Supplemental Material

Substrate specificity and mutational analysis of *Kluyveromyces lactis* γ-toxin, a eukaryal tRNA anticodon nuclease

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Supplemental Figure S1, S2

Supplemental Tables S1, S2

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MAATTAREEFFLCYDLIRYLKQYEKTGESKLVEQTFFNSIKNLDINSREYMELVYNKIAG  60
ISNERNKFENIYKDGDSISQVVERAVSEKKLTFGLNGKGLYVPENGEPRLKGYASIIERI  120
TLDLMEVYSIKGLNDIPRDIEFNMEKIRQERYNQMKEALNSVEGYKGKIVASDSDWCFKD  180
PQGNRITDFDSINKELGLGRRDVKLDKGHDDLIKLCTEKIDSMNNLQNGKCV          232
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Figure S1. **The amino acid sequence of the intracellular version of *K. lactis* γ-toxin**. The amino acids that have been subjected here and previously to alanine scanning are highlighted. Tests of the mutant alleles for toxicity *in vivo* in *S. cerevisiae* discriminated essential residues (green) and nonessential residues (yellow).

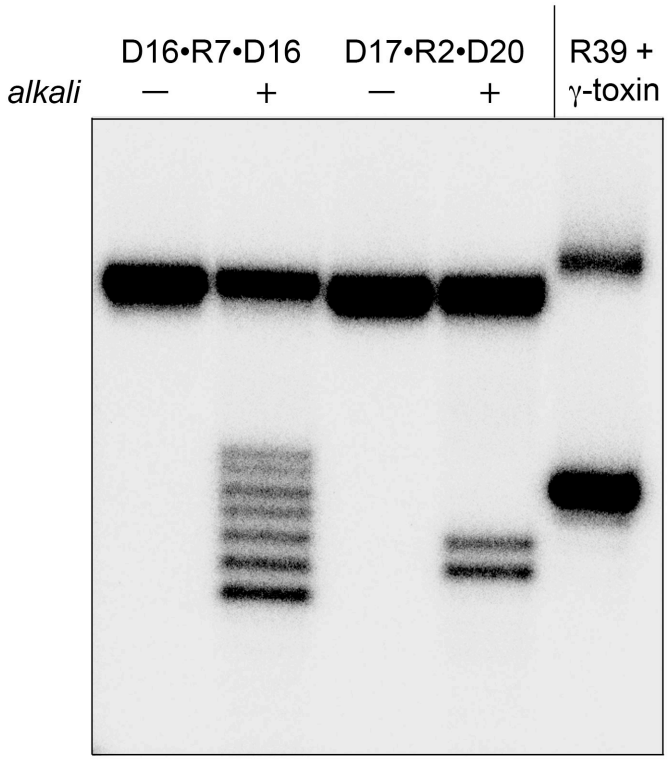

Figure S2. **Partial alkaline hydrolysis of chimaeric RNA-DNA stem-loops**. The 5' ^{32}P-labeled D16•R7•D16 and D17•R2•D20 oligonucleotides were incubated in a solution of 40 mM $NaHCO_3$, 60 mM Na_2CO_3 (pH 10.2) for 12 min at 95˚C (alkali +). The partially cleaved RNAs were then resolved by denaturing PAGE in parallel with untreated D16•R7•D16 and D17•R2•D20 oligonucleotides (alkali –) and with R39 oligonucleotide that had been incubated with γ-toxin.

Table S1

Mutational effects at tyrosine residues

γ-toxin allele	Toxicity
WT	yes
Y14A	no
Y14F	no
Y14L	no
Y14S	no
Y19A	yes
Y23A	no
Y23F	yes
Y23L	no
Y23S	no
Y50A	no
Y50F	no
Y50L	no
Y50S	no
Y55A	no
Y55F	yes
Y55L	no
Y55S	no
Y72A	no
Y72F	yes
Y72L	no
Y72S	no
Y101A	no
Y101F	no
Y101L	no
Y101S	no
Y113A	no
Y113F	no
Y113L	no
Y113S	no
Y128A	yes
Y152A	no
Y152F	yes
Y152L	no
Y152S	no
Y165A	no
Y165F	no
Y165L	no
Y165S	no

Table S2

Mutational effects at aspartate residues

γ-toxin allele	Toxicity
WT	yes
D15A	no
D15E	±
D15N	no
D44A	no
D44E	yes
D44N	yes
D74A	yes
D76A	no
D76E	±
D76N	no
D123A	no
D123E	yes
D123N	no
D135A	yes
D139A	yes
D173A	no
D173E	yes
D173N	no
D175A	no
D175E	yes
D175N	no
D180A	no
D180E	no
D180N	no
D188A	no
D188E	±
D188N	±
D190A	±
D202A	no
D202E	no
D202N	no
D206A	no
D206E	no
D206N	no
D210A	yes
D211A	yes
D221A	yes