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SUPPLEMENTARY INFORMATION

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RNA delivery by extracellular vesicles in mammalian cells and its applications

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Supplementary Table 1. Monitoring cellular fate of extracellular vesicles/nanoparticles and their cargo.

Extracellular vesicle delivery of functional mRNA:	**References**
Extracellular vesicle-mediated translation of combined *Gaussia* luciferase and metabolic biotinylation (GlucB) mRNA was determined by the addition of extracellular vesicles to recipient cells that were either treated or not treated with cycloheximide to inhibit translation. A subsequent increase in luminescence in recipient cells was attributed to the translation of mRNA.	[1]
Cre mRNA transfer through extracellular vesicles was demonstrated through RT-PCR detection of the transcript in the extracellular vesicle, while the protein was not confirmed by Western blot or ELISA. Extracellular vesicle transfer resulted in recombination events in the brains of ROSA-LacZ mice, supporting functional Cre RNA translation.	[2]
Escape from Endosome:	
Fluorescence	
DiD was used to label the envelope of HIV-1 and the signal was retained as it fused to the endosomal membrane while the released GFP signal of the nucleocapsid was no longer evident as it diffused throughout the cytoplasm, suggesting escape of content from the endosome.	[3]
Incubating recipient cells with cell impermeable dye Calcein within a synthetic nanoparticle showed cytoplasmic distribution of Calcein attributed to escape from endosomes. In controls lacking the nanoparticle, Calcein appeared punctate as though it was retained in endosomal compartments.	[4]
Alexa-647-labelled siRNA contained within lipoplexes was transferred to recipient cells labelled with galactin-8-GFP to monitor endosome rupture. In separate experiments GFP was tagged to endosomal markers EEA1, Rab5 and Rab7 with RNA release occurring in maturing endosomes. Further, the siRNA cargo exhibited a knockdown of GFP in the recipient cells demonstrating a functional effect.	[5,6]
Recipient cells were labelled with CellMask Deep Red and received donor CD63-GFP extracellular vesicles. CellMask Deep Red was utilized to identify endosomal compartments and localization of CD63-GFP within these compartments was visualized suggesting retention of many vesicles within endosomes.	[7]
Donor extracellular vesicles were labelled with DiD and recipient cells were labelled with membrane dye FM4-64 to assess co-localization of signal. This study also labelled lysosomes with dextran to assess fate of extracellular vesicles contained within the endosomal pathway suggesting recycling of extracellular vesicle lipids to cell periphery and fate of extracellular vesicle proteins in the lysosome.	[8]
CFSE is a cell permeable dye that is retained in cells once hydrolysed. Donor cells treated with CFSE secreted extracellular vesicles containing the dye and when transferred to recipient cells the dye was found to co-localize with	[9]

labelled EEA and LAMP1 showing particles present within the endosomal pathway.	
A mutated variant of pHluorin was fused to an outer loop of CD63 as well as a pH insensitive protein, mScarlet. By combining both fluorophores acidification of endocytosed extracellular vesicles was visualized showing uptake of extracellular vesicles into the endosomes of recipient cells.	[10]
GFPβ11-TAT was added to recipient cells stably expressing the corresponding GFPβ1-10 fragment and rescue of fluorescence was associated with the peptide escaping from the endosome.	[11]
Extracellular vesicles were labelled with R18 dye to determine fusion of the particles with recipient cells. In this same study, extracellular vesicles were also labelled with pHrodo to monitor uptake of particles via phagocytosis.	[12]
Luminescence	
Enduren™ is a substrate that requires esterases to convert it to the functional coelenterazine-h. When converted, it can then be activated by *Renilla* luciferase fused to CD63 or CD9 to allow quantitation of internalization of extracellular vesicles labelled with these fusion proteins.	[13]
Exosomes loaded with DMNPE-caged-luciferin that is retained within the vesicles and then transferred to recipient cells expressing cytosolic luciferase resulted in bioluminescence supporting release of exosome cargo.	[12]

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