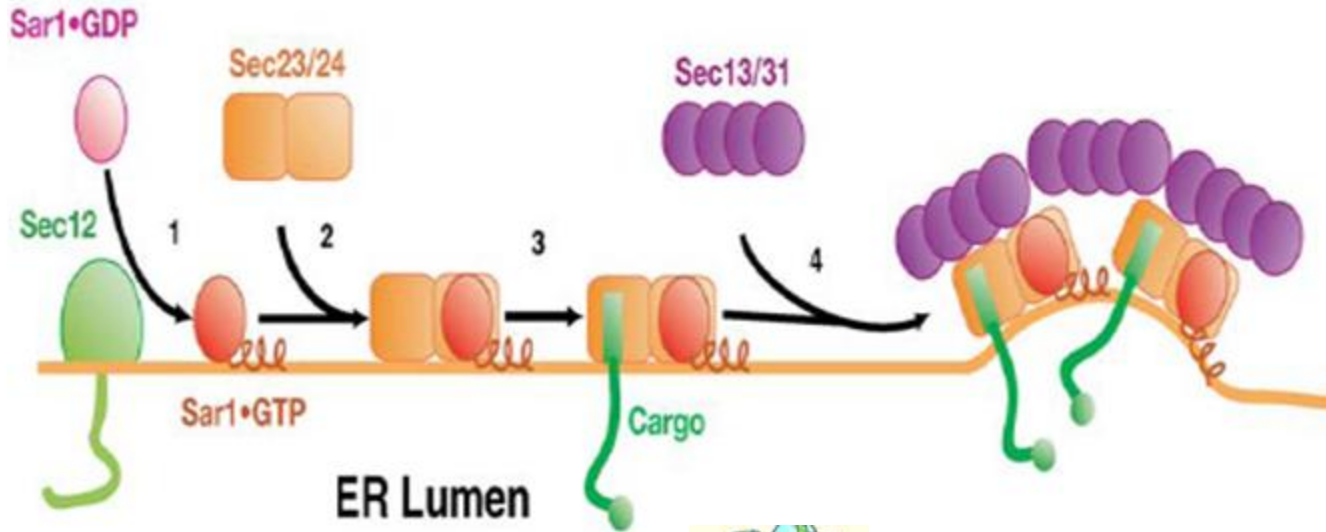


Genes and proteins required for secretion of large particles and miRNAs

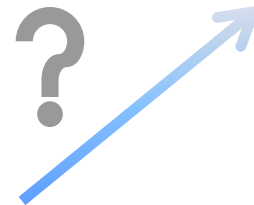
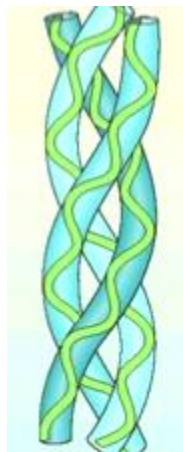
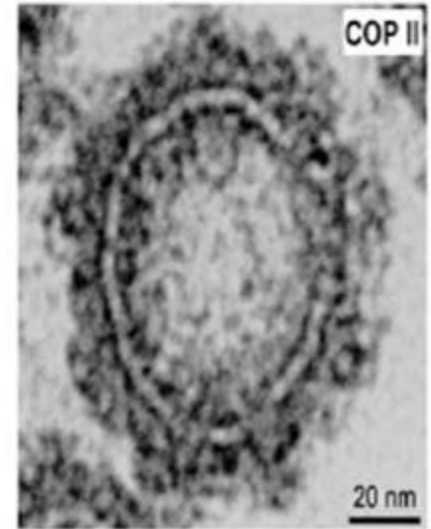
KITP course
August 20, 2015

Randy Schekman
Department of Molecular and Cell Biology
Howard Hughes Medical Institute
University of California, Berkeley

How does a COPII vesicle accommodate large cargo?

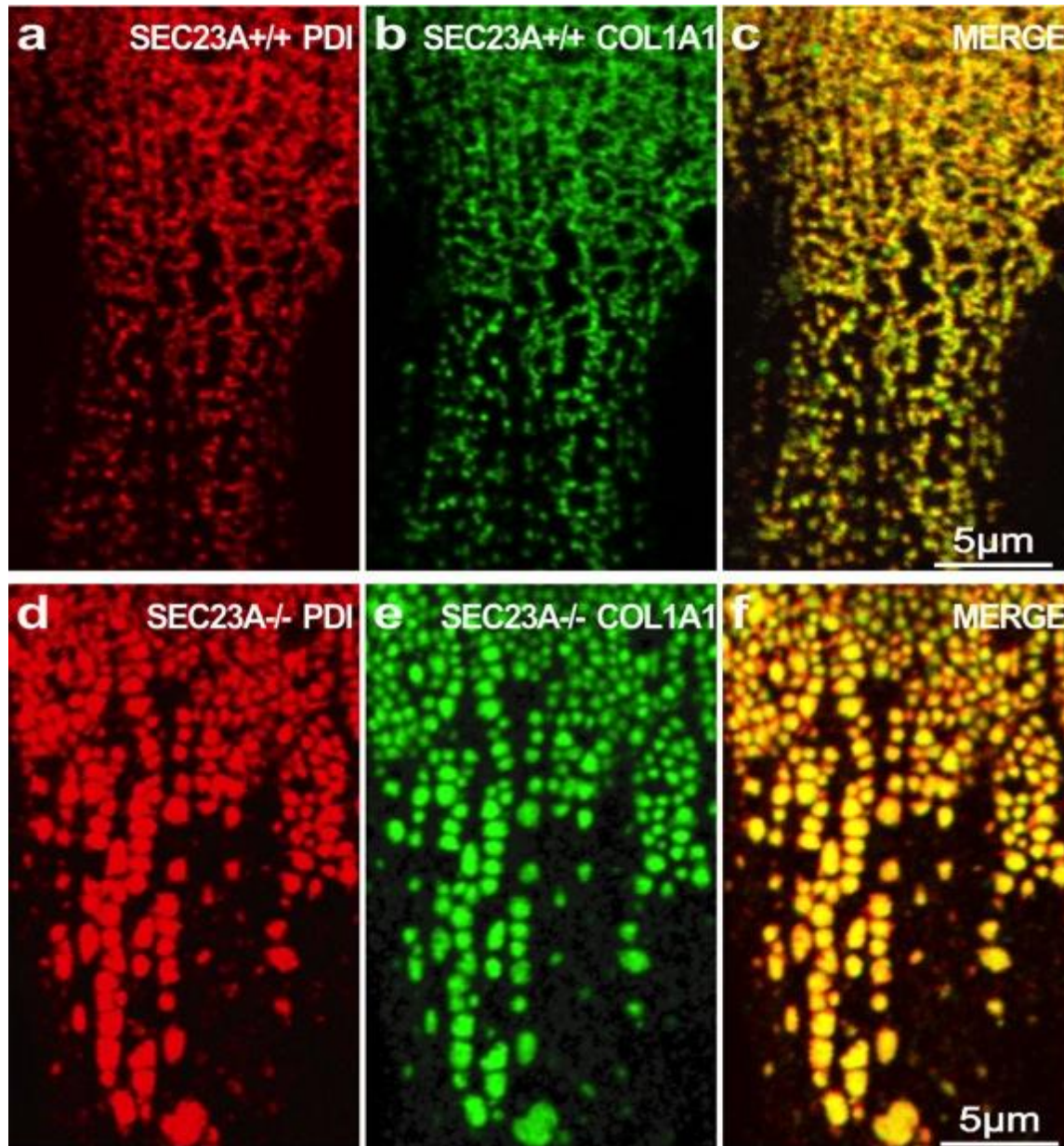


60-70 nm



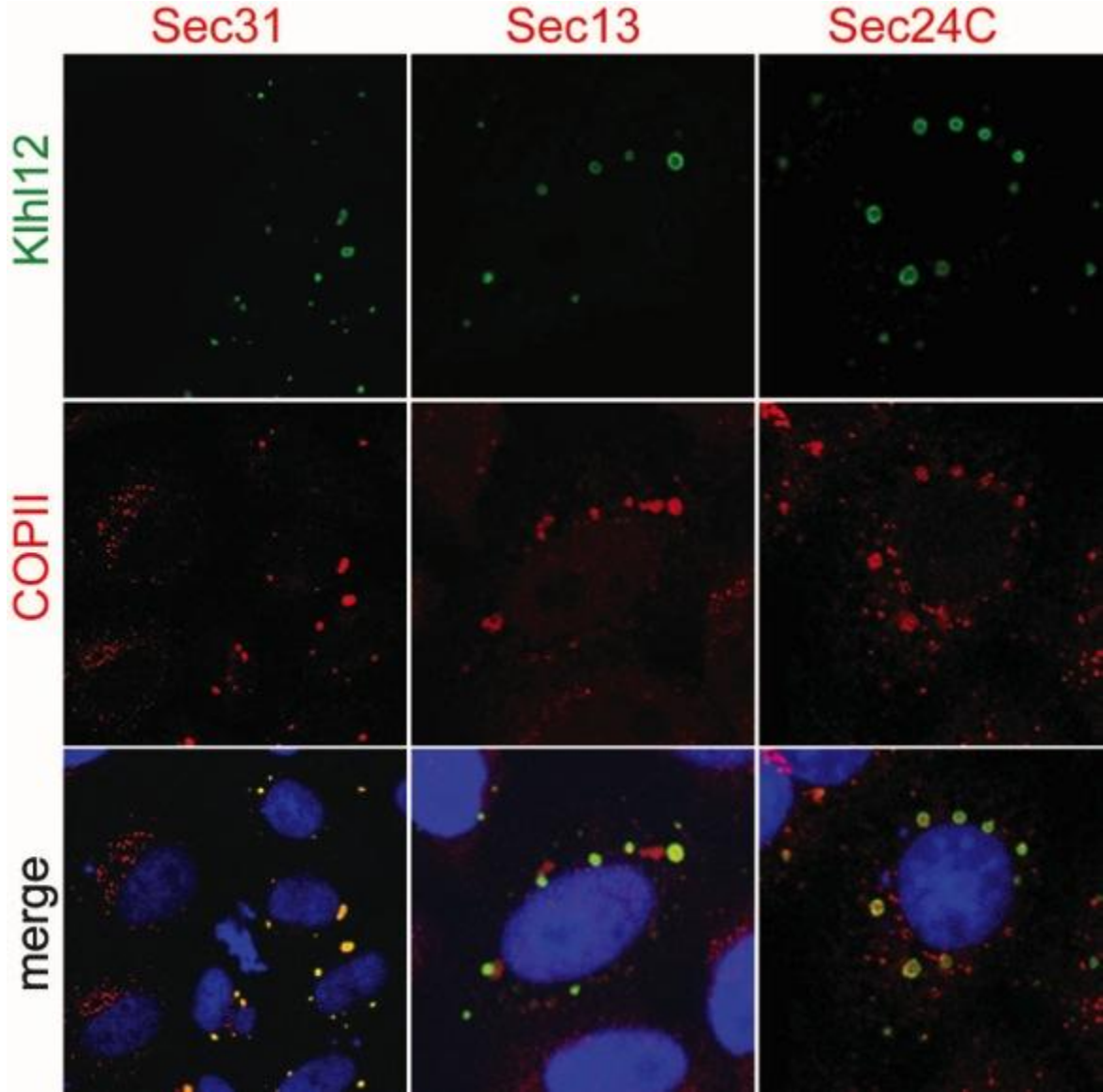
Procollagen (soluble cargo)
300 nm

Collagen accumulates in the ER in CLSD mutant fibroblasts

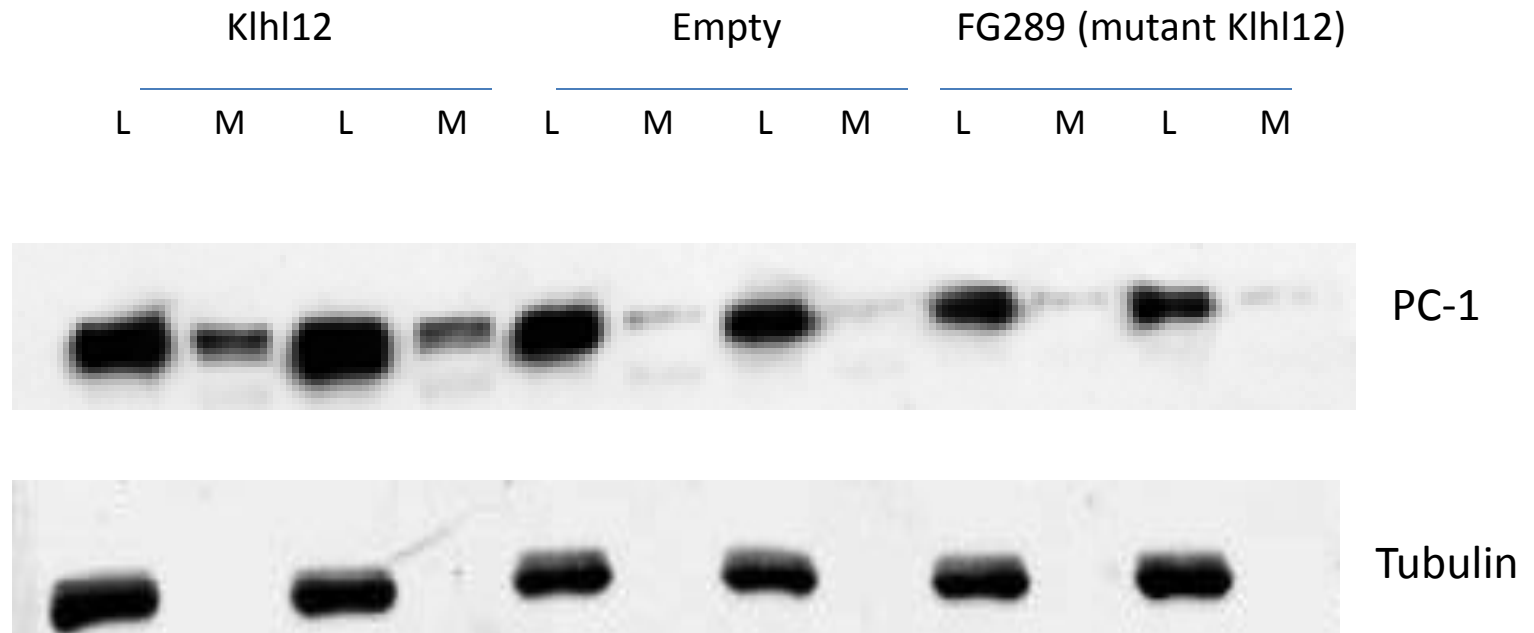




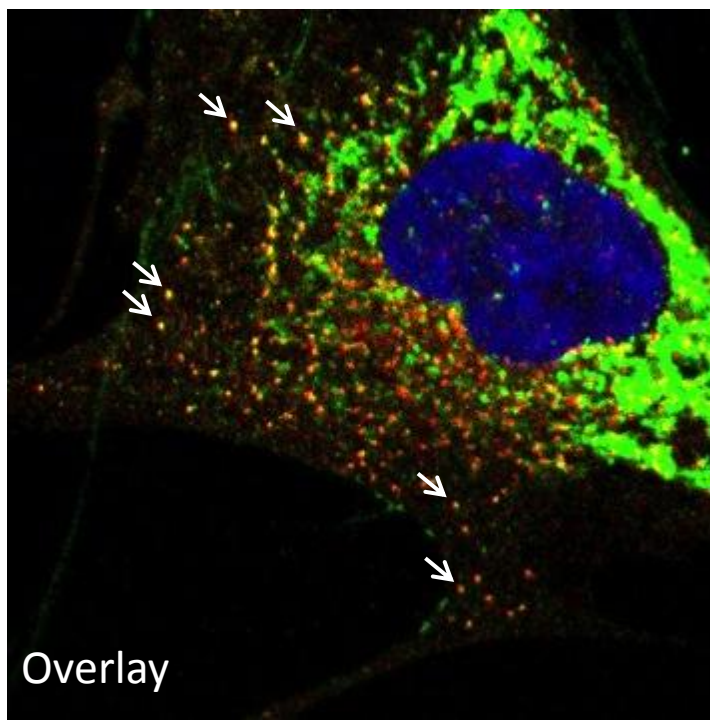
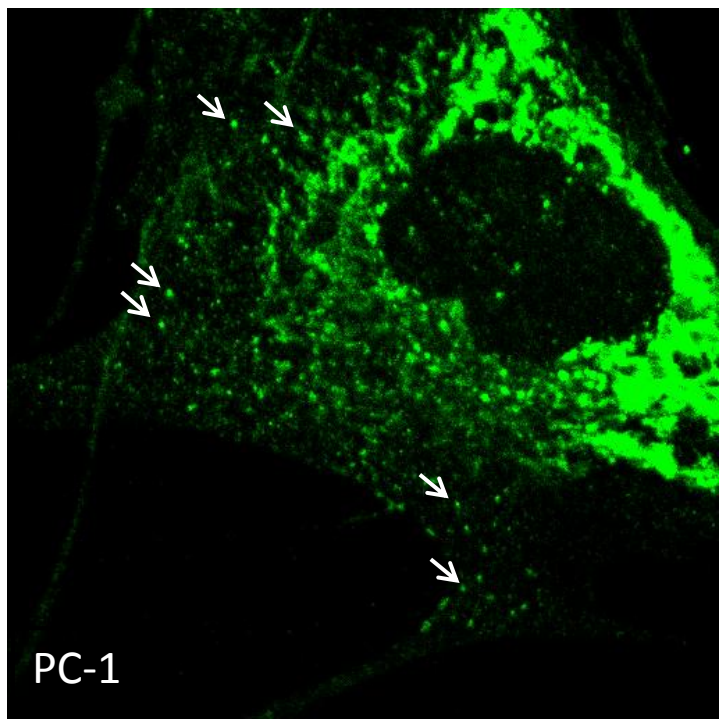
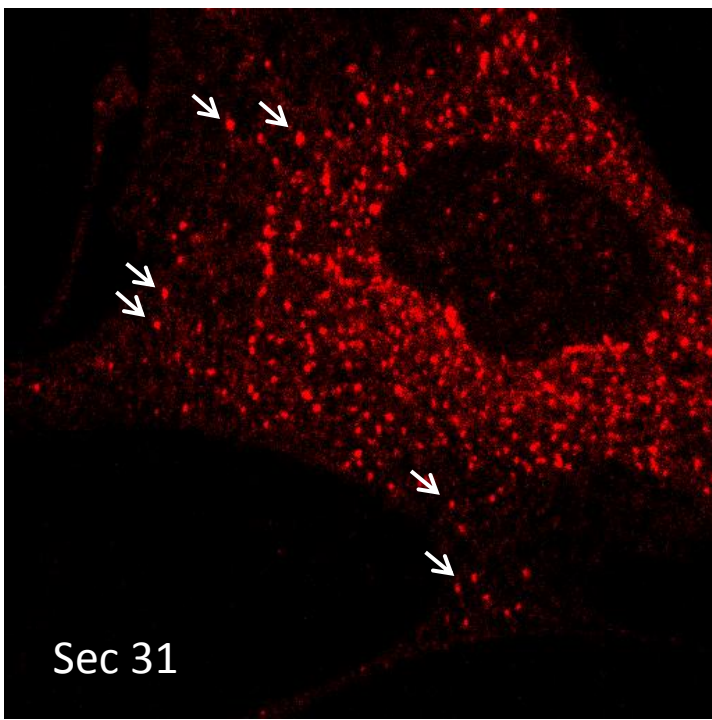
Cul3^{Klh12} increases the size of COPII vesicles



Overexpression of wt but not mutant Khl12 promotes collagen secretion

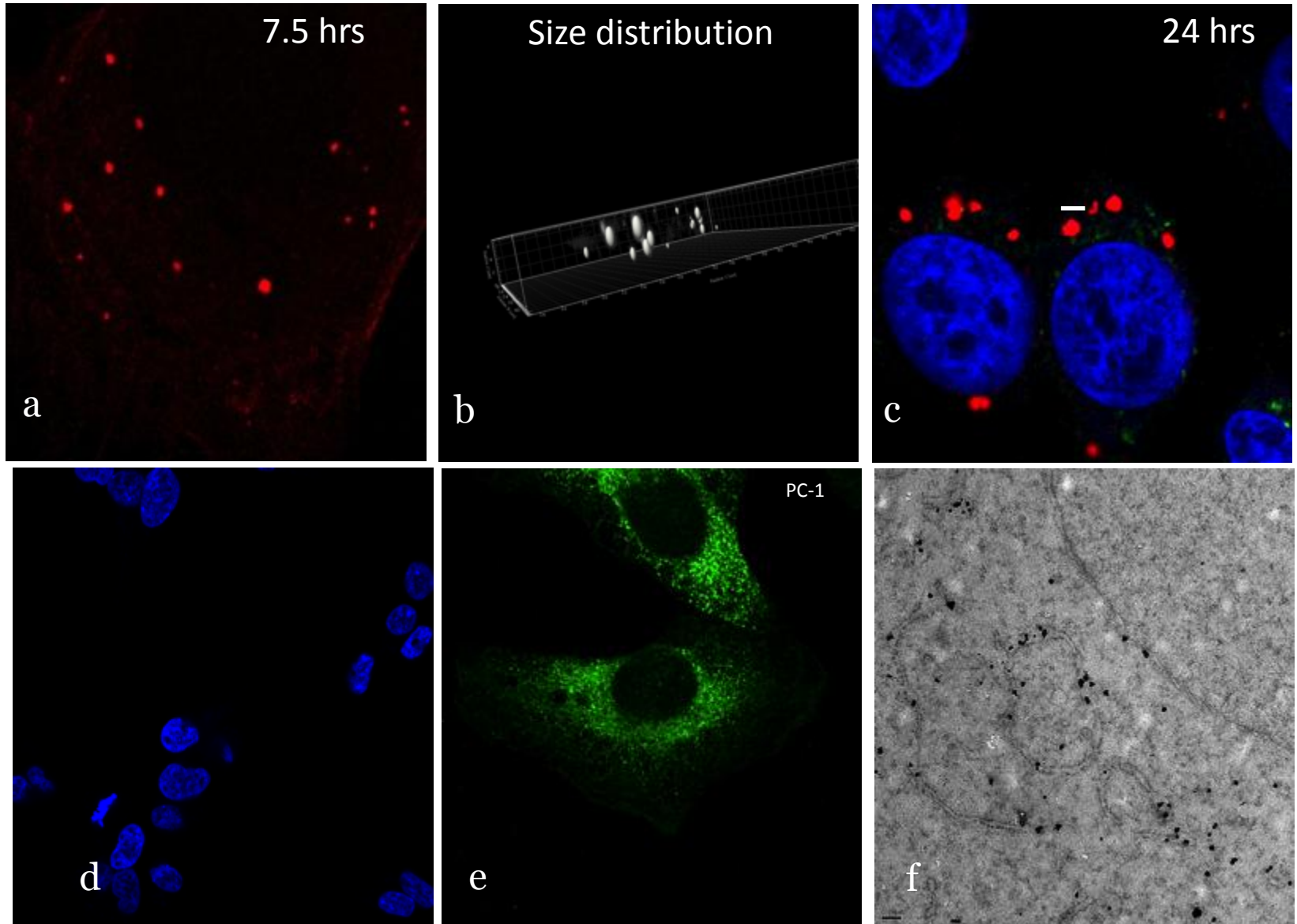


L, lysate ; M, media

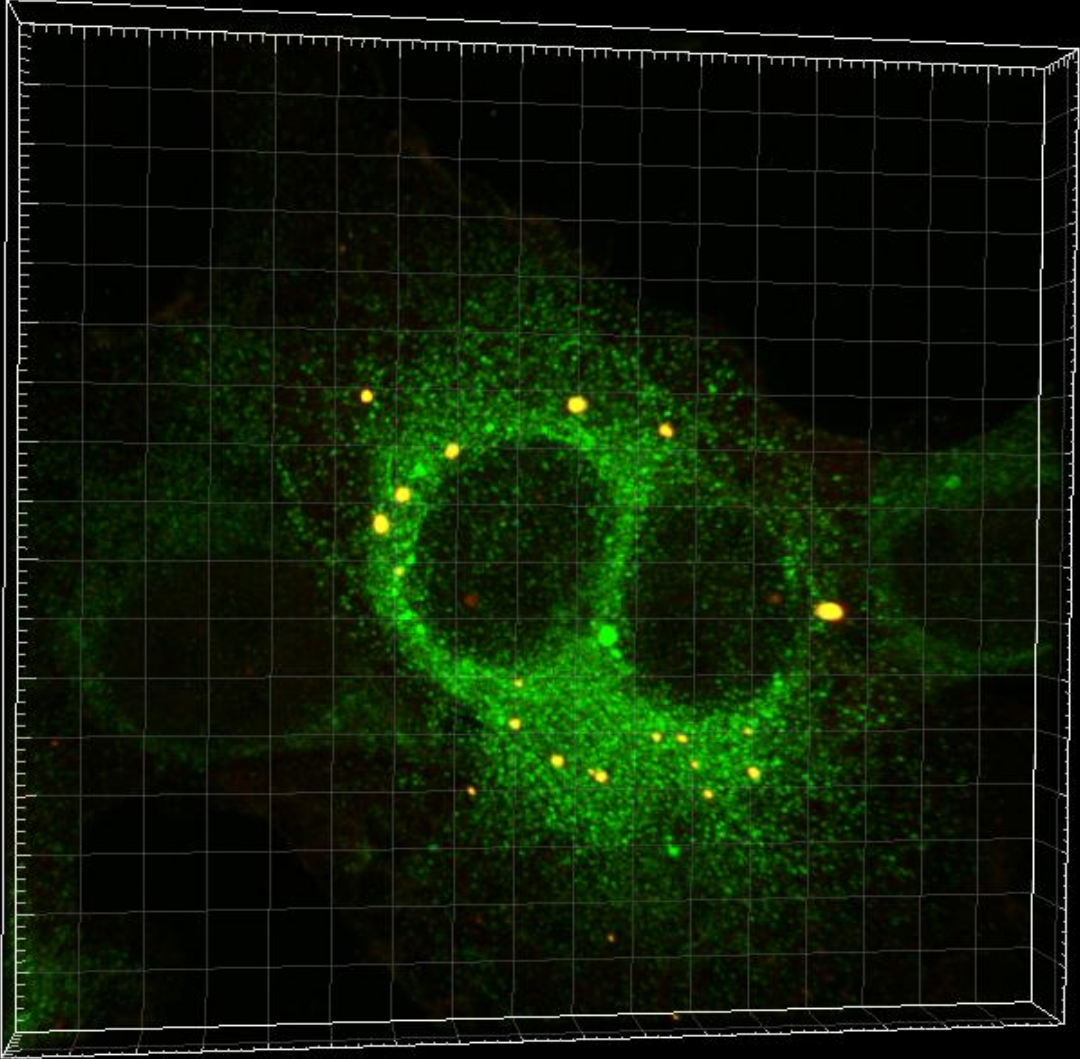


Sec31/PC-1 co-localization in IMR90 fibroblasts

Generation of doxycycline inducible Human Fibrosarcoma (HTPC KI) cell line stably transfected with PC-1 for structure/function analysis of COPII megacarriers

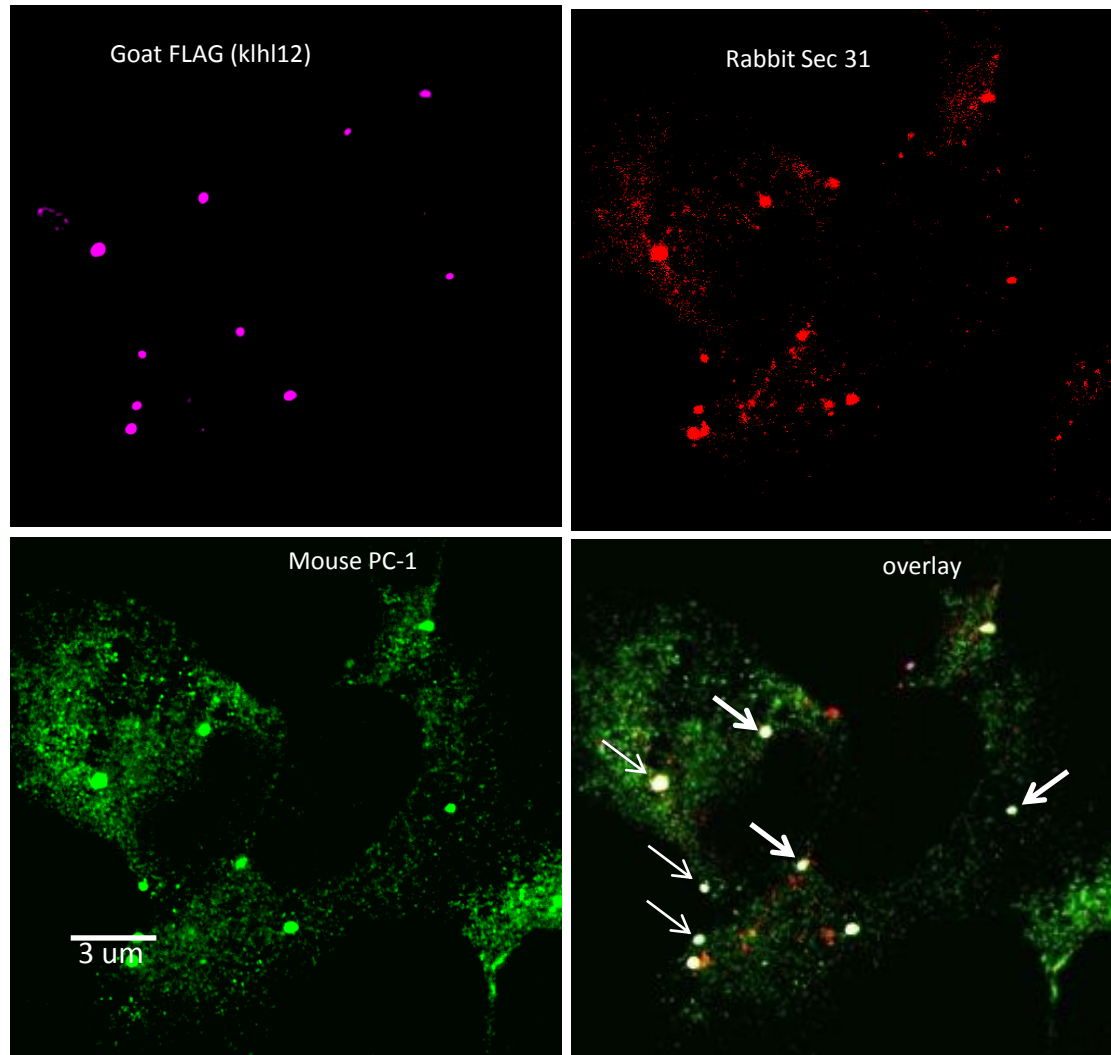


Double Immunofluorescence labeling showing co-localization of khl12/PC-1

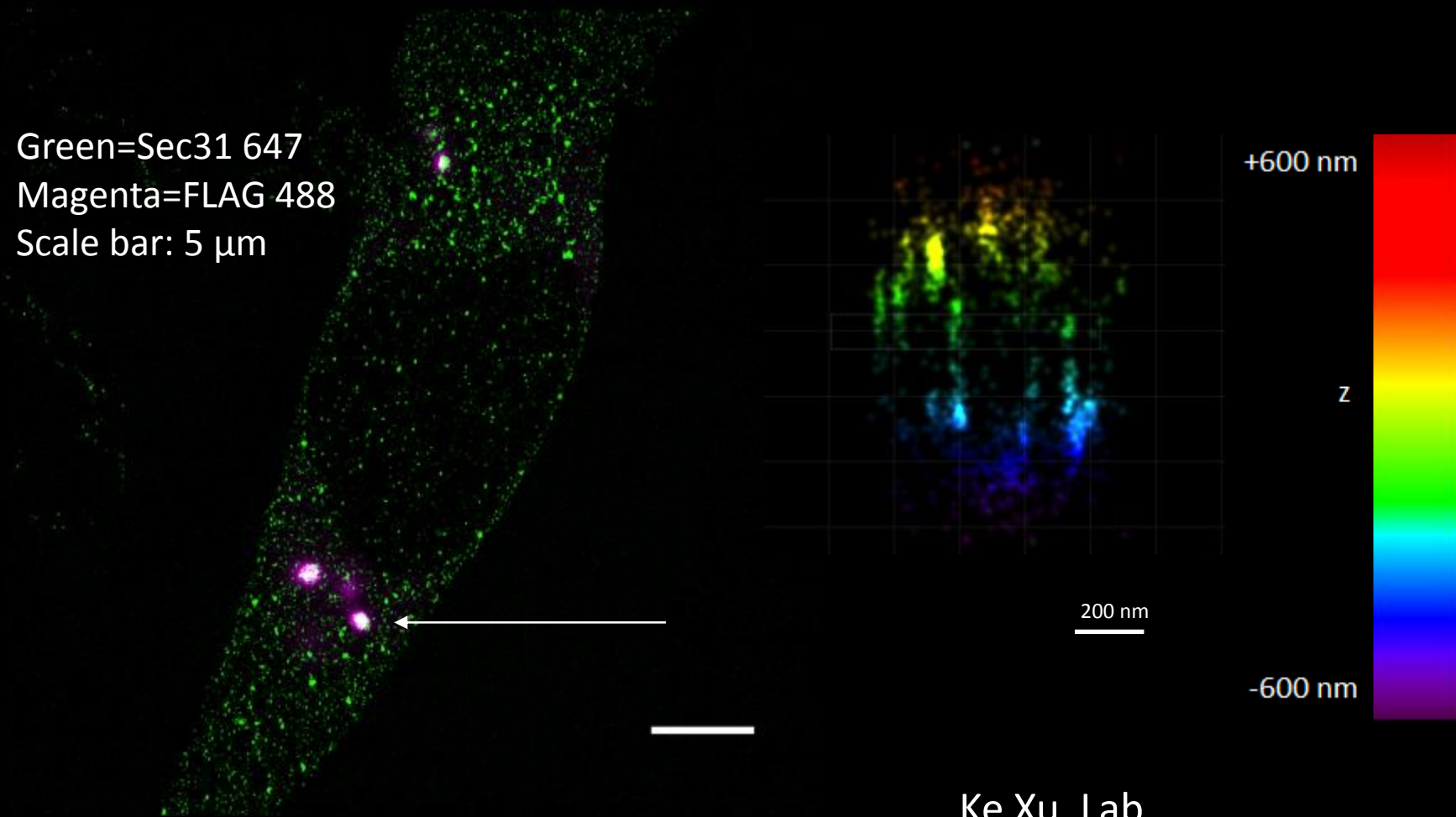


15.0 um

Triple Immunofluorescence labeling showing co-localization of klhl12/PC-1/Sec31

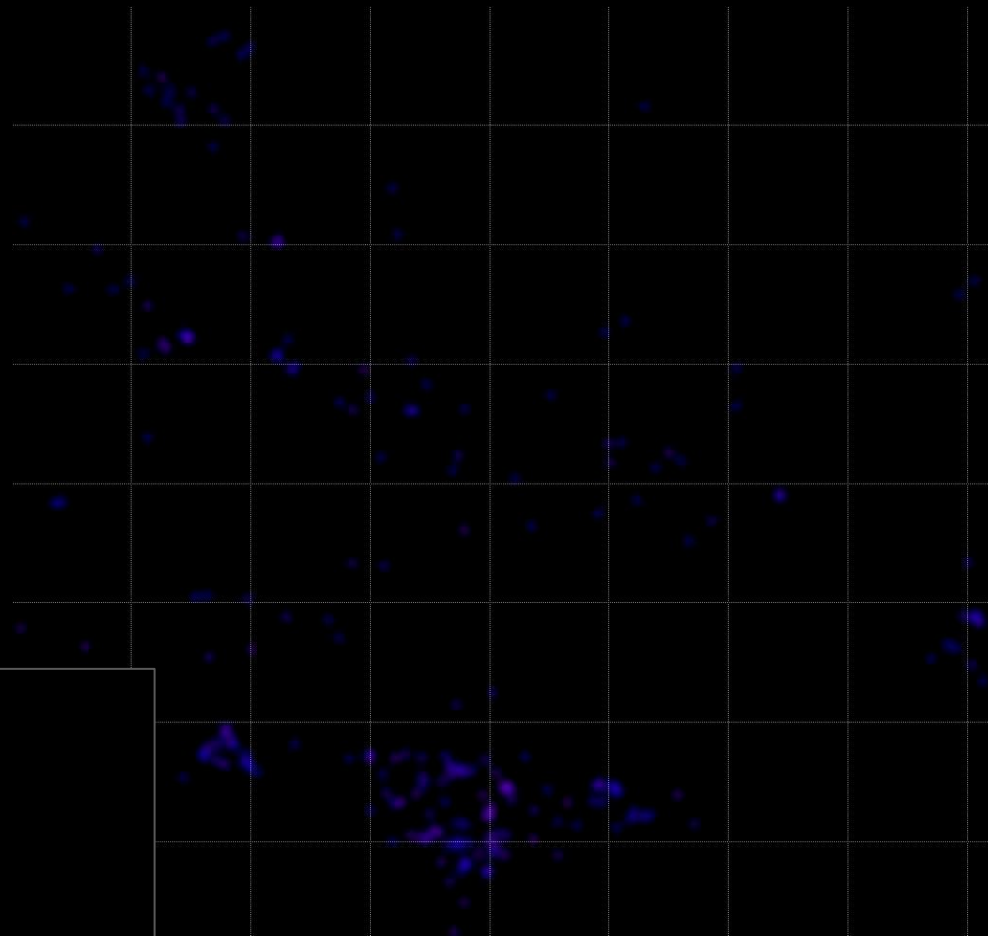
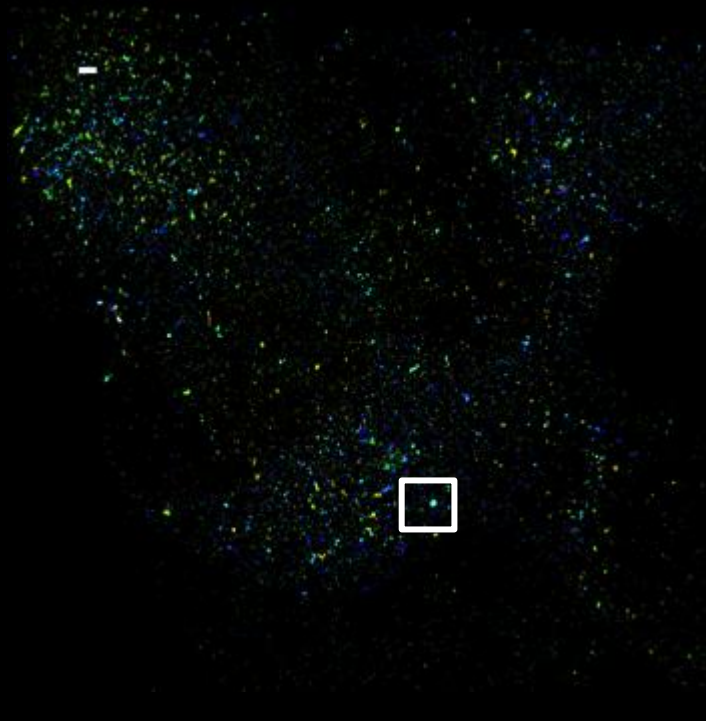


SUPER-RESOLUTION 3D STORM IMAGING OF ENLARGED COPII VESICLES

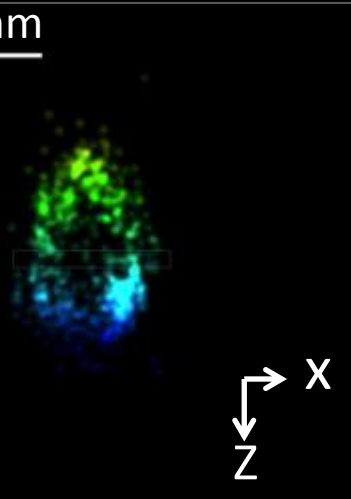


Ke Xu Lab
Dept. of Chemistry, UC Berkeley

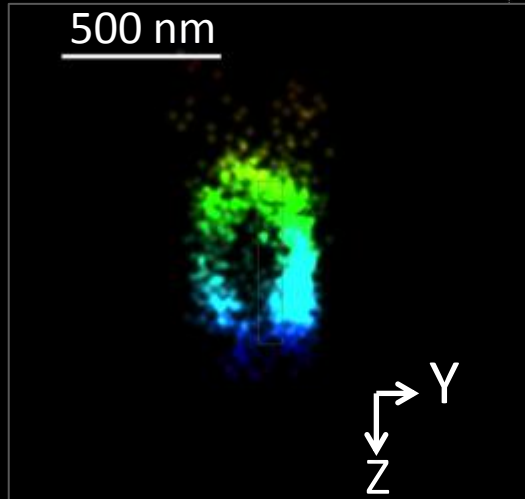
SUPER-RESOLUTION 3D STORM IMAGING REVEALS A HOLLOW CAGE



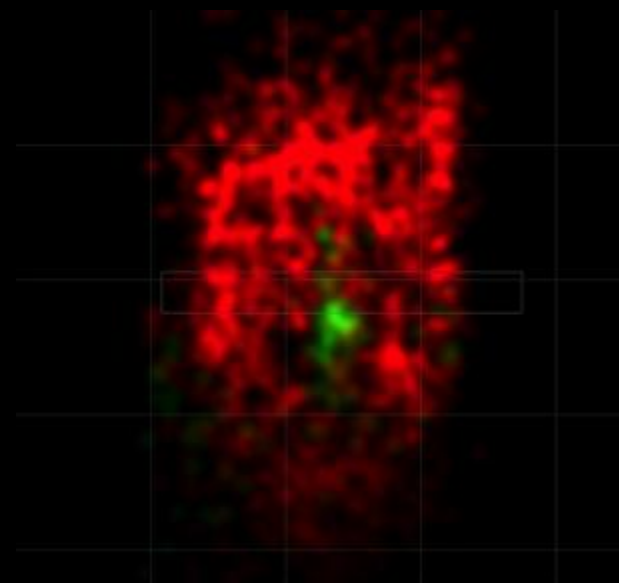
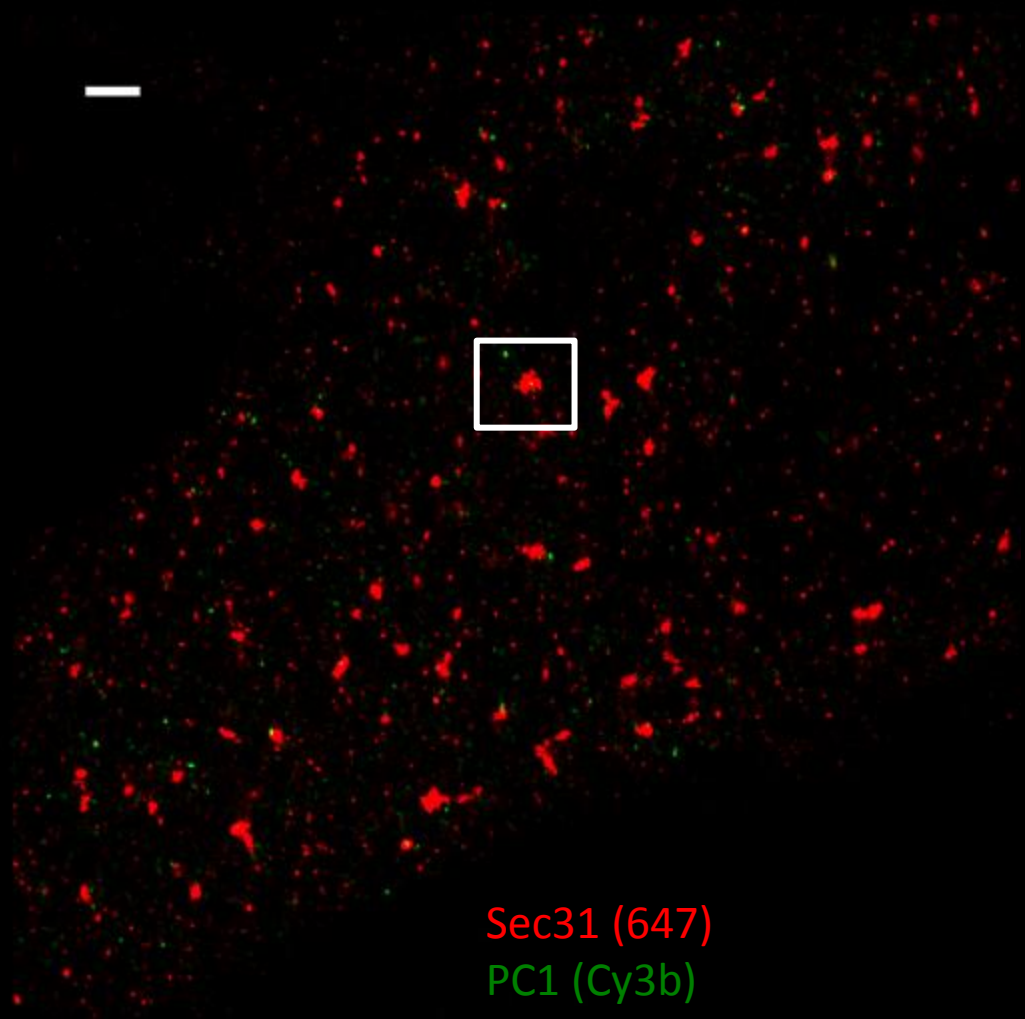
500 nm



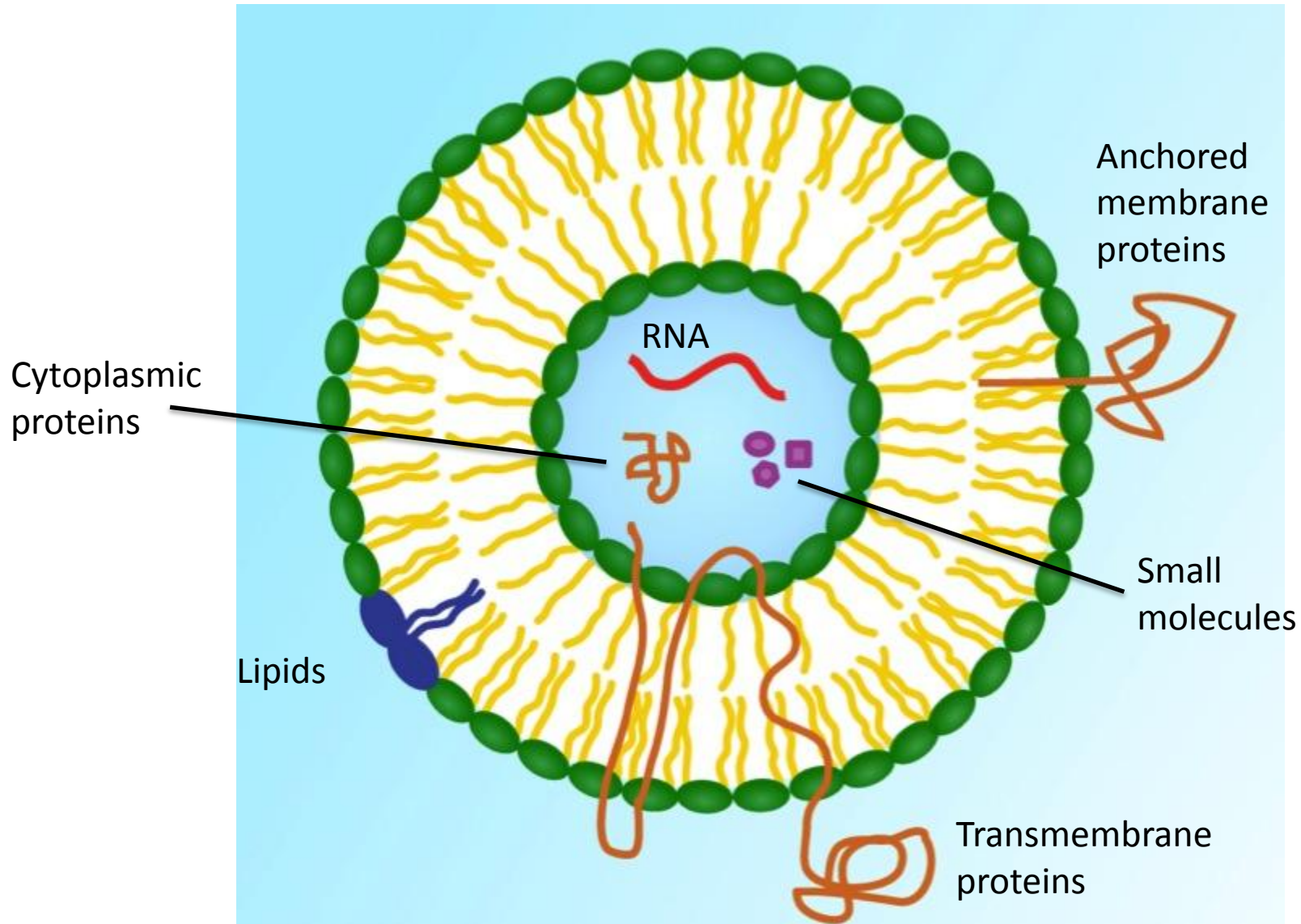
500 nm



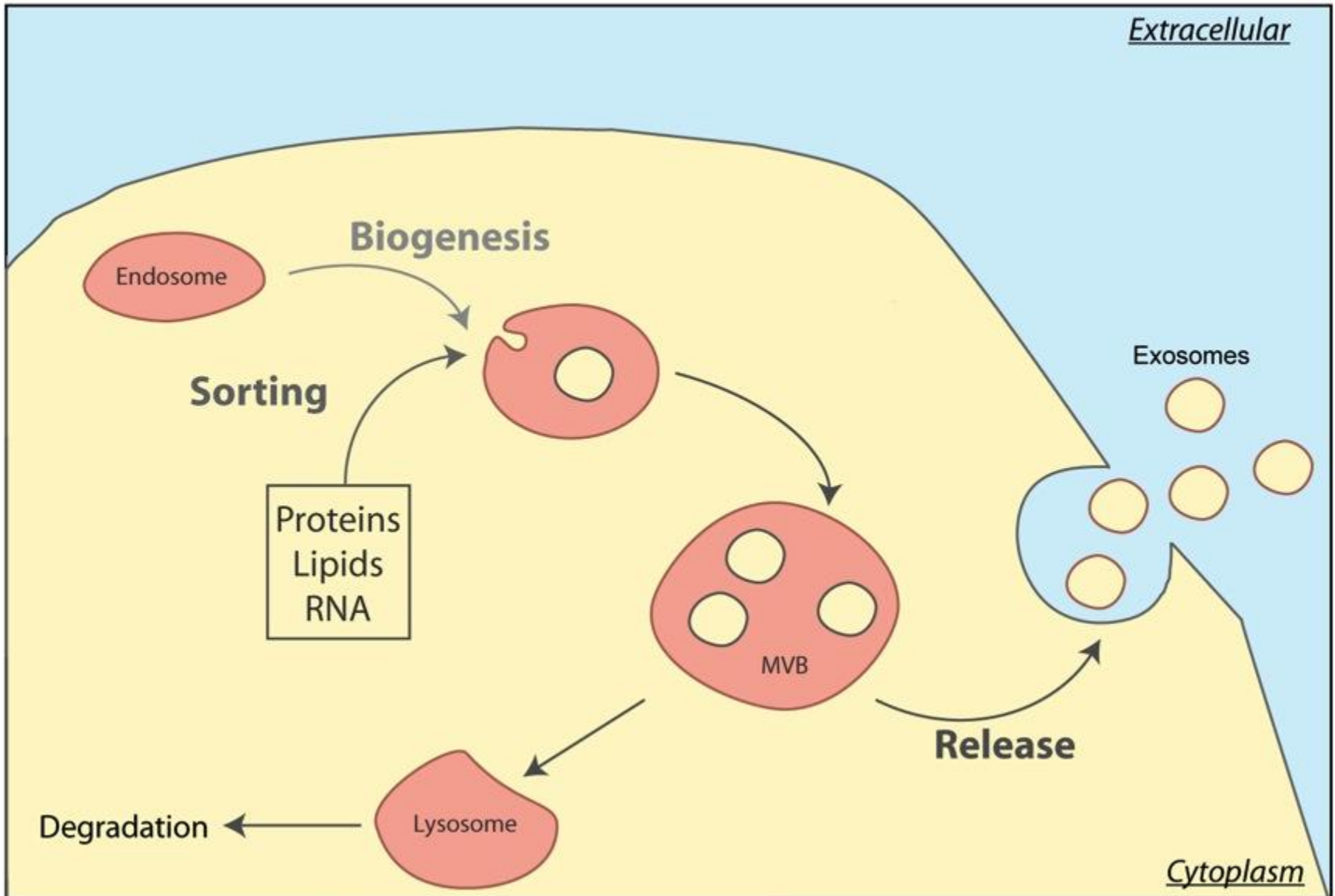
2 COLOR 3D STORM IMAGING REVEALS A CAGE ENCAPSULATING PROCOLLAGEN



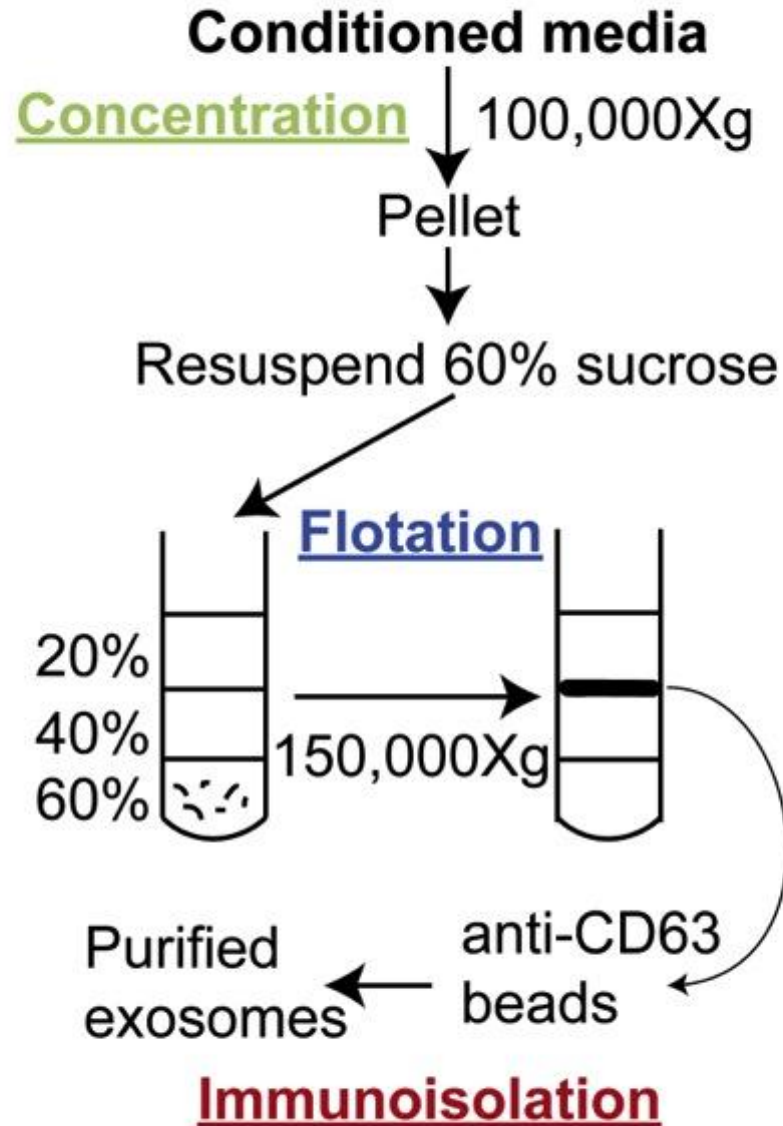
Extracellular vesicles as carriers of unconventional signals



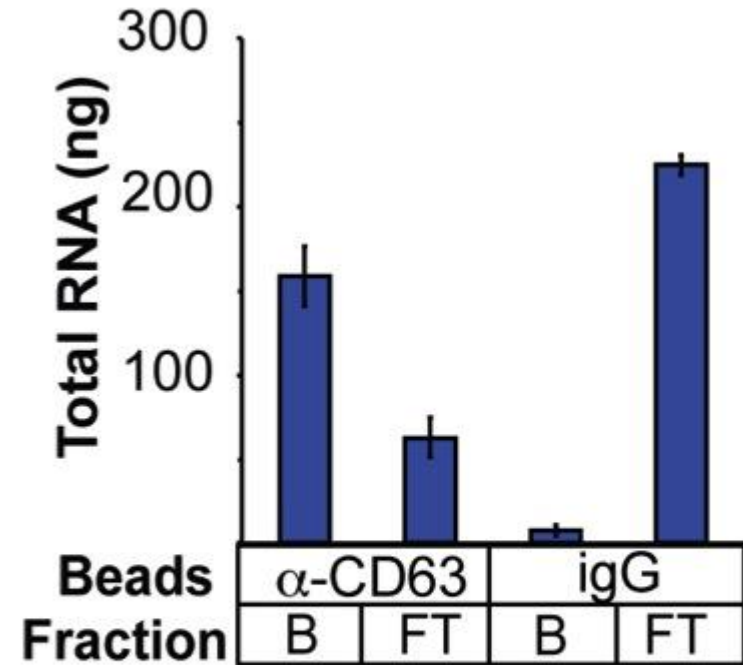
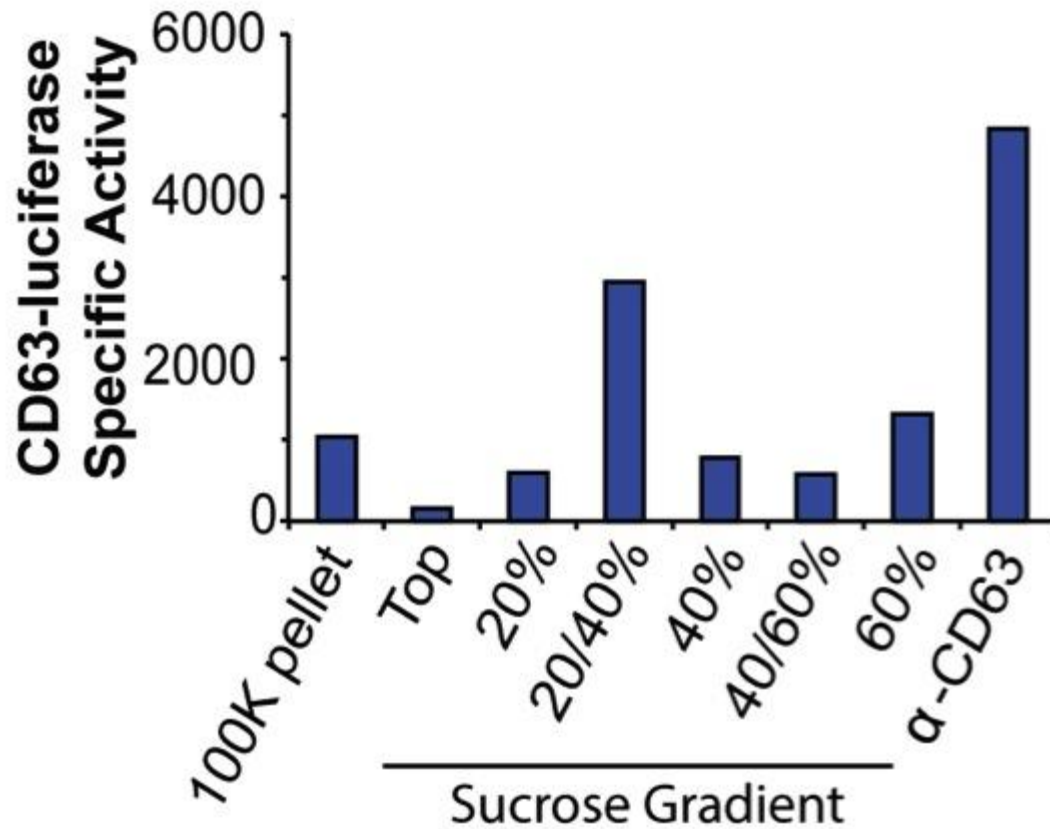
Origin and secretion of exosomes



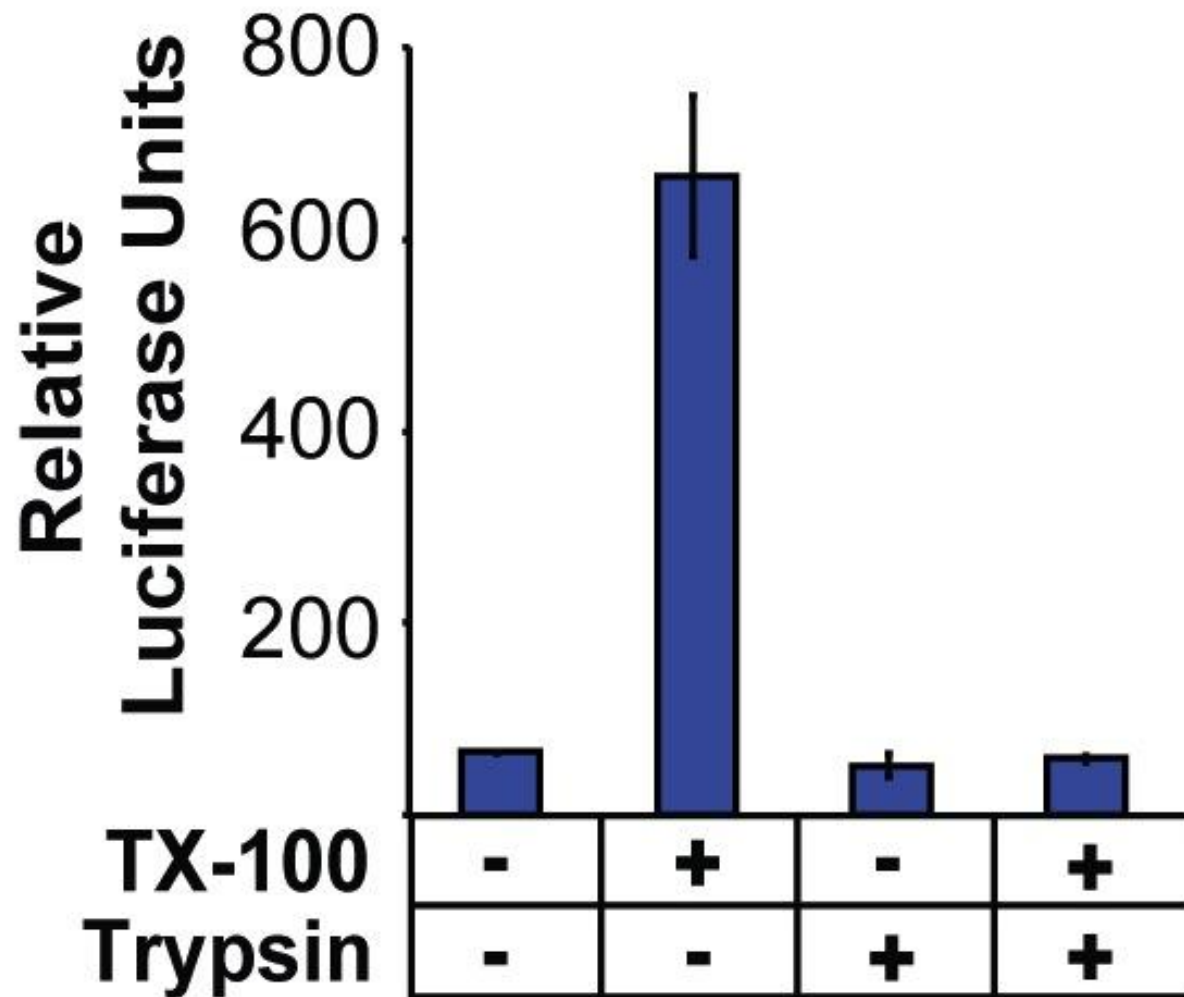
Purification of CD63 exosomes



CD63-luciferase and RNA in purified exosomes

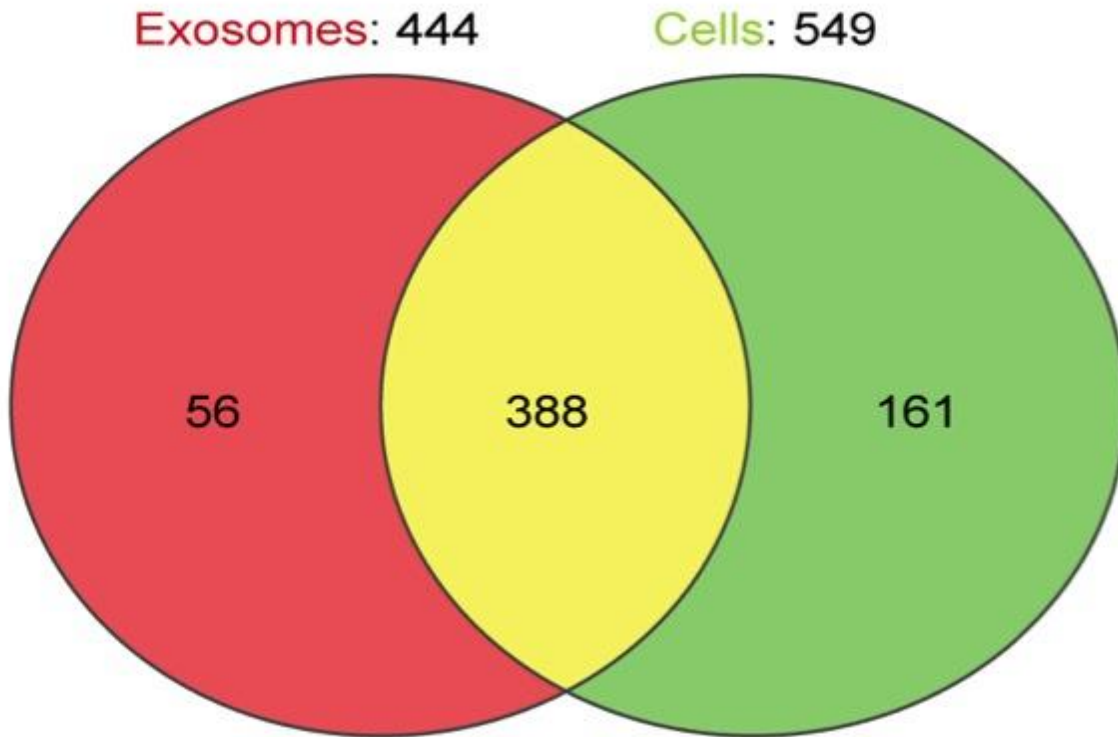


Luciferase latent in CD63 exosomes



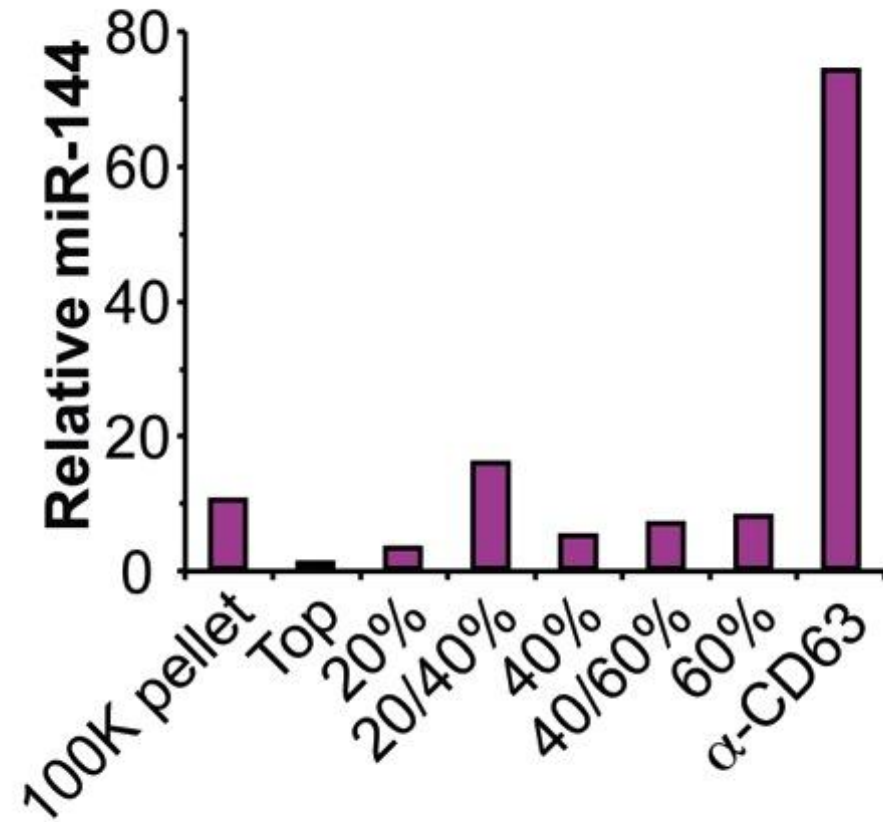
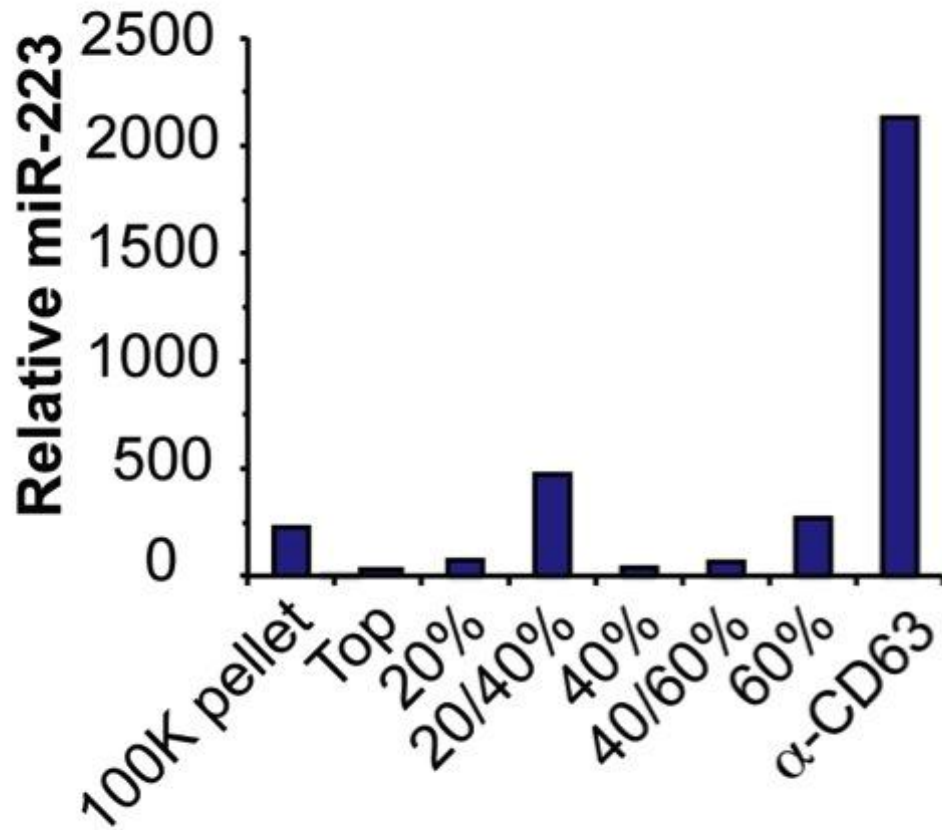
CD63-luciferase
topology in exosomes

Distribution of miRNAs in exosomes and cells

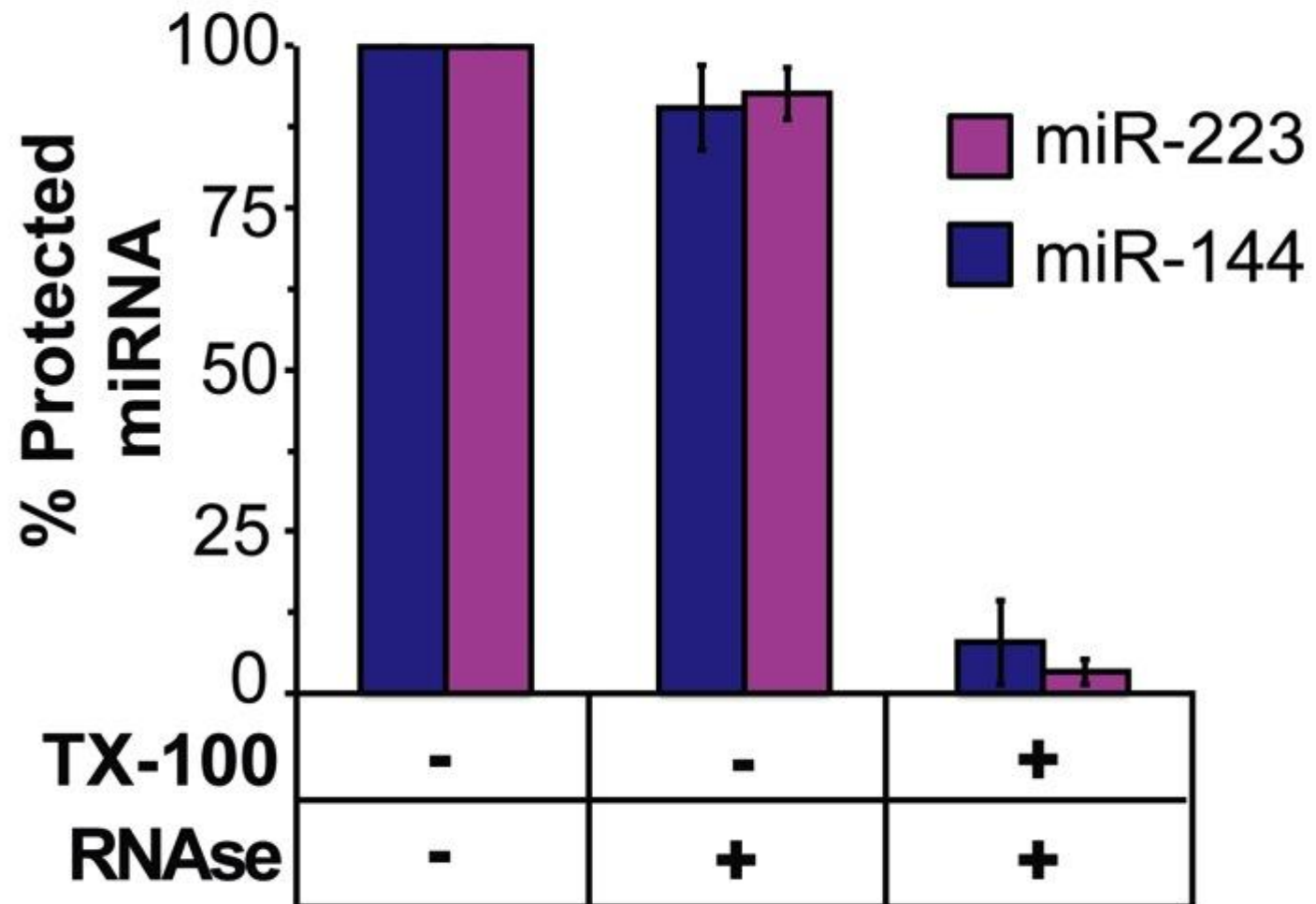


<u>Unique to Exosome Library</u>	
<u>miRNA</u>	<u>RPM</u>
MIR223	26.32
MIR4466	2.84
MIR382	1.78
MIR363	1.42
MIR493	1.42
MIR208	1.42
MIR3125	1.07

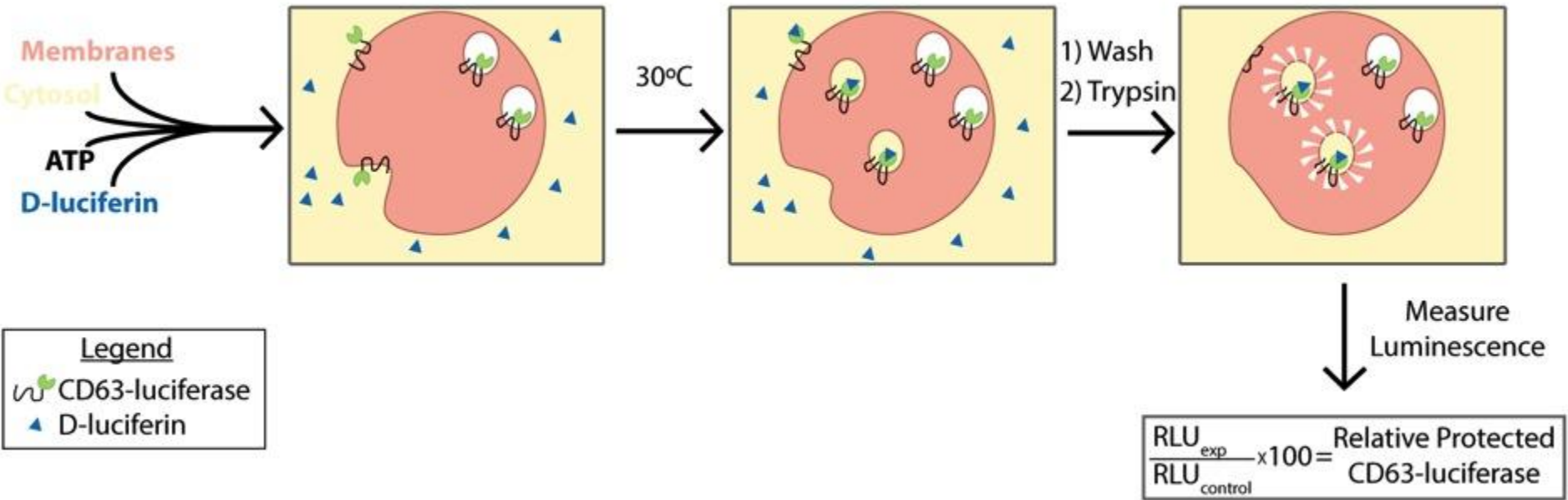
Co-purification of CD63 and miRNAs



miRNAs in detergent-sensitive vesicles

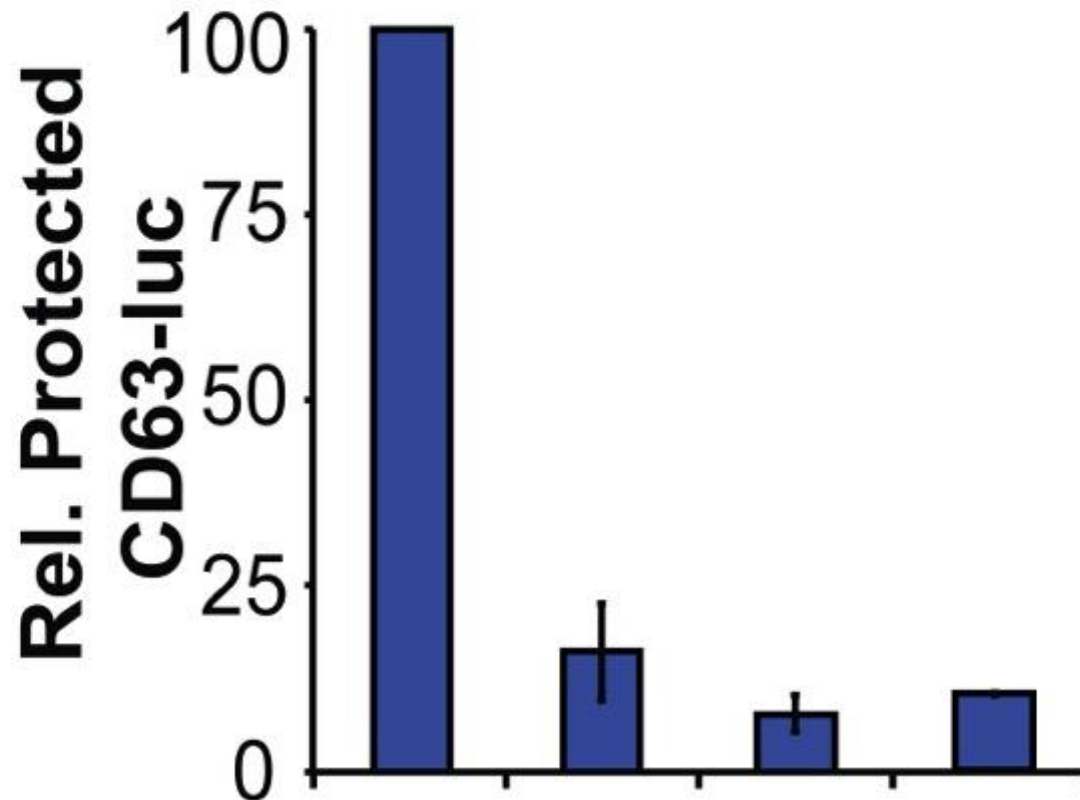


Cell-free exosome biogenesis

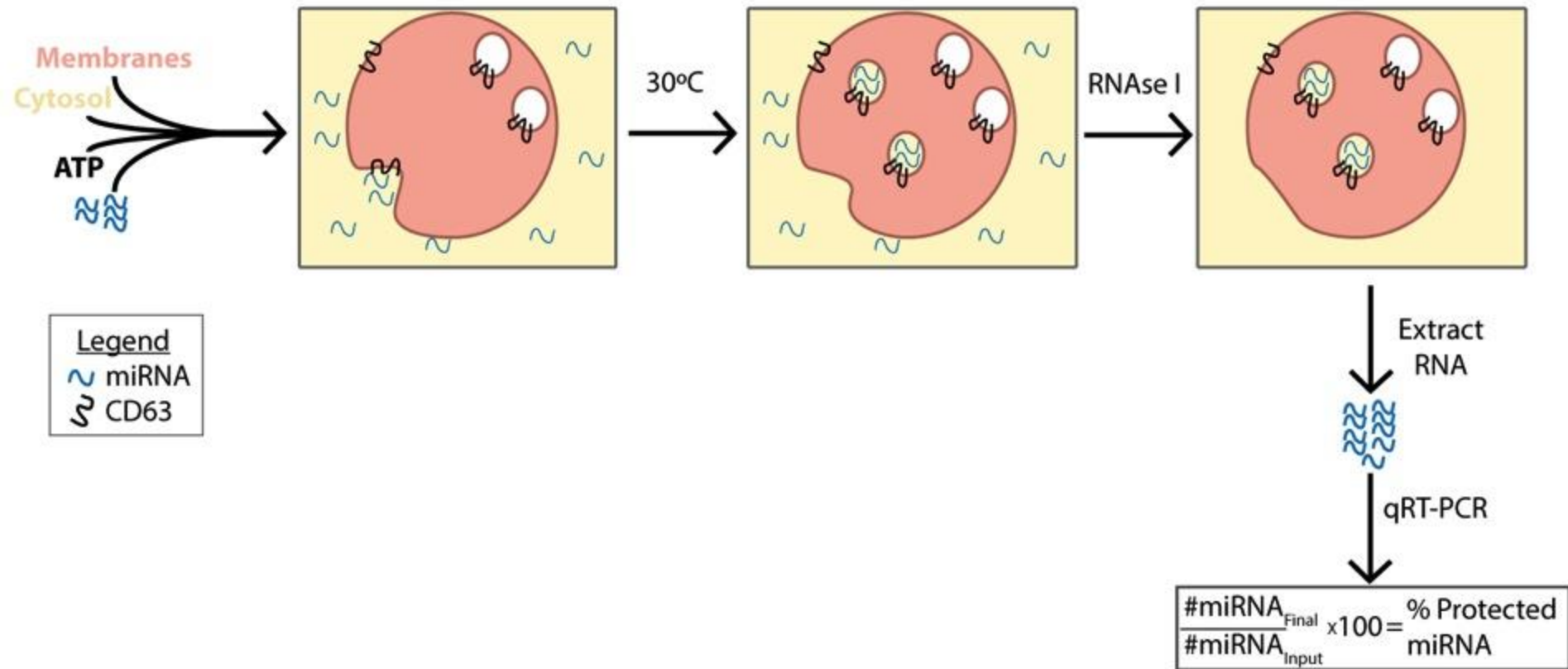


Exosome biogenesis requires cytosol and incubation at 30C

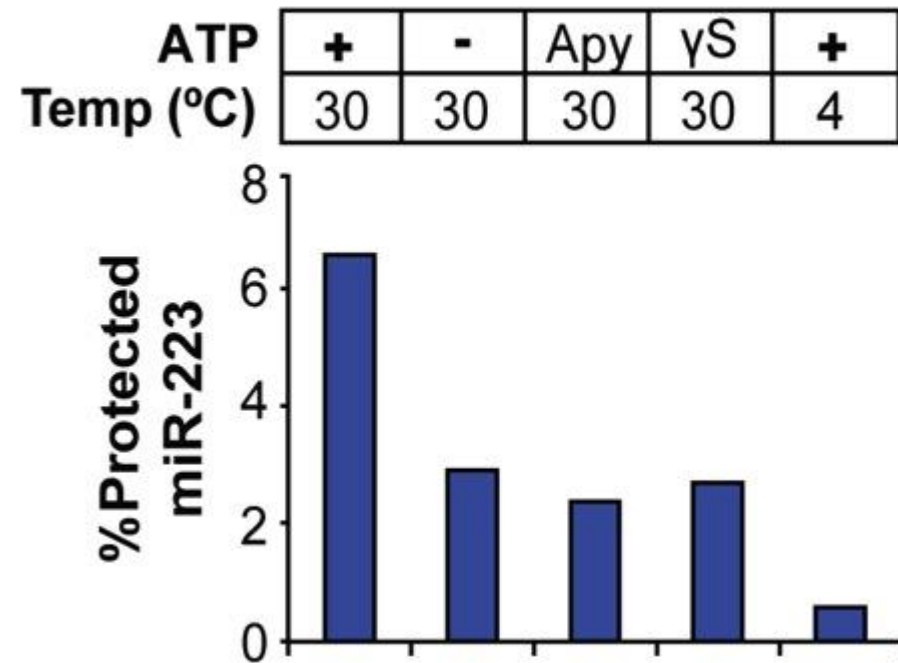
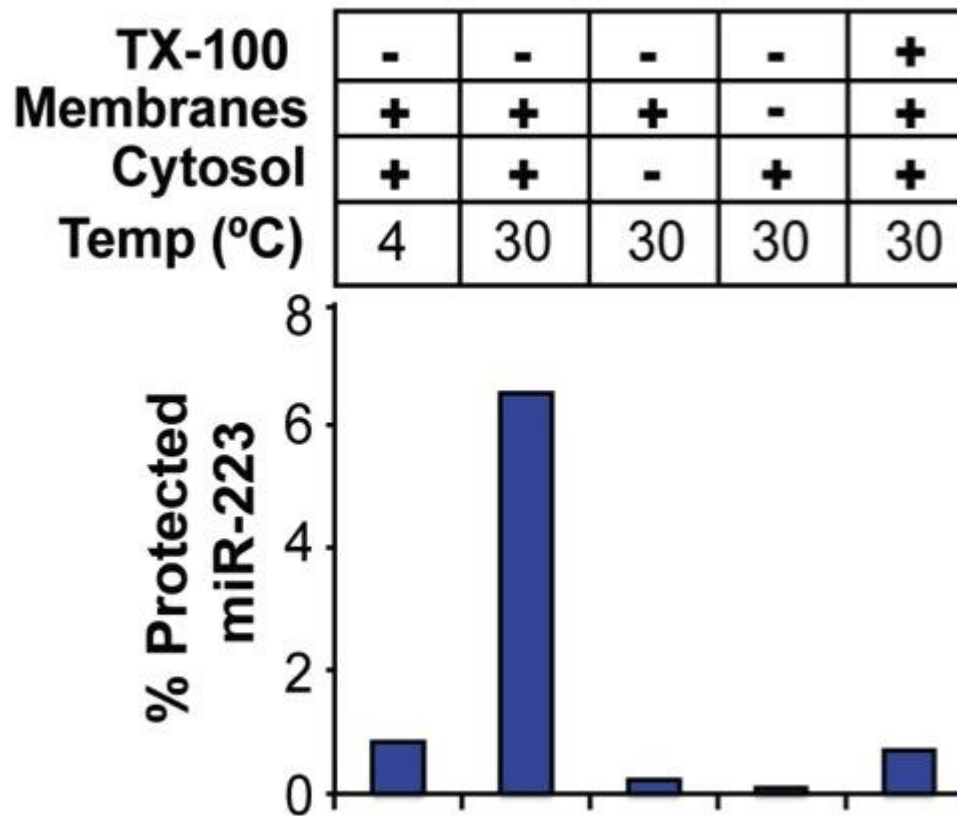
TX-100	-	-	+	-
Cytosol	+	-	+	+
Temp (°C)	30	30	30	4



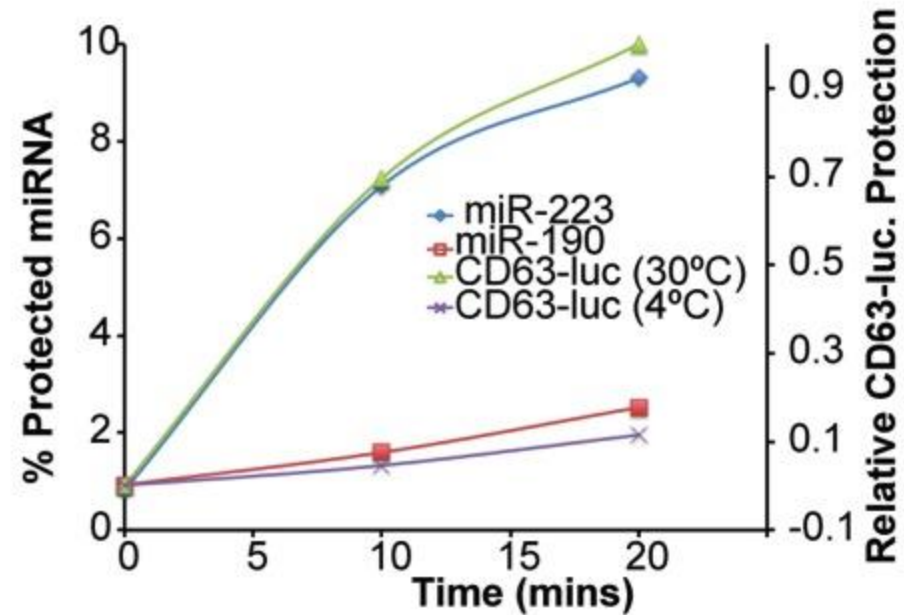
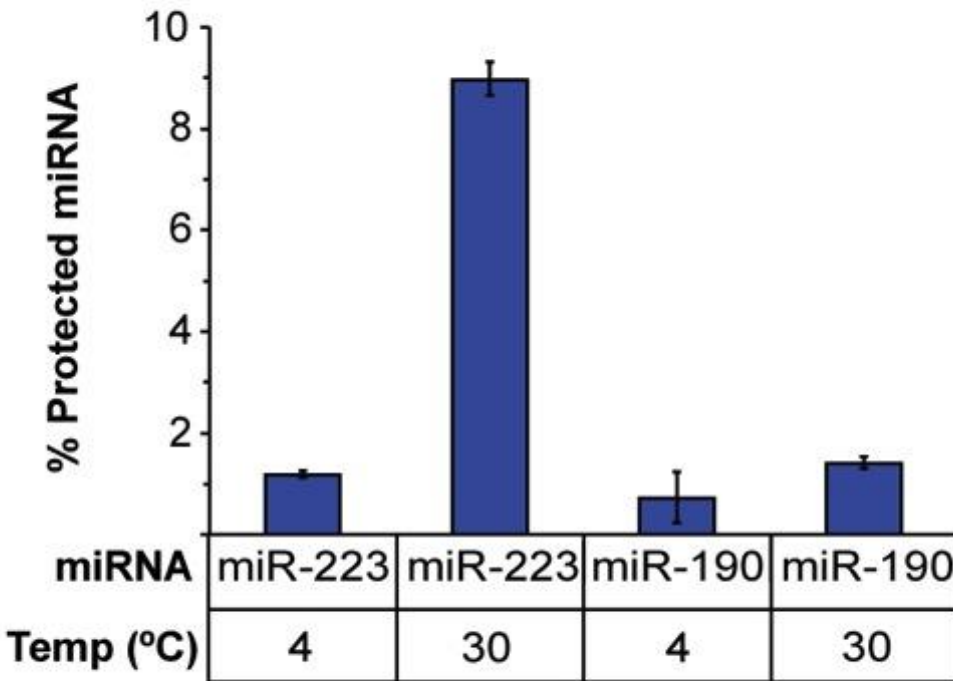
Cell-free miRNA packaging into exosomes



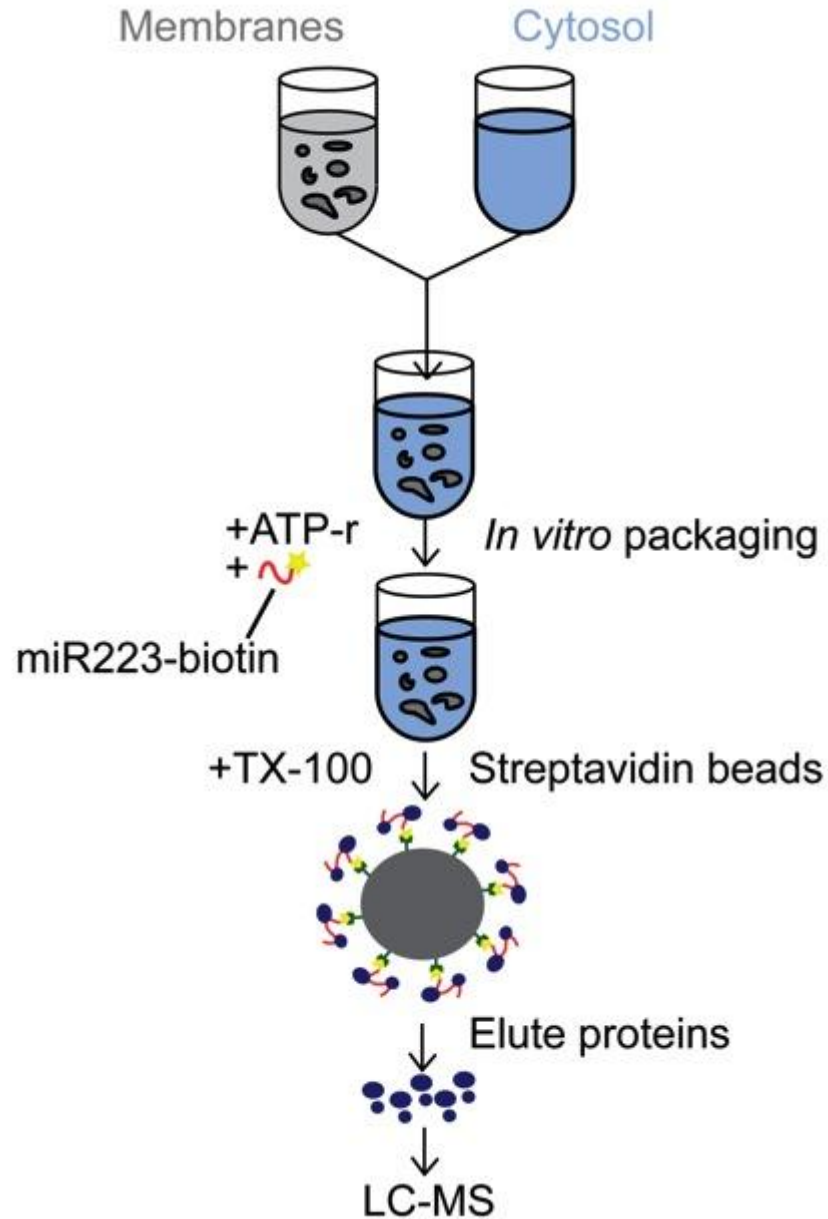
*miRNA packaging stimulated by cytosol,
incubation at 30C and ATP*



miRNA packaging selective



Isolation of miRNA-protein complexes



scientific report

Y-box protein-1 is actively secreted through a non-classical pathway and acts as an extracellular mitogen

Björn C. Frye^{1,2}, Sarah Halfter^{1*}, Sonja Djudjaj¹, Philipp Muehlenberg¹, Susanne Weber¹, Ute Raffetseder¹, Abdelaziz En-Nia¹, Hanna Knott¹, Jens M. Baron³, Steven Dooley⁴, Jürgen Bernhagen² & Peter R. Mertens^{1,5+}*

- Secreted YBX1 is protease protected
- Protease protection is detergent sensitive



Suggests it is secreted in a vesicle

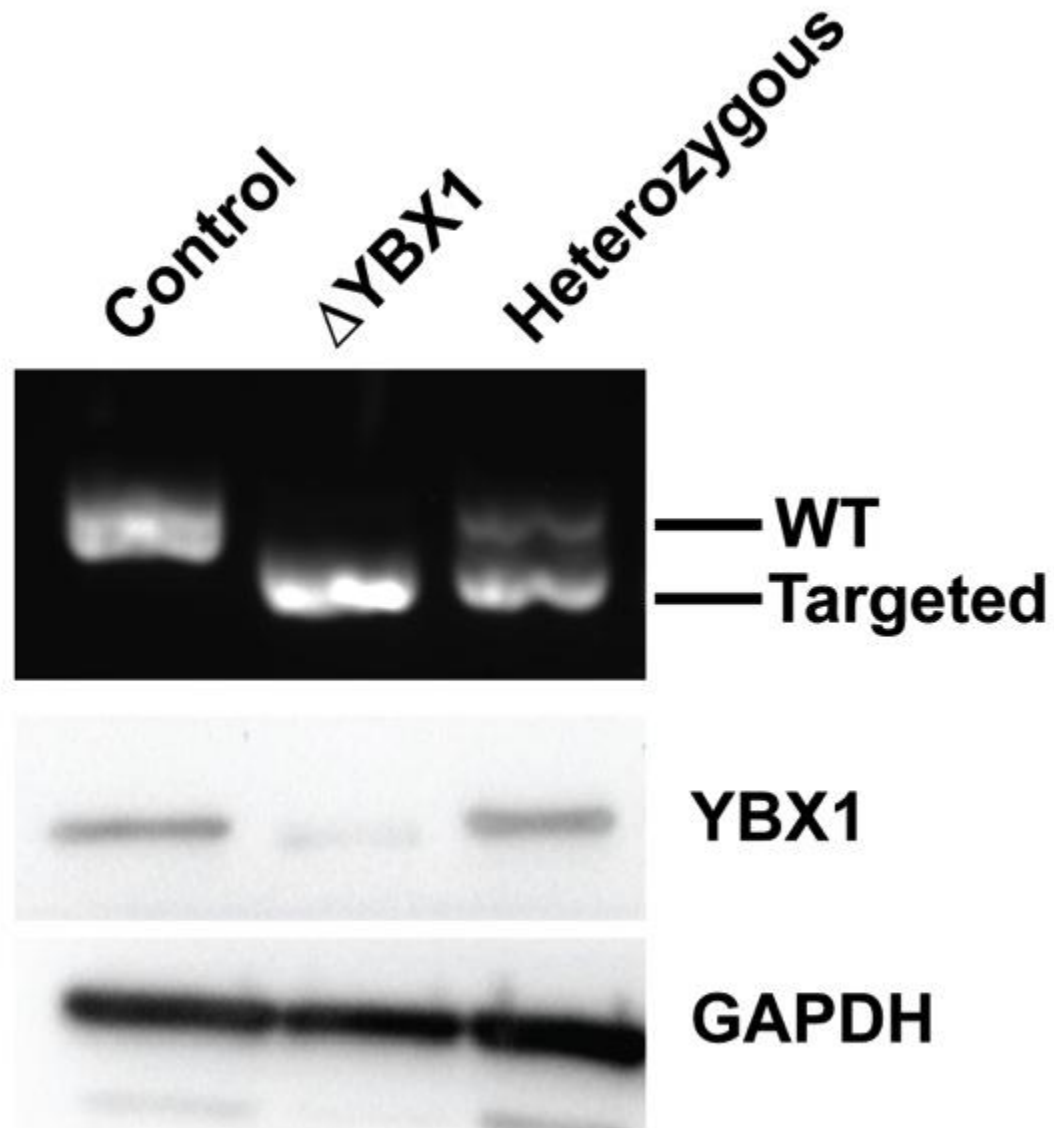
*YBX1 packaging stimulated by miR223,
cytosol, membranes and incubation at 30C*

miR223-biotin	+	+	+	+	-
Temp (°C)	30	30	30	4	30
Membranes	+	+	-	+	+
Cytosol	+	-	+	+	+
RNAse	+	+	+	+	+

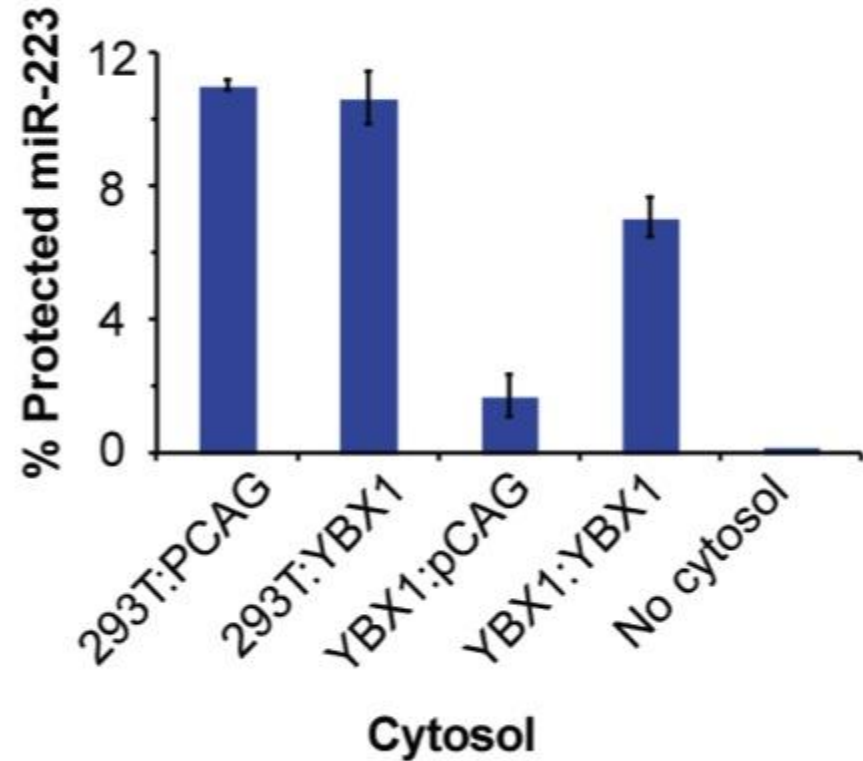
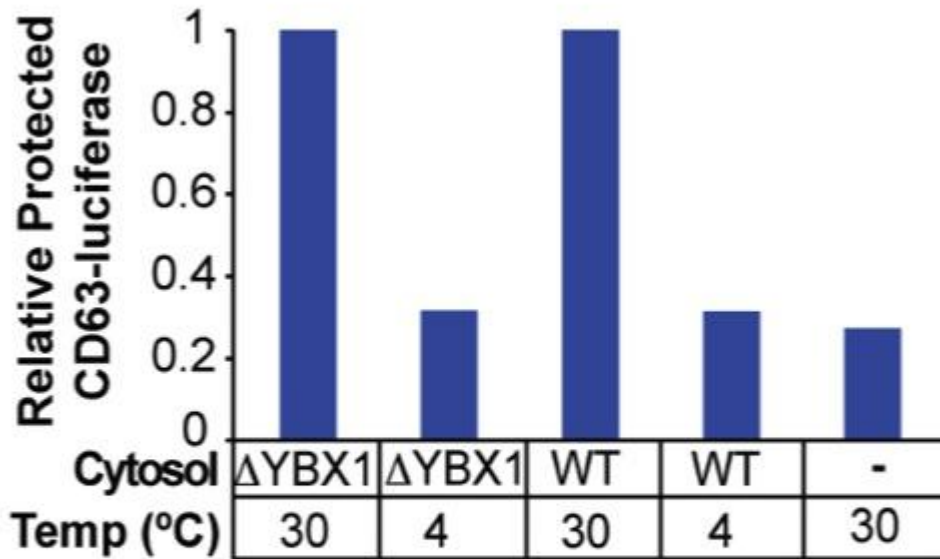
YBX1



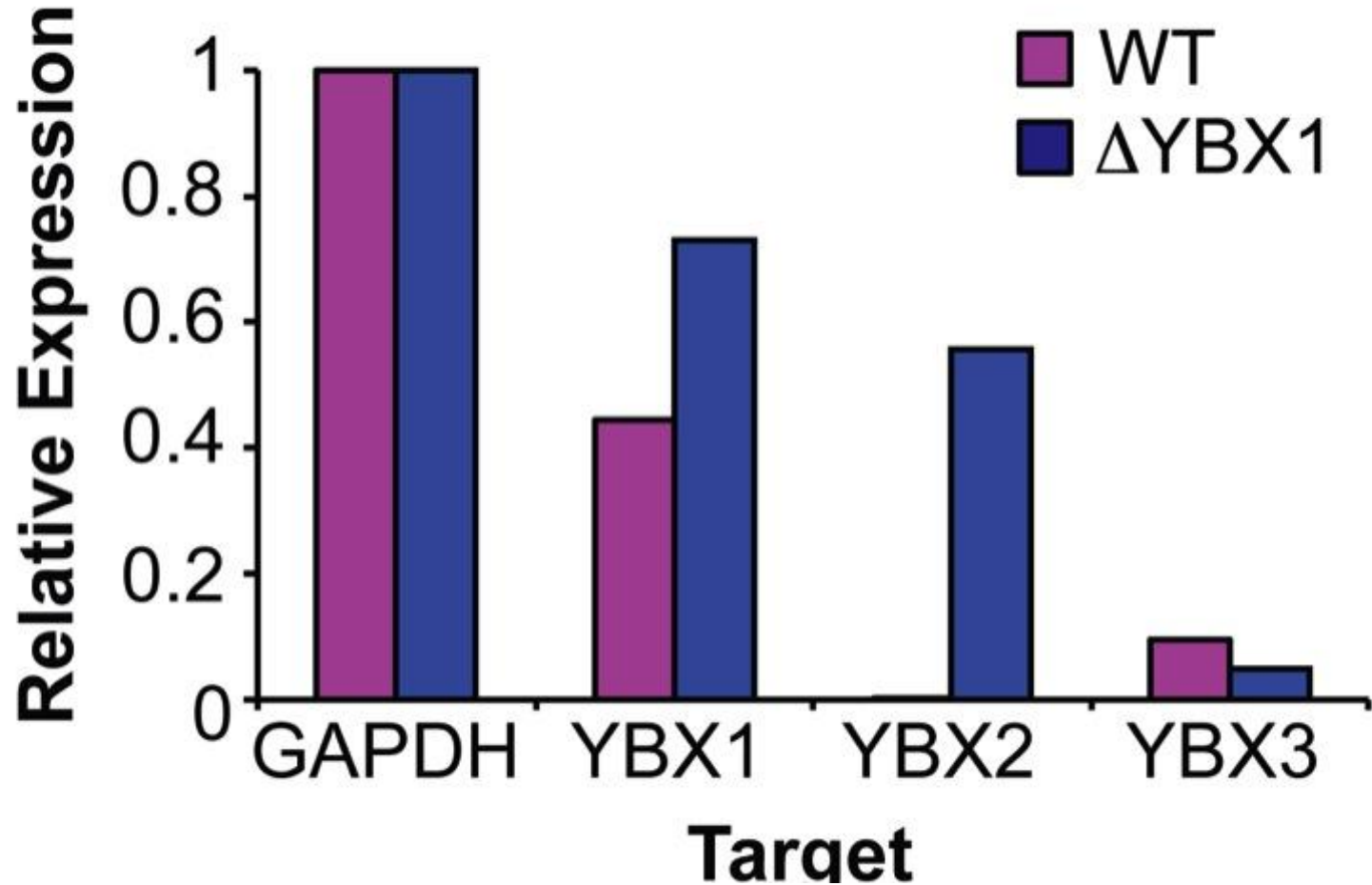
Knockout of YBX1



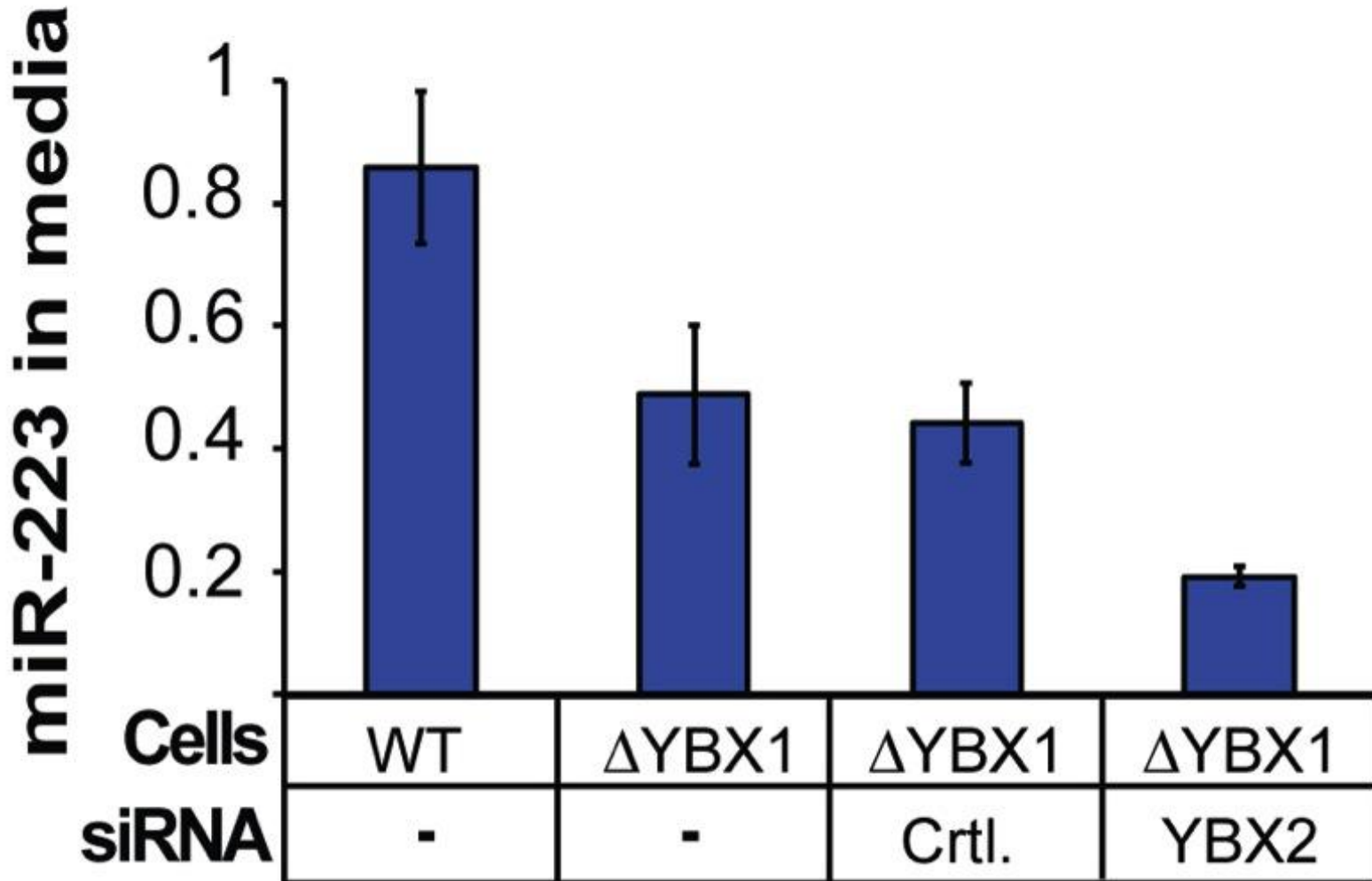
YBX1 required for packaging of miR-223 but not of CD63-luciferase

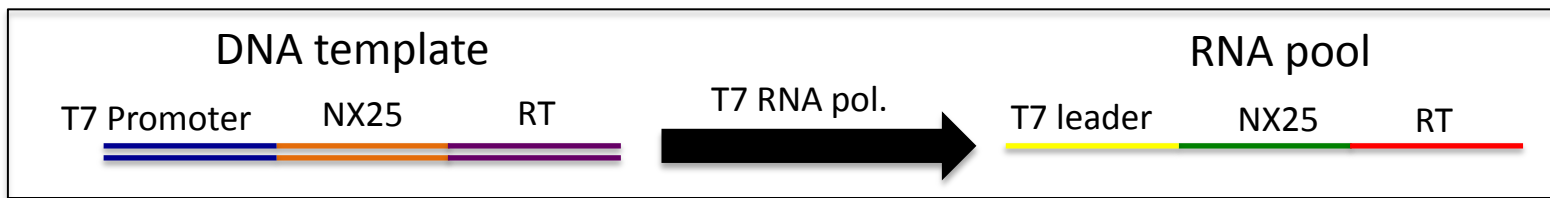
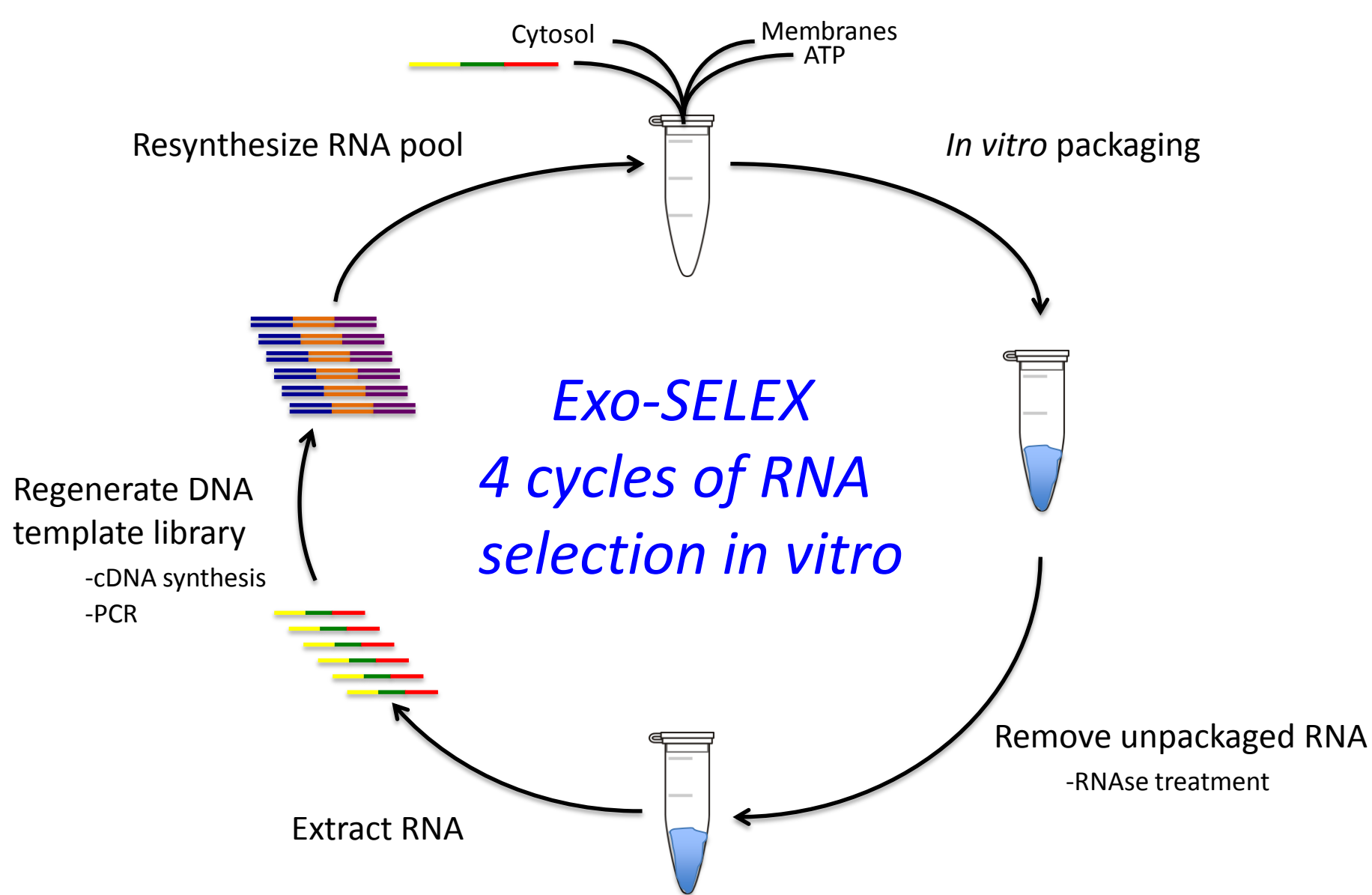


Upregulation of YBX2 mRNA in Δ YBX1 cells



Y-box proteins control miRNA secretion in vivo

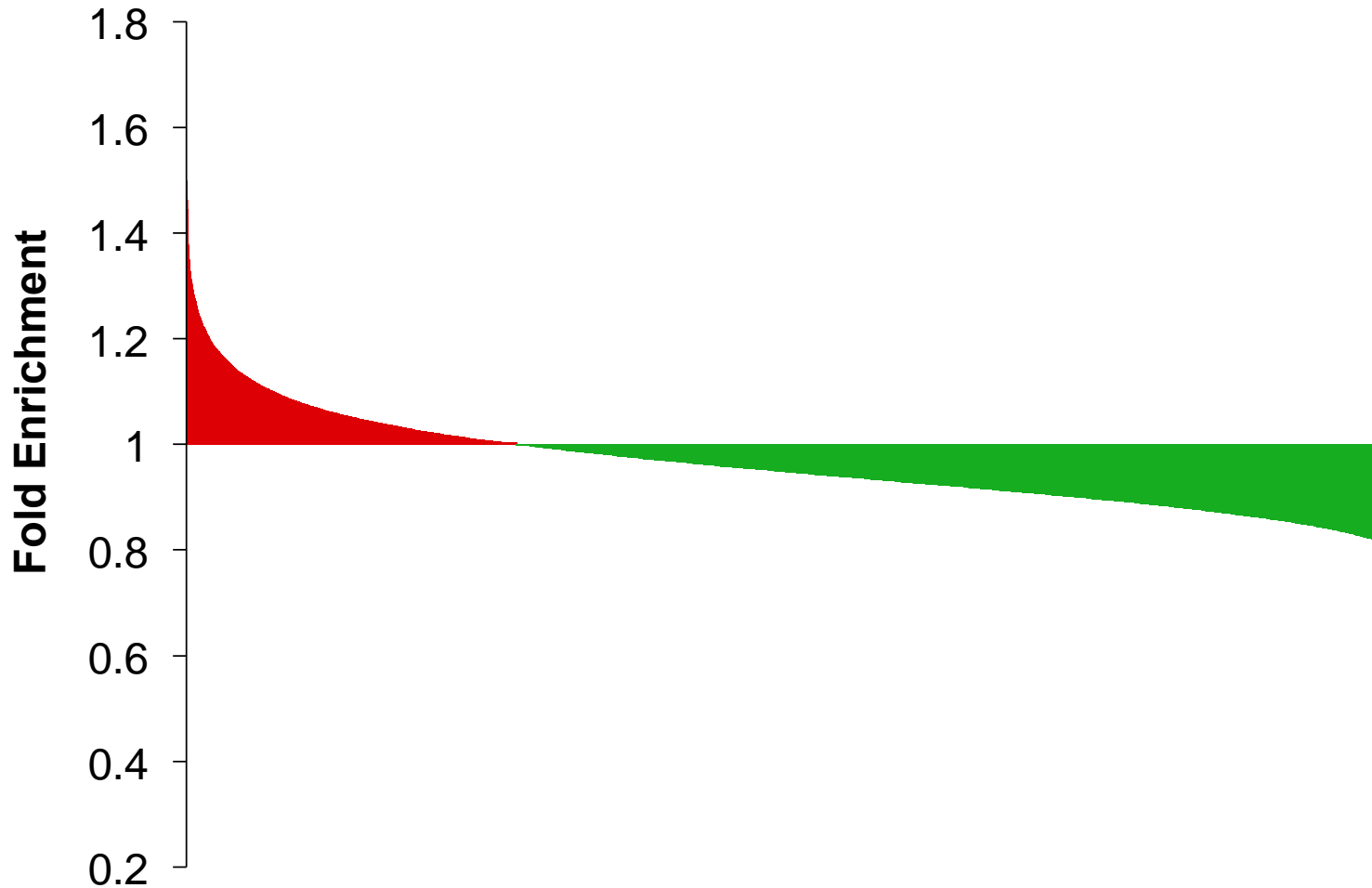




8mers become enriched during Exo-SELEX

Replicate 1

■ Enriched 8mers ■ Depleted 8mers



Consensus motif – found in mi223, not in mi190

Replicate 1

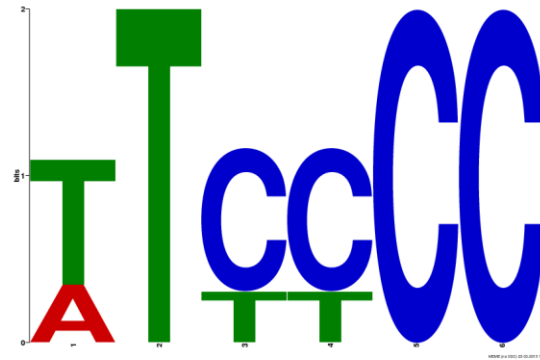


$E_{\text{value}}=1\text{e-}55$

Exosomal miR-223

Cellular miR-190a

Replicate 2



$E_{\text{value}}=1\text{e-}37$

UGUCAGUUUGUCAAAUACCCCA

UGAUAUGUUUGAUUAUUGGU

Test of consensus signal

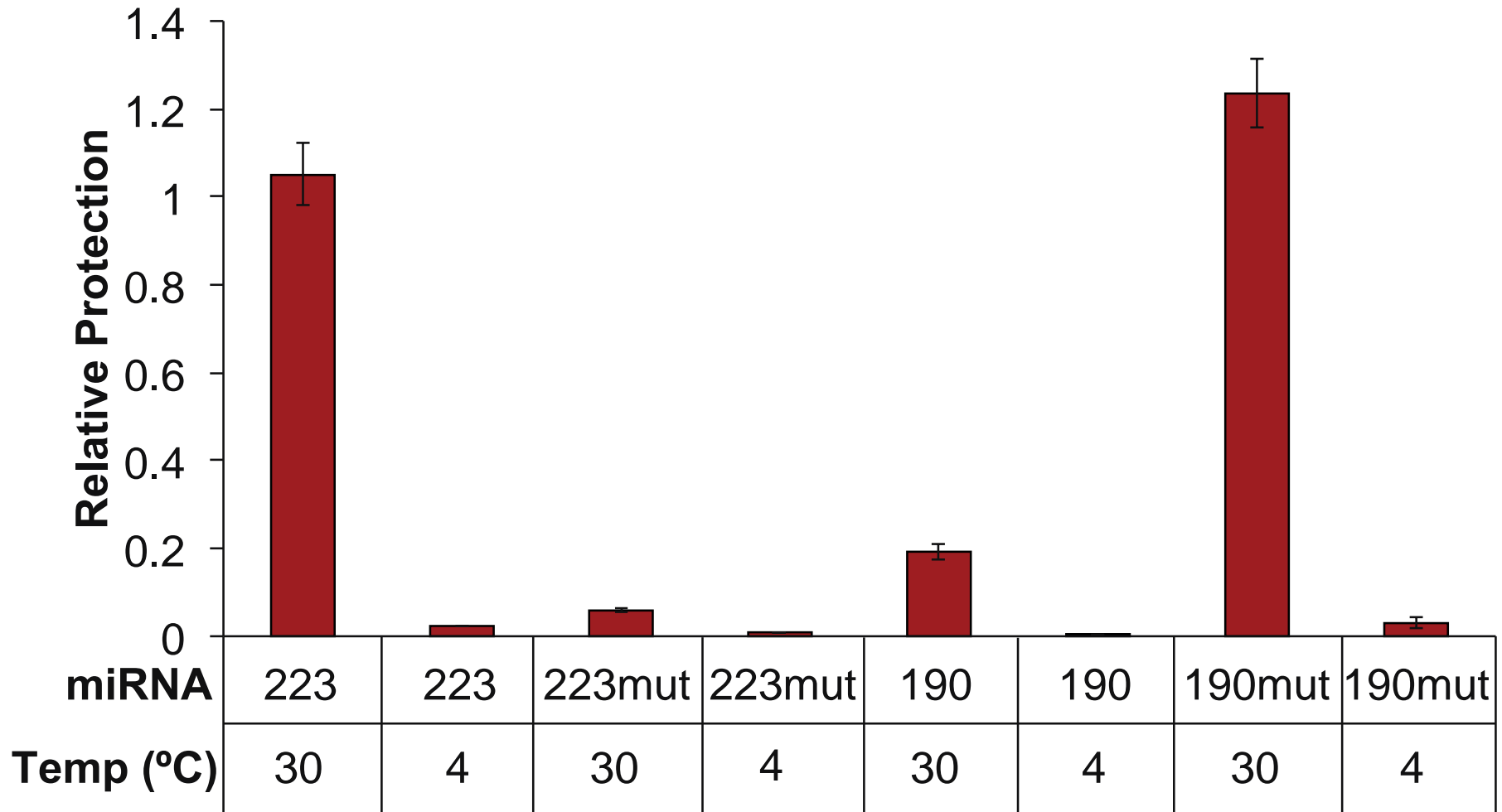
miR-223 UGUCAGUUUGUCAAAUACCCCA

miR-223mut UGUCAGUUUGUCAAAUGUUGGA

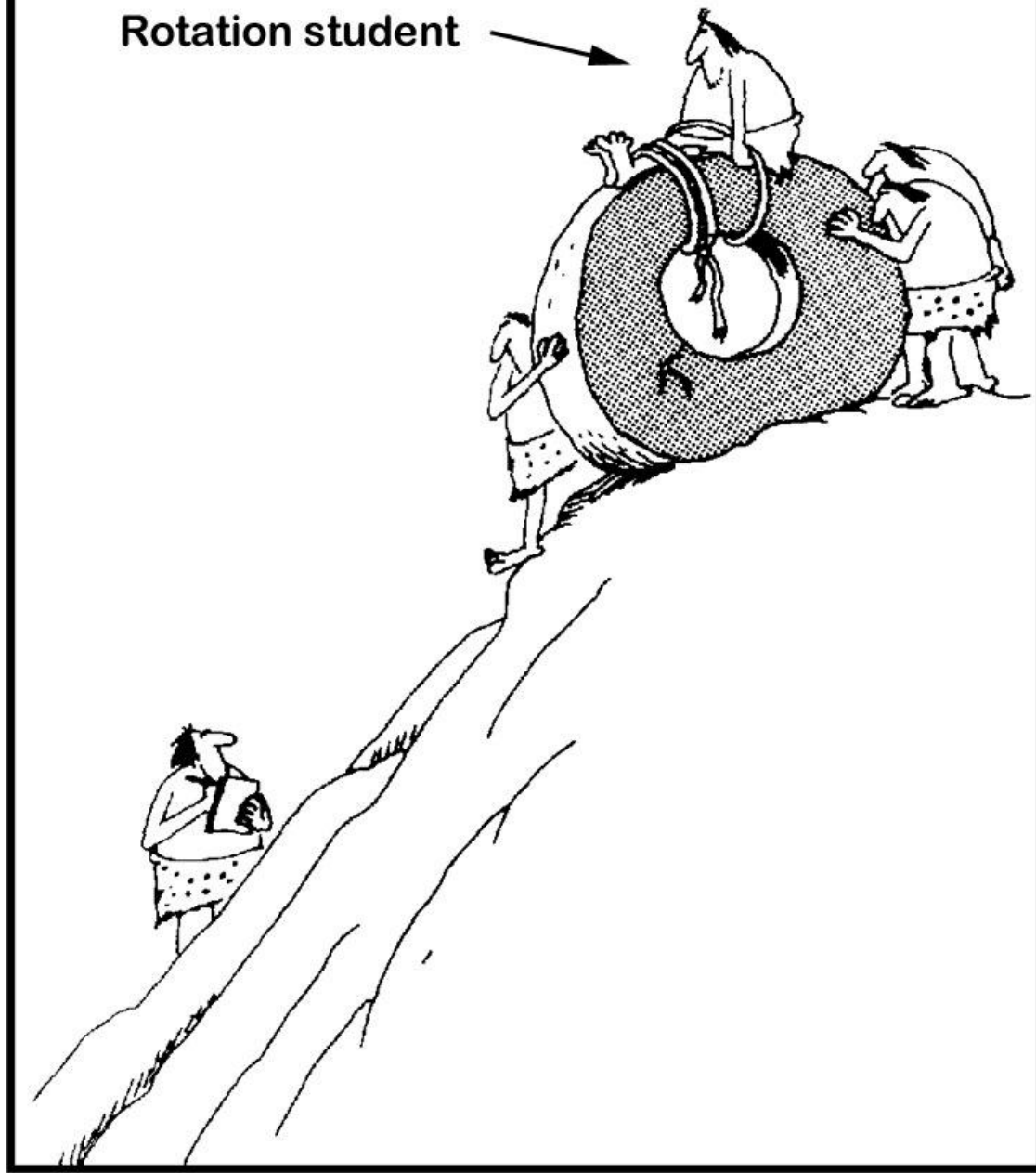
miR-190a UGAUAUGUUUGAUAUAUUAGGU

miR-190amut UGAUAUGUUUGAUAUAUACCCA

Cytosine enriched motifs are required for miRNA sorting to exosomes



Rotation student



Early experiments in transport

MicroRNA Packaging into Exosomes

