Supporting Information

Accessory factors are required for DNA polymerase δ to overcome protein barriers during DNA synthesis

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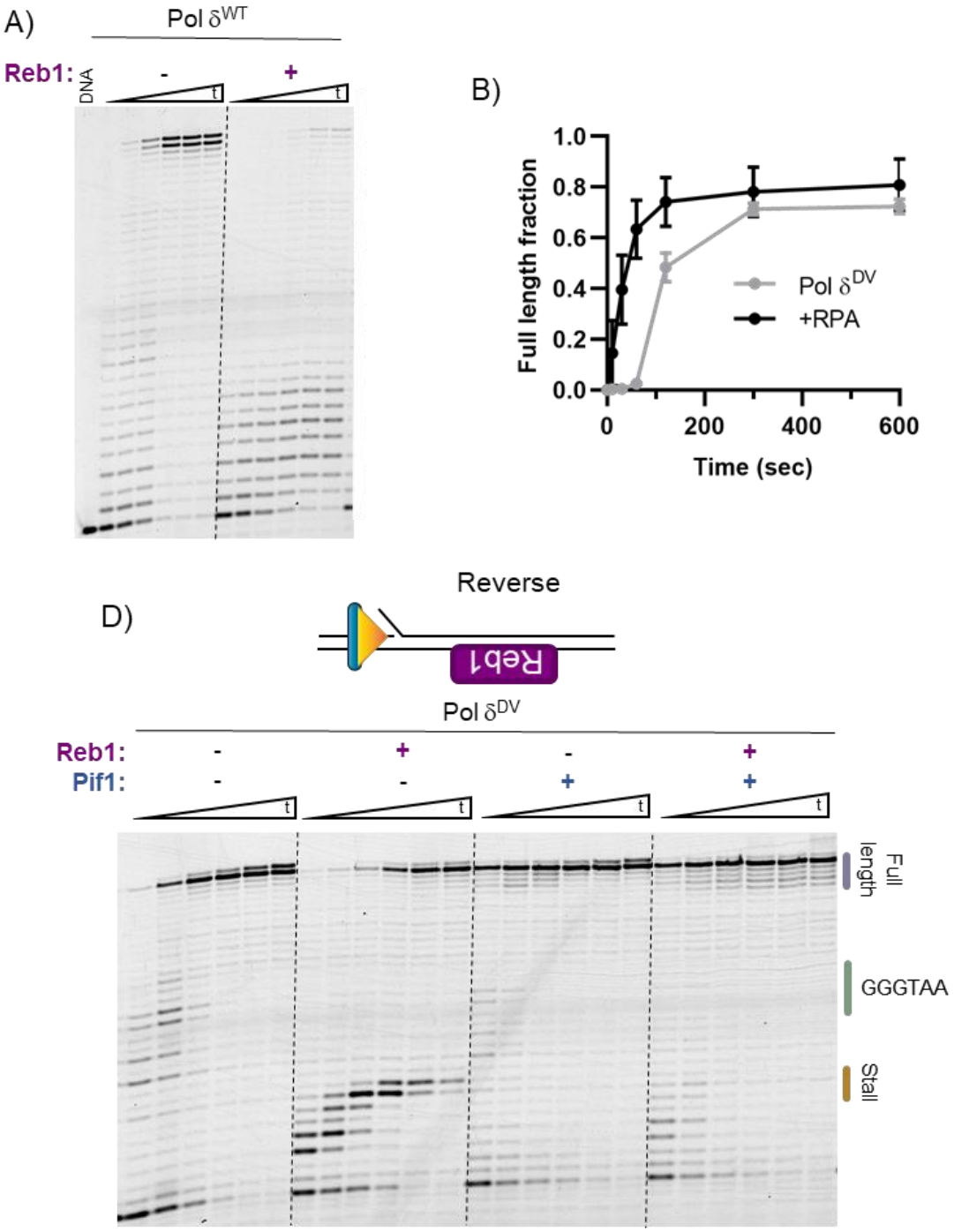
Table S1
Supplementary Figures S1 to S3

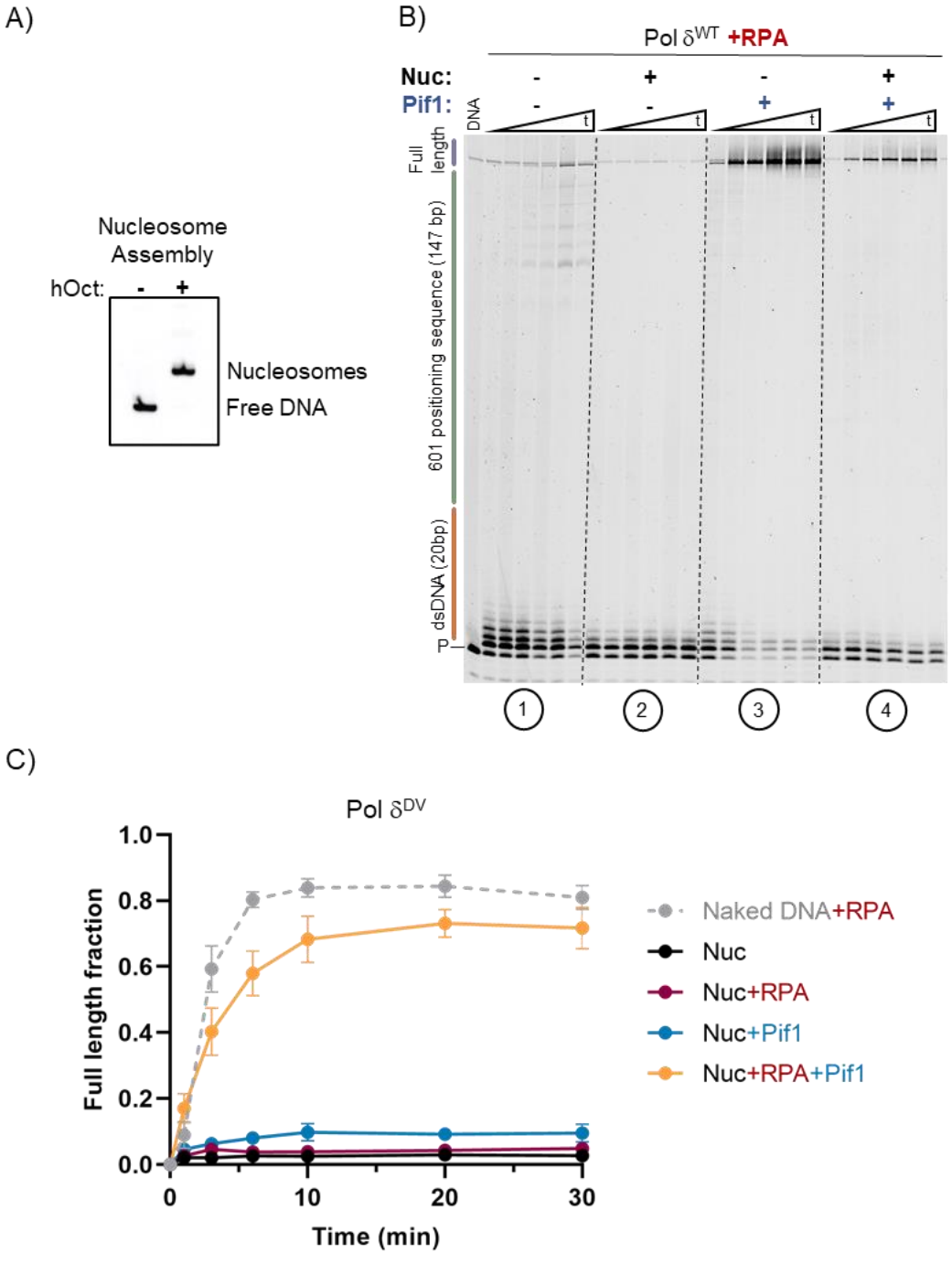
Supplementary Table S1. Sequences of oligonucleotides used to generate the tested DNA substrates.

DNA substrate name	**DNA sequence (5'3'):** (key: **DNA modification**; Binding Site)
Cy3-primer	**Cy3-**CCGCCGCGGAACTTATTAGTG
Reb1_for_top	TTTTTTTTTTTTTTTTTTTTTTTTCTGACACATGAGCTATATTACCCGGAGTACA
Reb1_for_bottom	TGTACTCCGGGTAATATAGCTCATGTGTCAGTCACTAATAAGTTCCGCGGCGG**-Bio**
Reb1_rev_top	TTTTTTTTTTTTTTTTTTTTTTTTCTGACACATCTCCGGGTAATATAGAGCTACA
Reb1_for_bottom	TGTAGCTCTATATTACCCGGAGATGTGTCAGTCACTAATAAGTTCCGCGGCGG**-Bio**
Tbf1_for_top	TTTTTTTTTTTTTTTTTTTTTTTTCTGACACATGAGCTCACCCTAACAGTACCCTAACTGC
Tbf1_for_bottom	GCAGTTAGGGTACTGTTAGGGTGAGCTCATGTGTCAGTCACTAATAAGTTCCGCGGCGG**-Bio**
Tbf1_rev_top	TTTTTTTTTTTTTTTTTTTTTTTTCGCTCGTTAGGGTAGTGTTAGGGTTGC
Tbf1_for_bottom	GCAACCCTAACACTACCCTAACGAGCGTCACTAATAAGTTCCGCGGCGG**-Bio**
Nuc_PCRprimer_for	**Bio-T-Cy3-**TGAATTCCTATTGAAGCAAACGCGTTTTACCTCAGC
Nuc_PCRprimer_rev_3ntTail	**(+/-Bio)-**AGAATCGGATGTATATATCTGACACGTGCCTGGAGAC
Nuc_PCRprimer_rev_50ntTail	**(+/-Bio)-**GGCGTAATCATGGTCATAGCTGTTTCCTGTGTG
Nuc_FullLength_3ntTail	**Bio-T-Cy3-**GAATTCCTATTGAAGCAAACGCGTTTTACCTCAGCACACTGCATGACATCATCGAGAATCCCGGTGCCGAGGCCGCTCAATTGGTCGTAGACAGCTCTAGCACCGCTTAAACGCACGTACGCGCTGTCCCCCGCGTTTTAACCGCCAAGGGGATTACTCCCTAGTCTCCAGGCACGTGTCAGATATATACATCCGATTCT**-(+/-Bio)**
Nuc_FullLength_50ntTail	**Bio-T-Cy3-**GAATTCCTATTGAAGCAAACGCGTTTTACCTCAGCACACTGCATGACATCATCGAGAATCCCGGTGCCGAGGCCGCTCAATTGGTCGTAGACAGCTCTAGCACCGCTTAAACGCACGTACGCGCTGTCCCCCGCGTTTTAACCGCCAAGGGGATTACTCCCTAGTCTCCAGGCACGTGTCAGATATATACATCCGATTCTAGAGTCGAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTG**-(+/-Bio)**

Supplementary Figure S1: **A)** Sequencing gels for primer extension assays using Pol δ^{WT} with and without a Reb1 block in the absence of RPA monitored over time (10", 30", 1', 2', 5', 10'). Dashed lines were added for visibility. **B)** Quantification of full-length product formation during primer extension assays performed using Pol δ^{DV} with and without RPA and no Reb1 block. (n=3) **C)** Representative primer extension assays performed on the DNA substrate with the Reb1 binding logo in the reverse orientation in the presence of RPA with and without Reb1 and Pif1. Dashed lines are added for visibility.



Supplemental Figure S2: A) Native PAGE gel monitoring the Cy3 labelled DNA. Nucleosome assembly is seen by the slower migration of the DNA substrate following salt dialysis in the presence of human octamer. **B)** Representative sequencing gels of primer extension assays using Pol δ^{WT} in the presence of RPA on a nucleosome free or nucleosome bound substrate with or without Pif1. Dashed lines are added for visibility. **C)** Quantification of full-length product formation from primer extension assays performed using Pol δ^{DV} without (Naked DNA) or with a nucleosome (Nuc), RPA, and/or Pif1. (n=3)



Supplemental Figure S3: A) Primer extension assays comparing nucleosome bound substrates with a short (3 bp; panels 1, 3, 5, 7) or long (50 bp; panels 2, 4, 6, 8) tail following the 601 nucleosome positioning sequence. Assays were performed using Pol δ^{DV} with and without RPA and/or Pif1. For each condition, the 3 bp and 50 bp tailed substrates were run on the same gel, either adjacent (dashed lines added for visibility) or at different locations on the gel (separated by gap). **B)** Cleavage products from FEN1 endonuclease activity of the P^{32} 5' radiolabelled flap with or without RPA.