

CAGI slide pack guidelines

I agree to my slides being used according to the following terms: *Kenneth A. Matreyek*

- Authors: please include THIS slide at the beginning of your presentation to indicate that you agree with reuse of your slides in CAGI presentations, according to the terms below, and digitally sign by writing your name above.
- To disseminate the CAGI results, I encourage all CAGI participants to remix these CAGI slides for inclusion in their oral and poster presentations
- CAGI participants may use or publish any content of these slides only in compliance with the CAGI Data Use Agreement
- CAGI participants may not cite or publish any content of these slides except with the written permission of the originating author or in the primary CAGI publications
- CAGI participants must acknowledge the original authors of these slides and CAGI 2013. Include the acknowledgement banner across the bottom
- CAGI participants must include the CAGI credits slide in their presentations
- Slides with a red slash (no remix) may not be included in a presentation unless all attendees are registered CAGI participants who signed the data use agreement
- Slides with a red X over the entire slide may not be used in any circumstances
- Slides with a blue slash (embargoed) may not be used unless the embargo is explicitly lifted
- Slides without slash or X may be tweeted unless flagged with the “No tweet” icon.
- CAGI participants using these slides must explain the “No tweet” icon, which means that the slide content should not be disseminated outside the presenting conference hall. If the presentation venue permits pervasive tweeting, you may not include these slides in your talk
- Note for slide authors: we expect all CAGI participants to abide by these restrictions, and will make best effort to ensure they are followed. However, but bear in mind that we have limited means of enforcing them, and therefore the restrictions cannot be guaranteed.



- Note: Some of the values changed from what was originally submitted to CAGI due to slight changes in data processing / analysis during the manuscript submission / review process. For the final dataset, please find the supplementary tables associated with the Nature Genetics manuscript (<https://www.nature.com/articles/s41588-018-0122-z>), or our VAMP-seq website <https://abundance.gs.washington.edu/shiny/stability/>

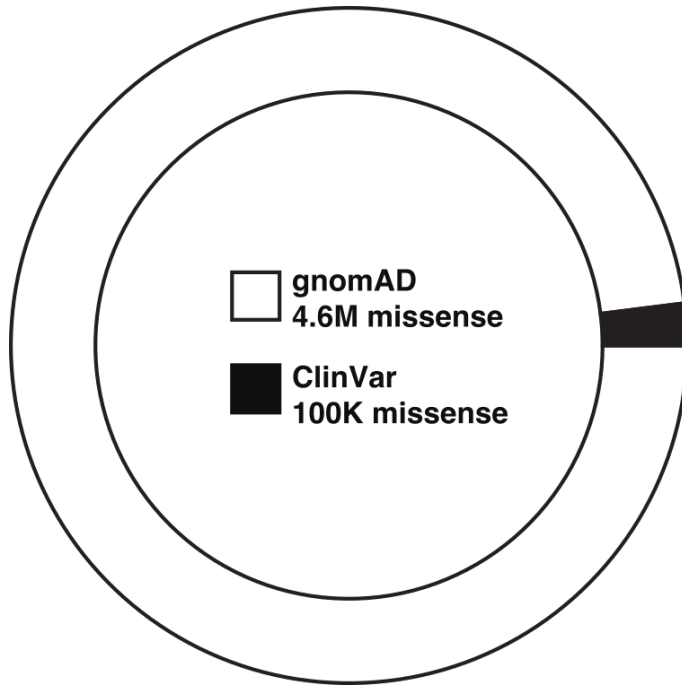
Multiplex assessment of protein variant abundance by massively parallel sequencing: PTEN and TPMT

Nature Genetics, June, 2018

Kenneth Matreyek
from the lab of Douglas Fowler

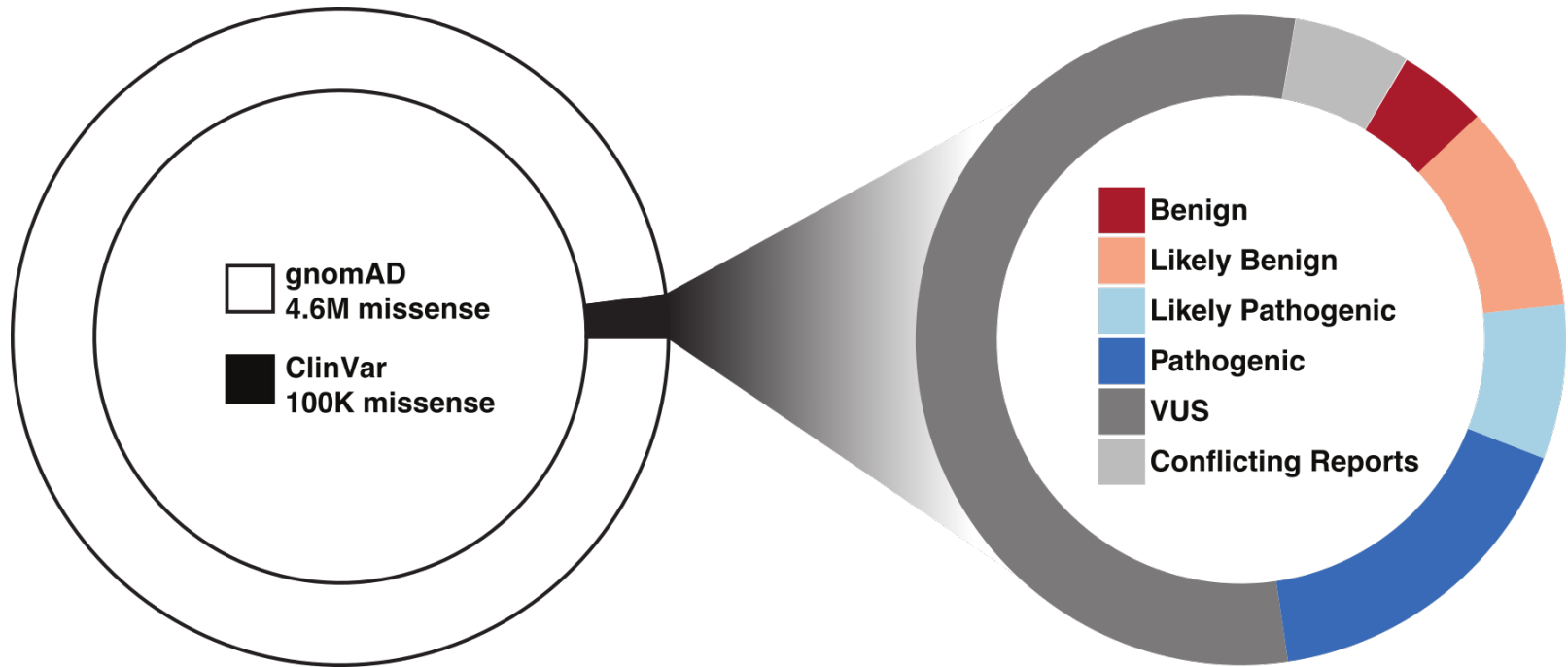
Department of Genome Sciences
University of Washington

Variant interpretation is a major problem in genetics



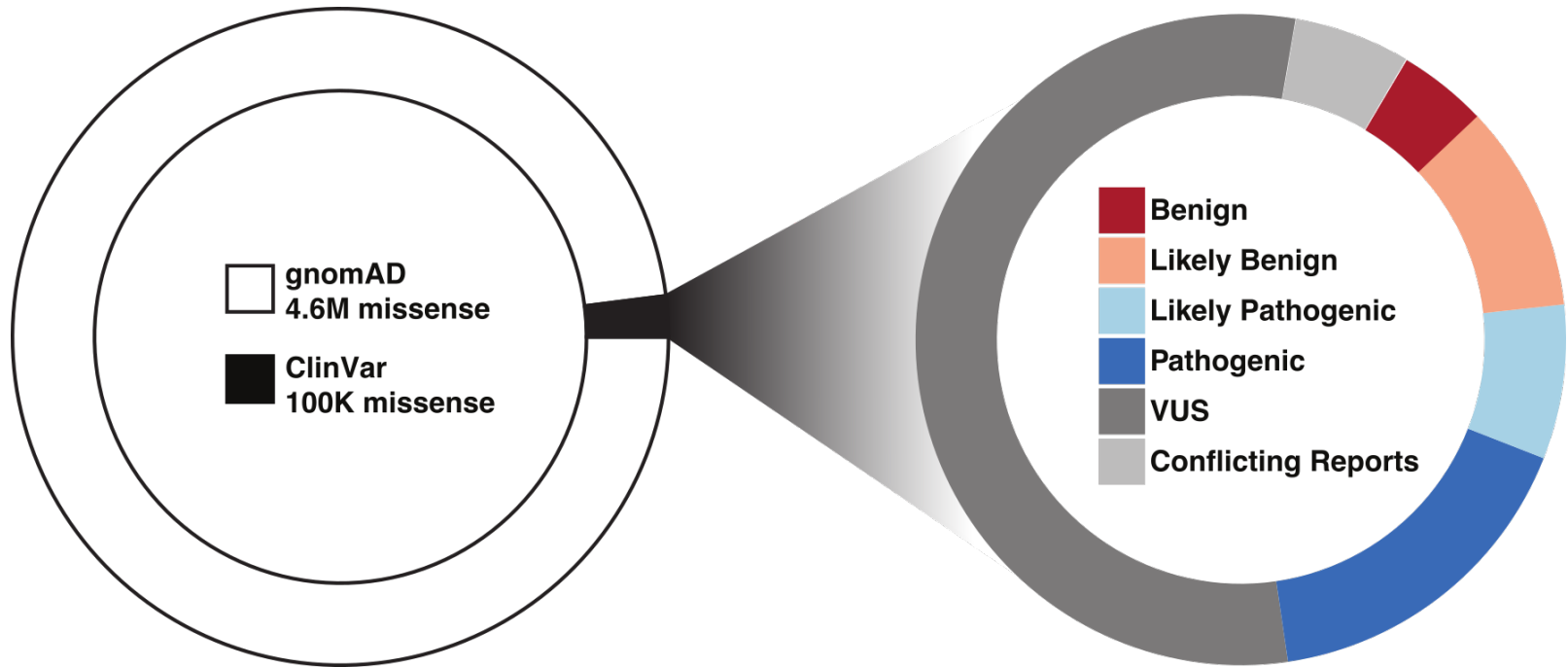
Starita *et al*, 2017, Am J Hum Genet

Variant interpretation is a major problem in genetics



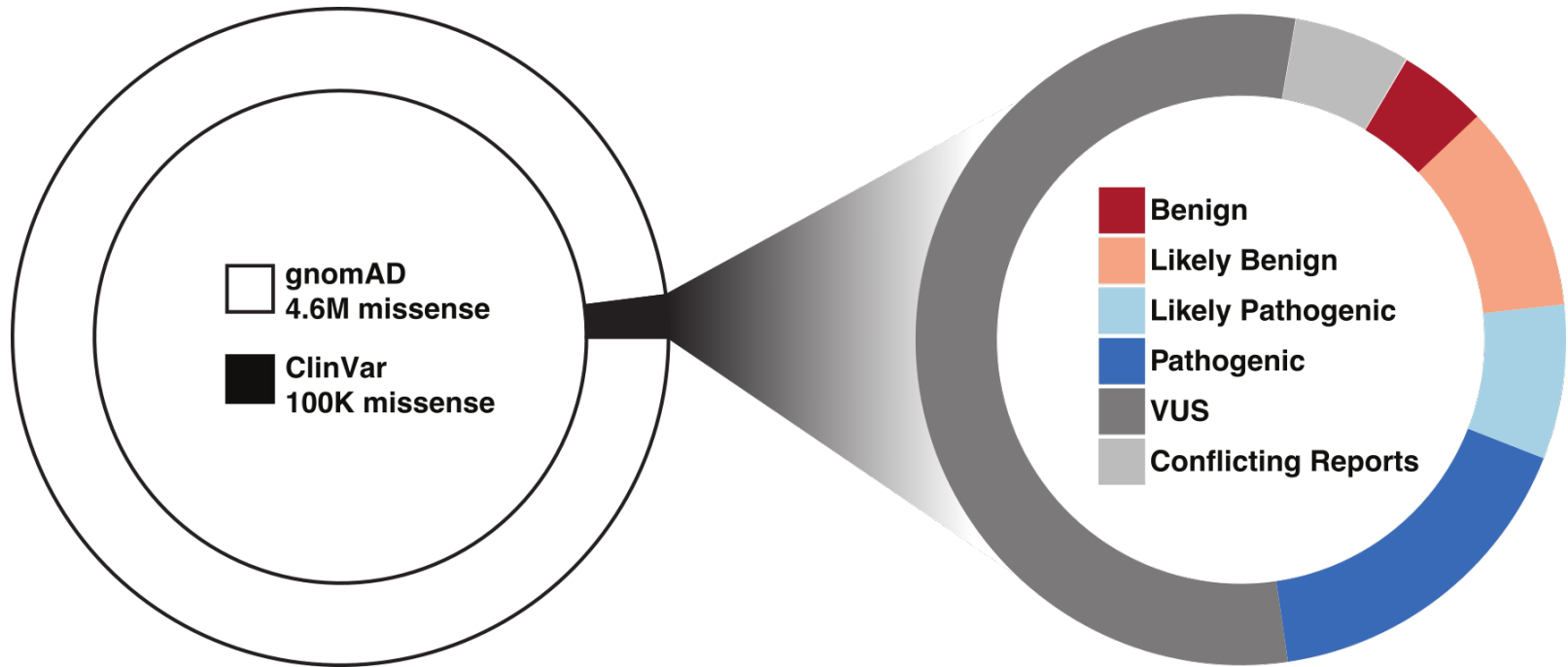
Starita *et al*, 2017, Am J Hum Genet

Variant interpretation is a major problem in genetics



Starita *et al*, 2017, Am J Hum Genet

Variant interpretation is a major problem in genetics

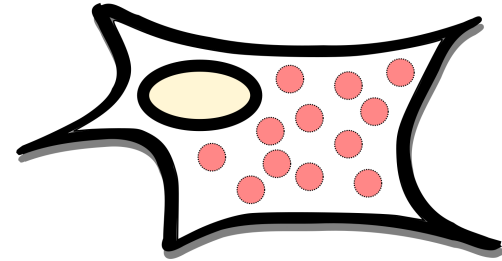


Starita *et al*, 2017, Am J Hum Genet

Goal: Create a generalizable massively-parallel assay for phenotyping protein variants

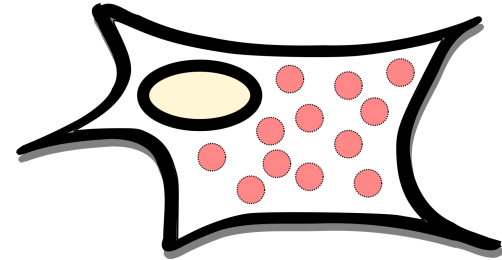
Proteins need to be correctly folded and stable to function

WT protein

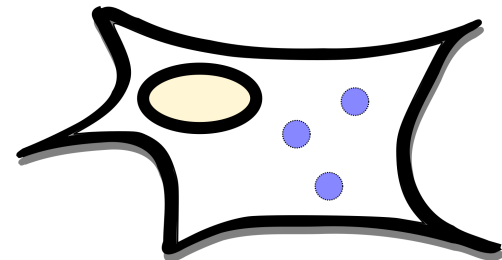
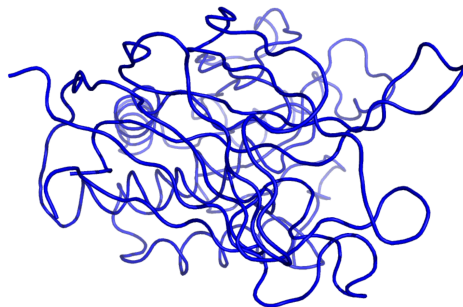


Destabilizing protein variations are a major cause of disease

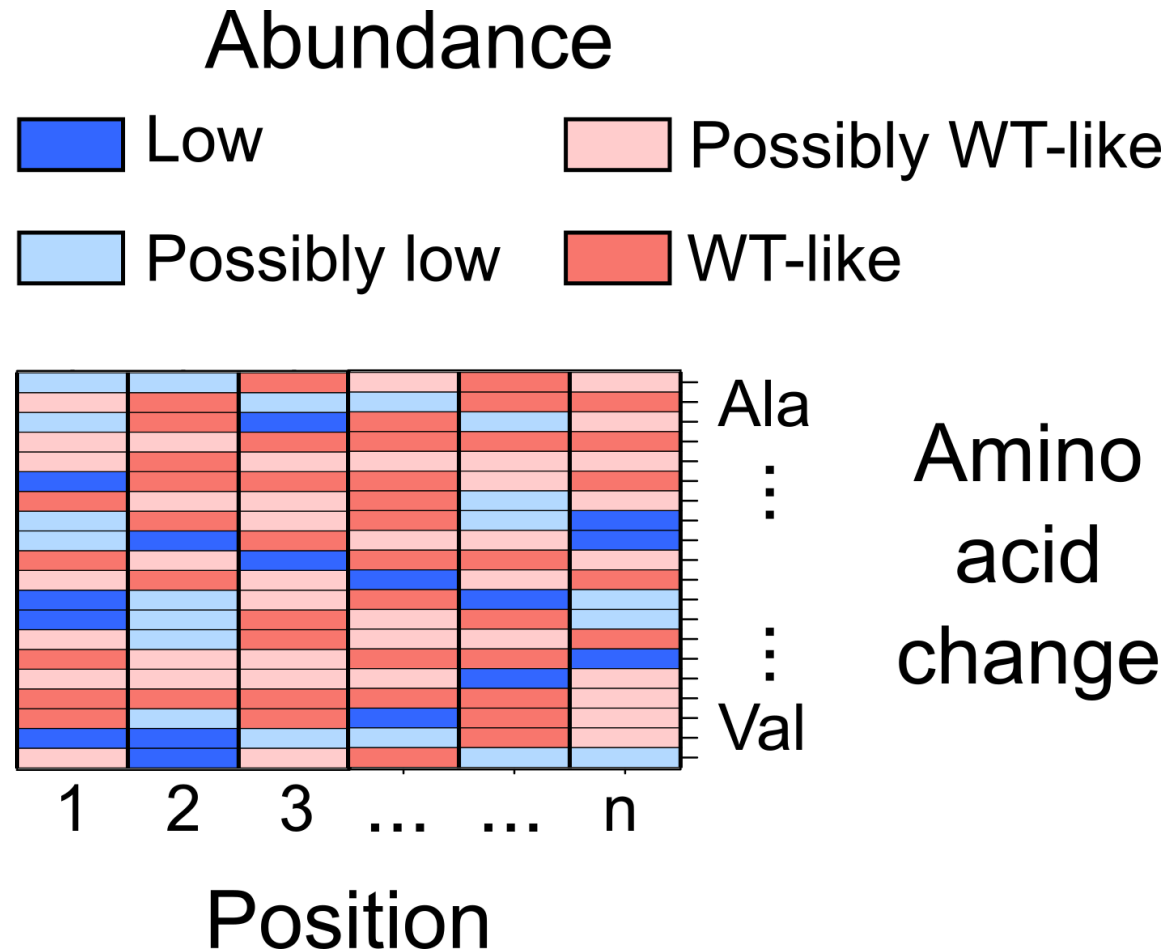
WT protein



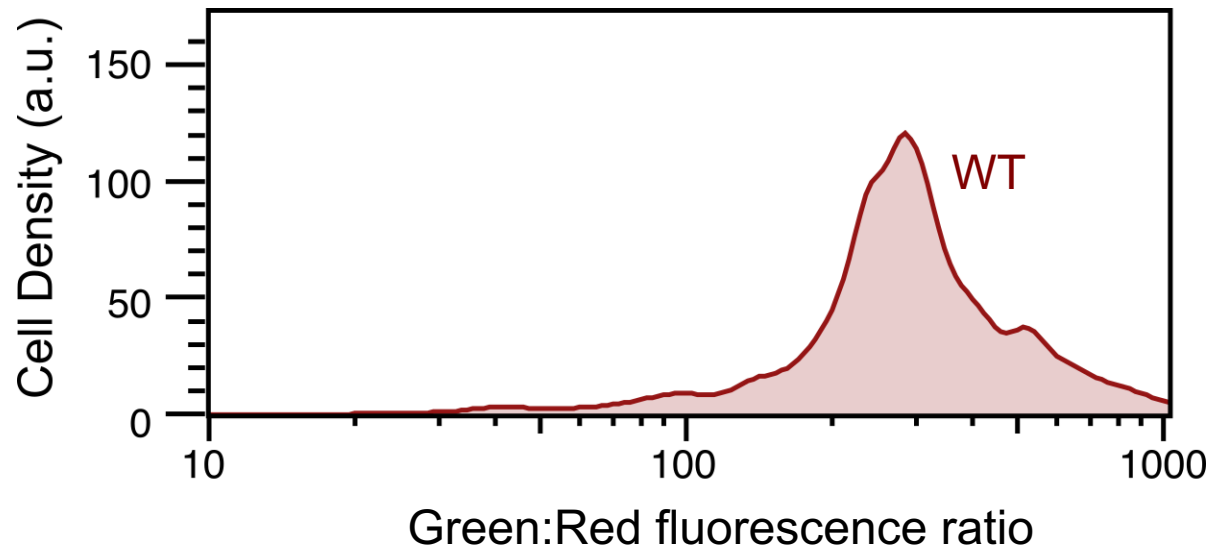
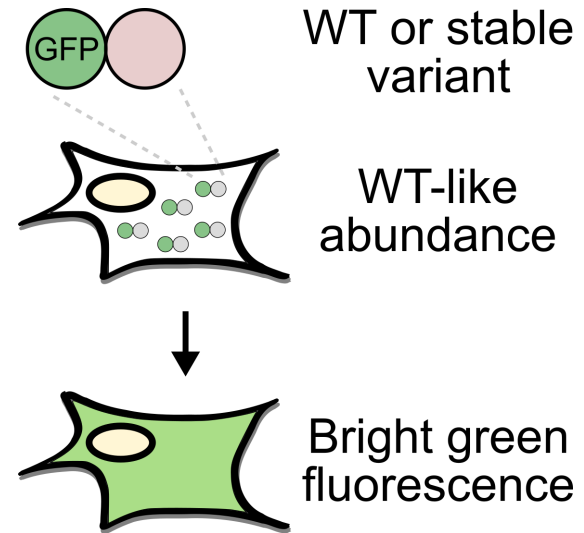
Misfolded /
unstable
variant



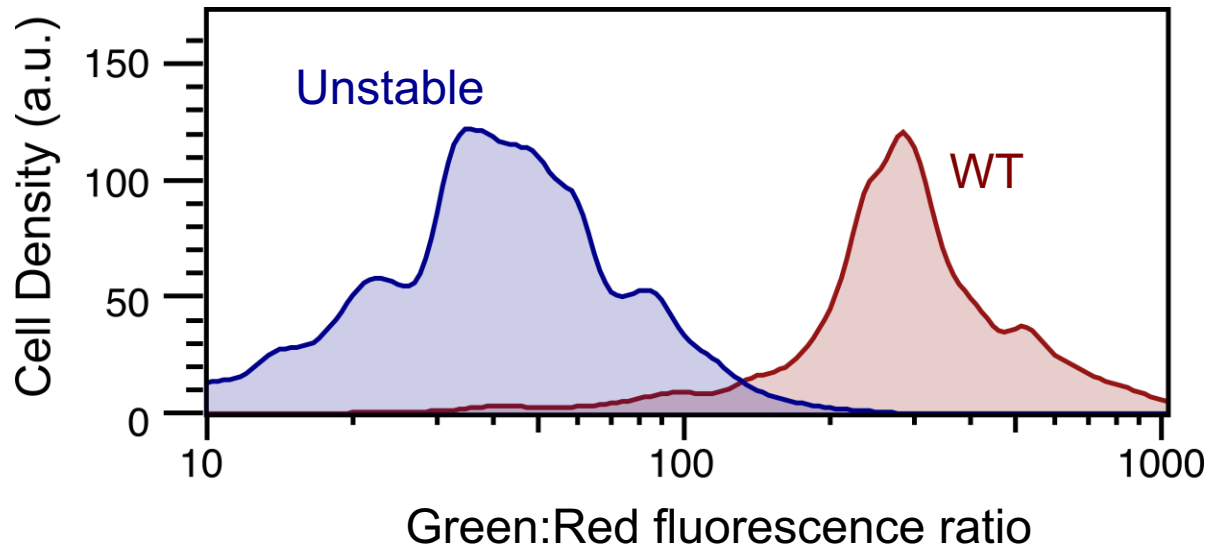
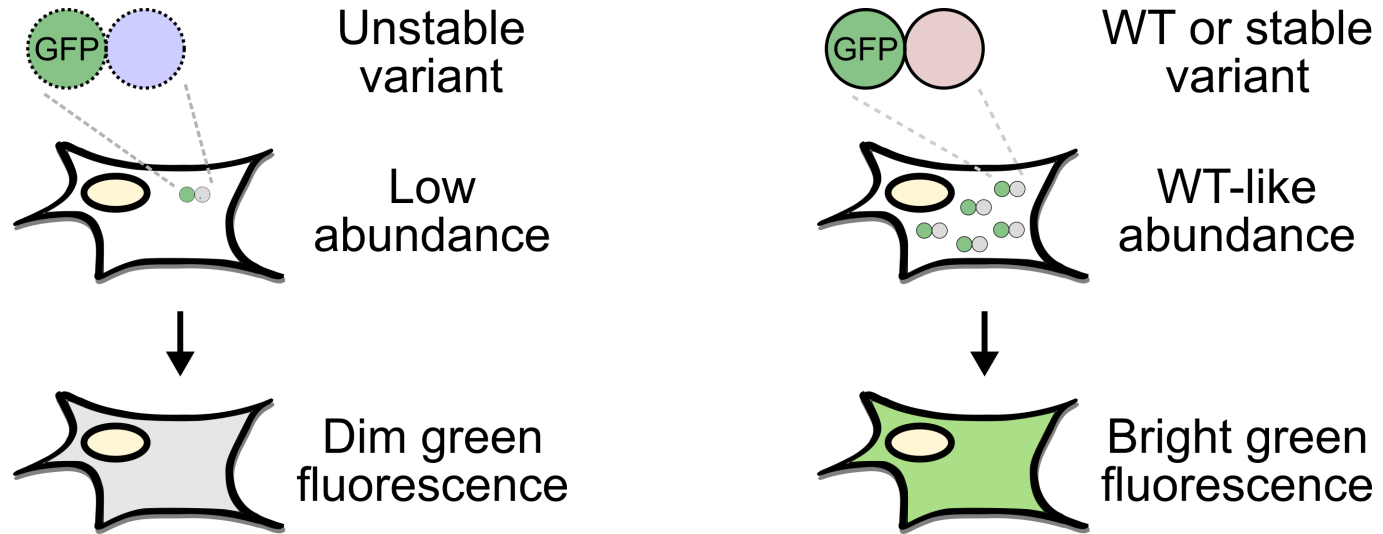
Characterizing protein Variant Abundance by Massively Parallel sequencing (VAMP-seq)



GFP fusions provide clear fluorescence-based readouts of variant stability and abundance

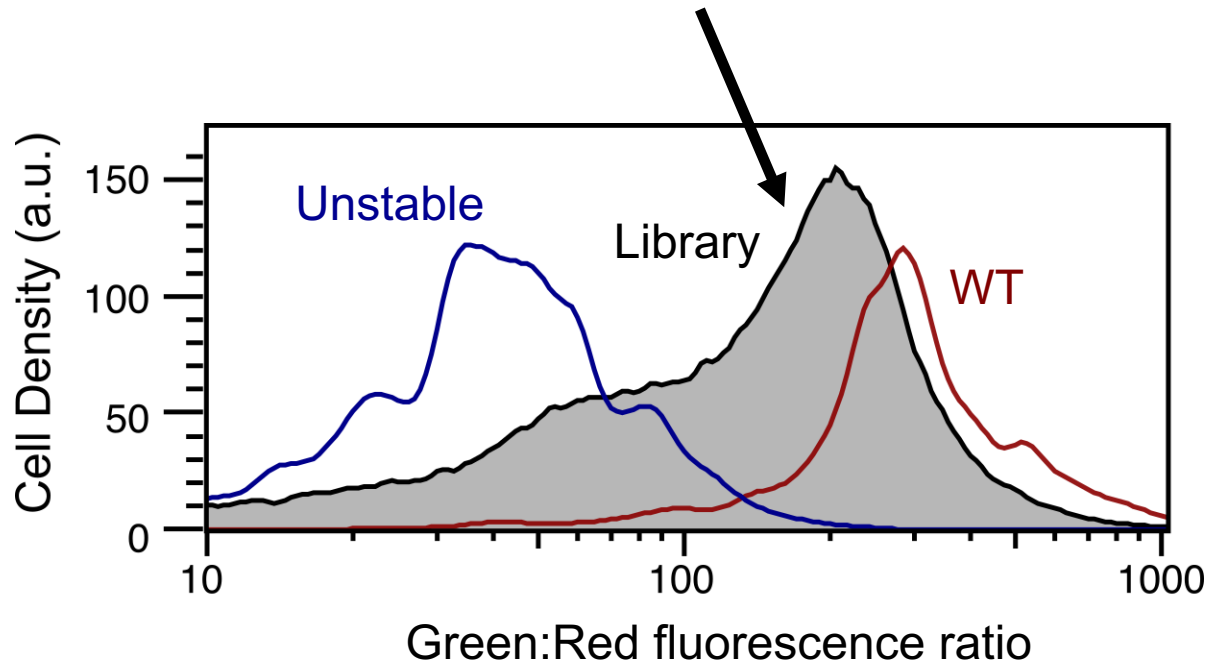
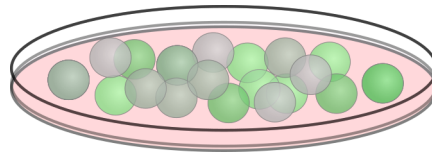


GFP fusions provide clear fluorescence-based readouts of variant stability and abundance



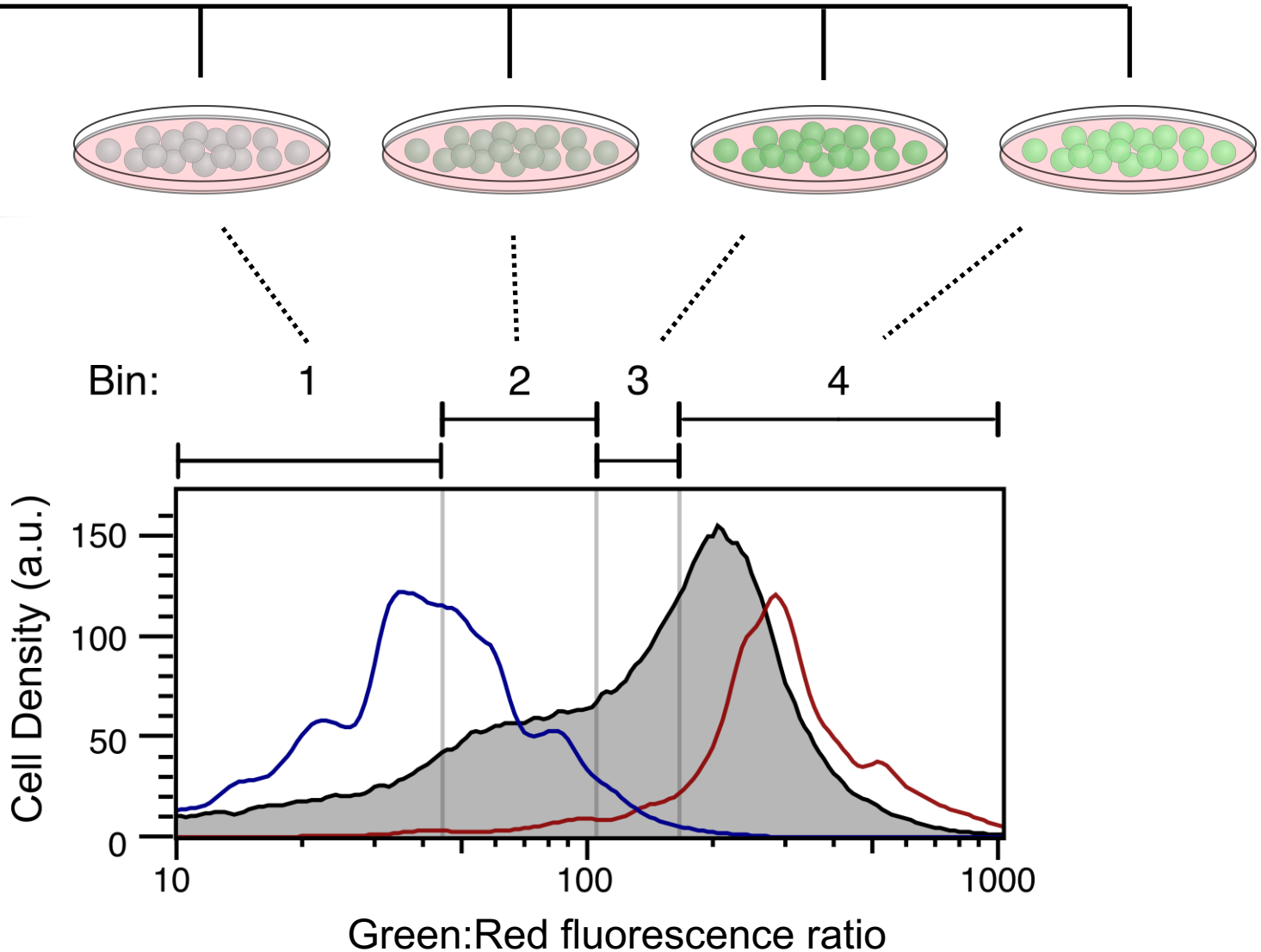
GFP fusions of a missense variant library span the assay fluorescence range

Thousands of missense variants

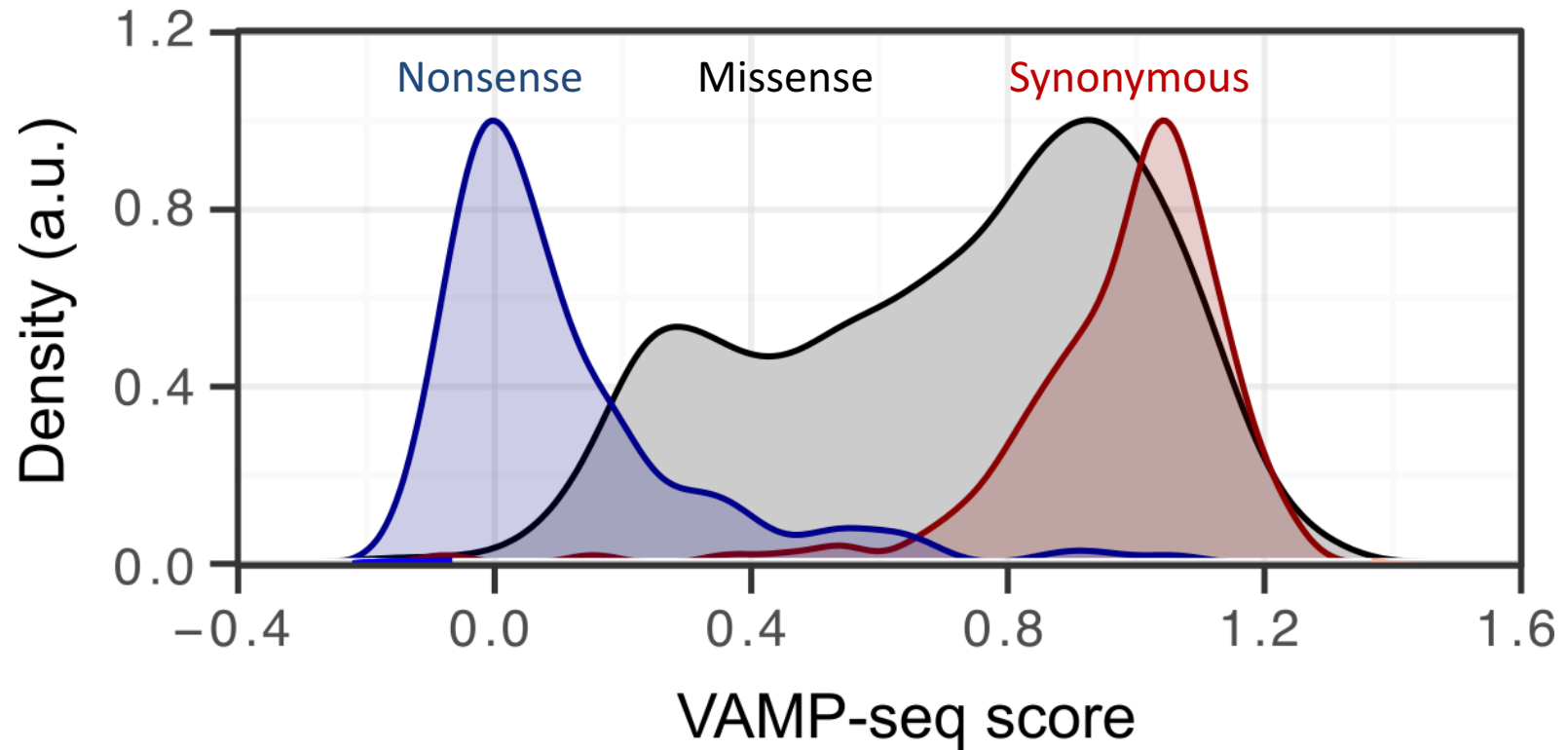


FACS and high throughput sequencing separates and counts the missense variant library based on protein abundance

High throughput sequencing



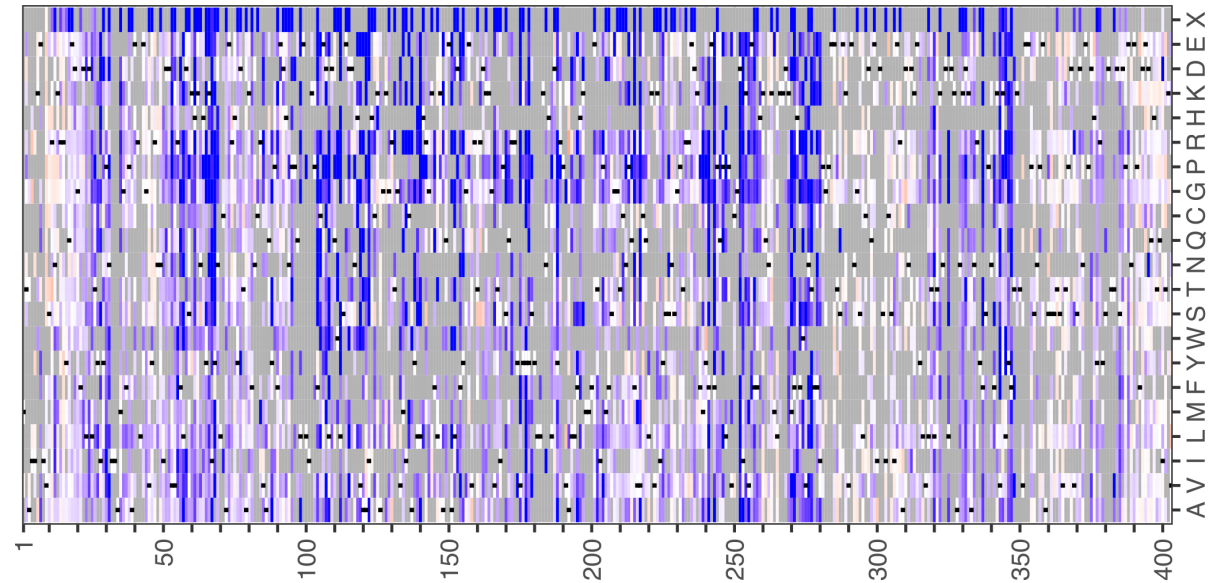
VAMP-seq scores show expected phenotypic distributions



The VAMP-seq genotype-phenotype map of protein abundance

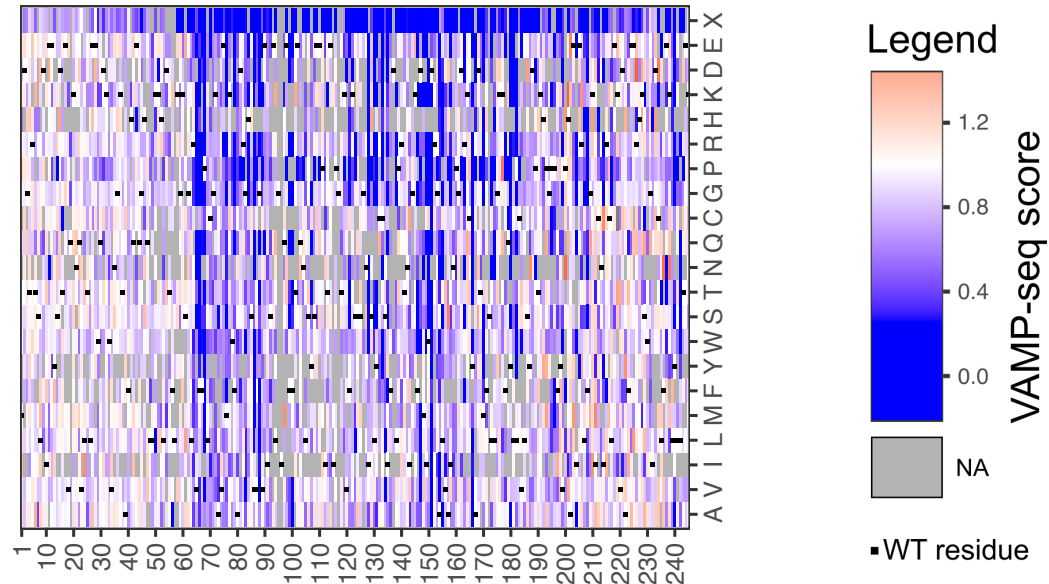
PTEN

4112 missense
140 nonsense
156 synonymous

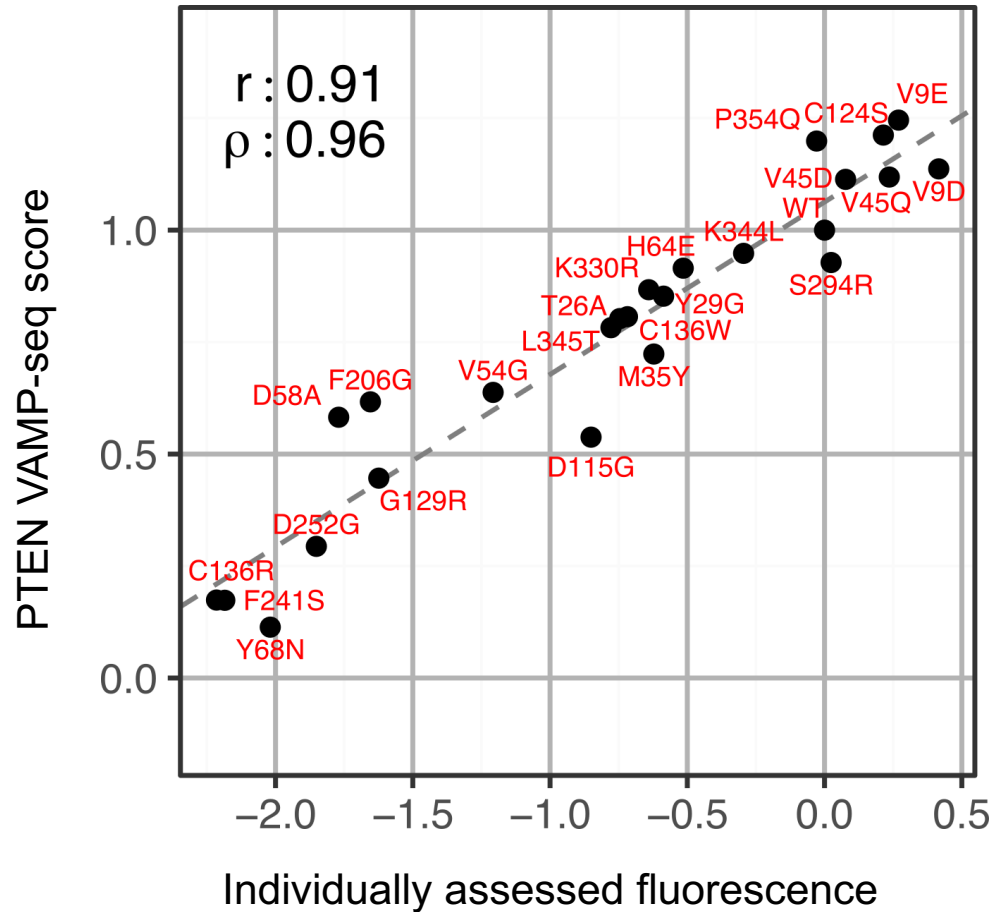


TPMT

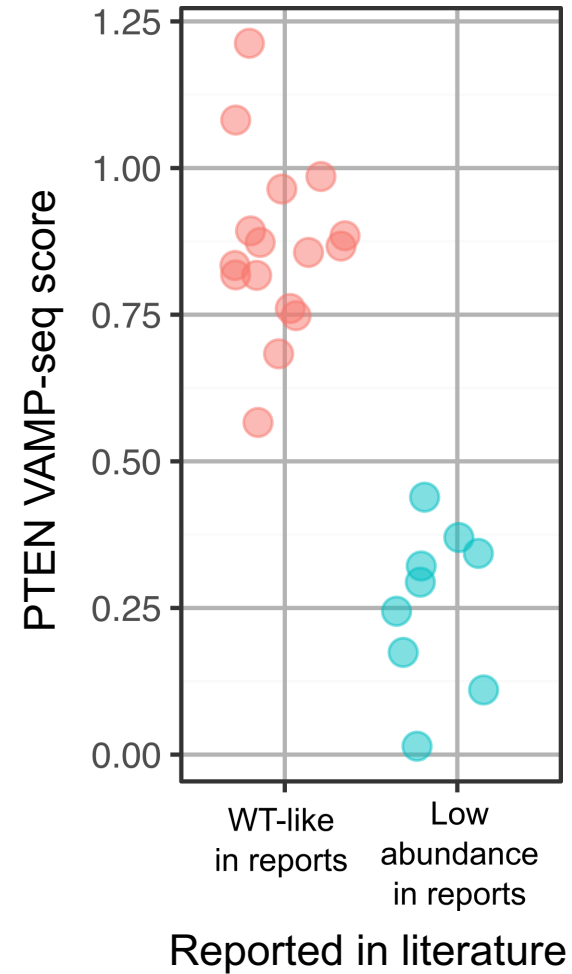
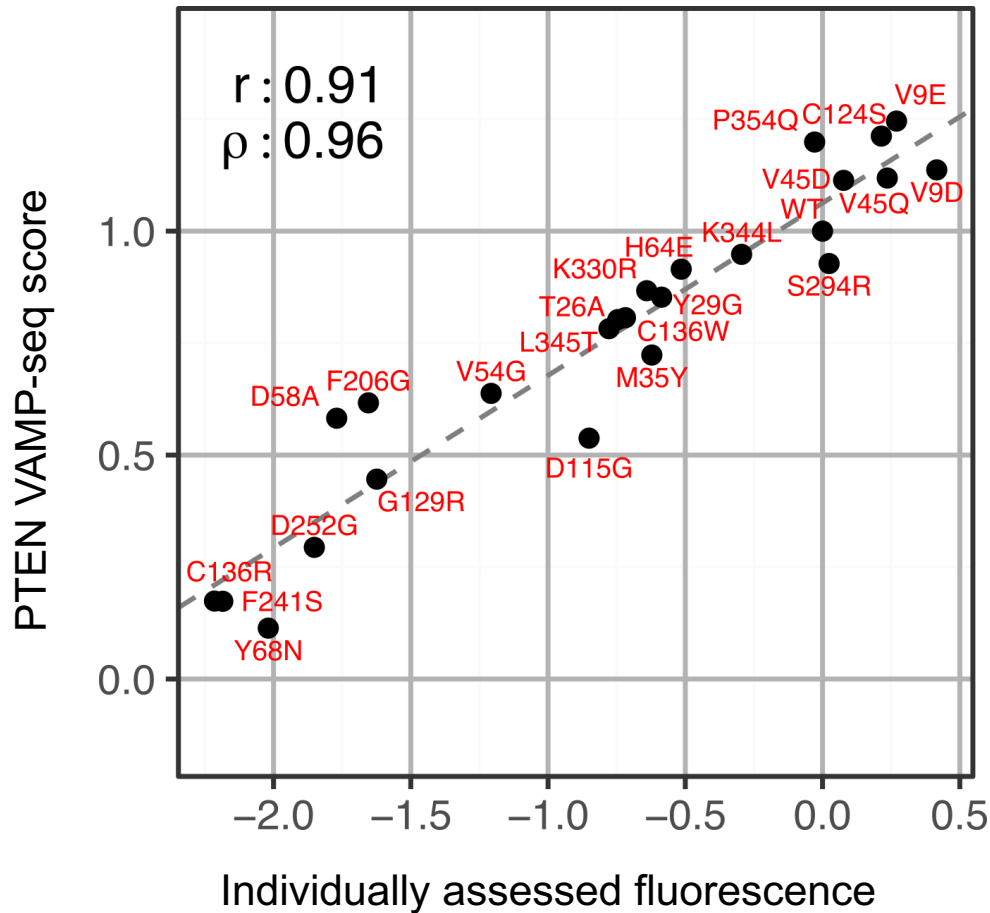
3689 missense
199 nonsense
139 synomyous



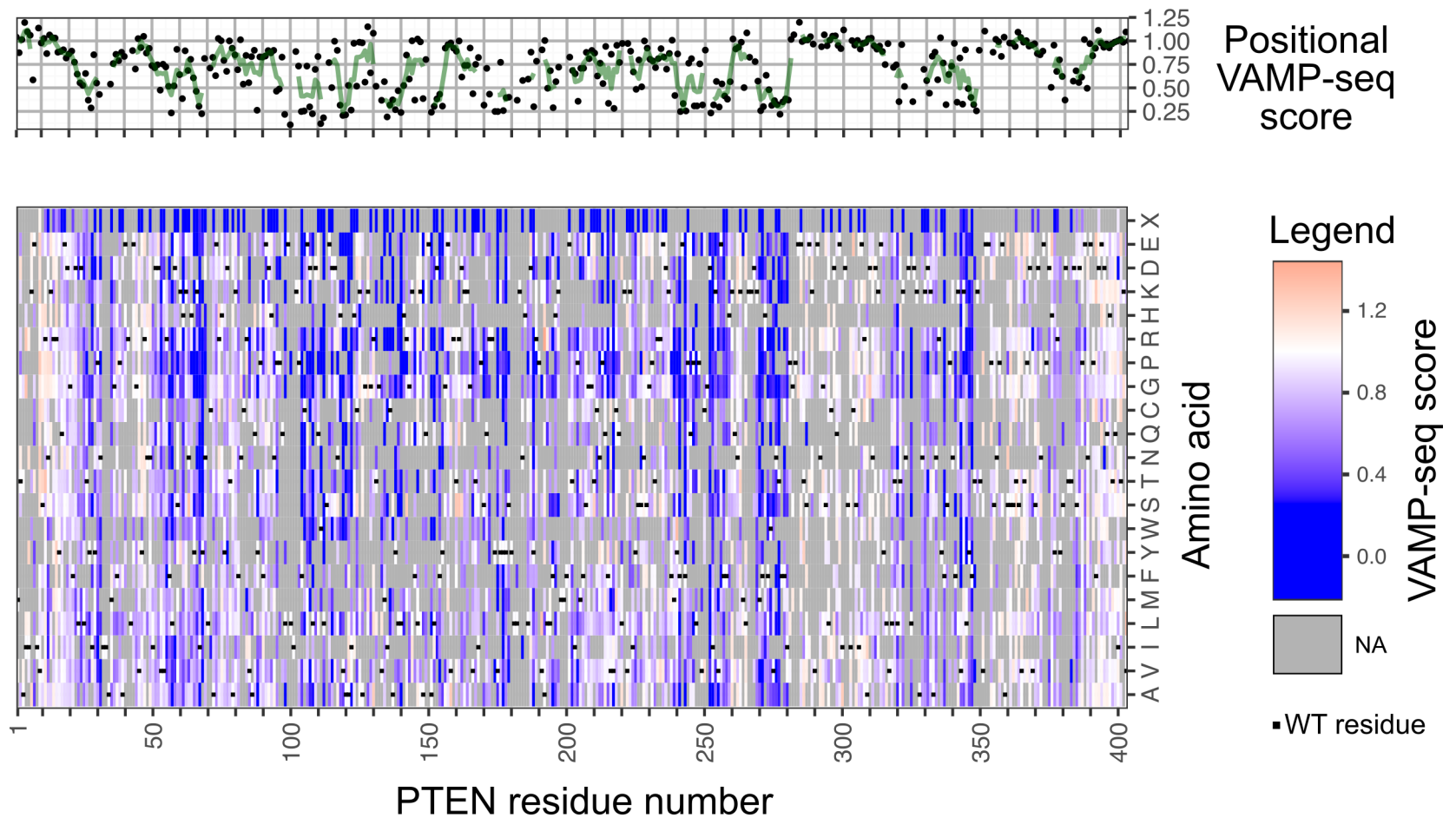
VAMP-seq scores correlate with individual variant assessments



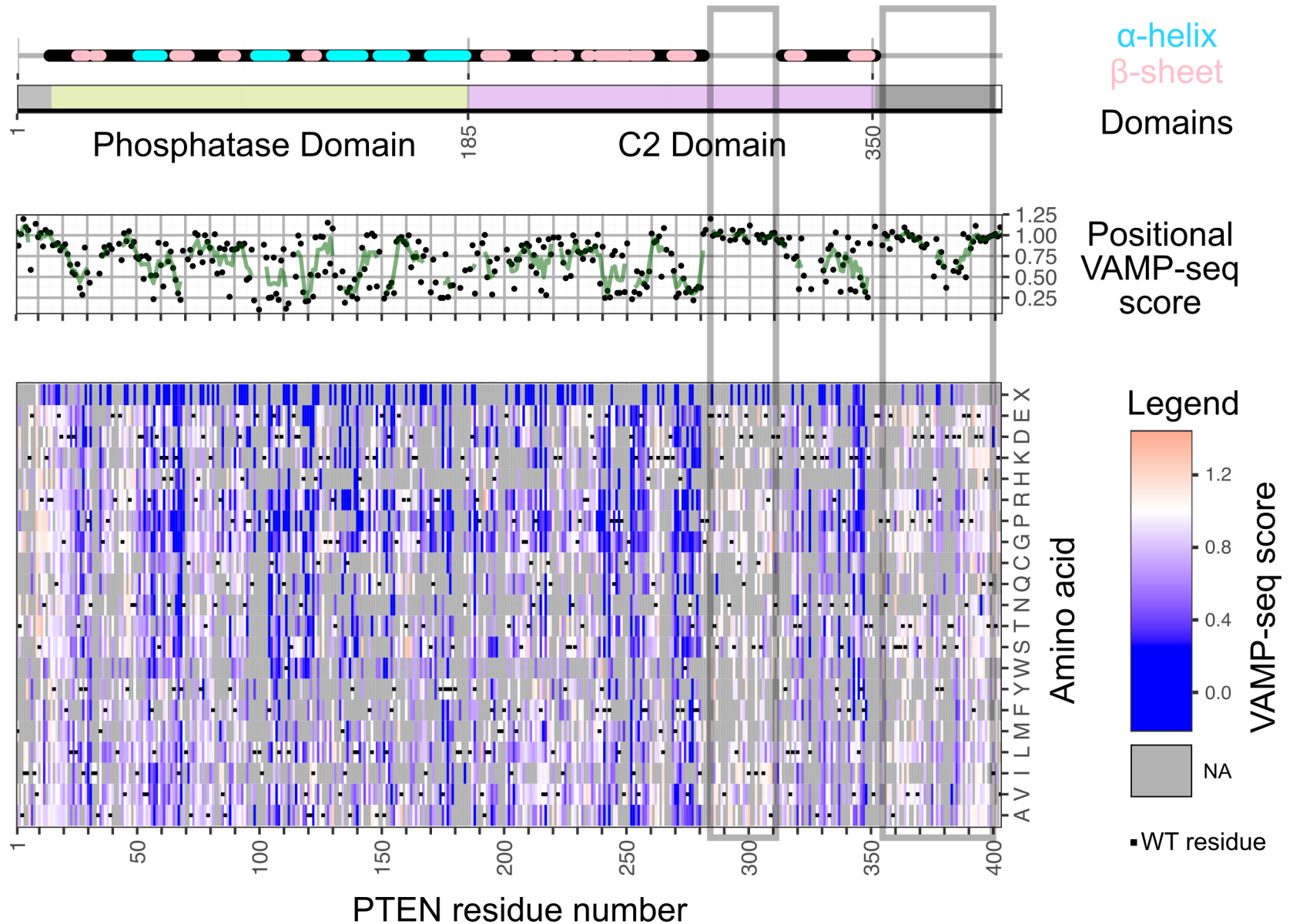
VAMP-seq scores corroborate observations from other labs



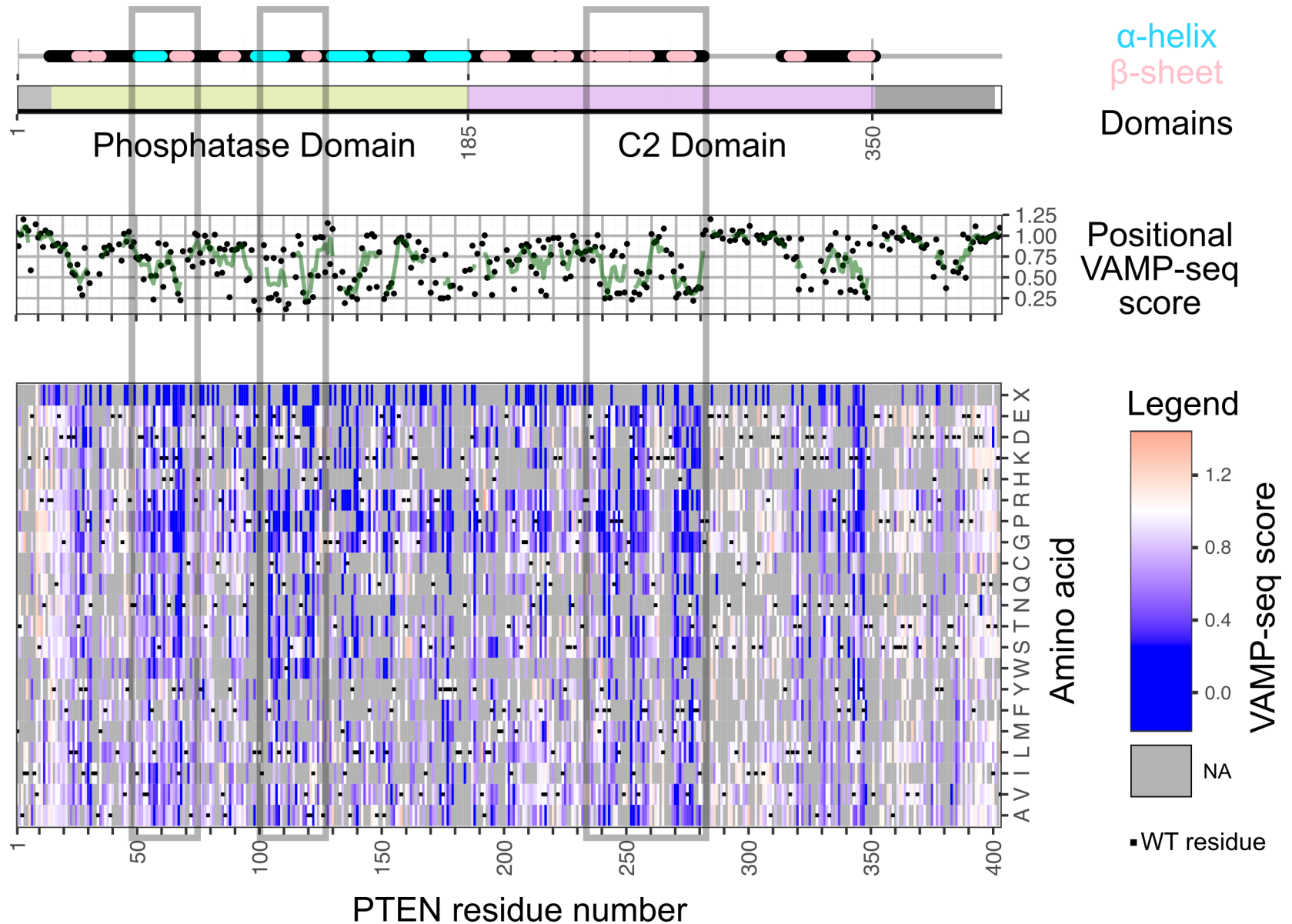
The VAMP-seq genotype-phenotype map of PTEN abundance



The VAMP-seq genotype-phenotype map of PTEN abundance



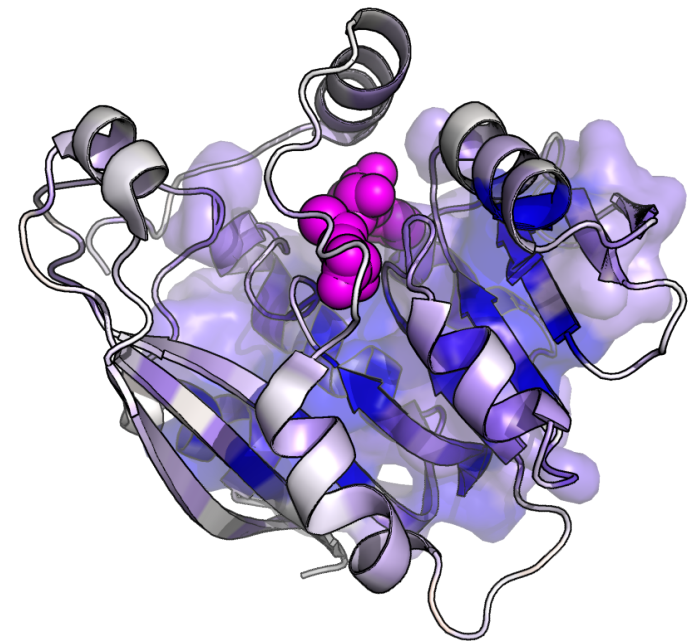
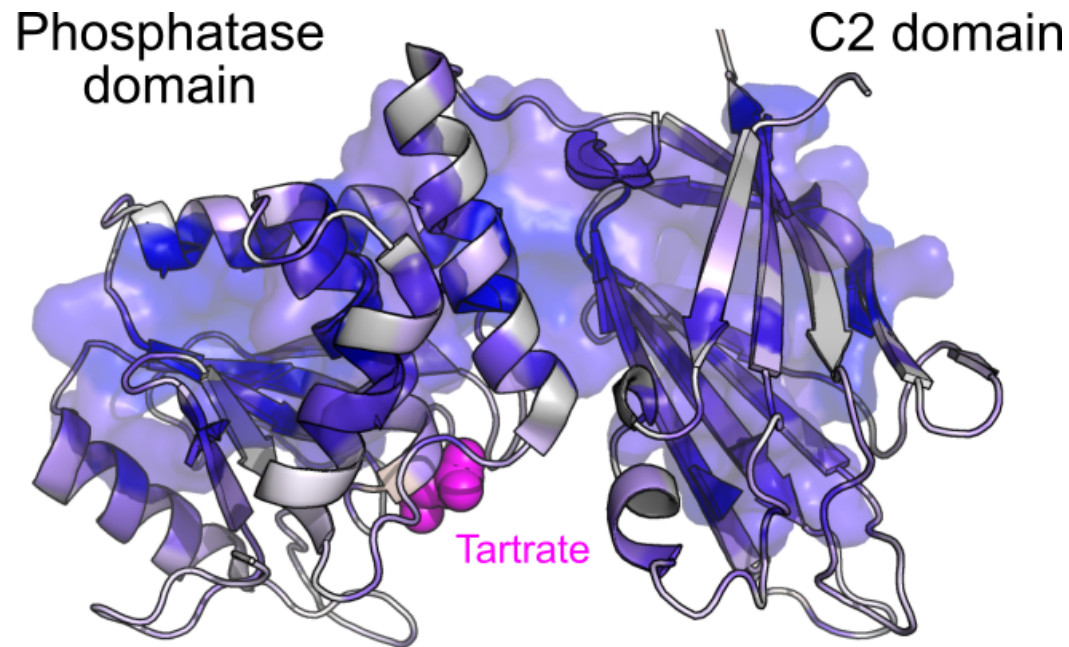
The VAMP-seq genotype-phenotype map of PTEN abundance



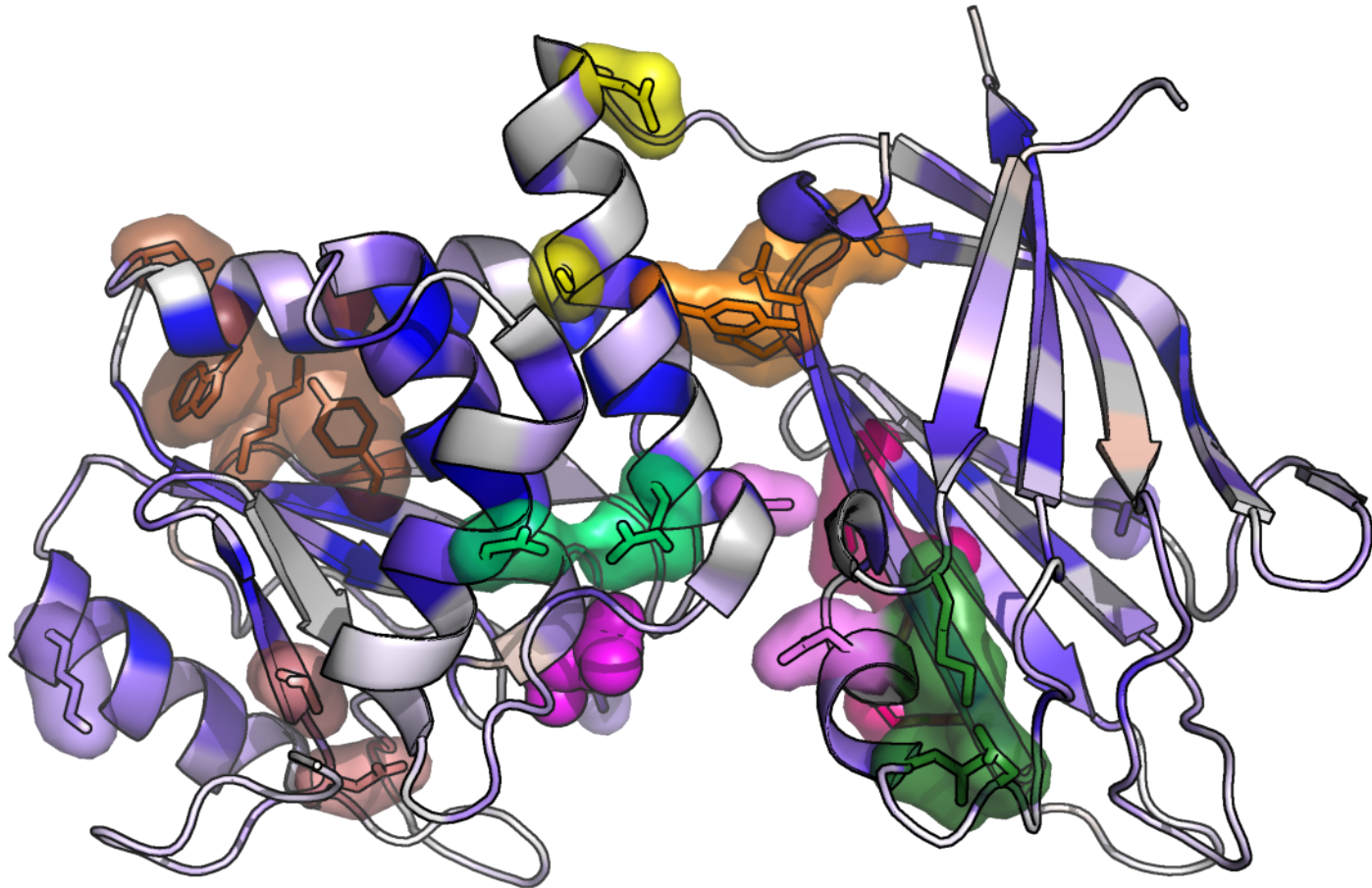
Variations in hydrophobic protein cores impact abundance

PTEN

TPMT



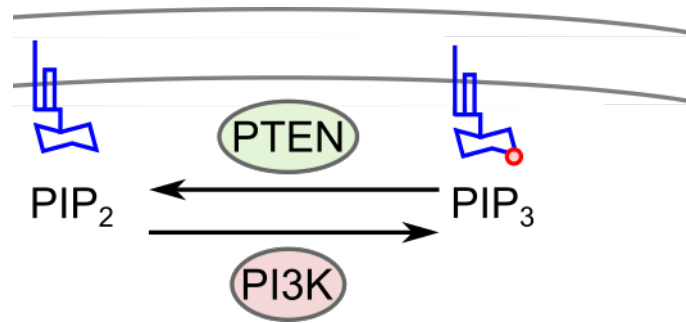
Hydrogen bonding residues likely critical for PTEN abundance



PTEN is a key negative regulator of cell growth

PTEN tumor suppressor protein

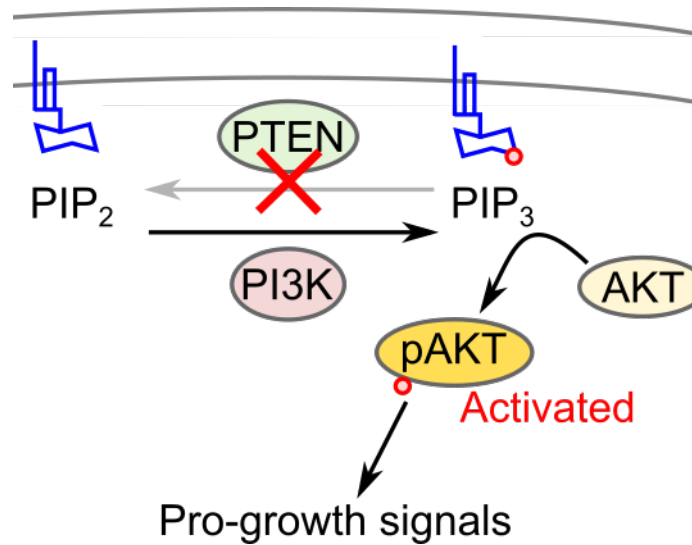
Growth-inhibitory phosphatase function



PTEN inactivation permits uncontrolled cell growth

PTEN tumor suppressor protein

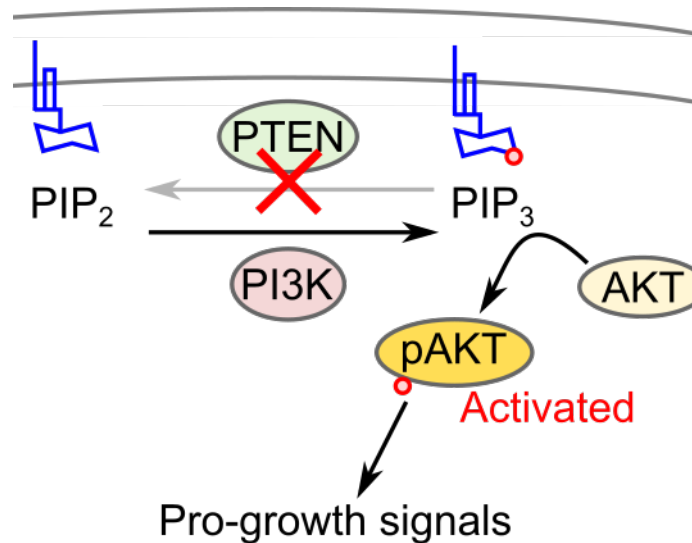
Growth-inhibitory phosphatase function



PTEN missense variation can have important clinical outcomes

PTEN tumor suppressor protein

Growth-inhibitory phosphatase function



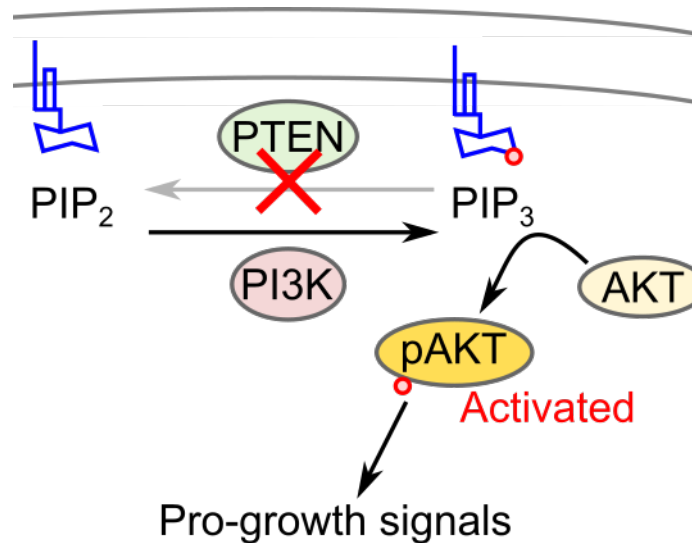
Clinically relevant:

Germline: PHTS (eg. Cowden Syndrome), autism

PTEN missense variation can have important clinical outcomes

PTEN tumor suppressor protein

Growth-inhibitory phosphatase function

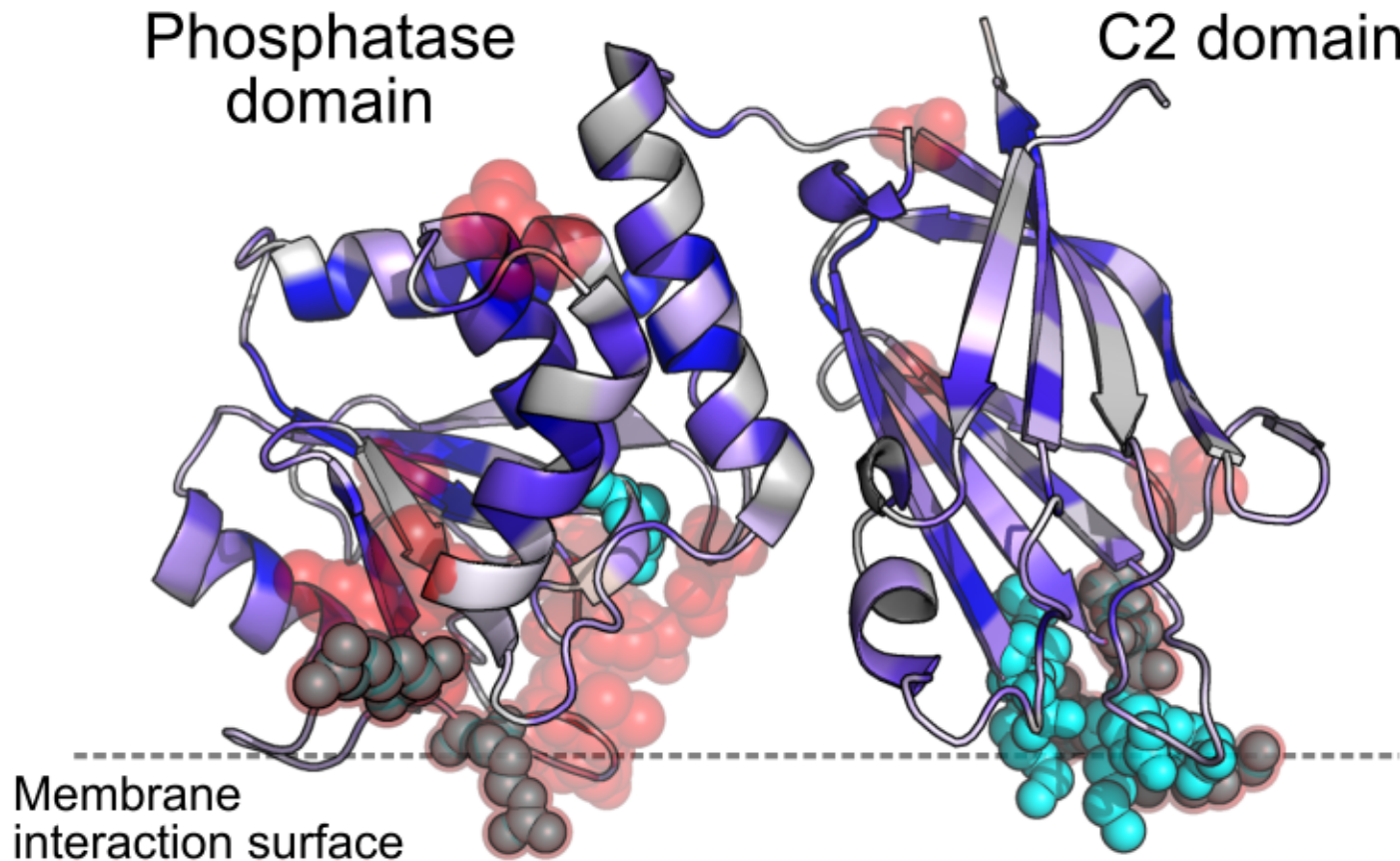


Clinically relevant:

Germline: PHTS (eg. Cowden Syndrome), autism

Somatic: Cancer (many types)

Loss of membrane interaction enhances PTEN abundance

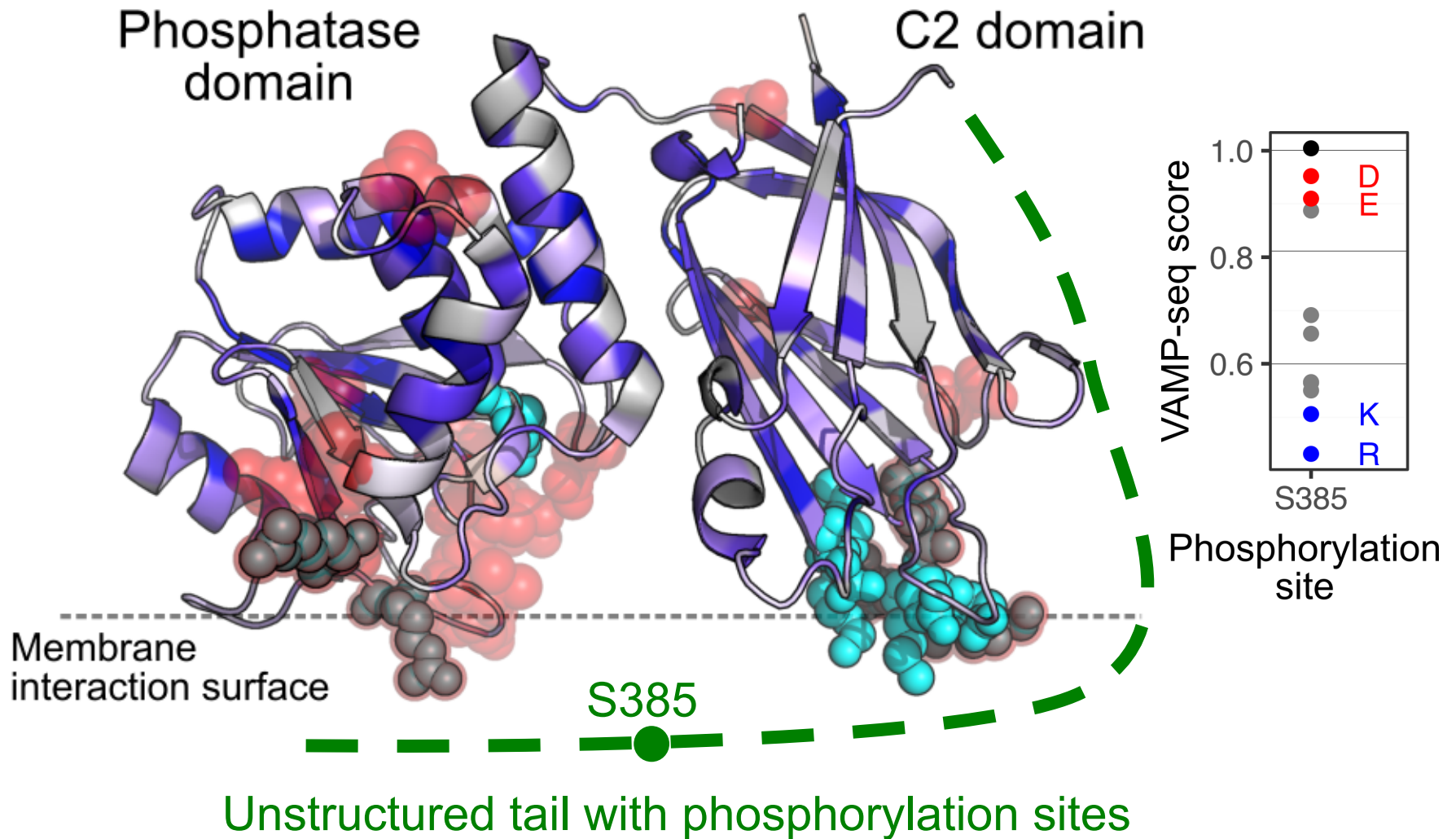


Red: Enhanced abundance positions

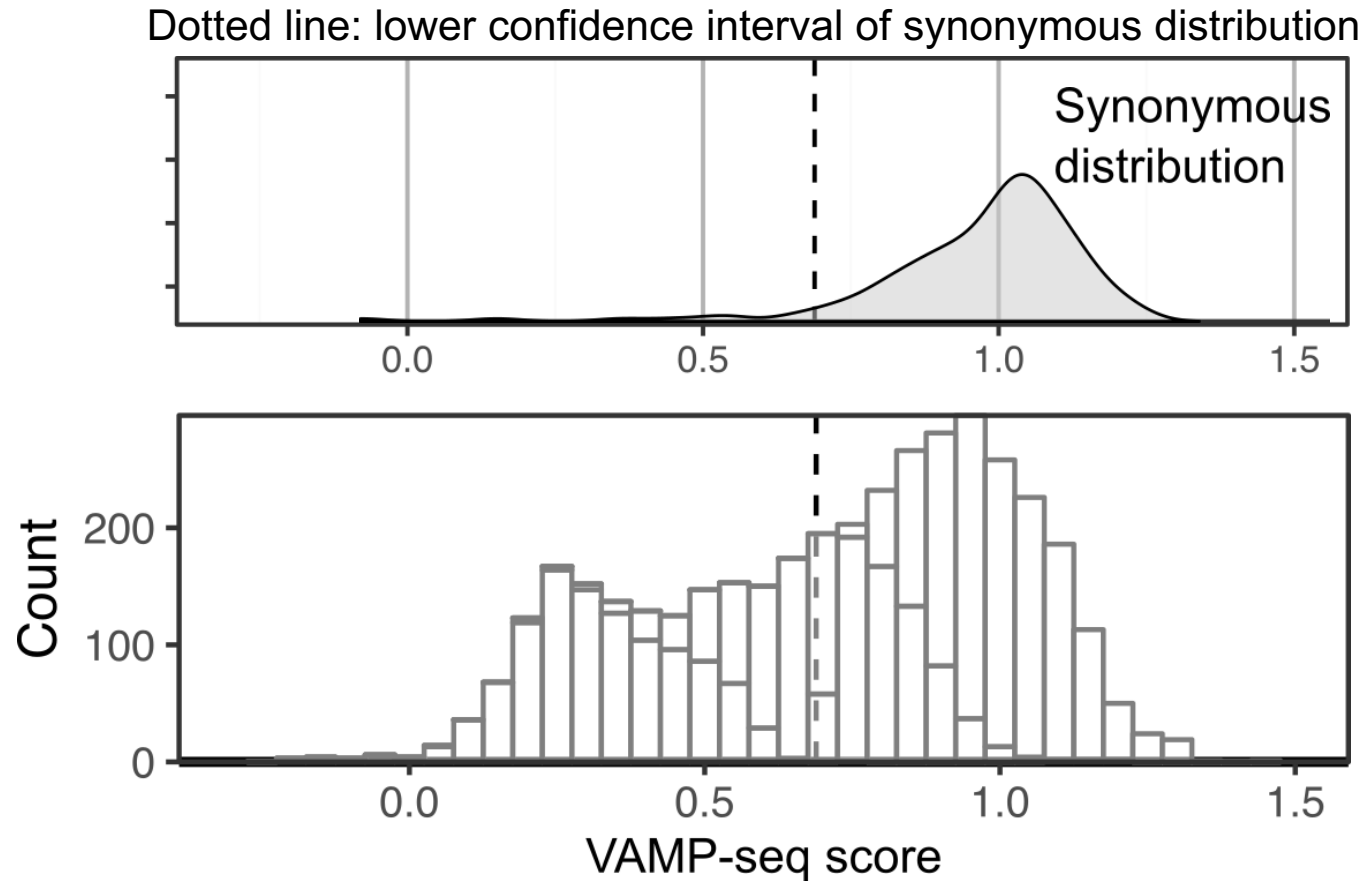
Cyan: Membrane interacting positions

Grey: Both (Enhanced and known membrane interacting)

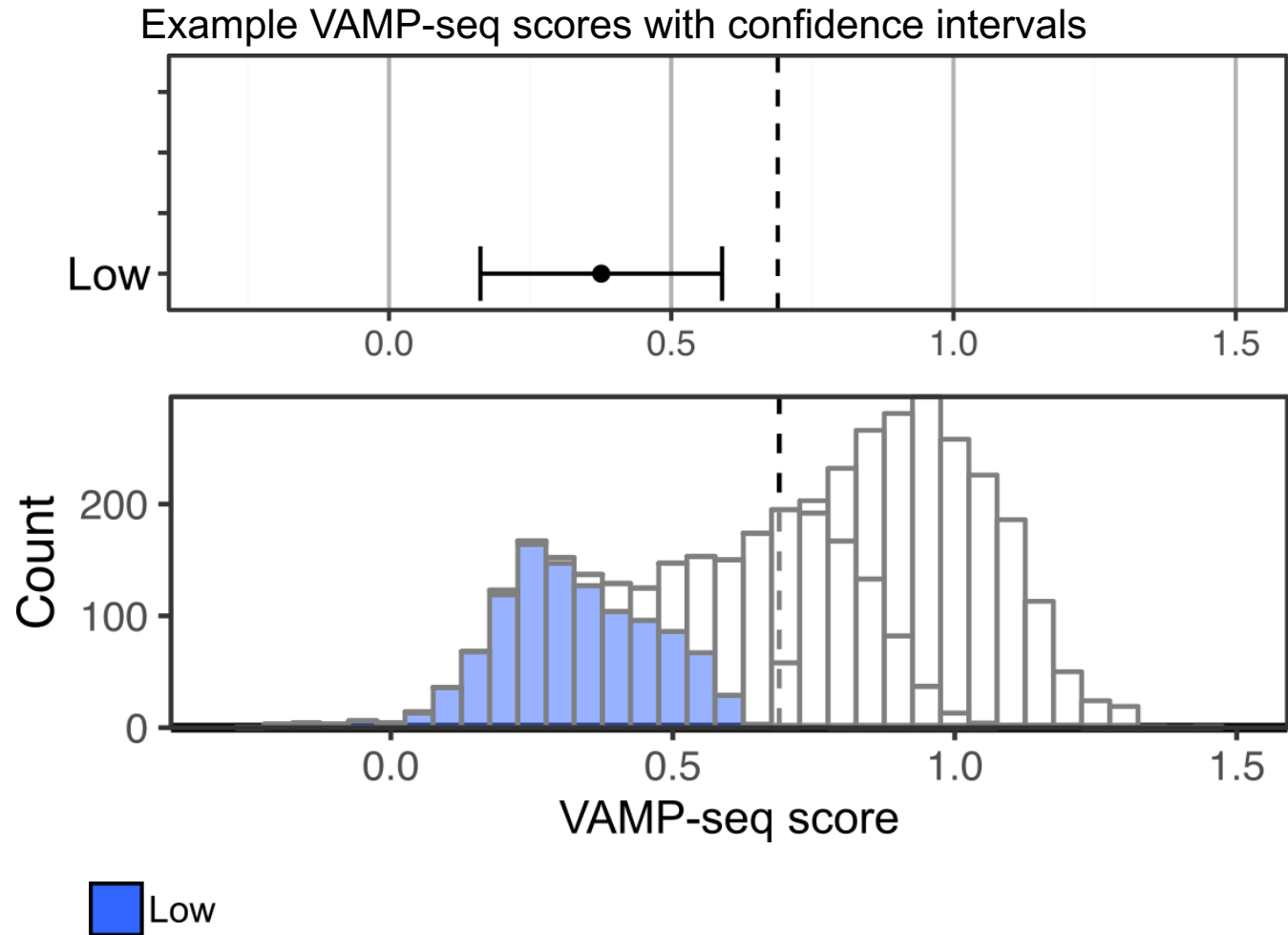
Loss of negative regulation enhances PTEN abundance



Low abundance variants are classified with high confidence

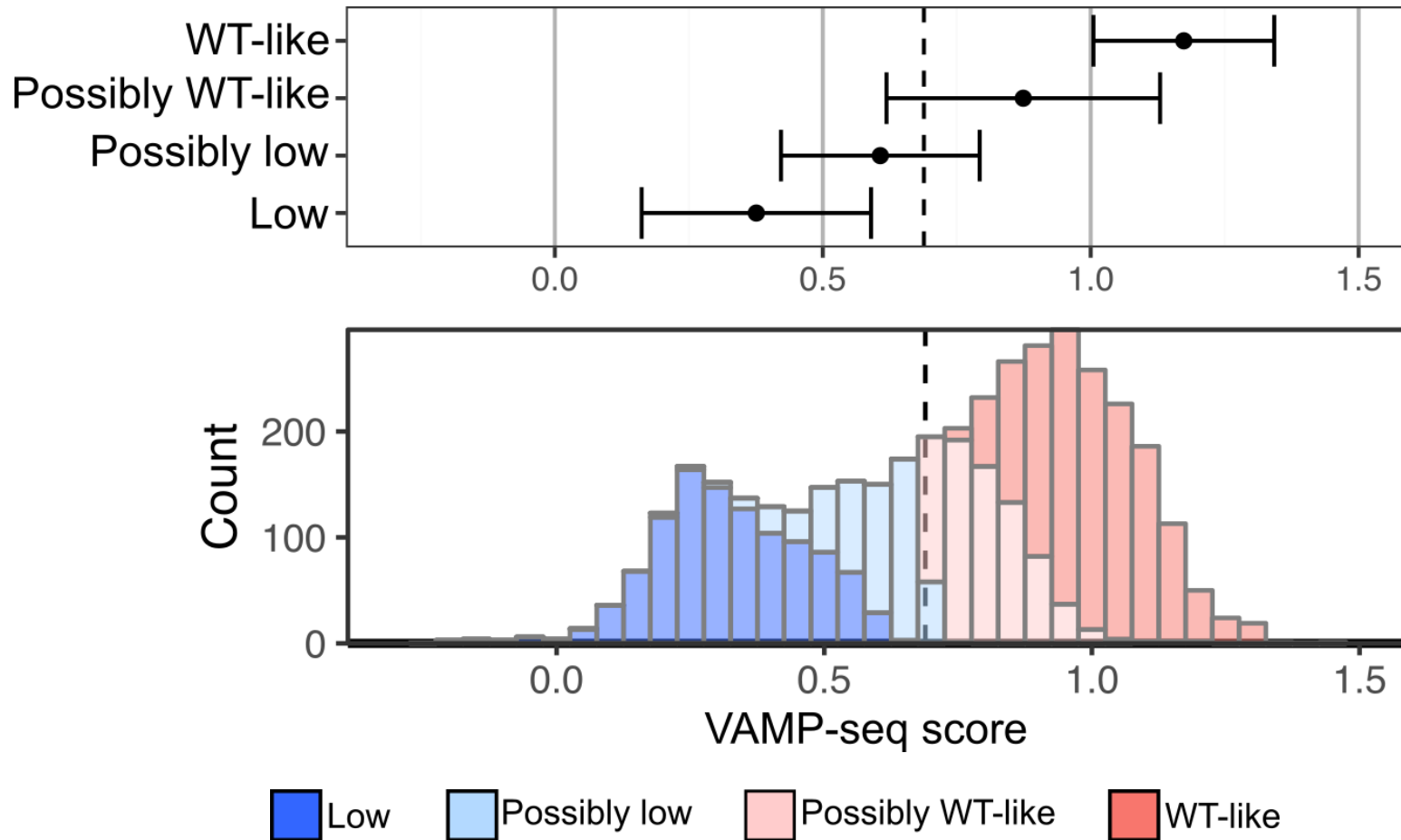


Low abundance variants are classified with high confidence

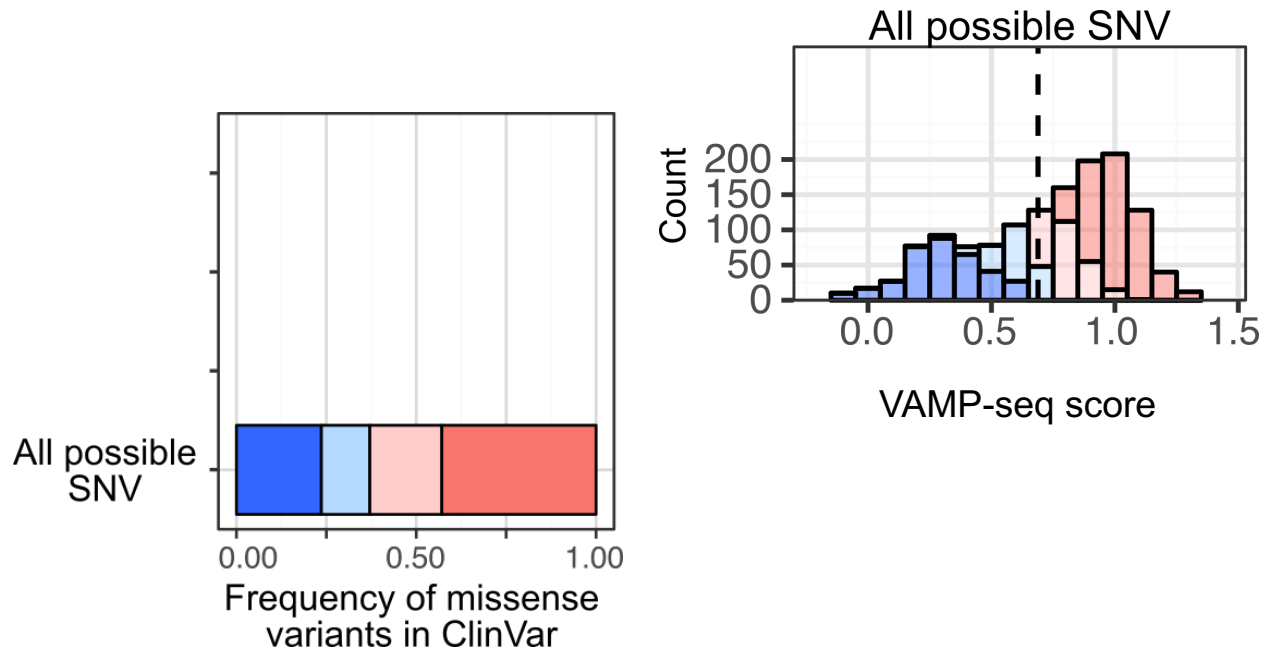


Remaining variant classes describe the spectrum of abundance phenotypes

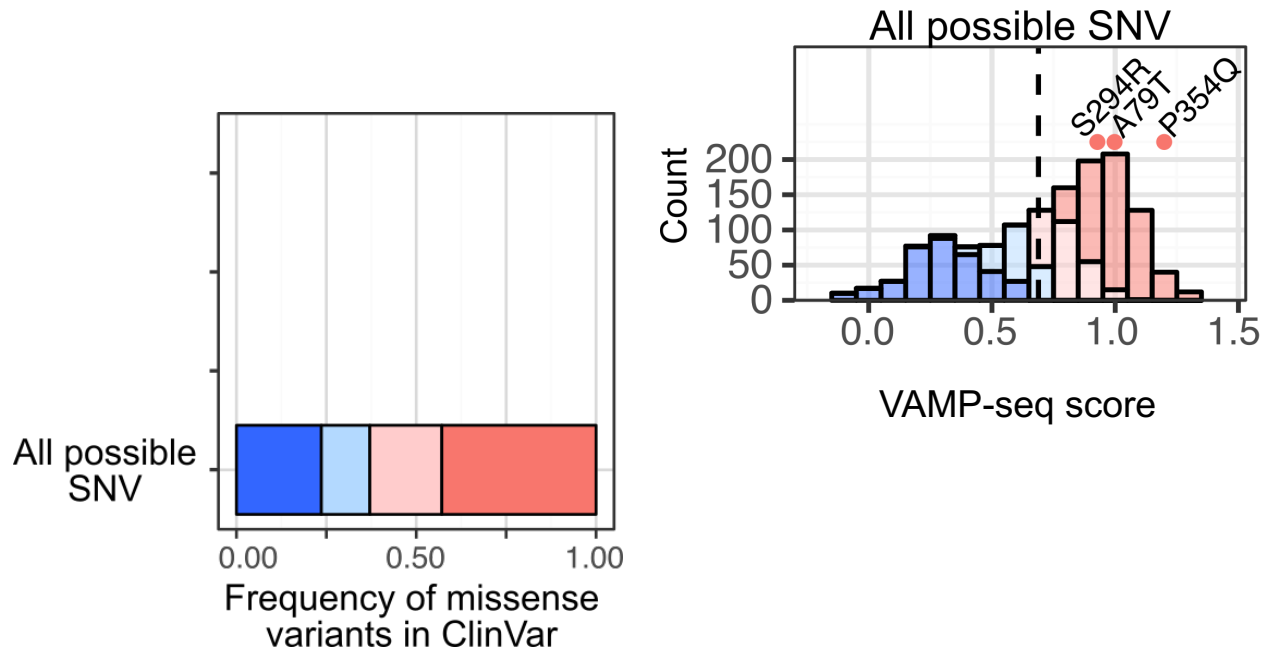
Example VAMP-seq scores with confidence intervals



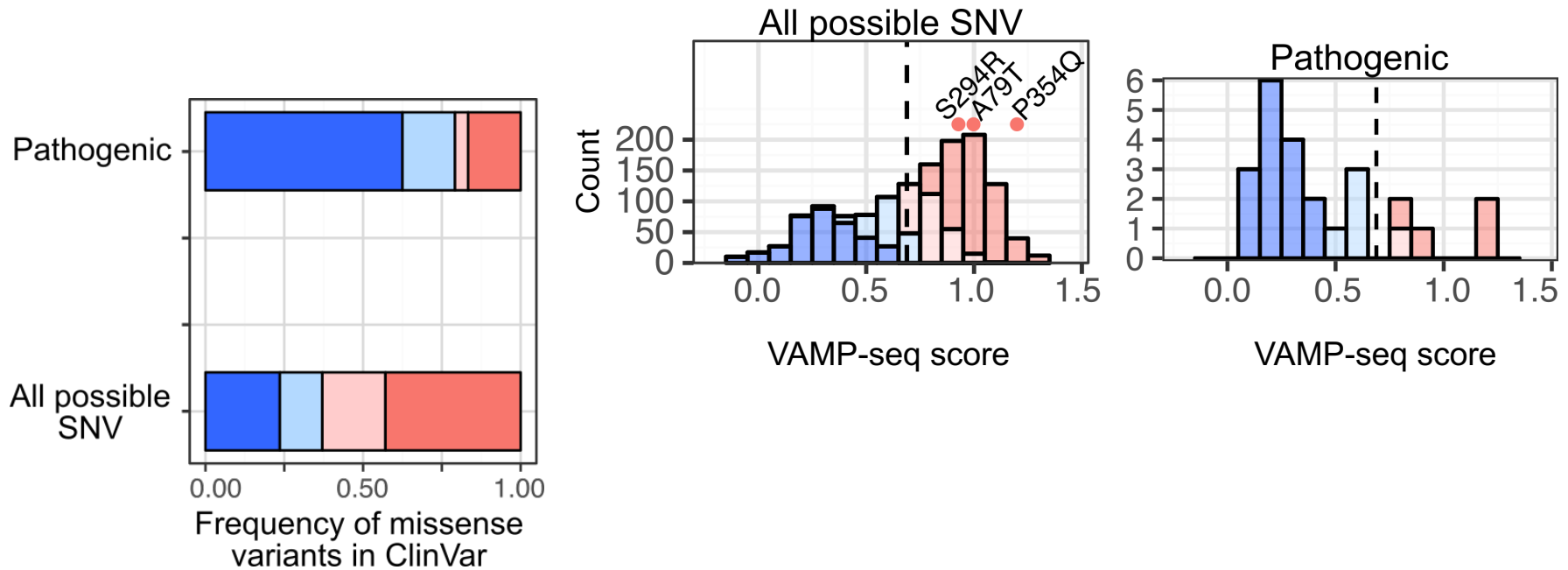
Utility of VAMP-seq for phenotyping PTEN missense variants



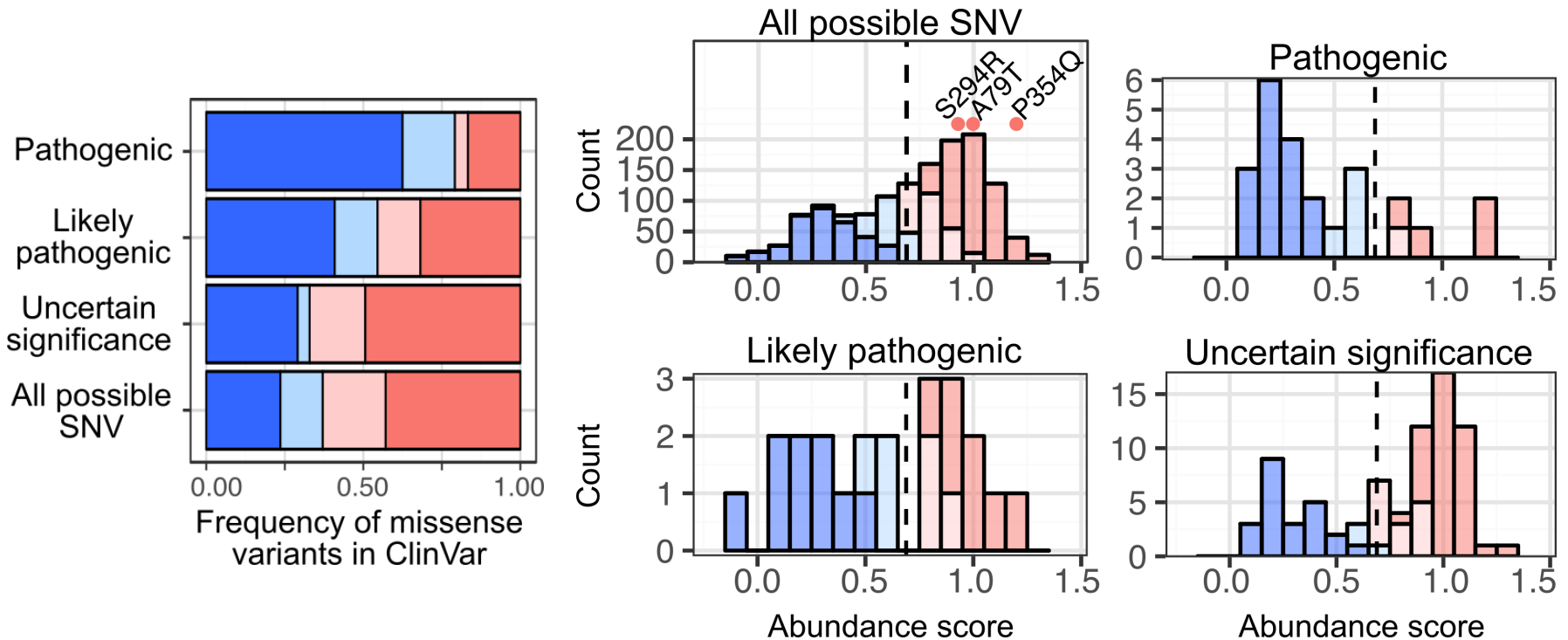
Low frequency natural variants exhibit WT-like abundance



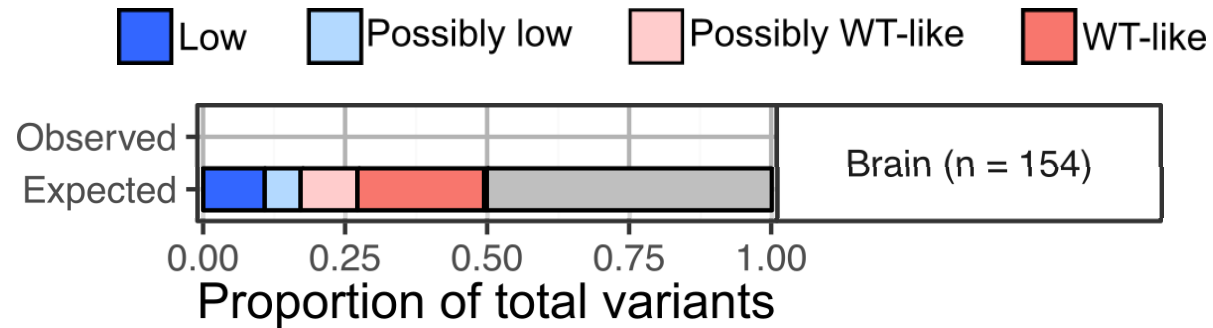
PTEN low-abundance missense variants are enriched in the ClinVar pathogenic class



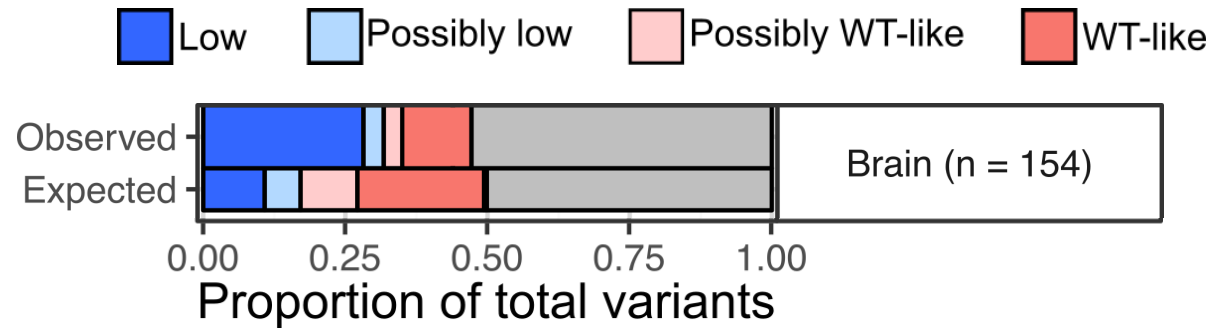
Low abundance variants are not enriched in other ClinVar classes



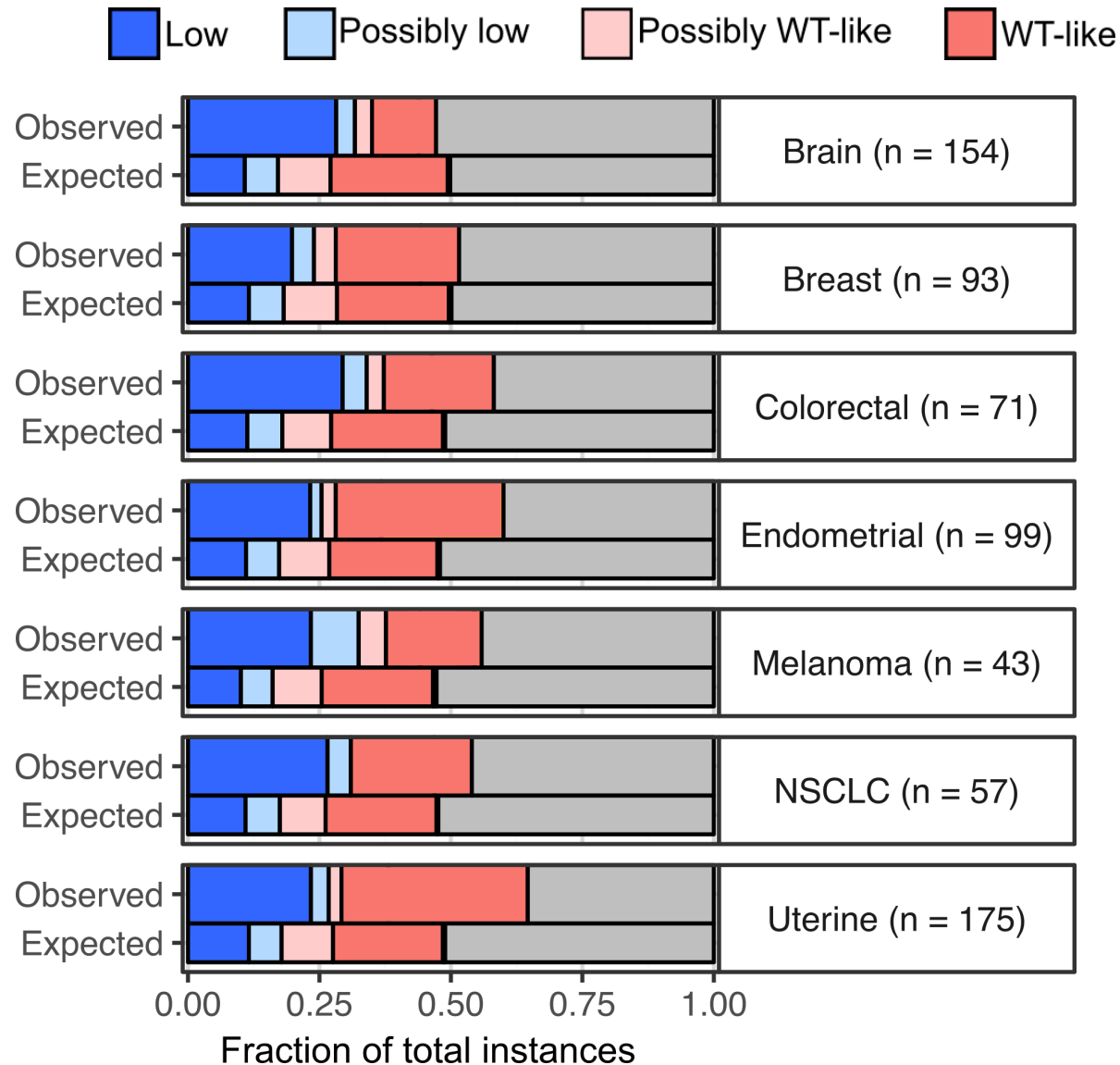
Assessment of PTEN abundance variants in cancers



Low abundance PTEN variants are enriched in all cancers

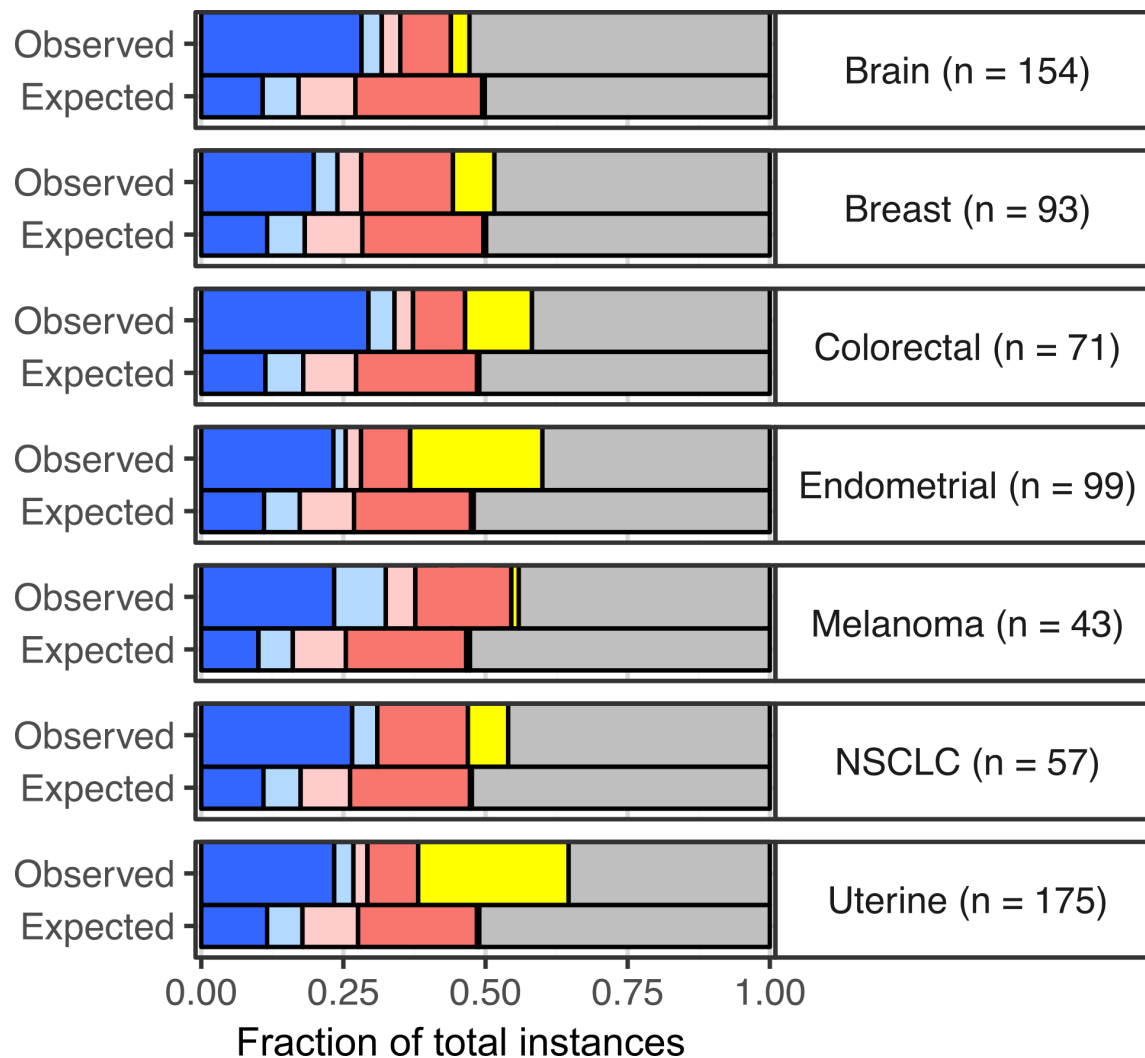


Low abundance PTEN variants are enriched in all cancers



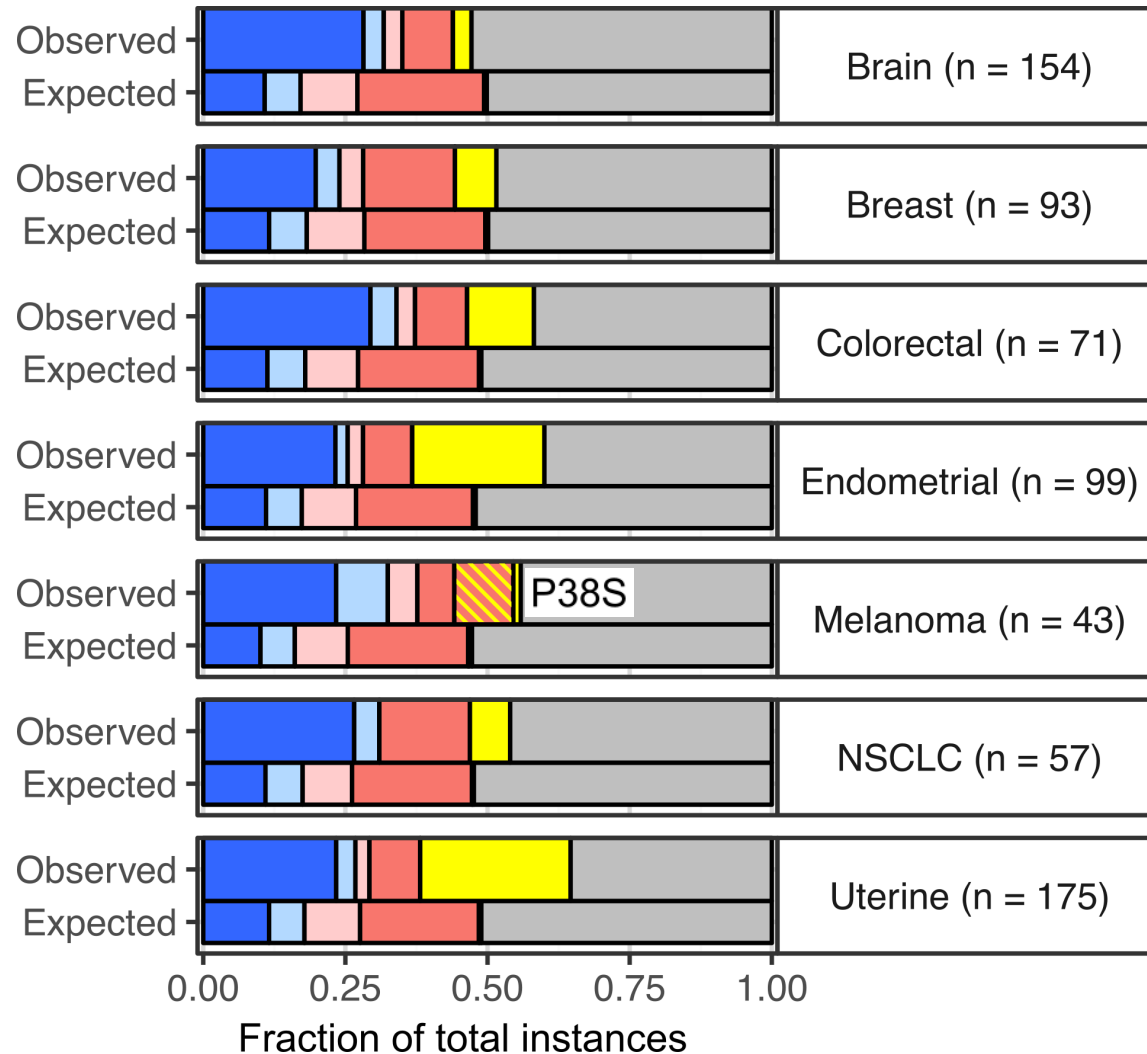
PTEN dominant negative variants are associated with more severe pathological phenotypes

Low Possibly low Possibly WT-like WT-like Known dominant negative (4)

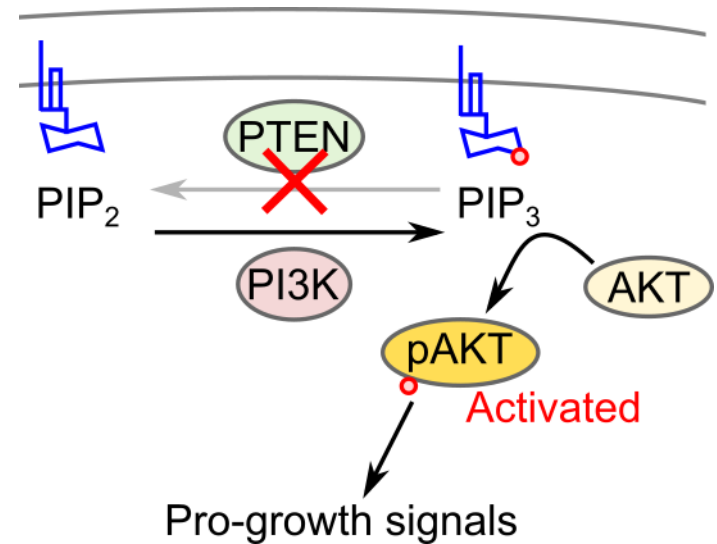
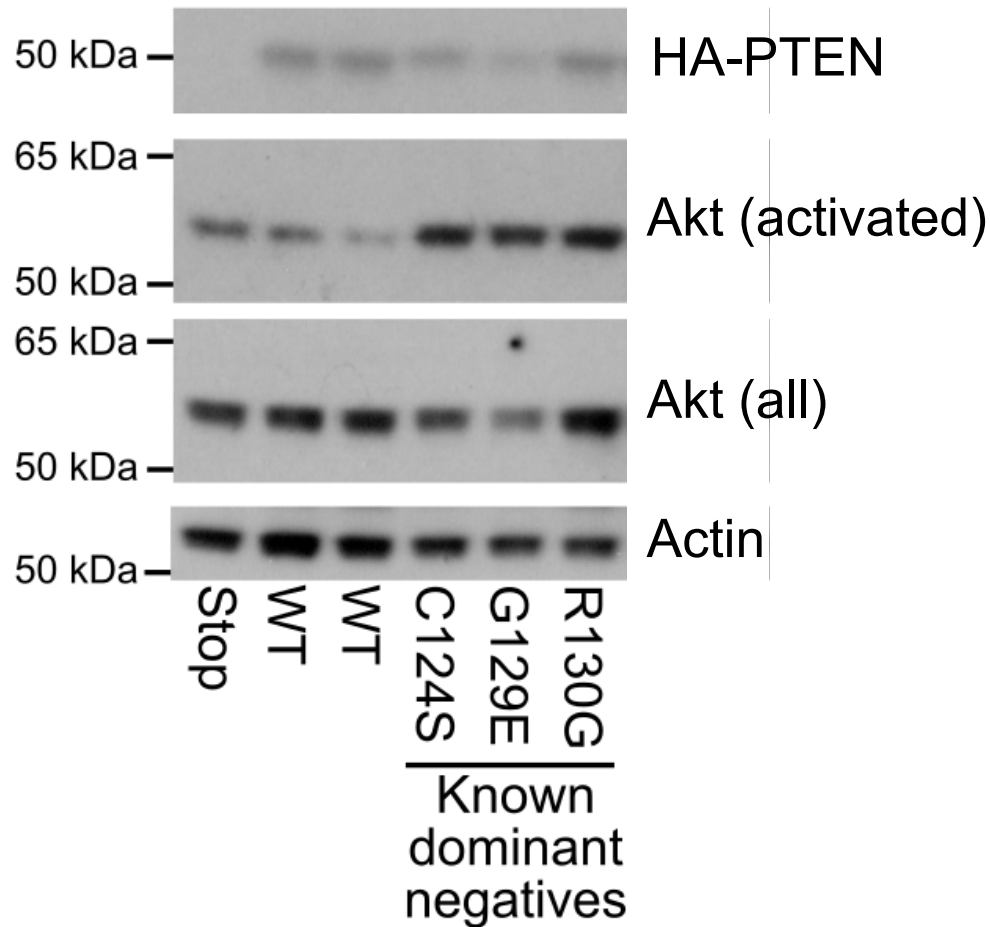


VAMP-seq identifies P38S as a possible dominant negative

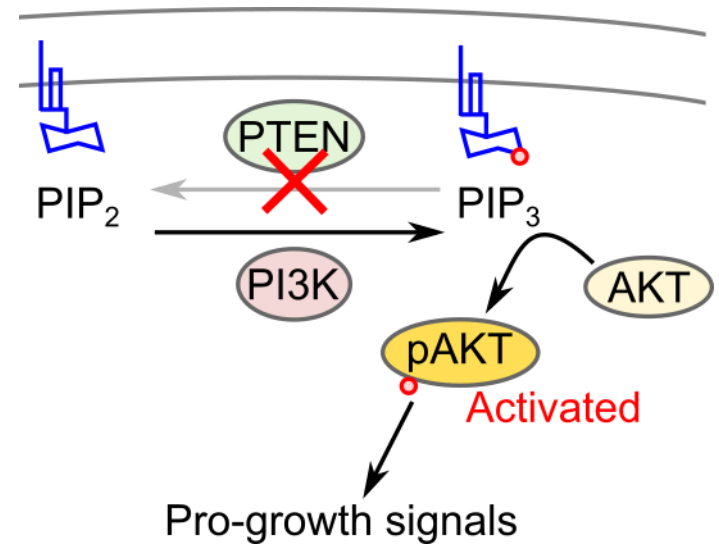
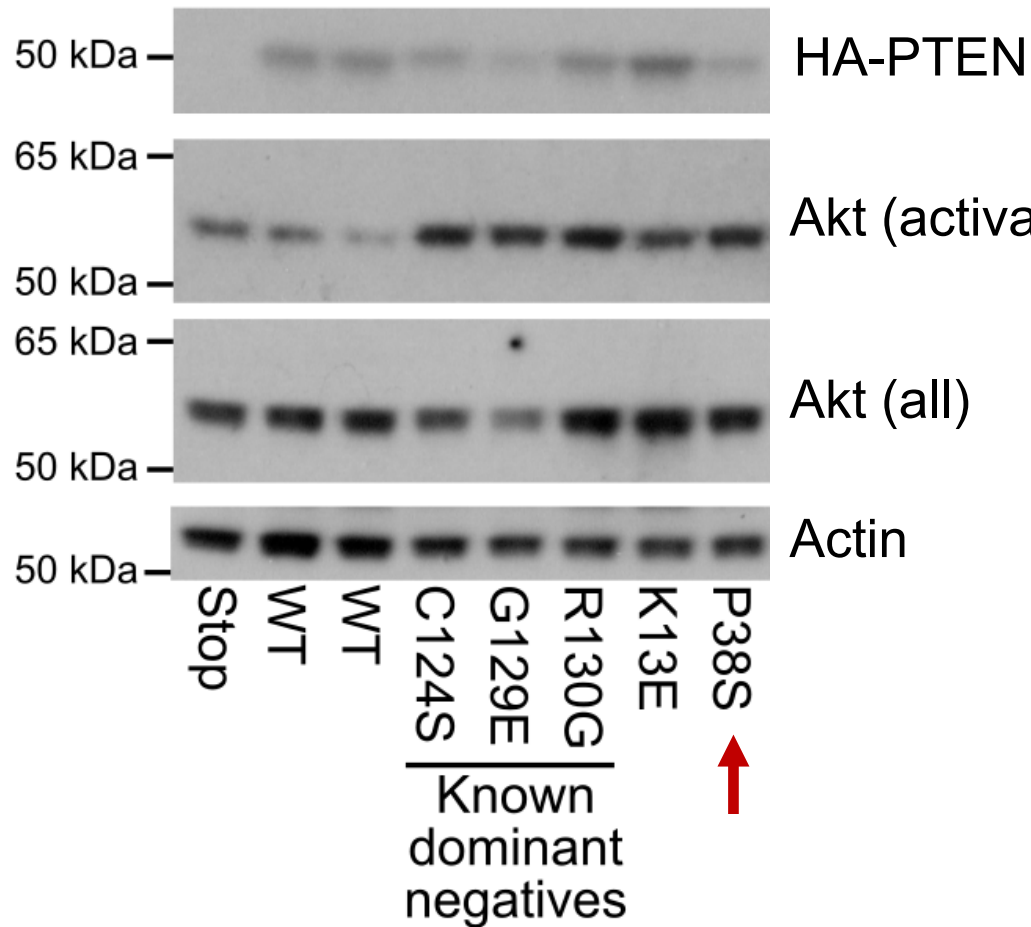
Low Possibly low Possibly WT-like WT-like Known dominant negative (4)



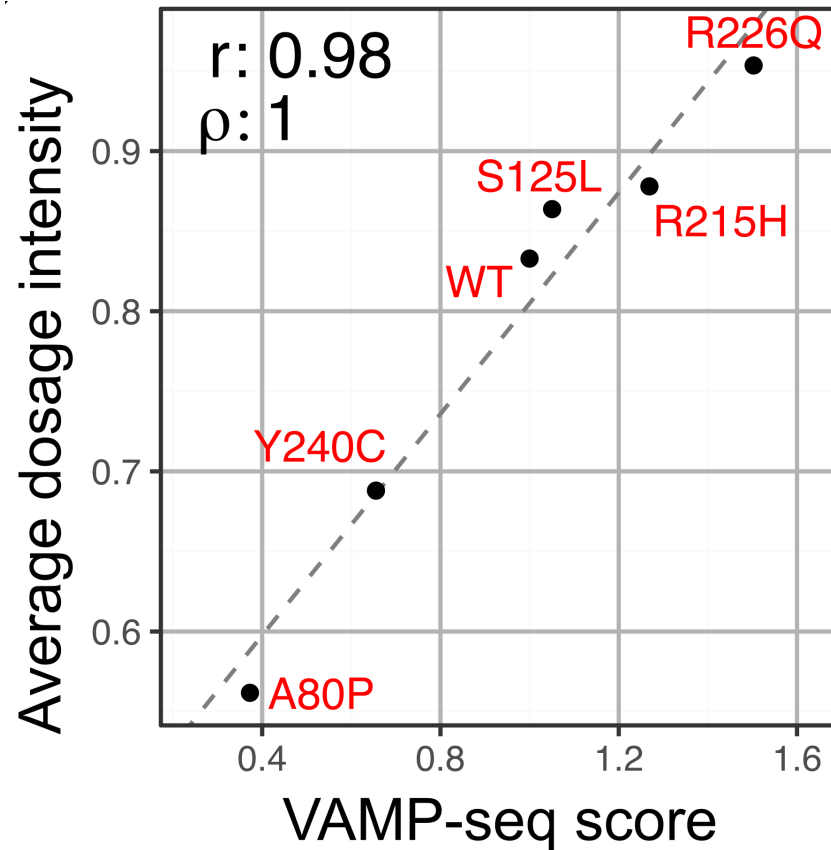
PTEN dominant negative variants result in elevated Akt activation



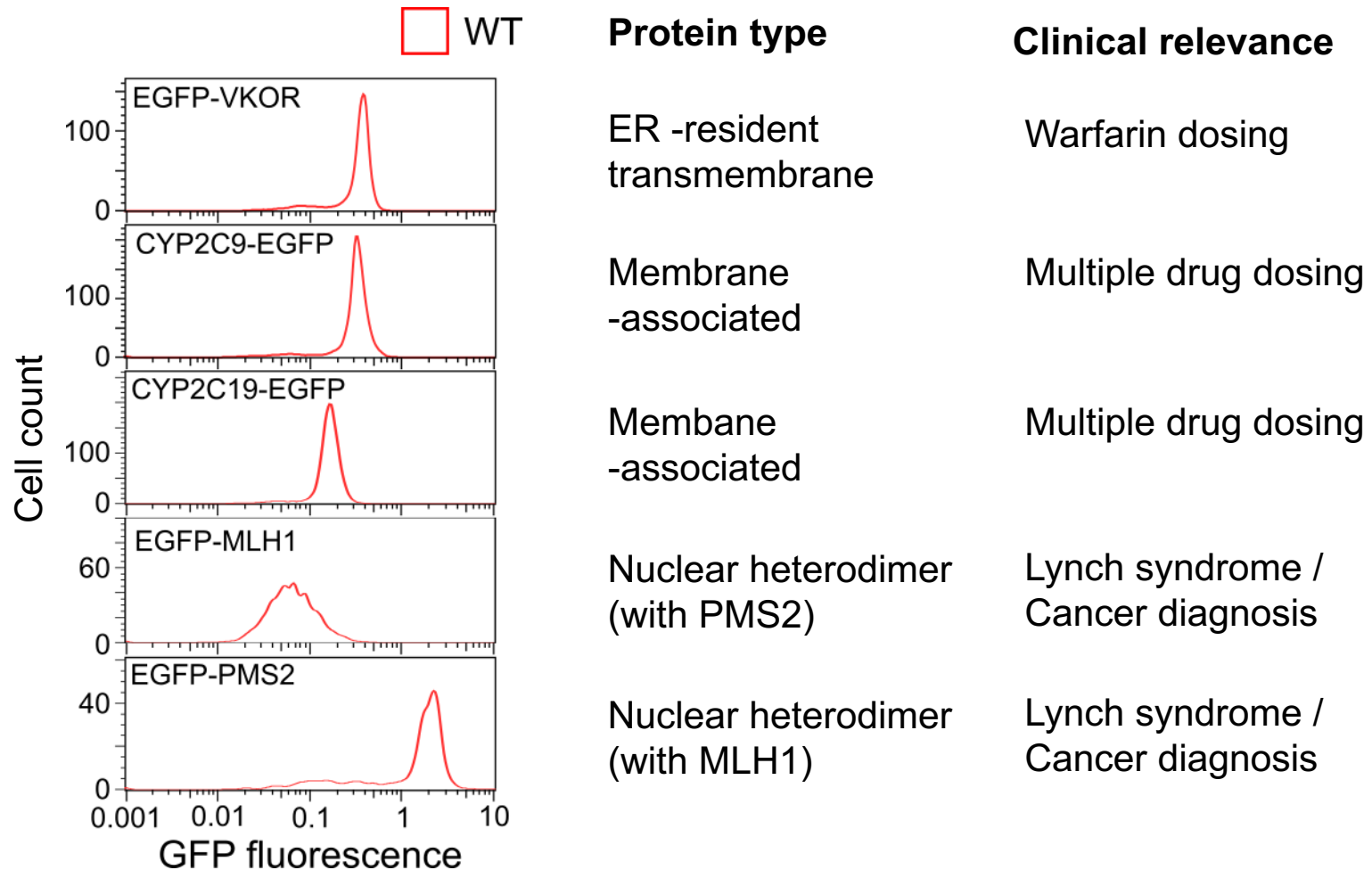
PTEN dominant negative variants result in elevated Akt activation



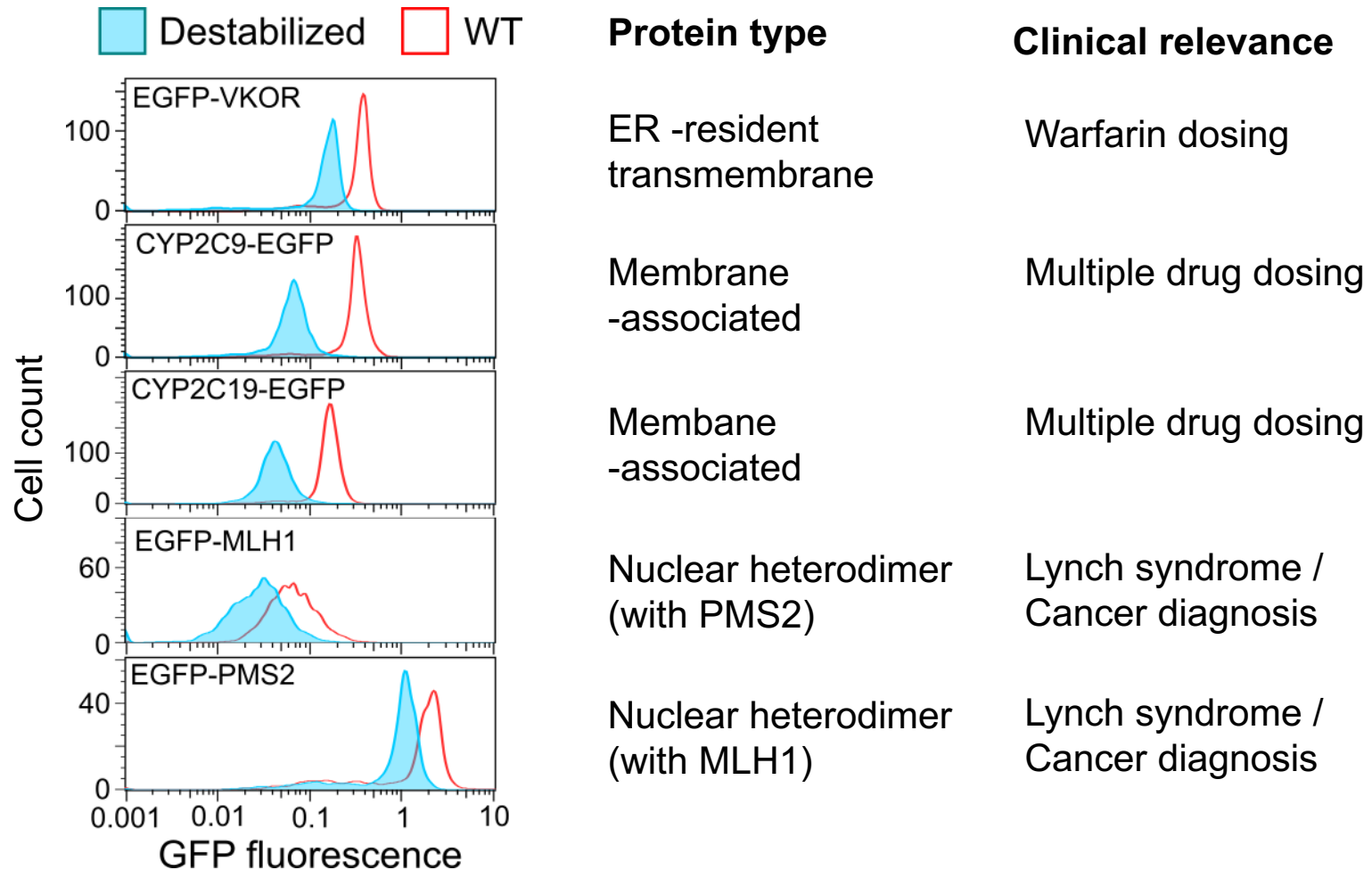
TPMT pharmacogene VAMP-seq scores correlate with chemotherapeutic dosing



VAMP-seq is likely applicable for diverse proteins / clinical applications



VAMP-seq is likely applicable for diverse proteins / clinical applications



Acknowledgments

Fowler lab:



Doug Fowler



Jason Stephany

Melissa Chiasson

Vanessa Gray

Arineh Khechaduri

Shendure lab:



Jay Shendure



Lea Starita

Beth Martin

Martin Kircher

Ron Hause

Jennifer Dines, Brian Shirts, Colin Pritchard
(UW Medical Genetics and UW Lab Medicine)

Support:



ACS Postdoctoral
Fellowship
(PF-15-221-01)

UW/FHCRC Training
Grant in Cancer
(2T32CA080416)