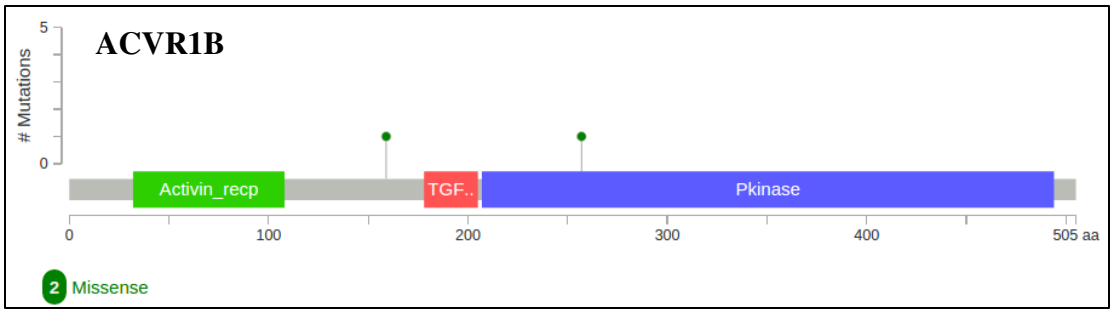
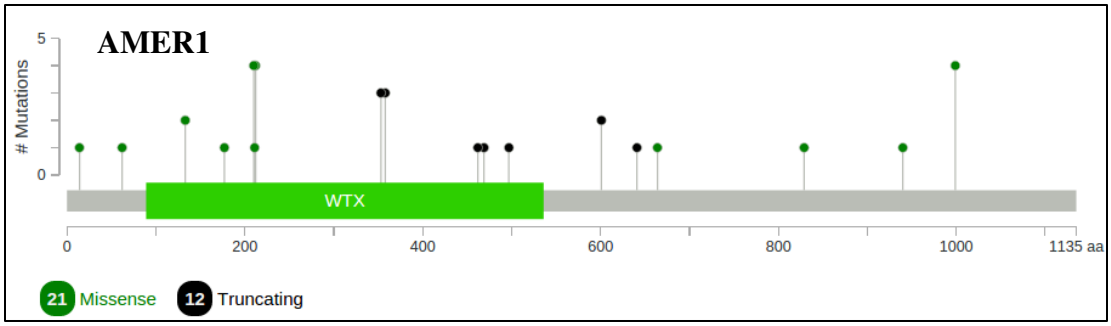
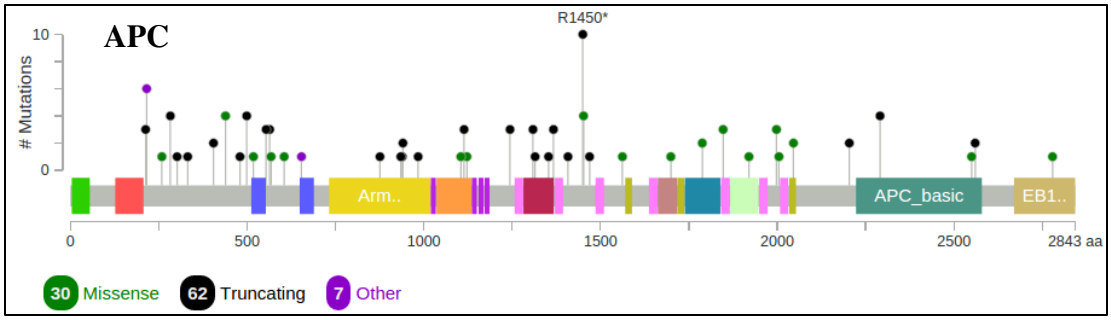
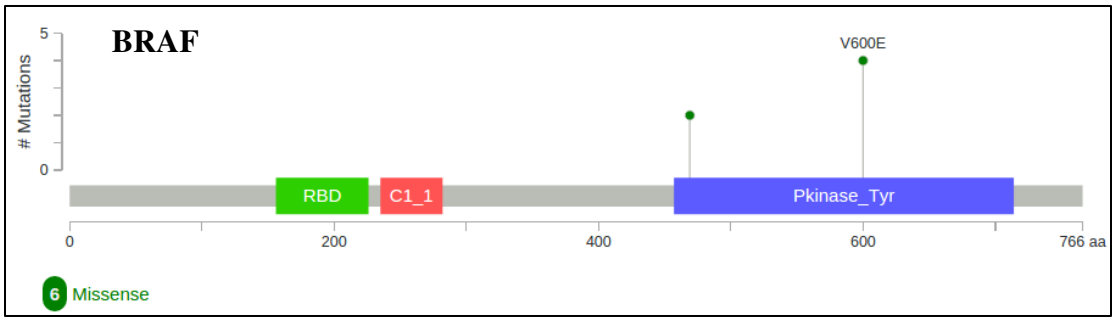
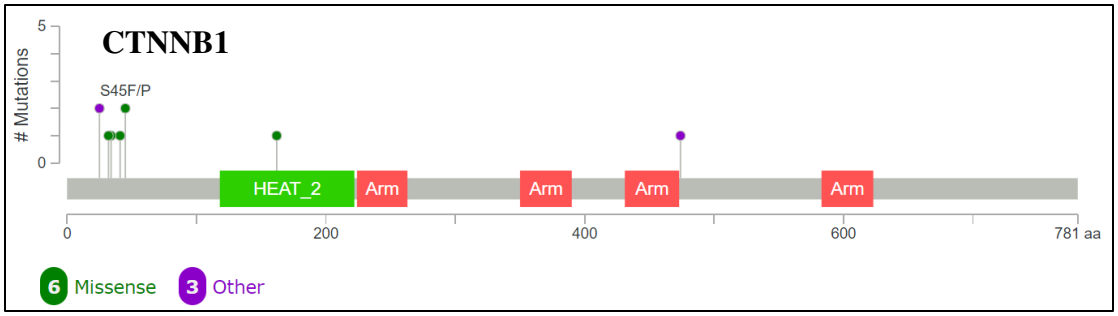
Supplementary Figure 1: MutationMapper illustrating the detected mutations (and their nature) represented on the linear proteins.

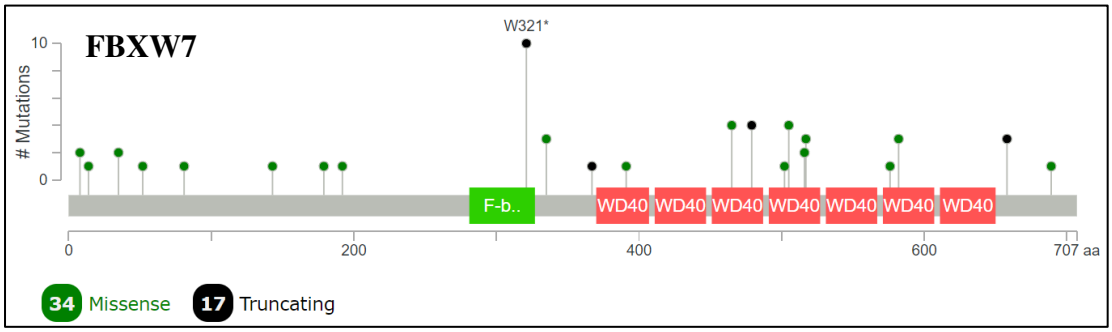


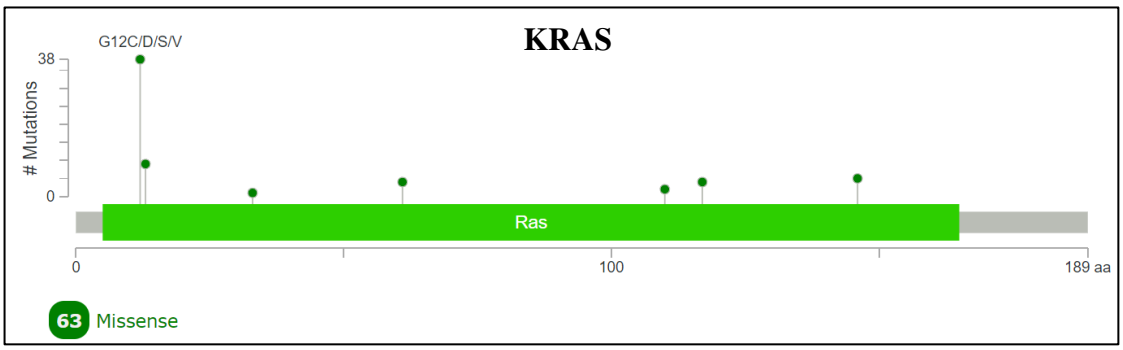


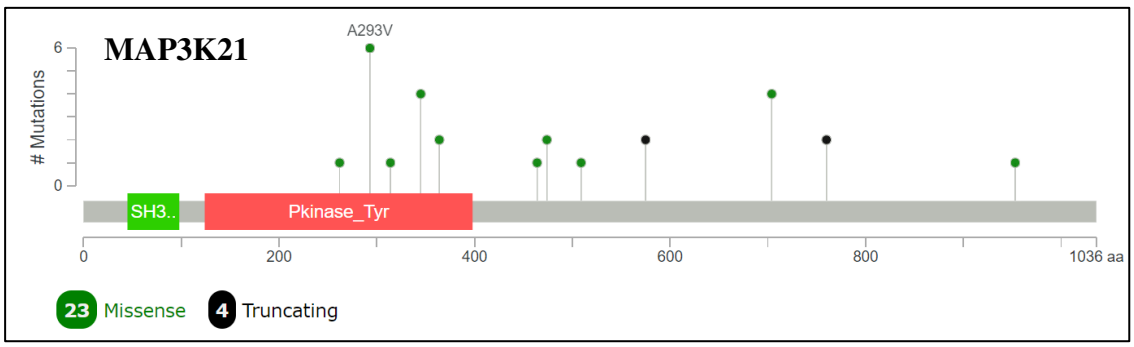


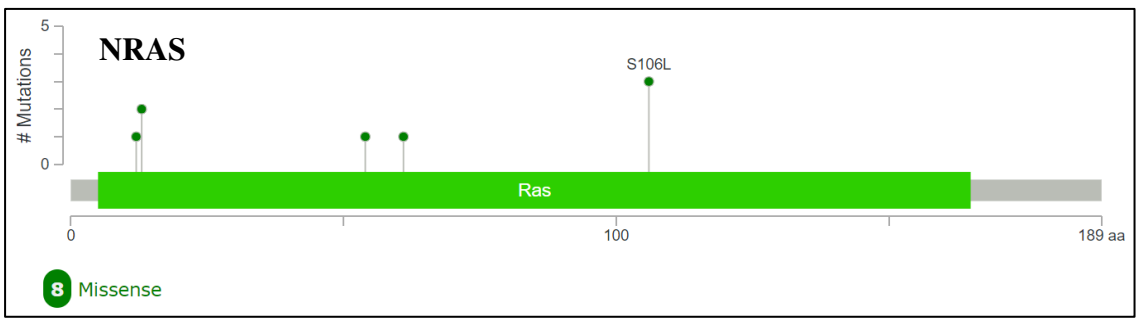


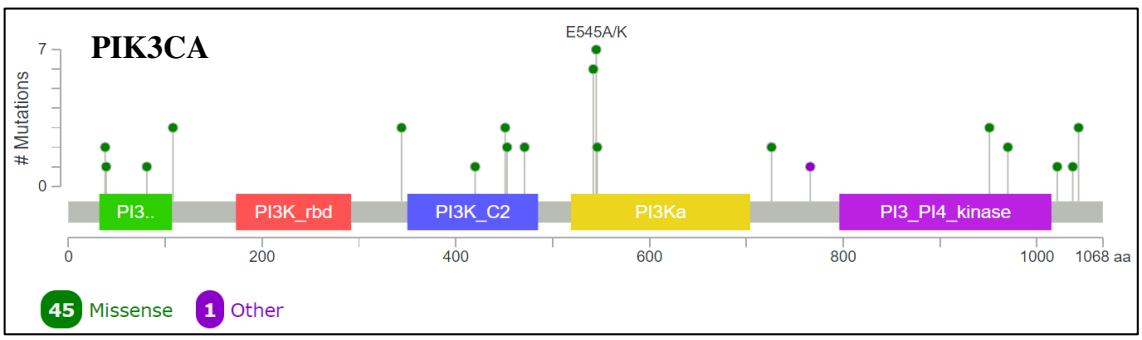


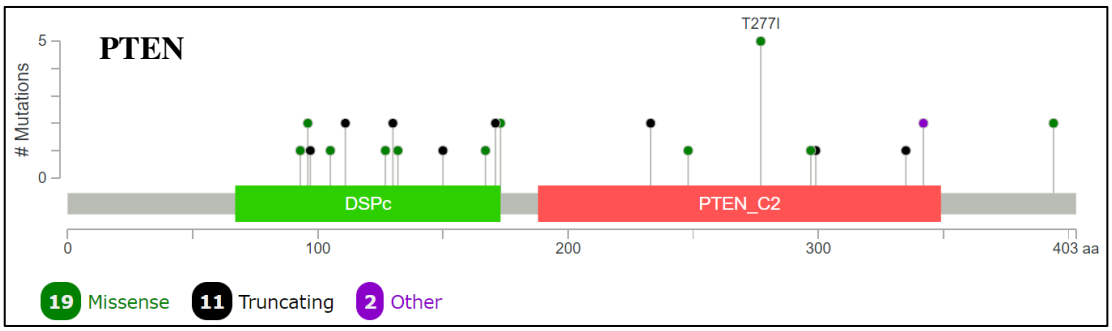

FBXW7
W321*
10
0
Mutations
F-b..
WD40
WD40
WD40
WD40
WD40
WD40
WD40
0
200
400
600
707 aa
34 Missense
17 Truncating

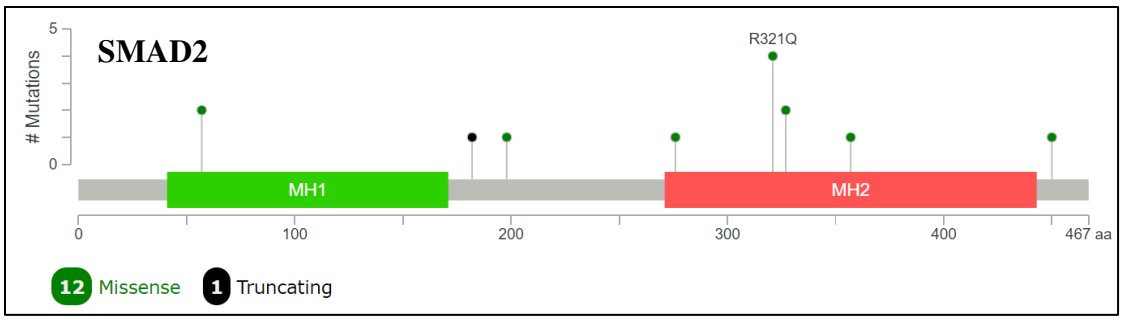

KRAS
G12C/D/S/V
38
0
Mutations
Ras
0
100
189 aa
63 Missense

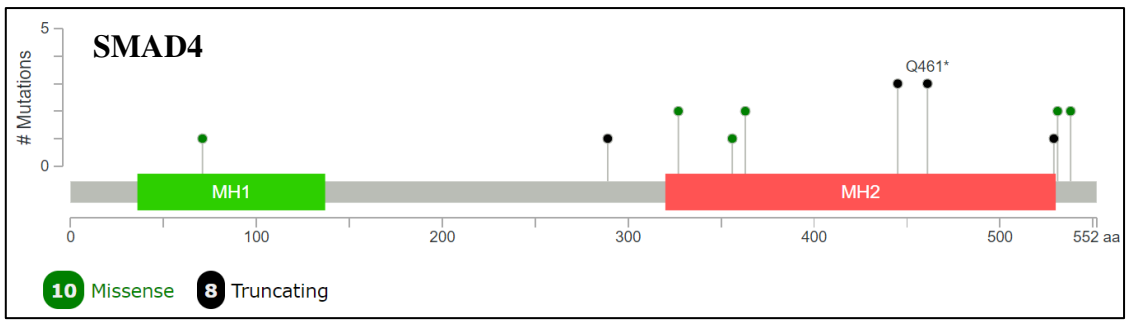

MAP3K21
A293V
6
0
Mutations
SH3..
Pkinase_Tyr
0
200
400
600
800
1036 aa
23 Missense
4 Truncating

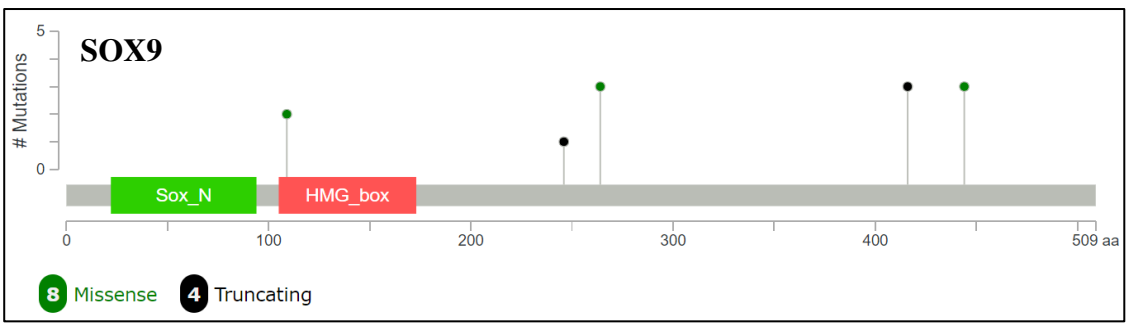

NRAS
S106L
5
0
Mutations
Ras
0
100
189 aa
8 Missense

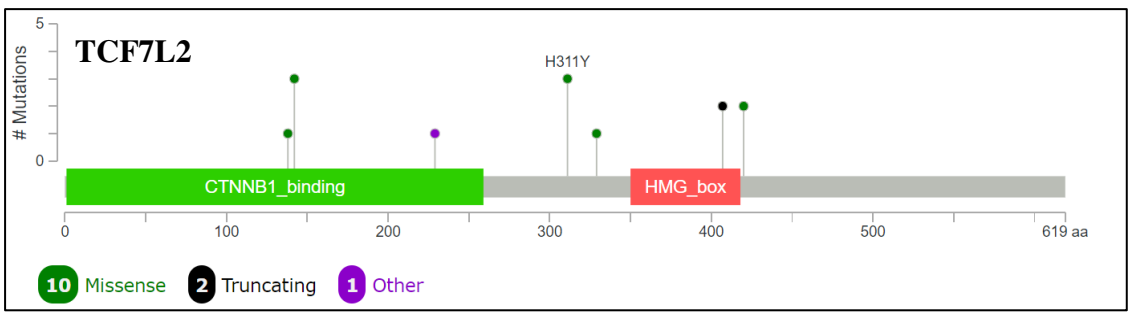

PIK3CA
E545A/K
7
0
Mutations
PI3..
PI3K_rbd
PI3K_C2
PI3Ka
PI3_PI4_kinase
0
200
400
600
800
1000
1068 aa
45 Missense
1 Other

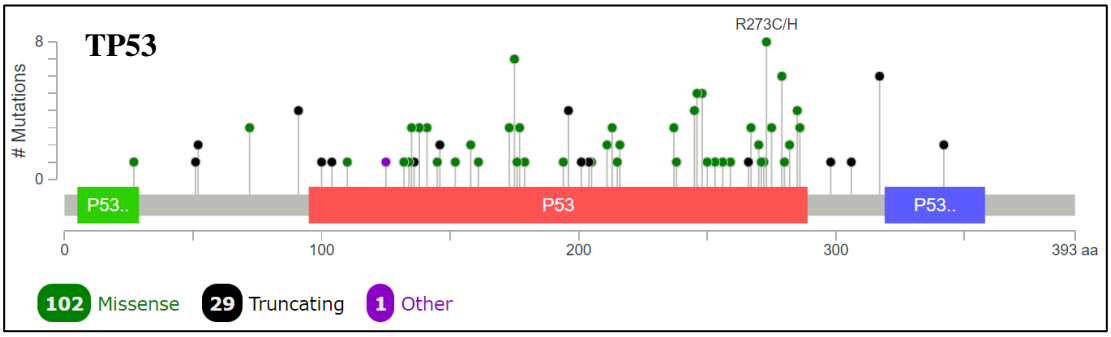

PTEN
T277I
Mutations
5
0
DSPc
PTEN_C2
0
100
200
300
403 aa
19 Missense
11 Truncating
2 Other


SMAD2
R321Q
Mutations
5
0
MH1
MH2
0
100
200
300
400
467 aa
12 Missense
1 Truncating

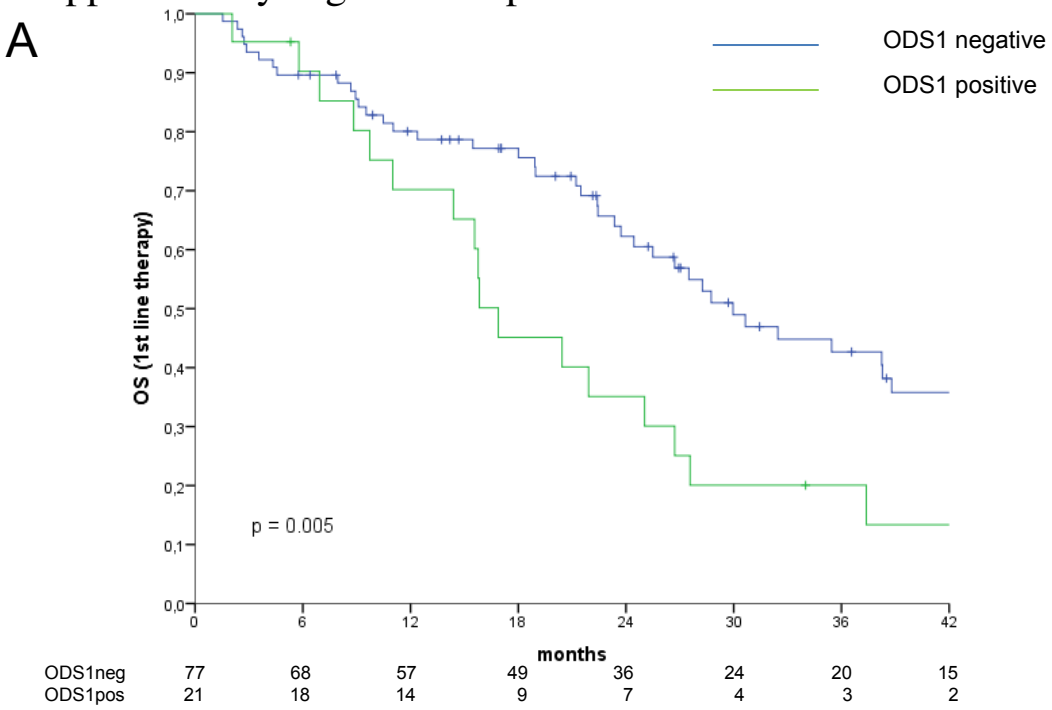

SMAD4
Q461*
Mutations
5
0
MH1
MH2
0
100
200
300
400
500
552 aa
10 Missense
8 Truncating

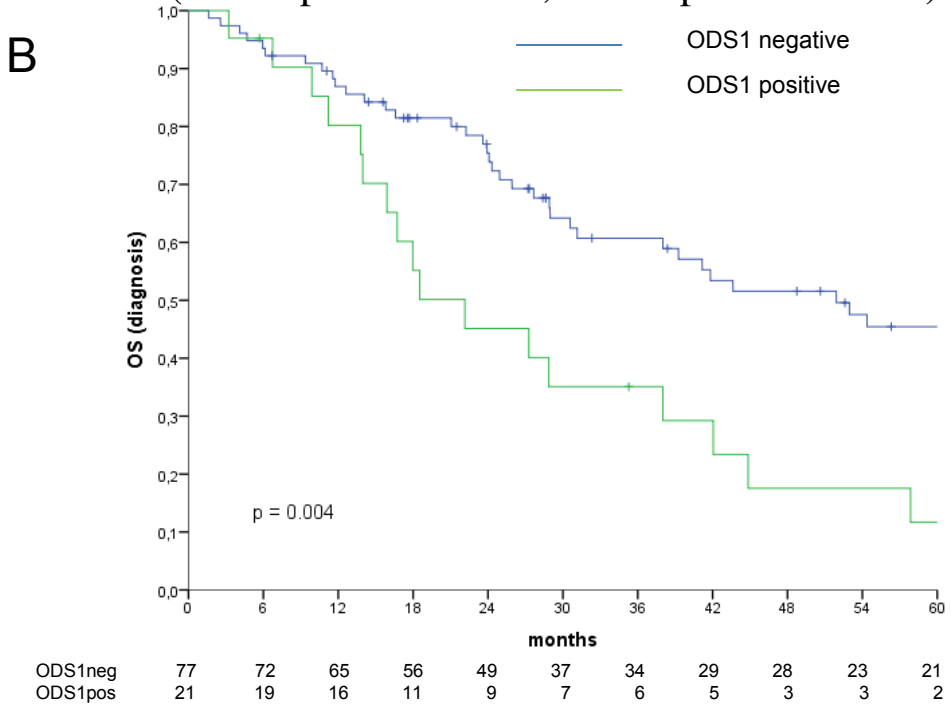

SOX9
Mutations
5
0
Sox_N
HMG_box
0
100
200
300
400
509 aa
8 Missense
4 Truncating

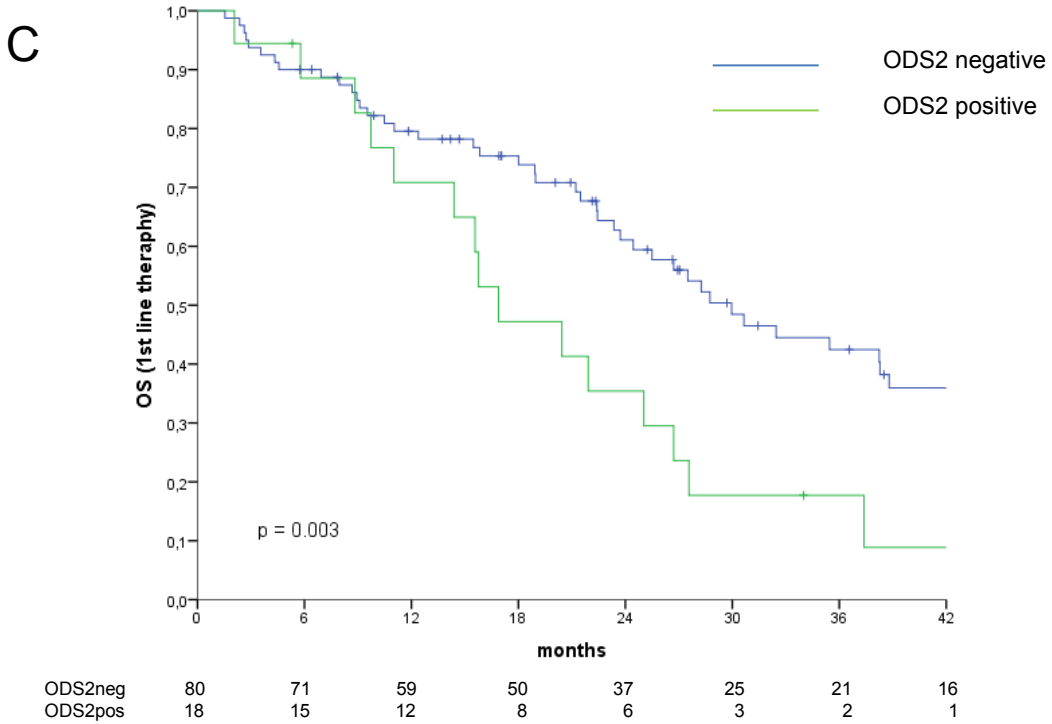

TCF7L2
H311Y
Mutations
5
0
CTNNB1_binding
HMG_box
0
100
200
300
400
500
619 aa
10 Missense
2 Truncating
1 Other

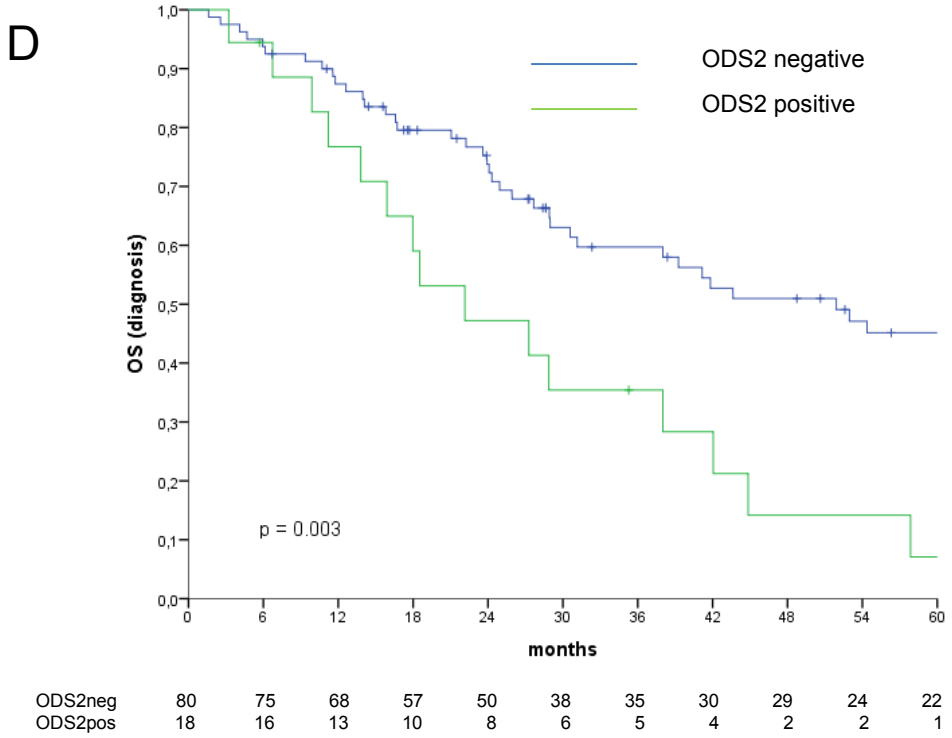

TP53
R273C/H
Mutations
8
0
P53..
P53
P53..
0
100
200
300
393 aa
102 Missense
29 Truncating
1 Other

Supplementary Figure 2: Kaplan-Meier survival curves of overall survival (ODS1 panel A and B; ODS2 panel C and D).









Supplementary Table 1: Mutation frequencies related to 16 CRC-associated genes observed in the present study (IRE-IFO) and in four publically available datasets (TCGA, DFCI, MSKCC, Genentech).

GENE (PATHWAY)	**IRE-IFO (N=98)**	TCGA (N=224)	DFCI (N=619)	MSKCC (N=69)	GENENTECH (N=72)
ACVR1B (TGF-β)	**1%**	6%	5%	0%	1.5%
AMER1 (WNT)	**7%**	11%	8%	9%	6%
APC (WNT)	**53%**	75%	57%	91%	40%
BRAF (RAS-MAPK)	**6%**	9%	20%	4%	8%
CTNNB1 (WNT)	**5%**	5%	6%	7%	7%
FBXW7 (WNT)	**21%**	17%	13%	9%	21%
KRAS (RAS-MAPK)	**58%**	42%	28%	55%	51%
MAP3K21 (RAS-MAPK)	**17%**	7%	4%	0%	0%
NRAS (RAS-MAPK)	**8%**	9%	4%	3%	3%
PIK3CA (PI3K-PTEN)	**33%**	20%	21%	22%	31%
PTEN (PI3K-PTEN)	**20%**	4%	8%	3%	6%
SMAD2 (TGF-β)	**8%**	7%	5%	0%	8%
SMAD4 (TGF-β)	**12%**	12%	11%	14%	24%
SOX9 (WNT)	**11%**	4%	10%	0%	3%
TCF7L2 (WNT)	**8%**	12%	7%	0%	8%
TP53 (p53 network)	**65%**	54%	50%	83%	56%

Supplementary Table 2: Statistically significant associations between the mutational status of 16 CRC-related genes and basal clinical features (N=98).

	Stage at diagnosis		Chi2 Test
	II-III	**IV**	**p-value**
	N(%)	**N(%)**	
AMERwt	29 (31.9)	62 (68.1)	0.047*
AMERmut	5 (71.4)	2 (28.6)	
PTENwt	23 (29.5)	55 (70.5)	0.032
PTENmut	11 (55.0)	9 (45.0)	
	ECOG PS		
	0	**1-2**	
	N(%)	**N(%)**	
CTNNB1wt	42 (45.2)	51 (54.8)	0.023*
CTNNB1mut	5 (100.0)	0 (0.0)	
TP53wt	11 (32.4)	23 (67.6)	0.024
TP53mut	36 (56.3)	28 (43.8)	
	Number of Metastatic Sites		
	1	**≥2**	
	N(%)	**N(%)**	
FBXW7wt	36 (46.8)	41 (53.2)	0.017
FBXW7mut	16 (76.2)	5 (23.8)	
	Side		
	Left	**Right + Transverse**	
	N(%)	**N(%)**	
KRASwt	28 (68.3)	13 (31.7)	0.039
KRASmut	27 (47.4)	30 (52.6)	
TP53wt	14 (41.2)	20 (58.8)	0.030
TP53mut	41 (64.1)	23 (35.9)	

* Fisher's Exact Test

Supplementary Table 3: Mutational status of the genes included in the OncoDriver signature 1 and 2 (ODS1 and ODS2).

GENE SIGNATURE	TP53	APC	AMER1	TCF7L2	FBXW7	SOX9	CTNNB1
ODS1	MUT	MUT	WT	WT	WT	Not Included	Not Included
ODS2	MUT	MUT	WT	WT	WT	WT	WT

Supplementary Table 4: Univariate and multivariate Cox regression models for progression-free survival (N=98). The multivariate Cox models were obtained by adjusting for all the variables tested in univariate analysis.

		Univariate Cox regression model		**Multivariate Cox regression model (ODS1 pos vs neg)**		**Multivariate Cox regression model (ODS2 pos vs neg)**	
		HR (95%CI)	**p-value**	**HR (95%CI)**	**p-value**	**HR (95%CI)**	**p-value**
ODS1	**Pos vs Neg**	2.42 (1.44-4.06)	0.001	2.16 (1.26-3.70)	0.005		
ODS2	**Pos vs Neg**	2.76 (1.59-4.80)	<0.001			2.56 (1.45-4.51)	0.001
Gender	**Female vs Male**	0.79 (0.51-1.24)	0.315	0.84 (0.53-1.35)	0.478	0.82 (0.51-1.30)	0.399
ECOG PS	**1-2 vs 0**	1.25 (0.82-1.89)	0.293	1.24 (0.81-1.90)	0.329	1.19 (0.78-1.83)	0.413
Side	**DX+TV vs SX**	0.93 (0.61-1.41)	0.730	0.90 (0.57-1.44)	0.675	0.87 (0.55-1.39)	0.559
Targeted Agent in first line therapy	**Yes vs No**	0.86 (0.57-1.31)	0.498	0.80 (0.51-1.27)	0.354	0.83 (0.53-1.31)	0.434
Surgery for metastatic disease	**Yes vs No**	0.63 (0.40-1.01)	0.054	0.60 (0.37-0.97)	0.039	0.59 (0.36-0.95)	0.032
Number of metastatic sites	**2-3 vs 1**	1.75 (1.14-2.68)	0.010	1.84 (1.17-2.89)	0.009	1.91 (1.21-3.00)	0.005

Supplementary Table 5: Uni- and multivariate Cox regression models for overall survival (N=98). The multivariate Cox models were obtained by adjusting for all the variables tested in univariate analysis.

		Univariate Cox regression model		**Multivariate Cox regression model (ODS1 pos vs neg)**		**Multivariate Cox regression model (ODS2 pos vs neg)**	
		HR (95%CI)	**p-value**	**HR (95%CI)**	**p-value**	**HR (95%CI)**	**p-value**
ODS1	**Pos vs Neg**	2.21 (1.26-3.88)	0.006	1.96 (1.07-3.59)	0.030		
ODS2	**Pos vs Neg**	2.34 (1.31-4.20)	0.004			2.22 (1.18-4.16)	0.013
Gender	**Female vs Male**	0.79 (0.47-1.33)	0.369	0.63 (0.36-1.11)	0.109	0.61 (0.35-1.08)	0.091
Stage at diagnosis	**IV vs II-III**	1.24 (0.74-2.08)	0.412	1.23 (0.72-2.12)	0.445	1.20 (0.70-2.05)	0.515
ECOG PS	**1-2 vs 0**	2.12 (1.28-3.52)	0.003	1.89 (1.10-3.25)	0.021	1.83 (1.06-3.14)	0.030
Side	**DX+TV vs SX**	1.07 (0.66-1.76)	0.777	0.86 (0.50-1.49)	0.588	0.83 (0.48-1.42)	0.491
Second line therapy	**Yes vs No**	0.29 (0.17-0.48)	<0.001	0.17 (0.09-0.32)	<0.001	0.16 (0.09-0.31)	<0.001
Surgery for metastatic disease	**Yes vs No**	0.58 (0.34-0.99)	0.046	0.49 (0.28-0.88)	0.016	0.50 (0.28-0.90)	0.020
Number of metastatic sites	**2-3 vs 1**	1.40 (0.85-2.33)	0.188	1.83 (1.05-3.17)	0.031	1.85 (1.07-3.20)	0.028
Targeted Agents	**Yes vs No**	0.66 (0.40-1.07)	0.094	0.83 (0.48-1.43)	0.503	0.81 (0.47-1.40)	0.449