Supporting Information

Library screening of cell-penetrating peptide for BY-2 cells, leaves of Arabidopsis, tobacco, tomato, poplar, and rice callus

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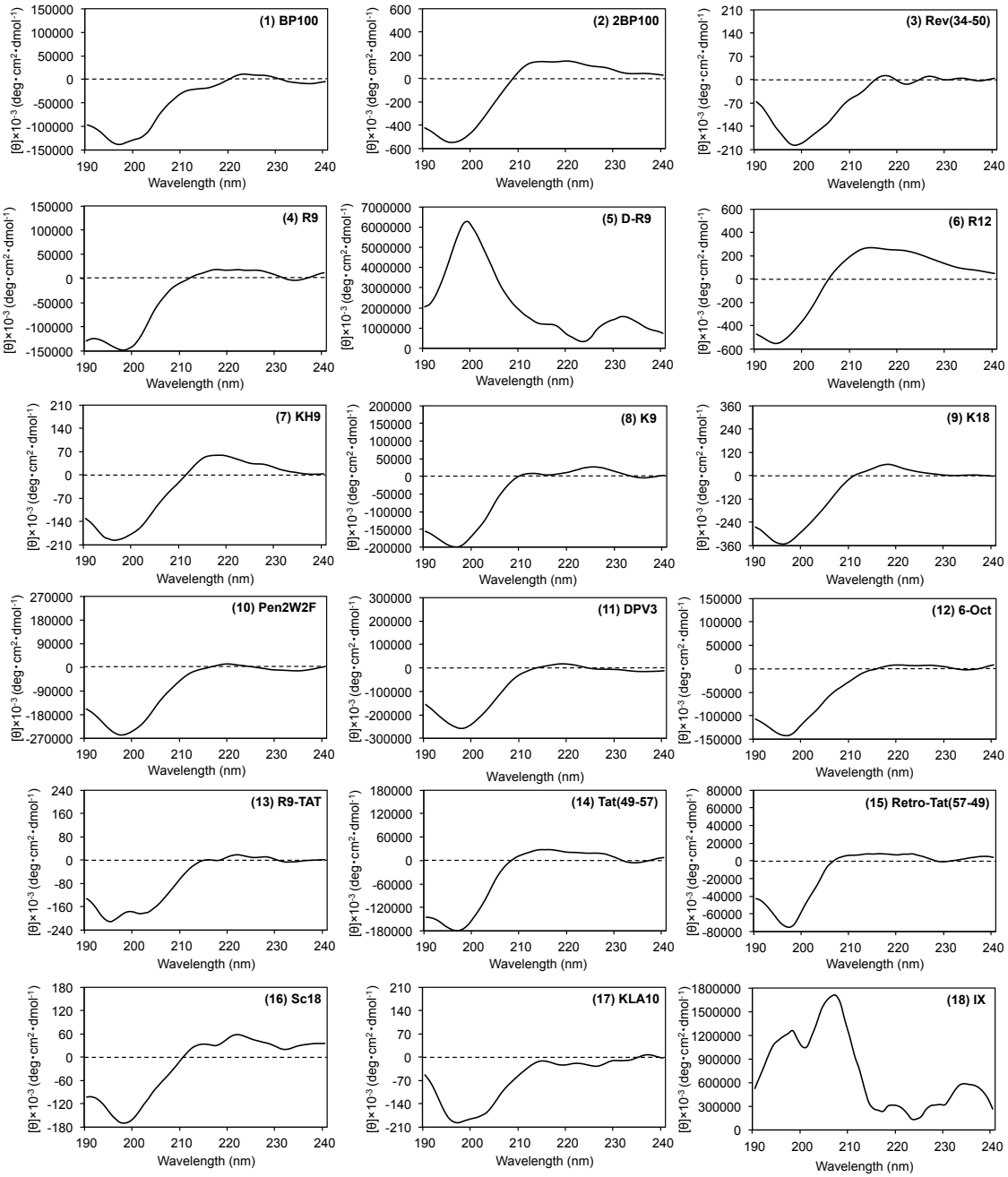
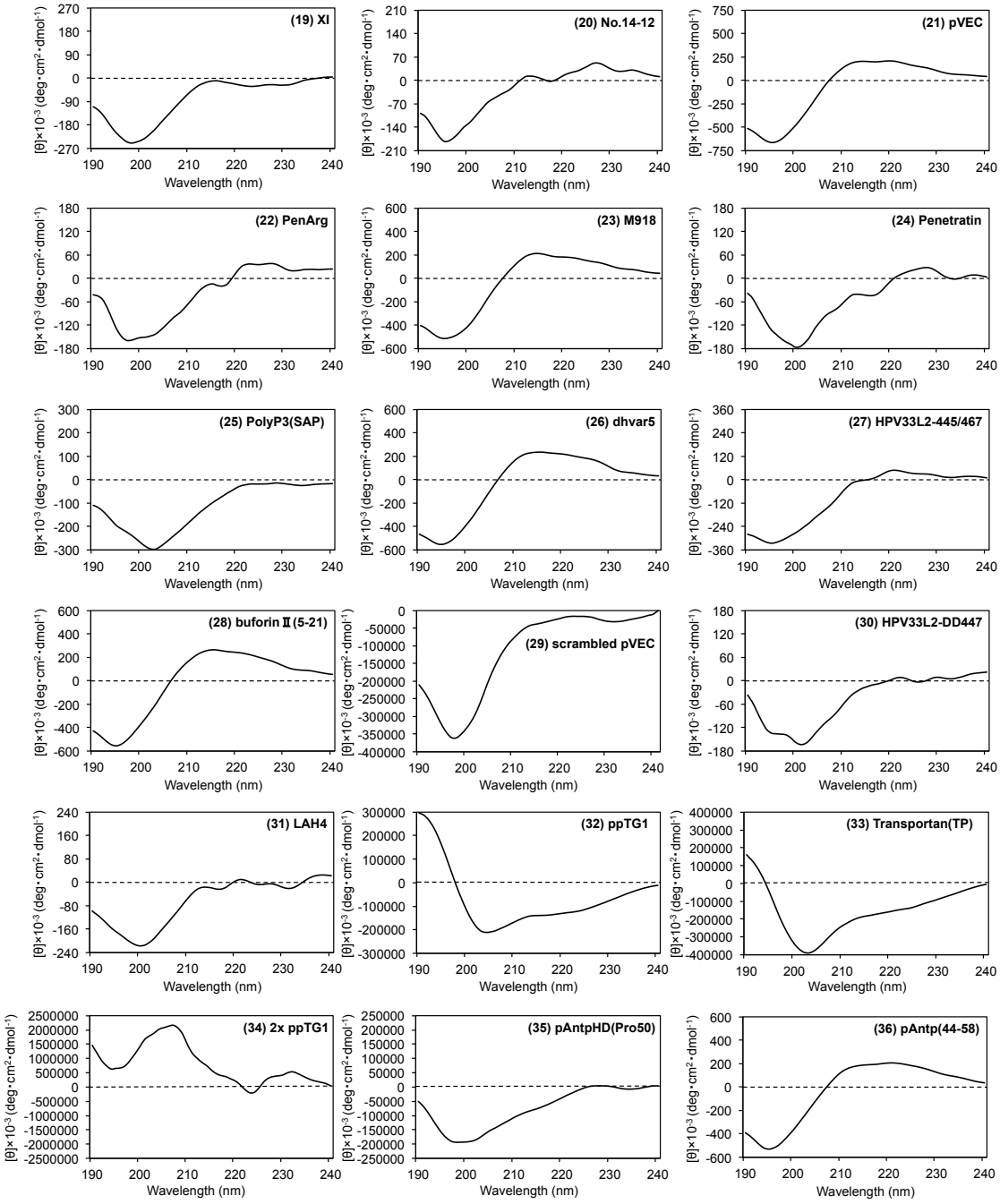
[2]Graduate School of Biological Sciences, Nara Institute of Science and Technology, Ikoma, Nara 630-0192, Japan.


(1) BP100
(2) 2BP100
(3) Rev(34-50)
(4) R9
(5) D-R9
(6) R12
(7) KH9
(8) K9
(9) K18
(10) Pen2W2F
(11) DPV3
(12) 6-Oct
(13) R9-TAT
(14) Tat(49-57)
(15) Retro-Tat(57-49)
(16) Sc18
(17) KLA10
(18) IX
[θ]×10-3 (deg·cm2·dmol-1)
Wavelength (nm)


(19) XI
(20) No.14-12
(21) pVEC
(22) PenArg
(23) M918
(24) Penetratin
(25) PolyP3(SAP)
(26) dhvar5
(27) HPV33L2-445/467
(28) buforin Ⅱ(5-21)
(29) scrambled pVEC
(30) HPV33L2-DD447
(31) LAH4
(32) ppTG1
(33) Transportan(TP)
(34) 2x ppTG1
(35) pAntpHD(Pro50)
(36) pAntp(44-58)
[θ]×10-3 (deg·cm2·dmol-1)
Wavelength (nm)

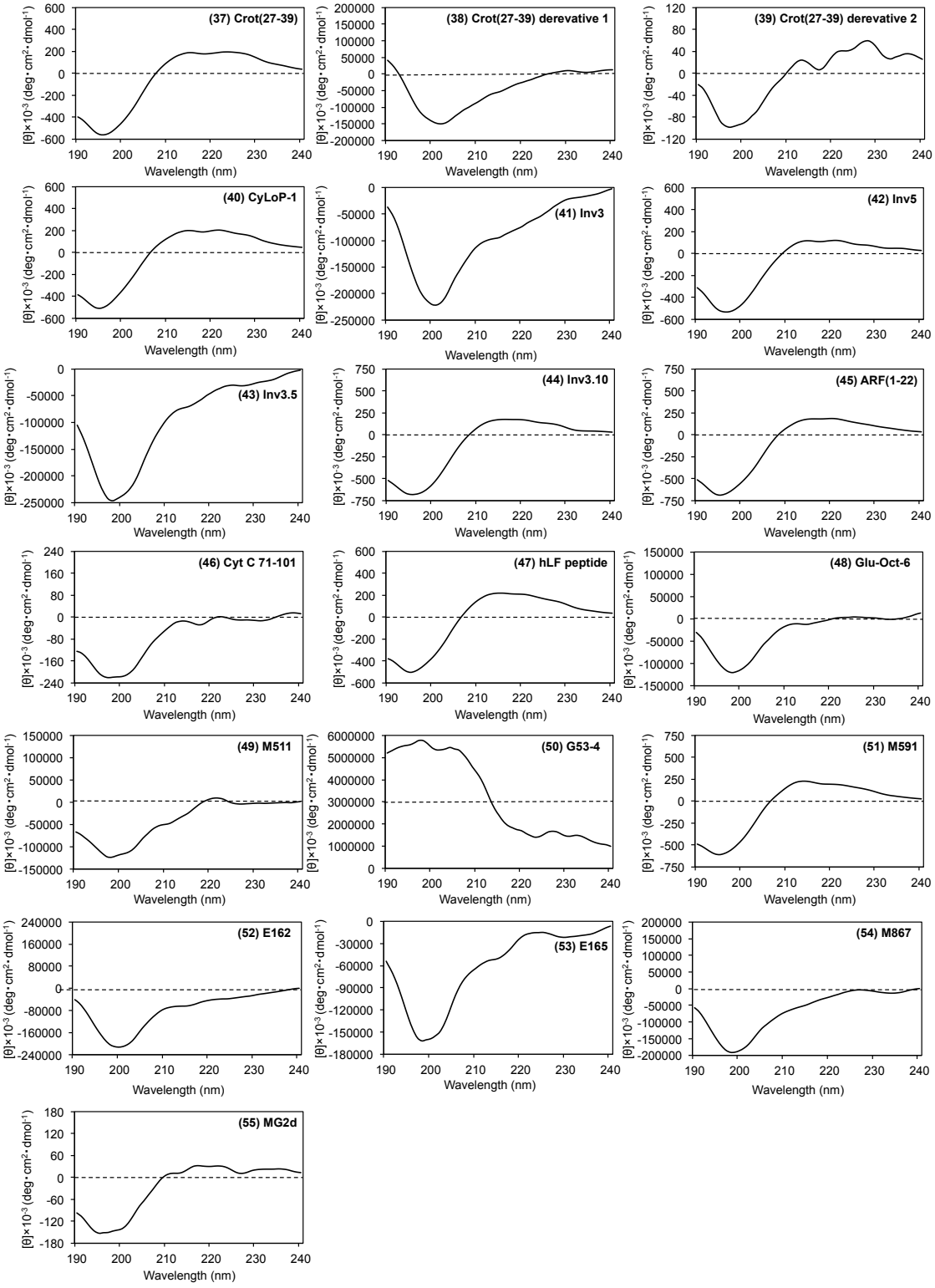

(37) Crot(27-39)
(38) Crot(27-39) derevative 1
(39) Crot(27-39) derevative 2
(40) CyLoP-1
(41) Inv3
(42) Inv5
(43) Inv3.5
(44) Inv3.10
(45) ARF(1-22)
(46) Cyt C 71-101
(47) hLF peptide
(48) Glu-Oct-6
(49) M511
(50) G53-4
(51) M591
(52) E162
(53) E165
(54) M867
(55) MG2d
[θ]×10-3 (deg·cm2·dmol-1)
Wavelength (nm)

Figure S1. CD spectra of 55 types of CPP. The type of CPP is shown by peptide number and name as listed in Table 1.

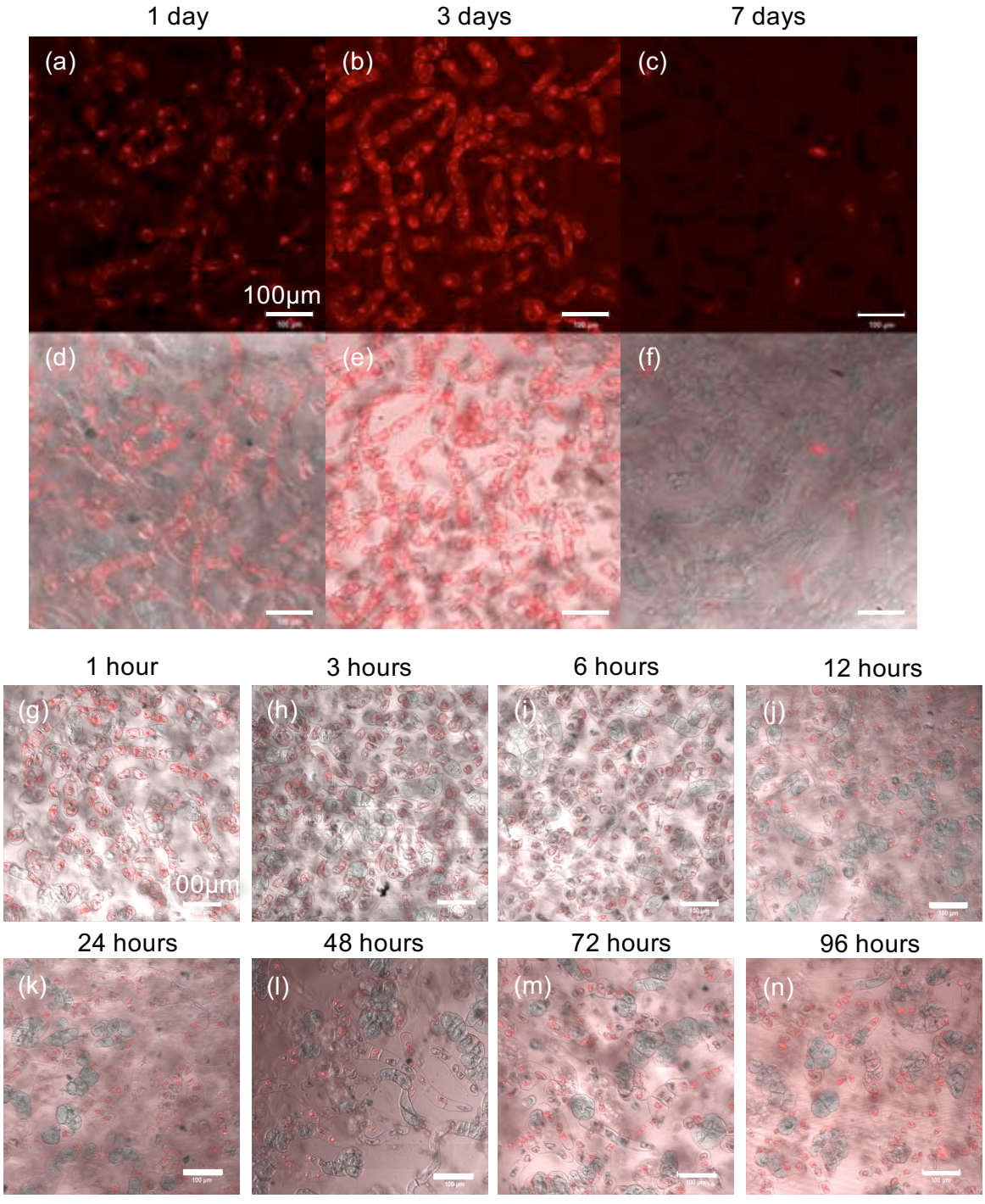
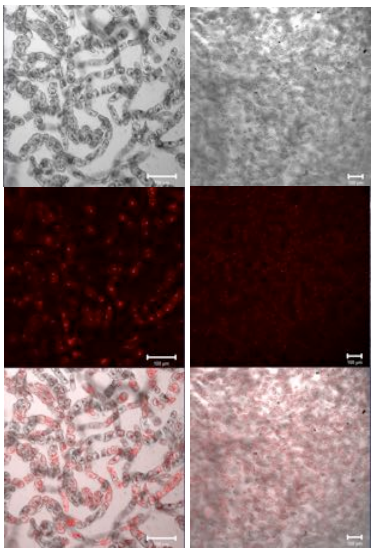
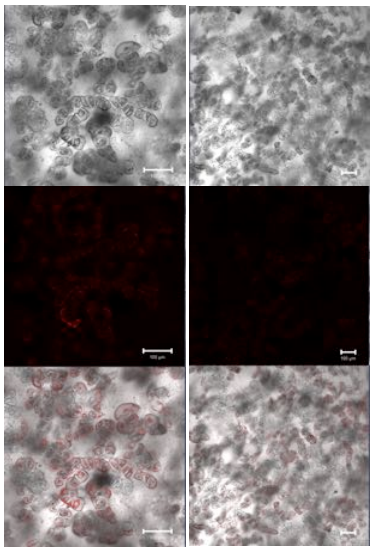


Figure S2. Effects of BY-2 growth phase on the cell penetrating efficiency of BP100 (a-f). BY-2 cells at 1-day (a,d), 3-day (b,e) and 7-day (c,f) after subculture were incubated with TAMRA-labelled BP100 for 3 h at 26ºC. (a-c) TAMRA fluorescence image and (d-

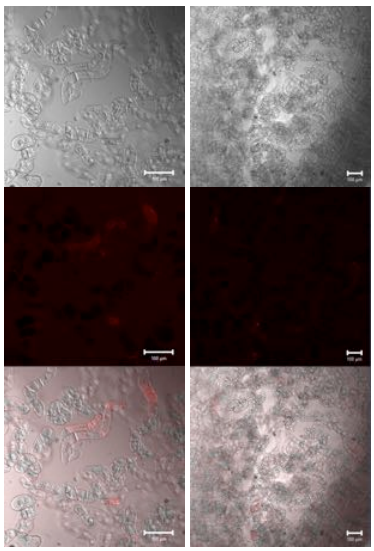
f) overlay image generated by CLSM. Scale bar, 100 μm. Time course CLSM observations of BY-2 cells incubated with TAMRA-labelled BP100 (g-n).



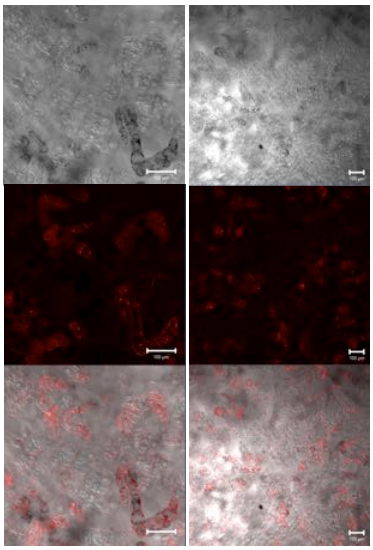
1. BP100



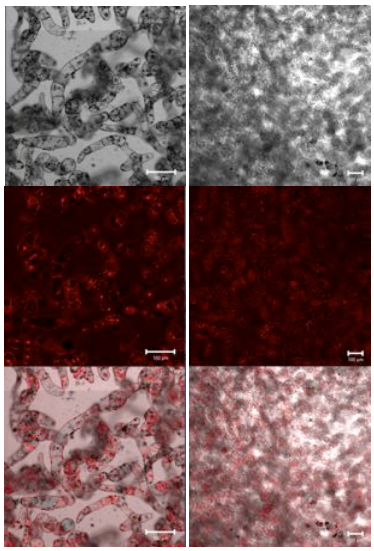
2. 2BP100



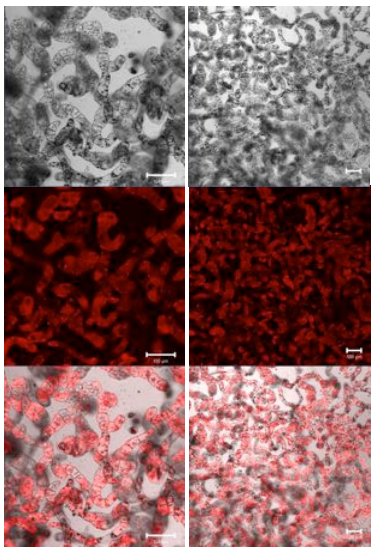
3. Rev(34-50)



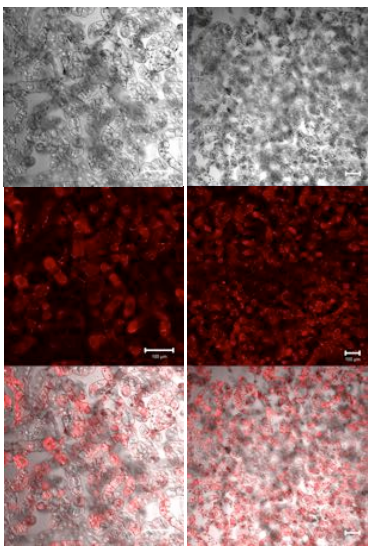
4. R9



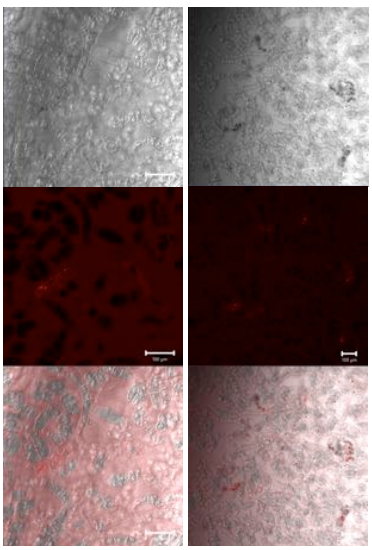
5. D-R9



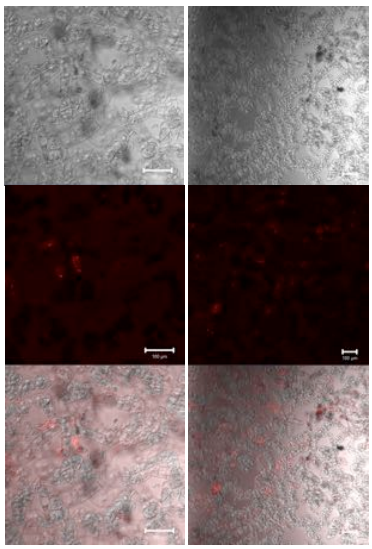
6. R12



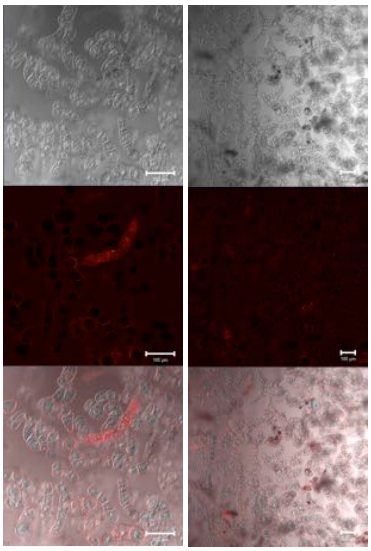
7. KH9



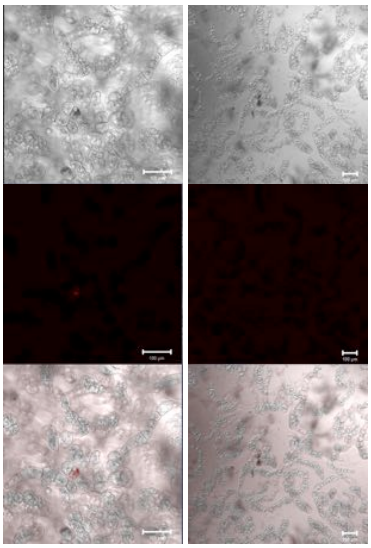
8. K9



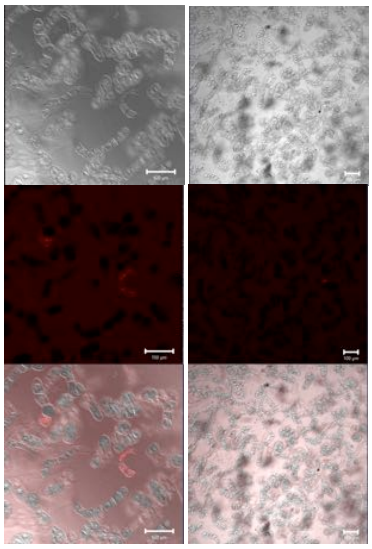
9. K18



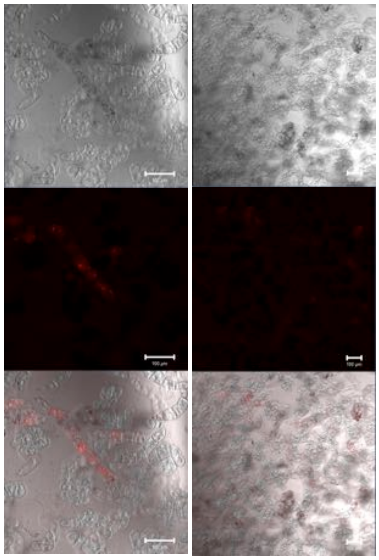
10. Pen2W2F



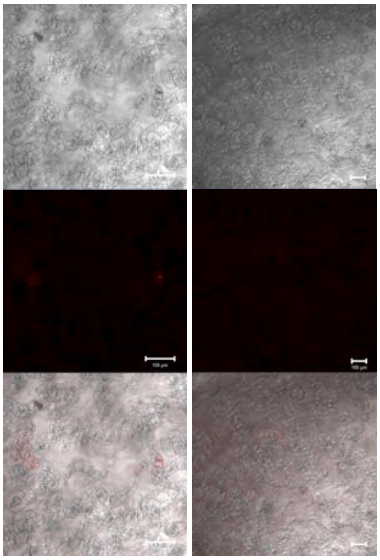
11. DPV3



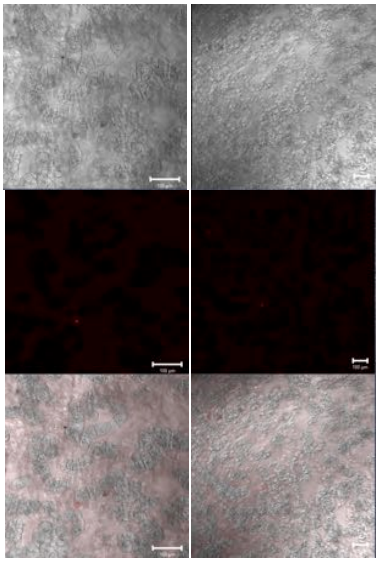
12. 6-Oct



13. R9-TAT



14. Tat(49-57)



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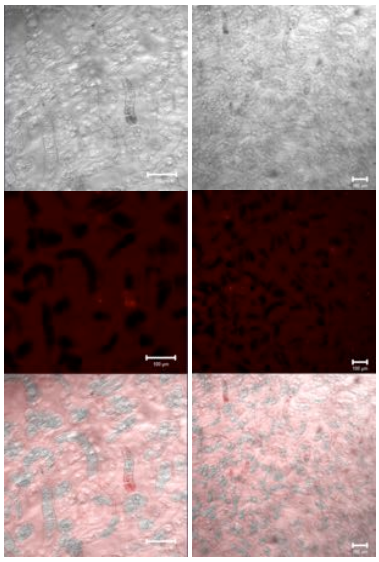
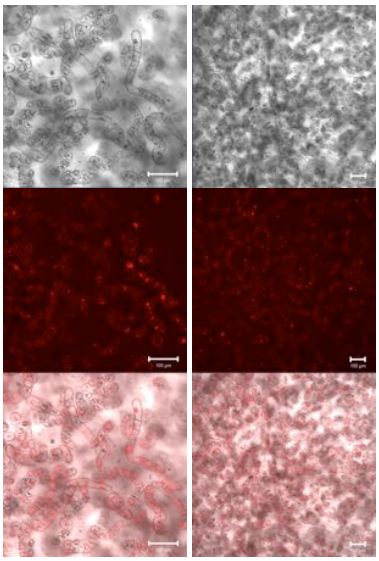


16. K18



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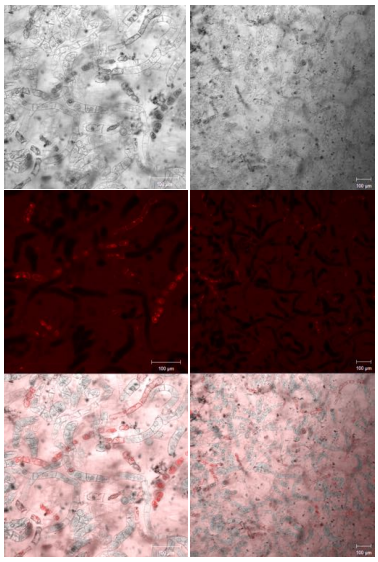
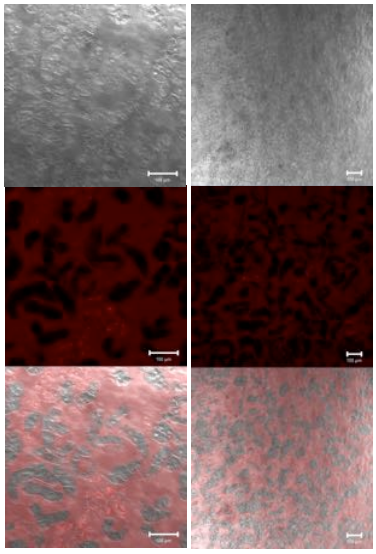
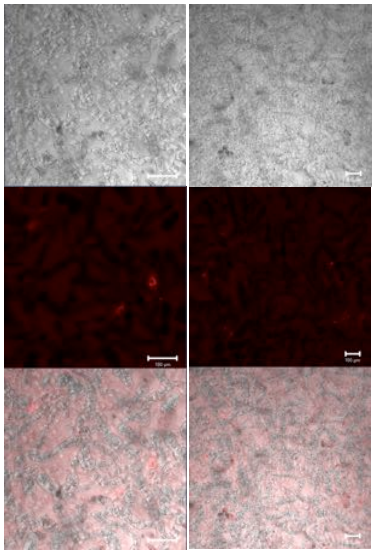


18. IX



19. XI



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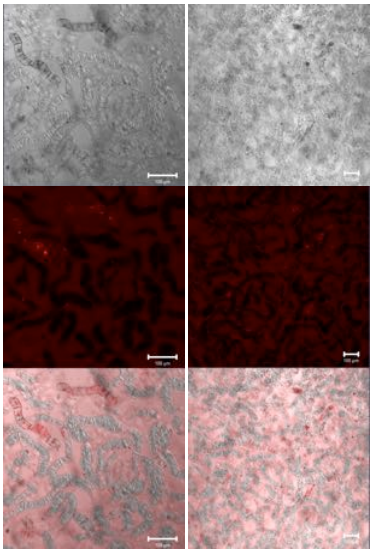

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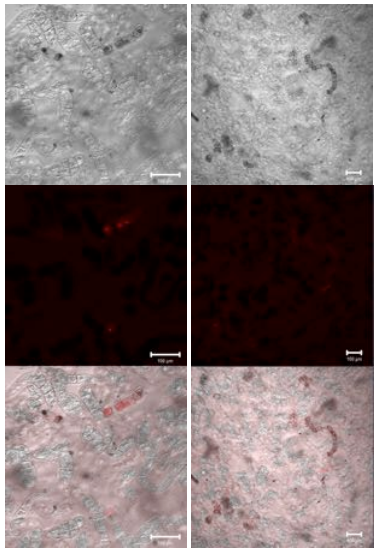
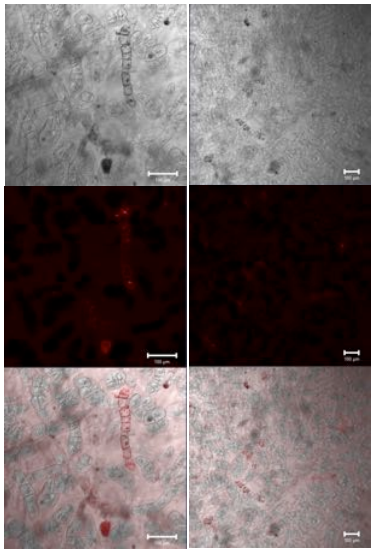
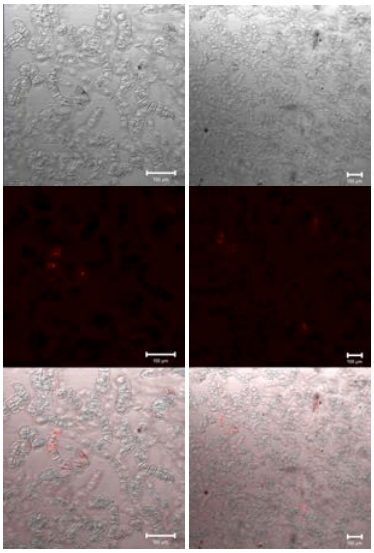


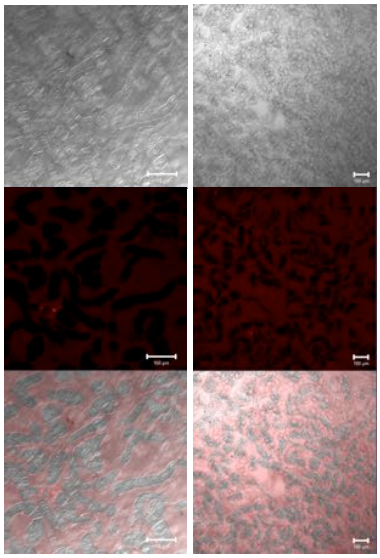
22. PenArg



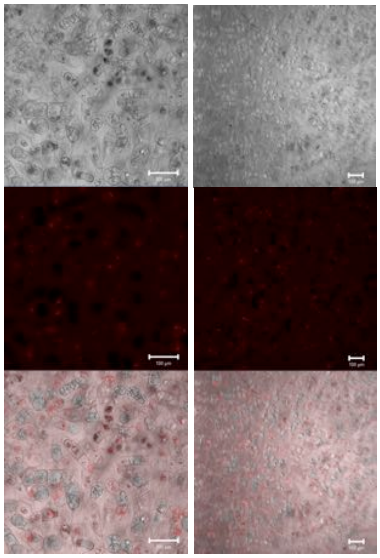
23. M918



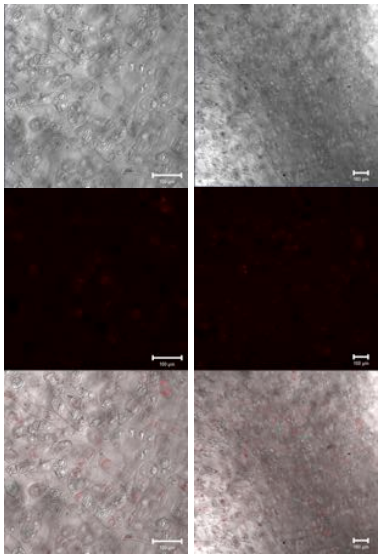
24. Penetratin



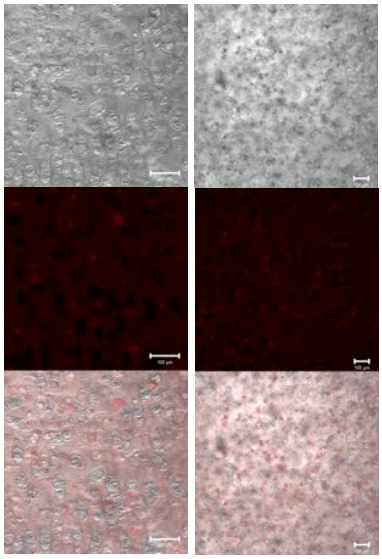
25. PolyP3(SAP)



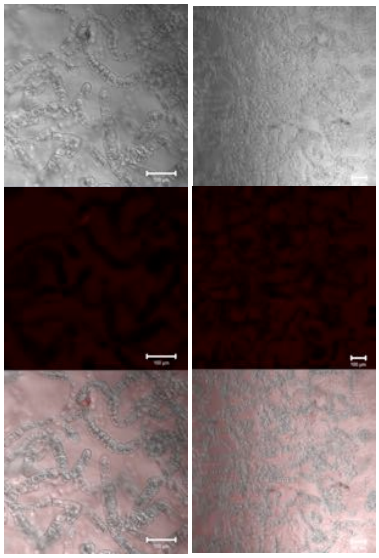
26. dhvar5



27.HPV33L2-445/467



28. Buforin Ⅱ(5-21)



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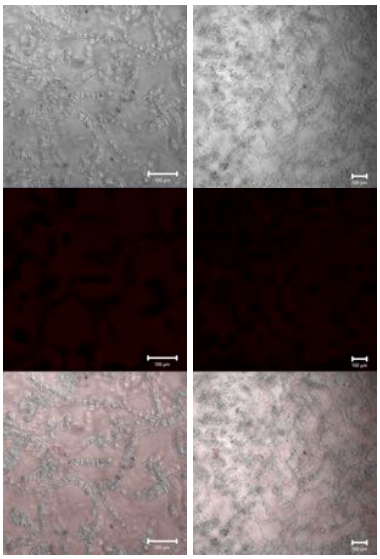
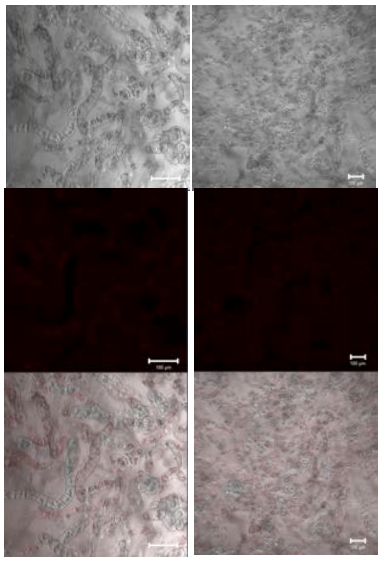
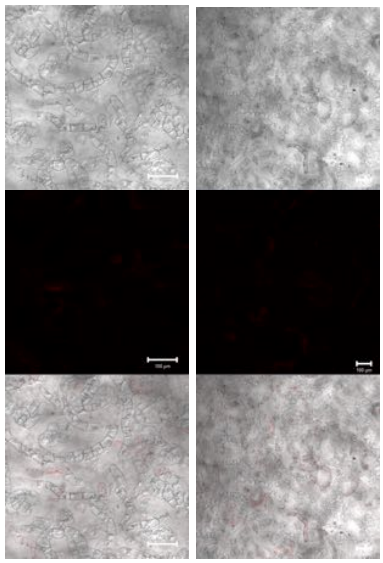


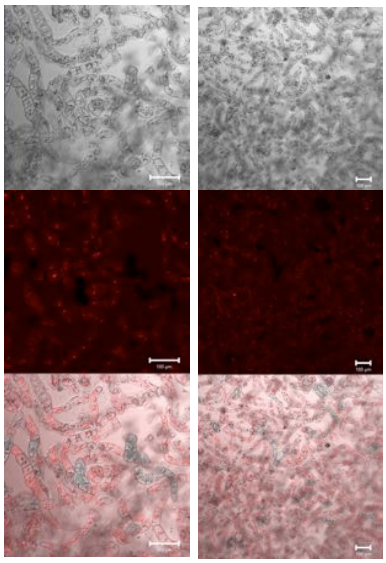
30. HPV33L2-DD447



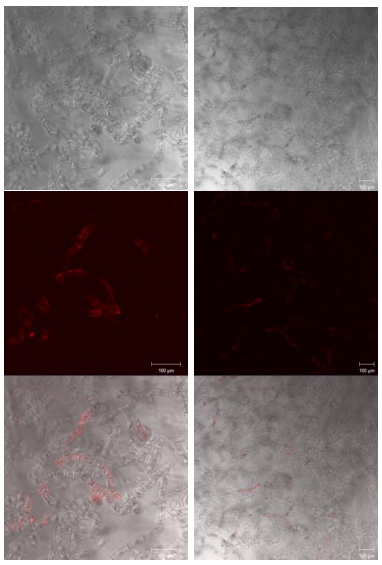
31. LAH4



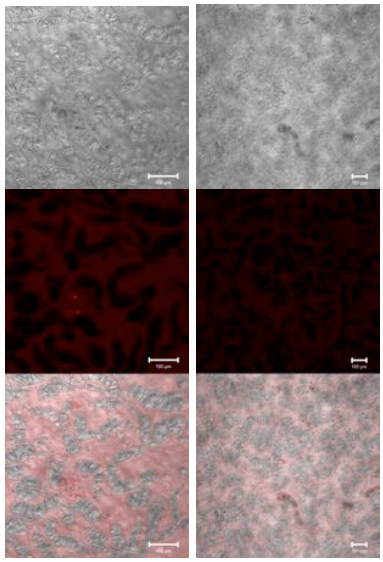
32. ppTG1



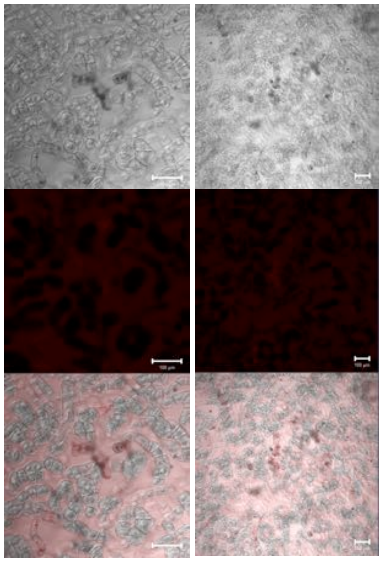
33. transportan(TP)



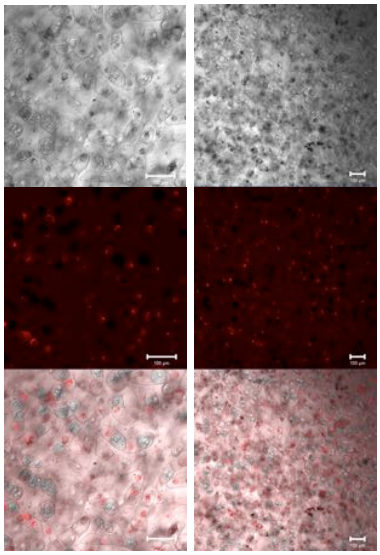
34. 2x ppTG1



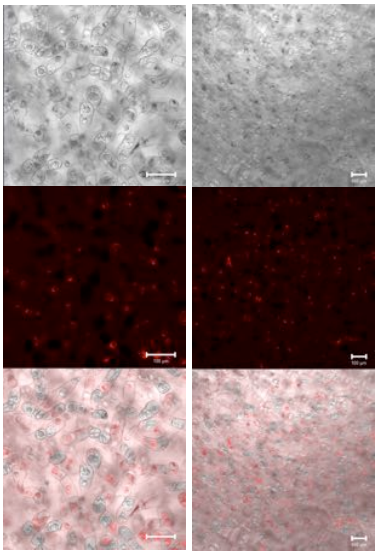
35. pAntpHD(Pro50)



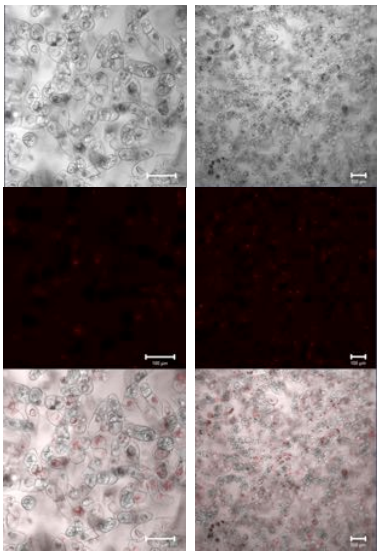
36. pAntp(44-58)



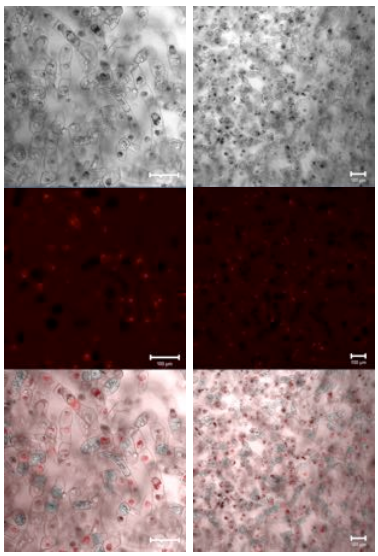
37. Crot(27-39)



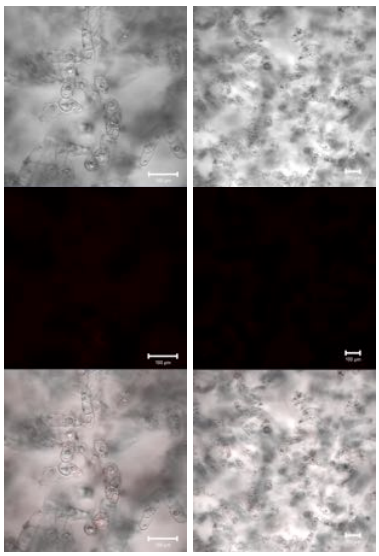
38. Crot(27-39) derevative1



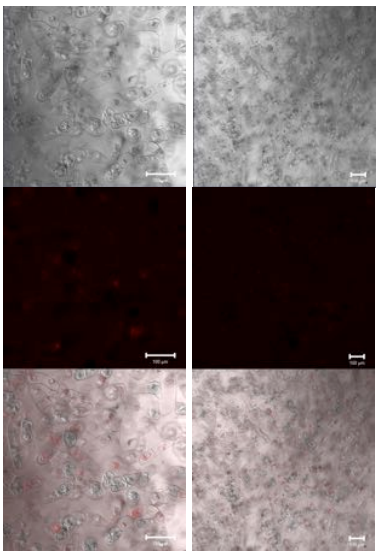
39. Crot(27-39) derevative2



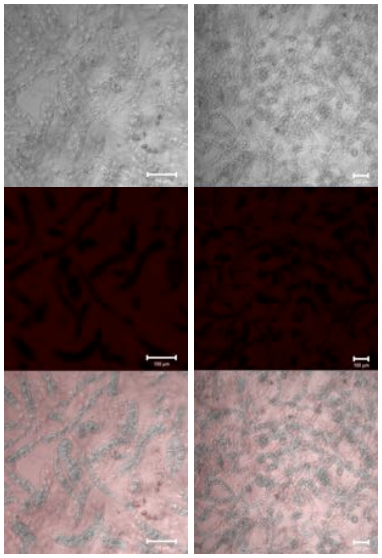
40. CyLoP-1



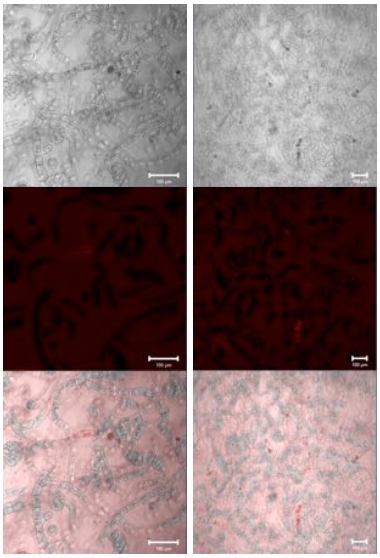
41. Inv3



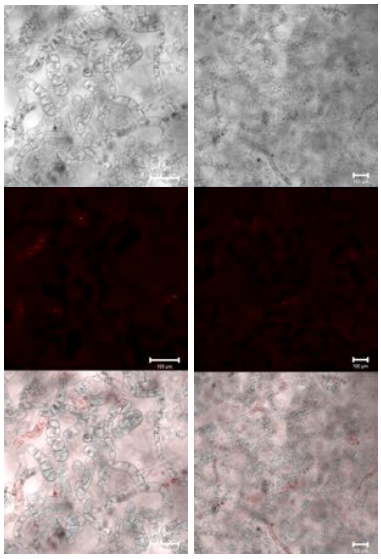
42. Inv5



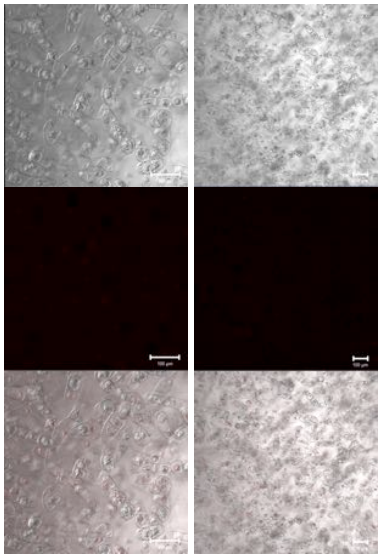
43. Inv3.5



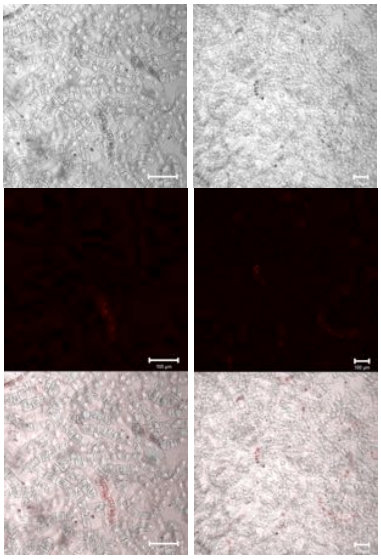
44. Inv3.10



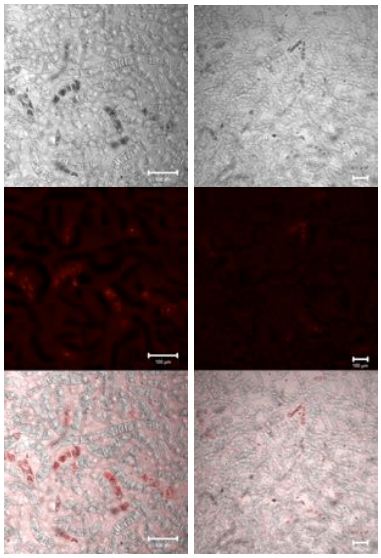
45. ARF(1-22)



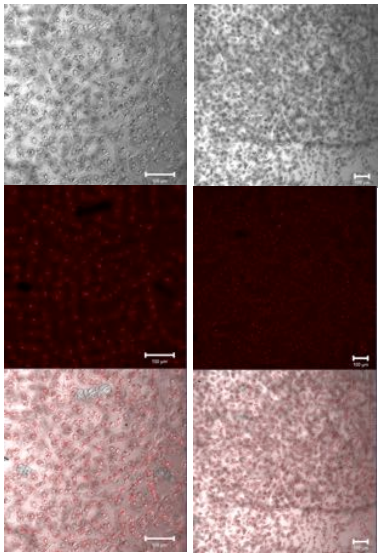
46. Cyt C 71-101



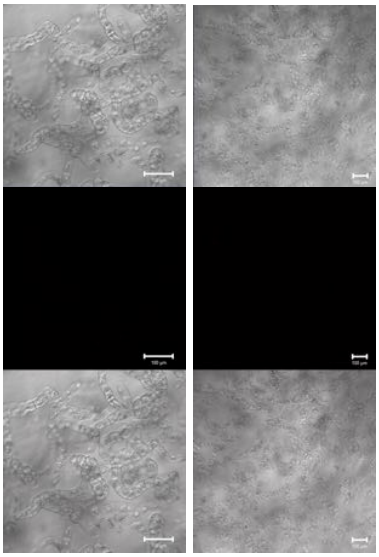
47. hLF peptide



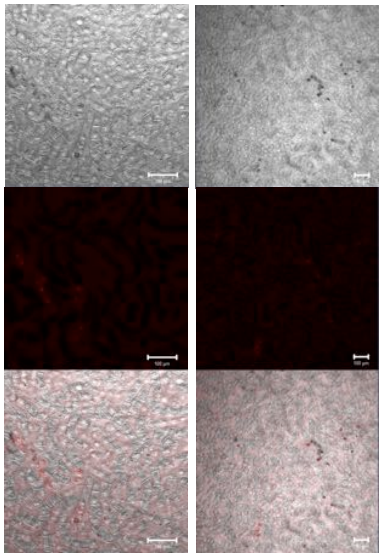
48. Glu-Oct-6



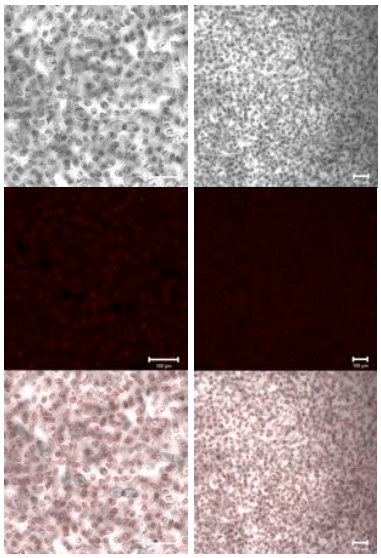
49. M511



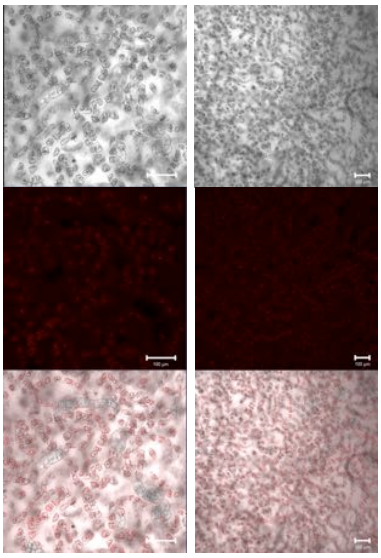
50. G53-4



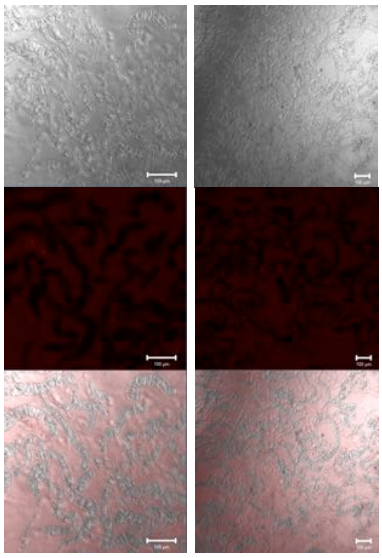
51. M591



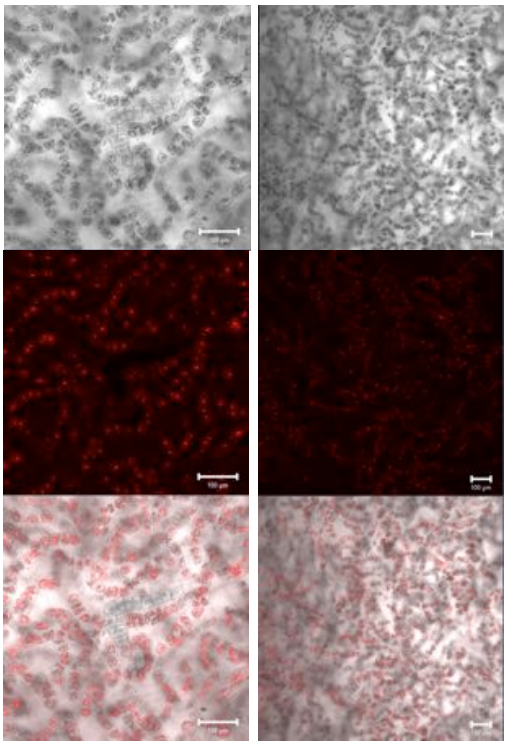
52. E162



53. E165



54. M867



55. MG2d

Figure S3. CLSM images of BY-2 cells incubated with 55 types of CPP for 3 hours at 26ºC. Top, middle and bottom images are DIC, TAMRA fluorescence and overlay images, respectively. The type of CPP is shown by peptide number and name as listed in Table 1. The peptide concentration was 100 μg/mL. The cell density was approximately 380 cells/μL.

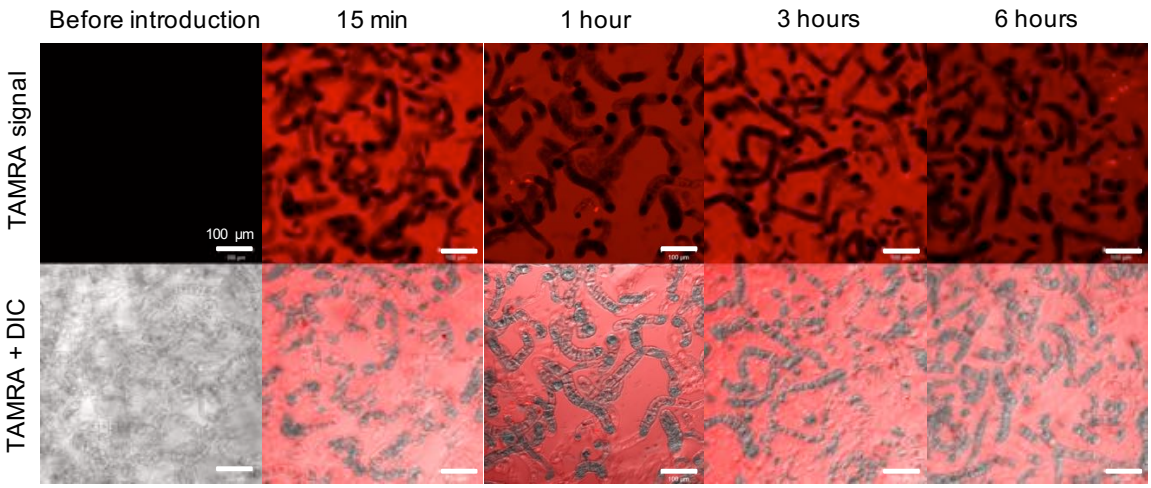


Figure S4. BY-2 cells before and after incubation with TAMRA alone for 15 min, 1 h, 3 h and 6 h at 26ºC. BY-2 cells at 3 days after subculture were used. The top and bottom images are TAMRA fluorescence image and overlay image of TAMRA signal and DIC image generated by CLSM. Scale bar, 100 μm.

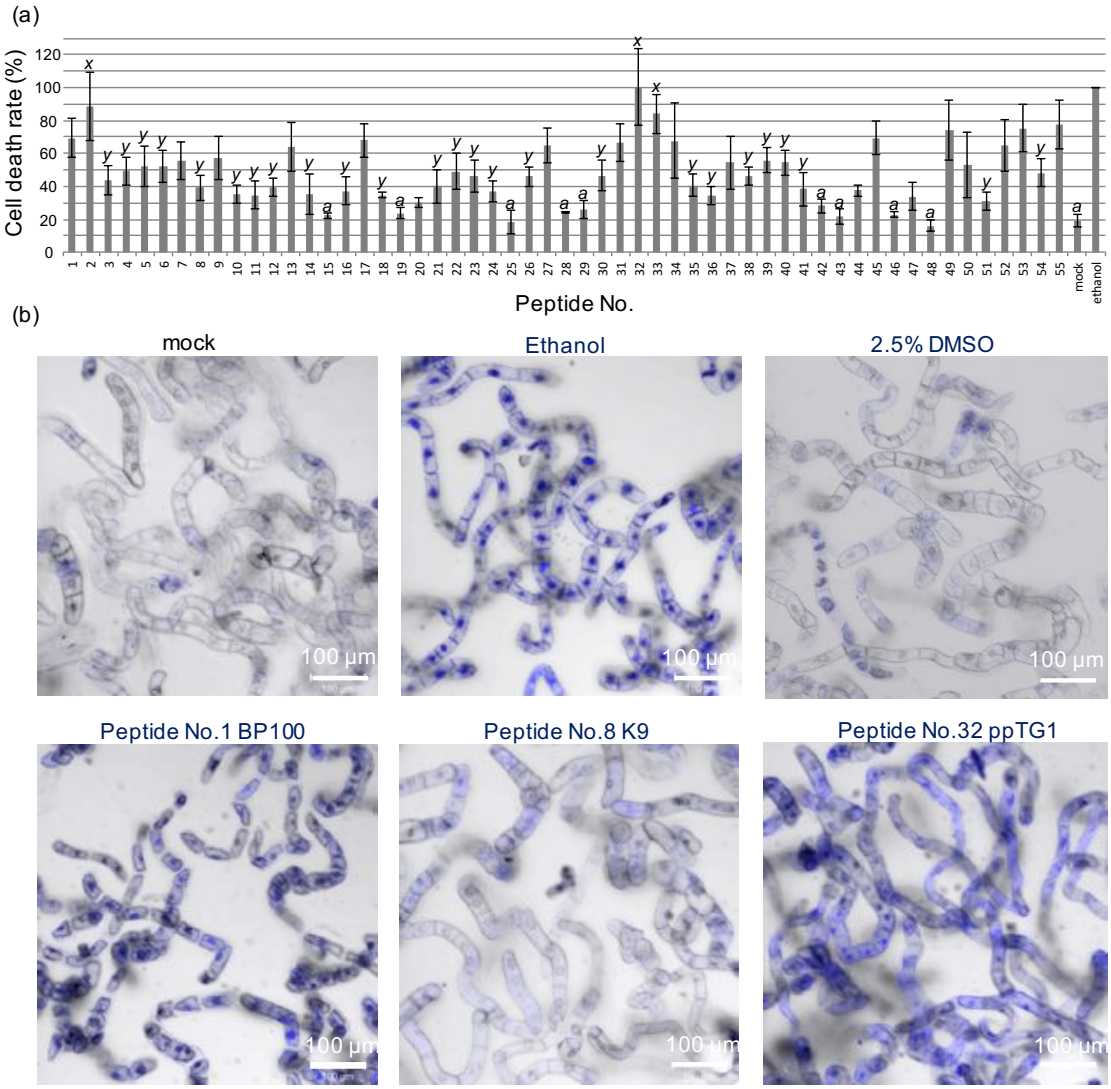


Figure S5. Evans blue assays with BY-2 cells. (a) BY-2 cell death rate of 55 types of CPPs. The type of CPP is shown as peptide number and is listed in Table 1. BY-2 cell was evaluated after a 3-h incubation at 26ºC. For cell death rate, the peptide concentration and cell density were 300 μg/mL and approximately 380 cells/μL, respectively. "Mock" indicates the result with BY-2 cell without CPP (live cells). "Ethanol" indicates the results of ethanol-treated BY-2 cell (dead cells). All data are expressed as the means ± S.D. from triplicate tests. The mean data labeled with different letters (*x* and *y*) are significantly different (Tukey's HSD test, $P < 0.05$). The mean data labeled with *a* are not significantly different from the mean value of the live cell. (b) CLSM overlay images of Evans Blue signals and DIC of BY-2 cells. Dead cells are indicated by blue color due to the Evans Blue staining. Non-stained cells are live cells.

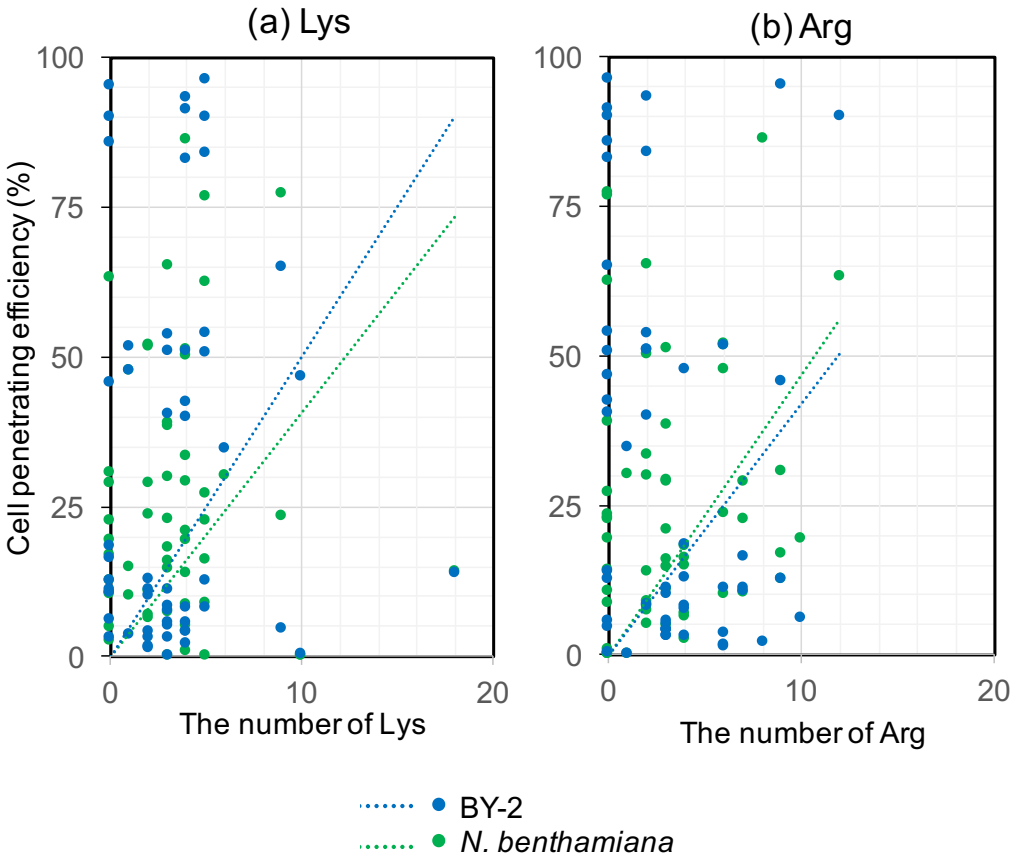


Figure S6. Relationship between the cell penetrating efficiencies into BY-2 cells and *N. benthamiana* leaves and the numbers of Lys (a) and Arg (b) in the amino acid sequences of CPPs. The broken lines are fitting curves using linear least squares analysis through the origin.

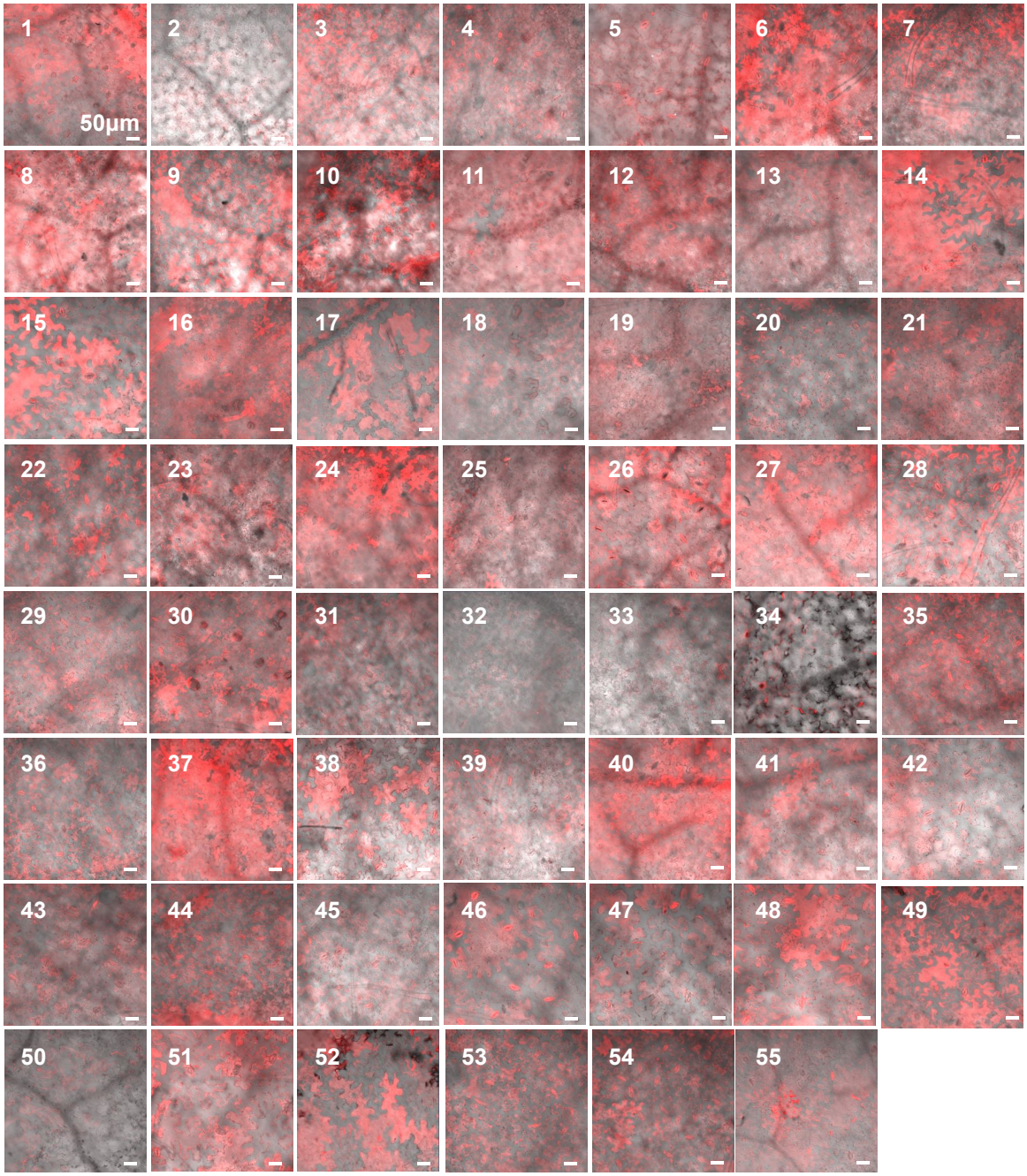


Figure S7. CLSM images of *N. benthamiana* leaf epidermal cells at 2 hours after infiltration of 55 types of CPP at 30ºC. The images are overlays of DIC (grey scale) and TAMRA fluorescence (red). The type of CPP is shown by peptide number and name as listed in Table 1.

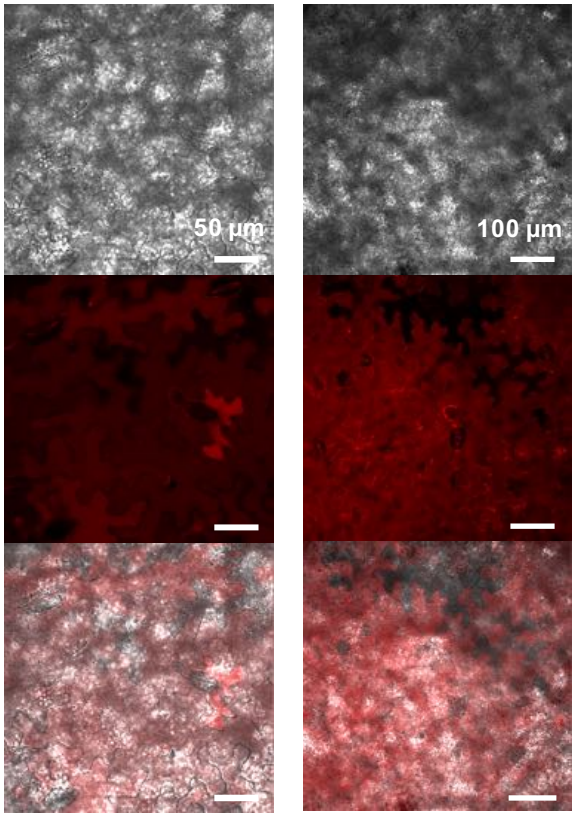
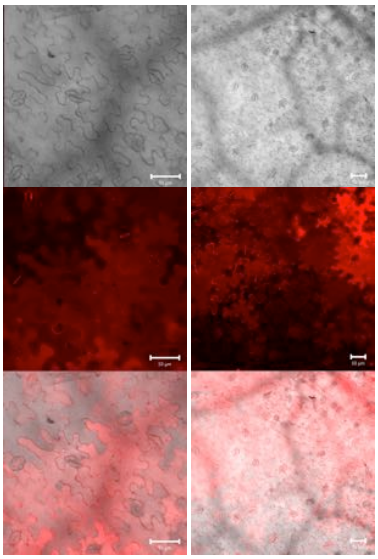
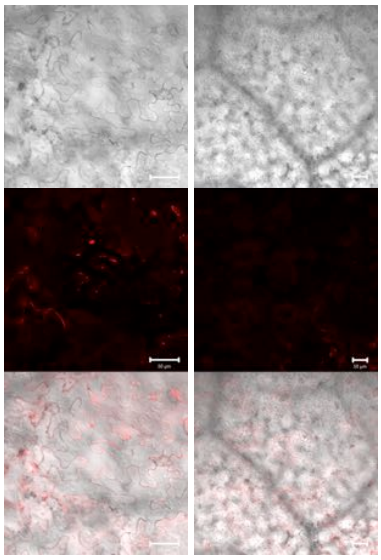


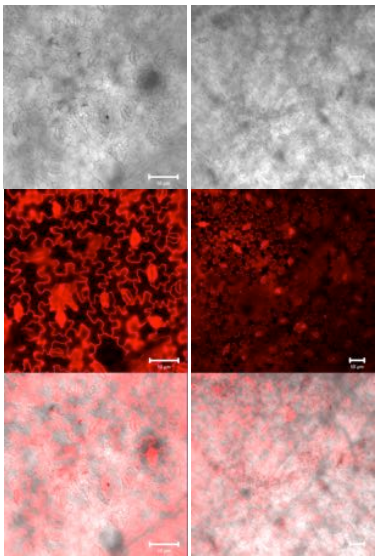
Figure S8. CLSM images of *N. benthamiana* cotyledons epidermal cells at 2 hours after infiltration of BP100 at 30ºC. Top, middle and bottom images are DIC, TAMRA fluorescence and overlay images, respectively.



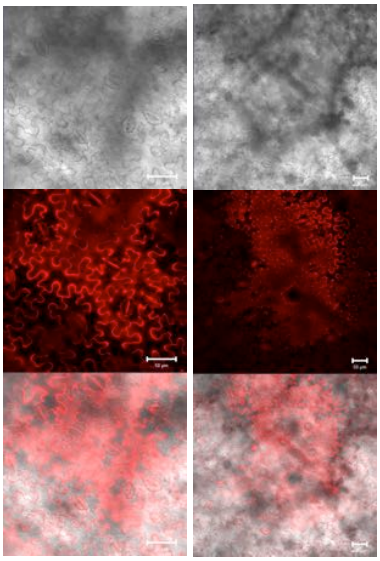
1. BP100



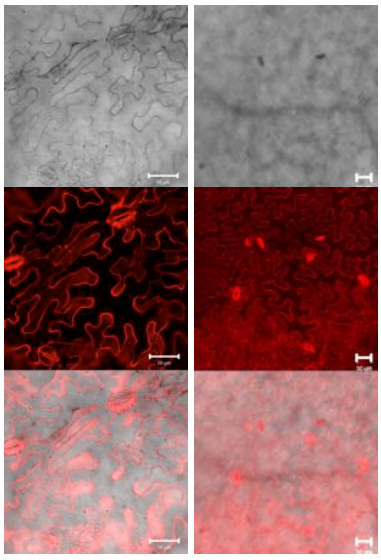
2. 2BP100



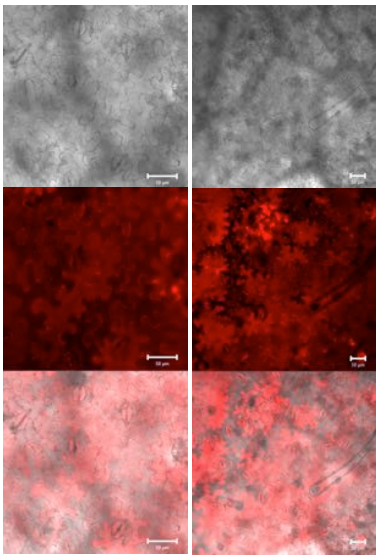
3. Rev(34-50)



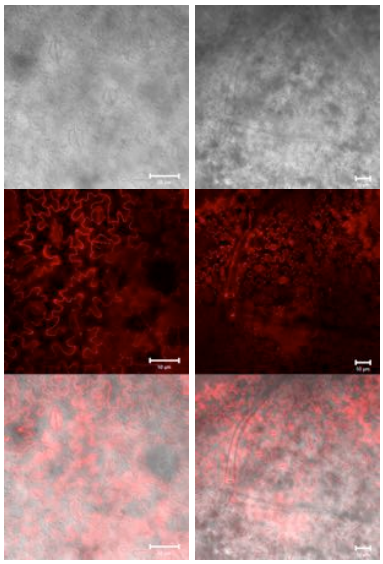
4. R9



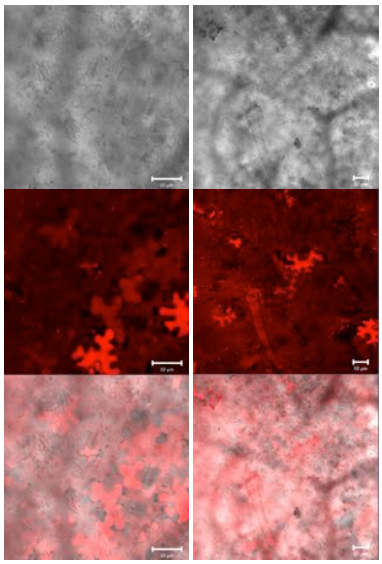
5. D-R9



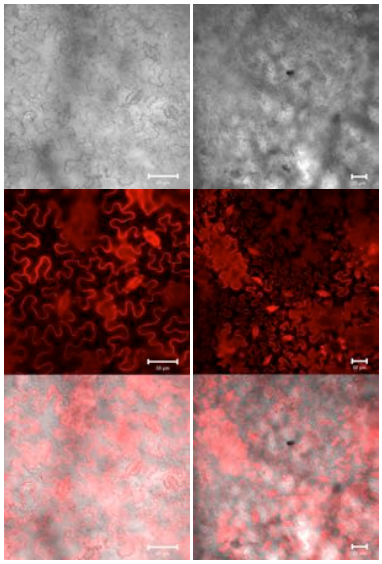
6. R12



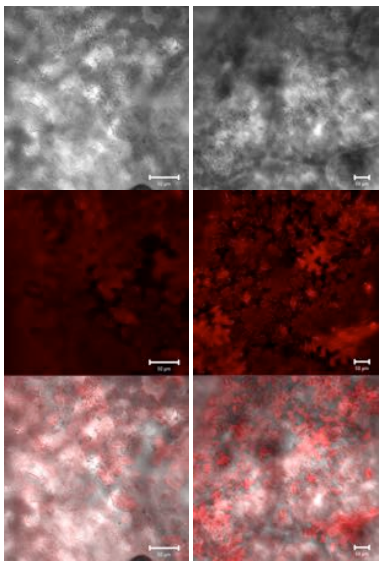
7. KH9



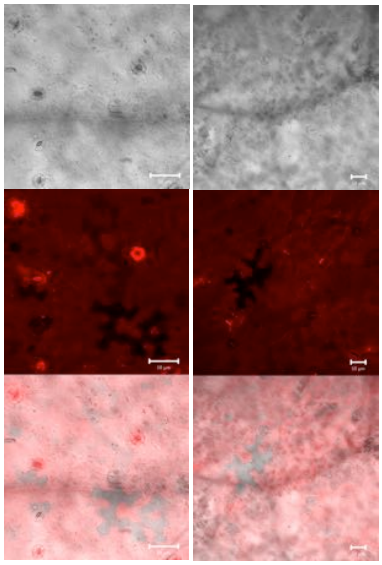
8. K9



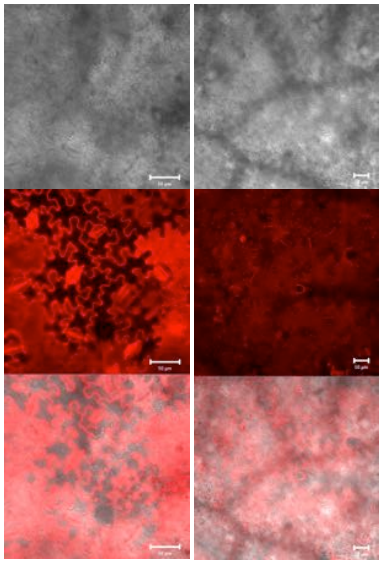
9. K18



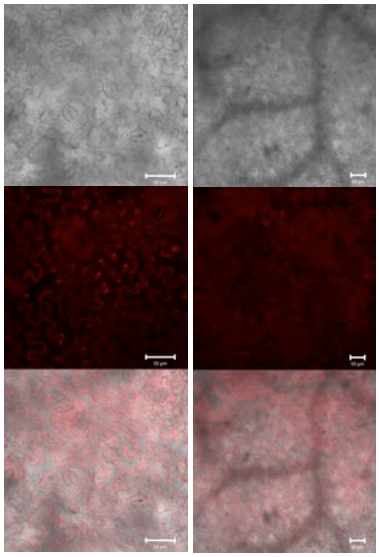
10. Pen2W2F



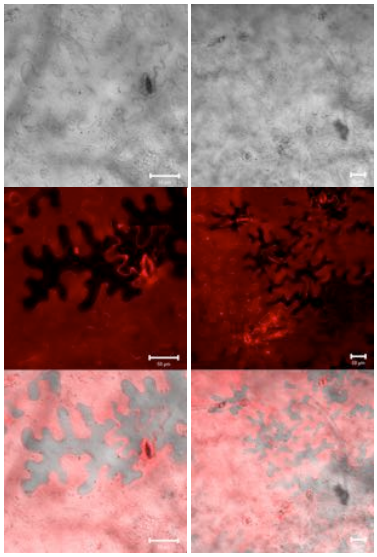
11. DPV3



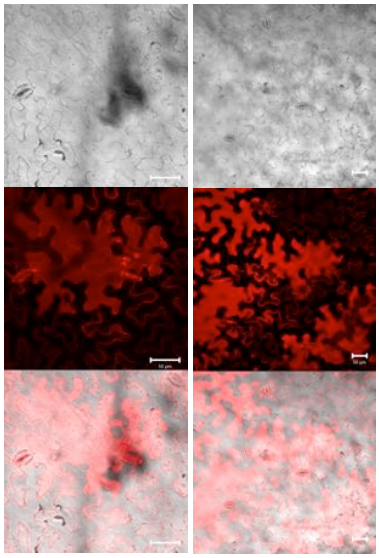
12. 6-Oct



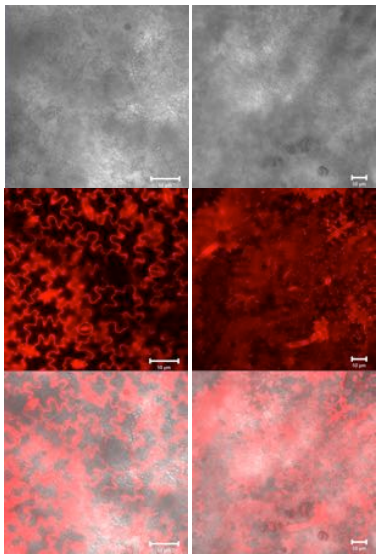
13. R9-TAT



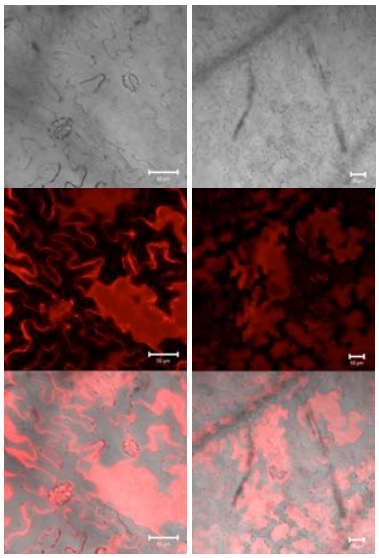
14. Tat(49-57)



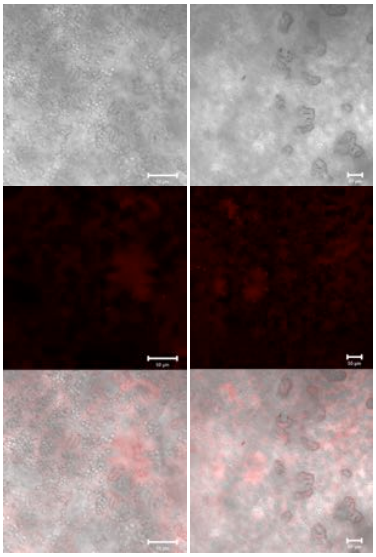
15. RetroTat(57-49)



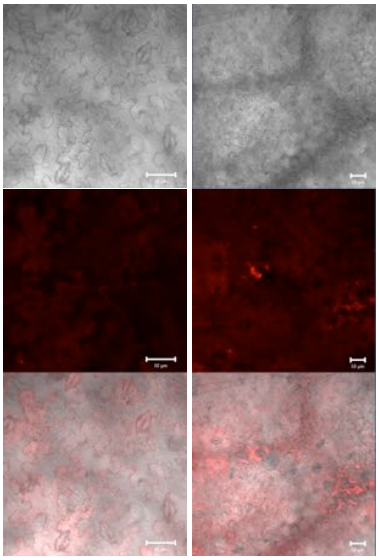
16. K18



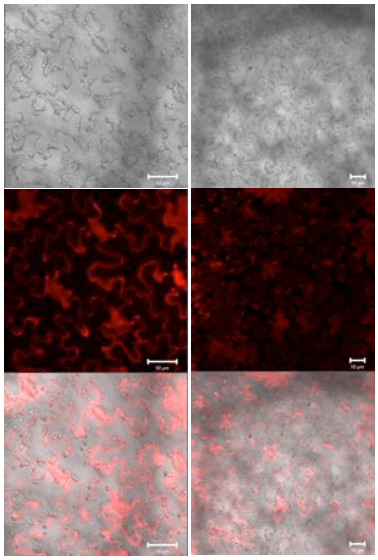
17. KLA10



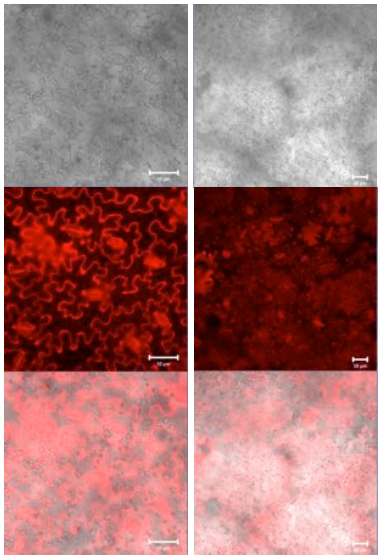
18. IX



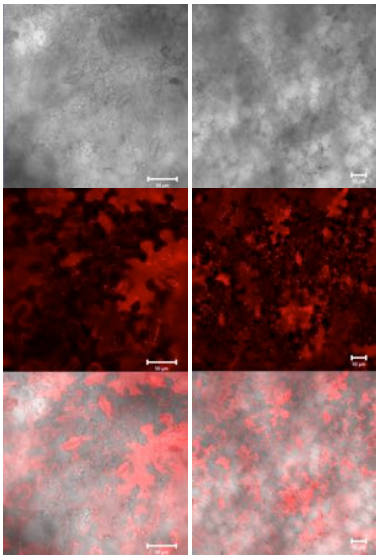
19. XI



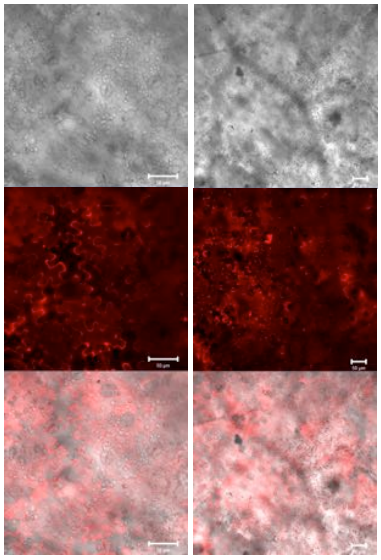
20. No.14-12



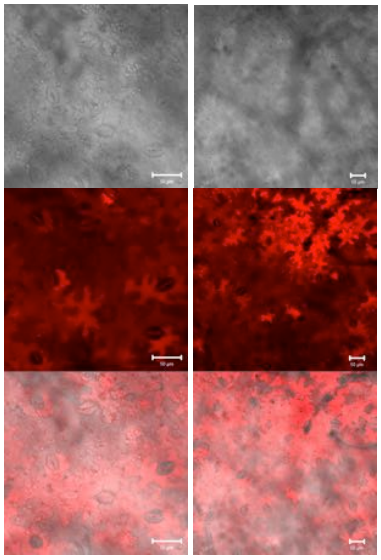
21. pVEC



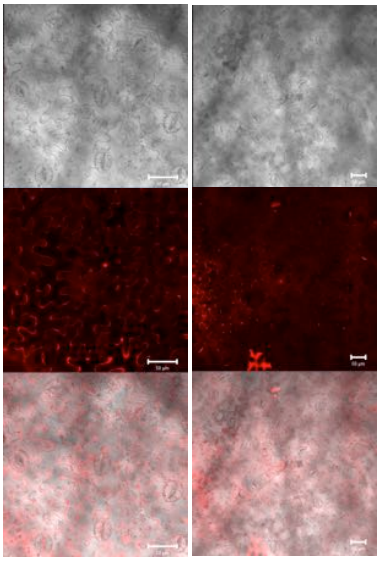
22. PenArg



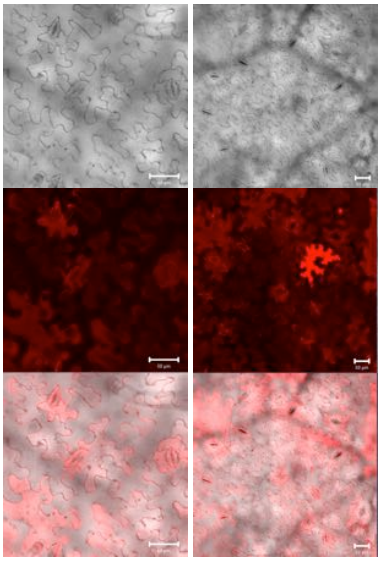
23. M918



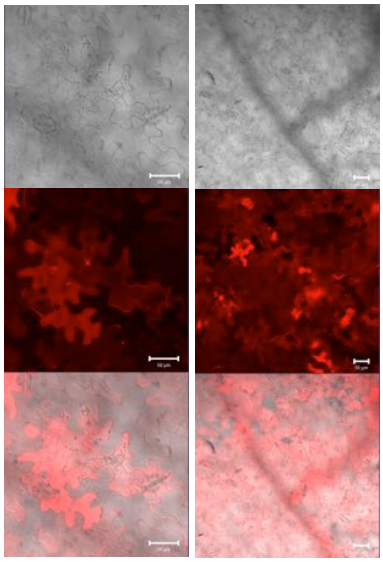
24. Penetratin



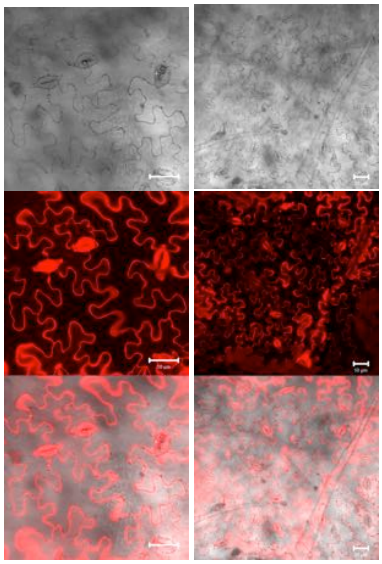
25. PolyP3(SAP)



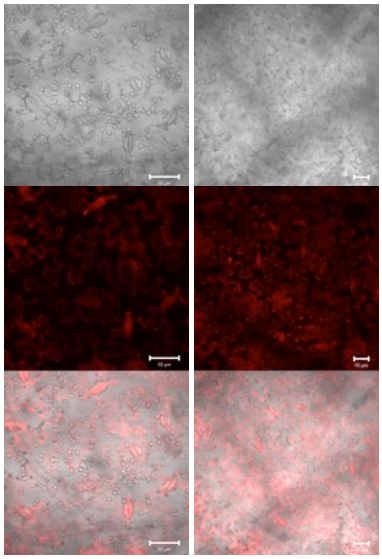
26. dhvar5



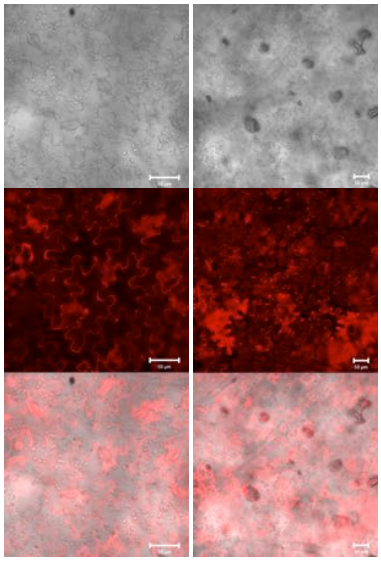
27.HPV33L2-445/467



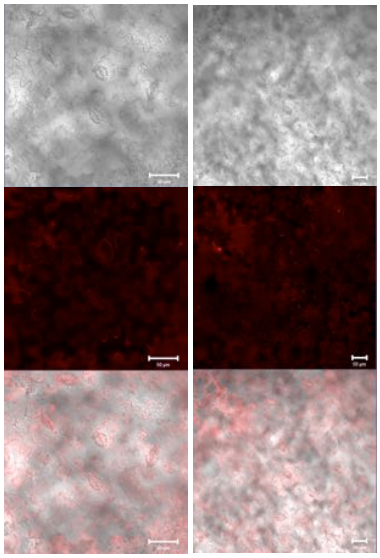
28. Buforin Ⅱ (5-21)



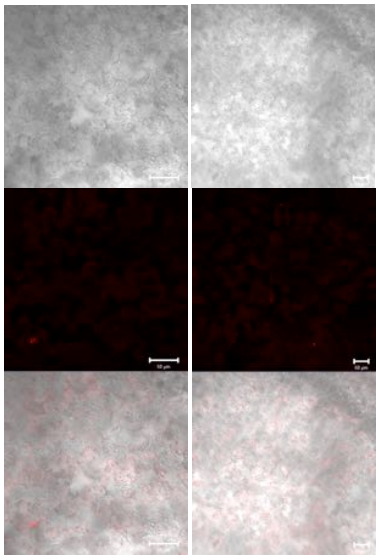
29. Scrambled pVEC



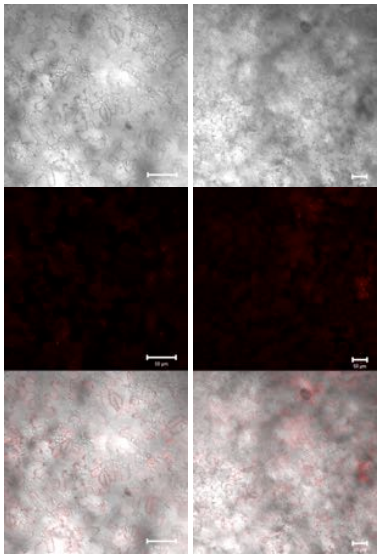
30. HPV33L2-DD447



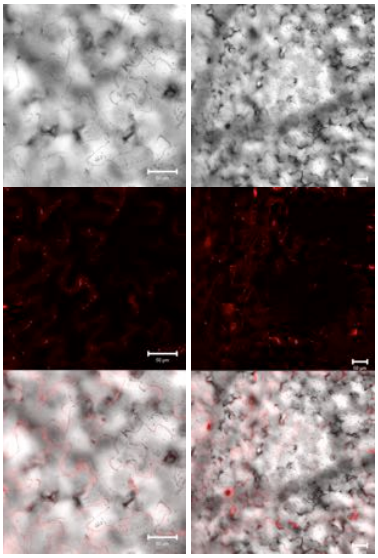
31. LAH4



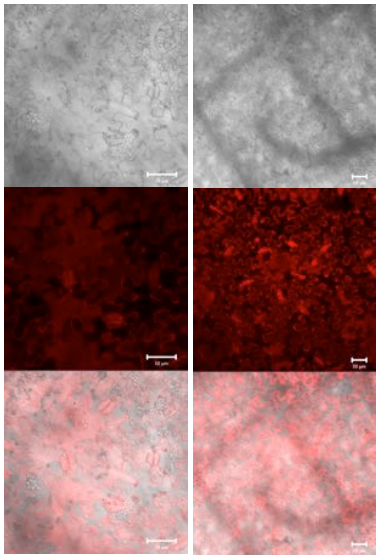
32. ppTG1



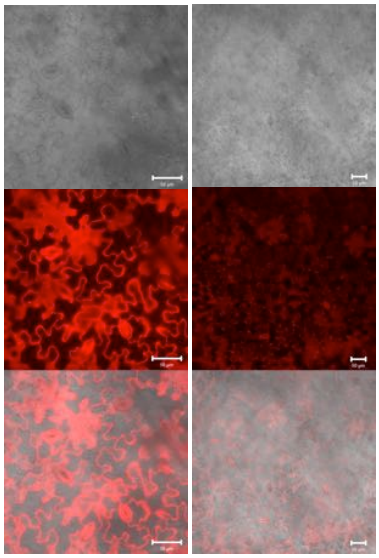
33. transportan(TP)



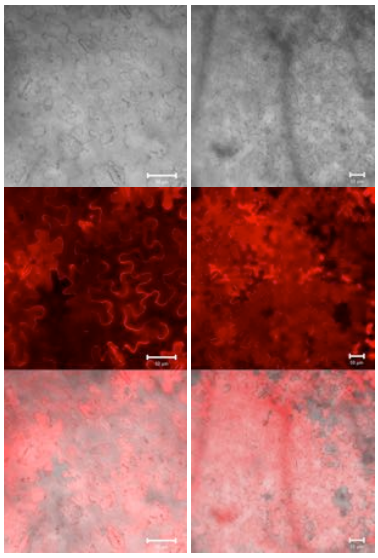
34. 2x ppTG1



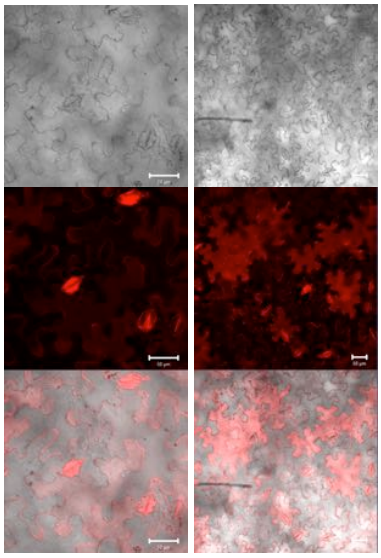
35. pAntpHD(Pro50)



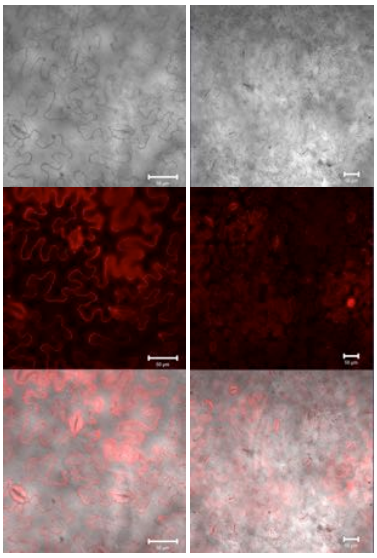
36. pAntp(44-58)



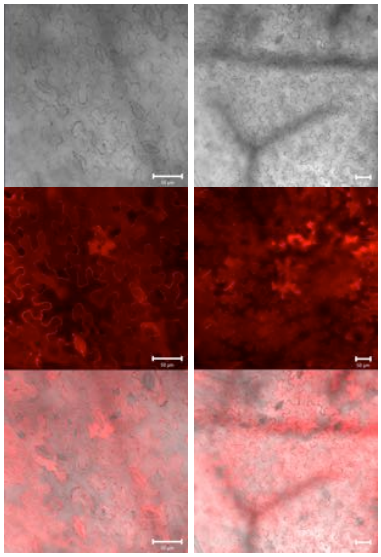
37. Crot(27-39)



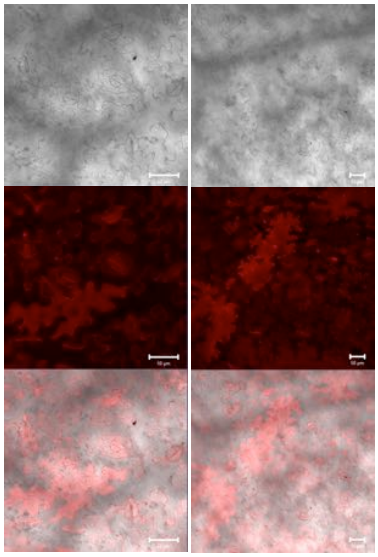
38. Crot(27-39) derevative1



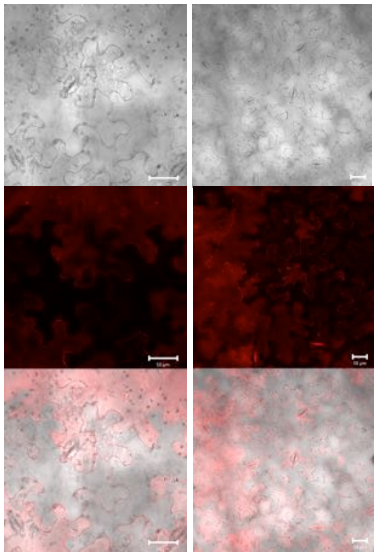
39. Crot(27-39) derevative2



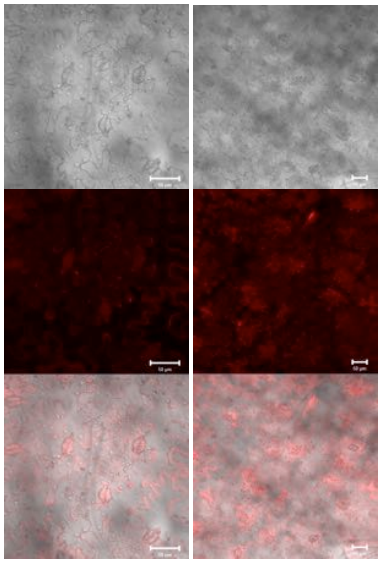
40. CyLoP-1



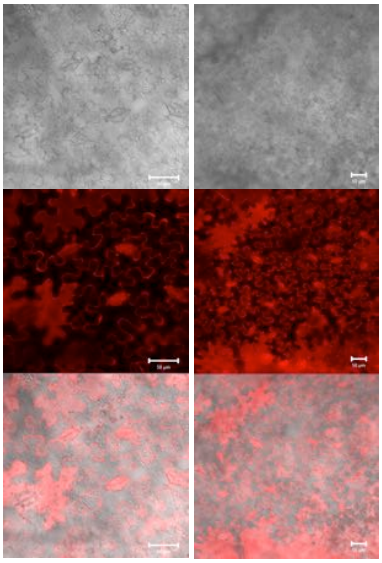
41. Inv3



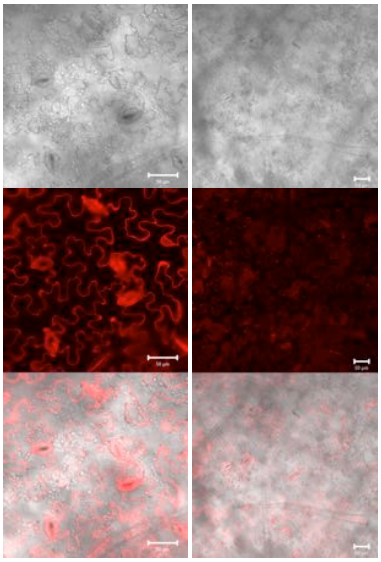
42. Inv5



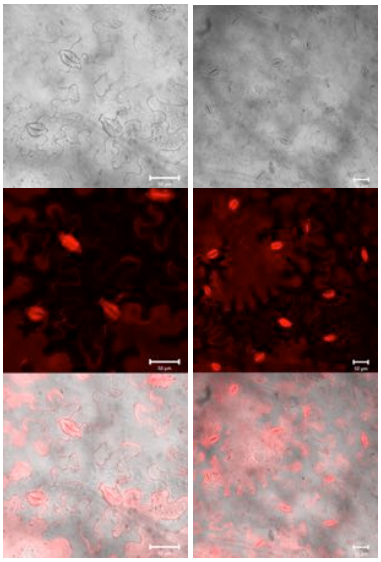
43. Inv3.5



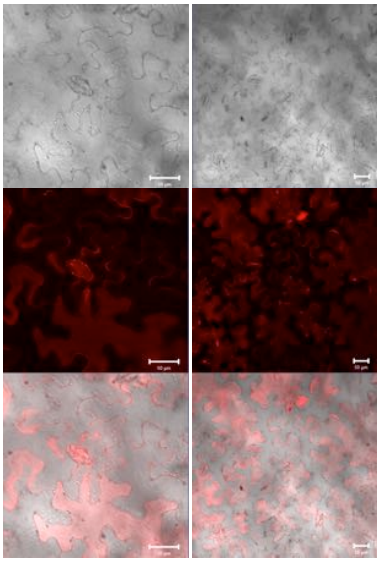
44. Inv3.10



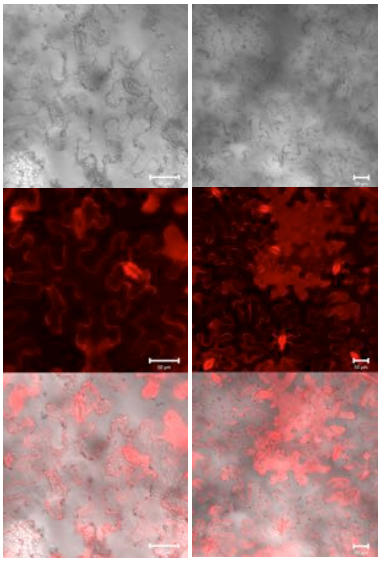
45. ARF(1-22)



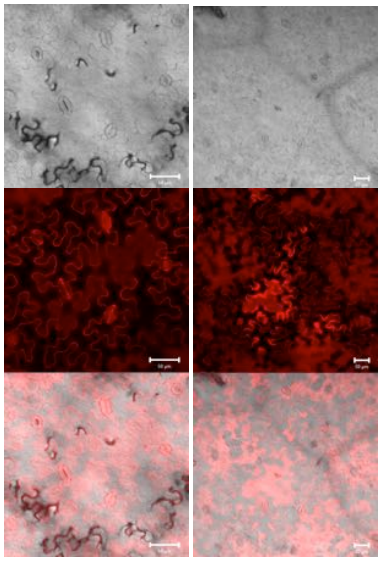
46. Cyt C 71-101



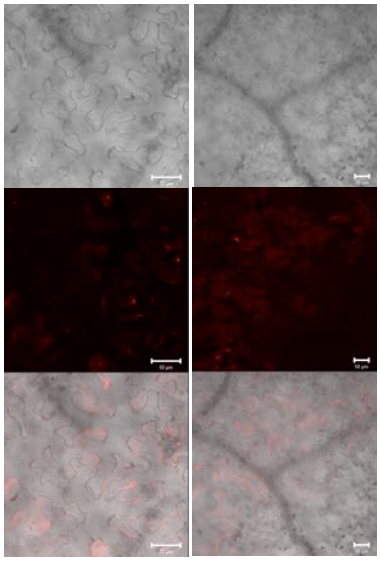
47. hLF peptide



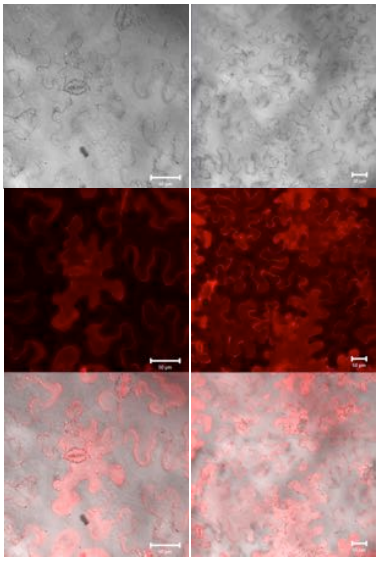
48. Glu-Oct-6



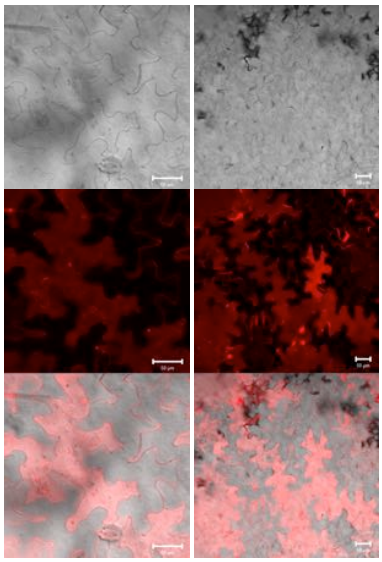
49. M511



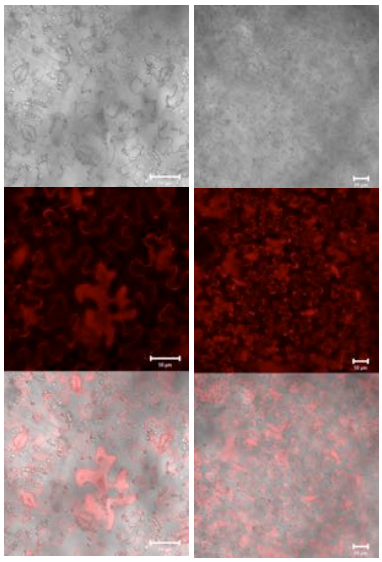
50. G53-4



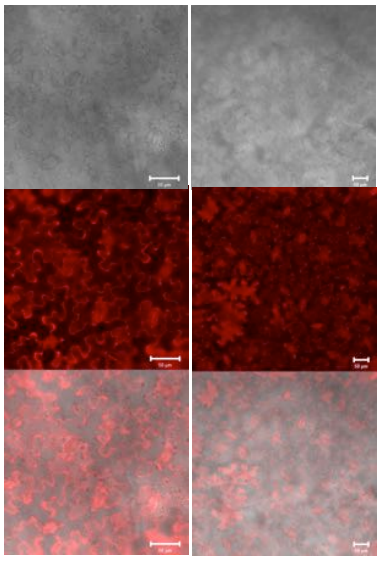
51. M591



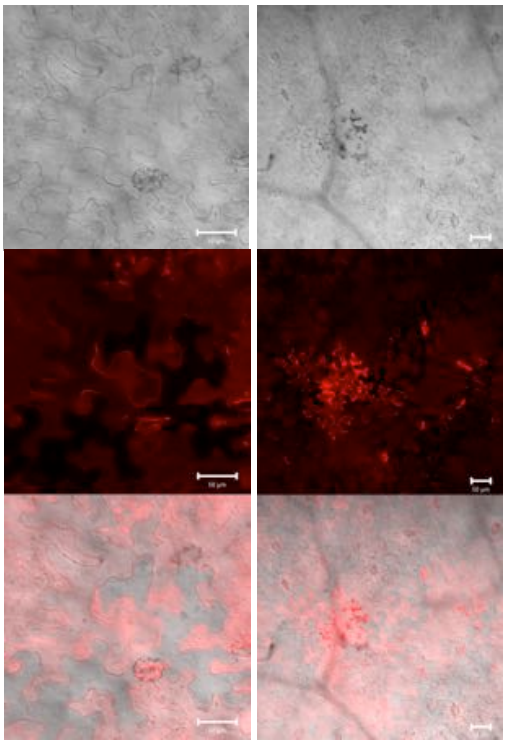
52. E162



53. E165



54. M867



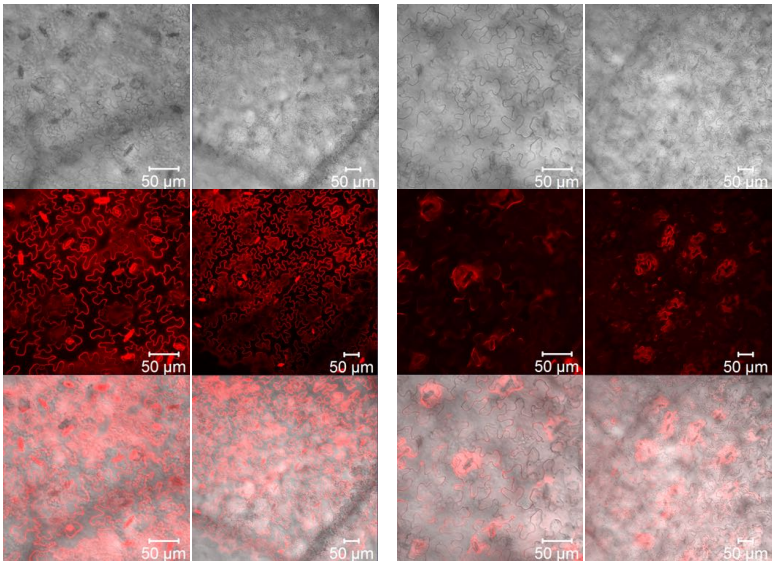
55. MG2d

Figure S9. CLSM images of *N. benthamiana* leaf epidermal cells at 2 hours after infiltration of 55 types of CPP at 30ºC. Top, middle and bottom images are DIC, TAMRA fluorescence and overlay images, respectively. The type of CPP is shown by peptide number and name as listed in Table 1.

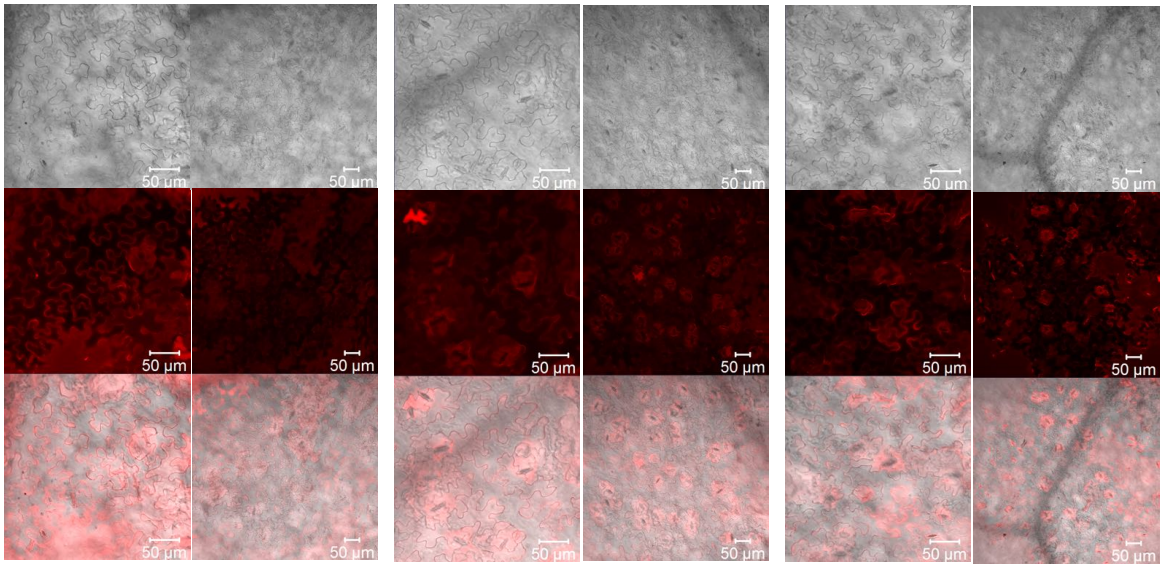
(a)

Arabidopsis thaliana

High efficiency peptides



5. D-R9 17. KLA10

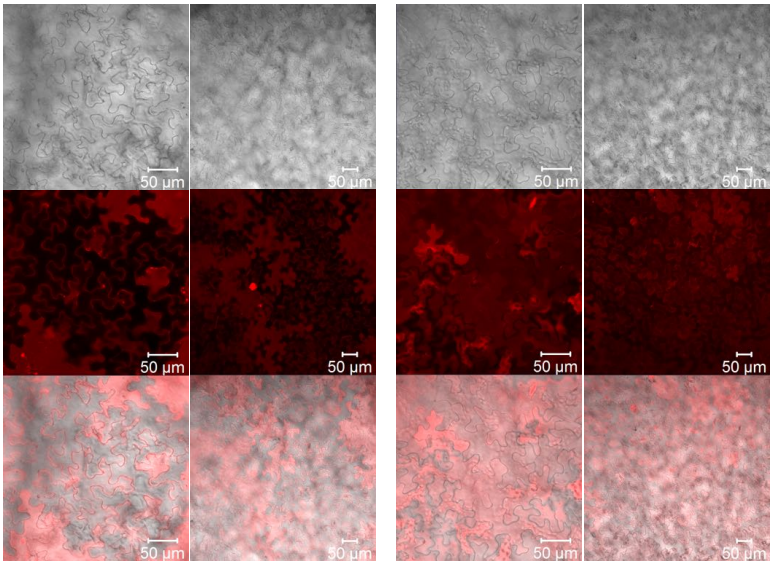


49. M511 52. E162 55. MG2d

(b)

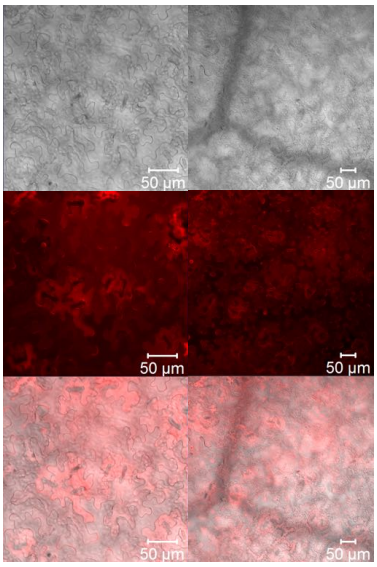
Arabidopsis thaliana

Middle efficiency peptides

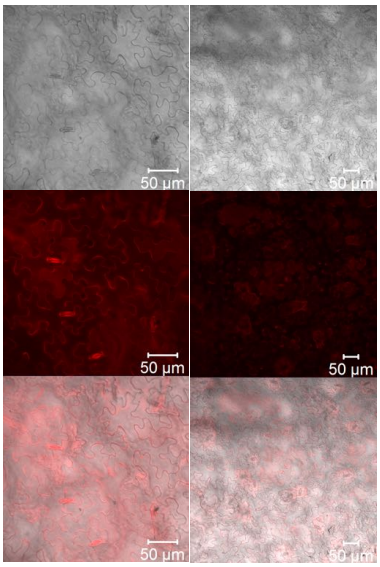


1. BP100

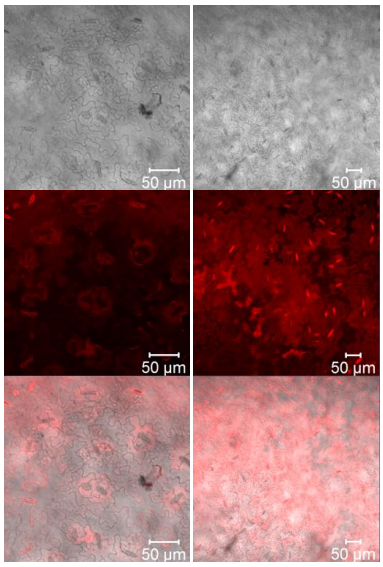
26. dhvar5



27.HPV33L2-445/467



37. Crot(27-39)



40. CyLoP-1

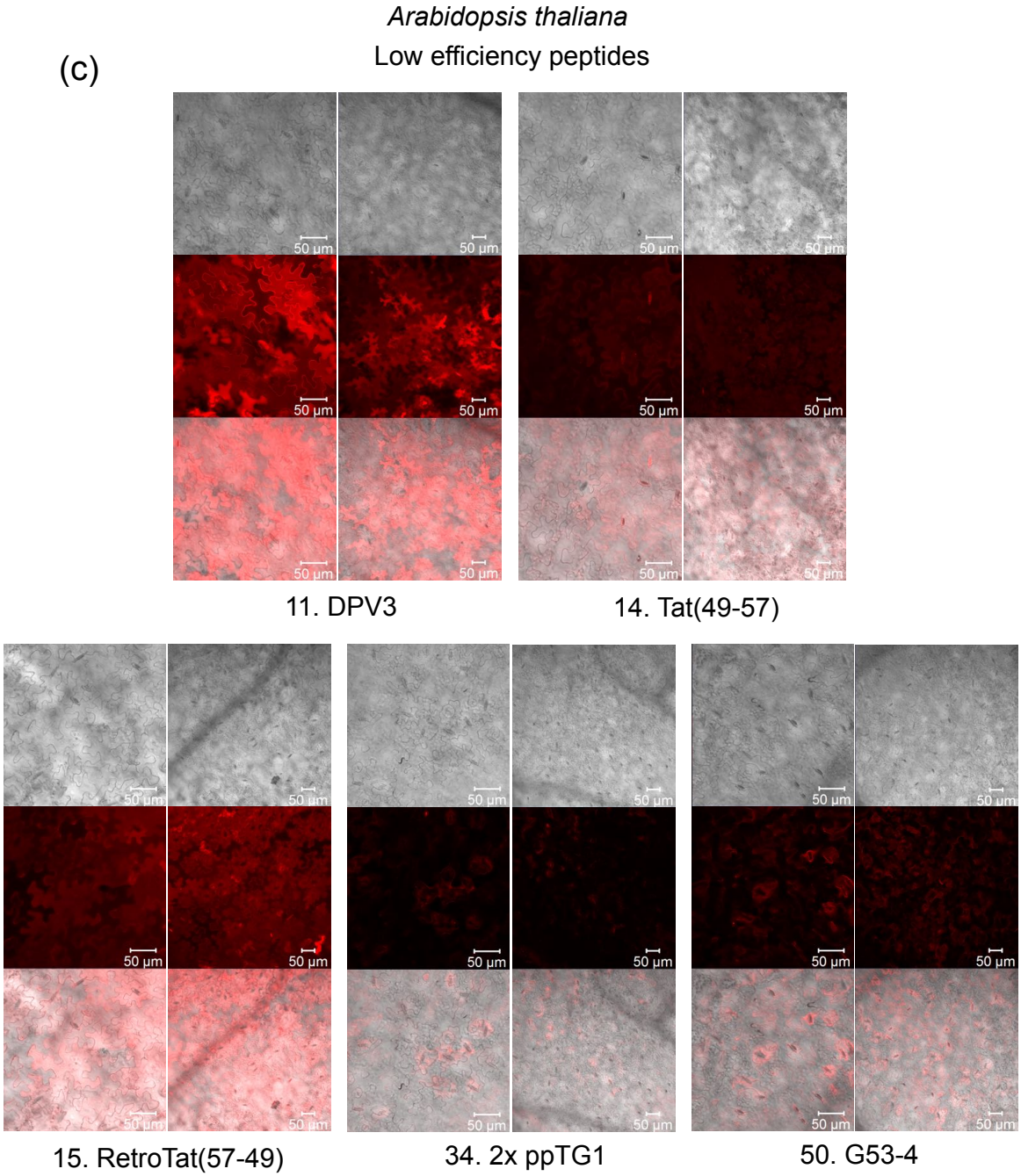
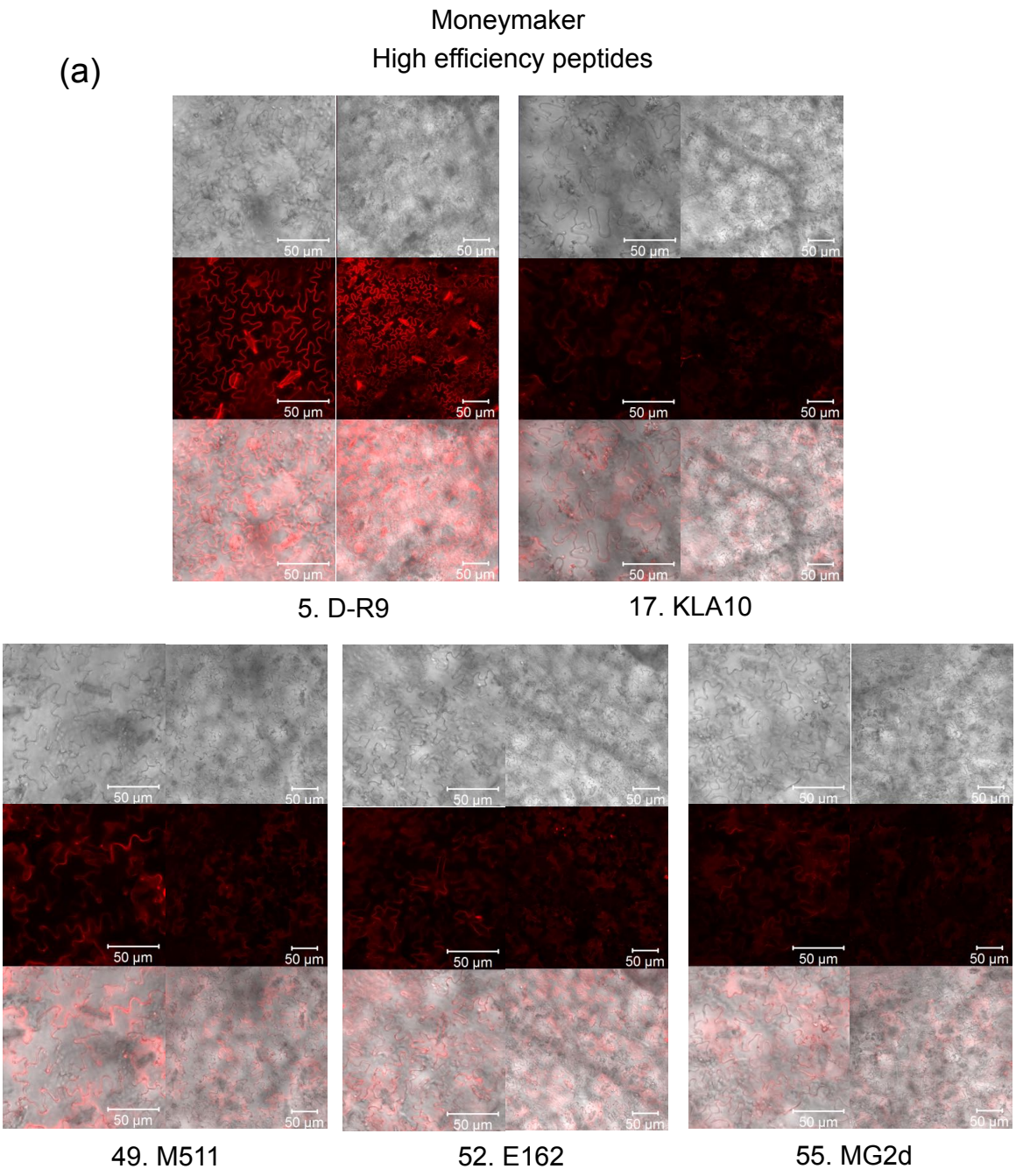


Figure S10. CLSM images of *A. thaliana* leaf epidermal cells at 2 hours after infiltration of three groups of CPPs at 21ºC. Based on the results with BY-2 cells, the high efficient (a), middle efficient (b) and low efficient CPPs (c) were classified and assayed. Top, middle and bottom

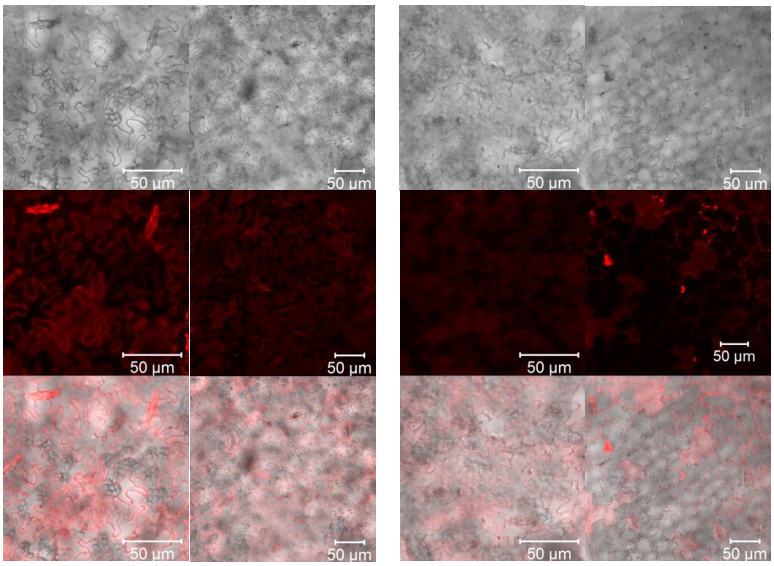
images are DIC, TAMRA fluorescence and overlay images, respectively. The type of CPP is shown by peptide number and name as listed in Table 1.



(b)

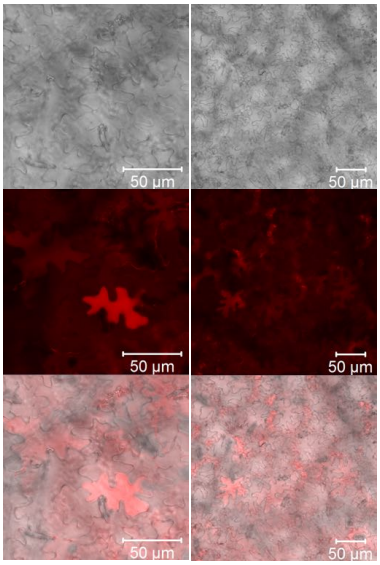
Moneymaker

Middle efficiency peptides

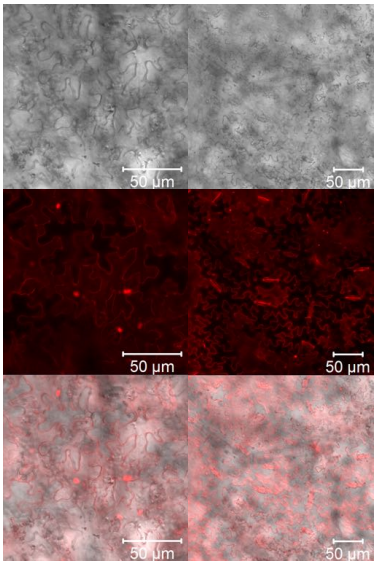


1. BP100

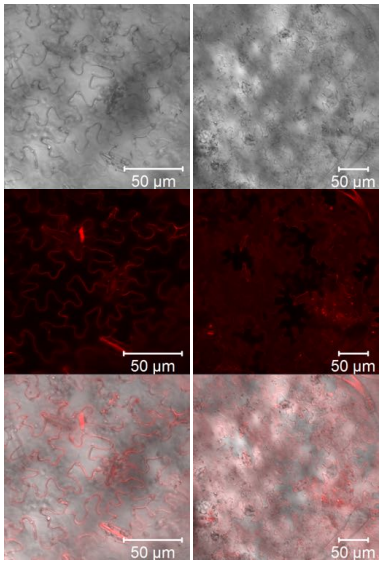
26. dhvar5



27.HPV33L2-445/467



37. Crot(27-39)



40. CyLoP-1

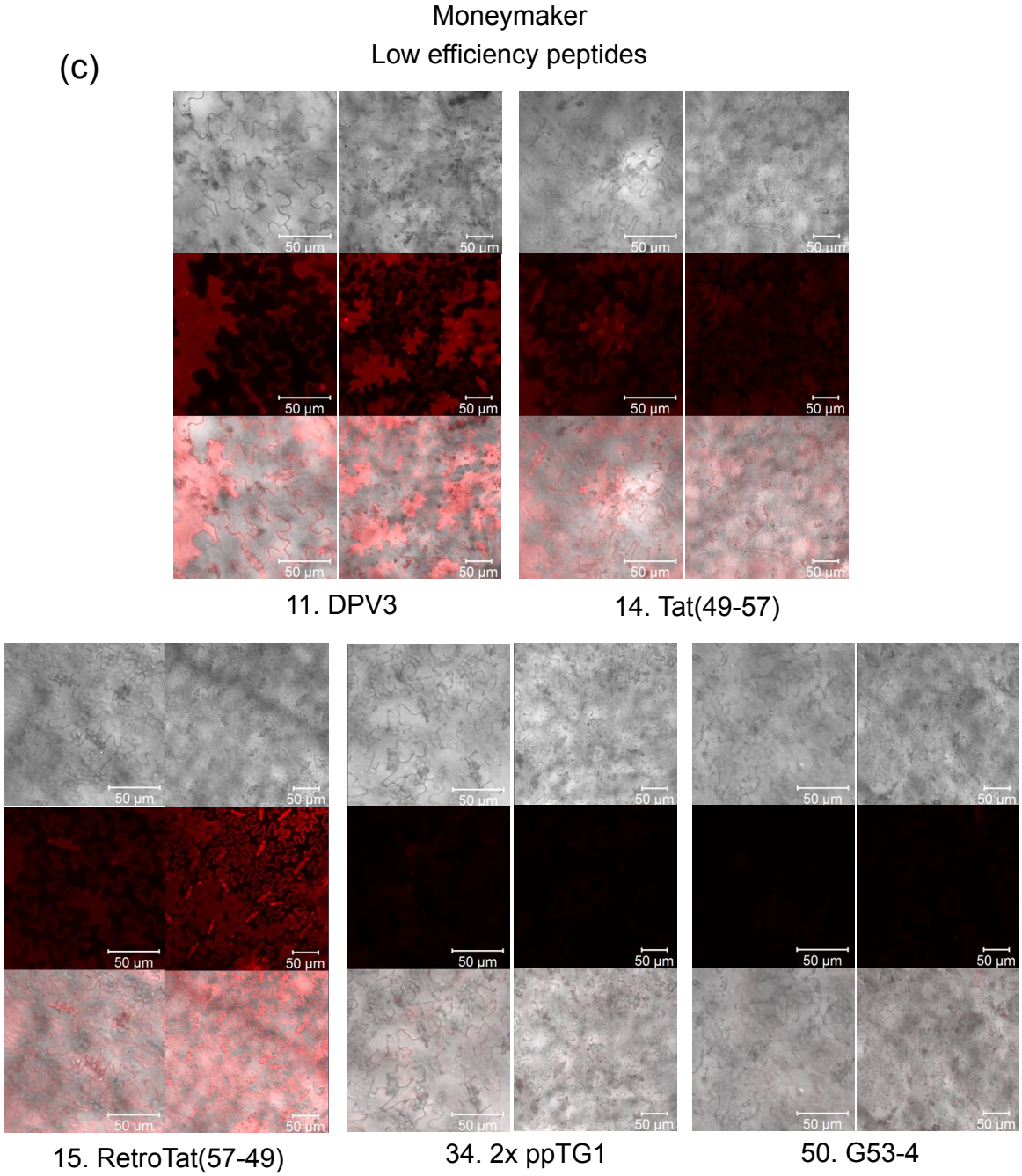
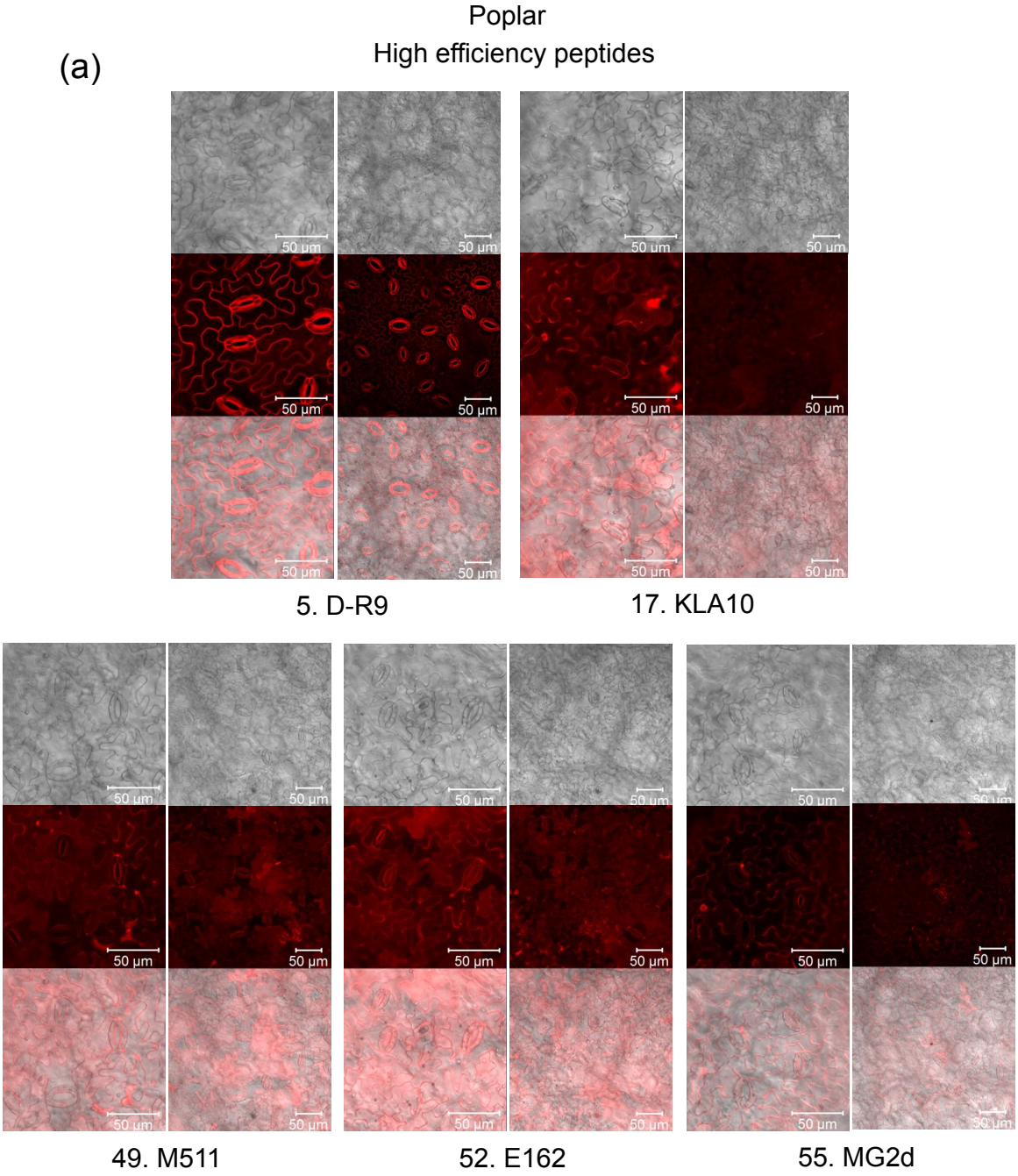


Figure S11. CLSM images of Moneymaker (*S. lycopersicum*) leaf epidermal cells at 2 hours after infiltration of three groups of CPPs at 26ºC. Based on the results with BY-2 cells, the high efficient (a), middle efficient (b) and low efficient CPPs (c) were classified and assayed with intact Moneymaker leaves. Top, middle and bottom images are DIC, TAMRA fluorescence and

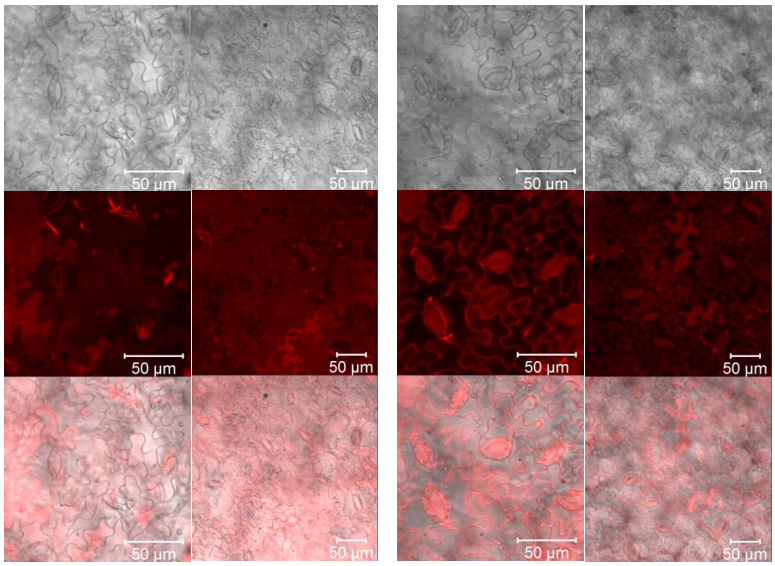
overlay images, respectively. The type of CPP is shown by peptide number and name as listed in Table 1.



(b)

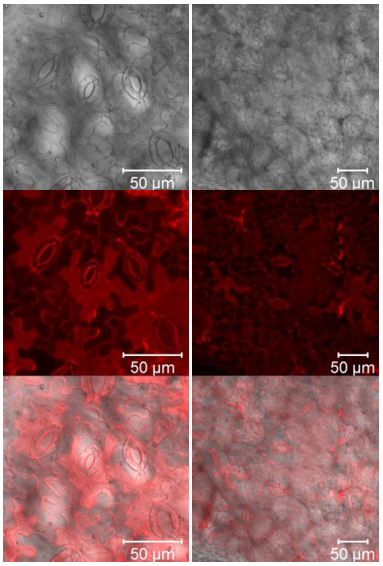
Poplar

Middle efficiency peptides

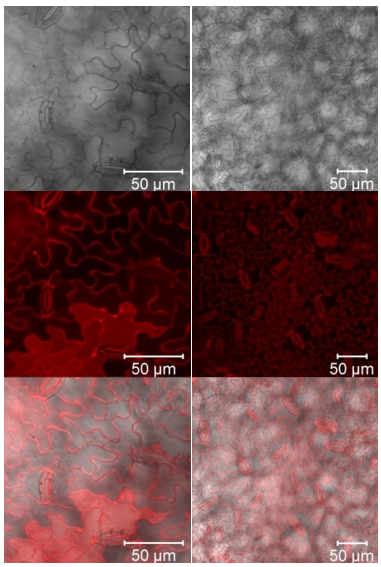


1. BP100

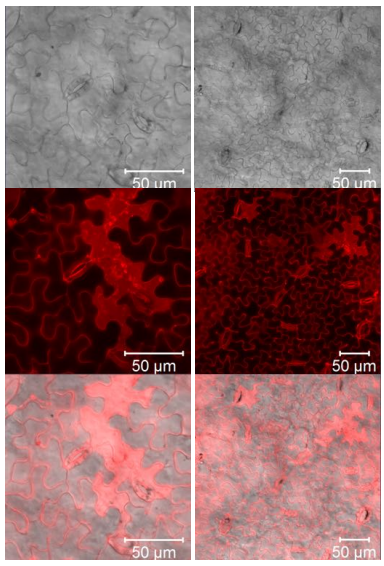
26. dhvar5



27.HPV33L2-445/467



37. Crot(27-39)

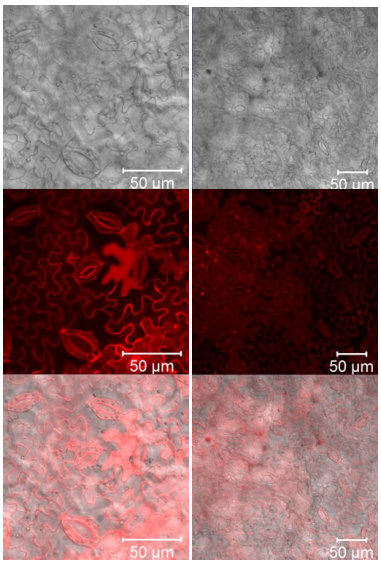


40. CyLoP-1

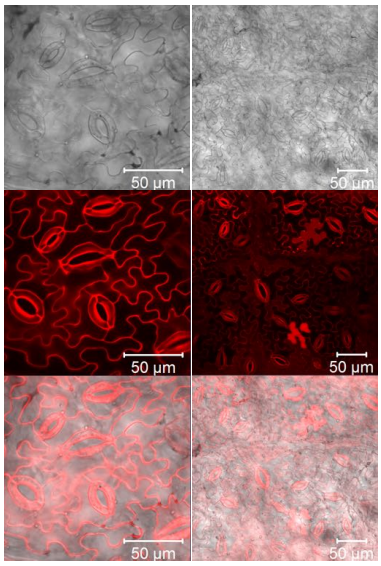
(c)

Poplar

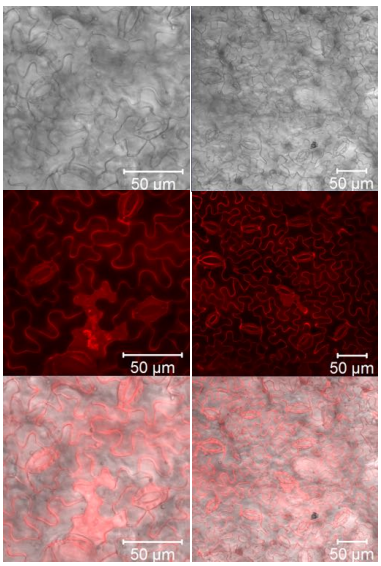
Low efficiency peptides



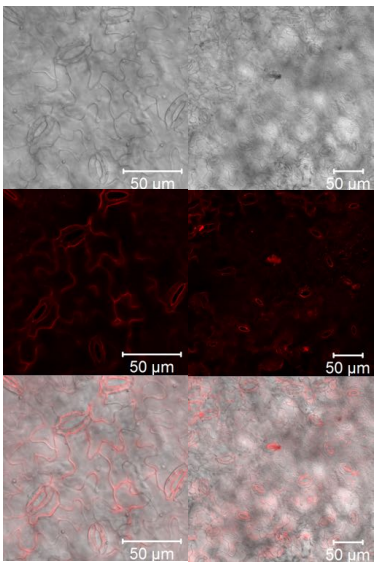
11. DPV3



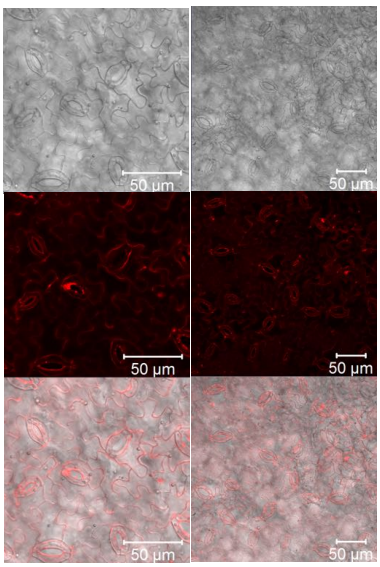
14. Tat(49-57)



15. RetroTat(57-49)

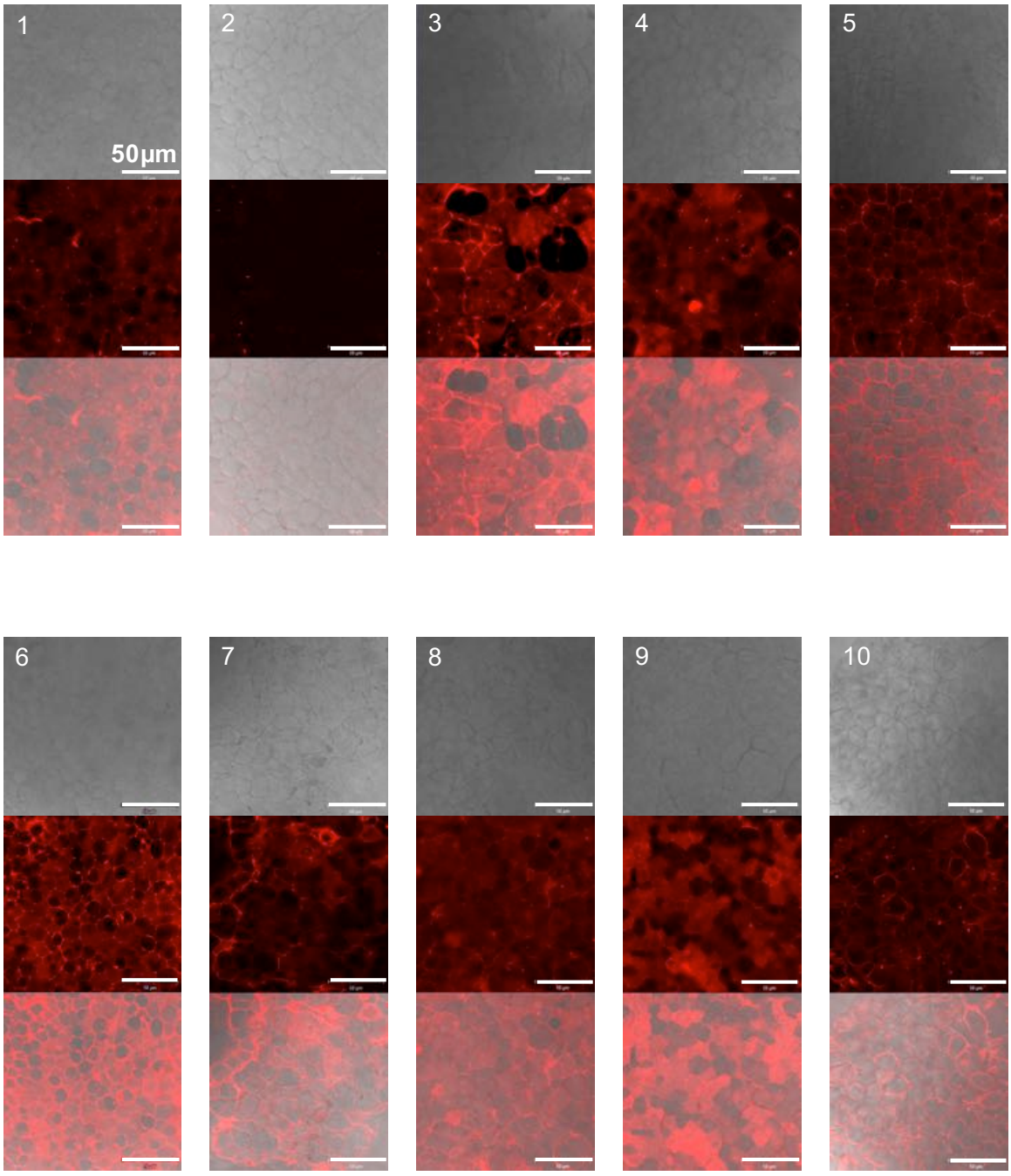


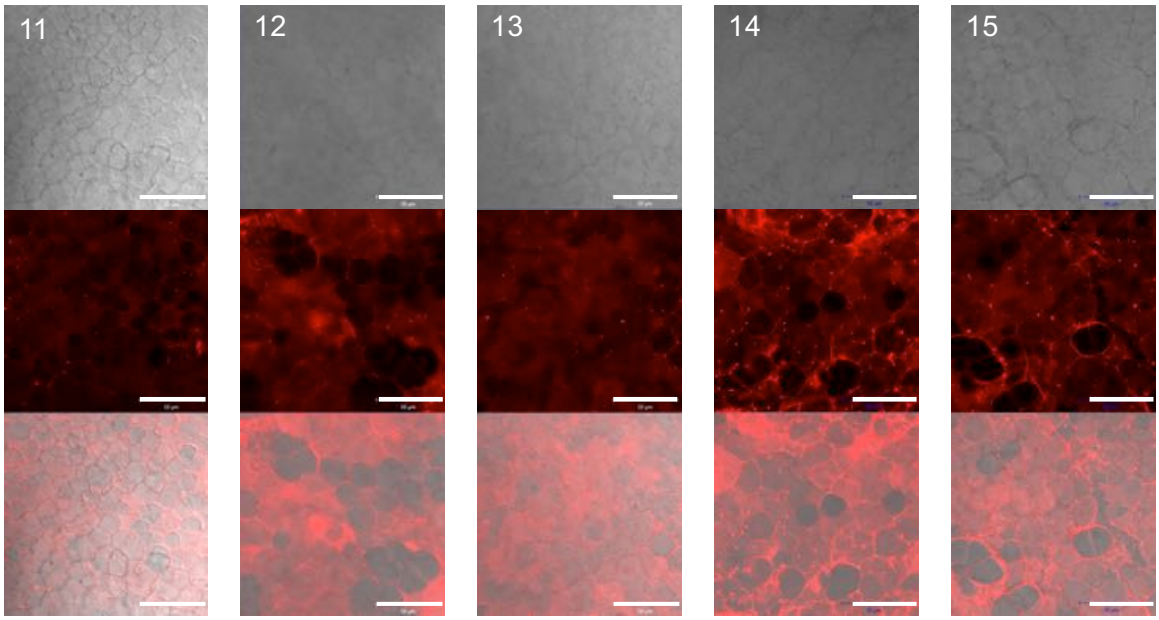
34. 2x ppTG1

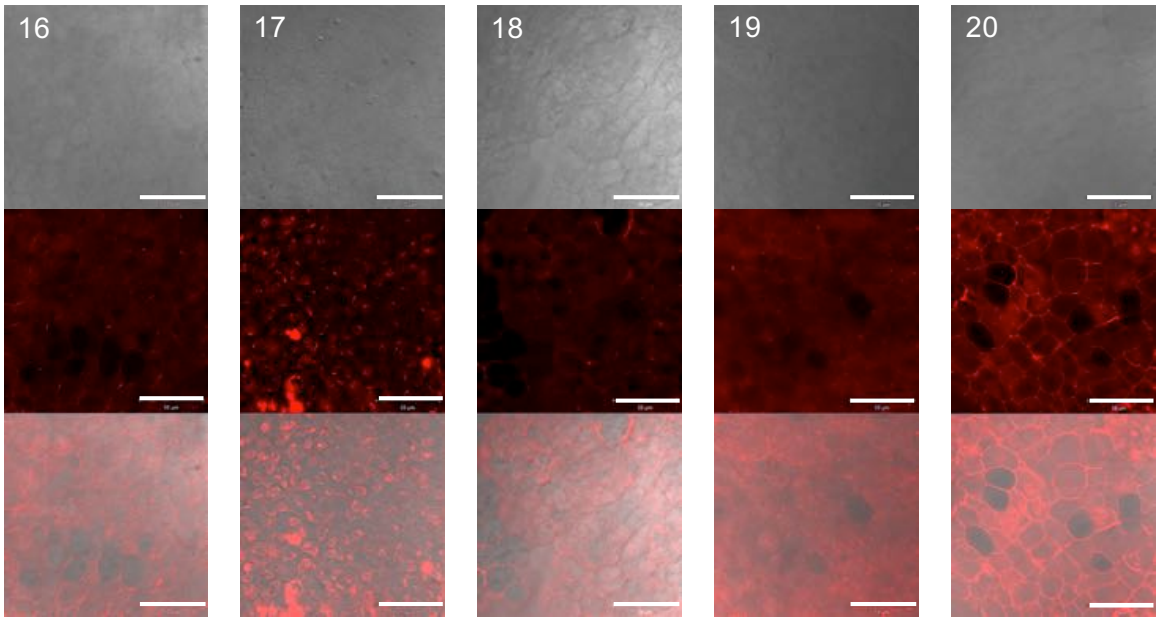


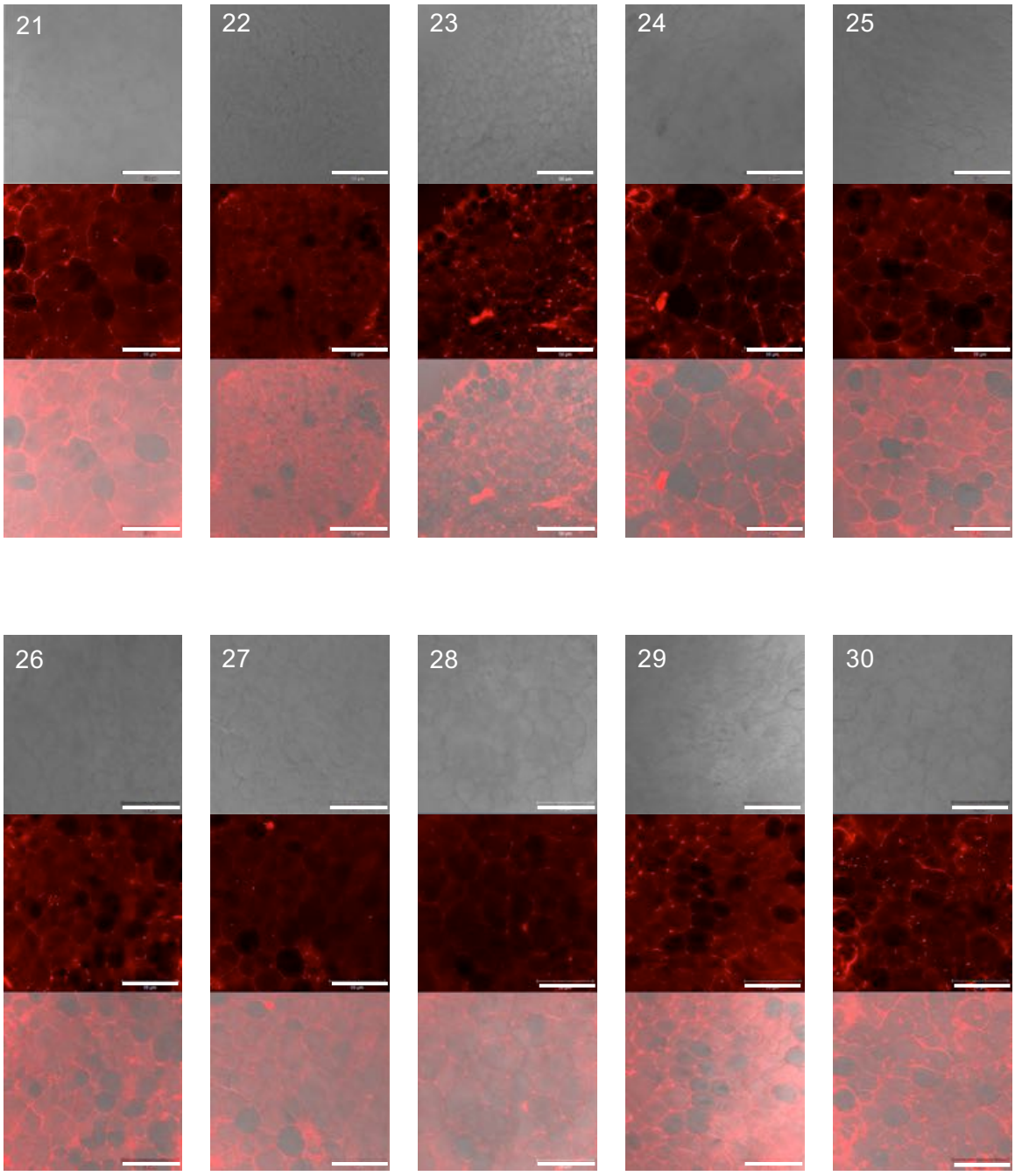
50. G53-4

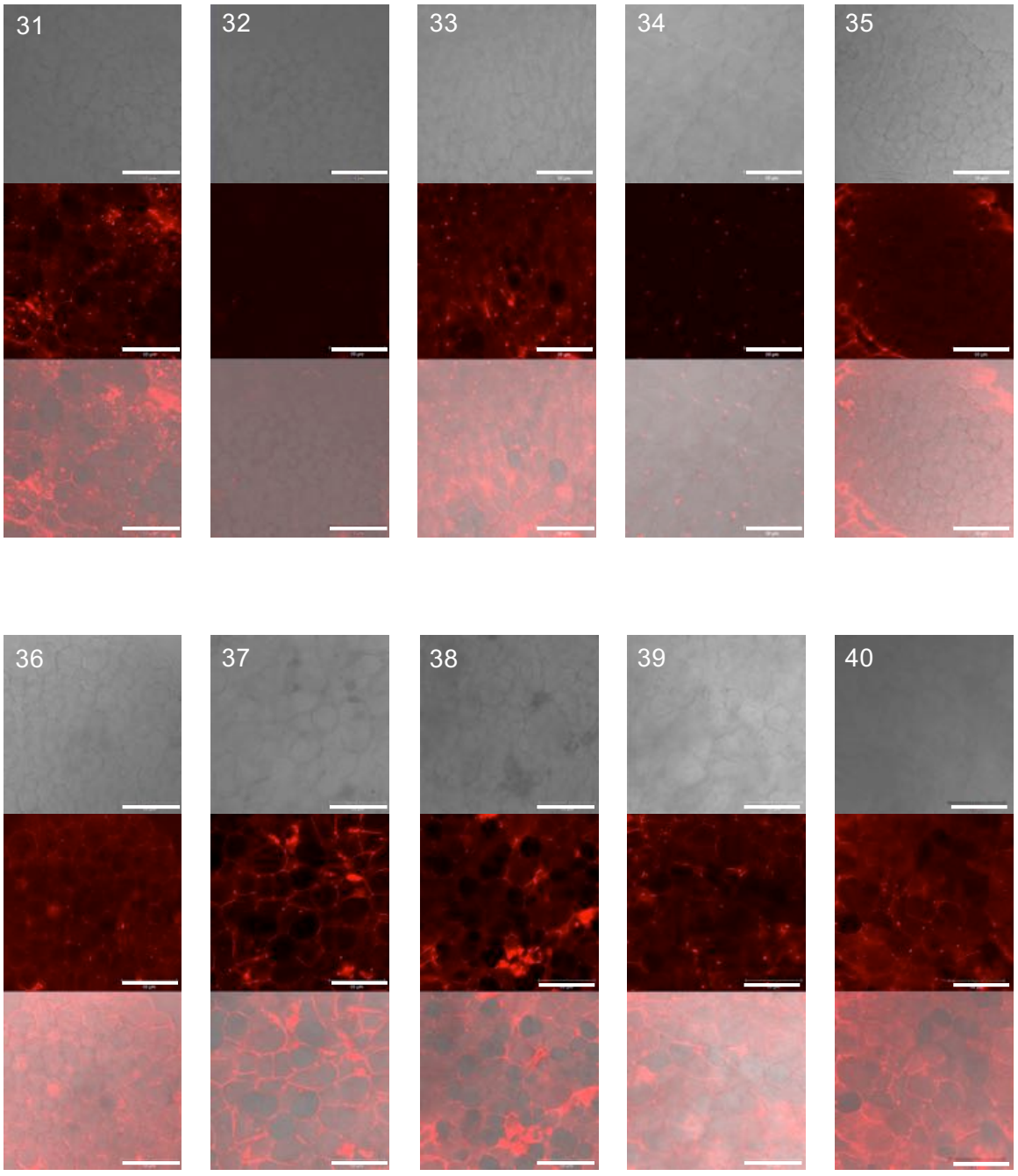
Figure S12. CLSM images of poplar leaf epidermal cells at 2 hours after infiltration of three groups of CPPs at 22ºC. Based on the results with BY-2 cells, the high efficient (a), middle efficient (b) and low efficient CPPs (c) were classified and assayed with intact poplar leaves. Top, middle and bottom images are DIC, TAMRA fluorescence and overlay images, respectively. The type of CPP is shown by peptide number and name as listed in Table 1.


1
50µm
2
3
4
5
6
7
8
9
10


11
12
13
14
15


16
17
18
19
20


21
22
23
24
25
26
27
28
29
30


31
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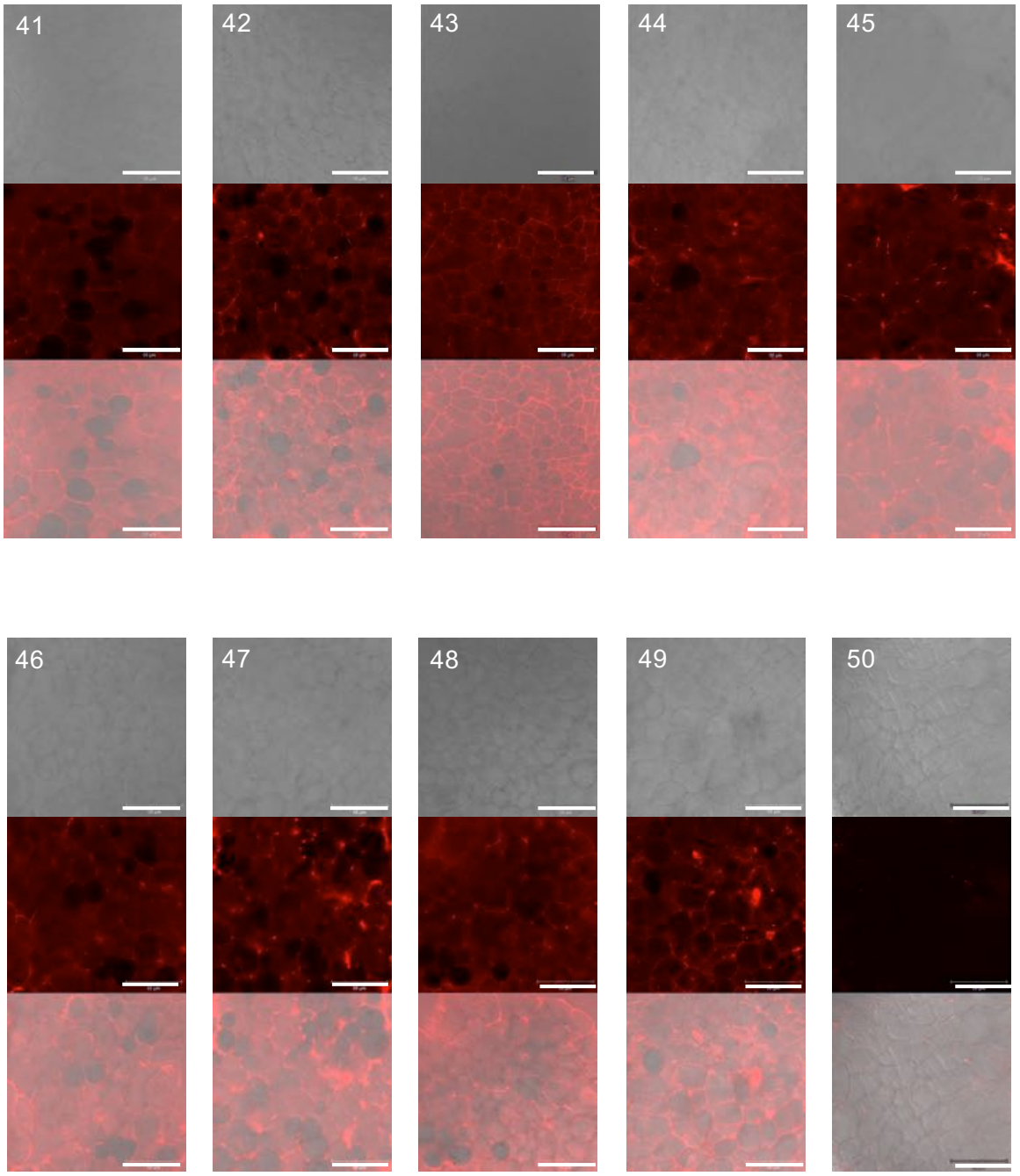
36
37
38
39
40


41
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47
48
49
50

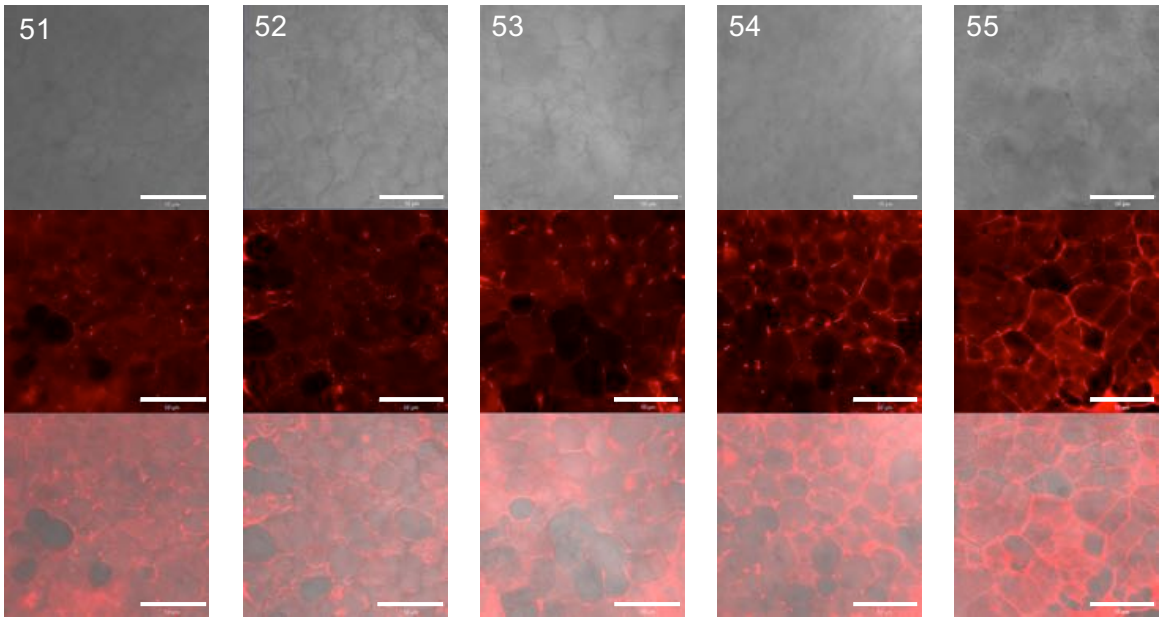


Figure S13. CLSM images of rice (*O. sativa*) callus at 2 hours after infiltration of 55 CPPs at 22ºC. Top, middle and bottom images are DIC, TAMRA fluorescence and overlay images, respectively. The type of CPP is shown by peptide number and name as listed in Table 1. Scale bar, 50 µm.

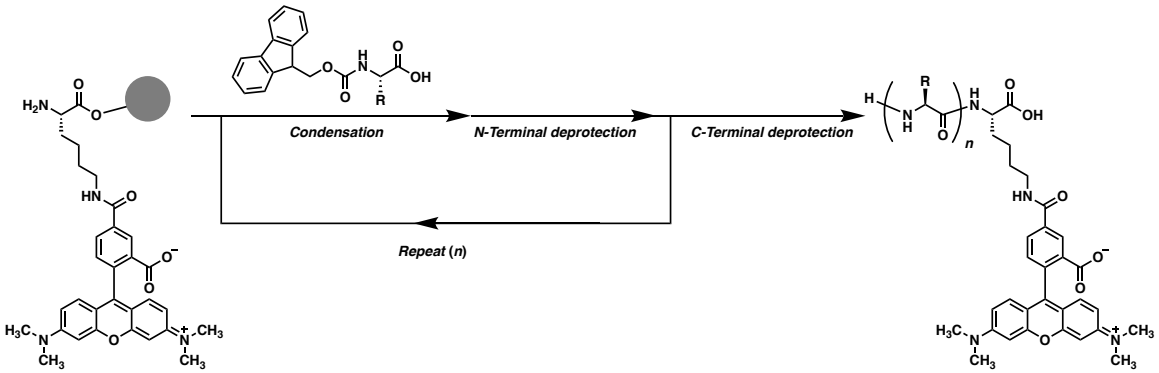
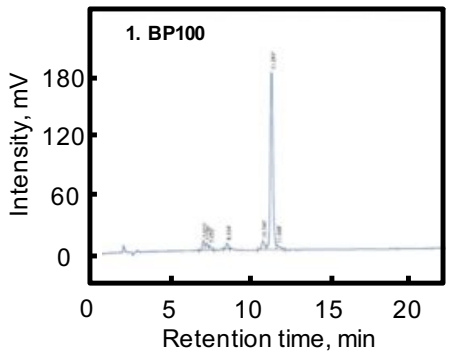
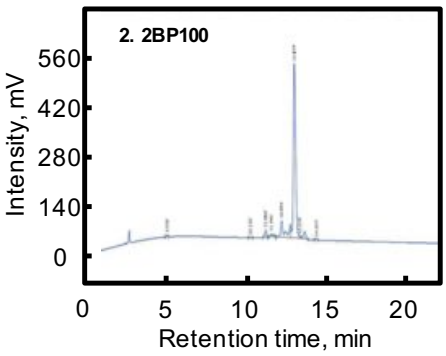
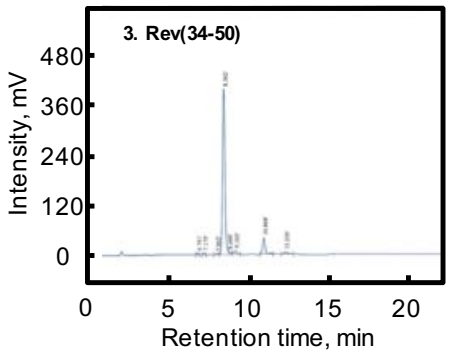
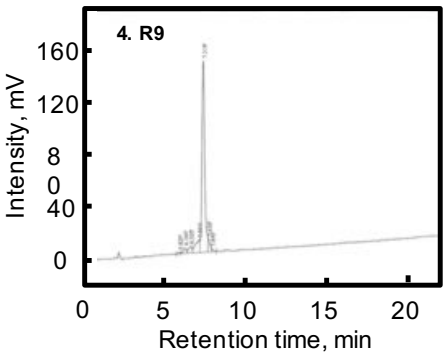


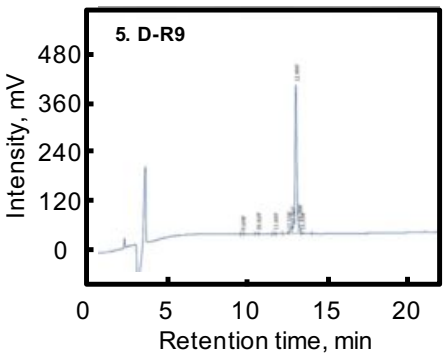
Figure S14. Reaction scheme to synthesize TAMRA-labeled CPP.

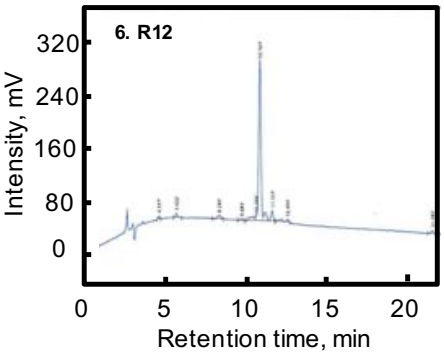

1. BP100
Intensity, mV
180
120
60
0
0
5
10
15
20
Retention time, min

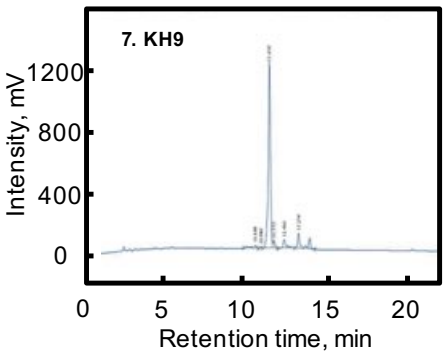

2. 2BP100
Intensity, mV
560
420
280
140
0
0
5
10
15
20
Retention time, min

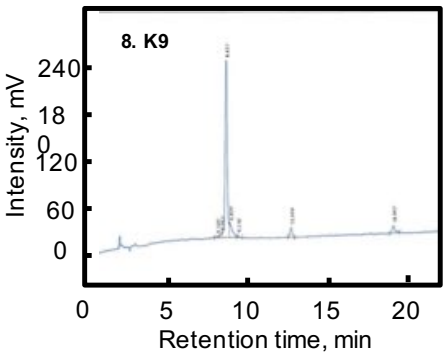

3. Rev(34-50)
Intensity, mV
480
360
240
120
0
0
5
10
15
20
Retention time, min

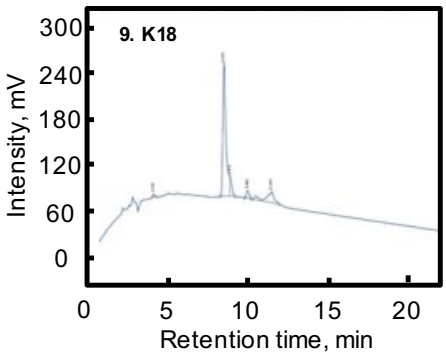

4. R9
Intensity, mV
160
120
8
0
40
0
0
5
10
15
20
Retention time, min

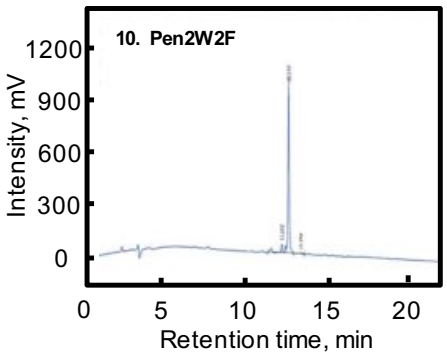

5. D-R9
Intensity, mV
480
360
240
120
0
0
5
10
15
20
Retention time, min

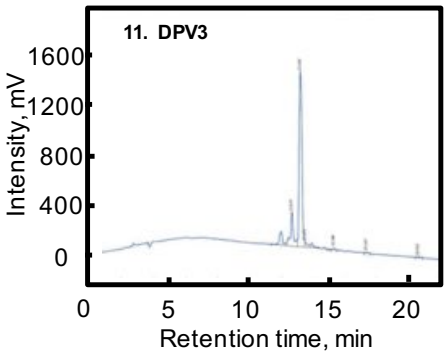

6. R12
Intensity, mV
320
240
160
80
0
0
5
10
15
20
Retention time, min

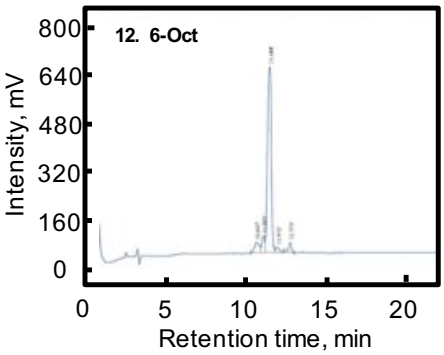

7. KH9
Intensity, mV
1200
800
400
0
0
5
10
15
20
Retention time, min

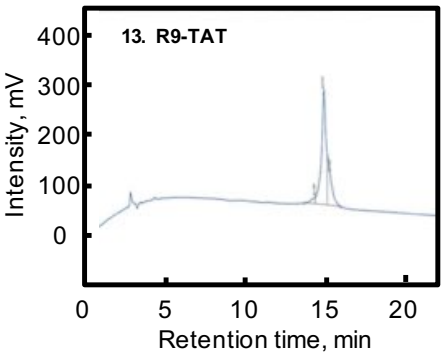

8. K9
Intensity, mV
240
18
0
120
60
0
0
5
10
15
20
Retention time, min

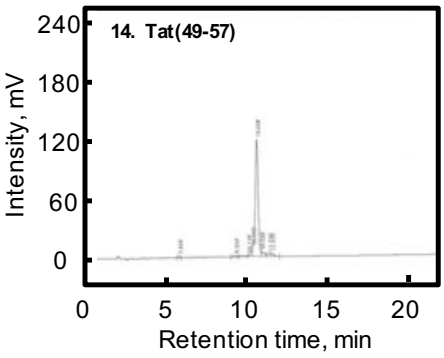

9. K18
Intensity, mV
Retention time, min

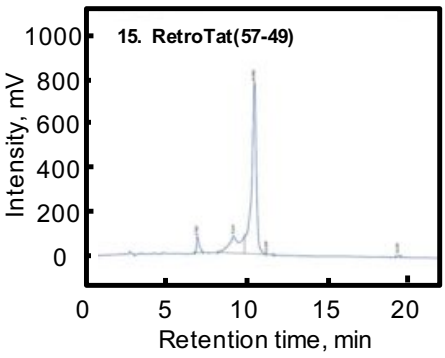

10. Pen2W2F
Intensity, mV
Retention time, min

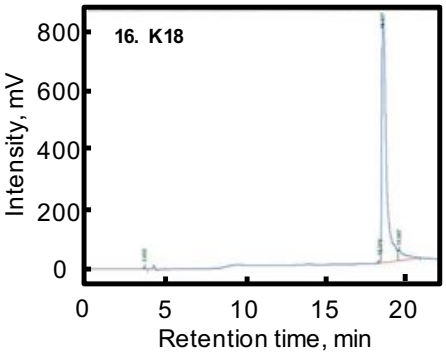

11. DPV3
Intensity, mV
Retention time, min

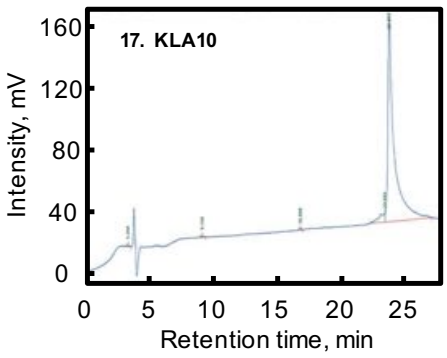

12. 6-Oct
Intensity, mV
Retention time, min

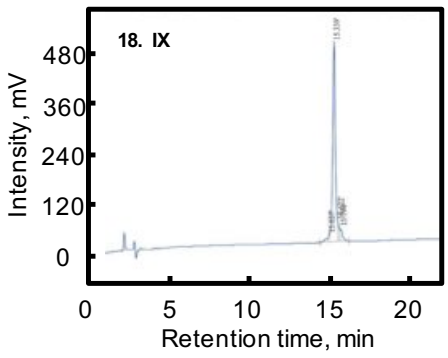

13. R9-TAT
Intensity, mV
Retention time, min

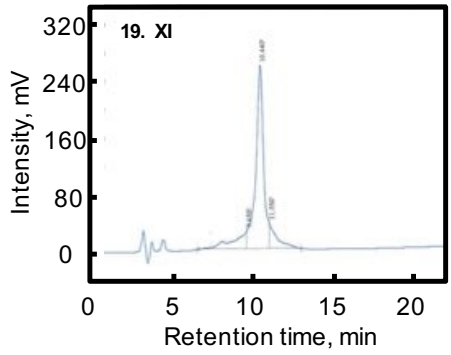

14. Tat(49-57)
Intensity, mV
Retention time, min

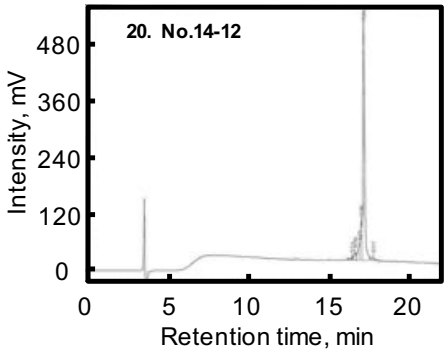

15. RetroTat(57-49)
Intensity, mV
Retention time, min

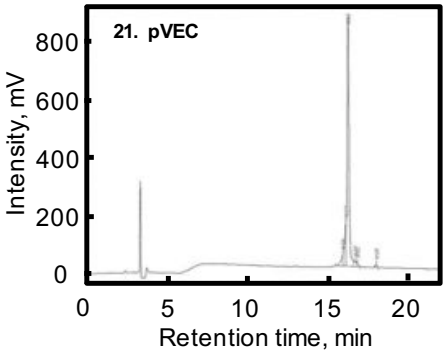

16. K18
Intensity, mV
Retention time, min

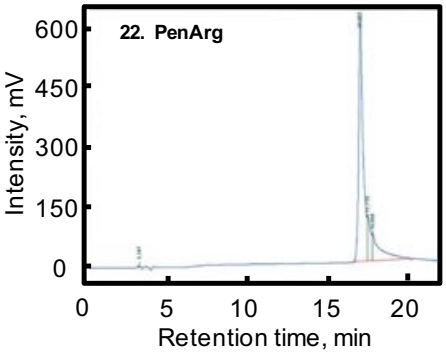

17. KLA10
Intensity, mV
Retention time, min

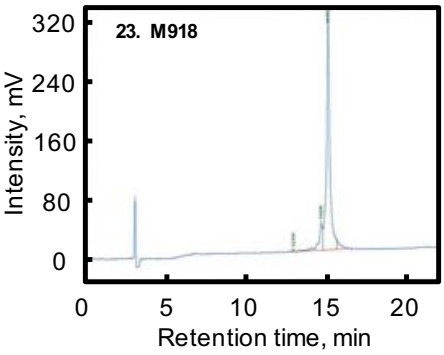

18. IX
Intensity, mV
Retention time, min

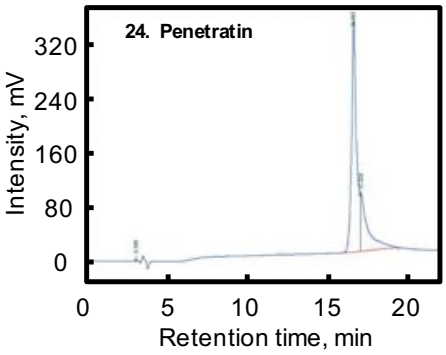

19. XI
Intensity, mV
Retention time, min

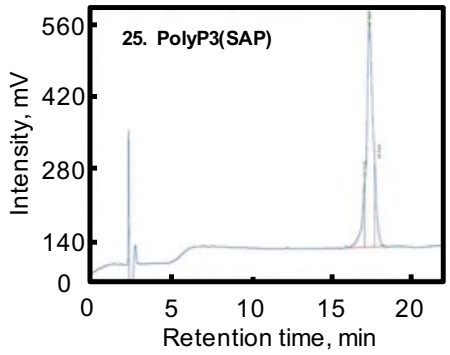

20. No.14-12
Intensity, mV
Retention time, min

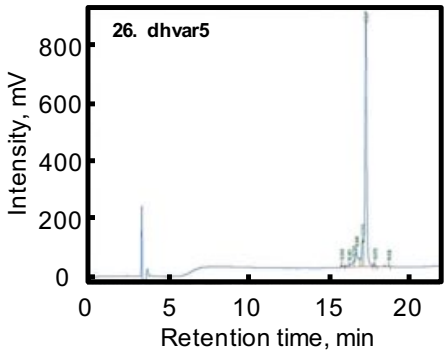

21. pVEC
Intensity, mV
Retention time, min

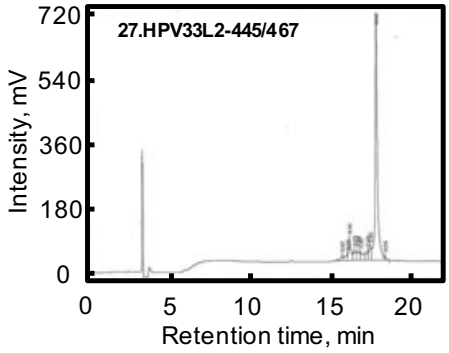

22. PenArg
Intensity, mV
Retention time, min

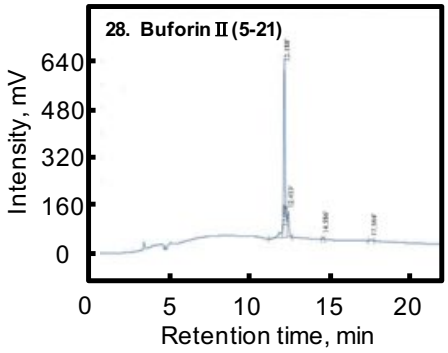

23. M918
Intensity, mV
Retention time, min

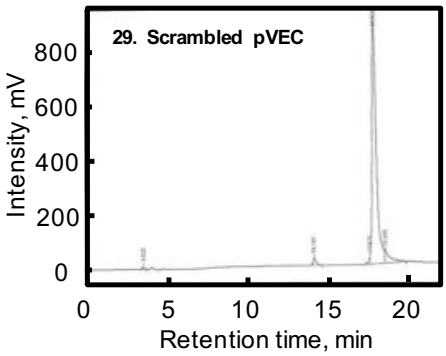

24. Penetratin
Intensity, mV
Retention time, min

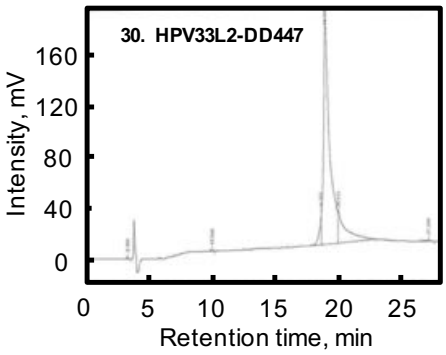

25. PolyP3(SAP)
Intensity, mV
560
420
280
140
0
0
5
10
15
20
Retention time, min

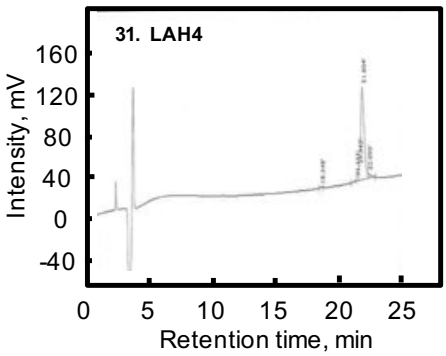

26. dhvar5
Intensity, mV
800
600
400
200
0
0
5
10
15
20
Retention time, min

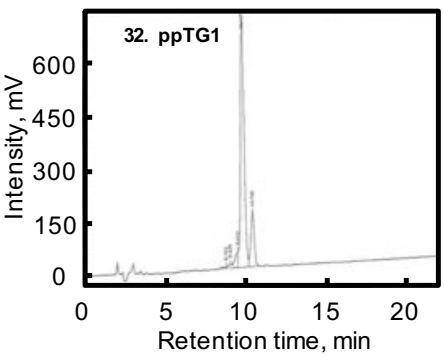

27.HPV33L2-445/467
Intensity, mV
720
540
360
180
0
0
5
10
15
20
Retention time, min

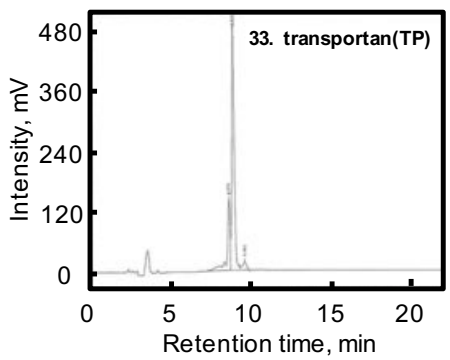

28. Buforin Ⅱ(5-21)
Intensity, mV
640
480
320
160
0
0
5
10
15
20
Retention time, min

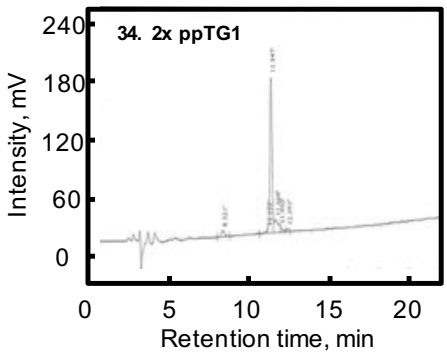

29. Scrambled pVEC
Intensity, mV
800
600
400
200
0
0
5
10
15
20
Retention time, min

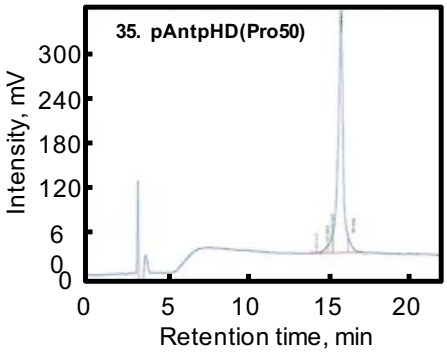

30. HPV33L2-DD447
Intensity, mV
160
120
80
40
0
0
5
10
15
20
25
Retention time, min

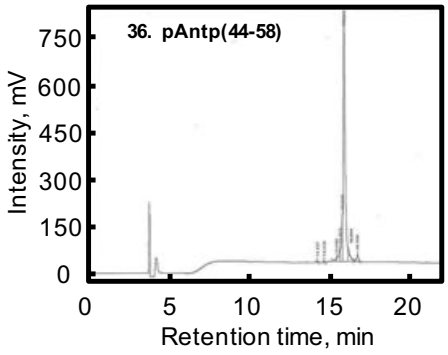

31. LAH4
Intensity, mV
160
120
80
40
0
-40
0
5
10
15
20
25
Retention time, min

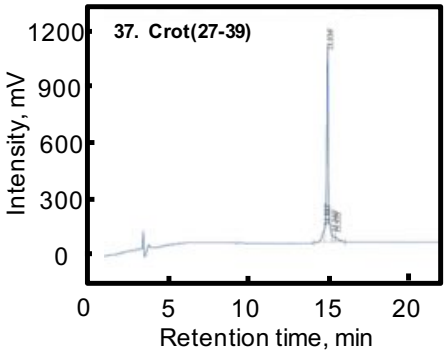

32. ppTG1
Intensity, mV
600
450
300
150
0
0
5
10
15
20
Retention time, min

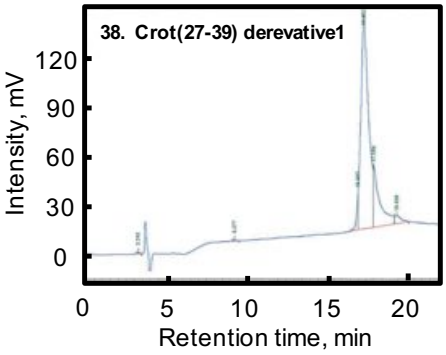

33. transportan(TP)
Intensity, mV
480
360
240
120
0
0
5
10
15
20
Retention time, min

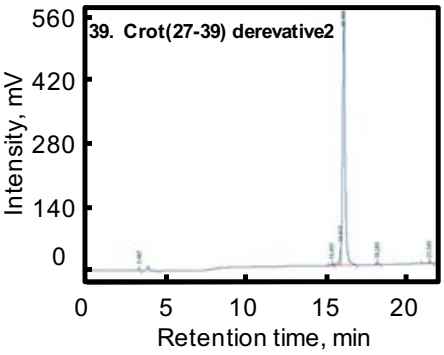

34. 2x ppTG1
Intensity, mV
240
180
120
60
0
0
5
10
15
20
Retention time, min

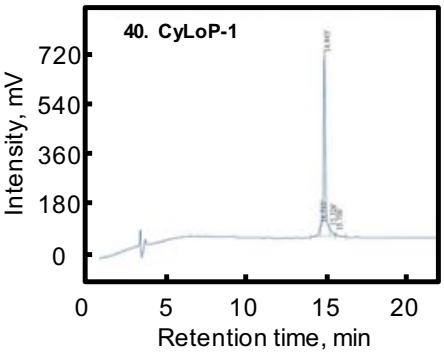

35. pAntpHD(Pro50)
Intensity, mV
300
240
180
120
6
0
0
5
10
15
20
Retention time, min

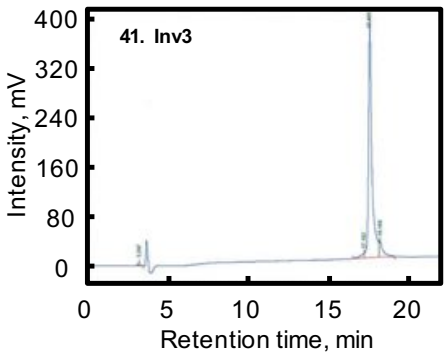

36. pAntp(44-58)
Intensity, mV
750
600
450
300
150
0
0
5
10
15
20
Retention time, min

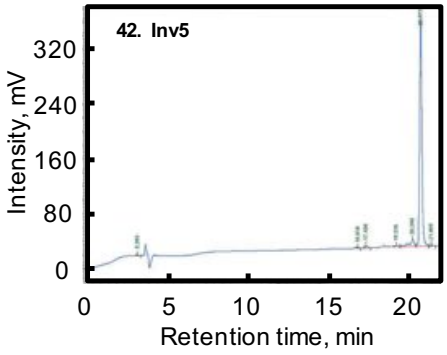

37. Crot(27-39)
Intensity, mV
1200
900
600
300
0
0
5
10
15
20
Retention time, min

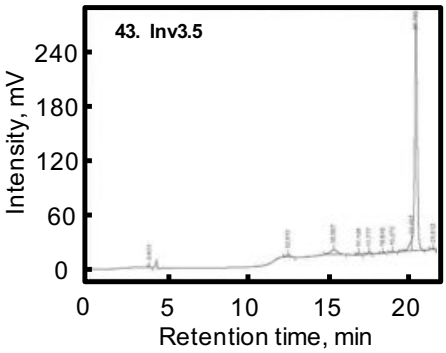

38. Crot(27-39) derevative1
Intensity, mV
120
90
60
30
0
0
5
10
15
20
Retention time, min

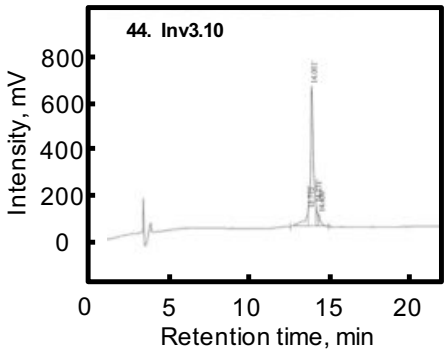

39. Crot(27-39) derevative2
Intensity, mV
560
420
280
140
0
0
5
10
15
20
Retention time, min

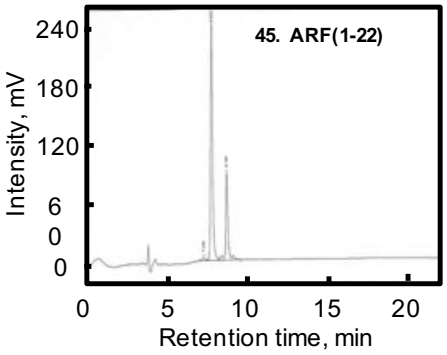

40. CyLoP-1
Intensity, mV
720
540
360
180
0
0
5
10
15
20
Retention time, min

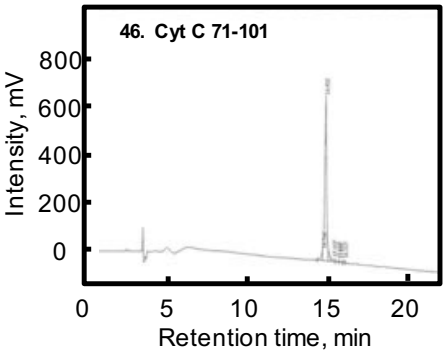

41. Inv3
Intensity, mV
400
320
240
160
80
0
0
5
10
15
20
Retention time, min

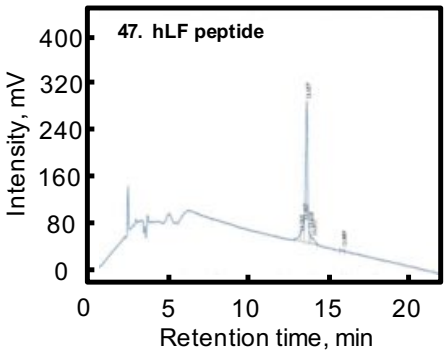

42. Inv5
Intensity, mV
320
240
160
80
0
0
5
10
15
20
Retention time, min

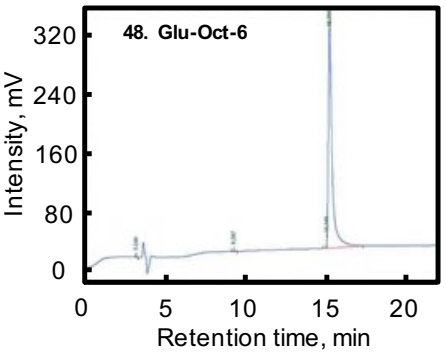

43. Inv3.5
Intensity, mV
240
180
120
60
0
0
5
10
15
20
Retention time, min


44. Inv3.10
Intensity, mV
800
600
400
200
0
0
5
10
15
20
Retention time, min


45. ARF(1-22)
Intensity, mV
240
180
120
6
0
0
0
5
10
15
20
Retention time, min


46. Cyt C 71-101
Intensity, mV
800
600
400
200
0
0
5
10
15
20
Retention time, min


47. hLF peptide
Intensity, mV
400
320
240
160
80
0
0
5
10
15
20
Retention time, min


48. Glu-Oct-6
Intensity, mV
320
240
160
80
0
0
5
10
15
20
Retention time, min

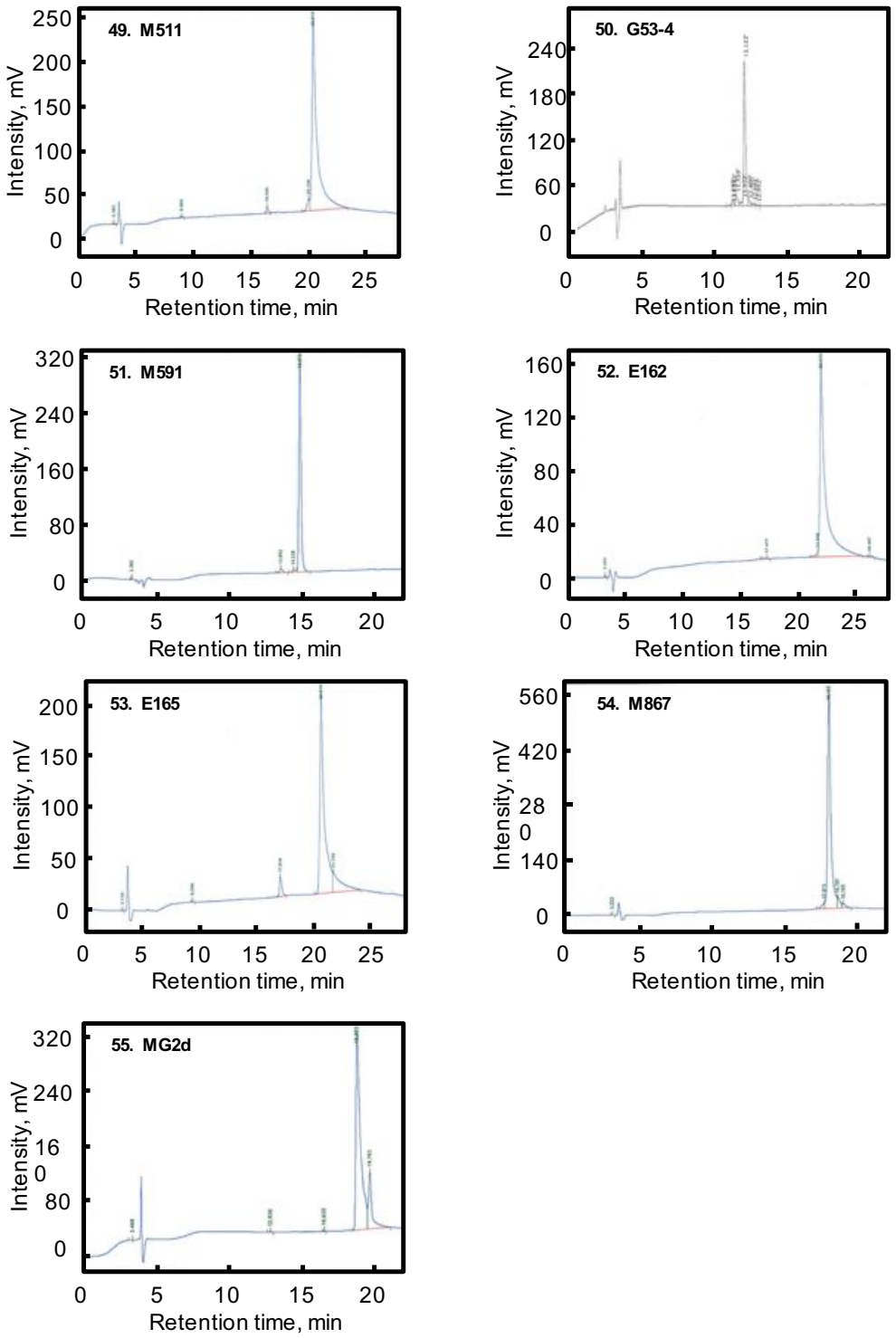


Figure S15. HPLC profiles of the peptides used in this study.

Table S1. Cell penetrating efficiency into various plant types of three groups of CPP. Based on the results with BY-2 cells, the high efficient (a), middle efficient (b) and low efficient CPPs (c) were classified and assayed with their leaves.

Peptide No.	Peptide	BY-2	*N. benthamiana*	*A. thaliana*	poplar	*S. lycopersicum*	*O. sativa*
5	D-R9	95.3±3.1	16.9±14.7	0.7±1.2	5.3±9.2	1.3±0.6	n.d.
17	KLA10	96.3±3.5	22.6±13.4	22.8±11.2	33.3±14.5	5.2±4.8	n.d.
49	M 511	90.0±4.6	27.1±10.0	21.8±8.4	30.7±18.0	3.0±1.7	n.d.
52	E162	93.3±4.5	33.3±5.9	25.5±16.1	22.0±16.4	3.7±2.7	n.d.
55	MG2d	91.3±5.5	19.3±10.5	25.4±8.2	2.7±4.6	18.0±2.2	n.d.
1	BP100	59.3±4.2	76.6±10.4	42.2±3.0	80.6±16.3	14.5±9.6	n.d.
26	dhvar5	54.0±2.6	62.6±25.3	44.3±16.1	12.6±9.9	13.4±4.5	n.d.
27	HPV33L2-445/467	51.7±2.1	47.7±7.5	70.2±7.8	27.1±12.5	65.5±19.6	n.d.
37	Crot(27-39)	51.0±4.6	50.1±13.8	10.1±1.5	21.7±12.2	21.8±9.6	n.d.
40	CyLoP-1	51.0±7.5	65.2±1.0	37.7±15.3	10.7±3.1	51.4±20.7	n.d.
11	DPV3	2.0±2.6	86.3±5.7	70.8±6.0	25.7±3.2	20.2±6.4	n.d.
14	Tat(49-57)	1.7±1.2	51.9±21.7	44.3±11.2	11.4±10.4	28.2±8.4	n.d.
15	Retro-Tat(57-49)	1.3±0.6	51.6±3.5	49.8±19.4	14.0±11.1	17.7±3.2	n.d.
34	2x ppTG1	0.3±0.6	n.d.	n.d.	n.d.	n.d.	n.d.
50	G53-4	n.d.	n.d.	3.1±3.4	21.3±12.2	n.d.	n.d.

n.d. means “not determined”.