Supplemental Data

The vital role of pol ζ and REV1 in mutagenic, but not correct, DNA synthesis across benzo[*a*]pyrene-dG and the recruitment of pol ζ by REV1 to a replication-stalled site

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Supplemental Tables

Table S1. TLS events for BPDE-dG and H-εdC in double and triple-gene knockout MEFs

DNA lesion	MEFs	TLS efficiency [a]	Total effective no. sequenced	No. and % of coding events detected				
				G*→T	G*→A	G*→G	G*→C	Others
BPDE -dG (G*)	Wild-type	1.68 (100%)	86 100%	63 73%	10 12%	8 9%	1 1%	4 5%
	Polk$^{-/-}$ *Polh*$^{-/-}$ *Poli*$^{-/-}$ (2091)	1.31 (78%)	95 100%	65 68%	14 15%	11 12%	1 1%	4 4%
	Polk$^{-/-}$ *Poli*$^{-/-}$	1.01 (60%)	95 100% [b]	51 54% [c]	21 22%	14 15%	4 4%	5 5%
	Polh$^{-/-}$ *Poli*$^{-/-}$	1.03 (61%)	94 100% [b]	41 44% [c]	23 24% [c]	14 15%	9 10% [c]	7 7%
				C*→T	C*→A	C*→G	C*→C	Others
H-εdC (C*)	Wild-type	0.802 (100%)	90 100%	54 60%	33 37%	2 2%	1 1%	0 0%
	Polk$^{-/-}$ *Polh*$^{-/-}$ *Poli*$^{-/-}$ (2091)	0.165 (21%)	94 100%	56 60%	35 37%	3 3%	0 0%	0 0%

Colors used in coding events correspond to those used in Fig. 2.
[a] Number of ampicillin/blasticidin-resistant transformants divided by that of kanamycin-resistant (internal control) transformants.
[b] The distribution of coding events are statistically significant at p<0.001 when compared with that in wild-type cells. Highlighted with a grey background.
[c] p<0.001 when compared with the corresponding event in wild-type cells. Highlighted with a grey background.

Table S2. TLS events for BPDE-dG in single-gene knockout MEFs

DNA lesion	MEFs	TLS efficiency [a]	Total effective no. sequenced	No. and % of coding events detected				
				G*→T	G*→A	G*→G	G*→C	Others
BPDE-dG (G*)	Wild-type	5.72 (100%)	92 100%	68 74%	14 15%	6 7%	1 1%	3 3%
	Rev1$^{-/-}$	2.34 (41%)	88 100% [b]	28 32% [c]	16 18%	42 48% [c]	1 1%	1 1%
	Rev3L$^{-/-}$	0.76 (13%)	94 100% [b]	6 6% [c]	0 0% [##]	88 94% [c]	0 0%	0 0%
	Rad18$^{-/-}$	1.50 (26%)	191 100% [b]	108 57% [c]	15 8%	55 29% [c]	3 2%	10 5%

Refer to the legend to Table S1.

Supplemental Figures

Figure S1. Genotyping of triple- and double-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 45 cycles using following primer sets. **A** and **B** (*Polh*), the wild-type, but not the mutant, *Polh* allele produced a 1.9-kb fragment with a primer set of 5'TTTCGATCTTTGGTTAGCCTCTCC and 5'GTAGTCTGGGGGGTTGAATC (**A**). The mutant, but not the wild-type, *Polh* allele, produced a 1.4-kb fragment with a primer set of 5'GTAGTCTGGGGGGTTGAATC and 5'GTCTGTTGTGCCCAGTCATAGC (**B**). Refer to ref. S1. **C** (*Polk*), the wild-type, but not the mutant, *Polk* allele produced a 300-bp fragment with a primer set of 5'GCACTGATCGATATGTCCATTTAGGG and 5'GTGAGACCAAACCTTGACAGGCTAAG. **D** (*Poli*), the wild-type and mutant *Poli* alleles produced 49- plus 39-bp fragments and a 88-bp fragment, respectively, following Taq$^{\alpha}$I digestion of PCR products amplified with a primer set of 5'-CAGTTTGCAGTCAAGGGCC and 5'-TCGACCTGGGCATAAAAGC. Refer to ref. S2. Two independent lines (91, TKO line #2091 and 95, TKO line #2095) of triple-gene knockout (*k/h/i*) MEFs were examined. "wt" represents a wild-type MEF. Size markers (M) are 1-kb ladder for **A** and **B**, 100-bp ladder for **C**, and 25-bp ladder for **D**. Diagnostic fragments are indicated by white arrows.

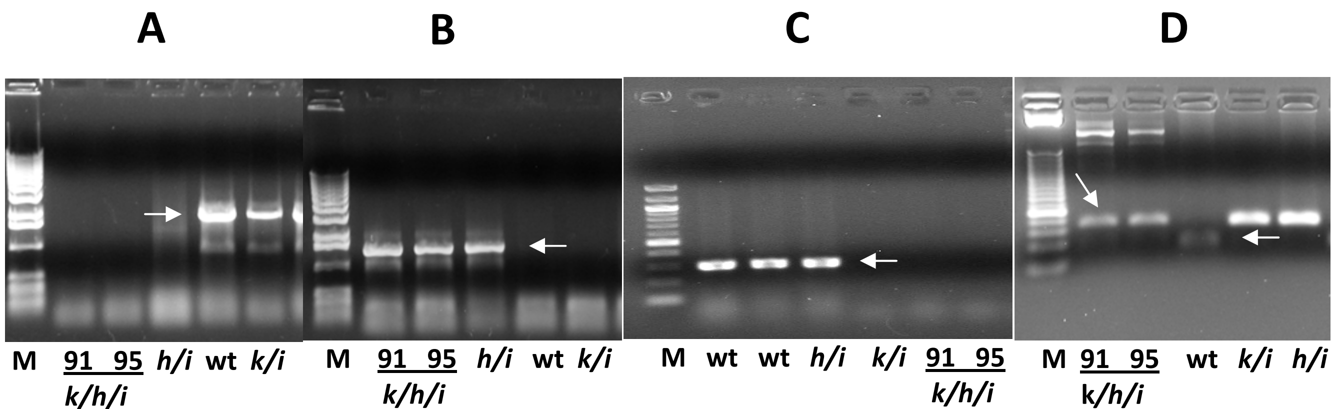


Figure S2. Clonogenic UV sensitivity assay of various Y-family polymerase-defective MEFs. Four hundred cells were seeded in a 10-cm plate and irradiated with UV-C. Cells were cultured for 10-14 days, after which colonies were counted with crystal violet staining. Dark blue, wild-type; light blue, *Polk*$^{-/-}$; green, *Polk*$^{-/-}$ *Poli*$^{-/-}$; pink, *Polh*$^{-/-}$; orange, *Polk*$^{-/-}$ *Polh*$^{-/-}$ *Poli*$^{-/-}$ (#2091); red, *Polk*$^{-/-}$ *Polh*$^{-/-}$ *Poli*$^{-/-}$ (#2095).

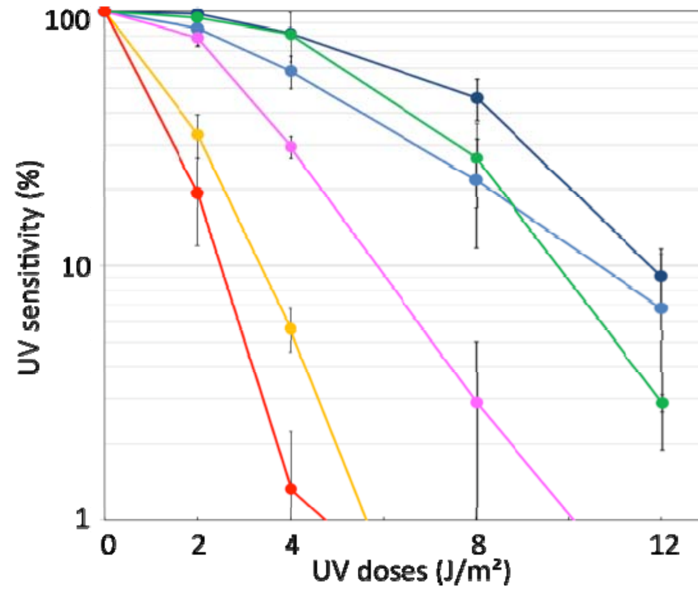
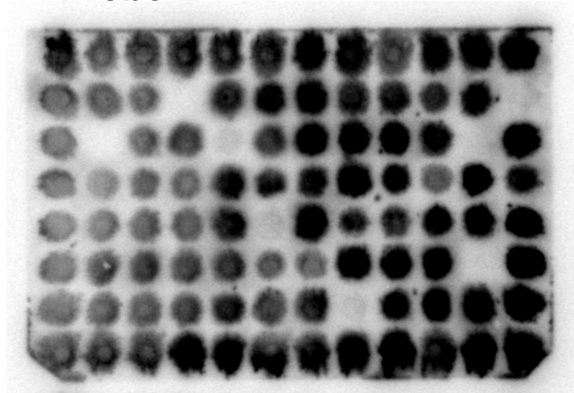
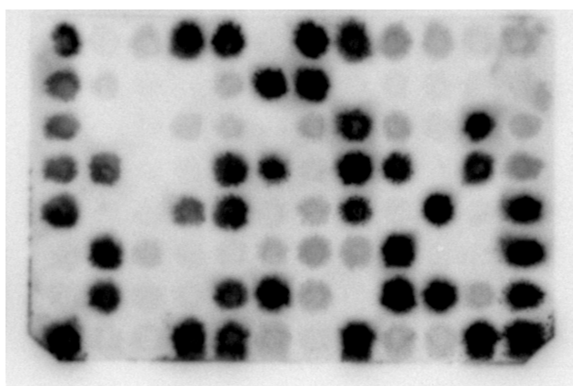


Figure S3. Examples of colony hybridization with oligonucleotide probes. Refer to Fig. 1 for the locations and sequences of oligonucleotide probes used. Colonies showing positive hybridization signals for both L and R probes are considered to be derived from TLS. *E. coli* transformants that did not hybridized with L or R probe were excluded from analysis. Probes T and C detect BPDE-dG→T and BPDE-dG→C transversions, respectively.

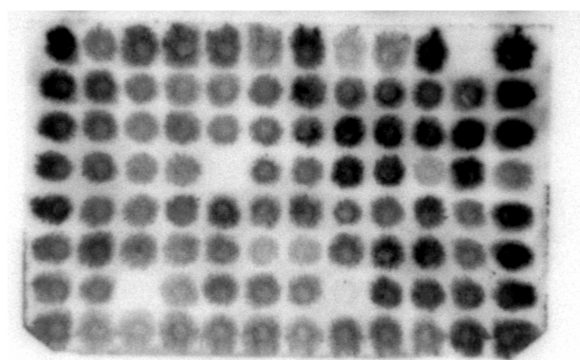
Probe L



Probe T



Probe R



Probe C

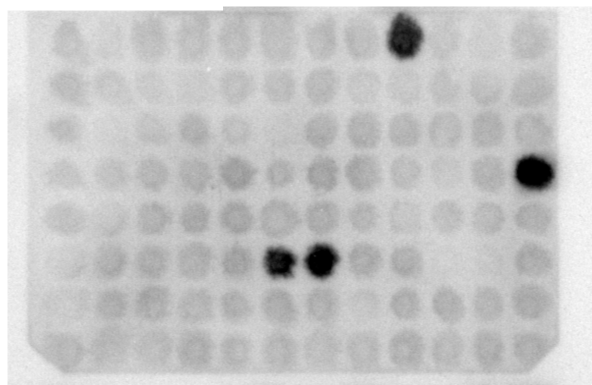


Figure S4. Immunoblot analysis of *Rev1*$^{-/-}$ mouse embryonic fibroblasts complemented with hREV1. Human REV1 (1251 amino acids) and its C-terminal deletion mutant (1-1140 amino acids) tagged with 3×FLAG (140 and 128 kDa, respectively) were expressed in *Rev1*$^{-/-}$ mouse cells. Anti-FLAG monoclonal antibody (M2, Sigma) was used to detect tagged hREV1. lane 1, protein marker; lane 2, *Rev1*$^{-/-}$ cells; lane 3, *Rev1*$^{-/-}$ cells complemented with hREV1 (1-1140); lane 4, *Rev1*$^{-/-}$ cells complemented with hREV1 (1-1251); *, hREV1 (1-1140); **, hREV1 (1-1251).

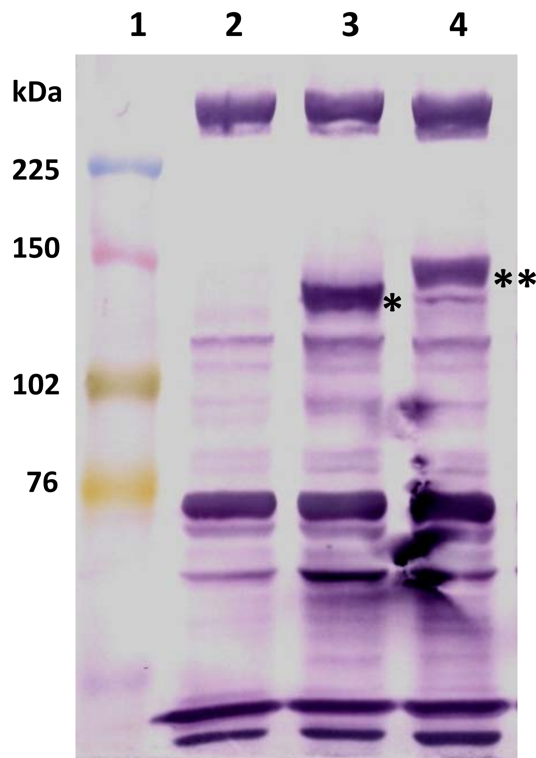
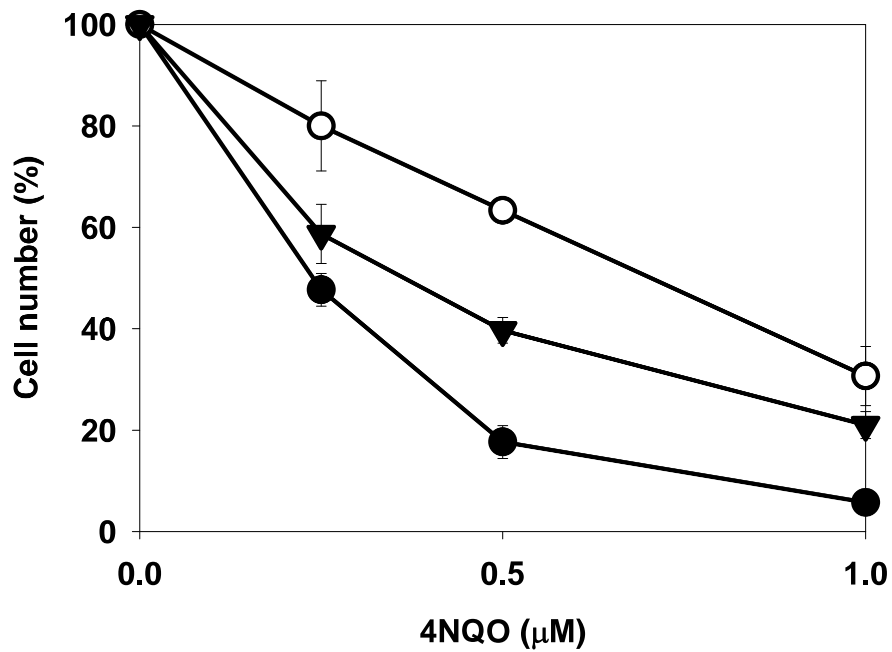


Figure S5. Sensitivity of *Rev1*$^{-/-}$ mouse embryonic fibroblasts complemented with hREV1 or C-terminal truncated hREV1 to 4-nitroquinoline-*N*-oxide (4NQO). Cells were treated with 0.25, 0.5, and 1 μM 4NQO for 1 h, after which the treatment medium was replaced with a fresh medium. Cells were cultured for 2 days, and the number of viable cells was determined by the Cell Titer 96 AQueous One Solution Cell Proliferation Assay (Promega). open circle, hREV1(1-1251); closed triangle, hREV1 C-terminal deletion mutant (1-1140); closed circle, *Rev1*$^{-/-}$ cells.



Supplemental References

S1. Ohkumo, T., Kondo, Y., Yokoi, M., Tsukamoto, T., Yamada, A., Sugimoto, T., Kanao, R., Higashi, Y., Kondoh, H., Tatematsu, M., Masutani, C., and Hanaoka, F. (2006) *Mol. Cell. Biol.* **26**, 7696-7706

S2. McDonald, J.P., Frank, E.G., Plosky, B.S., Rogozin, I.B., Masutani, C., Hanaoka, F., Woodgate, R., and Gearhart, P.J. (2003) *J. Exp. Med.* **198**, 635-643