Supplementary Tables

	A431 chloride salt	A431 DTT/f	CHO DTT/f	T test A431 DTT/f & chloride salt	T test A431 DTT/f & CHO DTT/f
FC	33.5±0.1	30.4±1.4	22.5±1.7	0.09	0.02
CE	0.6±0.1	0.6±0.04	7.5±0.4	0.7	7.1E-05
MG	0.2±0.05	0.4±0.01	0.2±0.04	0.04	0.03
DG	0.6±0.2	0.7±0.06	1.3±0.1	0.5	0.03
TG	0.2±0.05	0.06±0.01	0.020±0.002	0.05	0.06
Cer	1.4±0.5	0.3±0.01	0.4±0.02	0.09	0.008
SM	4.7±0.4	5.3±0.4	6.7±0.5	0.4	0.1
dhSM	1.2±0.04	1.2±0.03	0.2±0.03	0.3	2.5E-05
GalCer	0.0003±0.00002	0.0004±1.6E-05	0.0002±1.3E-05	0.03	0.001
Sulf	0.003±0.0007	0.003±0.0001	0.002±7.8E-05	0.2	0.006
GlcCer	0.2±0.05	0.09±0.009	0.2±0.02	0.3	0.01
LacCer	0.05±0.02	0.05±0.01	0.3±0.08	0.9	0.03
GM3	0.2±0.04	0.2±0.05	1.4±0.1	0.6	0.0009
PA	0.1±0.01	0.2±0.02	0.06±0.02	0.01	0.02
PC	28.9±0.5	35.2±1.4	38.9±3.2	0.01	0.3
PCe	7.4±2.2	4.2±0.5	8.9±1.8	0.2	0.07
PE	2.7±0.6	4.1±0.2	2.6±0.2	0.09	0.005
PEp	9.1±0.7	6.9±0.2	2.1±0.09	0.04	2.7E-05
PG	0.2±0.1	0.1±0.002	0.1±0.006	0.4	0.008
PI	4.0±0.9	4.2±0.6	2.9±0.3	0.9	0.1
PS	3.9±0.4	4.5±0.2	2.4±0.1	0.3	0.002
LPC	0.5±0.2	0.9±0.6	0.7±0.4	0.5	0.7
LPCe	0.03±0.02	0.04±0.02	0.06±0.05	0.8	0.7
LPE	0.09±0.02	0.2±0.01	0.2±0.03	0.004	0.3
LPEp	0.1±0.1	0.02±0.003	0.01±0.003	0.3	0.2
LPI	0.02±0.01	0.02±0.003	0.07±0.02	0.8	0.08
BMP	0.2±0.06	0.1±0.006	0.02±0.002	0.4	0.0001
APG	0.004±0.0006	0.004±3.2E-05	0.002±0.0003	0.5	0.01
NAPE	4.4E-05±1.5E-05	4.7E-05±3.4E-06	4.1E-05±6.6E-06	0.8	0.5
NAPEp	0.0006±0.0002	0.0006±6.3E-05	0.0002±2.1E-05	0.9	0.004
NAPS	0.008±0.003	0.002±0.0003	0.001±0.0001	0.1	0.02

Table S1. Lipid and cholesterol composition of A431 chloride salt vesicles, A431 DTT/formaldehyde vesicles, and CHO DTT/formaldehyde vesicles (see also Figure 1).

	A431 chloride salt	A431 DTT/f	CHO DTT/f	T test A431 DTT/f & chloride salt	T test A431 DTT/f & CHO DTT/f
MG	0.3±0.08	0.5±0.01	0.3±0.06	0.05	0.02
DG	0.9±0.3	1.0±0.09	1.8±0.2	0.6	0.02
TG	0.3±0.08	0.08±0.02	0.03±0.004	0.05	0.07
Cer	2.0±0.7	0.5±0.02	0.6±0.02	0.09	0.01
SM	7.2±0.6	7.7±0.6	9.6±0.5	0.6	0.07
dhSM	1.8±0.05	1.7±0.7	0.3±0.05	0.1	0.0001
GalCer	0.0004±0.00003	0.0005±0.00002	0.0003±0.00002	0.04	0.0005
Sulf	0.004±0.0001	0.004±0.0001	0.003±0.0002	0.5	0.006
GlcCer	4±0.07	0.1±0.01	0.3±0.02	0.2	0.007
LacCer	0.08±0.02	0.08±0.02	0.4±0.1	0.9	0.03
GM3	0.3±0.06	0.3±0.08	1.9±0.1	0.7	0.0003
PA	0.1±0.01	0.3±0.02	0.09±0.04	0.02	0.02
PC	43.8±0.8	51.0±1.4	55.5±3.1	0.01	0.2
PCe	11.3±3.3	6.0±0.7	12.9±3.1	0.2	0.09
PE	4.0±0.9	6.0±0.4	3.8±0.4	0.1	0.01
PEp	13.9±1.1	10.0±0.4	3.0±0.2	0.03	0.00008
PG	0.3±0.1	0.1±0.005	0.20±0.004	0.4	0.002
PI	6.1±1.4	6.1±0.9	4.2±0.3	1.0	0.1
PS	5.9±0.6	6.5±0.4	3.5±0.1	0.4	0.001
LPC	0.7±0.3	1.3±0.9	1.0±0.6	0.5	0.8
LPCe	0.04±0.02	0.05±0.03	0.09±0.07	0.8	0.6
LPE	0.1±0.03	0.3±0.02	0.3±0.04	0.006	0.2
LPEp	0.2±0.2	0.02±0.005	0.01±0.005	0.3	0.2
LPI	0.03±0.02	0.03±0.004	0.1±0.03	0.9	0.08
BMP	0.3±0.09	0.2±0.01	0.03±0.003	0.3	0.0004
APG	0.006±0.0009	0.005±0.0001	0.003±0.0006	0.4	0.02
NAPE	0.00007±0.00002	0.00007±5.7E-06	0.00006±7.80E-06	0.9	0.4
NAPEp	0.0009±0.0002	0.0008±0.00009	0.0002±0.00002	0.8	0.003
NAPS	0.01±0.004	0.003±0.00009	0.002±0.0003	0.1	0.02

Table S2. Lipid composition of A431 chloride salt vesicles, A431 DTT/formaldehyde vesicles, and CHO DTT/formaldehyde vesicles, recalculated without cholesterol content by re-scaling the LC-MS data shown in Table S1 and Figure 1.

Supplementary Figures

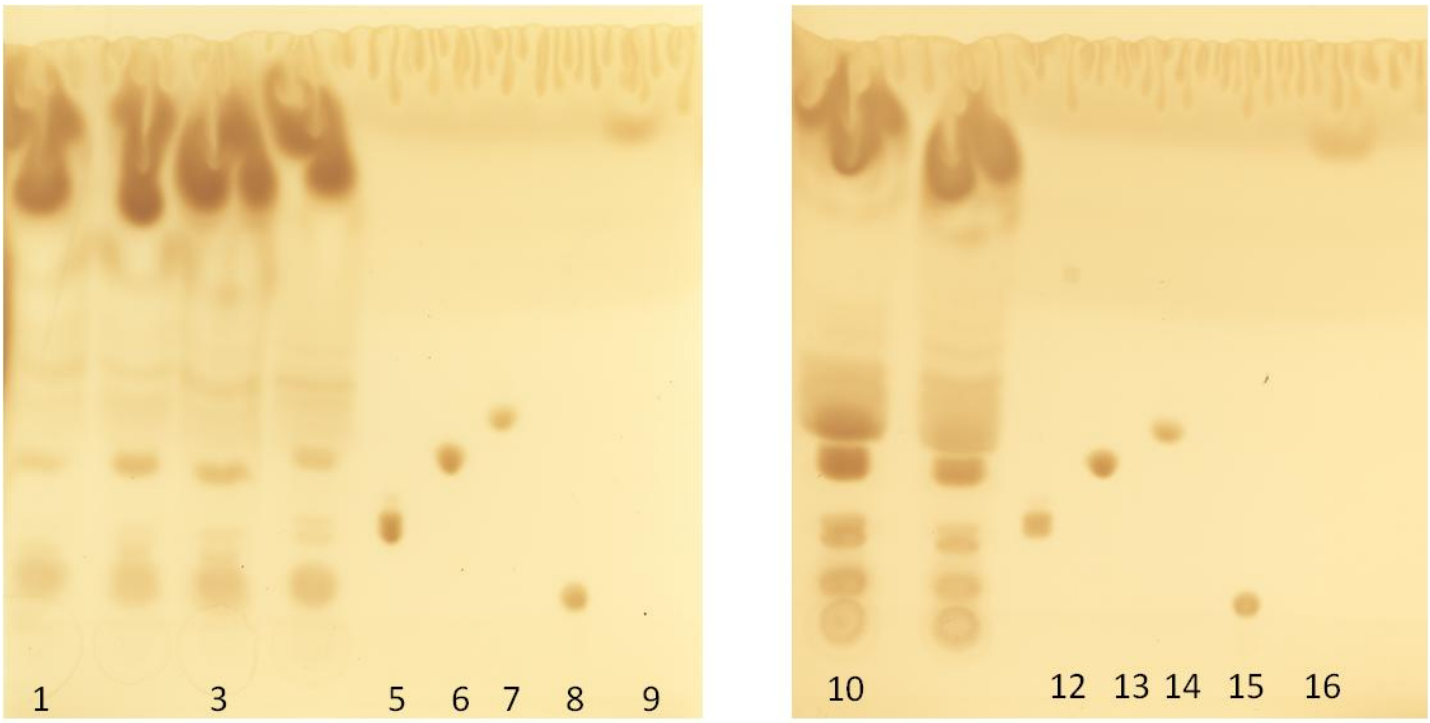
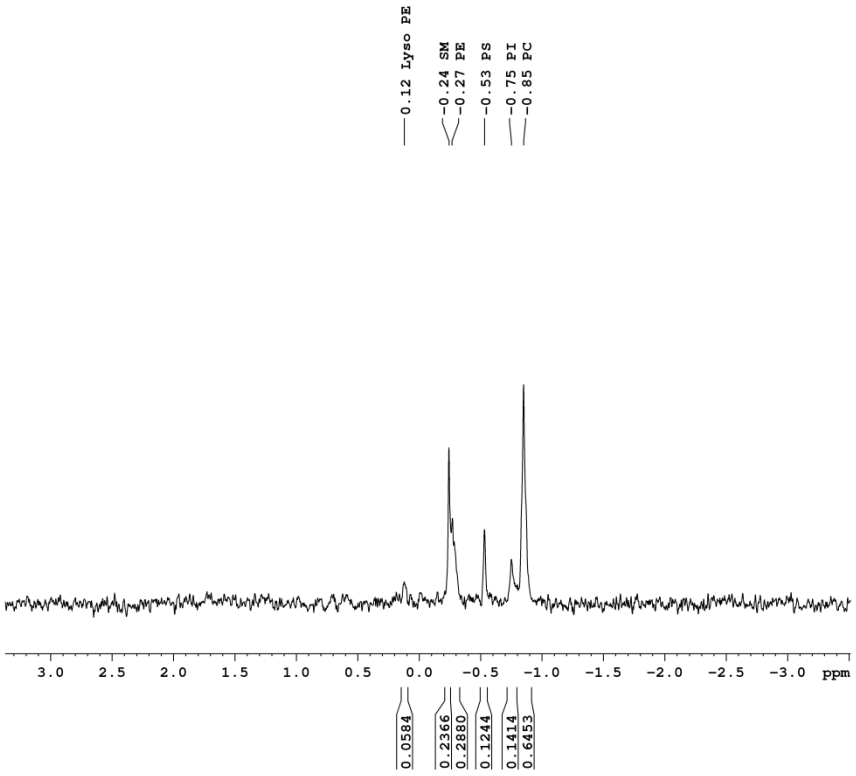


Figure S1. Thin-layer chromatogram of lipid extracts from different vesicle preparations. **Lane 1:** CHO, DTT/formaldehyde method. **Lane 3:** A431, DTT/formaldehyde method. **Lane 5:** Sphingomyelin (SM1 and SM2). **Lane 6:** Phoshatidylcholine (PC), **Lane 7:** Phoshatidylethanolamine (PE). **Lane 8:** Phoshatidylserine (PS). **Lane 9:** Cholesterol (CL). **Lane 10:** A431, chloride salt osmotic method. **Lane 12:** Sphingomyelin (SM1 and SM2). **Lane 13:** Phoshatidylcholine (PC), **Lane 14:** Phoshatidylethanolamine (PE). **Lane 15:** Phoshatidylserine (PS). **Lane 16:** Cholesterol (CL). **Thin layer chromatography results for lipid extracts from the three types of vesicles.** Qualitatively, the results support the MS-LC data, as the TLC analysis identifies PC, PE, PS, SM, and Cholesterol.

A.



B.

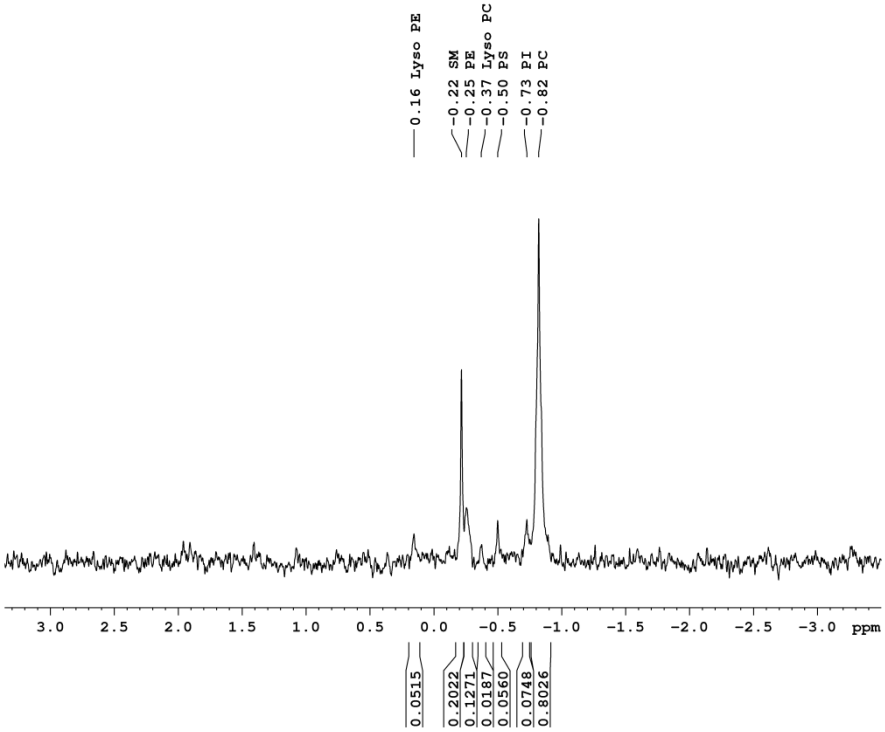


Figure S2. ^{31}P NMR spectra (acquired by Avanti Polar Lipids Analytical Services) for (A) A431 chloride salt vesicles sample and (B) A431 DTT/formaldehyde vesicle sample.

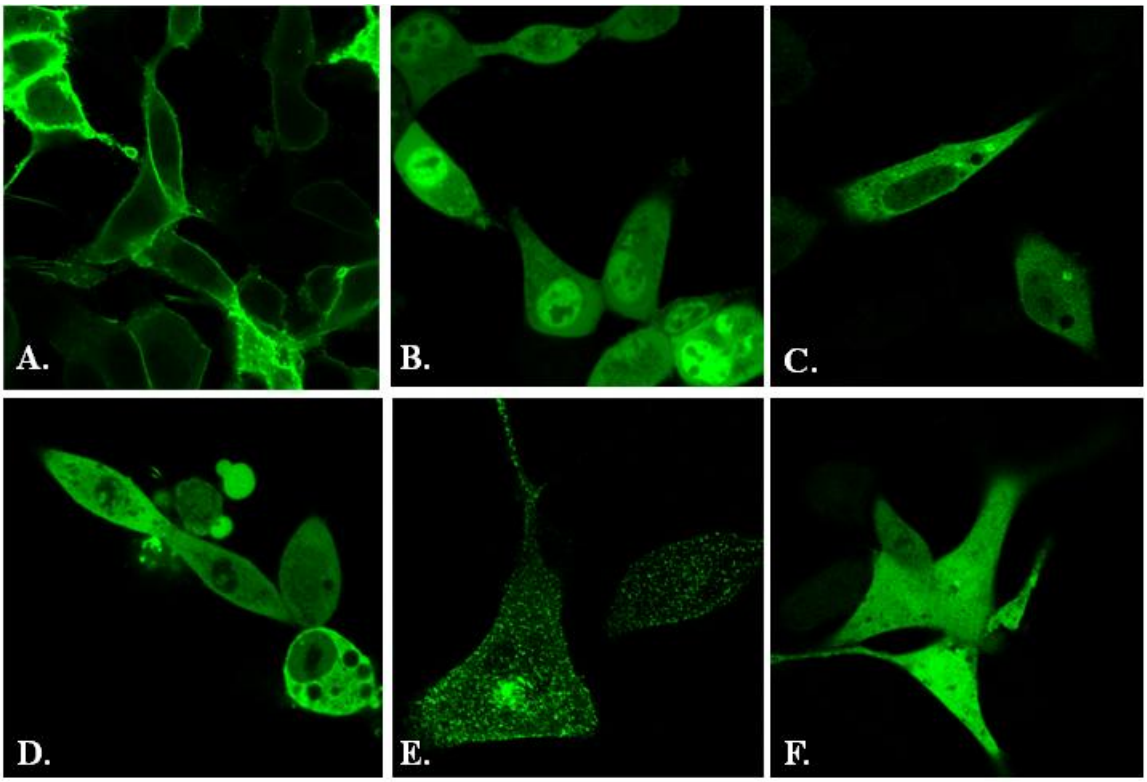


Figure S3. CHO cells expressing the soluble cytoplasmic proteins used in the leakage assays: **(A)** Plcδ1-PH-GFP (MW~45 kDa), **(B)** Grb2-Venus (MW~60 kDa), **(C)** PKCϴ-GFP (MW~120kDa), **(D)** Venusx6 (MW~160 kDa), **(E)** Intersectin II-GFP (MW~ 170 kDa) and **(F)** Plcɣ-GFP (MW~210 kDa).

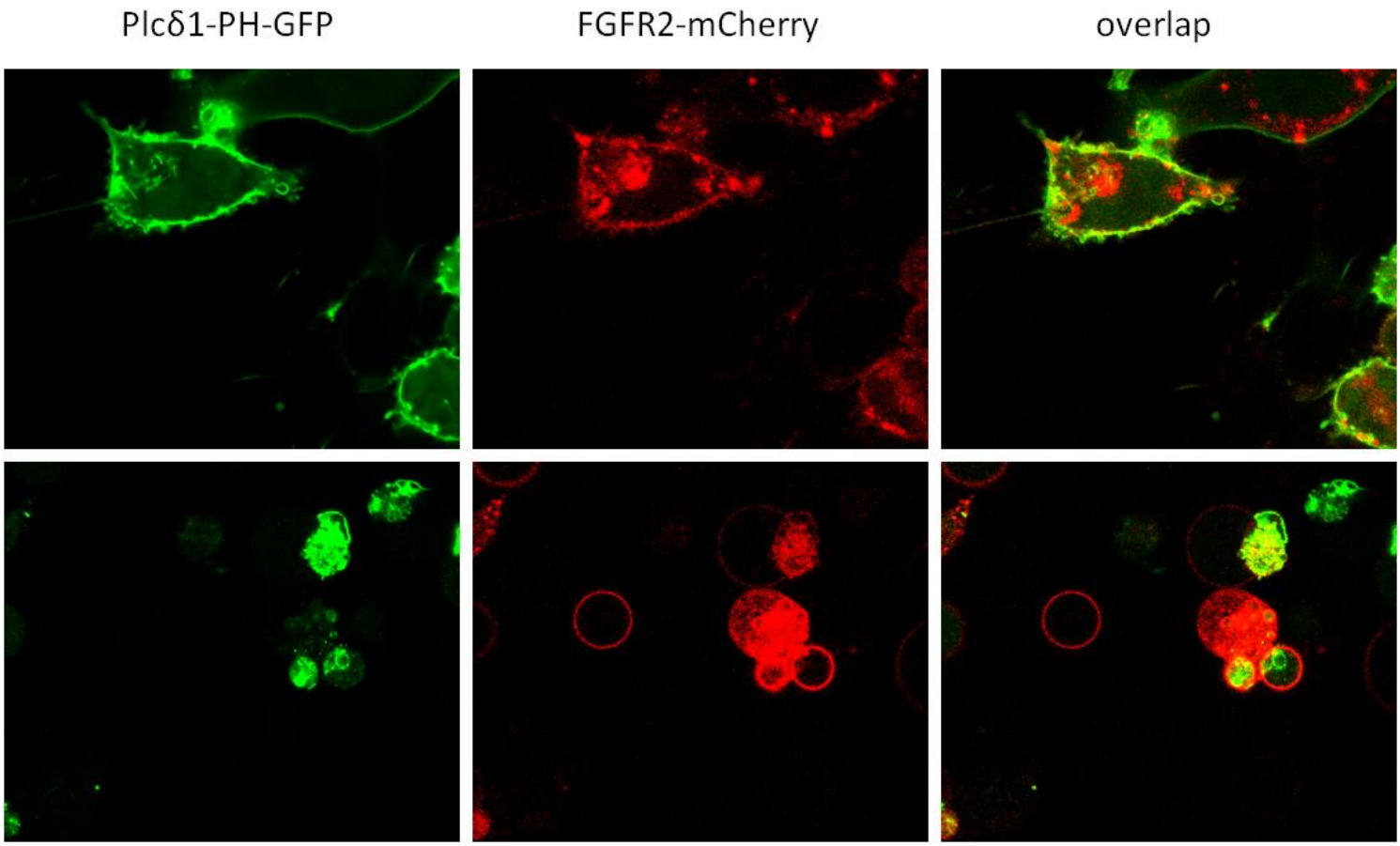


Figure S4. Top panel: Chinese Hamster Ovary (CHO) cells co-expressing FGFR2-mCherry and Plcδ1-PH-GFP. Bottom panel: Plcδ1-PH-GFP (MW~45 kDa) in not retained inside CHO chloride salt vesicles.

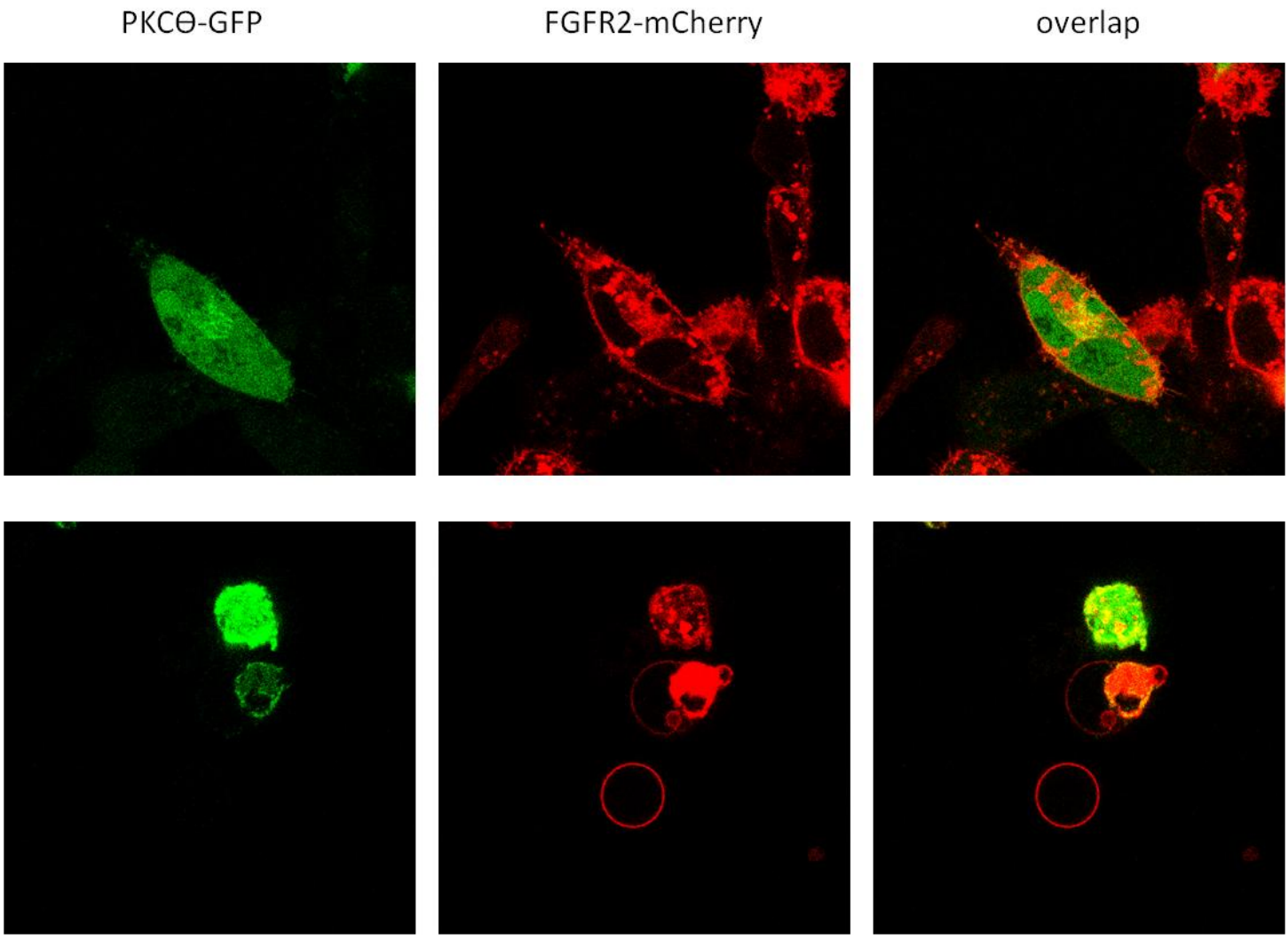


Figure S5. Top panel: CHO cells co-expressing PKCϴ-GFP and FGFR2-mCherry. Bottom panel: PKCϴ-GFP (MW~ 120kDa) is not retained inside CHO chloride salt vesicles.

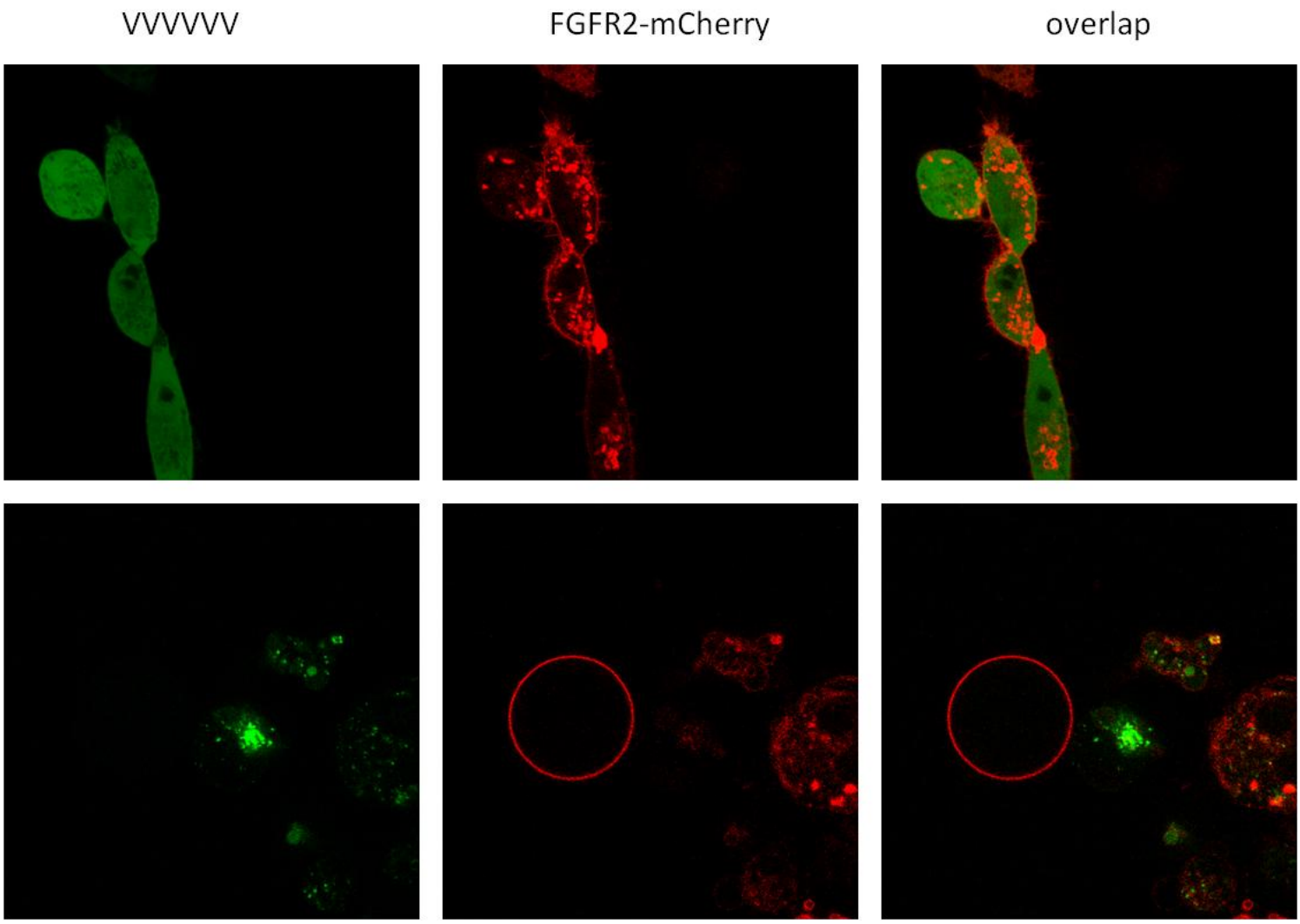


Figure S6. Top panel: Chinese Hamster Ovary (CHO) co-expressing VVVVVV (Venus x 6) and FGFR2-mCherry. Bottom panel: Venus x 6 (MW~ 160kDa) is not retained in CHO chloride salt vesicles.

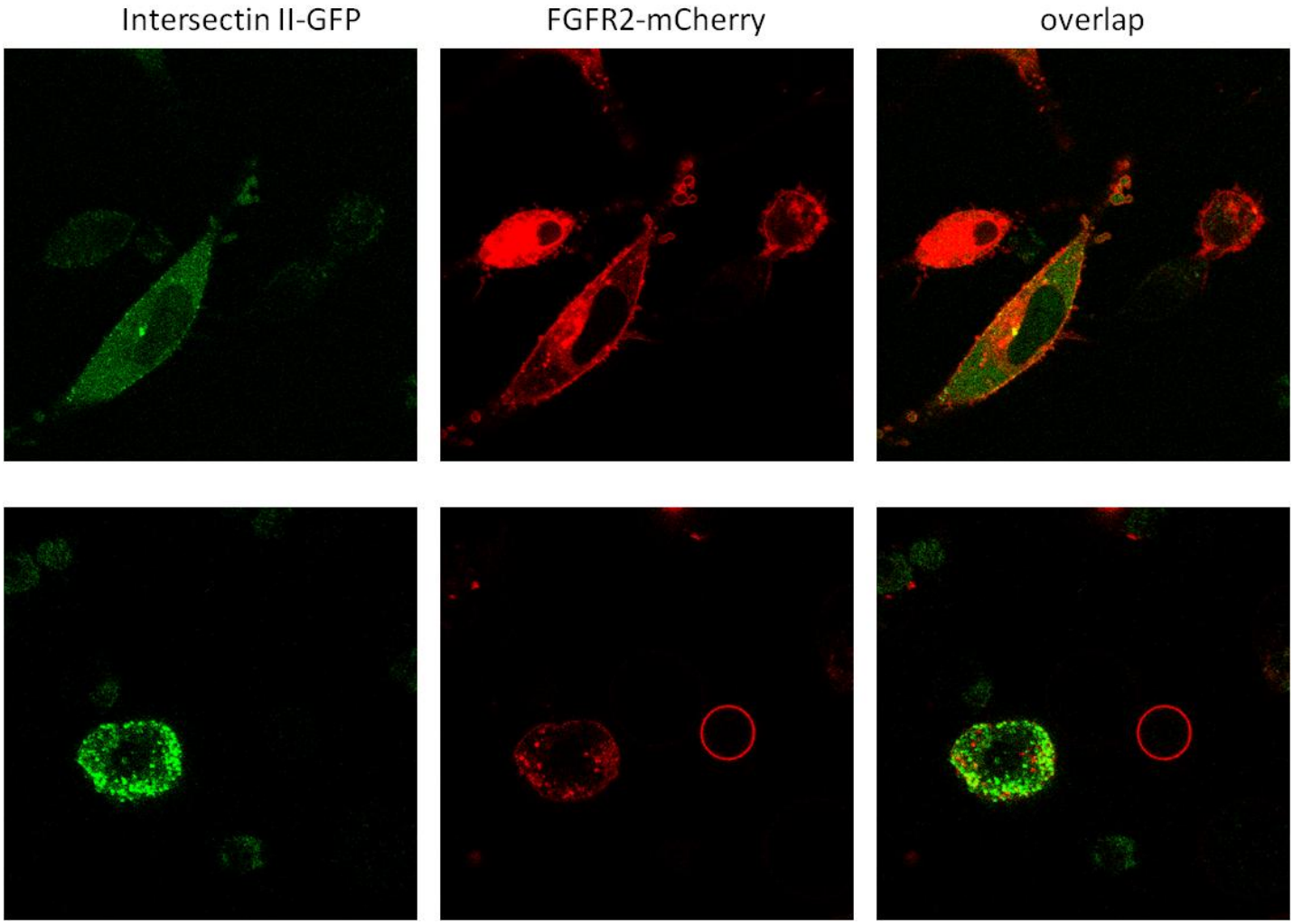


Figure S7. Top panel: CHO cells co-expressing Intersectin II-GFP and FGFR2-mCherry. Bottom panel: Intersectin II-GFP (MW~ 170kDa) is not retained inside CHO chloride salt vesicles.