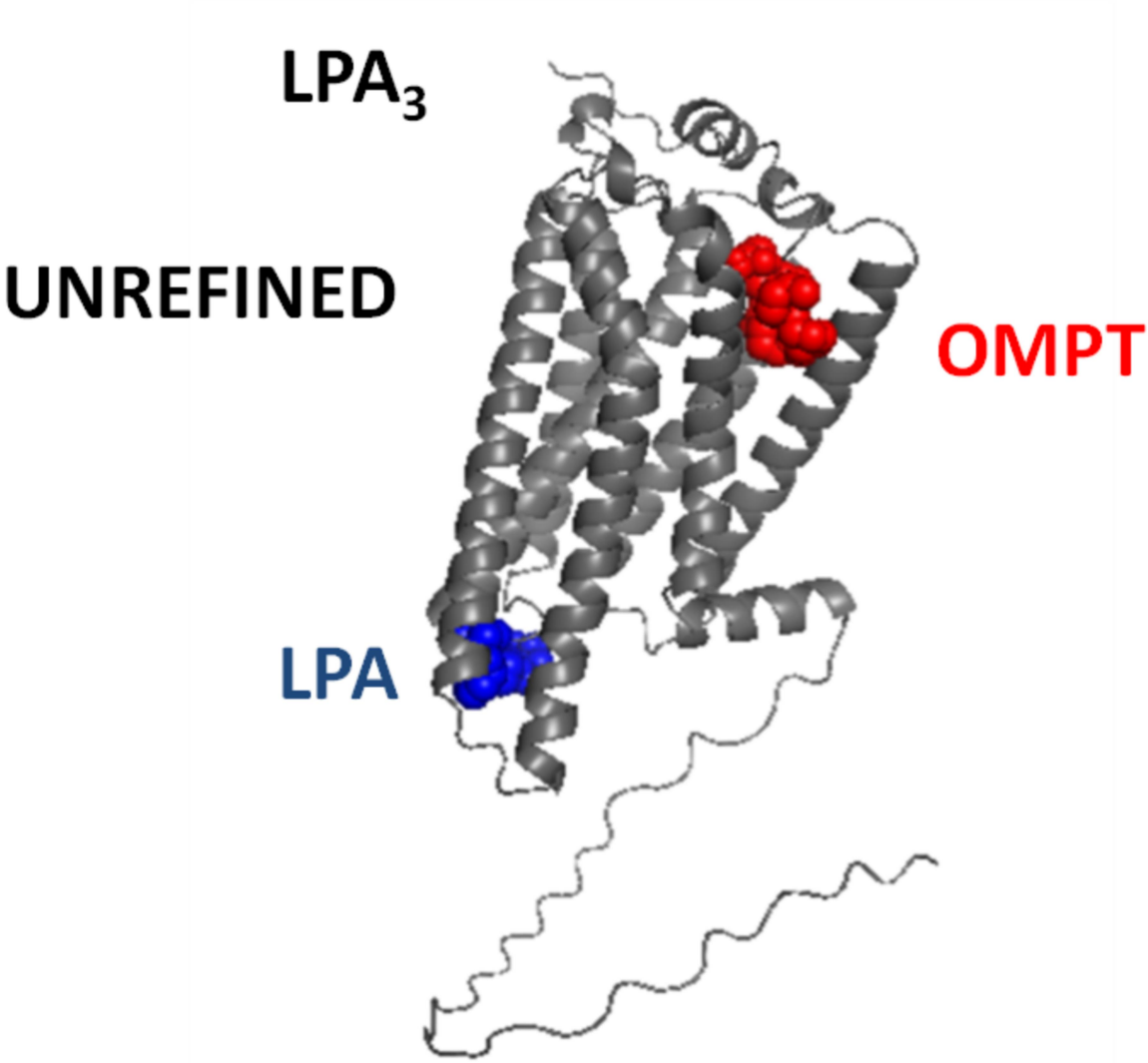
SUPPLEMENTARY MATERIAL

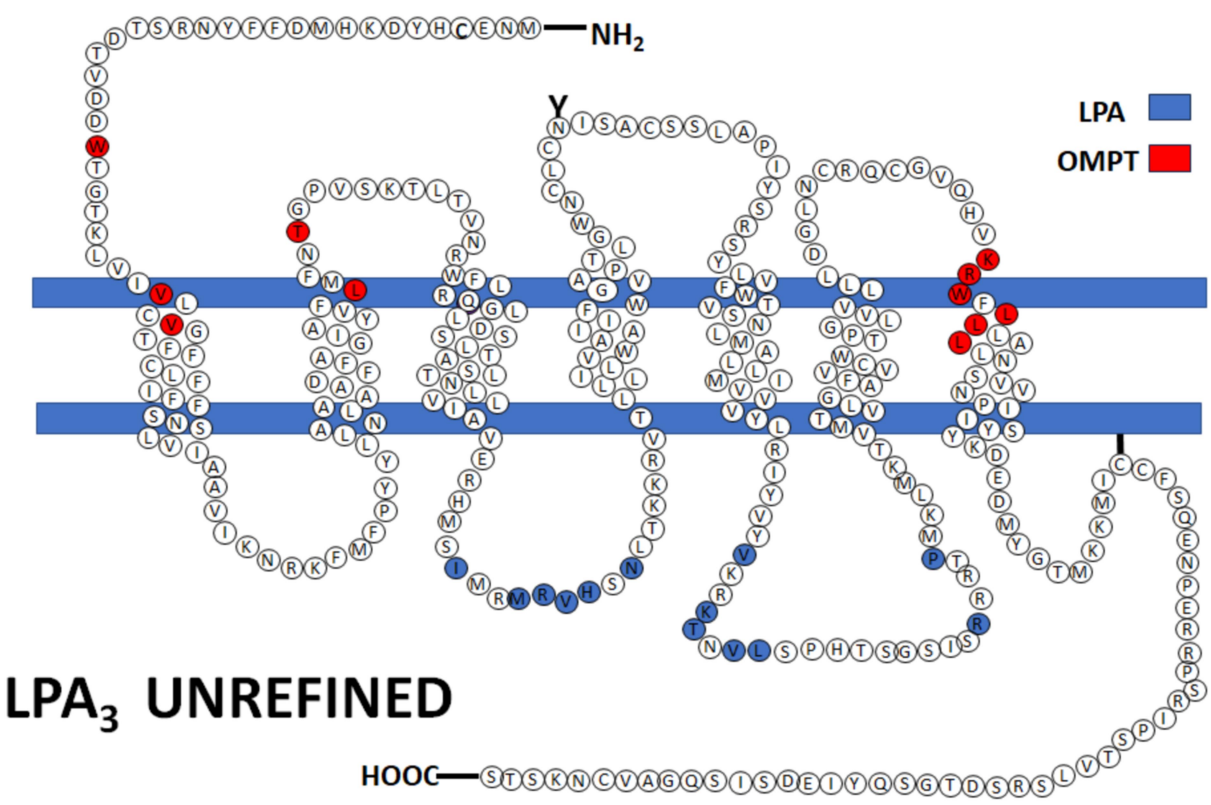
Supplementary Figure S1. Alignment of the FASTA sequence of the LPA_3 receptor (Q9UBY5) with the 3D model programs I-Tasser (model 01.pdb chainA_s001), Swiss Model (model1) and AlphaFold (AF-A v4).



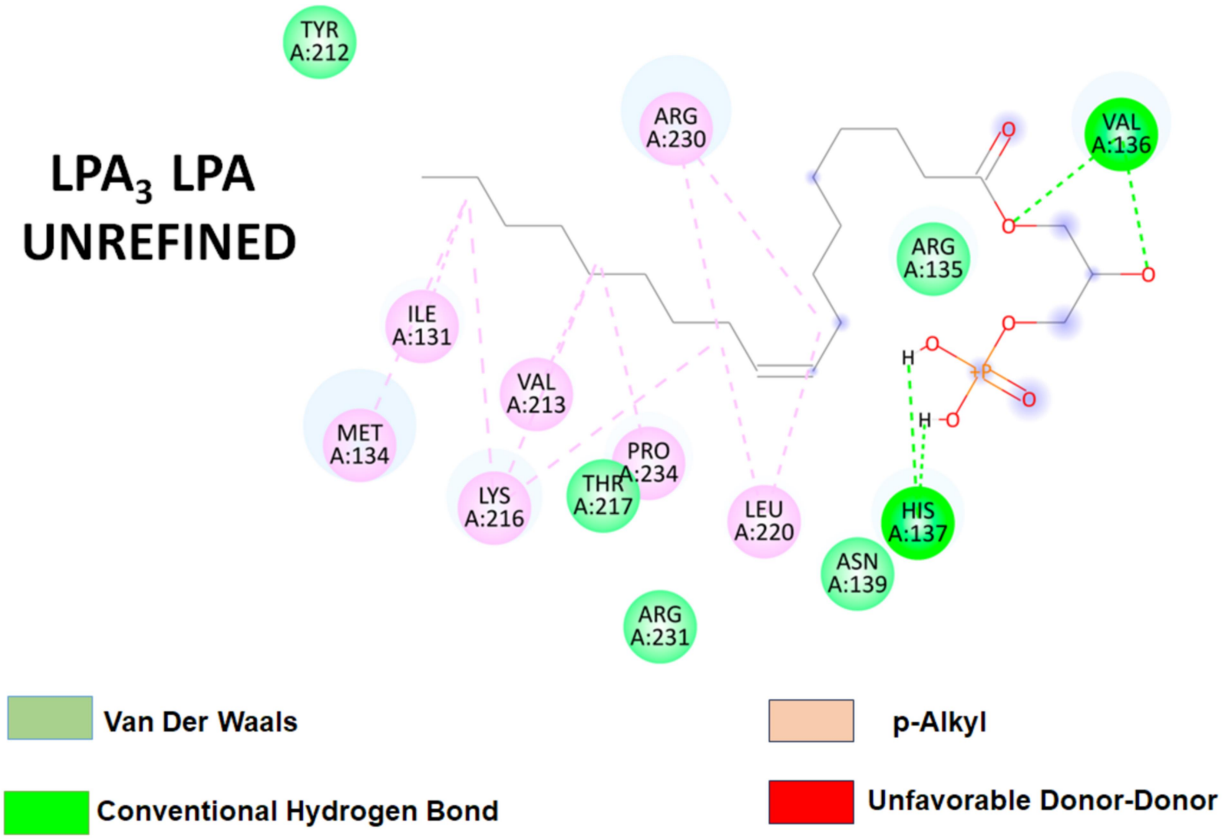
Supplementary Figure S2. Images resulting from the LPA_3-LPA (blue) and LPA_3-OMPT (red) docking analysis using PyMol.



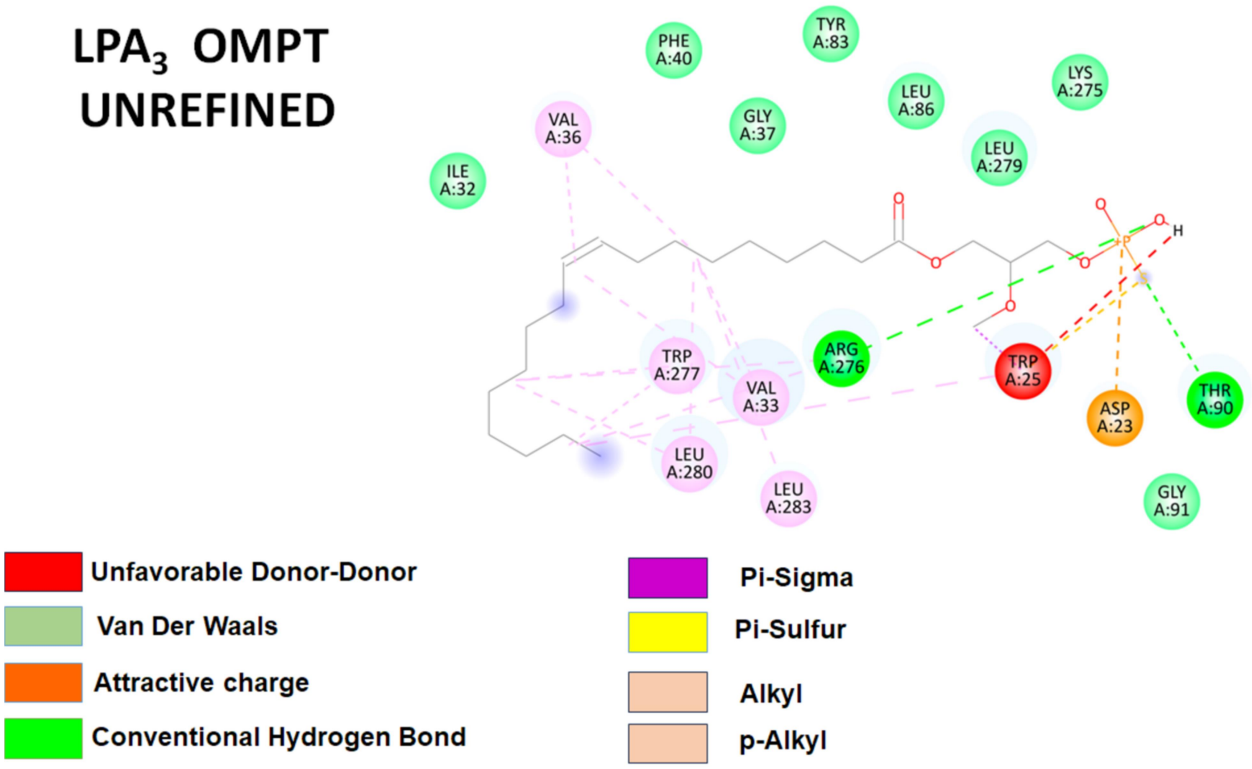
Supplementary Figure S3. Cartoon showing the LPA_3 receptor sites interacting with LPA (blue) and OMPT (red).



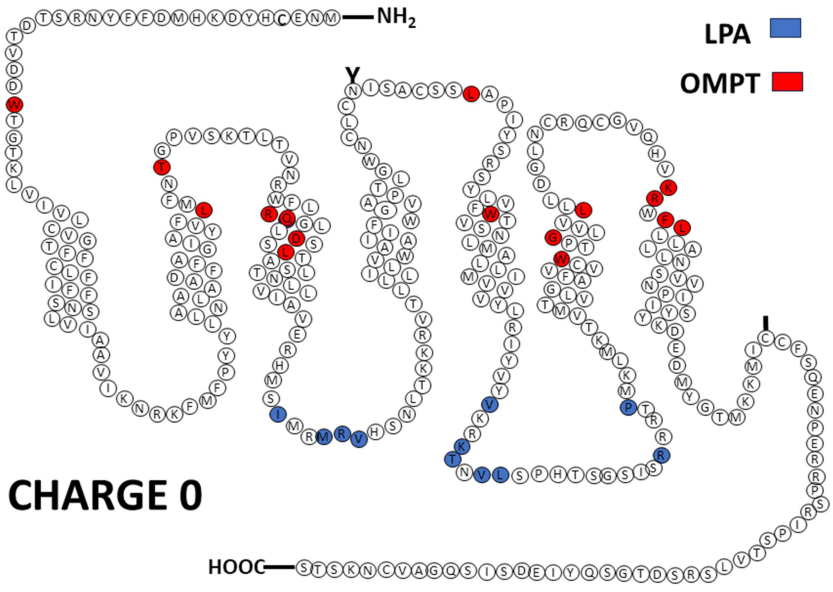
Supplementary Figure S4. The 2D diagram shows interactions observed at the LPA_3 binding site (Lower Cavity) with LPA (Discovery Studio Visualizer); critical amino acids contributing to interactions are shown in circles.

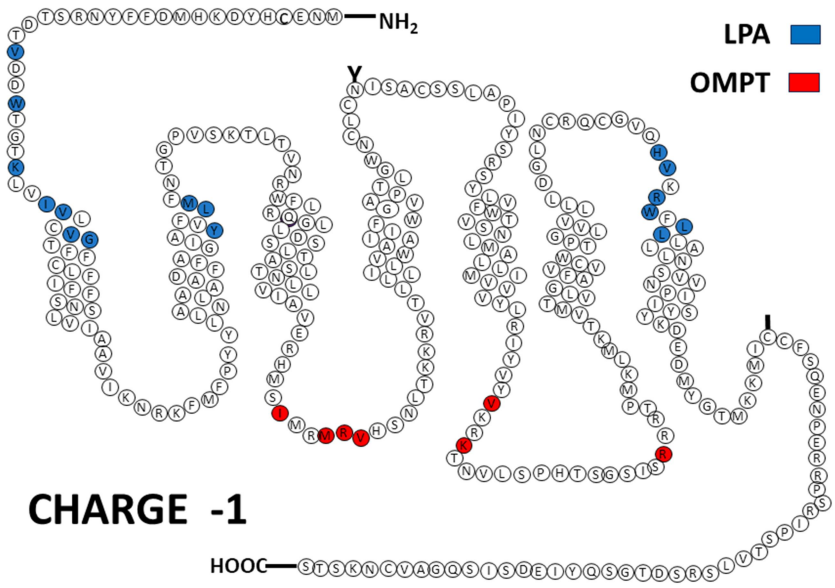


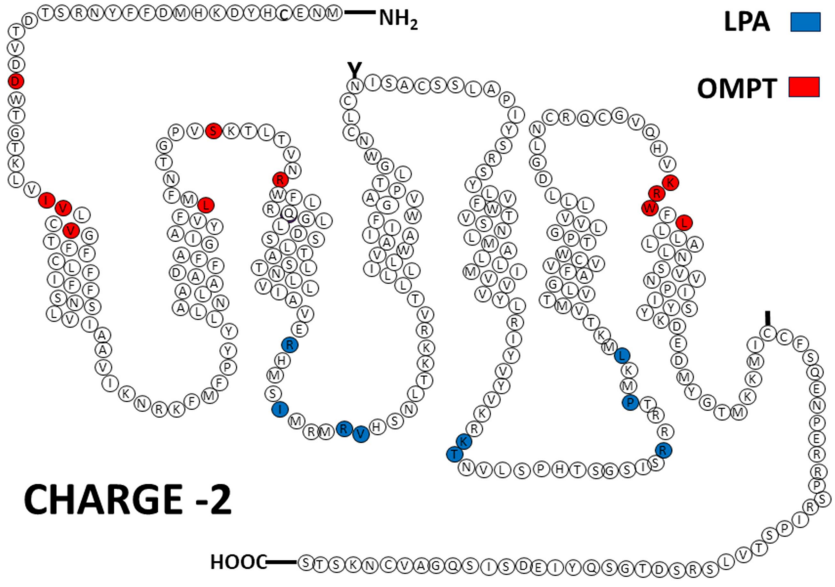
Supplementary Figure S5. The 2D diagram shows interactions at the LPA_3 binding site (Upper Cavity) with OMPT (Discovery Studio Visualizer). Critical amino acids contributing to interactions are shown in circles.



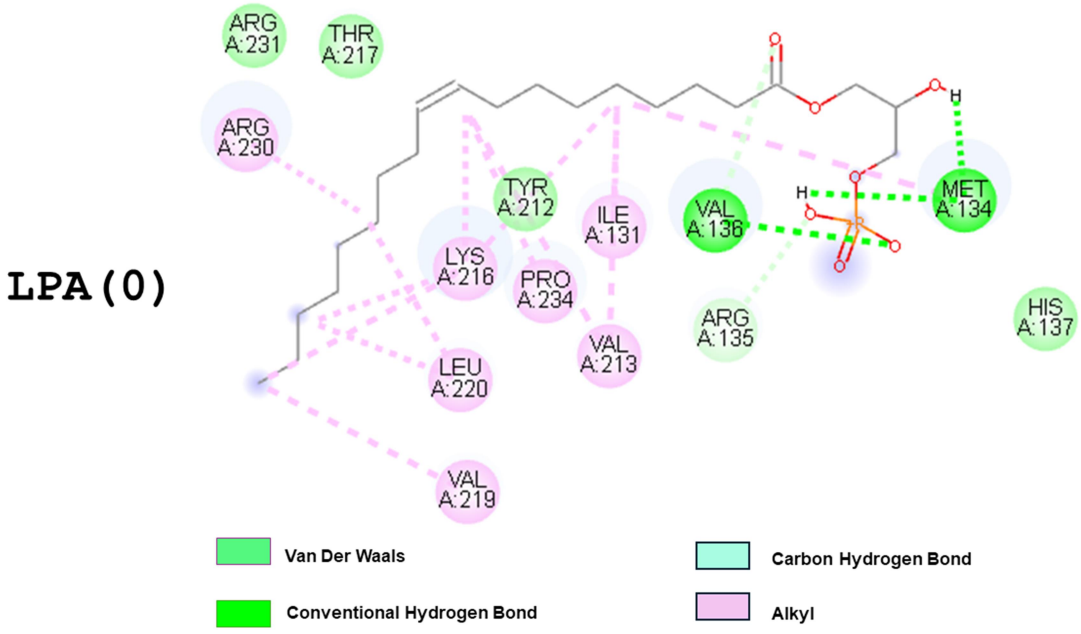
Supplementary Figure S6. Cartoons showing the refined LPA_3 receptor sites interacting with LPA (blue) and OMPT (red). Charges are indicated in the Figures.

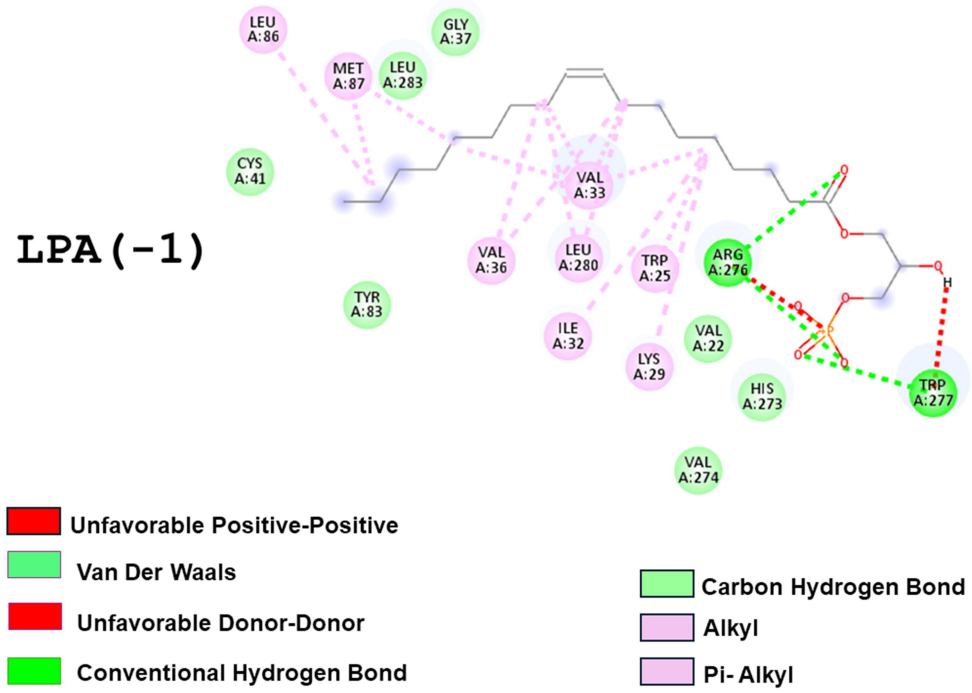



NH2
LPA
OMPT
CHARGE -1
HOOC

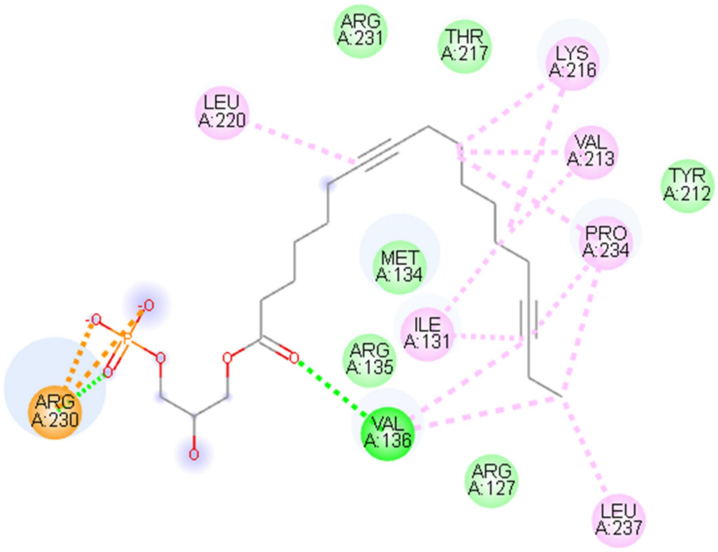

NH2
LPA
OMPT
CHARGE -2
HOOC

Supplementary Figure S7. The 2D diagram shows interactions at the LPA_3 binding site with LPA or OMPT. In the individual images, Ligand and Charge are indicated (Discovery Studio Visualizer). Critical amino acids contributing to interactions are shown in circles.




LPA(-1)
LEU A:86
GLY A:37
MET A:87
LEU A:283
CYS A:41
VAL A:33
VAL A:36
LEU A:280
TRP A:25
ARG A:276
TYR A:83
ILE A:32
VAL A:22
LYS A:29
HIS A:273
TRP A:277
VAL A:274
Unfavorable Positive-Positive
Van Der Waals
Unfavorable Donor-Donor
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Alkyl
Pi-Alkyl

LPA(-2)



Van Der Waals

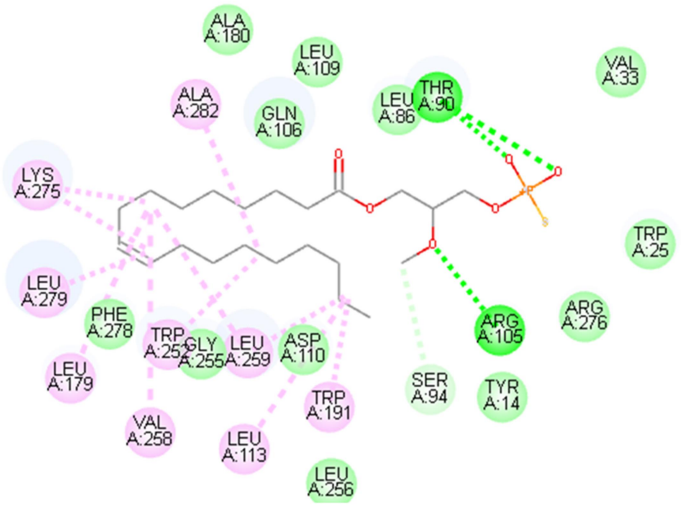
Attractive Charge

Conventional Hydrogen Bond

Carbon Hydrogen Bond

Alkyl

OMPT (0)



Van Der Waals

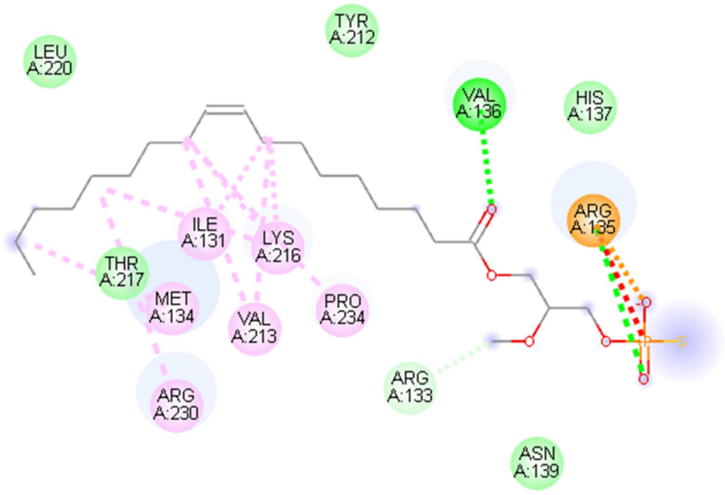
Conventional Hydrogen Bond

Carbon Hydrogen Bond

Alkyl

Pi-Alkyl

OMPT (-1)



Van Der Waals

Salt Bridge

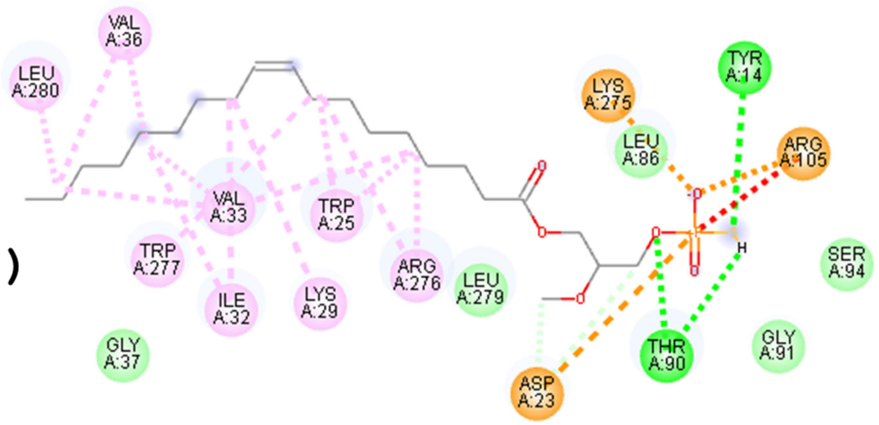
Conventional Hydrogen Bond

Carbon Hydrogen Bond

Unfavorable Positive-Positive

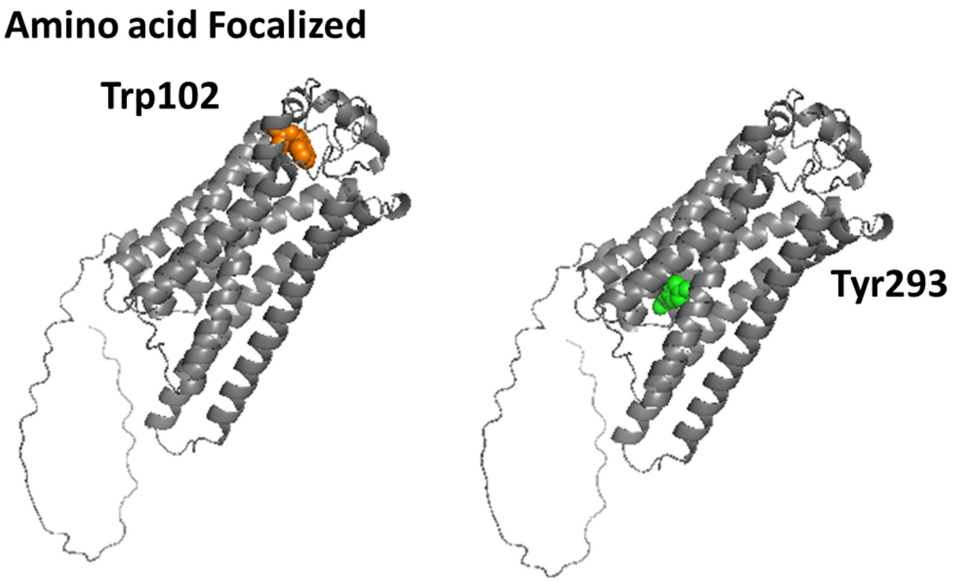
Alkyl

OMPT(-2)

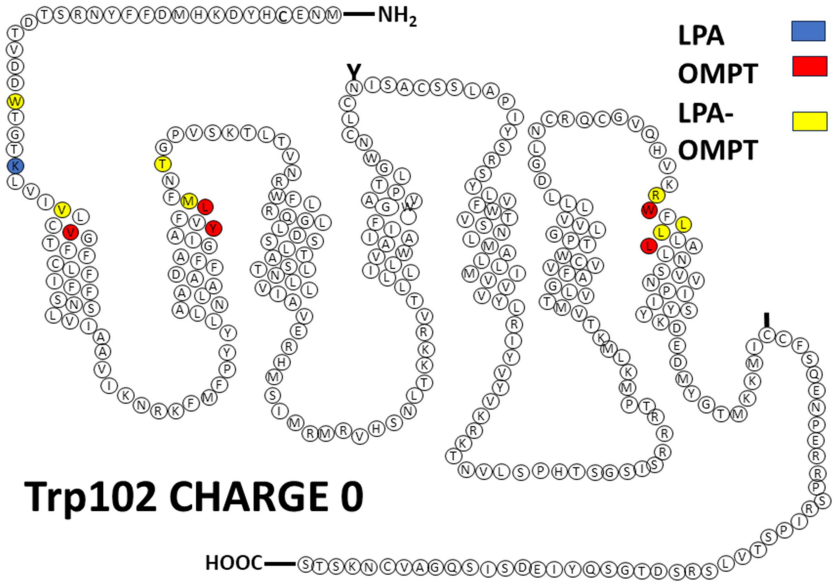


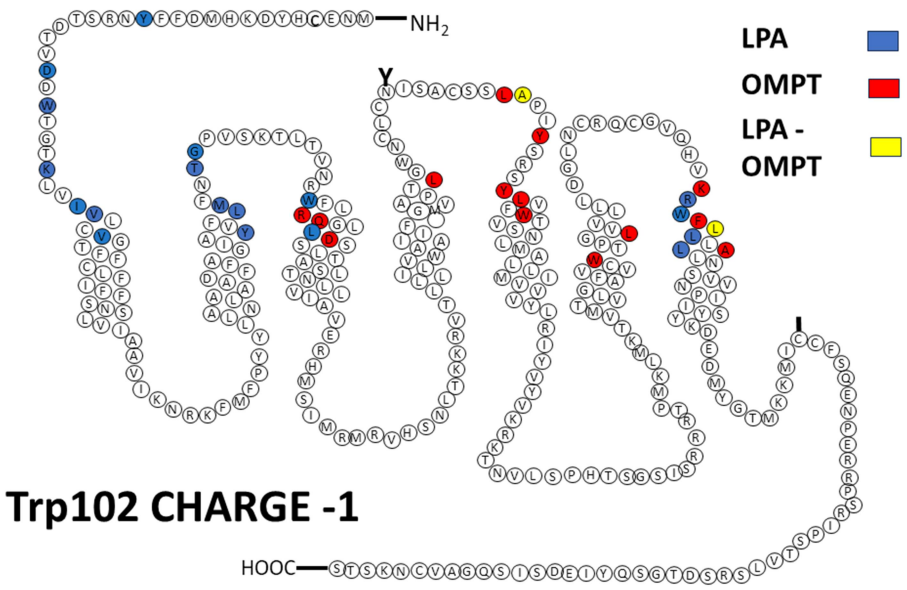
- Van Der Waals
- Salt Bridge
- Attractive Charge
- Conventional Hydrogen Bond
- Carbon Hydrogen Bond
- Unfavorable Positive-Positive
- Alkyl
- Pi-Alkyl

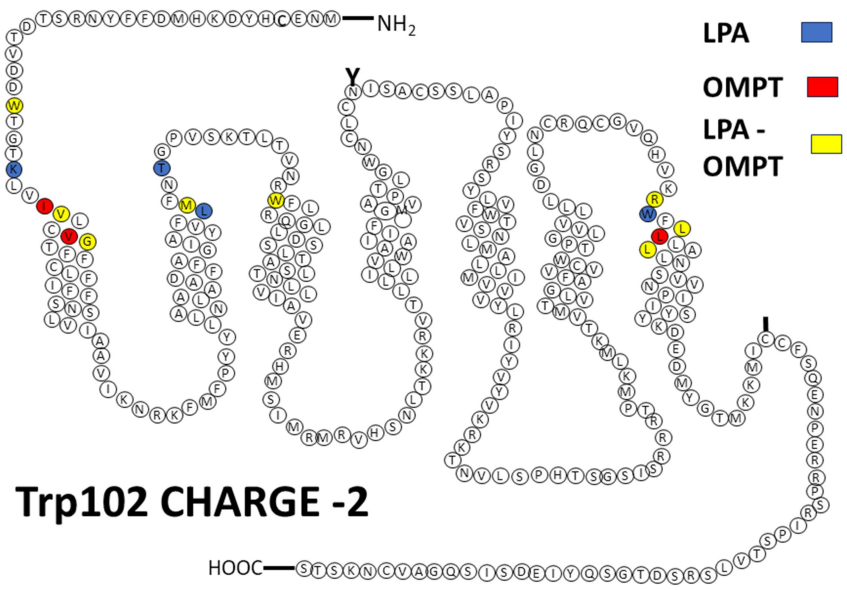
Supplementary Figure S8. Localization of the amino acids at the LPA_3 receptor to be focused. Trp102, orange; Tyr293, green.



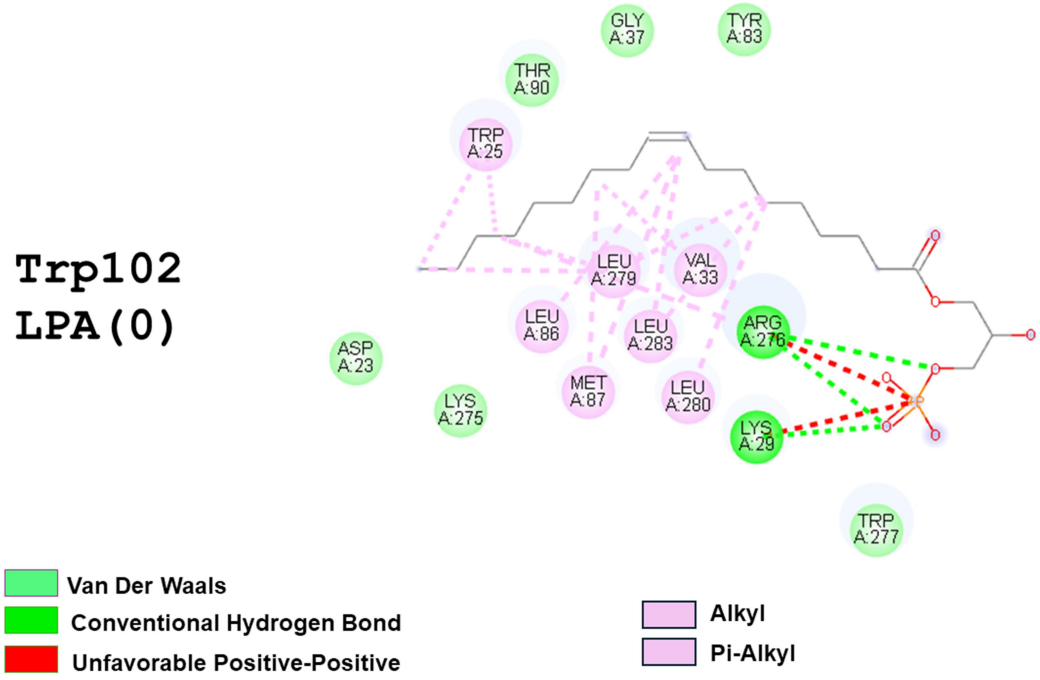
Supplementary Figure S9. Cartoons showing the LPA_3 receptor (Trp102-focused) sites interacting with LPA (blue) and OMPT (red). Charges are indicated in the Figures.

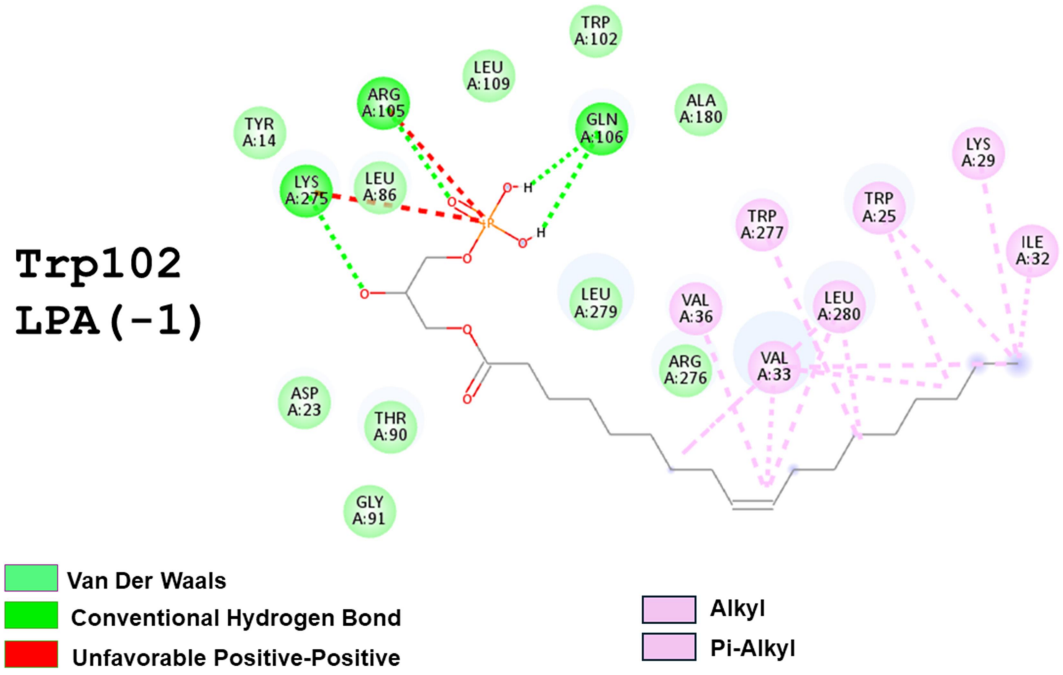



LPA
OMPT
LPA - OMPT
Trp102 CHARGE -1

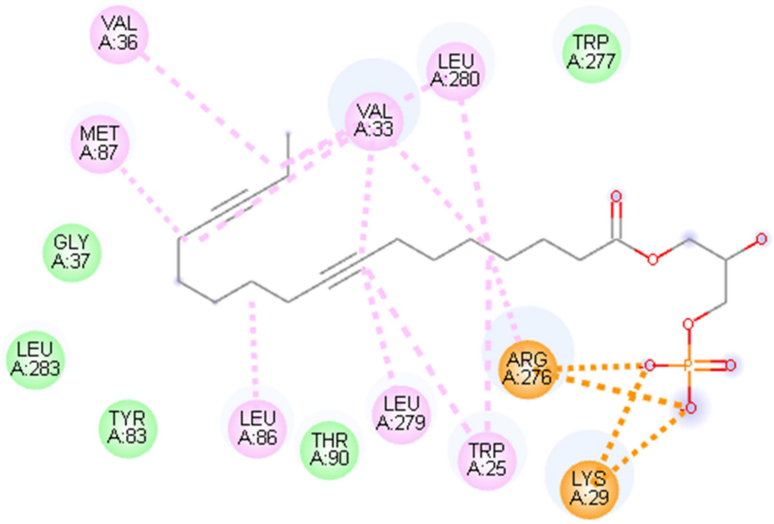

LPA
OMPT
LPA - OMPT
NH2
HOOC
Trp102 CHARGE -2

Supplementary Figure S10. The 2D diagram shows interactions of the LPA_3 (Trp102) structure with LPA or OMPT. In the individual images, Ligand and Charge are indicated (Discovery Studio Visualizer). Critical amino acids contributing to interactions are shown in circles.



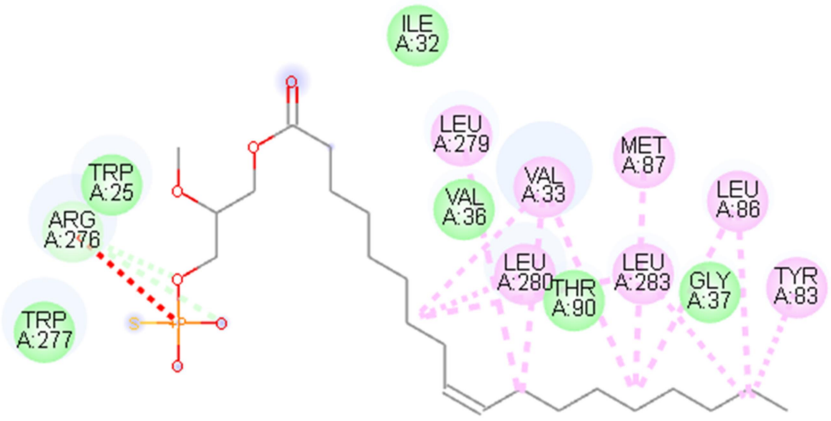

Trp102
LPA(-1)
TRP A:102
LEU A:109
ARG A:105
ALA A:180
TYR A:14
GLN A:106
LYS A:29
LYS A:275
LEU A:86
TRP A:277
TRP A:25
ILE A:32
LEU A:279
VAL A:36
LEU A:280
ARG A:276
VAL A:33
ASP A:23
THR A:90
GLY A:91
Van Der Waals
Conventional Hydrogen Bond
Unfavorable Positive-Positive
Alkyl
Pi-Alkyl

Trp102
LPA(-2)



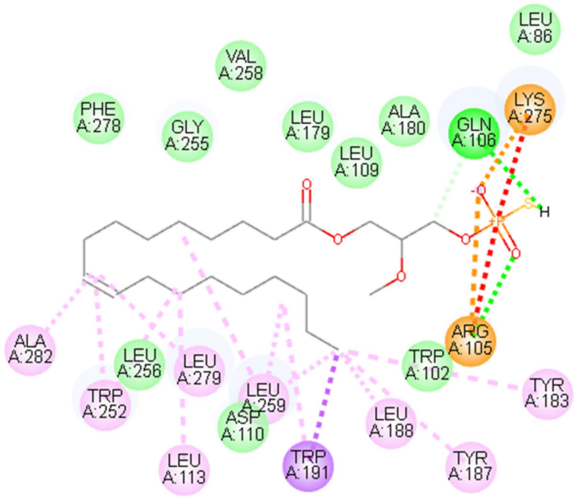
Van Der Waals
Salt Bridge
Attractive Charge
Carbon Hydrogen Bond
Alkyl
Pi-Alkyl

Trp102
OMPT(0)



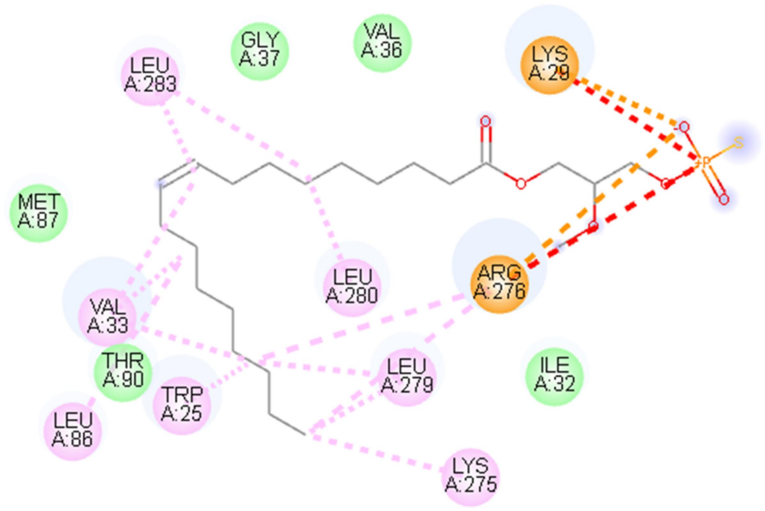
Van Der Waals
Carbon Hydrogen Bond
Unfavorable Positive-Positive
Alkyl
Pi-Alkyl

Trp102
OMPT(-1)



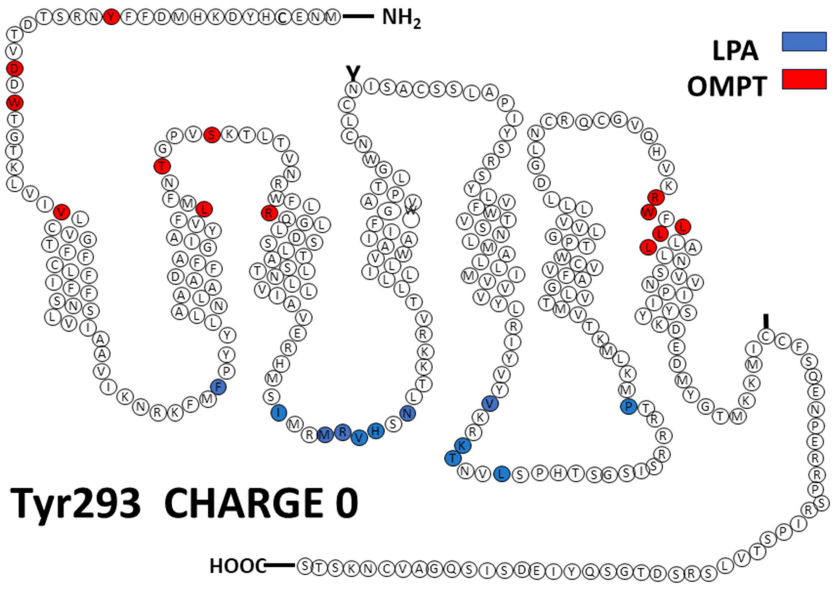
- Van Der Waals
- Attractive Charge
- Conventional Hydrogen Bond
- Carbon Hydrogen Bond
- Unfavorable Positive-Positive
- Pi-Sigma
- Alkyl
- Pi-Alkyl

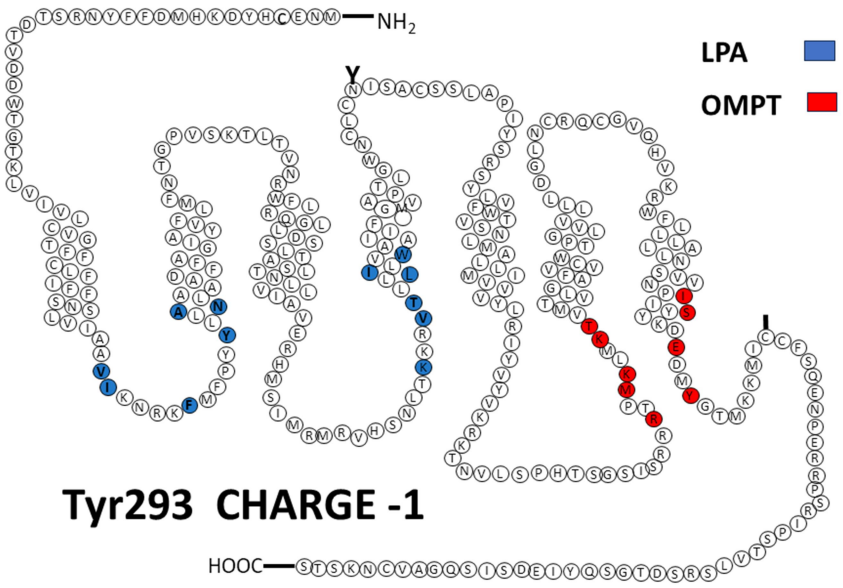
Trp102
OMPT(-2)

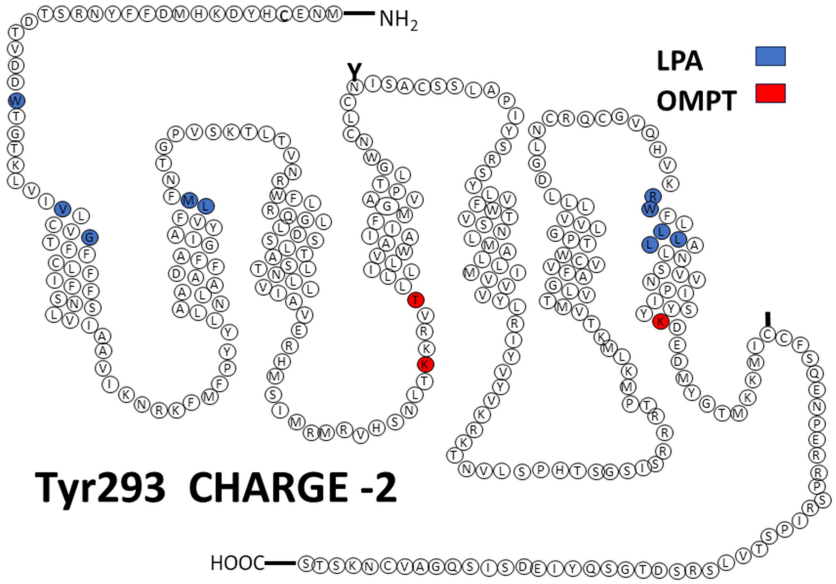


Van Der Waals
Salt Bridge
Attractive Charge
Carbon Hydrogen Bond
Alkyl
Pi-Alkyl

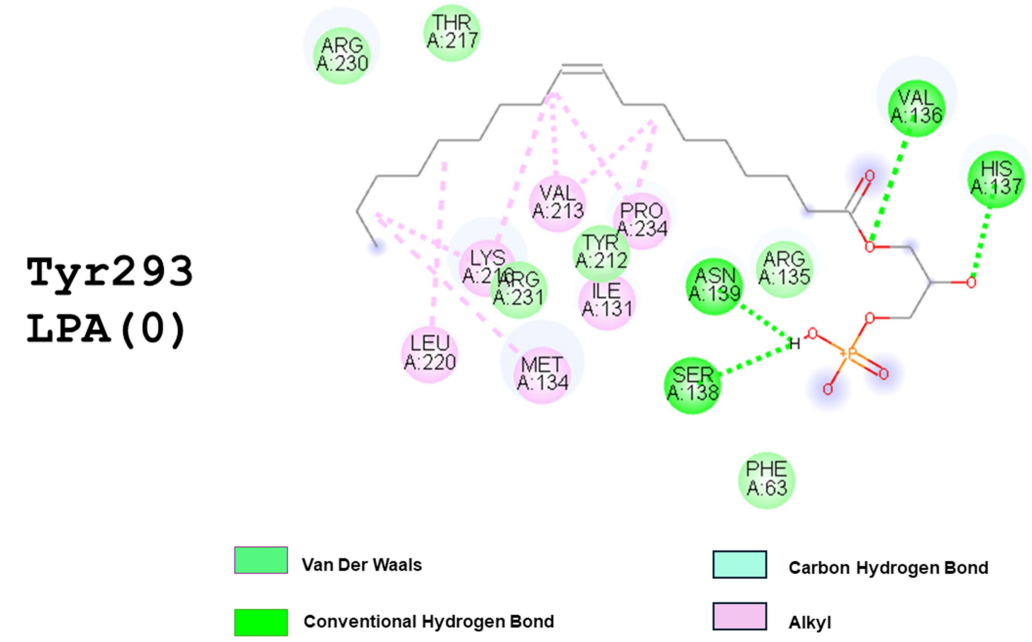
Supplementary Figure S11. Cartoons showing the LPA_3 receptor (Tyr293-focused) sites interacting with LPA (blue) and OMPT (red). Charges are indicated in the Figures.



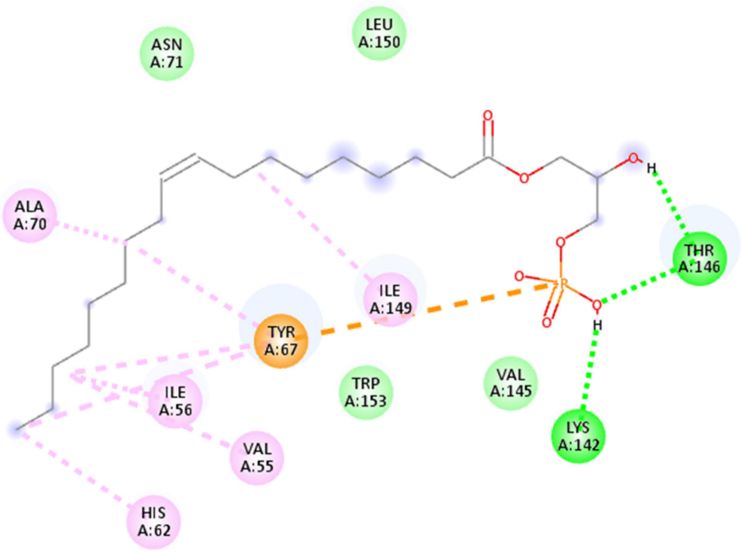

LPA
OMPT
Tyr293 CHARGE -1
HOOC
NH2


NH2
LPA
OMPT
Tyr293 CHARGE -2
HOOC

Supplementary Figure S12. The 2D diagram shows interactions of the LPA_3 (Tyr293) structure with LPA or OMPT. In the individual images, Ligand and Charge are indicated (Discovery Studio Visualizer). Critical amino acids contributing to interactions are shown in circles.

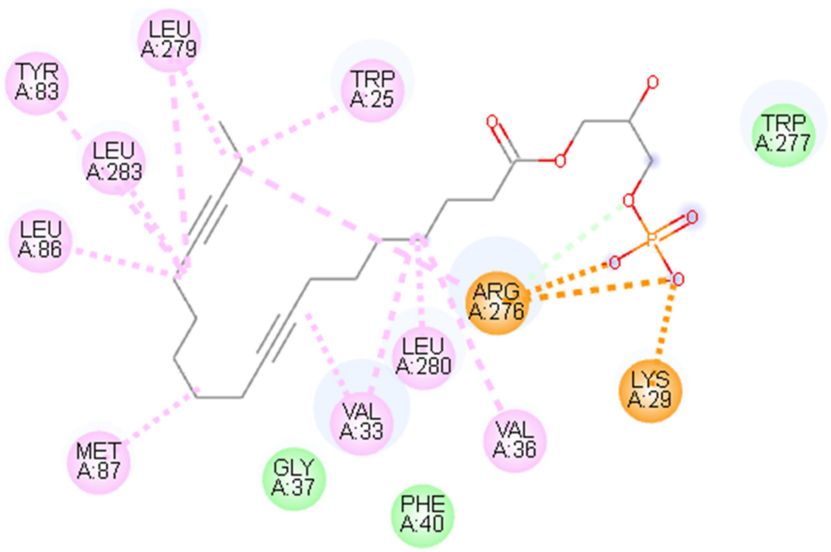


Tyr293
LPA(-1)



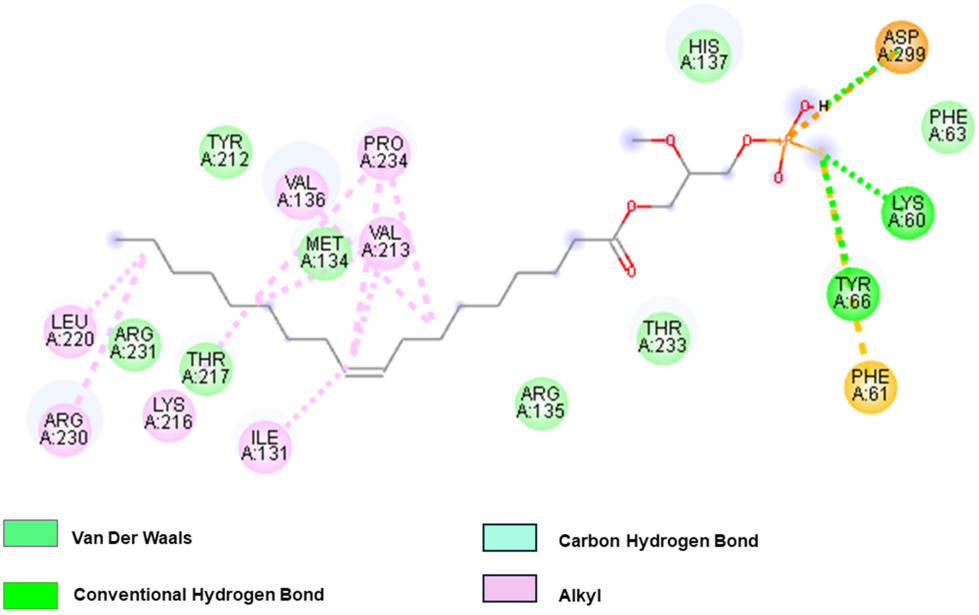
Van Der Waals
Conventional Hydrogen Bond
Pi-Cation
Alkyl
Pi-Alkyl

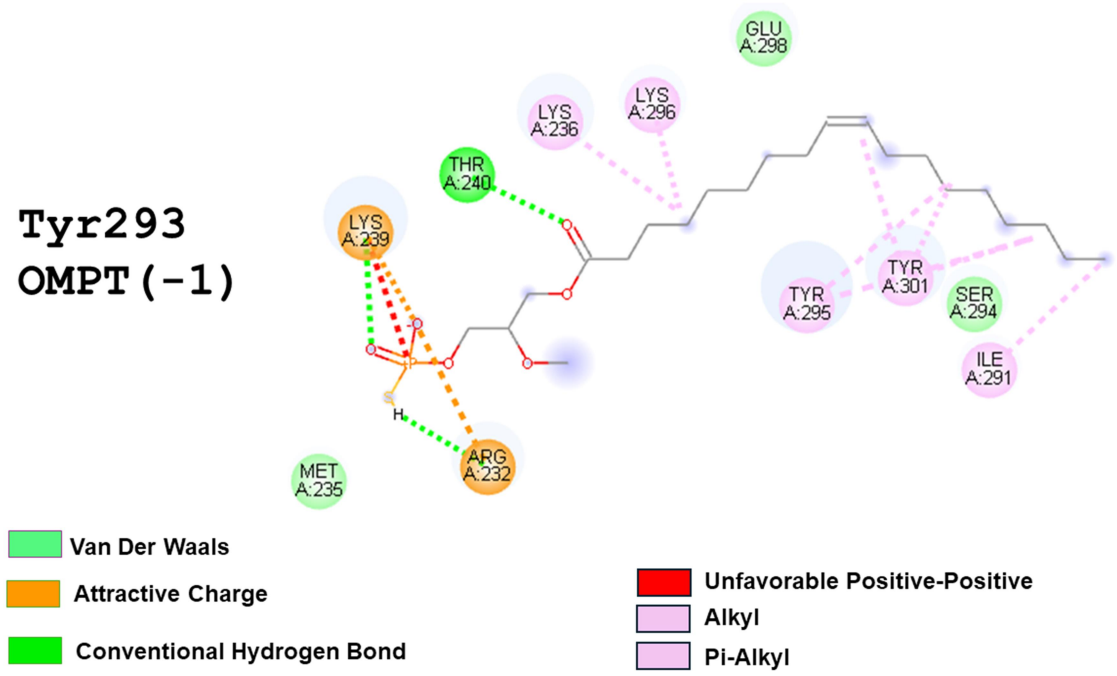
Tyr293
LPA(-2)

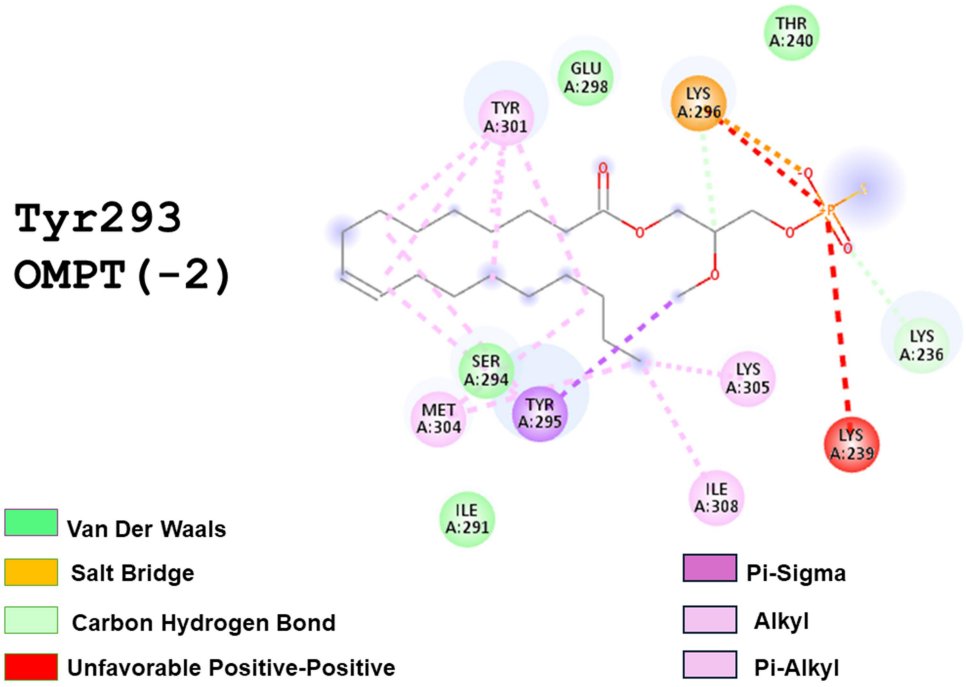


Van Der Waals
Salt Bridge
Attractive charge
Carbon Hydrogen Bond
Pi-Alkyl
Alkyl

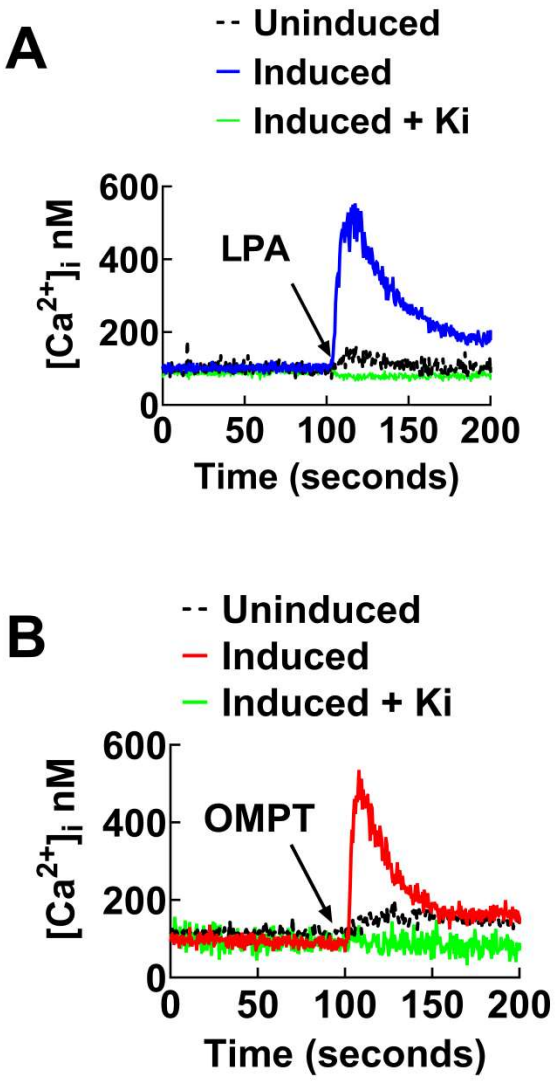
Tyr 293
OMPT(0)



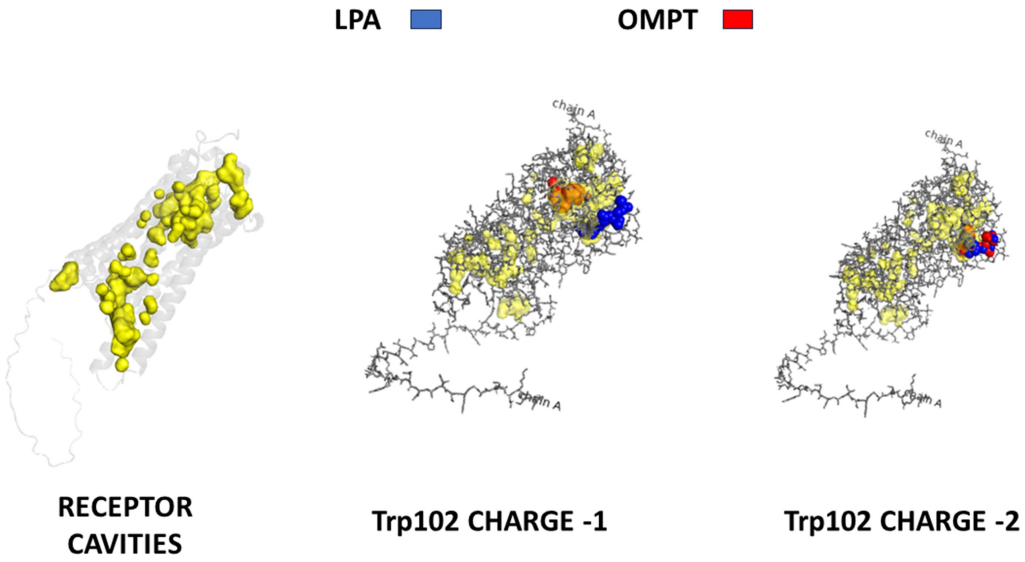

Tyr293
OMPT(-1)
GLU A:298
LYS A:296
LYS A:236
THR A:240
LYS A:239
TYR A:301
TYR A:295
SER A:294
ILE A:291
MET A:235
ARG A:232
Van Der Waals
Attractive Charge
Conventional Hydrogen Bond
Unfavorable Positive-Positive
Alkyl
Pi-Alkyl


Tyr293
OMPT(-2)
THR A:240
GLU A:298
LYS A:296
TYR A:301
LYS A:236
SER A:294
LYS A:305
MET A:304
TYR A:295
LYS A:239
ILE A:308
ILE A:291
Van Der Waals
Salt Bridge
Carbon Hydrogen Bond
Unfavorable Positive-Positive
Pi-Sigma
Alkyl
Pi-Alkyl

Supplementary Figure S13. Calcium tracings of cells transfected with the LPA_3 receptors that were or were not induced. In panel A, cells were stimulated with 1 μM LPA, whereas in panel B, 1 μM OMPT was the stimulus. Where indicated, 1 μM Ki16425 was added before the agonist. Data are representative of 3 experiments performed on different days with essentially identical results.



Supplementary Figure S14. LPA_3 receptor cavities were evidenced using PyMol. In the left panel, the cavities found are indicated in yellow. In the middle (Trp102-focused, Charge -1) and right (Trp102-focused, Charge -1) panel, the position of the ligands (LPA, blue, and OMPT, red) are indicated. The change in ligand color is due to merging with the cavity's yellow color.



Supplementary Table S1. Structural evaluation of the 3D models generated using the three most frequently used homology modeling servers (in green, the best values).

Server	Supplem	MolProbity: Protein Geometry/ Ramachandran favored	SAVESv6.0: ERRAT	SAVESv6.0: VERIFY
SWISS-MODEL		91.45%	94.8052	33.14%
I-TASSER		78.92%	83.4302	38.81%
AlphaFold		95.18%	94.3038	29.01%

Supplementary Table S2. Blind docking for LPA and OMPT at the unrefined LPA_3 receptor structure with a 126 Å3 grid box centered on the protein.

Ligand	ΔG (kcal/mol)	Sites of ligand-receptor interaction
LPA	-3.98	Leu220, Thr217, Arg230, Lys216, Val213, Met134, Ile131, Arg135, Pro234, Asn139, Val136, His137
OMPT	-5.31	Leu86, Leu283, Leu279, Leu280, Lys275, Val33, Val36, Thr90, Trp25, Arg276, Trp277