Supplementary Information

Bioengineering fluorescent virus-like particle/RNAi nanocomplexes act synergistically with temozolomide to eradicate brain tumors

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Experimental methods

Vectors for Qβ-coat protein (QβCP) and fluorescent protein co-expression:

DNA sequence of fluorescent protein (GFP or mCherry) and QβCP were cloned into pCDF-Duet-1 vector to form protein expression pCDF-Duet-GFP-QβCP or pCDF-Duet-mCherry-QβCP for coexpression. Vectors were transformed to *E. coli* cell line BL21 competent cells for protein coexpression to generate green-fluorescence gVLP (GFP) or red-fluorescence mVLP (mCherry protein).

RNAi scaffold construction:

The sequence of human miR-30B pre-miRNA, fused to the QβCP binding hairpin, was used as a scaffold for 22-mer sequences from targeting genes c-MET (sequences are shown in Fig. S1) with stem loops. Scaffolds were constructed containing specific RNAi sequences and their complements. Template DNAs, including the T7 promoter (T7p) and T7 terminator (T7t), were constructed by R-PCR. The DNA sequences encoding T7p-RNAi scaffold-T7t ($RNAi_{C\text{-}MET}$) were cloned into pET28-b(+) (Novagen) to generate QβCP binding RNAi scaffold expression vectors.

Production and purification of fluorescent VLP/RNAi nanocomplexes:

E. coli BL21 cells harboring the appropriate plasmids were grown in either lysogeny broth (LB) or NZY solution supplemented with antibiotic (kanamycin or streptomycin) at 50 μg/mL, respectively. Starter culture was grown for 18 h at 37°C and used to inoculate 1L of expression culture. One nanomolar IPTG was performed as a protein expression reagent at an OD_{600} of 0.8–1.0 in culture solution (LB; BD, LOT: 244620, France) overnight at 37°C. The overnight culture was harvested by centrifugation at 6,500 g and resuspended in 20 mL of phosphate-buffered saline (PBS; pH = 7.4) and then lysed by sonication. The lysate was centrifuged for 30 min at 23,000 g, followed by precipitation with ammonium sulfate to obtain crude VLP-based samples (VLP_{WT}, gVLP, mVLP, gVLP/$RNAi_{c\text{-}MET}$, mVLP/$RNAi_{c\text{-}MET}$, and gVLP/$RNAi_{Luc}$). Crude VLP-based samples were resuspended in PBS buffer and followed by 20% w:v PEG8000/2M NaCl precipitation to obtain pure virus-like particle (VLP). VLP were resuspended in 1 mL of PBS buffer and extracted with 1:1 n-butanol:chloroform. The VLP-based samples from the aqueous layer were purified by step sucrose gradient ultracentrifugation and then precipitated with 20% w:v PEG8000/2M NaCl solution and resuspended in 25 mL of PBS buffer, followed by exhaustive dialysis (SnakeSkin® Dialysis Tubing, 10,000 MWCO. Thermo, LOT: QD213952, USA) against PBS buffer (pH =7.4) for 48 h. The obtained pure VLP-based samples were concentrated using protein concentrate filter tubes (Amicon Ultra-15 Centrifugal Filter Units. 100,000 MWCO. Merck Millipore, LOT: R6EA45140, Ireland). The final concentration of VLPs was assessed using a Pierce BCA Protein Assay kit (Thermo, LOT: PD202250, USA).

Conjugation of CPP and ApoEP to gVLP/RNAi (or mVLP/RNAi):

Functional peptides (Cys-CPP and K16-ApoEP) were conjugated on the surface of gVLP/RNAi (or mVLP/RNAi) to enhance cell uptake and BBB penetration. The Cys-CPP peptides (KYGRRRQRRKKRG-cys-SH) were conjugated on the gVLP/RNAi (or mVLP/RNAi) by sulfosuccinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1carboxylate (sulfo-

SMCC; Sigma-Aldrich, St. Louis, MO, USA) as a crosslinker. Briefly, 5 μl of sulfo-SMCC solution (10 mg/mL in DI-H_2O) was mixed with 2 μM of gVLP/RNAi (or mVLP/RNAi) in 600 μL of PBS (pH 7.4) for 30 min at 25°C in the dark, and then purified using a filter column (Amicon Ultra-15 Centrifugal Filter Units. 100,000 MWCO. Merck Millipore, LOT: R6EA45140, Ireland) with PBS buffer. Subsequently, the maleimide-terminated gVLP/RNAi (or mVLP/RNAi) were reacted with 30 μL of Cys-CPP solution (0.3 mg/mL) at 25°C for 2 h in the dark, and then purified again using the abovementioned procedure to obtain TAT@gVLP/RNAi (or TAT@mVLP/RNAi). For further ApoEP modification, 2 mM TAT@gVLP/RNAi (or TAT@mVLP/RNAi) in 500 μL of MES buffer (pH=5.5) was reacted with a mixture of 9.8 mg of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC; Sigma-Aldrich, St. Louis, MO, USA) and 10.4 mg of *N*-hydroxysulfosuccinimide (sulfo-NHS; Sigma-Aldrich, St. Louis, MO, USA) at 10°C for 1.5 h in the dark to activate the carboxylic groups. After being washed with MES buffer three times, excess ApoE-peptides were added to the activated TAT@gVLP/RNAi (or TAT@mVLP/RNAi) at room temperature for another 4 h and then washed with PBS buffer (pH = 7.4) to obtain dP@gVLP/RNAi (or dP@mVLP/RNAi).

To confirm the successful conjugation of peptides on gVLP/RNAi (or mVLP/RNAi), the dP@gVLP/RNAi (or dP@mVLP/RNAi) were mixed with 2-Mercaptoethanol (Sigma-Aldrich, St. Louis, MO, USA) and incubated at 95°C for disulfide-bond breaking and protein denaturing. The denatured samples were analyzed by SDS-PAGE (12%) electrophoresis then stained using Coomassie Brilliant Blue R-250 Dye (Sigma-Aldrich, St. Louis, MO, USA).

RNAi scaffold extraction from gVLP/RNAi$_{c\text{-}MET}$ and dP@gVLP/RNAi$_{c\text{-}MET}$:

Total RNA from VLP-based samples containing RNAi scaffold or without were extracted using the following protocol: 2 mg of VLP-based samples were mixed with 100 mM 2-BME and 62.5 μL of 20% SDS each and adjusted with PBS (pH = 7.4) to a total

volume of 250 µL. This was left to stand at room temperature for 15 min and then added to 250 µL of acid phenol-chloroform (pH 5.5) and centrifuged at 14,000 rpm for 15 min. The supernatant was discarded and then 25 µL of 5 M ammonium acetate and 675 µL of ethanol were added. The RNA pellet was resuspended in DEPC water and quantified using a Nano-Drop spectrometer (ThermoFisher Scientific, USA).

In vitro transcription of RNAi$_{c\text{-}MET}$:

DNA containing T7$_p$–RNAi$_{c\text{-}MET}$-T7$_t$ was amplified by PCR with primers RNAi-Fwd and RNAi–Rev and used as a template for in vitro transcription. Approximately 1 µg of DNA was transcribed for 4 h at 37℃ with the MEGAscript High Yield Transcription Kit (Applied Biosystems) followed by incubation with TURBO DNase for 15 min at 37℃. RNA was pelleted by ammonium acetate precipitation, washed with 80% ethanol and lyophilized. Pellets were resuspended in nuclease-free water and purified on illustra™ NAP-5™ columns.

SDS-PAGE electrophoresis:

The SDS-PAGE gel 12% was used for protein separation and characterization. The 2-BME was added into samples and incubated at 95℃ for 10 min for disulfide-bond breaking and protein denaturing. The treated proteins were put on ice for 5 min and 20 µL (3 µg of protein) was loaded in each well. A voltage of 80 V for 15 min was used for stacking gel and 100V for 70 min for separating gel. Gels were stained by Coomassie Brilliant Blue R-250 Dye.

Urea gel electrophoresis:

RNAs extracted from VLP-based samples were adjusted to a concentration of 1 µg/µL as stock solution. To prepare a gel running sample, 2 µl of stock RNA solution (*in vitro* transcribed RNAi scaffold or RNAs extracted from gVLP, gVLP/RNAi$_{c\text{-}MET}$, dP@gVLP/RNAi$_{c\text{-}MET}$) was added into 38 µL of RNA loading dye (#B0363A, NEB, USA).

The RNAs were incubated at 90℃ for 10 min for denaturing and put on ice for 5 min to cool. Subsequently, they were separated in 8% urea denaturing gel (0.5 μg /well) at 100V for 80 min and stained with SYBR green II RNA Gel stain (CAT: 50523, Lonza, USA).

Transmission electron microscopy (TEM):

The diameter and morphology were analyzed using TEM (75 keV accelerating voltage). VLP-based samples were prepared by pipetting 5 μL onto Formvar-coated copper mesh grids (200 mesh, Ted Pella, Redding, CA, USA) for 5 min, followed by exposure to 8 μL of a solution of uranyl acetate (15 mg/mL in DI-H_2O) for 2 min as a negative stain. Excess stain was then removed, and the grids were left to dry in air overnight.

Cellular uptake studies:

GBM U87 cells were seeded into a 12-well plate (approximately 10^6 cells/well) and incubated for 24 h in Dulbecco's Modified Eagle's Medium (DMEM) with 2.2 mg/mL of sodium carbonate, 10% FBS, 50 μg/mL of penicillin, and 50 μg/mL of streptomycin at 37°C. All cells were grown and maintained at 37°C in 5% CO_2. The gVLP/$RNAi_{c\text{-}MET}$ or dP@gVLP/$RNAi_{c\text{-}MET}$ were added to the U87 cells to a final concentration of 500 nM. Treated cells were incubated for 0, 2, 6, 12, or 24 h, and stained with Hoechst 33342 (Sigma-Aldrich, St. Louis, MO, USA) for 10 min after being washed with PBS. Images were acquired on a Nikon® fluorescence microscope (Eclipse Ti-S Inverted Microscope System, Nikon®, Japan).

Furthermore, we investigated the cellular uptake efficiency of gVLP/$RNAi_{c\text{-}MET}$ or dP@gVLP/$RNAi_{c\text{-}MET}$ in GBM U87 cells. Cells were seeded into a 12-well plate (approximately 10^6 cells/well), incubated at 37°C for 24 h in DMEM, and then incubated with gVLP/$RNAi_{c\text{-}MET}$ or dP@gVLP/$RNAi_{c\text{-}MET}$ at a final concentration of 0.5 μM for 2, 4, 6, 12, and 24 h. After being washed three times with PBS, cells were detached, pelleted, and

resuspended in PBS. The flow data calculated the number and intensity of cells with GFP signals; 10,000 cells were collected in each data. Experiments were performed using Novocyte™ flow cytometry, and the data were analyzed using NovoExpress® (ACEA Biosciences, San Diego CA, USA).

In vitro toxicity of VLPs:

GBM U87 cells were cultured in DMEM supplemented with 2.2 mg/mL of sodium carbonate, 10% FBS, 50 μg/mL of penicillin, and 50 μg/mL of streptomycin. Approximately 5,000 cells per well were cultured in 96-well plates for 24 h. Various types of VLP-based samples (VLP_{WT}, $gVLP/RNAi_{c\text{-}MET}$, $dP@gVLP/RNAi_{c\text{-}MET}$) were added to GBM U87 cells with final concentrations of 0.2, 0.4, 0.6, 0.8, and 1 μM followed by incubation for 48 h. The culture medium was removed and the cells were incubated in 120 μL of XTT solution for 2 h. After that, 100 μL of XTT solution from each well was transferred to another 96-well counting plate. The survival of U87 cells at different time points was evaluated by OD at 490 nm.

GBM U87 cell luciferase knock down experiment:

Luciferase-expressing pGL4 luciferase Reporter Vector pGL4.51[luc2 CMV Neo] plasmid-bearing U87 cell line was seeded into a 96-well plate (approximately 5,000 cells/well) and allowed to grow for 24 h. The cells were treated with dP@gVLP or $dP@gVLP/RNAi_{c\text{-}MET}$ at a final concentration of 0.5 μM in DMEM. After 24 h of incubation, the supernatant was removed and fresh DMEM was added in each well for incubation for another 36 and 48 h. After incubation, the DMEM medium was removed and 10 μL of lysis buffer was added to each well. After cells were lysed, 50 μL of luciferase substrate (Luciferase Assay System, Promega, USA) was added and sent to an M2 ELISA Reader

(Molecular Device, USA) for luminescence measurement, as well as to a ChemiDoc™ XRS imaging system (BIO-RAD, USA) for imaging.

Western blot analysis of c-MET protein expression:

GBM U87 cells were seeded into a 12-well plate (approximately 10^6 cells/well) and incubated for 24 h in DMEM with 2.2 mg/mL of sodium carbonate, 10% FBS, 50 μg/mL of penicillin, and 50 μg/mL of streptomycin at 37°C in 5% CO_2. Subsequently, different samples [naked $siRNA_{c\text{-}MET}$ (100 nM), dP@gVLP (0.5 μM), dP@gVLP/$RNAi_{c\text{-}MET}$ (0.5 μM)] were added to the GBM U87 cells and incubated at 37°C for 48 h, respectively. For the delivery of naked $siRNAi_{c\text{-}MET}$, the naked $siRNAi_{c\text{-}MET}$ was transfected into GBM U87 cells using $T20^{TM}_{293T}$ transfection reagent (ALISA BIOSCIENCE LLC). The cells were lysed using a PRO-PREP™ protein extraction kit (iNtRON biotechnology, CAT:17081.1, USA). Thirty micrograms of protein lysates were separated by 10% SDS-PAGE and then transferred to 0.22-μm pore PVDF membranes. The membranes were blocked with 5% fat-free milk for 2 h at room temperature, followed by incubation with primary antibodies against target proteins (c-MET antibody [C3], C-term, 1:500, Gene Tex, CAT: GTX100637)) for 1 h at room temperature. An anti-GAPDH antibody (clone2D9. 1:5000, ORIGENE, CAT:TA802519) was used as a control. After incubation, the membranes were washed with TBST buffer four times and incubated with HRP-conjugated secondary antibody (goat anti-Mouse IgG (H+L)-HRP, 1:10,000, LeadGENE, CAT: 20101/20102. Or goat anti-Rabbit IgG (H+L)-HRP. 1:10,000, LeadGENE, CAT: 20201/20202) for 40 min at room temperature. Signals were visualized using a ChemiDocTM XRS imaging system and quantified using ImageJ.

Scratch assay:

GBM U87 cells were seeded into a 12-well plate (approximately 10^6 cells/well) and incubated for 24 h in DMEM with 2.2 mg/mL of sodium carbonate, 10% FBS, 50 μg/mL of penicillin, and 50 μg/mL of streptomycin. Subsequently, 0.5 μM dP@gVLP or $siRNA_{c\text{-}MET}$ or dP@gVLP/$RNAi_{c\text{-}MET}$ was added and the cells were grown for another 48 h. Cells were scratched at four random positions using a 200 μL pipette tip to generate a wound and washed in PBS buffer three times. Next, 1 mL of fresh DMEM was added to the cells for a further 24 h of incubation before pictures were taken to analyze the recovery. Quantitation was performed using ImageJ and all data were normalized to blank as 100%.

Reversion of temozolomide (TMZ) resistance by dP@gVLP/$RNAi_{c\text{-}MET}$*:*

GBM U87 cells were cultured in DMEM supplemented with 2.2 mg/mL of sodium carbonate, 10% FBS, 50 μg/mL of penicillin, and 50 μg/mL of streptomycin. Approximately 5,000 cells per well were cultured in 96-well plates for 24 h. Different samples [naked $siRNA_{c\text{-}MET}$ (100 nM) or dP@gVLP (0.5 μM) or dP@gVLP/$RNAi_{c\text{-}MET}$ (0.5 μM)] were added to the GBM U87 cells with various concentrations of TMZ together and incubated at 37°C. After 24, 48, and 72 h of incubation, the culture medium was removed, and the cells were incubated in 120 μL of XTT solution for 2 h. Afterwards, 100 μL of XTT solution from each well was transferred to another 96-well counting plate. The survival of GBM U87 cells at different time points was evaluated by OD at 490 nm.

For the stability studies of naked $siRNA_{c\text{-}MET}$ and packaged $RNAi_{c\text{-}MET}$, 100 nM naked $siRNA_{c\text{-}MET}$ or 0.5 μM dP@gVLP/$RNAi_{c\text{-}MET}$ was incubated with GBM U87 cells first for 36 h followed by TMZ treatment for another 24 h. For the delivery of naked $siRNAi_{c\text{-}MET}$, the naked $siRNAi_{c\text{-}MET}$ was transfected to GBM U87 cells using T20 transfection reagent. Subsequently, the culture medium was removed, and the cells were incubated in 120 μL of XTT solution for 2 h. Next, 100 μL of XTT solution from each well was transferred to

another 96-well counting plate. The survival of U87 cells at different time points was evaluated by OD at 490 nm.

In-vivo BBB penetration studies of dP@mVLP/RNAi$_{c\text{-}MET}$ using tyrosine hydroxylase (TH) immunohistochemistry:

The distribution of mVLP/RNAi$_{c\text{-}MET}$ or dP@mVLP/RNAi$_{c\text{-}MET}$ in mice brains was confirmed by mCherry immunohistochemical staining. All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Chang Gung University (Taoyuan, Taiwan), and approved by the Institutional Animal Care and Use Committee (approval number: CGU15-075) of Chang Gung University (Taoyuan, Taiwan). Mice were raised in a room with a thermostat at 26°C. The Nu/Nu mice weighing approximately 25–30 g (5–6 weeks old) were tested to confirm the efficacy of the proposed approach. Intracranial brain tumors were induced by transplantation of luciferase-stable U87 cells in mice brains. Briefly, cultured luciferase-stable U87 cells (5×10^5 cells/mouse) were injected over a 2-min period into brain tissue using a syringe, and needle withdrawal was performed over a period of 30 sec. The mice received 5 μM mVLP/RNAi$_{c\text{-}MET}$ or dP@mVLP/RNAi$_{c\text{-}MET}$ administration through tail-vein injection after 7 days of GBM U87 cell transplantation. After 16 h of injection, the brains of the nude mice were removed and post-fixed in 10% neutral buffered formalin for 24 h. Fixed brains were then embedded in paraffin. Serial coronal sections of brain (10-μm thick) were obtained and deparaffinized. Sections were incubated in sodium citrate buffer (10 mM sodium citrate, 0.05% Tween 20, pH 6.0) in boiling water for 30 min. After being rinsed with PBS, sections were transfer to 3% H_2O_2 to suppress endogenous peroxidase activity. Sections were then blocked with 10% goat neutral serum in PBST for 1 h and incubated overnight with rabbit polyclonal anti-mCherry antibody (ab183628, 1:100, Abcam). After being rinsed in PBS, the sections were incubated with secondary goat anti-rabbit IgG-HRP antibody for 1 h at room temperature. The activity

of HRP was visualized and counted by DAB substrate (Thermo Fisher Scientific) by optical microscope. The microscope images were read as gray level and integrated.

Blood biochemical analysis and immunoassay:

One group of mice ($n = 3$) were injected with 5 μM dP@mVLP/RNAi$_{c\text{-}MET}$ per mouse through the tail vein; another group of uninjected mice served as the control; 1-mL blood samples were collected by retro-orbital venous plexus puncture at 0 min (before injection) and 7 days after injection. Clotted blood samples were centrifuged at 7,000 rpm for 20 min to obtain serum for blood biochemistry analysis and immunoassay.

Biodistribution of dP@mVLP/RNAi$_{c\text{-}MET}$ through tail-vein injection:

Mice ($n = 3$) were injected with 5 μM dP@mVLP/RNAi$_{c\text{-}MET}$ per mouse through the tail vein, and then all mice were euthanized at 8 h postinjection. We collected tumors and major organs including hearts, livers, lungs, kidneys, normal brains, and brain tumors, for visualizing with an IVIS Spectrum Maestro In-Vivo Imaging System (XENOGEN IVIS 100) to quantify the accumulated amounts in major organs using a maximum excitation wavelength of 580 nm and a maximum emission wavelength of 630 nm.

In vivo antitumor efficiency of genetically regulated chemotherapy promotion:

All animal experiments were approved by the Animal Committee of Chang Gung University and adhered to the experimental animal care guidelines. Mice were raised in a room with a thermostat at 26°C. The Nu/Nu mice weighing approximately 25–30 g (5–6 weeks old) were tested to confirm the efficacy of the proposed approach. Intracranial brain tumors were induced by transplantation of luciferase-stable U87 cells in mice brains. Briefly, cultured luciferase-stable U87 cells (5×10^5 cells/mouse) were injected over a 2-min period into brain using a syringe, and needle-withdrawal was conducted over another period of 0.5 min. A total of 40 mice were used, and the experiments were divided into four groups. In

group 1 (n =10), the mice received no further treatment after transplantation of luciferase-stable U87 cells, and these mice served as the control. In group 2 (n =10), the mice received oral administration of 5 mg/kg of TMZ at 5 P.M. three times every 2 days after 4 days of luciferase-stable U87 cell transplantation. In group 3 (n = 10), the mice received an i.v. injection with 10 nmole/kg of mVLP/RNAi$_{c\text{-}MET}$ at 9 A.M. followed by oral administration with 5 mg/kg of TMZ at 5 P.M. in the same day after 4 days of luciferase-stable U87 cell transplantation, and the treatment was repeated three times every 2 days. In group 4 (n = 10), the mice received an i.v. injection with 10 nmole/kg of dP@mVLP/RNAi$_{c\text{-}MET}$ at 9 A.M. followed by oral administration of 5 mg/kg of TMZ at 5 P.M. in the same day after 4 days of luciferase-stable U87 cell transplantation, and the treatment was repeated three times every 2 days. The luciferase-stable U87 cell activity and region of interest (ROI) of the brain tumor in mice were detected with the IVIS Spectrum Maestro In-Vivo Imaging System to analyze tumor progression. The survival time was calculated from the day of luciferase-stable U87 cell inoculation (0 day) to the day of death. Kaplan–Meier survival curves were plotted for each group. The body weight of mice was monitored at determined time intervals.

Statistical analysis:

Data were expressed as mean ± SD on the basis of at least three independent experiments. Statistical analysis was performed using a Student's *t*-test. The differences were considered statistically significant at p values < 0.05 or p values < 0.005.

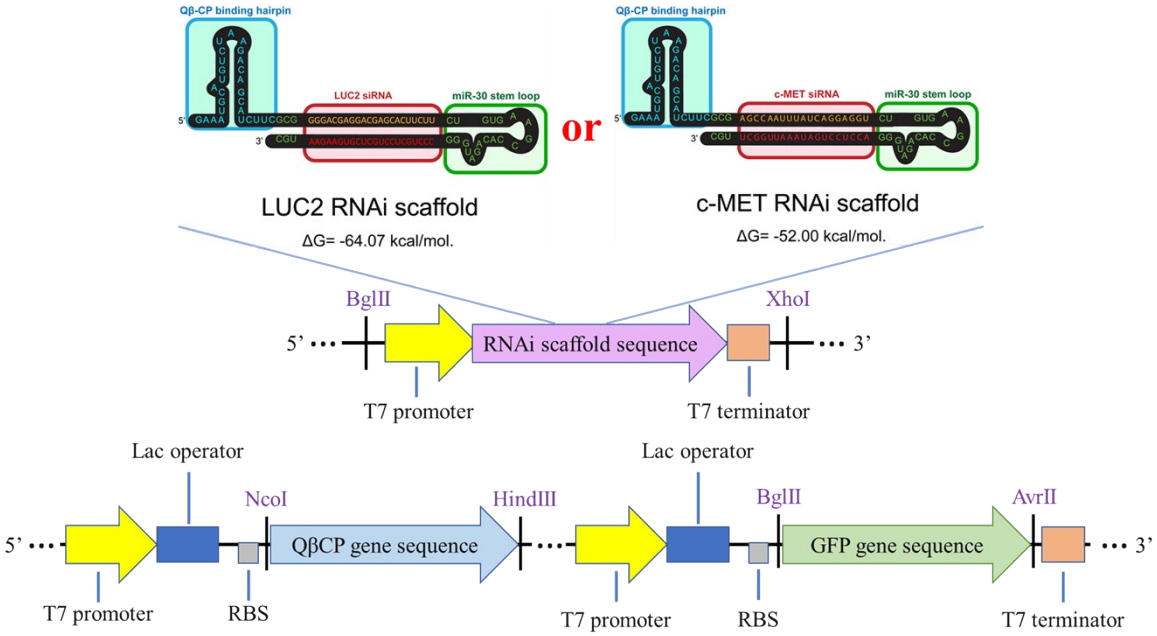


Fig. S1. Sequence maps of dual plasmid system. The Qβ RNAi scaffold folds in two hairpins and then coassembles with QβCP *in vivo*. A single RNA molecule forms the Qβ hairpin (blue) linked to a miR-30B stem loop (green), containing the functional RNA duplex (yellow/red). The functional RNA duplex encodes miRNA or siRNA. The scaffold incorporates any 22-base pair sequence, depending on the target of c-MET or luciferase mRNA.

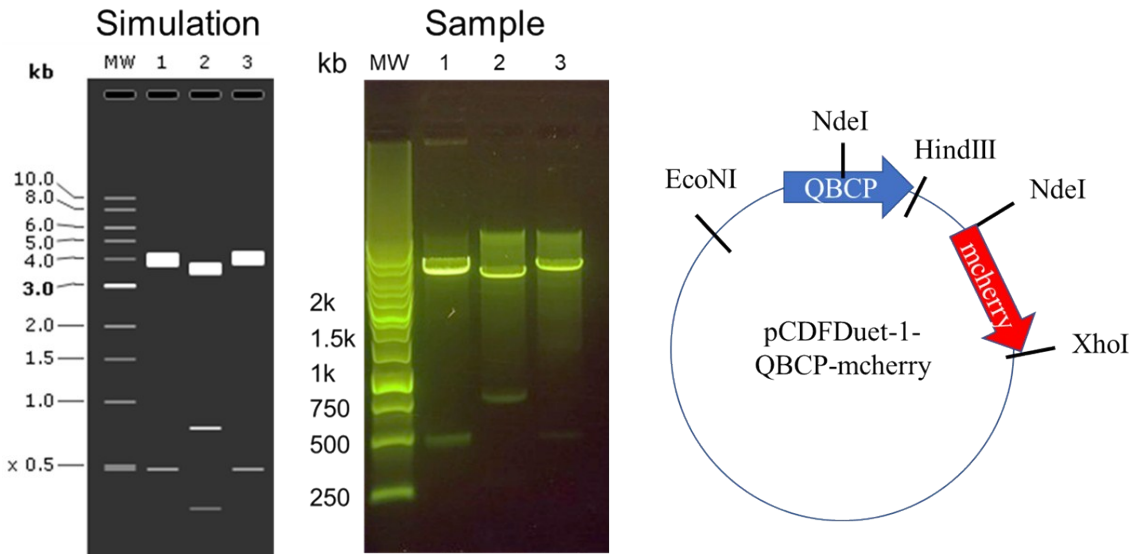


Fig. S2. Plasmid construction characterization through agarose gel analysis assay, both a software simulation and real sample analysis with 1% agarose gel. The map of pCDFDuet-1-QβCP-mCherry plasmid is shown on the right side.

- Lane 1: pCDFDuet1-QBCP-GFP restricted with EcoNI + HindIII demonstrates a large band (4210 bp), which indicates the linearized pCDFDuet1 plasmid and a 504 bp fragment as the QβCP (with T7 promoter) at the multiple cloning site I (MCSI).
- Lane 2: Constructed tVLP expressing pCDFDuet-1-QβCP-mCherry plasmid restricted with NdeI + XhoI, generating two fragments. A 3768 bp fragment indicates the linearized pCDFDuet-1 plasmid. A 781 bp fragment indicates the mCherry DNA sequence. A 291 bp fragment indicates the QβCP restricted in the middle to the start of mCherry sequence.
- Lane 3: Constructed tVLP expressing pCDFDuet-1-QβCP-mCherry plasmid restricted with EcoNI + HindIII as Lane 1 shows similar fragments, which indicates the successful construction of a plasmid with both QβCP and mCherry protein.

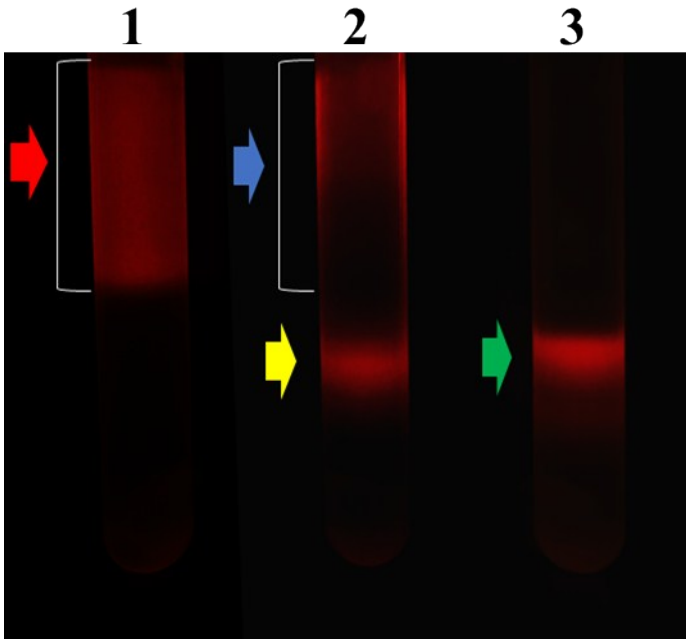


Fig. S3. Use of sucrose density gradient ultracentrifugation to concentrate and partially purify mVLP/RNAi$_{c\text{-}MET}$. 1: mCherry proteins (red arrow) produced via *E. coli* expression; 2: mVLP/RNAi$_{c\text{-}MET}$ purified using high ionic strength solution (PEG8000/2M NaCl); 3: pure mVLP/RNAi$_{c\text{-}MET}$ were purified again by dialysis with PBS (pH=7.4) using 100K MWCO protein concentration tube. Red arrow: pure mCherry proteins; blue arrow: absorbed mCherry proteins and impurities; yellow arrow: crude mVLP/RNAi$_{c\text{-}MET}$; green arrow: pure mVLP/RNAi$_{c\text{-}MET}$.

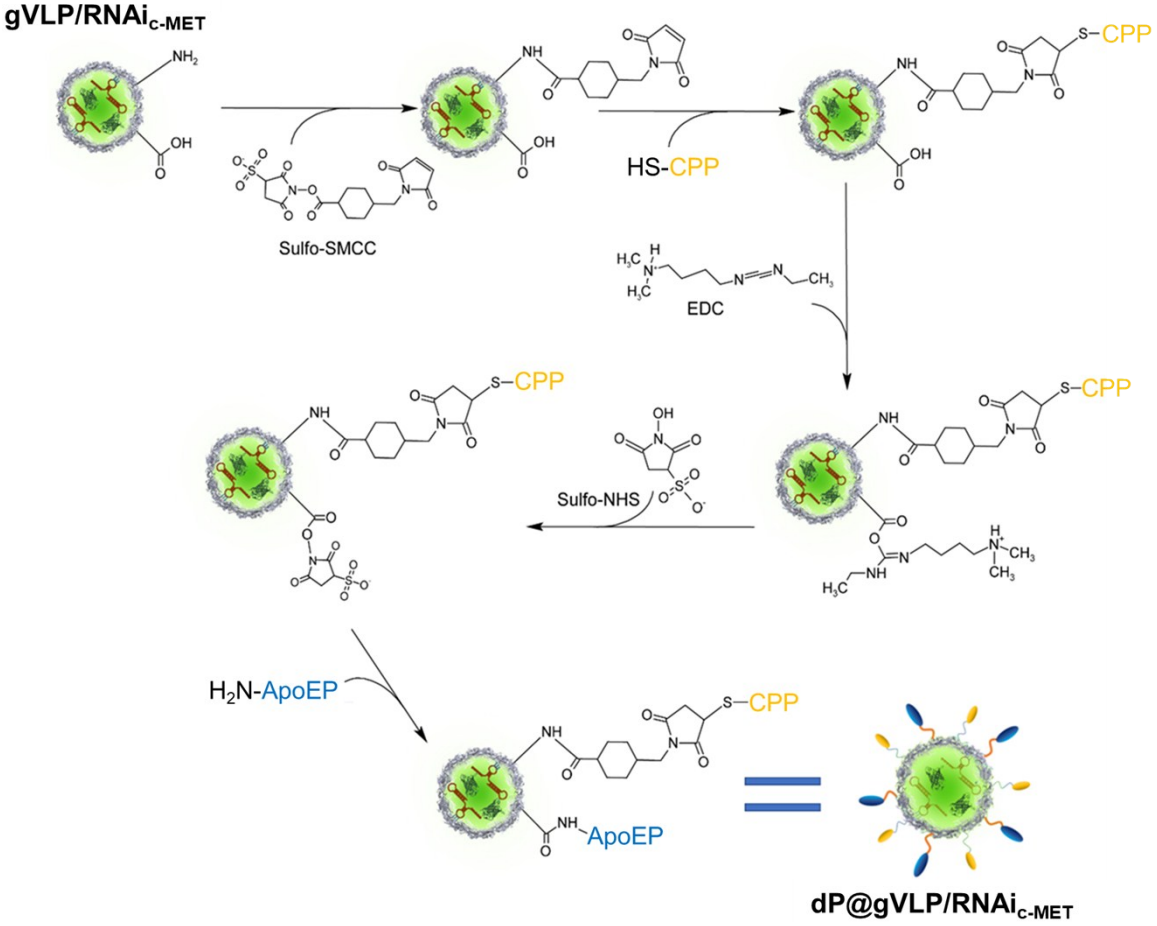
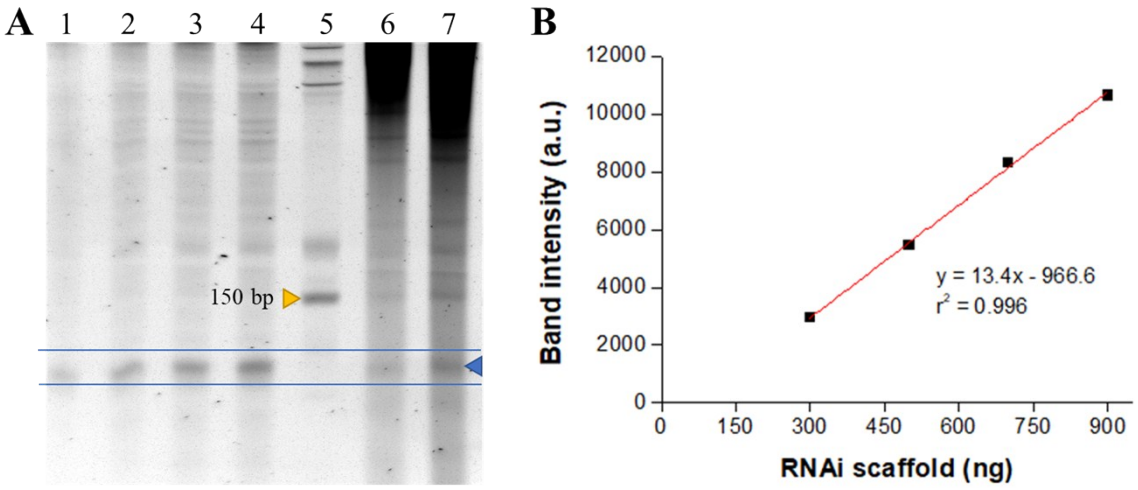


Fig. S4. Chemical structural formula of each component and preparation of the dual CPP and ApoEP modified gVLP/RNAi$_{c\text{-}MET}$ (dP@gVLP/RNAi$_{c\text{-}MET}$).



1: 300 ng *in vitro* transcribed RNAi scaffold

2: 500 ng *in vitro* transcribed RNAi scaffold

3: 700 ng *in vitro* transcribed RNAi scaffold

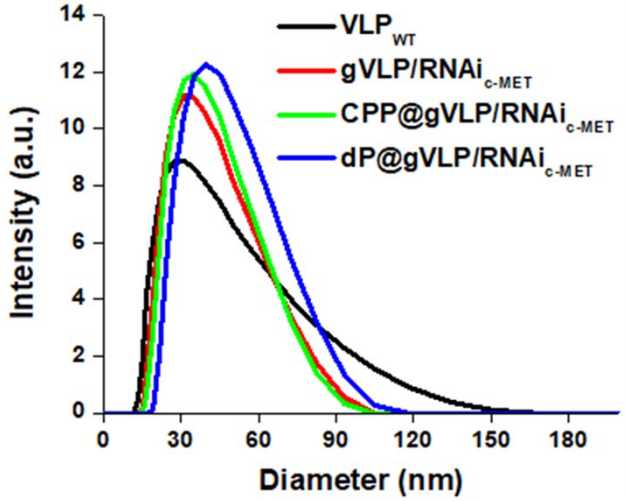
4: 900 ng *in vitro* transcribed RNAi scaffold

5: ssRNA ladder

6: 500 ng total RNAs extracted from gVLP/RNAi$_{c\text{-}MET}$

7: 1000 ng total RNAs extracted from gVLP/RNAi$_{c\text{-}MET}$

Fig. S5. (A) Urea denaturing PAGE analysis of RNAi scaffold prepared by *in vitro* transcription and RNAs extraction from gVLP/RNAi$_{c\text{-}MET}$. (B) Linear calibration curve between the band intensity against the amount of *in vitro* transcribed RNAi scaffold (300 ng-900 ng).



Sample type	Size average (nm)	S.D. (nm)
VLP_{WT}	30.4	± 0.2
$gVLP/RNAi_{c\text{-}MET}$	32.5	± 0.3
$CPP@gVLP/RNAi_{c\text{-}MET}$	39.5	± 0.5
$dP@gVLP/RNAi_{c\text{-}MET}$	42.7	± 0.9

Fig. S6. Characterization of VLP_{WT}, $gVLP/RNAi_{c\text{-}MET}$, $CPP@gVLP/RNAi_{c\text{-}MET}$, and $dP@gVLP/RNAi_{c\text{-}MET}$ by dynamic light scattering.

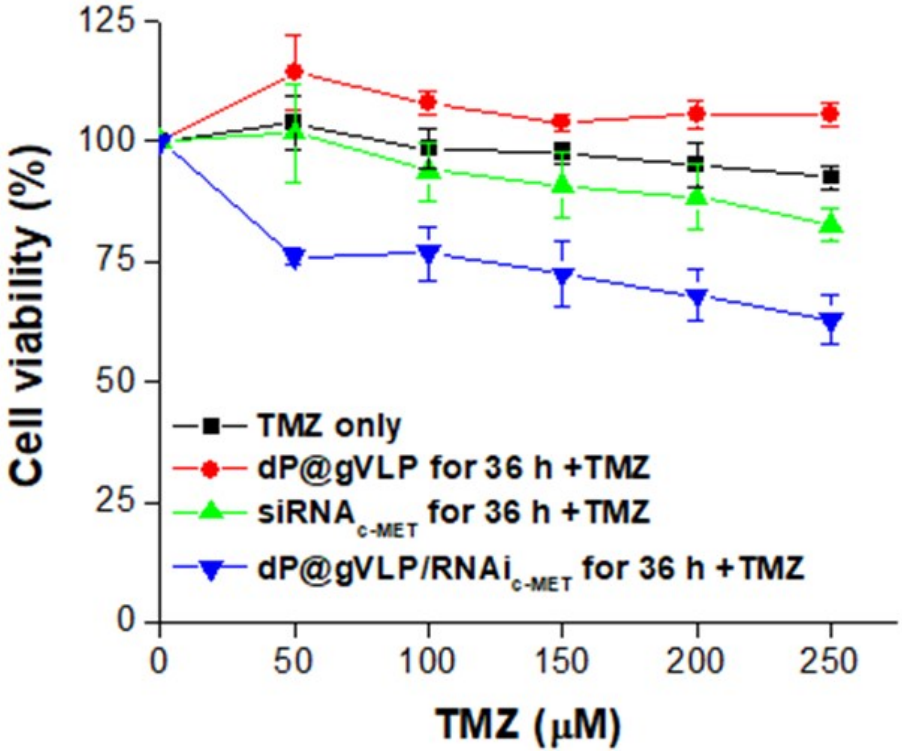


Fig. S7. c-Met knockdown inhibits GBM U87 cell proliferation *in vitro* for the packaged $RNAi_{c\text{-}MET}$ stability test. GBM U87 cells were pretreated with dP@gVLP (no $RNAi_{c\text{-}MET}$) or naked $siRNA_{c\text{-}MET}$ or dP@gVLP/$RNAi_{c\text{-}MET}$ for 36 h followed by treatment of 5 mg/kg of TMZ for a further 24 h. Cell viability was analyzed using an XTT assay kit to identify the stability of packaged $RNAi_{c\text{-}MET}$. Values are expressed as means ± SD (n = 8).

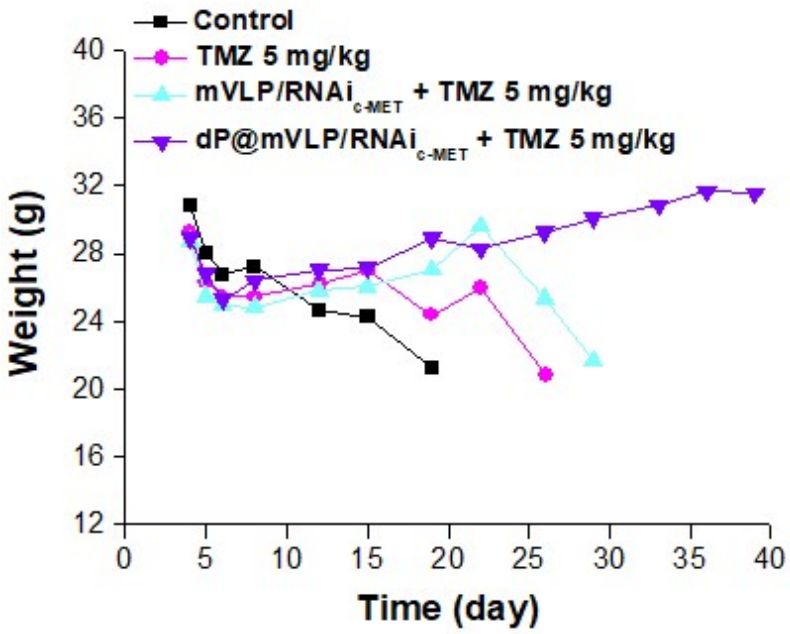


Fig. S8. Body weight of mice treated with phosphate-buffered saline (control), 5 mg/kg of free TMZ (oral), mVLP/RNAi$_{c\text{-}MET}$ (i.v.) + 5 mg/kg of TMZ (oral), and dP@mVLP/RNAi$_{c\text{-}MET}$ (i.v.) + 5 mg/kg of TMZ (oral).