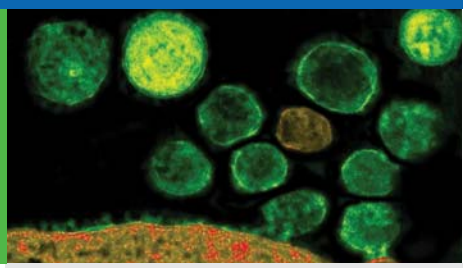
System Biosciences
Accelerating discoveries through innovations

Exosome Research

XStamp™ Exosome Targeting Technology

Engineer exosome surfaces for protein ligand display



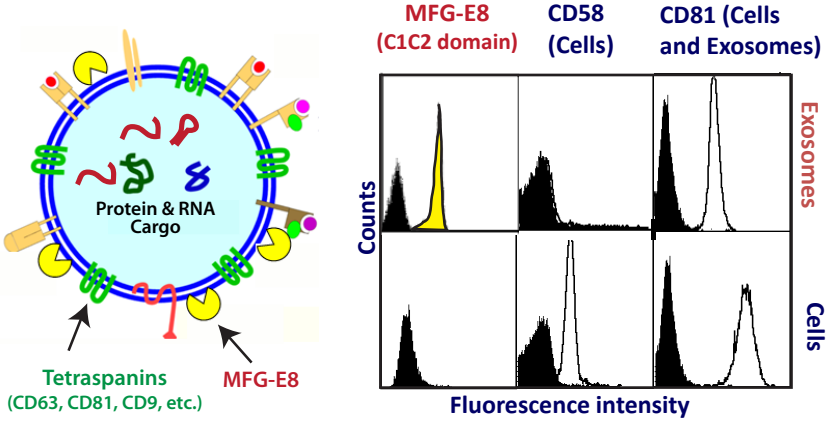
Program Exosomes with Specific Addresses

Exosomes are extracellular nanoshuttles that facilitate communication between cells and can be engineered as therapeutic shuttles to deliver biological molecules or drugs to target disease cells. SBI has developed an exosome surface display system that enables desired protein sequences to be placed efficiently on the surfaces of engineered exosomes called the "XStamp" technology. The patented XStamp technology is based upon a C-terminal fusion of the C1C2 domain from MFG-E8. Protein sequences that are fused to the XStamp tag will efficiently display the protein ligand fusion on the surfaces of secreted exosomes. The technology can be used to place cellular "addresses" on exosomes that send them to specific destinations for cargo delivery.

Highlights

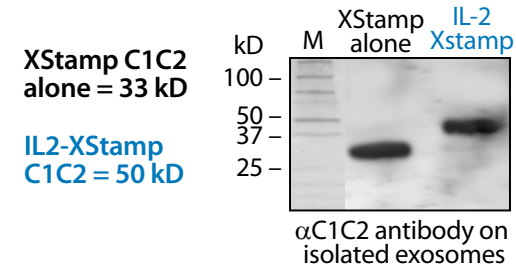
- Display proteins on the surfaces of secreted exosomes
- Coat exosomes with targeting ligands
- Target engineered exosomes to specific cellular destinations
- Create stable cell lines producing XStamp exosomes with any display protein for targeting

MFG-E8 Localizes to Exosome Surfaces (C1C2 domain)



Fluorescently-labeled antibodies for MFG-E8, CD58 and CD81 were used in combination for FACs analysis. The CD58 marker is a known cell surface marker that is absent on exosomes. CD81 is known to be present both in cells and exosomes. The FACs data show that MFG-E8 is exclusively detected on exosomes and not present in the cells.

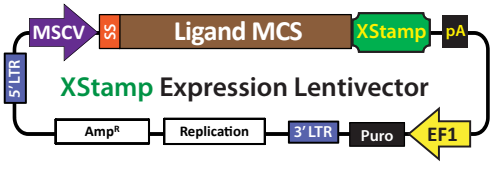
XStamp Western Blot



The empty, cloning XStamp lentivector (cat# XSTP710PA-1) and the human IL2-XStamp lentivector (cat#XST726PA-1) were transfected into HEK293 cells. The secreted exosomes were collected after 48 hours and analyzed by Western blot analysis for the presence of the engineered XStamp proteins in exosome lysates. The IL-2 XStamp construct is catalog# XSTP726PA-1.

The XStamp System

To take advantage of the localization of MFG-E8 on exosomes, the C1C2 domain (XStamp domain) of the protein's gene was cloned into SBI's MSCV-MCS-EF1-Puro lentivector and a 5' secretion signal sequence (SS) was placed within the multiple cloning site. The protein ligand chosen to display on exosomes is cloned into the MCS and fused to the C1C2 domain. The XStamp lentivector catalog # XSTP710PA-1 also features a downstream EF1-Puromycin cassette for selection and stable cell line development. The lentivector constructs can be used in transient transfection expression studies as well as for packaging into lentivirus to stably transduce cells to create cellular factories producing engineered exosomes.



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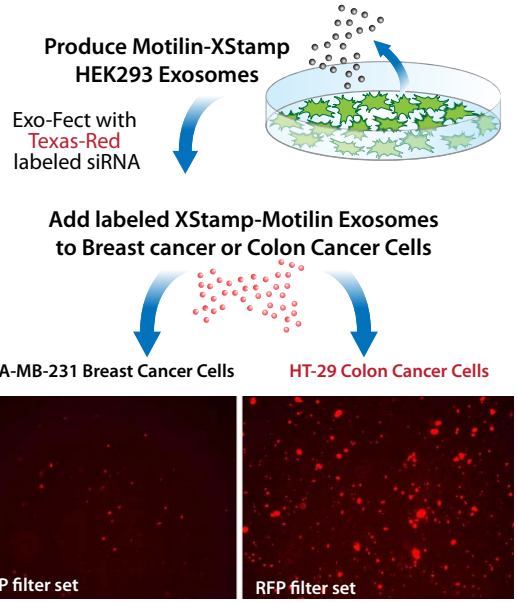
XStamp™ Exosome Targeting Technology

Motilin-XStamp for GI tract targeting

Motilin is a 22-amino acid polypeptide hormone that binds to the Motilin receptor which is exclusively expressed in the intestine. To test the Motilin-XStamp construct (catalog# XSTP720PA-1), it was transfected into HEK293 cells and after 48 hours, the exosomes were collected using ExoQuick-TC. The next day, the XStamp-Motilin exosomes were Exo-Fected with a Texas-Red-labeled siRNA to monitor exosome docking and delivery. The transfected XStamp-Motilin exosomes were then added to MDA-MB-231 Breast Cancer Cells (motilin receptor negative) and to HT-29 Colon Cancer Cells (motilin receptor positive). The cells were imaged after 24 hours for uptake of the Texas-Red-labeled siRNA delivery from the XStamped exosomes. The HT-29 colon cancer cells that are motilin receptor positive took up the XStamp-Motilin exosomes at a much higher rate than the MDA-MB-231 Breast Cancer (motilin receptor negative) cells.

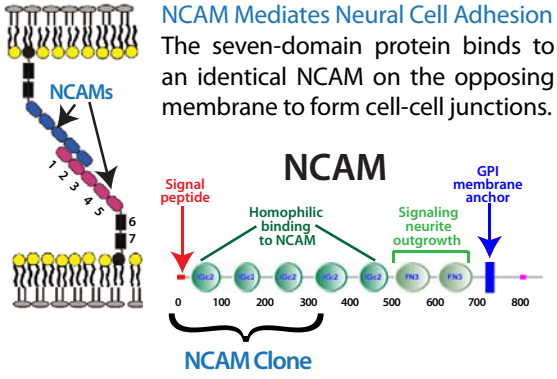


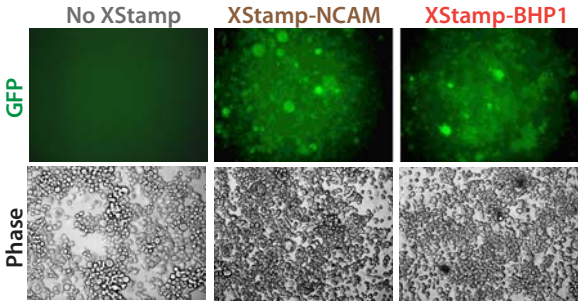
Targeting Neurons using XStamps and Exosomes

An NCAM-XStamp fusion was constructed which incorporated the first 300 amino acids (Signal peptide plus IGc2 domains 1-3) of the mouse NCAM gene translationally fused to the C1C2 XStamp display tag (catalog# XSTP721PA-1). In parallel, a Brain Homing Peptide (BHP1, from "*Organ targeting in vivo using phage display peptide libraries.*" Pasqualini R, et al. Nature (6572):364-6) was fused to the C1C2 XStamp domain to create the BHP1-XStamp construct (catalog# XSTP722PA-1).

NCAM-XStamp and Brain Homing Peptide 1 for Neural Targeting

The NCAM-XStamp construct (catalog# XSTP721PA-1) and the BHP1-XStamp construct (catalog# XSTP722PA-1), were transfected separately into mouse MSCs along with SBI's XPack-GFP construct (catalog# XPAK530PA-1) which packages GFP into the interior of exosomes for fluorescent tracking. After 48 hours, the exosomes were collected. Equal amounts (100 ug) of Control (No XStamp), NCAM-XStamp or BHP1-XStamp MSC exosomes (all labeled with XPack-GFP) were added to Neuro2a neuroblastoma cells in culture. The neurons were imaged for phase and GFP signals after a 24 hour incubation with the various exosomes to monitor GFP delivery mediated by the XStamp ligand coats. Taken together, this is known as the "***Pack and Stamp***" system, which can be adapted for any cargo and any delivery target.





Neuro2a neuroblastoma cells

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SBI System Biosciences

System Biosciences, Inc.
265 North Whisman Rd.
Mountain View, CA 94043

Toll Free: 888.266.5066
Fax: 650-968-2277
Email: info@systembio.com
www.systembio.com