Suppl. Fig. 1s

The oligonucleotides used in this study. The mutations are indicated by bold underlined letters.

Suppl. Fig. 2s

PAR (100 μM) was added to a DnaI solution (10 μM) and different aliquots of the DnaI/PAR mixture were then prepared in the presence of increasing concentrations (0.1–1.6 mM) of *p*-chloromercuribenzoic acid. The absorbance at 500 nm (A_{500}) was measured for all the aliquots. The Zn^{2+} ions released from DnaI by PAR were coordinated by PMBA and the resulting PMBA- Zn^{2+} complex absorbed light 500 nm manifested by an increase of A_{500} (left graph). Finally, increasing concentrations of EDTA were added in the aliquot with the highest concentration of PMBA (highest A_{500}) and the A_{500} was monitored (right graph). Gradual decrease in A_{500} indicated the extraction of the metal from the PMBA- Zn^{2+} complex by EDTA.

Suppl. Fig. 3s

CD spectra of all mutant proteins (5 μM) compared to wtDnaI, as indicated. All mutant proteins produced spectra comparable to the wtDnaI indicating proper overall folding.

Suppl. Fig. 4s

Comparison of the ATPase activities of wtDnaI and K174A. The top panel shows a TLC plate with the reaction products from a time course in the presence of 16.7 nM [γ-^{32}PATP] and 8 μM of either wt DnaI (left) or K174A (right), as indicated. The % hydrolysis was calculated and the comparative graphs are shown in the bottom panel. Lane C indicates a negative control, ATP without any proteins and incubated for 90 min in the same buffer. The activity of the K174A mutant is significantly reduced compared to wtDnaI.

Suppl. Fig. 5s

DnaI exhibits single turnover ATPase activity.

TLC-based ATPase assays at constant ATP concentration (50 μM) and varying DnaI concentrations (0.1, 1, 5, 10, 25 and 50 μM; bars 1-6, respectively). All reactions were carried out as described in the 'Materials and Methods' section using 100 μM ATP supplemented with 16.65 nM γ-^{32}P-ATP for 2 hours at 37^{o}C. The white bars indicate the concentration of DnaI and the grey bars the concentration of the hydrolysed ATP. All reactions were carried out in triplicate and error bars are indicated. A representative TLC plate from these reactions is also shown; lane C indicates a control reaction in the absence of DnaI.

The data indicate that at 0.1 μM DnaI, 0.1 μM ATP is hydrolysed but as the DnaI concentration is increased to 1, 5, 10, 25 and 50 μM the ATP hydrolysed is 0.7, 2.9, 3.9, 8.8 and 14.4 μM, respectively.

Mutagenesis:

C67AF 5'-CAAAGCAAGAA**GCC**TCCTATTGTTCGGAA-3'

C67AR 5'-TTCCGAACAATAGGA**GGC**ATTCTTGCTTTG-3'

C70AF 5'-AATTGCTCCTAT**GCT**TCGGAAGATGA-3'

C70AR 5'-TCATCTTCCGA**AGC**ATAGGAGCAATT-3'

C76AF 5'-GAAGATGAAAAC**GCC**AACAATTTGTTG-3'

C76AR 5'-CAACAAATTGTT**GGC**GTTTTCATCTTC-3'

H84AF 5'-TTGGAGGGCTAC**GCT**CCGAAGCT-3'

H84AR 5'-AGCTTCAA**AGC**GTAGCCCTCCAA-3'

C101AF 5'-GAGTATTACGAA**GCT**CCAGTCAAACGG-3'

C101AR 5'-CCGTTTGACTGG**AGC**TTCGTAATACTC-3'

K174AF 5'-TTTGGGGTAGGA**GCG**ACGTTTATGCTCGCT-3'

K174AR 5'-AGCGAGCATAAACGT**CGC**TCCTACCCCAAA-3'

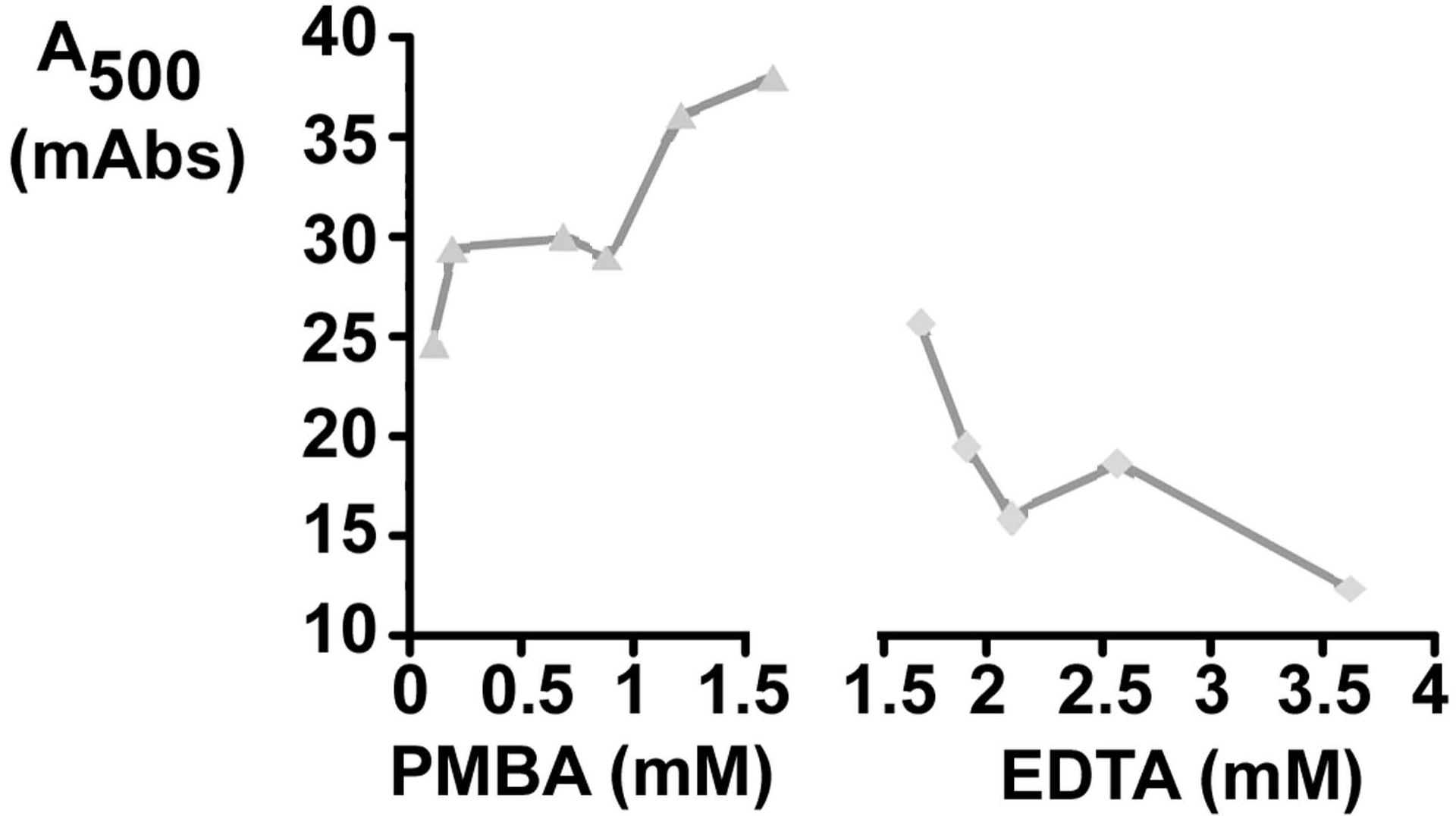
Gel shifts:

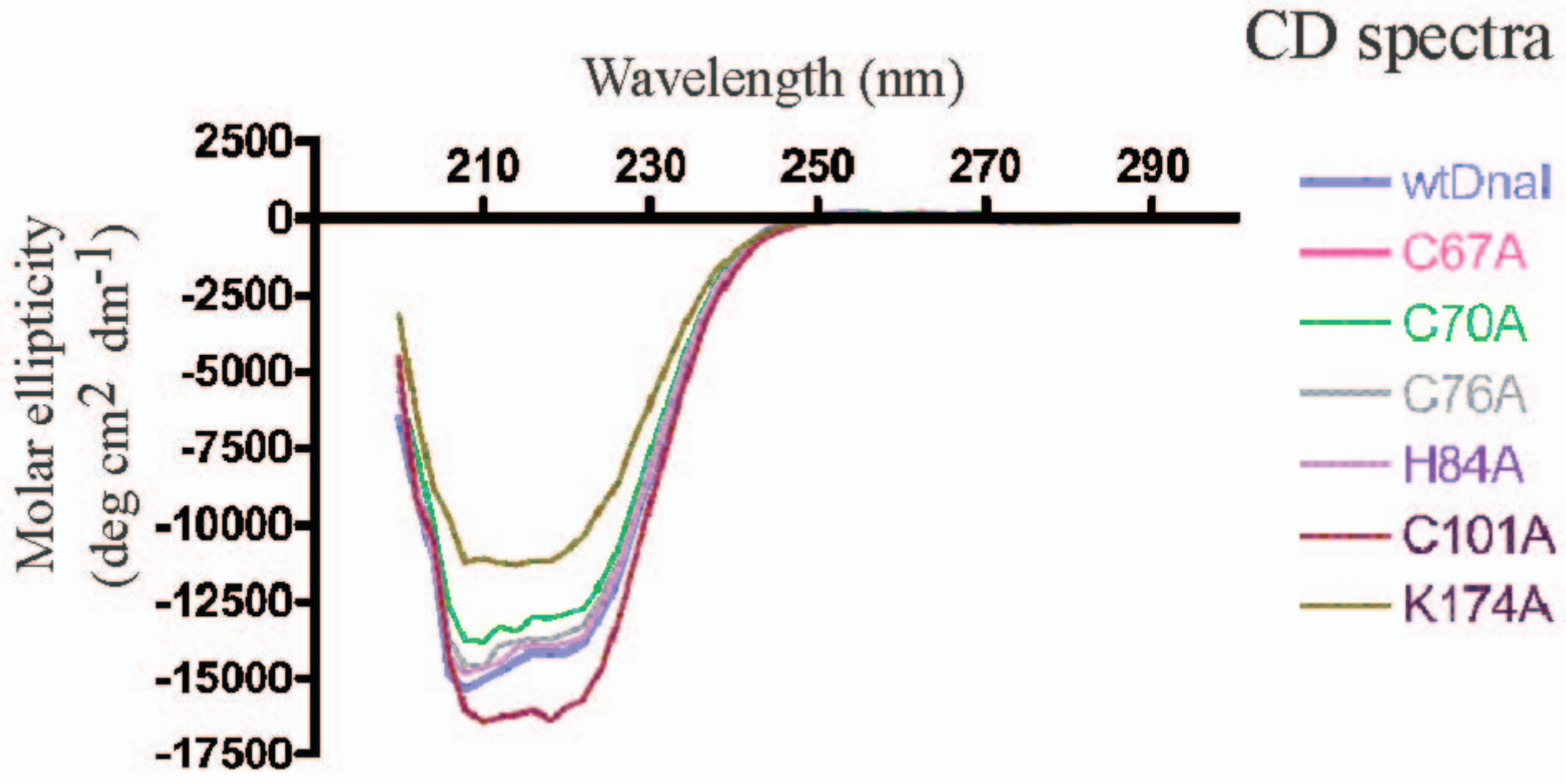
5'-GTCGGATCCTCTAGACAGCTCCATGATCACTGGCAC
TGGTAGAATTCGGC-3'

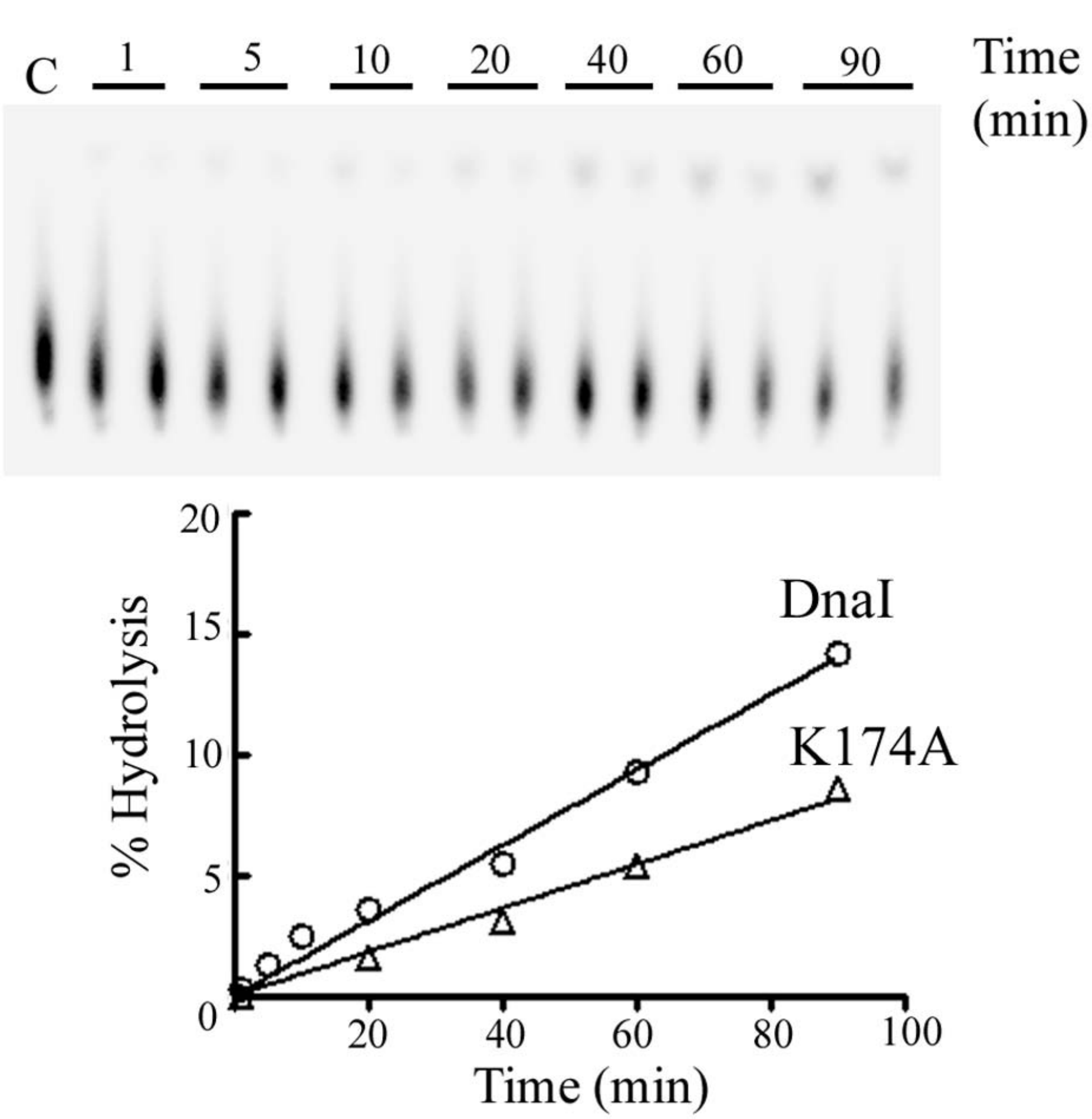
5'-GCCGAATTCTACCAGTGCCAGTGATGGACATCTTT
GCCCACGTTGACCCA-3'

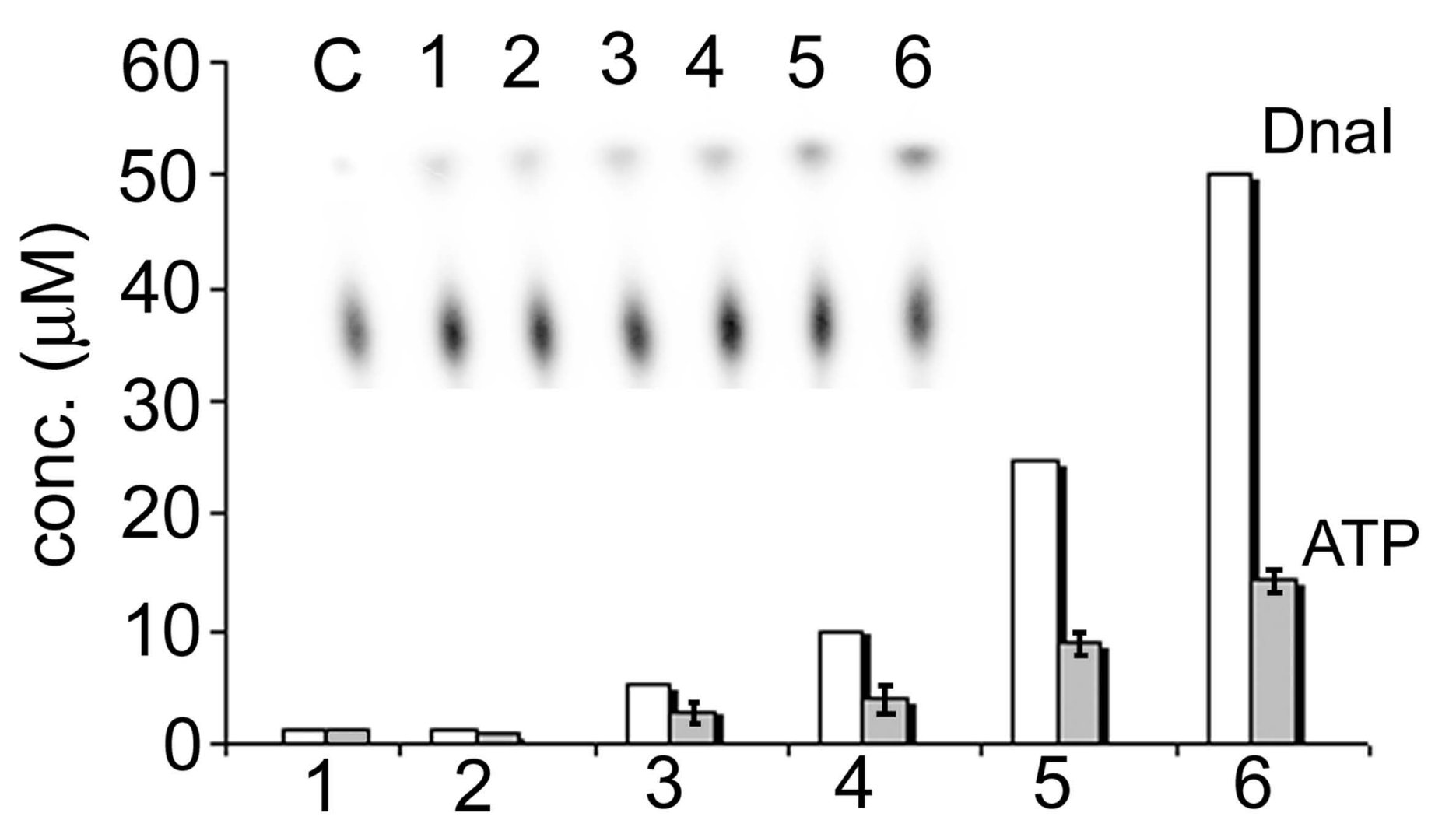
TLC assays:

5'-TGCATGCCTGCAGGTCGACTCTAGAGGATCCCC-3'


A500
(mAbs)
40
35
30
25
20
15
10
0
0.5
1
1.5
PMBA (mM)
1.5
2
2.5
3
3.5
4
EDTA (mM)


CD spectra
Wavelength (nm)
210
230
250
270
290
2500
0
-2500
-5000
-7500
-10000
-12500
-15000
-17500
Molar ellipticity
(deg cm2 dm-1)
wtDnaI
C67A
C70A
C76A
H84A
C101A
K174A


C
1
5
10
20
40
60
90
Time (min)
% Hydrolysis
0
5
10
15
20
DnaI
K174A
20
40
60
80
100
Time (min)


C 1 2 3 4 5 6
60
50
40
30
20
10
0
conc. (μM)
DnaI
ATP
1
2
3
4
5
6