1 Investigating the specificity of the dehydration and cyclization reactions

2 in engineered lanthipeptides by synechococcal SyncM

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15 **Supporting Information for Publication**
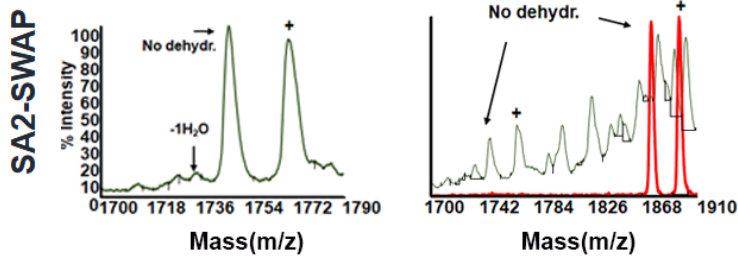16

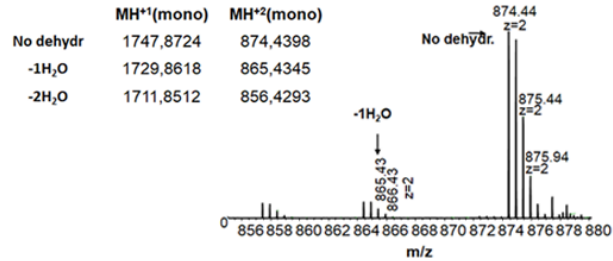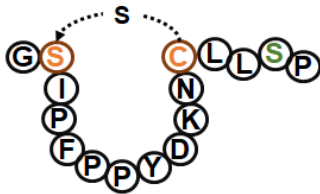17 **FIGURES AND TABLES**

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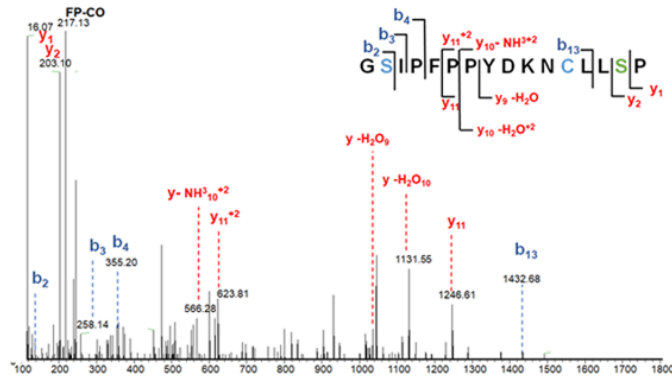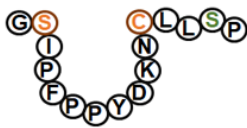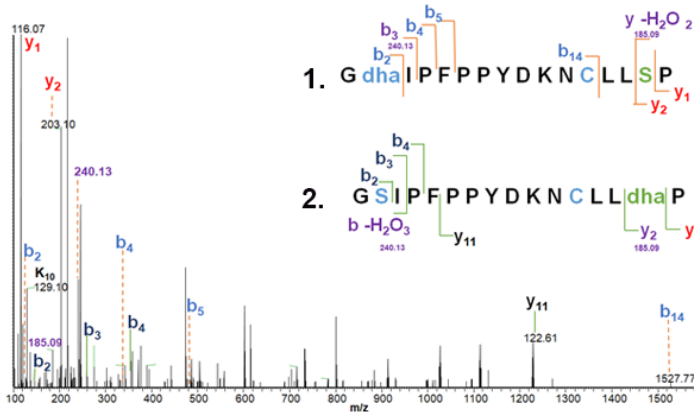
Supplementary Figure S1. Overview of SA2-SWAP of SyncA2 mutants. Dehydration and cyclization state analysis. A) MALDI-TOF spectra of SA2-SWAP; the dehydration state is depicted by arrows. Ring analysis with N-ethylmaleimide (NEM, red spectra correspond after NEM reaction). (+) Na-adduct B) LC-MS spectra. Detected masses

26   were found to correspond to non-dehydrated and -1H_2O peptides. C) MS^2 spectra of

27   the non-dehydrated peptide. Ion fragments of the unmodified peptide are indicated. D)

28   MS^2 spectra of the -1H_2O peptide. Ion fragments of -1H_2O unmodified peptide are

29   indicated. Two options for dehydration were found. Form 1 with the S2 dehydrated.

30   Form 2 with the S15 dehydrated. Ion fragments of each form are differentiated with

31   color. (1-Orange, 2-Dark blue, same ion-Red). Masses that could correspond to both

32   peptides are identified in purple.

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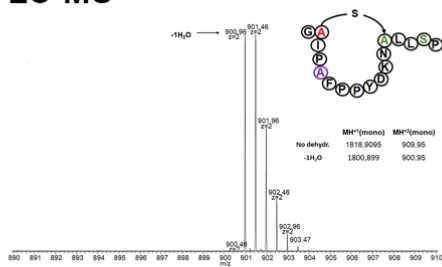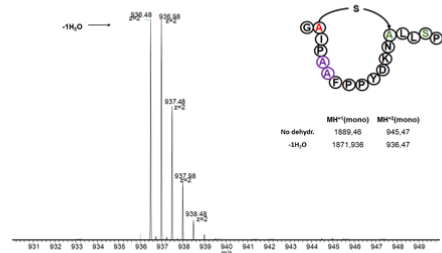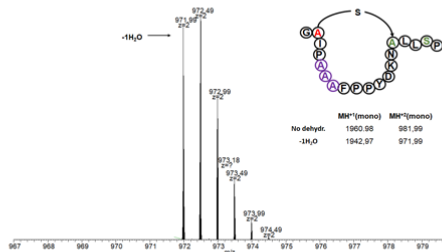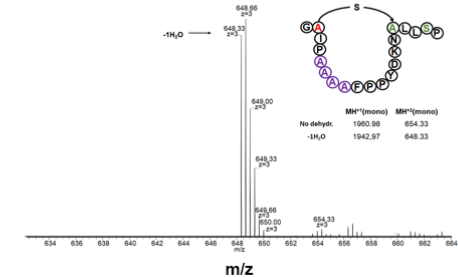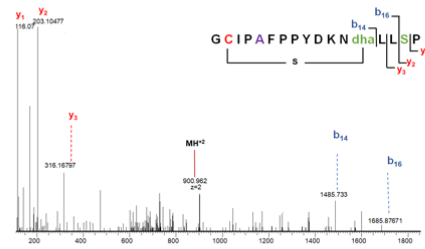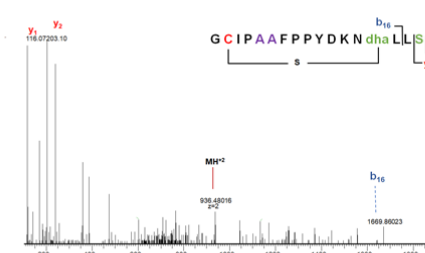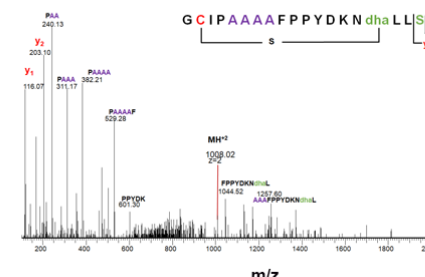
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37 **Supplementary Figure S2**. LC-MS/MS spectra of SyncA2 macrocycle variants. A)

38 Shows the LC-MS chromatograms. Expected dehydration was found in all mutants.

39 SA2-+1A, SA2-+2A, SA2-+3A and SA2-+4[a]. B) Ring formation analysis showing MS[2]

40 spectra of each macrocycle mutant: SA2-+1A, SA2-+2A, SA2-+3A, and SA2-+4[a]

41 Fragmented ions, corresponding with the two-time mass charge in each mutant, are

42 depicted. A lanthionine ring was installed by SyncM in all variants.

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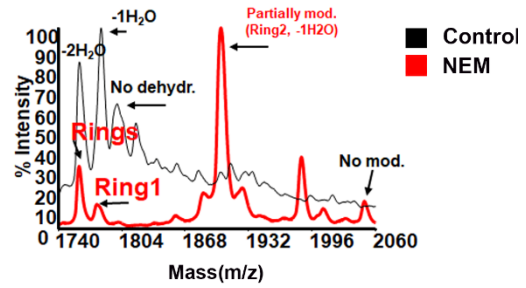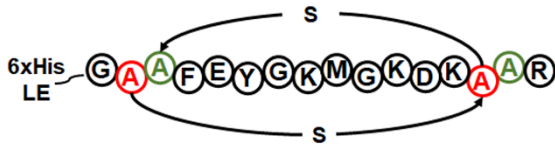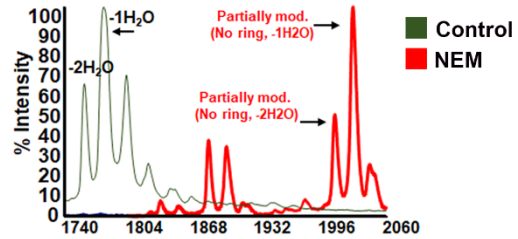
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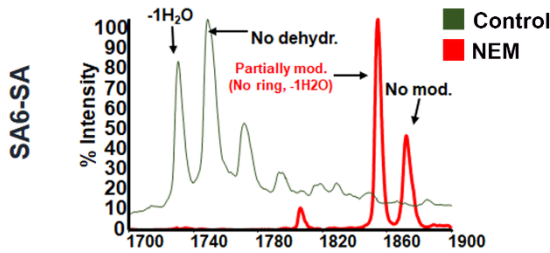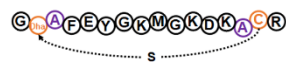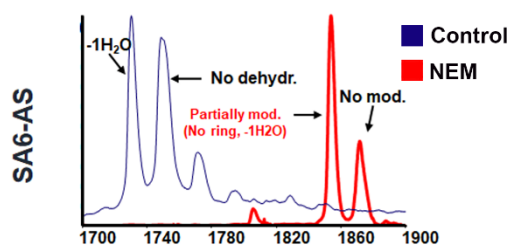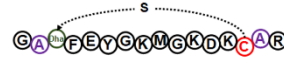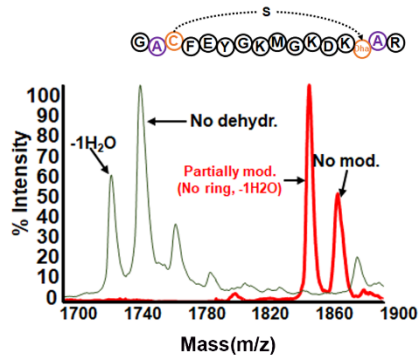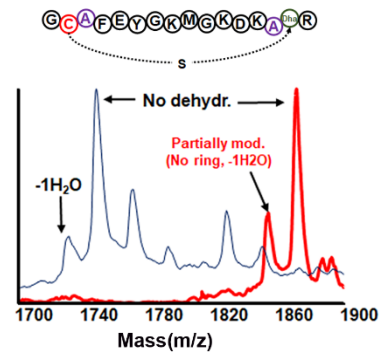
Supplementary Figure S3. Ring analysis of SyncA6 mutants with the NEM reaction.

A) SyncA6 control. B) Mutants with a change in the dehydration and ring direction. C)

Single-ring mutants. Red lines correspond to spectra after NEM reaction and back line

50 (black, green and blue) depicts control spectra. No ring was formed in any of the

51 mutants.

A) MALDI-TOF non-cyclized
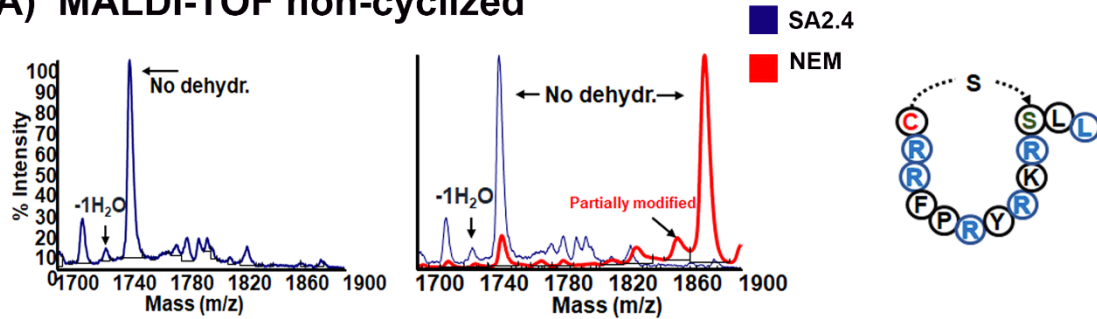
B) Ring analysis
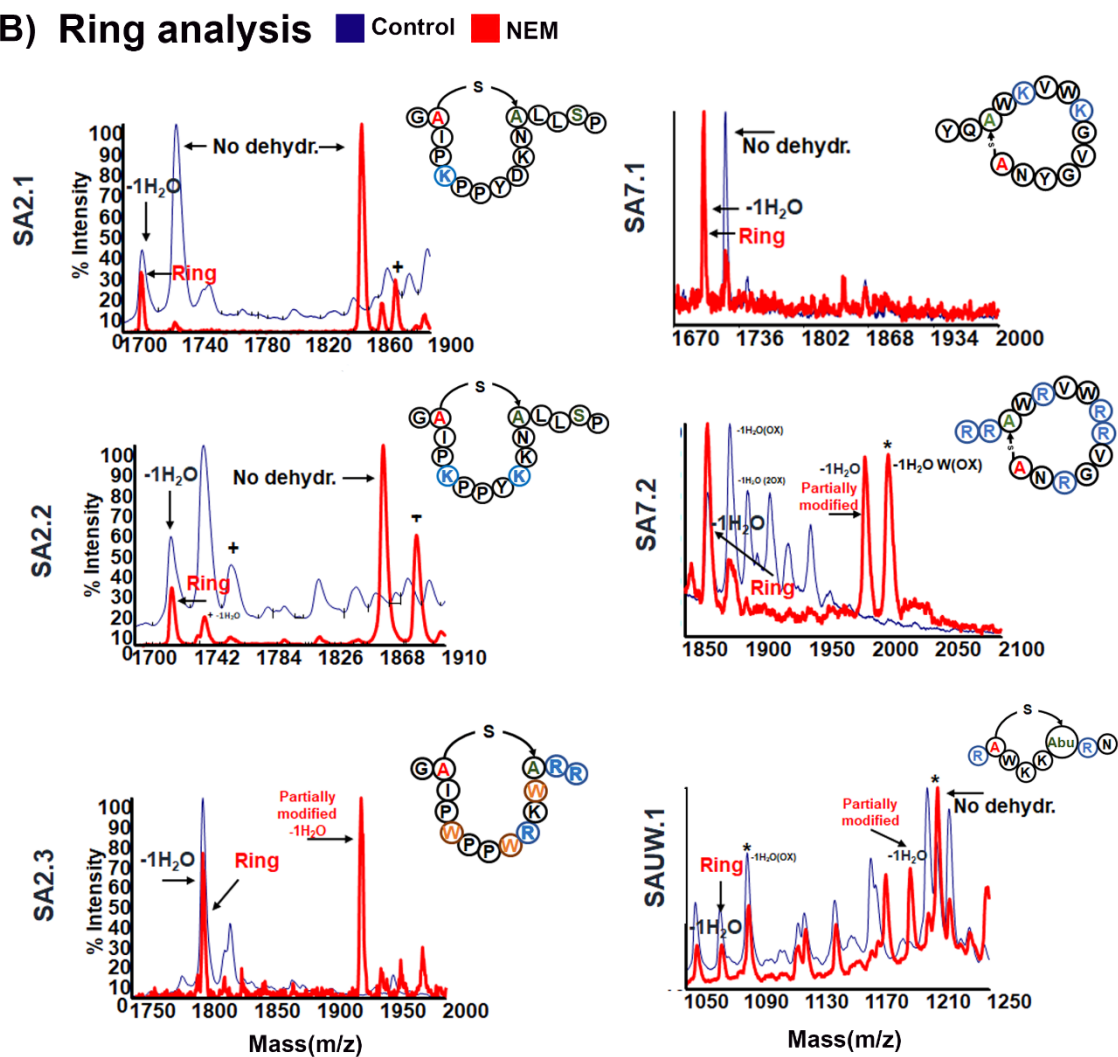
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53 **Supplementary Figure S4.** Ring analysis of single-ringed engineered SyncA's with

54 the NEM reaction. (+) Na adduct . A) The SA2.4 MALDI-TOF dehydration state is

Supplementary Figure S5. LC-MS Spectra of SyncA2 engineered single-ringed SyncA's. A) SA2.1 B) SA2.2, C) SA2.3, D) SA7. E) SAUW.1.

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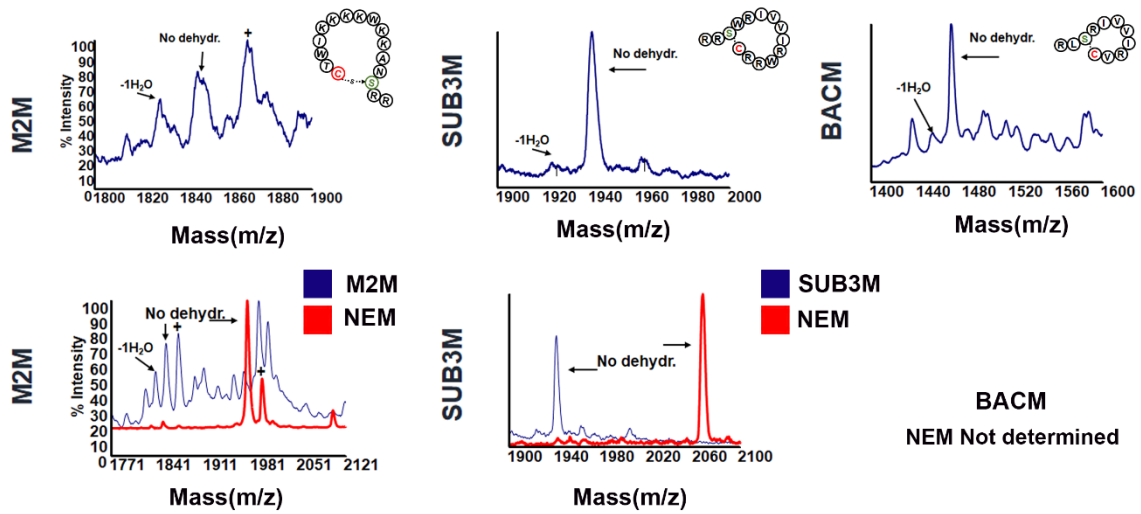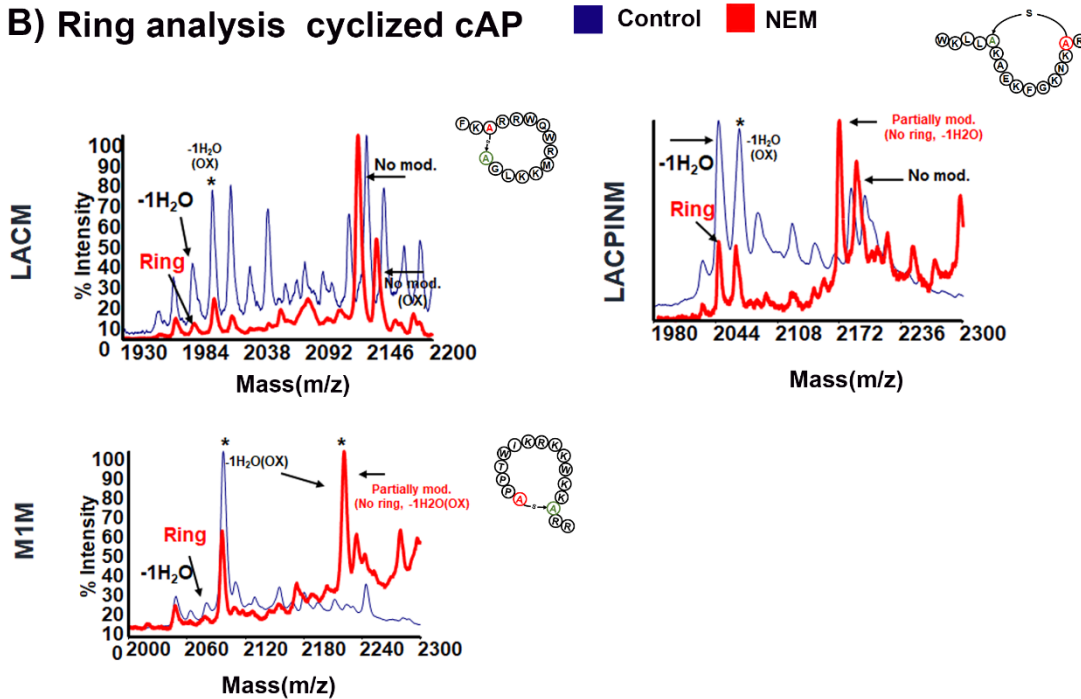
Supplementary Figure S6. Ring analysis of non-prochlorosin peptides co-expressed with SyncM. A) MALDI-TOF and NEM of non-modified AMPs B) Ring analysis of cAMP with NEM. LACM, M1M, and LACPINM.

A) LANM

	MH^{+1}(mono)	MH^{+4}(mono)
No dehydr.	2010,09	503,27
-1H$_2$O	1992,0683	498,77
-1H$_2$O(OX)	2008,0633	502,77
-1H$_2$O(2OX)	2024,0582	506,77

B) LACPINM

	MH^{+1}(mono)	MH^{+4}(mono)
No dehydr.	2064,15	516,79
-1H$_2$O	2046,14	512,29
-1H$_2$O(OX)	2062,14	516,29

C) M1M
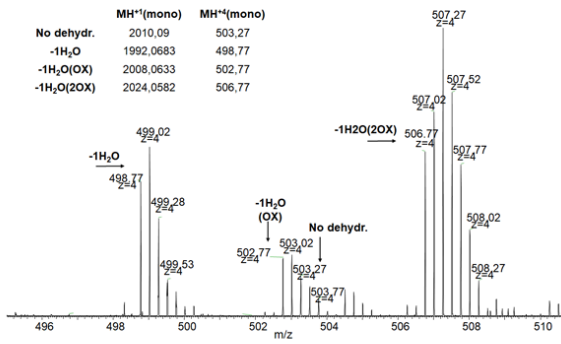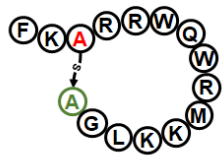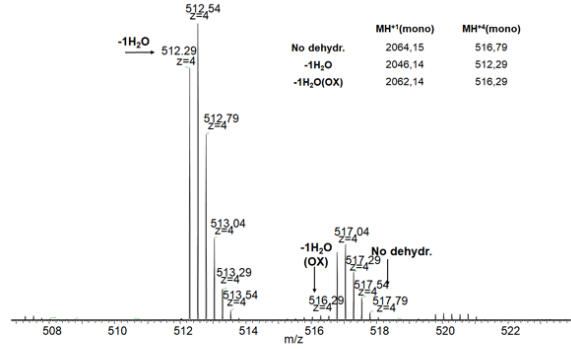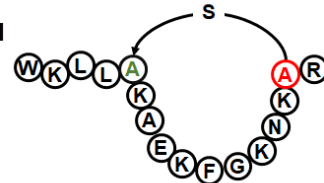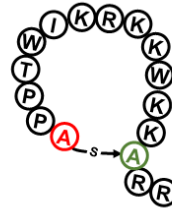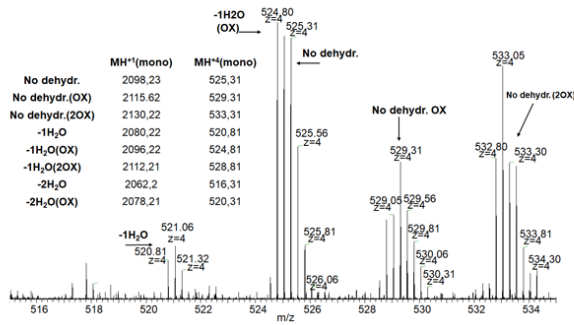
	MH^{+1}(mono)	MH^{+4}(mono)
No dehydr.	2098,23	525,31
No dehydr.(OX)	2115.62	529,31
No dehydr.(2OX)	2130,22	533,31
-1H$_2$O	2080,22	520,81
-1H$_2$O(OX)	2096,22	524,81
-1H$_2$O(2OX)	2112,21	528,81
-2H$_2$O	2062,2	516,31
-2H$_2$O(OX)	2078,21	520,31

Supplementary Figure S7 LC-MS analysis of non-prochlorosin peptides co-expressed with SyncM A) LACTO_+S15, B) LACPIN_S16C- and C) SyncAUW.1.

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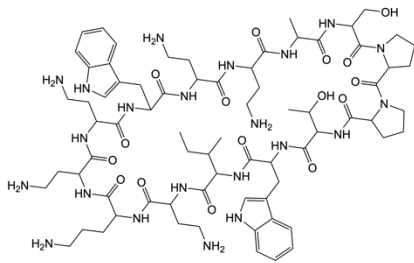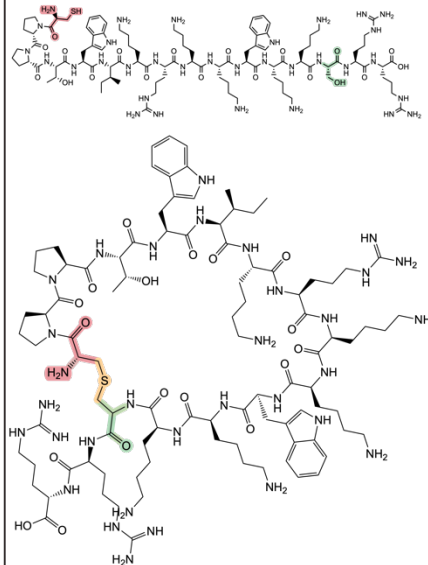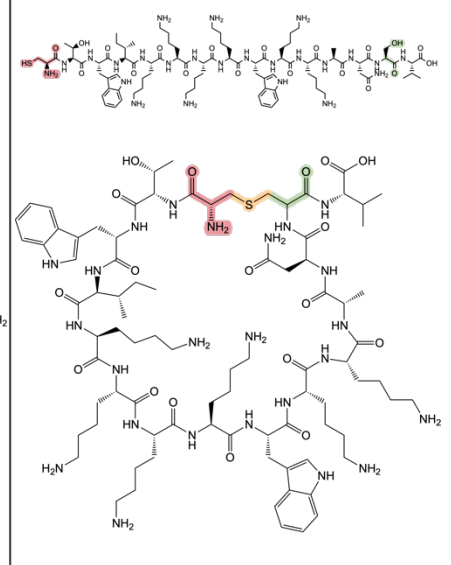
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76 **Supplementary figure S8. Chemical structure of Murepavadin[1] and proposed**

77 **chemical structure of M1M and M2M with the lanthionine ring.** A) Murepavadin

78 sequence and known structure; B) M1M amino acid sequence, linear chemical

79 structure Cys (Highlighted in Red) and Ser (Highlighted in green) and macrocyclic

80 structure after SyncM (lanthionine ring in yellow); C) M1M amino acid sequence, linear

81 chemical structure Cys (Highlighted in Red) and Ser (Highlighted in green) and

82 macrocyclic structure after SyncM (lanthionine ring in yellow).

#	Core Peptide Sequence	AA[a]	Ala	Ile	Val	Leu	Met	Phe	Trp	Tyr	Pro	Gly	Cys	Ser	Thr	Asn	Gln	Arg	Lys	His	Asp	Glu	CHARGE
1	TIPPIVNWIG EKMDKGCA GTLFAASYT SCVAMGTAN KECG	40	5	3	2	1	2	1	1	1	2	5	3	2	4	2	0	0	3	0	1	2	0
SA1	AGSANPQG GDPWCTWD FPICINHK	24	2	2	0	0	0	1	2	0	3	3	2	1	1	2	1	0	1	1	2	0	1
SA2	**GCIPFPPYD KNSLLSP**	16	0	1	0	2	0	1	0	1	4	1	1	2	0	1	0	0	1	0	1	0	0
4	RRCDSCGIW GWIGMAKTC KGPTQG	24	1	2	0	0	1	0	2	0	1	5	3	1	2	0	1	2	2	0	1	0	3
5	IPGVATTPQL THVLSNLTS QNPDFFNGF DFFSSLTNPI DKPPIPGTIE RINYLLKTLQ DIVAITVKDS QYPYQKSTS	96	3	8	4	9	0	6	0	3	8	4	2	10	11	5	7	3	5	1	6	1	1
6	TLSCFAIDGR QQLTSRKSC GDCVLGRAL SCLLLGSYA KQKR	22	2	0	1	5	0	0	0	1	0	3	2	2	0	0	1	2	2	0	1	0	3
7	**TNILNSAGFC NDNPESRSL QP**	21	1	1	0	2	0	1	0	0	2	1	1	3	1	4	1	1	0	0	1	1	-1
8	EYECLCHCT WDDVTSCTV NTWGLG	0	0	0	2	2	0	0	2	1	0	2	0	0	0	0	0	0	0	0	0	0	-4

	Sequence																						
9	GACPSTFTID ACAATGCNP KDL	22	4	1	0	1	0	1	0	0	2	2	3	1	3	1	0	0	1	0	2	0	-1
10	AASAIVGDC QSQEGHVCF SEGEVVCGK R	28	3	1	4	0	0	1	0	0	0	4	3	3	0	0	2	1	1	1	1	3	-2
11	TAWSNSCY	8	1	0	0	0	0	0	1	1	0	0	1	2	1	1	0	0	0	0	0	0	0
12	CDIELVSLCS KTHNVLC	17	0	1	2	3	0	0	0	0	0	0	3	2	1	1	0	0	1	1	1	1	-1
13	GLSIWGCTE CVDGTFHCK RCDLSNLF	26	0	1	1	3	0	2	1	0	0	3	4	2	2	1	0	1	1	1	2	1	-1
14	NSWGWCRG FTGLEGCSA WCNRFCHG RQTR	29	1	0	0	1	0	2	3	0	0	5	4	2	2	2	1	4	0	1	0	1	3
15	EYTGNDTGP TNMYGVCHI PKCPEWAAS KG	29	2	1	1	0	1	0	1	2	3	4	2	1	3	2	0	0	2	1	1	2	-1
16	SGWAGYKP GQACLGVDT	26	3	0	1	1	1	1	1	1	4	5	1	2	1	0	1	0	1	0	1	1	-1
SA9	MAPPSPGEF YLSYDGSER TFGCGGKGC GWVEPPTKI GGGECRDN KLR	38	0	1	1	2	0	1	1	2	2	9	3	2	2	1	0	3	3	0	2	3	1
18	GVRGGTAAY MCPTDRNKT CKPVATCPG LK	21	3	0	2	1	1	0	0	1	3	4	1	1	0	0	0	0	0	0	0	0	4
19	AEGGWCQL FPVPTACQV TWN	20	2	0	2	1	0	1	2	0	2	2	2	0	2	1	2	0	0	0	0	1	-1
20	DSGGDYLSY AGCDTGRCG ACSWGCPG	26	2	0	0	1	0	0	1	2	1	7	4	3	1	0	0	1	0	0	3	0	-2
21	LDALKQCNF TGTCWDTLL	18	1	0	0	4	0	1	1	0	0	1	2	0	3	1	1	0	1	0	2	0	-1
SA18	SDTNWITEV SKCPWWRR S	18	0	1	1	0	0	0	3	0	1	0	1	3	2	1	0	2	1	0	1	1	1
SA13	CWMNTCWG YGPGTAAAP DWN	20	3	0	0	0	1	0	3	1	2	3	2	0	2	2	0	0	0	0	1	0	-1

	Sequence																						
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25	ANICTGCQIATTCIDDSQILQYTNSG	26	2	4	0	1	0	0	0	1	0	2	3	2	4	2	3	0	0	0	2	0	-2
26	GACEVLTGPVIESYVGDSWHDYCCRIT	27	1	2	3	1	0	0	1	2	1	3	3	2	2	0	0	1	0	1	2	2	-3
SA4	EGSWRAGWGPCRVRLSMSPQHKNCRG	26	1	0	1	1	1	0	2	0	2	4	2	3	0	1	1	4	1	1	0	1	4
SA5	EQCPLNTGCPLHTGWWCTGVLVDQQP	40	2	1	2	3	0	0	2	0	3	3	3	2	4	2	0	0	3	0	1	2	-1
SA8	HRSAASNISDDETLTDFGCKDYERY	17	0	0	0	1	0	1	0	2	0	1	1	1	2	0	0	1	1	0	4	2	-4
SA3	NKSHYYCEPIPAPFA	15	2	1	0	0	0	1	0	2	3	0	1	1	0	1	0	0	1	1	0	1	0
31	EPTPWDPEWYN	11	0	0	0	0	0	0	2	1	3	0	0	0	1	1	0	0	0	0	1	2	-3
32	ECGNVSCVNIYLLTLGKDKEVEL	23	0	1	3	4	0	0	0	1	0	2	2	1	1	2	0	0	2	0	1	3	-2
33	KVSDFCTSDIVSYCCW	16	0	1	2	0	0	1	1	1	0	0	3	3	1	0	0	0	1	0	2	0	-1
34	ASWSGPMWNTTCIGG	15	1	1	0	0	1	0	2	0	1	3	1	2	2	1	0	0	0	0	0	0	0
35	DYCTVETATVCYIREPGC	25	1	1	2	0	0	0	0	3	1	2	4	1	4	0	0	1	0	0	3	2	-4
36	DTSDYGCRSYNWASWCSE	11	1	0	0	0	0	0	2	1	0	0	1	3	0	1	0	1	0	0	0	1	0
SA12	YYCACTRIPVYNHTN	15	1	1	1	0	0	0	0	3	1	0	2	0	2	2	0	1	0	1	0	0	1
38	GTGRHTCGTGSCGCFCSIYEC	24	0	1	0	0	0	1	0	1	0	5	2	1	1	2	1	0	1	1	2	0	0
39	ICRQSCIWRSIFKAAPE	17	2	3	0	0	0	1	1	0	1	0	2	2	0	0	1	2	1	0	0	1	2
4	VGGVCGAFTSEANGTP	16	2	0	2	0	0	1	0	0	1	4	1	1	2	1	0	0	0	0	0	1	-1
41	GTDVNHCYFATAYGPHTCSGACAK	24	4	0	1	0	0	1	0	2	1	3	3	1	3	1	0	0	1	2	1	0	0
42	TACALRVGTVFIGSCWN	17	2	1	2	1	0	1	1	0	0	2	2	1	2	1	0	1	0	0	0	0	1

ID	Sequence																						
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45	ANQSFEYGAIGQCLPPCPMSH	21	2	1	0	1	1	1	0	1	3	2	2	2	0	1	2	0	0	1	0	1	-1
46	ANSCDILTAIVWDSYMGTCFICNKH	25	2	3	1	1	1	1	1	1	0	1	3	2	2	2	0	0	1	1	2	0	-1
47	SETNCGYPTKFCMVCATCEHRVVYTQCQ	28	1	0	3	0	1	1	0	2	1	1	5	1	4	1	2	1	1	1	0	2	0
48	GHCLTNSPLAGGCTGWLRI	16	1	1	0	3	0	0	1	0	1	4	1	2	0	1	0	0	1	0	1	0	1
49	NSDNKHCYYYTSGIYTRCDD	20	0	1	0	0	0	0	0	4	0	1	2	2	2	2	0	1	1	1	3	0	-1
5	DITWDFVSTLVTACADRTMHCCKK	24	2	1	2	1	1	1	1	0	0	0	3	1	4	0	0	1	2	1	3	0	0
51	ALQSTIQSENCTFRGANCQL	20	2	1	0	2	0	1	0	0	0	1	2	2	2	2	3	1	0	0	0	1	0
52	KDGTCTKCVTECKGRYC	17	0	0	1	0	0	0	0	1	0	2	4	0	3	0	0	1	3	0	1	1	2
53	KKPKLEYSNTSLACGC	16	1	0	0	2	0	0	0	1	1	1	2	2	1	1	0	0	3	0	0	1	2
SA15	VTLKYKNWCYCTAL	14	1	0	1	2	0	0	1	2	0	0	2	0	2	1	0	0	2	0	0	0	2
55	RGFSYLPQECVTDYLCG	17	0	0	1	2	0	1	0	2	1	2	2	1	1	0	1	1	0	0	1	1	-1
SA6	GCSFEYGKMGKDKCSR	16	0	0	0	0	1	1	0	1	0	3	2	2	0	0	0	1	3	0	1	1	2
SA16	RIVCVSFGSLFIRRGKLS	18	0	2	2	2	0	2	0	0	0	2	1	3	0	0	0	3	1	0	0	0	4
SA11	CDIELVSLCSKTHNVLC	24	0	1	2	3	0	0	0	0	0	0	3	1	2	0	1	2	2	0	1	0	-1
59	CYNWKSARDY	10	1	0	0	0	0	0	1	2	0	0	1	1	0	1	0	1	1	0	1	0	1
6	FECSFPCKLSIGGPG	15	0	1	0	1	0	2	0	0	2	3	2	2	0	0	0	0	1	0	0	1	0
61	LGPKVSAGSLYCLCPG	16	1	0	1	3	0	0	0	1	2	3	2	2	0	0	0	0	1	0	0	0	1
62	TCFCFSVACRLDTGITFR	18	1	1	1	1	0	3	0	0	0	1	3	1	3	0	0	2	0	0	1	0	1

	Sequence	Total																				Charge[c]
63	WRCSVGPQTNKQNWTQALCCQKGQI	25	1	1	1	1	0	0	2	0	1	2	3	1	2	2	5	1	2	0	0	0	3
SA7	YQSWDVWDGVGYNC	14	0	0	2	0	0	0	2	2	0	2	1	1	0	1	1	0	0	0	2	0	-2
65	ADTLCCPVRCGQTFSCIA	18	2	1	1	1	0	1	0	0	1	1	4	1	2	0	1	1	0	0	1	0	0
66	GAETACLTNNTCFEAQACCYFTL	23	4	0	0	2	0	2	0	1	0	1	4	0	4	2	1	0	0	0	0	2	-2
67	KDYVGRTLTQGKCGC	15	0	0	1	1	0	0	0	1	0	3	2	0	2	0	1	1	2	0	1	0	2
68	NCQRCNHKSACSATYVPCVVLTEI	96	2	1	3	1	0	0	0	1	1	0	2	10	11	5	7	3	5	1	6	1	1
69	ECIGGGRGRCSFVSPQSERGGVGDEK	26	0	1	2	0	0	1	0	0	1	7	2	3	0	0	1	3	1	0	1	3	0
70	CACFKKTEIWFIR	13	1	2	0	0	0	2	1	0	0	0	2	0	1	0	0	1	2	0	0	1	2
71	FGTCFIGRNTTDGKVRITWYWV	22	0	2	2	0	0	2	2	1	0	3	1	0	4	1	0	2	1	0	1	0	2
72	ACIDCFTRYGTDEEVTLIDD	20	1	2	1	1	0	1	0	1	0	1	2	0	3	0	0	1	0	0	4	2	-5
SA1	AGSANPQGEDPWCTWDYPICINHK	24	2	2	0	0	0	0	2	1	3	2	2	1	1	2	1	0	1	1	2	1	-2
74	GHGVTFYNPCSTLIMTLKCCTKMLAVCPK	29	1	1	2	3	2	1	0	1	2	2	4	1	4	1	0	0	3	1	0	0	3
75	GQAKQSIRVCRRTLKS	16	1	1	1	1	0	0	0	0	0	1	1	2	1	0	2	3	2	0	0	0	5
76	ESCPTVPICGNMNFGCCTSTGV	22	0	1	2	0	1	1	0	0	2	3	4	2	3	2	0	0	0	0	0	1	-1
77	QFGSYCMCHHKTLCKNDSVDL	21	0	0	1	2	1	1	0	1	0	1	3	2	1	1	1	0	2	2	2	0	0
78	ACPSTFTIDACAATGCNPKDL	22	4	1	0	1	0	1	0	0	2	1	2	2	0	0	1	2	2	0	1	0	-1
SA3	WCGNACTNWKWSNGAGTGC	19	2	0	0	0	0	0	3	0	0	4	3	1	2	3	0	0	1	0	0	0	1
	Total of AA's	1783	102	75	80	94	18	56	57	66	86	178	178	131	149	82	55	66	87	25	89	59	Charge[c] %

		A	I	V	L	M	F	W	Y	P	G	C	S	T	N	Q	R	K	H	D	E		
Percentage %		5,9	4,3	4,6	5,4	1,0	3,2	3,3	3,8	4,9	10	10	7,6	8,6	4,7	3,2	3,8	5,0	1,5	5,1	3,4	-	43
Aminoacid presence in core peptides[b]		55	50	48	49	16	42	36	45	43	64	76	66	59	50	30	40	correct or not	23	50	40	+	37
Percentage %		70	63	61	62	20	54	46	57	54	81	96	84	75	63	38	51	65	29	63	51	N[d]	20

[a] Total aminoacid per core sequence

[b] Count of times an amino acid is observed in SyncA's core.

[c] Summary of net charge[2] found in SyncA's. Percentage

[d] Neutral charge (N)

Supplementary Table S2. Summary of plasmids and strains used in this study. All the used plasmids were cloned into *L. lactis* NZ9000.

Strain	Mutations	Characteristic	References
Micrococcus flavus		Sensitive strain	[3]
Lactococus lactis NZ9000	*pepN:nisRK*	Nisin inducible strain used in cloning and peptides expression	[4]
pNZ-nisP, pIL253		NisP soluble producer strain, Cm^R , Ery^R	[3]
pNZe-NisP8H		Ery^{R,} Cm$^{R.}$ NisP producer strain	[3]
pTLR-SyncM		SyncM lanthipeptide synthase cloned under nisin inducible promoter (P_{nis} promoter), Ery^R	[6]
pNZ8048		Nisin inducible expression vector, Cm^R,	[5]
pNZ8048-SyncA2		SyncA2 cloned under P_{nis} promoter in pNZ8048 Cm^R	[6]
pNZ8048-SA2 -S12A	S12A	SA2 -S12A cloned under P_{nis} promoter, Cm^R	
pNZ8048-SA2-SWAP	Ring(C2S/S12C)	SA2-SWAP cloned under P_{nis} promoter, Cm^R	This work
pNZ8048-SA2-+1A	+1A (P4_F5insA)	under P_{nis} promoter, Cm^R	This work

pNZ8048- SA2-+2A	+2A (P4_F5insAA)	under P_{nis} promoter, Cm^R	This work
pNZ8048- SA2-+3A	+3A (P4_F5insAAA)	under P_{nis} promoter, Cm^R	This work
pNZ8048- SA2-+4A	+4A (P4_F5insAAAA)	under P_{nis} promoter, Cm^R	This work
pNZ8048-SyncA6		SyncA6 cloned under under P_{nis} promoter, Cm^R	[5]
pNZ8048-SyncA6- SA6-SC	Ring1(C2S/S15C)-Ring2(S3C /C14S)	SA6-SC cloned under P_{nis} promoter, Cm^R	This work
pNZ8048-SyncA6- SA6-AS	ΔRing1(C2A/S15A)	SA6-AS cloned under P_{nis} promoter, Cm^R	This work
pNZ8048-SyncA6- SA6-AC	ΔRing1 (C2A/S15A)-Ring2(S2C /C14S)	SA6-AC cloned under P_{nis} promoter Cm^R	This work
pNZ8048-SyncA6- SA6-CA	ΔRing2(S2A/C14S)	SA6-CA cloned under P_{nis} promoter Cm^R	This work
pNZ8048-SyncA6- SA6-SA	Ring1(C2S/S15) - ΔRing2(S2A/C14S)	SA6-SA cloned under P_{nis} promoter Cm^R	This work
pNZ8048-SyncA2LE- SA2.1	F5K	SA2.1 cloned after SA2- LEADER -ASPR under P_{nis} promoter, Cm^R	This work
pNZ8048-SyncA2LE- SA2.2	F5K_D9K	SA2.2 cloned after SA2- LEADER -ASPR under P_{nis} promoter, Cm^R	This work
pNZ8048-SyncA2LE- SA-2.3	F5W_Y8W_D9R_L13R_L14R-ΔS15-ΔP16	SA-2.3 cloned after SA2-LEADER-ASPR under P_{nis} promoter, Cm^R	This work
pNZ8048-SyncA2LE- SA-2.4	Most of the structure change	SA-2.4 cloned after SA2- LEADER -ASPR pNZ8048, under P_{nis} promoter, Cm^R	This work
pNZ8048-SyncA2LE- SA-2.5	Most of the structure change	SA-2.5 cloned after SA2-LEADER ASPR pNZ8048, under P_{nis} promoter, Cm^R	This work
pNZ8048-SyncA2LE- SAUW.1	Q1R_N7R	SAUW.1cloned after SA2- LEADER -ASPR under P_{nis} promoter, Cm^R	This work
pNZ8048-LACM	insS15[7]	LACM cloned after SA2- LEADER -ASPR pNZ8048, under P_{nis} promoter, Cm^R Ery^R, syncM cloned in pTLR under P_{nis} promoter	This work

pNZ8048- LACPINM	S16C[7]	LACPINM cloned after SA2- LEADER -ASPR pNZ8048, under P_{nis} promoter, Cm^R	This work
pNZ8048- -BACM	C3S[8]	BACM cloned after SA2- LEADER -ASPR pNZ8048, under P_{nis} promoter, Cm^R	This work
pNZ8048 - SUB3M	insS3_insC14[9]	SUB3M cloned after SA2- LEADER -ASPR pNZ8048, under P_{nis} promoter, Cm^R	This work
pNZ8048 - M1M	Murepavadin mimic[1]	M1M cloned after SA2- LEADER -ASPR pNZ8048, under P_{nis} promoter, Cm^R	This work
pNZ8048 - M2M	Murepavadin mimic	M2M cloned after SA2- LEADER -ASPR pNZ8048, under P_{nis} promoter, Cm^R	This work
pNZ8048-SyncA7		under P_{nis} promoter Cm^R	[6]
pNZ8048-SyncA7LE- SyncA7.1	D5K_D8K	Desired core peptide was cloned after SA7- LEADER GG pNZ8048, under P_{nis} promoter, Cm^R Ery^R, syncM cloned in pTLR under P_{nis} promoter	This work
pNZ8048-SyncA7LE- SyncA7.2	-Y1R_G2R_D5R_D8R_Y12R	Desired core peptide was cloned after SA7-LE LEADER GG pNZ8048, under P_{nis} promoter, Cm^R Ery^R, syncM cloned in pTLR under P_{nis} promoter	This work
pNZ8048-SyncA2LE- SA2.2, pTLR-SyncM	F5K_D9K	Desired core peptide was cloned after SA2- LEADER -ASPR pNZ8048, under P_{nis} promoter, Cm^R Ery^R, syncM cloned in pTLR under P_{nis} promoter	This work
pNZ8048-SyncA2LE- SA-2.3, pTLR-SyncM	F5W_Y8W_D9R_L13R_L14R- ΔS15-ΔP16	Desired core peptide was cloned after SA2- LEADER-ASPR pNZ8048, under P_{nis} promoter, Cm^R Ery^R, syncM cloned in pTLR under P_{nis} promoter	This work
pNZ8048-SyncA2LE- SA-2.4, pTLR-SyncM	Most of the structure change	Desired core peptide was cloned after SA2- LEADER -ASPR pNZ8048, under P_{nis} promoter, Cm^R Ery^R, syncM cloned in pTLR under P_{nis} promoter	This work

pNZ8048-SyncA2LE- SA-2.5, pTLR-SyncM	Most of the structure change	Desired core peptide was cloned after SA2-LEADER ASPR pNZ8048, under P_{nis} promoter, Cm^R Ery^R, *sync*M cloned in pTLR under P_{nis} promoter	This work
pNZ8048-SyncA2LE- SAUW.1, pTLR-SyncM	-Q1R_N7R	Desired core peptide was cloned after SA2-LEADER -ASPR pNZ8048, under P_{nis} promoter, Cm^R Ery^R, *sync*M cloned in pTLR under P_{nis} promoter	This work
pNZ8048-LACM_, pTLR-SyncM	_+S15[7]	Desired core peptide was cloned after SA2-LEADER -ASPR pNZ8048, under P_{nis} promoter, Cm^R Ery^R, *sync*M cloned in pTLR under P_{nis} promoter	This work
pNZ8048- LACPINM, pTLR-SyncM	S16C[7]	Desired core peptide was cloned after SA2-LEADER -ASPR pNZ8048, under P_{nis} promoter, Cm^R Ery^R, *sync*M cloned in pTLR under P_{nis} promoter	This work
pNZ8048- -BACM, pTLR-SyncM	C3S [8]	Desired core peptide was cloned after SA2-LEADER -ASPR pNZ8048, under P_{nis} promoter, Cm^R Ery^R, *sync*M cloned in pTLR under P_{nis} promoter	This work
pNZ8048-SUB3M, pTLR-SyncM	+S3+C14[9]	Desired core peptide was cloned after SA2-LEADER -ASPR pNZ8048, under P_{nis} promoter, Cm^R Ery^R, *sync*M cloned in pTLR under P_{nis} promoter	This work
pNZ8048- M1M, pTLR-SyncM	Murepavadin mimic[1]	Desired core peptide was cloned after SA2-LEADER -ASPR pNZ8048, under P_{nis} promoter, Cm^R Ery^R, *sync*M cloned in pTLR under P_{nis} promoter	This work
pNZ8048 - M2M, pTLR-SyncM	Murepavadin mimic	Desired core peptide was cloned after SA2-LEADER -ASPR pNZ8048, under P_{nis} promoter, Cm^R Ery^R, *sync*M cloned in pTLR under P_{nis} promoter	This work

pNZ8048-SyncA7		under P$_{nis}$ promoter Cm^R	6
pNZ8048-SyncA7LE-SyncA7.1	-D5K_D8K	Desired core peptide was cloned after SA7-LEADER GG pNZ8048, under P$_{nis}$ promoter, Cm^R Ery^R, *sync*M cloned in pTLR under P$_{nis}$ promoter	This work
pNZ8048-SyncA7LE-SyncA7.2	-Y1R_G2R_D5R_D8R_Y12R	Desired core peptide was cloned after SA7-LE LEADER GG pNZ8048, under P$_{nis}$ promoter, Cm^R Ery^R, *sync*M cloned in pTLR under P$_{nis}$ promoter	This work

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