Supplementary Figure 1. Foundation Medicine Reporting Of Secondary Germline Findings

a

GERMLINE BANNER

VARIANTS TO CONSIDER FOR FOLLOW-UP GERMLINE TESTING IN SELECT CANCER SUSCEPTIBILITY GENES

Findings below have been previously reported as pathogenic germline in the ClinVar genomic database and were detected at an allele frequency of >10%. See appendix for details.

BRCA2 - S1982fs*22 ..... p. 4

This report does not indicate whether variants listed above are germline or somatic in this patient. In the appropriate clinical context, follow-up germline testing would be needed to determine whether a finding is germline or somatic.

b

PPGV W/ CLINVAR EVIDENCE

POTENTIAL GERMLINE IMPLICATIONS

The alteration seen here is one of several BRCA1/2 founder mutations seen with disproportionately high frequency in the Ashkenazi Jewish population, the most common mutations being BRCA1 185delAG (E23fs*17), BRCA1 5382insC (Q1756fs*74), and BRCA2 6174delT (S1982fs*22)[111]. These variants have been described in the ClinVar database as pathogenic germline mutations associated with hereditary breast and ovarian cancer syndrome (ClinVar, Sep 2022)[112]. Follow-up germline testing would be needed to distinguish whether the finding in this patient is somatic or germline. Inactivating germline mutations in BRCA1 or BRCA2 are associated with autosomal dominant hereditary breast and ovarian cancer[113-114], and the lifetime risk of breast and ovarian cancer in BRCA2 mutation carriers has been estimated to be as high as >80% and 23%, respectively[115]. Elevated risk for other cancer types, including gastric, pancreatic, prostate, and colorectal, has also been identified, with an increase in risk ranging from 20 to 60%[116]. The estimated prevalence of deleterious germline BRCA1/2 mutations in the general population is between 1:400 and 1:800, with an approximately 10-fold higher prevalence in the Ashkenazi Jewish population[115,117-122]. In the appropriate clinical context, germline testing of BRCA2 is recommended.

c

PPGV W/O CLINVAR EVIDENCE

POTENTIAL GERMLINE IMPLICATIONS

Inactivating germline mutations in BRCA1 or BRCA2 are associated with autosomal dominant hereditary breast and ovarian cancer[89-90], and the lifetime risk of breast and ovarian cancer in BRCA1/2 mutation carriers has been estimated to be as high as 87% and 44%, respectively[91]. Elevated risk for other cancer types, including gastric, pancreatic, prostate, and colorectal, has also been identified, with an increase in risk ranging from 20 to 60%[92]. The estimated prevalence of deleterious germline BRCA1/2 mutations in the general population is between 1:400 and 1:800, with an approximately 10-fold higher prevalence in the Ashkenazi Jewish population[91,93-98]. In the appropriate clinical context, germline testing of BRCA1 is recommended.

d

MSI-H BIOMARKER

POTENTIAL GERMLINE IMPLICATIONS

While approximately 80% of MSI-H tumors arise due to somatic inactivation of an MMR pathway protein, about 20% arise due to germline mutations in one of the MMR genes[21], which are associated with a condition known as Lynch syndrome (also known as hereditary nonpolyposis colorectal cancer or HNPCC)[34]. Lynch syndrome leads to an increased risk of colorectal, endometrial, gastric, and other cancers[34-36] and has an estimated prevalence in the general population ranging from 1:600 to 1:2000[37-39]. Therefore, in the appropriate clinical context, germline testing of MLH1, MSH2, MSH6, and PMS2 is recommended.

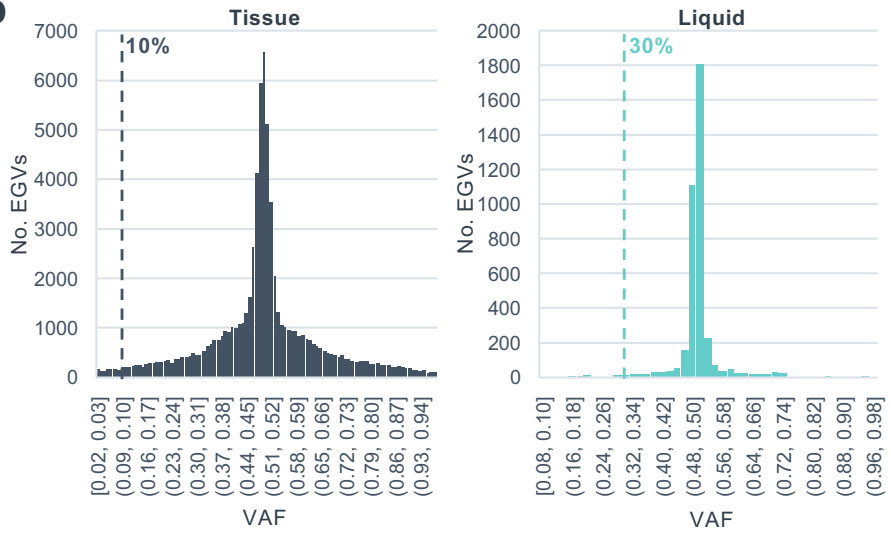
Example Foundation Medicine reporting of secondary germline findings: a) Germline Banner report feature located on Page 1 of Professional Services; b) 'Potential Germline Implications' discussion in Germline Findings section for PPGV reported in ClinVar; c) 'Potential Germline Implications' discussion in Germline Findings section for PPGV not reported in ClinVar; and d) 'Potential Germline Implications' discussion in Biomarker Findings section based on detection of MSI-H biomarker. MSI-H, Microsatellite Instability-High; PPGV, Potential Pathogenic Germline Variants.

Supplementary Figure 2. Development & Validation Of PPGV VAF Thresholds

a Exemplar Germline Variants (EGV)

Gene	Coding Sequence Effect	Protein Sequence Effect
BRCA1	114G>A	K38K
BRCA1	591C>T	C197C
BRCA1	1067A>G	Q356R
BRCA1	1971A>G	Q657Q
BRCA1	2077G>A	D693N
BRCA1	2458A>G	K820E
BRCA1	2521C>T	R841W
BRCA1	3119G>A	S1040N
BRCA1	3418A>G	S1140G
BRCA1	4039A>G	R1347G
BRCA1	4535G>T	S1512I
BRCA1	4956G>A	M1652I
BRCA2	125A>G	Y42C
BRCA2	865A>C	N289H
BRCA2	1365A>G	S455S
BRCA2	1788T>C	D596D
BRCA2	2229T>C	H743H
BRCA2	2971A>G	N991D
BRCA2	3264T>C	P1088P
BRCA2	3516G>A	S1172S
BRCA2	4068G>A	L1356L
BRCA2	4090A>C	I1364L
BRCA2	4258G>T	D1420Y
BRCA2	5199C>T	S1733S
BRCA2	5418A>G	E1806E
BRCA2	5704G>A	D1902N
BRCA2	5744C>T	T1915M
BRCA2	6100C>T	R2034C
BRCA2	6347A>G	H2116R
BRCA2	7017G>C	K2339N
BRCA2	7319A>G	H2440R
BRCA2	7469T>C	I2490T
BRCA2	8149G>T	A2717S
BRCA2	8182G>A	V2728I
BRCA2	8460A>C	V2820V
BRCA2	8567A>C	E2856A
BRCA2	8830A>T	I2944F
BRCA2	8851G>A	A2951T
BRCA2	9730G>A	V3244I
BRCA2	9976A>T	K3326*
BRCA2	10110G>A	R3370R
BRCA2	10234A>G	I3412V
TP53	108G>A	P36P
TP53	139C>T	P47S
TP53	639A>G	R213R

b

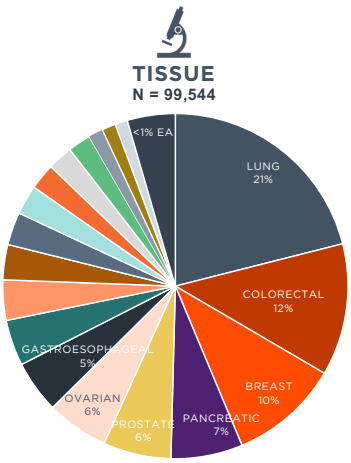


c VAF Threshold Validation

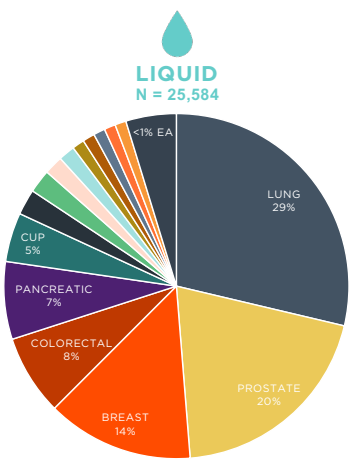
Test Type	VAF Threshold	Cancer Type	% EGV w/VAF Above Threshold	No. Of Variants
F1CDx® / FoundationOne®	>10% VAF	Pan-Cancer	98.2%	82,162
		Lung	98.6%	13,535
		Breast	98.2%	8,055
		Prostate	98.2%	2,510
		Ovary	96.6%	4,728
		Glioma	97.7%	2,565
F1LCDx™ / FoundationOne®Liquid	>30% VAF	Pan-Cancer	98.1%	3,969
		Lung	98.7%	1,360
		Breast	98.7%	393
		Prostate	100.0%	284
		Ovary	100.0%	79
		Glioma	100.0%	19

a) The VAF distribution of a set of exemplar germline variants (EGV) was plotted in both tissue and liquid CGP samples with the goal of identifying a threshold below which germline variants are unlikely to be present on tumor CGP. A set of common variants was chosen for this analysis as they would be expected to occur rarely as somatic variants in cancer. Variants in *BRCA1*, *BRCA2*, and *TP53* were chosen since these genes are frequently under loss of heterozygosity (LOH) in tumors. Variants with high population allele frequencies were excluded as they are frequently homozygous. Variants with VAF <2% and >98% were also excluded as they are unlikely to be heterozygous germline in a given sample. b) Based on this analysis, a threshold of >10% VAF for tissue CGP (FoundationOne®CDx, FoundationOne®) and >30% VAF for liquid CGP (FoundationOne®Liquid CDx, FoundationOne®Liquid) was selected. c) Applying these thresholds, there is high confidence that >95% of germline variants are identified across tumor CGP assays and across cancer types. CGP, Comprehensive Genomic Profiling; F1CDx®, FoundationOne®CDx; F1LCDx™, FoundationOne®Liquid CDx; VAF, Variant Allele Frequency.

Supplementary Figure 3. Cancer Types Profiled Using Tissue & Liquid Tumor CGP



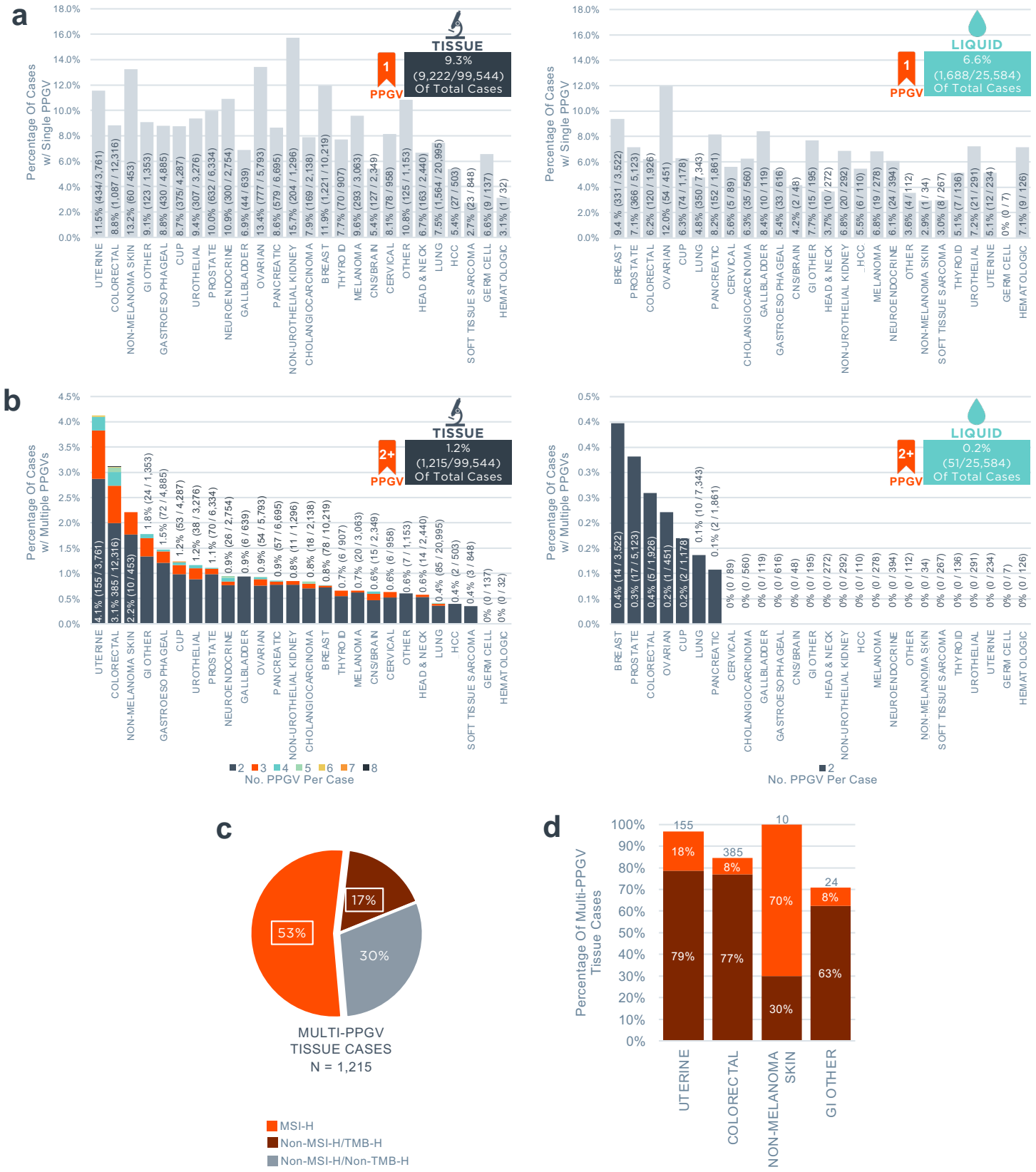
CUP	4%
UTERINE	4%
UROTHELIAL	3%
MELANOMA	3%
NEUROENDOCRINE	3%
HEAD & NECK	2%
CNS/BRAIN	2%
CHOLANGIOCARCINOMA	2%
GI OTHER	1%
NON-UROTHELIAL KIDNEY	1%
OTHER	1%
CERVICAL	<1%
THYROID	<1%
SOFT TISSUE SARCOMA	<1%
GALLBLADDER	<1%
LIVER	<1%
NON-MELANOMA SKIN	<1%
GERM CELL	<1%
HEMATOLOGIC	<1%



GASTROESOPHAGEAL	2%
CHOLANGIOCARCINOMA	2%
OVARIAN	2%
NEUROENDOCRINE	2%
NON-UROTHELIAL KIDNEY	1%
UROTHELIAL	1%
MELANOMA	1%
HEAD & NECK	1%
SOFT TISSUE SARCOMA	1%
UTERINE	<1%
GI OTHER	<1%
THYROID	<1%
HEMATOLOGIC	<1%
GALLBLADDER	<1%
OTHER	<1%
LIVER	<1%
CERVICAL	<1%
CNS/BRAIN	<1%
NON-MELANOMA SKIN	<1%
GERM CELL	<1%

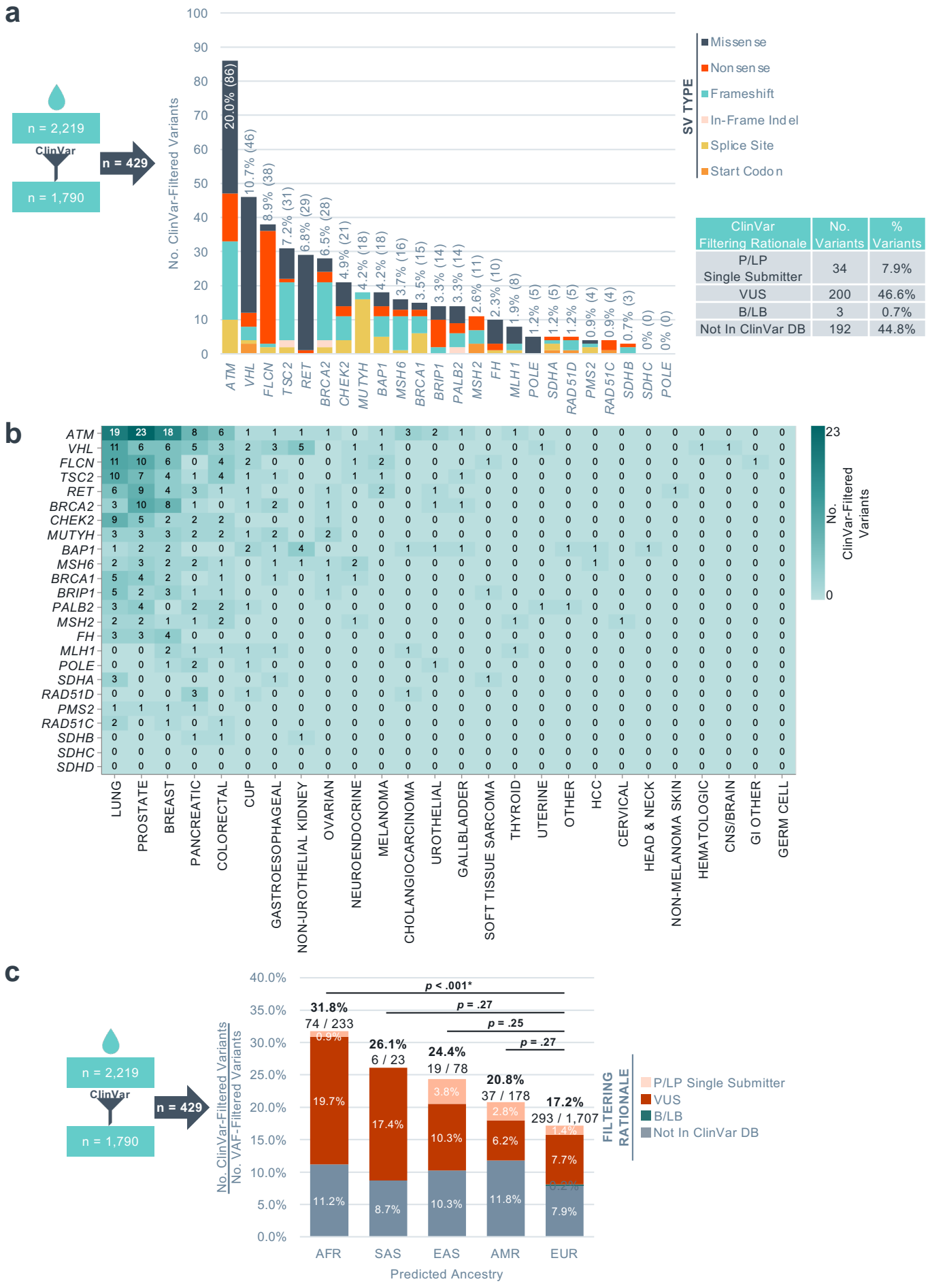
Percentages reflect the % of total tissue or liquid cases profiled, respectively.

Supplementary Figure 4. Multi-PPGV Samples Are Partially Explained By Underlying MSI-H/TMB-H



a) Percentage of cases in tissue (*Left*) and liquid (*Right*) CGP cohorts in which a single PPGV was identified. b) Percentage of cases in tissue (*Left*) and liquid (*Right*) CGP in which multiple PPGVs were identified. c) MSI and TMB status of the 1,215 tissue CGP cases with multiple PPGV. TMB-H was defined as ≥10 Mut/Mb. d) The MSI-H and Non-MSI-H/TMB-H breakdown for cancer types with the highest percentage (>1.5%) of cases with multiple PPGVs are shown. MSI-H, Microsatellite Instability-High; PPGV, Potential Pathogenic Germline Variant; TMB-H, Tumor Mutational Burden-High.

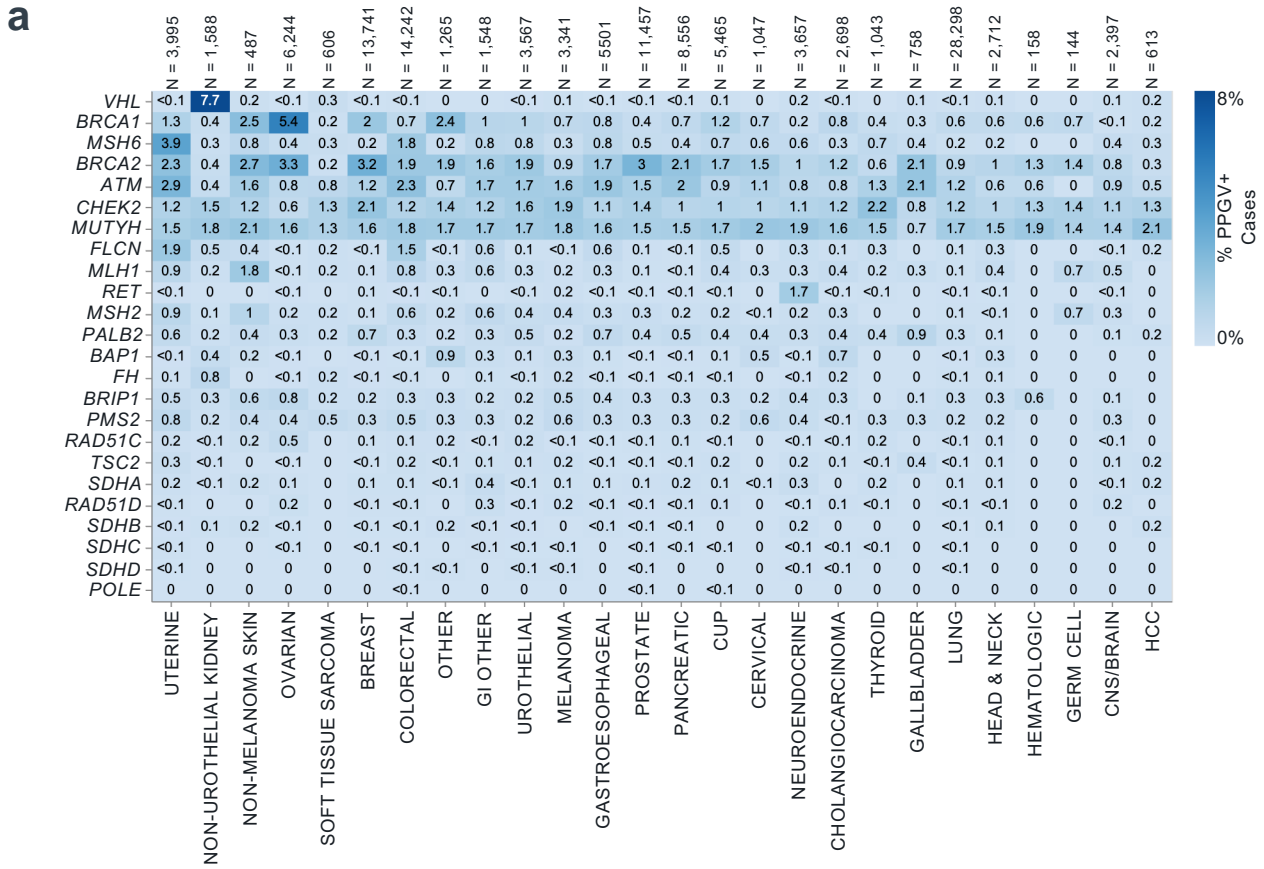
Supplementary Figure 5. Filtering Of Liquid Tumor CGP Variants On The Basis Of ClinVar Classification



a) Pan-cancer distribution of ClinVar-filtered variants from liquid tumor CGP cases across 24 germline banner CSGs and rationale for filtering out (Table). 429 SV across 422 cases were excluded. b) Pan-tumor landscape of ClinVar-filtered variants from liquid CGP cases in 24 germline banner genes. Values in the heatmap reflect the number of filtered variants. The x-axis is arranged by the total number of filtered variants per cancer type. The y-axis is arranged by the total number of filtered variants per CSG. c) Association of predicted patient ancestry and ClinVar-filtered variants. Percentages reflect the ratio of variants removed after the ClinVar filtering step to variants retained after the VAF filtering step, i.e., the ratio of ClinVar-filtered to pre-ClinVar-filtered variants for each ancestry. Statistical analysis was performed using Fisher's Exact Test with the Benjamini-Hochberg Procedure for p-value multiple hypothesis corrections. AFR, African; AMR, Admixed American; B/LB, Benign/Likely Benign; CGP, Comprehensive Genomic Profiling; CSG, Cancer Susceptibility Gene; DB, Database; EAS, East Asian; EUR, European; P/LP, Pathogenic/Likely Pathogenic; PPGV, Potential Pathogenic Germline Variant; SAS, South Asian; SV, Short Variant; VUS, Variant Of Uncertain Significance.

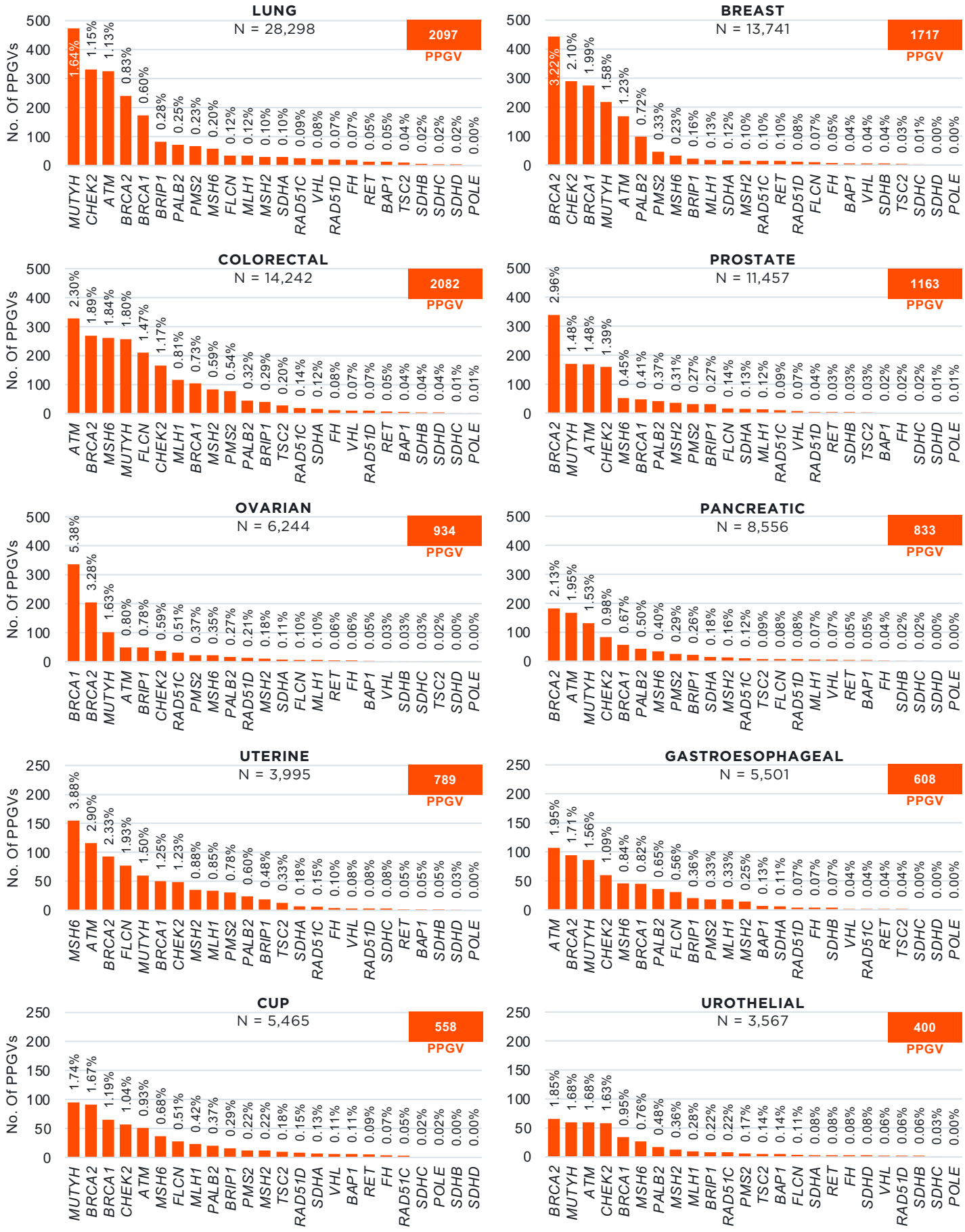
Supplementary Figure 6. Pan-Tumor Landscape Of PPGVs Across 24 Germline Banner CSGs (Tissue & Liquid)

a



Supplementary Figure 6. Pan-Tumor Landscape Of PPGVs Across 24 Germline Banner CSGs (Tissue & Liquid)

b



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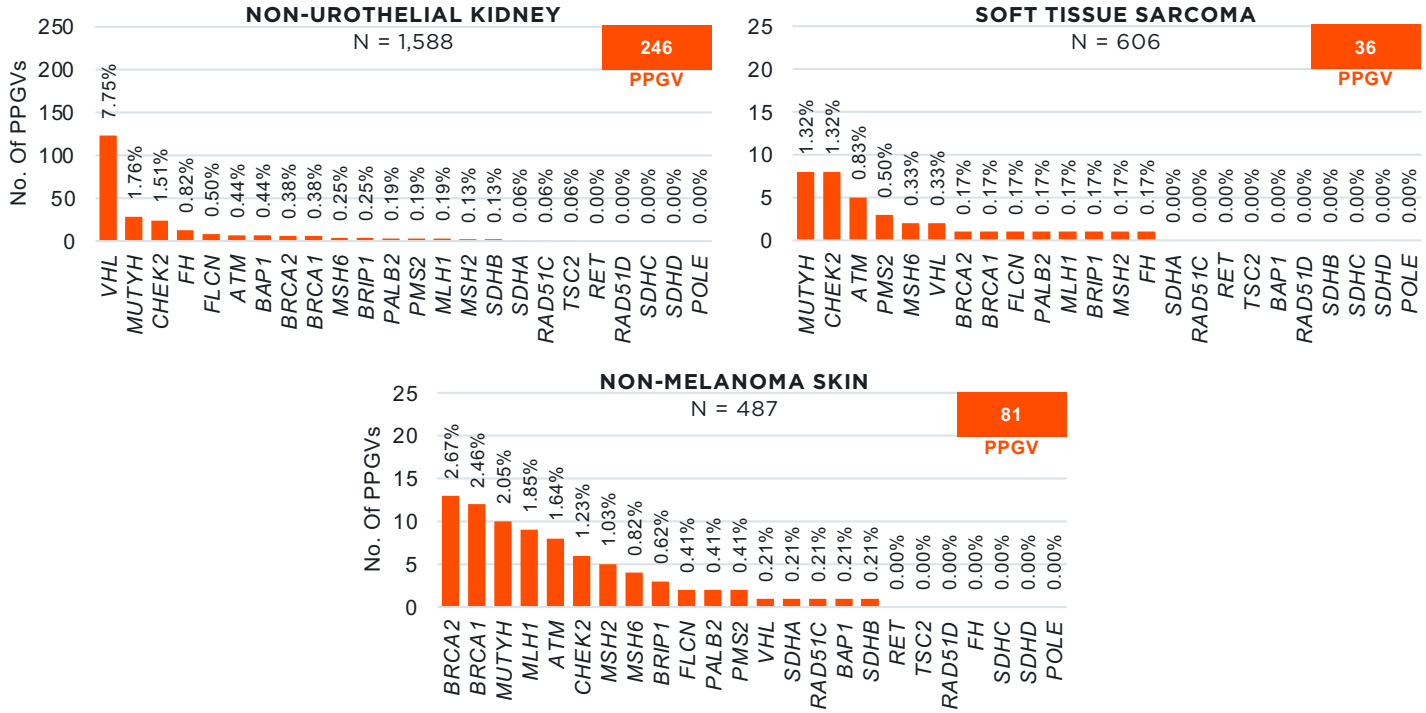

a) Values in the heatmap reflect the % of total cases per cancer type (N) with PPGVs detected in combined tissue and liquid CGP. The x-axis is arranged by the overall % of PPGV+ cases per cancer type (refer to **Figure 2A**). The y-axis is arranged according to the maximum cancer type-specific prevalence for each gene. b) Long tail plots showing the distribution of PPGVs across the 24 germline banner CSGs in cancer types/groupings with either PPGV prevalence ≥10% and/or PPGV incidence ≥500 samples (n = 13). Percentages represent gene-specific PPGV prevalence in the given cancer type. CSG, Cancer Susceptibility Gene; PPGV, Potential Pathogenic Germline Variant.

Supplementary Figure 7. 'Off-Tumor' Cancer Types With Founder Mutation PPGVs (Related To Figure 2B)

a *BRCA1* E23fs*17, C61G, Q1756fs*74

'OFF-TUMOR' TYPE	NO. CASES	% CASES
LUNG	21	11.4%
CUP	14	7.6%
GASTROESOPHAGEAL	11	6.0%
UROTHELIAL	8	4.3%
OTHER	6	3.3%
COLORECTAL	6	3.3%
CHOLANGIOCARCINOMA	5	2.7%
UTERINE	5	2.7%
NON-UROTHELIAL KIDNEY	2	1.1%
MELANOMA	2	1.1%
HCC	1	0.5%
CNS/BRAIN	1	0.5%
NEUROENDOCRINE	1	0.5%
HEAD & NECK	1	0.5%

b *BRCA2* E1308*, S1982fs*22

'OFF-TUMOR' TYPE	NO. CASES	% CASES
LUNG	7	5.6%
COLORECTAL	7	5.6%
GASTROESOPHAGEAL	6	4.8%
UTERINE	3	2.4%
CUP	3	2.4%
HEMATOLOGIC	2	1.6%
GI OTHER	2	1.6%
UROTHELIAL	2	1.6%
OTHER	2	1.6%
HEAD & NECK	2	1.6%
MELANOMA	1	0.8%
CERVICAL	1	0.8%

c *ATM* V2424G

'OFF-TUMOR' TYPE	NO. CASES	% CASES
LUNG	18	41.9%
PROSTATE	4	9.3%
COLORECTAL	4	9.3%
OTHER	2	4.7%
GASTROESOPHAGEAL	2	4.7%
CUP	2	4.7%
MELANOMA	1	2.3%
UROTHELIAL	1	2.3%
CHOLANGIOCARCINOMA	1	2.3%

d *CHEK2* T367fs*15

'OFF-TUMOR' TYPE	NO. CASES	% CASES
LUNG	108	19.8%
PROSTATE	66	12.1%
CUP	25	4.6%
MELANOMA	20	3.7%
GASTROESOPHAGEAL	17	3.1%
PANCREATIC	17	3.1%
UROTHELIAL	16	2.9%
NON-UROTHELIAL KIDNEY	13	2.4%
UTERINE	12	2.2%
OVARIAN	10	1.8%
CHOLANGIOCARCINOMA	9	1.7%
NEUROENDOCRINE	9	1.7%
THYROID	8	1.5%
CNS/BRAIN	7	1.3%
GI OTHER	5	0.9%
CERVICAL	4	0.7%
HEAD & NECK	4	0.7%
OTHER	4	0.7%
SOFT TISSUE SARCOMA	2	0.4%
GALLBLADDER	1	0.2%
HEMATOLOGIC	1	0.2%
HCC	1	0.2%
NON-MELANOMA SKIN	1	0.2%

e *MUTYH* Biallelic Y165C, G382D

'OFF-TUMOR' TYPE	NO. CASES	% CASES
LUNG	5	13.2%
BREAST	4	10.5%
PROSTATE	3	7.9%
GI OTHER	2	5.3%
MELANOMA	1	2.6%
PANCREAS	1	2.6%
NEUROENDOCRINE	1	2.6%
CUP	1	2.6%

COLORECTAL (n = 20)	BIALLELIC *MUTYH* PPGV	NO. CASES	% CASES
	Y165C/G382D	6	30%
	G382D/G382D	5	25%
	Y165C/OTHER	4	20%
	G382D/OTHER	4	20%
	Y165C/Y165C	1	5%
	TOTAL	20	100%

NON-COLORECTAL (n = 18)	BIALLELIC *MUTYH* PPGV	NO. CASES	% CASES
	G382D/G382D	7	39%
	Y165C/G382D	6	33%
	G382D/OTHER	3	17%
	Y165C/Y165C	1	6%
	Y165C/OTHER	1	6%
	TOTAL	18	100%

Percentages reflect the % of total cases harboring the indicated founder mutation(s) across all cancer types in combined tissue and liquid CGP. For biallelic *MUTYH* PPGV, homozygous versus compound heterozygous status for colorectal versus non-colorectal cases is provided.

Supplementary Table 1. Variants Highlighted In Text & Figures

GENE	NCBI REFERENCE SEQUENCE	CODING SEQUENCE EFFECT	PROTEIN SEQUENCE EFFECT
ATM	NM_000051	c.7271T>G	p.V2424G
BRCA1	NM_007294	c.68_69delAG	p.E23fs*17
BRCA1	NM_007294	c.181T>G	p.C61G
BRCA1	NM_007294	c.971delG	p.S324fs*17
BRCA1	NM_007294	c.5266_5267insC	p.Q1756fs*74
BRCA2	NM_000059	c.2937delA	p.I979fs*12
BRCA2	NM_000059	c.3922G>T	p.E1308*
BRCA2	NM_000059	c.5946delT	p.S1982fs*22
CHEK2	NM_007194	c.1100delC	p.T367fs*15
MUTYH	NM_001048171	c.494A>G	p.Y165C
MUTYH	NM_001048171	c.1145G>A	p.G382D