



1. Ntr P2 P1

L ₂ A	QAK GLPVC-
	-2 -1

K ₁ A	QLA GLPVC-
	-2 -1

2. Cyclotide P2 P1

R ₂₈ A	CTAN GLPSLAA
	28 29
R ₂₈ K	CTKN GLPSLAA
	28 29
N ₂₉ Q	CTRQ GLPSLAA
	28 29

3. Ctr truncation

GLP*	TRN GLP
	30 31 32
GL*	TRN GL
	30 31
G*	TRN G
	30

4. Ctr P1' mutants

G ₃₀ A	TRN ALPSLAA
	30 31 32
G ₃₀ F	TRN FLPSLAA
	30 31 32
SLP*	TRN SLP
	30 31 32

5. Ctr P2' mutants

L ₃₁ A	TRN GAPSLAA
	30 31 32
L ₃₁ I	TRN GIPS LAA
	30 31 32

6. Ctr P3' mutant

GLA*	TRN GLA
	30 31 32

7. Ctr shuffling

GGL*	TRN GGL
	30 31 32
ALA*	TRN ALA
	30 31 32
LGP*	TRN LGP
	30 31 32
GP*	TRN GP
	30 31

8. L31 Ctr truncation

GAPSL	TRN GAPSL
	30 31 32 33 34
GAPSL	TRN GAPS
	30 31 32 33
GAP*	TRN GAP
	30 31 32
GIP*	TRN GIP
	30 31 32

9. L31A mutants

L ₃₁ A, ΔP ₃₂	TRN GAS LAA
	30 31 32 33 34 35 36
L ₃₁ A, P ₃₂ A	TRN GAAS LAA
	30 31 32 33 34 35 36
L ₃₁ A, P ₃₂ N	TRN GANS LAA
	30 31 32 33 34 35 36
L ₃₁ A, L ₃₄ A	TRN GAPS AAA
	30 31 32 33 34 35 36

Supp Fig 2: Oak1 mutants constructed for evaluation of the residues required for enzymatic cyclization. 1) mutants with changes in the Ntr. 2) mutants with changes within the cyclotide domain. 3) truncation mutants of the Ctr to determine the minimum number of amino acids for cyclization. 4) mutants of the P1' position of the Ctr. 5) mutants of the P2' position of the Ctr. 6) mutant of the P3' position of the Ctr. 7) shuffling of the Ctr. 8) truncation mutants of the Ctr containing a substitution at position L31. 9) Double mutants containing the L31A substitution and additional substitutions within the Ctr.