Supporting Information:

Interrogation of Base Pairing of the Spiroiminodihydantoin Diastereomers Using the α-Hemolysin Latch

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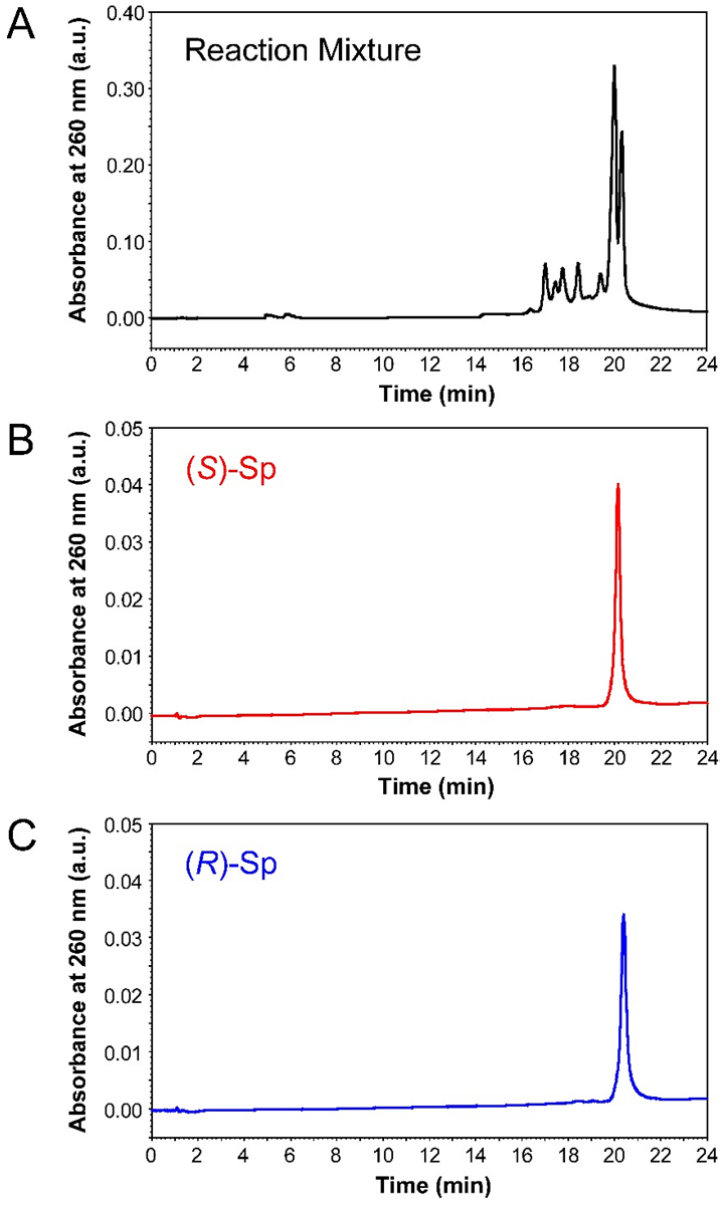

Figure S1. HPLC chromatograms of the reaction mixture (A, black) and the purified oligomers containing (*S*)-Sp (B, red) or (*R*)-Sp (C, blue).

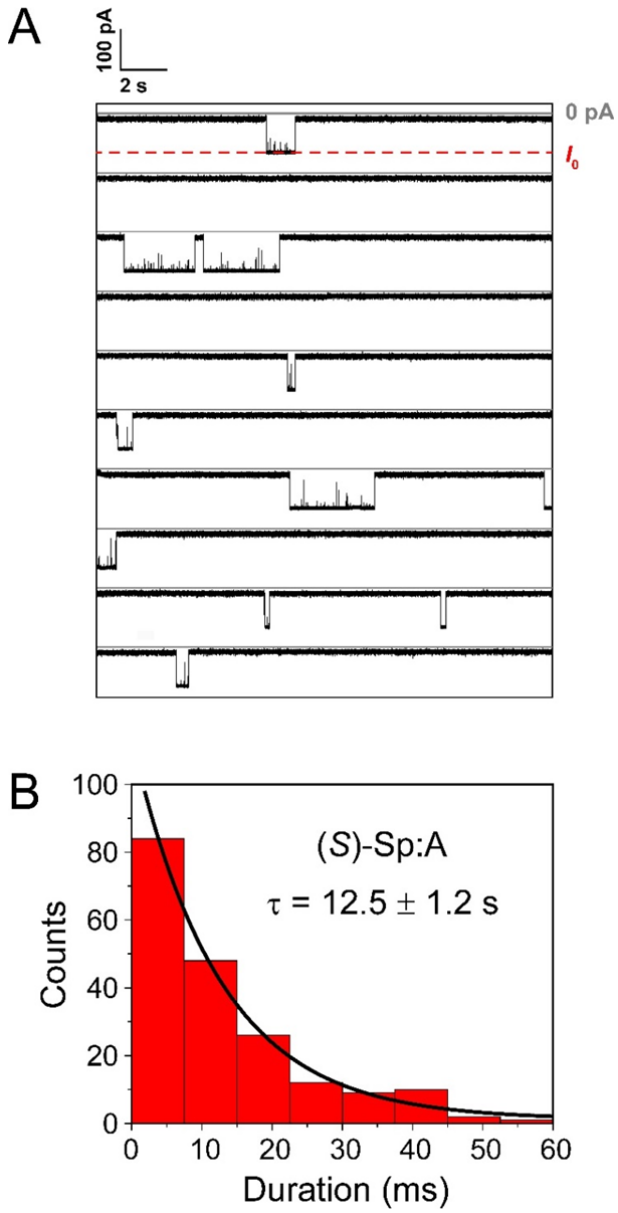

Figure S2. Uninterrupted *i–t* trace (A) and corresponding duration histogram (B) for (*S*)-Sp:A-containing duplex in the αHL nanopore. The unzipping lifetime (τ) was obtained by fitting the duration histogram (B) with a first order exponential decay function (the error is based on three individual experiments, in which each experiment comprised over 200 events). Typically, the duplex structure stays inside the αHL channel for tens of seconds before unzipping. The traces were recorded in 1.00 M KCl, 10 mM KP_i, pH 7.4 at 100 mV (*trans vs. cis*) and 22 ± 1 °C.

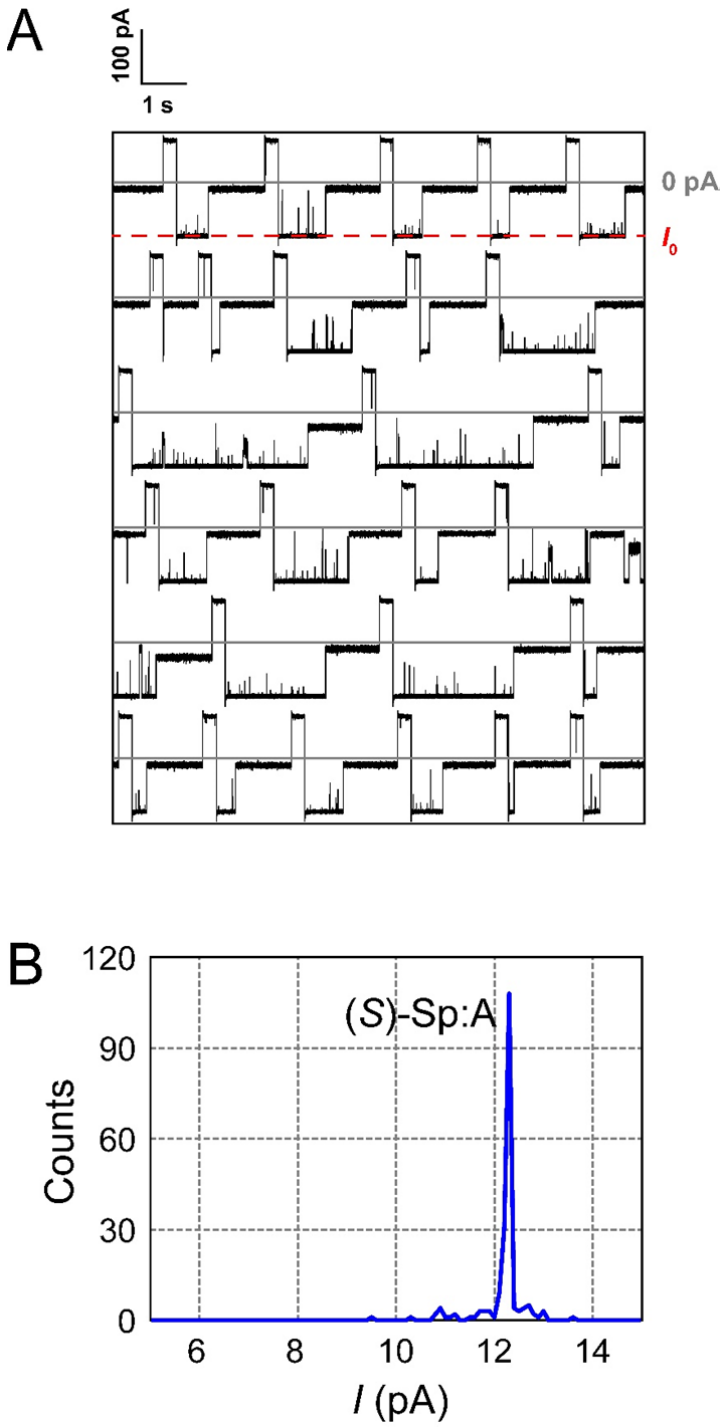

Figure S3. Representative *i–t* trace (A) and current histogram (B) for (*S*)-Sp:A-containing duplex in the αHL nanopore. The single peak in the current histogram supports a predominant base pairing configuration between (*S*)-Sp and A. The traces were recorded in 1.00 M KCl, 10 mM KP_i, pH 7.4 at 100 mV (*trans vs. cis*) and 22 ± 1 °C.

Table S1. Current blocks normalized by the open channel current. The data were obtained in 1.00 M KCl, 10 mM KP_i, pH 7.4 at 100 mV (*trans vs. cis*) and 22 ± 1 °C.

Analyte	$\Delta I/I_0$
(*R*)-Sp:A *vs.* (*S*)-Sp:A	0.004 ± 0.001
(*R*)-Sp:G *vs.* (*S*)-Sp:G	0.008 ± 0.001
(*R*)-Sp:T *vs.* (*S*)-Sp:T	0.004 ± 0.001
(*R*)-Sp:C *vs.* (*S*)-Sp:C	0.003 ± 0.001
(*S*)-Sp:A *vs.* T:A	0.026 ± 0.001
(*R*)-Sp:A *vs.* T:A	0.029 ± 0.001
(*S*)-Sp:G *vs.* T:A	0.022 ± 0.002
(*R*)-Sp:G *vs.* T:A	0.029 ± 0.001
(*S*)-Sp:T *vs.* T:A	0.022 ± 0.002
(*R*)-Sp:T *vs.* T:A	0.025 ± 0.002
(*S*)-Sp:C *vs.* T:A	0.021 ± 0.001
(*R*)-Sp:C *vs.* T:A	0.022 ± 0.001

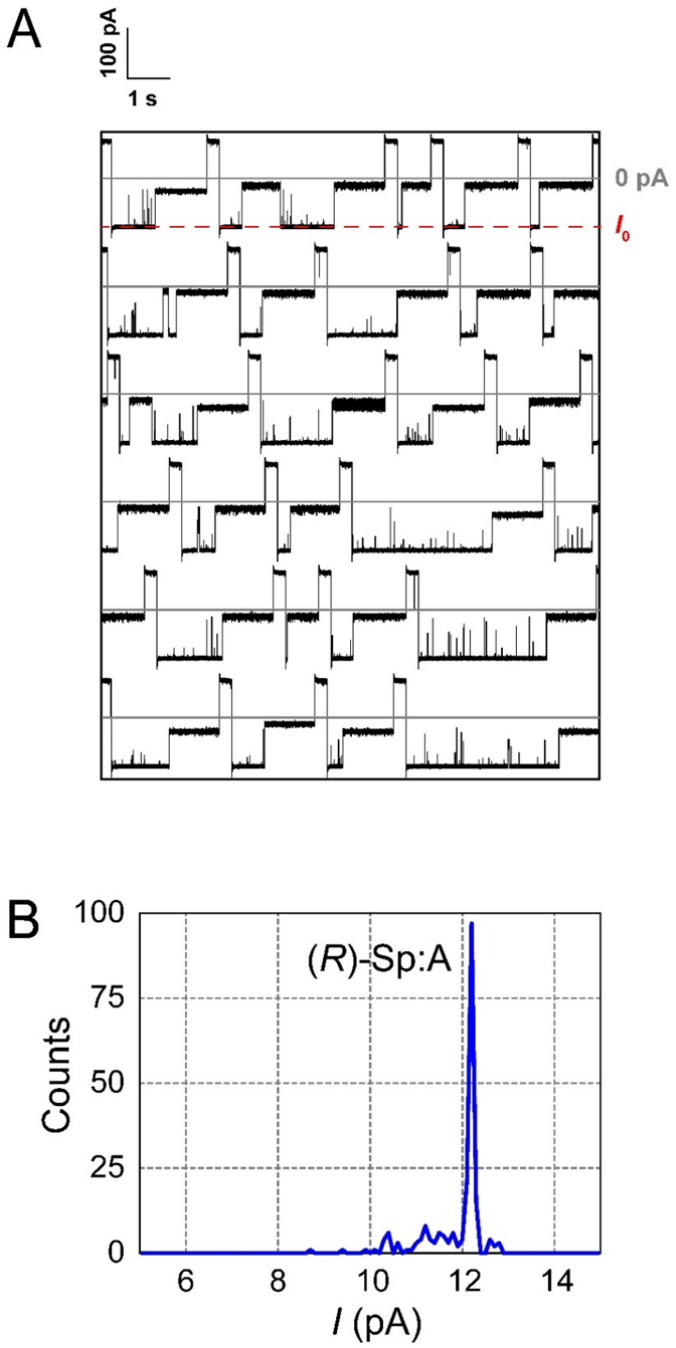

Figure S4. Representative *i–t* trace (A) and current histogram (B) for (*R*)-Sp:A-containing duplex in the αHL nanopore. The single peak in the current histogram supports a predominant base pairing configuration between (*R*)-Sp and A, similar to the base pair *S*-Sp:A (Figure S3). The traces were recorded in 1.00 M KCl, 10 mM KP_i, pH 7.4 at 100 mV (*trans vs. cis*) and 22 ± 1 °C.

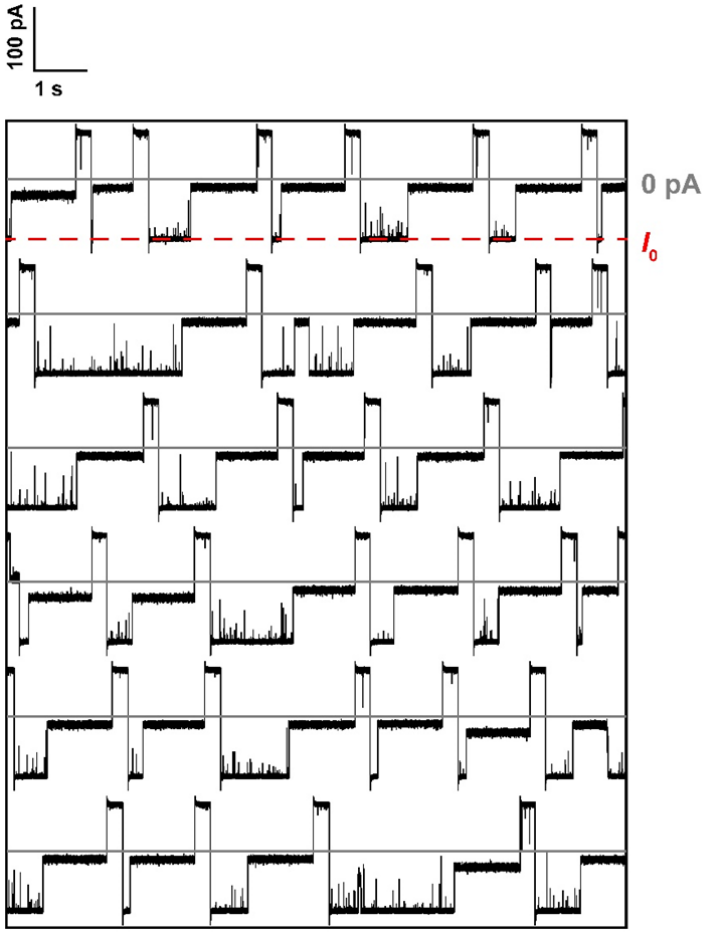

Figure S5. Additional sample of *i–t* trace for a mixture of (*S*)-Sp:A- and (*R*)-Sp:A-containing duplexes in the αHL nanopore. The traces were recorded in 1.00 M KCl, 10 mM KP_i, pH 7.4 at 100 mV (*trans vs. cis*) and 22 ± 1 °C. These data were used to construct the current histogram and current–noise density plot shown in Figure 4C and Figure 4D of the main text.

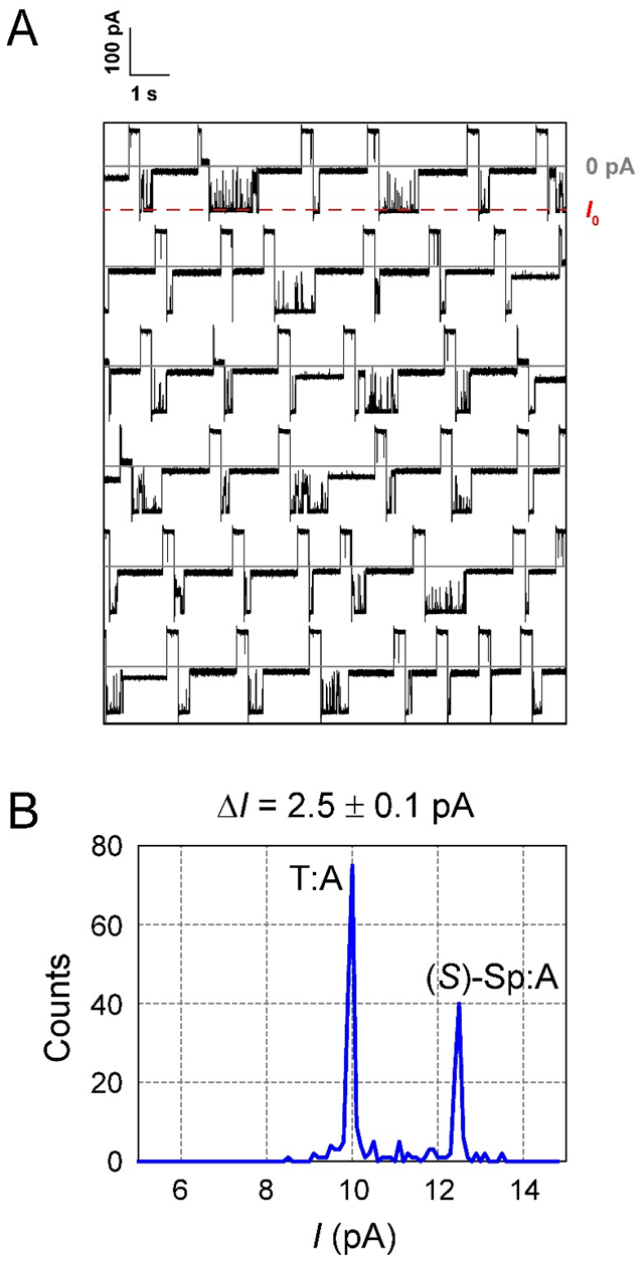

Figure S6. Representative *i–t* trace (A) and current histogram (B) for (*S*)-Sp:A-containing duplex *vs.* the T:A internal standard in the αHL nanopore. The 2:1 peak ratio reflects the twofold greater concentration of the T:A standard. The current difference between (*S*)-Sp:A duplex and the T:A internal standard is 2.5 ± 0.1 pA (the error is based on three individual experiments, in which each experiment comprised over 200 events). The traces were recorded in 1.00 M KCl, 10 mM KP_i, pH 7.4 at 100 mV (*trans vs. cis*) and 22 ± 1 °C.

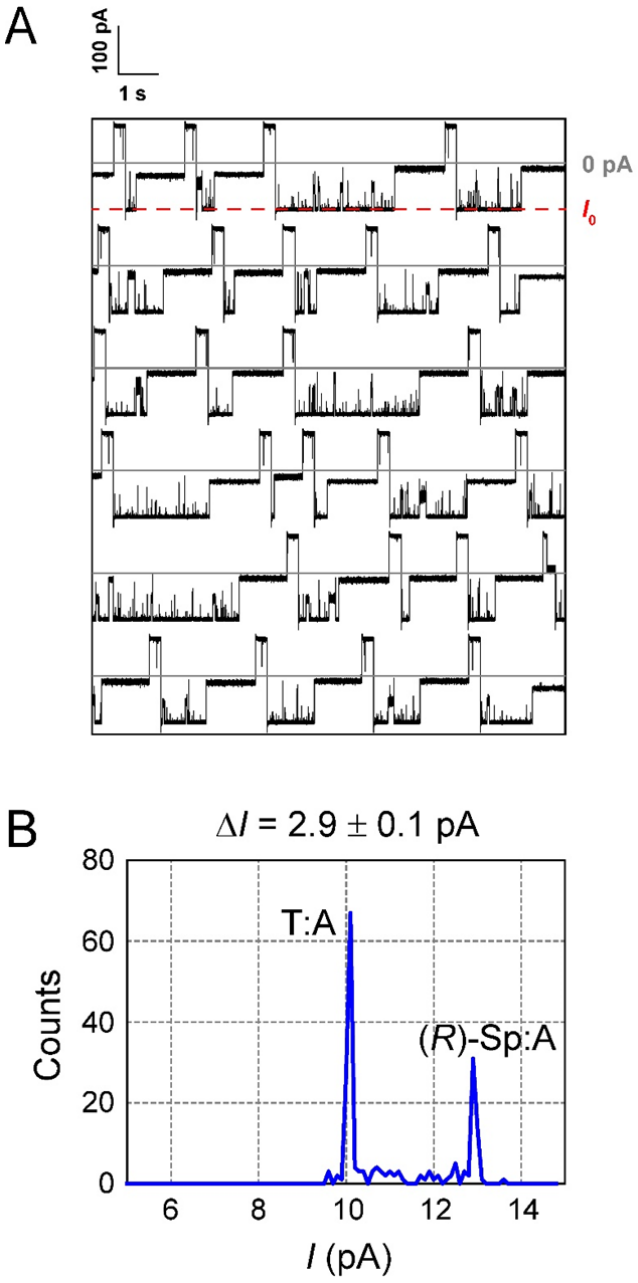

Figure S7. Representative *i–t* trace (A) and current histogram (B) for (*R*)-Sp:A-containing duplex *vs.* the T:A internal standard in the αHL nanopore. The 2:1 peak ratio reflects the twofold greater concentration of the T:A standard. The current difference between (*R*)-Sp:A duplex and the T:A internal standard is 2.9 ± 0.1 pA (the error is based on three individual experiments, in which each experiment comprised over 200 events). The traces were recorded in 1.00 M KCl, 10 mM KP_i, pH 7.4 at 100 mV (*trans vs. cis*) and 22 ± 1 °C.

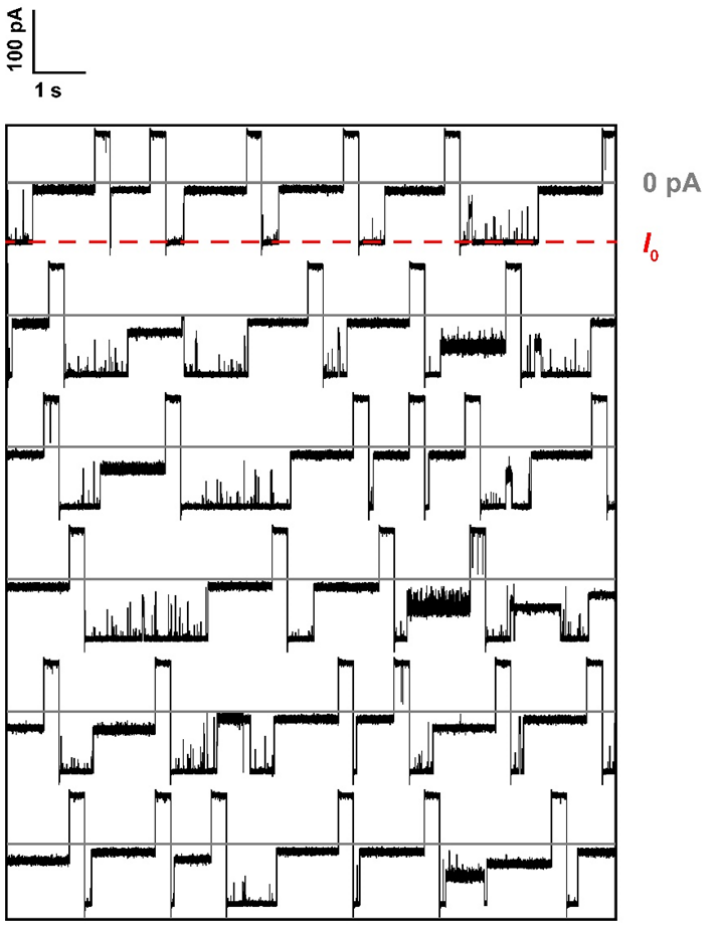

Figure S8. Representative *i–t* trace for a mixture of (*S*)-Sp:G- and (*R*)-Sp:G-containing duplexes in the αHL nanopore. The traces were recorded in 1.00 M KCl, 10 mM KP_i, pH 7.4 at 100 mV (*trans vs. cis*) and 22 ± 1 °C. These data were used to construct the current histogram shown in Fig 5A of the main text.

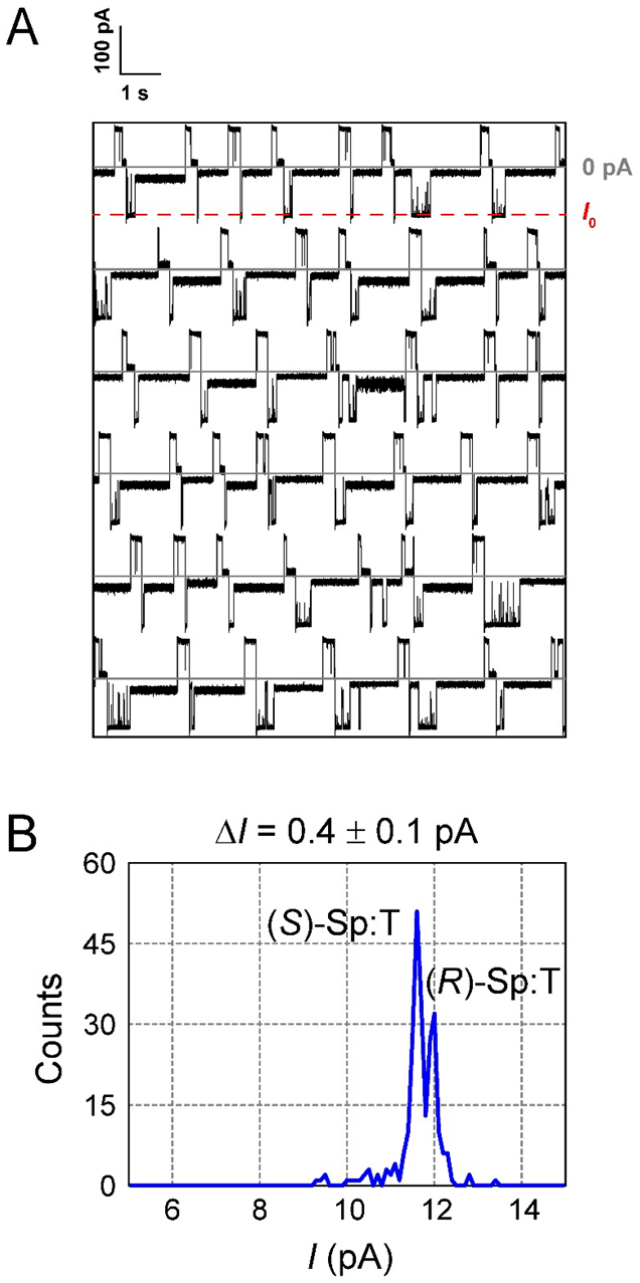

Figure S9. Representative *i–t* trace (A) and current histogram (B) for a mixture of (*S*)-Sp:T- and (*R*)-Sp:T-containing duplexes in the αHL nanopore. The 2:1 peak ratio reflects the twofold greater concentration of (*S*)-Sp:T duplex. The current difference between (*S*)-Sp:T and (*R*)-Sp:T duplexes is 0.4 ± 0.1 pA (the error is based on three individual experiments, in which each experiment comprised over 200 events). The traces were recorded in 1.00 M KCl, 10 mM KP_i, pH 7.4 at 100 mV (*trans vs. cis*) and 22 ± 1 °C.

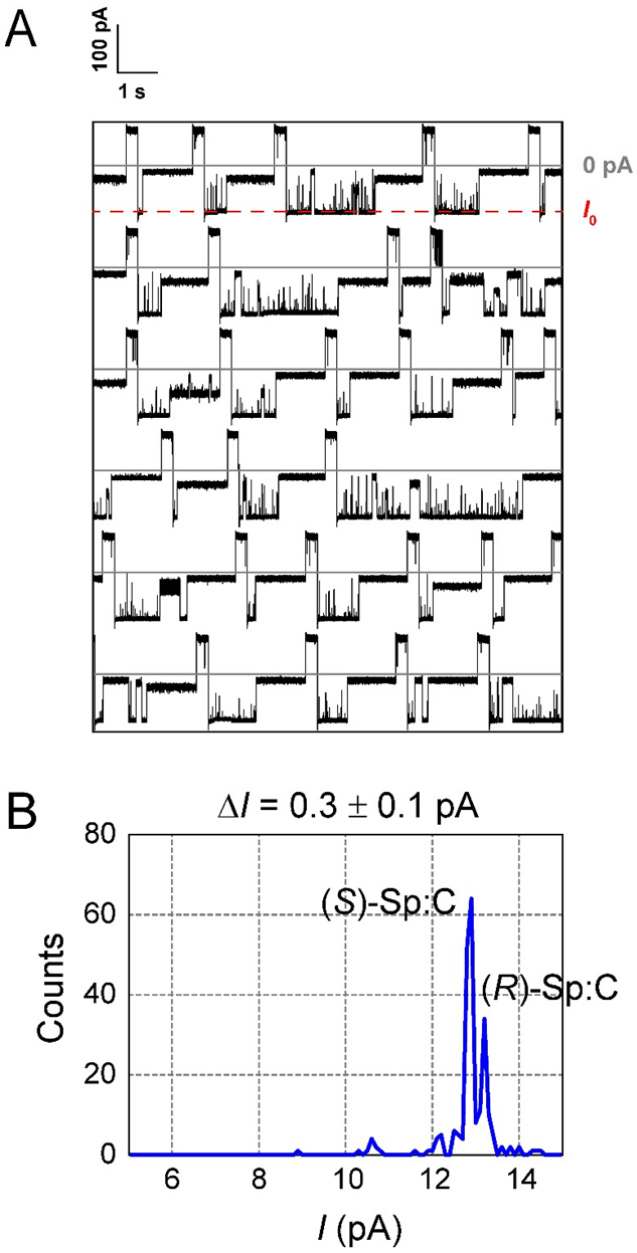

Figure S10. Representative *i–t* trace (A) and current histogram (B) for a mixture of (*S*)-Sp:C- and (*R*)-Sp:C-containing duplexes in the αHL nanopore. The 2:1 peak ratio reflects the twofold greater concentration of (*S*)-Sp:C duplex. The current difference between (*S*)-Sp:C and (*R*)-Sp:C duplexes is 0.3 ± 0.1 pA (the error is based on three individual experiments, in which each experiment comprised over 200 events). The traces were recorded in 1.00 M KCl, 10 mM KP_i, pH 7.4 at 100 mV (*trans vs. cis*) and 22 ± 1 °C.

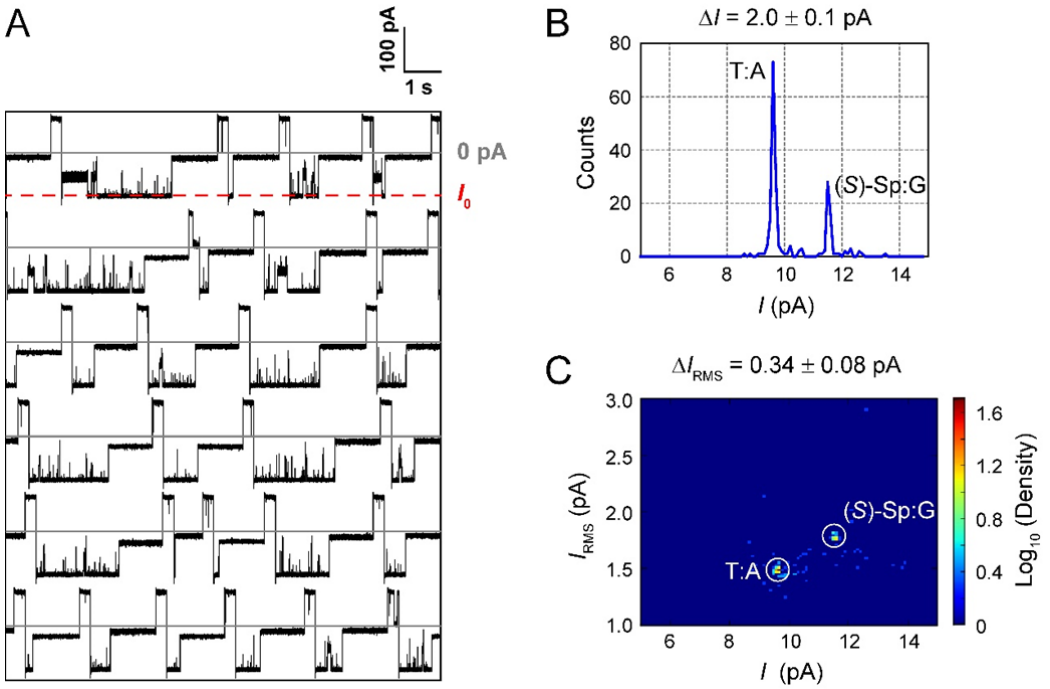

Figure S11. Representative *i–t* trace (A), current histogram (B), and current–noise density plot (C) for (*S*)-Sp:G-containing duplex *vs.* the T:A internal standard in the αHL nanopore. The 2:1 peak ratio in the current histogram reflects the twofold greater concentration of the T:A standard. The current (B) and noise (C) differences between (*S*)-Sp:G duplex and the T:A internal standard are 2.0 ± 0.1 pA and 0.34 ± 0.08 pA, respectively (the errors are based on three individual experiments, in which each experiment comprised over 200 events). The traces were recorded in 1.00 M KCl, 10 mM KP_i, pH 7.4 at 100 mV (*trans vs. cis*) and 22 ± 1 °C.

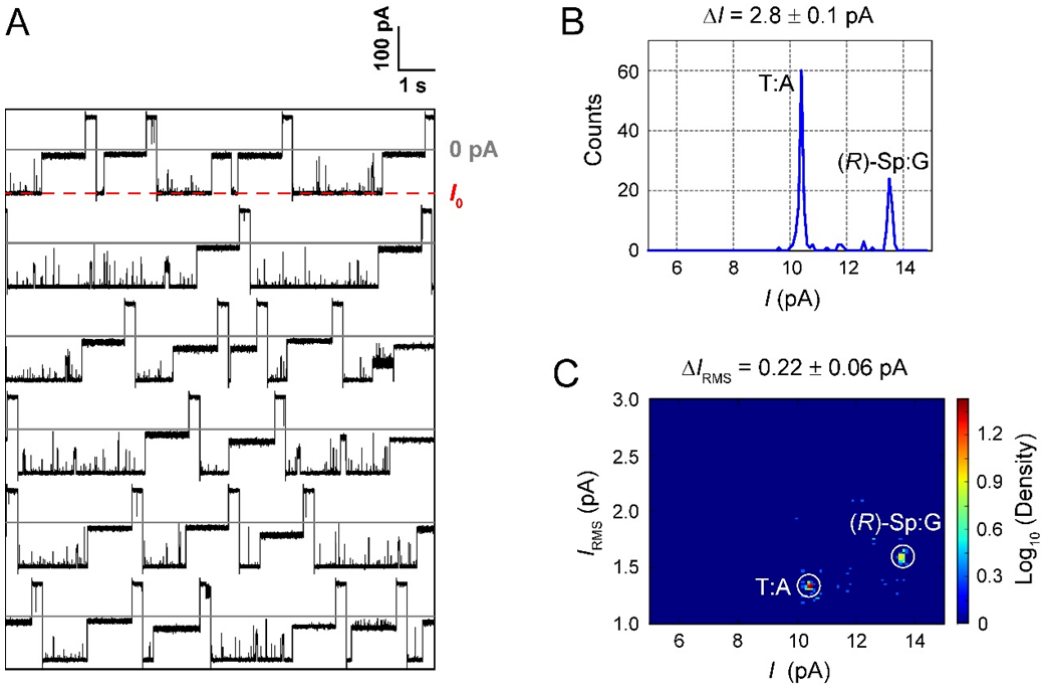

Figure S12. Representative *i*–*t* trace (A), current histogram (B), and current–noise density plot (C) for *R*-Sp:G-containing duplex *vs.* the T:A internal standard in the αHL nanopore. The 2:1 peak ratio in the current histogram reflects the twofold greater concentration of the T:A standard. The current (B) and noise (C) differences between *R*-Sp:G duplex and the T:A internal standard are 2.8 ± 0.1 pA and 0.22 ± 0.06 pA, respectively (the errors are based on three individual experiments, in which each experiment comprised over 200 events). The traces were recorded in 1.00 M KCl, 10 mM KP_i, pH 7.4 at 100 mV (*trans vs. cis*) and 22 ± 1 °C.

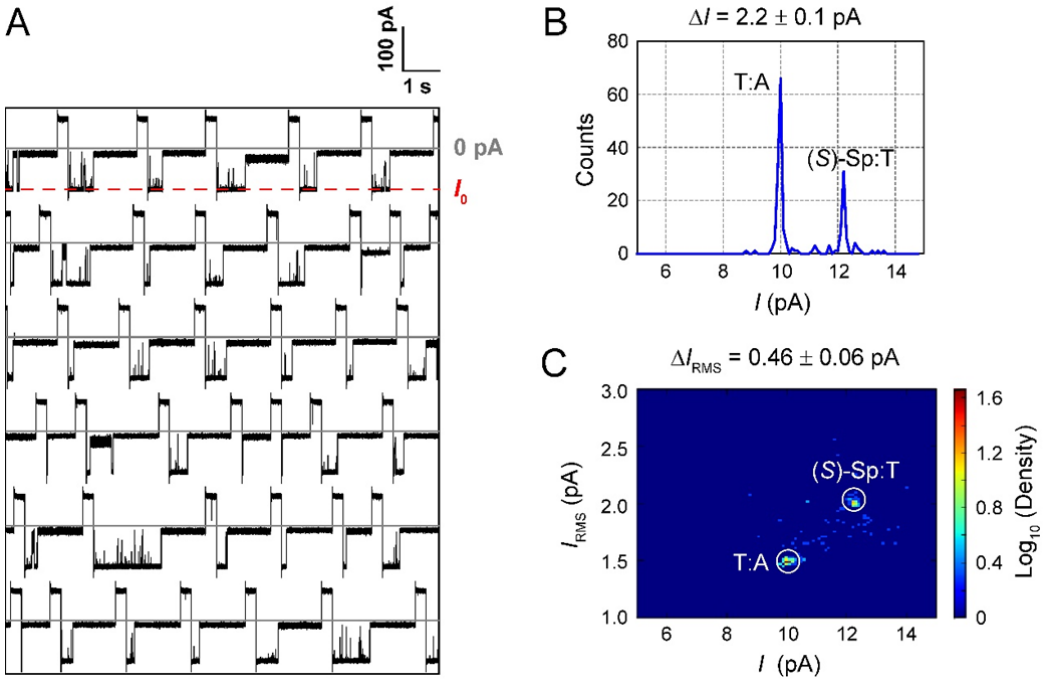

Figure S13. Representative *i*–*t* trace (A), current histogram (B), and current–noise density plot (C) for (*S*)-Sp:T-containing duplex *vs.* the T:A internal standard in the αHL nanopore. The 2:1 peak ratio in the current histogram reflects the twofold greater concentration of the T:A standard. The current (B) and noise (C) differences between (*S*)-Sp:T duplex and the T:A internal standard are 2.2 ± 0.1 pA and 0.46 ± 0.06 pA, respectively (the errors are based on three individual experiments, in which each experiment comprised over 200 events). The traces were recorded in 1.00 M KCl, 10 mM KP_i, pH 7.4 at 100 mV (*trans vs. cis*) and 22 ± 1 °C.

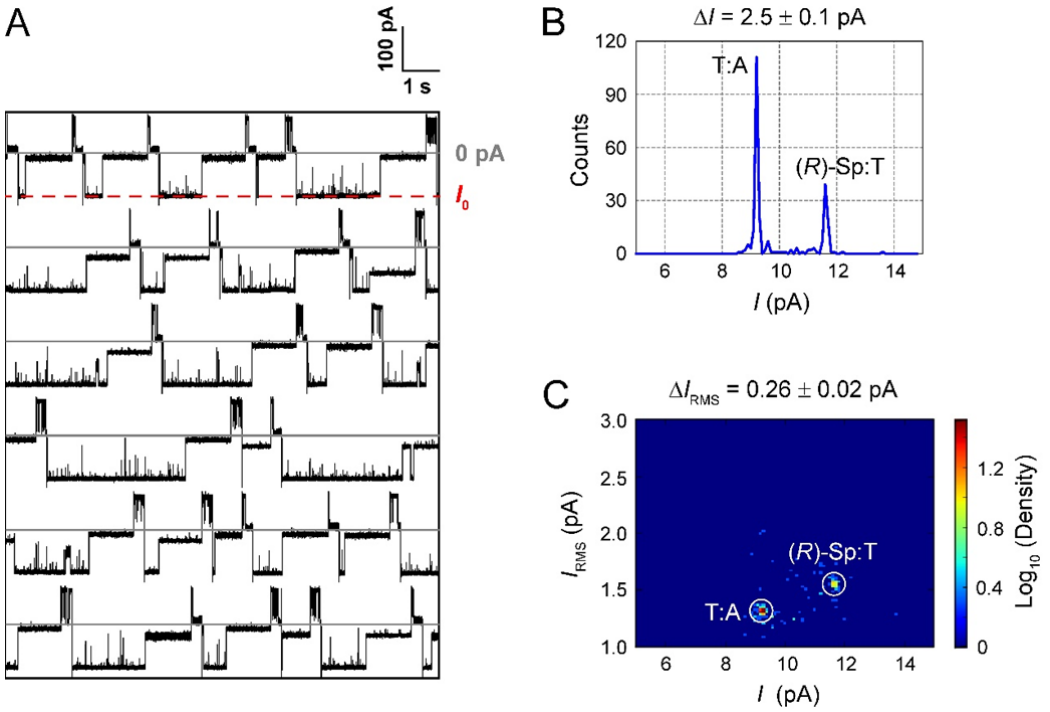

Figure S14. Representative *i–t* trace (A), current histogram (B), and current–noise density plot (C) for (*R*)-Sp:T-containing duplex *vs.* the T:A internal standard in the αHL nanopore. The 2:1 peak ratio in the current histogram reflects the twofold greater concentration of the T:A standard. The current (B) and noise (C) differences between (*R*)-Sp:T duplex and the T:A internal standard are 2.5 ± 0.1 pA and 0.26 ± 0.02 pA, respectively (the errors are based on three individual experiments, in which each experiment comprised over 200 events). The traces were recorded in 1.00 M KCl, 10 mM KP_i, pH 7.4 at 100 mV (*trans vs. cis*) and 22 ± 1 °C.

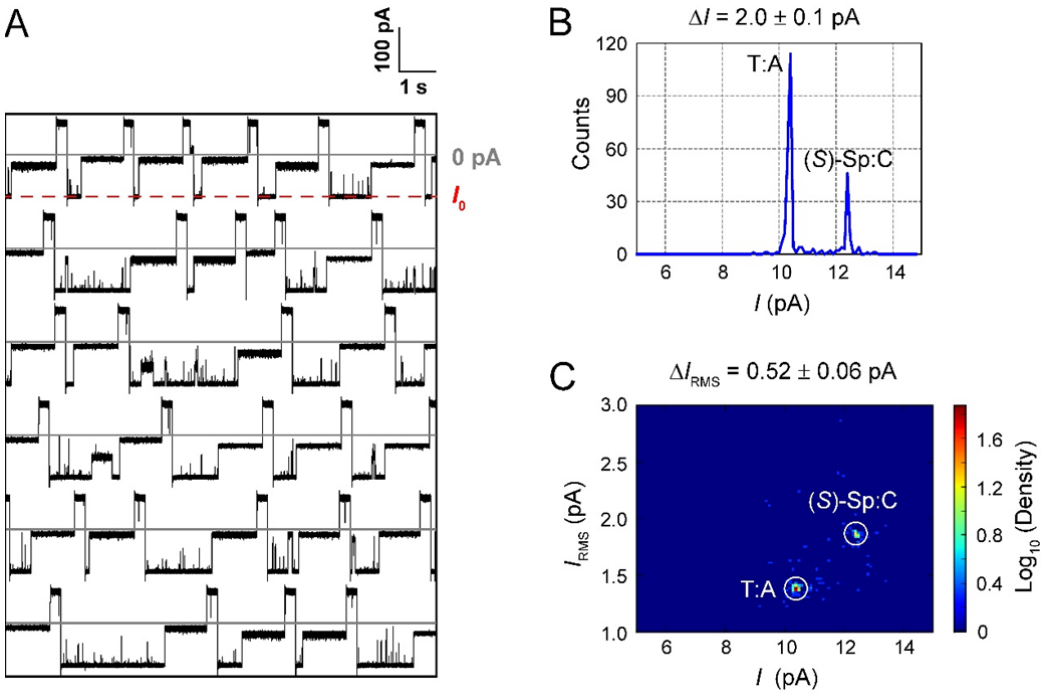

Figure S15. Representative *i*–*t* trace (A), current histogram (B), and current–noise density plot (C) for (*S*)-Sp:C-containing duplex *vs.* the T:A internal standard in the αHL nanopore. The 2:1 peak ratio in the current histogram reflects the twofold greater concentration of the T:A standard. The current (B) and noise (C) differences between (*S*)-Sp:C duplex and the T:A internal standard are 2.0 ± 0.1 pA and 0.52 ± 0.06 pA, respectively (the errors are based on three individual experiments, in which each experiment comprised over 200 events). The traces were recorded in 1.00 M KCl, 10 mM KP_i, pH 7.4 at 100 mV (*trans vs. cis*) and 22 ± 1 °C.

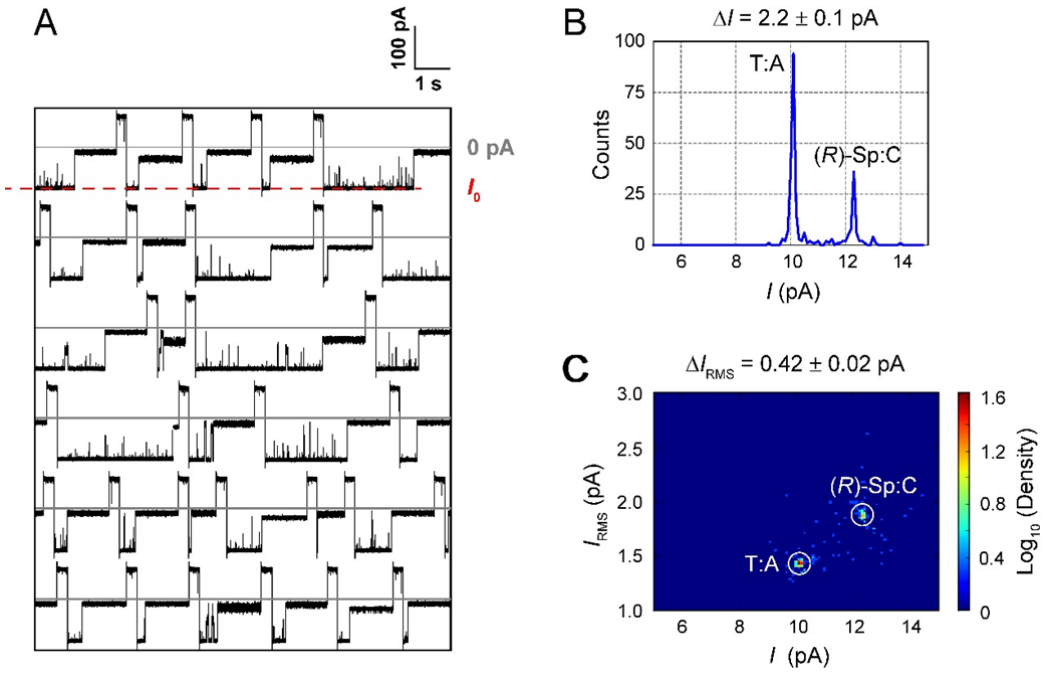

Figure S16. Representative *i*–*t* trace (A), current histogram (B), and current–noise density plot (C) for (*R*)-Sp:C-containing duplex *vs.* the T:A internal standard in the αHL nanopore. The 2:1 peak ratio in the current histogram reflects the twofold greater concentration of the T:A standard. The current (B) and noise (C) differences between (*R*)-Sp:C duplex and the T:A internal standard are 2.2 ± 0.1 pA and 0.42 ± 0.02 pA, respectively (the errors are based on three individual experiments, in which each experiment comprised over 200 events). The traces were recorded in 1.00 M KCl, 10 mM KP_i, pH 7.4 at 100 mV (*trans vs. cis*) and 22 ± 1 °C.

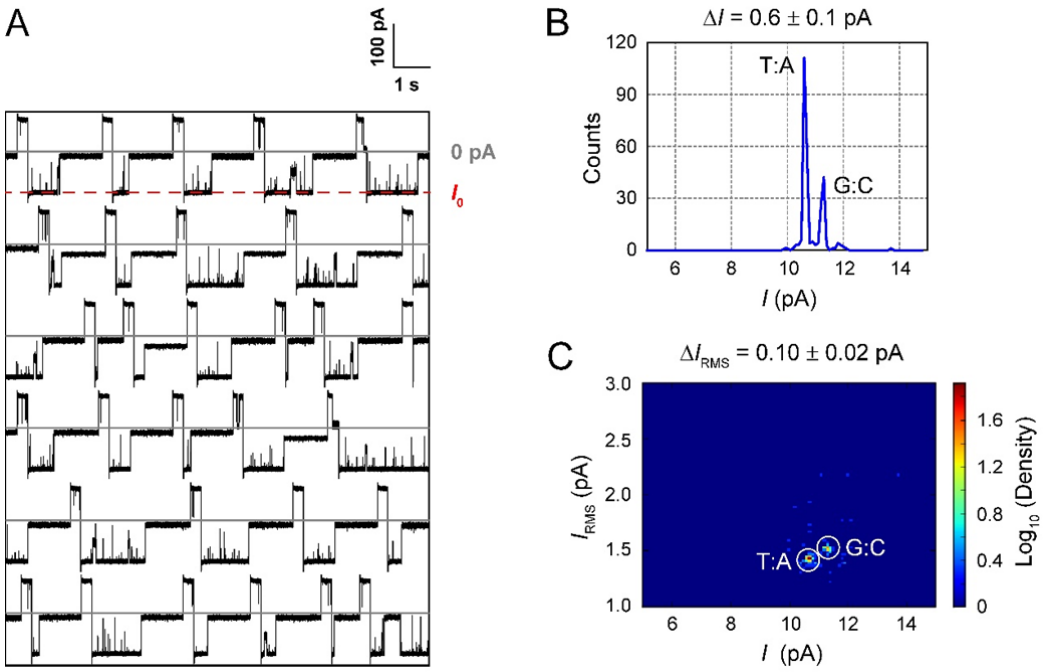

Figure S17. Representative *i*–*t* trace (A), current histogram (B), and current–noise density plot (C) for G:C-containing duplex *vs.* the T:A internal standard in the αHL nanopore. The 2:1 peak ratio in the current histogram reflects the twofold greater concentration of the T:A standard. The current (B) and noise (C) differences between G:C duplex and the T:A internal standard are 0.6 ± 0.1 pA and 0.10 ± 0.02 pA, respectively (the errors are based on three individual experiments, in which each experiment comprised over 200 events). The traces were recorded in 1.00 M KCl, 10 mM KP_i, pH 7.4 at 100 mV (*trans vs. cis*) and 22 ± 1 °C.

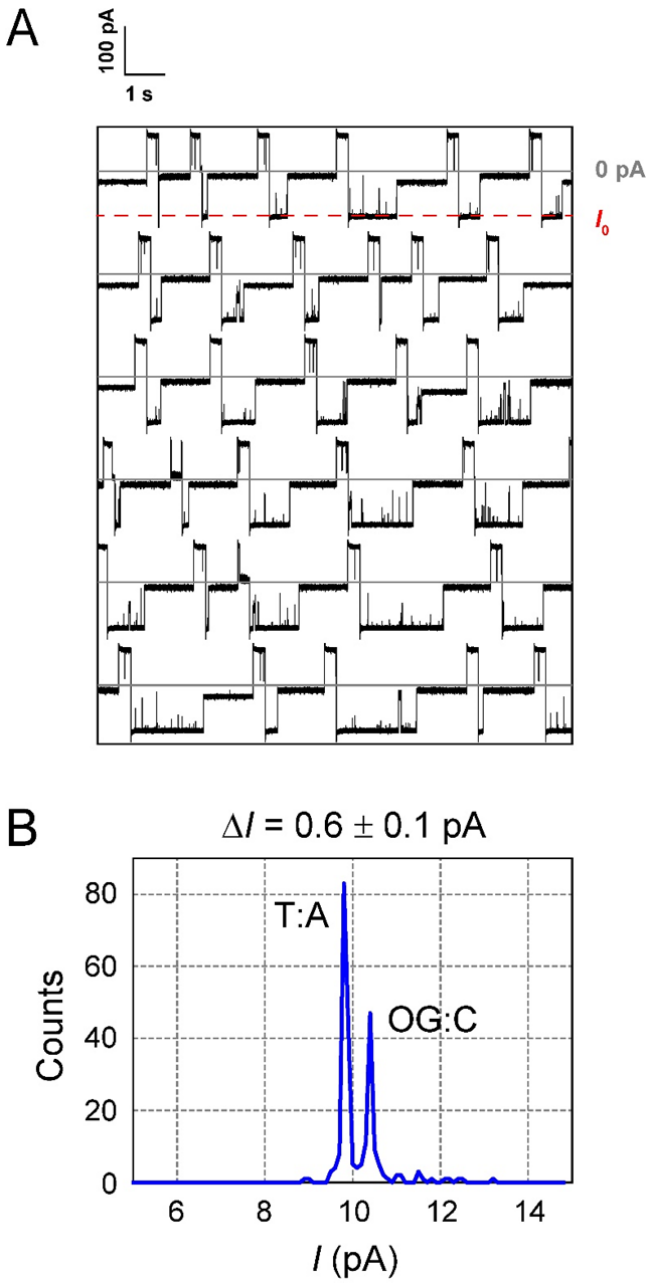

Figure S18. Representative *i–t* trace (A) and current histogram (B) for OG:C-containing duplex *vs.* the T:A internal standard in the αHL nanopore. The 2:1 peak ratio reflects the twofold greater concentration of the T:A standard. The current difference between OG:C duplex and the T:A internal standard is 0.6 ± 0.1 pA (the error is based on three individual experiments, in which each experiment comprised over 200 events). The traces were recorded in 1.00 M KCl, 10 mM KP_i, pH 7.4 at 100 mV (*trans vs. cis*) and 22 ± 1 °C.

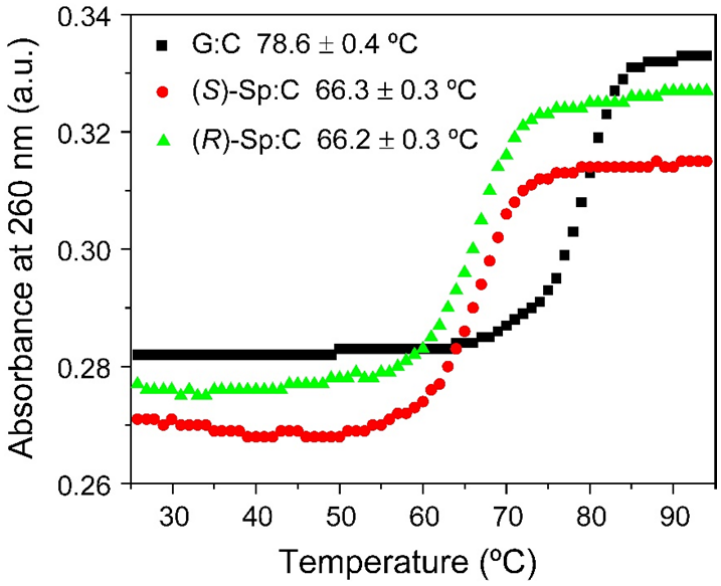

Figure S19. Melting curves for the duplexes containing G:C (black square), (*S*)-Sp:C (red circle), or (*R*)-Sp:C (green triangle) base pair. The data were collected in 1.00 M KCl, 10 mM KPi, pH 7.4 with a DNA concentration of 0.75 μM. Note that the $(CAT)_{10}$ tail was removed from the sequences in the T_m experiments.