Supporting Information

Optimization of Ultra-Fast Proteomics using an LC-Quadrupole-Orbitrap Mass Spectrometer with Data-Independent Acquisition

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Figure S4. The levels of EGFR and proteins annotated with “signaling by receptor tyrosine kinase (R-HSA-9006934)” by LPA(14:0) stimulation.

Table S1. The number of identified proteins and peptides in triplicate runs for selected nine DIA methods by analyzing DIA-NN. (.xlsx)

Table S2. The number of identified proteins and peptides in triplicate runs for selected nine DIA methods by analyzing Scaffold DIA. (.xlsx)

Table S3. The information of annotated pathway with statistical significance for each stimulation. (.xlsx)

Table S4. Summary of cell stimulation assay using eight chemical substances. (.xlsx)

Figure S1. Representative total ion chromatogram of 5-min gradient LC-MS/MS.

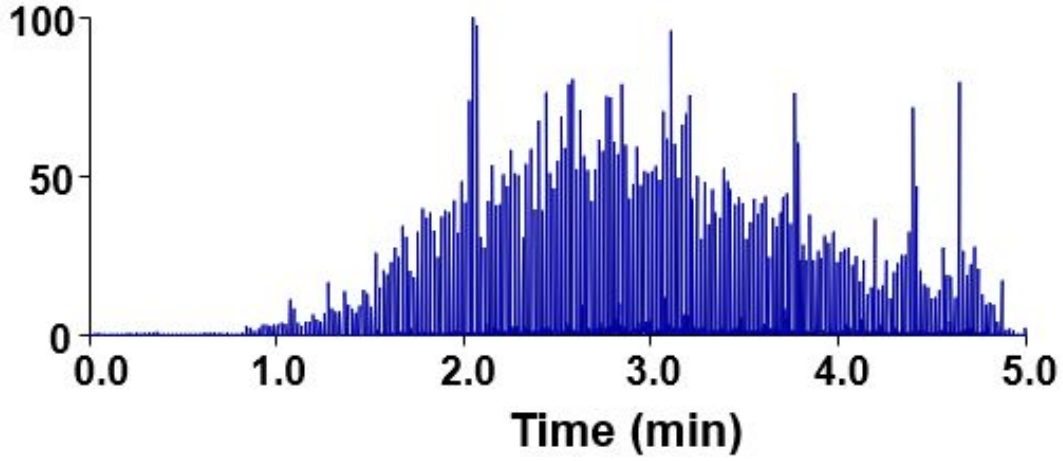


Figure S2. The re-analysis of data obtained from selected nine methods by analyzing Scaffold DIA. The bar graphs indicate the number of identified (A) proteins and (B) peptides using 500 ng cell digest with triplicate runs. Nine methods are comprising with narrow precursor mass ranges (120 Da: Res15k/W4/650–770, Res30k/W8/650–770, Res30k/W8/600–720, Res30k/W8/550–670, and Res30k/W8/500–620) and wide precursor mass ranges (240 Da; Res15k/W8/650–890, Res15k/W8/600–840, Res15k/W8/550–790, and Res15k/W8/500–740).

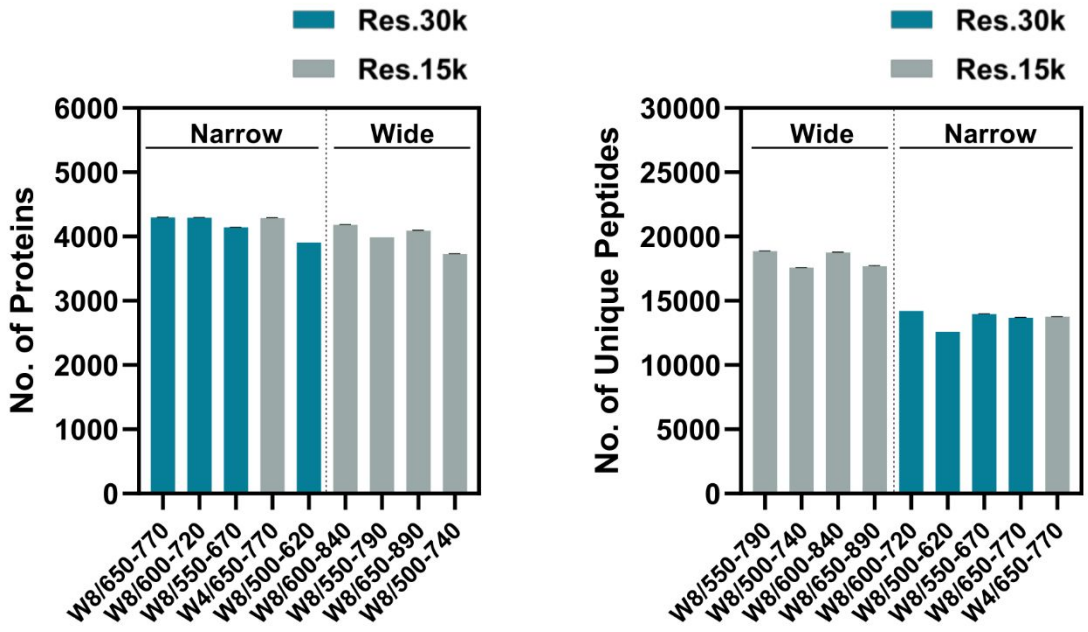
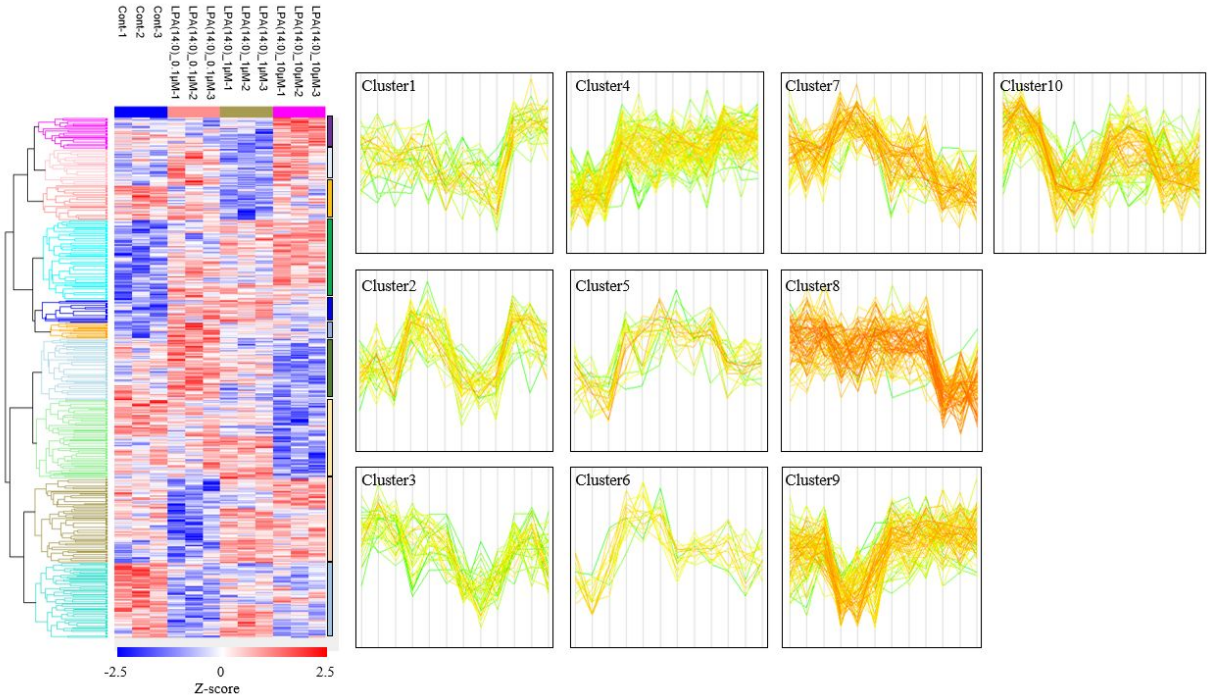


Figure S3. Hierarchical clustering and GO enrichment analysis of HEK293T cell proteins stimulated by (A) LPA(14:0), (B) S1P, (C) Sph, (D) PI3P, (E) PI4P, (F) PI5P, (G) IP3, and (IP4).

(A) LPA(14:0)



(B) S1P

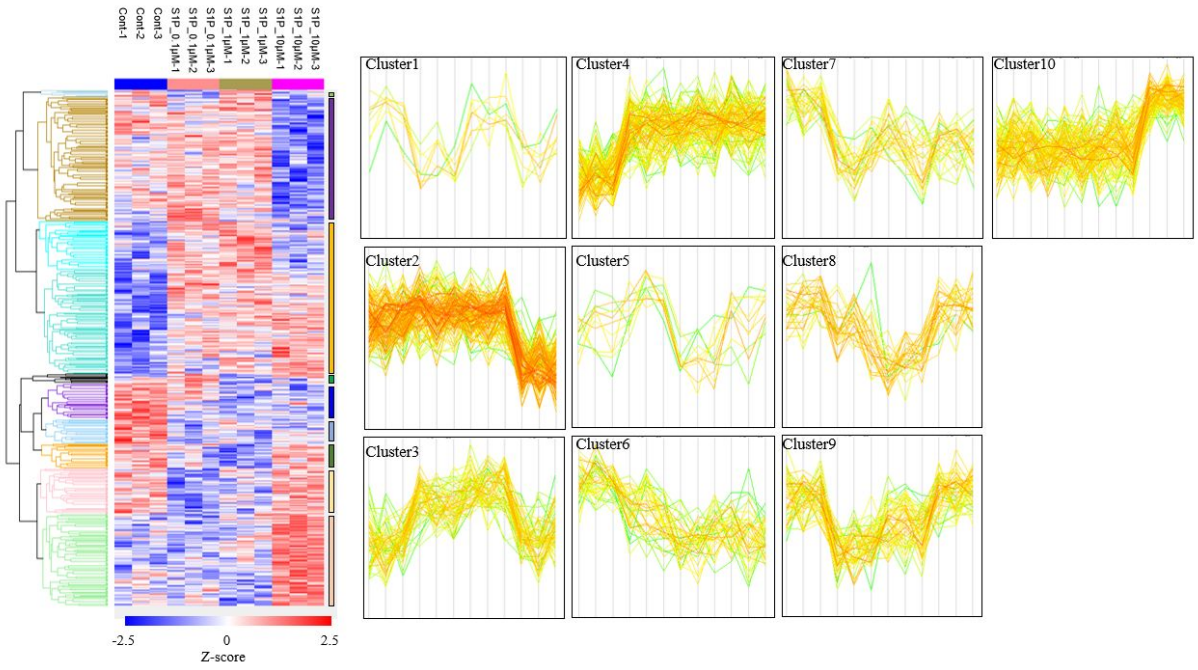
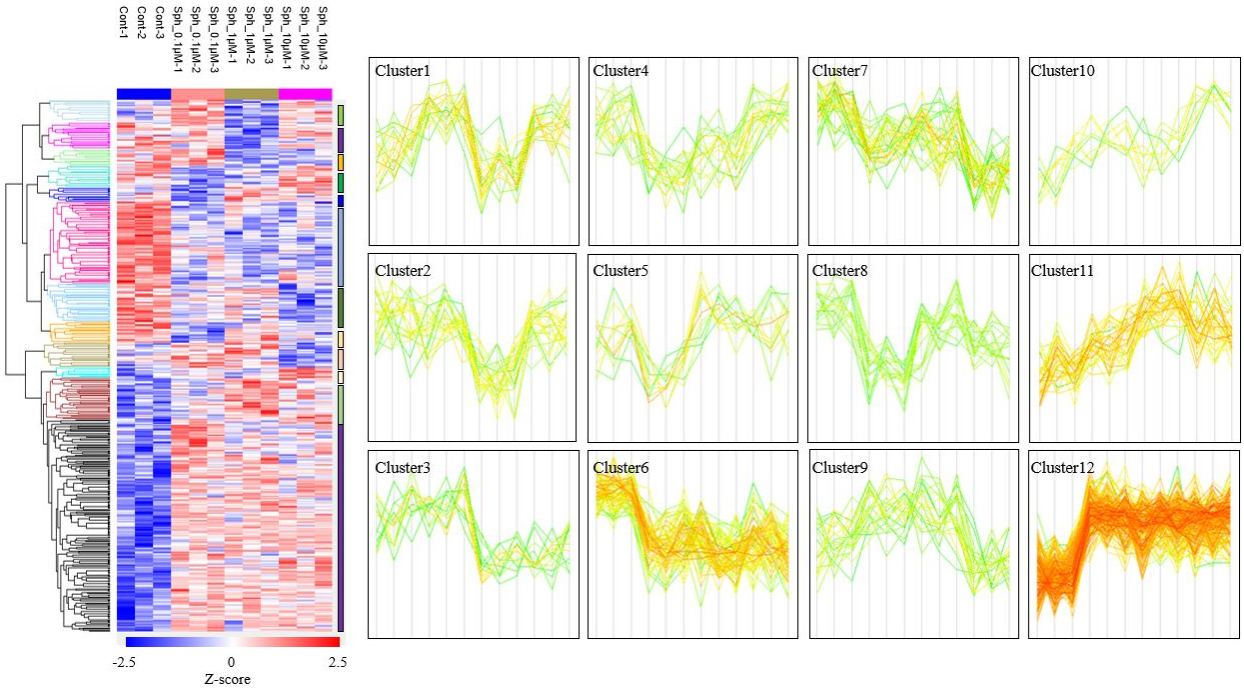


Figure. S3 continue

(C) Sph



(D) PI3P

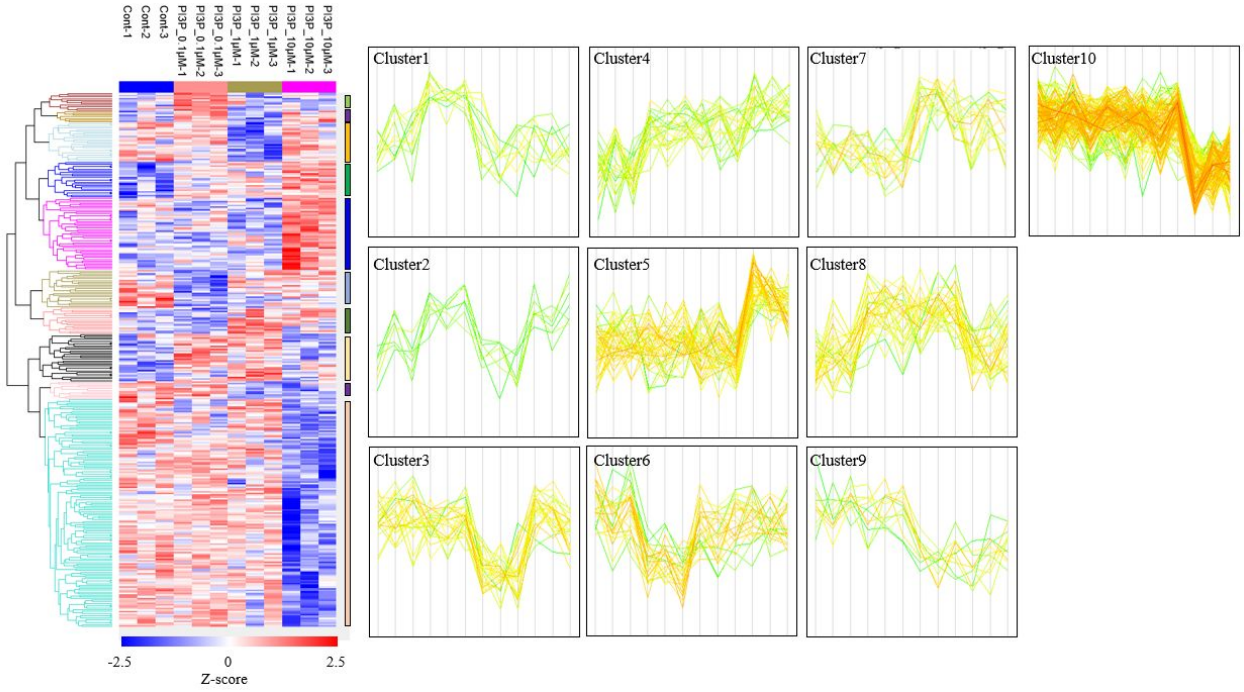
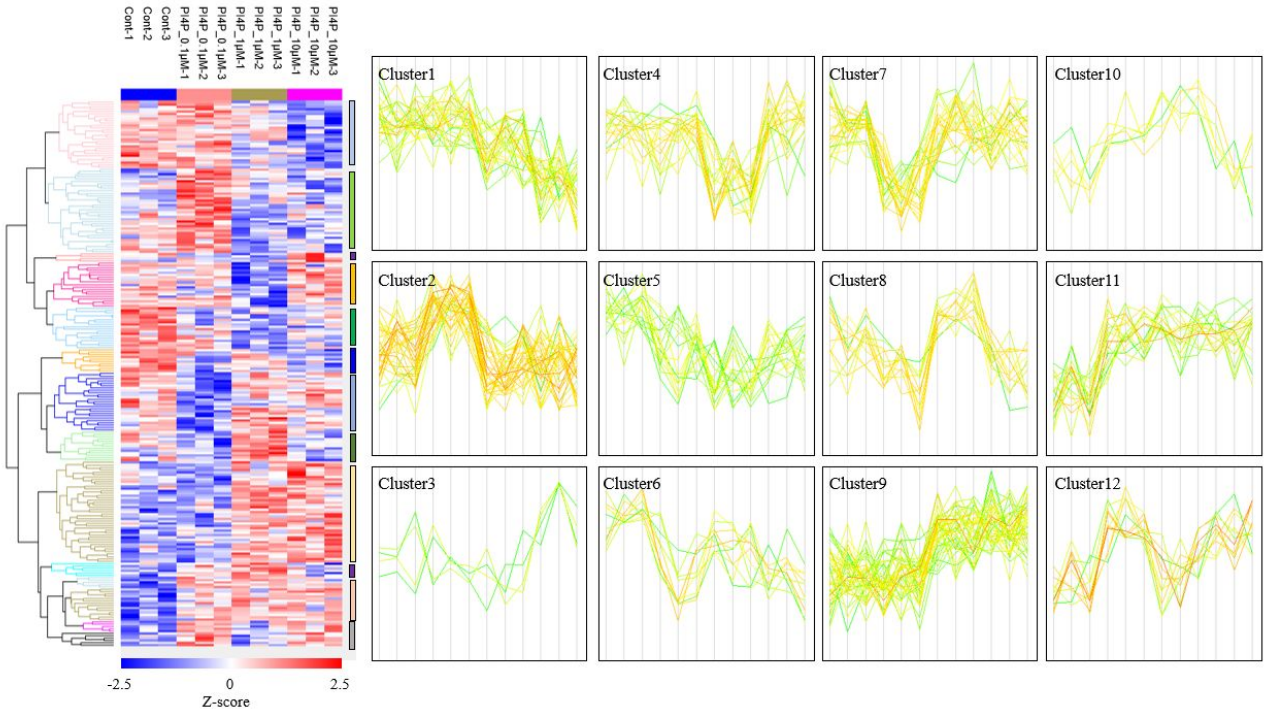


Figure. S3 continue

(E) PI4P



(F) PI5P

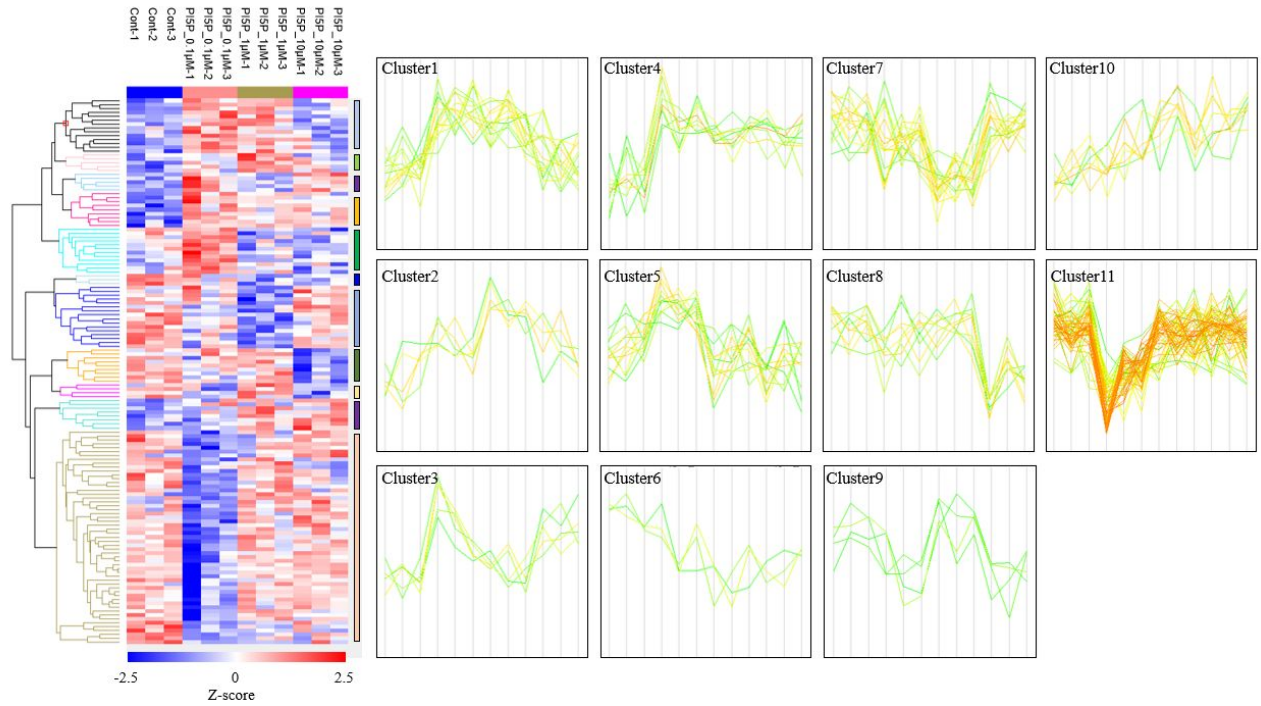
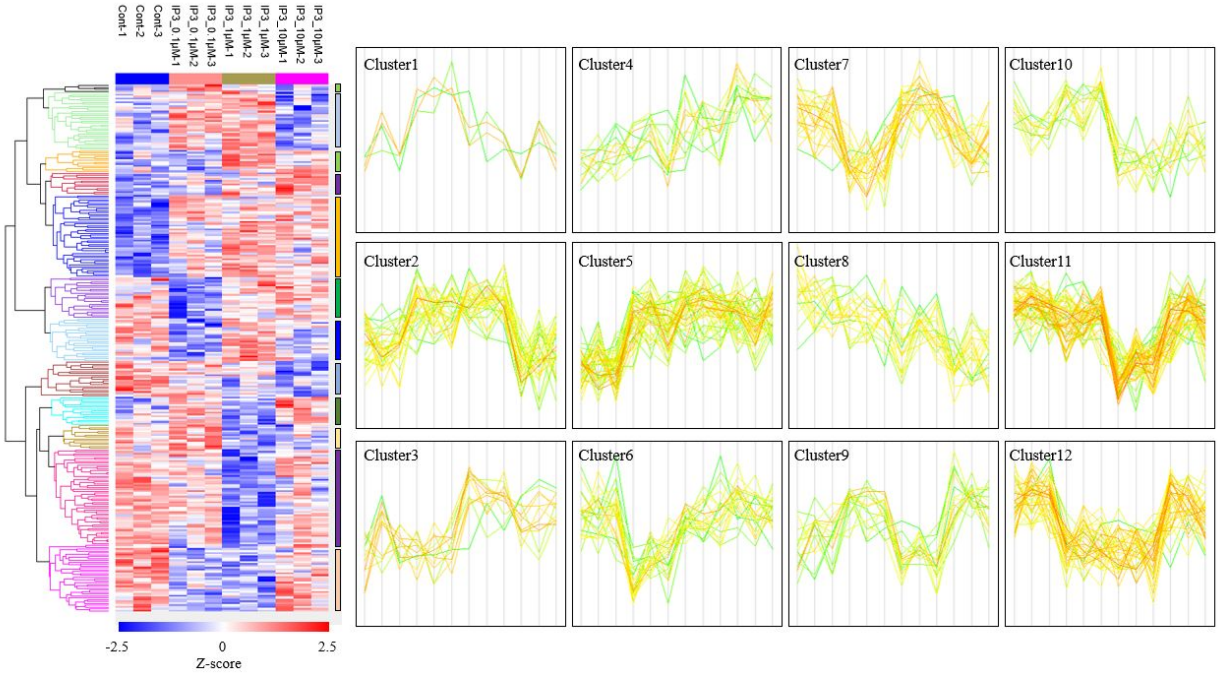


Figure. S3 continue

(G) IP3



(H) IP4

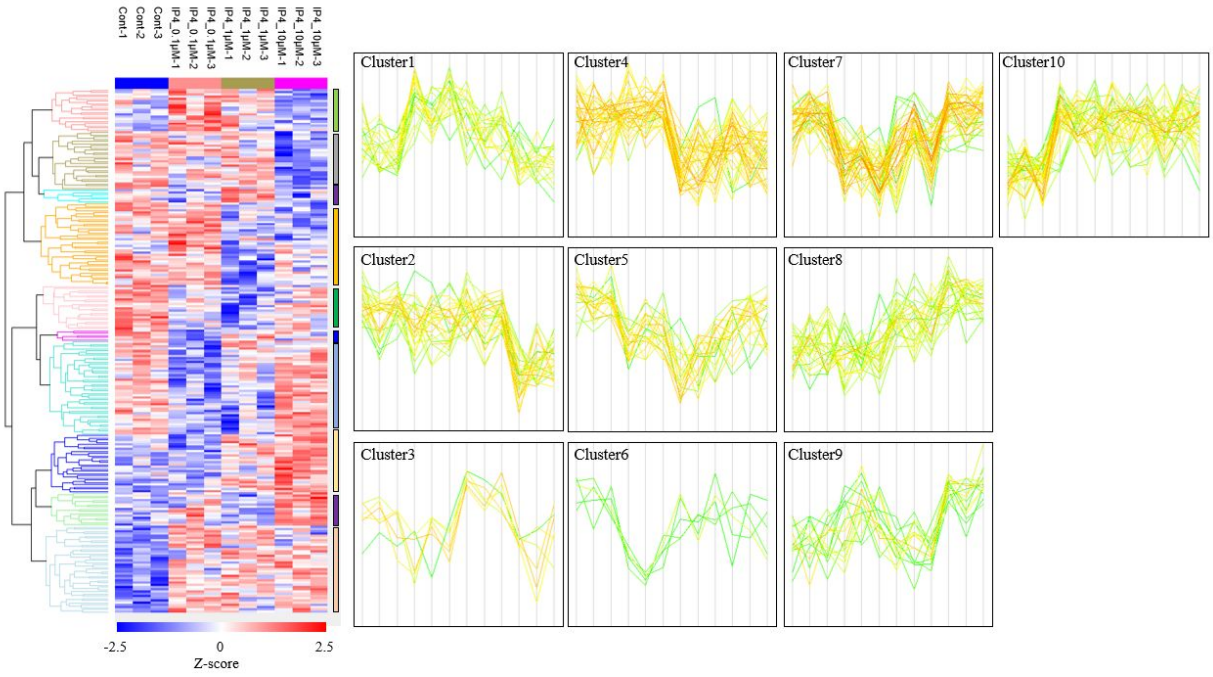


Figure S4. The levels of EGFR and proteins annotated with "signaling by receptor tyrosine kinase (R-HSA-9006934)" by LPA(14:0) stimulation. Statistical analysis was performed using one-way ANOVA followed by post-hoc Turkey's multiple comparison test (*$p < 0.05$; **$p < 0.01$).