Supporting Information for:

Mutations in the Proteolipid Subunits of the Vacuolar H^+-ATPase Provide Resistance to Indolotryptoline Natural Products.

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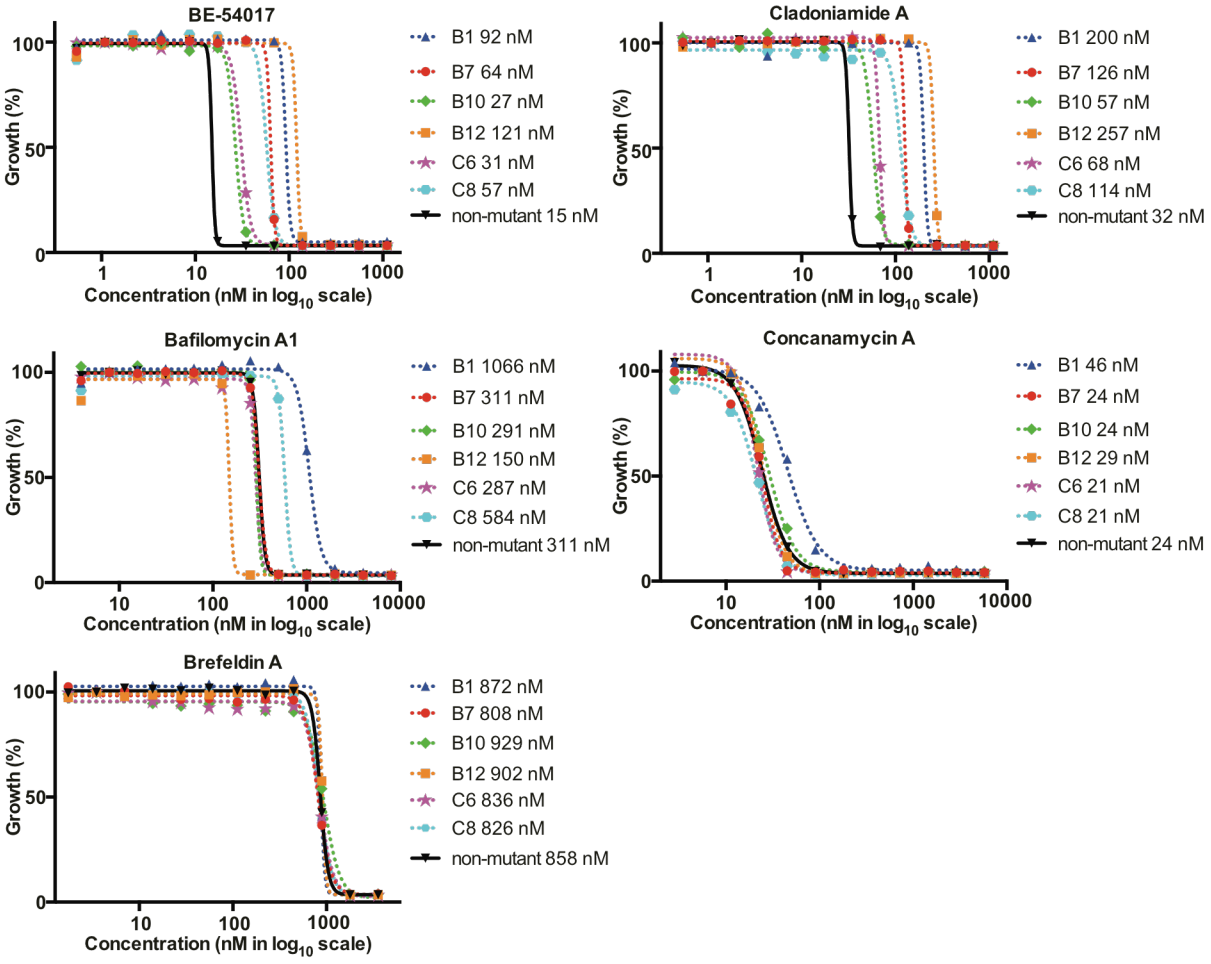
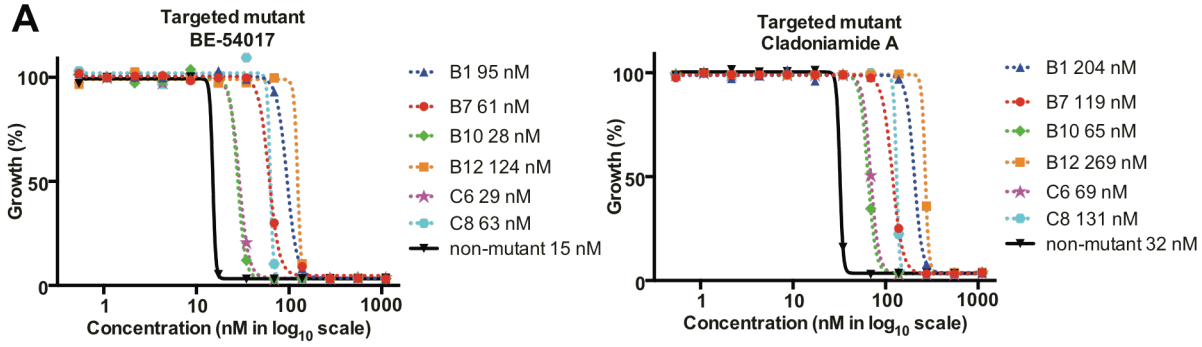


Figure S1. Whole-cell cytotoxicity dose response curves of compounds tested in this study in resistant mutant and un-mutagenized MDR-sup *S. pombe* strains. The IC_{50} values determined from these dose response curves are noted on the legend of each graph. See also Table S3.

A



B

Mutant ID		B1	B7	B10	B12	C6	C8
Fold difference in IC_{50} (relative to non-mutant)	BE	x6.3	x4.1	x1.9	x8.3	x1.9	x4.2
	Cla	x6.4	x3.7	x2.0	x8.4	x2.2	x4.1

Figure S2. Targeted mutants. **A)** Dose response curves of indolotryptoline compounds tested in target mutagenized and un-mutagenized MDR-sup *S. pombe* strains. The IC_{50} values determined from these dose response curves are noted on the legend of each graph. **B)** Fold difference in IC_{50} of the targeted mutants relative to the un-mutagenized control. Abbreviations: BE, BE-54017; Cla, cladoniamide A. See also Table S3.

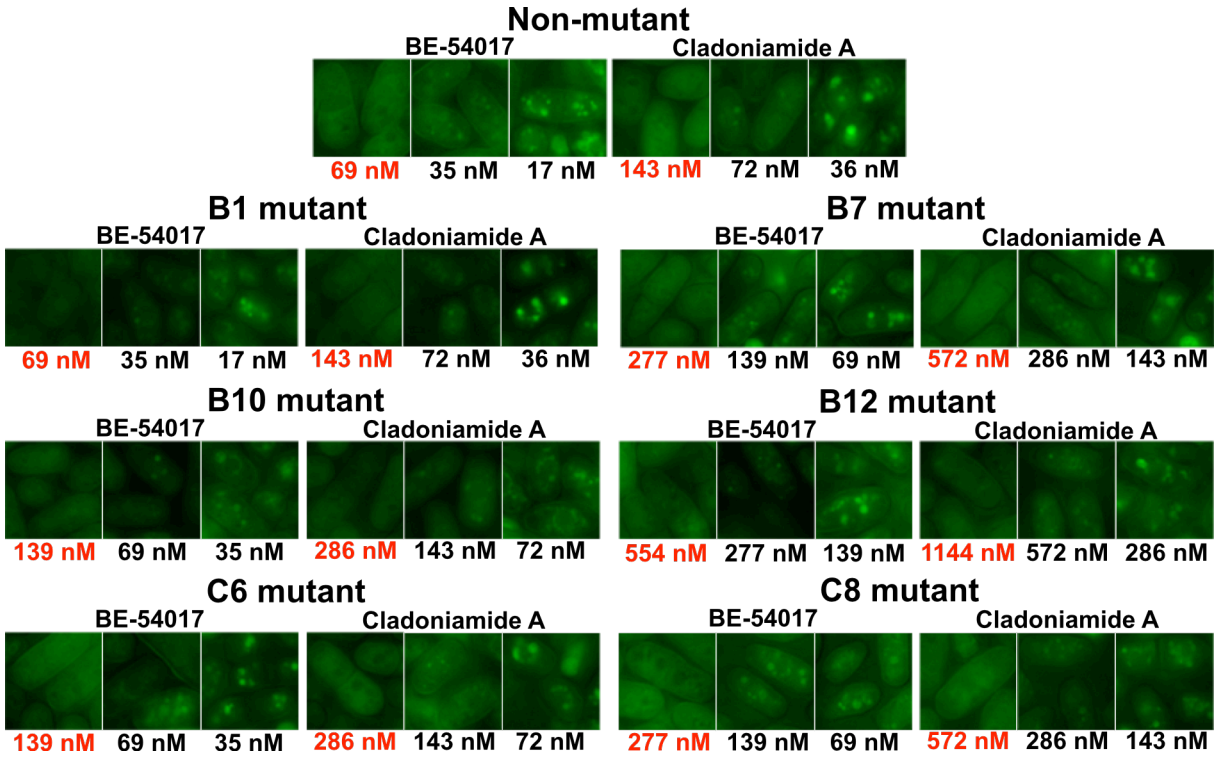


Figure S3. Fluorescent images of quinacrine-stained resistant mutants or un-mutagenized MDR-sup *S. pombe* upon incubation with either BE-54017 or cladoniamide A. The minimal inhibitory concentration (MIC) for *in vivo* V-ATPase activity was defined as the minimal concentration of compound at which no fluorescent puncta was observed in >95% of the cells and indicated for each assay as red text. See also Table S3.

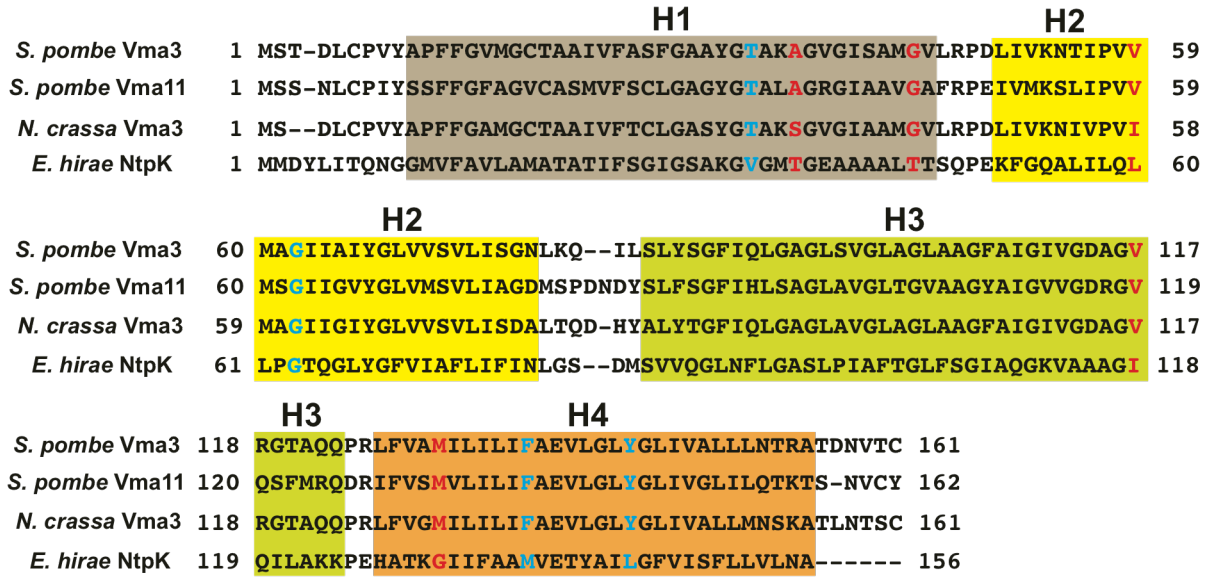


Figure S4. Protein sequence alignment of *Schizosaccharomyces pombe*, *Neurospora crassa* (used for plecomacrolide resistance), and *Enterococcus hirae* (used for crystal structure) proteolipid subunits. Regions that constitute the four helix bundle are highlighted (H1 brown, H2 yellow, H3 green, H4 orange). Residues that confer resistance to indolotryptolines in this study (red) and plecomacrolides from a previous study (blue) are colored.

Table S1. *Schizosaccharomyces pombe* strain list

Name	Genotype
SAK1	h+; *ade6-M210 leu1 ura4-D18*
SAK84	h+; *ade6 leu1 pap1::kanr bfr1::hygr pmd1::natr caf5::kanr mfs1::natr erg5* dnf2**
SAK690	h-; *ade6 leu1 pap1::kanr bfr1::hygr pmd1::natr caf5::kanr mfs1::natr erg5* dnf2**

* indicates frameshift mutation

Table S2. PCR primer list.

Name	Sequence
seq[a]-vma3-F	CGACATTGTAAAAGCCAGCT
seq-vma3-R	TCCCACCATAGAGATTCTC
seq-vma11-F	CAACGAAATACTACATCGACA
seq-vma11-R	TGATTAGCCTTAGAGAAAGTC
seq-zhf1-F	ATATAGCAAGTTTGCGCCTC
seq-zhf1-R	GTGACACAATAGATTAACCACG
mut[b]-vma3-F	CGATACGACATTGTAAAAGCC
mut-vma3-R	CGTGAAGTACATGCTTATACG
mut-vma11-F	AGAACTTGTGCCAAAAGTCC
mut-vma11-R	GCCTTAGAGAAAGTCAACAAG
mut-zhf1-F	TTGTGGTAAACGCGATTAGTG
mut-zhf1-R	CTAACGAGAAGAATCAAACC

(a) seq = primer used for gene sequencing. (b) mut = primer used for creating homologous recombination cassettes.

Table S3. Summary of IC_{50}/MIC values.

A) IC_{50} (in nM) of randomly mutagenized resistant mutant strains (as in Figure 3 and S1).

Mutant ID	BE-54017	Cladoniamide A	Bafilomycin A1	Concanamycin A	Brefeldin A
B1	92	200	1066	46	872
B3	87	179	N/D	N/D	N/D
B5	94	199	N/D	N/D	N/D
B7	64	126	311	24	808
B10, C12	27	57	291	24	929
B11	29	65	N/D	N/D	N/D
B12	121	257	150	29	902
C3	91	191	N/D	N/D	N/D
C6	31	68	287	21	836
C8	57	114	584	21	826
non-mutant	15	32	311	24	858
8 remaining clones	93*	194*	N/D	N/D	N/D

N/D = not determined; number in asterisk (*) represents the average value.

B) IC_{50} (in nM) of targeted mutants (as in Figure S2).

Mutant ID	BE-54017	Cladoniamide A
B1	95	204
B7	61	119
B10	28	65
B12	124	269
C6	29	69
C8	63	131

C) MIC (in nM) of whole-cell V-ATPase activity (as in Figure 4 and S3).

Mutant ID	BE-54017	Cladoniamide A
B1	69	143
B7	277	572
B10	139	286
B12	554	1144
C6	139	286
C8	277	572
non-mutant	69	143

D) IC_{50} (in nM) of unmutagenized strain in the presence of zinc (as in Figure 5).

[$ZnCl_2$]	BE-54017	Cladoniamide A	Bafilomycin A1	Concanamycin A	Brefeldin A
0.01 mM	13	30	300	26	877
0.05 mM	7.5	19	191	19	830
0.1 mM	4.5	11	150	15	857
0.2 mM	3.3	7.6	76	11	802