

Membrane blebbing plays important roles in cell migration, cytokinesis, apoptosis, as well as extracellular vesicle formation for intercellular communication. During budding of the plasma membrane (PM) to form extracellular vesicles, phosphatidylserine is often translocated from the PM inner leaflet to the outer leaflet, indicative of lipid scrambling that results in loss of native membrane asymmetry. Using micrometer-sized giant plasma membrane vesicles (GPMV) as a readout of PM vesiculation, we found that knocking out (KO) the lipid scramblase TMEM16F reduced lipid scrambling and dramatically inhibited GPMV formation, in line with previous observations. TMEM16F repletion could rescue GPMV formation, indicating that lipid scrambling is necessary for membrane budding. We hypothesized three mechanisms to explain this surprising observation, namely that lipid scrambling: (1) depletes PI(4,5)P₂ from the inner leaflet leading to detachment of the PM from the actin cytoskeleton, (2) makes PM bilayers softer and easier to bend, (3) externalizes lipids that stabilize highly curved bud necks. Depletion of PI(4,5)P₂ was indeed observed during vesiculation, however this occurred in both wild type and KO cells, implying that detachment of PM from actin cytoskeleton due to PIP₂ depletion is not sufficient to make large vesicles. Next, through fluorescence lifetime imaging microscopy of a lipid packing reporter (Di4), we found that lipid scrambling leads to a decrease of PM packing, potentially making the membrane softer for bending and thus facilitating membrane budding. Finally, we observed that lipid scrambling resulted in externalization of phosphatidylethanolamine (PE). This PE was preferentially located at the highly curved vesicle necks, suggesting that the intrinsic curvature of PE stabilizes this highly curved membrane structure. Thus, we conclude that lipid scrambling changes membrane biophysical properties, potentially explaining mechanisms underlying vesicle-budding in physiological contexts including apoptotic cell removal, bone mineralization, fertilization, and blood coagulation.