Supplementary Online Content

Iams WT, Mackay M, Ben-Shachar R, et al. Concurrent tissue and circulating tumor DNA molecular profiling to detect guideline-based targeted mutations in a multicancer cohort. *JAMA Netw Open.* 2024;7(1):e2351700. doi:10.1001/jamanetworkopen.2023.51700

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

eMethods.

Data ethics statement

Data contained within the Tempus multimodal database has been collected in accordance with applicable law, and is not generally obtained pursuant to a prospective clinical study conducted based on study-specific informed written consent and supervised by an Institutional Review Board. Data may originate from protected health information that Tempus Labs, Inc. receives in the course of providing services, and is de-identified in accordance with federal HIPAA regulation so that it is proprietary, de-identified data that is no longer considered protected health information under HIPAA and can be used to facilitate research and development to benefit the next generation of patients. Tempus Labs, Inc. has additionally been granted an IRB exemption (Advarra Pro00072742) permitting the use of de-identified clinical, molecular, and multimodal data in order to derive or capture results, insights, or discoveries. This study follows the Strengthening the Reporting Studies of Observational Studies in Epidemiology (STROBE) guidelines for cohort studies.

Cohort sample requirements

Our cohort consisted of 3209 de-identified advanced solid tumor patient records from the Tempus multimodal database with a primary diagnosis of non-small cell lung cancer (NSCLC; n=1302), breast (n=660), prostate (n=324), or colorectal cancer (CRC; n=923) sequenced between May 2020 and Dec. 2022. Diagnoses were derived from either abstraction of clinical documents, electronic health records, or NGS order forms. Additionally, patients were required to have (i) Stage IV disease, (ii) a tumor biopsy with minimum 20% tumor purity based on pathological review, (iii) liquid and solid-tumor biopsies collected within 30 days of each other, and (iv) both ctDNA and solid-tumor assays successfully pass quality metrics, resulting in a patient report. In the event that a patient had more than one pair of assays that met the inclusion criteria, the pair of assays that occurred closest in time to one another were selected. The four cancer types assessed were selected based on existing guidelines support, or having a large number of patients that met the study inclusion/exclusion criteria defined above. Patients who had no genomic results detected by either test or who were under the age of 19 were excluded from this analysis. Across the cohort, the median age was 65.3 years, 52.8% of patients were female, and the majority of patients (65.5%) had adenocarcinoma histology (**eTable 1**). The median time between tumor and blood collection was 12.0 days.

Tissue-based sequencing

Tissue-based sequencing was performed via the Tempus xT assay (v4), which includes DNA-seq of 648 genes with 500x coverage and enhanced whole-exome capture RNA-seq. This assay identifies short nucleotide variants (SNVs), insertions/deletions (INDELs), copy number variants (CNVs), select genomic rearrangements, and microsatellite instability (MSI) status

using DNA isolated from formalin-fixed paraffin-embedded (FFPE) tumor tissue. Normal-matched tissue was also sequenced for all patients in the cohort, to an average coverage of 150x, which can distinguish incidental germline mutations from somatic variants. Full details on this assay have been previously published[1,2].

ctDNA sequencing

ctDNA profiling was performed using the Tempus xF assay, a non-invasive, liquid biopsy panel of 105 (v2) or 523 (v3) genes focused on detection of oncogenic and resistance mutations, including SNVs and INDELs, CNVs, genomic rearrangements, and MSI status. Full details on this assay have been previously published[3].

Tumor purity

Solid-tissue sample metadata including tumor purity (a ratio of tumor to normal nuclei) was determined by board certified pathologists prior to DNA extraction from formalin-fixed paraffin-embedded (FFPE) tumor tissue.

Guideline-based variant identification and concordance assessment

Clinically actionable variants were identified using indication-matched recommendations from NCCN guidelines for all four cancer types included in the study (**eTable 2**). All actionable variants were detectable in both the Tempus xT and Tempus xF assays. For each assay, variants were considered as "detected" only if they were present at frequencies above the limit-of-detection for the respective assay: VAF of 5% and 10% for SNVs and INDELs in Tempus xT, respectively, and 0.25% and 0.5% for SNVs and INDELs in Tempus xF, respectively.

Patient concordance was defined as the percent of patients with no unique findings by ctDNA or by tissue testing, i.e. concordance rate = (N - u) / N where u is patients with unique findings and N is the total number of patients. Similarly, variant concordance was defined as the number of variants found in both ctDNA and tissue testing divided by all detected variants within both assays. Positive percent agreement (PPA) was defined as the percent agreement of ctDNA testing with tissue, i.e. PPA = $|S \cap C| / |S|$, where S is the set of solid variants and C is the set of ctDNA variants.

When assessing the actionable cohort specifically (defined above), we grouped variants according to whether they were: i) detected by both assays (concordant), ii) uniquely detected in ctDNA (ctDNA-unique), or iii) uniquely detected in tissue (solid-unique). These latter two categories were further grouped and referenced as assay-unique when relevant. The percent increases that we report are calculated as the number of patients with ctDNA-unique variants in the indicated gene divided by the number of patients with a solid-tissue variant in that gene, multiplied by 100 to become a percentage.

CHIP and germline variant detection

Patient inclusion criteria include solid tissue profiling with a matcher normal. Patient's normal tissue are primarily derived from plasma and buffy coat from streck tubes, isolated and used for sequencing, and used to remove germline variants from both solid (xT) and liquid (xF) results. Although the vast majority of patients follow this protocol, the xT assay is designed to use other sample sources for normal control as necessary. <1% of patients normal tissues were derived from saliva and these were manually inspected to ensure that none of the ctDNA-unique variants were from prominent CHIP genes (*ATM, KRAS, NRAS*). All variants that were detected in ctDNA but not solid-tumor tissue, but which were present within the normal sample were removed from analysis as potential CHIP variants; this process removed 12 total variants (0.85% of all ctDNA-unique variants).

eReferences.

1. Beaubier N, Tell R, Lau D, et al. Clinical validation of the Tempus xT next-generation targeted oncology sequencing assay. *Oncotarget*. 2019;10(24):2384-2396.

2. Beaubier N, Bontrager M, Huether R, et al. Integrated genomic profiling expands clinical options for patients with cancer. *Nat Biotechnol*. 2019;37(11):1351-1360.

3. Finkle JD, Boulos H, Driessen TM, et al. Validation of a liquid biopsy assay with molecular and clinical profiling of circulating tumor DNA. *NPJ Precis Oncol*. 2021;5(1):63.

eTable 1. Overview of Cohort Demographics and Clinical Features for Individual Cancer Types and the Combined Cohort

Characteristic	NSCLC	Colorectal	Breast	Prostate	Combined
Number of patients	1302	923	660	324	3209
Age at stage 4 diagnosis, median, 95% quantiles	68.0, (49.3 - 83.8)	61.2, (40.2 - 82.9)	62.4, (39.1 - 80.7)	69.4, (55.1 - 83.7)	65.3, (43.3 - 83.3)
Sex					
Female, n (%)	649 (49.8%)	391 (42.4%)	653 (98.9%)	0 (0%)	1693 (52.8%)
Male, n (%)	653 (50.2%)	532 (57.6%)	7 (1.1%)	324 (100.0%)	1516 (47.2%)
Race					
White	674.0 (51.8%)	411 (44.5%)	327 (49.5%)	127 (39.2%)	1539 (48.0%)
Black or African American	109.0 (8.4%)	70 (7.6%)	63 (9.6%)	48 (14.8%)	290 (9.0%)
Hispanic or Latino	43.0 (3.3%)	60 (6.5%)	53 (8.0%)	23 (7.1%)	179 (5.6%)
Asian	36.0 (2.8%)	28 (3.0%)	21 (3.2%)	10 (3.1%)	95 (3.0%)
Other Race	29.0 (2.2%)	38 (4.1%)	25 (3.8%)	5 (1.5%)	97 (3.0%)
Unknown Race	411.0 (31.5%)	316 (34.3%)	171 (25.9%)	111 (34.3%)	1009 (31.4%)
Smoking Status					
Current or former smoker n (%)	946 (72.7%)	343 (37.2%)	175 (26.5%)	134 (41.3%)	1598 (49.8%)
Never smoker	236 (18.1%)	384 (41.6%)	313 (47.4%)	135 (41.7%)	1068 (33.3%)
Unknown	120 (9.2%)	196 (21.2%)	172 (26.1%)	55 (17.0%)	543 (16.9%)
Histology					
Adenocarcinoma n (%)	937 (72.0%)	829 (89.8%)	93 (14.1%)	242 (74.7%)	2101 (65.5%)
Other n (%)	365 (28.0%)	94 (10.2%)	567 (85.9%)	82 (25.3%)	1108 (34.5%)

Time between biopsy collections days, median, 95% quantiles	12.0, (2.0 - 27.0)	13.0, (2.0 - 28.0)	11.0, (1.0 - 28.0)	12.0, (0.0 - 28.0)	12.0, (1.0 - 28.0)

eTable 2. List of Indication-Matched, Guidelines-Based Variants Used to Assess Actionability

Cancer	Gene	Variant	Variant type
Breast Cancer	BRCA1	Pathogenic variants	SNV/Indel
Breast Cancer	BRCA2	Pathogenic variants	SNV/Indel
Breast Cancer	ERBB2 (HER2)	Pathogenic variants	SNV/Indel
Breast Cancer	ERBB2 (HER2)	Amplification	Copy Number Variant
Breast Cancer	ESR1	Pathogenic variants	SNV/Indel
Breast Cancer	PIK3CA	Pathogenic variants	SNV/Indel
Breast Cancer		MSI High	Biomarker
Colorectal Cancer	BRAF	V600E	SNV/Indel
Colorectal Cancer	ERBB2 (HER2)	Amplification	Copy Number Variant
Colorectal Cancer	KRAS	Pathogenic variants	SNV/Indel
Colorectal Cancer	NRAS	Pathogenic variants	SNV/Indel
Colorectal Cancer		MSI High	Biomarker
Non-Small Cell Lung Cancer	ALK	ALK Fusion	Fusion
Non-Small Cell Lung Cancer	BRAF	V600E	SNV/Indel
Non-Small Cell Lung Cancer	EGFR	L858R	SNV/Indel
Non-Small Cell Lung Cancer	EGFR	L861Q	SNV/Indel
Non-Small Cell Lung Cancer	EGFR	S768I	SNV/Indel
Non-Small Cell Lung Cancer	EGFR	T790M	SNV/Indel
Non-Small Cell Lung Cancer	EGFR	Exon 19 Deletion	SNV/Indel
Non-Small Cell Lung Cancer	EGFR	Exon 19 Insertion	SNV/Indel
Non-Small Cell Lung Cancer	EGFR	Exon 20 Insertion	SNV/Indel
Non-Small Cell Lung Cancer	ERBB2 (HER2)	Pathogenic variants	SNV/Indel
Non-Small Cell Lung Cancer	KRAS	G12C	SNV/Indel
Non-Small Cell Lung Cancer	MET	Exon 14 Skipping	Splicing Variant
Non-Small Cell Lung Cancer	MET	MET Amplification	Copy Number Variant
Non-Small Cell Lung Cancer	RET	RET Fusion	Fusion
Non-Small Cell Lung Cancer	ROS1	ROS-1 Fusion	Fusion

Prostate Cancer	ATM	Pathogenic variants	SNV/Indel
Prostate Cancer	BARD1	Pathogenic variants	SNV/Indel
Prostate Cancer	BRCA1	Pathogenic variants	SNV/Indel
Prostate Cancer	BRCA2	Pathogenic variants	SNV/Indel
Prostate Cancer	BRIP1	Pathogenic variants	SNV/Indel
Prostate Cancer	CDK12	Pathogenic variants	SNV/Indel
Prostate Cancer	CHEK1	Pathogenic variants	SNV/Indel
Prostate Cancer	CHEK2	Pathogenic variants	SNV/Indel
Prostate Cancer	FANCL	Pathogenic variants	SNV/Indel
Prostate Cancer	PALB2	Pathogenic variants	SNV/Indel
Prostate Cancer	RAD51B	Pathogenic variants	SNV/Indel
Prostate Cancer	RAD51C	Pathogenic variants	SNV/Indel
Prostate Cancer	RAD51D	Pathogenic variants	SNV/Indel
Prostate Cancer		MSI High	Biomarker
All Solid Tumors	NTRK1	NTRK1-Fusion	Fusion
All Solid Tumors	NTRK2	NTRK2-Fusion	Fusion
All Solid Tumors	NTRK3	NTRK3-Fusion	Fusion

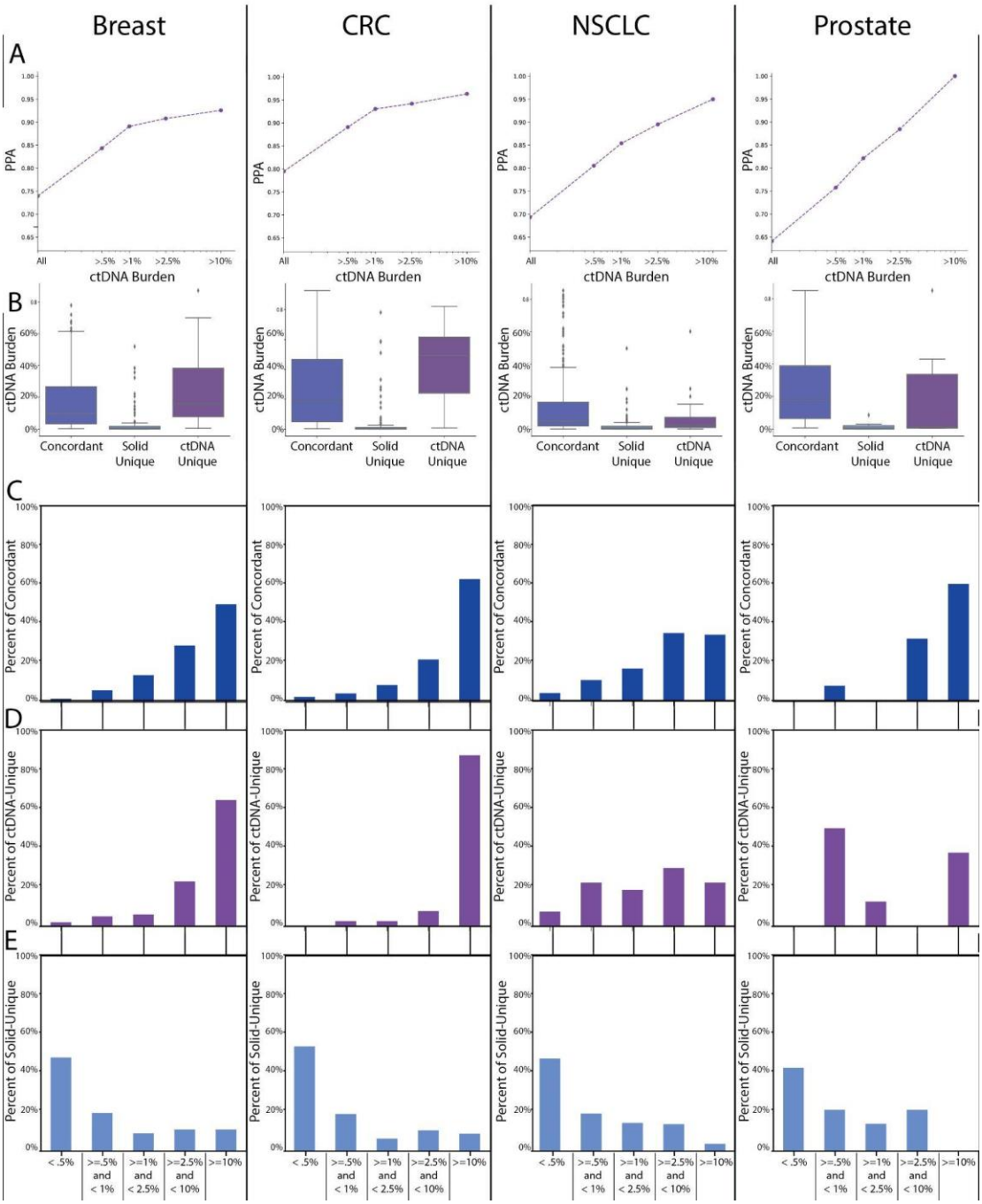
eTable 3. Overview of Actionable Variant Counts per Patient for Each Cancer Type and in the Combined Cohort

Cancer type	Patients with 1 variant (n)	Patients with 2 variants (n)	Patients with >2 variants (n)
Breast Cancer	243	77	32
Colorectal Cancer	519	42	4
Non-Small Cell Lung Cancer	467	23	0
Prostate Cancer	35	6	0
Combined	1264	148	36

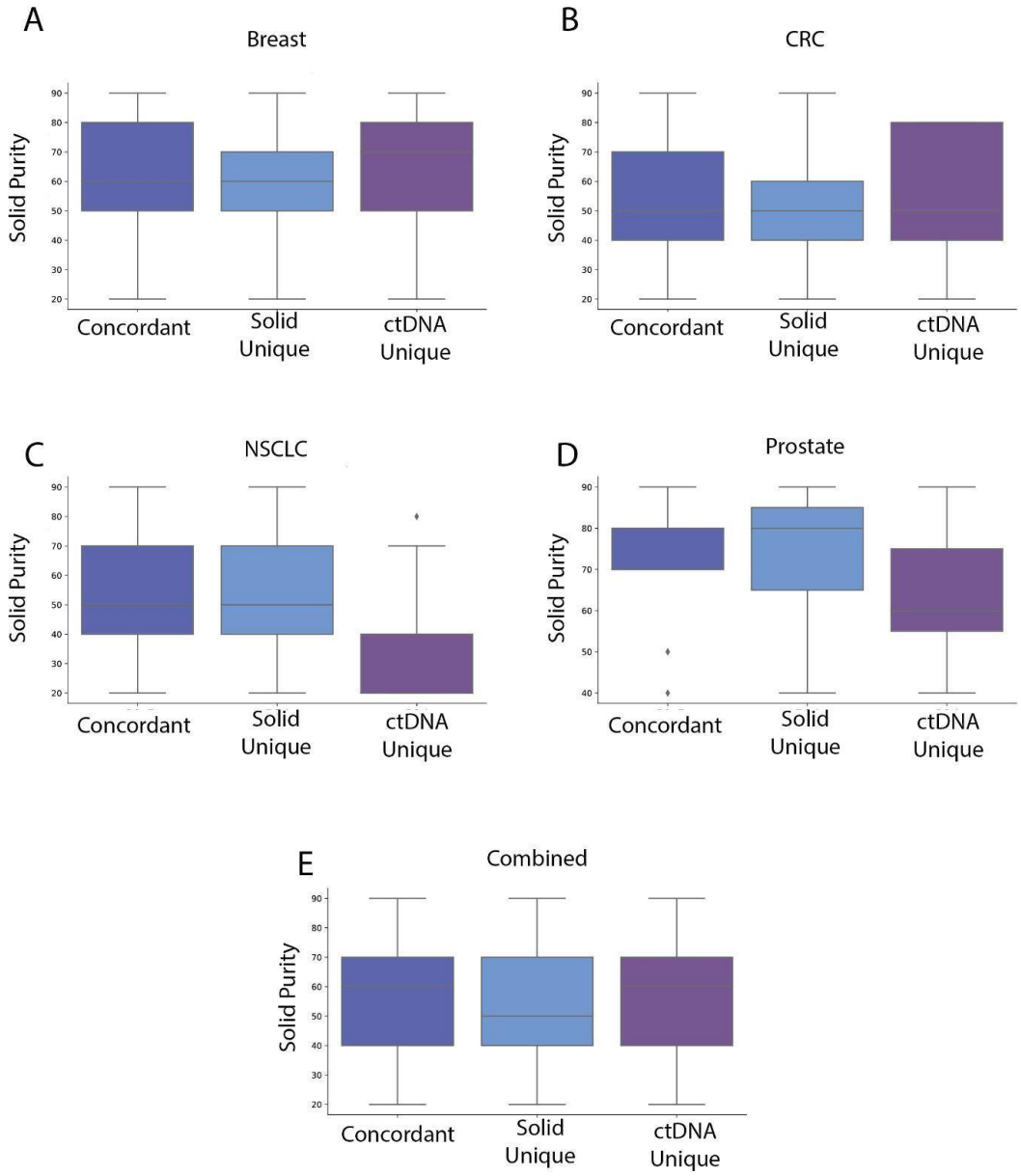
eTable 4. Overview of Actionable Variant Counts Within an Individual Gene at the Patient-Level for Each Cancer Type, Highlighting That Many Patients Have More Than One Actionable Variant Identified Within the Same Gene

Cancer type	Gene	Patients with actionable variant in gene (n)	Patients with 1 actionable variant in gene (n)	Patients with >1 actionable variant in gene (n)	Patients with >1 actionable variants in gene (%)
Breast Cancer	BRCA1	15	15	0	0.00%
Breast Cancer	BRCA2	17	15	2	11.76%
Breast Cancer	ERBB2	45	45	0	0.00%
Breast Cancer	ESR1	116	92	24	20.69%
Breast Cancer	IDH1	1	1	0	0.00%
Breast Cancer	PIK3CA	245	223	22	8.98%
Colorectal Cancer	BRAF	72	72	0	0.00%
Colorectal Cancer	ERBB2	20	20	0	0.00%
Colorectal Cancer	KRAS	439	429	10	2.28%
Colorectal Cancer	NRAS	48	45	3	6.25%
Colorectal Cancer	NTRK1	1	1	0	0.00%
NSCLC	ALK	41	41	0	0.00%
NSCLC	BRAF	28	28	0	0.00%
NSCLC	EGFR	206	196	10	4.85%
NSCLC	ERBB2	19	18	1	5.26%
NSCLC	KRAS	157	157	0	0.00%
NSCLC	MET	46	44	2	4.35%
NSCLC	NTRK1	1	1	0	0.00%
NSCLC	ROS1	2	2	0	0.00%
Prostate Cancer	ATM	13	13	0	0.00%
Prostate Cancer	BRCA1	4	4	0	0.00%
Prostate Cancer	BRCA2	10	9	1	10.00%
Prostate Cancer	PALB2	1	1	0	0.00%
Prostate Cancer	RAD51C	2	2	0	0.00%

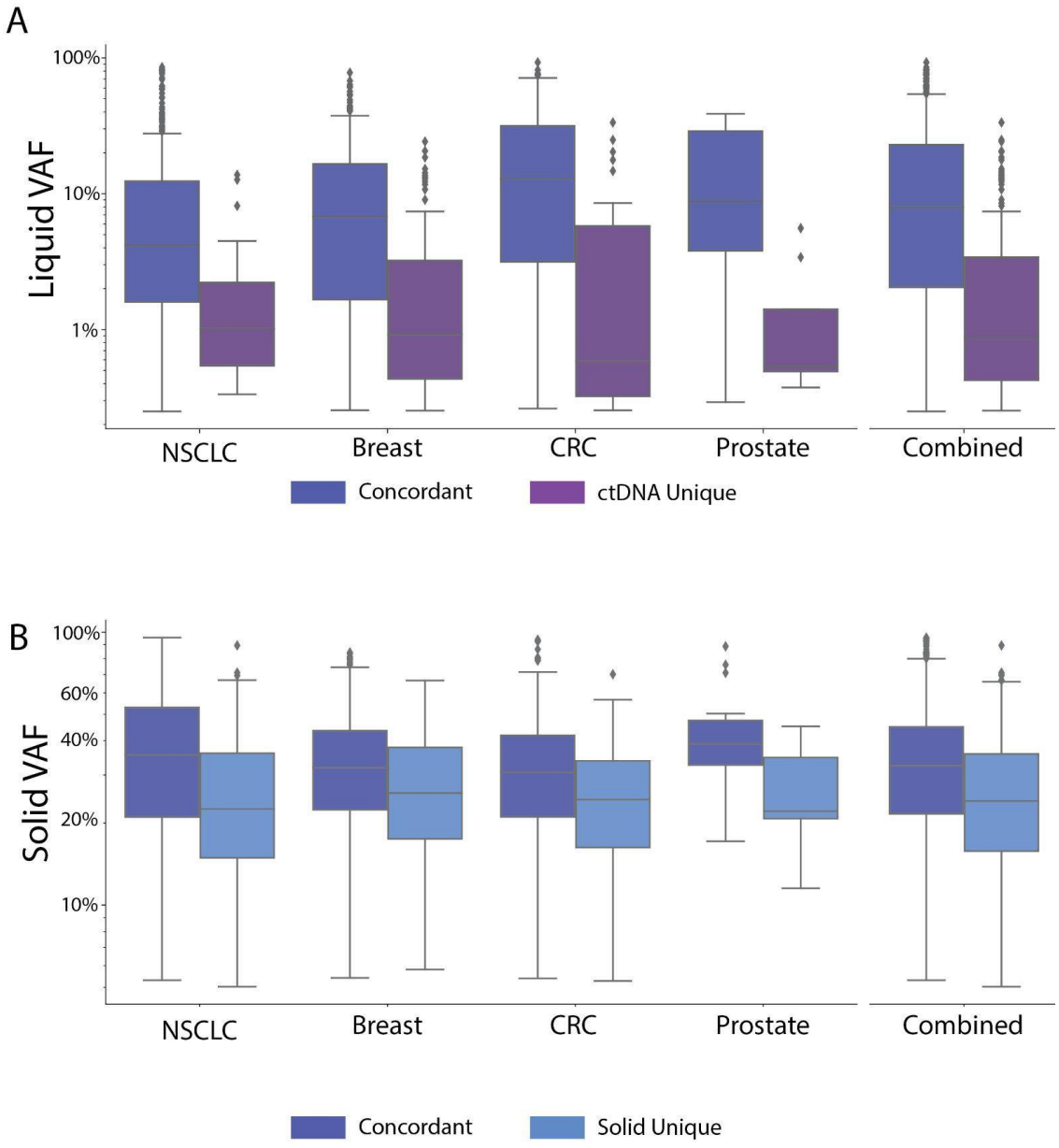
eFigure 1. (**A**) Using Solid-Tissue Results as the Comparator, Positive Percent Agreement (PPA) Is Shown for Different Thresholds of ctDNA Burden. (**B**) Distributions of ctDNA Burden Tumor Shedding Values for Concordant, ctDNA-Unique, and Solid-Unique Variants. (**C,D,E**) Proportion of all Concordant (C), ctDNA-Unique (D), and Solid-Unique (E) Variants at Varying ctDNA Burden Thresholds. As in Main text Fig. 2, with data stratified according to cancer type.



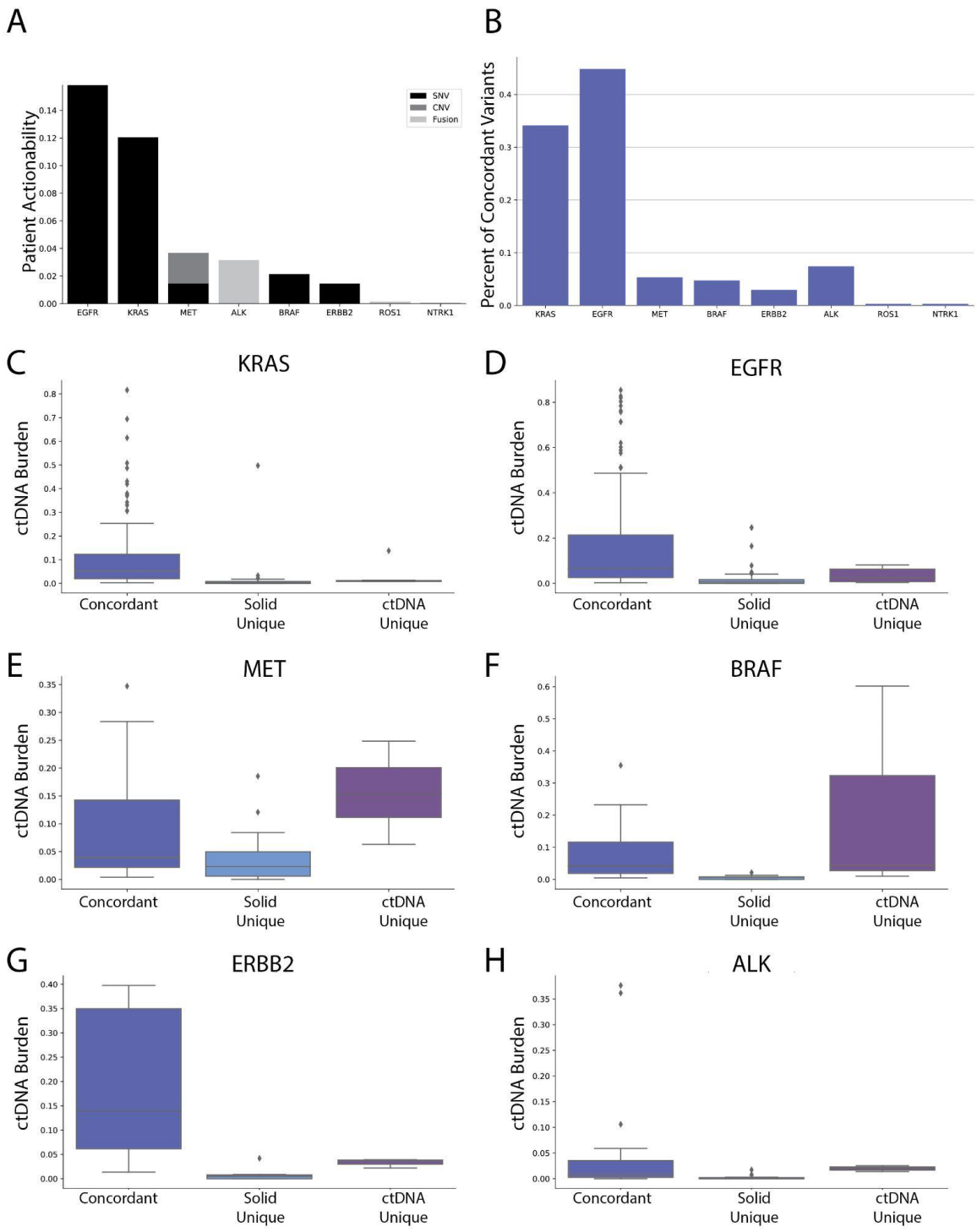
eFigure 2. Distributions of Solid-Tumor Purity Values for Concordant, Solid-Unique, and ctDNA-Unique Variants. Results shown separately for (A) Breast, (B) CRC, (C) NSCLC (p = 3.9e-5, Kruskal-Wallis test, Bonferroni corrected), (D) prostate and (E) the combined cohort.



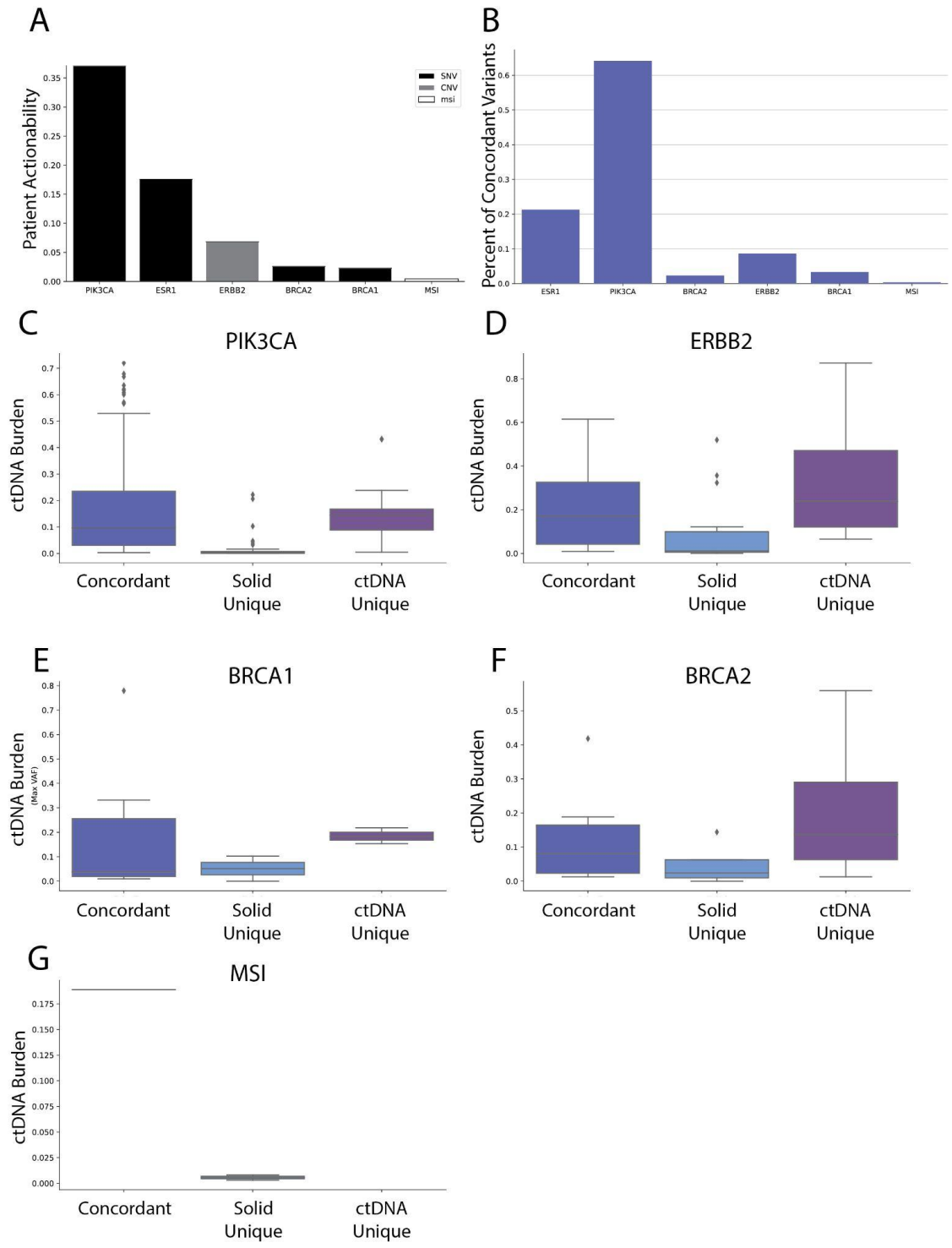
eFigure 3. Comparison of VAFs by Assay Concordance. (A) Concordant (with respective VAF coming from the ctDNA assay) and ctDNA unique variants (for combined, p = 1.15e-30, Mann Whitney U test). (B) Concordant (with respective VAF coming from the solid-tumor assay) and solid-tissue-unique variants (for combined, p = 1.13e-12, Mann Whitney U test).



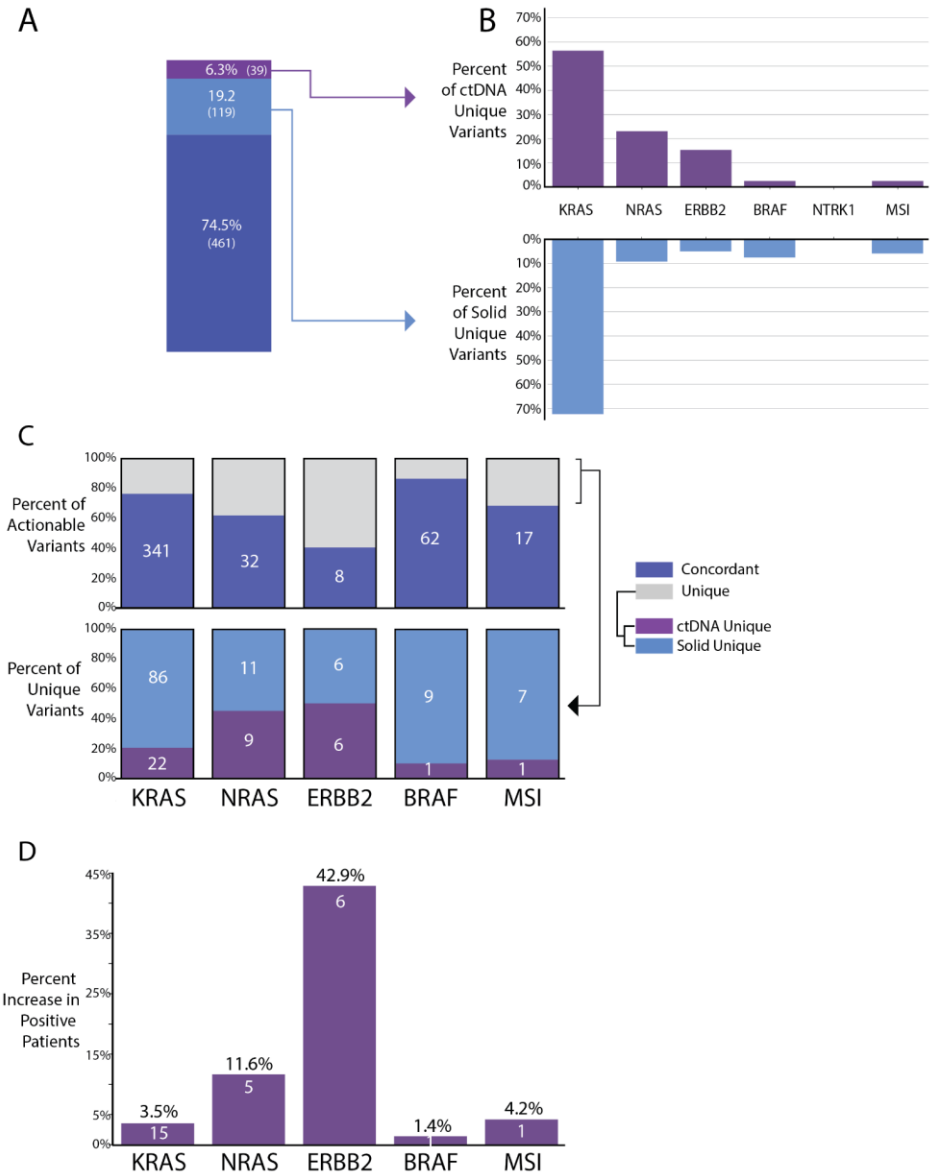
eFigure 4. (A) Summary of the Gene-Specific Actionable Variant Prevalence in the Actionable NSCLC cohort. (B) Concordance Values Between ctDNA and Solid-Tissue at the Individual Gene Level in the NSCLC Cohort. The ctDNA Burden Values for Individual Variant Types Stratified According to Gene: (C) *KRAS*, (D) *EGFR*, (E) *MET*, (F) *BRAF*, (G) *ERBB2*, and (H) *ALK*.



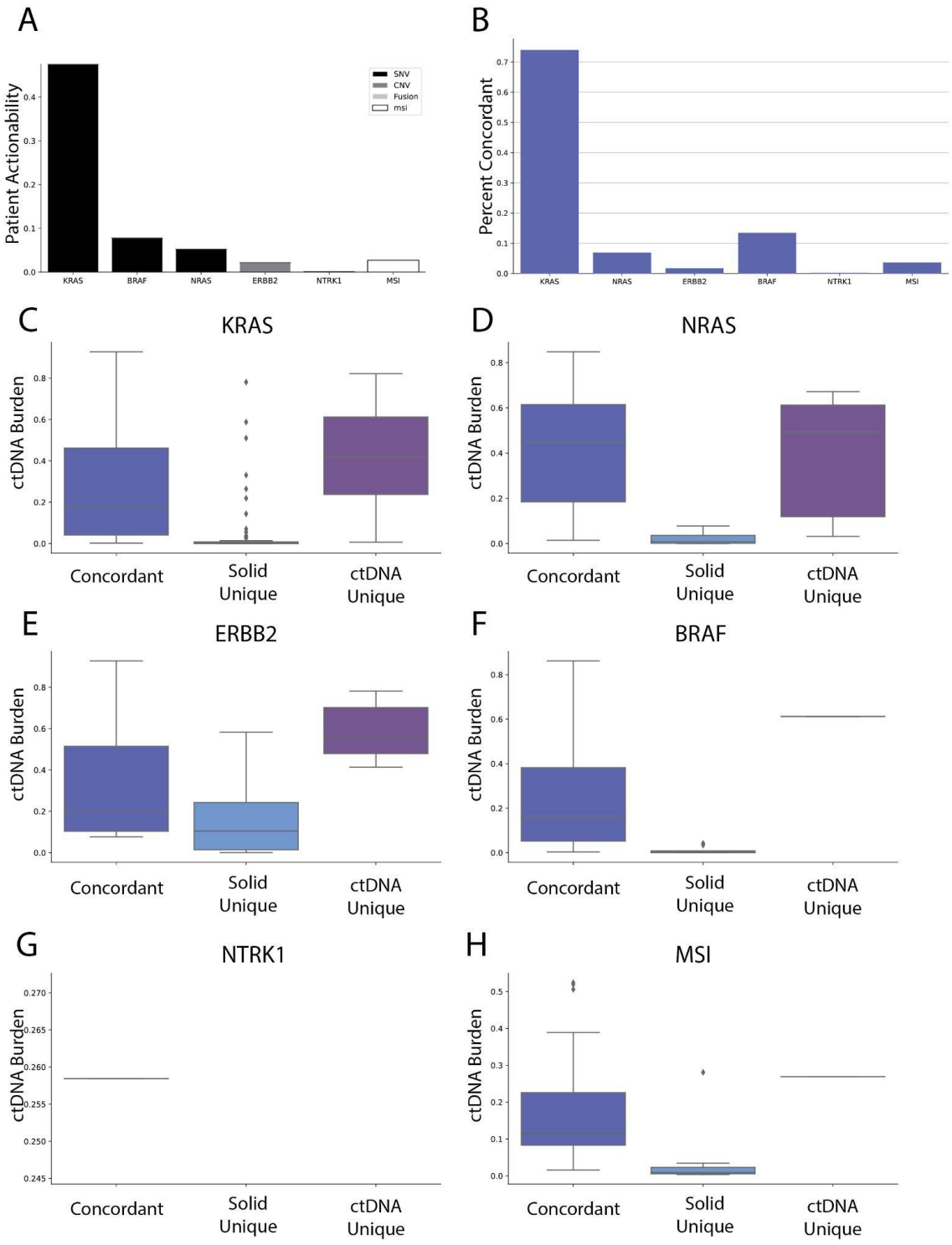
eFigure 5. (A) Summary of the Gene-Specific Actionable Variant Prevalence in the Actionable Breast Cancer Cohort. (B) Concordance Values Between ctDNA and Solid-Tissue at the Individual Gene Level in the Breast Cancer Cohort. The ctDNA Burden Values for Individual Variant Types Stratified According to Gene/Biomarker: (C) *PIK3CA*, (D) *HER2*, (E) *BRCA1*, (F) *BRCA2* and (G) MSI.



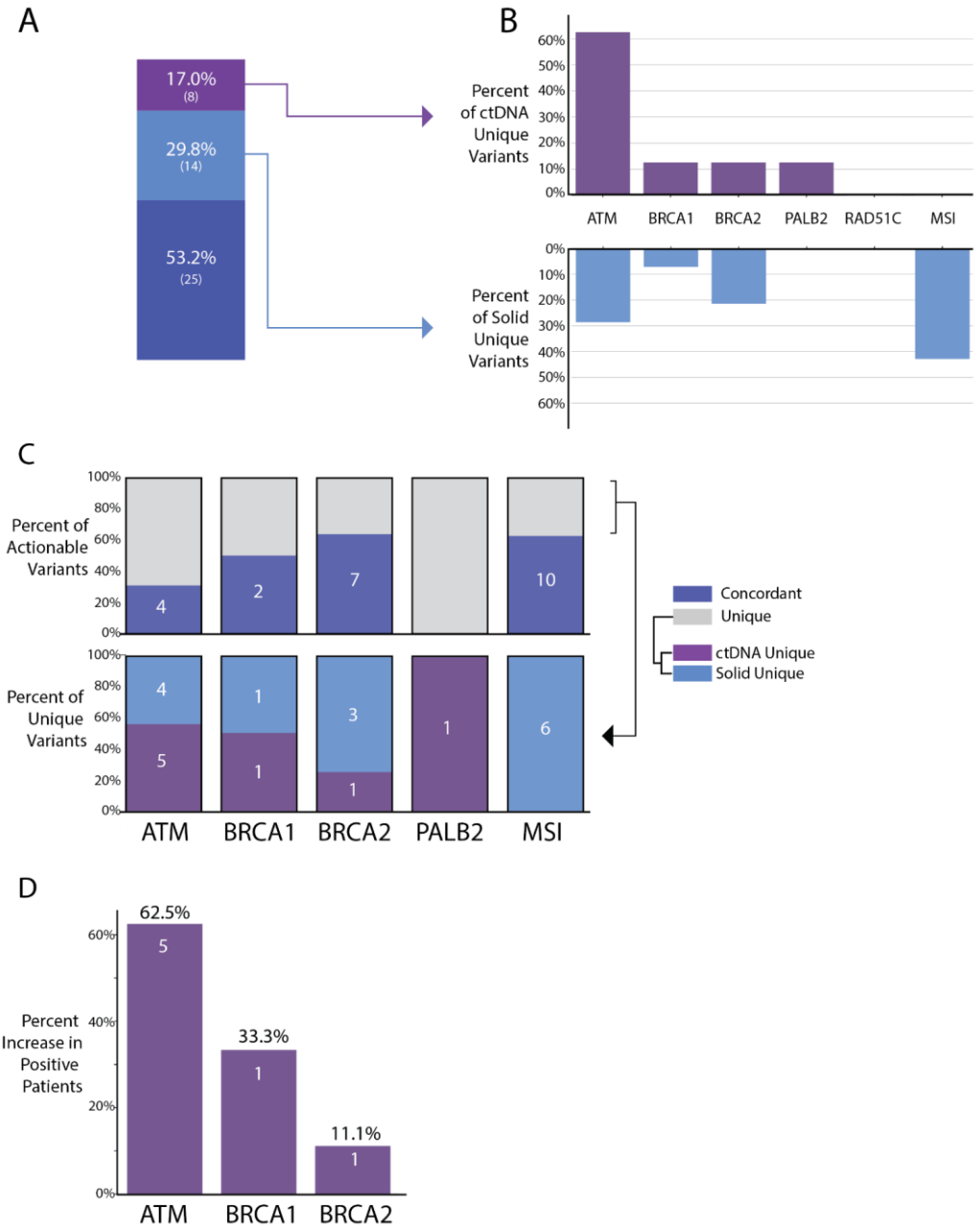
eFigure 6. (A) Distribution of Actionable Variants in CRC by Type: Concordant (Dark Blue), ctDNA-Unique (Purple), Solid-Unique (Light Blue). (B) Proportion of All ctDNA-Unique Variants That Were Detected in the Indicated Genes (Top), and the Proportion of All Solid-Unique Variants That Were Detected in the Indicated Genes (Bottom). Between Solid-Unique and ctDNA-Unique Variants, P = 0.016; Between ctDNA-Unique and Concordant Variants P = 3.42e-08; Between Solid-Unique and Concordant Variants P = 0.079 (FET). (C) At the Individual Gene Level, Variants Detected in the CRC Cohort Are Classified as Either Concordant or Assay-Unique (Top), With Concordance Varying Across Genes. Assay-Unique Variants Are Separately Categorized According to Whether They Were Uniquely Detected by ctDNA or Solid-Tissue Testing (Bottom). (D) Per Gene, the Percent Increase in the Number of Patients With an Actionable Finding Identified by Concurrent Testing Compared to Solid-Tissue Testing Alone.



eFigure 7. (A) Summary of the Gene-Specific Actionable Variant Prevalence in the Actionable CRC Cohort. (B) Concordance Values Between ctDNA and Solid-Tissue at the Individual Gene Level in the CRC Cohort. The ctDNA Burden Values for Individual Variant Types Stratified According to Gene/Biomarker: (C) *KRAS*, (D) *NRAS*, (E) *ERBB2*, (F) *BRAF*, (G) *NTRK1* and (H) MSI.



eFigure 8. (A) Distribution of Actionable Variants in Prostate Cancer by Type: Concordant (Dark Blue), ctDNA-Unique (Purple), Solid-Unique (Light Blue). (B) Proportion of all ctDNA-Unique Variants That Were Detected in the Indicated Genes (Top), and the Proportion of All Solid-Unique Variants That Were Detected in the Indicated Genes (Bottom). (C) At the Individual Gene Level, Variants Detected in the Prostate Cancer Cohort Are Classified as Either Concordant or Assay-Unique (Top), With Concordance Varying Across Genes. Assay-Unique Variants Are Separately Categorized According to Whether They Were Uniquely Detected by ctDNA or Solid-Tissue Testing (Bottom). (D) Per Gene, the Percent Increase in the Number of Patients With an Actionable Finding Identified by Concurrent Testing Compared to Solid-Tissue Testing Alone



eFigure 9. (A) Summary of the Gene-Specific Actionable Variant Prevalence in the Actionable Prostate Cancer Cohort. (B) Concordance Values Between ctDNA and Solid-Tissue at the Individual Gene Level in the Prostate Cancer Cohort. The ctDNA Burden Values for Individual Variant Types Stratified According to Gene/Biomarker: (C) *ATM*, (D) *BRCA1*, (E) *BRCA2*, (F) *PALB2*, (G) *RAD51C* and (H) MSI