LineUp

Identifying Deleterious Mutations Using Protein Domain Alignment

Brady Bernard

Shmulevich Group

The Cancer Genome Atlas

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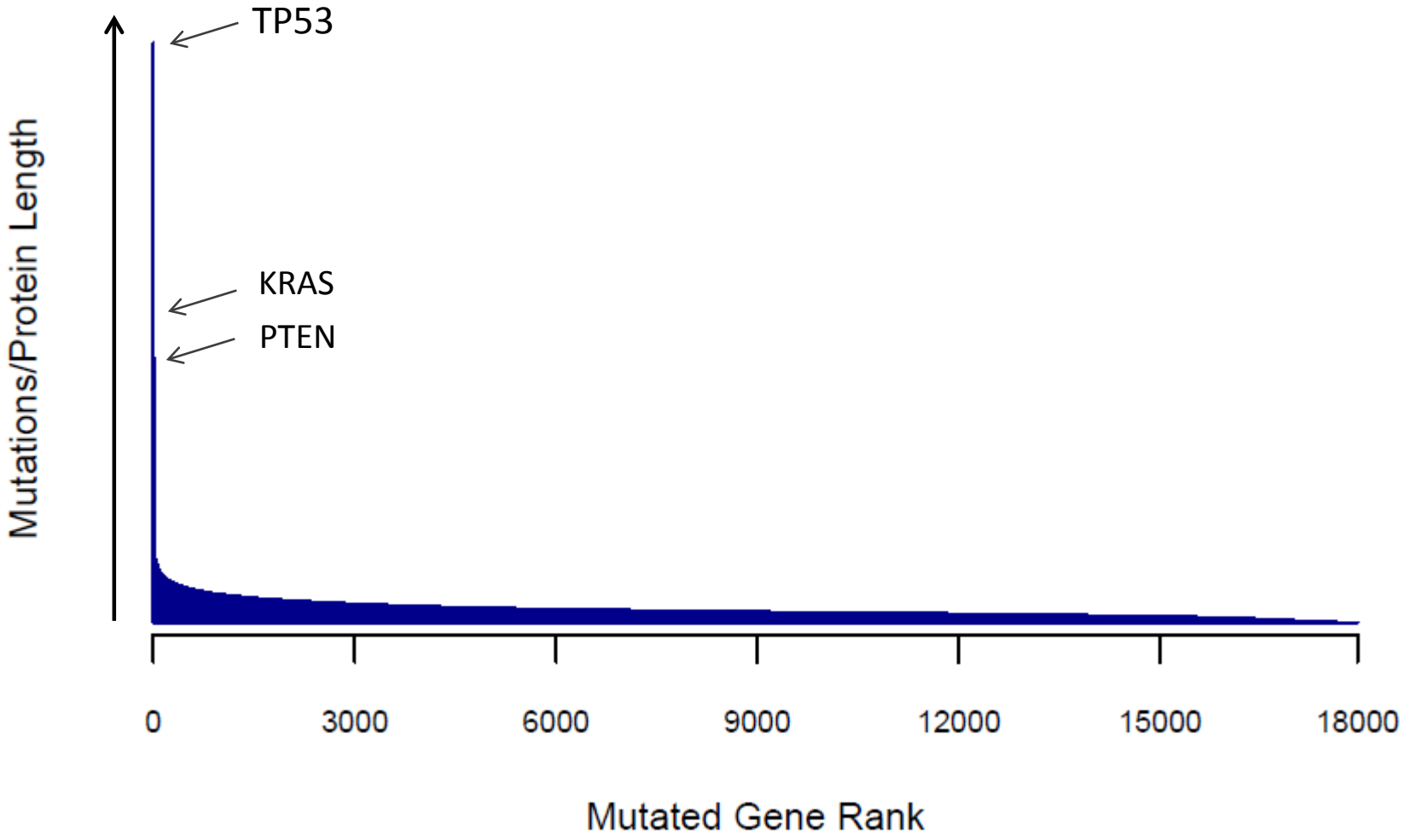
Normalized mutation frequencies

Twenty tumor types



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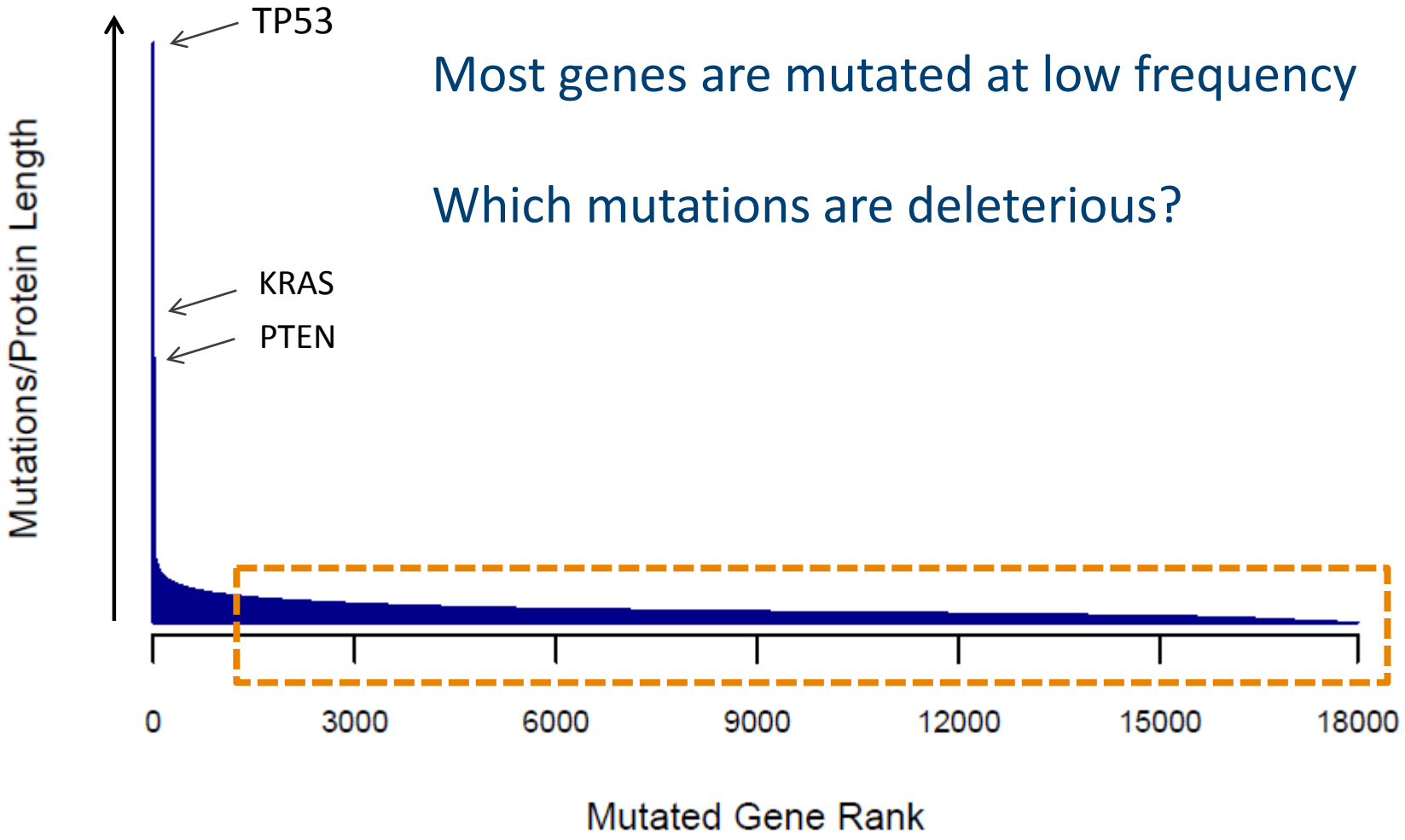
Normalized mutation frequencies
Twenty tumor types



Most genes are mutated at low frequency

Which mutations are deleterious?

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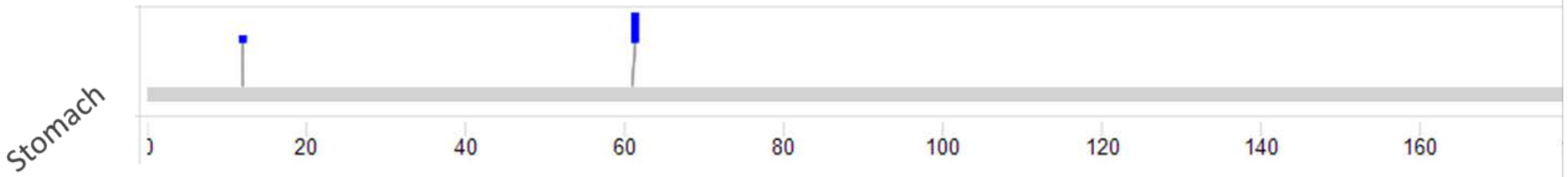
Example protein mutation spectrum



$\log_2$ scaled counts per position

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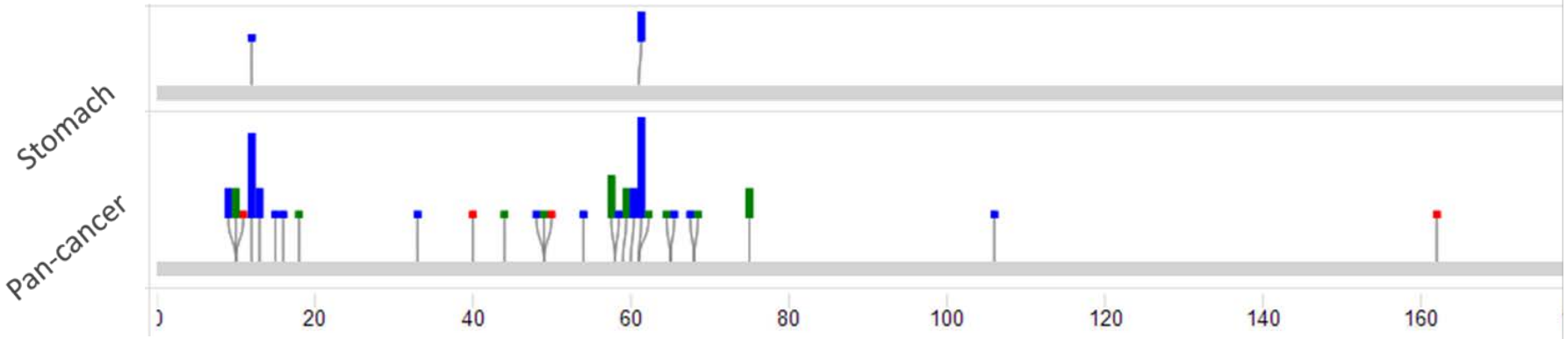
Pan-cancer mutation spectrum



$\log_2$ scaled counts per position

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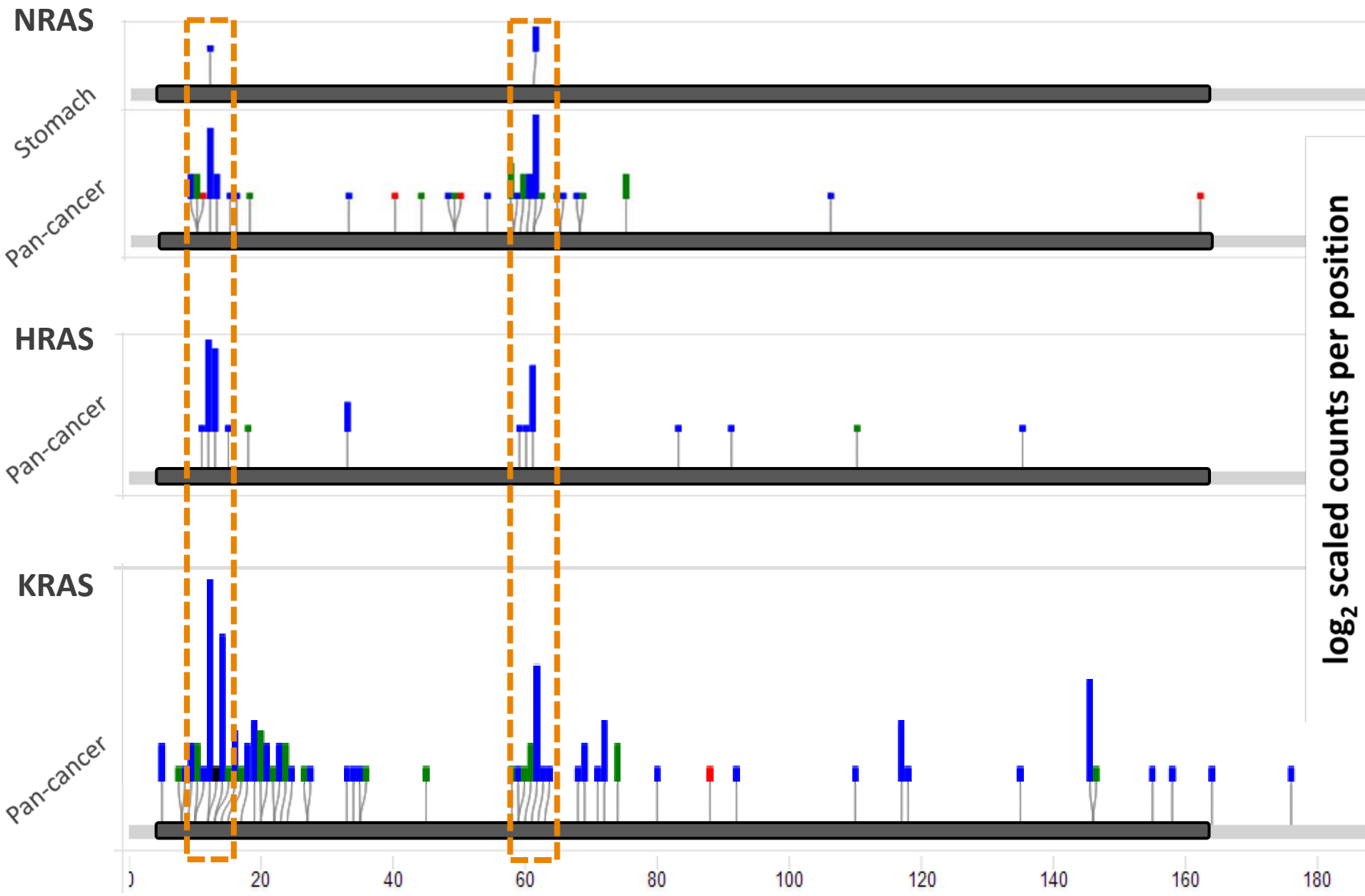
Pan-cancer Ras domain mutation spectra



Ras domain structure alignment

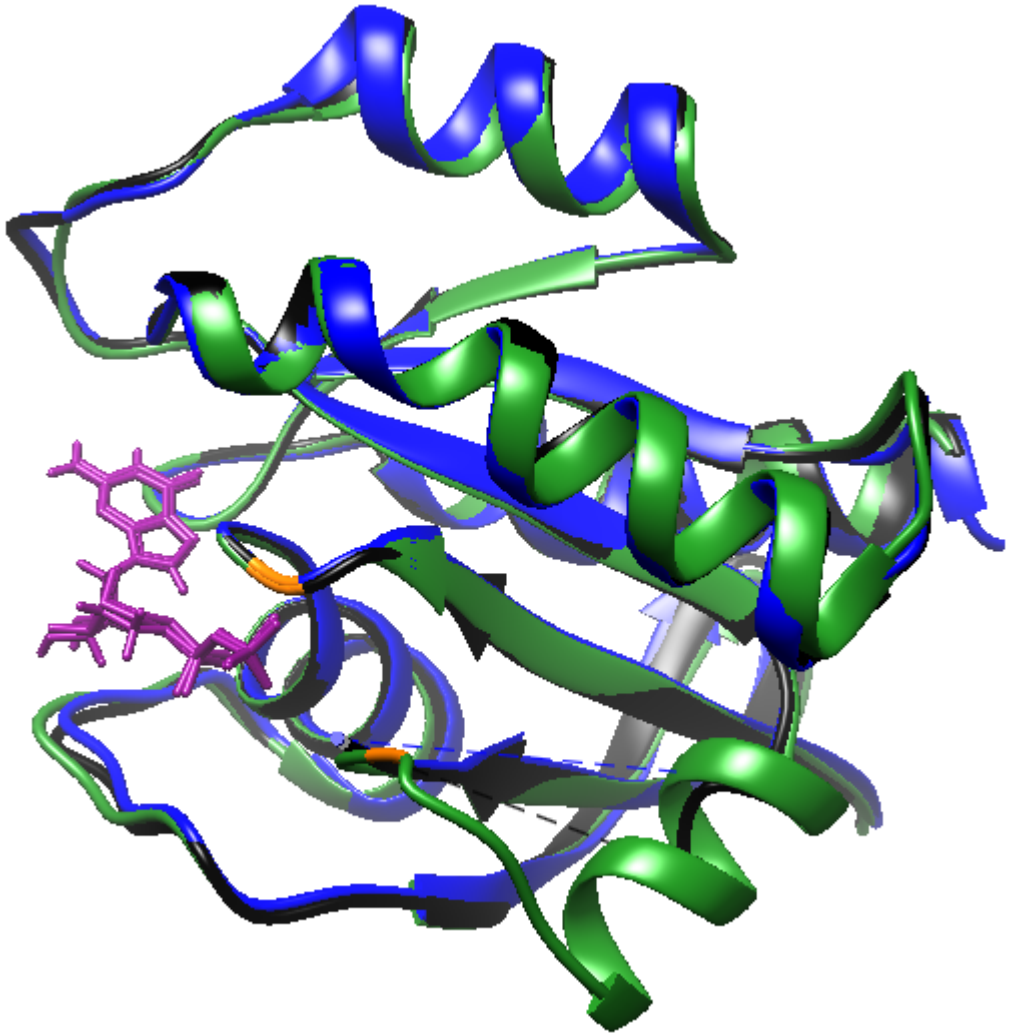
KRAS

NRAS

HRAS

GDP

Hotspots



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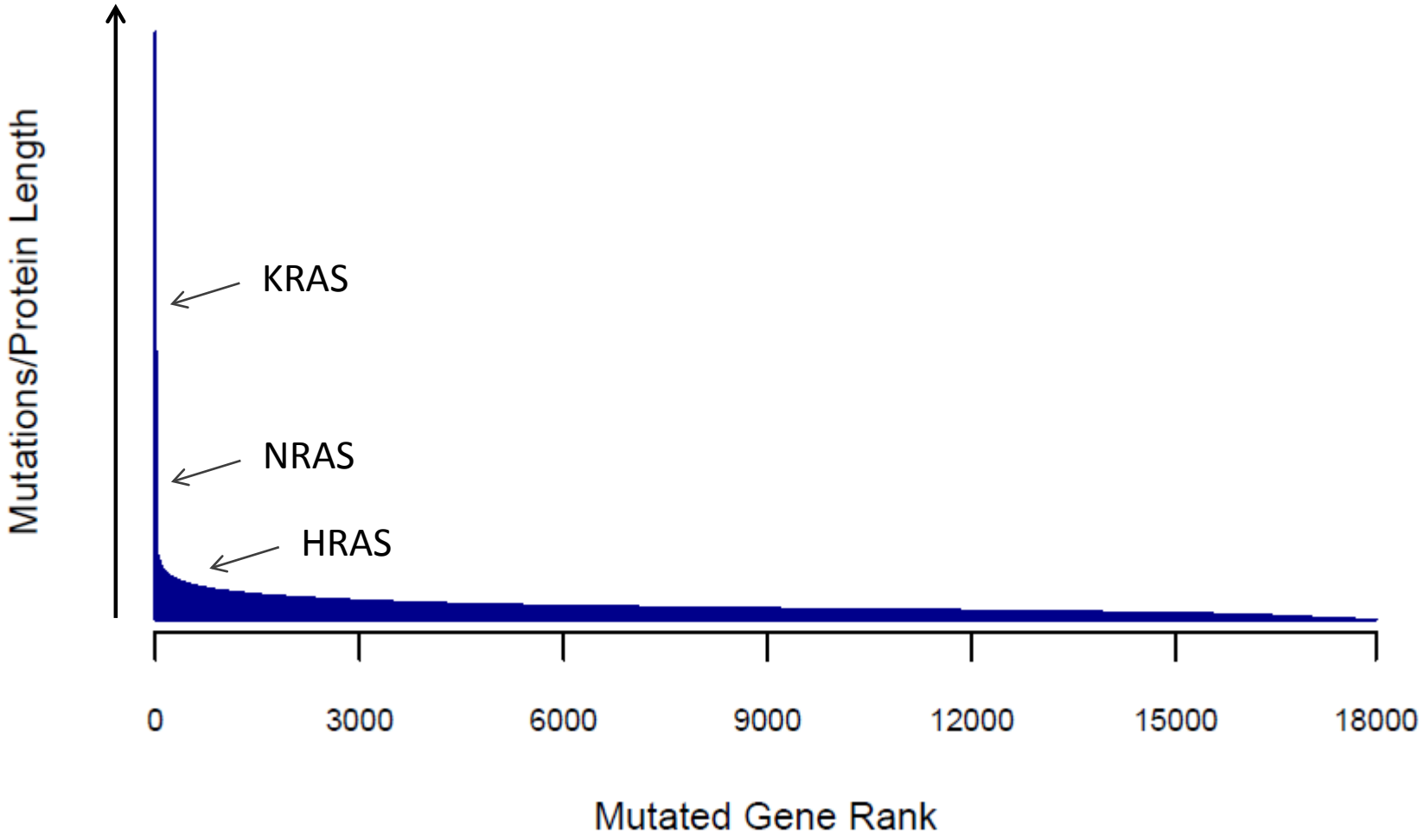

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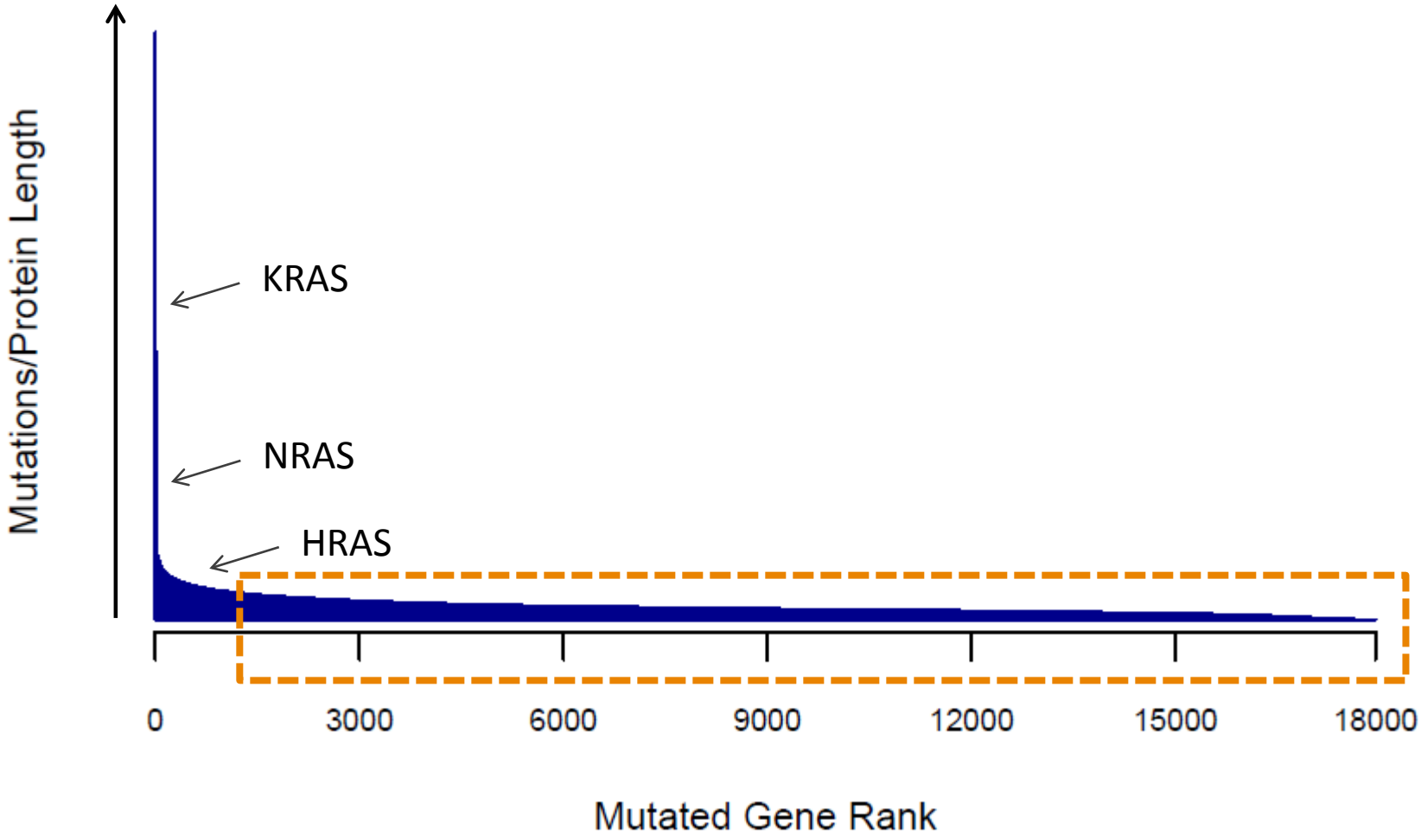

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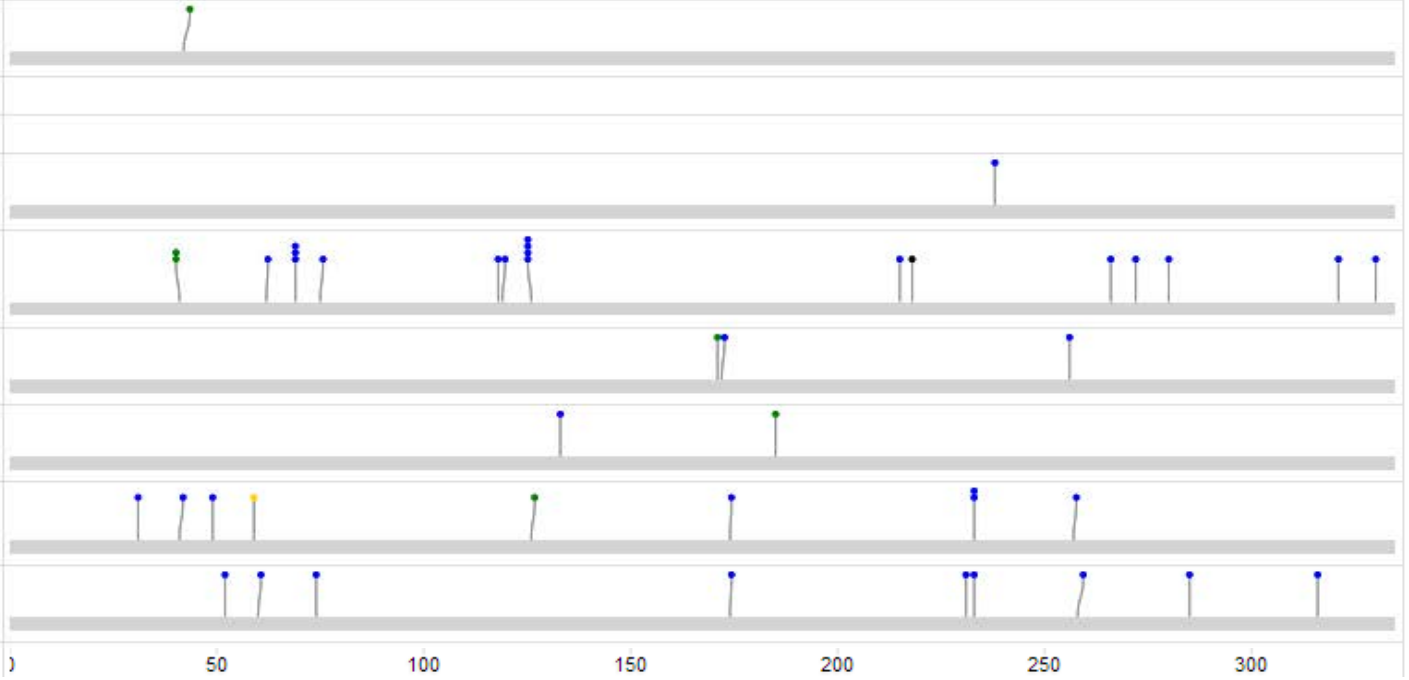
Typical pan-cancer mutation spectrum

Tumor Type	MutSig Rank	Samples (#)
BLCA	9638	1
BRCA	952	0
GBM	14085	0
KIRC	2051	1
LUAD	925	14
LUSC	9703	2
OV	3421	2
STAD	1118	8
UCEC	2211	7



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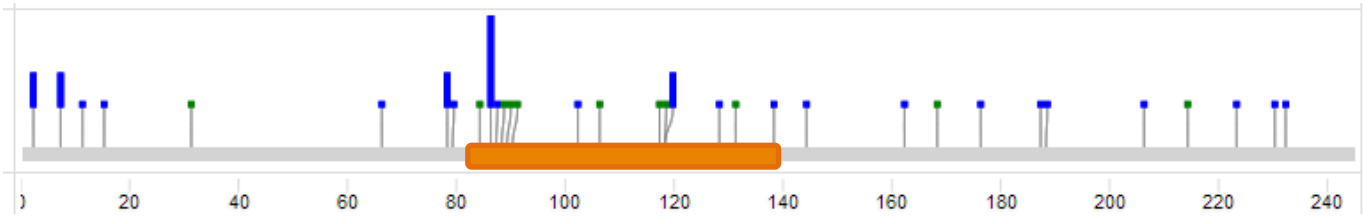
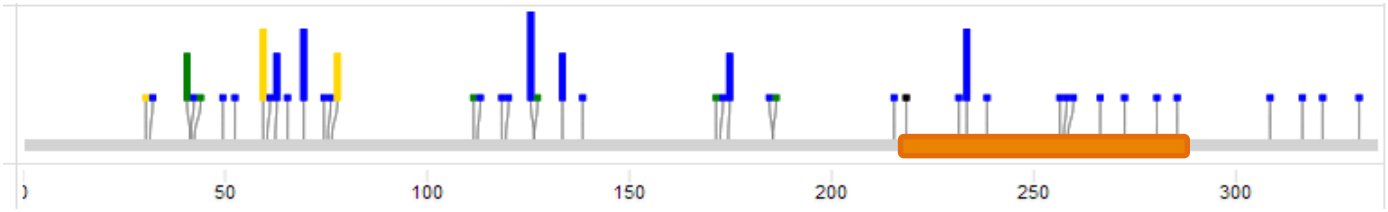
LineUp Approach

Gene A
Pan-cancer





Gene B
Pan-cancer

- Align sequences from all matching domains across all tumor types
- Evaluate missense mutation frequencies
- Applies to ~40% of missense mutations

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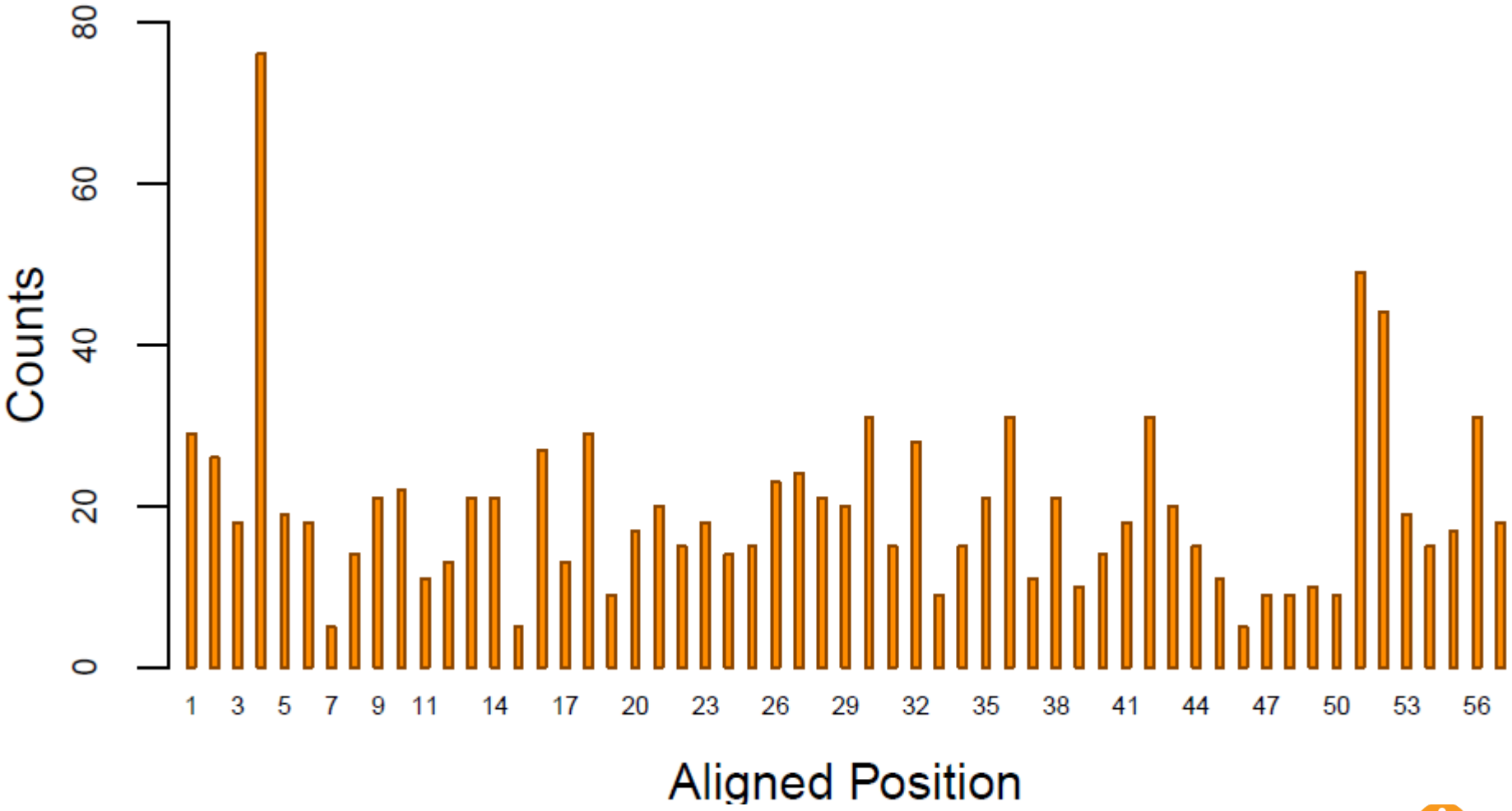
Mutations per position for two hundred aligned Homeobox domains across twenty tumor types



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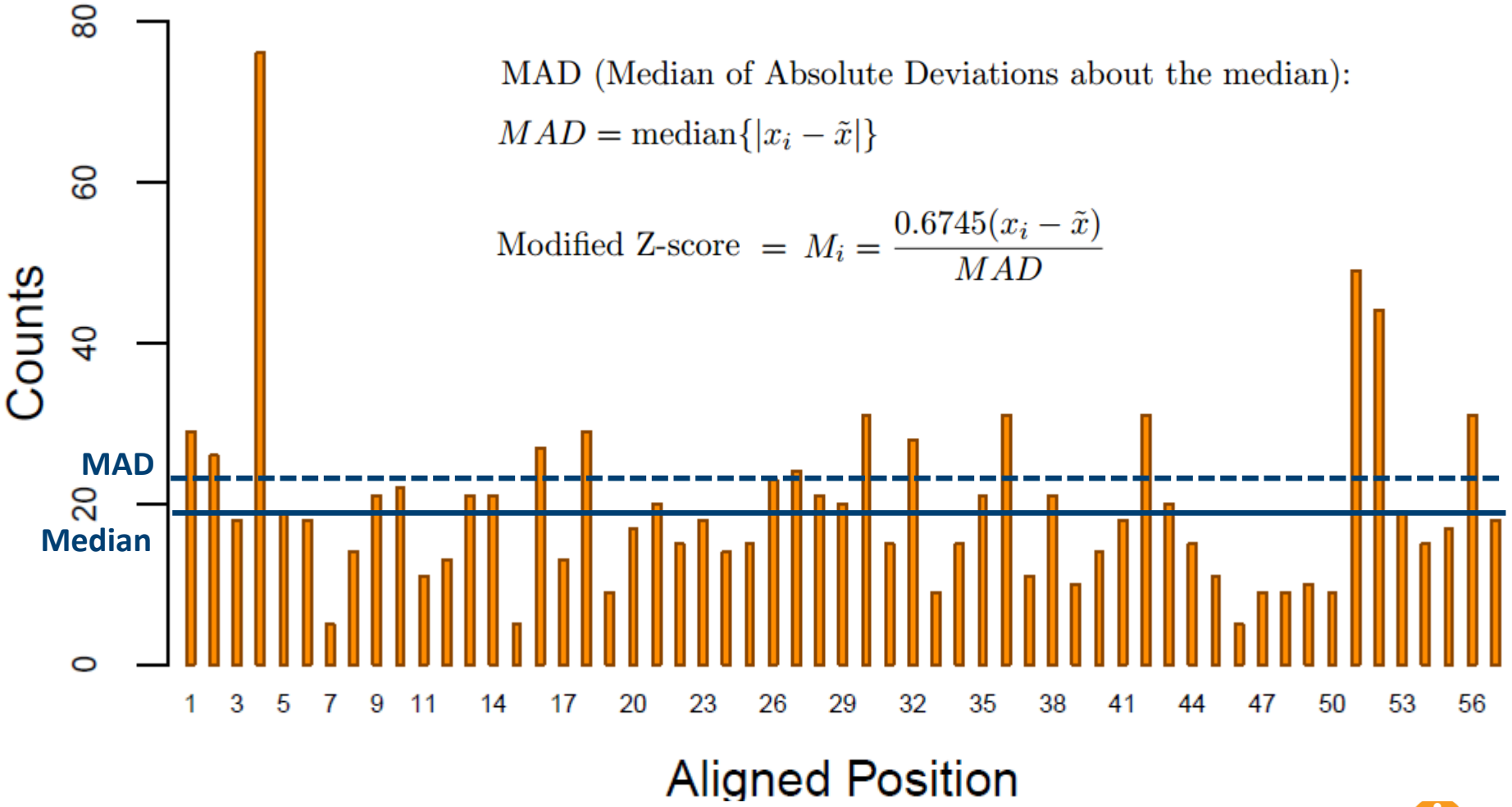
Identifying outliers: Modified Z-score



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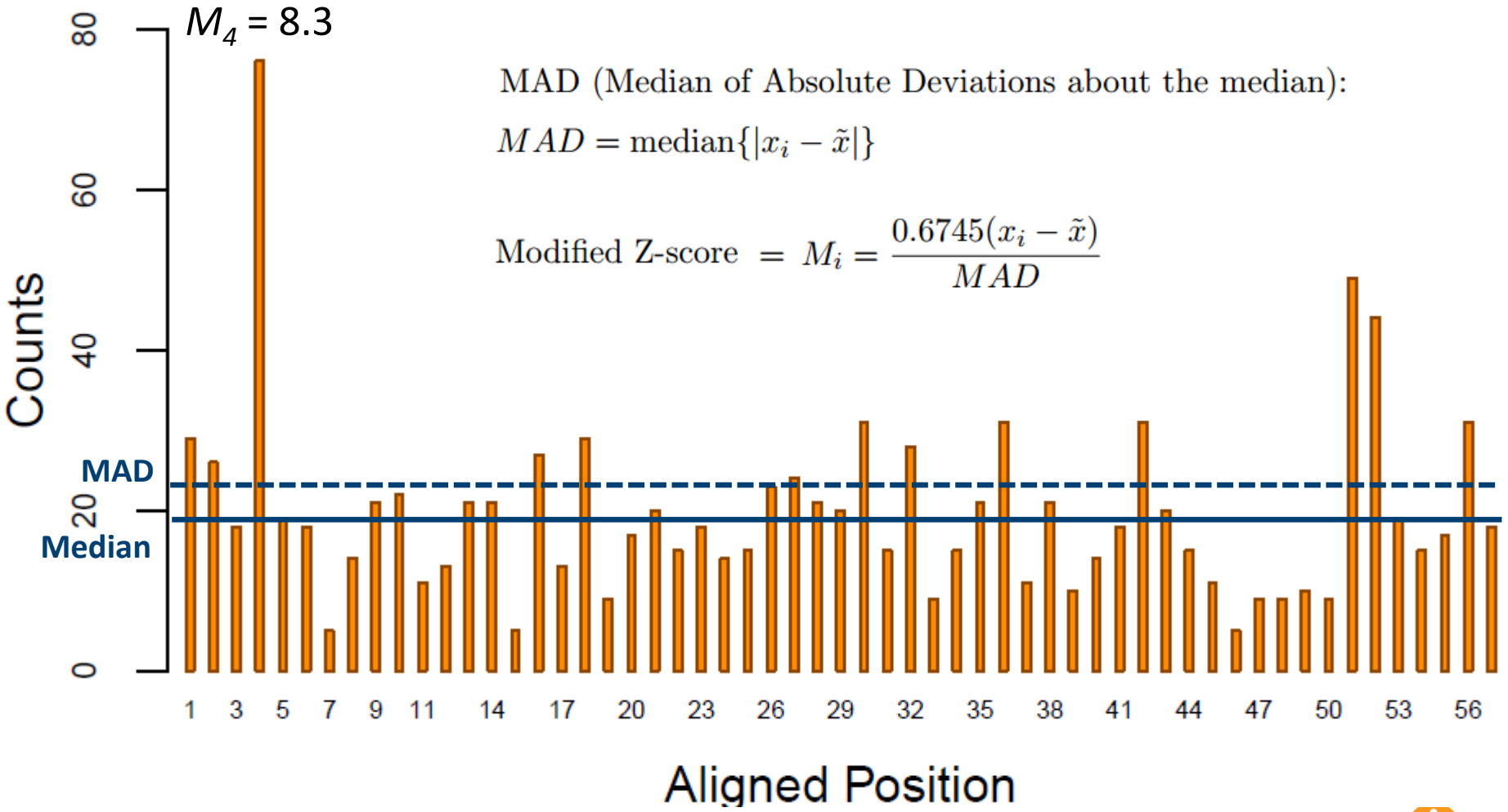

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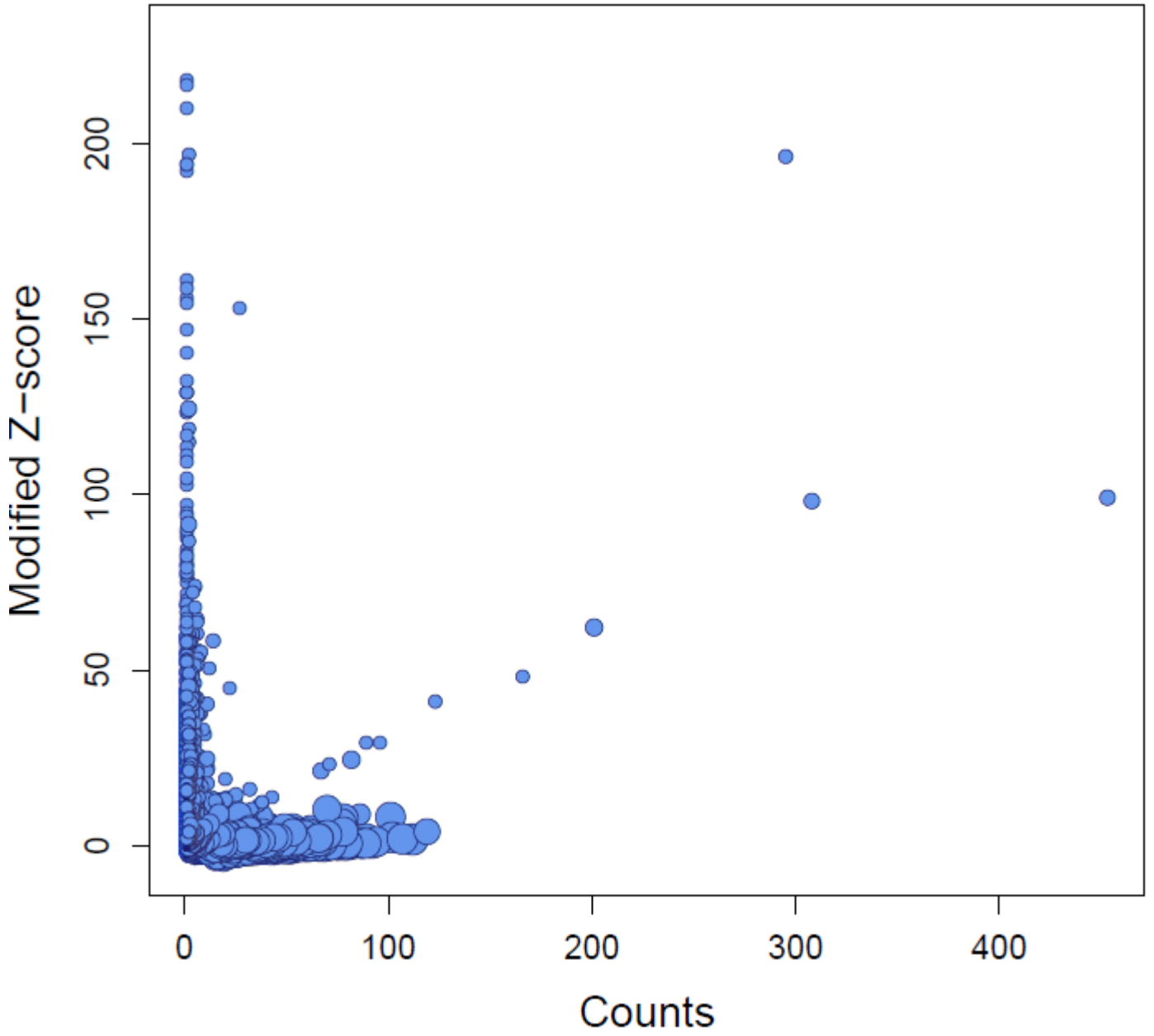
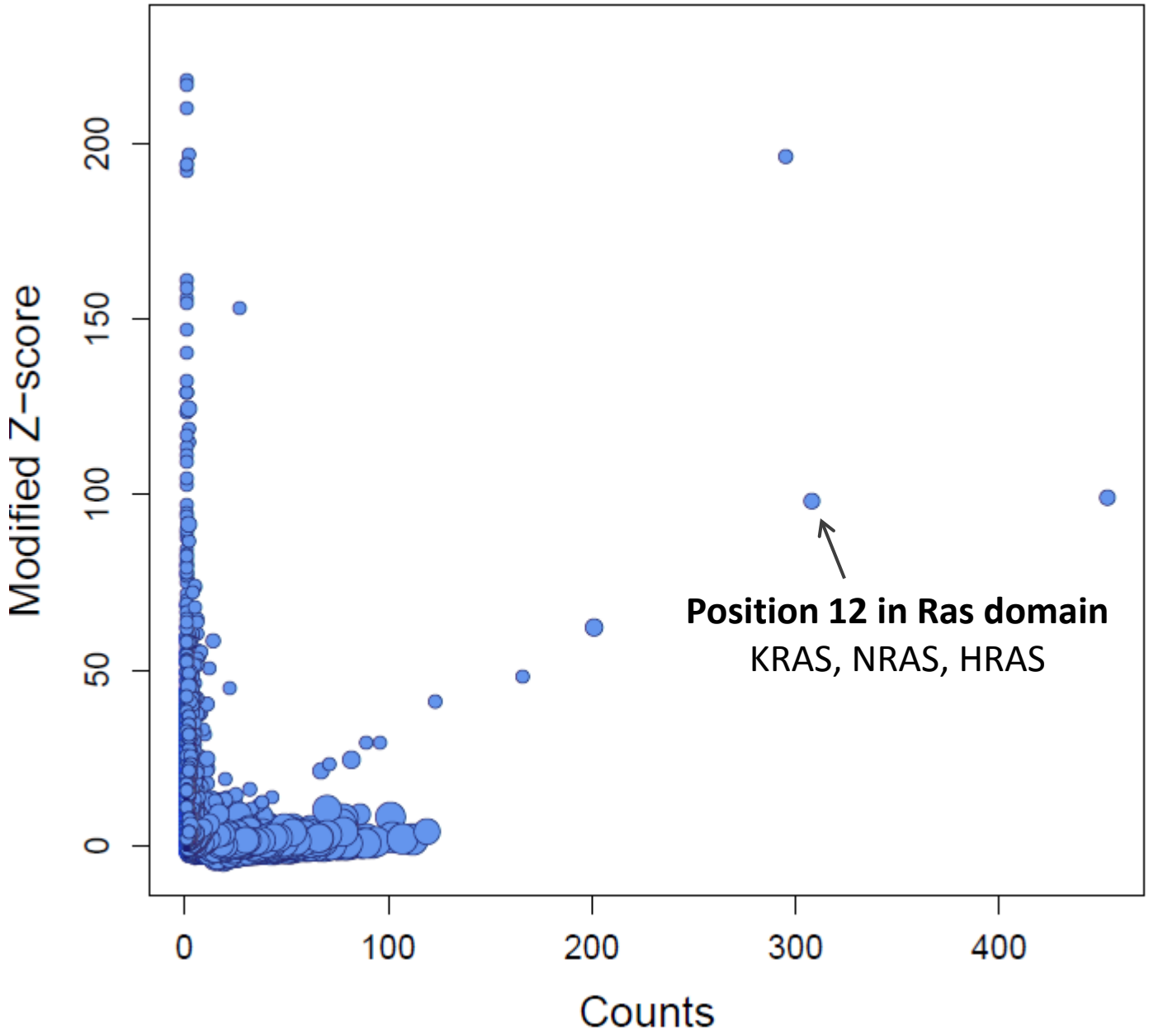
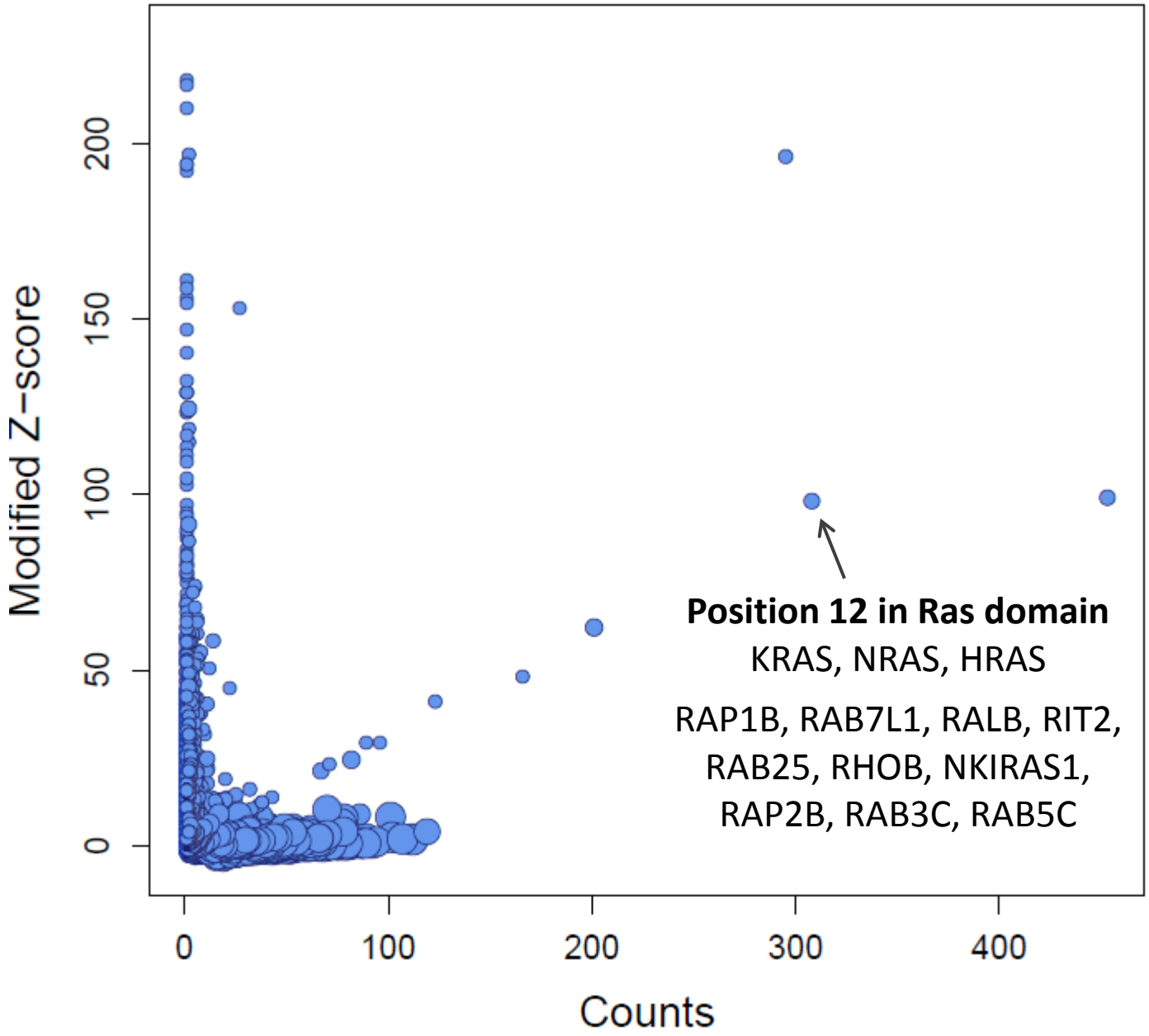
All positions within all domains



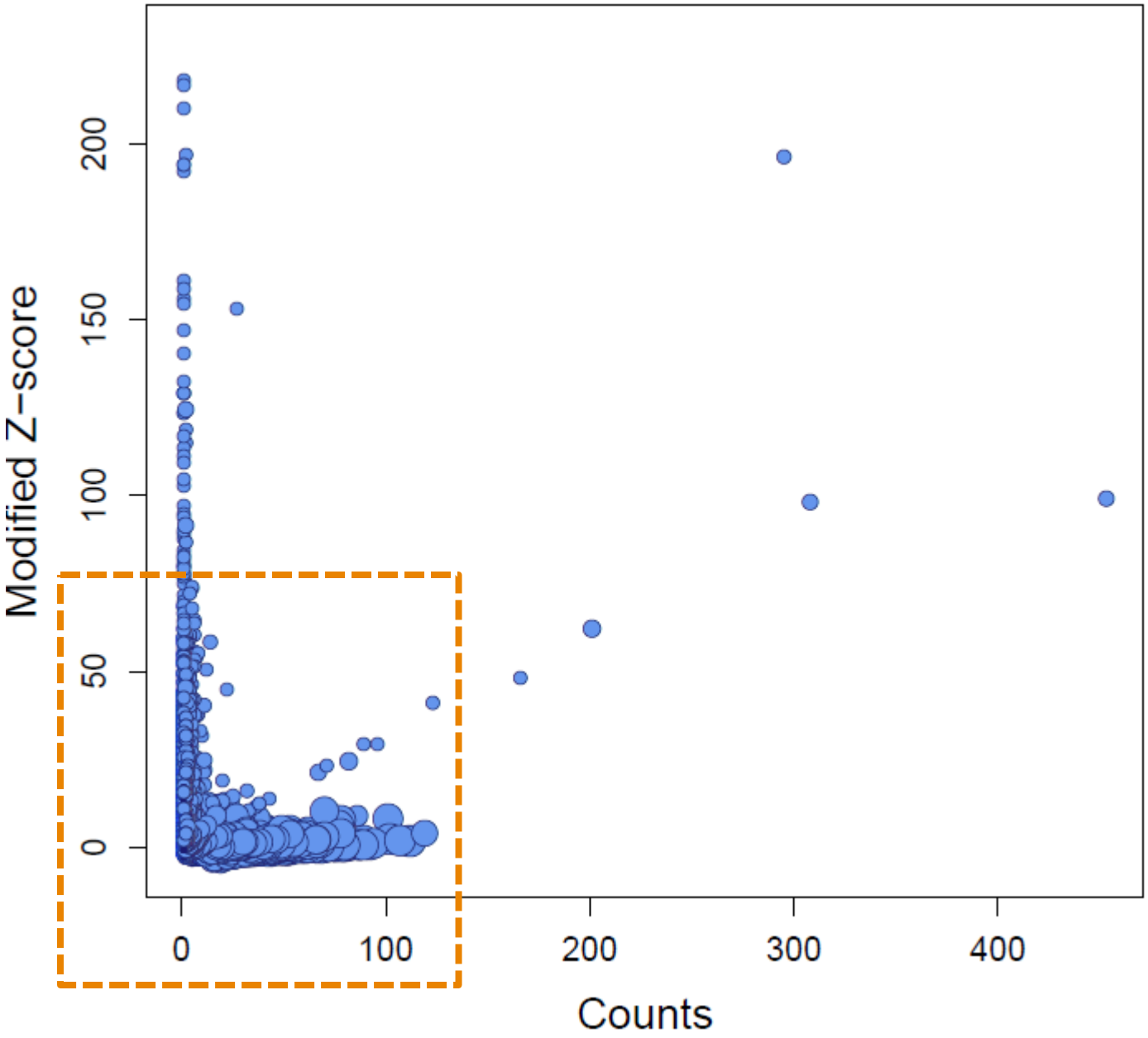
High Modified Z-score and counts for Ras domain



Multiple other Ras genes mutated at the same position



Investigating genes with low mutation frequency



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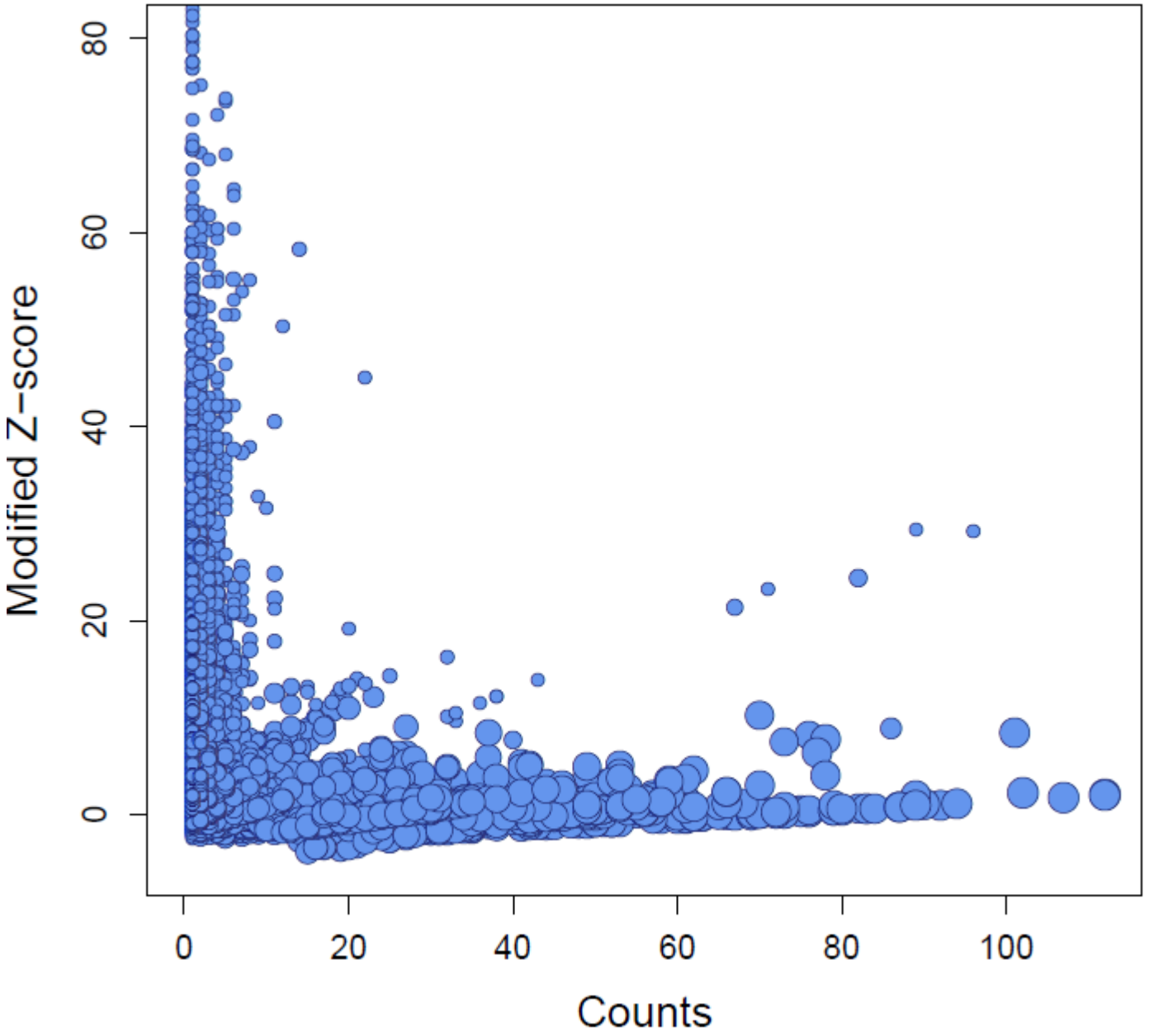
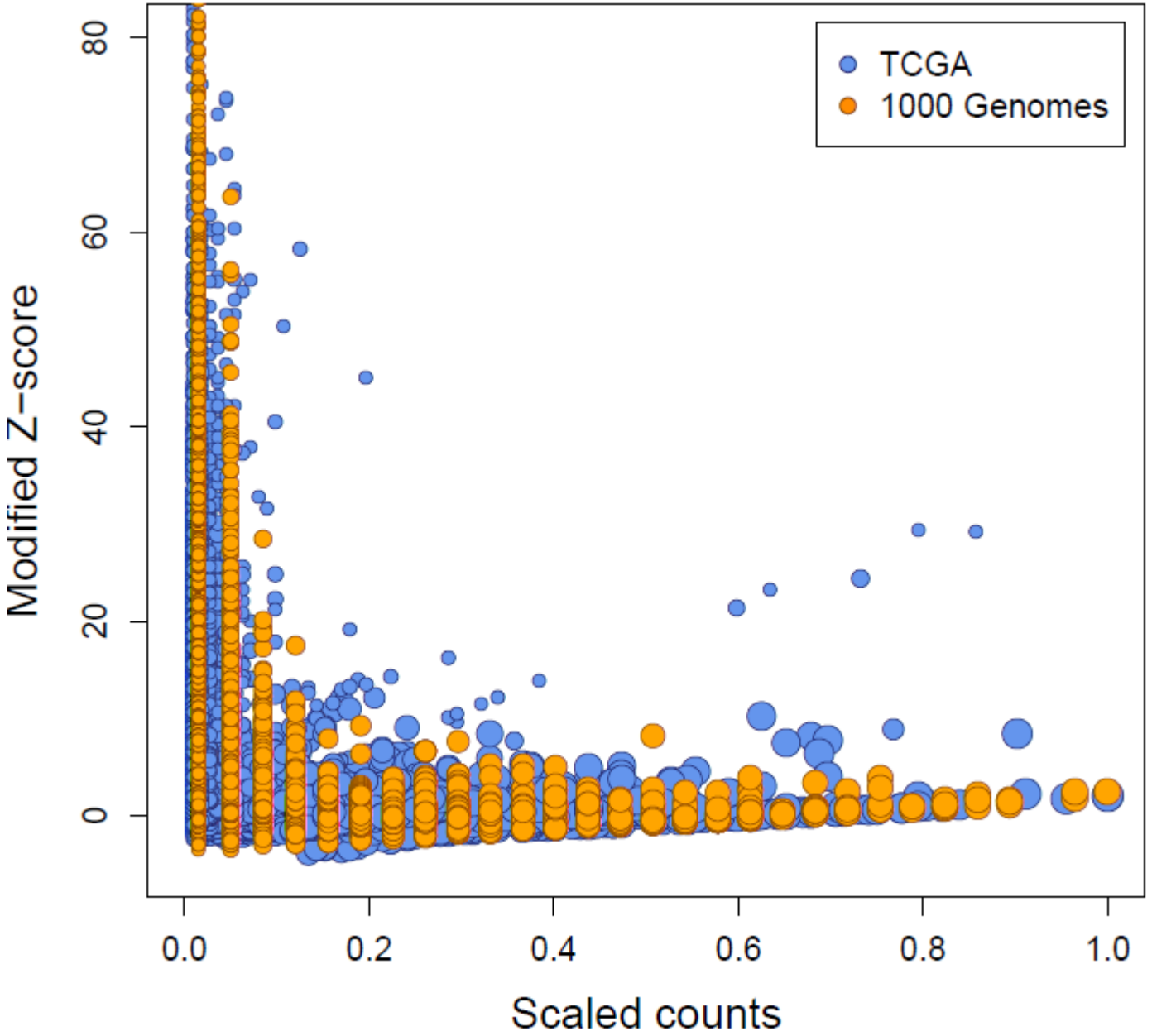
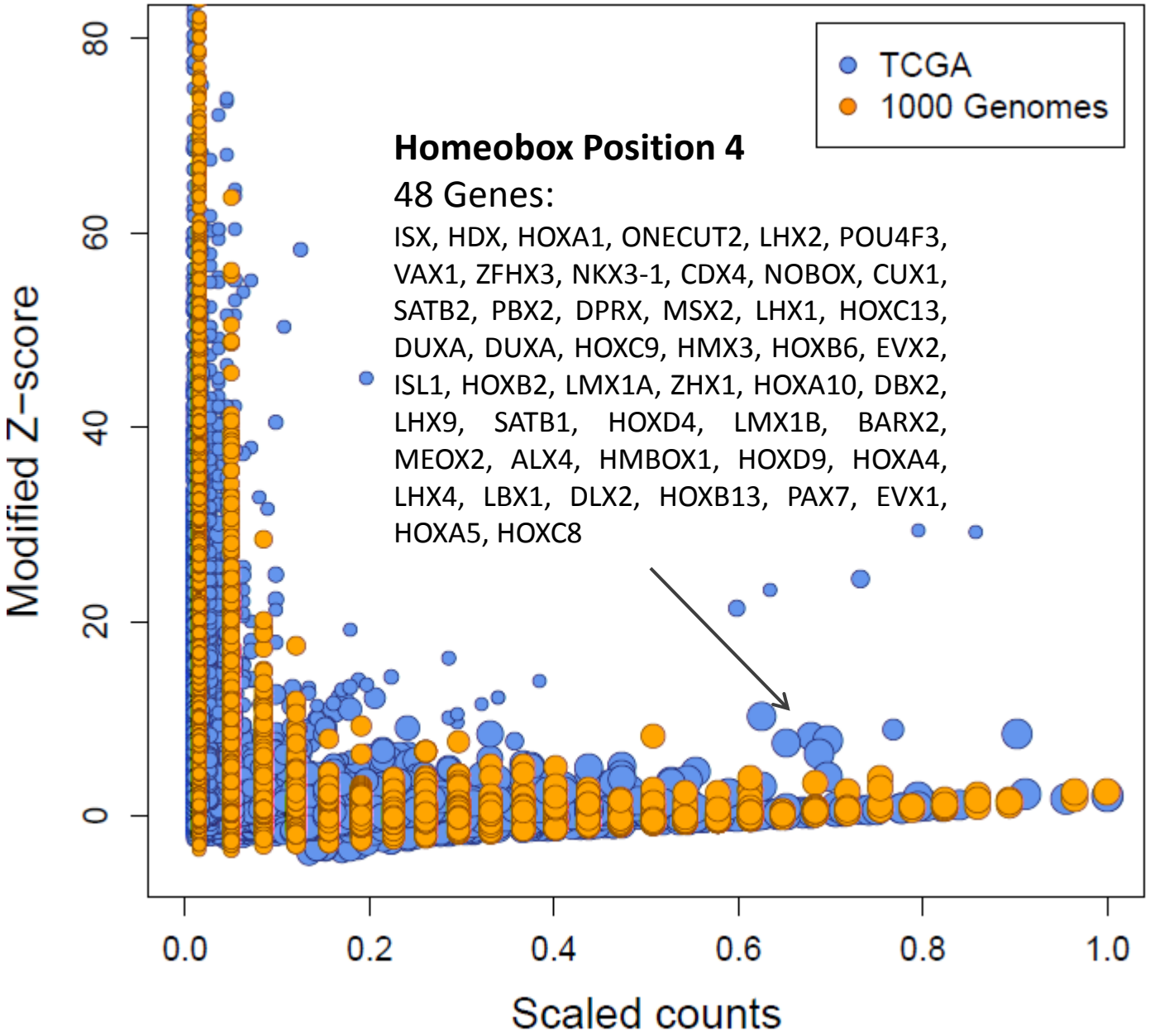
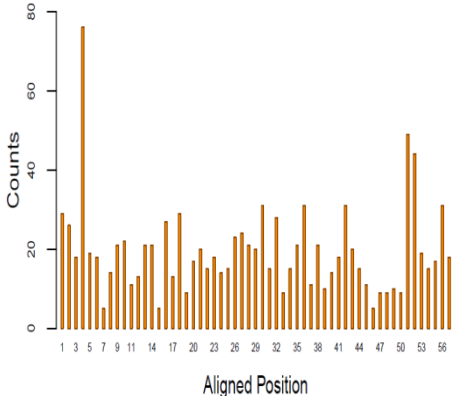


Background expectation:
Comparing TCGA to 1000 Genomes



Outlier position within Homeobox domain





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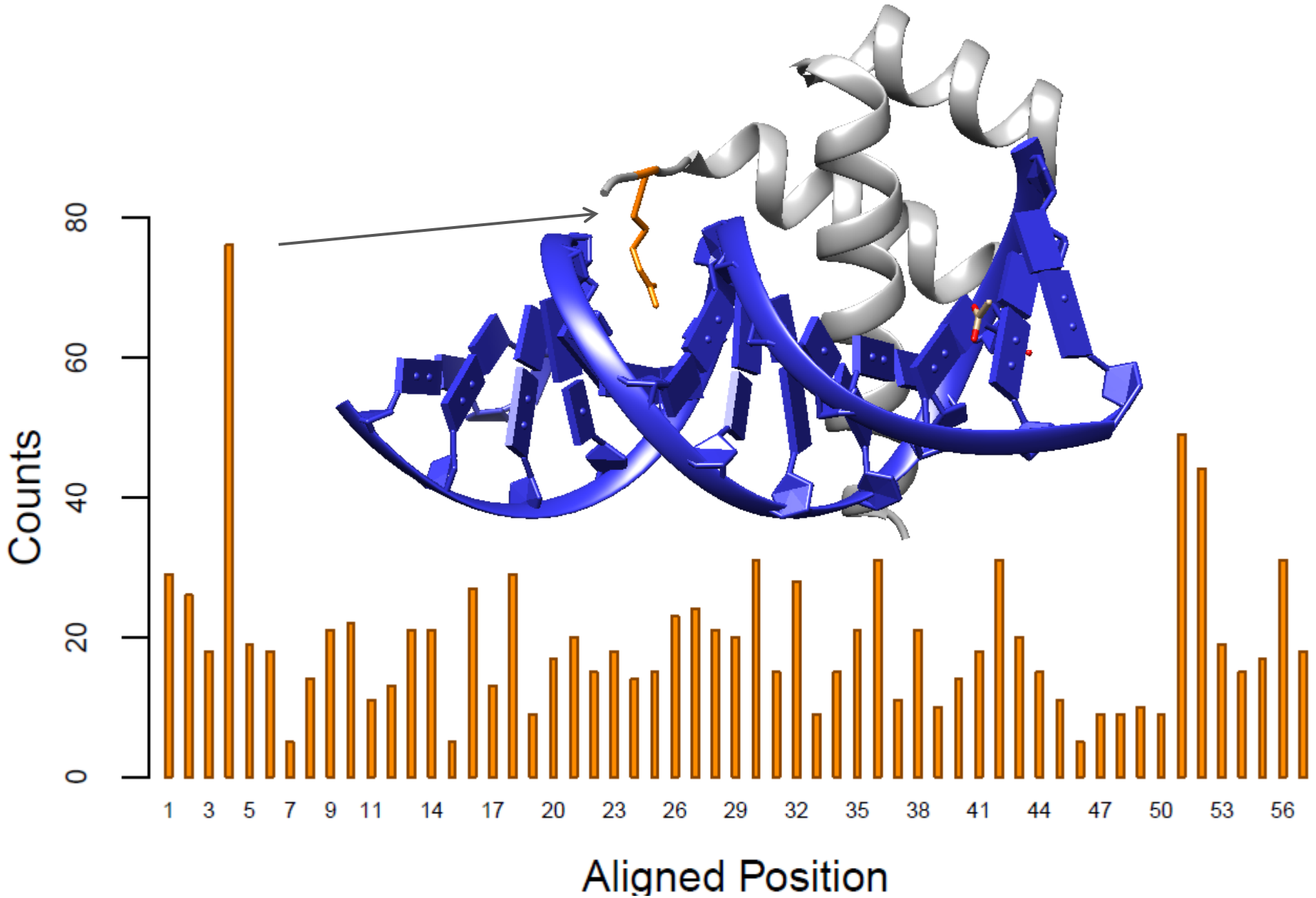
Structural interpretation



Summary and Next Steps

- We have comprehensively evaluated mutations at all positions within all domains to identify low frequency but likely deleterious mutations
- Hotspots outside of domains and mutations that broadly disrupt structure and function not addressed
  - Integration with other methods is essential
- Functional validation of low frequency events in such data sets remains challenging
- As cancer and normal genome data size increases, more robust normalization per position per domain can be achieved

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Acknowledgements

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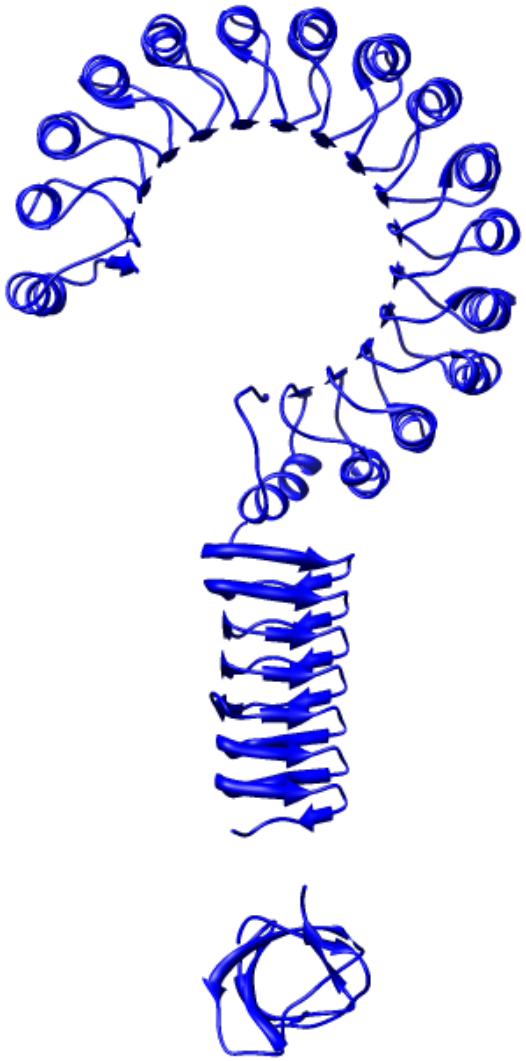

2bnh: alpha-beta horseshoe

1hv9: left-handed beta helix

1m30: SH3-like barrel

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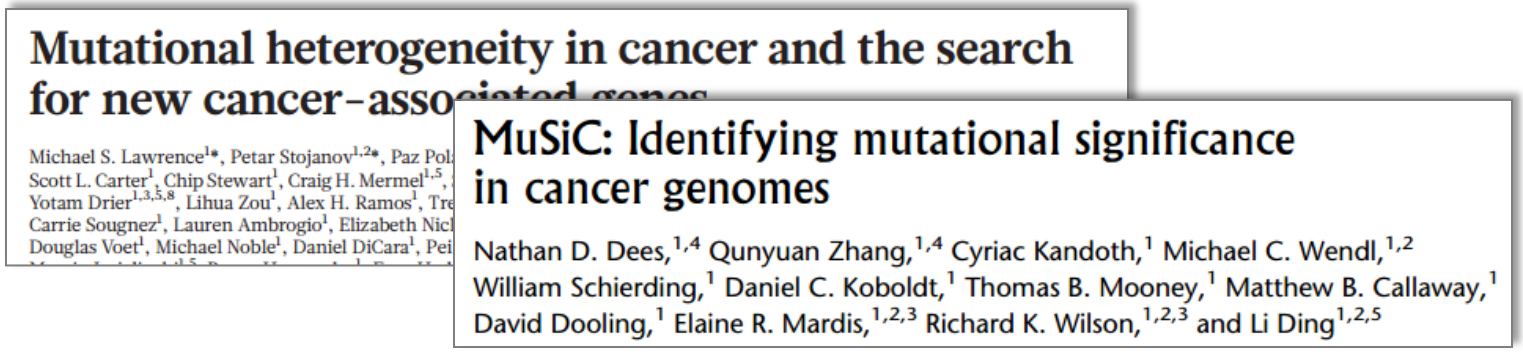
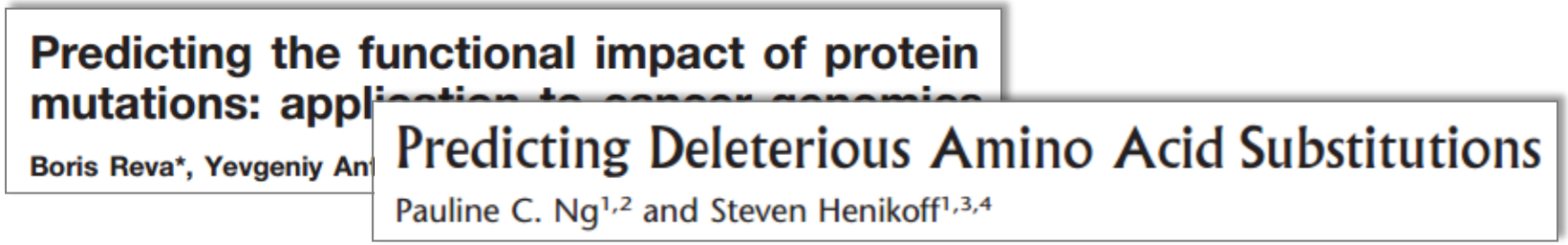
Example approaches and methods

- Statistical assessment of multiple samples (MutSig, MuSiC, …)



- Sequence conservation (MutationAssessor, SIFT, …)



- Machine learning and classification (PolyPhen-2, CHASM, …)