

Supplementary Figures

Supplementary Figure 1. Structural identity for each mutant obtained from randomly mutating a nucleotide in one of the other three.

Supplementary Figure 2. Difference in free energy between the WT and the MT with structural identity = 100%.

Supplementary Figure 3. Structural identity distributions for the viral (A) and non-viral (B) IRES sequences.

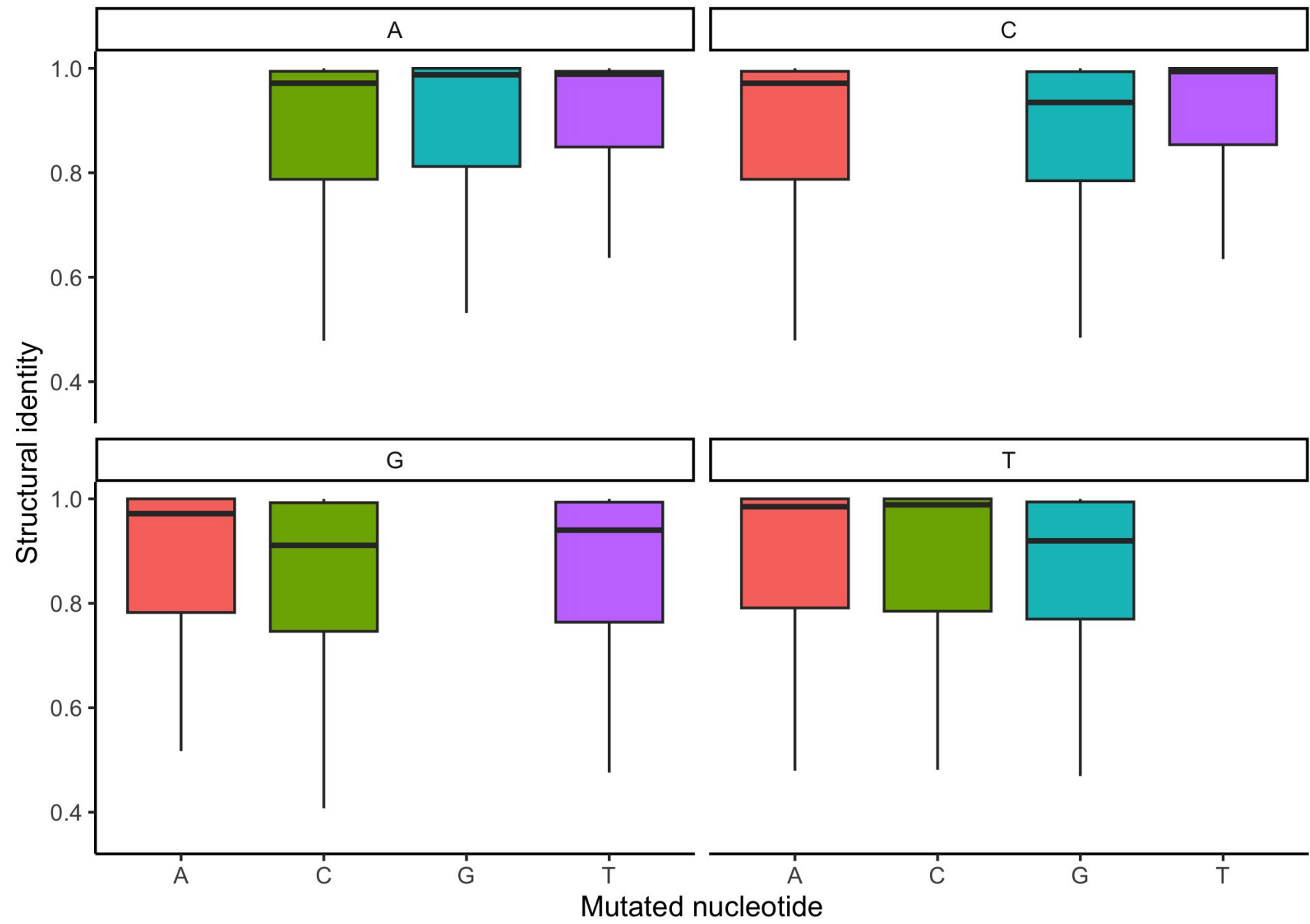
Supplementary Figure 4. Enrichment of known ITAFs in IRES interactions. A. ITAF enrichment calculated at different thresholds of RBPs ranked by *cat*RAPID score compared to 10 random interactomes. B. Distribution of *cat*RAPID binding propensity score of the 2064 RBPs binding to VEGF and IGF2 IRESs.

Supplementary Figure 5. Frequency of mutations of one nucleotide into another as beneficial mutations selected with the above criteria.

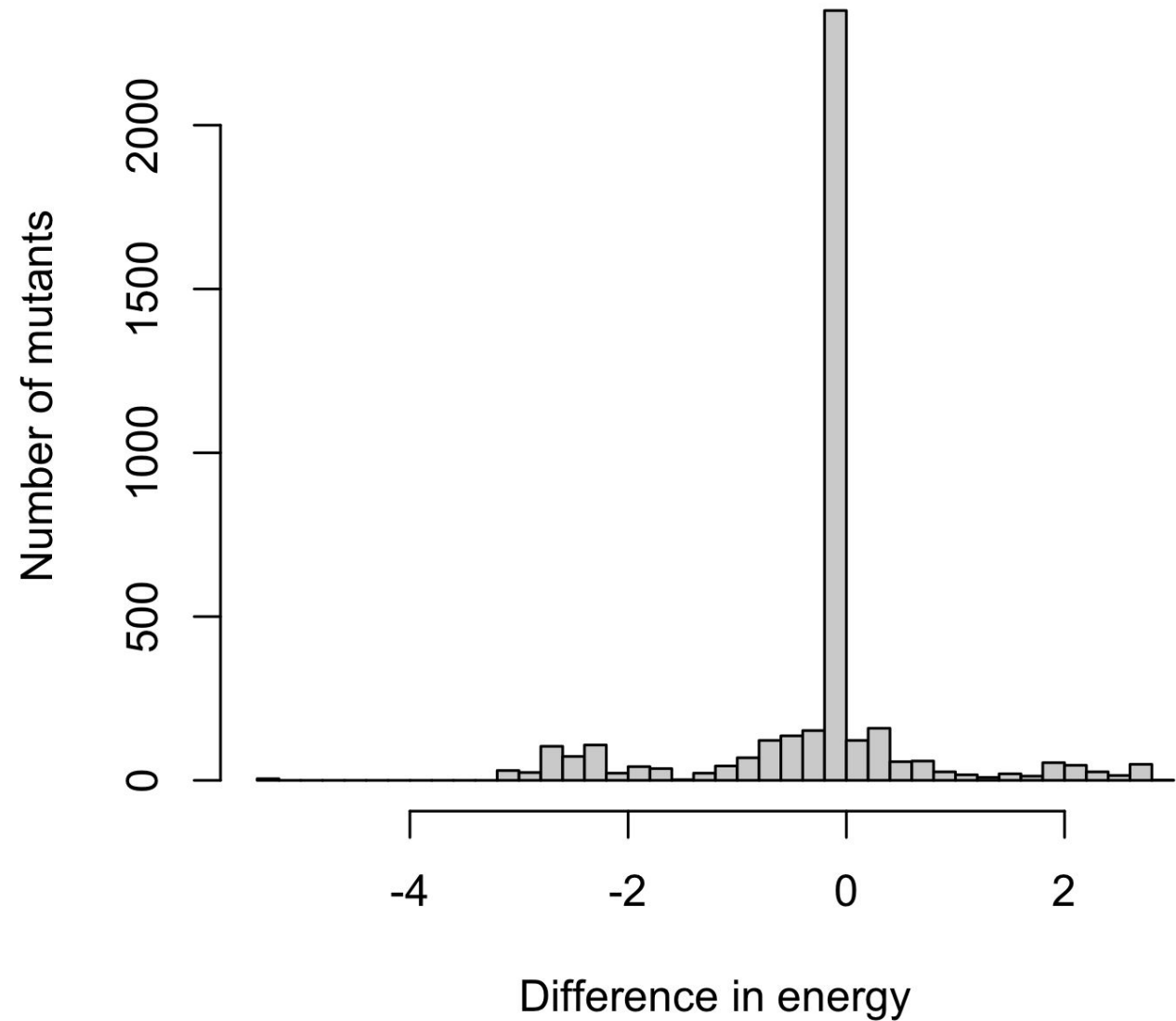
Supplementary Figure 6. Presence of beneficial mutations in the 5' (first 50 nt) and 3' (last 50 nt) of IRES sequences.

Supplementary Figure 7. GO enrichment of the 50 highest-ranked RBPs by change in binding propensity (MutA/MutD sequences vs WT).

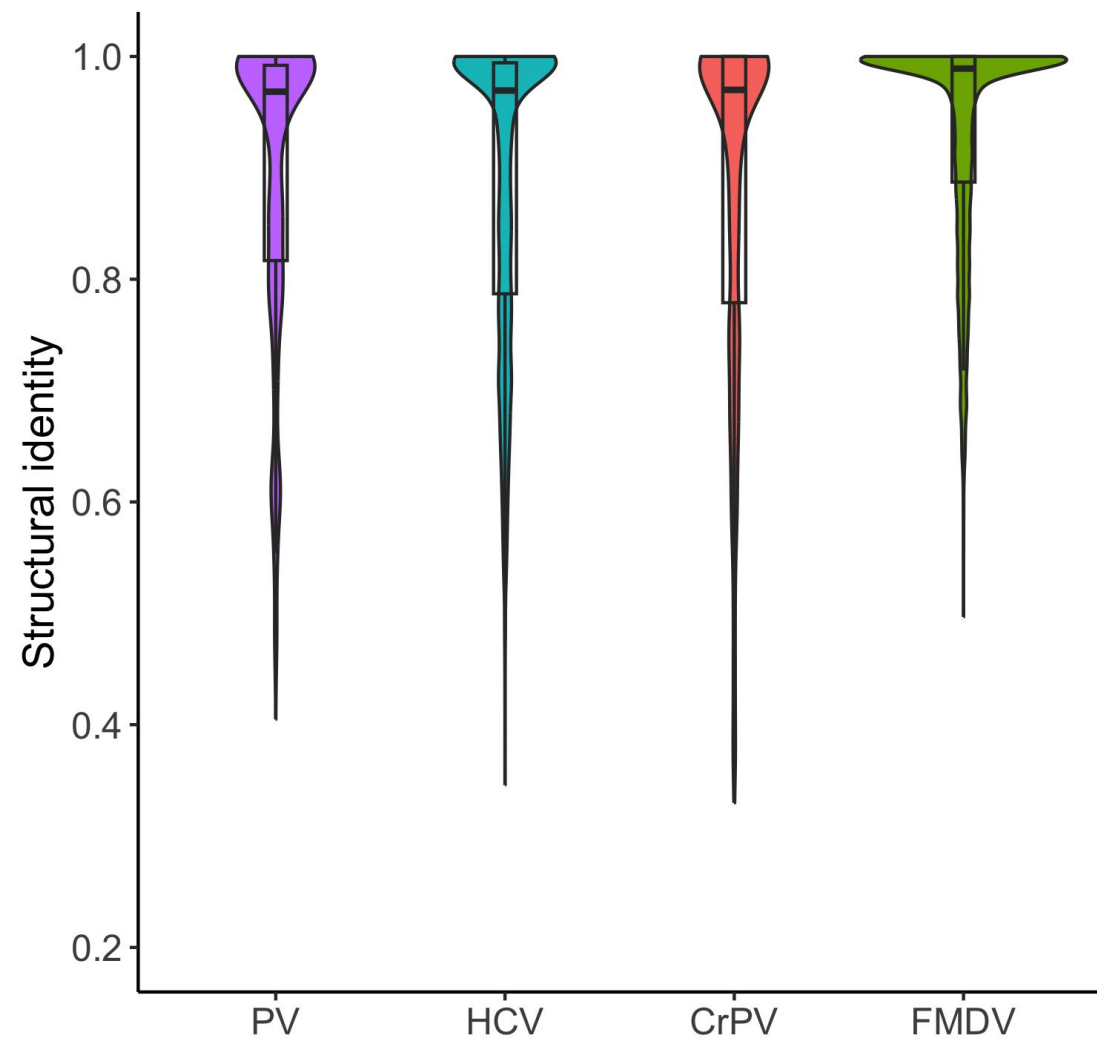
Supplementary Figure 1



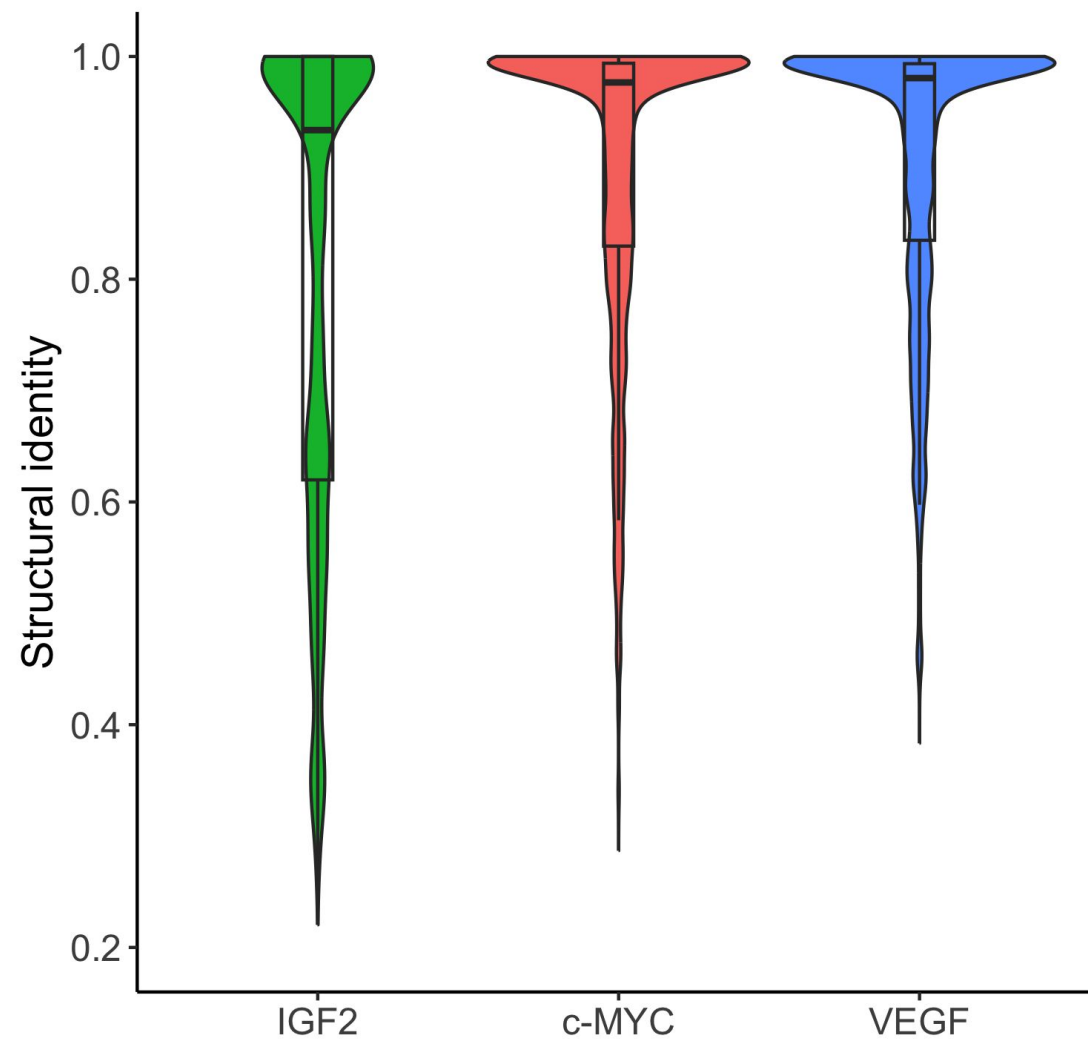
Supplementary Figure 2



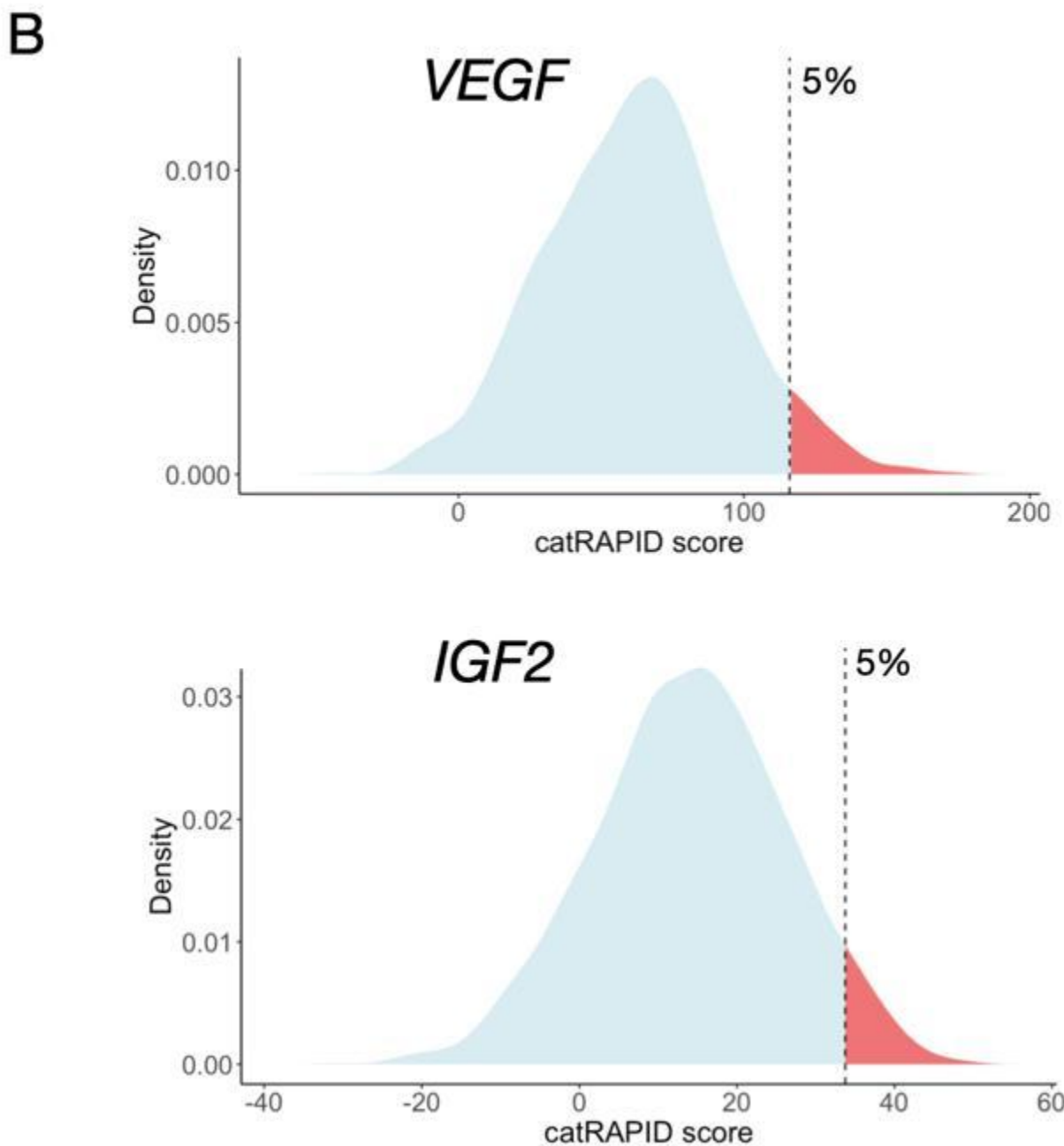
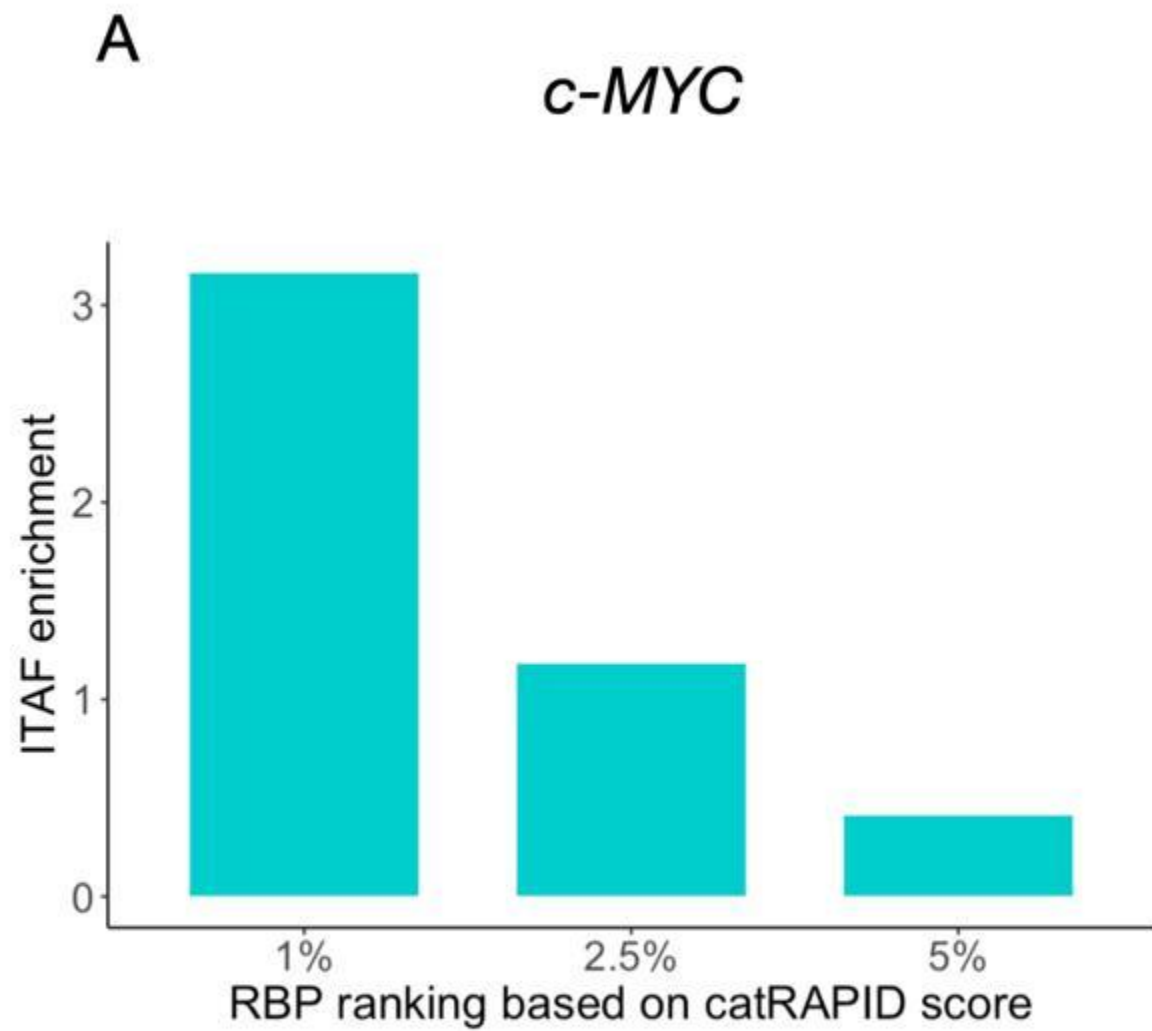
A



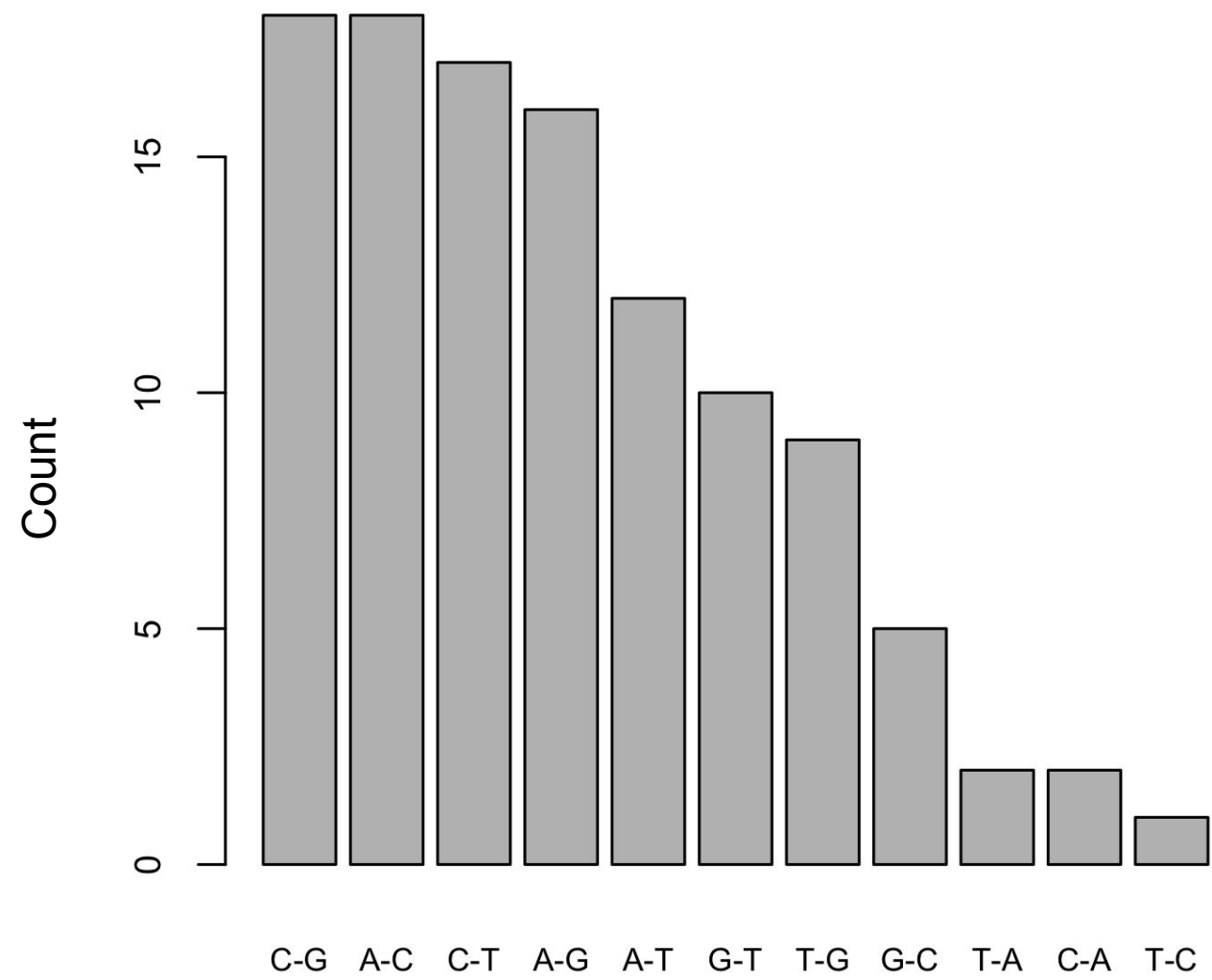
B



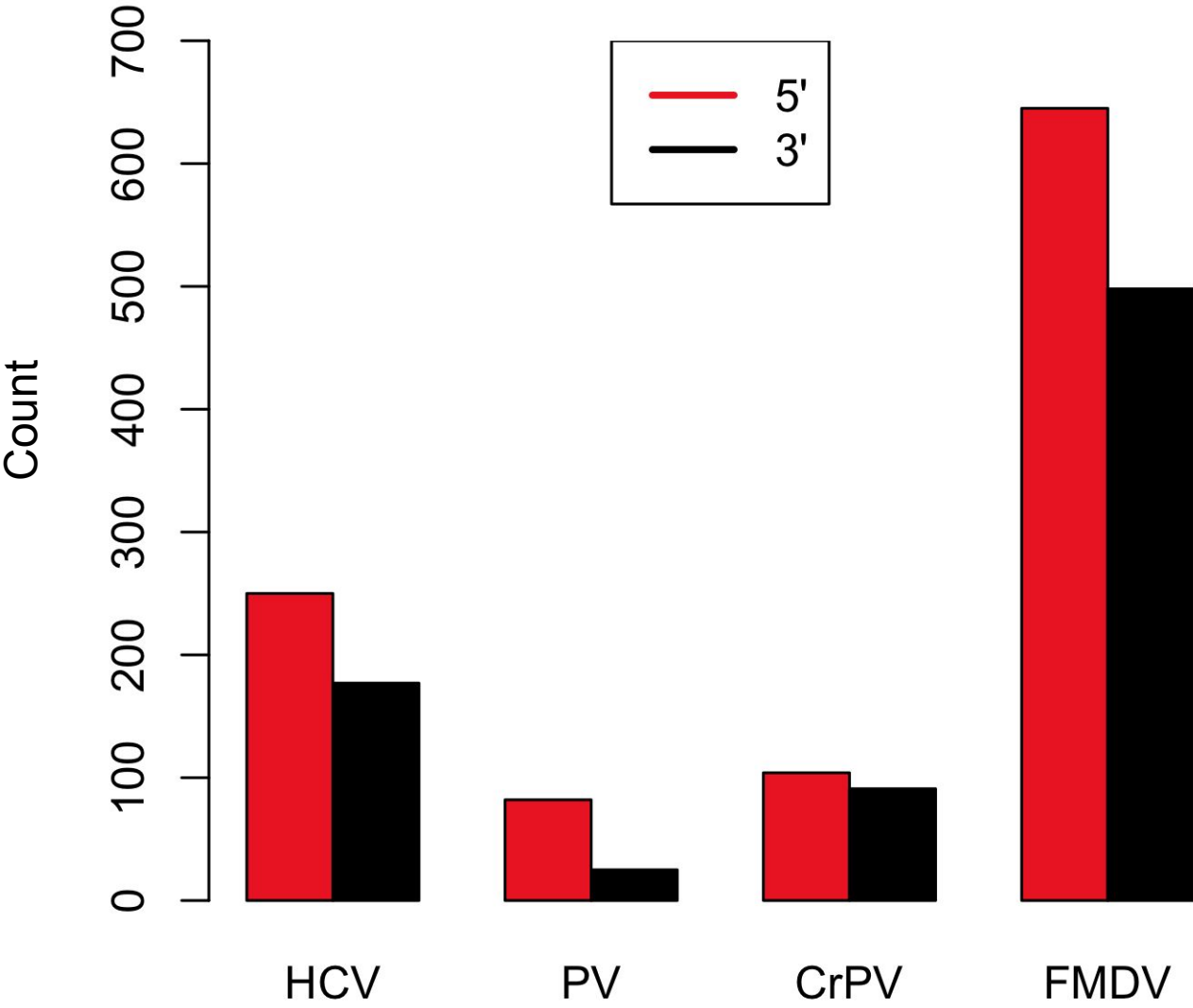
Supplementary Figure 4



Structural identity > 0.95; ΔG Mut < ΔG WT; Structural content > 2%



Supplementary Figure 6



MutD

Term Name	Padj (query_1)
RNA binding	5.984×10 ⁻¹⁷
nucleic acid binding	1.211×10 ⁻¹⁰
organic cyclic compound binding	1.572×10 ⁻⁹
glutamyl-tRNA synthase (glutamine-hydrolyzi...	8.072×10 ⁻⁸
mRNA binding	1.355×10 ⁻²
translation regulator activity	1.402×10 ⁻²
catalytic activity, acting on RNA	2.294×10 ⁻²
regulation of mRNA metabolic process	1.486×10 ⁻⁶
regulation of mRNA stability	1.596×10 ⁻⁴
regulation of RNA stability	2.524×10 ⁻⁴
regulation of mRNA catabolic process	3.026×10 ⁻⁴
mRNA metabolic process	3.525×10 ⁻⁴
translation	5.518×10 ⁻⁴
ribosome biogenesis	6.544×10 ⁻⁴
mRNA catabolic process	1.925×10 ⁻³
regulation of translation	2.518×10 ⁻³
RNA catabolic process	7.667×10 ⁻³
RNA metabolic process	1.197×10 ⁻²
nucleic acid catabolic process	1.328×10 ⁻²
gene expression	1.513×10 ⁻²
negative regulation of translation	1.557×10 ⁻²
glutamyl-tRNAIn biosynthesis via transamid...	2.421×10 ⁻²
nucleobase-containing compound metabolic pr...	2.621×10 ⁻²
post-transcriptional regulation of gene express...	2.912×10 ⁻²
organelle lumen	1.948×10 ⁻⁵
membrane-enclosed lumen	1.948×10 ⁻⁵
intracellular organelle lumen	1.948×10 ⁻⁵
nucleoplasm	1.217×10 ⁻⁴
intracellular membrane-bounded organelle	9.743×10 ⁻⁴
nuclear lumen	1.479×10 ⁻³
glutamyl-tRNA(Gln) amidotransferase complex	3.366×10 ⁻³
nucleus	4.273×10 ⁻³
intracellular organelle	6.049×10 ⁻³
ribonucleoprotein granule	7.011×10 ⁻³
membrane-bounded organelle	1.622×10 ⁻²
P-body	1.667×10 ⁻²
organelle	4.503×10 ⁻²

MutA

Term Name	padj (query_1)
RNA binding	1.488×10 ⁻¹²
nucleic acid binding	1.053×10 ⁻⁹
organic cyclic compound binding	1.422×10 ⁻⁸
mRNA binding	7.675×10 ⁻⁶
translation factor activity, RNA binding	4.810×10 ⁻⁵
translation regulator activity, nucleic acid bindi...	1.863×10 ⁻⁴
translation regulator activity	7.333×10 ⁻⁴
catalytic activity, acting on RNA	2.294×10 ⁻²
molecular condensate scaffold activity	3.559×10 ⁻²
3'-5'-RNA exonuclease activity	4.598×10 ⁻²
translation	5.715×10 ⁻⁸
mitochondrial gene expression	5.406×10 ⁻⁶
mitochondrial translation	2.831×10 ⁻⁵
biosynthetic process	8.405×10 ⁻⁴
macromolecule biosynthetic process	8.666×10 ⁻⁴
gene expression	9.890×10 ⁻⁴
ribosome biogenesis	1.138×10 ⁻²
translational initiation	1.606×10 ⁻²
regulation of translation	3.624×10 ⁻²
rRNA metabolic process	4.620×10 ⁻²
positive regulation of stress granule assembly	4.928×10 ⁻²
organelle lumen	9.303×10 ⁻⁶
intracellular organelle lumen	9.303×10 ⁻⁶
membrane-enclosed lumen	9.303×10 ⁻⁶
intracellular anatomical structure	8.019×10 ⁻³
cytoplasmic stress granule	1.201×10 ⁻²
mitochondrial matrix	2.291×10 ⁻²

Supplementary Figure 7