Methods and protocols for a double extraction of knock-out PMM1 (-/-PMM1) in Arg141His/Phe119LeuPMM2 patient-derived fibroblasts (+/+PMM1):

This protocol refers to the double polar and lipophilic metabolite extraction of fibroblast samples starting from a cell pellet in an Eppendorf tube.

**Sample preparation**:

* PMM2 fibroblasts received at the lab frozen in dry ice.
* Cells were immortalized as described in [Monticelli *et al*.,2022] and grown in RPMI medium supplemented with 10% Fetal Bovine Serum, 2 mM glutamine, 0.5 mg/mL penicillin, 0.5 mg/mL streptomycin, and non-essential amino acids at 37 °C in 5% humidified CO2.
* -/-PMM1 was generated by the insertion of a puromycin resistance cassette into the first exon of PMM1, using the Origene kit KN202004.
* Following the manufacturer's instructions, puromycin -up to 0.3 ng/mL- was used for the selection, and resistant cells were analysed via PCR to verify the correct insertion. Then positive cells were grown (-/-PMM1) in parallel with their controls (+/+PMM1).
* Cells from confluent 150 cm2 plates were washed with PBS and enzymatically detached using trypsin, then pelleted in PBS and washed again for 3 times.
* Metabolites extraction was performed using methanol:chloroform:water 1:1:1 protocol, as suggested by [Beckonert *et al*., 2007].
* Polar and nonpolar fractions were transferred into glass vials and the solvents were removed under a nitrogen stream at room temperature and stored at -80°C until they were analysed.

**Sample transfer to NMR tubes**:

* For NMR analysis, only polar fractions were resuspended in 630 uL of phosphate buffer saline (PBS, pH 7.4), containing 10% 2H2O (to provide a field frequency lock) and 1 mM sodium 3-trimethylsylyl [2,2,3,3-2H4] propionate (TSP) as a chemical shift reference for 1H spectra. The final volume of 700 uL for each sample was then tranferred in a 1.7mm SampleJet NMR tube.

NMR run:

* The Samplejet tube racks are put in the autosampler at 4oC

NMR settings pulseprog and experiments 1D + 2D: zgesgp, TOCSY, HSQC.

**References:**

M. Monticelli, L. Liguori, M. Allocca, A. Bosso, G. Andreotti, J. Lukas, M.C. Monti, E. Morretta, M.V. Cubellis, B. Hay Mele, Drug Repositioning for Fabry Disease: Acetylsalicylic Acid Potentiates the Stabilization of Lysosomal Alpha-Galactosidase by Pharmacological Chaperones, International Journal of Molecular Sciences 23 (2022) 5105. https://doi.org/10.3390/ijms23095105.

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