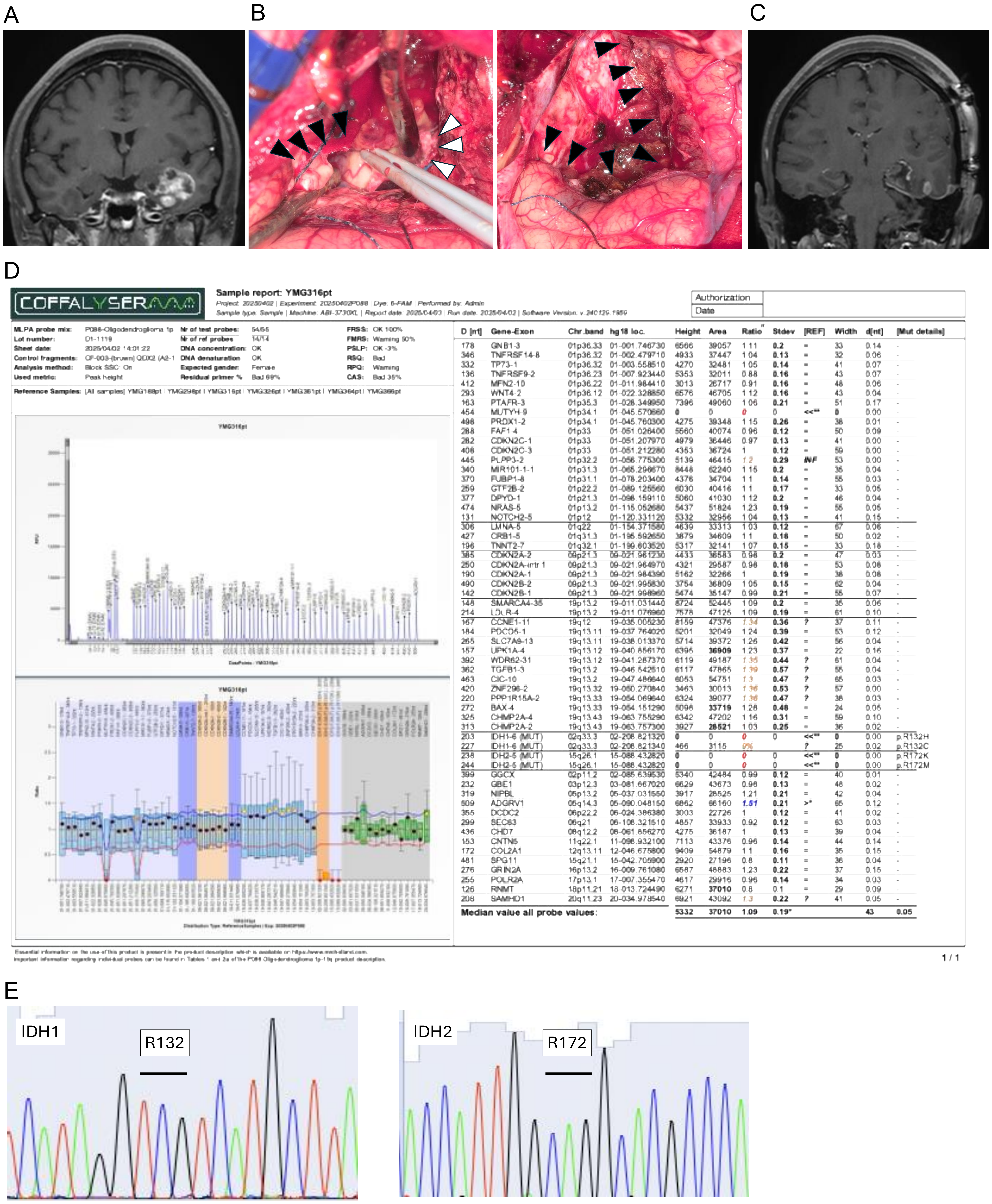
Supplementary Figure 1



COFFALYSER

Sample report: YMG316pt

Project: 20250402 | Experiment: 20250402P088 | Dye: 6-FAM | Performed by: Admin
Sample type: Sample | Machine: ABI-3730XL | Report date: 2025/04/03 | Run date: 2025/04/02 | Software Version: v.240129.1959

Authorization	
Date	

MLPA probe mix:	P088-Oligodendroglioma 1p	**Nr of test probes:**	54/55	**FRSS:**	OK 100%
Lot number:	D1-1119	**Nr of ref probes**	14/14	**FMRS:**	Warning 50%
Sheet date:	2025/04/02 14:01:22	**DNA concentration:**	OK	**PSLP:**	OK -3%
Control fragments:	CF-003-[brown] QDX2 (A2-1	**DNA denaturation**	OK	**RSQ:**	Bad
Analysis method:	Block SSC: On	**Expected gender:**	Female	**RPQ:**	Warning
Used metric:	Peak height	**Residual primer %**	Bad 69%	**CAS:**	Bad 35%

Reference Samples: [All samples] YMG168pt | YMG296pt | YMG316pt | YMG326pt | YMG361pt | YMG364pt | YMG366pt

D [nt]	Gene-Exon	Chr.band	hg18 loc.	Height	Area	Ratio	Stdev	[REF]	Width	d[nt]	[Mut details]
178	GNB1-3	01p36.33	01-001.746730	6566	39057	1.11	**0.2**	=	33	0.14	-
346	TNFRSF14-8	01p36.32	01-002.479710	4933	37447	1.04	**0.13**	=	32	0.06	-
332	TP73-1	01p36.32	01-003.558510	4270	32481	1.05	**0.14**	=	41	0.07	-
136	TNFRSF9-2	01p36.23	01-007.923440	5353	32011	0.88	**0.16**	=	43	0.07	-
412	MFN2-10	01p36.22	01-011.984410	3013	26717	0.91	**0.16**	=	48	0.06	-
293	WNT4-2	01p36.12	01-022.328850	6576	46705	1.12	**0.16**	=	43	0.04	-
163	PTAFR-3	01p35.3	01-028.349950	7396	49060	1.06	**0.21**	=	51	0.17	-
454	MUTYH-9	01p34.1	01-045.570660	**0**	0	*0*	0	<<**	**0**	0.00	-
498	PRDX1-2	01p34.1	01-045.760300	4275	39348	1.15	**0.26**	=	38	0.01	-
288	FAF1-4	01p33	01-051.026400	5560	40074	0.96	**0.12**	=	50	0.09	-
282	CDKN2C-1	01p33	01-051.207970	4979	36446	0.97	**0.13**	=	41	0.00	-
408	CDKN2C-3	01p33	01-051.212280	4353	36724	1	**0.12**	=	59	0.00	-
445	PLPP3-2	01p32.2	01-056.775300	5139	46415	*1.2*	**0.29**	*INF*	53	0.00	-
340	MIR101-1-1	01p31.3	01-065.296670	8448	62240	1.15	**0.2**	=	35	0.04	-
370	FUBP1-8	01p31.1	01-078.203400	4376	34704	1.1	**0.14**	=	55	0.03	-
259	GTF2B-2	01p22.2	01-089.125560	6030	40416	1.1	**0.17**	=	33	0.05	-
377	DPYD-1	01p21.3	01-098.159110	5060	41030	1.12	**0.2**	=	46	0.04	-
474	NRAS-5	01p13.2	01-115.052680	5437	51824	1.23	**0.19**	=	55	0.05	-
131	NOTCH2-5	01p12	01-120.331120	5332	32956	1.04	**0.13**	=	41	0.15	-
306	LMNA-5	01q22	01-154.371580	4639	33313	1.03	**0.12**	=	67	0.06	-
427	CRB1-5	01q31.3	01-195.592650	3879	34609	1.1	**0.16**	=	50	0.02	-
196	TNNT2-7	01q32.1	01-199.603520	5317	32141	1.07	**0.15**	=	33	0.18	-
385	CDKN2A-2	09p21.3	09-021.961230	4433	36583	0.98	**0.2**	=	47	0.03	-
250	CDKN2A-intr.1	09p21.3	09-021.964970	4321	29587	0.98	**0.18**	=	53	0.08	-
190	CDKN2A-1	09p21.3	09-021.984390	5162	32266	1	**0.19**	=	38	0.08	-
490	CDKN2B-2	09p21.3	09-021.995830	3754	36809	1.05	**0.15**	=	62	0.04	-
142	CDKN2B-1	09p21.3	09-021.998960	5474	35147	0.99	**0.21**	=	55	0.07	-
148	SMARCA4-35	19p13.2	19-011.031440	8724	52445	1.09	**0.2**	=	35	0.06	-
214	LDLR-4	19p13.2	19-011.076960	7578	47125	1.09	**0.19**	=	61	0.10	-
167	CCNE1-11	19q12	19-035.005230	8159	47376	*1.34*	**0.36**	?	37	0.11	-
184	PDCD5-1	19q13.11	19-037.764020	5201	32049	1.24	**0.39**	=	53	0.12	-
265	SLC7A9-13	19q13.11	19-038.013370	5714	39372	1.26	**0.42**	=	56	0.04	-
157	UPK1A-4	19q13.12	19-040.856170	6395	**36909**	1.23	**0.37**	=	22	0.16	-
392	WDR62-31	19q13.12	19-041.287370	6119	49187	*1.35*	**0.44**	?	61	0.04	-
362	TGFB1-3	19q13.2	19-046.542510	6117	47865	*1.39*	**0.57**	?	55	0.04	-
463	CIC-10	19q13.2	19-047.486640	6053	54751	*1.3*	**0.47**	?	65	0.03	-
420	ZNF296-2	19q13.32	19-050.270840	3463	30013	*1.36*	**0.53**	?	57	0.00	-
220	PPP1R15A-2	19q13.33	19-054.069640	6324	39077	*1.36*	**0.47**	?	38	0.03	-
272	BAX-4	19q13.33	19-054.151290	5098	**33719**	1.28	**0.48**	=	24	0.05	-
325	CHMP2A-4	19q13.43	19-063.755290	6342	47202	1.16	**0.31**	=	59	0.10	-
313	CHMP2A-2	19q13.43	19-063.757300	3927	**28521**	1.03	**0.25**	=	36	0.02	-
203	IDH1-6 (MUT)	02q33.3	02-208.821320	**0**	0	*0*	0	<<**	**0**	0.00	p.R132H
227	IDH1-6 (MUT)	02q33.3	02-208.821340	466	3115	*9%*		?	25	0.02	p.R132C
238	IDH2-5 (MUT)	15q26.1	15-088.432820	**0**	0	*0*	0	<<**	**0**	0.00	p.R172K
244	IDH2-5 (MUT)	15q26.1	15-088.432820	**0**	0	*0*	0	<<**	**0**	0.00	p.R172M
399	GGCX	02p11.2	02-085.639530	5340	42484	0.99	**0.12**	=	40	0.01	-
232	GBE1	03p12.3	03-081.667020	6629	43673	0.98	**0.13**	=	48	0.02	-
319	NIPBL	05p13.2	05-037.031550	3917	28525	1.21	**0.21**	=	42	0.04	-
509	ADGRV1	05q14.3	05-090.048150	6862	66160	***1.51***	**0.21**	>*	65	0.12	-
355	DCDC2	06p22.2	06-024.386380	3003	22726	1	**0.12**	=	41	0.02	-
299	SEC63	06q21	06-108.321510	4857	33933	0.92	**0.12**	=	63	0.03	-
436	CHD7	08q12.2	08-061.856270	4275	36187	1	**0.13**	=	39	0.04	-
153	CNTN5	11q22.1	11-098.932100	7113	43376	0.96	**0.14**	=	44	0.14	-
172	COL2A1	12q13.11	12-046.675800	9409	54879	1.1	**0.16**	=	35	0.15	-
481	SPG11	15q21.1	15-042.705900	2920	27196	0.8	**0.11**	=	36	0.04	-
276	GRIN2A	16p13.2	16-009.761080	6587	48883	1.23	**0.22**	=	37	0.15	-
255	POLR2A	17p13.1	17-007.355470	4617	29916	0.96	**0.14**	=	34	0.03	-
126	RNMT	18p11.21	18-013.724490	6271	**37010**	0.8	0.1	=	29	0.09	-
208	SAMHD1	20q11.23	20-034.978540	6921	43092	*1.3*	**0.22**	?	41	0.05	-
Median value all probe values:				**5332**	**37010**	**1.09**	**0.19***		**43**	**0.05**	

Essential information on the use of this product is present in the product description which is available on https://www.mrcholland.com.
Important information regarding individual probes can be found in Tables 1 and 2a of the P088 Oligodendroglioma 1p-19q product description.

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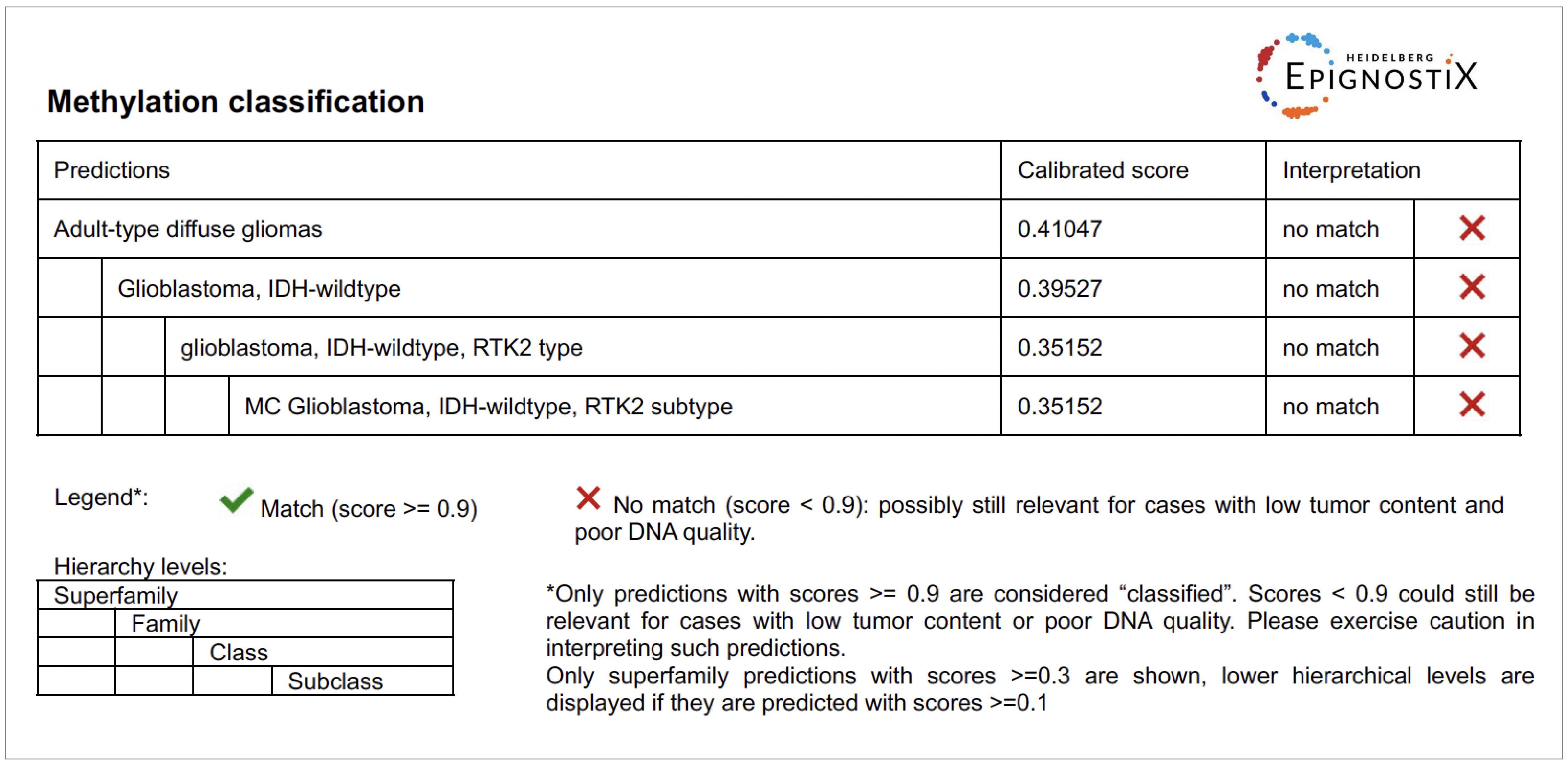
Supplementary Figure 1

(A) Preoperative coronal T1-weighted contrast-enhanced MRI **(B)** Intraoperative image: Black arrowheads indicate the parahippocampal gyrus, and white arrowheads denote the hippocampus. **(C)** Postoperative coronal T1-weighted contrast-enhanced MRI **(D)** MLPA analysis of sample YMG316pt performed using Coffalyser. The upper panel displays the electropherogram of probe signals, and the lower panel shows normalized copy number ratios. Ratios near 1.0 indicate normal copy number, while values below 0.7 suggest deletions. In the lower panel, probes corresponding to chromosome arms 1p (e.g., 1p36, 1p32) and 19q (e.g., 19q13) show ratios close to 1.0, without evidence of combined whole-arm loss. These findings are further supported by the numerical table on the right, where the ratio values for 1p and 19q probes remain within the normal range. The results do not support the presence of 1p/19q codeletion and are not consistent with oligodendroglioma. **(E)** Sanger sequencing of IDH1 and IDH2 hotspot regions. The chromatograms show wild-type sequences at codon R132 of IDH1 (left) and codon R172 of IDH2 (right), indicating the absence of pathogenic mutations in either gene.

Supplementary Figure 2

A



HEIDELBERG EPIGNOSTIX

Methylation classification

Predictions				Calibrated score	Interpretation	
Adult-type diffuse gliomas				0.41047	no match	✗
	Glioblastoma, IDH-wildtype			0.39527	no match	✗
		glioblastoma, IDH-wildtype, RTK2 type		0.35152	no match	✗
			MC Glioblastoma, IDH-wildtype, RTK2 subtype	0.35152	no match	✗

Legend*: ✓ Match (score >= 0.9) ✗ No match (score < 0.9): possibly still relevant for cases with low tumor content and poor DNA quality.

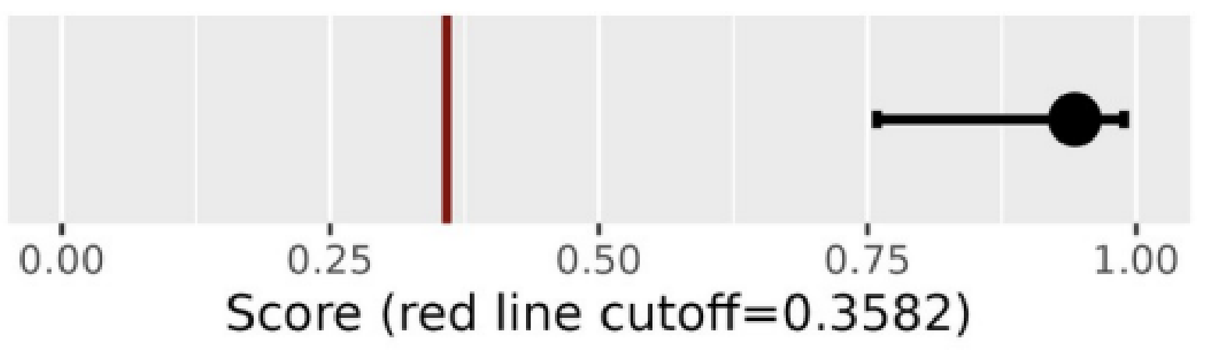
Hierarchy levels:

Superfamily			
	Family		
		Class	
			Subclass

*Only predictions with scores >= 0.9 are considered "classified". Scores < 0.9 could still be relevant for cases with low tumor content or poor DNA quality. Please exercise caution in interpreting such predictions.
Only superfamily predictions with scores >=0.3 are shown, lower hierarchical levels are displayed if they are predicted with scores >=0.1

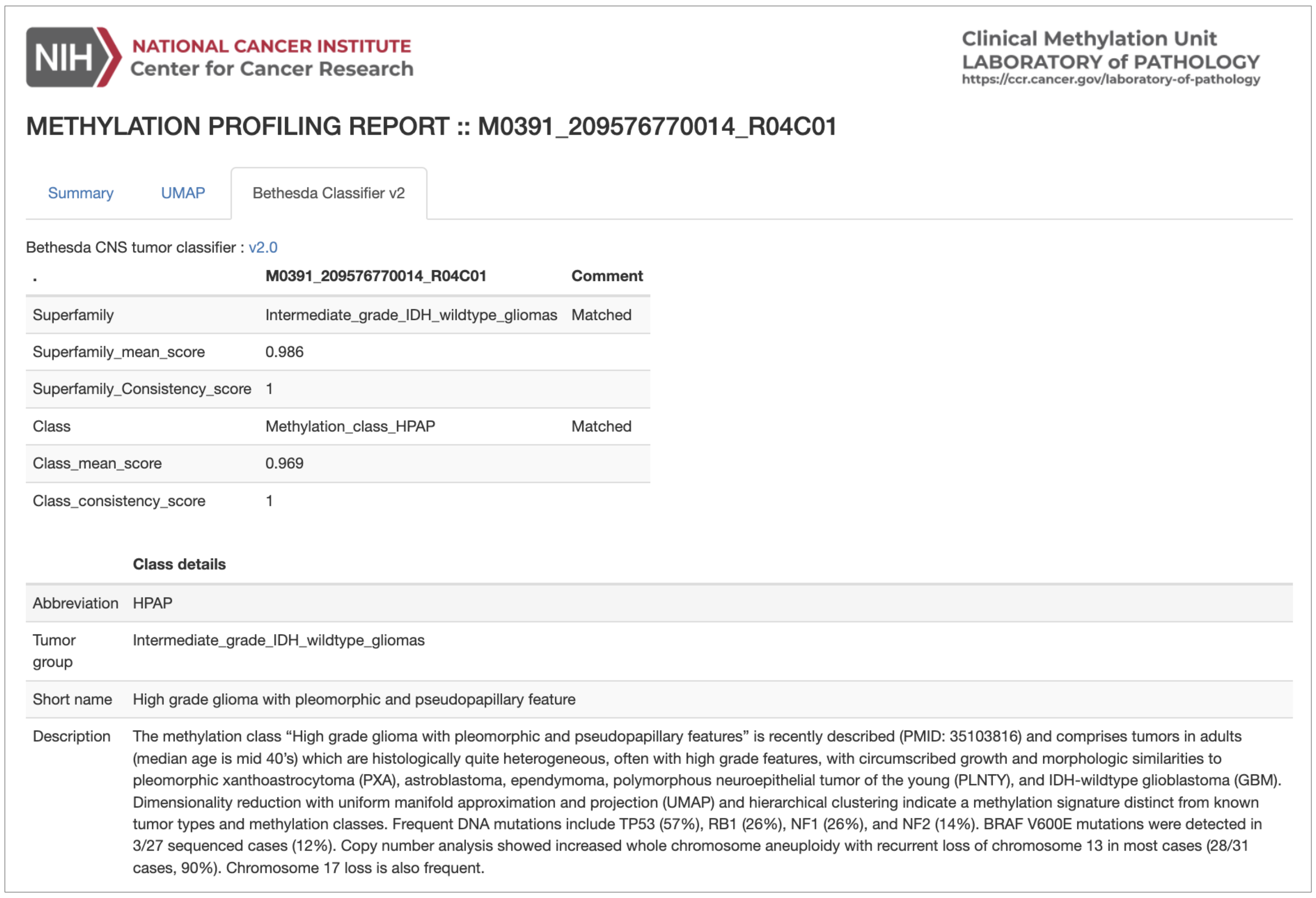
B

MGMT promotor status prediction



Status	Estimated	CI lower	CI upper
methylated	0.94288	0.75869	0.9886

C



NIH NATIONAL CANCER INSTITUTE Center for Cancer Research

Clinical Methylation Unit
LABORATORY of PATHOLOGY
https://ccr.cancer.gov/laboratory-of-pathology

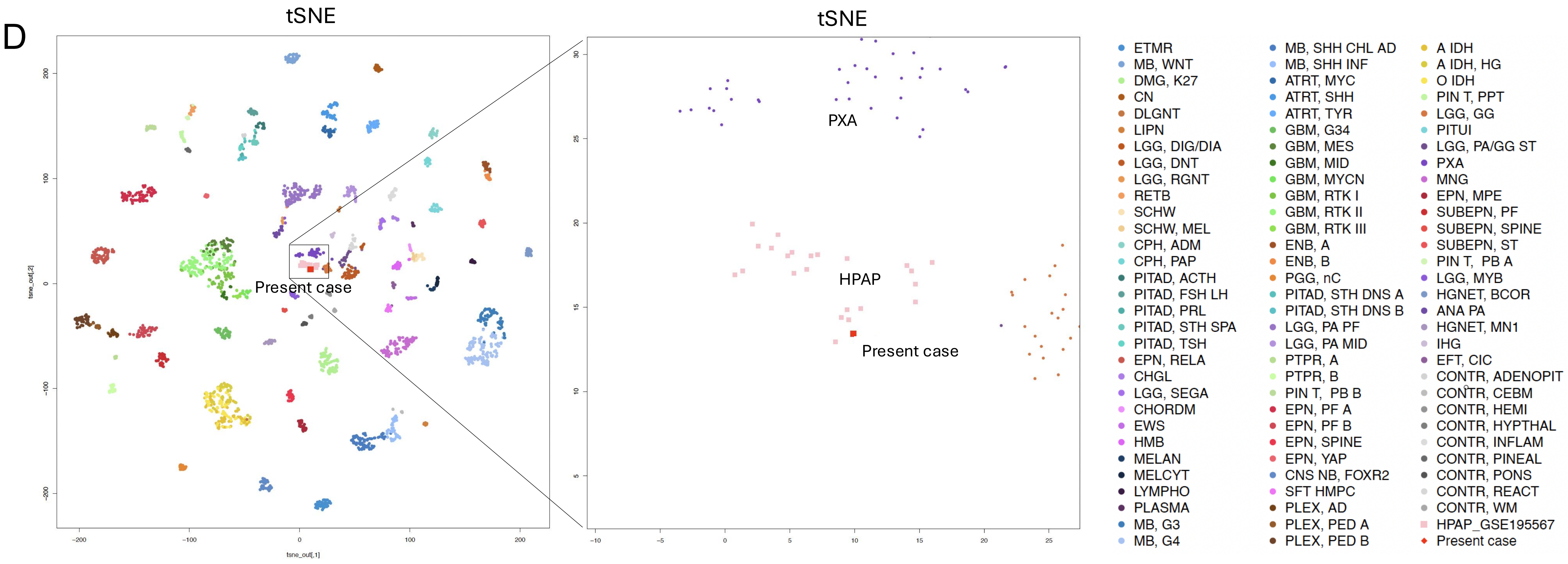
METHYLATION PROFILING REPORT :: M0391_209576770014_R04C01

Bethesda CNS tumor classifier : v2.0

.	M0391_209576770014_R04C01	Comment
Superfamily	Intermediate_grade_IDH_wildtype_gliomas	Matched
Superfamily_mean_score	0.986	
Superfamily_Consistency_score	1	
Class	Methylation_class_HPAP	Matched
Class_mean_score	0.969	
Class_consistency_score	1	

	Class details
Abbreviation	HPAP
Tumor group	Intermediate_grade_IDH_wildtype_gliomas
Short name	High grade glioma with pleomorphic and pseudopapillary feature
Description	The methylation class "High grade glioma with pleomorphic and pseudopapillary features" is recently described (PMID: 35103816) and comprises tumors in adults (median age is mid 40's) which are histologically quite heterogeneous, often with high grade features, with circumscribed growth and morphologic similarities to pleomorphic xanthoastrocytoma (PXA), astroblastoma, ependymoma, polymorphous neuroepithelial tumor of the young (PLNTY), and IDH-wildtype glioblastoma (GBM). Dimensionality reduction with uniform manifold approximation and projection (UMAP) and hierarchical clustering indicate a methylation signature distinct from known tumor types and methylation classes. Frequent DNA mutations include TP53 (57%), RB1 (26%), NF1 (26%), and NF2 (14%). BRAF V600E mutations were detected in 3/27 sequenced cases (12%). Copy number analysis showed increased whole chromosome aneuploidy with recurrent loss of chromosome 13 in most cases (28/31 cases, 90%). Chromosome 17 loss is also frequent.

D



Supplementary Figure 2

(**A**)Methylation classification results using the Heidelberg Classifier: According to the Heidelberg CNS tumor classifier (v12.8) , the present case did not match any known methylation classes with high confidence (all calibrated scores < 0.9). Although the tumor showed some similarity to adult-type diffuse gliomas, particularly the glioblastoma, IDH-wildtype (RTK2 subtype), none of the predicted classes exceeded the classification threshold. **(B)** MGMT promoter methylation status estimation: The calculated score was 0.94288, which is well above the established threshold (0.3582, red line), indicating a methylated MGMT promoter. **(C)** Methylation Classifier Report Identifying HPAP (Bethesda CNS classifier v2.0): Methylation profiling using the Bethesda CNS classifier v2.0 identified the present tumor as belonging to the methylation class "HPAP" (High-grade glioma with pleomorphic and pseudopapillary features), with high confidence (class mean score = 0.969). **(D)** t-distributed stochastic neighbor embedding (t-SNE) visualization of genome-wide DNA methylation profiling. (Left) Unsupervised clustering based on DNA methylation data from the 2801 reference cohort and 23 HPAP samples. HPAP cluster is indicated pink squares labeled "HPAP_GSE195567" and the present case is highlighted in red. (Right) Enlarged view showing that the present case clusters within the HPAP group, supporting its classification as HPAP.