

Table S1. Cell lineage markers for analyzing cell types in organoids

Markers	Expression	Localization	Application in Organoids
SOX2	Stem Cells	Nuclear	Qian et al., 2016, Qian et al., 2020; Bhaduri et al., 2020; Tang et al., 2021; Watanabe et al., 2022
PAX6	Early Progenitors	Nuclear	Pasca et al., 2015; Pollen et al., 2019; Bhaduri et al., 2020; Tang et al., 2021; Watanabe et al., 2022
EOMES	Intermediate Progenitors	Nuclear	Pasca et al., 2015; Pollen et al., 2019; Qiang et al., 2020; Bhaduri et al., 2020; Watanabe et al., 2022
HOPX	Outer Radial Glia	Nuclear	Bhaduri et al., 2020; Qiang et al., 2020; Dong et al., 2020
NeuN	Mature neurons	Nuclear	Pasca et al., 2015; Dong et al., 2020
TBR1	Deep layer neurons (layer VI)	Nuclear	Pasca et al., 2015; Dong et al., 2020; Watanabe et al., 2022;
BCL11B	Deep layer neurons (layer V)	Nuclear	Pasca et al., 2015; Pollen et al., 2019; Velasco et al., 2019; Qiang et al., 2020; Bhaduri et al., 2020; Dong et al., 2020; Watanabe et al., 2022
SATB2	Upper layer neurons (layer IV)	Nuclear	Pasca et al., 2015; Pollen et al., 2019; Velasco et al., 2019; Qiang et al., 2020; Bhaduri et al., 2020; Tang et al., 2021; Watanabe et al., 2022
POU3F2	Upper layer neurons (layer III)	Nuclear	Pasca et al., 2015; Dong et al., 2020
DCX	Immature neurons	Cytosolic	Dong et al., 2020; Huang et al., 2022
TUJ1	Differentiating neurons	Cytosolic	Dong et al., 2020; Tang et al., 2021
MAP2	Neuronal dendrites	Cytosolic	Pasca et al., 2015; Velasco et al., 2019; Dong et al., 2020; Tang et al., 2021; Huang et al., 2022
GFAP	Astrocytes	Cytosolic	Pasca et al., 2015; Velasco et al., 2019; Huang et al., 2022; Watanabe et al., 2022
S100B	Astrocytes	Cytosolic	Velasco et al., 2019; Huang et al., 2022

Table S2. Software and methods used for quantitative analysis of cell types in organoids

	Overall Area/Volume	Cell Counting	Layer Thickness	Intensity Measurements
ImageJ	Yes	Yes	Yes	Yes
MatLab	Yes	Yes	No	Yes
Imaris	Yes	Yes	Yes	Yes
CellProfiler	Yes	Yes	No	Yes

Table S3. Tissue clearing and analysis methods

Clearing Methods	Type of solvent	Microscopy	Data Analysis	Examples
ClearT2	Hydrophilic reagent	Zeiss AxioImager M2 microscope with Apotome, Leica SP8 confocal, light-sheet	ImageJ or 3D imaging software(Amira, Imaris, NeuroLucida, etc.)	Boutin and Hoffman-Kim, 2015; Grist et al., 2016; Nurnberg et al., 2020; Diosdi et al., 2021
Sca/e	Hydrophilic reagent	Confocal, light-sheet microscope	ImageJ or 3D imaging software(Amira, Imaris, NeuroLucida, etc.)	Boutin and Hoffman-Kim, 2015; Diosdi et al., 2021
SeeDB	Hydrophilic reagent	Confocal, Light-Sheet microscope	ImageJ or 3D imaging software(Amira, Imaris, NeuroLucida, etc.)	Boutin and Hoffman-Kim, 2015; Grist et al., 2016
Sca/eSQ	Hydrophilic reagent	Leica SP8 confocal	ImageJ or 3D imaging software(Amira, Imaris, NeuroLucida, etc.)	Grist et al., 2016
SHIELD	Hydrogel embedding	Light-Sheet microscope	SCOUT pipeline	Albanese et al., 2020
CLARITY	Hydrogel embedding	Light-Sheet microscope	ImageJ or 3D imaging software(Amira, Imaris, NeuroLucida, etc.)	Shnaider and Pristyazhnyuk, 2021
CUBIC	Hydrophilic reagent	Light-Sheet microscope	ImageJ or 3D imaging software(Amira, Imaris, NeuroLucida, etc.)	Diosdi et al., 2021, Meng et al., 2023
Fructose-glycerol	Hydrophilic reagent	Confocal, Multi-Photon, Light-Sheet microscope	ImageJ or 3D imaging software(Amira, Imaris, NeuroLucida, etc.)	Dekkers et al., 2019
iDISCO	Organic	Leica SP8 confocal	ImageJ or 3D imaging software(Amira, Imaris, NeuroLucida, etc.)	Birey et al., 2017

Table S4. Methods used for metabolic assessment.

Listed in order of increasing complexity and cost

* indicates high recommendation for entry level analysis of organoid metabolism

	Assay	Example	Reference
Terminal Assays	Histology/immunofluorescence	TOM20, GORASP2, ARCN1, PGK1	Bhaduri 2020 (PMID 31996853), Le 2021 (PMID: 34223837)
	qPCR*	Unfolded protein response genes (e.g. PERK, XBP1s)	Pasca 2019 (PMID: 31061540)
	Seahorse XF	Mitochondrial activity	Zhou 2019 (PMID: 30814486), Le 2021 (PMID: 34223837), Ludikhuize 2021 (PMID: 33778780)
	Transcriptomics	Enzyme expression	Vertesy 2022 (PMID: 35919947), Huang 2022 (PMID: 35141969), Pollen 2019 (PMID: 30735633), many others
	Metabolomics	Metabolome content (whole and spatially resolved)	Gomez-Giro 2019 (PMID: 31888773), Notaras 2021 (PMID: 34158620), Sun 2023 (PMID: 37164975)
	Proteomics	ER stress response and unfolded protein response genes	Gonzalez-Bohorquez 2022 (PMID: 34996870)
	Imaging mass spectrometry	Spatial mapping of metabolites and lipids	Cappuccio 2023 (PMID: 37304070)
Live Assays	Fluorescent reporters*	redox potential of NAD ⁺ /NADH	Martynov 2018 (PMID: 30279147), Jiang et al 2017 (PMID: 28703127), Jiang et al 2019 (PMID: 30358421)
		glutathione status	
	Fluorescence lifetimes imaging microscopy (FLIM)	relative amount of the free or enzyme-bound form of NAD(P)H	Okkelman 2020 (PMID: 31935648), Meleshina 2016 (PMID: 26911347), Meleshina 2017 (PMID: 28129796), Stringari 2012 (PMID: 22891156), Kashirina 2021 (PMID: 34829971)
	Genetically encoded pH sensors	SypHer-2	Kashirina 2021 (PMID: 34829971)
	NMR	Amino acids, sugars, lactate	Sapir 2021 (PMID: 34577579)
	Oxygen-sensitive phosphorus nanoparticles	Oxygen levels	Cho 2021 (PMID: 34354063), Choi 2012 (PMID: 22240511)
Live and Terminal Assays Available	Biochemical assays/plate reader assays*	Lactate level, glucose level	Zhou 2019 (PMID: 30814486), Cho 2021 (PMID: 34354063)