

Molecular dynamics simulations reveal membrane lipid interactions of the full-length lymphocyte specific kinase Lck

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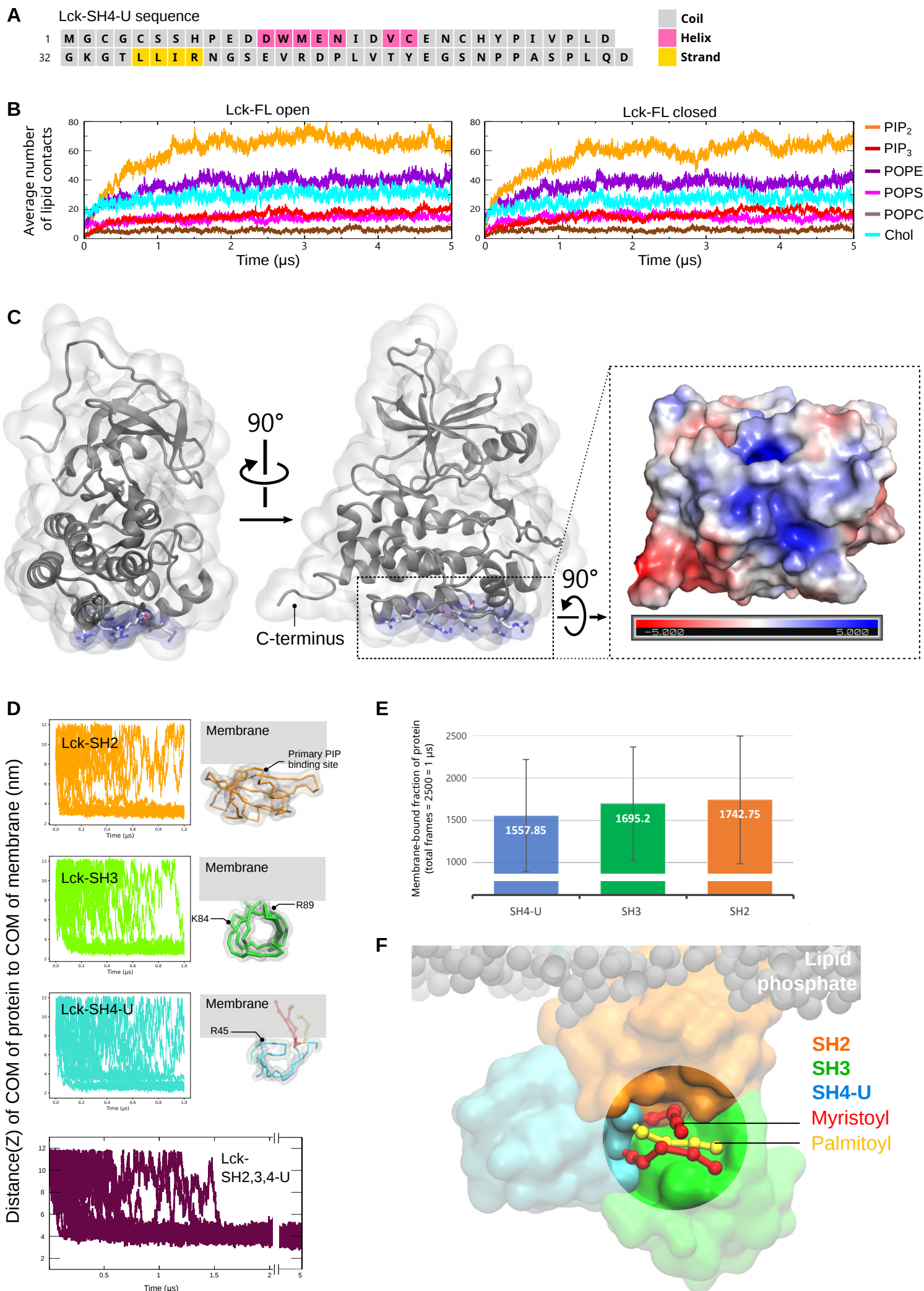


Fig S1. Secondary structure prediction of Lck-SH4-U, membrane binding of individual Lck-SH domains, and electrostatics of the kinase domain. (A) Secondary structure prediction of Lck-SH4-U by PSIPRED 4.0 server. **(B)** Non-normalized number of contacts between Lck and lipid headgroups versus time averaged from all 20 CGMD simulations from the Lck-FL open (left) and Lck-FL closed simulations (right). **(C)** Residues in the kinase domain forming a flat cationic patch. Their electrostatic profile was calculated using in the ± 5 kT/e range and at pH 7.0 using the PDB2PQR and APBS tools. Electronegative and electropositive regions are indicated by red and blue intensities respectively. **(D)** Distance between the center of mass (COM) of protein to COM of membrane in all 20 simulation replicates: SH4-U (cyan), SH3 (green), SH2 (orange), Lck-SH2,3,4-U (maroon). The most observed membrane-bound orientations of the individually simulated Lck-SH domains are also shown. Residues interacting most with the membrane in these simulations are labelled. **(E)** Average number of frames in the individual Lck-SH simulations that the protein stayed unbound (red) or bound (green) to the membrane. **(F)** A snapshot from one of the Lck-SH2,3,4-U simulations highlighting the binding pocket of the SH4 lipid tails (near the SH2-SH3 linker loop region) when they did not insert into the membrane.

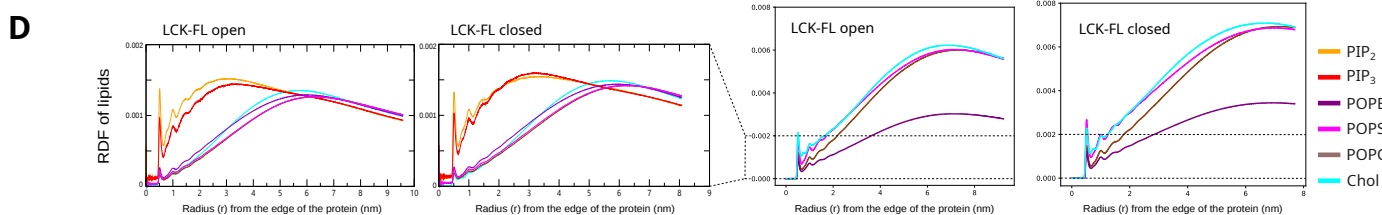
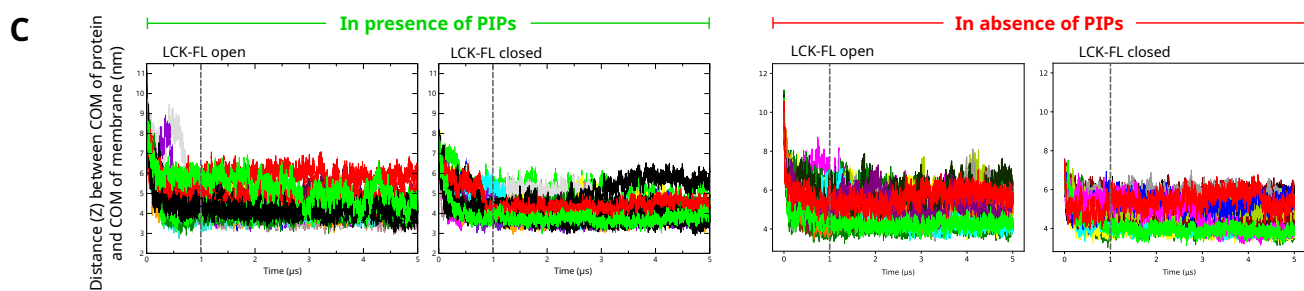
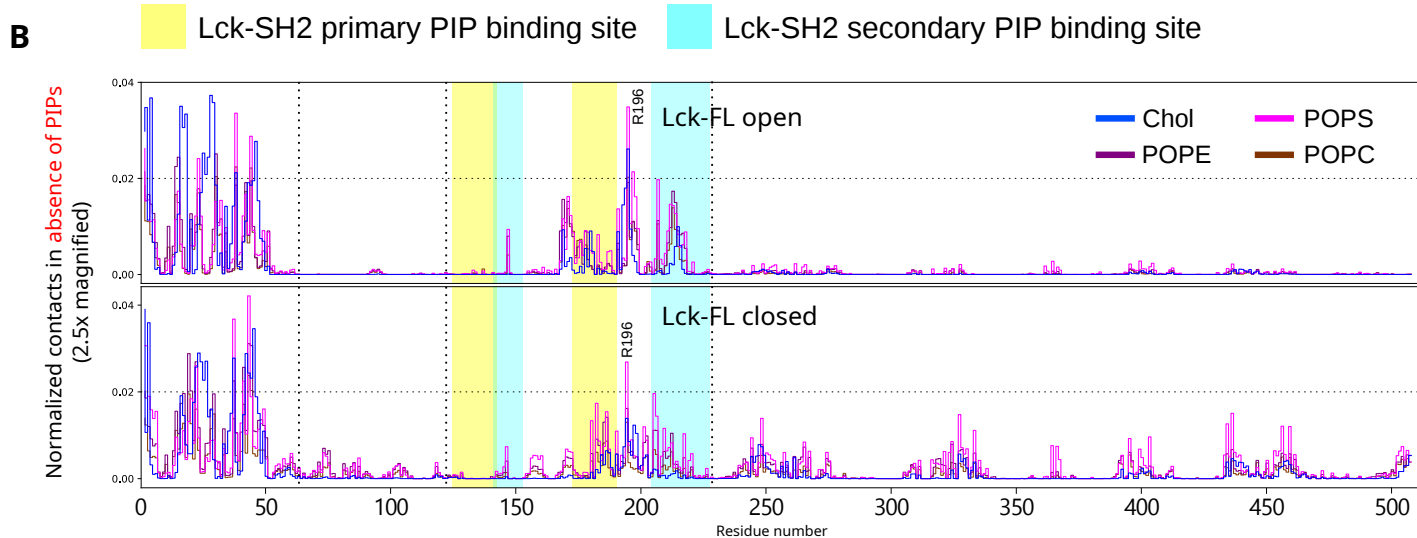
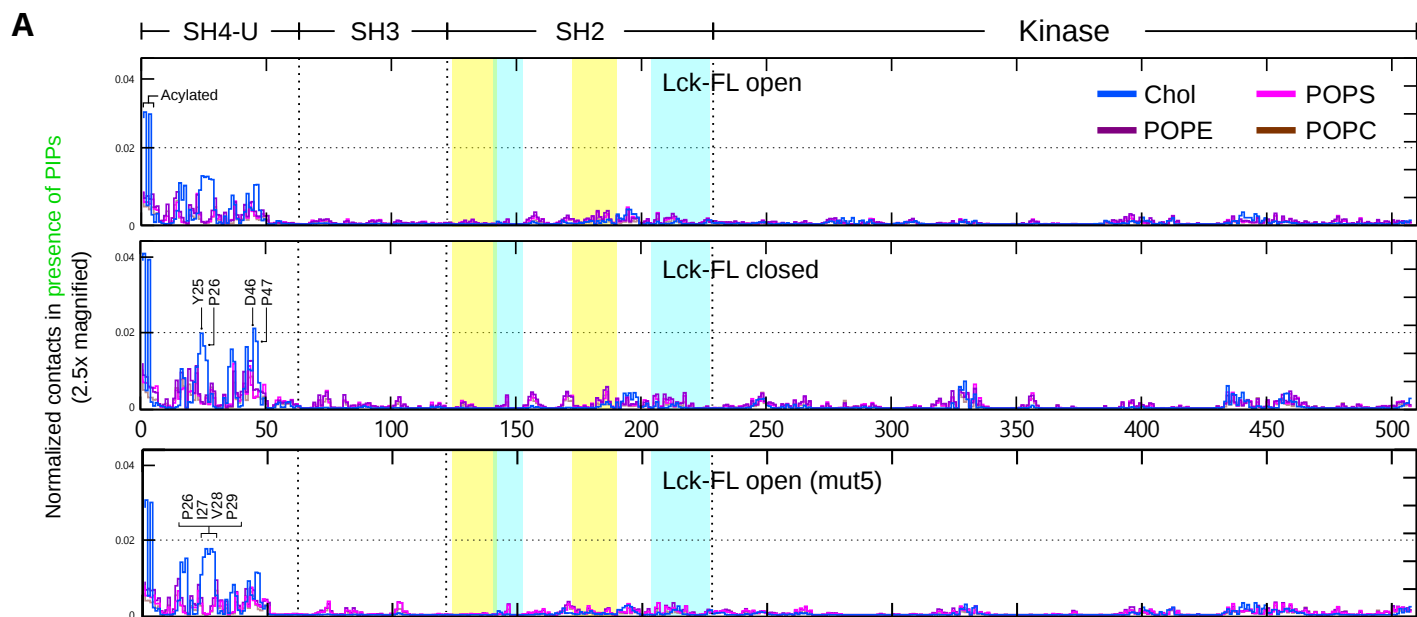
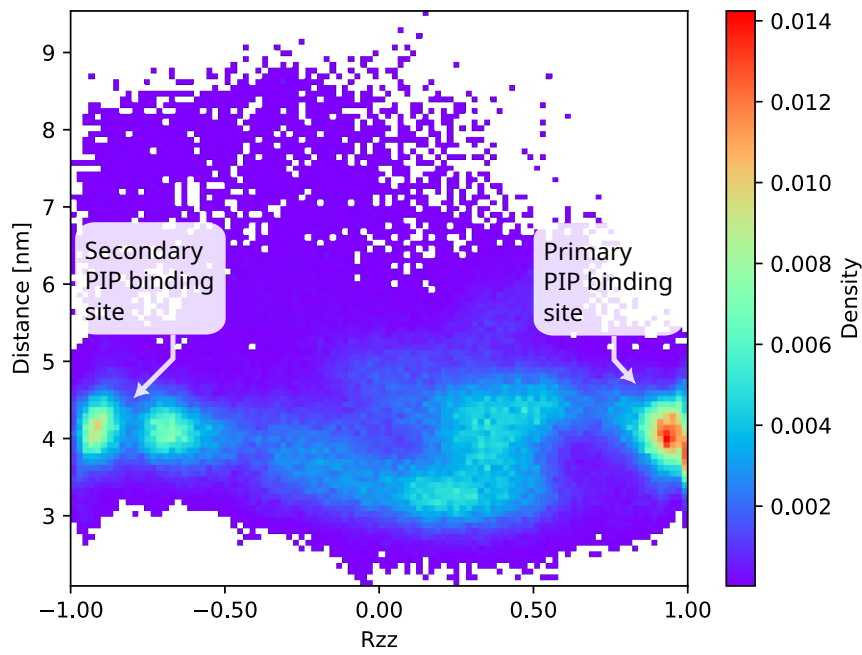


Fig S2. A comparison of lipid interactions of Lck-FL open and closed conformations in the presence and absence of PIP lipids in the membrane. (A) Normalized lipid interactions calculated from the Lck-FL open, closed, and open (mut5) simulations in the presence of PIP lipids and **(B)** absence of PIP lipids in the membrane. **(C)** Distance between the center of mass (COM) of Lck-FL and COM of the membrane versus time in the presence (left) and absence of PIPs (right) in the membrane. The line drawn at 1 μ s is to indicate that Lck-FL binds to the membrane by this point in time in all simulations. **(D)** The normalized radial distribution function (RDF) of all lipid types around Lck-FL calculated throughout the simulation time from all 20 CGMD simulations combined. The RDF data enables comparison between Lck-FL open and closed conformations, and also between the presence and absence of PIPs in the membrane. The RDF is normalized by the total number of lipids in the membrane of that system to enable comparison between the open and closed conformations of Lck-FL.

A SH2 orientation (Lck-FL open)
(all 20 MD simulation replicates combined)



B SH2 orientation (Lck-FL open)
(all 20 MD simulation replicates individually analysed)

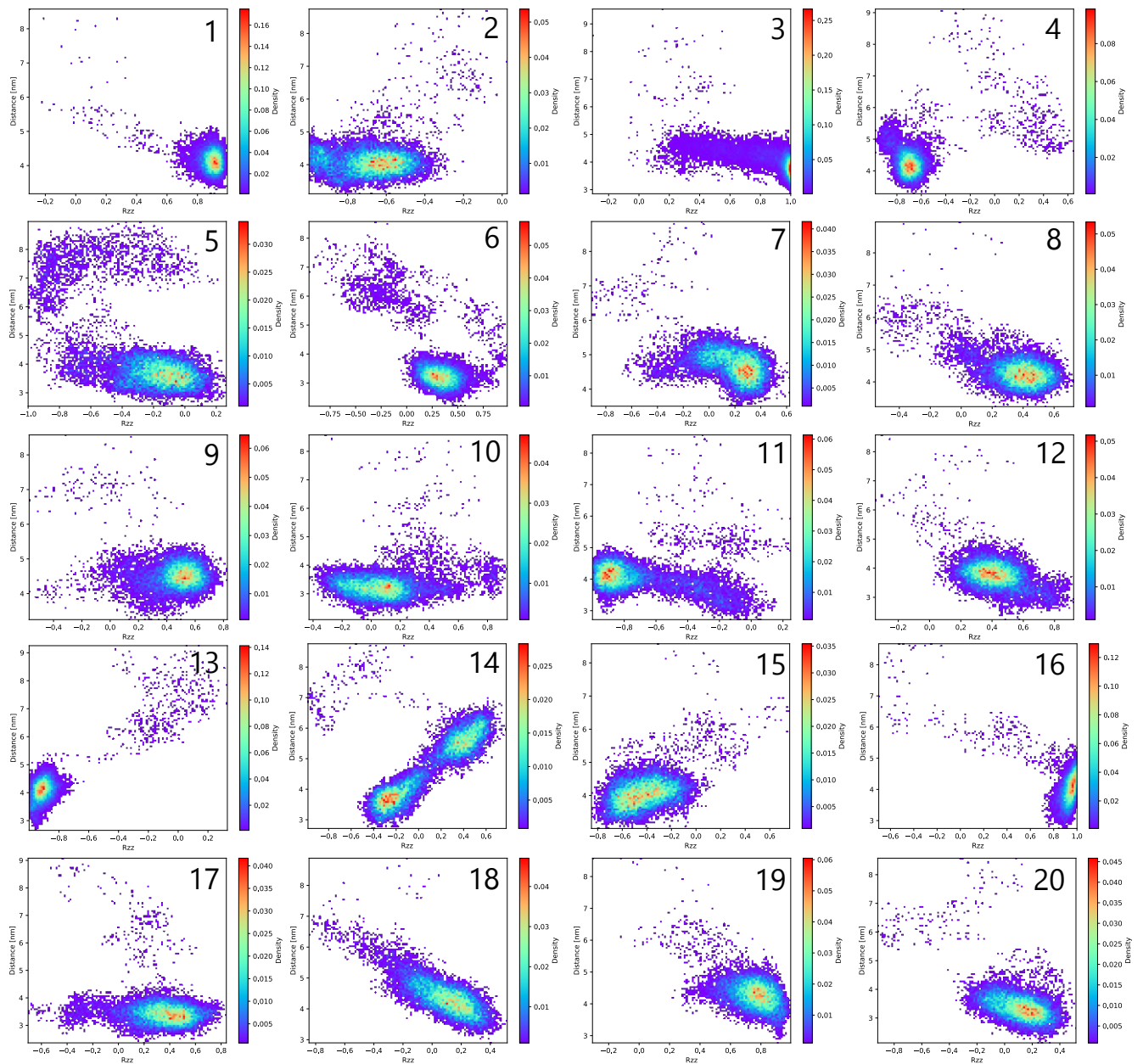
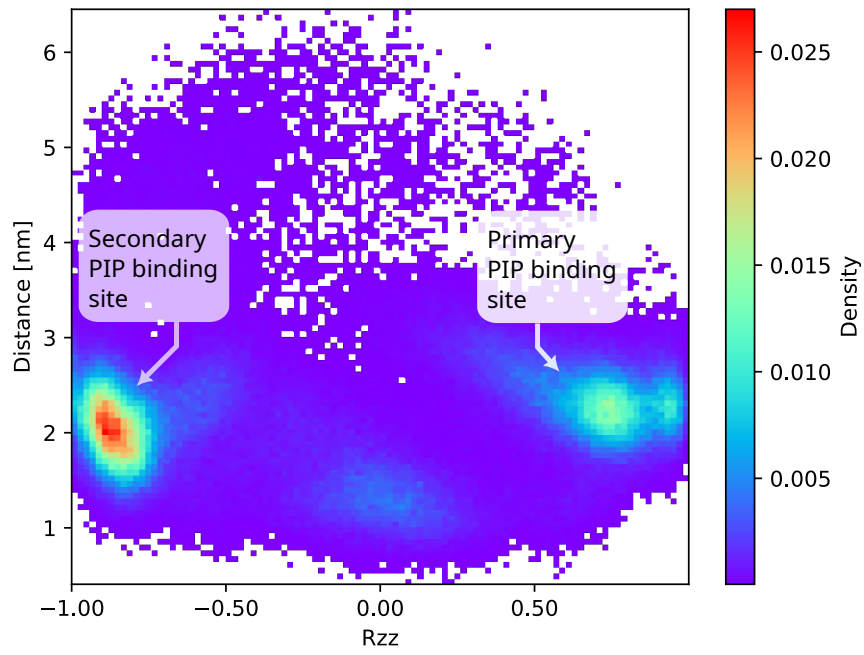


Fig S3. The membrane-bound orientation of the Lck-SH2 domain in the Lck-FL open conformation in the presence of PIPs in the membrane (A) as shown from all CGMD simulations combined and **(B)** in each CGMD simulation replicate individually. In all these plots, the X axis represents the ZZ component of the rotational matrix of the protein, and the Y axis represents the distance between the center of mass of the protein and the center of mass of the membrane along the Z axis (perpendicular to the membrane).

A SH2 orientation (Lck-FL closed)
(all 20 MD simulation replicates combined)



B SH2 orientation (Lck-FL closed)
(all 20 MD simulation replicates individually analysed)

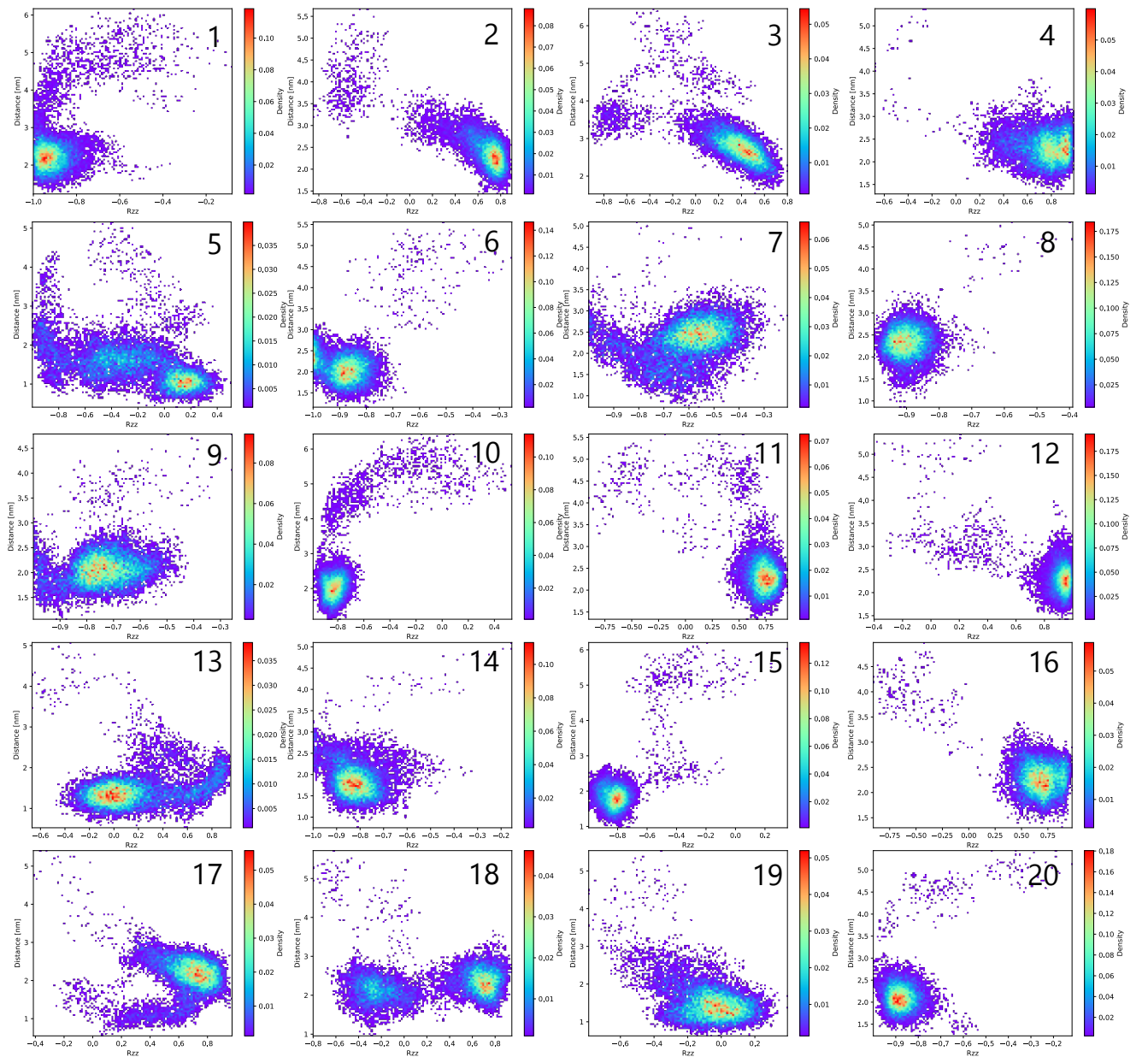


Fig S4. The membrane-bound orientation of the Lck-SH2 domain in the Lck-FL closed conformation in the presence of PIPs in the membrane (A) as shown from all CGMD simulations combined and **(B)** in each CGMD simulation replicate individually. In all these plots, the X axis represents the ZZ component of the rotational matrix of the protein, and the Y axis represents the distance between the center of mass of the protein and the center of mass of the membrane along the Z axis (perpendicular to the membrane).

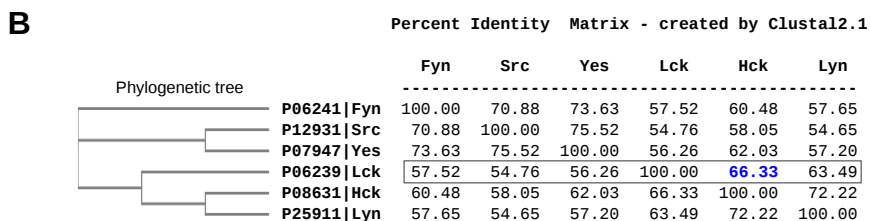
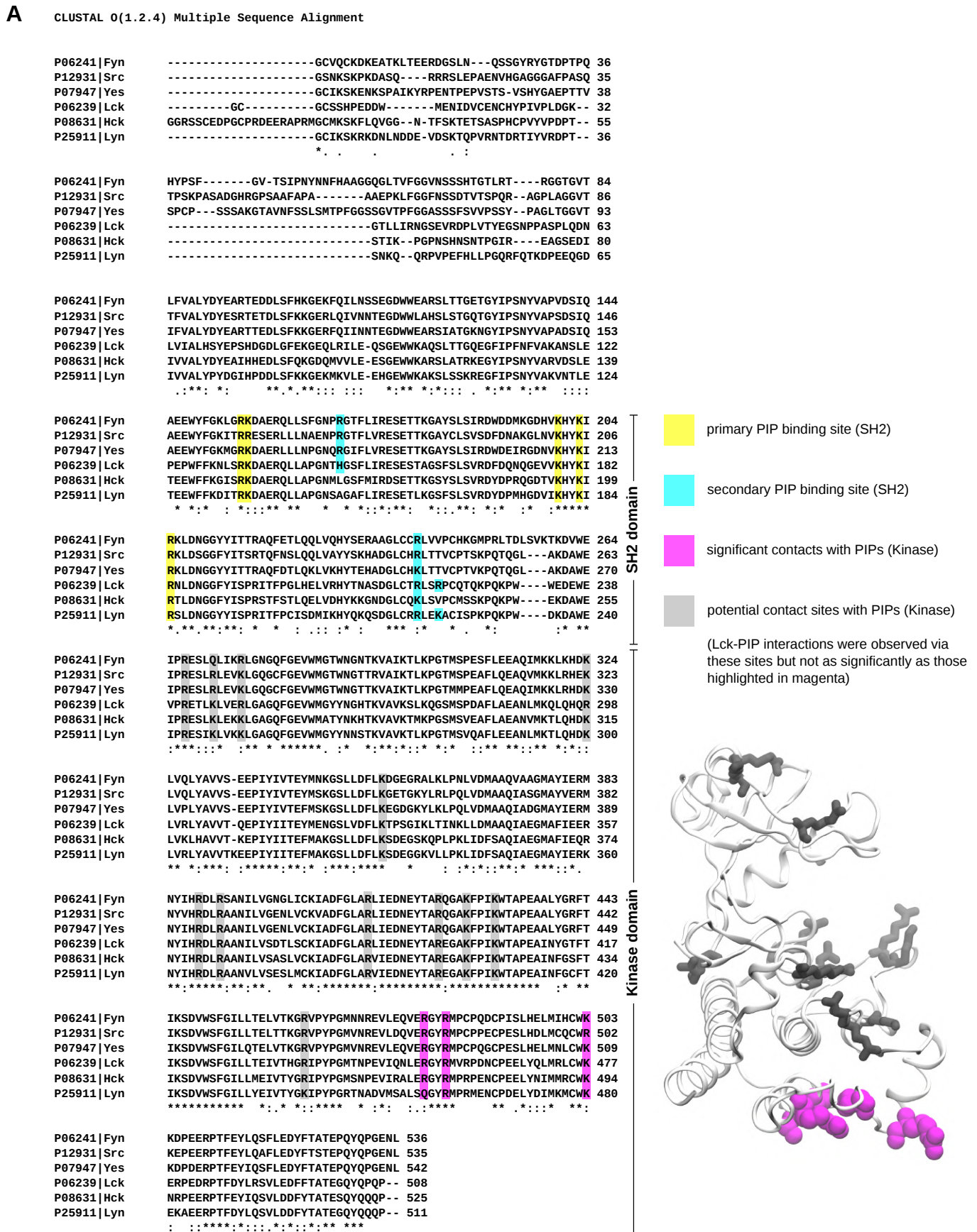


Fig S5. Multiple sequence alignment and the identity of the full-length Lck with other members of the Src family of kinases. **(A)** Multiple sequence alignment, obtained by Clustal Omega, highlighting the residues in the SH2 and kinase domains that mediated Lck-PIP interactions. These residues are observed to be conserved. Note that the myristoylated G2 residue is considered as the first residue, hence all residues are shifted one position behind i.e., 'n-1'. Refer Fig 3E to visualize the primary and secondary PIP lipid binding sites of Lck-SH2. The figure shown on the right is a cartoon representation of the kinase domain with significant PIP contacting residues shown as magenta spheres, and potentially contacting residues shown as grey sticks. **(B)** Schematic phylogenetic tree showing the evolution of Src family of kinases and their identity matrix (calculated in %). This suggests that Lck is most similar to Hck compared to other Src family members. The protein sequences were obtained from Uniprot (whose IDs are listed beside the respective protein names).

Movies

S1 Movie. CGMD simulation displaying insertion of myristoylated and palmitoylated lipid tails of the SH4 domain into the membrane. Phospholipid tails are shown as transparent grey spheres and their headgroups as transparent coloured spheres (POPC: brown, POPE: purple, PIP₂: orange, PIP₃: red). The myristoylated and palmitoylated residues are shown as yellow and red ball and sticks respectively. Lck-SH4-U backbone is shown as white bonds, and its PIP lipid binding residues R39, R45 are shown as blue surfaces.