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An Innovation Solution of Functional Metabolomics Reveals the Central role of Pentose Phosphate Pathway in Resident Thymic Macrophages

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Introduction

Among of the Multi-omics, the functional metabolomics is a powerful technique for understanding biological systems by measuring the abundance of metabolites, however, data interpretation is often complicated by a lack of dynamic information. The innovation targeted metabolomics combine multi-methods were developed to improve the analytical performance by considering peak shape, separation and metabolite coverage of Central carbon metabolism, Energy metabolism, and Amino acid metabolism to cover over hundreds of metabolites using UHPLC/Q-TOF systems in body fluids, tissue or cultured cell line of biological origin. This study was applied the solution for the research of the bone marrow-derived macrophages (BMDMs) reveal the pentose phosphate pathway (PPP) that T cells enter the apoptotic process after failing to pass the selection, which would stress the thymus's crowded environment if the dead cells were not efficiently removed.

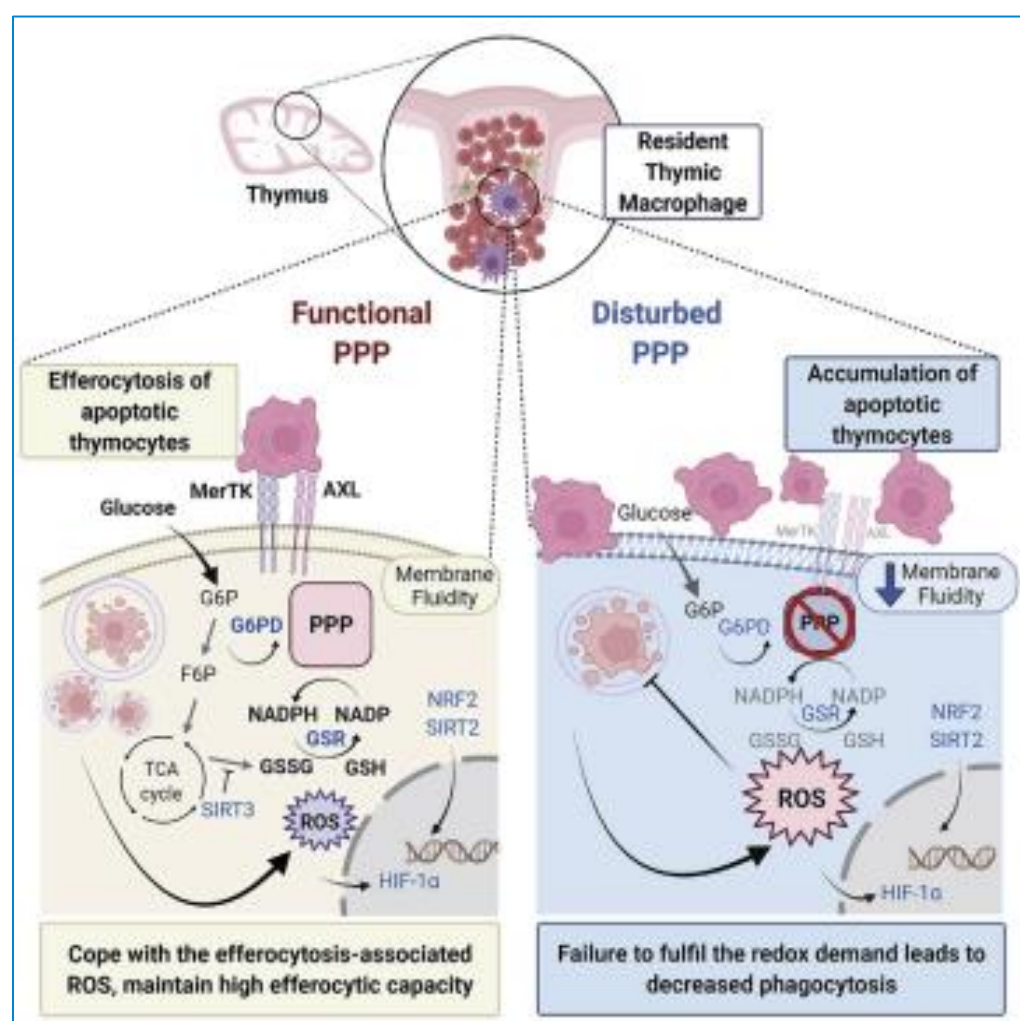


Figure 1. The thymic macrophages (TM ϕ s) population and reveal that PPP is a crucial metabolic adaptation to cope with oxidative stress and support efferocytosis.

Experimental

The high coverage 143 metabolites in six biological key pathways: Glycolysis · PPP · TCA cycle · Amino acid metabolism · Energy metabolism and others. All functional metabolites have built the database and library with Agilent PCDL manager software. (Table 1)

Pathway metabolism	Library
Glycolysis	17
PPP	11
TCA	16
Amino Acid (AA)	46
Energy metabolism (NTP/NS)	35
Others	17
Internal standard	1

- **NTP: nucleotide**
- **NS: nucleoside**
- **ISTD: 2 or 4-Chloro-phenylalanine**

Figure 2. Totally, 143 metabolites were shown the amount of each pathway.

1290 Infinity II UHPLC Method 1

Column: Amide, 2.1X100mm, 1.7 μ m.

Mobile phase A (MPA): 15mM NH₄CO₂H and 0.3% NH₄OH in water

Mobile phase B (MPB): 15mM NH₄CO₂H and 0.3% NH₄OH in 90% acetonitrile (v/v) aqueous

Column Oven: 40 °C

The linear gradient was used with flow rate, 0.3 mL min⁻¹ and the total run time was 20min.

6546 LC/Q-TOF System

Ion source: Dual-AJS

Nebulizer gas: 45psi

Dry gas: 8 L min⁻¹

Dry gas Temperature: 280°C

Sheath gas: 10 L min⁻¹

Sheath gas Temperature: 325°C

Nozzle voltage: 250V (Positive); 1000V (Negative)

Capillary voltage: 3500V (Positive & Negative)

Fragmentor Voltage: 120V

Scan Mass Range: 50-1200 *m/z*

Results and Discussion

Generally, a significant group of nucleotide metabolites were strong retained, poor peak shapes or undetected with inappropriate or incomplete elution, because the part of phosphate group will encounter chelate effect from the metal in LC flow path. The easy, quick and convenient solution is to add 0.5mM of medronic acid (MA) into sample solution and improved the part of the separation chromatography and peak shape, especially sugar phosphate metabolites and related energy metabolism. (Figure 3 and 4)

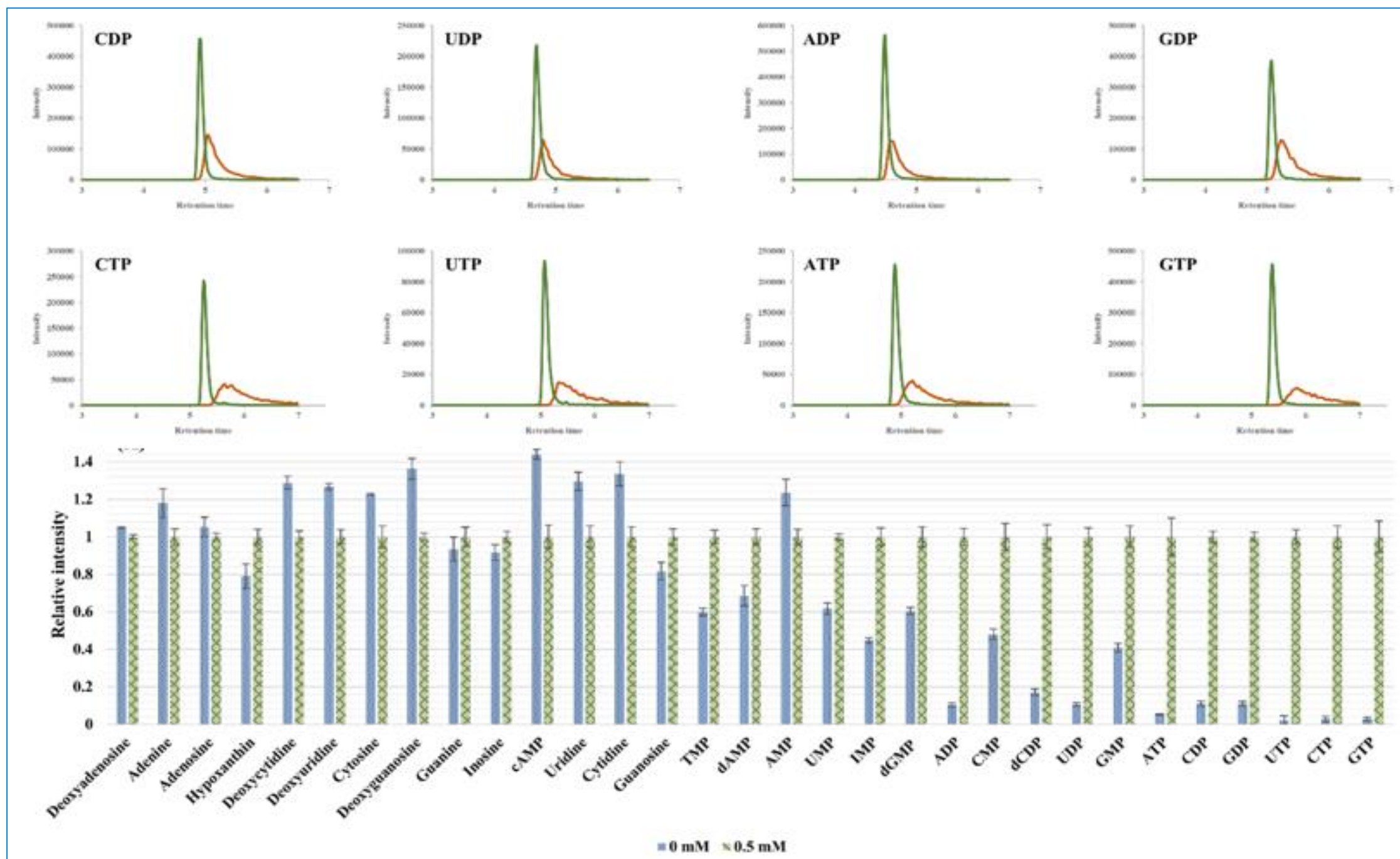


Figure 3. Comparison of the signal abundance without or with MA and show the peak shape improvement for nucleotide di and tri-phosphate metabolites.

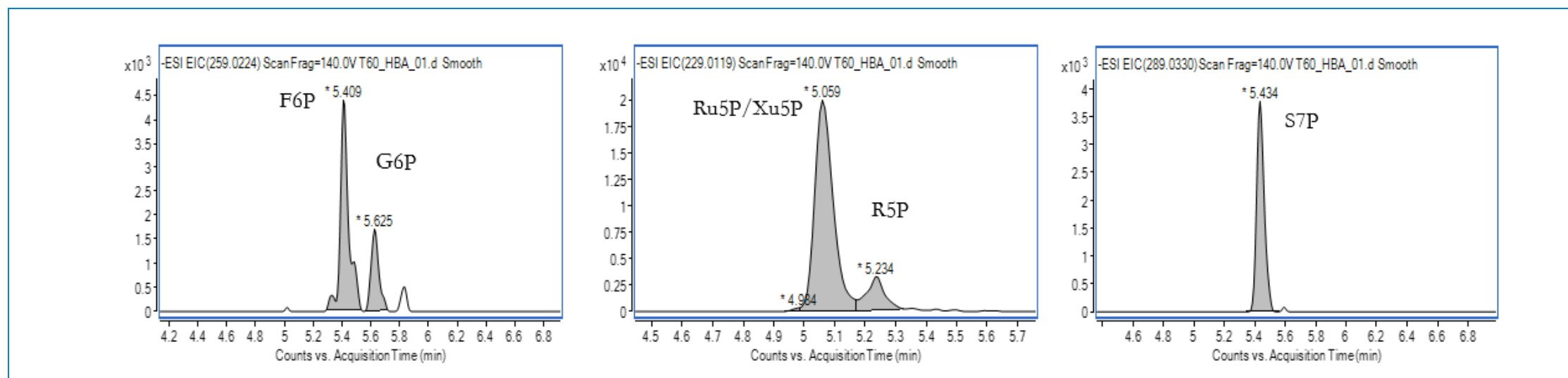


Figure 4. The well separation and chromatography of sugar phosphate metabolites (PPP) in the stem cell extract.

Results and Discussion

The high coverage solution of functional pathways were applied to the experience of efferocytosis with fluxomics analysis. The murine bone marrow-derived macrophages (BMDMs) were treated and pulsed with $^{13}\text{C}_6$ -Glucose to follow the metabolic reprogramming. (Figure 5) The tracing experiment was performed under normoxia (20% O_2) and the thymic microenvironment-mimicking condition (5% O_2). The glucose flux to glycolysis and generating of 3PG was not altered by efferocytosis. Instead, an apparent increase of PPP intermediates, including Ru5P/Xu5P, R5P and S7P was observed in the presence of active efferocytosis. (Figure 6)

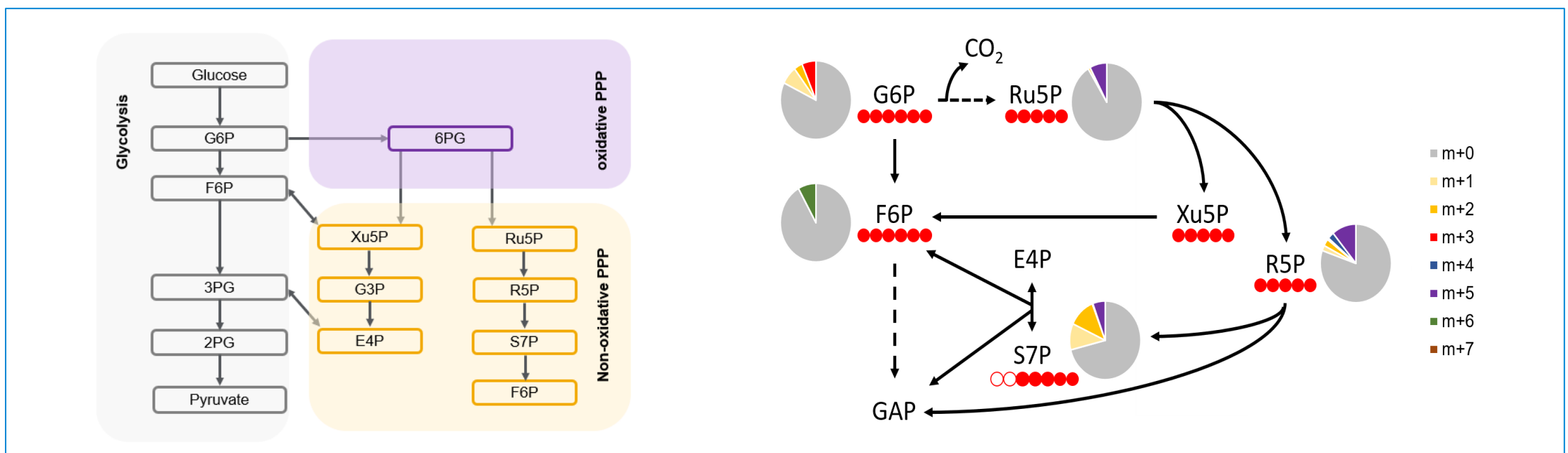


Figure 5. Simplified illustration of glycolysis and PPP. The result of flux analysis showed that the treatment led to a preferential usage of PPP in BMDMs.

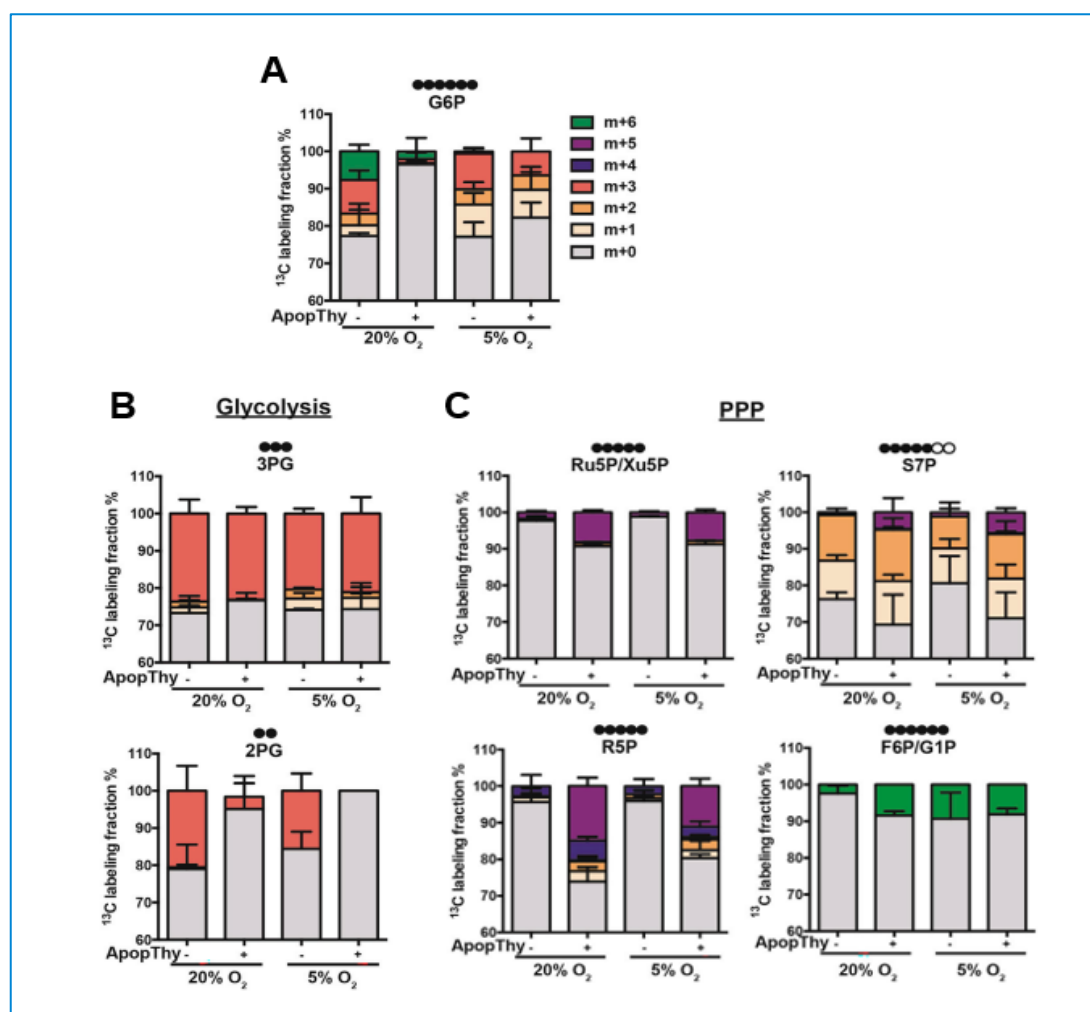


Figure 6. (A-C) The BMDMs were treated with apoptosis cells and then pulse with tracer for 120min, and subjected to LC/Q-TOF analysis. (n=3)

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Conclusions

The initial results have shown that the addition of modifier to the sample solution benefits the analytical sensitivity and separation of low-level sugar phosphate metabolites, which can be used routinely in functional metabolomics analyses. The $\text{TM}\phi\text{s}$ rely on the pentose phosphate pathway to overcome the oxidative stress brought by phagocytosing apoptotic thymocytes.

References

1. Trace Phosphate improve ZIC-pHILIC Peak Shape, Sensitivity, and Coverage for Untargeted Metabolomics. *J. Proteome Res.* (2018)17:3537.
2. Targeted Determination of Tissue Energy Status by LC-MS/MS. *Anal. Chem.* (2019) 91:5881.



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