OMTO, Volume *12*

Supplemental Information

Measles Virus-Based Treatments Trigger a Pro-inflammatory Cascade and a Distinctive Immunopeptidome in Glioblastoma

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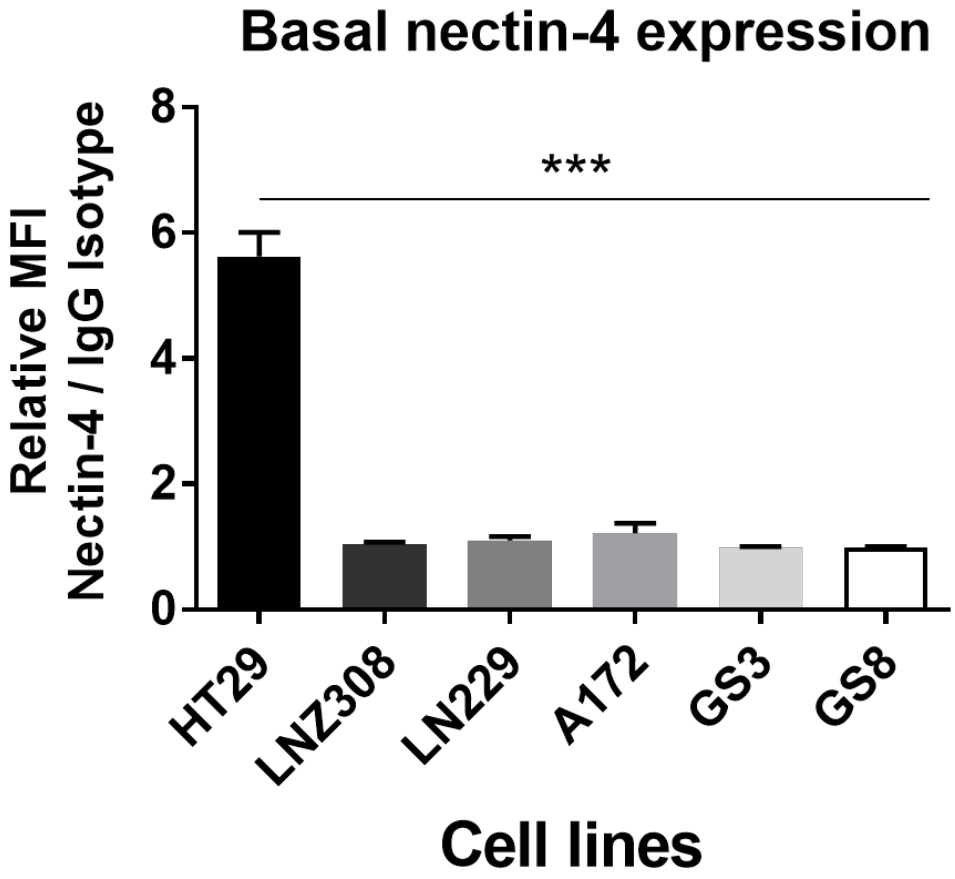
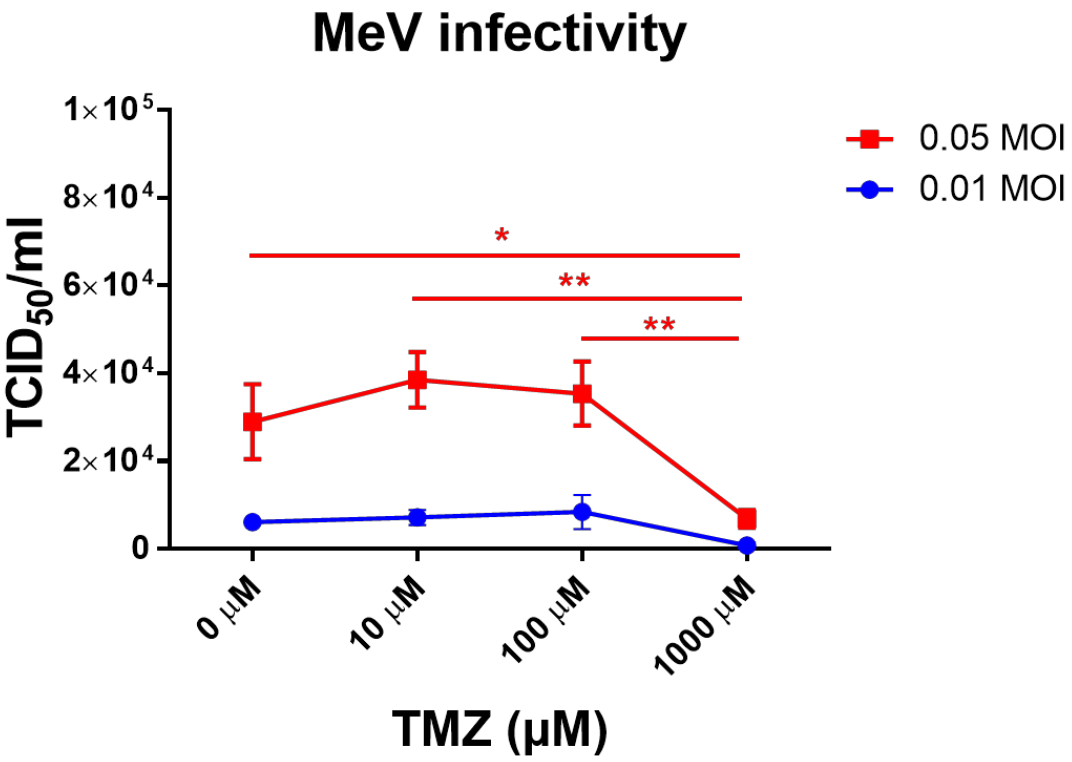
Supplemental Information

Supplemental Figures

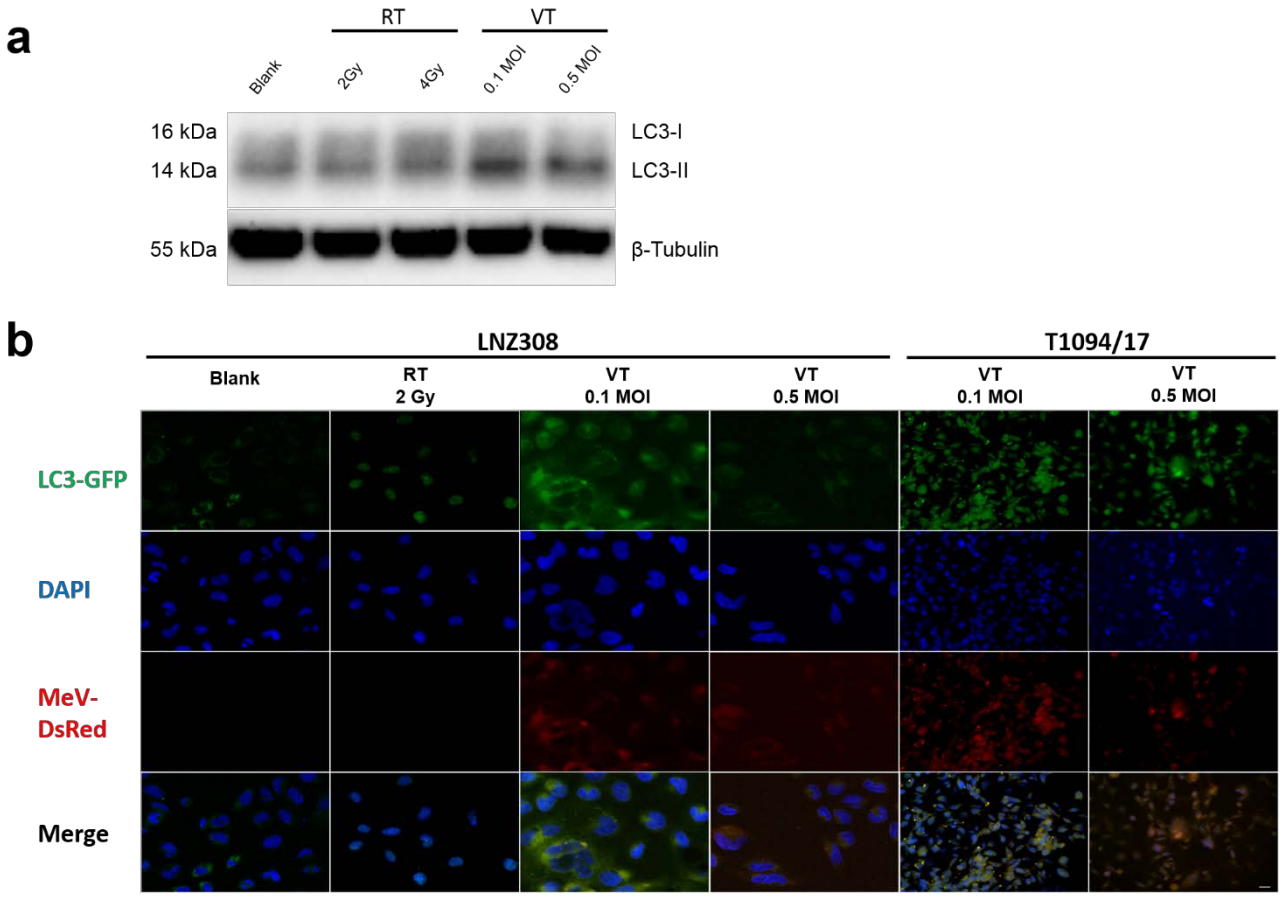
Supplemental Figure S1



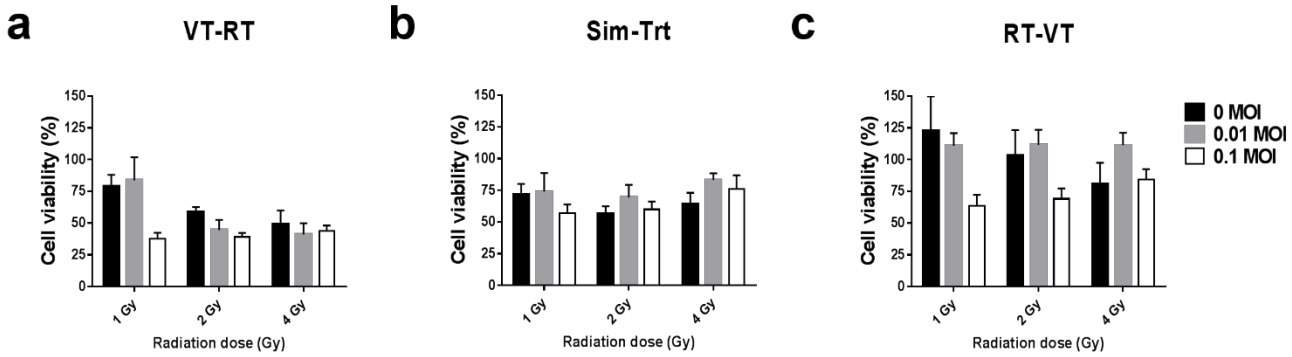
Supplemental Figure S2



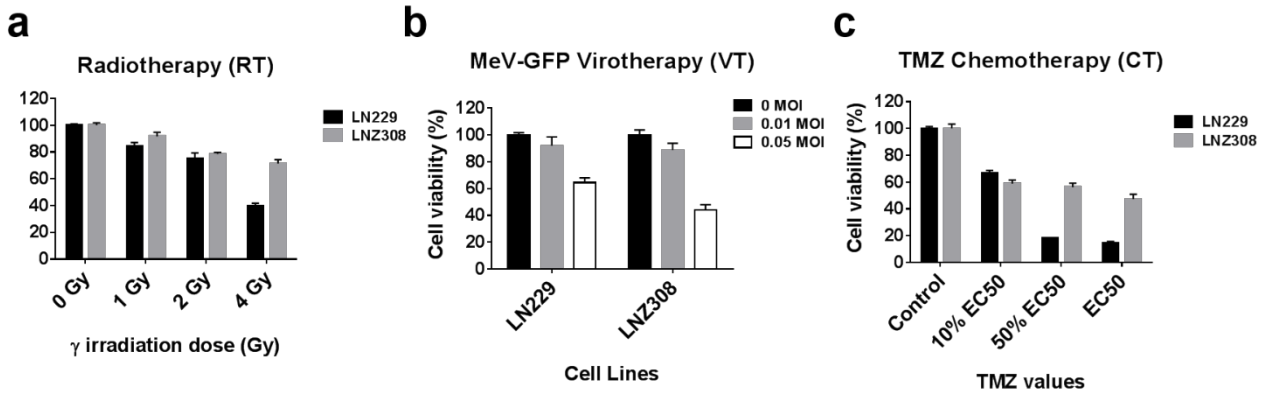
Supplemental Figure S3



Supplemental Figure S4



Supplemental Figure S5



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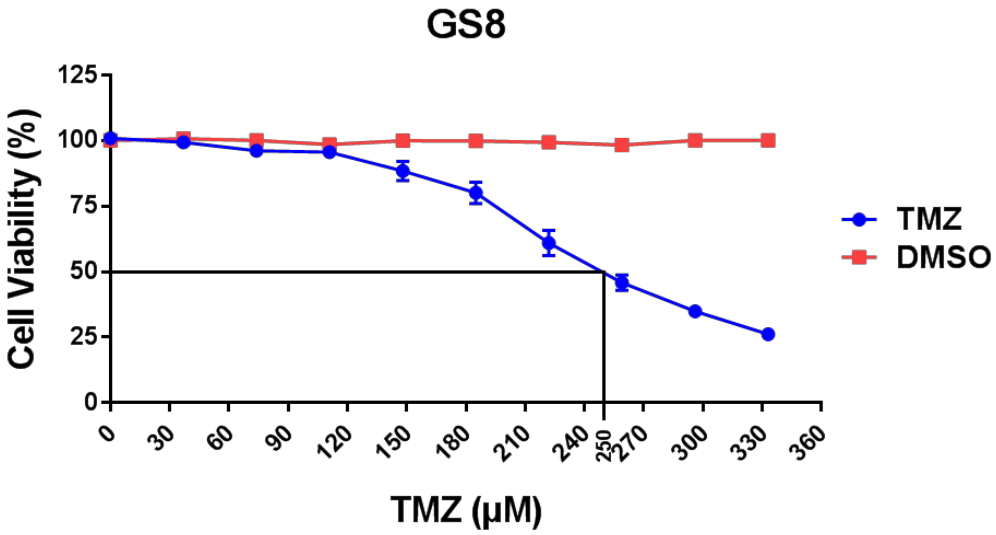

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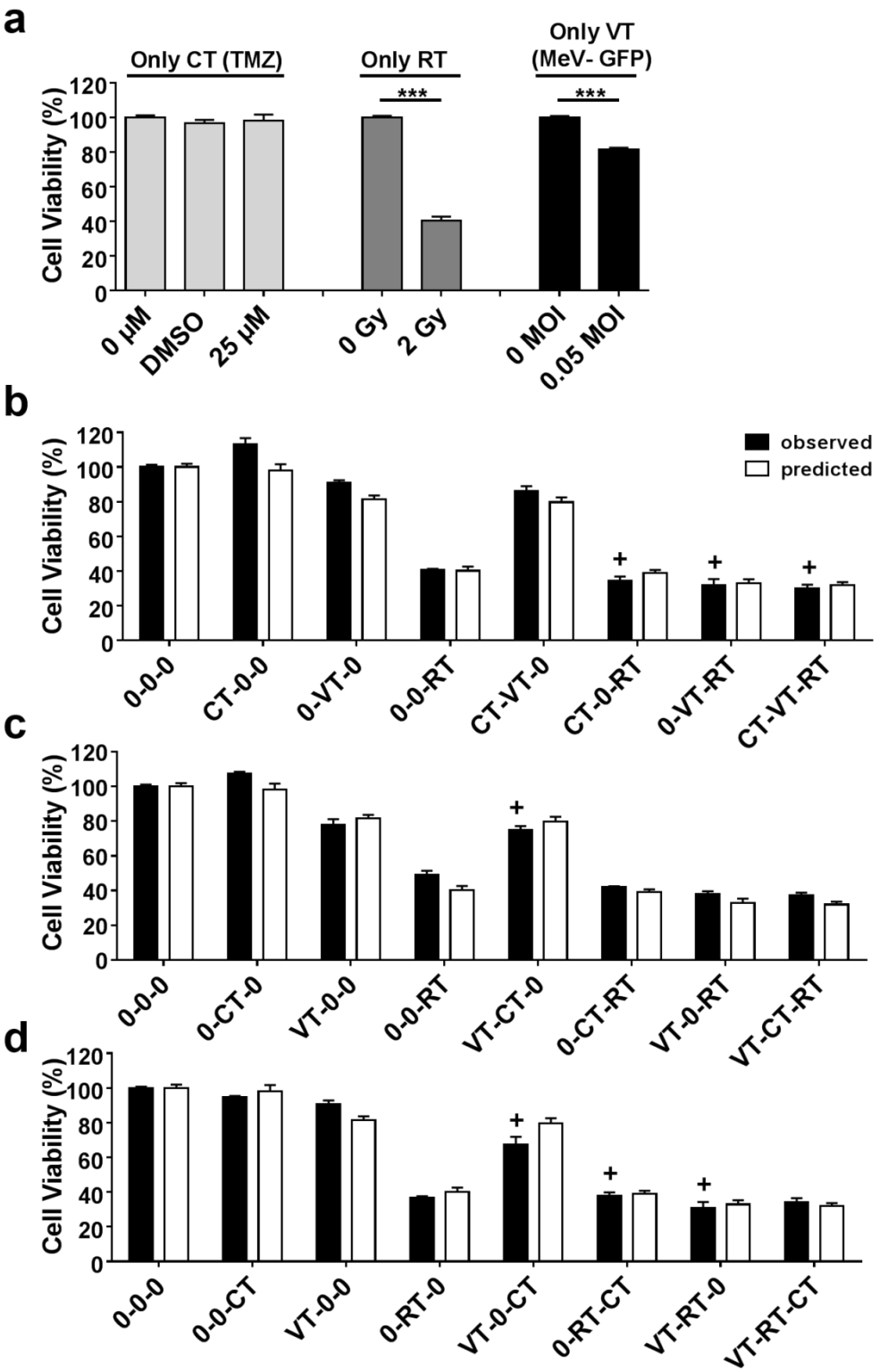

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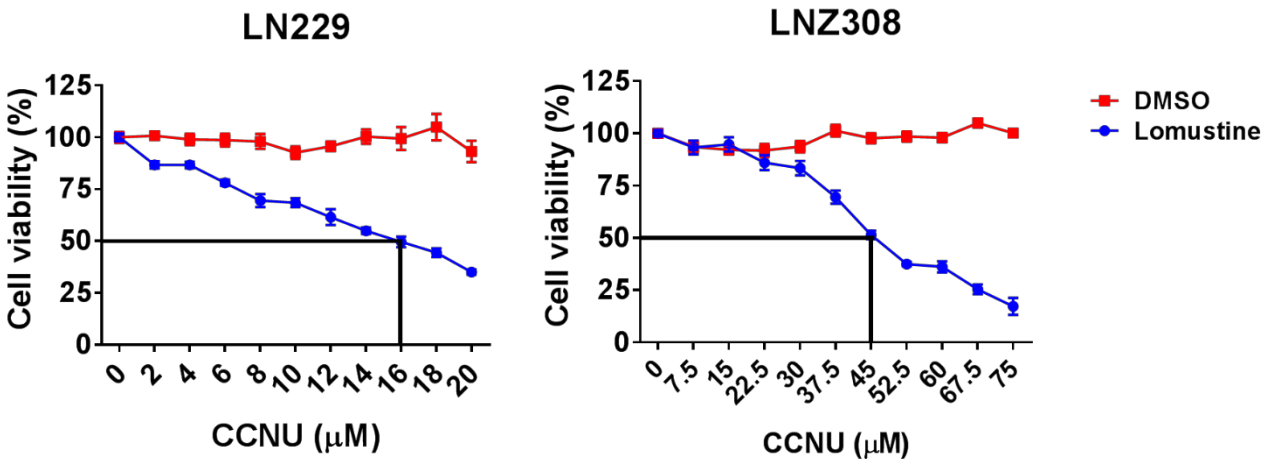
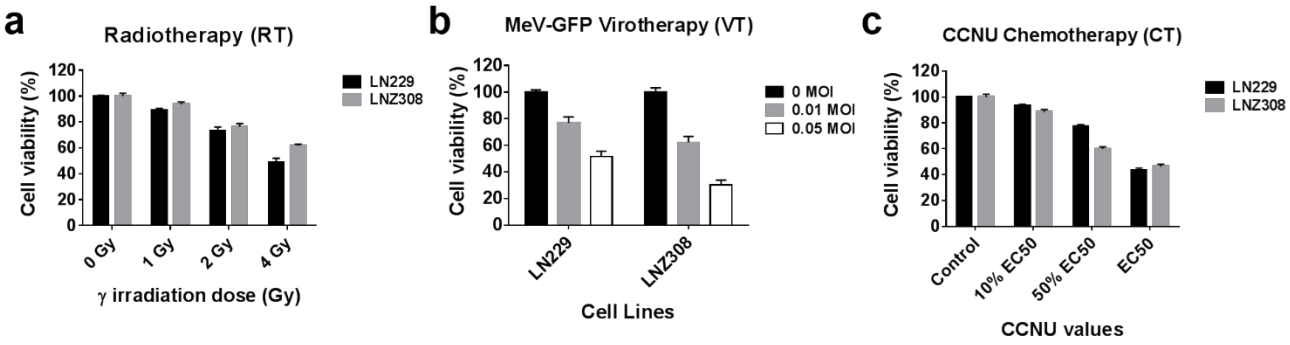


Supplemental Figure S9



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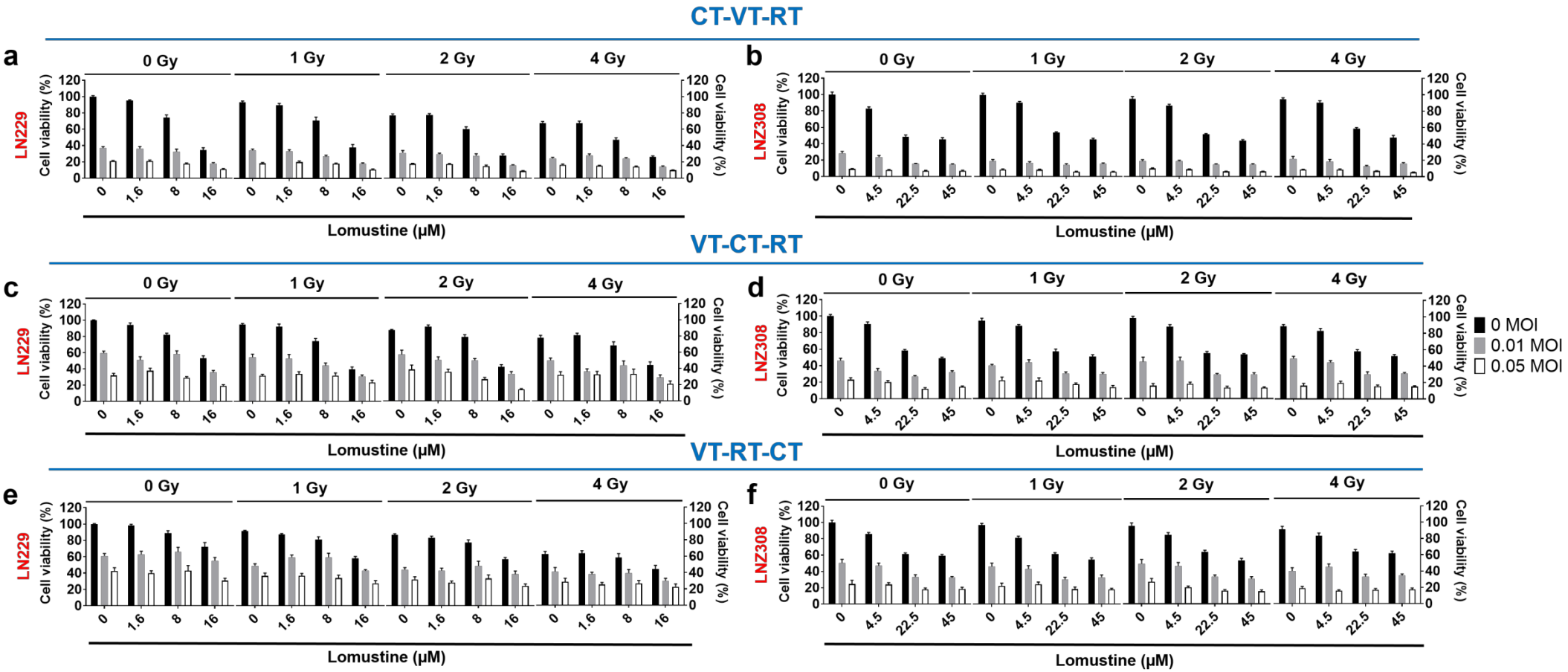

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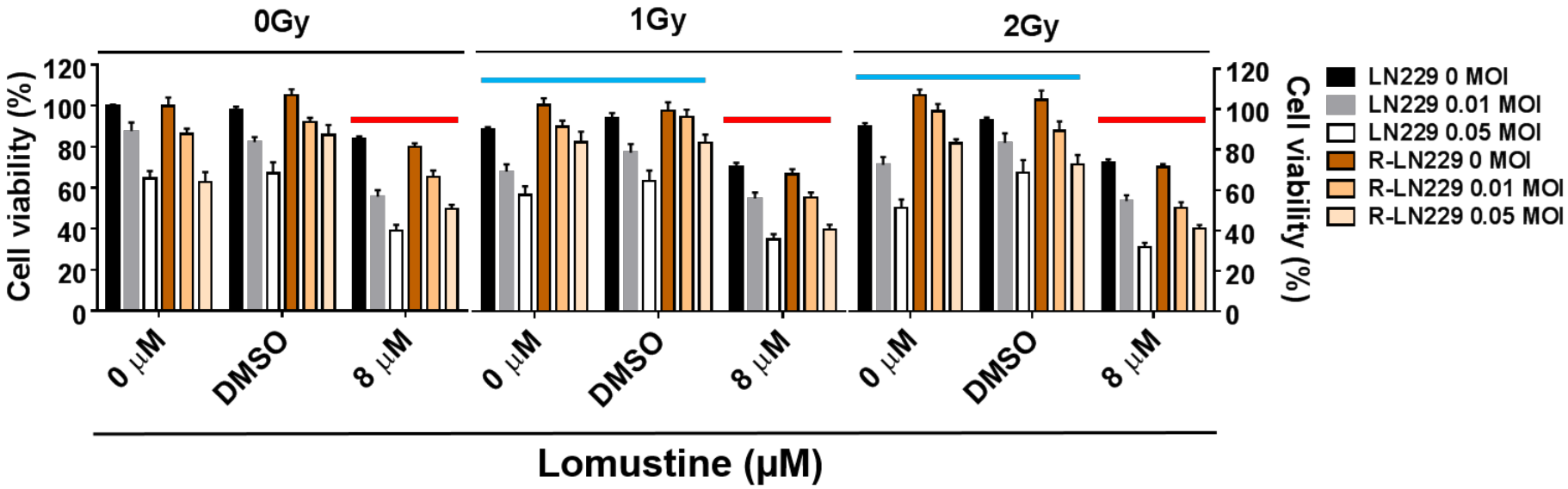
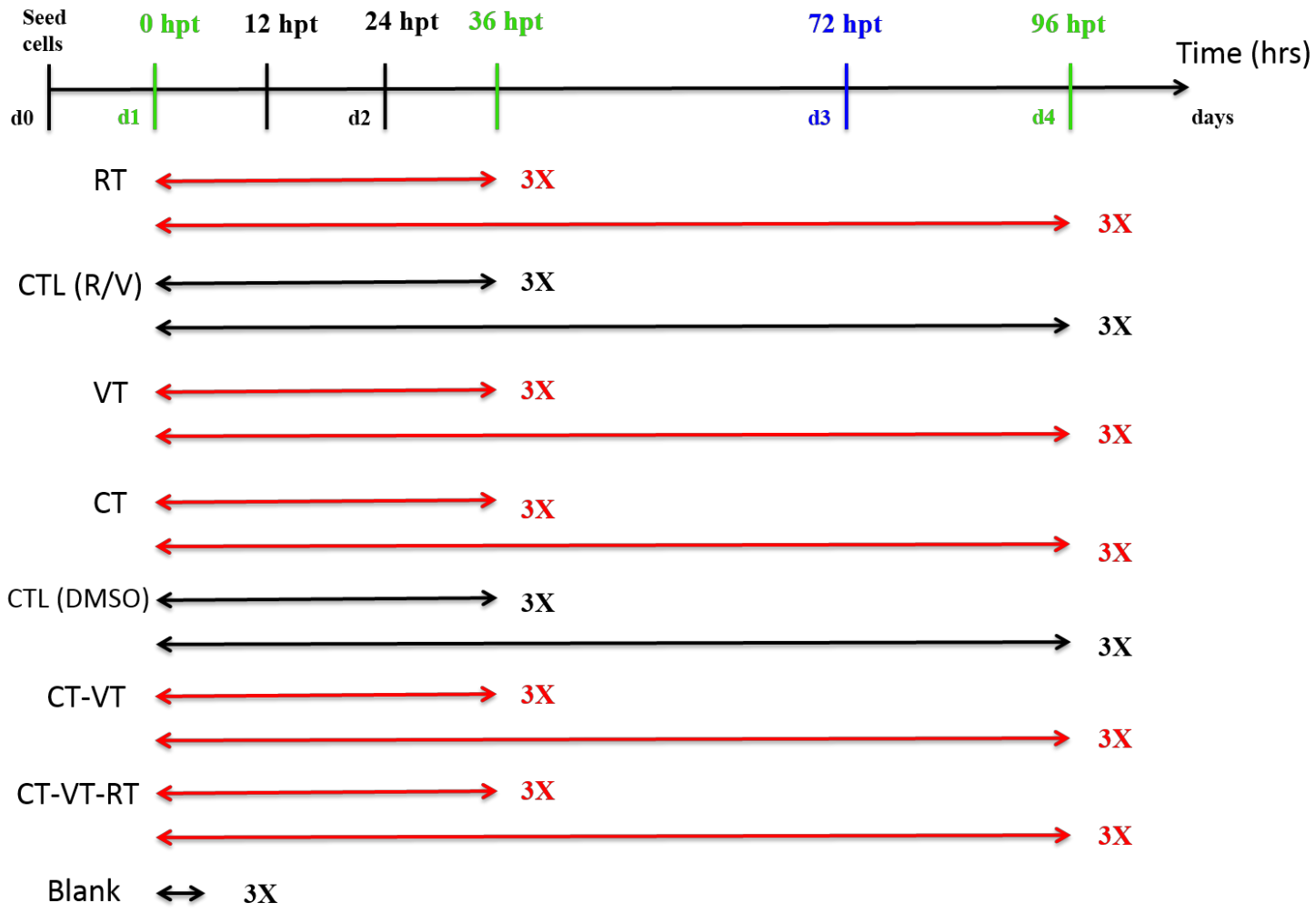


Supplemental Figure S12



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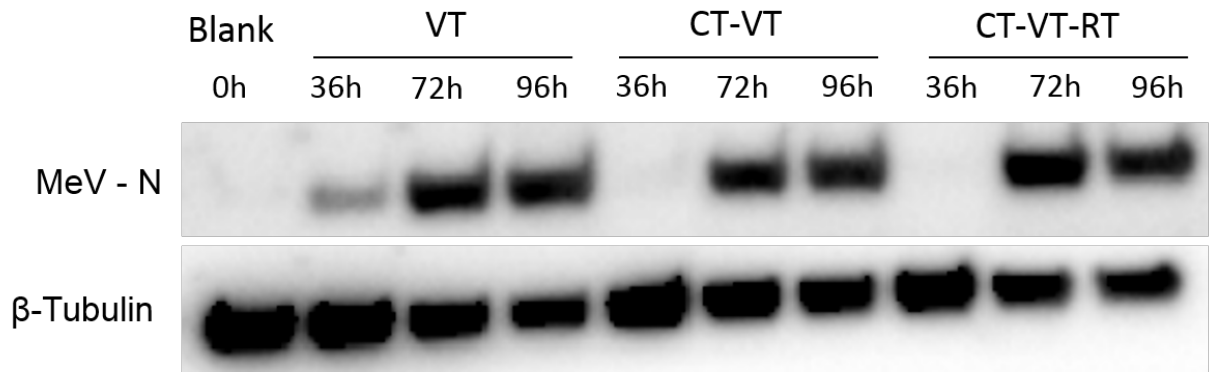

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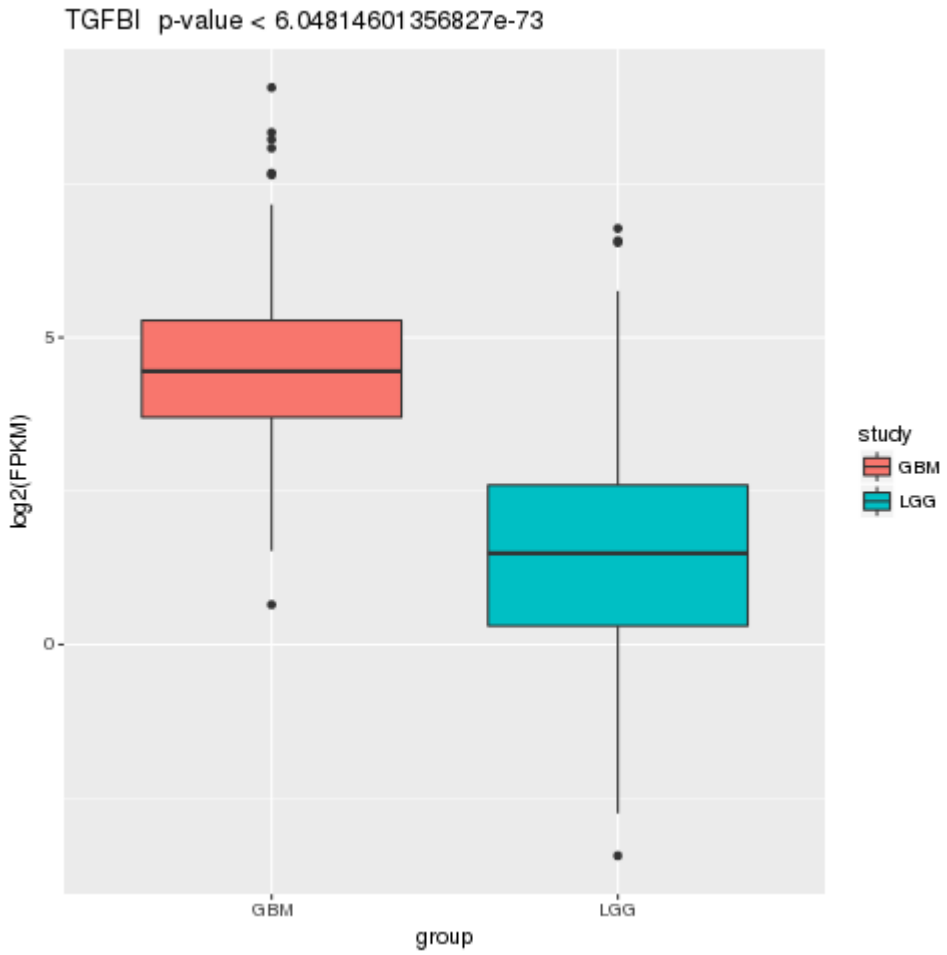

Supplemental Figure Legends

Supplemental Figure S1: Nectin-4 expression in glioma cells. No basal nectin-4 expression was observed in glioma cells and glioma stem-like cells as determined via flow cytometry along with positive control HT-29 cells, a nectin-4^+ colorectal adenocarcinoma cell line. Receptor expression depicted as relative MFI compared to isotype control and expressed as Mean ± SEM, n = 3. One-way ANOVA with Dunnett's multiple comparison test; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Supplemental Figure S2: Measles viral infectivity post TMZ treatment in LNZ308 glioma cells. MeV infectivity increased after treatment with doses of 10 μM and 100 μM TMZ while significant decrease in viral titers were observed post treatment with 1000 μM TMZ in LNZ308 at a viral dose of 0.05 MOI. Minimal differences in viral infectivity were noticed upon TMZ treatment at a lower viral dose of 0.01 MOI. Two-way ANOVA with Tukey's multiple comparison test; *, $p < 0.05$; **, $p < 0.01$. Data expressed as Mean ± SEM, n = 3.

Supplemental Figure S3: Induction of autophagy upon MeV infection. a, Immunoblot analyses reveals conversion of LC3-I to LC3-II (upper panel) upon MeV infection (VT) in contrast to radiotherapy (RT); β-tubulin served as loading control (lower panel). **b,** VT initiates autophagic flux as demonstrated by an increased GFP-tagged LC3 expression in LNZ308 and primary GBM T1094/17 cells while basal expression seen in Blank untreated cells and upon radiotherapy (RT).

Supplemental Figure S4: Virotherapy followed by irradiation (VT-RT) is more efficient than other regimen in LN229. Cell viabilities assessed post sequential treatments with MeV and VT in **a,** VT-RT showing maximal synergistic potency in comparison to **b,** Simultaneous treatment (Sim-Trt) of MeV with RT or **c,** RT followed by VT, which shows less cytotoxic efficacy. Black, grey and white bars indicate viral doses in indicated multiplicity of infection (MOI) of 0, 0.01 or 0.1. Data expressed as Mean ± SEM, n = 6.

Supplemental Figure S5: Montherapies using RT, MeV or TMZ. LN229 and LNZ308 cells were treated with monotherapies of **a,** γ-irradiation (RT), **b,** MeV virotherapy (VT) or **c,** TMZ chemotherapy (CT). The cell viability post monotherapies were used to calculate predicted values of combinatorial treatment using Chou-Talalay fractional product method. Data expressed as Mean ± SEM, n = 9.

Supplemental Figure S6: Determination of EC_{50} value of TMZ for GS8 cells. Depicted is the survival of GS8 cells incubated with different concentrations of TMZ. The EC_{50} for GS8 was identified to be 250 µM, employing appropriate solvent (DMSO) controls. TMZ and DMSO are depicted by blue and red dose-response curves respectively. Data expressed as Mean ± SEM, n = 9.

Supplemental Figure S7: Cytotoxic survival assay in GS8 using TMZ as alkylating agent in combination with VT or RT. a, Monotherapies with TMZ (CT), γ-irradiation (RT), or MeV (VT). **b - d,** Black bars indicate observed values and white bars indicate calculated predicted values in the corresponding permutated regimen. Regimen marked with "0" in a certain position of the sequence indicate absence of this respective treatment modality, while CT = 25 µM, VT = 0.05 MOI, RT = 2 Gy were employed at the pre-determined sequence of that respective regimen. For example, in CT-0-RT, no virus was used, while TMZ concentration in CT was 25 µM, and cells were irradiated for RT with a dose of 2 Gy. Similarly, the sequence of treatments is indicated in each regimen, e.g. VT-x-x indicates a regimen initiated with VT, while x-VT-x indicates that virotherapy came second 12 h after the first treatment in this respective protocol. Thereby, we can visualize all observed monotherapies, dual therapies, and triple therapies with '+' indicating synergy when comparing observed vs. predicted cytotoxicity. **b,** CT-VT-RT is the only regimen to elicit synergy as a triple regimen, while **d,** the sequence VT-RT-CT revealed synergy in dual therapies, but not as a triple regimen. Data expressed as Mean ± SEM, n = 9.

Supplemental Figure S8: Determination of EC_{50} value of lomustine (CCNU) for LN229 and LNZ308 cells. Depicted is the survival of LN229 or LNZ308 cells incubated with different concentrations of CCNU. Cell viability of LN229 or LNZ308 cells after incubation with different concentrations of CCNU or DMSO (control). The EC_{50} for LN229 and LNZ308 were identified to be

16 µM and 45 µM, respectively. Blue line indicates cell viability post CCNU, while red line depicts viability under DMSO control treatment. Data expressed as Mean ± SEM, n = 6.

Supplemental Figure S9: Monotherapies using lomustine (CCNU) as alkylating agent. LN229 and LNZ308 cells were treated with monotherapies of **a,** γ-irradiation (RT), **b,** MeV virotherapy (VT), and **c,** CCNU chemotherapy (CT). The cell viability post monotherapies were used to calculate predicted values of combinatorial treatment using fractional product method. Data expressed as Mean ± SEM, n = 9.

Supplemental Figure S10: Anti-glioma effect of CT-VT-RT using CCNU as chemotherapeutic agent. CT-VT-RT is synergistic in **a,** LN229 and **b,** LNZ308 cells. VT-CT-RT showed poor combinatorial efficacy in **c,** LN229 and **d,** LNZ308 cells. Similarly, regimen VT-RT-CT exhibited poor combinatorial efficacy in **e,** LN229 and **f,** in LNZ308. Data expressed as Mean ± SEM, n = 9.

Supplemental Figure S11: Anti-glioma activity of CT-VT-RT in TMZ-resistant cells (R-LN229) using CCNU as alkylating agent. Black, grey, and white bars indicate cell viability post CT-VT-RT treatment in parental LN229 cells. Orange and brown bars indicate cell viability post CT-VT-RT treatment in temozolomide-resistant R-LN229 cells. Addition of CCNU rescued the resistant effect (red lines) in synergistic regimen as opposed to aggressive proliferative effect (blue lines) in the absence of CCNU (0 µM or DMSO) with increasing doses of γ-irradiation. Abbreviations: Prd, predicted. Data expressed as Mean ± SEM, n = 9.

Supplemental Figure S12: Schematic representation of treatments and time points of samples considered for RNASeq. LNZ308 cells were treated with monotherapies (RT - 2 Gy, CT - 130 µM TMZ, VT - 0.05 MOI), double regimen (CT-VT, 130 µM TMZ - 0.05 MOI) and synergistic triple regimen (CT-VT-RT, 130 µM TMZ - 0.05 MOI - 2 Gy) along with CTL (DMSO) as control for CT initiated regimens and CTL (R/V) serving as control for RT and VT regimens. Blank (0 hpt) was used as basal control for expression. RNA and protein lysates were harvested at 0 hpt, 36 hpt, 72 hpt, and 96 hpt post respective treatments. Only time points marked in green were further processed for RNA sequencing (0 hpt, 36 hpt, and 96 hpt) as indicated with double-headed arrows. Samples at time-point

72 hpt (in blue) were collected, but solely used for validation of transcriptome data. “3X” indicate that all treatments were carried out in biological triplicates.

Supplemental Figure S13: Delayed STAT1 signaling augmenting efficient viral proliferation in CT-VT-RT. Immunoblot analyses reveal viral proliferation as observed with MeV nucleocapsid (N) expression (upper panel) in all VT-containing regimens and similar expression in CT-VT-RT at 72 hpt despite viral infection 12 h later than VT alone; while β-tubulin served as loading control (lower panel).

Supplemental Figure S14: Expression profile of TGFBI analysed in TCGA dataset. TGFBI was significantly overexpressed in glioblastoma patients (GBM) in comparison to patients with low grade gliomas (LGG). The TGFBI expression represented in terms of log2 (FPKM). Abbreviations: FPKM, fragments per kilobase of transcript per Million mapped reads. p-value: 6.04×10^{-73}.

Supplemental Tables

Supplemental Table ST1: Table depicting synergy observed with CT-VT-RT regimen in LN229 including 2 Gy radiation. Synergy is depicted with "+", antognism with "-" and additive effect as "0". (Abbreviations: CT, chemotherapy; GFP, green fluorescent protein; MeV, measles virus; MOI, multiplicity of infection; RT, radiotherapy; TMZ, temozolomide; VT, virotherapy)

CT-VT-RT LN229		VT (MeV-GFP)								
RT 2 Gy		0 MOI			0.01 MOI			0.05 MOI		
		Mean Observed	Mean Predicted	Synergy /Anta-gonism	Mean Observed	Mean Predicted	Synergy /Anta-gonism	Mean Observed	Mean Predicted	Synergy /Anta-gonism
CT (TMZ)	0 µM	76.13	75.08	0	52.85	69.04	+	35.49	47.86	+
	9 µM	48.44	49.68	+	34.72	45.30	+	19.62	31.69	+
	45 µM	13.47	13.36	0	11.81	12.35	+	6.97	8.56	+
	90 µM	12.40	10.81	0/-	10.93	9.91	0/-	7.45	6.83	0

Supplemental Table ST2: Table detailing calculation with low synergy potential observed in LN229 treated including VT-CT-RT at 2 Gy radiation. Synergy is depicted with "+", antognism with "-" and additive effect as "0". (Abbreviations: CT, chemotherapy; GFP, green fluorescent protein; MeV, measles virus; MOI, multiplicity of infection; RT, radiotherapy; TMZ, temozolomide; VT, virotherapy)

VT-CT-RT LN229		**VT (MeV-GFP)**								
RT 2 Gy		**0 MOI**			**0.01 MOI**			**0.05 MOI**		
		Mean Observed	**Mean Predicted**	**Synergy /Anta-gonism**	**Mean Observed**	**Mean Predicted**	**Synergy /Anta-gonism**	**Mean Observed**	**Mean Predicted**	**Synergy /Anta-gonism**
CT (TMZ)	**0 µM**	84.74	75.08	-	62.81	69.04	+	35.98	47.86	+
	9 µM	62.57	49.68	-	48.95	45.30	-	35.37	31.69	-
	45 µM	25.78	13.36	-	22.65	12.35	-	15.24	8.56	-
	90 µM	22.49	10.81	-	21.52	9.91	-	15	6.83	-

Supplemental Table ST3: Table detailing calculation with low synergy potential observed in LN229 treated with VT-RT-CT including 2 Gy radiation. Synergy is depicted with "+", antognism with "-" and additive effect as "0". **(**Abbreviations: CT, chemotherapy; GFP, green fluorescent protein; MeV, measles virus; MOI, multiplicity of infection; RT, radiotherapy; TMZ, temozolomide; VT, virotherapy.)

VT-RT-CT LN229		**VT (MeV-GFP)**								
RT 2 Gy		**0 MOI**			**0.01 MOI**			**0.05 MOI**		
		Mean Observed	**Mean Predicted**	**Synergy /Anta-gonism**	**Mean Observed**	**Mean Predicted**	**Synergy /Anta-gonism**	**Mean Observed**	**Mean Predicted**	**Synergy /Anta-gonism/ Anta-gonism**
CT (TMZ)	**0 µM**	64.60	75.08	+	48.32	69.04	+	36.59	47.86	+
	9 µM	55.60	49.68	-	38.63	45.30	+	34.33	31.69	-
	45 µM	25.68	13.36	-	22.29	12.35	-	14.12	8.56	-
	90 µM	24.84	10.81	-	24.25	9.91	-	16.15	6.83	-

Table ST4: Table depicting synergy observed with CT-VT-RT regimen in LNZ308 treated including 2 Gy radiation. Synergy is depicted with "+", antognism with "-" and additive effect as "0". (Abbreviations: CT, chemotherapy; GFP, green fluorescent protein; MeV, measles virus; MOI, multiplicity of infection; RT, radiotherapy; TMZ, temozolomide; VT, virotherapy.)

CT-VT-RT LNZ308		VT (MeV-GFP)								
RT 2 Gy		0 MOI			0.01 MOI			0.05 MOI		
		Mean Observed	Mean Predicted	Synergy /Anta-gonism	Mean Observed	Mean Predicted	Synergy /Anta-gonism	Mean Observed	Mean Predicted	Synergy/Anta-gonism
CT (TMZ)	0 µM	101.50	78.97	-	79.47	69.28	-	30.52	34.33	+
	130 µM	68.52	46.52	-	53.48	40.64	-	11.79	20.34	+
	650 µM	60.09	44.52	-	35.92	39.36	+	9.64	19.15	+
	1300 µM	48.66	37.30	-	33.33	32.60	0	9.78	16.18	+

ST5: Table detailing calculation with low synergy potential observed in LNZ308 treated with VT-CT-RT including 2 Gy radiation. Synergy is depicted with "+", antognism with "-" and additive effect as "0". (Abbreviations: CT, chemotherapy; GFP, green fluorescent protein; MeV, measles virus; MOI, multiplicity of infection; RT, radiotherapy; TMZ, temozolomide; VT, virotherapy.)

VT-CT-RT LNZ308		VT (MeV-GFP)								
RT 2 Gy		0 MOI			0.01 MOI			0.05 MOI		
		Mean Observed	Mean Predicted	Synergy /Anta-gonism	Mean Observed	Mean Predicted	Synergy /Anta-gonism	Mean Observed	Mean Predicted	Synergy/Anta-gonism
CT (TMZ)	0 µM	92.86	78.97	-	71.99	69.28	-	31.30	34.33	+
	130 µM	67.82	46.52	-	56.57	40.64	-	25.70	20.34	-
	650 µM	48.96	44.52	-	45.10	39.36	-	25.38	19.15	-
	1300 µM	41.80	37.30	-	37.16	32.60	-	32.19	16.18	-

Table ST6: Table detailing calculation with low synergy observed in LNZ308 treated with VT-RT-CT including 2 Gy radiation. Synergy is depicted with "+", antognism with "-" and additive effect as "0". (Abbreviations: CT, chemotherapy; GFP, green fluorescent protein; MeV, measles virus; MOI, multiplicity of infection; RT, radiotherapy; TMZ, temozolomide; VT, virotherapy.)

VT-RT-CT LNZ308		VT (MeV-GFP)								
RT 2 Gy		0 MOI			0.01 MOI			0.05 MOI		
		Mean Observed	Mean Predicted	Synergy /Anta-gonism	Mean Observed	Mean Predicted	Synergy /Anta-gonism	Mean Observed	Mean Predicted	Synergy/Anta-gonism
CT (TMZ)	0 µM	93.95	78.97	-	83.29	69.28	-	30.60	34.33	-
	130 µM	70.42	46.52	-	56.76	40.64	-	26.01	20.34	-
	650 µM	60.38	44.52	-	41.23	39.36	-	23.52	19.15	-
	1300 µM	42.22	37.30	-	40.11	32.60	-	23.62	16.18	-

Supplemental Table ST7

Gene	Forward Primer (5'→ 3')	Reverse Primer (5'→ 3')
ARF1	GACCACGATCCTCTACAAGC	TCCCACACAGTGAAGCTGATG
DDX58	AGACAAAGATGAAGAGAGCAGGA	GCTCGGACATTGCTGAAGAAG
HLA-A	GAGTATTGGGACCAGGAGACA	CGTCGCAGCCATACATTATCTG
HLA-B	TGAGATGGGAGCCGTCTTC	CTACACATCACAGCAGCGAC
IFIH1	CGGATATAAAGAATGTAACATTGTTATC	ATGAGCATACTCCTCTGGTTTCA
IFIT1	CCTCCTTGGGTTCGTCTACA	GGCTGATATCTGGGTGCCTA
IFN-β	GTCTCCTCCAAATTGCTCTCC	CAGTATTCAAGCCTCCCATTCA
ISG15	ATGGGCTGGGACCTGACG	GCCGATCTTCTGGGTGATC
MX1	CGCTGGTGCTGAAACTGAAGA	GCGATGGCATTCTGGGCTTTA
MX2	AGTTCAGAATGGAGCAGATGG	ACCGAAGACTCATTACTGGGAA
OAS1	CACAGAACTACAGAGAGACTTC	CAAGCATAGACCGTCAGGAG
OAS2	GACTTCTCCCAACCTGGATAATG	CTGTCAATCTGCTCTAGGAAGC
STAT1	CAGAACAGAGAACACGAGACCA	GTTCAGTGACATTCAGCAACTCTA
TAP1	AAAGACACTCAACCAGAAGGAG	GCCCACCAATGTAGAGGATTC