

Supplementary Information

Organic-inorganic covalent selenium reversing ischemic reperfusion injury

Zushuang Xiong ^{1,2,3,#}, Guanning Huang ^{1,2,#}, Jia-Run Huang ², Ying Liu ¹, Lizhen He ^{1,*},

Tianfeng Chen ^{1,2,3,*}

¹ Department of Neurology and Stroke Center, The First Affiliated Hospital, Jinan University, Guangzhou 510632, China.

² Department of Chemistry, State Key Laboratory of Bioactive Molecules and Druggability Assessment, Key Laboratory for Regenerative Medicine of Ministry of Education, Key Laboratory of Viral Pathogenesis & Infection Prevention and Control, Jinan University, Guangzhou 510632, China.

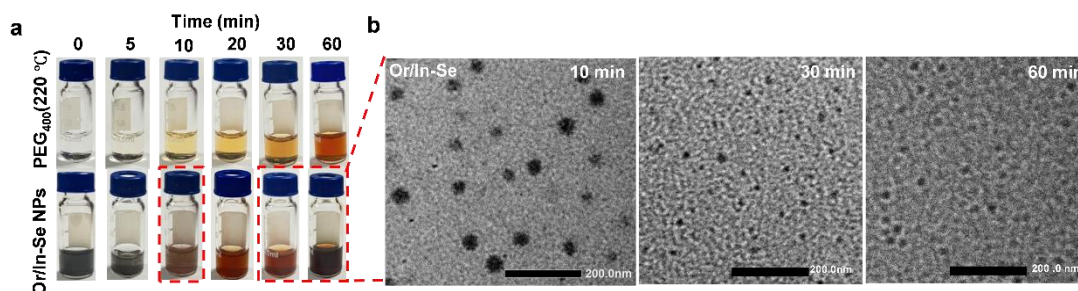
³ Guangdong Provincial Key Laboratory of Tumor Interventional Diagnosis and Treatment, Zhuhai People's Hospital (Zhuhai Hospital Affiliated with Jinan University), Jinan University, Guangdong 519000, China.

These authors contributed equally to this work: Zushuang Xiong and Guanning Huang.

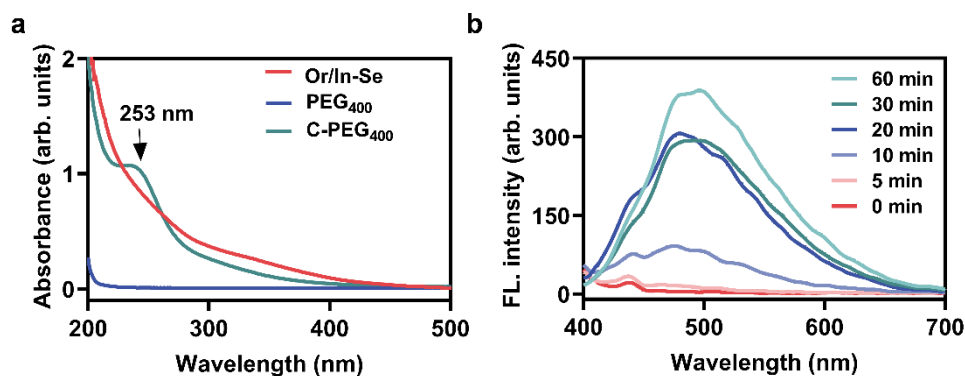
* Corresponding author.

hlz6371@jnu.edu.cn, tchentf@jnu.edu.cn

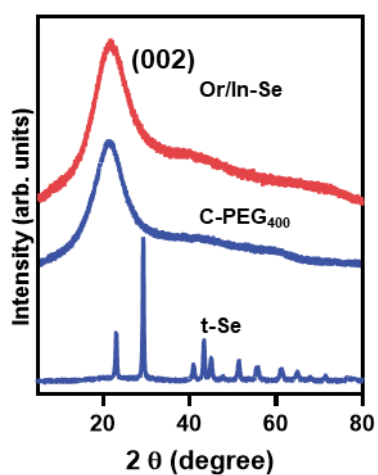
Supplementary Figures



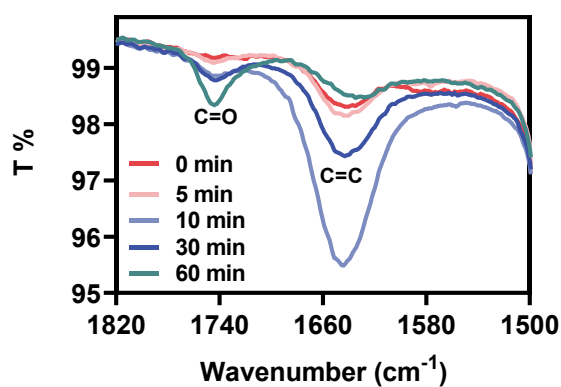
Supplementary Figure 1. **a** Digital images for the reaction system of free PEG₄₀₀ and Or/In-Se NPs reaction system under 220 °C at different time points. **b** TEM images of Or/In-Se NPs at different reaction time. Experiments were repeated three times independently with similar results.



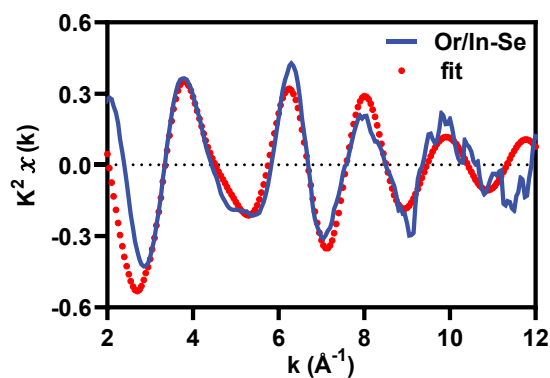
Supplementary Figure 2. **a** UV-Vis spectra of the free PEG₄₀₀ at room temperature, the Or/In-Se NPs and PEG₄₀₀ system under 220 °C at 60 min (C-PEG₄₀₀). **b** Fluorescence spectra of the Or/In-Se NPs reaction system at different time points.



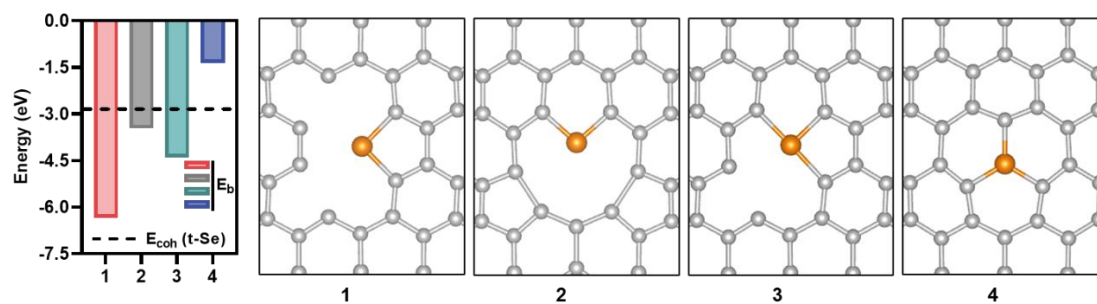
Supplementary Figure 3. XRD spectra for the Or/In-Se NPs, C-PEG₄₀₀ and t-Se.



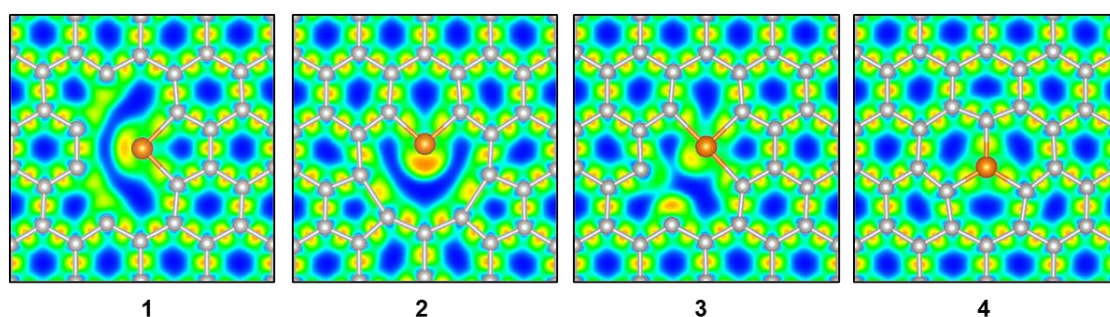
Supplementary Figure 4. FT-IR spectra for Or/In-Se NPs reaction system at different time points.



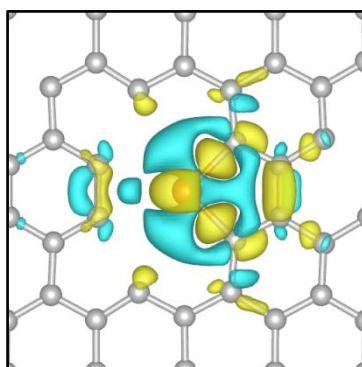
Supplementary Figure 5. Se K-edge EXAFS (line) and fit (points) for the sample, shown in k^2 weighted k -space.



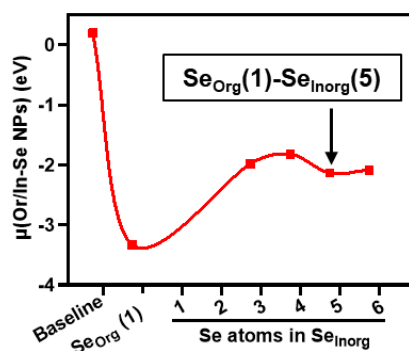
Supplementary Figure 6. Chemical structure for composites of Se atom with different sites on carbon dots and corresponding cohesive energy.



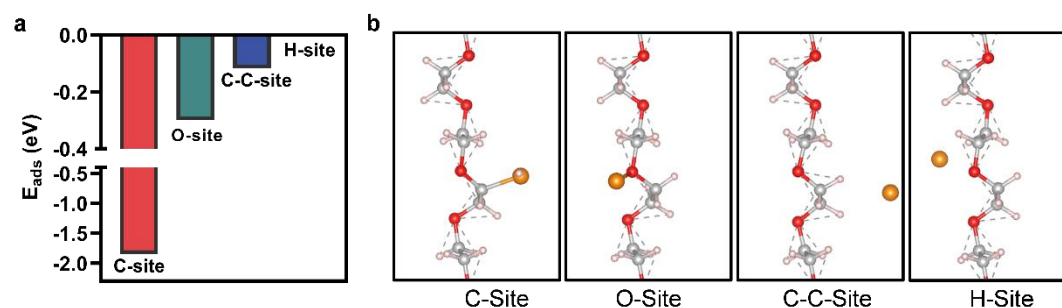
Supplementary Figure 7. ELF images for composites of Se atom with different sites on carbon dots.



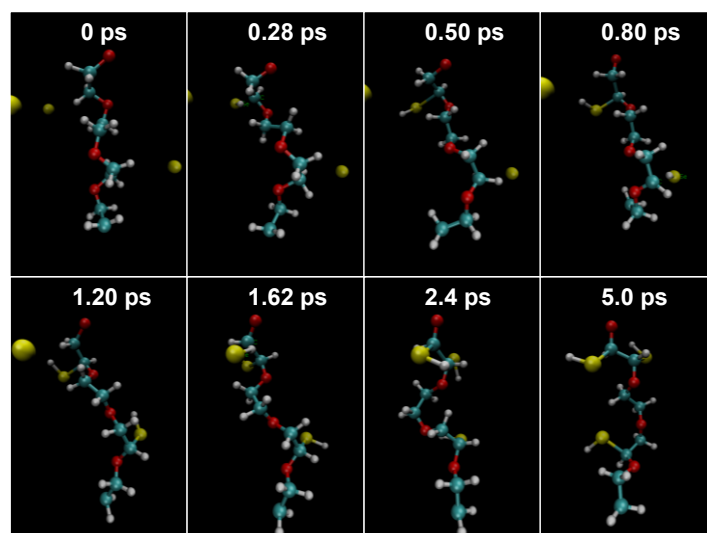
Supplementary Figure 8. Electron density difference of composites of Se atom with graphene.



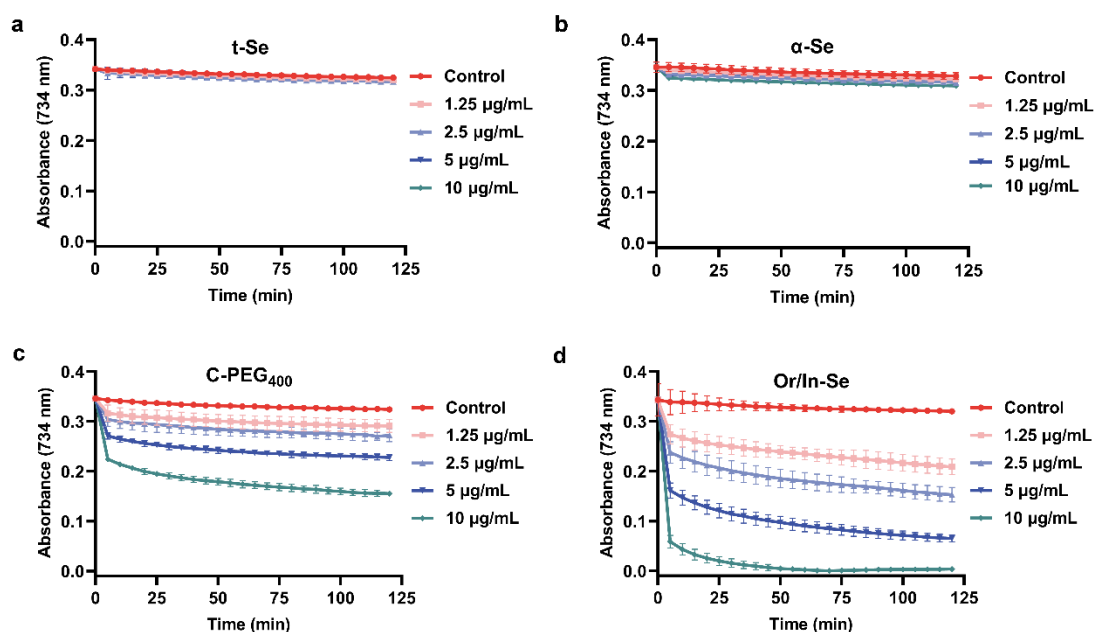
Supplementary Figure 9. The chemical potential for the simulated Or/In-Se NPs, $\mu(\text{Or/In-Se NPs})$, containing different Se atoms, $\text{Se}_{\text{Inorg}}(x)$. When the Se atom number is 1 which will form C-Se firstly to be Se_{Org} . The total number of Se atoms in Or/In-Se NPs ($\text{Se}_{\text{Total}} = \text{Se}_{\text{Org}}(1) + \text{Se}_{\text{Inorg}}(x)$).



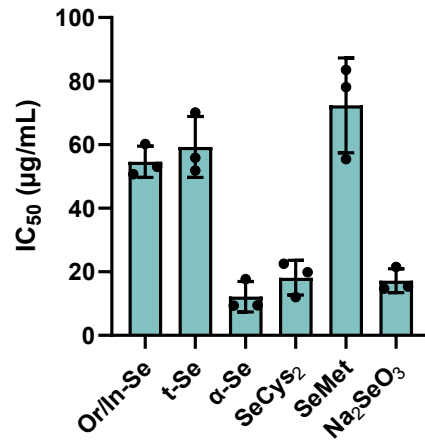
Supplementary Figure 10. **(a)** Binding energy and **(b)** images for Se atom to bind with C, O, C-C and H sites in the PEG chain.



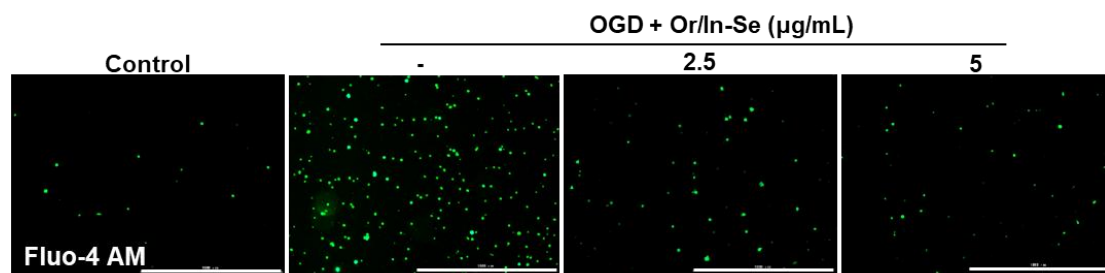
Supplementary Figure 11. Chemical structure of the composites of Se atoms attached to PEG chain at different times in AIMD.



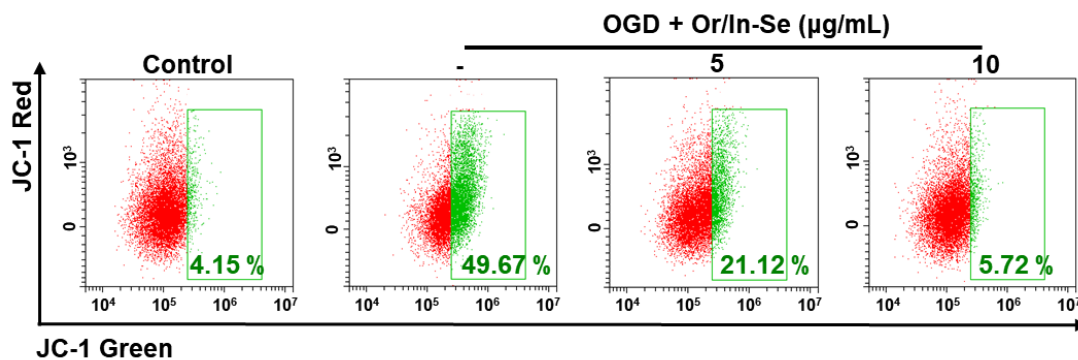
Supplementary Figure 12. Absorption of ABTS free radical scavenging at 734 nm for (a) t-Se, (b) α -Se, (c) C-PEG₄₀₀ and (d) Or/In-Se NPs within 2 h (mean $\pm$ SD, n=3 biological replicates).



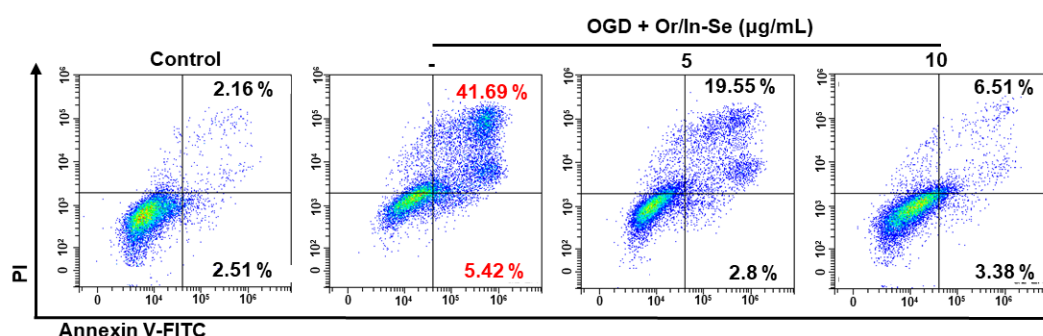
Supplementary Figure 13. IC₅₀ value of different Se species to SH-SY5Y cells for 72 h (mean ± SD, n=3 biological replicates).



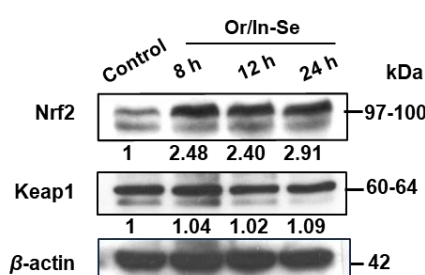
Supplementary Figure 14. Fluorescence images of SH-SY5Y cells labeled by Fluo-4 AM under OGD model with different concentration of Or/In-Se NPs (n=3 biological replicates, scale bar: 1000 µm).



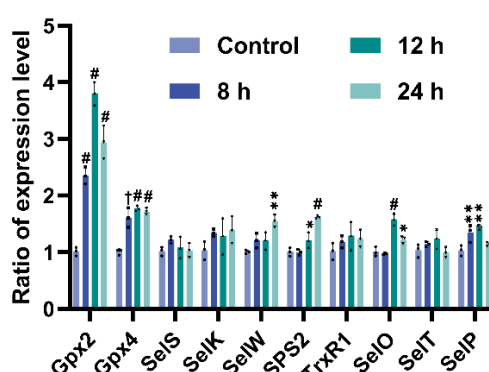
Supplementary Figure 15. Mitochondrial membrane potentials detected by JC-1 probe in SH-SY5Y OGD-cells model with different concentration treatment of Or/In-Se NPs (n=3 biological replicates).



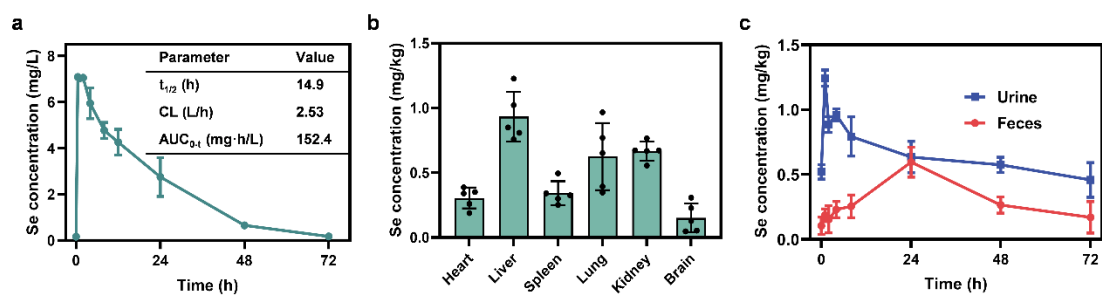
Supplementary Figure 16. Apoptotic cell ratio in SH-SY5Y OGD-cells model with Or/In-Se NPs-treatment detected by Annexin-V/PI double staining and analyzed by flow cytometry (n=3 biological replicates).



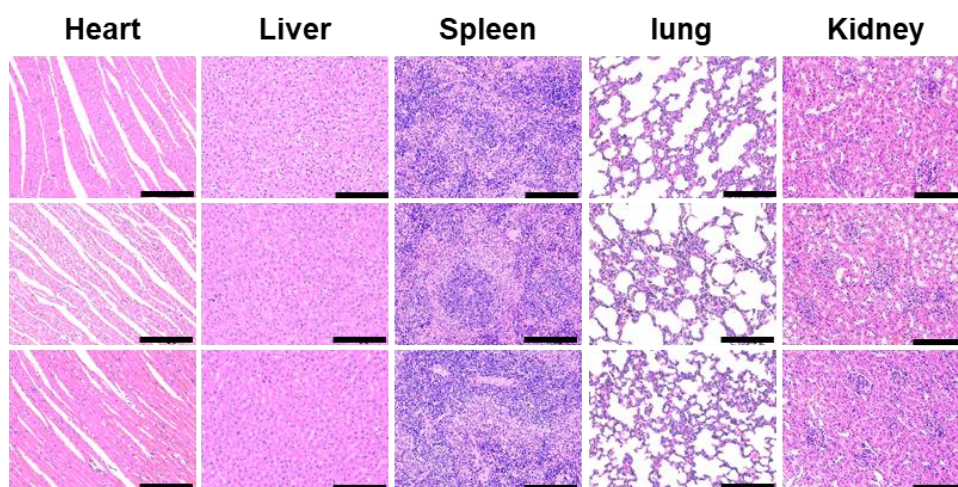
Supplementary Figure 17. Expression level of Nrf2 and Keap1 in SH-SY5Y cells after incubation with Or/In-Se NPs (10 µg/mL) for 8, 12 and 24 h, n=3 biological replicates.



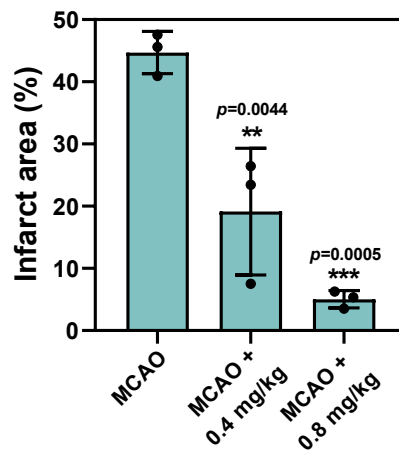
Supplementary Figure 18. Quantification and statistical analysis of selenoproteins in SH-SY5Y cells after incubation with Or/In-Se NPs (10 µg/mL) for 8, 12 and 24 h (mean $\pm$ SD, n=3 biological replicates, compared with Control group). The specific p values are as followed (One-Way ANOVA test, * p <0.05, ** p <0.01, † p <0.001, # p <0.0001, compared with the Control group): Gpx2 (8 h: # p =0.000083; 12 h: # p =0.00000031; 24 h: # p =0.0000056), Gpx4 (8 h: † p =0.00017; 12 h: # p =0.000029; 24 h: # p =0.000052), SelW (24 h: ** p =0.0011), SPS2 (12 h: * p =0.033; 24 h: # p =0.000046), SelO (12 h: # p =0.000033; 24 h: * p =0.020), SelP (8 h: ** p =0.0083; 12 h: ** p =0.0014).



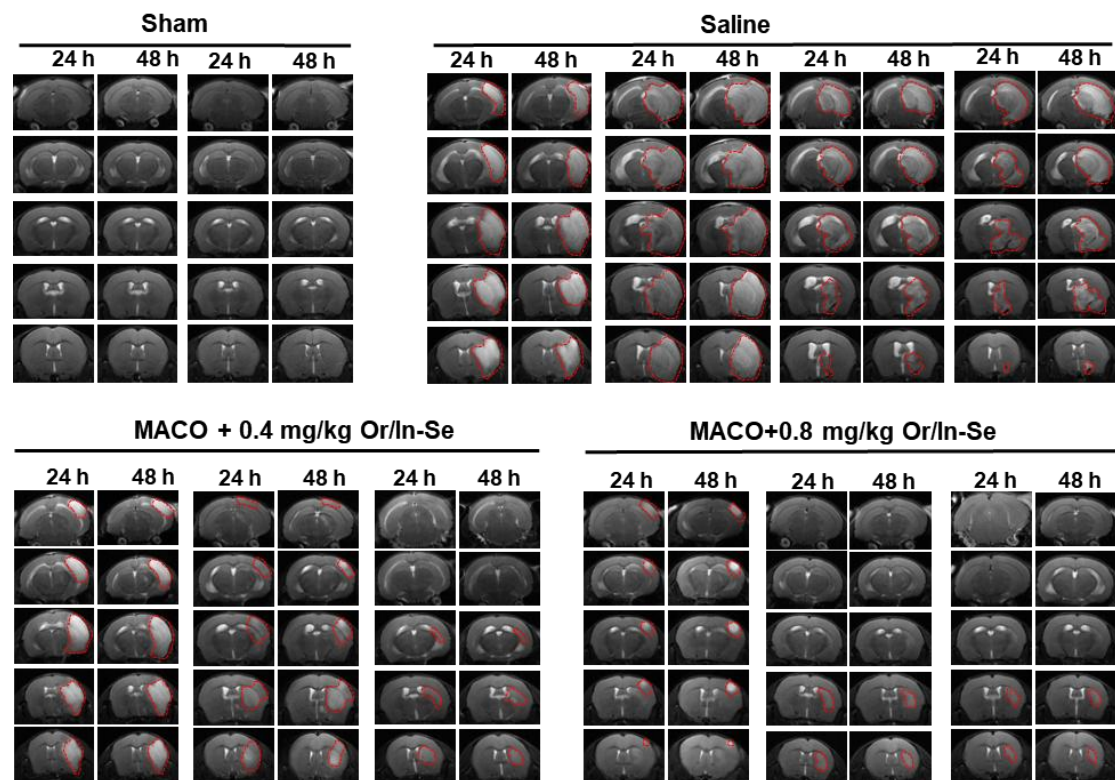
Supplementary Figure 19. The concentration of Se in (a) blood serum, (b) main organs, and (c) urine and feces of Or/In-Se NPs treated mice at different time points after injection of Or/In-Se NPs (2 mg/kg) (mean $\pm$ SD, n=5 biologically independent samples).



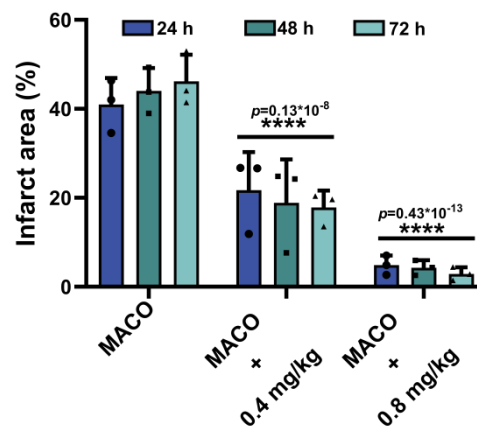
Supplementary Figure 20. H&E staining of the main organs in Or/In-Se NPs treated mice (n=3 biologically independent samples, scale bar: 50 μ m).



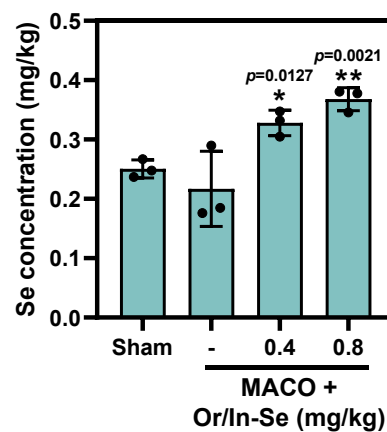
Supplementary Figure 21. Infarct areas of the treated MCAO mice quantified the TTC staining images by ImageJ (mean $\pm$ SD, $n=3$ biologically independent samples, One-Way ANOVA test, ** $p<0.01$, *** $p<0.001$, compared with MCAO group).



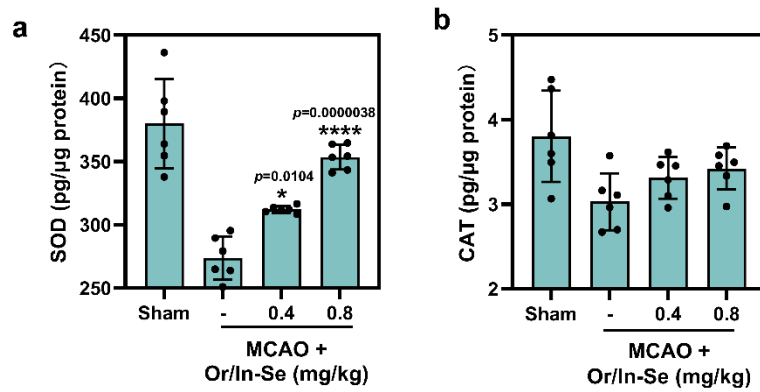
Supplementary Figure 22. MRI T2 images for monitoring the progression of infarct areas in the treated MCAO mice at time points of 24 h and 48 h (Red cycle: Infarct area, $n=3$ biologically independent samples).



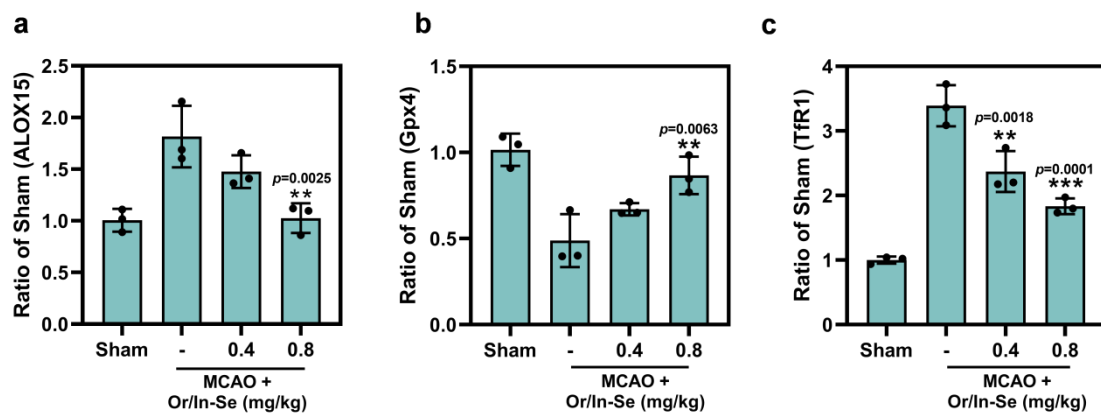
Supplementary Figure 23. Corresponding infarct areas of the treated MCAO mice quantified the maximum cross section of MRI data at different treated time points (mean $\pm$ SD, n=3 biologically independent samples, One-Way ANOVA test, **** p <0.0001, compared with MCAO group).



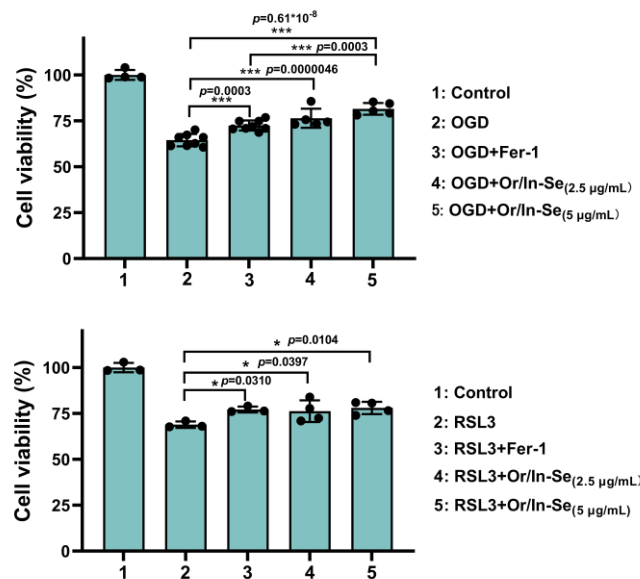
Supplementary Figure 24. Se content in brain tissues of MCAO mice with different treatments (mean $\pm$ SD, n=3 biologically independent samples, One-Way ANOVA test, * p <0.05, ** p <0.01, compared with MCAO group).



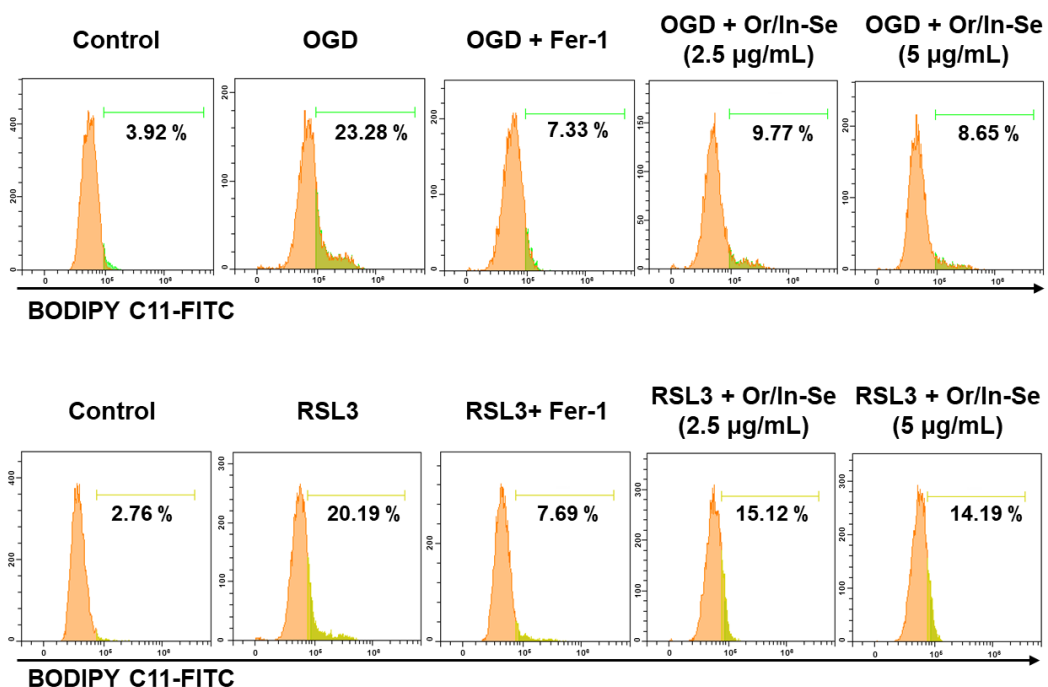
Supplementary Figure 25. Concentration of (a) SOD and (b) CAT in brain homogenate on the injured side of MCAO mice with different treatments (mean $\pm$ SD, $n=6$ biologically independent samples, One-Way ANOVA test, $*p<0.05$, $****p<0.0001$, compared with MCAO group).



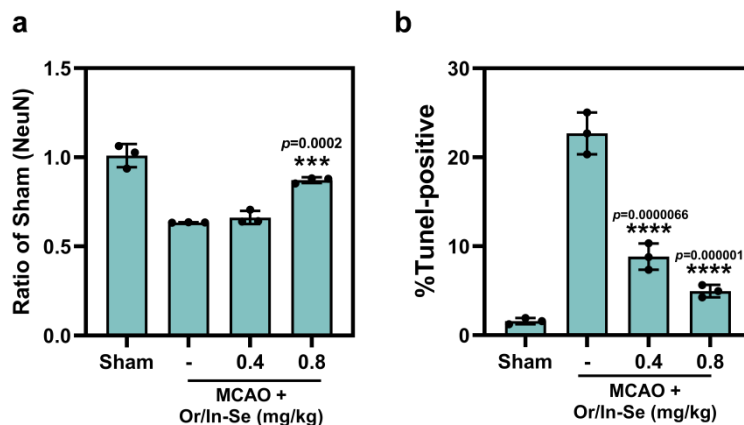
Supplementary Figure 26. Immunofluorescence quantification of (a) ALOX15, (b) Gpx4 and (c) TfR1 images of brain tissues in MCAO mice with different treatments (mean $\pm$ SD, $n=3$ biologically independent samples, One-Way ANOVA test, $*p<0.05$, $**p<0.01$, $***p<0.001$, compared with MCAO group).



Supplementary Figure 27. Cell viability of SH-SY5Y cells with treatment of Fer-1 (5 μ M) and Or/In Se NPs (2.5 and 5 μ g/mL) under OGD (6 h) model and RSL3 (10 μ M) model (mean $\pm$ SD, $n > 3$ biological replicates, One-Way ANOVA test, * $p < 0.05$, *** $p < 0.001$, compared with 2 group).



Supplementary Figure 28. Lipid peroxidation level of cells treated with Fer-1 (5 μ M) and Or/In Se NPs (2.5 and 5 μ g/mL) under OGD model (6 h) and RSL3 (10 μ M) model ($n = 3$ biological replicates).



Supplementary Figure 29. Immunofluorescence quantification of **(a)** NeuN and **(b)** TUNEL images of brain tissues in MCAO mice with different treatments (mean $\pm$ SD, $n=3$ biologically independent samples, One-Way ANOVA test, *** $p<0.001$, **** $p<0.0001$, compared with MCAO group).

Supplementary Tables

Supplementary Table 1. Fitting parameters of Se K-edge EXAFS for collected data of Or/In-Se NPs.

Paths	Se-C	Se-Se
CN	1.9 $\pm$ 0.1	2.1 $\pm$ 0.2
R/ $\text{\AA}$	1.99	2.31
σ^2	0.001	0.019

Supplementary Table 2. Fitting parameters for Se K-edge EXAFS of Or/In-Se NPs with simulated model of Se_{Org}(1)-Se_{Inorg}(4), Se_{Org}(1)-Se_{Inorg}(5) and Se_{Org}(1)-Se_{Inorg}(6).

Fitting model of inorganic	Scattering pair	CN	R (Å)	σ^2	ΔE_0 (eV)	R factor
Se _{Org} (1)-Se _{Inorg} (4)	Se-C	1.79±0.04	2.00±0.10	0.0011	8.56±0.39	0.002
	Se-Se	4.12±1.35	2.32±0.14	0.0252		
Se _{Org} (1)-Se _{Inorg} (5)	Se-C	1.95±0.09	1.99±0.09	0.0028	7.73±0.44	0.009
	Se-Se	2.07±0.22	2.32±0.01	0.0172		
Se _{Org} (1)-Se _{Inorg} (6)	Se-C	1.74±0.04	2.00±0.09	0.0009	8.60±0.32	0.003
	Se-Se	3.92±0.28	2.33±0.04	0.0247		

The condition of parameters fitted in **Tables 1** and **2** are as follows. S_0^2 was obtained from Se foil and fixed as 0.7156. ΔE_0 was returned a value of 6.004±0.3616 eV. Data range $3.0 \leq k \leq 11.0 \text{ Å}^{-1}$, $1.5 \leq R \leq 2.5 \text{ Å}$. The number of variable parameters is 3, out of a total of 6.3662 independent data points, R factor for this fit is 0.35%. The Debye-Waller factors and delta R s are obtained based on the guessing parameters and constrained for Se-C and Se-Se.

Supplementary Table 3. The blood biochemical detections for the mice after injection of Or/In-Se NPs for 72 h (n=3 biological replicates).

Parameters	Unit	Or/In-Se NPs	Reference
ALT	U/L	50.25±8.15	21.53-61.75
AST	U/L	154.31±26.99	41.47-195.65
γ-GT	U/L	0.84±0.04	0.58-6.81
BUN	mg/dL	22.97±3.44	9.75-22.71
UA	μmol/L	61.18±13.82	58.38-122.65
CR	μmol/L	31.89±6.41	10.90-118.07
CK	U/L	480.98±24.39	58.24-530.38
T-CHO	mmol/L	2.39±0.35	1.13-3.48
TG	mmol/L	0.67±0.13	0.34-2.19
HDL-C	mmol/L	0.93±0.17	0.31-0.73
LDL-C	mmol/L	0.26±0.03	0.09-0.24
GLU	mmol/L	8.76±0.76	4.98-15.95

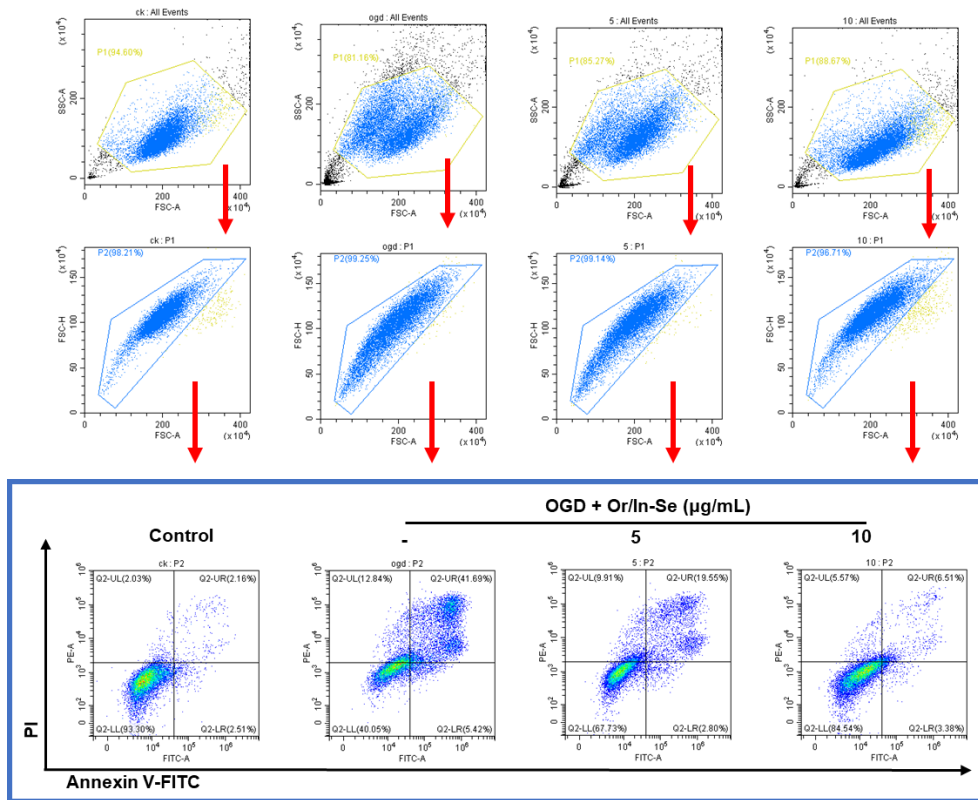
Supplementary Table 4. Oligonucleotides used in this study.

Primer name	Sequence (from 5' to 3')
H- <i>GAPDH</i> Forward	CCATGTTTCGTCATGGGTGTGAACCA
H- <i>GAPDH</i> Reverse	GCCAGTAGAGGCAGGGATGATGTTC
H- <i>SELENOH</i> Forward	TCTTCGTTGCCCAGTTTC
H- <i>SELENOH</i> Reverse	TCAGCCTTACGCTTCCTC
H- <i>SELENOK</i> Forward	CGGACAAGTGTTGGACAGC
H- <i>SELENOK</i> Reverse	GCCACGCAGATGATTGATT
H- <i>SELENOM</i> Forward	AACCGCCTAAAGGAGGTGAAG
H- <i>SELENOM</i> Reverse	GATGCGCTCTAGTTCCTCGTA
H- <i>SELENON</i> Forward	TGGCCTTTTTCTCTTTTCC
H- <i>SELENON</i> Reverse	GACAGAACAAGACCCTGTTA
H- <i>SELENOO</i> Forward	AAGGTCTCCTACTTGCCGTTCA
H- <i>SELENOO</i> Reverse	GAGTTCTCCTAGTTATTCTA
H- <i>SELENOP</i> Forward	TGTGATAACTTGGGACAGTG
H- <i>SELENOP</i> Reverse	TCATTGTTCCATTCTGAGGG
H- <i>SELENOS</i> Forward	AGAAGATTGAAATGTGGGACA
H- <i>SELENOS</i> Reverse	CGCCTCCTTCACCAGACAA
H- <i>SELENOT</i> Forward	ATCCTTTTGCTTTCTTTGGC
H- <i>SELENOT</i> Reverse	CTGGTTCTCAATCATGTTGC
H- <i>SELENOW</i> Forward	CCGTCCGAGTCGTTTATT
H- <i>SELENOW</i> Reverse	GTAGCCATCGOCTTTCTT
H- <i>TXNRD1</i> Forward	GGGACAGAATGATAGAAGC
H- <i>TXNRD1</i> Reverse	CTAAACCAATACCAGCAAG
H- <i>TXNRD3</i> Forward	TGGACTACGTGGAACCTTCTC
H- <i>TXNRD3</i> Reverse	CGCCTGGTGCATCAGCT
H- <i>TXNRD2</i> Forward	AAAACATTAGTGGTGGGTG
H- <i>TXNRD2</i> Reverse	GGAGAAGGATTAAGCATA
H- <i>GPX1</i> Forward	TCGGTGTATGCCTTCTCGG
H- <i>GPX1</i> Reverse	CGTTCTCCTGATGOCCAAA
H- <i>GPX2</i> Forward	GATGACCCATTTTOCCTCAT
H- <i>GPX2</i> Reverse	ATGTCAGGCTCAATGTTGAT
H- <i>GPX3</i> Forward	CCATTCCGGTCTGGTCATT
H- <i>GPX3</i> Reverse	CCTGGTCCGACATACTTG
H- <i>GPX4</i> Forward	AGGGAGTAACGAAGAGATCA
H- <i>GPX4</i> Reverse	GATGAGGAACTTGGTGAAGT
H- <i>SELENOF</i> Forward	CTCATTGAGAACGGGCCATG
H- <i>SELENOF</i> Reverse	ATCAACAGGATCCCAAGG CA

Supplementary Table 5. Oligonucleotides used in this study.

Primer name	Sequence (from 5' to 3')
M- <i>GAPDH</i> Forward	GGGAAGCCCATCACCATC
M- <i>GAPDH</i> Reverse	CGGCCTCACCCCATTTG
M- <i>TXNRD1</i> Forward	CCTATGTCGCCTTGGAATGTGC
M- <i>TXNRD1</i> Reverse	ATGGTCTCCTCGCTGTTGTGG
M- <i>TXNRD2</i> Forward	CTTTGCAAGATGCCAAGAAA
M- <i>TXNRD2</i> Reverse	TCATGGCCTCCCAGTTGT
M- <i>GPX2</i> Forward	GCAACCAGTTCGGACATCAGGA
M- <i>GPX2</i> Reverse	GAGGGAGAATGGGTCGTCATAAGG
M- <i>GPX3</i> Forward	ATTTGGCTTGGTCATTCTGG
M- <i>GPX3</i> Reverse	CCACCTGGTCGAACATACTTG
M- <i>GPX4</i> Forward	TCTGTGTAAATGGGGACGATGC
M- <i>GPX4</i> Reverse	TCTCTATCACCTGGGGCTCCTC
M- <i>SELENOS</i> Forward	AGAAGATTGAAATGTGGGACA
M- <i>SELENOS</i> Reverse	CGCCTCCTTCACCAGACAA
M- <i>SELENOT</i> Forward	GAGTACATGCGGGTTATCAG
M- <i>SELENOT</i> Reverse	CACCTGTTGACATACACTGG
M- <i>SELENOV</i> Reverse	CTTGATCCGTGTGATGTACT
M- <i>SELENOV</i> Forward	CCACAAACACTTCAAACCTCC
M- <i>SELENOW</i> Forward	GGTGCCTCCCCAGAATCTAC
M- <i>SELENOW</i> Reverse	TGGGGGAATTCAGAGAGAGA
M- <i>DIO3</i> Forward	GGAGGTTGTCCGACCTGAT
M- <i>DIO3</i> Reverse	GTCCCTTGTGCGTAGTCGAG

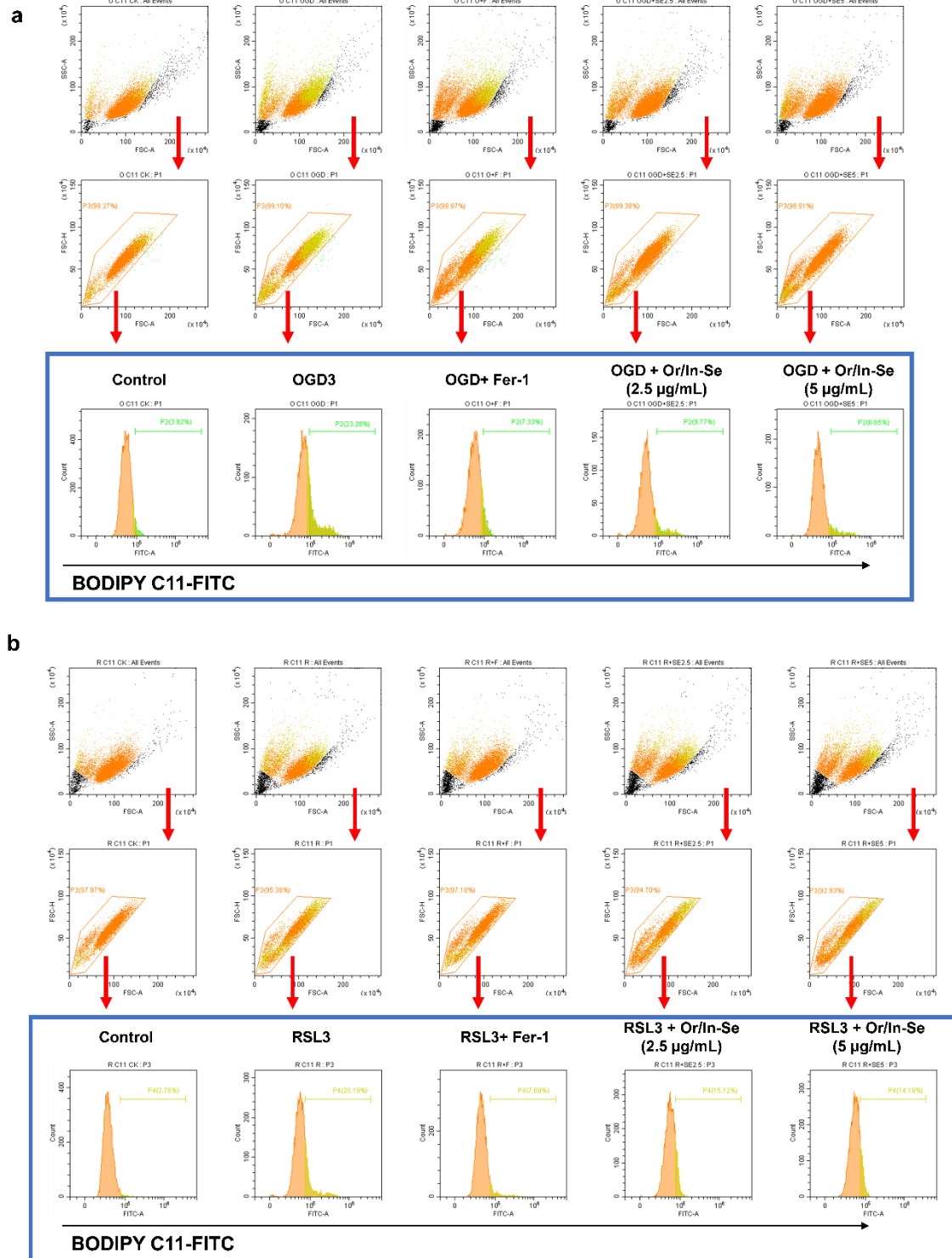
Source Data for Supplementary Figure 16



Supplementary Figure 30. The gating strategy for the flow cytometry of cell apoptosis.

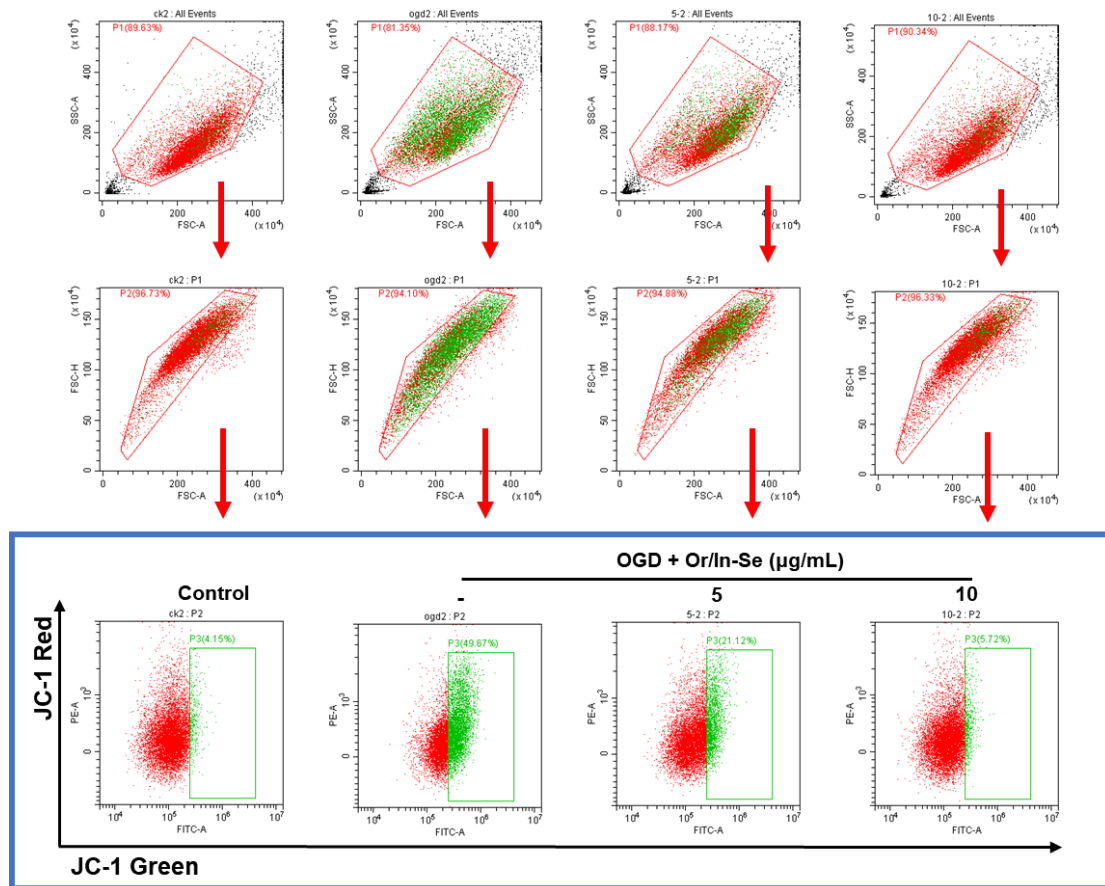
The data in the blue line box was shown in Supplementary Figure 16.

Source Data for Supplementary Figure 28



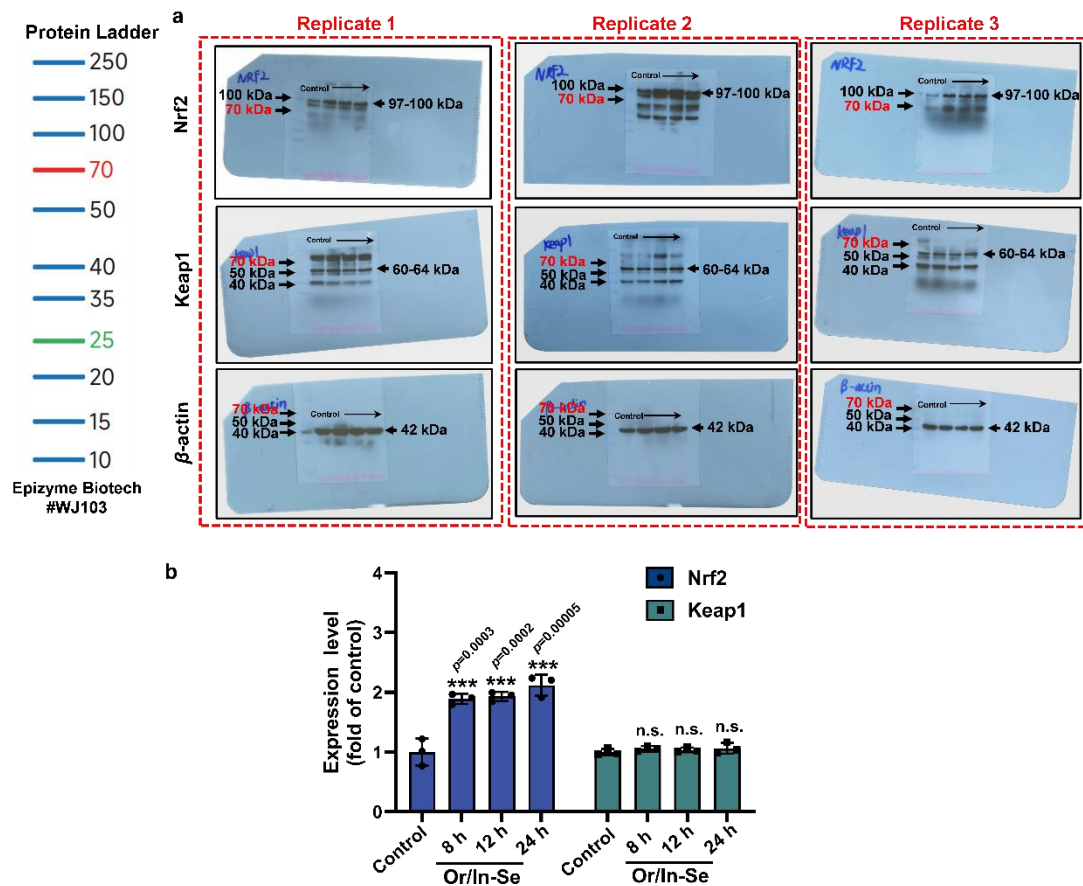
Supplementary Figure 31. The gating strategy for the flow cytometry of lipid peroxidation level in **(a)** OGD and **(b)** RSL model. The data in the blue line box was shown in Supplementary Figure 28.

Source Data for Supplementary Figure 15



Supplementary Figure 32. The gating strategy for the flow cytometry of mitochondrial membrane potentials. The data in the blue line box was shown in Supplementary Figure 15.

Source Data for Supplementary Figure 17



Supplementary Figure 33. a Uncropped Western Blotting images with protein ladders and **(b)** quantification and statistical analysis of Nrf2 and Keap1. The replicate 1 was used as representative results in Supplementary Figure 17 (mean $\pm$ SD, $n=3$ biological replicates, One-Way ANOVA test, *** $p<0.001$, compared with the Control group, n.s., no significance compared with Control group).