Supplementary Data

Specific interactions between the *Candida albicans* ABC transporter Cdr1p ectodomain and a D-octapeptide derivative inhibitor

Kyoko Niimi[1], David R. K. Harding[2], Ann R. Holmes[1], Erwin Lamping[1], Masakazu Niimi[1,3], Joel D. A. Tyndall[4], Richard D. Cannon[1] and Brian C. Monk[1*]

[1]The Sir John Walsh Research Institute, University of Otago, Dunedin, New Zealand. [2]The Centre for Separation Science, Massey University, Palmerston North, New Zealand. [3]Department of Bioactive Molecules, National Institute of Infectious Diseases, Tokyo, Japan. [3]National School of Pharmacy, University of Otago, Dunedin, New Zealand.

***Corresponding author**: Department of Oral Sciences and the Sir John Walsh Research Institute, School of Dentistry, University of Otago, P.O. Box 647, 310 Great King Steet, Dunedin, New Zealand

Tel: +64 3 479 7099, Fax: +64 3 479 7078

Email: brian.monk@otago.ac.nz

Figure legends

Fig S1. Checkerboard assays for *Saccharomyces cerevisiae* strains overexpressing *S. cerevisiae* Pdr5p and heterologous proteins from *Candida albicans* (Ca) or *C. glabrata* (Cg). A, AD/PDR5; B, AD/CaCDR2; C, AD/CgCDR1; D, AD/CgCDR2; E, AD/CaMDR1; F, AD/CaERG11. Checkerboard assays were carried out as described in Experimental Procedures.

Fig S2. Checkerboard assays for isogenic *Candida albicans* strains. A, CAI4; B. MML33 (homozygous *CDR1* deletant). Checkerboard assays were carried out as described in Experimental Procedures.

Fig S3. TRITC-labeled RC21, RC22 and RCC3 are excluded from yeast. Yeast strains (AD/pABC3 or AD/CDR1-EGFP) were grown in CSM medium at pH 6.8 to mid-log phase (OD_{600nm} = 1), washed by centrifugation and then incubated in 10 μM TRITC-labeled RC21, RC22 or RC23 in fresh CSM medium for 5 minutes at room temperature and viewed by confocal microscopy.

Fig S4. RC21 does not appear to affect the folding or localization of CaCdr1p-EGFP chimeras. An overnight culture of AD/CDR1-EGFP cells was grown in fresh CSM medium at pH 6.8 from 0.1 OD_{600nm} for 6 h in the presence or absence of 2.5 μM RC21v3. The cells were grown to the same density in this time (~1.0 OD_{600nm}) and viewed by confocal microscopy as a series of z-sections.

Fig S5. Inhibition of wild type and RC21 chemosensitization suppressor mutant CaCdr1p by RC21v3. Plasma membranes were prepared and CaCdr1p ATPase activity determined at pH 7.5 without ATP protection as described in Experimental Procedures. The RC21 chemosensitization suppressor mutants are affected by the mutations described in Table S1.

Fig S1

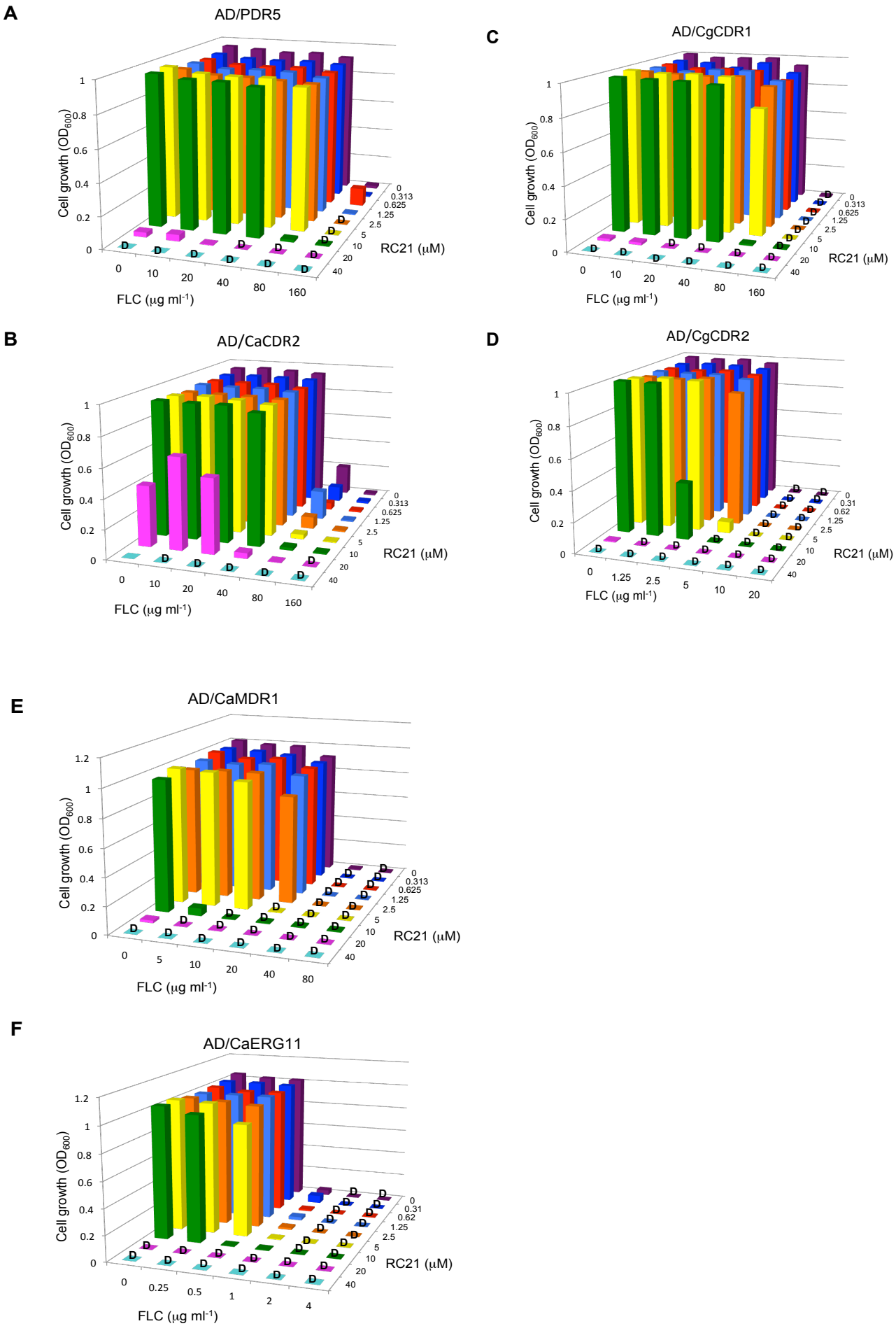

A
AD/PDR5
Cell growth (OD600)
FLC (μg ml-1)
RC21 (μM)
B
AD/CaCDR2
C
AD/CgCDR1
D
AD/CgCDR2
E
AD/CaMDR1
F
AD/CaERG11

Fig S2

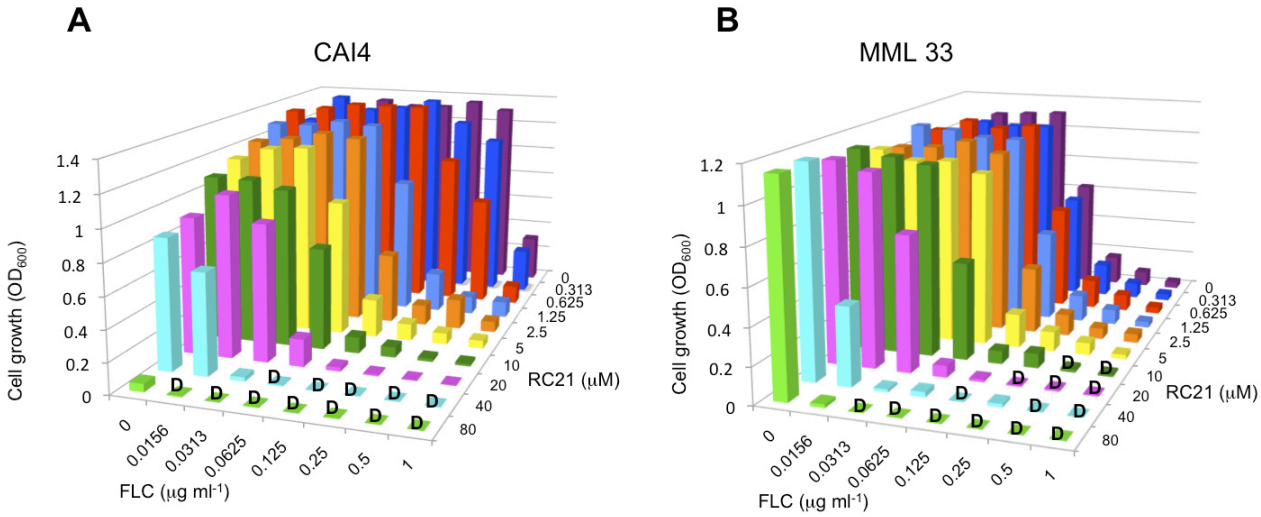

A
CAI4
Cell growth (OD600)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
0.0156
0.0313
0.0625
0.125
0.25
0.5
1
FLC (μg ml-1)
0
0.313
0.625
1.25
2.5
5
10
20
40
80
RC21 (μM)
D
B
MML 33
Cell growth (OD600)
1.2
1
0.8
0.6
0.4
0.2
0
FLC (μg ml-1)
RC21 (μM)

Fig S3

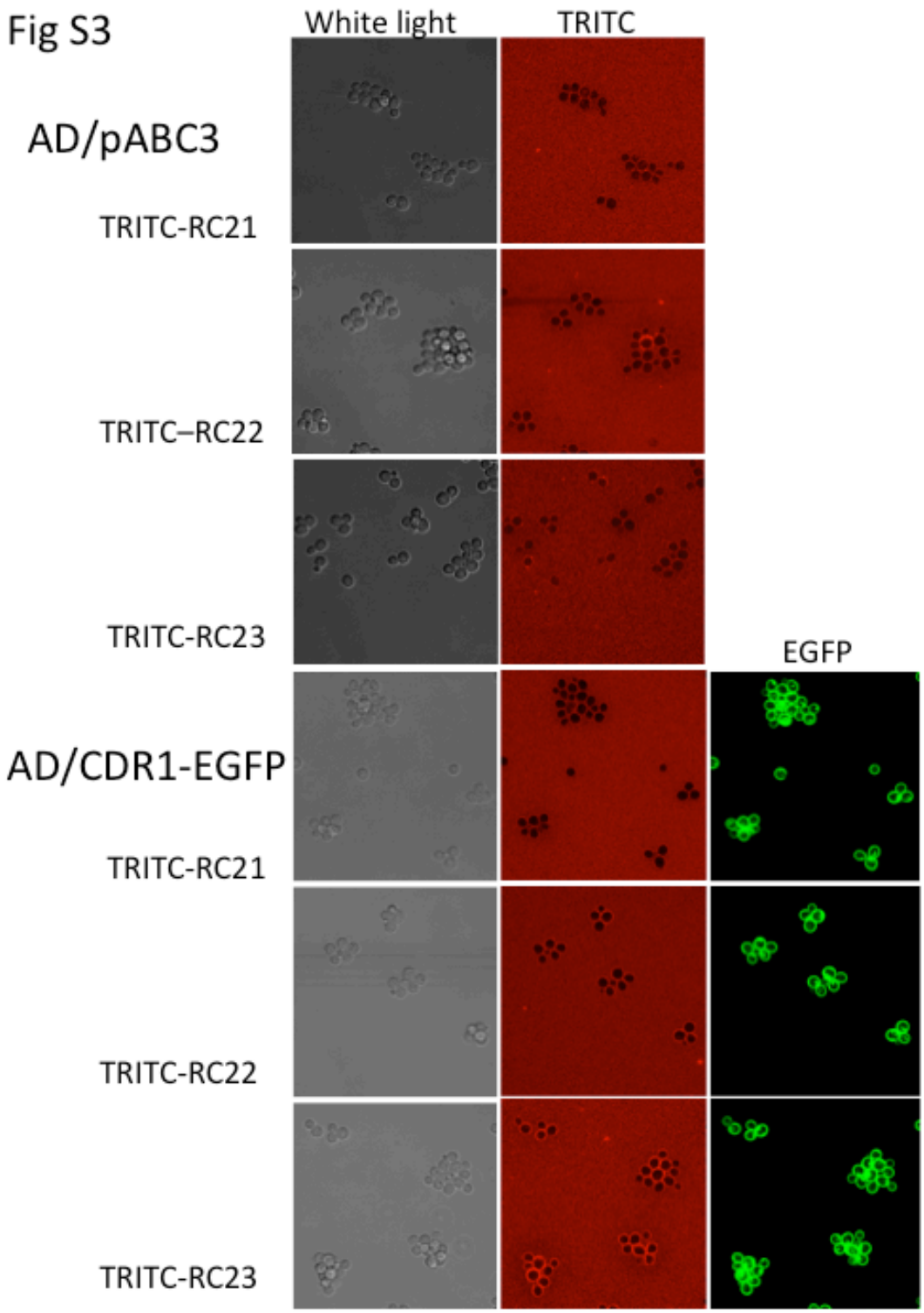
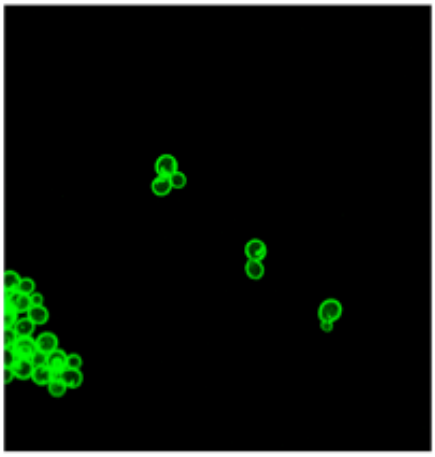

White light
TRITC
AD/pABC3
TRITC-RC21
TRITC–RC22
TRITC-RC23
EGFP
AD/CDR1-EGFP
TRITC-RC21
TRITC-RC22
TRITC-RC23

Fig S4

AD/CDR1-EGFP
(control)



AD/CDR1-EGFP
+2.5 µM RC21v3

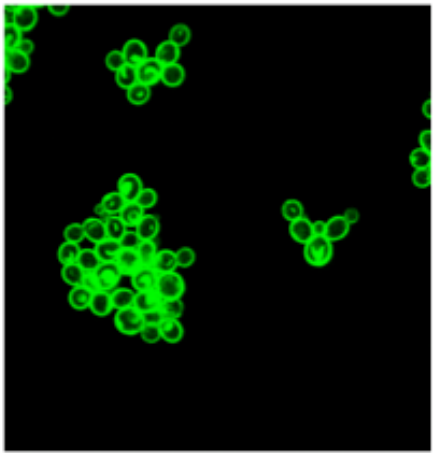


Fig S5

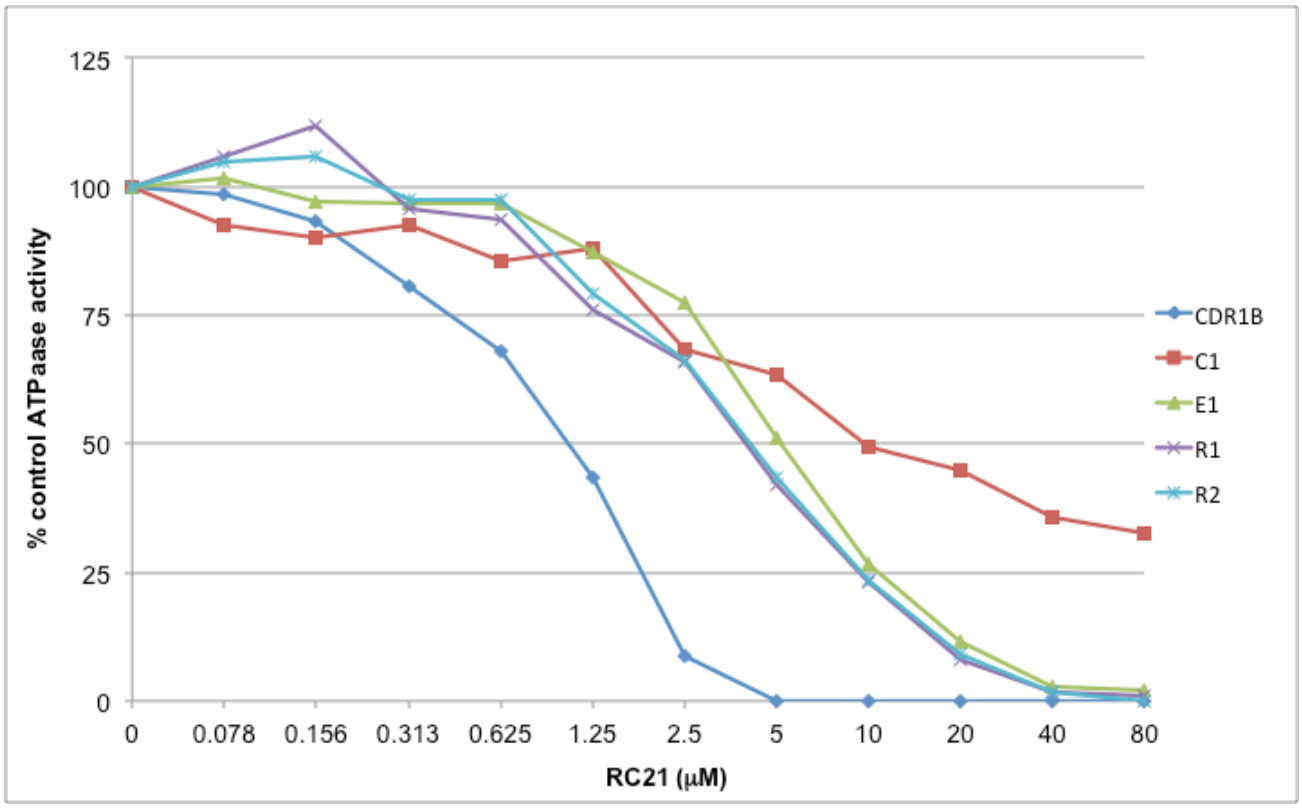

125
100
75
50
25
0
% control ATPaase activity
0
0.078
0.156
0.313
0.625
1.25
2.5
5
10
20
40
80
RC21 (μM)
CDR1B
C1
E1
R1
R2

Table S1

IC_{50} of RC21v3 for Cdr1p-ATPase activity in plasma membrane fractions prepared from RC21 chemosensitization suppressor mutants

		IC_{50} of RC21v3 (μM)		
Strain	Amino acid change	Assay 1	Assay 2	Average
CDR1 B	Wild type	1.1	1.1	1.1 (100)
C1	Q1226K	8.9	9.7	9.3 (68)
E1	V674F	6.2	5.5	5.9 (97)
R1	Q714K	4.9	4.3	4.6 (99)
R2	Q1445K	3.0	4.2	3.6 (99)

ATPase inhibition assays were performed in duplicate at pH 7.5 without ATP protection in two separate experiments of which Fig S5 is a representative example. The mean % inhibition of CaCdr1p by RC21v3 at 40 μM is given in brackets.