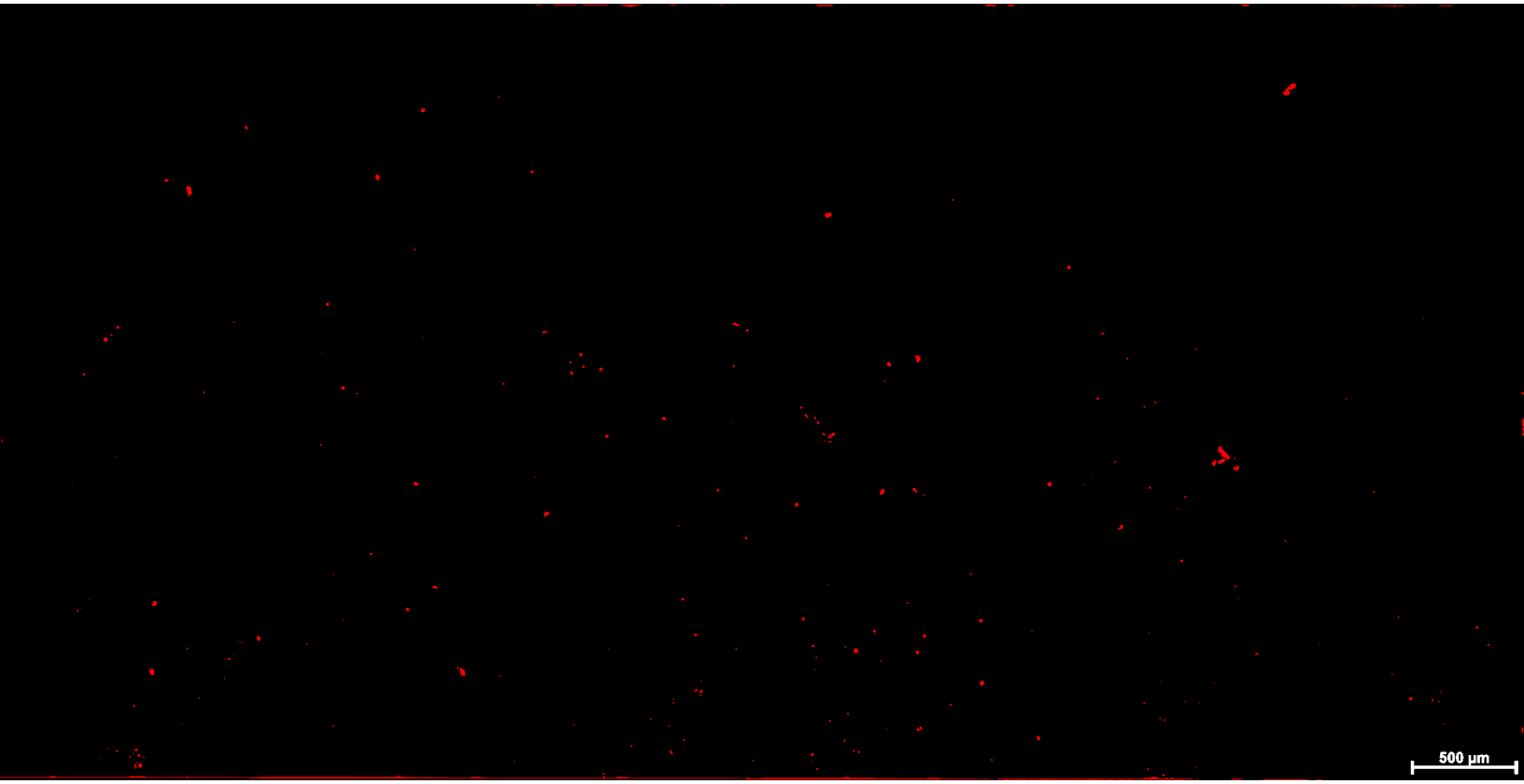
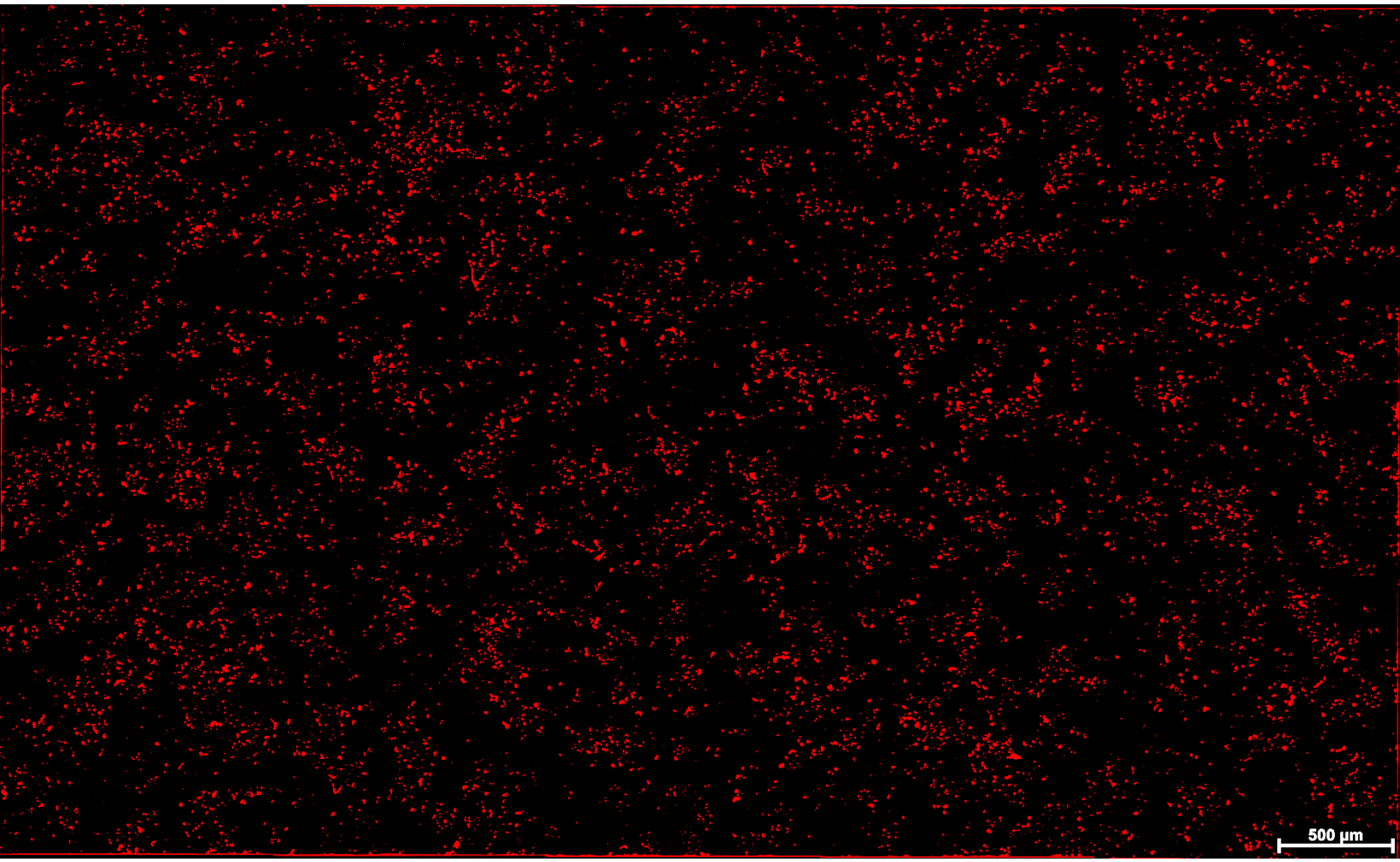
Supplemental Table 1: Patient Characteristics

Patient ID	Tissue Type	Occurrence	Tumor Category	Age	Race	Gender	Received Any Sort of Treatment Pre Surgery
101	Metastatic poorly differentiated squamous cell carcinoma from lung to brain	Metastatic Tumor	Adult Metastatic	60	White	Male	Yes
102	Metastatic HER2-positive breast carcinoma to brain	Metastatic Tumor	Adult Metastatic	44	White	Female	Yes
103	Metastatic poorly differentiated adenocarcinoma from lung to brain	Metastatic Tumor	Adult Metastatic	70	White	Female	Yes
104	*Tisue Not Obtained - No Remaining Tissue*						
105	*Tisue Not Obtained - After Hours Surgery*						
106	Meningioma, CNS WHO grade 2	Primary Tumor	Adult Low-Grade	49	Hispanic/latino	Female	No
107	*Tisue Not Obtained - After Hours Surgery*						
108	Astrocytoma, IDH-mutant, CNS WHO grade 4	Recurrent Tumor	Adult High-Grade	39	White	Male	Yes
109	Low-grade glioneuronal tumor, not otherwise specified (NOS)	Primary Tumor	Adult Low-Grade	25	White	Female	No
110	*Tisue Not Obtained - After Hours Surgery*						
111	Meningioma, CNS WHO grade 1	Primary Tumor	Adult Low-Grade	48	White	Female	No
112	*Tisue Not Obtained - After Hours Surgery*						
113	Glioblastoma IDH-wildtype CNS WHO grade 4	Primary and Recurrent Tumor	Adult High-Grade	70	Black	Male	No
114	*Tisue Not Obtained - After Hours Surgery*						
115	Pilocytic astrocytoma, CNS WHO grade 1	Primary Tumor	Pediatric Low-Grade	6	White	Male	No
116	*Tisue Not Obtained - No Remaining Tissue*						
117	*Tisue Not Obtained - After Hours Surgery*						
118	*Tisue Not Obtained - After Hours Surgery*						
119	Metastatic squamous cell carcinoma of lung to brain	Metastatic Tumor	Adult Metastatic	78	Black	Male	No
120	Metastatic breast carcinoma to brain, ER-positive, HER2-equivocal	Metastatic Tumor	Adult Metastatic	75	White	Female	Yes
121	*Tisue Not Obtained - No Remaining Tissue*						
122	Astrocytoma, IDH-mutant, CNS WHO grade 3	Primary Tumor	Adult High-Grade	54	White	Male	No
123	Glioblastoma IDH-wildtype CNS WHO grade 4	Recurrent Tumor	Adult High-Grade	57	Hispanic/latino	Female	Yes
124	Glioblastoma IDH-wildtype CNS WHO grade 4	Primary Tumor	Adult High-Grade	53	White	Male	No
125	Glioblastoma IDH-wildtype CNS WHO grade 4	Primary Tumor	Adult High-Grade	45	White	Male	No
126	Glioblastoma IDH-wildtype CNS WHO grade 4	Primary Tumor	Adult High-Grade	77	Black	Female	No
127	Metastatic triple-negative breast carcinoma to brain	Metastatic Tumor	Adult Metastatic	55	White	Female	Yes
128	Glioblastoma IDH-wildtype CNS WHO grade 4	Primary Tumor	Adult High-Grade	68	White	Female	No
129	*Tisue Not Obtained - Patient Removed from Study Prior to Surgery*						
130	*Tisue Not Obtained - No Remaining Tissue*						
131	*Tisue Not Obtained - Patient Removed from Study Prior to Surgery*						
132	*Tisue Not Obtained - After Hours Surgery*						
133	Glioblastoma IDH-wildtype CNS WHO grade 4	Primary Tumor	Adult High-Grade	80	White	Male	No
134	*Tisue Not Obtained - After Hours Surgery*						
135	*Tisue Not Obtained - After Hours Surgery*						
136	*Tisue Not Obtained - After Hours Surgery*						
137	*Tisue Not Obtained - After Hours Surgery*						
138	Glioblastoma IDH-wildtype CNS WHO grade 4	Recurrent Tumor	Adult High-Grade	52	Black	Male	No
139	*Tisue Not Obtained - After Hours Surgery*						
140	Glioblastoma, IDH-wildtype, CNS WHO grade 4	Recurrent Tumor	Adult High-Grade	62	White	Female	Yes
141	Radiation necrosis	Metastatic Tumor	Pseudoprogression	61	Black	Male	Yes
142	Posterior fossa ependymoma group PFA CNS WHO grade 3	Primary Tumor	Pediatric High-Grade	1	Black, Filipino	Male	No
143	Pleomorphic xanthoastrocytoma, BRAF V600E-mutant, CNS WHO grade 2	Primary Tumor	Pediatric High-Grade	8	Black	Female	No
144	Astrocytoma, IDH-mutant, CNS WHO grade 4	Recurrent Tumor	Adult High-Grade	21	Black	Male	Yes
145	*Tisue Not Obtained - After Hours Surgery*						
146	*Tisue Not Obtained - No Remaining Tissue*						
147	Metastatic ovarian carcinoma to brain	Metastatic Tumor	Adult Metastatic	52	White	Female	Yes
148	*Tisue Not Obtained - After Hours Surgery*						
149	*Tisue Not Obtained - No Remaining Tissue*						
150	Atypical teratoid/rhabdoid tumor CNS WHO grade 4	Primary Tumor	Pediatric High-Grade	5	Asian Indian	Male	No
151	Medulloblastoma, SHH-activated and TP53-wildtype, CNS WHO grade 4	Primary Tumor	Pediatric High-Grade	18	Hispanic/Latino	Female	No
152	Glioblastoma IDH-wildtype CNS WHO grade 4	Recurrent Tumor	Adult High-Grade	65	White	Female	Yes
153	Glioblastoma, IDH-wildtype, BRAF V600E-mutant, CNS WHO grade 4	Recurrent Tumor	Adult High-Grade	48	White	Male	Yes
154	*Tisue Not Obtained - After Hours Surgery*						
155	Medulloblastoma, SHH-activated and TP53-wildtype, CNS WHO grade 4	Primary Tumor	Pediatric High-Grade	4	White	Male	No
156	*Tisue Not Obtained - Tissue Designated to Interventional Trial*						
157	Metastatic melanoma to brain	Metastatic Tumor	Adult Metastatic	69	White	Female	No
158	Glioblastoma IDH-wildtype CNS WHO grade 4	Primary Tumor	Adult High-Grade	68	White	Male	No
159	Pilocytic astrocytoma, CNS WHO grade 1, BRAF-KIAA1549 fusion-positive	Primary Tumor	Pediatric Low-Grade	8	White	Female	No
160	Atypical teratoid/rhabdoid tumor CNS WHO grade 4	Primary Tumor	Pediatric High-Grade	1	White	Male	No

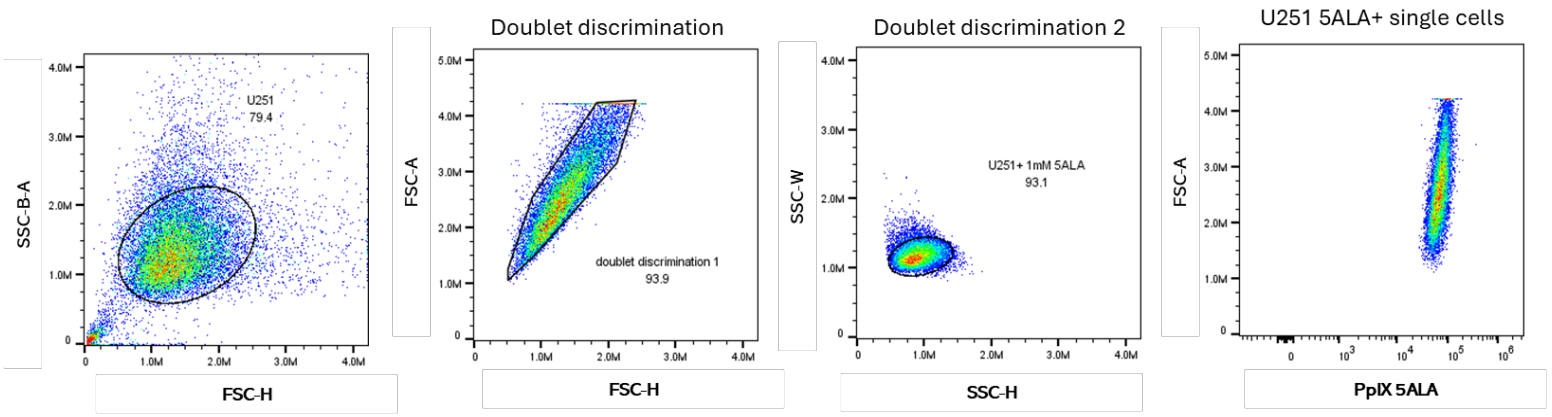
Supplemental Figure 1: High Mag Images of 5-ALA Fluorescence



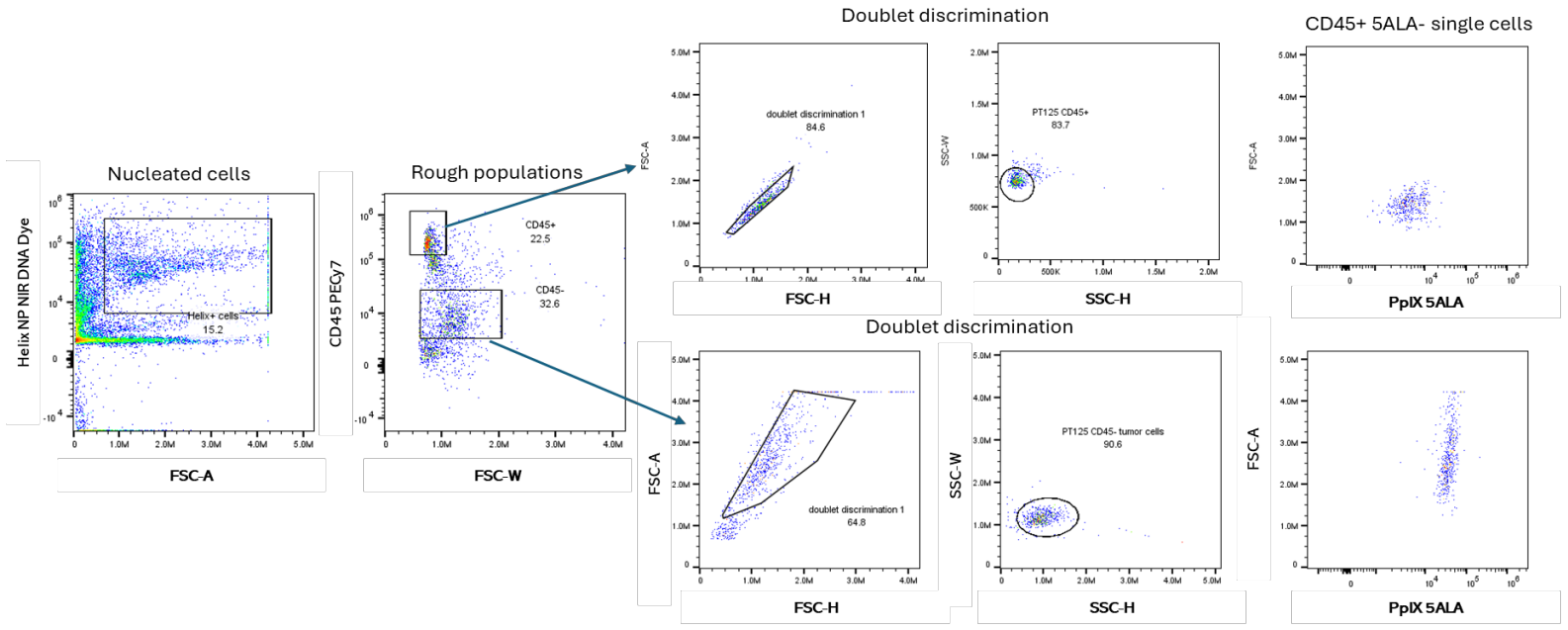


Supplemental Figure 2: Gating Strategies to Measure 5-ALA by Flow Cytometry

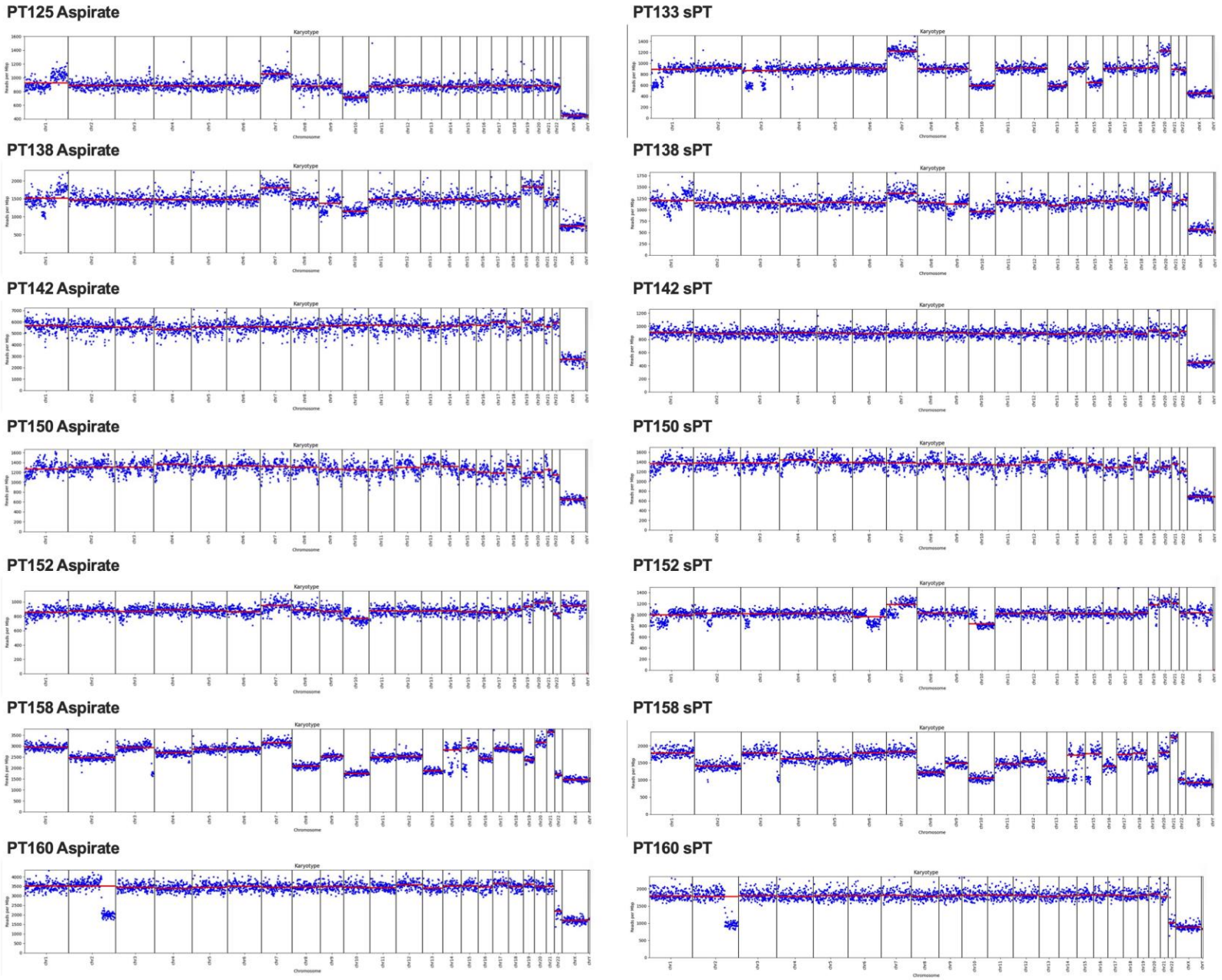
Gating strategy, U251 cell line



Gating strategy, PT125 glioblastoma



Supplemental Figure 3: Nanopore Karyotypes



Supplemental Table 2: Nanopore Sequencing Experiment-Wise Downsampling

Sample	Mean Sequencing Quality	Individual Bases Aligned	Average Genomic Coverage	Number of Sturgeon CG Sites Used For Classification	Classification	Classification Probability	Estimated Tumor Purity	Other Copy Number Alterations	Suspected Chromosome-Level Copy Number Alterations
138 sPT	35.6	16,804,587,742	7.270x	424449	Mesenchymal Glioblastoma	0.96375	45.85%	EGFR Amplification	+1q, +7, -9p, -10, +19, +20
138 aspir	37.6	16,812,674,259	7.169x	425162	Mesenchymal Glioblastoma	0.97306	39.15%	EGFR Amplification	+1q, +7, -9p, -10, +19, +20
142 sPT	12	16,809,030,735	7.275x	413841	Posterior Fossa Ependymoma Group A	0.99780	N/A	N/A	N/A
142 aspir	31.9	16,819,305,399	7.042x	426054	Posterior Fossa Ependymoma Group A	0.99958	N/A	N/A	N/A
150 sPT	38.5	16,804,979,727	7.296x	422803	MYC Subtype Atypical Teratoid Rhabdoid Tumor	0.99857	84.17%	Deletion upstream of SMARCB1	N/A
150 aspir	38.8	16,809,171,416	6.988x	422576	MYC Subtype Atypical Teratoid Rhabdoid Tumor	0.99822	88.89%	Deletion upstream of SMARCB1	N/A
152 sPT	40.8	16,814,608,178	6.834x	424409	Mesenchymal Glioblastoma	0.93908	36.55%	EGFR Amplification	+7q, -10q, +19, +20, +21
152 aspir	40	16,814,080,785	6.766x	424205	Mesenchymal Glioblastoma	0.94791	22.31%	EGFR Amplification	+7, -10q, +19, +20, +21, +X
158 sPT	37.7	16,814,209,230	6.839x	422855	Mesenchymal Glioblastoma	0.96712	87.83%	N/A	-2, -4, +7, -8, -9, -10, -11, -12, -13, -15p, -16, -19, +20, +21, -22
158 aspir	42.1	16,811,818,147	6.906x	424208	Mesenchymal Glioblastoma	0.95245	82.48%	N/A	-2, -4, +7, -8, -9, -10, -11, -12, -13, -15p, -16, -19, +20, +21, -22
160 sPT	38.7	16,774,054,022	6.863x	424456	MYC Subtype Atypical Teratoid Rhabdoid Tumor	0.99903	90.27%	SMARCB1 deletion	-2q, -22
160 aspir	37.8	16,824,111,541	6.898x	425021	MYC Subtype Atypical Teratoid Rhabdoid Tumor	0.99898	79.54%	SMARCB1 deletion	-2q, -22

Supplemental Table 3: Nanopore Sequencing Patient-Wise Downsampling

Sample	Mean Sequencing Quality	Individual Bases Aligned	Average Genomic Coverage	Number of Sturgeon CG Sites Used For Classification	Classification	Classification Probability	Estimated Tumor Purity	Other Copy Number Alterations	Suspected Chromosome-Level Copy Number Alterations
125 aspir	32.1	12,571,938,283.00	5.53x	416187	Mesenchymal Glioblastoma	0.83270	0.3803611738	TBD	+1q, +7, -10
133 sPT	33.3	7,654,025,304.00	3.38x	373128	RTK II Glioblastoma	0.93515	0.6779279279	TBD	-1p, +7, -10, -13, -15, +20
138 sPT	35.6	29,508,475,046.00	12.761x	427597	Mesenchymal Glioblastoma	0.98162	0.450608931	EGFR Amplification	+1q, +7, -9p, -10, +19, +20
138 aspir	37.6	20,574,415,760.00	8.776x	426793	Mesenchymal Glioblastoma	0.96807	0.3838209983	EGFR Amplification	+1q, +7, -9p, -10, +19, +20
142 sPT	12	16,809,030,735.00	7.275x	413841	Posterior Fossa Ependymoma Group A	0.99780	N/A	N/A	N/A
142 aspir	31.9	63,662,400,145.00	26.662x	427823	Posterior Fossa Ependymoma Group A	0.99970	N/A	N/A	N/A
150 sPT	38.5	18,045,458,021.00	7.835x	424069	MYC Subtype Atypical Teratoid Rhabdoid Tumor	0.99857	0.8532110092	Deletion upstream of SMARCB1	N/A
150 aspir	38.7	28,922,515,158.00	12.028x	427181	MYC Subtype Atypical Teratoid Rhabdoid Tumor	0.99848	0.8524173028	Deletion upstream of SMARCB1	N/A
152 sPT	40.8	22,886,649,835.00	9.303x	427000	Mesenchymal Glioblastoma	0.92693	0.3628741347	EGFR Amplification	+7q, -10q, +19, +20, +21
152 aspir	40	21,235,814,380.00	8.551x	426658	Mesenchymal Glioblastoma	0.79445	0.2334827071	EGFR Amplification	+7, -10q, +19, +20, +21, +X
158 sPT	37.8	40,618,731,758.00	16.541x	427584	Mesenchymal Glioblastoma	0.93853	0.8626104024	N/A	-2, -4, +7, -8, -9, -10, -11, -12, -13, -15p, -16, -19, +20, +21, -22
158 aspir	42.1	61,051,766,600.00	25.080x	427675	Mesenchymal Glioblastoma	0.96932	0.8666666667	N/A	-2, -4, +7, -8, -9, -10, -11, -12, -13, -15p, -16, -19, +20, +21, -22
160 sPT	38.7	58,884,019,618.00	24.110x	427602	MYC Subtype Atypical Teratoid Rhabdoid Tumor	0.99903	0.8787121843	SMARCB1 deletion	-2q, -22
160 aspir	37.8	65,140,936,906.00	26.713x	427756	MYC Subtype Atypical Teratoid Rhabdoid Tumor	0.99886	0.7470638785	SMARCB1 deletion	-2q, -22

Supplemental Table 4: Nanopore Sequencing Classification Status

Sample	Nanopore Classification	Clinical Classification	Classification Status
125 aspir	Mesenchymal Glioblastoma	Glioblastoma, IDH-Wild Type, WHO Grade 4	Molecular Refinement
133 sPT	RTK II Glioblastoma	Glioblastoma, IDH-Wild Type, WHO Grade 4	Molecular Refinement
138 sPT	Mesenchymal Glioblastoma	Glioblastoma, IDH-Wild Type, WHO Grade 4	Molecular Refinement
138 aspir	Mesenchymal Glioblastoma	Glioblastoma, IDH-Wild Type, WHO Grade 4	Molecular Refinement
142 sPT	Posterior Fossa Ependymoma Group A	Posterior Fossa Ependymoma Group A	Matched Clinical Diagnosis
142 aspir	Posterior Fossa Ependymoma Group A	Posterior Fossa Ependymoma Group A	Matched Clinical Diagnosis
150 sPT	MYC Subtype Atypical Teratoid Rhabdoid Tumor	Atypical Teratoid Rhabdoid Tumor WHO Group 4	Molecular Refinement
150 aspir	MYC Subtype Atypical Teratoid Rhabdoid Tumor	Atypical Teratoid Rhabdoid Tumor WHO Group 4	Molecular Refinement
152 sPT	Mesenchymal Glioblastoma	Glioblastoma, IDH-Wild Type, WHO Grade 4	Molecular Refinement
152 aspir	Mesenchymal Glioblastoma	Glioblastoma, IDH-Wild Type, WHO Grade 4	Molecular Refinement
158 sPT	Mesenchymal Glioblastoma	Glioblastoma, IDH-Wild Type, WHO Grade 4	Molecular Refinement
158 aspir	Mesenchymal Glioblastoma	Glioblastoma, IDH-Wild Type, WHO Grade 4	Molecular Refinement
160 sPT	MYC Subtype Atypical Teratoid Rhabdoid Tumor	Atypical Teratoid Rhabdoid Tumor WHO Group 4	Molecular Refinement
160 aspir	MYC Subtype Atypical Teratoid Rhabdoid Tumor	Atypical Teratoid Rhabdoid Tumor WHO Group 4	Molecular Refinement