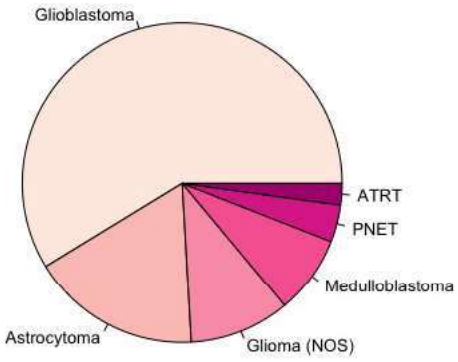
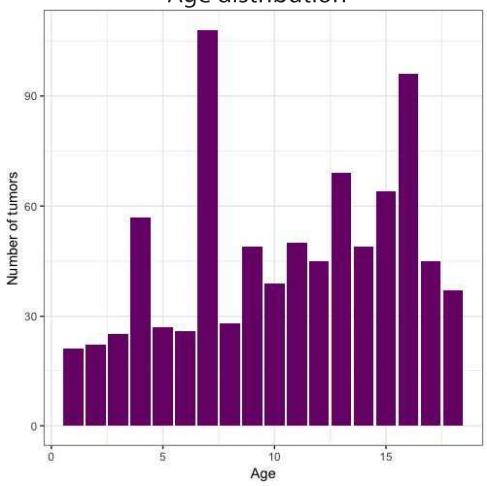
Cohort of 1215 pediatric cancers by subtype

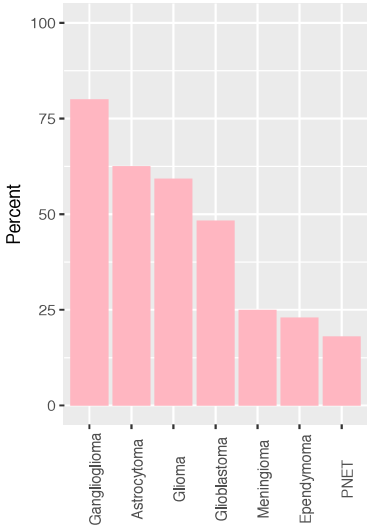
Brain tumors, n = 219



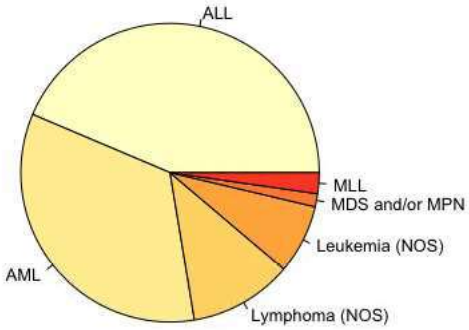
Age distribution



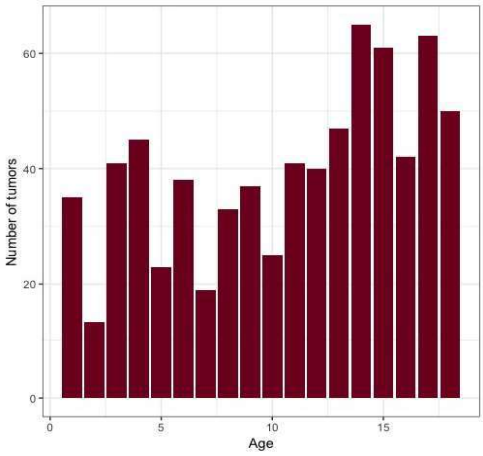
% of RAS/MAPK mutant tumors by subtype



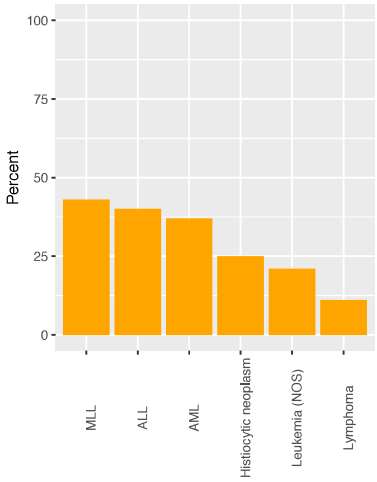
Heamatological malignancies, n = 217



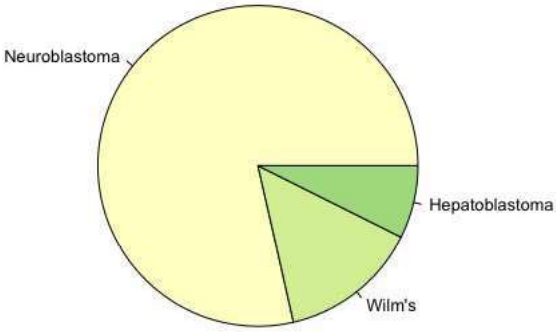
Age distribution



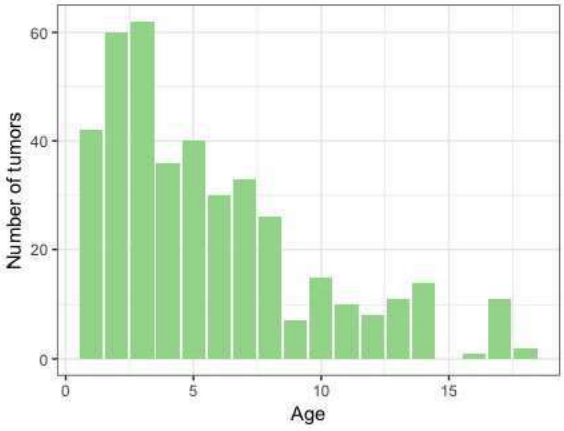
% of RAS/MAPK mutant tumors by subtype



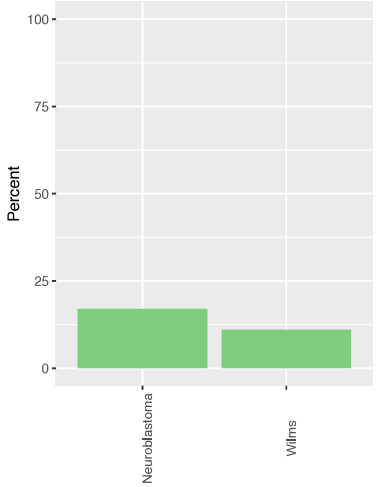
Extracranial embryonal, n = 214



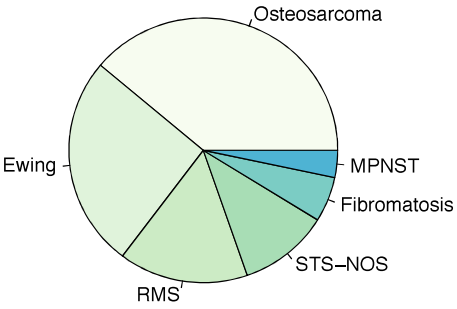
Age distribution

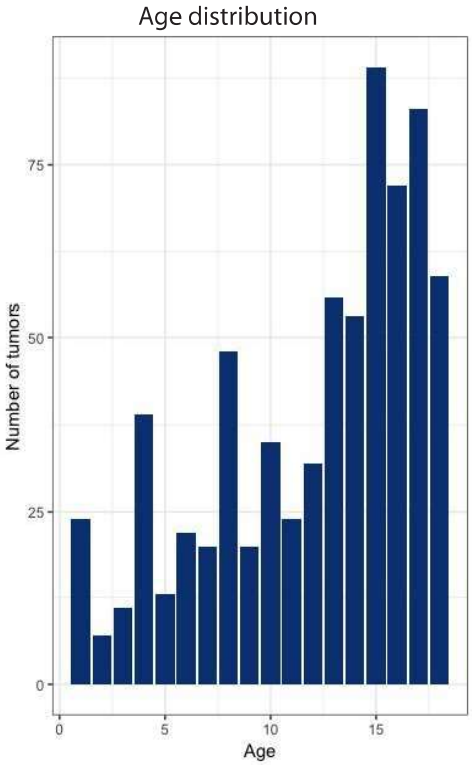


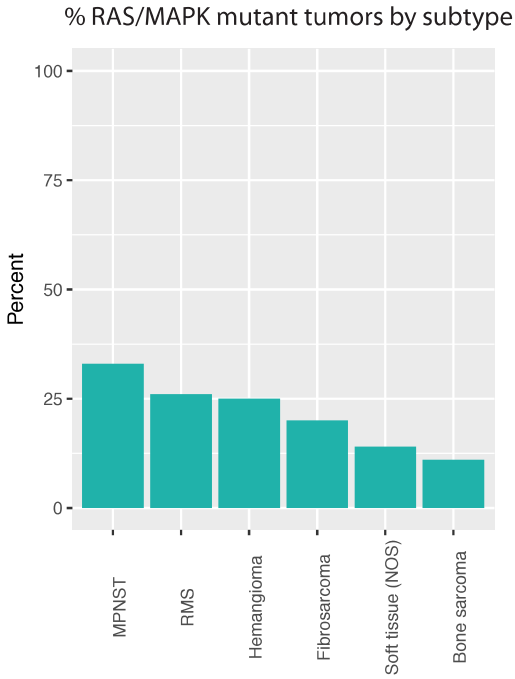
% of RAS/MAPK mutant tumors by subtype



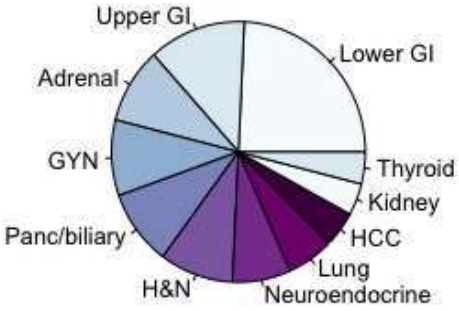
Sarcoma, n = 288

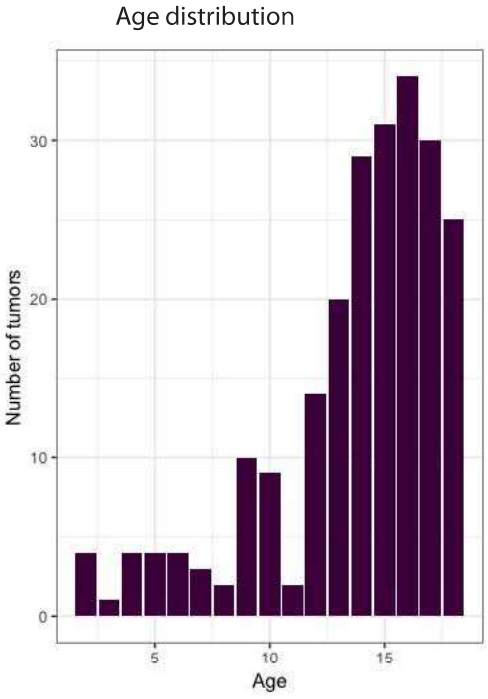


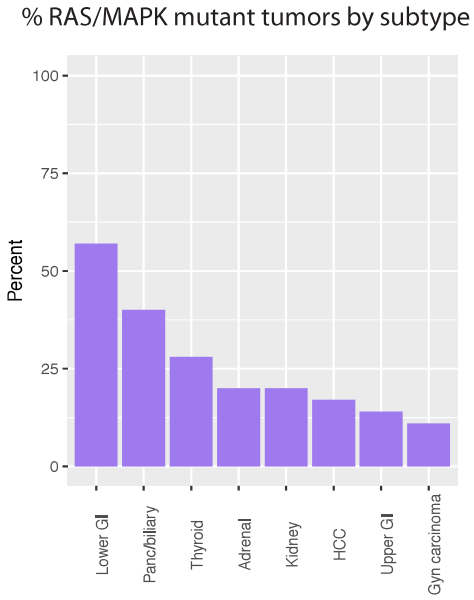




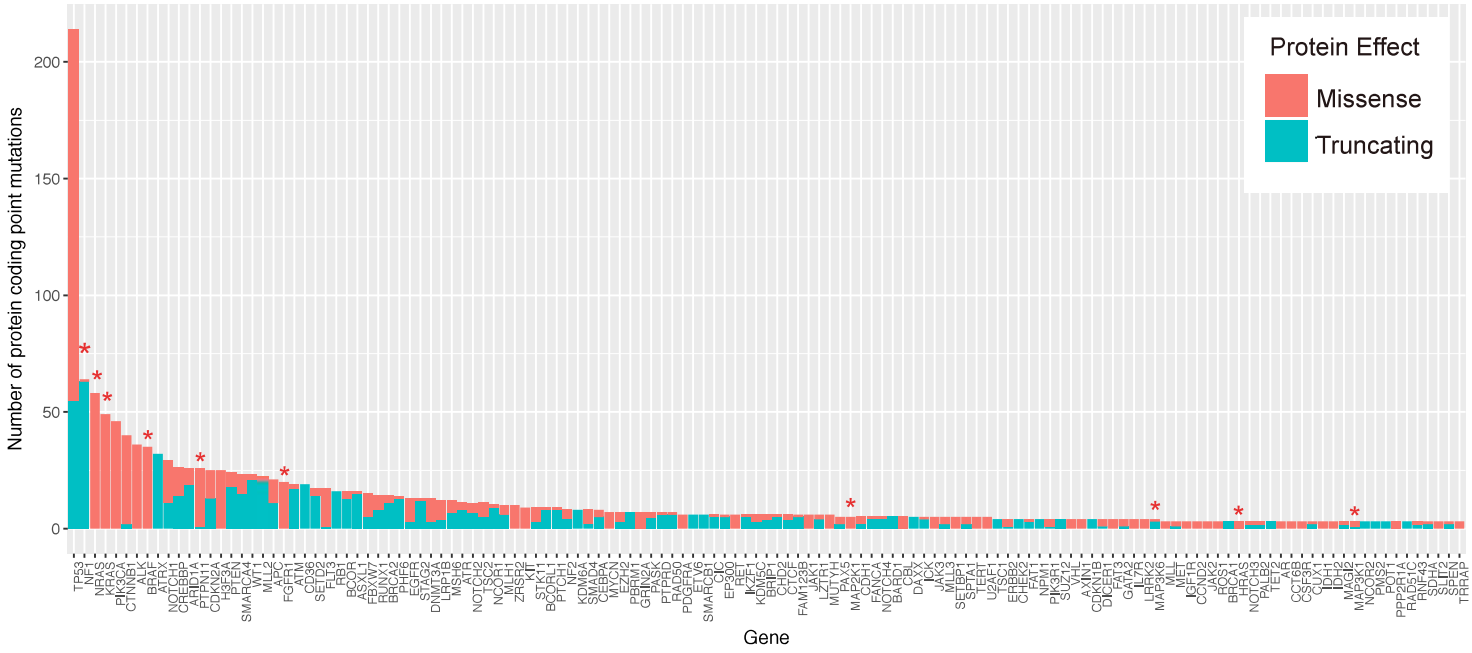
Carcinoma, n = 83







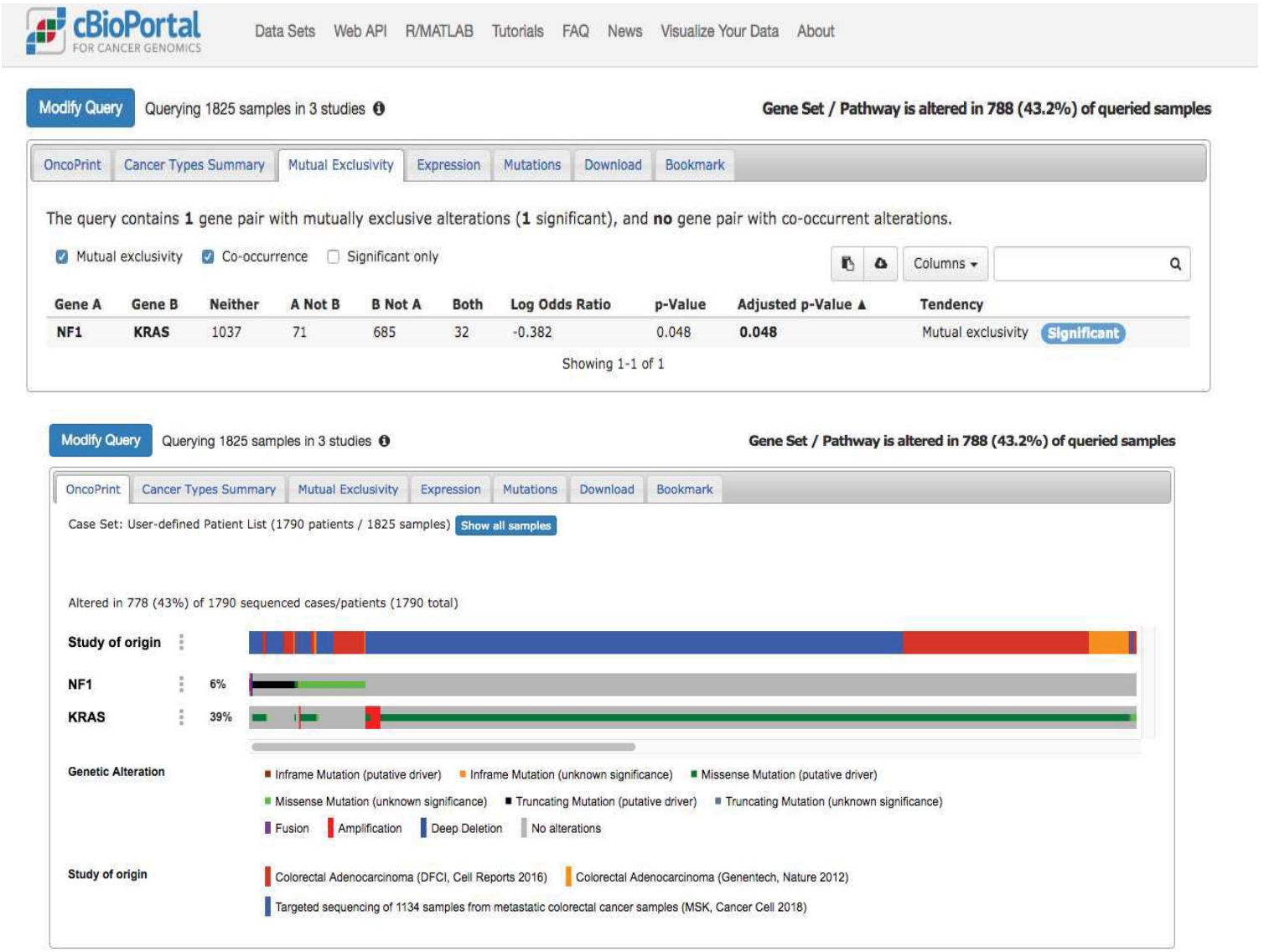
Supplemental Figure 1: Major subtypes in 1215 pediatric cancers (Ages 0-18). Colored bar charts display age distribution per subtype and grey barcharts display frequency of RAS/MAPK pathway mutations



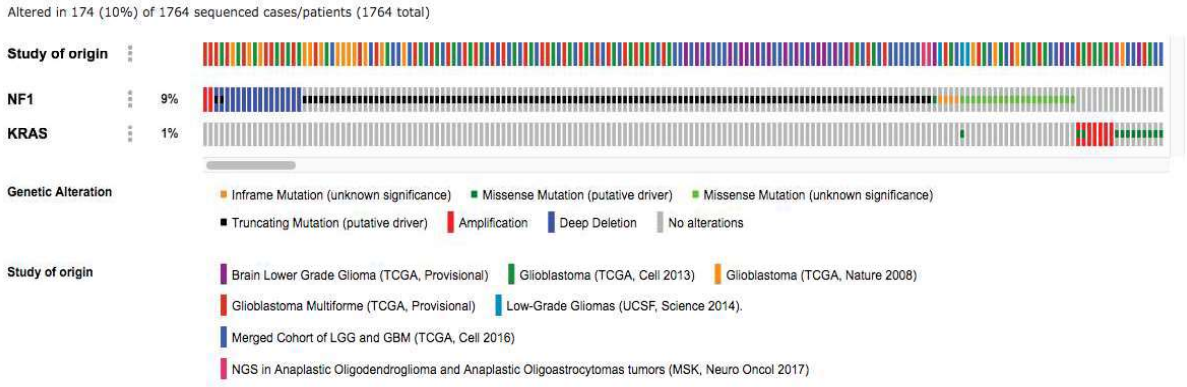
Supplemental Figure 2: Bar plot displaying frequency of protein coding point mutations resulting in a single amino acid change or truncation in 1215 pediatric tumors. Genes with less than 3 occurrences in the cohort were excluded.

* = RAS/MAPK pathway actors

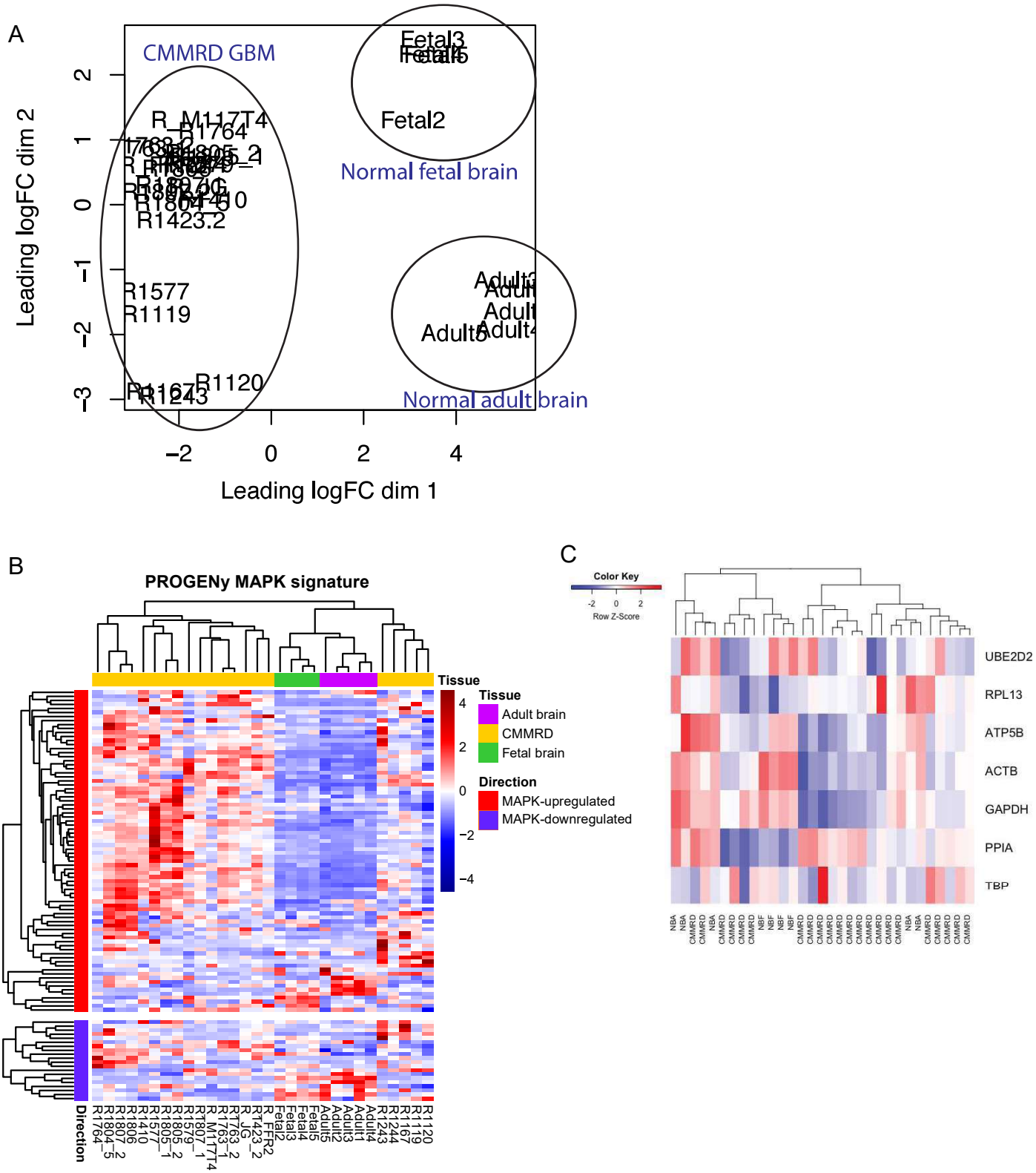
A



B



Supplemental Figure 3: Confirmatory tissue specific preferences for MAPK pathway mutations in 1825 colorectal cancers (A) and 1764 gliomas (B). KRAS dominance is observed in GI vs. NF1 dominance in CNS tumors, mimicking pediatric data.

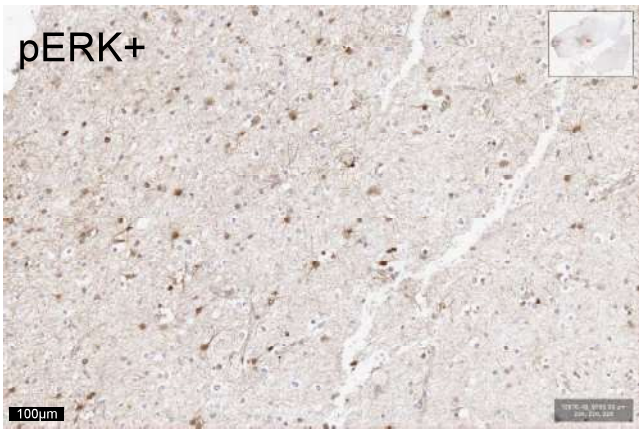


Supplemental Figure 4: A) Multidimensional scaling plot demonstrating clustering of CMMRD tumors, normal fetal brain, and normal adult brain based on RNA sequencing count data. CMMRD tumors cluster distinctly from normal fetal and adult brain. Normal fetal brain and adult brain also show distinct expression patterns. B) Unsupervised clustering of CMMRD tumors (n=22), normal fetal brains (n=4), and normal adult brains (n=5) based on expression of PROGENy 100 MAPK pathway transcriptional output signature genes. C) Control clustering for all tumors based on expression of a set of randomly selected housekeeping genes demonstrates that clustering distinctly does not occur nonspecifically.

Replication Repair Deficient (RRD) hypermutant brain tumors

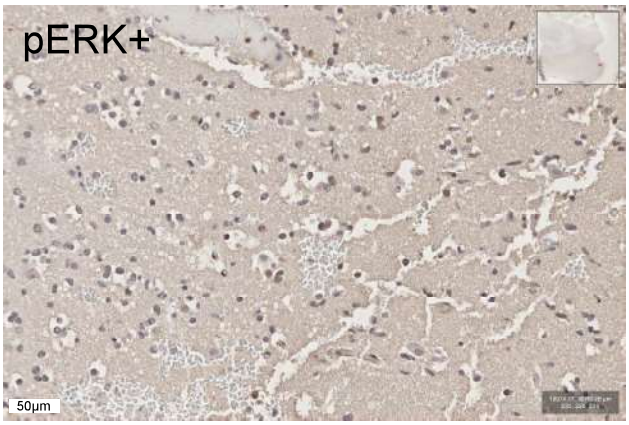
1. Patient ID: MMR138
Tumor Type: Anaplastic Astrocytoma
Germline: *POLE* P436R mutation
Mutation burden: 191 mut/mb

RAS/MAPK Gene	AA Change	VAF
NRAS	S106A	0.08
NRAS	E98K	0.08
MAP3K1	S418Y	0.05
MAP2K1	D67N	0.07
NF1	T1843A	0.1



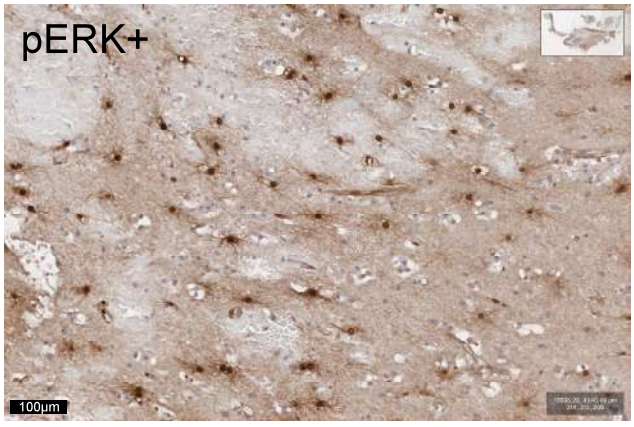
2. Patient ID: MMR142
Tumor Type: Anaplastic Oligodendrioglioma
Germline: *MSH2* Exon 1-6 deletion
Mutation Burden: 194 mut/mb

RAS/MAPK Gene	AA Change	VAF
MAP3K1	P1115Q	0.07
MAP3K1	P1493H	0.06



3. Patient ID: MMR101
Tumor Type: Glioblastoma
Germline: Biallelic *PMS2* deletion
Mutation Burden: 541 mut/mb

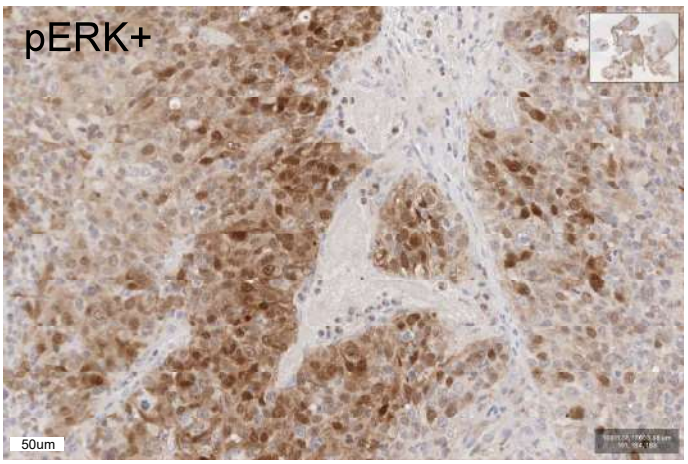
RAS/MAPK Gene	AA Change	VAF
NF1	A463T	0.39
NF1	G2003X	0.14
NF1	A2753T	0.11
NF1	S413X	0.06
RAF1	A628T	0.25
RAF1	N140T	0.12
MAP2K2	R371W	0.06



Replication Repair Deficient (RRD) hypermutant brain tumors

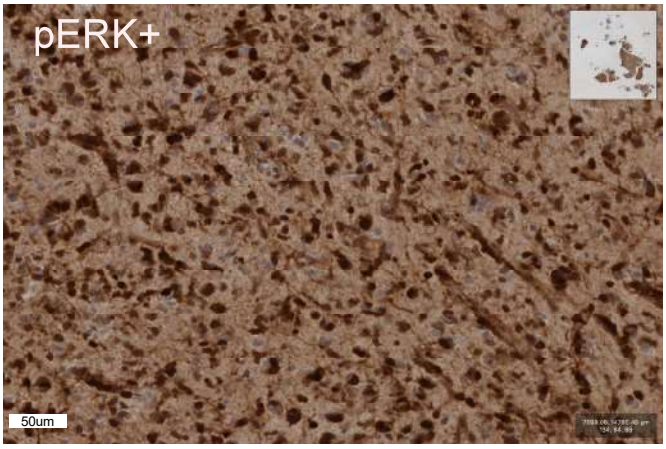
4. Patient ID: MMR100
Tumor Type: Glioblastoma Multiforme
Germline: Biallelic *PMS2* deletion
Mutation burden: 496 mut/mb

RAS/MAPK Gene	AA Change	VAF
NF1	A198S	0.42
NF1	R1416M	0.42
NF1	R2237X	0.38
NF1	R720Q	0.37
NF1	T1013M	0.05
NF1	L121I	0.04
MAP3K2	Q429H	0.53
MAP2K2	R164M	0.42
MAPK1	P268S	0.39
BRAF	D213N	0.11
KRAS	T50I	0.07
MAPK3	D192N	0.05



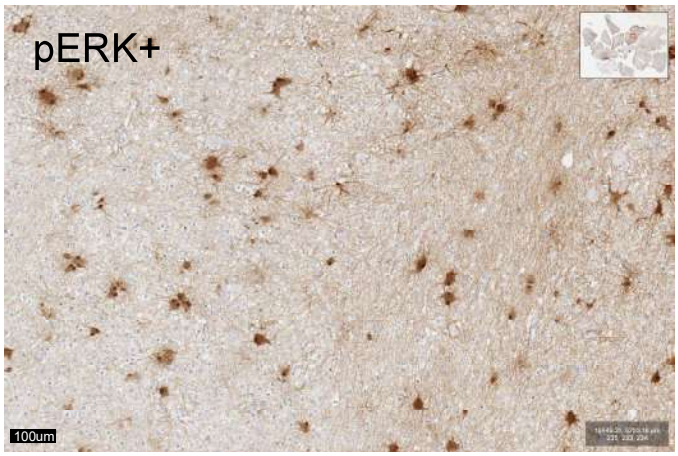
5. Patient ID: MMR190
Tumor Type: Glioblastoma Multiforme
Germline: Biallelic *PMS2* mutation
Mutation burden: 183 mut/mb

RAS/MAPK Gene	AA Change	VAF
NF1	I1679V	0.51
NF1	R156H	0.47
NF1	E152*	0.44
NF1	A422T	0.411
NF1	R652H	0.25
NF1	Q1775*	0.14
NF1	M747I	0.08
NF1	R816*	0.07
NF1	A548D	0.07
NF1	A887T	0.04
HRAS	V109M	0.33
HRAS	D105Y	0.08
HRAS	R68W	0.02
MAP3K1	D806N	0.49
MAP3K1	V906I	0.49
MAP3K1	R1368K	0.12
MAP3K1	R306H	0.04



6. Patient ID: MMR152
Tumor Type: Glioblastoma Multiforme
Germline: Biallelic MMR
Mutation burden: 182 mut/mb

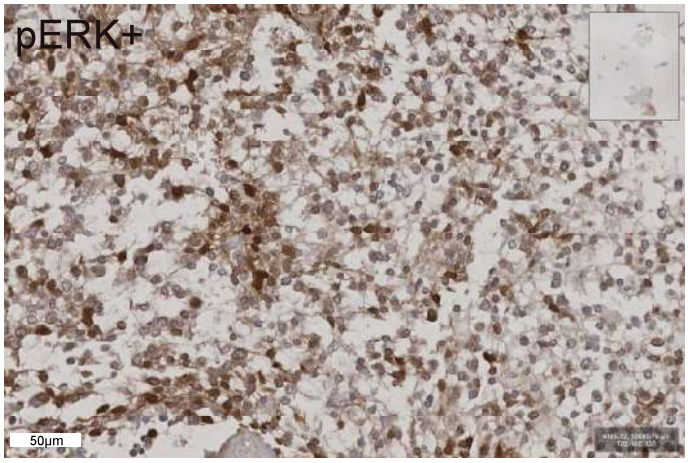
RAS/MAPK Gene	AA Change	VAF
NRAS	E63K	0.34
NF1	A2737V	0.18



Replication Repair Deficient (RRD) hypermutant brain tumors

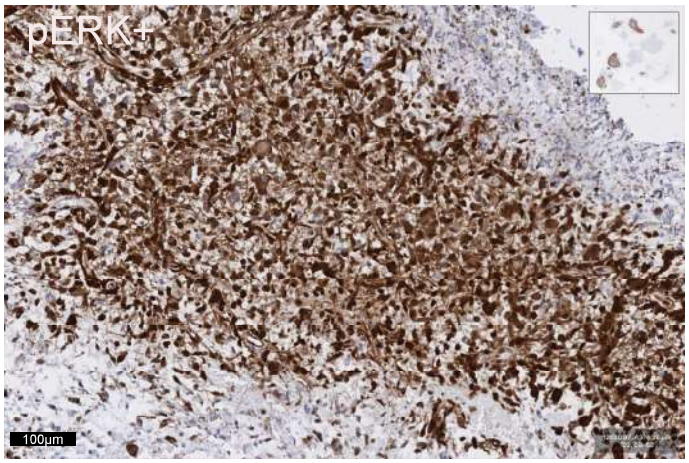
7. Patient ID: MMR134
Tumor Type: Glioblastoma Multiforme
Germline: Biallelic *PMS2* mutation
Mutation burden: 50 mut/mb

RAS/MAPK Gene	AA Change	VAF
NF1	R1554M	0.48
NF1	G1692E	0.48
NF1	A2603T	0.47
MAP3K1	P301T	0.44
MAP3K1	L639M	0.45
BRAF	G104R	0.42
MAP2K1	E333D	0.48



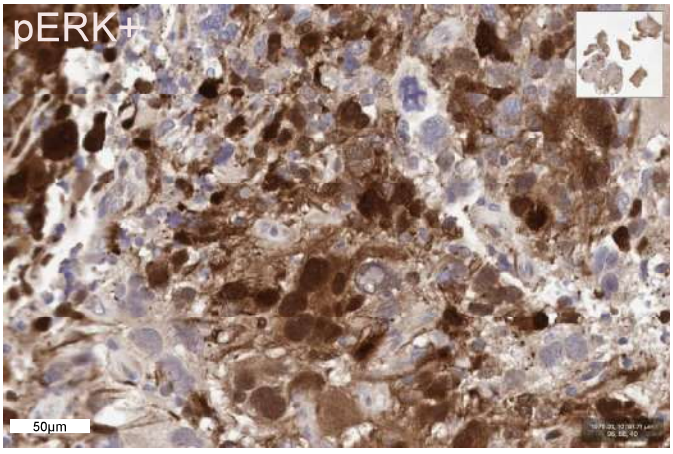
8. Patient ID: MMR1293
Tumor Type: Glioblastoma Multiforme
Germline: Biallelic MMR
Mutation burden: 161 mut/mb

RAS/MAPK Gene	AA Change	VAF
NF1	G2024X	0.28
NF1	T1546I	0.26
NF1	S2817P	0.22
MAPK1	A352V	0.54



9. Patient ID: MMR1273
Tumor Type: Glioblastoma Multiforme
Germline: Biallelic PMS2 mutations, *DICER1* mutation
Mutation burden: 121 mut/mb

RAS/MAPK Gene	AA Change	VAF
NF1	V1212I	0.345

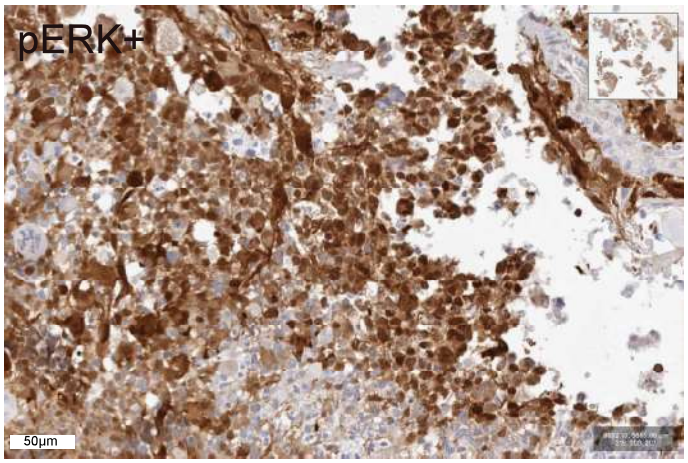


Replication Repair Deficient (RRD) hypermutant brain tumors

Patient ID: MMR109
Tumor Type: Glioblastoma Multiforme
Germline: Biallelic *MSH2* mutations
Mutation burden: Not available

RAS/MAPK Gene AA Change VAF

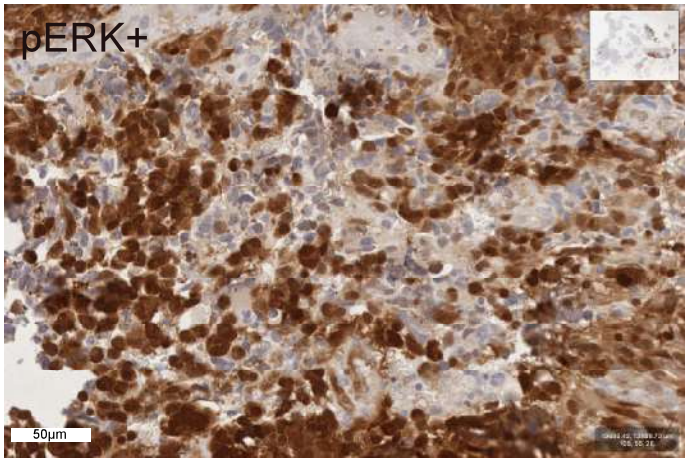
Not available



Patient ID: MMR110
Tumor Type: Glioblastoma Multiforme
Germline: Biallelic *MSH2* mutations
Mutation burden: Not available

RAS/MAPK Gene AA Change VAF

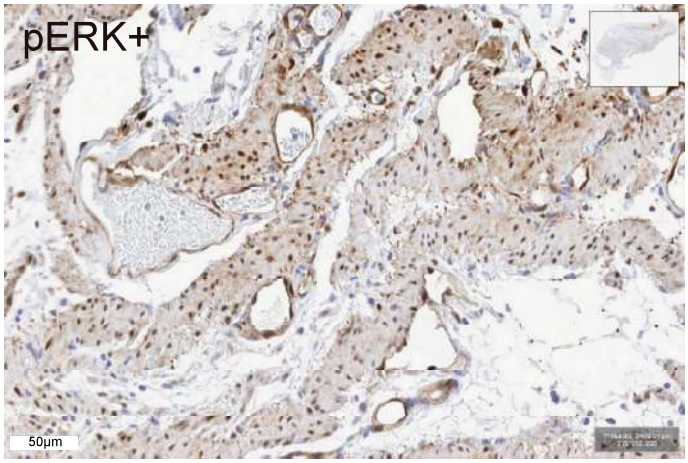
Not available



Replication Repair Deficient (RRD) hypermutant colorectal tumors

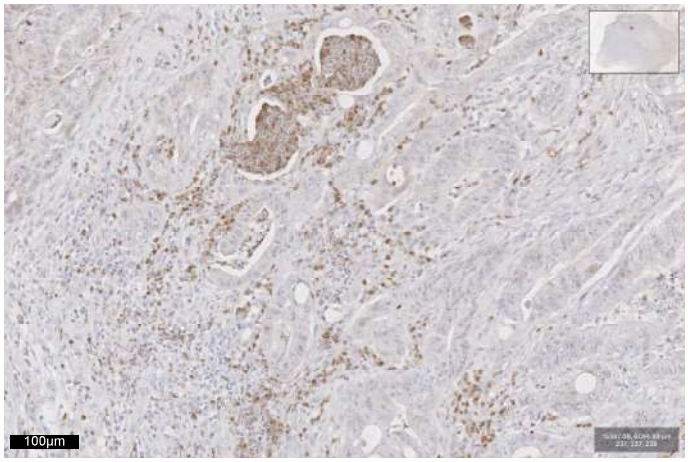
12. Patient ID: MMR66
Tumor Type: Colorectal Adenocarcinoma
Germline:
Mutation burden: 99 mut/mb

RAS/MAPK Gene	AA Change	VAF
NRAS	Y137C	0.1
MAP3K1	A660V	0.14



13. Patient ID: MMR138
Tumor Type: Colorectal Adenocarcinoma
Germline: *POLE* P436R mutation
Mutation burden: 495 mut/mb

RAS/MAPK Gene	AA Change	VAF
NRAS	S106A	0.28
NRAS	E98K	0.27
NRAS	K170T	0.08
KRAS	Q22H	0.2
MAP3K1	S418Y	0.1
MAP2K1	D67N	0.25
NF1	N45D	0.16
NF1	G57S	0.16
NF1	C673G	0.18
NF1	G949X	0.18
NF1	T1843A	0.19
NF1	R2083C	0.15

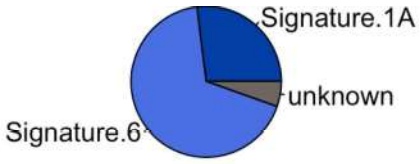


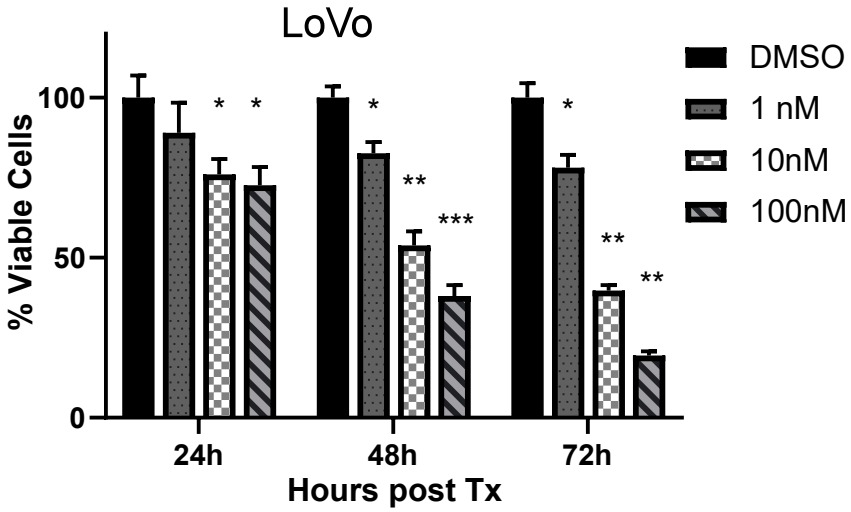
Supplemental Figure 5: Immunohistochemical staining of 10 hypermutant replication repair deficient gliomas and 1 hypermutant replication repair deficient colorectal cancers. Germline status is shown, along with various mutations in RAS/MAPK pathway genes and their variant allele fractions. Black bars indicate 100µm; white scale bars indicate 50µm.

LoVo Mutation Burden: 66 mut/mb

RAS/MAPK pathway mutations

Gene	AA Change	Type
KRAS	G13D	Missense
NF1	R1696Q	Missense
RAF1	V98A	Missense

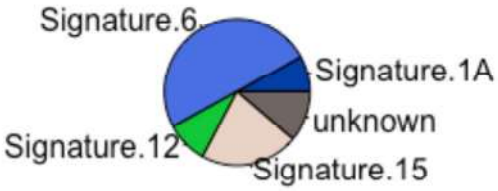


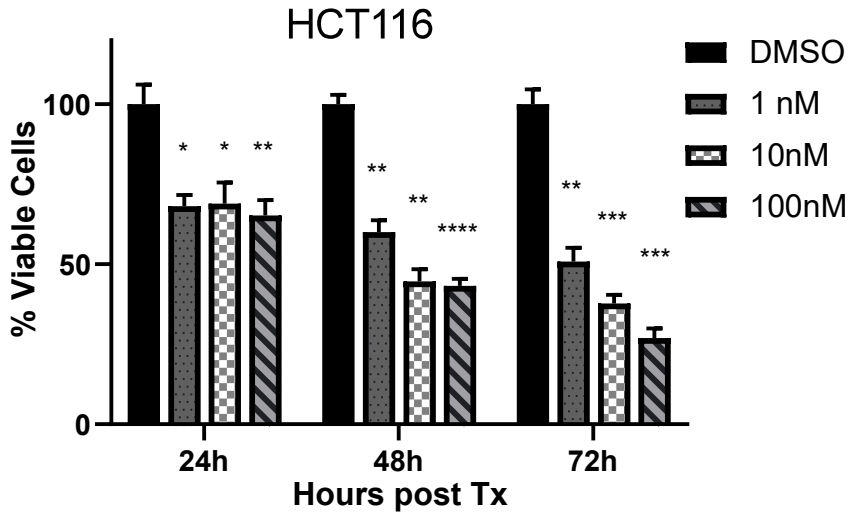


HCT-116 Mutation Burden: 88 mut/mb

RAS/MAPK pathway mutations

Gene	AA Change	Type
KRAS	G13D	Missense
MAP3K1	R351C	Missense
NF1	T676fs	Frameshift



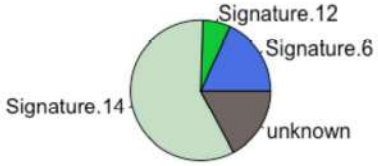


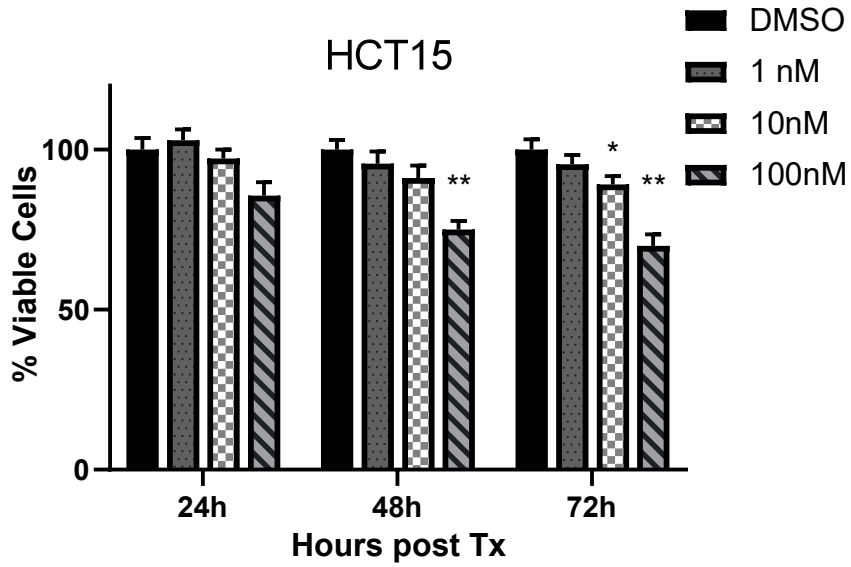
HCT15 Mutation Burden: 212 mut/mb

Gene	AA Change	Type
POLD1	R689W	Missense

RAS/MAPK pathway mutations

Gene	AA Change	Type
KRAS	G13D	Missense
MAP3K1	p.P1474H	Missense
MAP3K1	p.A552V	Missense
MAP3K4	p.I484V	Missense
MAP3K4	p.K814R	Missense
MAP3K6	p.P870Q	Missense
NF1	p.A74D	Missense
NF1	p.P2477S	Missense
NF1	p.A2617V	Missense





Supplemental Figure 6: Three established hypermutant colorectal cancer MMR-deficient cell lines (LoVo, HCT116, and HCT15) harbor several RAS/MAPK pathway promoting mutations and COSMIC signatures related to MMR and/or polymerase exonuclease deficiency (*left*). Mutational signatures reflect MMR deficiency as a source of hypermutation. Cell lines were treated with Trametinib concentrations ranging from 1nM - 100 nM and viable cells were measured using a hemocytometer at 24, 48, and 72 hours post treatment (*right*). Cell number percentages were normalized to DMSO. Three technical replicates were analysed and are shown as mean and SD. Statistical significance was assessed by ANOVA (*p < 0.05; **p < 0.01; ***p < 0.001; and ****p < 0.0001).

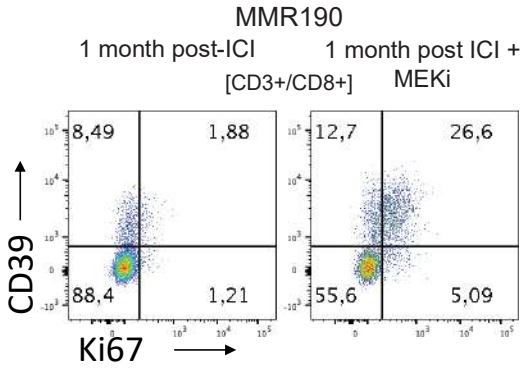
A

MMR190 - Glioblastoma multiforme

Mutaional Burden = 328 mut/mb

Gene	AA change	Allelic Frequency
NF1	R156H	0.526
NF1	A422T	0.424
NF1	M747I	0.087
NF1	E1582*	0.458
NF1	Q1775*	0.094
MAP3K1	R377fs	0.235
MAP3K2	T293A	0.333
PTPN11	R502W	0.575

B



Supplemental Figure 7: Combination anti-PD1 and MEK inhibition promotes an anti-tumor immune response. A) Mutational burden and RAS/MAPK pathway mutations detected by exome sequencing of GMB tumor from patient MMR190. B) Expression of CD39 on $Ki67^{+}CD3^{+}CD8^{+}$ PBMCs using flow cytommetry post anti-PD1 treatment alone (left) and combination anti-PD1 + Trametinib treatment (right). * = stopgain mutation; fs = frameshift mutation; ICI = anti-PD1; MEKi = Trametinib.