Supplementary Materials for

A Non-Canonical Interface of SNX9 PX Domain Selectively Sequesters PI(3,4)P2 Lipids, Protecting Them from Hydrolysis

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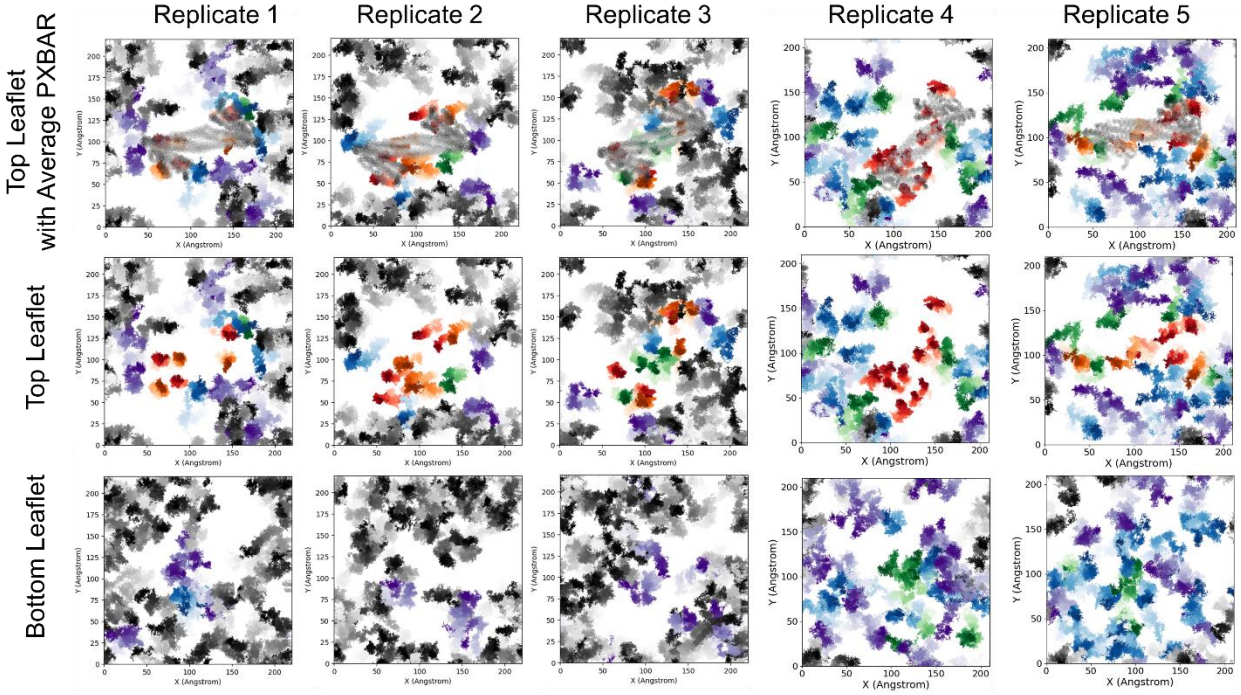
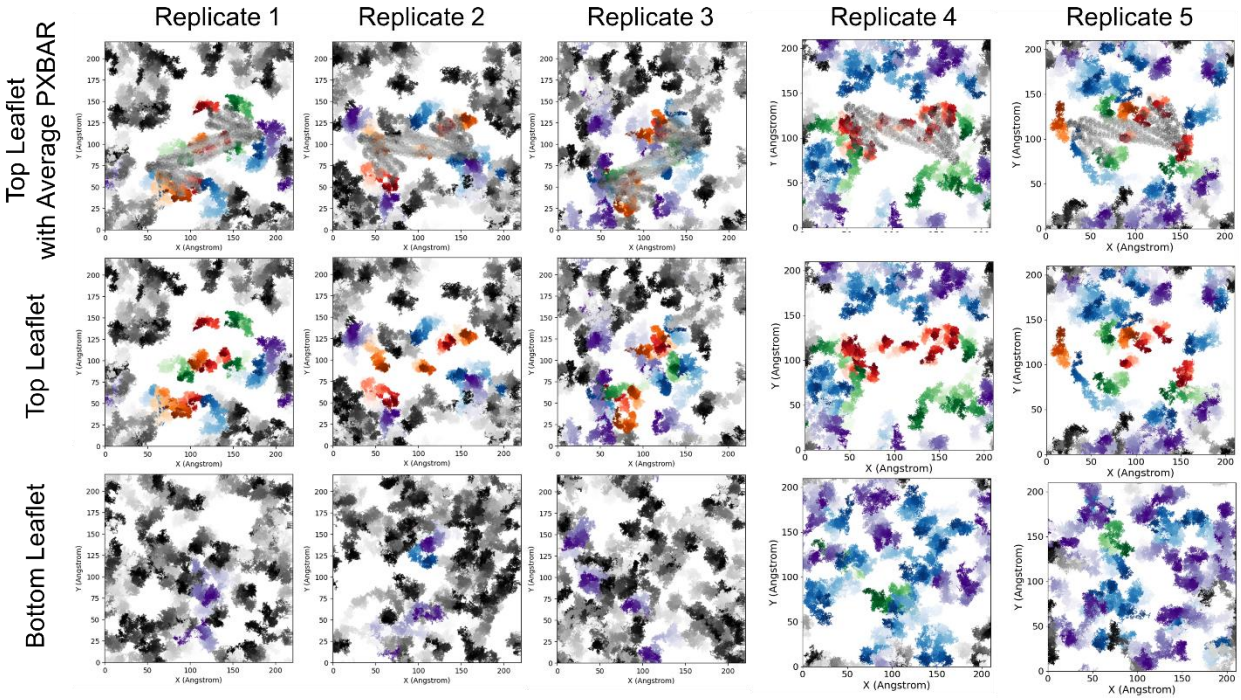
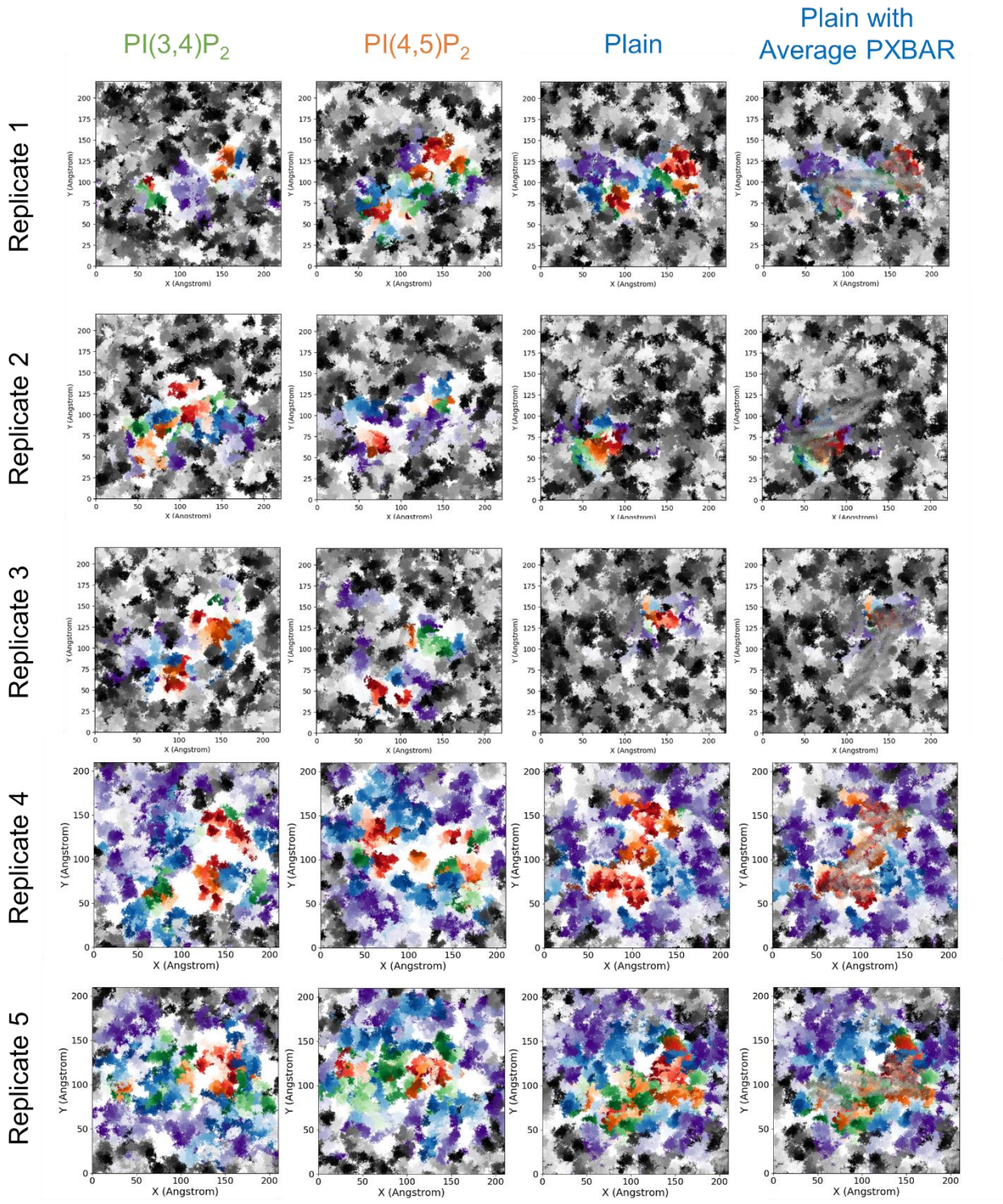


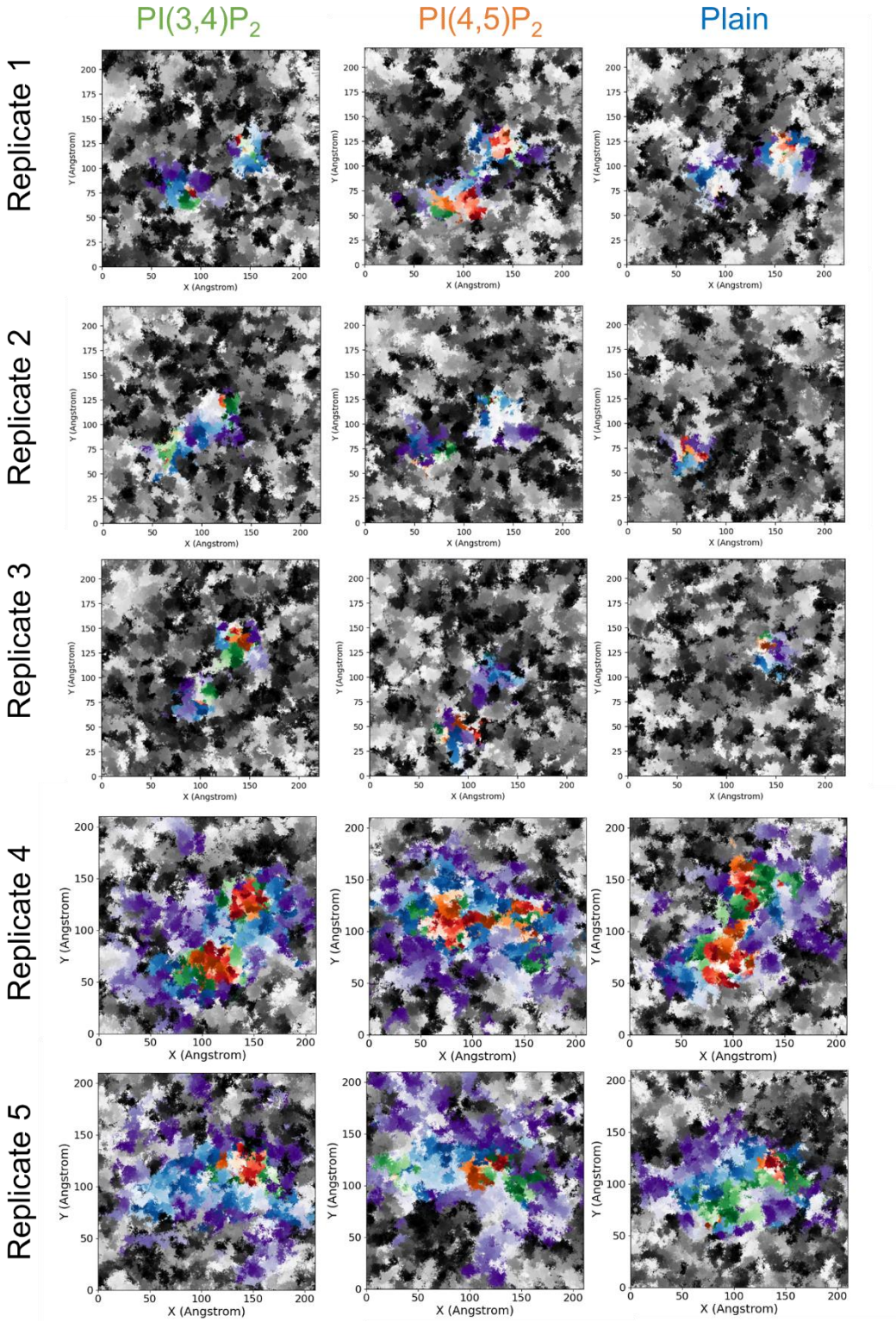
Supplementary Figure 1. PI(3,4)P_2 trajectory trace plots for all replicates. Coloring is based on distance traveled by each lipid (see Fig. 5D).



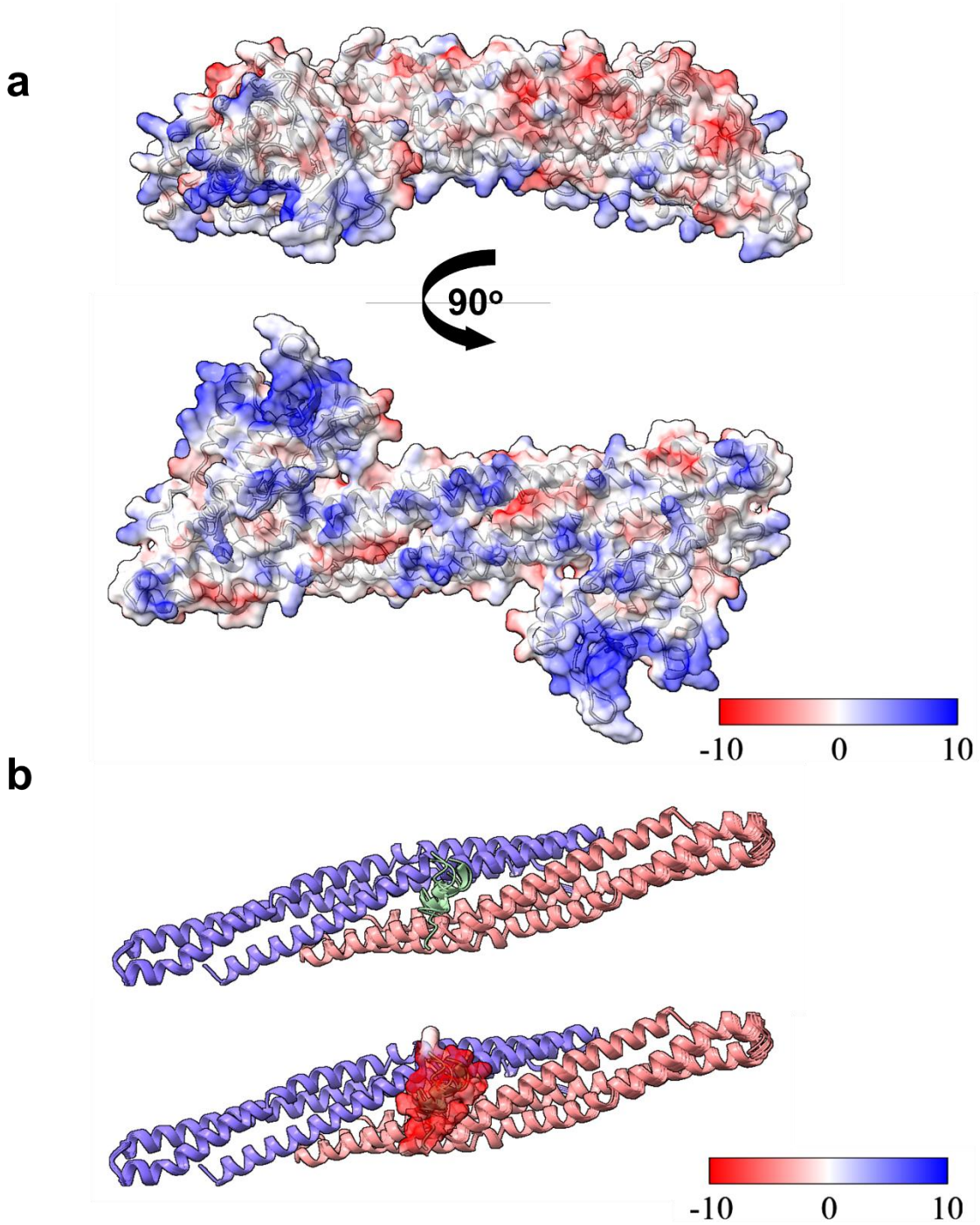
Supplementary Figure 2. PI(4,5)P_2 trajectory trace plots for all replicates. Coloring is based on distance traveled by each lipid (see Fig. 5D).



Supplementary Figure 3. DOPS trajectory trace plot for the top leaflet in all replicates. Coloring is based on distance traveled by each lipid (see Fig. 5D).



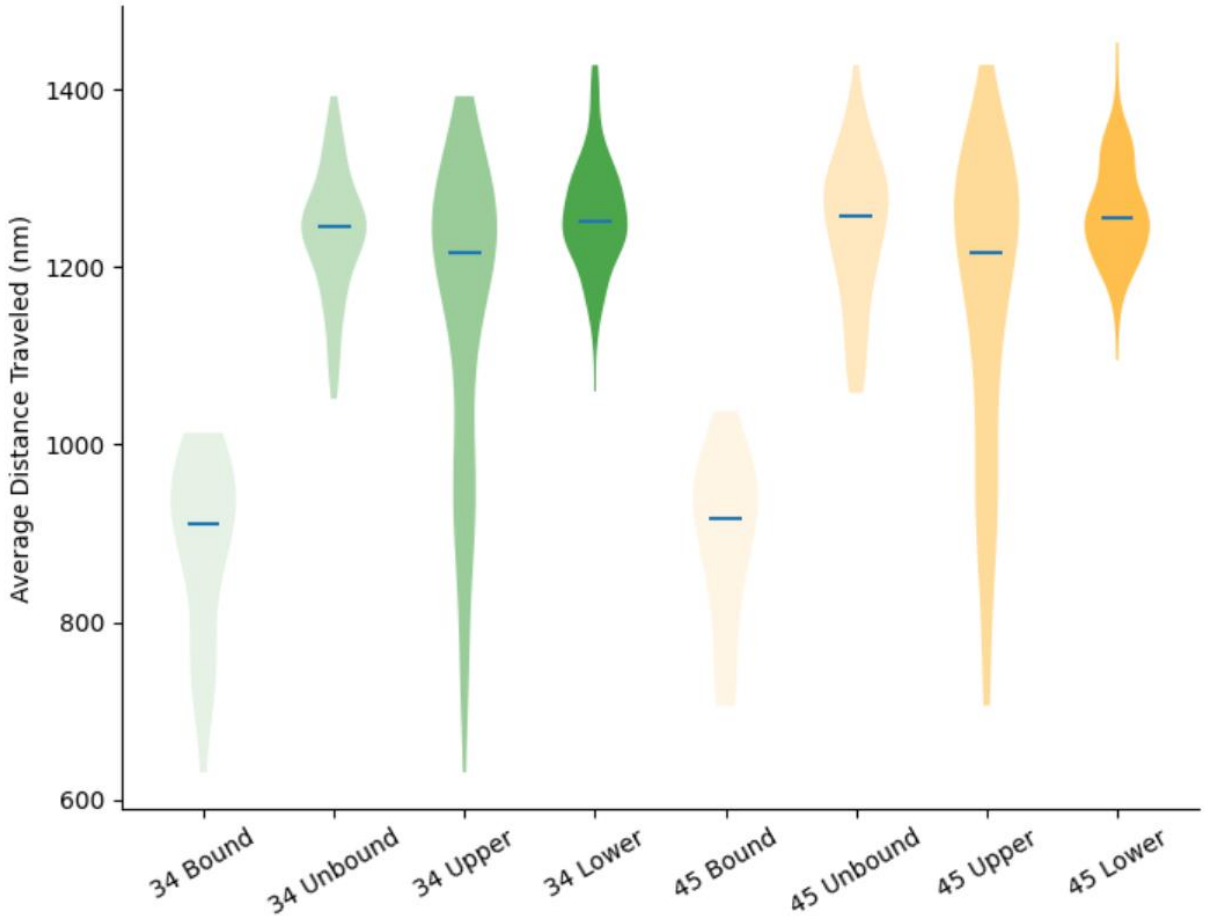
Supplementary Figure 4. DOPC trajectory trace plot for the top leaflet in all replicates. Coloring is based on distance traveled by each lipid (see Fig. 5D).



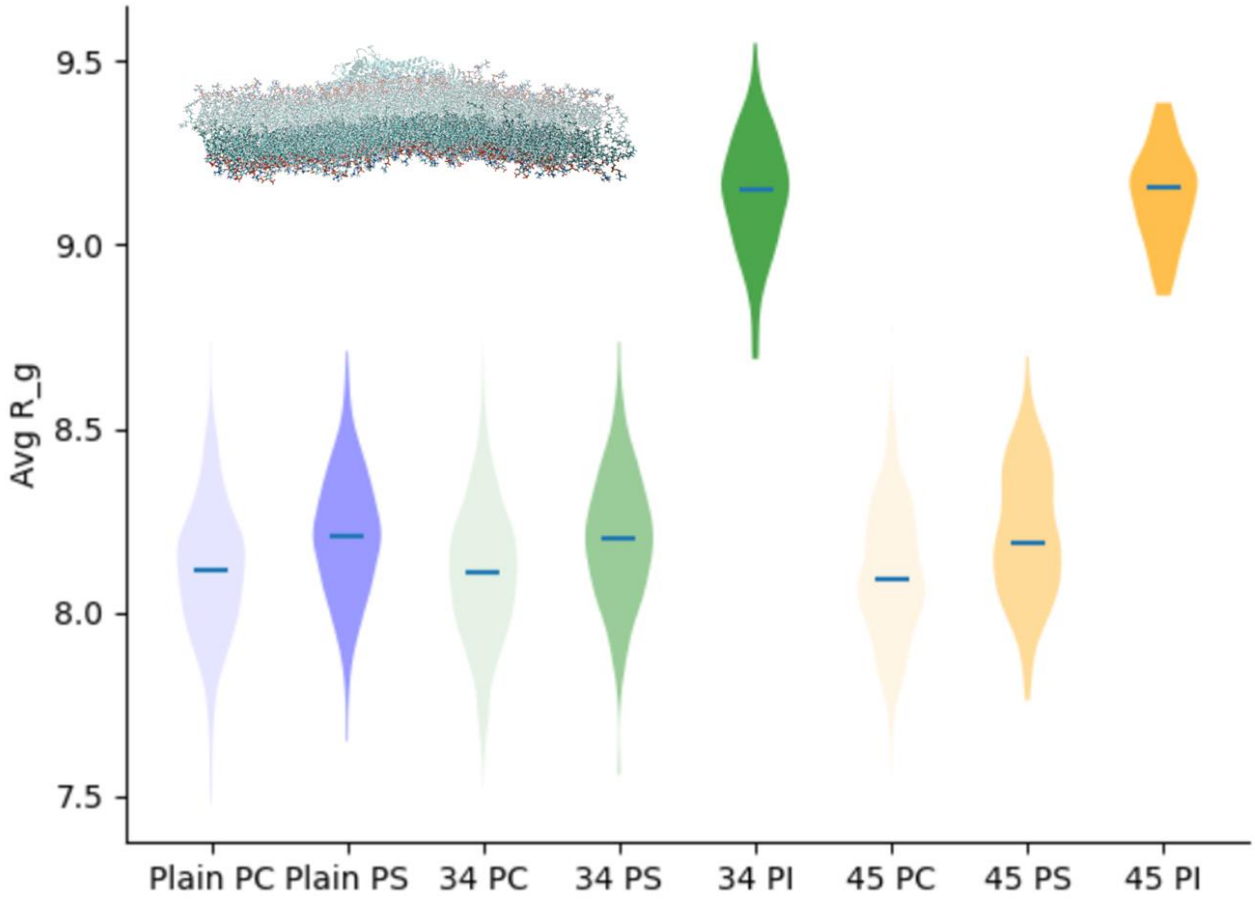
Supplementary Figure 5. Electrostatic Map of the PXBAR domain and autoinhibition motif. **(A)** Electrostatic surface map shown transparently over a cartoon representation of the SNX9-PXBAR domain from the side (top) and membrane binding interface (bottom). **(B)** Cartoon (top) and surface (bottom) depiction of predicted BAR domain (blue/pink) and autoinhibition motif (green) interaction from AlphaFold2 [Main Text Ref 39]. Visualization generated with ChimeraX [Main Text Ref 58].

	PI(3,4)P_2				
	BAR	**PX1**	**PX2**	***Total***	***PX avg***
Replicate 1	3.53	4.17	3.56	11.26	3.87
Replicate 2	4.76	3.24	2.93	10.93	3.09
Replicate 3	5.98	3.56	3.99	13.53	3.78
Replicate 4	5.66	2.00	4.15	11.81	3.08
Replicate 5	3.75	4.31	0.63	8.69	2.47
	PI(4,5)P_2				
	BAR	**PX1**	**PX2**	***Total***	***PX avg***
Replicate 1	5.31	2.55	4.60	12.46	3.56
Replicate 2	3.11	3.25	2.79	9.15	3.02
Replicate 3	6.08	4.22	3.28	13.58	3.75
Replicate 4	2.43	4.09	4.90	11.42	4.50
Replicate 5	3.06	2.46	1.08	6.60	1.77

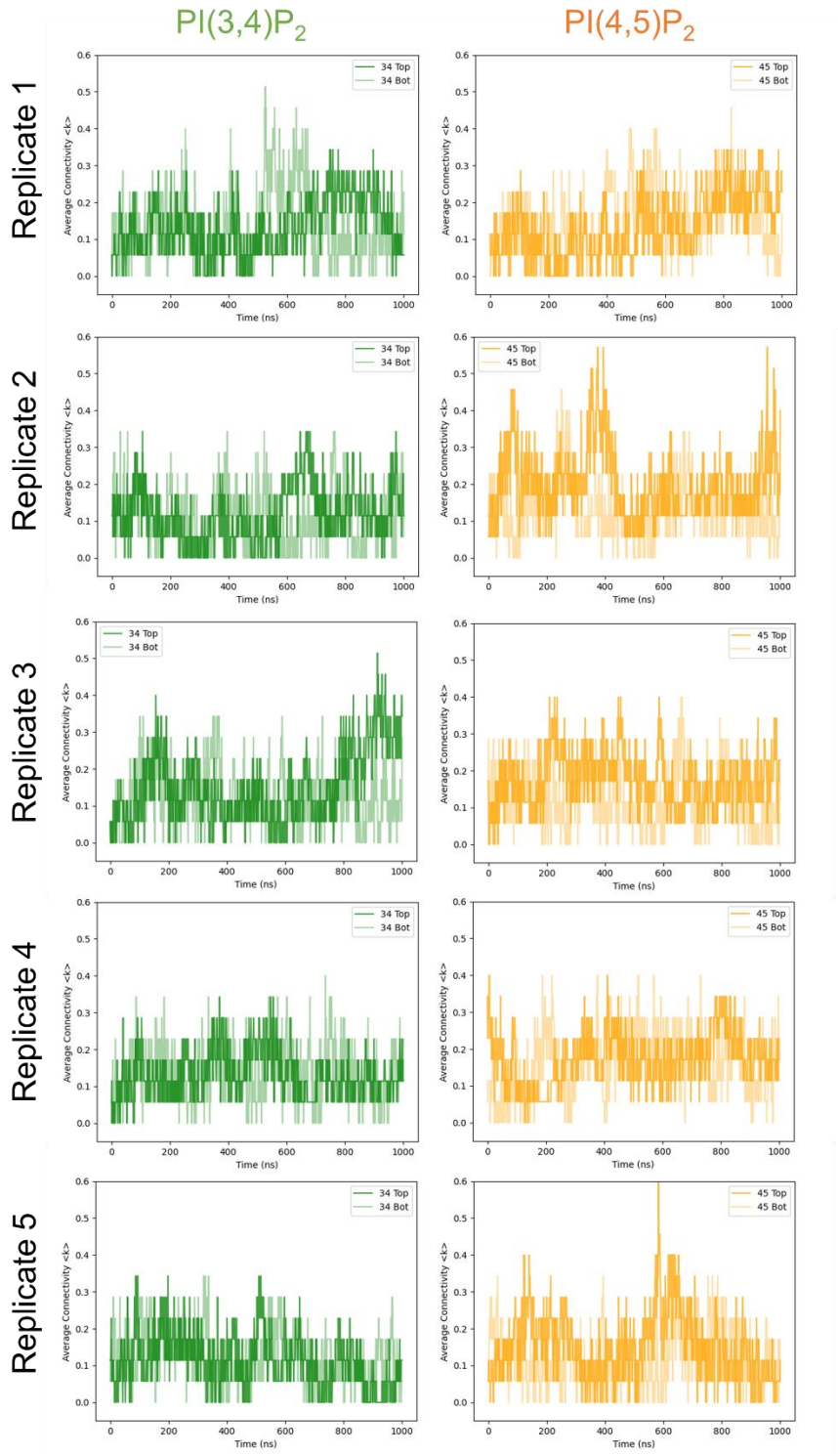
Supplementary Table 1. PIP_2 Occupancy values for the SNX9 PX-BAR dimer. Occupancy values based on number of lipids within distance threshold over course of simulation production time – see Fig. 6A for threshold metric visualization.



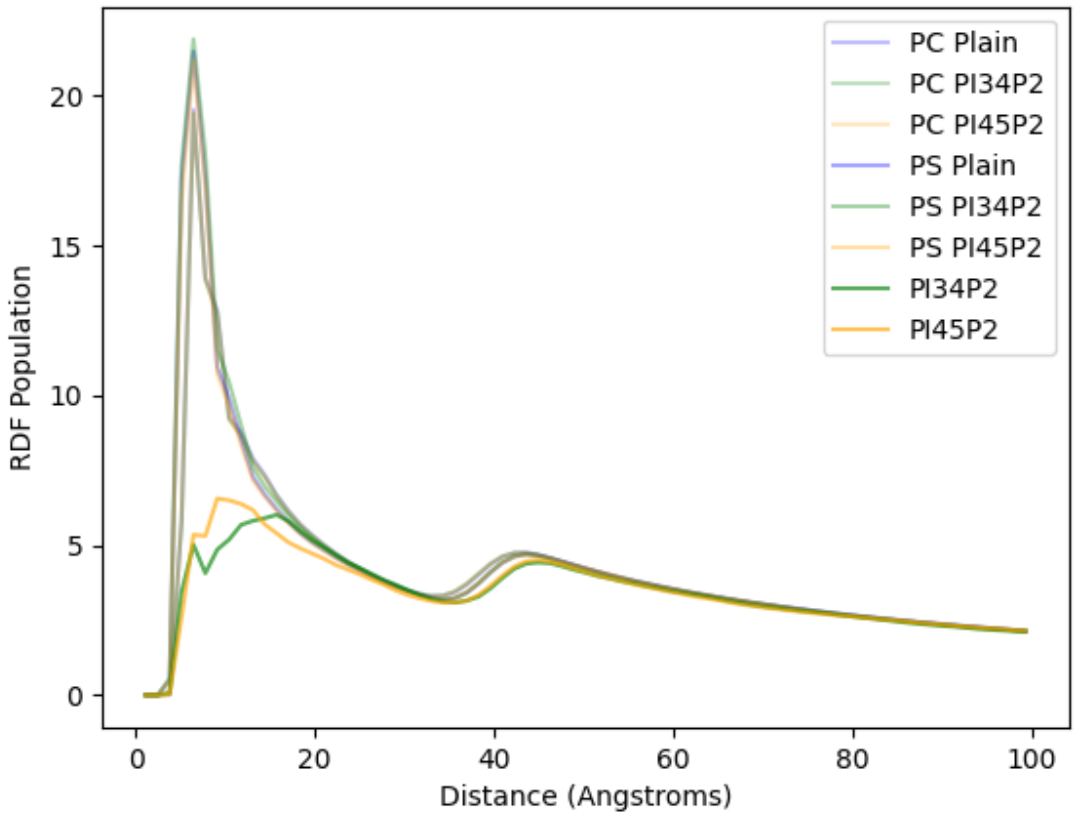
Supplementary Figure 6. Composite violin plot of average distance traveled of PIP_2 lipids from all five replicas. “Bound” and “unbound” refer to lipids in the upper leaflet that are either within the threshold distance (see Fig. 6A) or outside, respectively.



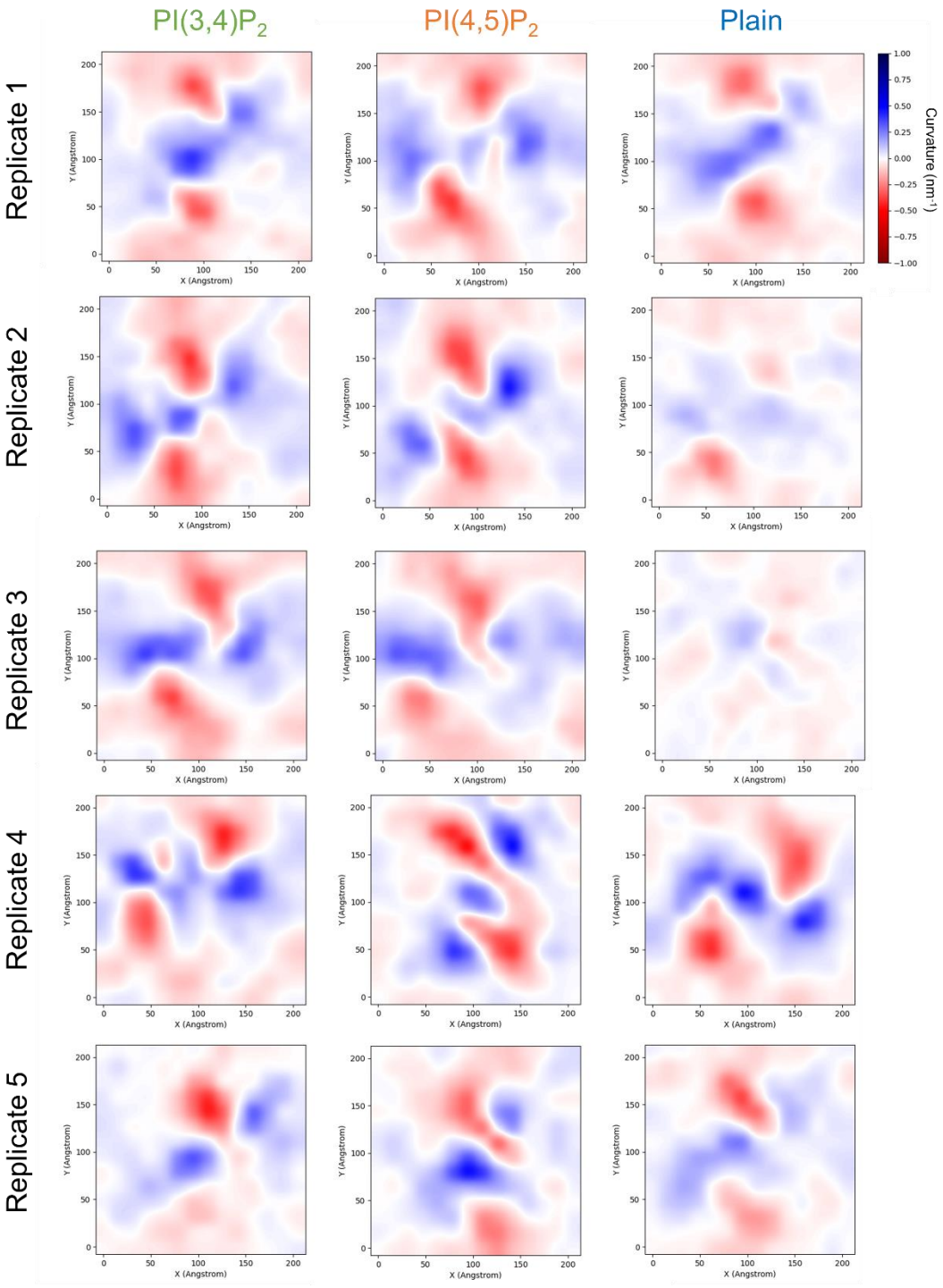
Supplementary Figure 7. Average Radius of Gyration of lipids in lower leaflets across all five replicates. Lower leaflet is defined as leaflet that does not have protein bound to it (see inset representation).



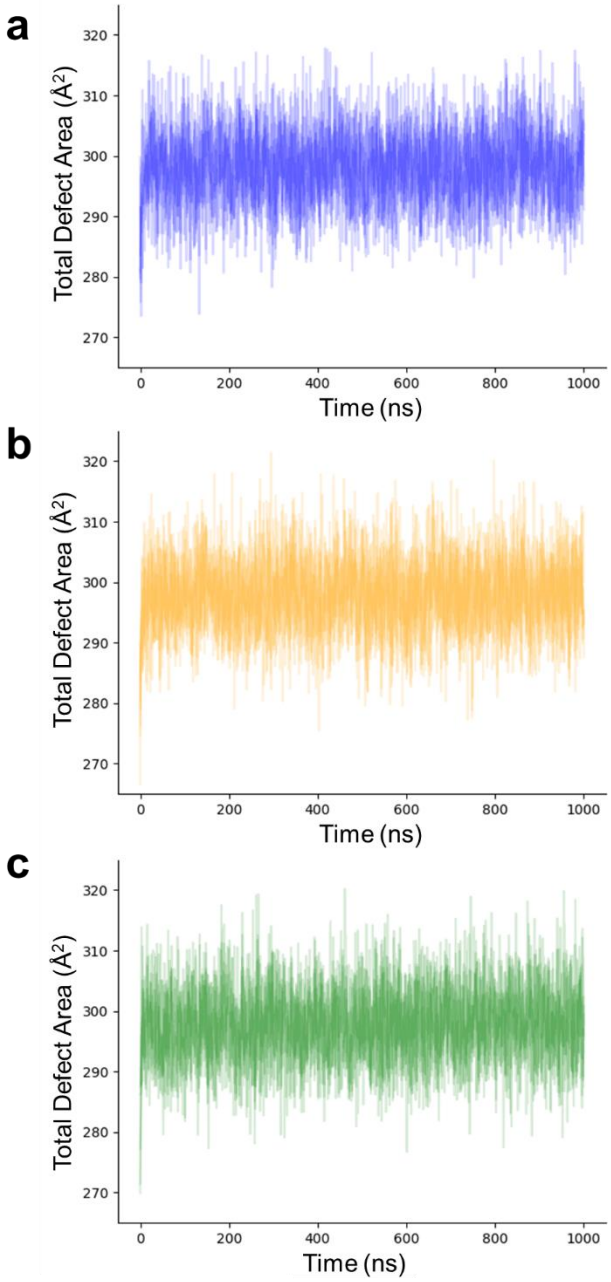
Supplementary Figure 8. Average connectivity <k> values for $PI(3,4)P_2$ and $PI(4,5)P_2$ as a function of time over the course of each replicate, based on analysis in Main Text Ref 41. Dark green or orange indicates the clustering values for top leaflet, while light green or orange indicates the clustering values for the lower leaflet.



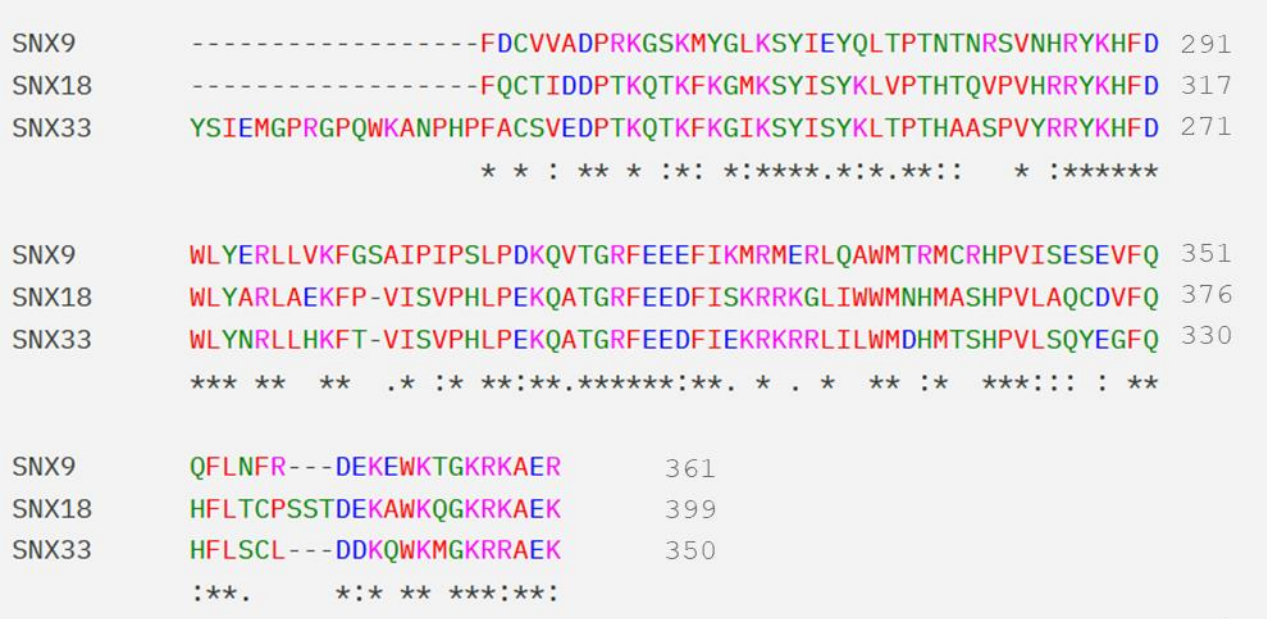
Supplementary Figure 9. Composite self-radial distribution function for all lipids.



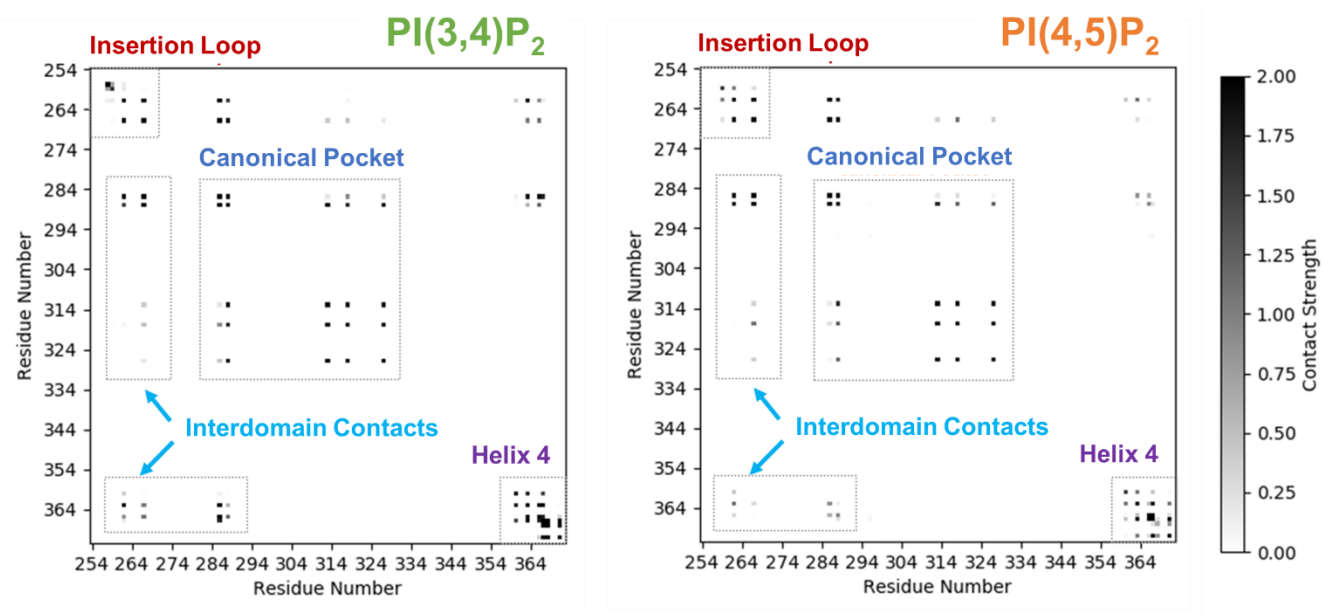
Supplementary Figure 10. Curvature analysis for the upper leaflet of each replicate.



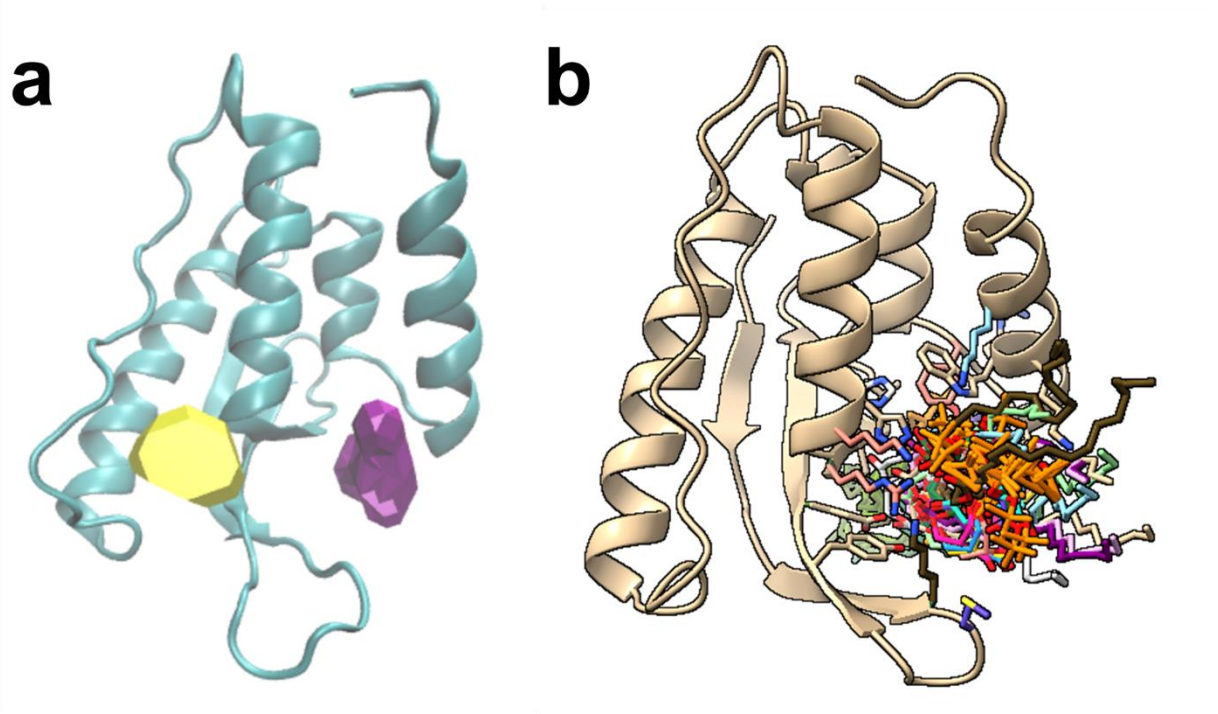
Supplementary Figure 11. Total membrane defect area for both the upper and lower membrane leaflets over the course of the simulation trajectories, based on Packmem script [1]. Individual trajectories for each replicate are plotted for the membranes without PIP_2 **(a)**, membranes with 5% $PI(4,5)P_2$ **(b)**, and membranes with 5% $PI(3,4)P_2$ **(c)**.



Supplementary Figure 12. Phylogeny analysis and sequence alignment of PX domains of closely related PXBAR containing SNX proteins, conducted using the CLUSTAL OMEGA MSA program[2]. MSA of PX domains of SNX9, SNX18, and SNX33, with color to indicate amino acid properties such as charge or hydrophobicity. * indicates an amino acid identically conserved in all three proteins; : indicates a higher conservation of property but not identity; and . indicates a lower conservation of property.



Supplementary Figure 13. Full PX domain PIP_2 contact networks, with $PI(3,4)P_2$ (*Left*) and $PI(4,5)P_2$ (*Right*). Contact metric based on Bjerrum distance (see Fig. 7b).



Supplementary Figure 14. Spatial analysis of PX binding pockets. **(a)** Cavity analysis [3] of the PX domain of 2RAK, visualized with VMD [Main Text Ref 59], demonstrates two major binding pockets with volumes of 745 $Å^3$ (yellow, canonical pocket) and 905 $Å^3$ (purple, helix four pocket). **(b)** Docking [4] of the ten most likely predicted PIP_2 positions for $PI(3,4)P_2$ and $PI(4,5)P_2$, visualized with ChimeraX [Main Text Ref 58].

Supplementary Citations

1 Gautier, R. *et al.* Packmem: A versatile tool to compute and visualize interfacial packing defects in lipid bilayers. *Biophys J* **115**, 436-444 (2018). https://doi.org/10.1016/j.bpj.2018.06.025

2 Madeira, F. *et al.* The EMBL-EBI Job Dispatcher sequence analysis tools framework in 2024. *Nucleic Acids Res* **52**, W521-W525 (2024). https://doi.org/10.1093/nar/gkae241

3 Durrant, J. D., Votapka, L., Sørensen, J. & Amaro, R. E. POVME 2.0: an enhanced tool for determining pocket shape and volume characteristics. *J Chem Theory Comput* **10**, 5047-5056 (2014). https://doi.org/10.1021/ct500381c

4 Singh, A., Copeland, M. M., Kundrotas, P. J. & Vakser, I. A. GRAMM web server for protein docking. *Methods Mol Biol* **2714**, 101-112 (2024). https://doi.org/10.1007/978-1-0716-3441-7_5