Supplementary Information

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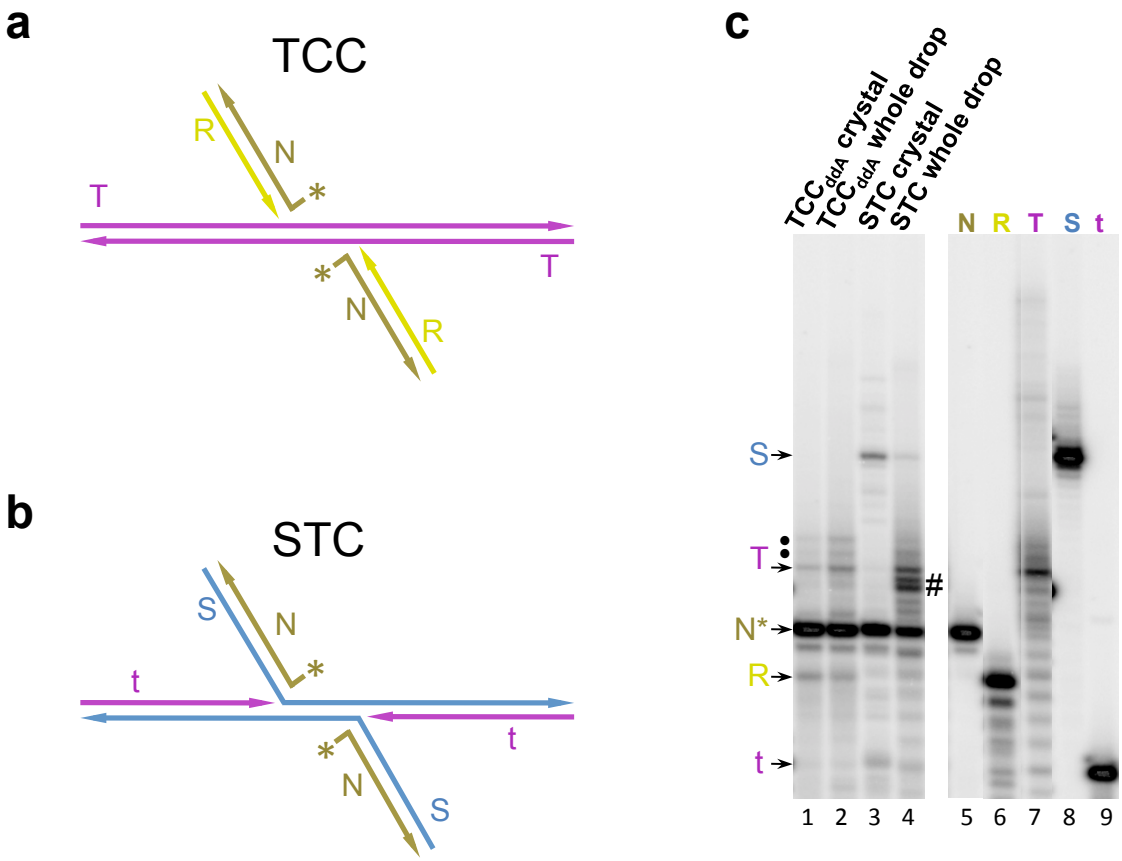
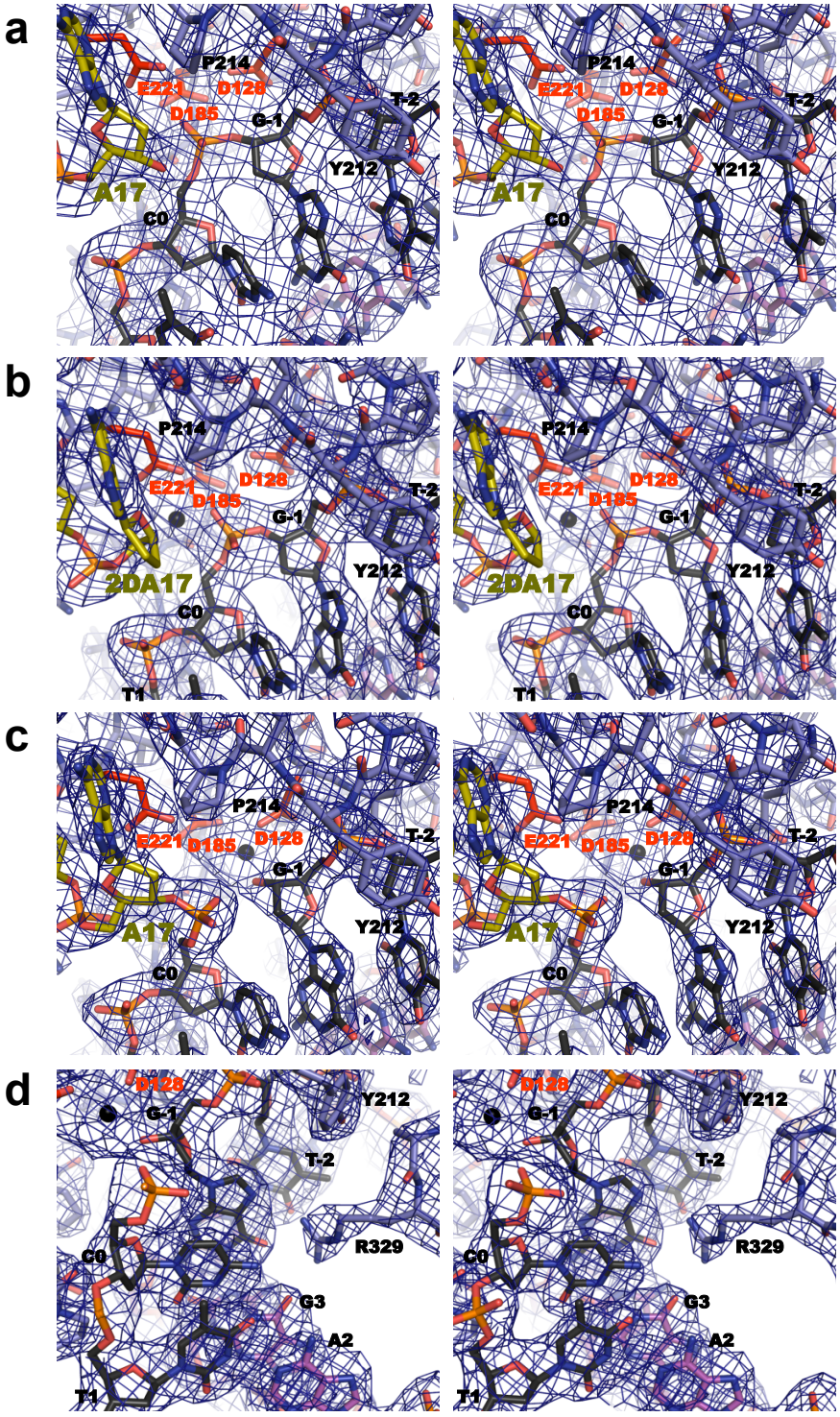
Supplementary Table 1. X-ray diffraction data collection and refinement statistics.

	STC	**TCC_{Apo}**	**TCC_{ddA}**
Data collection			
Space group	$P4_12_12$	$P4_12_12$	$P4_12_12$
Cell dimensions *a*, *b*, *c* (Å)	159.9, 159.9, 125.1	161.0, 161.0, 127.9	160.0, 160.0, 127.8
α,β,γ (°)	90	90	90
Resolution (Å)	40-2.81 (2.96-2.81)*	40-3.32 (3.50-3.32)	40-2.97 (3.13-2.97)
R_{merge} (%)	8.8 (99.8)	9.0 (99.0)	9.8 (76.3)
$I/\sigma(I)$	15.9 (2.0)	15.9 (2.0)	9.7 (2.0)
Completeness (%)	99.1 (99.7)	99.8 (100.0)	99.6 (100)
Redundancy	5.7	6.4	4.6
Refinement			
Resolution	40-2.81	40-3.32	40-2.97
No. reflections:			
work	37,526	24,011	32,775
free	1,959	1,288	1,710
R_{work}/R_{free} (%)	20.8/24.0	22.3/25.9	23.1/26.3
No. atoms:			
protein	4,323	4,162	4,162
DNA	1,098	1,060	1,059
water/ion	7	6	8
Average B ($Å^2$):			
protein	61.3	114.5	82.0
DNA	63.6	121.1	87.0
R.m.s. deviations:			
bond lengths (Å)	0.009	0.009	0.008
bond angles (°)	1.45	1.37	1.31

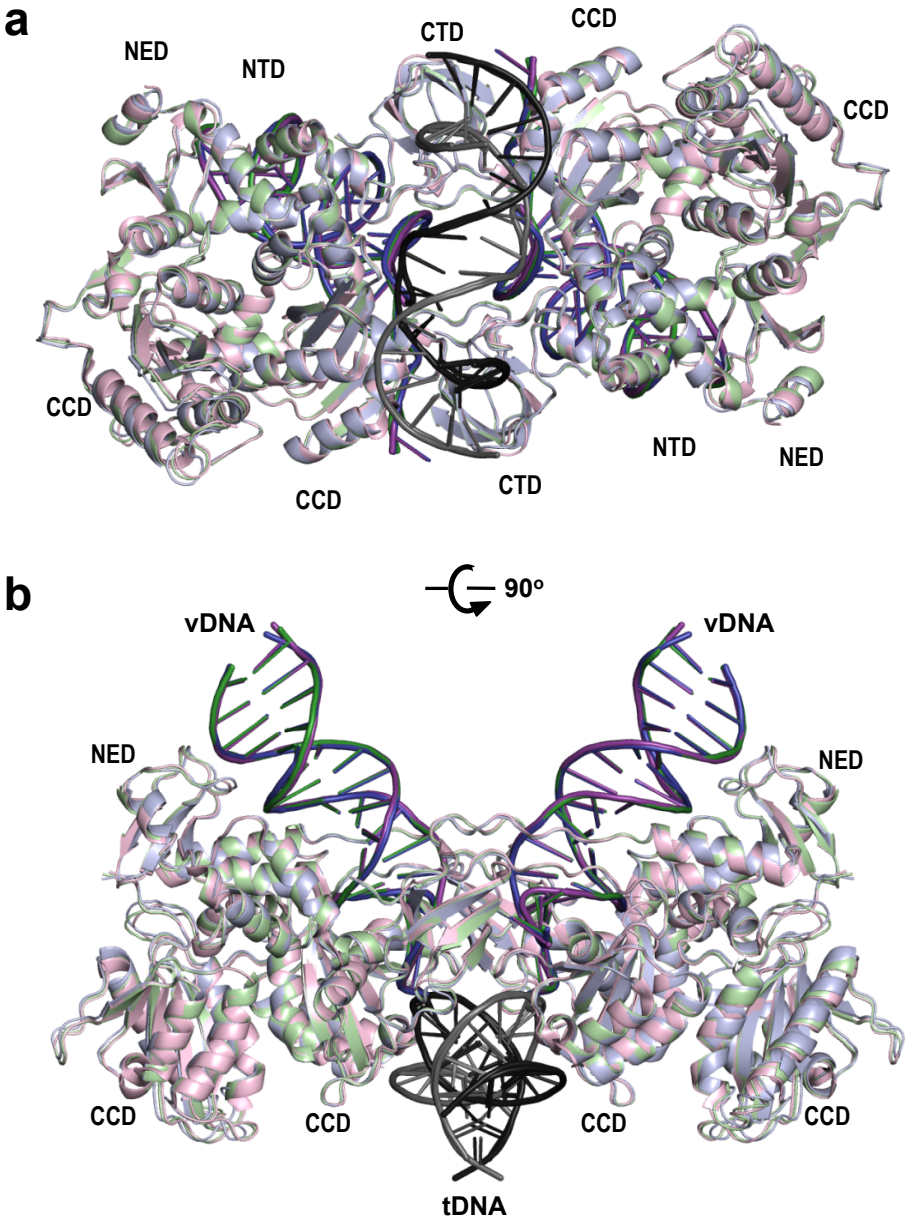
Each dataset was collected from a single crystal.
*Values in parentheses are for the highest resolution shell.



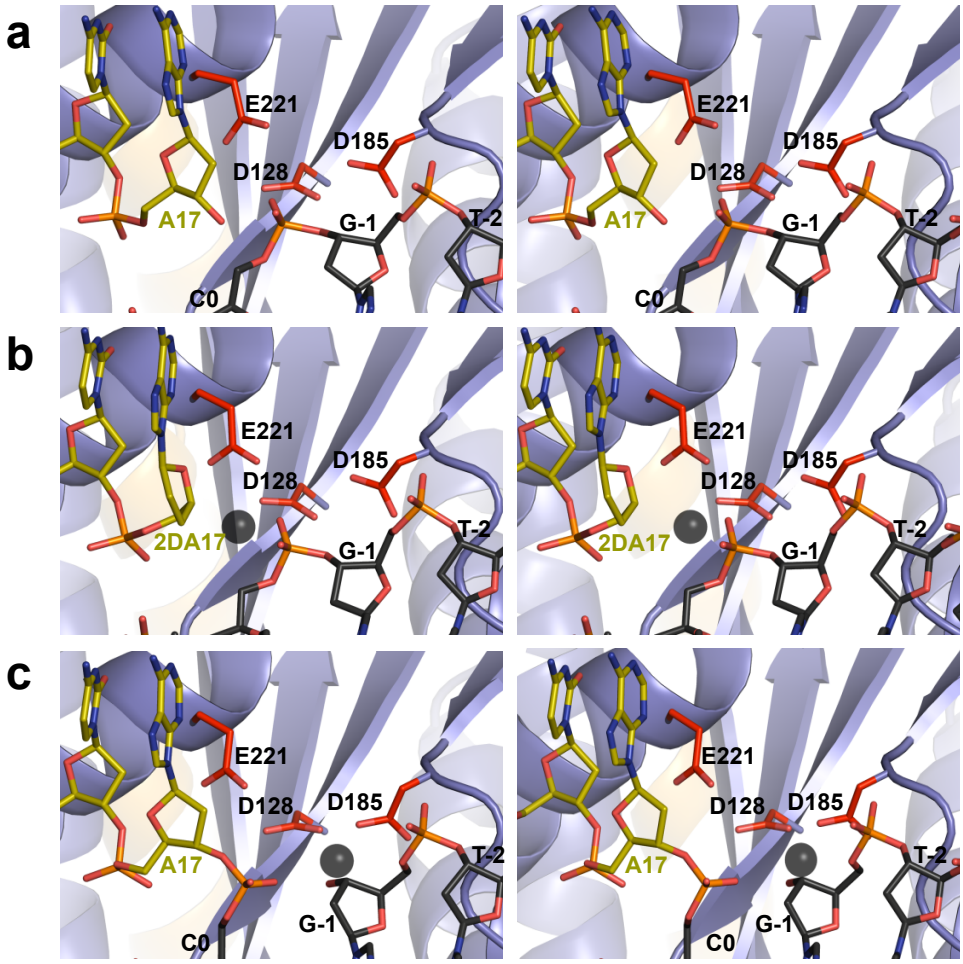
Supplementary Figure 1 | Strand transfer during crystallization. a-b, Schematics illustrating DNA species within TCC (**a**) and STC (**b**). Individual DNA chains are shown as lines: reactive viral DNA strand (R, yellow), non-transferred viral DNA strand (N, brown), tDNA (T, magenta), the product of symmetric strand transfer (S, blue), post-strand transfer tDNA fragment (t, magenta). Arrowheads indicate 3′ termini of the DNA strands. Stars indicate overhanging 5′ ends of the non-transferred viral DNA strands. **c**, Denaturing PAGE analysis of DNAs from crystallization drops. Individual crystals (lanes 1 and 3) or entire drops (lanes 2 and 4) from crystallization conditions used to obtain TCC_{ddA} (lanes 1 and 2) or STC (lanes 3 and 4) crystals were deproteinized, 5′ ^{32}P-labeled using T4 polynucleotide kinase, separated in a denaturing 17% polyacrylamide gel and detected by autoradiography. Lanes 5-9 contained sequence-matched electrophoresis migration standards. Migration positions of various DNA species are indicated to the left of the gel. Dots indicate additional tDNA bands consistently observed due to stable secondary structures (compare lanes 1, 2, and 7). Note that non-transferred viral DNA strand is phosphorylated by T4 polynucleotide kinase much more efficiently than the remaining DNAs due to its overhanging 5′-end within TCC and STC (panels **a** and **b**). Non-symmetric strand transfer products (indicated with a hash symbol) detected in crystallization drops are selectively excluded from the STC crystals (compare lanes 3 and 4).



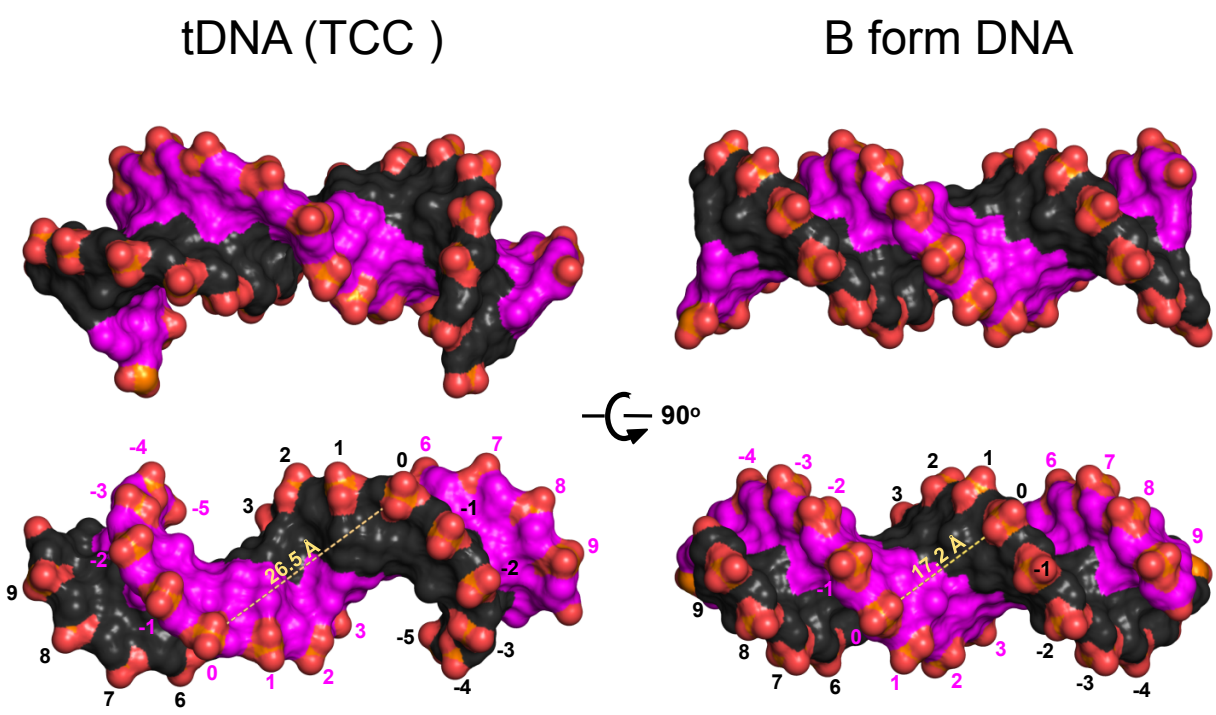
Supplementary Figure 2 | Examples of final electron density maps. The active site regions of TCC_{Apo} (**a**), TCC_{ddA} (**b**), and STC (**c**) structures are shown in stick representation. The viral DNA is shown in yellow, the tDNA strand targeted by the active site in dark grey and the complementary tDNA strand in magenta. Side chains of active site residues Asp128, Asp185, and Glu221 (red), Pro214 and Tyr212 are indicated. (**d**) The region around Arg329 in the STC crystal structure is shown as sticks. Grey spheres are Mg atoms. The sigma weighted *2Fo-Fc* density maps are shown as blue mesh, contoured at 1.5σ (panels **a** and **b**), 2σ (**c**) and 1σ (**d**).



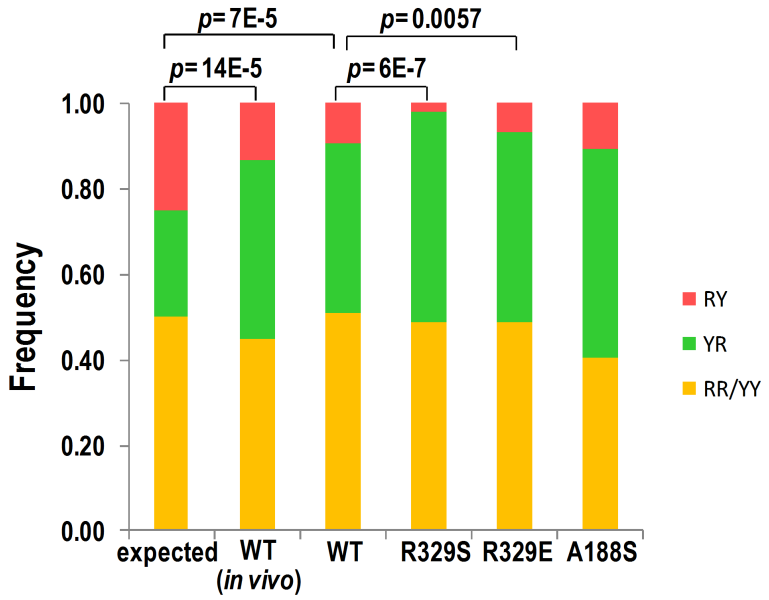
Supplementary Figure 3 | Superposition of the intasome, TCC, and STC structures. Structures of the intasome (PDB ID 3L2R) (pink), TCC_{Apo} (purple), and STC (green) are shown as cartoons, viewed along (**a**) or perpendicular (**b**) to the principal two-fold axis.



Supplementary Figure 4 | Stereo views on the active sites in the TCC_{Apo} (a), TCC_{ddA} (b), and STC (c) crystal structures. IN active site residues (Asp128, Asp185, and Glu221) are shown as red sticks. The transferred strand of viral DNA and the tDNA are represented as sticks and coloured yellow and dark grey, respectively.

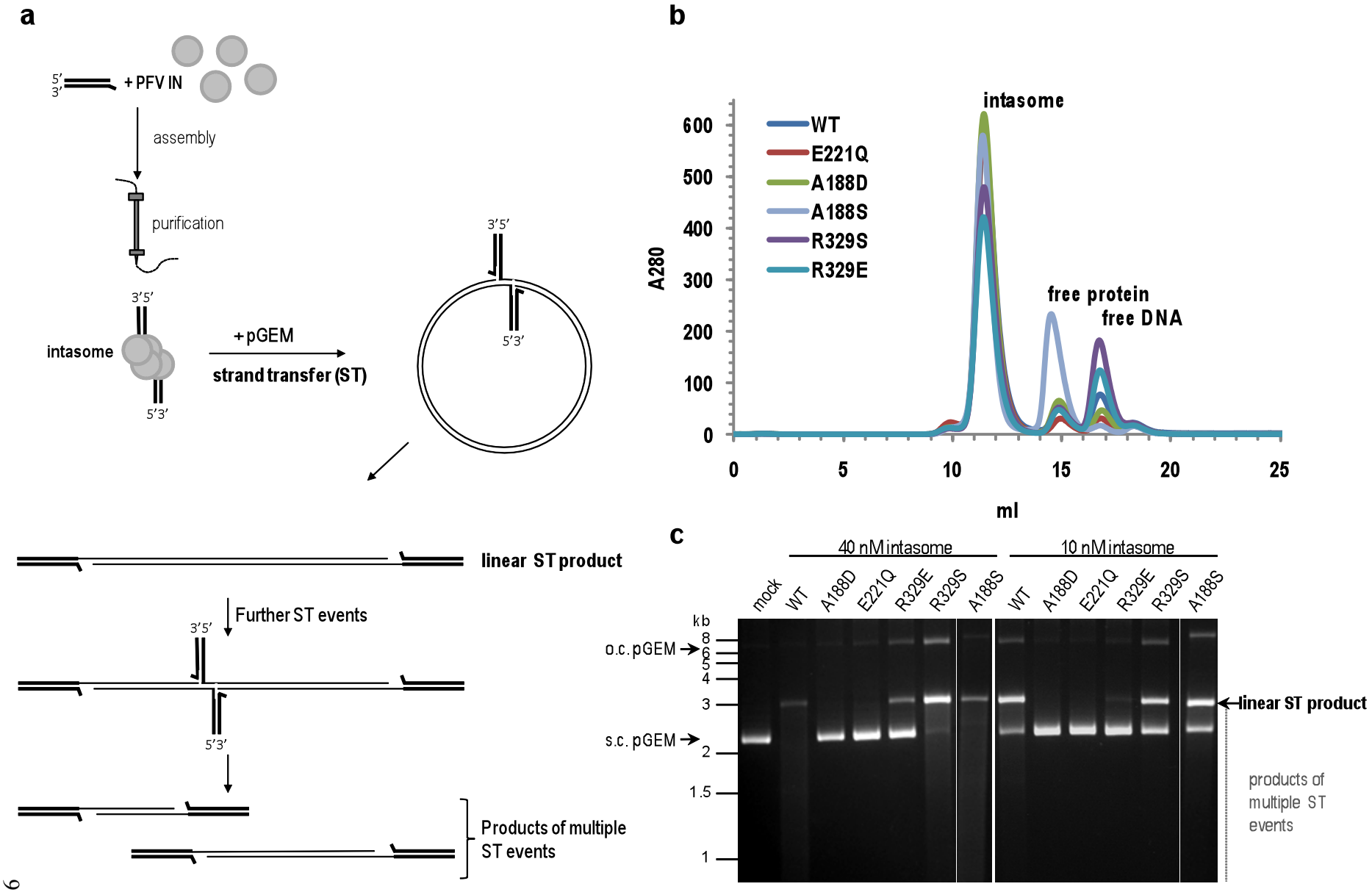


Supplementary Figure 5 | Comparison of the tDNA conformation within TCC with an ideal B form DNA duplex. Two alternative views on the tDNA duplex as found in the TCC_{ddA} structure (**left**) or ideal B-form DNA of the same sequence (**right**). The DNA is shown in a space-fill mode with opposing strands in magenta and dark grey, except for phosphate groups, which are coloured by atom (oxygen, red; phosphorous, orange). The orientation of tDNA in the upper left image is conserved from Fig. 2c. Dashed lines indicate distances between phosphorous atoms targeted by strand transfer events during concerted integration; nucleotides are numbered as in Fig. 1a.

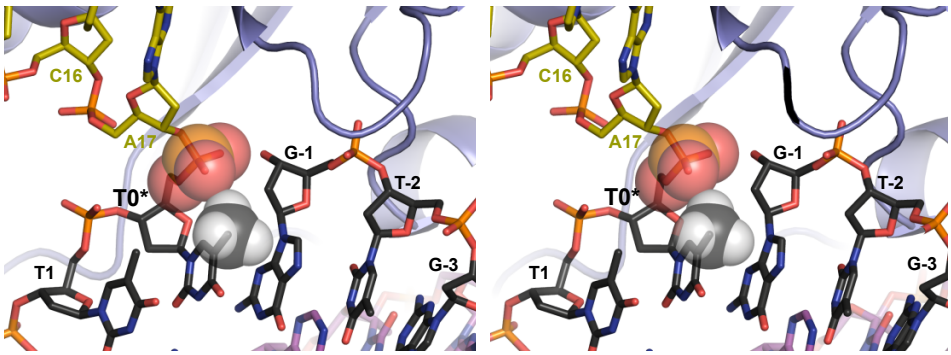


Supplementary Figure 6 | PFV IN is biased towards flexible dinucleotides at the centre of the integration site. The stacked bar graphs show expected or actual frequencies of integration sites with RR or YY (RR/YY, yellow), YR (green), and RY (red) dinucleotides at positions +1 and +2; *p*-values are indicated.

Supplementary Figure 7, Maertens G. *et al*



Supplementary Figure 7 | Strand transfer reactions with PFV intasomes. (**a**) The scheme illustrates PFV intasome preparation and the strand transfer reaction. (**b**) Size exclusion chromatogram of WT and mutant intasome assembly mixtures. (**c**) Strand transfer assays with purified WT and mutant intasomes, used at 10 nM or 40 nM concentration (indicated); resulting products separated by agarose gel electrophoresis were detected by staining with ethidium bromide. Migration of the open circular (o.c.) and super coiled (s.c.) pGEM DNA forms are indicated to the left of the gel image. Migration of the linear concerted strand transfer (ST) product, and the products of multiple concerted ST events are shown to the right.



Supplementary Figure 8 | STC model with thymidine at tDNA position 0. Shown is a stereo view of DNA in the STC crystal structure with cytosine at position 0 replaced with thymidine. The methyl group at position C5 of the thymine base and the phosphate group of the tDNA are shown as grey and red Van der Waals spheres, respectively. Viral and tDNA are shown as sticks in yellow and dark grey, respectively. Chain A of IN is shown as cartoon.