Supplementary Information

Molecular Mechanism of S1P Binding and Activation of the $S1P_1$ Receptor

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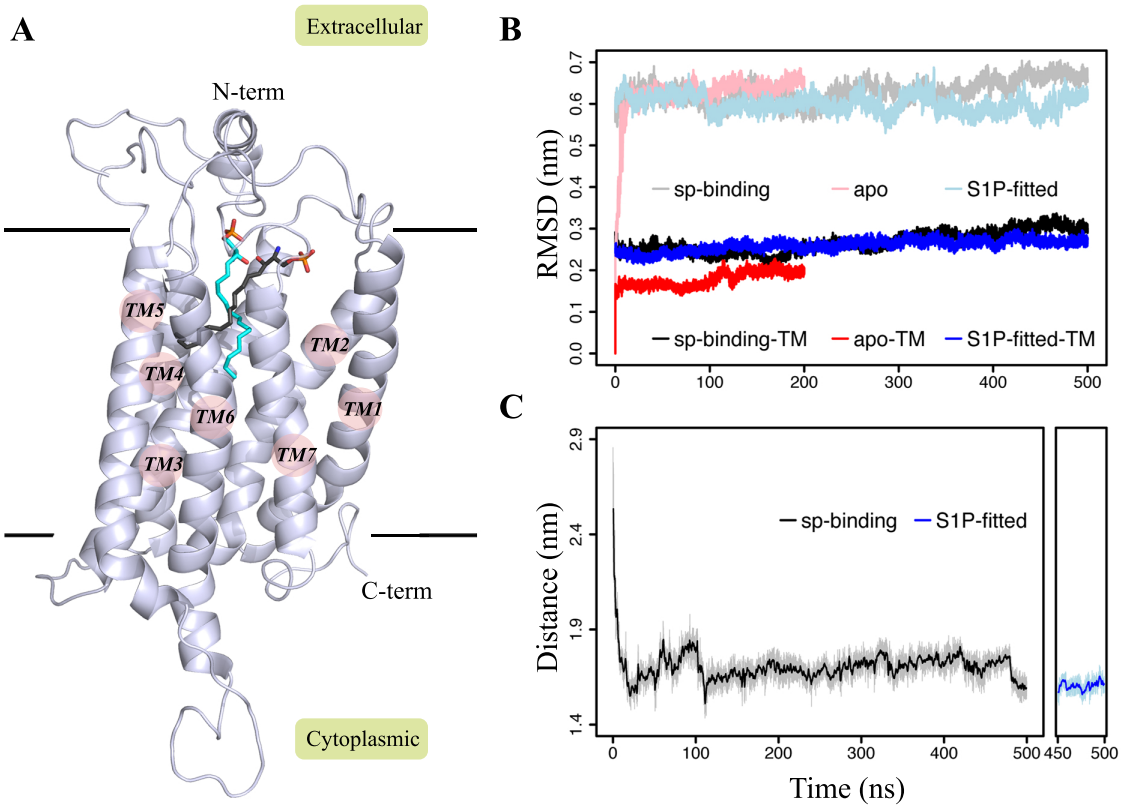

Figure S1. Root mean square deviations (RMSDs) of the receptor and the S1P binding depth. (**A**) Ribbon diagram structure of $S1P_1$. The last snapshot of S1P binding at 500 ns in the sp-binding simulation is shown in black and is colored in cyan for the in the S1P-fitted simulation at 500 ns. (**B**) RMSD of the receptor relative to the crystal structure for the whole protein and transmembrane region during the simulation times of spontaneous S1P binding (sp-binding, shown in grey and black, respectively), the apo $S1P_1$ simulation (shown in light red and red, respectively), and the S1P-fitted simulation (shown in light blue and blue, respectively). (**C**) Distance between the COM of S1P and the COM of the helical bundle of the receptor (residues 42-330) observed in the sp-binding simulation (black) and the S1P-fitted simulation (blue).

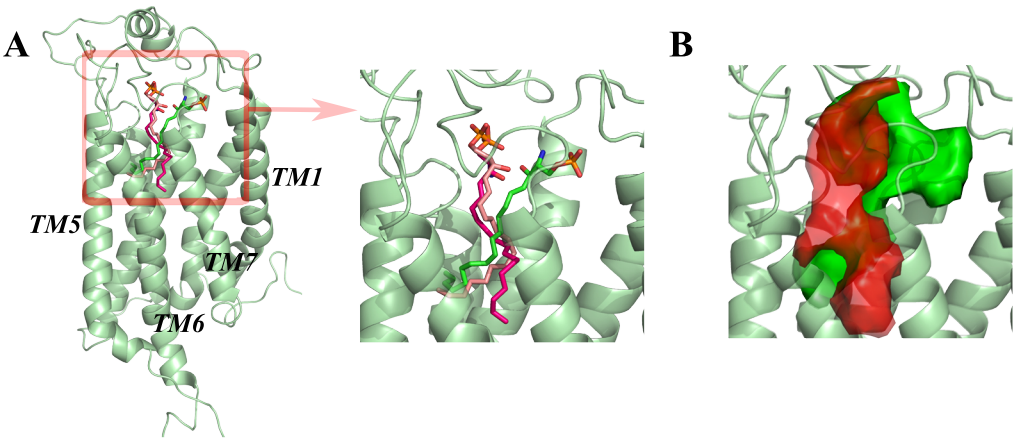

Figure S2. Comparison of S1P binding poses. (**A**) The S1P position in the S1P sp-binding simulation at 200 ns (pink) and 500 ns (magenta) in addition to the fit in the S1P-fitted simulation (green). (**B**) The final binding pocket volumes were 516 $Å^3$ in the sp-binding simulation (red) and 529 $Å^3$ in the S1P-fitted simulation (green).

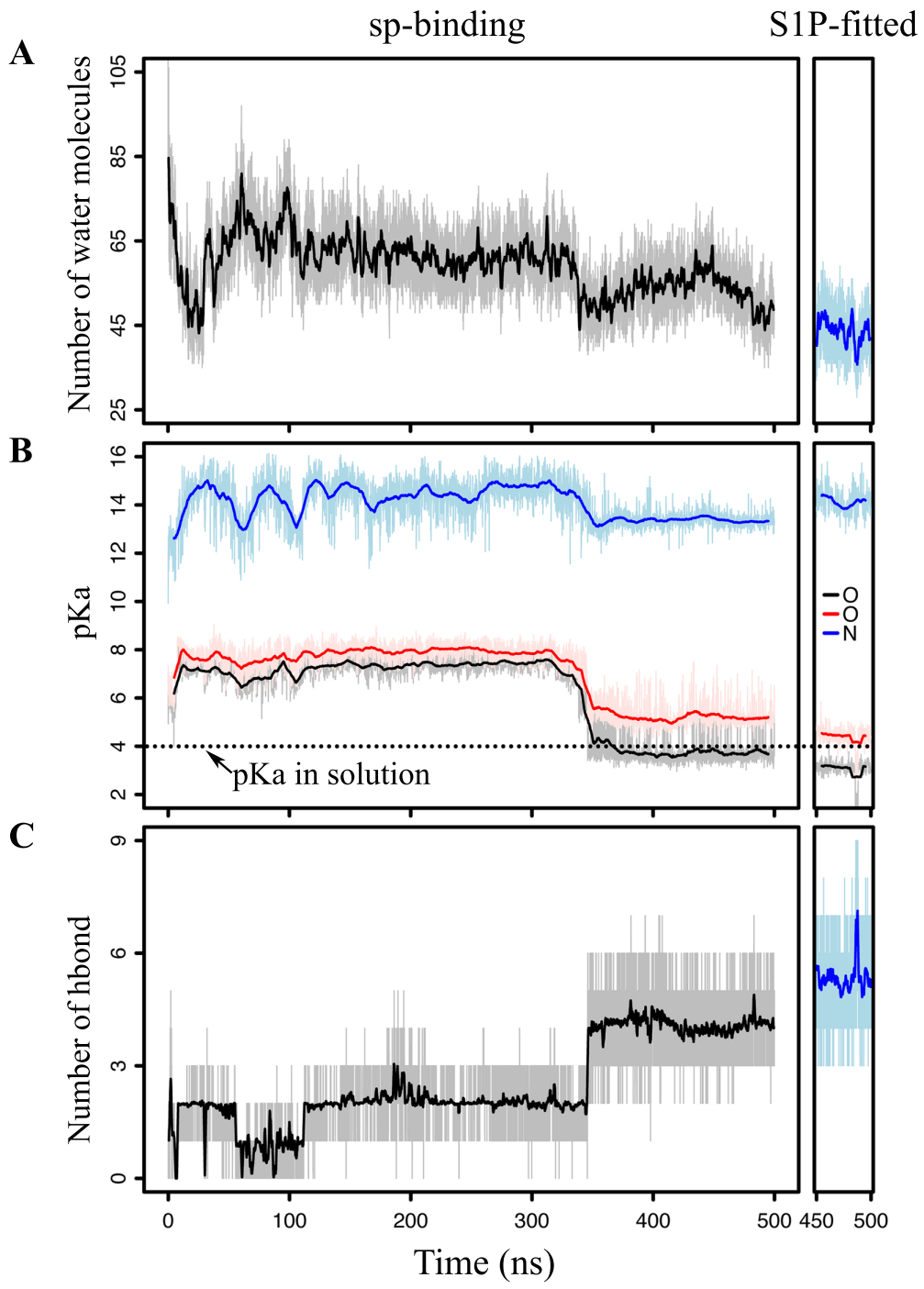

Figure S3. S1P solvation, preferred protonation state, and interactions within the receptor through simulation time. Plots on the left show data from the S1P sp-binding simulation and those on the right show data from the S1P-fitted simulation. (**A**) Number of water molecules within 4 Å of S1P. (**B**) pKa of S1P's titratable

groups including oxygen atoms from the phosphate moiety (indicated in black and red) and the nitrogen atom from the amino group (shown in blue). The dotted line shows the pKa of the phosphate moiety of S1P in solution. (**C**) Number of hydrogen bonds between S1P and the $S1P_1$ receptor.

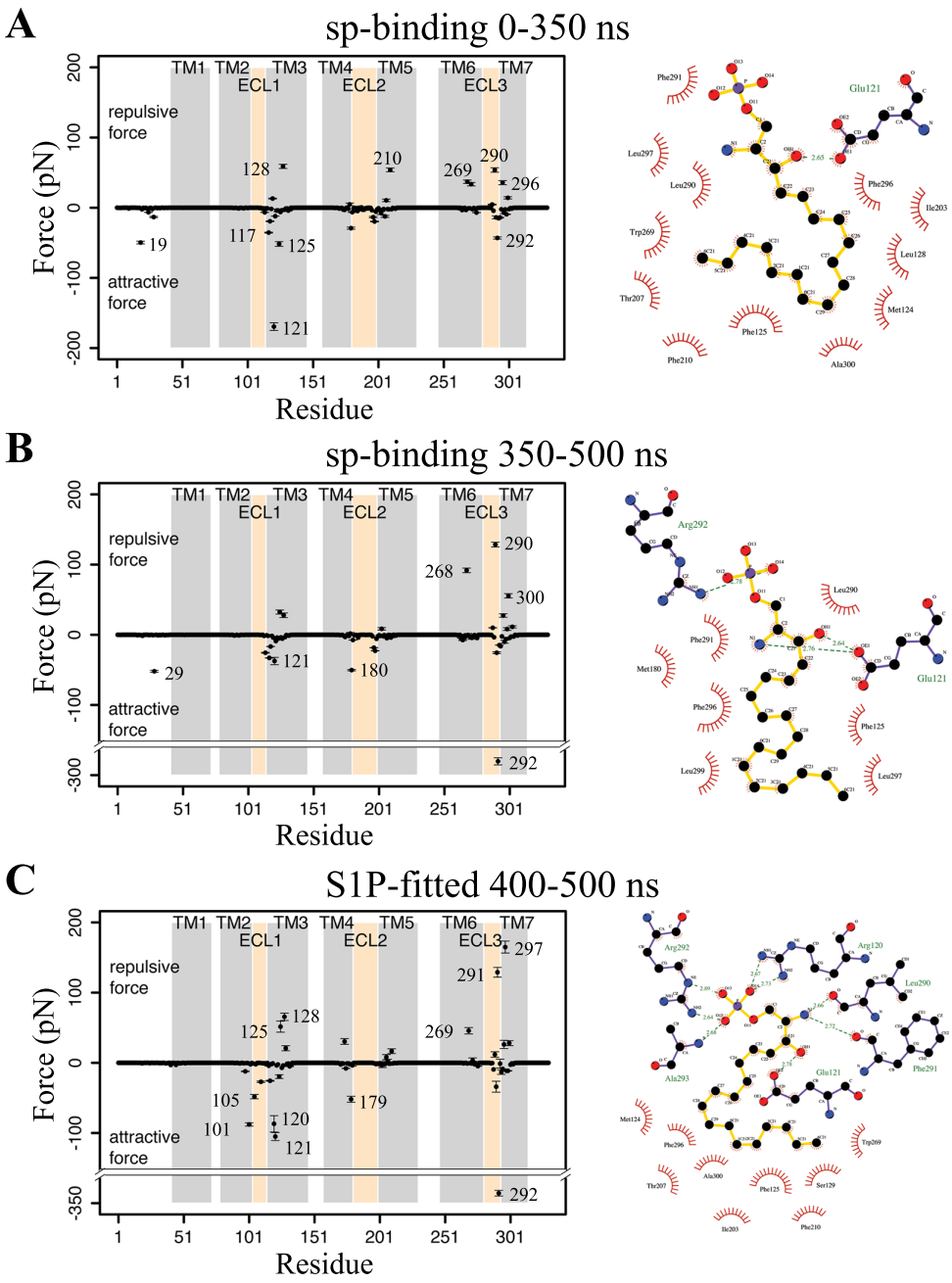

Figure S4. S1P-S1P$_1$ receptor intermolecular interactions. The time-averaged residue-wise forces calculated between S1P and individual residues of the S1P$_1$ receptor are shown on the left. The schematic 2-D representations of the ligand-protein interaction diagram (via LigPlot) are shown on the right for the **A**) sp-binding (0-

350 ns), **B**) sp-binding (350-500 ns), and **C**) S1P-fitted (400-500 ns) simulations. S1P exerts the most forces on the three ECLs and on the upper half of the TM3 helix. In the 2-D plot, hydrogen bonds are shown in green and nonbonded contacts are shown in red. S1P is highlighted by golden sticks, and $S1P_1$ residues by purple sticks. Heavy atoms are color-coded with carbon in black, nitrogen in blue, and oxygen in red.

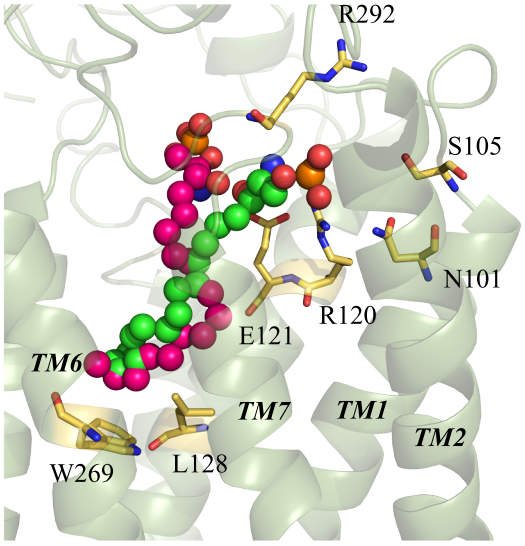

Figure S5. Residues essential for S1P binding. The residues that experienced large intermolecular residue-wise forces between S1P and individual residues of the $S1P_1$ receptor are shown in a zoomed in view of the $S1P_1$ receptor.

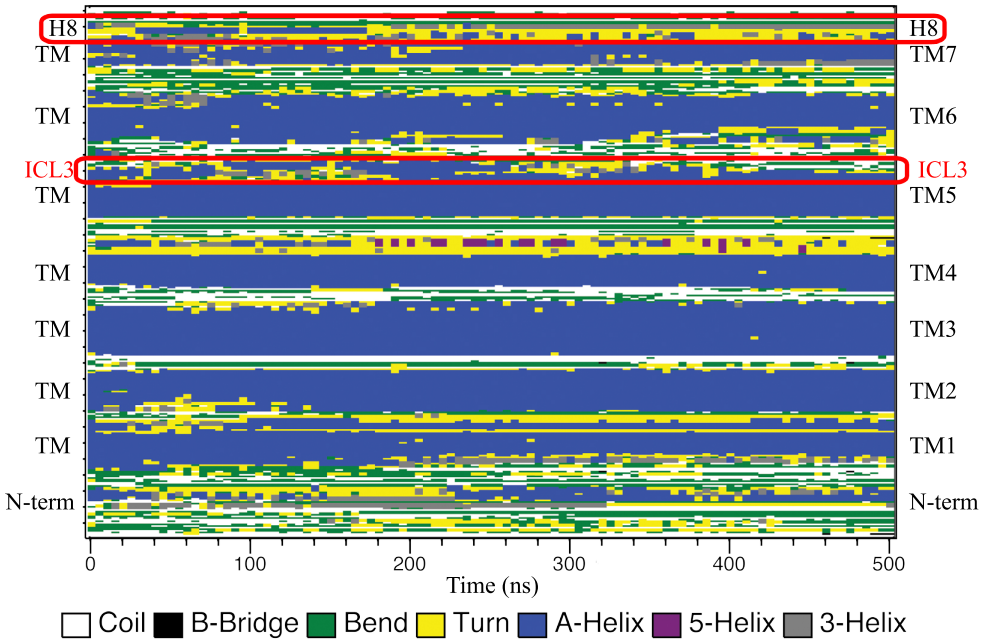

Figure S6. Stability of the S1P$_1$ secondary structure during the sp-binding simulation. Red boxes denote instances where Helix 8 (H8) lost the helical structure after 200 ns in the simulation, and where ICL3 became partially helical over the simulation.