Devnote-Cx43 liposomes

Status	Shipped
Tags	
Researchers	Ⓨ Yen-Yu Hsu
Keywords	
Summary	The research investigates the localization of Cx43-EGFP in liposome membranes and its functionality as a hemichannel using dye leakage assays. Initial results show successful expression and localization of Cx43-EGFP, although expression levels are low. Observations indicate that liposomes with Cx43 exhibit reduced fluorescence and yield compared to controls, suggesting potential dye leakage through Cx43 channels.

Overview

*Summary of the research or experiment, particularly the **why** of the work.*

We're trying to see

(1) If PURE expressed Cx43 can localize to the liposome membranes

(2) If the Cx43-EGFP can function as a hemichannel using dye leakage assay

Contents

Notebook

MATERIALS

Lipids

POPC: 16:0-18:1 PC (POPC)

Chol: cholesterol (plant)

Deuterated lipid: 16:0-d31-18:1 PC

Rhod-PE

Glucose

@August 27, 2025

We want to see if Cx43-EGFP can be expressed using NEB PURE and localize to liposome membranes.

REACTION SETUP

Lipid-in-oil solution (~5mg/ ml): 74.5% POPC + 29.95% Chol + 0.05% Rhod

1. Add 1 ml mineral oil in the 2mL small glass jar
2. Add lipids shown in the above table into the glass jar on top of the mineral oil

3. Vortex the lipid-in-oil mixture for 10 secs
4. Put the glass jar in the bead-loaded hot bath at ~55c for 3 hrs (keep the jar uncovered without lids)
5. Place the jar (with lid) containing lipid-in-oil solution at RT for 10 mins before using

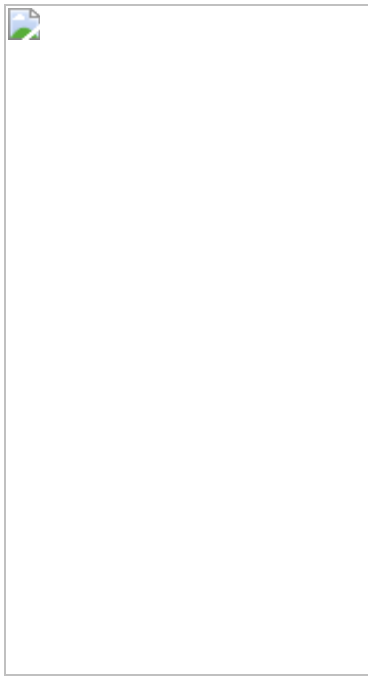
Outer solution: 10 mM HEPES-KOH and 10 mM NaCl solution in 700 mM Glucose

Inner solution:

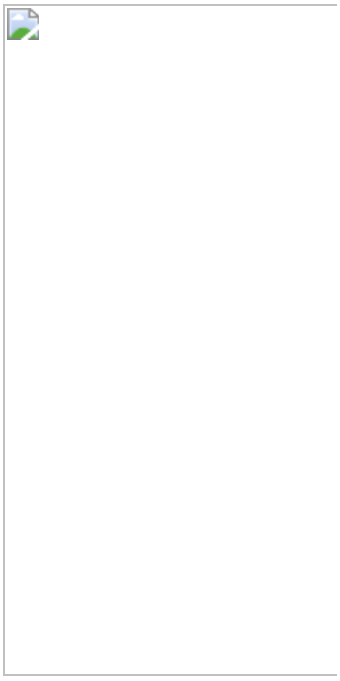
Sample	+ Cx43	- Cx43
Sol A	8	8
Sol B	6	6
RNase Inhibitor	1	1
DNA (Cx43-deGFP)	2	0
Optiprep	0.67	0.67
H2O	2.33	4.33
Total	20	20

RESULTS

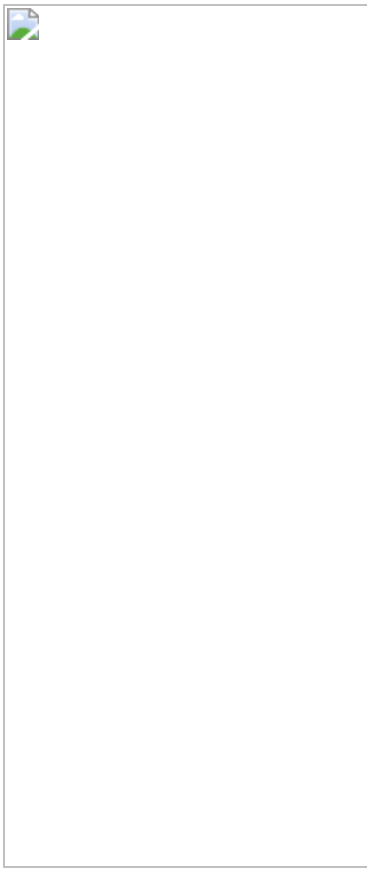
▼ Control



561: 80 ms



488: 200 ms



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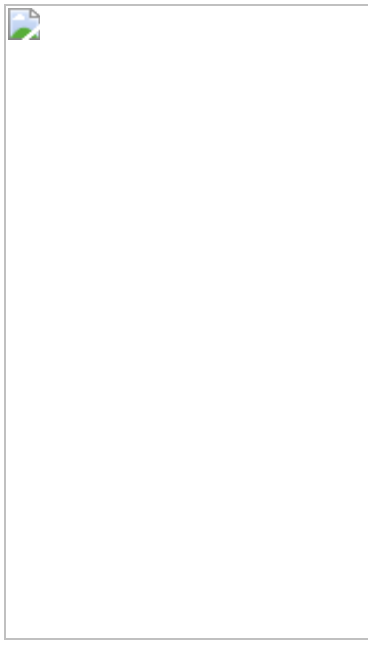

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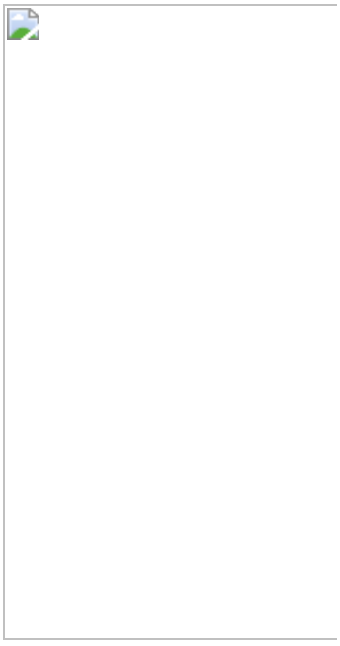

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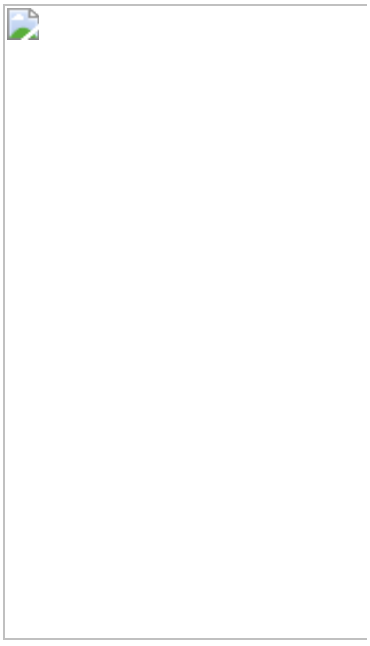

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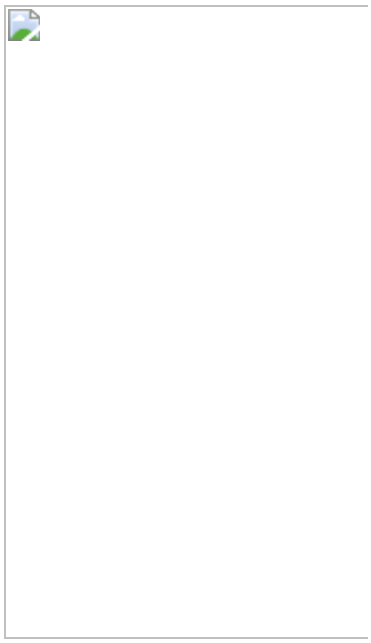

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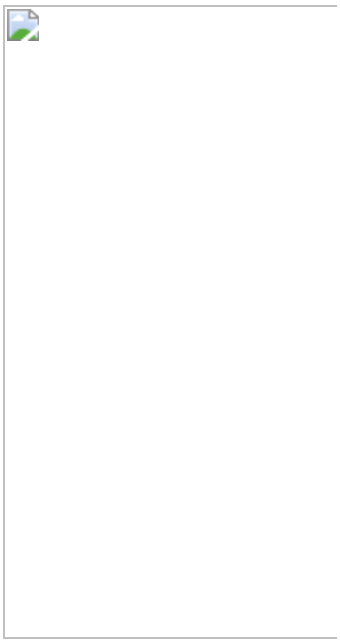

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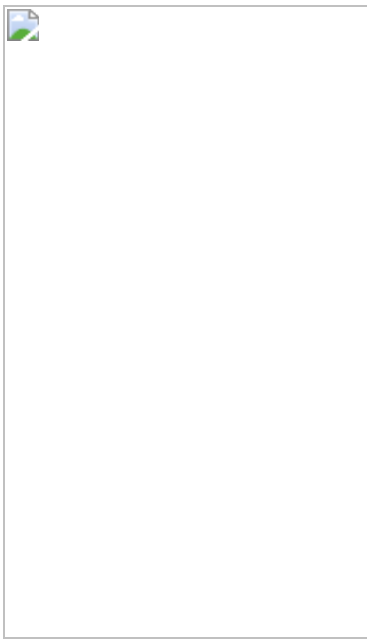

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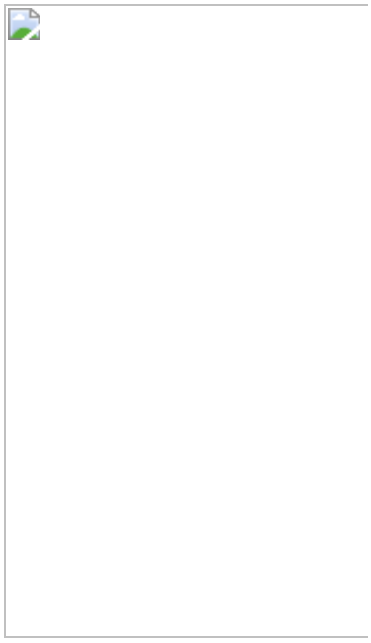

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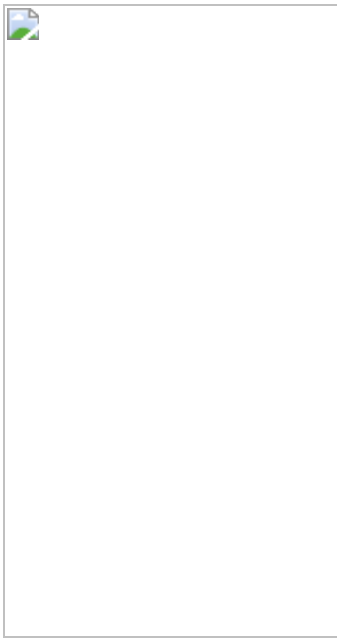

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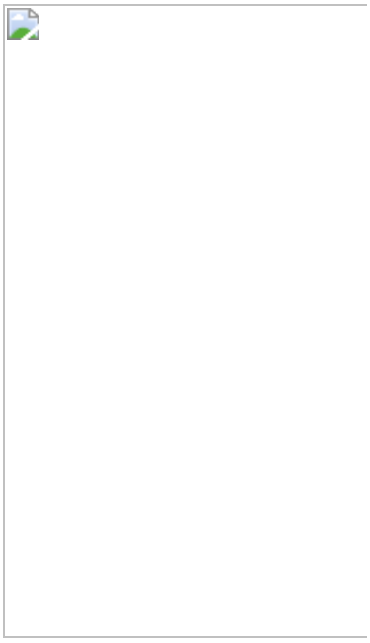
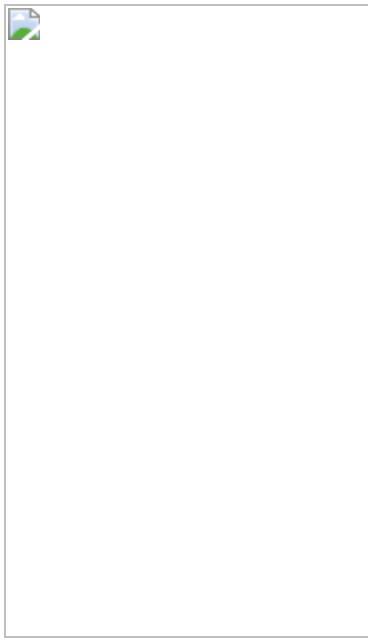


561+488

▼ Sample



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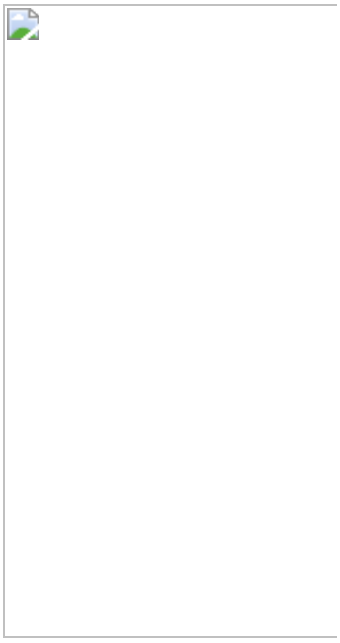

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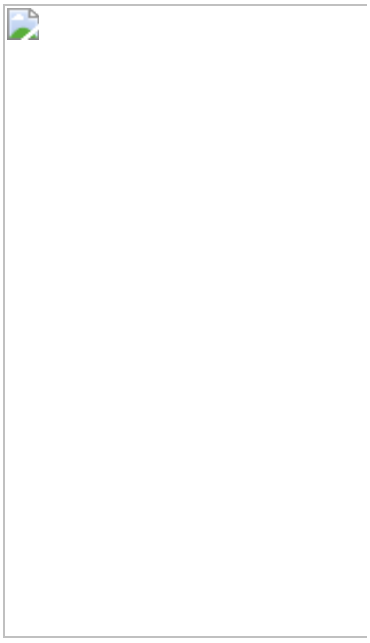

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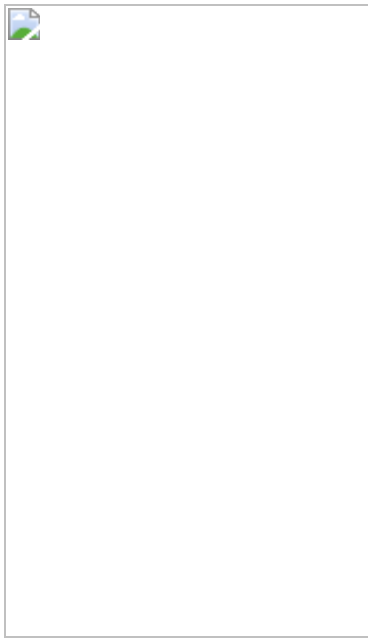

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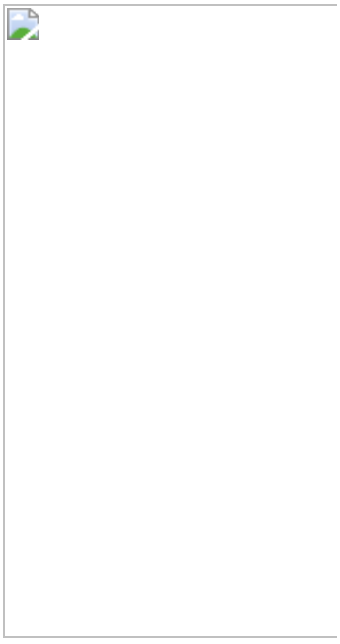

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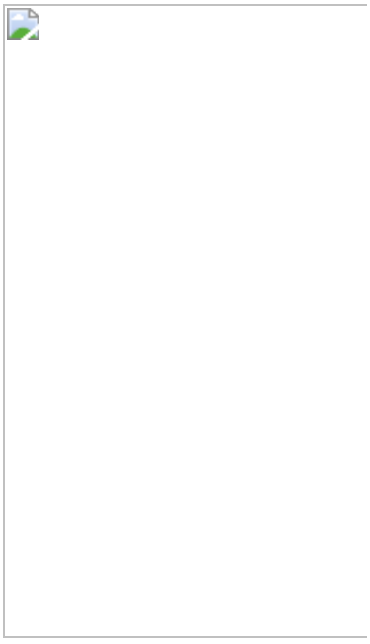

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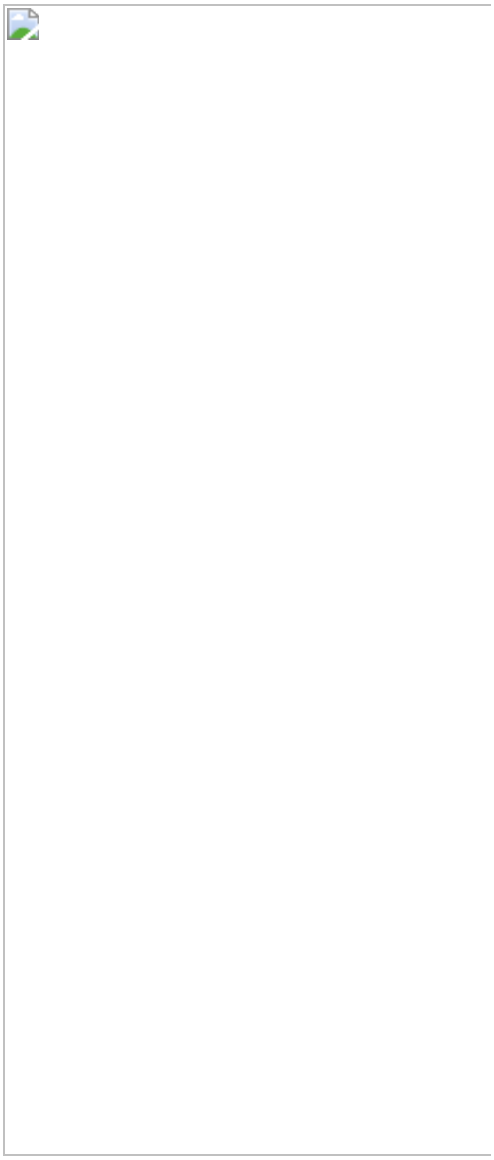
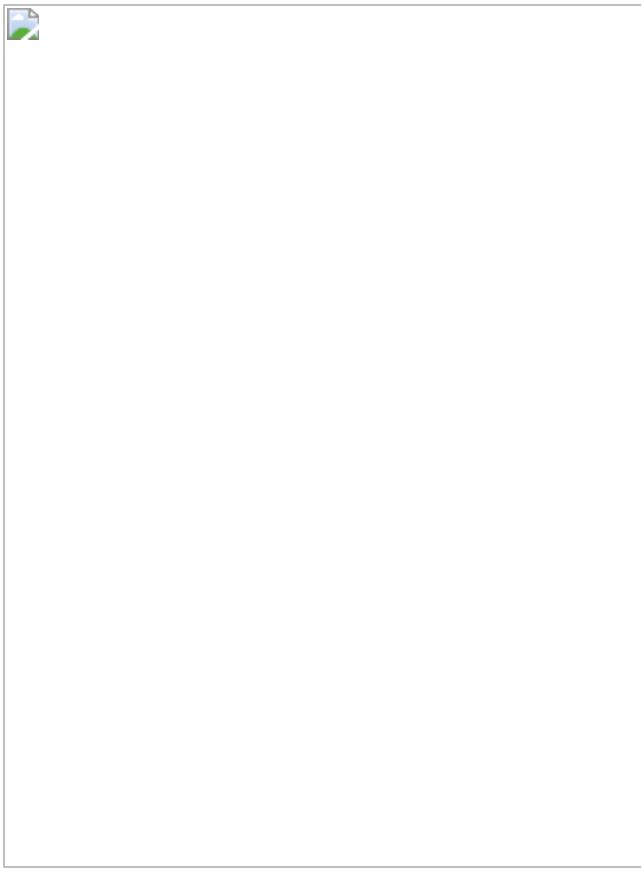


3 hrs of expression



3 hrs of expression

▼ Cells - Samples

S1: 561 channel

S2: 561 channel

S3: 561 channel

S4: 561 channel

S5: 561 channel

S6: 561 channel

S4: 488 channel

S5: 488 channel

S6: 488 channel

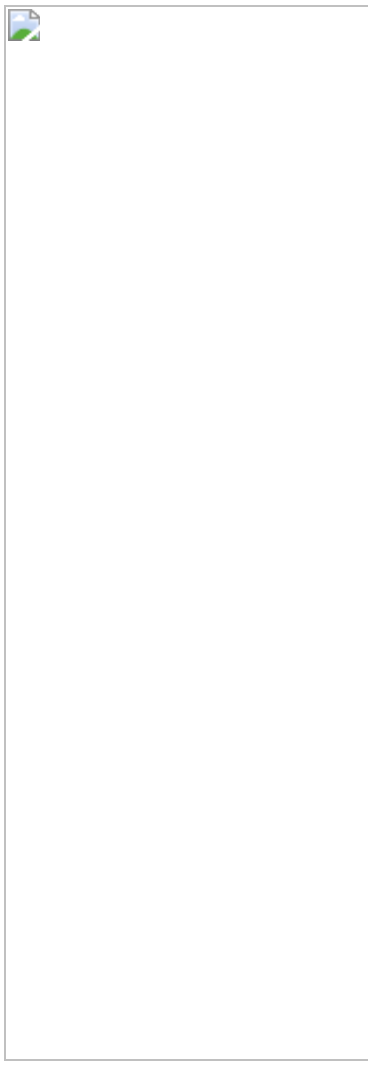
S1: 488 channel

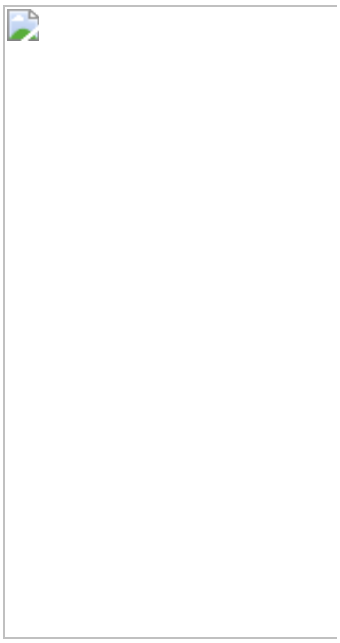
S2: 488 channel

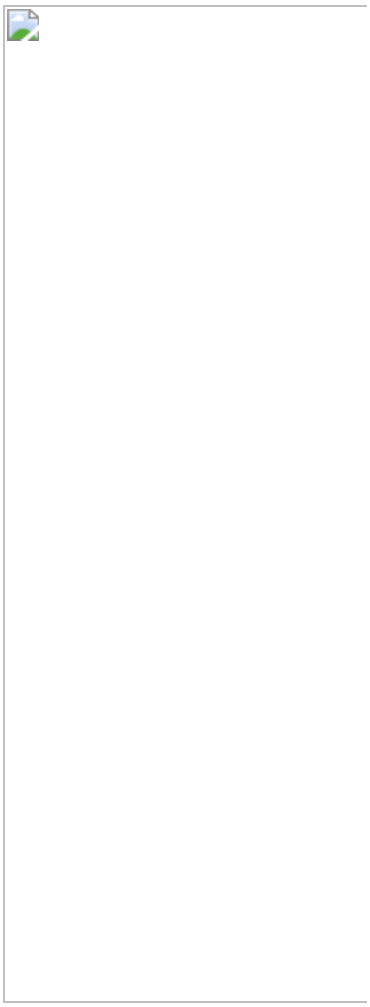
S3: 488 channel

561 channel: exp 80 ms; 488 channel: exp 200 ms.

▼ Data







CONCLUSION

Cx43-EGFP was successfully expressed using the NEB PURE system and localized to liposome membranes. However, the expression level appears to be low, as indicated by dim green fluorescence. In addition, some liposomes didn’t show green fluorescent ring.

OBSERVATIONS

Although the overall expression level was low, we observed clear localization of Cx43-EGFP on liposome membranes. In contrast, control liposomes lacking Cx43

showed no green fluorescent rings under identical imaging conditions using the 488 nm channel (200 ms exposure).

@August 29, 2026

Repeat the experiment from @August 28, 2025

REACTION SETUP

Lipid-in-oil solution (~5mg/ ml): 74.5% POPC + 29.95% Chol + 0.05% Rhod

1. Add 1 ml mineral oil in the 2mL small glass jar

2. Add lipids shown in the above table into the glass jar on top of the mineral oil
3. Vortex the lipid-in-oil mixture for 10 secs
4. Put the glass jar in the bead-loaded hot bath at ~55c for 3 hrs (keep the jar uncovered without lids)
5. Place the jar (with lid) containing lipid-in-oil solution at RT for 10 mins before using

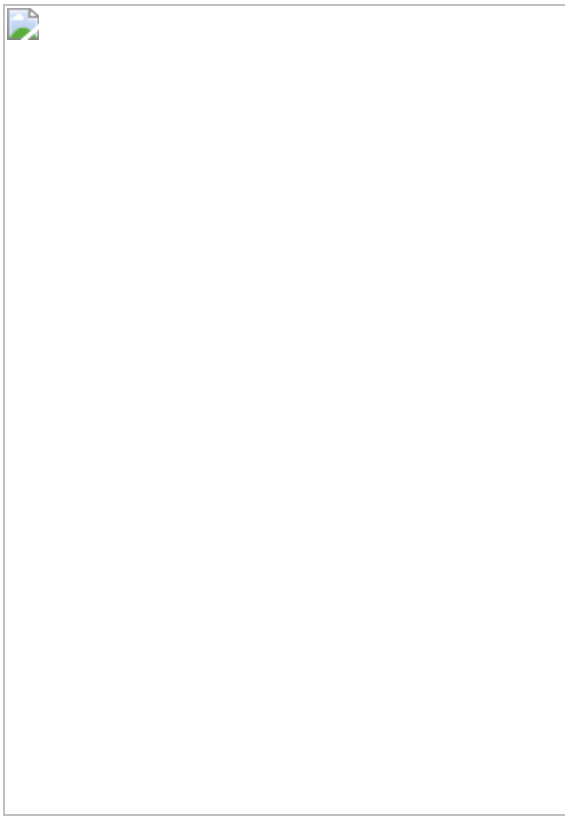
Outer solution: 10 mM HEPES-KOH and 10 mM NaCl solution in 700 mM Glucose

Inner solution:

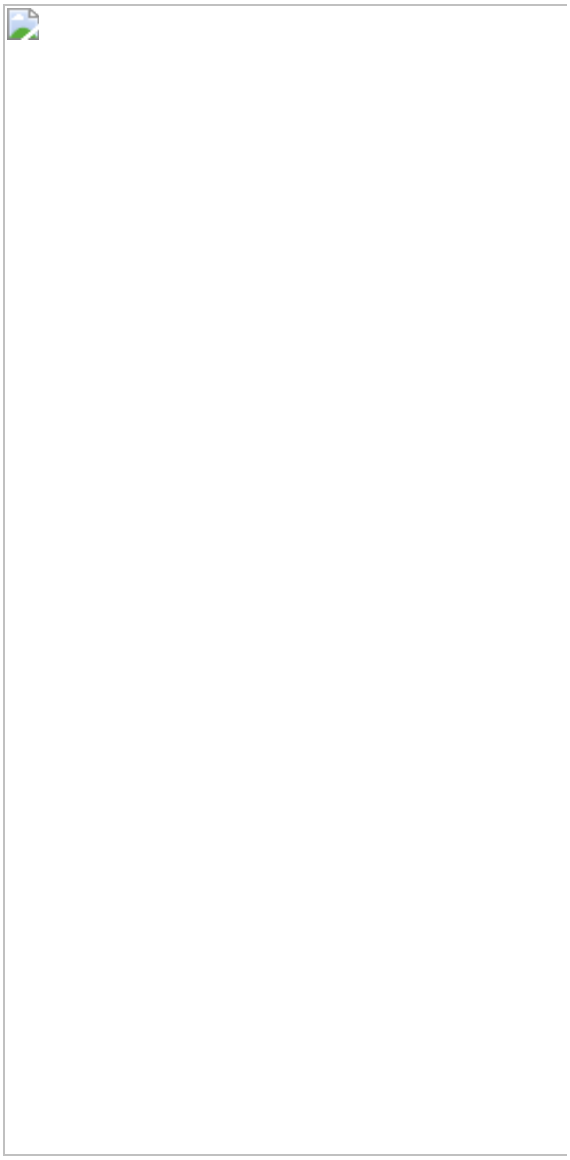
Sample	+ Cx43	- Cx43
Sol A	8	8
Sol B	6	6
RNase Inhibitor	1	1
DNA (Cx43)	2	0
Optiprep	0.67	0.67
Alexa Fluor 647 (1mM)	0.1	0.1
H2O	2.23	4.23
Total	20	20

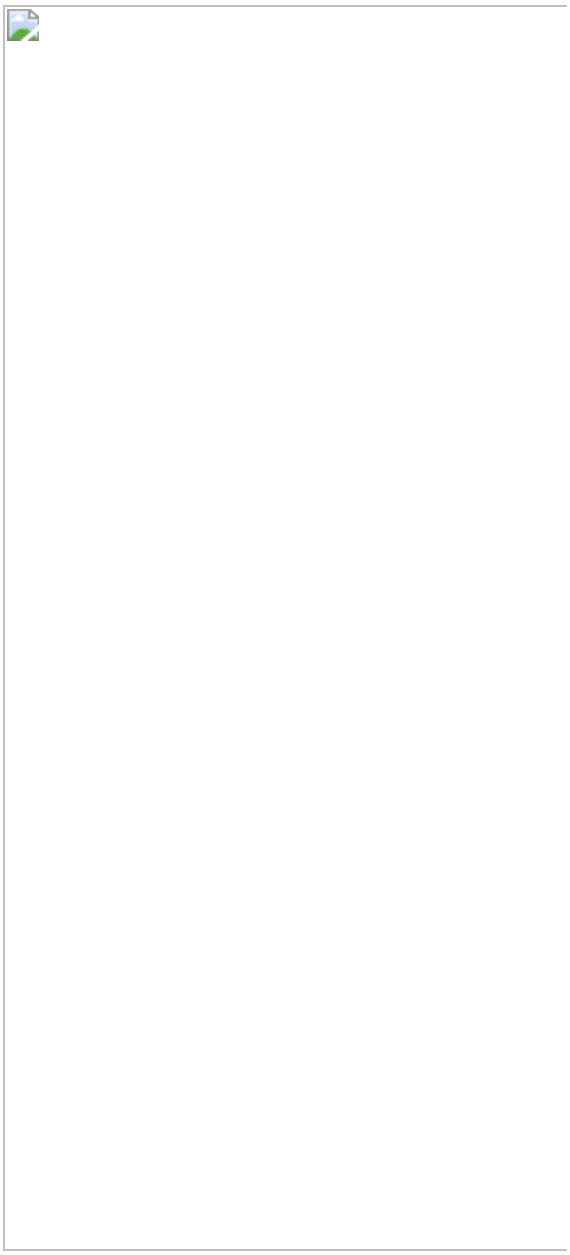
RESULTS

▼ Figures



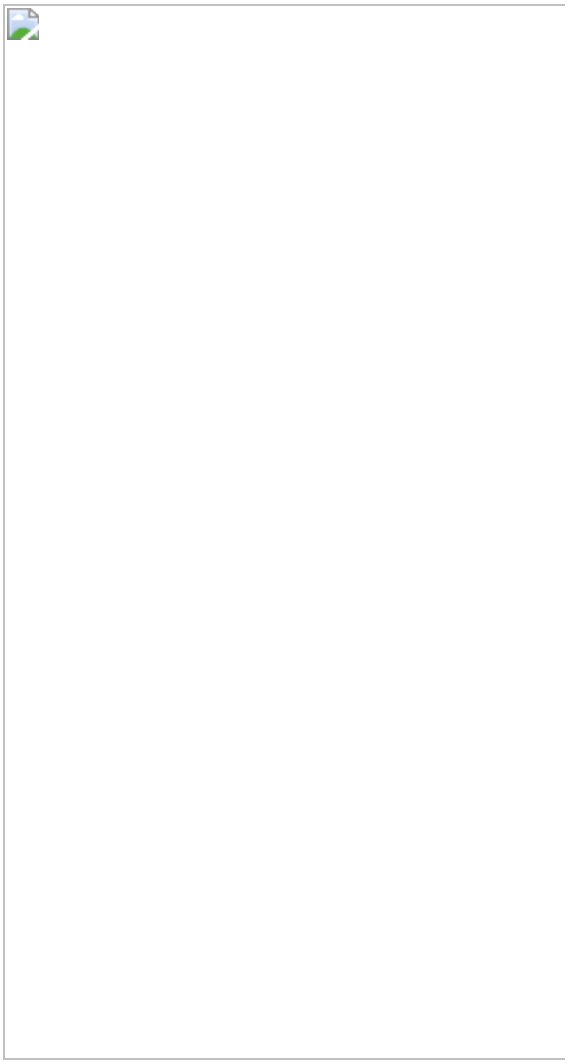
Liposome fluorescence intensity



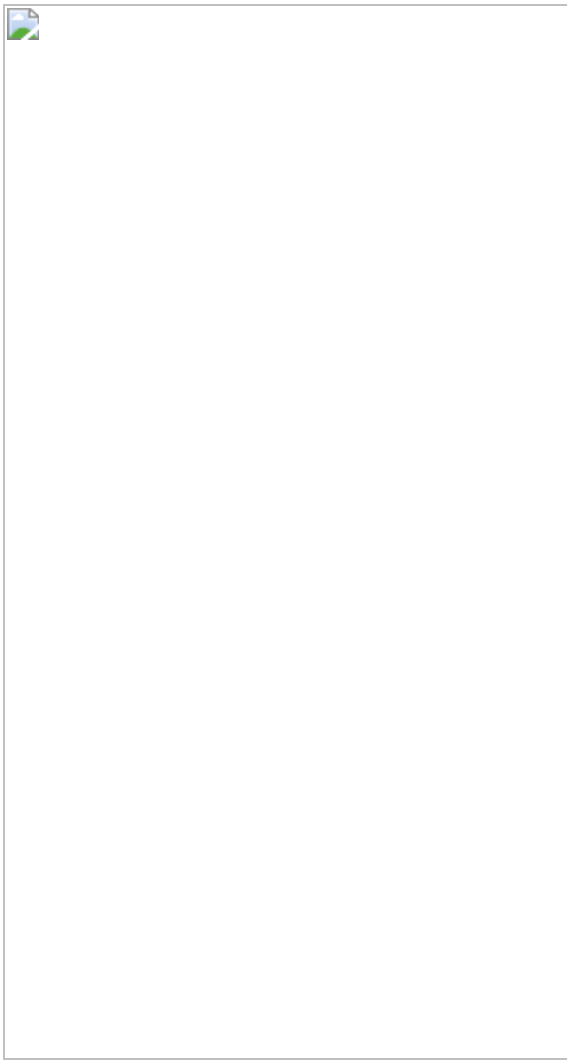


Top 1% liposomes

▼ Sample



Startpoint

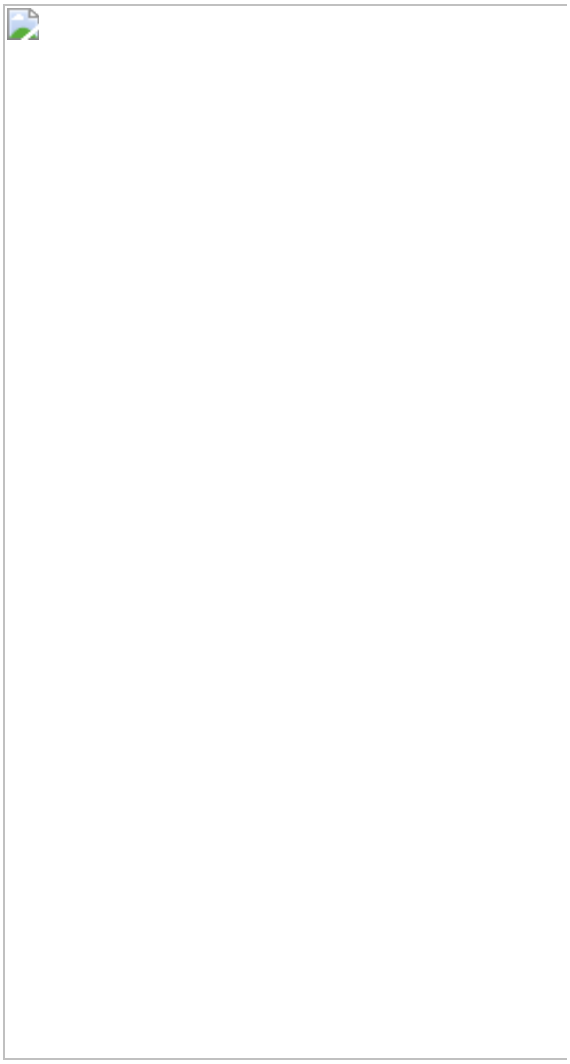


Endpoint

Timeseries: Every 10 mins

▼ Control

Startpoint



Endpoint

Timeseries: Every 10 mins

Timeseries: Every 10 mins

OBSERVATIONS

- Liposomes containing Cx43 show lower yield and smaller average size compared to liposomes without Cx43.
- The average dye fluorescence of liposomes with Cx43 is markedly reduced relative to controls, suggesting dye leakage through Cx43 channels.
- A higher proportion of non-fluorescent liposomes is observed in the Cx43-containing samples compared to those without Cx43.