Supplemental Material:

Rev1 promotes replication through UV lesions in conjunction with DNA polymerases η, ι, and κ, but not with DNA polymerase ζ

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Supplemental Table S1. Effects of siRNA knockdown of Polη or Rev1 on TLS opposite a *cis-syn* TT dimer carried on the leading strand DNA template in wild type human fibroblasts and complementation of Polη or Rev1 depleted cells by vector expressing siRNA resistant (siR) form of Polη or Rev1

siRNA	Vector expressing	# kan^+ colonies	# blue colonies among kan^+	TLS (%)
NC	-	440	110	25.0
Polη	-	198	22	11.1
Polη	Flag-WT-Polη	284	33	11.6
Polη	Flag-WT-siR-Polη	302	77	25.5
Rev1	-	258	20	7.8
Rev1	Flag-WT-Rev1	230	17	7.4
Rev1	Flag-WT-siR-Rev1	246	68	27.6

Supplemental Table S2. Effects of siRNA knockdown of Rev1 on mutation frequencies and nucleotides inserted opposite a *cis-syn* TT dimer carried on the leading or lagging strand DNA template in NER defective XPA human fibroblasts

cis-syn TT dimer	# of *Kan*+ blue colonies sequenced[1]	Nucleotide inserted				Mutation frequency %
		A	G	C	T	
Leading strand						
NC siRNA	238 (5)[1]	233	3 (3' T)[2]	-	2 (5' T)	2.2
Rev1 siRNA	408 (15)	383	6 (5' T) 5 (3' T)	-	4 (3'T)	3.8
Lagging strand						
NC siRNA	285 (9)	276	4 (5' T) 2 (3 'T)	-	2 (3' T)	2.8
Rev1 siRNA	242 (13)	229	6 (5 'T) 3 (3' T)	-	4 (3' T)	5.4

1. Numbers of colonies where TLS occurred by insertion of a nucleotide other than an A opposite the *cis-syn* TT dimer are shown in parenthesis.

2. The site where mutation occurred, 3'T or the 5'T of the *cis*-syn TT dimer is indicated in parenthesis.

Supplemental Table S3. Effects of siRNA knockdown of Rev1 on mutation frequencies and nucleotides inserted opposite a (6-4) TT photoproduct carried on the leading or lagging strand DNA template in NER defective XPA human fibroblasts

(6-4) TT photoproduct	# of *Kan*+ blue colonies sequenced[1]	Nucleotide inserted					Mutation frequency %
		A	G	C	T	Other	
Leading strand							
NC siRNA	432 (6)[1]	426	2 (5' T)[2] 1 (3' T)	-	2 (3' T)	1	2.2
Rev1 siRNA	334 (0)	334	-	-	-	-	0
Lagging strand							
NC siRNA	398 (6)	392	3 (5' T) 1 (3 'T)	-	1 (3' T)	1	1.6
Rev1 siRNA	320 (0)	229	-	-	-	-	0

1. Numbers of colonies where TLS occurred by insertion of a nucleotide other than an A opposite the (6-4) TT photoproduct are shown in parenthesis.

2. The site where mutation occurred, 3'T or the 5'T of the (6-4) TT photoproduct is indicated in parenthesis.

Supplemental Table S4. UV induced mutation frequencies in the *cII* gene in (BBMEF) mouse cells carrying the pNeo vector which expresses no photolyase; hence, UV induced mutations result from replication through both the CPDs and (6-4) photoproducts in these cells

siRNA	UV[1]	PR[2]	Mutation frequency[3] ($x10^{-5}$)
NC[4]	-	-	18.6 ± 4.2
NC	+	+	52.6 ± 5.2
Pol η	+	+	95.8 ± 6.3
Pol κ	+	+	31.5 ± 5.6
Rev3	+	+	34.1 ± 4.5
Rev1	+	+	69.8 ± 4.2

1. 5 J/m^2 of UVC (254 nm) light.
2. Photoreactivation with UVA (360nm) light for 3 h.
3. Mutation frequency data were obtained from averages of 7 independent experiments.
4. NC, negative control siRNA.

Supplemental Table S5. UV induced mutation frequencies in the *cII* gene in $Rev1^{+/+}$, $Rev1^{+/-}$, and $Rev1^{-/-}$ mouse primary embryonic fibroblasts

Cell line	UV[1]	Mutation frequency[2] ($x10^{-5}$)
$Rev1^{+/+}$	-	4.6 ± 1.2
$Rev1^{+/-}$	-	5.2 ± 1.4
$Rev1^{-/-}$ (#1)[3]	-	5.8 ± 1.5
$Rev1^{+/+}$	+	31.6 ± 2.8
$Rev1^{+/-}$	+	40.8 ± 3.2
$Rev1^{-/-}$ (#1)	+	57.2 ± 3.8
$Rev1^{-/-}$ (#2)	+	54.6 ± 2.4

1. 5 J/m^2 of UVC (254nm) light.
2. Mutation frequencies were determined from the average of 3 independent experiments.
3. Two different $Rev1^{-/-}$ primary MEF cell lines (#s 1 and 2) were used for these experiments.

Supplemental Table S6. Effects of siRNA depletion of Rev1 and Rev3 on the frequency of gap filling opposite the (6-4) TT photoproduct in human fibroblasts

siRNA	No. of Zeo^R colonies	No. of Amp^R colonies	Gap-filling opposite (6-4) TT photoproduct % Amp^R/Zeo^R
NC	338	75	22.0
Rev1	315	26	8.3
Rev3	311	25	8.0
Rev1 + Rev3	426	34	8.0

Supplemental Table S7. Effects of siRNA depletion of Polη, Rev1, and Rev3 on the frequency of gap filling opposite the *cis-syn* TT dimer in human fibroblasts

siRNA	No. of Zeo^R colonies	No. of Amp^R colonies	Gap-filling opposite *cis-syn* TT dimer % Amp^R/Zeo^R
NC	220	137	62.3
Polη	168	30	17.9
Rev1	236	142	60.2
Rev3	208	120	57.6

Supplemental Table S8. Mutagenicity of TLS opposite (6-4) TT photoproduct carried in the gapped plasmid in human fibroblasts

siRNA	# of *Amp*^{R} colonies sequenced	Nucleotide inserted					Mutation frequency
		A	G	C	T	other	
NC	288 (35)[1]	253	12 (3' T)[2]	0	12 (3' T) 5 (5'T)	6[3]	12.1

1. Numbers of colonies in parenthesis where TLS incurred a mutational event.
2. The site where mutations occurred, 3'T or the 5'T of the (6-4) TT photoproduct is indicated in parenthesis.
3. These mutational events occurred in bases flanking the TT photoproduct.

Supplementary Figure Legends

Figure S1. SV40 plasmid for TLS assays. The plasmid carries the wild type *Kan*+ gene on the lesion-containing strand and the *Kan*- gene on the other DNA strand. In the *lacZ'* gene, the lesion-containing DNA strand harbors an MfeI restriction site, and it encodes functional β-galactosidase (β-gal), while the opposite strand harbors an SpeI site containing a +1 frameshift, making it non-functional for β-gal. TLS through the *cis-syn* T^T dimer (CPD) results in the formation of *Kan*+ blue colonies. Template switch results in continuing replication of the *Kan*+ strand by using the *lacZ'* strand harboring the SpeI sequence as a template. This process generates colonies that are *Kan*+ white. Thus, with this assay system, *Kan*+ blue colonies result from TLS whereas *Kan*+ white colonies would derive from template switching in NER-defective cells. Mismatch repair has no effect in this plasmid system.

Figure S2. Efficient siRNA knockdown of Rev1 in human and mouse fibroblasts. (A) Western blot analyses of siRNA knockdown of TLS Pols in human fibroblasts. β-tubulin was used as the loading control. (B) Normal human fibroblasts (MRC5) expressing 3x-Flag-wild type or 3x-Flag-siRNA resistant form (SiR) of Polη or Rev1 were treated with siRNA for 48 h. The efficiency of siRNA depletion was determined by Western blot with anti-Flag antibody (Sigma). β-tubulin was used as the loading control. (C) Western blot analysis of siRNA knock down of TLS Pols in mouse embryonic fibroblasts. β-tubulin was used as the loading control.

Figure S3. Growth retardation of Rev1$^{-/-}$ mice and UV sensitivity of Rev1$^{-/-}$ MEFs. (A) One month old wild type (right) and Rev1$^{-/-}$ mice (left) are shown. Rev1$^{-/-}$ mice display a growth retardation phenotype. (B) The integration site of the gene trapping vector in the first intron of

WT Rev1 and the resulting mutant Rev1 allele are shown. Genotype analysis of $Rev1^{+/+}$ (WT). $Rev1^{+/-}$, and $Rev1^{-/-}$ mice by PCR amplification with the primers IST10468C11F and LTR-rev. No PCR products are detected in wild type mouse tail genomic DNA. M, size marker; dH_20, water control for PCR. Loss of Rev1 protein in $Rev1^{-/-}$ MEFs was verified by Western blot analysis using mouse Rev1 antibodies. (C) UV survival of $Rev1^{+/+}$(WT), $Rev1^{+/-}$, and $Rev1^{-/-}$ mouse primary embryonic fibroblasts irradiated in PBS buffer with 10 J/m^2 of UVC light. Cells were incubated for an additional 48 h after UV irradiation. UV survival was determined by the MTT assay. The data represent the mean and standard deviation of results of three independent experiments.

Figure S4. Requirement of Rev1 for the accumulation of Y-family Pols into replication foci in UV damaged MEFs. (A) SV40-transformed $Rev1^{+/+}$ MEFs were transfected with GFP-Polη or GFP-Polκ. After 20 h, cells were treated with 20 J/m^2 UVC. Representative images of GPF-Polη and GFP-Polκ foci are shown on the left and quantification of cells containing these foci is shown on the right. Error bars represent the standard deviation of three independent experiments. (B) SV40-transformed $Rev1^{-/-}$ MEFs were transfected with GFP-Polη or GFP-Polκ. Reduction of GPF-Polη and GFP-Polκ foci are shown on the left and quantification of cells containing these foci is shown on the right. Error bars represent the standard deviation of three independent experiments. (C) Rev7 foci formation in UV treated primary $Rev1^{+/+}$ or $Rev1^{-/-}$ MEFs. UV induced Rev7 foci in the primary $Rev1^{+/+}$ or $Rev1^{-/-}$ MEFs are shown on the left and quantification of mouse Rev7 foci is shown on the right. Error bars represent the standard deviation of three independent experiments.

Figure S5. Requirement of Pols η, ι, and κ for the accumulation of Rev1 into replication foci in UV damaged human fibroblasts. (A) Reduction of Rev1 foci in wild type cells treated with Polη siRNA and UV irradiated at 20 J/m^2. (B) Quantification of UV induced Rev1 foci in wild type cells treated with control (NC) siRNA, Polη siRNA, or Rev7 siRNA. (C) UV induced assembly of Rev1 into replication foci is abrogated in XPV cells upon siRNA depletion of both Polι and Polκ.

Figure S6. Requirement of Rad18 for the accumulation of TLS Pols into replication foci in UV damaged human fibroblasts. (A) Rad18 siRNA knock down efficiency in normal human fibroblasts (MRC5). (B) Reduction of Polη and Rev1 foci in MRC5 fibroblasts treated with Rad18 siRNA and UV irradiated at 20 J/m^2. (C) Quantification of UV induced GFP-Polη, GFP-Polκ, GFP-Rev1, and GFP-Rev7 foci in MRC5 fibroblasts treated with control (NC) or Rad18 siRNA.

Figure S7. Plasmid system for analyzing TLS opposite DNA lesions in a gapped plasmid. Two plasmids, one [pGAP-(6-4)PP)] carrying the DNA lesion (T^T) opposite a gap of 11 nts and the other (pGAP-ND) with a gap in undamaged DNA are co-transfected in pre-siRNA treated normal human fibroblast (HF) cells. After transfection, the plasmid DNA is isolated from cells and transformed into XL-1 blue *E. coli* cells. Transformed *E. coli* cells are grown on LB/zeocin and LB/ampicillin plates. Gap filling frequency is determined by the ratio of Amp^R/Zeo^R colonies. The (6-4) TT photoproduct (Supplemental Table S6) or the *cis-syn* TT dimer (Supplemental Table S7) were present in the same sequence context as reported previously (Yoon et al., 2009, Proc. Natl. Acad. Sci., USA 106:18219; Yoon et al., 2010, Genes Dev 24:123).

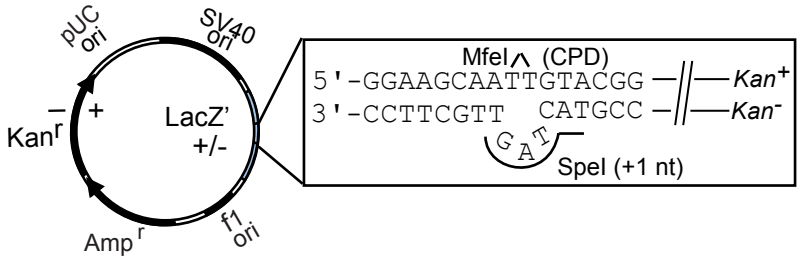
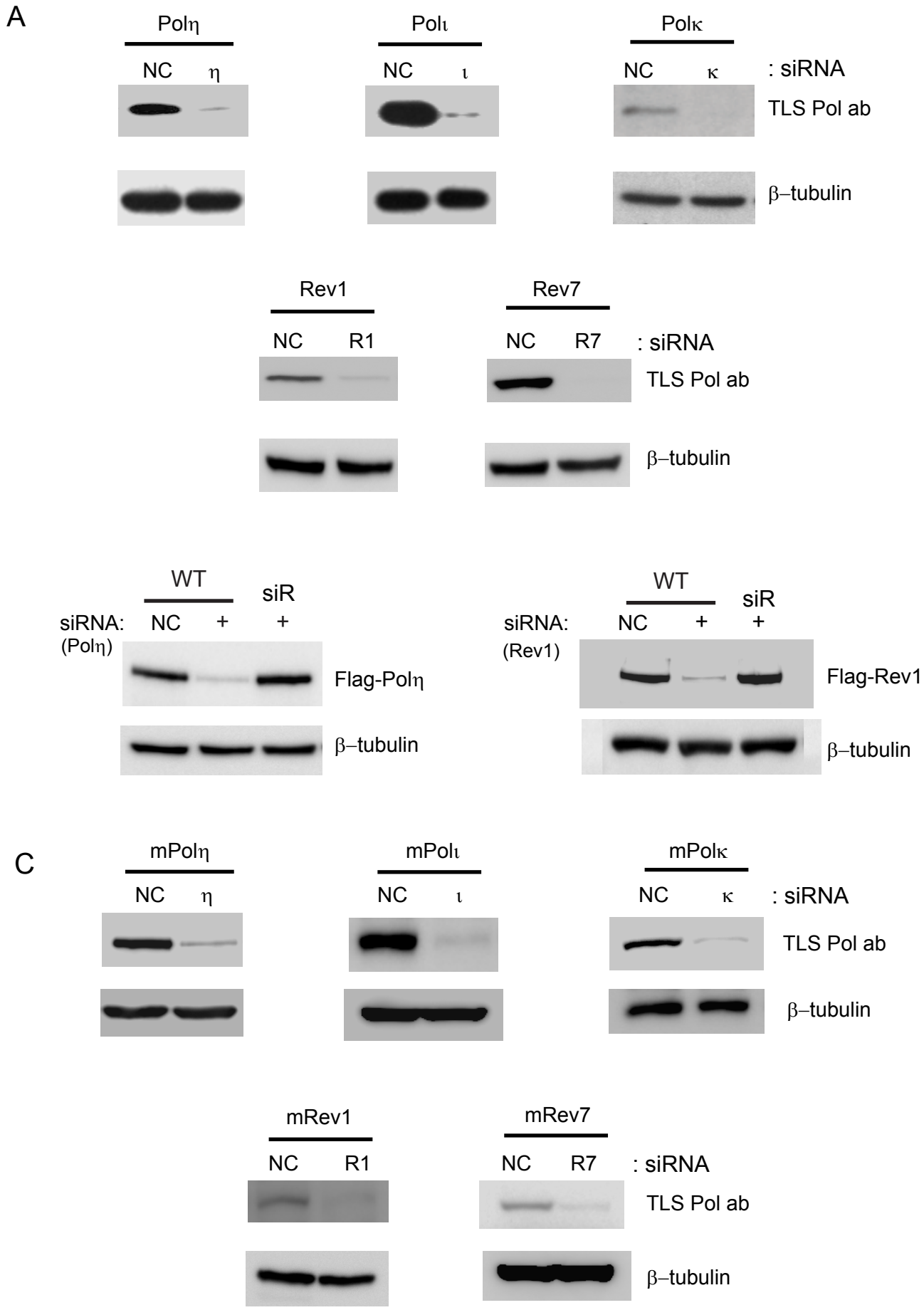

pUC ori
SV40 ori
Kan^r
–
+
LacZ'
+/-
Amp^r
f1 ori
MfeI^ (CPD)
5'-GGAAGCAATTGTACGG
3'-CCTTCGTT CATGCC
Kan+
Kan-
G A T
SpeI (+1 nt)

Figure S1


A
Polη
Polι
Polκ
NC
η
NC
ι
NC
κ
: siRNA
TLS Pol ab
β–tubulin
Rev1
Rev7
NC
R1
NC
R7
: siRNA
TLS Pol ab
β–tubulin
B
WT
siR
siRNA:
(Polη)
NC
+
+
Flag-Polη
β–tubulin
WT
siR
siRNA:
(Rev1)
NC
+
+
Flag-Rev1
β–tubulin
C
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mPolι
mPolκ
NC
η
NC
ι
NC
κ
: siRNA
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R1
NC
R7
: siRNA
TLS Pol ab
β–tubulin

Figure S2

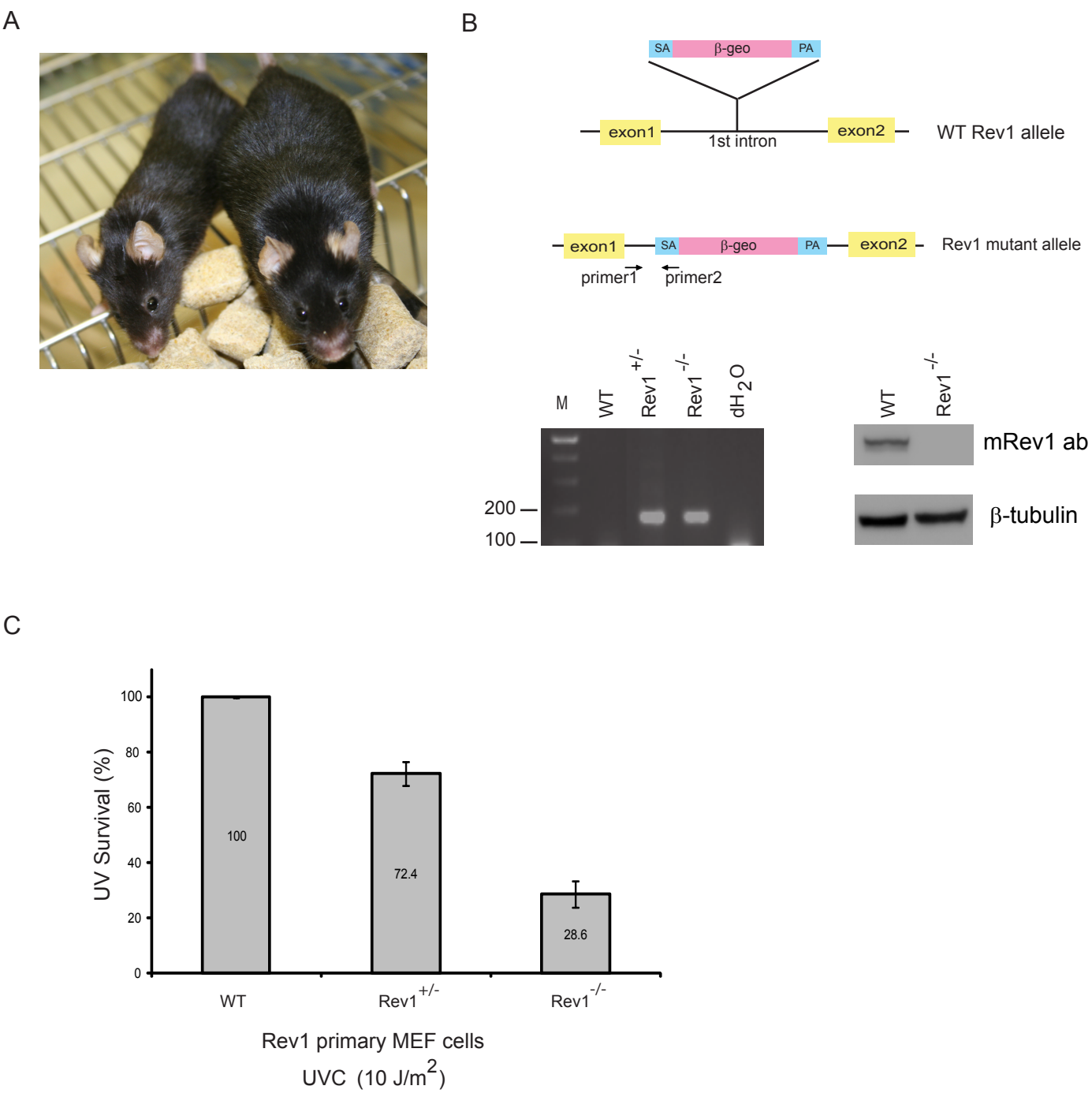

A
B
SA
β-geo
PA
exon1
1st intron
exon2
WT Rev1 allele
exon1
SA
β-geo
PA
exon2
Rev1 mutant allele
primer1
primer2
M
WT
Rev1+/-
Rev1-/-
dH2O
200
100
WT
Rev1-/-
mRev1 ab
β-tubulin
C
UV Survival (%)
100
80
60
40
20
0
100
72.4
28.6
WT
Rev1+/-
Rev1-/-
Rev1 primary MEF cells
UVC (10 J/m2)

Figure S3

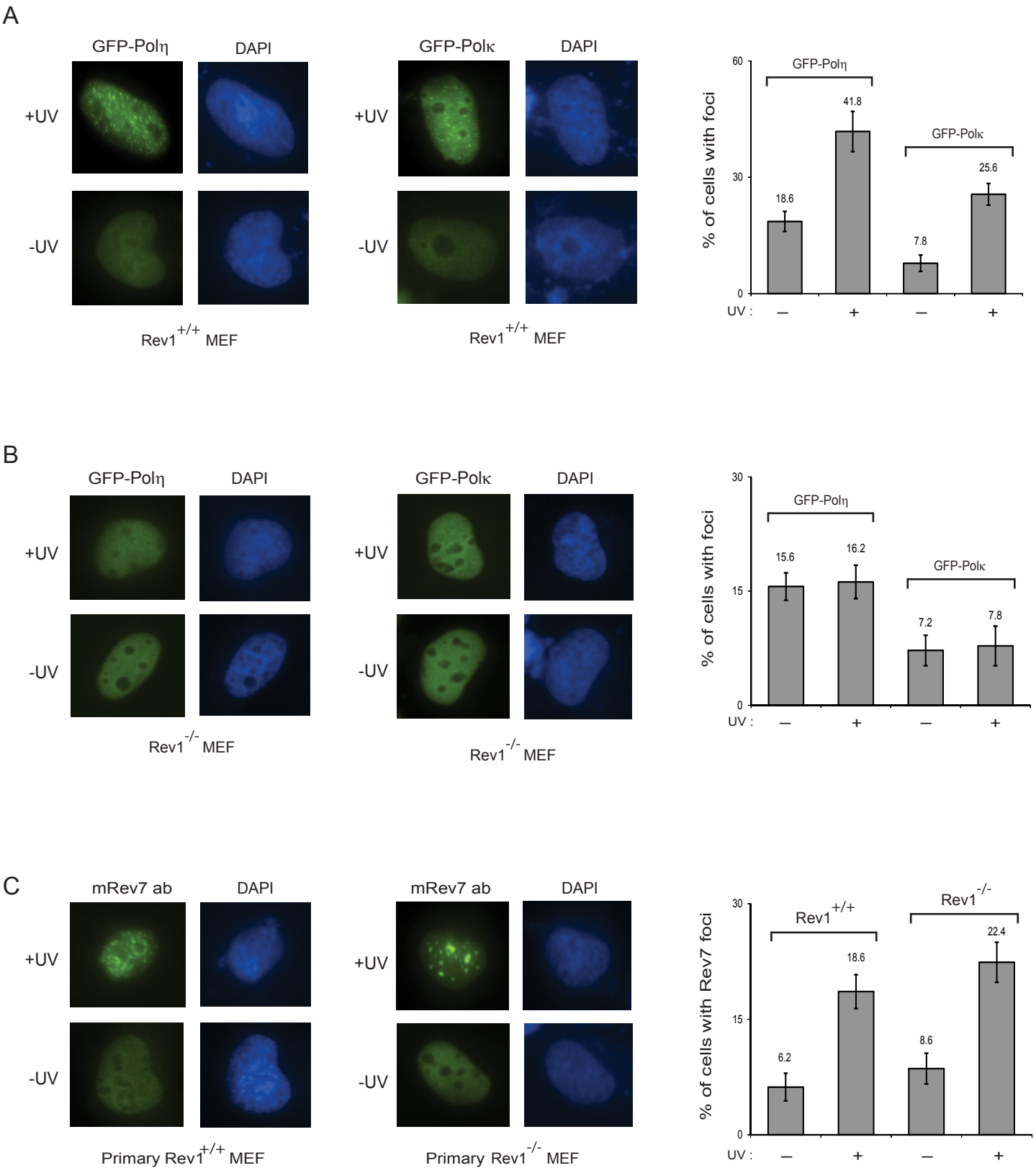

A
GFP-Polη
DAPI
+UV
-UV
Rev1+/+ MEF
GFP-Polκ
DAPI
+UV
-UV
Rev1+/+ MEF
% of cells with foci
GFP-Polη
41.8
GFP-Polκ
25.6
18.6
7.8
60
30
0
UV : — + — +
B
GFP-Polη
DAPI
+UV
-UV
Rev1-/- MEF
GFP-Polκ
DAPI
+UV
-UV
Rev1-/- MEF
% of cells with foci
GFP-Polη
15.6
16.2
GFP-Polκ
7.2
7.8
30
15
0
UV : — + — +
C
mRev7 ab
DAPI
+UV
-UV
Primary Rev1+/+ MEF
mRev7 ab
DAPI
+UV
-UV
Primary Rev1-/- MEF
% of cells with Rev7 foci
Rev1+/+
Rev1-/-
18.6
22.4
6.2
8.6
30
15
0
UV : — + — +

Figure S4

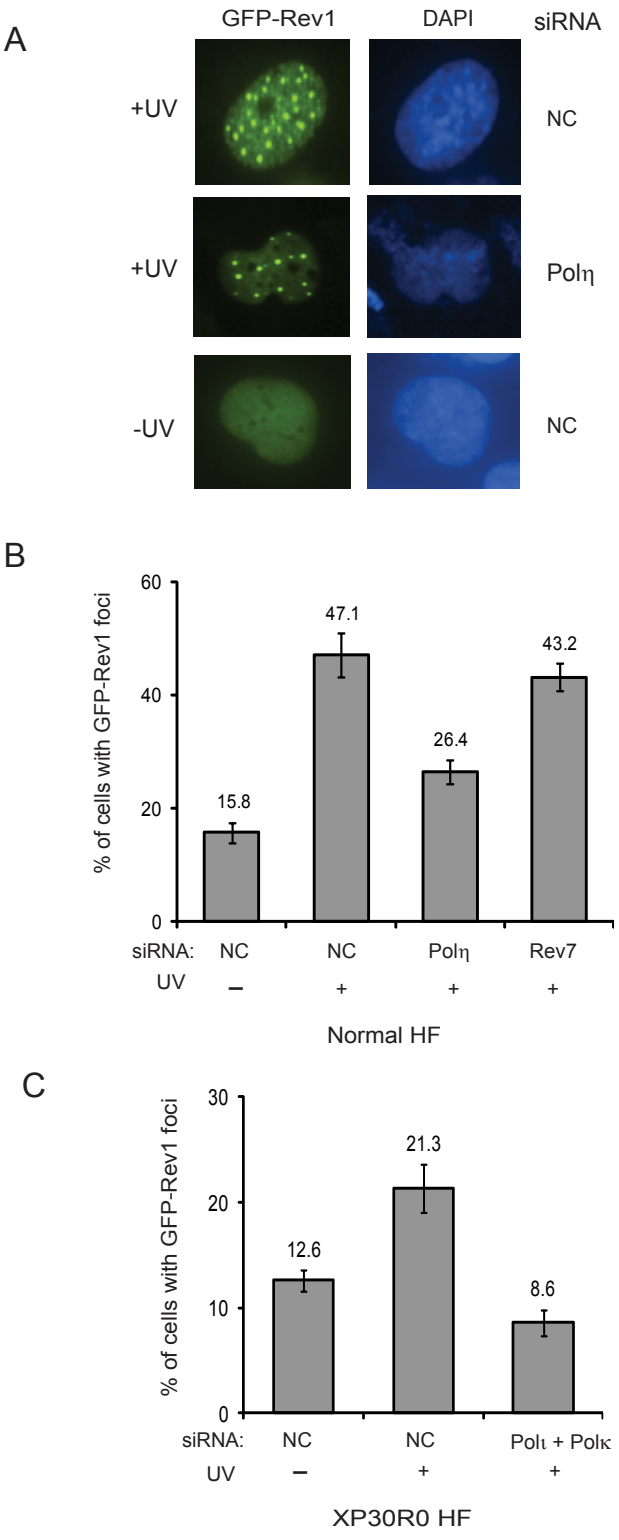
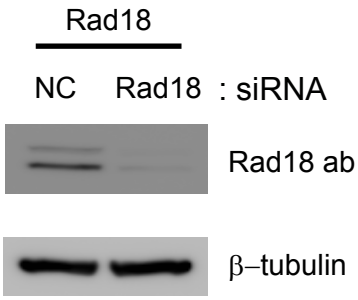

A
GFP-Rev1
DAPI
siRNA
+UV
NC
+UV
Polη
-UV
NC
B
% of cells with GFP-Rev1 foci
60
40
20
0
47.1
43.2
26.4
15.8
siRNA:
NC
NC
Polη
Rev7
UV
–
+
+
+
Normal HF
C
% of cells with GFP-Rev1 foci
30
20
10
0
21.3
12.6
8.6
siRNA:
NC
NC
Polι + Polκ
UV
–
+
+
XP30R0 HF

Figure S5

A



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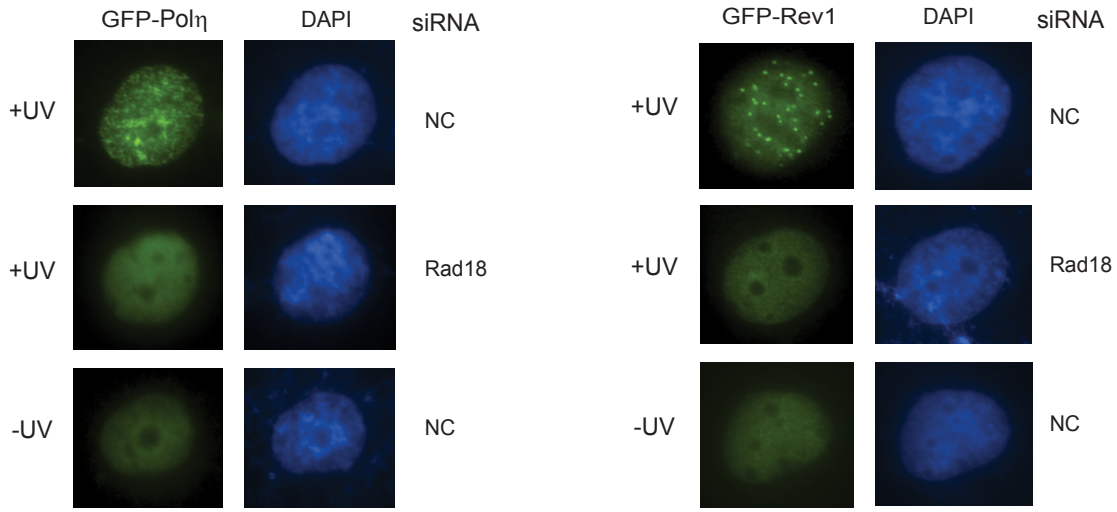

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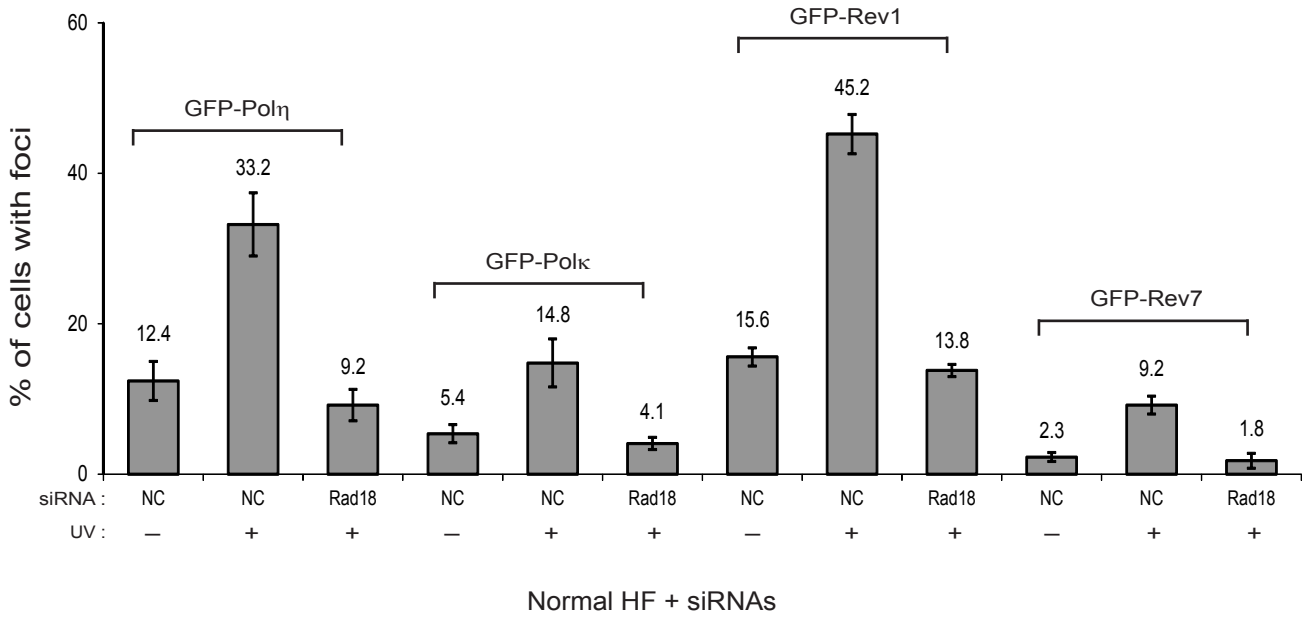

Figure S6

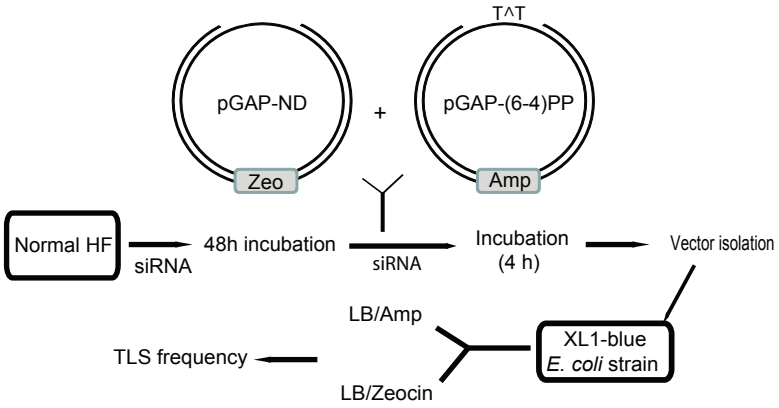
T^T
pGAP-ND
+
pGAP-(6-4)PP
Zeo
Amp
Normal HF
siRNA
48h incubation
siRNA
Incubation
(4 h)
Vector isolation
LB/Amp
XL1-blue
E. coli strain
TLS frequency
LB/Zeocin

FIGURE S7