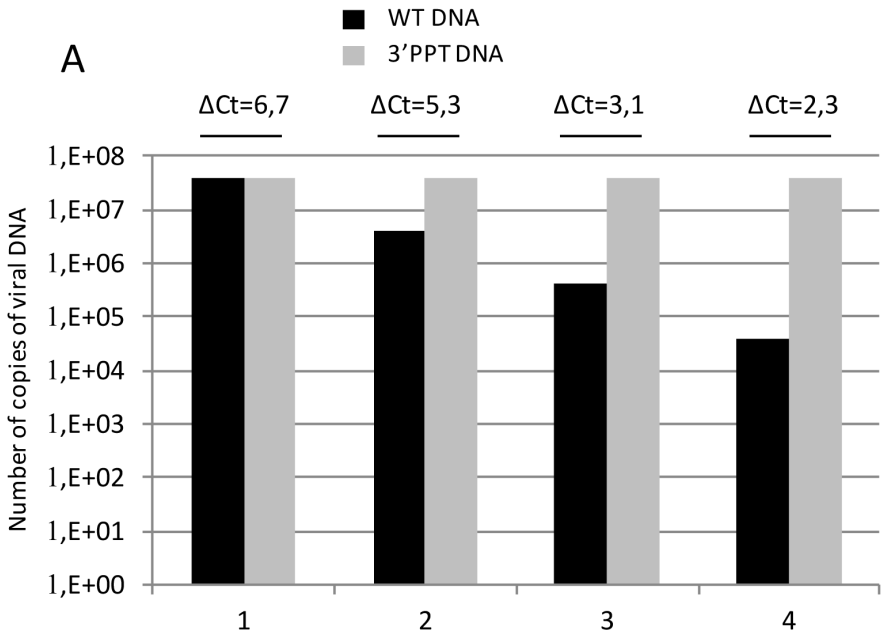
Supplementary Figures

Figure S1.



B

Samples	Number of insertion	Gene	Non Gene
WT d2	8000	70	30
WT d3	5000	68	32
3'-PPT d7	120	?	?
3'-PPT+DTG d9	125	?	?

Figure S1. **Increasing the sensitivity of integrated viral DNA detection among nonintegrated viral DNA.** (A) A known amount of integrated viral DNA from a WT infection (4×10^7 copies of integrated viral DNA) was diluted in a constant amount of viral DNA from a 3'-PPT mutant infection (4×10^7 copies of viral DNA). Integrated viral DNA quantification was performed increasing the number of cycles for the first Alu-LTR PCR. Black column: integrated WT viral DNA. Grey column: 3'-PPT viral DNA. 1: 4×10^7 copies of integrated WT DNA mixed with 4×10^7 copies of 3'-PPT mutant DNA. 2: 4×10^6 copies of integrated WT DNA mixed with 4×10^7 copies of 3'-PPT mutant DNA. 3: 4×10^5 copies of integrated WT DNA mixed with 4×10^7 copies of 3'-PPT mutant DNA. 4: 4×10^4 copies of integrated WT DNA mixed with 4×10^7 copies of 3'-PPT mutant DNA. The ΔCt are indicated. (B) Integration site analysis. DNA from cells infected with WT or 3'-PPT mutant viruses were analyzed for integrated viral DNA sites. All experiments were repeated three times.

Figure S2

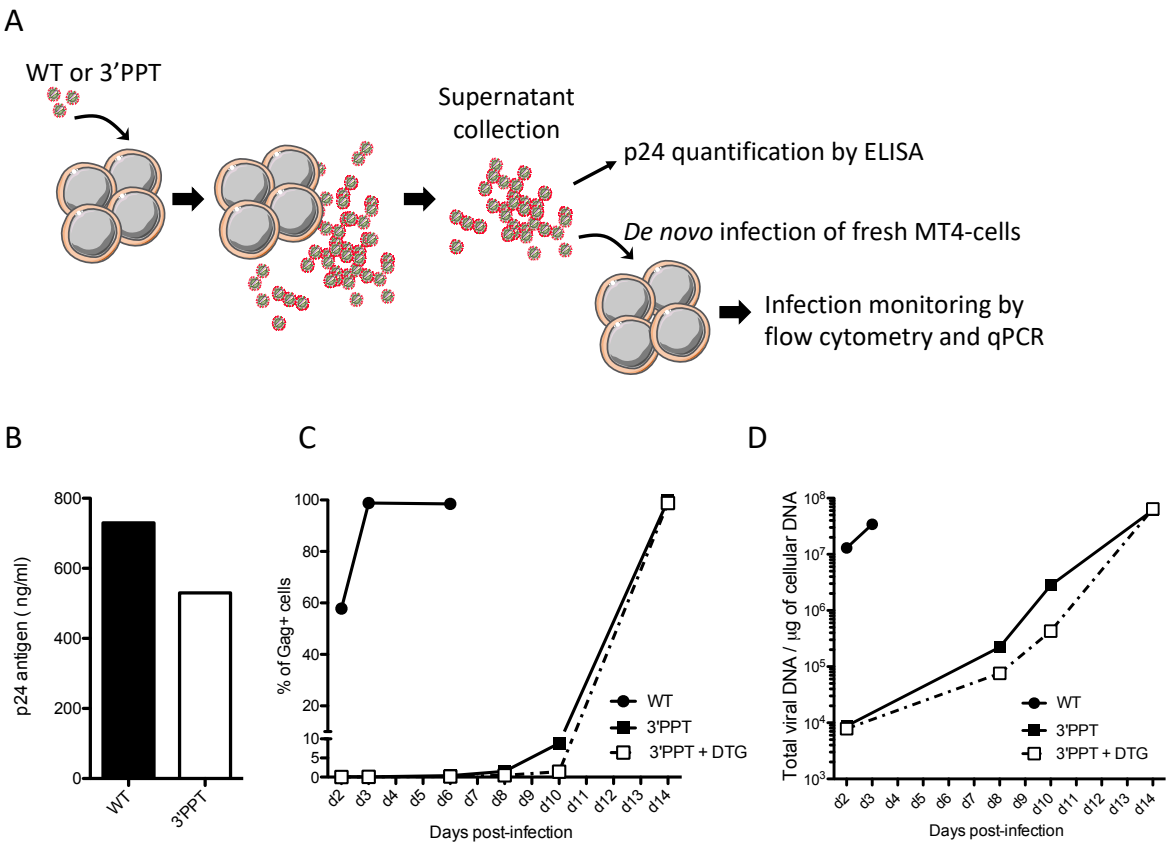


Figure S2. **The 3'-PPT mutant produces infectious viral particles.** (A-D) MT4-cells were infected with WT or 3'-PPT mutant. Supernatants were collected from infected cells at day 2 for WT infection and day 7 for 3'-PPT infection. (B) p24 antigen in supernatants were quantified by ELISA. (C-D) Fresh MT4-cells were infected with these supernatants in absence or presence of 500 nM DTG for the 3'-PPT supernatant. De novo infection was monitored in MT4 cells by intracellular staining of gag antigen using an anti-Gag antibody conjugated to phycoerythrin (PE) (C) and by quantification of total viral DNA by qPCR (D).

Figure S3

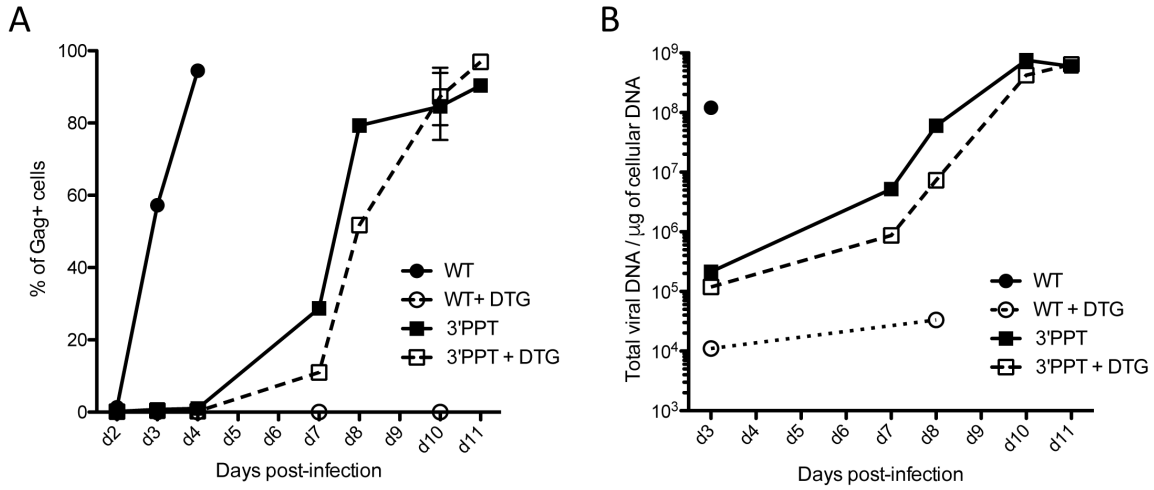


Figure S3. Replication of the 3'-PPT mutant virus in CEMss cells. CEMss cells were infected with WT or 3'-PPT mutant viruses with or without 500 nM DTG. (A) The percentage of infected cells during the course of infection was followed by intracellular staining of gag antigen using an anti-Gag antibody conjugated to phycoerythrin (PE). The graph represents the mean ± SD of two independent experiments. (B) Total viral DNA was quantified by qPCR and was normalized against the cellular DNA content (in µg).

Figure S4

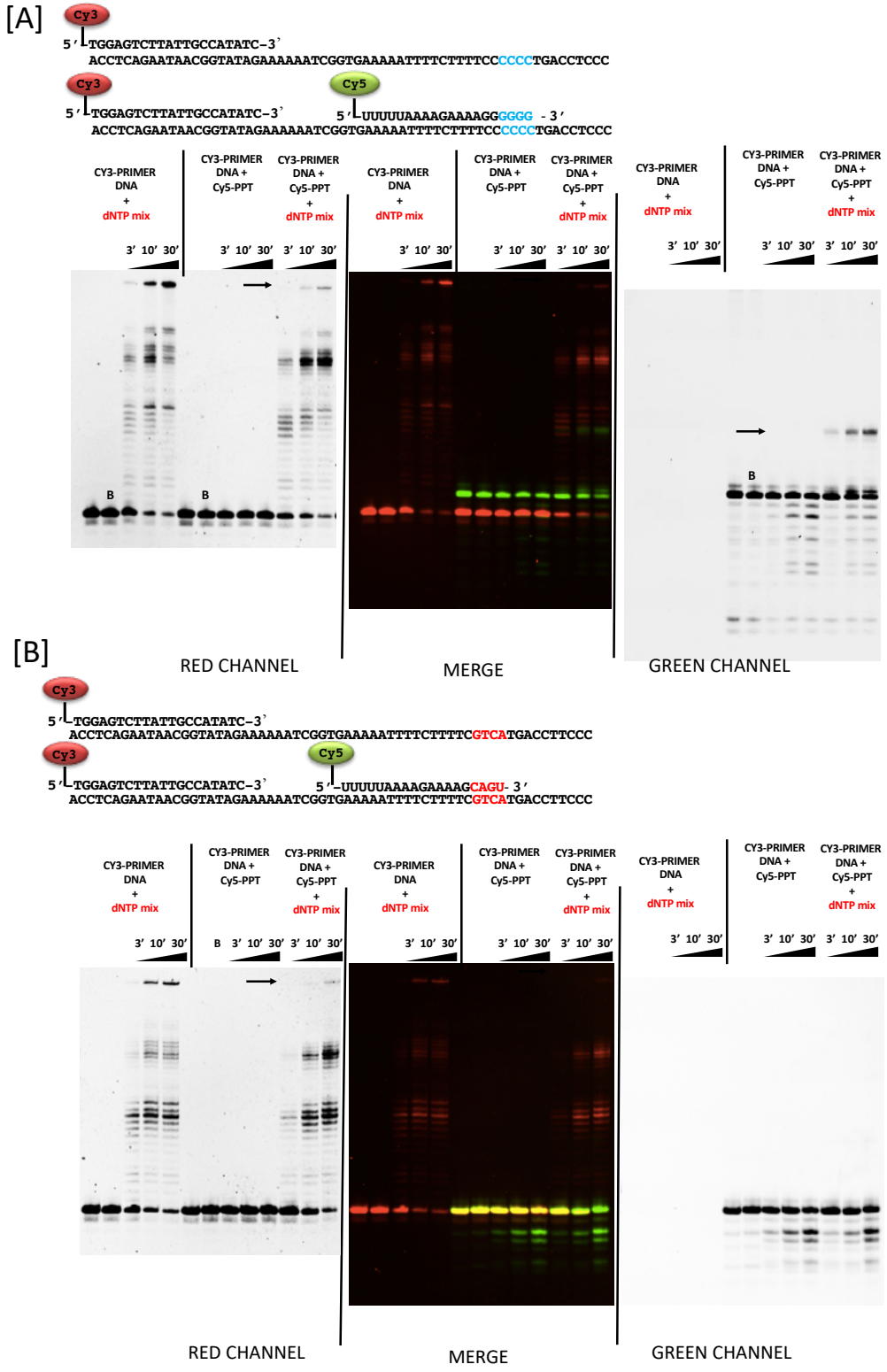


Figure S4. PPT processing by HIV-1 WT RT. Elongation and strand displacement WT 3'-PPT (A) and mutated 3'-PPT (B). Reaction products resolved by high voltage denaturing 15% polyacrylamide gel electrophoresis (Acrylamide/bisacrylamide ratio 19:1, 7M Urea in Tris Borate EDTA buffer 1X) and visualized by fluorescent dual channel imaging (ChemiDoc Biorad). Bands were analyzed and quantified by ImageLabTM version 6.0.1.

Figure S5

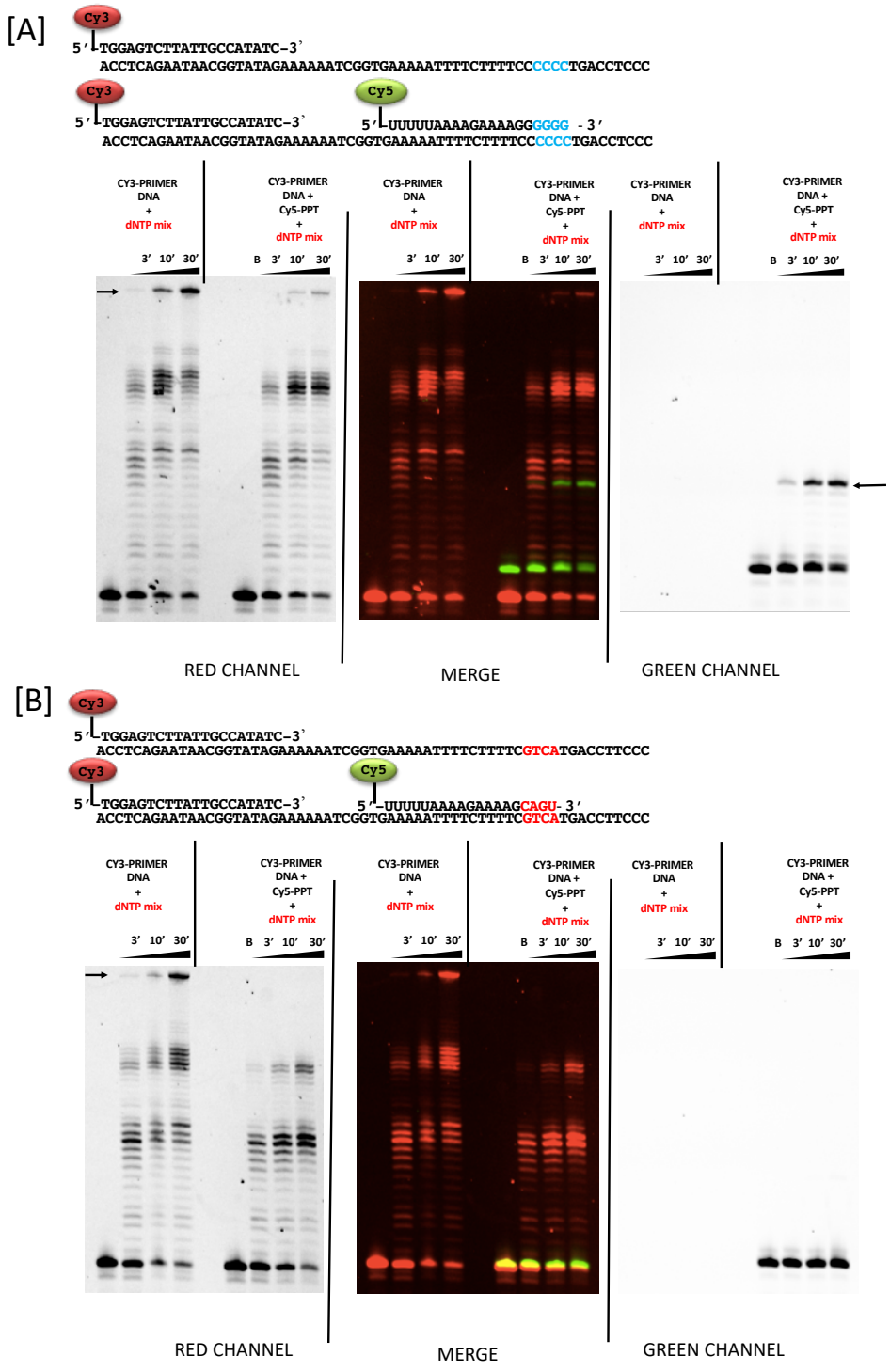


Figure S5. PPT processing by HIV-1 RT E478Q. Elongation and strand displacement WT 3'-PPT (A) and mutated 3'-PPT (B). Reaction products resolved by high voltage denaturing 15% polyacrylamide gel electrophoresis (Acrylamide/bisacrylamide ratio 19:1, 7M Urea in Tris Borate EDTA buffer 1X) and visualized by fluorescent dual channel imaging (ChemiDoc Biorad). Bands were analyzed and quantified by ImageLabTM version 6.0.1.

Figure S6.

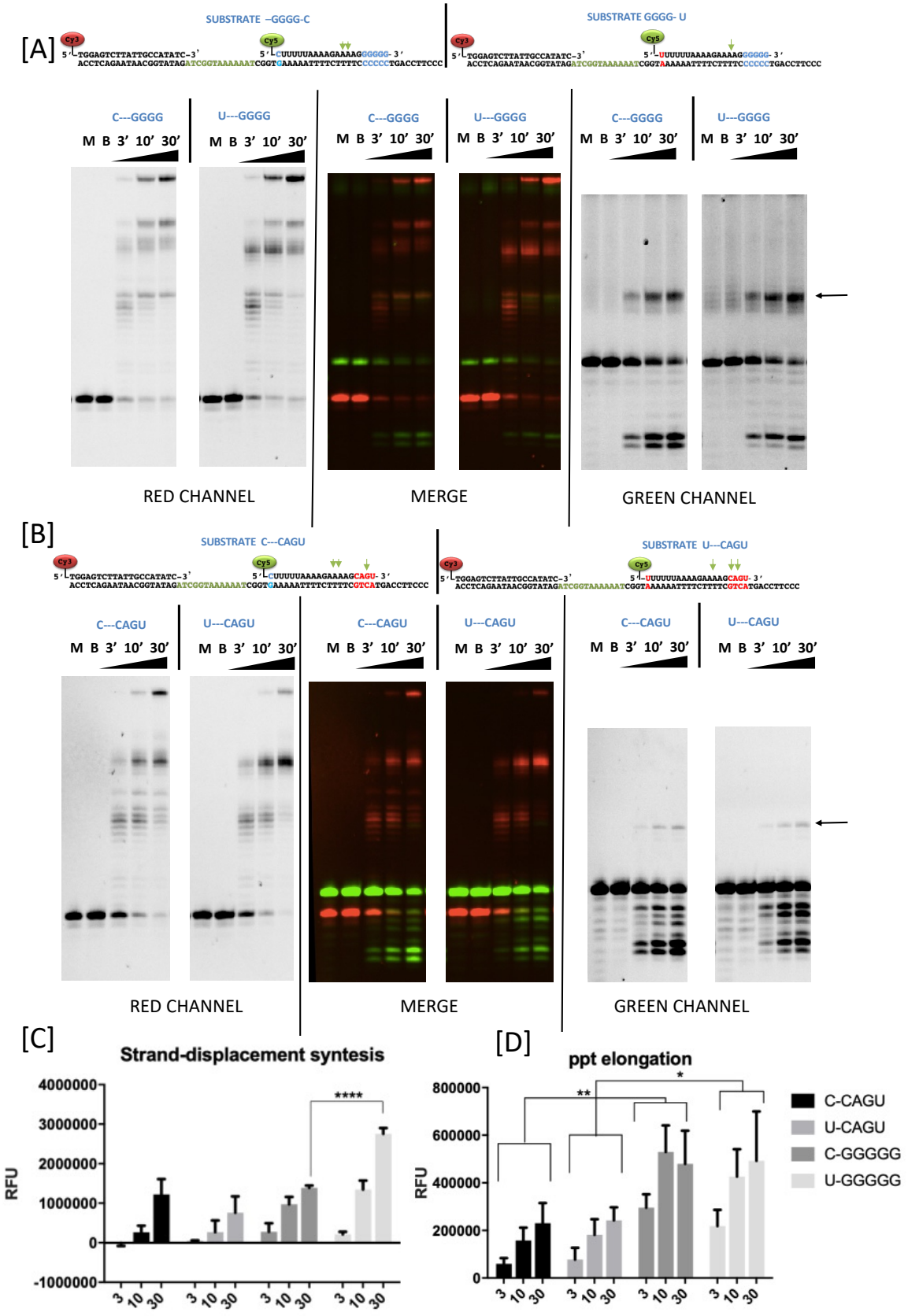


Figure S6. Evaluation of effect of C->U substitution. Elongation and strand displacement WT 3'-PPT (A) and mutated 3'-PPT (B) either with or without C->U substitution at -6 of 3'-PPT

sequence. Reaction products resolved by high voltage denaturing 15% polyacrylamide gel electrophoresis (Acrylamide/bisacrylamide ratio 19:1, 7M Urea in Tris Borate EDTA buffer 1X) and visualized by fluorescent dual channel imaging (ChemiDoc Biorad). Bands were analyzed and quantified by ImageLabTM version 6.0.1. Mean ± standard deviation of two independent experiments; p values were calculated by paired, two-tailed t tests using GraphPad Prism 6.01 software (GraphPad Software, Inc.; San Diego, CA, USA). Figures were drawn with GraphPad Prism 6 version 6.01.