Electronic Supplementary Material (ESI) for RSC Advances.

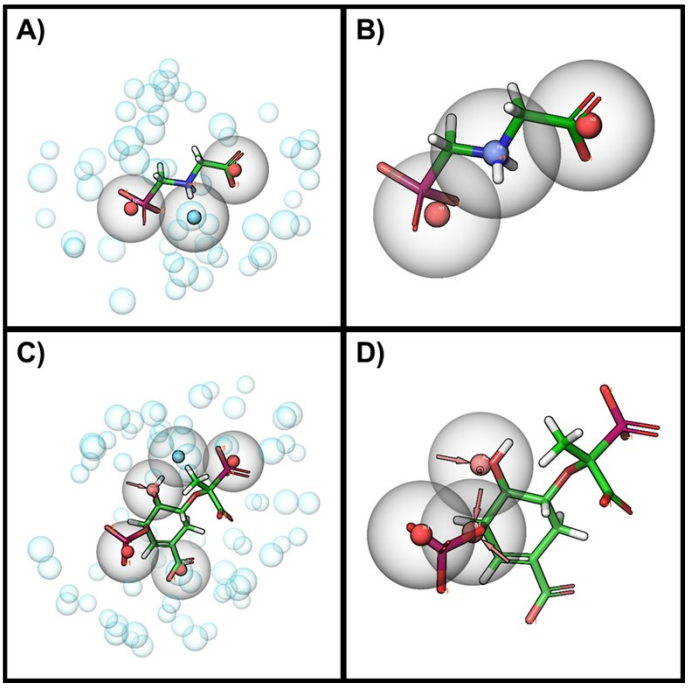

Figure S1. Pharmacophore models considered in this study. A) pharmacophore hypothesis generated from the glyphosate-EPSPS interactions (PDB ID: 3FJZ); B) pharmacophore model based on the overlay of multiple active conformations of glyphosate (PDB IDs: 3FJZ, 2QFU, 2GGA, 2AAY, 1G6S, 3NVS, 3SLH, 1RF6); C) pharmacophore hypothesis built from the intermediate state-like inhibitor (ISLI)-EPSPS interactions (PDB ID: 2PQC); D) pharmacophore model based on the overlay of multiple active conformations of ISLI (PDB IDs: 2PQC, 2PQD). Light blue sphere indicates H-bond donors, light red – H-bond acceptor, blue – positive ionic group, red – negative ionic group. All models were visualized in Schrodinger Maestro 12.6.

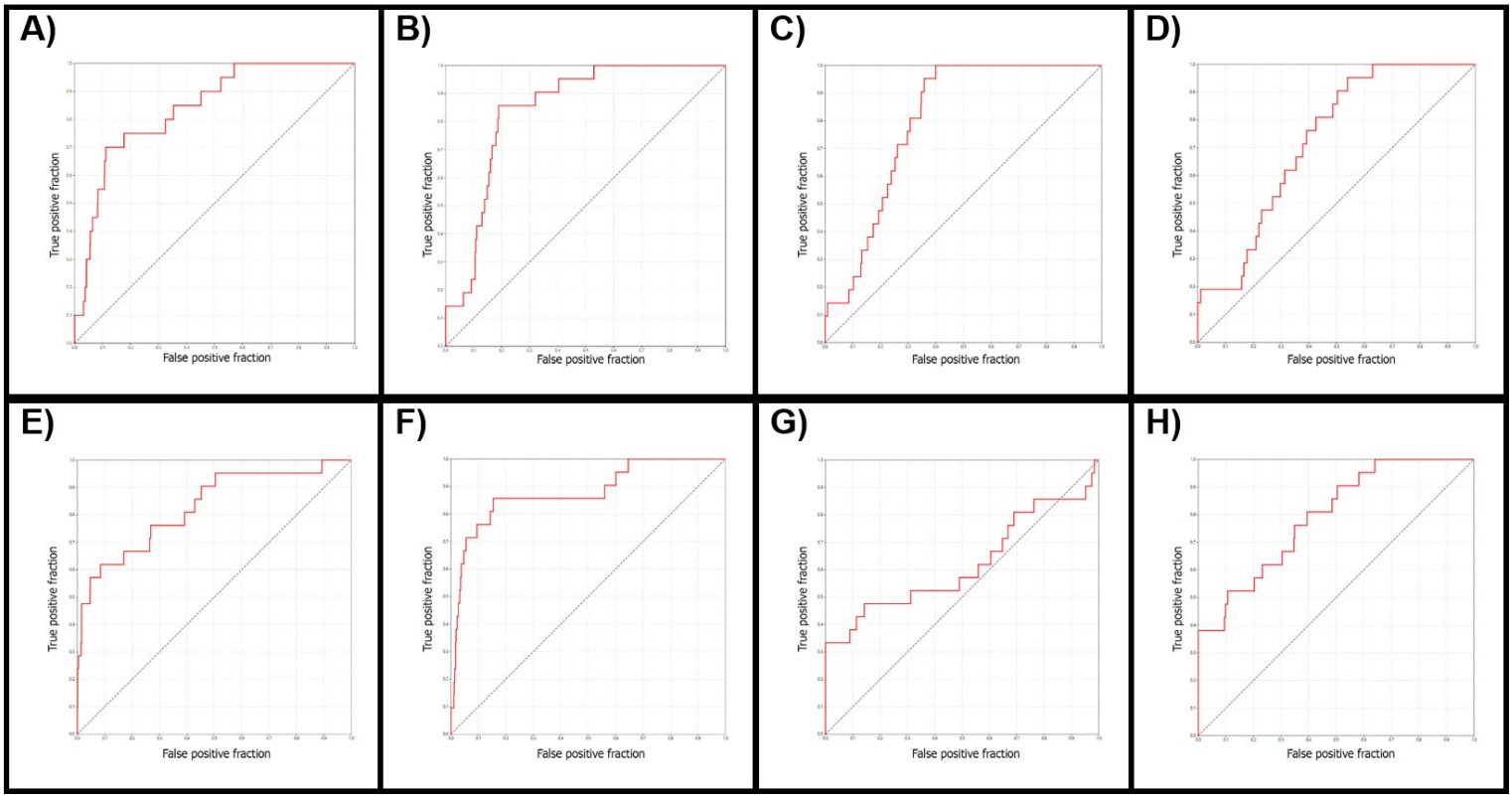

Figure S2. ROC curves built for the different pharmacophore models to estimate their screening performance and make prediction about their future performance in actual screening. A) and B) ROC curves for the glyphosate-interaction-based pharmacophore screened against DUDE and RADER validation datasets, respectively; C) and D) ROC curves for the glyphosate active conformations-based pharmacophore screened against DUDE and RADER validation datasets, respectively; E) and F) ROC curves for the ISLI-interaction-based pharmacophore screened against DUDE and RADER validation datasets, respectively; G) and H) ROC curves for the ISLI active conformations-based pharmacophore screened against DUDE and RADER validation datasets, respectively.

Number	ChEMBL id	Name	Inhibitory potency (K_i)
1	CHEMBL95406	(N-Phosphonomethyl-hydrazino)-acetic acid	0.61 μM
2	CHEMBL96010	N-Nitroso-N-(phosphonomethyl)glycine	2.20 μM
3	CHEMBL97331	2-{3-carboxy-5-[carboxy(phosphono)methoxy]phenoxy}propanedioic acid	1.30 μM
4	CHEMBL97756	2-[methyl(phosphonomethyl)amino]acetic acid	78 μM
5	CHEMBL98618	{[(2-methoxy-2-oxoethyl)amino]methyl}phosphonic acid	1.00 μM
6	CHEMBL98868 (CHEMBL95764)	2-[(phosphonomethyl)amino]acetic acid (glyphosate)	0.16-52 μM
7	CHEMBL1627013 (CHEMBL1159978)	(3R,4S,5R)-5-[(1S)-1-carboxy-1-phosphonoethoxy]-4-hydroxy-3-(phosphonooxy)cyclohex-1-ene-1-carboxylic acid	0.11-5 nM
8	CHEMBL609383	(3R,4S,5R)-5-[(carboxymethyl)(phosphonomethyl)amino]-4-hydroxy-3-(phosphonooxy)cyclohex-1-ene-1-carboxylic acid	7.4-13.0 μM

9	CHEMBL1627015 (CHEMBL1206855)	(3R,4S,5R)-5-[(1S)-1-carboxy-2,2-difluoro-1-(phosphonooxy)ethoxy]-4-hydroxy-3-(phosphonooxy)cyclohex-1-ene-1-carboxylic acid	4 nM
10	CHEMBL337302	(2E)-6,7-dihydroxy-2-[(2-hydroxy-4-methoxyphenyl)methylidene]-1-benzofuran-3-one	0.10 μM
11	CHEMBL334500	(2E)-2-{[4-(diethylamino)phenyl]methylidene}-6,7-dihydroxy-1-benzofuran-3-one	0.65 μM
12	CHEMBL330146	2-[hydroxy(phosphonomethyl)amino]acetic acid	0.65 μM
13	CHEMBL329115	2-[3-carboxy-5-(dicarboxymethoxy)phenoxy]propanedioic acid	2.50 μM
14	CHEMBL318618	(3R,4S,5R)-5-(carboxymethoxy)-4-hydroxy-3-(phosphonooxy)cyclohex-1-ene-1-carboxylic acid	1.50 μM
15	CHEMBL313253	3-[carboxylato(hydrogen phosphonato)methoxy]-5-(hydrogen phosphonatooxy)benzoic acid	0.16 μM
16	CHEMBL287334	(3R,4S,5R)-5-(carboxylatomethoxy)-3-(hydrogen phosphonatooxy)-4-hydroxycyclohex-1-ene-1-carboxylic acid	1.50 μM
17	CHEMBL1627011 (CHEMBL1205445)	3-[carboxy(phosphono)methoxy]-5-(phosphonooxy)benzoic acid	0.16 μM
18	CHEMBL1206828	3,5-bis(phosphonooxy)benzoic acid	0.78 μM
19	CHEMBL319218	2-{[(1R,5R,6S)-3-carboxy-5-(carboxyformamido)-6-hydroxycyclohex-2-en-1-yl]oxy}propanedioic acid	5.20 μM
20	CHEMBL314765	3-carboxy-5-(hydrogen phosphonatooxy)phenyl hydrogen phosphate	0.78 μM
21	CHEMBL1205418 (CHEMBL1627010)	(3R,4S,5R)-5-[(1S)-1-carboxy-2,2,2-trifluoro-1-(phosphonooxy)ethoxy]-4-hydroxy-3-(phosphonooxy)cyclohex-1-ene-1-carboxylic acid	32 nM
22	CHEMBL1205355 (CHEMBL88396)	(3R,4R,5R)-5-(carboxymethoxy)-4-hydroxy-3-(phosphonooxy)cyclohex-1-ene-1-carboxylic acid	1.50 μM
23	CHEMBL1160691	4-[hydroxy(phosphono)methyl]-1H-pyrrole-2-carboxylic acid	14.5 nM
24	CHEMBL1160690	(3R,4S)-4-hydroxy-3-(phosphonooxy)cyclohex-1-ene-1-carboxylic acid	13 μM
25	CHEMBL88396	(3R,4R,5R)-5-(carboxylatomethoxy)-3-(hydrogen phosphonatooxy)-4-hydroxycyclohex-1-ene-1-carboxylic acid	1.5 μM

Table S1. List of compounds from ChEMBL database with demonstrated inhibitory potency against EPSPS enzyme used to generate the validation datasets.
Remark: all K_i values are for *E. coli* K12 EPSPS (id of the target – CHEMBL5033).

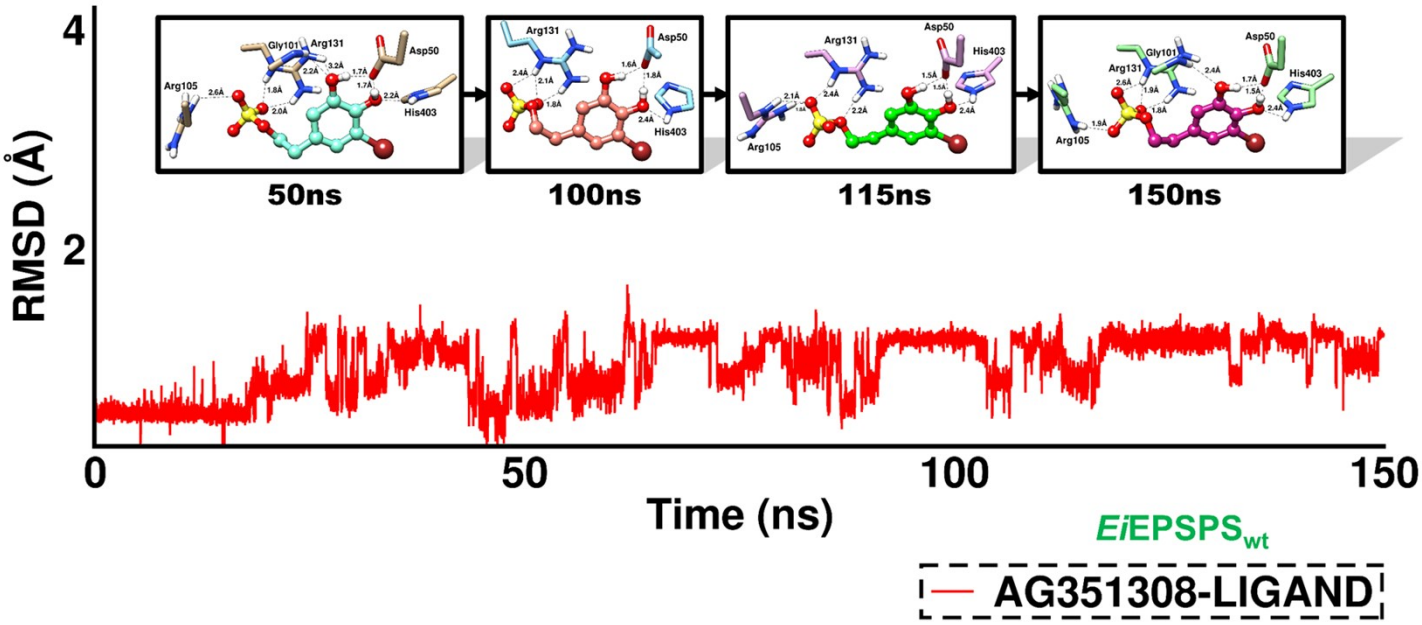

Figure S3. RMSD plots of all ligand atoms in the WT *Ei*EPSPS (red) and its poses in 50 ns, 100 ns, 115 ns and 150 ns analyzed over 150-ns MD simulation.

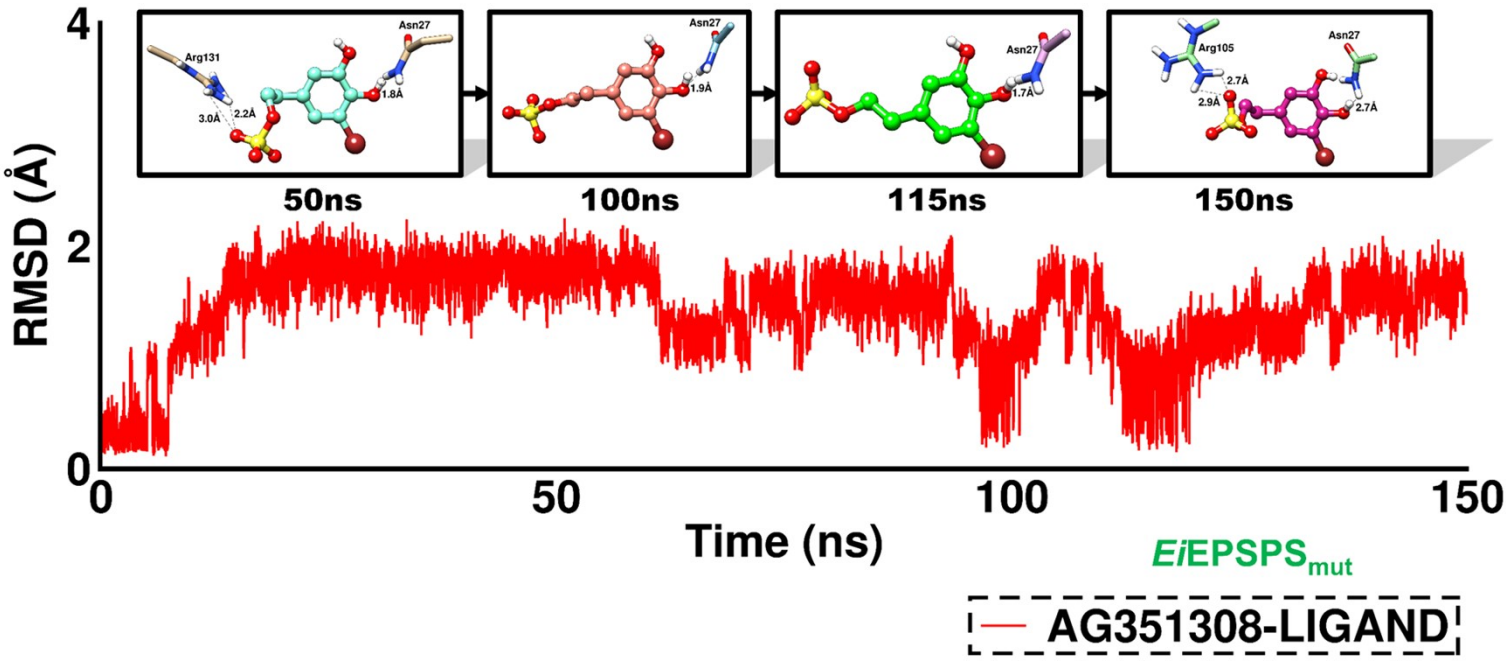

Figure S4. RMSD plots of all ligand atoms in the MUT *Ei*EPSPS (red) and its poses in 50 ns, 100 ns, 115 ns and 150 ns analyzed over 150-ns MD simulation.

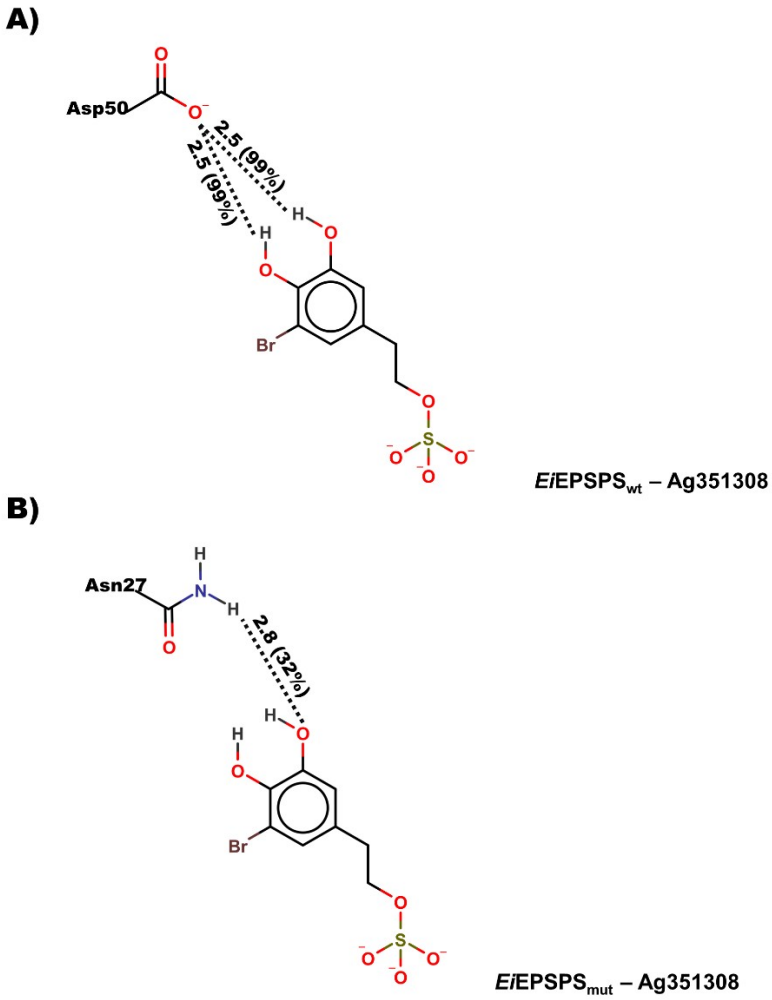

Figure S5. The 2D diagram of the A) *Ei*EPSPS$_{wt}$ and B) *Ei*EPSPS$_{mut}$ complexed with AG351308, showing the average distances of the main intermolecular interactions with occupancy value ≥ 50% obtained over the MD simulation.

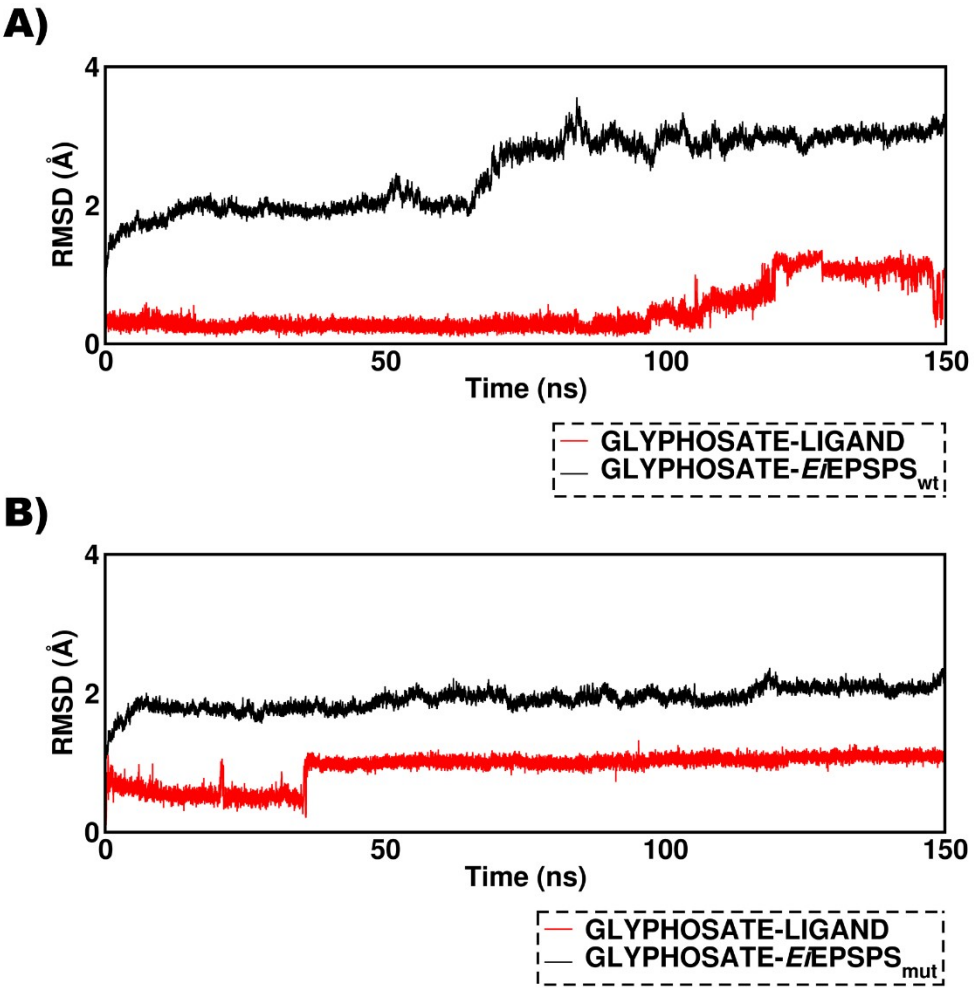

Figure S6. RMSD plots of the backbone atoms in the WT and MUT ligand complexes (black) and only ligands (red) analyzed over 150 ns of MD simulation.

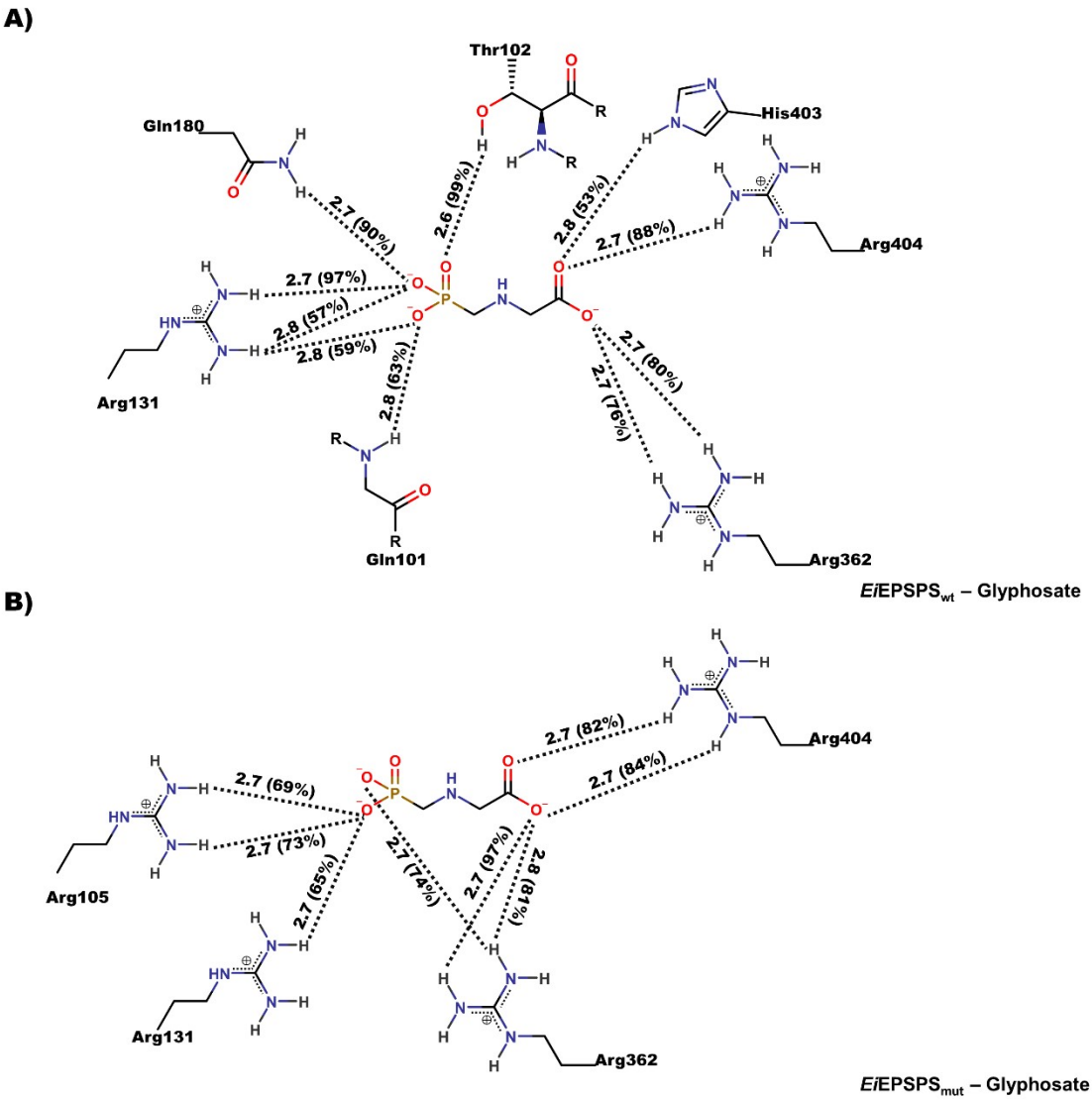

Figure S7. The 2D diagram of the A) *Ei*EPSPS$_{wt}$ and B) *Ei*EPSPS$_{mut}$ complexed with Glyphosate, showing the average distances of the main intermolecular interactions with occupancy value ≥ 50% obtained over the MD simulation.

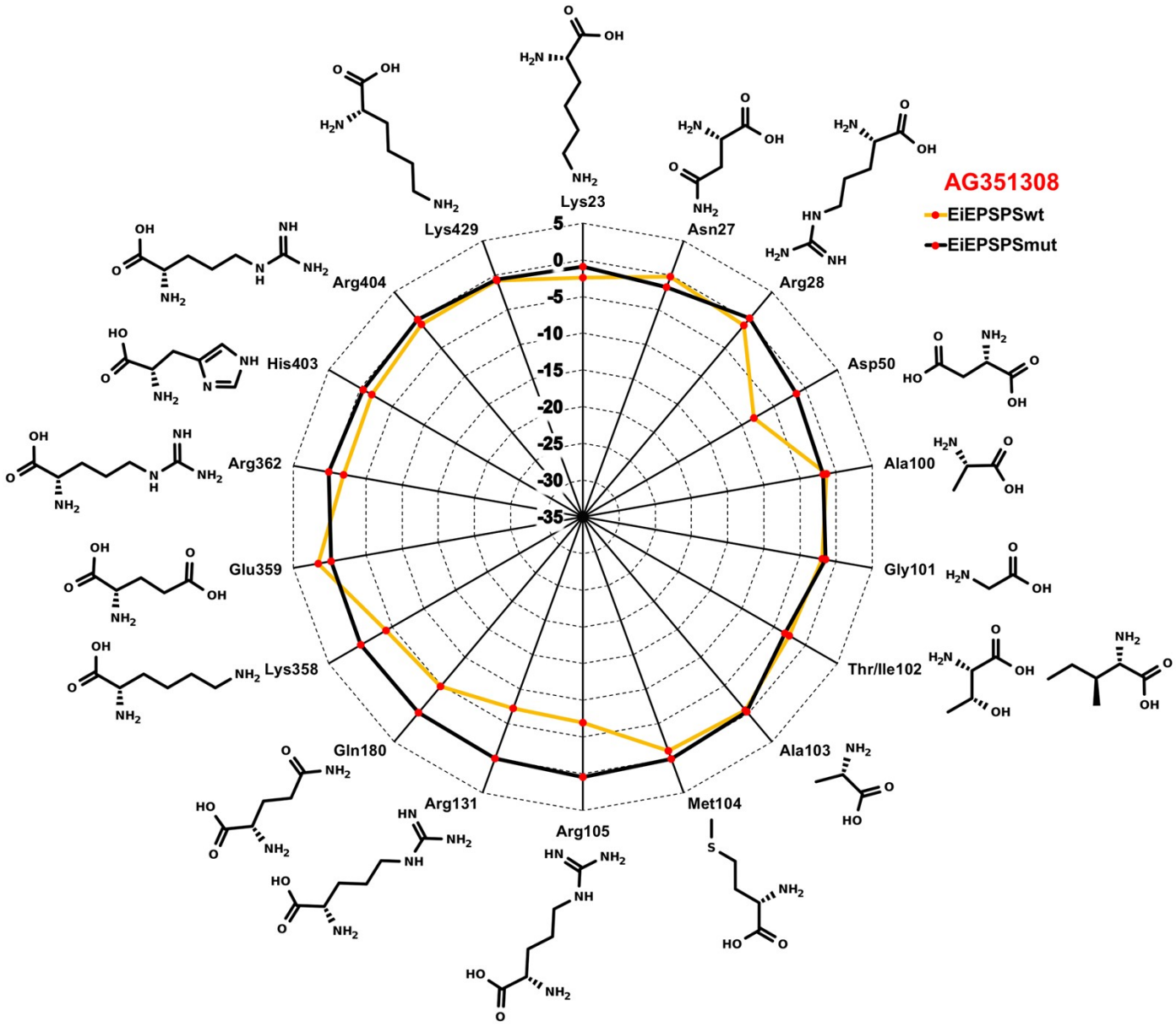

Figure S8. Per-residue energy decomposition analysis of main residues with energy decomposition value ≤ or ≥ 0.5 kcal/mol of of A) Glyphosate and B) AG332841 complexed with *Ei*EPSPS$_{wt}$ (yellow) and *Ei*EPSPS$_{mut}$ (black) structures.