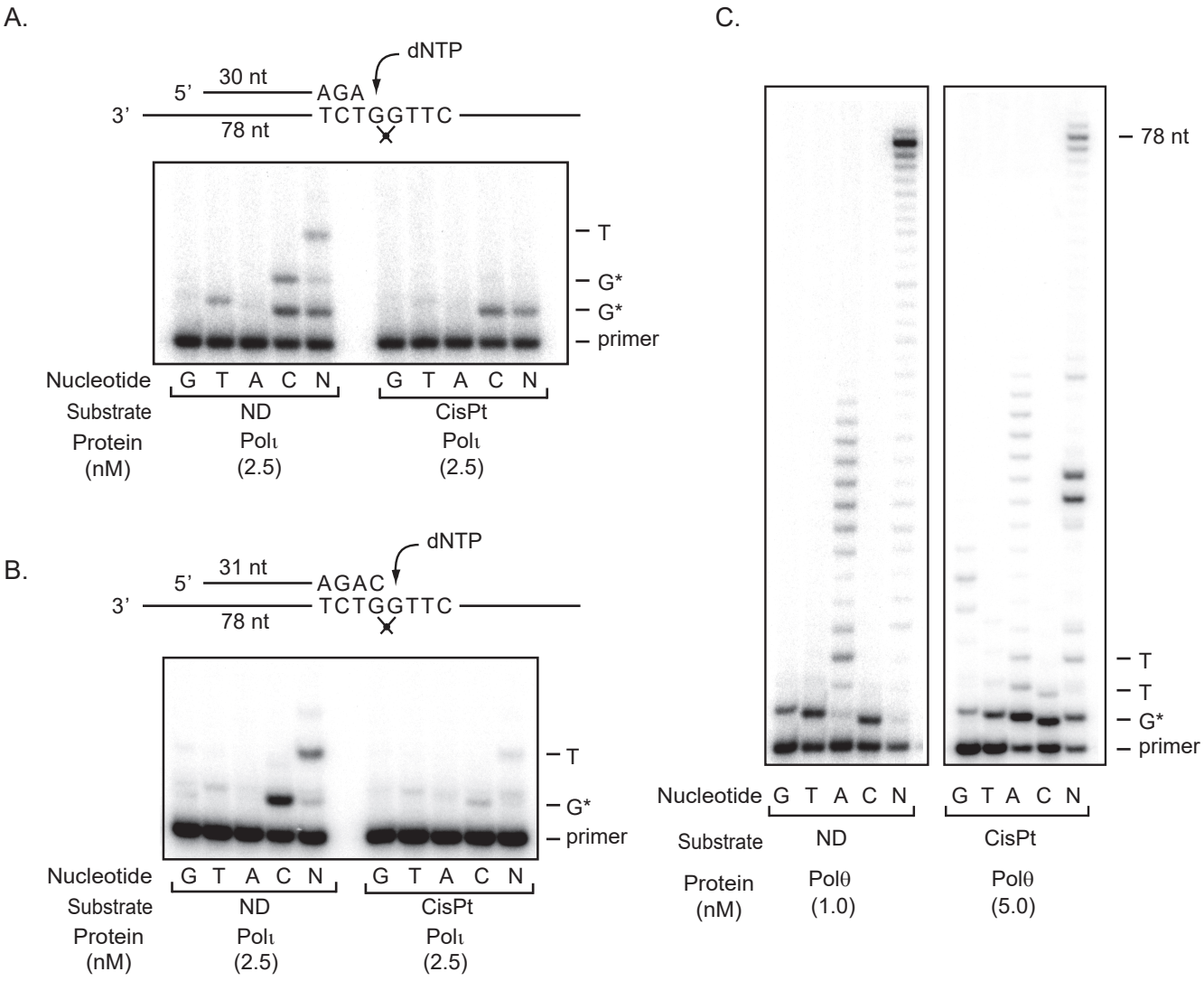

Figure S4. TLS opposite a cisplatin GG intrastrand crosslink by human DNA polymerases Polι and Polθ. A. Deoxynucleotide incorporation opposite the 3' G of a cisplatin GG intrastrand crosslink or a corresponding unadducted G by Polι. A diagrammatic representation of the DNA substrate is shown, wherein the 78mer template is on the bottom and either contains 2 normal G nucleotides or a cisplatin GG intrastrand crosslink at the equivalent position, as indicated by the X. The 30mer primer is on top and the terminus initiates DNA synthesis at the first G of the GG crosslink. Assays were performed using the standard DNA polymerase conditions, and protein concentrations are indicated in the figure. Reactions contained either the undamaged DNA (ND) or the GG cisplatin DNA crosslink (cis Pt) as indicated underneath, and contained either dGTP, dTTP, dATP, dCTP or all 4 nucleotides as indicated in lanes marked by G, T, A, C or N. The position of the 30mer primer and the extension products are shown on the right. **B**. Nucleotide incorporation opposite the 5' G of the un-adducted GG sequence or the corresponding G of the cisplatin crosslink by Polι. The DNA substrate is depicted as shown in **A.,** except that the primer terminus directs incorporation opposite the second G of the GG crosslink. Reactions were carried out as in **A. C.** Deoxynucleotide incorporation opposite the 5' G of a cisplatin GG intrastrand crosslink and extension of synthesis thereafter or a corresponding unadducted G by Polθ. The DNA substrate is the same as shown in **B.** Reactions were carried out as is in A, using protein concentrations shown below. Reactions contained either G, T, A, C or all four (N) deoxynucleotides as indicated. The position of the primer and reaction products are given on the right.

Supplemental_Fig_S5.pdf

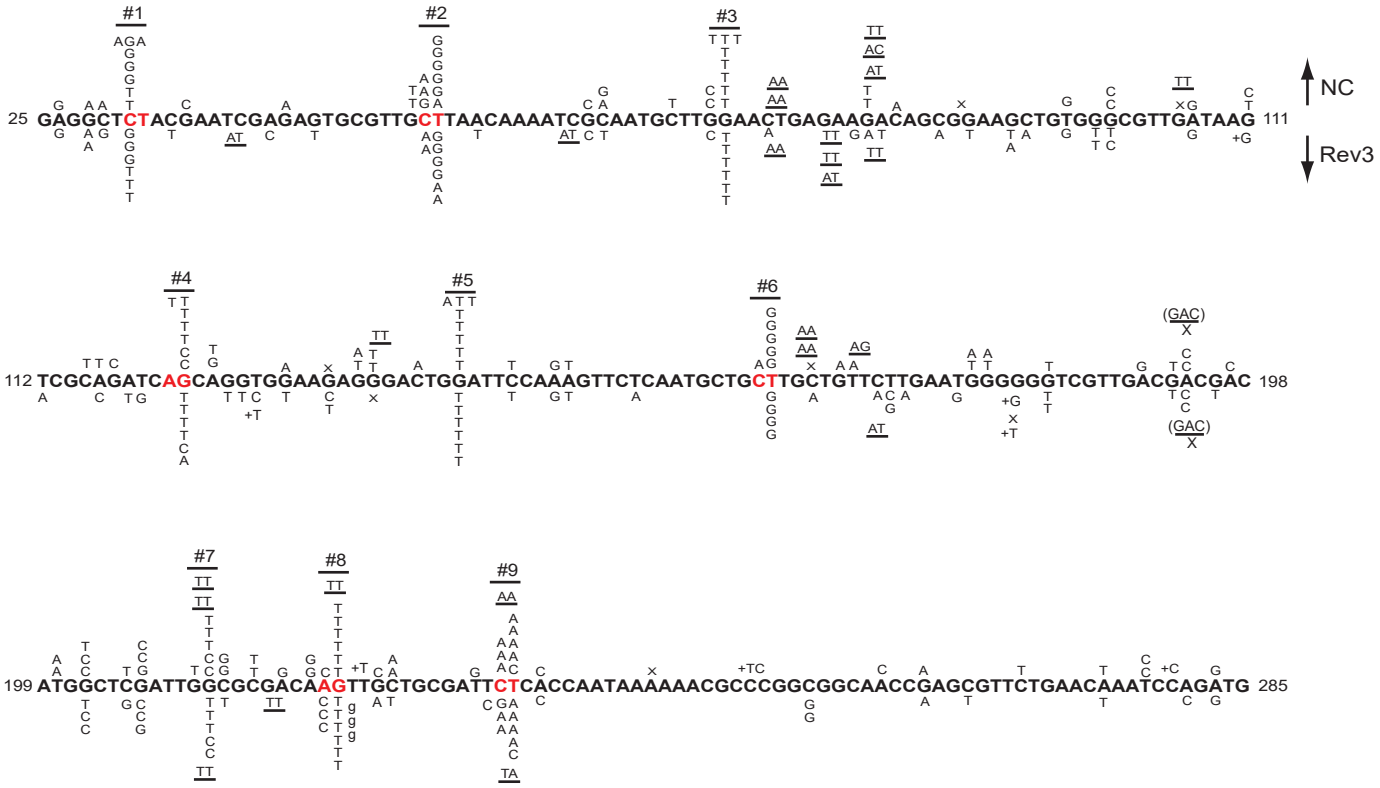

Figure S5. Cisplatin induced mutation spectra in the cII gene in Rev3 depleted BBMEFs. Mutational spectra of cisplatin induced mutations in control siRNA (NC) treated cells are shown above the sequence and mutational spectra in cells treated with Rev3 siRNA are shown below the sequence.

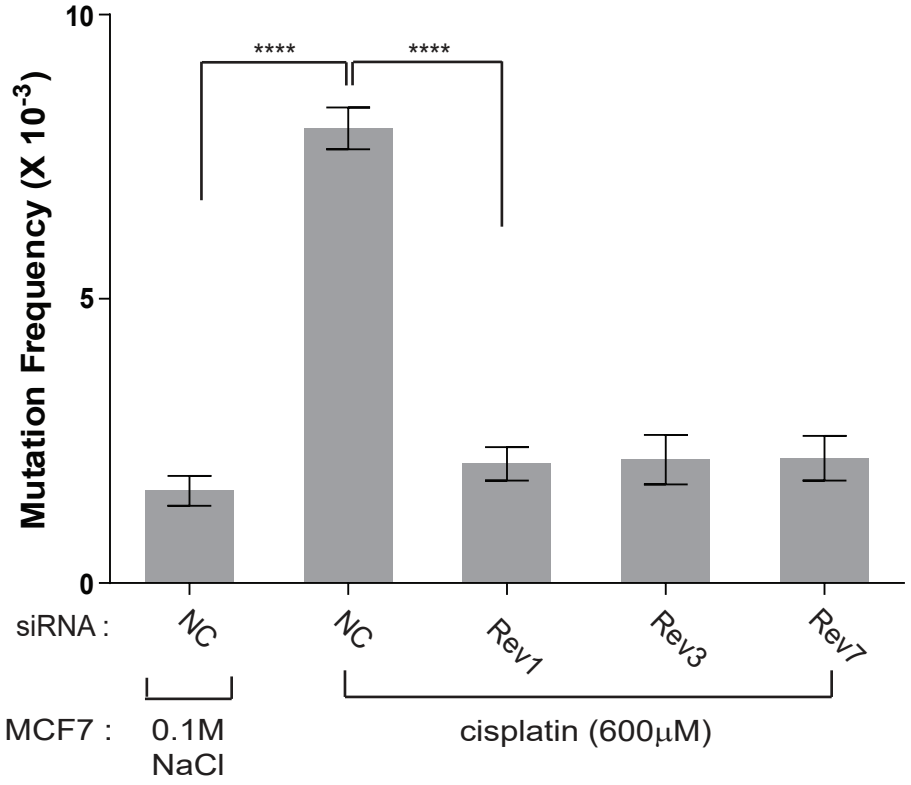

Figure S6. Cisplatin induced mutation frequency analysis in the *supF* gene in MCF7 cells. Cisplatin (600μM for 2h) induced mutation frequencies in the *supF* gene in MCF7 breast cancer cells depleted for Rev1, Rev3, or Rev7. Mutation frequencies are shown as the average of four independent experiments. Error bars indicate the standard deviation; student's two-tailed t-test p values, ****, $p<0.0001$.

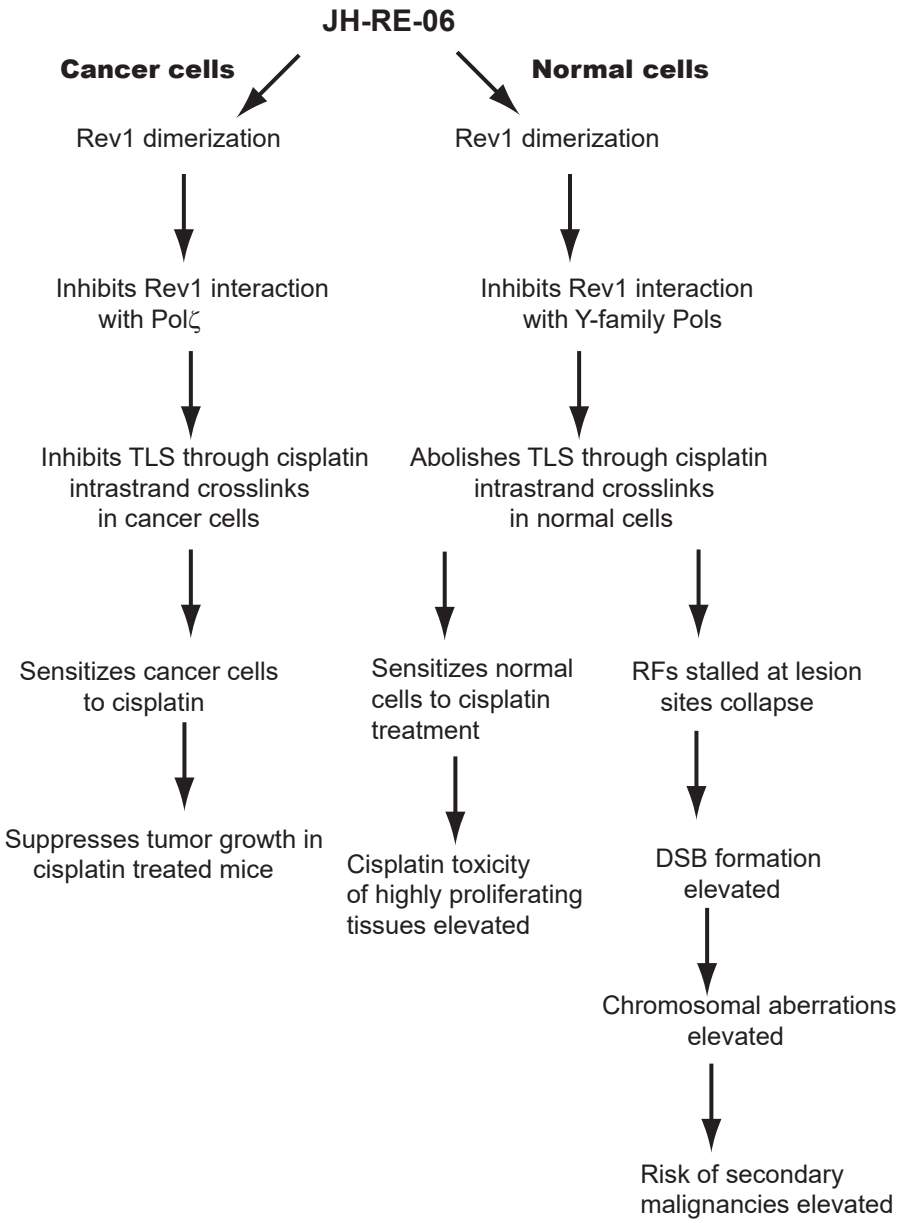

Figure S7. Disparate effects of JH-RE-06 on cancer *vs.* normal cells in response to cisplatin treatment. Whereas by inactivating Rev1 interaction with Polζ, JH-RE-06 would inhibit TLS through cisplatin adducts in cancer cells, by inactivating Rev1 interaction with Y-family Pols, JH-RE-06 would abrogate TLS through cisplatin adducts in normal cells. Thereby, JH-RE-06 would enhance the toxicity and tumorigenicity of normal tissues to cisplatin treatment.