Supplementary Information

A minimum catalytic unit for synthesis of $InsP_6$ and 5-PP-$InsP_5$ in Arabidopsis

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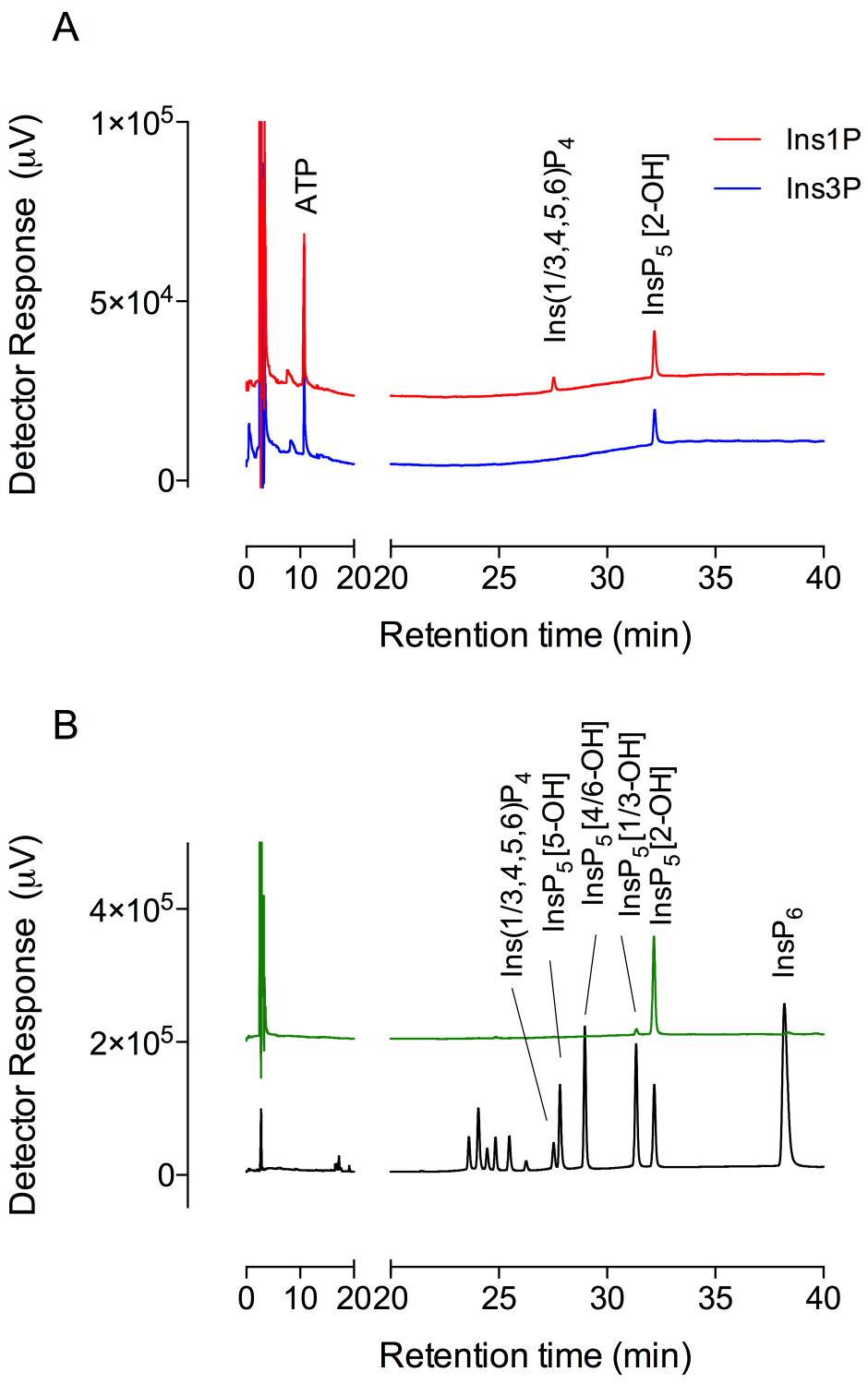

Figure S1. Analysis of Ins1P and Ins3P phosphorylation. Assays stopped by the addition of NaF, EDTA, pH 10, were eluted on a CarboPac PA200 column. **A**, products generated from Ins1P (red trace) share retention time with standards: Ins(1/3,4,5,6)P_4 (identified in an hydrolysate of $InsP_6$, black trace in B) and Ins(1,3,4,5,6)P_5 ($InsP_5$ [2-OH], green trace in B). Products generated from Ins3P (blue trace) share retention time with $InsP_5$ [2-OH]. The position of elution of ATP is shown. The gradient of methanesulfonic acid used was : time (min), % B (0.6 M MeSA); 0,0; 25,25; 100,38; 45,100. The chromatography shown in the figure has been repeated on more than ten occasions. Ins1P, Ins3P and EDTA elute in the solvent front at c. 2.5-3 minutes.

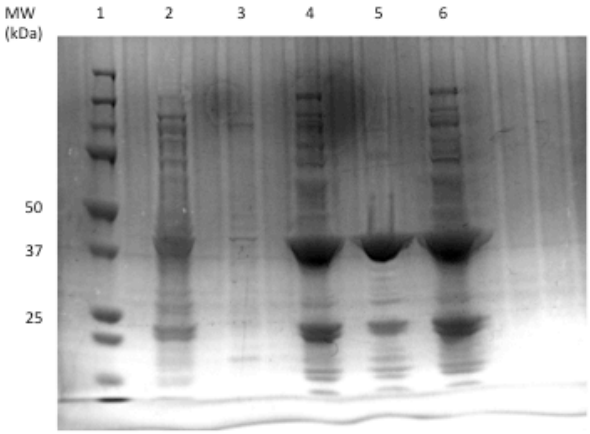

Figure S2. SDS-PAGE of pET23a: *Af*IPS, expressed in Rosetta™ 2 (DE3)pLysS (Novagen). Lanes (interspersed with unloaded lanes): 1, Ladder; 2, NiNTA wash A (50 mM NaH_2PO_4 pH 7.5, 300 mM NaCl, 20 mM imidazole); 3, NiNTA wash B (50 mM NaH_2PO_4 pH 7.5, 300 mM NaCl, 250 mM imidazole); 4, 60°C in 2 mM DTT for 30 mins; 5, 80°C in 2 mM DTT for 30 mins; 6, 40°C for 1 hr then 60°C for 30 mins.