Probing metabolic pathways in a single cell

Taking the next big leap past bulk population metabolomic readouts

Most current assays to investigate metabolic pathways in vitro tend to be difficult, expensive and restricted to bulk analysis of heterogeneous populations of cells in culture. While these methods are extremely valuable, they are limited in their ability to illuminate what is happening at the single cell level. John E. Connolly and his team recently developed Met-Flow, a method using monoclonal antibodies and flow cytometry to investigate the metabolic state of individual immune cells by staining rate-limiting targets that are key steps in metabolic pathways. BD Biosciences is excited to enable deeper insights into this growing field by offering a broad collection of conjugated Met-Flow antibodies.

Glycolysis

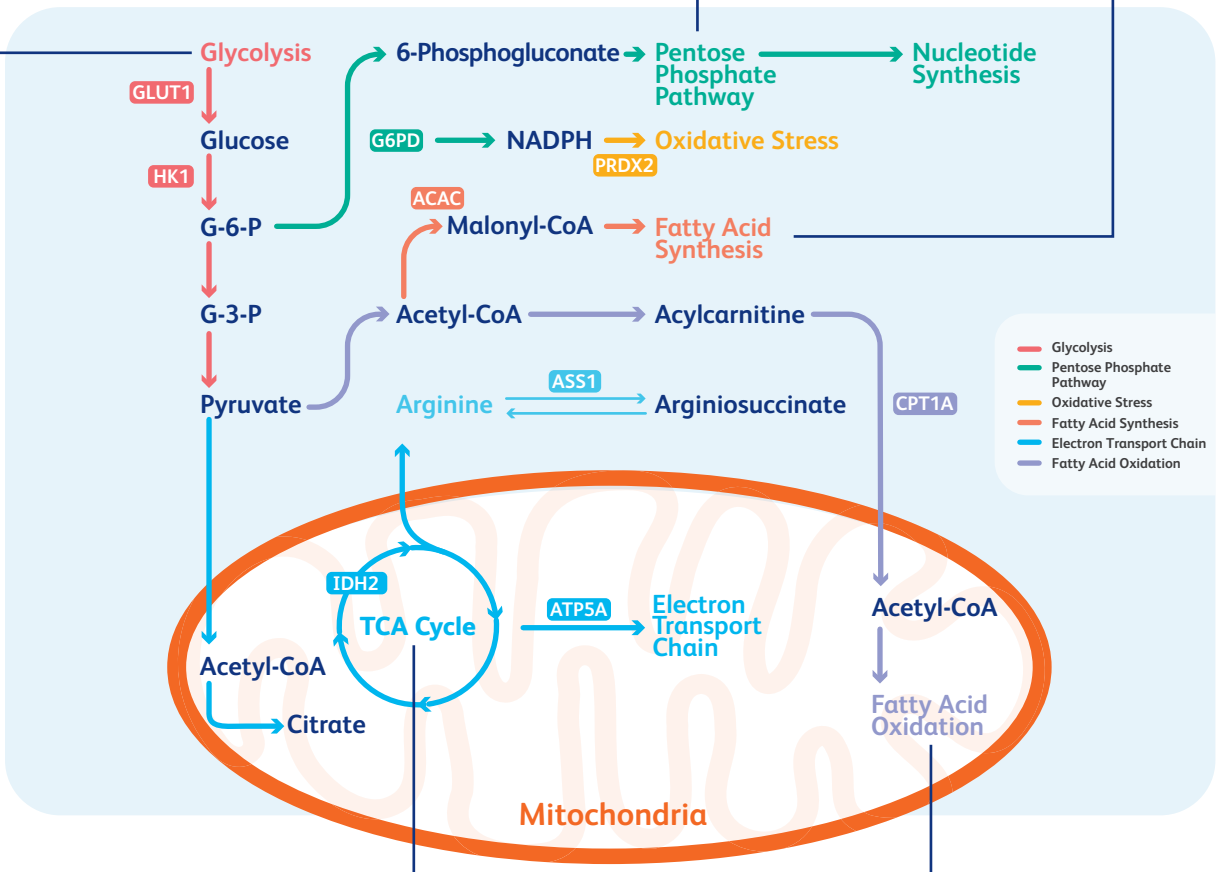
- **Inflammatory M1 macrophage** — Promotes phagocytosis and secretion of pro-inflammatory cytokines
- **Effector T cell** — Fuels proliferation and supports pro-inflammatory cytokine secretion

Pentose phosphate pathway

- **Inflammatory M1 macrophage** — Initiates synthesis of nucleotides and ROS

Fatty acid synthesis

- **Inflammatory M1 macrophage** — Drives proliferation and supports secretion of pro-inflammatory cytokines
- **Effector T cell** — Fuels proliferation and regulates effector cytokines



TCA cycle

- **M2 macrophage** — Supports oxidative phosphorilation
- **Memory CD8 T cell** — Sustains memory cell phenotype
- **Regulatory T cell** — Associated with suppressive function

Fatty acid oxidation

- **M2 macrophage** — Inhibits inflammatory signals and drives M2 phenotype
- **Memory CD8 T cell** — Supports memory cell formation and survival
- **Regulatory T cell** — Facilitates T_{reg} development and immune tolerance

Image was adapted from Ahl PJ, Hopkins RA, Xiang WW, et al. Met-Flow, a strategy for single-cell metabolic analysis highlights dynamic changes in immune subpopulations. *Commun Biol.* 2020;3(1):305. doi: 10.1038/s42003-020-1027-9

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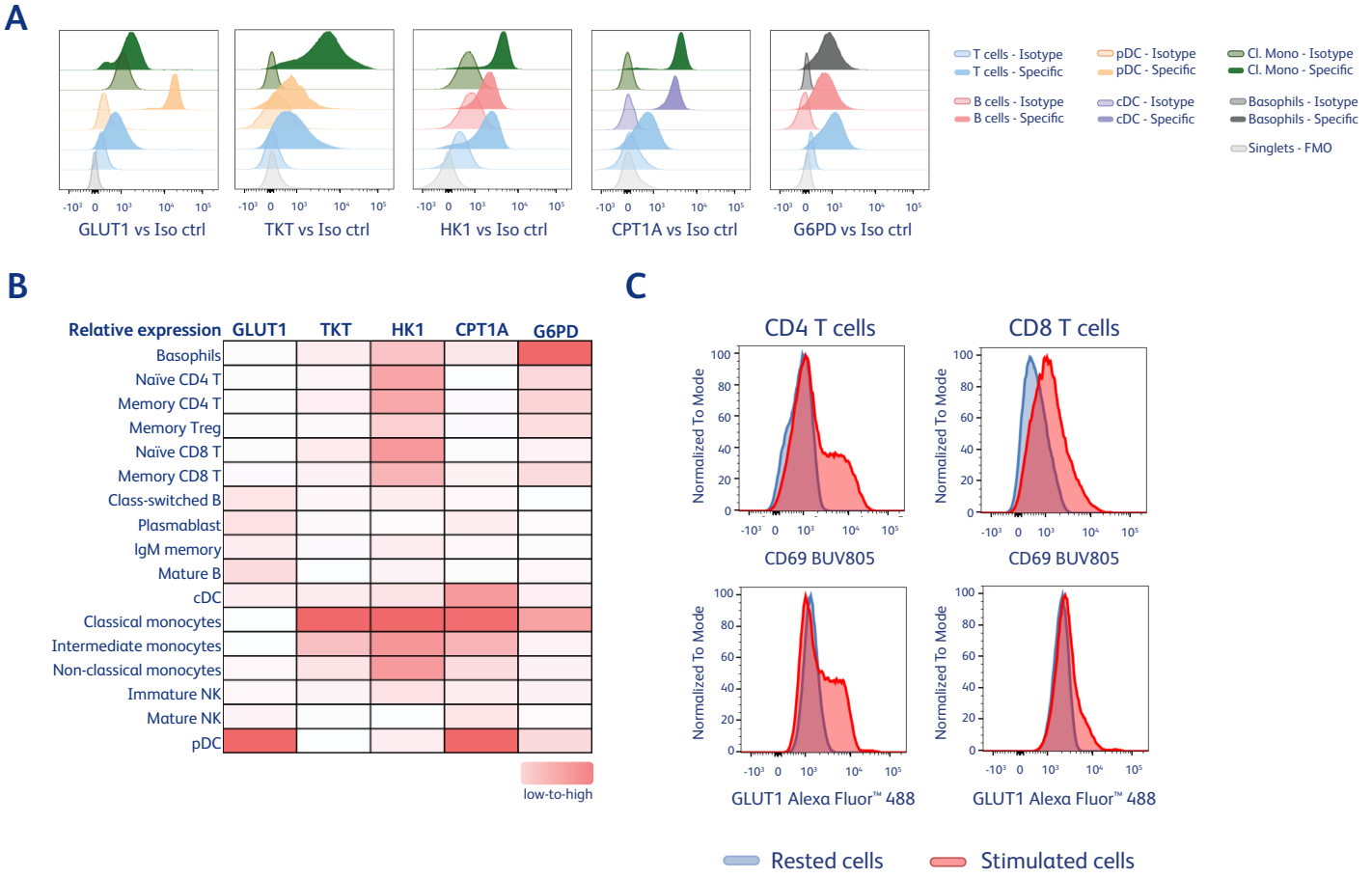
Expression of metabolic proteins in immune cell populations

Just like in other cells in the body, immune cells require energy to carry out their various functions, such as proliferation, cytokine production and antigen presentation. Here we demonstrate simultaneous analysis of different metabolic proteins, using validated fluorochrome-conjugated antibodies. High-parameter spectral flow cytometry analyses revealed a broad expression of GLUT1, TKT, HK1, CPT1A and G6PD across multiple human immune cell populations. Additionally, we show upregulation of the glucose transporter GLUT1 in activated CD69⁺ T cells. It is clear by the growing body of literature that the effect of the glycolytic pathway on T cell activation and cells of the immune system is important for both the understanding of basic immune function and also helping to fuel early immunotherapy discoveries.



Flow cytometry assay for assessment of metabolic proteins in human peripheral blood mononuclear cells. A. PBMCs stained with a 30-color panel of antibodies including anti-GLUT1 and four metabolic enzymes. The histogram overlays show isotype control or specific staining in selected populations. **B.** Heat map representing the relative expression of the indicated metabolic proteins in different cell subsets in a scale of low to high stain index values. **C.** Rested or 24-hour-stimulated PBMCs stained with a 24-color panel for assessment of GLUT1 expression on activated T cells.

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