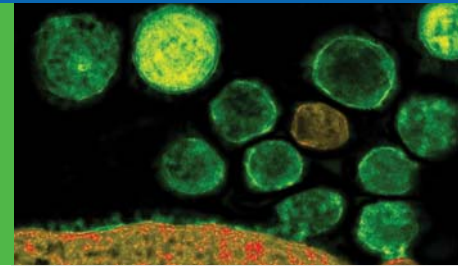


# XPack™ Exosome Protein Engineering

Tag fusion proteins for incorporation into secreted exosomes



## Engineer Exosome Protein Cargo

The XPack technology allows for cell-mediated generation of ready to use exosomes packed with any protein of choice. These exosomes can then be used to efficiently deliver proteins to target cells to alter or supplement biological pathways or be used to study exosome trafficking *in vivo*.

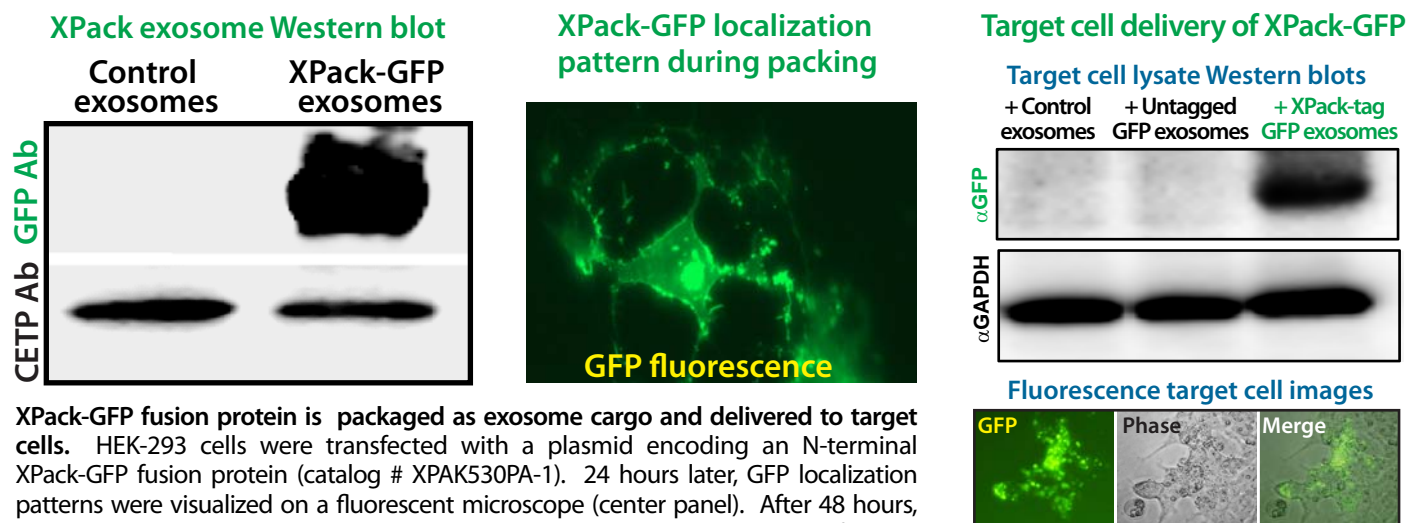
Exosomes are secreted nanoshuttles that facilitate communication between cells and organs and are found in various biofluids including blood, urine, amniotic fluid, breast milk, malignant ascites fluid, and cerebrospinal fluid (CSF). Their natural function as cell to cell communication vehicles makes them attractive for use as therapeutic shuttles to deliver biological molecules or drugs to target disease cells.

SBI has optimized a specific peptide sequence that targets a protein to the interior exosomal membrane, allowing the fusion protein to be packaged into exosomes for secretion. This “XPack” peptide sequence has been incorporated into the XPack cloning and expression lentivectors, allowing for reporter proteins or any protein of interest to be loaded into exosomes within a producer cell line. These engineered exosomes can then be isolated and delivered to target recipient cells. Additionally, stable XPack cell lines have been generated that serve as a constant source of exosomes loaded with reporter protein cargo, enabling tracking of exosome dynamics in both human cells and *in vivo* murine models. Purified, ready to use exosomes from these stable cell lines are also available as part of the comprehensive XPack system.

## Highlights

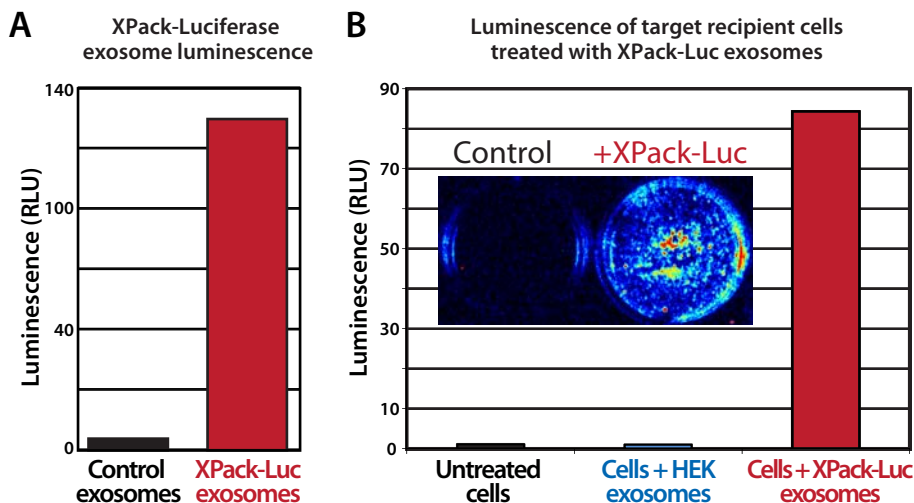
- Package reporter proteins into secreted exosomes to track cargo delivery
- Express and package any protein to create biotherapeutic exosomes
- Generate bioluminescent exosomes for tracking delivery *in vivo*
- Create stable cell lines producing XPack exosomes with any protein cargo

## Example Data for XPack-GFP Loaded Exosomes



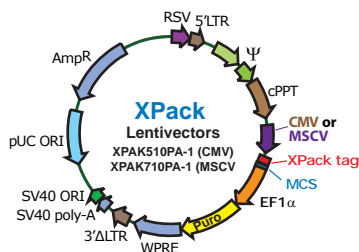
XPack-GFP fusion protein is packaged as exosome cargo and delivered to target cells. HEK-293 cells were transfected with a plasmid encoding an N-terminal XPack-GFP fusion protein (catalog # XPAK530PA-1). 24 hours later, GFP localization patterns were visualized on a fluorescent microscope (center panel). After 48 hours, exosomes were isolated using ExoQuick-TC® and analyzed by Western blot for GFP loading (upper left panel). XPack-GFP exosomes were also added to target HEK-293 cells and 24 hours later, cells were imaged for GFP delivery into target cells and then lysed for Western blot analysis (right panels).

## Use the XPack Technology to Create Bioluminescent Exosomes



### XPack Delivers Luciferase to Target Cells

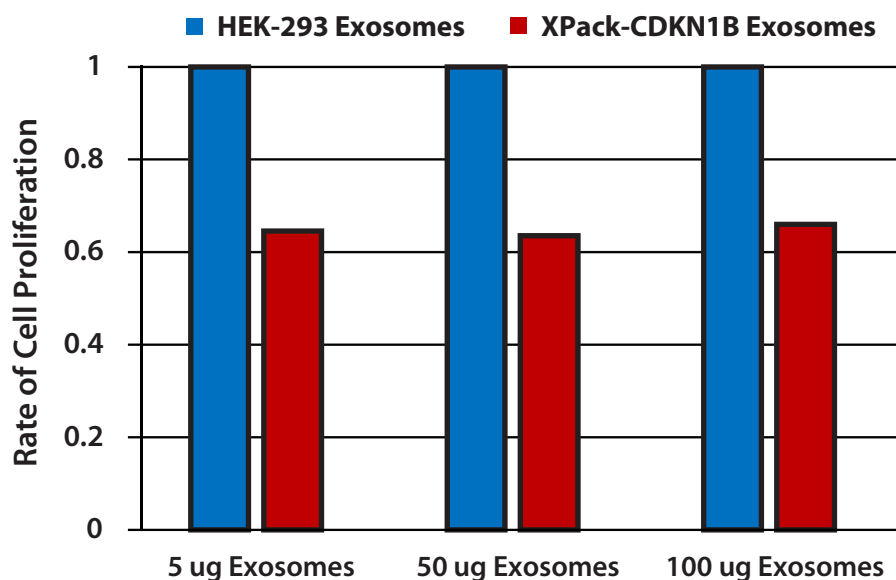
The XPack-Luciferase fusion protein (~62 kD) is packaged as exosome cargo and can be delivered to target cells. HEK-293 cells were first transfected with the XPack-Luc plasmid (cat# XPAK532PA-1) encoding the XPack-Luciferase fusion protein. The secreted exosomes were collected from the media after 48 hours using ExoQuick-TC. Luciferase levels of exosomes from control cells and XPack-Luc producer cells were quantitated using standard luminescence assays (Panel A). XPack-Luciferase exosomes (250 ug) from were then added to HEK-293 target cells in culture in a 6-well plate format. Luciferase activities were measured after 24 hours and data are shown in Panel B. Control cells were either not treated with exosomes (Untreated cells) or with exosomes from untransfected HEK-293 cells (Cells + HEK exosomes). The image inset is from imaging live HEK-293 cells treated with either Control or XPack-Luc exosomes. Approximately 400K cells were incubated with 150 ng/ml D-Luciferin substrate for 2 minutes at ambient temperature prior to signal imaging.



### XPack Lentivector Features

- CMV or MSCV promoter options
- Built-in XPack N-terminal tag
- EF1-Puromycin selection cassette
- High titer lentivirus backbone

## XPack-CDKN1B Exosomes Alter Target Cell Proliferation



### Custom XPack Construction and Exosome Loading Services Available!

#### XPack Delivered Proteins are Functional:

An XPack fusion to the cell cycle inhibitor CDKN1B was transfected into HEK-293 cells. Exosomes were isolated from the media using ExoQuick-TC after 48 hours. A range of 5 ug to 100 ug of XPack-CDKN1B exosomes were then added to HEK-293 target cells in culture at 24 hour intervals for a total of 72 hours. Exosomes from non-transfected cells were used as a control. Cell division was quantified using an MTT Assay (Millipore catalog # CT02) and signals were normalized to HEK-293 cells treated with control exosomes.

## We Also Offer Custom Services

System Biosciences offers a wide-range of custom services to support your research, allowing you to spend less time making tools, and more time making discoveries. To learn more, visit our website at [www.systembio.com/service](http://www.systembio.com/service) or call us at 888-266-5066.