Expanded View Figures

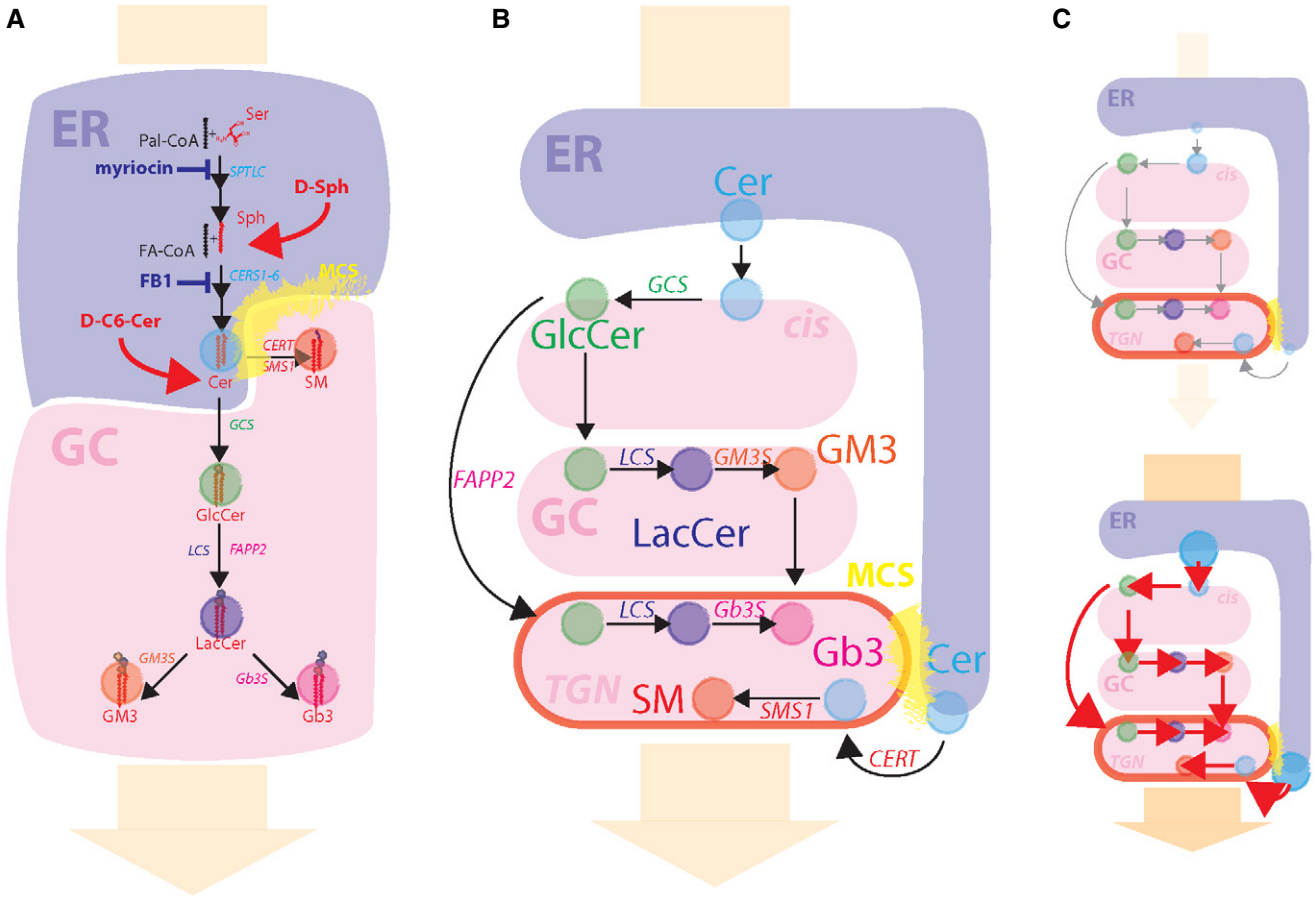

Figure EV1. Intracellular organization of SL metabolism.

A Schematic representation of SL synthetic reactions happening at the ER (blue shaded area) and at the Golgi complex (GC; red shaded area). The metabolic positions where SL synthetic inhibitors act (blue) and where cell-permeable SL precursors enter SL metabolism (red) are indicated. The regions highlighted in yellow indicate ER–TGN MCSs. The orange arrow in the background indicates the direction of the biosynthetic flow.

B Schematic representation of the sub-Golgi distribution of SL metabolic enzymes and associated reactions.

C Experimental scheme for decreased (up) and increased (down) SL flow obtained by the use of cell-permeable SL precursors or SL synthetic inhibitors.

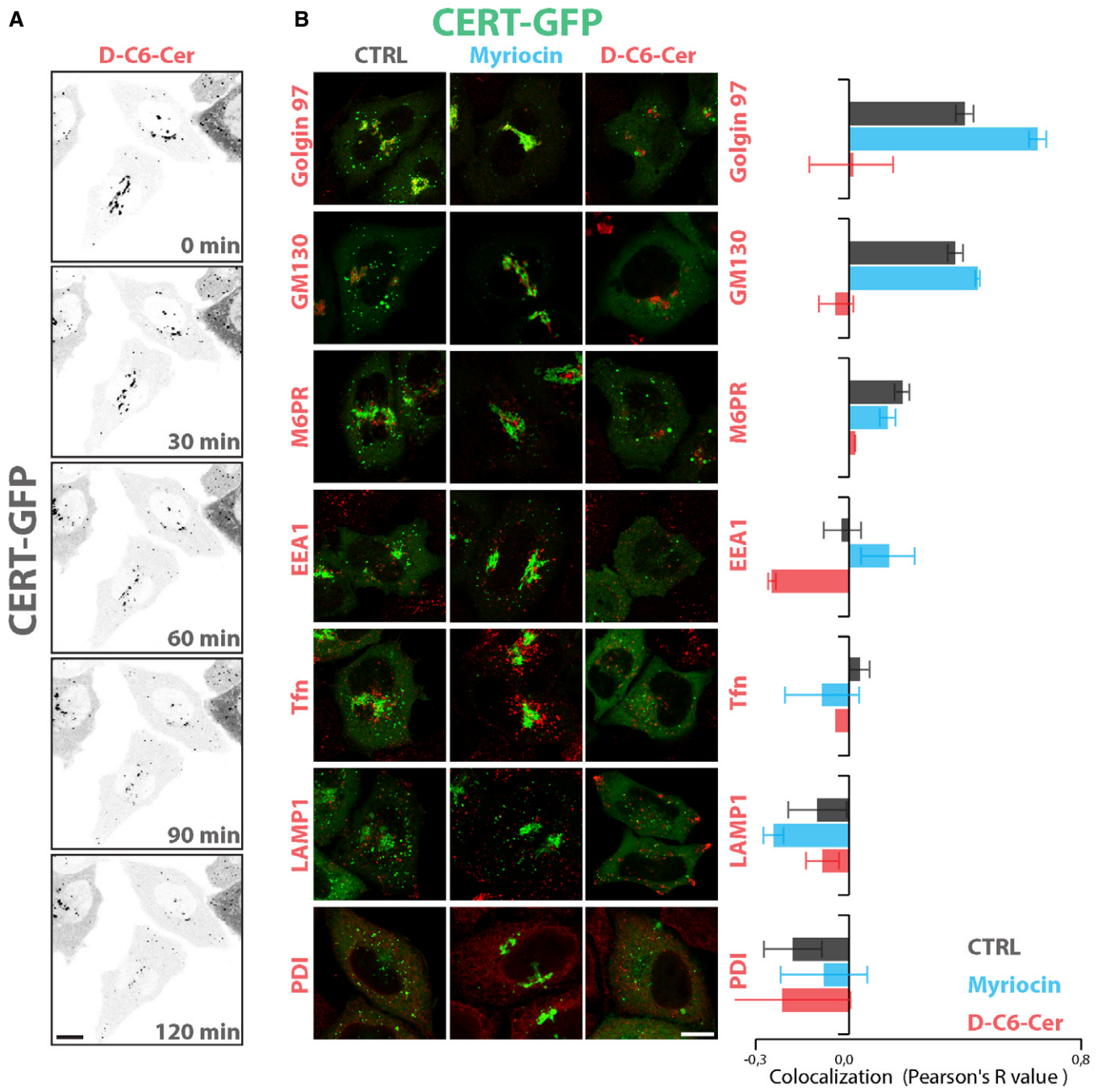

Figure EV2. The association of GFP-CERT with TGN membranes depends on SL flow.

A GFP-CERT localization in HeLa cells, pre-treated with myriocin (2.5 μM, 24 h) and then treated with D-C6-Cer (10 μM) for 2 h. Images are selected frames from Movie EV1. Scale bar, 10 μm.

B GFP-CERT co-localization with the indicated organelle markers in HeLa cells either non-treated (CTRL, grey), treated with myriocin (2.5 μM, 24 h) (myriocin, cyan) or pre-treated with myriocin (2.5 μM, 24 h) and then treated with D-C6-Cer (10 μM) for 2 h (D-C6-Cer; red). Left panels: representative images. Right panel: co-localization values calculated as Pearson's correlation coefficients (R-values) between GFP-CERT and the indicated markers under the different treatments. Scale bar, 10 μm. Data are means ± SEM from at least 3 independent experiments where at least 10 cells per experiment were considered.

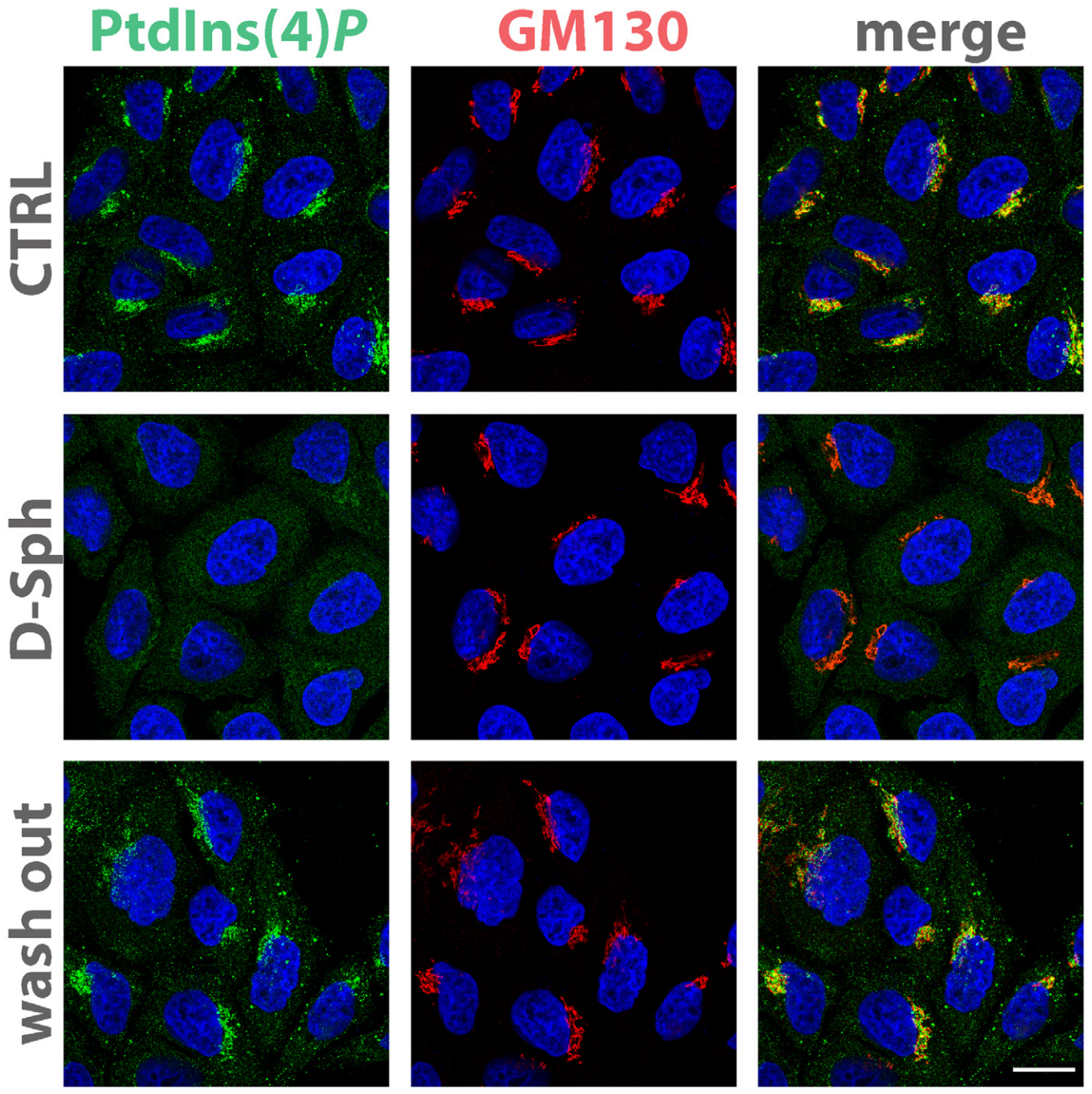

Figure EV3. SL flow controls PtdIns(4)*P* levels at the Golgi.
Cells treated either with vehicle (EtOH), with D-Sph (30 μM) for 30 min or with D-Sph (30 μM) and washed out for 4 h were fixed and permeabilized as in Fig 3A and stained with DAPI (blue), an anti-GM130 antibody (red) and anti-PtdIns(4)*P* antibody (green). Scale bar, 10 μm.

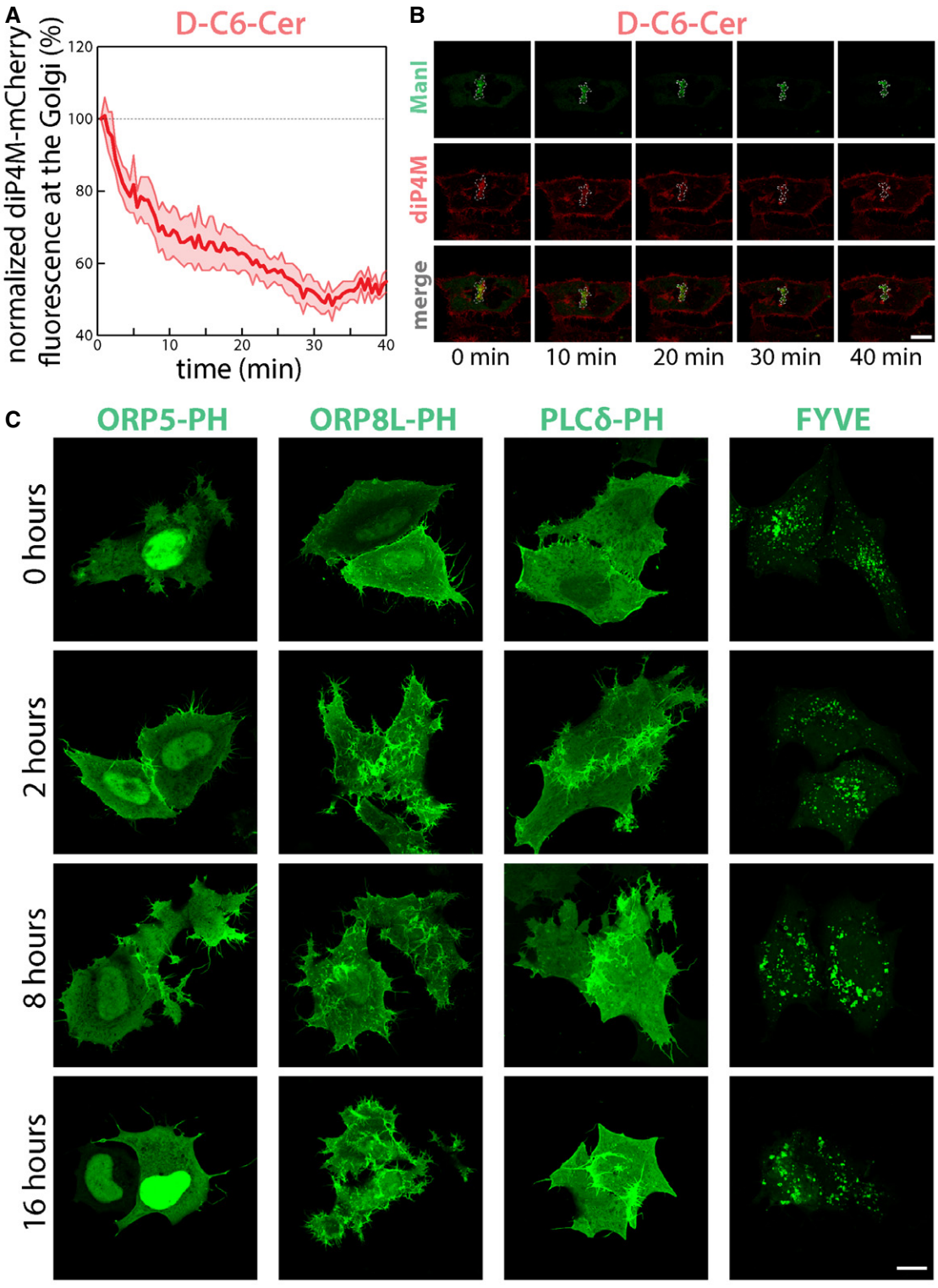

Figure EV4. Sustained SL flow specifically controls PtdIns(4)*P* at the TGN.

A HeLa cells transfected with plasmids encoding the PtdIns(4)*P* binding protein diP4M-SidM-mCherry (diP4M-mCherry, red) and the Golgi enzyme Man1-YFP (MANI, green) were treated with D-C6-Cer (10 μM) and imaged for the indicated time. The amount of diP4M-mCherry associated with the Golgi region was quantified. Data are mean ± SEM.

B Selected frames from Movie EV6 are reported; dashed line indicates Golgi perimeter. Scale bar, 10 μm.

C Cells transfected with GFP-tagged ORP5-PH, ORP8L-PH, PLCδ-PH or FYVE were treated with D-C6-Cer (10 μM) for the indicated times, fixed and imaged. Scale bar, 10 μm.

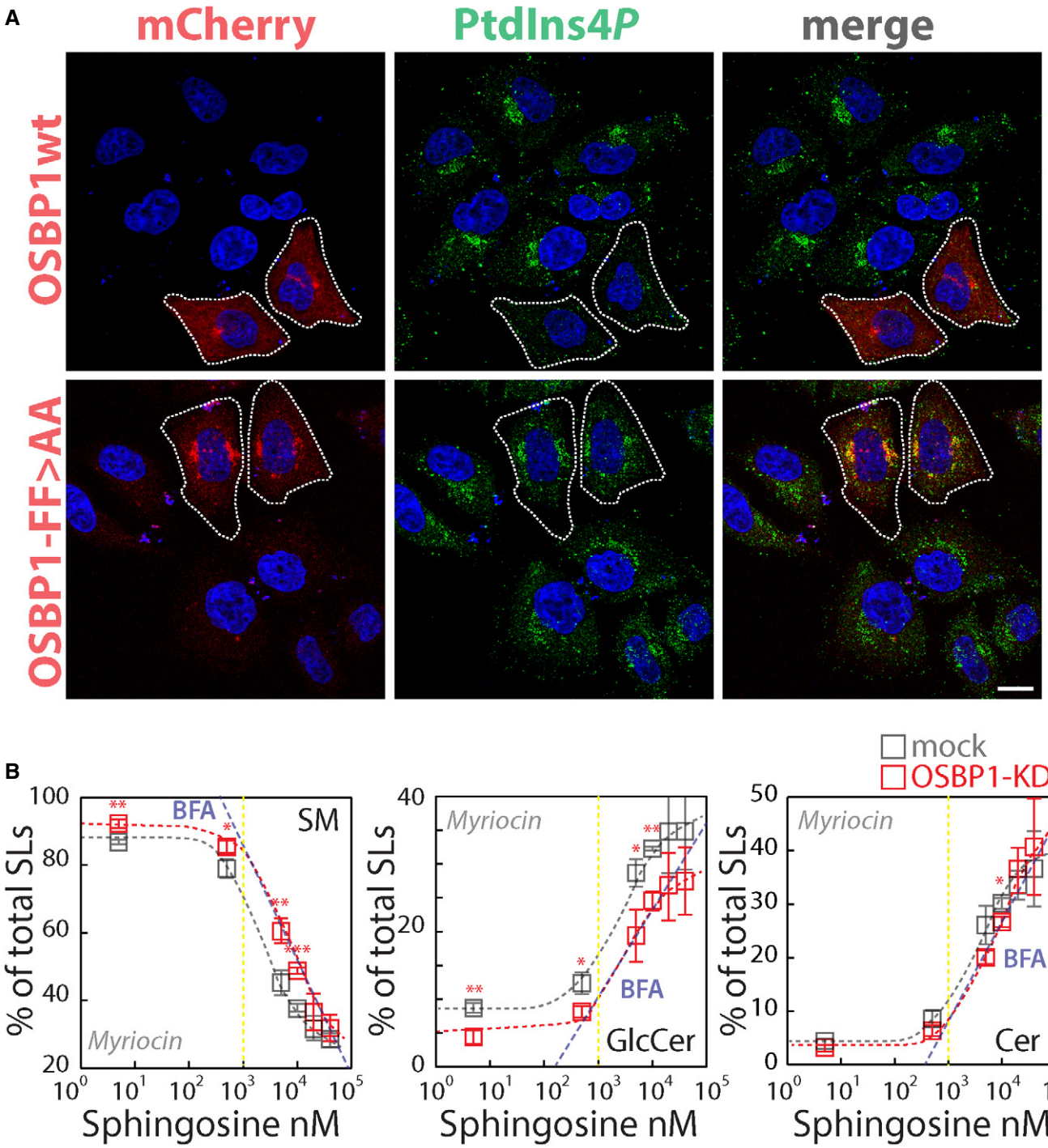

Figure EV5. OSBP1 mediates PtdIns(4)*P* relocation to the ER to control SL homeostasis at the TGN.

A Cells transiently expressing mCherry-OSBP1 wt or the mCherry-OSBP1-FF>AA mutant were fixed and permeabilized as in Fig 3 and stained with anti-PtdIns(4)*P* (green). The dashed line indicates mCherry-OSBP1-transfected cells. Scale bar, 10 μm.

B Cells KD for OSBP1 were treated with myriocin (2.5 μM) for 24 h and fed with increasing concentrations of D-Sph for 2 h. ^{3}H-D-Sph (≈5 nM) was mixed as a tracer with its non-radioactive counterpart. The percentage of total radioactivity associated with SM (left panel), GlcCer (middle panel) and Cer (right panel) in mock (grey) and OSBP1-KD cells (red) was quantitated after lipid extraction and HPTLC separation. The blue dashed line represents the values obtained under BFA treatment (from Fig 1B). Data are means ± SEM from at least 4 independent experiments. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; according to two-tailed Student's t-test.

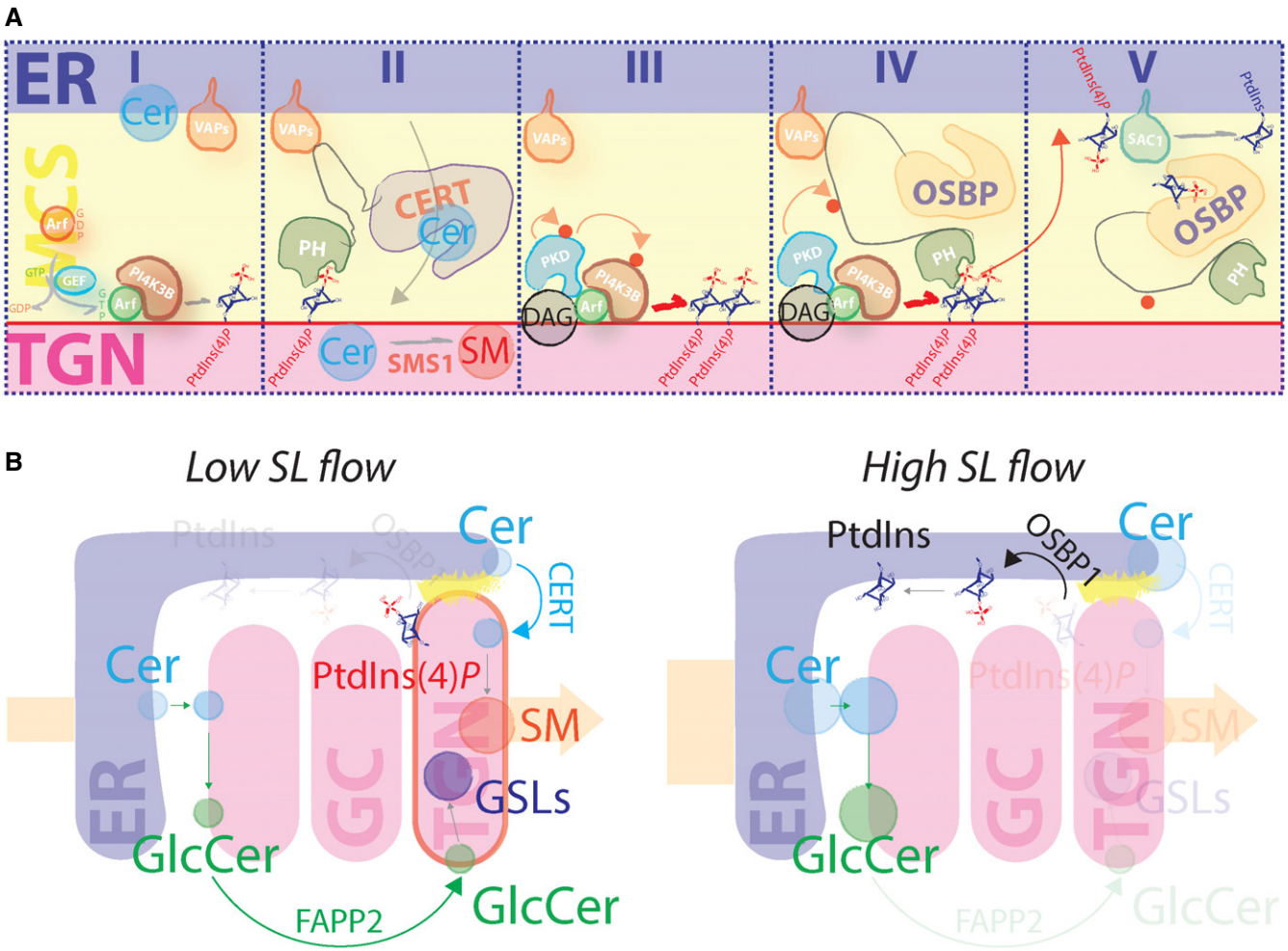

Figure EV6. SL flow to the Golgi controls phosphoinositide turnover at the TGN.

A Schematic representation of SL-controlled phosphoinositide turnover at the ER–TGN MCSs. Phase I: ARF1 recruits PI4KIIIβ to the TGN to produce PtdIns(4)*P*. Phase II: CERT transfers Cer from the ER to the TGN by contacting VAPs at the ER and PtdIns(4)*P* at the TGN. CERT-transferred Cer is converted to SM by SMS1. Phase III: SMS1-produced DAG recruits (together with ARF) and locally activates PKD. PKD phosphorylates itself and PI4KIIIβ to increase PtdIns(4)*P* production. Phase IV: OSBP1 is recruited by PtdIns(4)*P*. PKD phosphorylates OSBP1. Phase V: phospho-OSBP1 efficiently transfers PtdIns(4)*P* to the ER for its dephosphorylation operated by Sac1.

B Schematic representation of TGN response to sustained SL flow. At low SL flow (left scheme), Cer and GlcCer are efficiently transported to the TGN by the PtdIns(4)*P* binding proteins CERT and FAPP2, respectively, to yield SM and complex GSLs. At high SL flow (right scheme), PtdIns(4)*P* is transported to the ER by OSBP1 and dephosphorylated to PtdIns. As a consequence, CERT and FAPP2 cannot deliver further SL precursors (Cer and GlcCer) to the TGN, resulting in reduced production of SM and complex GSLs.