Supporting Information for

CRISPR-Cas12a-Empowered Electrochemical Biosensor for Rapid and Ultrasensitive Detection of SARS-CoV-2 Delta Variant

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Supplementary Tables and Figures

Table S1 crRNA and reporters used in this study. crRNA is designed according to "CRISPR-SHERLOCK" method published by Feng Zhang *et al*. [S1]

Name	Sequence (5'-3')
crRNA-Delta	UAAUUUCUACUAAGUGUAGAUAAGTTTTCCAAGTGCACTTG
MB-ssDNA reporter	SH-CTCAACttattattACGAAC-MB
FAM-ssDNA reporter	FAM- CTCAACttattattACGAAC-BHQ

Table S2 Sequences from SARS-CoV-2 (Origin/Delta), Middle East respiratory syndrome-related coronavirus (MERS), Influenza A virus (H1N1/H3N2), Influenza B virus, Human Respiratory Syncytial Virus (HRSV). In the sequence of SARS-CoV-2 Origin, the mutation site labelled in red indicates the specific mutation in Delta variant (D950N: 24410 G > A)

Name	Inserted Sequences (5'-3')
SARS-CoV-2 Origin (NC_045512.2, S gene, 24192-24643)	TGTTAGCGGGTACAATCACTTCTGGTTGGACCTTTGGTGCAGGTGCT GCATTACAAATACCATTTGCTATGCAAATGGCTTATAGGTTTAATGG TATTGGAGTTACACAGAATGTTCTCTATGAGAACCAAAAATTGATTG CCAACCAATTTAATAGTGCTATTGGCAAAATTCAAGACTCACTTTCT TCCACAGCAAGTGCACTTGGAAAACTTCAAG(Delta:G>A)ATGTGGTC AACCAAAATGCACAAGCTTTAAACACGCTTGTTAAACAACTTAGCTC CAATTTTGGTGCAATTTCAAGTGTTTTAAATGATATCCTTTCACGTCT TGACAAAGTTGAGGCTGAAGTGCAAATTGATAGGTTGATCACAGGC AGACTTCAAAGTTTGCAGACATATGTGACTCAACAATTAATTAGAGC TGCAGAAATCAGAGCTTCTGCTAATCTTGCTGCTACT
MERS (NC_019843.3, 24275-24938)	TGTTAATATGGAAGCCGCGTATACTTCATCTTTGCTTGGCAGCATAG CAGGTGTTGGCTGGACTGCTGGCTTATCCTCCTTTGCTGCTATTCCAT TTGCACAGAGTATCTTTTATAGGTTAAACGGTGTTGGCATTACTCAA CAGGTTCTTTCAGAGAACCAAAAGCTTATTGCCAATAAGTTTAATCA GGCTCTGGGAGCTATGCAAACAGGCTTCACTACAACTAATGAAGCT TTTCAGAAGGTTCAGGATGCTGTGAACAACAATGCACAGGCTCTATC CAAATTAGCTAGCGAGCTATCTAATACTTTTGGTGCTATTTCCGCCT CTATTGGAGACATCATACAACGTCTTGATGTTCTCGAACAGGACGCC CAAATAGACAGACTTATTAATGGCCGTTTGACAACACTAAATGCTTT TGTTGCACAGCAGCTTGTTCGTTCCGAATCAGCTGCTCTTTCCGCTCA ATTGGCTAAAGATAAAGTCAATGAGTGTGTCAAGGCACAATCCAAG CGTTCTGGATTTTGCGGTCAAGGCACACATATAGTGTCCTTTGTTGT AAATGCCCCTAATGGCCTTTACTTCATGCATGTTGGTTATTACCCTA GCAACCACATTGAGGTTGTTTCTGCTTATGGTCTTTGCGATGCAGCT AACCCT
Influenza A virus, H1N1 (NC_026431.1)	ATGAGTCTTCTAACCGAGGTCGAAACGTACGTTCTTTCTATCATCCC GTCAGGCCCCCTCAAAGCCGAGATCGCGCAGAGACTGGAAAGTGTC TTTGCAGGAAAGAACACAGATCTTGAGGCTCTCATGGAATGGCTAA AGACAAGACCAATCTTGTCACCTCTGACTAAGGGAATTTTAGGATTT GTGTTCACGCTCACCGTGCCCAGTGAGCGAGGACTGCAGCGTAGAC GCTTTGTCCAAAATGCCCTAAATGGGAATGGGGACCCGAACAACAT GGATAGAGCAGTTAAACTATACAAGAAGCTCAAAAGAGAAATAAC GTTCCATGGGGCCAAGGAGGTGTCACTAAGCTATTCAACTGGTGCA CTTGCCAGTTGCATGGGCCTCATATACAACAGGATGGGAACAGTGA CCACAGAAGCTGCTTTTGGTCTAGTGTGTGCCACTTGTGAACAGATT GCTGATTCACAGCATCGGTCTCACAGACAGATGGCTACTACCACCA ATCCACTAATCAGGCATGAAAACAGAATGGTGCTGGCTAGCACTAC GGCAAAGGCTATGGAACAGATGGCTGGATCGAGTGAACAGGCAGC GGAGGCCATGGAGGTTGCTAATCAGACTAGGCAGATGGTACATGCA ATGAGAACTATTGGGACTCATCCTAGCTCCAGTGCTGGTCTGAAAGA TGACCTTCTTGAAAATTTGCAGGCCTACCAGAAGCGAATGGGAGTG CAGATGCAGCGATTCAAGTGATCCTCTCGTCATTGCAGCAAATATCA TTGGGATCTTGCACCTGATATTGTGGATTACTGATCGTCTTTTTTTCA AATGTATTTATCGTCGCTTTAAATACGGTTTGAAAAGAGGGCCTTCT ACGGAAGGAGTGCCTGAGTCCATGAGGGAAGAATATCAACAGGAA

	CAGCAGAGTGCTGTGGATGTTGACGATGGTCATTTTGTCAACATAGA GCTAGAGTAA
Influenza A virus, H3N2 (U51247.1)	ATGAATCCAAATCAAAAGATAATAACAATTGGCTCTGTTTCTCTCAC TATTGCCACAATATGCTGCCTTATGCAAATTGCCATCCTGGTAACTA CTGTAACATTACATTTCAAGCAATATGAATGCAACTCCCCCCCAAAC AACCAAGTAATGCTGTGTGAACCAACAATAATAGAAAGAAACATAA CAGAGATAGTGTATCTGACCAACACCACCATAGAGAAAGAAGTATG CCCCAAACTAGCAGAATACAGAAATTGGTCAAAGCCGCAATGTAAA ATTACAGGATTTGCACCTTTTTCTAAGGACAATTCAATTCGGCTTTCC GCTGGTGGAGACATTTGGGTGACAAGAGAACCTTATGTGTCATGCG ATCCTGGCAAGTGTTATCAATTTGCCCTTGGACAGGGAACAACACTA AACAACAGGCATTCAAATGACACAGTACATGATAGGACCCCTTATC GAACCCTATTGATGAATGAGTTGGGTGTTCCATTTCATTTGGGAACC AAGCAAGTGTGCATAGCATGGTCCAGCTCAAGTTGTCACGATGGAA AAGCATGGCTGCATGTTTGTGTAACTGGGCATGATGAAAATGCAAC TGCTAGCTTCATTTACGATGGGAGGCTTGTAGATAGTATTGGTTCAT GGTCCAAAAATATCCTCAGGACCCAGGAGTCGGAATGCGTTTGTAT CAATGGAACTTGTACAGTAGTAATGACTGATGGAAGTGCTTCAGGA AGAGCTGATACTAAAATACTATTCATTGAAGAGGGGAAAATCGCTC ATATTAGCCCATTGTCAGGAAGTGCTCAGCATGTCGAGGAGTGCTCC TGTTATCCTCGATATCCTGGTGTCAGATGTGTCTGCAGAGACAACTG GAAAGGCTCCAATAGGCCCATCGTAGATATAAATGTGAAAGATTAT AGCATTGTTTCCAGTTATGTGTGCTCAGGACTTGTTGGAGACACAGC CAGAAAAAACGACAGCTCCAGCAGTAGCTATTGCCGGAATCCTAAC AATGAGAAAGGGAGTCATGGAGTGAAAGGCTGGGCCTTTGATGATG GAAATGATGTGTGGATGGGAAGAACGATCAGCGAGAAGTTACGCTC AGGTGATGAAACCTTCAAAGTCATTGGAGGCTGGTCCAAACCTAAC TCCAAATTGCAGATAAATAGGCAAGTCATAGTTGACAGAGGTAATA GGTCCGGTTATTCTGGTATTTTCTCTGTTGAAGGCAAAAGCTGCATC AATCGGTGCTTTTATGTGGAGTTGATAAGGGGAAGGAAACAGGAAA CTGAAGTCTGGTGGACCTCAAACAGTATTGTTGTGTTTTGTGGCACC TCAGGTACATATGGAACAGGCTCATGGCCTGATGGGGCGGACATCA ATCTCATGCCTATATAAA
Influenza B virus segement 7 (NC_002210.1)	AGCAGAAGCACGCACTTTCTTAAAATGTCGCTGTTTGGAGACACAAT TGCCTACCTGCTTTCACTAATAGAAGATGGAGAAGGCAAAGCAGAA CTAGCTGAAAAATTACACTGTTGGTTCGGTGGGAAAGAATTTGACCT AGATTCTGCTTTGGAATGGATAAAAAACAAAAGGTGCCTAACTGAT ATACAAAAAGCACTAATTGGTGCCTCTATATGCTTTTTAAAACCCAA AGACCAAGAAAGAAAAAGGAGATTCATCACAGAGCCCCTGTCAGG AATGGGAACAACAGCAACAAAGAAGAAAGGCCTAATTCTAGCTGA GAGAAAAATGAGAAGATGTGTAAGCTTTCATGAAGCATTTGAAATA GCAGAAGGCCACGAAAGCTCAGCATTACTATATTGTCTTATGGTCAT GTACCTAAACCCTGAAAACTATTCAATGCAAGTAAAACTAGGAACG CTCTGTGCTTTATGCGAGAAACAAGCATCGCACTCGCATAGAGCCCA TAGCAGAGCAGCAAGGTCTTCGGTACCTGGAGTAAGACGAGAAATG CAGATGGTTTCAGCTATGAACACAGCAAAGACAATGAATGGAATGG GAAAGGGAGAAGACGTCCAAAAACTAGCAGAAGAGCTGCAAAACA ACATTGGAGTGTTGAGATCTCTAGGAGCAAGTCAAAAGAATGGAGA AGGAATTGCCAAAGATGTAATGGAAGTGCTAAAACAGAGCTCTATG GGAAATTCAGCTCTTGTGAGGAAATACTTATAATGCTCGAACCACTT CAGATTCTTTCAATTTGTTCTTTCATTTTATCAGCTCTCCATTTCATG GCTTGGACAATAGGGCATTTGAATCAAATAAAAAGAGGGGTAAACT TGAAAATACAAATAAGGAATCCAAATAAGGAGGCAATAAACAGAG AGGTGTCAATTCTGAGACACAATTACCAAAAGGAAATCCAAGCCAA

	AGAAACAATGAAGAAAATACTCTCTGACAACATGGAAGTATTGGGT GACCACATAGTAGTTGAAGGGCTTTCAACTGATGAGATAATAAAAA TGGGTGAAACAGTTTTGGAGGTGGAAGAATTGCAATGAGCCCAATT TTCACTGTATTTCTTACTATGCATTTAAGCAAATTGTAATCAATGTCA GTGAATAAAACTGGAAAAAGTGCGTTGTTTCTACT
HRSV, Human orthopneumovirus Subgroup A, (NC_038235.1)	GGGGCAAATACAAAGATGGCTCTTAGCAAAGTCAAGTTGAATGATA CACTCAACAAAGATCAACTTCTGTCATCCAGCAAATACACCATCCAA CGGAGCACAGGAGATAGTATTGATACTCCTAATTATGATGTGCAGA AACACATCAATAAGTTATGTGGCATGTTATTAATCACAGAAGATGCT AATCATAAATTCACTGGGTTAATAGGTATGTTATATGCGATGTCTAG GTTAGGAAGAGAAGACACCATAAAAATACTCAGAGATGCGGGATAT CATGTAAAAGCAAATGGAGTAGATGTAACAACACATCGTCAAGACA TTAATGGAAAAGAAATGAAATTTGAAGTGTTAACATTGGCAAGCTT AACAACTGAAATTCAAATCAACATTGAGATAGAATCTAGAAAATCC TACAAAAAAAATGCTAAAAGAAATGGGAGAGGTAGCTCCAGAATAC AGGCATGACTCTCCTGATTGTGGGATGATAATATTATGTATAGCAGC ATTAGTAATAACTAAATTAGCAGCAGGGGACAGATCTGGTCTTACA GCCGTGATTAGGAGAGCTAATAATGTCCTAAAAAATGAAATGAAAC GTTACAAAGGCTTACTACCCAAGGACATAGCCAACAGCTTCTATGA AGTGTTTGAAAAACATCCCCACTTTATAGATGTTTTTGTTCATTTTGG TATAGCACAATCTTCTACCAGAGGTGGCAGTAGAGTTGAAGGGATT TTTGCAGGATTGTTTATGAATGCCTATGGTGCAGGGCAAGTGATGTT ACGGTGGGGAGTCTTAGCAAAATCAGTTAAAAATATTATGTTAGGA CATGCTAGTGTGCAAGCAGAAATGGAACAAGTTGTTGAGGTTTATG AATATGCCCAAAAATTGGGTGGTGAAGCAGGATTCTACCATATATT GAACAACCCAAAAGCATCATTATTATCTTTGACTCAATTTCCTCACT TCTCCAGTGTAGTATTAGGCAATGCTGCTGGCCTAGGCATAATGGGA GAGTACAGAGGTACACCGAGGAATCAAGATCTATATGATGCAGCAA AGGCATATGCTGAACAACTCAAAGAAAATGGTGTGATTAACTACAG TGTACTAGACTTGACAGCAGAAGAACTAGAGGCTATCAAACATCAG CTTAATCCAAAAGATAATGATGTAGAGCTTTGAGTTAATAAAAAA

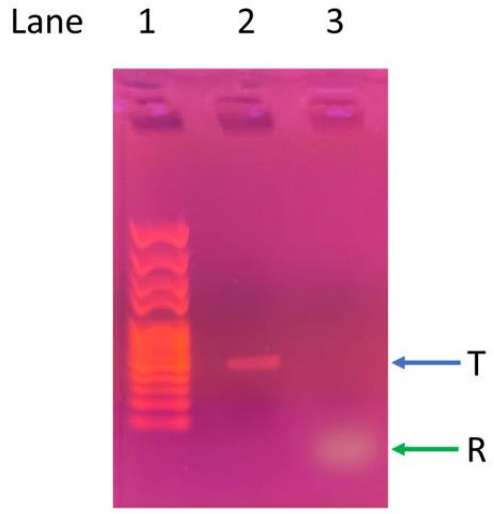

Fig. S1 Agarose gel electrophoresis was performed to verify the trans- and cis- cleavage. Lane 1: marker, Lane 2: 10 nM DNA template, Lane 3: CRISPR reaction with 10 nM DNA template. “T” indicates the location of DNA template, and “R” indicates the location of freed FAM

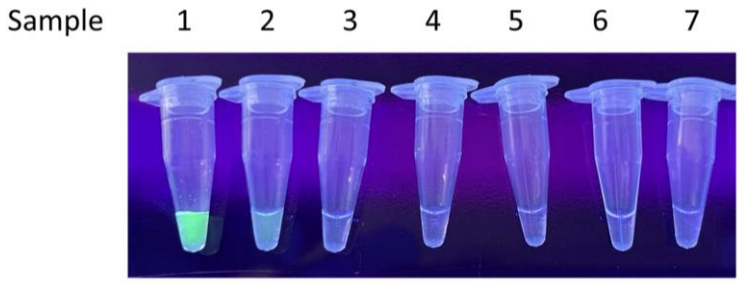

Fig. S2 Original photograph of all the tubes with CRISPR reactions. Sample 1: 10 nM DNA template; Sample 2: 1 nM DNA template; Sample 3: 100 pM DNA template, Sample 4: 10 pM DNA template, Sample 5: 1 pM DNA template, Sample 6: 100 fM DNA template; Sample 7: Negative control

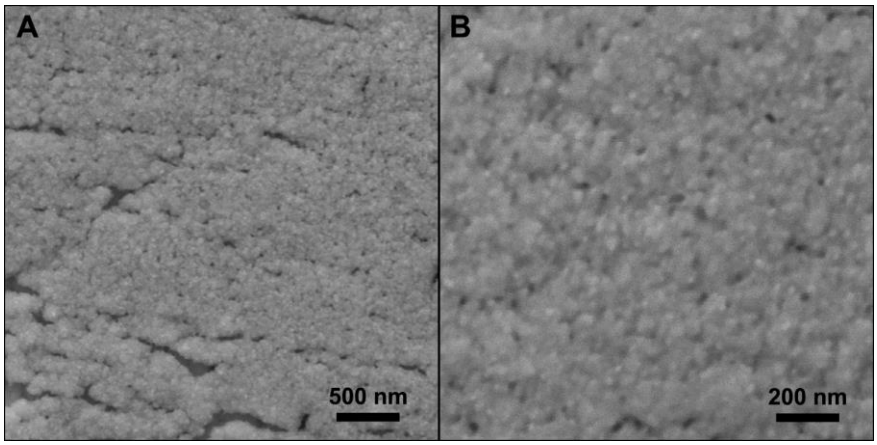

Fig. S3 SEM image of the electro-deposited AuNPs on the AuE

The SEM image was obtained from the electrode directly for accuracy with the assistance of customized AuE electrode, in which the top part of the electrode can be separated from the body of the electrode for further characterization.

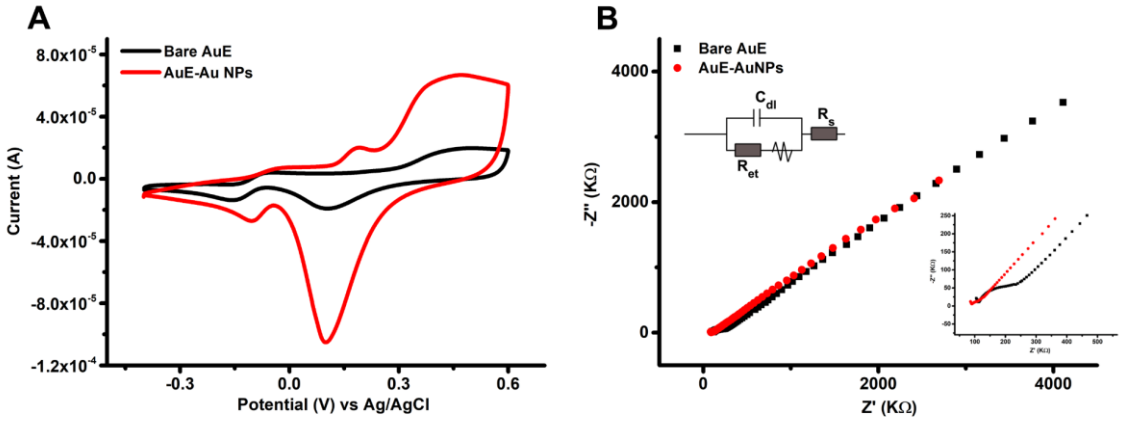

Fig. S4 CV (**A**) and EIS (**B**) curves of bare AuE and AuE-AuNPs

The CV was carried out in 0.5 M KOH with a potential range from -0.4 to 0.6 V vs Ag/AgCl at the scan rate of 100 mV/s. EIS was performed in 0.1 M KCl solution contenting 5 mM $[Fe(CN)_6]^{3-/4-}$, biased potential of 0.23 V (vs. Ag/AgCl) in the frequency range of 0.01–10^5 Hz, and 5 mV amplitude.

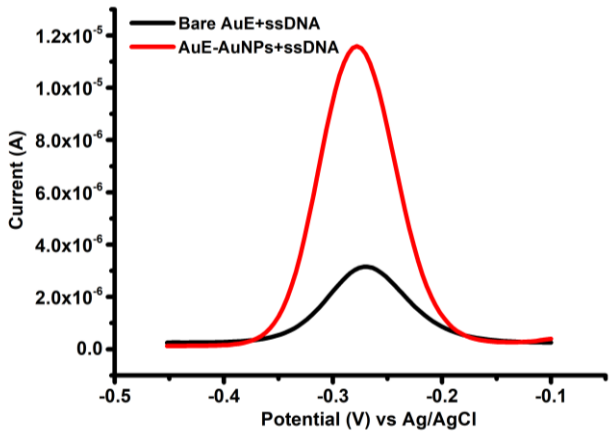

Fig. S5 The SWV of MB-ssDNA modified bare AuE and MB-ssDNA modified AuE-AuNPs

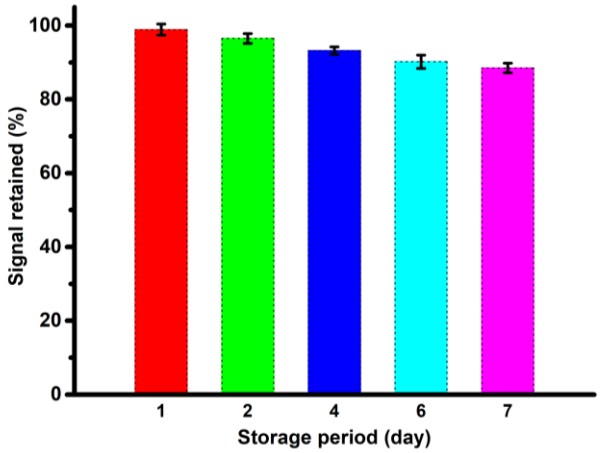

Fig. S6 Evaluation of the storage ability of the optimized ssDNA electrode surface. 100% signal was obtained through SWV test on the freshly prepared ssDNA electrode. The prepared ssDNA covered sensors were stored at 4 °C in the dark under nitrogen protection. Error bars represent standard derivation obtained in three parallel experiments

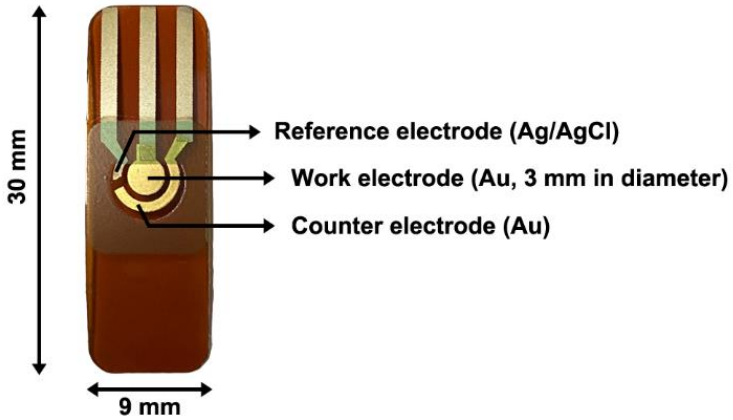

Fig. S7 Configuration of the screen-printed electrode

Supplementary Reference

[S1] M.J. Kellner, J.G. Koob, J.S. Gootenberg, O.O. Abudayyeh, F. Zhang, SHERLOCK. nucleic acid detection with CRISPR nucleases. Nat. Protoc. **14**, 2986–3012 (2019). https://doi.org/10.1038/s41596-019-0210-2