Electronic Supplementary Material (ESI) for Molecular BioSystems

Supplementary Data

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Characterizing the laulimalide/peloruside binding site using site-directed mutagenesis of *TUB2* in *S. cerevisiae*

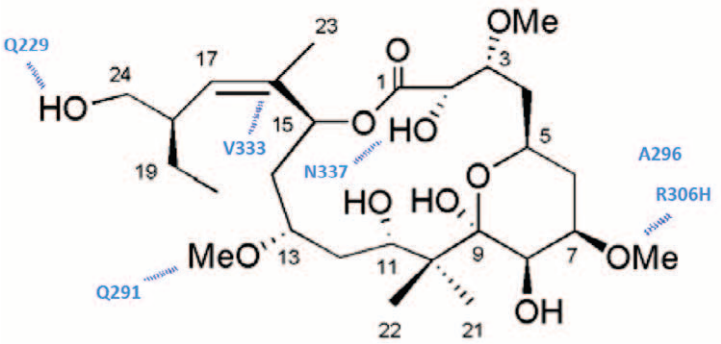

Figure S1. Peloruside A interactions with β-tubulin based on computer modeling.
Q229, N337 and Q291 are H-donors; whereas, R306 is an H-acceptor. The choice of amino acid residues for β-tubulin mutagenesis was adopted from unpublished computer modelling studies carried out by Paul Teesdale-Spittle (Victoria University of Wellington) and Pahk Thepchatri and Jim Snyder (Emory University, Atlanta, GA).

Table S1. Predicted PelA-sensitive sites on β-tubulin.

Functional Group	Residues	Importance	Predicted effect
7 OMe	R306	Direct interaction with R306	Resistance to peloruside
2 OH	N337	H-bond	Resistance to peloruside
13 OMe	Q291	H-bond	Resistance to peloruside
16 Me	V333	weak hydrophobic bond	Resistance to laulimalide but have less of an effect on peloruside

Sites on β-tubulin likely to be involved in peloruside binding.

Table S2. Properties of amino acids used in this study for peloruside A.

Residues	Abbrev.	Residue property	Altered Residue	Abbrev.	Residue property
Ala296	A	Acyclic, small, neutral, hydrophobic	Thr	T	Acyclic, medium, neutral, polar
Arg306	R	Acyclic, basic, charged, large, polar, positive	His	H	Aromatic, basic, charged, cyclic, large, neutral, polar, positive
Arg282	R	Acyclic, basic, charged, large, polar, positive	Gln	Q	Acyclic, neutral, large, polar
Asn337	N	Acyclic, neutral, medium, polar	Leu	L	Acyclic, neutral, large, aliphatic, hydrophobic,
Gln291	Q	Acyclic, neutral, large, polar	Met	M	Acyclic, neutral, large, hydrophobic
Val333	V	Acyclic, neutral, medium, aliphatic	Trp	W	Aromatic, neutral, large, cyclic, hydrophobic

Chemical properties of the amino acids predicted to be involved in peloruside binding and the properties of the amino acids replaced by site-directed mutations.

Table S3. Primer sequences for *TUB2* point mutations

Primer A (forward) have a sequence as follows:

***tub2*_A296T**

5'-TTTAGATCTTTGACTGTCCCTGAATTAACACAGCAAATGTTTGATACCAAGAACATGA-3'

***tub2*_R306H**
5'-CAGCAAATGTTTGATGCCAAGAACATGATGGCTGCTGCCGATCCACATAACGGTA
GAT-3'

***tub2*_A296T + R306H**
5'-TTTAGATCTTTGACTGTCCCTGAATTAACACAGCAAATGTTTGATACCAAGAACAT
GATGGCTGCTGCCGATCCACATAACGGTAGAT-3'

***tub2*_R282Q**
5'-ATGGTCGGCTACGCTCCATTGACGGCAATTGGCTCTCAATCATTTCAATCTTTGACTG-3'

***tub2*_Q291M**
5'-ATTGGCTCTCAATCATTTAGATCTTTGACTGTCCCTGAATTAACAATGCAAATGTTTG-3'

***tub2*_V333W**
5'-AGAGGTAAAGTTTCCGTTAAGGAGGTGGAAGATGAAATGCATAAATGGCAATCTAAAA-3'

***tub2*_N337L**
5'-TCCGTTAAGGAGGTGGAAGATGAAATGCATAAAGTGCAATCTAAACTCTCAGACTATT-3'

Primer B (Reverse)
5'-GGGTATTCTGGGCCTCCATGTCTATATTCACTAATACTCGGGGTGT-3'

Primer C (Forward)
5'-ACACCCCGAGTATTAGTGAATATAGACATGGAGGCCCAGAATACCC-3'

(Blue-coloured letters designate homology to nat-resistance cassette region on the plasmid, and black-coloured letters are the region homolog to gDNA)

Primer D (Reverse)
5'-AAATCCCTGATCTGCGTAATATTGCAAGTTCTTTTTTATCGGCCCCAGTATAGCGACCAGCATTCAC-3'

(Green colour coded letters represent homology to natMX-cassette region on the plasmid (as explained earlier); however, black colour letters represent homology to gDNA)

Transformation confirmation primer:

Forward primer: Primer A (400 bp upstream of point mutation)
5'-GATGGCCACCTTCTCCGT-3'

Reverse primers:

A296T
5'-AGCCATCATGTTCTTGGC-3' (WT) **or**
5'-AGCCATCATGTTCTTGGT-3' (μ)

R306H
5'-AAGGTATCTACCGTTTCT-3' (WT) **or**
5'-AAGGTATCTACCGTTATG-3' (μ)

R282Q
5'-AGGGACAGTCAAAGATCT-3' (WT) **or**
5'-AGGGACAGTCAAAGATTG-3' (μ)

Q291M
5'-GGCATCAAACATTTGCTG-3' (WT) **or**
5'-GGCATCAAACATTTGCAT-3' (μ)

V333W
5'-TGAGTTTTTAGATTGCAC-3' (WT) **or**
5'-TGAGTTTTTAGATTGCCA-3' (μ)

N337L
5'-CACGAAATAGTCTGAGTT-3' (WT) **or**
5'-CACGAAATAGTCTGAGAG-3' (μ)

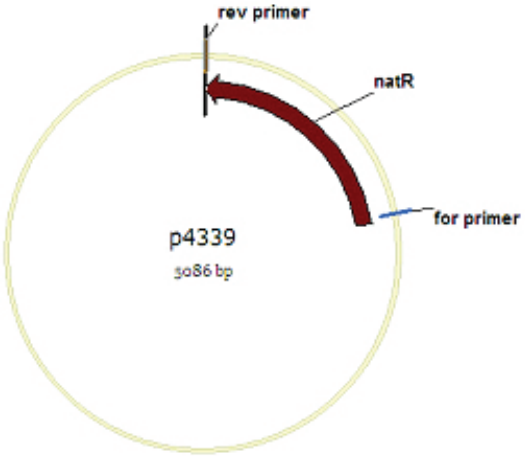

Figure S2. The NatMX cassette

Frozen bacterial cultures containing the plasmid p4339 for the NatMX cassette were streaked on LB plates containing 100 µg/mL ampicillin and grown at 37°C overnight with shaking. The following day, a single colony was inoculated in 2 mL LB medium containing 100 µg/mL ampicillin and grown at 37°C overnight with shaking. The plasmid purification was accomplished using the QIAprep® Miniprep Kit (Qiagen) according to the manufacturer's protocol. The NatMX cassette was then PCR-amplified using forward primer C and reverse primer D (See Table S3 above).

LB Medium (1 L) **LB**

Bacto-Tryptone	10 g	
Bacto-yeast extract	5 g	
NaCl	10 g	
Bacto-agar	15 g	(to LB plates)

The 100 mg/ml filter-sterilised ampicillin antibiotic was added after autoclaving and cooling of the medium to approximately 60°C.

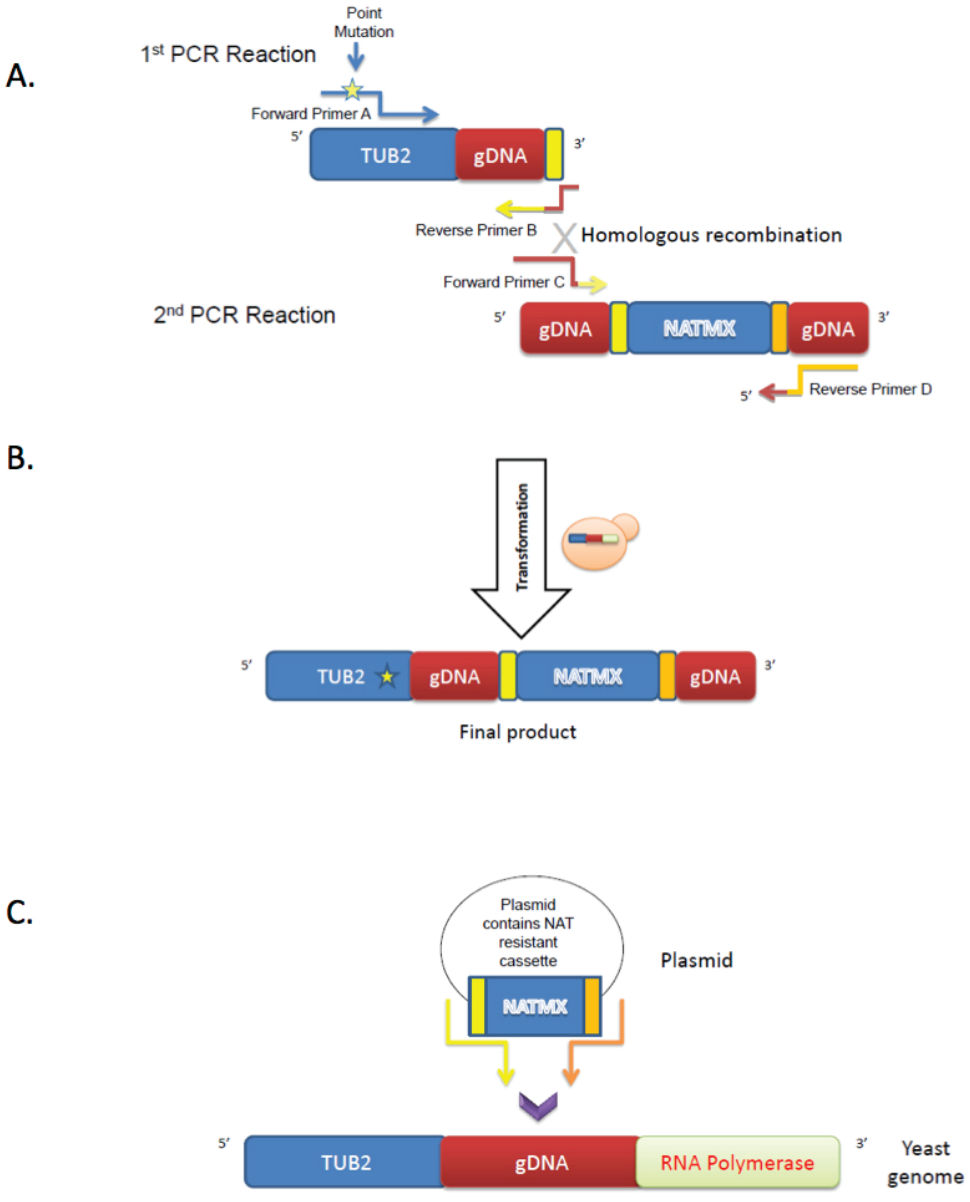

Figure S3. Overlap PCR design for site-directed yeast mutations.
(A) Two overlap PCR reactions were carried out separately. The first reaction (primers A and B) was designed to introduce the point mutation (yellow star), and the second PCR reaction (primers C and D) was designed to amplify and insert the NatMX cassette. The two PCR products were then inserted into the yeast genome at the *TUB2* locus by homologous recombination. (B) The final product that was used to generate the mutant TUB2 strain. (C) Schematic procedure for insertion of the NatMX cassette into the yeast genome downstream of the *TUB2* gene.

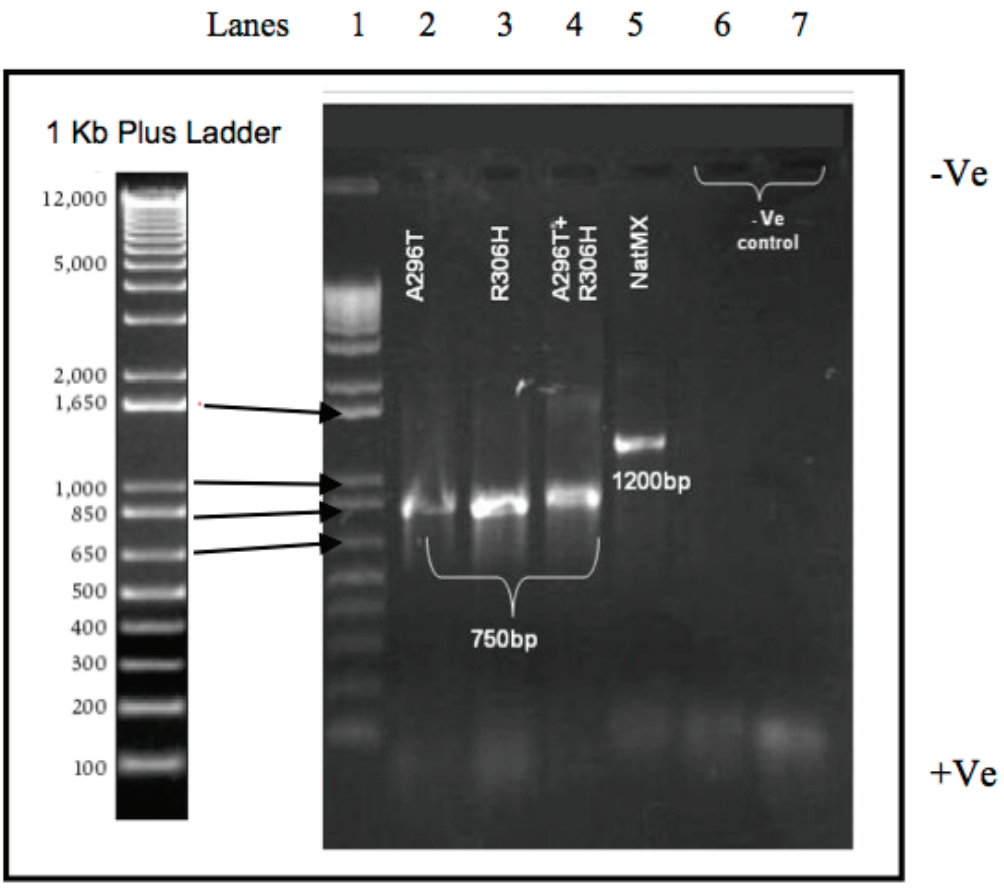

Figure S4. Size confirmation of single A296T and R306H point mutations, double mutant, and NatMX cassette PCR products.
Lane 1= 1 Kb plus DNA ladder, **Lane 2**= A296T PCR product, **Lane 3**= R306H PCR product, **Lane 4**= the combined double mutant PCR product, **Lane 5**= NatMX PCR product, **Lane 6 & 7**= negative controls (tubes with only PCR mix and no DNA template i.e. NatMX or BY4741 DNA). Fragment sizes were in the expected range: 750 bp for *TUB2* mutations and 1200 bp for NatMX.