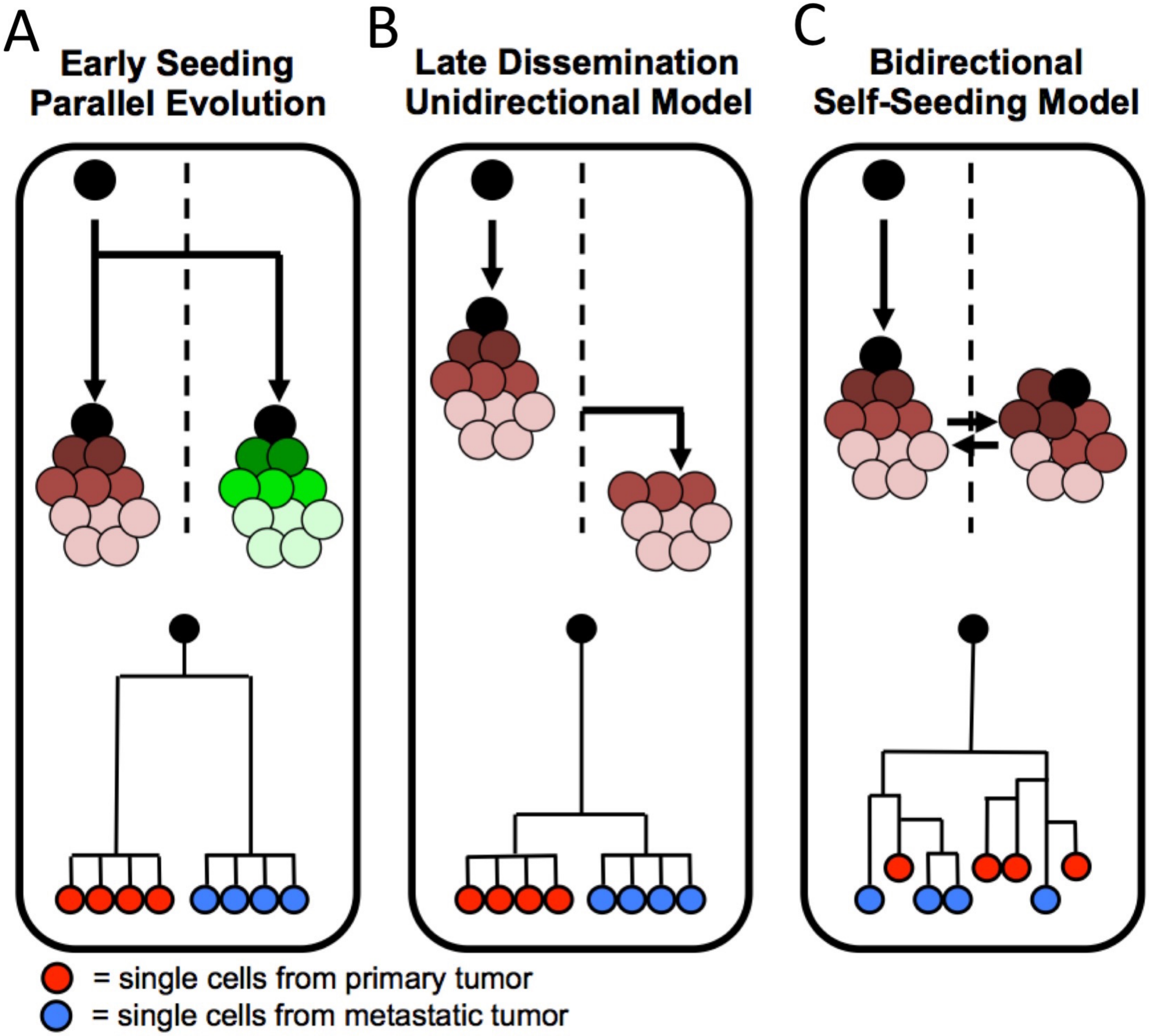
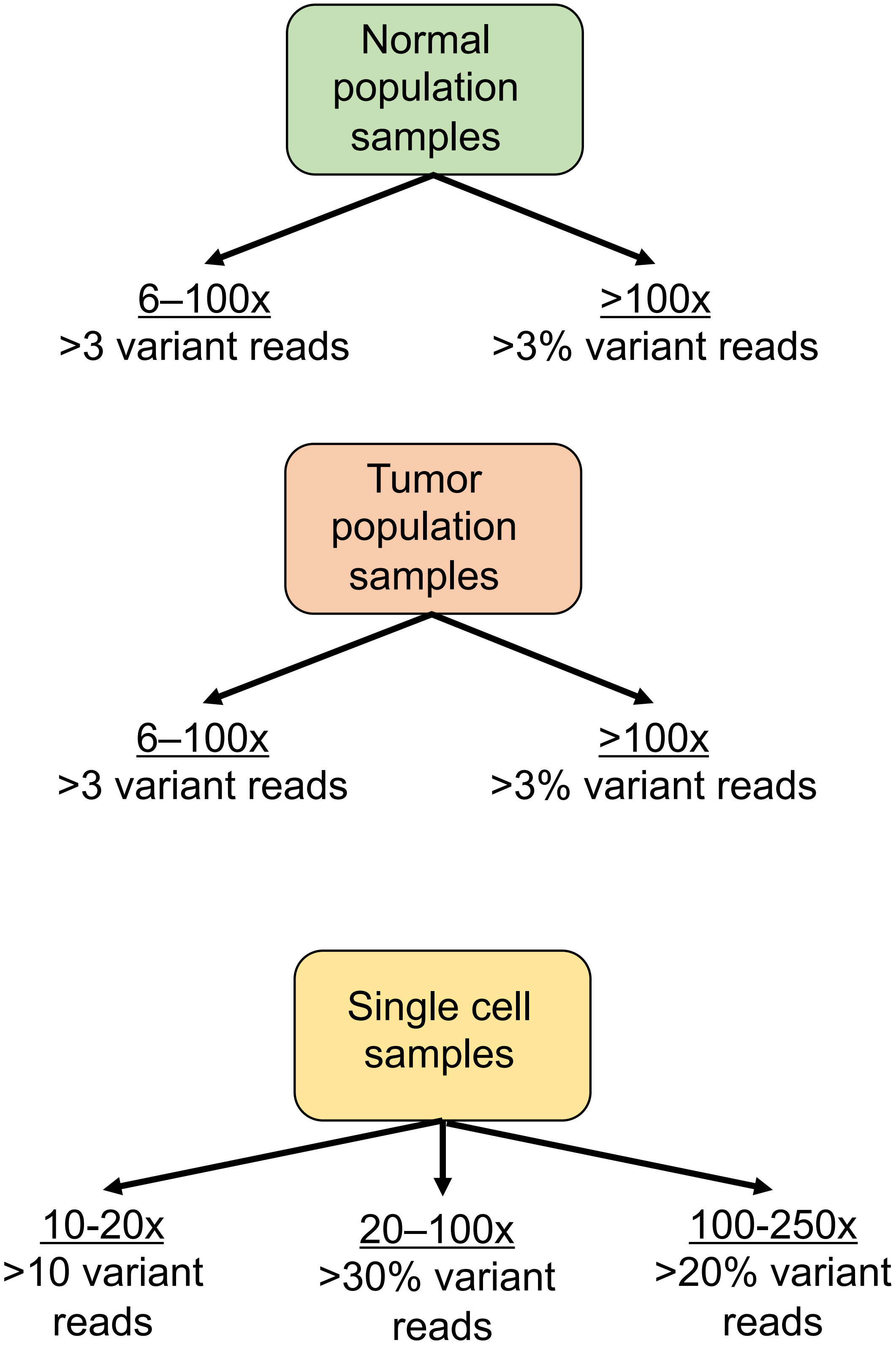
Supplementary Figure 1



Supplementary Figure 1 - Models of Metastasis

Illustrations of the general models of metastasis and corresponding phylogenetic lineages. (A) Early seeding and parallel evolution model (B) Late dissemination model (C) self-seeding model and bidirectional trafficking of tumor cells between the primary and metastatic organ sites.

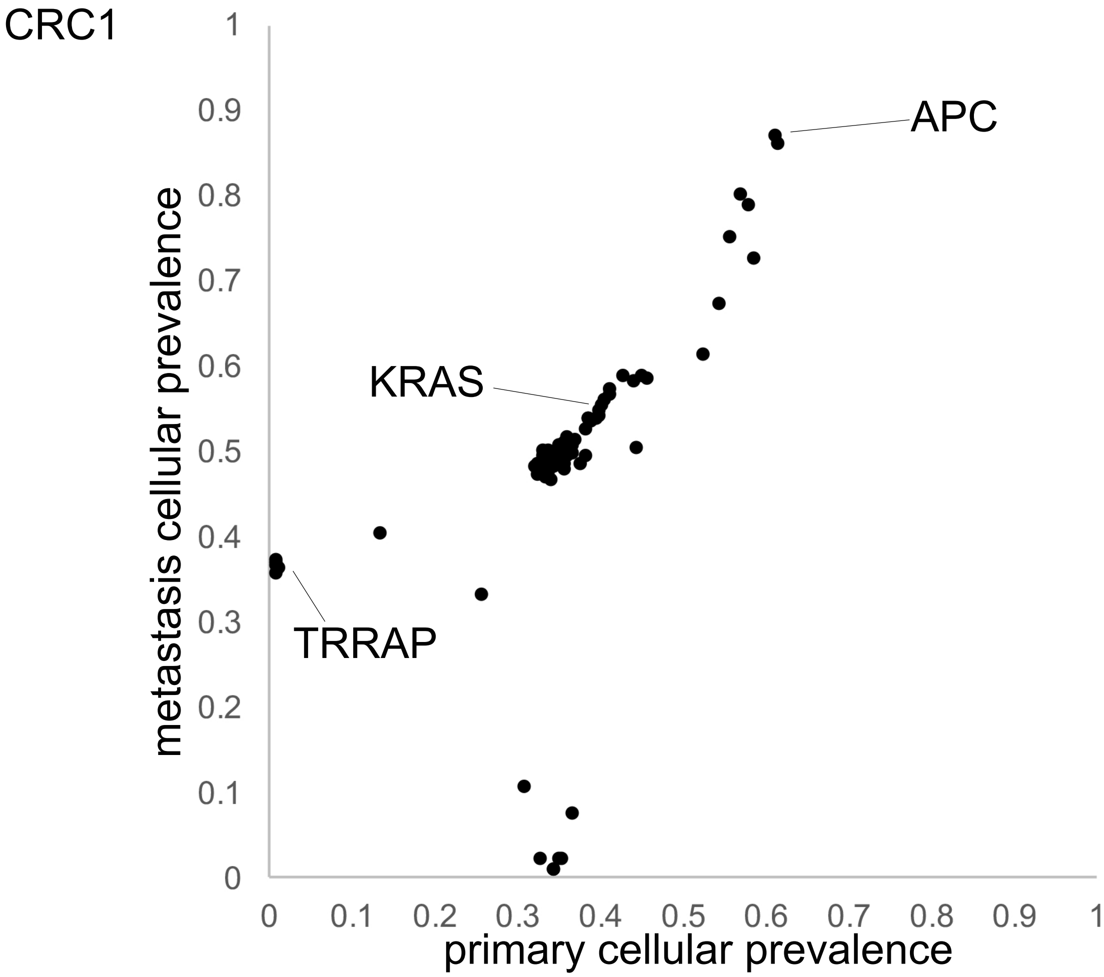
Supplementary Figure 2 -Variant Detection Filtering Criteria

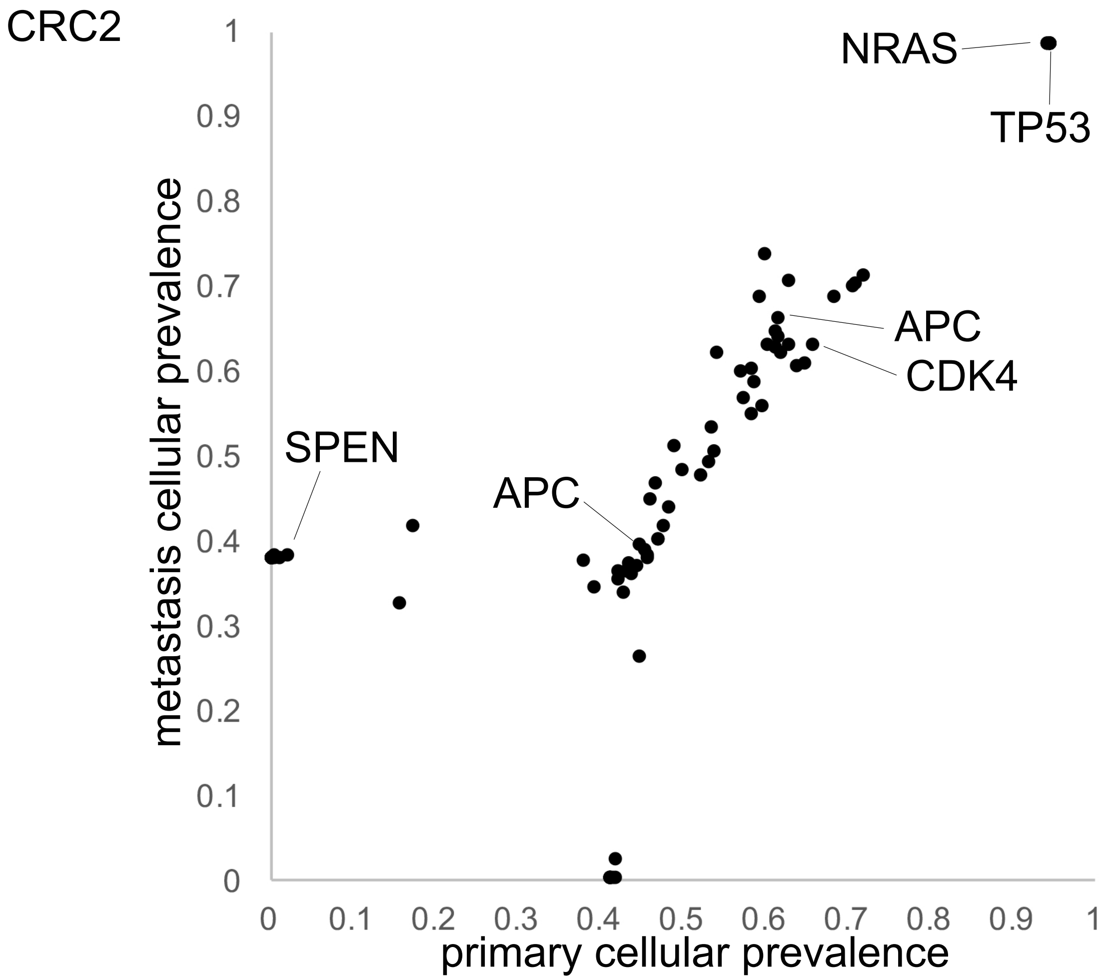


Supplementary Figure 2 – Variant Detection Filtering

This flow chart shows the coverage depths and filtering criteria used for variant in the single cell sequencing and bulk sequencing data.

Supplemental Figure 3



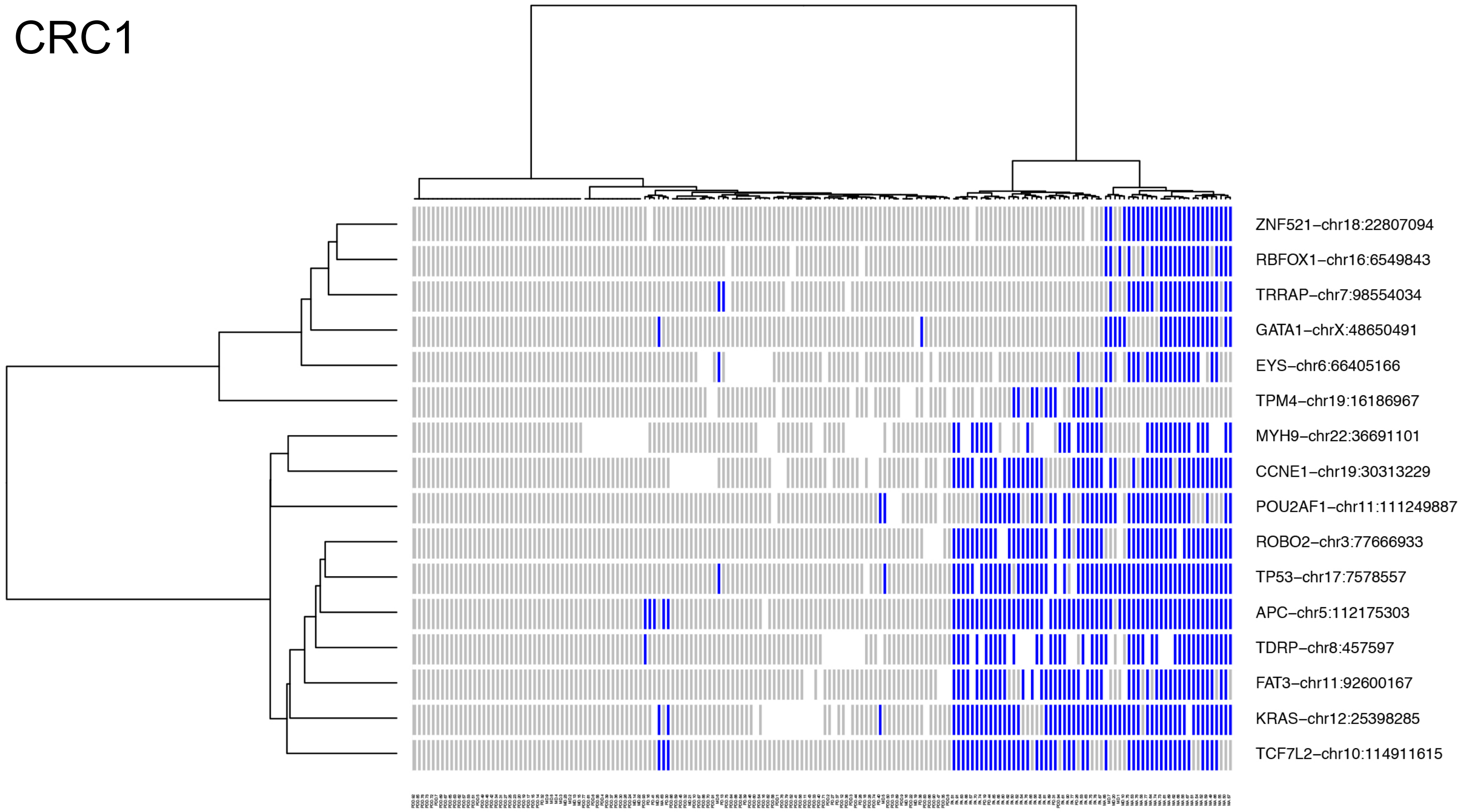


Supplementary Figure 3 – Copy number-adjusted Variant Allele Frequencies

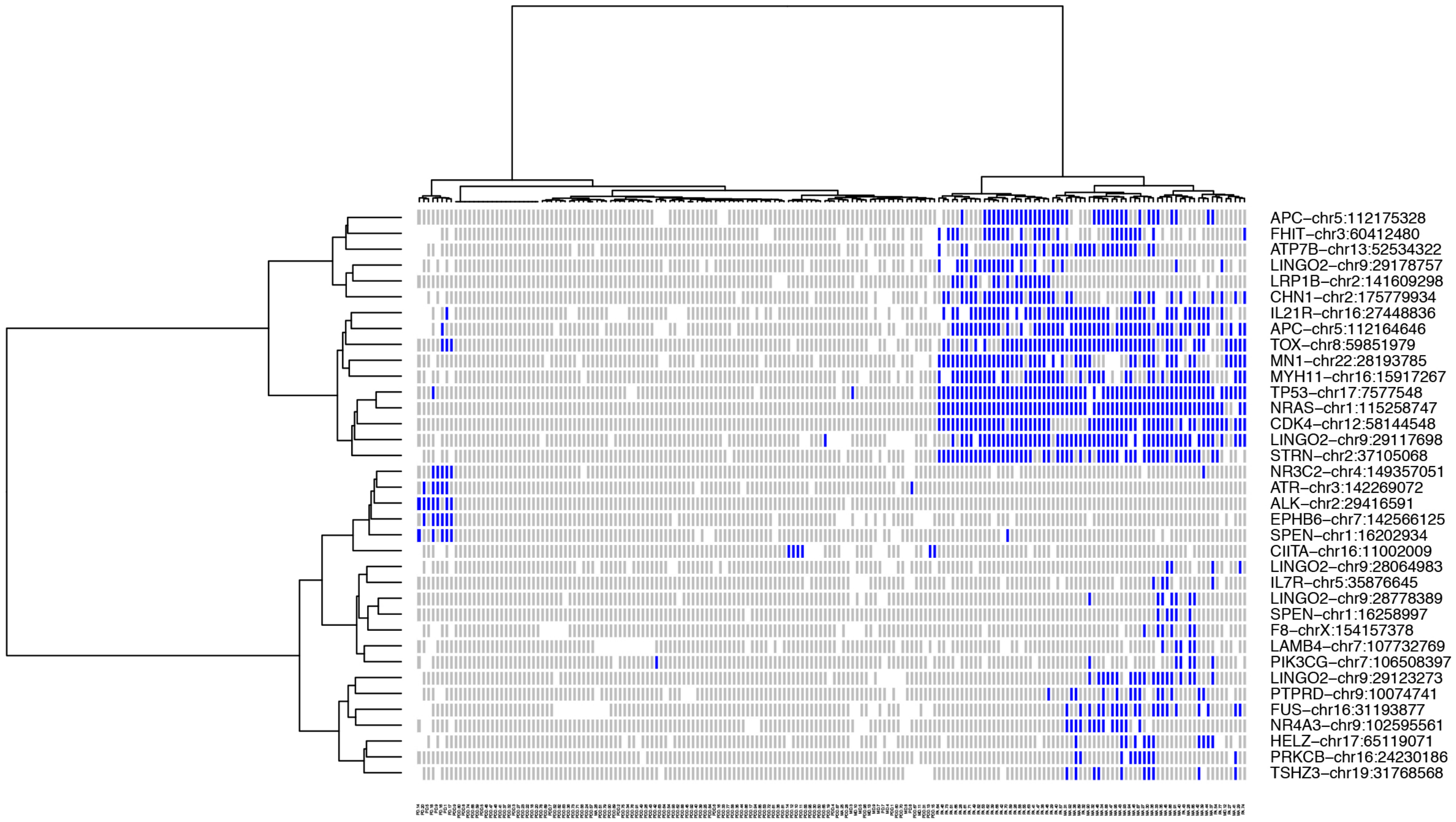
Mutations detected in both CRC1 (a) and CRC2 (b) were computed using PyClone to normalize copy number states and calculate cellular prevalence for each variant.

Supplemental Figure 4

CRC1



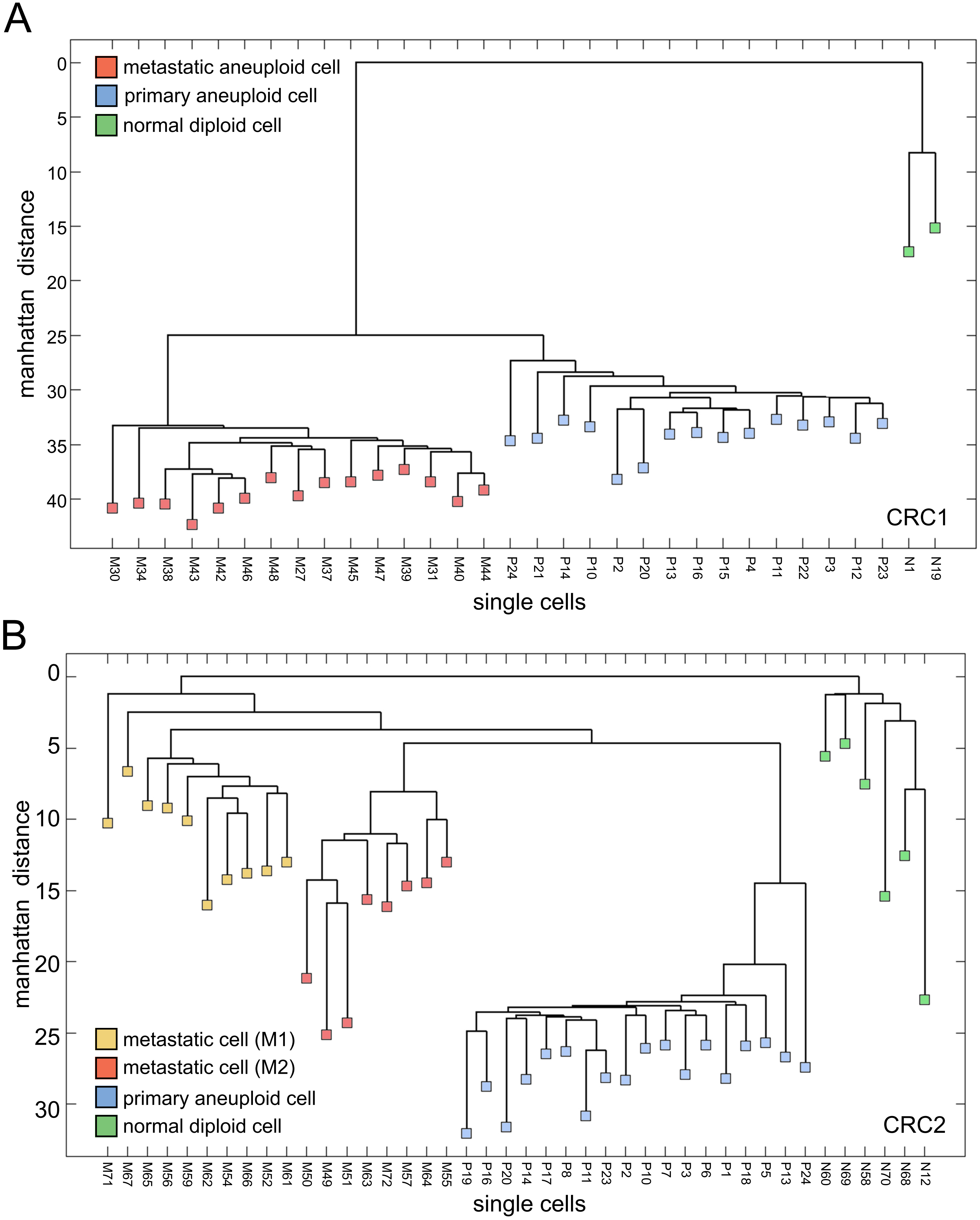
CRC2



Supplementary Figure 4 – Mutation Heatmaps with Dendrograms

Hierarchical clustered mutation heatmaps for patients CO5 (a) and CO8 (b) are shown with the dendrograms on the x-axis and y-axis.

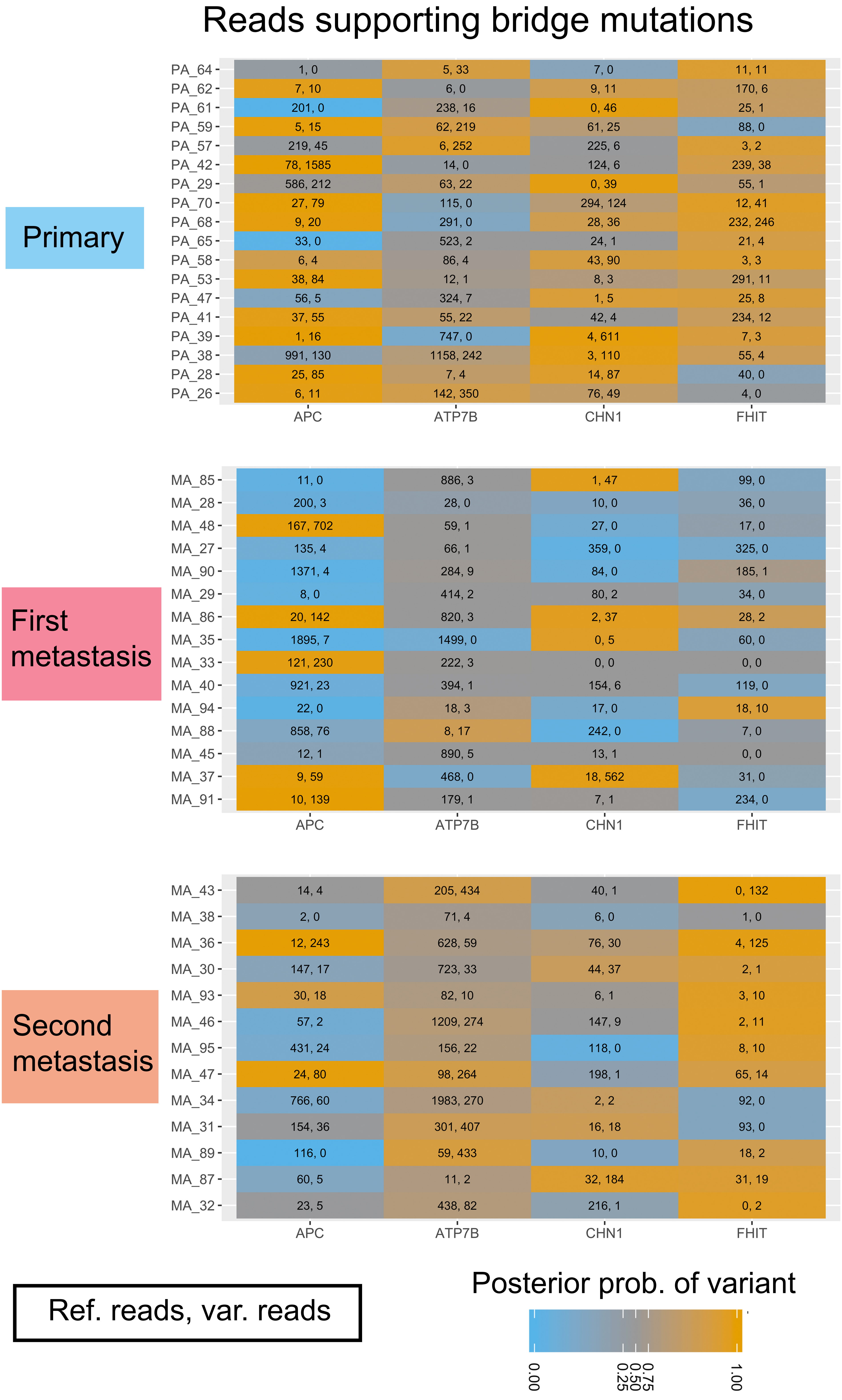
Supplemental Figure 5



Supplementary Figure 5 – Balanced Minimum Evolution Copy Number Trees

Copy number trees for patient CRC1 (a) and CRC2 (b) were constructed from single cell copy number data using the FastME balanced minimum evolution algorithm (Method).

Supplemental Figure 6



Reads supporting bridge mutations

Primary

	APC	ATP7B	CHN1	FHIT
PA_64	1, 0	5, 33	7, 0	11, 11
PA_62	7, 10	6, 0	9, 11	170, 6
PA_61	201, 0	238, 16	0, 46	25, 1
PA_59	5, 15	62, 219	61, 25	88, 0
PA_57	219, 45	6, 252	225, 6	3, 2
PA_42	78, 1585	14, 0	124, 6	239, 38
PA_29	586, 212	63, 22	0, 39	55, 1
PA_70	27, 79	115, 0	294, 124	12, 41
PA_68	9, 20	291, 0	28, 36	232, 246
PA_65	33, 0	523, 2	24, 1	21, 4
PA_58	6, 4	86, 4	43, 90	3, 3
PA_53	38, 84	12, 1	8, 3	291, 11
PA_47	56, 5	324, 7	1, 5	25, 8
PA_41	37, 55	55, 22	42, 4	234, 12
PA_39	1, 16	747, 0	4, 611	7, 3
PA_38	991, 130	1158, 242	3, 110	55, 4
PA_28	25, 85	7, 4	14, 87	40, 0
PA_26	6, 11	142, 350	76, 49	4, 0

First metastasis

	APC	ATP7B	CHN1	FHIT
MA_85	11, 0	886, 3	1, 47	99, 0
MA_28	200, 3	28, 0	10, 0	36, 0
MA_48	167, 702	59, 1	27, 0	17, 0
MA_27	135, 4	66, 1	359, 0	325, 0
MA_90	1371, 4	284, 9	84, 0	185, 1
MA_29	8, 0	414, 2	80, 2	34, 0
MA_86	20, 142	820, 3	2, 37	28, 2
MA_35	1895, 7	1499, 0	0, 5	60, 0
MA_33	121, 230	222, 3	0, 0	0, 0
MA_40	921, 23	394, 1	154, 6	119, 0
MA_94	22, 0	18, 3	17, 0	18, 10
MA_88	858, 76	8, 17	242, 0	7, 0
MA_45	12, 1	890, 5	13, 1	0, 0
MA_37	9, 59	468, 0	18, 562	31, 0
MA_91	10, 139	179, 1	7, 1	234, 0

Second metastasis

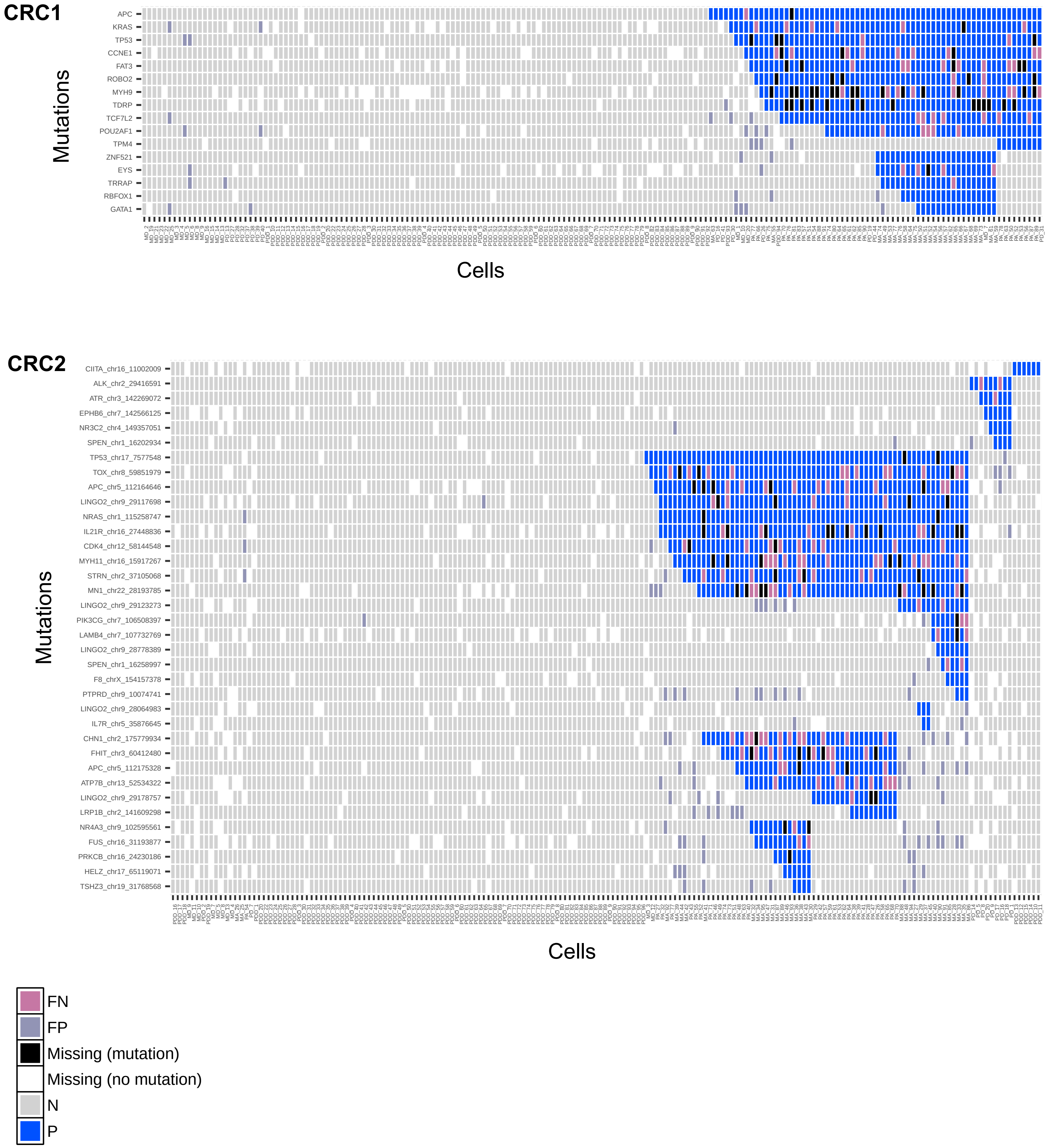
	APC	ATP7B	CHN1	FHIT
MA_43	14, 4	205, 434	40, 1	0, 132
MA_38	2, 0	71, 4	6, 0	1, 0
MA_36	12, 243	628, 59	76, 30	4, 125
MA_30	147, 17	723, 33	44, 37	2, 1
MA_93	30, 18	82, 10	6, 1	3, 10
MA_46	57, 2	1209, 274	147, 9	2, 11
MA_95	431, 24	156, 22	118, 0	8, 10
MA_47	24, 80	98, 264	198, 1	65, 14
MA_34	766, 60	1983, 270	2, 2	92, 0
MA_31	154, 36	301, 407	16, 18	93, 0
MA_89	116, 0	59, 433	10, 0	18, 2
MA_87	60, 5	11, 2	32, 184	31, 19
MA_32	23, 5	438, 82	216, 1	0, 2

Ref. reads, var. reads

Posterior prob. of variant

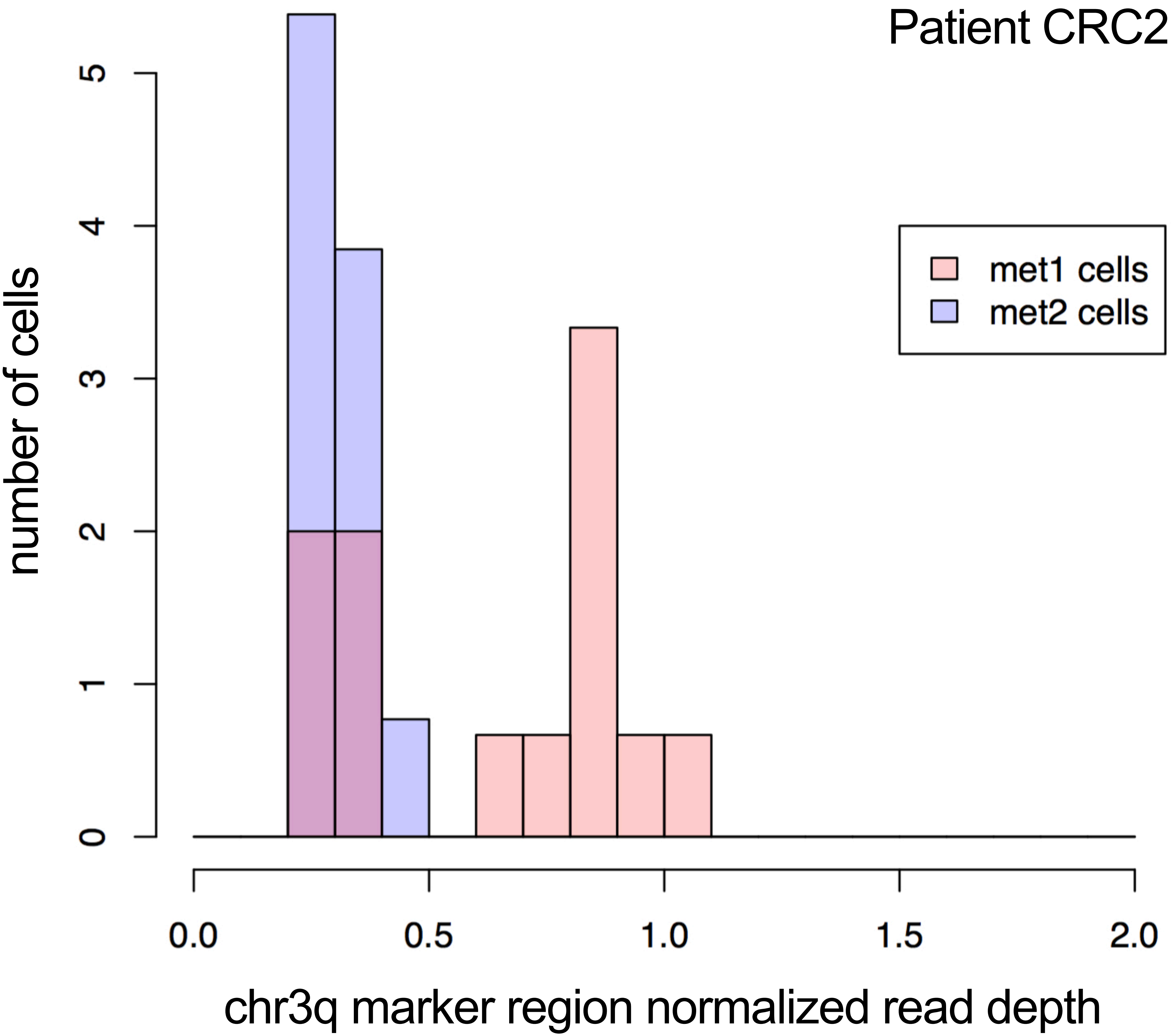
Supplementary Figure 6 – Probability Heatmap of Bridge Mutations in CRC2

Heatmap of the reference and variant read counts of the four bridge mutations (*APC*, *ATP7B*, *CHN1* and *FHIT*) in patient CRC2 are listed for the primary and metastatic tumor cells. The metastatic tumor cells are shown separately for the groups defined as ‘first metastasis’ and ‘second metastasis’ by the SCITE tree. Each variant is colored in the heatmap based on the corresponding posterior probability value.


CRC1
Mutations
APC
KRAS
TP53
CCNE1
FAT3
ROBO2
MYH9
TDRP
TCF7L2
POU2AF1
TPM4
ZNF521
EYS
TRRAP
RBFOX1
GATA1
Cells
CRC2
Mutations
CIITA_chr16_11002009
ALK_chr2_29416591
ATR_chr3_142269072
EPHB6_chr7_142566125
NR3C2_chr4_149357051
SPEN_chr1_16202934
TP53_chr17_7577548
TOX_chr8_59851979
APC_chr5_112164646
LINGO2_chr9_29117698
NRAS_chr1_115258747
IL21R_chr16_27448836
CDK4_chr12_58144548
MYH11_chr16_15917267
STRN_chr2_37105068
MN1_chr22_28193785
LINGO2_chr9_29123273
PIK3CG_chr7_106508397
LAMB4_chr7_107732769
LINGO2_chr9_28778389
SPEN_chr1_16258997
F8_chrX_154157378
PTPRD_chr9_10074741
LINGO2_chr9_28064983
IL7R_chr5_35876645
CHN1_chr2_175779934
FHIT_chr3_60412480
APC_chr5_112175328
ATP7B_chr13_52534322
LINGO2_chr9_29178757
LRP1B_chr2_141609298
NR4A3_chr9_102595561
FUS_chr16_31193877
PRKCB_chr16_24230186
HELZ_chr17_65119071
TSHZ3_chr19_31768568
Cells
FN
FP
Missing (mutation)
Missing (no mutation)
N
P

Supplementary Figure 7 – Genotype Matrices Calculated from SCITE Trees
Heatmaps of genotype matrices with inferred errors based on the placement of single cells in the SCITE trees. False negative (FN) errors indicates sites in which the variant is called in the theoretical genotype but called as non-variant. False positive (FP) errors indicates a site which is non-variant in the theoretical genotype but called as variant. Missing (mutation) indicates that a site is variant in the theoretical genotype, but was excluded due to low coverage depth. This case is plotted with the same color as a false negative error, since it also represents an unobserved variant. Missing (no mutation) indicates sites that are non-variant in the theoretical genotype but excluded due to low coverage depth. Negative and positive represent concordance between the theoretical and observed genotypes in the absence and presence of a variant, respectively.

Supplemental Figure 8



Supplementary Figure 8 – Copy number concordance with single cell sequencing data in CRC2

Two copy number subpopulations were detected in the metastasis (M1 and M2) of CRC2 and two subpopulations detections by mutational profiling in the SCITE tree (first and second metastasis). However, the correspondence of the subpopulations between these data was unclear. A region of chromosome 3q was identified that servers as an effective marker for distinguishing the two subclones of the CRC2 metastasis (M1 and M2) detected using single-cell copy number profiling. To determine the amplification of 3q in the mutation data (metastasis 1 or 2), we calculated the median normalized read counts of 3q for each single cell sequenced. The 3q marker divides the mutation data into two groups, which correspond to the cells in the first and second metastasis (met1 and met2) as determined by the SCITE tree. The met1 subpopulation in the mutation data showed amplification of 3q suggesting that it corresponded to the M1 copy number subpopulation, while the met2 subpopulation corresponded to the M2 copy number subpopulation.

Supplementary Figure 9 – Integrated Mutation and Copy Number Trees

Mutation trees were calculated from single cell data using SCITE and copy number aberrations were annotated based on the FastME results and tissue of origin. In the case of CRC2 metastasis, where two subclones coexist, the correspondence between CNV subclones and SNV subclones was determined on the basis of read depth of the SNV-genotyped cells in a genomic region (3p) that distinguishes the CNV subclones (Supp. Figure 8). *Amp* indicates amplification, while *Del* indicates a deletion.

Supplementary Table 1

CRC1	Depth (x)	Breadth
Normal colon	59	0.9596
Normal liver	63	0.9417
Primary Diploid	63	0.9684
Primary Aneuploid	80	0.9819
Metastatic Diploid	49	0.9744
Metastatic Aneuploid	70	0.9786
mean	64	0.9674

CRC2	Depth (x)	Breadth
Normal colon	111	0.9812
Normal liver	83	0.9776
Primary Diploid	93	0.9805
Primary Aneuploid	93	0.98
Metastatic Diploid	46	0.9756
Metastatic Aneuploid	96	0.9798
mean	87	0.9791

Supplementary Table 1 - Coverage performance of population exome sequencing data

This table shows the coverage depth and coverage breadth (physical coverage at sites with at least 1x depth) for each exome population sample from patients CRC1 and CRC2.

Supplementary Table 2.1 - CRC1

Primary	Metastasis	Chrom	Position	Gene Name	NCBI Ref Seq	Ref	Var	Mutation	Base Change	Amino Acid	Cancer Gene	Polyphen	SIFT	mtaster	COSMIC
☐	☐	chr9	95784648	FGD3	NM_001083536	G	A	nonsynonymous	c.G1534A	p.E512K	NA	0.352	0.07	1	COSM1598552,COSM1598553
☐	☐	chr10	131641447	EBF3	NM_001005463	G	A	nonsynonymous	c.C1121T	p.A374V	NA	0.998	0.04	1	COSM1204867,COSM1204868
☐	☐	chr19	50214113	CPT1C	NM_001199752	C	T	nonsynonymous	c.C1865T	p.T622M	NA	0.99	0.04	1	NA
☐	☐	chr19	5244392	PTPRS	NM_002850	C	T	nonsynonymous	c.G1090A	p.V364I	NA	0.56	0.33	1	NA
☐	☐	chr15	42438030	PLA2G4F	NM_213600	C	T	nonsynonymous	c.G1606A	p.G536S	NA	0.998	0.01	0.988	NA
☐	☐	chr8	27779273	SCARA5	NM_173833	C	T	nonsynonymous	c.G731A	p.R244Q	NA	0.041	0.44	1	NA
☐	☐	chr16	511409	RAB11FIP3	NM_014700	G	T	nonsynonymous	c.G720T	p.K240N	NA	0.982	0.13	1	NA
☐	☐	chr2	27552346	GTF3C2	NM_001035521	G	A	nonsynonymous	c.C1777T	p.R593W	NA	0.99	0.01	0.999	NA
☐	☐	chr17	3772840	CAMKK1	NM_032294	C	T	nonsynonymous	c.G1282A	p.E428K	NA	0.929	0.03	1	NA
☐	☐	chr7	75050957	POM121C	NM_001099415	C	T	nonsynonymous	c.G2578A	p.A860T	NA	0	0.46	0.987	NA
☐	☐	chrX	48650491	GATA1	NM_002049	C	A	nonsynonymous	c.C461A	p.S154X	GATA1	NA	1	1	NA
☐	☐	chr9	116931070	COL27A1	NM_032888	G	A	nonsynonymous	c.G1235A	p.R412H	NA	0	0.55	1	COSM1201862
☐	☐	chrX	7811288	VCX	NM_013452	C	G	nonsynonymous	c.C44G	p.T15R	NA	0.004	0.51	1	NA
☐	☐	**chr10**	**114911615**	**TCF7L2**	**NM_001146274**	**C**	**A**	**nonsynonymous**	**c.C1133A**	**p.A378E**	**TCF7L2**	**1**	**0**	**1**	**NA**
☐	☐	**chr22**	**39629508**	**PDGFB**	**NM_002608**	**T**	**C**	**nonsynonymous**	**c.A182G**	**p.D61G**	**PDGFB**	**0.916**	**0.07**	**0.998**	**NA**
☐	☐	chr19	2216629	DOT1L	NM_032482	C	T	nonsynonymous	c.C2273T	p.P758L	DOT1L	0.283	0	0.963	NA
☐	☐	chr7	36492153	ANLN	NM_001284301	G	T	nonsynonymous	c.G3208T	p.V1070F	NA	1	0.05	1	NA
☐	☐	chr11	111249887	POU2AF1	NM_006235	G	A	nonsynonymous	c.C16T	p.P6S	POU2AF1	0	NA	0.989	NA
☐	☐	chr12	42512910	GXYLT1	NM_173601	T	A	nonsynonymous	c.A378T	p.R126S	NA	0.999	0.11	1	NA
☐	☐	chr15	41056381	GCHFR	NM_005258	G	A	nonsynonymous	c.G3A	p.M1I	NA	0.851	0	1	NA
☐	☐	chr1	181686322	CACNA1E	NM_000721	G	A	nonsynonymous	c.G1409A	p.R470H	NA	0.999	0.03	1	COSM1247132,COSM1247131
☐	☐	chr7	75050891	POM121C	NM_001099415	T	C	nonsynonymous	c.A2644G	p.T882A	NA	0	0.42	1	NA
☐	☐	chr16	16103671	ABCC1	NM_004996	C	A	nonsynonymous	c.C264A	p.D88E	NA	0.702	0.47	1	NA
☐	☐	chr17	27383288	PIPOX	NM_016518	A	C	nonsynonymous	c.A1138C	p.S380R	NA	0.326	0.24	0.925	NA
☐	☐	chr17	39978517	FKBP10	NM_021939	C	T	nonsynonymous	c.C1606T	p.R536C	NA	1	0.02	1	NA
☐	☐	**chr5**	**112175303**	**APC**	**NM_001127510**	**C**	**T**	**nonsynonymous**	**c.C4012T**	**p.Q1338X**	**APC**	**NA**	**NA**	**1**	**COSM13129**
☐	☐	chr17	72739280	RAB37	NM_001006638	G	A	nonsynonymous	c.G259A	p.A87T	NA	1	0	1	COSM1479973,COSM1479972,COSM1479971
☐	☐	chr11	6470323	TRIM3	NM_001248006	C	A	nonsynonymous	c.G2170T	p.D724Y	NA	1	0	1	NA
☐	☐	**chr1**	**197111575**	**ASPM**	**NM_001206846**	**G**	**T**	**nonsynonymous**	**c.C1807A**	**p.H603N**	**ASPM**	**0.976**	**0.4**	**1**	**NA**
☐	☐	**chr12**	**25398285**	**KRAS**	**NM_004985**	**C**	**A**	**nonsynonymous**	**c.G34T**	**p.G12C**	**KRAS**	**1**	**0.01**	**1**	**COSM1140136,COSM516**
☐	☐	chr11	67012738	KDM2A	NM_012308	C	T	nonsynonymous	c.C1642T	p.R548W	NA	0.999	0.02	0.997	NA
☐	☐	chr4	109672135	ETNPPL	NM_031279	C	T	nonsynonymous	c.G658A	p.G220R	NA	1	NA	1	NA
☐	☐	chr19	58320385	ZNF552	NM_024762	C	T	nonsynonymous	c.G247A	p.A83T	NA	0.079	0.51	1	NA
☐	☐	chr1	111957412	OVGP1	NM_002557	G	A	nonsynonymous	c.C1711T	p.R571C	NA	0.958	0.02	1	COSM1648330,COSM1648331
☐	☐	chr20	43851625	SEMG2	NM_003008	C	G	nonsynonymous	c.C1352G	p.T451R	NA	0.003	0.84	1	NA
☐	☐	chr9	43625849	SPATA31A6	NM_001145196	C	G	nonsynonymous	c.G2838C	p.E946D	NA	0.488	0.38	1	NA
☐	☐	chr4	187455223	MTNR1A	NM_005958	G	A	nonsynonymous	c.C673T	p.R225C	NA	0.002	0.12	1	NA
☐	☐	chr4	114278764	ANK2	NM_001148	C	T	nonsynonymous	c.C8990T	p.S2997F	NA	0.874	0.02	1	NA
☐	☐	chr2	114512750	SLC35F5	NM_025181	G	A	nonsynonymous	c.C265T	p.L89F	NA	0.999	0.06	1	NA
☐	☐	chr19	9048227	MUC16	NM_024690	A	G	nonsynonymous	c.T33404C	p.V11135A	NA	0	0	NA	NA
☐	☐	chr4	96761627	PDHA2	NM_005390	C	T	nonsynonymous	c.C326T	p.S109L	NA	0.846	0.29	0.99	COSM1261822
☐	☐	**chr2**	**152515652**	**NEB**	**NM_001271208**	**T**	**G**	**nonsynonymous**	**c.A6002C**	**p.K2001T**	**NEB**	**1**	**0.05**	**1**	**NA**
☐	☐	chr18	61570307	SERPINB2	NM_001143818	A	G	nonsynonymous	c.A1016G	p.N339S	NA	0.939	0.01	1	NA
☐	☐	chr11	111177170	COLCA2	NM_001271458	G	A	nonsynonymous	c.G209A	p.R70Q	NA	NA	NA	NA	NA
☐	☐	chr19	30313229	CCNE1	NM_001238	C	T	nonsynonymous	c.C923T	p.S308L	NA	1	0.7	1	NA
☐	☐	chr5	169535601	FOXI1	NM_144769	G	T	nonsynonymous	c.G838T	p.G280C	NA	1	0	1	NA
☐	☐	chr3	38739105	SCN10A	NM_001293306	C	T	nonsynonymous	c.G5603A	p.R1868H	NA	0.171	0.1	0.988	NA
☐	☐	chr17	56270739	EPX	NM_000502	C	A	nonsynonymous	c.C178A	p.Q60K	NA	0.002	1	0.902	NA
☐	☐	chr17	72832510	TMEM104	NM_017728	C	T	nonsynonymous	c.C1175T	p.P392L	NA	1	0.56	1	NA
☐	☐	chr11	72945731	P2RY2	NM_002564	C	T	nonsynonymous	c.C527T	p.A176V	NA	0.001	0.44	1	NA
☐	☐	chr17	12905805	ELAC2	NM_001165962	G	A	nonsynonymous	c.C1051T	p.L351F	NA	0.999	0.23	1	NA
☐	☐	chr4	2691303	FAM193A	NM_001256666	A	G	nonsynonymous	c.A1529G	p.H510R	NA	0.999	0.06	1	NA
☐	☐	chr1	27332541	FAM46B	NM_052943	C	T	nonsynonymous	c.G1172A	p.R391H	NA	0	0.18	0.995	NA
☐	☐	chr9	137620520	COL5A1	NM_000093	C	T	nonsynonymous	c.C791T	p.T264M	NA	0.744	0.04	1	COSM2157265
☐	☐	chr10	81697853	SFTPD	NM_003019	C	T	nonsynonymous	c.G883A	p.A295T	NA	0.19	0.2	1	NA
☐	☐	chr16	80718449	CDYL2	NM_152342	G	A	nonsynonymous	c.C602T	p.A201V	NA	0	0.53	1	NA
☐	☐	chr10	82348482	SH2D4B	NM_001145719	C	A	nonsynonymous	c.C445A	p.Q149K	NA	0.996	0.07	0.983	NA
☐	☐	chr15	86838537	AGBL1	NM_152336	C	T	nonsynonymous	c.C2134T	p.R712W	NA	1	0	0.887	COSM3690677
☐	☐	chr3	187387985	SST	NM_001048	C	T	nonsynonymous	c.G95A	p.R32H	NA	1	0.18	1	NA
☐	☐	chr5	149677523	ARSI	NM_001012301	C	T	nonsynonymous	c.G964A	p.G322S	NA	1	0.01	1	NA
☐	☐	chr5	141237011	PCDH1	NM_032420	G	A	nonsynonymous	c.C3125T	p.S1042L	NA	1	0	1	NA
☐	☐	chrX	48207025	SSX3	NM_021014	C	T	nonsynonymous	c.G481A	p.E161K	NA	0	1	1	NA
☐	☐	chr17	42854546	ADAM11	NM_002390	G	A	nonsynonymous	c.G1694A	p.R565H	NA	0.996	0.02	1	NA
☐	☐	chr5	137766019	KDM3B	NM_016604	C	T	nonsynonymous	c.C4975T	p.R1659C	NA	1	0	1	COSM175846
☐	☐	chr18	22807094	ZNF521	NM_015461	T	C	nonsynonymous	c.A788G	p.K263R	ZNF521	0.071	0.58	0.702	NA
☐	☐	chr1	6681651	PHF13	NM_153812	G	A	nonsynonymous	c.G857A	p.R286H	NA	0.999	0.03	1	NA
☐	☐	chrX	38135983	RPGR	NM_000328	C	T	nonsynonymous	c.G1948A	p.V650M	RPGR	0.009	0.15	1	NA
☐	☐	chr5	54423155	CDC20B	NM_152623	G	A	nonsynonymous	c.C919T	p.R307W	NA	0.999	0	1	NA
☐	☐	chrX	135958730	RBMX	NM_002139	C	A	nonsynonymous	c.G473T	p.G158V	RBMX	0.802	0.09	0	NA
☐	☐	chr7	5415673	TNRC18	NM_001080495	G	A	nonsynonymous	c.C2791T	p.Q931X	NA	NA	0.08	1	NA
☐	☐	chr7	142458526	PRSS1	NM_002769	A	G	nonsynonymous	c.A161G	p.N54S	NA	0	0.51	0	NA
☐	☐	chr17	39394674	KRTAP9-8	NM_031963	A	G	nonsynonymous	c.A371G	p.N124S	NA	0.006	1	1	NA
☐	☐	chr2	131797751	ARHGEF4	NM_015320	C	T	nonsynonymous	c.C910T	p.R304C	NA	0.999	0	1	NA
☐	☐	chr1	36564618	COL8A2	NM_005202	C	T	nonsynonymous	c.G664A	p.G222R	NA	0	0.45	0.803	NA
☐	☐	chr13	109792732	MYO16	NM_015011	C	A	nonsynonymous	c.C4106A	p.S1369Y	NA	0.845	NA	1	NA
☐	☐	chr5	156279	PLEKHG4B	NM_052909	G	A	nonsynonymous	c.G1234A	p.V412I	NA	0.968	0.45	1	COSM1221148
☐	☐	chr2	166929996	SCN1A	NM_006920	C	T	nonsynonymous	c.G136A	p.E46K	NA	0.941	0.03	1	COSM1325785,COSM1325784
☐	☐	chr22	24581995	SUSD2	NM_019601	G	A	nonsynonymous	c.G1351A	p.G451R	NA	1	0	1	NA
☐	☐	chr5	140594292	PCDHB13	NM_018933	A	C	nonsynonymous	c.A597C	p.K199N	NA	0.992	0.01	1	NA
☐	☐	chr22	20460526	RIMBP3	NM_015672	C	T	nonsynonymous	c.G776A	p.R259H	NA	0.995	0.26	1	NA
☐		chrX	101912757	GPRASP1	NM_001099410	A	T	nonsynonymous	c.A3916T	p.N1306Y	NA	1	0.01	1	NA
☐		chr19	15292500	NOTCH3	NM_000435	G	T	nonsynonymous	c.C2679A	p.S893R	NA	1	0	1	NA
☐		chr1	152279527	FLG	NM_002016	T	C	nonsynonymous	c.A7835G	p.D2612G	NA	0.949	0.65	1	NA
☐		chr15	44038842	PDIA3	NM_005313	C	G	nonsynonymous	c.C105G	p.F35L	NA	1	0.02	1	NA
☐		chr4	170042033	SH3RF1	NM_020870	C	G	nonsynonymous	c.G1454C	p.G485A	NA	1	0	1	NA
☐		chr11	62294315	AHNAK	NM_001620	G	C	nonsynonymous	c.C7574G	p.A2525G	AHNAK	0	0.04	0.996	NA
	☐	chr5	173035291	BOD1	NM_001159651	G	A	nonsynonymous	c.C367T	p.R123C	NA	0	0.05	1	NA
	☐	chr7	98554034	TRRAP	NM_003496	A	G	nonsynonymous	c.A6034G	p.M2012V	NA	0.001	0.31	0.996	NA
	☐	chr4	106755675	GSTCD	NM_024751	A	G	nonsynonymous	c.A1327G	p.T443A	NA	0	1	1	NA
	☐	**chr11**	**130785060**	**SNX19**	**NM_014758**	**A**	**T**	**nonsynonymous**	**c.T775A**	**p.W259R**	**NA**	**1**	**0.05**	**1**	**NA**

Supplementary Table 2.2

Primary	Metastasis	Chrom	Position	Gene Name	NCBI Ref Seq	Ref	Var	Mutation	Base Change	Amino Acid	Cancer Gene	Polyphen	SIFT	mutationtaster	COSMIC
✔	✔	chr16	10525156	ATF7IP2	NM_001256160	G	T	nonsynonymous	c.G679T	p.V227L	NA	0.033	0.27	0.971	NA
✔	✔	chr8	51465694	SNTG1	NM_001287813	G	T	nonsynonymous	c.G765T	p.W255C	NA	1	0	1	NA
✔	✔	chr3	38648271	SCN5A	NM_001160160	C	A	nonsynonymous	c.G1029T	p.K343N	NA	1	0	0.99	NA
✔	✔	chr5	666171	TPPP	NM_007030	G	A	nonsynonymous	c.C379T	p.R127X	NA	NA	1	1	NA
✔	✔	chr6	56883251	BEND6	NM_152731	G	T	nonsynonymous	c.G745T	p.D249Y	NA	1	0	1	NA
✔	✔	chr1	46290133	MAST2	NM_015112	T	G	nonsynonymous	c.T206G	p.L69R	MAST2	0.028	0.84	1	NA
✔	✔	chr4	114279178	ANK2	NM_001148	G	T	nonsynonymous	c.G9404T	p.G3135V	NA	0.998	0	1	NA
✔	✔	chr20	60585112	TAF4	NM_003185	G	A	nonsynonymous	c.C1751T	p.S584L	NA	0.495	0.29	0.882	NA
✔	✔	chr10	84718709	NRG3	NM_001165972	C	T	nonsynonymous	c.C1159T	p.Q387X	NA	NA	0.04	1	NA
✔	✔	chr8	59851979	TOX	NM_014729	T	C	nonsynonymous	c.A293G	p.H98R	NA	0.851	NA	0.996	NA
✔	✔	chr3	136076689	STAG1	NM_005862	C	T	nonsynonymous	c.G2938A	p.D980N	NA	1	0.34	1	NA
✔	✔	chr7	32909384	KBTBD2	NM_015483	T	C	nonsynonymous	c.A1445G	p.N482S	NA	0.003	0.76	1	NA
✔	✔	chr1	228504485	OBSCN	NM_001271223	C	T	nonsynonymous	c.C16232T	p.T5411M	NA	0.995	0.13	1	NA
✔	✔	chr2	215880335	ABCA12	NM_015657	G	A	nonsynonymous	c.C881T	p.P294L	NA	0	0.24	1	NA
✔	✔	chr11	117299235	DSCAML1	NM_020693	T	G	nonsynonymous	c.A6151C	p.T2051P	NA	0	0.06	1	NA
✔	✔	**chr12**	**58144548**	**CDK4**	**NM_000075**	**C**	**A**	**nonsynonymous**	**c.G523T**	**p.V175F**	**CDK4**	**0.964**	**0**	**1**	**NA**
✔	✔	chr4	186545169	SORBS2	NM_021069	C	T	nonsynonymous	c.G1402A	p.E468K	NA	1	0.05	1	NA
✔	✔	**chr16**	**15917267**	**MYH11**	**NM_002474**	**G**	**A**	**nonsynonymous**	**c.C347T**	**p.T116M**	**MYH11**	**1**	**0**	**1**	**COSM290683,COSM290682**
✔	✔	chr8	77767363	ZFHX4	NM_024721	C	T	nonsynonymous	c.C8206T	p.P2736S	NA	0.037	0.89	0.999	NA
✔	✔	chr12	9254240	A2M	NM_000014	C	T	nonsynonymous	c.G1297A	p.G433S	NA	0.017	0.8	1	NA
✔	✔	chr1	103471858	COL11A1	NM_080630	A	G	nonsynonymous	c.T1349C	p.V450A	NA	0.042	0.96	0.998	NA
✔	✔	chr8	106814316	ZFPM2	NM_012082	A	G	nonsynonymous	c.A2006G	p.D669G	NA	0.877	0.24	1	NA
✔	✔	chr1	3645901	TP73	NM_001204187	G	A	nonsynonymous	c.G1085A	p.R362Q	NA	1	0.01	1	NA
✔	✔	chr19	8613192	MYO1F	NM_012335	G	T	nonsynonymous	c.C1111A	p.R371S	NA	0.036	0.63	0.913	NA
✔	✔	chr1	217975125	SPATA17	NM_138796	A	C	nonsynonymous	c.A938C	p.K313T	NA	0.221	0.15	0.995	NA
✔	✔	chr5	16694700	MYO10	NM_012334	G	A	nonsynonymous	c.C3580T	p.R1194C	NA	1	0	1	NA
✔	✔	chr7	48563978	ABCA13	NM_152701	G	A	nonsynonymous	c.G14186A	p.R4729H	NA	0.003	0.23	1	NA
✔	✔	chr11	117279728	CEP164	NM_014956	G	T	nonsynonymous	c.G3732T	p.W1244C	NA	1	0.18	0.996	NA
✔	✔	chr11	44069747	ACCSL	NM_001031854	C	T	nonsynonymous	c.C161T	p.S54L	NA	0	0.36	1	NA
✔	✔	chr12	112701998	HECTD4	NM_001109662	G	A	nonsynonymous	c.C2206T	p.R736C	NA	0.007	0	1	NA
✔	✔	chr11	66392695	RBM14	NM_006328	G	A	nonsynonymous	c.G1348A	p.A450T	NA	0.999	0.01	1	NA
✔	✔	chr6	50810945	TFAP2B	NM_003221	C	T	nonsynonymous	c.C1223T	p.P408L	NA	1	0.03	1	NA
✔	✔	chr15	28520057	HERC2	NM_004667	G	A	nonsynonymous	c.C637T	p.R213X	NA	NA	1	1	NA
✔	✔	chr20	5283324	PROKR2	NM_144773	G	T	nonsynonymous	c.C517A	p.L173M	NA	0.989	0.02	0.999	NA
✔	✔	**chr5**	**112164646**	**APC**	**NM_001127510**	**G**	**T**	**nonsynonymous**	**c.G1720T**	**p.E574X**	**APC**	**NA**	**0.09**	**1**	**NA**
✔	✔	chr10	27687804	PTCHD3	NM_001034842	C	T	nonsynonymous	c.G1723A	p.D575N	NA	0.242	0.41	1	NA
✔	✔	chr11	119053871	NLRX1	NM_001282358	G	A	nonsynonymous	c.G2651A	p.R884Q	NA	0.07	0.34	1	NA
✔	✔	chr11	48373961	UNKNOWN		A	T	nonsynonymous			NA	NA	NA	NA	NA
✔	✔	chr10	52603881	A1CF	NM_001198820	T	G	nonsynonymous	c.A125C	p.E42A	NA	0.998	0.04	1	NA
✔	✔	chr8	59059734	FAM110B	NM_147189	C	A	nonsynonymous	c.C945A	p.S315R	NA	0.999	NA	1	NA
✔	✔	**chr1**	**115258747**	**NRAS**	**NM_002524**	**C**	**A**	**nonsynonymous**	**c.G35T**	**p.G12V**	**NRAS**	**0.613**	**0.01**	**1**	**COSM566**
✔	✔	chr19	52130463	SIGLEC5	NM_003830	G	C	nonsynonymous	c.C1321G	p.L441V	NA	0.799	0.85	1	NA
✔	✔	chr15	41810233	RPAP1	NM_015540	A	T	nonsynonymous	c.T3943A	p.F1315I	NA	0.995	0	1	NA
✔	✔	chr22	28193785	MN1	NM_002430	G	T	nonsynonymous	c.C2747A	p.T916N	MN1	0.13	0.16	0.768	NA
✔	✔	chr2	179426759	TTN	NM_001267550	C	A	nonsynonymous	c.G84100T	p.E28034X	NA	NA	0	1	NA
✔	✔	chr11	32635763	CCDC73	NM_001008391	G	A	nonsynonymous	c.C2101T	p.P701S	NA	0.003	0.49	0.997	NA
✔	✔	chr9	131483555	ZDHHC12	NM_032799	G	A	nonsynonymous	c.C709T	p.R237C	NA	0.998	0.13	1	NA
✔	✔	chr17	17129519	FLCN	NM_144606	G	A	nonsynonymous	c.C367T	p.Q123X	FLCN	NA	0.29	1	NA
✔	✔	chr11	65978634	PACS1	NM_018026	C	A	nonsynonymous	c.C564A	p.N188K	NA	0.999	0	1	NA
✔	✔	chr21	30934019	GRIK1	NM_175611	G	A	nonsynonymous	c.C2237T	p.T746M	NA	1	0.04	1	COSM1253702,COSM1253701
✔	✔	chr6	163956109	QKI	NM_206853	C	G	nonsynonymous	c.C498G	p.I166M	QKI	0.112	0.01	1	NA
✔	✔	chr2	37105068	STRN	NM_003162	G	A	nonsynonymous	c.C1289T	p.T430M	NA	1	0.02	1	NA
✔	✔	chr20	35444571	SOGA1	NM_080627	T	C	nonsynonymous	c.A1274G	p.D425G	NA	1	0.03	1	NA
✔	✔	chrX	133700173	PLAC1	NM_021796	T	G	nonsynonymous	c.A540C	p.Q180H	NA	0.02	0	1	NA
✔	✔	chr2	226447238	NYAP2	NM_020864	G	A	nonsynonymous	c.G1105A	p.V369M	NA	0.997	0.08	1	NA
✔	✔	chr7	48391820	ABCA13	NM_152701	C	T	nonsynonymous	c.C10424T	p.S3475L	NA	0.611	0.02	1	NA
✔	✔	chr12	75601447	KCNC2	NM_001260499	C	T	nonsynonymous	c.G317A	p.R106Q	NA	1	0	1	NA
✔	✔	chr11	120811150	GRIK4	NM_001282473	T	A	nonsynonymous	c.T1571A	p.I524N	NA	1	0	1	NA
✔	✔	chr2	167145040	SCN9A	NM_002977	T	G	nonsynonymous	c.A1221C	p.E407D	NA	1	0.03	0.998	NA
✔	✔	chr1	205631135	SLC45A3	NM_033102	C	T	nonsynonymous	c.G1078A	p.A360T	SLC45A3	0.02	0.16	0.919	NA
✔	✔	chr1	152275373	FLG	NM_002016	C	T	nonsynonymous	c.G11989A	p.G3997R	NA	0.981	0.53	1	NA
✔	✔	chrX	17819893	RAI2	NM_001172743	G	T	nonsynonymous	c.C238A	p.P80T	NA	1	0.01	1	NA
✔	✔	chr7	94293611	PEG10	NM_001172438	G	A	nonsynonymous	c.G971A	p.R324H	NA	0.998	0.21	1	NA
✔	✔	chr6	7374272	CAGE1	NM_001170692	C	A	nonsynonymous	c.G780T	p.E260D	NA	0.004	1	1	NA
✔	✔	**chr5**	**112175328**	**APC**	**NM_001127510**	**C**	**A**	**nonsynonymous**	**c.C4037A**	**p.S1346X**	**APC**	**NA**	**NA**	**1**	**COSM19084**
✔	✔	chr10	49929315	WDFY4	NM_020945	G	A	nonsynonymous	c.G359A	p.R120Q	NA	1	0.06	0.783	NA
✔	✔	chr15	43574260	TGM7	NM_052955	G	A	nonsynonymous	c.C1133T	p.S378F	NA	1	0.01	1	NA
✔	✔	**chr17**	**7577548**	**TP53**	**NM_001276761**	**C**	**T**	**nonsynonymous**	**c.G616A**	**p.G206S**	**TP53**	**1**	**0**	**1**	**COSM1640833,COSM121036**
✔		chr6	90422940	MDN1	NM_014611	C	T	nonsynonymous	c.G7145A	p.S2382N	MDN1	0.239	0.17	0.883	NA
✔		chrX	32536138	DMD	NM_000109	T	G	nonsynonymous	c.A2255C	p.K752T	DMD	0.002	0.08	1	NA
✔		chr15	56122103	NEDD4	NM_001284340	C	G	nonsynonymous	c.G3901C	p.V1301L	NA	1	0	1	NA
✔		chr22	40417962	FAM83F	NM_138435	T	A	nonsynonymous	c.T1448A	p.I483N	NA	0.003	0.01	1	NA
✔		chr21	43691270	ABCG1	NM_207629	C	G	nonsynonymous	c.C368G	p.T123R	NA	1	NA	1	NA
✔		chr11	101771267	ANGPTL5	NM_178127	C	A	nonsynonymous	c.G555T	p.M185I	NA	1	0.12	1	NA
✔		chr10	15821128	FAM188A	NM_024948	C	G	nonsynonymous	c.G1201C	p.E401Q	NA	0.993	0.54	1	NA
	✔	chr19	9071750	MUC16	NM_024690	T	G	nonsynonymous	c.A15696C	p.K5232N	NA	0.349	0	NA	NA
	✔	chr1	225156539	DNAH14	NM_001145154	T	C	nonsynonymous	c.T730C	p.Y244H	NA	0.019	0.58	1	NA
	✔	chr6	153345483	RGS17	NM_012419	A	C	nonsynonymous	c.T358G	p.L120V	NA	0.64	0.01	1	NA
	✔	chr5	178140358	ZNF354A	NM_005649	A	T	nonsynonymous	c.T521A	p.L174H	NA	0.005	0	1	NA
	✔	chr1	211192300	KCNH1	NM_002238	A	C	nonsynonymous	c.T857G	p.L286R	NA	1	0.26	1	NA
	✔	chr5	106762962	EFNA5	NM_001962	G	T	nonsynonymous	c.C374A	p.S125Y	NA	1	0.08	1	NA
	✔	**chr1**	**16258997**	**SPEN**	**NM_015001**	**G**	**C**	**nonsynonymous**	**c.G6262C**	**p.D2088H**	**SPEN**	**0.996**	**0.02**	**0.988**	**NA**
	✔	chr21	19638334	CHODL	NM_001204178	A	C	nonsynonymous	c.A575C	p.K192T	NA	0.001	NA	0.538	NA
	✔	chr2	21228452	APOB	NM_000384	A	C	nonsynonymous	c.T11288G	p.L3763R	NA	0.999	0	1	NA
	✔	chr22	26898017	TFIP11	NM_012143	T	A	nonsynonymous	c.A650T	p.E217V	NA	0.914	0.08	1	NA
	✔	chr2	11716651	GREB1	NM_148903	G	C	nonsynonymous	c.G627C	p.W209C	NA	1	0	1	NA
	✔	chrX	154157378	F8	NM_000132	C	G	nonsynonymous	c.G4687C	p.V1563L	F8	0	0.89	1	NA
	✔	chr22	46930524	CELSR1	NM_014246	C	T	nonsynonymous	c.G2544A	p.M848I	NA	0.016	0.17	0.997	NA
	✔	**chr5**	**127866348**	**FBN2**	**NM_001999**	**G**	**T**	**nonsynonymous**	**c.C376A**	**p.R126S**	**FBN2**	**0.997**	**0.24**	**1**	**NA**
	✔	chr12	25260947	LRMP	NM_001204127	T	A	nonsynonymous	c.T1453A	p.L485M	NA	0.997	0.05	0.998	NA
	✔	chr14	20711786	OR11H4	NM_001004479	T	C	nonsynonymous	c.T836C	p.L279S	NA	0.051	0.27	1	NA
	✔	chr12	102811648	IGF1	NM_001111285	C	A	nonsynonymous	c.G536T	p.R179M	NA	0.999	0	1	NA
	✔	chr2	128624549	AMMECR1L	NM_031445	A	G	nonsynonymous	c.T746C	p.I249T	NA	1	0	1	NA
	✔	chr5	140573225	PCDHB10	NM_018930	T	A	nonsynonymous	c.T1100A	p.V367D	NA	0.999	0	1	NA
	✔	chr6	18197451	KDM1B	NM_153042	A	C	nonsynonymous	c.A737C	p.K246T	NA	0.651	0.11	1	NA
	✔	**chr16**	**31193877**	**FUS**	**NM_001170937**	**C**	**T**	**nonsynonymous**	**c.C82T**	**p.Q28X**	**FUS**	**NA**	**0.08**	**1**	**NA**
	✔	chr14	45639916	FANCM	NM_020937	G	C	nonsynonymous	c.G2127C	p.Q709H	NA	0.001	0.2	1	NA
	✔	chr7	92120681	PEX1	NM_001282678	C	T	nonsynonymous	c.G2719A	p.E907K	NA	0.65	0.19	1	NA
	✔	chr5	38943059	RICTOR	NM_001285440	T	G	nonsynonymous	c.A3959C	p.Y1320S	NA	0.437	0	1	NA
	✔	chr4	119035963	NDST3	NM_004784	A	C	nonsynonymous	c.A1072C	p.T358P	NA	0.998	0.01	1	NA
	✔	chr1	186097285	HMCN1	NM_031935	G	A	nonsynonymous	c.G12766A	p.V4256M	NA	0.999	0.01	1	NA
	✔	chr8	55539448	RP1	NM_006269	A	C	nonsynonymous	c.A3006C	p.E1002D	NA	0.807	0.11	1	NA
	✔	chrX	36329023	CXorf30	NM_001098843	C	T	nonsynonymous	c.C601T	p.R201C	NA	0.007	0.14	1	NA
	✔	chrX	114398248	LRCH2	NM_001243963	T	G	nonsynonymous	c.A1454C	p.Q485P	NA	0.89	0.46	1	NA
	✔	chr11	45671752	CHST1	NM_003654	G	T	nonsynonymous	c.C722A	p.A241D	NA	0.993	0.44	1	NA
	✔	chr15	22958342	CYFIP1	NM_014608	A	C	nonsynonymous	c.A1985C	p.E662A	NA	1	0.05	1	NA
	✔	chr4	158224914	GRIA2	NM_000826	A	G	nonsynonymous	c.A440G	p.K147R	NA	0.971	NA	1	NA
	✔	**chr9**	**90321594**	**DAPK1**	**NM_001288731**	**C**	**T**	**nonsynonymous**	**c.C3608T**	**p.T1203M**	**DAPK1**	**0.856**	**0.03**	**1**	**NA**

Supplementary Table 2.3

Primary	Metastasis	Chrom	Positions	Gene Name	NCBI Ref Seq	Ref	Var	Mutation	Case Change
✓	✓	chr3	47888401	DHX30	NM_014966	C	T	synonymous SNV	c.C1722T
✓	✓	chr17	19318460	RNF112	NM_007148	C	T	synonymous SNV	c.C1236T
✓	✓	chr14	69995041	PLEKHD1	NM_001161498	C	A	synonymous SNV	c.C1426A
✓	✓	chr9	136573457	SARDH	NM_001134707	G	A	synonymous SNV	c.C1422T
✓	✓	chr16	72991729	ZFHX3	NM_006885	C	T	synonymous SNV	c.G2316A
✓	✓	chr19	39906041	PLEKHG2	NM_022835	G	A	synonymous SNV	c.G441A
✓	✓	chr22	36691101	MYH9	NM_002473	C	A	synonymous SNV	c.G3507T
✓	✓	chr20	2736298	EBF4	NM_001110514	G	A	synonymous SNV	c.G1554A
✓	✓	chr20	42144040	L3MBTL1	NM_032107	G	A	synonymous SNV	c.G696A
✓	✓	chr11	92600167	FAT3	NM_001008781	G	A	synonymous SNV	c.G11919A
✓	✓	chr13	36744857	CCDC169-SOHLH2	NM_001198910	C	T	synonymous SNV	c.G1299A
✓	✓	chr1	215775446	KCTD3	NM_016121	G	A	synonymous SNV	c.G1041A
✓	✓	chr19	5231460	PTPRS	NM_002850	G	A	synonymous SNV	c.C2016T
✓	✓	chr20	8745967	PLCB1	NM_182734	C	T	synonymous SNV	c.C2892T
✓	✓	chrX	47433829	SYN1	NM_133499	G	A	synonymous SNV	c.C1554T
✓	✓	chr16	84014711	NECAB2	NM_019065	G	A	synonymous SNV	c.G438A
✓	✓	chrX	53114852	TSPYL2	NM_022117	C	T	synonymous SNV	c.C1278T
✓		chr1	17266463	CROCC	NM_014675	C	T	synonymous SNV	c.C1683T
✓		chr3	75714853	FRG2C	NM_001124759	G	A	synonymous SNV	c.G510A
✓		chr2	109382170	RANBP2	NM_006267	A	G	synonymous SNV	c.A5175G
✓		chr1	247200886	ZNF670	NM_033213	C	T	synonymous SNV	c.G1035A
✓		chrX	51075841	NUDT10	NM_153183	A	G	synonymous SNV	c.A24G
✓		chr17	39767744	KRT16	NM_005557	A	G	synonymous SNV	c.T624C
✓		chr2	207620124	MDH1B	NM_001039845	C	T	synonymous SNV	c.G519A
	✓	chr1	74819758	FPGT-TNNI3K	NM_001199327	C	T	synonymous SNV	c.C1464T
	✓	chr1	54675662	MRPL37	NM_016491	G	T	synonymous SNV	c.G690T
	✓	chr22	37482336	TMPRSS6	NM_001289000	C	T	synonymous SNV	c.G960A
	✓	chr17	56057986	VEZF1	NM_007146	T	G	synonymous SNV	c.A954C
	✓	chr10	70892791	VPS26A	NM_004896	C	T	synonymous SNV	c.C141T
	✓	chr1	82416003	LPHN2	NM_001297705	G	C	synonymous SNV	c.G1329C
	✓	chr14	105410001	AHNAK2	NM_138420	A	C	synonymous SNV	c.T11787G
	✓	chr16	2152949	PKD1	NM_001009944	A	G	synonymous SNV	c.T8814C
	✓	chr10	46968591	SYT15	NM_031912	G	A	synonymous SNV	c.C345T
	✓	chr5	122522784	PRDM6	NM_001136239	C	T	synonymous SNV	c.C1677T
	✓	chr1	75037305	ERICH3	NM_001002912	C	T	synonymous SNV	c.G4089A
	✓	chr2	132236963	TUBA3D	NM_080386	C	T	synonymous SNV	c.C309T

Supplementary Table 2.4

Primary	Metastasis	Chrom	Positions	Gene Name	NCBI Ref Seq	Ref	Var	Mutation	Case Change
✔	✔	chr9	125377121	OR1Q1	NM_012364	C	T	synonymous SNV	c.C105T
✔	✔	chr4	138452430	PCDH18	NM_019035	C	T	synonymous SNV	c.G813A
✔	✔	chr2	201284122	SPATS2L	NM_001282743	G	T	synonymous SNV	c.G168T
✔	✔	chr3	130649319	ATP2C1	NM_001199180	A	G	synonymous SNV	c.A168G
✔	✔	chr10	98144513	TLL2	NM_012465	G	A	synonymous SNV	c.C2025T
✔	✔	chr15	85407757	ALPK3	NM_020778	G	A	synonymous SNV	c.G5190A
✔	✔	chr5	131543488	P4HA2	NM_001142598	G	A	synonymous SNV	c.C993T
✔	✔	chr11	27077130	BBOX1	NM_003986	G	T	synonymous SNV	c.G153T
✔	✔	chrX	82763698	POU3F4	NM_000307	G	A	synonymous SNV	c.G366A
✔	✔	chr6	33740438	LEMD2	NM_001143944	A	T	synonymous SNV	c.T573A
✔	✔	chr16	27448836	IL21R	NM_021798	C	T	synonymous SNV	c.C180T
✔	✔	chr13	27998976	GTF3A	NM_002097	C	T	synonymous SNV	c.C102T
✔	✔	chr2	220396566	ASIC4	NM_182847	C	T	synonymous SNV	c.C1050T
✔	✔	chr2	220379560	ASIC4	NM_182847	C	G	synonymous SNV	c.C495G
✔	✔	chr3	3887633	LRRN1	NM_020873	C	T	synonymous SNV	c.C1308T
✔	✔	chr11	77090350	PAK1	NM_001128620	C	T	synonymous SNV	c.G375A
✔	0	chr9	122004349	BRINP1	NM_014618	G	T	synonymous SNV	c.C555A
✔	0	chr6	42075218	C6orf132	NM_001164446	T	G	synonymous SNV	c.A432C
✔	0	chr12	81004322	PTPRQ	NM_001145026	C	A	synonymous SNV	c.C4320A
✔	0	chr19	58549366	ZSCAN1	NM_182572	C	G	synonymous SNV	c.C162G
✔	0	chr7	6547862	GRID2IP	NM_001145118	G	A	synonymous SNV	c.C2298T
✔	0	chr4	37962070	PTTG2	NM_006607	C	A	synonymous SNV	c.C15A
0	✔	chr6	28093407	ZSCAN16	NM_025231	G	C	synonymous SNV	c.G186C
0	✔	chr1	156641628	NES	NM_006617	A	G	synonymous SNV	c.T2352C
0	✔	chr14	73763919	NUMB	NM_001005743	C	T	synonymous SNV	c.G309A
0	✔	chr10	135026321	KNDC1	NM_152643	C	T	synonymous SNV	c.C4338T
0	✔	chr1	153748219	SLC27A3	NM_024330	C	T	synonymous SNV	c.C387T
0	✔	chrX	148044425	AFF2	NM_001169123	T	C	synonymous SNV	c.T2841C
0	✔	chr5	140562218	PCDHB16	NM_020957	C	A	synonymous SNV	c.C84A
0	✔	chr19	1785228	ATP8B3	NM_001178002	G	A	synonymous SNV	c.C3351T
0	✔	chr15	54435225	UNC13C	NM_001080534	T	C	synonymous SNV	c.T2994C
0	✔	chr2	231223717	SP140L	NM_138402	A	G	synonymous SNV	c.A309G
0	✔	chr16	24966014	ARHGAP17	NM_001006634	T	A	synonymous SNV	c.A762T

Supplementary Table 2 - Nonsynonymous detected by population exome sequencing

These tables list all nonsynonymous somatic mutations detected in the primary and/or metastatic tumors of CRC1 (ST2.1) and CRC2 (ST2.2). The annotations for each mutation are as follows: chromosome positions, reference/variant alleles, nucleotide change and amino acid change, COSMIC mutations, Cancer Gene Census, Polyphen, SIFT and MutationTaster.

Supplementary Table 3

						normal - Deep Sequencing						primary - Deep Sequencing								Met - Exome Sequencing	
sample	**chr**	**pos**	**gene**	**ref**	**var**	**A**	**C**	**G**	**T**	**total**	**vaf**	**A**	**C**	**G**	**T**	**total**	**primary_vaf**	**Bayesian_NP**	**deepSNV pval**	**met_vaf**	**Bayesian_NP**
CO5	5	173035291	BOD1	G	A	64	39	5106	146	5355	###########	37	29	3322	102	3490	0.010601719	0.954	0.84657359	0.3	0.01
	7	98554034	TRRAP	A	G	1445168	1008	318	302	1446796	0.000219796	1466151	496	303	348	1467298	0.000206502	0.994	0.21665346	0.31	0
	4	106755675	GSTCD	A	G	1500362	671	574	295	1501902	0.000382182	1632261	868	422	352	1633903	0.000258277	0.993	1	0.33	0
sample	**chr**	**pos**	**gene**	**ref**	**var**	**A**	**C**	**G**	**T**	**total**	**vaf**	**A**	**C**	**G**	**T**	**total**	**primary_vaf**	**Bayesian_NP**	**deepSNV pval**	**met_vaf**	**Bayesian_NP**
CO8	6	153345483	RGS17	A	C	3613	36	11	37	3697	0.009737625	5247	49	32	52	5380	0.009107807	0.964	0.59657359	0.22	0.008
	5	178140358	ZNF354A	A	T	821913	246	283	278	822720	0.000337904	785945	259	260	298	786762	0.000378768	0.984	0.124077842	0.3	0
	1	211192300	KCNH1	A	C	1921380	1481	731	416	1924008	0.000769747	2023907	1193	631	325	2026056	0.000588829	0.998	0.345142699	0.34	0
	5	106762962	EGNA5	G	T	334	66	1290095	548	1291043	0.000424463	519	128	1838594	713	1839954	0.00038751	0.993	0.84657359	0.27	0
	22	26898017	TFIP11	T	A	592	929	1192	1448993	1451706	0.000407796	508	884	765	1232711	1234868	0.00041138	0.991	1	0.45	0.024
	2	11716651	GREB1	G	C	699	343	1636714	1299	1639055	0.000209267	560	303	1218420	965	1220248	0.00024831	0.999	0.000689522	0.25	0
	22	46930524	CELSR1	C	T	428	2007773	458	739	2009398	0.000367772	885	1412564	455	627	1414531	0.000443256	0.997	0.341147711	0.33	0
	5	127866348	FBN2	G	T	219	88	880028	295	880630	0.000334987	148	60	495614	215	496037	0.000433435	0.989	0.838476316	0.36	0.001
	12	25260947	LRMP	T	A	399	703	1706	1445118	1447926	0.000275567	464	899	1619	1940018	1943000	0.000238806	0.977	0.512890212	0.32	0
	14	20711786	OR11H4	T	C	141	203	232	1246055	1246631	0.000162839	126	171	137	1125174	1125608	0.000151918	0.999	1	0.38	0
	2	128624549	AMMECR1L	A	G	1767704	838	518	749	1769809	0.000292687	2565317	1216	723	1247	2568503	0.000281487	0.996	1	0.21	0
	5	140573225	PCDHB10	T	A	308	662	1010	1521724	1523704	0.000202139	319	413	696	1299987	1301415	0.000245118	0.996	0.499686185	0.4	0.001
	6	18197451	KDM1B	A	C	2844026	2683	730	529	2847968	0.000942075	2771445	1922	653	453	2774473	0.000692744	0.998	1	0.22	0.002
	16	31193877	FUS	C	T	1868	2704367	610	692	2707537	0.000255583	2042	2658991	633	688	2662354	0.000258418	1	1	0.29	0
	8	55539448	RP1	A	C	1662490	377	402	241	1663510	0.000226629	530243	80	126	75	530524	0.000150794	0.995	0.84657359	0.23	0.004
	X	114398248	LRCH2	T	G	43	71	68	371793	371975	0.000182808	24	30	46	185557	185657	0.000247769	0.994	0.84657359	0.38	0
	11	45671752	CHST1	G	T	858	1482	1437757	1213	1441310	0.000841595	358	618	589642	678	591296	0.001146634	0.985	0.135600977	0.43	0
	9	90321594	DAPK1	C	T	2035	3215837	1613	1235	3220720	0.000383455	1670	2292808	1079	947	2296504	0.000412366	0.986	1	0.3	0

Supplementary Table 3 - Targeted deep-sequencing read counts of metastatic-specific mutations

This table lists the reference and variant read counts for each nucleotide position in the primary tumor samples and matched normal tissue. The variant allele frequency was calculated using the nucleotide variant that was detected in the bulk exome sequencing data.

Supplementary Table 4.1

Sample	Coverage Depth	Cov Breadth	Sample Type
NC-pop	216	0.9845	population
NL-pop	127	0.9819	population
PD-pop	179	0.9833	population
PA-pop	162	0.9826	population
MD-pop	224	0.9845	population
MA-pop	213	0.9844	population
PD-13	108	0.9327	single cell
PD-16	134	0.9574	single cell
PD-19	97	0.9714	single cell
PD-26	108	0.9525	single cell
PD-27	110	0.9326	single cell
PD-28	103	0.937	single cell
PD-30	152	0.9634	single cell
PD-31	126	0.972	single cell
PD-32	132	0.9456	single cell
PD-37	84	0.8984	single cell
PD-38	131	0.9158	single cell
PD-39	111	0.9504	single cell
PD-40	266	0.9621	single cell
PD-41	96	0.9398	single cell
PD-42	1	0.4786	single cell
PD-44	97	0.9703	single cell
PA-50	243	0.9733	single cell
PA-51	168	0.9592	single cell
PA-52	116	0.9502	single cell
PA-53	133	0.9541	single cell
PA-54	127	0.953	single cell
PA-56	165	0.9474	single cell
PA-57	110	0.9164	single cell
PA-61	159	0.9599	single cell
PA-62	108	0.9547	single cell
PA-63	162	0.9492	single cell
PA-64	113	0.9552	single cell
PA-65	163	0.9641	single cell
PA-66	120	0.9501	single cell
PA-70	147	0.9458	single cell
PA-74	127	0.9519	single cell
PA-75	117	0.9549	single cell
PA-76	139	0.9565	single cell
PA-77	148	0.9516	single cell
PA-78	119	0.9569	single cell
PA-80	107	0.9495	single cell
PA-81	156	0.9365	single cell
PA-83	143	0.9581	single cell
PA-85	129	0.9468	single cell
PA-86	127	0.7879	single cell
PA-87	159	0.9622	single cell
PA-88	132	0.9557	single cell
PA-89	135	0.9512	single cell
PA-90	155	0.9575	single cell
PA-91	120	0.9613	single cell
MD-1	154	0.891	single cell
MD-2	168	0.9631	single cell
MD-3	135	0.9545	single cell
MD-4	147	0.9531	single cell
MD-5	148	0.9531	single cell
MD-6	120	0.9587	single cell
MD-7	155	0.9665	single cell
MD-8	133	0.9343	single cell
MD-9	147	0.961	single cell
MD-10	174	0.9539	single cell
MD-13	154	0.9548	single cell
MD-14	168	0.9515	single cell
MD-15	131	0.9599	single cell
MD-16	165	0.9639	single cell
MD-19	150	0.9562	single cell
MD-20	150	0.9561	single cell
MD-21	150	0.9575	single cell
MD-22	140	0.9578	single cell
MD-23	141	0.9187	single cell
MD-25	161	0.9665	single cell
MA-49	152	0.9632	single cell
MA-50	160	0.9701	single cell
MA-51	138	0.9653	single cell
MA-52	126	0.952	single cell
MA-53	152	0.9639	single cell
MA-54	169	0.9644	single cell
MA-55	160	0.9641	single cell
MA-56	162	0.9674	single cell
MA-57	152	0.9649	single cell
MA-58	146	0.9631	single cell
MA-59	165	0.9695	single cell
MA-61	156	0.9615	single cell
MA-62	164	0.9629	single cell
MA-63	168	0.9616	single cell
MA-64	144	0.9631	single cell
MA-65	161	0.9508	single cell
MA-66	182	0.9653	single cell
MA-67	144	0.9626	single cell
MA-68	159	0.9664	single cell
MA-69	160	0.9681	single cell
MA-73	149	0.9663	single cell
MA-74	115	0.9629	single cell
MA-75	133	0.9651	single cell
MA-76	154	0.9628	single cell
MA-77	134	0.9596	single cell
PDD-1	140	0.8984	single cell
PDD-2	136	0.9433	single cell

PDD-3	140	0.939	single cell
PDD-4	119	0.9404	single cell
PDD-5	154	0.956	single cell
PDD-6	166	0.9331	single cell
PDD-7	144	0.9529	single cell
PDD-8	148	0.9488	single cell
PDD-9	146	0.9488	single cell
PDD-10	141	0.9522	single cell
PDD-11	154	0.9533	single cell
PDD-12	152	0.9442	single cell
PDD-13	165	0.9465	single cell
PDD-14	147	0.941	single cell
PDD-15	171	0.9556	single cell
PDD-16	87	0.9343	single cell
PDD-17	144	0.9448	single cell
PDD-18	122	0.9227	single cell
PDD-19	145	0.9435	single cell
PDD-20	128	0.9396	single cell
PDD-21	3	0.7138	single cell
PDD-22	153	0.9463	single cell
PDD-23	289	0.9703	single cell
PDD-24	109	0.929	single cell
PDD-25	162	0.9482	single cell
PDD-26	163	0.9474	single cell
PDD-27	289	0.966	single cell
PDD-28	124	0.8906	single cell
PDD-29	2	0.6277	single cell
PDD-30	142	0.9437	single cell
PDD-31	136	0.9485	single cell
PDD-32	154	0.9561	single cell
PDD-33	140	0.9521	single cell
PDD-34	173	0.9568	single cell
PDD-35	170	0.9516	single cell
PDD-36	155	0.945	single cell
PDD-37	130	0.9379	single cell
PDD-38	121	0.8928	single cell
PDD-39	171	0.9535	single cell
PDD-40	122	0.9455	single cell
PDD-41	125	0.9452	single cell
PDD-42	111	0.9434	single cell
PDD-43	147	0.9492	single cell
PDD-44	135	0.9421	single cell
PDD-45	151	0.9457	single cell
PDD-46	143	0.9528	single cell
PDD-47	127	0.9263	single cell
PDD-48	125	0.9338	single cell
PDD-49	143	0.9504	single cell
PDD-50	165	0.9532	single cell
PDD-51	145	0.9541	single cell
PDD-52	139	0.9388	single cell
PDD-53	146	0.9505	single cell
PDD-54	117	0.9409	single cell
PDD-55	129	0.9456	single cell
PDD-56	120	0.9448	single cell
PDD-57	141	0.9356	single cell
PDD-58	118	0.9091	single cell
PDD-59	123	0.9256	single cell
PDD-60	133	0.9339	single cell
PDD-61	138	0.948	single cell
PDD-62	146	0.9271	single cell
PDD-63	171	0.9431	single cell
PDD-64	60	0.9258	single cell
PDD-65	155	0.9517	single cell
PDD-66	143	0.9517	single cell
PDD-67	169	0.9497	single cell
PDD-68	183	0.9517	single cell
PDD-69	177	0.9514	single cell
PDD-70	156	0.9328	single cell
PDD-71	149	0.9511	single cell
PDD-72	149	0.9448	single cell
PDD-73	170	0.9585	single cell
PDD-74	139	0.9352	single cell
PDD-75	76	0.925	single cell
PDD-76	145	0.9543	single cell
PDD-77	181	0.9494	single cell
PDD-78	133	0.9254	single cell
PDD-79	158	0.9557	single cell
PDD-80	76	0.6989	single cell
PDD-81	72	0.7596	single cell
PDD-82	122	0.9169	single cell
PDD-83	121	0.9357	single cell
PDD-84	164	0.9479	single cell
PDD-85	178	0.9591	single cell
PDD-86	125	0.9439	single cell
PDD-87	107	0.9439	single cell
PDD-88	110	0.9434	single cell
PDD-89	93	0.9313	single cell
PDD-90	87	0.9395	single cell
PDD-91	112	0.9447	single cell
PDD-92	96	0.932	single cell
PDD-93	84	0.9216	single cell
PDD-94	109	0.9184	single cell
PDD-95	63	0.6785	single cell
PDD-96	2	0.6465	single cell

Supplementary Table 4.2

Sample	Coverage Depth	Cov Breadth	Sample Type
NC-pop	127	0.9808	population
NL-pop	103	0.9803	population
PD-pop	109	0.9812	population
PA-pop	105	0.981	population
MD-pop	27	0.8479	population
MA-pop	132	0.9803	population
PD-1	671	0.9801	single cell
PD-5	115	0.8786	single cell
PD-7	103	0.8334	single cell
PD-8	104	0.8734	single cell
PD-9	130	0.8708	single cell
PD-14	115	0.882	single cell
PD-16	191	0.9286	single cell
PD-17	123	0.8624	single cell
PD-18	175	0.912	single cell
PD-20	103	0.8476	single cell
PA-26	133	0.9193	single cell
PA-27	123	0.924	single cell
PA-28	173	0.9299	single cell
PA-29	114	0.9147	single cell
PA-31	1	0.441	single cell
PA-32	118	0.9223	single cell
PA-38	325	0.9688	single cell
PA-39	124	0.9311	single cell
PA-40	148	0.9304	single cell
PA-41	145	0.9251	single cell
PA-42	131	0.9162	single cell
PA-43	149	0.9223	single cell
PA-46	125	0.9118	single cell
PA-47	144	0.9457	single cell
PA-48	159	0.931	single cell
PA-49	158	0.9205	single cell
PA-51	128	0.9412	single cell
PA-52	160	0.9402	single cell
PA-53	157	0.9359	single cell
PA-54	145	0.9266	single cell
PA-55	124	0.9408	single cell
PA-57	179	0.936	single cell
PA-58	142	0.9342	single cell
PA-59	127	0.9153	single cell
PA-61	123	0.9214	single cell
PA-62	114	0.9242	single cell
PA-63	166	0.9403	single cell
PA-64	147	0.9384	single cell
PA-65	167	0.9397	single cell
PA-68	133	0.9376	single cell
PA-70	208	0.9498	single cell
PA-71	145	0.946	single cell
PA-73	119	0.9227	single cell
PA-74	166	0.9435	single cell
PA-77	151	0.939	single cell
MD-3	75	0.8073	single cell
MD-4	66	0.8225	single cell
MD-5	74	0.8005	single cell
MD-7	61	0.8173	single cell
MD-8	67	0.8226	single cell
MD-9	76	0.8369	single cell
MD-10	135	0.9078	single cell
MD-11	135	0.8899	single cell
MD-12	149	0.9232	single cell
MD-13	151	0.9075	single cell
MA-25	145	0.9058	single cell
MA-26	144	0.8986	single cell
MA-27	125	0.9264	single cell
MA-28	119	0.9432	single cell
MA-29	124	0.929	single cell
MA-30	142	0.9448	single cell
MA-31	314	0.9645	single cell
MA-32	142	0.9459	single cell
MA-33	115	0.9346	single cell
MA-34	164	0.9461	single cell
MA-35	155	0.9374	single cell
MA-36	165	0.9487	single cell
MA-37	130	0.9203	single cell
MA-38	133	0.8998	single cell
MA-39	171	0.9334	single cell
MA-40	148	0.9364	single cell
MA-41	132	0.9154	single cell
MA-42	180	0.9281	single cell
MA-43	129	0.9222	single cell
MA-44	150	0.9428	single cell
MA-45	148	0.9313	single cell
MA-46	151	0.9219	single cell
MA-47	162	0.9283	single cell
MA-48	154	0.9463	single cell
MA-85	118	0.9252	single cell
MA-86	143	0.9418	single cell
MA-87	147	0.9322	single cell
MA-88	139	0.928	single cell
MA-89	114	0.9448	single cell
MA-90	148	0.9464	single cell
MA-91	139	0.9473	single cell
MA-92	124	0.9124	single cell
MA-93	112	0.9138	single cell
MA-94	122	0.9208	single cell
MA-95	121	0.9189	single cell
PDD-1	151	0.917	single cell
PDD-2	127	0.9418	single cell

PDD-3	144	0.9524	single cell
PDD-4	183	0.9564	single cell
PDD-5	188	0.9571	single cell
PDD-6	160	0.9537	single cell
PDD-7	148	0.9572	single cell
PDD-8	133	0.9531	single cell
PDD-9	162	0.954	single cell
PDD-10	113	0.9349	single cell
PDD-11	119	0.9384	single cell
PDD-12	138	0.9486	single cell
PDD-13	10	0.8517	single cell
PDD-14	139	0.9482	single cell
PDD-15	163	0.8968	single cell
PDD-16	88	0.9274	single cell
PDD-17	145	0.9472	single cell
PDD-18	129	0.9464	single cell
PDD-19	167	0.951	single cell
PDD-20	164	0.9555	single cell
PDD-21	2	0.4965	single cell
PDD-22	160	0.9533	single cell
PDD-23	350	0.9698	single cell
PDD-24	136	0.9463	single cell
PDD-25	115	0.9545	single cell
PDD-26	147	0.9482	single cell
PDD-27	230	0.9696	single cell
PDD-28	110	0.9292	single cell
PDD-29	0	0.266	single cell
PDD-30	121	0.9397	single cell
PDD-31	89	0.833	single cell
PDD-32	162	0.9505	single cell
PDD-33	158	0.9371	single cell
PDD-34	160	0.9542	single cell
PDD-35	160	0.9558	single cell
PDD-36	135	0.9484	single cell
PDD-37	145	0.9548	single cell
PDD-38	128	0.9469	single cell
PDD-39	165	0.9566	single cell
PDD-40	154	0.9483	single cell
PDD-41	124	0.9514	single cell
PDD-42	134	0.9516	single cell
PDD-43	115	0.9446	single cell
PDD-44	155	0.955	single cell
PDD-45	197	0.9565	single cell
PDD-46	151	0.9518	single cell
PDD-47	127	0.9432	single cell
PDD-48	172	0.9533	single cell
PDD-49	125	0.936	single cell
PDD-50	166	0.9516	single cell
PDD-51	151	0.933	single cell
PDD-52	167	0.9401	single cell
PDD-53	115	0.9413	single cell
PDD-54	86	0.9211	single cell
PDD-55	112	0.9384	single cell
PDD-56	112	0.914	single cell
PDD-57	115	0.9415	single cell
PDD-58	158	0.9518	single cell
PDD-59	124	0.9378	single cell
PDD-60	15	0.88	single cell
PDD-61	133	0.944	single cell
PDD-62	143	0.9429	single cell
PDD-63	143	0.9276	single cell
PDD-64	118	0.9386	single cell
PDD-65	165	0.9451	single cell
PDD-66	121	0.7515	single cell
PDD-67	135	0.8572	single cell
PDD-68	166	0.9536	single cell
PDD-69	135	0.9489	single cell
PDD-70	115	0.9211	single cell
PDD-71	129	0.9493	single cell
PDD-72	137	0.931	single cell
PDD-73	118	0.9426	single cell
PDD-74	145	0.9484	single cell
PDD-75	147	0.9131	single cell
PDD-76	132	0.9339	single cell
PDD-77	116	0.9453	single cell
PDD-78	152	0.9408	single cell
PDD-79	163	0.9454	single cell
PDD-80	151	0.9512	single cell
PDD-81	146	0.9455	single cell
PDD-82	166	0.9458	single cell
PDD-83	129	0.9382	single cell
PDD-84	109	0.9146	single cell
PDD-85	129	0.9198	single cell
PDD-86	145	0.9334	single cell
PDD-87	97	0.9233	single cell
PDD-88	119	0.9308	single cell
PDD-89	82	0.9125	single cell
PDD-90	102	0.9138	single cell
PDD-91	108	0.9374	single cell
PDD-92	118	0.9329	single cell
PDD-93	89	0.9183	single cell
PDD-94	76	0.9165	single cell
PDD-95	86	0.9298	single cell
PDD-96	134	0.8979	single cell

Supplementary Table 4 – Single Cell Mutational Data Coverage Metrics
Coverage depth and breadth metrics for the single cell and bulk exome samples sequenced from patient CRC1 (ST4.1) and patient CRC2 (ST4.2). Cells highlighted in red were excluded from analysis by QC due to insufficient coverage.

Supplementary Table 5.1

chromosome	position	genes	primary exome allele frequency	primary single cell frequency
chr5	112175303	APC	0.56	0.93
chr10	114911615	TCF7L2	0.30	0.86
chr11	92600167	FAT3	0.31	0.64
chr11	111249887	POU2AF1	0.20	0.64
chr12	25398285	KRAS	0.44	0.64
chr19	30313229	CCNE1	0.30	0.79
chr22	39629508	MYH9	0.22	0.29

chromosome	position	genes	metastasis exome allele frequency	metastasis single cell frequency
chr5	112175303	APC	0.94	1.00
chr10	114911615	TCF7L2	0.33	0.80
chr11	92600167	FAT3	0.42	0.68
chr11	111249887	POU2AF1	0.42	0.72
chr12	25398285	KRAS	0.68	0.80
chr19	30313229	CCNE1	0.25	0.80
chr22	39629508	MYH9	0.34	0.64
chrX	48650491	GATA1	0.28	0.64
chr7	98554034	TRRAP	0.31	0.84
chr18	22807094	ZNF521	0.43	0.96

Supplementary Table 5.2

chromosomes	positions	genes	primary exome allele frequency	primary single cell frequency
chr1	115258747	NRAS	0.96	0.85
chr2	37105068	STRN	0.33	0.79
chr5	112164646	APC	0.58	0.56
chr5	112175328	APC	0.34	0.56
chr8	59851979	TOX	0.4	0.68
chr12	58144548	CDK4	0.68	0.76
chr16	15917267	MYH11	0.54	0.71
chr16	27448836	IR21R	0.58	0.71
chr17	7577548	TP53	0.88	0.82
chr22	28193785	MN1	0.48	0.82

chromosomes	positions	genes	metastasis exome allele frequency	metastasis single cell frequency
chr1	16258997	SPEN	0.14	0.26
chr1	115258747	NRAS	0.89	0.95
chr2	37105068	STRN	0.69	0.36
chr5	112164646	APC	0.54	0.63
chr5	112175328	APC	0.46	0.38
chr8	59851979	TOX	0.80	0.33
chr12	58144548	CDK4	0.69	0.66
chr16	15917267	MYH11	0.57	0.37
chr16	27448836	IR21R	0.69	0.68
chr16	31193877	FUS	0.57	0.29
chr17	7577548	TP53	0.80	1.00
chr22	28193785	MN1	0.43	0.25
chrX	154157378	F8	0.17	0.30

Supplementary Table 5 – Mutational concordance of bulk exome and single cell mutation frequencies

For the mutations that are covered with targeted probes in both the population exome platform and single cell T1000 platform in patients CO5 (ST5.1) and CO8 (ST5.2) we calculated and compared the mutation frequencies in this table.