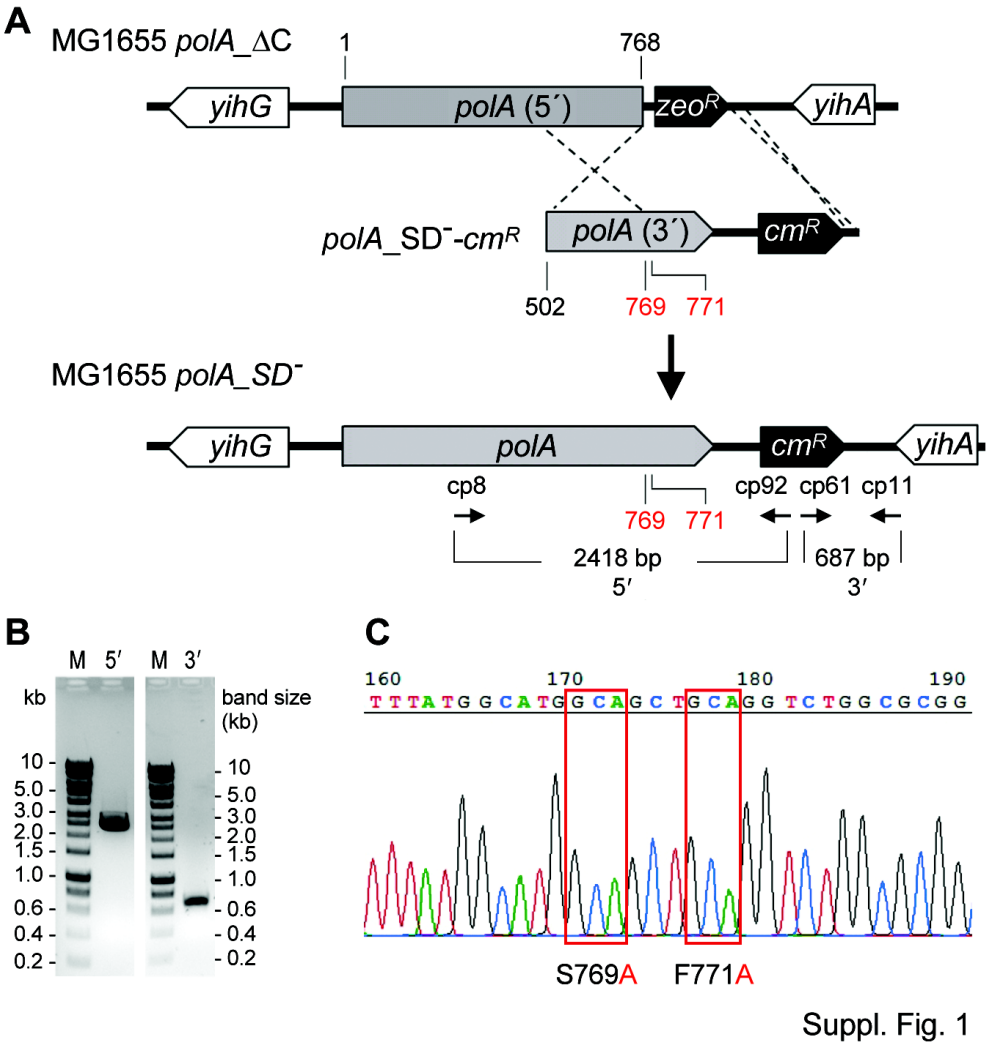

Suppl. Fig. 1

Fig. S1. Introduction of S769(AGT)→A769(GCA) and F771(TTC)→A771(GCA) mutations into chromosomal *polA* gene of *E. coli* MG1655. **A**. Codon replacement within the chromosomal *polA* gene done in two recombineering steps. First, the 3' *polA* region (codon 769 to the ochre stop codon) was replaced with a Zeo^R cassette. In a second recombineering step, the *polA*_SD$^-$ ::*cat* allele replaced the Zeo^R cassette and introduced the altered S769A (AGT → GCA) and F771A (TTC → GCA) codons into the *polA* locus. **B**. PCR-amplification of genomic fragments from MG1655 *polA*_SD$^-$ ::*cat* with primers cp8 and cp92 and cp61 and cp11 (Table 3) respectively, yielded fragments of 2418 bp and 687 bp characteristic for a successful *polA*_SD$^-$ ::*cat* insertion. M, DNA size markers. **C**. Trace DNA sequencing file displaying the intended AGT →GCA (Ser769) and TTC→GCA (F771) *polA* mutations in the genome of *E. coli* MG1655 *polA*_SD$^-$ ::*cat*.

Supplementary Table 1: Plasmids used in this study

Strain	Relevant Characteristics	Source or Reference
pRed/ET	Red/ET expression plasmid; pSC101 based; Ap^R	Gene Bridges
pSKpolAint	pBluescript derivative with 3'-end of the wild-type *polA* gene and closely linked *cat* gene; Ap^R, Cm^R	[1]
pJM993	pSKpolAint derivative: 3'- end of *polA* encodes S769(AGT)→A769 (GCA) and F771(TTC) → A771(GCA) point mutations and closely linked *cat* gene; Ap^R, Cm^R	This work
pGB2	low-copy-number, pSC101-derived vector: Spc^R	[2]
pRW134	pGB2 derivative expressing *umuD'C* from the native *umuDC* promoter; Spc^R	[3]
pJM963	pRW134 derivative expressing *umuD'* and *umuC*_Y11A from the native *umuDC* promoter: Spc^R	[4]

Supplementary Table 2: Oligonucleotides used in this study

Name	Sequence	Source and Reference
cp6	5'-CAGATGTCACCTTGCAGTTGC	BioSpring
cp8	5'-AATGGCAGCGAAGCTCGAGC	BioSpring
cp11	5'-GGCGGTAGACAGCAATATCG	BioSpring
cp61	5'-GGCTTCCATGTCGGCAGAATG	BioSpring
cp92	5'-TATCAACGGTGGTATATCCAG	BioSpring
p2	5'-GTGACAGCTTATGTTGCTTACTTACGAAAAAAGGCAT GTTCAGGCGAATCTTACGCCCCGCCCTGCCACTC	BioSpring
p3	5'-AGCAACGCCGTAGCGCGAAAGCGATCAACTTTGGTCT GATTTATGGCATGAATTAACCCTCACTAAAGGGCG	BioSpring
P4	5'-GTGACAGCTTATGTTGCTTACTTACGAAAAAAGGCAT GTTCAGGCGAATCTAGCACGGAGTTCATTAGGGCTC	BioSpring
polA-FD424	5'-CGGACTGGATACGCTGTATGC	Lofstrand/[1]
polA-RD424	5'- CTTCCAGTACCTCTTCCGACG	Lofstrand/[1]
polA-RBamHI	5'-GCTCGGATCCTTTAGTGCGCCTGATCCCAGT	Lofstrand
polA_F2105	5'-GCCAGAGGATTATGTGATTGT	Lofstrand
SSP1	5'-ATGGTACGGACGTGCTT	Lofstrand
SSP1-27	5'-ATGGTACGGACGTGCTTTAGTCGTTAA	Lofstrand
FLAPU	5'-UAGTCGTTAATCATTAGTACCAGTATCGACAG	Lofstrand
XAG49[a]	5'-CTGTCGATACTGGTACTAATGA**X**TAACGACTAAAGCA CGTCCGTACCAT	Lofstrand

[a]:**X** denotes the location of the synthetic abasic site

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