DNA Polymerase zeta is a major determinant of resistance to platinum-based chemotherapeutic agents Shilpy Sharma, Nicholas A. Shah, Ariell M. Joiner, Katelyn H. Roberts, and Christine E. Canman



*Figure S1 Time-dependent and dose-dependent induction of*  $\gamma$ -H2AX *in REV3-deficient HeLa cells treated with platinating agents.* Control or REV3-specific siRNA-transfected cells were treated with the indicated concentrations of drug for one hour. Cells were fixed 8, 24, or 48 hrs post treatment and stained for  $\gamma$ -H2AX (green) and DAPI (blue) as described in materials and methods. Representative images from one experiment are shown.

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*Figure S2. Cisplatin-induced hyper-phosphorylation of Chk1 and RPA32 in REV3-deficient cells is dependent upon the ATR kinase.* HeLa cells were transfected with Control, REV3, ATR, or a combination of REV3 and ATR-specific siRNA overnight. Cells were allowed to recover 24 hrs and then treated with 10 µM cisplatin for 1 hr. Cells were harvested 24 hrs later and subjected to SDS-PAGE and immunoblotted with the indicated antibodies. A) Levels of ATR protein 48 hours after siRNA transfection. B) The phosphorylation status of Chk1 and RPA32 (serines 4 and 8) were examined in untreated and cisplatin-treated HeLa cells transfected with the indicated siRNAs. Representative images from two independent experiments are shown. ATR-specific siRNA was obtained from Qiagen, Valencia, CA (HP Validated siRNA, SI02664347). Polyclonal antibody specific for ATR was obtained by Abcam, Cambridge, MA.

*Figure S3, S4, S5, and S6. Time-dependent and dose-dependent induction and resolution of DSBs in REV3-deficient cells treated with cisplatin, oxaliplatin, picoplatin, and satraplatin.* HeLa cells were transfected with control or REV3-specific siRNA, and then treated with the indicated concentrations of drug for one hour. Cells were fixed 8, 24, or 48 hrs post treatment and stained for S1981P-ATM (green) and 53BP1 (red) as described in materials and methods. Nuclei were counterstained with DAPI. Representative images from a single experiment are shown:

Figure S3 Cisplatin, page 3 Figure S4 Oxaliplatin, page 4 Figure S5 Picoplatin, page 5 Figure S6 Satraplatin, page 6

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		aiCa	otrol	Picoplatin (1 $\mu$ M)				
	DAPI	P-ATM	53BP1	Merge	DAPI	P-ATM	53BP1	Merge
8 Hrs	- 		**************************************					
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48 Hrs								
	siControl			Picoplatin (25 μM)				
	DAPI	P-ATM	53BP1	Merge	DAPI	P-ATM		Merge
8 Hrs	· · ·		en e		۲۰۱۹ ۲۰۱۹ ۲۰۱۹ - ۲۰۱۹			
24 Hrs								
48 Hrs	• • • • • •							
	siControl			Picoplatin (75 μM) siREV3				
	DAPI	P-ATM	53BP1	Merge	DAPI	P-ATM	53BP1	Merge
8 Hrs					80 3			
24 Hrs			* 6	* Ø Ø				
48 Hrs	●		* (s) (s)	<ul> <li>A</li> </ul>	د <sup>رو</sup> به ه			

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