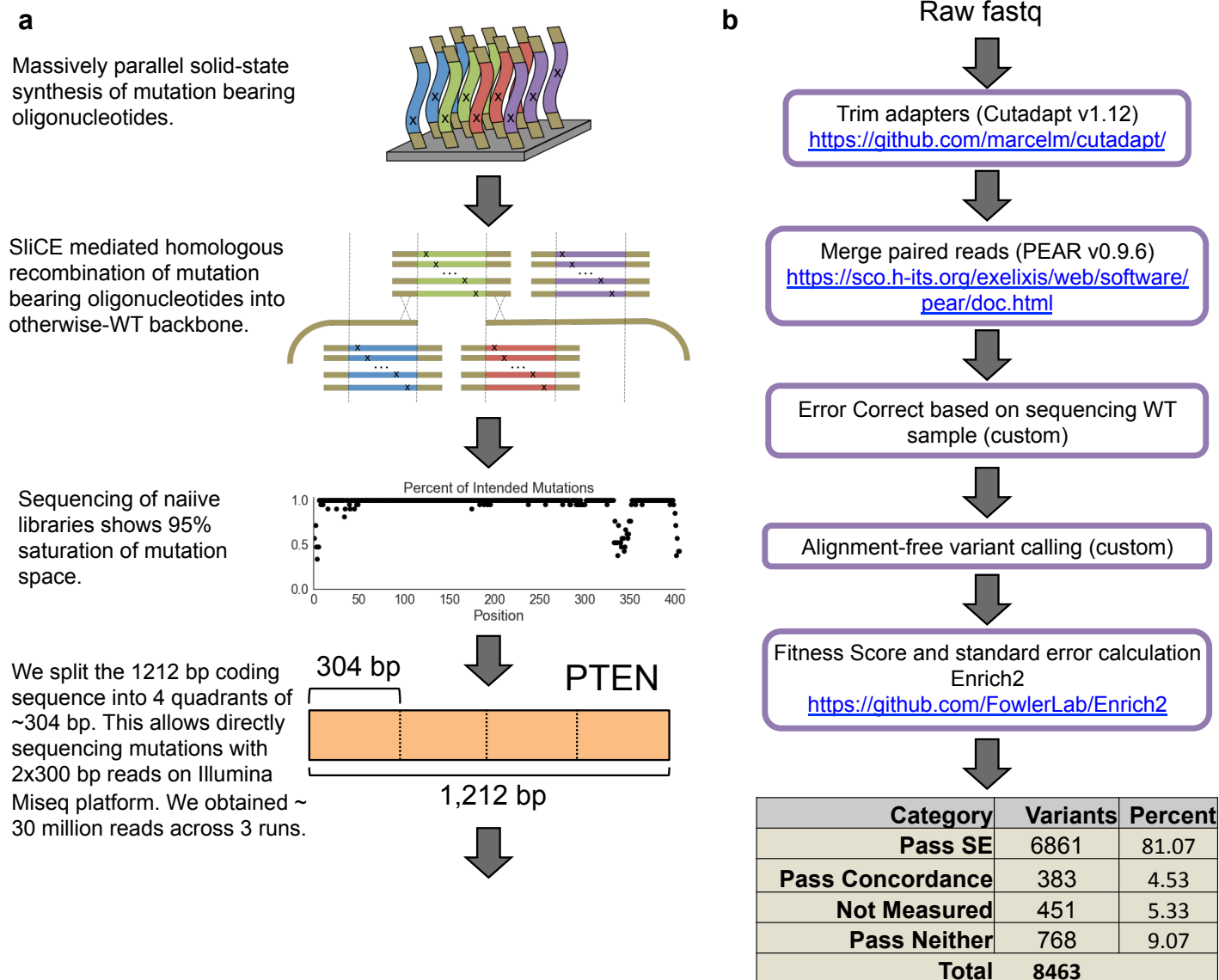
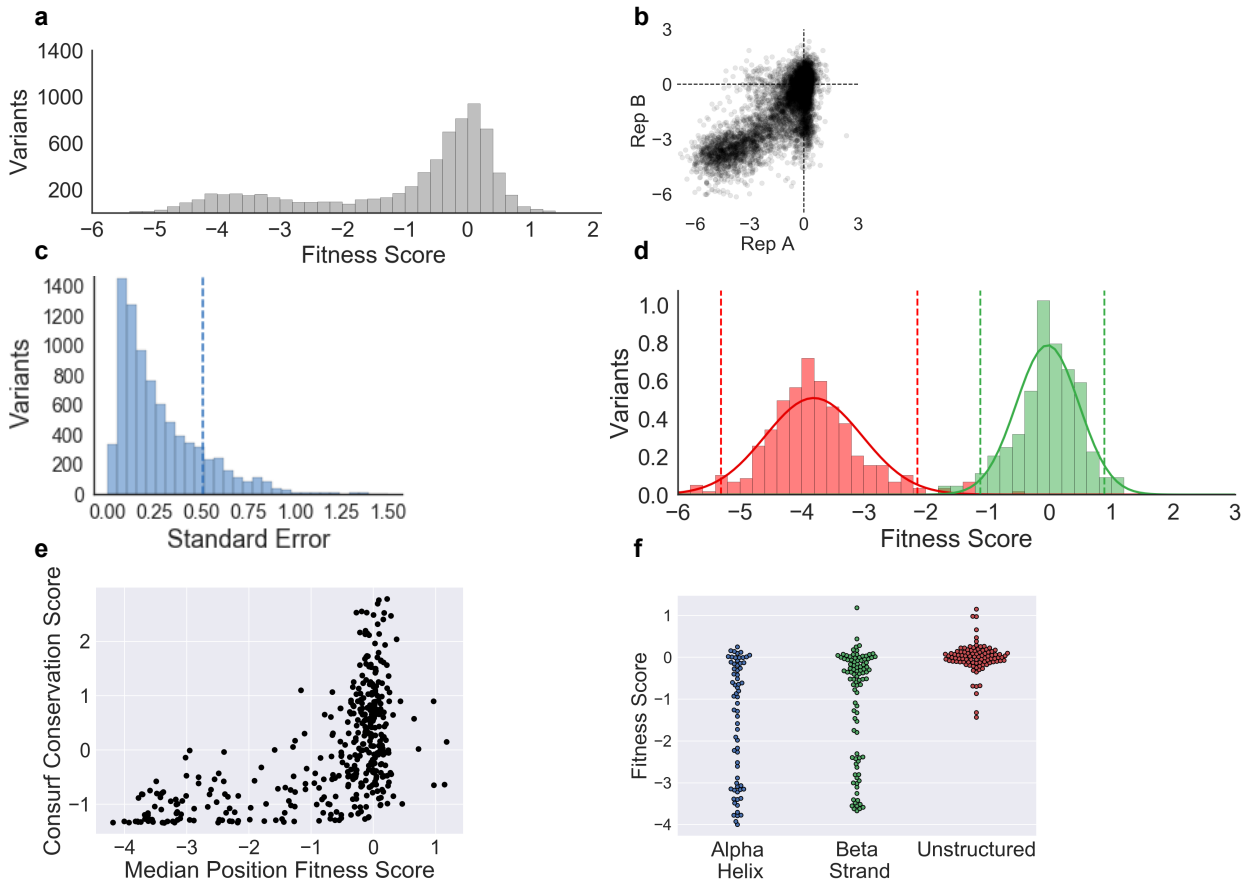


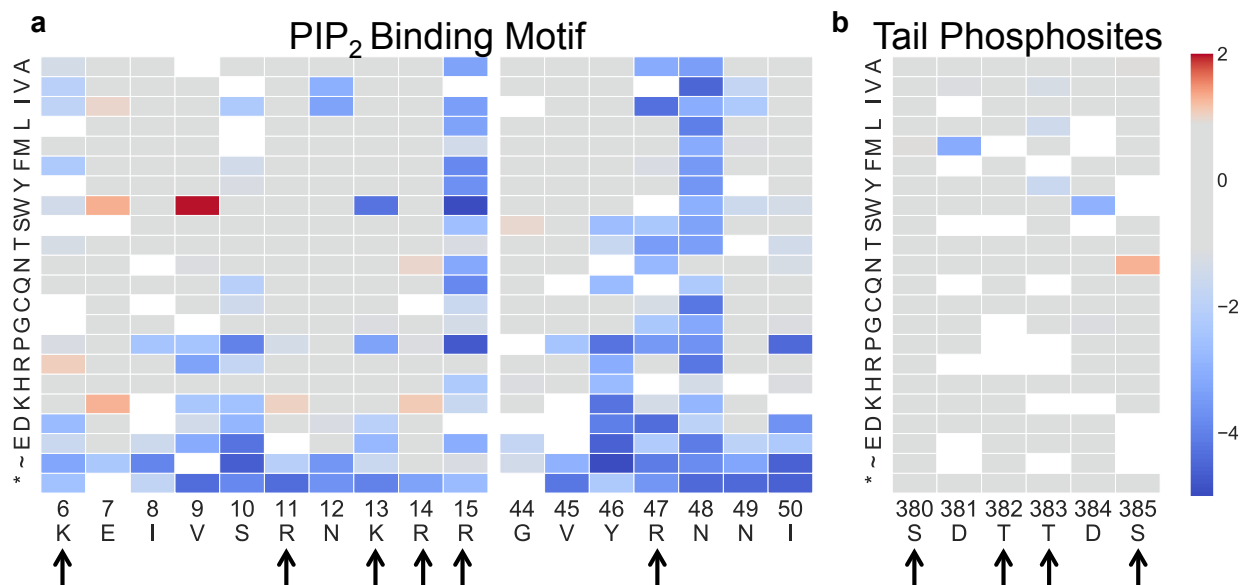
Supplementary Fig. 1 | Optimization of humanized yeast assay for liquid culture induction and selection. We performed a pilot experiment with ~400 variants (single tile) to determine when effect size was maximized under induction conditions. We sequenced the input library (pre-induction) and p110 α and PTEN induced populations at indicated time points. At each time point, five million yeast cells were passaged to fresh induction medium and the remainder used for DNA extraction. Displayed are the relative read counts of each variant, plotted in the same order as input. Effect size reaches a plateau at 48 hours, which we then used as the selected time points for the rest of the experiments in this study.



Supplementary Fig. 2 | Schematic overview of mutagenesis and computational workflow. **a**, We generated a saturation mutagenesis library by incorporating single-mutation-bearing oligonucleotides into an otherwise wild-type backbone. Oligos were synthesized on solid-state arrays (CustomArray) in 31 individual tiles/pools. Oligo tiles were PCR amplified separately. Long range PCRs of otherwise wild-type plasmid with custom primers for each tile were used as template for SliCE mediated homologous recombination. We divided the protein coding sequence into 4, ~300 bp fragments/quadrants so that we could cover each entire mutation-bearing segment with 2x300 base-pair (bp) paired-end sequencing reads. Mutagenized plasmids were transformed into bacteria. Clones from individual mutagenesis tiles were pooled by quadrant and transformed into yeast for functional assays. **b**, Overview of the computational pipeline for processing reads and obtaining fitness scores. Variant predictions were considered high-confidence if passing a standard error (SE) filter or showing concordant effects between two biologic replicates (Methods).

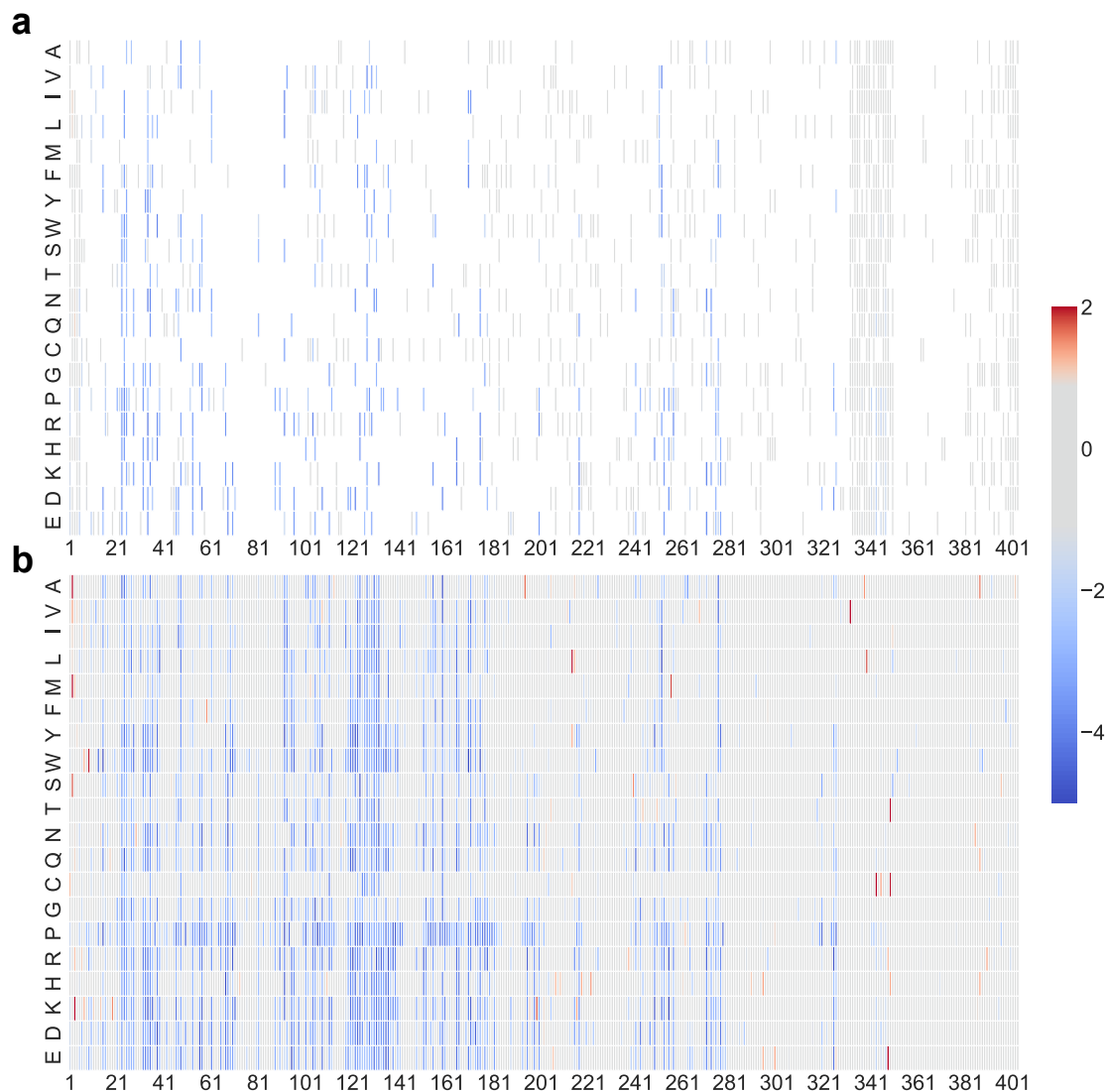


Supplementary Fig. 3 | Overview of PTEN saturation mutagenesis dataset and relative fitness scores. a, Distribution of fitness effects for all high-confidence variants (7,244) derived from two biologic replicates, with three technical replicates each. **b**, Biological replicates show high correlation (Pearson's $r = 0.76$). **c**, Distribution of standard errors for measured variants. High-confidence variants to the left of the dashed line have 95% confidence intervals less than or equal to one natural-log fold change. **d**, The distributions of truncating mutations (excluding those in the regulatory tail) (red, left) and synonymous wild-type like mutations (green, right) are shown. Dashed lines indicate the two-tailed 95th percentile limits for synonymous and truncating variants. **e**, The median fitness score of all high-confidence scores at each position is correlated with the evolutionary conservation at that position (Spearman $\rho = 0.58$). Evolutionary conservation for all positions was obtained with ConSurf (<http://consurf.tau.ac.il/2016/>), using following options: "Amino-Acids", "No known protein structure", "No MSA", and default homolog search parameters. **f**, Comparison of median fitness scores for positions in alpha helices, beta strands, or unstructured regions. Alpha helix and beta strand assignments obtained through STRIDE (<http://webclu.bio.wzw.tum.de/cgi-bin/stride/db.py>) for pdb structure 1D5R. Unstructured positions are those absent from the crystal structure (1-13, 282-312, 352-403).



Supplementary Fig. 4 | Evaluation of mutation effects within the PTEN predicted PIP₂ binding motif and tail phosphosites. **a**, Fitness scores highlighting positively charged residues in PIP₂ binding domain (K6, R11, K13, R14, R15) as well as R47, with neighboring residues. K13, R15, and R47 are the most critical in our assay. **b**, Fitness scores for C-terminal regulatory tail phosphosites (S380, T382, T383, S385) and neighboring positions.

sampled from our high-confidence dataset and retrained models at each indicated percent map completeness. The maximum value is 82.3%, which is the percent map completeness that our high-confidence missense dataset represents. 5 replicates were performed at each point % map completeness. Options same as above, except `random_state=None`.



Supplementary Fig. 6 | A comprehensive functional map of predicted effects of PTEN mutations using imputed scores. a, We trained the random forest algorithm on 6,300 missense variants that were measured with low standard error (95% confidence interval < 1 fitness score). We omitted single residue deletions and nonsense mutations. We then predicted the fitness score of the remaining 1,357 variants. Imputed values are colored according to their fitness score. Variants used in the training are white. **b**, Complete sequence function map with high-confidence measurements in addition to imputed values.