Precise regulation of protein assembly at specialized membrane domains is essential for diverse cellular functions including synaptic transmission. However, it incompletely understood how protein clustering at the plasma membrane is initiated, maintained and controlled. Protein palmitoylation, a common reversible lipidation, regulates protein targeting to the plasma membrane. Such modified proteins are enriched in these specialized membrane domains. Recently, we found that endogenous palmitoylated PSD-95 is partitioned into multiple discrete subdomains (nanodomains) in a dendritic spine in cultured hippocampal neurons. PSD-95 in nanodomains undergoes continuous de/repalmitoylation cycles driven by local palmitoylating activity, ensuring the maintenance of compartmentalized PSD-95 clusters within individual spines. Acutely induced plasma membrane insertion of DHHC2 palmitoylating enzyme triggers specific accumulation of PSD-95 at the plasma membrane, and this plasma membrane-inserted DHHC2 is essential for postsynaptic nanodomain formation. Furthermore, we obtained the candidate for the membrane-bound enzyme to depalmitoylate PSD-95 and disperse synaptic PSD-95 clusters. We propose that synaptic palmitoylation machinery defines subsynaptic nanodomains through constituting local palmitoyl cycles on PSD-95 and determines the geometry of postsynaptic densities.