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Supplementary information

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Adaptive model-guided protein evolution with sparse data optimizes compact eukaryotic genome editors

In the format provided by the authors and unedited

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1. Fanzor2 Homolog Discovery and Phylogenetic Analysis

1.1 Overview

To characterize the evolutionary landscape of Fanzor2 (Fz2) proteins across all domains of life, we performed comprehensive homology searches followed by phylogenetic reconstruction. The pipeline consisted of four stages: (i) seed-based homology search, (ii) filtering and de-replication, (iii) multiple sequence alignment, and (iv) maximum-likelihood tree inference.

1.2 Database Generation

1.2.1 Seed Sequence Selection

We compiled a seed set of known or putative Fanzor2 protein sequences from each domain of life, Eukaryota, Bacteria, Archaea, as well as from large eukaryotic viruses. Seeds were selected from prior literature and databases as representative Fz2 homologs (distinguished from Fz1 clade sequences). The seed set included:

- Eukaryotic Fanzor2 proteins previously identified in fungi and protists
- Bacterial and archaeal TnpB homologs classified as Fanzor2-like
- Viral-encoded Fanzor2 homologs from nucleocytoplasmic large DNA viruses

Each seed protein was annotated with its source domain to enable domain-specific searches. The complete list of seed sequences is provided in Supplementary Data 1.

1.2.2 BLASTp Homology Search

Using the seed sequences as queries, we performed exhaustive homology searches against the NCBI non-redundant (nr) protein database using BLASTp (NCBI BLAST+ v2.12). Searches were performed using BLASTp (NCBI BLAST+ v2.12) with an E-value cutoff of 1×10^{-2} and a maximum of 200 target sequences per query.

Searches were domain-focused: eukaryotic seeds were used to identify Fz2 candidates in eukaryotes, bacterial seeds for bacteria, and so forth. This strategy ensured broad coverage of homologs in each lineage while minimizing taxonomic bias. For seeds with sparse protein hits, tblastn searches against the nr/nt nucleotide database were performed to uncover unannotated genes in metagenomes.

All hits meeting the E-value threshold were retrieved in tabular format (CSV) with the following fields recorded: accession, organism, alignment length, percentage identity, query coverage, and E-value.

1.2.3 Filtering and De-replication

Raw BLAST results were filtered through a multi-step pipeline to produce a high-quality, non-redundant Fz2 candidate set:

1. Exact duplicate removal: Identical sequences retrieved from multiple seeds or appearing in overlapping searches were removed.

2. Subsequence pruning: If a candidate protein was entirely contained as a subsequence of another (longer) protein, the shorter sequence was discarded as likely a fragment or redundant isoform.
3. Identity clustering: Hits with >95% sequence identity to another hit (based on BLAST pairwise identity over the aligned region) were clustered, retaining only one representative per cluster.
4. Length filter: Sequences shorter than 200 amino acids were removed as likely incomplete fragments.
5. Stop codon filter: Sequences containing internal stop codons were excluded.
6. Catalytic motif filter: Candidates were screened for the conserved RuvC catalytic triad (Asp-Glu-Asp) by motif scanning using custom scripts and manual inspection of preliminary alignments.

1.2.4 Database Statistics

The final dataset comprised approximately 1600 high-confidence Fz2 candidate sequences predominantly 300–500 amino acids in length, consistent with full-length TnpB-family nucleases.

1.3 Multiple Sequence Alignment

1.3.1 Alignment Generation

All curated Fz2 candidate sequences were aligned using MAFFT v7.505 with the following command:

```
mafft --auto Fz2_candidates_nr.fasta > Fz2_aligned.fasta
```

The --auto strategy selected the L-INS-i iterative refinement algorithm given the number and divergence of sequences. Default gap opening (1.53) and extension (0.123) costs were used.

1.3.2 Alignment Trimming

The raw alignment was trimmed to remove positions with low information content using trimAl v1.4:

```
trimal -in Fz2_aligned.fasta -out Fz2_trimmed.fasta -gappyout
```

The -gappyout mode automatically eliminates columns that are mostly gaps or highly ambiguous while retaining well-aligned core regions.

1.3.3 Manual Curation

After automated trimming, we manually inspected the alignment to ensure proper alignment of critical functional residues:

- The RuvC catalytic triad (Asp-Glu-Asp) was confirmed to be aligned across all sequences
- Columns corresponding to obvious alignment errors at termini were removed

1.4 Phylogenetic Tree Inference

1.4.1 Initial Tree with FastTree

An initial topology was generated using FastTree v2.1.11 to obtain a starting tree and identify outlier sequences:

```
FastTree -wag -cat 20 -gamma Fz2_trimmed.fasta > Fz2_initial.tre
```

Parameters: -wag: WAG amino acid substitution model; -cat 20: CAT approximation with 20 rate categories; -gamma: Gamma20-based likelihood rescaling.

1.4.2 Long-Branch Detection and Removal

The FastTree topology was inspected for long-branch artifacts. Following established criteria, sequences with branch length ≥1.5 substitutions per site were flagged as potential outliers. Unless such sequences showed significant similarity to other Fz2/TnpB sequences (indicating a real divergent clade), they were pruned from the dataset. This procedure removed 12 outlier sequences.

1.4.3 Maximum-Likelihood Inference with IQ-TREE

For the final high-accuracy phylogeny, we used IQ-TREE 2 v2.2.0:

```
iqtree2 -s Fz2_trimmed.fasta -m MFP -B 1000 -bnni -t Fz2_initial.tre -pre Fz2_final --runs 5
```

Parameters: -m MFP: ModelFinder to select the best-fit substitution model; -B 1000: 1,000 ultrafast bootstrap replicates; -bnni: Reduce bootstrap support inflation by accounting for model violation; -t: Starting tree from FastTree; --runs 5: Five independent runs from different random seeds.

1.5 Tree Rooting and Annotation

1.5.1 Rooting Strategy

The tree was midpoint-rooted and visualized relative to the branch containing the functionally characterized Naegleria Fanzor2 effectors. This rooting was used for visualization and should not be interpreted as definitive evidence of basal placement or evolutionary origin.

1.5.2 Metadata Annotation

The rooted tree was uploaded to the Interactive Tree of Life (iTOL) web server for annotation. The following metadata were mapped to each leaf: Taxonomic domain (Eukaryota, Bacteria, Archaea, Viruses), Sequence length (amino acids), Source organism. Leaves were color-coded by domain using distinct color strips on the perimeter.

1.6 Tree Visualization

1.6.1 Radial Tree with Annotation Rings

Final publication-quality visualization was generated using ToyTree and matplotlib in Python. The rooted Newick tree was loaded and rendered as a circular phylogram with all branch lengths preserved. Two concentric annotation rings were added around the tree circumference.

Inner ring (taxonomic domain): Color-filled segments encircle the tree, colored by domain of origin.

Outer ring (protein length): Radial bars indicate protein length for each sequence. Bar length is proportional to amino acid count (range: 300-500 aa).

1.6.2 Ring Generation

Ring positions were calculated by parsing the Nexus translate table from iTOL to determine the ordering of leaves around the circle. Bars were drawn at corresponding polar coordinates for each sequence using custom matplotlib scripts.

1.6.3 Final Assembly

The tree graphic and rings were merged by exporting the ToyTree base tree as SVG, overlaying the matplotlib-generated rings (with transparent background), and finalizing in Adobe Illustrator.

2 EvoMax: Computational Methods for Mutation Prediction

2.1 Overview

EvoMax predicts beneficial single-site mutations by combining (i) a Gaussian process regressor (GPR) trained on experimental activity measurements to learn the mapping between mutation features and protein function, (ii) a protein language model (PLM) to score mutations based on evolutionary plausibility, and (iii) a structure-conditioned inverse folding model (ESM-IF) to filter candidates for structural compatibility with the native protein fold. Unlike zero-shot approaches that rely solely on PLM fitness scores or structural models alone, EvoMax learns protein-specific activity landscapes from a small number of experimentally characterized mutations (the low-N regime) and integrates empirical, evolutionary, and structural information through a hierarchical two-stage ranking procedure.

The core insight is that PLMs such as ESM-2 encode evolutionary and biophysical regularities but do not directly predict protein activity, and structural models such as ESM-IF assess fold compatibility but not functional performance. We observed a weak correlation between ESM-2 masked-mutant probability scores and measured Fanzor2 nuclease activities, consistent with observations in antibody and enzyme optimization. By training a regression model using experimental data and combining its predictions with complementary evolutionary and structural signals, EvoMax learns a task-specific mapping that generalizes to untested mutations.

2.2 Training Data

2.2.1 Mutation Selection

The GPR was trained on 209 single-site mutations of NlovFz2. Training mutations were selected through two approaches:

Rational design: Mutations were selected based on structural and evolutionary considerations, targeting positions in or near the active site and residues showing conservation patterns suggestive of functional importance.

Literature curation: Previously characterized variants from Wei et al. (2026) were included to leverage existing experimental data.

Stop codons and frameshift mutations were excluded from analysis.

2.2.2 Activity Measurement

Activity was measured using a fluorescence reporter assay. Published and newly generated measurements used two and three biological replicates, respectively. Activity values were expressed as fold-change relative to wild-type:

$$Fold - change = \frac{\overline{F}_{\text{variant}}}{\overline{F}_{\text{wild-type}}}$$

where $\bar{F}$ denotes the mean fluorescence intensity across replicates.

2.3 Mutation Representation

We represent each single-site mutation as a tuple $m = (p, a_{\mathrm{wt}}, a_{\mathrm{mut}})$, where $p \in \{1, \ldots, L\}$ is the position in the protein sequence of length L, $a_{\mathrm{wt}} \in \mathcal{A}$ is the wild-type amino acid, and $a_{\mathrm{mut}} \in \mathcal{A}$ is the mutant amino acid, with $\mathcal{A}$ denoting the set of 20 canonical amino acids.

2.3.1 BLOSUM-Informed Features

We encode mutations using the BLOSUM62 substitution matrix $B \in \mathbb{R}^{20\times 20}$, which captures evolutionary substitution frequencies and biochemical similarity between amino acids. The raw BLOSUM62 matrix is normalized to the unit interval:

$$\tilde{B}(a, a') = \frac{B(a, a') - B_{min}}{B_{max} - B_{min}}$$

where $B_{min} = -4$ and $B_{max} = 11$ are the minimum and maximum entries of the BLOSUM62 matrix, respectively.

2.4 PLM-Based Mutation Scoring

2.4.1 Masked-Mutant Probability Scoring

For each candidate mutation $m = (p, a_{wt}, a_{mut})$, we use the probability assigned to the mutant residue under ESM-2's masked language model objective:

$$S_{\mathrm{PLM}}(m) = P_{\mathrm{ESM-2}}(a_{\mathrm{mut}} \mid x_{\backslash p})$$

where $x_{\backslash p}$ denotes the wild-type sequence with position p replaced by the [MASK] token. The probability $P_{\mathrm{ESM-2}}(a \mid x_{\backslash p})$ is the softmax output of ESM-2 at the masked position. We used ESM-2 with 650M parameters (esm2_t33_650M_UR50D).

2.4.2 Interpretation

Higher values of S_{PLM} indicate that ESM-2 considers the mutant residue more plausible in the local sequence context. This score reflects evolutionary plausibility: mutations to residues commonly observed at homologous positions in natural sequences receive higher scores.

2.5 Gaussian Process Regression Model

EvoMax utilizes a Gaussian Process Regressor (GPR) as its top-layer model to learn the relationship between mutation features and activity. Given a training set $\mathcal{D} = \{(m_i, y_i)\}_{i=1}^{N}$ of N experimentally characterized mutations with measured activities $y_i \in \mathbb{R}$, the GPR defines a posterior distribution over the activity of any candidate mutation.

2.5.1 Kernel Design

We designed a custom kernel function that measures similarity between mutations based on biochemical properties of the amino acid substitution:

$$k(m_i, m_j) = \alpha \cdot \frac{1}{2}[\tilde{B}(a_{wt}^{(i)}, a_{wt}^{(j)}) + \tilde{B}(a_{mut}^{(i)}, a_{mut}^{(j)})] \cdot \delta_{p_i,p_j}$$

where:

- $\tilde{B}(\cdot,\cdot)$ is the normalized BLOSUM62 substitution matrix
- δ_{p_i,p_j} is the Kronecker delta, equal to 1 if $p_i = p_j$ and 0 otherwise
- α is the kernel amplitude hyperparameter

The kernel has two interpretable components:

Biochemical Similarity: The term $\frac{1}{2}[B(a_{wt}^{(i)}, a_{wt}^{(j)}) + B(a_{mut}^{(i)}, a_{mut}^{(j)})]$ measures similarity between two mutations based on their wild-type and mutant residues.

Position Gating: The Kronecker delta δ_{p_i,p_j} restricts the kernel to compare only mutations at the same position. This reflects the biological observation that substitution effects are highly position dependent.

2.5.2 Posterior Predictions

Given the training data $\mathcal{D}$, the GPR posterior mean provides the predicted activity for a candidate mutation m*:

$$S_{GPR}(m*) = E[y* \mid \mathcal{D}] = k_*^\top (K + \sigma^2{}_n I)^{-1} y$$

where:

- $k_* \in \mathbb{R}^N$ with $[k_*]_i = k(m*, m_i)$ is the kernel vector between the test mutation and training mutations
- $K \in \mathbb{R}^{N \times N}$ with $[K]_{ij} = k(m_i, m_j)$ the training kernel matrix
- σ_n^2 is the observation noise variance
- $y = (y_1, \ldots, y_N)^\top$ is the vector of observed activities

The posterior variance provides calibrated uncertainty estimates:

$$Var[y* \mid \mathcal{D}] = k(m*, m*) - k_*^\top (K + \sigma^2{}_n I)^{-1} k_*$$

2.6 Structural Filtering via Inverse Folding

To identify mutations likely to destabilize the protein fold, we filter top candidates using structure-conditioned inverse folding models.

2.6.1 Inverse Folding Likelihood

Given backbone coordinates $\mathcal{C} = \{(r_i^N, r_i^{C\alpha}, r_i^C)\}_{i=1}^L$ and a mutant sequence x', an inverse folding model computes the conditional probability $P(x' \mid \mathcal{C})$. Each mutant sequence is scored by its average per-residue log-likelihood:

$$S_{\mathrm{IF}}(x') = \frac{1}{|\mathcal{R}|} \sum_{i \in \mathcal{R}} \log P(x'_i \mid \mathcal{C}, x'_{<i})$$

where $\mathcal{R}$ denotes the set of residues with complete backbone coordinates, and the probability is computed autoregressively.

2.6.2 Model and Structure

We used ESM-IF1 (142M parameters; esm_if1_gvp4_t16_142M_UR50), a GVP-Transformer architecture that processes backbone geometry using geometric vector perceptrons.

Structural scoring used: AlphaFold3-predicted structures.

2.6.3 Interpretation

Inverse folding scores serve as a filter for fold compatibility, not as a predictor of improved activity. Higher (less negative) ESM-IF1 log-likelihoods indicate better structural compatibility; scores were normalized without sign inversion before weighted integration.

2.7 Score Normalization

Raw scores from each model are on different scales. We apply the same robust median-IQR normalization to each model score:

Robust median-IQR normalization. For GPR, ESM-2, and ESM-IF scores, we use the median and interquartile range (IQR):

$$\tilde{s} = \frac{S - median(S)}{IQR(S)}$$

where *s* denotes an individual raw score, *S* denotes the corresponding model-specific score distribution, IQR(*S*) = *Q*75(*S*) - *Q*25(*S*), and *Qp* denotes the *p*-th percentile. For Stage 1, component scores were normalized over the full single-mutant candidate set; for Stage 2, the three component scores were re-normalized over the Stage 1 shortlist before weighted aggregation.

2.8 Two-Stage Ranking

EvoMax employs a two-stage ranking procedure to balance computational efficiency with prediction accuracy.

2.8.1 Stage 1: Sequence-Based Filtering

All single-site mutations (*L* × 19 candidates for a protein of length *L*) are scored with GPR and ESM-2. The Stage 1 score is computed as:

$$S_{stage1}(m) = w_{GPR}^{(1)} \cdot \tilde{S}_{GPR}(m) + w_{PLM}^{(1)} \cdot \tilde{S}_{PLM}(m)$$

The top 1.5% of candidates ranked by the Stage 1 score are advanced to Stage 2.

2.8.2 Stage 2: Structure-Conditioned Re-Ranking

Shortlisted candidates are scored with ESM-IF for structural compatibility. The final consensus score combines all three components:

$$S_{final}(m) = w_{GPR}^{(2)} \cdot \tilde{S}_{GPR}(m) + w_{PLM}^{(2)} \cdot \tilde{S}_{PLM}(m) + w_{IF}^{(2)} \cdot \tilde{S}_{IF}(m)$$

After two-stage ranking, candidates for experimental validation in Rounds 1 and 2 were drawn from the EvoMax-prioritized ranking. Position-level deduplication was used where appropriate to broaden residue coverage, while allowing multiple substitutions at the same residue when they were experimentally or structurally informative. Candidate sets were selected across a range of EvoMax score ranks to increase positional and structure-informed diversity in experimental validation. Round 3 candidates were selected directly from the top-ranked EvoMax outputs.

2.9 *Z*-score calculation for activity-prediction association analyses

For visualization and correlation analyses in Fig. 4e and Supplementary Fig. S3, predicted scores were converted to standard *Z*-scores after candidate nomination. These *Z*-scores were used only for plotting and rank-association analyses and were distinct from the robust median-IQR normalization used internally for EvoMax score integration. For the EvoMax activity-prediction analysis in Fig. 4e, the top 100 EvoMax-prioritized candidates from each optimization round were used as the round-specific normalization background, reflecting the candidate pool considered for experimental nomination. Scores were transformed as $Z = (x - \mu_{\text{round}})/\sigma_{\text{round}}$, where x is the EvoMax-predicted score and μ_{round} and σ_{round} are the mean and standard deviation of the corresponding top 100 candidate pool. For ESM-2 and ESM-IF ablation analyses in Supplementary Fig. S3, component scores were standardized using their corresponding full single-mutant score distributions to ensure inclusion of all experimentally tested Round 1 variants. For the BLOSUM62 baseline, substitution-matrix scores were standardized across the experimentally tested variants from the three optimization rounds before calculating Spearman's rank correlation with experimentally measured activity.

3 Benchmarking

3.1 EVOLVEpro

To benchmark EvoMax against EVOLVEpro, we generated comparison predictions using the EVOLVEpro pipeline (https://github.com/mat10d/EvolvePro). All EVOLVEpro modules and functions were executed out-of-the-box without any modifications to the core algorithmic framework.

Baseline language model representations for the NaloFz2 target protein were extracted using the ESM-2 650M parameter model (esm2_t33_650M_UR50D), generating mean embeddings for all possible single-amino-acid variants. For the first experimental round, we utilized the native EVOLVEpro zero-shot module, specifically the suggest_initial_mutants function, to nominate the initial variant set for experimental testing. For Rounds 2 and 3, variants were generated using the evolve experimental function.

3.2 AiCE

We implemented the AiCE framework to independently nominate high fitness mutations for NaloFz2. The implementation followed the official repository (https://github.com/ScorpioLea/AiCE). Unless otherwise specified, the mathematical framework for all generations and analyses remained identical to those described by the published work.

Following the official implementation of the AiCE-ProteinMPNN configuration, we generated a pool of ~1,000 sequences for the NaloFz2 structure, which was sufficient to identify a consistent top-tier of mutations with frequency scores (f_{max}) exceeding 0.95. The target structural template was derived from an AlphaFold 3 prediction of the NaloFz2 protein.
To maintain compatibility with the mkdssp binary used for secondary structure assignment within AiCE, the AlphaFold 3 PDB output underwent a strict sanitation process to remove MODEL/ENDMDL tags and non-coordinate metadata. Standard AiCE filtering parameters were applied, utilizing a global threshold of β=0.8 for rigid structural elements and a flexible-region threshold of γ = 0.5, as suggested in the official AiCE GitHub repository.

3.3 ProteinGym DMS

To evaluate EvoMax's Round 1 prediction against established zero-shot models and a Gaussian Process Regressor, we benchmarked EvoMax's Round 1 ensemble (90% GPR, 5% ESM-2, 5% ESM-IF) on 196 deep mutational scanning (DMS) datasets from the ProteinGym benchmark suite. These datasets span diverse protein families, including enzymes, viral proteins, transcription factors, and signaling proteins.

3.3.1 Scoring procedure

For each dataset, single-site variants were scored by four methods. For this ProteinGym benchmark only, ESM-2 and ESM-IF1 scores were obtained as pre-computed zero-shot predictions from the ProteinGym score repository. The ESM-2 benchmark scores were masked-marginal log-likelihood ratios from the 650M-parameter model, whereas the ESM-IF1 benchmark scores were average per-residue inverse-folding log-likelihoods conditioned on experimentally determined or AlphaFold-predicted structures. These pre-computed benchmark scores were not recalculated. The ESM-2 benchmark scores are distinct from the masked-mutant probabilities used in the NaloFz2 EvoMax ranking pipeline. GPR predictions were generated for each dataset independently using the same BLOSUM62-based Gaussian Process Regressor described in Section 2.5, evaluated under 5-fold cross-validation so that each variant received an out-of-fold prediction from a model that had never observed it during training. The EvoMax R1 ensemble score was then computed by converting each of the three component scores to percentile ranks within the dataset and combining them using the Round 1 preset weights.

3.3.2 Evaluation Metric

For each method and dataset, we compute the Spearman rank correlation coefficient (ρ) between predicted scores and experimentally measured DMS enrichment values. Statistical comparisons between methods were performed using two-sided Wilcoxon signed-rank tests across the 196 paired observations.

4 Software and Computational Resources

4.1 Phylogenetic Analysis Software

Supplementary Methods Table 1: Software for phylogenetic analysis.

Task	Software Version
Homology search	NCBI BLAST+ 2.12
Multiple sequence alignment	MAFFT 7.505
Alignment trimming	trimAl 1.4
Initial tree inference	FastTree 2.1.11
Maximum-likelihood inference	IQ-TREE 2 2.2.0
Tree annotation	iTOL Web (v6)

4.2 EvoMax Software

Supplementary Methods Table 2: Software and model versions for EvoMax.

Component	Software/Model Version
Programming language	Python 3.11
Deep learning framework	PyTorch 2.6.0 (CUDA 12.4)
PLM scoring	ESM-2 esm2_t33_650M_UR50D (650M params)
ESM library	fair-esm 2.0.0
Regression	scikit-learn (GPR) 1.5.2
Structure scoring	ESM-IF1 esm_if1_gvp4_t16_142M_UR50 (142M params)
Sequence I/O	Biopython 1.84
Tokenization	Transformers 5.0.0

4.3 Computational Resources

EvoMax computations were performed on an NVIDIA A100 GPU (40 GB). The full prediction pipeline, including ESM-2 scoring of all single-site mutations, GPR training and inference, and ESM-IF1 structural scoring of top candidates, completes in approximately 1 minute for a protein of ~400 residues.

4.4 Code Availability

Code for EvoMax is available at: https://github.com/Jackson-Gold/EvoMax

5 Structural visualizations

Structural models were visualized using UCSF ChimeraX (version 1.10).

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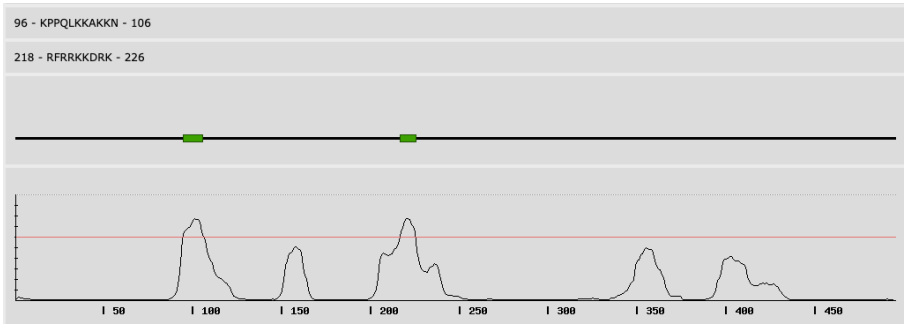
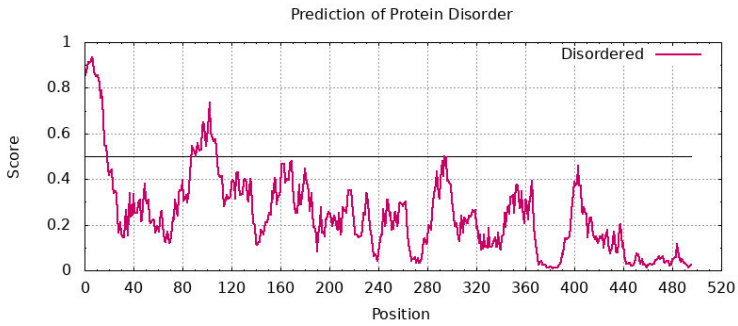
Supplementary Figures



1a. NLS prediction of NaloFz2



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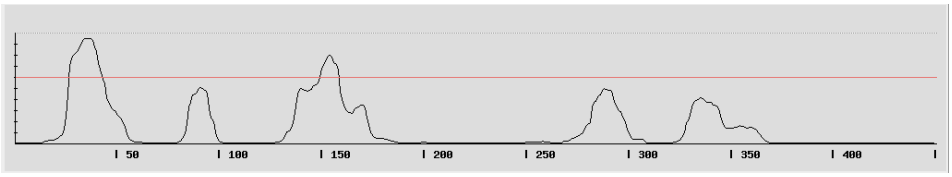
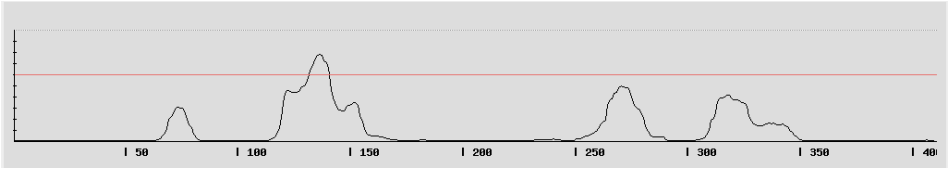
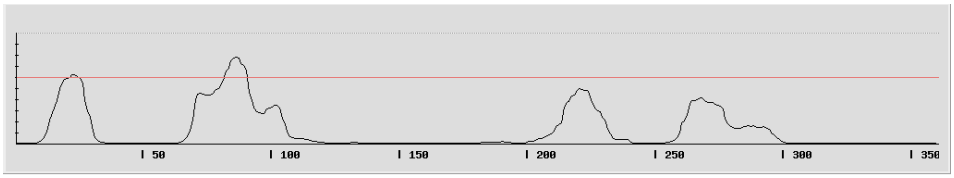


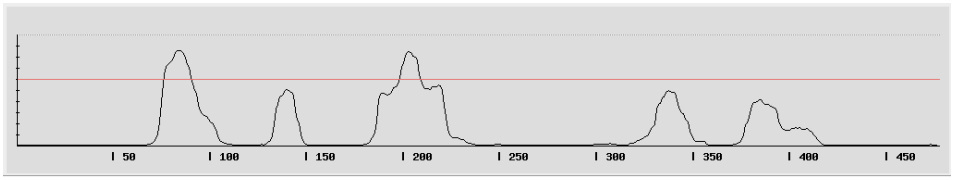
1c. NLS prediction of M1



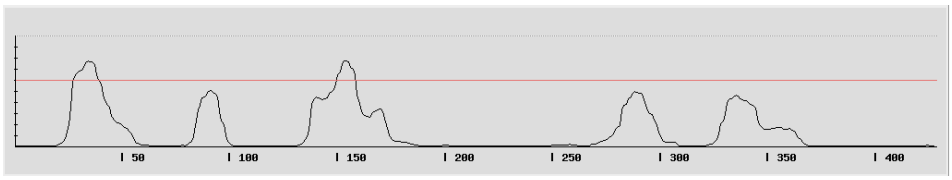
1d. NLS prediction of M2



1e. NLS prediction of M5

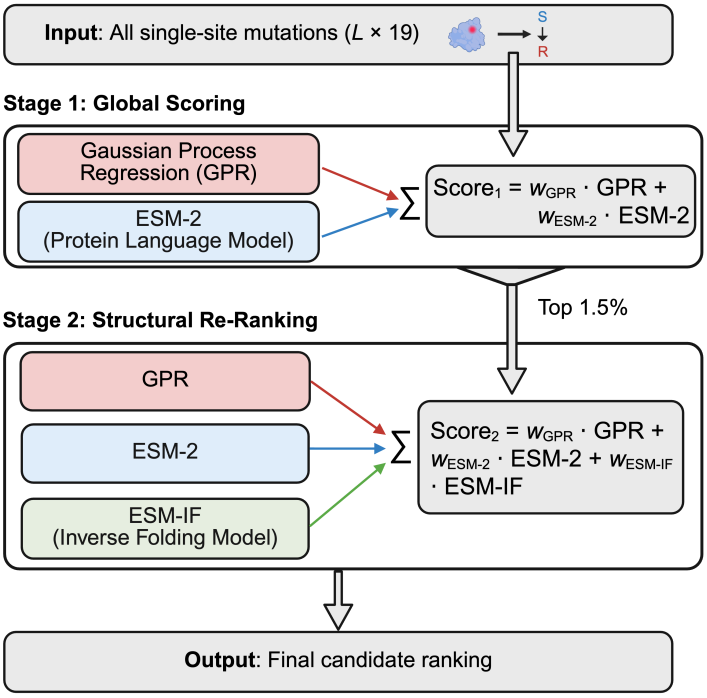


1f. NLS prediction of M8

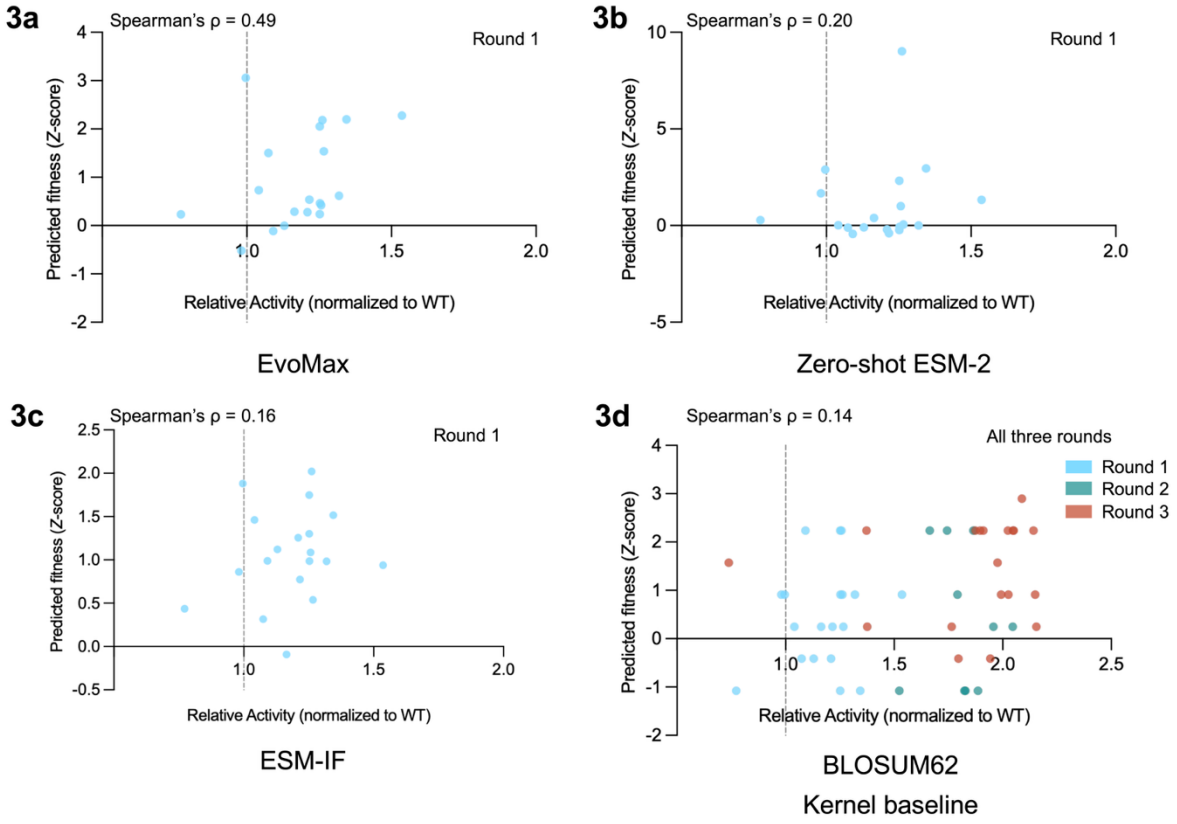


1g. NLS prediction of M9

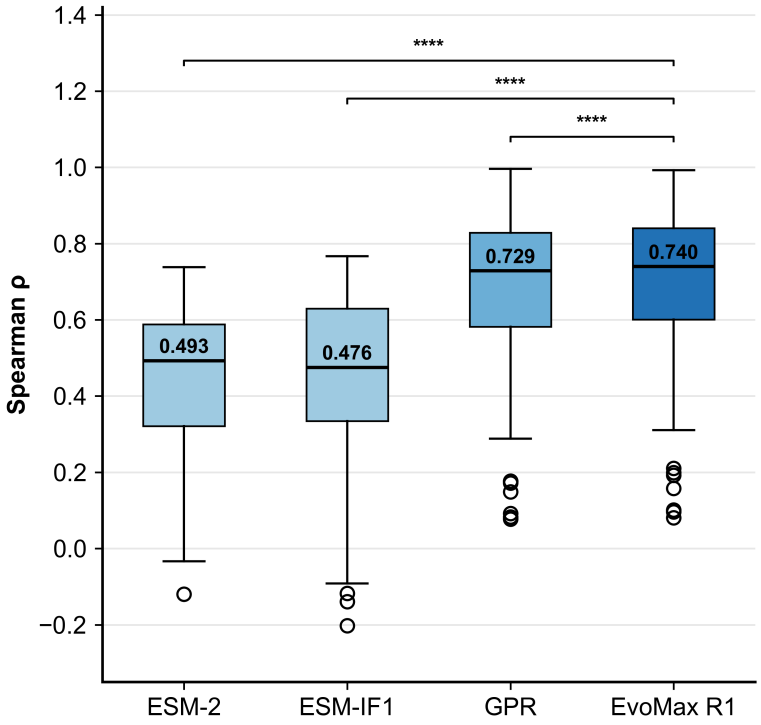
Supplementary Fig. S1. Computational prediction of NTDs in Fz2 orthologs
Probability distribution of potential NLS elements across the NaloFz2 (**a**), M1 (**c**), M2 (**d**), M5 (**e**), M8 (**f**), M9 (**g**) protein sequence as predicted by NLStradamus[1]. The default cutoff at 0.6 is used to call significant NLS-like elements. **b**, Prediction of the disorder region across the NaloFz2.



Supplementary Fig. S2. Schematic overview of the EvoMax two-stage ranking pipeline
All possible single-site mutations are scored in Stage 1 using a weighted combination of Gaussian Process Regression (GPR) and ESM-2 masked-mutant probabilities. The top 1.5% of candidates are advanced to Stage 2, where they are re-ranked using a three-component score that adds ESM-IF folding predictions to the GPR and ESM-2 features, producing the final candidate ranking for experimental validation. Created in BioRender. Vure, P. (2026) https://BioRender.com/7zhysuy.

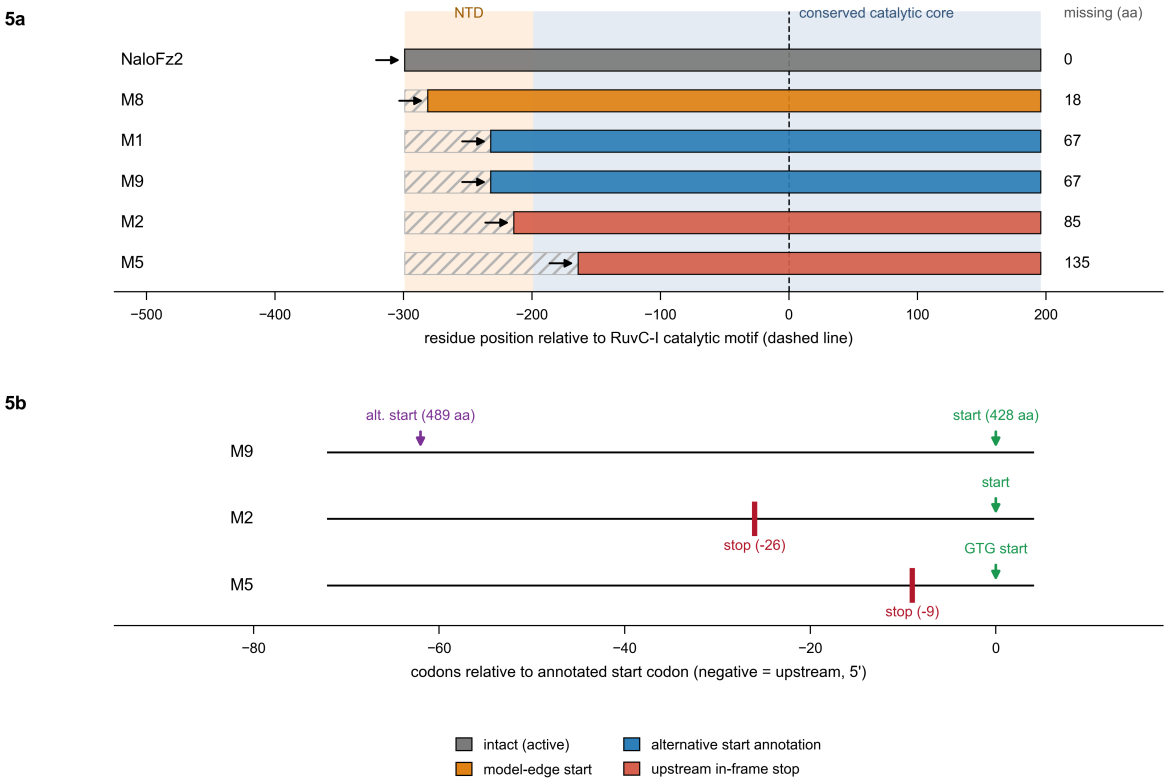


Supplementary Fig. S3. Retrospective ablation analysis of EvoMax pipeline components
a–c, Association between measured NaloFz2 activity and predicted fitness *Z*-scores for Round 1 candidates using the full EvoMax pipeline (a), zero-shot ESM-2 masked-mutant probability scores (b), and ESM-IF inverse-folding scores (c). d, Association between measured activity and BLOSUM62 kernel baseline *Z*-scores across all three directed-evolution rounds. Dashed vertical lines indicate WT-normalized activity = 1. *Z*-score calculation details are described in Supplementary Methods. Two-sided Spearman rank correlations were: a, $\rho = 0.486178$, $P = 0.0348068$ ($n = 19$); b, $\rho = 0.202721$, $P = 0.405215$ ($n = 19$); c, $\rho = 0.160597$, $P = 0.511319$ ($n = 19$); and d, $\rho = 0.142006$, $P = 0.335648$ ($n = 48$).

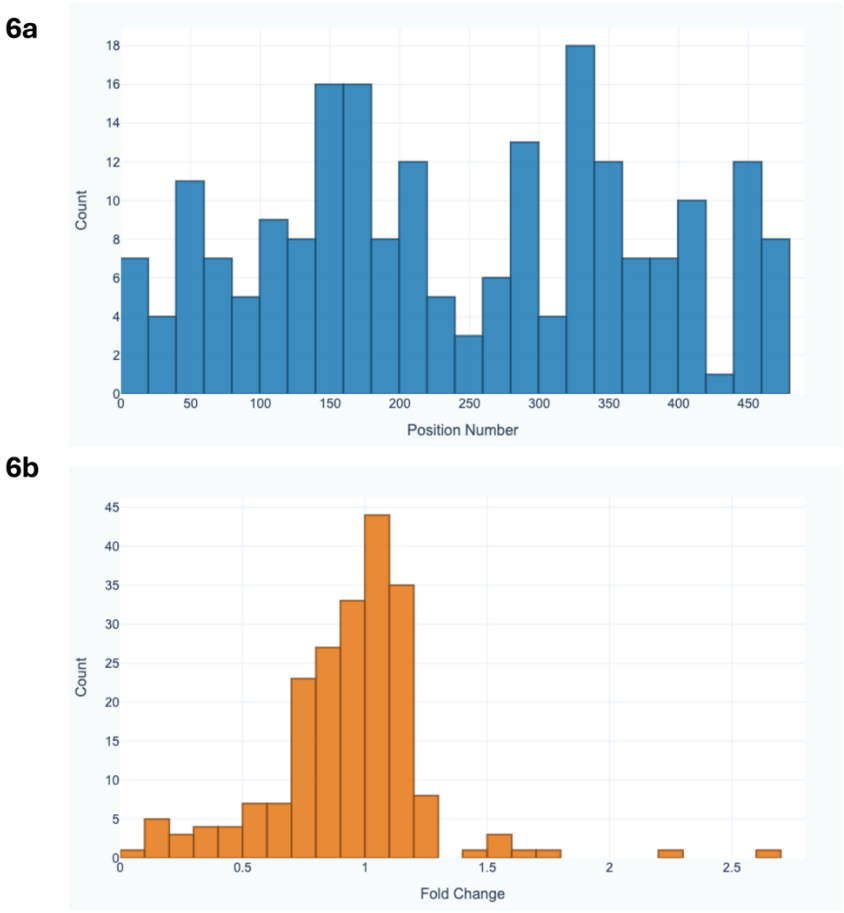


Supplementary Fig. S4. DMS benchmarking on ProteinGym Datasets

Model evaluation on 196 DMS datasets. Spearman rank correlation (ρ) between predicted and experimentally measured variant fitness across 196 deep mutational scanning datasets from the ProteinGym benchmark. Pairwise comparisons were performed using two-sided paired Wilcoxon signed-rank tests across the 196 matched datasets, with Holm adjustment for three comparisons. ****$P < 0.0001$. Exact raw P values were: EvoMax R1 versus ESM-2, $P = 8.078 \times 10^{-42}$; EvoMax R1 versus ESM-IF1, $P = 1.516 \times 10^{-47}$; and EvoMax R1 versus GPR, $P = 1.352 \times 10^{-53}$. Corresponding Holm-adjusted P values were: EvoMax R1 versus ESM-2, $P = 8.078 \times 10^{-42}$; EvoMax R1 versus ESM-IF1, $P = 3.032 \times 10^{-47}$; and EvoMax R1 versus GPR, $P = 4.055 \times 10^{-53}$.

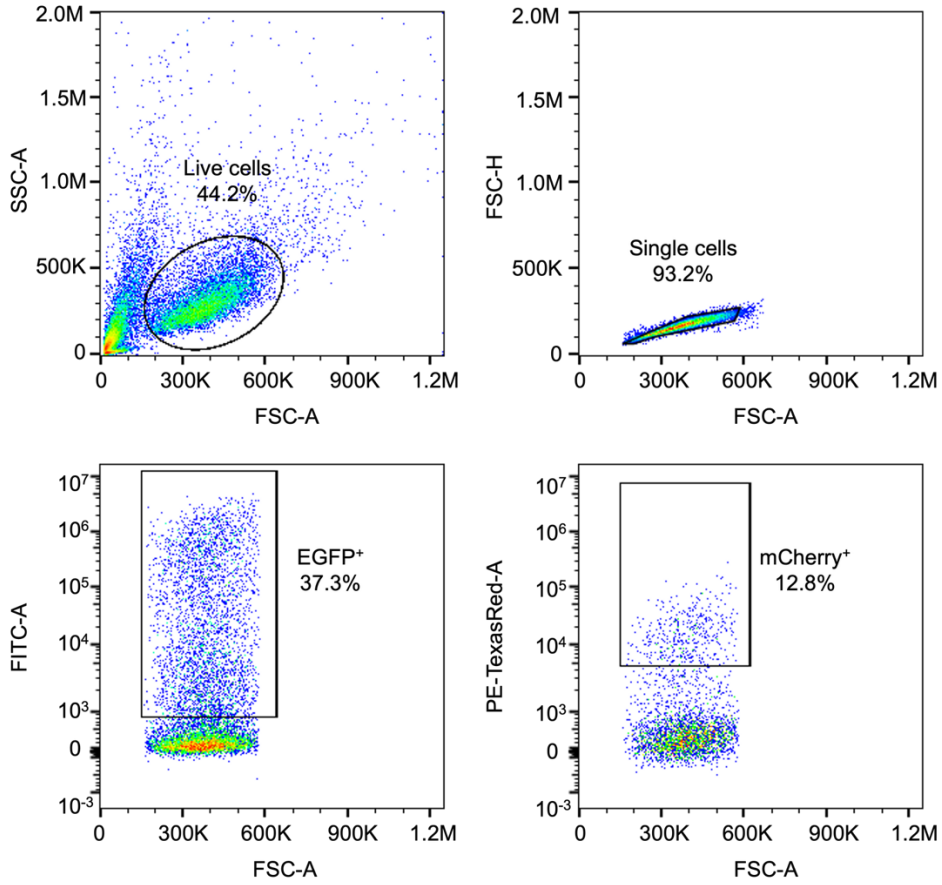


Supplementary Fig. S5. Genomic context of apparent NTD truncations in inactive Fz2 orthologs
a, NaloFz2 (active, full-length) and the inactive orthologs M8, M1, M9, M2, and M5 aligned at the conserved RuvC-I motif (dashed line). Solid bars, annotated protein sequence; hatched bars, *N*-terminal sequence absent relative to NaloFz2; black arrows, annotated start codon; right-hand numbers, residues absent *N*-terminal to RuvC-I; bar color, category (key). Background shading marks the NTD and the conserved catalytic core. **b**, In-frame coding potential upstream of the annotated start codon (codon 0) for M9, M2 and M5. Green and purple arrows, annotated and alternative in-frame start codons; red bars, nearest upstream in-frame stop codon. M9 is annotated at two in-frame start codons (yielding 428- and 489-residue proteins) with no disrupting upstream stop; M2 and M5 have upstream in-frame stop codons (at codons -26 and -9; M5 uses a GTG start).



Supplementary Fig. S6. GPR dataset statistics

a, Distribution of single-site mutations across the NlovFz2 protein sequence, showing coverage across the full length of the protein. **b**, Distribution of fold-change values (relative to wild-type activity) for the 209 training mutations. The 209-mutation training set summarized in Supplementary Fig. S6 was compiled from newly generated measurements shown in Extended Data Fig. 5a and previously published NlovFz2 mutational measurements reported by Wei et al. (2026).



Supplementary Fig. S7. FACS gating strategy for mCherry-positive cells

Live cells were gated by side scatter area versus forward scatter area (SSC-A vs. FSC-A). Singlets were selected by forward scatter height versus forward scatter area (FSC-H vs. FSC-A). EGFP-positive cells were gated by FITC area versus forward scatter area (FITC-A vs. FSC-A). mCherry-positive cells were selected by PE-TexasRed area versus forward scatter area (PE-TexasRed-A vs. FSC-A). Gates were defined using non-transfected cells as a negative control. The gating example is representative of the strategy applied consistently across all flow-cytometry assays, each performed in at least three independent biological replicates.

References

A. N. Nguyen Ba, A. Pogoutse, N. Provart, A. M. Moses, NLStradamus: A simple Hidden Markov Model for nuclear localization signal prediction. BMC Bioinformatics 10, 202 (2009).

Wei, Y. et al. Engineering eukaryotic transposon-encoded Fanzor2 system for genome editing in mammals. Nat. Chem. Biol. 22, 48–57 (2026).

Supplementary Table 1. Sequences of engineered ωRNAs

ωRNA	RNA sequence (Bases deleted from the WT ωRNA-NaloFz2 sequence are indicated by strikethrough.)
WT-ωRNA-NaloFz2	gagcaccuugugugcguuggguccuuuccccuggcuuucacucuuugaguguuugcuugcaagaucagcacuuuguucgauuuguuuguuuuguucaaauuguugauuuaaauaaaggcg
SL1-GAAA	gagcacgaaaugugcguuggguccuuuccccuggcuuucacucuuugaguguuugcuugcaagaucagcacuuuguucgauuuguuuguuuuguucaaauuguugauuuaaauaaaggcg
SL2 Δ1bp	gagcaccuugugugcguuggguccuuuccccuggcuuucacu~~u~~uuu~~g~~aguguuugcuugcaagaucagcacuuuguucgauuuguuuguuuuguucaaauuguugauuuaaauaaaggcg
SL2 Δ1bp AU-GC	gagcaccuugugugcguuggguccuuuccccuggcuuucacg~~u~~uuu~~g~~cguguuugcuugcaagaucagcacuuuguucgauuuguuuguuuuguucaaauuguugauuuaaauaaaggcg
SL2 Δ2bp	gagcaccuugugugcguuggguccuuuccccuggcuuucac~~uc~~uuu~~ga~~guguuugcuugcaagaucagcacuuuguucgauuuguuuguuuuguucaaauuguugauuuaaauaaaggcg
SL2 Δ3bp	gagcaccuugugugcguuggguccuuuccccuggcuuuca~~cuc~~uuu~~gag~~guguuugcuugcaagaucagcacuuuguucgauuuguuuguuuuguucaaauuguugauuuaaauaaaggcg
SL2 Δ3bp AU-GC	gagcaccuugugugcguuggguccuuuccccuggcuuucg~~cuc~~uuu~~gag~~cguuugcuugcaagaucagcacuuuguucgauuuguuuguuuuguucaaauuguugauuuaaauaaaggcg
SL2 Δ4bp	gagcaccuugugugcguuggguccuuuccccuggcuuuca~~cuuc~~uuu~~gagu~~guuugcuugcaagaucagcacuuuguucgauuuguuuguuuuguucaaauuguugauuuaaauaaaggcg
SL2-GAAA	gagcaccuugugugcguuggguccuuuccccuggcuuucacucgaaagaguguuugcuugcaagaucagcacuuuguucgauuuguuuguuuuguucaaauuguugauuuaaauaaaggcg
SL3 Δ1bp	gagcaccuugugugcguuggguccuuuccccuggcuuucacucuuugaguguuugcuugcaagaucagcacuuuguucg~~a~~uuuguuuguuu~~u~~uguucaaauuguugauuuaaauaaaggcg
SL3 Δ1bp U-C	gagcaccuugugugcguuggguccuuuccccuggcuuucacucuuugaguguuugcuugcaagaucagcacuuuguucg~~a~~uuuguuuguuu~~u~~cguucaaauuguugauuuaaauaaaggcg
SL3 Δ2bp	gagcaccuugugugcguuggguccuuuccccuggcuuucacucuuugaguguuugcuugcaagaucagcacuuuguuc~~ga~~uuuguuuguu~~uu~~guucaaauuguugauuuaaauaaaggcg
SL3 Δ5bp	gagcaccuugugugcguuggguccuuuccccuggcuuucacucuuugaguguuugcuugcaagaucagcacuuug~~uucga~~uuuguuuguu~~uugu~~caaauuguugauuuaaauaaaggcg
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SL3 Δ9bp U-G	gagcaccuugugugcguuggguccuuuccccuggcuuucacucuuugaguguuugcuugcaagaucagcac~~uuguucga~~uuuguuuguu~~uugua~~guguugauuuaaauaaaggcg
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SL3 Δ12bp G-A	gagcaccuugugugcguuggguccuuuccccuggcuuucacucuuugaguguuugcuugcaagaucaa~~cauuuguucga~~uuuguuuguu~~uugua~~uugauuuaaauaaaggcg
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Supplementary Table 2. Mutations in NaloFz2 variants

Mutation	Amino acid sequence (mutations different from WT-NaloFz2 sequence are indicated in red font)
WT-NaloFz2	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYTVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
E8R	MKRSRED**R**PTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYTVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
P9R	MKRSREDE**R**THPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYTVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
E70R	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVI**R**SKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYTVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
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G156D	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSK**D**VKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYTVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
K159R	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVK**R**NKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYTVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
K161R	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKN**R**KNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYTVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
D172R	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNN**R**NYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYTVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
N201K	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAF**K**TNFEKKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYTVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
E205R	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNF**R**KKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYTVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
A209R	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKK**R**GTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYTVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
T211M	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAG**M**IDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYTVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
L242M	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSG**M**YSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYTVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF

Supplementary Table 2. Mutations in NaloFz2 variants

Mutation	Amino acid sequence (mutations different from WT-NaloFz2 sequence are indicated in red font)
V280R	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQ**R**LDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYTVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
C332K	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLC**K**AYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYTVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
C349R	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRH**R**KRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYTVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
K396N	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQ**N**MVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYTVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
Y456K	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHI**K**TVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
T457R	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIY**R**VGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
G156D-E205R	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSK**D**VKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNF**R**KKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYTVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
N201K-E205R	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAF**K**TNF**R**KKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYTVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
E205R-A209R	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNF**R**KKK**R**GTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYTVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
E205R-T211M	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNF**R**KKKAG**M**IDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYTVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
E205R-C332K	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNF**R**KKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLC**K**AYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYTVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
E205R-C349R	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNF**R**KKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRH**R**KRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYTVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
C332K-C349R	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLC**K**AYDKLQSKWSQPEVRH**R**KRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYTVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
N201K-E205R-C332K = FanzMAX v1	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAF**K**TNF**R**KKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLC**K**AYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYTVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF

Supplementary Table 2. Mutations in NaloFz2 variants

Mutation	Amino acid sequence (mutations different from WT-NaloFz2 sequence are indicated in red font)
N201K-E205R-C349R	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFKTNFRKKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHRKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYTVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
E205R-A209R-C332K	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFRKKKRGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCKAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYTVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
E205R-A209R-C349R	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFRKKKRGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHRKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYTVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
E205R-T211M-C332K	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFRKKKAGMIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCKAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYTVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
E205R-T211M-C349R	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFRKKKAGMIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHRKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYTVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
V74R-FanzMAX v1	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPRFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFKTNFRKKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCKAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYTVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
D172Q-FanzMAX v1	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNQNYQTENQWVVNTPYDVRDAAAIELLTAFKTNFRKKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCKAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYTVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
V182R-FanzMAX v1	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVRNTPYDVRDAAAIELLTAFKTNFRKKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCKAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYTVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
N201R-FanzMAX v1	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFRTNFRKKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCKAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYTVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
T211Q-FanzMAX v1	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFKTNFRKKKAGQIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCKAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYTVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
C332R-FanzMAX v1	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFKTNFRKKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCRAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYTVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
C349Q-FanzMAX v1	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFKTNFRKKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCKAYDKLQSKWSQPEVRHQKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYTVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
C349K-FanzMAX v1	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFKTNFRKKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCKAYDKLQSKWSQPEVRHKKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYTVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
K412R-FanzMAX v1	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFKTNFRKKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCKAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARRMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYTVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF

Supplementary Table 2. Mutations in NaloFz2 variants

Mutation	Amino acid sequence (mutations different from WT-NaloFz2 sequence are indicated in red font)
T457Q-FanzMAX v 1 = FanzMAX v2	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFKTNFRKKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCKAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYQVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
E129Q-FanzMAX v 2	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQQRTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFKTNFRKKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCKAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYQVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
N203A-FanzMAX v 2	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFKTAFRKKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCKAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYQVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
R220K-FanzMAX v 2	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFKTNFRKKKAGTIDKFMIRFKRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCKAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYQVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
R221S-FanzMAX v 2	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFKTNFRKKKAGTIDKFMIRFRSKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCKAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYQVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
R247G-FanzMAX v2	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFKTNFRKKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIGNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCKAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYQVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
N271G-FanzMAX v2	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFKTNFRKKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLGHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCKAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYQVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
L299I-FanzMAX v2	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFKTNFRKKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVAIDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCKAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYQVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
K318E-FanzMAX v 2	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFKTNFRKKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTEWGSGGAERIGRLCKAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYQVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
Y334L-FanzMAX v 2	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFKTNFRKKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCKALDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYQVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
I361L-FanzMAX v2	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFKTNFRKKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCKAYDKLQSKWSQPEVRHCKRYKYKRAGRRLQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYQVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
I368L-FanzMAX v2	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFKTNFRKKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCKAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNLVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYQVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
D371E-FanzMAX v 2	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFKTNFRKKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCKAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDELHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYQVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
Q395S-FanzMAX v 2	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFKTNFRKKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCKAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETSKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIYQVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF

Supplementary Table 2. Mutations in NaloFz2 variants

Mutation	Amino acid sequence (mutations different from WT-NaloFz2 sequence are indicated in red font)
H402K-FanzMAX v 2	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAF**K**TNF**R**KKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLC**K**AYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKL**K**RRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIY**Q**VGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
R404K-FanzMAX v 2	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAF**K**TNF**R**KKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLC**K**AYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHR**K**INSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIY**Q**VGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
H432Y-FanzMAX v 2	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAF**K**TNF**R**KKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLC**K**AYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKARE**Y**PWTHIYIVNEAYTSKTCSCCGHIY**Q**VGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
I437V-FanzMAX v 2	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAF**K**TNF**R**KKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLC**K**AYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTH**V**YIVNEAYTSKTCSCCGHIY**Q**VGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
E462K-FanzMAX v 2	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAF**K**TNF**R**KKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLC**K**AYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIY**Q**VGSS**K**VFRCPSCGSIFDRDINAARNILLRFLTTHRISF
A479G-FanzMAX v 2	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAF**K**TNF**R**KKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLC**K**AYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIY**Q**VGSSEVFRCPSCGSIFDRDIN**G**ARNILLRFLTTHRISF
E129Q-R221S-FanzMAX v2	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNE**Q**Q**R**TTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAF**K**TNF**R**KKKAGTIDKFMIRFR**S**KKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLC**K**AYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIY**Q**VGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
R220K-R221S-FanzMAX v2	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAF**K**TNF**R**KKKAGTIDKFMIRF**KS**KKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLC**K**AYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIY**Q**VGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
R221S-Q395S-FanzMAX v2 = FanzMAX v3	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAF**K**TNF**R**KKKAGTIDKFMIRFR**S**KKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLC**K**AYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFET**S**KMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIY**Q**VGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
R221S-R404K-FanzMAX v2	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAF**K**TNF**R**KKKAGTIDKFMIRFR**S**KKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLC**K**AYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHR**K**INSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIY**Q**VGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
R221S-H432Y-FanzMAX v2	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAF**K**TNF**R**KKKAGTIDKFMIRFR**S**KKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLC**K**AYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKARE**Y**PWTHIYIVNEAYTSKTCSCCGHIY**Q**VGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
R221S-E462K-FanzMAX v2	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARW IYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAF**K**TNF**R**KKKAGTIDKFMIRFR**S**KKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR GENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLC**K**AYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLL HKAREHPWTHIYIVNEAYTSKTCSCCGHIY**Q**VGSS**K**VFRCPSCGSIFDRDINAARNILLRFLTTHRISF

Supplementary Table 3. Mutations in NlovFz2 variants

Mutation	Amino acid sequence (mutations different from WT-NlovFz2 sequence are indicated in red font)
WT-NlovFz2	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
G49R	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNK**R**CTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
C50R	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKG**R**TDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
T51R	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGC**R**DGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
D52R	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCT**R**GWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
G53R	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTD**R**WFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
W54R	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDG**R**FNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
F55R	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGW**R**NFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
N56R	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWF**R**FSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
F57R	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFN**R**SQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
Q101K	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKG**K**KTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
Q105R	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTP**R**KFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
E121R	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPN**R**QERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
N128K	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTL**K**QWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF

Supplementary Table 3. Mutations in NlovFz2 variants

Mutation	Amino acid sequence (mutations different from WT-NlovFz2 sequence are indicated in red font)
N164K	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVV**K**NDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
N177K	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVV**K**TPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
E189R	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAI**R**LLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
A193R	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLT**R**FNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
N195K	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAF**K**TNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
T196R	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFN**R**NFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
E199R	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNF**R**KKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
A203R	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKK**R**GTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
G204R	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKA**R**TIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
T205R	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAG**R**IDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
I206R	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGT**R**DKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
D207R	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTI**R**KFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
F209G	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDK**G**MIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
D218R	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKK**R**RKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF

Supplementary Table 3. Mutations in NlovFz2 variants

Mutation	Amino acid sequence (mutations different from WT-NlovFz2 sequence are indicated in red font)
D221R	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRK**R**HFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
R226Q	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVL**Q**CKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
H229Y	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCK**Y**WKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
Y255H	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQ**H**DSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
G279R	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIR**R**ENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
E280R	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRG**R**NQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
P295L	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALD**L**GVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
C326K	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLC**K**AYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
A327R	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCC**R**YDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
W335R	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSK**R**SQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
S336R	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKW**R**QPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
Q337R	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWS**R**PEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
P338R	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQ**R**EVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
E339R	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQP**R**VRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF

Supplementary Table 3. Mutations in NlovFz2 variants

Mutation	Amino acid sequence (mutations different from WT-NlovFz2 sequence are indicated in red font)
V340R	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPE**R**RHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
C343R	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRH**R**KRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
Y348R	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYK**R**KRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
Q356R	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRI**R**QKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
N361R	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIR**R**IVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
D364K	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIV**K**DLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
D364R	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIV**R**DLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
Q389R	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFET**R**KMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
K390N	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQ**N**MVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
L395R	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKK**R**HRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
H396R	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKL**R**RRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
A404K	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKT**K**RKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
Y450K	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHV**K**TVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
T451R	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVY**R**VGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF

Supplementary Table 3. Mutations in NlovFz2 variants

Mutation	Amino acid sequence (mutations different from WT-NlovFz2 sequence are indicated in red font)
E456K	MEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCL EFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAP QHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREH PWTHIYIVNEAYTSKTCSCCGHVYTVGSS**K**VFRCPSCGSIFDRDINGARNILLRFLTTHRISF

Supplementary Table 4. On-Target genomic sites, guide sequences and primer sequences

Target	TAM	Guide sequence	NGS-Primer-F (5'-3')	NGS-Primer-R (5'-3')
ALDH1A3	ACCG	ATGATCCTCTGCAGAGAATC	GGCAGGGCCTGGGCTAGCTG	CAGGTCCAAATAATTGATAA
B2M	ACCG	TCACCTGTCTCCAAGCCAGC	GATAGCCTCCAGGCCAGAAAG	ACCCAGTCTAGTGCATGCCTTC
CA2	ACCG	ATTTCTGCTACTCACTGCAC	ATTCCAGCAATCTGAAAGAG	AGGTAAGCAGTATAAATGGG
CXCR4-Site 1	ACCG	AAAGGAGTTTTCTTGACCAT	CAAACGTTTGAACTTAGAGCGCAGC	TACGTTTGCAAACAGCGTGCAAG
CXCR4-Site 2	CCCG	AAAGGAGTTTTCTTGACCAT	N/A	N/A
CXCR4-Site 3	CCCG	TACTCCAAAAAAGGGTCACC	CAAACGTTTGAACTTAGAGCGCAGC	TACGTTTGCAAACAGCGTGCAAG
CXCR4-Site 4	ACCG	CATCTGGAGAACCAGCGGTT	AGCAGGTAGCAAAGTGACGCCGAG	GAGTACGGGTACCTCCAATGT
DMD	ACCG	ACAAGGGTAGGTAACACATA	GGAAAGGGTGAAGCTACAGG	CCCTGTCTTTTTTCCATGGAGG
DYRK1A	ACCG	TGTTAGCCAGGATGGTCTCG	TCATACCTTTCTCCTGCCTC	CCCAAAGACCATACGCCTAT
EMX1-Site 1	ACCG	GGACCCTCTCCATTTCTACC	TGCAGCCCCCGCACTCCTTCTT	AGTAACTTCTAAACAGCCCCGCGC
EMX1-Site 2	ACCG	CTTCTTCGGCCACCGCTTCC	ACCGGGACCCTCTCCATTTCTACC	AGCCTTCCCGGCAGTAACTTCTAAA
EMX1-Site 3	ACCG	GAGGACAAAGTACAAACGGC	AGTTTCTCATCTGTGCCCCTCCCT	AGTCATTGGAGGTGACATCGATGTC
FANCF	ACCG	AGGGCCTGGAAGTTCGCTAA	ATGCAGCTCGTTACCACCTGGTGCA	GCTTTGGTCGGCATGGCCCCATTC
HPRT1	ACCG	CCGTAACTCTAGCACTTAGC	GTTGTTTTGGCAATGACCTG	CTCCGTTAATAGCACTGCTCTTTACC
MSTN	ACCG	TTGGGGTAAGATACCTTTGT	CACTCCCTTGATACTCTTAC	ACCAGGAGAAGATGGGCTGGTA
PCSK9-Site 1	ACTG	CTGCTGCTGCTGCTGCTGCT	AGGACAGCAACCTCTCCCCT	AGAGGAAACAGCACCGCACC
PCSK9-Site 2	GCCG	CTGCCACTGCTGCTGCTGCT	AGGACAGCAACCTCTCCCCT	AGAGGAAACAGCACCGCACC
PCSK9-Site 3	ACCG	TCAGCTCCAGGCGGTCCTGG	AGGACAGCAACCTCTCCCCT	AGAGGAAACAGCACCGCACC
VEGFA-Site 1	GCCG	CCCCACAGCCCGAGCCGGAG	AGACAGTGCTCCAGCCGCGC	CAAGGCTCCAATGCACCCAA
VEGFA-Site 2	ACCG	CCGGAGCGCGGCGTGAGCCC	AAGTCGAGGAAGAGAGAGAC	GGACGAAAAGTTTCAGTGCGA
VEGFA-Site 3	ACCG	CCGCCTCACCCGTCCATGAG	TTCTGGGCTGTTCTCGCTTC	TCGGCGAGCTACTCTTCCTC

Supplementary Table 5. Off-Target genomic sites, guide sequences and primer sequences

Target	Guide sequence	NGS-Primer-F (5'-3')	NGS-Primer-R (5'-3')
CXCR4-OT1	CCGGAAGGAGTTATTTTTGAGCAT	TCGGCCTCCCAAAGTGCTGGGATTA	AAGAGCTGAAAGTCACTACTGGGAG
CXCR4-OT2	CCGAAAGAAGTTTTTCTAGAGCAT	TCAGCCTCCCAAAGTGCTGGGATTA	TTGAGCCAGCAACTACACTCCTAGG
CXCR4-OT3	CCGAAAGGCAGTTGTATTGACCCT	CAGGCATATCTTTTCACCTGCTCTA	CCTGAAGGAATATTACTGGGGGCCT
CXCR4-OT4	CCGAAGAGCAGTTTGCTTGACCCT	CCTTGGTTGATTTGGCAGAGCATTT	TCCCTATTCGCTCATTCCAGGGTAC
CXCR4-OT5	CCGACAGCAGATTTTTTTGACCAT	CTTGGCCTCCTAAAATGTTGGGATT	CTTTAAGCTCTGTTGGACTTGACTG
CXCR4-OT6	CCGTAATGGAGGTTTCTTTACCAT	GCATCCTCAATAGGGAAGTTACAGG	GCCACTGACTTAACCAGCATTGACT
CXCR4-OT7	CCGAACGCAGTTGTTCTTGACCGT	TGGTAAGGCATACTAAAACCACCAC	GGCCTCAAGCGATCCTCCCACCCCA
CXCR4-OT8	CCGATATGGAGTTTTCTTGCCCAG	GGCTCACCCTCTCCTCTGTTTTAAG	GGCTGAGGCGGGAGAATCACTTGAA
VEGFA-OT1	CCGCCGCCACCGCCCGCGCCGGAG	CGCCCACCCGTCAGTCTCT	AGGCCGCTTCGCTGCTTGA
VEGFA-OT2	CCGCCCAACAGGCCCGAGGCGGAG	ACGCCCAACAGAACCGTTAGGACCA	GAGAGAGCTGCTAGGGCGGTTT
VEGFA-OT3	CCGCCCCTCAGCCTGCAGCCGGAG	CACGGAGCCCAAACAGGTACCTCT	TTATTCCTGTGCGAGTAAGGAGCGC
VEGFA-OT4	CCGCCCCCCAGCCCCGAGCCGGCG	ATCAAAACGCAAAACCCCGGCTGAC	AAGGAGGGAACGGAATCTGCCGTTA
PCSK9-OT1	CCGCCAGCTCCCGGCAGTGCTGG	AATGTACGCTCCATGAAGGC	GGGAAGATAAACATCTAGTTCC
PCSK9-OT2	CCGACAGCTCCAGGAGGACATGG	GTTAACAGGCCAATCATTCC	GGGACTCTAGAAATCTATGTC
PCSK9-OT3	CCGGTGGCTCCAGGCGTTCCTGG	TTAAATAGCATCTGGAAGGG	CTCTTGAAGATATGACATGC
PCSK9-OT4	CCGTCTGCACCAAGCAGTCCTGG	GTCATTGGTTTCAGATTTTGCC	TTCTTTCTTCCTTTCCCTGC
PCSK9-OT5	CCGTGAGCTCAAGGAGGTCCAGG	GGTAGGCACCGATACAGCAG	GCAGGAAGGTCTATTACTGT
PCSK9-OT6	CCGCCAGCTGCAGGCGCTCCTGT	CTGGGGCAAAAGGAGTGCTC	TTGTTCACAGGTGGTATTGG
DMD-OT1	CCGAGAATGGTAGCTAGCACATA	AGCGCGCCTCTGCCCGCACCT	GCAGAATGTGCAGCTGATGG
DMD-OT2	CCGACCTGGGTAGGAAAAACACA	CCTCCGTAGCATTTATCAGT	CTGAGCACCTATTATATGCC
DMD-OT3	CCGACCAGAGAAGGTAAAACACA	TAAGGGGTTGCCAGAGCCAT	GCAGAAGAGAAGACACAGAG
DMD-OT4	CCGAGAAGGGTAGGTCCCACATT	CATCCAAACTTCCAAGCAAG	AACAGTGCTGAACTGGCCAG
DMD-OT5	CCGACCAGGGAAGATAACACACC	ACAGGGAGAATAACTACTCC	AGAGGGCCCTTTTCGGTGCA
DMD-OT6	CCGACAGAGAGAGGAAACACATA	TGTGGGCATCTGCCACTTCC	CGAGGGCAGGCTAAGCTGCA

Supplementary Table 6. Protein sequence of Fanzor2 candidates

Name	Amino acid sequence
NaloFz2	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTA RWIYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSSEPLPEELQYDSIIIKNKLNHYYLCIPQV LDIRGENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYR FKQRLLHKAREHPWTHIYIVNEAYTSKTCSCCGHIYTVGSSEVFRCPSCGSIFDRDINAARNILLRFLTTHRISF
M1	MIESKPVFDVPLNVHHSKTENVAFDNSKKPPQLKKAKKKQKSAQKFQADKSMKIRLYSNEQERTTLNQWMGTTRWIYNRCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFKTNFEKK KAGMIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGQYSFIRNIKSAEPLPMELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVR HCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKSKEHPWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFHCPSCGSIFDRDINAARNILLRF LTTHRISFLAPCVLGLPHLFDLFVLFKLLI
M2	MSTHNKYRINFGHVQEIPFMSLISTTTRDAQTDGSTFLYPNEQERTTLNQWMGTARWIYNKCLEFTHNFKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGMIDKFMIRFRRKKDR KDHFVLHCKHWKKKSGLYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAPQHSGQVVGLDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKI RNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREHPWTHIYIVNEAYTSKTCSCCGQIYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
M3	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLSVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPKEQERTTLNQWMGTA RWIYNKCLEFTHNFKGVKKNKKNFRTVVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFKTNFEKKKAGTIDKFMIRLRRKKDRKDHFVLHCKHWKKKNGLYSFIRNIKSAEPLLEELQYDSIIIKNKLNRYYLCIPQV LEIRGENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYR FKQRLLHKAREHPWTHIYIVNEAYTSKTCSCCGHVYTVGLSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
M4	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLSVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPKEQERTTLNQWMGTA RWIYNKCLEFTHNFKGVKKNKKNFRTVVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFKTNFEKKKAGTIDKFMIRLRRKKDRKDHFVLRCKHWKKKNGLYSFIRNIKSAEPLLEELQYDSIIIKNKLNRYYLCIPQV LEIRGENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYR FKQRLLHKAREHPWTHIYIVNEAYTSKTCSCCGHVYTVGLSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
M5	MDGNCSLDLQQVFRIHNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGMIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYL CIPQVLDIRGENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLT WSHYRFKQRLLHKAREHPWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHGISF
M6	MKRSREDEPTHPPTNPSLAHGIIPFWDEYSQQVSDELWACSRDSFHEFNQYNNKGCTDRWFKFSQFTVIESKPVFDVPLNVHYSITENVAFDNSKKPPQLKKAKKNQKTPQKFQADKSLKIRLYPNEQERTTLNQWMGTA RWIYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAANELLTAFKTNFEKKKAGMIDKFMIRFRRKKDRKDHFVLHCKHWKKKTGLYSFIRNIKSAKPLPEELQYDSIIIKNKLNHYYLCIPQV LDIRGENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLLKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYR FKQRLLHKAREHPWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
M7	MKRSREDEPTHPPTNPSLAHGIIPFWNEYSQQVSDKLWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESKPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKTPQKLQADKSLKIRLYPNEQERTTLNQWMGTA RWIYNKCLEFTHNFKDVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGMIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGLYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQV LDIRGENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYR FKQRLLHKAREHPWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHGISF
M8	MNLLILLLIHLLHTASFHSWACSRDSFHEFNQYNNKGCTDGWFNFSQFTVIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKGQKTPQKFQADKSMKIRLYPNEQERTTLNQWMGTARWIYNKCLEFTNKSKGVK KNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKKKAGTIDKFMIRFRRKKDRKDHFVLRCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAPQHSGQVVA LDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVRHCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHRRINSKTARKMLTWSHYRFKQRLLHKAREHPWTHIY IVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRFLTTHRISF
M9	MIESQPVFDVPLNVHHSITENVAFDNSKKPPQLKKAKKNQKVAQKFQADKSLKIRLYPNEQERTTLNQWMGTARWIYNKCLEFTNKSKGVKKNKKNFRTFVVNNDNYQTENQWVVNTPYDVRDAAAIELLTAFNTNFEKK KAGTIDKFMIRFRRKKDRKDHFVLHCKHWKKKSGMYSFIRNIKSAEPLPEELQYDSIIIKNKLNHYYLCIPQVLDIRGENQAPQHSGQVVALDPGVRTFQTTFDLNGYSTKWGSGGAERIGRLCCAYDKLQSKWSQPEVR HCKRYKYKRAGRRIQQKIRNIVDDLHKKLCLWLCRNYQVILLPSFETQKMVKKLHKRINSKTARKMLTWSHYRFKQRLLHKAREHPWTHIYIVNEAYTSKTCSCCGHVYTVGSSEVFRCPSCGSIFDRDINGARNILLRF LTTHRISF
XP_017987766.1	MTILPNKKRQATLKSKLVLQQGNDLYKKPLDIPSWTSFKLIDHGQEAGDQVLIKDIKQGGSSDGTGTKDLWKPKTIRIYPTRKQREIFQLWLNATRHMYNLAVAERIRMGHEAKENGTKVKLKLKHLREVTPLKKSFDYE KYPPLMYLKQVPFLIKDNGLRDFDKDYRTNLAKSKKSLRPFKIHFKKVKDRQSMTVEKKVWGLTRGVYHDVFATNVLRSKEPLPEKLPHDSRLIKEYGKWFLVIPFEARGRMPKTDKSINAVAVDPGVRTFLTTYDSSQR VMEIGEDGYLKLQHLDRRLRSIQSELYKLRKKSPLSKDDRKRKIRNRRKVAIRLYQRKKNLANDIHRKACILLASKYNYILIPVLNFHNFKKTNRRVRANLANLKHCLFVQRLKNKAQHFENTTVIEVDEEFTTKTCSSC GVRSDIGASKIFKCDNCESVFDRDHNAAKNILLKYLGEVE
NP_986403.1	MPIFAAKKRQATLKKKLVLQQGDELKKESLNIPSWAPFKLIDHGQAANDQVLIPQKPEVPGDALKDHWTPKTVRIYPTSKQKEILHLWLTATRHMYNLAVAERIRLGKEAKENGTKVKMKLKHLREVMPLKKNFDYERDP KLTYLKNVPFLIKDNGLRDFDKDYRTNLAKSKNTLRPFKIHFKKIKDKQSMTVEKKVWGLSRGIYHDVFRKGAFRSTEPLPEQLPHDSRLIREHGKWFLVIPYETSGLMPKKEAINAVAVDPGVRTFLTTYDSSQRVLEI GEDGYLKLQHLDRRLRSLQSEIHKLRSKGIVLSKEDRKRKIRNRRTVISRLYQRKKNLANDVHRKASHLLASKYNYILIPVLNFHNFKKTNRRVKANLANMKHCLFVQRLKNKASHFENTTVIEVDEEFTTKTCSLCGVR SNIGASKVFKCDNCDATFDRDHNAAKNILLKYLGEID
KAK7493728.1	MKRTYSATKSSLTLWTAASVKTTSAPKVVTTFSGWMKKILPTRAETSLTLINPADIADPSPPKKKAKKTTPATPKPTLRIYKIGLRPSPAQRKTLNACIVAANFAYNQCVHLVQHKVCKPHLYDLQKIVAKMKTPEDINH RYAPDRDGWFWKSSTIVRLLATKDFCAAYKAIVSNKKKDVAVIKYKTYDDPEAINPLSGLFGCQKQYATVTQAGLRLLPRLFGKDPIPLVKKKLKVATIDHDFKIEKTSKGKFVLCLTVECSLLRRVKPPAPLFEDGYIH ACGIDPGVRSFVTVYDPTRQDCYQFGTSAQKAERLDPITNAIDNWNSFVDQHRDKAPPTAIESWSRKTKKLWYKLKNQVRSLHDQVIAHLLGAYNFISLGKLDVSCFRRGTTAKSTNRWLRIYRHFEFRTKLLARVEGTD NCRVEITDERWTSKTCGMCRSIHRELGAKELFECPNCHYTCHRDVHGARNILLRSFGQFPV
KAK7500073.1	MKRTYSATKSSLTLWTAASVKTTSAPKVVTTFSGWMKKILPTRAETSLTLINPADIADPSPPKKKAKKTTPATPKPTLRIYKIGLRPSPAQRKTLNACIVAANFAYNQCVHLVQHKVCKPHLYDLQKIVAKMKTPEDINH RYAPDRDGWFWKSSTIVRLLATKDFCAAYKAIVSNKKKDVAVIKYKTYDDPEAINPLSGLFGCQKQYATVTQAGLRLLPRLFGKDPIPLVKKKPKVATIDHDFKIEKTSKGKFVLCLTVECSLLRRVKPPAPLFEDGYIH ACGIDPGVRSFVTVYDPTRQDCYQFGTSAQKAERLDPITNAIDNWNSFVDQHRDKAPPTAIESWSRKTKKLWYKLKNQVRSLHDQVIAHLLGAYNFISLGKLDVSCFRRGTTAKSTNRWLRIYRHFEFRTKLLARVEGTD NCRVEITDERWTSKTCGMCRSIHRELGAKELFECPNCHYTCHRDVHGARNILLRSFGQFPV

Supplementary Table 6. Protein sequence of Fanzor2 candidates

Name	Amino acid sequence
XP_003682942.1	MTIVVQNQAKKRKTSNSKTTKPYWNEDAAHISQQWGLNTILPTAAKNLSQKITSDSWFNVVKHPQVPVINDSLNLPLSNGVKEEITRARKIRIYPTNSQKETLKKWFGCQRYIYNRALKLHRDGMAMTIKGLRQTLLNRD TNILEPQEQWLNDYSYDLKDEALRDLVKNYSSNMAKLKNTGVPFKLRFKSKKAPAQTLSVLKKHWNKRTKSFWSKVFSSSKMRSAEVLPEKLLMDSRLQRTRNNKFYLILPVVGEEIVRKMKADKFIFIDPGIRTFLTGY DSGQNVVEIGKNAVVRIEKLKRRRRQLQSKLAKLRKHKKRQNHRKALHRLDEKIQHIVRDMHKKTASYLCKNYENIFLPKLNYHQCTRLTRKTRSSMATIAHCCFHDRLTMKAEQFHSTSVHEVEEDYTSKTCSSCGNLK DDLGSNKIYSCSSCSSVFDRDFNAAKNIMLKYICEHLMASGGSSISSALSVRNVGFAMMGPGPFVH
KAH3786489.1	MKRKREHFTLWDAANVHKHKSMWYWWAYIRRKDMVNHEKTDCDVMQLLQSASVKKQKTQSDKFLTSFSVGLRPTKHQKQVLNEMLRVSNYTYNWCLWLVNEKGLKPHLFELQKVVCKTNASDVDPYYRMENDNWFFNNKM STIKLTSCKNFCTSYKSAKSLKSKLKRPMSVSNIIQGSFCVQKLFIRRLSDKDVSIDNTQMQNRYICMMPDNFEKRSNPKERFLKLAKPITKIPPIDHDVKIVKRADGMFIMNIPCDPKYTRRNASNDTIEKRVCGIDPG GRTFATVYDPIDCCVFQVGIKEDKQYVISKLHNKIDHAHMHLTKAQNKKQQQAARERIVSLKKTHLKLKTFVEDIHLKLSSHLVKEYQYVALGKINVAPLVKKKHLSKRAKRDVLYWQHYRFRQRLTNRATNTECILEVQ NEAYTSKTCGVCGTINKNLEKSETFYCDNCKYNTHRDVNGARNILLKSLRMFPFVNSQ

Supplementary Table 7. ωRNA sequences of Fanzor2 candidates

Name	RNA sequence
NaloFz2	GAGCACCUUGUGUGCGUUGGGUCCUUUCCCCUGGCUUUCACUCUUUGAGUGUUUGCUUGCAAGAUCAGCACUUUGUUCGAUUUGUUUGUUUUGUUCAAAUUGUUGAUUUAAAUAAAGGCG
M1	UAGCACCUUGUGUGUUGGGUCUUCCCCACCUGUUCGAUUUGUUUGUUUUGUUCAAAUUGUUGAUUUAAAUAAAGGCG
M2	GAGCACCUUGUGUGUUGGGUCUUCCCCACCUUGUGUGCGUUGGGUCCUUUCCCCUGGCUUUCACUCUUUGAGUGUUUGCUUGCAAGAUCAGCACUUUGUUCGAUUUGUUUGUUUUGUUCAAAUUGUUGAUUUAAAUAAAG GCG
M3	GAGCACCUUGUGUGUUGGGUCUUCCCCACCUUGUGUGCGUUGGGUCCUUUCCCCUGGCUUUCACUCUUUGAGUGUUUGCUUGCAAGAUCAGCACUUUGUUCGAUUUGUUUGUUUUGUUCAAAAUGUUGAUUUAAAUAAAG GCG
M4	GAGCACCUUGUGUGUUGGGUCUUCCCCACCUUGUGUGCGUUGGGUCCUUUCCCCUGGCUUUCACUCUUUGAGUGUUUGCUUGCAAGAUCAGCACUUUGUUCGAUUUGUUUGUUUUGUUCAAAAUGUUGAUUUAAAUAAAG GCG
M5	GAGCACCAUGUGUUUUAGGUCUUCCCCACCUUGUGUGCGUUGGGUCCUUUCCCCUGACUUUCACUCUUUGAGUGUUUGCUGCAAGAUCAGCACUUUGUUCGAUUUGUUUGUUUUGUUCAAAUUGUUGAUUUAAAUAAAGG CG
M6	GAGCACCUUGUGUGUUGGGUCUUCCCCACCUUGUGUACGUUGGGUCCUUUCCCCUGGCUUUCACUCUUUGAGUGUUUGCUUGCAAGAUCAGCACUUUGUUCGAUUUGUUUGUUUUGUUCAAAUUGUUGAUUUAAAUAAAG GCG
M7	GAGCACCUUGUGUGUUGGGUCUUCCCCACCUUGUGUGCGUUGGGUCCUUUCCCCUGGCUUUCACUCUUUGAGUGUUUGCUUGCAAGAUCAGCACUUUGUUCGAUUUGUUUGUUUUGUUCAAAUUGUUGAUUUAAAUAAAG GCG
M8	GAGCACCUUGUGUGUUGGGUCUUCCCCACCUUGUGUGCGUUGGGUCCUUUCCCCUGGCUUUCACUCUUUGAGUGUUUGCUUGCAAGAUCAGCACUUUGUUCGAUUUGUUUGUUUUGUUCAAAUUGUUGAUUUAAAUAAAG GCG
M9	GAGCACCUUGUGUGUUGGGUCUUCCCCACCUUGUGUGCGUUGGGUCCUUUCCCCUGGCUUUCACUCUUUGAGUGUUUGCUUGCAAGAUCAGCACUUUGUUCGAUUUGUUUGUUUUGUUCAAAUUGUUGAUUUAAAUAAAG GCG
XP_017987766.1	UCGUGAGUUAAAUUUGUAUUACCUUUUUCGGAAAUGCUGGCAAUAUUUCCCUUUUUUUAAUUAACAAGAUUGCCAAGGUUGCAGAAAGCCAGCCUAAAACAGGAGUCAUCAUAUCAUAUC
NP_986403.1	UCAUGAGUUAAUUUUGUUUUUACCUUUUCGGGAAAUGCUGGCAAUCUAAUUAAUCACUGAUCUGGCAUACUAAGUUAUGCAGCCAUCUCUUCUGAGAACGGUUCGUCCGUCAAAUGUAUA
KAK7493728.1	UUUUUCCUUGGCCCAAGGAUUGCCAAACACCACUCAAAUCCUCGUUCAGGGGCCUCGAGUGGCUGGGCAUAUAUGGGUUAUAGACGCUUACCGAAAU
KAK7500073.1	UUUUUCCUUGGCCCAAGGAUUGCCAAACACCACUCAAAUCCUCGUUCAGGGGCCUCGAGUGGCUGGGCAUAUAUGGGUUAGAAACGAAAGAGGAUGCGCUGUUGUUUGCUGUACAUGGUUGUCUCAGCUGAAAU
XP_003682942.1	GUGGUGGAUCUUCCAUUUCUUCUGCUUUAAGUGUACGAAAUGUUGGAUUUGCCAUGAUGGGUCCCGGCCCCUUCGUACAUUAGUACGUUGUUAGUUCUUCGUGAAUUUUACCGGGCGGGCGACGUCGUAUUAAGCGACGC CCUUCGCUGCGAUUCGGGCGGCUUCCCAACAAAGAAUAAGAAACGUUUACAAUGGGCCCCAGAUAUACCUAUAUAAAGACAAGUGACACUCUGUAGUUAGCAAUC
KAH3786489.1	AAUUAGGUCGAUCCAUCCUAAGUUCGAAAGUUGAUUGCUGGGCAAUUUUCUGCCGAAUGAUAUGGGUUAUGACACUACGUUAGUGAUUGUGUGCC

Supplementary Table 8. Amino acid sequence of La tested in this study

Gene name	Gene sequence (amino acid sequences)
Arabidopsis thaliana	MSSLPLRSGEKMESPSISDAVPLHAPEDATADFSQPQSPLHEVDSFPVTESSDDVVVNVSEIPNLSPSDDDFDHERNSGEDRDQDHGENLAETDVVVVVPIDELKKKIIRQVEYYFSDENLPTDKFLLNAMKR NKKGFVPISTIATFHKMKKLTRDHALIVSALKESSLLVVSADEKKVKRLSPLPEIKDPKIFTVLVENLPEDHSDENIREIFGKAGSIKSVSICDPNATEESEKGGKKEHFIRTRLHAFVEYETVEAAEKAVAT LNNEQDWRNGLRVKLLEQAAGKFAQRRPARKEVDKEKDTTGRVHDQTGGDKNKKTREHQNHRLHHSDNPADDDGGNKNGNKGRGGGQGRRQNHQGGNGIGHGTASSSSHHNYHPVEVSKRPPGPRMPDGTRGF TMGRGKVIPPPTSTQTSHEV
Caenorhabditis elegans	MGETAAAVHDDADQKIIKQLEYYFGNINLPRDKFLQEKLKEDDGWVPITTMLNFNRLASISKDTEKIANAVKNSGSGIISVSEDNQKIRRNEENPVPENSLEYWQKIKHRTVYMKGFSTDTQLDDIIQWANQF GETENVLMRRLKPGDRTFKGSVFITYKTREEAEAAQKAEVKFGETELTKMMQDEYWTLKNKETKEARAANKAAKSAKNTSEAIESEKAQHAVRYEKGLILAVDGLGPDSTIDSIKTFFKLHGSVGYVAHENGG KTAEIRFNNDSEGAQKAWDNAVAAGTDGKVILQEAELSARVLDGEEEEKYWTEFNARKNQRQGGGRGGRGGRGGGRGGRGGRGGRGGRGGRGDRRGDKRSANDEGADGSDEPKAKRTVFAEDGAPAAAAAE
Drosophila melanogaster	MAEVAETPSVEAQEEVAQPAEAQVLEAKNGDAKKDPAPAAEEAAGGFTKQERAIIRQVEYYFGDANLNRDKFLREQIGKNEDGWVPLSVLVTFKRLASLSTDLSEIVAALNKSEEGLVEISEDKLSLRRHPER PIPEHNEERRKEIQERTAYAKGFPLDSQISELLDFTANYDKVVNLTMRNSYDKPTKSYKFKGSIFLTFETKDQAKAFLEQEKIVYKERELLRKWQVDYLKEKQEEYAQKNEKRKNKKEAKPEPAFELPKNAIV VFEGAPETSSREEIRERFEKIKDFEVAYIEFAKGETKGSVRLTEADAAEKYIAKVEEGKLKFNDEVSLSLRKATEEEEKEFIDKAIEFMKKRRDFTRNKGKRFNRKRHGGNDHKHGGGKKARGD
Mus musculus	MAENGDNEKMTALEAKICHQIEYYFGDFNLPRDKFLKEQIKLDEGWVPLETMIKFNRLNRLTTDFNVIVQALSKSKAKLMEVSADKTKIRRSPSRPLPEVTDEYKNDVKNRSVYIKGFPTDATLDDIKEWLDD KGQILNIQMRRTLHKTFKGSIFAVFDSIQSAKKFVEIPGQKYKDTNLLILFKEDYFAKKNEERKQSKVEAKLKAKQEHEGRHKPGSTETRALEGKMGCLLKFSGDLDDQTCREDLHFLFSNHGEIKWVDFARG AKEGIILFKEKAKEALEKARNANNGNLLLRNKKVTWKVLEGHAEKEALKKITDDQQESLNKWKSKGGHAGGRFKGSHVFTAARRFKGKGKGNRPGYAGAPKGRGQFHGRRTRFDDDDRRRGPMKRGRDGRDRE EPASKHKKRENGARDK
Oryza sativa Japonica Group	MDLQDPAADAVAAEPLTVAETDDQPPPPAPPLEAEGVVVAEEDPLPHPPLEVVEEDVAPVVAEAGAAAVPMEPSEAGAGGVVLTDELCDRIVKQVEYYFSDENLPTDEFLIKFVKKNKQGFVPIGVIASFRRM KKLSQDLSIIEAALRTSSKLVVSPDGKRVRRLHPLPNNELNDVKKRIVVVENLPSDFSMESIHAKFETVGKIAKITIHDQHSVGESATIKNHDIMLSNKVHALVEYETAEAAEKAAITLNDGSNWRTGMKVRL AKRTVGSGKHNQSSKENQAIQGQPSKVEQQMVSEKNGGTDSVESAFDNENVNSNISHEDMHQHQKVNAKGGRKGRYKSQGRGQIQQNTSGQGHVSSPATSGSDHVNKPIPGPRMPDGTRGFTMGRGKSPTFQK GDNAEE
Nicotiana tabacum	MEADGEGVIPVPSHSHSVSSPPHDDDHPDFPPVGSPESHFIDDLSQPSDQHDAQVTPTLLTDDLRNKIVKQVEYYFSDENLPTDKFLLKYVMRDREGFVPVKVIASFRKVKKHTKDTAIIAAALRESSLLVVS SDGTKVKRLHPLPLSEIIDPKVCTVLVENLPEDHSVDNLRRIFGHAGNVKHITIRDPHTERDSRKCTTAEKLLSSKLHALVEYNTVEAAEKAVAILNDEQDWRFGLRVKLLKKTNKPGQNNKGWRDPDSERNK NVQASDQAVNVKNHSSEHRVLQNKPGQSKKGGSYPDSDRNTNIQASDLAVNEEHHSNEHHIDSQDEEEGDHRSKETIGEHAQKEKNEHRVQTRNRSRGRRNKRGTNGHGHGTTSYNHAVEPSKPPPGPRMPDG TRGFAMGRGRPLSSSPS
Fungi	MADEQKPVTAQATNAEAEKDVQNVLAELKGEAEVPVNGTEKEEAKEASADKSEEAEEARIVAAAAKLGEKSEKSENGNQAEDRGRANRRRNESKFDPSTQKETDDPVEIRKQVEFYFSDSNLPMDKFLLSKVG GSKNNPVPLELLHSFKRMRRFQPFSAIVEALKNSETLELTDNDTAVRRKVPLPETVKDTHDPTVVKVFEDKAMARSIYAKGFGEETPTTQLDIEAFFAPYGPINAIRLRRTNEKYFKGSVFVEFASEEKQKAF LELDPMPKWKDQELIIKSKKDYCDEKVREIEAGRVKPSGGRGRGGARGRGGRGGRGGRGGRRDNNRDWRERRDADQKQGFKNGQDEQPERREVQKDARYSGVPVVQSTADAGQKRTREDEASAEHPAKKVDTK E
Human	MAENGDNEKMAALEAKICHQIEYYFGDFNLPRDKFLKEQIKLDEGWVPLEIMIKFNRLNRLTTDFNVIVEALSKSKAELMEISEDKTKIRRSPSKPLPEVTDEYKNDVKNRSVYIKGFPTDATLDDIKEWLED KGQVLNIQMRRTLHKAFKGSIFVVFDSIESAKKFVETPGQKYKETDLLILFKDDYFAKKNE

Supplementary Table 9. AAV plasmid sequence

AAV 1.0 AAV-pTTR-yLa-FanzMAX v1-pcsk9-site1	https://benchling.com/s/seq-eyeRadLYCMX24AVbcJoI?m=slm-XimWID3RKop5c74yEYRw
AAV 2.0 AAV-pTTR-FanzMAX v2-hLa-WPRE-bgH polyA (2 sites)	https://benchling.com/s/seq-Cajso9rc8e51yGIOd12L?m=slm-7yppx6SrlusY0Z1TxARe
AAV 3.0 AAV-pTBG-FanzMAX v3-hLa-WPRE-bgH polyA	https://benchling.com/s/seq-KD6myaJbWkqsBQedTxUG?m=slm-fXPu4i5tGyM3l5TkPQV9

Supplementary Table 10. Construct key with protein, ωRNA, and fusion versions

Variant	Protein	ωRNA scaffold	La fusion
WT-NaloFz2 (+ native ωRNA)	WT NaloFz2	Native ωRNA	None
WT-NaloFz2 (+ enωRNA v2)	WT NaloFz2	enωRNA v2	None
FanzMAX v1	WT NaloFz2 + E205R + N201K + C332K	enωRNA v2	None
FanzMAX v2	FanzMAX v1 + T457Q	enωRNA v2	None
FanzMAX v3	FanzMAX v2 + R221S + Q395S	enωRNA v2	None
FanzMAX v3-hLa	FanzMAX v3	enωRNA v2	C-terminal hLa
AAV 1.0	FanzMAX v1	enωRNA v2 (single-guide)	N-terminal yLa
AAV 2.0	FanzMAX v2	enωRNA v2 (dual-guide)	C-terminal hLa
AAV 3.0	FanzMAX v3	enωRNA v2 (single-guide)	C-terminal hLa
NlovFz2max	WT NlovFz2 + C326K + E199R + H229Y + K390N	enωRNA v2	None
yLa-NlovFz2max	NlovFz2max	enωRNA v2	N-terminal yLa