SUPPLEMENTARY MATERIALS

Clinicopathologic and Genomic Analysis of *TP53*-Mutated Endometrial Carcinomas

Momeni-Boroujeni et al.

SUPPLEMENTARY METHODS

Analysis of prognostic effects of genomic alterations in non-hypermutant *TP53*-mutated endometrial cancers (ECs):

Parametric and semi-parametric survival studies such as Cox-proportional hazards regression analysis are the standard tools for studying survival variables with censoring. These tools establish a direct link between variable(s) and survival and allow for easy interpretation and inference (1). However, using these tools require that the variable(s) of interest be determined for the link with survival to be investigated. While investigating links between a limited number of variables and survival is practical using such regression models, they become rapidly impractical as more variables are included. Specifically, for interrogating the effects of genomic alterations on survival, where often hundreds of gene alterations need to be investigated, alternative approaches are required; survival trees are one of such alternative approaches. The idea behind the survival trees approach is to recursively partition the covariate space to form groups of subjects that have similar outcomes. The success of partitions is measured with different impurity indices with exhaustive searches of possible partitioning solutions performed to minimize node impurity; the nodes are then partitioned into children nodes and the process continues until a stopping criterion is met (often node size). The final tree is then produced by either pruning and selection of the trees or using an ensemble approach (2). For survival outcomes in nodes, the Kaplan-Meier estimate of the survival function is reported for each terminal node (3). In order to investigate the effects of genomic alterations on survival of p53abn ECs, an alteration matrix was produced for all the 410-468 genes included in the MSK-IMPACT assay, whereby, if a gene had any alterations (mutations or copy number alterations) in a tumor sample, that gene was assigned a score of '1' in the matrix for that sample and if no alteration was found a score of '0' was assigned. Subsequently, to identify molecular subgroups associated with overall survival (OS) and disease-free survival (DFS) effects, survival tree analysis using a recursive partitioning approach (4) was performed by employing the packages 'rpart' and 'randomForestSRC' (5) in R following the principles explained above (1), with single altered genes and random combinations of altered genes (altered in at least 20 patients) used as input variables and stopping rule set to a node size that had at least 10% of the p53abn EC patients who had initial treatment planning at MSK (n=18/185). The genomic alterations with predicted survival effect were then assessed by a multivariate Cox-regression model.

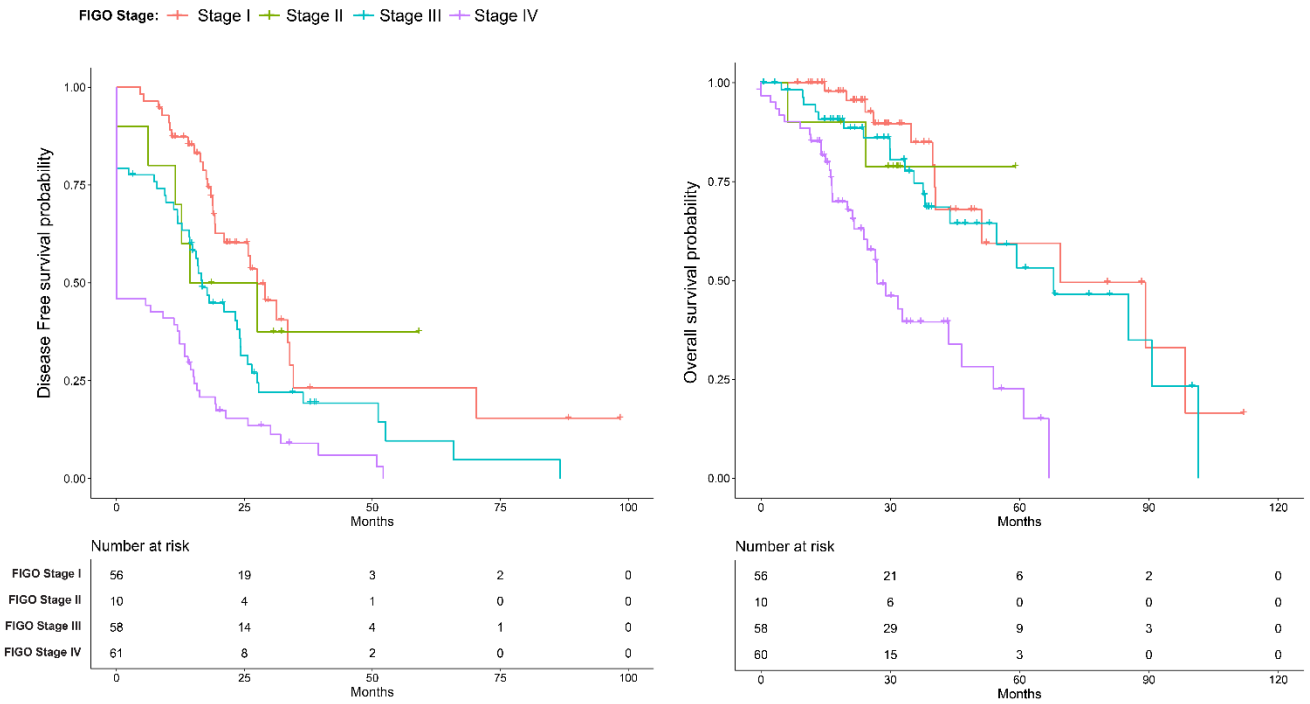
While no single gene alteration was found to represent a survival node in our approach, we found that tumors harboring alterations in any combination of the *SMARCA4* (deletion/mutation), *FGFR2* (amplification/mutation), *CIC* (mutation) and *CDK12* (amplification/mutation) genes (altered in 41/185 cases, 22.2%) had significantly worse prognosis (Log-rank, OS p=0.0001, DFS p=0.016). Multivariate Cox Proportional Hazards analysis showed that the HR for these alterations for OS, after controlling for clinical stage and tumor histology, was 3.0 (p=0.00003). Conversely, alterations in any combination of the *KMT2C*, *PTPRD*, *BCL2L1*, and *RIT1* genes (altered in 28/185 cases, 15.1%) were associated with better OS, with an adjusted HR of 0.19 (p=0.021; Log-rank, OS p=0.0017, DFS p=0.34; Supplementary Figure S5; Supplementary Tables S1 and S2).

References

1. Bou-Hamad I, Larocque D, Ben-Ameur H. A review of survival trees. Statistics surveys 2011;5:44-71.
2. LeBlanc M, Crowley J. Survival trees by goodness of split. Journal of the American Statistical Association 1993;88:457-67.
3. Bou-hamad I, Larocque D, Ben-Ameur H, Mâsse LC, Vitaro F, Tremblay RE. Discrete-time survival trees. Canadian Journal of Statistics 2009;37:17-32.
4. Zhou Y, McArdle JJ. Rationale and Applications of Survival Tree and Survival Ensemble Methods. Psychometrika 2015;80:811-33.
5. Ishwaran H, Kogalur UB, Blackstone EH, Lauer MS. Random survival forests. The annals of applied statistics 2008;2:841-60.

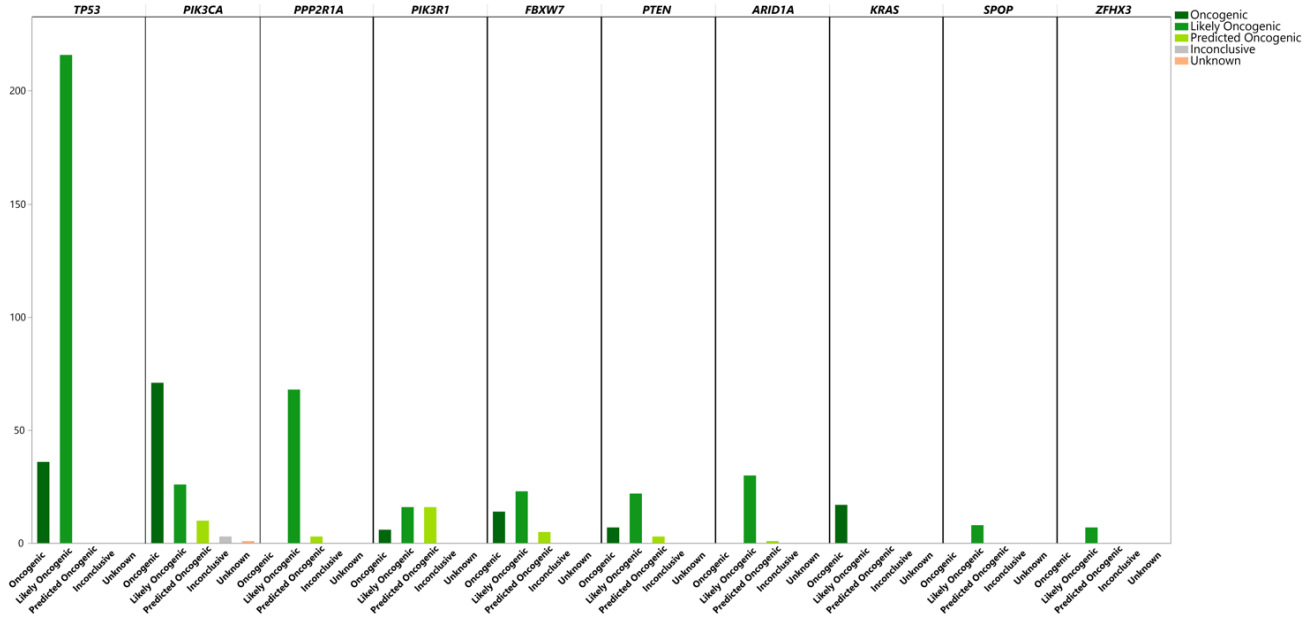
SUPPLEMENTARY FIGURES

Supplementary Figure S1



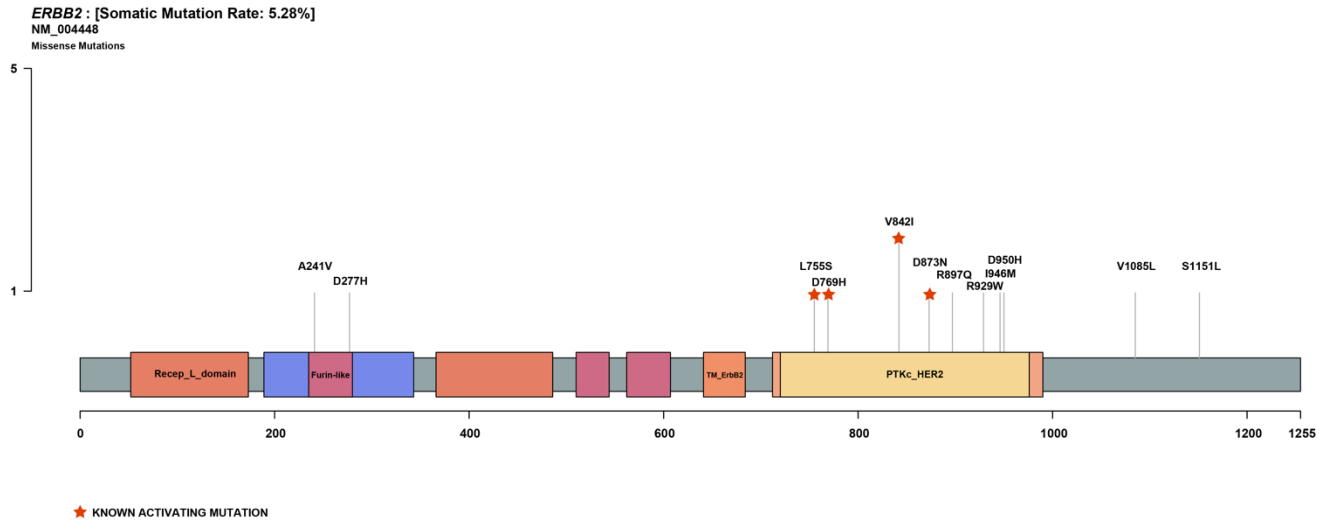
Supplementary Figure S1: Outcome in non-hypermutant *TP53*-mutated endometrial cancers according to stage.
Kaplan-Meier curves for disease-free survival (left) and overall survival (right) of TP53-mutated endometrial cancers according to FIGO stage.

Supplementary Figure S2



Supplementary Figure S2. Mutation function predictions of recurrently mutated genes in non-hypermutant *TP53*-mutated endometrial carcinomas.
Barplot showing the oncogenic predictions of the somatic mutations identified in recurrently altered genes in *TP53*-mutated endometrial carcinomas. Oncogenicity color-coded according to the legend.

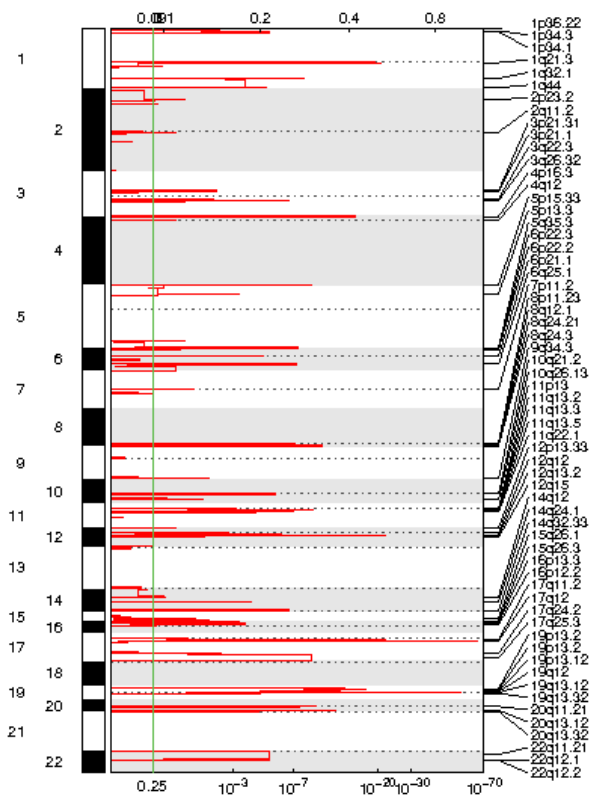
Supplementary Figure S3



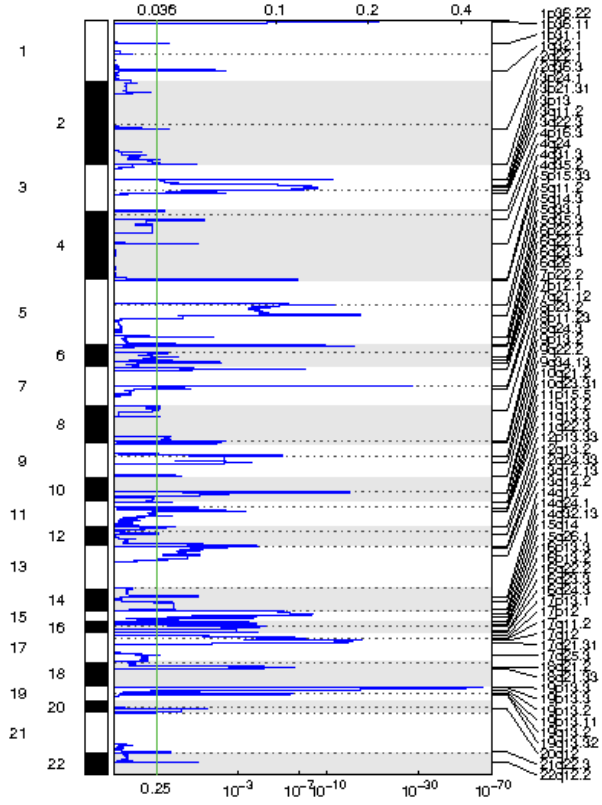
Supplementary Figure S3. Distribution of somatic *ERBB2* mutations in non-hypermutant *TP53*-mutated endometrial carcinomas.
Lollipop plot displaying the somatic missense mutations identified in *ERBB2* in *TP53*-mutated endometrial carcinomas. Stars highlight gain-of-function mutations that were previously shown to be associated with sensitivity to anti-HER2 treatment in other tumor types.

Supplementary Figure S4

A

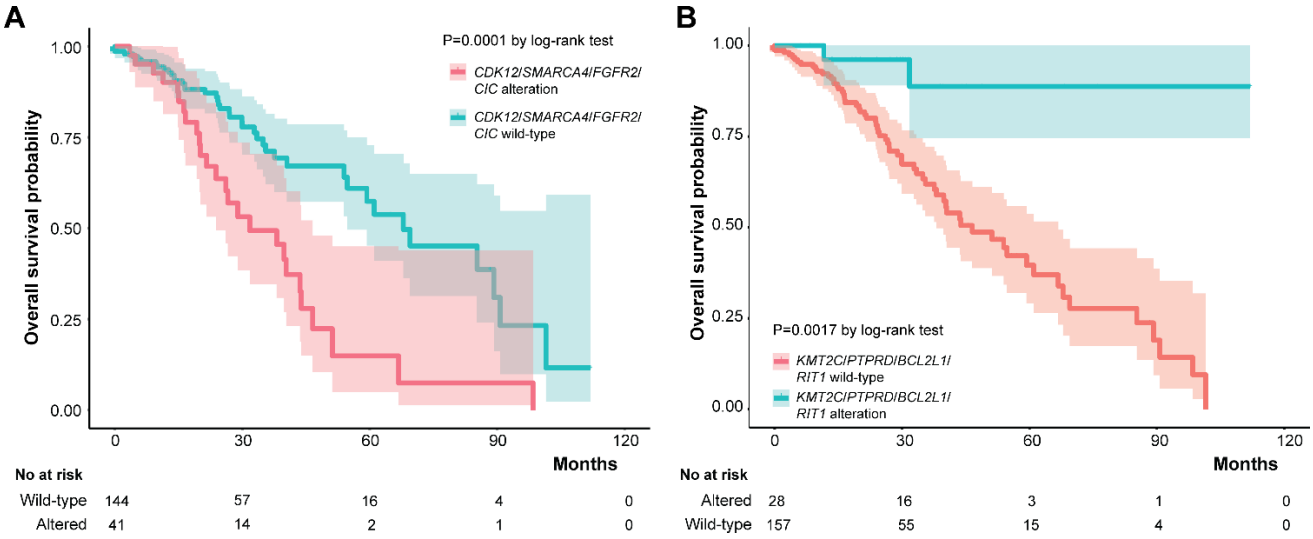


B



Supplementary Figure S4: Copy number alterations in non-hypermutant *TP53*-mutated endometrial carcinomas.
Qplots displaying the most recurrent copy number alterations in *TP53*-mutated endometrial carcinomas. (**A**) Commonly amplified loci and (**B**) commonly deleted loci.

Supplementary Figure S5



Supplementary Figure S5. Exploratory hypothesis-generating analysis of the association of genetic alterations with outcome in non-hypermutant *TP53*-mutated endometrial cancers. (**A**) Kaplan-Meier curve for overall survival of *TP53*-mutated endometrial cancers with and without *SMARCA4*, *FGFR2*, *CIC* and *CDK12* gene alterations. (**B**) Kaplan-Meier curve for overall survival of *TP53*-mutated endometrial cancers with and without *KMT2C*, *PTPRD*, *BCL2L1* and *RIT1* gene alterations.

SUPPLEMENTARY TABLES

Supplementary Table S1. Regression summaries of multivariate Cox regression disease-free survival analysis.

Variable	Regression Coefficient (B)	Standard Error of B	HAZARD Ratio (Exp(B))	Wald (Z-value)	P-value
High FIGO stage (stages IIIC-IV)	1.0187	0.1863	2.7695	5.469	4.52e-08
KMT2C/*PTPRD*/*BCL2L1*/*RIT1* gene alteration	0.3758	0.2349	1.4562	1.600	0.10964
CDK12/*SMARCA4*/*FGFR2*/*CIC* gene alteration	0.4560	0.2083	1.5777	2.188	0.02863
Serous morphology (versus other histologic types)	0.5022	0.1865	1.6524	2.693	0.00708
ERBB2/ *CCNE1* alteration	0.3290	0.2089	1.3896	1.575	0.11531
Adjuvant Therapy	-0.7246	0.4845	0.2902	-2.497	0.0125

Supplementary Table S2. Regression summaries of multivariate Cox regression overall survival analysis.

Variable	Regression Coefficient (B)	Standard Error of B	HAZARD Ratio (Exp(B))	Wald (Z-value)	P-value
High FIGO stage (stages IIIC-IV)	0.9012	0.2796	2.4626	3.223	0.00127
KMT2C/*PTPRD*/*BCL2L1*/*RIT1* gene alteration	-1.6638	0.7239	0.1894	-2.298	0.02154
CDK12/*SMARCA4*/*FGFR2*/*CIC* gene alteration	1.1087	0.2685	3.0304	4.129	3.64e-05
Serous morphology (versus other histologic types)	0.4828	0.2602	1.6206	1.856	0.06350
ERBB2/ *CCNE1* alteration	-0.1034	0.3014	0.9017	-0.343	0.73145
Adjuvant Therapy	-0.3403	0.7115	0.4167	-0.817	0.41404