Table S1: Oligos used for *delitto perfetto*

Relevant allele	Oligo sequence corresponding to the coding (non-transcribed) strand of *pTET-lys2FΔA746NR*
pTET-lys2FΔA746NR,(CCCTT)$_2$net-1 (detects 4-bp deletions when Top1 sites are 5-bp apart)	5′-CGAGCTAGCT GAATCAATTC AAAGTTGCCA AGCATCCCTT CCCTTTGCAA GATCTGGAAA GGAGGCCTCA GTTGTTCCGT TTGGCC
pTET-lys2FΔA746NR,(CCCTTCCTT) (detects 4-bp deletions when Top1 sites are 4-bp apart)	5′-TTGACGAGCT AGCTGAATCA ATTCAAAGTT GCCAAGATCG CATCCCTTCC TTTGCAAAGA TCTGGAAAGG AGGCCTCAGT TGTTCCGTTT GGCCTGTCTG
pTET-lys2FΔA746NR,(CCCTTTTCCCTT) (detects 7-bp deletions when Top1 sites are 7-bp apart)	5′-TGACGAGCTA GCTGAATCAA TTCAAAGTTG CCAAGATCGC ATCCCTTTTC CCTTTGCAAA GATCTGGAAA GGAGGCCTCA GTTGTTCCGT TTGGCCTGTC
pTET-lys2FΔA746NR,(CCCTT)$_3$ (detects 5-bp deletions)	5′-ACGAGCTAGC TGAATCAATT CAAAGTTGCC AAGATCAGCA TCCCTTCCCT TCCCTTTGCA AAGATCTGGA AAGGAGGCCT CAGTTGTTCC GTTTGGCCTG

Table S2. Deletion rates at the $(CCCTT)_2$ hotspot and derivatives

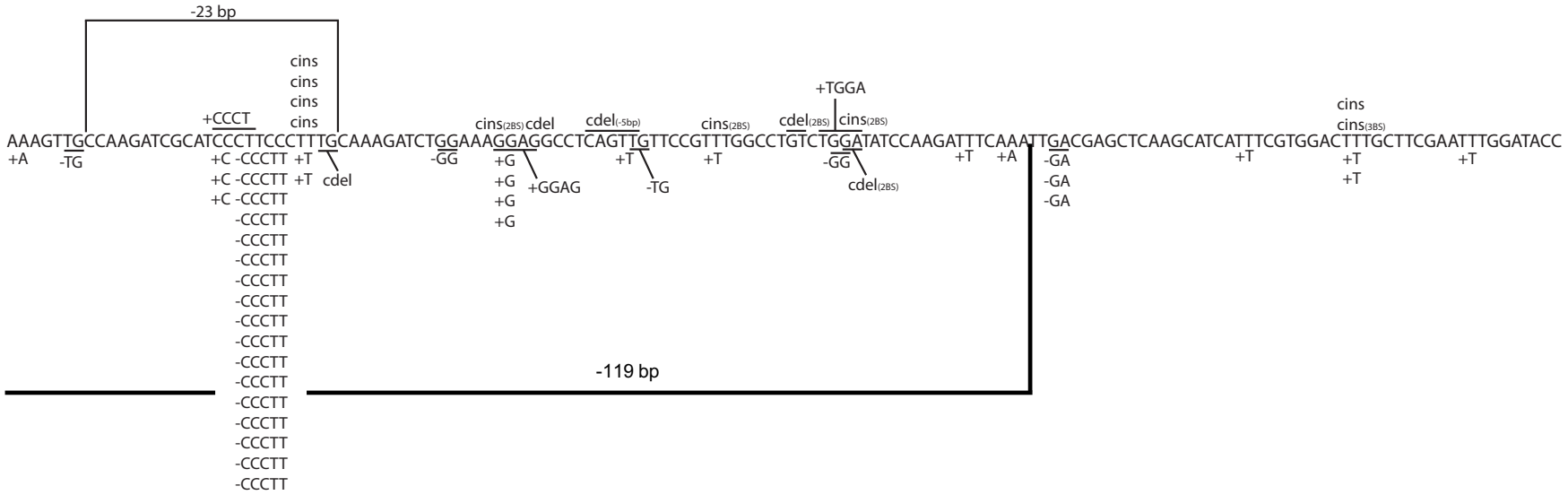
Repeat sequence	Relevant genotype	Strain (SJR#)	Lys^+ rate x 10^{-10} (95% CI)	Deletion fraction	Deletion rate x 10^{-10}
$(CCCTT)_2$ 5-bp deletion	*WT*	3464	277 (222-351)	17/61	77.2
	rnh201Δ	3478	1360 (1250-1570)	7/46	210
	rnh201Δ top1Δ	3640	1070 (967-1440)	0/45	<24
	rnh201Δ pol2-M644G	3605	10100 (9510-11600)	42/87	4880
	rnh201Δ pol2-M644G top1Δ	3628	2210 (1890-2500)	0/36	<61.4
CCCTTCCTT 4-bp deletion	*WT*	3747	830 (675-1080)	39/46	704
	rnh201Δ pol2-M644G	4289	18000 (16500-19900)	40/46	15700
	rnh201Δ pol2-M644G top1Δ	4290	1590 (1370-2020)	0/48	<33
CCCTTTTCCCTT 7-bp deletion	*WT*	3749	233 (167-278)	1/45	5.18
	rnh201Δ pol2-M644G	4291	4770 (4270-5500)	6/150	191
	rnh201Δ pol2-M644G top1Δ	4292	2050 (1680-2250)	0/64	<32
$(CCCTT)_3$ 5-bp deleton	*WT*	3745	960 (771-1220)	42/46	877
	top1Δ	3773	387 (316-417)	19/46	160
	rnh201Δ	3746	3640 (3280-4270)	16/47	12405
	rnh201Δ top1Δ	3770	2180 (1590-2810)	4/47	186
	rnh201Δ pol2-M644G	4287	32200 (29200-35600)	40/47	27400
	rnh201Δ pol2-M644G top1Δ	4288	5250 (4530-5830)	3/46	342
$(CCCTT)_2$ 4-bp deletion	*WT*	3946	134 (94.7-171)	3/68	5.91
	rnh201Δ	3947	969 (886-1250)	1/78	14.9
	rnh201Δ pol2-M644G	4778	3960 (3530-4210)	0/105	<38
	rnh201Δ pol2-M644G top1Δ	4779	1940 (1650-2460)	0/104	<19

When no events were observed, rates were calculated assuming 1 event.

CI, 95% confidence interval

Figure S1

A Wild type $(CCCTT)_2$, N=61



B *rnh201Δ* $(CCCTT)_2$, N=46

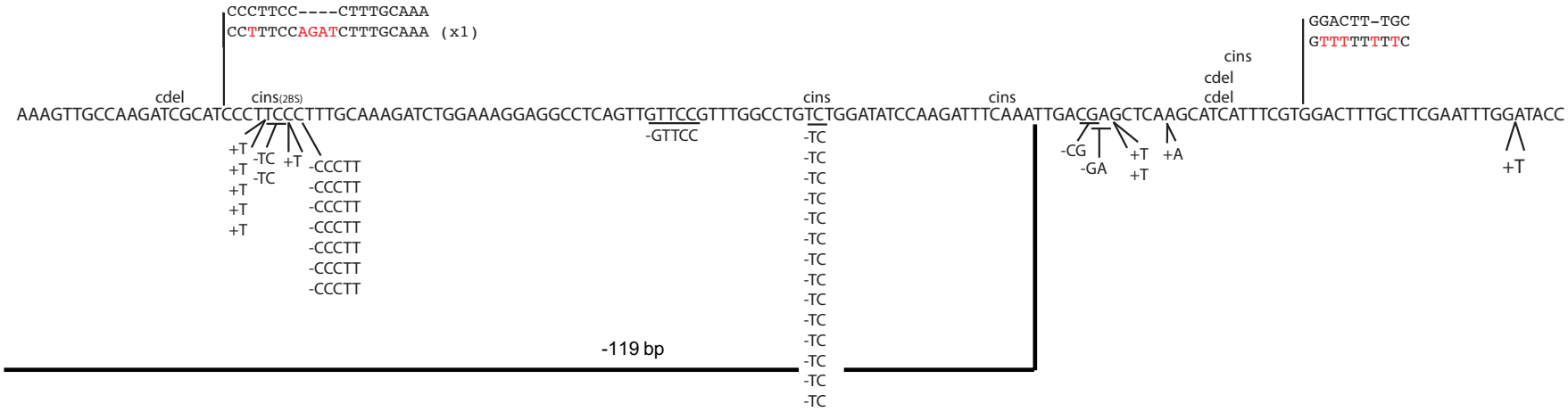


Figure S2

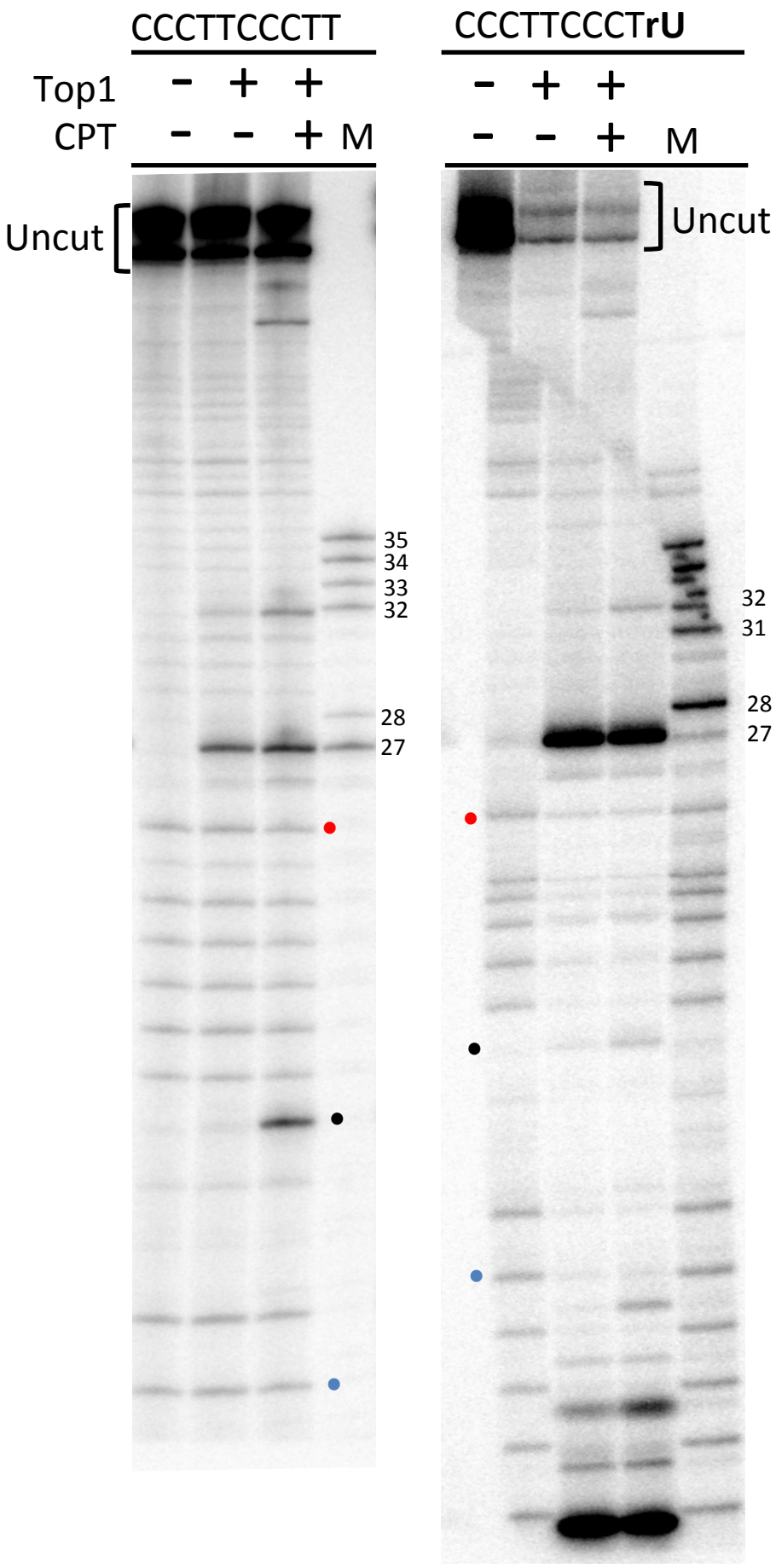

CCCTTCCCTT
CCCTTCCCTrU
Top1
CPT
- + +
- - + M
- + +
- - + M
Uncut
Uncut
35
34
33
32
28
27
32
31
28
27

Figure S3

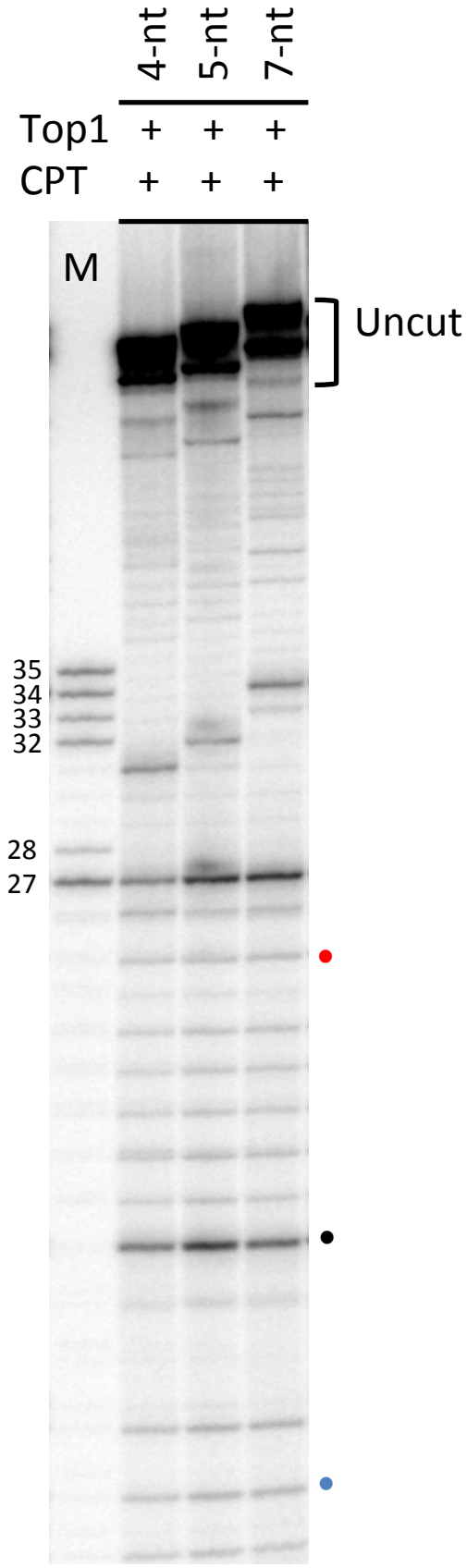
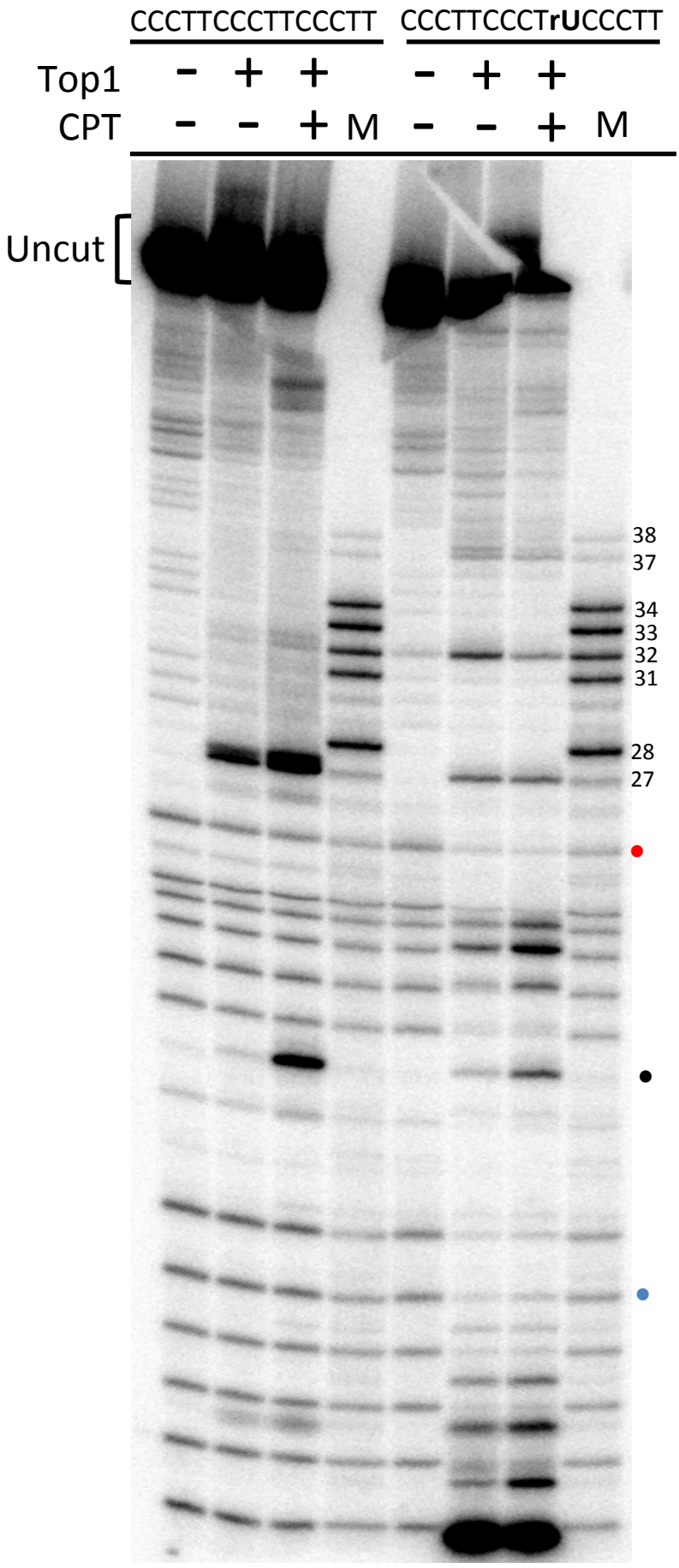


Figure S4


CCCTTCCCTTCCCTT
CCCTTCCCTrUCCCTT
Top1
CPT
- + + - + +
- - + M - - + M
Uncut
38
37
34
33
32
31
28
27

SUPPLEMENTARY FIGURE LEGENDS

Figure S1: **Mutation spectra of *pTET-lys2::(CCCTT)$_2$* revertants in WT and *rnh201Δ* backgrounds.** A portion of the *pTET-lys2::(CCCTT)$_2$* coding sequence is shown. "+" and "-" indicate small insertions and deletions, respectively, of the indicated bases. "cins" and "cdel" indicate complex insertion or deletions, respectively, where the selected frameshift is accompanied by a base substitution(s) within 10 bp. Deletions larger than 5 bp are indicated by a bracket. N, number of independent revertants sequenced.

Figure S2: Mapping of Top1 incision sites in (CCCTT)$_2$ and CCCTTCCCTrU.
Shown are the original autoradiographs for the data in Figure 2C. As expected for relatively irreversible cleavage at rU, there is a decrease in uncut substrate and an increase in the 27-nt product with the CCCTTCCCTrU substrate. Red, black and blue dots indicate fragments of the same size on the two gels. The black dot indicates the position of a CPT-sensitive cleavage product. Uncut, intact substrate; M; size markers; CPT; camptothecin.

Figure S3: Mapping of Top1 incision sites in (CCCTT)$_2$, CCCTTCCTT and CCCTTTTCCCTT. Shown are the original autoradiographs for the data in Figure 3A. Red, black and blue dots indicate fragments of the same size as in Figure S2. Uncut, intact substrate; M, size markers; CPT, camptothecin.

Figure S4: Mapping of Top1 incision sites in (CCCTT)$_3$ and CCCTTCCCTrUCCCTT. Shown are the original autoradiographs for the data in Figure 6B. Substitution of rU for the terminal thymine of the central repeat enhances the 37-nt and 32-nt cleavage products. Red, black and blue dots indicate fragments of the same size as in Figure S2. Uncut, intact substrate; M, size markers; CPT, camptothecin.