

## Supporting Information

### Energetics of Base-Flipping at a DNA Mismatch Site Confined at the Latch Constriction of $\alpha$ -Hemolysin

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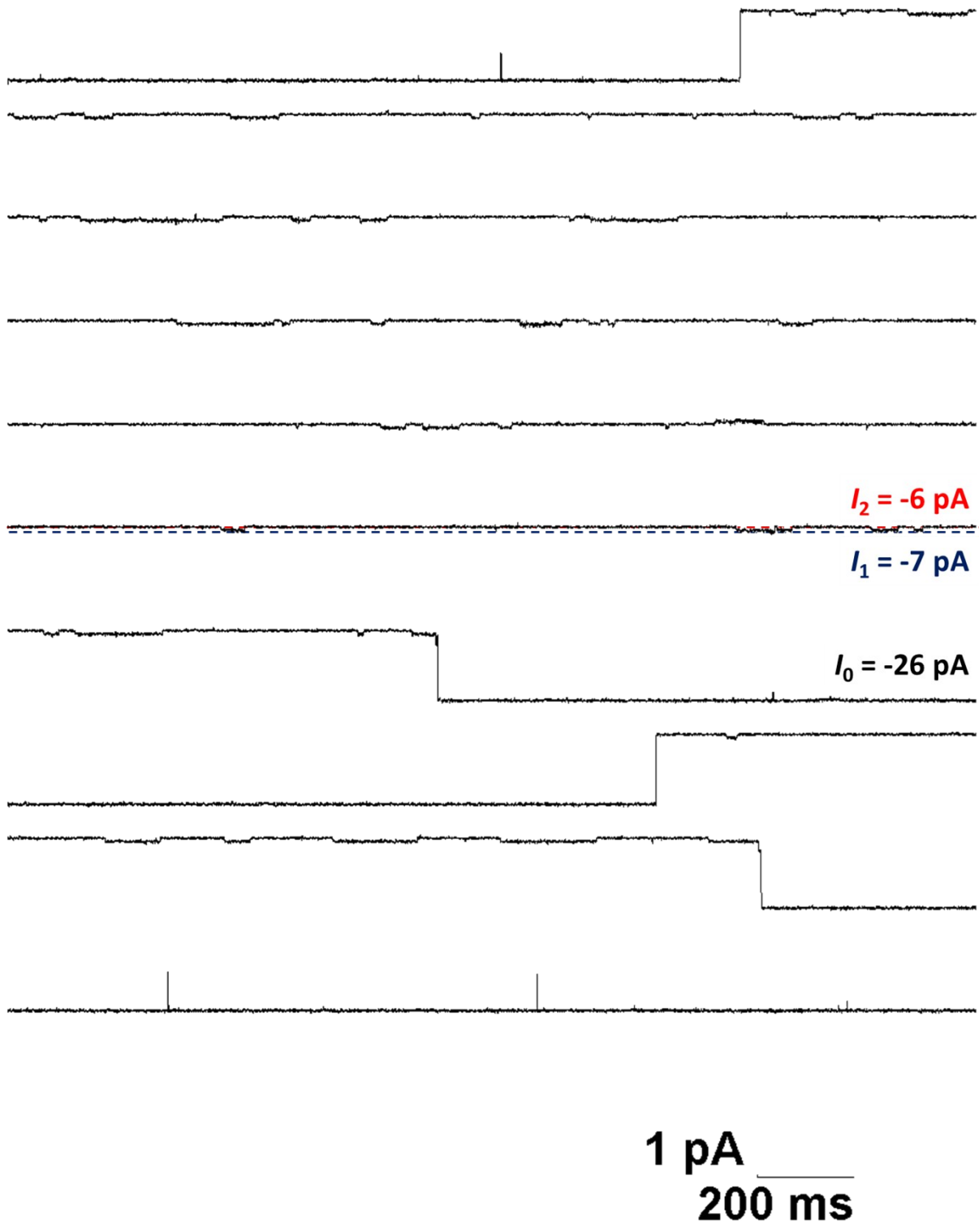
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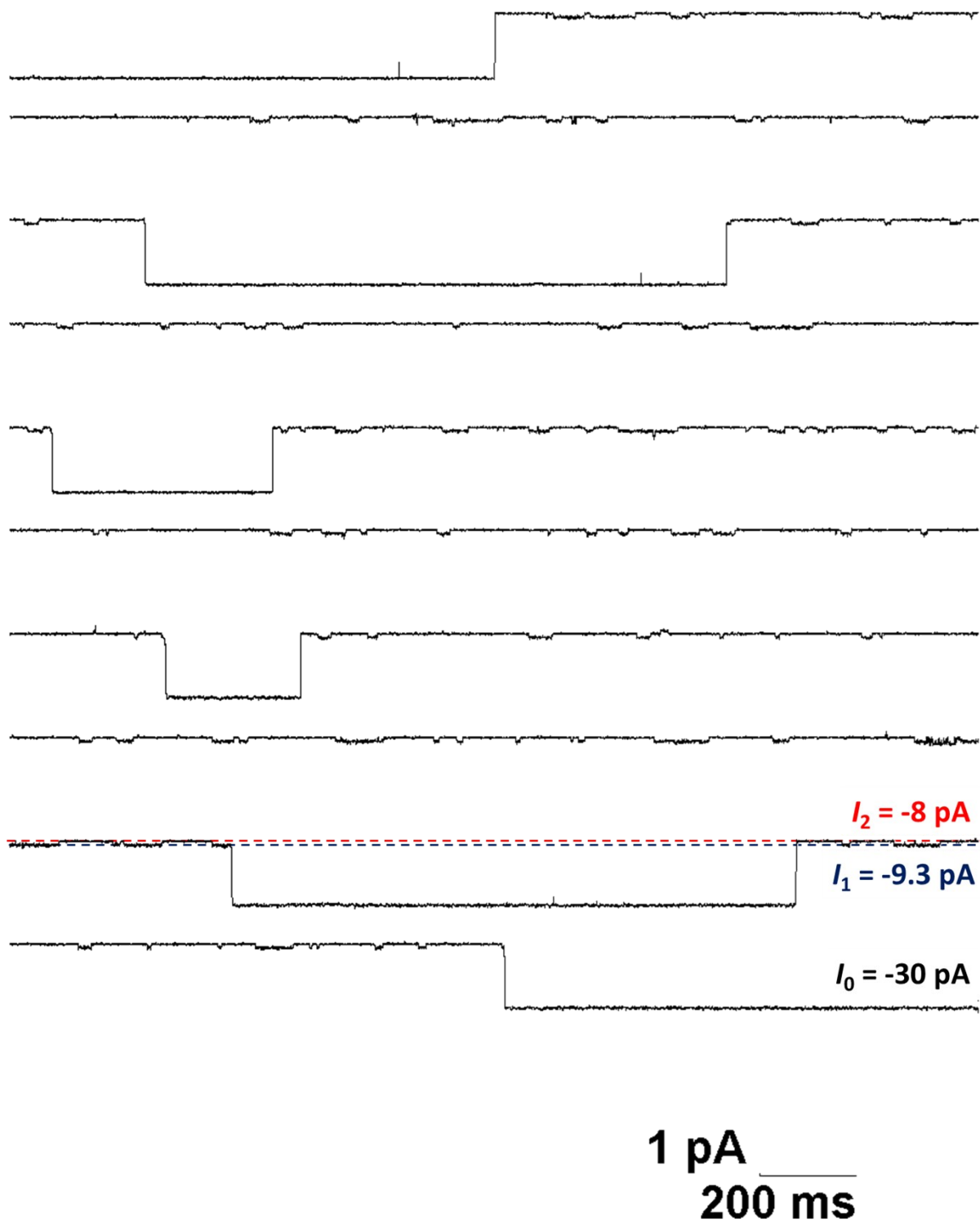
1. Effect of temperature on capture and analysis of the CC<sub>9</sub> duplex.....S1
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### Effect of temperature on capture and analysis of the CC<sub>9</sub> duplex

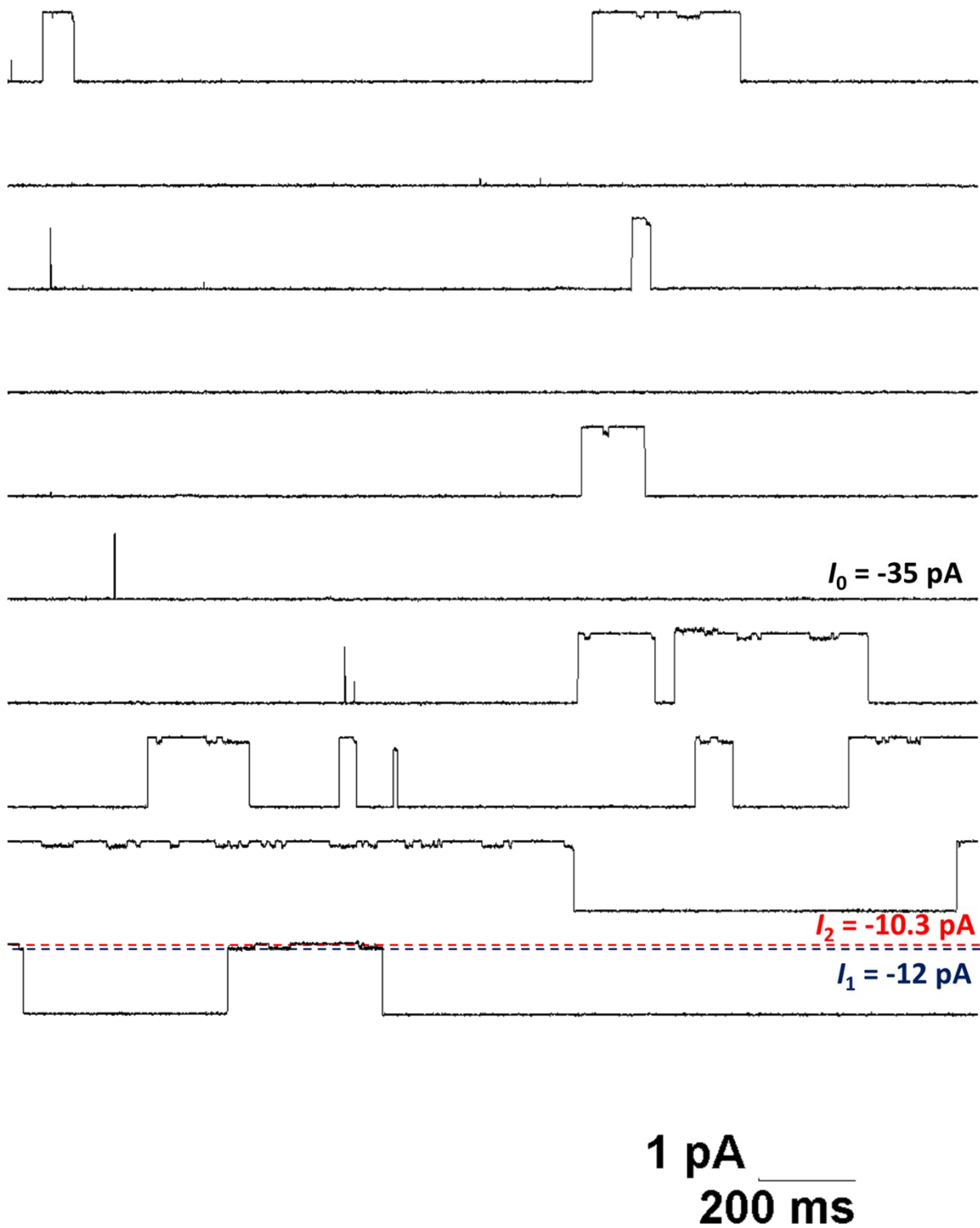
Figures S1-S6 are examples shown as an accompaniment to Figure 2 in the main text. Each shows approximately 20 s of recorded data from the same  $\alpha$ HL channel but at different temperatures. Labels for  $I_0$  (open channel current), and  $I_1$  and  $I_2$  (current states observed when DNA is inside  $\alpha$ HL) have been added as a guide to eye.



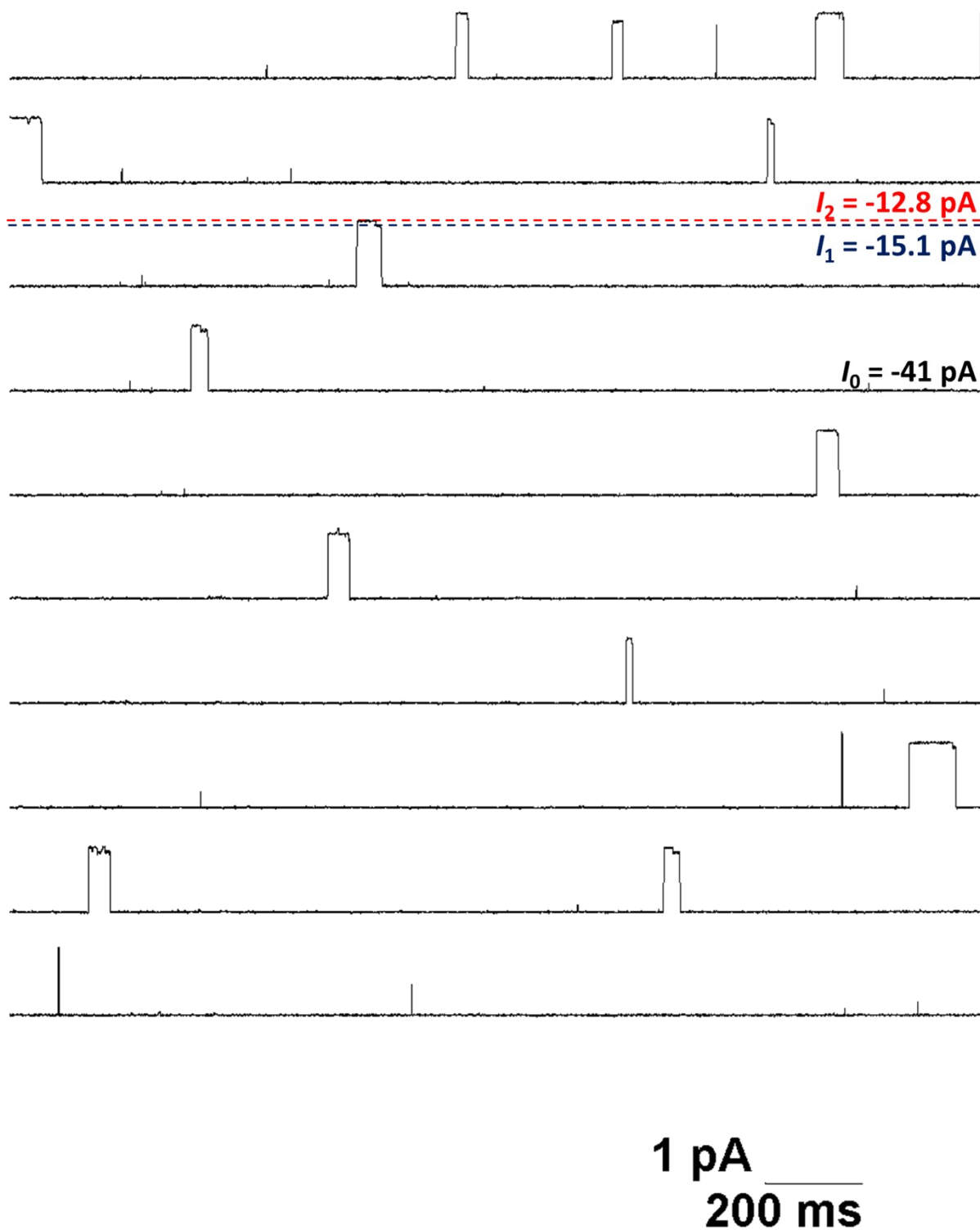
**Figure S1.** Extended current-time trace demonstrating capture and analysis of the CC<sub>9</sub> duplex at 18 °C. Data were recorded in a 10 mM phosphate buffer (pH 7.5) with 0.25 M KCl. A potential of 120 mV was applied across the  $\alpha$ HL channel.



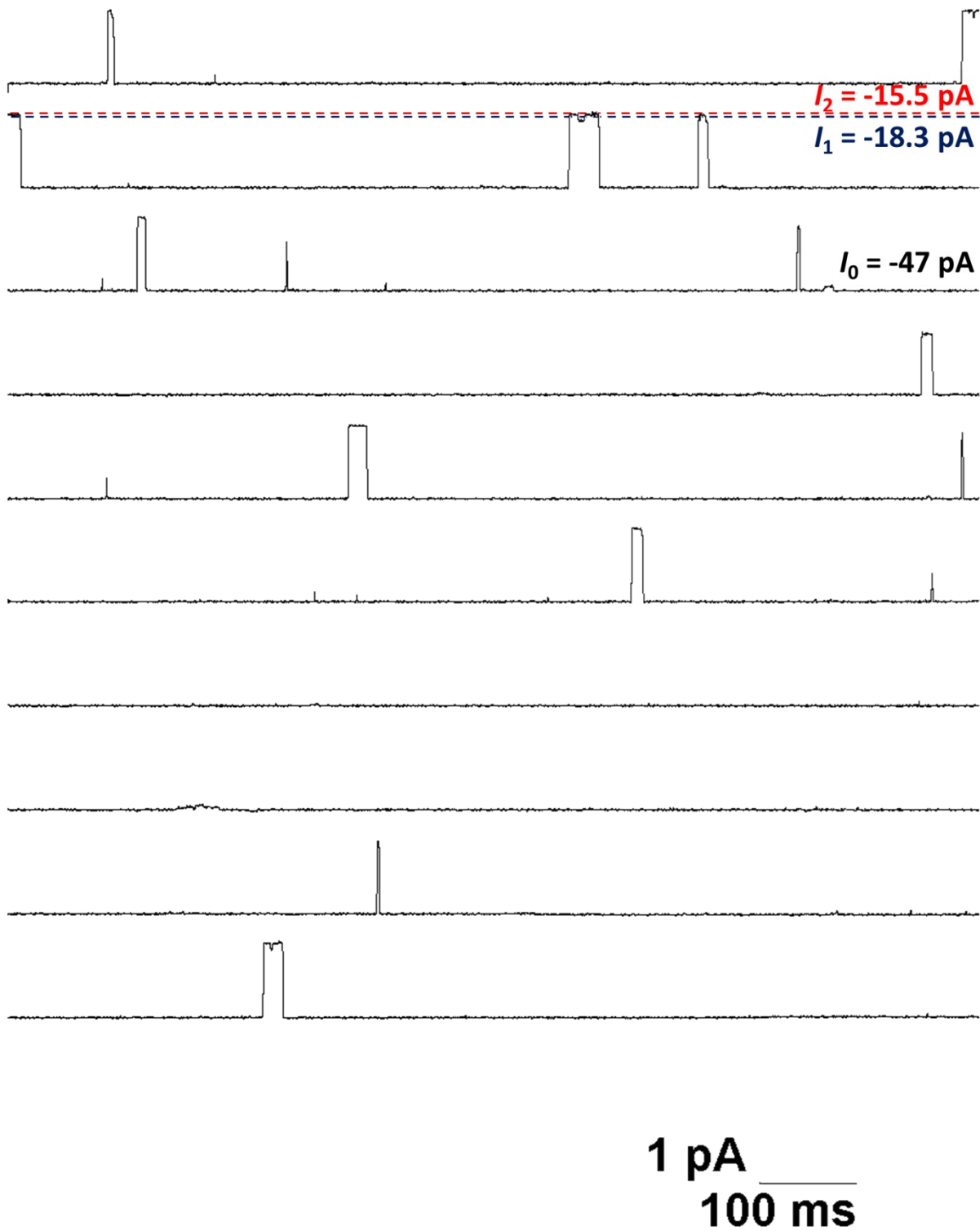
**Figure S2.** Extended current-time trace demonstrating capture and analysis of the CC<sub>9</sub> duplex at 20 °C. Data were recorded in a 10 mM phosphate buffer (pH 7.5) with 0.25 M KCl. A potential of 120 mV was applied across the  $\alpha$ HL channel.



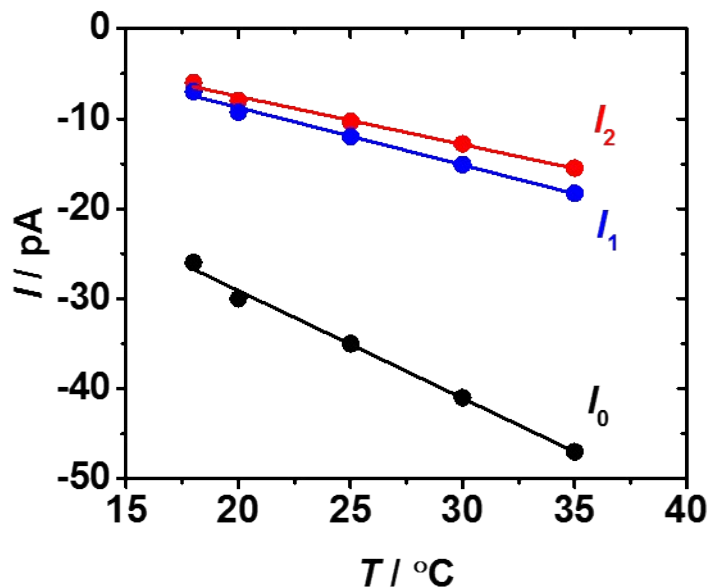
**Figure S3.** Extended current-time trace demonstrating capture and analysis of the  $CC_9$  duplex at 25 °C. Data were recorded in a 10 mM phosphate buffer (pH 7.5) with 0.25 M KCl. A potential of 120 mV was applied across the  $\alpha$ HL channel.



**Figure S4.** Extended current-time trace demonstrating capture and analysis of the  $\text{CC}_9$  duplex at  $30^\circ\text{C}$ . Data were recorded in a  $10 \text{ mM}$  phosphate buffer ( $\text{pH } 7.5$ ) with  $0.25 \text{ M KCl}$ . A potential of  $120 \text{ mV}$  was applied across the  $\alpha\text{HL}$  channel.



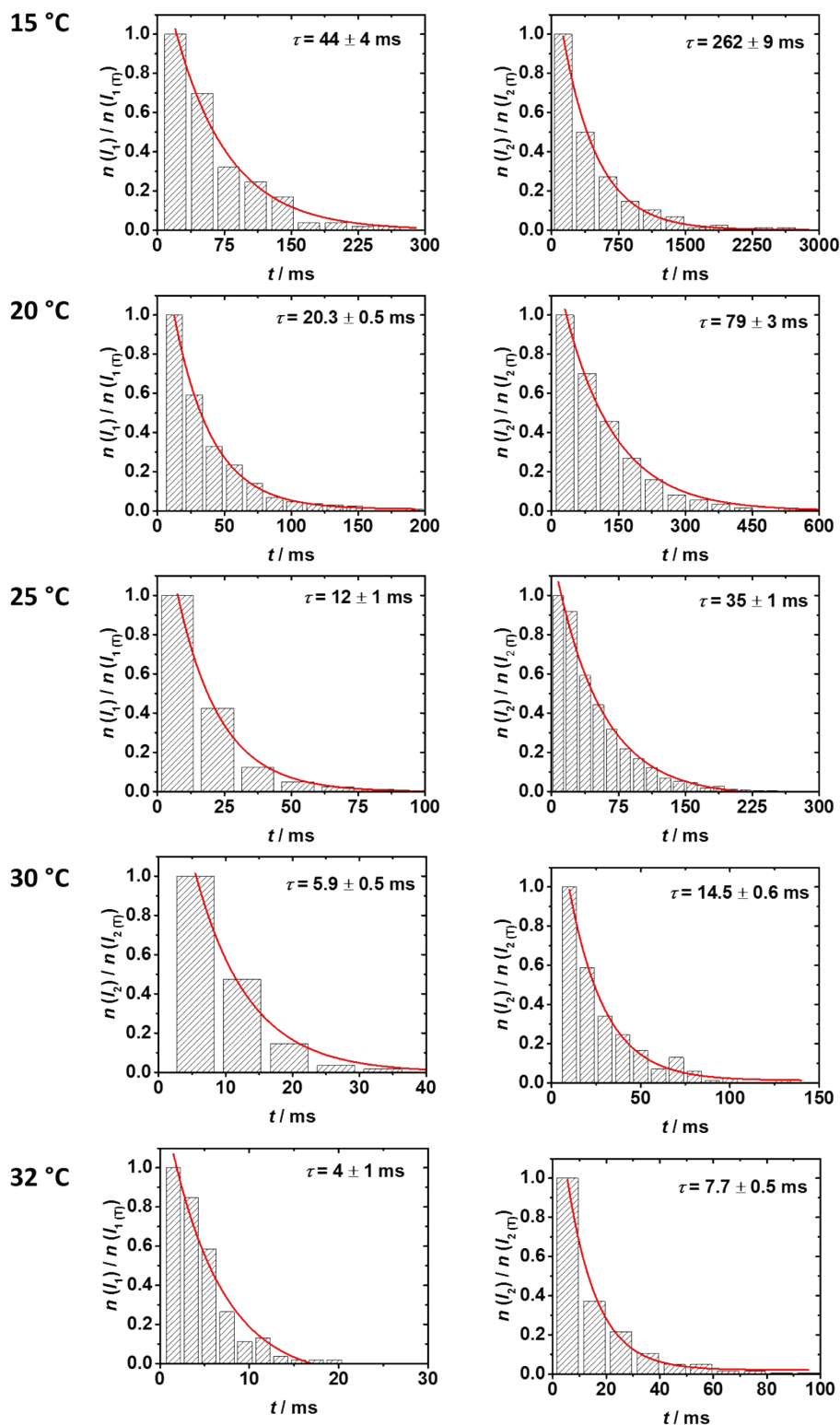
**Figure S5.** Extended current-time trace demonstrating capture and analysis of the  $\text{CC}_9$  duplex at 35 °C. Data were recorded in a 10 mM phosphate buffer (pH 7.5) with 0.25 M KCl. A potential of 120 mV was applied across the  $\alpha\text{HL}$  channel.



**Figure S6.** The residual current of states  $I_1$ ,  $I_2$ , and  $I_0$  increase approximately linearly with temperature. Data are shown for measurements from a single  $\alpha$ HL protein channel, recorded at different temperatures (Figures S1-S5). States  $I_1$  and  $I_2$  correspond to the flipped-in and flipped-out states, respectively.  $I_0$  corresponds to the open channel current. Data were recorded in a 10 mM phosphate buffer (pH 7.5) with 0.25 M KCl. A potential of 120 mV was applied across the  $\alpha$ HL channel.

## State lifetime histograms as a function of temperature

Figure S7 shows the kinetic data from which the lifetime constants for states  $I_1$  and  $I_2$  were derived at each temperature, as an accompaniment to Figure 3 from the main text. Data for  $T = 18^\circ\text{C}$  and  $35^\circ\text{C}$  are shown in Figure 3.



**Figure S7.** Lifetime histograms of the states  $I_1$  and  $I_2$  as a function of temperature. Data at  $18^\circ\text{C}$  and  $35^\circ\text{C}$  are shown in Figure 2 (main text).