Supplementary Tables and Figures

Functional Characterization of Brain Tumor-Initiating Cells and Establishment of GBM Preclinical Models that Incorporate Heterogeneity, Therapy, and Sex Differences

Cesar A. Garcia[1,2], Adip G. Bhargav[1,2,3], Mieu Brooks[1,2], Paola Suarez-Meade[1,2], Sujan K. Mondal[1,2], Natanael Zarco[1,4], Karim ReFaey[1], Mark Jentoft[5], Erik H. Middlebrooks[1,6], Matija Snuderl[7], Anna Carrano[1,4], Hugo Guerrero-Cazares[1,4], Paula Schiapparelli[1,2], Rachel Sarabia-Estrada[1,2], Alfredo Quiñones-Hinojosa[1,2]

[1]Department of Neurosurgery, Mayo Clinic, Jacksonville, FL, USA
[2]Brain Tumor Stem Cell Laboratory, Mayo Clinic, Jacksonville, FL, USA
[3] Mayo Clinic Alix School of Medicine, Mayo Clinic, Rochester, MN, USA
[4]Neurogenesis and Brain Tumors Laboratory, Mayo Clinic, Jacksonville, FL, USA
[5]Department of Pathology, Mayo Clinic, Jacksonville, FL, USA
[6]Department of Radiology, Mayo Clinic, Jacksonville, FL, USA
[7]Department of Pathology, NYU Langone Health, New York, NY, USA

Corresponding Author
Alfredo Quiñones-Hinojosa, MD, FAANS, FACS
William J. and Charles H. Mayo Professor, Chair
Neurologic Surgery
Mayo Clinic
4500 San Pablo Rd.
Jacksonville, FL 32224, USA
Phone: (904)-956-3435
Email: Quinones-Hinojosa.Alfredo@mayo.edu

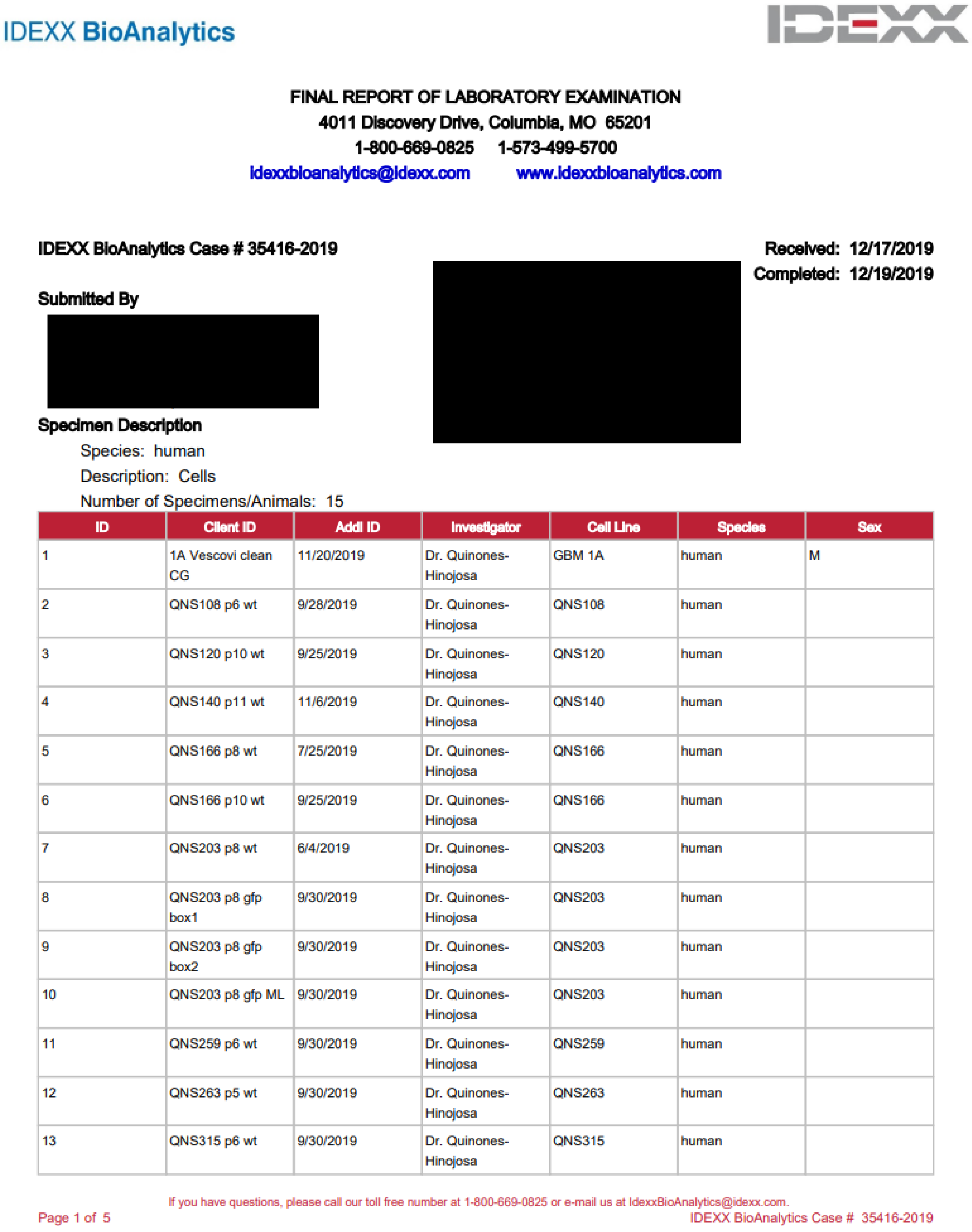
Table S1. Material List

Material	Manafacturer	Catalogue Number
Leica low profile blades 819	Leica	14035838925
Tissue-Tek® O.C.T. Compound	Sakura	4583
Polyclonal Rabbit Anti-Glial Fibrillary Acidic Protein	Dako	Z0334
Purified anti-Tubulin ß3 (TUB3) Clone: TUJ1	BioLegend	801201
MS x Nestin, clone 10C2 Human Specific	EMD Millipore	MAB5326
Goat Serum	Sigma	G9023-10ml
Alexa Fluor ® 488 goat anti-mouse IgG	Invitrogen	A11001
Alexa Fluor ® 594 goat anti-rabbit IgG	Invitrogen	A11012
Isoflurane, USP	Piramal Critical Care	NDC 66794-017-25
PBS, 1x	Corning Cellgro	21-040-CV
Fisherbrand Microscope Cover Glass	Fisher Scientific	12-545-J
Round Glass Cover Slip, German Glass, Size: 15mm diameter	Electron Microscopy Sciences	72196
Poly-L-ornithine solution	Sigma	P49757-50ml
Gem21 NeuroPlex™ Serum-Free, w/o Vitamin A	Gemini Bio-Products	400-161
Human EGF	PeproTech, INC.	AF-100-15-1MG
Human FGF-Basic	PeproTech, INC.	AF-100-18B-1MG
DMSO	Sigma	D1435-500ml
Formaldehyde (Methanol Free), 10% UltraPure EM Grade	Polysciences, Inc.	04018-1
Ethylene Glycol	EMSURE®	1.09621.1000
DAPI	Thermo Scientific	62248
Cell Culture Plate, 96 well	costar®	3595
Accutase	Sigma	A6964-500
6.5mm Transwell® with 8.0 µm Pore Polycarbonate Membrane Insert, Sterile	Corning Costar®	3422
Nunc Non-treated T25 Easyflask, Filter cap (white)	Thermo Scientific	169900
Nunc Cell Culture treated T25 EasyFlask, Filter cap (blue)	Thermo Scientific	156367
Nunc Non-treated T75 Easyflask, Filter cap (white)	Thermo Scientific	156800
Nunc Cell Culture treated T75 EasyFlask, Filter cap (blue)	Thermo Scientific	156499
Cultrex Mouse Laminin I	Fisher Scientific	3400-010-01
CD133/1 antibody	Miltenyi Biotec	130-098-829
Isotype Control Antibody, Mouse IgG1 PE	Miltenyi Biotec	130-113-200
HEK293 cells	ATCC®	CRL-3216™
CyQUANT™ Cell Proliferation Assay Kit, for cells in culture	Invitrogen	C7026
Temozolomide	Selleckchem	S1237
Recombinant human TRAIL protein	Abcam	ab168898
Recombinant human BMP4-4	PeproTech	120-05ET
Hamilton Syringes 10µl Model 1701 NSYR, Cemented NDL, 26ga, 2 in, point style 3	Hamilton	80085
Lipofectamine 3000	Thermo Fisher	L3000015
D-Luciferin, Potassium Salt	Gold Biotechnology	LUCK-5G
psPAX2, pMD.2, and the plasmid pHAGE PGK-GFP-IRES-LUC-W	Addgene	46793
Lenti-X™ p24 Rapid Titer Kit	Takara	632200
TC plate 6 well standard F	sarstedt	83.3920.
Lenti-X Concentrator reagent	Clontech	631231
Target2™ PES (Polyethersulfone) Syringe Filters 0.45µm	ThermoFisher Scientific	F2500-14
Pump 11 Elite	Haravard Apparatus	70-4507
Homeothermic Monitoring System	Haravard Apparatus	55-7020
Olympus SZ2-STB2 surgical scope	Olympus	4H05300
1 mm Bone Drill	Ideal Micro-Drill	67-1000 (3052)
Cell Strainer 40 µm nylon mesh	Fisherbrand®	22363547
500 ml Vacuum Filter/Storage Bottle System, 0.22µm Pore 33.2 cm^2 PES Membrane, sterile, 12/Case	Corning	431097
Corning Costar® TC-Treated Multiple Well Plates	Corning Costar®	3526
Diamond™ White Glass Microscope Slides	Globe Scientific INC.	1380-30
psPAX2	Addgene	12260
pMD.2	Addgene	12259
p24 ELISA Kit	Takara Bio	632200
Qiagen Dneasy® Blood & Tissue Kit (50)	Qiagen	69504
Anti-IDH1 R132H/DIA-H09-RUO	dianova	DIA-H09-RUO
Novacastra^{TM} Liquid Mouse Monoclonal Antibody p53 Protein (DO-7)	Leica	NCL-L-p53-DO7
ATRX (D-5)	SANTA CRUZ BIOTECHNOLOGY, I	sc-55584
Anti-Nuclei Antibody, clone 3E1.3 (Human Nuclei)	Millipore Sigma	MAB4383
Anti-Ki-67 Antibody, clone Ki-S5	Millipore Millipore Sigma	MAB4190

Table S2. Equipment and Software

Equipment/Software
The X-RAD SmART© irradiator
Zeiss LSM 800 Confocal System Based on Axio Observer for Z1 Motorized Inverted Microscope
Arivis Vision4D x64 3.0
Synergy HTX multi-mode reader
Zen 2.3 System
Zeiss Confocal Microscope (Observer Z, LSM 800, X-Cite 120 LED Boost)
Vi-Cell™ XR Cell Viability Analyzer
Leica CM1950 Cryostat
Leica Aperio AT2
IVIS® Spectrum XENOGEN

Figure S1: Cell Line STR Fragment Authentication Report from IDEXX



IDEXX BioAnalytics

FINAL REPORT OF LABORATORY EXAMINATION
4011 Discovery Drive, Columbia, MO 65201
1-800-669-0825 1-573-499-5700
idexxbioanalytics@idexx.com www.idexxbioanalytics.com

IDEXX BioAnalytics Case # 35416-2019

Received: 12/17/2019
Completed: 12/19/2019

Submitted By

[redacted]

[redacted]

Specimen Description

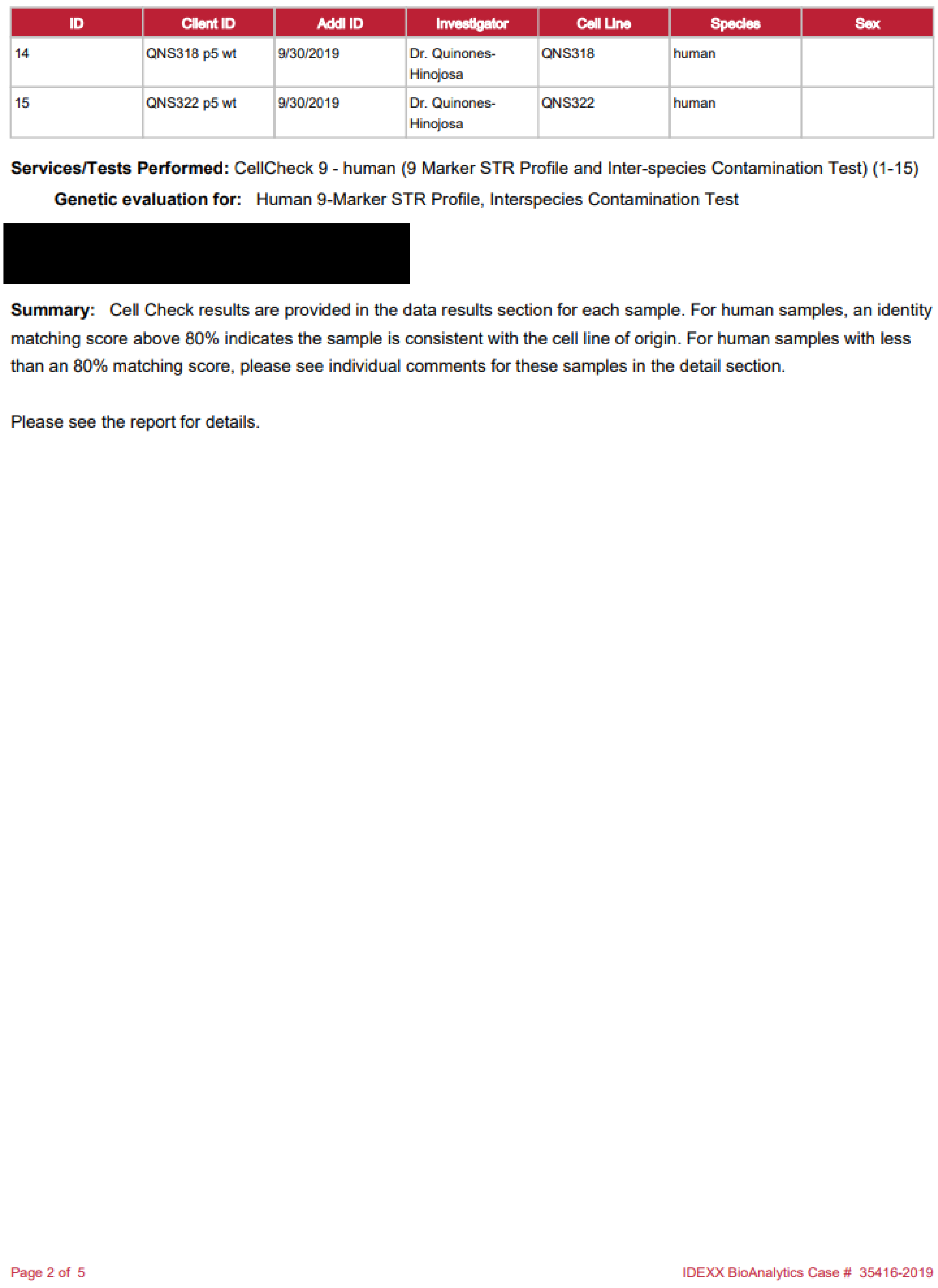
Species: human
Description: Cells
Number of Specimens/Animals: 15

ID	Client ID	Addl ID	Investigator	Cell Line	Species	Sex
1	1A Vescovi clean CG	11/20/2019	Dr. Quinones-Hinojosa	GBM 1A	human	M
2	QNS108 p6 wt	9/28/2019	Dr. Quinones-Hinojosa	QNS108	human	
3	QNS120 p10 wt	9/25/2019	Dr. Quinones-Hinojosa	QNS120	human	
4	QNS140 p11 wt	11/6/2019	Dr. Quinones-Hinojosa	QNS140	human	
5	QNS166 p8 wt	7/25/2019	Dr. Quinones-Hinojosa	QNS166	human	
6	QNS166 p10 wt	9/25/2019	Dr. Quinones-Hinojosa	QNS166	human	
7	QNS203 p8 wt	6/4/2019	Dr. Quinones-Hinojosa	QNS203	human	
8	QNS203 p8 gfp box1	9/30/2019	Dr. Quinones-Hinojosa	QNS203	human	
9	QNS203 p8 gfp box2	9/30/2019	Dr. Quinones-Hinojosa	QNS203	human	
10	QNS203 p8 gfp ML	9/30/2019	Dr. Quinones-Hinojosa	QNS203	human	
11	QNS259 p6 wt	9/30/2019	Dr. Quinones-Hinojosa	QNS259	human	
12	QNS263 p5 wt	9/30/2019	Dr. Quinones-Hinojosa	QNS263	human	
13	QNS315 p6 wt	9/30/2019	Dr. Quinones-Hinojosa	QNS315	human	

If you have questions, please call our toll free number at 1-800-669-0825 or e-mail us at IdexxBioAnalytics@idexx.com.

Page 1 of 5

IDEXX BioAnalytics Case # 35416-2019



ID	Client ID	Addl ID	Investigator	Cell Line	Species	Sex
14	QNS318 p5 wt	9/30/2019	Dr. Quinones-Hinojosa	QNS318	human	
15	QNS322 p5 wt	9/30/2019	Dr. Quinones-Hinojosa	QNS322	human	

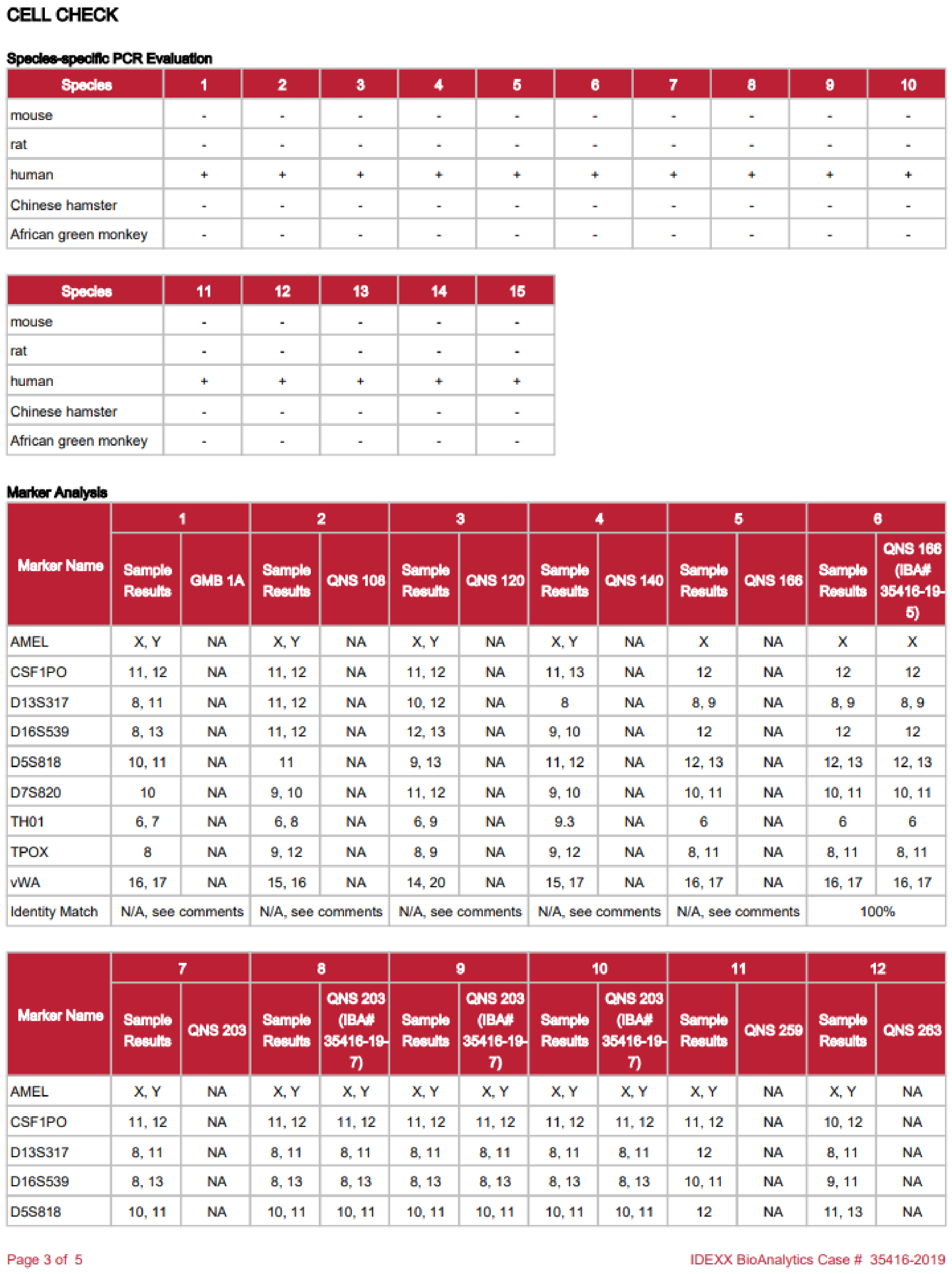
Services/Tests Performed: CellCheck 9 - human (9 Marker STR Profile and Inter-species Contamination Test) (1-15)

Genetic evaluation for: Human 9-Marker STR Profile, Interspecies Contamination Test

[redacted]

Summary: Cell Check results are provided in the data results section for each sample. For human samples, an identity matching score above 80% indicates the sample is consistent with the cell line of origin. For human samples with less than an 80% matching score, please see individual comments for these samples in the detail section.

Please see the report for details.



CELL CHECK

Species-specific PCR Evaluation

Species	1	2	3	4	5	6	7	8	9	10
mouse	-	-	-	-	-	-	-	-	-	-
rat	-	-	-	-	-	-	-	-	-	-
human	+	+	+	+	+	+	+	+	+	+
Chinese hamster	-	-	-	-	-	-	-	-	-	-
African green monkey	-	-	-	-	-	-	-	-	-	-

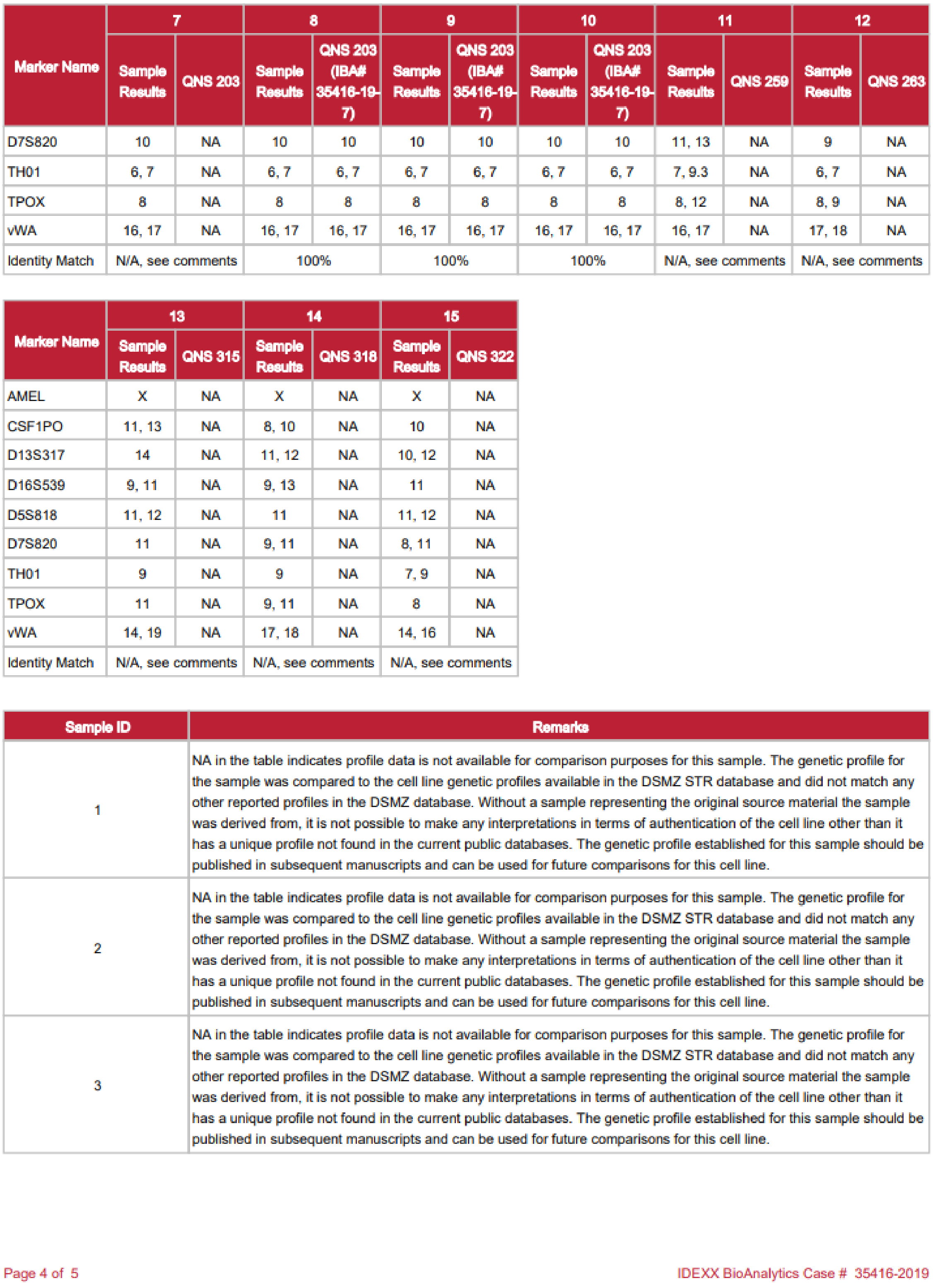
Species	11	12	13	14	15
mouse	-	-	-	-	-
rat	-	-	-	-	-
human	+	+	+	+	+
Chinese hamster	-	-	-	-	-
African green monkey	-	-	-	-	-

Marker Analysis

Marker Name	1		2		3		4		5		6	
	Sample Results	GMB 1A	Sample Results	QNS 108	Sample Results	QNS 120	Sample Results	QNS 140	Sample Results	QNS 166	Sample Results	QNS 166 (IBA# 35416-19-5)
AMEL	X, Y	NA	X, Y	NA	X, Y	NA	X, Y	NA	X	NA	X	X
CSF1PO	11, 12	NA	11, 12	NA	11, 12	NA	11, 13	NA	12	NA	12	12
D13S317	8, 11	NA	11, 12	NA	10, 12	NA	8	NA	8, 9	NA	8, 9	8, 9
D16S539	8, 13	NA	11, 12	NA	12, 13	NA	9, 10	NA	12	NA	12	12
D5S818	10, 11	NA	11	NA	9, 13	NA	11, 12	NA	12, 13	NA	12, 13	12, 13
D7S820	10	NA	9, 10	NA	11, 12	NA	9, 10	NA	10, 11	NA	10, 11	10, 11
TH01	6, 7	NA	6, 8	NA	6, 9	NA	9.3	NA	6	NA	6	6
TPOX	8	NA	9, 12	NA	8, 9	NA	9, 12	NA	8, 11	NA	8, 11	8, 11
vWA	16, 17	NA	15, 16	NA	14, 20	NA	15, 17	NA	16, 17	NA	16, 17	16, 17
Identity Match	N/A, see comments		N/A, see comments		N/A, see comments		N/A, see comments		N/A, see comments		100%	

Marker Name	7		8		9		10		11		12	
	Sample Results	QNS 203	Sample Results	QNS 203 (IBA# 35416-19-7)	Sample Results	QNS 203 (IBA# 35416-19-7)	Sample Results	QNS 203 (IBA# 35416-19-7)	Sample Results	QNS 259	Sample Results	QNS 263
AMEL	X, Y	NA	X, Y	X, Y	X, Y	X, Y	X, Y	X, Y	X, Y	NA	X, Y	NA
CSF1PO	11, 12	NA	11, 12	11, 12	11, 12	11, 12	11, 12	11, 12	11, 12	NA	10, 12	NA
D13S317	8, 11	NA	8, 11	8, 11	8, 11	8, 11	8, 11	8, 11	12	NA	8, 11	NA
D16S539	8, 13	NA	8, 13	8, 13	8, 13	8, 13	8, 13	8, 13	10, 11	NA	9, 11	NA
D5S818	10, 11	NA	10, 11	10, 11	10, 11	10, 11	10, 11	10, 11	12	NA	11, 13	NA

Page 3 of 5
IDEXX BioAnalytics Case # 35416-2019



Marker Name	7		8		9		10		11		12	
	Sample Results	QNS 203	Sample Results	QNS 203 (IBA# 35416-19-7)	Sample Results	QNS 203 (IBA# 35416-19-7)	Sample Results	QNS 203 (IBA# 35416-19-7)	Sample Results	QNS 259	Sample Results	QNS 263
D7S820	10	NA	10	10	10	10	10	10	11, 13	NA	9	NA
TH01	6, 7	NA	6, 7	6, 7	6, 7	6, 7	6, 7	6, 7	7, 9.3	NA	6, 7	NA
TPOX	8	NA	8	8	8	8	8	8	8, 12	NA	8, 9	NA
vWA	16, 17	NA	16, 17	16, 17	16, 17	16, 17	16, 17	16, 17	16, 17	NA	17, 18	NA
Identity Match	N/A, see comments		100%		100%		100%		N/A, see comments		N/A, see comments	

Marker Name	13		14		15	
	Sample Results	QNS 315	Sample Results	QNS 318	Sample Results	QNS 322
AMEL	X	NA	X	NA	X	NA
CSF1PO	11, 13	NA	8, 10	NA	10	NA
D13S317	14	NA	11, 12	NA	10, 12	NA
D16S539	9, 11	NA	9, 13	NA	11	NA
D5S818	11, 12	NA	11	NA	11, 12	NA
D7S820	11	NA	9, 11	NA	8, 11	NA
TH01	9	NA	9	NA	7, 9	NA
TPOX	11	NA	9, 11	NA	8	NA
vWA	14, 19	NA	17, 18	NA	14, 16	NA
Identity Match	N/A, see comments		N/A, see comments		N/A, see comments	

Sample ID	Remarks
1	NA in the table indicates profile data is not available for comparison purposes for this sample. The genetic profile for the sample was compared to the cell line genetic profiles available in the DSMZ STR database and did not match any other reported profiles in the DSMZ database. Without a sample representing the original source material the sample was derived from, it is not possible to make any interpretations in terms of authentication of the cell line other than it has a unique profile not found in the current public databases. The genetic profile established for this sample should be published in subsequent manuscripts and can be used for future comparisons for this cell line.
2	NA in the table indicates profile data is not available for comparison purposes for this sample. The genetic profile for the sample was compared to the cell line genetic profiles available in the DSMZ STR database and did not match any other reported profiles in the DSMZ database. Without a sample representing the original source material the sample was derived from, it is not possible to make any interpretations in terms of authentication of the cell line other than it has a unique profile not found in the current public databases. The genetic profile established for this sample should be published in subsequent manuscripts and can be used for future comparisons for this cell line.
3	NA in the table indicates profile data is not available for comparison purposes for this sample. The genetic profile for the sample was compared to the cell line genetic profiles available in the DSMZ STR database and did not match any other reported profiles in the DSMZ database. Without a sample representing the original source material the sample was derived from, it is not possible to make any interpretations in terms of authentication of the cell line other than it has a unique profile not found in the current public databases. The genetic profile established for this sample should be published in subsequent manuscripts and can be used for future comparisons for this cell line.

Sample ID	Remarks
4	NA in the table indicates profile data is not available for comparison purposes for this sample. The genetic profile for the sample was compared to the cell line genetic profiles available in the DSMZ STR database and did not match any other reported profiles in the DSMZ database. Without a sample representing the original source material the sample was derived from, it is not possible to make any interpretations in terms of authentication of the cell line other than it has a unique profile not found in the current public databases. The genetic profile established for this sample should be published in subsequent manuscripts and can be used for future comparisons for this cell line.
5	NA in the table indicates profile data is not available for comparison purposes for this sample. The genetic profile for the sample was compared to the cell line genetic profiles available in the DSMZ STR database and did not match any other reported profiles in the DSMZ database. Without a sample representing the original source material the sample was derived from, it is not possible to make any interpretations in terms of authentication of the cell line other than it has a unique profile not found in the current public databases. The genetic profile established for this sample should be published in subsequent manuscripts and can be used for future comparisons for this cell line.
7	NA in the table indicates profile data is not available for comparison purposes for this sample. The genetic profile for the sample was compared to the cell line genetic profiles available in the DSMZ STR database and did not match any other reported profiles in the DSMZ database. Without a sample representing the original source material the sample was derived from, it is not possible to make any interpretations in terms of authentication of the cell line other than it has a unique profile not found in the current public databases. The genetic profile established for this sample should be published in subsequent manuscripts and can be used for future comparisons for this cell line.
11	NA in the table indicates profile data is not available for comparison purposes for this sample. The genetic profile for the sample was compared to the cell line genetic profiles available in the DSMZ STR database and did not match any other reported profiles in the DSMZ database. Without a sample representing the original source material the sample was derived from, it is not possible to make any interpretations in terms of authentication of the cell line other than it has a unique profile not found in the current public databases. The genetic profile established for this sample should be published in subsequent manuscripts and can be used for future comparisons for this cell line.
12	NA in the table indicates profile data is not available for comparison purposes for this sample. The genetic profile for the sample was compared to the cell line genetic profiles available in the DSMZ STR database and did not match any other reported profiles in the DSMZ database. Without a sample representing the original source material the sample was derived from, it is not possible to make any interpretations in terms of authentication of the cell line other than it has a unique profile not found in the current public databases. The genetic profile established for this sample should be published in subsequent manuscripts and can be used for future comparisons for this cell line.
13	NA in the table indicates profile data is not available for comparison purposes for this sample. The genetic profile for the sample was compared to the cell line genetic profiles available in the DSMZ STR database and did not match any other reported profiles in the DSMZ database. Without a sample representing the original source material the sample was derived from, it is not possible to make any interpretations in terms of authentication of the cell line other than it has a unique profile not found in the current public databases. The genetic profile established for this sample should be published in subsequent manuscripts and can be used for future comparisons for this cell line.
14	NA in the table indicates profile data is not available for comparison purposes for this sample. The genetic profile for the sample was compared to the cell line genetic profiles available in the DSMZ STR database and did not match any other reported profiles in the DSMZ database. Without a sample representing the original source material the sample was derived from, it is not possible to make any interpretations in terms of authentication of the cell line other than it has a unique profile not found in the current public databases. The genetic profile established for this sample should be published in subsequent manuscripts and can be used for future comparisons for this cell line.
15	NA in the table indicates profile data is not available for comparison purposes for this sample. The genetic profile for the sample was compared to the cell line genetic profiles available in the DSMZ STR database and did not match any other reported profiles in the DSMZ database. Without a sample representing the original source material the sample was derived from, it is not possible to make any interpretations in terms of authentication of the cell line other than it has a unique profile not found in the current public databases. The genetic profile established for this sample should be published in subsequent manuscripts and can be used for future comparisons for this cell line.

Page 5 of 5

IDEXX BioAnalytics Case # 35416-2019

Figure S1. Cell line authentication was conducted with IDEXX BioAnalytics by performing Short Tandem Repeat (STR) Fragment analysis where GBM cell lines BTIC lines were compared to the DSMZ STR database. A collection of other cell lines stored in our laboratory's bank were included in the analysis and compared to our primary GBM cell lines. GBM cell lines were unique, human-derived, and matched the sex of the original patient donors.

Table S3. Patient Characteristics

Characteristics	**QNS108♂**	**QNS120♂**	**QNS140♂**	**QNS166♀**	**QNS315♀**	**QNS509♀**
Demographics						
Age at Diagnosis	63 yo	59 yo	64 yo	59 yo	63 yo	75 yo
Sex	Male	Male	Male	Female	Female	Female
Diagnosis*	GBM	GBM	GBM	GBM	GBM	GBM
Tumor Location						
Lobe	Left parieto-temporal	Left parieto-temporal	Right temporal	Left inferior parietal	Right temporal	Right
Proximal or Distal (P/D) to Ventricle	P	P	P	P	P	P
Molecular Markers						
IDH Mutant	-	-	-	-	-	-
MGMT Methylation	-	+	+	-	+	-
ATRX nuclear expression	+	+	+	+	+	+
p53 intact	+	+	+	+	+	+

Abbreviations: yo= years old, GBM=glioblastoma, IDH= Isocitrate Dehydrogenase, MGMT=O6-methylguanine DNA Methyltransferase
*Diagnosis based on World Health Organization criteria, P= proximal, D= distal

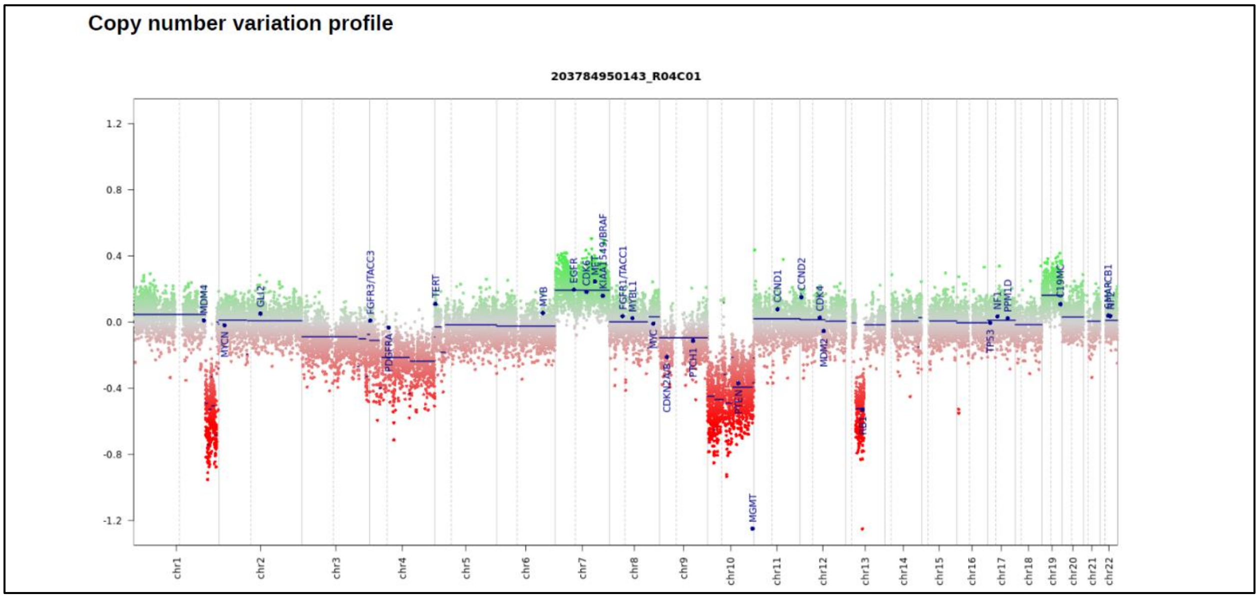
Table S4. Epigenetic Classification

Cell Line	Epigenetic CNS Tumor Classification (*Callibrated score)	GBM Subclass (*Calibrated score, TCGA classification)	MGMT Methylation Status
QNS 108 ♂	GBM IDH-WT (0.99)	RTK II (0.87, Classical) + RTK I (0.1, Proneural)	unmethylated
QNS 120 ♂	GBM IDH-WT (0.98)	RTK II (0.75, Classical) + Mesenchymal (0.16, Mesenchymal)	methylated
QNS 140 ♂	GBM IDH-WT (0.93)	RTK II (0.67, Classical) + RTK I (0.24, Proneural)	methylated
QNS 166 ♀	GBM IDH-WT (0.96)	RTK II (0.82, Classical) + RTK I (0.11, Proneural)	methylated
QNS 315 ♀	GBM IDH-WT (0.98)	RTK II (0.91, Classical) + Mesenchymal (0.05, Mesenchymal)	methylated
QNS 509 ♀	GBM IDH-WT (0.98)	RTK II (0.74, Classical) + RTK I (0.14, Proneural)	methylated

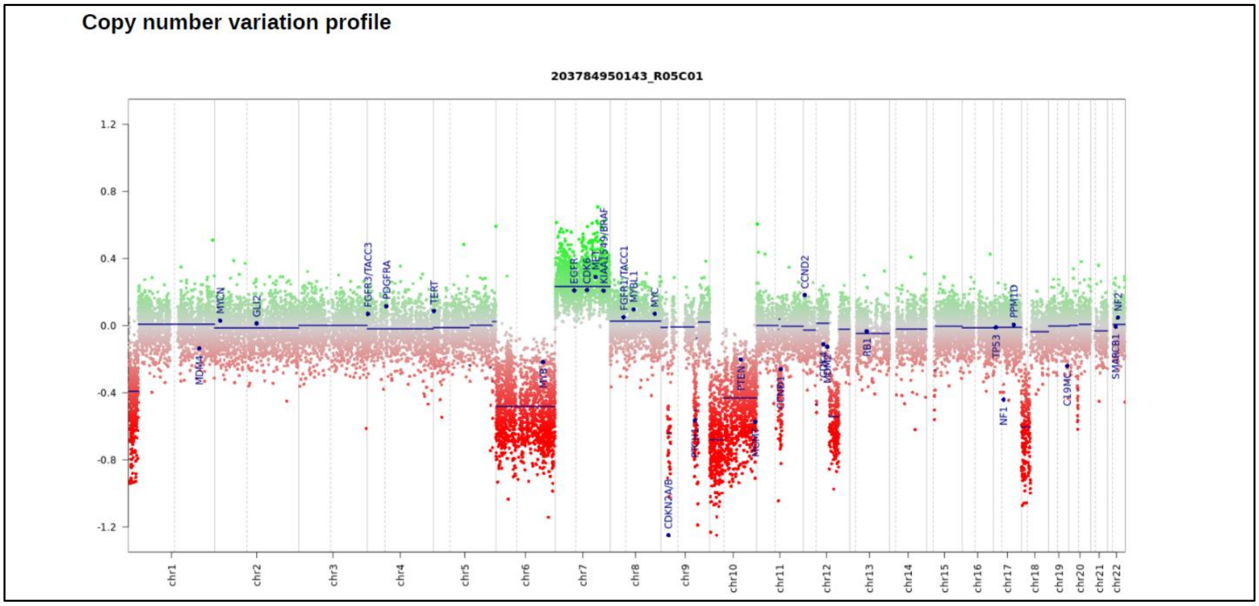
*Calibrated score designation based on subtyping analysis. CNS tumor match >= 0.9 and subclass match >= 0.5.

Figure S2: Copy Number Variation (CNV) Profiles

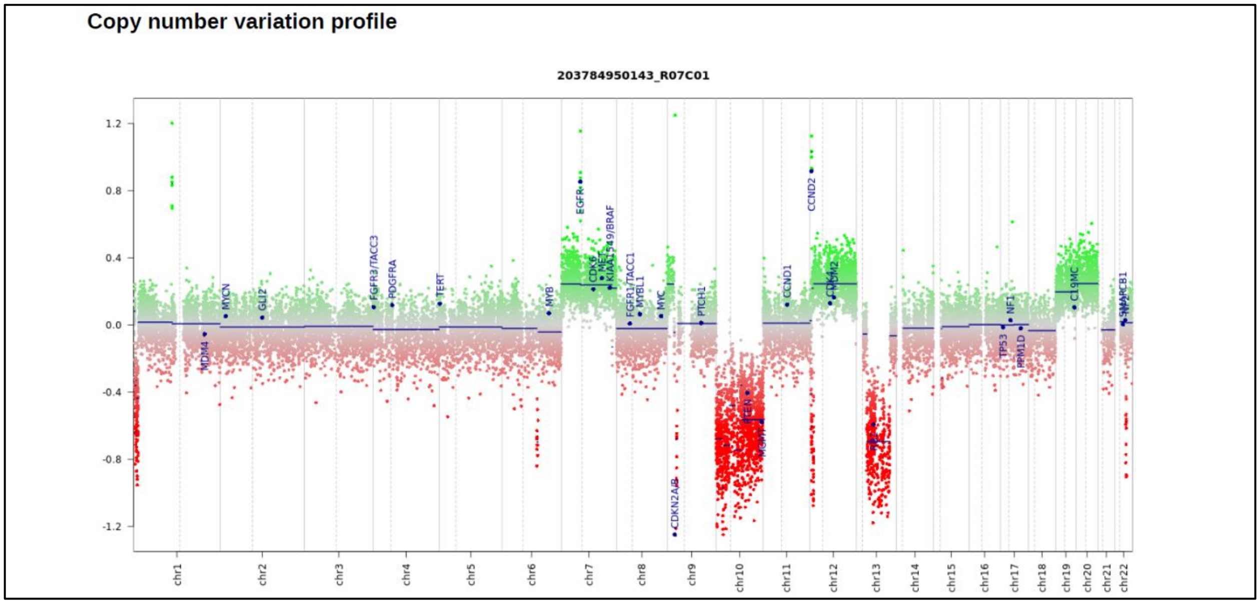
QNS108



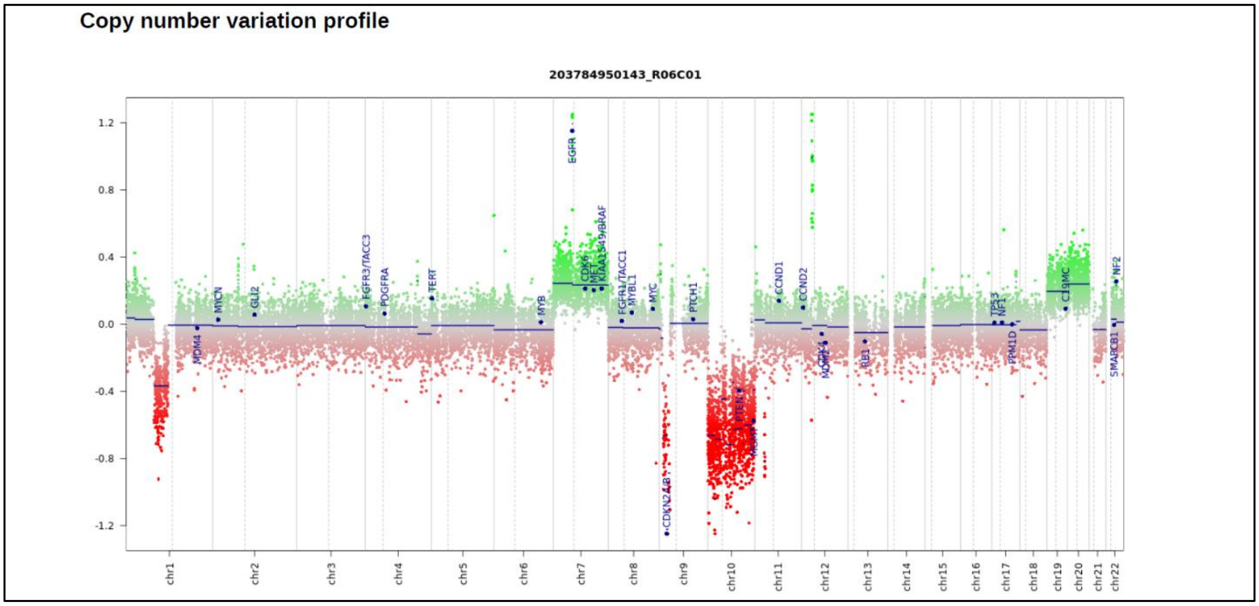
QNS120



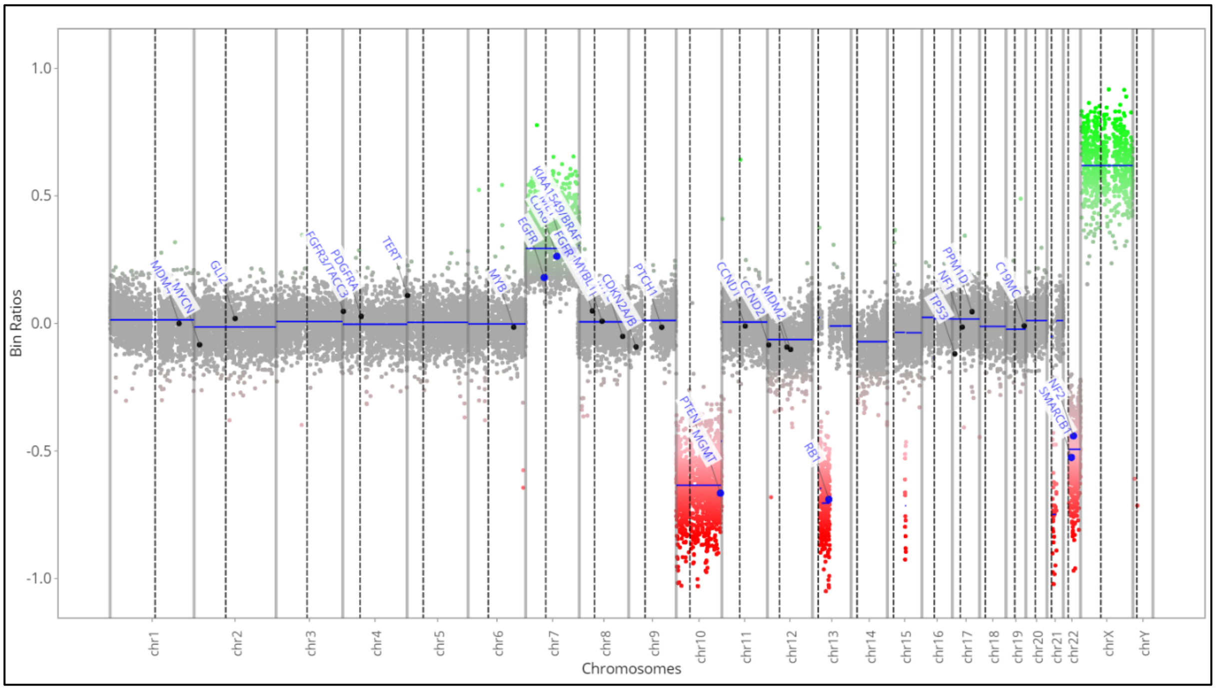
QNS140



QNS166



QNS315



QNS509

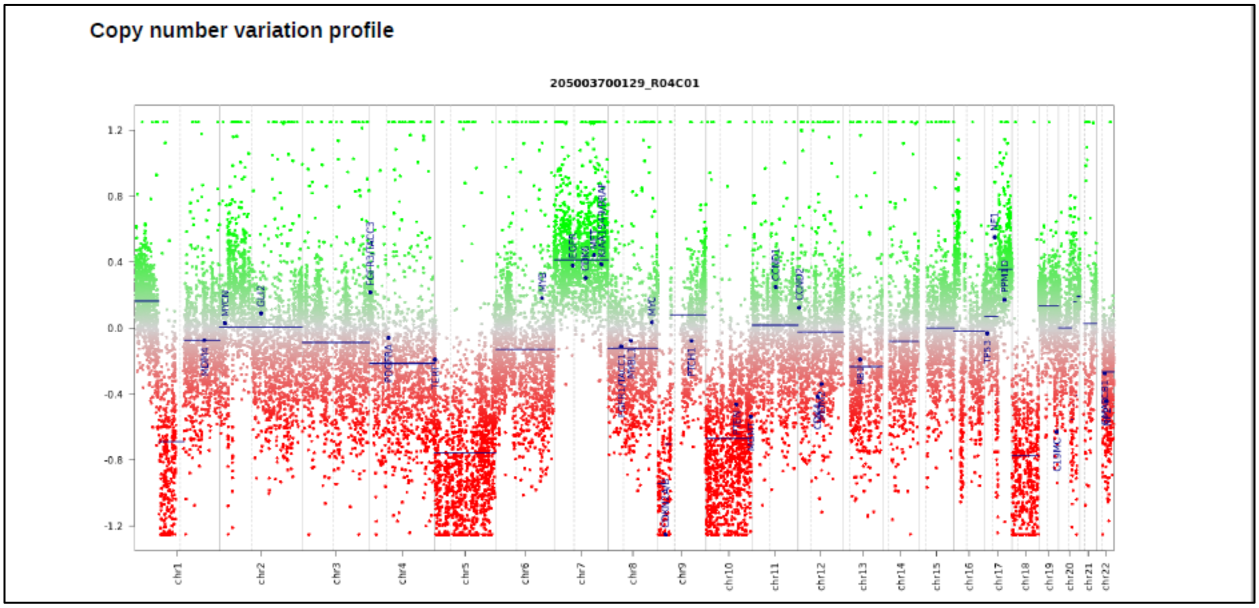


Figure S2: Chromosome copy number variation profiles for each characterized GBM tumor cell line. Gain of copy numbers are represented in green and loss of coy numbers are represented in red, all relative to the baseline at 0. All cell lines matched expected GBM CNV profiles.

Figure S3: CD133+ Expression of GBM Tumor Cell Lines Compared to Isotype (IgG1 PE) Control

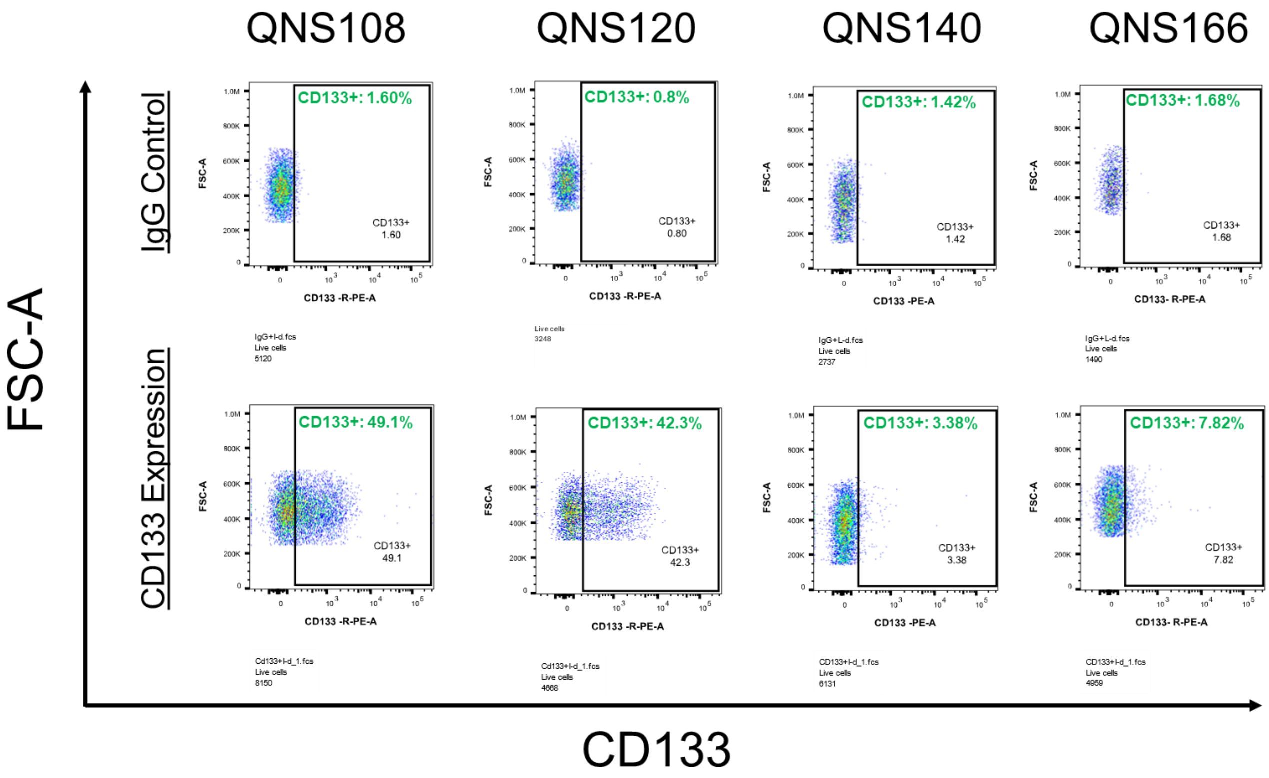


Figure S3: Expression of the stem marker CD133 by GBM tumor cell lines was compared to expression of IgG1 PE isotype control for validation of flow cytometry experiments.

Figure S4:

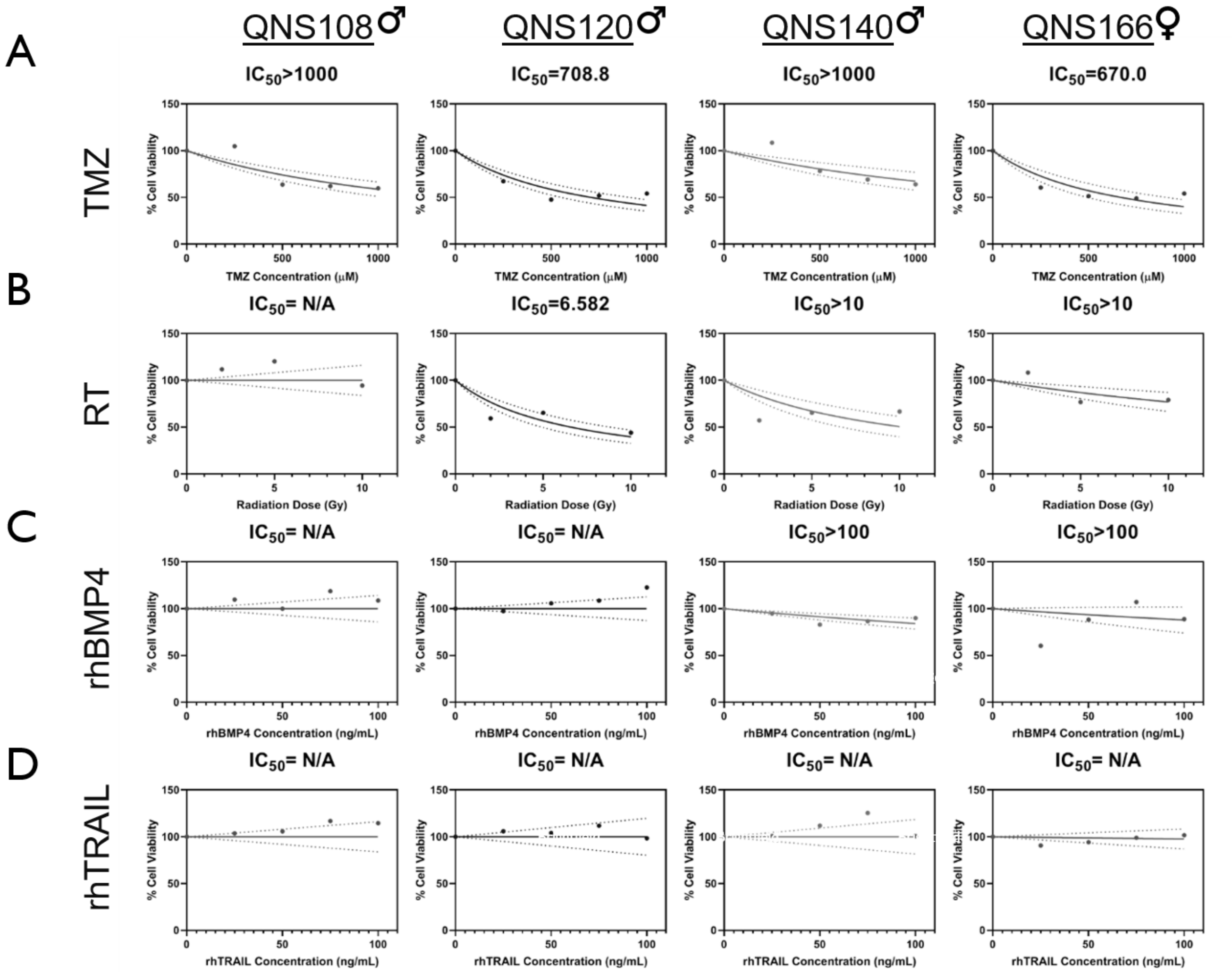


Figure S4. GBM tumor cells demonstrate varying sensitivity to different therapeutic strategies. **A)** Differential sensitivity to TMZ. **B)** Differential sensitivity to radiation therapy (RT). **C)** Differential sensitivity to recombinant human bone morphogenetic protein 4 (rh*BMP4*). **D)** Differential sensitivity to recombinant human TNF-related apoptosis-inducing ligand (rhTRAIL).

Table S5. Summary of Functional Characteristics

Summary Table of Functional Characteristics						
Functional Characteristics	**Cell Line**					
	QNS108	**QNS120**	**QNS140**	**QNS166**	**QNS315**	**QNS509**
Sphere Count (# SFU)	180	62	17	35	NA	NA
Sphere Diameter (µm)	98.06 µm	71.86 µm	58.82 µm	74.87 µm	NA	NA
% CD133 Expression	62.05%	22.74%	2.86%	12.72%	NA	NA
Mean # of Migrating Cells	2.22	66.36	0.8	13.78	15.07	1.07
***in vitro* Growth Kinetics Doubling Time (hrs)**	59.14 hrs	83.92 hrs	136.15 hrs	115.78 hrs	N/A	N/A
***in vivo* Growth Slope for Male Mice (k)**	1.527	0.3139	-0.005216	-0.04773	0.8303	0.07183
***in vivo* Growth Slope for Female Mice (k)**	1.616	0.3706	-0.03789	-0.04773	0.9541	0.09378

SFU = Sphere Forming Unit,

Figure S5: Histology for QNS108 and QNS120

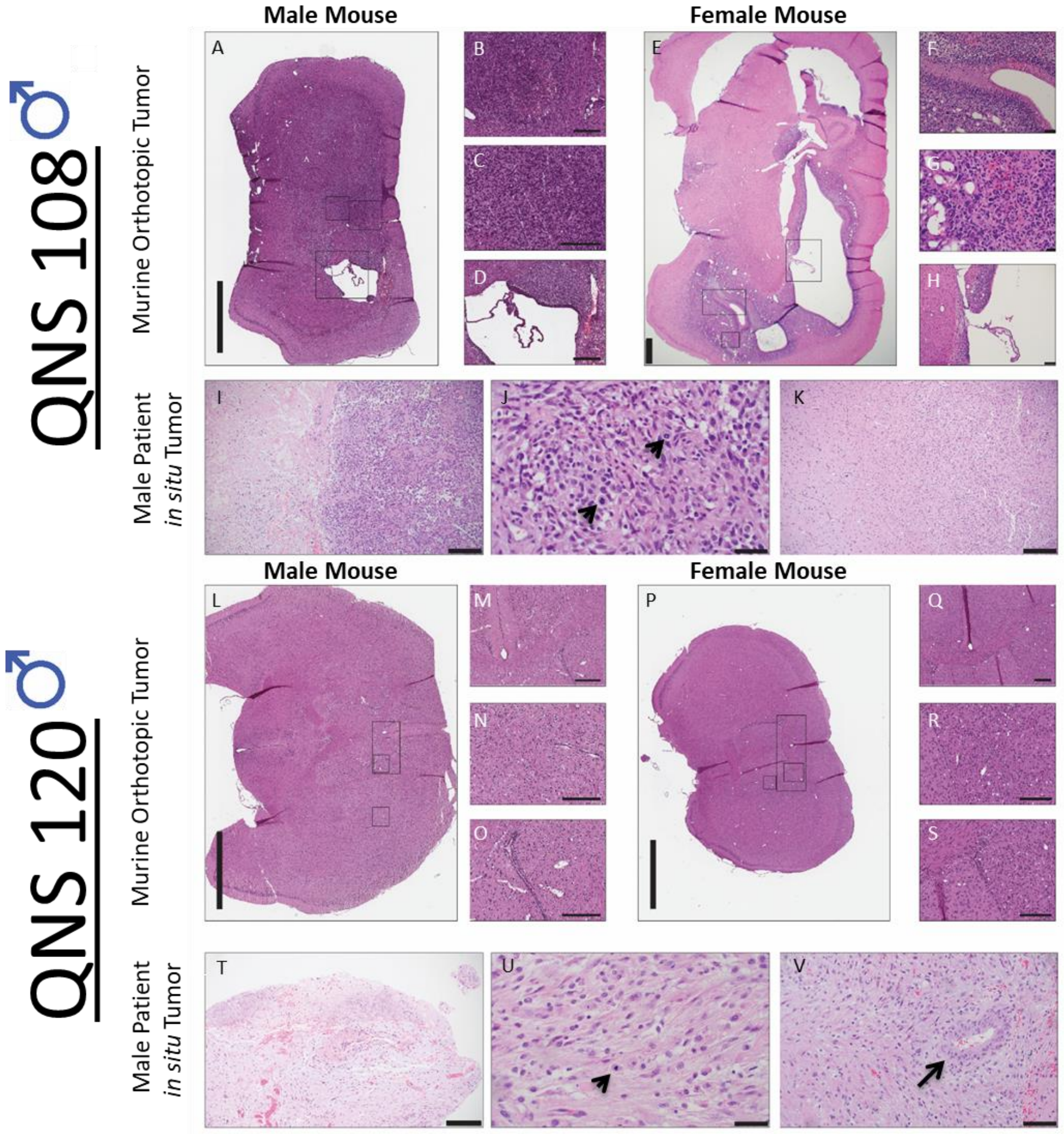


Figure S5. H&E stains of QNS108 and QNS120 BTIC orthotopic tumor models recapitulate patient GBM tumor features. **A-D)** Male murine QNS108 whole brain (**A**) with features of pseudopalisading necrosis (**B**), mitotic figures (**C**), and tumor cells proximal to ventricles (**D**), (scale bars: A=2 mm, B-C=200 µm, D=300 µm). **E-H)** Female murine QNS108 whole brain (**E**) with features of pseudopalisading necrosis (**F**), mitotic figures (**G**), and tumor cells proximal to ventricles (**H**), (scale bars: E-F=500 µm, G=200 µm, H=100 µm). **I-K)** QNS108 *in situ* Patient tumor histology with evidence of necrosis (**I**), mitotic figures (indicated by arrows), (**J**), and invasion throughout the parenchyma (**K**), (scale bars: I-J =200µm, K=50µm). **L-O)** Male murine QNS120 whole brain (**L**) with features of migratory tumor cells (**M**), mitotic figures (**N**), and tumor cells proximal to ventricles (**O**), (scale bars: L=2 mm, M=300 µm, N-O=200 µm). **P-S)** Female murine QNS120 whole brain (**P**) with features of migratory tumor cells (**Q**), mitotic figures and hypercellularity (**R**), and tumor cells proximal to ventricles (**S**), (scale bars: P=2 mm, Q=300 µm, R-S=200 µm). **T-V)** QNS120 *in situ* Patient tumor histology with evidence of necrosis (**T**), mitotic figures (indicated by arrow), (**U**), and vascular proliferation (indicated by arrow), (**V**), (scale bars: T=200 µm, U=50µm, V=100µm).

Figure S6: QNS108 *in vivo* Expression of Human Nuclei, Proliferation, Stem Cell, and Astrocytic Markers

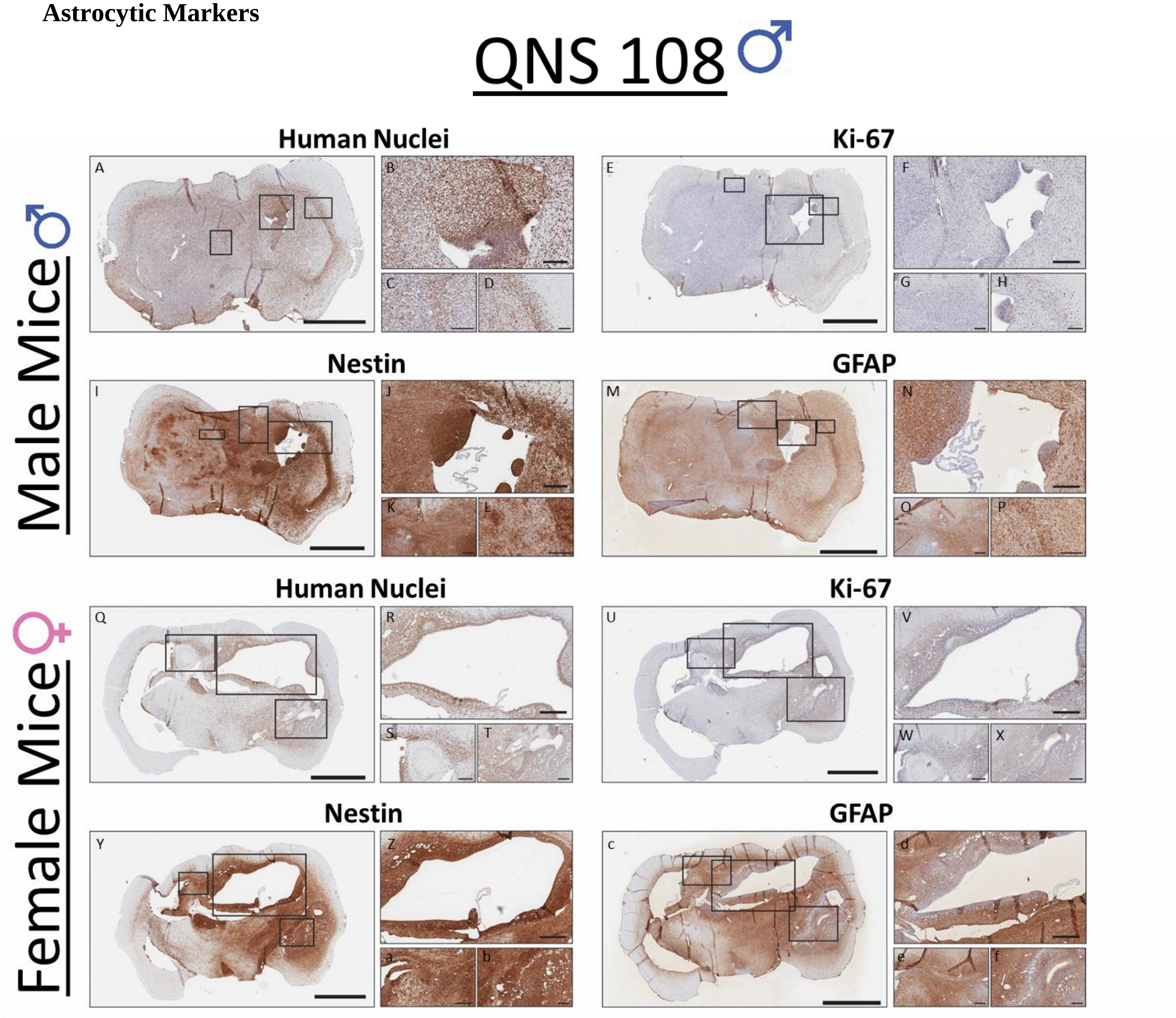


Figure S6. QNS108 BTIC orthotopic tumors implanted into male and female mice expressed stem cell, astrocytic, and proliferation markers. **A-D)** Expression of Human Nuclei in male murine brains (scale bars: A=2mm, B-D=200 µm). **E-H)** Expression of Ki-67 in male murine brains (scale bars: E=2mm, F=400 µm, G-H=200 µm). **I-L)** Expression of Nestin in male murine brains (scale bars: I=2 mm, J=300 µm, K-L=200 µm). **M-P)** Expression of GFAP in male murine brains (scale bars: M=2 mm, N-P=200 µm). **Q-T)** Expression of Human Nuclei in female murine brains (scale bars: Q=2 mm, R=500 µm, S-T=300 µm). **U-X)** Expression of Ki-67 in female murine brains (scale bars: U=2 mm, V=500 µm, W=300 µm, X=400 µm). **Y-b)** Expression of Nestin in female murine brains (scale bars: Y=2 mm, Z=500 µm, a-b=200 µm). **c-f)** Expression of GFAP in female murine brains (scale bars: c=2 mm, d=400 µm, e-f=200 µm).

Figure S7: QNS120 *in vivo* Expression of Human Nuclei, Proliferation, Stem Cell, and Astrocytic Markers

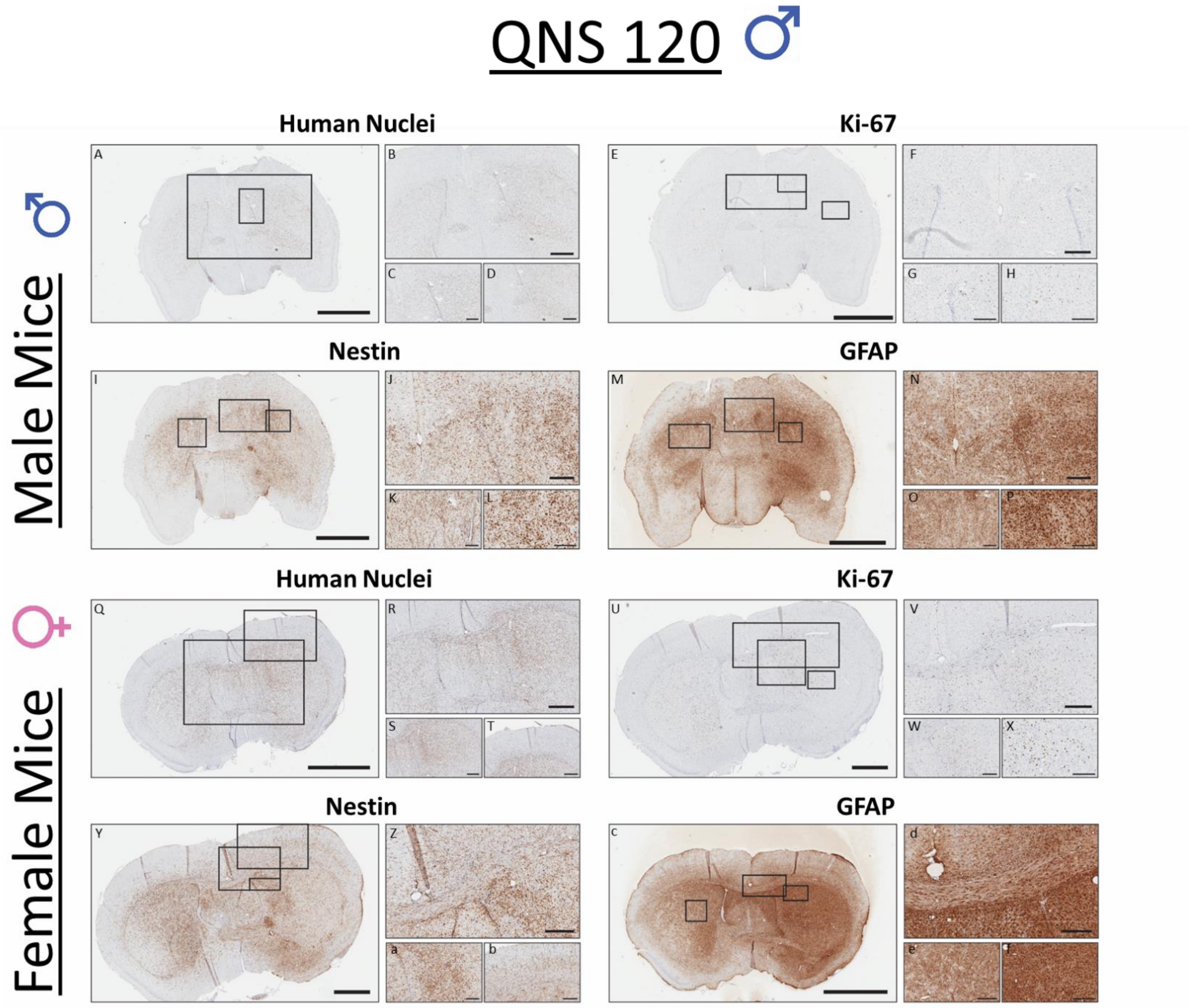


Figure S7. QNS120 BTIC orthotopic tumors implanted into male and female mice expressed stem cell, astrocytic, and proliferation markers. **A-D)** Expression of Human Nuclei in male murine brains (scale bars: A=2 mm, B= 700 µm, C=300 µm, D=500 µm). **E-H)** Expression of Ki-67 in male murine brains (scale bars: E=2 mm, F= 300 µm, G-H=200 µm). **I-L)** Expression of Nestin in male murine brains (scale bars: I=2 mm, J=300 µm, K=200 µm, L=200 µm). **M-P)** Expression of GFAP in male murine brains (scale bars: M=2 mm, N= 300 µm, O-P=200 µm). **Q-T)** Expression of Human Nuclei in female murine brains (scale bars: Q=2 mm, R=600 µm, S=400 µm, T=400 µm). **U-X)** Expression of Ki-67 in female murine brains (scale bars: U=1 mm, V=400 µm, W=300 µm, X=200 µm). **Y-b)** Expression of Nestin in female murine brains (scale bars: Y=1 mm, Z=300 µm, a=200 µm, b= 300 µm). **c-f)** Expression of GFAP in female murine brains (scale bars: c=2 mm, d-f=200 µm).

Figure S8: Histology for QNS166 and QNS140

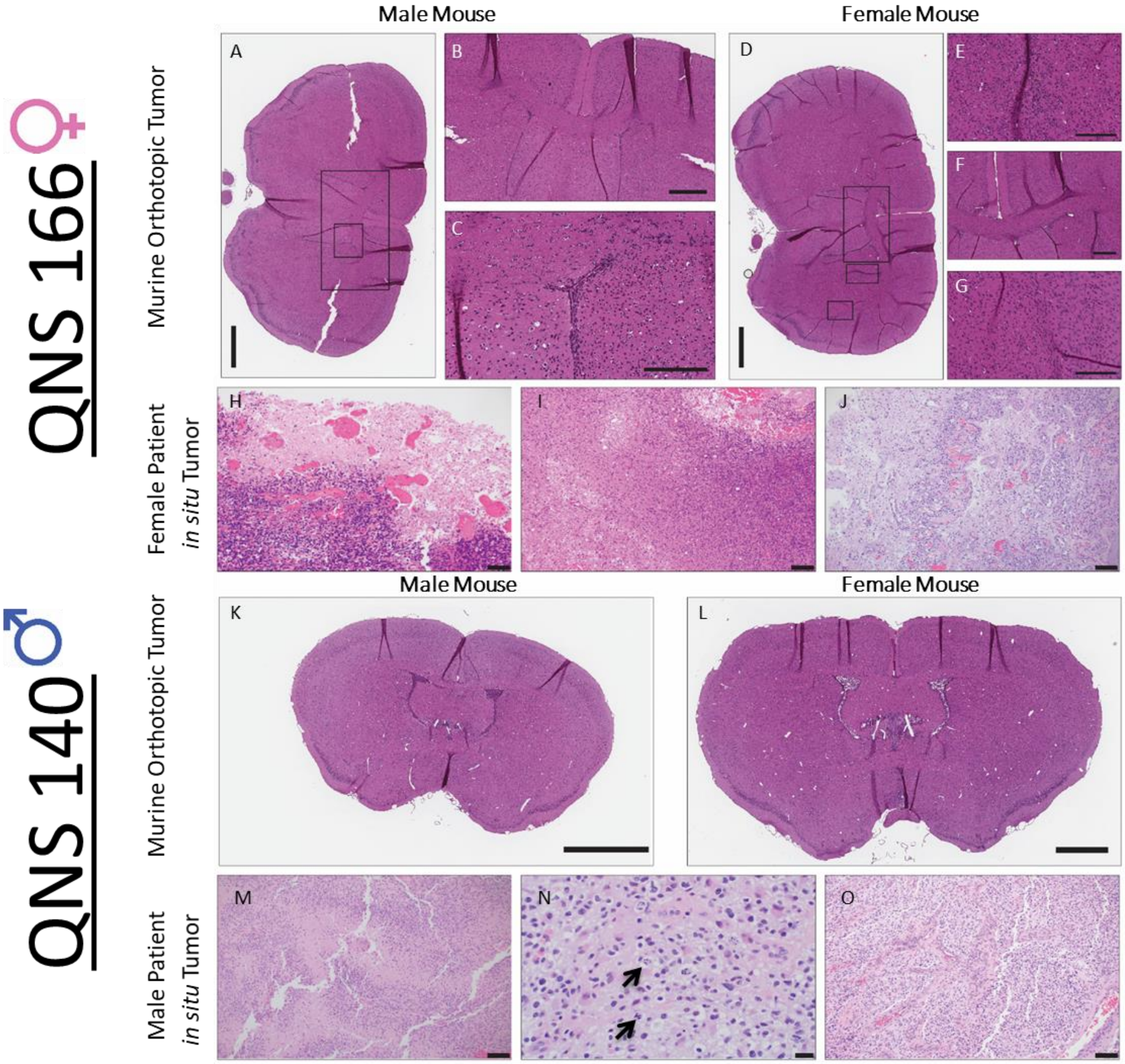


Figure S8. H&E stains of QNS140 and QNS 166 orthotopic murine tumors did not recapitulate patient tumor growth. **A-C)** Male murine QNS166 whole brain (**A**) with view of corpus callosum (**B**), and ventricle (**C**) where minimal tumor cells were observed (scale bars: A=900 µm, B= 500 µm, C=200 µm). **D-G)** Female murine QNS166 whole brain (**D**) with view of tumor cell injection site (**E**), corpus callosum (**F**), and cluster of cells towards outer right hemisphere of brain (**G**), (scale bars: D=900 µm, E=200 µm, F=300 µm, G=200 µm). **H-J)** QNS166 patient *in situ* brain tumor histology with necrosis (**H**), invasion (**I**), and hypervascularity (**J**), (scale bars: H=50 µm, I=100 µm, J=100 µm). **K)** Male murine QNS140 whole brain with no observed tumor growth (scale bar: K=2 mm). **L)** Female murine QNS140 whole brain with no observed tumor growth (scale bars: L=1 mm). **M-O)** QNS140 patient *in situ* brain tumor histology with necrosis (**M**), mitotic figures (indicated by arrows), (**N**), and hypervascularity (**O**), (scale bars: M=100 µm, N=20 µm, O=100 µm).

Figure S9: Histology for QNS315 and QNS509

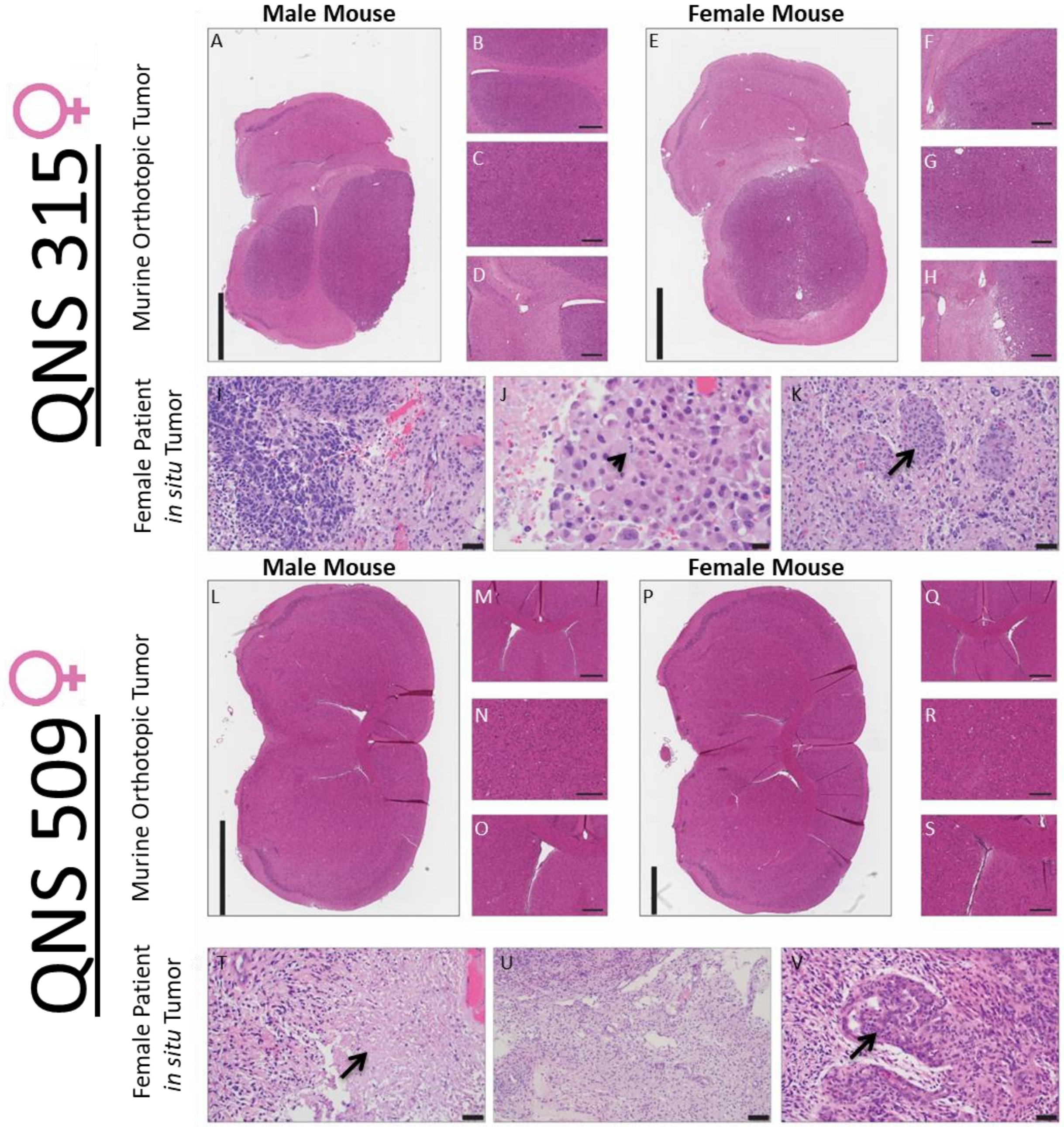


Figure S9. H&E stains of QNS315 and QNS509 orthotopic murine tumors and *in situ* patient histology. **A-D)** Male murine QNS315 whole brain (**A**) with views of corpus callosum (**B**), hypercellularity (**C**), and ventricles (**D**), (scale bars: A=2 mm, B=500 µm, C-D=300 µm). **E-H)** Female murine QNS315 whole brain (**E**) with views of corpus callosum (**F**), hypercellularity (**G**), and ventricles (**H**), (scale bars: E=2 mm, F-H=300 µm.) **I-K)** QNS315 patient *in situ* histology with a primitive appearing component (**I**), presence of mitotic figures (**J**), and vascular proliferation (**K**), (scale bars: I=50 µm, J=20 µm, K=50 µm). **L-O)** Male murine QNS509 whole brain (**L**) with views of corpus callosum (**M**), hypercellularity (**N**), and the ventricle (**O**), (scale bars: L=2 mm, M=500 µm, N=200 µm, O=300 µm). **P-S)** Female murine QNS509 whole brain (**P**) with views of corpus callosum (**Q**), hypercellularity (**R**), and ventricle (**S**), (scale bars: P=1 mm, Q=500 µm, R=200 µm, S=200 µm). **T-V)** QNS509 patient *in situ* histology with necrosis (**T**), tumor cell infiltration (**U**), and vascular proliferation (**V**), (scale bars: T= 50 µm, U=100 µm, V=50 µm).