

Supplementary material

Pathogenic Alterations in *PIK3CA* and *KMT2C* are Frequent and Independent Prognostic Factors in Anal Squamous Cell Carcinoma Treated with Salvage Abdominoperineal Resection

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Supplementary Table 4: Distribution of pathogenic variants in frequently altered genes.

Hugo Symbol	N in HPV-positive (%)	N in HPV-negative (%)	N total (%)	p-value ¹	Adjusted p-value ²
<i>TP53</i>	7 (4.96)	10 (58.82)	17 (10.76)	1.38E-07	2.78E-05
<i>TERT</i>	4 (2.84)	8 (47.06)	12 (7.59)	1.20E-06	1.21E-04
<i>CDKN2A</i>	1 (0.71)	3 (17.65)	4 (2.53)	0.00	0.26
<i>ARID1A</i>	2 (1.42)	2 (11.76)	4 (2.53)	0.06	1.00
<i>PIK3CA</i>	39 (27.66)	1 (5.88)	40 (25.32)	0.07	1.00
<i>KMT2D</i>	23 (16.31)	0 (0)	23 (14.56)	0.14	1.00
<i>NFE2L2</i>	6 (4.26)	2 (11.76)	8 (5.06)	0.21	1.00
<i>FBXW7</i>	19 (13.48)	0 (0)	19 (12.03)	0.23	1.00
<i>ATRX</i>	2 (1.42)	1 (5.88)	3 (1.9)	0.29	1.00
<i>CCND1</i>	2 (1.42)	1 (5.88)	3 (1.9)	0.29	1.00
<i>KDM6A</i>	2 (1.42)	1 (5.88)	3 (1.9)	0.29	1.00
<i>NF1</i>	2 (1.42)	1 (5.88)	3 (1.9)	0.29	1.00
<i>NSD1</i>	2 (1.42)	1 (5.88)	3 (1.9)	0.29	1.00
<i>SETD2</i>	2 (1.42)	1 (5.88)	3 (1.9)	0.29	1.00
<i>ATM</i>	3 (2.13)	1 (5.88)	4 (2.53)	0.37	1.00
<i>ARID2</i>	4 (2.84)	1 (5.88)	5 (3.16)	0.44	1.00
<i>BRCA2</i>	4 (2.84)	1 (5.88)	5 (3.16)	0.44	1.00
<i>FAT2</i>	4 (2.84)	1 (5.88)	5 (3.16)	0.44	1.00
<i>MGA</i>	4 (2.84)	1 (5.88)	5 (3.16)	0.44	1.00
<i>CASP8</i>	6 (4.26)	1 (5.88)	7 (4.43)	0.56	1.00
<i>NOTCH1</i>	9 (6.38)	0 (0)	9 (5.7)	0.60	1.00
<i>TGFBR2</i>	10 (7.09)	0 (0)	10 (6.33)	0.60	1.00
<i>KMT2C</i>	22 (15.6)	2 (11.76)	24 (15.19)	1.00	1.00
<i>FAT1</i>	15 (10.64)	2 (11.76)	17 (10.76)	1.00	1.00
<i>EP300</i>	12 (8.51)	1 (5.88)	13 (8.23)	1.00	1.00
<i>PTEN</i>	7 (4.96)	0 (0)	7 (4.43)	1.00	1.00
<i>PIK3R1</i>	6 (4.26)	0 (0)	6 (3.8)	1.00	1.00
<i>PBRM1</i>	5 (3.55)	0 (0)	5 (3.16)	1.00	1.00
<i>RB1</i>	5 (3.55)	0 (0)	5 (3.16)	1.00	1.00
<i>SOX2</i>	5 (3.55)	0 (0)	5 (3.16)	1.00	1.00
<i>STK11</i>	5 (3.55)	0 (0)	5 (3.16)	1.00	1.00
<i>TP53BP1</i>	5 (3.55)	0 (0)	5 (3.16)	1.00	1.00
<i>BRCA1</i>	4 (2.84)	0 (0)	4 (2.53)	1.00	1.00
<i>FAT4</i>	4 (2.84)	0 (0)	4 (2.53)	1.00	1.00
<i>MED12</i>	4 (2.84)	0 (0)	4 (2.53)	1.00	1.00
<i>RAD21</i>	4 (2.84)	0 (0)	4 (2.53)	1.00	1.00
<i>SRCAP</i>	4 (2.84)	0 (0)	4 (2.53)	1.00	1.00
<i>ZNF750</i>	4 (2.84)	0 (0)	4 (2.53)	1.00	1.00
<i>BCORL1</i>	3 (2.13)	0 (0)	3 (1.9)	1.00	1.00
<i>BLM</i>	3 (2.13)	0 (0)	3 (1.9)	1.00	1.00
<i>BRAF</i>	3 (2.13)	0 (0)	3 (1.9)	1.00	1.00
<i>BRIP1</i>	3 (2.13)	0 (0)	3 (1.9)	1.00	1.00
<i>CREBBP</i>	3 (2.13)	0 (0)	3 (1.9)	1.00	1.00
<i>CTCF</i>	3 (2.13)	0 (0)	3 (1.9)	1.00	1.00
<i>ERBB2</i>	3 (2.13)	0 (0)	3 (1.9)	1.00	1.00
<i>INPP4B</i>	3 (2.13)	0 (0)	3 (1.9)	1.00	1.00
<i>KMT2B</i>	3 (2.13)	0 (0)	3 (1.9)	1.00	1.00
<i>MEF2C</i>	3 (2.13)	0 (0)	3 (1.9)	1.00	1.00
<i>NCOR1</i>	3 (2.13)	0 (0)	3 (1.9)	1.00	1.00
<i>NOTCH4</i>	3 (2.13)	0 (0)	3 (1.9)	1.00	1.00
<i>PKD1</i>	3 (2.13)	0 (0)	3 (1.9)	1.00	1.00
<i>SAMD9L</i>	3 (2.13)	0 (0)	3 (1.9)	1.00	1.00
<i>TET2</i>	3 (2.13)	0 (0)	3 (1.9)	1.00	1.00

N: number of patients with at least one alteration in this pathway

¹: Fisher's exact test

²: Benjamini and Hochberg method

Supplementary Table 5: Distribution of pathogenic variants in frequently altered biological pathways

Pathways	N in HPV-positive (%)	N in HPV-negative (%)	N total (%)	p-value ²	Adjusted p-value ²
Chromatine remodelling	67 (47.52)	8 (47.06)	75 (47.47)	1.0000	1.0000
PI3K/AKT/mTOR	60 (42.55)	1 (5.88)	61 (38.61)	0.0029	0.0358
DNA Repair	38 (26.95)	6 (35.29)	44 (27.85)	0.5672	1.0000
Regulation of gene expression	32 (22.7)	4 (23.53)	36 (22.78)	1.0000	1.0000
Notch	32 (22.7)	1 (5.88)	33 (20.89)	0.2016	1.0000
Genome Integrity	15 (10.64)	12 (70.59)	27 (17.09)	1.97E-07	4.93E-06
RTK-Ras	22 (15.6)	3 (17.65)	25 (15.82)	0.7347	1.0000
Hippo	21 (14.89)	2 (11.76)	23 (14.56)	1.0000	1.0000
others	16 (11.35)	2 (11.76)	18 (11.39)	1.0000	1.0000
Cell cycle	14 (9.93)	4 (23.53)	18 (11.39)	0.1081	0.9006
Apoptosis	12 (8.51)	1 (5.88)	13 (8.23)	1.0000	1.0000
TGF-β	12 (8.51)	0 (0)	12 (7.59)	0.3653	1.0000
Immunity	10 (7.09)	0 (0)	10 (6.33)	0.6020	1.0000
Oxydative stress	7 (4.96)	2 (11.76)	9 (5.7)	0.2498	1.0000
Adhesion-migration	8 (5.67)	0 (0)	8 (5.06)	0.6007	1.0000
Myc	5 (3.55)	1 (5.88)	6 (3.8)	0.5008	1.0000
Epigenetics	4 (2.84)	0 (0)	4 (2.53)	1.0000	1.0000
MAPK	3 (2.13)	1 (5.88)	4 (2.53)	0.3687	1.0000
Wnt	3 (2.13)	1 (5.88)	4 (2.53)	0.3687	1.0000
Cellular metabolisme	3 (2.13)	0 (0)	3 (1.9)	1.0000	1.0000
mRNA processing	3 (2.13)	0 (0)	3 (1.9)	1.0000	1.0000
Protein processing	2 (1.42)	0 (0)	2 (1.27)	1.0000	1.0000
JAK-STAT	1 (0.71)	0 (0)	1 (0.63)	1.0000	1.0000
SHH	1 (0.71)	0 (0)	1 (0.63)	1.0000	1.0000

N: number of patients with at least one alteration in this pathway

¹: Fisher's exact test

²: Benjamini and Hochberg method

Supplementary Table 6: Univariate analysis of the association between histopathological, clinical, and biological variables and survival measures in HPV-positive cases.

Characteristic	Overall survival				Disease-free survival				Metastase-free survival			
	N	Event N	Median survival (months) ¹	p-value ²	N	Event N	Median survival (months) ¹	p-value ²	N	Event N	Median survival (months) ¹	p-value ²
Age	134	57		0.061	140	77		0.006	140	69		0.011
<65	88	43	126		92	59	18		92	53	31	
≥65	46	14	183		48	18	—		48	16	—	
Sex	134	57		0.8	140	77		0.6	140	69		>0.9
Female	92	38	132		96	55	30		96	46	63	
Male	42	19	183		44	23	49		44	23	49	
ypT	132	55		0.009	138	75		0.042	138	67		0.025
ypT1-2	65	18	—		69	31	—		69	26	—	
ypT3-4	67	37	89		69	44	18		69	41	29	
ypN	133	57		<0.001	139	77		<0.001	139	69		<0.001
ypN0	106	36	183		109	52	273		109	46	273	
ypN+	27	21	54		30	25	11		30	23	19	
ypM	134	57		<0.001	140	77		<0.001	140	69		<0.001
ypM0	128	51	161		133	70	41		133	63	63	
ypM1	6	6	28		7	7	2.1		7	6	2.1	
Differentiation	133	56		0.2	138	75		0.6	138	67		0.7
Low	33	11	—		34	17	36		34	15	—	
Moderate/high	100	45	126		104	58	30		104	52	54	
Stage	126	56		0.029	129	72		0.2	129	64		0.3
I	11	6	183		11	6	20		11	6	24	
II	61	27	99		62	34	36		62	30	54	
III	52	21	290		54	30	19		54	26	273	
IV	2	2	33		2	2	6.6		2	2	15	
Resection margin	134	57		<0.001	140	77		<0.001	140	69		<0.001
R0	101	31	183		107	48	273		107	42	273	
R1	33	26	55		33	29	10		33	27	20	
HIV status	119	51		0.8	125	70		0.6	125	62		>0.9
Negative	100	42	132		104	59	20		104	51	38	
Positive	19	9	—		21	11	54		21	11	54	
Vascular embols	133	56		<0.001	139	76		0.008	139	68		0.013
No	80	23	221		85	39	—		85	34	—	
Yes	53	33	58		54	37	14		54	34	28	
Pre-operative treatment	133	57		0.3	139	77		0.2	139	69		0.5
Chemoradiotherapy	100		126		104	59	19		104	51	50	
Radiotherapy	33		183		35	18	49		35	18	63	
PIK3CA (all)	134	57		0.048	140	77		0.2	140	69		0.021
WT	97	41	183		101	51	50		101	43	273	
Mutated	37	16	87		39	26	20		39	26	29	
PIK3CA (exon 9/20)	134	57		0.004	140	77		0.044	140	69		0.006
WT	104	39	183		109	55	49		109	47	273	
Mutated	30	18	58		31	22	18		31	22	20	
PIK3CA (non exon 9/20)	134	57		0.3	140	77		0.5	140	69		0.8
WT	127	55	132		132	73	30		132	65	54	
Mutated	7	2	—		8	4	49		8	4	49	
KMT2D	134	57		0.025	140	77		0.11	140	69		0.032
WT	113	44	183		117	61	36		117	53	273	
Mutated	21	13	62		23	16	19		23	16	24	
KMT2C	134	57		0.005	140	77		0.002	140	69		<0.001
WT	114	44	183		118	59	50		118	52	273	
Mutated	20	13	55		22	18	10		22	17	16	
FBXW7	134	57		0.039	140	77		0.3	140	69		0.12
WT	115	47	161		121	66	32		121	58	54	
Mutated	19	10	132		19	11	9.5		19	11	20	
FAT1	134	57		0.4	140	77		0.2	140	69		0.3
WT	119	48	183		125	67	36		125	60	54	
Mutated	15	9	126		19	10	11		19	9	20	
EP300	134	57		0.2	140	77		0.4	140	69		0.8
WT	122	50	161		128	69	32		128	62	54	
Mutated	12	7	60		12	8	13		12	7	33	
TGFB2	134	57		>0.9	140	77		0.7	140	69		0.8
WT	125	54	132		130	73	30		130	65	50	
Mutated	9	3	—		10	4	—		10	4	—	
NOTCH1	134	57		0.11	140	77		0.065	140	69		0.044
WT	126	56	126		131	75	24		131	68	45	
Mutated	8	1	142		9	2	—		9	1	—	
TP53	134	57		0.6	140	77		0.6	140	69		0.4
WT	127	53	132		133	73	31		133	65	54	
Mutated	7	4	183		7	4	11		7	4	24	
PTEN	134	57		0.063	140	77		0.037	140	69		0.09
WT	127	52	161		133	71	36		133	64	54	
Mutated	7	5	62		7	6	8.7		7	5	24	

N: Number of cases with non missing data for the variable

WT: wild-type

¹ —: Median survival not reached

²: Log-rank test

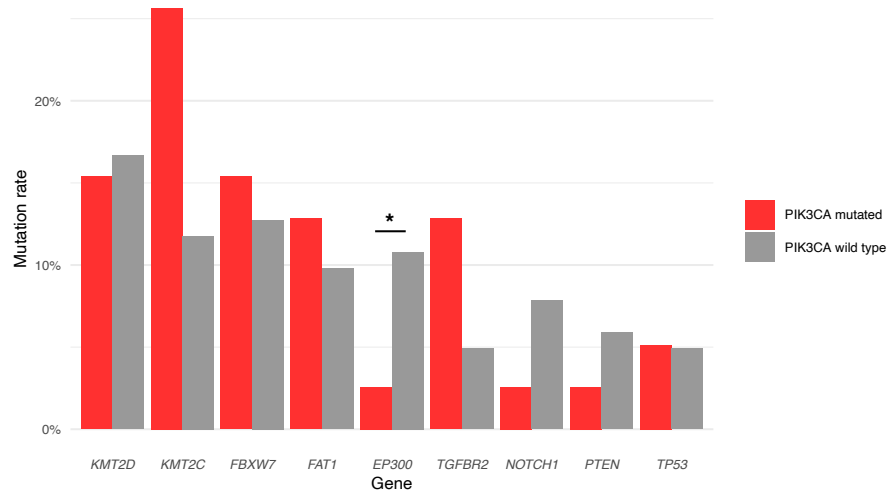
Supplementary Table 7: Univariate analysis of the association between histopathological, clinical, and biological variables and survival measures in HPV-negative cases.

Characteristic	Overall survival				Disease-free survival				Metastase-free survival			
	N	Event N	Median survival (months) ¹	p-value ²	N	Event N	Median survival (months) ¹	p-value ²	N	Event N	Median survival (months) ¹	p-value ²
Age	16	6	—	0.5	17	8	—	0.6	17	6	—	0.4
	12	5	—		12	6	21		12	5	—	
	4	1	—		5	2	—		5	1	—	
Sex	16	6	—	0.4	17	8	—	>0.9	17	6	—	0.5
	5	1	—		5	2	—		5	1	—	
	11	5	75		12	6	21		12	5	—	
ypT	16	6	—	0.5	17	8	—	0.4	17	6	—	0.5
	9	3	—		10	4	—		10	3	—	
	7	3	69		7	4	21		7	3	—	
ypN	15	5	—	<0.001	16	7	—	0.065	16	5	—	0.012
	14	4	—		15	6	—		15	4	—	
	1	1	17		1	1	6.9		1	1	6.9	
Differentiation	16	6	—	0.016	17	8	—	0.2	17	6	—	0.073
	2	2	27		2	2	14		2	2	14	
	14	4	—		15	6	—		15	4	—	
Stage	14	5	—	0.2	15	7	—	0.3	15	5	—	0.2
	3	0	—		3	0	—		3	0	—	
	6	4	42		7	5	16		7	4	21	
	5	1	—		5	2	—		5	1	—	
Resection margin	15	6	—	0.1	16	8	—	<0.001	16	6	—	<0.001
	14	5	—		15	7	21		15	5	—	
	1	1	24		1	1	1		1	1	1.7	
HIV status	16	6	—	0.3	17	8	—	0.2	17	6	—	0.3
	14	6	—		15	8	21		15	6	—	
	2	0	—		2	0	—		2	0	—	
Vascular embols	16	6	—	0.5	17	8	—	0.4	17	6	—	0.7
	12	5	—		13	7	21		13	5	—	
	4	1	—		4	1	—		4	1	—	
Pre-operative treatment	15	6	—	0.12	16	8	—	0.3	16	6	—	0.2
	7	1	—		7	2	—		7	1	—	
	8	5	42		9	6	16		9	5	21	
TP53	16	6	—	0.041	17	8	—	0.2	17	6	—	0.016
	6	0	—		7	2	—		7	0	—	
	10	6	42		10	6	15		10	6	13	
TERT	16	6	—	0.11	17	8	—	0.2	17	6	—	0.033
	9	2	—		10	4	—		10	2	—	
	10	4	37		7	4	9		7	4	13	
CDKN2A	16	6	—	0.5	17	8	—	0.4	17	6	—	0.2
	13	4	—		14	6	—		14	4	—	
	3	2	47		3	2	9		3	2	13	

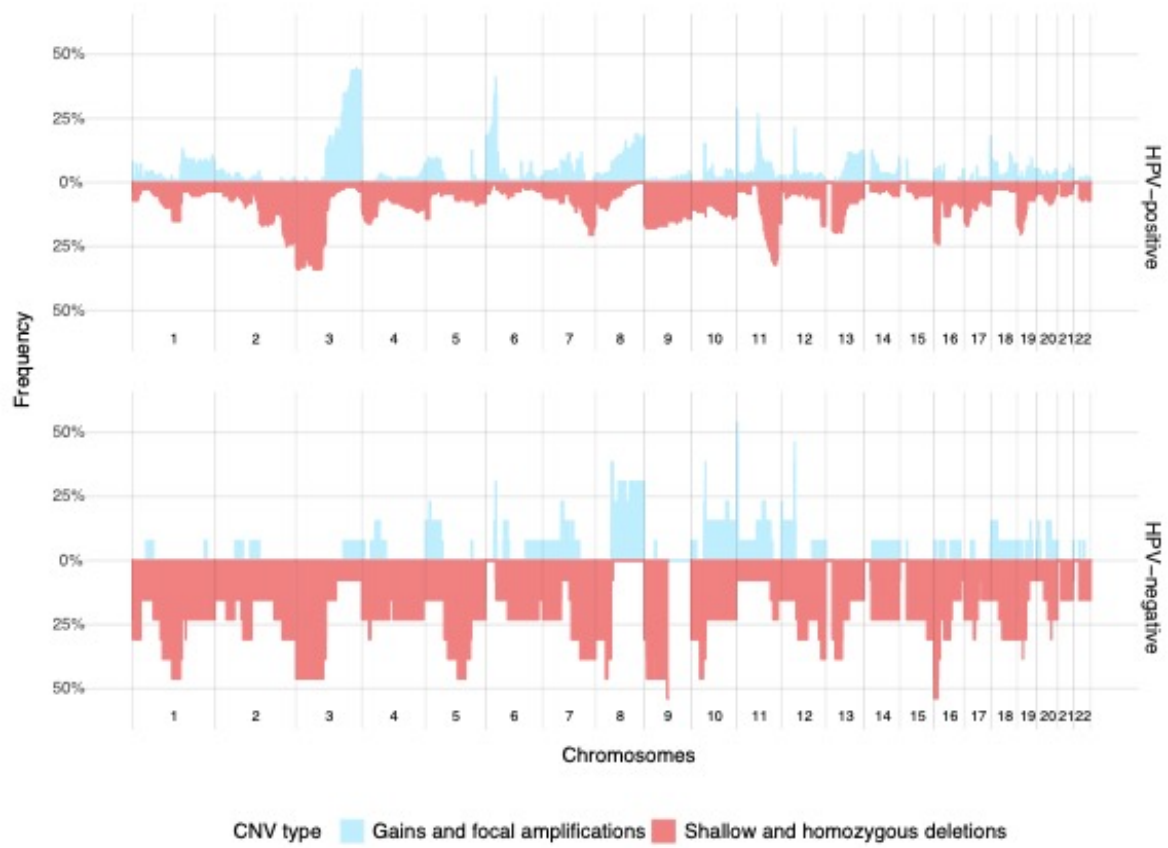
WT: wild-type

¹ — : Median survival not reached

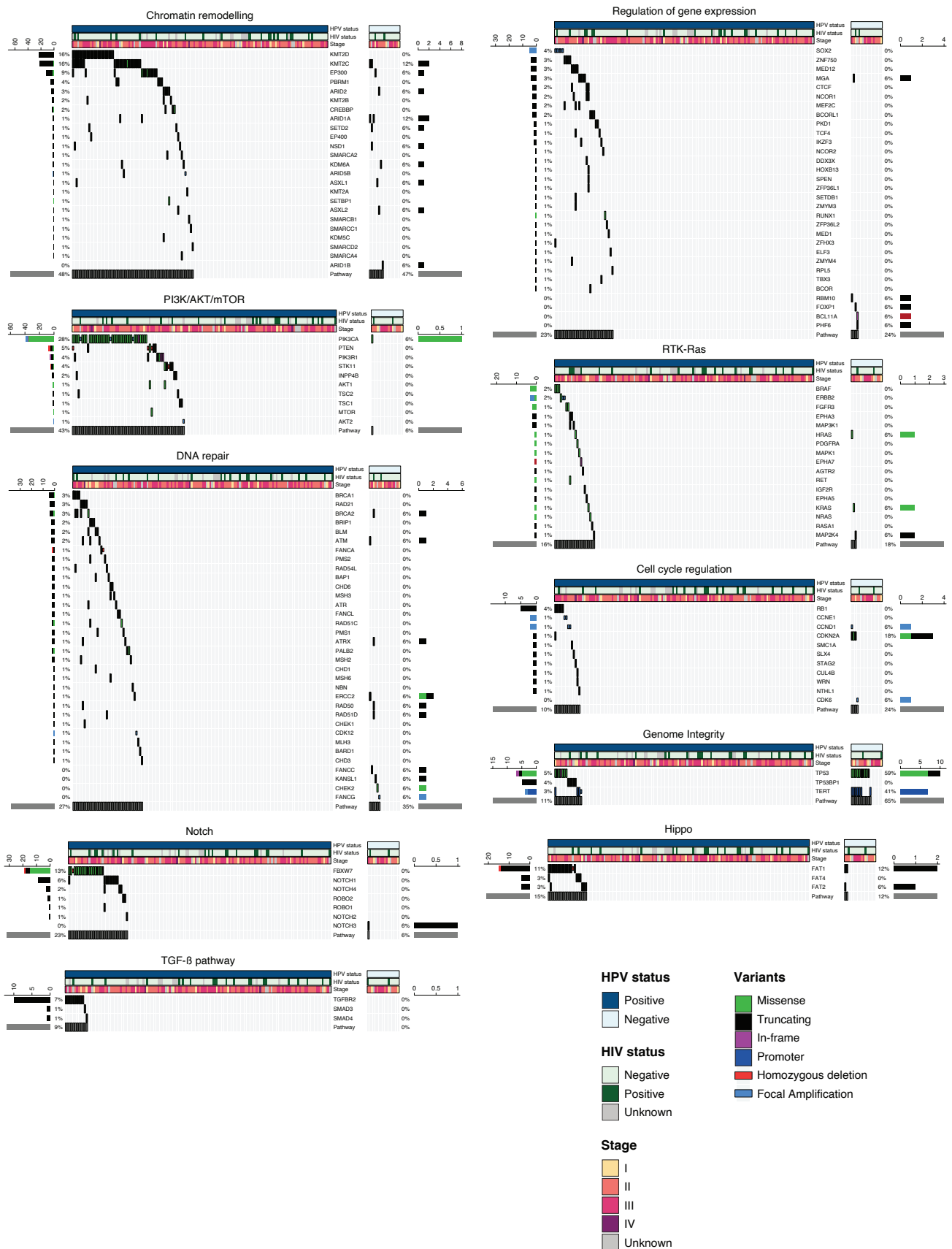
² : Log-rank test



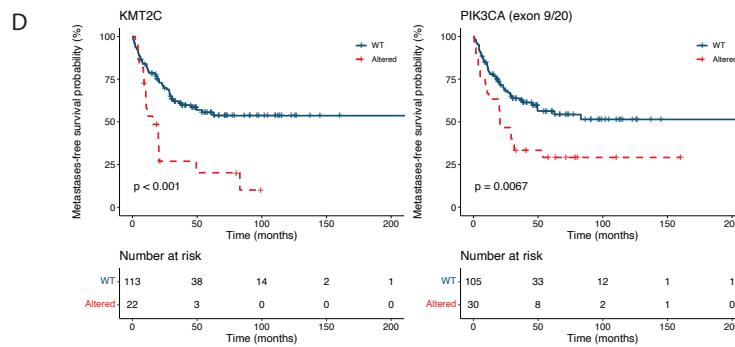
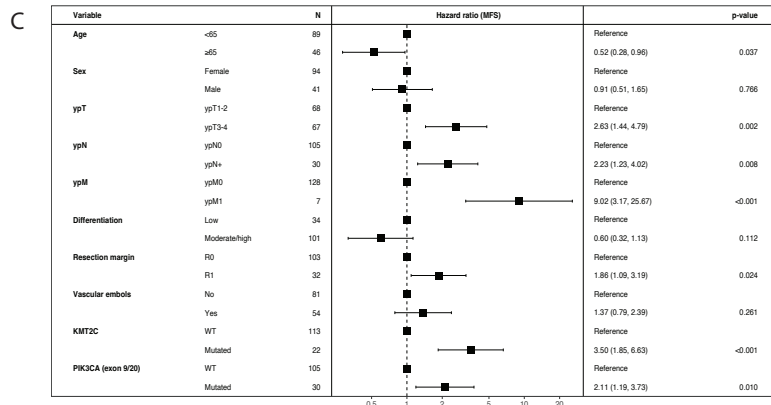
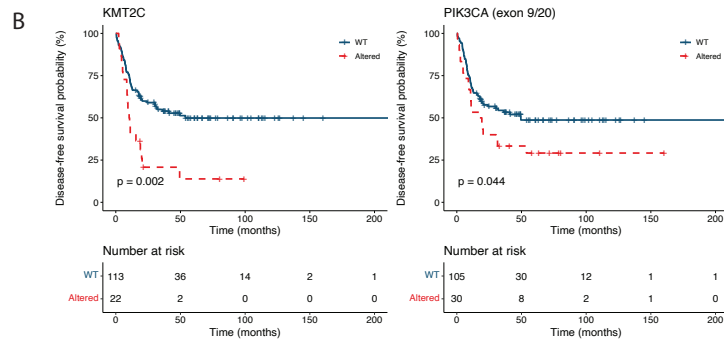
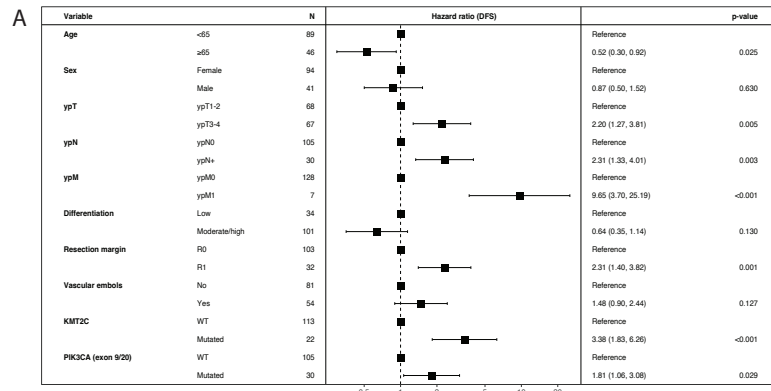
Supplementary Figure 1: Frequency of alterations in frequently mutated genes by *PIK3CA* status.* : adjusted p-value < 0.05.



Supplementary Figure 2: Genome-wide profile of CNV frequencies. Samples are separated by viral status.



Supplementary Figure 3: Genomic landscape of frequently altered biological pathways and cellular processes in ASCC. Samples are separated by viral status. Truncating variants include non-sense and frame-shift variants and substitution at splice-sites in tumor suppressor genes. Only variants with established pathogenicity (determined as described in the Material and Method section) are represented.



Supplementary Figure 4: KMT2C and PIK3CA exon 9/20 alterations are associated with shorter DFS and MFS. A: Cox multivariate model of DFS; B: Kaplan-Meier curves and risk tables of DFS stratified by KMT2C and PIK3CA exon 9/20 status; C: Cox multivariate model of MFS; D: Kaplan-Meier curves and risk tables of MFS stratified by KMT2C and PIK3CA exon 9/20 status. N: The p-value of the log-rank test comparing survival probabilities is reported on the graph. WT: wild type.