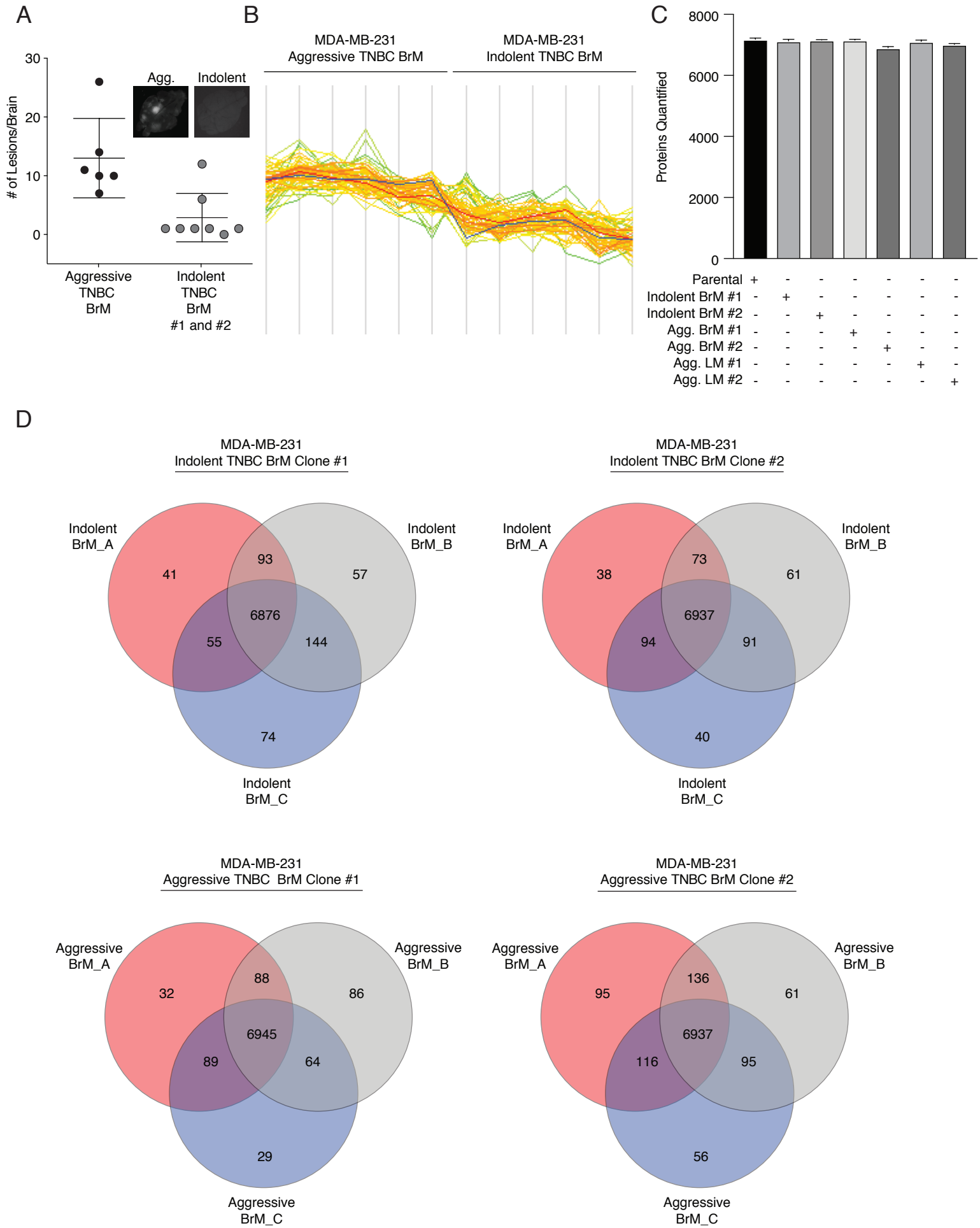
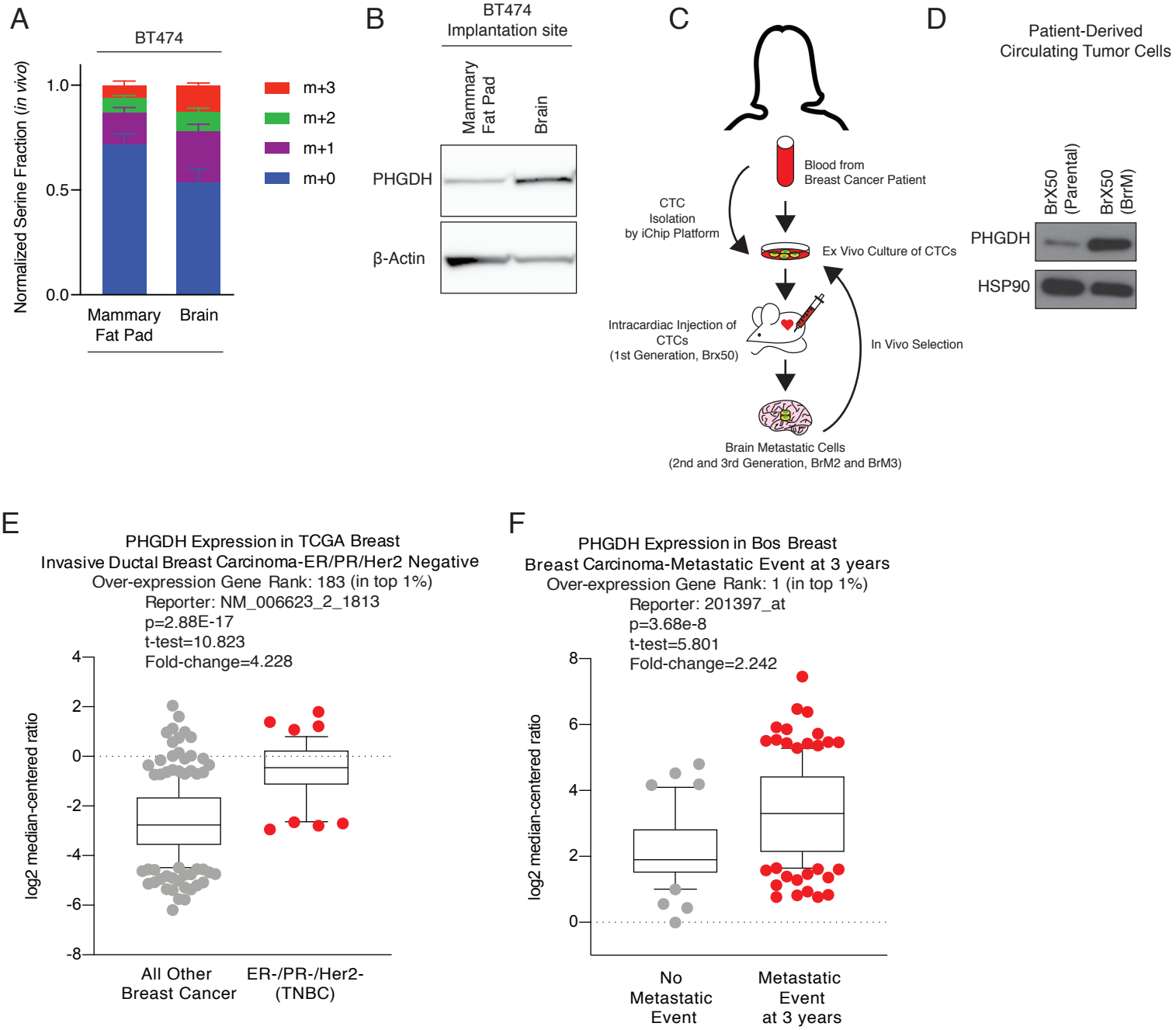
Supplementary Figure S1:



Supplementary Figure S1, Related to Figure 1.

(A) Number of aggressive or indolent metastatic lesions per brain quantified with a fluorescence dissection microscope.
(B) PHGDH peptide intensity from aggressive and indolent TNBC BrM cells.
(C) Number of proteins quantified per proteome sample.
(D) Venn diagram depicting number of differentially expressed proteins within replicates. Less than 1.5% of quantified proteins show no overlap within each group.

Supplementary Figure S2:



Supplementary Figure S2, Related to Figure 2.

(A) *In vivo* fractional labeling of U-^{13}C-glucose-derived m+3 serine from BT474 tumors implanted into the brain (ICr) or mammary fat pad (MFP).
(B) Western blot of PHGDH expression in tumors derived from BT474 tumors implanted into the brain (ICr) or mammary fat pad (MFP).
(C) Schematic of patient derived CTC isolation and selection of Brx50 BrM cells.
(D) Western blot of PHGDH expression from patient derived circulating tumor cells.
(E) Oncomine TCGA breast cancer expression data comparing PHGDH expression levels in triple-negative versus all other breast cancer subtypes.
(F) Oncomine expression data of PHGDH expression in patients with metastatic event compared to patients who did not have metastases at 3 years.

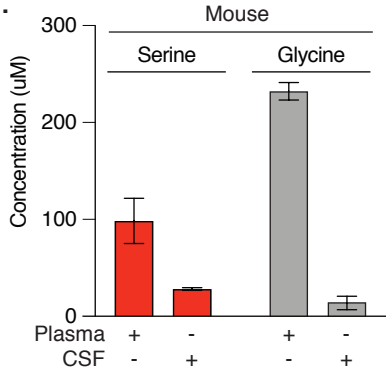
Supplementary Figure S3:

A

Mouse	[Amino Acid] (μM)	
	Serine	Glycine
Plasma	109	246
CSF	37	34
Brain ISF	9.8	7.8

See legend for references

B



C

Working concentrations

Media	[Amino Acid] (μM)	
	Serine	Glycine
Plasma	170	240
CSF	25	5
-Ser/-Gly	0	0

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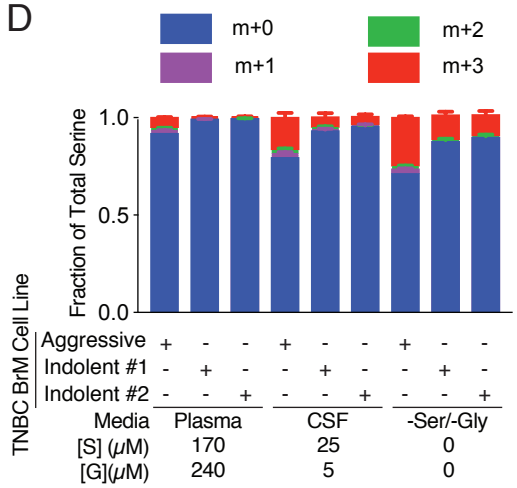

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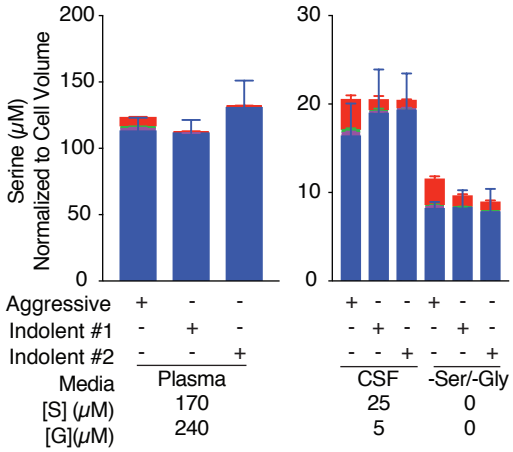

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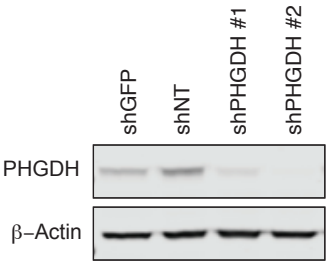

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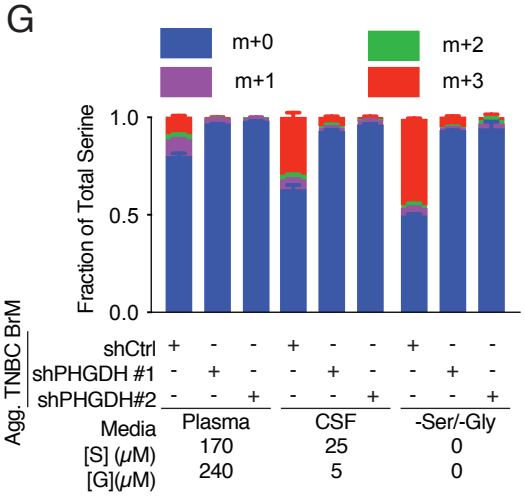

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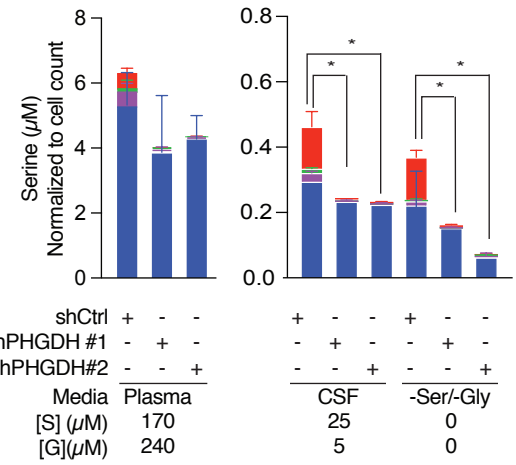

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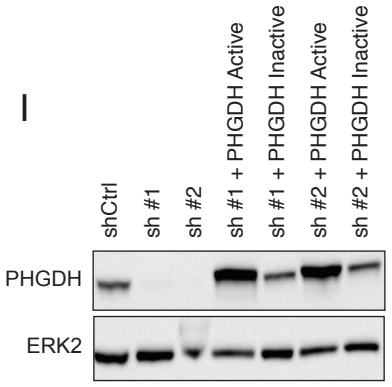

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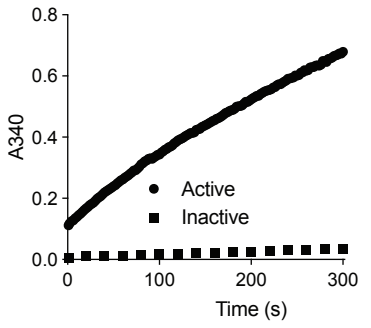

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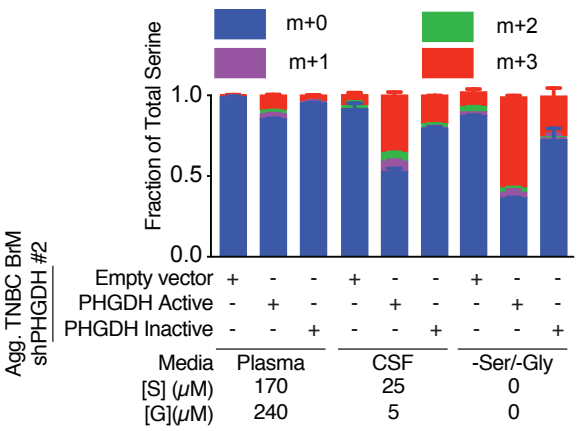

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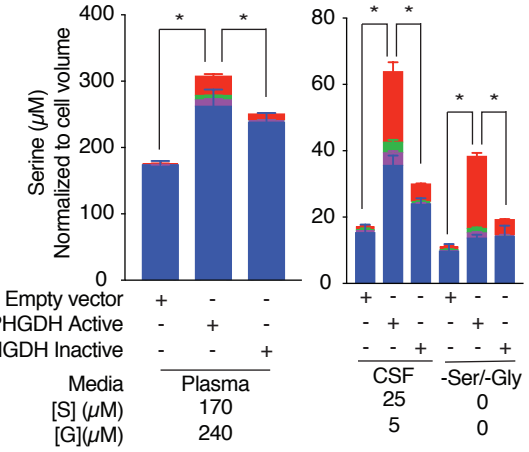


Supplementary Figure S3, Related to Figure 3.

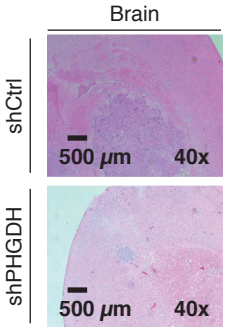
(A) Published concentrations of serine and glycine in mouse plasma, cerebrospinal fluid (CSF), and the brain interstitial fluid (ISF) (20,23).
(B) Concentrations of serine and glycine in plasma or CSF collected from 6-week old female mice.
(C) Working concentrations of serine and glycine used in Plasma, CSF, and -Ser/-Gly cell culture media.
(D) Fractional labeling of U-^{13}C-glucose-derived serine from aggressive and indolent TNBC BrM cells cultured in plasma, CSF, and -Ser/-Gly media.
(E) Total concentrations of unlabeled, exogenous serine and m+3, glucose-derived serine in aggressive and indolent TNBC BrM cells cultured in plasma, CSF, and -Ser/-Gly media.
(F) Western blot of PHGDH expression in aggressive TNBC BrM cells transduced with control shRNAs (shGFP and shNT; referred to as shCtrl) or shRNAs targeting PHGDH (shPHGDH #1 and shPHGDH #2).
(G) Fractional labeling of U-^{13}C-glucose derived serine from aggressive TNBC BrM cells transduced with shCtrl or shRNAs targeting PHGDH (shPHGDH #1 or shPHGDH #2) and cultured in plasma, CSF, or -Ser/-Gly media.
(H) Total serine concentration, normalized to cell counts, from fractional labeling of U-^{13}C- glucose derived serine from aggressive TNBC BrM cells transduced with shCtrl or shPHGDH cultured in plasma, CSF, or -Ser/-Gly media.
(I) Western blot of PHGDH depleted aggressive TNBC BrM cells, expressing empty vector (EV), catalytically active PHGDH, or catalytically inactive PHGDH.
(J) *In vitro* enzymatic activity assay of catalytically active PHGDH and inactive mutant PHGDH (D175N, R236K, H283A). NADH is measured by absorbance at 340 nm.
(K) Fractional labeling of U-^{13}C-glucose-derved m+3 serine from PHGDH depleted aggressive TNBC BrM cells, expressing an empty vector (EV) control, catalytically active PHGDH or inactive mutant (D175N, R236K, H283A) PHGDH. Cells were cultured in plasma, CSF, and -Ser/-Gly media.
(L) Total serine concentration, normalized to cell volume, in PHGDH depleted aggressive TNBC BrM cells rescued with catalytically active and inactive PHGDH.

Supplementary Figure S4:

A

H1975 Aggressive NSCLC BrM

Total Metastasis Area / Total Brain Area (H&E)	0-10%	>10%
shCtrl	1 (17%)	5 (83%)
shPHGDH	5 (71%)	2 (29%)



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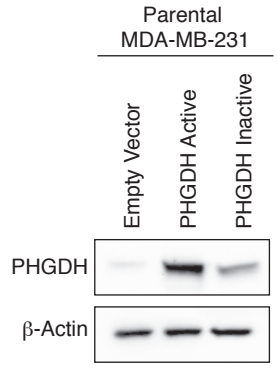
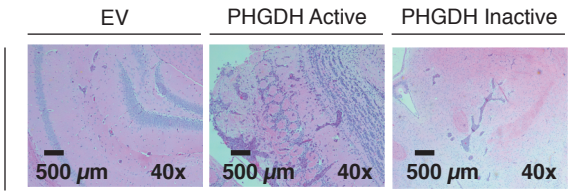


C

Parental MDA-MB-231 (Intracardiac Injection)

Total Metastasis Area / Total Brain Area (H&E)	0-10%	>10%	
Expression Construct: EV	8 (100%)	0 (0%)	*
PHGDH Active	0 (0%)	8 (100%)	*
PHGDH Inactive	3 (60%)	2 (40%)	



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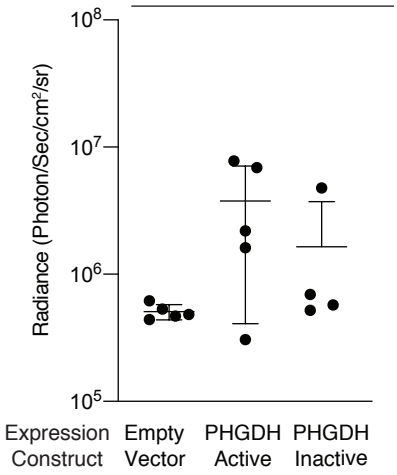
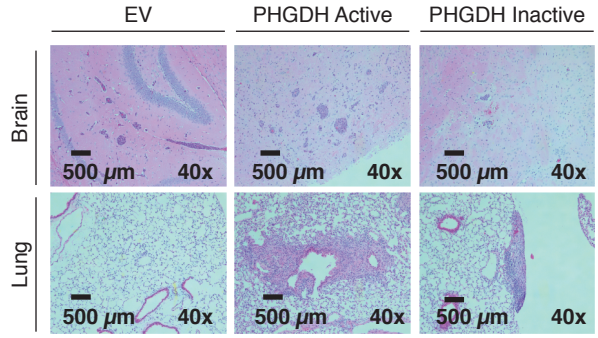


E

Parental MDA-MB-231 (Tail Vein Injection)

Total Metastasis Area / Total Tissue Area (H&E)	Brain 0-10%	Brain >10%	Lung 0-10%	Lung >10%
Expression Construct: EV	4 (100%)	0 (0%)	1 (25%)	3 (75%)
PHGDH Active	5 (100%)	0 (0%)	3 (60%)	2 (40%)
PHGDH Inactive	4 (80%)	1 (20%)	3 (60%)	2 (40%)



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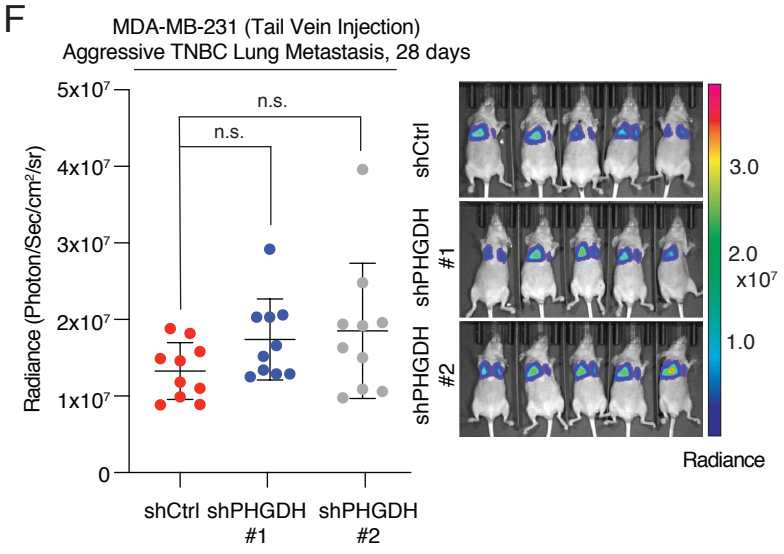
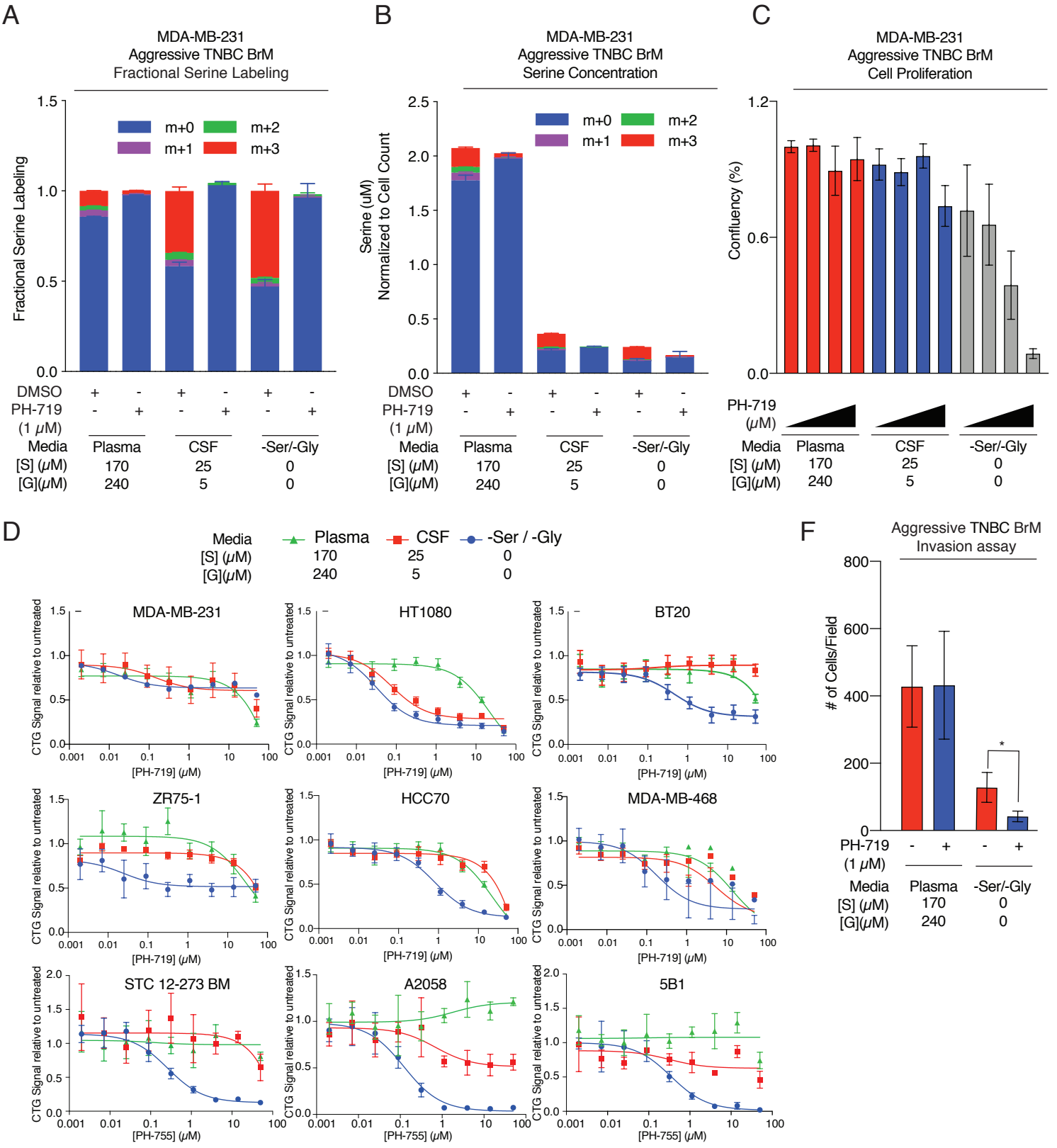


Supplementary Figure S4, Related to Figure 4.

(A) Histological confirmation by H&E staining of brain metastasis burden following injection of H1975 aggressive NSCLC BrM cells (Figure 4C).
(B) Western blot of parental MDA-MB-231 cells expressing empty vector (EV), or a virus containing catalytically active or catalytically inactive PHGDH.
(C) Histological confirmation by H&E staining of brain metastasis burden (Figure 4E-G). Mice were injected with parental MDA-MB-231 cells transduced with an empty vector virus (EV), or a virus containing catalytically active or catalytically inactive PHGDH.
(D) Quantification of radiance 70 days post tail vein injection of parental MDA-MB-231 cells transduced with an empty vector virus (EV), or a virus containing catalytically active or catalytically inactive PHGDH.
(E) Histological confirmation by H&E staining of lung and brain metastasis burden following tail vein injection of parental MDA-MB-231 cells an empty vector virus (EV), or a virus containing catalytically active or catalytically inactive PHGDH (Supplementary Fig. S4D).
(F) Quantification of radiance following tail vein injection of MDA-MB-231 aggressive TNBC lung metastatic cells transduced with shCtrl or shPHGDH, measured 28 days post injection.

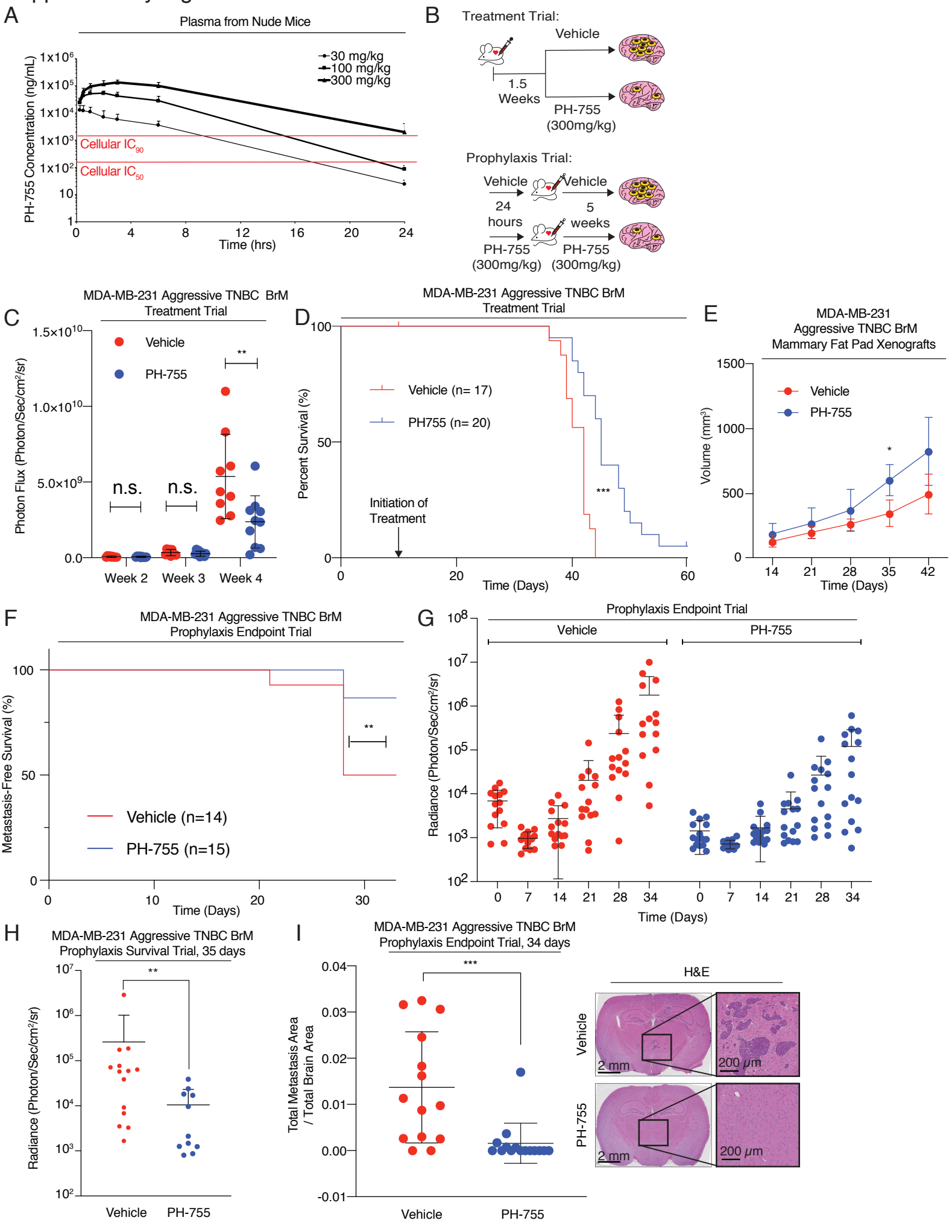
Supplementary Figure S5:



Supplementary Figure S5, Related to Figure 5.

(A) Fractional labeling of U-^{13}C-Glucose derived serine from aggressive TNBC BrM cells grown in Plasma, CSF, or -Ser/-Gly media, and treated with DMSO or 1μM of PH-719.
(B) Total serine concentration, normalized to cell counts, in aggressive TNBC BrM cells treated with DMSO or 1 μM PH-719.
(C) Proliferative capacity of aggressive TNBC BrM cells cultured in Plasma, CSF, or -Ser/-Gly media and treated with DMSO, 10nM, 100nM, or 1μM of PH-719.
(D) PH-719 dose response curves of MDA-MB-231, MDA-MB-468, HT1080, HCC70, BT20, and ZR-75-1 cells grown in plasma, CSF, or -Ser/-Gly media, and PH-755 dose-response curves of STC 12-273 BM, A2058, and 5B1 brain-trophic melanoma cell lines grown in plasma, CSF, or -Ser/Gly media.
(E) Western blot of PHGDH expression in MDA-MB-231, ZR-75-1, HT1080, HCC70, BT20, MDA-MB-468, A2058 and patient-derived Short Term Culture 12-273 Melanoma Brain Metastasis (STC 12-273BM) cells. Dashed lines denote non-adjacent Western blot lanes.
(F) Cell invasion assay of aggressive TNBC BrM cells cultured in media with plasma or -Ser/-Gly media, and treated with DMSO or 1μM of PH-719.

Supplementary Figure S6:



Supplementary Figure S6, Related to Figure 6.

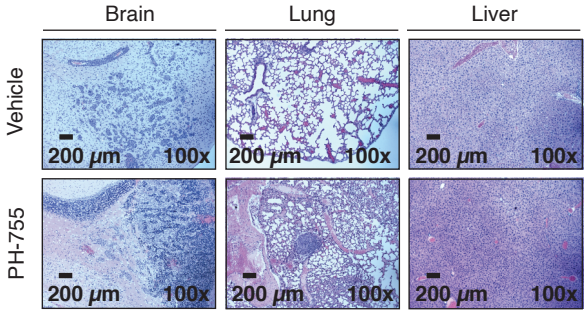
(A) Half-life of PH-755 in female nude mice following oral administration.
(B) Schematic of mouse clinical trials in which mice were either injected with aggressive brain metastatic cells, and treated 1.5 weeks later with vehicle or 300mg/kg of PH-755 twice daily (treatment trial), or where PH-755 treatment preceded the injection of aggressive brain metastatic cells (prophylaxis trial).
(C) Quantified total photon flux by bioluminescence imaging of aggressive TNBC brain metastasis-bearing mice treated over time with vehicle control or 300mg/kg of PH-755 twice daily.
(D) Kaplan-Meier plot showing disease-specific survival of aggressive TNBC-brain metastasis bearing mice treated with PH-755 started 1.5 weeks after intracardiac injection. Significance tested using log-rank test.
(E) Tumor volume measurement of aggressive TNBC BrM cells established as xenografts in the 4^{th} mammary fat pad (250,000 cells/injection) following treatment with vehicle control or 300mg/kg of PH-755 twice daily.
(F) Metastasis-free survival of mice from prophylaxisendpoint trial treated with vehicle or 300 mg/kg of PH-755 twice daily. Mice were defined to have brain metastases once the total brain photon flux exceeded brain photon flux measured on the day of injection.
(G) Quantified total photon flux by IVIS bioluminescence imaging of brain metastasis bearing mice from the prophylaxis endpoint trial treated over 5 weeks with vehicle control or 300 mg/kg of PH-755 twice daily.
(H) Quantified total photon flux by IVIS bioluminescence imaging of mice bearing aggressive TNBC BrM that were prophylactically treated with PH-755. This study was run with a survival endpoint and had significantly decreased brain burden in the treatment arm by day 35.
(I) Histological H&E Quantification of aggressive TNBC BrM lesions by H&E staining from mice in prophylaxis trial at week 5. Mice were treated with vehicle control or 300 mg/kg of PH-755 twice daily. Tumor areas were measured and quantified by two independent observers.

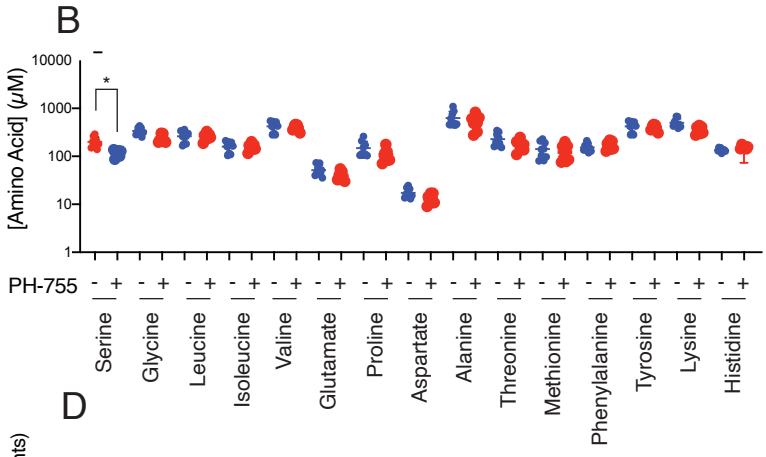
Supplementary Figure S7:

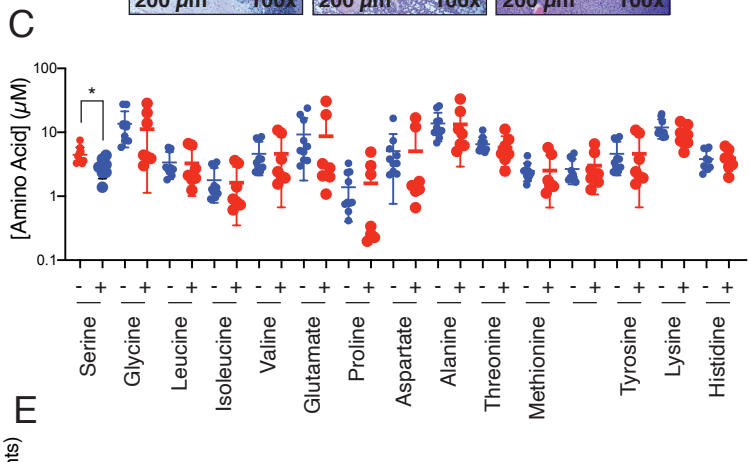
A

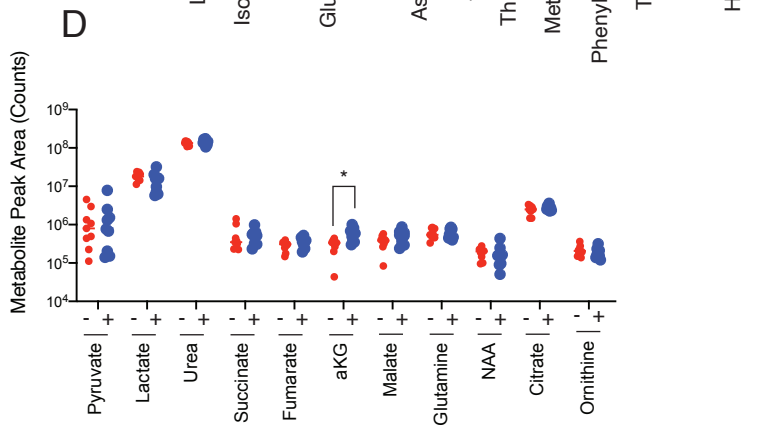
MDA-MB-231 Aggressive TNBC BrM Prophylaxis Survival Trial
Tissues collected at Humane Endpoint (35-65 days)

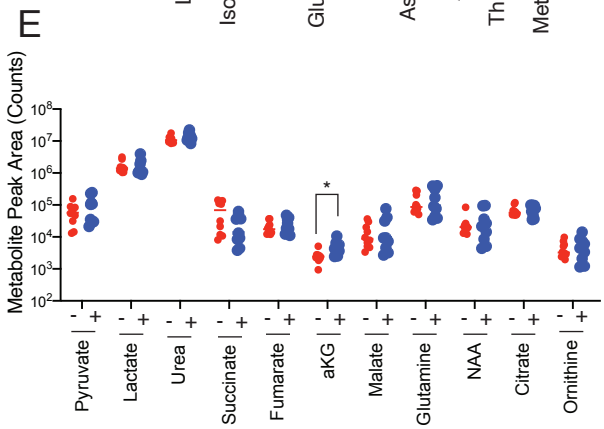
Total Metastasis Area / Total Organ Area (H&E)	Brain		Lung		Liver	
	0-10%	>10%	0-10%	>10%	0-10%	>10%
Vehicle	5 (33%)	10 (67%)	14 (93%)	1 (7%)	15 (100%)	0 (0%)
PH-755	8 (67%)	4 (33%)	12 (100%)	0 (0%)	12 (100%)	0 (0%)

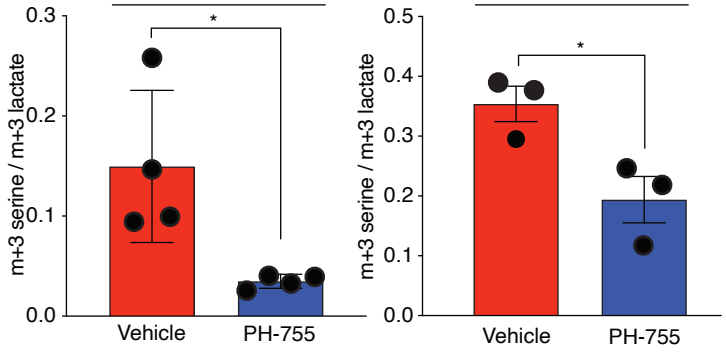


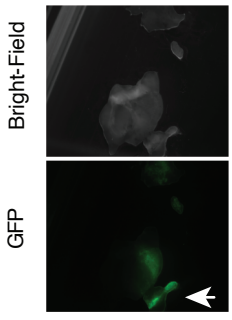


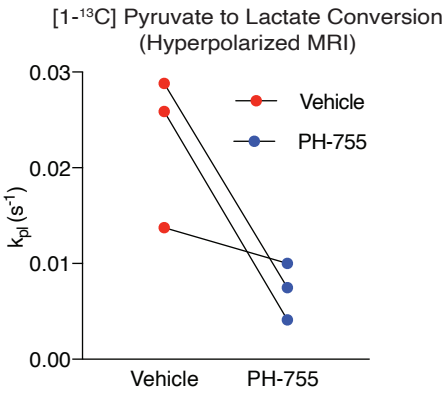


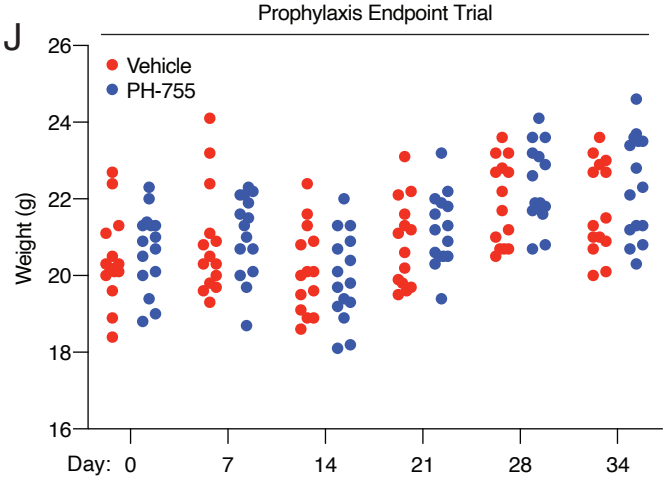


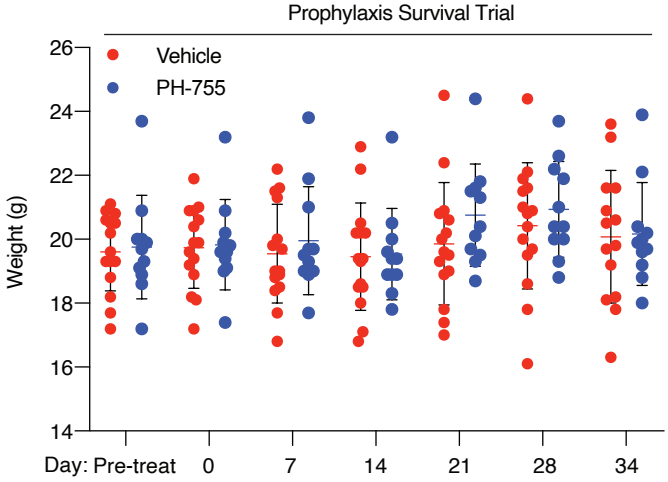








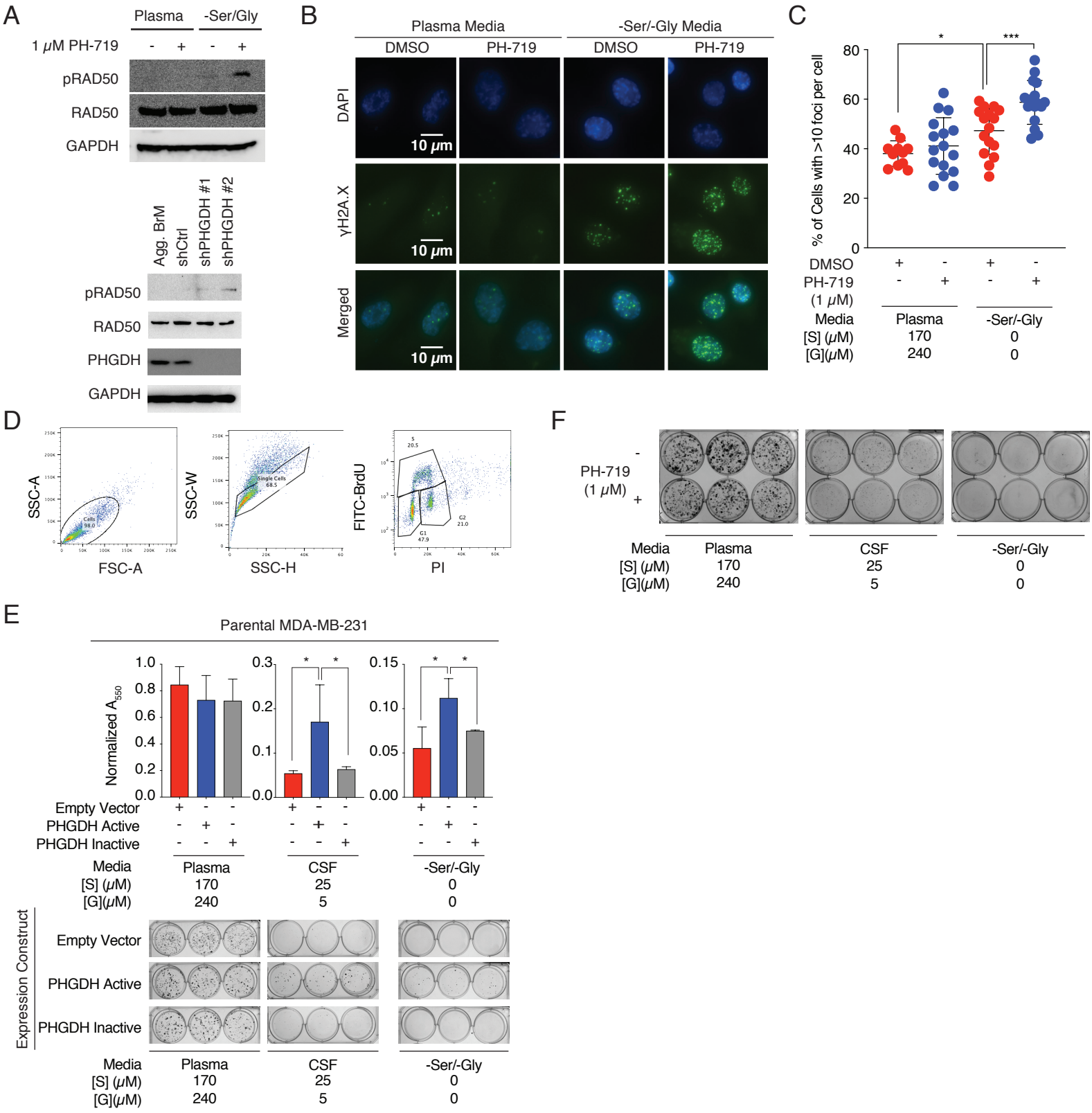




Supplementary Figure S7, Related to Figure 6.

(A) Histological confirmation by H&E staining of metastasis burden in the PH-755 prophylaxis survival trial (Figure 6F). Mice were sacrificed when they reached humane endpoints.
(B) Plasma and **(C)** CSF amino acid concentrations from mice 6 hours after treatment with vehicle control or 300 mg/kg of PH-755.
(D) Plasma and **(E)** CSF metabolite counts from mice 6 hours after treatment with vehicle control or 300 mg/kg of PH-755.
(F) Fractional labeling of U-^{13}C-Glucose-derived m+3 serine, normalized to fraction of U-^{13}C-Glucose-derived lactate, from the plasma of mice 6 hours after treatment with vehicle control or 300mg/kg of PH-755.
(G) Fraction of U-^{13}C-Glucose-derived m+3 serine, normalized to fraction of U-^{13}C-Glucose-derived lactate, from brain metastatic lesions isolated from mice 6 hours after treatment with vehicle control or 300mg/kg of PH-755.
(H) Representative image of isolated GFP-positive brain metastatic lesions following excision. Arrow indicates representative tumors used for Supplementary Fig. 4G, with minimal surrounding brain tissue.
(I) [1-^{3}C] Pyruvate Hyperpolarized Magnetic Resonance Imaging of pyruvate to lactate conversion *in vivo* with PH-755 treatment.
(J) Weight of individual mice plotted over the course of two 5-week PH-755 prophylaxis trials. Mice were treated with vehicle control or 300mg/kg twice daily of PH-755.

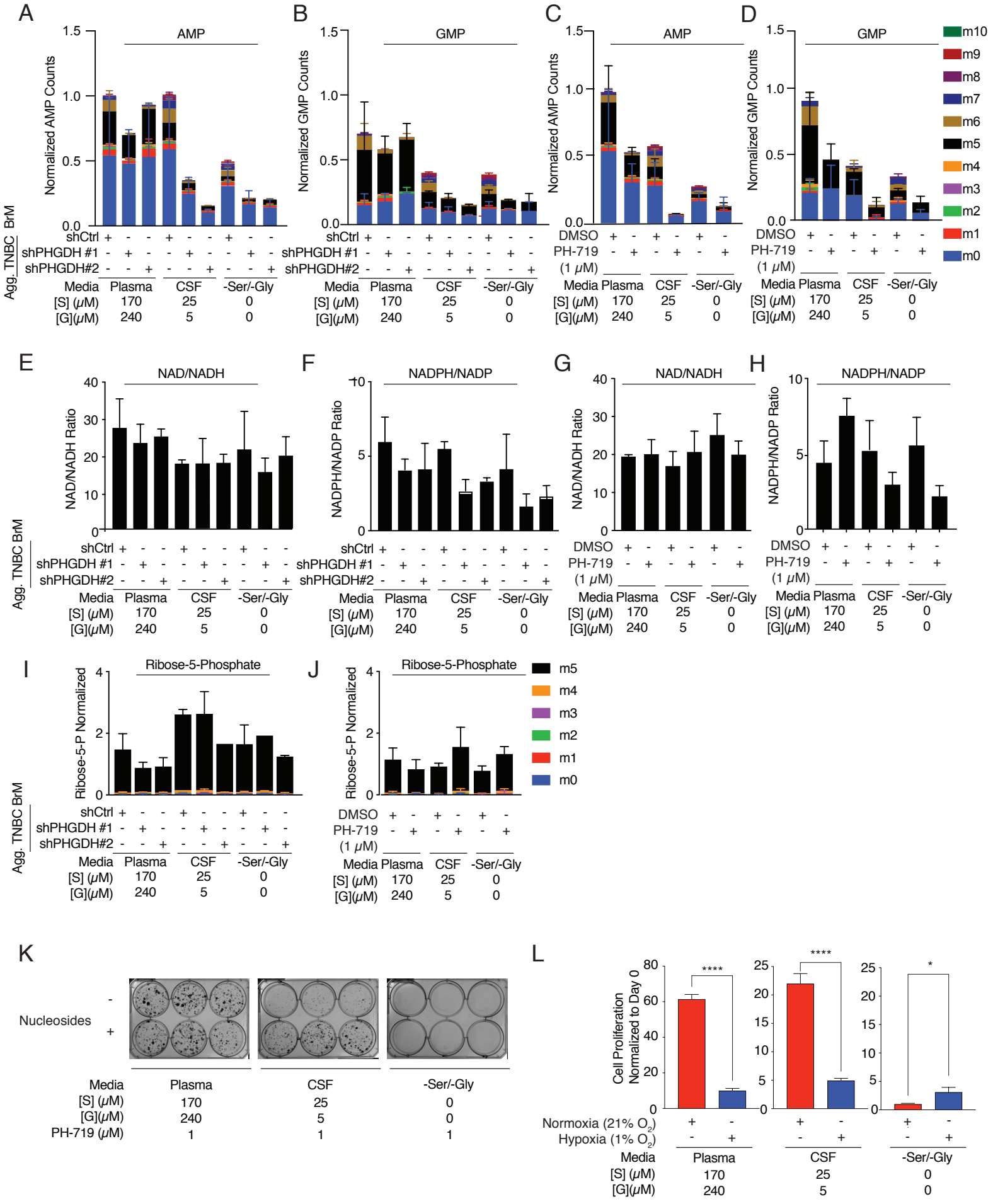
Supplementary Figure S8:



Supplementary Figure S8, Related to Figure 7.

(A) In MDA-MB-231 aggressive TNBC BrM Cells, pharmacological and genetic inhibition of PHGDH both induce RAD50 phosphorylation, a marker of DNA damage, selectively in -Ser/-Gly media. Detection by western blot.
(B) Immunofluorescence of γH2A.X foci, a marker of DNA damage, in the nuclei of aggressive TNBC BrM cells grown in plasma media or -Ser/-Gly media, and either in the absence orpresence of the PHGDH inhibitor PH-719.
(C) Quantitation of γH2A.X foci per cell in (B).
(D) FACS gating strategy for cell cycle analysis.
(E) Colony formation assay of MDA-MB-231 cells expressing empty vector, catalytically active PHGDH, or catalyt-ically inactive PHGDH in plasma, CSF, or -Ser/-Gly media.
(F) Colony formation assay of aggressive TNBC BrM cells in the absence or presence of PH-719 cultured in plasma, CSF, or -Ser/-Gly media.

Supplementary Figure S9:



Supplementary Figure S9, Related to Figure 7.

All metabolomics data are from MDA-MB-231 aggressive TNBC BrM cells.
(A) Quantitation of AMP pools in aggressive TNBC BrM cells following PHGDH suppression in plasma, CSF, or -Ser/-Gly media.
(B) Quantitation of GMP pools in aggressive TNBC BrM cells following PHGDH suppression in plasma, CSF, or -Ser/-Gly media.
(C) Quantitation of AMP pools in aggressive TNBC BrM cells following PHGDH inhibition in plasma, CSF, or -Ser/-Gly media.
(D) Quantitation of GMP pools in aggressive TNBC BrM cells following PHGDH inhibition in plasma, CSF, or -Ser/-Gly media.
(E) NAD/NADH ratio in aggressive TNBC BrM cells expressing shCtrl or shPHGDH in plasma, CSF, or -Ser/-Gly media.
(F) NADPH/NADP ratio in aggressive TNBC BrM cells expressing shCtrl or shPHGDH in plasma, CSF, or -Ser/-Gly media.
(G) NAD/NADH ratio in aggressive TNBC BrM cells following PHGDH inhibition with PH-719 in plasma, CSF, or -Ser/-Gly media.
(H) NADPH /NADP ratio in aggressive TNBC BrM cells following PHGDH inhibition with PH-719 in plasma, CSF, or -Ser/-Gly media.
(I) Ribose-5-phosphate levels in aggressive TNBC BrM cells following PHGDH suppression in plasma, CSF, or -Ser/-Gly media.
(J) Ribose-5-phosphate levels in aggressive TNBC BrM cells following PHGDH inhibition in plasma, CSF, or -Ser/-Gly media.
(K) Colony formation assay of aggressive TNBC BrM cells in the presence or absence of nucleosides cultured in in plasma, CSF, or -Ser/-Gly media with PH-719.
(L) Proliferation of aggressive TNBC BrM cells in hypoxia (1% O_2) or normoxia (21% O_2) in plasma, CSF, or -Ser/-Gly media.

Supplementary Table S1. shRNA sequences used in this work.

Name	TRC Number (if applicable)	Sequence
shNT	N/A	CCGGCAACAAGATGAAGAGCACCAACTCGAGTTGGTGCTCTTCATCTTGTTGTTTTT
shGFP	TRCN0000559399	CCGGACAACAGCCACAACGTCTATACTCGAGTATAGACGTTGTGGCTGTTGTTTTTTG
shPHGDH #1	TRCN0000233029	CCGGCAGGACTGTGAAGGCCTTATTCTCGAGAATAAGGCCTTCACAGTCCTGTTTTTG
shPHGDH #2	TRCN0000028520	CCGGGCTTCGATGAAGGACGGCAAACTCGAGTTTGCCGTCCTTCATCGAAGCTTTTT