Literature Report

Reporter: Wang Guangying
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Organelle membrane-specific chemical labeling and dynamic imaging in living cells

Tomonori Tamura[1,2], Alma Fujisawa[1,2], Masaki Tsuchiya[1,2], Yuying Shen[1], Kohjiro Nagao[1], Shin Kawano[3,4], Yasushi Tamura[5], Toshiya Endo[3,4], Masato Umeda[1] and Itaru Hamachi[1,2] ✉
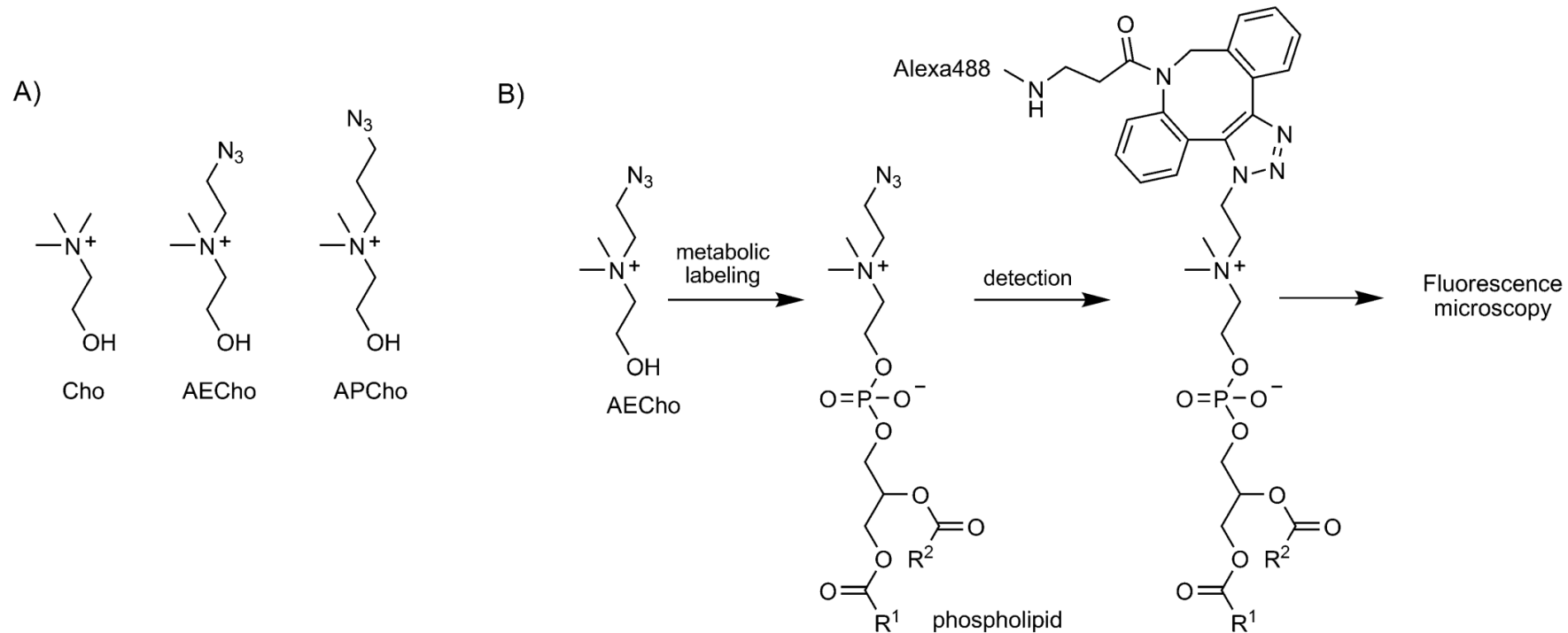
Intracellular Transport of Phosphatidylcholine to the Plasma Membrane

Pulse labeling of CPLs

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A) Cho, AECho, APCho

B) AECho → (metabolic labeling) → N3 phospholipid → (detection) → Alexa488 labeled → Fluorescence microscopy
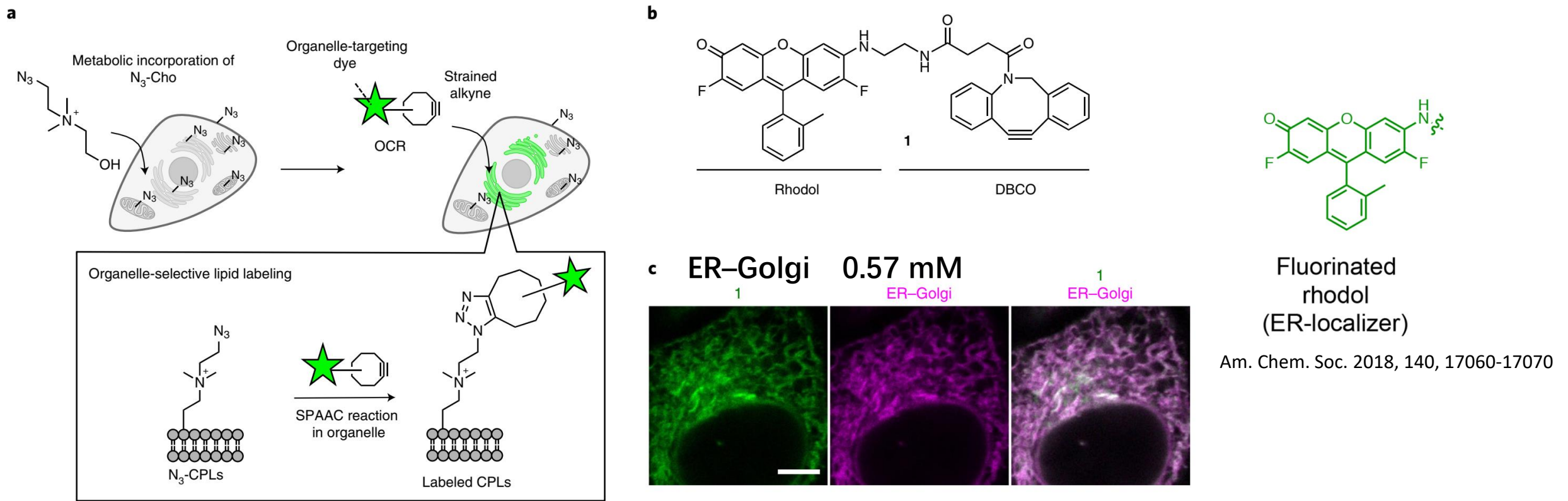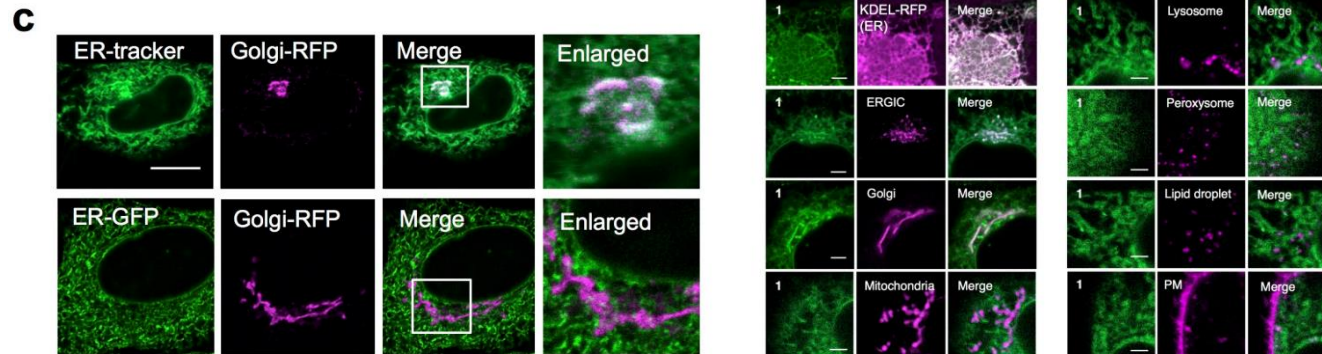
ChemBioChem 2015, 16, 472 − 476

Fig. 1 | ER–golgi-selective PC labeling and imaging in live cells. **a**, Schematic of organelle-selective lipid labeling with OCR. **b**, Molecular structure of the ER–Golgi OCR **1**. **c**, CLSM images of a HeLa cell treated with **100 nM 1** (green) for 15 min. **ER-Tracker** (magenta) was used for imaging both the ER and Golgi because it stains both in HeLa cells (Supplementary Fig. 1c). Scale bar. 5 um

Supplementary Figure 1. Spectroscopic properties and intracellular localization of 1.

Am. Chem. Soc. 2018, 140, 17060-17070

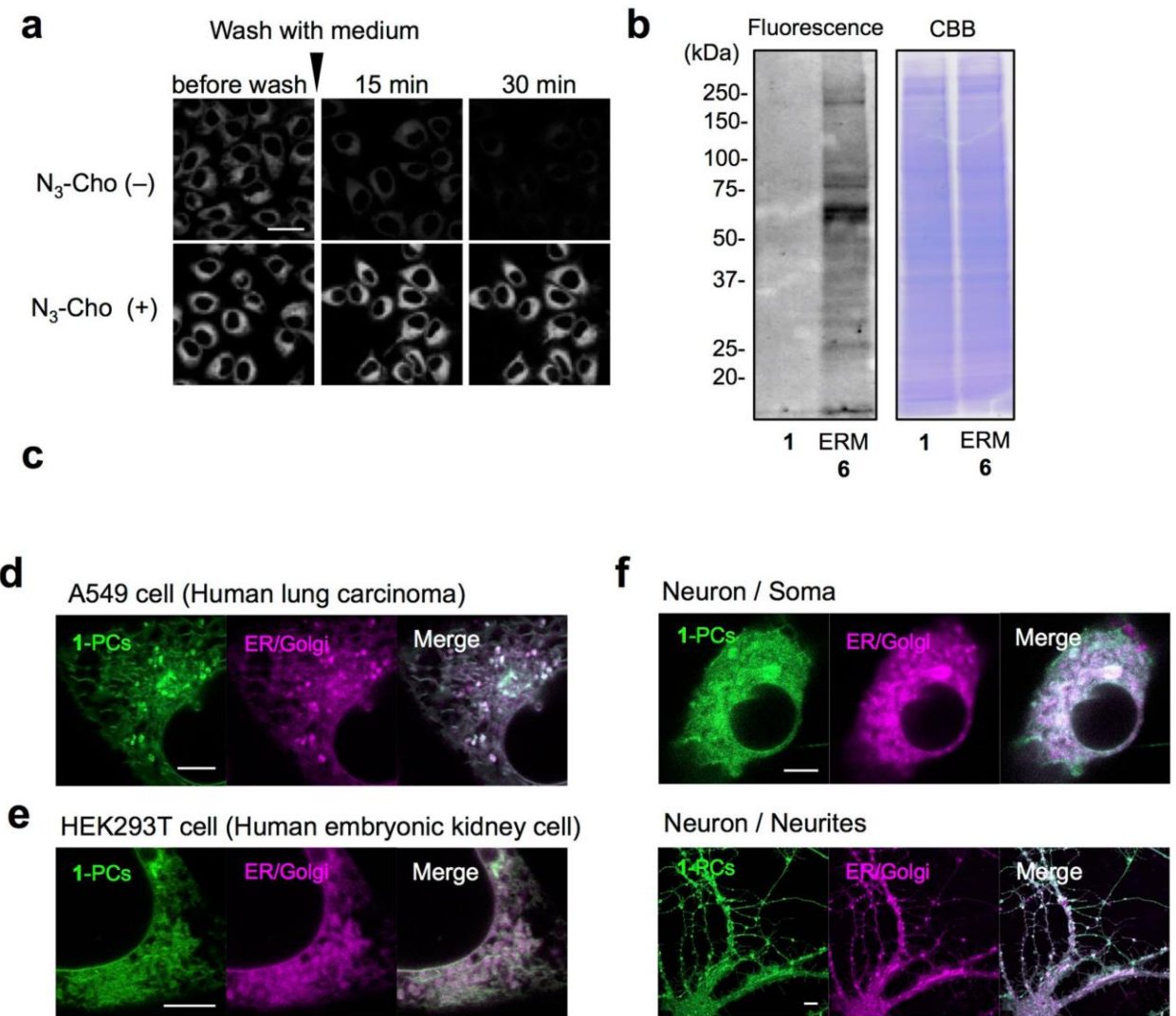**ER, the ER–Golgi, Golgi,** mitochondria, lysosomes, peroxisomes, lipid droplets and PMs
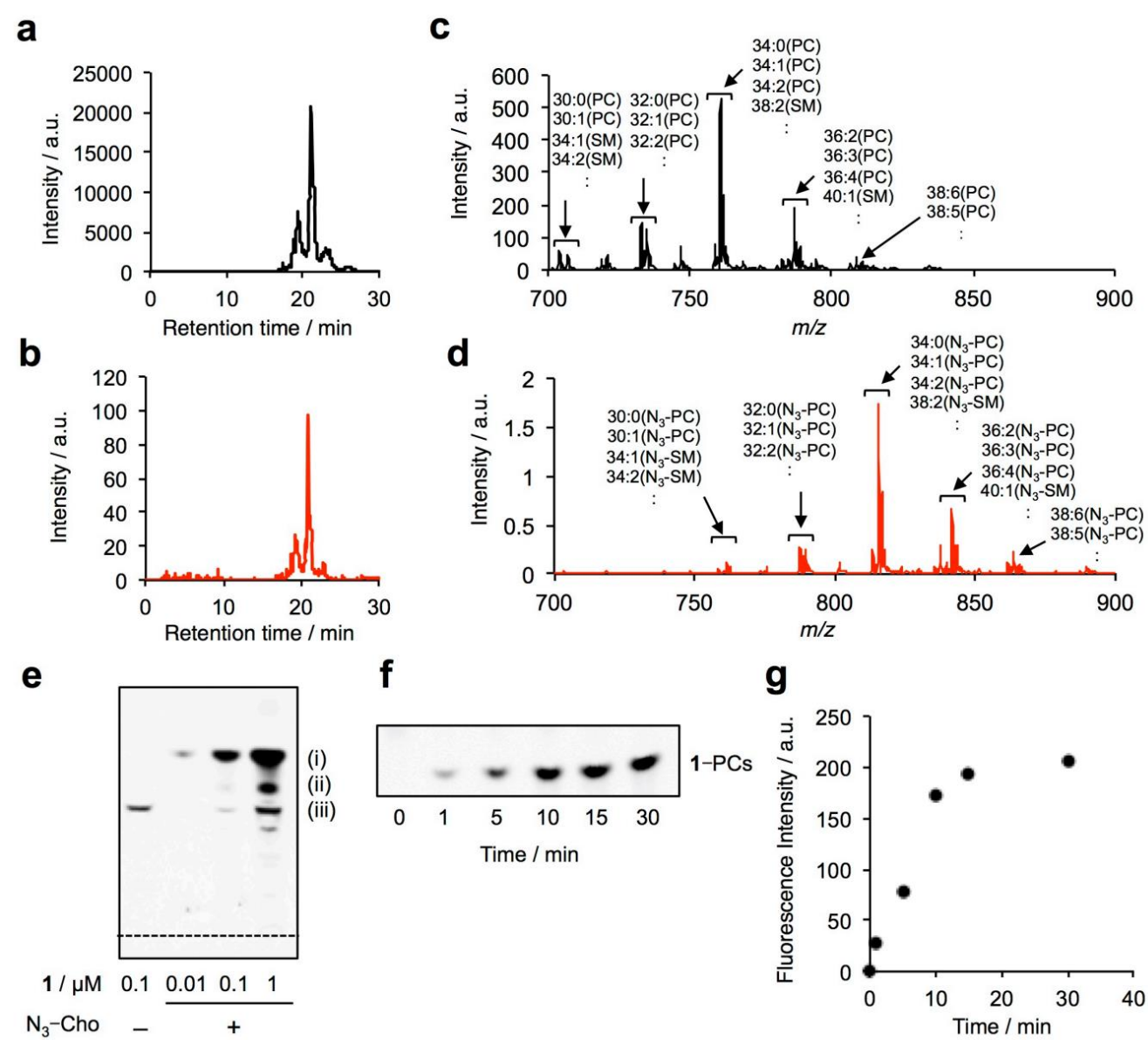
Supplementary Figure 2. LC-MS and TLC analysis of metabolic incorporation of N3-Cho and subsequent spatially limited strain-promoted alkyne-azide cycloaddition (SPAAC) reaction.
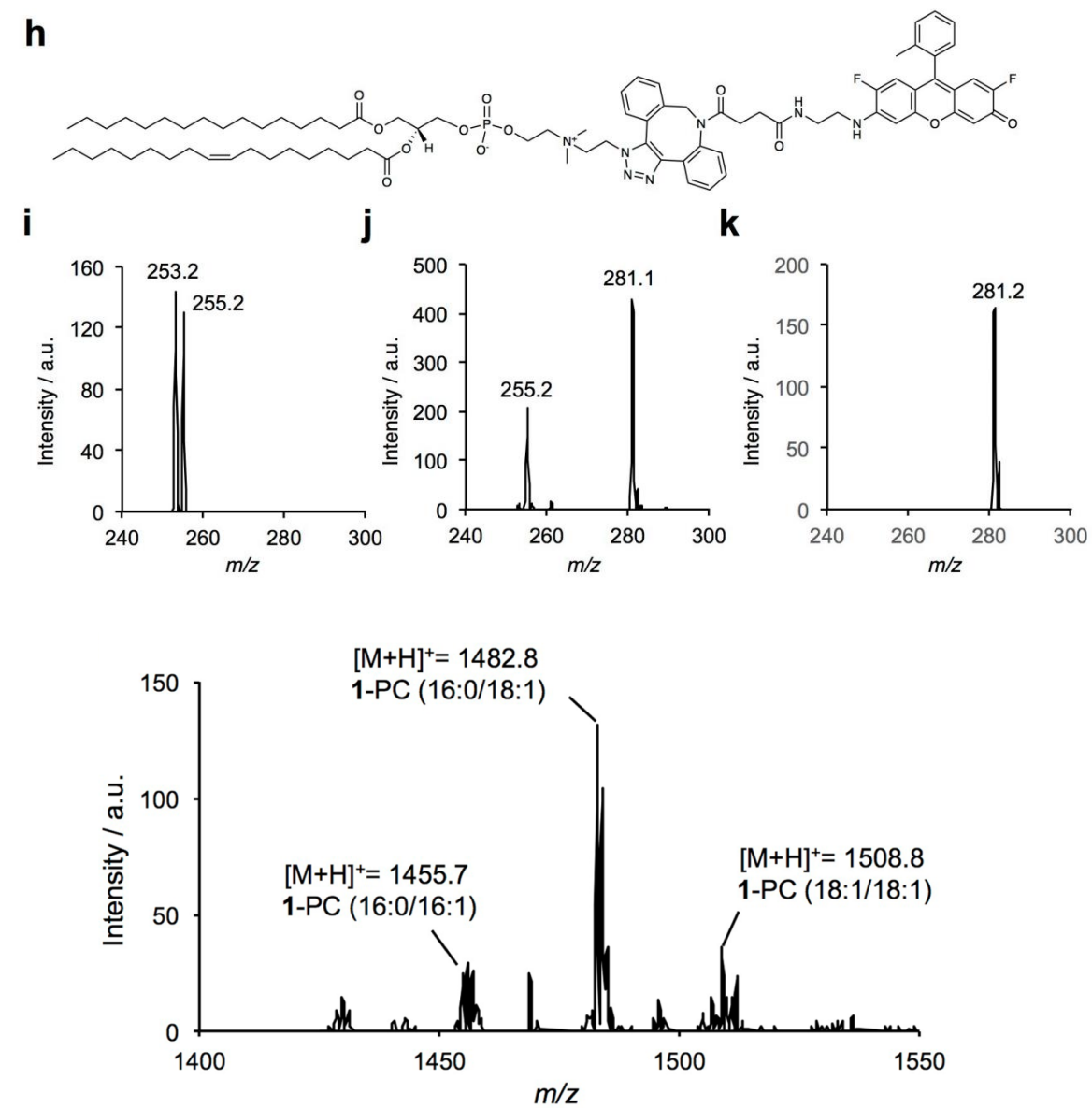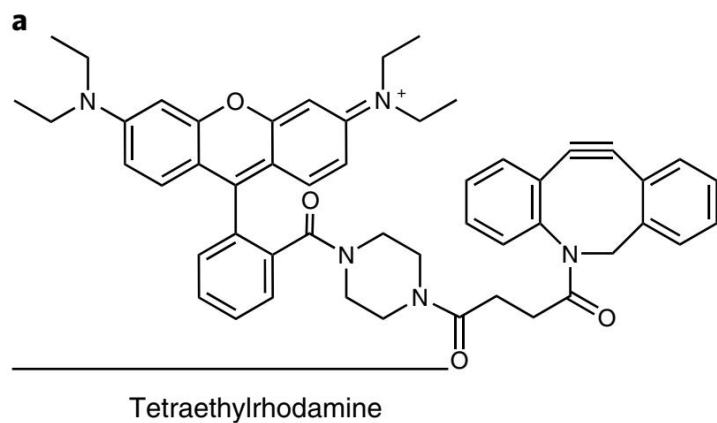
Extended Data Fig. 1 | Product analysis of ER/golgi-selective phosphatidylcholine (PC) labelling in live cells.
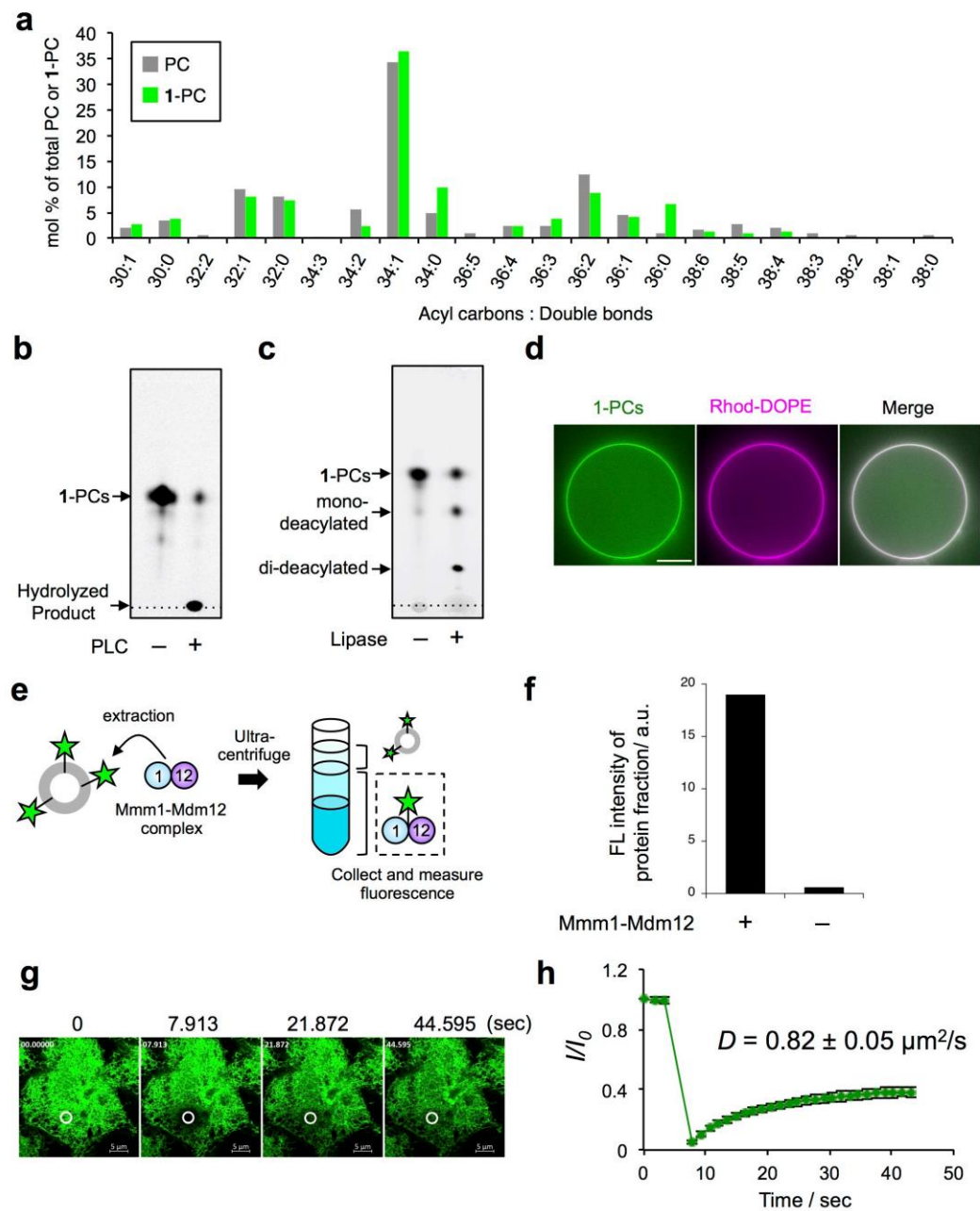
Supplementary Figure 3. Biochemical and physical properties of 1-PCs.

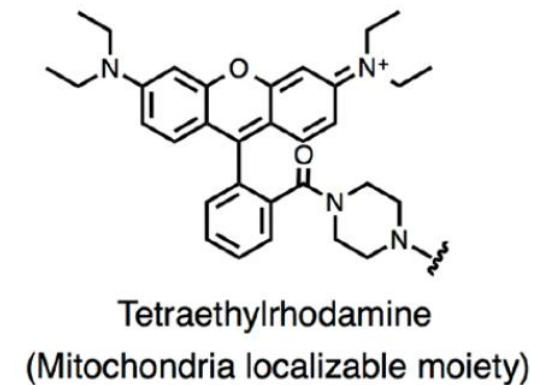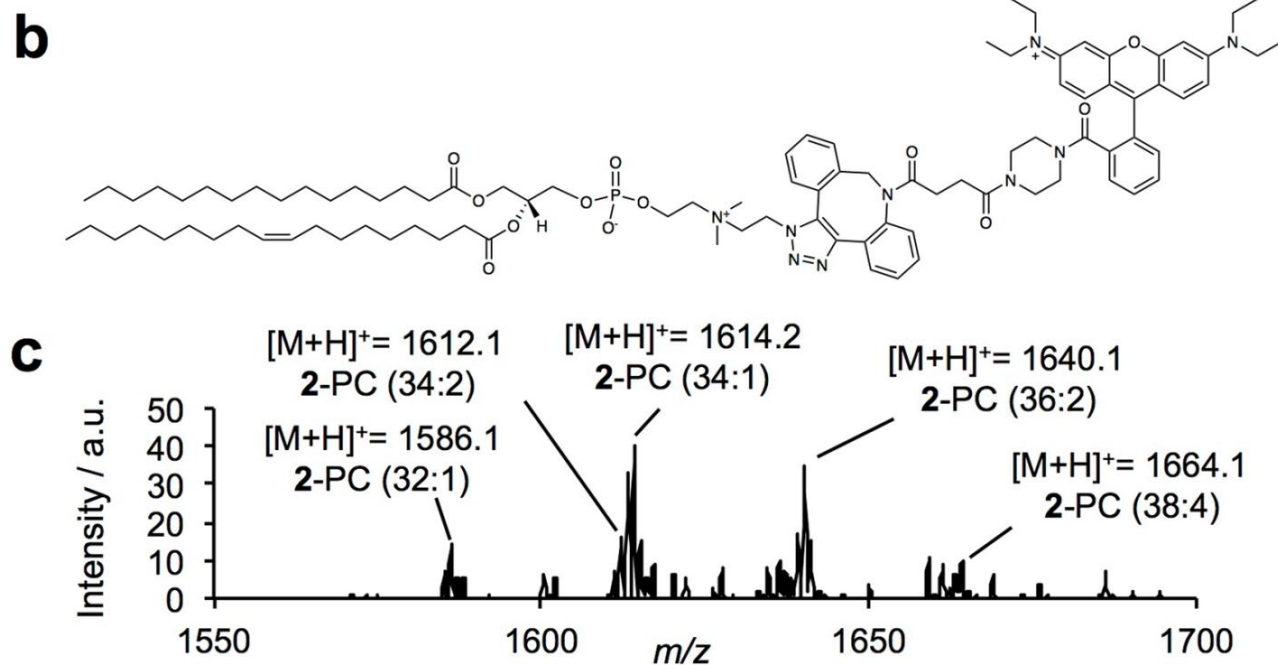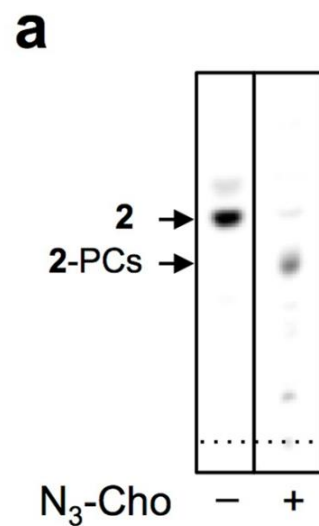**Extended Data Fig. 2 |** ER/golgi-specific PC labelling with **1**

a

Tetraethylrhodamine

2

b

2–PC

Rhodamine 123

2–PC
Rhodamine 123

Tetraethylrhodamine
(Mitochondria localizable moiety)

Fig. 2 | Mitochondria-selective PC labeling and imaging

J. Am. Chem. Soc. 2019, 141, 2782-2799

a

2
2-PCs

N$_3$-Cho   −   +

b

c

$[M+H]^+ = 1612.1$
2-PC (34:2)

$[M+H]^+ = 1614.2$
2-PC (34:1)

$[M+H]^+ = 1640.1$
2-PC (36:2)

$[M+H]^+ = 1586.1$
2-PC (32:1)

$[M+H]^+ = 1664.1$
2-PC (38:4)

Intensity / a.u.

50
40
30
20
10
0

1550   1600   1650   1700

m/z

Extended Data Fig. 3 | labelling and imaging of PCs in mitochondria with 2
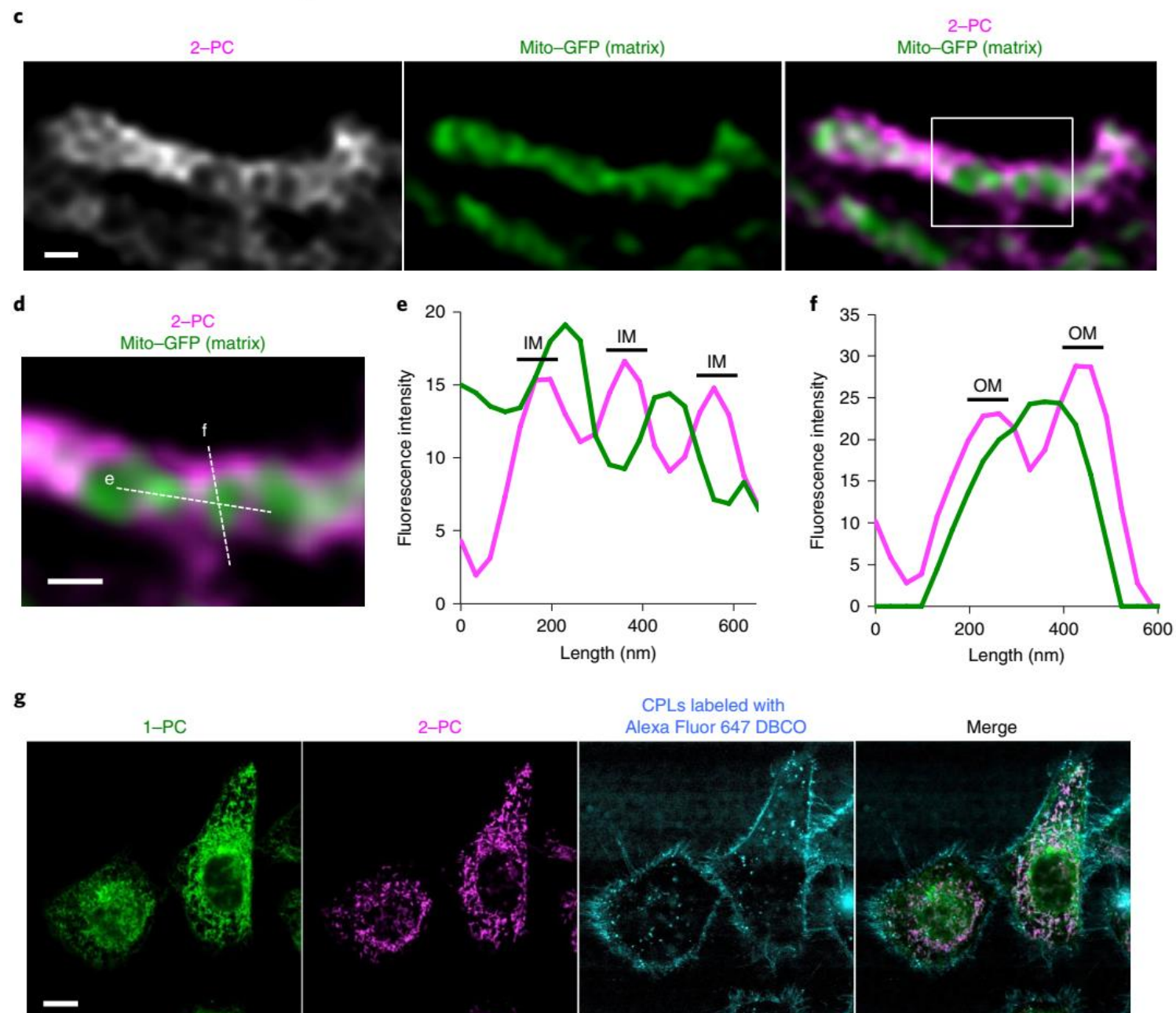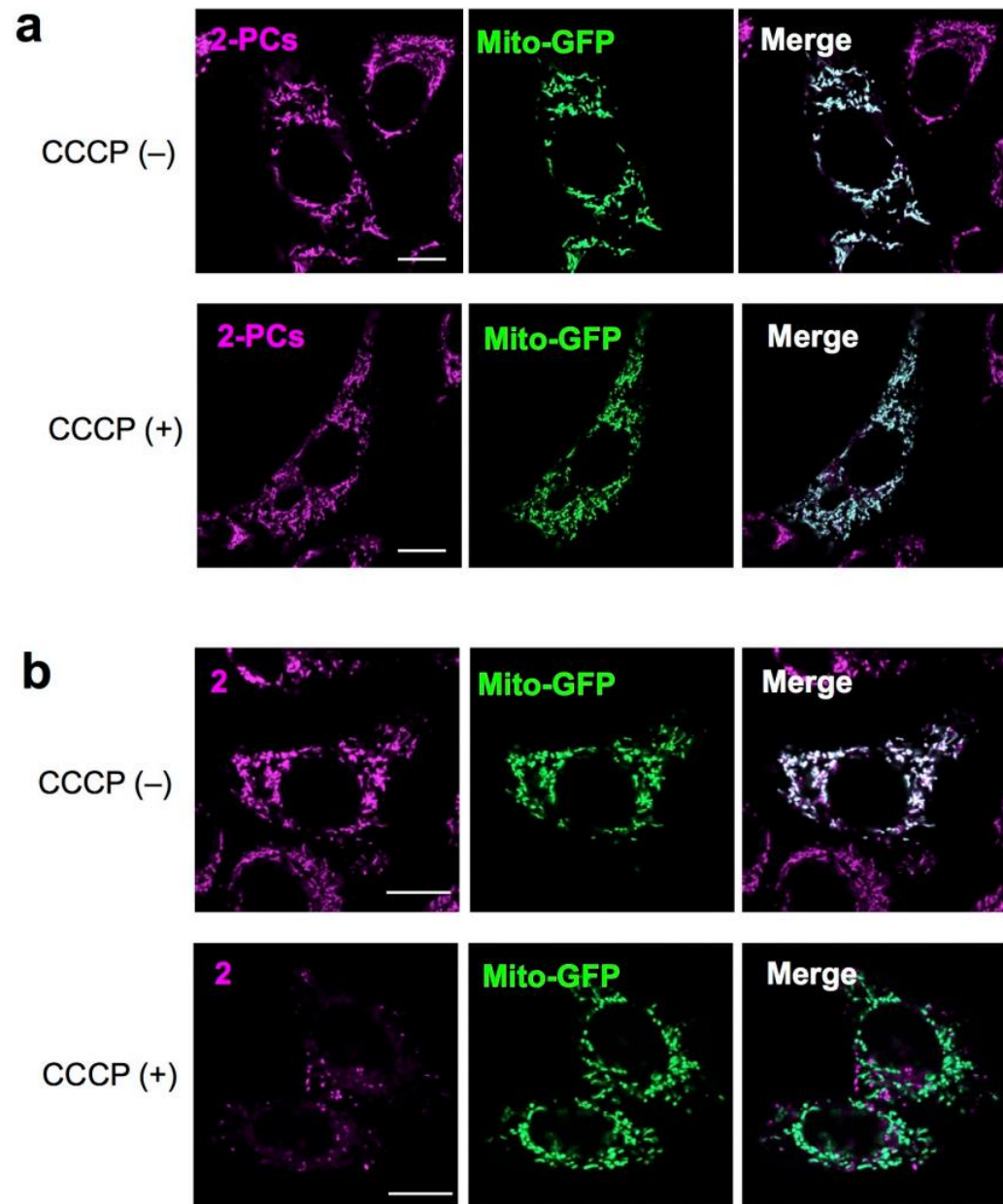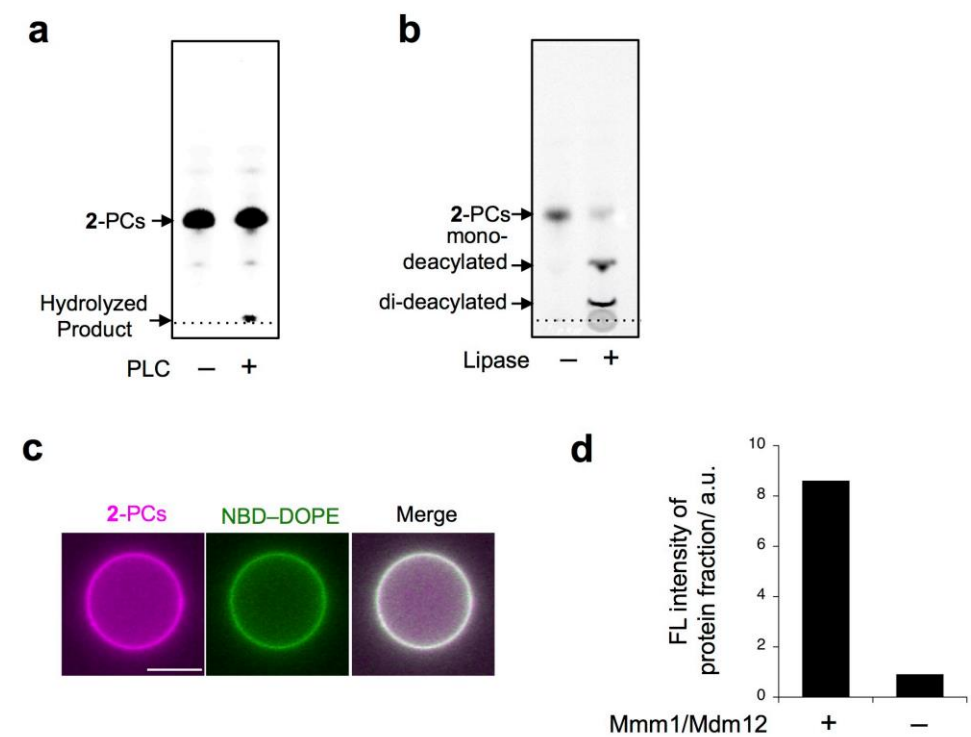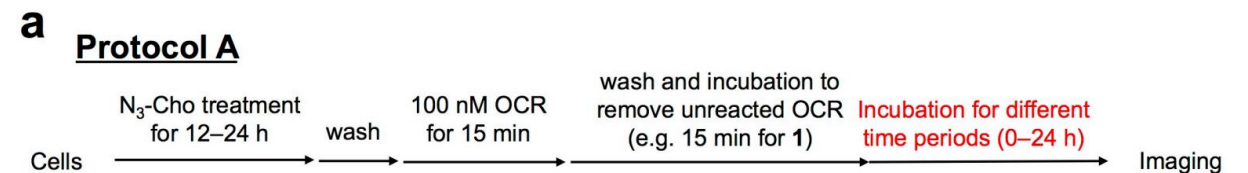
Fig. 2 | Mitochondria-selective PC labeling and imaging

Supplementary Figure 5. Mitochondrial retention of 2-PCs does not depend on the membrane potential.

Supplementary Figure 6. Biochemical and physical properties of 2-PCs.

Protocol A

Supplementary Figure 7. Two different protocols for fluorescent imaging of interorganelle translocation of labelled PCs.
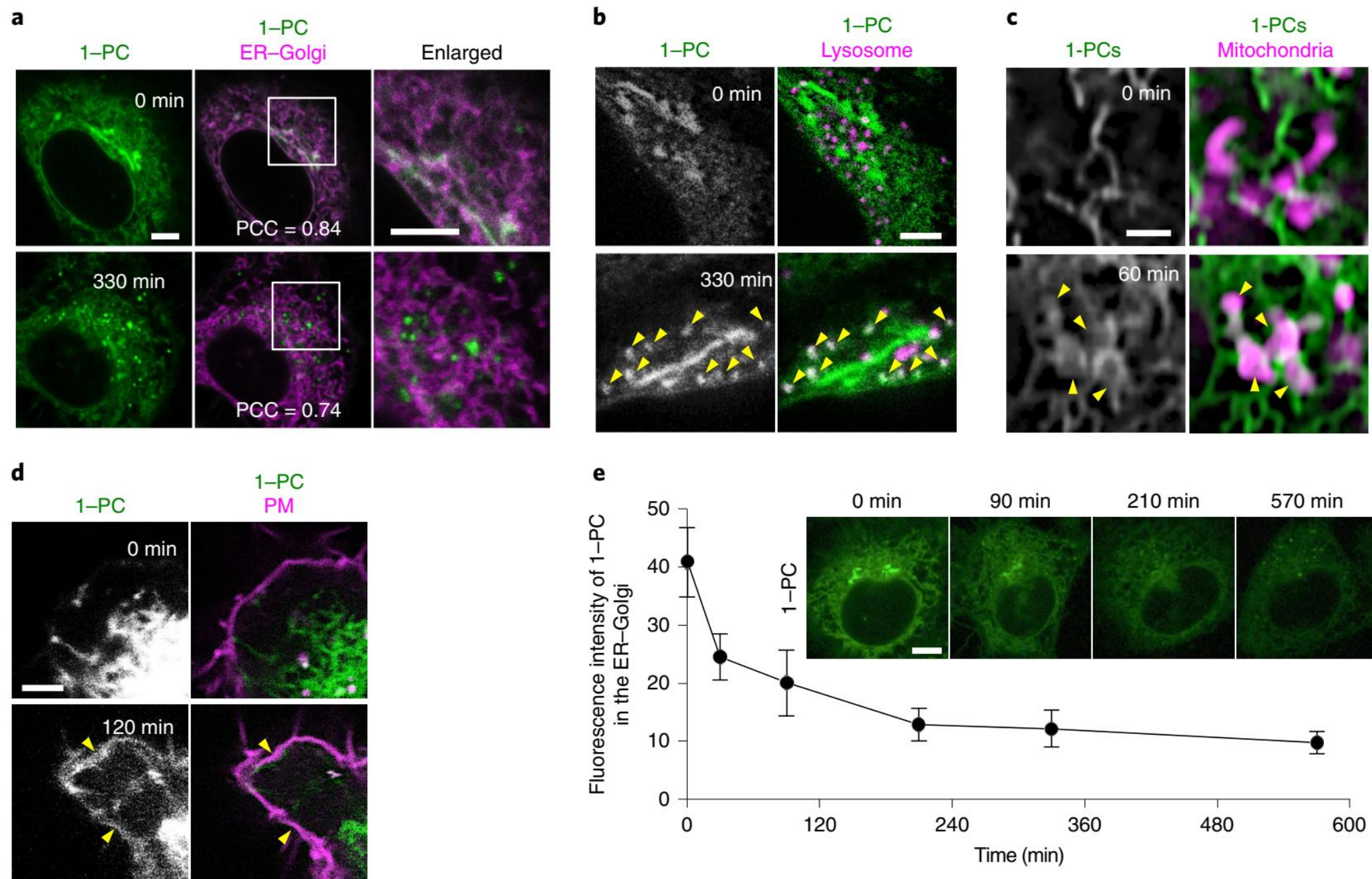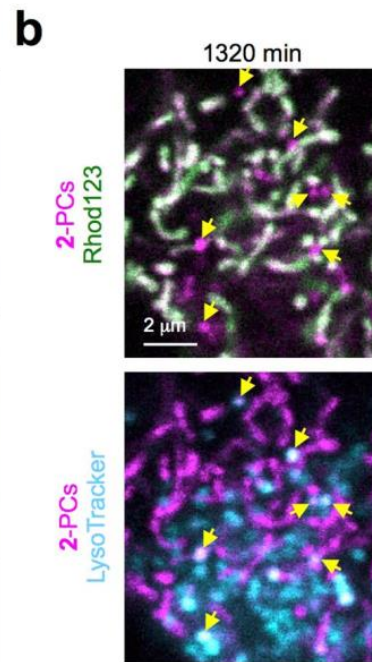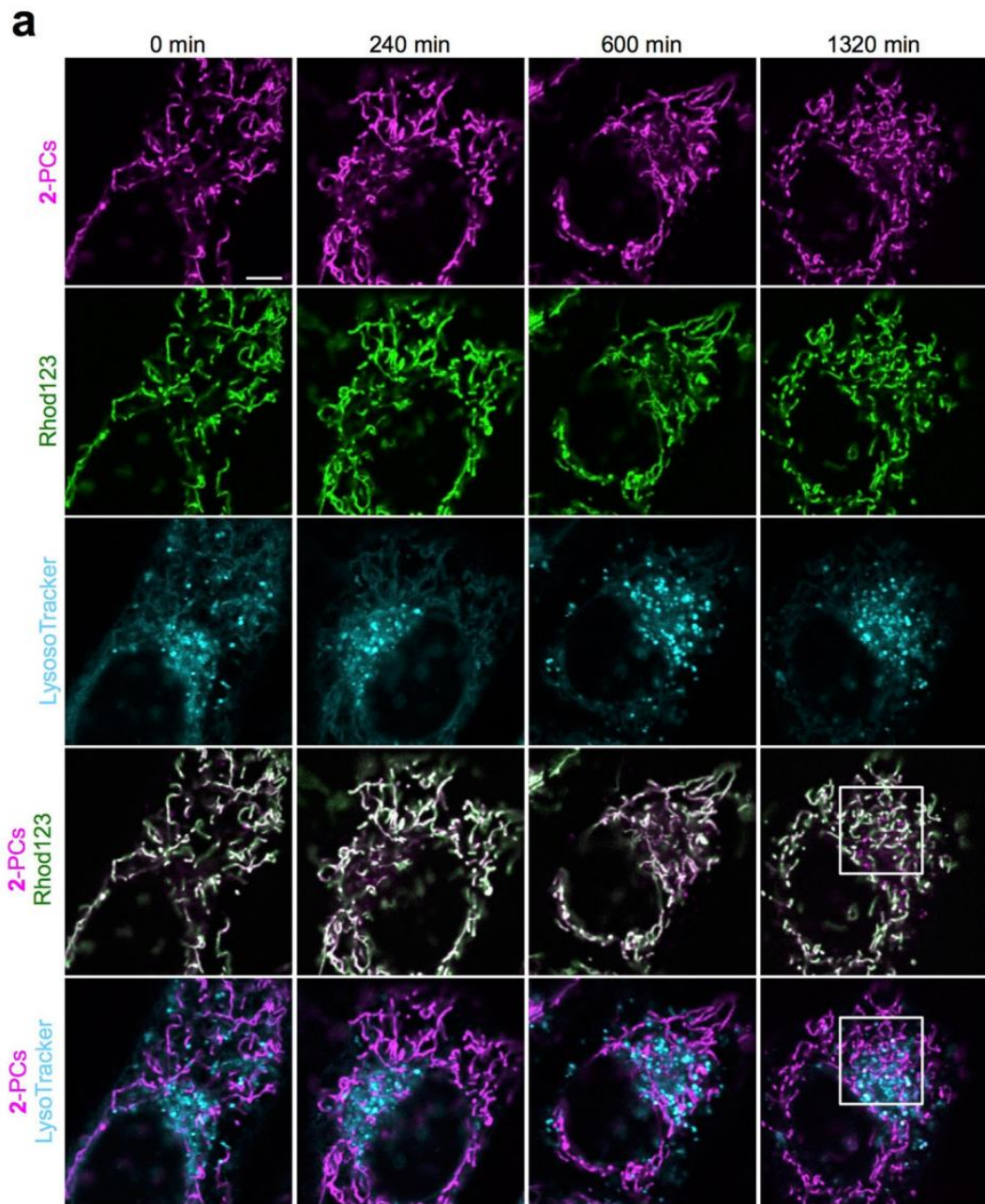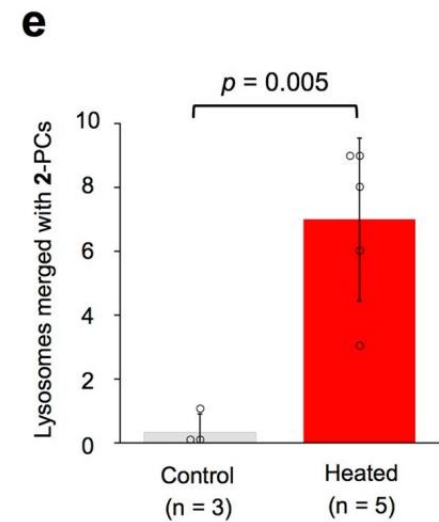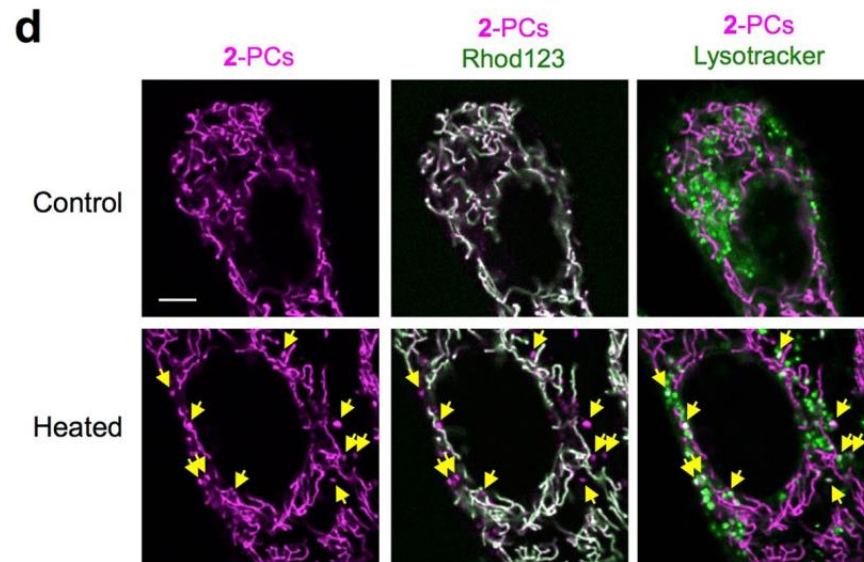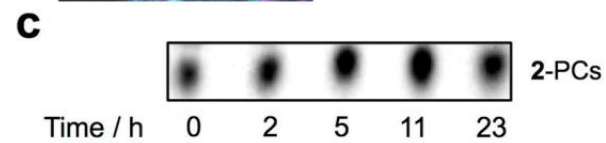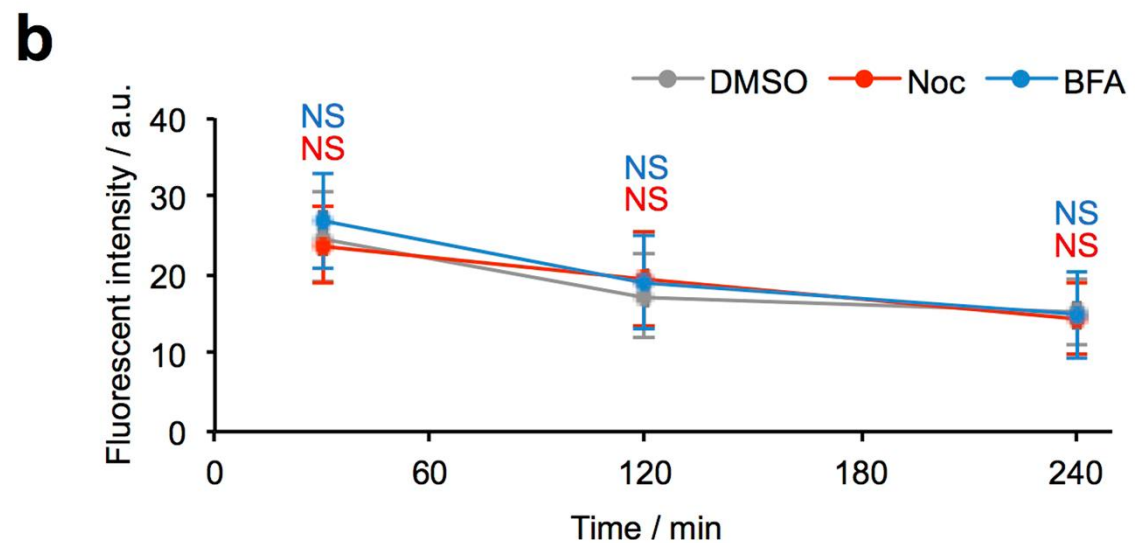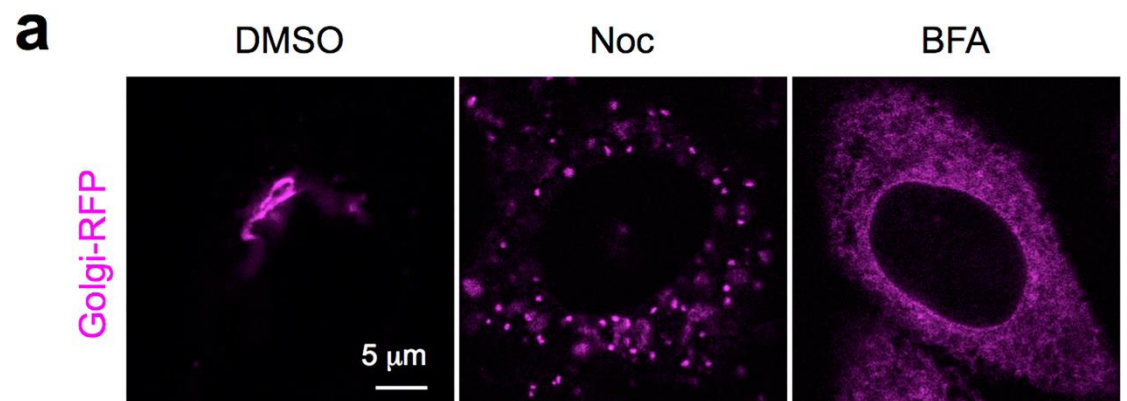
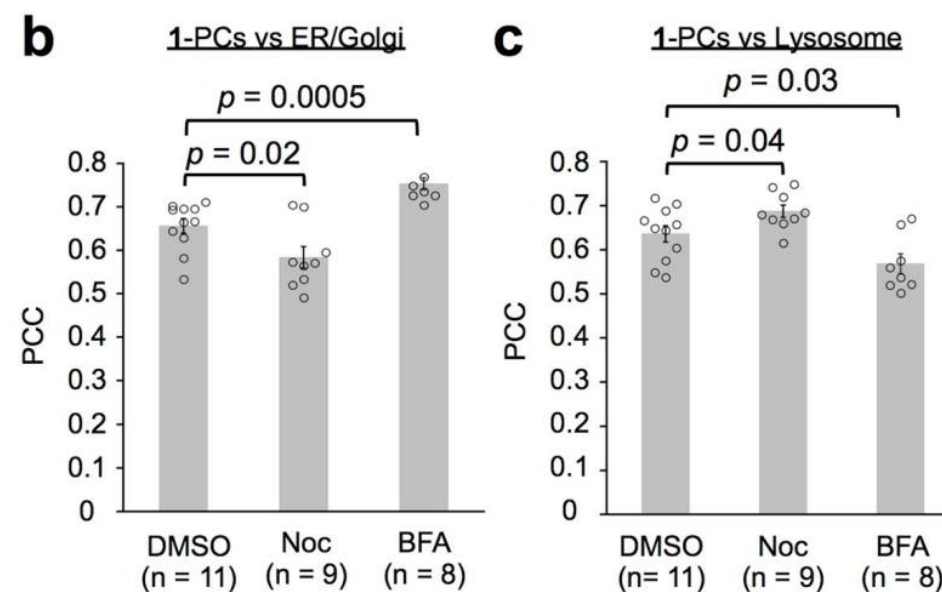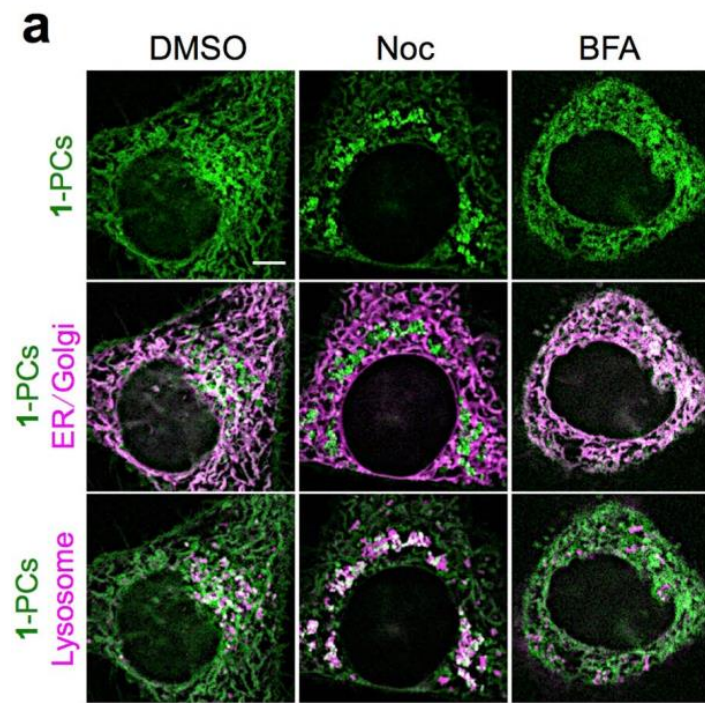**Fig. 3 |** live-cell tracing of interorganelle translocation of 1–PC

Extended Data Fig. 7 | Translocation of 2-PCs from mitochondria to lysosomes.

Extended Data Fig. 8 | Effects of Noc and BFA on interorganelle lipid transport

Extended Data Fig. 9 | Interorganelle lipid transport is affected by treatment of nocodazole (Noc) and brefeldin A (BFA)