## Supporting Information 2

# Rapid Analysis of Protein Farnesyltransferase Substrate Specificity using Peptide Libraries and Isoprenoid Diphosphate 

## Analogs

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Figure S9. Screening of enzymatic prenylation of an $\mathrm{RAGCVa}_{2} X$ library. Each membrane was prenylated with OPP-C15-alkyne by rPFTase ( $55 \mathrm{ug} / \mathrm{mL}$ ), clicked with biotin-azide and then visualized by SP-AP in BCIP solution.

Spot identity:
1-20: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)E 21-40: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)Q 41-60: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)D 61-80: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)N 81-100: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)R 101-120: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)K 121-140: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)H 141-160: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)A 161-180: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)V 181-200: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)I 201-220: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)L 221-240: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)F 241-260: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)Y 261-280: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)W 281-300: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)G 301-320: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)C 321-340: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)M 341-360: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)S 361-380: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)T 381-399: SVI(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,S,T,W,Y,V)

401-410: CTRK, CQRK, CKCI, CSEI, CIII, CKYI, CASL, CTIL, CSGL, CAIL 411-420: CIIL, CIDL, CPFW, CHDE, CRME, CKGE, CCLD, CHHD, CDDD, CALD 421-430: CWKD, CDPN, CRNR, CQGK, CLAK, CRVK, CIGK, CIHH, CYNA, CTVA 431-440: CSNA, CPKA, CYDA, CNDV, CMYV, CFIF, CIQF, CNAG, CCVC, CSIM 441-450: CCIM, CTIM, CIIM, CAIM, CTLM, CCPS, CDFS, CIIS, CCCS, CKQS 451-460: CIKS, CTDS, CKCT, CKQQ, CCIQ, CASQ, CDDY, CAPY, CADY, CPNY
(a)



Figure S10. Screening of enzymatic prenylation of an RAGCCa ${ }_{2} X$ library. Each membrane was prenylated with OPP-C15-alkyne by rPFTase ( $55 \mathrm{ug} / \mathrm{mL}$ in (a), $182 \mathrm{ug} / \mathrm{mL}$ in (b)), clicked with biotin-azide and then visualized by SP-AP in BCIP solution.
Spot identity:
1-20: CC(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)E
21-40: CC(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)Q
41-60: CC(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)D
61-80: CC(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)N
81-100: CC(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)R
101-120: CC(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)K

121-140: CC(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)H 141-160: CC(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)A 161-180: CC(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)V 181-200: CC(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)I 201-220: CC(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)L 221-240: CC(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)F 241-260: CC(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)Y 261-280: CC(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)W 281-300: CC(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)G 301-320: CC(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)C 321-340: CC(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)M 341-360: CC(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)S 361-380: CC(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)T 381-399: SCI(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,S,T,W,Y,V) 401-410: CTRK, CQRK, CKCI, CSEI, CIII, CKYI, CASL, CTIL, CSGL, CAIL 411-420: CIIL, CIDL, CPFW, CHDE, CRME, CKGE, CCLD, CHHD, CDDD, CALD 421-430: CWKD, CDPN, CRNR, CQGK, CLAK, CRVK, CIGK, CIHH, CYNA, CTVA 431-440: CSNA, CPKA, CYDA, CNDV, CMYV, CFIF, CIQF, CNAG, CCVC, CSIM 441-450: CCIM, CTIM, CIIM, CAIM, CTLM, CCPS, CDFS, CIIS, CCCS, CKQS 451-460: CIKS, CTDS, CKCT, CKQQ, CCIQ, CASQ, CDDY, CAPY, CADY, CPNY


Figure S11. Screening of enzymatic prenylation of an $\mathrm{RAGCVa}_{2} \mathrm{X}$ library. Each membrane was prenylated with OPP-C15-alkyne by CaPFTase ( $4.2 \mathrm{ug} / \mathrm{mL}$ ), clicked with biotin-azide and then visualized by SP-AP in BCIP solution.
Spot identity:
1-20: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)E
21-40: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)Q
41-60: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)D
61-80: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)N
81-100: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)R
101-120: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)K

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121-140: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)H
141-160: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)A
161-180: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)V
181-200: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)I
201-220: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)L
221-240: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)F
241-260: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)Y
261-280: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)W
281-300: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)G
301-320: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)C
321-340: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)M
341-360: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)S
361-380: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)T
381-399: SVI(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,S,T,W,Y,V)
401-410: CTRK, CQRK, CKCI, CSEI, CIII, CKYI, CASL, CTIL, CSGL, CAIL
411-420: CIIL, CIDL, CPFW, CHDE, CRME, CKGE, CCLD, CHHD, CDDD, CALD
421-430: CWKD, CDPN, CRNR, CQGK, CLAK, CRVK, CIGK, CIHH, CYNA, CTVA
431-440: CSNA, CPKA, CYDA, CNDV, CMYV, CFIF, CIQF, CNAG, CCVC, CSIM
441-450: CCIM, CTIM, CIIM, CAIM, CTLM, CCPS, CDFS, CIIS, CCCS, CKQS
451-460: CIKS, CTDS, CKCT, CKQQ, CCIQ, CASQ, CDDY, CAPY, CADY, CPNY
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(a)

1
(b)


Figure S12. Screening of enzymatic prenylation of an $R A G C V a_{2} X$ library. Each membrane was prenylated with OPP-C15-alkyne by yPFTase ( $2.7 \mathrm{ug} / \mathrm{mL}$ ) , clicked with biotin-azide and then visualized by SP-AP in BCIP solution.
Spot identity:
(a)

1-20: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)E
21-40: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)Q

41-60: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)D 61-80: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)N 81-100: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)R 101-120: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)K 121-140: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)H 141-160: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)A 161-180: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)V 181-200: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)I 201-220: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)L 221-240: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)F 241-260: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)W 261-280: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)G 281-300: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)C 301-320: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)M 321-340: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)S 341-360: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)T 361-370, 421-430: CTRK, CQRK, CKCI, CSEI, CIII, CKYI, CASL, CTIL, CSGL, CAIL 371-380, 431-440: CIIL, CIDL, CPFW, CHDE, CRME, CKGE, CCLD, CHHD, CDDD, CALD 381-390, 441-450: CWKD, CDPN, CRNR, CQGK, CLAK, CRVK, CIGK, CIHH, CYNA, CTVA 391-400, 451-460: CSNA, CPKA, CYDA, CNDV, CMYV, CFIF, CIQF, CNAG, CCVC, CSIM 401-410, 461-470: CCIM, CTIM, CIIM, CAIM, CTLM, CCPS, CDFS, CIIS, CCCS, CKQS 411-420, 471-480: CIKS, CTDS, CKCT, CKQQ, CCIQ, CASQ, CDDY, CAPY, CADY, CPNY 481-499: SVI(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,V)
(b)

1-20: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)E 21-40: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)Q 41-60: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)D 61-80: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)N 81-100: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)R 101-120: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)K 121-140: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)H 141-160: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)A 161-180: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)V 181-200: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)I 201-220: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)L 221-240: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)F 241-260: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)Y 261-280: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)W

281-300: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)G
301-320: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)C
321-340: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)M
341-360: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)S
361-380: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)T
381-399: SVI(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,S,T,W,Y,V)
401-410: CTRK, CQRK, CKCI, CSEI, CIII, CKYI, CASL, CTIL, CSGL, CAIL
411-420: CIIL, CIDL, CPFW, CHDE, CRME, CKGE, CCLD, CHHD, CDDD, CALD
421-430: CWKD, CDPN, CRNR, CQGK, CLAK, CRVK, CIGK, CIHH, CYNA, CTVA 431-440: CSNA, CPKA, CYDA, CNDV, CMYV, CFIF, CIQF, CNAG, CCVC, CSIM 441-450: CCIM, CTIM, CIIM, CAIM, CTLM, CCPS, CDFS, CIIS, CCCS, CKQS 451-460: CIKS, CTDS, CKCT, CKQQ, CCIQ, CASQ, CDDY, CAPY, CADY, CPNY


Figure S13. Screening of enzymatic prenylation of an $\mathrm{RAGCVa}_{2} X$ library. Each membrane was prenylated with OPP-C10-alkyne by rPFTase ( $40 \mathrm{ug} / \mathrm{mL}$ ), clicked with biotin-azide and then visualized by SP-AP in BCIP solution.

Spot identity:
1-20: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)E
21-40: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)Q
41-60: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)D
61-80: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)N
81-100: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)R

101-120: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)K 121-140: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)H 141-160: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)A 161-180: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)V 181-200: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)I 201-220: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)L 221-240: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)F 241-260: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)Y 261-280: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)W 281-300: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)G 301-320: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)C 321-340: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)M 341-360: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)S 361-380: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)T 381-399: SVI(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,S,T,W,Y,V) 401-410: CTRK, CQRK, CKCI, CSEI, CIII, CKYI, CASL, CTIL, CSGL, CAIL 411-420: CIIL, CIDL, CPFW, CHDE, CRME, CKGE, CCLD, CHHD, CDDD, CALD 421-430: CWKD, CDPN, CRNR, CQGK, CLAK, CRVK, CIGK, CIHH, CYNA, CTVA 431-440: CSNA, CPKA, CYDA, CNDV, CMYV, CFIF, CIQF, CNAG, CCVC, CSIM 441-450: ССIM, СТIM, CIIM, CAIM, CTLM, CCPS, CDFS, CIIS, CCCS, CKQS 451-460: CIKS, CTDS, CKCT, CKQQ, CCIQ, CASQ, CDDY, CAPY, CADY, CPNY
(a)

(b)

1

Figure S14. Screening of enzymatic prenylation of an $\mathrm{RAGCVa}_{2} \mathrm{X}$ library. Each membrane was prenylated with OPP-C5-alkyne by rPFTase ( $55 \mathrm{ug} / \mathrm{mL}$ ), clicked with biotin-azide and then visualized by SP-AP in BCIP solution. (a) The membrane is from Intavis (b) The membrane is from Whatman540 Spot identity:
1-20: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)E
21-40: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)Q
41-60: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)D
61-80: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)N

```
81-100: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)R
101-120: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)K
121-140: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)H
141-160: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)A
161-180: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)V
181-200: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)I
201-220: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)L
221-240: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)F
241-260: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)Y
261-280: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)W
281-300: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)G
301-320: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)C
321-340: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)M
341-360: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)S
361-380: CV(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V)T
381-399: SVI(A,R,N,D,C,Q,E,G,H,I,L,K,M,F,S,T,W,Y,V)
401-410: CTRK, CQRK, CKCI, CSEI, CIII, CKYI, CASL, CTIL, CSGL, CAIL
411-420: CIIL, CIDL, CPFW, CHDE, CRME, CKGE, CCLD, CHHD, CDDD, CALD
421-430: CWKD, CDPN, CRNR, CQGK, CLAK, CRVK, CIGK, CIHH, CYNA, CTVA
431-440: CSNA, CPKA, CYDA, CNDV, CMYV, CFIF, CIQF, CNAG, CCVC, CSIM
441-450: CCIM, CTIM, CIIM, CAIM, CTLM, CCPS, CDFS, CIIS, CCCS, CKQS
451-460: CIKS, CTDS, CKCT, CKQQ, CCIQ, CASQ, CDDY, CAPY, CADY, CPNY
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RAGCCIE $\left(\mathrm{M}+\mathrm{H}^{+} \mathrm{m} / \mathrm{z}\right.$ calc. $\left.=751.323\right)$



RAGCCII ( $\mathrm{M}+\mathrm{H}^{+} \mathrm{m} / \mathrm{z}$ calc. $=735.364$ )




RAGCCIM $\left(\mathrm{M}+\mathrm{H}^{+} \mathrm{m} / \mathrm{z}\right.$ calc. $\left.=753.320\right)$



Figure S15. MALDI analysis of peptides produced by SPOT synthesis performed to verify the production of the desired product. The photocleavable moiety shown in Scheme S 2 was used as the linker. The individual spot was treated under UV lamp ( 365 nm ) in $\mathrm{CH}_{3} \mathrm{CN} / \mathrm{H}_{2} \mathrm{O}$ ( $\mathrm{v} / \mathrm{v}=5 / 95$ ) for 2 h . The spots were washed with $\mathrm{CH}_{3} \mathrm{CN}$ and $\mathrm{CH}_{3} \mathrm{OH}$. The combined solvents were removed in vacuo. The peptides (RAGCCIX, $\mathrm{X} \neq \mathrm{P}$ ) were re-dissolved in $\mathrm{CH}_{3} \mathrm{CN} / \mathrm{H}_{2} \mathrm{O}(\mathrm{v} / \mathrm{v}=50 / 50)$ with $0.1 \% \mathrm{TFA}$. The $\mathrm{m} / \mathrm{z}$ shown was manually calibrated by an internal standard $\left(3 \mathrm{XCCA}+\mathrm{H}^{+}\right)$. Peptides ending with CCIC, CCIG, CCIW either had low signal intensity or cannot be found.

