Electronic supplementary information (ESI)

Mass spectrometry-based quantitation combined with time-dependent metabolomics to discover metabolic features in human neurogenesis using neural constructs generated from neural progenitor cells

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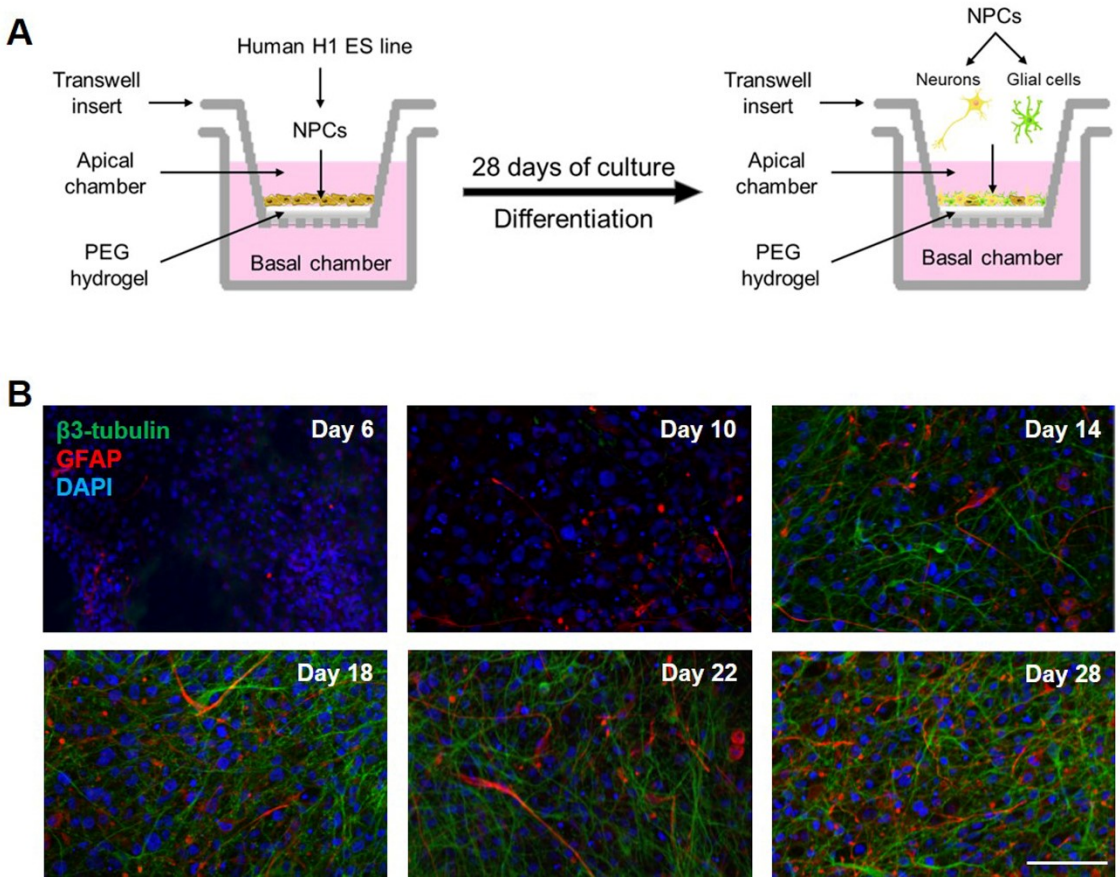

Fig. S1 (A) Schematic representation of the human neural constructs generated from neural progenitor cells; (B) representative immunofluorescence images illustrating β3-tubulin (neurons, green), GFAP (glial cells, red), and DAPI (nuclei, blue) expression for neural constructs after 6 days, 10 days, 14 days, 18 days, 22 days and 28 days of culture on PEG hydrogels (Scale bar: 50 μm).

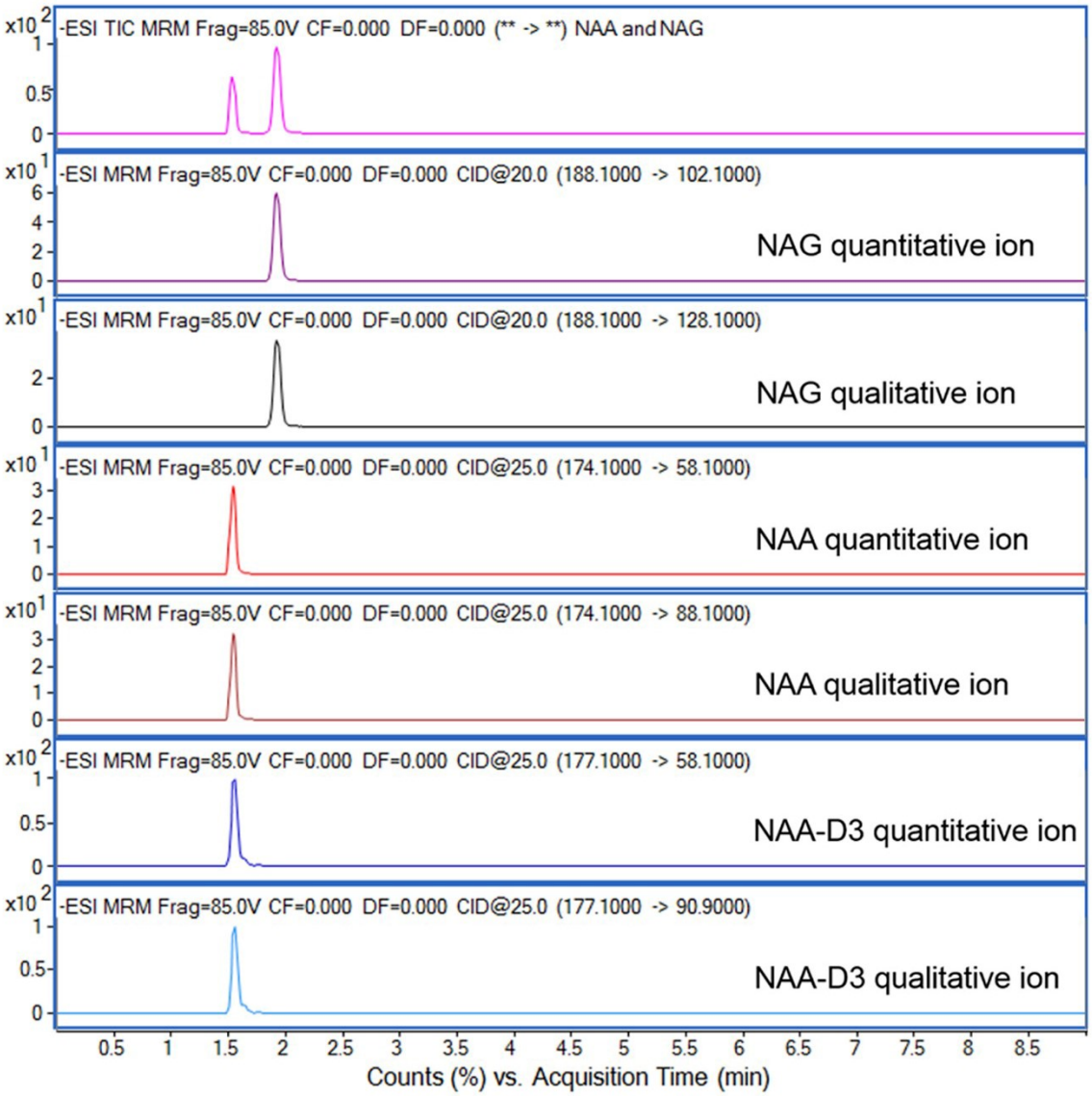

Fig. S2 The typical MRM chromatograms of NAA, NAG and NAA-D3. The transitions of *m/z* 188.1→102.1 and *m/z* 174.1→58.1 were used for the quantitation of NAG and NAA, respectively; the ions at *m/z* 128.1 and *m/z* 88.1 were selected as qualitative ions for NAG and NAA. NAA-D3 was employed as the internal standard for quantitation.

Table S1 Optimized MRM parameters for NAA and NAG quantitation by using LC-QqQ MS

Analyte	Quantitation			Confirmation		
	Precursor ion (*m/z*)	Product ion (*m/z*)	Collision energy	Precursor ion (*m/z*)	Product ion (m/z)	Collision energy
NAA	174.1	58.1	25 V	174.1	88.1	25 V
NAG	188.1	102.1	20 V	188.1	128.1	20 V
NAA-D3 (IS)	177.1	58.1	25 V	177.1	90.9	25 V

Table S2 The precisions and recoveries for the measurements of NAA and NAG in blank medium by LC-MS/MRM method

Analyte	Intra-day precision RSD (%, n=5)			Inter-day precision RSD (%, n=5)			Recovery (%, n=3)		
	Low	Medium	High	Low	Medium	High	Low	Medium	High
NAA	(10 nM)	(100 nM)	(1000 nM)	(10 nM)	(100 nM)	(1000 nM)	(10 nM)	(100 nM)	(1000 nM)
	8.8	3.1	3.5	9.7	6.9	7.9	88.8	92.9	101.0
NAG	(2 nM)	(20 nM)	(200 nM)	(2 nM)	(20 nM)	(200 nM)	(2 nM)	(20 nM)	(200 nM)
	11.4	8.9	3.0	12.6	8.0	9.3	103.1	104.5	95.6

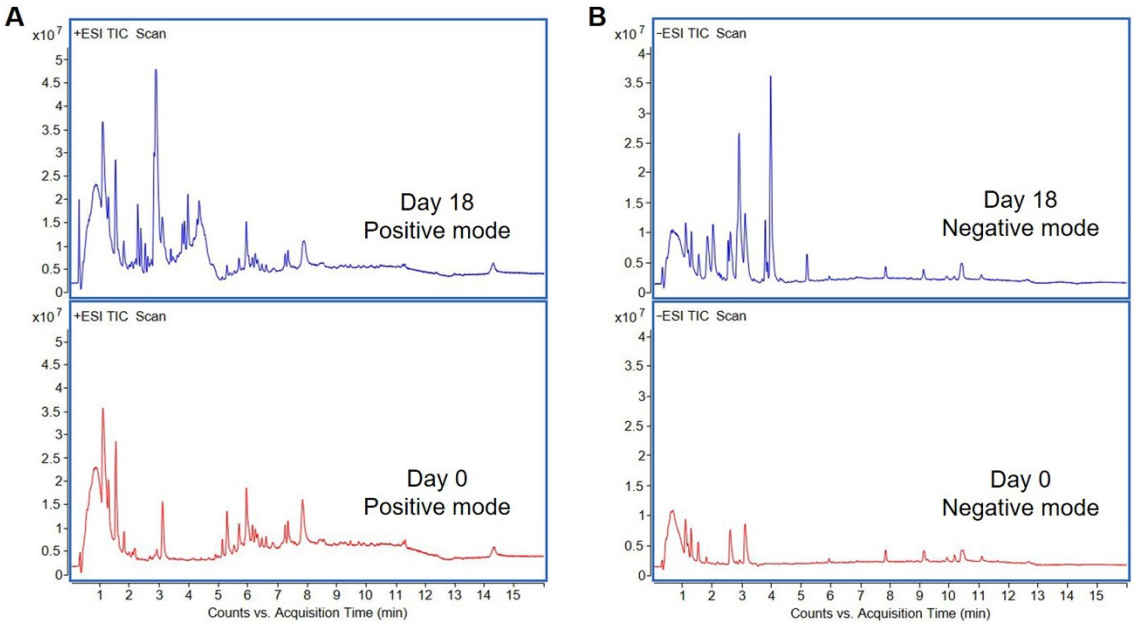

Fig. S3 Typical LC-MS total-ion chromatograms of day 18 and day 0 (control) samples acquired in both positive (A) and negative (B) ion modes.

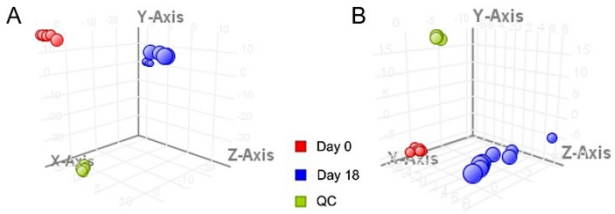

Fig. S4 PCA plots of day 0 (red), day 18 (blue), and QC (green) samples under both positive (A) and negative (B) modes, with the first three principal components accounting for 91.2% (A) and 87.9% (B) of the variance in analysis. Each dot represents a sample and each color represents the type of the sample.

Table S3 Metabolite profiling and comparison between the sample groups (day 10, day 18, day 28) and control group (day 0) (Fold change > 1.5, $p < 0.05$)

Ionization mode	Samples	Total features	Total significantly changed features	Total increased features	Total decreased features
	Day 10 vs Day 0	13803	2483	1829	654
LC−ESI$^+$	Day 18 vs Day 0	15732	3929	3344	585
	Day 28 vs Day 0	15850	4224	3639	585
	Day 10 vs Day 0	858	342	317	25
LC−ESI$^-$	Day 18 vs Day 0	865	320	278	42
	Day 28 vs Day 0	852	333	280	53