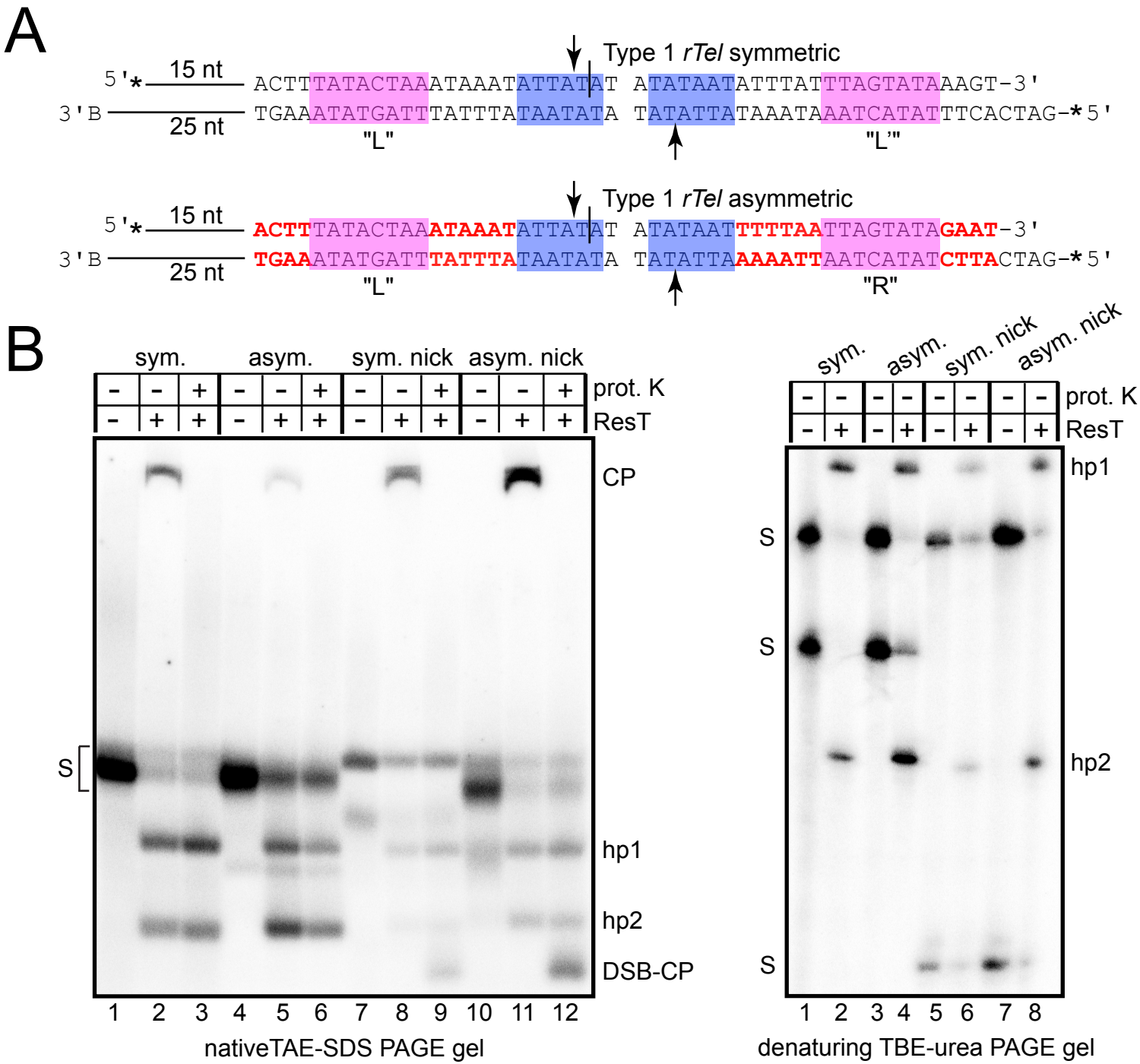
Supplementary Table 1. Oligonucleotides used in this study.

Name (OGCB)	Length (nt)	Sequence
75	65	5′-TCCTCTAACCATTGCTATTTATACTAATTAATAATAAATTAATTTATTATCTTTTAG TATAATGA-3′
77	65	5′-TCCTCTAACCATTGCACTTTATACTAAATAAATATTATATATATAATTTTTAATTAG TATAGAAT-3′
79	39	5′-TCCTCTAACCATTGCTATTTATACTAATTAATAATAAAT-3′
80	26	5′-TAATTTATTATCTTTTAGTATAATGA-3′
81	38	5′-TCCTCTAACCATTGCACTTTATACTAAATAAATATTAT-3′
82	27	5′-ATATATAATTTTTAATTAGTATAGAAT-3′
85	69	5′-GATCTCATTATACTAAAAGATAATAAATTAATTTATTATTAATTAGTATAAATAGCA ATGGTTAGAGGA-3′BIOTIN
86	74	5′-GATCTCATTATACTAAAAGATAATAAATTAATTTATTATTAATTAGTATAAATAGCA ATGGTTAGAGGAATCAT-3′BIOTIN
87	79	5′-GATCTCATTATACTAAAAGATAATAAATTAATTTATTATTAATTAGTATAAATAGCA ATGGTTAGAGGAATCATATCAT-3′BIOTIN
88	69	5′-GATCATTCTATACTAATTAAAAATTATATATATAATATTTATTTAGTATAAAGTGCA ATGGTTAGAGGA-3′BIOTIN
89	74	5′-GATCATTCTATACTAATTAAAAATTATATATATAATATTTATTTAGTATAAAGTGCA ATGGTTAGAGGAATCAT-3′BIOTIN
90	79	5′-GATCATTCTATACTAATTAAAAATTATATATATAATATTTATTTAGTATAAAGTGCA ATGGTTAGAGGAATCATATCAT-3′BIOTIN
91	65	5′-TCCTCTAACCATTGCTATTTATACTAATTAATAATAAATTAATTTATTATCTTTTAG TATAATGA-3′
92	28	5′-GATCTCATTATACTAAAAGATAATAAAT-3′
93	51	5′-TAATTTATTATTAATTAGTATAAATAGCAATGGTTAGAGGAATCATATCAT -3′BIOTIN
94	65	5′-TCCTCTAACCATTGCTCATTATACTAAAAGATAATAAATTAATTTATTATTAATTAG TATAAATA-3′
95	79	5′-GATCTATTTATACTAAATTATAATAAATTAATTTATTATCTTTTAGTATAATGAGCA ATGGTTAGAGGAATCATATCAT-3′BIOTIN
96	49	5′-TAGTATAGTATCCTCTAACCATTGCTATTTATACTAATTAATAATAAAT-3′
97	64	5′-AGGAGATTGGAACGTGAAATATGATTTATTTATAATATATATATTAAAAATTAATCA TATCTTA-3′
98	69	5′-CTAGTAAGATATGATTAATTTTTAATATATATATTATAAATAAATCATATTTCACGT TACCAATCTCCT-3′
99	65	5′-TCCTCTAACCATTGCACTTTATACTAAATAAATATTATATATATAATATTTATTTAG TATAAAGT-3′
100	65	5′-GATCACTTTATACTAAATAAATATTATATATATAATATTTATTTAGTATAAAGTGCA ATGGTTAGAGGAATCATATCAT-3′
101	39	5′-TCCTCTAACCATTGCACTTTATACTAAATAAATATTATA-3′
102	26	5′-TATATAATATTTATTTAGTATAAAGT-3′

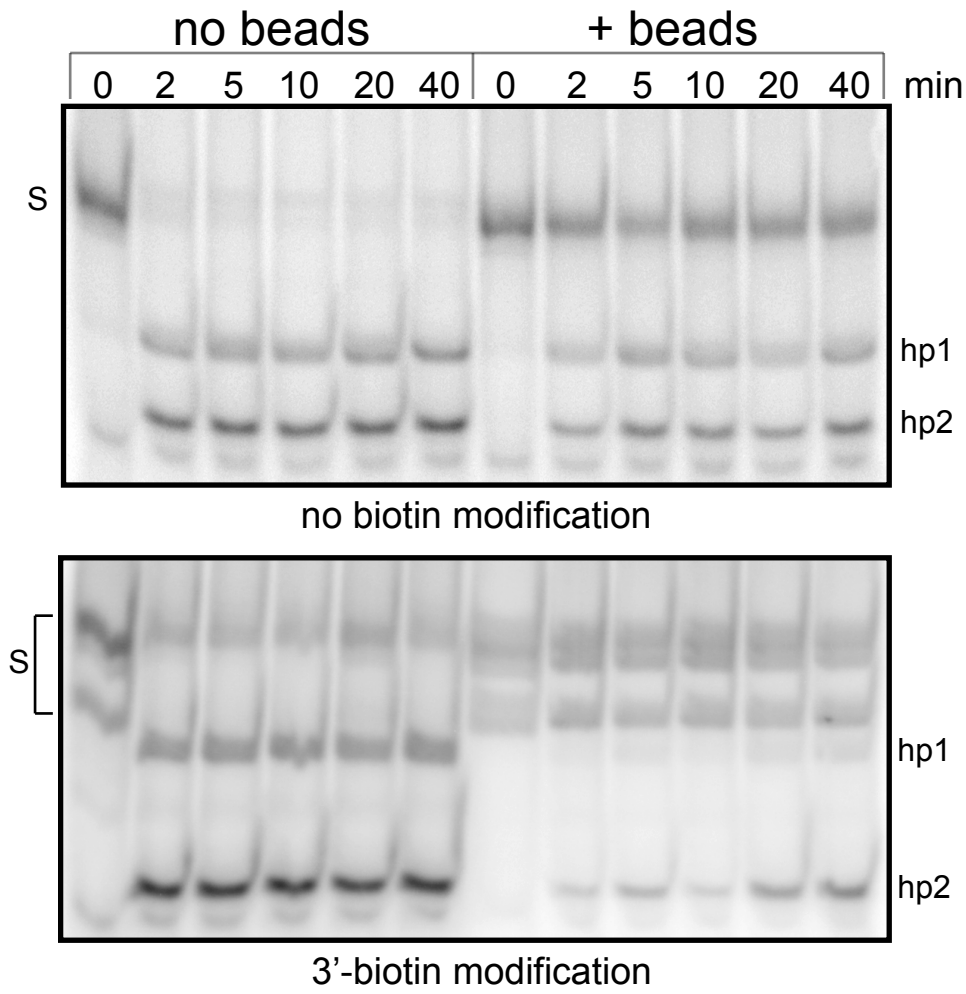
Supplementary Fig. 1. Confirmation that asymmetric *rTel* substrates yield the same products as their 'natural' symmetric counterparts. A) A schematic of the substrates used. The top substrate represents an asymmetric *rTel* with the "L" half sequence derived from the sequence of the telomeres lp17R and lp56R and the "R" side sequence derived from the ChromR telomere (31). Such asymmetric *rTels* were designed to facilitate substrate construction free of contamination with self-annealed hairpins that would complicate the interpretation of the results with immobilized versions of these substrates. The resulting asymmetric *rTels* now have distinct "L" and "R" sides where the sequence of different Type 1 *rTels* varies (red letters). The bottom substrate represents a 'natural' symmetric *rTel* that would result from replication of a hairpin telomere with the "L" sequence. The magenta and blue boxes highlight sequences found in all Type 1 *rTels*; the arrows indicate the scissile phosphates and the black lines through the sequence represent the position of nicks introduced into the *rTels* to produce nicked 'suicide' versions of the substrates. 15 bp of non-telomeric sequence was added the left side and the biotin moiety (B) is on the terminus of an additional 10 nt of ssDNA. These extensions were added to eliminate steric clash and to increase the gel mobility differences of the substrate and products. The asterisks on the 5' ends represent ^{32}P-endlabels.
B) 10% native TAE-SDS PAGE and 10% denaturing TBE-urea PAGE analysis of 10 min. telomere resolution reactions with the substrates in A) (see conditions for Fig. 5). In the reaction key above the gels it is indicated whether the ResT was included in the incubation and whether after the reaction the reaction was treated with protease K. Protease K removes ResT covalently attached to the DNA in the cleavage product (CP; see schematic in Fig. 2B). On the gel labels S is substrate; hp1 & hp2 are the hairpin telomere products; CP is the cleavage product that has not yet formed a hp; DSB-CP is the double-strand break cleavage product left by protease K digestion of CP; * marks the position of contaminating self-annealed hairpins and partially annealed nicked *rTels* present in the constructions of the symmetric *rTels*.

Supplementary Fig. 2. Effect of adding streptavidin beads to reactions with substrates with and without the 3'-biotin moiety. 10% native TAE-SDS PAGE analysis of timecourse reactions with a Type 1 *rTel* (Fig. 2). The top panel shows reactions of the substrate without the 3'-biotin modification under conditions without bead addition (left) and with bead addition (right). The bottom panel shows the same reaction conditions using the same substrate carrying the 3'-biotin modification on the bottom strand. On the gel labels S is substrate. hp1 & hp2 are the hairpin telomere products. The reaction of the 3'-biotin containing substrate in the presence of beads is visualized without separation of the bead and supernatant fractions; the whole reaction is boiled to liberate the unreacted substrate and hp1 from the beads (because of this treatment the unreacted substrate migrates as two bands on the gel).

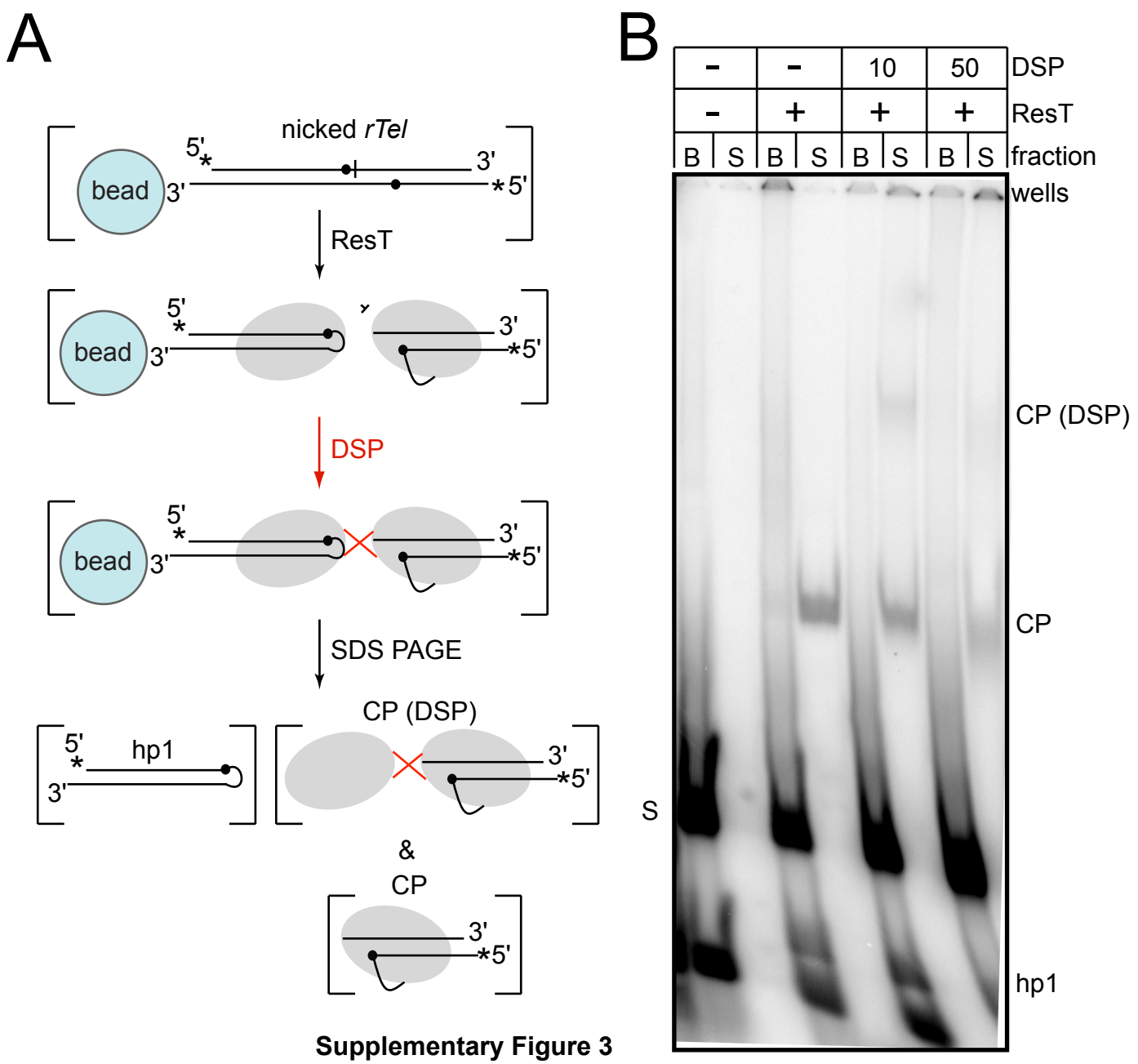
Supplementary Fig. 3. DSP-crosslinking a second ResT protomer to the ResT protomer trapped in the cleavage product (CP) produced by reaction with an immobilized nicked *rTel*.
A) Schematic representation of the protein-protein crosslinking experiment with the Type 1 nicked *rTel* used in Figs. 2 & 4 (see Fig. 2A & B). ResT is incubated with the immobilized nicked *rTel* under conditions that maximize the yield of the cleavage product (CP) in which ResT is covalently attached to the radiolabeled DNA (no glycerol in the reaction buffer; 30^{o}C, 30 min. incubation; see also Fig. 5). Dithiobis (succinimidyl propionate) (DSP) was added to induce crosslinking. The resulting crosslinked species are visualized on 4.5% TAE-SDS PAGE. ResT is represented by shaded ovals, the line connecting the ResT to the scissile phosphate (●) indicates the covalent 3'-phosphotyrosine linkage trapped by cleavage next to the nick on the top strand. CP and CP (DSP) are covalent ResT-DNA complexes with the radiolabeled half-site derived from the right side of the *rTel* after cleavage; crosslinking is visualized by virtue of attachment to this half-site. hp1 is the hairpin telomere derived from the left side of the *rTel*.
B) 4.5% TAE-SDS PAGE analysis of the results of DSP crosslinking a reaction with a Type 1 nicked *rTel*. The key above the gel indicates whether ResT has been added to the incubation and if DSP treatment has followed the reaction (two concentrations of DSP were used; 10 μg/ml & 50 μg/ml). B denotes recovery from the bead fraction and S from the supernatant (after addition of SDS). The gel labels are explained in A). A 20 cm long vertical gel apparatus was used.



Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3