Supplementary Materials

Supplementary Figure S1

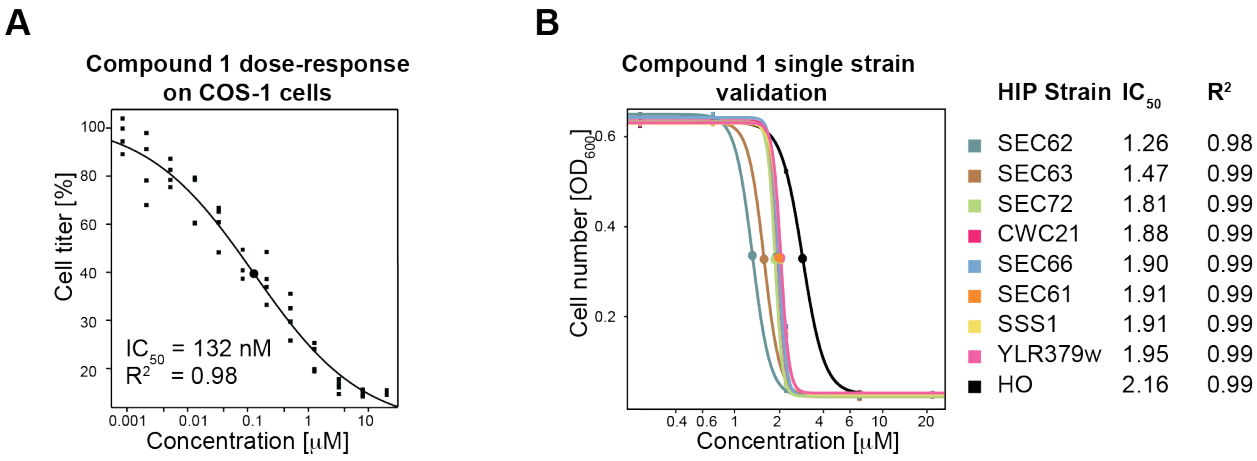

Figure S1. Validation of individual HIP strains identified to be hypersensitive to compound 1.

Dose response curves and IC_{50} values confirm hypersensitivity to compound 1 of diploid strains specifically haploid for translocon components compared to HO control strain in the same order as observed in the HIP profile shown in Fig. 2. r^2 values reflect the quality of the fit to a sigmoid curve function.

Supplementary Figure S2

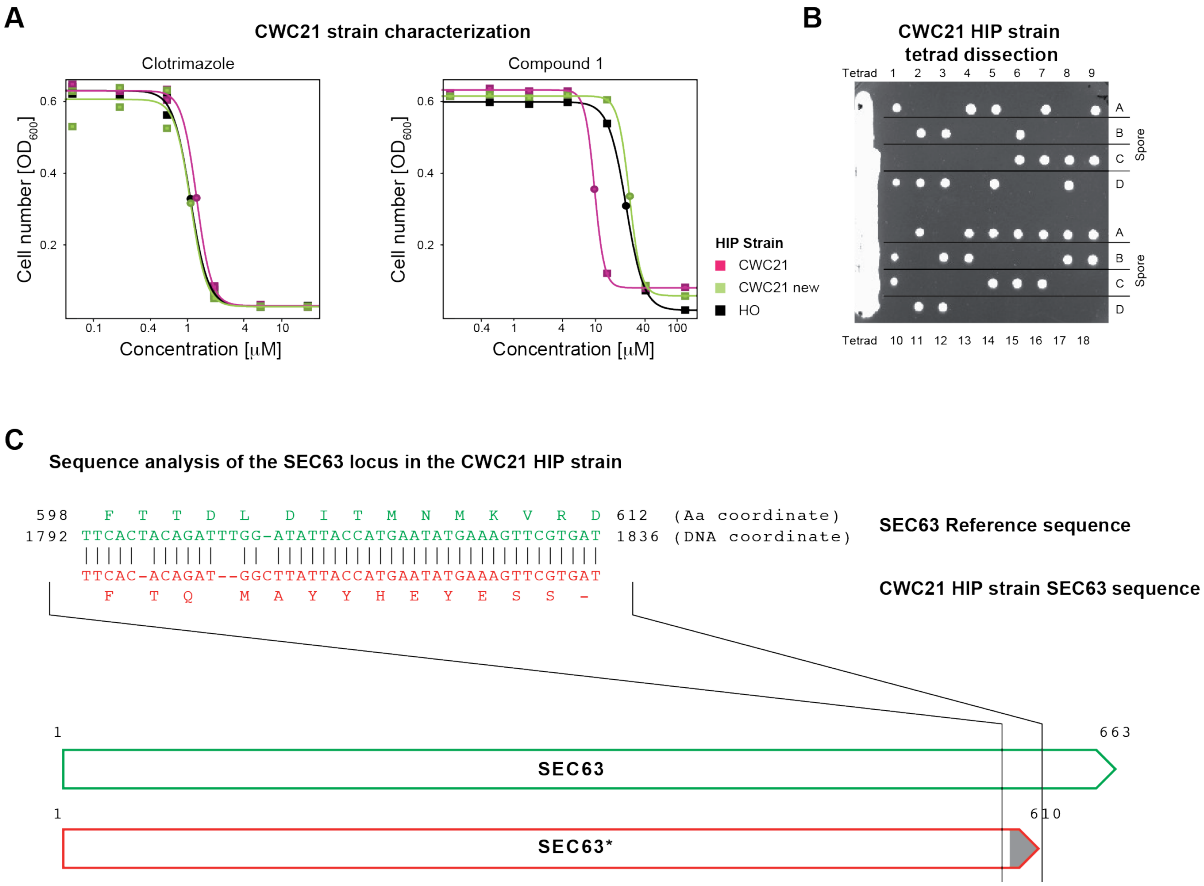

Figure S2. The *CWC21* gene is not linked to translocon function, but the CWC21 HIP strain in the deletion collection carries a background mutation in the SEC63 gene.

(A) The CWC21 HIP strain from the yeast deletion collection, but not a freshly generated CWC21 HIP strain, is hypersensitive to compound 1 targeting the SEC61 translocon, but not against the Erg11 inhibitor clotrimazole, an example of an unrelated inhibitor. (B) Sporulation and tetrad dissection of the CWC21 HIP strain yielded only 2 viable spores, even though CWC21 is a nonessential gene. (Further analysis revealed that viability is not linked to the *cwc21::KanMX* marker, data not shown). (C) Sequencing the *SEC61*, *SEC62*, and *SEC63* genes in the CWC21 HIP strain revealed heterozygous mutations in the C-terminal part of SEC63, leading to amino acid changes and protein truncation.

Supplementary Figure S3

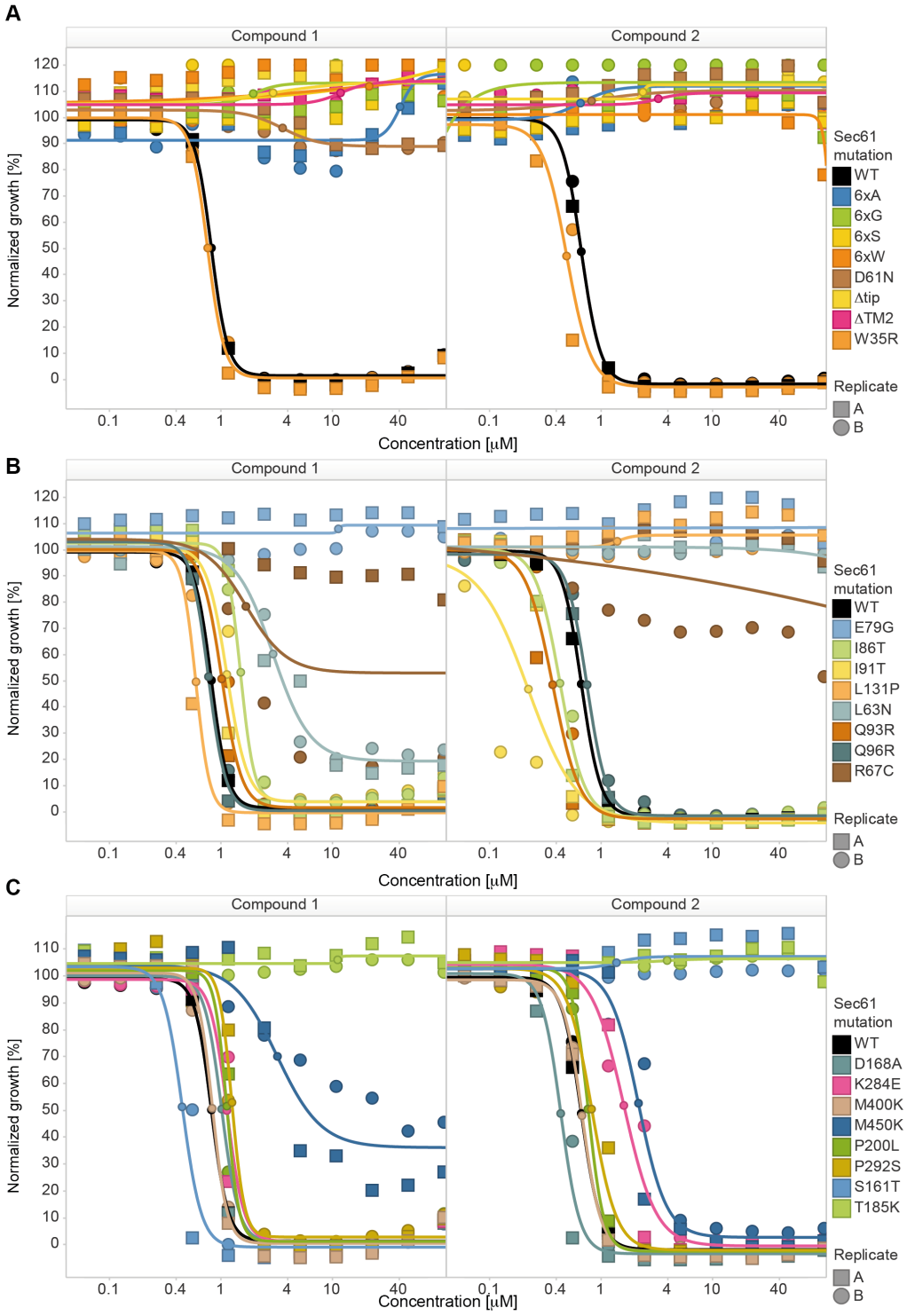

Figure S3. Sensitivity of previously described Sec61p mutants to compounds 1 and 2. Dose-response curves of yeast strains expressing the indicated Sec61p point mutants described previously (Junne *et al.*,

2006; Junne *et al*., 2007), for compounds 1 and 2. Each panel shows Sec61p wild-type and eight mutants tested against each compound. Replicate analyses are distinguished by squares and circles. Resulting IC_{50} and r^2 values are listed in Table 2.

Table S1. DNA and Protein sequences of genes depicted in Fig. 1C

Orf2 : L-pipecolate oxidase
Orf3 : NRPS
Orf4 : 2-hydroxyacid dehydrogenase
Orf5 : 2-isopropylmalate synthase

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Strucutural characterization of compound 1.

High-resolution mass spectrometry (Bruker qTOF maxis 3G):
Found: 592.91764 ($M+H^{2+}$); calc. for $C_{63}H_{109}N_9O_{12}$+H: 592.9171.

Table S2. ^{1}H and ^{13}C assignments for compound 1

Aminoacid	**NH, (N-CH_3), [^{13}C], ppm**	**α-H, [^{13}C] ppm**	**β-H, [^{13}C] ppm**	**γ-H, [^{13}C] ppm**	**δ-H, [^{13}C] ppm**	**ε-H, [^{13}C] ppm**	**C=O [^{13}C] ppm**
2-Hydroxy-5-methyl hexanoic acid [1]		5.26, [71.8]	1.74, 1.68 [28.8]	1.34, 1.28 [33.6]	1.55 [27.9]*	0.8-0.9 [23.1, 23.0]**	[169.9]
Pipecolic acid [2]		5.01 [52.2]	2.26, 1.33 [24.8]	1.61, 1.34 [20.8]	1.70, 1.34 [25.5]	4.07, 2.98 [43.5]	[169.6]
2-Amino-5-methyl hexanoic acid [3]	6.68	4.50 [51.4]	1.66, 1.50 [26.2]	1.34 [35.6]	1.55 [27.7]*	0.8-0.9 [22.9, 22.8]**	[170.9]
5-Methyl-2(methylamino)hexanoic acid [4]	(2.77) [29.4]	5.19 [53.4]	1.73, 1.45 [26.2]	1.00, 0.96 [34.4]	1.55 [27.6]*	0.8-0.9 [22.9, 22.6]**	[169.7]
N-Methyl Threonine [5]	(2.79) [29.8]	5.20 [57.6]	3.86 [64.3]	1.05 [20.6] OH:4.55			[170.1]
Pipecolic acid [6]		4.70 [52.4]	1.77, 1.68 [25.1]	1.61, 1.50 [18.0]	1.74, 1.61 [22.9]	3.97, 3.61 [41.9]	[172.4]
5-Methyl-2(methylamino)hexanoic acid [7]	(2.55) [28.1]	4.63 [59.9]	1.94, 1.55 [25.9]	1.15, 0.96 [34.9]	1.55 [27.6]*	0.8-0.9 [22.6, 22.3]**	[169.3]
Alanine [8]	8.78	4.91 [45.2]	1.19 [18.3]				[171.8]
Pipecolic acid [9]		5.36 [49.4]	1.89, 1.38 [26.1]	2.17, 1.57 [20.2]	1.45 [26.4]	3.81, 1.91 [43.5]	[171.1]
N-Methyl-Isoleucine [10]	(2.63) [29.2]	4.96 [58.3]	1.81 [33.5]	CH_2 1.19, 0.93 [23.6]	0.79 [10.4]	γ-CH_3 0.83 [15.2]	[171.7]

NMR spectra were recorded in d6-DMSO on a Bruker AV-III-600 NMR spectrometer (Fällanden, Switzerland) using a 1.7 mm TXI Cryoprobe. All assignments are based on chemical shifts and homo- and hetero nuclear correlations.

* 5-Methyl-(2-x)-hexanoicacid , position δ-CH, shifts interchangeable
**5-Methyl-(2-x)-hexanoicacid , position ε-CH_3, shifts interchangeable