Supplementary Online Content

Necchi A, Spiess PE, Costa de Padua T, et al. Genomic profiles and clinical outcomes of penile squamous cell carcinoma with elevated tumor mutational burden. *JAMA Netw Open*. 2023;6(12):e2348002. doi:10.1001/jamanetworkopen.2023.48002

This supplementary material has been provided by the authors to give readers additional information about their work.

eTable. Clinical characteristics of the routine clinical outcomes cohort

Characteristic	**All patients N (%)**	**Chemotherapy* N (%)**	**Mono ICI N (%)**
N	30	20	10
Age at start of therapy (years; median [IQR])	61.5 (52.2; 70.8)	60.5 (51.8; 70)	69.0 (61; 81)
ECOG Performance Status:			
• 0	6 (20.0)	3 (15)	3 (30)
• 1	14 (46.7)	9 (45)	5 (50)
• ≥ 2	2 (6.67)	2 (10)	0
• Unknown	8 (26.7)	6 (30)	2 (20)
NLR:			
• NLR < 2.5	1 (3.33)	0	1 (10)
• NLR ≥ 2.5	21 (70)	13 (65)	8 (80)
• Unknown	8 (26.7)	7 (35)	1 (10)
Stage at diagnosis:			
• Stage I-III	10 (33.3)	7 (35)	3 (30)
• Stage IV	9 (30)	7 (35)	2 (20)
• Unknown/not documented	11 (36.7)	6 (3)	5 (50)
Prior receipt of systemic antineoplastic therapy:			
• Did not have prior systemic therapy	14 (46.7)	10 (50)	4 (40)
• Had prior systemic therapy	16 (53.3)	10 (50)	6 (60)
SES (quintile):**			
• 1-2 - Lowest SES	11 (36.7)	6 (30)	5 (50)
• 3	11 (36.7)	<10 (<50)	<5 (<50)

• 4-5 - Highest SES	5 (16.7)	<5 (<25)	<5 (<50)
• Unknown	3 (10)	<5 (<25)	<5 (<50)
Race (self-reported):			
• White	21 (70)	14 (70)	7 (70)
• Other/unknown	9 (30)	6 (30)	3 (30)
Genomic ancestry:			
• EUR	18 (60)	10 (50)	8 (80)
• Other	9 (30)	9 (45)	0
• Unknown	3 (10)	1 (5)	2 (20)
TMB status:			
• TMB-L	21 (70)	16 (80)	5 (50)
• TMB-H	4 (13.3)	2 (10)	2 (20)
• TMB-VH	2 (6.67)	1 (5)	1 (10)
• Unknown	3 (10)	1 (5)	2 (20)
MSI status:			
• MSS	25 (83.3)	19 (95)	6 (60)
• MSI-H	2 (6.67)	0	2 (20)
• Unknown	3 (10)	1 (5)	2 (20)
Genomic LOH status:			
• High gLOH	1 (3.33)	1 (5)	0
• Low gLOH	19 (63.3)	12 (60)	7 (70)
• Unknown	10 (33.3)	7 (35)	3 (30)
PD-L1 status (22C3 TPS):			
• PD-L1 negative	7 (23.3)	4 (20)	3 (30)
• PD-L1 high positive	1 (3.33)	1 (5)	0
• Unknown	22 (73.3)	15 (75)	7 (70)
HPV strain detected:			

• HPV-16	6 (20)	3 (15)	3 (30)
• HPV-33	1 (3.33)	0	1 (10)
• HPV-6	2 (6.67)	2 (10)	0
• Not detected	21 (70)	15 (75)	6 (60)
Smoking status:			
• History of smoking	20 (66.7)	16 (80)	4 (40)
• No history of smoking	10 (33.3)	4 (20)	6 (60)

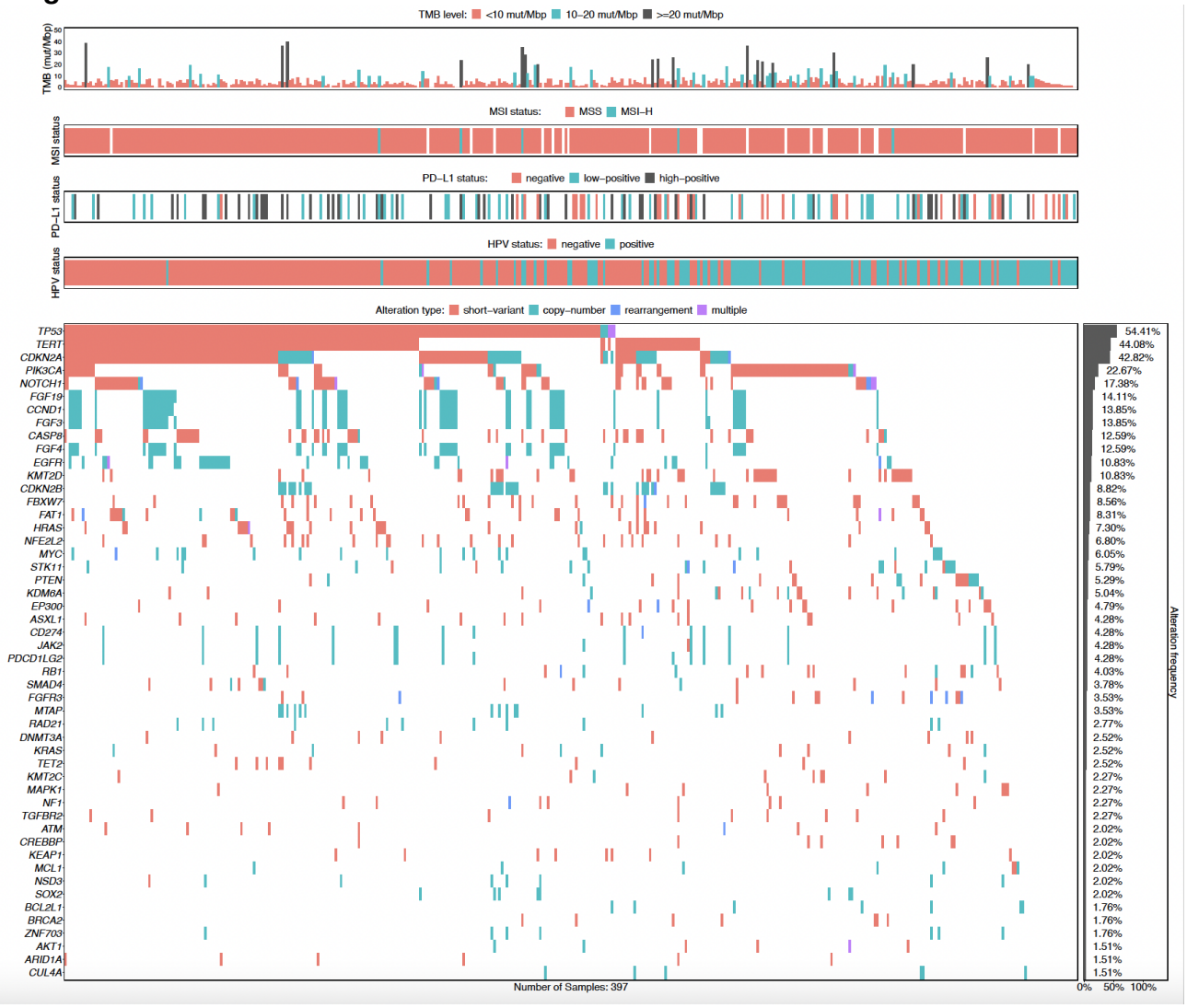
Abbreviations: ECOG: Eastern Cooperative Oncology Group; HPV: human papillomavirus; ICI: immune-checkpoint inhibitors; IQR: interquartile range; LOH: loss of heterozygosity; Mono: monotherapy; MSI-H: microsatellite instability high; MSS: microsatellite stable; NLR: neutrophil-to-lymphocyte ratio; PD-L1: programmed-cell death-ligand-1; SES: socioeconomic status; TMB: tumor mutational burden (H: high; L: low; VH: very high);

* Two patients who received targeted therapy (cetuximab) are counted in the chemotherapy group.

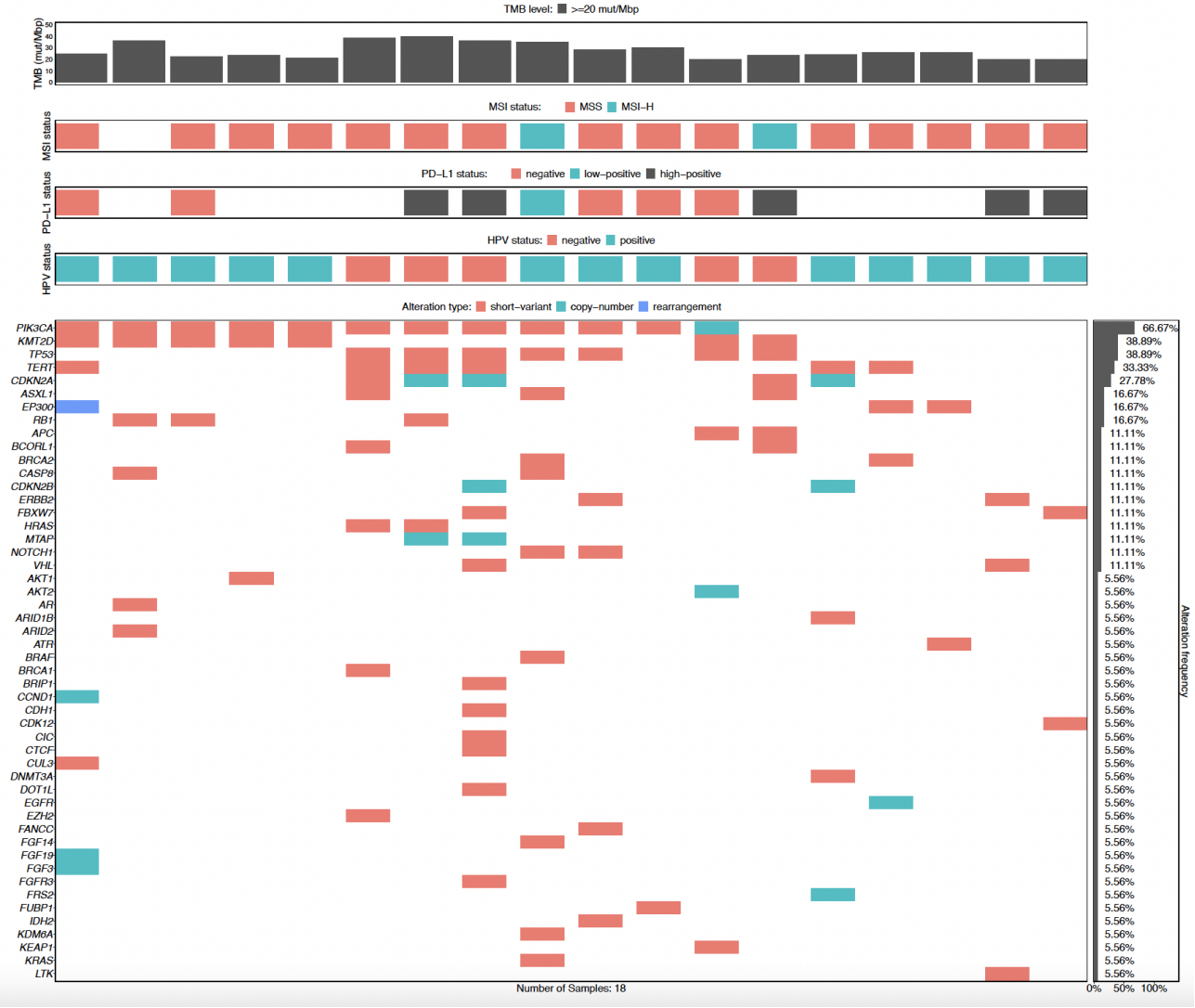
** To protect patient privacy and reduce the risk of re-identification, some SES categories have been merged and/or had their counts masked.

eFigure 1 Tile plot showing the distribution, type and frequency of single gene alterations* occurring in the entire population (A) or in the population of patients with TMB-very high PSCC (B)

eFigure 1A

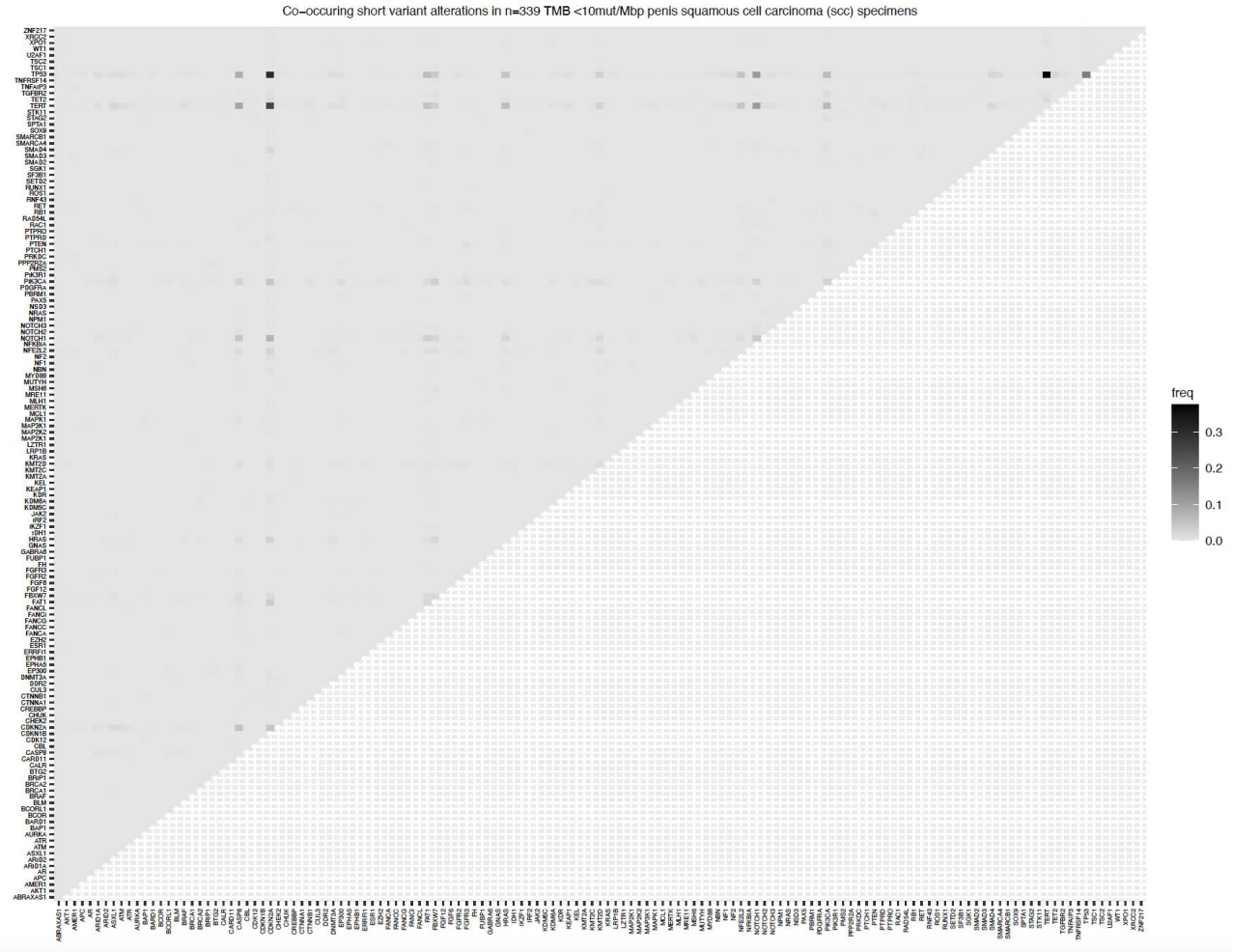


eFigure 1B

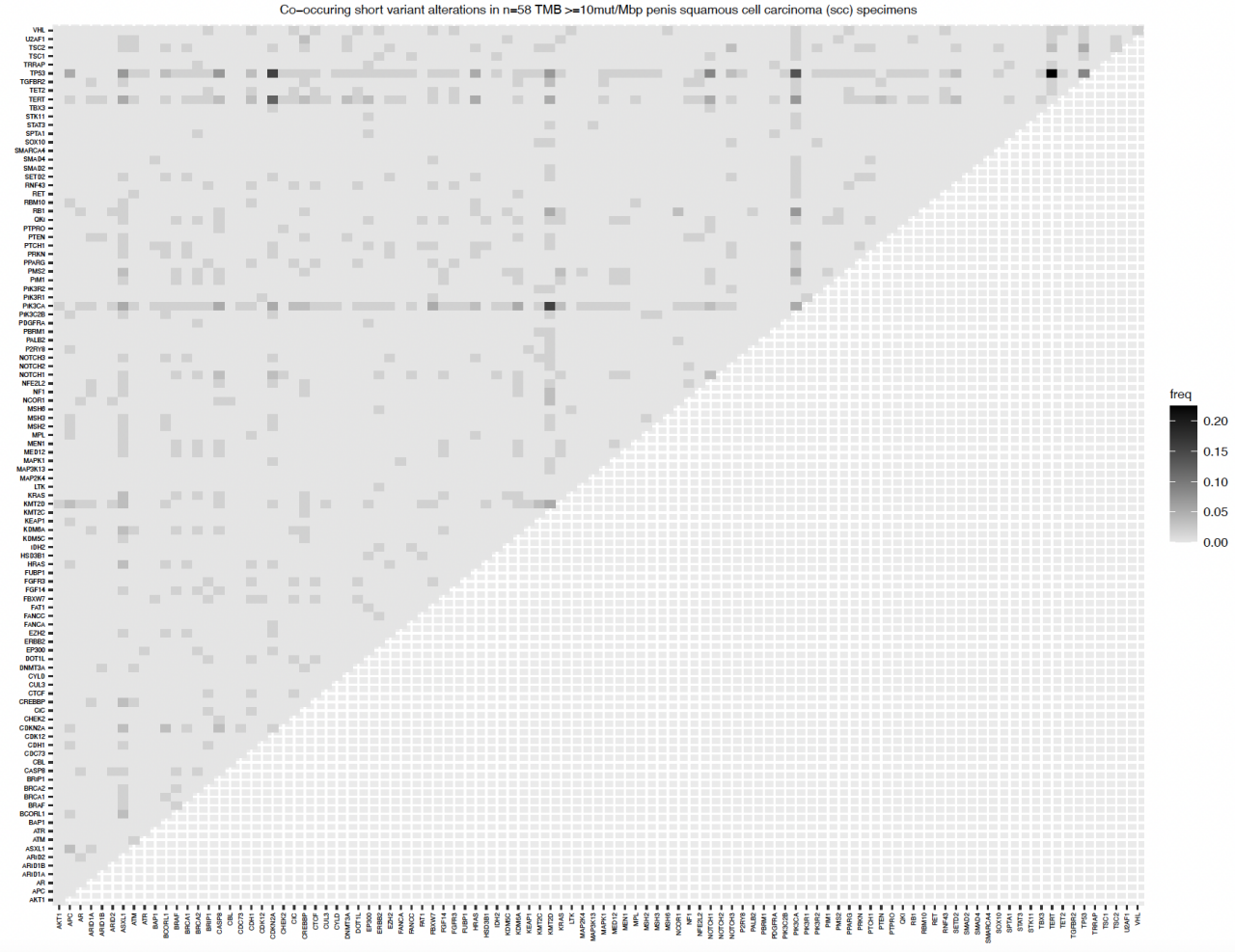


eFigure 2. Tile plot displaying the frequency of pairwise co-occurring short variant alterations in the cohort of TMB-low (A) and TMB-high + very high (B) PSCC

eFigure 2A

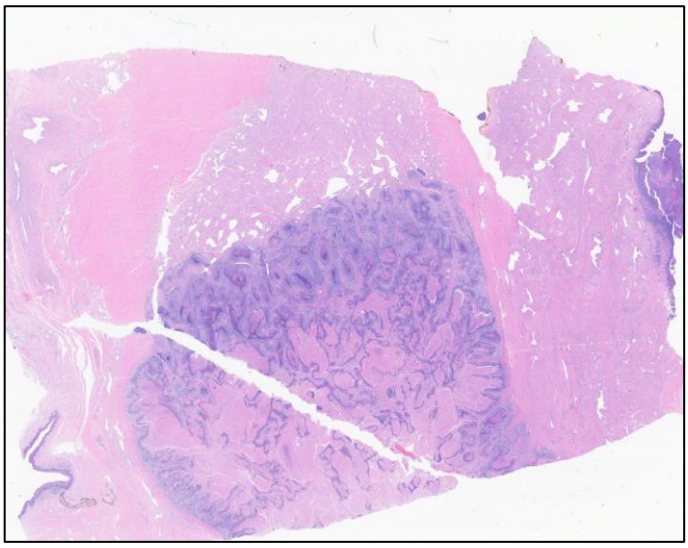


eFigure 2B

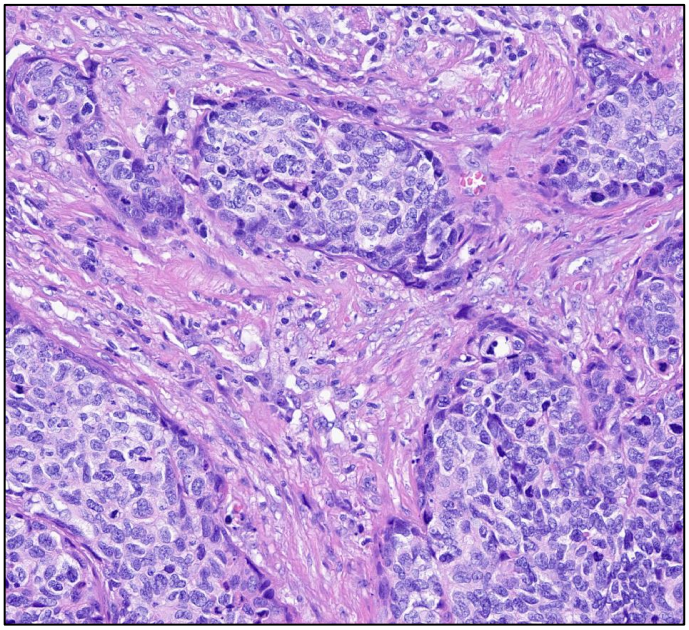


eFigure 3. Low magnification (A) and high magnification (B) images of the primary PSCC which was used for sequencing are shown. Comprehensive genomic profiling revealed a missense extra-cellular domain E265 ERBB2 mutation present in 44% of reads as seen in the IGV (Integrated Genomics Viewer) view (C).

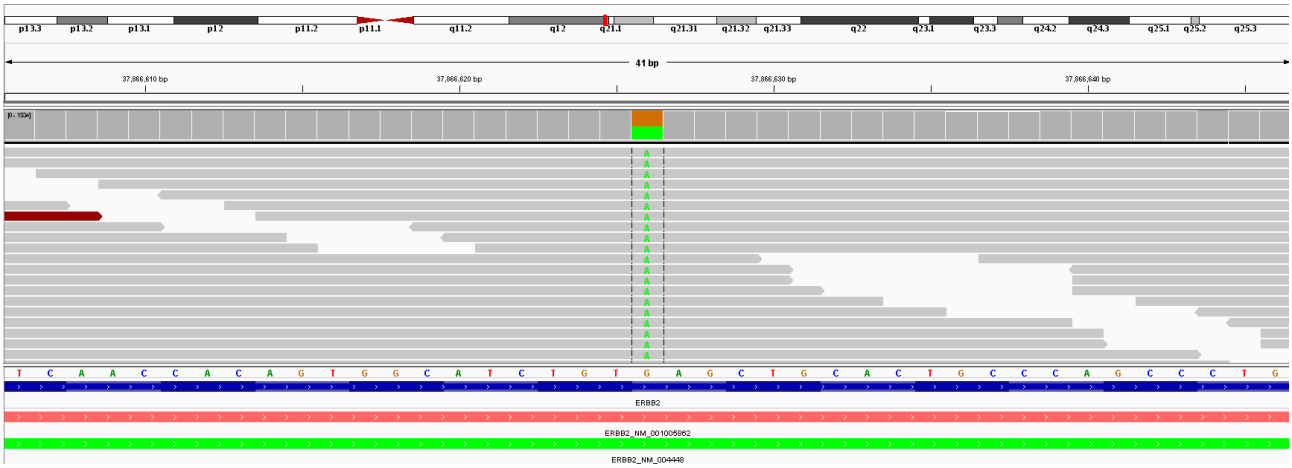
eFigure 3A



eFigure 3B

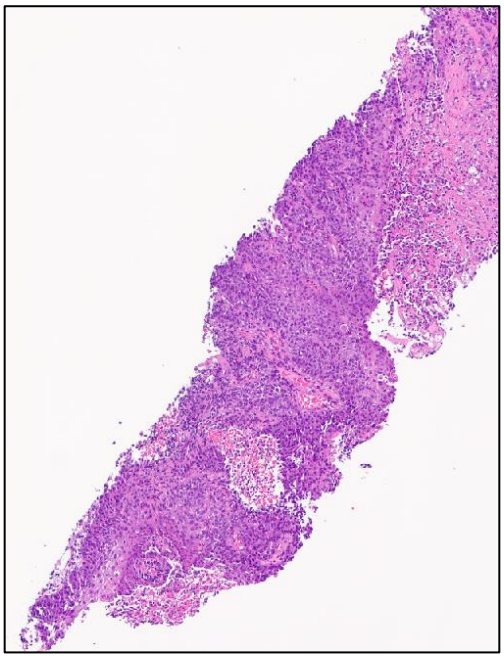


eFigure 3C

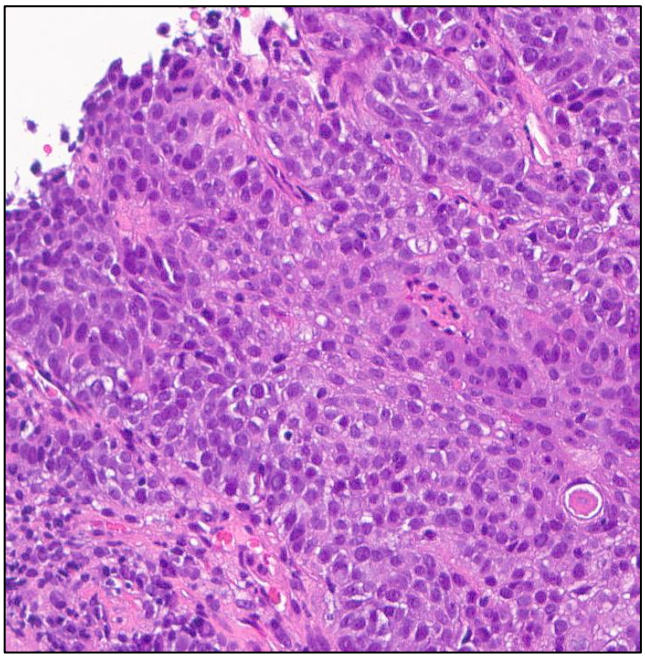


eFigure 4. Low magnification (A) and high magnification (B) images of the primary PSCC which was used for sequencing are shown. Comprehensive genomic profiling revealed a NOTCH1 R2327W missense mutation present in 28% of reads, as seen in the IGV (Integrated Genomics Viewer) view (C).

eFigure 4A



eFigure 4B



eFigure 4C