Supplementary Materials

Integrated multi-level analysis reveals the neurotoxic mechanisms of BDE-209 in zebrafish brain

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Figures

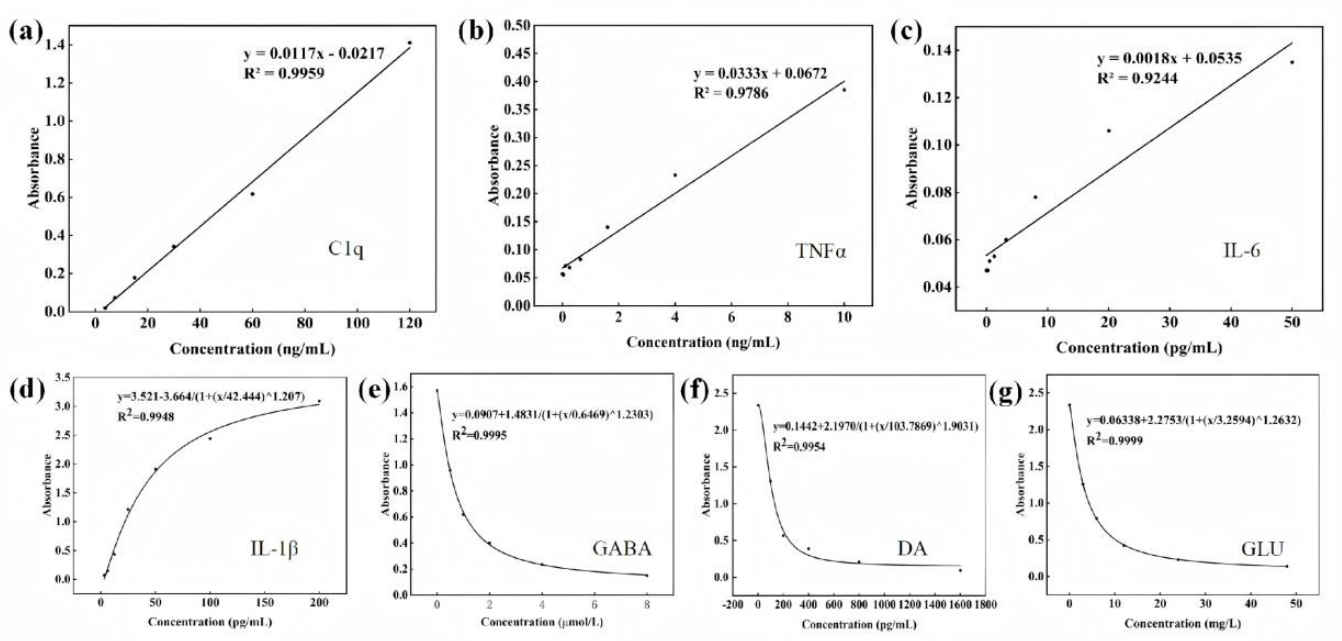


Fig. S1. Standard curves used for the determination of enzyme activities, oxidative stress–related and immune-related indices, and neurotransmitter contents. (a) Complement component (C1q), (b) Tumor necrosis factor-alpha (TNFα), (c) interleukin-6 (IL-6), (d) Interleukin-1 beta (IL-1β), (e) γ-aminobutyric acid transaminase (GABA), (f) Dopamine (DA), and (g) Glutamate (GLU).

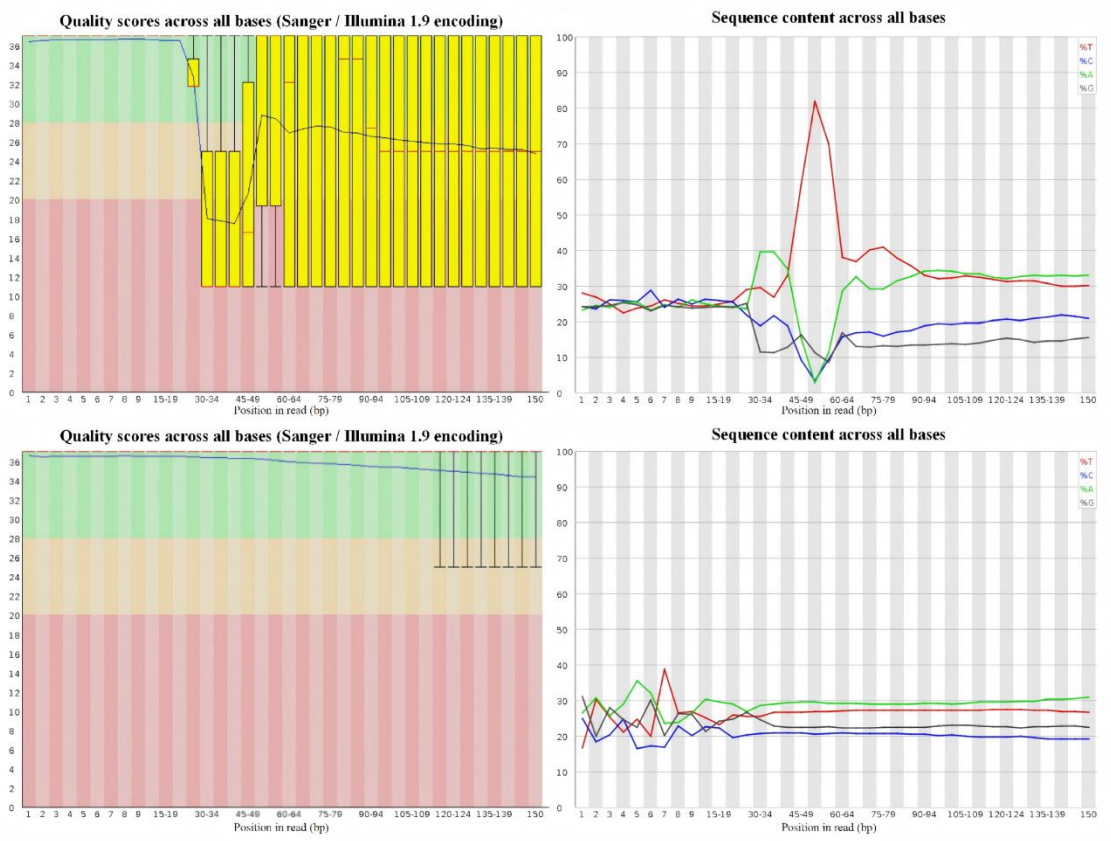


Fig. S2. Base quality score distribution and base composition distribution of single-cell RNA sequencing data from zebrafish brain samples in the control group. The first row shows the results for sample R1, and the second row shows those for sample R2. The panels on the left display base quality score distributions, while the panels on the right show base composition distributions.

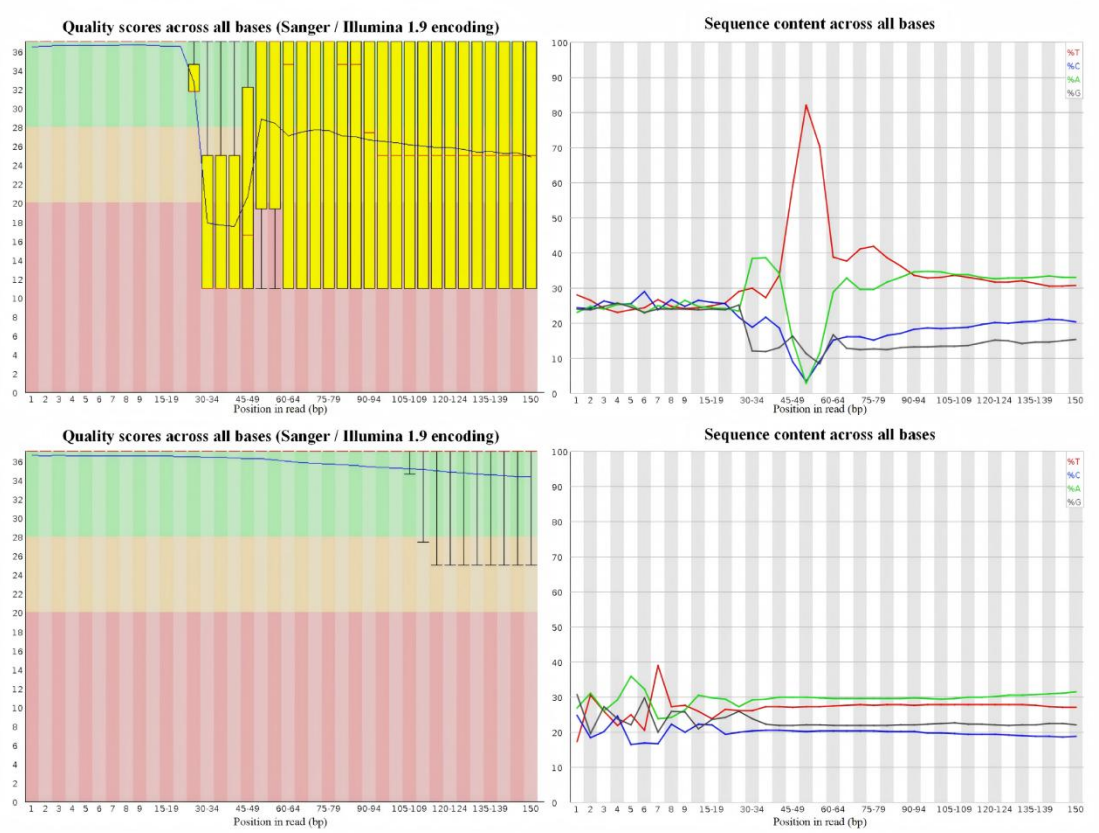


Fig. S3. Base quality score distribution and base composition distribution of single-cell RNA sequencing data from zebrafish brain samples in the 5 μg/L BDE-209 group. The first row shows the results for sample R1, and the second row shows those for sample R2. The panels on the left display base quality score distributions, while the panels on the right show base composition distributions.

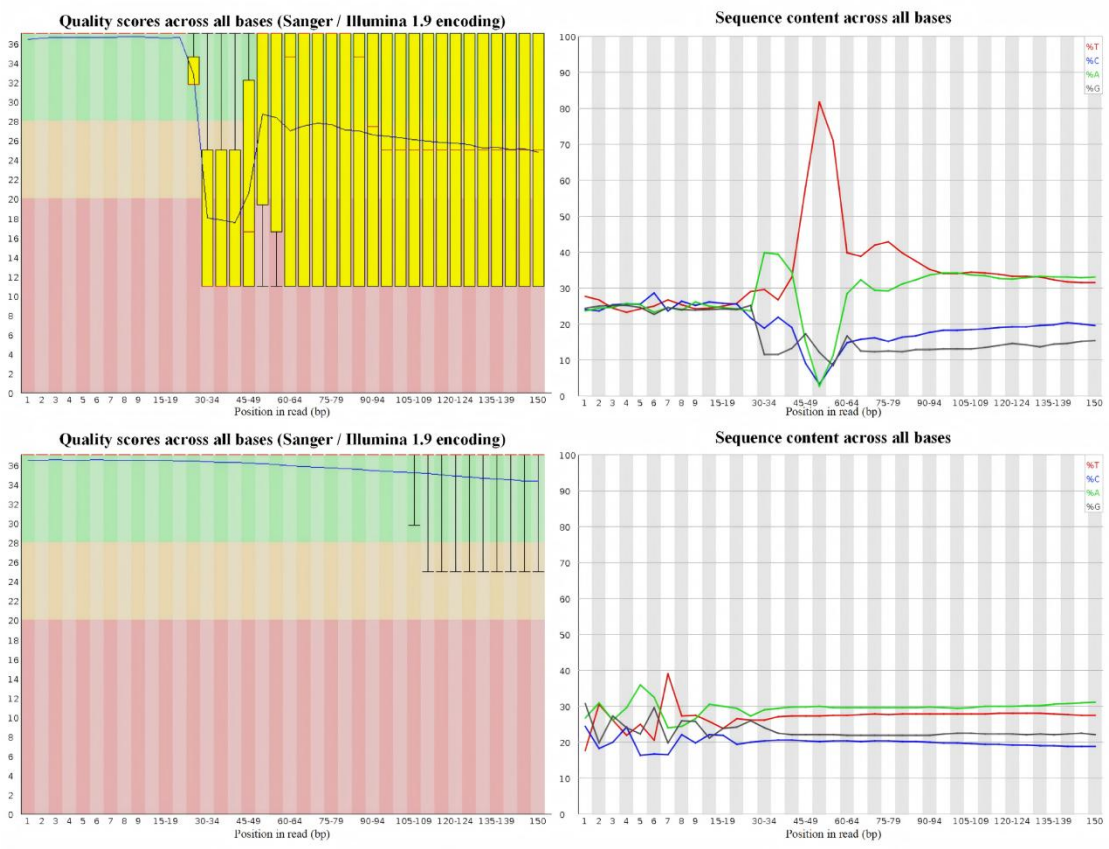


Fig. S4. Base quality score distribution and base composition distribution of single-cell RNA sequencing data from zebrafish brain samples in the 500 μg/L BDE-209 group. The first row shows the results for sample R1, and the second row shows those for sample R2. The panels on the left display base quality score distributions, while the panels on the right show base composition distributions.

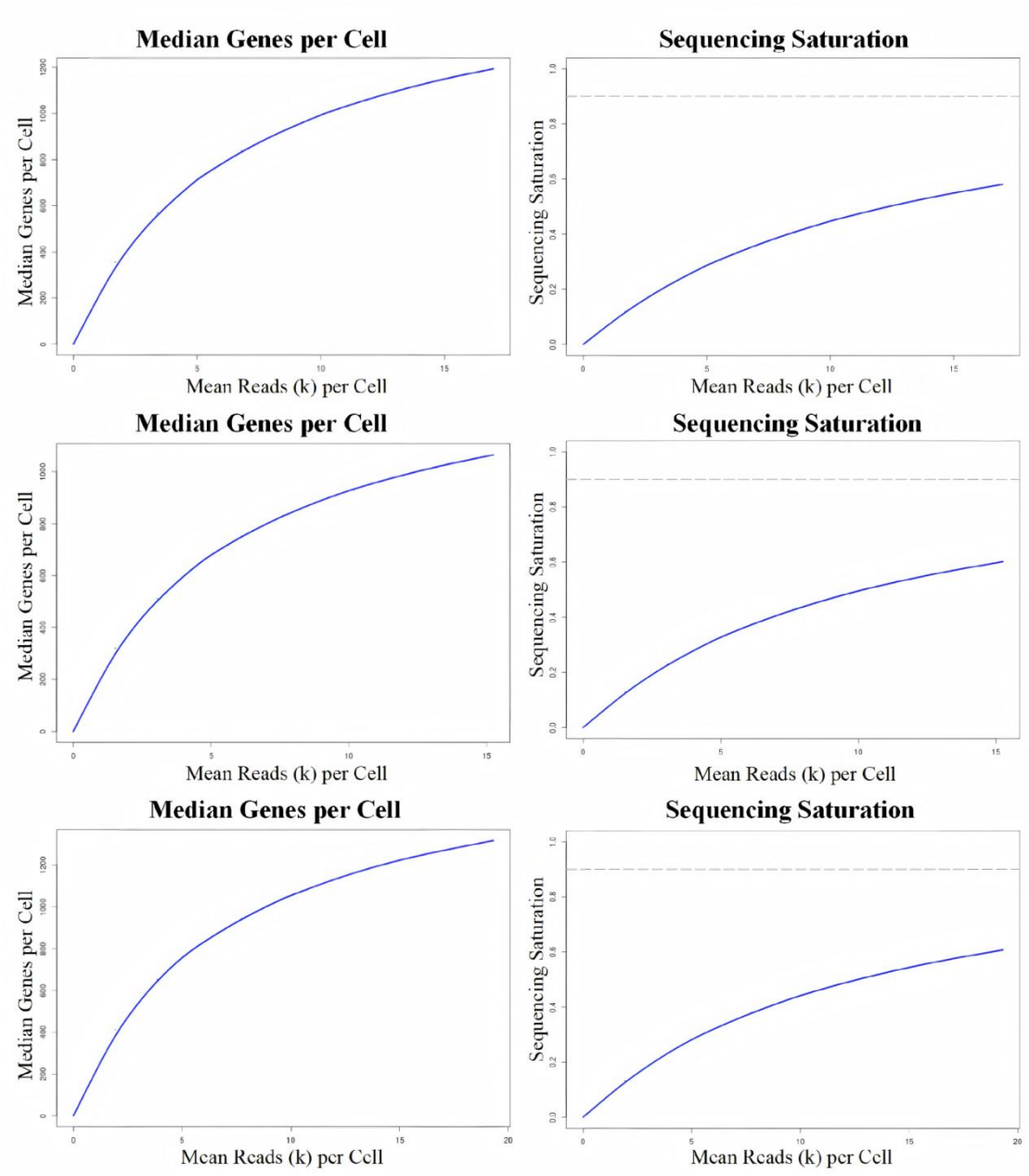


Fig. S5. Sequencing saturation curves of single-cell RNA sequencing data from zebrafish brain samples. The three rows represent the control group, the 5 μg/L BDE-209 exposure group, and the 500 μg/L BDE-209 exposure group, respectively. In each row, the plots on the left show the relationship between average sequencing depth per cell (x-axis) and sequencing saturation (y-axis), while the plots on the right display the relationship between average sequencing depth per cell (x-axis) and the median

number of genes detected per cell (y-axis).

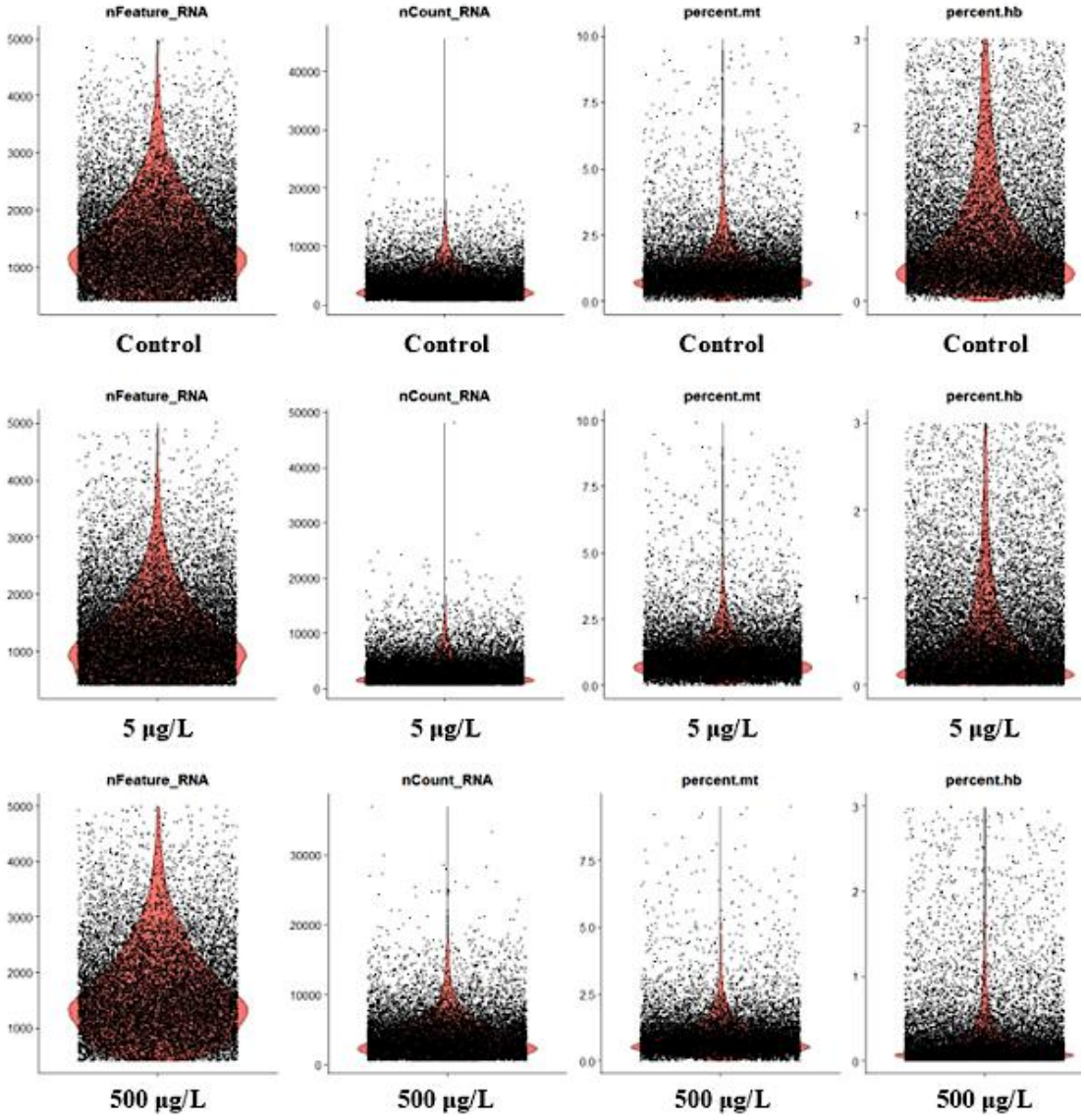


Fig. S6. Quality control of raw single-cell RNA sequencing data from zebrafish brain samples in the control and BDE-209 exposure groups, including filtering based on the number of detected genes, total gene expression counts, mitochondrial gene proportion, and hemoglobin gene proportion.

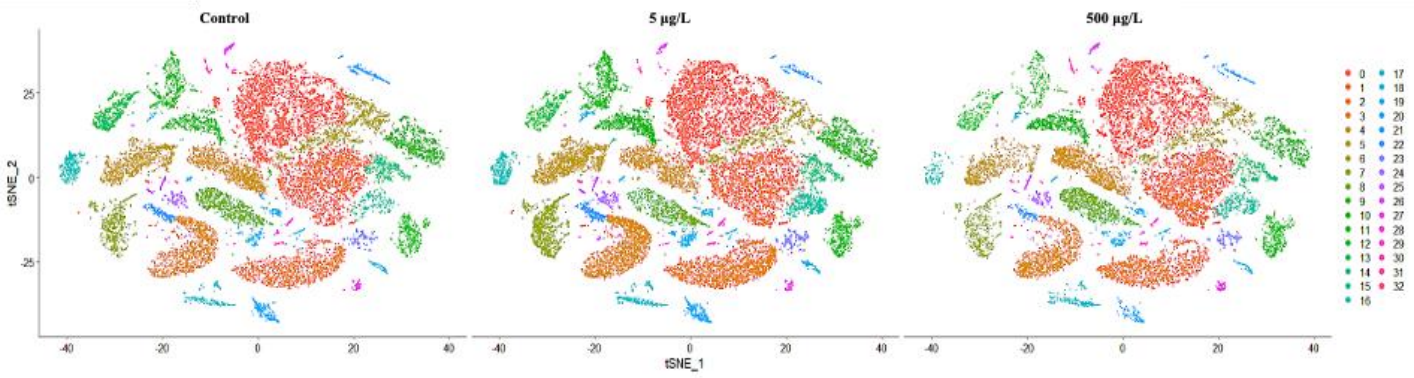


Fig. S7. Cell clusters identified from single-cell RNA sequencing of zebrafish brain samples in the control group and two BDE-209 exposure groups (5 and 500 μg/L). Different colors on the right indicate the 32 distinct subclusters identified across all groups.

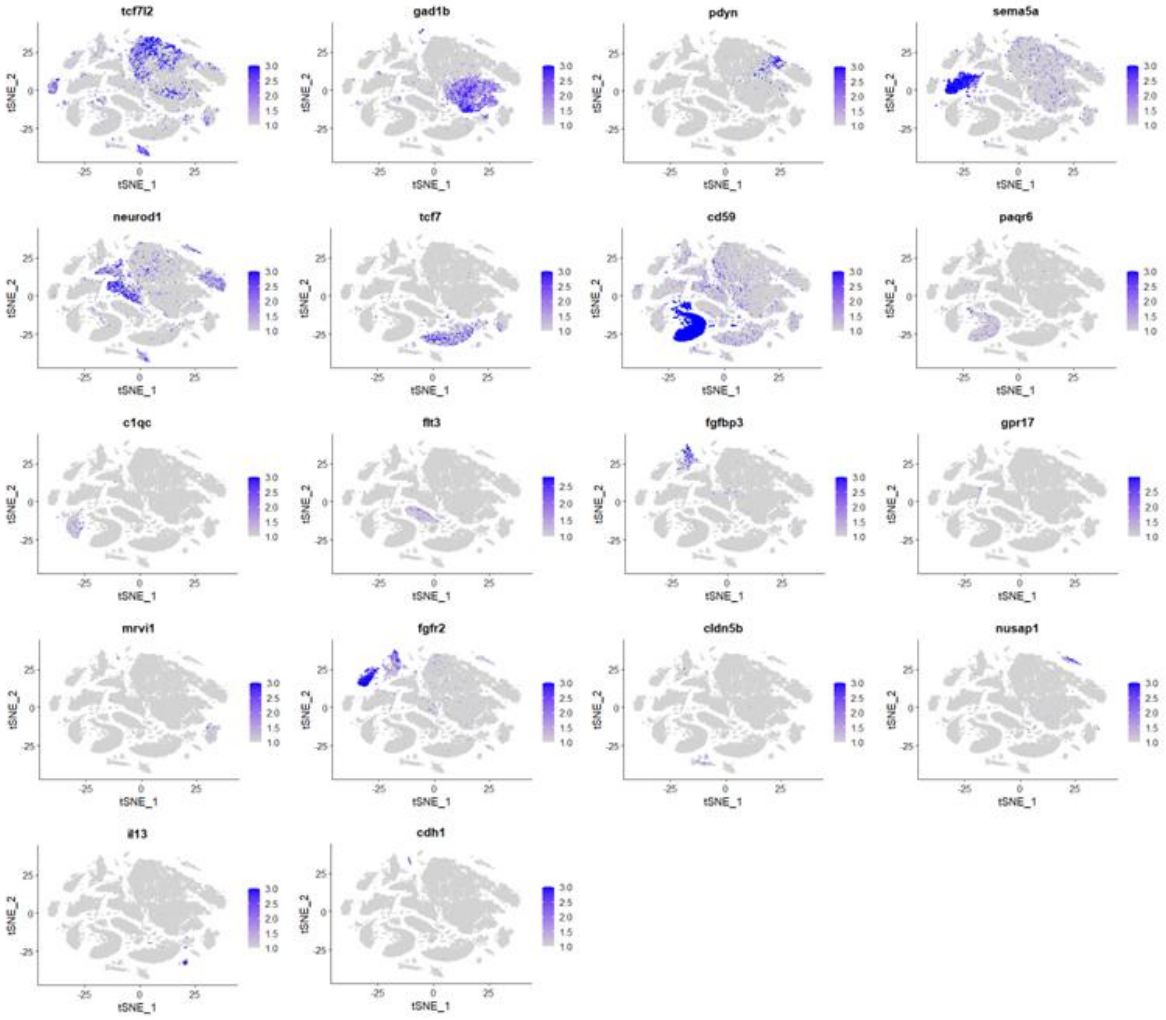


Fig. S8. Heatmaps showing the expression of marker genes for 14 identified cell clusters in zebrafish brain samples. The coordinate axes of all heatmaps correspond to the two-dimensional spatial coordinates generated by the t-SNE algorithm, with color gradients ranging from gray to blue indicating increasing gene expression levels.

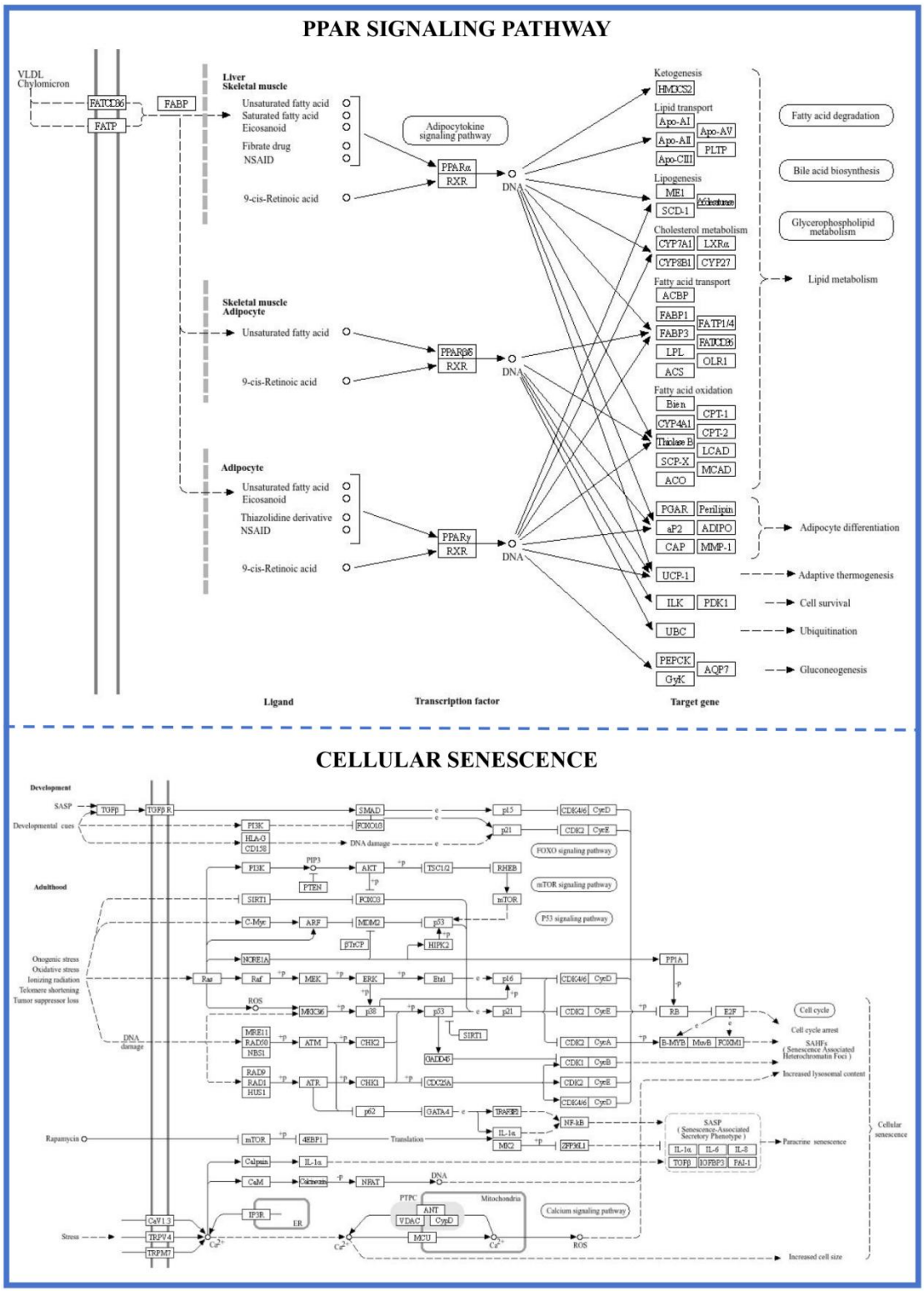


Fig. S9. KEGG pathways "PPAR signaling pathway" and "Cellular senescence" enriched in the macrophages of zebrafish brain after exposures to 5 and 500 μg/L BDE-209.

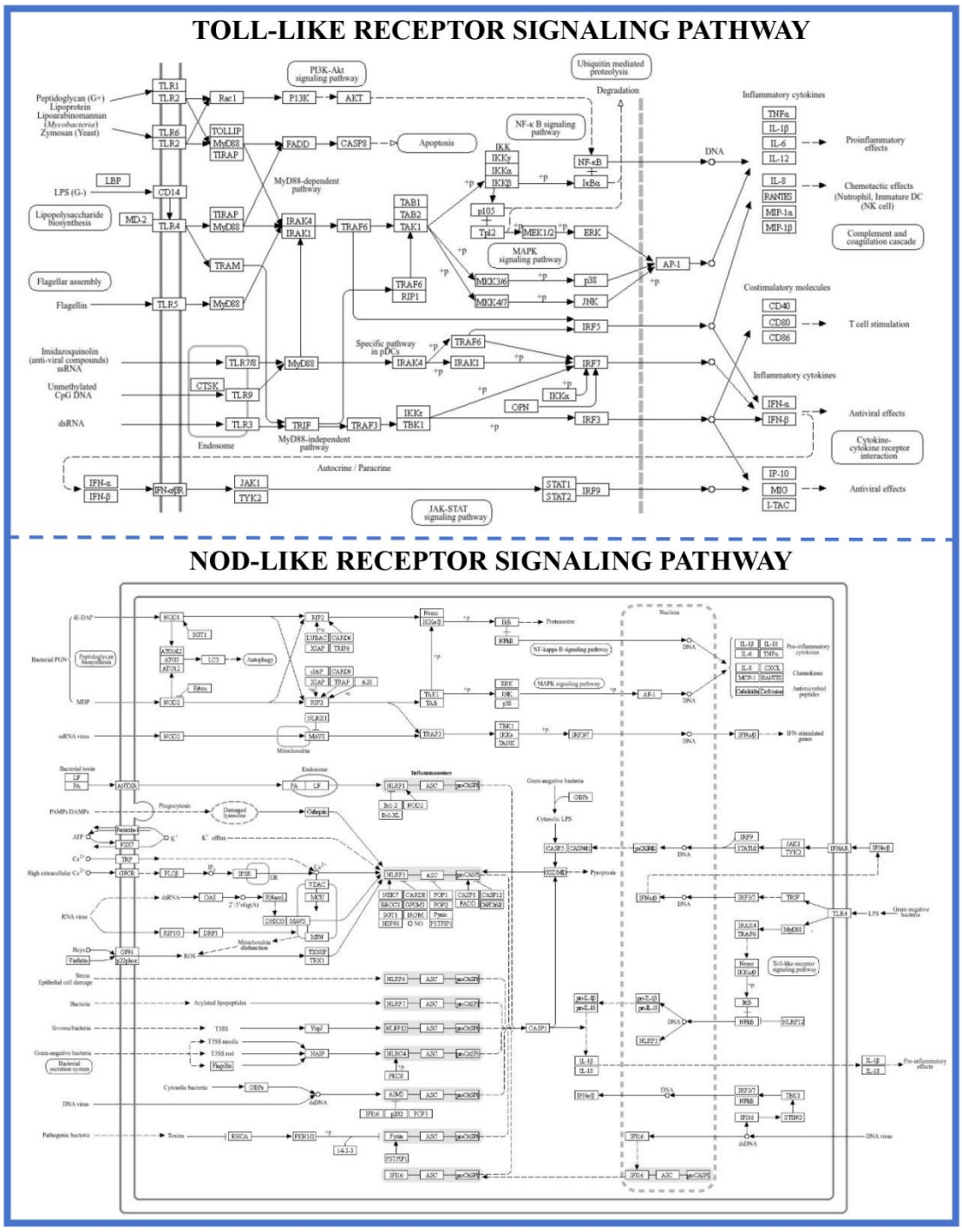


Fig. S10a. KEGG pathways "Toll-like receptor signaling pathway", "NOD-like receptor signaling pathway", "C-type lectin receptor signaling pathway", "RIG-I-like receptor signaling pathway", and "Salmonella infection" enriched in the natural killer T cells of zebrafish brain after exposures to 5 and 500 μg/L BDE-209.

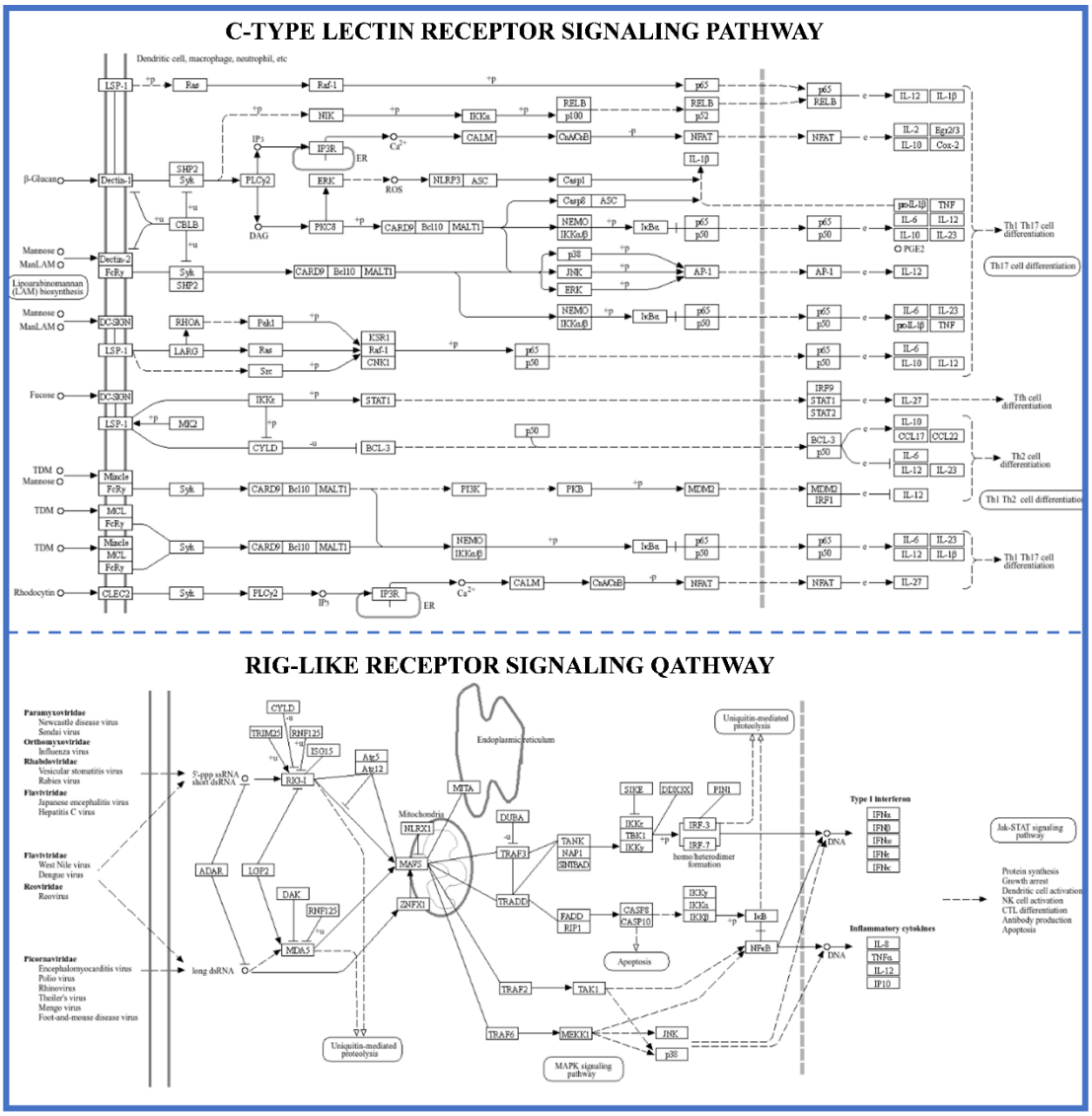


Fig. S10b. KEGG pathways “Toll-like receptor signaling pathway”, “NOD-like receptor signaling pathway”, “C-type lectin receptor signaling pathway”, “RIG-I-like receptor signaling pathway”, and “Salmonella infection” enriched in the natural killer T cells of zebrafish brain after exposures to 5 and 500 μg/L BDE-209.

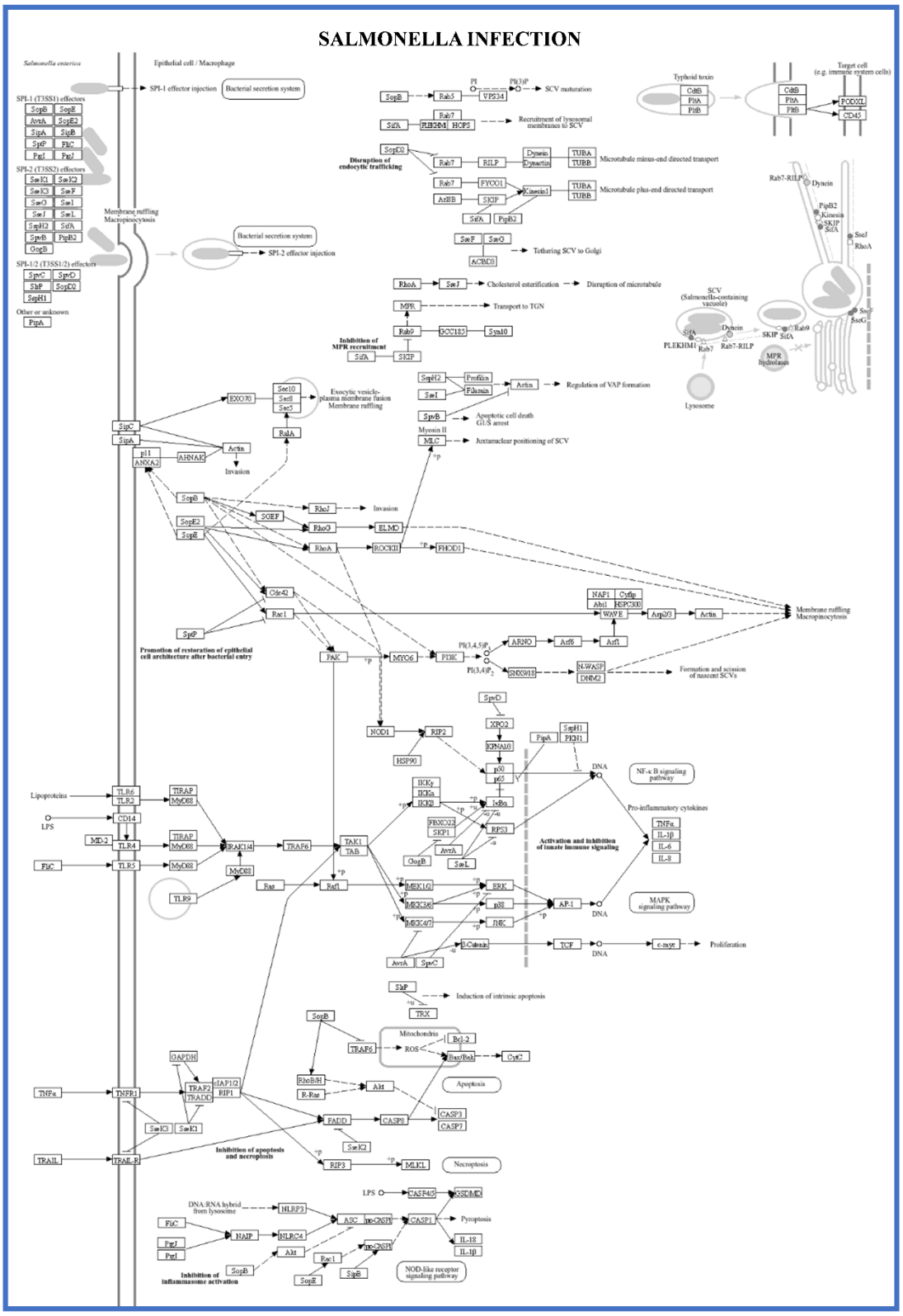


Fig. S10c. KEGG pathways “Toll-like receptor signaling pathway”, “NOD-like receptor signaling pathway”, “C-type lectin receptor signaling pathway”, “RIG-I-like

receptor signaling pathway", and "Salmonella infection" enriched in the natural killer T cells of zebrafish brain after exposures to 5 and 500 μg/L BDE-209.

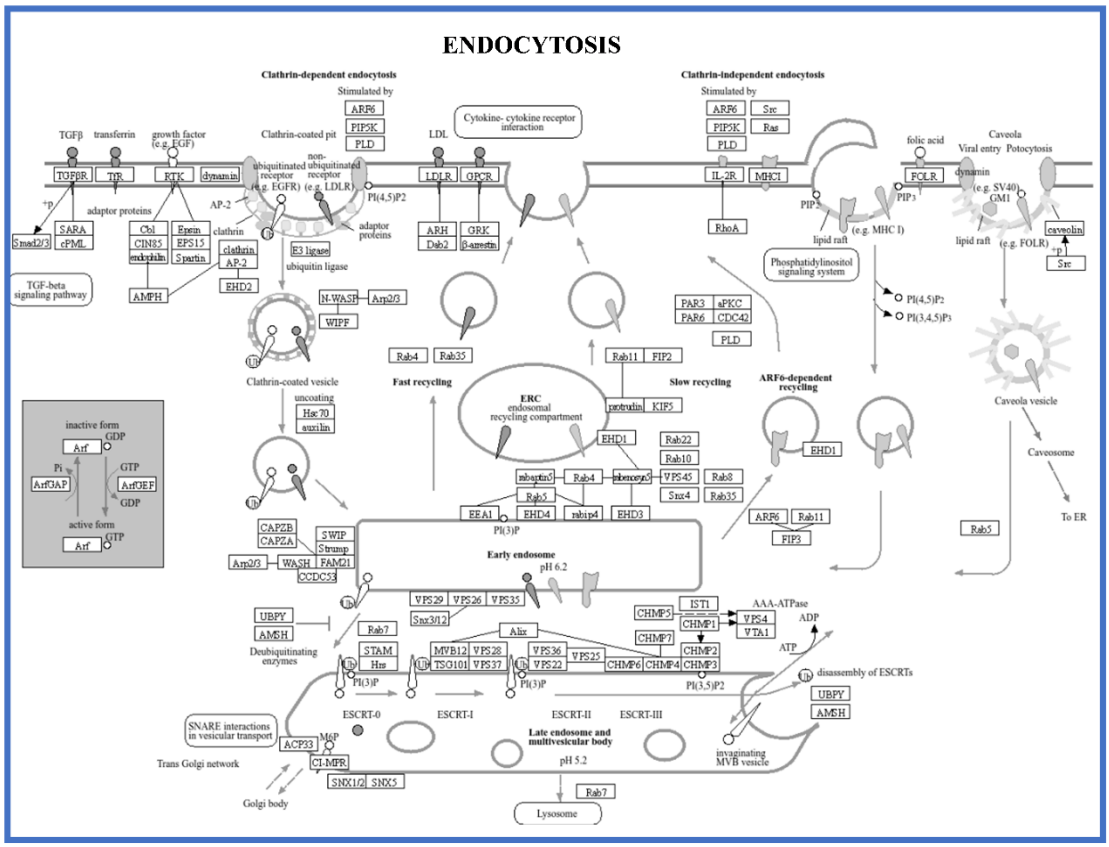


Fig. S11. KEGG pathway "Endocytosis" enriched in the excitatory neurons of zebrafish brain after exposures to 5 and 500 μg/L BDE-209.

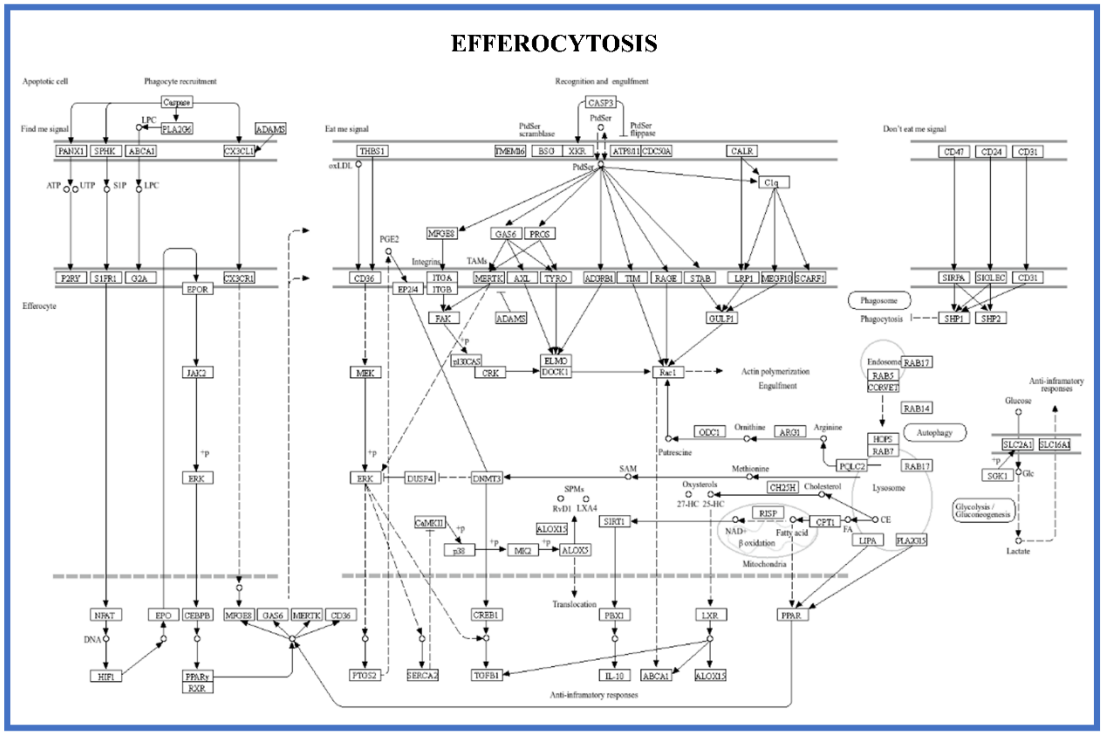


Fig. S12. KEGG pathway "Efferocytosis" enriched in the inhibitory neurons of zebrafish brain after exposures to 5 and 500 μg/L BDE-209.

Tables

Table S1. Detailed water quality parameters detected from the tank water for zebrafish culture.

		Temperature (℃)	pH	ORP	Conductivity (μS/cm)	Resistivity (MΩ·cm)	TDS (ppm)	Salinity (PSU)
Day1	Control	23.78	7.63	325.9	427	0.0023	214	0.20
	5 μg/L	23.42	8.22	326.0	428	0.0023	214	0.20
	500 μg/L	23.04	8.18	325.1	428	0.0023	214	0.20
Day2	Control	24.52	7.79	293.4	484	0.0021	242	0.23
	5 μg/L	25.33	7.91	290.4	484	0.0021	242	0.23
	500 μg/L	25.12	8.03	287.1	482	0.0021	241	0.23
Day3	Control	25.59	7.30	298.2	478	0.0021	240	0.22
	5 μg/L	25.73	7.98	293.6	482	0.0021	241	0.23
	500 μg/L	25.94	8.04	291.9	481	0.0021	240	0.23
Day4	Control	24.54	8.01	284.9	489	0.0021	242	0.23
	5 μg/L	25.54	8.02	280.8	477	0.0021	238	0.23
	500 μg/L	25.49	8.03	275.9	481	0.0021	240	0.23
Day5	Control	24.20	7.89	295.5	466	0.0022	233	0.22
	5 μg/L	25.16	8.08	289.2	482	0.0021	241	0.23
	500 μg/L	25.73	8.21	284.9	479	0.0021	239	0.23
Day6	Control	24.34	7.94	355.8	482	0.0021	241	0.23
	5 μg/L	25.19	8.15	365.1	486	0.0021	243	0.23

	500 μg/L	25.47	8.23	360.1	482	0.0021	241	0.23
	Control	23.70	7.86	367.2	480	0.0021	240	0.23
Day7	5 μg/L	24.18	7.94	370.1	484	0.0021	242	0.23
	500 μg/L	24.26	8.03	367.6	479	0.0021	239	0.23
	Control	24.56	7.99	328.0	488	0.0021	244	0.23
Day8	5 μg/L	25.28	8.03	329.1	491	0.0021	245	0.23
	500 μg/L	25.75	8.14	323.9	491	0.0020	245	0.23
	Control	24.25	7.34	378.8	492	0.0021	246	0.23
Day9	5 μg/L	24.35	8.09	405.3	498	0.0020	249	0.24
	500 μg/L	24.83	8.13	394.2	495	0.0020	247	0.24
	Control	24.47	7.99	407.4	500	0.0020	250	0.24
Day10	5 μg/L	25.05	8.02	407.1	503	0.0020	251	0.24
	500 μg/L	25.50	8.08	401.2	499	0.0020	250	0.24
	Control	23.46	8.01	367.3	495	0.0020	248	0.24
Day11	5 μg/L	24.45	8.03	362.5	499	0.0020	250	0.24
	500 μg/L	25.55	8.09	258.0	498	0.0020	249	0.24
	Control	23.84	8.01	356.8	509	0.0020	254	0.24
Day12	5 μg/L	24.38	8.07	355.1	505	0.0020	252	0.24
	500 μg/L	24.68	8.10	350.9	499	0.0020	250	0.24
	Control	24.42	8.02	385.7	515	0.0020	259	0.24
Day13	5 μg/L	24.68	8.11	387.0	509	0.0020	255	0.24
	500 μg/L	24.46	8.19	384.8	508	0.0020	254	0.24

	Control	24.03	8.01	410.5	510	0.0020	255	0.24
Day14	5 μg/L	24.96	8.07	414.2	517	0.0020	258	0.25
	500 μg/L	25.62	8.11	410.8	510	0.0020	256	0.24
	Control	24.21	7.99	389.0	507	0.0020	253	0.24
Day15	5 μg/L	24.67	7.98	390.9	513	0.0020	256	0.24
	500 μg/L	25.00	8.05	386.3	512	0.0020	256	0.24
	Control	24.68	7.75	332.5	517	0.0020	258	0.25
Day16	5 μg/L	25.12	7.85	333.9	524	0.0019	262	0.25
	500 μg/L	25.01	7.93	331.9	518	0.0019	259	0.25
	Control	23.84	7.85	380.1	514	0.0020	257	0.25
Day17	5 μg/L	24.40	7.92	394.7	520	0.0019	260	0.25
	500 μg/L	24.48	7.98	394.1	516	0.0019	258	0.25
	Control	23.96	7.78	346.5	510	0.0020	255	0.25
Day18	5 μg/L	24.27	7.72	347.1	522	0.0019	261	0.25
	500 μg/L	24.78	7.69	350.9	517	0.0019	258	0.25
	Control	23.62	8.07	310.7	512	0.0020	256	0.24
Day19	5 μg/L	24.13	8.02	302.1	521	0.0019	261	0.25
	500 μg/L	24.63	7.99	307.8	516	0.0019	258	0.25
	Control	23.92	7.96	305.6	516	0.0019	258	0.25
Day20	5 μg/L	24.08	7.93	314.3	520	0.0020	259	0.25
	500 μg/L	24.49	7.88	320.8	515	0.0019	258	0.25
Day21	Control	24.01	7.99	311.6	516	0.0020	257	0.25

5 μg/L	24.13	8.01	321.3	513	0.0019	259	0.25
500 μg/L	24.50	8.02	307.9	514	0.0019	258	0.25

Table S2. Summary of raw data output and quality control from single-cell RNA sequencing of zebrafish brains in the control and two BDE-209 exposure groups (5 and 500 μg/L). UMI, Unique Molecular Identifier.

Sample	Raw data (Gb)	Raw Reads	Number of Reads	Valid Barcodes	Q30 Bases in Barcode	Q30 Bases in RNA Read	Q30 Bases in UMI
Control	98.92	329740233	329740233	96.3%	97.5%	92.9%	97.3%
5 μg/L	108.39	361312075	361312075	96.5%	97.5%	92.7%	97.3%
500 μg/L	102.58	341938440	341938440	96.7%	97.5%	92.4%	97.4%

Table S3. Statistics of the number of effective cells obtained from single-cell RNA sequencing of zebrafish brains in the control and two BDE-209 exposure groups (5 and 500 μg/L). UMI, Unique Molecular Identifier.

Sample	Barcode Count in Whitelist	Detected Barcode Count	Detected Barcode UMI Count	Estimated Barcode Count	Estimated Barcode UMI Count
Control	1528515	1021217	79904970	19417	65846223
5 μg/L	1407030	916830	84162580	23706	71612799
500 μg/L	1414451	953685	79758101	17712	68128090