Table S1. The effects of siRNA knockdowns of Polθ and other TLS Pols on replicative bypass of a *cis-syn* TT dimer or a (6-4) TT photoproduct carried on the leading or lagging DNA strand template in XPV human fibroblasts (Related to Table 1)

		Leading strand			Lagging strand		
UV lesion	siRNA	Number of *Kan*$^+$ colonies	Number of blue colonies among *Kan*$^+$	TLS (%)	Number of *Kan*$^+$ colonies	Number of blue colonies among *Kan*$^+$	TLS (%)
cis-syn TT dimer	NC	387	48	12.4	495	52	10.5
	Polθ	356	11	3.1	256	6	2.3
(6-4) TT PP	NC	482	90	18.6	542	89	16.3
	Polι	475	51	10.7	485	46	9.5
	Polθ	612	59	9.6	640	60	9.4
	Polθ + Polι	646	66	10.2	582	57	9.8
	Rev3	584	51	8.7	564	42	7.4
	Polθ + Rev3	420	18	4.3	395	15	3.8

Table S2. Effects of siRNA knockdown of Polθ on mutation frequencies and nucleotides inserted opposite a *cis-syn* TT dimer or a (6-4) TT photoproduct carried on the leading or lagging strand DNA template in XPA human fibroblasts (Related to Table 2)

DNA lesion	Lesion containing DNA strand	siRNA	# of *Kan*$^+$ blue colonies sequenced	Nucleotide inserted					Mutation frequency %
				A	G	C	T	Other	
cis-syn TT dimer	Leading	NC	340 (7)[a]	333	4 (3' T)[b]	-	3 (5' T)	-	2.1
		Polθ	470 (0)	470	-	-	-	-	0
	Lagging	NC	374 (9)	365	4 (5' T) 2 (3' T)	-	3 (3' T)	-	2.4
		Polθ	524 (0)	524	-	-	-	-	0
(6-4) TT PP	Leading	NC	432 (6)	426	2 (5' T) 1 (3' T)	-	2 (3' T)	1	2.2
		Polθ	408(0)	408	-	-	-	-	0
	Lagging	NC	398 (6)	392	3 (5' T) 1 (3' T)	-	1 (3' T)	1	1.6
		Polθ	412 (0)	412	-	-	-	-	0

a. Numbers of colonies where TLS occurred by insertion of a nucleotide other than an A are shown in parenthesis.
b. The site where mutation occurred, 3'T or 5'T of the UV lesion is indicated in parenthesis.

Table S3. Effects of catalytically active (WT) Polθ or catalytically inactive D2540A, E2541A mutant Polθ (1708-2590) on TLS opposite a *cis-syn* TT dimer or a (6-4) TT photoproduct carried on the leading strand in normal human fibroblasts (Related to Tables 1 and 2)

DNA lesion	siRNA	Vector expressing	Number of *Kan*$^+$ colonies	Number of blue colonies among *Kan*$^+$ colonies	TLS (%)
cis-syn TT dimer	NC	No Polθ (control)	408	88	21.6
		1708-2590 (WT Polθ)	384	80	20.8
		1708-2590 (mutant Polθ)	445	87	19.6
	Polθ	No Polθ (control)	424	46	10.8
		1708-2590 (WT Polθ)	312	64	20.5
		1708-2590 (mutant Polθ)	584	56	9.6
(6-4) TT PP	NC	No Polθ (control)	394	74	18.8
		1708-2590 (WT Polθ)	402	80	19.9
		1708-2590 (mutant Polθ)	445	78	17.5
	Polθ	No Polθ (control)	337	30	8.9
		1708-2590 (WT Polθ)	410	76	18.5
		1708-2590 (mutant Polθ)	486	41	8.4

Table S4. Viable offspring of Polθ$^{+/-}$ Polη$^{+/-}$ x Polθ$^{+/-}$ Polη$^{+/-}$ intercrosses (Related to Figure 5)

Live pups	θ$^{-/-}$ η$^{-/-}$	θ$^{-/-}$ η$^{+/-}$	θ$^{-/-}$ η$^{+/+}$	θ$^{+/-}$ η$^{-/-}$	θ$^{+/-}$ η$^{+/-}$	θ$^{+/-}$ η$^{+/+}$	θ$^{+/+}$ η$^{-/-}$	θ$^{+/+}$η$^{+/-}$	θ$^{+/+}$ η$^{+/+}$	Total
Observed	4	14	3	15	42	10	5	9	5	107
Expected	6.7	13.4	6.7	13.4	26.7	13.4	6.7	13.4	6.7	107

Table S5. Primer sequences for RT-PCR in Polθ and Polη knockout MEFs (Related to Figures 2 – 4)

Gene	Primer Sequences
GAPDH	Forward: 5′- ATTGTGCACATCCAGGCGG -3′ Reverse: 5′- CCTCCTTCATGGACATGGG -3’
Polη	Forward: 5′- GAAGCCCGAGCATTTGGTG -3′ Reverse: 5′- GCCTCTCCTCAAGTTCCAG -3’
Polθ	Forward: 5′- CGGTAGCTGTCACTAGGTTG -3′ Reverse: 5′- CAGAGTCTAGTCCTGAAGGTG -3’