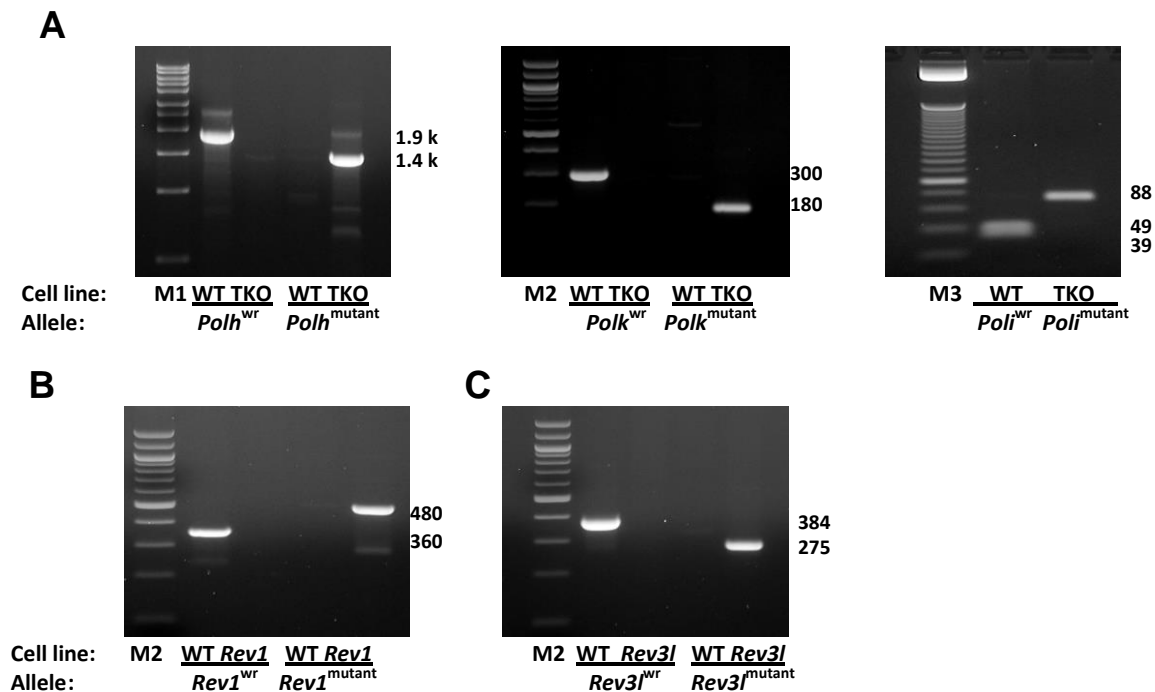Figure S1. Genotyping of *Polh Polk Poli* triple-gene (TKO) (A), *Rev1* (B) and *Rev3l* (C) single gene knockout MEFs by genomic PCR

PCR was performed at 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 min with 40 cycles using primer sets listed below: "WT" and "TKO" represent a wild-type and Polη Polκ Polι triple-gene knockout MEFs, respectively. Size markers: M1, 1-kb ladder; M2, 100-bp ladder; and M3, 25-bp ladder

| Allele	PCR primer (5'→3')	Length (base pair) of PCR product
Polh wild type	TTTCGATCTTTGGTTAGCCTCTCC GTAGTCTGGGGGGTTGAATC	1.9 k
Polh mutant	GTAGTCTGGGGGGTTGAATC GTCTGTTGTGCCCAGTCATAGC	1.4 k
Polk wild type	GCACTGATCGATATGTCCATTTAGGG GTGAGACCAAACCTTGACAGGCTAAG	300
Polk mutant	GGGCCAGCTCATTCCTCCACTCATGATC CAGGCTGCAGGGTTGGAAACAGCCACAC	180
Poli wild type	CAGTTTGCAGTCAAGGGCC TCGACCTGGGCATAAAAGC	49 + 39[a]
Poli mutant	CAGTTTGCAGTCAAGGGCC TCGACCTGGGCATAAAAGC	88[a]
Rev1 wild type	ATTGTGAGTCTCTAGCGTTTGTCCCTC TCCTGGCTGGAATTGAAATTCTAGGACAC	360
Rev1 mutant	ATTGTGAGTCTCTAGCGTTTGTCCCTC GCTTCCATTGCTCAGCGGTG	480
Rev3l wild type	GTGCTGAGAAAGCTCATGTCTAGGC GATTGCCTTCCCTATCTGTCCTTCATTG	384
Rev3l mutant	GATTGCCTTCCCTATCTGTCCTTCATTG CTAAAGCGCATGCTCCAGACT	275

[a] Generated by Taq$^\alpha$I digestion of PCR products

A

Cell line:	M1	WT TKO	WT TKO
Allele:		*Polh*^{wr}	*Polh*^{mutant}

1.9 k
1.4 k

Cell line:	M2	WT TKO	WT TKO
Allele:		*Polk*^{wr}	*Polk*^{mutant}

300
180

Cell line:	M3	WT	TKO
Allele:		*Poli*^{wr}	*Poli*^{mutant}

88
49
39

B

Cell line:	M2	WT *Rev1*	WT *Rev1*
Allele:		*Rev1*^{wr}	*Rev1*^{mutant}

480
360

C

Cell line:	M2	WT *Rev3l*	WT *Rev3l*
Allele:		*Rev3l*^{wr}	*Rev3l*^{mutant}

384
275

Figure S2. TLS efficiency in 5'A<u>A</u>T context in gene knockout MEFs
TLS efficiency in wild-type MEFs was set to 100 %.
*$p<0.001$ when compared with a value for wild-type (WT) MEFs.
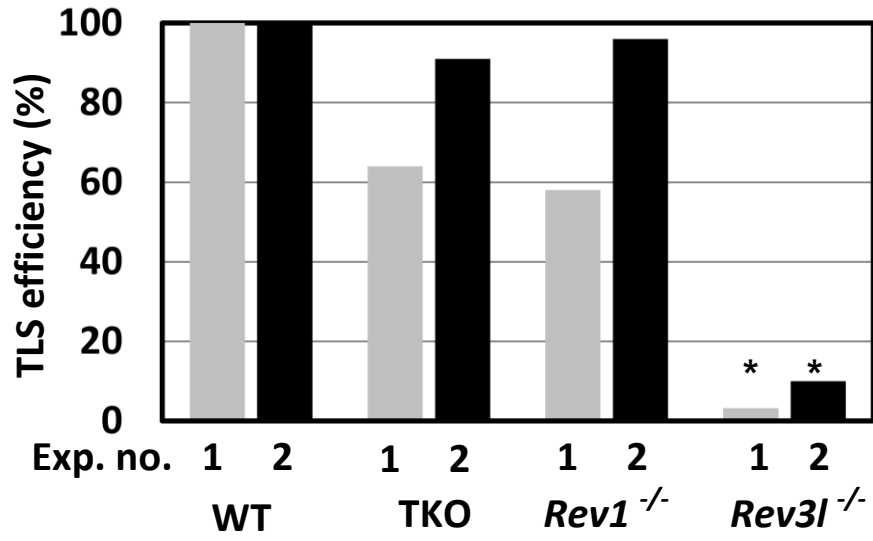
Table S1. Targeted coding specificity of translesion DNA synthesis across dA-AL-I

Host cells	Exp.	Sequence context	Total no. sequenced	[dA-AL-I] →					
				A	T	G	C	Δ	Others
Wild-type	1	C**A**G	181	71 (39%)	88 (49%)[a]	0 (0%)	4 (2%)	5 (3%)	13 (7%)
		A**A**T	181	121 (67%)	44 (24%)[a]	7 (4%)	3 (2%)	3 (2%)	3 (2%)
	2	C**A**G	189	76 (40%)	96 (51%)[b]	2 (1%)	1 (0.5%)	6 (3%)	8 (4%)
		A**A**T	183	123 (67%)	45 (25%)[b]	4 (2%)	3 (2%)	7 (4%)	1 (0.5%)
TKO	1	C**A**G	94	33 (35%)	45 (48%)[c]	0 (0%)	2 (2%)	1 (1%)	13 (14%)
		A**A**T	92	58 (63%)	32 (35%)[c]	1 (1%)	0 (0%)	0 (0%)	1 (1%)
	2	C**A**G	93	27 (29%)	60 (65%)[d]	1 (1%)	2 (2%)	0 (0%)	3 (3%)
		A**A**T	89	60 (67%)	25 (28%)[d]	0 (0%)	2 (2%)	2 (2%)	0 (0%)
Rev3l [-/-]	1	C**A**G	90	49 (54%)	0 (0%)	0 (0%)	3 (3%)	36 (40%)	2 (2%)
		A**A**T	96	84 (88%)	0 (0%)	0 (0%)	0 (0%)	7 (7%)	5 (5%)
	2	C**A**G	70	18 (26%)	0 (0%)	0 (0%)	30 (43%)	20 (29%)	2 (3%)
		A**A**T	78	46 (59%)	0 (0%)	0 (0%)	0 (0%)	30 (39%)	2 (3%)
Rev1 [-/-]	1	C**A**G	92	34 (37%)	44 (48%)[e]	0 (0%)	0 (0%)	5 (5%)	9 (10%)
		A**A**T	88	49 (56%)	34 (39%)[e]	1 (1%)	1 (1%)	0 (0%)	3 (3%)
	2	C**A**G	91	36 (40%)	46 (51%)[f]	0 (0%)	5 (6%)	0 (0%)	4 (4%)
		A**A**T	90	58 (64%)	27 (30%)[f]	1 (1%)	2 (2%)	2 (2%)	0 (0%)

Exp. No. 1 and 2 show the results of two independent experiments for each MEFs. The results in 5'C$\underline{\text{A}}$G context were adopted from Table 1. [a, b, d, f] $p<0.001$, [c] $p<0.01$, [e] $p>0.05$ when values in 5'C$\underline{\text{A}}$G and 5'A$\underline{\text{A}}$T are compared with each other. 'Others' includes untargeted and multiple mutations.