Supplemental Table S1. Genetic alterations in G3/MMR glioblastoma cases

Only pathogenic or likely pathogenic mutations are included in this table.
Colored font: recurrent mutations
VAF, Variant allele fraction
VAF MMR in germline range: red font
VAF MMR in germline range with genetic testing confirmation: bold blue font
Pathway:
MMR, mismatch repair; RTK, receptor tyrosine kinase; MAPK, mitogen-activated protein kinase; PI3K, phosphatidylinositol 3-OH kinase; DDR, DNA damage repair; Chr Rm, chromatin remodeling; TF, transcription factor; SHH, sonic hedgehog; Wnt, wingless-type.

Case	Gene	Mutation/fusion	Amino acid	Effect	VAF %	NM	Pathway
M62							
	MSH6	c.3439-2A>G		Splice	50	NM_000179	MMR
	MSH6	c.3261delC	p.F1088fs	Frameshift	21	NM_000179	MMR
	MSH2	c.687dupA	p.A230fs	Frameshift	11	NM_000251	MMR
	NF1	c.2033dupC	p.I679fs	Frameshift	28	NM_001042492	MAPK
	PTPN11	c.1381G>A	p.A461T	Missense	22	NM_002834	MAPK
	PIK3CA	c.263G>A	p.R88Q	Missense	24	NM_006218	PI3K
	TERT	c.2072G>A	p.R691H	Missense	5	NM_198253	Telomere
	CDKN2A	c.202G>A	p.A68T	Missense	22	NM_000077	Cell cycle
	TP53	c.817C>T	p.R273C	Missense	24	NM_000546	p53
	TP53	c.473G>A	p.R158H	Missense	24	NM_000546	p53
	ATR	c.6221+1G>T		Splice	23	NM_001184	DDR
	FANCM		p.R579C	Missense	10	NM_020937	DDR
	DNMT3A	c.1046 _1047 insT	p.F350fs	Frameshift	25	NM_022552	Chr Rm
	DNMT3A	c.1790G>A	p.R597Q	Missense	20	NM_022552	Chr Rm
	SMARCA4	c.3403C>T	p.R1135W	Missense	20	NM_001128844	Chr Rm
	SMARCA4	c.2818G>A	p.E940K	Missense	25	NM_001128844	Chr Rm
	SMARCA4	c.1093G>C	p.E365Q	Missense	24	NM_001128844	Chr Rm
	EP300		p.G1179D	Missense	6	NM_001429	Chr Rm
M68							
	MSH6	c.3261delC	p.F1088fs	Frameshift	86	NM_000179	MMR
	FLT1	c.1294G>A	p.E432K	Missense	40	NM_002019	RTK
	NF1	c.4600C>T	p.R1534*	Nonsense	14	NM_001042492	MAPK
	PIK3CA	c.263G>A	p.R88Q	Missense	41	NM_006218	PI3K
	PTEN	c.379G>A	p.G127R	Missense	36	NM_000314	PI3K
	PTEN	c.492+3_492+4del		Splice	42	NM_000314	PI3K
	TSC2	c.3209C>T	p.T1070M	Missense	42	NM_000548	PI3K
	MTOR	c.4730C>T	p.A1577V	Missense	16	NM_004958	PI3K
	NOTCH1	c.1123G>A	p.A375T	Missense	40	NM_017617	NOTCH
	SMO	c.815C>T	p.A272V	Missense	26	NM_005631	SHH
	MYH11	c.5195G>A	p.R1732H	Missense	37	NM_002474	Cytoskeleton
	PTPRT	c.3218G>T	p.G1073V	Missense	41	NM_133170	Cell adhesion
	ATRX	c.3145dup	p.I1049fs	Frameshift	83	NM_000489	Telomere
	CDKN2A	Homozygous loss		Expression loss			Cell cycle
	CDKN2B	Homozygous loss		Expression loss			Cell cycle
	TP53	c.817C>T	p.R273C	Missense	37	NM_000546	p53
	TP53	c.916C>T	p.R306*	Nonsense	39	NM_000546	p53

	SMARCA4	c.1199C>T	p.A400V	Missense	25	NM_001128849	Chr Rm
	HNF1B	c.1004C>A	p.P335H	Missense	39	NM_000458	TF
F70							
	MSH2	c.942+3A>T		Splice	**50**	NM_000251	MMR
	CSF1R	c.2446C>T	p.R816C	Missense	41	NM_005211	RTK
	RET	c.2905C>T	p.R969W	Missense	40	NM_020975	RTK
	CBL	c.1256G>A	C419Y	Missense	71	NM_005188	RTK
	CBLB	c.1267G>A	p.V423M	Missense	21	NM_170662	RTK
	KRAS	c.40G>A	p.V14I	Missense	72	NM_033360	MAPK
	PTPN11	c.1492C>T	p.R498W	Missense	34	NM_002834	MAPK
	PIK3R1	c.1684C>T	p.R562C	Missense	6	NM_181523	PI3K
	PIK3CA	c.263G>A	p.R88Q	Missense	35	NM_006218	PI3K
	PTEN	c.697C>T	p.R233*	Nonsense	44	NM_000314	PI3K
	PTEN	c.867del	p.V290fs	Frameshift	12	NM_000314	PI3K
	TSC2	c.5257C>T		Splice	40	NM_000548	PI3K
	TSC2	c.2749C>T	p.R917W	Missense	9	NM_000548	PI3K
	TP53	c.638G>A	p.R213Q	Missense	42	NM_000546	p53
	TP53	c.742C>T	p.R248W	Missense	42	NM_000546	p53
	MRE11	c.1532del	p.N511fs	Frameshift	15	NM_005591	DDR
	CHD2	c.1719G>A		Splice	38	NM_001271	DDR
	CHD2	c.4172_4173dup	p.Q1392fs	Frameshift	38	NM_001271	DDR
	FUS	c.1542-7_1542-5del		Splice	36	NM_004960	DDR/splicing
	DNMT3A	c.958C>T	p.R320*	Nonsense	38	NM_022552	Chr Rm
	SMARCA1	c.595G>T	p.E199*	Nonsense	31	NM_001282874	Chr Rm
	PBRM1	c.835dup	p.I279fs	Frameshift	65	NM_018313	Chr Rm
	SETD2	c.4408C>T	p.P1470S	Missense	74	NM_014159	Chr Rm
	ASXL1	c.1717C>T		Splice	50	NM_015338	Chr Rm
	BCOR	c.1805del	p.P602fs	Frameshift	47	NM_001123385	Chr Rm/TF
	KEAP1	c.1430dup	p.F478fs	Frameshift	40	NM_012289	TF
	HNF1A	c.864del	p.P291fs	Frameshift	36	NM_000545	TF
	PAX5	c.350G>A	p.R117Q	Missense	7	NM_016734	TF
	CDK8	c.727del	p.T243fs	Frameshift	68	NM_001260	Transcription
	TAF1	c.1913G>A	p.R638H	Missense	36	NM_001286074	Transcription
	MKI67	c.4991_4992dup	p.E1665fs	Frameshift	36	NM_002417	Cell cycle
M72							
	MSH6	c.2615_2616dup	G873fs	Frameshift	47	NM_000179	MMR
	RERG	c.223C>T	p.R75*	Nonsense	23	NM_032918	MAPK
	GNAS	c.292A>G	p.N98D	Missense	14	NM_000516	MAPK
	PTEN	c.389G>A	p.R130Q	Missense	22	NM_000314	PI3K
	TSC2	c.1831C>T	p.R611W	Missense	32	NM_000548	PI3K
	TSC2	c.2220+1G>A		Splice	21	NM_000548	PI3K
	RPTOR	c.3709C>T	p.R1237C	Missense	21	NM_020761	PI3K
	PLCG1	c.2120G>A		Splice	14	NM_002660	PLCg
	NOTCH1	c.6074A>T	p.D2025V	Missense	25	NM_017617	NOTCH
	SMO	c.703G>A	p.A235T	Missense	13	NM_005631	SHH
	TP53	c.524G>A	p.R175H	Missense	26	NM_000546	p53
	TP53	c.542G>A	p.R181H	Missense	16	NM_000546	p53
	HELB	c.1566_1568delinsCC	p.E522fs	Frameshift	29	NM_033647	DDR
	HELB	c.186C>T		Splice	22	NM_033647	DDR
	POLQ	c.1564C>T	p.R522*	Nonsense	23	NM_199420	DDR

	ZUFSP	c.960C>T		Splice	17	NM_145062	DDR
	TIMELESS	c.2113C>T	p.R705*	Nonsense	17	NM_003920	DDR
	YY1	c.550_551del	p.S184fs	Frameshift	25	NM_003403	ChrRm/TF
	BAX	c.265C>T	p.R89*	Nonsense	26	NM_001291428	Apoptosis
M79							
	MSH2	Deletion				NM_000251	MMR
	MSH6	Deletion				NM_000179	MMR
	RET	c.2080C>T	p.R694W	Missense	9	NM_020975	RTK
	MAP2K4	c.533C>A	A178E	Missense	28	NM_001281435	MAPK
	PIK3CA	c.263G>A	p.R88Q	Missense	10	NM_006218	PI3K
	PTEN	c.968dup	p.N323fs	Frameshift	45 LOH	NM_000314	PI3K
	PDK1	c.1148G>A	p.R383H	Missense	29	NM_001278549	PI3K
	APC	c.2802_2805del	p.Y935fs	Frameshift	29	NM_000038	Wnt
	NOTCH1	c.4780C>T	p.R1594W	Missense	29	NM_017617	NOTCH
	ATRX	c.5406dup	p.R1803fs	Frameshift	64	NM_000489	Telomere
	RB1	c.180_181del	p.C61fs	Frameshift	43 LOH	NM_000321	Cell cycle
	TP53	c.844C>T	p.R282W	Missense	34	NM_000546	p53
	TP53	c.524G>A	p.R175H	Missense	32	NM_000546	p53
	CHEK1	c.484C>T	p.R162C	Missense	30	NM_001274	DDR
	FANCM	c.1397-5_1397-2dup		Splice	5	NM_020937	DDR
	ARID1B	c.1927-3del		Splice	32	NM_020732	Chr Rm
	BRD4	c.338G>A	p.R113H	Missense	27	NM_058243	Chr Rm
	KMT2C	c.2719C>T	p.R907*	Nonsense	7	NM_170606	Chr Rm
	BCOR	c.2580dup	p.R861fs	Frameshift	6	NM_001123385	Chr Rm/TF

Supplemental Table S2. MMR mutations in G1-G7 glioblastoma subgroups

Mutation color code: green, MUTYH; purple, POLE.

Mutations are pathogenic or likely pathogenic unless mentioned otherwise.
VUS, Variant of unknown significance.

VAF, Variant allele fraction; Mutations in the germline or somatic range are in red or blue, respectively.
When somatic mutations (e.g. TERT, PIK3CA) show VAF within 40-50%, the font for MMR mutations is black.
*Somatic mutations that appear only in one of multiple foci sequenced for the same patient.

MGCs, multinucleated giant cells: only 2 cases highlighted in yellow show MGCs and TP53 mutations. Both cases classify in the G5/PDGFRA molecular subgroup.

Case	TMB	Gene	Mutation	Amino acid	Effect	VUS	VAF%	NM	T53 status	MGCs	MMR IHC
G1/EGFR-amplified											
M66/TMZ	70								wild-type	Absent	
		MSH2	c.1216C>T	p.R406*	Nonsense		43	NM_000251			
		MSH6	c.2897C>T	p.T966I	Missense	VUS	38	NM_000179			
F58/TMZ	30								wild-type	Absent	
		MSH6	c.1447G>A	p.V483M	Missense		12	NM_000179			
		MSH2	c.1219C>T	p.L407F	Missense	VUS	12	NM_000251			
		MLH3	c.1744G>A	p.E582K	Missense	Benign	11	NM_001040108			
		MUTYH	c.226G>A	p.V76I	Missense	Benign	19	NM_001128425			
		POLE	c2588G>A	p.G863D	Missense	VUS	11	NM_006231			
M53/SC	75.4								wild-type	Absent	
		MSH2	c.2500G>T	p.A834S	Missense		7	NM_000251			
		MSH2	c.1550C>A	p.A517E	Missense	VUS	5	NM_000251			
		POLH	c.716C>A	p.S239*	Nonsense		5	NM_006502			
		POLH	c.1893C>A	p.D631E	Missense	Benign	5	NM_006502			
M31	4.2								mutant	Absent	
		MLH3	c.3292del	p.R1098fs	Frameshift		43	NM_001040108			
M56	5.2								mutant	Absent	Retained
		MSH4	c.101del	p.G34fs	Frameshift		41*	NM_002440			

		MSH4	c.2440G>C	p.V814L	Missense		34*	NM_002440			
M60	3.6								wild-type	Absent	
		MLH1	c.1154G>A	p.R385H	Missense		11	NM_000249			
M67	5.3								wild-type	Absent	
		POLE	c.4772G>A	p.W1591*	Nonsense		11	NM_006231			
G2/FGFR3											
M60	0.8								wild-type	Absent	
		MSH6	c.3018C>G	p.Y1006*	Nonsense		36	NM_000179			
F62	5.8								wild-type	Absent	
		MUTYH	c.1187G>A	p.G396D	Splice		46	NM_001128425			
G3/NF1											
M64	6.3								mutant	Absent	
		MSH6	c.2392C>G	p.L798V	Missense		47	NM_000179			
F86	5.3								wild-type	Present	
		MSH2	c.2211-5T>G		Splice	VUS	42	NM_000251			
G5/PDGFRA											
M64	1.7								mutant	Present	
		MUTYH	c.1147del	p.A385fs	Frameshift		47	NM_001128425			
F73	6.3								wild-type	Absent	
		MSH2	c.2072T>C	p.I691T	Missense	VUS	44	NM_000251			
		MSH2	c.2503A>G	p.N835D	Missense	VUS	42	NM_000251			
M77	5.3								mutant	Present	
		MSH3	c.2335C>T	p.R779C	Missense		58	NM_002439			
G6/Multi-RTK											
M40	8.9								mutant	Focal post-radiation	MSH2/MSH6 retained MLH1/PMS2 patchy attenuated
		MSH3	c.172_189dup	p.A58_P63dup	In-frame insertion	Benign	16	NM_002439			
M69	7.4								wild-type	Absent	
		MSH2	c.2267C>G	p.T756S	Missense	VUS	46	NM_000251			

Supplemental Table S3. Mutation % frequency in glioblastoma subgroups for major pathways.

Gene	G3/MMR n=5	GBM–MMR* n=213	G1/EGFR↑ n=60	G1/EGFRm n=10	G2/FGFR3 n=7	G3/NF1 n=51	G4/RAF n=6	G5/PDGFRA n=15	G6/Multi-RTK n=22	G7/Other n=42
MAPK path	100	80.3	100	100	100	100	100	100	100	0
PTEN	80	55.9	53.3	70.0	57.1	56.9	66.7	26.7	54.5	64.3
PIK3CA	80	15.0	15.0	0	14.3	17.6	0	26.7	13.6	14.3
PIK3CA R88Q	100	2.9	0	0	0	0	0	0	0	16.7
PI3K path[2]	100	74.2	73.3	70.0	71.4	76.5	66.7	53.3	68.2	85.7
TERT	0[1]	84.8	94.8	80.0	85.7	80.4	83.3	60.0	77.3	90.5
CDKN2A↓	20.0	60.8	78.3	40.0	71.4	70.6	80.0	66.7	54.5	26.2
G1 phase[3]	60.0	86.8	88.3	100	100	84.3	80.0	93.3	81.8	83.3
TP53	100	33.3	15.0	60.0	14.3	31.4	16.7	33.3	50.0	52.4
TP53 R mut	100	26.1	22.2	16.7	0	30.8	0	20.0	0	40.9
p53 path[4]	100	52.6	31.7	60.0	42.9	41.2	33.3	66.7	77.3	81.0
DDR path[5]	80.0	19.4	15.5	30	28.6	19.6	16.7	26.7	18.2	19.0
SWI/SNF[6]	80.0	8.0	10.0	0	0	13.7	16.7	6.7	9.1	0
Other ChRm[7]	80.0	22.6	20.0	10.0	85.7	18.0	0	20.0	31.8	23.8

GBM, glioblastoma; ↑, gene amplification; ↓, homozygous CN loss; m, point mutation; path, pathway; R mut, Arg mutations; DDR, DNA damage response; ChRm, chromatin remodeling.

PIK3CA R88Q and TP53 R mutations are calculated as % from the number of cases with mutant PIK3CA and TP53, respectively.

*All GBM cases without G3/MMR subgroup cases.

[1]In one case, whole exome NGS was performed which did not include promoter regions (see Figure 4B).

[2]% cases with at least one alteration in *PTEN*, *PIK3CA*, *PIK3R1*, or *MTOR*.

[3]% cases with *CDKN2A*, *CDK4*/*6* or *RB1* alterations.

[4]% cases with *TP53, MDM2*, *MDM4*, *RPL5* and *PPM1D or XPO1* alterations.

[5]% cases with pathogenic or likely pathogenic mutations or in various genes involved in DNA repair by homologous recombination (*ATM, ATR, CHEK1, BRCA2, BRCA1, BRIP1, GEN1, CHD4, HELB, MRE11, NBN, RECQL4, RAD51, ERCC3, ERCC5, FANCI, FANCM*) or nonhomologous end joining *(PRKDC, ASTE1, CHD2, SHPRH, PARP2)*; the hypermutator genes involved in nucleotide excision repair are not included (see Supplemental Table S2).

[6]% cases with SWI/SNF complex *ARID1A*, *ARID1B*, *ARID2*, *SMARCA1*, *SMARCA4, SMARCD1* or *PBRM1* pathogenic mutations.

[7]% cases with either *YEATS4* amplification or *DNM3TA*, *TET2*, *EP300*, *KDM5C*, *KDM6A*, *KMT2C*, *KMT2D, NPM, BCOR, YY1, ASXL1* or *CREBBP* pathogenic mutations.

Supplemental Table S4. RTK fold overexpression in G3/MMR tumors.

RTK, receptor tyrosine kinase.

Overexpression values ≥4 fold are colored in red.

*Mean +/- SEM for amplified *EGFR, PDGFRA* and *MET* expression from the G1/EGFR-amplified, G5/PDGFRA and G6/Multi-RTK subgroups, respectively, is shown in red for comparison.

RTKs	**M62**	**M68**	**F70**	**M72**	**M79**	*G1/EGFR↑ n = 30	*G5/PDGFRA n = 11	*G6/MET n = 5
EGFR	1.1	2.1	1.2	3.5	1.3	27.8 +/- 3.7		
ERBB2	1.0	1.6	0.5	2.9	6.7			
ERBB4	5.2	0.6	1.8	0.0	0.1			
MET	1.8	0.8	2.1	25.0	2.1			51.8 +/- 14.6
FGFR1	1.7	2.2	7.4	4.9	0.1			
FGFR2	5.8	1.7	0.9	0.2	2.2			
CSF1R	3.9	0.5	0.6	1.2	1.0			
PDGFRA	2.0	4.3	5.9	1.7	2.5		46.0 +/- 8.5	
KDR	0.8	2.8	6.9	0.1	2.4			
NTRK3	1.1	0.9	4.5	0.0	0.5			
RET	1.4	1.0	5.2	0.1	0.5			
ALK	2.0	4.1	1.4	0.7	1.4			
EPHA3	0.8	3.8	1.9	5.2	0.3			
EPHA5	1.4	0.7	9.1	0.5	0.5			
EPHB4	0.7	0.8	0.5	6.3	2.0			
DDR2	2.8	1.2	0.6	5.0	0.8			

Supplemental Table S5. FOS-JUN transcription factors fold overexpression in G3/MMR tumors.

Overexpression values ≥3 fold are colored in red.

Transcription factors	M62	M68	F70	M72	M79
FOS	4.4	1.5	2.1	6.4	5.3
FOSB	3.9	0.7	1.1	25.0	10.9
FOSL1	1.6	1.7	4.9	23.1	4.6
FOSL2	1.5	2.2	1.7	6.4	3.0
JUN	1.5	2.0	2.6	6.9	3.4
JUNB	4.4	1.2	1.6	5.9	3.4
JUND	1.1	0.5	0.3	1.0	0.9

Supplemental Table S6. Fold overexpression of feedback inhibitors of the canonical MAPK-PI3K pathways in G3/MMR tumors.

Overexpression values ≥2 fold are colored in red.

MAPK-PI3K feedback Inhibitors	M62	M68	F70	M72	M79
DUSP1	3.1	0.6	1.2	5.5	2.0
DUSP4	0.4	0.7	1.3	19.6	1.4
DUSP5	3.3	2.4	6.9	7.8	2.0
DUSP6	1.2	1.2	3.7	5.6	1.6
ERRFI1	0.7	2.1	1.3	10.3	1.3
SPRY1	1.8	2.8	4.5	1.8	2.1
SPRY4	0.4	2.1	4.3	2.7	2.7