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**2'-O-Methyl modified guide RNA promotes the single nucleotide polymorphisms (SNPs)
discrimination ability of CRISPR-Cas12a systems**

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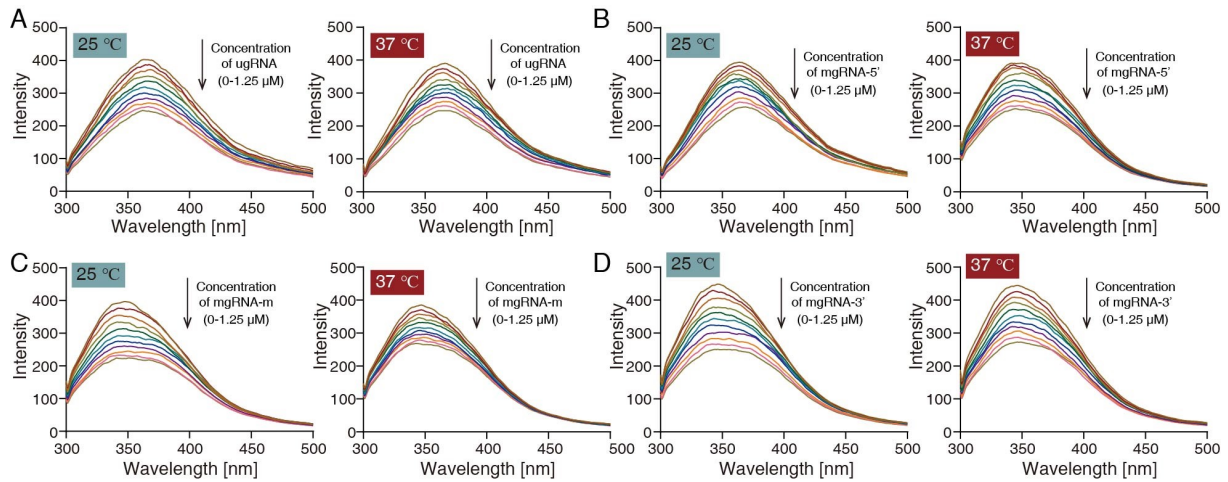


Figure S1. Fluorescence spectra of Cas12a binding with various concentrations of gRNAs (0 – 1.25 μM) at 25 $^{\circ}\text{C}$ and 37 $^{\circ}\text{C}$. A) Cas12a binding with ugRNA at 25 $^{\circ}\text{C}$ (left) and 37 $^{\circ}\text{C}$ (right). B) Cas12a binding with mgRNA-5' at 25 $^{\circ}\text{C}$ (left) and 37 $^{\circ}\text{C}$ (right). C) Cas12a binding with mgRNA-m at 25 $^{\circ}\text{C}$ (left) and 37 $^{\circ}\text{C}$ (right). D) Cas12a binding with mgRNA-3' at 25 $^{\circ}\text{C}$ (left) and 37 $^{\circ}\text{C}$ (right).

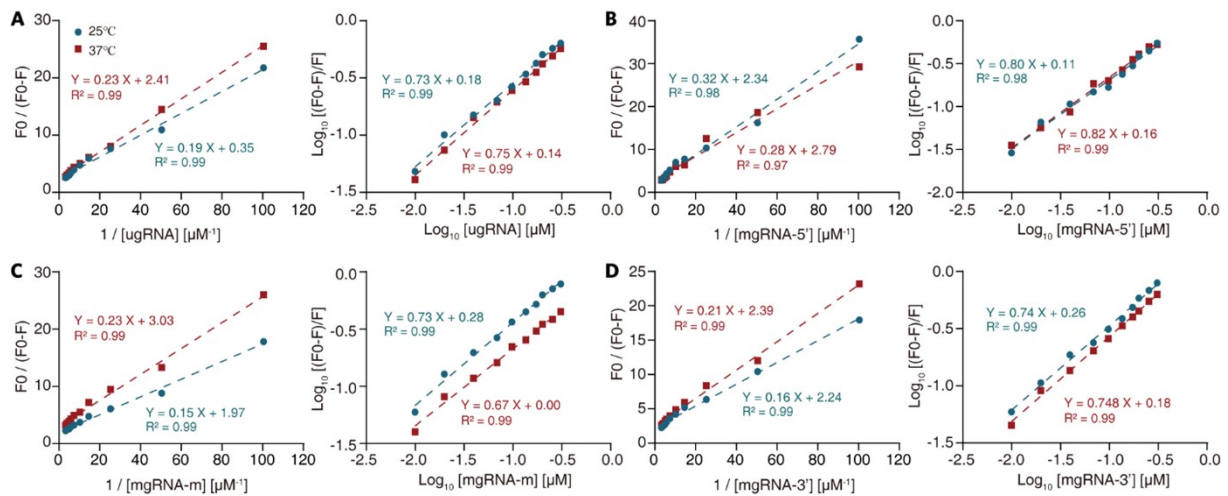


Figure S2. Stern-Volmer plots of Cas12a binding with gRNAs at 25 $^{\circ}\text{C}$ and 37 $^{\circ}\text{C}$. A) Cas12a interacting with ugRNA. B) Cas12a binding with mgRNA-5'. C) Cas12a binding with mgRNA-m. D) Cas12a binding with mgRNA-3'.

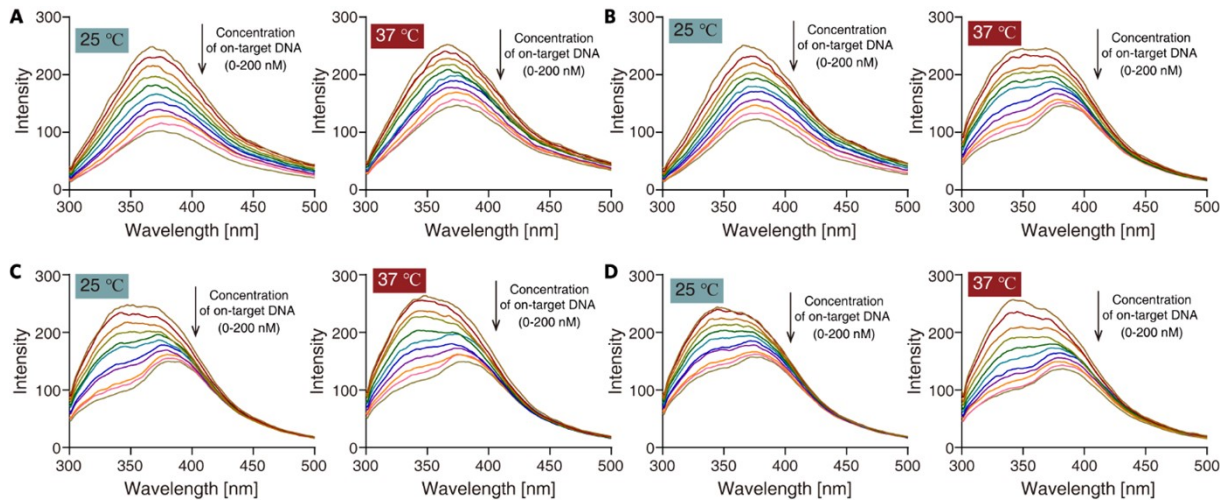


Figure S3. Fluorescence spectra of Cas12a/gRNA complex binding with various concentrations of off-target DNA (0 – 200 nM) at 25 °C and 37 °C. A) Cas12a binding with ugRNA at 25 °C (left) and 37 °C (right). B) Cas12a binding with mgRNA-5' at 25 °C (left) and 37 °C (right). C) Cas12a binding with mgRNA-m at 25 °C (left) and 37 °C (right). D) Cas12a binding with mgRNA-3' at 25 °C (left) and 37 °C (right).

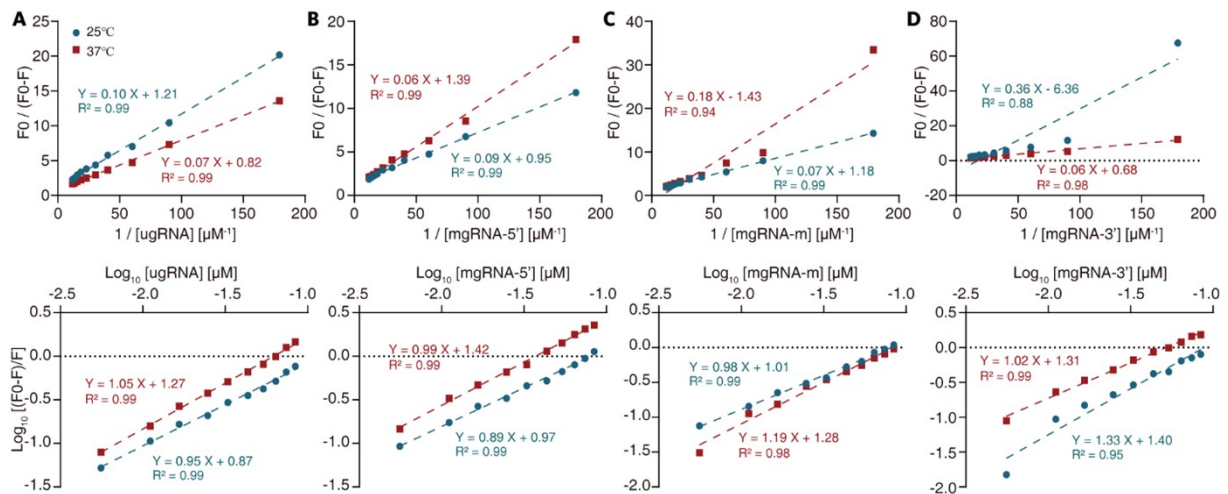


Figure S4. Stern-Volmer plots of Cas12a/gRNA complex interacting with off-target DNA at 25 °C and 37 °C. A) Cas12a interacting with ugRNA. B) Cas12a binding with mgRNA-5'. C) Cas12a binding with mgRNA-m. D) Cas12a binding with mgRNA-3'.

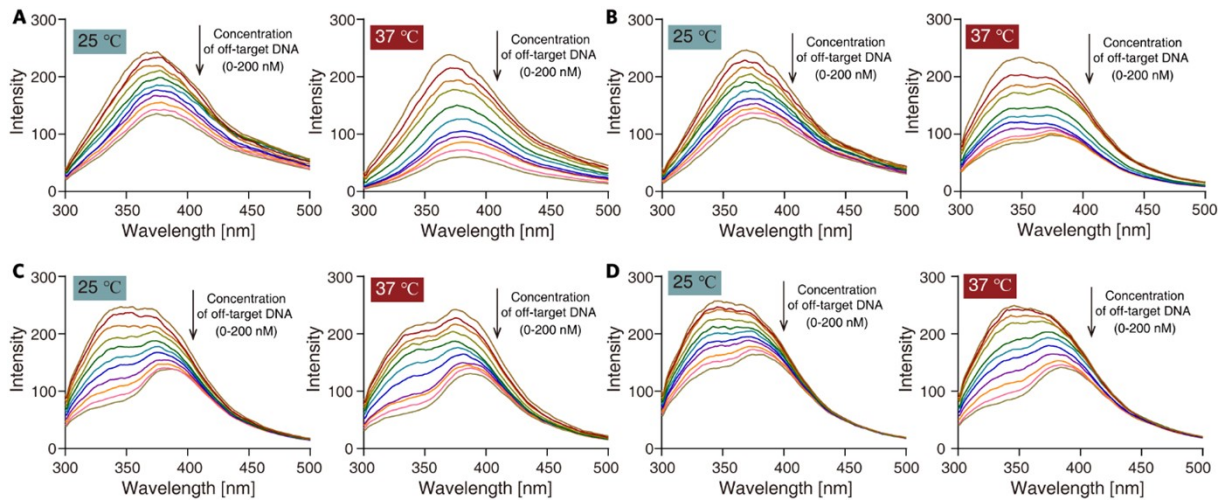


Figure S5. Fluorescence spectra of Cas12a/gRNA complex binding with various concentrations of off-target DNA (0 – 200 nM) at 25 °C and 37 °C. A) Cas12a/ugRNA binding with off-target DNA at 25 °C (left) and 37 °C (right). B) Cas12a/mgRNA-5' binding with off-target DNA at 25 °C (left) and 37 °C (right). C) Cas12a/mgRNA-m binding with off-target DNA at 25 °C (left) and 37 °C (right). D) Cas12a/mgRNA-3' binding with off-target DNA at 25 °C (left) and 37 °C (right).

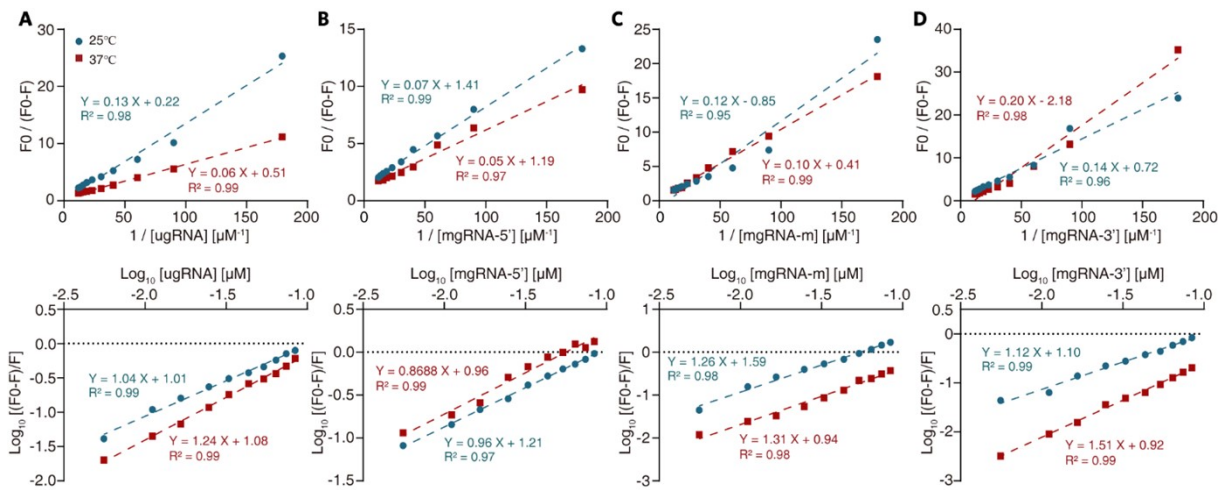


Figure S6. Stern-Volmer plots of Cas12a/gRNA complex interacting with off-target DNA at 25 °C and 37 °C. A) Cas12a interacting with ugRNA. B) Cas12a binding with mgRNA-5'. C) Cas12a binding with mgRNA-m. D) Cas12a binding with mgRNA-3'.

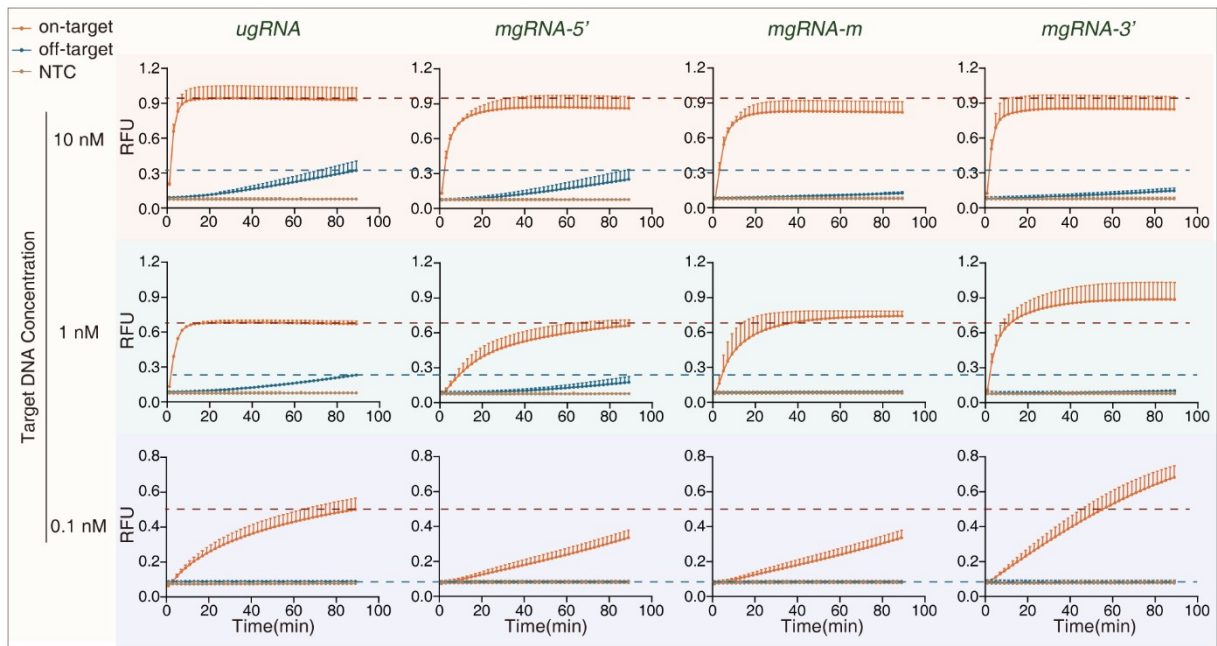


Figure S7. Fluorescence Kinetics of Cas12a's specificity tests. On-target (HBV B-type) DNA signal is shown in orange, off-target (HBV C-type) DNA signal is in blue, and non-target control (NTC) signal is in brown. The final on-target signal for ugRNA at 90 min is highlighted with red dot line, while the off-target signal is marked with cyan dot line. Target DNA concentration at 10 nM, 1 nM and 0.1 nM were tested for all gRNAs.

Table S1. Gibbs free energy results of the Cas12a protein interacting with gRNA and target DNA at 37 °C

Group	ΔG^0_1 [kJ·mol ⁻¹]	$\Delta G^0_{2-[on]}$ [kJ·mol ⁻¹]	$\Delta G^0_{2-[off]}$ [kJ·mol ⁻¹]	ΔG [kJ·mol ⁻¹]	$\Delta G^0_1 + \Delta G^0_{2-[on]}$ [kJ·mol ⁻¹]
ugRNA	-36.45	-43.16	-42.03	1.13	-79.61
mgRNA-5' ^{a)}	-36.57	-44.05	-42.81	1.24	-80.62
mgRNA-m ^{b)}	-35.63	-43.22	-41.21	2.01	-78.85
mgRNA-3' ^{c)}	-36.69	-43.40	-41.08	2.32	-80.09

^{a)} mgRNA-5' has 2'-OMe modifications at the 5'-end;

^{b)} mgRNA-m has 2'-OMe modifications at the middle region;

^{c)} mgRNA-3' has 2'-OMe modifications at the 3'-end.

Table S2. PCR primers and ssDNA fluorescence reporter used in this study

Name	Sequence
HBV PCR forward primer	5'-GGACTTCTCTCAATTTTCTAGGG-3'
HBV PCR reverse primer	5'-ACTTTCCAATCAATAGG-3'
SARS-CoV-2 PCR reverse primer	5'-GTGATGCTGTCCGTGATCCA-3'
SARS-CoV-2 PCR reverse primer	5'-AGCGCATATACCTGCACCAA-3'
ssDNA fluorescence reporter	5'-HEX-AAAAAAAAAAAAA-BHQ1-3'

Table S3. Guide RNAs (gRNAs) used in this study

Name	Whole sequence (from 5' to 3') ^{a)}	2'-O-Methyl modification positions
ugRNA	UAAUUUCUACUAAGUGUAG AUUCAUCUUCCUCUGCAUCCUG	\
mgRNA-5'	UAA UUUCUACUAAGUGUAG AUUCAUCUUCCUCUGCAUCCUG	1 , 2 , 3
mgRNA-m	UAAUUUCUACUAAGUGUAG AU UCA UCUUCCUCUGCAUCCUG	22 , 23 , 24
mgRNA-3'	UAAUUUCUACUAAGUGUAG AUUCAUCUUCCUCUGCAUC UG	39 , 40 , 41
ugRNA-11 th	UAAUUUCUACUAAGUGUAG AUUACAGCAACAUGAGGGAAAC	\
mgRNA-11 th -5'	UAAUUUCUACUAAGUGUAG AU UAC AGCAACAUGAGGGAAAC	1 , 2 , 3
mgRNA-11 th -m	UAAUUUCUACUAAGUGUAG AU UAC AGCAACAUGAGGGAAAC	22 , 23 , 24
mgRNA-11 th -3'	UAAUUUCUACUAAGUGUAG AUUACAGCAACAUGAGGG AAAC	39 , 40 , 41
ugRNA-19 th	UAAUUUCUACUAAGUGUAG AUGGUAUACAUUUAAACCCUCA	\
mgRNA-19 th -5'	UAA UUUCUACUAAGUGUAG AUGGUAUACAUUUAAACCCUCA	1 , 2 , 3
mgRNA-19 th -m	UAAUUUCUACUAAGUGUAG AU GGU AUACAUUUAAACCCUCA	22 , 23 , 24
mgRNA-19 th -3'	UAAUUUCUACUAAGUGUAG AUGGUAUACAUUUAAACCC UCA	39 , 40 , 41

^{a)} The 2'-Ome modified nucleotides are marked in red.

Table S4. Plasmids used in this study

Name	Plasmid ID (catalog number)
HBV B-genotype	AF100309
HBV C-genotype	AF461363
SARS-CoV-2 wild-type	GS-200519_A001
SARS-CoV-2 D614G mutant	GS-200519_A012