Supporting Information

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A

```
gct tag gat cat cga gga t W M X gc tcg gtg caa ttc agc gg
cga atc cta gta gct cct a Z M'Y cg agc cac gtt aag tcg cc
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B

1	2	3	4	5	6	7	8	9	10
g	t	t	W	M	X	g	a	c	g
c	a	a	Z	M′	Y	c	t	g	c
20	19	18	17	16	15	14	13	12	11

Fig. S1. Mismatched duplex DNA substrates. The representative 41-mer G/T mismatched DNA substrate used for the ATPase and TIR experiments (*A*) and 10-mer G/T mismatched DNA duplex used for thermal denaturation and NMR analysis (*B*) are shown. The position of mismatch and nearest neighbor flanking sequence are shown (*W*, *X*, *Y*, and *Z* positions). This resulted in 16 different nearest-neighbor sequence contexts for each mismatch. For DNA substrates examined by TIR, thermal denaturation, and NMR the nearest neighbors flanking the mismatched bases in the 2 × 3′-purine sequence context, where W is cytosine, X is adenine, Y is thymine, and Z is guanine, and in the 2 × 3″-pyrimidine sequence context, where W is adenine, X is cytosine, Y is guanine, and Z is thymine.

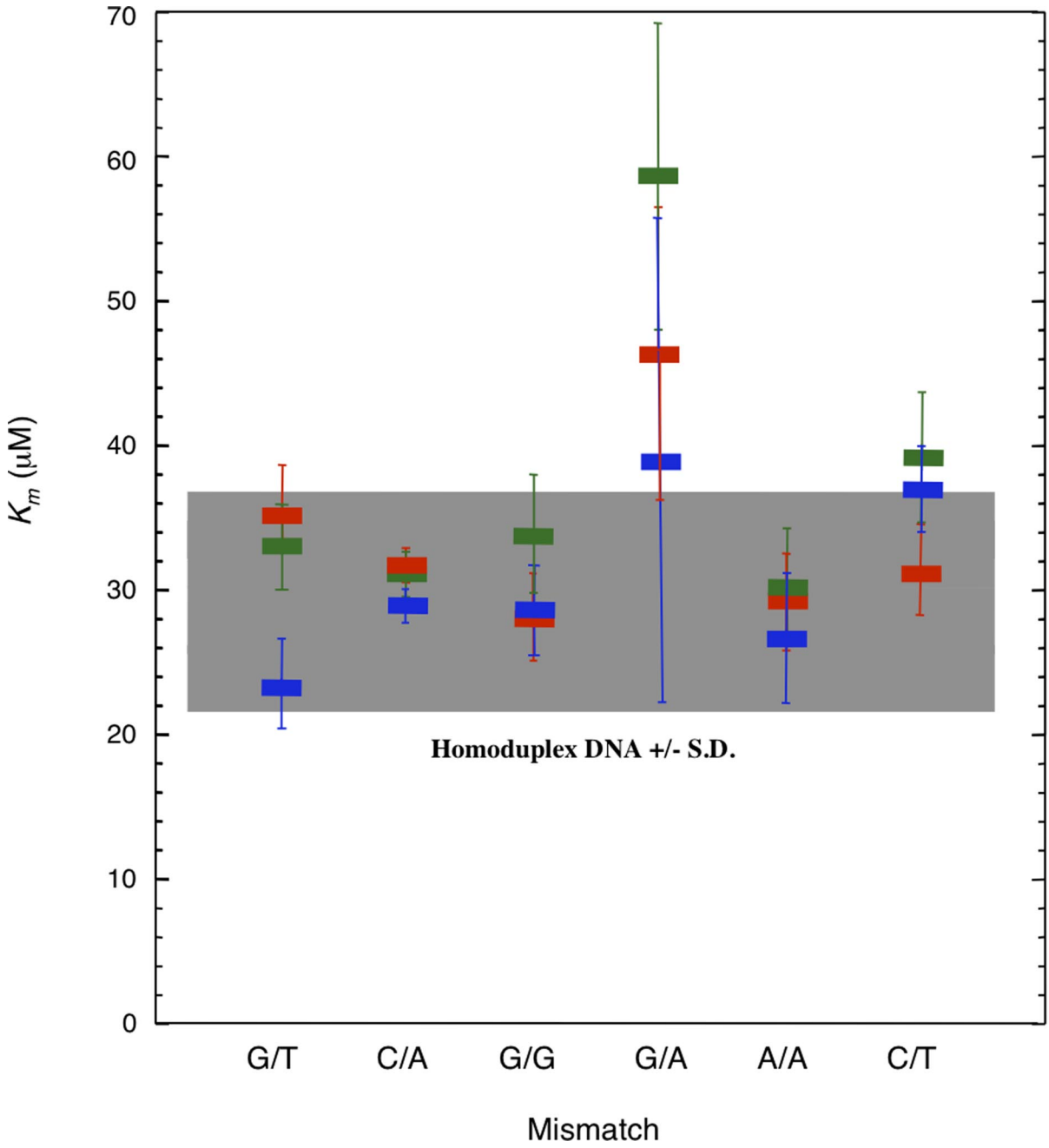

Fig. S2. The effect of nearest-neighbor sequence context on the K_m of the mismatch-dependent hMSH2-hMSH6 ATPase. The red bar corresponds to the average K_m of the 2 × 3′-purine sequence context; the green bar corresponds to the average K_m of the 1 × 3′-purine plus 1 × 3′-pyrimidine sequence context; the blue bar corresponds to the average K_m of the 2 × 3′-pyrimidine sequence context. Gray box indicates K_m (± standard deviation) by a homoduplex DNA containing an A/T or G/C base pair at the mismatch site and generally indicates the background of end-activation due to the use of linear open-ended oligonucleotides.

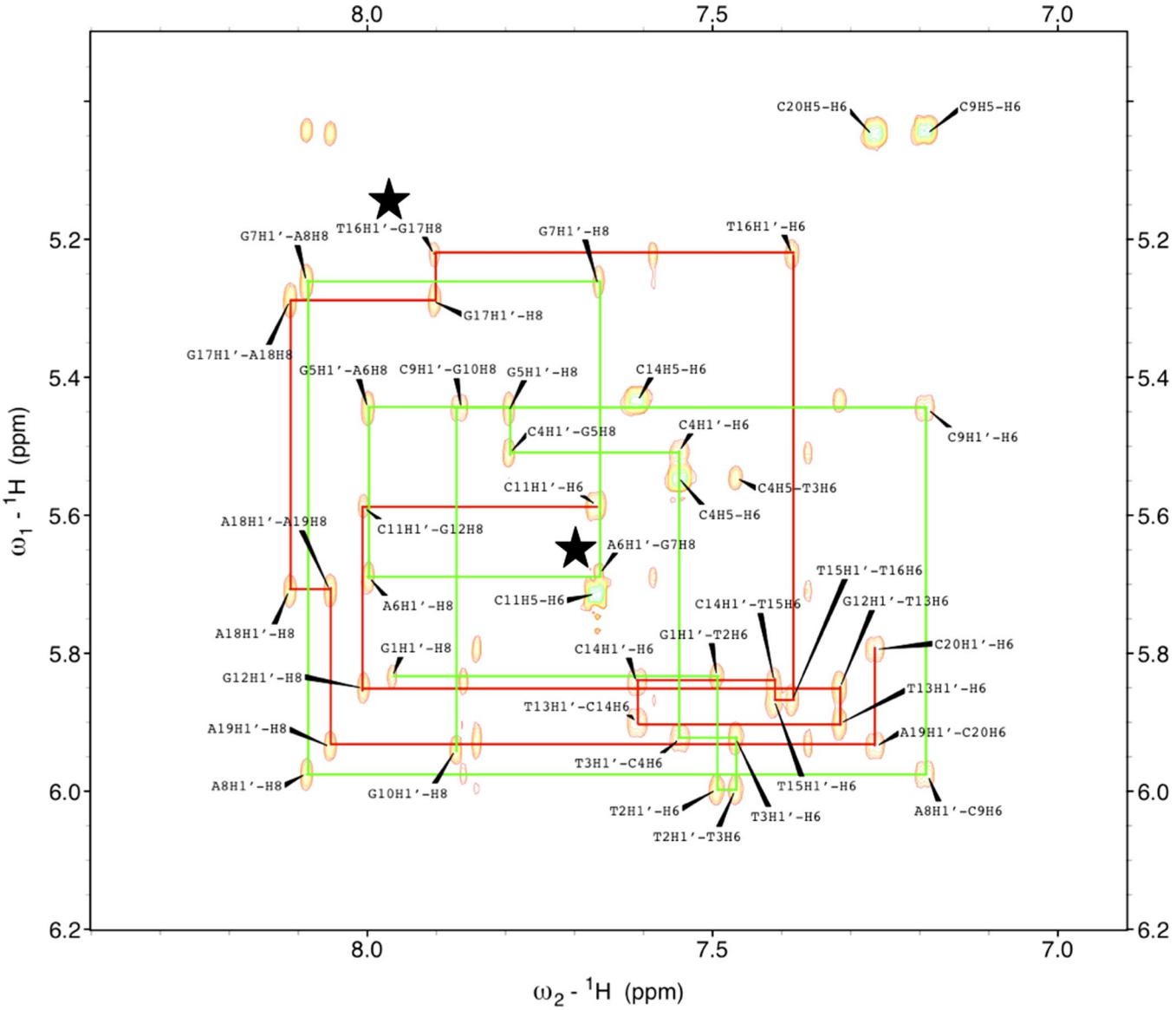

Fig. S3. ^{1}H NMR NOESY (T_m = 250 ms) spectra of the base proton–H1′ region for G/T and G/A mismatched duplexes in CxA and AxC sequence context. G/T mismatch in the CxA sequence context is shown. The base-H1′ sugar connectivity is marked by lines (top strand, green; bottom strand, red). Stars indicate broken or weak NOE.

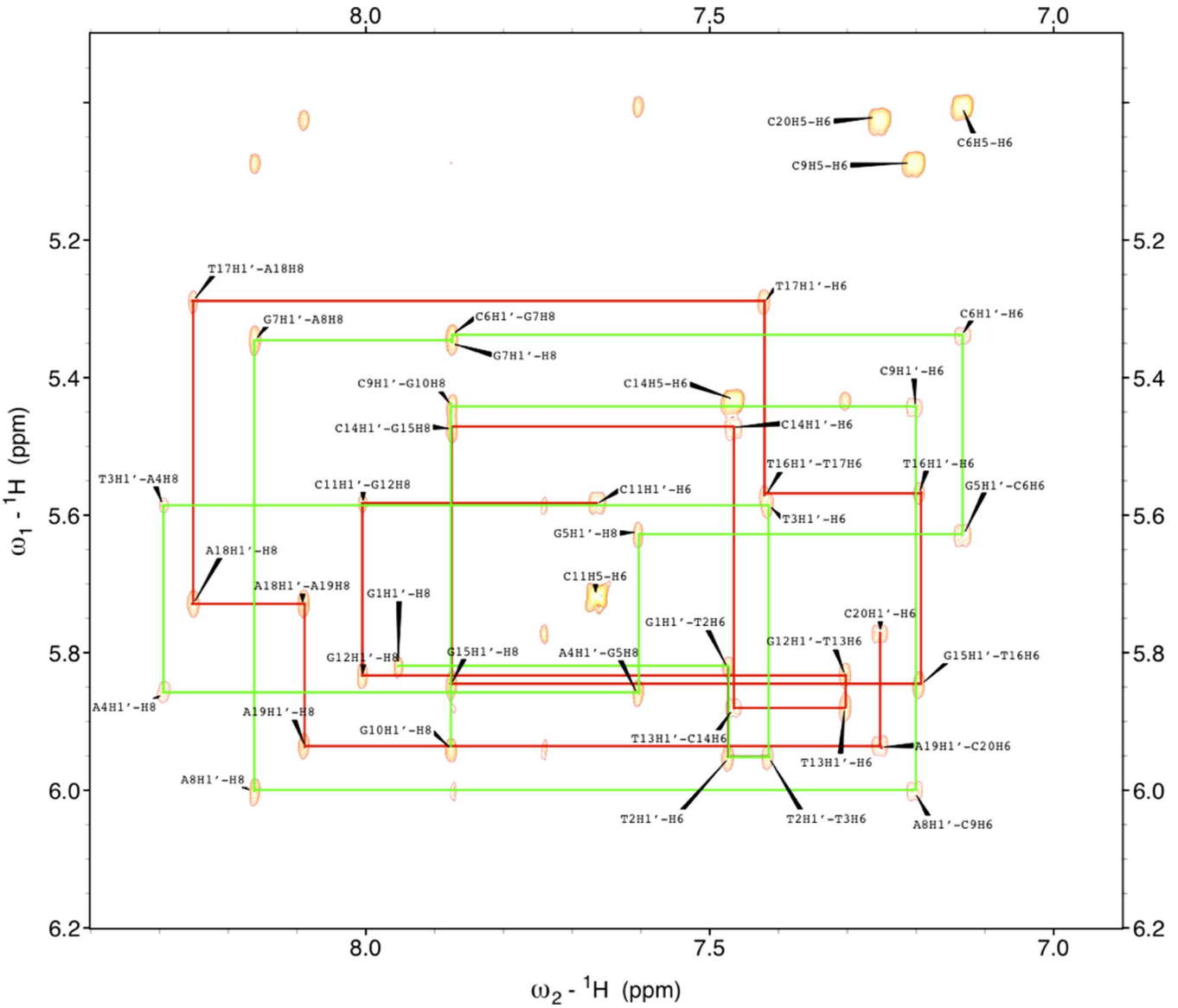

Fig. S4. ^{1}H NMR NOESY (T_m = 250 ms) spectra of the base proton–H1′ region for G/T and G/A mismatched duplexes in CxA and AxC sequence context. GT mismatch in the AxC sequence context is shown. The base-H1′ sugar connectivity is marked by lines (top strand, green; bottom strand, red). Stars indicate broken or weak NOE.

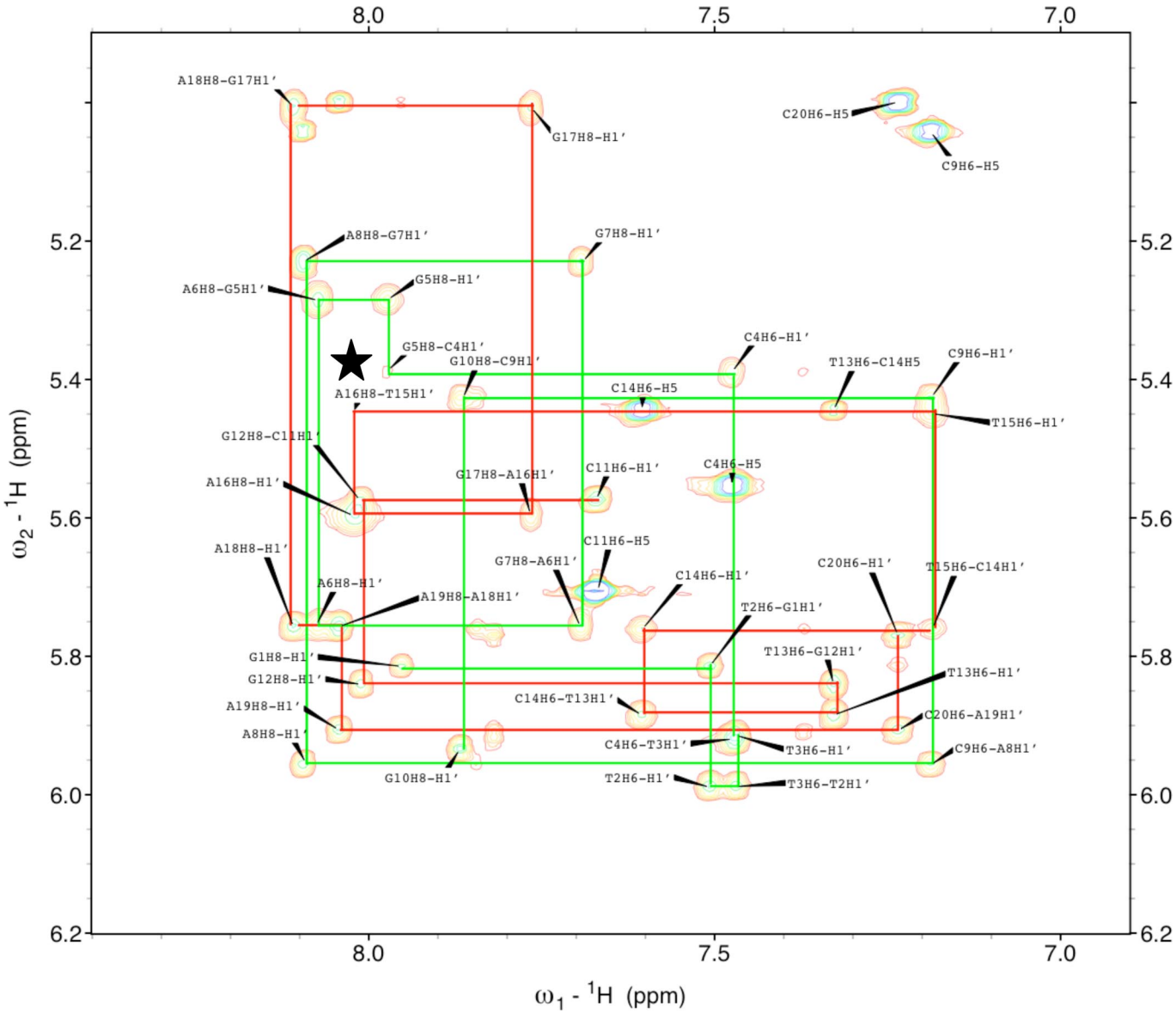

Fig. S5. ^{1}H NMR NOESY (T_m = 250 ms) spectra of the base proton–H1′ region for G/T and G/A mismatched duplexes in CxA and AxC sequence context. G/T mismatch in the CxA sequence context is shown. The base-H1′ sugar connectivity is marked by lines (top strand, green; bottom strand, red). Stars indicate brokenor weak NOE.

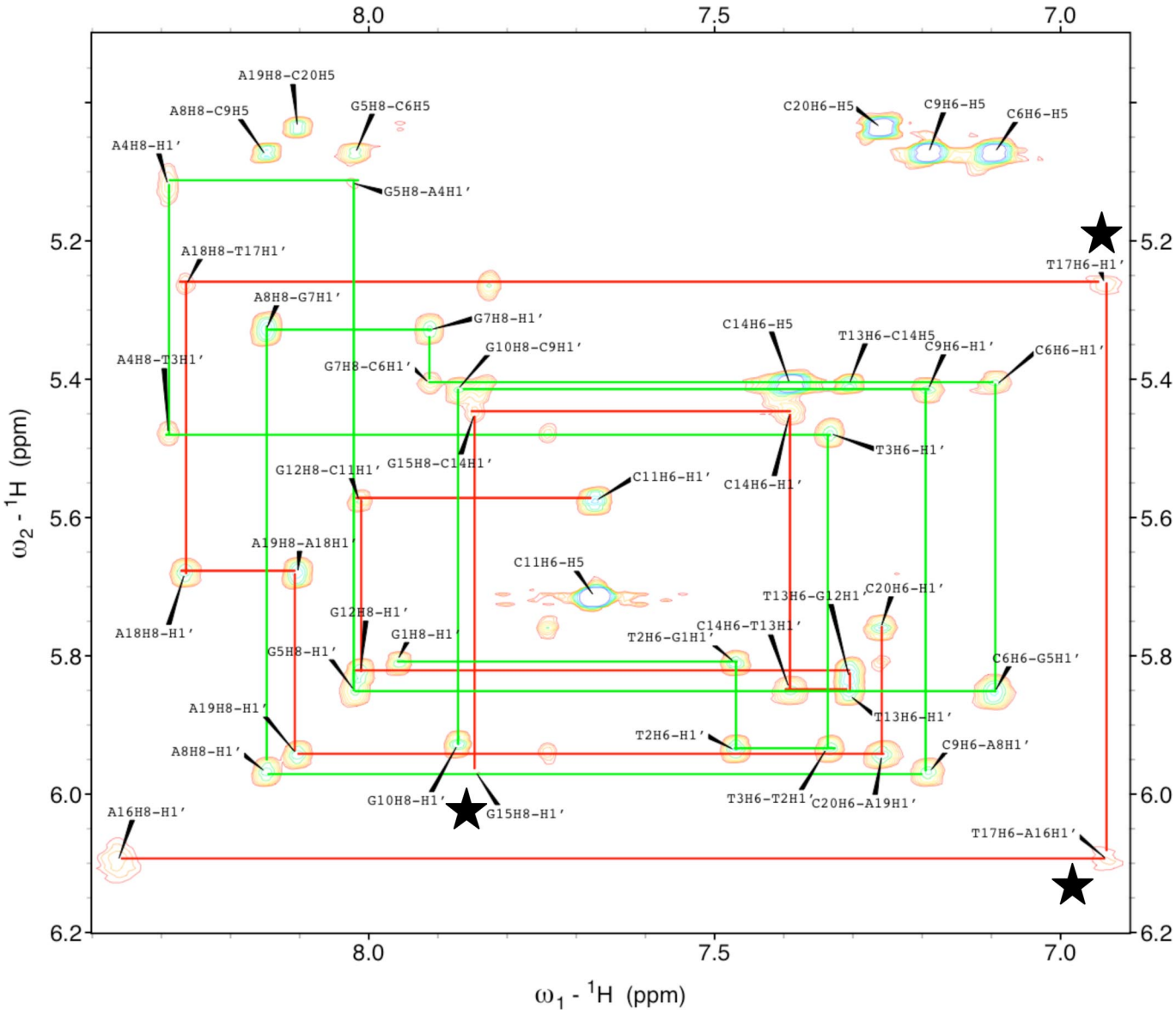

Fig. S6. ^{1}H NMR NOESY (T_m = 250 ms) spectra of the base proton–H1′ region for G/T and G/A mismatched duplexes in CxA and AxC sequence context. G/T mismatch in the CxA sequence context is shown. The base-H1′ sugar connectivity is marked by lines (top strand, green; bottom strand, red). Stars indicate brokenor weak NOE.

Other Supporting Information Files

Table S1 (PDF)
Table S2 (PDF)
Table S3 (PDF)
Table S4 (PDF)
Table S5 (PDF)
Table S6 (PDF)