

Methods

Metabolomics

For H1 hESCs, the extracellular metabolite dynamics was analyzed during primed to naïve conversion in the bioreactor. For this, the media of H1 hESCs in the bioreactor was gradually switched from mTESR1 to RSeT media within 6 days of culture (D2 to D6) and the media was collected every day for the analysis. For H9 hESCs, the extracellular metabolites were analyzed in the spent media of primed and established naïve (P5) hPSCs in the bioreactor, and the media of each primed or naïve hPSCs was collected at day four of bioreactor culture. The metabolite extraction was started using LC-MS or HPLC grade methanol (Sigma-Aldrich, 1.06035). Briefly, a 950 μ L of pre-chilled 50% MeOH/H₂O was added to a 50 μ L of the bioreactor-collected media (making a D20 dilution), and the samples were incubated on ice for 30 min to allow for full extraction. Macromolecules were then pelleted by centrifugation at max speed (~18,000-21,000 g) for 10 mins in a bench top centrifuge (preferably chilled) to extract the supernatant. Further, the extracted samples were stored in -80 prior to running HPLC-MS. For the analysis, 200-400 μ L of the extracted supernatant from each sample was transferred into individual wells in 96 well plates and were run in HPLC-MS. General metabolomics runs were performed on a Q Exactive™ Hybrid Quadrupole-Orbitrap™ Mass Spectrometer (Thermo-Fisher, IQLAAEGAAPFALGMAZR) coupled to a Vanquish™ Flex UHPLC System, Integrated biocompatible system (Thermo-Fisher, IQLAAAGABHFAPUMBHV). Chromatographic separation was achieved on a Zorbax SB-C18 UHPLC column (2.1mm x 50mm x 1.8 μ m, Agilent, Part number 822700-902) using a binary solvent system at a flow rate of 600 μ L/min. Solvent A, 10mM tributylamine, 10mM acetate pH 7.5 in 97/3% (v/v) mass spectrometry grade water/methanol; Solvent B, mass spectrometry grade acetonitrile. A sample injection volume of 2 μ L was used. The mass spectrometer was run in negative full scan mode at a resolution of 140,000 scanning from 70-1000m/z. Data was processed using MAVEN. Statistical analysis and visual representation of the data was done in R and MATLAB.