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Supplementary Materials for

Prediction of immunotherapy response using mutations to cancer protein assemblies

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

Supplementary Text

Model performance in Samstein cohort

It has been previously suggested that comparing patients with similar predicted risks to compute CI measures does not lead to clinically meaningful predictions (*77*). To reduce the number of comparisons between patients with similar predicted risks, we considered a threshold of 30%, where patients with top and bottom 30% of the predicted risk scores were selected (totaling 60% of the test dataset) (Fig. S6). To test the robustness of our approach, we recomputed the predictive performance across different thresholds (Fig. S6). To complement the analysis of protein assemblies in NeST, we also evaluated AMBs for gene sets documented in Reactome (*78*) and GO-Slim (*79*). We found that neither pathway database improved prediction performance compared to using NeST in the discovery cohort (Fig. S7).

In-vivo CRISPR screening in mice

To functionally validate the importance of the assemblies in modulating immunotherapy response, we used a public CRISPR screening data in a lung cancer model (*37*). Lewis lung carcinoma cell line was used for the CRISPR screens. A recent study suggested that Lewis lung carcinoma is similar to human lung adenocarcinoma (*80*), which allows investigation of this screening data suitable for our study. STARS algorithm (*81*) was used to compute the gene-level statistical significance of CRISPR screening results. We tested for significant differences (significance score) in single-guide RNA abundance between untreated and anti-PD-1 + anti-CTLA4-treated immunocompetent mice. The resulting significance scores were used to calculate the enrichment of an assembly by running a Gene Set Enrichment Analysis (GSEA) (*82*). We used the GSEApy (*83*) python package to compute assembly-level significance (FDR corrected p-value), identifying 77 enriched assemblies at a False Discovery Rate (FDR) of 0.25 (Fig. 4C).

Relating AMB predictions to immune phenotypes

We used the AMB model to calculate the predicted ICI responses of each patient in the TCGA lung adenocarcinoma (TCGA-LUAD) (*40*) and lung squamous cell carcinoma (TCGA-LUSC) (*41*) cohorts, as these cohorts include matched somatic mutation, mRNA expression, and histology imaging data. We designated the 20% of TCGA patients with the lowest predicted risk scores as "predicted responders" (low-risk patients) and the others as "predicted non-responders" (high-risk patients). To compute the association between these AMB predictions and immune infiltration phenotypes, we considered 8 individual features that were generated from gene expression or histology. The gene expression features included inferred proportions of (i) CD8+ T cells, (ii) M1 macrophages, (iii) CD4+ T cells and (iv) regulatory T cells, all obtained from a previous study (*84*). We additionally computed the expression of signals related to tumor-associated macrophages, by leveraging a set of genes (*6*, *85*) for which high mRNA expression is indicative of the feature. We conducted a single-sample gene set enrichment analysis (ssGSEA) using the GSEApy Python package (*83*) to score each gene set in each tumor sample.

Quantification of tumor-infiltrating lymphocytes using histology

To analyze hematoxylin and eosin (H&E)-stained histology image-based features (Fig. 5), we used the Lunit SCOPE IO software to classify the tumor microenvironment based on the spatial distribution and density of tumor-infiltrating lymphocytes (TILs) (*39*). Lunit SCOPE IO is composed of two deep learning models: a cell detection model and a tissue segmentation model. The cell detection model detects the location of lymphocytes and tumor cells, which employs a DeepLabV3+ (*86*) architecture with a Resnet-34 (*87*) backbone. The tissue segmentation model determines if a pixel belongs to cancer epithelium, cancer stroma, or background regions, using the same architecture as the cell detection model. The cell detection model was developed with

patches extracted from 3,334 whole-slide images (WSIs; N=2,485 for training and N=849 for tuning) and the tissue segmentation model was developed with patches extracted from 15,830 WSIs (*N*=15,004 for training, and *N*=826 for tuning). To perform spatial analysis of heterogeneous TIL distributions within WSI of various sizes, each WSI was divided into 0.25 mm^2 grids, with the immune phenotype of each grid classified based on the following criteria (*88*, *89*): "Inflamed," if the TIL density within the total cancer epithelial area (CA) in the grid is ≥ 130/mm^2; "Immune-Excluded," if the TIL density within the total CA is < 130/mm^2 and that within the total cancer stroma (CS) is ≥ 260/mm^2; or "Immune-Desert," if the TIL densities are below threshold in both the total CA and CS within the grid. The final WSI-level Inflamed score, Immune-Excluded score, and Immune-Desert score were calculated by dividing the number of grids having that respective phenotype over the total number of grids analyzed.

Calculating associations between assembly-level AMB and TIL features

To test the association between immune infiltration phenotypes and AMB status of an assembly (Supplementary Fig. 3F-H), we conducted a non-linear regression (Random Forest Regression from Scikit-learn Python package (90)) that aims to predict immune phenotypes using AMB levels of 13 important assemblies. To determine the importances of assemblies in predicting immune phenotypes (Supplementary Fig. 3F-H), we computed SHapley Additive exPlanations (SHAP) values (91). Default settings of "Explainer" function from the SHAP Python package were used to measure feature importances (91). To visualize associations across assembly mutational status and immune phenotypes, we used the plotly Python package (92) to draw a Sankey diagram (Fig. 5E).

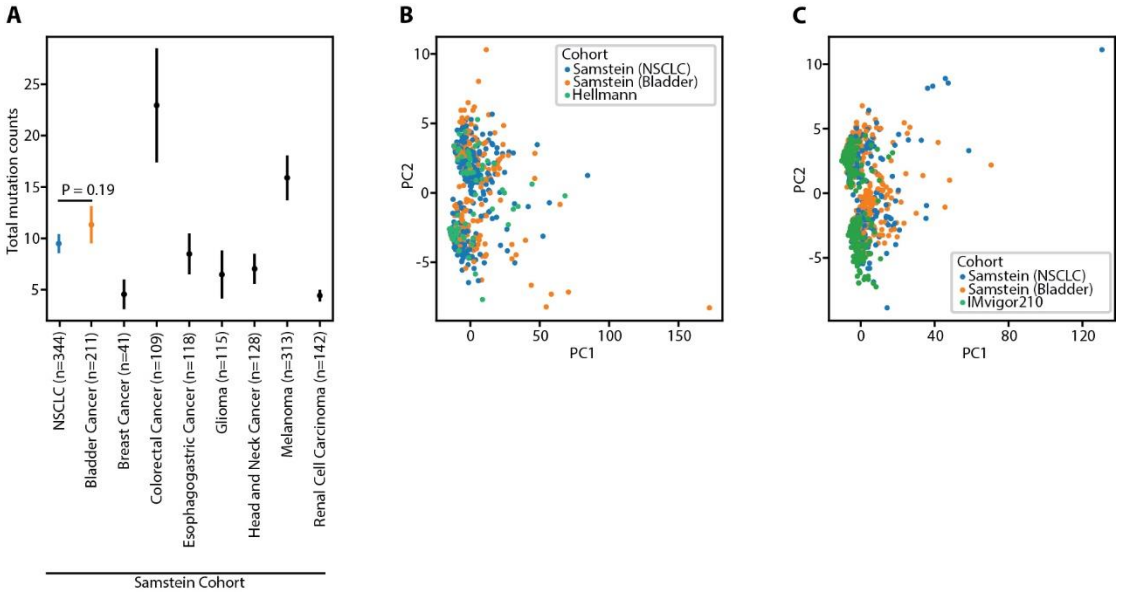

Fig. S1. Supplemental characterization of immunotherapy cohorts. A Distribution of total mutation counts for patients in the Samstein cohort. Error bars represent 95% confidence intervals. Mann-Whitney U test used to compute p-value. **B-C** Principal component (PC) analysis using the profile of AMBs across all assemblies, with PC1 and PC2 of each patient plotted for Samstein cohort and **B** Hellmann cohort or **C** IMvigor210 cohort. NSCLC: Non-Small Cell Lung Cancer.

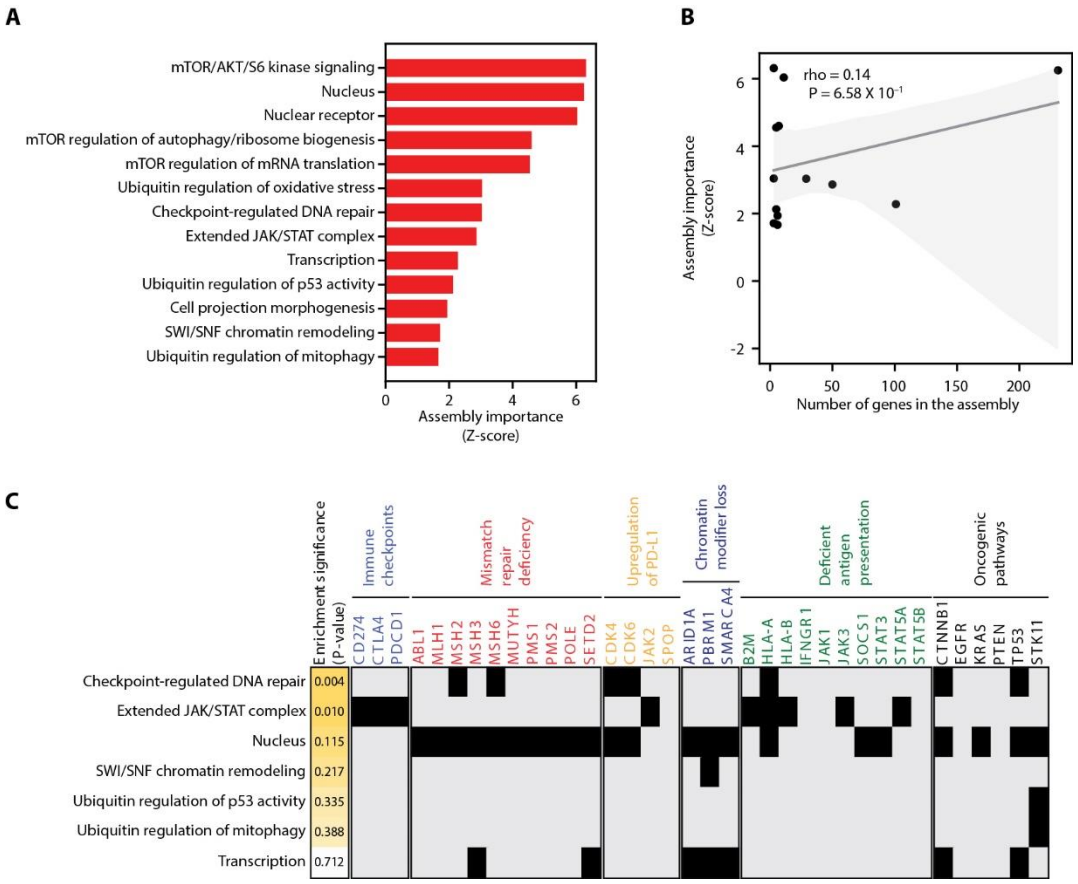

Fig. S2. Supplemental analysis of important assemblies. **A** Z-score-converted assembly importance scores from the model. Important assemblies shown ($z \geq 1.64$). **B** Importance versus size of important protein assemblies. Spearman correlation and significance of correlation is shown. Grey area represents 95% confidence of the fitted linear line. **C** Correspondence between important protein assemblies (rows) and previously reported single-gene biomarkers of ICI response (columns). Black squares indicate mapping of genes to assemblies. Enrichment of an assembly for the previously reported biomarkers is also shown (hypergeometric p-value, leftmost column).

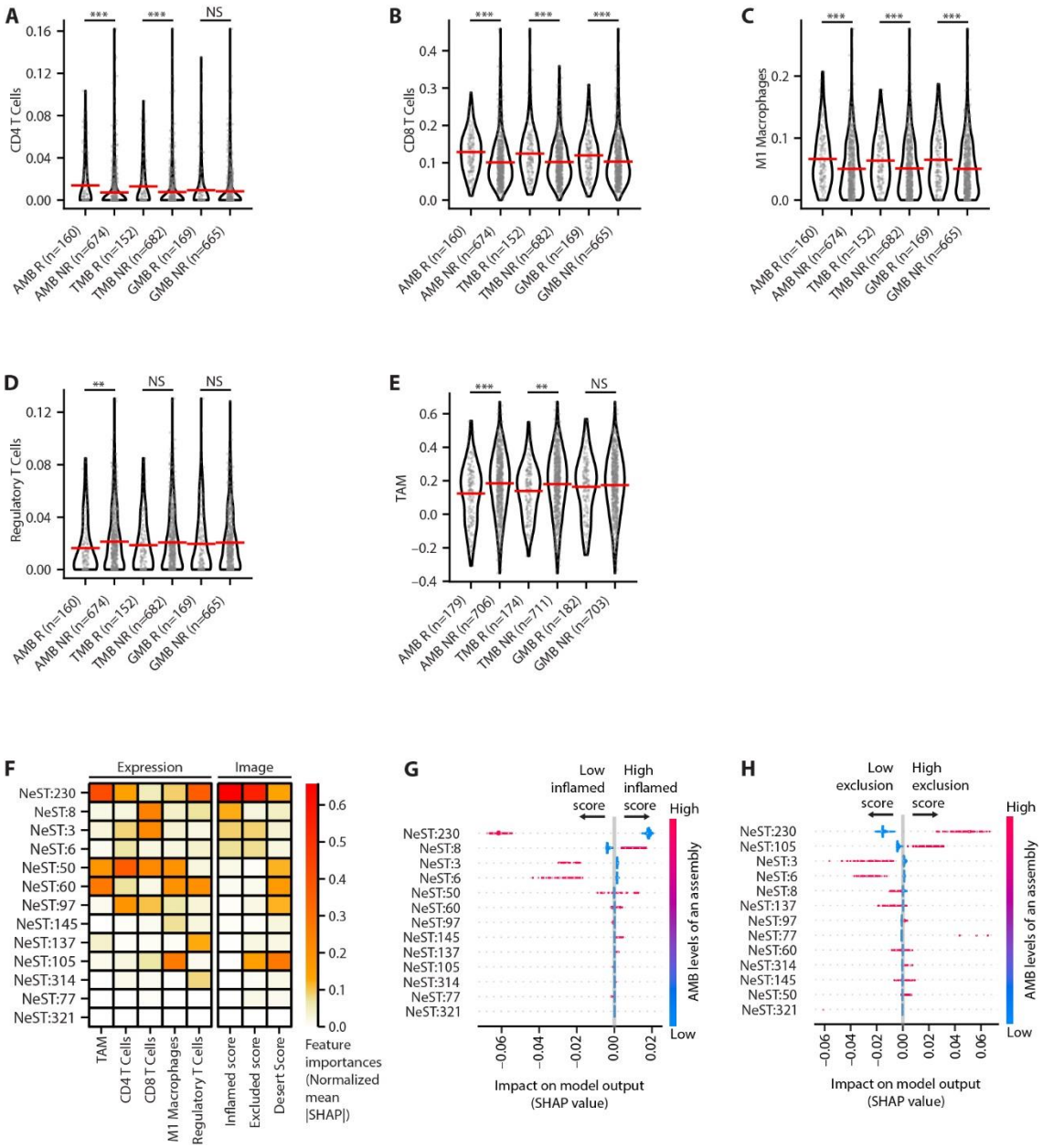

Fig. S3. Relation of AMB model to independent immunogenic features. A-E AMB predictions are correlated with various immunogenic features, which includes expression profiles related to **A** CD4 T cells, **B** CD8 T cells, **C** M1 macrophages, **D** Regulatory T cells and **E** Tumor associated macrophages (TAM). Mann-Whitney U test was used to compute statistical significance. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS $P > 0.05$. Red horizontal bars indicate the mean. **F** Feature importances of 13 important assemblies in predicting immune phenotypes. Feature importances were computed using SHAP values (average of absolute SHAP values)(*91*). Feature importance scores were normalized by dividing each importance score with the total sum of importance scores. **G, H** Raw SHAP values for predicting **G** inflamed or **H** exclusion scores. Each dot represents the AMB level of an assembly in a patient.

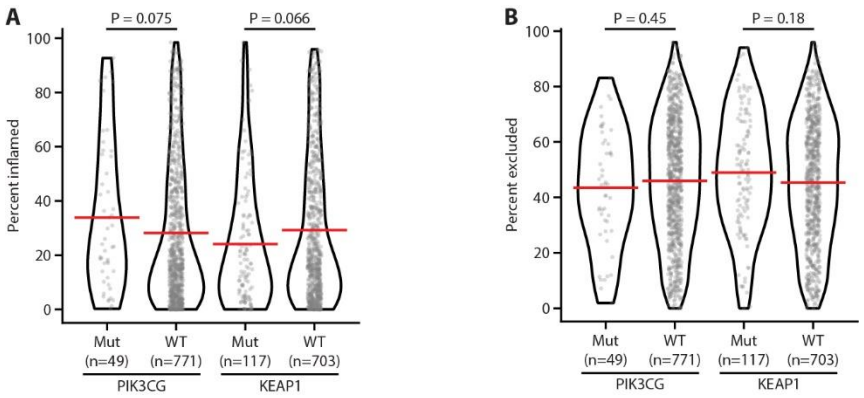

Fig. S4. Relation of individual gene mutations to immunogenic features. A, B Association between mutated (Mut) or wild-type (WT) genes and immune phenotypes from H&E stained image in the TCGA dataset. Mann-Whitney U test was used to compute statistical significance.

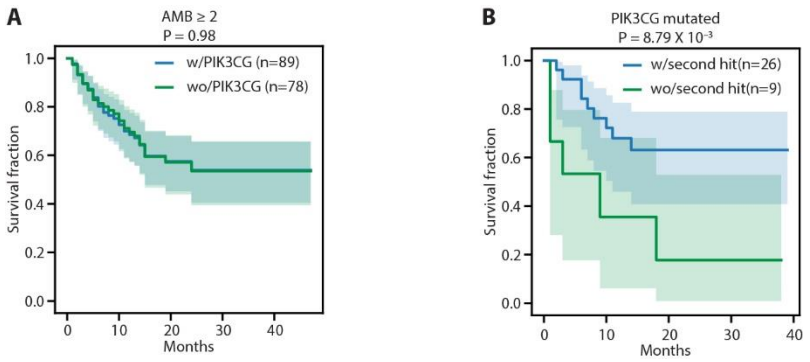

Fig. S5. Impact of PIK3CG on NeST:8 AMB's performance. A Predictive performance in the Samstein cohort when using patients with NeST:8 AMB>=2, stratified by PIK3CG mutated (w/PIK3CG) or not mutated (wo/PIK3CG). Log-rank test was used to compute statistical significance. **B** Predictive performance among patients with PIK3CG mutations, stratified by either having an additional mutation in NeST:8 components (w/second hit) or without additional mutation (wo/second hit).

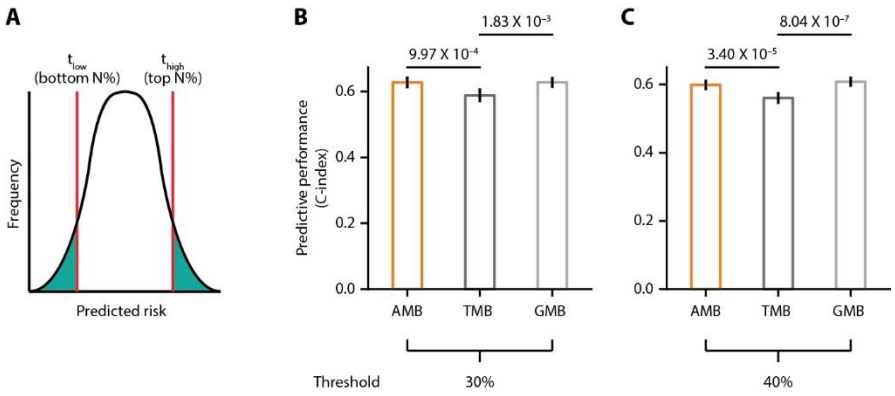

Fig. S6. Prediction of overall survival in Samstein cohort across varying thresholds. A Top N% and bottom N% of the patients were used to compute concordance index (C-index). **B-D** Assessment of predictive performance across varying thresholds for N, spanning **B** 30%, **C** 40%. To measure predictive performance, random 90/10 splits were used (90% train/10% test, 100 independent iterations). The Mann-Whitney U test was used to compute statistical significance.

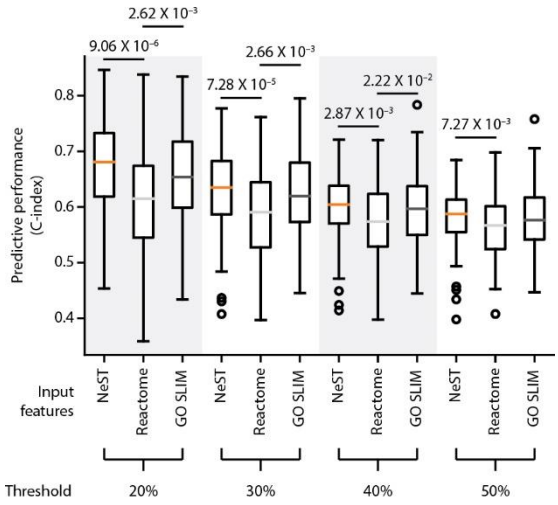

Fig. S7. Prediction across sources of cancer pathway knowledge. A Predictive performance in the Samstein cohort when using AMBs computed in three ways, using assemblies in NeST, pathways in Reactome, or terms in the Gene Ontology (GO) SLIM database. To measure predictive performance, a random 90/10 split of samples was used (100 independent iterations) to alot training versus testing datasets, respectively. Predictive performance was measured using concordance index (C-index). Box plot shows median value and interquartile range (IQR) as bounds of the box. Whiskers of the box plot extends from the box to upper/lower quartile $\pm$ IQR $\times$ 1.5. Mann-Whitney U test was used to compute statistical significance.

Data S1. (separate file)

Best hyper-parameters identified during Monte Carlo cross validation in the Samstein cohort

Data S2. (separate file)

Predicted risk scores in Hellmann cohort

Data S3. (separate file)

Predicted risk scores in IMvigor210 cohort

Data S4. (separate file)

Number of overlapping assemblies between (i) important assemblies from AMB model and (ii) CRISPR enriched assemblies

Data S5. (separate file)

Gene Set Enrichment Analysis (GSEA) results from CRISPR analysis

Data S6. (separate file)

Predicted responders and non-responders using AMB or GMB scores in TCGA

Data S7. (separate file)

Predicted responders and non-responders using TMB levels in TCGA

Data S8. (separate file)

TIL scores measured using immunohistology images in TCGA

Data S9. (separate file)

Enrichment of gene expression levels of tumor-associated macrophage markers using single sample GSEA (ssGSEA) in TCGA

Data S10. (separate file)

Feature importances of NeST assemblies from AMB model

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