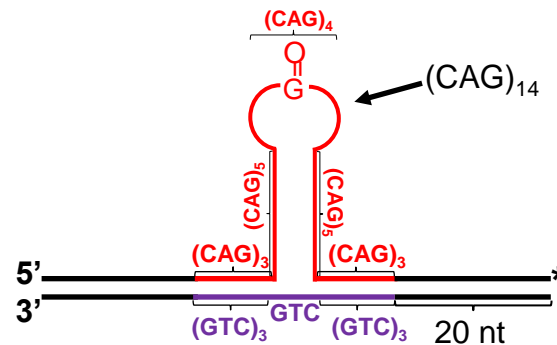
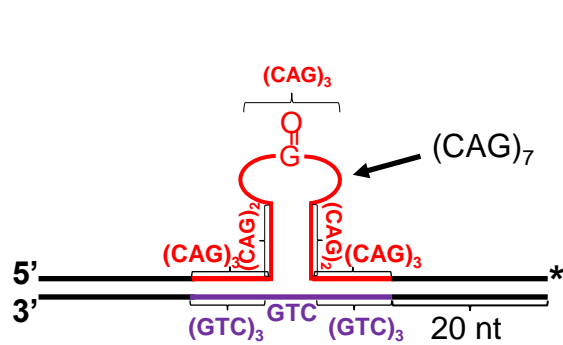


**Table S1 Oligonucleotide sequence**

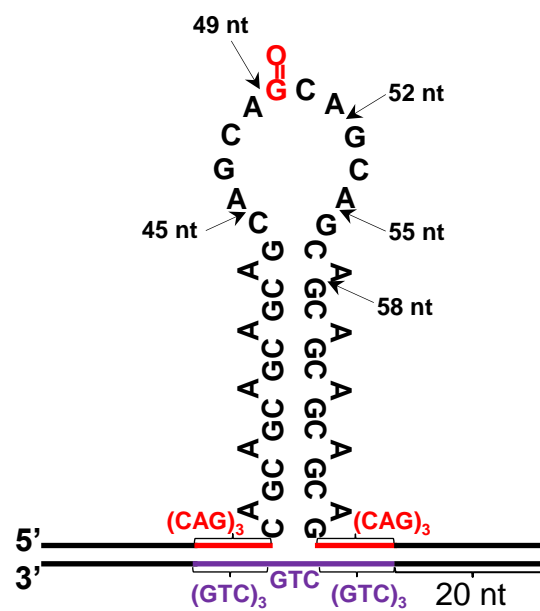
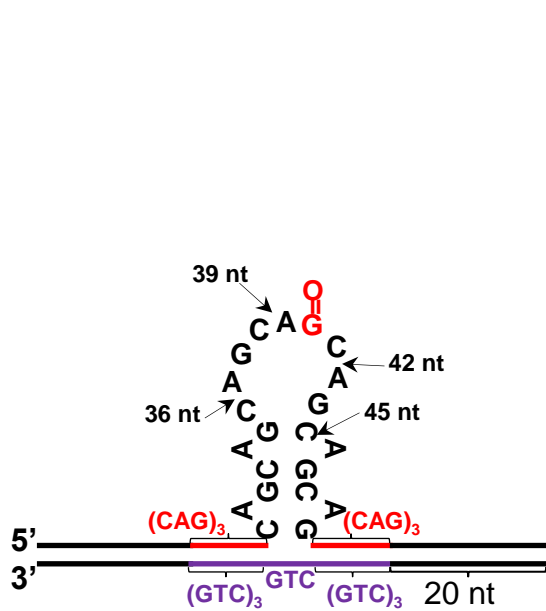
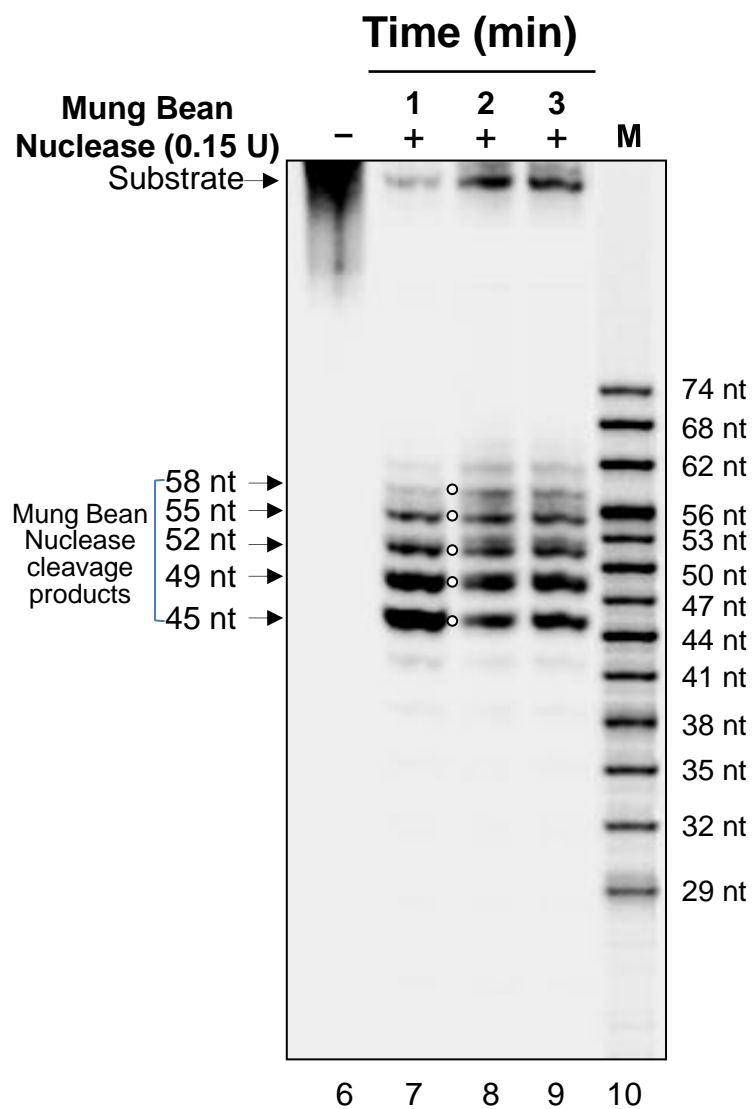
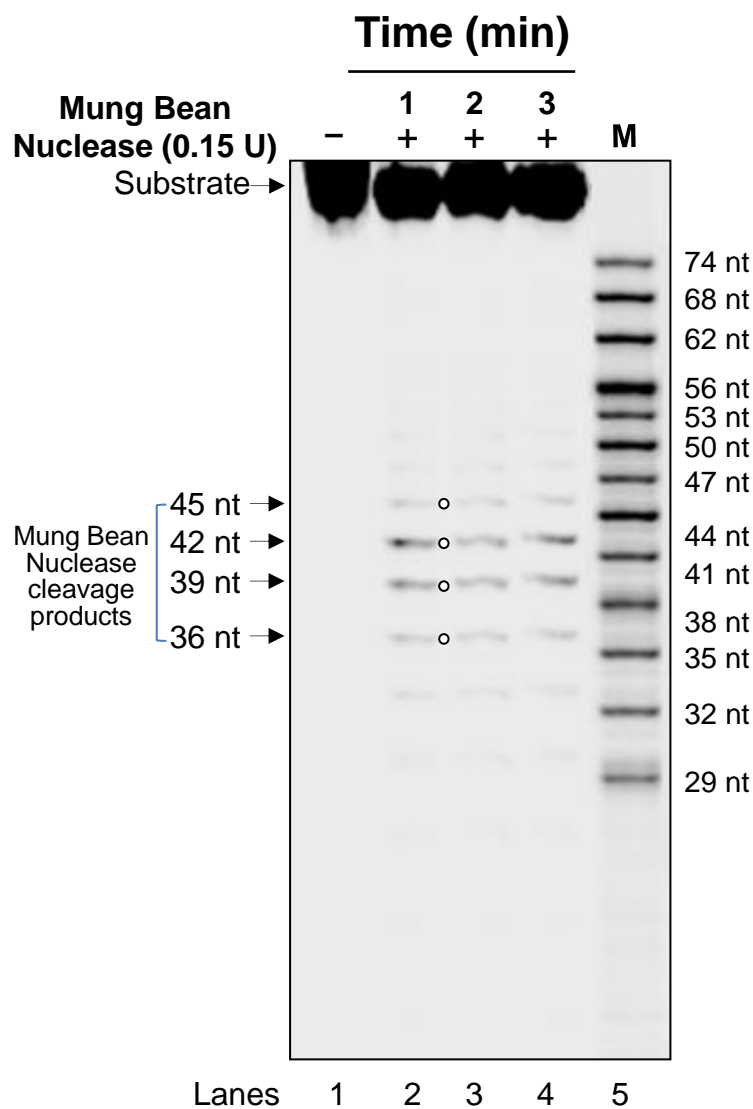
Oligonucleotides	nt	Sequence (5' –3')
Downstream		
D1	40	p <b>F</b> CAG CAG CAG CAG CAG CAG TA CGT AGA CTT ACT CAT TGC
D2	50	p <b>F</b> CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG TA CGT AGA CTT ACT CAT TGC
D3	79	CGA GTC ATC TAG CAT CCG TA CAG CAG CAG CAG CAG CAG CA <b>8oxo-G</b> CAG CAG CAG CAG CAG TA CGT AGA CTT ACT CAT TGC
D4	100	CGA GTC ATC TAG CAT CCG TA CAG CAG CAG CAG CAG CAG CAG CAG CAG CA <b>8oxo-G</b> CAG CAG CAG CAG CAG CAG CAG CAG CAG TA CGT AGA CTT ACT CAT TGC
D5	78	CGA GTC ATC TAG CAT CCG TA CAG CAG CAG CAG CAG CAG CA <b>F</b> CAG CAG CAG CAG CAG TA CGT AGA CTT ACT CAT TGC
D6	100	CGA GTC ATC TAG CAT CCG TA CAG CAG CAG CAG CAG CAG CAG CAG CAG CA <b>F</b> CAG CAG CAG CAG CAG CAG CAG CAG CAG TA CGT AGA CTT ACT CAT TGC
Template		
T1	61	GCA ATG AGT AAG TCT ACG TAC TGC TGC TGC TGC TGC TGC TGT ACG GAT GCT AGA TGA CTC G
Upstream		
U1	40	CGA GTC ATC TAG CAT CCG TA CA GCA GCA GCA GCA GCA GCA
U2	49	CGA GTC ATC TAG CAT CCG TA CAG CAG CAG CAG CAG CAG CAG CAG CA

F: tetrahydrofuran, THF 8-oxoG: 8-oxoguanine

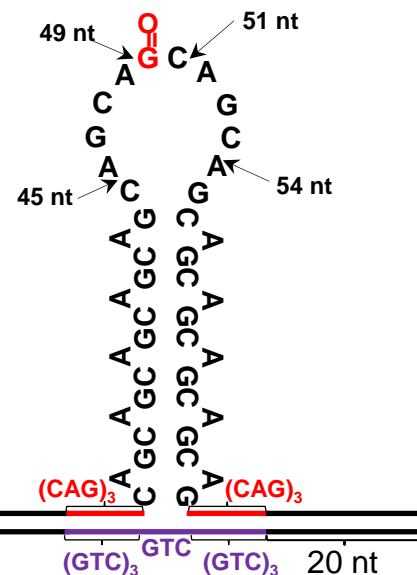
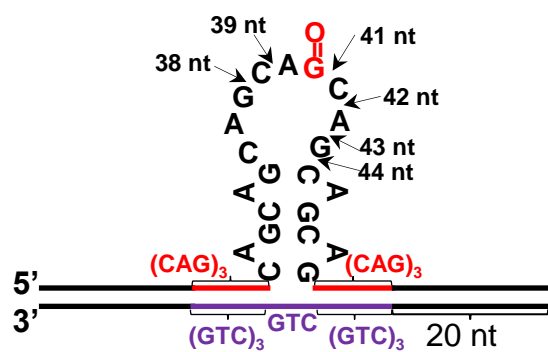
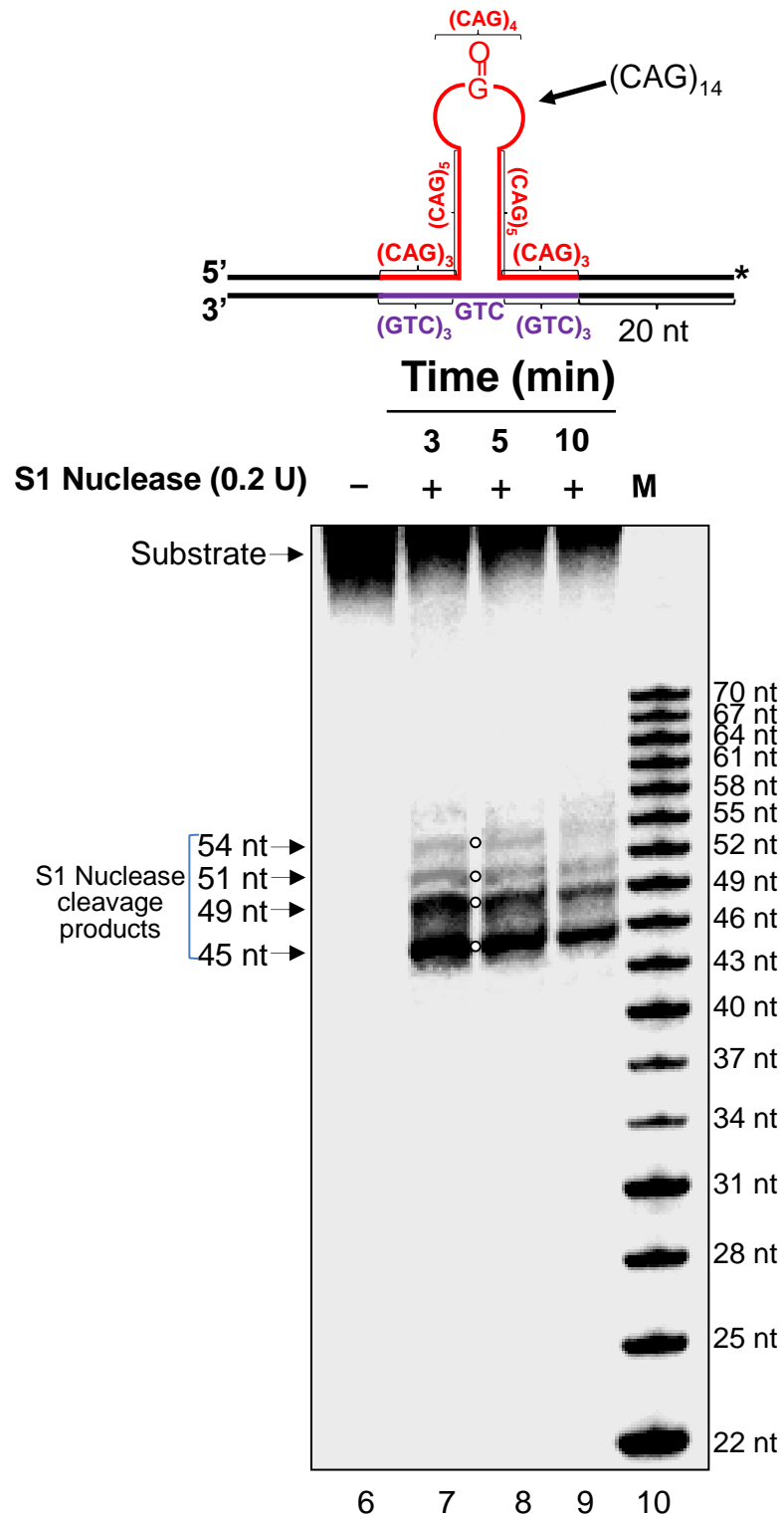
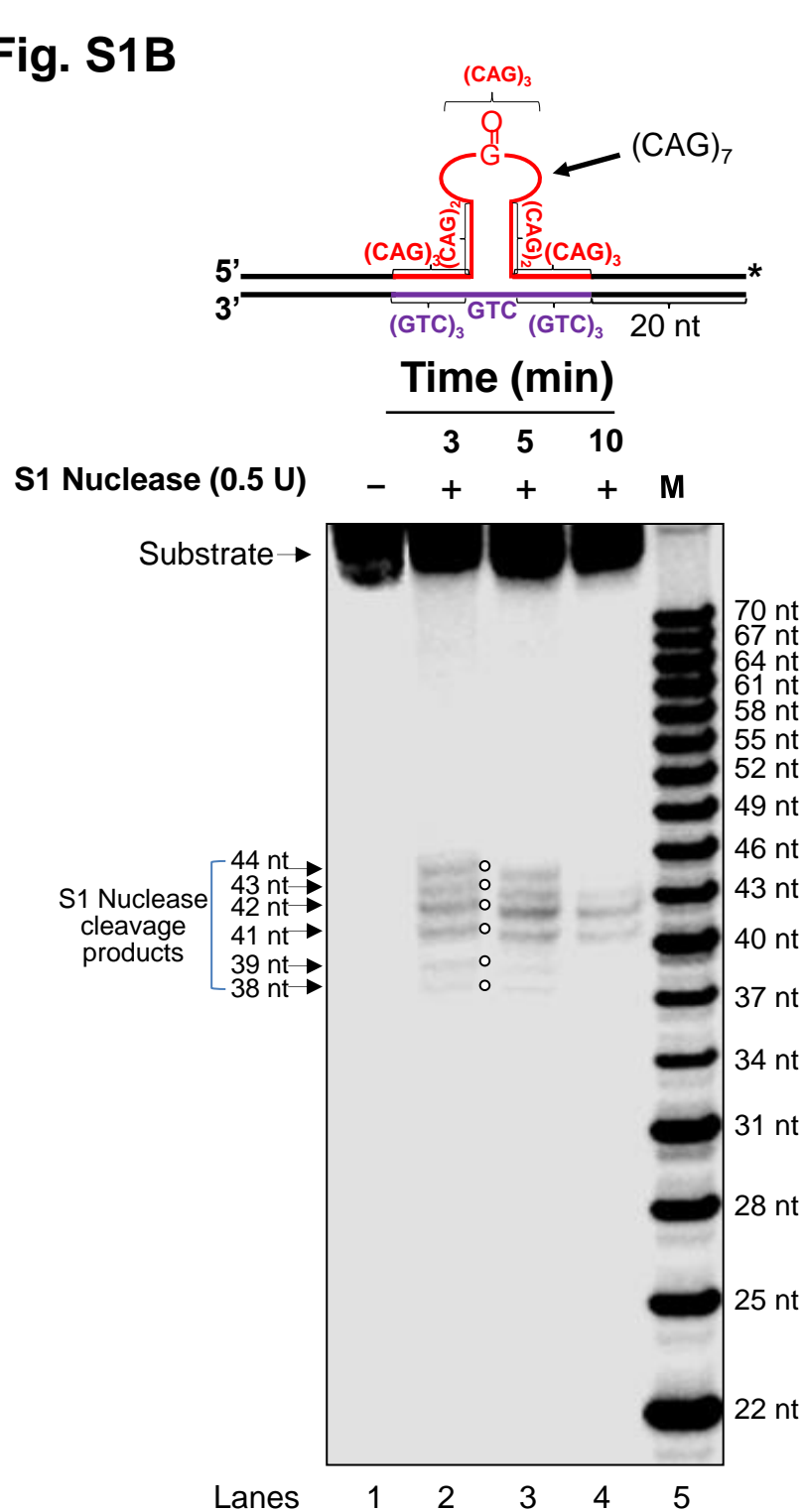
**Fig. S1A**



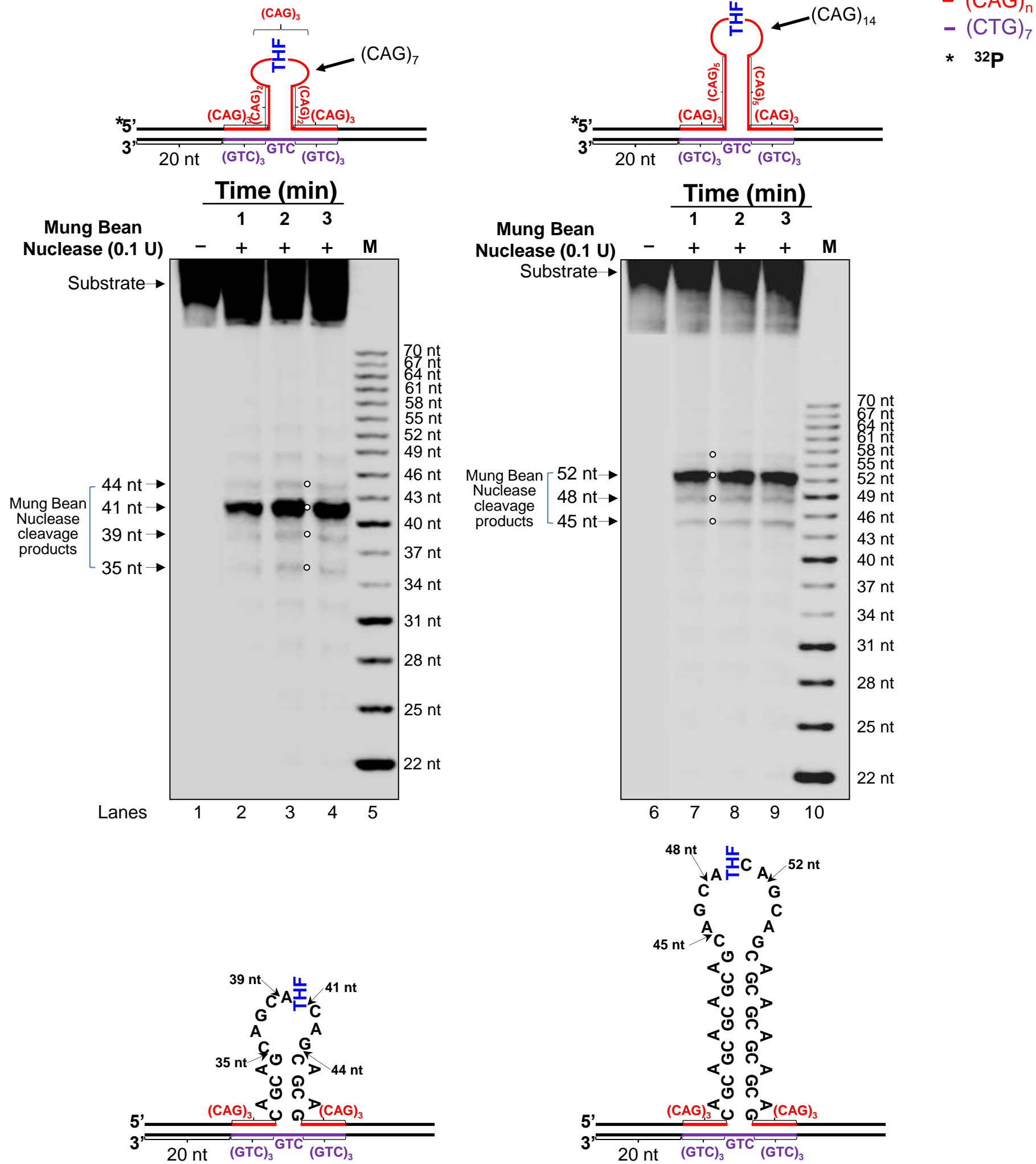
- (CAG)<sub>n</sub>  
 - (CTG)<sub>7</sub>  
 \* 32P



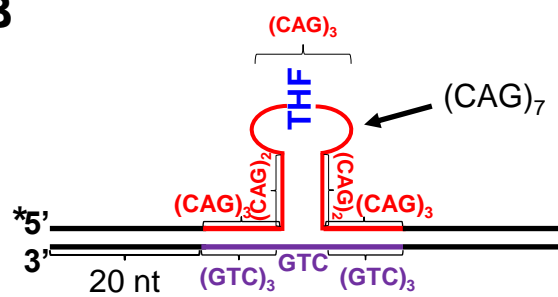
**Fig. S1B**



**Fig. S2A**



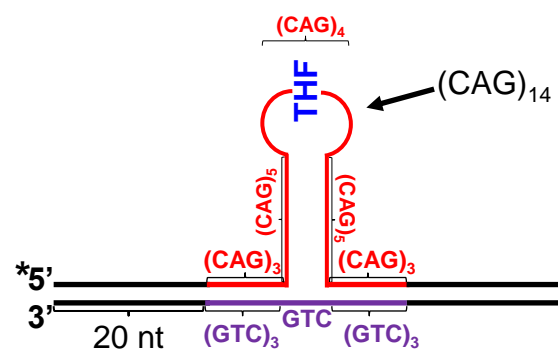
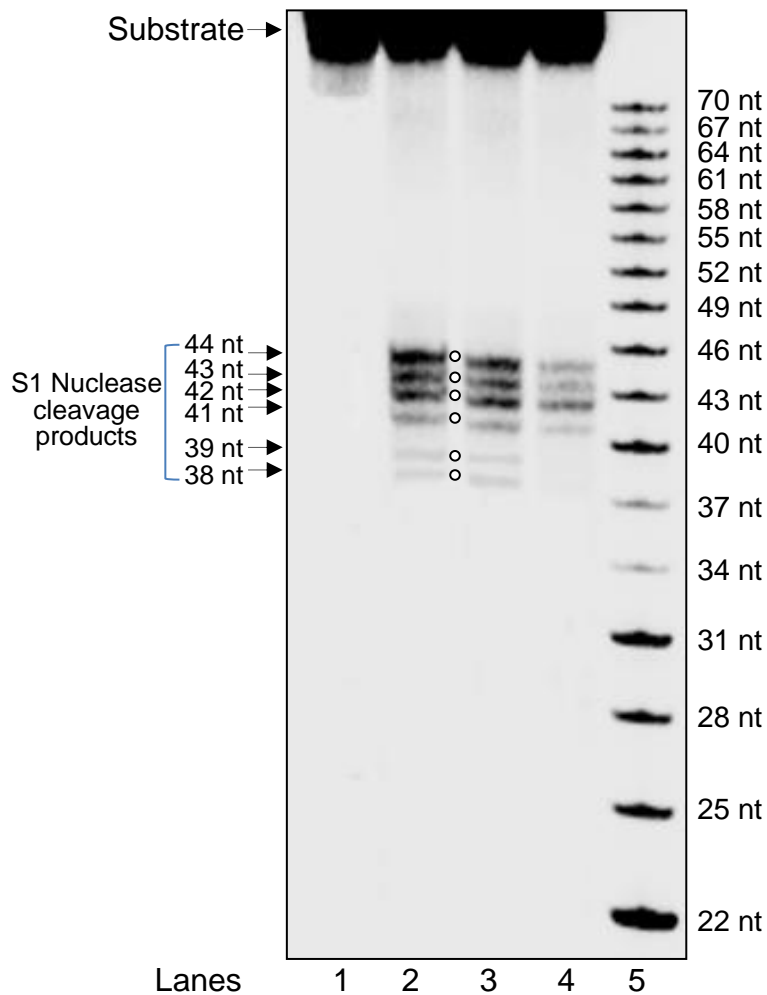
**Fig. S2B**



**Time (min)**

3 5 10

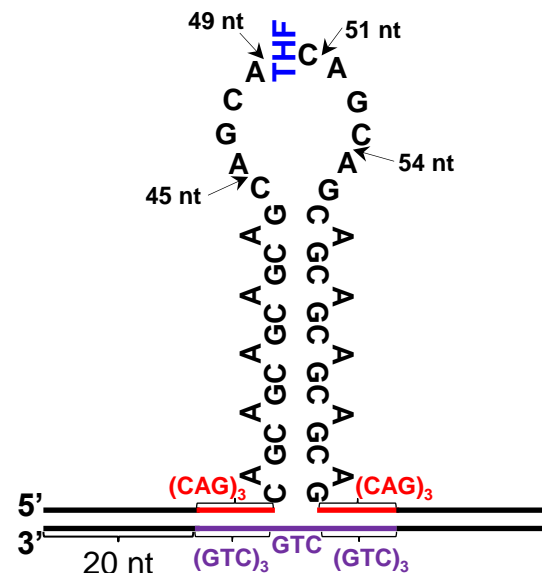
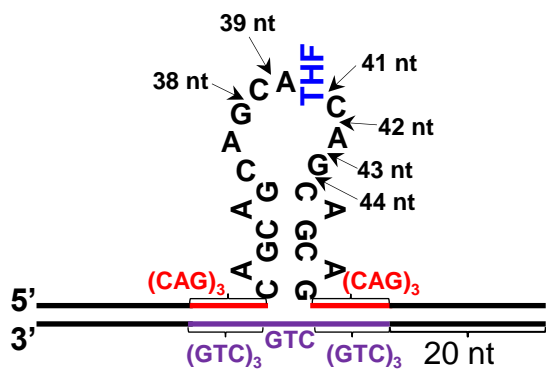
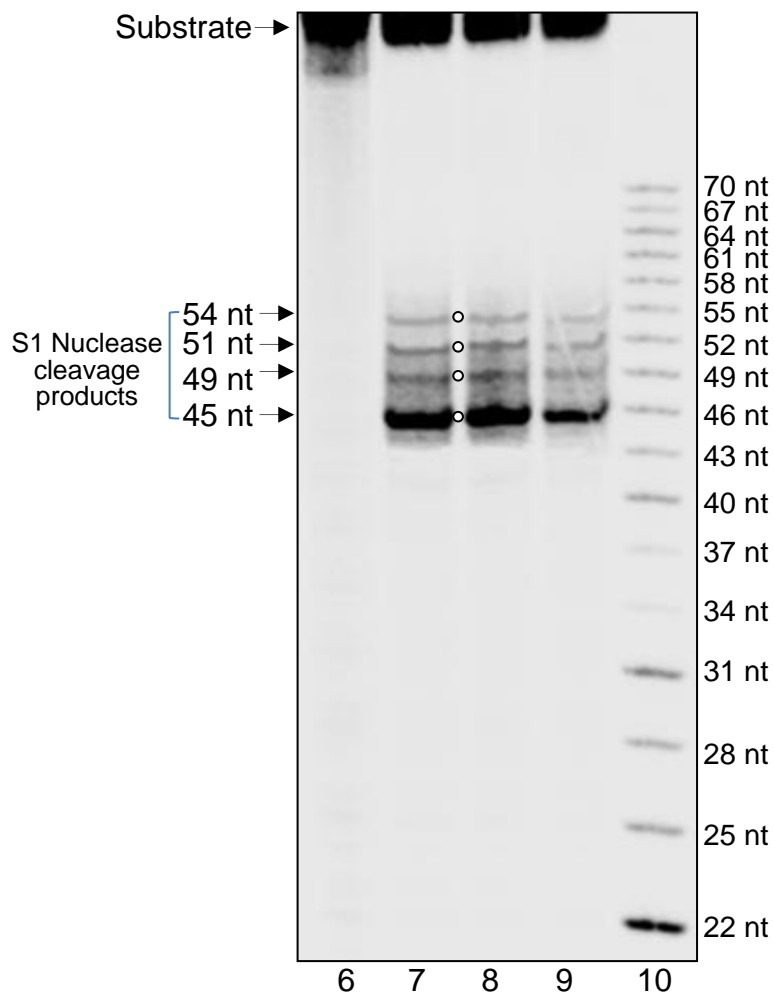
**S1 Nuclease (0.5 U)** - + + + M



**Time (min)**

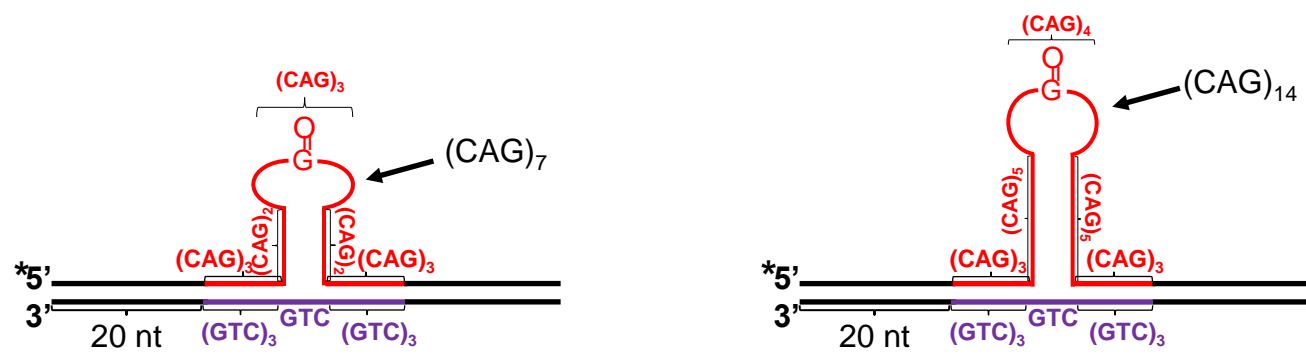
3 5 10

**S1 Nuclease (0.2 U)** - + + + M



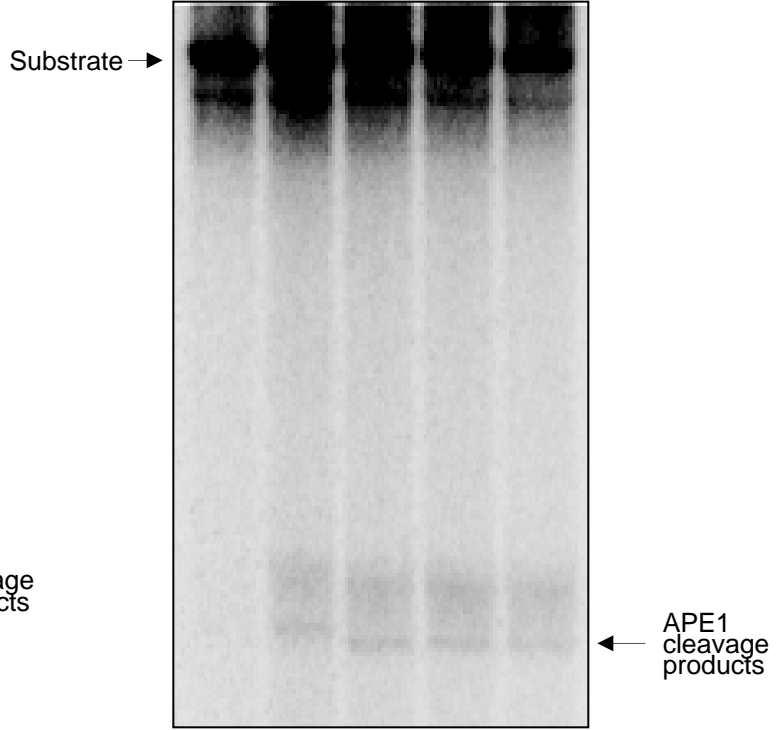
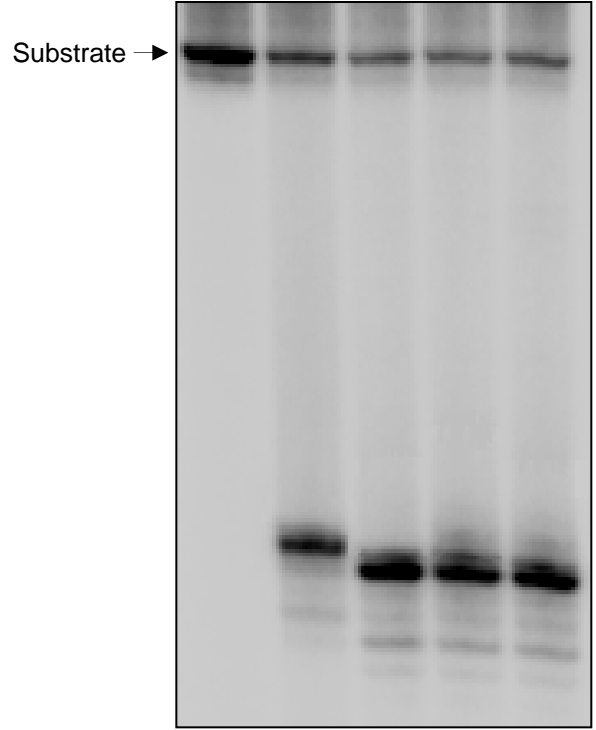
**Fig. S3**

- (CAG)<sub>n</sub>  
 - (CTG)<sub>7</sub>  
 \* <sup>32</sup>P



LIG I (5 nM)	-	-	-	-	+
Pol β (5 nM)	-	-	-	+	+
APE1 (50 nM)	-	-	+	+	+
OGG1 (10 nM)	-	+	+	+	+

LIG I (5 nM)	-	-	-	-	+
Pol β (5 nM)	-	-	-	+	+
APE1 (50 nM)	-	-	+	+	+
OGG1 (10 nM)	-	+	+	+	+



Lanes 1 2 3 4 5

6 7 8 9 10

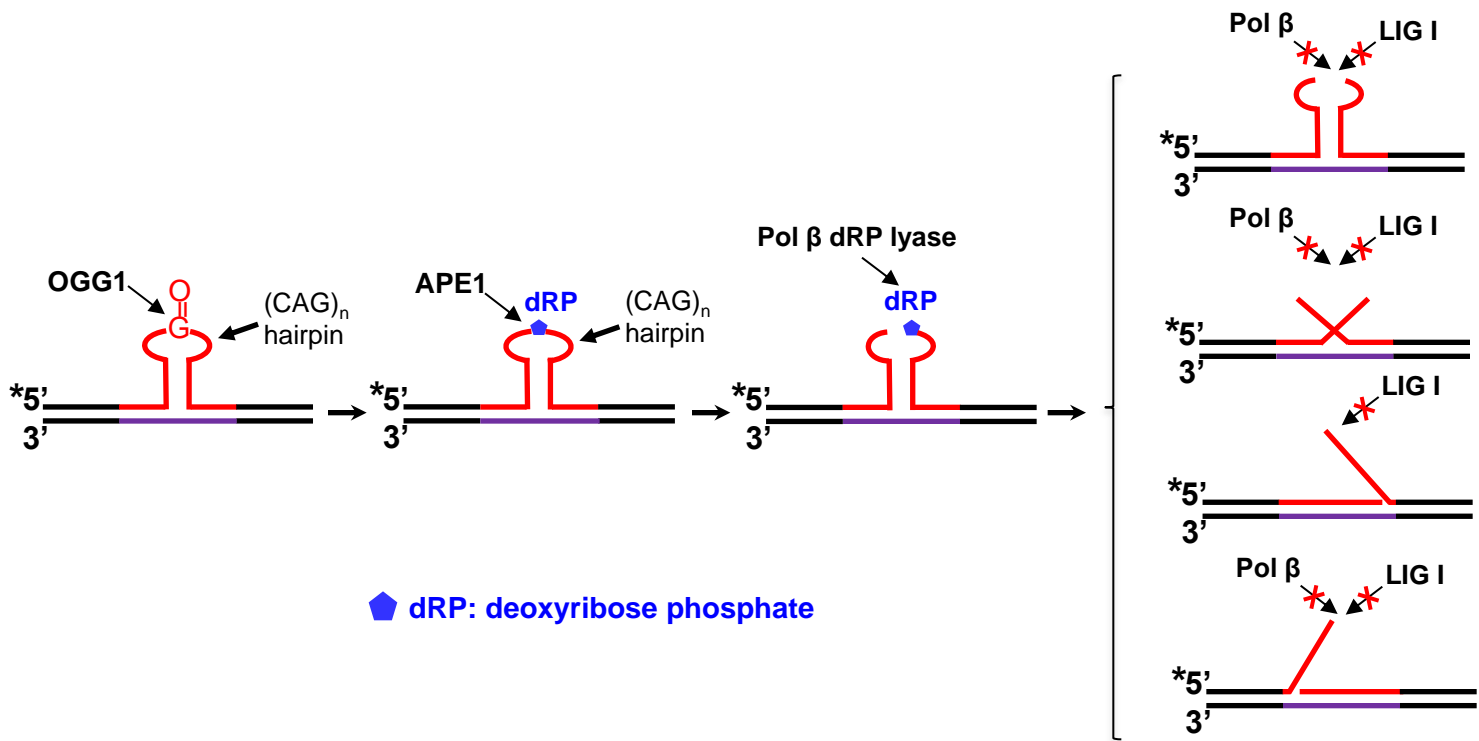
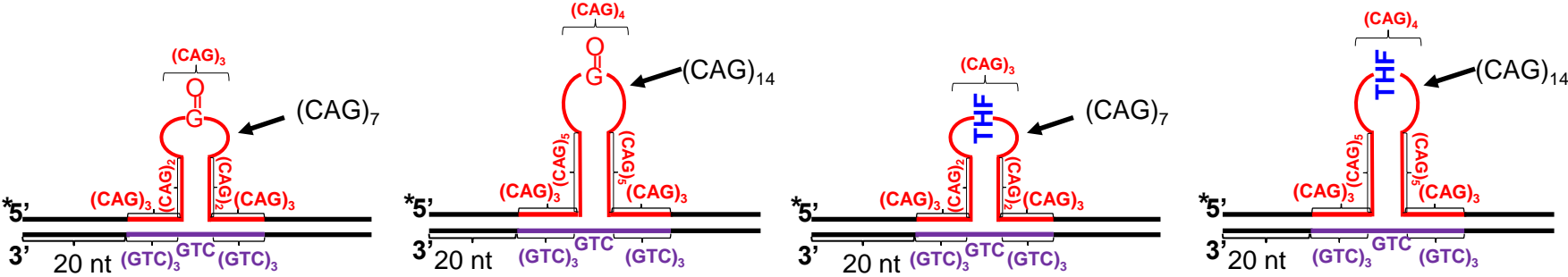
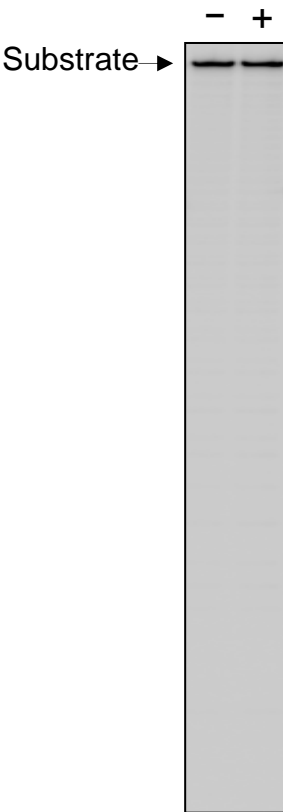
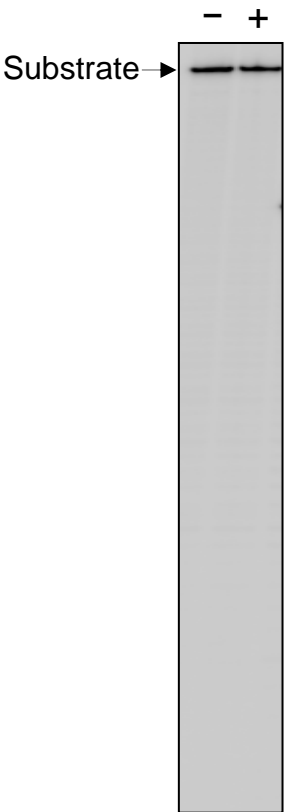
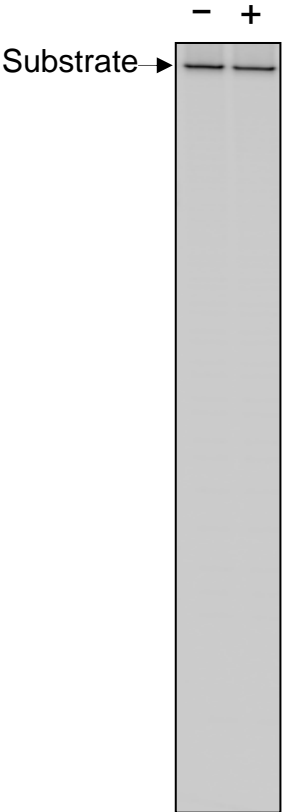


Fig. S4



Mus81/Eme1  
(250 nM)

Substrate →



Lanes

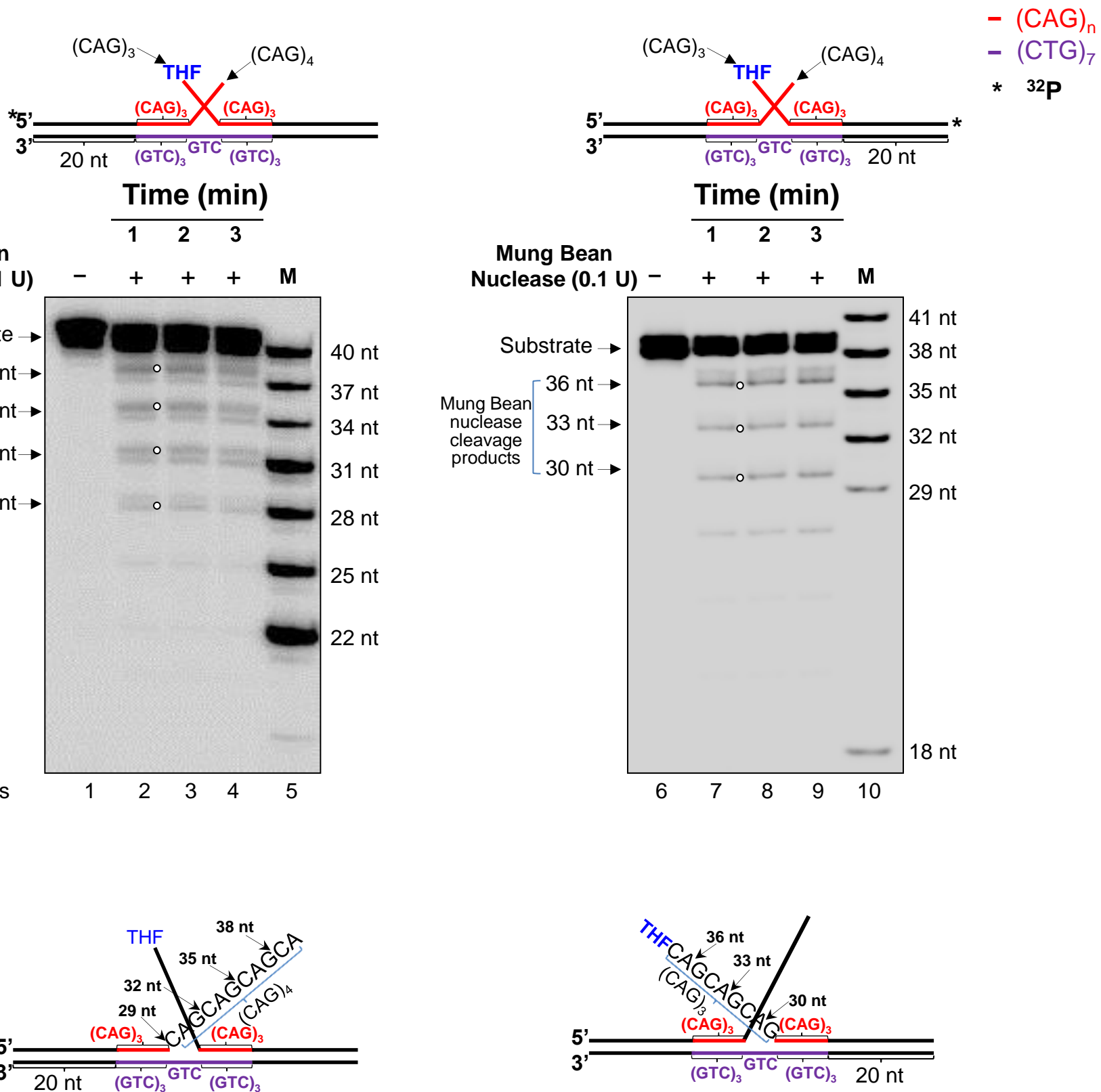
1 2

3 4

5 6

7 8

**Fig. S5**





**Fig. S6**

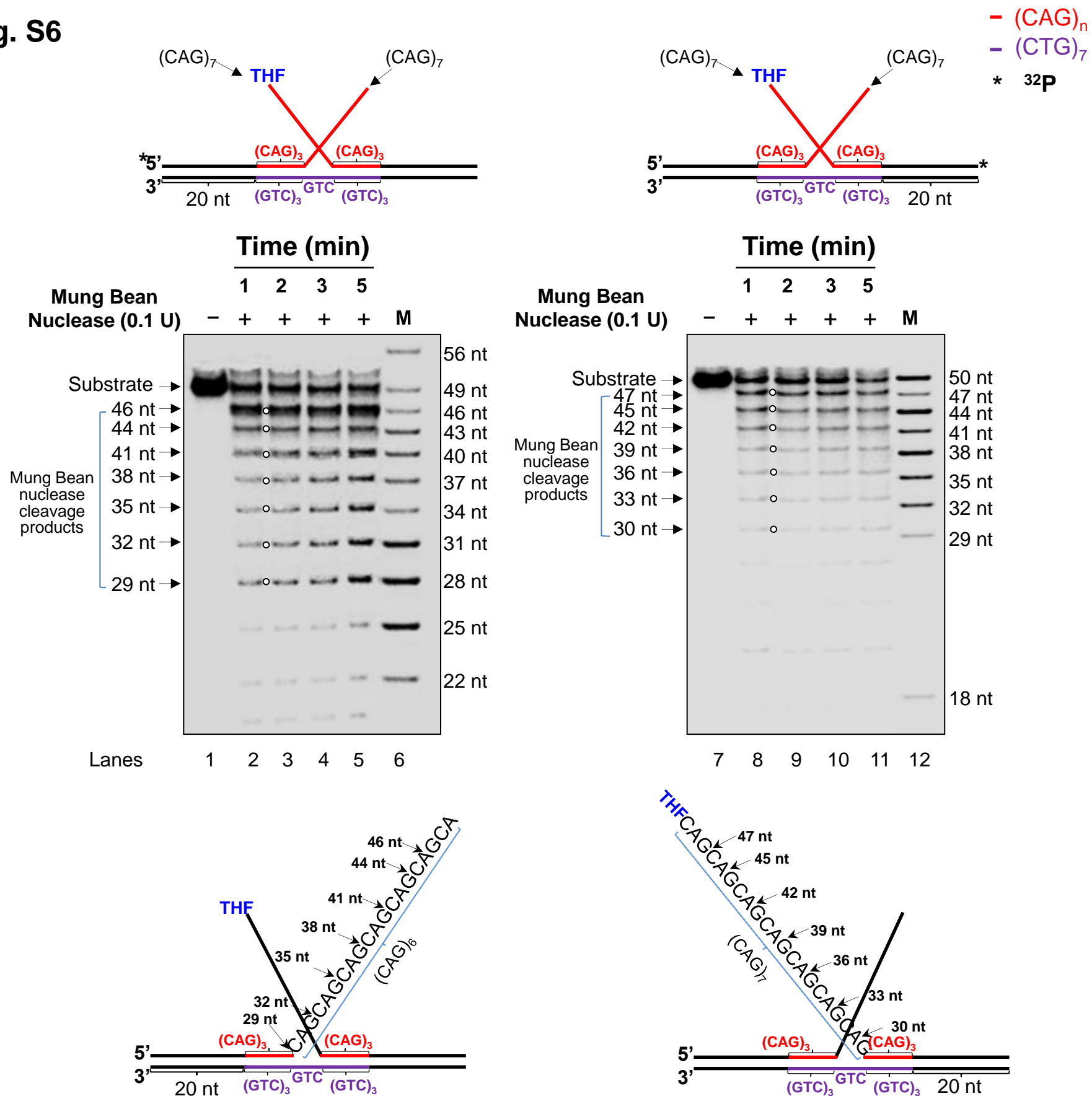
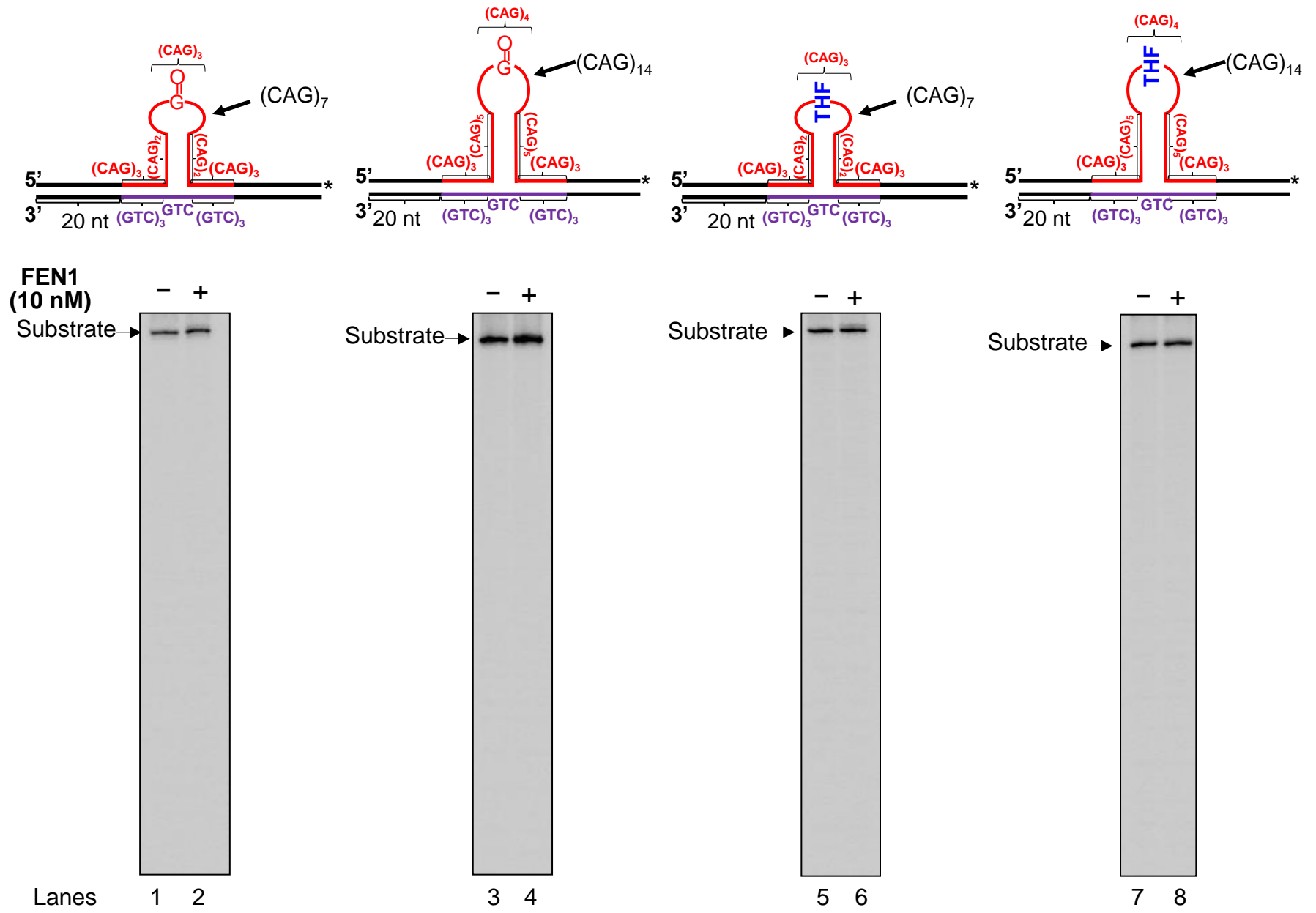


Fig. S7

\* 32P



## Supplementary Data

### Supplementary Figure S1. Hairpin probing of (CAG)<sub>7</sub>- and (CAG)<sub>14</sub>-8-oxoG hairpin substrates

(A) The damaged strand of the (CAG)<sub>7</sub>- or (CAG)<sub>14</sub>-8-oxoG hairpin substrate was radiolabeled at the 3'-end. Substrates were incubated with 0.15 U of Mung Bean Nuclease at 1-, 2- and 3-min time intervals (lanes 2-4, and lanes 7-9). Lanes 1 and 6 represent the undigested substrate. Lanes 5 and 10 represent 3'-radiolabeled synthesized size markers (M) with 29 nt, 32 nt, 35 nt, 38 nt, 41 nt, 44 nt, 47 nt, 50 nt, 53 nt, 56 nt, 62 nt, 68 nt and 74 nt, respectively. (B) The damaged strand of the (CAG)<sub>7</sub>- or (CAG)<sub>14</sub>-8-oxoG substrates was radiolabeled at 5'-end. Substrates were incubated with 0.2 U or 0.5 U of S1 Nuclease at 3-, 5- and 10-min time intervals (lanes 2-4, and lanes 7-9). Lanes 1 and 6 represent the undigested substrate. Lanes 5 and 10 represent 5'-radiolabeled synthesized size markers (M) with 22 nt, 25 nt, 28 nt, 31 nt, 34 nt, 37 nt, 40 nt, 43 nt, 46 nt, 49 nt, 52 nt, 55 nt, 58 nt, 61 nt, 64 nt, 67 nt, and 70 nt, respectively. For all the experiments, 200 nM of substrate was used. Arrows and white dots indicate the major digestion products. The substrates are illustrated schematically above the gels. Hairpins deduced by a specific nuclease cleavage pattern and the nuclease digestion sites are illustrated schematically below the gels.

### Supplementary Figure S2. Hairpin probing of (CAG)<sub>7</sub>- and (CAG)<sub>14</sub>-THF hairpin substrates

(A) The (CAG)<sub>7</sub>- and (CAG)<sub>14</sub>-THF hairpin substrates were radiolabeled at the 5'-end of the damaged strand. Substrates (200 nM) were incubated with 0.1 U of Mung Bean Nuclease at 1-, 2- and 3-min time intervals (lanes 2-4 and lanes 7-9). Lanes 1 and 6 represent the undigested substrate. Lanes 5 and 10 represent 5'-radiolabeled synthesized size markers (M) with 22 nt, 25 nt, 28 nt, 31 nt, 34 nt, 37 nt, 40 nt, 43 nt, 46 nt, 49 nt, 52 nt, 55 nt, 58 nt, 61 nt, 64 nt, 67 nt, and 70 nt, respectively. (B) 5'- radiolabeled substrates were incubated with 0.2 U or 0.5 U of S1 Nuclease at 3-, 5- and 10-min time intervals (lanes 2-4, and lanes 7-9). Lanes 1 and 6 represent the undigested substrate. Lanes 5 and 10 represent 5'-radiolabeled synthesized size markers (M) with 22 nt, 25 nt, 28 nt, 31 nt, 34 nt, 37 nt, 40 nt, 43 nt, 46 nt, 49 nt, 52 nt, 55 nt, 58 nt, 61 nt, 64 nt, 67 nt, and 70 nt, respectively. Arrows and white dots indicate the major nuclease digestion products. The substrates are illustrated schematically above the gels. Hairpins deduced by a specific nuclease cleavage pattern and the nuclease digestion sites are illustrated schematically below the gels.

### Supplementary Figure S3. Short-patch BER of (CAG)<sub>7</sub>- and (CAG)<sub>14</sub>-8-oxoG hairpin

### **containing substrates**

Short-patch BER of an 8-oxoG in the loop region of a CAG hairpin was examined by incubating the (CAG)<sub>7</sub>- and (CAG)<sub>14</sub>-8-oxoG substrates (25 nM) with OGG1 (10 nM) alone (lanes 2 and 7), or OGG1 (10 nM) along with APE1 (50 nM) (lanes 3 and 8) or OGG1 (10 nM) along with both APE1 (50 nM) and pol β (5 nM) (lanes 4 and 9) or OGG1 (10 nM) along with APE1 (50 nM), pol β (5 nM) and LIG (5 nM) (lanes 5 and 10) at 37°C for 30 min. Lanes 1 and 6 correspond to substrates only. Substrates were <sup>32</sup>P-labeled at the 5'-end of their damaged strands and are illustrated schematically above the gels. The scheme of short-patch BER of an 8-oxoG in the hairpin substrates is illustrated below the gels.

### **Supplementary Figure S4. Mus81/Eme1 cleavage of a hairpin**

The (CAG)<sub>7</sub>-8-oxoG/THF and (CAG)<sub>14</sub>-8-oxoG/THF substrates were radiolabeled at the 5'-end of the hairpin containing strand. Substrates (25 nM) were incubated with 250 nM purified Mus81/Eme1 at 37°C for 30 min (lanes 2, 4, 6 and 8). Lanes 1, 3, 5 and 7 represent substrate alone. Substrates are illustrated schematically above the gels.

### **Supplementary Figure S5. Flap probing of (CAG)<sub>3</sub>/(CAG)<sub>4</sub> double-flap substrate**

A substrate with a 3'-(CAG)<sub>4</sub> flap and a 5'-(CAG)<sub>3</sub>-THF flap was radiolabeled at the 5'-end (left panel) of the upstream strand or the 3'-end (right panel) of the downstream strand for probing the formation of an upstream 3'-flap and a downstream 5'-flap. The substrate (200 nM) was incubated with 0.1 U of Mung Bean Nuclease at 1-, 2- and 3-min time intervals (lanes 2-4 and lanes 7-9). Lanes 1 and 6 represent an undigested substrate. Lanes 5 and 10 represent 5'-radiolabeled synthesized size markers (M) with 22 nt, 25 nt, 28 nt, 31 nt, 34 nt, 37 nt, and 40 nt, respectively (left panel) and 3'-radiolabeled size markers (M) with 18 nt, 29 nt, 32 nt, 35 nt, 38 nt, and 41 nt, respectively. Arrows and white dots indicate the major nuclease digestion products. The substrates are illustrated schematically above the gels. Double-flaps deduced by a specific nuclease cleavage pattern and the nuclease digestion sites are illustrated schematically below the gels.

### **Supplementary Figure S6. Flap probing of (CAG)<sub>7</sub> double-flap substrate**

A substrate with a 3'-(CAG)<sub>7</sub>- and 5'-(CAG)<sub>7</sub>-THF flap was radiolabeled at the 5'-end of the upstream, strand (left panel) or the 3'-end of the downstream strand (right panel). The substrate (200 nM) was incubated with 0.1 U of Mung Bean Nuclease at 1-, 2-, 3-, and 5-min time intervals (lanes 2-5 and lanes 8-11). Lanes 1 and 7 represent an undigested substrate. Lane 6 represents 5'-

radiolabeled synthesized size markers (M) with 22 nt, 25 nt, 28 nt, 31 nt, 34 nt, 37 nt, 40 nt, 43 nt, 46 nt, 49 nt and 56 nt for probing the formation of a 3'-flap. Lane 12 represents 3'-radiolabeled size markers (M) with 18 nt, 29 nt, 32 nt, 35 nt, 38 nt, 41 nt, 44 nt, 47 nt and 50 nt for probing the formation of a 5'-flap. Arrows and white dots indicate the major nuclease digestion products. Substrates are illustrated schematically above the gels. Double-flaps deduced by a specific nuclease cleavage pattern and the nuclease digestion sites are illustrated schematically below the gels.

**Supplementary Figure S7. FEN1 cleavage of a hairpin**

The (CAG)<sub>7</sub>-8-oxoG/THF and (CAG)<sub>14</sub>-8-oxoG/THF hairpin substrates were labeled at the 3'-end of the hairpin-containing strand. Substrates (25 nM) were incubated with 10 nM purified FEN1 at 37°C for 30 min (lanes 2, 4, 6 and 8). Lanes 1, 3, 5 and 7 represent a substrate alone. Substrates are illustrated schematically above the gels.