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B cells Using Calcium Signaling for Specific and Rapid Detection of *Escherichia coli* O157:H7

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A rapid and sensitive detection technology is highly desirable for specific detection of *E. coli* O157:H7, one of the leading bacterial pathogens causing foodborne illness. In this study, we reported the rapid detection of *E. coli* O157:H7 by using calcium signaling of the B cell upon cellular membrane anchors anti-*E. coli* O157:H7 IgM. The binding of *E. coli* O157:H7 to the IgM on B cell surface activates the B cell receptor (BCR)-induced Ca^{2+} signaling pathway and results in the release of Ca^{2+} within seconds. The elevated intracellular Ca^{2+} triggers Fura-2, a fluorescent Ca^{2+} indicator, for reporting the presence of pathogens. The Fura-2 is transferred to B cells before detection. The study demonstrated that the developed B cell based biosensor was able to specifically detect *E. coli* O157:H7 at the low concentration within 10 min in pure culture samples. Finally, the B cell based biosensor was used for the detection of *E. coli* O157:H7 in ground beef samples. With its short detection time and high sensitivity at the low concentration of the target bacteria, this B cell biosensor shows promise in future application of the high throughput and rapid food detection, biosafety and environmental monitoring.

48 3,000 ,
78 [1 3]. I
U S [4]. I , *Cam l bac e jej ni*, *Li e ia m n c gene*, *Salm nella en e ica*, *E. c li* O157:H7 - *E. c li* (-O157 STEC), *Vib i* . [5]. *E. c li* O157:H7
, , , , , ,
(38%)[6]. ,
-
C - (CBB) [7].
CBB [8,9], [10] [11 15].
. B , , , ,
CBB , B , , T , [16 24]. A , B
-
BCR (B C R) T [25]. M B
(P -2E9) *Li e ia*, O
Bacill [13,25,26]. R *e al.*[11] B

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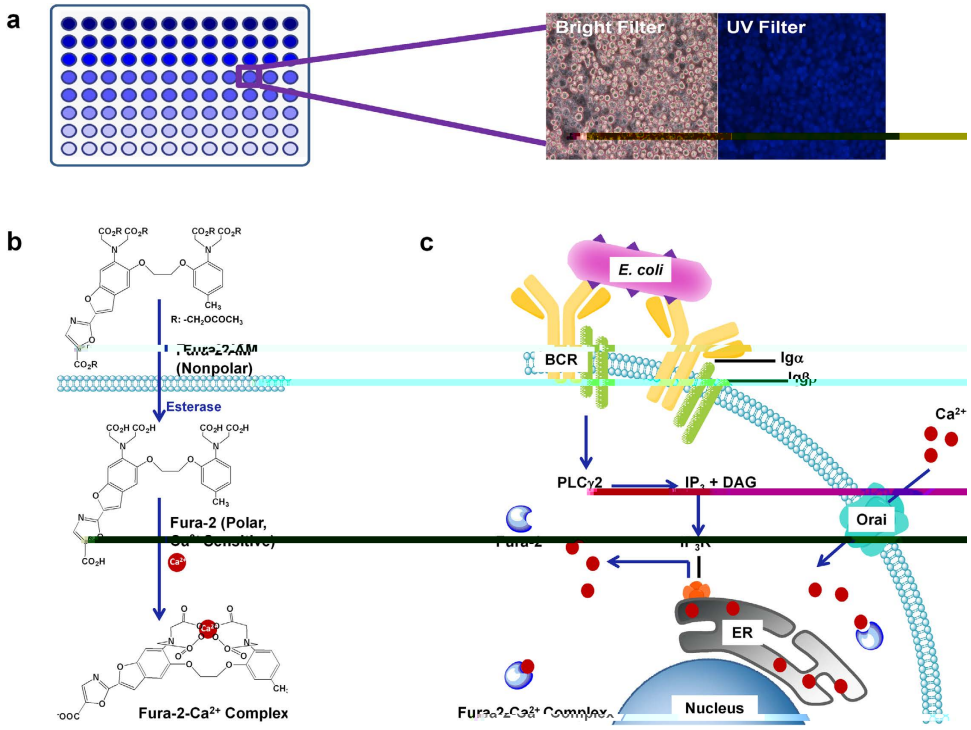

Figure 1. P B . (**a**). B . (**b**). S F -2 C 2+ . F -2 AM C 2+ . O , AM F -2- (C 2+ ,) , C 2+. (**c**). B . B , - B (BCR) , , C 2+ . F -2 340/380 .

5 [11]. B *E. c li* O157:H7 500 CFU/ (<1) [27,28], . O B BCR C 2+ , B [29]. A - . F -2 (C 2+) [30,31]. (340:380) C 2+ [32,33]. I [34 36]. [37 39]. H , , F -2 B . I B F -2 B *E. c li* O157:H7 . C 2+- , F -2 BCR- C 2+ B . C 2+- F -2 [31]. I F -2 C 2+. F -2 340 C 2+ 380 C 2+. I 340/380 C 2+[30]. *E. c li* O157:H7 .

Results

Principles of the B cell biosensor. C F -2 C 2+ B , C 2+- B *E. c li* O157:H7 . A F . 1 , F -2 B 96- , - (340:380) . F -2 (AM)

C 2+ . O , AM F -2- (C 2+ ,) C 2+ (F . 1). B *E. c li* O157: H7. BCR- C 2+ , B BCR- C 2+ [29]. A F . 1 , B BCR[25]. BCR - (I) I α I β. C BCR [40]. BCR- PLC-γ2 4,5- (PIP_2) 1,4,5- (IP_3) (DAG). IP_3 IP_3 (IP_3R), (ER) [41]. ER C 2+ , O , - (CRAC), CRAC . , C 2+ C 2+ ER C 2+ [29,42,43]. IP_3 - , C 2+ , - [44 46].

Optimization of the B cell biosensor. B , C 2+ . MARC 29F8 (MA) LPS *E. c li* O157:H7[47]. U , B ELISA (E -). ELISA S I . ELISA (S F . S1) B (12 B . I - 5 12) B B . *E. c li* O157:H7 B (SEM). R SEM F . 2 . F -2 (*FR*) 1 . A 10^3 CFU/ L *E. c li* O157:H7 , 0 . A F . 2 , *FR* *E. c li* O157:H7, (HBSS), . I , C 2+- (Ca_{min}) C 2+- (Ca_{ma}) . R *FR C n l* Ca_{min} Ca_{ma} , . F -2 *FR C n l* (F . 2) - 0 60 . F -2 , [32]. T C 2+ , C 2+ C 2+ - B *E. c li* O157:H7 (10^1-10^9 CFU/ L). P - , F . 2 . I NF (F -2), B F -2, . NF6 (F -2, 10^6 / L) NF5 (F -2, 10^5 / L) (< 0.0001). I NC (C 2+), B F -2 C 2+ M 2+- . (> 0.05) NF5 (F -2, 10^5 / L) NF6. I C (C 2+), B F -2 C 2+ M 2+ . C6 (C 2+, 10^6 / L) C5 (C 2+, 10^5 / L) (= 0.0025). S F -2 10^6 / L B , 10^6 / L B . F -2 NF6 NC6 (C 2+, 10^6 / L) (= 0.0324) C6 (C 2+, 10^6 / L) (< 0.0001). A C 2+ (= 0.0006) NC6 C 2+ M 2+- C6, C 2+ M 2+ .

Detection of *E. coli* O157:H7 in pure culture. B *E. c li* O157:H7 10^1 10^5 CFU/ L . HBSS *E. c li* O157:H7. C , *E. c li* O157:H7 10^2-10^5 CFU/ L. A F . 3 - 10^1 10^3 CFU/ L. A , - =0.0565 + 0.6753 (R^2 = 0.96). LPS B (10^1-10^7 CFU/ L), LPS (F . 3). A , =0.03183 + 0.6532 (R^2 = 0.83).

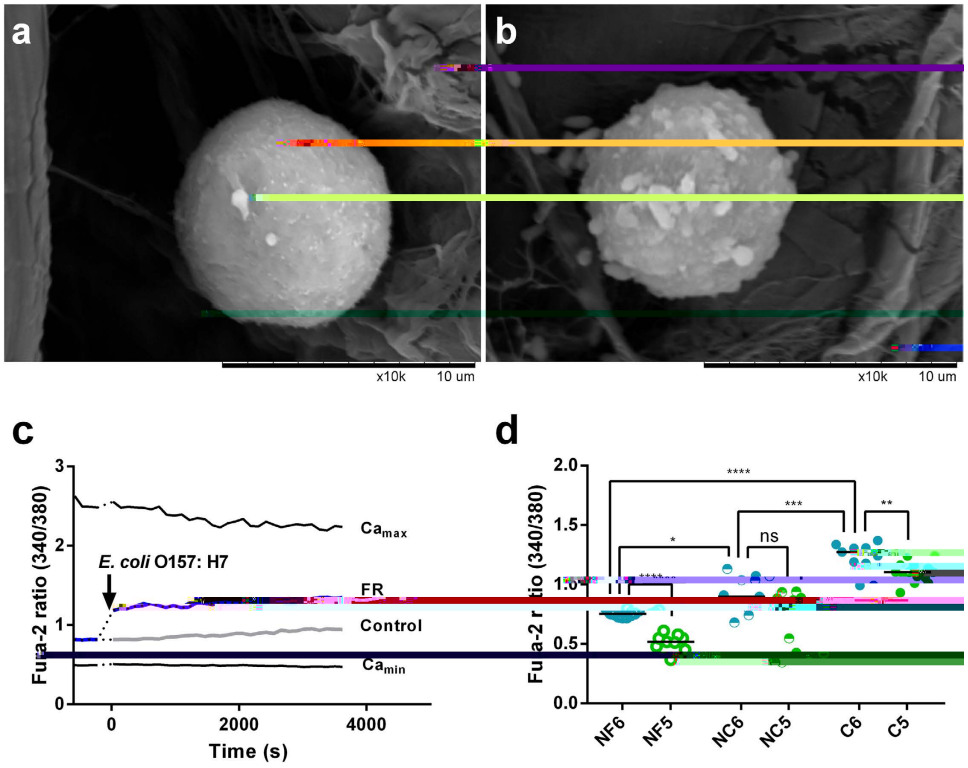

Figure 2. O B . (**a**). SEM B (×10000). (**b**). SEM B *E.c li* O157:H7 (×10000). (**c**). R F -2 340/380 . (**d**). C F -2 . D *E. c li* O157:H7 (10^1-10^9 CFU/ L) . B - . (- , > 0.05, * < 0.05, ** < 0.01, *** < 0.001, **** < 0.0001, = 9) NF6: B (F -2, C $^{2+}$ M $^{2+}$ HBSS), 10^6 / L. NF5: B (F -2, C $^{2+}$ M $^{2+}$ HBSS), 10^5 / L. NC6: B (F -2, C $^{2+}$ M $^{2+}$- HBSS), 10^6 / L. NC5: B (F -2, C $^{2+}$ M $^{2+}$- HBSS), 10^5 / L. C6: B (F -2, C $^{2+}$ M $^{2+}$ HBSS), 10^6 / L. C5: B (F -2, C $^{2+}$ M $^{2+}$ HBSS), 10^5 / L.

S *E. c li* O157:H7 , B - E *E. c li* 2 (O157 -O157) , EHEC (-O157), . E 7 6 - (F . 3). - S T S5.

Li e ia m n c gene, *S.* T *V. a ahaem l ic* - , B (MA) LPS *E. c li* O157 N *Salm nella* . B (L , NH, USA) - I M (C : BE0087) B MARC 29F8. M 29F8 LPS 12- 16- D - LPS[47]. *L. m n c gene V. a ahaem l ic* G - G - A *S.* T , O- . N *Salm nella S. g de be g* - I ( ). *S.* T D (I: ,)[48].

R O C (ROC) (F . 3). F -2 *E. c li* O157:H7 ATCC 43888 - 30 , . ROC . ROC (AUR) 0.7319, 0.7690, 0.8484, 0.7817 0.7885, *E. c li* O157:H7 10^1, 10^2, 10^3,10^4 10^5 CFU/ L. A AUR *E. c li* O157:H7 0.7

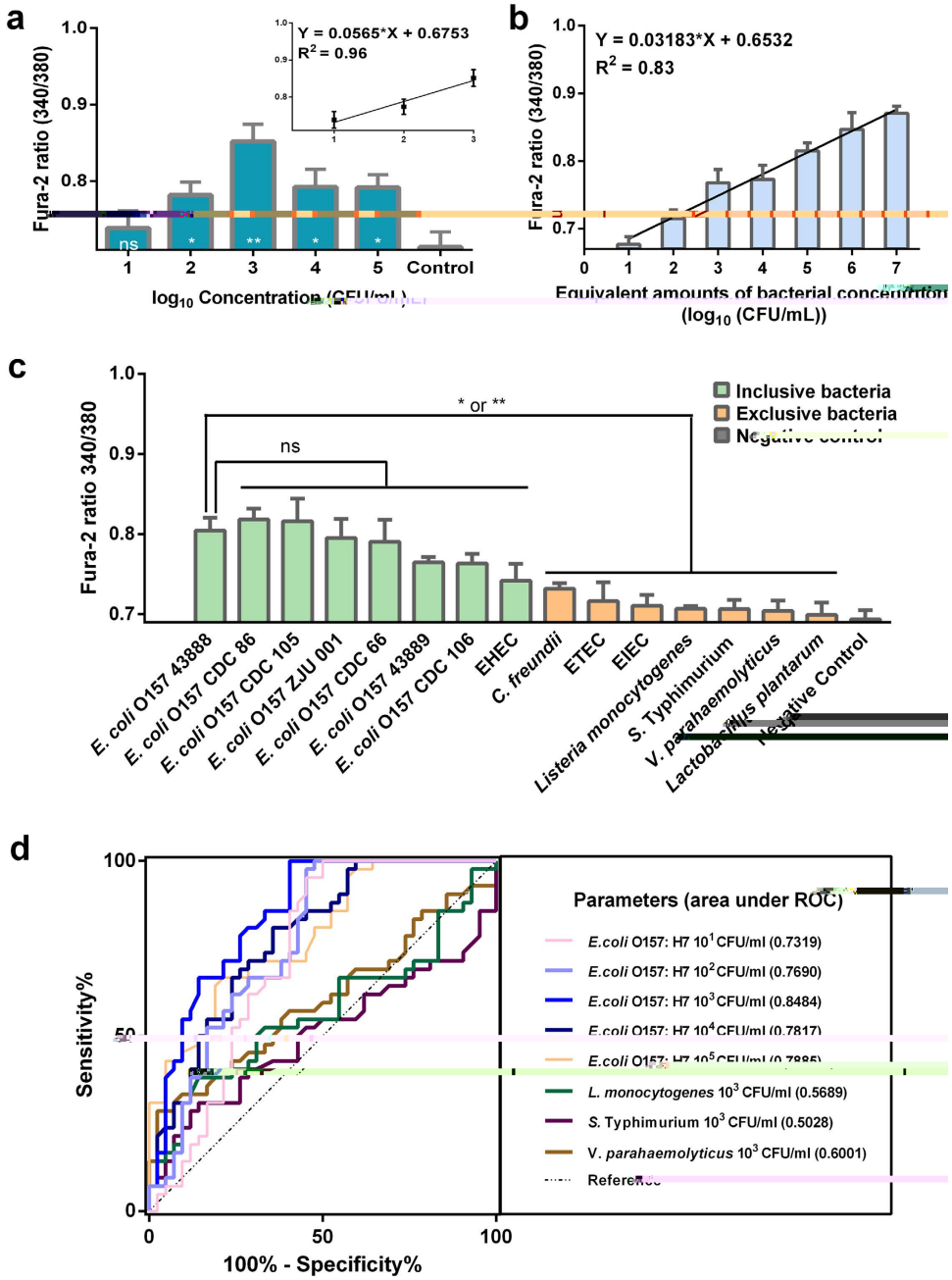

Figure 3. D *E. c li* O157:H7 . (**a**). P ' , F -2 , *E. c li* O157:H7 . I : L *E. c li* O157:H7 10^1 10^3 CFU/ L. A ± . . . - . (- , >0.05, * <0.05, ** <0.01, =4). (**b**). P ' , F -2 , LPS *E. c li* O157:H7 10^1 10^7 CFU/ L. A ± . . . - . (- , >0.05, * <0.05, ** <0.01, =3). (**c**). I B ' - 10^3 CFU/ L. A ± . . . - . (- , >0.05, * <0.05, ** <0.01, =3). (**d**). ROC 30 .

0.7319 0.8484, B . I AUR 0.7817 0.7885 *E. c li* O157:H7 10^4 CFU/ L 10^5 CFU/ L, 10^3 CFU/ L *L. m n c gene*, *S.* T , *V. a ahaem l ic* , AUR 0.5689, 0.5028 0.6001,

Detection of *E. coli*

I *E. c li* O157:H7 , B
E. c li O157:H7 (10^1-10^3 CFU/ L). *E. c li* O157:H7 10^4 CFU/ L 10^5 CFU/ L, *E. c li* B -[50], BCR- C $^{2+}$. T ,
LPS *E. c li* O157:H7 B . A F . 3 ,
LPS (10^1-10^7 CFU/ L). I
B - T [51].

I *E. c li* O157:H7 , -
B .
O MA B O- LPS G - , EHEC (-O157) G -
L. m n c gene Lac bacill lan a m, I F . 3 ,
- (-) -
(S T S5), , *E. c li* O157:H7

B ROC F . 3 , B
10
10 30 . S
E. c li O157:H7 10^1 CFU/ L,
E. c li O157:H7 10^2-10^5 CFU/ L.
F , B 5.9×10^2
CFU/ L. A 10 . I
10^2 10^4 CFU/ L,
(SPR)[52], - (ELISA)[53], [54]
(QCM)[55]. *E. c li* O157:H7 7 CFU
- (SPCE) 70 [56], 10 CFU/ L
- - (LRSP-FS) 40 [57].
, 67 CFU/ L
- (MNP) 8 [58], 500 CFU/ CANAR ( )
) 5 [11]. I 30μL
, B 18 *E. c li* O157:H7. I ,
B L.
,
, .
F , B 8.6×10^2
CFU/ L (26 CFU 30μL) *E. c li* O157:H7 .
A , -
. I
B .
B C $^{2+}$ F -2
C $^{2+}$ - B *E. c li* O157:H7 .
B ‘ ’
. ,
, F -2.
T , B ,
.
I B MARC 29 8 C $^{2+}$ - F -2
. B *E. c li* O157:H7 ,
C $^{2+}$ BCR C $^{2+}$
. B C $^{2+}$ F -2
B .
B
10^2 CFU/ L 10 .
B *E. c li* O157:H7
B , -
.

Methods

Reagents. D , E , (DMEM), D , E , (DMEM PR), - (FBS), H , (HBSS), C $^{2+}$ M $^{2+}$- HBSS, MEM - (MEM NAA), 0.4% T B S , F -2/AM 0.5 M EDTA L T (C , CA, USA). A BD (S , MD, USA). G I M-HRP ( ), L (LPS) A D (A D S , USA), I B (G - , K) S B (S , C), A S (S . L , MO, USA). A - (DI) M D -8 (B , MA, USA).

B cell lines and culture conditions. B MARC 29F8 ATCC (A T C C , M , A; ATCC CRL-2508). B , B , I M. I DMEM 4 M L- 1.5 /L , 4.5 /L , 1 % MEM NAA 10% - FBS. 37 C 7% CO_2. L - - MARC 29F8 (5 10) 1:10 72 . MARC 29F8 DMEM 10% - FBS T-25 T-75 (F , O , USA) (72) . TC10 A C C (B -R , H , CA, USA) , .

Bacterial strains and culture conditions. B A T C (ATCC), C C I C C (CICC), C N C M C C (CMCC), C C D C P (P CDC) U (JU). S T S1 T S2.

Lac bacill lan a m MRS BHI 37 C, - *Li ia m n c gene* 48 24 , - T S A (TSA). S I .

Scanning electron microscopy (SEM) imaging SEM (S I) H TM1000 (SEM) (H , J).

Preparation of a B cell biosensor. B B C $^{2+}$ - F -2, C $^{2+}$ F -2[33]. I , C $^{2+}$, - MARC 29F8 DMEM PR 37 C 5 . B HBSS DMEM (PR) . 4.5 F -2/AM 10^6 30 37 C 30 L HBSS . A 15 - F -2/AM, , 30 L HBSS C $^{2+}$ M $^{2+}$- HBSS .

Detection of *E. coli* O157:H7 in pure culture. B 30 μ F -2 MARC 29F8 B 96- , 30 μ . T , (340 380) F -2 MARC 29F8 0.1% T -100 4.5 M - (EDTA), F 340- 380- (F340 F380) 510 S H1 H M -M M (B T , , T, USA). D 120 . D 340/380 C $^{2+30}$. *E. c li* O157:H7 ATCC 43888 HBSS 6.2×10^1 6.2×10^9 5.9×10^1 5.9×10^5 , E LPS *E. c li* O157:H7 ATCC 43888 B . S I .

T 8 7 10^3 CFU/ L , (S T S1 T S2). *E. c li* O157:H7 ATCC 43888 HBSS F -2 B

Detection of *E. coli* O157:H7 in ground beef. T B , - . , *E. c li* O157:H7 - . T - 225 HBSS F (L I ., Q , C) S 400 (S , N , UK) 1 . . O *E. c li* O157:H7 10^3, 10^4, 10^5 CFU/ L 9 , *E. c li* O157:H7 10^2, 10^3, 10^4 CFU/ L. *E. c li* O157:H7 HBSS . F -2 B .

Statistical analysis. D ± . . ( ) - . G P P (G P , S D , CA). P - .

Optimization of the B cell biosensor *E. c li* O157: H7 U - . < 0.05 .

References

1. S , E. & M , B. E. F D A S N (F N) 2012: U S . *Clin. Infec . Di .* **54** S 5, S381 384 (2012).
2. S , E., G , P. M., A , F. J., T , . . & H , . M. F U S . *Eme g. Infec . Di .* **17,** 16 22 (2011).
3. S , E. *e al.* F U S . *Eme g. Infec . Di .* **17,** 7 15 (2011).
4. S , . L. E U S . *J. F d P .* **75,** 123 131 (2012).
5. A -C , T. *e al.* D - DNA . *Talan a* **101,** 405 412 (2012).
6. S M. C *e al.* I - F , 10 U.S. S , 2006 2013. *M b. M al. Wee l e .* **63,** 328 332 (2014).
7. A , P., S , A., D , N. & C A. B . *Bi en . Bi elec n.* **28,** 1 12 (2011).
8. , D. M., , A. J. & T , A. P. - . *Bi en* **4,** 299 311 (1989).
9. N , T. *e al.* G *fabA fab* : *Anal. Chem.* **78,** 4952 4956 (2006).
10. F , T. *e al.* L . *Bi en . Bi elec n.* **21,** 2263 2269 (2006).
11. , T. H. *e al.* A B - . *Science* **301,** 213 215 (2003).
12. M . M., , ., B , L. G. & A , . D - - . *Anal. Chem.* **76,** 4156 4161 (2004).
13. M , *e al.* C , - . *Lab. In e .* **85,** 1429 1439 (2005).
14. L , Q. *e al.* D - . *Bi en . Bi elec n.* **22,** 3224 3229 (2007).
15. B , P., L , D., , J. P., , J. L. & B , A. . A - - B- . *Lab. In e .* **88,** 196 206 (2008).
16. B , P. & B , A. . M - . *T end Bi echn l.* **27,** 179 188 (2009).
17. C , L.-E., C , S., , . & , M. P . *Elec chim. Ac a* **53,** 6690 6697 (2008).
18. E , A. ., M , T. H. & P , P. S. B . *Ad . A l. Mic bi l.* **64,** 137 166 (2008).
19. H , T. C : C . *Anal. Chim. Ac a* **568,** 211 216 (2006).
20. , S. E. C - . *F n . D g De . Di c e* **2,** 225 239 (2005).
21. , G. T. E . *P. IEEE* **91,** 915 929 (2003).
22. , S. M. *e al.* A *Li e ia m n c gene* : - HT-29 . *In . J. F d Mic bi l.* **68,** 33 44 (2001).
23. C , T., N , . M. ., B , C., T , J. & H , D. D - . *Bi en . Bi elec n.* **23,** 1024 1031 (2008).
24. L , B., L , P. & C , B. T. A - - . *Sen . Ac a . B-Chem.* **114,** 559 564 (2006).
25. S , A. *e al.* L BC . *Imm ni* **17,** 451 462 (2002).
26. B , A. & , D. A *Li e ia . Le . A l. Mic bi l.* **26,** 305 310 (1998).
27. , T. CANA , . *Bi . Mic . and Nan em C nfe ence: BMN' 06,* S F . N , USA: IEEE. (2006, J . 15-18)
28. P , M. S. *e al.* - . *Linc ln Lab. J.* **17,** 63 84 (2007).
29. , L. B. & F , B. D. B- . *Imm n l. e .* **231,** 265 277 (2009).
30. G , G., P , M. & T , . . A C $^{2+}$. *J. Bi l. Chem.* **260,** 3440 3450 (1985).
31. N , E. -2 C C . *Ne ha mac l g* **34,** 1423 1442 (1995).
32. M , A., M , D., M , J. & P , T. F -2 C $^{2+}$. *J. Cell Bi l.* **105,** 2145 2155 (1987).

33. H , . A., H , C., H , . & L , D. G. *Calci m Signaling P c l* 2 , **Vol. 312** (. L , D.G.) C . 2, 37 45 (H P , 2006).
34. P , A. B. & P J. S - . *Ph i l. e .* **85,** 757 810 (2005).
35. , U. B. & S , . C - . *Ph i l. e .* **82,** 769 824 (2002).
36. S , D., L , . & E , T. P P2 . *F n . Cell. Ne ci.* **8,** 1 18 (2014).
37. H , F., I , . & S , B. C $^{2+}$ - C $^{2+}$. *Bi h . J.* **70,** 1069 1081 (1996).
38. N , J., M , M., E -D , G. C. & , H. S - NMDA - C $^{2+}$. *Ne n* **46,** 609 622 (2005).
39. G , C. & , A. I . *Ne n* **73,** 862 885 (2012).
40. H , J., T , T., L , L., S , H. & , M. M B- I M . *Na e* **343,** 760 762 (1990).
41. , . *e al.* BAN BC - IP$_3$. *EMBO J.* **21,** 83 92 (2002).
42. P , M. *e al.* O 1 C AC . *Na e* **443,** 230 233 (2006).
43. F , S. & P , M. C STIM1 . *Na . S c . M l. Bi l.* **20,** 918 919 (2013).
44. O - , M. & , A. C . *C . O in. Imm n l.* **20,** 250 258 (2008).
45. F , S. C . *Na . e . Imm n l.* **7,** 690 702 (2007).
46. , S. G. & B , . S. - C . *J. Bi l. Chem.* **272,** 15045 15048 (1997).
47. , . B., H , ., , J. E., L , E. T. & , J. P *E che ichia c li* O157. *J. Clin. Mic bi l.* **35,** 679 684 (1997).
48. L , O., S , A. & , O. I O S E . *Bac e i l. e .* **30,** 192 255 (1966).
49. , C. A. *e al.* I CD20 B C . *J. Bi l. Chem.* **283,** 16971 16984 (2008).
50. A , A. O. *e al.* C - -2. *Science* **285,** 736 739 (1999).
51. F , S. *e al.* B . *Science* **312,** 738 741 (2006).
52. O , B.- ., , .- ., B , ., L , & C , J.- D *E che ichia c li* O157: H7 . *J. Mic bi l. Bi echn l.* **12,** 780 786 (2002).
53. , N., H , M. & S , H.-C. N - (ELISA) T *E. c li* . *Anal. Chim. Ac a* **590,** 224 231 (2007).
54. G , T., U , J., T , S.-I. & B , A. . F - A -F *E che ichia c li* O157: H7 . *Sen* **6,** 796 807 (2006).
55. S , .-L. & L , . A - *E che ichia c li* O157: H7. *Bi en . Bi elec n.* **19,** 563 574 (2004).
56. S , E. B. & A , E. C. - *E che ichia c li* O157: H7. *Bi en . Bi elec n.* **26,** 2208 2214 (2011).
57. H , C.-J., D , J., S , A. & , L - - *E. c li* O157: H7. *Anal. Chem.* **83,** 674 677 (2011).
58. L , . *e al.* N *E. c li* O157: H7. *IEEE T an . Nan echn l.* **11,** 676 681 (2012).

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Author Contributions

L. R. . R. . L. . B.- K., S. J., K. . F. - . L. .

Additional Information

Supplementary information ://

Competing financial interests: .

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