Supplementary Data

Functional RNAs: Combined Assembly and Packaging in VLPs

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A. Primer and construct sequences

Table S1. Primers used to generate Qβ CP, GFP, and RNAi constructs. Restriction sites are underlined: CP-Fwd: NcoI; CP-Rev: AvrII; GFP-Fwd: BglII; GFP-Rev: BlpI; RNAi-Fwd: BglII; RNAi-Rev: XhoI.

Primer name	Primer sequence
CP-Fwd	5'-GTGGCCATGGCAAATTAGAGACTGTTACTT-3'
CP-Rev	5'-CACCCCTAGGTCAATACGCTGGGTTC-3'
GFP-Fwd	5'-GTGGTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAG ATATACCATGGCTAGCAAAGGAGAAGAACTCT-3'
GFP-Rev	5'-CACCGCTCAGCTCAGTTGTACAGTTCATCCATGCC-3'
RNAi-Fwd	5'-GTGGAGATCTTAATACGACTCACTATAGGG-*3'*
RNAi-Rev	5'-CACCCTCGAGCAAAAAACCC-3'

Table S2. RNAi scaffold sequences used to inhibit GFP and Pan-Ras protein expression. The T7 promoter and T7 terminator are in italics, Qβ RNA hairpin is in blue, stem-loop derived from miR-30b is in green and the targeting sequence and its complement are in red. BglII/XhoI restriction sites are underlined.

$RNAi_{GFP}$ sequence
5'-GTGGAGATCT*TAATACGACTCACTATAGGG*AAATGCATGTCTAAGACAGC ATCTTCGCGCAACATTCTGGGACACAAATTGCTGTGAAGCCACAGATGGGCAATTTG TGTCCCAGAATGTTGTGC*CTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGG GTTTTTTG*CTCGAGGGTG-3'
$RNAi_{let-7}$ sequence
5'-GTGGAGATCT*TAATACGACTCACTATAGGG*AAATGCATGTCTAAGACAGC ATCTTCGCGAACTGTACAAACTACTACCTCACTGTGAAGCCACAGATGGGTGAGGTA GTAGTTTGTACAGTTTGC*CTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGG GTTTTTTG*CTCGAGGGTG-3'

B. Additional data

1. Characterization of VLP-GFP uptake by human cells (Figure S1) and *in vivo* packaging of GFP in VLPs (Figure S2-S3)

Qβ VLP_{647} with endogenous RNA enter DU-145 cells (Figure S1). Co-expression of Qβ CP and GFP constructs in *E. coli* produces GFP-containing VLPs (Figure S2). GFP is spontaneously packaged inside VLPs. GFP packaging and expression *in vivo* is sufficient to confer observable fluorescence (Figure S3). VLP-GFPs are internalized by PC3 cells (Figure 3e-f), regardless the presence or absence of surface decoration (Figure 3b-c). Previous studies showed that *in vivo* packaging of GFP in VLPs is mediated by RNA aptamers[1]. Here we show GFP packaging in VLPs simply by expressing GFP and CP in *E. coli*.

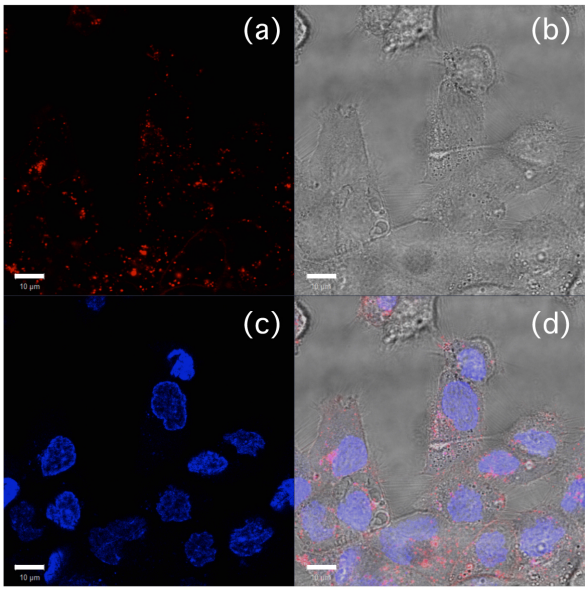


Figure S1. Internalization of VLP_{647} by DU-145 cells: DU-145 cells were incubated with VLP_{647} (100 nM) for 24 h. Images were taken by confocal microscopy. (a) DyLight 647-conjugated VLPs, (b) bright field (c) nuclei, (d) merged confocal signals. The nuclei are stained blue with DAPI. VLP conjugated with DyLight 647 is magenta. Scale bar = 10 μm.

Figure S2. Characterization of *in vivo* prepared GFP-containing Qβ-VLPs by 10% SDS-PAGE. The molecular weight of Qβ CP monomer is 14.5 kDa. Lane 1: ladder, Lane 2: Qβ CP monomer from purified wild-type Qβ-VLPs, Lane 3: *in vivo* –prepared, GFP-containing Qβ-VLPs. The molecular weight of GFP (A) is 28.3 kDa. An unknown *E. coli* protein (B) is encapsidated simultaneously with GFP in VLPs.

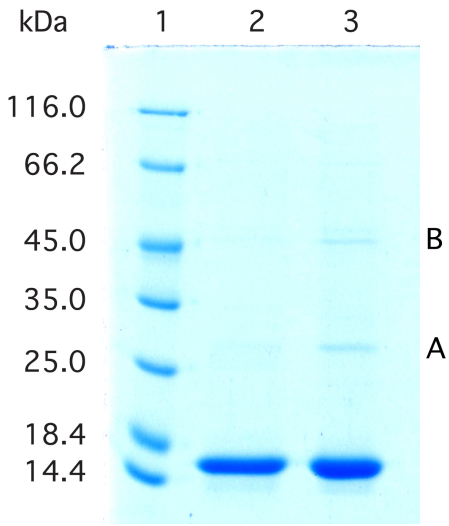
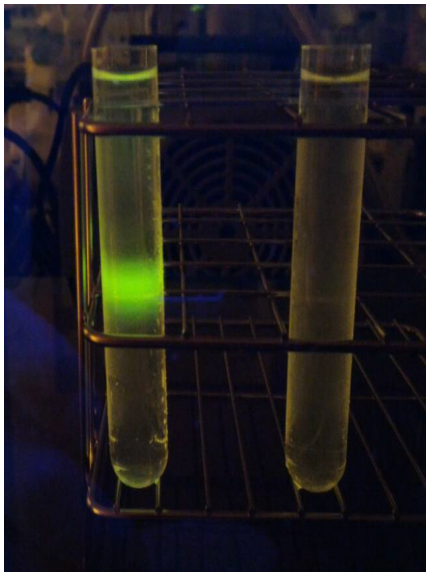


Figure S3. Characterization of *in vivo* –prepared, GFP-containing Qβ VLPs by sucrose density gradient ultracentrifugation. *E. coli* produced GFP-containing VLPs form a discrete layer of green fluorescence signal after ultracentrifugation. The wild-type VLPs (control) do not show fluorescence.



2. RNAi activity of *in vivo* VLP-RNAi$_{GFP}$ assembly (Figure S4-S5)

Qβ-CP and RNAi$_{GFP}$ were co-expressed in *E. coli* to spontaneously assemble functional VLPs. Undecorated VLPs show RNAi activity that inhibits GFP expression in HeLa cells.

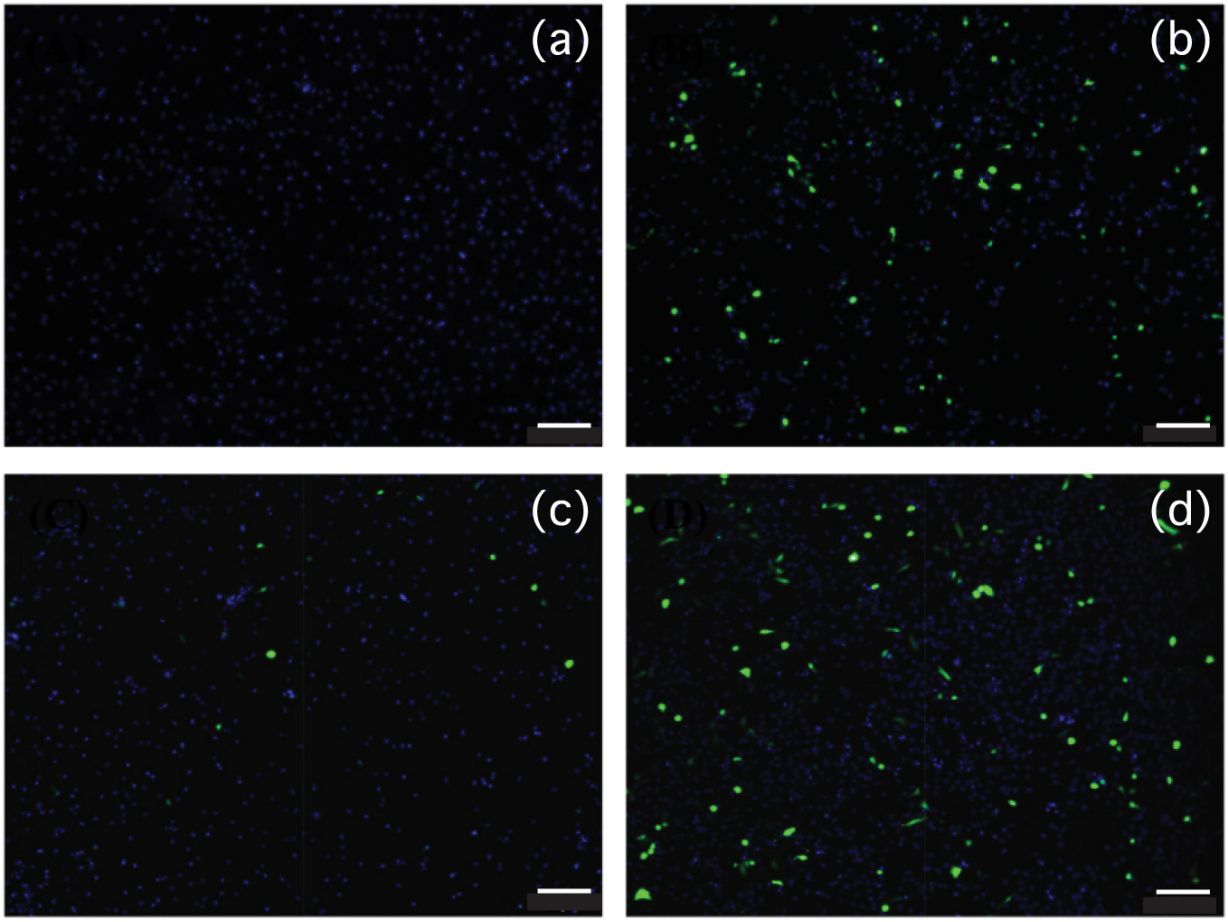


Figure S4. Suppression of gene expression by VLP-RNAi$_{GFP}$ in HeLa cells. HeLa cells were treated with (a) PBS, (b) a plasmid the expresses GFP, (c) VLP-RNAi$_{GFP}$ after transfection with GFP-expressing plasmid, or (d) VLPs after GFP-expressing plasmid transfection. Cells were incubated with 360 nM VLP-RNAi$_{GFP}$ or VLPs in the absence of transfection reagents for 24 h. Images were taken 24 h after the addition of VLP-RNAi$_{GFP}$. Green spots indicate cells that express GFP. Blue spots show the nuclei, stained with DAPI. The scale bar = 200 μm.

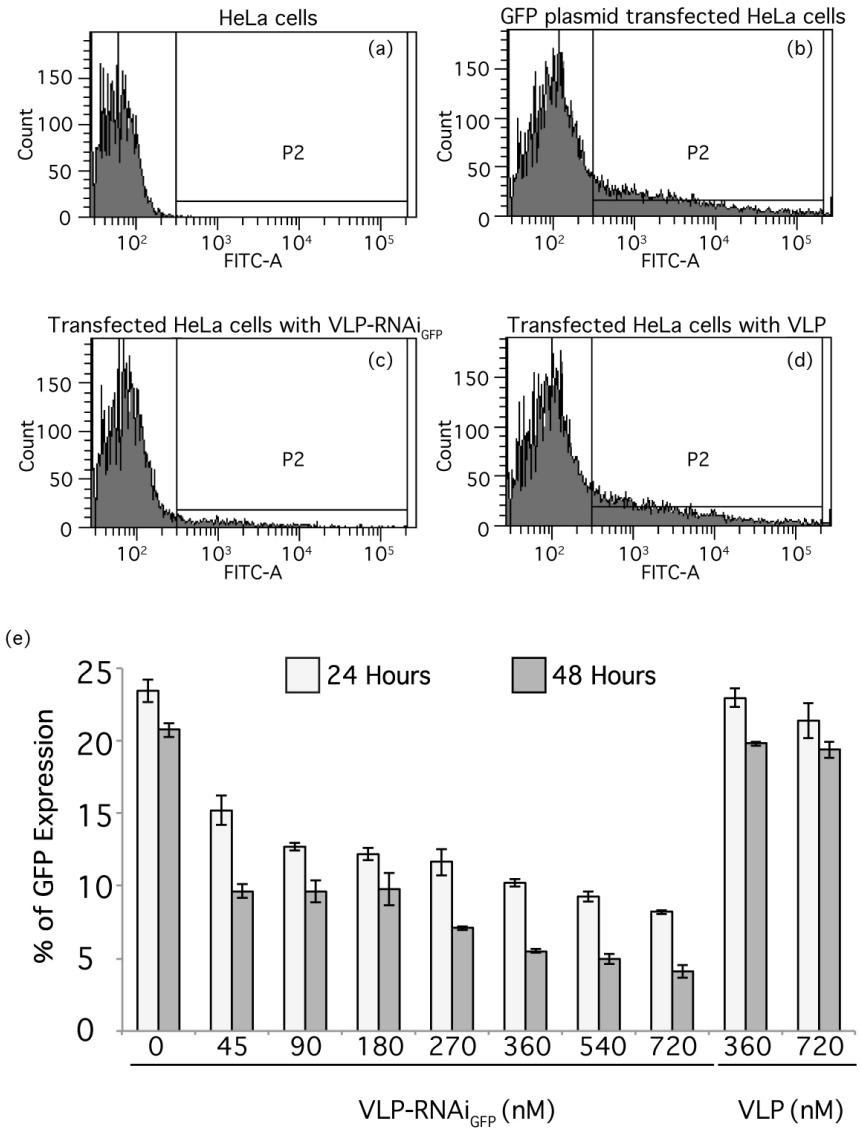


Figure S5. Inhibition of GFP expression in HeLa cells quantified by flow cytometry. (a) HeLa cells; (b) HeLa cells transfected with GFP expressing plasmid; (c) HeLa cells transfected with GFP expressing plasmid co-cultured with VLP-RNAi$_{GFP}$; (d) HeLa cells transfected with GFP expressing plasmid co-cultured with VLPs. P2 represents the fluorescent signal of GFP. The negative control consists of VLPs without RNAi$_{GFP}$. (e) Gene expression levels of GFP in HeLa cells was determined after treatment with various concentrations of VLP-RNAi$_{GFP}$ for 24 h (white) and 48 h (grey). The GFP gene expression levels are reported as percentage of cells that fluoresce. The negative control contains VLPs without RNAi$_{GFP}$. Values are means ± SD (n = 3).

C. Reagents

Green fluorescent protein (GFP) was purchased from EMD Millipore. DyLight 633 Amine-Reactive kit was purchased from Thermo Scientific. DNA oligos were ordered from Eurofins MWG Operon, Inc. NZY broth media was purchased from TEKNOVA. Recombinant human dicer enzyme was purchased from Genlantis. Polyallomer centrifuge tubes were purchased from Beckman Coulter. Amicon® Ultra centrifugal filters (100 kDa MWCO) were purchased from Millipore. Spectra/Por® dialysis tubing (15 kDa MWCO) was purchased from Spectrum® Laboratories, Inc. Sec-Butanol and chloroform were purchased from Fisher Scientific. Ammonium sulfate was purchased from ICN Biomedicals. HeLa cells were obtained from the American Type Culture Collection. U87 human glioblastoma cells were a gift from Dr. Kuo-Chen Wei at the Department of Neurosurgery (Chang Gung Memorial Hospital, Taiwan). Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Primary Pan-Ras antibodies were purchase from Santa Cruz biotechnology. Secondary antibody, horseradish peroxidase-conjugated goat anti-mouse IgG was purchase from PerkinElmer. Glass bottom microwell dishes were purchased from MatTek. XTT kit was purchased from Sigma. All other reagents were analytical grade. All DNA constructs were confirmed by sequence analysis (Eurofins MWG Operon).

D. References

1 Rhee, J. K. *et al.* Colorful Virus-Like Particles: Fluorescent Protein Packaging by the Qbeta Capsid. *Biomacromolecules* **12**, 3977-3981, (2011).