Supporting Information

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SI Materials and Methods

Construction of the Gag Destination Vector. The Gag coding sequence was cloned by PCR from RCAS-GFP (1) using the following PCR primers: RCAS (BsiE1)-F: 5′-TATTACGGCCGGCCACCATGGAAGCCGTCATT AAGGTGATTT CGTCCG CGTG-3′ and RCAS (Kpn1)-R: 5′-TTATATAGGTACCTGCCTGGCCACGG CCCC GAAGAGAGACCTCTTCCTG-3′. The PCR product was digested with BsiE1 and Kpn1 and was ligated to pDest 472 digested with Sac II and KpnI. This plasmid was designated pDest 472-Gag.

Construction of Gag-GFP and Gag-Cre Fusion. To construct pDest 472-Gag-GFP, an entry clone plasmid containing the GFP coding region (pEL100) was used for transfer to pDest 472-Gag, using recombinational cloning (Gateway; Life Technologies). To generate the Gag-Cre fusion, an entry clone containing Cre (pEL-Cre) was used for cloning into pDest472-Gag by recombinational cloning. The construct was designated as pDest 472-Gag-Cre.

Transduction of the PC3-Lox1-GFP-Lox2-RFP Reporter Cell Line. The PC3 (human prostate carcinoma) reporter cell line containing the stably integrated CMV-Lox1-GFP-Lox2 RFP expression cassette was transduced with 1 mL of VLPs (~10^6 VLPs/mL) corresponding to each experimental construct, or with purified His-Cre or TAT-Cre recombinase at a final concentration of 2 μM. For all such experiments, six-well plates with 1×10^5 cell per well in 2 mL of complete culture medium (DMEM + 10% FCS) were used. At 72 h posttransduction, cells were analyzed using fluorescence microscopy.

Transduction of the PC3-Lox1-GFP-Lox2-RFP Reporter Cell Line with VLPs Containing Gag-Cre Recombinase. The PC3 reporter cell line, which contains the stably integrated pCMV-Lox1-GFP-Lox2 RFP expression cassette, was transduced with 1 mL of VLPs corresponding to each experimental construct. At 72 h posttransduction, cells were analyzed using the Olympus IX81 fluorescence microscopy system.

Construction of Gag-Fcy::Fur. To generate the cytotoxic enzyme Fcy::Fur fusion clone, pGag-RSV-GFP was digested with ApaI plus NotI. The Fcy::Fur coding sequence was amplified by PCR from pORF5-Fcy::Fur (InvivoGen) using the following PCR primers: Fcy-Apa1-F: 5′-TATTTAGGGCCC GTCACAGGAG GCATGGCTTCAAAGTG 3′ and Fur-Not1-R: 5′-AATAAAGCGGCCGCTTAGA CACAGTAGTATCTGTCCCCAAAG-3′. The PCR product was digested with Apa1/Not1 and ligated to Apa1/Not1 digested with pGag-RSV-GFP, resulting in pGag-RSV-Fcy::Fur.

Generation of the NA Tag Vector. The NA tag vector, which is used to display ligands on the surface of VLPs, was prepared by cloning a portion of influenza NA into pDsRed1N1 (Clontech) as a Nhe1 and EcoR1 fragment. The truncated form of the influenza NA domain was synthesized and optimized for mammalian expression (GeneArt) as follows: 5′-GGCTAGCCCACCATGAATCCAAACCAGAAAATAATAACCATTGGGTCAATCTGTATGGTAGTCGGAATAATTAGCCTAATATTGCAAATAGGAAATATAATCTCAATATGGATTAGCCATTCAATTCAAACCGGAAATCAAAACCATACTGGAATATGCAACCAAGGCAGCATGAATTC-3′). The restriction sites NheI and EcoRI are underlined. This generated the plasmid pCMV-NA-Tag.

Cloning of Mouse IFN-γ as an NA Fusion Protein. The coding sequence for the mature form of mouse IFN-γ was amplified by PCR from the pORF5-mIFNg plasmid (InvivoGen) using the following oligonucleotides: IFN-γ (m) EcoR1-F: 5′ATTATAGAATTCACACGGCACAGTCATTGAAAGCCTAGAA-3′ and IFN-γ (m) Not1 R: 5′-TTATATTGCGGCCGCGCTCAGCAGCGACTCCTTTTCCG- CTTCCTG-3′. The restriction sites are underlined. The PCR fragment was digested with EcoR1 and Not1 and ligated into pCMV-NA-Tag digested with the same restriction enzymes. The resulting construct was designated as pCMV-NA-mIFN-γ.

Generation of the pCMV-HA-Tag Vector. Influenza hemmagglutinin (HA) was synthesized and optimized for mammalian expression (GeneArt; Invitrogen). HA was synthesized as an EcoRI/NotI fragment: 5′GAATTCAATGGGACTTATGATTATCCAAAATATTCAGAAGAATCAAAGTTGAACAGGGAAAAGATAGATGGAGTGAAATTGGAATCAATGGGGGTGTATCAGATTCTGGCGATCTACTCAACTGTCGCCAGTTCACTGGTGCTTTTGGTCTCCCTGGGGGCAATCAGTTTCTGGATGTGTTCTAATGGGTCTTTGCAGTGCAGAATATGCATCTGAGCGGCCGC-3′. The fragment was cloned into pDsRed1N1 (Clontech) digested with EcoRI and Not I. This construct was designated as pCMV-HA-Tag.

Cloning of Mouse IFN-γ as an HA Fusion Protein. The coding sequence for mature mouse IFN-γ was amplified from the pORF5-mIFN-γ plasmid (InvivoGen) using the following primers: IFN-γ (m) Nhe1-F: 5′-ATTATTAATGCTAGC CACCATGGCCAACGCTACACACTGCATCTTG 3′ and INF-γ (m) EcoR1-R: 5′ ATAATTATGAATTCGCAGCGACTCCTTTTCCGCTTCCTGAGGCTGGATTCC 3′. The restriction sites are underlined. The PCR fragment was digested with NheI and EcoRI and ligated into pCMV-HA-Tag plasmid digested with the same restriction enzymes to generate pCMV-HA(m)IFN-γ.

Cloning of Human TRAIL Ligand as an NA-TRAIL Fusion Protein. The amino acid sequence of human TRAIL (114–281 aa) was obtained from the Web site of Calbiochem-EMD Biosciences. The sequence was optimized for mammalian expression and synthesized commercially (see below; GeneART; Invitrogen). An His-6 tag was included in the sequence to facilitate detection with an anti-His antibody. 5′***GAATTC***TCATCACCATCATCACCATGTGCGCGAACGCGGCCCGCAGCGCGTGGCGGCGCATATTACCGGCACCCGCGGTCGCAGCAACACCCTGAGCAGCCCGAACAGCAAAAACGAAAAAGCGCTGGGCCGCAAAATTAACAGCTGGGAAAGCAGCCGCAGCGGCCATAGCTTTCTGAGCAACCTGCATCTGCGCAACGGCGAACTGGTGATTCATGAAAAAGGCTTTTATTATATTTATAGCCAGACCTATTTTCGCTTTCAGGAAGAAATTAAAGAAAACACCAAAAACGATAAACAGATGGTGCAGTATATTTATAAATATACCAGCTATCCGGATCCGATTCTGCTGATGAAAAGCGCGCGCAACAGCTGCTGGAGCAAAGATGCGGAATATGGCCTGTATAGCTTTATCAGGGCGGCATTTTTGAACTGAAAGAAAACGATCGCATTTTTGTGAGCGTGACCAACGAACATCTGATTGATATGGATCATGAAGCGAGCTTTTTTGGCGCGTTTCTGGTGGGCTGATGA***GCGGCCGC***-3′. The restriction sites EcoRI and NotI are in bold italics; the 6 histidine tag is underlined. The synthesized fragment was digested with EcoRI and NotI and cloned into the pCMV-NA-Tag vector digested with the same restriction enzymes. The resulting plasmid was designated pNA-TRAIL.

Human Split Caspase 8B as Gag-Fusion. The plasmid pORF9-hCACP8b (InvivoGen) containing the sequence for human caspase 8b was used to generate caspase 8 clones p10 and p18. The fragments, p10 and p18, were cloned as Gag fusion proteins. To clone p10 of human caspase 8, the following primers were used: p10-Apa1-F: 5′-TATATTAGGGCCCTTATCATCACCTCAAACGAGATATATCCCG GAT-3′ and p10-Sal1-R: 5′-TAATATGTCGACTTATCAATCAGAAGGGAAGACAAGTTTTTTTC-3′. For the p18 fragment amplification, the following primers were used: p18-Apa1-F: 5′-TATATTGGCCCGGGCCCGACTTCAGCAGAAATCTTTATGATATTGGGGA-3′ and p18-Sal1-R: 5′-ATATATGTCGACTATCATTAATCAGTCTCAACAGGTATACCTTTCTG-3′. Following amplification, the PCR fragments were cloned as ApaI-SalI fragments into pDest472-Gag. The plasmids were designated pdest472-Gag-p10 and pDest472-Gag-p18, respectively.

Transduction of the PC3 Cell Line with VLPs Consisting of Gag-Fcy::Fur. On the day of transduction, culture medium was supplemented with 5-fluorocytosine (5FC) at 60 μg/mL and with 1 mL of VLPs corresponding to each experimental construct. After 5 d posttransduction, cell viability was measured using the Cedex (Innovatis) cell analysis system.

Transduction of the PC3 Cell Line with VLPs Consisting of Gag-GFP and Surface-Displayed Human TRAIL. On the day of transduction, culture media was supplemented with soluble TRAIL ligand (Calbiochem) at 100 ng/mL or with 1 mL of VIPs corresponding to each experimental construct in a total volume of 2 mL. After 24 h posttransduction, cell viability was measured using the Cedex (Innovatis) cell analysis system.

Transduction of the PC3 Cell Line with VLPs Consisting of Gag Split (H) Caspase 8b. On the day of transduction, culture medium was supplemented with 1 mL of VLPs corresponding to each experimental construct in a total volume of 2 mL. After 48 h posttransduction, cell viability was measured using the Cedex (Innovatis) cell analysis system.

Western Blotting. A total of 1.5 mL of cellular supernatant containing VLPs was overlaid on top of a 20% sucrose solution (400 μL) in 2.2-mL microcentrifuge tubes. The contents were centrifuged at 12,000 × *g* for 40 min at 4 °C. The resulting pellet was resuspended in a total volume of 50 μL of sample loading buffer supplemented with reducing reagent (Bond-Breaker TCEP Solution; Thermo Scientific) and heated at 75 °C for 10 min. Samples were resolved via PAGE and transferred to nitrocellulose membranes, which were probed with anti-CA (anti–p27-HRP IgG 583301; Charles River Laboratories). Membranes were then subjected to the bioluminescent SuperSignal West Femto detection system (ThermoFisher). The same membranes were reprobed using IgG specific for the presence of the Cre recombinase (mAb clone 7.23 MMS-106P; Covance) or the His-tag (mAb anti–6His-HRP, MABO50H; R&D Systems).

Titration of VLP by ELISA. Titration of VLP was done by ELISA by using a known titer of retrovirus as a reference. Anti-p27 (ASLV capsid) antibody was used for this determination. A 96-well high-binding capacity plate (Costar 9018) was coated with 100 μL of anti-p27 IgG diluted 1:1,000 in sodium carbonate/sodium bicarbonate buffer at pH 9.4 and stored overnight at 4 °C. The wells were washed with 200 μL of PBS/0.05% Tween-20 and blocked with 200 μL of blocking solution (PBS/0.05% Tween-20 and 5% BLOTTO) for 2 h at room temperature. Plates were rinsed with PBS/Tween-20 three times, and 100 μL of cell culture supernatants containing 0.05% Tween-20 was dispensed into each well; the plates were sealed with tape and incubated at 37 °C for 1 h. After incubation, plates were washed four times with 200 μL of wash buffer (PBS + 0.05% Tween-20), and 100 μL of anti-p27 HRP IgG (1:1,000 dilution in PBS + 0.005% Tween-20 + 5% BLOTTO) was added to each well. Plates were again sealed and incubated at 37 °C for 1 h. Finally, plates were washed again four times with 200 μL of wash solution, treated with colorimetric substrate (ABTS Peroxidase Substrate; KPL), and analyzed on a FluoroStar-Optima (BMG) plate reader.

Flow Cytometry. Immortalized C57BL6 macrophages were established by infection of primary mouse bone marrow cells using the J2 recombinant retrovirus as described previously (2). To each well of a six-well dish, 1 mL of cells (1×10^6 cells/mL in 10% DMEM) was added. To control wells, 1 mL of 10% DMEM was added. For a positive control, 500 U/mL of mouse IFN-γ (Peprotech) was added, and 1 mL of VLP ($1 \times 1 \times 10^6$) was added to the test wells. The cells were then incubated for 30 min at 37 °C. The cells were removed from the wells by scraping and transferred to 5 mL polystyrene round-bottom tubes and pelleted at 1,200 × *g* for 5 min at 4 °C. The media was removed and the cells were resuspended in 1 mL of 1× PBS. To fix the cells, 125 μL of 16% paraformaldehyde was added (final concentration of 2%) and the cells were incubated for 10 min at room temperature. The cells were pelleted at 1,200 × *g* for 5 min at 4 °C and the media removed. To the cell pellet, 1 mL of cold methanol was added, and the cells resuspended by vortex and incubated for 30 min at 4 °C. The cells were pelleted at 1,200 × *g* for 5 min at 4 °C, the media removed, and the cells then washed 2× with 500 μL of FACS buffer (1× PBS, 1% BSA, and 1% sodium azide). The cells were resuspended with 100 μL of FACS buffer and stained with 7.5 μL of anti–phospho-STAT-1 PE (BD) for 30 min at 4 °C. The cells were then washed 2× with 500 μL of FACS buffer and resuspended with 300 μL of FACS buffer for analysis on the flow cytometer.

Fluorescence Microscopy. The HEK293T cells were seeded at 2×10^5 cells per chamber in a two-chambered dish (Lab-Tech II; Nalge Nunc International) in 500 μL of complete culture medium. The next day, the culture medium was supplemented with 500 μL culture media containing VLPs expressing Gag-GFP or VLPs expressing Gag-GFP pseudotyped with the VSV-G envelope. The cells were exposed to VLPs for 24 h and analyzed by fluorescence (1,000× oil immersion) microscopy using an Olympus IX81 microscope.

1. Weldon RA, Jr., Erdie CR, Oliver MG, Wills JW (1990) Incorporation of chimeric gag protein into retroviral particles. *J Virol* 64:4169–4179.

2. Blasi E, Radzioch D, Merletti L, Varesio L (1989) Generation of macrophage cell line from fresh bone marrow cells with a myc/raf recombinant retrovirus. *Cancer Biochem Biophys* 10:303–317.

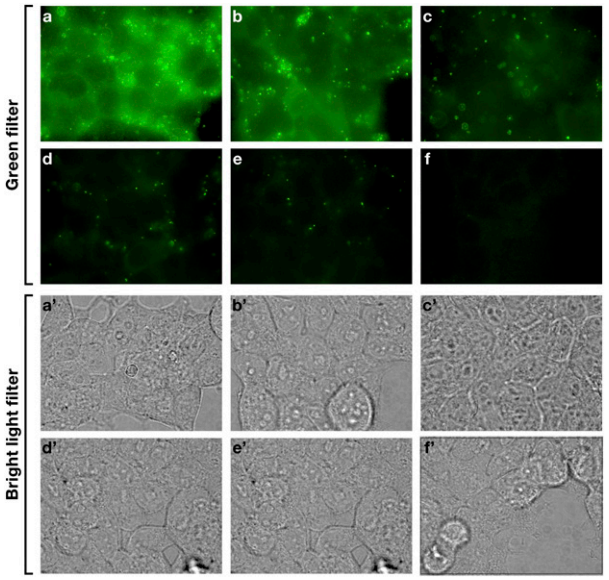

Fig. S1. Transduction of 293T cells with various dilution of VLP. 293T cells were plated at 200,000 cells per well. VLPs prepared in the presence of Gag-GFP pseudotyped with VSV-G envelope were added to cells at different VLP-to-cell ratio. (*A*) 5:1; (*B*) 2.5:1; (*C*) 1.25:1; (*D*) 0.625:1; (*E*) 0.313:1; and (*F*) nontransduced. (*A′–F′*) Corresponding bright fields of the cells.

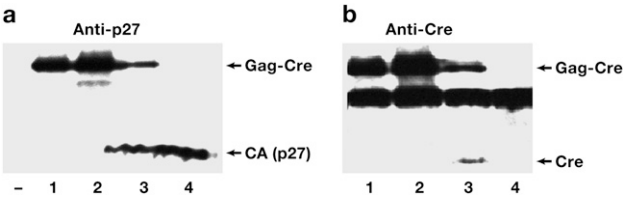

Fig. S2. Western blot analysis. Culture supernatants containing VLPs were analyzed using anti-p27 (CA) and anti-Cre recombinase antibodies. Lane (–), complete culture medium; lane 1, Gag-Cre; lane 2, Gag-Cre + VSV-G; lane 3, Gag-Cre + VSV-G + Gag-Protease; lane 4, Gag-Protease + VSV-G. The large band in the middle of the anti-Cre Western blot is a host protein that cross-reacts with the antibody.

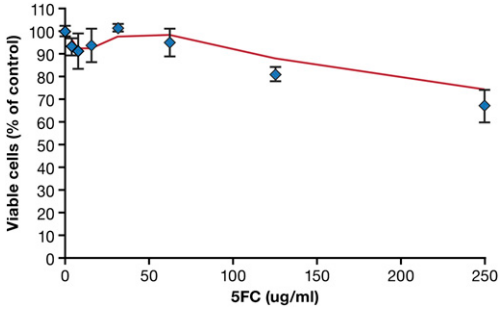

Fig. S3. PC3 sensitivity to 5-fluorocytosine (5-FC) cytotoxicity. Indicated amount of 5FC was added to the culture media and cell viability was determined.

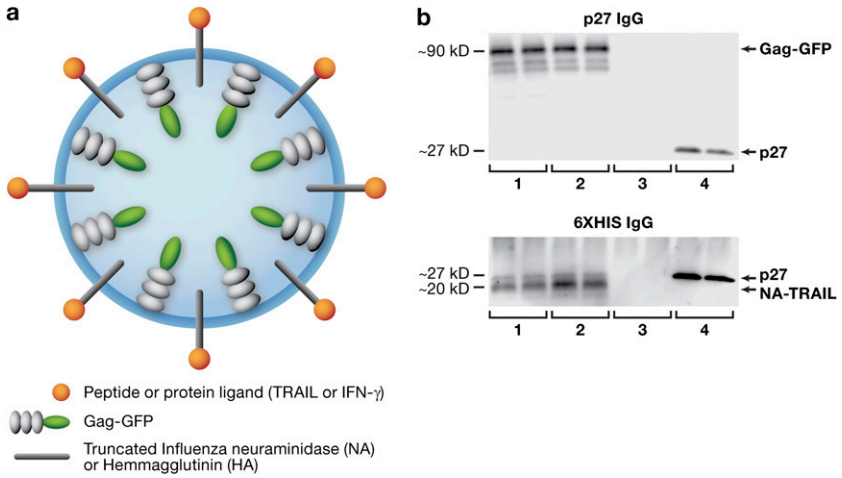

Fig. S4. Cell-specific ligands fused to neuraminidase or hemmagglutinin. (*A*) Graphic representation of VLPs consisting of Gag-POI (Gag-GFP in this case) and a surface ligand (fused to NA or HA). (*B*) Western blots analysis for Gag-GFP (*Upper*) in VLP and NA-TRAIL on the VLP (*Lower*). Detection of Gag-GFP in VLPs was done using p27 (CA) antibodies (lanes 1–3 in duplicates); lane 4, mature RCAS vector particles (positive control for processed CA). Detection of NA-TRAIL on VLPs was done using anti-His antibodies (all in duplicates); Gag-GFP + NA-TRAIL (lane 1), Gag-GFP + NA-TRAIL + VSV-G (lane 2), carrier DNA (lane 3), RCAS (positive control for p27; lane 4).

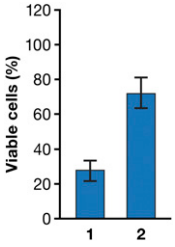

Fig. S5. Cell viability determination of VLP-TRAIL and filtered VLP-TRAIL. Culture supernatant containing VLP carrying Gag-GFP-NA-TRAIL (1) and the eluate of the same culture supernatant (2) that passed through a 300-kDa cutoff membrane were used for cell viability assay.

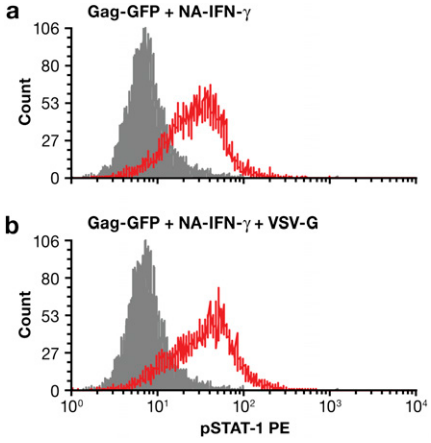

Fig. S6. Murine macrophage cells respond to VLPs carrying mouse IFN-γ. The mouse macrophage cell line was treated with VLPs containing NA murine IFN-γ either in the presence or absence of VSV-G envelope. Positive and negative controls, as well as results, are the same as those used in Fig. 8. Cells were analyzed for the presence of phospho-STAT1 by flow cytometry. (*A*) NA–INF-γ + VSV-G; (*B*) Gag-GFP + NA–IFN-γ + VSV-G.