

Supplementary Predictive genetic biomarkers for the development of peritoneal metastases in colorectal cancer

Supplementary 1

Figure A. Overview of genes included in the TruSight Oncology 500 panel. [Illumina, Inc. (n.d.)].

DNA content										
ABL1	BRD4	CUX1	FAM175A	GATA6	IGF1	MAP3K13	NOTCH4	POLE	RPTOR	TAF1
ABL2	BRIP1	CXCR4	FAM46C	GEN1	IGF1R	MAP3K14	NPM1	PPARG	RUNX1	TBX3
ACVR1	BTG1	CYLD	FANCA	GID4	IGF2	MAP3K4	NRAS	PPM1D	RUNX1T1	TCEB1
ACVR1B	BTB	DAXX	FANCC	GLI1	IKBKE	MAPK1	NRG1	PPP2R1A	RYBP	TCF3
AKT1	C11orf30	DCUN1D1	FANCD2	GNA11	IKZF1	MAPK3	NSD1	PPP2R2A	SDHA	TCF7L2
AKT2	CALR	DDR2	FANCE	GNA13	IL10	MAX	NTRK1	PPP6C	SDHA2	TERC
AKT3	CARD11	DDX41	FANCF	GNAQ	IL7R	MCL1	NTRK2	PRDM1	SDHB	TERT
ALK	CASP8	DHX15	FANCG	GNAS	INHBA	MDC1	NTRK3	PREX2	SDHC	TET1
ALOX12B	CBFB	DICER1	FANCI	GPR124	INHBA	MDM2	NUP93	PRKAR1A	SDHD	TET2
ANKRD11	CBL	DIS3	FANCL	GPS2	INPP4A	MDM4	NUTM1	PRKCI	SETBP1	TFE3
ANKRD26	CCND1	DNAJB1	FAS	GREM1	INPP4B	MED12	PAK1	PRKDC	SETD2	TFRC
APC	CCND2	DNMT1	FAT1	GRIN2A	INSR	MEF2B	PAK3	PRSS8	SF3B1	TGFBF1
AR	CCND3	DNMT3A	FBXW7	GRM3	IRF2	MEN1	PAK7	PTCH1	SH2B3	TGFBF2
ARAF	CCNE1	DNMT3B	FGF1	GSK3B	IRF4	MET	PALB2	PTEN	SH2D1A	TMEM127
ARFRP1	CD274	DOT1L	FGF10	H3F3A	IRS1	MGA	PARK2	PTPN11	SHQ1	TMPRSS2
ARID1A	CD276	E2F3	FGF14	H3F3B	IRS2	MITF	PARP1	PTPRD	SLIT2	TNFAIP3
ARID1B	CD74	EED	FGF19	H3F3C	JAK1	MLH1	PAX3	PTPRS	SLX4	TNFRSF14
ARID2	CD79A	EGFL7	FGF2	HGF	JAK2	MLL	PAX5	PTPRT	SMAD2	TOP1
ARID5B	CD79B	EGFR	FGF23	HIST1H1C	JAK3	MLLT3	PAX7	QKI	SMAD3	TOP2A
ASXL1	CDG73	EIF1AX	FGF3	HIST1H2BD	JUN	MPL	PAX8	RAB35	SMAD4	TP53
ASXL2	CDH1	EIF4A2	FGF4	HIST1H3A	KAT6A	MRE11A	PBRM1	RAC1	SMARCA4	TP63
ATM	CDK12	EIF4E	FGF5	HIST1H3B	KDM5A	MSH2	PDCD1	RAD21	SMARCB1	TRAF2
ATR	CDK4	EML4	FGF6	HIST1H3C	KDM5C	MSH3	PDCD1LG2	RAD50	SMARCD1	TRAF7
ATRX	CDK6	EP300	FGF7	HIST1H3D	KDM6A	MSH6	PDGFRA	RAD51	SMC1A	TSC1
AURKA	CDK8	EPCAM	FGF8	HIST1H3E	KDR	MST1	PDGFRB	RAD51B	SMC3	TSC2
AURKB	CDKN1A	EPHA3	FGF9	HIST1H3F	KEAP1	MST1R	PDK1	RAD51C	SMO	TSHR
AXIN1	CDKN1B	EPHA5	FGFR1	HIST1H3G	KEL	MTOR	PDPK1	RAD51D	SNCAIP	U2AF1
AXIN2	CDKN2A	EPHA7	FGFR2	HIST1H3H	KIF5B	MUTYH	PGR	RAD52	SOC3	VEGFA
AXL	CDKN2B	EPHB1	FGFR3	HIST1H3I	KIT	MYB	PHF6	RAD54L	SOX10	VHL
B2M	CDKN2C	ERBB2	FGFR4	HIST1H3J	KLF4	MYC	PHOX2B	RAF1	SOX17	VTCN1
BAP1	CEBPA	ERBB3	FH	HIST2H3A	KLHL6	MYCL1	PIK3C2B	RANBP2	SOX2	WSP3
BARD1	CENPA	ERBB4	FLCN	HIST2H3C	KMT2B	MYCN	PIK3C2G	RARA	SOX9	WT1
BBC3	CHD2	ERCC1	FLI1	HIST2H3D	KMT2C	MYD88	PIK3C3	RASA1	SPEN	XIAP
BCL10	CHD4	ERCC2	FLT1	HIST3H3	KMT2D	MYO1D	PIK3CA	RB1	SPOP	XPO1
BCL2	CHEK1	ERCC3	FLT3	HLA-A	KRAS	NAB2	PIK3CB	RBM10	SPTA1	XRCC2
BCL2L1	CHEK2	ERCC4	FLT4	HLA-B	LAMP1	NBN	PIK3CD	RECQL4	SRC	YAP1
BCL2L11	CIC	ERCC5	FOXA1	HLA-C	LATS1	NCOA3	PIK3CG	REL	SRSF2	YES1
BCL2L2	CREBBP	ERG	FOXL2	HNF1A	LATS2	NCOR1	PIK3R1	RET	STAG1	ZBTB2
BCL6	CRKL	ERRF1	FOXO1	HNRNP	LMO1	NEGR1	PIK3R2	RFWD2	STAG2	ZBTB7A
BCOR	CRLF2	ESR1	FOXP1	HOXB13	LRP1B	NF1	PIK3R3	RHEB	STAT3	ZFXH3
BCORL1	CSF1R	ETS1	FRS2	HRAS	LYN	NF2	PIM1	RHOA	STAT4	ZNF217
BCR	CSF3R	ETV1	FUBP1	HSD3B1	LZTR1	NFE2L2	PLCG2	RICTOR	STAT5A	ZNF703
BIRC3	CSNK1A1	ETV4	FYN	HSP90AA1	MAGI2	NFKBIA	PLK2	RIT1	STAT5B	ZRSR2
BLM	CTCF	ETV5	GABRA6	ICOSLG	MALT1	NKX2-1	PMAIP1	RNF43	STK11	
BMPR1A	CTLA4	ETV6	GATA1	ID3	MAP2K1	NKX3-1	PMS1	ROS1	STK40	
BRAF	CTNNA1	EWSR1	GATA2	IDH1	MAP2K2	NOTCH1	PMS2	RPS6KA4	SUFU	
BRCA1	CTNNB1	EZH2	GATA3	IDH2	MAP2K4	NOTCH2	PNRC1	RPS6KB1	SUZ12	
BRCA2	CUL3	FAM123B	GATA4	IFNGR1	MAP3K1	NOTCH3	POLD1	RPS6KB2	SYK	
RNA content*										
ABL1	BCL2	CSF1R	ESR1	EWSR1	FLI1	KIF5B	MSH2	NRG1	PAX7	RAF1
AKT3	BRAF	EGFR	ETS1	FGFR1	FLT1	KIT	MYC	NTRK1	PDGFRA	RET
ALK	BRCA1	EML4	ETV1	FGFR2	FLT3	MET	NOTCH1	NTRK2	PDGFRB	ROS1
AR	BRCA2	ERBB2	ETV4	FGFR3	JAK2	MLL	NOTCH2	NTRK3	PIK3CA	RPS6KB1
AXL	CDK4	ERG	ETV5	FGFR4	KDR	MLLT3	NOTCH3	PAX3	PPARG	TMPRSS2

Figure B. Boxplots of **(A)** median unstable MSI sites (%) and **(B)** median TMB.

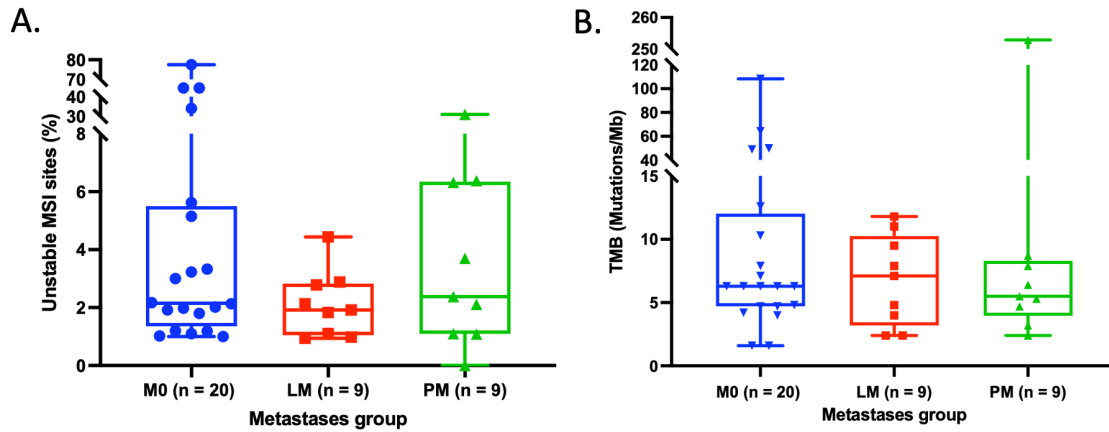


Table A. Overview of tumor sample characteristics

	M0 (N = 20)	LM (N = 10)	PM (N = 9)	p value
Tumor cell percentage (%) – median (Q1 – Q3)	75.00 (62.50 – 78.75)	55.00 (37.50 – 70.00)	70.00 (50.00 – 75.00)	0.011^{a*}
DNA fragmentation (bp) – median (Q1 – Q3)	300 (300 – 400)	300 (300 – 400)	300 (300 – 300)	0.216 ^a
RNA fragmentation (DV200) – median (Q1 – Q3)	n = 10 45.44 (53.16 – 56.16)	n = 10 54.07 (41.94 – 62.48)	n = 9 54.95 (34.23 – 64.75)	0.462 ^a

^aKruskall-Wallis Test; *pairwise comparison showed a significant difference ($p = 0.002$) between LM and M0 group, which remained significant after Bonferroni correction ($p = 0.006$).

M0, no metastases; LM, liver metastases; PM, peritoneal metastases; bp, base pairs; Q1 – Q3, quartile 1 – quartile 3

Table B. (1) Overview of DNA sequencing (likely) pathogenic variant types* and amplifications of total cohort

	Total freq.	M0 (N = 20)		LM (N = 9)		PM (N = 9)		Total pat.	p value ^a
		#	n (%)	#	n (%)	#	n (%)		
Missense	142	74	20 (100)	23	8 (89)	45	9 (100)	37	0.474
Frameshift	114	91	14 (70)	9	5 (56)	14	6 (67)	25	0.899
Nonsense	71	32	18 (90)	15	8 (89)	24	8 (89)	34	1.000
InFrameDeletion	6	5	5 (25)	0	-	1	1 (11)	6	0.271
SpliceAcceptorSNV	7	2	2 (10)	1	1 (11)	4	1 (11)	4	1.000
SpliceDonorSNV	6	3	3 (15)	0	-	3	2 (22)	5	0.397
IntronicDeletion	4	4	3 (15)	0	-	0	-	3	0.403
InFrameInsertion	2	0	-	2	2 (22)	0	-	2	0.102
SpliceDonorInsertion	1	1	1 (5)	0	-	0	-	1	1.000
CdsStartSNV	1	0	-	0	-	1	1 (11)	1	0.474
Amplification**	11	2	2 (10)	7	3 (33)	2	1 (11)	6	0.375

^aFisher-Freeman-Halton Exact Test; *Allele frequency ≥ 5%; ** Fold change ≥ 3 Pat., patients; freq., frequency

(2) Overview of DNA sequencing (likely) pathogenic variant types* and amplifications after MSI samples exclusion

	Total freq.	M0 (N = 16)		LM (N = 9)		PM (N = 8)		Total pat.	p value ^a
		#	n (%)	#	n (%)	#	n (%)		
Missense	75	34	16 (100)	23	8 (89)	18	8 (100)	32	0.515
Frameshift	35	14	11 (69)	9	6 (67)	12	6 (75)	23	1.000
Nonsense	44	22	14 (88)	15	9 (100)	7	7 (88)	30	0.591
InFrameDeletion	4	3	3 (19)	0	-	1	1 (13)	4	0.533
SpliceAcceptorSNV	2	1	1 (6)	1	1 (11)	0	-	2	1.000
SpliceDonorSNV	2	1	1 (6)	0	-	1	1 (13)	2	0.727
InFrameInsertion	2	0	-	2	2 (22)	0	-	2	0.121
Amplification**	11	2	2 (13)	7	3 (33)	2	1 (13)	6	0.499

^aFisher-Freeman-Halton Exact Test; *Allele frequency ≥ 5%; ** Fold change ≥ 3; Pat., patients; freq., frequency

Table C. Detailed overview of (likely) pathogenic DNA mutations and amplifications

Patient (M group)	Gene	cDNA	Protein	Allele frequency	Exon	Consequence
1 (M0)	APC	c.4135G>T	p.E1379*	54%	2	Nonsense
	TP53	c.817C>T	p.R273C	40%	8	Missense
2 (M0)	APC	c.2910delT	p.S970Rfs*10	29%	16	Frameshift
		c.4057G>T	p.E1353*	13%	16	Nonsense
	RAC1	c.608_610delAGA	p.K203delK	15%	7	InFrameDeletion
	GATA4	c.848G>A	p.R283H	13%	4	Missense
	NF1	c.3805_3808dupGACT	p.S1270*fs*1	5%	28	Nonsense
	CUL3	c.944A>T	p.E315V	5%	7	Missense
	3 (M0)	APC	c.4012C>T	p.Q1338*	43%	16
NRAS		c.181C>A	p.Q61K	26%	3	Missense
AR		c.1174C>T	p.P392S	100%	1	Missense
ATM		c.2921+1G>A	.	30%	-	SpliceDonorSNV
PARK2		c.736A>C	p.S246R	24%	7	Missense
CREBBP		c.1289G>A	p.C430Y	18%	5	Missense
ATM		c.9150delA	p.G3051Dfs*24	17%	63	Frameshift
B2M		c.89A>G	p.Y30C	12%	2	Missenese
4 (M0)	APC	c.2635C>T	p.Q879*	34%	16	Nonsense
	KRAS	c.35G>T	p.G12V	23%	2	Missense
	TP53	c.586C>T	p.R196*	30%	6	Nonsense
	GATA3	c.1163A>G	p.K388R	45%	6	Missense
	PRKDC	c.10412_10416delTAGAA c.10404_10405delTC	p.I3471Tfs*50 p.Q3469Dfs*53	4% 5%	74	Frameshift
5 (M0)	APC	c.2545_2546delGA c.4240delG	p.D849*fs*1 p.V1414*fs*1	23%	16	Frameshift Frameshift
		TP53	c.844C>T	p.R282W	29%	8
	TGFBR1	c.643C>T	p.R215*	25%	4	Nonsense

7 (M0)	APC	c.4285C>T	p.Q1429*	27%	16	Nonsense
	KRAS	c.38_39delinsAT	p.G13D	37%	2	Missense
	HRAS	c.187_189delGAG	p.E63delE	25%	3	InFrameDeletion
	APC	c.4280delC	p.P1427Lfs*46	36%	16	Frameshift
	TP53	c.586C>T	p.R196*	2%	6	Nonsense
		c.743G>A	p.R248Q	52%	7	Missense
	SMAD4	c.1082G>A	p.R361H	30%	9	Missense
	FLI1	c.493A>C	p.K165Q	76%	4	Missense
8 (M0)	SOX9	c.1231C>T	p.Q411*	25%	3	Nonsense
	APC	c.3910delA	p.I1304*fs*1	17%	16	Frameshift
		c.904C>T	p.R302*	31%	9	Nonsense
	TP53	c.476C>T	p.A159V	43%	5	Missense
	SMARCB1	c.1121G>A	p.R374Q	42%	9	Missense
9 (M0)	CDK8	c.103G>A	p.V35I	34%	1	Missense
	APC	c.3925G>T	p.E1309*	66%	16	Nonsense
	TP53	c.6735_676dupCCTAGGTTG	p.G226Afs*2	19%	7	Frameshift
		c.673-1G>T	-	36%	-	SpliceAcceptorSNV
	PIK3C2B	c.4382_4383insT	p.E1463*fs*1	47%	30	Nonsense
	MUTYH	c.536A>G	p.Y179C	17%	7	Missense
	PAK3	c.807delG	p.L269Ffs*14	5%	8	Frameshift
10 (M0)	NRAS	.	.	.	.	Amplification
	APC	c.3964G>T	p.E1322*	75%	16	Nonsense
	BRAF	c.1024delA	p.I342Ffs*30	5%	8	Frameshift
	TP53	c.818G>A	p.R273H	73%	8	Missense
	POLD1	c.2954G>C	p.R985P	18%	24	Missense
	BARD1	c.1811-3dupT	.	6%	-	SpliceAcceptorInsertion
	FAT1	c.5916delC	p.S1972Rfs*6	5%	10	Frameshift
	APC	c.3907C>T	p.Q1303*	56%	16	Nonsense

11 (M0)	TP53	c.641A>G	p.H214R	51%	6	Missense
	SMAD3	c.733G>A	p.G245R	58%	6	Missense
12 (M0)	BRAF	c.1799T>A	p.V600E	10%	15	Missense
	PIK3CA	c.3128T>C	p.M1043T	10%	21	Missense
MSI TMB	PIM1	c.83G>T	p.G28V	48%	2	Missense
	ESR1	c.805C>T	p.R269C	44%	16	Missense
	RNF43	c.1976delG	p.G659Vfs*41	22%	8	Frameshift
	PTEN	c.649G>T	p.V217F	14%	7	Missense
	MRE11A	c.1757C>A	p.S586*	14%	15	Nonsense
	ZNF217	c.724delA	p.T242Lfs*30	13%	2	Frameshift
	PAK3	c.539-1G>T	.	12%	-	SpliceAcceptorSNV
	ARID1A	c.1636delC	p.Q546Rfs*73	10%	3	Frameshift
	CIC	c.3347dupC	p.S1117Kfs*34	10%	14	Frameshift
	HNF1A	c.864delG	p.P291Qfs*51	10%	4	Frameshift
	MGA	c.1246_1249delAACA	p.N416Vfs*7	10%	3	Frameshift
	CHD4	c.3283C>T	p.R1095C	9%	22	Missense
	ZBTB2	c.484C>T	p.Q162*	9%	3	Nonsense
	GNAS	c.349G>A	p.V117M	8%	5	Missense
	FLI1	c.490G>C	p.G164R	8%	4	Missense
	POLE	c.3510dupA	p.L1171Tfs*6	7%	29	Frameshift
	JAK1	c.1645C>T	p.R549*	7%	11	Nonsense
29 (M0)	PIK3CA	c.3062A>G	p.Y1021C	19%	21	Missense
		c.3139C>T	p.H1047Y	16%		Missense
MSI TMB	BRAF	c.1799T>A	p.V600E	20%	15	Missense
	FAM175A	c.796+1G>A	.	54%	-	SpliceDonorSNV
	BARD1	c.1921C>T	p.R641*	27%	10	Nonsense
	ABL1	c.929C>T	p.P310L	25%	6	Missense
	XRCC2	c.94_97delTTTG	p.F32Lfs*29	22%	2	Frameshift
	TET2	c.436_437delGA	p.D146*fs*1	21%	3	Frameshift
	FLT3	c.2955_2956delITC	p.P986Afs*164	21%	24	Frameshift
	PAX7	c.906delC	p.Y303Tfs*64	21%	6	Frameshift
	ATM	c.8432dupA	p.S2812Vfs*3	21%	58	Frameshift
	ZRSR2	c.938G>T	p.G313V	20%	11	Missense
	MGA	c.4357delT	p.Y1453Mfs*27	20%	13	Frameshift

	DNMT3A	c.2503A>G	p.T835A	20%	22	Missense
	MGA	c.4357delT	p.Y1453Mfs*27	20%	13	Frameshift
	EML4	c.387delA	p.E130Rfs*43	19%	4	Frameshift
	FAT1	c.4603C>T	p.R1535*	19%	9	Nonsense
	BLM	c.3210+9dupA	.	18%	-	SpliceDonorInsertion
	BRCA2	c.9253delA	p.T3085Qfs*19	18%	24	Frameshift
	CDKN2A	c.85C>T	p.R29W	18%	1	Missense
	SOX9	c.1121dupC	p.Q375Afs*203	17%	3	Frameshift
	CSF3R	c.1404delC	p.S469Afs*22	17%	11	Frameshift
	IRS1	c.2456delG	p.G819Dfs*124	17%	1	Frameshift
	MAGI2	c.2846-3delT	.	17%	-	IntronicDeletion
	SOX9	c.1121dupC	p.Q375Afs*203	17%	3	Frameshift
	CSF3R	c.1404delC	p.S469Afs*22	17%	11	Frameshift
	ERBB3	c.694G>A	p.A232T	17%	6	Missense
	FOXL2	c.576delC	p.K193Sfs*78	16%	1	Frameshift
	GATA2	c.818dupG	p.P274Tfs*8	16%	3	Frameshift
	SETBP1	c.1261delA	p.R421Dfs*23	16%	4	Frameshift
	ALK	c.2633G>A	p.G878D	15%	16	Missense
	BCORL1	c.1929delC	p.M644Cfs*4	13%	3	Frameshift
	AXIN2	c.1994dupG	p.N666Qfs*41	13%	8	Frameshift
	ALOX12B	c.1303G>A	p.V435I	12%	10	Missense
	TGFBR1	c.557C>T	p.T186M	9%	3	Missense
	CARD11	c.1663delC	p.R555Gfs*45	8%	3	Frameshift
	CASP8	c.1173delC	p.I392Sfs*4	8%	8	Frameshift
	ATRX	c.6111-3delT	.	7%	-	IntronicDeletion
	NOTCH4	c.529delC	p.Q177Rfs*75	6%	4	Frameshift
	FAM175A	c.796+1G>A	.	54%	-	SpliceDonorSNV
	RNF43	c.1976delG	p.G659Vfs*41	9%	8	Frameshift
		c.354delC	p.C119Afs*39	20%	2	Frameshift
		c.349dupC	p.R117Pfs*8	16%	2	Frameshift
14 (M0)	BARD1	c.1921C>T	p.R641*	27%	10	Nonsense
	PIK3CA	c.3062A>G	p.Y1021C	19%	21	Missense
		c.3139C>T	p.H1047Y	16%		Missense
	BRAF	c.1799T>A	p.V600E	20%	15	Missense

	ATRX	c.6111-3delT	.	7%	-	IntronicDeletion
	MAGI2	c.2846-3delT	.	17%	-	IntronicDeletion
	RBM10	c.534delT	p.F178Lfs*88	38%	6	Frameshift
	KRAS	c.40G>A	p.V14I	21%	2	Missense
	TP53	c.383dupC	p.A129Cfs*20	24%	5	Frameshift
	ALK	c.523_540delAGTTGGTGGATTCGCCAA	p.S175_Q180delSWWIRQ	43%	1	InFrameDeletion
	PBRM1	c.2566C>T	p.R856W	23%	17	Missense
	AKT3	c.740G>A	p.R247H	19%	9	Missense
	JUN	c.930_931delGA	p.K311Sfs*6	17%	1	Frameshift
	AXIN2	c.1228dupA	p.T410Nfs*52	16%	6	Frameshift
	FGFR1	.	.	.	.	Amplification
16 (M0) MSI TMB	BRAF	c.1799T>A	p.V600E	25%	15	Missense
	PIK3CA	c.328_330delGAA	p.E110delE	21%	2	InFrameDeletion
	TP53	c.455delC	p.P152Rfs*18	38%	5	Frameshift
	DDR2	c.1552delG	p.V518Cfs*9	46%	14	Frameshift
	FOX L2	c.18delC	p.E7Sfs*143	39%	1	Frameshift
	CDKN2A	c.202G>A	p.A68T	37%	2	Missense
	MEN1	c.1366G>T	p.V456L	34%	10	Missense
	CREBBP	c.1816C>T	p.H606Y	33%	8	Missense
	FANCE	c.967C>T	p.Q323*	32%	4	Nonsense
	KDM5C	c.1354G>A	p.G452S	32%	10	Missense
	BCR	c.2115+2T>C	.	32%	-	SpliceDonorSNV
	BCOR	c.2428C>T	p.R810*	31%	4	Nonsense
	LRP1B	c.11869dupA	p.R3957Kfs*11	31%	77	Frameshift
	CUL3	c.2231G>A	p.R744H	30%	16	Missense
	JAK2	c.544G>T	p.A182S	30%	6	Missense
	IRF2	c.86A>C	p.K29T	29%	2	Missense
	MSH6	c.3163G>A	p.A1055T	29%	4	Missense
	RARA	c.89delC	p.P30Lfs*12	29%	2	Frameshift
	ZBTB7A	c.522delC	p.A175Rfs*43	29%	2	Frameshift
	GNAS	c.1066C>T	p.R356C	28%	13	Missense
	EED	c.1124A>C	p.K375T	28%	10	Missense
	TSHR	c.16_17dupTT	p.L6Ffs*53	28%	2	Frameshift

BRCA2	c.36delT	p.F12Lfs*13	27%	2	Frameshift
IL7R	c.799delA	p.R267Gfs*28	27%	6	Frameshift
KDM5A	c.23delG	p.G8Afs*58	27%	1	Frameshift
ATRX	c.6111-3delT	.	27%	-	IntronicDeletion
POLD1	c.342delG	p.P116Hfs*53	26%	4	Frameshift
HNRNPK	c.746G>A	p.R249H	26%	11	Missense
RPTOR	c.3010C>T	p.Q1004*	26%	25	Nonsense
ETV6	c.1226A>T	p.K409M	25%	7	Missense
FGFR3	c.1206dupC	p.K403Qfs*93	25%	9	Frameshift
SOCS1	c.150delC	p.G51Afs*34	24%	2	Frameshift
SOX9	c.66delC	p.S23Afs*38	24%	1	Frameshift
GLI1	c.821delG	p.G274Afs*6	23%	8	Frameshift
ICOSLG	c.718_719delGA	p.D240Qfs*103	23%	5	Frameshift
IRF4	c.623delC	p.P208Qfs*9	23%	5	Frameshift
EPHA3	c.2538delC	p.M847Wfs*10	22%	15	Frameshift
MAP2K1	c.320T>C	p.I107T	22%	3	Missense
KIF5B	c.1052dupA	p.N351Kfs*2	21%	11	Frameshift
TOP2A	c.3613delA	p.T1205Hfs*19	21%	28	Frameshift
SPTA1	c.4866G>A	p.W1622*	19%	34	Nonsense
AXIN1	c.1034delC	p.P345Hfs*69	31%	4	Frameshift
	c.792delC	p.G265Efs*149	26%	2	Frameshift
AXIN2	c.2011delC	p.R671Afs*18	8%	8	Frameshift
	c.1994delG	p.G665Afs*24	14%		Frameshift
BCORL1	c.220delG	p.A74Qfs*42	28%	3	Frameshift
	c.5041_5042delCC	.P1681Rfs*4	26%	12	Frameshift
NOTCH4	c.4322delC	p.P1441Lfs*17	27%	24	Frameshift
	c.2125delC	p.Q709Sfs*22	23%	13	Frameshift
RASA1	c.625_626delAG	p.S209*fs*1	26%	2	Frameshift
	c.2513delA	p.N838Mfs*4	24%	19	Frameshift
RNF43	c.1976delG	p.G659Vfs*41	28%	8	Frameshift
	c.468delG	p.L157*fs*1	30%	4	Frameshift
PAX5	c.295delA	p.I99Sfs*60	21%	3	Frameshift
	c.81delT	p.F27Lfs*2	21%	2	Frameshift

	JAK1	c.2580delA	p.K860Nfs*16	25%	19	Frameshift
		c.1289dupC	p.L431Vfs*22	30%	9	Frameshift
		c.425delA	p.K142Rfs*26	24%	5	Frameshift
17	TP53	c.574C>T	p.Q192*	55%	6	Nonsense
(M0)	CTNNB1	c.121A>G	p.T41A	52%	3	Missense
26	APC	c.3807_3808delAT	p.I1269Mfs*6	26%	16	Frameshift
(M0)	TP53	c.817C>T	p.R273C	17%	8	Missense
19	KRAS	c.35G>T	p.G12V	26%	2	Missense
(M0)	BRAF	c.1406G>C	p.G469A	22%	11	Missense
	PIK3CA	c.335T>A	p.I112N	12%	2	Missense
MSI	RNF43	c.1976delG	p.G659Vfs*41	51%	8	Frameshift
TMB	ATRX	c.6111-3delT	.	47%	-	IntronicDeletion
	GATA1	c.842C>T	p.A281V	45%	5	Missense
	PARK2	c.1298A>C	p.H433P	41%	12	Missense
	RAD54L	c.304delA	p.R102Gfs*25	27%	5	Frameshift
	CTCF	c.1928delC	p.P643Qfs*43	26%	11	Frameshift
	TGFBR2	c.1157T>C	p.L386P	26%	5	Missense
	PRKCI	c.665C>G	p.S222*	25%	8	Nonsense
	PREX2	c.141G>A	p.S47S	25%	1	Synonymous
	CDKN1B	c.214_215dupGG	p.K73Afs*47	24%	1	Frameshift
	NBN	c.247dupA	p.M83Nfs*23	24%	3	Frameshift
	MAGI2	c.104delG	p.G35Afs*13	24%	1	Frameshift
	MLL	c.2318delC	p.P773Rfs*8	24%	3	Frameshift
	MUTYH	c.596delG	p.G199Afs*41	23%	8	Frameshift
	IDH2	c.202_204delGAG	p.E68delE	22%	2	InFrameDeletion
		c.1620delA	p.K540Nfs*32	22%	17	Frameshift
	MSH3	c.2582delA	p.N861Mfs*6	19%	19	Frameshift
	SETD2	c.843dupA	p.E282Rfs*9	17%	3	Frameshift
23	APC	c.3253A>T	p.K1085*	26%	16	Nonsense
(M0)		c.4585C>T	p.Q1529*	19%		Nonsense
	KRAS	c.35G>T	p.G12V	33%	2	Missense
	SMAD4	c.1256G>T	p.G419V	40%	10	Missense
	TP53	c.722C>A	p.S241Y	38%	7	Missense

	FAM123B	c.1834G>T	p.E612*	34%	2	Nonsense
	IRS1	c.2512C>T	p.Q838*	18%	1	Nonsense
21 (LM)	APC	c.3860_3879dupTAGGATGTAATCAGACGACA	p.Q1294*fs*1	21%	16	Nonsense
	TP53	c.844_849dupCGGCGC	p.R282_R283dupRR	29%	8	InFrameInsertion
22 (LM)	APC	c.4254_4269delAAGCCCCAGTGATCTT	p.S1419Qfs*49	51%	16	Frameshift
	NRAS	c.182A>T	p.Q61L	55%	3	Missense
	TP53	c.817C>T	p.R273C	36%	8	Missense
	SMAD4	c.1067C>A	p.P356H	41%	9	Missense
	SOX9	c.1319dupA	p.Y440*fs*1	55%	3	Nonsense
	PAX8	c.275T>C	p.I92T	50%	4	Missense
	GPS2	c.139G>T	p.E47*	35%	3	Nonsense
	RAF1	c.770C>T	p.S257L	28%	7	Missense
13 (LM)	APC	c.3871C>T	p.Q1291*	78%	16	Nonsense
	SMAD4	c.170_171delTA	p.L57Yfs*12	10%	2	Frameshift
	TP53	c.524G>A	p.R175H	80%	5	Missense
	KRAS	c.35G>C	p.G12A	79%	2	Missense
	BRCA2	.	.	.	.	Amplification
	LAMP1	.	.	.	.	Amplification
20 (LM)	APC	c.3927_3931delAAAGA	p.E1309Dfs*4	8%	16	Frameshift
		c.2413C>T	p.R805*	36%	16	Nonsense
	TP53	c.493C>T	p.Q165*	51%	5	Nonsense
	PIK3R1	c.1389_1390insATT	p.Y463_D464insI	39%	11	InFrameInsertion
	H3F3A	c.350G>A	p.R117H	36%	3	Missense
	PBRM1	c.3463G>T	p.E1155*	22%	23	Nonsense
	SOX9	c.437T>A	p.L146Q	26%	2	Missense
24 (LM)		c.611dupT	p.K205Qfs*47	34%	2	Frameshift
	APC	c.3294T>A	p.C1098*	15%	16	Nonsense
		c.3871C>T	p.Q1291*	16%		Nonsense
	KRAS	c.35G>A	p.G12D	25%	2	Missense
	PIK3CA	c.3127A>G	p.M1043V	11%	21	Missense
		c.344G>T	p.R115L	10%	2	Missense

25 (LM)	SMAD4	c.1577A>C	p.E526A	25%	12	Missense
	MED12	c.97G>C	p.E33Q	48%	1	Missense
	PAK3	c.1201G>T	p.A401S	12%	12	Missense
	SPTA1	c.3136C>T	p.R1046*	15%	22	Nonsense
	ATRX	c.4318-2A>G	.	8%	-	SpliceAcceptorSNV
	NF1	c.5050delT	p.S1684Pfs*14	2%	37	Frameshift
	APC	c.4222G>T	p.E1408*	21%	16	Nonsense
	TP53	c.916C>T	p.R306*	17%	8	Nonsense
	KRAS	c.35G>A	p.G12D	23%	2	Missense
	CCND1	.	.	.	.	Amplification
27 (LM)	FGF3	.	.	.	.	Amplification
	APC	c.637C>T	p.R213*	57%	6	Nonsense
	TP53	c.817C>T	p.R273C	61%	8	Missense
	RAD50	c.1232_1242delCCAACCAACTG	p.A411Dfs*3	17%	8	Frameshift
	MLH1	c.1964T>C	p.I655T	55%	17	Missense
	CDKN2A	c.301G>T	p.G101W	51%	2	Missense
	FGF23	.	.	.	.	Amplification
	FGF6	.	.	.	.	Amplification
28 (LM)	KRAS	.	.	.	.	Amplification
	APC	c.2825dupA	p.N942Kfs*4	32%	16	Frameshift
		c.4216C>T	p.Q1406*	35%	16	Nonsense
	KRAS	c.34G>T	p.G12C	34%	2	Missense
	BCORL1	c.2350C>T	p.R784*	79%	3	Nonsense
	SOX9	c.1113_1117dupGGCAC	p.P373Rfs*12	34%	3	Frameshift
	PARK2	c.476C>T	p.P159L	32%	4	Missense
	MITF	c.836G>A	p.R279Q	18%	8	Missense
30 (LM)	NOTCH1	c.7541_7542delCT	p.P2514Rfs*4	19%	34	Frameshift
	FAT1	c.3744_3754delGTTCTACAAAA	p.K1248Nfs*5	5%	5	Frameshift
	FAM123B	c.994delA	p.I332*fs*1	77%	2	Frameshift
	ATM	c.9022C>T	p.R3008C	36%	63	Missense
	APC	c.3927_3931delAAAGA	p.E1309Dfs*4	17%	16	Frameshift

31 (PM)		c.1495C>T	p.R499*	23%	12	Nonsense
	ATM	c.5216dupA	p.N1739Kfs*10	34%	35	Frameshift
	PAX3	c.290G>A	p.R97H	20%	2	Missense
	MDM4	.	.	.	.	Amplification
	ERBB2	.	.	.	.	Amplification
32 (PM)	BRAF	c.1799T>A	p.V600E	39%	15	Missense
	TP53	c.637C>T	p.R213*	56%	6	Nonsense
	SOX2	c.804_805delinsAC	p.D269H	42%	1	Missense
	RHEB	c.275+2T>G	.	22%	-	SpliceDonorSNV
	INSR	c.3560C>T	p.T1187M	16%	20	Missense
33 (PM)	APC	c.3934G>T	p.G1312*	56%	16	Nonsense
	KRAS	c.35G>A	p.G12D	46%	2	Missense
	SMAD4	c.1201dupT	p.C401Lfs*3	35%	10	Frameshift
	TP53	c.383delC	p.P128Lfs*42	40%	5	Frameshift
34 (PM)	APC	c.4214_4247del TTCAGAGTGAACCATGCAGTGGGAATGGTAAGTGG	p.V1405Afs*3	18%	16	Frameshift
	TP53	c.224dupC	p.A76Cfs*73	29%	4	Frameshift
	FLCN	c.616A>G	p.K206E	32%	6	Missense
	TCF7L2	c.268C>T	p.Q90*	27%	3	Nonsense
35 (PM) TMB MSI	APC	c.3778C>T	p.Q1260*	26%	16	Nonsense
		c.4654G>T	p.E1552*	22%		Nonsense
	KRAS	c.175G>A	p.A59T	17%	3	Missense
	NRAS	c.510dupA	p.L171Tfs*5	18%	5	Frameshift
	PIK3CA	c.929G>A	p.R310H	10%	5	Missense
	ERCC5	c.2974C>T	p.R992*	48%	15	Nonsense
	MSH6	c.3772C>T	p.Q1258*	44%	8	Nonsense
	FAM123B	c.1756C>T	p.R586*	39%	2	Nonsense
	H3F3A	c.350G>A	p.R117H	33%	3	Missense
	GNAQ	c.477-2A>G	.	29%	-	SpliceAcceptorSNV
	KAT6A	c.2323C>T	p.R775C	28%	15	Missense
	ERBB3	c.695C>T	p.A232V	27%	6	Missense
	FLT3	c.2480T>C	p.I827T	26%	20	Missense

LRP1B	c.9032C>T	p.S3011L	26%	56	Missense
BRD4	c.3223C>T	p.Q1075*	26%	15	Nonsense
PHOX2B	c.323C>T	p.A108V	26%	2	Missense
EGFR	c.2135T>C	p.F712S	25%	18	Missense
GNA11	c.656G>A	p.C219Y	24%	5	Missense
TRAF7	c.91G>A	p.E31K	24%	3	Missense
HRAS	c.291-1G>T	.	24%	-	SpliceAcceptorSNV
LATS1	c.2785C>T	p.Q929*	24%	7	Nonsense
PARK2	c.844C>T	p.Q282*	24%	7	Nonsense
WISP3	c.382G>A	p.E128K	24%	2	Missense
ABL1	c.908G>T	p.G303V	23%	6	Missense
CREBBP	c.5470G>A	p.A1824T	23%	31	Missense
IDH1	c.874A>G	p.T292A	23%	8	Missense
SMARCB1	c.1097G>A	p.R366H	23%	8	Missense
IGF1R	c.1101delG	p.N368Ifs*17	23%	4	Frameshift
SYK	c.583C>T	p.R195*	23%	4	Nonsense
ARID1A	c.5503C>T	p.Q1835*	22%	20	Nonsense
FGFR2	c.1547C>T	p.A516V	22%	11	Missense
TGFBR2	c.1658G>A	p.R553H	22%	8	Missense
MAPK3	c.171-1G>A	.	22%	-	SpliceAcceptorSNV
NF2	c.774G>A	p.W258*	21%	8	Nonsense
NOTCH1	c.4721T>C	p.L1574P	21%	26	Missense
PPP6C	c.442C>T	p.R148*	21%	5	Nonsense
BCL6	c.1948C>T	p.R650*	21%	9	Nonsense
GNAS	c.325G>A	p.A109T	20%	5	Missense
MITF	c.836G>A	p.R279Q	20%	8	Missense
SMAD3	c.3G>A	.	20%	1	CdsStartSNV
EED	c.540G>A	p.M180I	20%	5	Missense
RARA	c.436C>T	p.Q146*	20%	4	Nonsense
FLCN	c.1579C>T	p.R527*	19%	14	Nonsense
JAK2	c.1310T>C	p.L437S	19%	10	Missense
TCF7L2	c.1318+1G>A	.	19%	-	SpliceDonorSNV
	c.1391G>T	p.R464M	21%	13	Missense
FLT4	c.3122G>A	p.R1041Q	18%	23	Missense
ASXL2	c.3799C>T	p.Q1267*	13%	13	Nonsense

	PAX3	c.62C>T	p.P21L	13%	1	Missense
	PTPRT	c.2857C>T	p.R953*	11%	20	Nonsense
	ALOX12B	c.1510G>A	p.A504T	9%	11	Missense
	CTNNA1	c.361C>T	p.R121*	5%	4	Nonsense
	PTEN	c.209+2T>C	.	5%	-	SpliceDonorSNV
		c.254-1G>A	.	5%	-	SpliceAcceptorSNV
36 (PM)	KRAS	c.34G>A	p.G12S	17%	2	Missense
	PIK3CA	c.1633G>A	p.E545K	13%	10	Missense
	TP53	c.455C>T	p.P152L	15%	5	Missense
	DDX41	c.647dupT	p.S217Ifs*4	47%	8	Frameshift
	MSH6	c.2081_2082delGC	p.C694Sfs*3	9%	4	Frameshift
	BRCA1	c.3862G>T	p.E1288*	8%	10	Nonsense
	DHX15	c.139_140insA	p.G47Efs*3	6%	2	Frameshift
	KIF5B	c.1026_1033delGAAGTATG	p.Y344Rfs*6	5%	11	Frameshift
37 (PM)	SMAD4	c.1572G>T	p.W524C	23%	12	Missense
	TP53	c.817C>T	p.R273C	33%	8	Missense
	BRAF	c.1799T>A	p.V600E	34%	15	Missense
38 (PM)	APC	c.4393_4394delAG	p.S1465Wfs*3	50%	16	Frameshift
	KRAS	c.35G>T	p.G12V	44%	2	Missense
	FAM123B	c.227delG	p.G76Dfs*24	62%	2	Frameshift
	KDM5C	c.1714_1731delAATCCCAACACCCTCATG	p.N572_M577delNPNTLM	6%	12	InFrameDeletion
	SLX4	c.634C>T	p.R212*	30%	3	Nonsense
40 (PM)	TP53	c.404G>T	p.C135F	57%	5	Missense
	MUTYH	c.1187G>A	p.G396D	43%	13	Missense
	PREX2	c.1158T>A	p.Y386*	38%	10	Nonsense
	AR	c.2471A>C	p.N824T	26%	7	Missense
	BRAF	c.1799T>A	p.V600E	25%	15	Missense
	FBXW7	c.943G>C	p.A315P	24%	6	Missense

Table D. Detailed overview percentage of mutated/amplified gene in each subgroup with statistical analysis

Gene	M0 (%) N = 20	LM (%) N = 9	PM (%) N = 9	P value^a
APC	70.0	88.88	55.55	0.331
TP53	65.0	66.66	66.66	1.000
BARD1	10.0	0.0	0.0	1.000
FAT1	10.0	0.0	0.0	1.000
POLD1	10.0	0.0	0.0	1.000
SMAD3	5.0	0.0	11.11	0.730
BRAF	20.0	0.0	33.33	0.219
ARID1A	5.0	0.0	11.11	0.730
CHD4	5.0	0.0	0.0	1.000
CIC	5.0	0.0	0.0	1.000
ESR1	5.0	0.0	0.0	1.000
GNAS	10.0	0.0	11.11	1.000
HNF1A	5.0	0.0	0.0	1.000
MGA	10.0	0.0	0.0	1.000
PIK3CA	20.0	11.11	22.22	1.000
PTEN	5.0	0.0	11.11	0.730
RNF43	20.0	0.0	0.0	0.153
MRE11A	5.0	0.0	0.0	1.000
POLE	5.0	0.0	0.0	1.000
FLI1	10.0	0.0	0.0	1.000
JAK1	10.0	0.0	0.0	1.000
PAK3	10.0	11.11	0.0	1.000
PIM1	5.0	0.0	0.0	1.000
ZBTB2	5.0	0.0	0.0	1.000
ZNF217	5.0	0.0	0.0	1.000
SMAD4	10.0	33.33	22.22	0.398
KRAS	30.0	55.55	44.44	0.467
IDH2	10.0	0.0	0.0	1.000
CSF1R	5.0	0.0	0.0	1.000
PIK3R1	5.0	11.11	0.0	0.730
RBM10	5.0	0.0	0.0	1.000
AXIN2	15.0	0.0	0.0	1.000
PBRM1	5.0	11.11	0.0	0.730
AKT3	5.0	0.0	0.0	1.000
ALK	10.0	0.0	0.0	1.000
JUN	5.0	0.0	0.0	1.000
AXIN1	5.0	0.0	0.0	1.000
BCOR	5.0	0.0	0.0	1.000
BRCA2	10.0	11.11	0.0	1.000
CDKN2A	10.0	11.11	0.0	1.000
CREBBP	10.0	0.0	11.11	1.000
CUL3	10.0	0.0	0.0	1.000
ETV6	5.0	0.0	0.0	1.000
FANCE	5.0	0.0	0.0	1.000
IRF2	5.0	0.0	0.0	1.000
JAK2	5.0	0.0	11.11	0.730
KDM5C	5.0	0.0	11.11	0.730
LRP1B	5.0	0.0	11.11	0.730
MEN1	5.0	0.0	0.0	1.000
RASA1	5.0	0.0	0.0	1.000

SOCS1	5.0	0.0	0.0	1.000
SOX9	15.0	33.33	0.0	0.170
BCORL1	10.0	11.11	0.0	1.000
EPHA3	5.0	0.0	0.0	1.000
GLI1	5.0	0.0	0.0	1.000
NOTCH4	10.0	0.0	0.0	1.000
BCR	5.0	0.0	0.0	1.000
DDR2	5.0	0.0	0.0	1.000
EED	5.0	0.0	11.11	0.730
FGFR3	5.0	0.0	0.0	1.000
FOXL2	10.0	0.0	0.0	1.000
HNRNPK	5.0	0.0	0.0	1.000
ICOSLG	5.0	0.0	0.0	1.000
IL7R	5.0	0.0	0.0	1.000
IRF4	5.0	0.0	0.0	1.000
KDM5A	5.0	0.0	0.0	1.000
KIF5B	5.0	0.0	11.11	0.730
MAP2K1	5.0	0.0	0.0	1.000
MSH6	5.0	0.0	22.22	0.267
PAX5	5.0	0.0	0.0	1.000
RARA	5.0	0.0	11.11	0.730
RPTOR	5.0	0.0	0.0	1.000
SPTA1	5.0	11.11	0.0	0.730
TOP2A	5.0	0.0	0.0	1.000
TSHR	5.0	0.0	0.0	1.000
ZBTB7A	5.0	0.0	0.0	1.000
ATRX	15.0	11.11	0.0	0.792
CTNNB1	5.0	0.0	0.0	1.000
CDKN1B	5.0	0.0	0.0	1.000
CTCF	5.0	0.0	0.0	1.000
GATA1	5.0	0.0	0.0	1.000
MSH3	5.0	0.0	0.0	1.000
MUTYH	10.0	0.0	11.11	1.000
NBN	5.0	0.0	0.0	1.000
SETD2	5.0	0.0	0.0	1.000
TGFBR2	5.0	0.0	11.11	0.730
RAD54L	5.0	0.0	0.0	1.000
IKBKE	5.0	0.0	0.0	1.000
MAGI2	10.0	0.0	0.0	1.000
MLL	5.0	0.0	0.0	1.000
PARK2	10.0	11.11	11.11	1.000
PRKCI	5.0	0.0	0.0	1.000
PREX2	5.0	0.0	11.11	0.730
GATA4	5.0	0.0	0.0	1.000
RAC1	5.0	0.0	0.0	1.000
H3F3A	0.0	11.11	11.11	0.218
GPS2	0.0	11.11	0.0	0.474
PAX8	0.0	11.11	0.0	0.474
RAF1	0.0	11.11	0.0	0.474
NRAS	10.0	11.11	11.11	1.000
FAM123B	5.0	11.11	11.11	0.328
IRS1	10.0	0.0	0.0	1.000
MED12	0.0	11.11	0.0	0.474
RAD50	0.0	11.11	0.0	0.474

MLH1	0.0	11.11	0.0	0.474
MITF	0.0	11.11	11.11	0.218
NOTCH1	0.0	11.11	11.11	0.218
ABL1	5.0	0.0	11.11	0.730
ATM	10.0	11.11	11.11	1.000
BLM	5.0	0.0	0.0	1.000
CASP8	5.0	0.0	0.0	1.000
DNMT3A	5.0	0.0	0.0	1.000
ERBB3	5.0	0.0	11.11	0.730
TET2	5.0	0.0	0.0	1.000
ZRSR2	5.0	0.0	0.0	1.000
FAM175A	5.0	0.0	0.0	1.000
ALOX12B	5.0	0.0	11.11	0.730
CARD11	5.0	0.0	0.0	1.000
CSF3R	5.0	0.0	0.0	1.000
EML4	5.0	0.0	0.0	1.000
FLT3	5.0	0.0	11.11	0.730
GATA2	5.0	0.0	0.0	1.000
PAX7	5.0	0.0	0.0	1.000
SETBP1	5.0	0.0	0.0	1.000
TGFBR1	10.0	0.0	0.0	1.000
XRCC2	5.0	0.0	0.0	1.000
AR	5.0	0.0	11.11	0.730
B2M	5.0	0.0	0.0	1.000
PAX3	0.0	0.0	22.22	0.102
INSR	0.0	0.0	11.11	0.474
RHEB	0.0	0.0	11.11	0.474
SOX2	0.0	0.0	11.11	0.474
FLCN	0.0	0.0	22.22	0.102
TCF7L2	0.0	0.0	22.22	0.102
ASXL2	0.0	0.0	11.11	0.474
EGFR	0.0	0.0	11.11	0.474
ERCC5	0.0	0.0	11.11	0.474
FGFR2	0.0	0.0	11.11	0.474
GNA11	0.0	0.0	11.11	0.474
IDH1	0.0	0.0	11.11	0.474
KAT6A	0.0	0.0	11.11	0.474
NF2	0.0	0.0	11.11	0.474
PPP6C	0.0	0.0	11.11	0.474
SMARCB1	5.0	0.0	11.11	0.730
TRAF7	0.0	0.0	11.11	0.474
PTPRT	0.0	0.0	11.11	0.474
BCL6	0.0	0.0	11.11	0.474
BRD4	0.0	0.0	11.11	0.474
FLT4	0.0	0.0	11.11	0.474
GNAQ	0.0	0.0	11.11	0.474
HRAS	5.0	0.0	11.11	0.730
IGF1R	0.0	0.0	11.11	0.474
LATS1	0.0	0.0	11.11	0.474
MAPK3	0.0	0.0	11.11	0.474
PHOX2B	0.0	0.0	11.11	0.474
SYK	0.0	0.0	11.11	0.474
WISP3	0.0	0.0	11.11	0.474
BRCA1	0.0	0.0	11.11	0.474

DHX15	0.0	0.0	11.11	0.474
DDX41	0.0	0.0	11.11	0.474
SLX4	0.0	0.0	11.11	0.474
GATA3	5.0	0.0	0.0	1.000
FBXW7	0.0	0.0	11.11	0.474
CDK8	5.0	0.0	0.0	1.000
PIK3C2B	5.0	0.0	0.0	1.000
LAMP1	0.0	11.11	0.0	0.474
FGFR1	5.0	0.0	0.0	1.000
CCND1	0.0	11.11	0.0	0.474
FGF3	0.0	11.11	0.0	0.474
FGF23	0.0	11.11	0.0	0.474
FGF6	0.0	11.11	0.0	0.474
MDM4	0.0	0.0	11.11	0.474
ERBB2	0.0	0.0	11.11	0.474

^aFisher-Freeman-Halton Exact test

Table E. Detailed overview percentage of mutated/amplified gene in each subgroup after MSI sample deletion, with statistical analysis

Gene	M0 (%) N = 16	LM (%) N = 9	PM (%) N = 8	P value^a
APC	87.5	88.89	50.0	0.134
TP53	75.0	66.67	75.0	0.886
BARD1	6.25	0.0	0.0	1.000
FAT1	6.25	0.0	0.0	1.000
POLD1	6.25	0.0	0.0	1.000
SMAD3	6.25	0.0	0.0	1.000
BRAF	0.0	0.0	37.5	0.010
PIK3CA	0.0	11.11	12.5	0.258
FLI1	6.25	0.0	0.0	1.000
PAK3	6.25	11.11	0.0	1.000
SMAD4	12.5	33.33	25.0	0.476
KRAS	31.25	55.56	37.5	0.588
IDH2	6.25	0.0	0.0	1.000
CSF1R	6.25	0.0	0.0	1.000
PIK3R1	6.25	11.11	0.0	1.000
RBM10	6.25	0.0	0.0	1.000
AXIN2	6.25	0.0	0.0	1.000
PBRM1	6.25	11.11	0.0	1.000
AKT3	6.25	0.0	0.0	1.000
ALK	6.25	0.0	0.0	1.000
JUN	6.25	0.0	0.0	1.000
BRCA2	0.0	11.11	0.0	0.515
CDKN2A	0.0	11.11	0.0	0.515
CREBBP	6.25	0.0	0.0	1.000
CUL3	6.25	0.0	0.0	1.000
KDM5C	0.0	0.0	12.5	0.242
SOX9	6.25	33.33	0.0	0.113
BCORL1	0.0	11.11	0.0	0.515
KIF5B	0.0	0.0	12.5	0.242
MSH6	0.0	0.0	12.5	0.242
SPTA1	0.0	11.11	0.0	0.515
ATRX	0.0	11.11	0.0	0.515
CTNNB1	6.25	0.0	0.0	1.000
MUTYH	6.25	0.0	12.5	0.727
PARK2	6.25	11.11	0.0	1.000
PREX2	0.0	0.0	12.5	0.242
GATA4	6.25	0.0	0.0	1.000
RAC1	6.25	0.0	0.0	1.000
H3F3A	0.0	11.11	0.0	0.515
GPS2	0.0	11.11	0.0	0.515
PAX8	0.0	11.11	0.0	0.515
RAF1	0.0	11.11	0.0	0.515
NRAS	12.5	11.11	0.0	0.789
FAM123B	6.25	11.11	12.5	1.000
IRS1	6.25	0.0	0.0	1.000
MED12	0.0	11.11	0.0	0.515
RAD50	0.0	11.11	0.0	0.515
MLH1	0.0	11.11	0.0	0.515
MITF	0.0	11.11	0.0	0.515
NOTCH1	0.0	11.11	0.0	0.515

ATM	6.25	11.11	12.5	1.000
TGFBR1	6.25	0.0	0.0	1.000
AR	6.25	0.0	12.5	0.727
B2M	6.25	0.0	0.0	1.000
PAX3	0.0	0.0	12.5	0.242
INSR	0.0	0.0	12.5	0.242
RHEB	0.0	0.0	12.5	0.242
SOX2	0.0	0.0	12.5	0.242
FLCN	0.0	0.0	12.5	0.242
TCF7L2	0.0	0.0	12.5	0.242
SMARCB1	6.25	0.0	0.0	1.000
HRAS	6.25	0.0	0.0	1.000
BRCA1	0.0	0.0	12.5	0.242
DHX15	0.0	0.0	12.5	0.242
DDX41	0.0	0.0	12.5	0.242
SLX4	0.0	0.0	12.5	0.242
GATA3	6.25	0.0	0.0	1.000
FBXW7	0.0	0.0	12.5	0.242
CDK8	6.25	0.0	0.0	1.000
PIK3C2B	6.25	0.0	0.0	1.000
LAMP1	0.0	11.11	0.0	0.515
FGFR1	6.25	0.0	0.0	1.000
CCND1	0.0	11.11	0.0	0.515
FGF3	0.0	11.11	0.0	0.515
FGF23	0.0	11.11	0.0	0.515
FGF6	0.0	11.11	0.0	0.515
MDM4	0.0	0.0	12.5	0.242
ERBB2	0.0	0.0	12.5	0.242
*Fisher-Freeman-Halton Exact test				

Supplementary 2: Analysis on total cohort (MSI + MSS samples)

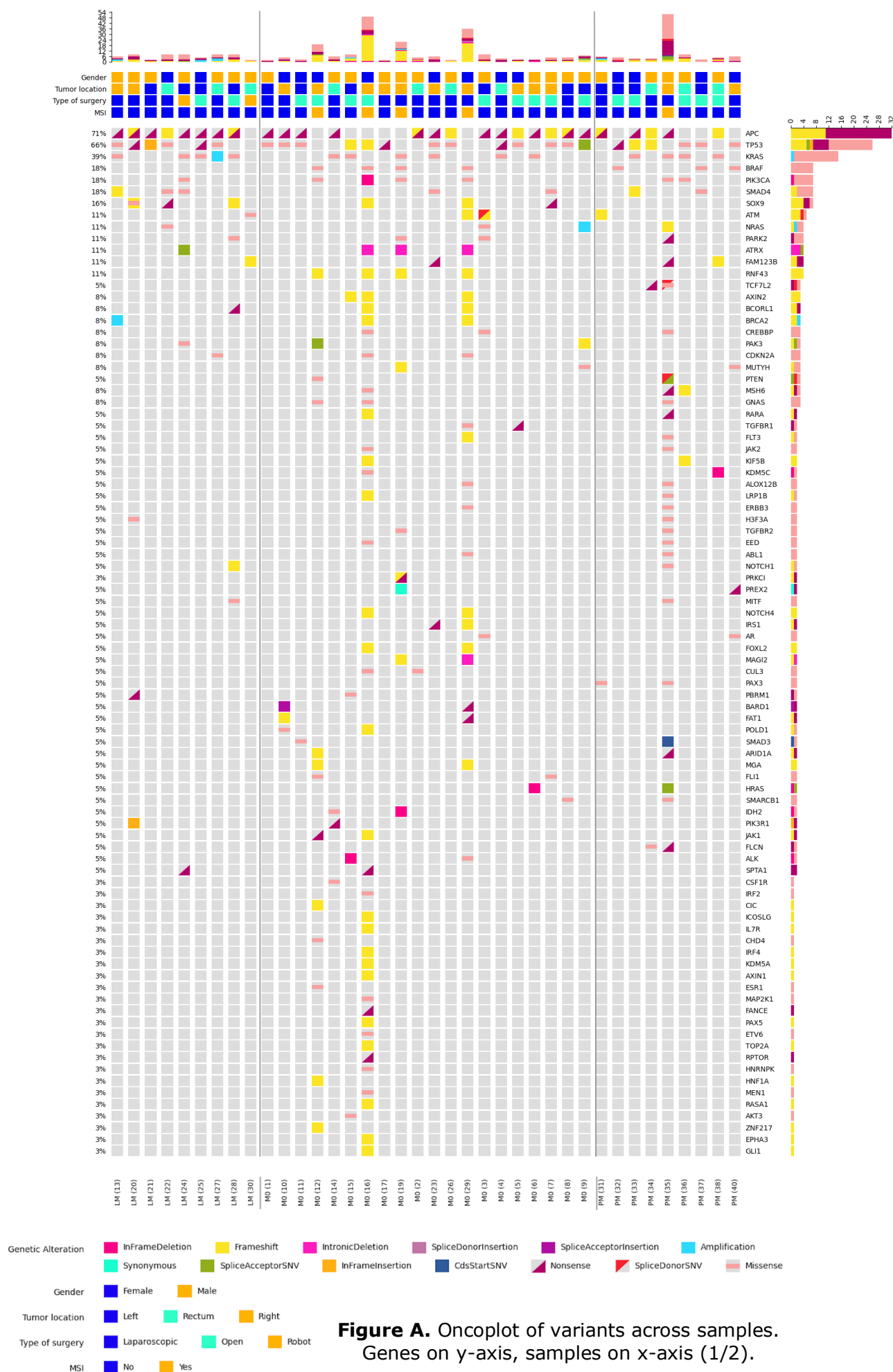
173/523 genes were (likely) pathogenic affected, resulting in 415 variant types (Figure S2 – A). Missense, frameshift, and nonsense mutations were most common. When comparing the occurrence of all variant types and amplifications, no significant differences were found.

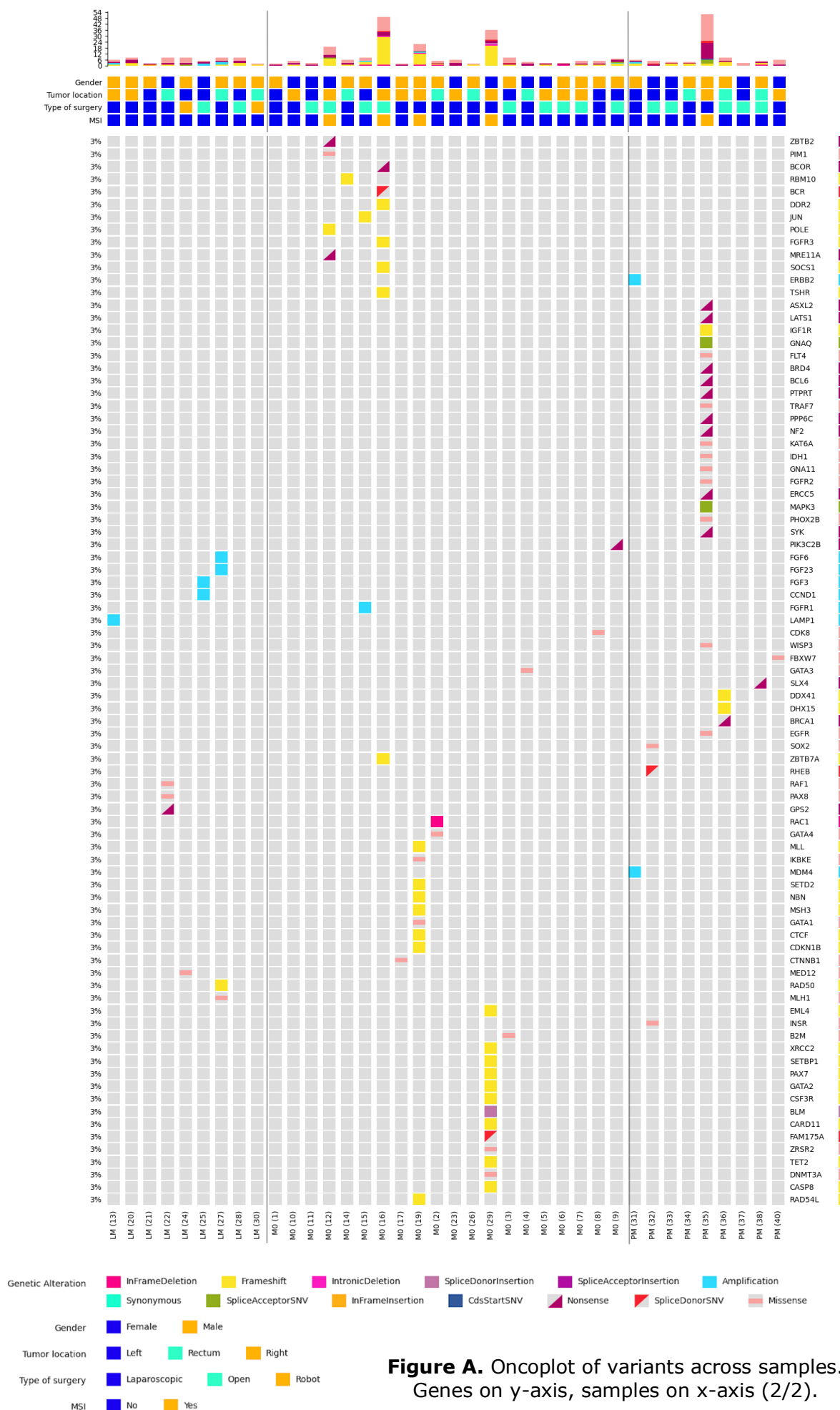
Well-known oncogenes related to CRC were investigated in each subgroup (Figure 2A). APC mutations occurred most in LM patients ($n = 8/9$, 88.89%), while TP53 mutations were equally divided into three groups. BRAF mutations did not occur in LM patients and were most present in PM patients ($n = 3/9$, 33.33%). PIK3CA and NRAS mutations were almost equally distributed. SMAD4 mutations and KRAS mutations were mostly found in LM groups ($n = 3/9$, 33.33% and $n = 5/9$, 55.56% respectively) and to a lesser extent in M0 groups. None of the gene alterations were significantly different when comparing all groups.

Although no gene variant was significantly higher in the PM group, we identified 34 genes which were solely affected in the PM group (Figure 2B). In total, 32 mutations and two amplifications occurred in 7/9 patients. The majority of the affected genes (91%) occurred in solely patient, except for three gene alterations (e.g., PAX3, FLCN and TCF7L2) in two PM patients, although none of these variants were on the same exon.

A total of 11 gene variants were solely detected in 6 LM patients (Figure 2C). Mutations in GPS2, PAX8, and RAF1 co-occurred in one patient, mutations in RAD50 and MLH1 were found in another patient. The same counts for amplifications of CCND1 and FGF3, and of FGF23 and FGF6. The MED12 mutation and LAMP1 amplification were identified in two different patients.

When taking the results of the LM and PM groups together, H3F3A, MITF, and NOTCH1 were mutated in one LM and one PM sample (Figure 2D). The latter occurred in the same PM patient, whilst two patients were involved in the LM group. Both H3F3A and MITF mutations were missense mutations, on the exact same location (exon 3 and 8 respectively). For NOTCH1 different variant types were involved.





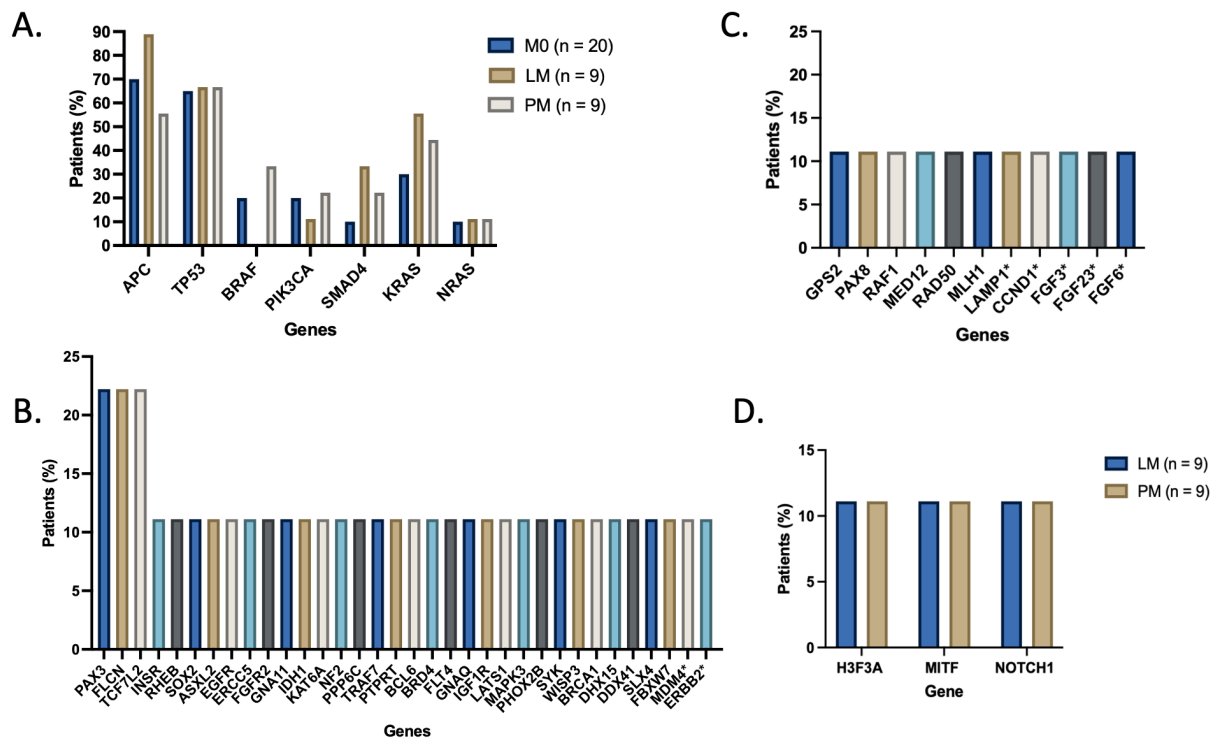


Figure 2. (A) Distribution of well-known oncogenes related to CRC between subgroups. **(B)** Genes mutated or amplified* in PM group only. **(C)** Genes mutated or amplified* in LM group only. **(D)** Genes mutated in LM and PM group (not in M0).

References

1. Mi H, Ebert D, Muruganujan A, Mills C, Albu L-P, Mushayamha T, et al. PANTHER version 16: a revised family classification, tree-based classification tool, enhancer regions and extensive API. Nucleic Acids Research. 2020;49(D1):D394-D403.
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3. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, et al. Gene Ontology: tool for the unification of biology. Nature Genetics. 2000;25(1):25-9.