SUPPLEMENTAL MATERIALS AND METHODS.

Toxicokinetic and toxicology studies in cynomolgus monkeys.

1. Test article. NU-0129 was stored frozen (-60°C to -80°C), and was stable under this condition. The vehicle used in preparation of the test article formulations and for administration to the control group was PBS, pH 7.4, and sterile water for injection, USP. The formulations were prepared using aseptic technique including the use of the laminar flow hood and sterile glassware and equipment. Test article formulations were prepared once as single formulations for each dose level for the cardiovascular and toxicology groups, and stored at room temperature.

2. Animals. Cynomolgus monkeys (Macaca fascicularis; of Chinese origin) were used as the test system on this study. This species of animal is recognized as appropriate for repeat-dose toxicity studies. The cynomolgus monkey was chosen because it is a widely used species for which significant historical control data are available. In order to have a sufficient number of samples to perform meaningful statistical analyses, and to differentiate between background versus test article-related findings, 3 animals/sex/group at the primary necropsy and 2 animals/sex/group at the recovery necropsy for the toxicology group animals was determined to be the minimum necessary to yield scientifically meaningful results. Two animals/sex/group in Groups 1 and 4 was the minimum needed for the cardiovascular assessment, to discern if there were any perceptible differences from the control group at 8 mg/kg of the test article while keeping the use of animals to a minimum. Cynomolgus monkeys (27 males and 27 females) were received in apparent good health from Covance Research Products Inc., Alice, TX. The monkeys were young adults, approximately 2-3 years old upon receipt. Each animal was examined by a veterinarian upon receipt and weighed the following day. Each animal was uniquely identified with a chest tattoo by the supplier prior to shipment and subcutaneous microchips (BMDS system) implanted in the hind

limbs during acclimation. Animals considered suitable for the study were housed for minimum of a 37-day acclimation. During this period, each animal was observed twice daily for mortality and changes in general appearance or behavior. Individual body weights were recorded and detailed physical examinations were performed periodically during acclimation. In addition, clinical examinations were performed for at least 6 consecutive days prior to randomization. Clinical pathology and radiotelemetry data were also recorded for animals during acclimation. Upon arrival, all animals were housed individually until compatible social housing groups were established for the toxicology group. Thereafter, the toxicology animals were housed in groups of 2-3 animals (by sex and group). The cardiovascular animals were housed individually throughout the study. All animals were maintained in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011). The animal facilities at WIL Research are accredited by AAALAC International. 22 days prior to the initiation of dose administration for the toxicology groups, all available monkeys were weighed and examined in detail for physical abnormalities. The animals judged suitable for assignment to the study were selected for use in a computerized randomization procedure based on body weight stratification in a block design. For toxicology groups (Groups 1-4), each group consisted of 5 males and 5 females. Each cardiovascular group (Groups 1C and 4C) consisted of 2 males and 2 females. The animals were approximately 2-3 years old at the initiation of dose administration. Individual body weights ranged from 2,394 g to 2,946 g for males, and from 2,212 g to 2,784 g for females in the toxicology groups at randomization, and from 2,458 g to 2,785 g for males and 2,287 g to 2,429 g for females in the cardiovascular groups prior to the initiation of the dose administration.

3. Surgical preparation for radiotelemetry. During acclimation 5 animals/sex were implanted with TL11M2-D70-PCT radiotelemetry transmitters according to WIL Research SOPs. The

radiotelemetry transmitters had a fluid filled catheter for blood pressure data collection (coated with an antithrombotic film to inhibit thrombus formation) with the tip of the catheter filled with a patented gel for blood pressure collection, and 2 ECG leads that were surgically implanted emulation a lead II configuration.

4.Test groups, dose levels and treatment regimen. The vehicle and test article formulations were administered via intravenous (bolus) injection into the saphenous vein using a winged-infusion set and appropriately sized syringe on study day 0. Each animal was dosed while properly restrained. The injection sites and surrounding area used for dosing (*i.e.*, insertion site and catheter tip) were marked with indelible ink and remarked as necessary for identification, and collected at the scheduled necropsies. A dose volume of 5 mL/kg was used for all animals. Individual doses were based on the most recently recorded body weights to provide the correct mg/kg dose. Adjusted doses became effective the day of collection of the weekly body weights. For the cardiovascular groups, the control group and test article-treated group animals were dosed alternatively (Group1C males, Group 4C males, Group 1C females, and Group 4C females), in order to maintain consistency between the 2 groups in the cardiovascular profile, to better distinguish between any test article-related changes compared to vehicle-related changes, and to rule out procedure-related changes. The selected route of administration for this study was intravenous (bolus) because the intravenous route is the intended clinical route of exposure.

5. Parameters evaluated. All animals were observed twice daily, once in the morning and once in the afternoon, for mortality and moribundity. Clinical examinations were performed once daily for at least 6 days prior to randomization, including the day of randomization, prior to dosing, and at 1-2 hours following dose administration. During the recovery period, the animals were observed once daily. The absence or presence of findings was recorded for individual animals at the

scheduled intervals. Detailed physical examinations were conducted on all animals at least weekly during acclimation, on the day of randomization, weekly (± 2 days) during the study period, and on the day of the scheduled necropsies for toxicology groups. Individual body weights were recorded at least weekly during acclimation, on the day of randomization, on study day 0 (prior to dosing), weekly (± 2 days) during the study period, and on the day prior to the scheduled necropsies for the toxicology groups (non-fasted). Mean body weights and mean body weight changes were calculated for the corresponding intervals. Final body weights (fasted) were recorded on the day of the scheduled necropsies for the toxicology groups. Blood and urine samples for clinical pathology evaluations (hematology, coagulation, serum chemistry, and urinalysis) were collected from all monkeys during acclimation, (study day -28) and at the scheduled necropsies (study day 2 and 14). The animals were fasted overnight prior to blood collection while using cage pans for urine collection. Blood was collected from a femoral vein into tubes containing potassium EDTA (hematology), sodium citrate (coagulation), or no anticoagulant (serum chemistry). The following parameters were evaluated: Total leukocyte count (WBC), Erythrocyte count (RBC), Hemoglobin (HGB), Hematocrit (HCT), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Platelet count (PLATELET), Prothrombin time (PT), Activated partial thromboplastin time (APTT), Reticulocyte count, Percent (RETIC), Absolute (RETIC ABSOLUTE), Mean platelet volume (MPV), Differential leukocyte count, Neutrophil (NEU), Lymphocyte (LYMPH), Monocyte (MONO), Eosinophil (EOS), Basophil (BASO), Large unstained cell (LUC), Fibrinogen, Red cell distribution width (RDW), Hemoglobin distribution width (HDW), Platelet estimate, Red cell morphology (RBC morphology), Albumin, Globulin [by calculation], Albumin/globulin ratio (A/G Ratio) [by calculation], Total bilirubin (Total Bili), Urea nitrogen, Creatinine, Alkaline phosphatase (ALP),

Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Gamma glutamyltransferase (GGT), Glucose, Total cholesterol (Cholesterol), Calcium Chloride, Phosphorus, Potassium, Sodium, Sorbitol dehydrogenase (SDH), Triglycerides (Triglyceride), Creatine kinase (CK), Lactate dehydrogenase (LDH), Bicarbonate (HCO3), Urinalysis (including Specific gravity (SG), pH, Urobilinogen (URO), Total volume (TVOL), Color (COL), Clarity (CLA), Protein (PRO), Glucose (GLU), Ketones (KET), Bilirubin (BIL), Occult blood (BLD), Leukocytes (LEU), Nitrites (NIT), Microscopy of sediment.

6. Toxicokinetics and toxicology groups. Blood samples for toxicokinetics were collected prior to dose administration and at approximately 3, 10, and 30 minutes, and 2, 8, and 24 hours after dose administration on study day 0. Blood was collected via a femoral vein into chilled tubes containing potassium (K2) EDTA. Blood samples were maintained on wet ice during collection and processing. An attempt was made to isolate plasma in a refrigerated centrifuge within 15 minutes of collection. Plasma samples were immediately flash-frozen on dry ice and stored frozen (-65°C to -85°C). The plasma samples were shipped on dry ice via overnight courier to Tandem Labs™, Salt Lake City, UT, for analysis using a non-GLP method. Interpretation of the toxicokinetic data was performed by WIL Research.

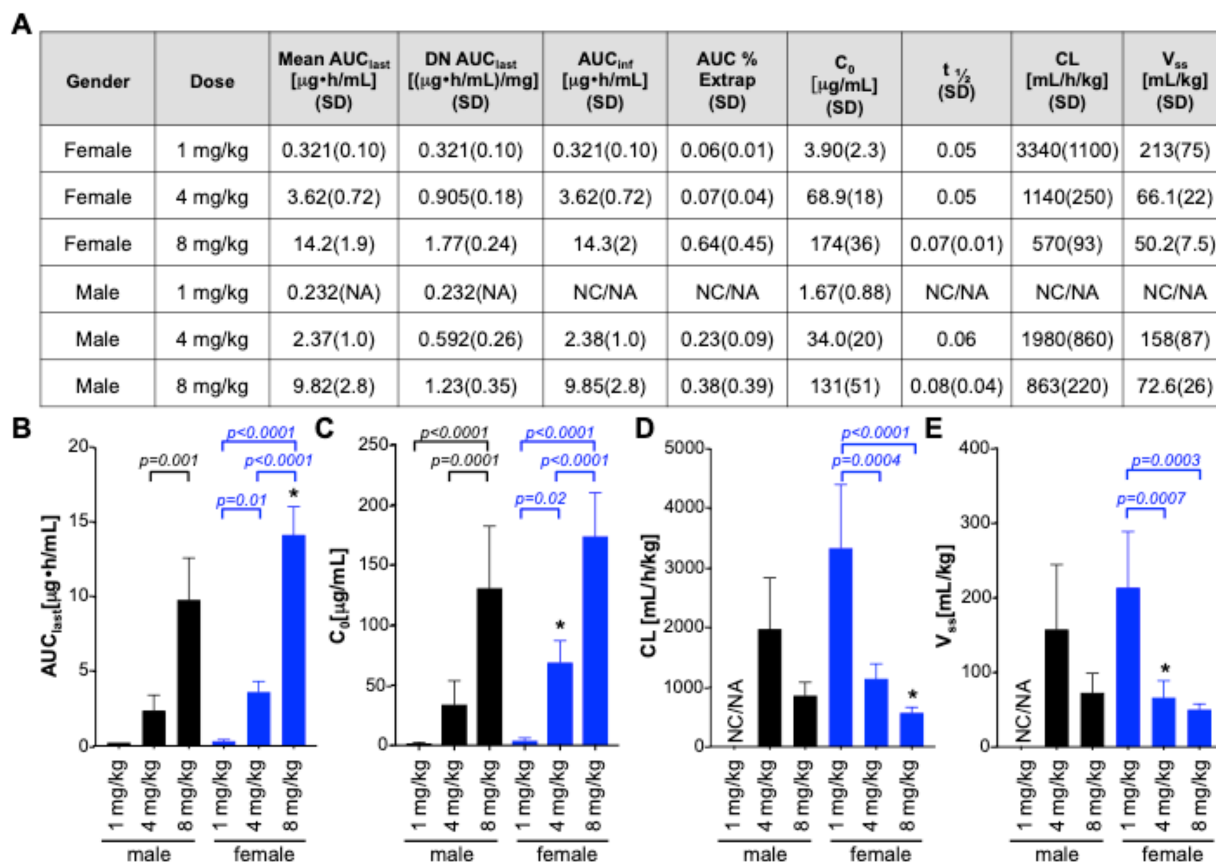
7. Radiotelemetry evaluations (cardiovascular groups). The radiotelemetry system (Data Sciences International, St. Paul, MN) consisted of large animal radiotelemetry transmitters (TL11M2-D70-PCT with arterial pressure, body temperature, and electrocardiographic waveform capabilities), receivers (RMC-1), and 1 or more data exchange matrices (DEM) that relayed information from the receivers to the computer. The transmitters had a fluid-filled catheter (coated with an antithrombotic film to inhibit thrombus formation) with the tip filled with a patented gel for collection of blood pressure, and 2 ECG leads, which were surgically implanted, emulating a

lead II configuration. On each day of dosing, after confirmation of a functional probe, baseline arterial blood pressure (systolic, diastolic, and mean), pulse pressure, heart rate, electrocardiographic (ECG) waveforms, and body temperature were collected continuously for approximately 1 hour prior to administration of vehicle or test article. Following administration of vehicle or test article, the above-listed parameters were collected continuously for approximately 24 hours and were averaged to appropriate time intervals for statistical analysis. The animals were observed daily (clinical observations were recorded) for 4 days following the final cardiovascular data collection on study day 1 and transferred to the WIL Research primate colony on study day 5.

8. Anatomic Pathology. A complete necropsy was conducted on all animals. Animals were anesthetized with ketamine prior to euthanasia by an intravenous injection of sodium pentobarbital followed by exsanguination. The necropsies included, but were not limited to, examination of the external surface, all orifices, and the cranial, thoracic, abdominal, and pelvic cavities including viscera. Organs weights for recorded.

9. Statistical Analysis. Each mean was presented with the standard deviation (SD), standard error (SE), and the number of animals (n) used to calculate the mean. All statistical tests were performed using WTDMS™ unless otherwise noted.

SUPPLEMENTARY FIGURES.



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Gender	Dose	Mean AUC$_{last}$ [µg•h/mL] (SD)	DN AUC$_{last}$ [(µg•h/mL)/mg] (SD)	AUC$_{inf}$ [µg•h/mL] (SD)	AUC % Extrap (SD)	C$_0$ [µg/mL] (SD)	t ½ (SD)	CL [mL/h/kg] (SD)	V$_{ss}$ [mL/kg] (SD)
Female	1 mg/kg	0.321(0.10)	0.321(0.10)	0.321(0.10)	0.06(0.01)	3.90(2.3)	0.05	3340(1100)	213(75)
Female	4 mg/kg	3.62(0.72)	0.905(0.18)	3.62(0.72)	0.07(0.04)	68.9(18)	0.05	1140(250)	66.1(22)
Female	8 mg/kg	14.2(1.9)	1.77(0.24)	14.3(2)	0.64(0.45)	174(36)	0.07(0.01)	570(93)	50.2(7.5)
Male	1 mg/kg	0.232(NA)	0.232(NA)	NC/NA	NC/NA	1.67(0.88)	NC/NA	NC/NA	NC/NA
Male	4 mg/kg	2.37(1.0)	0.592(0.26)	2.38(1.0)	0.23(0.09)	34.0(20)	0.06	1980(860)	158(87)
Male	8 mg/kg	9.82(2.8)	1.23(0.35)	9.85(2.8)	0.38(0.39)	131(51)	0.08(0.04)	863(220)	72.6(26)

fig. S1. Toxicokinetic parameters of NU-0129 as assessed in cynomolgus monkeys. (A) Summary of toxicokinetic parameters. AUC$_{last}$ = estimate of area under the concentration vs. time curve from time of dosing to the last detectable level; DN AUC$_{last}$ = dose normalized AUC$_{last}$; AUC$_{inf}$ = estimate of area under plasma concentration vs. time curve dosing to infinity; C$_0$ = estimated analyte concentration immediately following IV administration; t$_{1/2}$ = half-life of analyte in plasma; CL= apparent systemic clearance for the analyte in plasma; V$_{ss}$ = apparent volume of distribution for the analyte in plasma at steady state. Of note, CL, V$_{SS}$, and terminal half-life were not calculable at 1 mg/kg for males. **(B-E)** AUC$_{last}$, C$_0$, CL, and V$_{ss}$ in female and male monkeys combined. Shown are mean values, ± standard deviations. *p* values indicated in the figure were

calculated via one-way ANOVA. Asterisks indicate statistically significant differences with $p<0.05$ between groups of male and female animals.

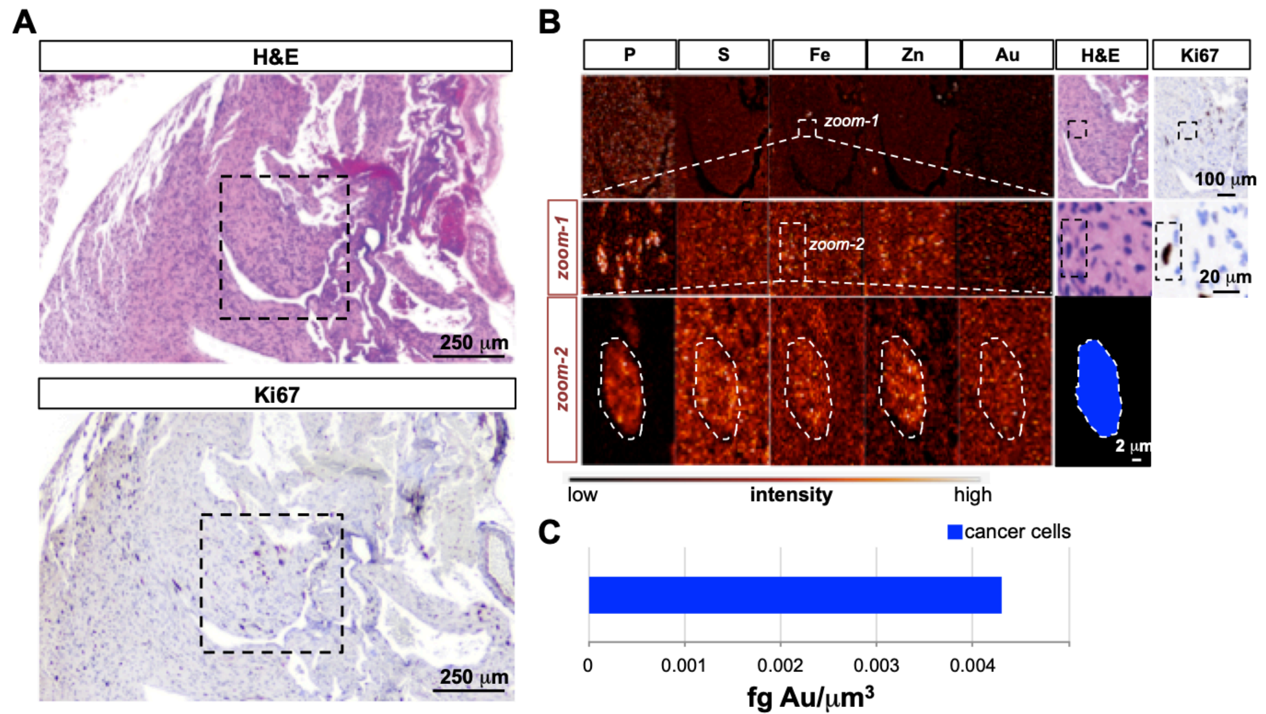

Fig. S2. XFM elemental maps of recurrent GBM tumor (patient ID 105). (A, D) Overview H&E and Ki67 stainings. **(B)** Phosphorus (P), sulfur (S), iron (Fe), zinc (Zn) and gold (Au) elemental maps and matched Ki67 stainings of GBM tumor sample. Zoomed areas in lower resolution scans are boxed. **(C)** Quantification of Au content in Ki67-positive glioma cells. Color bar indicates pixel concentrations for each element ranging from black (no signal) to white (highest signal for a given element).

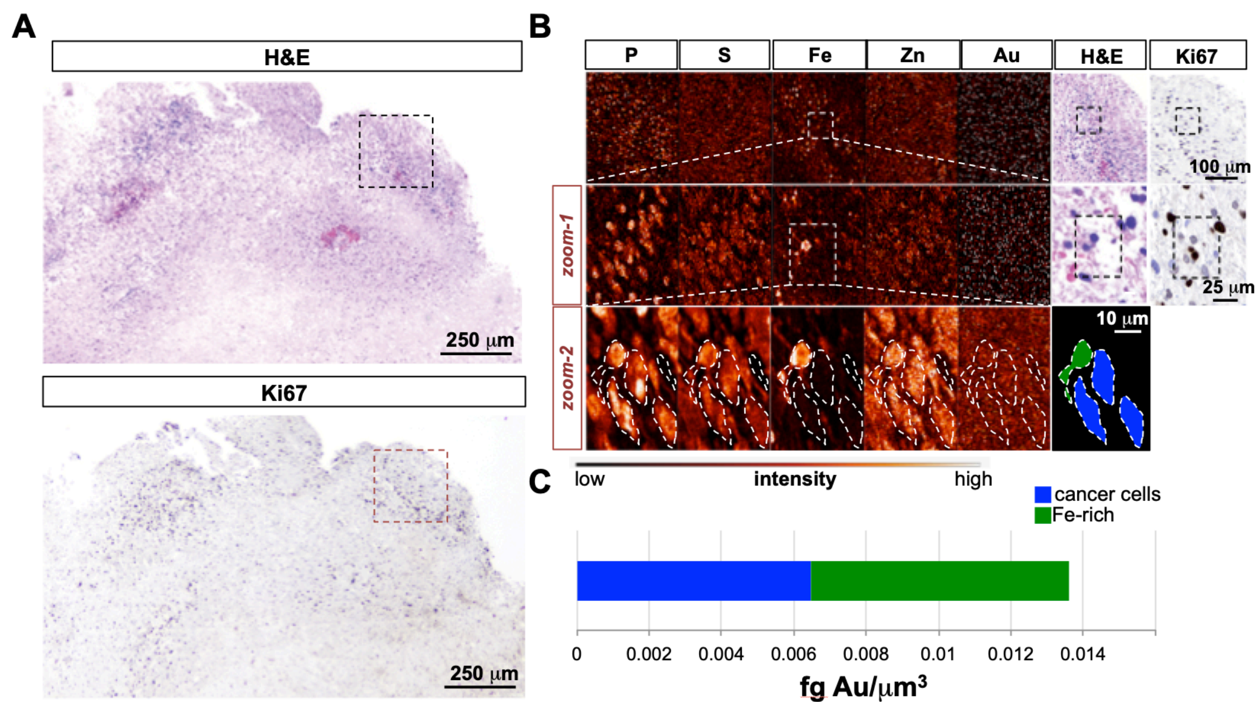

Fig. S3. XFM elemental maps of recurrent GBM tumor (patient ID 101). (A, D) Overview H&E and Ki67 stainings. **(B)** Phosphorus (P), sulfur (S), iron (Fe), zinc (Zn) and gold (Au) elemental maps and matched Ki67 stainings of GBM tumor sample. Zoomed areas in lower resolution scans are boxed. **(C)** Quantification of Au content in Ki67-positive glioma cells and Fe-rich cells. Color bar indicates pixel concentrations for each element ranging from black (no signal) to white (highest signal for a given element).

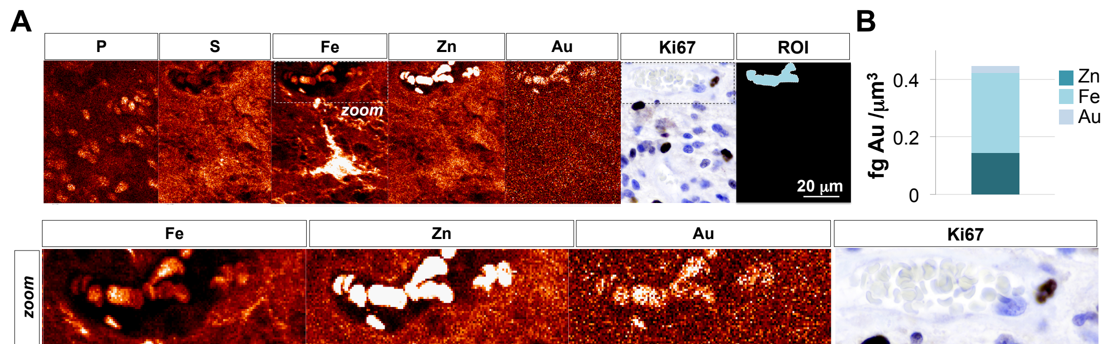

fig. S4. XFM elemental maps of recurrent GBM tumors identify Fe and Zn as markers for RBCs in blood vessels. (A) Phosphorus (P), sulfur (S), iron (Fe), zinc (Zn) and gold (Au) elemental maps and matching Ki67 staining of tumor from patient 107, demonstrating Zn-enrichment in blood cells. **(B)** Quantification of Au, Zn and Fe content associated with tumor blood vessels. Color bar indicates pixel concentrations for each element ranging from black (no signal) to white (highest signal for a given element).

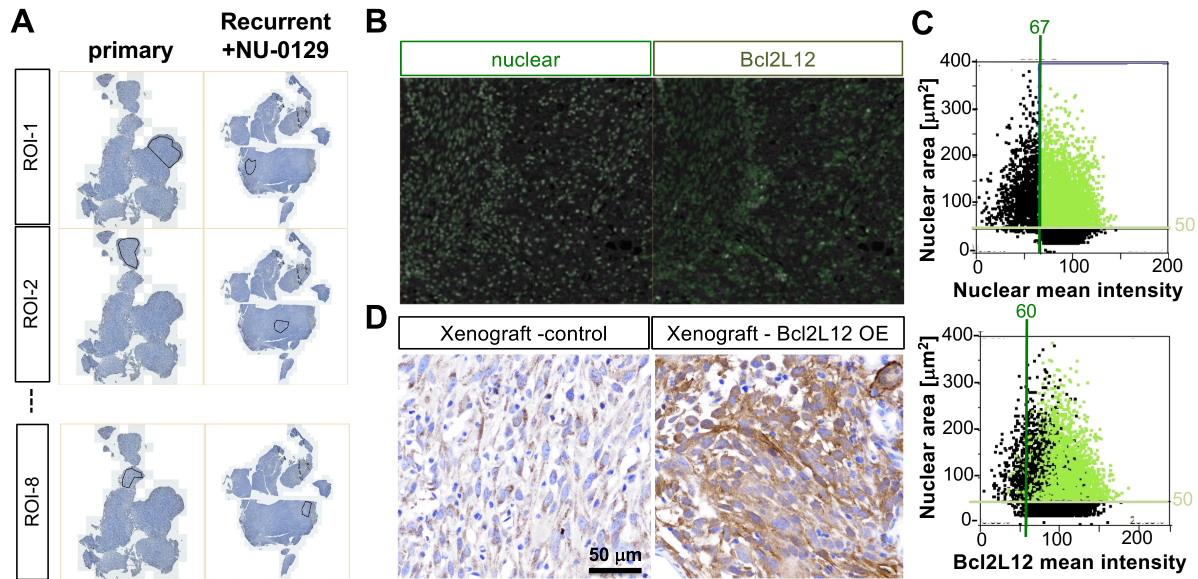

Fig. S5. ROI selection for IHC staining quantification. (A) A minimum of 8 ROIs indicated by the boxed areas were selected per tumor sample. **(B)** Representative nuclear and Bcl2L12 stainings. **(C)** ROIs were required to have above background Bcl2L12 signal (relative mean Bcl2L12 staining intensity > 60) and large nuclei (nuclear area >50 µm^2 and nuclear staining intensity > 67). **(D)** Bcl2L12 IHC using anti-Bcl2L12 (Thermo Fisher PA5-54267) on U87MG xenografts expressing vector control or Bcl2L12.

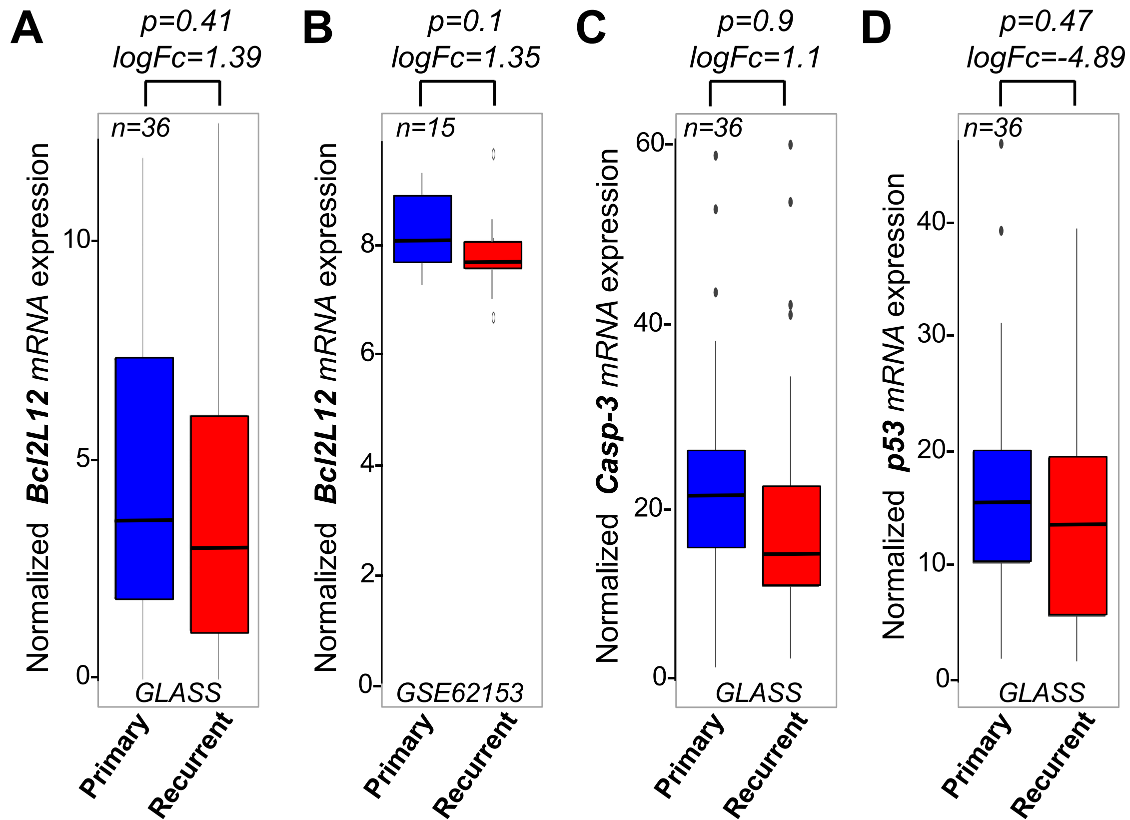

Fig. S6. mRNA expression of *Bcl2L12*, *wild-type p53* and *caspase-3* in primary versus recurrent GBM. (A-B) *In silico* analysis of *Bcl2L12* mRNA expression in primary versus recurrent GBM using GLASS consortium and GSE62153 datasets. **(C)** Longitudinal changes in *caspase-3* mRNA expression during recurrence. **(D)** *Wild-type p53* transcript levels in primary versus recurrent GBM. We used the limma package in R for differential gene expression analyses. Limma uses an empirical Bayes model to produce standard errors, *t*-statistics and raw *p* values.

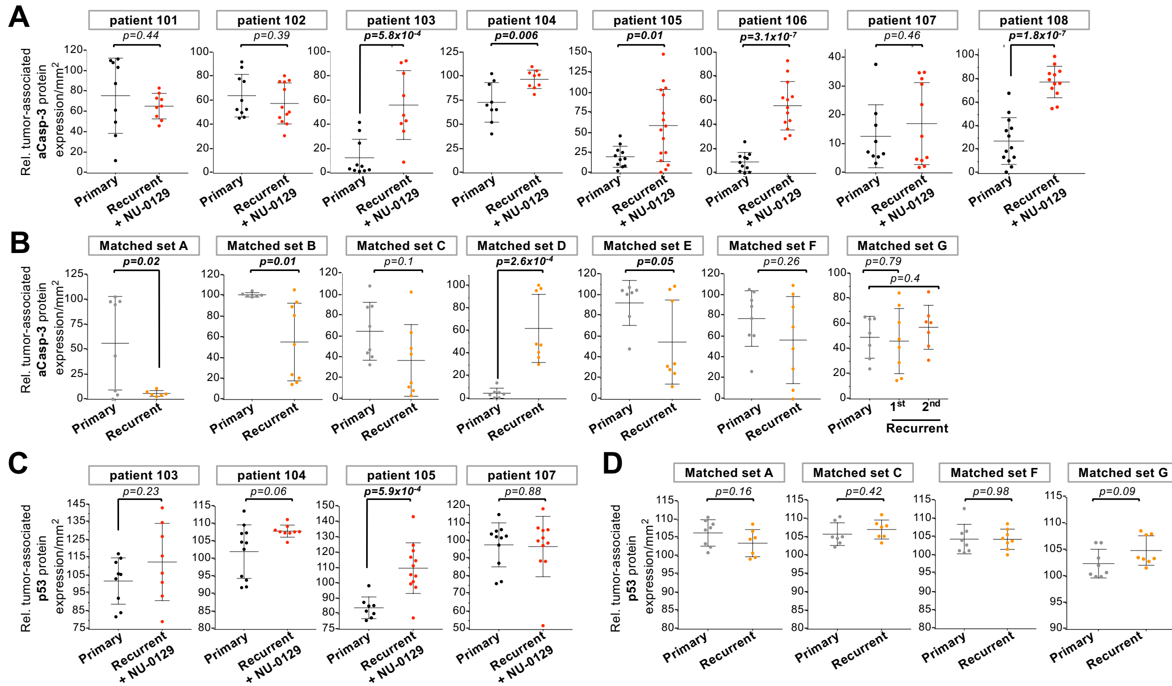

Fig. S7. Protein expression of active caspase-3 and wild-type p53 in individual matched newly diagnosed and recurrent on trial and trial-unrelated GBM. (A-B) Histoquest quantification of IHC staining for active caspase-3 in matched primary and NU-0129-treated recurrent GBM tumors ***(A)***, and in trial-unrelated matched primary and recurrent GBM tumors ***(B)***. A minimum of 8 ROIs were selected per tumor samples. Shown is the mean ± standard deviations. **(C-D)** Histoquest quantification of IHC staining for p53 in matched primary and NU-0129-treated recurrent GBM tumors ***(C)***, and in trial unrelated matched primary and recurrent GBM tumors ***(D)***. A minimum of 8 ROIs were selected per tumor samples. Shown is the mean ± standard deviations.

siRNA Duplex Purity					
Sample	**0 months**	**3 months**	**6 months**	**9 months**	**12 months**
-20 ± 5°C	89%	97%	96%	96%	90%
25 ± 2°C 60% ± 5% RH		96%	95%	93%	89%

Particle size [λ_{max}/600nm]					
Sample	**0 months**	**3 months**	**6 months**	**9 months**	**12 months**
-20 ± 5°C	3.21	3.53	3.34	3.76	3.49
25 ± 2°C 60% ± 5% RH		1.91	2.06	2.0	1.75

	siRNA content [OD/ml]				
Sample	**0 months**	**3 months**	**6 months**	**9 months**	**12 months**
20 ± 5°C	5.2	3.3	3.42	3.64	3.63
25 ± 2°C 60% ± 5% RH		2.1	2.29	2.19	2.51

table S1. NU-0129 stability and integrity. NU-0129 drug substance stability, as assessed by quantifying siRNA duplex purity, nanoconjugate size and siRNA content. RH, relative humidity.