

## Supporting Information

### Crystal Structures of Type-II Inositol Polyphosphate 5-Phosphatase INPP5B with Synthetic Inositol Polyphosphate Surrogates Reveal New Mechanistic Insights for the Inositol 5-Phosphatase Family

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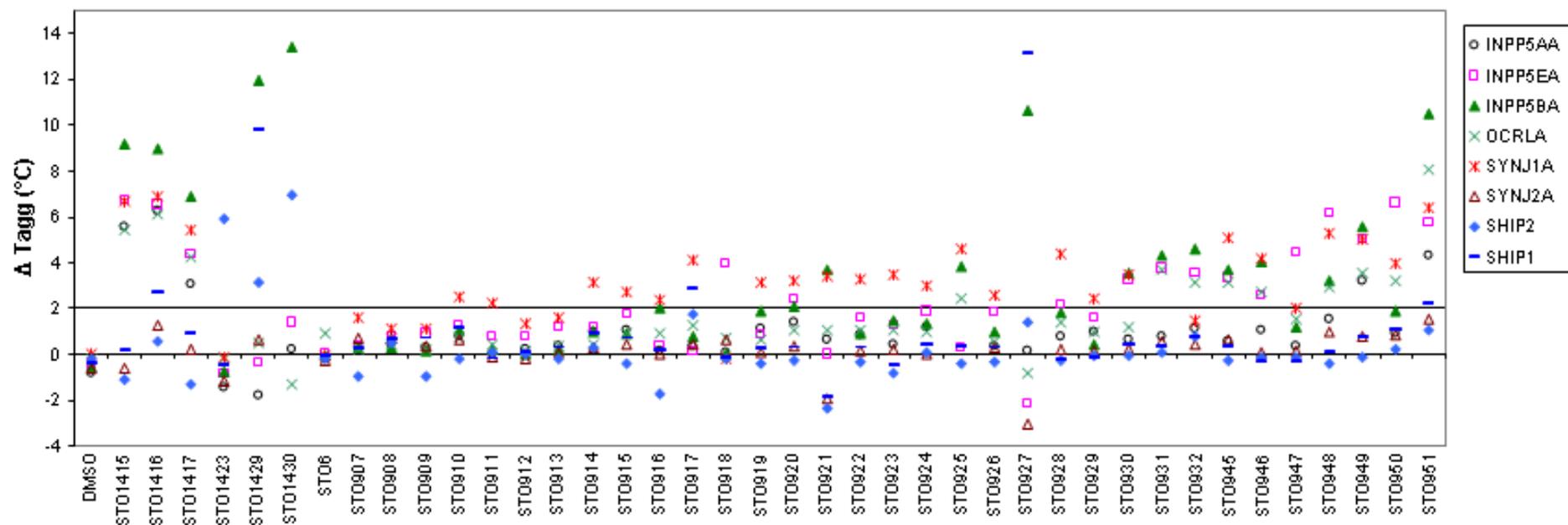
Supporting Information Scheme 1 shows a scatter graph of forty compounds (**Supporting Information Table 1, Figure S1A, S1B and S1C**) that are potential candidates for co-crystallisation with eight of the ten possible inositol polyphosphate 5-phosphatase enzymes. The INPP5B protein also has to be pure (>95%) otherwise reproducibility and measuring  $\Delta T_{agg}$  (temperature of aggregation corresponding to the midpoint of the transition) [1] is a problem since any impurity affects the shape of the transition curve. The pixel intensities were then processed using the appropriate software and then these intensities were plotted against temperature to give an individual  $\Delta T_{agg}$ , for each protein and since there is a temperature change, this value can be measured. Thus, by measuring the denaturation and subsequent aggregation of the unfolded protein by monitoring the static light scattering we obtained a  $\Delta T_{agg}$  value.

## Reference

1. Eronina, T., Borzova, V., Maloletkina, O., Kleymenov, S., Asryants, R., Markossian K. and Kurganov, B., (2011) A protein aggregation based test for screening of the agents affecting thermostability of proteins. *PLOS ONE*, 6, e22154.

## Thermal Shift Screen of Ligands against Eight Inositol 5-Phosphatase Enzymes

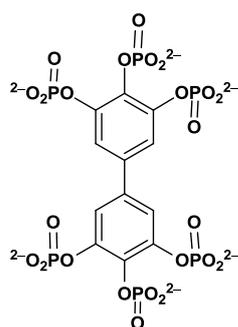
### Supporting Information Scheme S1



Inositol polyphosphate and benzene and biphenyl polyphosphate compounds that interact with inositol 5-phosphatase enzymes **INPP5A** (Type I 5-phosphatase), **INPP5E** (inositol polyphosphate 5-phosphatase 72 kDa), **INPP5B** (type II inositol 5-phosphatase), **OCRL** (Low oculocerebrorenal syndrome protein, inositol polyphosphate 5-phosphatase **OCRL-1** or **INPP5F**, SYNJ1 (Synaptojanin 1), **SYNJ2** (Synaptojanin 2), SHIP2 (SH2-containing 5'-inositol phosphatase 2), **SHIP1** (SH2-containing 5'-inositol phosphatase 1).

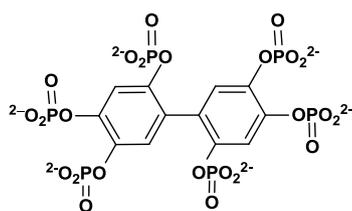
## Supporting Information Table S1 (Compound Names)

STO_ID	<u>Compound Name</u>
STO1415	BiPh(3,3',4,4',5,5')P <sub>6</sub>
<b>STO1416</b>	<b>BiPh(2,2',4,4',5,5')P<sub>6</sub></b>
STO1417	(Ethane-1,2-diylbis(oxy))bis(benzene-5,4,2,1-tetrayl) hexakisphosphate
<b>STO1423</b>	<b>(S)-3-(4-chlorobenzoyloxy)-N-(1-phenylethyl)thiophene-2-carboxamide</b>
STO1429	PtdIns(4,5)P <sub>2</sub> (di-octanoyl)
<b>STO1430</b>	<b>PtdIns(4)P (di-octanoyl)</b>
STO6	<i>myo</i> -Inositol
<b>STO907</b>	<b>D-Ins(4,5)P<sub>2</sub></b>
STO908	Glucose 3,4-bisphosphate
<b>STO909</b>	<b>Ins(1,3)P<sub>2</sub></b>
STO910	D-Ins(1,4,5)P <sub>3</sub>
<b>STO911</b>	<b>D-Ins(2,4,5)P<sub>3</sub></b>
STO912	D-2-deoxy-Ins(1,4,5)P <sub>3</sub>
<b>STO913</b>	<b>D-3-deoxy-Ins(1,4,6)P<sub>3</sub></b>
STO914	DL-Ins(1,3,4)P <sub>3</sub>
<b>STO915</b>	<b>D-1-deoxy-Ins(3,4,6)P<sub>3</sub></b>
STO916	Ins(1,3,5)P <sub>3</sub>
<b>STO917</b>	<b>PtdIns(3,5)P<sub>2</sub> (di-palmitoyl)</b>
STO918	L-Thr(1,2,4)P <sub>3</sub>
<b>STO919</b>	<b>D-Ins(1,3,4,5)P<sub>4</sub></b>
STO920	D-6-deoxy-Ins(1,3,4,5)P <sub>4</sub>
<b>STO921</b>	<b>DL-6-O-benzoyl-Ins(1,3,4,5)P<sub>4</sub></b>
STO922	D-Ins(3,4,5,6)P <sub>4</sub>
<b>STO923</b>	<b>D-<i>chiro</i>-Ins(2,3,4,5)P<sub>4</sub></b>
STO924	Ins(1,3,4,6)P <sub>4</sub>
<b>STO925</b>	<b><i>neo</i>-Ins(1,3,4,6)P<sub>4</sub></b>
STO926	D-Ins(1,4,5,6)P <sub>4</sub>
<b>STO927</b>	<b>PtdIns(3,4,5)P<sub>3</sub> (di-octanoyl)</b>
STO928	Ins(1,3,4,5,6)P <sub>5</sub>
<b>STO929</b>	<b><i>scyllo</i>-Ins(1,2,3,4,5)P<sub>5</sub></b>
STO930	2-O-Benzyl-Ins(1,3,4,5,6)P <sub>5</sub>
<b>STO931</b>	<b><i>neo</i>-InsP<sub>6</sub></b>
STO932	D- <i>chiro</i> -InsP <sub>6</sub>
<b>STO945</b>	<b>Bz(1,2,4)P<sub>3</sub></b>
STO946	Bz(1,3,5)P <sub>3</sub>
<b>STO947</b>	<b>Bz(1,2,3)P<sub>3</sub></b>
STO948	Bz(1,2,3,4)P <sub>4</sub>
<b>STO949</b>	<b>Bz(1,2,4,5)P<sub>4</sub></b>
STO950	Bz(1,2,3,5)P <sub>4</sub>
<b>STO951</b>	<b>BiPh(2,3',4,5',6)P<sub>5</sub></b>



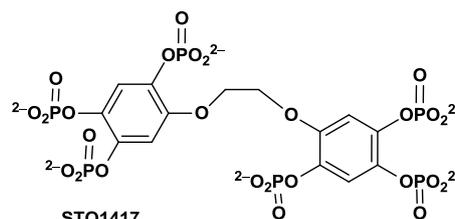
BiPh(3,3',4,4',5,5')P<sub>6</sub>

STO1415



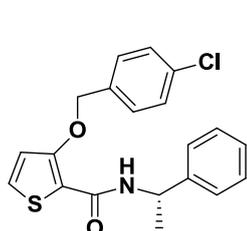
Biphenyl 2,2',4,4',5,5'-hexakisphosphate

STO1416



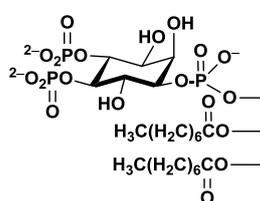
STO1417

Ethane-1,2-diylbis(oxy)bis(benzene-5,4,2,1-tetrayl) hexakisphosphate. (1,2,4-Dimer)



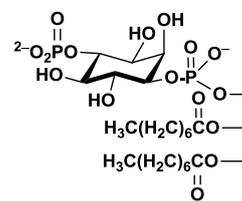
AS1949490

STO1423 specific SHP2 inhibitor



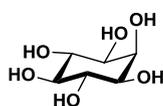
STO1429

PtdIns(4,5)P<sub>2</sub> (dioctanoyl)



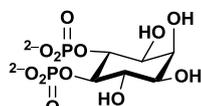
STO1430

PtdIns(4)P (dioctanoyl)



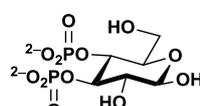
STO6

myo-Inositol



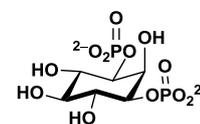
STO907

D-Ins(4,5)P<sub>2</sub>



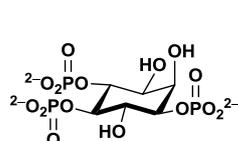
STO908

Glucose 3,4-bisphosphate



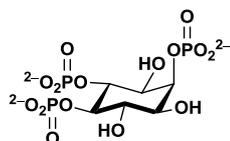
STO909

Ins(1,3)P<sub>2</sub>



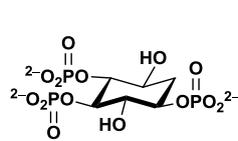
STO910

D-Ins(1,4,5)P<sub>3</sub>



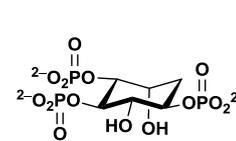
STO911

D-Ins(2,4,5)P<sub>3</sub>



STO912

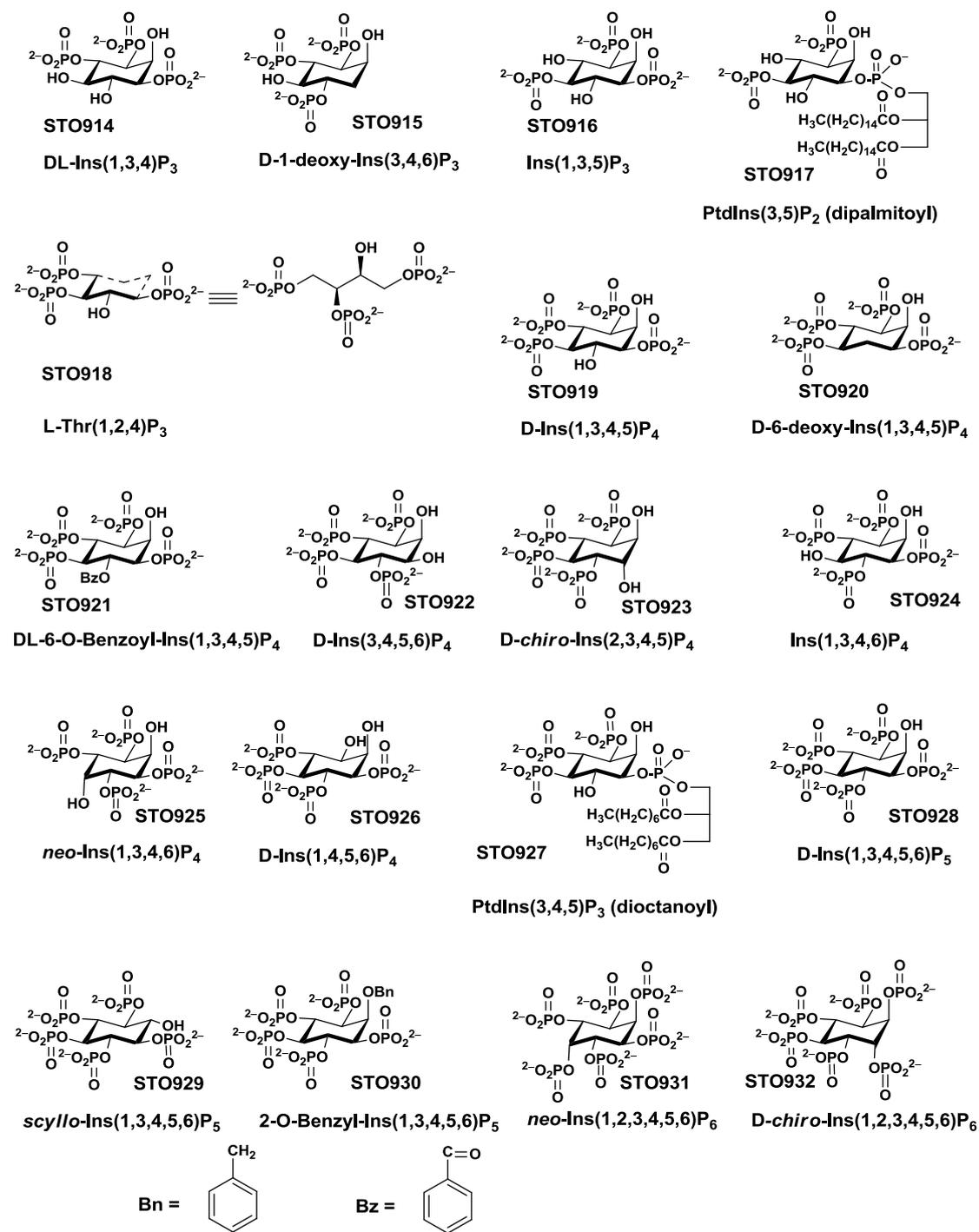
D-2-deoxy-Ins(1,4,5)P<sub>3</sub>



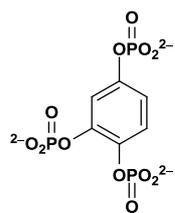
STO913

D-3-deoxy-Ins(1,4,6)P<sub>3</sub>

Figure S1A

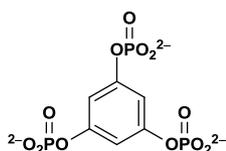


**Figure S1B**



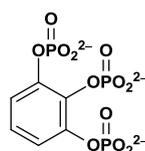
STO945

Bz(1,2,4)P<sub>3</sub>



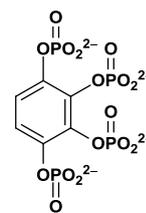
STO946

Bz(1,3,5)P<sub>3</sub>



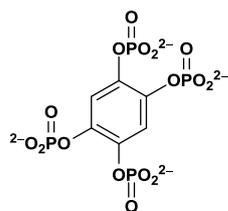
STO947

Bz(1,2,3)P<sub>3</sub>



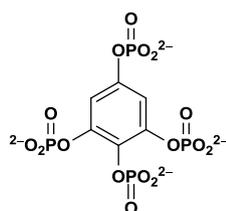
STO948

Bz(1,2,3,4)P<sub>4</sub>



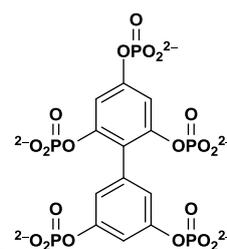
STO949

Bz(1,2,4,5)P<sub>4</sub>



STO950

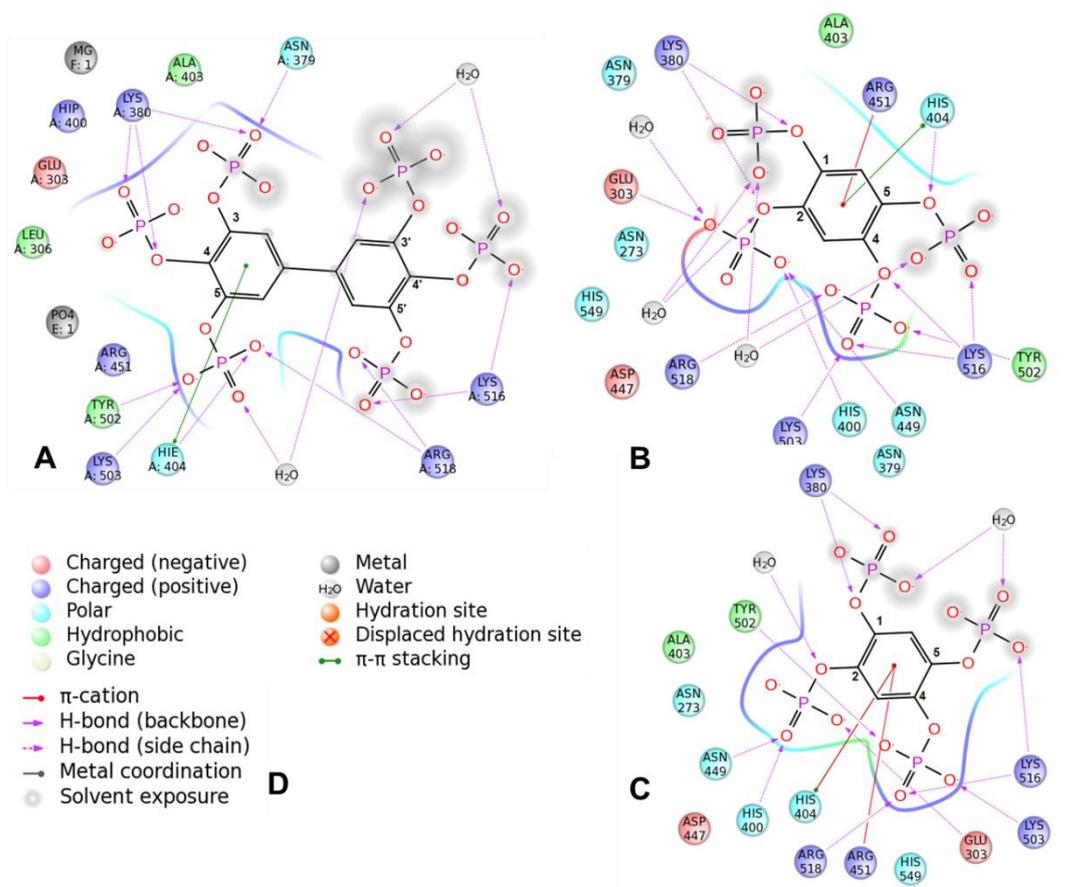
Bz(1,2,3,5)P<sub>4</sub>



STO951

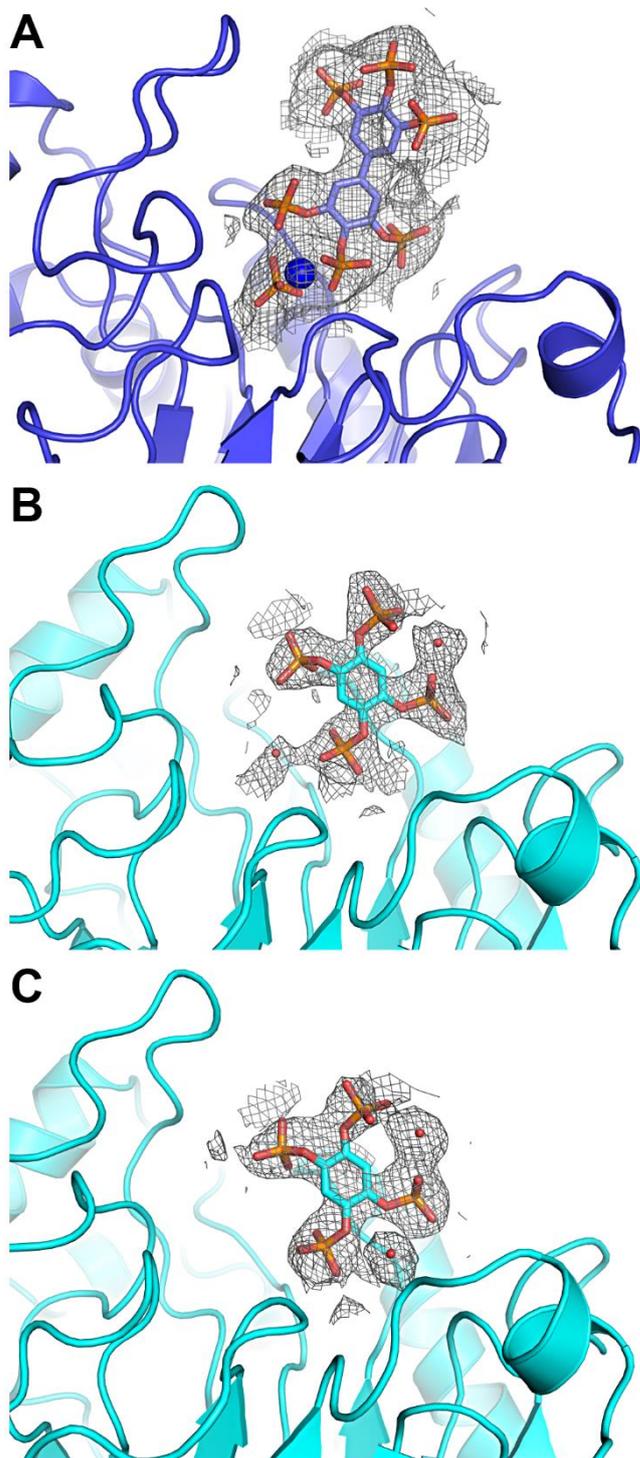
BiPh(2,3',4,5',6)P<sub>5</sub>

Figure S1C



## Supporting Information Figure S2

(Supporting Information Figure S2 panel A) Ligand interaction diagram showing the amino acid residues of INPP5B interacting with BiPh(3,3',4,4',5,5')P<sub>6</sub>. Panel B & C shows the pose of Bz(1,2,4,5)P<sub>4</sub> in two chains. First, (Supporting Information Figure S2 panel B) is associated with chain A and second, (Supporting Information Figure S2 panel C) is associated with chain B of the protein structure of the INPP5B–Bz(1,2,4,5)P<sub>4</sub> complex where chain A and chain B are virtually identical within the unit cell. (Supporting Information Figure S2 panel D) shows the key for the interactions of the diagrams.



**Supporting Information Figure S3.**

**Figure S3. Electron density maps around BiPh(3,3',4,4',5,5')P<sub>6</sub> and Bz(1,2,4,5)P<sub>4</sub> in their respective complexes with INPP5B.** Inhibitors are shown as sticks surrounded by 2mF<sub>o</sub>-DF<sub>c</sub> electron density maps (contoured at 1σ) while the protein backbone is displayed as cartoons. **A.** BiPh(3,3',4,4',5,5')P<sub>6</sub> complex with INPP5B. **B** and **C** are Bz(1,2,4,5)P<sub>4</sub> in complex with INPP5B. Complex with protein chain A and protein

chain B are represented in panel B and C, respectively. Magnesium ion and inorganic phosphate are displayed as a sphere and sticks, respectively in panel A. Water molecules present in the vicinity of the inhibitors are displayed as red spheres in panel B and C.

**Supporting Information Table S2.**

	INPP5B– PtdIns(3,4) P <sub>2</sub>	INPP5B– BiPh(3,3',4, 4',5,5')P <sub>6</sub>	INPP5B– Bz(1,2,4,5) P <sub>4</sub>	SHIP2– BiPh(2,3',4, 5',6)P <sub>5</sub>	SPsynaptoja nin– Ins(1,4)P <sub>2</sub>	Apo- SPsynaptoja nin	INPP5E	OCRL
INPP5B– PtdIns4P	<b>0.08</b> (276)	<b>0.25</b> (278)	<b>0.27</b> (273)	<b>0.51</b> (187)	<b>0.64</b> (225)	<b>0.67</b> (228)	<b>0.65</b> (200)	<b>0.38</b> (264)
INPP5B– PtdIns(3,4)P <sub>2</sub>	-	<b>0.26</b> (270)	<b>0.28</b> (272)	<b>0.51</b> (184)	<b>0.62</b> (222)	<b>0.66</b> (228)	<b>0.66</b> (203)	<b>0.38</b> (263)
INPP5B– BiPh(3,3',4,4',5,5')P <sub>6</sub>	-	-	<b>0.34</b> (268)	<b>0.55</b> (181)	<b>0.63</b> (221)	<b>0.66</b> (223)	<b>0.77</b> (190)	<b>0.45</b> (265)
INPP5B– Bz(1,2,4,5)P <sub>4</sub>	-	-	-	<b>0.53</b> (183)	<b>0.67</b> (228)	<b>0.72</b> (232)	<b>0.67</b> (200)	<b>0.45</b> (265)
SHIP2– BiPh(2,3',4,5',6)P <sub>5</sub>	-	-	-	-	<b>0.59</b> (189)	<b>0.61</b> (192)	<b>0.66</b> (169)	<b>0.62</b> (196)
SPsynaptojanin– Ins(1,4)P <sub>2</sub>	-	-	-	-	-	<b>0.17</b> (312)	<b>0.69</b> (188)	<b>0.73</b> (232)
Apo-SPsynaptojanin	-	-	-	-	-	-	<b>0.71</b> (189)	<b>0.77</b> (237)
INPP5E	-	-	-	-	-	-	-	<b>0.71</b> (176)

Rmsd (Å) for the C $\alpha$  atoms that structurally superimpose (number of C $\alpha$  shown in brackets) between the 5-phosphatase structures calculated using the PYMOL programme.

Supporting Information Table S3A.

	INPP5B– PtdIns4P	INPP5B– PtdIns(3,4)P <sub>2</sub>	INPP5B– BiPh(3,3',4,4',5,5')P <sub>6</sub>	INPP5B– Bz(1,2,4,5)P <sub>4</sub>	Synaptojanin– Ins(1,4)P <sub>2</sub>	SHIP2– BiPh(2,3',4,5',6)P <sub>5</sub>
Conserved 1-phosphate position	1-phosphate  <b>Asn-379</b> <b>Lys-380</b> 3 waters	1-phosphate  <b>Asn-379</b> <b>Lys-380</b> 2 waters	3-phosphate  <b>Asn-379</b> <b>Lys-380</b>	1-phosphate  <b>Lys-380</b> 2 water	Not conserved	3'-phosphate  <b>Lys-541</b> Ser-564 (bb) Arg-571
Conserved 4-phosphate position	4-phosphate  <b>Tyr-502</b> <b>Lys-503</b> <b>Lys-516</b> <b>Arg-518</b> 3 waters	4-phosphate  <b>Tyr-502</b> <b>Lys-503</b> <b>Lys-516</b> <b>Arg-518</b> 2 waters	5-phosphate  His-404 <b>Tyr-502</b> <b>Lys-503</b>  Arg-518 Mg <sup>2+</sup> 1 water	4-phosphate  <b>Tyr-502</b> <b>Lys-503</b> <b>Lys-516</b> <b>Arg-518</b>	4-phosphate  <b>Tyr-794</b> <b>Lys-795</b>  Arg-810 4 waters	2-phosphate  Gly-565 (bb) Arg-611 <b>Tyr-661</b>  Arg-682 Asn-684 1 water
Conserved 5- and 6- hydroxyl position	5-hydroxyl  <b>Glu-303</b> Mg <sup>2+</sup> 3 waters	5-hydroxyl  1 water	4-phosphate  <b>Glu-303</b> <b>Lys-380</b> His-400 Mg <sup>2+</sup>	2-phosphate  <b>Glu-303</b> <b>Lys-380</b> His-400 Asn-449 Arg451 2 water	Not conserved	5'-phosphate  <b>Lys-541</b> Ser-564 1 water
	6-hydroxyl  <b>Glu-303</b> <b>Lys-380</b>	6-hydroxyl  <b>Lys-380</b>				

Supporting Information Table S3B.

	<b>INPP5B– PtdIns4P</b>	<b>INPP5B– PtdIns(3,4)P<sub>2</sub></b>	<b>INPP5B– BiPh(3,3',4,4',5,5')P<sub>6</sub></b>	<b>INPP5B– Bz(1,2,4,5)P<sub>4</sub></b>	<b>Synaptojanin– Ins(1,4)P<sub>2</sub></b>	<b>SHIP2– BiPh(2,3',4,5',6)P<sub>5</sub></b>
<b>Other interactions</b>	<b>2-hydroxyl</b>  <b>1 water</b>	<b>2-hydroxyl</b>  <b>1 water</b>	<b>3'-phosphate</b>  <b>1 water</b>	<b>5-phosphate</b>  <b>His-404</b> <b>Lys-516</b> <b>1 water</b>	<b>1-phosphate</b>  <b>Tyr-704(bb)</b> <b>1 water</b>	<b>4-phosphate</b>  <b>Asn-566</b> <b>Asn-566 (bb)</b> <b>Thr-683 (bb)</b>
	<b>3-hydroxyl</b>  <b>1 water</b>	<b>3-phosphate</b>  <b>His-404</b>	<b>4'-phosphate</b>  <b>Lys-516</b> <b>1 water</b>		<b>2-hydroxyl</b>  <b>1 water</b>	<b>6-phosphate</b>  <b>None</b>
	<b>Fatty acid</b>  <b>1 water</b>	<b>Fatty acid</b>  <b>1 water</b>	<b>5'-phosphate</b>  <b>Lys-516</b> <b>Arg-518</b>		<b>3-hydroxyl</b>  <b>1 water</b>	
					<b>5-hydroxyl</b>  <b>Arg-744</b> <b>Tyr-794</b>	
					<b>6-hydroxyl</b>  <b>Arg-744</b>	

**Tables S4A and S4B** Show the ligand–protein interactions for the INPP5B–PtdIns(4)P, INPP5B–PtdIns(3,4)P<sub>2</sub>, INPP5B–BiPh(3,3',4,4',5,5')P<sub>6</sub>, INPP5B–Bz(1,2,4,5)P<sub>4</sub>, SPsynaptojanin–Ins(1,4)P<sub>2</sub> and SHIP2–BiPh(2,3',4,5',6)P<sub>5</sub> complexes. Residues in equivalent positions in the structures are colored, bb = backbone.