Midbrain differentiation and organoid maintaining

hiPSCs were cultured in Stemflex^{TM} medium (ThermoFisher) at 37°C, with 5% CO2 in a humidified incubator, as previously described (Sarrafha et al., 2021). For the organoid aggregation and differentiation, 125-ml disposable spinner flasks (Corning, VWR) were placed on a nine-position stir plate (Dura-Mag) at a speed of 65 rpm, as previously reported, starting with dissociated 40x10^6 hPSCs in Stemflex + Rhok inhibitor Y- 27632 (4 μM). Differentiation was initiated when spheres reached 300-500 μm by dual-SMAD inhibition with SB431542 (R&D Systems, 10 μM), LDN193189 (Stemgent, 100 nM), B27-Vit A and N2 in DMEM-F12, to unsure the proper size range spheres were filtered using a set of 300 and 500 μm filters (pluriSelect). Midbrain-specific patterning for midbrain NPCs organoids was the addition of CHIR99021 (Stemgent, 3 μM), Purmorphamine (STEMCELL, 2 μM), and SAG (Abcam, 1 μM) (Kriks et al., 2011). Post patterning Neural maturation medium was DMEM F12 medium containing N2, B27-VitA, 20 ng/mL GDNF (R&D Systems), 20 ng/mL BDNF (R&D Systems), 0.2 mM ascorbic acid (Sigma), 0.1 mM dibutyryl cAMP (Biolong), 10 μM DAPT (Cayman Chemical). For long-term maintenance (after day 35), the spheres were transferred to ultralow attachment plates (Corning, VWR) at 5 spheres per ml of media. The medium for long-term culture was DMEM F12 medium containing N2, B27-VitA, 10 ng/ML GDNF (R&D Systems), 10 ng/mL BDNF (R&D Systems), 0.2 mM ascorbic acid (Sigma).

LC-MS/MS with the hybrid metabolomics

Midbrain organoids from WT (CTR) and KO (DJ1 KO) were subjected to an LCMS analysis to detect and quantify known peaks at different days post differentiation. A metabolite extraction was carried out on each sample based on a previously described method (Pacold et al., 2016). The LC column was a Millipore TMZIC-pHILIC (2.1x150 mm, 5μm) coupled to a Dionex Ultimate 3000TM system and the column oven temperature was set to 25oC for the gradient elution. A flow rate of 100μL/min was used with the 10 mM ammonium carbonate in water (A), pH 9.0, and acetonitrile (B). The gradient profile was 80-20% B (0-30min), 20-80% B (30-31min), 80-80% B (31-42min). Injection volume was set to 2 μL for all analyses (42min total run time per injection). MS analyses were carried out by coupling the LC system to a Thermo Q Exactive HFTM mass spectrometer operating in heated electrospray ionization mode (HESI). Method duration was 30 min with polarity switching data-dependent Top 5method for both positive and negative modes. Spray voltage for both positive and negative modes was 3.5kV and the capillary temperature was set to 320 ºC with a sheath gas rate of 35, aux gas of 10, and max spray current of 100 μA. The full MS scan for both polarities utilized 120,000 resolution with an AGC target of 3e^6 and a

maximum IT of 100 ms, and the scan range was from 67-1000 m/z. Tandem MS spectra for both positive and negative modes used a resolution of 15,000, AGC target of 1 e^5, maximum IT of 50 ms, isolation window of 0.4 m/z, isolation offset of 0.1 m/z, fixed first mass of 50 m/z, and 3-way multiplexed normalized collision energies (nCE) of 10, 35, 80. The minimum AGC target was 1e4 with an intensity threshold of 2e5. All data were acquired in profile mode.

Pacold, M.E., Brimacombe, K.R., Chan, S.H., Rohde, J.M., Lewis, C.A., Swier, L.J.Y.M., Possemato, R., Chen, W.W., Sullivan, L.B., Fiske, B.P., et al. (2016). A PHGDH inhibitor reveals coordination of serine synthesis and one-carbon unit fate. Nat. Chem. Biol. *12*, 452–458.