

Supplementary Material

Panels and models for accurate prediction of tumor mutation burden in tumor samples

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SUPPLEMENTARY DATA

Supplementary Data 1. Accuracy of panels and models for TMB prediction

Supplementary Data 2. Suggested panels for TMB prediction in 14 cancer types, classified by Mb to be sequenced

Supplementary Data 3. Filters applied to the training and validation datasets

Supplementary Data 4. External validation dataset

Supplementary Data 5. Filters applied to the consensus panels and models

SUPPLEMENTARY TABLES

Supplementary Table 1. Total number of samples used in the training dataset and types of tumor where >80% samples were included in the dataset.


Selection	Genes		Exons	
	Total samples	Types of tumor with > 80% samples	Total samples	Types of tumor with > 80% samples
“our set”	22166	26	21035	23
CGC	22153	30	22153	30
FO-panel	20495	23	20495	23

Supplementary Table 2. Number of consensus panels after filtering

Conditions to be accepted		Consensus panels	Cancer type
		7,242	25
i)	R^2 model ≥ 0.6	6,988	25
ii)	RSE model \leq median TMB in each cancer type	6,459	25
iii)	R^2 internal validation ≥ 0.6 & R^2 internal validation $\geq R^2$ model - 0.1	5,556	22
iv)	RSE internal validation \leq median TMB & RSE internal validation $\leq 1.25 \cdot$ RSE model	2,978	20
v)	R^2 external validation ≥ 0.6	2,301	14

SUPPLEMENTARY FIGURES

DATASET

 WGS and WES data
v84 Select exonic somatic mutations

Filter tumors with

- Inconsistencies
- < 10 mutant samples

24726 samples for **42** tumors

Evaluation of 6 strategies

Variable Type	X	Selection Type
Gene	X	Our
Exon		FO-panel
		CGC

After gene selection by strategy, tumors >80% of initial samples with mutations in selected genes, were analysed

22279 samples for **30** tumors

PANELS

Panels and associated linear regression models developed by strategy and tumor

Genes were aggregated to the panels by steps.

Linear regression models were fitted with the experimental TMB

731958 panels for **30** tumors

Panel selection models

- $R^2 \geq 0.6$
- $RSE < TMB$ cancer


354644 panels for **25** tumors

Consensus panels and associated models were made with the previously selected models as seeds

7242 panels for **25** tumors

VALIDATION

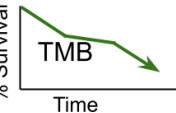
Internal Validation
BOOTSTRAP 1000 times
2978 panels for **20** tumors

External Validation
4075 samples from articles
+
 v90 1945 COSMIC samples
2301 panels for **14** tumors

One panel selected per tumor for TMB prediction, based on performance
25 additional panels of different sizes also reported
39 panels for **14** tumors

Immunotherapy response evaluation of selected models

Melanoma and NSCLC } % Survival

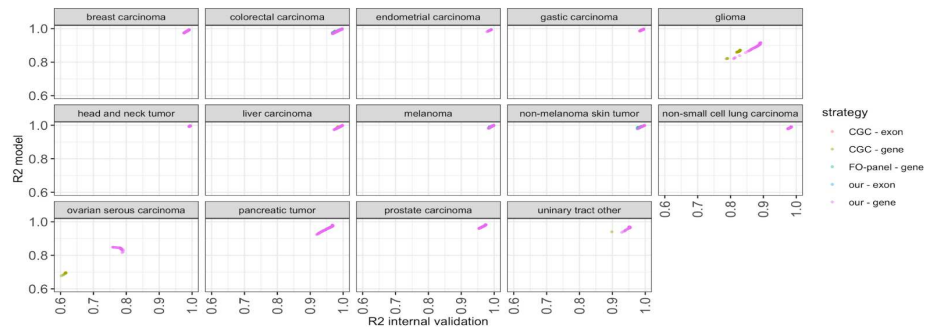


TMB

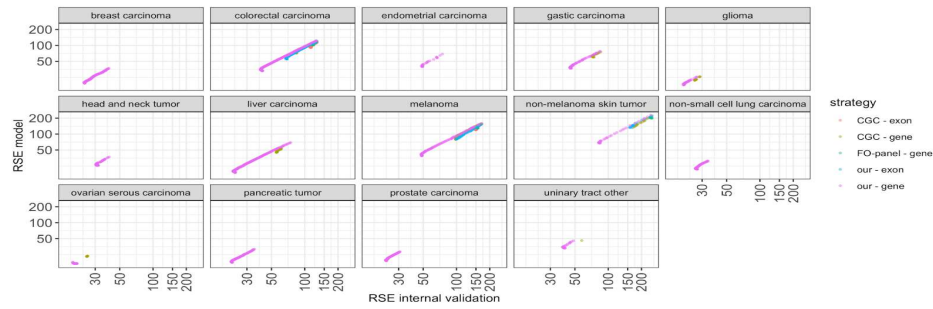
Time

Supplementary Figure 1. Workflow describing the bioinformatics-based approach used to select cancer-specific panels for TMB prediction

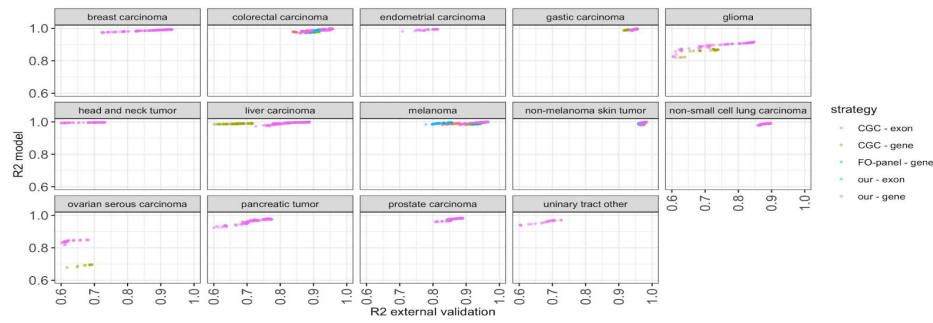
a



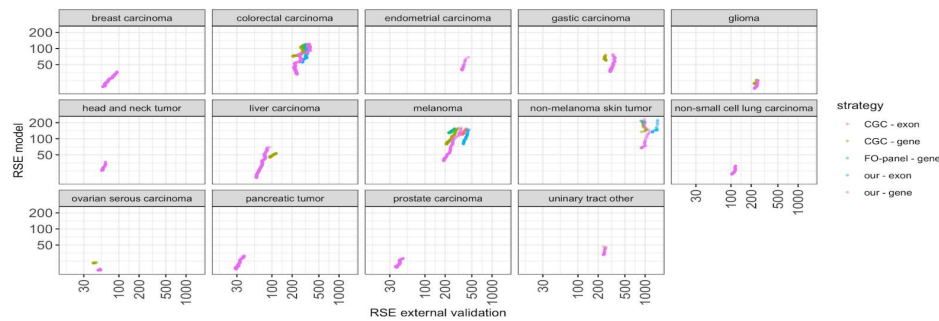
b



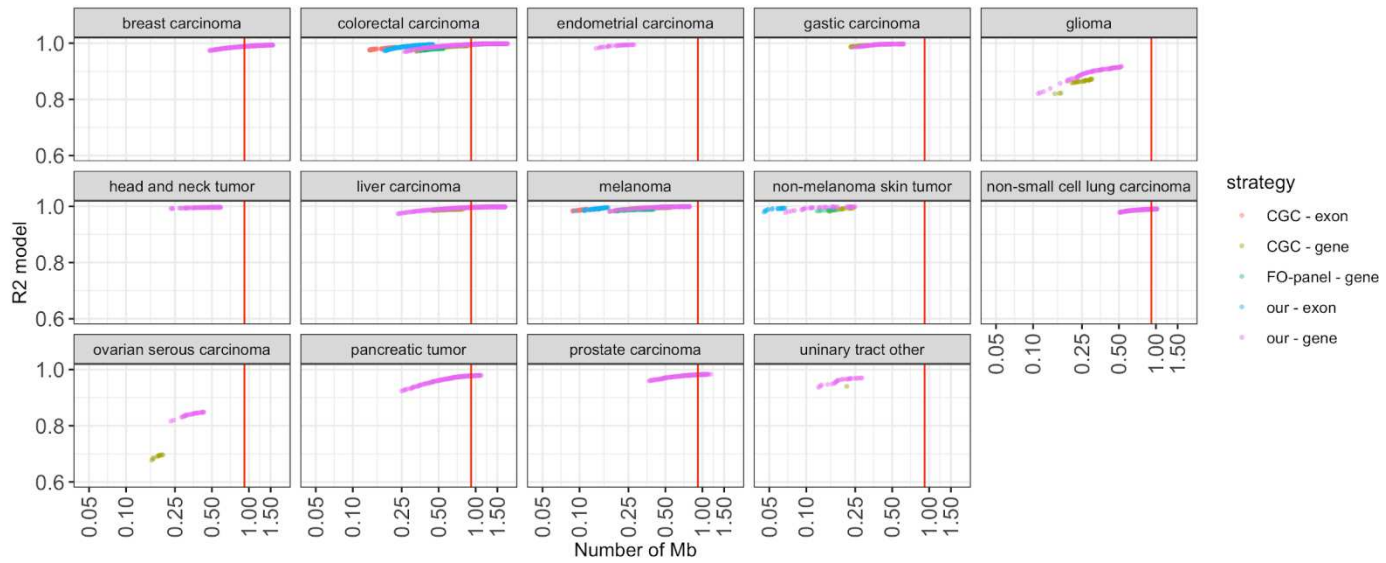
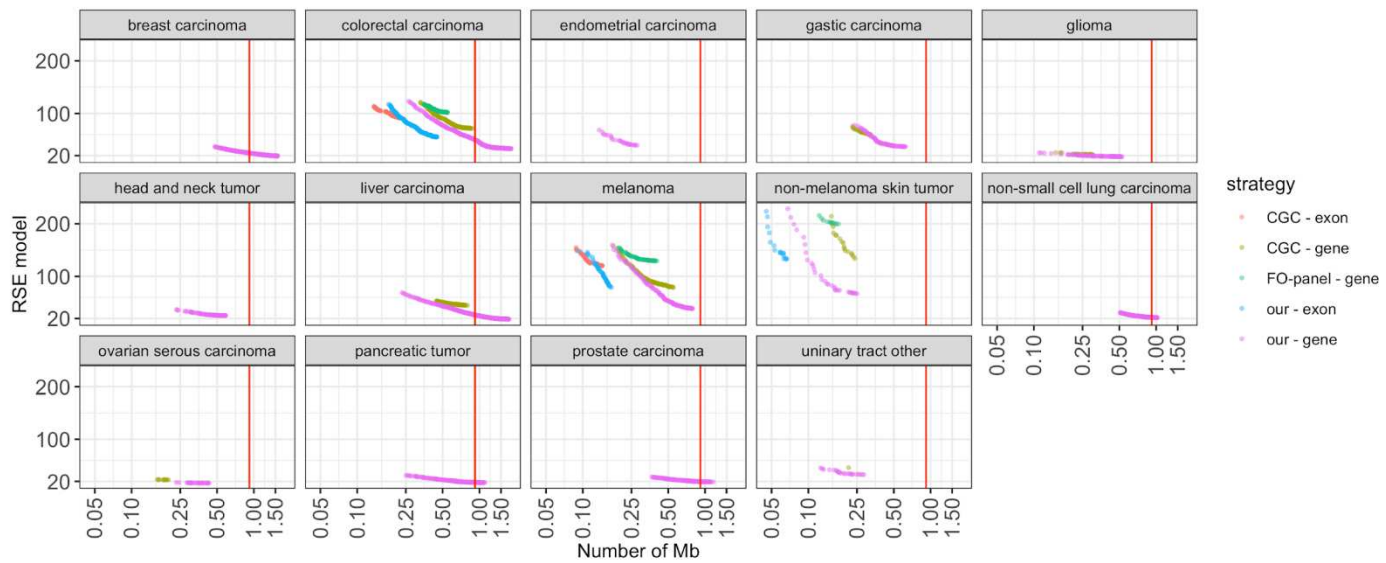
c



d



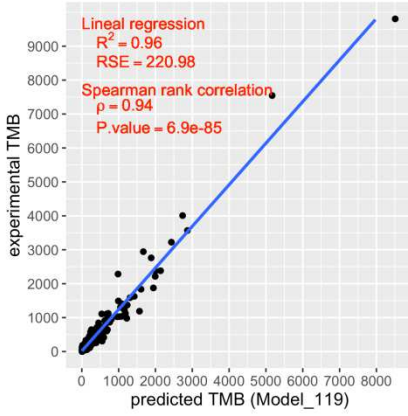
Supplementary Figure 2. Accuracy of the panels and associated models for TMB prediction. a: R^2 , internal validation; **b:** RSE, internal validation; **c:** R^2 , external validation; **d:** RSE, external validation. Each dot represents a model, colors indicate initial datasets.

a**b**

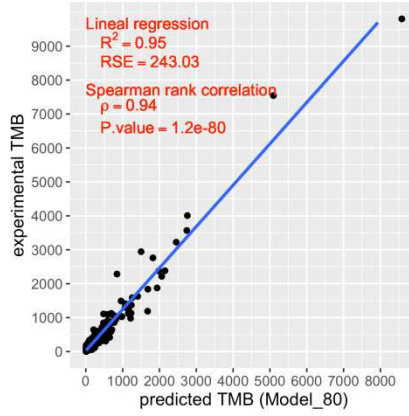
Supplementary Figure 3. Characteristics of the panels for TMB prediction. **a:** R2 and **b:** RSE of the panels vs. length (in Mb). Each dot represents a model, colors indicate initial datasets.

a

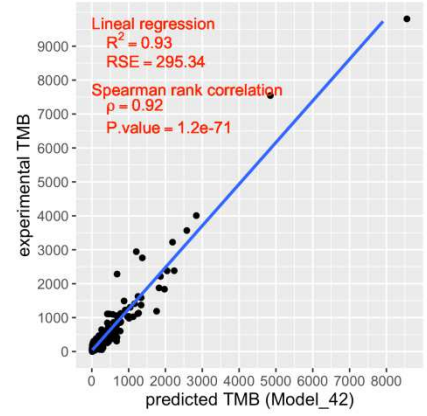
Model_119



Model_80

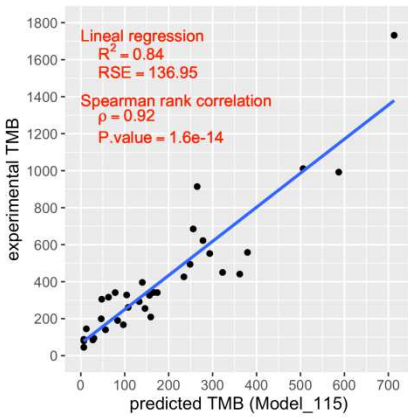


Model_42

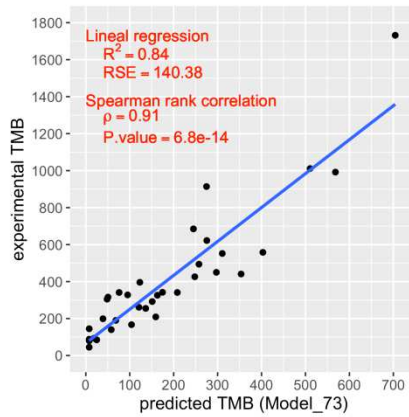


b

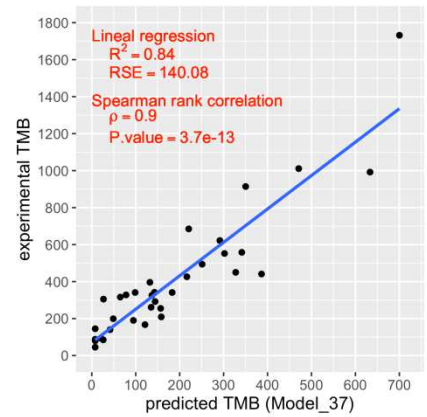
Model_115



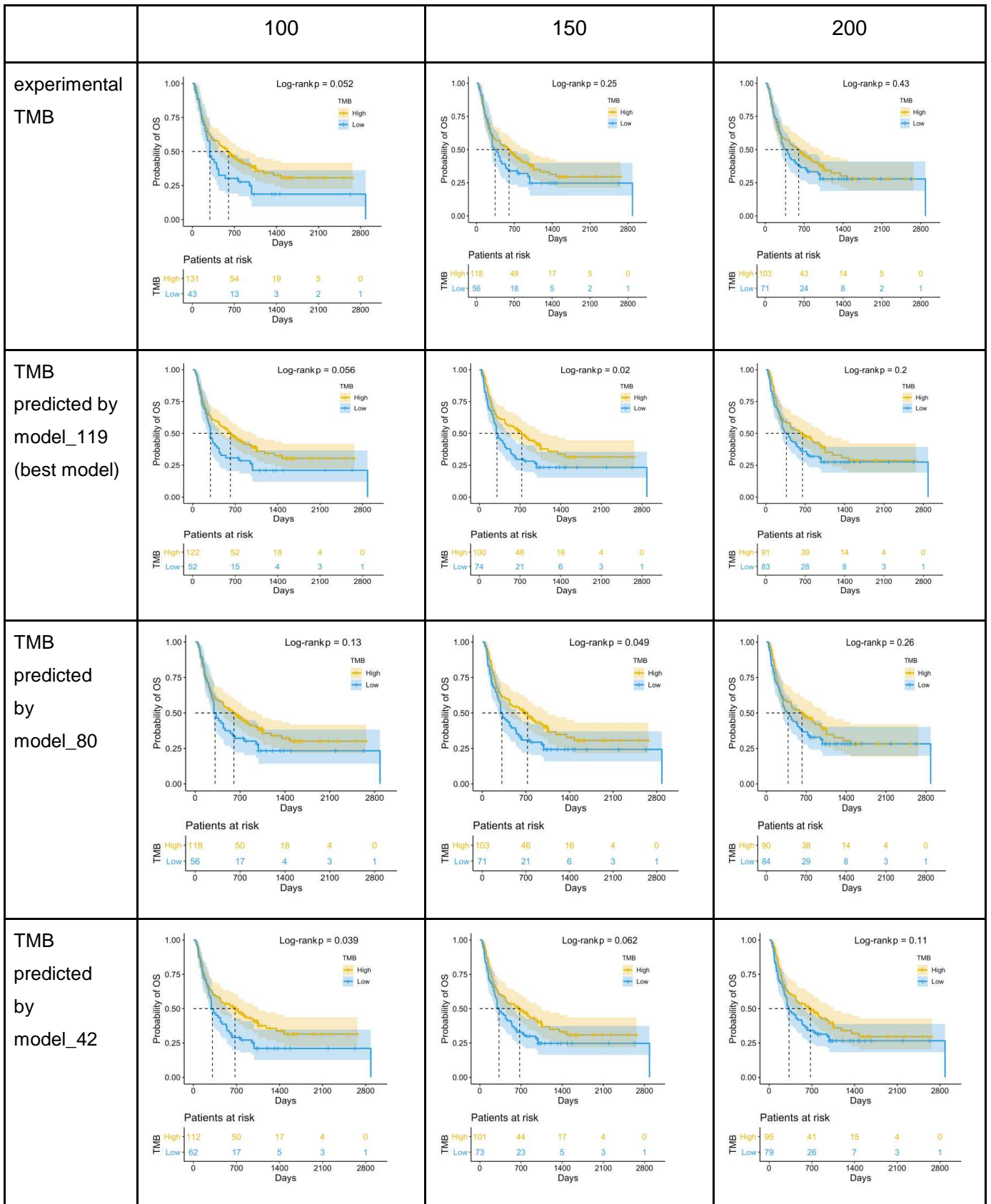
Model_73



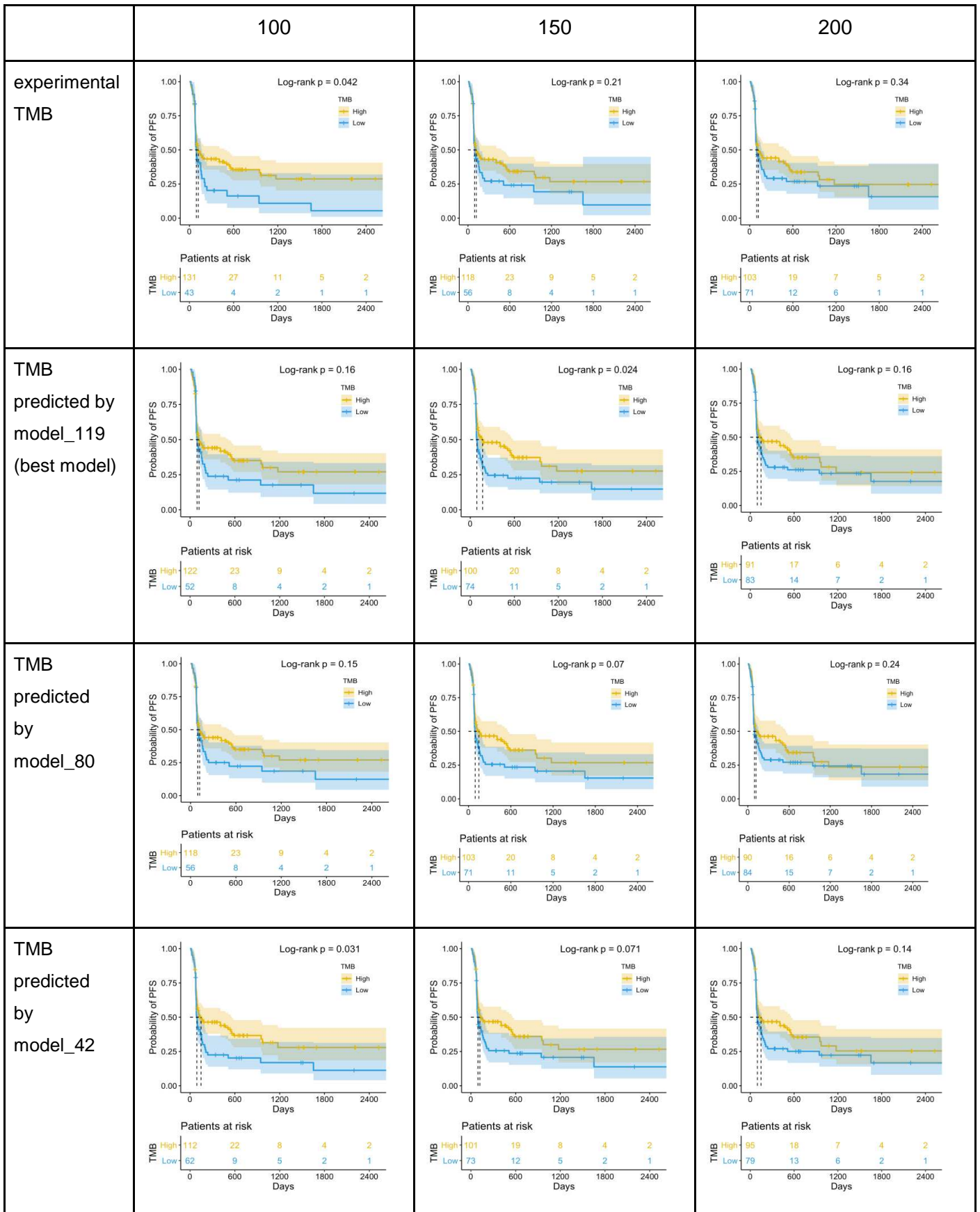
Model_37



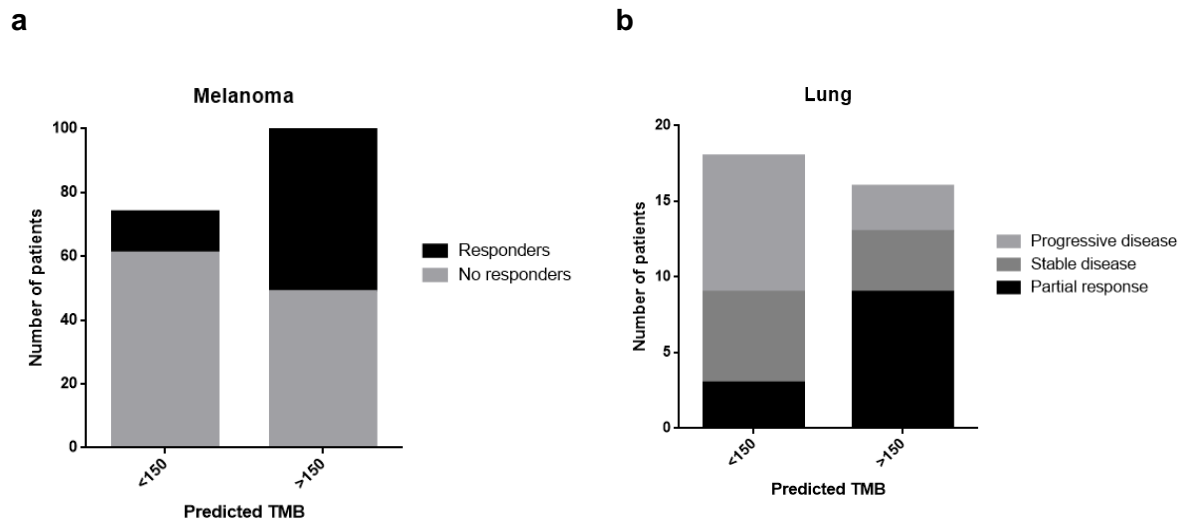
Supplementary Figure 4. Correlation between experimental TMB and the TMB predicted with 3 suggested panels and associated models in two cohorts of melanoma (a) and lung-non small cell patients (b).



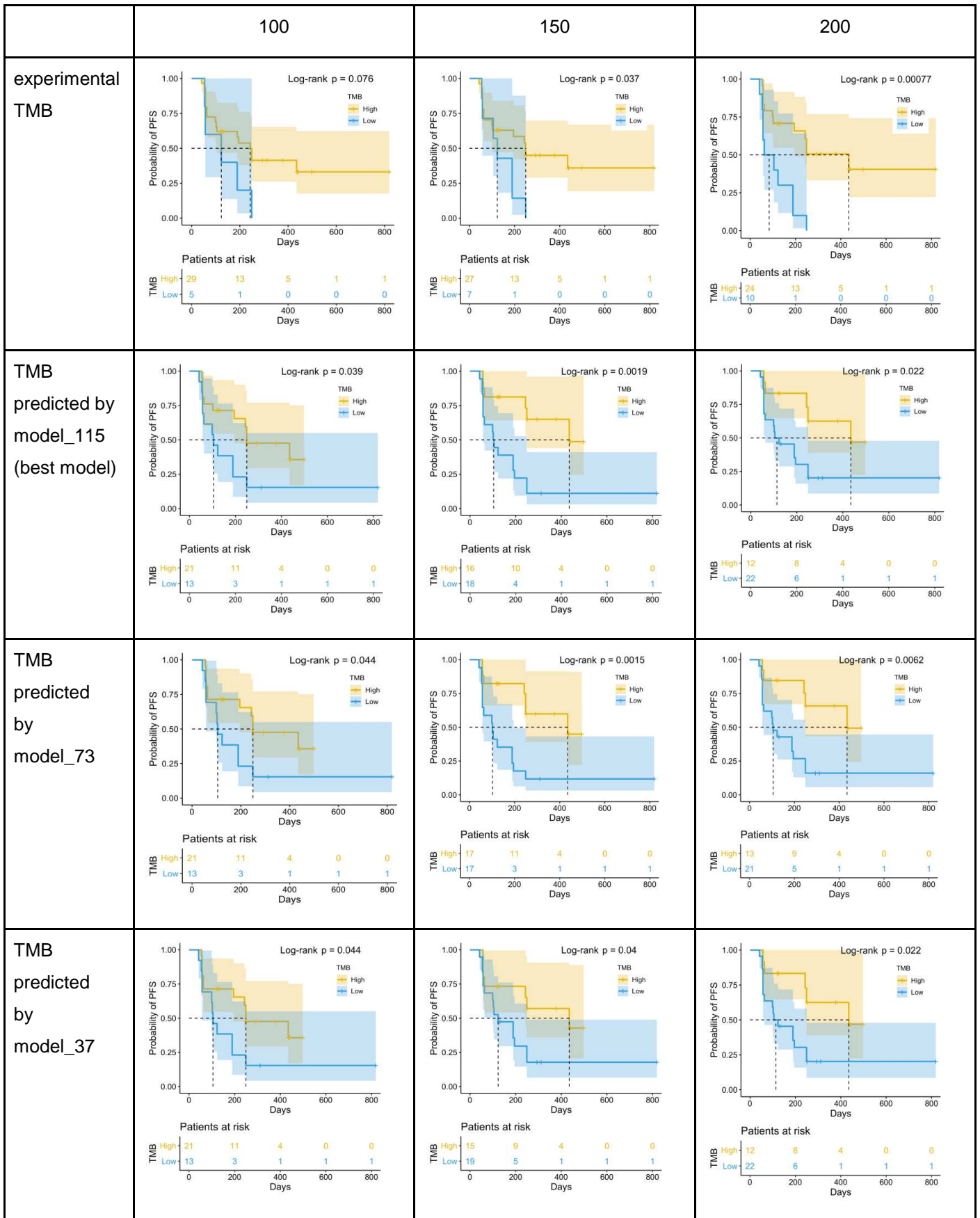
Supplementary Figure 5. Kaplan-Meier plot of OS in a cohort of 174 metastatic melanoma patients, treated with ICB therapy, stratified according to predicted TMB with 100, 150, and 200 cut-off values).



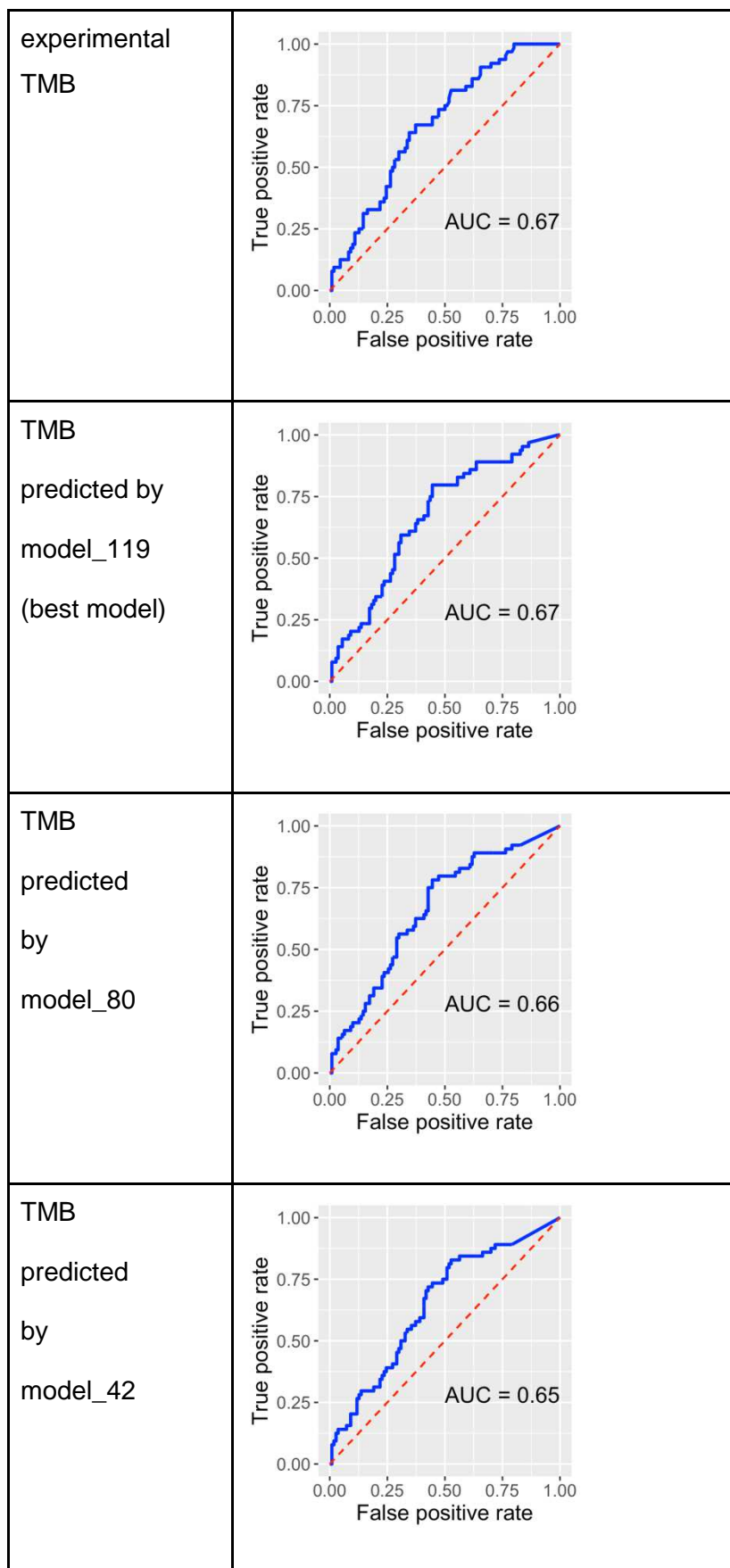
Supplementary Figure 6: Kaplan-Meier plot of PFS in 174 metastatic melanoma patients, treated with ICB therapy, stratified according to predicted TMB (100, 150, and 200 cut-off).



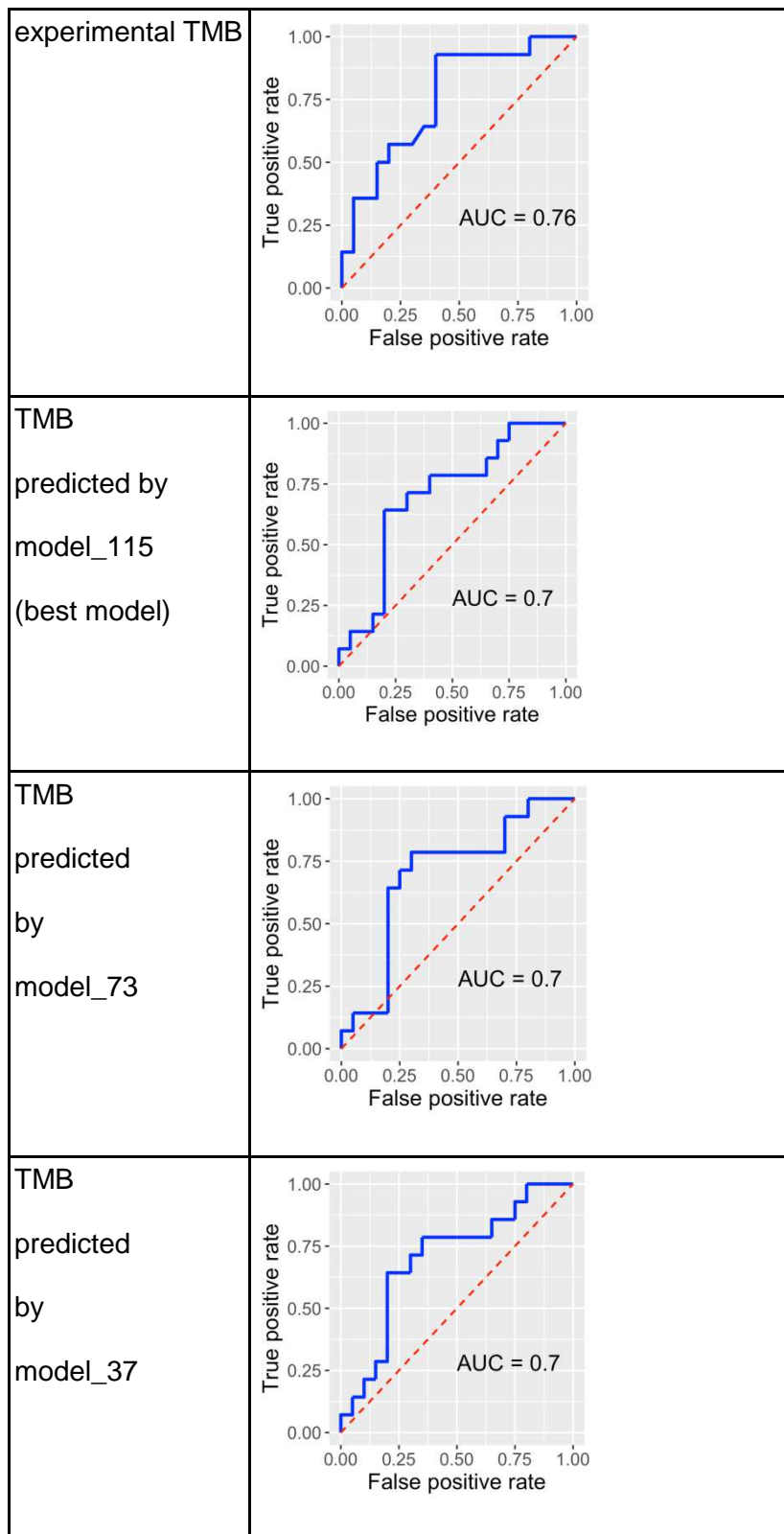
Supplementary Figure 7: Response to ICB therapy to 174 metastatic melanoma (a) and NSCLC (b) patients, stratified according to predicted TMB (cut-off, 150 mut/Mb)



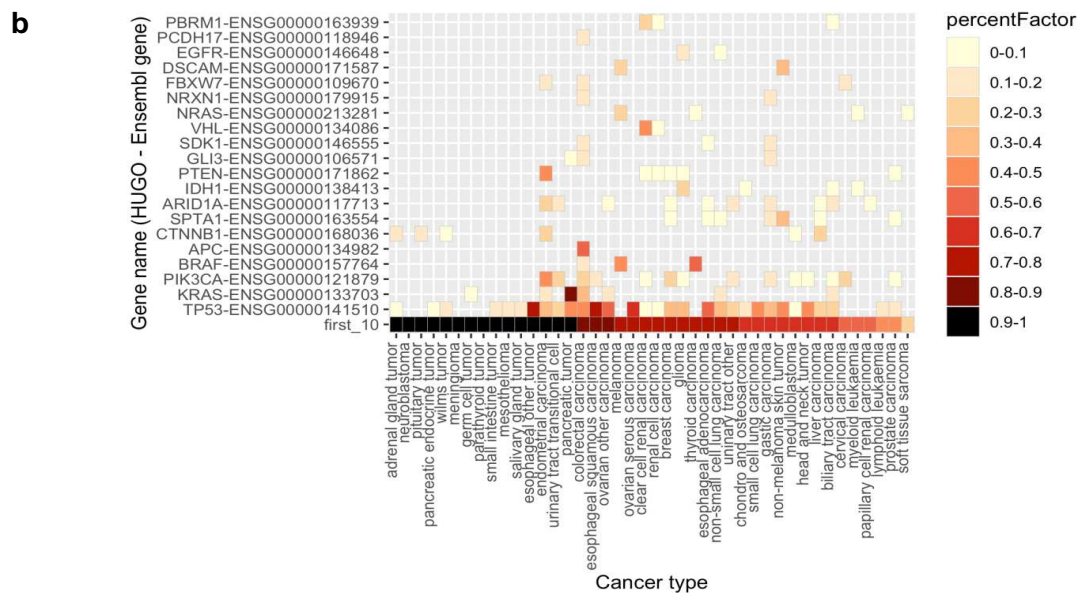
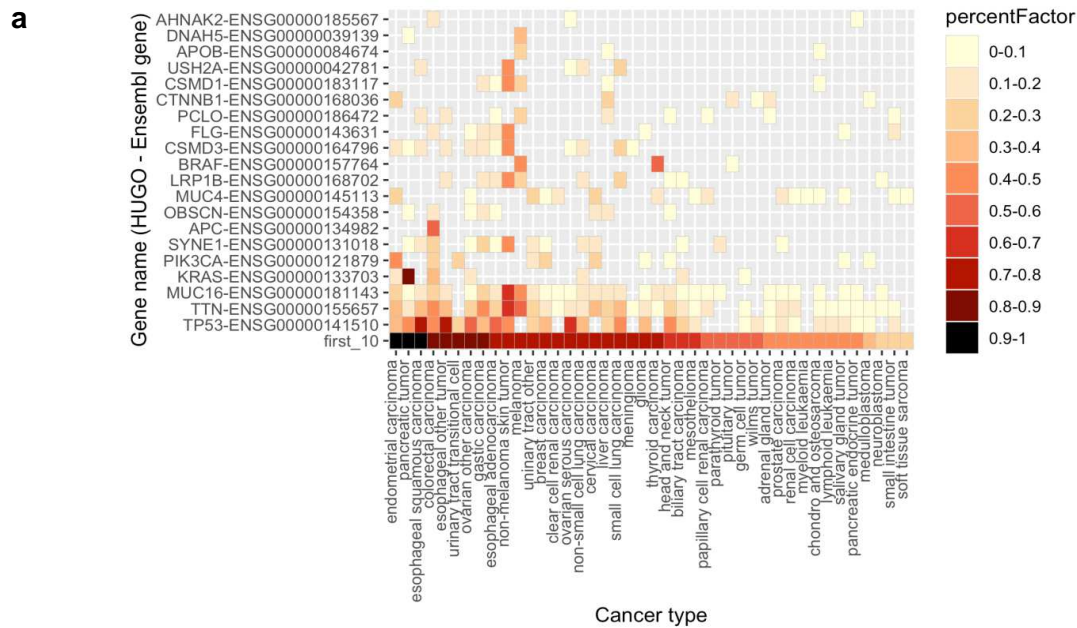
Supplementary Figure 8. Kaplan-Meier plot of PFS in 34 metastatic NSCLC patients, treated with ICB therapy, stratified according to predicted TMB (100, 150, and 200 cut-off).



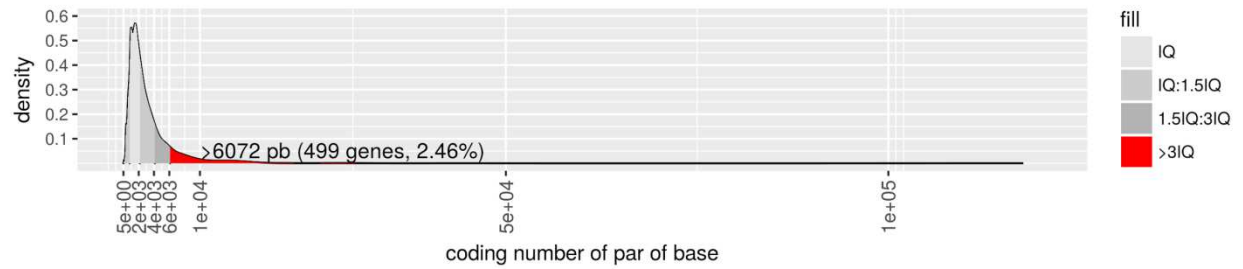
Supplementary Figure 9: ROC curve of predicted TMB and type of response to ICB treatment in a cohort of 174 metastatic melanoma patients



Supplementary Figure 10: ROC curve of predicted TMB and type of response to ICB treatment in a cohort of 34 metastatic NSCLC patients

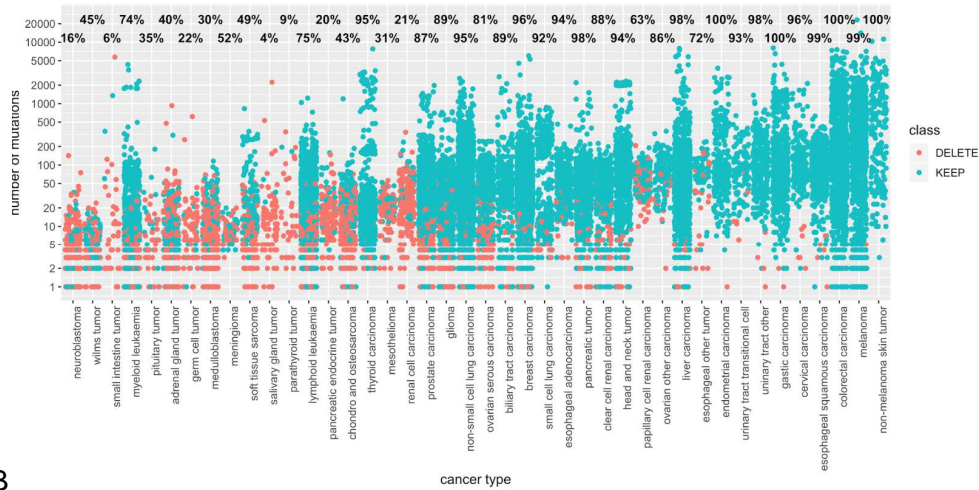


Supplementary Figure 11. Top mutant genes per cancer type before (a) and after penalization by length (b). The intensity of the colors indicates the percentage of mutant samples. To improve clarity, only 20 of the total 157 (a) and 195 (b) top 10 mutant genes are represented

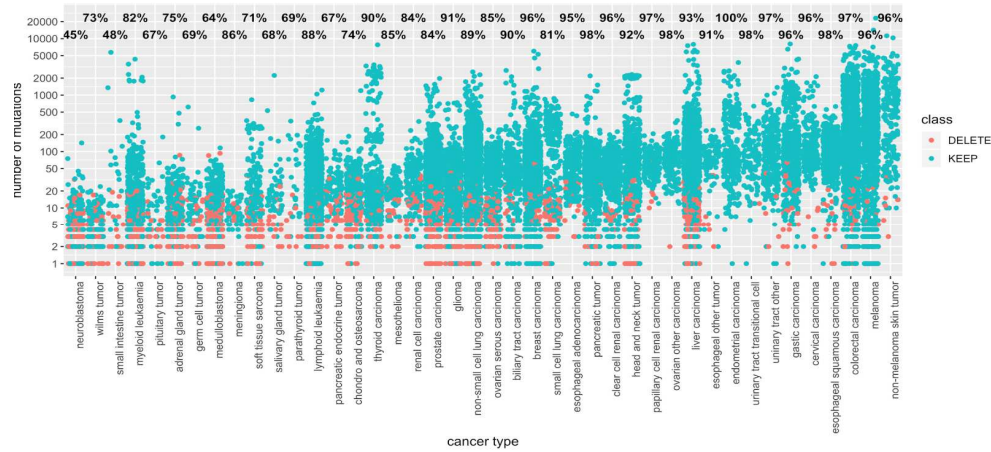


Supplementary Figure 12. Distribution of coding base-pairs among human genes (n= 20,291). 499 genes (2.46%) have coding regions longer than 3*IQ distance (in red)

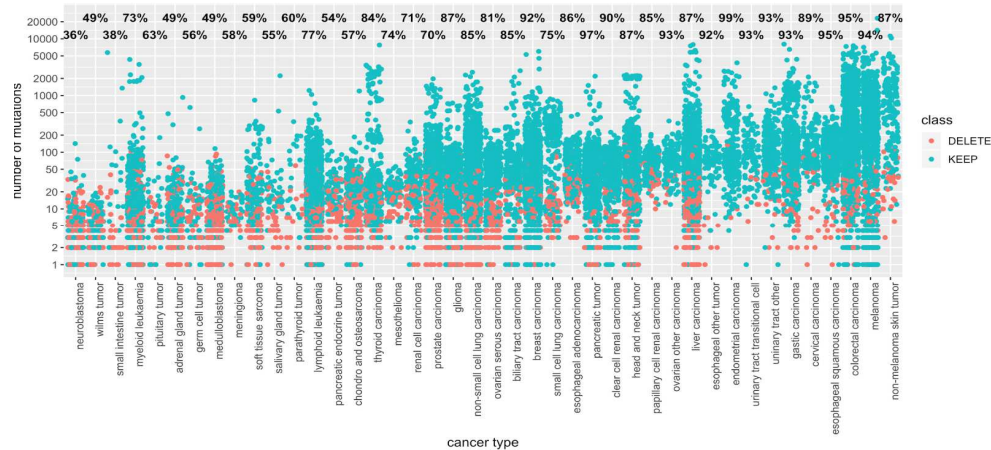
A



B



C:



Supplementary Figure 13. Samples conserved and excluded in our analyses by cancer type and strategy: Each dot represents a conserved sample (blue) or a deleted sample (red). Numbers on top of each cancer type are the percentage of conserved samples. **A:** “our-exon” selection; **B:** CGC gene/exon selection; **C:** FO-panel gene/exon selection