14-helical β-peptides elicit toxicity against *C. albicans* by forming pores in the cell membrane and subsequently disrupting intracellular organelles

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Table S1. Retention times and minimum inhibitory concentrations (MIC) against *C. albicans* for β-peptides used in this study, Related to Figure 1,.

Peptide	RT[a] (min ± SD)	MIC[b] (μg/mL)	MIC[b] (μM)	Peptide	RT[a] (min ± SD)	MIC[b] (μg/mL)	MIC[b] (μM)
1	21.13 ± 0.04	8	5.0	1-NBD	29.43 ± 0.08	16	9.6
2	22.03 ± 0.07	8	4.7	2-NBD	31.04 ± 0.07	8	4.6
3	15.49 ± 0.01	512	335	3-NBD	21.07 ± 0.04	64	40.5
Magainin 2		512	3390	1-Cy5	28.89 ± 0.05	4	1.9
Melittin		4	1.1				

[a] The average value obtained from three independent analytical RP-HPLC measurements. [b] The median value obtained from three independent experiments with triplicate measurements in each. SD denotes standard deviation (n = 3).

Table S2. The phospholipid ratios and sizes of SUVs were characterized using ^{31}P NMR and dynamic light scattering, Related to Figure 2. Calcein (70 mM) encapsulated SUVs ([lipid] = 36 μmol) were prepared three times on different days. Errors denote standard deviation (n = 3).

Vesicle composition	Theoretical ratio (%)	Quantitated ratio (NMR)	31P NMR (%)	DLS (Dynamic Light Scattering)		
		31P (%)		Intensity Averaged Diameter (nm)	Number Averaged Diameter (nm)	Effective Diameter (nm)
PC PE	60 40	59.0 ± 0.7 41.0 ± 0.7	4(	96.9	25.6	75.8 ± 2.0
PC PE	80 20	80.6 ± 1.8 19.4 ± 1.8	21	129.2	11.5	86.2 ± 2.8
PC PG	60 40	61.2 ± 0.4 38.8 ± 0.4	38.5	76.2	11.5	54.6 ± 1.4

Table S3. The phospholipid and ergosterol ratios and sizes of SUVs were characterized using ^{31}P NMR, ^{1}H NMR and dynamic light scattering, Related to Figure 3. The SUVs ([lipid] = 36 μmol for PC/PE (60:40) and PC/PG (60:40), [lipid] = 54 μmol for PC/PE (50:30:20)) were prepared three times on different days. Errors denote standard deviation (n = 3).

Vesicle composition	Theoretical ratio (%)	Quantitated ratio (NMR)		31P NMR (%)	DLS (Dynamic Light Scattering)		
		31P (%)	1H (%)		Intensity Averaged Diameter (nm)	Number Averaged Diameter (nm)	Effective Diameter (nm)
PC PE	60 40	60.3 ± 1.1 39.7 ± 1.1	N.D.	41	91.0	13.5	65.1 ± 3.4
PC PG	60 40	61.2 ± 0.5 38.8 ± 0.5	N.D.	38.7	52.8	16.8	47.7 ± 4.5
PC PE Ergosterol	50 (62.5) 30 (37.5) 20	62.0 ± 0.9 38.0 ± 0.9	84.8 ± 0.8 15.2 ± 0.8	38	74.6	15.9	73.0 ± 8.4

Table S4. Partition coefficients and critical concentrations obtained for NBD-labeled β-peptides with SUVs of the indicated lipid compositions, Related to Figure 3.

Peptide	Partition Coefficient ($10^4 M^{-1}$ ± SD)			Critical Concentration (nM ± SD)		
	1-NBC	2-NBD	3-NBD	1-NBC	2-NBD	3-NBD
PC:PE (60:40)	5.0 ± 2.0 42.5 ± 1.9	5.7 ± 7.0 63.1 ± 10.7	1.8 ± 0.2	13.7 ± 5.1	17.8 ± 4.2	None
PC:PG (60:40)	9.2 ± 3.8 95.3 ± 0.1	4.2 ± 0.1 92.0 ± 1.5	1.1 ± 0.1 29.5 ± 2.8	11.2 ± 5.4	15.8 ± 2.9	11.5 ± 2.1
PC:PG :Ergosterol (50:30:20)	25.6 ± 3.6	38.9 ± 5.3	1.2 ± 0.1	None	None	None

Values represent means and SD denotes standard deviation (n = 3).

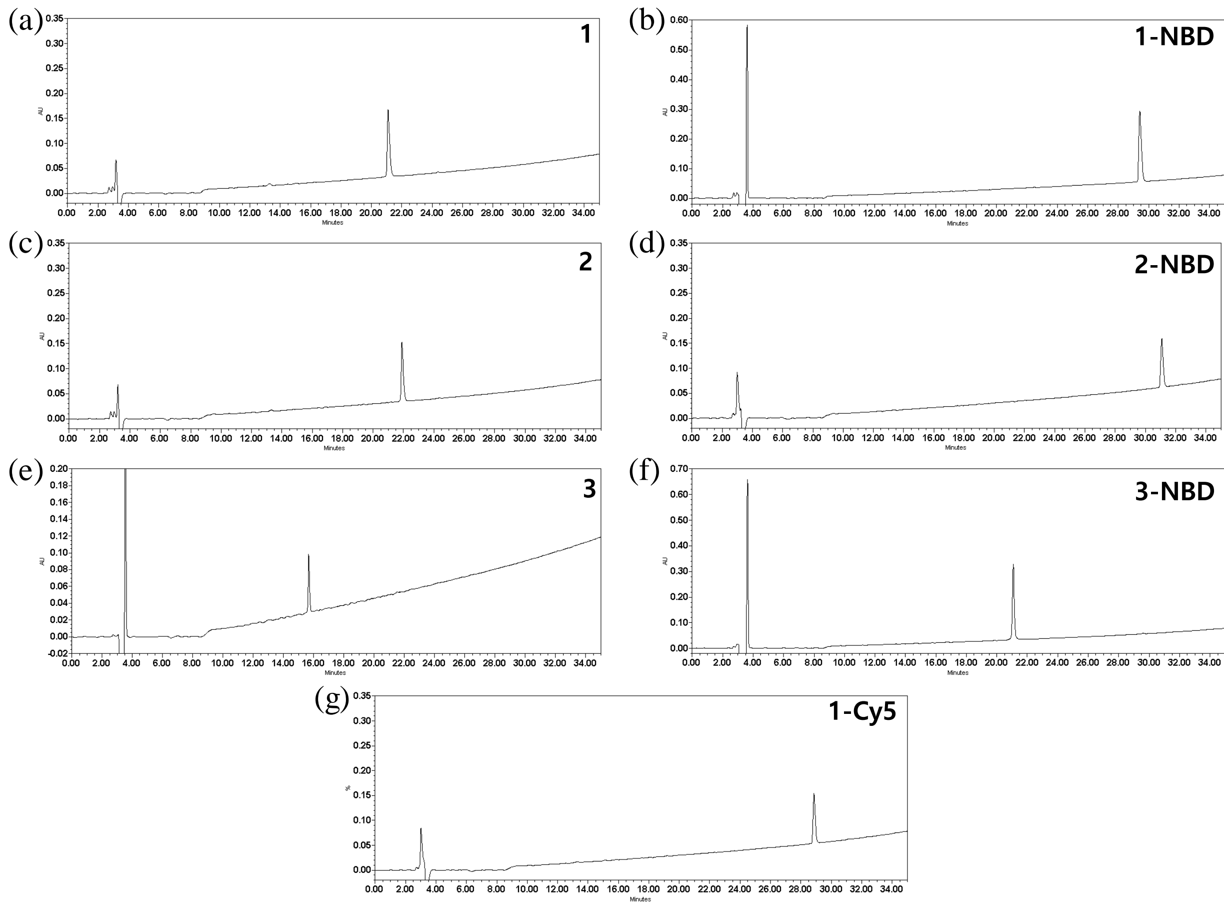


Figure S1. Reverse phase HPLC analysis of β-peptide retention times, Related to Figure 1. A C18 column (Waters, X-bridge) was used to quantify retention time in triplicate with a gradient of 20-80% CH_3CN in water containing 0.1% TFA over 5-35 min.

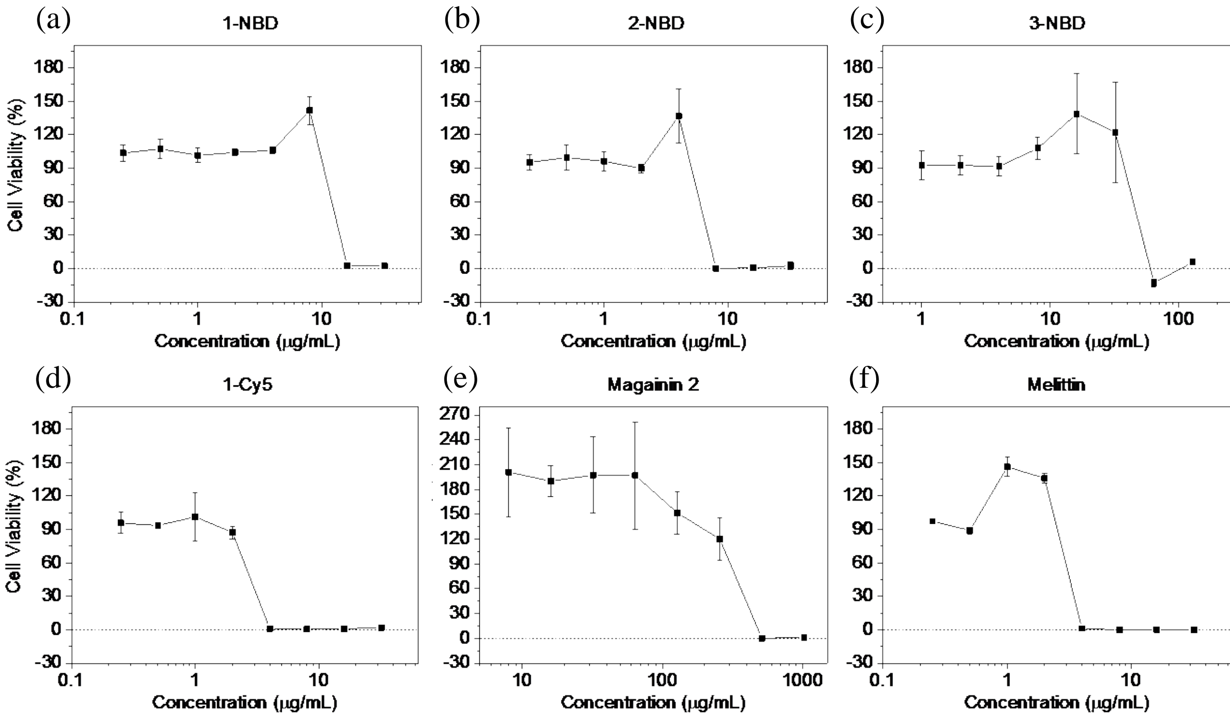


Figure S2. Concentration-dependent growth inhibition of planktonic *C. albicans* by β-peptides, magainin 2 and melittin, Related to Figure 1. *C. albicans* cells (10^3 cells/mL) were incubated with β-peptides for 48 h and β-peptide susceptibility was assessed using an XTT assay to quantify the absorbance at 490 nm. Percent viability is the concentration in β-peptide treated samples normalized to untreated samples. Data points are the averages of three independent experiments of three replicates each. Error bars denote standard deviations (n = 3).

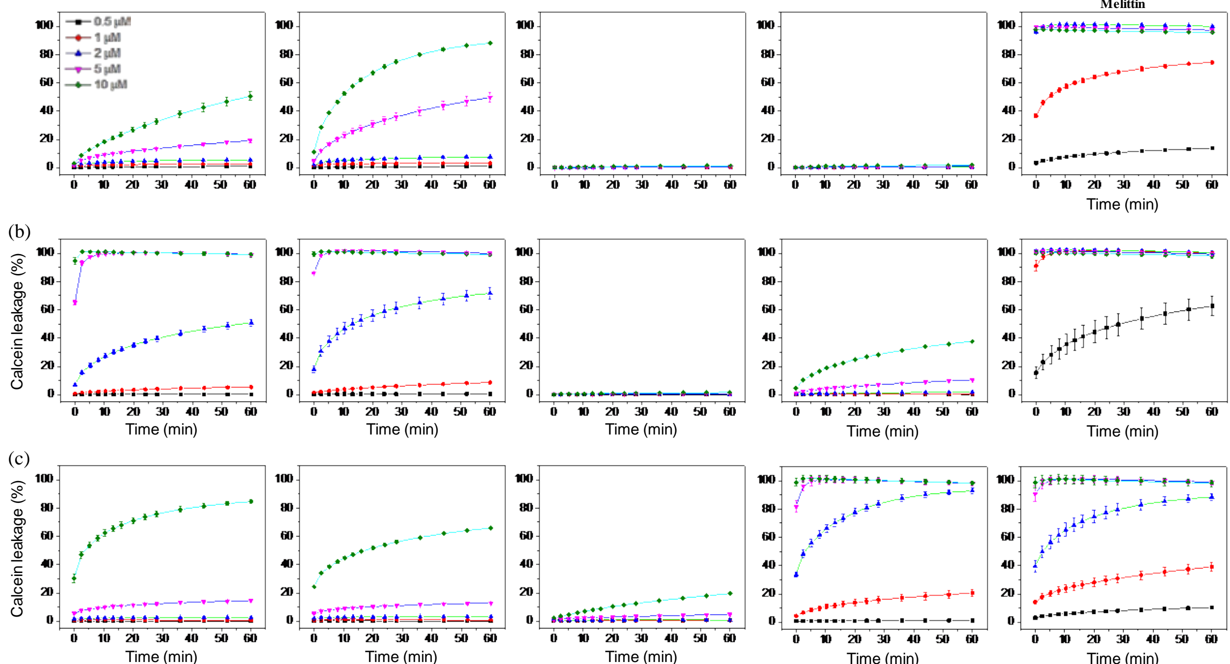


Figure S3. The time course of calcein leakage from (a) PC/PE (60:40) SUVs, (b) PC/PE (80:20) SUVs, and (c) PC/PG SUVs (60:40) induced by β-peptides and control peptides, Related to Figure 2. The leakage of calcein from SUVs ([lipid] = 130 μM) was quantified through time by monitoring the fluorescence of released calcein (ext. 495 / em. 515 nm) after exposure to the indicated concentrations of β-peptides, magainin 2 and melittin over 1 h. Data points are the averages of three independent experiments and error bars denote standard deviation (n = 3).

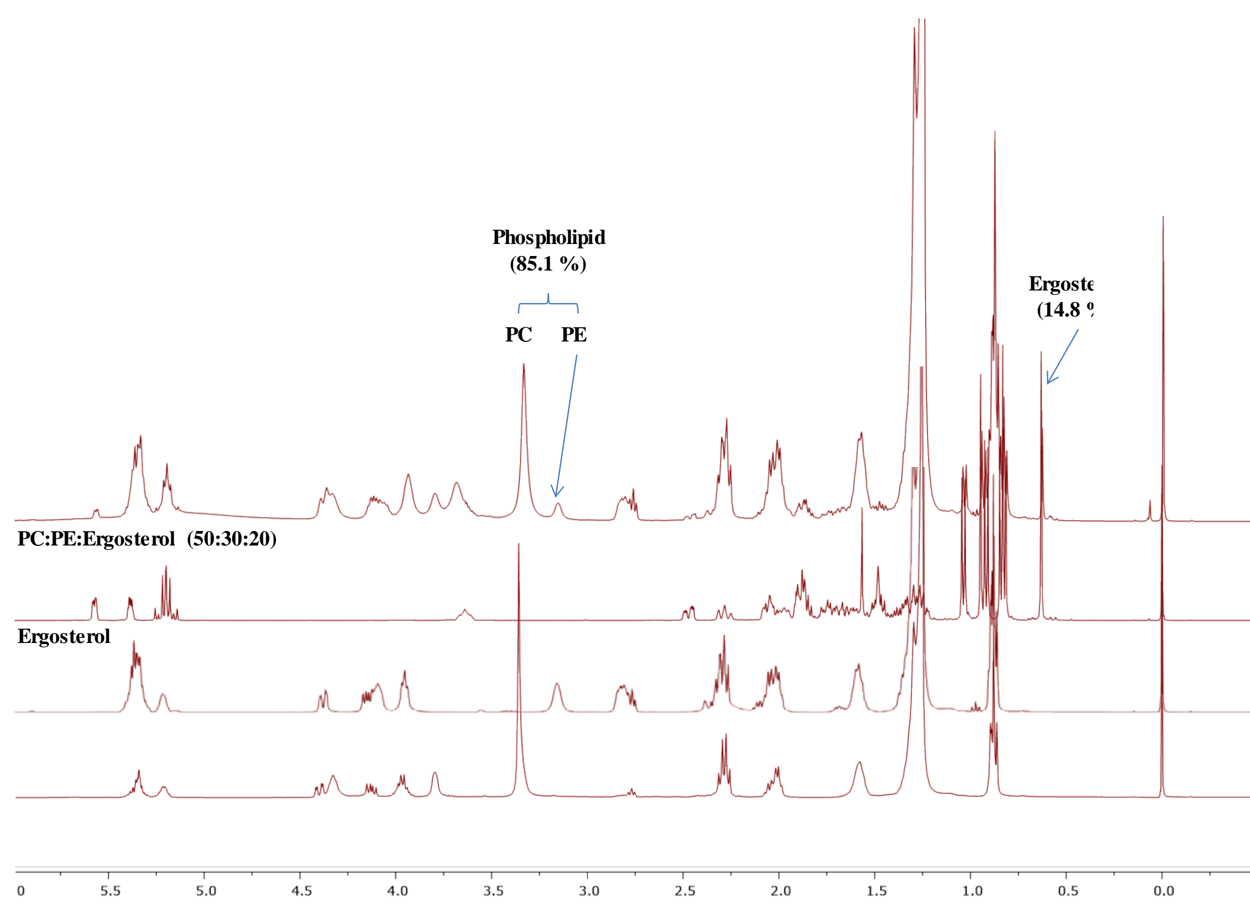


Figure S4. ^{1}H NMR quantification of phospholipid and ergosterol concentrations in SUVs, Related to Figure 3.

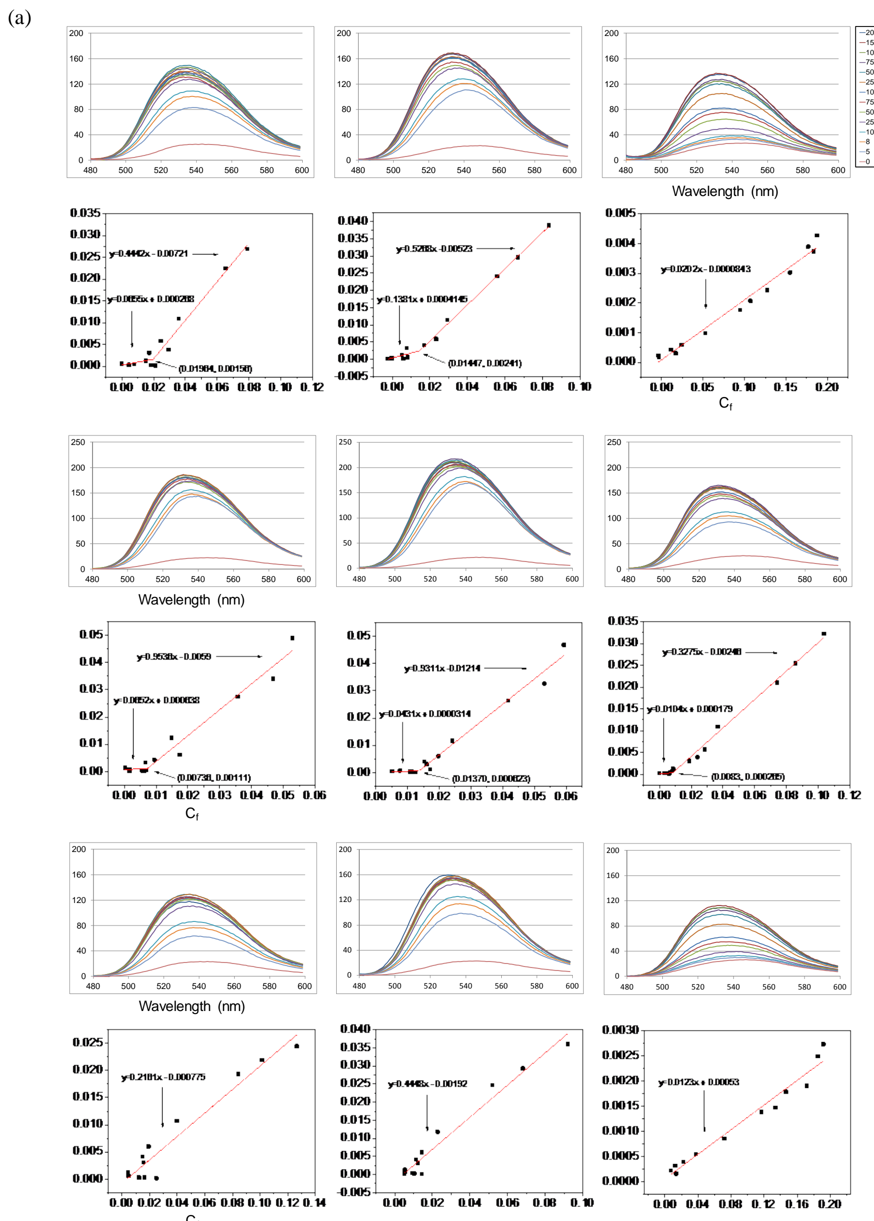


Figure S5. Fluorescence emission spectra of 0.2 μM NBD-labeled β-peptides, Related to Figure 3. Spectra were determined in the presence of PC/PE (60:40) SUVs (a and b), PC/PG SUVs (60:40) (c and d), and PC/PE/ergosterol (50:30:20) SUVs (e and f) ([lipid] = 5 to 2000 μM) in TBS buffer. Fluorescence spectra (ext. 468 nm) were scanned from 480 – 600 nm. (b, d, and f) Binding isotherms were obtained by plotting X_b* (molar ratio of bound peptide per 60% of lipid) vs C_f (equilibrium concentration of free peptide in the solution). The partition coefficient is the slope of the binding isotherm and the critical concentration is the intersection of two slopes of the binding isotherm. (a and b), (c and d), and (e and f) are the result of a single representative experiment selected from triplicate experiments performed on different days.

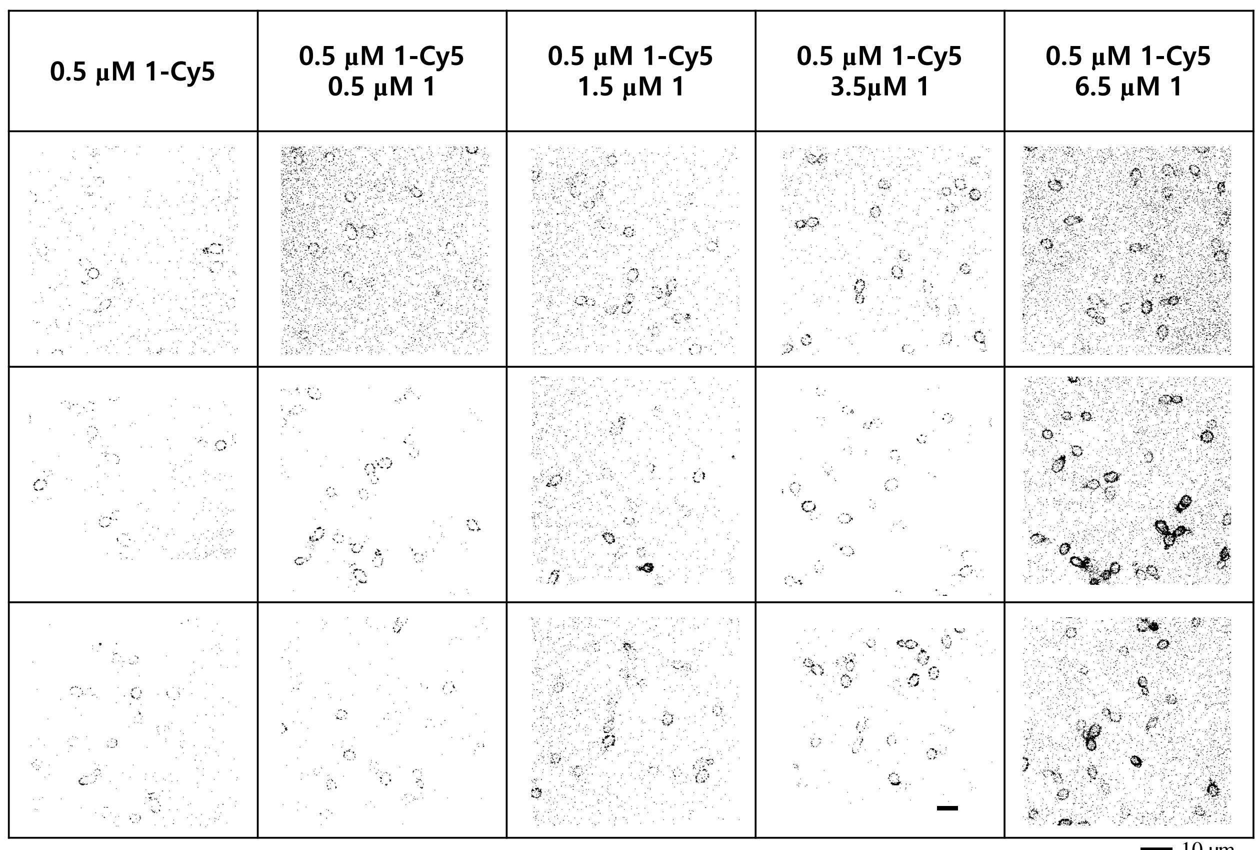


Figure S6. β-peptide 1-Cy5 insertion on the plasma membrane of *C. albicans* was imaged by CLSM after 3 min incubation with the mixture of 0.5 µM β-peptide 1-Cy5 (0.5 µM) and various concentrations of unlabeled β-peptide 1 (0, 0.5, 1.5, 3.5, 6.5 µM) in DPBS, Related to Figure 4. Scale bar indicates 10 µm.

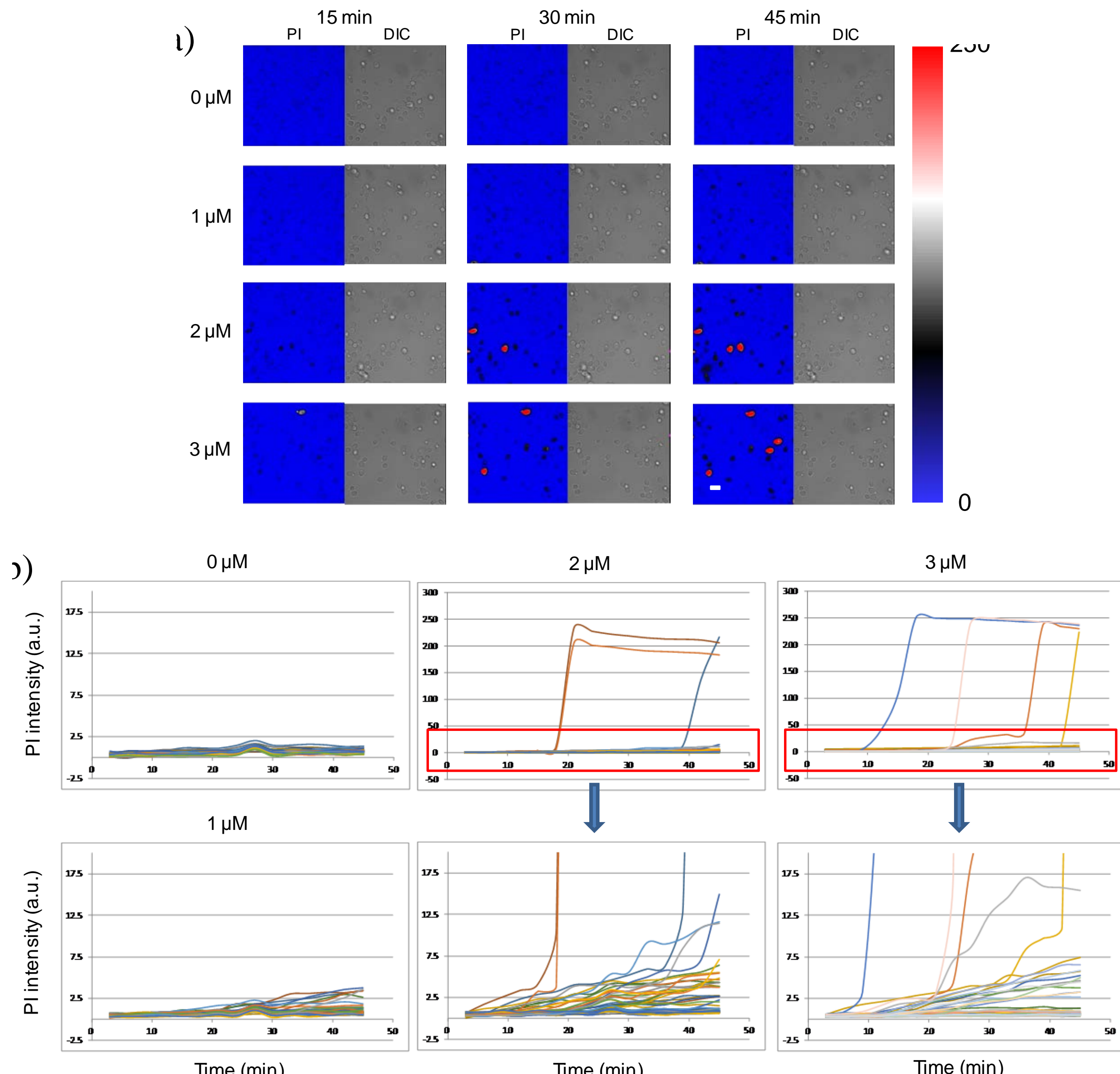
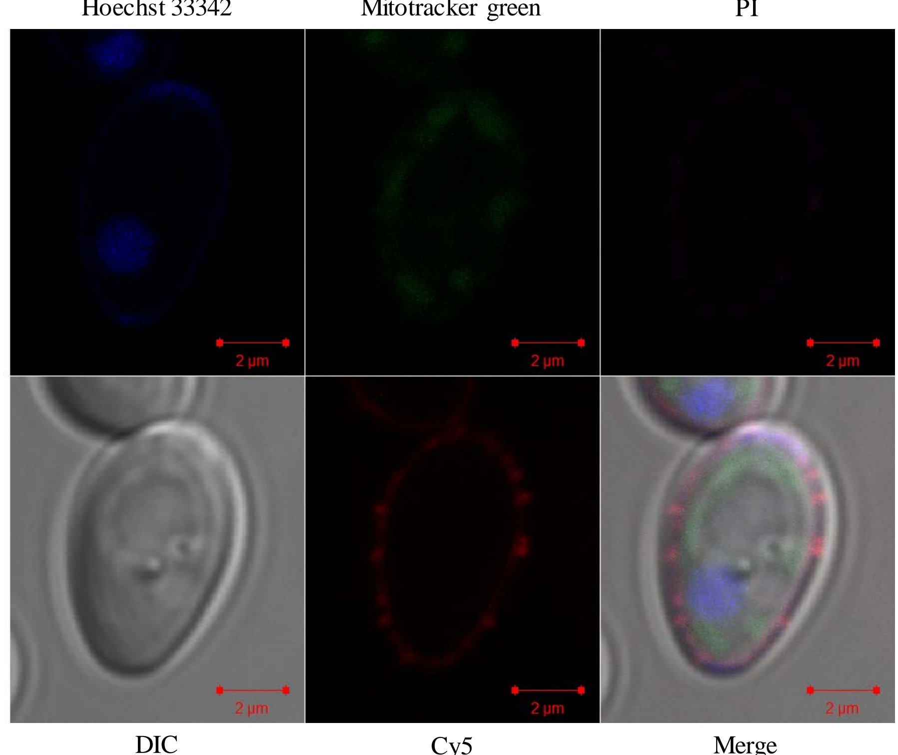


Figure S7. PI uptake by live *C. albicans*, Related to Figure 6. (a) DIC and fluorescence images of *C. albicans* were taken by CLSM after 15, 30, and 45 min incubation with 10 μg/mL PI and indicated concentrations of β-peptide 1 (0, 1, 2, or 3 μM) in DPBS (related to Figure 6). Red cells are lysed dead cells which were excluded from the analysis to calculate PI intensity. Black cells represent live cells which have taken up PI. Scale bar indicates 5 μm (b) The time course of PI uptake during 45 min of incubation with PI and β-peptides at the indicated concentrations. PI intensity of each individual cells, represented by different lines on the plots, was monitored every 3 min.



Movie S1. Time-lapse microscopy of β-peptide 1-Cy5 localization in a live *C. albicans,* Related to Figure 7*.* Cells were incubated with 2.0 µM β-peptide **1-Cy5**, 3.0 µM unlabeled β-peptide **1** and organelle specific fluorescence dyes: PI for DNA, Hoechst 33342 for the nucleus DNA, and Mitotracker green for mitochondria. A CLSM image was taken every 40 s for 15 - 20 min. Scale bar indicates 2 µm.