

Detection and genomic analysis of BRAF fusions in Juvenile Pilocytic Astrocytoma through the combination and integration of multi-omic data - Supplemental Figures (1-4) and Tables (1-6)

Supplemental Figure legends

Sup Fig 1 Linked-reads data supporting *BRAF* fusions, visualized in Loupe, of tandem duplication resulting in the canonical *KIAA1549-BRAF* fusion in JPA_4, JPA_5, JPA_6

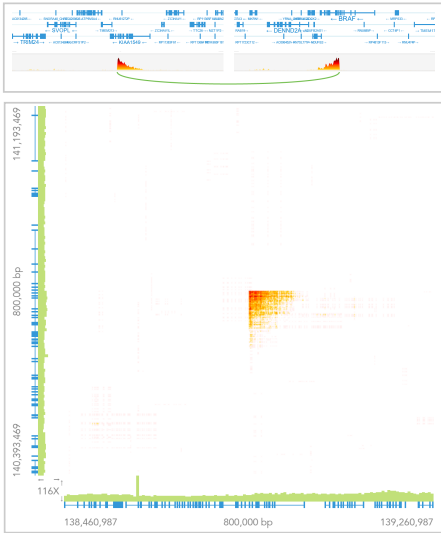
Sup Fig 2 Interaction matrices showing interactions between (a) *KIAA1549* and *BRAF* in fused and non-fused JPAs, (b) *PTPRZ1* and *BRAF* in JPA_1 and (c) *GNAI1* and *BRAF* in JPA_9

Sup Fig 3 (a) UMAP clustering of JPAs based on the compartment scores for the entire genome (50kb bins) shows clustering of JPAs. (b) Correlation matrix showing hierarchical clustering by compartment score over entire genome (50kb bins) shows clustering of JPAs. (c) Outer, Compartment scores for adult astrocytes and JPAs across chromosomes 3 and 7. Inner, most common *RAF* fusions in JPAs as well as novel fusions described in this paper. (d) Compartment score over breakpoints in *KIAA1549* and *BRAF* in JPA_2, and in *PTPRZ1* and *BRAF* in JPA_1 showing that both genes are in open chromatin regions in the matched Hi-C data

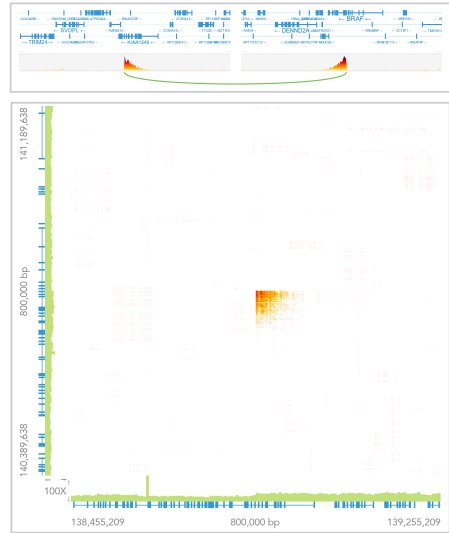
Sup Fig 4 (a) Expression of *BRAF* across all developmental time points regardless of cell type. (b) Average expression of *BRAF* across all cell types in the mouse cerebellum single-cell atlas at time points E16 and E18. (c) Percent cells expressing *BRAF* across all cell types in the mouse cerebellum single-cell atlas at time points E16 and E18. (d) Expression of *BRAF*, *RAF1*, recurrent and novel fusion partners in all cell types in the mouse cerebellum single-cell atlas at time points E16 and E18

Supplemental Figure 1.

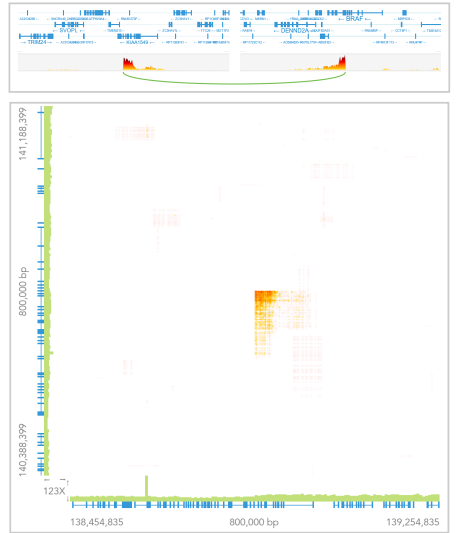
JPA_4
Tandem duplication resulting in
KIAA1549-BRAF
(EX16-EX9,HAP_ALLELIC_FRAC=1.0)



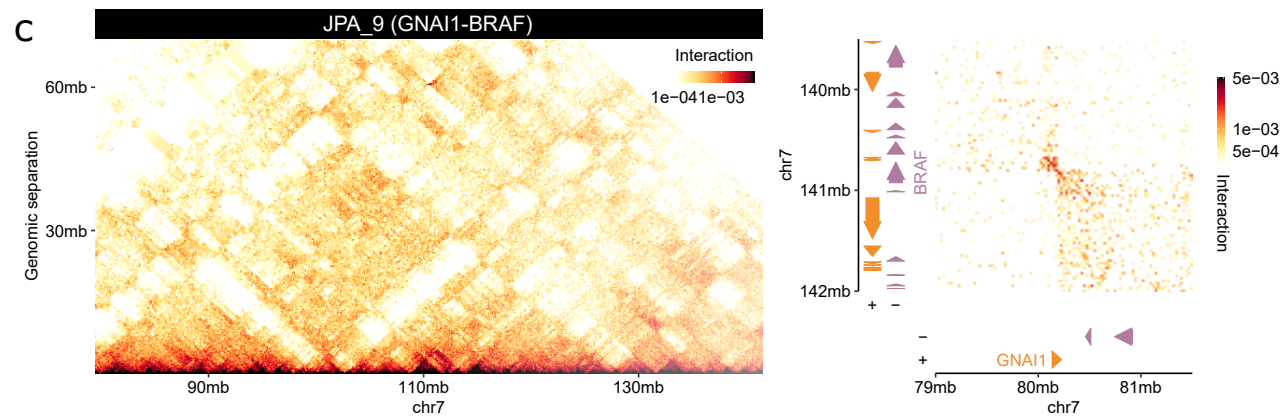
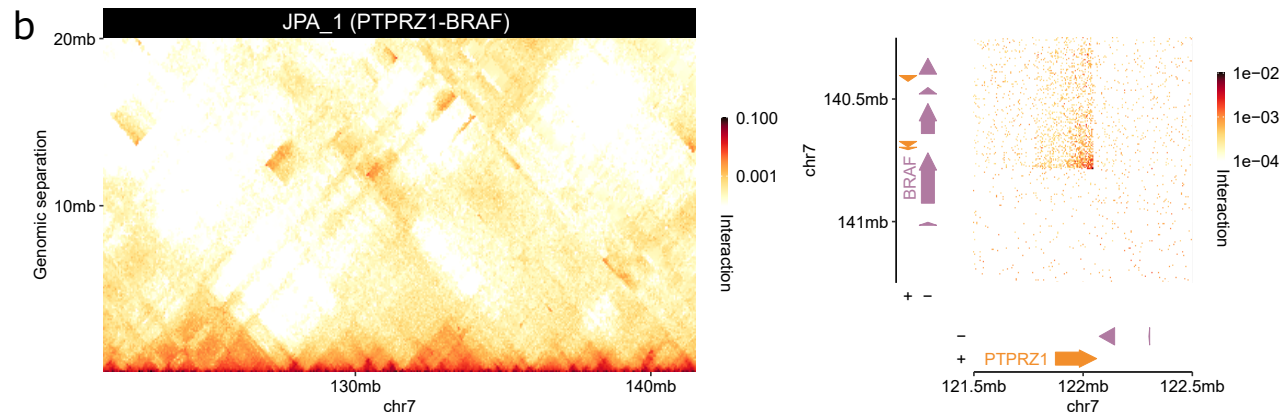
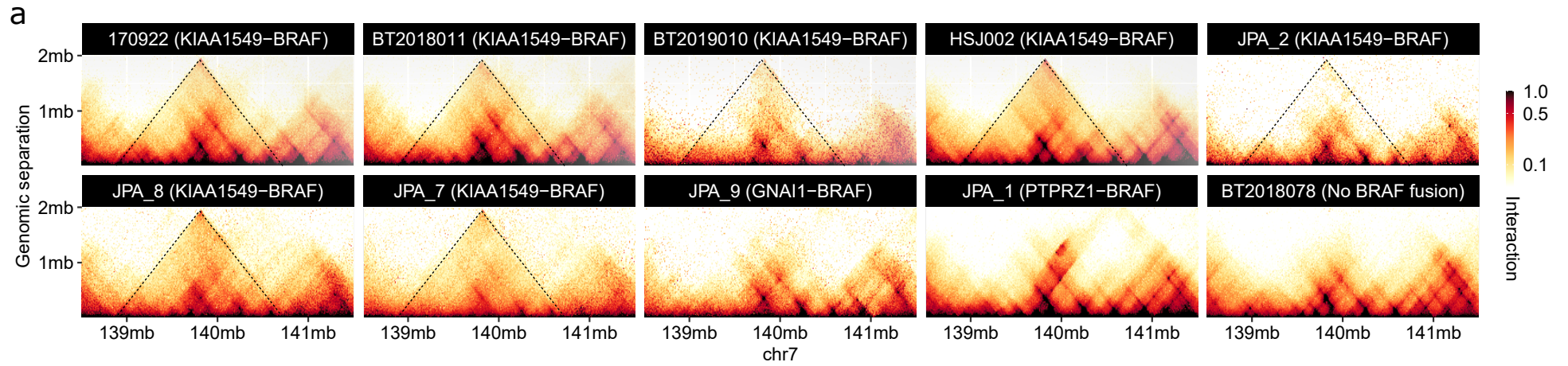
JPA_5
Tandem duplication resulting in
KIAA1549-BRAF
(EX16-EX9, HAP_ALLELIC_FRAC=0.928)



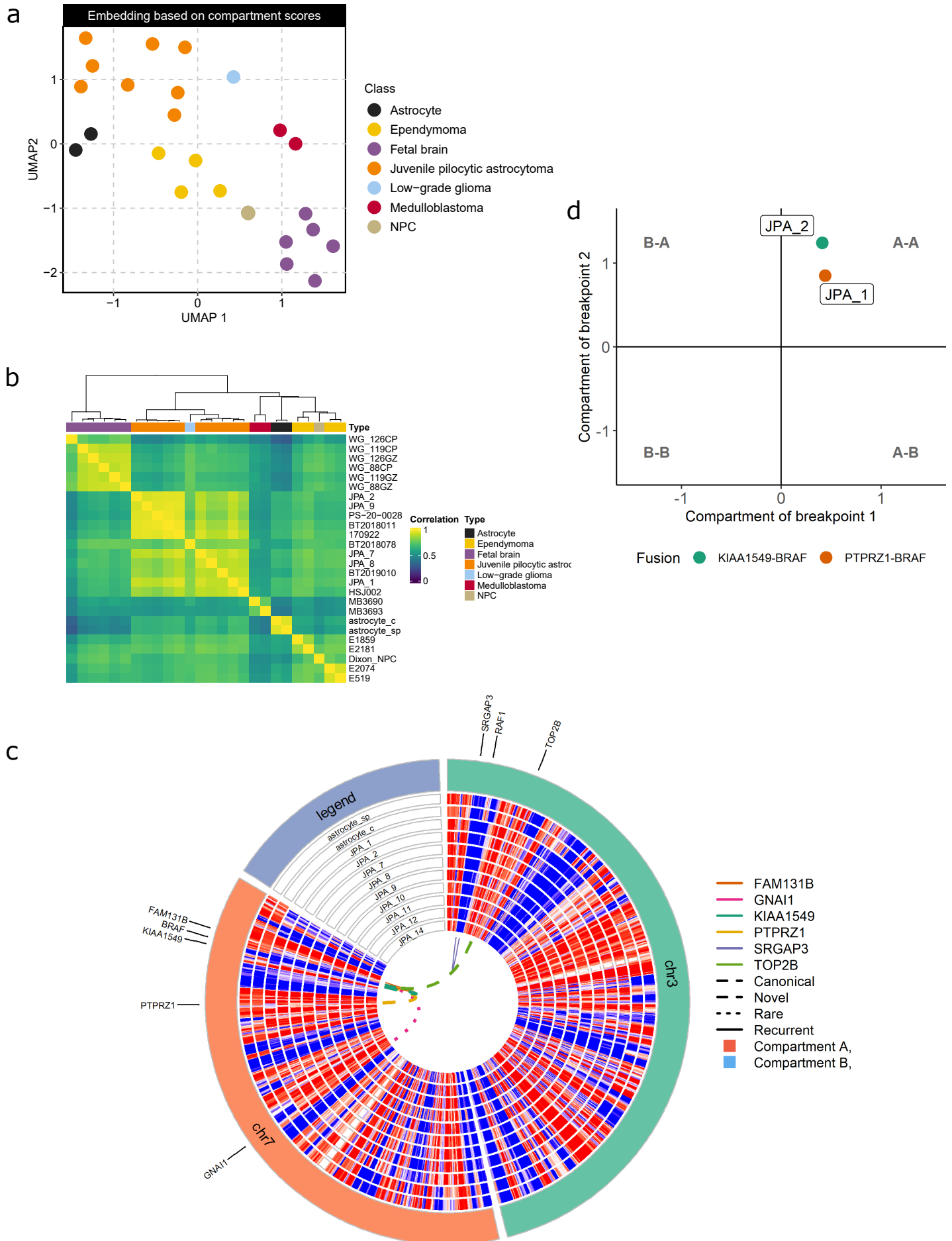
JPA_6
Tandem duplication resulting in
KIAA1549-BRAF
(EX16-EX9, HAP_ALLELIC_FRAC=0.8)



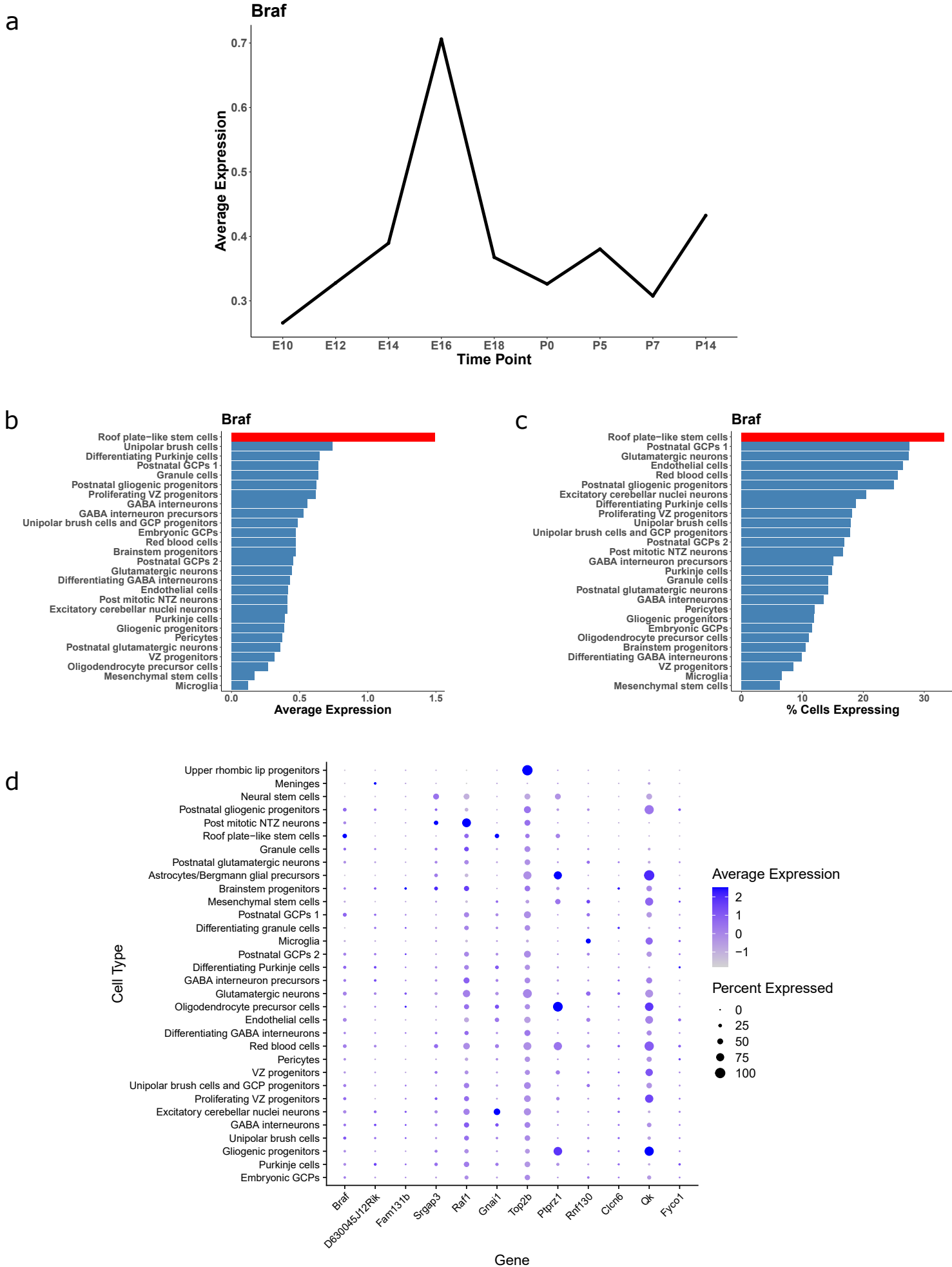
Supplemental Figure 2.



Supplemental Figure 3.



Supplemental Figure 4.



Supplemental Table 1. Sample description and detection of BRAF fusions across technologies

Sample Information				Linked-reads					RNA-Seq			Hi-C
Patient	Diagnosis	Tumor Location	5' Fusion Partner	LongRanger	GROC_SV	LinkedSV	NAIBR	SvABA	STAR-Fusion	Arriba	InFusion	Hi-C
JPA_1	JPA	Cerebellum	PTPRZ1	Yes	No	No	Yes	No	Yes	Yes	Yes	Yes
JPA_2	JPA	Left cerebellum	KIAA1549	Yes	No	No	Yes	Yes	No	Yes	Yes	Yes
JPA_3	JPA	Posterior	TOP2B	Yes	Yes	No	Yes	Yes	Yes	Yes	No	-
JPA_4	JPA	Thalamic Midbrain	KIAA1549	Yes	Yes	No	Yes	Yes	No	Yes	No	-
JPA_5	JPA	Posterior fossa	KIAA1549	Yes	Yes	No	Yes	Yes	No	Yes	No	-
JPA_6	JPA	Posterior fossa	KIAA1549	Yes	Yes	No	Yes	No	No	Yes	No	-
JPA_7	JPA	Ventricle	KIAA1549	-	-	-	-	-	No	Yes	No	Yes
JPA_8	Recurrence of JPA_7		KIAA1549	-	-	-	-	-	-	-	-	Yes
JPA_9	JPA	Spinal	GNAI1	-	-	-	-	-	Yes	Yes	No	Yes
HSJ-002	JPA	Cerebellum	KIAA1549	-	-	-	-	-	No	Yes	No	Yes
BT2018011	JPA	Posterior fossa	KIAA1549	-	-	-	-	-	-	-	-	Yes
170922	JPA	Trigone	KIAA1549	-	-	-	-	-	-	-	-	Yes
BT2019010	JPA	Posterior fossa	KIAA1549	-	-	-	-	-	-	-	-	Yes
BT2018078	LGG	Cortex	No Fusion	-	-	-	-	-	-	-	-	Yes

Supplemental Table 2. Fusion calls in RNA-Seq data

Sample	Star-Fusion	Arriba	InFusion	Total calls
JPA_1	10	851	107	968
JPA_2	1	859	45	905
JPA_3	1	505	28	534
JPA_4	0	779	157	936
JPA_5	1	1103	81	1185
JPA_6	1	1124	171	1296
JPA_7	1	515	34	550
JPA_9	1	635	41	677
HSJ-002	1	404	24	429

Supplemental Table 3. SV calls in linked-read data

Sample	Tissue	GROC_SV	LinkedSV	LongRange	NAIBR	SvABA
JPA_1	Blood	41	148	215	3313	401366
JPA_1	Tumor	40	157	1290	9605	9947
JPA_2	Blood	15	122	144	5198	59193
JPA_2	Tumor	10	228	957	5668	1971
JPA_3	Blood	31	96	254	6764	40187
JPA_3	Tumor	4	47	691	2669	2403
JPA_4	Blood	15	92	144	7778	30027
JPA_4	Tumor	3	141	382	4061	3514
JPA_5	Blood	22	103	164	4708	31948
JPA_5	Tumor	3	99	706	3155	556
JPA_6	Blood	22	71	145	4684	36062
JPA_6	Tumor	11	334	1211	3068	3320

Supplemental Table 4. Extraction method and sample metrics as calculated by LongRanger

	Tumor								Blood							
Sample	Extraction & Size selection method	Mean depth	Average molecule length	DNA in Molecules >20kb	DNA in Molecules >100kb	SNPs Phased	N50 Phase Block	Number of large SVs	Extraction & Size selection method	Mean depth	Average molecule length	DNA in Molecules >20kb	DNA in Molecules >100kb	SNPs Phased	N50 Phase Block	Number of large SVs
JPA_1	Chemagen & BluePippin PacBio 20kb	77.1X	19kb	49.7%	3.03%	99.5%	460kb	1378	MagAttract & BluePippin BPLUS10	69.7X	42kb	86.9%	3.94%	99.60%	1,946kb	186
JPA_2	MagAttract	38.7X	58kb	90.20%	16.7%	98.90%	2,981kb	986	MagAttract	32.7X	96kb	95.00%	49.1%	99.00%	8,996kb	151
JPA_3	Chemagen & BluePippin PacBio 20kb	38.8X	31kb	78.90%	1.75%	99.0%	816kb	661	MagAttract & HLS HMW HighPass 300kb	31.4X	79kb	94.50%	33.5%	98.9%	5,580kb	265
JPA_4	MagAttract	33.1X	85kb	95.30%	39.40%	99.4%	5,874kb	398	MagAttract	29.4X	104kb	96.20%	55.2%	99.3%	8,018kb	141
JPA_5	MagAttract	27.6X	64kb	93.30%	22.00%	98.90%	3,067kb	681	MagAttract	35.1X	84kb	94.90%	38.6%	99.00%	5,828kb	141
JPA_6	MagAttract	36.8X	62kb	89.20%	22.30%	99.30%	3,888kb	1273	MagAttract	30.4X	76kb	94.10%	33.2%	99.3%	4,605kb	121

Supplemental Table 5. Quality metrics for Hi-C datasets

Sample	Resolution	total_sequenced_abs	normal_paired_abs	normal_paired_pct	chimeric_paired_abs	chimeric_paired_pct	alignable_abs	alignable_pct	hic_contact_s_abs	hic_contact_s_pct	ligation_motif_pct	inter_chrom_abs	inter_chrom_pct	intra_chrom_abs	intra_chrom_pct	short_range_abs	short_range_pct	long_range_abs	long_range_pct
JPA_1	4900	648298450	434151379	66.97	172596578	26.62	606747957	93.59	413876697	71.79	34.47	90133632	15.63	323743065	56.16	120844319	20.96	202898561	35.2
JPA_2	17000	521941348	381214913	73.04	108938726	20.87	490153639	93.91	117676846	48.43	15.58	21018373	8.65	96658473	39.78	71942152	29.61	24716234	10.17
JPA_7	14800	299896108	136896650	45.65	107227035	35.75	244123685	81.4	176270792	74.7	55.68	72731084	30.82	103539708	43.88	39900390	16.91	63639169	26.97
JPA_8	12200	291618237	152941765	52.45	85616955	29.36	238558720	81.81	147045245	67.8	40.56	45537216	21	101508029	46.8	37328144	17.21	64179749	29.59
JPA_9	16850	351523017	210792802	59.97	87226064	24.81	298018866	84.78	148145954	51.99	25.15	55833407	19.59	92312547	32.39	44334593	15.56	47977733	16.84
HSJ002	4850	759595762	562263832	74.02	138897082	18.29	701160914	92.31	402037505	61.13	18.18	112588791	17.12	289448714	44.01	97622344	14.84	191826080	29.17
BT2018011	5550	487812373	245369596	50.3	201712579	41.35	447082175	91.65	319948048	83.02	55.38	101523797	26.34	218424251	56.67	50983489	13.23	167440693	43.45
170922	5900	510715657	247098337	48.38	215059964	42.11	462158301	90.49	315888184	83.34	57.15	108013239	28.5	207874945	54.84	61782464	16.3	146092344	38.54
BT2019010	42500	54872613	27603607	50.3	23221998	42.32	50825605	92.62	36439117	83	56.82	11154120	25.41	25284997	57.59	6460655	14.72	18824330	42.88
BT2018078	7600	365683475	178617177	48.84	154954061	42.37	333571238	91.22	244592496	83.59	58.06	69628651	23.8	174963845	59.79	46626070	15.93	128337700	43.86

Supplemental Table 6. Comparison of RNA-Seq, 10x Genomics Linked-Reads and Hi-C protocols

Technology	Preparation of input material	Amount of starting material	Hands on time for preparation of input material	Time for library preparation	Total turnaround time (not including sequencing)	Reagent Cost per library	Input Requirements	Recommended Sequencing depth	Recommended Sequencing Length
RNA-Seq	RNA extraction with RNeasy Mini Kit (1h) QC by Qubit and TapeStation (1h)	10–30mg	2 hours	~10.5 hours	2 days	\$	100-1000ng total RNA	50-100 million paired reads	75-150bp
10X linked-Reads	HMW DNA extraction with circulomics kit (2h) Recommended to allow HMW DNA to sit and resuspend overnight QC by Qubit and Femto Pulse (1h prep, 3h run time)	5-10mg	3 hours (overnight incubation for resuspension is recommended)	8 hours	3 days	\$\$	10ng HMW DNA	425 million paired reads	150bp
Hi-C from fresh/frozen tissue	Dovetail® Omni-C Kit (Steps 1-3) Tissue dissociation with liquid Nitrogen and Crosslinking (2h) Lysate QC (2h) Proximity Ligation (4.5h)	20mg	9.5 hours	5 hours	2 days	\$\$\$	150ng purified DNA	300 million paired reads	150bp
Hi-C from FFPE	Arima-HiC+ FFPE Kit Dewaxing (1h) Digestion (3h) Overnight incubation Reverse Cross-Linking (15min) Overnight incubation Purification and QC (1h)	5mm ³ from 5-10µm FFPE tissue section	5.5 hours	4 hours	3 days	\$\$\$	125ng-2000ng purified, fragmented and size selected DNA	600 million paired reads	150bp