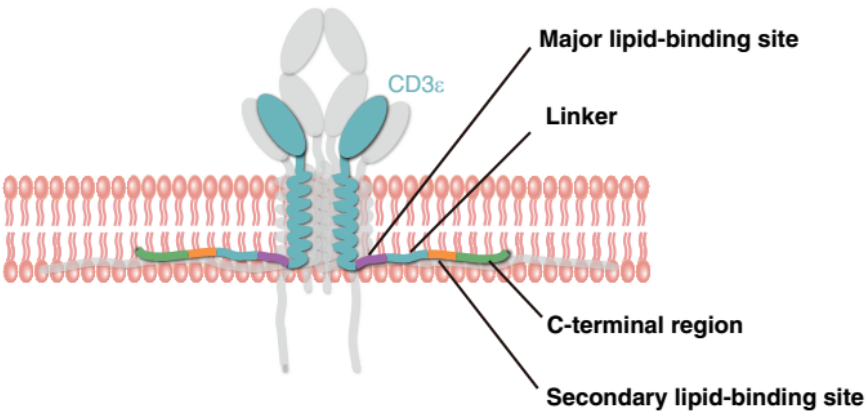
A



B

Conformational state	I	M1	II	M2	III
Major lipid-binding site (BRS)	on	on	on	on	off
Linker	on	on	on	off	off
Secondary lipid-binding site (PRS + proximal half ITAM)	on	on	off	off	off
C-terminal region (distal half ITAM)	on	off	off	off	off

C

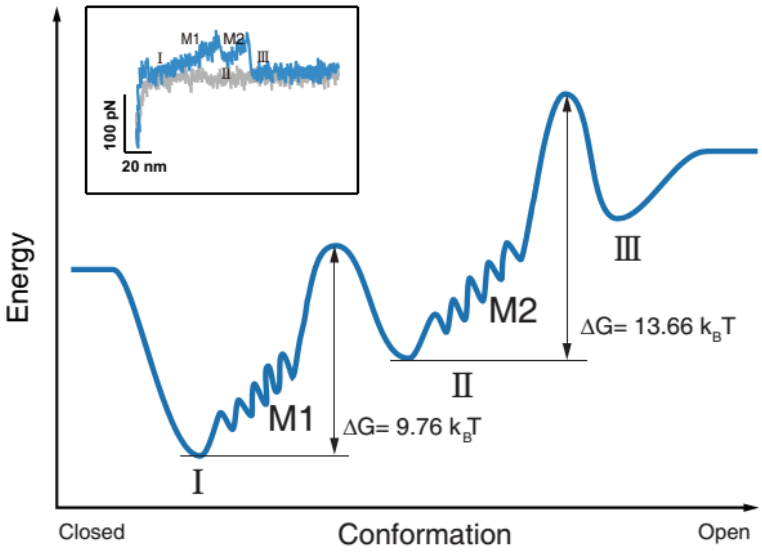


Figure S6 Multiple kinetic intermediates between the closed and open conformations of the CD3ε cytoplasmic domain. (**A**) Four regions of CD3ε cytoplasmic domain. (**B**) The multiple conformations of the CD3ε cytoplasmic domain are categorized into three stable states (I, II, III) and two potential transient states (M1 and M2): State I, all four regions of CD3ε cytoplasmic domain are on the membrane; State II, both the C-terminal region and the secondary lipid-binding site are off the membrane whereas the linker and the major lipid-binding site are on the membrane; State III, all four regions are off the membrane. In between State I and II, there might be a group of transient intermediates, together designated as State Mixture 1 (M1), having a conformational state at which the two lipid-binding sites and the linker are associated with the membrane but the C-terminal region is heterogeneously dissociated from the membrane. In between State II and III, there might be another group of transient intermediates, together designated as State Mixture 2 (M2), having a conformational state at which the C-terminal region and the secondary lipid-binding site are dissociated from the membrane while the major lipid-binding site is still associated with the membrane, along with the heterogeneous dissociation of the linker from the membrane. (**C**) Free energy landscape illustrating the dynamic process of the closed-to-open conformational transition of the CD3ε cytoplasmic domain.