SUPPLEMENTARY INFORMATION for:

Interactions of the Auxilin-1 PTEN-like Domain with Model Membranes Result in Nanoclustering of Phosphatidyl Inositol Phosphates

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SUPPLEMENTARY FIGURES:

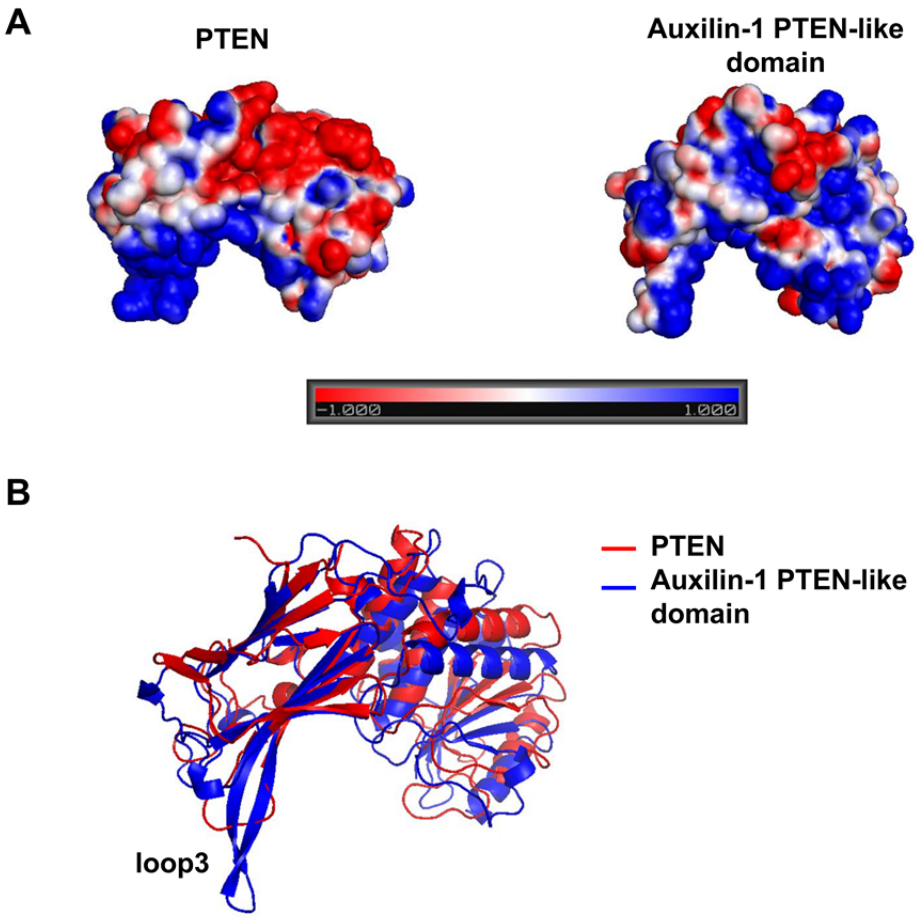

Figure S1:
A. Electrostatic surfaces of the PTEN and the auxilin-1 PTEN-like domain. Blue indicates a positive surface and red a negative surface. B. Alignment of the C2 domains from the PTEN and the auxilin-1 PTEN-like domain crystal structures. The loop3 which was shown to drive the association with the membrane is highlighted.

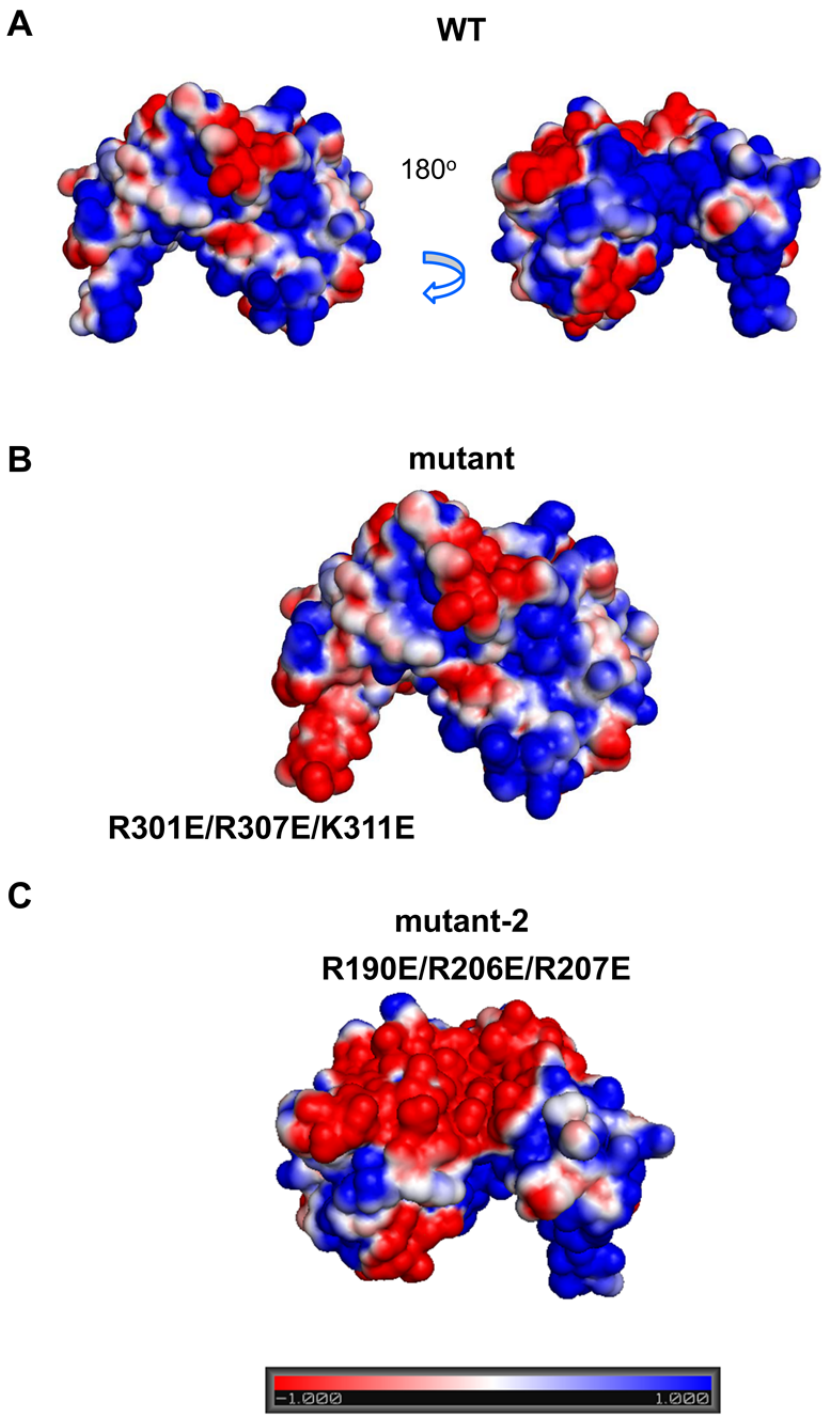

Figure S2:
Electrostatic surface of the auxilin-1 PTEN-like domain for the R301E/R307E/K311E mutant (B) and the R190E/R206E/R207E mutant (C). Blue indicates a positive surface and red a negative surface. The electrostatic surface of the wild type auxilin-1 PTEN-like domain is shown in A for comparison.

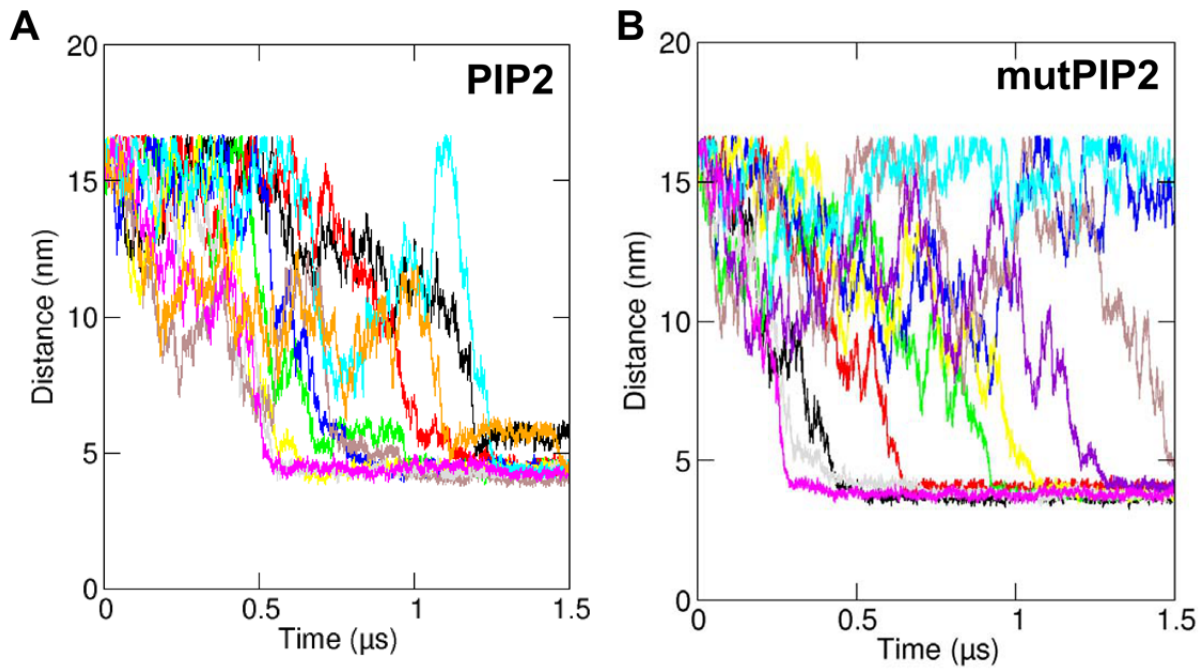

Figure S3:
A,B. Progress of the simulations with the wild type (A; PIP2 simulation in Table 1) and the mutated form (B; mutPIP2 simulation in Table 1) of Auxilin. The progress of the simulations is shown as the separation between the centres of mass of the protein and the bilayer as a function of time. The ten different colours represent the ten different repeat simulations performed for each system.

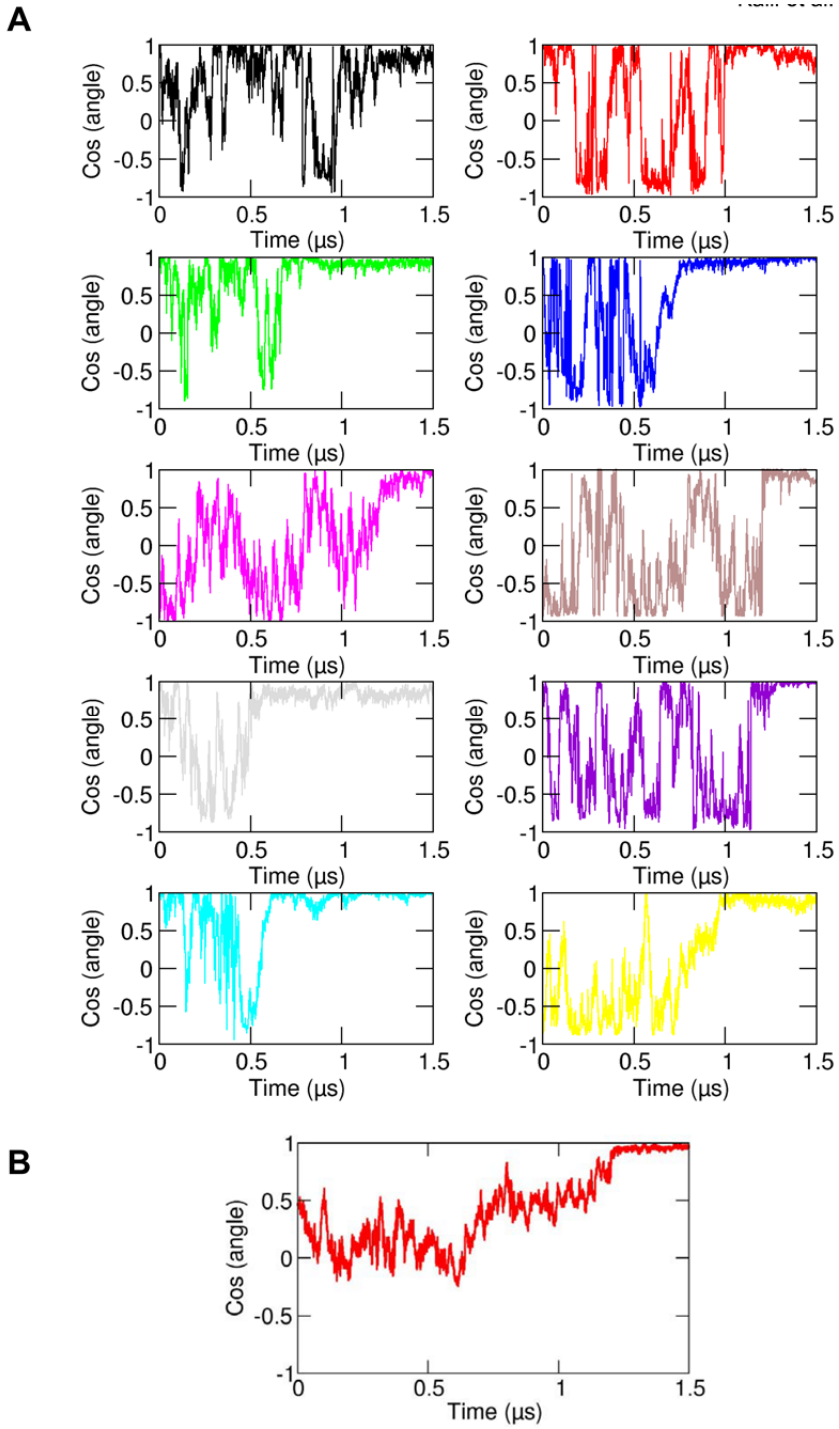

Figure S4:

A. The cosine of the angle between the plane of the protein and the bilayer plane is shown for the ten individual simulations of the PIP2 system as function of time (shown using the same colour coding as in Supplementary Fig. 3A). B. Average cosine of the angle between the plane of the protein and the bilayer plane (over all the simulations in A) as a function of time.

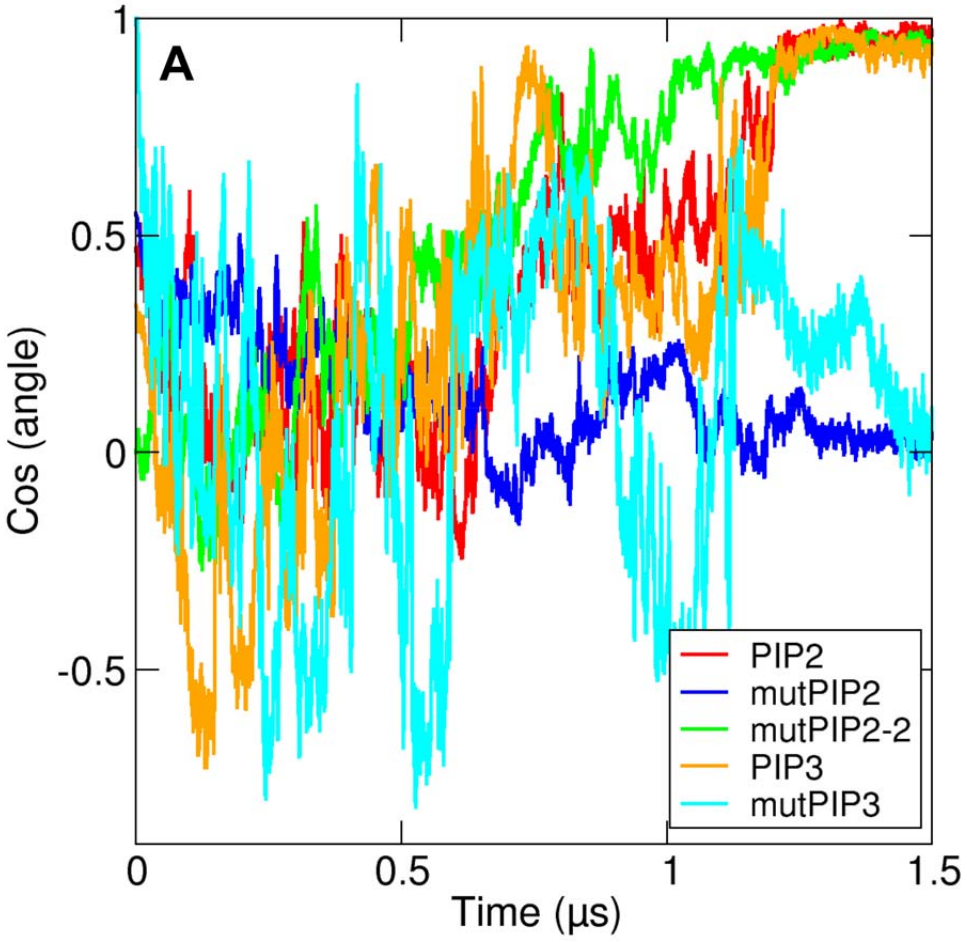

Figure S5:
A. Average (across all simulations which resulted in an Auxilin/bilayer complex) value of the angle between the plane of the protein and the plane of the bilayer for the PIP2 (red), mutPIP2 (blue), mutPIP2-2 (green), PIP3 (orange) and mutPIP3 (cyan) simulations.

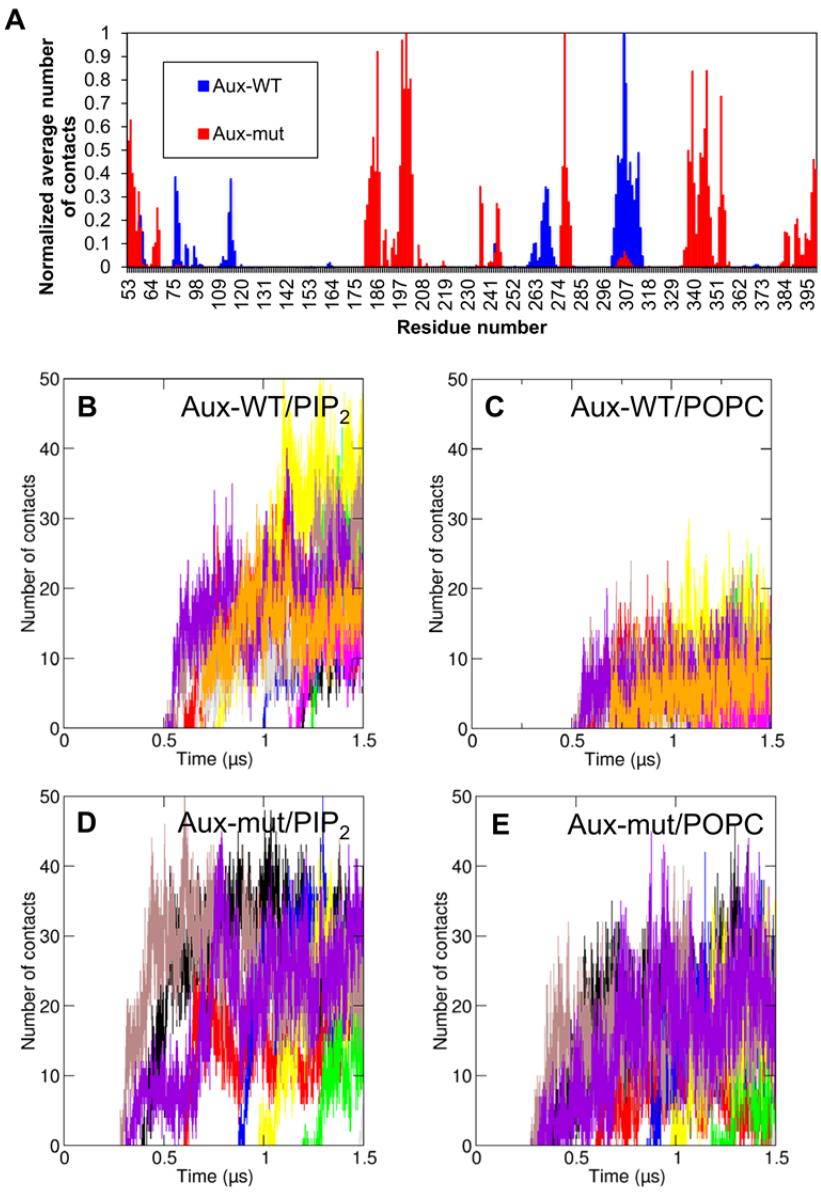

Figure S6:

A. Normalized average number of contacts (using a cut-off distance of 7.5 Å) between the Auxilin and the lipids in the bilayer (across all repeats of the PIP2 and mutPIP2 simulations where an Auxilin/bilayer complex was formed). The contacts of the wild type Auxilin are shown in blue and the contacts of the mutated form of Auxilin in red. B, C, D, E. Number of contacts of the Auxilin-1 with the PIP_2 (B, D) and POPC (C, E) lipids shown as a function of simulation time for the PIP2 (B, C) and the mutPIP2 (D, E) simulations (see Table 1 for more information). The different colours represent the different repeat simulations performed.

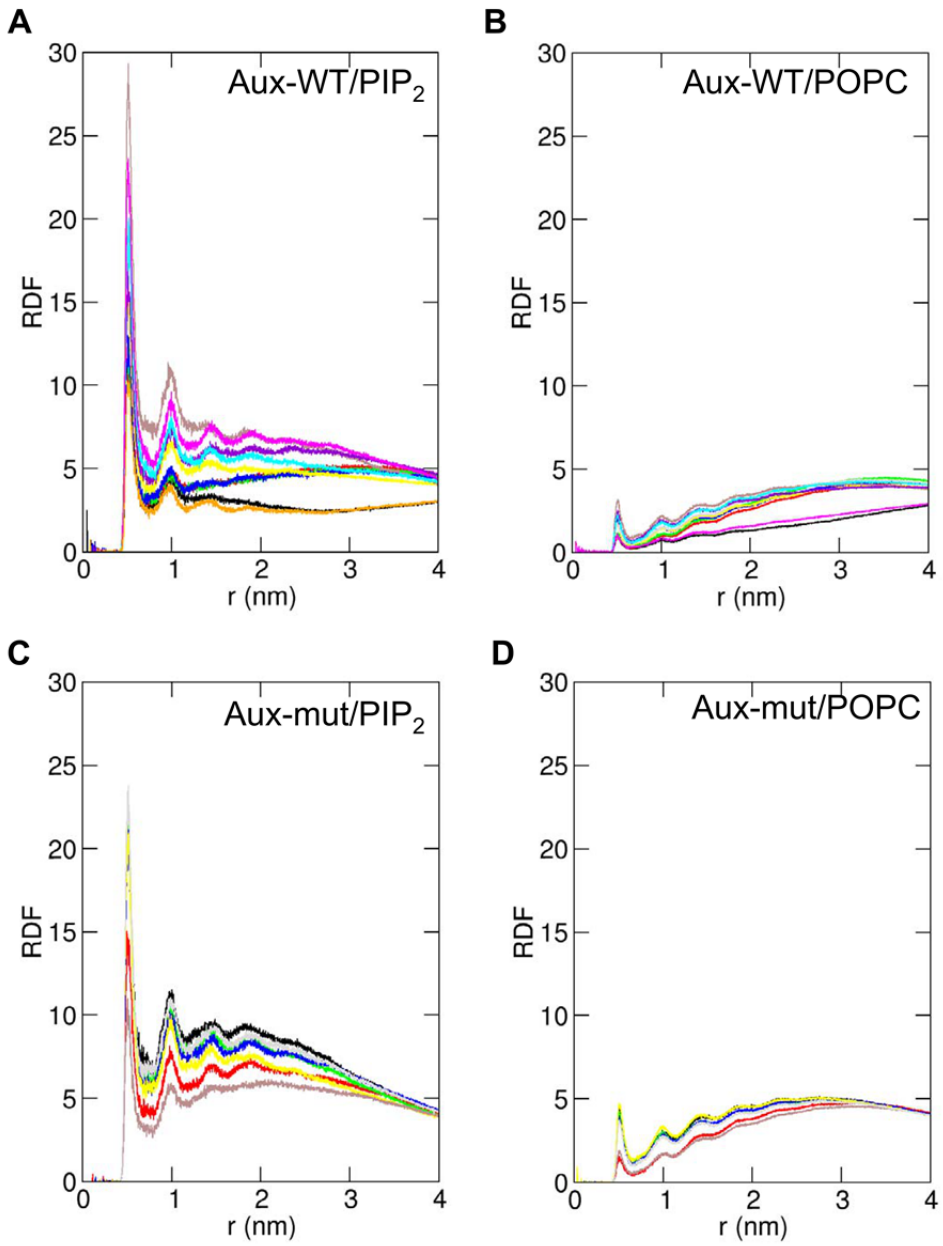

Figure S7:
A,B,C,D. The lipid radial distribution function for the PIP2 (A,B) and the mutPIP2 (C,D) simulations. The distribution for each lipid type (i.e. POPC and PIP_2 lipids) for all repeat simulations which resulted in an Auxilin/bilayer complex is shown separately. The radial distributions were calculated around the Auxilin-1 PTEN domain.

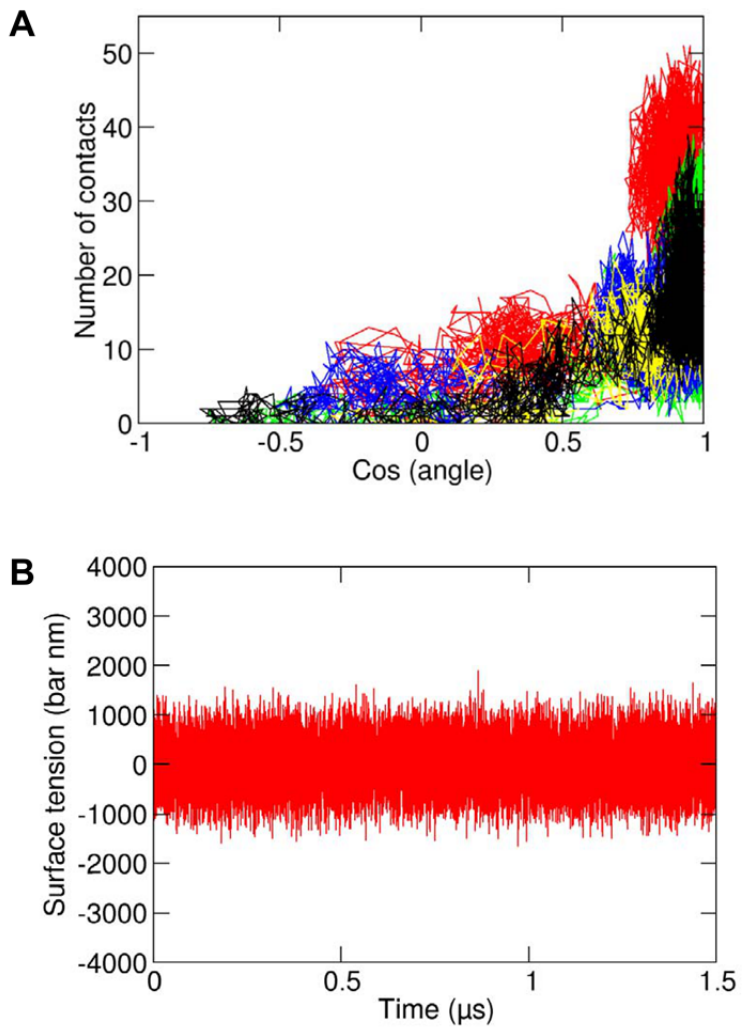

Figure S8:
A. Number of contacts (using a cut-off distance of 7.5 Å) between the protein and the PIP_2 lipids for the simulations with the POPC/PIP_2 bilayer in which the rotation of the protein on the bilayer surface was observed as a function of the angle between the protein and the bilayer. This analysis suggests that the clustering of PIP_2 lipids occurs after Auxilin adopts a stable productive orientation relative to the bilayer. B. Bilayer surface tension from one of the simulations of the PIP2 system.

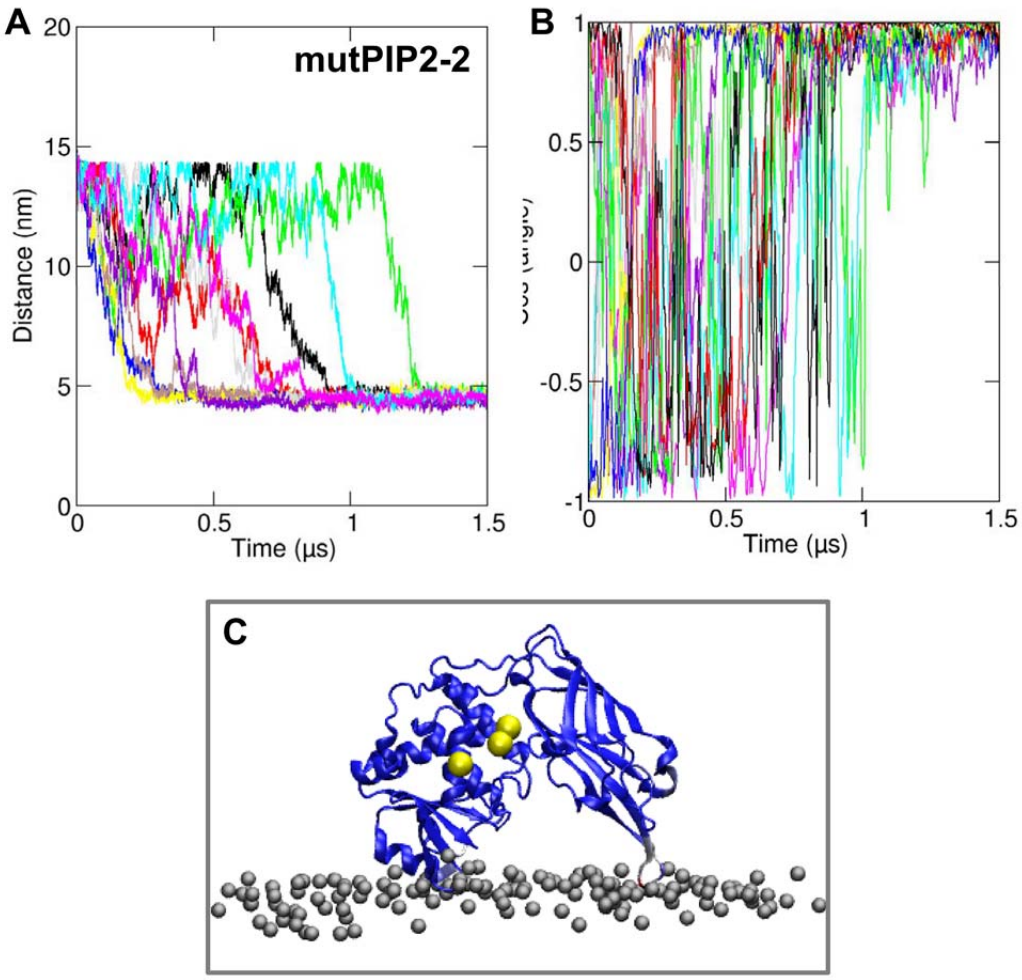

Figure S9:

A. Separation between the centres of mass of Auxilin-1 and the bilayer as a function of time for the simulation with the second mutated form of Auxilin and a POPC/PIP_2 bilayer (simulation mutPIP2-2 in Table 1). The ten different colours represent the ten different repeat simulations performed. B. The cosine of the angle between the plane of the protein and the bilayer plane is shown for the same system as function of time for the simulations that yielded an Auxilin/bilayer complex (shown using the same colour coding as in A). This analysis revealed that the productive binding mode of the wild type Auxilin was also observed when a triple mutation in an area which is not in the Auxilin/bilayer interface was made. C. Normalized average number of contacts (using a cut-off distance of 7.5 Å) between the Auxilin and the lipids in the bilayer (across all repeats of the CG-MD simulations with the PIP_2/POPC bilayer; simulation mutPIP2-2 in Table 1). The normalized average number of contacts was mapped on the Auxilin-1 productive orientation. Blue indicates a low number, white indicates a medium number and red a large number of contacts. The bilayer phosphate atoms are shown as grey spheres. The backbone particles of the mutated residues are shown in yellow spheres.

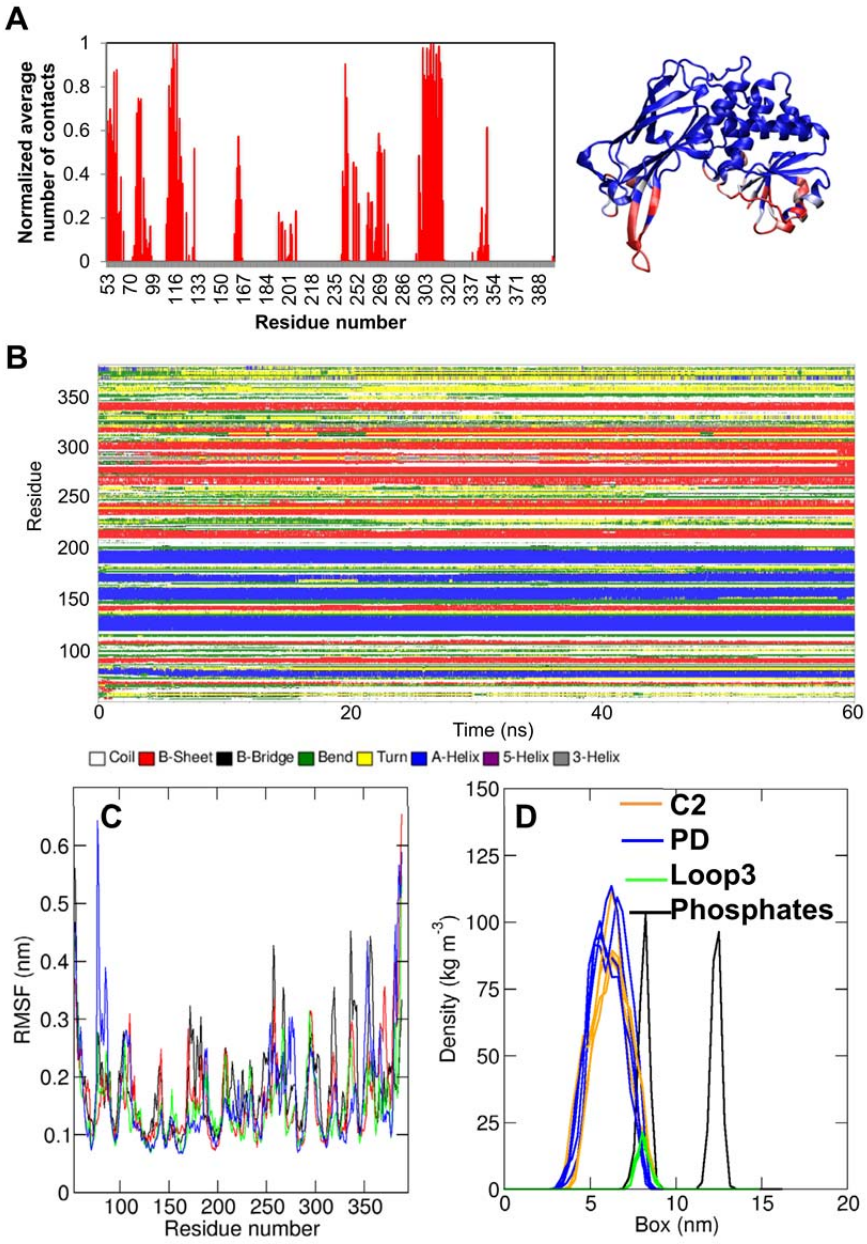

Figure S10:

A. Normalized average number of contacts (using a cut-off distance of 3.5 Å) between the Auxilin and the lipids in the bilayer (across all repeats of the AT-MD simulations with the PIP_2/POPC bilayer; simulation PIP2-AT in Table 1). The normalized average number of contacts was mapped on the Auxilin-1 crystal structure. Blue indicates a low number, white indicates a medium number and red a large number of contacts. B. Secondary structure analysis during one of the AT-MD simulations. C. Root means square fluctuation (RMSF) per residue for the same systems as in A. The RMSFs for the four repeats are shown in four different colors. D. Density profiles along the membrane normal for different parts of Auxilin for the four repeats of the PIP2-AT system. The density for the Auxilin C2 domain is shown in orange, for the PD is shown in blue and for the loop 3 is shown in green. The black lines demonstrate the density of the bilayer phosphate atoms.

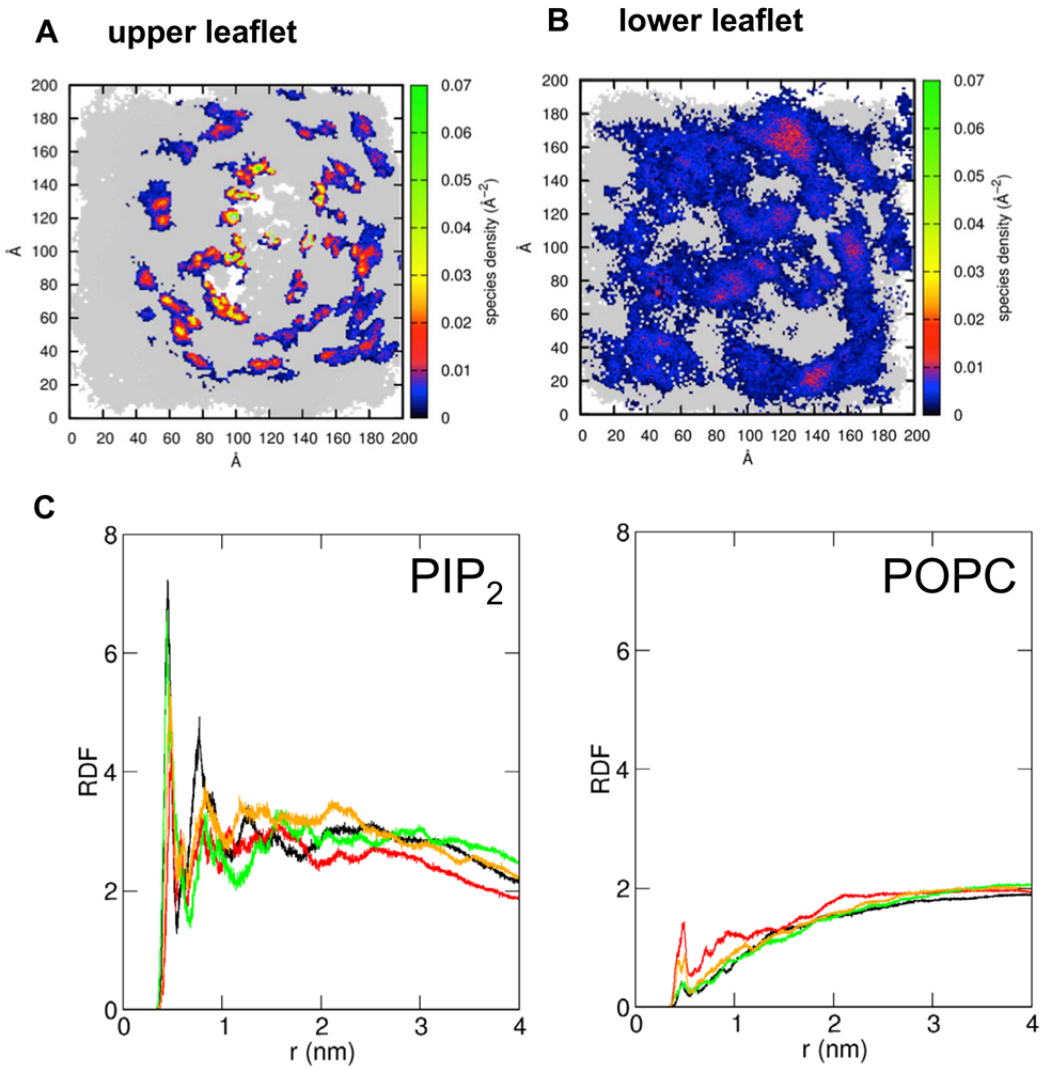

Figure S11:

A,B. Spatial distribution density in the (**A**) upper and (**B**) lower bilayer plane of the PIP_2 headgroups, from the atomistic simulations of the auxilin-1 PTEN-like domain bound to a 15% PIP_2/85%POPC bilayer (simulation PIP2-AT). In the upper leaflet, to which the auxilin PTEN-like domain was bound, clustering of the PIP_2 headgroups can be seen while in the lower leaflet no evidence of PIP_2 clustering is observed. For this analysis all simulation frames were fitted using the protein as a reference structure and the positions of the PIP_2 headgroups were calculated for the whole duration of the simulations. The density of the PIP_2 headgroups is coloured from blue (low) through red to green (high). The white regions indicate the footprint of the protein on the lipid bilayer surface. C. The lipid radial distribution function for all the PIP2-AT simulations. The distribution for each lipid type for all repeat simulations is shown separately (PIP_2 is on the left side and POPC is on the right side). The radial distributions were calculated around the Auxilin-1 PTEN domain.

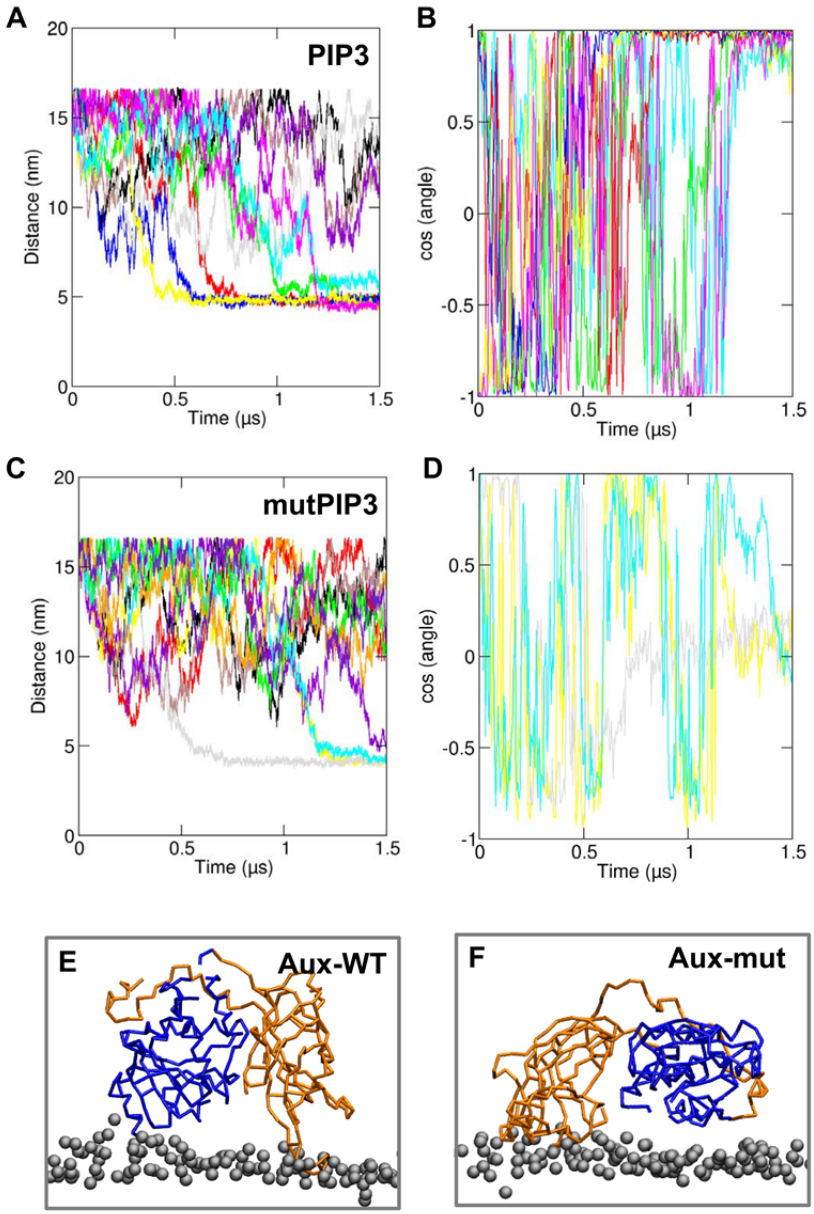

Figure S12:

A,C. Separation between the centres of mass of Auxilin-1 and the bilayer as a function of time for the simulation with the wild type Auxilin (A) and the mutated form of Auxilin (C) and a POPC/PIP_3 bilayer (simulation PIP3 and mutPIP3 in Table 1). The ten different colours represent the ten different repeat simulations performed. B,D. The cosine of the angle between the plane of the protein and the bilayer plane is shown for the same systems as function of time for the simulations that yielded an Auxilin/bilayer complex (shown using the same colour coding as in A and C). This analysis revealed that the mutations in Auxilin perturbed the binding mode observed to the wild type simulations. E, F. Final snapshot from one of the simulations with the wild type Auxilin (E) and the mutated form of Auxilin (F) demonstrating the final orientation of Auxilin relative to the bilayer in the PIP3 (E) and mutPIP3 (F) simulations. The C2 domain is shown in orange, the PD in blue and the lipid phosphate atoms in grey.

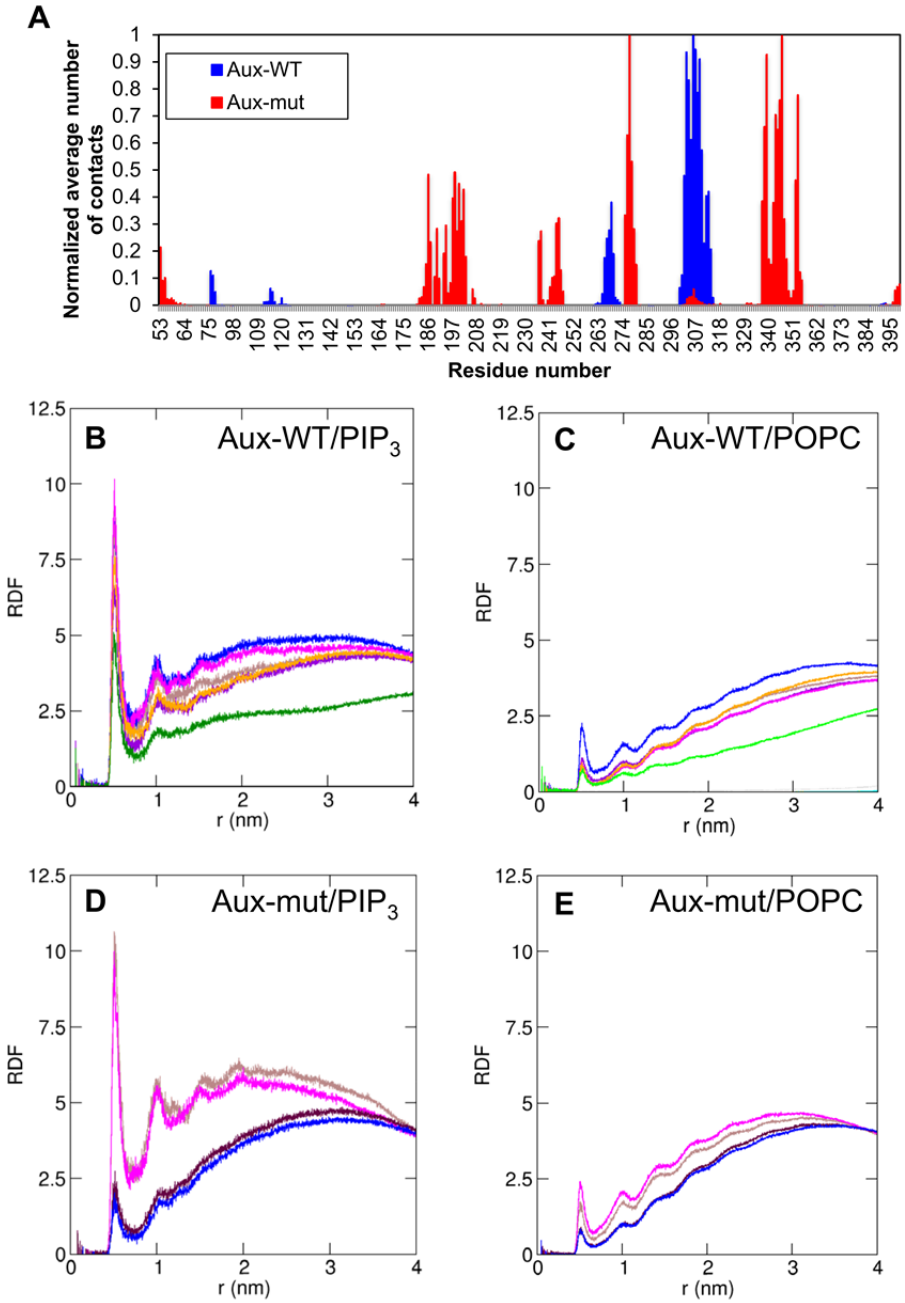

Figure S13:

A. Normalized average number of contacts (using a cut-off distance of 7.5 Å) between the Auxilin and the lipids in the bilayer (across all repeats of the PIP3 and mutPIP3 simulations which formed a complex with the bilayer). The contacts of the wild type Auxilin is shown in blue and the contacts of the mutated form of Auxilin in red. B,C,D,E. The lipid radial distribution function for the PIP3 (B,C) and the mutPIP3 (D,E) simulations. The distribution for each lipid type for all repeat simulations which resulted in an Auxilin/bilayer complex is shown separately. The radial distributions were calculated around the Auxilin-1 PTEN domain.

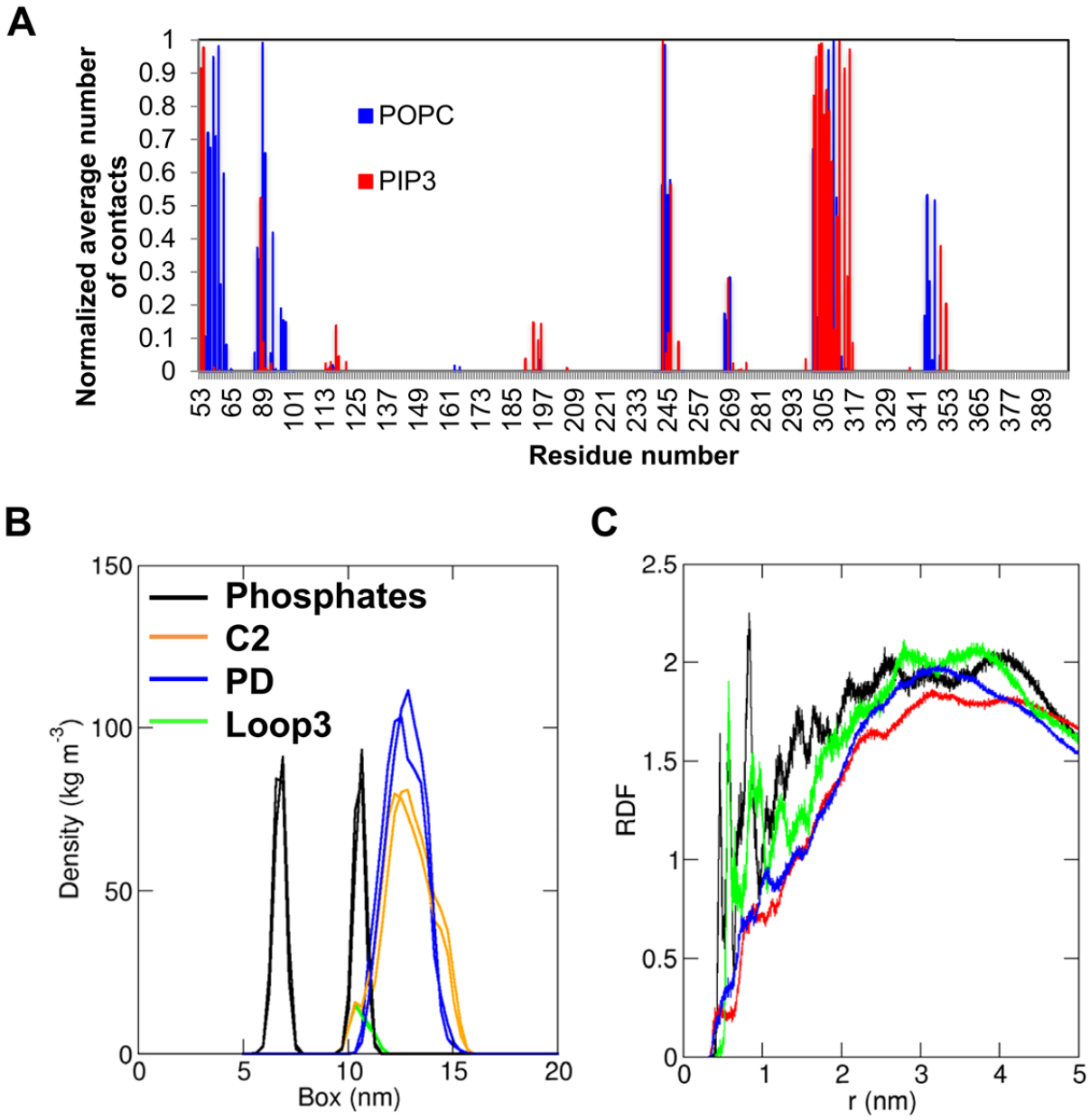

Figure S14:

A. Normalized average number of contacts (using a cut-off distance of 3.5 Å) between the Auxilin and the lipids in the bilayer (across all repeats of the AT-MD simulations with the PIP_3/POPC bilayer; simulation PIP3-AT in Table 1). B. Density profiles along the membrane normal for different parts of Auxilin for the two repeats of the PIP3-AT simulation. The Auxilin C2 domain is shown in orange, the PD is shown in blue and the loop 3 is shown in green. The black lines demonstrate the density of the bilayer phosphate atoms. C. The lipid radial distribution function for all the AT-PIP3 simulations. The distribution for POPC lipids is shown in blue and red and for PIP_3 in green and black. The radial distributions were calculated around the Auxilin-1 PTEN domain.

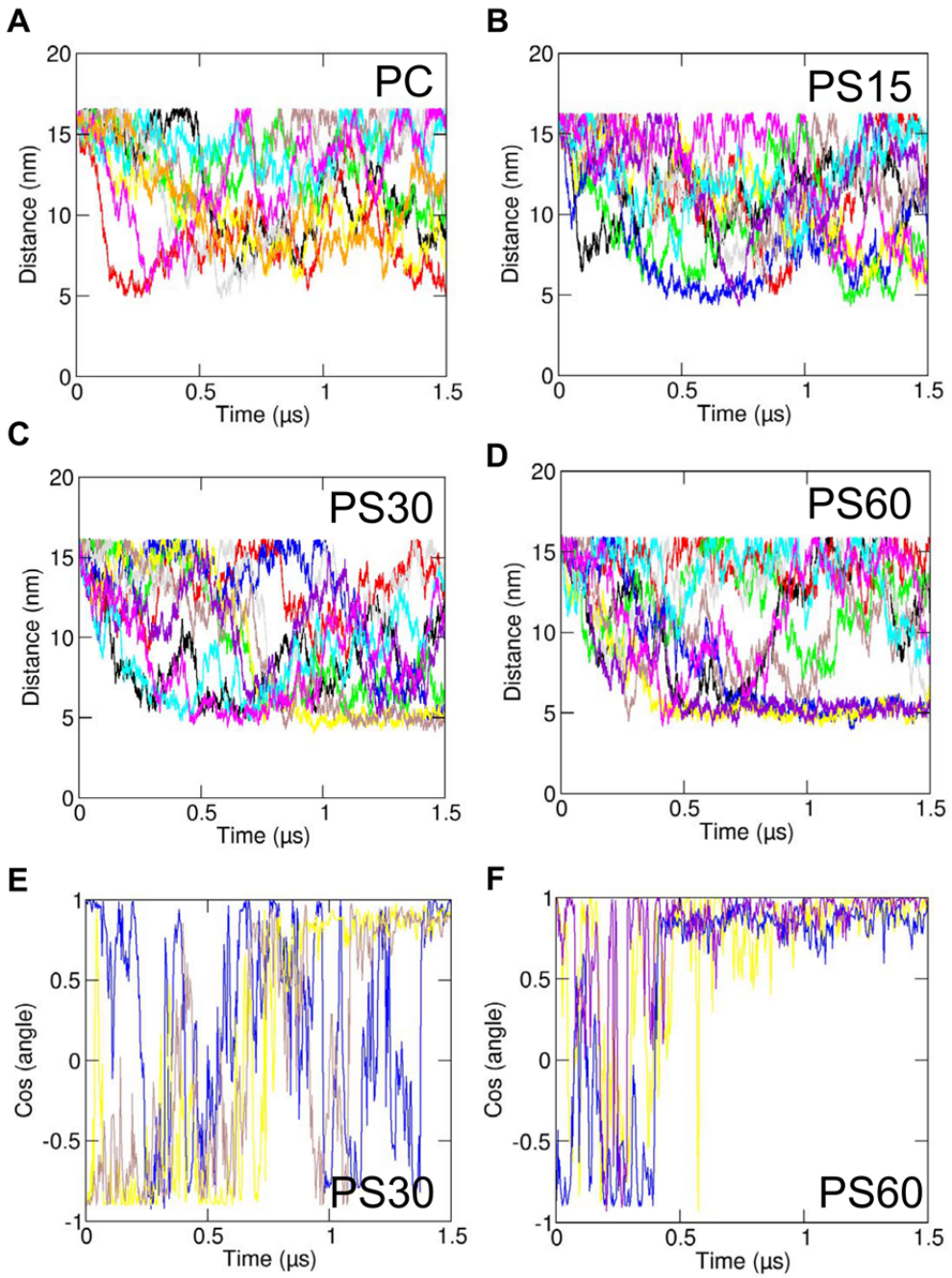

Figure S15:
Progress of the simulations of the **A** PC, **B** PS15, **C** PS30 and **D** PS60 simulations (see Table 1 for details) shown as the distances between the centre of mass of the protein and the centre of mass of the bilayer as a function of time. E,F. The cosine of the angle between the plane of the protein and the bilayer plane is shown for the **E** PS30 and **F** PS60 systems as function of time for the simulations that yielded an Auxilin/bilayer complex (shown using the same colour coding as in C and D).

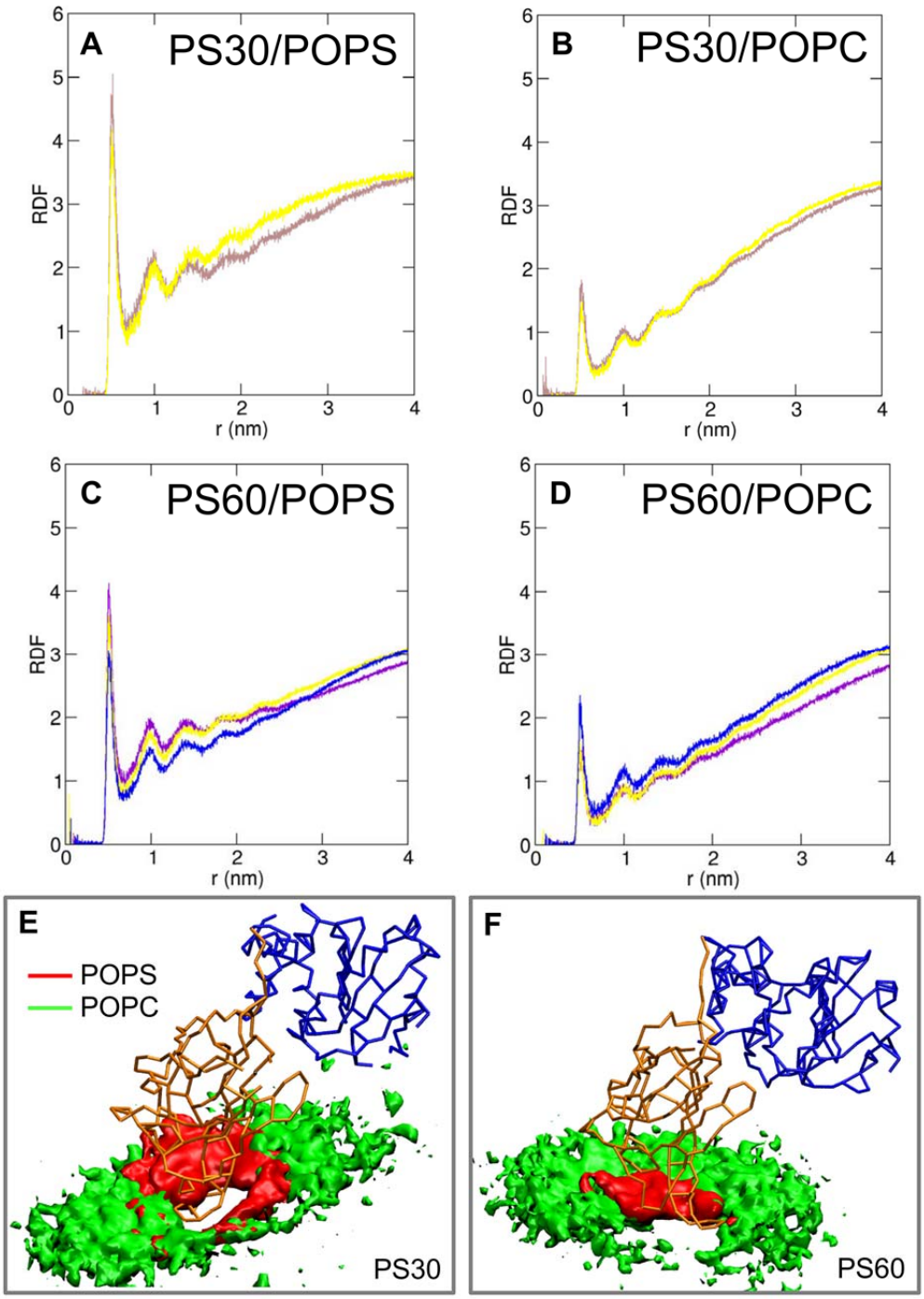

Figure S16:

A,B,C,D. The lipid radial distribution function for the PS30 (A,B) and the PS60 (C,D) simulations. The distribution for each lipid type for all repeat simulations which resulted in an Auxilin/bilayer complex is shown separately. The radial distributions were calculated around the Auxilin-1 PTEN domain. E,F. Average lipid headgroup densities around Auxilin for the POPS and POPC molecules from the *PS30* simulation (E) and the *PS60* simulation (F). The POPS lipids density is shown in red and the POPC density in green.