

**Supplemental materials of “N-3 polyunsaturated fatty acids prevent the D-galactose induced cognitive impairment by up-regulating the levels of 5-hydroxymethylcytosine in mouse brain”**

Supplemental Figures S1

Supplemental Figures S2

Supplemental Figures S3

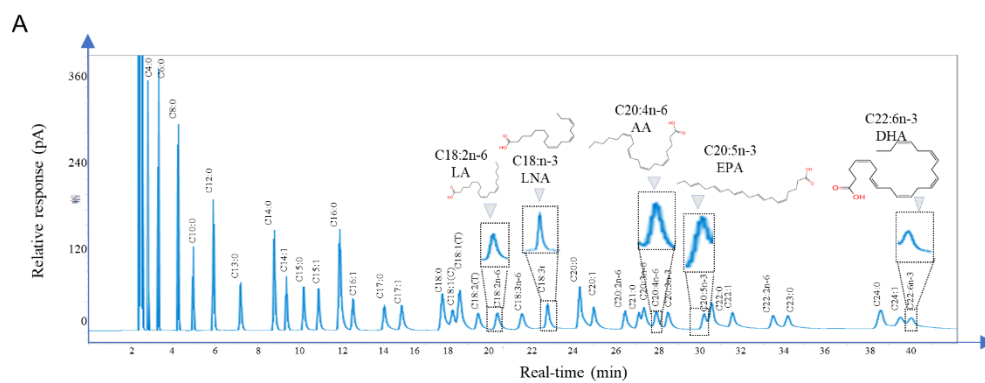
Supplemental Table.1

Supplemental Table.2

Supplemental Table.3

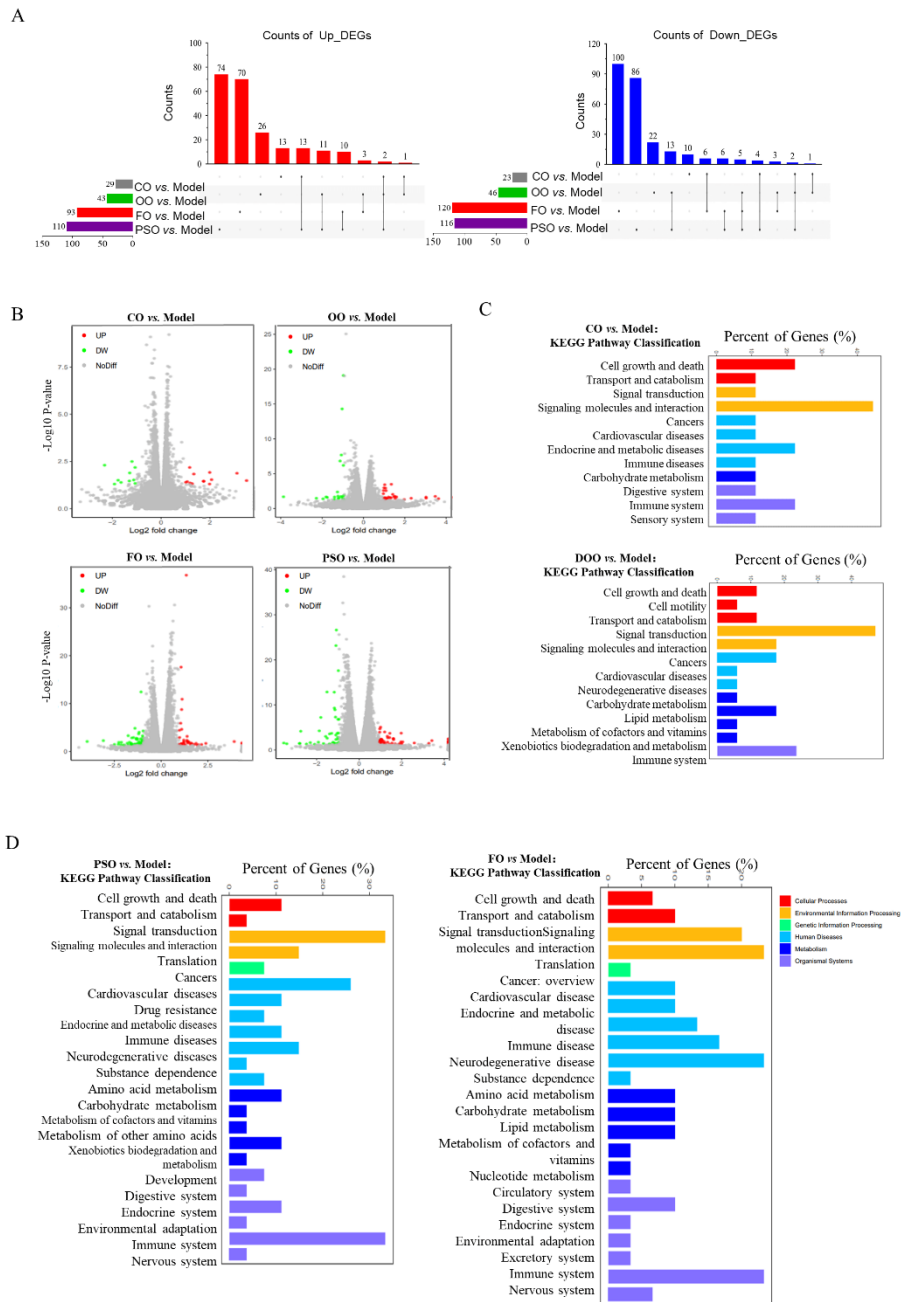
Supplemental Table.4

## Supplemental Figures S1

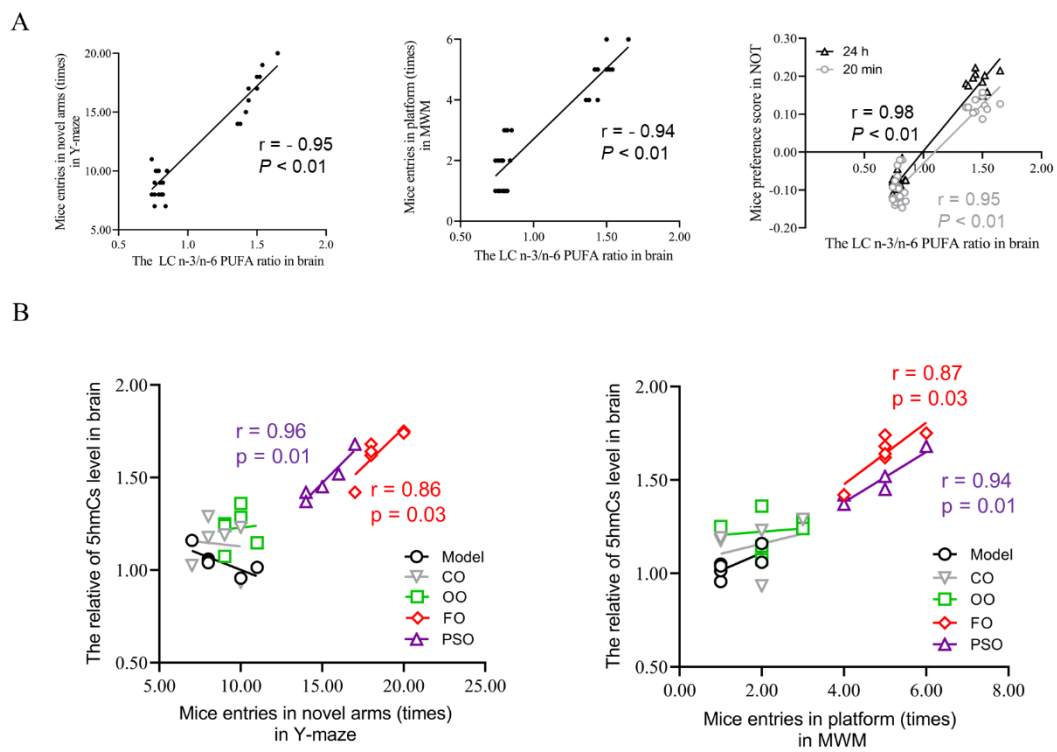


**Figure S1 (A)** Fatty Acid Methyl Ester (FAME) Standards detected by Gas chromatogram. C18:3 LNA and C22:6 DHA's structures are indicated by grey arrows.

## Supplemental Figures S2



### Supplemental Figures S3



**Figure S3** (A) Correlation of LC n-3/n-6 PUFA ratio in brain (x-axis) and mice behavior preference (y-axis) in the Y-maze, MWM and NOT. (B) Correlation of mice behavior preference in Y-maze, MWM performance (x-axis) with DNA 5hmCs level in brain (y-axis). Pearson's correlation is a measure of a linear correlation in the data,  $n = 5$  or  $6$  mice/group.

Supplemental Table.1 Nutrition ingredients of standard diet

Ingredient	g/kg	kcal/g	kcal%
Casein	200.00	3.58	20.36
Corn Starch	300.00	3.60	30.72
Sucrose	310.00	4	35.26
Maltodextrin	75	4	8.53
Cellulose	47	0	0
Soybean Oil	15	9	3.83
Mineral Mix	11.4	0	0
Vitamin Mix	11.4	4	1.30
Total	969.8	28.18	100

Supplemental Table.2 oils for each oil/diet

	Soybean oils	Corn oil	Olive oil	Fish oil	Peony seed oil
kcal/g	9.00	8.84	8.99	8.95	8.83

Supplemental Table.3 Information of target gene's primer

Gene name	Sequence ( 5'-> 3')	Genebank Accession
<i>Fads1</i>	F: AGCACATGCCATACAACCATC	NM_146094
	R: TTTCCGCTGAACCACAAAATAGA	
<i>Fads2</i>	F: TCATCGGACACTATTCGGGAG	NM_019699
	R: GGGCCAGCTCACCAATCAG	
<i>Elovl2</i>	F: CACGTACCTGCTCTCGATATGG	NM_019423
	R: TGTGATTGCGAGGTTATACAAGG	
<i>Elovl5</i>	F: CAGATCACCGTGCTCCATGTC	NM_134255
	R: CTGTTGAGTGTCGCACCAAA	
<i>Actb</i>	F: GGCTGTATTCCCCTCCATCG	NM_007393
	R: CCAGTTGGTAACAATGCCATGT	

Forward primer; R: Reverse primer

Supplemental Table.4 The raw dates of RNA-sequencing analysis

Samples	RawReads	RawBases	CleanReads	CleanBases	ValidBases	Q30	GC
Model_1	49.06M	7.36G	48.59M	7.13G	96.84%	95.20%	46.83%
Model_2	46.99M	7.05G	46.52M	6.79G	96.30%	95.18%	46.55%
Model_3	47.37M	7.10G	46.91M	6.85G	96.45%	95.31%	47.04%
Model_4	47.29M	7.09G	46.85M	6.84G	96.42%	95.24%	47.02%
CO_1	48.64M	7.30G	48.24M	7.01G	96.06%	95.81%	47.39%
CO_2	47.83M	7.17G	47.44M	6.84G	95.36%	95.85%	47.29%
CO_3	48.58M	7.29G	48.20M	6.95G	95.37%	96.00%	47.66%
CO_4	49.83M	7.47G	49.44M	7.16G	95.81%	95.86%	47.44%
OO_1	41.79M	6.27G	41.38M	6.05G	96.55%	95.34%	46.27%
OO_2	47.61M	7.14G	47.16M	6.90G	96.57%	95.41%	46.34%
OO_3	47.58M	7.14G	47.12M	6.86G	96.16%	95.43%	46.15%
OO_4	47.51M	7.13G	47.04M	6.87G	96.36%	95.46%	46.34%
PSO_1	47.95M	7.19G	47.48M	6.92G	96.26%	95.54%	46.52%
PSO_2	47.51M	7.13G	47.06M	6.91G	96.96%	95.39%	46.25%
PSO_3	47.58M	7.14G	47.12M	6.83G	95.66%	95.52%	46.67%
PSO_4	45.77M	6.87G	45.32M	6.59G	95.97%	95.27%	46.25%
FO_1	49.98M	7.50G	49.47M	7.19G	95.89%	95.43%	46.53%
FO_2	47.98M	7.20G	47.51M	6.94G	96.40%	95.30%	46.71%
FO_3	48.82M	7.32G	48.33M	7.03G	96.02%	95.10%	46.79%
FO_4	51.71M	7.76G	51.19M	7.45G	96.02%	95.34%	46.54%

Note: Q30 notes the sequencing error recognition rate was 0.1%.