

Analysis of Inositol Phosphate Metabolism by Capillary Electrophoresis Electrospray Ionization Mass Spectrometry

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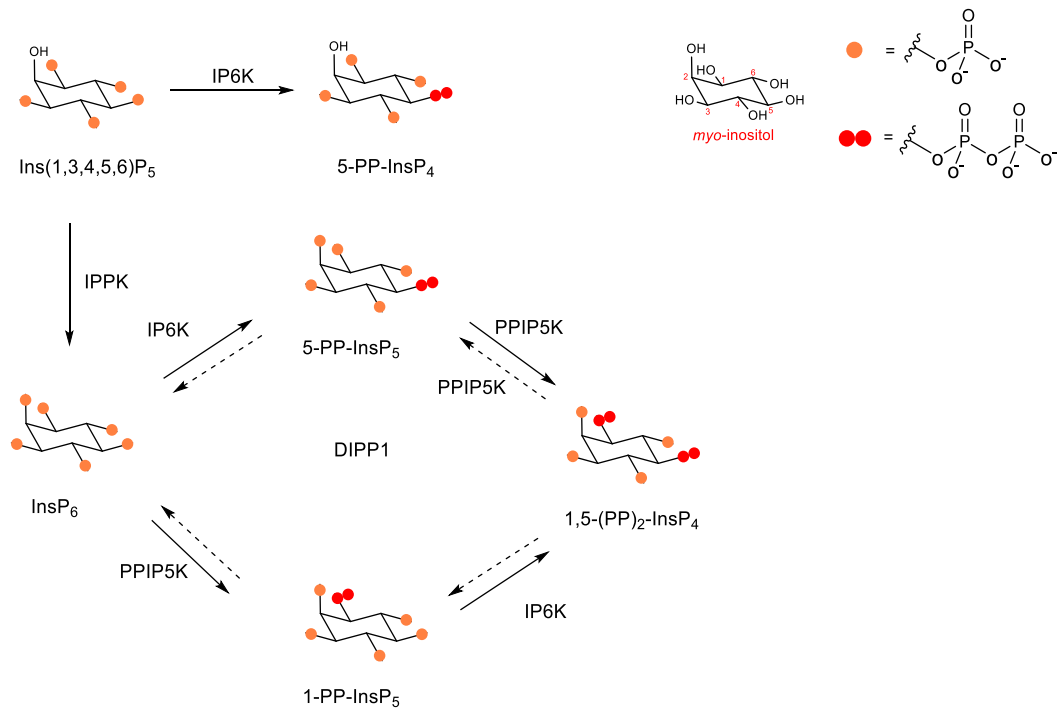
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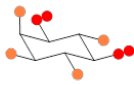
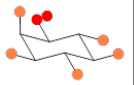
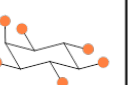

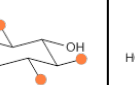
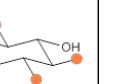
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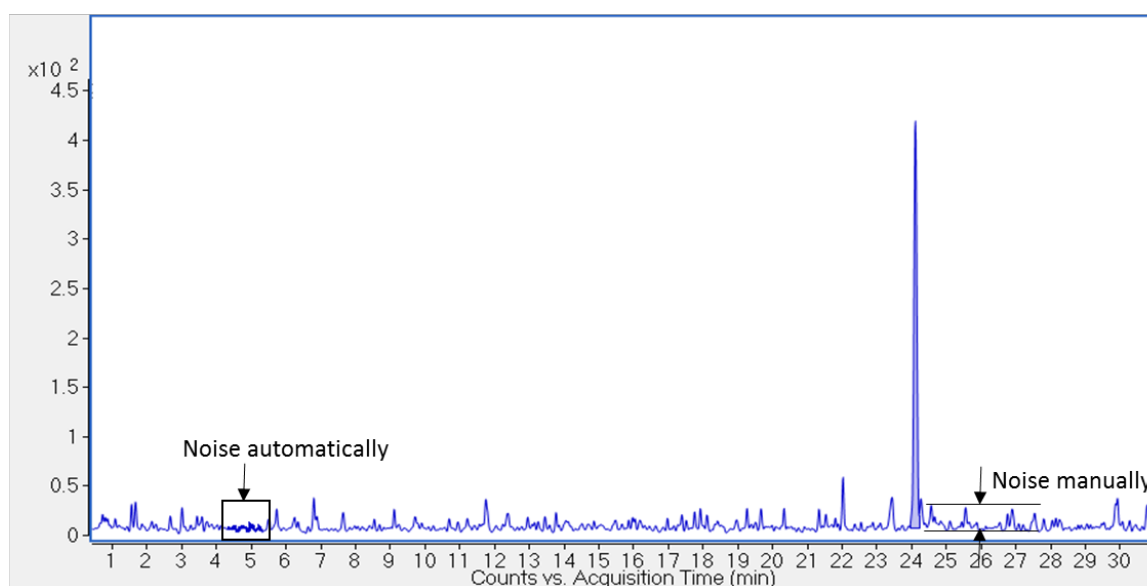


Supplementary Figure 1 Metabolism of inositol pyrophosphates in mammals. IPPK: inositol pentakisphosphate 2-kinase, IP6K: inositol hexakisphosphate kinase, PPIP5K: diphosphoinositol pentakisphosphate kinase, DIPP1: diphosphoinositol polyphosphate phosphohydrolase 1.

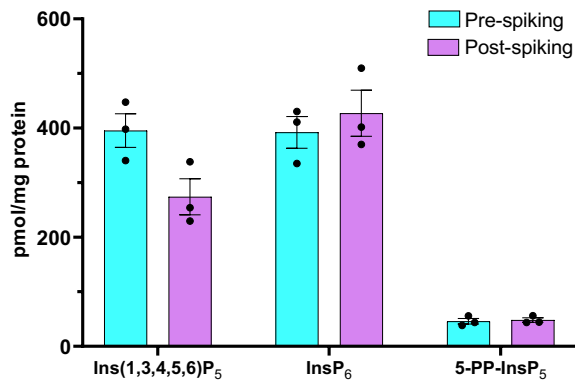
a

	 InsP ₈	 InsP ₇	 InsP ₆	 InsP ₅	 InsP ₄	 InsP ₃
LOD (nM)	75	45	45	75	150	150
LOQ (nM)	250	150	150	250	500	500

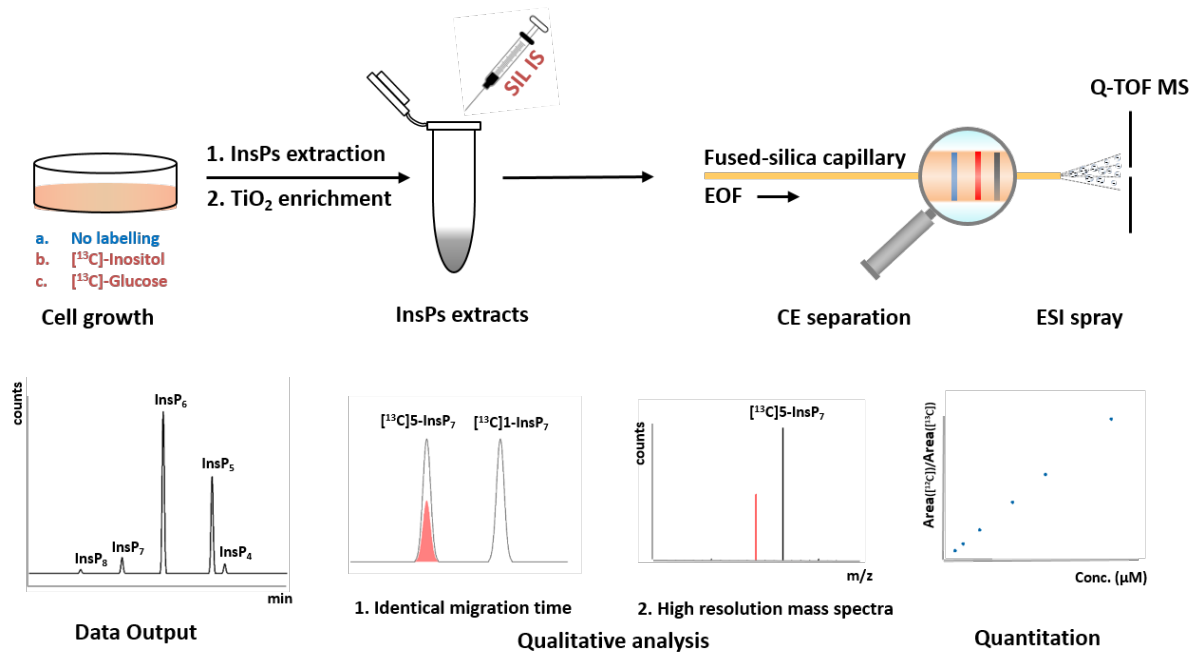
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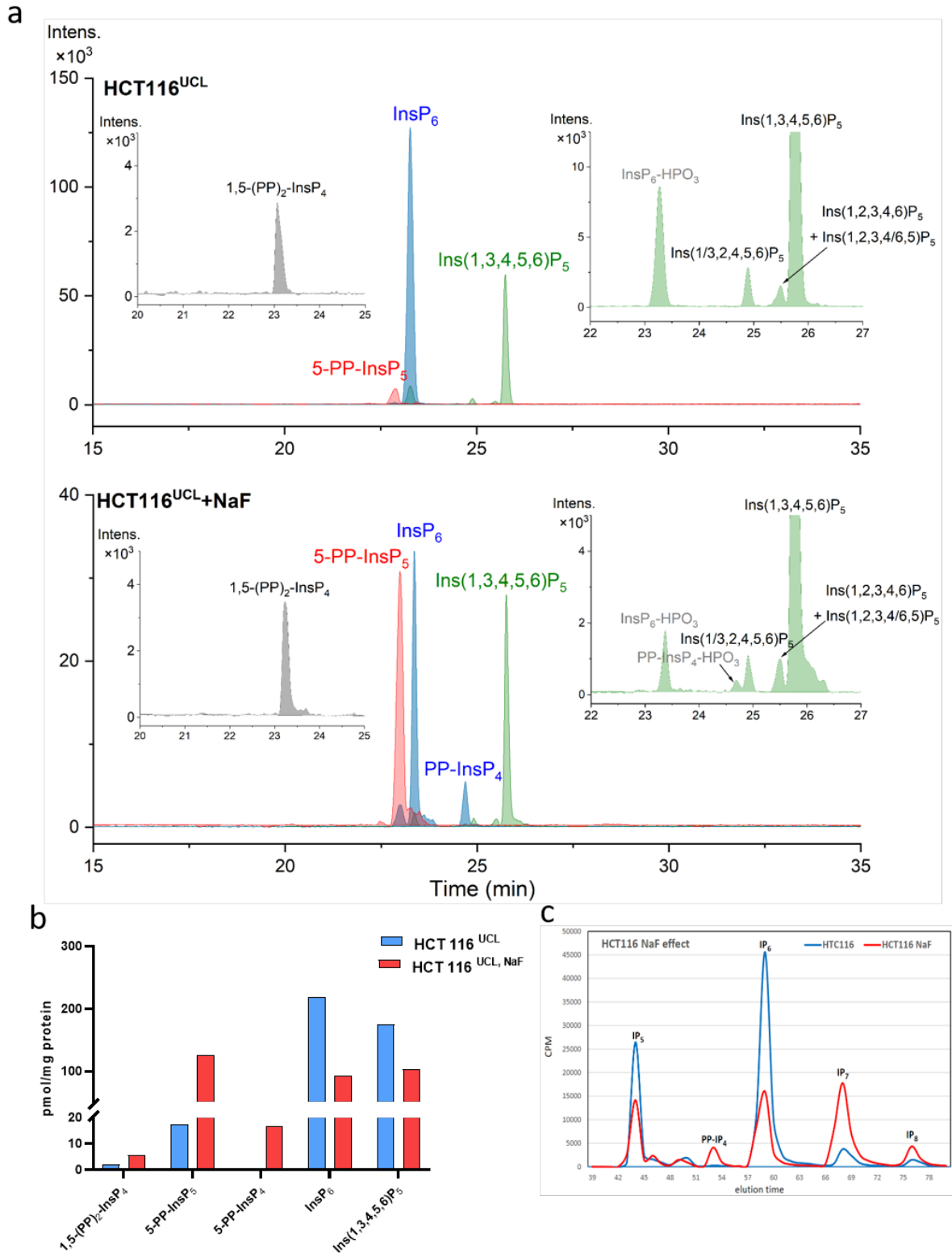
Supplementary Figure 2 (a) Limit of detection (LOD) and limit of quantitation (LOQ) for InsPs. LOD and LOQ was obtained based on signal-to-noise (S/N) approach: determination of S/N is performed by comparing signals from analytes with known low concentration with baseline noise, typical S/N ratio is 3:1 for LODs and 10:1 for LOQs; **(b)** EIE of 250 nM InsP₆ and example for baseline noise calculation. Noise was manually selected, while the baseline segments for estimation of noise automatically selected and calculated by Data analysis software is artificial.



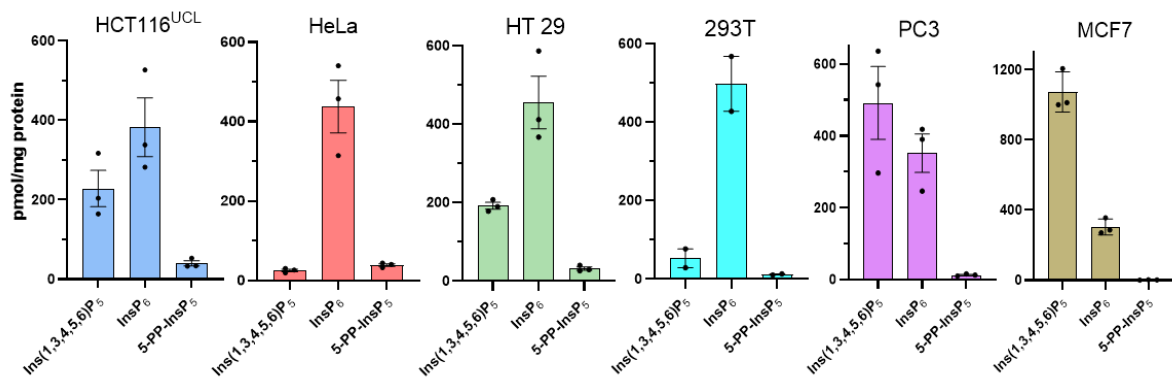
Supplementary Figure 3 Recovery of 5-PP-InsP and InsP₆ from mammalian cell extracts with the TiO₂ extraction procedure. 20 μM [¹³C₆]Ins(1,3,4,5,6)P₅, 20 μM [¹³C₆]InsP₆ and 5 μM [¹³C₆]5-PP-InsP₅ were spiked into HCT116^{NiH} cell extracts before the measurement step (post-spiking), or added to culture dishes containing HCT116^{NiH} cells before the perchloric acid extraction step (pre-spiking). The applied InsP purification protocol showed full recovery for 5-PP-InsP₅(105%) and InsP₆ (109%), and good recovery for Ins(1,3,4,5,6)P₅ (69%). Data are means ± SEM from three independent experiments, individual values are shown with dots. Source data are provided as a Source Data file.



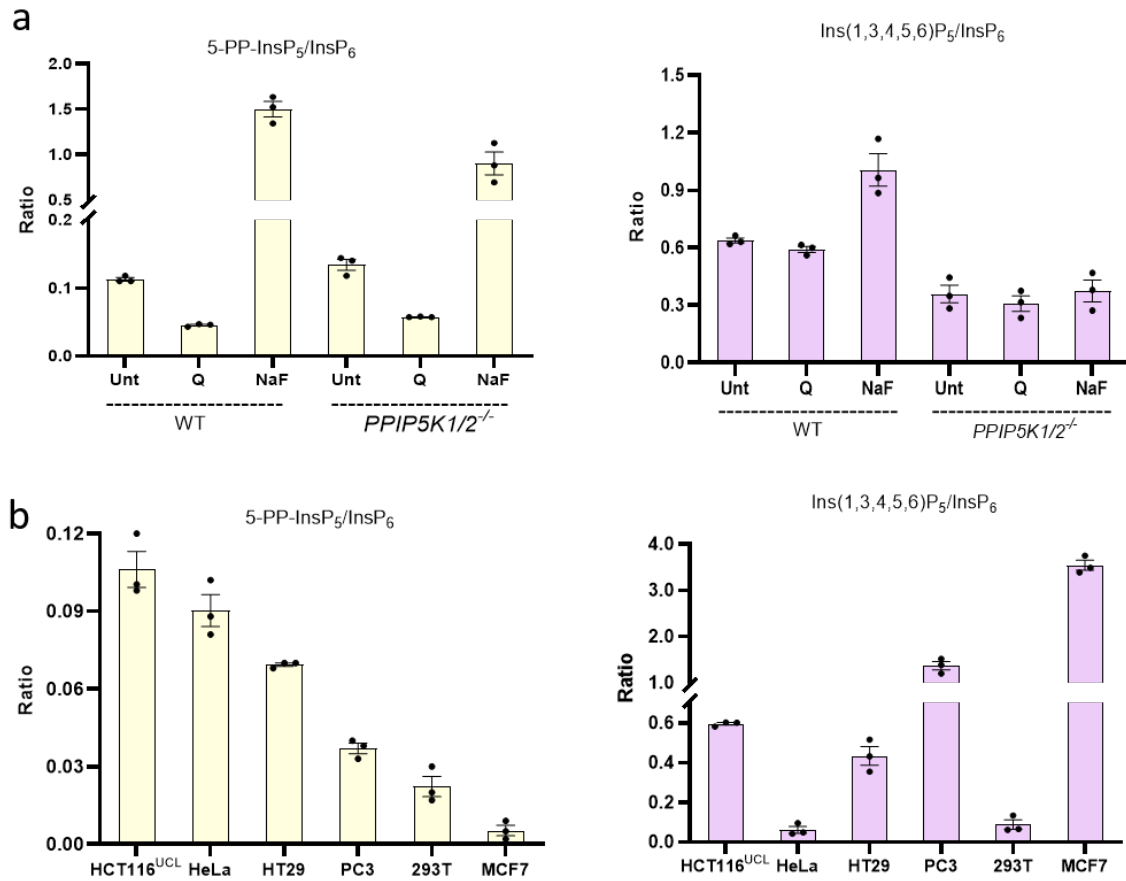
Supplementary Figure 4 Inositol phosphates extraction and analysis workflow for CE-MS.



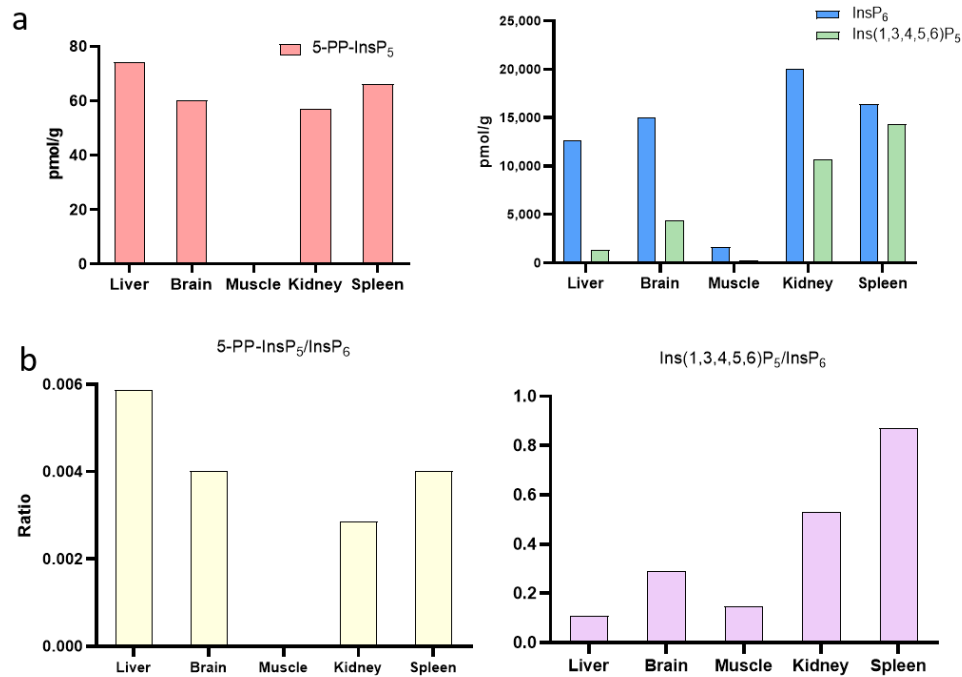
20 μM [$^{13}\text{C}_6$]Ins(1,3,4,5,6)P₅, 20 μM [$^{13}\text{C}_6$]InsP₆, 10 μM [$^{13}\text{C}_6$] 5-PP-InsP₅, 1 μM [$^{13}\text{C}_6$] 1-PP-InsP₅ and 1 μM [$^{13}\text{C}_6$] 1,5-(PP)₂-InsP₄ were spiked. **(b)** CE-ESI-MS results for inositol (pyro)phosphates (amount normalized by protein content) in HCT116^{UCL} cells showing the effect of NaF treatment; **(c)** SAX-HPLC analysis of [^3H]-inositol-labeled HCT116^{UCL} cells for NaF treatment. Source data are provided as a Source Data file.



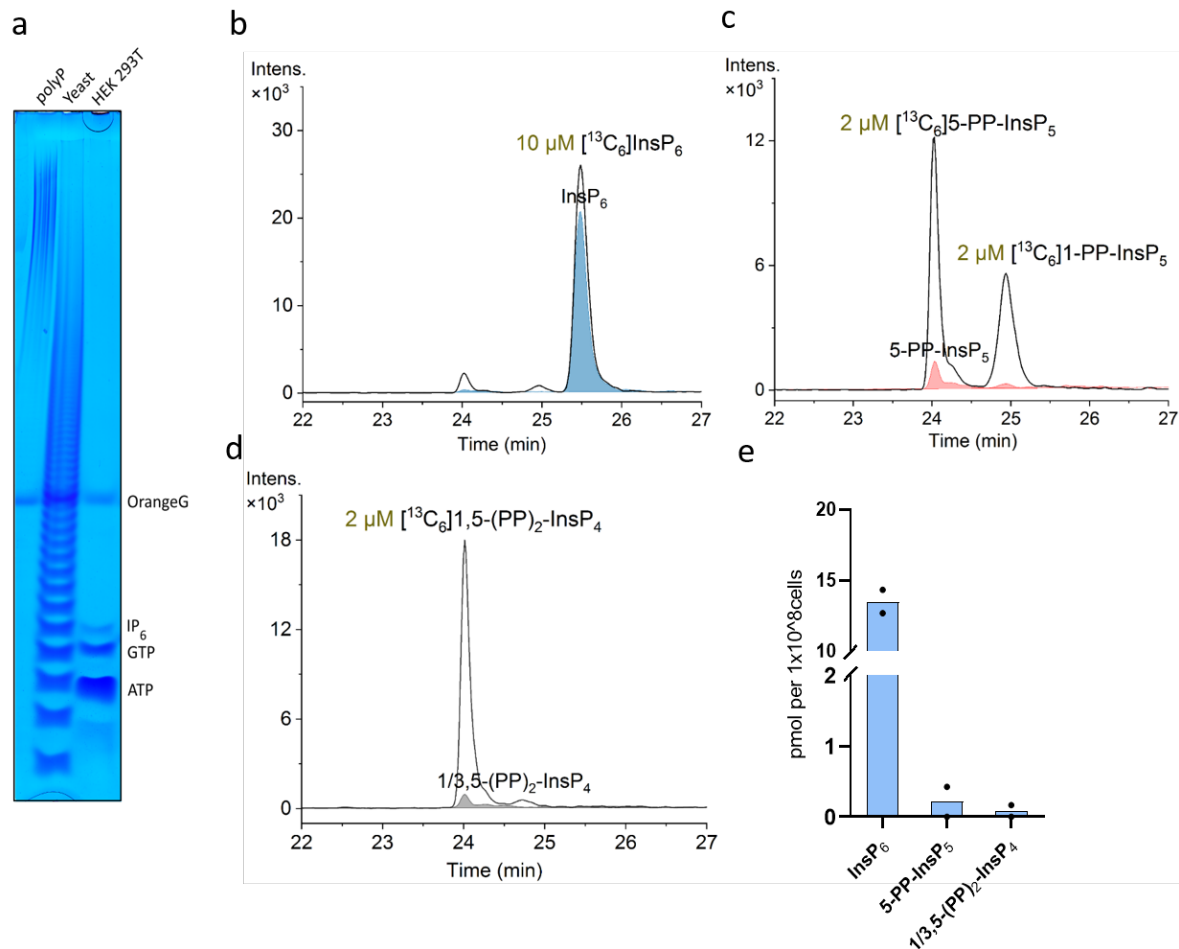
Supplementary Figure 6 InsP levels normalized by protein content in human cell lines: HeLa, HCT116^{UCL}, HT29, PC3, 293T and MCF7. The results vary between 25-1080 pmol/mg protein Ins(1,3,4,5,6)P₅, 300-500 pmol/mg protein InsP₆, and 1-40 pmol/mg protein 5-PP-InsP₅ across the cells tested. Data are means ± SEM from three independent experiments. Source data are provided as a Source Data file.



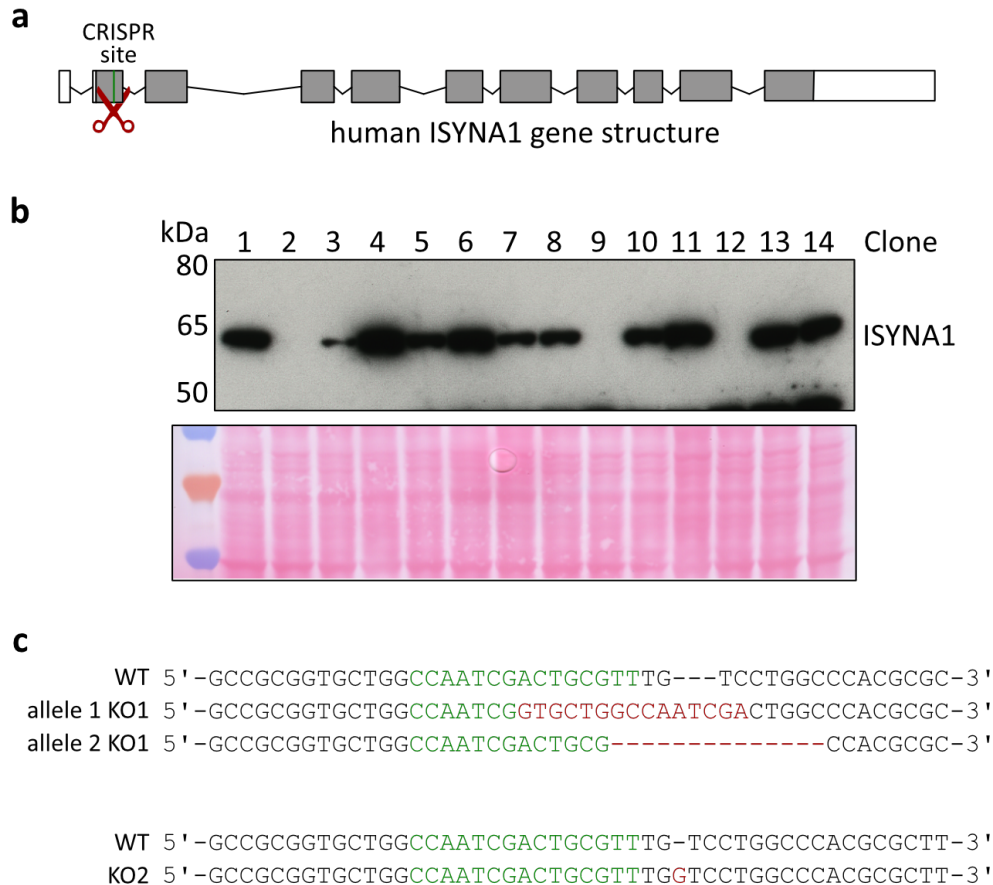
Supplementary Figure 7 Ratios of InsPs and PP-InsPs in different cell lines. **(a)** Ratio of 5-PP-InsP₅/InsP₆ (yellow) and Ins(1,3,4,5,6)P₅/InsP₆ (purple) in HCT116^{NIH} and HCT116^{NIH}PPIP5K^{-/-} cells after treatment with NaF (10 mM for 60 min) or the inositol polyphosphate kinase (IPMK) inhibitor quercetin (Q; 2.5 μM for 30 min); **(b)** Ratio of 5-PP-InsP₅/InsP₆ (yellow) and Ins(1,3,4,5,6)P₅/InsP₆ (purple) in HCT116^{UCL}, HeLa, HT29, PC3, 293T and MCF7 cells. Data are means ± SEM from three independent experiments. Source data are provided as a Source Data file.



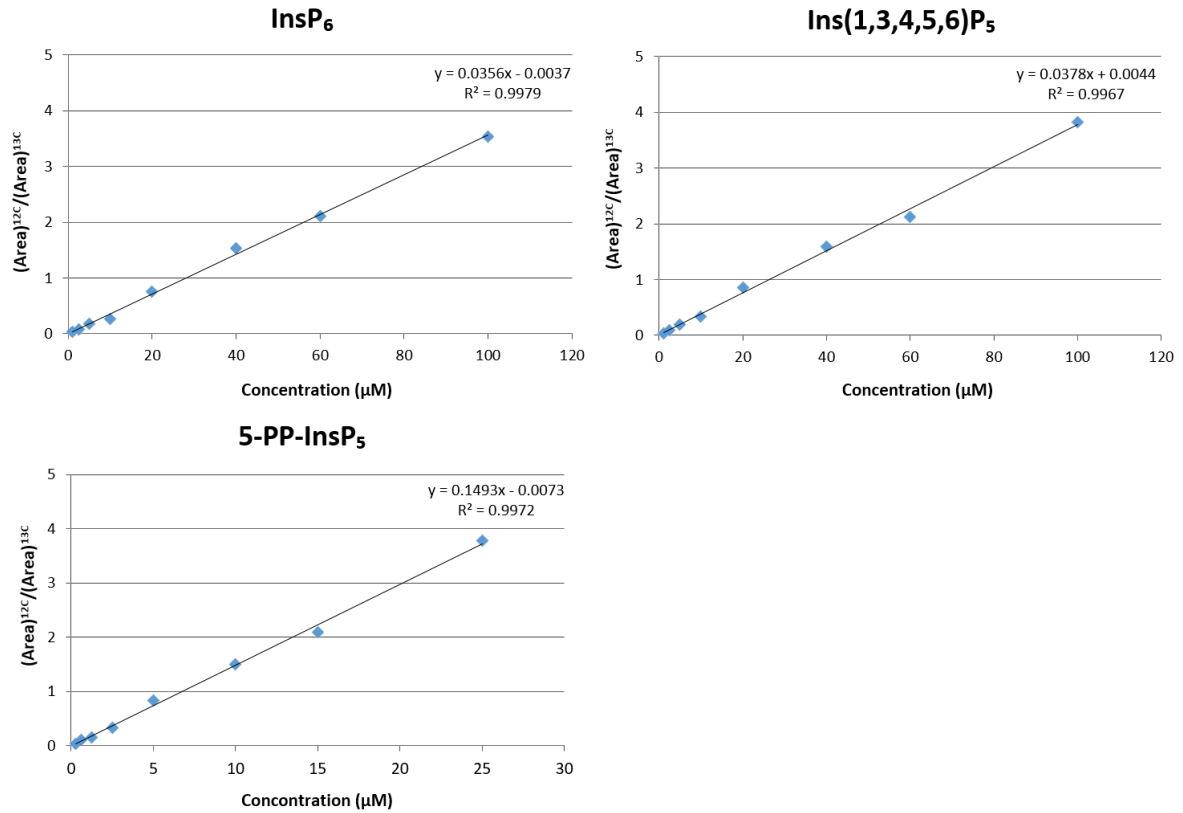
Supplementary Figure 8 Inositol phosphates and pyrophosphates in organs. **(a)** Amount of inositol (pyro)phosphates in different mouse organs (normalized by organ weight), including liver, brain, muscle, kidney and spleen; **(b)** Ratio of 5-PP-InsP₅/InsP₆ (yellow) and Ins(1,3,4,5,6)P₅/InsP₆ (purple) in different mouse organs. Standards of 4 μM [¹³C₆]5-PP-InsP₅, 120 μM [¹³C₆]InsP₆, and 20 μM [¹³C₆]Ins(1,3,4,5,6)P₅ were spiked into organ extracts for quantitation. Source data are provided as a Source Data file.



Supplementary Figure 9 CE-ESI-MS analysis of inositol (pyro)phosphates in wild type *Saccharomyces cerevisiae*. **(a)** Yeast extract analyzed by PAGE. A 33% PAGE gel was loaded with poly standard, TiO₂ purified wild type yeast *Saccharomyces cerevisiae* (5 OD₆₀₀) extract and TiO₂ purified HEK 293T (10cm plate 80% confluent) extract. After running the phosphate-rich metabolites were visualized by Toluidine staining. The very abundant polyP present in yeast is revealed by the distinctive ladder. The polyP ladder conceals the InsP and nucleotide signals that are conversely easily detectable in the HEK 293T cell extract. The result is representative of an experiment repeated at least four times. polyP loaded lane was used to define samples order; **(b-d)** EIEs of InsP₆, InsP₇ and InsP₈ along with spiked 10 μM [¹³C₆]InsP₆, 2 μM [¹³C₆]5-PP-InsP₅, 2 μM [¹³C₆]1-PP-InsP₅, and 2 μM [¹³C₆]1,5-(PP)₂-InsP₄, respectively; **(e)** Amounts of inositol (pyro)phosphates (normalized by cell counts) in *S. cerevisiae*. Data are means from independent duplicates. Source data are provided as a Source Data file.



Supplementary Figure 10 Generation of ISYNA1 knockout clones. **(a)** Schematic structure of the human ISYNA1 gene, with exons shown in grey, indicating the Alt-R CRISPR targeted region; **(b)** Western blotting for ISYNA1 revealed its absence in three of the HCT116^{UCL}-derived clones tested. Ponceau red staining confirmed similar protein loading. We chose to focus on two independent clones we called *ISYNA1*^{-/-}KO1 and *ISYNA1*^{-/-}KO2; **(c)** The mutations around the CRISPR site were determined by Sanger sequencing. Sequencing found that KO1 has an insertion on one allele and a deletion on the other. All sequences from clone KO2 showed a single nucleotide insertion. Source data are provided as a Source Data file.



Supplementary Figure 11 Calibration curves for quantitation of Ins(1,3,4,5,6)P₅, InsP₆ and 5-PP-InsP₅, in mammalian cells. Standards were spiked with 5 µM [¹³C₆] 5-PP-InsP₅, 20 µM [¹³C₆] InsP₆ and 20 µM [¹³C₆] Ins(1,3,4,5,6)P₅. The ratios of peak areas for Ins(1,3,4,5,6)P₅, InsP₆ and 5-PP-InsP₅ with respect to internal standards [(Area)¹²C/(Area)¹³C] are plotted against the concentrations of the standards. Source data are provided as a Source Data file.