Supporting information

Visual detection of HPV16 using a photoactivatable CRISPR-Cas12 system

Xiaoya Gu[a], Zhe Ma[b], Lin Zhou[a], Na Li[a], Shijiang Yu[b], Fu Wang*[b, c] and Ruifang An*[a]

[a] Department of Obstetrics and Gynecology, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an Jiaotong University, Xi'an 710061, China

[b] Institute of Medical Engineering, School of Basic Medical Sciences, Xi'an Jiaotong University, Xi'an 710061, China

[c] The Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi 710004, China

***Corresponding author to the following:**

Ruifang An, The First Affiliated Hospital of Xi'an Jiaotong University, E-mail: anruifang@xjtu.edu.cn

Fu Wang, Xi'an Jiaotong University, E-mail: wangfu@xjtu.edu.cn

Materials and instruments

The Lachnospiraceae bacterium ND2006 Cas12a (LbCas12a) was purchased from Magigen Biotechnology Co., Ltd (Guangzhou, China). The RPA reaction was performed using the Basic DNA Amplification Kit from Amp-Future (Jiangsu, China). The plasmid containing the HPV16-L1 gene (pUC19-HPV16 L1) was obtained from Tsingke Biotechnology (Beijing, China). Primers, TBA11-FQ and crRNAs were synthesized by Sangon Biotechnology (Shanghai, China). Total DNA was extracted from the cervical swab sample using the TIANamp viral genomic DNA/RNA Kit according to the instructions (DP315-F; Tiangen Biochemical Technology, Beijing, China). Hieff UNICON® Universal Blue qPCR SYBR Green Master Mix was purchased from Yeasen Biotechnology Co., Ltd. (Shanghai, China). All the sequences are provided in Table S1.

CRISPR-Cas12a

Briefly, 20 μl of a CRISPR/Cas12a reaction buffer (5 mM Tris−HCl (pH9.0), 15 mM $MgCl_2$,10 mM NaCl, 0.01% IGEPAL CA−630 (v/v))) consisted of 20 nM LbCas12a, 20 nM crRNA(crRNA/3-crRNA/3N-crRNA)，800 nM DNA-FQ reporter, and a varying amount of target DNA. After being exposed to 365 nm of 35 W for 30 seconds at a height of 5 cm from the tube or not, the mixture was incubated at 37℃ in the dark for 20 minutes. Finally, the fluorescence intensity/fluorescence spectrum was obtained using a microplate reader (FAM FQ: λex: 488/10 nm, λem: 518/10 nm).

CRISPR-Cas12a assisted by RPA assay (two-step)

For RPA, the Amp-Future Basic Kit was used, which contains 29.4 μl of 1XRPA reaction buffer, an RPA reaction pellet, 0.4 μM forward/reverse primer, 14 mM MgOAc, and a varying amount of target DNA. The reaction was incubated at 37℃ for 30 minutes. Then，5 μl of the products were transferred to a new tube containing 20 nM LbCas12a, 20 nM crRNA（crRNA/3-crRNA/3N-crRNA），800 nM DNA-FQ reporter, and 20 μl CRISPR/Cas12a reaction buffer. The mixture was irradiated with or without 365 nm of 35 W for 30 seconds at a height of 5 cm from the tube, and then incubated at 37℃ in the dark for 20 minutes. Finally, the fluorescence intensity/fluorescence spectrum was obtained using a microplate reader (FAM FQ: λex: 488/10 nm, λem: 518/10 nm).

CRISPR-Cas12a supported by RPA assay (one-pot)

The reactions of CRISPR-Cas12a detection and RPA amplification reactions were

performed in a one-pot solution. Briefly, 50 μl of one-pot reaction is consisted of 29.4 μl of 1XRPA reaction buffer, one RPA reaction pellet, 0.4 μM of forward/reverse primer, 16 mM of MgOAc, 20 nM of LbCas12a, 20 nM of crRNA(crRNA/3-crRNA/3N-crRNA)，800 nM DNA-FQ reporter，5 μL 10× NEBufferr and a varying amount of target DNA. The reaction was incubated at 37℃ for 50 minutes in the dark. After 30 minutes, the reaction was exposed or not to 365 nm of 35 W for 30 seconds at a height of 5 cm from the tube. Finally, the fluorescence intensity/fluorescence spectrum was obtained using a microplate reader (FAM FQ: λex: 488/10 nm, λem: 518/10 nm).

One-pot photoactivatable CRISPR-Cas12a combined with RPA with tube-in-tube structure

In simple terms, a 50 μl of one-pot reaction consists of 35 μl photoactivatable CRISPR/Cas12a reaction in the PCR tube (20 nM LbCas12a, 20 nM 3N-crRNA, 800 nM DNA-FQ reporter, 1XCRISPR/Cas12a reaction buffer) and 15 μl RPA reaction in the inner tube (9.8 μl of 1XRPA mix (29.4 μl of 1XRPA reaction buffer was used to resuspend an RPA reaction pellet and then one third of the mixture was used), 0.4 μM forward/reverse primer, 16 mM MgOAc, a varying amount of target DNA). The tube-in-tube structure was incubated at 37℃ for 30 min in the dark. It was then centrifuged (600 rpm) for 1 min or shaken vigorously to transfer the components in the inner cannula to the outer cannula，mixed well, and irradiated with at 365 nm of 35 W for 30 seconds at a height of 5 cm from the tube. Finally, after incubation at 37℃ for 20 minutes in the dark, the result can be distinguished with the aid of the eye using UV light at 302 nm，analyzed by ImageJ software.

Clinical specimen preparation

This study has been approved by The Biomedical Ethics Committee of Health Science Center of Xian Jiaotong University. Clinical cervical swab samples obtained from the The First Affiliated Hospital of Xi'an Jiaotong University (No. XJTU1AF2023LSK-502) were used for the assay. To extract the target nucleic acid from a human clinical swab sample, 1 ml of the sample was centrifuged at 1000g for 2 min, then the methanol-containing supernatant was removed, leaving cell particles. The suspended cell particles were washed 3 times with ddH_2O. Total DNA was extracted from the cell pellets using the TIANamp viral genomic DNA/RNA kit. After purification, HPV DNA was used directly for detection or stored in a freezer at -20 °C.

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Table S1. Sequences used for assay.

Name	Sequences (from 5′ to 3′)
HPV16 crRNA	UAAUUUCUACUAAGUGUAGAUGAAUACAUUUACCUGACCCC
HPV16 3-crRNA	UAAUUUCUACUAAGUGUAGAUGAAUACAUUUACCUGACCCC dTdAdTdTdAdTdT
HPV16 3N-crRNA	UAAUUUCUACUAAGUGUAGAUGAA(dT-NPOM)ACA(dT-NPOM)UUACC(dT-NPOM)GACCCCdTdAdTdTdAdTdT
HPV16-L1 RPA-F1	TTTTCAGATGTCTCTTTGGCTGCCTAGTGA
HPV16-L1 RPA-R1	TGACCACGACCTACCTCAACACCTACACAG
HPV16-L1 RPA-F2	TTTTCAGATGTCTCTTTGGCTGCCTAGTGA
HPV16-L1 RPA-R2	CTGACCACGACCTACCTCAACACCTACACA
HPV16-L1 RPA-F3	TTTTCAGATGTCTCTTTGGCTGCCTAGTGA
HPV16 -L1RPA-R3	CTAATGGCTGACCACGACCTACCTCAACAC
HPV16 -L1RPA-F4	CTGTCCCAGTATCTAAGGTTGTAAGCACGG
HPV16 -L1RPA-R4	GCATAAGCACTAGCATTTTCTGTGTCATCC
HPV16 -L1RPA-F5	CTGTCCCAGTATCTAAGGTTGTAAGCACGG
HPV16 -L1RPA-R5	CTGACCACGACCTACCTCAACACCTACACA
HPV16 -L1RPA-F6	CATGCAGGAACATCCAGACTA
HPV16-L1 RPA-R6	GACCTACCTCAACACCTACACAG
HPV16 -L1RPA-F7	CATGCAGGAACATCCAGACT
HPV16 -L1RPA-R7	GACCTACCTCAACACCTACACAG
HPV16 -L1RPA-F8	CTGTCCCAGTATCTAAGGTTGTAAGCACGG
HPV16-L1 RPA-R8	CTAATGGCTGACCACGACCTACCTCAACAC
HPV16 -L1 PCR-F	GACATTTATTTAATAGGGCTGGTGC
HPV16-L1 PCR-R	CCTGGGATGTTACAAACCTATAAGTATC
ssDNA-FQ	FAM-TTATT-BHQ1
Indicator	TATTATTATTATTTATTATTTATTATTATT

HPV16-L1 plasmid (from 5′to 3′)

ATGCAGGTGACTTTTATTTACATCCTAGTTATTACATGTTACGAAAACGACGTAAACG
TTTACCATATTTTTTTTCAGATGTCTCTTTGGCTGCCTAGTGAGGCCACTGTCTACTTG
CCTCCTGTCCCAGTATCTAAGGTTGTAAGCACGGATGAATATGTTGCACGCACAAACA
TATATTATCATGCAGGAACATCCAGACTACTTGCAGTTGGACATCCCTATTTTCCTATT
AAAAAACCTAACAATAACAAAATATTAGTTCCTAAAGTATCAGGATTACAATACAGG
GTATTTAGAATACATTTACCTGACCCCAATAAGTTTGGTTTTCCTGACACCTCATTTTA
TAATCCAGATACACAGCGGCTGGTTTGGGCCTGTGTAGGTGTTGAGGTAGGTCGTGGT
CAGCCATTAGGTGTGGGCATTAGTGGCCATCCTTTATTAAATAAATTGGATGACACAG
AAAATGCTAGTGCTTATGCAGCAAATGCAGGTGTGGATAATAGAGAATGTATATCTA
TGGATTACAAACAAACACAATTGTGTTTAATTGGTTGCAAACCACCTATAGGGGAAC
ACTGGGGCAAAGGATCCCCATGTACCAATGTTGCAGTAAATCCAGGTGATTGTCCAC
CATTAGAGTTAATAAACACAGTTATTCAGGATGGTGATATGGTTGATACTGGCTTTGG
TGCTATGGACTTTACTACATTACAGGCTAACAAAAGTGAAGTTCCACTGGATATTTGT
ACATCTATTTGCAAATATCCAGATTATATTAAAATGGTGTCAGAACCATATGGCGACA
GCTTATTTTTTTATTTACGAAGGGAACAAATGTTTGTTAGACATTTATTTAATAGGGCT
GGTGCTGTTGGTGAAAATGTACCAGACGATTTATACATTAAAGGCTCTGGGTCTACTG
CAAATTTAGCCAGTTCAAATTATTTTCCTACACCTAGTGGTTCTATGGTTACCTCTGAT
GCCCAAATATTCAATAAACCTTATTGGTTACAACGAGCACAGGGCCACAATAATGGC
ATTTGTTGGGGTAACCAACTATTTGTTACTGTTGTTGATACTACACGCAGTACAAATA
TGTCATTATGTGCTGCCATATCTACTTCAGAAACTACATATAAAAATACTAACTTTAA
GGAGTACCTACGACATGGGGAGGAATATGATTTACAGTTTATTTTTCAACTGTGCAAA
ATAACCTTAACTGCAGACGTTATGACATACATACATTCTATGAATTCCACTATTTTGG
AGGACTGGAATTTTGGTCTACAACCTCCCCCAGGAGGCACACTAGAAGATACTTATA
GGTTTGTAACATCCCAGGCAATTGCTTGTCAAAAACATACACCTCCAGCACCTAAAG
AAGATCCCCTTAAAAAATACACTTTTTGGGAAGTAAATTTAAAGGAAAAGTTTTCTGC
AGACCTAGATCAGTTTCCTTTAGGACGCAAATTTTTACTACAAGCAGGATTGAAGGCC
AAACCAAAATTTACATTAGGAAAACGAAAAGCTACACCCACCACCTCATCTACCTCT
ACAACTGCTAAACGCAAAAAACGTAAGCTGTAA

Table S2. HPV16 visual detection results.

CRISPR-Cas12a results	Clinical results		Total	Positive predictive agreement	Negative predictive agreement
	Positive	Negative			
Positive	14	1	15	14/15（93.33%）	30/30（100%）
Negative	0	30	30		

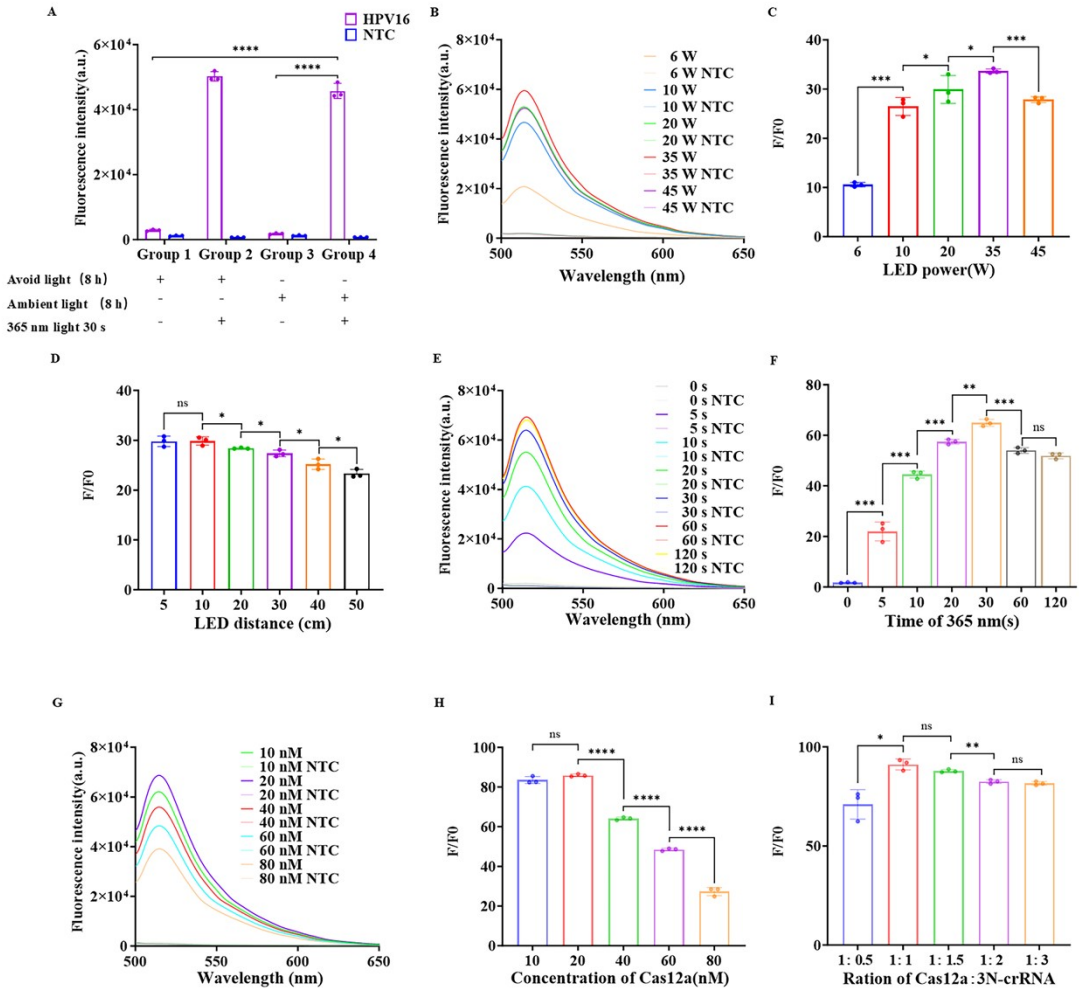

Figure S1. Optimization of photoactivatable CRISPR-Cas12a system. (A) Stability study: Photoactivatable crRNA (3N-crRNA) was pretreated without light or exposed to light for 8 h at room temperature. (B) Investigation of the light intensity required to restore the activity of the photoactivatable CRISPR/Cas12a system. (C) Quantification of the fluorescence intensity in (B). (D) Optimization of the LED distance to the tube. (E) Investigation of the light time required to restore the activity of the photoactivatable CRISPR/Cas12a system. Subsequently, Photoactivatable CRISPR/Cas12a was then reacted with or without UV light at 365 nm of 35 W for 30 seconds at a height of 5 cm from the tube. (F) Quantification of the fluorescence intensity in (E). (G) Optimization of the concentration of LbCas12a. (H) Quantification of the fluorescence intensity in (G). (I) Optimization of the ratio of LbCas12a to 3N-crRNA($^{ns}P > 0.05$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$)

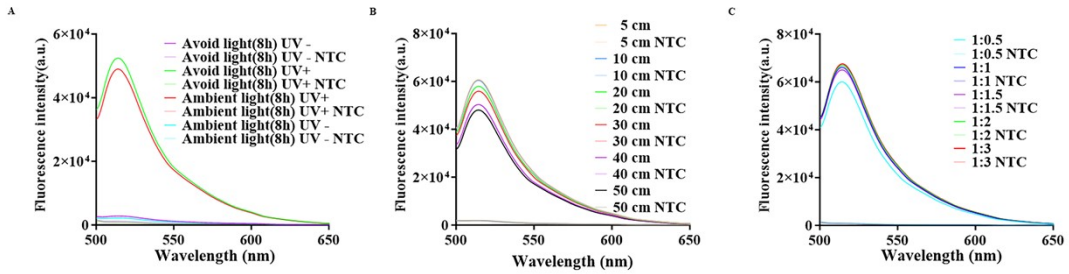

Figure S2. **Fluorescence spectroscopy results.** (A) Photoactivatable crRNA (3N-crRNA) was pretreated without light or exposed to light for 8 hours at room temperature. (B) Optimization of LED distance to the tube (C) Optimization of the ratio of LbCas12a to photoactivatable crRNA (3N-crRNA). (NTC: No target control)

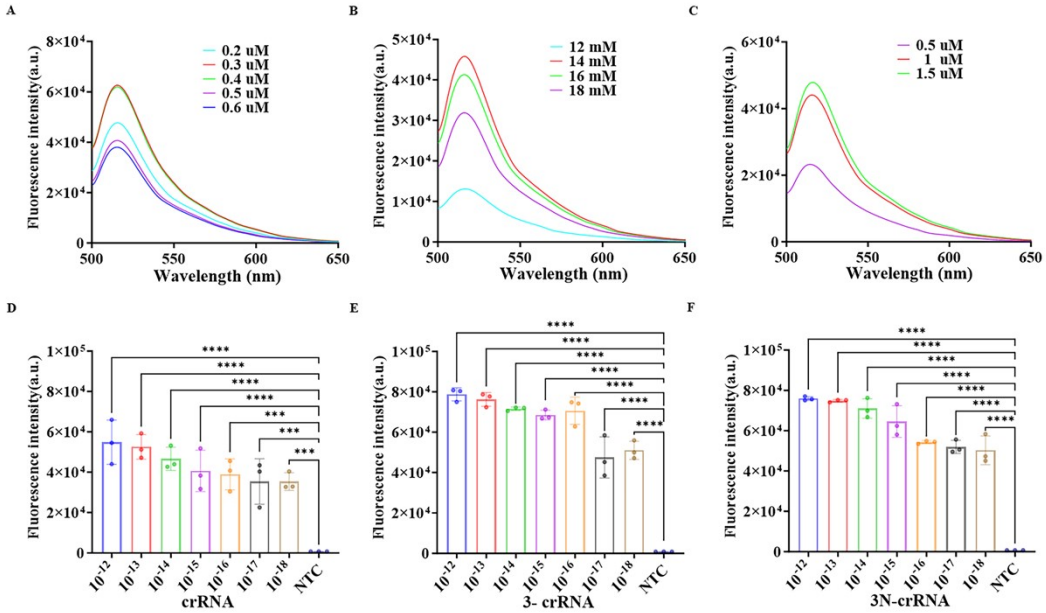

Figure S3. **Conventional CRISPR/Cas12a combined with RPA assay.** (A) Optimization of primer concentration. (B) Optimization of MgOAc concentration. (C) Optimization of ssDNA-FQ probe concentration. (D) Detection efficiency with normal crRNA. (E) Detection efficiency with engineered crRNA (3-crRNA). (F) Detection efficiency with photoactivatable crRNA (3N-crRNA). (NTC: No target control, ****$P < 0.0001$)

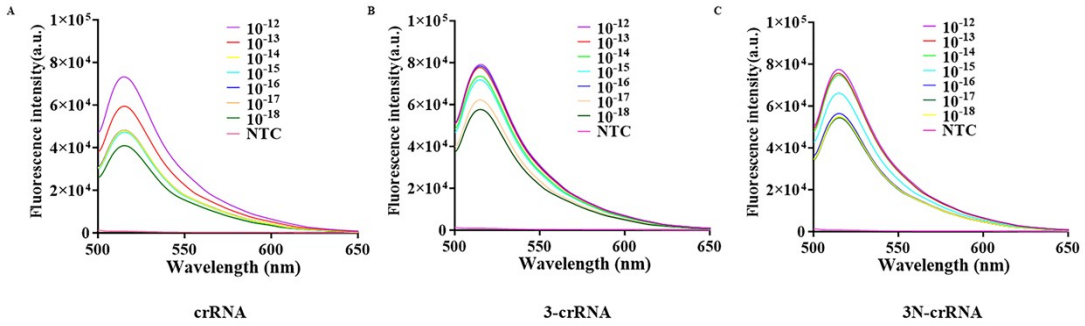

Figure S4. **Fluorescence spectroscopy results of conventional CRISPR/Cas12a combined with RPA assays.** (A) Detection efficiency with crRNA. (B) Detection efficiency with engineered crRNA(3-crRNA). (C) Detection efficiency with photoactivatable crRNA (3N-crRNA). (NTC: No target control)

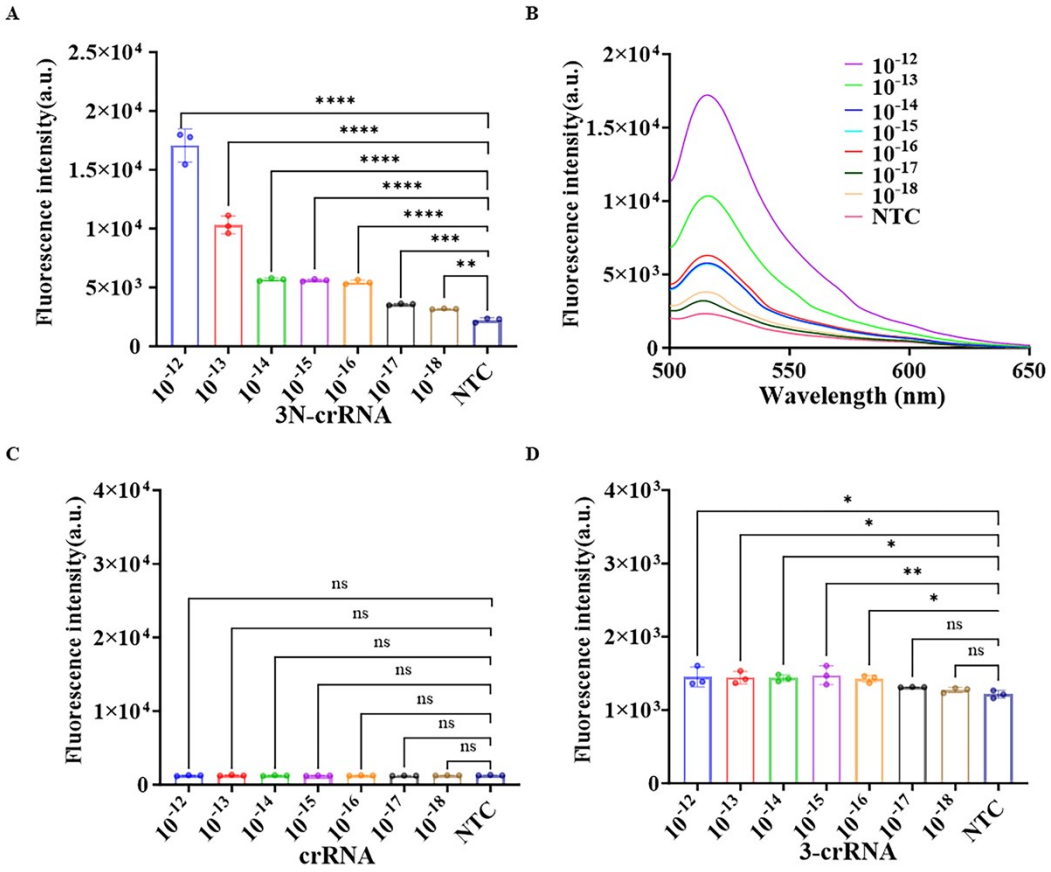

Figure S5. **Comparison of detection efficiency with different crRNA**. (A, B) Detection efficiency (10^{-12}-10^{-18} M) with photoactivatable crRNA (3N-crRNA). (C) Detection efficiency with ordinary crRNA. (D) Detection efficiency with engineered crRNA (3-crRNA). (NTC: No target control, $^{ns}P > 0.05$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$)

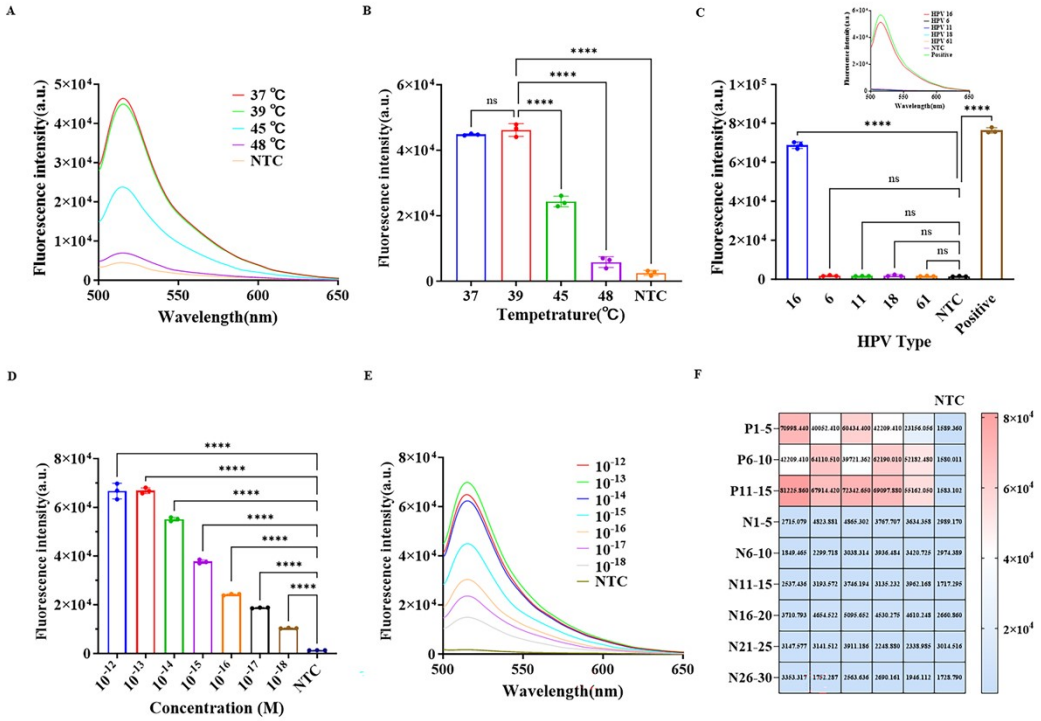

Figure S6. **HPV16 detection (the fluorescence intensity) by photoactivatable CRISPR-Cas12 with tube-in-tube structure.** (A) The effect of different temperatures on the fluorescence spectroscopy. (B) Quantification of the fluorescence intensity in (A). (C) Specificity of one-pot photoactivatable CRISPR/Cas12a tube-in-tube structure. (D) Sensitivity of photoactivatable CRISPR-Cas12 with tube-in-tube structure. (E) Fluorescence spectroscopy results of (E). (F) Photoactivatable CRISPR-Cas12 with tube-in-tube structure was further applied to the detect of HPV16 in real clinical samples. (NTC: No target control，Positive: HPV16-L1 plasmids, N:Negative clinical samples validated by qPCR, P: Positive clinical samples validated by qPCR，ns $P > 0.05$， **** $P < 0.0001$)

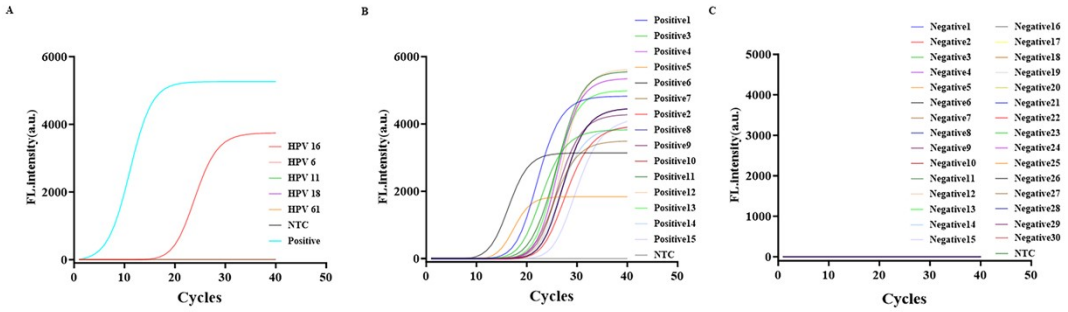

Figure S7. **qPCR results of clinical samples.** (A) qPCR results from specificity clinical samples and positive (HPV16-L1 plasmids). (B) qPCR results from 15 HPV16-positive clinical samples. (C) qPCR results from 30 HPV16 negative clinical samples. (NTC: No target control)