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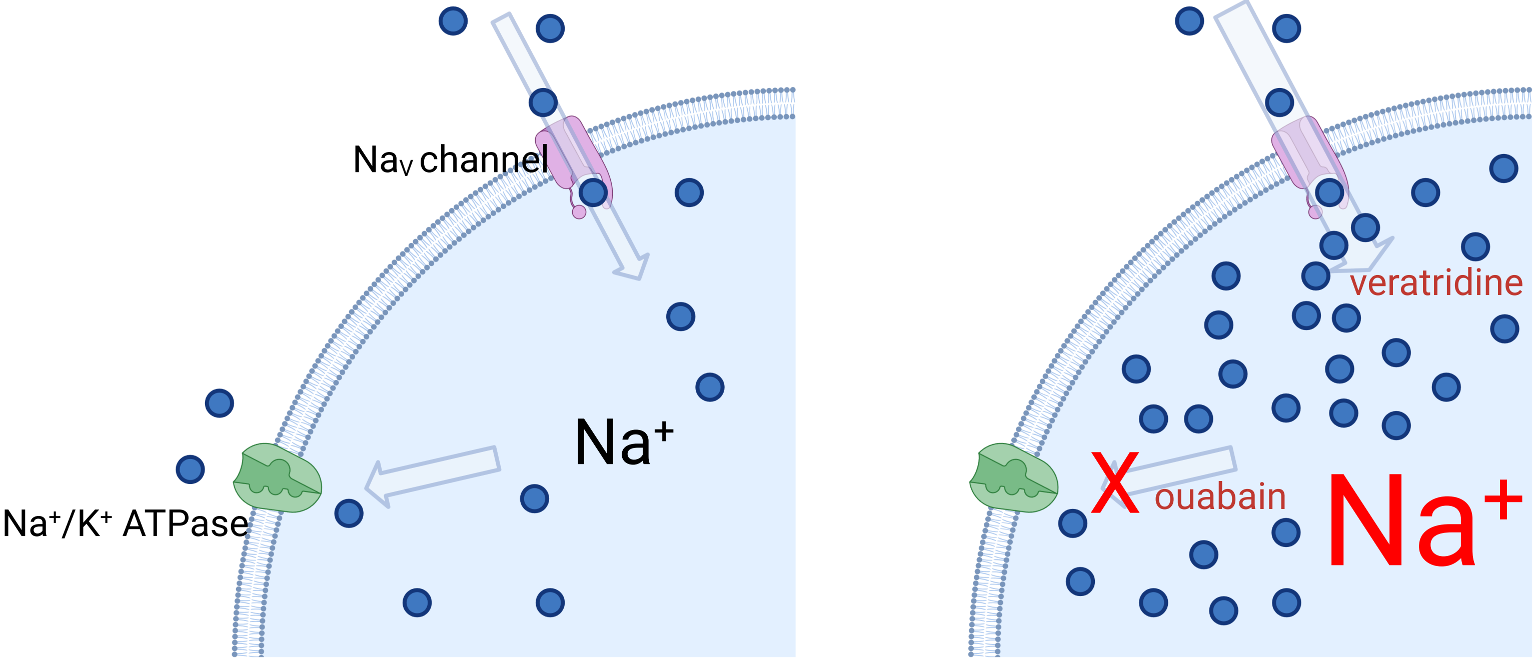
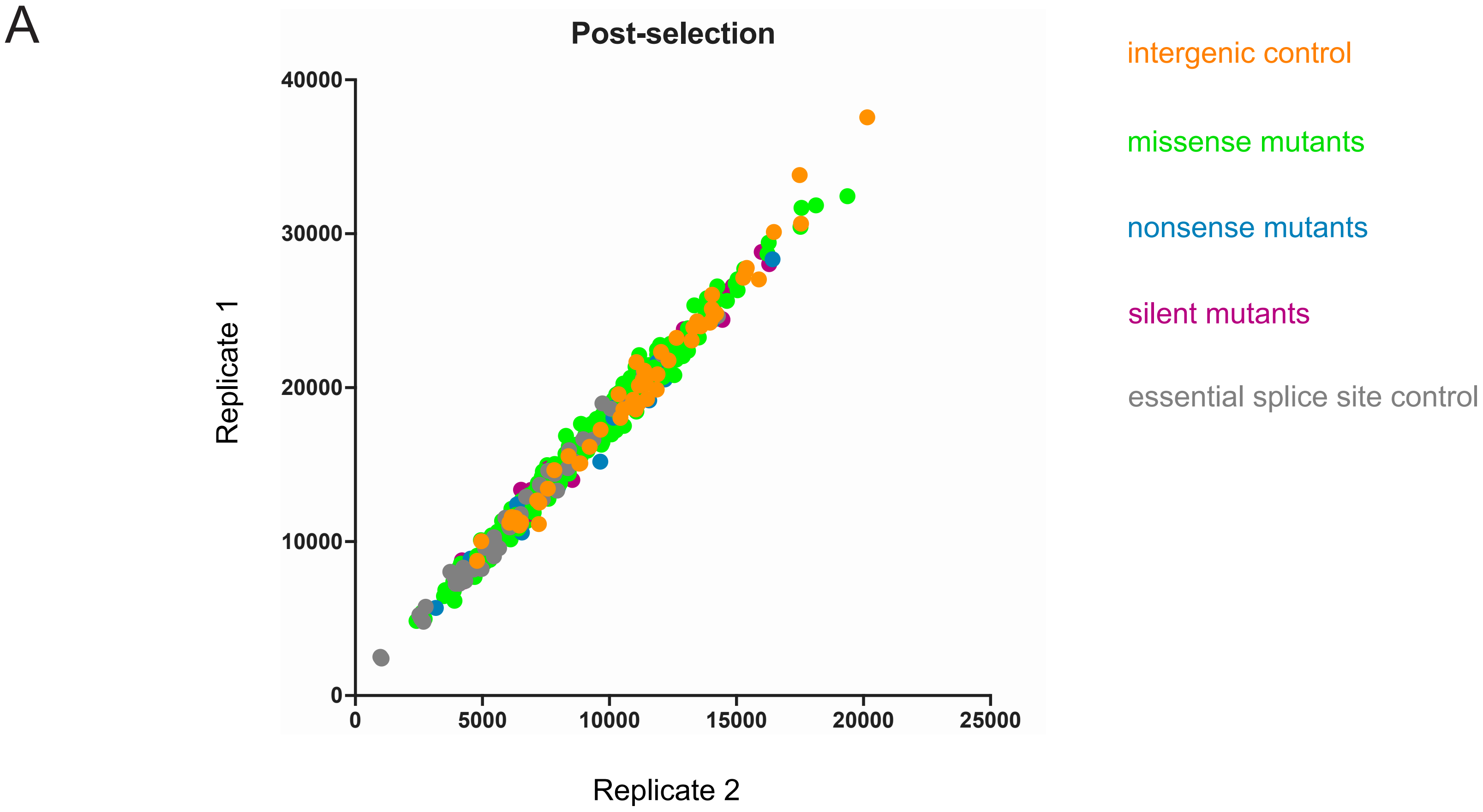
Supplemental information

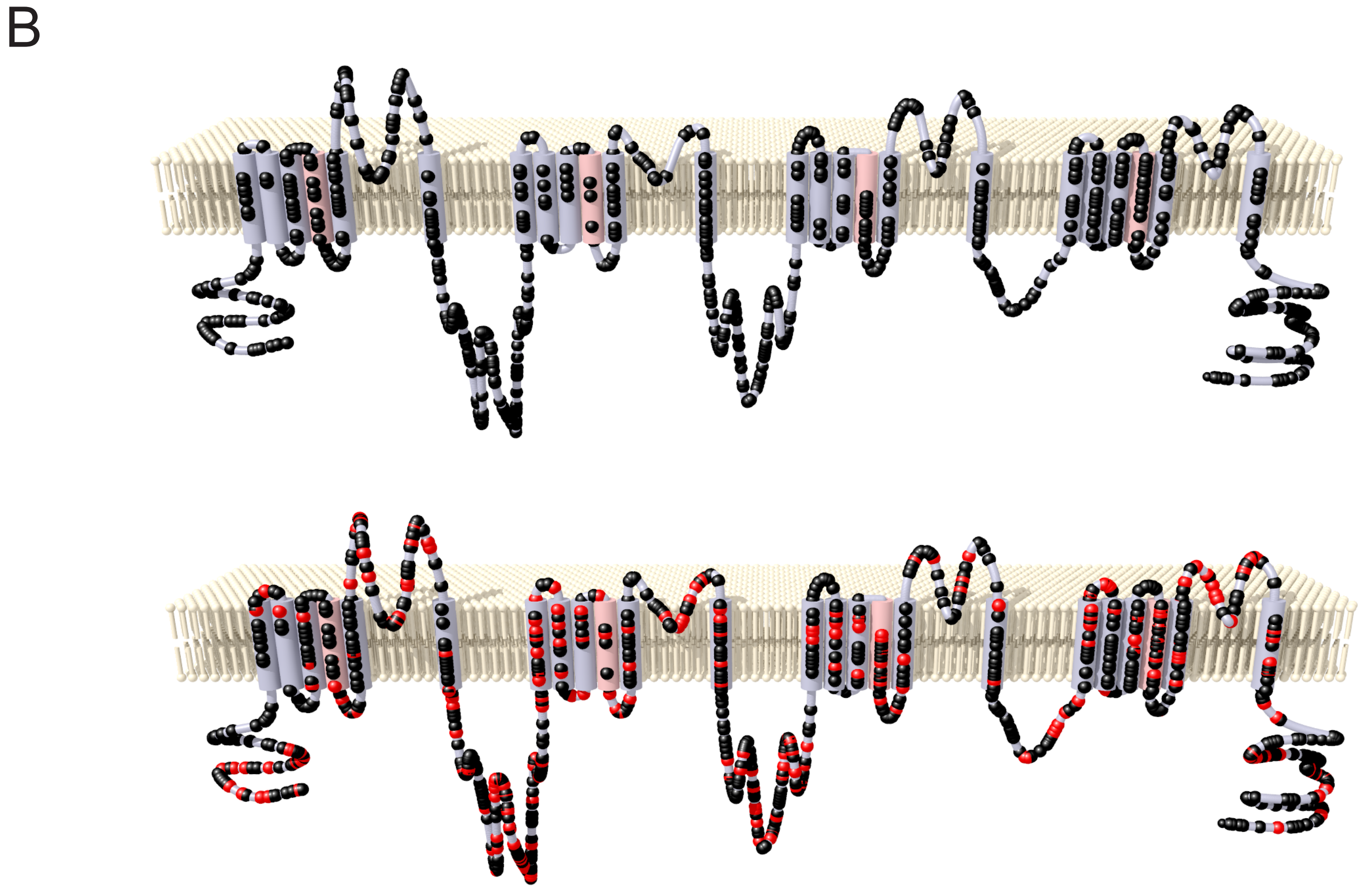
Scanning mutagenesis of the voltage-gated sodium channel $Na_V1.2$ using base editing

Juan Lorenzo B. Pablo, Savannah L. Cornett, Lei A. Wang, Sooyeon Jo, Tobias Brünger, Nikita Budnik, Mudra Hegde, Jean-Marc DeKeyser, Christopher H. Thompson, John G. Doench, Dennis Lal, Alfred L. George Jr., and Jen Q. Pan


Na$_V$ channel
Na$^+$/K$^+$ ATPase
Na$^+$
veratridine
X
ouabain
Na$^+$

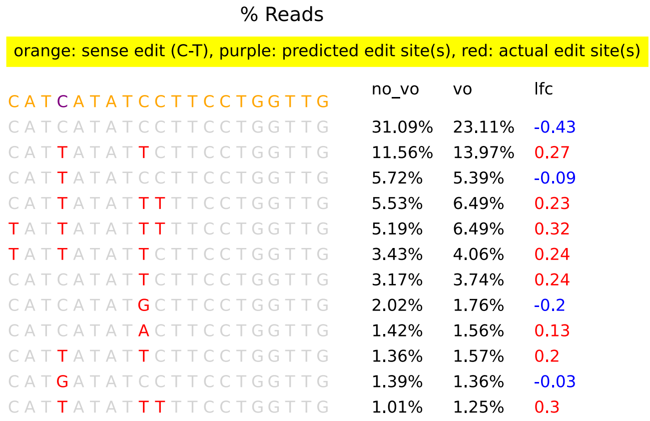
Supplemental Figure 1. Schematic showing the mechanism of toxicity induced by veratridine and ouabain. Veratridine increases $Na_V1.2$ dependent influx of sodium ions while ouabain blocks sodium ion efflux through the blockade of the Na^+/K^+ ATPase. Figure created using Biorender.com.


A
Post-selection
Replicate 1
Replicate 2
0
10000
20000
30000
40000
0
5000
10000
15000
20000
25000
intergenic control
missense mutants
nonsense mutants
silent mutants
essential splice site control


B

Supplemental Figure 2. Design of the pooled library for base editing. A) Scatter plots of sequencing counts for each individual gRNA (colored dot). Two independent replicates of the screen are plotted against each other. The color code for different types of gRNAs is indicated. B) (Top) A membrane topology map of the Na_V1.2 protein shows the distribution of ClinVar missense variants (as of February 2023). (Bottom) The locations of gRNAs predicted to make missense mutations (red dots) superimposed on the ClinVar missense variants (black dots).

I1761I

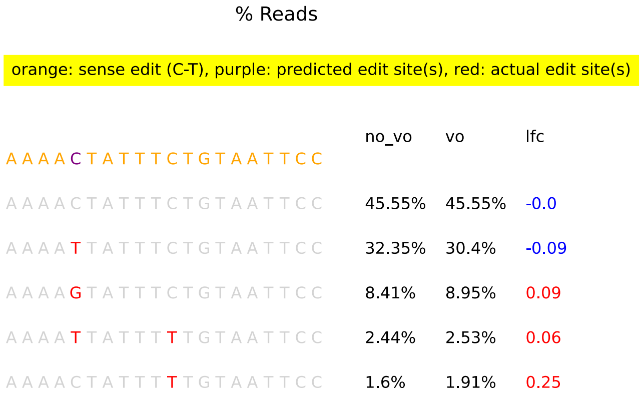


% Reads

orange: sense edit (C-T), purple: predicted edit site(s), red: actual edit site(s)

	no_vo	vo	lfc
CATCATATCCTTCCTGGTTG			
CATCATATCCTTCCTGGTTG	31.09%	23.11%	-0.43
CATTATATTCTTCCTGGTTG	11.56%	13.97%	0.27
CATTATATCCTTCCTGGTTG	5.72%	5.39%	-0.09
CATTATATTTTTCCTGGTTG	5.53%	6.49%	0.23
TATTATATTTTTCCTGGTTG	5.19%	6.49%	0.32
TATTATATTCTTCCTGGTTG	3.43%	4.06%	0.24
CATCATATTCTTCCTGGTTG	3.17%	3.74%	0.24
CATCATATGCTTCCTGGTTG	2.02%	1.76%	-0.2
CATCATATACTTCCTGGTTG	1.42%	1.56%	0.13
CATTATATTCTTCCTGGTTG	1.36%	1.57%	0.2
CATGATATCCTTCCTGGTTG	1.39%	1.36%	-0.03
CATTATATTTTTCCTGGTTG	1.01%	1.25%	0.3

T227I

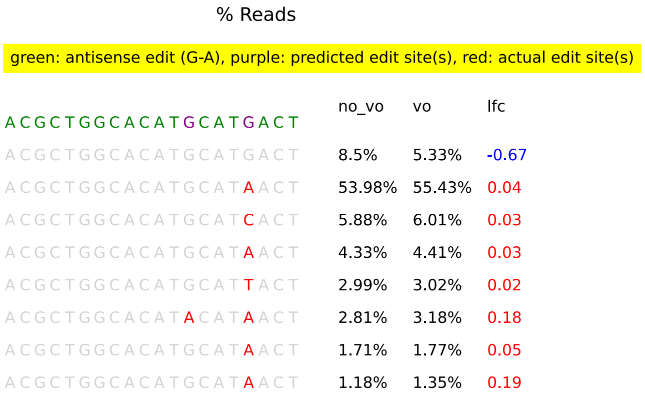


% Reads

orange: sense edit (C-T), purple: predicted edit site(s), red: actual edit site(s)

	no_vo	vo	lfc
AAAACTATTTCTGTAATTCC			
AAAACTATTTCTGTAATTCC	45.55%	45.55%	-0.0
AAAATTATTTCTGTAATTCC	32.35%	30.4%	-0.09
AAAAGTATTTCTGTAATTCC	8.41%	8.95%	0.09
AAAATTATTTTTGTAATTCC	2.44%	2.53%	0.06
AAAACTATTTTTGTAATTCC	1.6%	1.91%	0.25

D927N, M925I

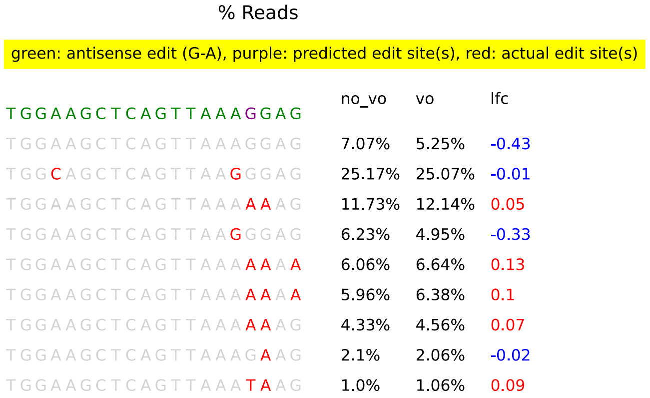


% Reads

green: antisense edit (G-A), purple: predicted edit site(s), red: actual edit site(s)

	no_vo	vo	lfc
ACGCTGGCACATGCATGACT			
ACGCTGGCACATGCATGACT	8.5%	5.33%	-0.67
ACGCTGGCACATGCATAACT	53.98%	55.43%	0.04
ACGCTGGCACATGCATCACT	5.88%	6.01%	0.03
ACGCTGGCACATGCATAACT	4.33%	4.41%	0.03
ACGCTGGCACATGCATTACT	2.99%	3.02%	0.02
ACGCTGGCACATACATAACT	2.81%	3.18%	0.18
ACGCTGGCACATGCATAACT	1.71%	1.77%	0.05
ACGCTGGCACATGCATAACT	1.18%	1.35%	0.19

G1744R

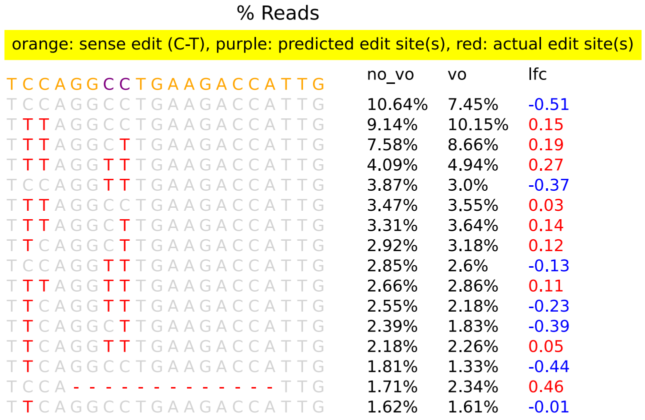


% Reads

green: antisense edit (G-A), purple: predicted edit site(s), red: actual edit site(s)

	no_vo	vo	lfc
TGGAAGCTCAGTTAAAGGAG			
TGGAAGCTCAGTTAAAGGAG	7.07%	5.25%	-0.43
TGGCAGCTCAGTTAAGGGAG	25.17%	25.07%	-0.01
TGGAAGCTCAGTTAAAAAAG	11.73%	12.14%	0.05
TGGAAGCTCAGTTAAGGGAG	6.23%	4.95%	-0.33
TGGAAGCTCAGTTAAAAAAA	6.06%	6.64%	0.13
TGGAAGCTCAGTTAAAAAAA	5.96%	6.38%	0.1
TGGAAGCTCAGTTAAAAAAG	4.33%	4.56%	0.07
TGGAAGCTCAGTTAAAGAAG	2.1%	2.06%	-0.02
TGGAAGCTCAGTTAAATAAG	1.0%	1.06%	0.09

G223G, L234L

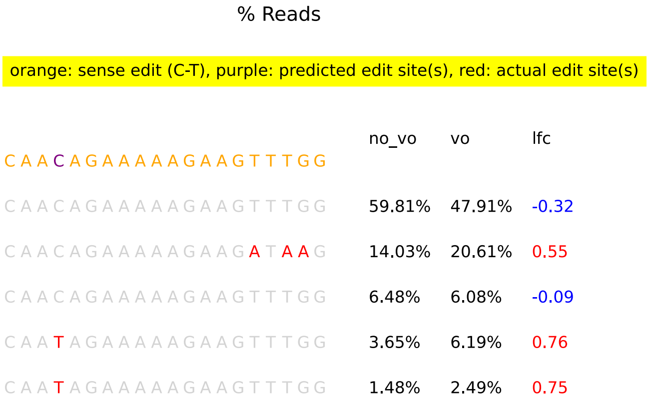


% Reads

orange: sense edit (C-T), purple: predicted edit site(s), red: actual edit site(s)

	no_vo	vo	lfc
TCCAGGCCTGAAGACCATTG			
TCCAGGCCTGAAGACCATTG	10.64%	7.45%	-0.51
TTTAGGCCTGAAGACCATTG	9.14%	10.15%	0.15
TTTAGGCTTGAAGACCATTG	7.58%	8.66%	0.19
TTTAGGTTTGAAGACCATTG	4.09%	4.94%	0.27
TCCAGGTTTGAAGACCATTG	3.87%	3.0%	-0.37
TTTAGGCCTGAAGACCATTG	3.47%	3.55%	0.03
TTTAGGCTTGAAGACCATTG	3.31%	3.64%	0.14
TTCAGGCTTGAAGACCATTG	2.92%	3.18%	0.12
TCCAGGTTTGAAGACCATTG	2.85%	2.6%	-0.13
TTTAGGTTTGAAGACCATTG	2.66%	2.86%	0.11
TTCAGGTTTGAAGACCATTG	2.55%	2.18%	-0.23
TTCAGGCTTGAAGACCATTG	2.39%	1.83%	-0.39
TTCAGGTTTGAAGACCATTG	2.18%	2.26%	0.05
TTCAGGCCTGAAGACCATTG	1.81%	1.33%	-0.44
TCCA-------------TTG	1.71%	2.34%	0.46
TTCAGGCCTGAAGACCATTG	1.62%	1.61%	-0.01

Q1479*

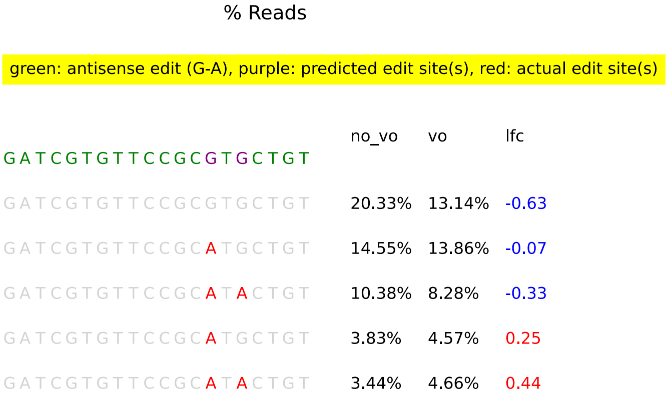


% Reads

orange: sense edit (C-T), purple: predicted edit site(s), red: actual edit site(s)

	no_vo	vo	lfc
CAACAGAAAAAGAAGTTTGG			
CAACAGAAAAAGAAGTTTGG	59.81%	47.91%	-0.32
CAACAGAAAAAGAAGATAAG	14.03%	20.61%	0.55
CAACAGAAAAAGAAGTTTGG	6.48%	6.08%	-0.09
CAATAGAAAAAGAAGTTTGG	3.65%	6.19%	0.76
CAATAGAAAAAGAAGTTTGG	1.48%	2.49%	0.75

V938I

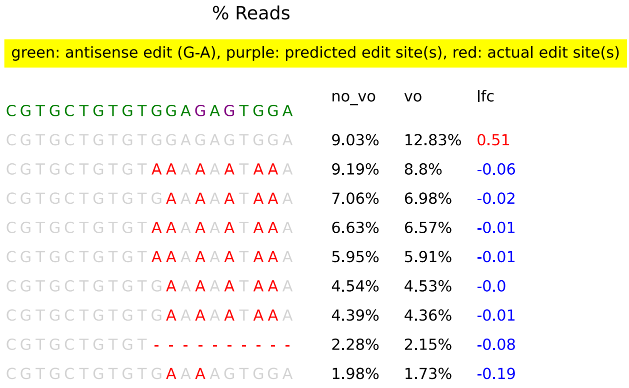


% Reads

green: antisense edit (G-A), purple: predicted edit site(s), red: actual edit site(s)

	no_vo	vo	lfc
GATCGTGTTCCGCGTGCTGT			
GATCGTGTTCCGCGTGCTGT	20.33%	13.14%	-0.63
GATCGTGTTCCGCATGCTGT	14.55%	13.86%	-0.07
GATCGTGTTCCGCATACTGT	10.38%	8.28%	-0.33
GATCGTGTTCCGCATGCTGT	3.83%	4.57%	0.25
GATCGTGTTCCGCATACTGT	3.44%	4.66%	0.44

E942K



% Reads

green: antisense edit (G-A), purple: predicted edit site(s), red: actual edit site(s)

	no_vo	vo	lfc
CGTGCTGTGTGGAGAGTGGA			
CGTGCTGTGTGGAGAGTGGA	9.03%	12.83%	0.51
CGTGCTGTGTAAAAAATAAA	9.19%	8.8%	-0.06
CGTGCTGTGTGAAAAATAAA	7.06%	6.98%	-0.02
CGTGCTGTGTAAAAAATAAA	6.63%	6.57%	-0.01
CGTGCTGTGTAAAAAATAAA	5.95%	5.91%	-0.01
CGTGCTGTGTGAAAAATAAA	4.54%	4.53%	-0.0
CGTGCTGTGTGAAAAATAAA	4.39%	4.36%	-0.01
CGTGCTGTGT----------	2.28%	2.15%	-0.08
CGTGCTGTGTGAAAAGTGGA	1.98%	1.73%	-0.19

Supplemental Figure 3. CRISPResso analysis of all the gRNAs analyzed in the secondary confirmation screen. The % abundance of each allele in total sequenced reads at each targeting locus without or with drug treatment are shown in column *no_vo* and *vo* respectively. Negative numbers indicate depletion and positive numbers enrichment. Alleles less frequent than 1% are not shown. Purple letters indicate the predicted edits. Sequence validation reveals edits (intended or unintended) underlying LOF/GOF phenotypes of missense and silent mutations, except for E942K where collateral mutations contributed to complex interpretation. lfc: log2(fold change)