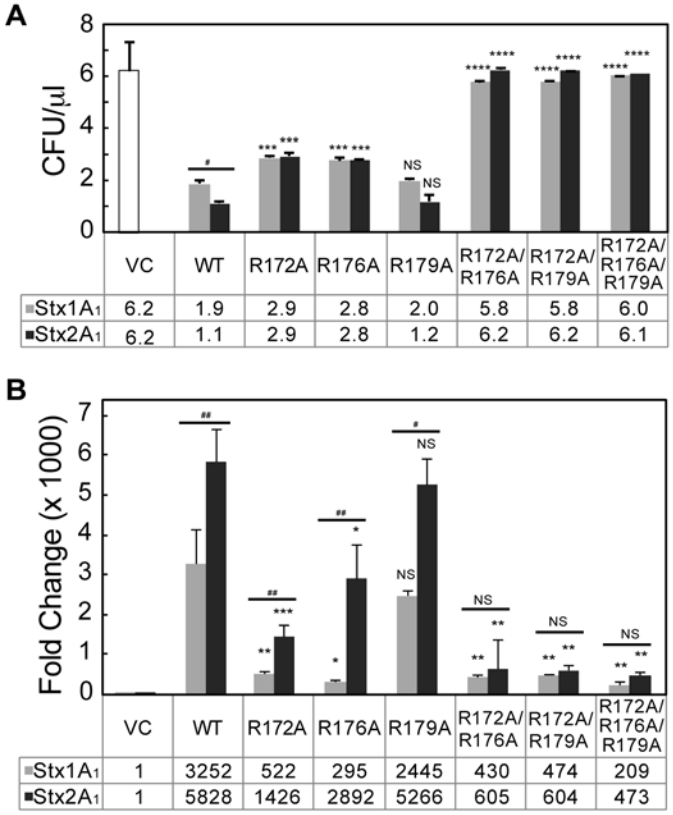
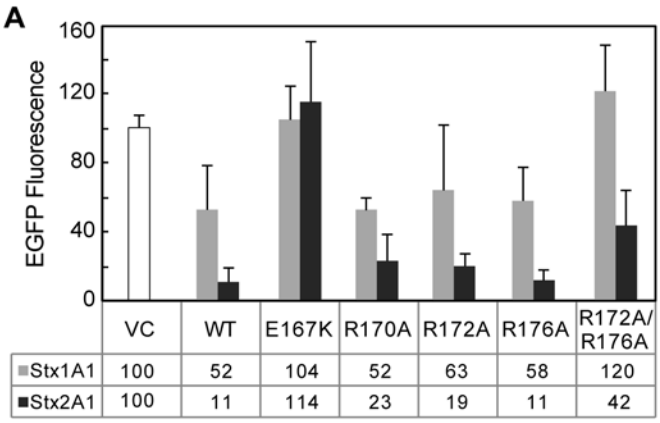


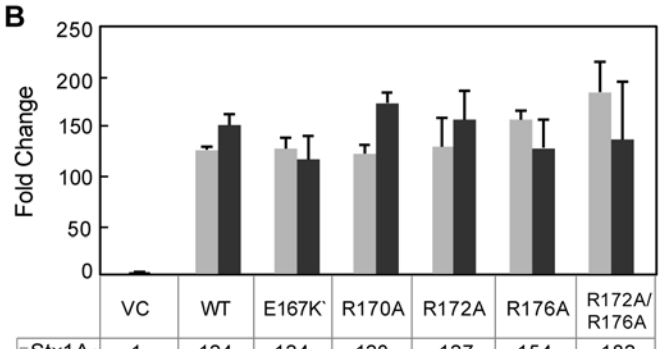
S1. (A) Viability and ribosome depurination in yeast expressing wild type (WT) or mutant $Stx1A_1$ or $Stx2A_1$. Yeast transformed with plasmids carrying WT or mutant $Stx1A_1$ (gray bars) or $Stx2A_1$ (black bars) and yeast harboring the empty vector (VC) were grown in SD medium supplemented with 2% glucose and transferred to SD medium supplemented with 2% galactose. At 0 and 4 hours post induction (hpi), a series of 10-fold dilutions were plated on media containing 2% glucose and grown at 30°C for 1-2 days. Colony forming units per ml (CFU/ml) were calculated at 4 hpi from at least 3 independent transformants. Error bars represent S. E. where n=3 independent experiments. Means of WT Stx1A1, Stx2A1 and Stx1A1 and Stx2A1 variants were significantly different using two-sample t-test (*means compared to respective WT; #means compared between Stx1A1 and Stx2A1. ***P< 0.001, ****P< 0.0001, $^{\#}$P< 0.05, NS= Not significant). (B) Depurination of yeast ribosomes. Total RNA (375 ng) isolated from 1 OD_{600} cells expressing wild type (WT) or mutant $Stx1A_1$ (gray bars) or $Stx2A_1$ (black bars) collected at 1 hpi was used to quantify the relative level of depurination

using qRT-PCR. The y-axis shows the fold change in depurination of toxin-treated samples over the control samples without toxin (VC). Error bars represent S. E. where n=3 replicates. Means of WT Stx1A1, Stx2A1 and Stx1A1 and Stx2A1 variants were significantly different using two-sample t-test (*means compared to respective WT; #means compared between Stx1A1 and Stx2A1. *P< 0.05, **P< 0.01, ***P< 0.001, $^{\#}$P< 0.05, $^{\#\#}$P< 0.01, NS= Not significant).

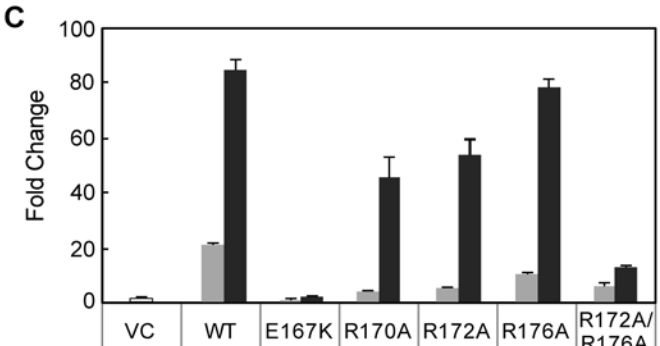
Supplementary Figure 2



	VC	WT	E167K	R170A	R172A	R176A	R172A/ R176A
Stx1A1	100	52	104	52	63	58	120
Stx2A1	100	11	114	23	19	11	42



	VC	WT	E167K	R170A	R172A	R176A	R172A/ R176A
Stx1A1	1	124	124	120	127	154	182
Stx2A1	1	148	113	171	153	126	134



	VC	WT	E167K	R170A	R172A	R176A	R172A/ R176A
Stx1A1	1	21	1	4	5	10	6
Stx2A1	1	84	2	45	53	78	12

S2 (A) Translation inhibition in mammalian cells by WT Stx1A1 or Stx2A1 or Stx1A1 and Stx2A1 variants. Vero cells were cotransfected with WT or mutant forms of Stx1A1

(gray bars) or Stx2A1 (black bars) and EGFP. Cells carrying the empty vector (VC) were used as controls. EGFP fluorescence was measured at 22h post transfection. Fluorescence measured in cells cotransfected with EGFP and empty vector was considered as 100 % and fluorescence in controls lacking EGFP plasmid as background. Experiment was repeated at least three times. A representative experiment is shown. Error bars represent S. E. where n=3 technical replicates. Statistical significance of means for WT Stx2A1 and Stx1A1 and variants were determined by using PROC GLM (Table S1). Only E167K and R172A/R176A variants were significantly different from WT ($P< 0.001$) (B) Shiga toxin gene expression in Vero cells. Total RNA (375 ng) isolated from Vero cells expressing $Stx1A_1$ (gray bars) or $Stx2A_1$ (black bars) collected at 23h post DNA exposure was used to quantify the relative levels of gene expression using qRT-PCR by the comparative CT method (ΔΔCT). The $Stx1A_1$ primers were Stx1_qPCR_F5' aatgtcgcatagtggaacctca 3' and Stx1_qPCR_R 5' aacatcgctcttgccacagac 3', while the Stx2 primers were Stx2_qPCR_F5' gtatacgatgacgccgggag 3' and Stx2_qPCR_R 5' attcgcccccagttcagagt 3'. β-actin was used as internal control. The y-axis shows the fold change in toxin-gene containing samples over the control samples without the toxin gene (VC). (C) Depurination of ribosomes in mammalian cells expressing WT or mutant forms of Stx1A1 and Stx2A1. Total RNA (375 ng) from Vero cells expressing WT or mutant forms of Stx1A1 (grey bars) or Stx2A1 (black bars) collected at 23h post DNA exposure was used to quantify the relative levels of depurination using qRT-PCR. The y-axis shows the fold change in depurination over the control samples (VC). The table shows the fold change in depurination levels in cells transfected with Stx1A1 and Stx2A1 relative to cells transfected with the empty vector. Experiment was repeated at least three times. A representative experiment is shown. Error bars represent S. E. where n=3 technical replicates. Statistical significance of means for WT Stx2A1 and Stx1A1 and variants were determined by using PROC GLM (Table S2). Means were significantly different between WT Stx2A1 and Stx2A1 variants ($P< 0.001$).

Table S1. EGFP fluorescence statistical significance of the contrasts*

Contrast	DF	Contrast SS	Mean Square	F value	Pr > F
Compare Stx1A1 E167K with WT	**1**	**1926072.2**	**1926072.2**	**23.4**	**<0.0001**
Compare Stx1A1 R170A with WT	**1**	**64729.9**	**64729.9**	**0.8**	**0.3764**
Compare Stx1A1 R172A with WT	**1**	**926894.9**	**926894.9**	**11.3**	**0.1009**
Compare Stx1A1 R176A with WT	**1**	**51003.4**	**51003.4**	**0.62**	**0.4322**
Compare Stx1A1 R172A/R176A with WT	**1**	**1119635.7**	**1119635.7**	**13.6**	**0.0003**
Compare Stx2A1 E167K with WT	**1**	**4538565.2**	**4538565.2**	**55.11**	**<0.0001**
Compare Stx2A1 R170A with WT	**1**	**40687.8**	**40687.8**	**0.5**	**0.4829**
Compare Stx2A1 R172A with WT	**1**	**161.7**	**161.7**	**0**	**0.9647**
Compare Stx2A1 R176A with WT	**1**	**207220.4**	**207220.4**	**2.5**	**0.1142**
Compare Stx2A1 R172A/R176A with WT	**1**	**851309.2**	**851309.2**	**10.3**	**0.0015**

*To test the differences of treatment means in data presented in Fig. S2A, PROC GLM in SAS was used to compute contrasts for pairwise comparisons between each variant and their respective WT in Stx1A1 and Stx2A1 (for trait EGFP Fluorescence, *in cell*). The Contrast statement in PROC GLM produces contrast sums of square, mean square, F value, and corresponding p value for each LS-mean difference comparison computed. Reported P values were adjusted for multiple comparisons using the Tukey-Kramer option within PROC GLM. There are highly significant differences between the variants and their respective WT in Stx1A1 and Stx2A1.

Table S2. Depurination statistical significance of the contrasts*

Contrast	DF	Contrast SS	Mean Square	F value	Pr > F
Compare Stx1A1 E167K with WT	**1**	**1460.7**	**1460.7**	**0.2**	**0.6597**
Compare Stx1A1 R170A with WT	**1**	**572.5**	**572.5**	**0.08**	**0.7827**
Compare Stx1A1 R172A with WT	**1**	**565.9**	**565.9**	**0.08**	**0.7839**
Compare Stx1A1 R176A with WT	**1**	**157.2**	**157.2**	**0.02**	**0.8851**
Compare Stx1A1 R172A/R176A with WT	**1**	**403.3**	**403.3**	**0.5**	**0.8169**
Compare Stx2A1 E167K with WT	**1**	**557806.8**	**557806.8**	**74.47**	**<0.0001**
Compare Stx2A1 R170A with WT	**1**	**324382.5**	**324382.5**	**43.31**	**<0.0001**
Compare Stx2A1 R172A with WT	**1**	**284548.8**	**284548.8**	**37.99**	**<0.0001**
Compare Stx2A1 R176A with WT	**1**	**87699.3**	**87699.3**	**11.71**	**0.0009**
Compare Stx2A1 R172A/R176A with WT	**1**	**597926.4**	**597926.4**	**79.83**	**<0.0001**

*To test the differences of treatment means in data presented in Fig. S2C, PROC GLM in SAS was used to compute contrasts for pairwise comparisons between each variant and their respective WT in Stx1A1 and Stx2A1 (for trait depurination). The Contrast statement in PROC GLM produces contrast sums of square, mean square, F value, and corresponding p value for each LS-mean difference comparison computed. Reported P values were adjusted for multiple comparisons using the Tukey-Kramer option within PROC GLM. There are highly significant differences between the variants and their respective WT in Stx2A1.