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Supporting Information

Exploring the Trans-Cleavage Activity of CRISPR-Cas12a (cpf1) for the Development of a Universal Electrochemical Biosensor

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Experimental Section

Fabrication of ssDNA Reporter surface

An array containing twenty sensors was first cleaned through an established procedure using potassium hydroxide, sulfuric acid and nitric acid.[1] Thiol linked ssDNA reporter was treated with 10 μM of tris(2-carboxyethyl)phosphine (TCEP) to reduce the S-S bond for 10 min in the dark at room temperature. The ssDNA reporter was then diluted to 1 μM using 10 mM Tris buffer containing 10 mM EDTA. 20 μL of the 1 μM ssDNA reporter was directly incubated onto the gold sensor for 1 hr in the dark at room temperature. The ssDNA immobilized sensor array was then cleaned by immersing in 10 mM Tris buffer for 5 min. After cleaning, the sensor array was immersed in 2 mM 6-mercaptoheaxnol (MCH) prepared in 10 mM Tris buffer for 30 min to passivate the surface and replace loosely tethered ssDNA reporter, forming a highly-aligned surface (Operation of MCH related steps should be conducted in a fume hood due to its toxicity). After the MCH treatment, the sensor array was then cleaned by immersing in 10 mM Tris buffer for 5 min. The cleaned sensor array was then dried by nitrogen gas and ready for treatment by CRISPR system. For a short storage period, the cleaned sensor array can be stored in 10 mM Tris buffer (containing 100 mM NaCl) at 4 °C.

In vitro Digestion of Cas12a-crRNA

Cas12a-crRNA duplex was prepared in a buffer prepared by nuclease free water containing 50 mM NaCl, 10 mM Tris-HCl, 15 mM MgCl2, 100 μg/ml BSA with a pH of 7.9. 30 nM of Cas12a-crRNA was assembled and incubated at 25°C for 10 min. Typically, for nucleic acid detection, 4 μL of sample was added into 26 μL of the Cas12a-crRNA duplex to form the Cas12a-crRNA-

target triplex and incubated for 10 min at room temperature. 20 μL of the Cas12a-crRNA-target triplex solution was applied to ssDNA reporter covered sensor for trans-cleavage activity at 37°C for 30 min. 80 U/mL of Proteinase k was applied to the CRISPR treated surface at 37°C for 15 min before the electrochemical analysis. For protein detection, 10 μL of 100 nM of aptamer was applied to treat 10 μL of sample (resulting in a 50 nM final concentration of aptamer) and incubated at room temperature for 30 min. E-CRISPR as described above was then applied for protein sample analysis with an elongated trans-cleavage period for 60 min.

On-Chip Electrochemical Analysis

After the on-chip CRISPR reaction, the sensors were cleaned by immersing the sensors into a 10 mM Tris buffer for 5 min. For electrochemical test, a 10 mM Tris buffer containing 100 mM NaCl was applied as the electrolyte. Square wave voltammetry (SWV) was applied before and after the treatment of Cas12a-crRNA-target triplex to obtain the change of current based on a potential range of -0.6V to -0.1V, a frequency of 25 Hz, an amplitude of 25 mV (variation of frequency (15 Hz-120 Hz) and amplitude (25 mV- 50 mV) did not present significant enhancement of the quantity of signal changed or the signal stability).

Clinical Sample-Mesenchymal stem cell (MSCs) culture and differentiation:

Cultures of human bone marrow-derived MSCs from healthy de-identified adult volunteer donors were established as previously described.[27] The bone marrow was collected using a procedure reviewed and approved by the University Hospitals of Cleveland Institutional Review Board; informed consent was obtained from all de-identified donors. Cells were expanded in DMEM-LG supplemented with 10% fetal bovine serum, supplemented with FGF2 (10 ng/ml of) for 14 days. Cells were trypsinized and then resuspended in chondrogenic differentiation medium consisting of DMEM-high glucose supplemented with 1% ITS+,10-7 M dexamethasone, 1mM sodium pyruvate, 120 mM ascorbic acid-2 phosphate, 100 mM nonessential amino acids, and 10 ng/mL TGF-β1 protein. Two hundred microliters of this cell suspension containing 250,000 cells was added per well of a 96-well polypropylene V-bottom, multi-well dish (Phenix Research). The multi-well plates were centrifuged at 500 g for 5 min and then incubated at 37 °C. The differentiation medium was changed every other day. Conditioned medium from these pellets was collected at different time points. Days 2 and 28 were chosen to use in the biosensor platform based on previous transcriptome data (RNAseq) showing a greater difference in TGF-β1 protein expression between days 2 and 28 (Figure S10). To activate the latent secreted TGF-β1 protein to the detectable form, 20 μL of 1 M HCl were added to 100 μL of conditioned medium and incubated for 10 minutes and then neutralized with 20 μL of 1.2 M NaOH/0.5 M HEPES. The samples were assayed immediately. This procedure ensures that only the secreted version of TGF-β1 protein assayed.

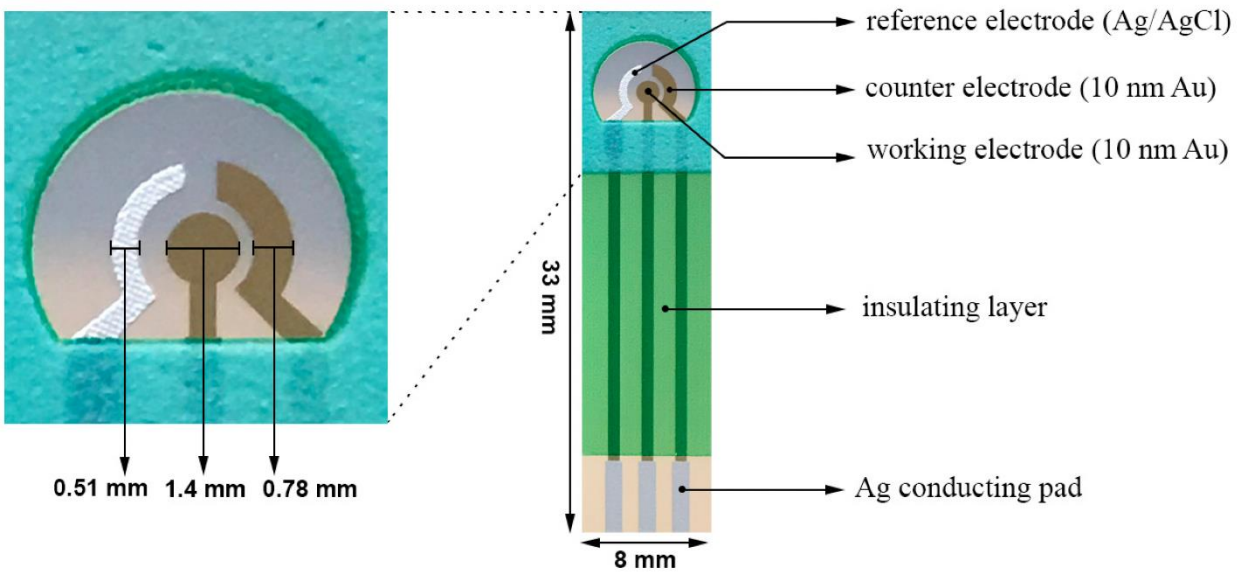

Figure S1. Configuration of the single-use sensor. An array of 100 sensors is fabricated in batch. Working and counter electrodes are fabricated by chemical vapor deposition. Reference electrode (Ag/AgCl) is fabricated by thick-film printing. The electrodes are aligned on polyethylene terephthalate (PET) substrate without any binder and patterned by laser ablation technique. 100 individual biosensors in 4 rows are fabricated on each PET sheet (355 × 280 mm^2). The overall dimensions of an individual biosensor are 33.0 × 8.0 mm^2. The surface area of the working electrode is 1.54 mm^2. The working electrode can accommodate 20-30 µL. The stability and the reproducibility of the sensor was evaluated previously. [1] The fabrication method and design of the sensors allows mass-production of single-use disposable sensors with a cost around $1.5/sensor. Therefore, this sensor platform is suitable for the development of a portable, cost-effective, electrochemical sensing system as a potential point-of-care diagnostic platform.

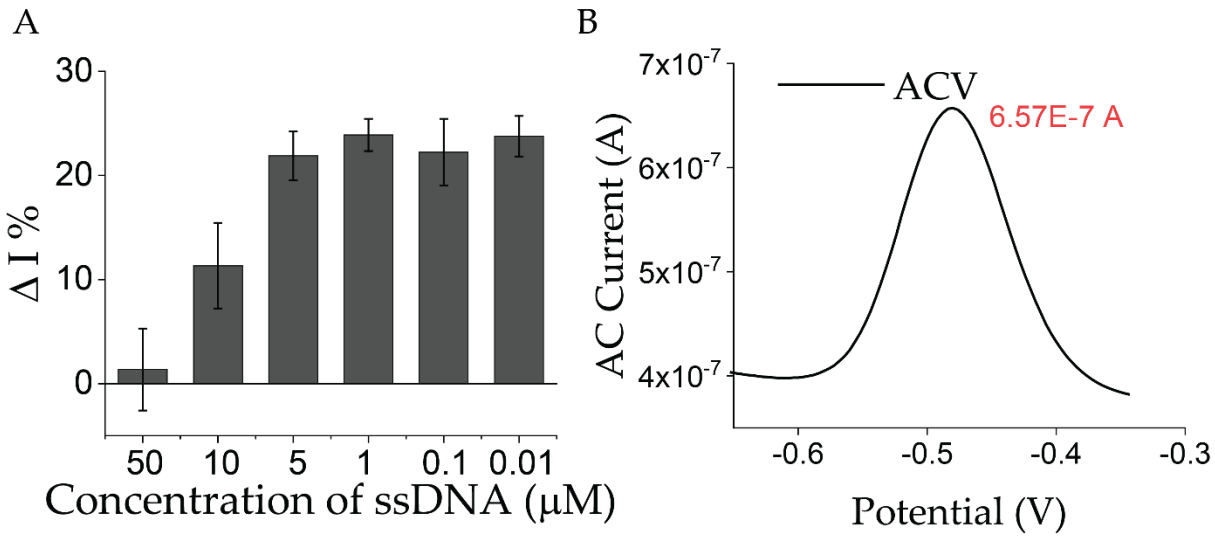

Figure S2. A) Evaluation of different concentrations of ssDNA reporter prepared interrogating electrode based on the trans-cleavage activity. The 10 nt ssDNA reporter was incubated on the electrode for 1 hr at room temperature in the dark. 2 mM of 3-mercaptopropanol (3-MCP) in 10 mM Tris buffer (containing 100 mM NaCl) was applied to passivate the ssDNA electrode for 30 min, forming an aligned electrode surface. 50 nM of LbCas12a-crRNA targeting HPV-16 was then applied for the detection of 50 nM of HPV-16 target based on the described procedure in the manuscript. The trans-cleavage activity was then investigated by SWV. The signal change acquired by comparison with the electrochemical signal based on mutated trans-cleavage activity. The ΔI% was compared between different concentrations of ssDNA reporter prepared electrode surface. **B)** Alternating current voltammetry (ACV) evaluation of the surface density of the 1 μM ssDNA reporter prepared electrode. The density was calculated based on the current output, the surface density of the probe was evaluated through the following equations,[2]

$$N_{total} = I/[2nf \frac{\sinh\left(\frac{nFE_{ac}}{RT}\right)}{\cosh\left(\frac{nFE_{ac}}{RT}\right)+1}]$$

, in which I was the value of alternating current output (peak current in ACV graph), E_{ac} was the reaction potential of methylene blue, N_{total} was the number (mol) of the species oxidized (probe

density), F was the faraday constant (96485.3 C mol^{-1}), f was the frequency of ACV setup, R was the gas constant (8.314 J K^{-1} mol^{-1}), n was the electron transferred (2e- for methylene blue reaction), T was the temperature (298 k). A peak current was observed to be 0.657 μA, therefore surface density was calculated to be 4 pmol/mm^2.

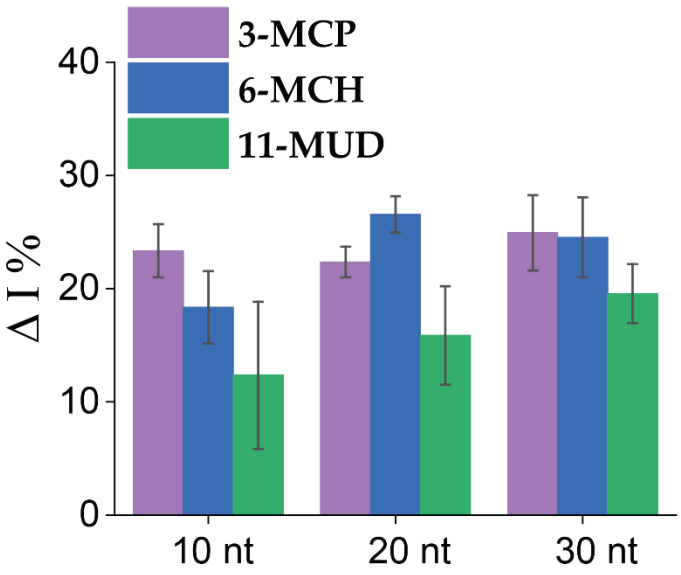

Figure S3. Evaluation of the effect of different passivation agents and different lengths of the ssDNA reporters on the probing of the trans-cleavage activity. 1 μM of the ssDNA reporter was incubated on the electrode surface for 1 hr at room temperature in the dark. 2 mM of 3-mercaptopropanol (3-MCP) or 6-mercaptoheaxnol (6-MCH) or 11-Mercapto-1-undecanol (11-MUD) prepared in 10 mM Tris buffer (containing 100 mM NaCl) was applied for passivation for 30 min at room temperature. 50 nM LbCas12a-crRNA-HPV16 triplex was applied to investigate the trans-cleavage on different performed electrode. The signal changes and standard errors of each passivation agent and ssDNA reporter pair were compared.

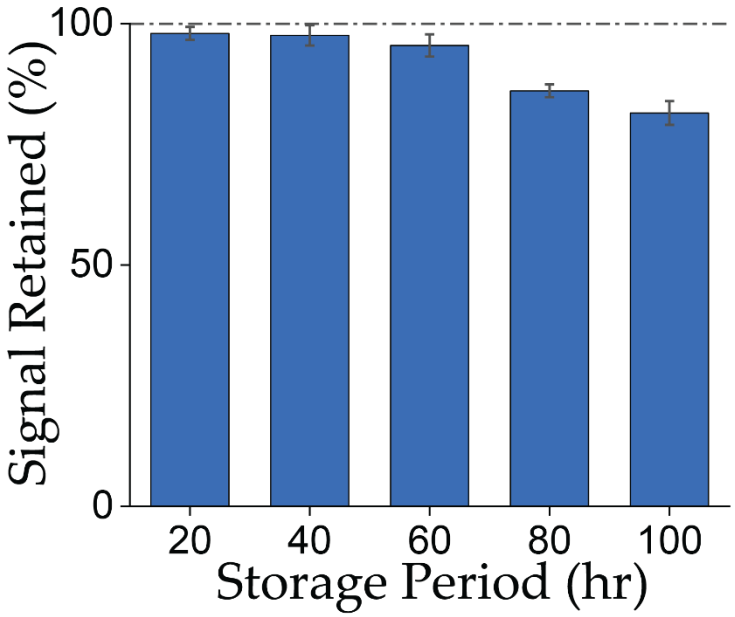

Figure S4. Evaluation of the storage ability of the optimized ssDNA electrode surface. 100% signal reference line (black dash line) was obtained through SWV test on the freshly prepared ssDNA electrode. The prepared ssDNA covered sensors were stored at 4 °C in a humidified environment.[3] Triplet tests with different sensors at each time point were performed to evaluate the signal retained with the increasing period of dry storage. After 60 hr of storage, significant signal decay (>10%) was observed. An approximate storage period was identified as 3 days after functionalization of the sensor with optimized ssDNA and self-assembled monolayer system.

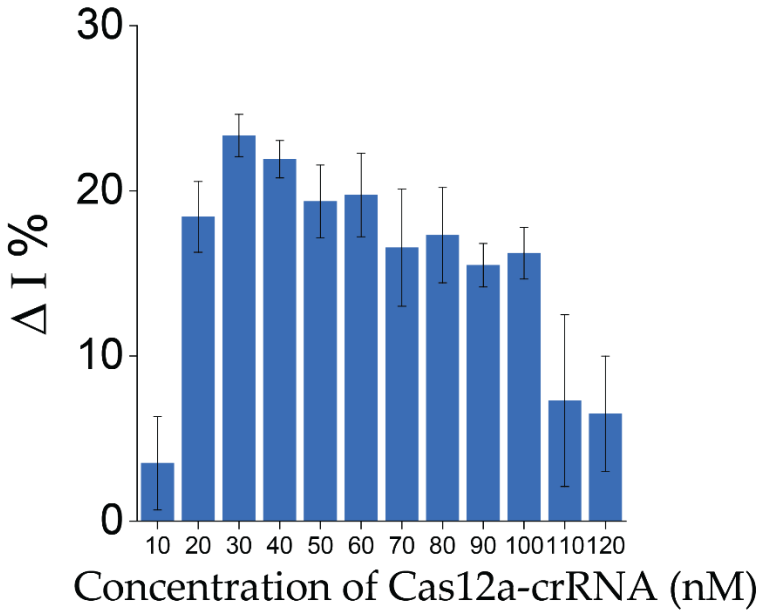

Figure S5. The concentration of Cas12a-crRNA in the 30 uL detection solution was evaluated for the optimized performance of trans-cleavage activity. Based on the optimized ssDNA surface density, a fixed concentration of HPV-16 target of 10x nM was applied as triggering DNA for the trans-cleavage activity on the sensor surface. A 30 min of trans-cleavage period at 37 °C was applied for the test. The change of signal % was compared between different concentrations of Cas12a-crRNA duplex. An optimized trans-cleavage activation concentration was identified to be 30 nM based on the signal stability and the degree of the change of signal. An increasing concentration (>100 nM) of Cas12a-crRNA would lead to significant hindrance in the diffusion and capture of cognate target, therefore decreasing the accessibility of ssDNA reporter to the Cas12a endonuclease. A low concentration (<30 nM) would decrease the available nucleases for trans-cleavage activity, hence decreasing the change of signal. In all, to activate the cleavage function for Cas12a-crRNA *in vitro* application, it is important to dilute to an ideal concentration for operation.

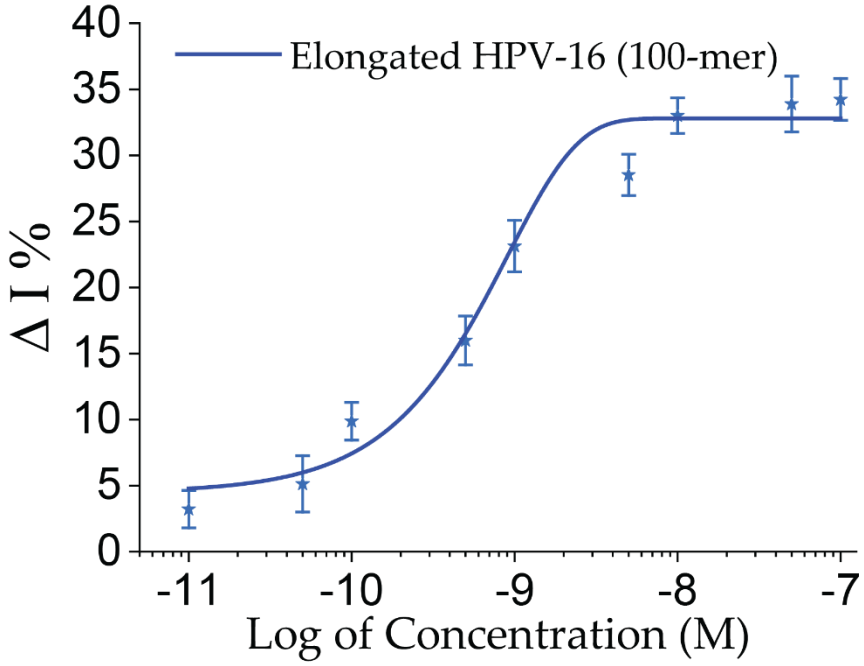

Figure S6. Evaluation the effect of the length of the target sequence on the *in vitro* trans-cleavage activity. Elongated HPV-16 (100-mer) was applied to the E-CRISPR. A IC-50 value of 0.62 nM and an experimental detection limit of 50 pM were obtained (n=3, SE=1.71%). The detection performance for elongated target is comparable with the detection performance of the original HPV-16 target (39-mer), indicating the length of the target would not influence the *in vitro* trans-cleavage activity of the CRIPSR Cas12a system.

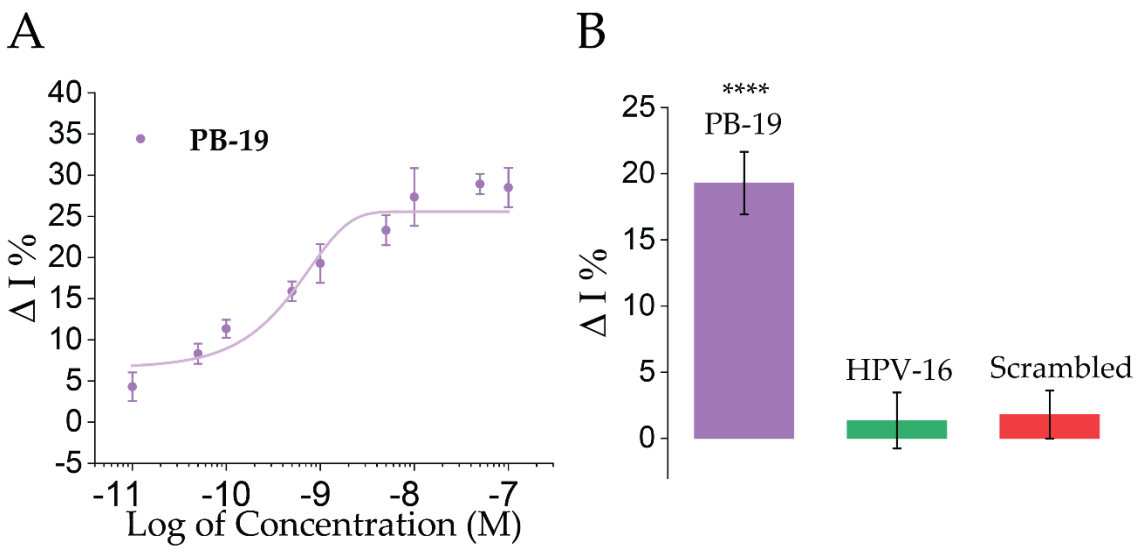

Figure S7. E-CRISPR detection on PB-19. A) Dose-response curve for the detection of single strand DNA virus, Parvovirus B19 (PB-19) (n=3, SE=1.84%). The detection procedure is the same as described for the detection of HPV-16. The ΔI% of PB-19 and HPV-16 are consistent based on the same concentration of the Cas12a-crRNA-target complex. This phenomenon indicates the high-consistency of the E-CRISPR system and demonstrates its great potential as a generalized platform for nucleic acid detection. **B)** Evaluation of the selectivity of E-CRISPR on PB-19 detection. 1 nM of PB-19 target signal was used to compare with the signal generated by 500 nM of HPV-16 or scrambled DNA sequences, which demonstrated a signal change (< 2%) less than the standard error of the PB-19 test (SE=2.36%), indicating the high-selectivity of programmable Cas12a-crRNA duplex (n=3, ****P<0.01, target vs. non-target).

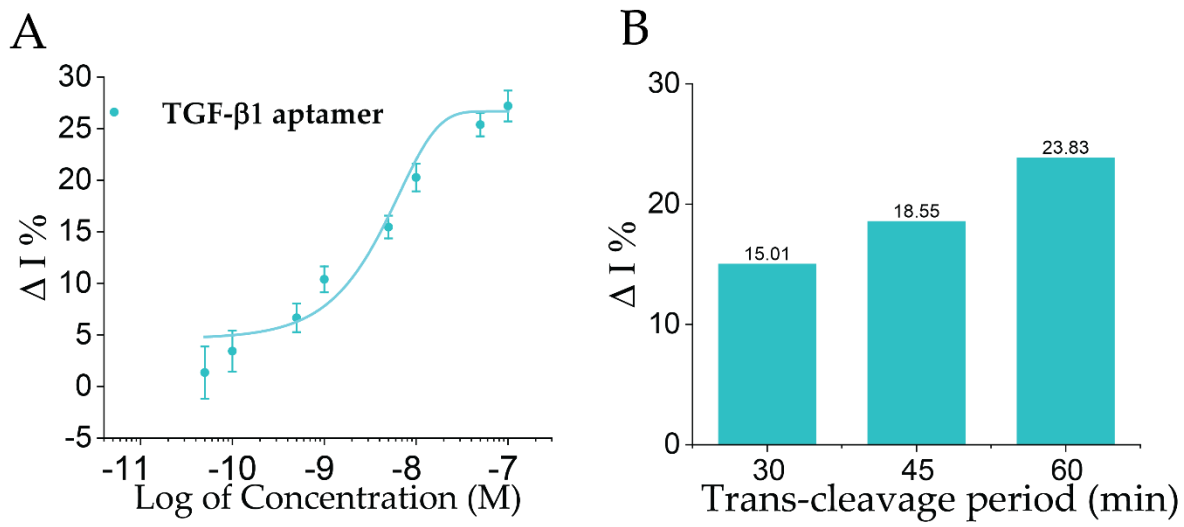

Figure S8. A) Dose-response curve for the detection of TGF-β1 aptamer (n=3, SE=1.53%). The detection procedure is the same as described for the detection of HPV-16. **B)** Comparison of current outputs difference between 50 nM and 1 nM (detection resolution) based on different trans-cleavage period for Cas12a-crRNA-TGF-β1 aptamer. The current change was calculated through the difference between the ΔI% of 50 nM and 1 nM TGF-β1 aptamer.

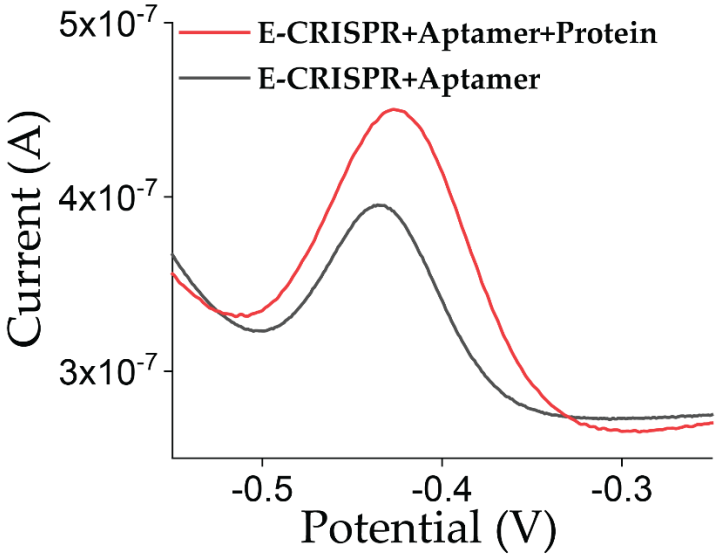

Figure S9. E-CRISPR SWV evaluation of blank cell lysate sample with 50 nM TGF-β1 protein (red line) and without TGF-β1 protein (black line) after treatment with 50 nM TGF-β1 aptamer.

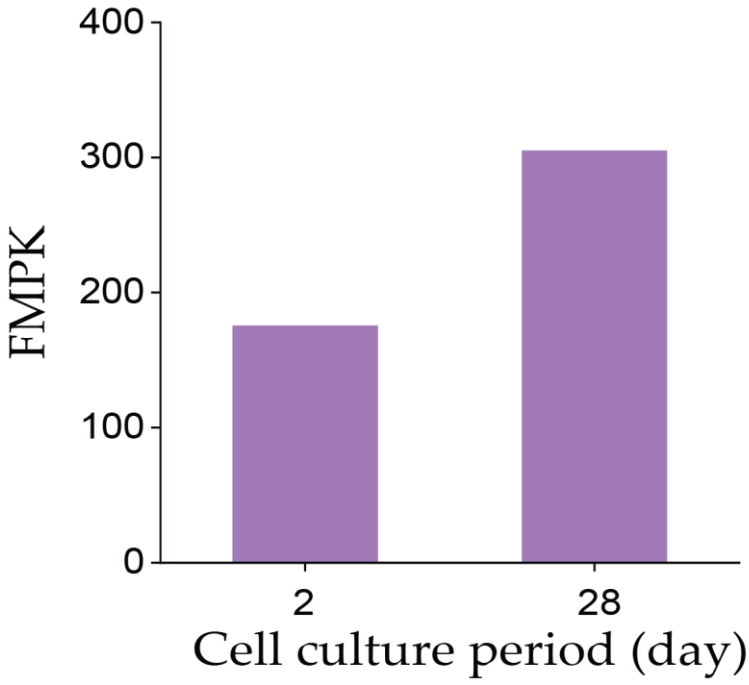

Figure S10. Transcriptome data (RNAseq) for TGF-β1 expression at day 2 and day 28.

Materials

crRNA	Sequence
AsCas12a-crRNA-HPV16	rUrArArUrUrUrCrUrArCrUrCrUrUrGrUrArGrArUrUrGrArArGrUrArGrArUrArUrGrGrCrArGrCrArC
LbCas12a-crRNA-HPV16	rUrArArUrUrUrCrUrArCrUrArArGrUrGrUrArGrArUrGrArArGrUrArGrArUrArUrGrGrCrArGrCrArC
LbCas12a-crRNA-PB-19	rUrArArUrUrUrCrUrArCrUrArArGrUrGrUrArGrArUrCrArGrCrUrUrCrCrArCrArArUrArGrUrGrGrArC
LbCas12a-crRNA-TGF-β1 aptamer	rUrArArUrUrUrCrUrArCrUrArArGrUrGrUrArGrArUrCrArGrUrCrCrGrGrArUrGrCrUrCrUrArGrArGrU
Target sequence	
HPV-16	AATATGTCATTATGTGCTGCCATATCTACTTCAGAAACT
Scrambled	TCAAAGACTTCATCTATACCGTCGTGTATTACTGTATAA
Elongated HPV-16	AATATGTCATTATGTGCTGCCATATCTACTTCAGAAACTACATATAAAAATACTAACTTTAAGG AGTACCTACGACATGGGGAGGAATATGATTTACAGT
Mismatch 1 HPV-16	AATATGTCATTATGTGCTGCCATATCTACTTCAGAATCT
Mismatch 2 HPV-16	AATATGTCATTATGTGCTGCCATATCTACTTCTGAAACT
Mismatch 3 HPV-16	AATATGTCATTATGTGCTGCCATATCTTCTTCAGAAACT
Mismatch 4 HPV-16	AATATGTCATTATGTGCTGCCAAATCTACTTCAGAAACT
Mismatch 5 HPV-16	AATATGTCATTATGTGCAGCCATATCTACTTCAGAAACT
PB-19	CATTATTAAGTCCACTATTGTGGAAGCTGCAAAAGCTATT
TGF-β1 aptamer	CGCTCGGCTTCACGAGATTCGTGTCGTTGTGTCCTGTACCCGCCTTGACCAGTCACTCTAGAGCATCCGGACTGGAAACT
Surface probe	
10 nt	/5ThioMC6-D/TTTTAATTTT/3MeBlN/
20 nt	/5ThioMC6-D/AAAAAAAAAAAAAAAAAAAA/3MeBlN/
30 nt	/5ThioMC6-D/AAAAAAAAAAAAAATTTAAAAAAAAAAAAA/3MeBlN/

All the nucleic acids tested was customized and purchased from Integrated DNA Technologies (IDT) (Iowa, USA). Cas12a endonuclease from Acidaminococcus sp. BV3L6 (AsCas12a) and nuclease free water were also purchased from IDT. Cas12a endonuclease from Lachnospiraceae bacterium ND2006, NEB buffer 2.1, Proteinase-K were obtained from New England BioLabs (MA, USA). Tris(2-carboxyethyl) phosphine hydrochloride (TECP), 3-mercaptopropanol, 6-mercaptohexanol, 11-mercapto-1-undecanol and all the other fundamental chemicals were purchased from Sigma Aldrich (MO, USA). Pooled healthy human serum was purchased from Innovative Research (MI, USA).

References

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[2] S. D. O'Connor, G. T. Olsen, S. E. Creager, *Journal of Electroanalytical Chemistry* **1999**, *466*, 197-202.

[3] F. Kuralay, S. Campuzano, J. Wang, *Talanta* **2012**, *99*, 155-160.