

Genome-Wide Quantitative Proteomic and Transcriptomic Analysis Reveals Post- Transcriptional Regulation of Mitochondrial Biogenesis in Human Hematopoiesis

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复旦大学生物医学研究院

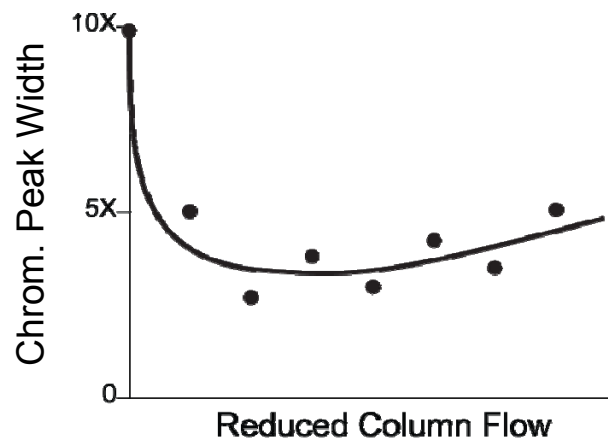
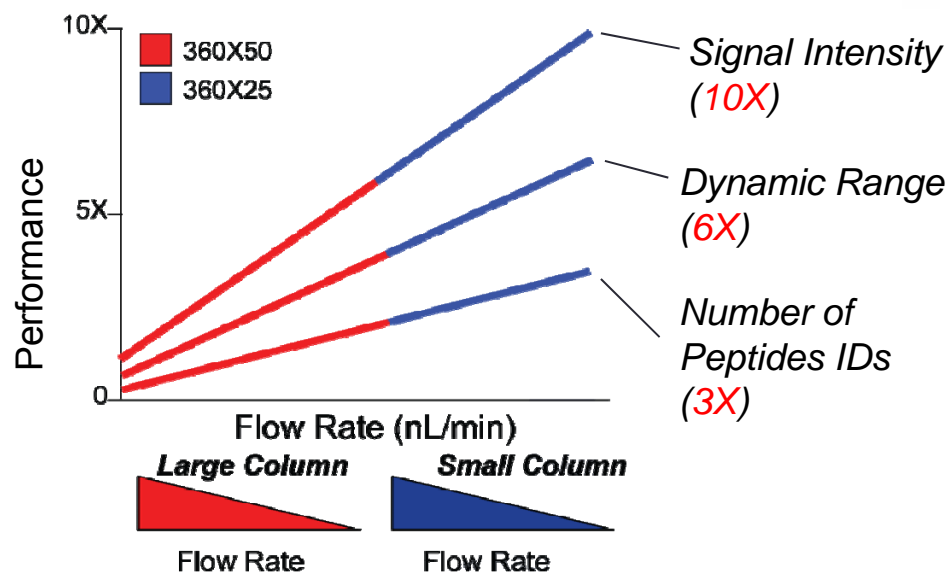
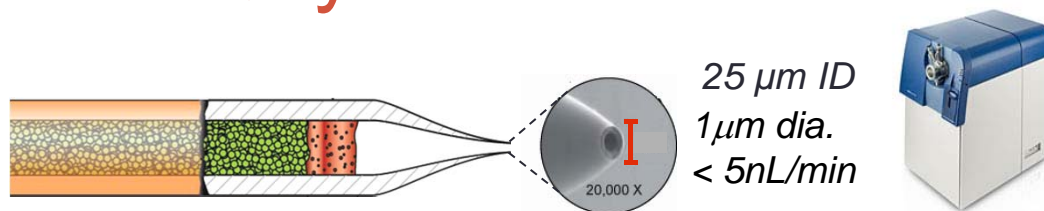
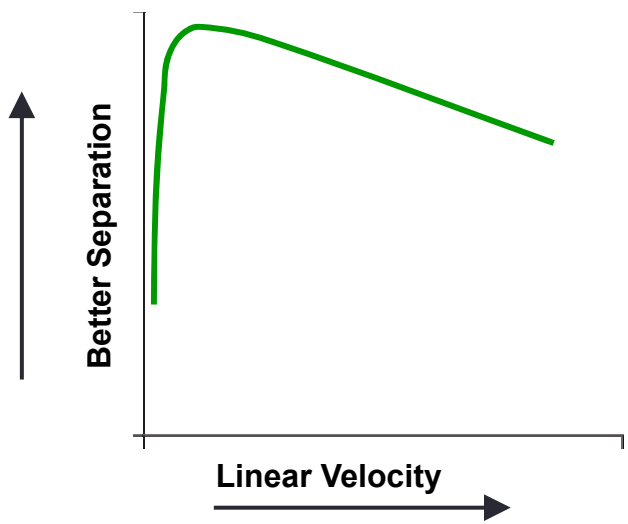
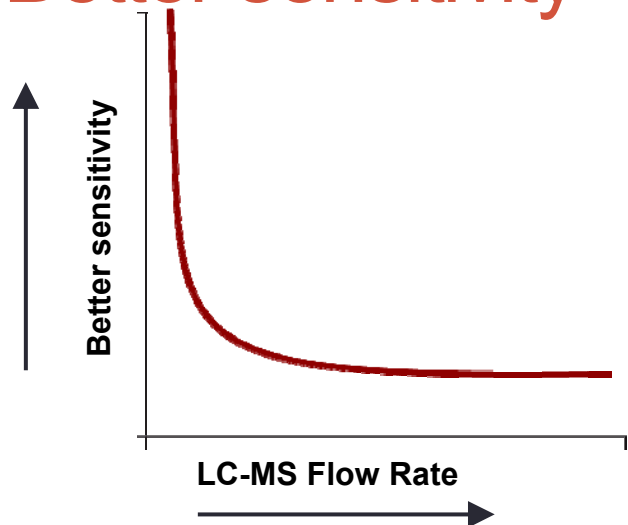
Outline

- The evolution of genome wide proteome quantification platform
- Genome wide proteome quantification platform study of red blood cell development
- Post-transcriptional regulation of mitochondrial biogenesis in human hematopoiesis

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The evolution of our GWPQ system: Better sensitivity



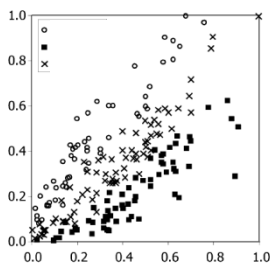
The evolution of our GWPQ system: Better separation

Starting point



25µm × 10cm

Ficarro, et al.,
Anal Chem
2009;81:3440-7



Zhou F, et al., *J Proteome Res.* 10, 2133-2139 (2010).

Finally! --2012

Fully automated
GWPQ platform
w/ 1-m Long Column



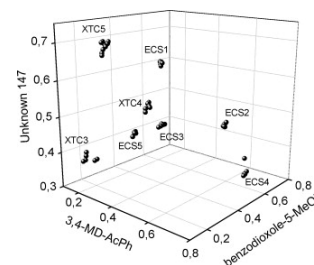
1D 1-m Long column



25µm × 100cm

Zhou F, et al., *Anal Chem*
84, 5133-5139 (2012).

3D
RP-SAX-RP



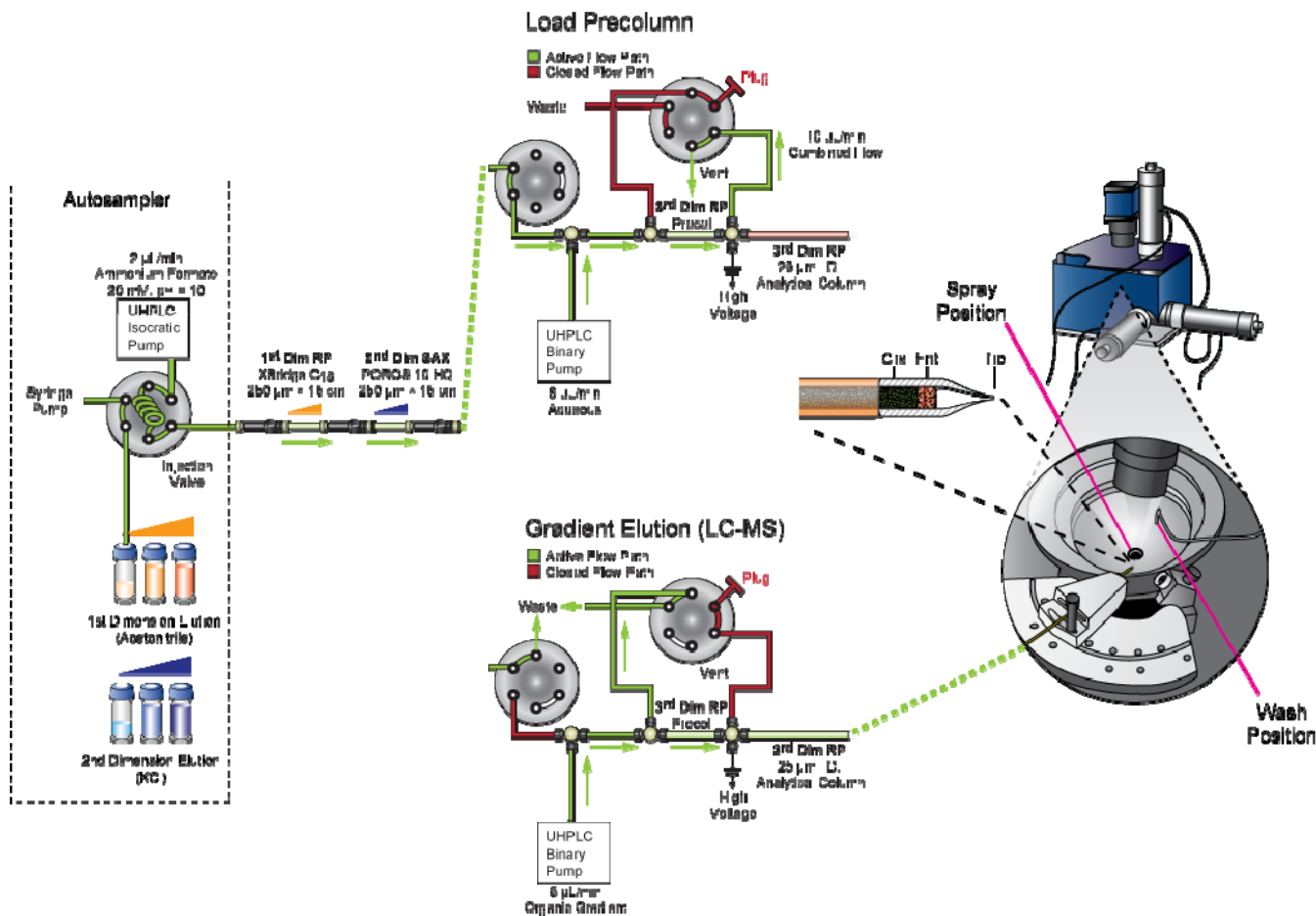
Zhou F, et al., *Anal Chem*
(2011) 83, 6996-7005

Fully automated GWPQ platform

Sample Loading, pH = 10
 1st Dimension RP, pH = 10
 2nd Dimension SAX, pH = 10

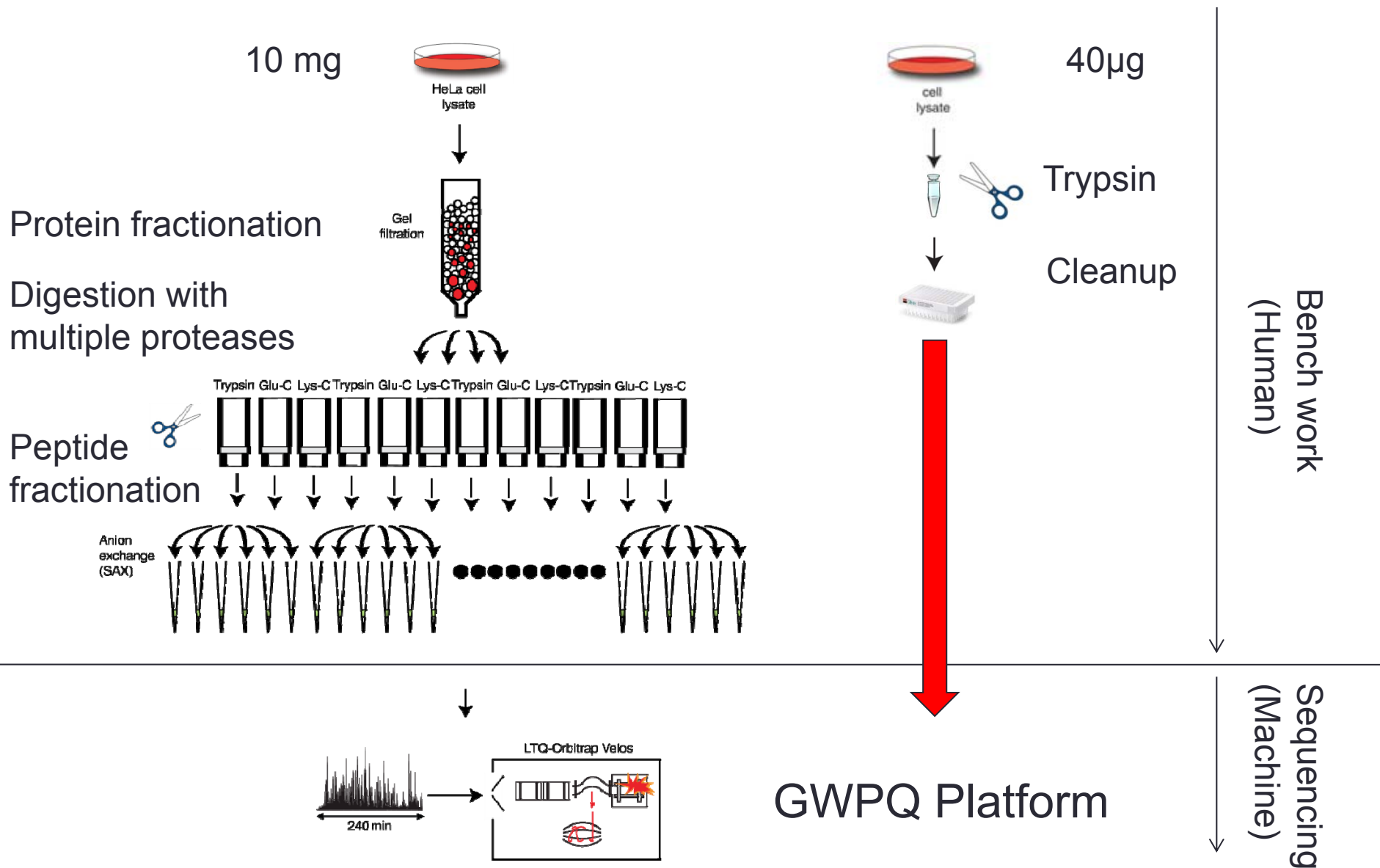
3rd Dimension RP, pH = 3
 Vented Column Configuration

Digital Picoview
 Electrospray Positioning Platform



*Fully automated
 (avoid Manual
 operation)*

*Fully online
 (avoid sample
 losses)*



Nagaraj, N. et al. *Mol. Syst. Biol.* 7 (2011).

Proteomics v.s. Ribosome profiling (part I)



Ribosome Profiling of Mouse Embryonic Stem Cells Reveals the Complexity and Dynamics of Mammalian Proteomes

Nicholas T. Ingolia,^{1,3,*} Liana F. Lareau,² and Jonathan S. Weissman¹

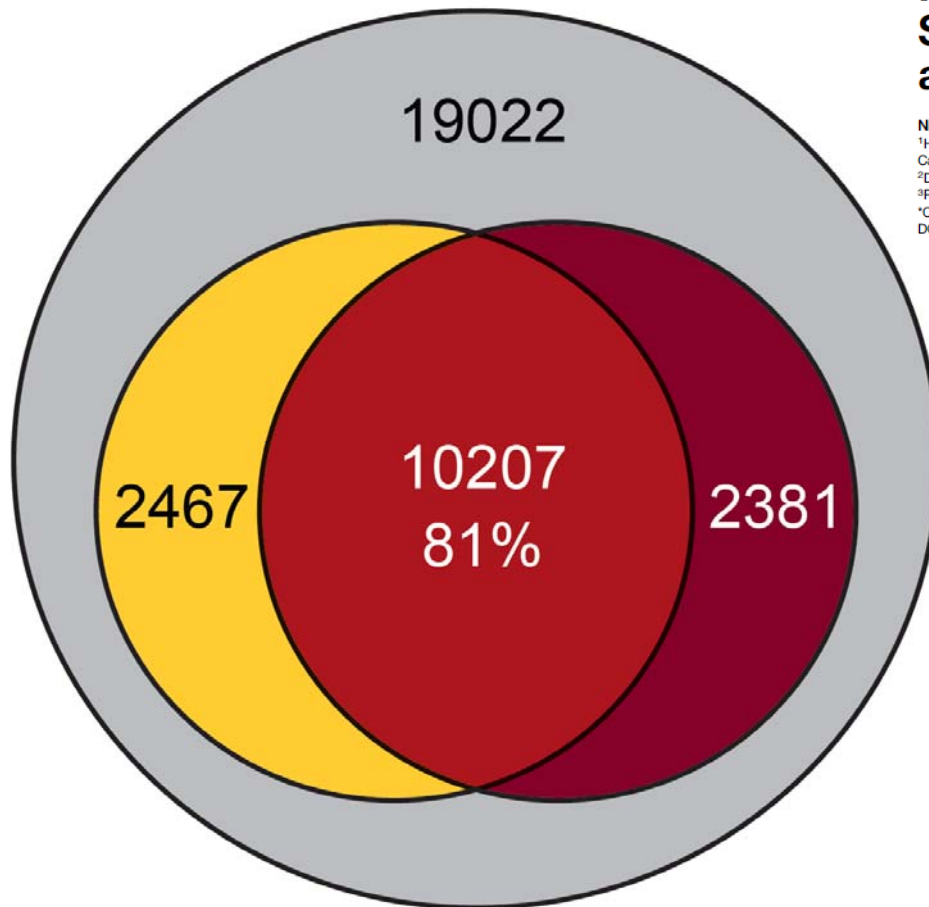
¹Howard Hughes Medical Institute, Department of Cellular and Molecular Pharmacology, University of California, San Francisco and California Institute for Quantitative Biosciences, San Francisco, CA 94158, USA




²Department of Biochemistry, Stanford University, Stanford, CA 94305, USA

³Present address: Department of Embryology, Carnegie Institution for Science, Baltimore, MD 21218, USA

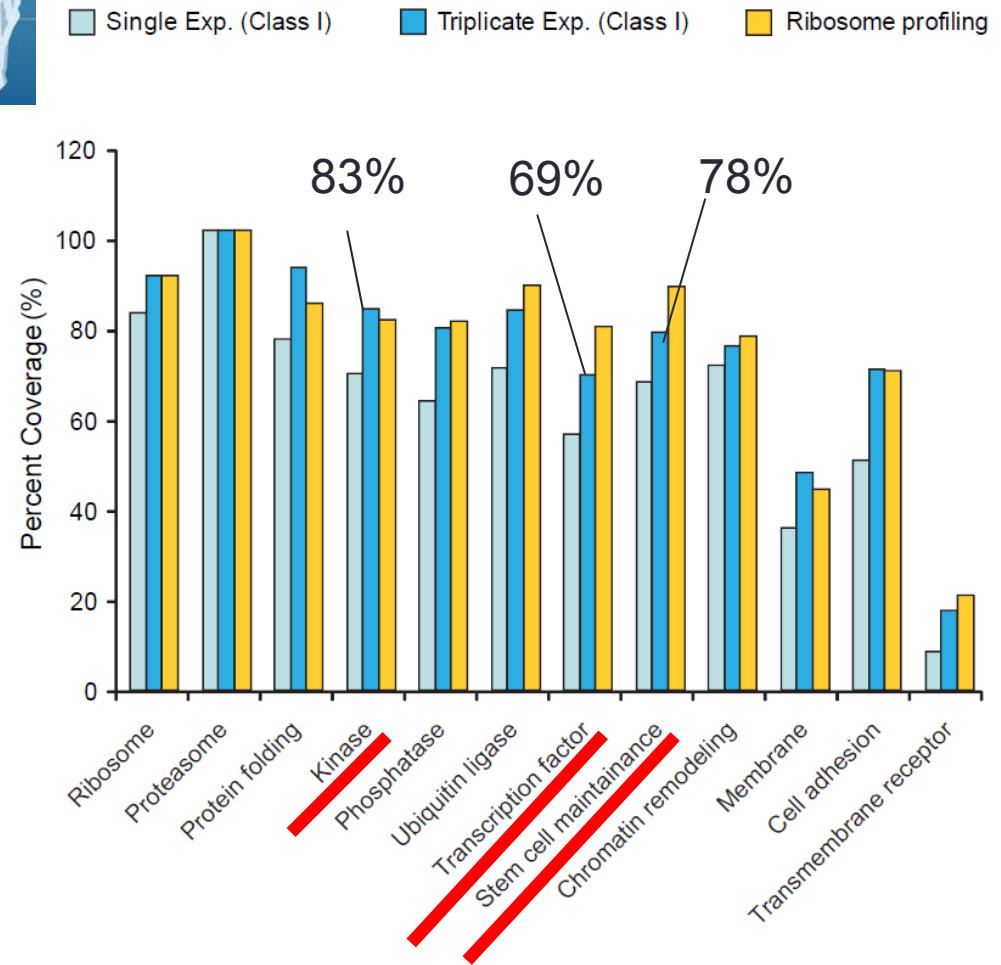
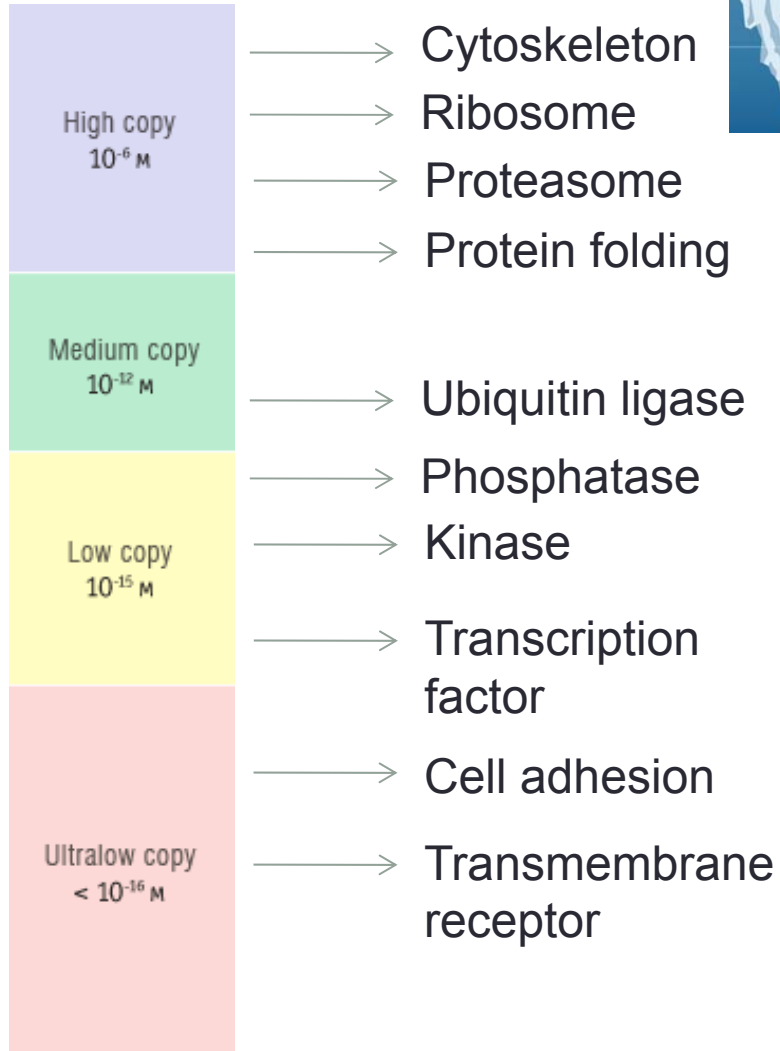
*Correspondence: ingolia@ciwemb.edu

DOI 10.1016/j.cell.2011.10.002



-  UCSC mouse database (mm9)
-  Ribosomal profiling
-  DEEP SEQ MS/MS (Class I + II)

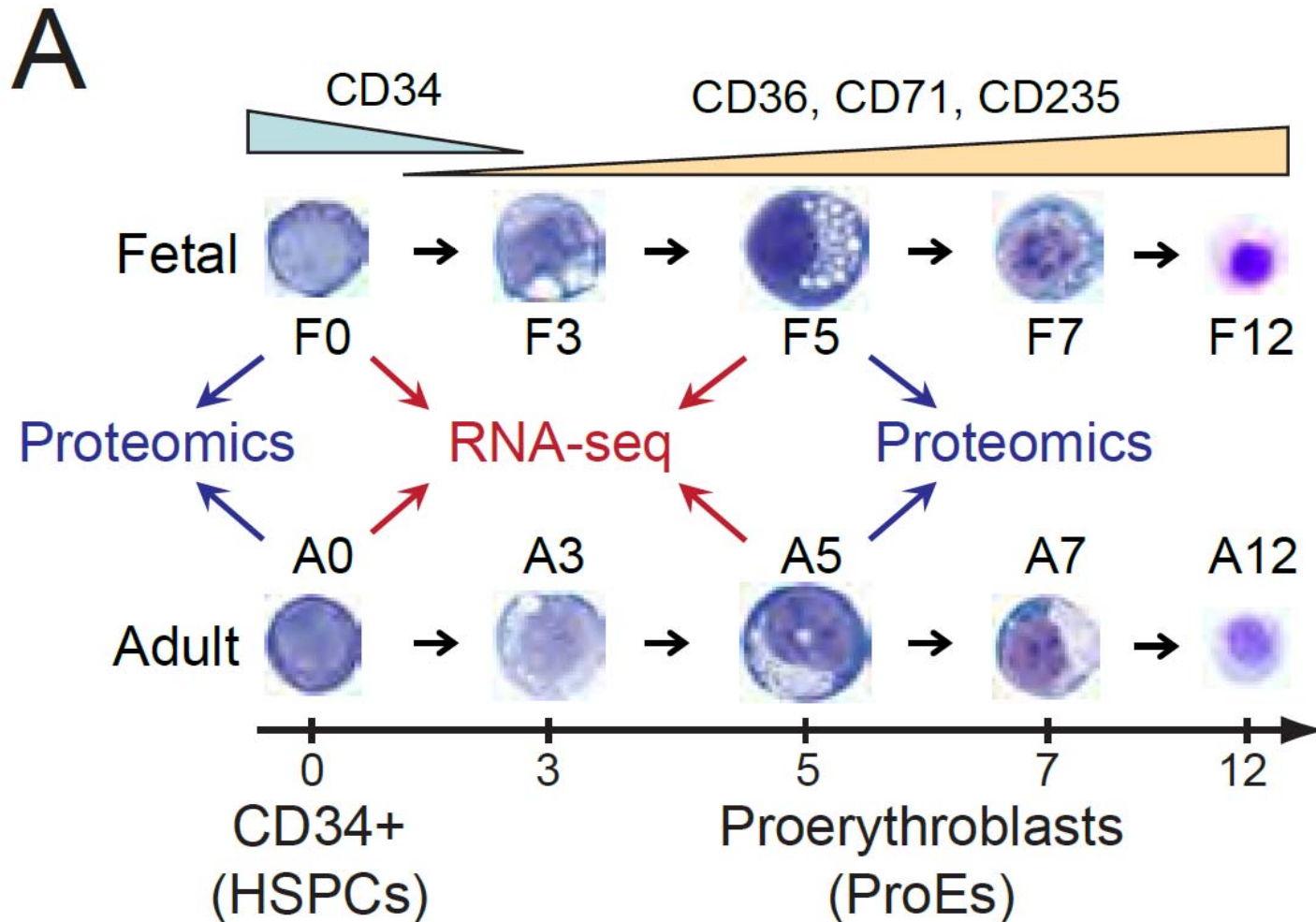
Coverage for GO functional database



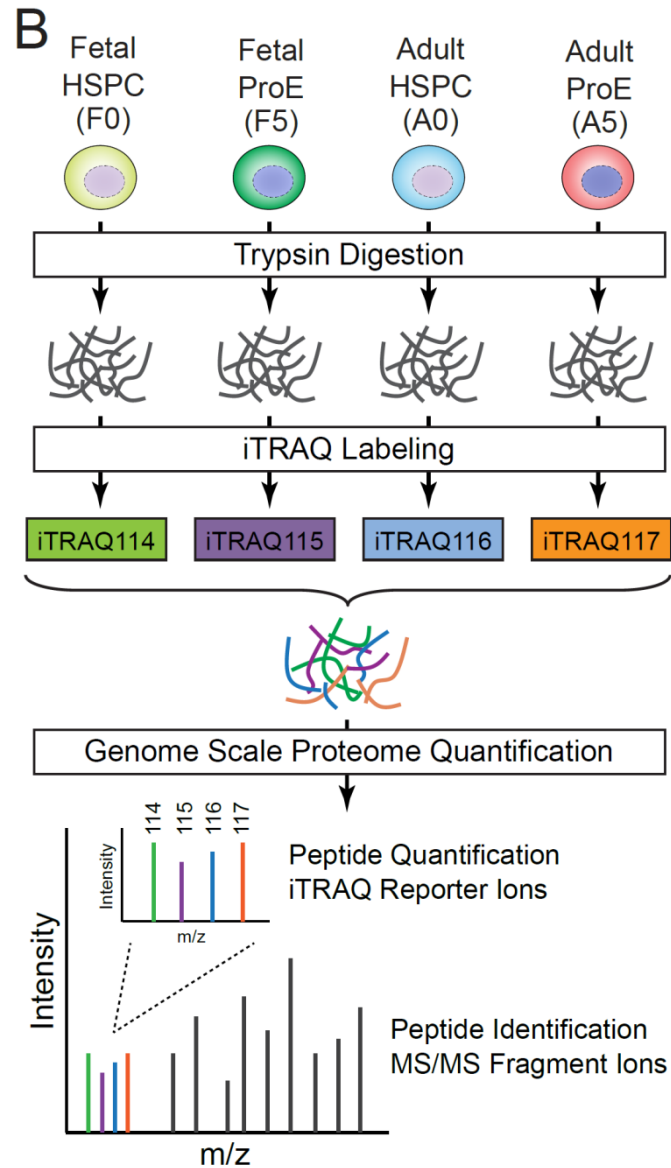
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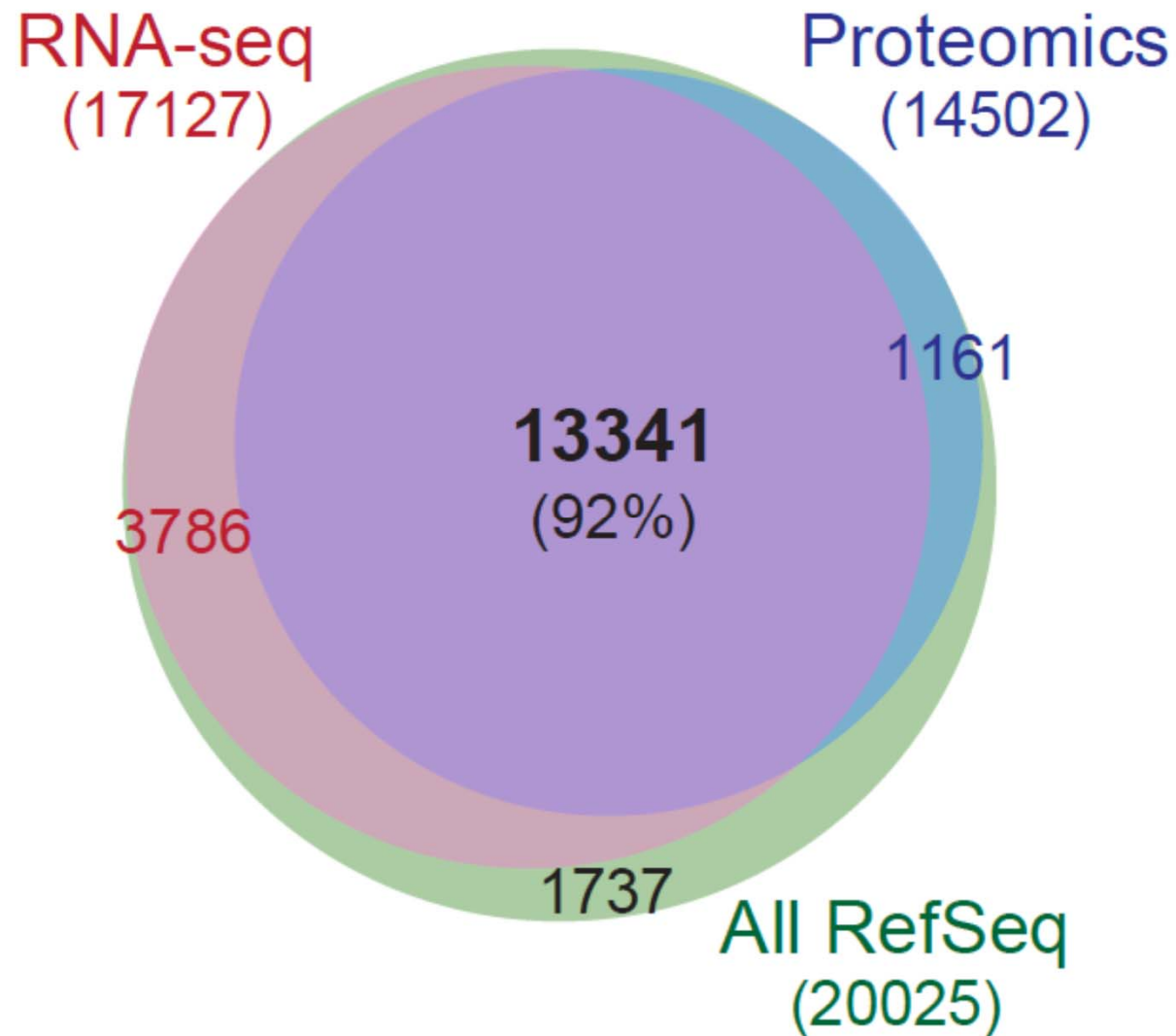
Genome-Wide Quantitative Proteomic and Transcriptomic Analysis for Hematopoiesis



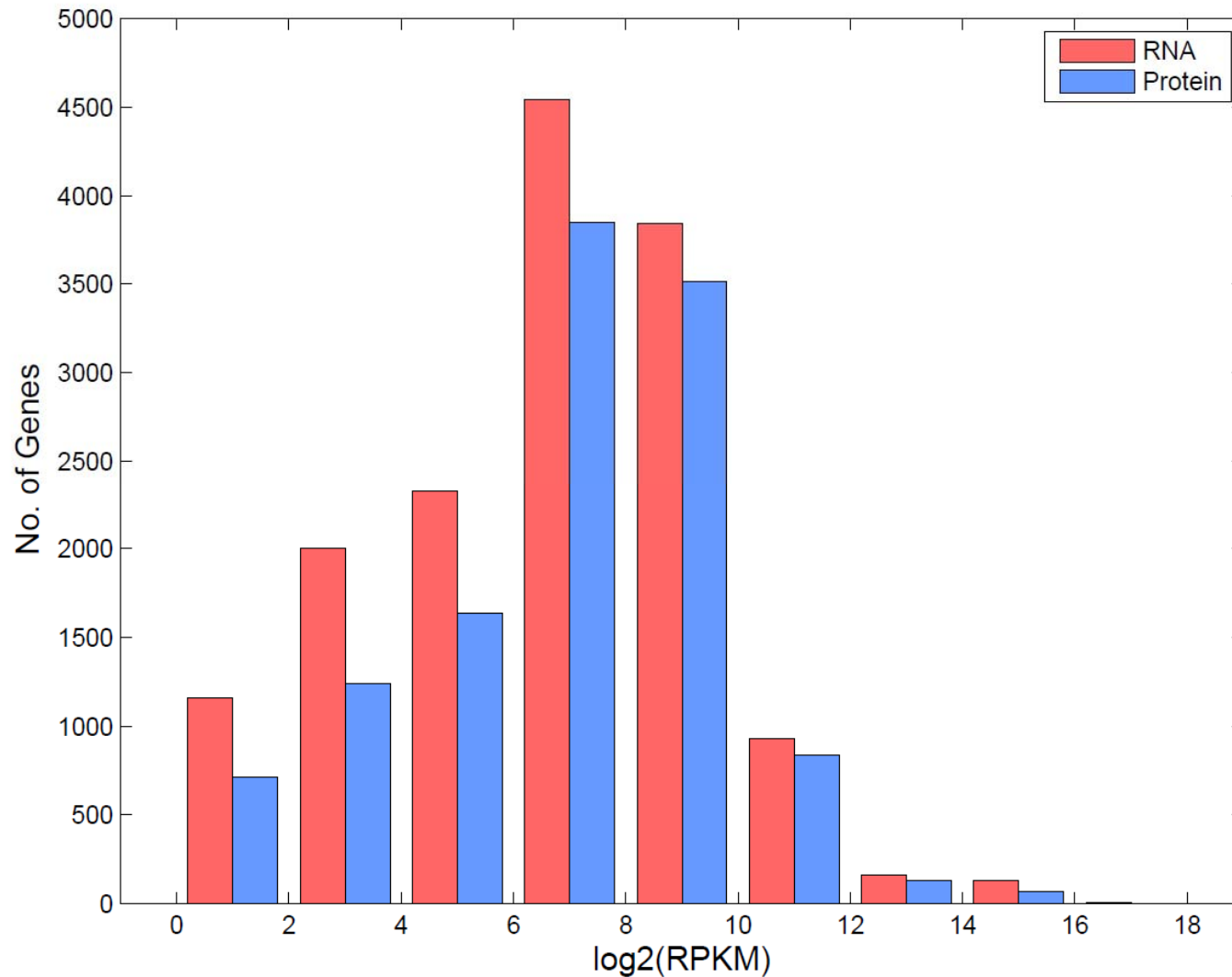
The Proteomic Experiment



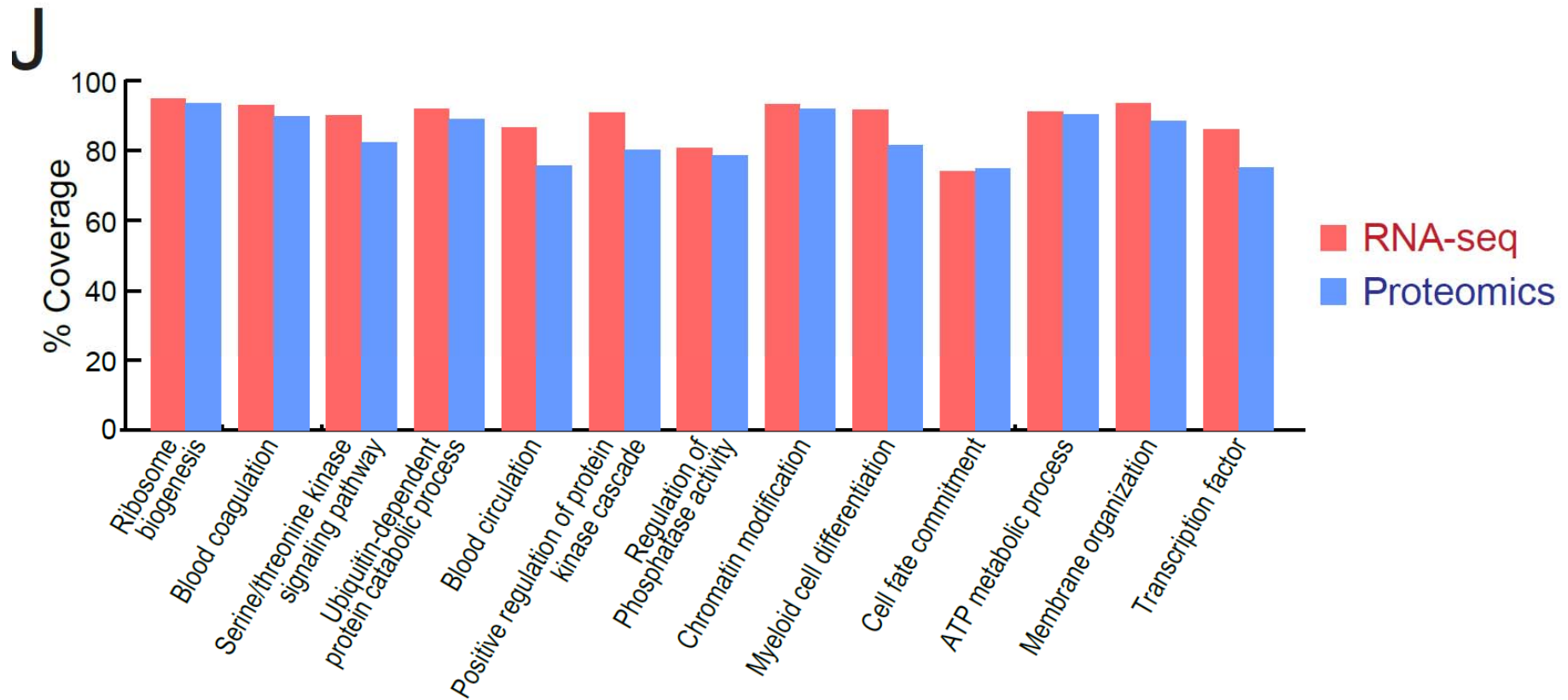
The Coverage: RNA-SEQ v.s. Proteomics



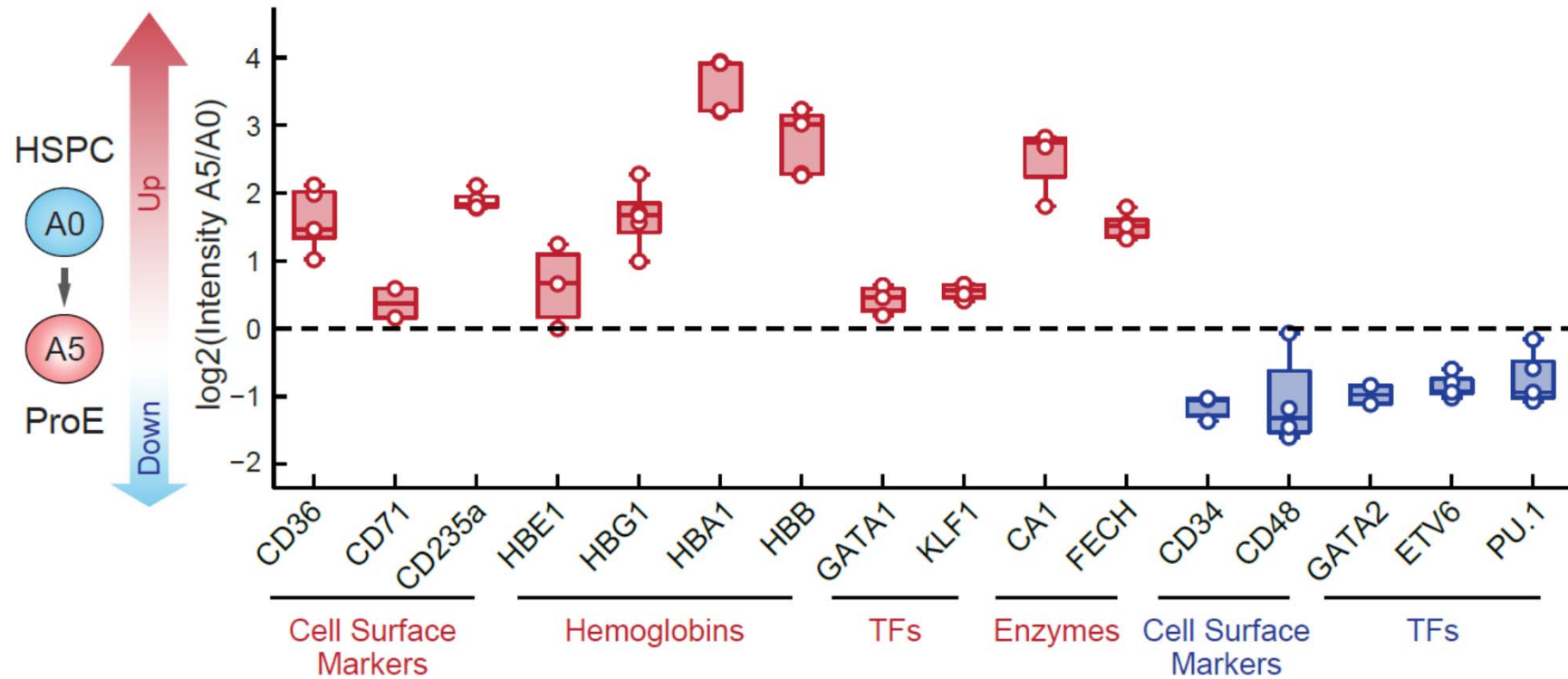
Proteomics can cover full RNA-SEQ dynamic range



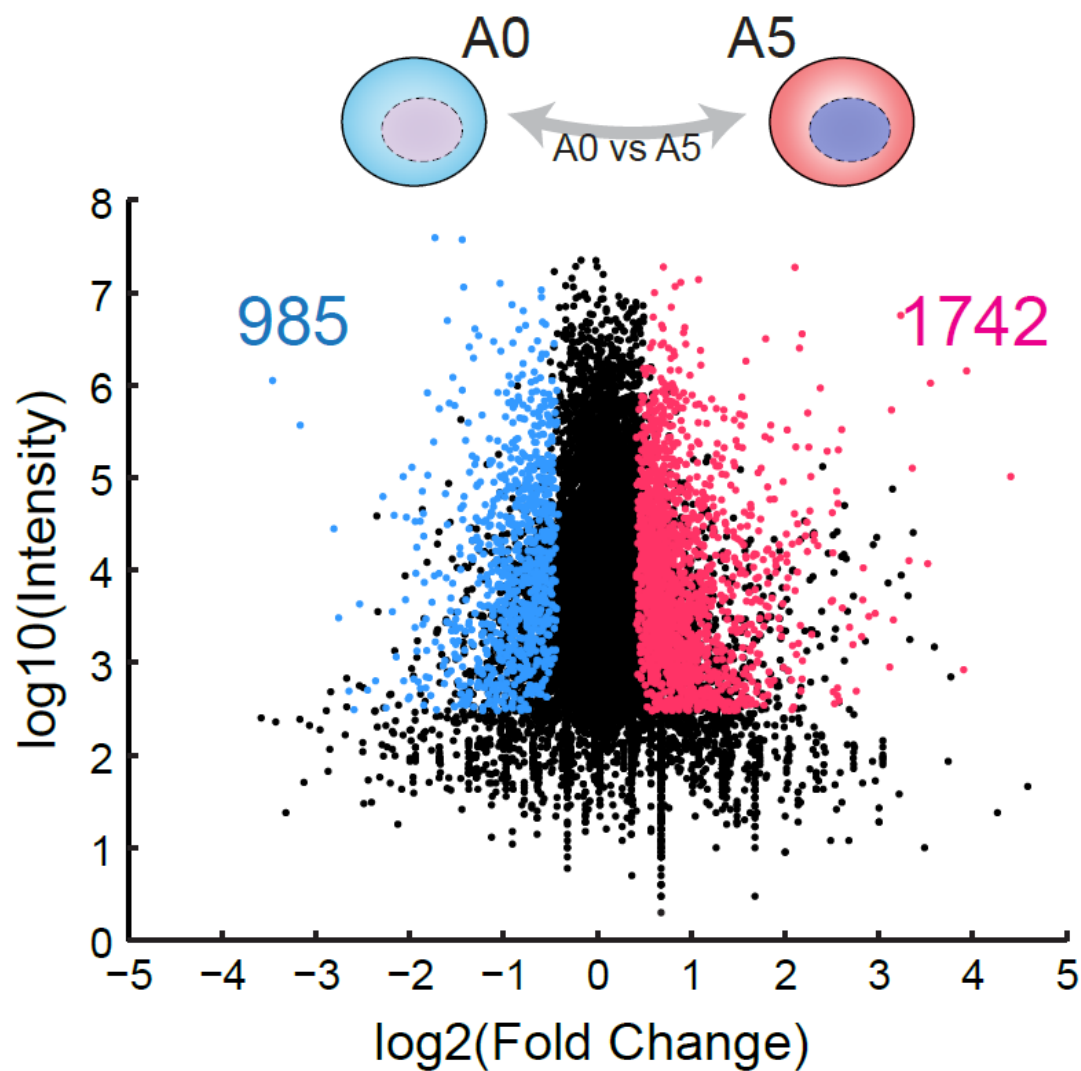
对不同类别的蛋白的覆盖率比较



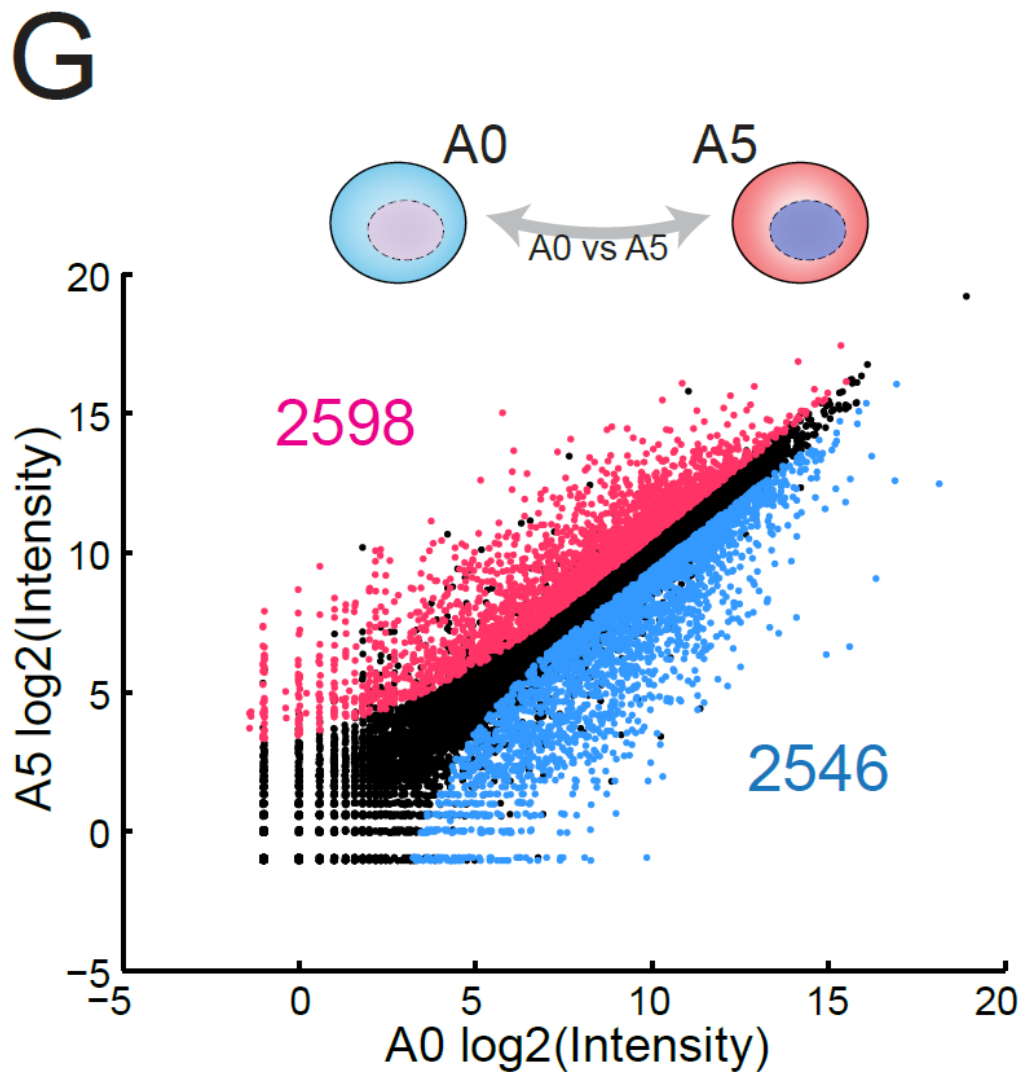
对已知红细胞发育 markers 的覆盖



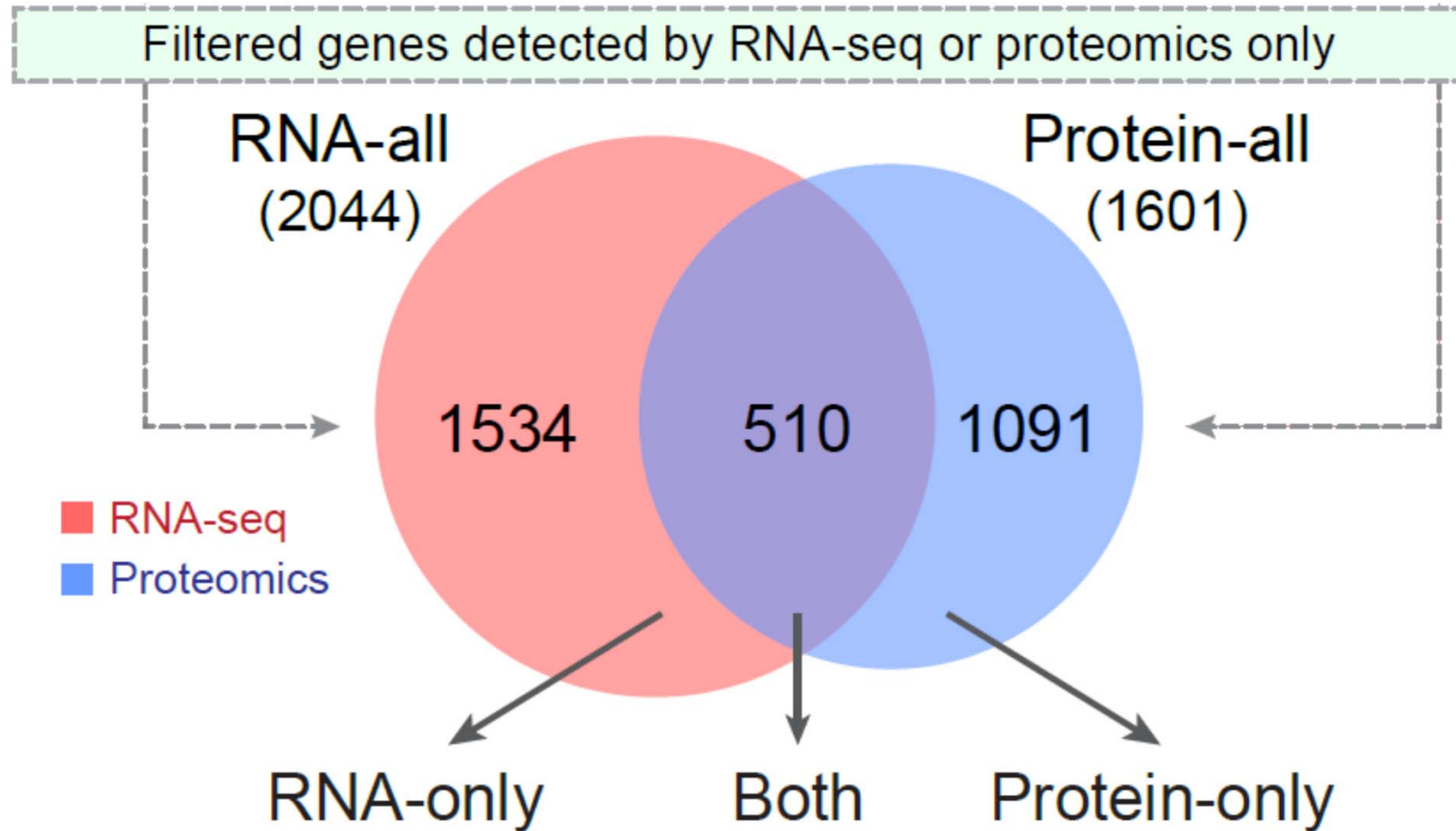
成人红细胞发育的蛋白质组定量变化



成人红细胞发育的转录组变化



蛋白质组与转录组变化的比较

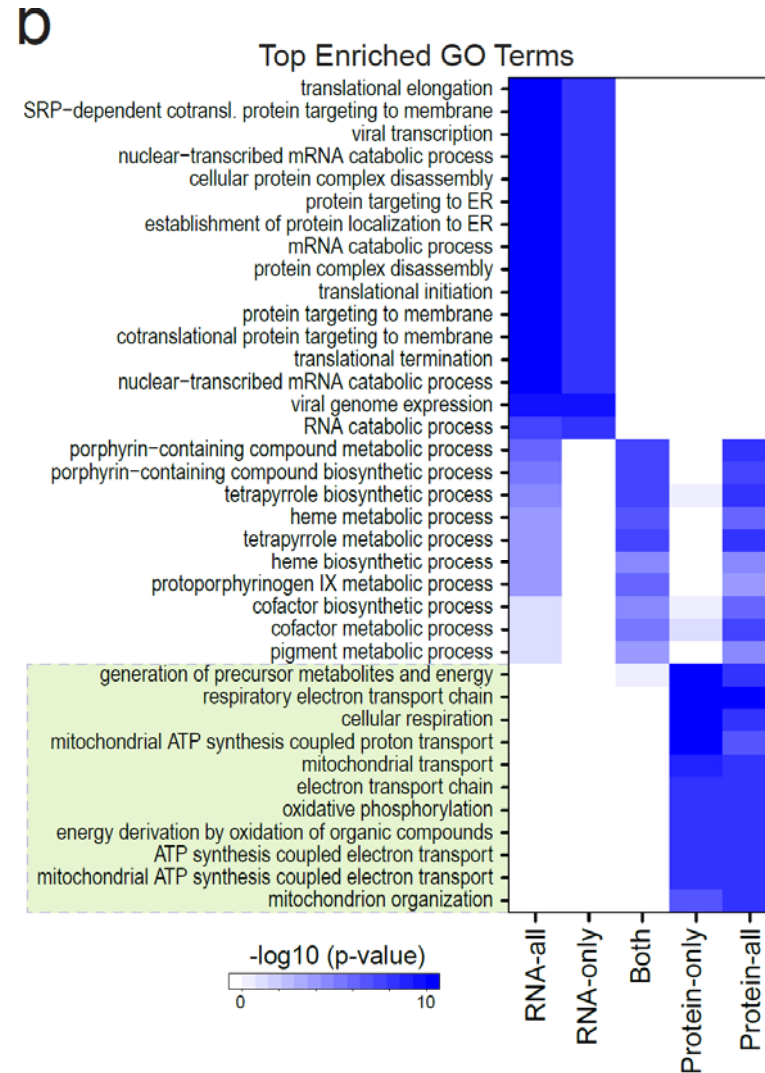


Outline

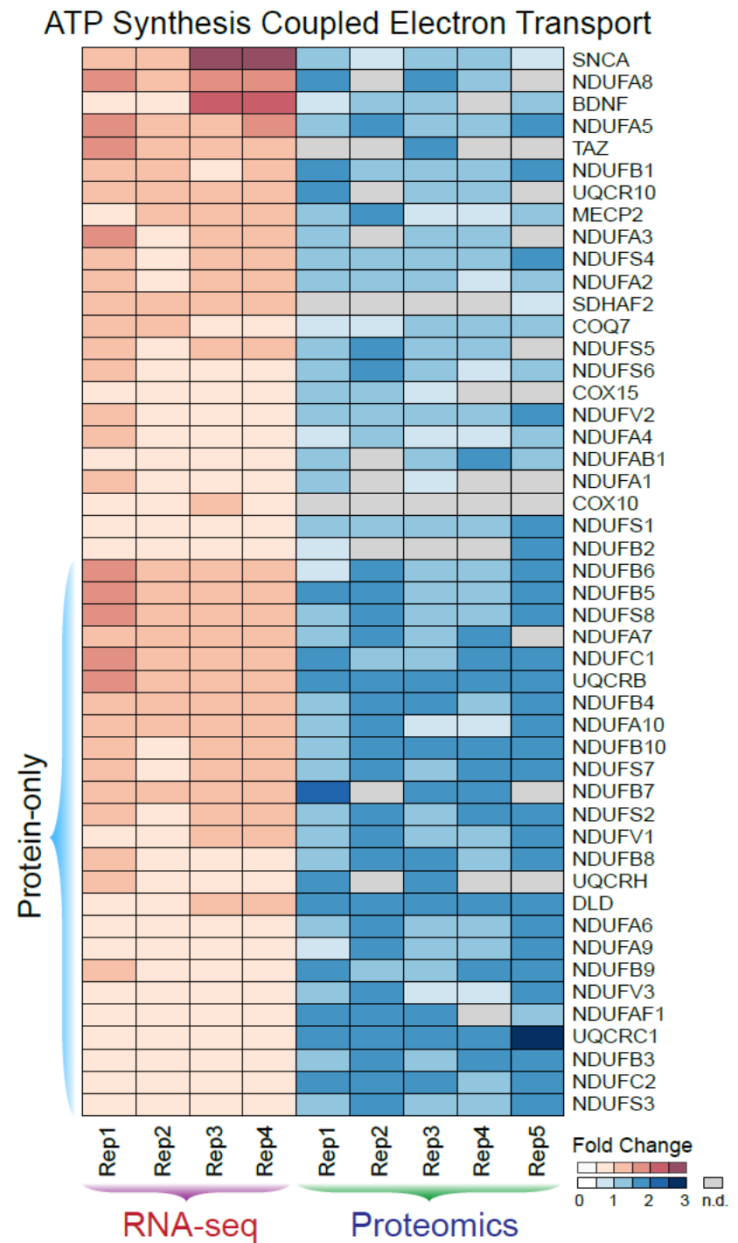
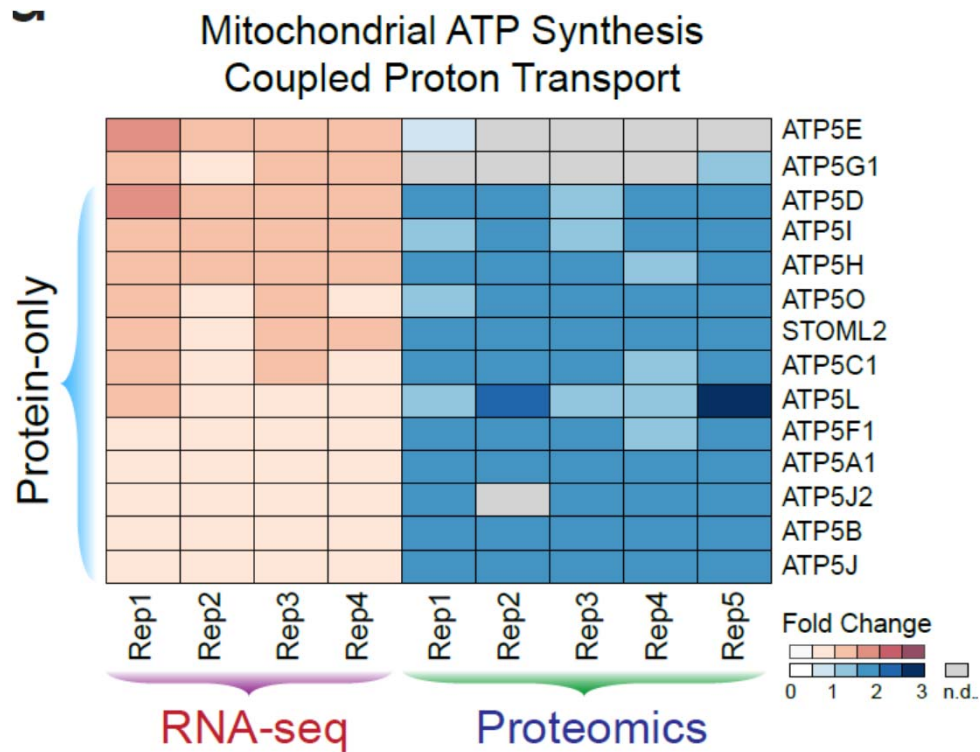
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对Protein-only的生物信息学分析

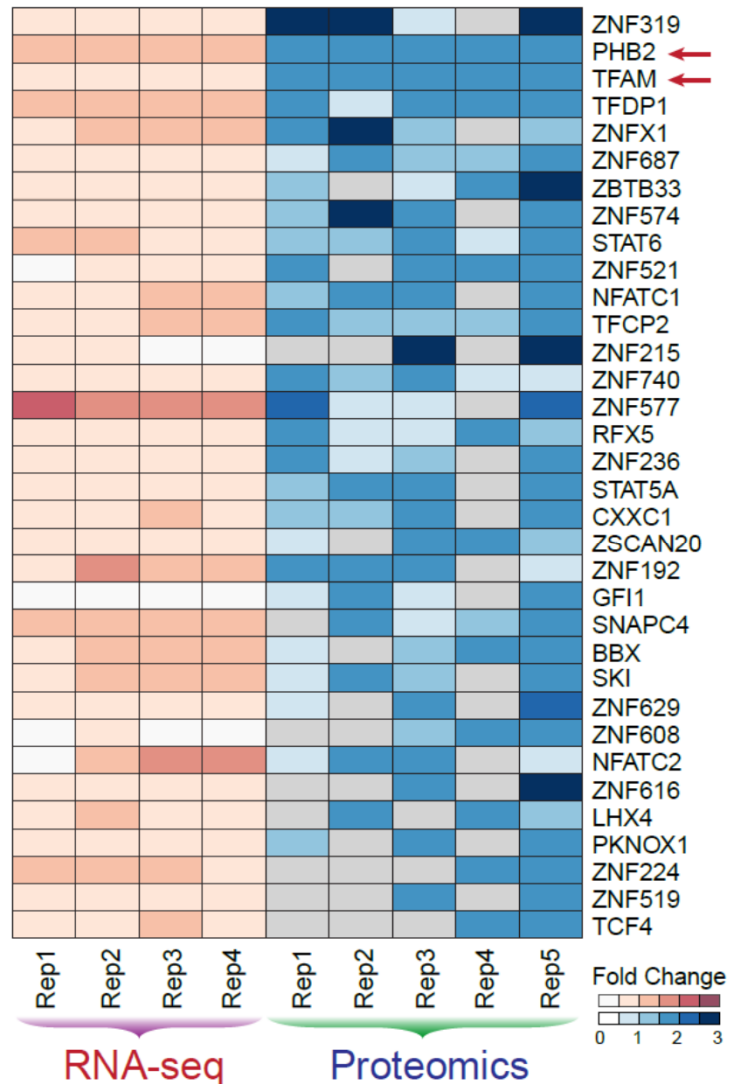
Gene ontology 分析



对GO分析结果的确认

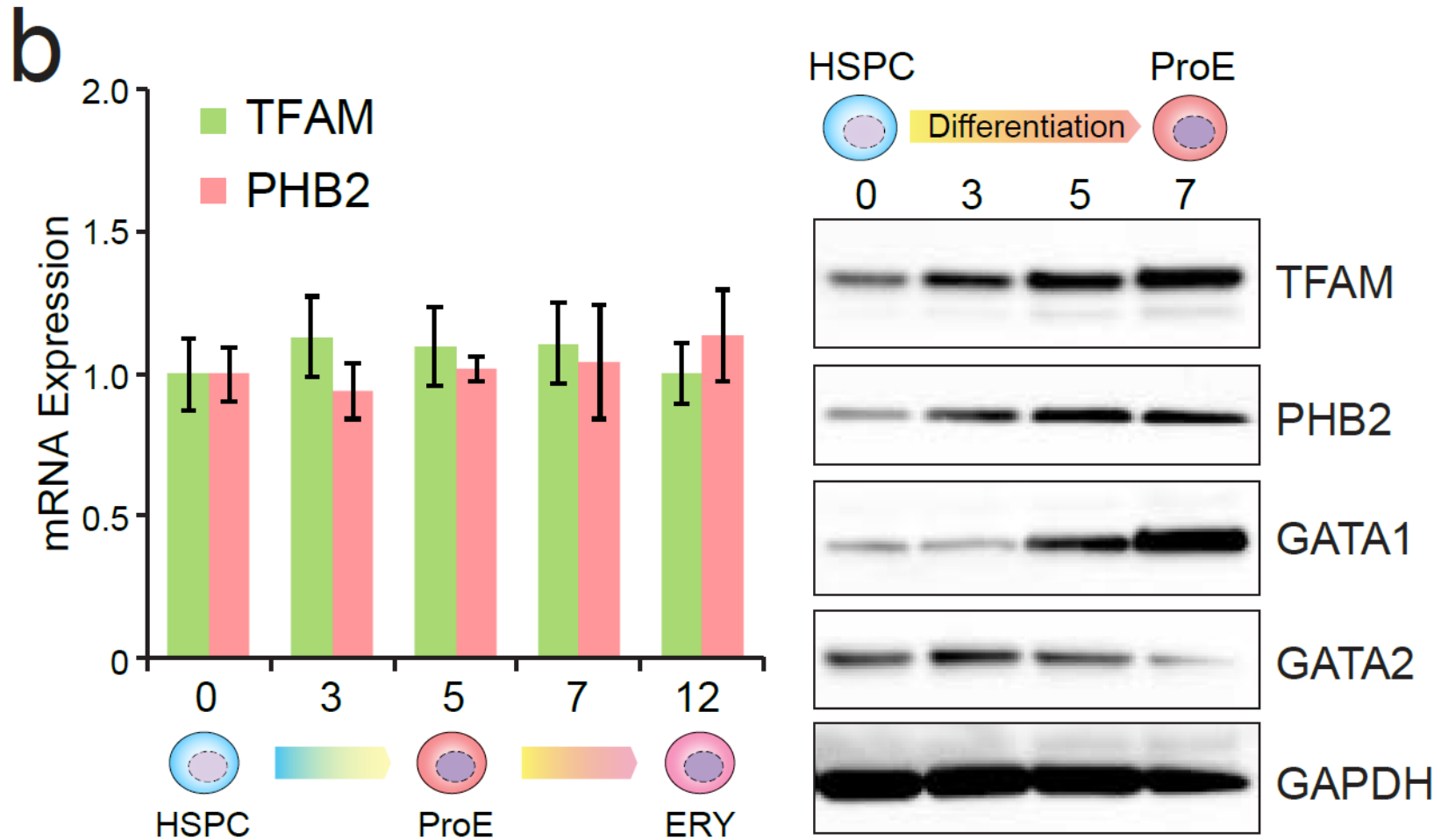


对Protein-only的转录因子分析

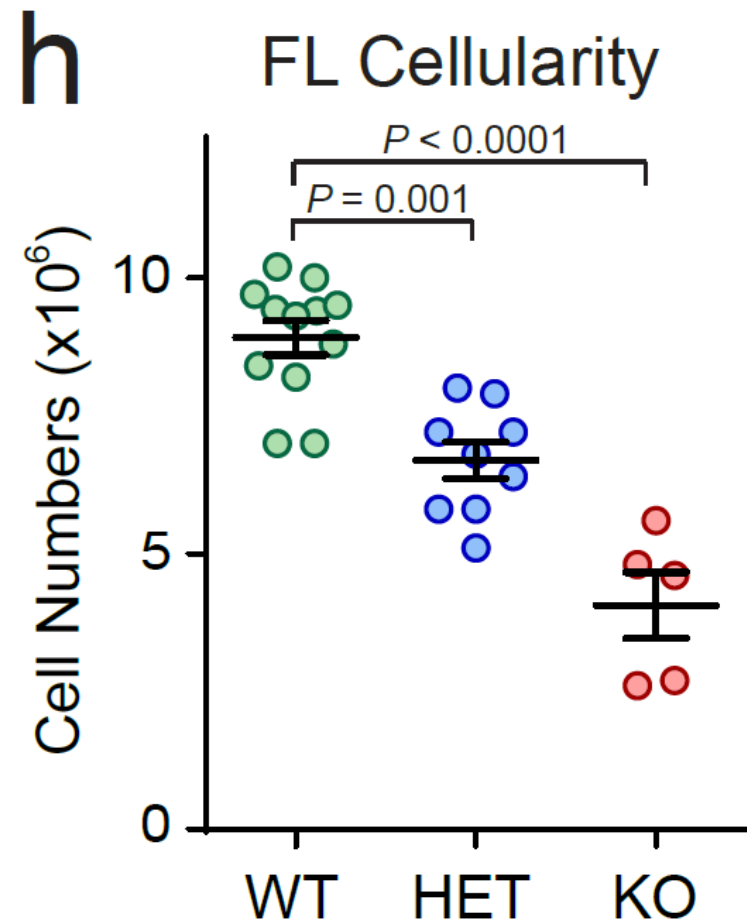
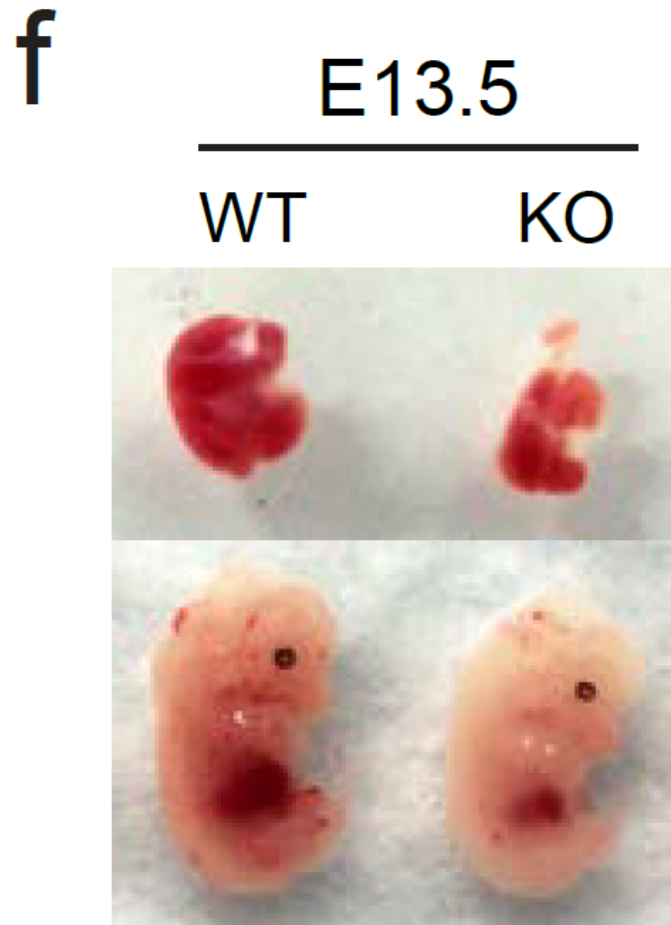


PHB2 and TFAM are Mitochondrial master transcription factors

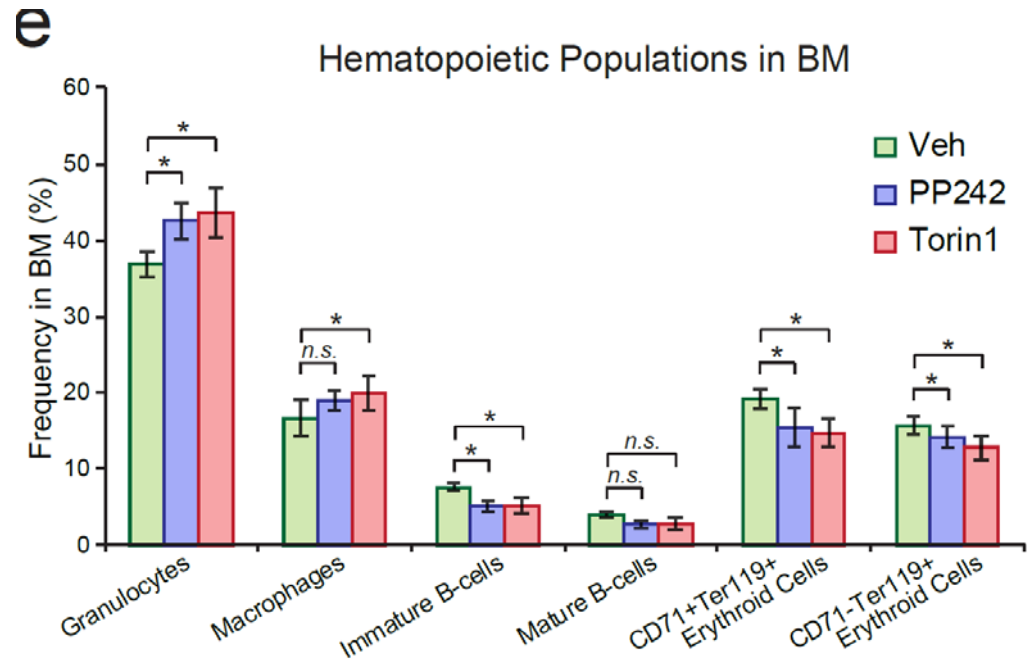
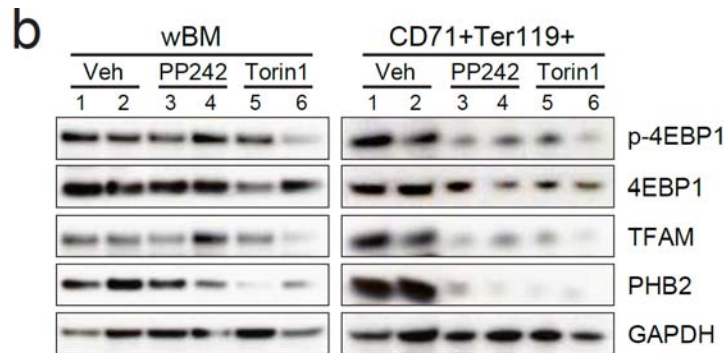
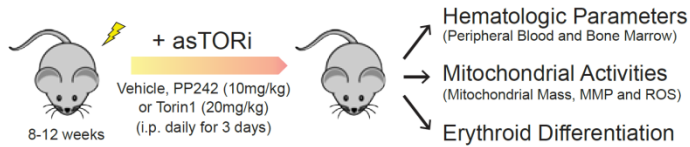
TFAM与PHB2只在蛋白水平的层面被调控



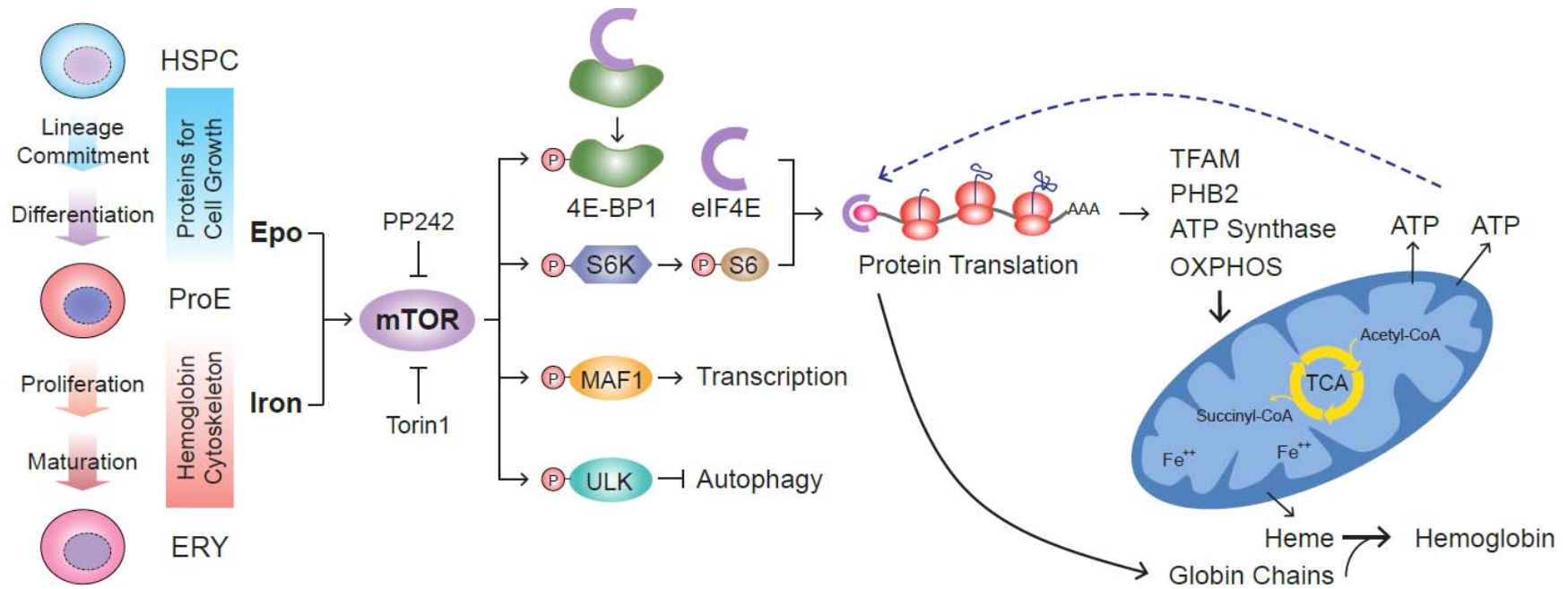
小鼠模型的验证



mTOR的抑制剂对小鼠红细胞发育的影响



模型



Ultrasensitive Genome wide Proteome Quantification (GWPPQ) Platform

- Achieve the Genome wide proteome coverage. The profiling depth is equivalent to Ribosomal profiling.
- Achieve ultrasensitive protein quantification down to 5 μg sample
- Also applied to post-translational modification (PTM) characterization such as phosphorylation, acetylation, methylation and glycosylation.
- Compatible with various proteome quantification methods. (iTRAQ, TMT, SILAC or Label free)



感谢大家的宝贵时间!

欢迎宝贵意见!