Supporting information

Differential fates of biomolecules delivered to target cells via extracellular vesicles

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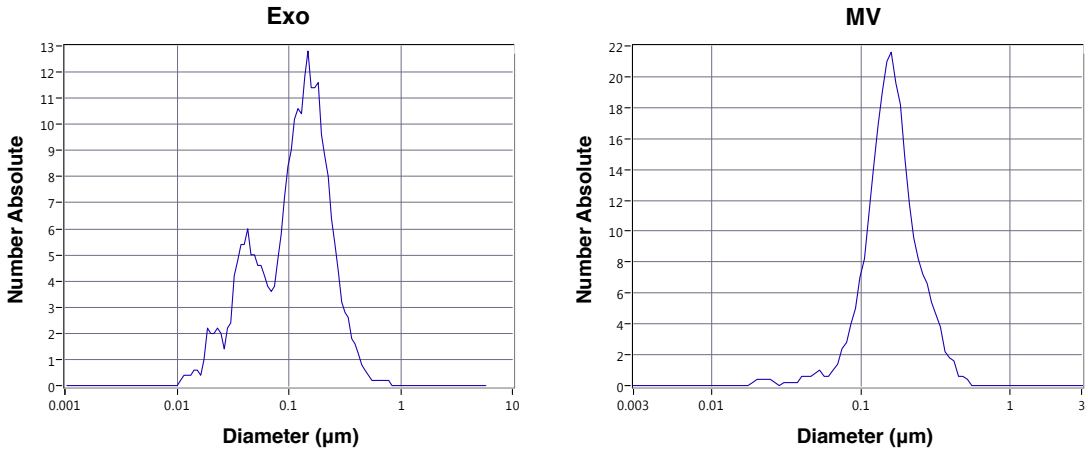

Fig. S1. Size distribution of exosomes and MVs measured by NTA.

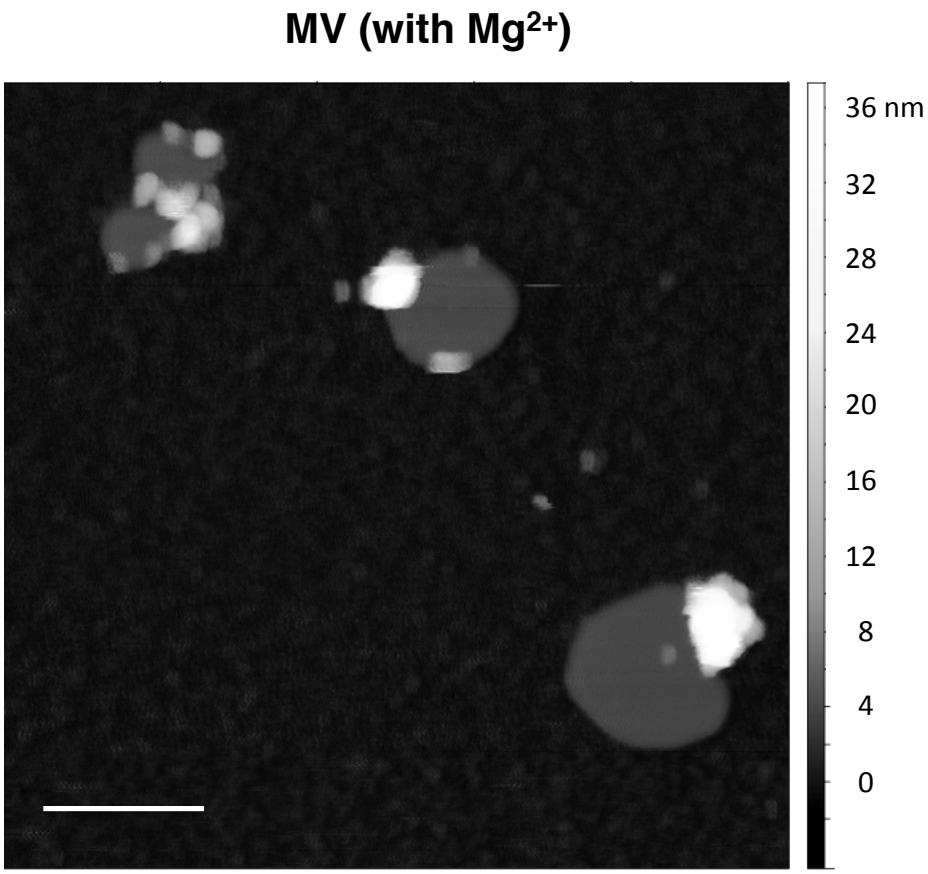

Fig. S2. Collapsed MVs in the presence of Mg^{2+}. Topographic AFM images of HEK293FT cell-derived MVs adsorbed to the mica surface in the presence of Mg^{2+}. All the MVs collapsed and showed lipid-bilayer spreading on the mica surface. Bar, 500 nm.

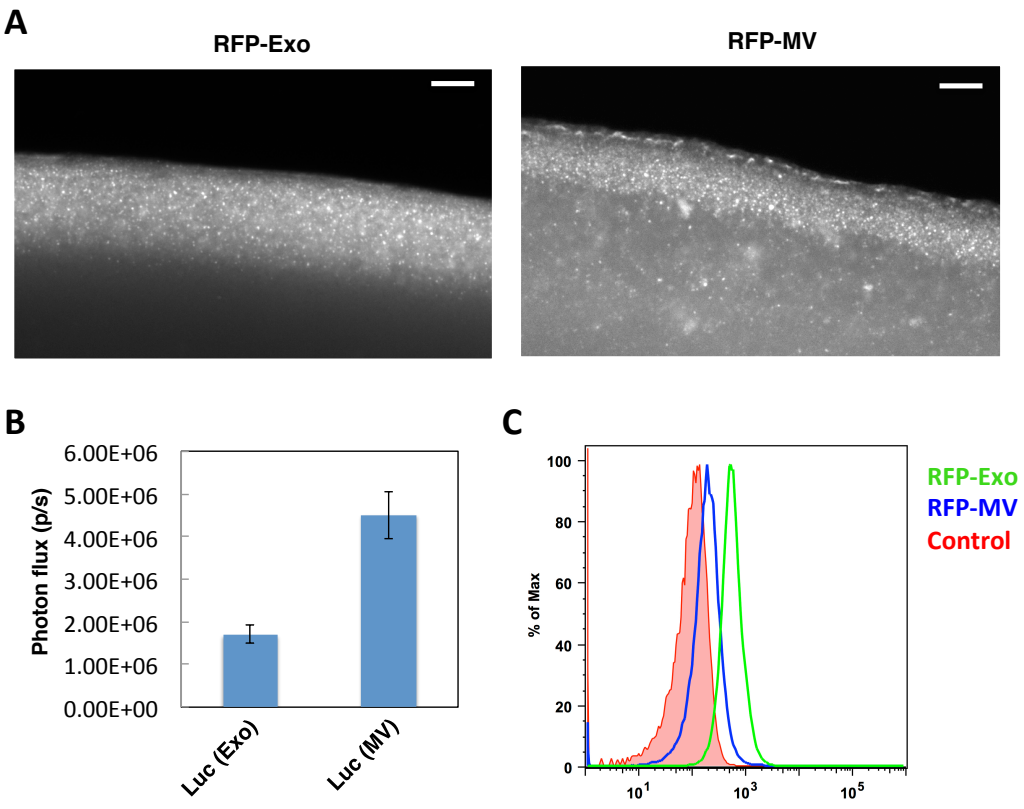

Fig. S3. Loading of reporter protein in EVs. (*A*) A drop of buffer containing isolated EVs derived from HEK293FT cells transiently transfected with Luc-RFP pDNA was loaded onto a cover glass. EV particles were detected by the fluorescence of the RFP portion of the fusion reporter protein. Bars, 20 µm. (*B*) Analysis of Luc protein loading into EVs by luciferase assay. 0.02 µg of the PEG-precipitated EVs were lysed and analyzed. Error bars represent s.d. (n = 6). (*C*) CD63 expression on the RFP-containing EVs was analyzed using anti-CD63-coated beads and detected by flow cytometry (n = 3).

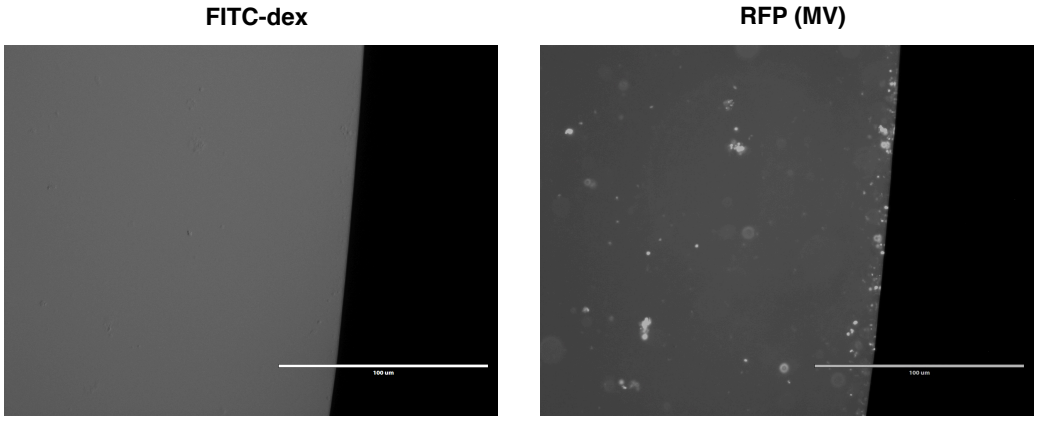

Fig. S4. Evaluation of nonspecific binding of FITC with MVs. MVs derived from HEK293FT cells transiently expressing Luc-RFP were incubated with FITC-dextran (100 μM). Nonspecific binding of FITC-dextran with MVs was evaluated by the fluorescence of the RFP (right) and FITC (left). Bars, 100 μm.

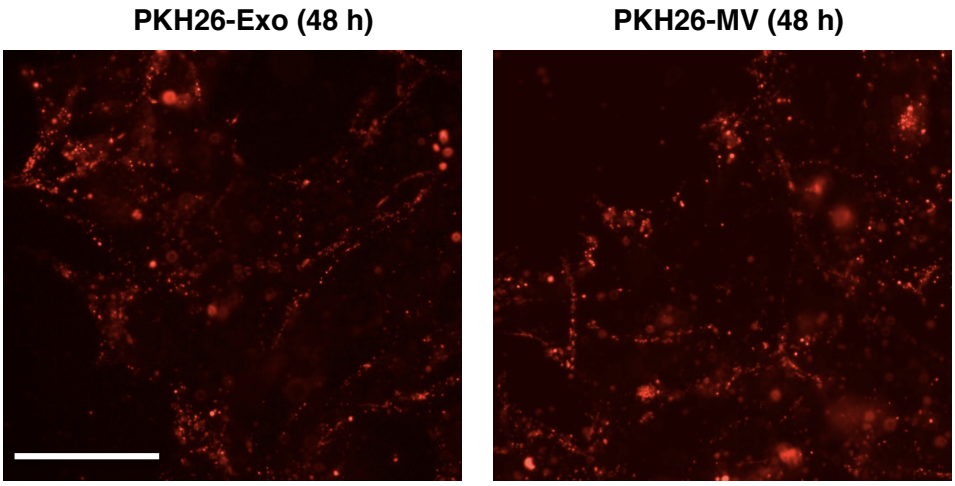

Fig. S5. Visualizing the uptake of EVs stained with lipophilic dye. EVs were labeled with the dye PKH26 (red) and incubated with HEK293FT cells. 24 h after removal of nonadherent EVs, the EVs taken up appeared to accumulate in intracellular compartments. Bar, 50 μm.

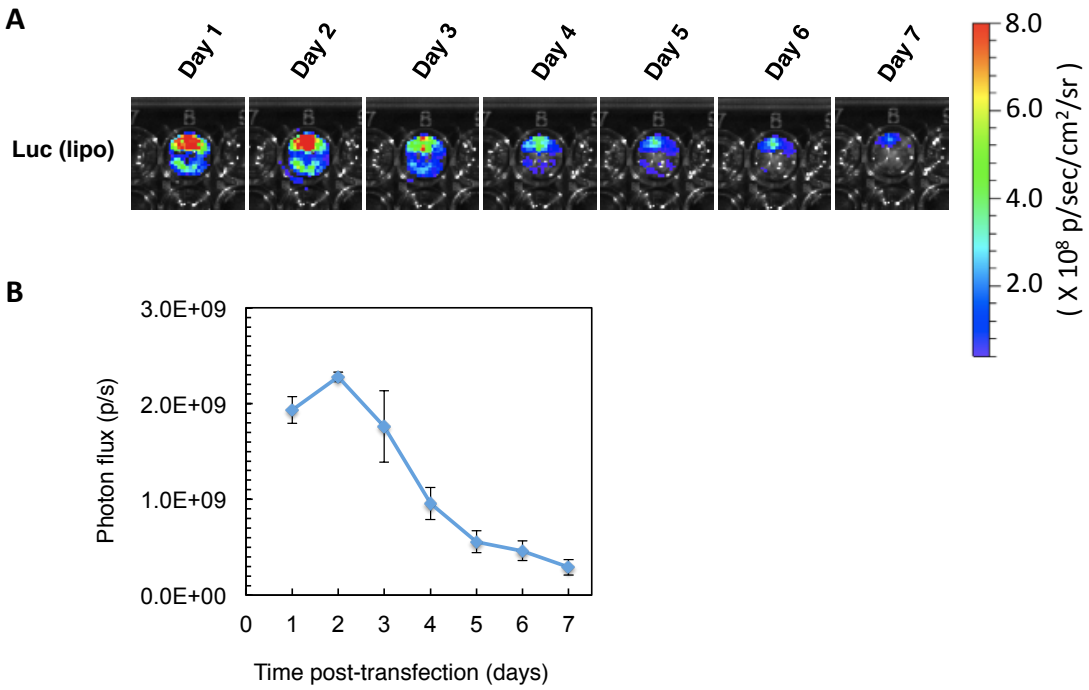

Fig. S6. Bioluminescence of cells transfected with Luc encoding pDNA using cationic lipids. (*A*) Bioluminescence of HEK293FT cells transfected with Luc encoding pDNA using Lipofectamine 2000. Representative wells are depicted. (*B*) Time course of (A). Error bars represent s.e.m. (n = 3). Color scale: radiance (x10^8 photons/cm^2/sec/sr).

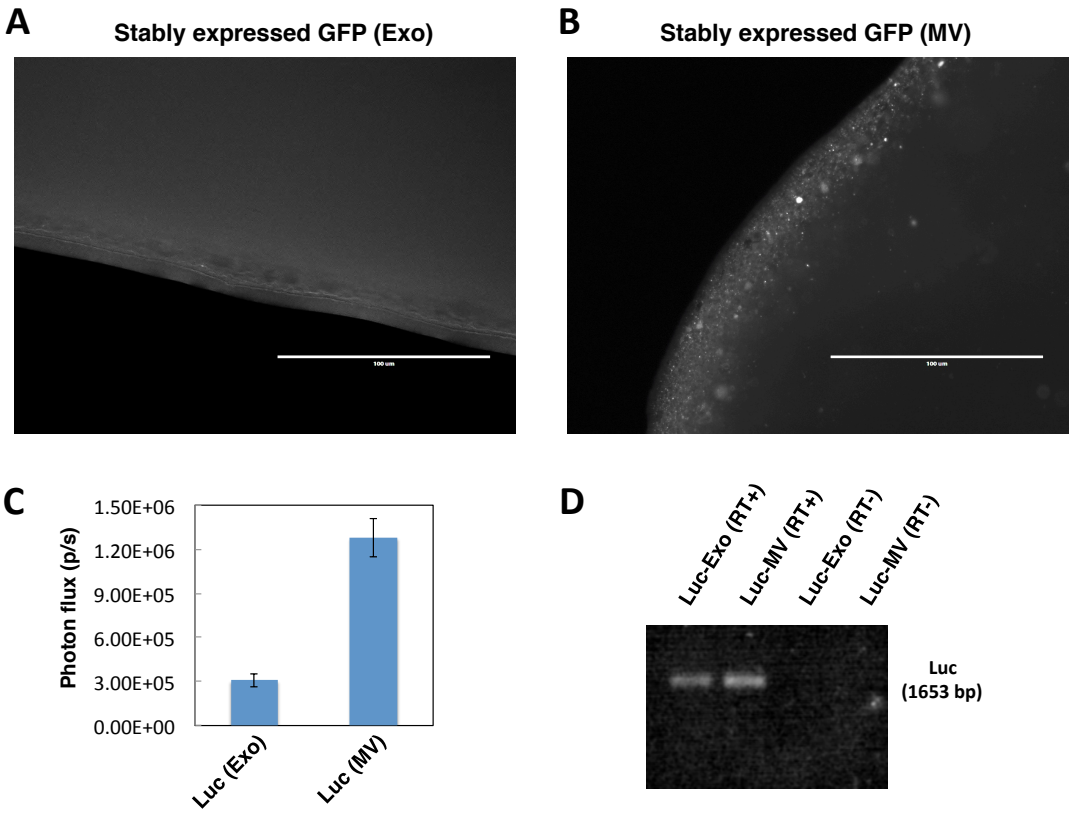

Fig. S7. Loading of reporter proteins and mRNAs into EVs. (*A*, *B*) A drop of buffer containing EVs isolated from 4T1 cells stably expressing Luc and EGFP was loaded onto a cover glass. Small particles were visualized by measuring GFP signal. Bars, 100 µm. (*C*) Analysis of Luc protein loading into EVs by luciferase assay. 0.18 µg of the PEG-precipitated EVs were lysed and analyzed. Error bars represent s.d. (n = 4). (*D*) Detection of full length *Luc* mRNAs in EVs derived from 4T1 cells stably expressing Luc. Nucleic acids isolated from EVs were treated with DNaseI and PCR was performed with or without RT.

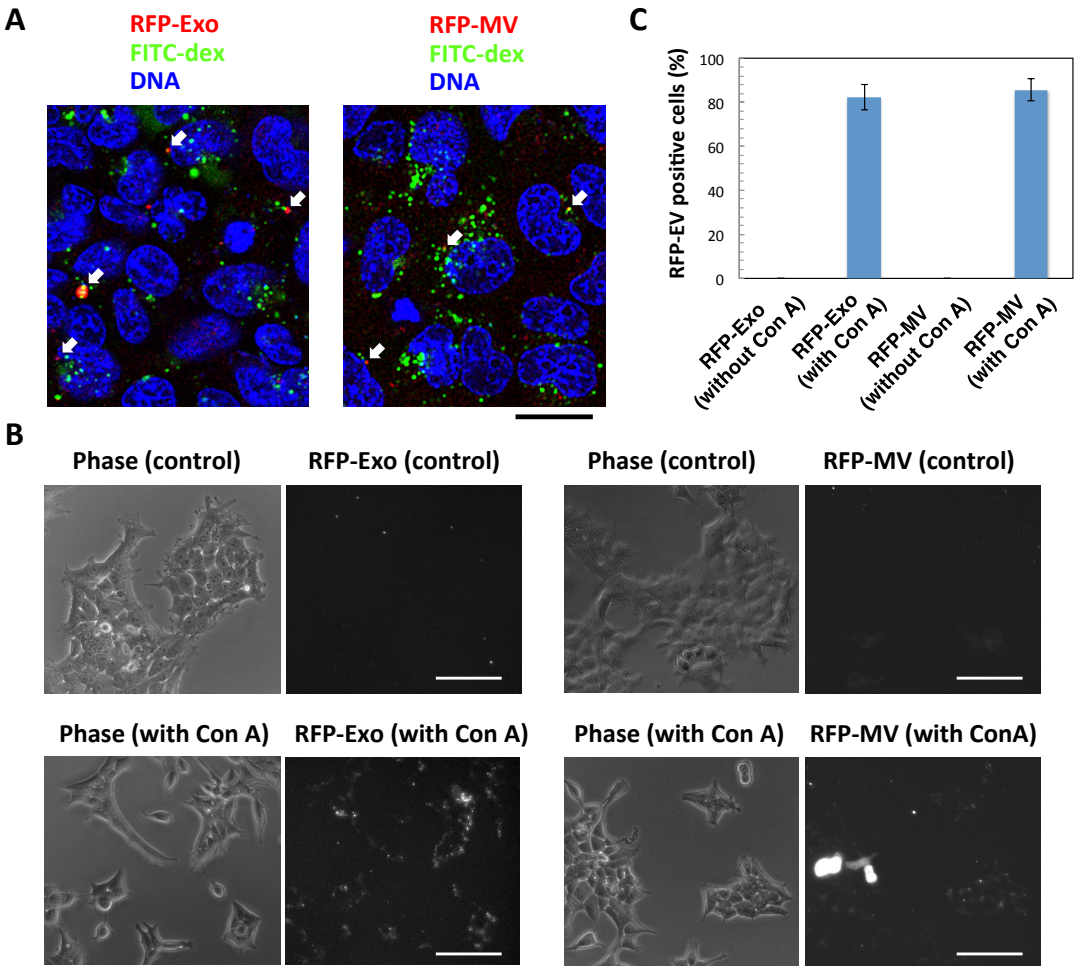

Fig. S8. Endosomal localization of EVs in recipient cells, and the role of lysosomal acidification on reporter protein degradation. (*A*) Long-term loading with FITC-dextran labeled endocytic compartments of HEK293FT cells. Arrows indicate that some punctate signals of RFP (red) merged with punctate signals of FITC (green). Blue indicates nuclei stained with Hoechst 33342. (*B*) Images of phase contrast and fluorescence of RFP-containing EVs in the recipient cells. HEK293FT cells were cultured with 0.46 μg (exosomes) or 0.37 μg (MVs) RFP-containing EVs for 24 h, and these cells were cultured for another 24 h in the presence or absence of 50 nM Con A. Bars, 100 μm. (*C*) Number of the cells exhibiting punctate RFP signals was counted at 48 h. 100 cells were analyzed. Error bars represent s.d. (n = 3).

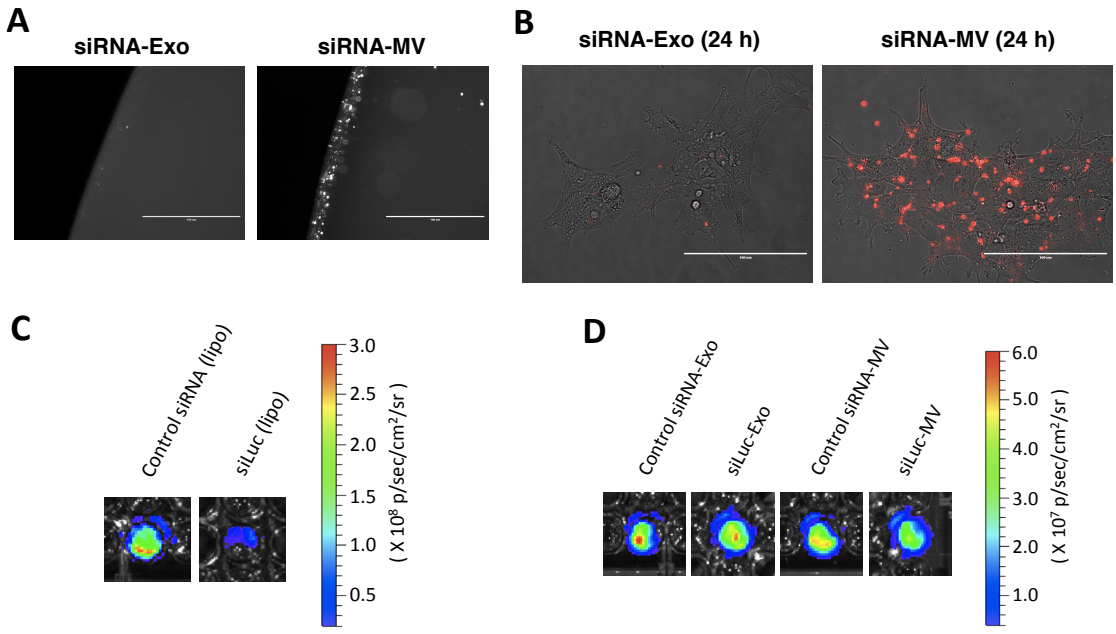

Fig. S9. Evaluation of small interfering RNA delivery via EVs. (*A*) A drop of buffer containing EVs derived from HEK293FT cells transiently transfected with fluorescently labeled siRNA (siGlo) was visualized by fluorescence microscopy. Bars, 100 µm. (*B*) Merged images of fluorescence and phase contrast images of HEK293FT cells treated with EVs from (A). The EVs were cultured with the recipient cells for 24 h prior to imaging. Bars, 100 µm. (*C*) Reporter HaCaTs were transfected with control siRNA or siLuc using Lipofectamine 2000. Color scale: radiance (x10^8 photons/cm^2/sec/sr). (*D*) Reporter HaCaTs treated with 5 µg siLuc-loaded EVs showed no significant suppression of Luc expression (n = 5). Color scale: radiance (x10^7 photons/cm^2/sec/sr).

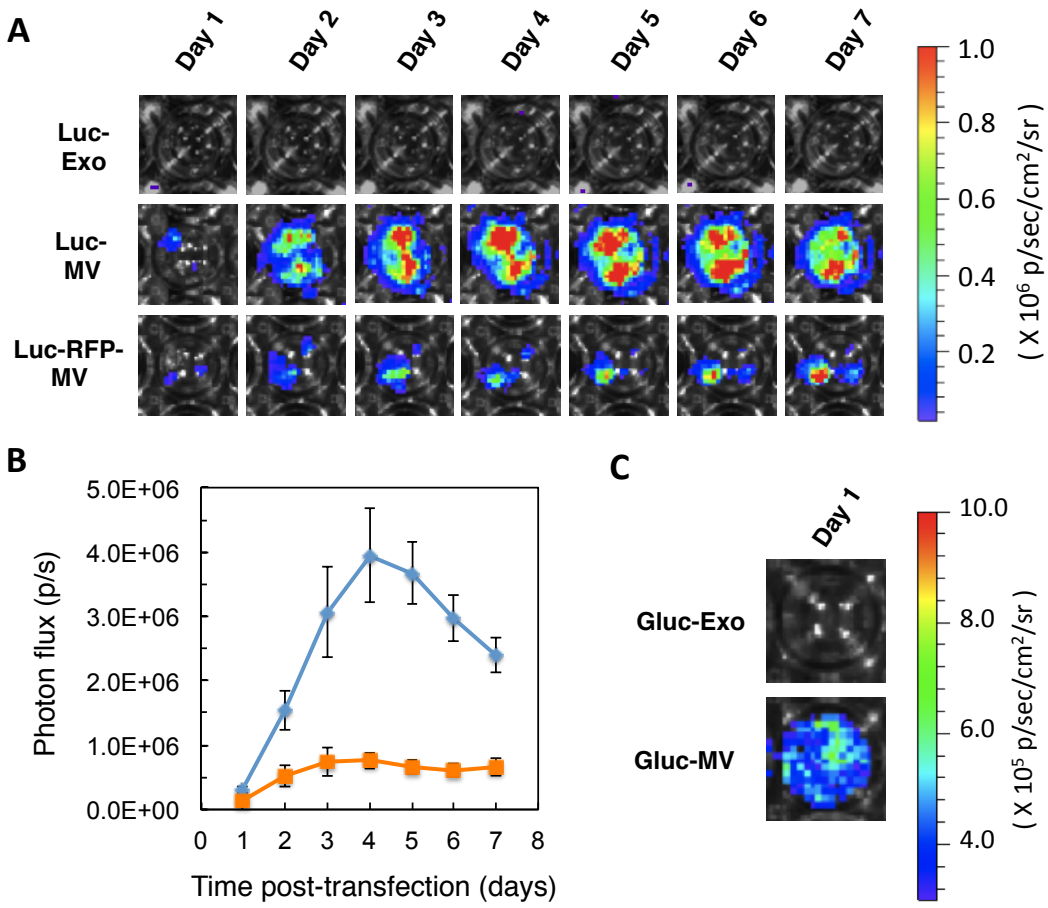

Fig. S10. Correlation between pDNA size and reporter molecule expression in MV recipient cells. (*A*) Luciferase expression in recipient cells treated with MVs derived from HEK293FT cells transfected with two luciferase expression vectors of different size, Luc (size: 7090 bp; middle panel) or Luc-RFP (8567 bp; bottom panel). Recipient cells treated with exosomes derived from HEK293FT cells expressing Luc are shown (top panel). Color scale: radiance (x10^6 photons/cm^2/sec/sr). (*B*) Time course of (*A*). Photon emission from recipient cells treated with Luc (blue) or Luc-RFP (orange) containing MVs was integrated for each well and plotted as photon flux (photons/sec) over time (days). Error bars represent standard error of the mean (s.e.m.) (n = 8). (*C*) GLuc was expressed in the recipient cells that took up MVs derived from HEK293FT cells trasfected with GLuc encoding pDNA (5947 bp). Incubation with exosomes from GLuc-expressing cells produced bioluminescence signal within the background range (n = 6).

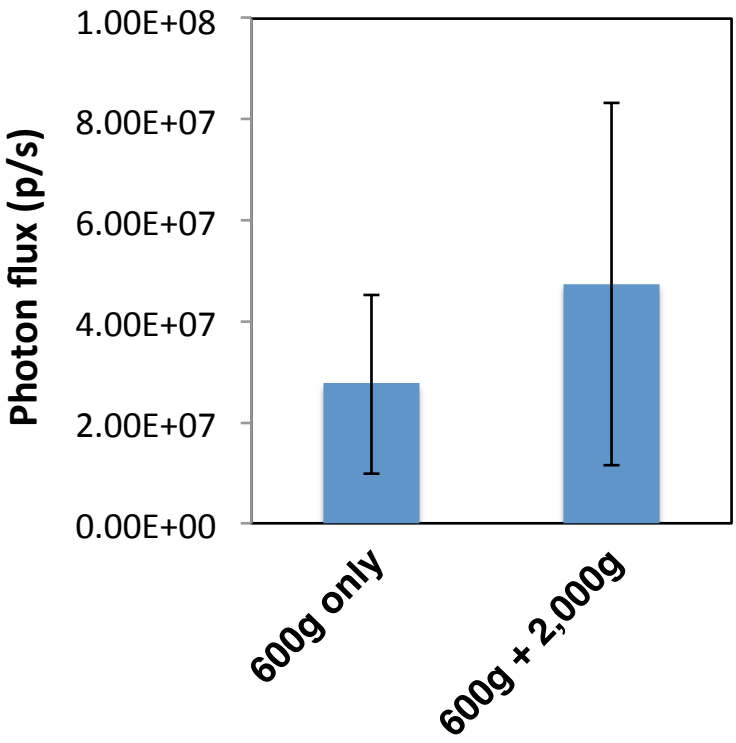

Fig. S11. Effects of ABs on MV-mediated biomolecule transfer. Conditioned media from the HEK293FT cells expressing Luc was centrifuged at only 600g or at 2,000g following 600g before isolating MVs. Both preparations led to Luc expression in the recipient cells without significant difference (n = 6).

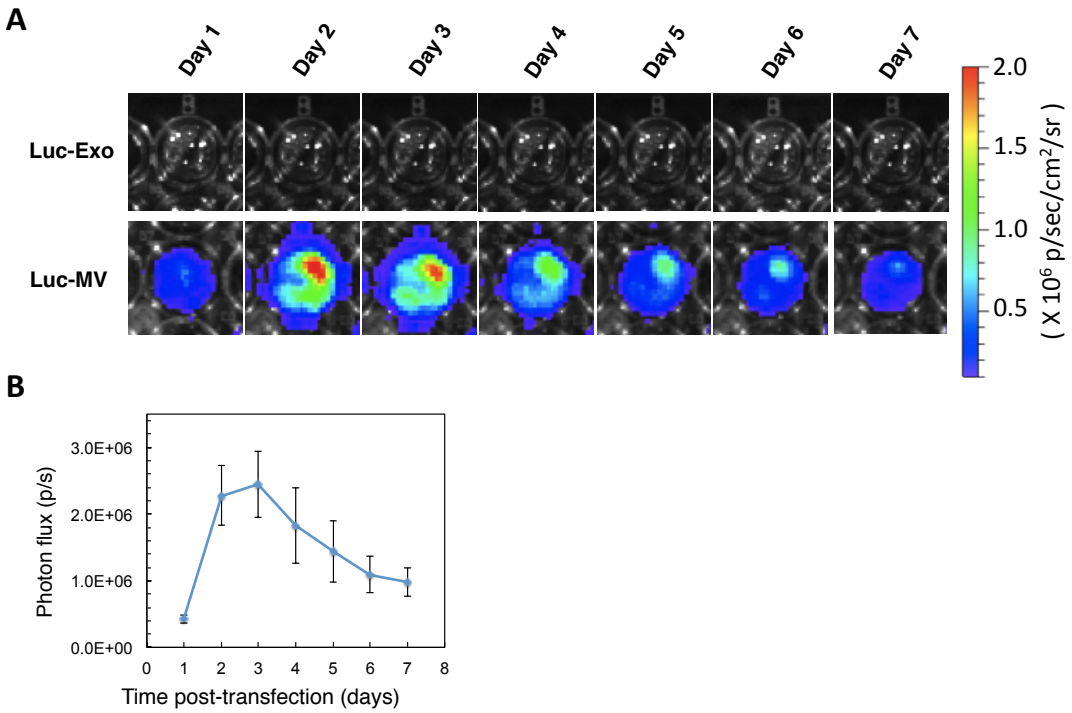

Fig. S12. pDNA delivery via MVs derived from breast tumor cells. (*A*) HEK293 cells were treated with EVs derived from 4T1 cells transiently expressing Luc. Expression of Luc in the recipient cells was measured by BLI as described above. Color scale: radiance (x10^6 photons/cm^2/sec/sr). (*B*) Time course of (A). Error bars represent s.e.m. (n = 6).

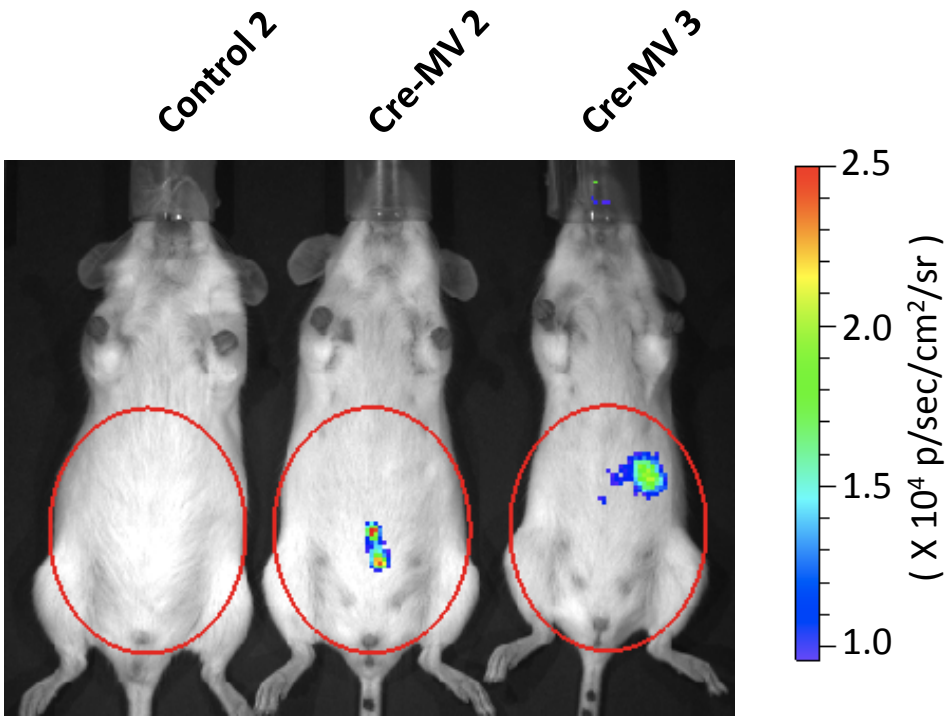

Fig. S13. Stable expression of Luc induced by tumor cell-derived MVs in reporter mice. Two mice showed significant Luc expression 21 days after the injection of Cre-MVs derived from Met-1 cells.