

Supplementary figures and
tables

- **Molecular diagnosis of diffuse glioma using a chip-based digital PCR system to analyze *IDH*, *TERT*, and *H3* mutations in the cerebrospinal fluid**

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Supplementary table 1

Assay data

mutation	Assay ID	product length(bp)	Forward Primer	Reverse Primer	Wild type probe	Mutation probe
IDH R132H	ANDJ4XD	88	GCAAAATCACATTATTGCCAACA	CTTGTGAGTGGATGGGTAAAACCT	AAGCATGACGACCTAT	TAAGCATGATGACCTATG
H3K27M	ANFVT27	107	CTTTGTCCCATTTTTTTCCTGTTT	GAAGCAACTGGCTACAAAAGCC	CGCACTCTTGCGAGC	CGCACTCATGCGAGC
TERT promoter mutation C228T	Hs000000092_rm	NA	NA	NA	NA	NA
TERT promoter mutation C250T	Hs000000093_rm	NA	NA	NA	NA	NA

NA: not available

Supplementary table 2

IDH1R132H mutation and H3K27M mutation

Stage 1	Stage 2		Stage 3	
96°C	56.0°C	98.0°C	60.0°C	10.0°C
10 min	2 min	30 s	2 min	∞
1x (Hold)	39x (Cycles)		1x (Hold)	

TERT promoter mutation C228T and C250T

Stage 1	Stage 2		Stage 3	
96°C	55.0°C	98.0°C	55.0°C	10.0°C
10 min	2 min	30 s	2 min	∞
1x (Hold)	54x (Cycles)		1x (Hold)	

Supplementary table 3

	1 reaction μL	Final concentration $\text{ng}/\mu\text{L}$
Digital PCR master mix v2	7.25	1x
Taqman Assay 20x (primer + probe)	0.75	1x
Nuclease free water	6.5-X	
Template DNA	X	X/14.5
Total	14.5	

X: Up to 6.5 μL

Supplementary table 4

Taqman Assay	X	Y
IDH1 R132H mutation	1200	4000
H3F3A K27M mutation	1400	4000
TERT promoter mutation C228T	350	1200
TERT promoter mutation C250T	400	2000

VIC threshold = mode + X

FAM threshold = mode + Y

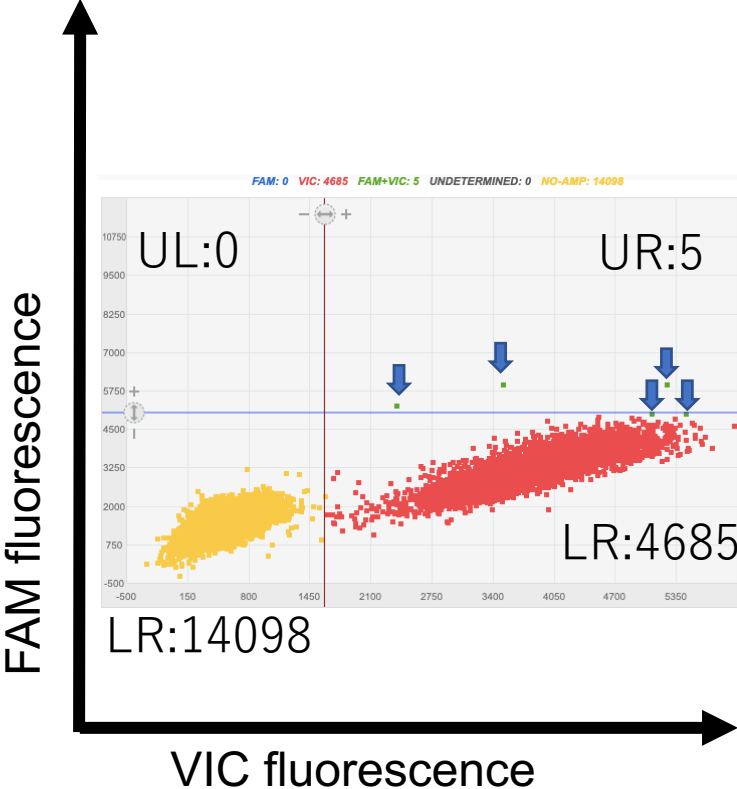
Supplementary table 5
Molecular diagnosis

Molecular diagnosis	WHO grading	Tumor DNA				N	matched	sensitivity
		IDH	TERT 228	TERT 250	H3 K27M			
GBM,IDH mut	IV	mut	wt	wt	wt	4	4	100%
GBM,IDH wt	IV	wt	mut	wt	wt	3	3	100%
			wt	mut				
DMG,H3K27M	IV	wt	wt	wt	mut	5	4	80%
AO,IDH mut	III	mut	mut	wt	wt	8	8	100%
			wt	mut				
AA,IDH mut	III	mut	mut	wt	wt	2	1	50%
AA,IDH wt	III	wt	mut	wt	wt	2	0	0%
			wt	mut				
OD,IDH mut	II	mut	mut	wt	wt	1	0	0%
			wt	mut				
DA,IDH mut	II	mut	wt	wt	wt	1	0	0%
DA,IDH wt	II	wt	mut	wt	wt	2	0	0%
			wt	mut				
GBMorAAorDA	-	wt	wt	wt	wt	6	6	100%

mut: mutant wt: wildtype GBM: glioblastoma DMG: diffuse midline glioma AO: anaplastic oligodendroglioma AA: anaplastic astrocytoma
OD: oligodendroglioma DA: diffuse astrocytoma

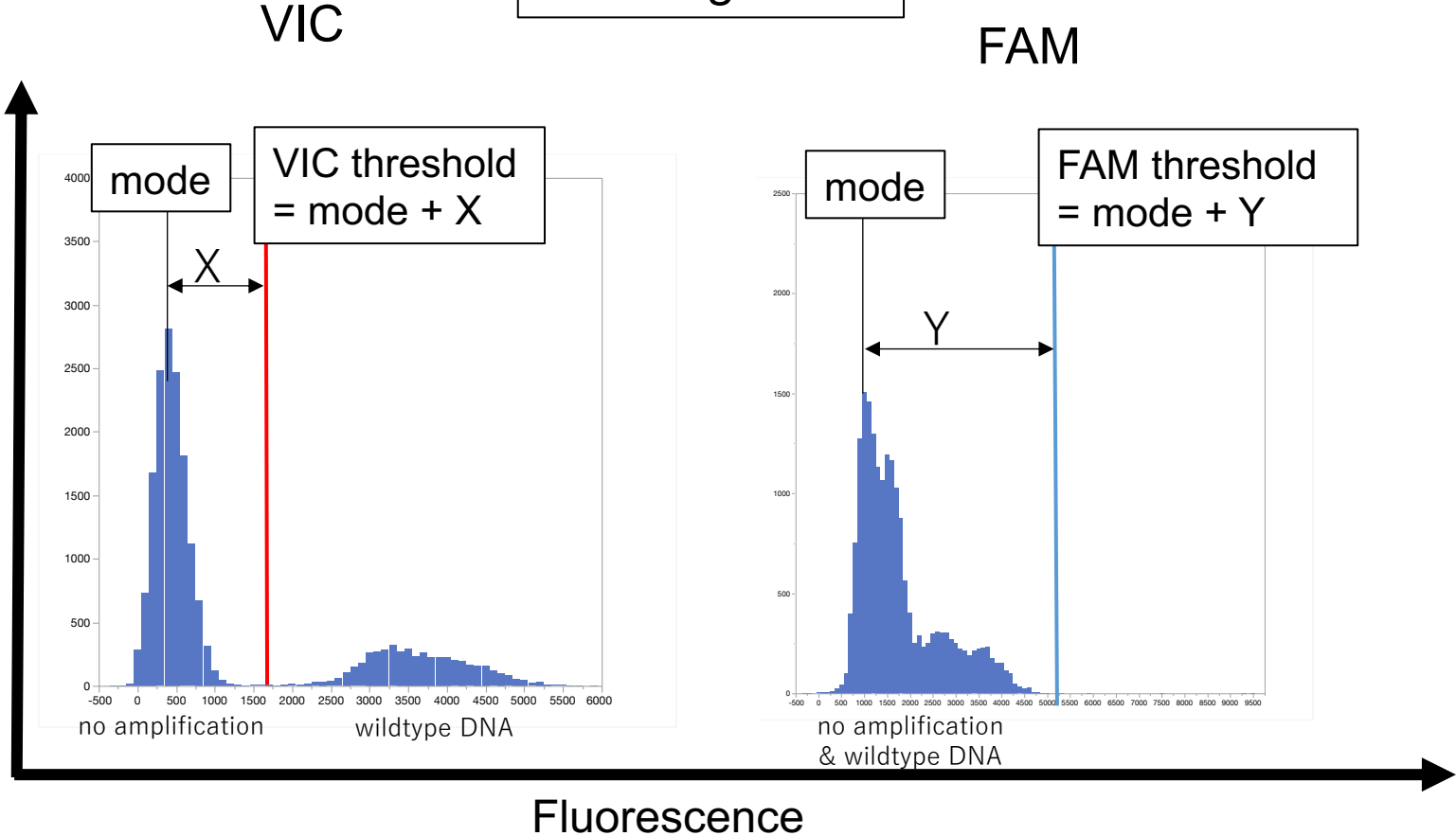
Supplementary Fig 1a

Scatter plot



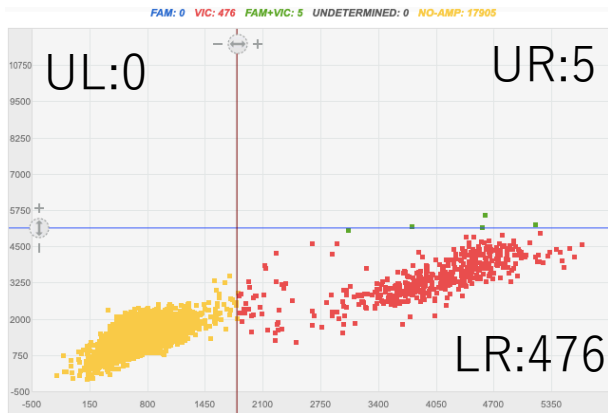
UL: Upper left
UR: Upper right
LL: Lower left
LR: Lower right

Histogram



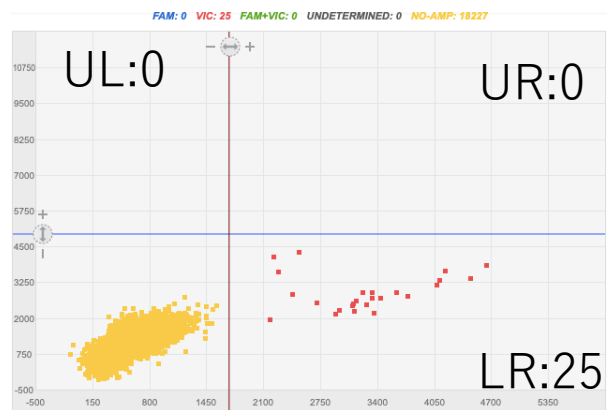
Supplementary Fig 1b

FC: 0.1 ng/ μ L



LL:17905

FC: 0.01 ng/ μ L



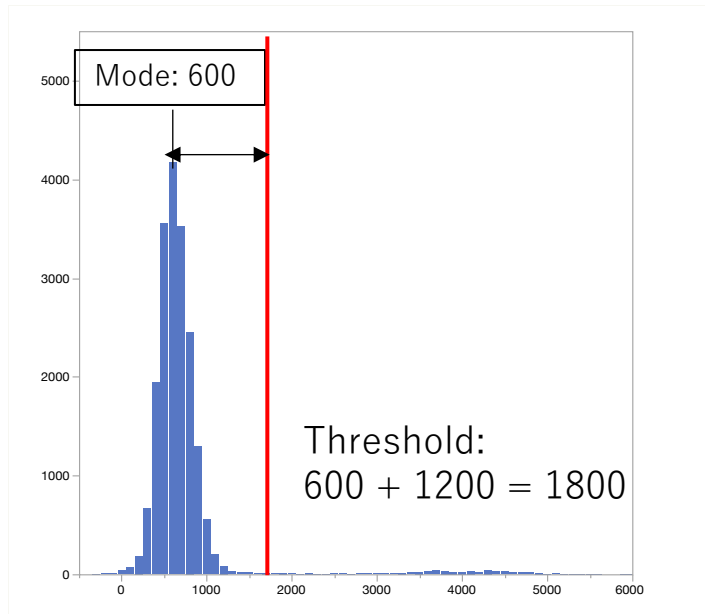
LL:18277

FAM fluorescence

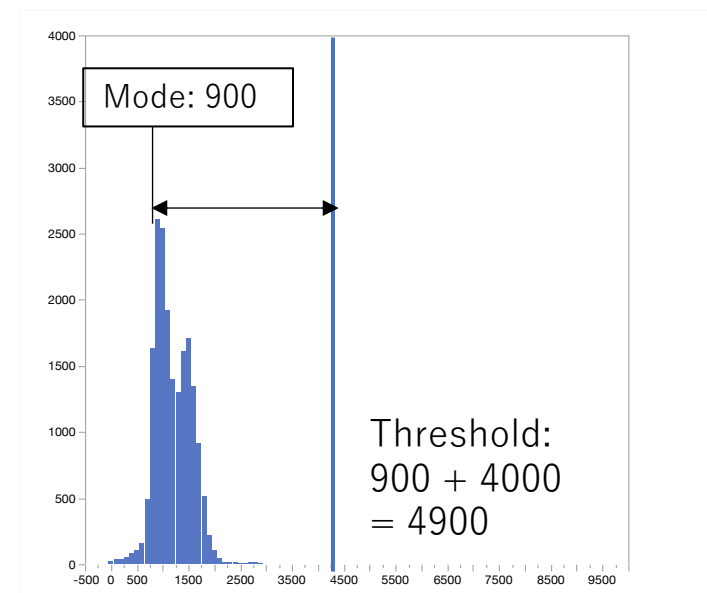
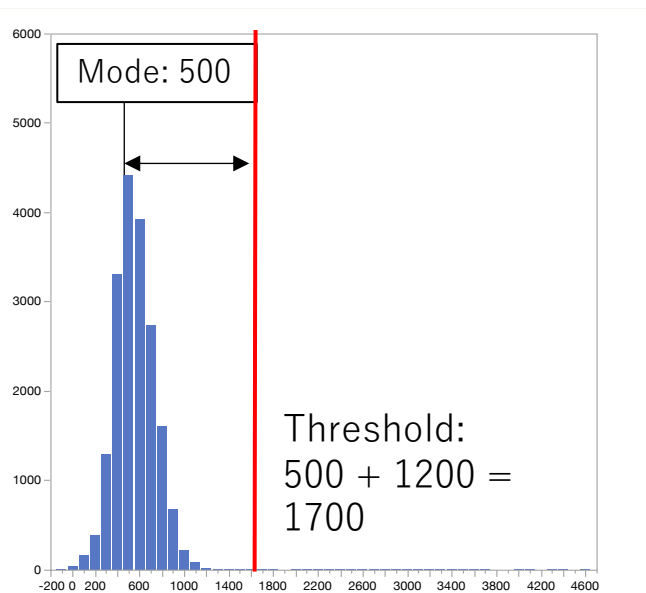
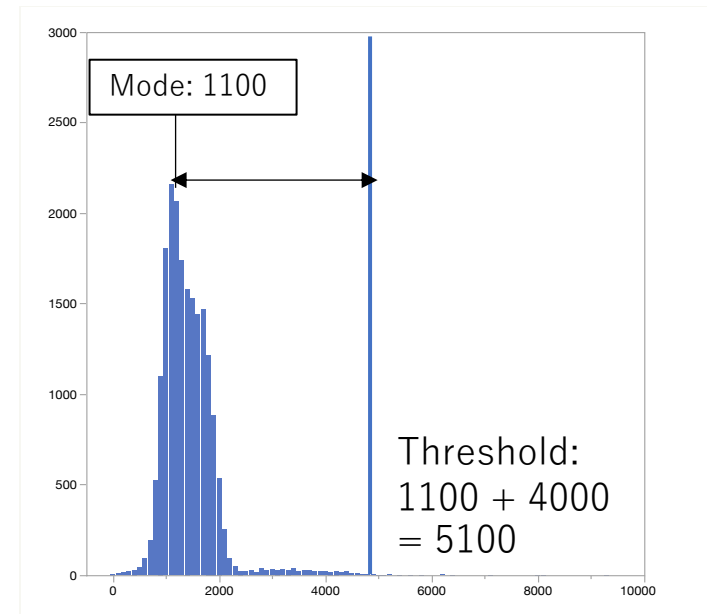
VIC fluorescence

Well counts

VIC



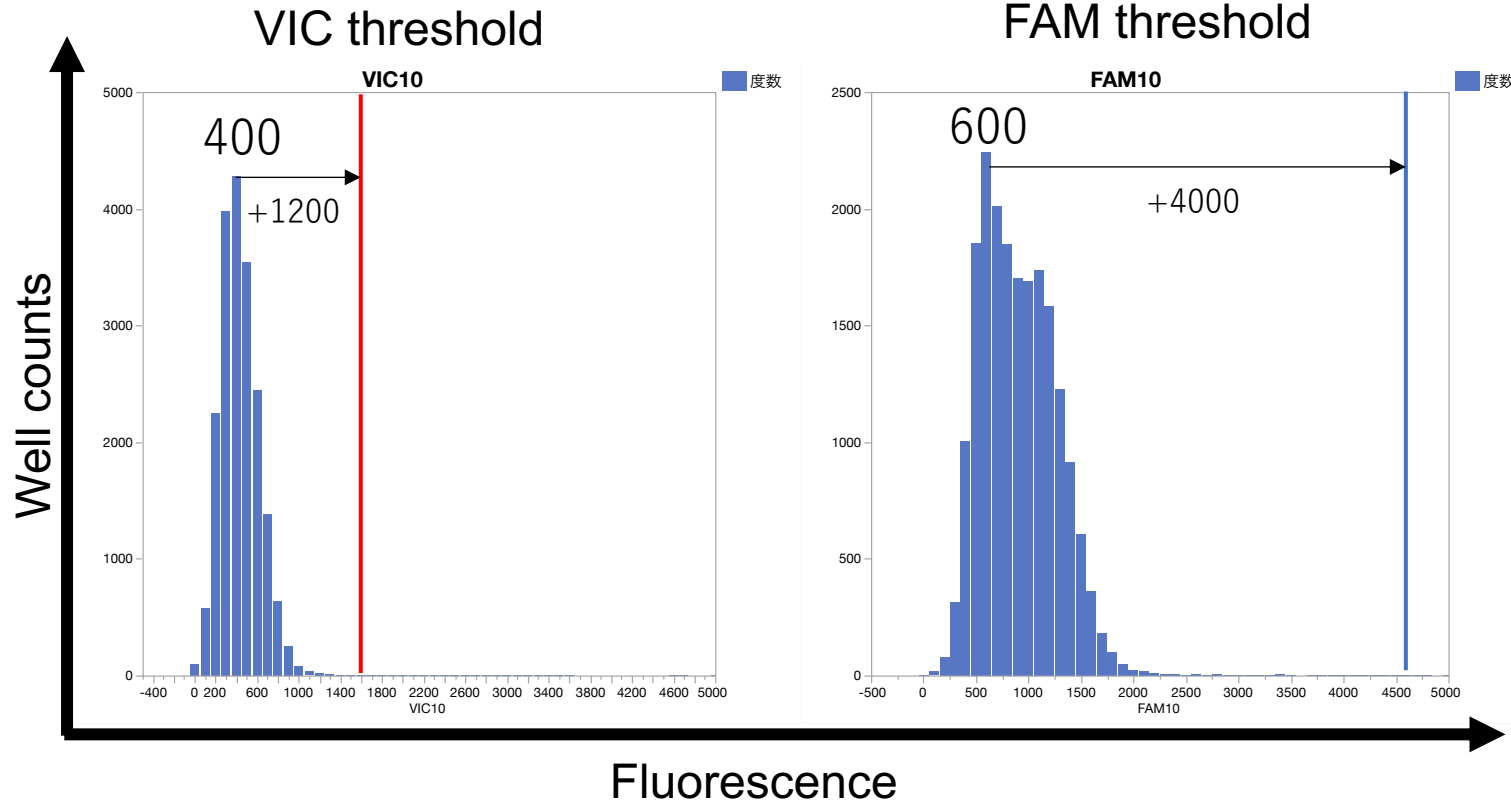
FAM



Fluorescence

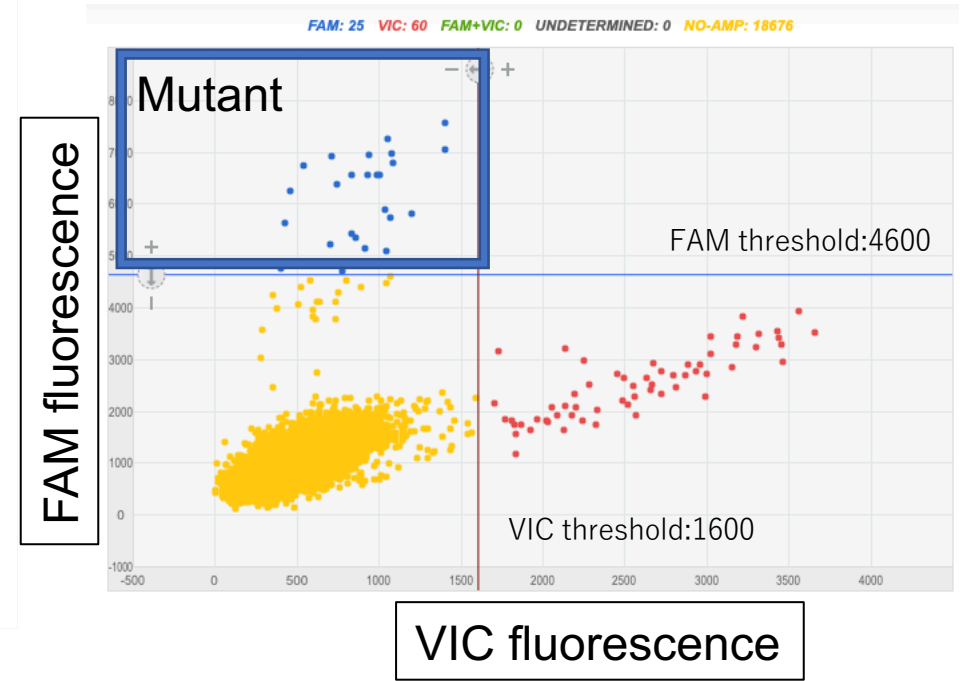
Supplementary Fig 1c

IDH1 R132H mutation assay



Mode: 400
VIC threshold:
Mode + 1200 = 1600

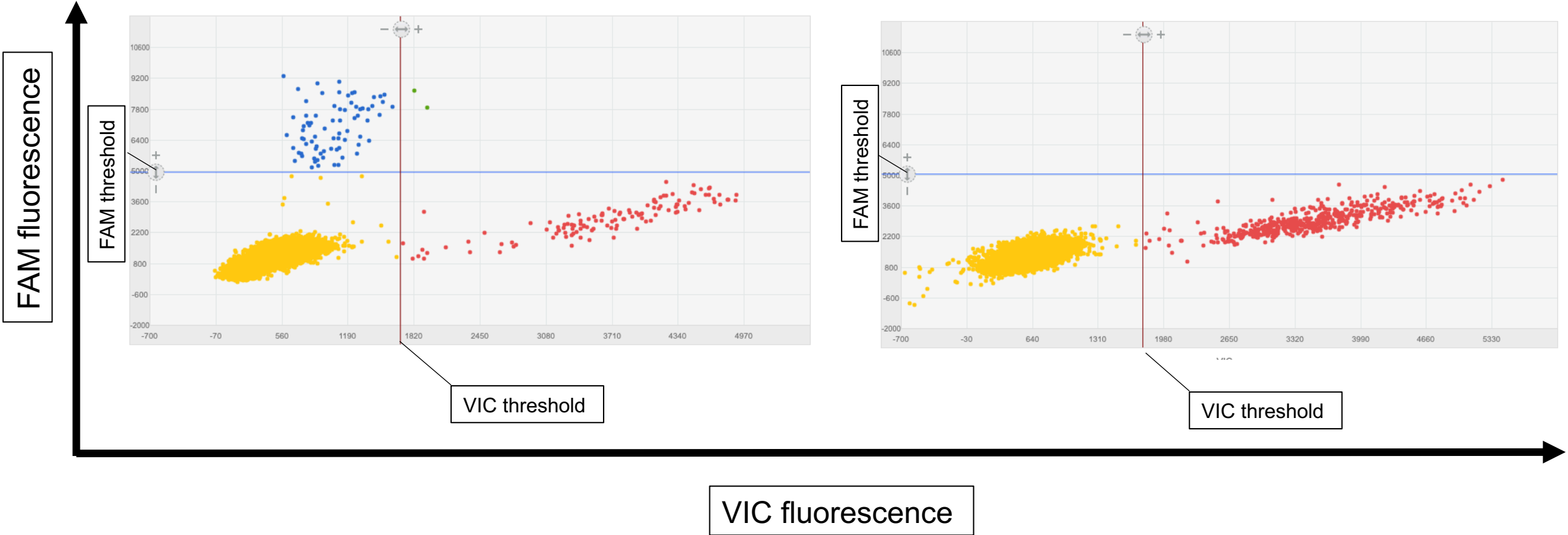
Mode: 600
FAM threshold:
Mode + 4000 = 4600



Supplementary Fig 2

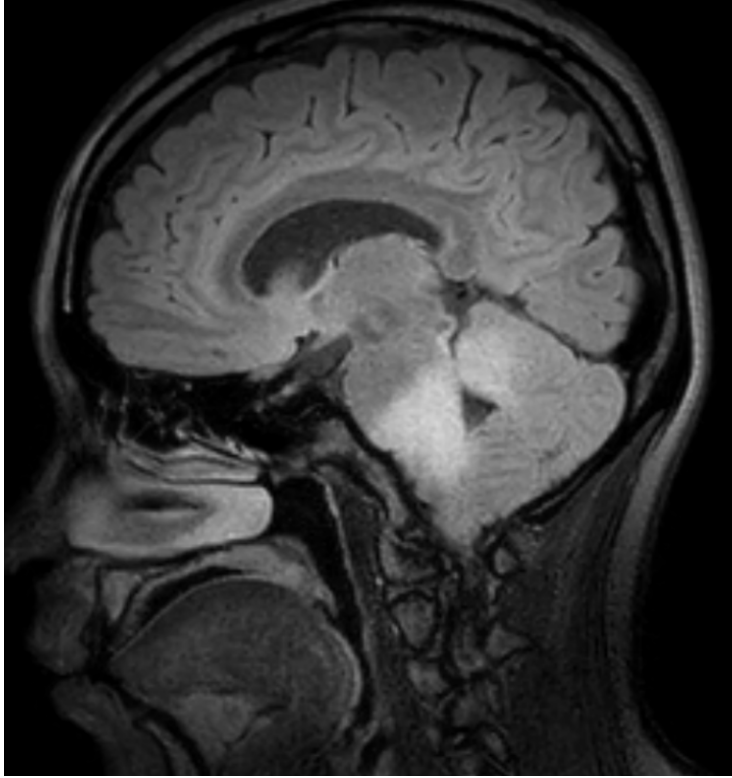
Mutant

Wild type

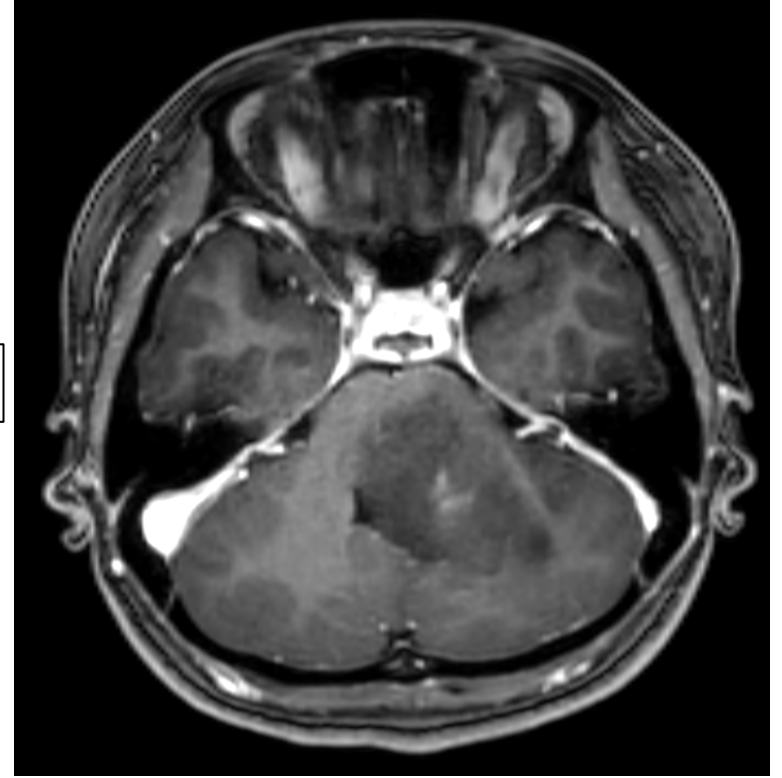


Supplementary
Fig 3a:
MRI imaging

FLAIR

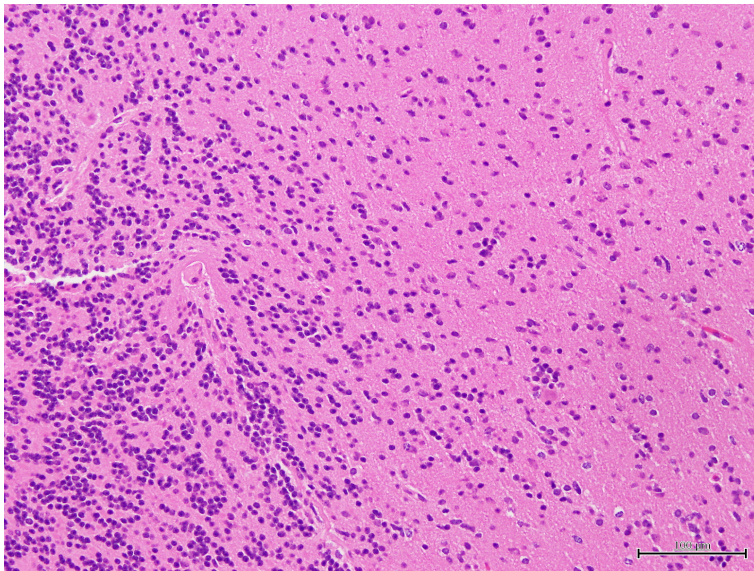


GdT1WI

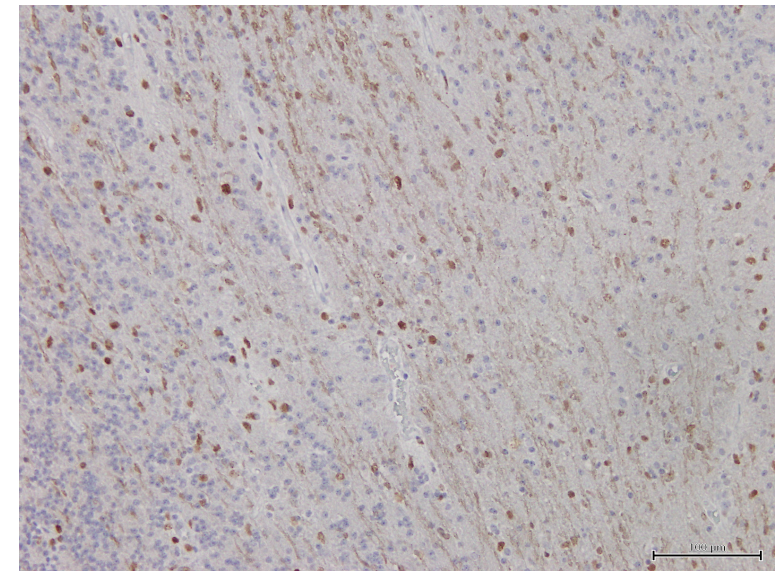


Pathological
finding

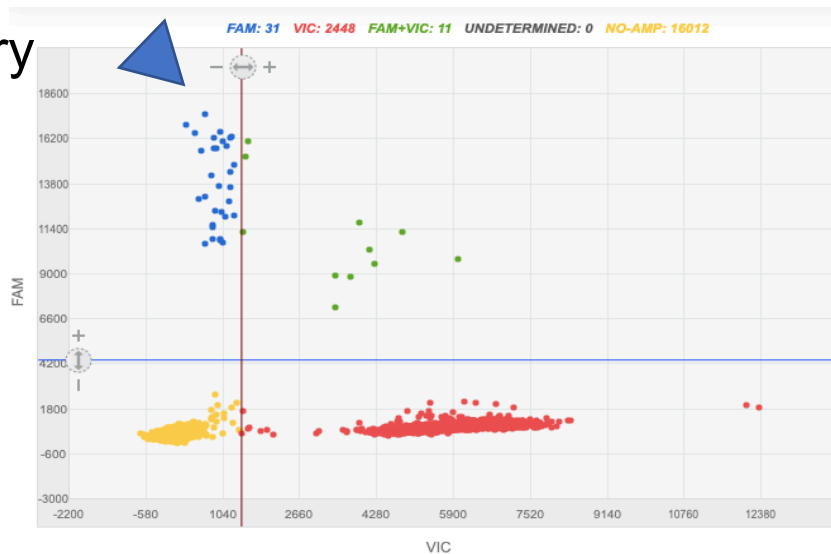
Supplementary
Fig 3b



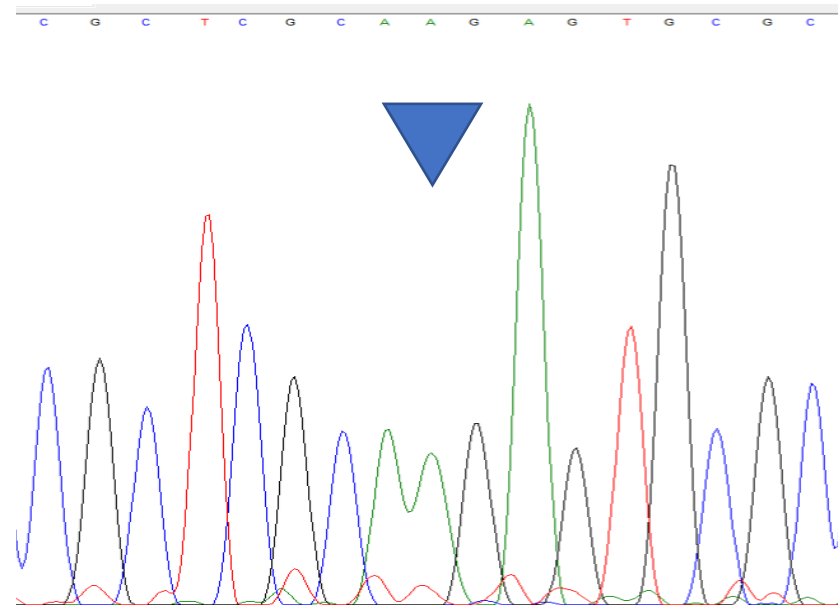
Supplementary
Fig 3c



Supplementary
Fig 3d:
Tumor DNA

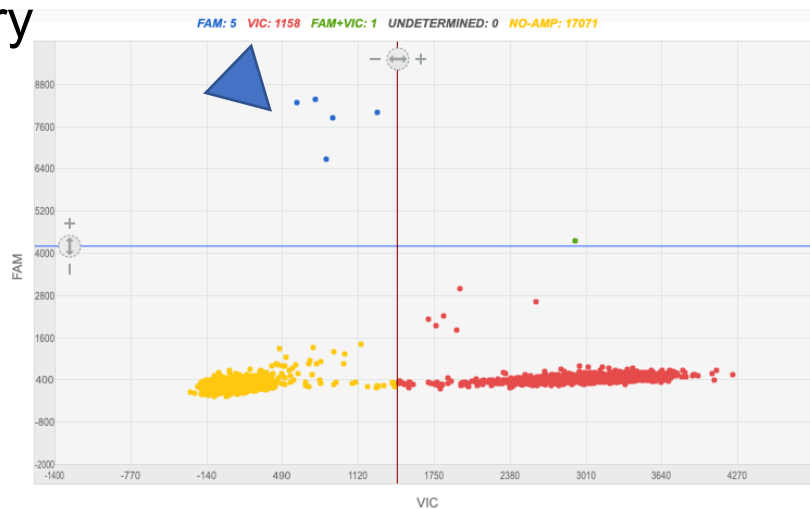


Digital PCR: positive

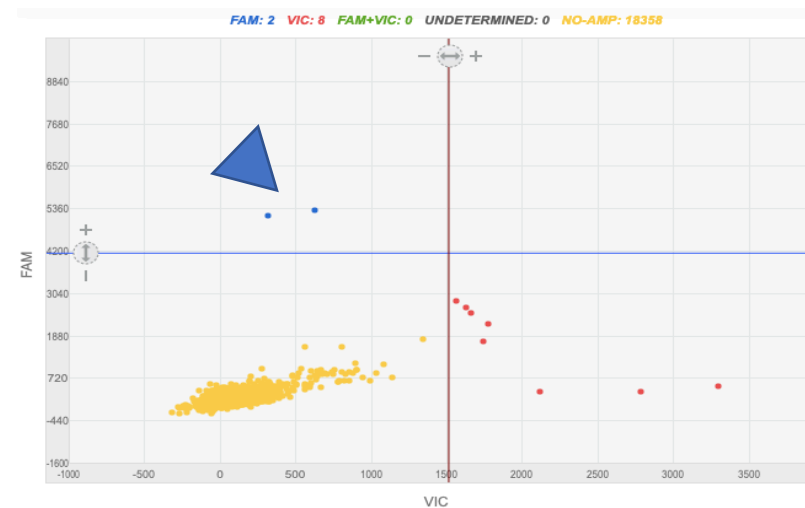


Sanger sequence: wild type

Supplementary
Fig 3e:
cfDNA in CSF



Intracranial CSF



Lumbar puncture CSF