Supplementary information

Fast and sensitive detection of viable *Escherichia coli* O157:H7 using microwell confined and propidium monoazide assisted digital CRISPR microfluidic platform

Weihong Yin, ‡ [a] Kai Hu, ‡ [a] Bingwen Yu, [ab] Tao Zhang, *[a] Haohua Mei, [ac] Bowen Zhang, [a] Zheyu Zou, [a] Liping Xia, [a] Yehong Gui, [ac] Juxing Yin, [a] Wei Jin, [ab] and Ying Mu *[ac]

a. Research Centre for Analytical Instrumentation, State Key Laboratory of Industrial Control Technology, Zhejiang University, Hangzhou 310027, P. R. China;

b. Huzhou Institute of Zhejiang University, Huzhou 313002, P. R. China;

c. College of Life Sciences, Zhejiang University, Hangzhou, 310058, P. R. China.

* Corresponding author: zhtao@zju.edu.cn ; muying@zju.edu.cn

‡ Weihong Yin, Kai Hu contributed to this work equally

Supplementary results

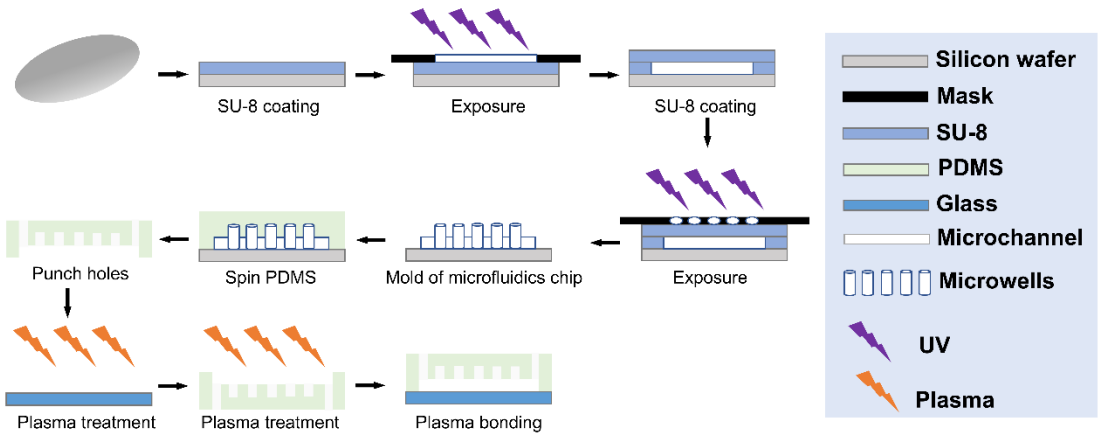

Figure S1 Fabrication processes of the molds and the microfluidic chips

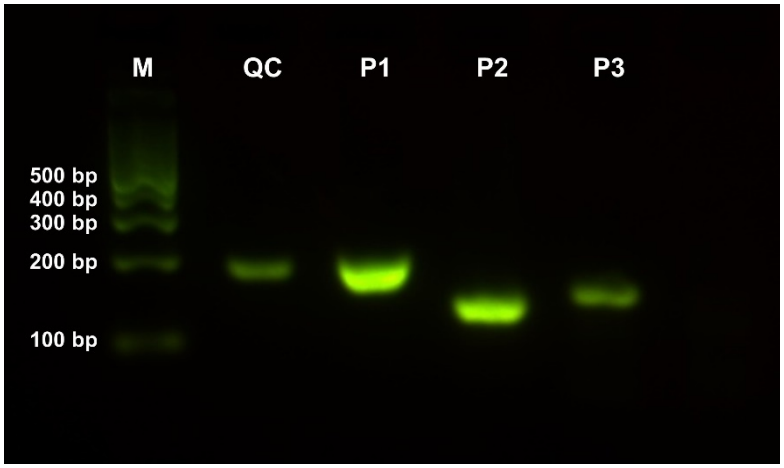

Figure S2 Agarose gel electrophoresis for RPA product detection. M represents DNA marker; QC represents quality Control; P1, P2 and P3 represents the amplification products of three different primer pairs.

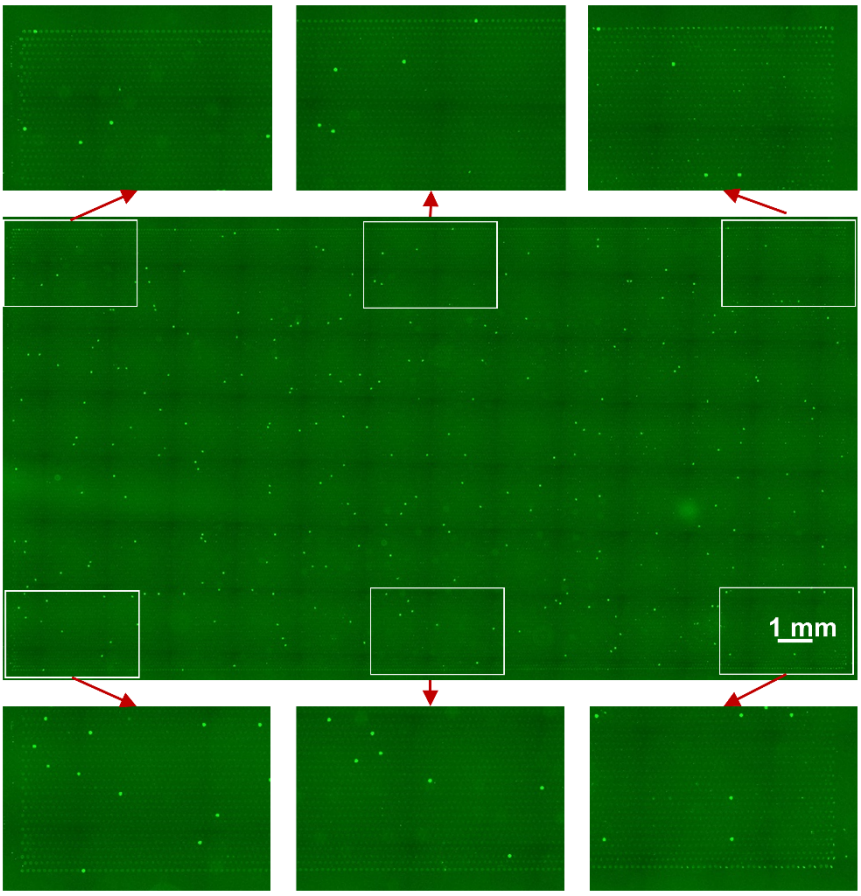

Figure S3 Fluorescence images of the chip at 37 ℃.

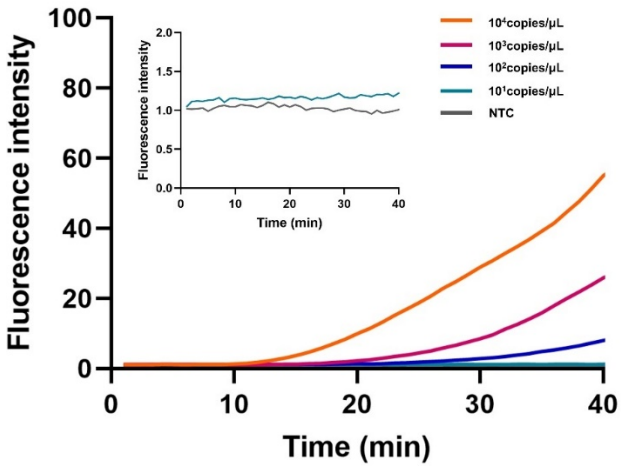

Figure S4 Real time fluorescence curve of the bulk RPA-CRISPR assay with serial dilutions of synthetic *rfbE* gene.

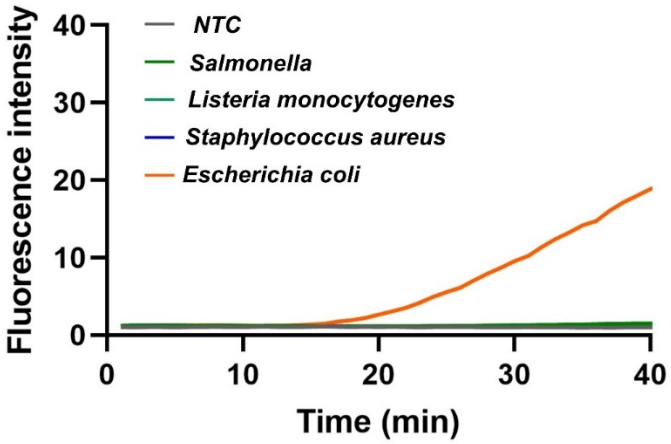

Figure S5 The specificity of bulk RPA-CRISPR assay.

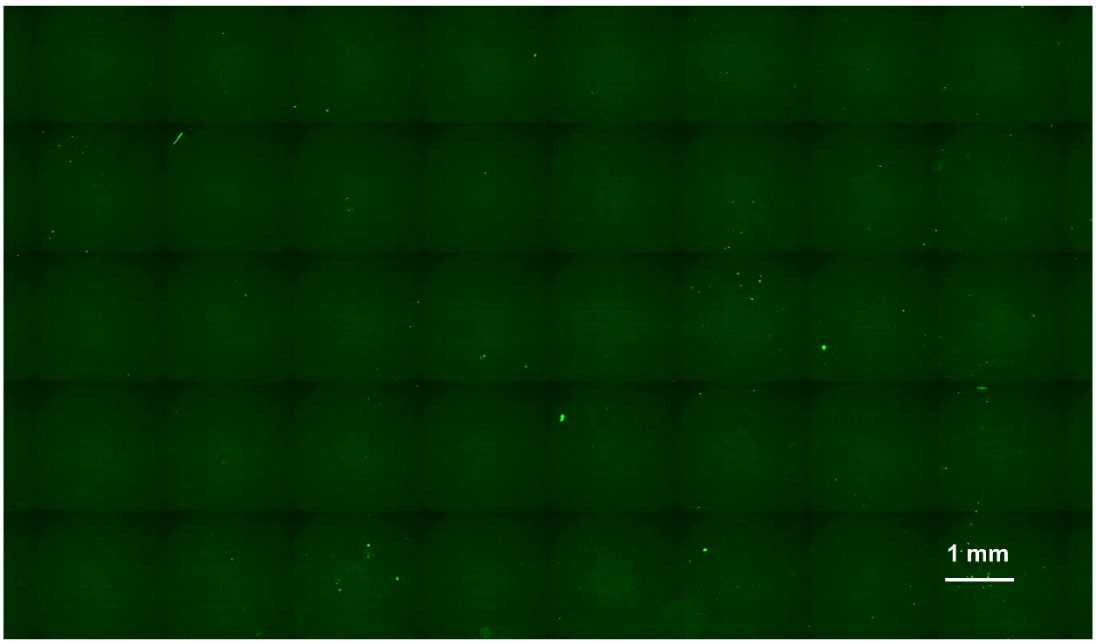

Figure S6 Fluorescence image captured at a concentration of 2.5×10^3 CFU/mL of *E coli*.

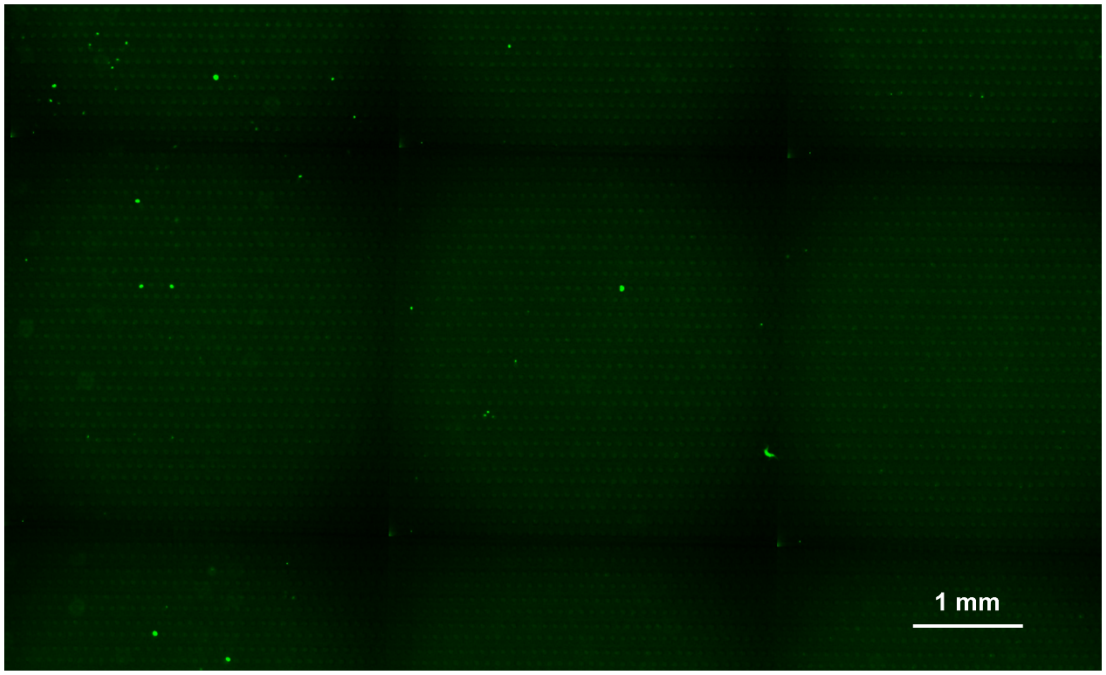

Figure S7 Fluorescence image captured at a concentration of 1×10^2 CFU/mL of *E. coli* O157:H7.

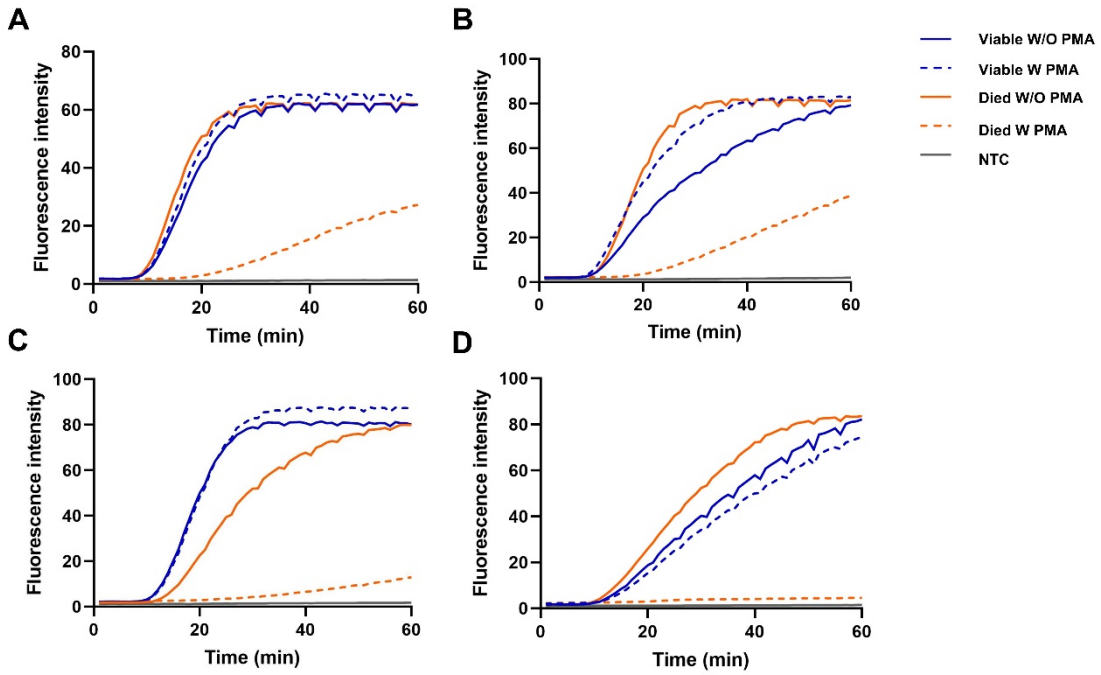

Figure S8 Real time fluorescence curves after treatment with different PMA concentrations. (A-D) 1,5,25 and 100 µM

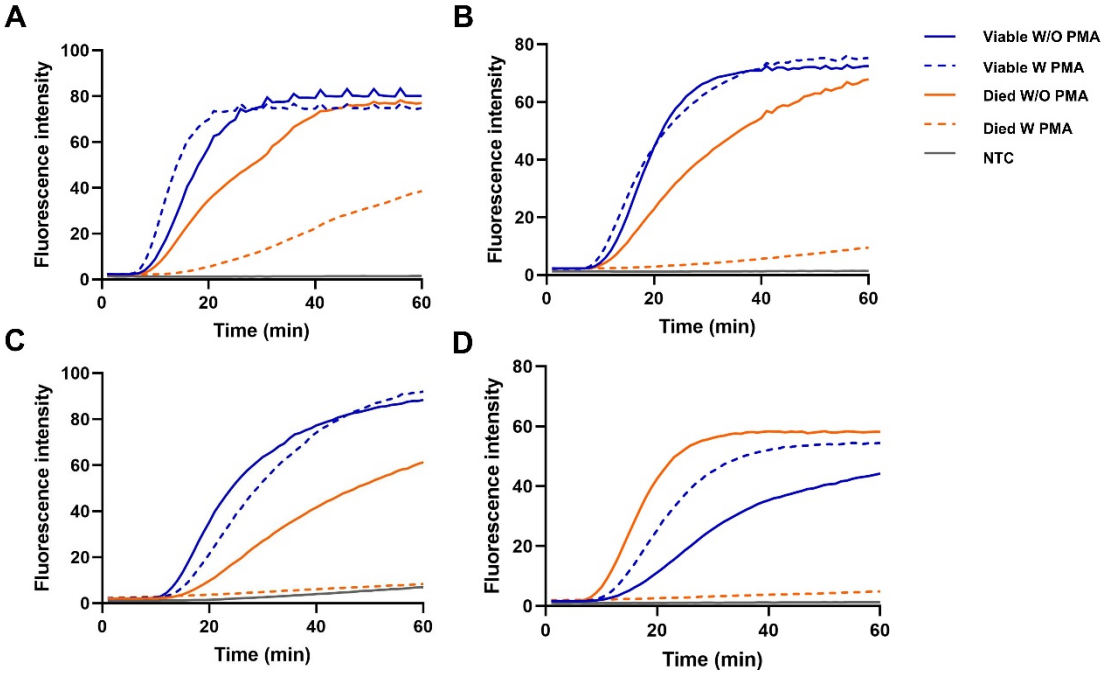

Figure S9 Real time fluorescence curves after processing with different exposure times. (A-D) 10,30,60 and 180s

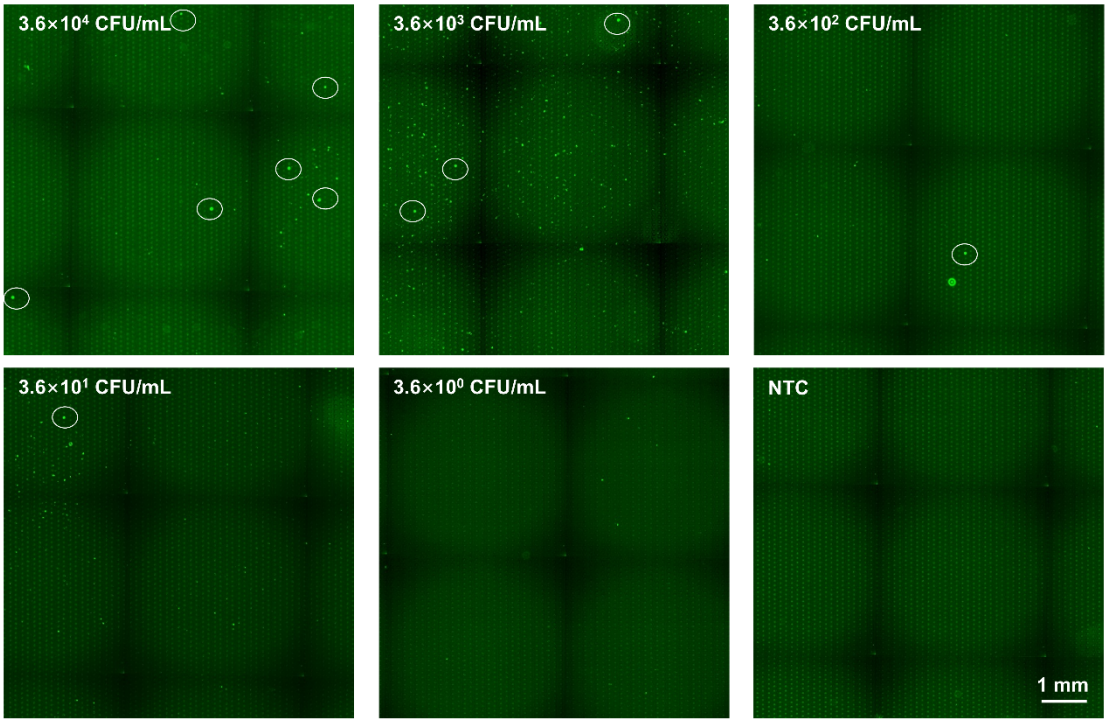

Figure S10 Fluorescence images of the MP-dCRISPR for *E. coli* O157:H7 detection at different concentrations

Table S1. The list of all used sequence in this study

Name	Sequence (5'-3')
rfbE gene	TCAACAGTCTTGTACAAGTCCACAAGGAAAGTAAAGATGTTTTTCACACTT ATTGGATGGTCTCAATTCTAACTAGGACCGCAGAGGAAAGAGAGGAATTA AGGAATCACCTTGCAGATAAACTCATCGAAACAAGGCCAGTTTTTTACCCT GTCCACACGATGCCAATGTACTCGGAAAAATATCAAAAGCACCCTATAGC TGAGGATCTTGGTTGGCGTGGAATTAATTTACCTAGTTTCCCCAGCCTATC GAATGAGCAAGTTATTTATATTTGTGAATCTATTAACGAATTTTATA
Forward primer1	GAATTAAGGAATCACCTTGCAGATAAACTCAT
Reverse primer1	ATTCACAAATATAAATAACTTGCTCATTCGATAG
Forward primer2	GATGCCAATGTACTCGGAAAAATATCAAAAG
Reverse primer2	TCGTTAATAGATTCACAAATATAAATAACTTGCT
Forward primer3	AAGGAAAGTAAAGATGTTTTTCACACTTATTG
Reverse primer3	TATAGGGTGCTTTTGATATTTTTCCGAGTACAT
crRNA	UAAUUUCUACUAAGUGUAGAUAGAUCCUCAGCUAUAGGGUG
Report probe	FAM-TTATT-BHQ1

Table S2. Comparison of currently reported digital CRISPR assays with the MP-dCRISPR assay

Assay name	NAA	CRISPR/Cas	Target	One-pot	Reaction time	Nuclear acid extraction	Ref.
MP-dCRISPR	RPA	Cas12a	*E. coli*	Yes	15 min	Extraction-free	This work
RADICA	RPA	Cas12a	SARS-CoV-2/EBV	Yes	40-60 min	Yes	1
deCOVID	RT-RPA	Cas12a	SARS-CoV-2	Yes	30 min	Yes	2
MEDICA	RPA	Cas13a	HPV16/HPV18	Yes	25 min	Yes	3
dWS-CRISPR	LAMP	Cas12a	SARS-CoV-2	Yes	90 min	Yes	4
DropCRISPR	LAMP	Cas12a	*Salmonella*	No	60 min	Extraction-free	5
3D-CRISPR assay	RPA	Cas12a	*Salmonella*	Yes	30 min	Extraction-free	6

References

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