

Figure. S1. Mutation prediction results on the SMH dataset by the classifiers trained with TCGA FFPE data. The area under the curves (AUCs) for the receiver operating characteristic (ROC) curves were presented for each classifier.

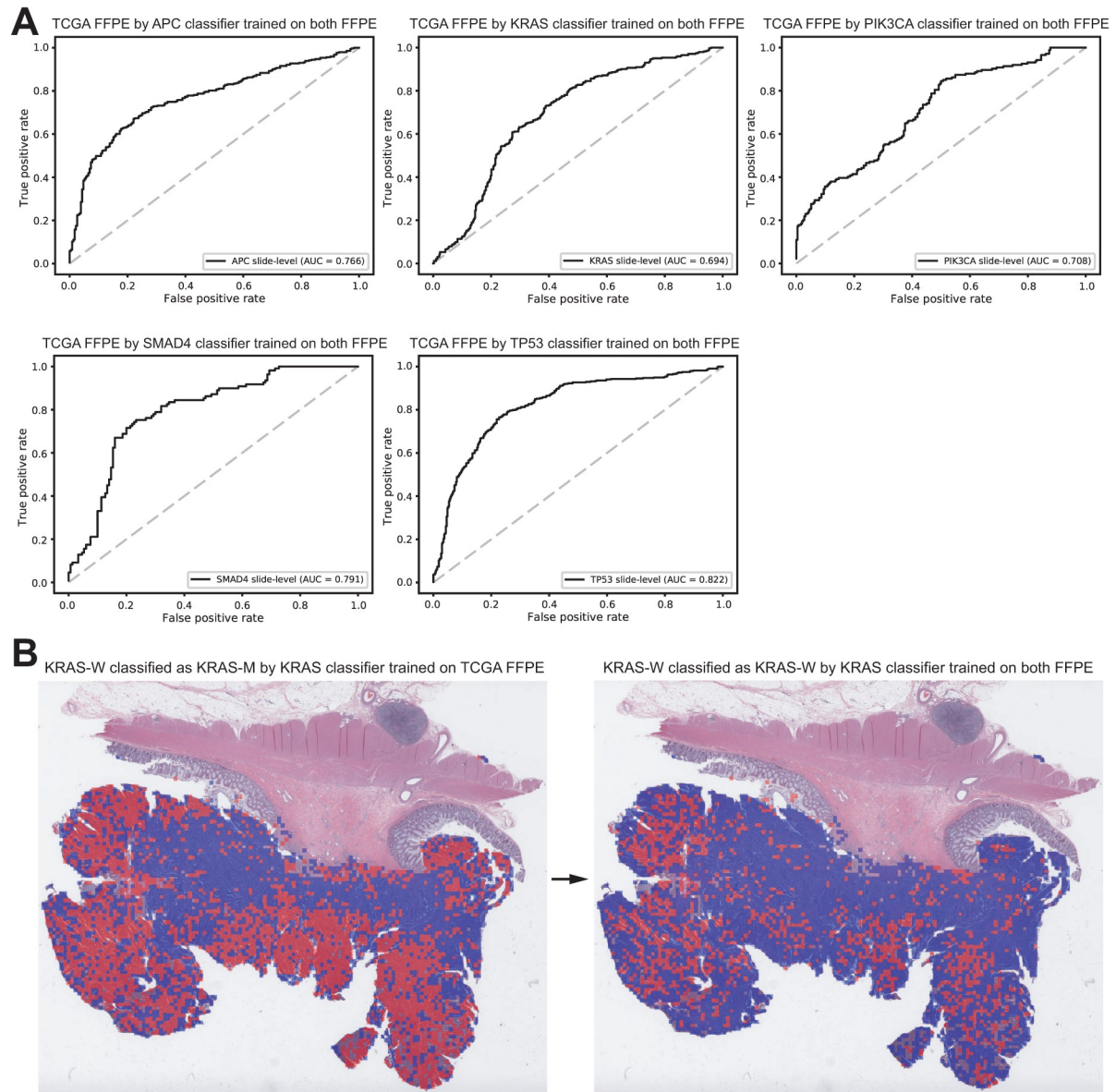


Figure. S2. Mutation prediction results on the TCGA FFPE dataset by the classifiers trained with both TCGA and SMH FFPE data. **A.** The area under the curves (AUCs) for the receiver operating characteristic (ROC) curves were presented for each classifier. **B.** A representative heatmap of whole slide image which was falsely classified by the classifier trained with TCGA data but correctly classified by the classifier trained with both data.

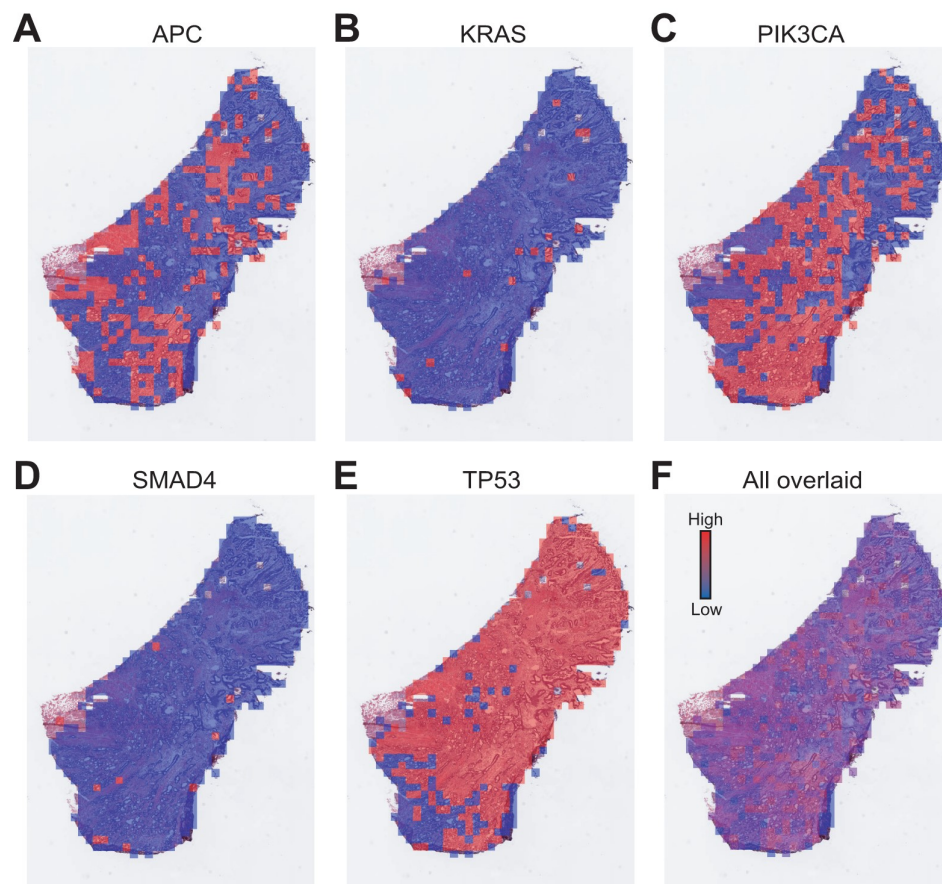


Figure. S3. Representative mutation prediction results on a frozen tissue slide in the TCGA dataset demonstrating overall mutational status. **A.** Heatmap for APC mutation prediction. **B.** Heatmap for KRAS mutation prediction. **C.** Heatmap for PIK3CA mutation prediction. **D.** Heatmap for SMAD4 mutation prediction. **E.** Heatmap for TP53 mutation prediction. **F.** Overlaid probability map of mutation prediction results for all five genes. Blue color indicates lowly mutated region of the tissue and red color indicates highly mutated region of the tissue.

SUPPLEMENTARY METHODS

Tumor Specimens and DNA extraction

CRC areas with rich tumor cell population (at least 70%) were selected for microdissection from unstained formalin-fixed, paraffin-embedded (FFPE) tissue of the patient's specimen. DNA was then extracted using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's recommendations.

Library preparation and sequencing

Library preparation was accomplished using an Ion Chef System and Oncomine Comprehensive Assay v3 (Thermo Fisher Scientific, San Francisco, CA, USA) according to the manufacturer's instructions. Genomic DNA was then amplified and targeted gene sequencing was performed using the Ion S5 XL sequencer (Thermo Fisher Scientific, San Francisco, CA, USA).

The sequencing data were analyzed by the Ion Torrent suite v5.10.2 (Life Technologies, San Francisco, CA, USA) using the variantCaller plugin. To eliminate base calling errors, several filtering steps were employed for generating final variant calling: minimum allele frequency of hotspot variant: $\geq 4\%$, minimum allele frequency of indel variant: $\geq 5\%$, minimum allele frequency of SNP variant: $\geq 5\%$, minimum read counts for fusions: ≥ 40 , CNV gain threshold: 4 and gain confidence level: 0.05. All identified variants were visually confirmed by the Integrative Genomics viewer (IGV 2.8.6).

Patient IDs of each mutation				
APC mutated	KRAS mutated	PIK3CA mutated	SMAD4 mutated	TP53 mutated
TCGA-DY-A1DG	TCGA-AA-3511	TCGA-D5-6931	TCGA-AF-6136	TCGA-CM-6677
TCGA-A6-2671	TCGA-F5-6812	TCGA-AA-A01V	TCGA-AA-3984	TCGA-A6-3807
TCGA-AA-3980	TCGA-AG-3583	TCGA-CM-4752	TCGA-DM-A28K	TCGA-AA-3517
TCGA-AF-2693	TCGA-DM-A1D4	TCGA-QG-A5YV	TCGA-AG-A02X	TCGA-A6-6649
TCGA-G4-6322	TCGA-AF-2689	TCGA-3L-AA1B	TCGA-AG-A008	TCGA-G4-6294
TCGA-AA-3506	TCGA-AA-3556	TCGA-D5-6541	TCGA-D5-7000	TCGA-AF-2689
TCGA-CM-4746	TCGA-AG-A02X	TCGA-G4-6628	TCGA-AZ-4614	TCGA-AZ-6603
TCGA-AD-6548	TCGA-CM-6674	TCGA-CM-4746	TCGA-G4-6322	TCGA-AZ-6605
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TCGA-AA-A02E	TCGA-AG-3909	TCGA-AY-6386	TCGA-AG-A020	TCGA-A6-2674
TCGA-DM-A28H	TCGA-5M-AAT6	TCGA-CM-5860	TCGA-AG-3887	TCGA-CA-6718
TCGA-AG-4022	TCGA-AG-3901	TCGA-AG-A00C	TCGA-AF-6672	TCGA-AG-A026
TCGA-A6-6652	TCGA-EI-6881	TCGA-EI-6917	TCGA-AA-3558	TCGA-AA-3869
TCGA-EF-5830	TCGA-AA-A00A	TCGA-F4-6461	TCGA-CA-5796	TCGA-AA-3542
TCGA-EI-6881	TCGA-5M-AAT4	TCGA-CK-6747	TCGA-CM-5868	TCGA-F5-6811
TCGA-CK-6746	TCGA-DY-A1H8	TCGA-AG-3878	TCGA-A6-5664	TCGA-AA-3549
TCGA-CM-5868	TCGA-AG-A014	TCGA-AG-A008	TCGA-AF-2690	TCGA-AU-3779
TCGA-AG-3591	TCGA-D5-6538	TCGA-AA-3947	TCGA-AY-6386	TCGA-DC-6683
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TCGA-AA-3685	TCGA-AG-A020	TCGA-F4-6809	TCGA-DY-A1DD	TCGA-AG-A01L
TCGA-AG-3885	TCGA-A6-4107	TCGA-AA-3977	TCGA-F5-6864	TCGA-AA-3511
TCGA-EI-6509	TCGA-D5-5539	TCGA-AA-A02Y	TCGA-AA-3496	TCGA-AA-3685
TCGA-AA-3521	TCGA-DM-A28M	TCGA-AZ-4315	TCGA-AA-3692	TCGA-AG-3587
TCGA-AA-3837	TCGA-F5-6861	TCGA-CM-6680	TCGA-AA-A00K	TCGA-D5-6924
TCGA-AZ-6599	TCGA-D5-5540	TCGA-NH-A50T	TCGA-AA-A020	TCGA-AG-A01N
TCGA-AG-A01N	TCGA-CM-4744	TCGA-AA-3489	TCGA-EI-6882	TCGA-DM-A0XD
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TCGA-CK-6748	TCGA-AA-A029	TCGA-AY-5543
TCGA-DM-A282	TCGA-CK-6748	TCGA-5M-AAT4
TCGA-A6-5667	TCGA-AA-3695	TCGA-DM-A1D9
TCGA-AA-3846	TCGA-DY-A1DF	TCGA-AG-4022
TCGA-D5-6932	TCGA-D5-6531	TCGA-AA-3980
TCGA-AY-6197	TCGA-AA-3939	TCGA-AA-3558

TCGA-AA-A01T	TCGA-AA-3672	TCGA-D5-6926
TCGA-G4-6303	TCGA-AY-5543	TCGA-AA-3492
TCGA-D5-6898	TCGA-AG-A02N	TCGA-AG-A032
TCGA-AG-3581	TCGA-AG-3731	TCGA-AA-3975
TCGA-AG-3593	TCGA-AA-A01Z	TCGA-A6-2675
TCGA-AH-6644	TCGA-NH-A8F8	TCGA-AA-3956
TCGA-CL-5917	TCGA-AF-6672	TCGA-AG-4001
TCGA-CM-6680	TCGA-AA-3520	TCGA-F5-6465
TCGA-F5-6571	TCGA-AA-3660	TCGA-AA-A00D
TCGA-D5-7000	TCGA-G4-6297	TCGA-AZ-6606
TCGA-A6-4105	TCGA-AG-3881	TCGA-AY-A54L
TCGA-DY-A1H8	TCGA-F4-6806	TCGA-AD-6965
TCGA-DM-A1D7	TCGA-AM-5820	TCGA-DC-4745
TCGA-F4-6808	TCGA-DM-A28G	TCGA-AF-6655
TCGA-F4-6809	TCGA-AA-3930	TCGA-AA-A00E
TCGA-AA-A00U	TCGA-CK-4948	TCGA-AF-2690
TCGA-G4-6293	TCGA-AA-A01I	TCGA-AA-3955
TCGA-AA-3854	TCGA-A6-2685	TCGA-AZ-4616
TCGA-CM-6167	TCGA-AA-3818	TCGA-AA-3848
TCGA-G4-6323	TCGA-AG-3999	TCGA-F4-6854
TCGA-AY-6386	TCGA-A6-2681	TCGA-CM-4752
TCGA-AA-3681	TCGA-AA-3663	TCGA-AY-4070
TCGA-A6-2686	TCGA-A6-6653	TCGA-A6-2681
TCGA-RU-A8FL	TCGA-F5-6571	TCGA-QG-A5YV
TCGA-DM-A28E	TCGA-CL-5917	TCGA-EF-5830
TCGA-NH-A50T	TCGA-AA-A02W	TCGA-AA-A004
TCGA-G4-6294	TCGA-AA-3715	TCGA-CK-5916
TCGA-AA-3956	TCGA-AA-3852	TCGA-DM-A28A
TCGA-AG-3612	TCGA-AA-A01R	TCGA-DM-A1DA
TCGA-A6-A56B	TCGA-EI-6510	TCGA-AD-6963
TCGA-G5-6641	TCGA-F4-6703	TCGA-AA-A01D
TCGA-CK-4952	TCGA-A6-5657	TCGA-G4-6320
TCGA-DM-A0XD	TCGA-F4-6463	TCGA-NH-A50V
TCGA-AA-A024	TCGA-AA-A00N	TCGA-AG-3581
TCGA-AG-A016	TCGA-AZ-6606	TCGA-F5-6702
TCGA-AG-3605		TCGA-DM-A28H
TCGA-AA-3667		TCGA-AG-A02N
TCGA-NH-A6GA		TCGA-AA-3562
TCGA-SS-A7H0		TCGA-NH-A6GA
TCGA-CA-5797		TCGA-AF-2691
TCGA-AD-6963		TCGA-AZ-6600
TCGA-G4-6309		TCGA-EI-6514
TCGA-D5-6924		TCGA-EI-6510
TCGA-AA-3693		TCGA-AF-A56L
TCGA-AA-A017		TCGA-AA-A010
TCGA-DC-6682		TCGA-EI-6883
TCGA-CK-5912		TCGA-AA-A02J
TCGA-AA-3519		TCGA-AA-A01K
TCGA-F5-6861		TCGA-AG-4015
		TCGA-AG-A00Y
		TCGA-EI-6882
		TCGA-DY-A1DC
		TCGA-DY-A1DF
		TCGA-DM-A1D8

TCGA-AG-A01Y
TCGA-AZ-4323
TCGA-A6-2671
TCGA-AG-3896
TCGA-AA-3552
TCGA-F5-6571
TCGA-DC-6160
TCGA-AG-4005
TCGA-CM-6679
TCGA-CM-6164
TCGA-AD-A5EJ
TCGA-AF-6672
TCGA-D5-6533
TCGA-EI-6509
TCGA-AG-3885
TCGA-AA-3968
TCGA-AY-6196
TCGA-AG-3612
TCGA-G4-6297
TCGA-CM-6172
TCGA-AA-3867
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TCGA-EI-7002
TCGA-AG-A014
TCGA-D5-6538
TCGA-AA-A000
TCGA-AG-3893
TCGA-AH-6544
TCGA-G4-6315
TCGA-AD-A5EK
TCGA-AG-3898
TCGA-WS-AB45
TCGA-AG-3732
TCGA-AG-3999
TCGA-AZ-4614
TCGA-CA-5797
TCGA-AA-3860
TCGA-A6-6137
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TCGA-AA-3531
TCGA-EI-6885
TCGA-AA-3667
TCGA-D5-6932
TCGA-EI-6512
TCGA-A6-5667
TCGA-AH-6643

TCGA-AG-3890
TCGA-CL-5918
TCGA-AA-A02H
TCGA-AF-2687
TCGA-DM-A1DB
TCGA-G4-6307
TCGA-AZ-4681
TCGA-EI-6917
TCGA-F4-6808
TCGA-DM-A1D4
TCGA-AG-3602
TCGA-CM-5862
TCGA-G4-6302
TCGA-AA-3842
TCGA-AA-3976
TCGA-AA-A01Z
TCGA-AA-3561
TCGA-DC-6154