Supporting Information

Co(II)-based metal–organic framework induces apoptosis through activating the HIF-1α/BNIP3 signaling pathway in microglial cells

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I. Materials and Methods

1. Reagents.

Methanol (Sinopharm Chemical Reagent Co., Ltd., Beijing, China), ethanol (Sinopharm Chemical Reagent Co., Ltd.), N,N-dimethylformamide (DMF) (Shanghai Macklin Biochemical Co., Ltd., Shanghai, China), $Co(NO_3)_2 \cdot 6H_2O$ (Sigma-Aldrich, St. Louis, USA), $FeCl_3 \cdot 6H_2O$ (Sinopharm Chemical Reagent Co., Ltd.), 1,4-benzenedicarboxylic acid (H_2BDC) (Alfa Aesar Chemicals Co., Ltd., Tianjin, China), $Cu(NO_3)_2 \cdot 3H_2O$ (Tianjin Fuchen Chemical Reagent Factory, Tianjin, China), Trimellitic acid (H_3BTC) (Shanghai Macklin Biochemical Co., Ltd.), $Mn(NO_3)_2 \cdot 4H_2O$ (Sinopharm Chemical Reagent Co., Ltd.), $Zn(NO_3)_2 \cdot 6H_2O$ (Alfa Aesar Chemicals Co., Ltd.), 2-Methylimidazole (2-MEIM) (Shanghai Macklin Biochemical Co., Ltd.) were used. All chemicals purchased were used without further purification.

2. Synthesis of MOFs

ZIF-67 [1]: 0.6 g of $Co(NO_3)_2 \cdot 6H_2O$ and 0.532 g of 2-MEIM were dissolved in 40 mL methanol, respectively. After sonication, the solutions were mixed and stirred for 24 h at room temperature. To get the product, the mixture was centrifuged for 10 min at 6000 g, and then washed several times with ethanol to remove the unreacted reagents until the supernatant was colorless. Finally, the as-synthesized ZIF-67 was freeze-dried and stored for further analysis.

MIL-101 (Fe) [2]: 0.675 g $FeCl_3 \cdot 6H_2O$ and 0.206 g H_2BDC were dissolved in 15 mL of DMF. The mixture solution was sonicated for 10-15 min to make the solid fully dissolved and then transferred to a Teflon-lined stainless steel autoclave and heated at 110 °C in a muffle oven for 20 h. Afterwards, the orange solid products were separated by centrifugation and washed with DMF and ethanol three times, respectively. At last, MIL-101 (Fe) were dried at 70 °C in an oven overnight.

Cu-BTC [3]: 0.363 g $Cu(NO_3)_2 \cdot 3H_2O$ and 0.21 g H_3BTC were dissolved in 20 mL of DMF. The mixture solution was stirred for 10 min to make the solid fully dissolved and then transferred to a Teflon-lined stainless steel autoclave and heated at 140 °C in a muffle oven for 24 h. After cooling down at room temperature, the solid product was recovered by

filtration, and washed repeatedly with DMF followed by methanol. Finally, Cu-BTC was dried at 80°C in an oven overnight.

MIL-100 (Mn) [4]: 0.502 g $Mn(NO_3)_2 \cdot 4H_2O$ and 0.399 g H_3BTC were dissolved in 18 mL of ethanol. The mixture solution was sonicated for 10 min to make the solid fully dissolved and then transferred to a Teflon-lined stainless steel autoclave and heated at 125 °C in a muffle oven for 2 h. After cooling to room temperature, the obtained product was collected, centrifuged and washed three times with ethanol. Then the product was dried at 70 °C and stored for analysis.

ZIF-8 [5]: 1.17 g $Zn(NO_3)_2 \cdot 6H_2O$ and 22.7 g 2-MEIM were dissolved in 88 mL of deionized water. The mixed solution was stirred for 5 min and aged for 24 h at room temperature. Afterwards, the white products were separated by centrifugation and washed with methanol three times. The washed products were dried at 80 °C overnight.

3. Characterization of MOFs

The morphologies and structural analysis of ZIF-67 (Co), ZIF-8 (Zn), MIL-100 (Mn), MIL-101 (Fe), and Cu-BTC were carried out by a transmission electron microscope (Philips CM10 100 kV microscope). The particle size distribution was evaluated using DMEM with 10% serum as a dispersion medium, and the samples were measured through dynamic light scattering (DLS) by a Photal ELS-8000 (OTSUKA Electronics, Osaka, Japan). X-ray diffraction (XRD) analysis is done with an X-ray source of Cu Kα radiation (X'Pert PRO MPD, PANalytical, Almelo, Holland). ζ-Potential was measured using dynamic light scattering (Zetasizer Nano 90, Malvern). Nanoparticle tracking analysis (NTA) was performed with a NanoSight LM20 (NanoSight, Amesbury, United Kingdom), equipped with a sample chamber with a 640-nm laser and a Viton fluoroelastomer O-ring. All measurements were performed at room temperature.

4. Cobalt dissolution test

The release rates of cobalt ions from ZIF-67 in DMEM with 10% serum at different concentrations and different time intervals were measured by ICP-MS (Agilent, California, USA). ZIF-67 was dispersed in the DMEM cell medium with 10% serum (5, 25, 50, and 100

μM) and incubated at 37 °C for 24 h. For ZIF-67 at 50 μM, the release content of cobalt was determined at 6, 12, 24, 48, and 96 h. After incubation, cell culture media were pipetted into a centrifuge tube and centrifuged at 13000 rpm for 15 min. The supernatant was collected and subject to the ICP-MS analysis.

II. Supplementary Figures

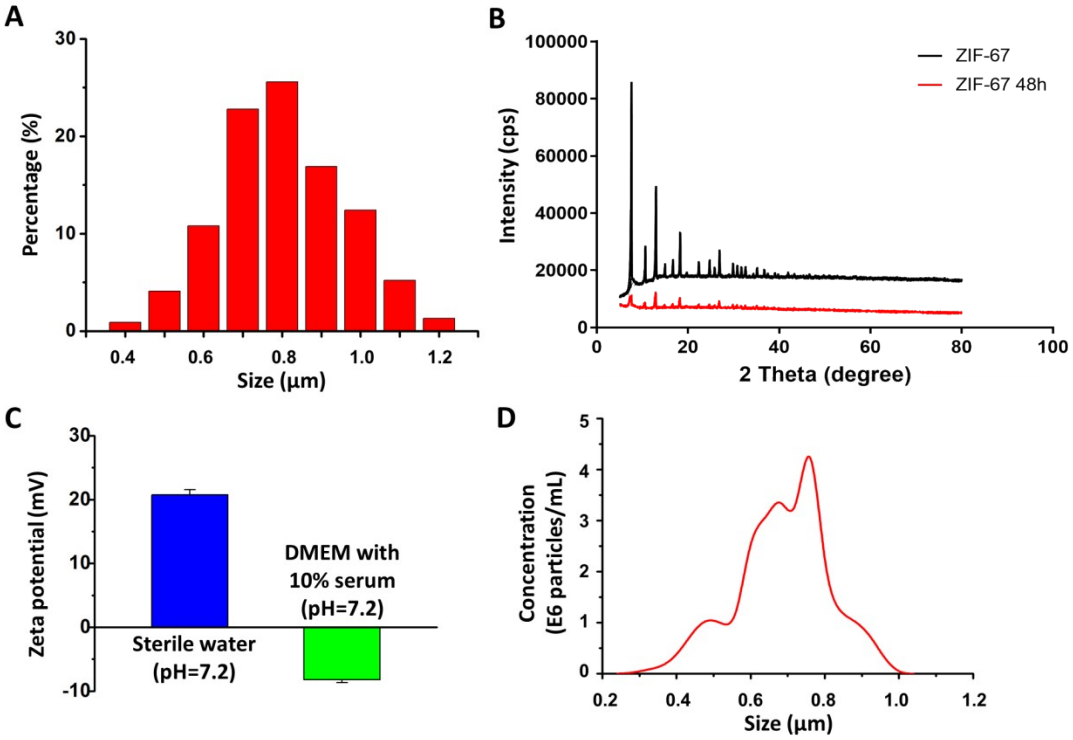

Figure S1. Characterization of ZIF-67. (A) TEM analysis of ZIF-67. (B) XRD patterns of ZIF-67 samples in DMEM with 10% serum. (C) The zeta potential of ZIF-67 in sterile water (pH=7.2) and in DMEM with 10% serum. (D) Size distribution of ZIF-67 measured by NTA.

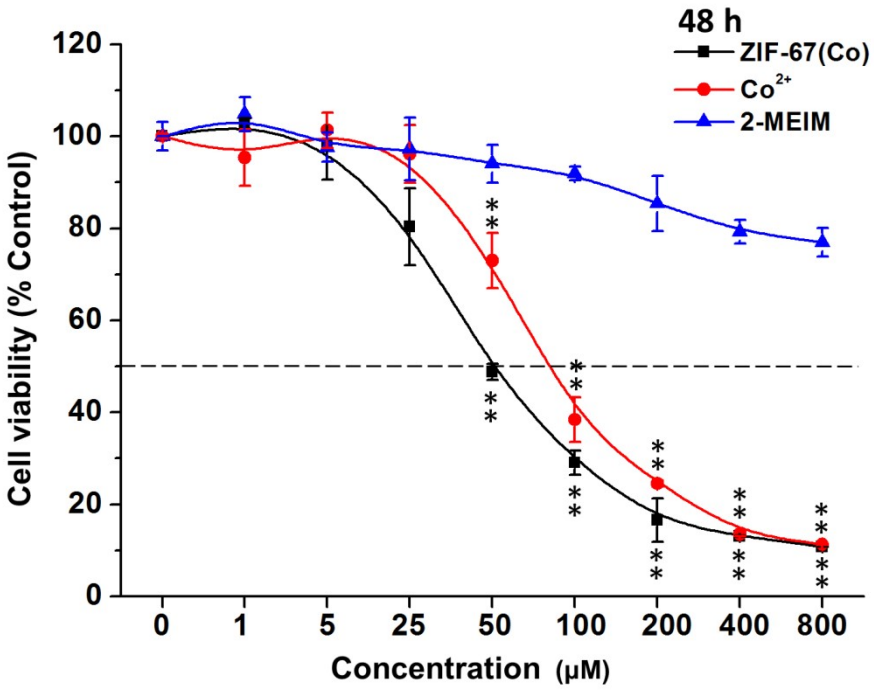

Figure S2. Cell viability of BV2 cells treated with ZIF-67, Co^{2+} and 2-MEIM for 48 h. Data are shown as means ± standard deviation (SD). *$p < 0.05$ and ** $p < 0.01$ compared with the control group.

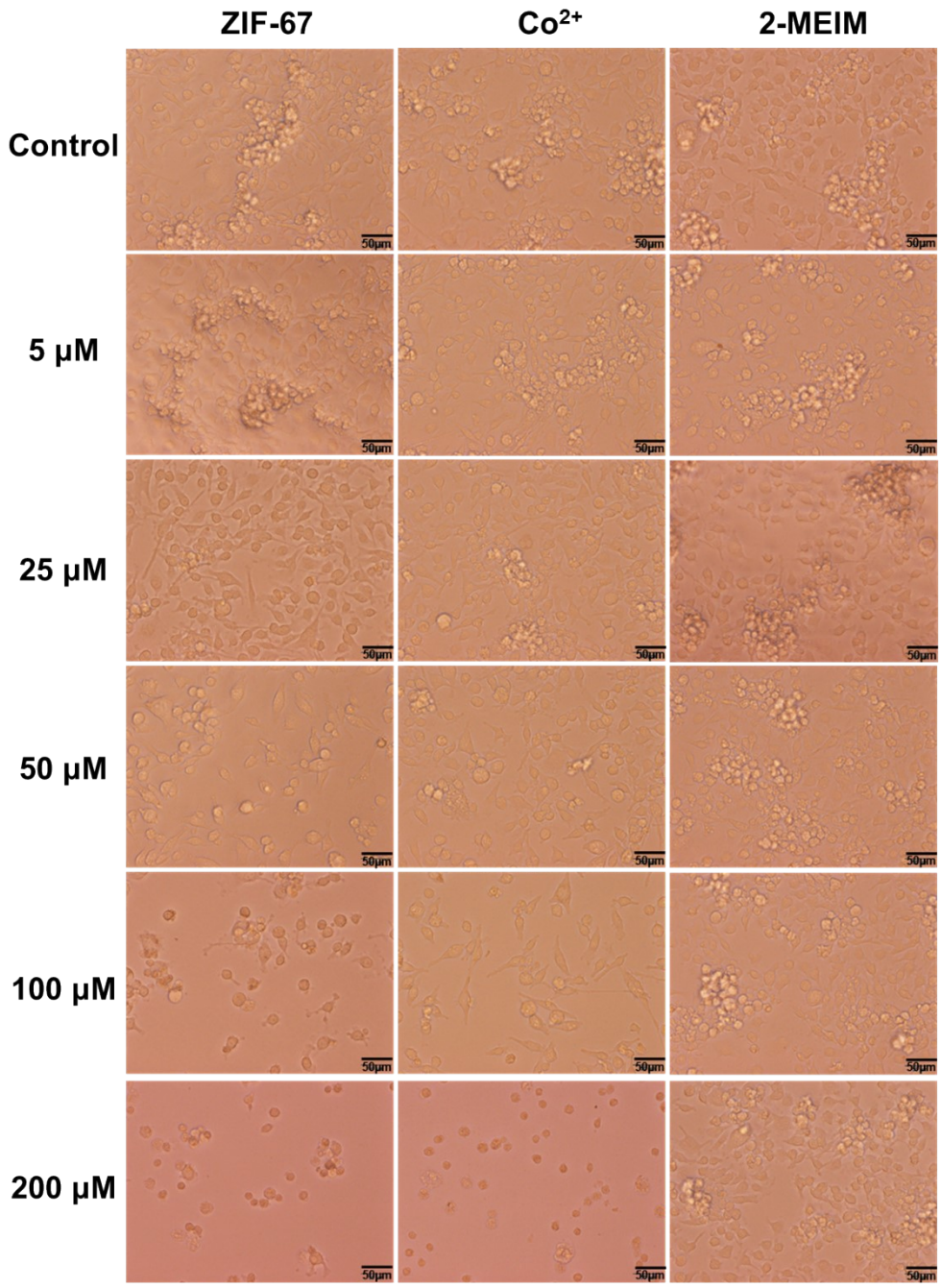

Figure S3. Morphological alterations in BV2 cells after 48 h of treatments with ZIF-67, Co^{2+} and 2-MEIM. Scale bar = 50 μm.

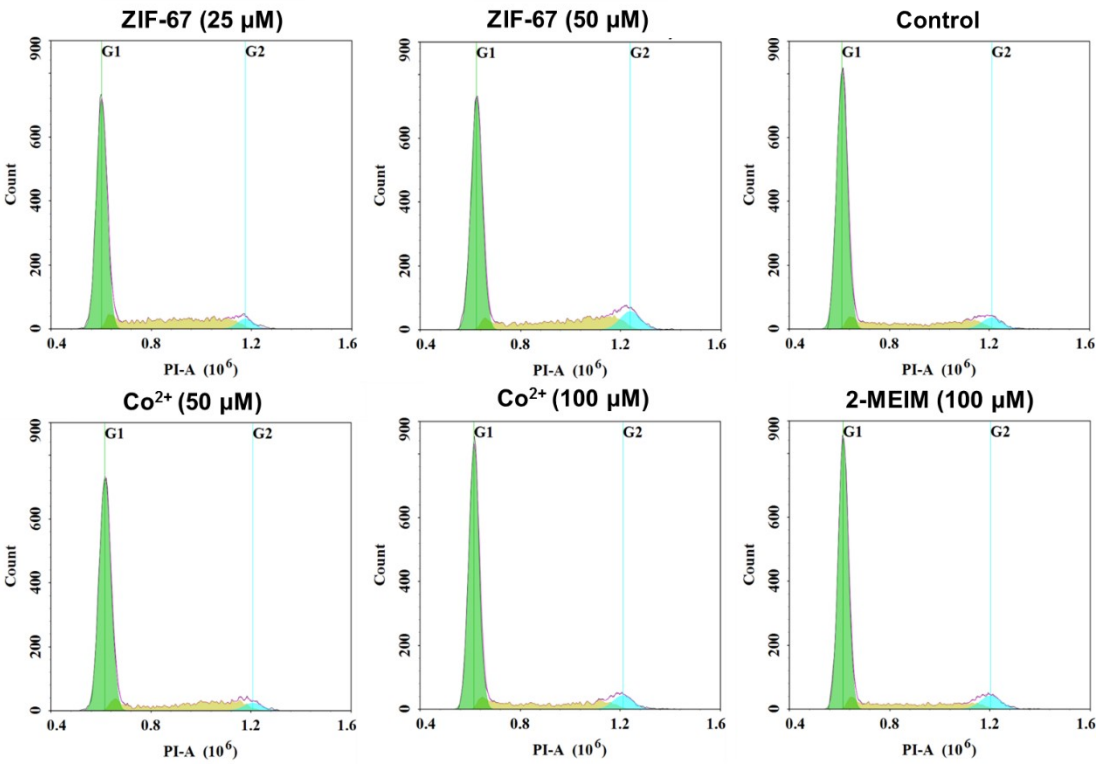

Figure S4. Distribution of cell cycle in BV2 cells after the ZIF-67, Co^{2+} and 2-MEIM treatments. Cells were harvested, stained with PI, and analyzed by flow cytometric analysis.

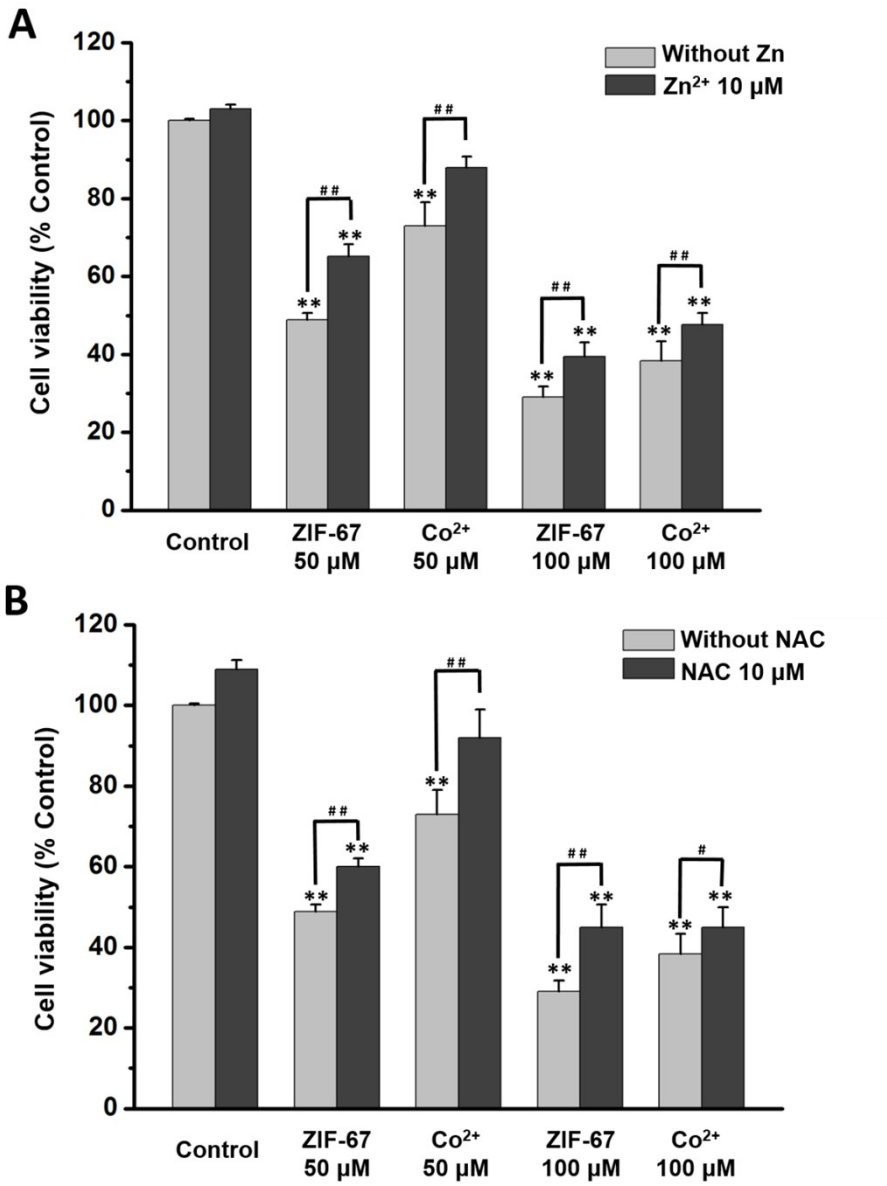

Figure S5. Protective effects of zinc ion (Zn^{2+}) and NAC against cytotoxicity in BV2 cells induced by ZIF-67 and Co^{2+}. The cells were exposed to 50 and 100 μM ZIF-67 and Co^{2+} alone or in combination with 10 μM Zn^{2+}/NAC for 48 h. Data are shown as means ± SD. ** $p < 0.01$ compared with the control group; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ compared with the relevant treatment group.

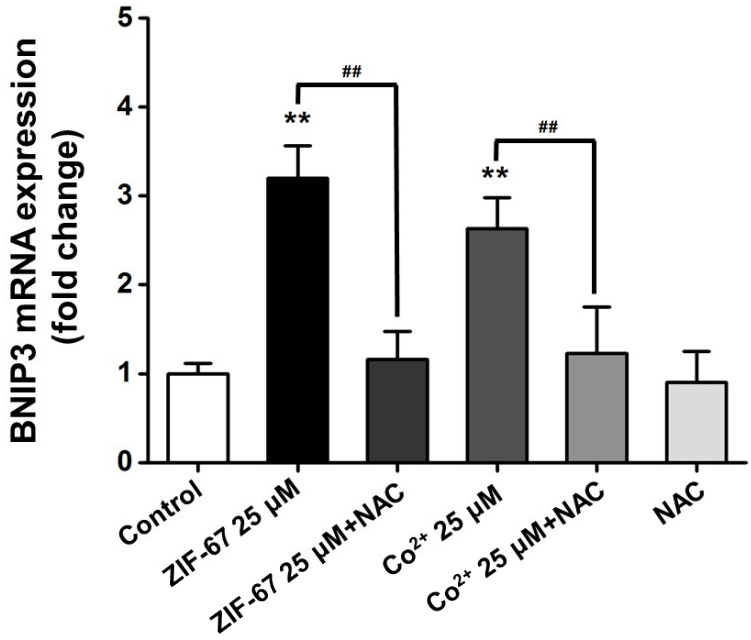

Figure S6. BNIP3 mRNA expressions in BV2 cells after 48 h of treatment with ZIF-67, Co^{2+} and NAC. Data are shown as means ± SD. *$p < 0.05$ and ** $p < 0.01$ compared with the control group; $^{\#\#}$ $p < 0.01$, compared with the ZIF-67 and Co^{2+} treatment groups.

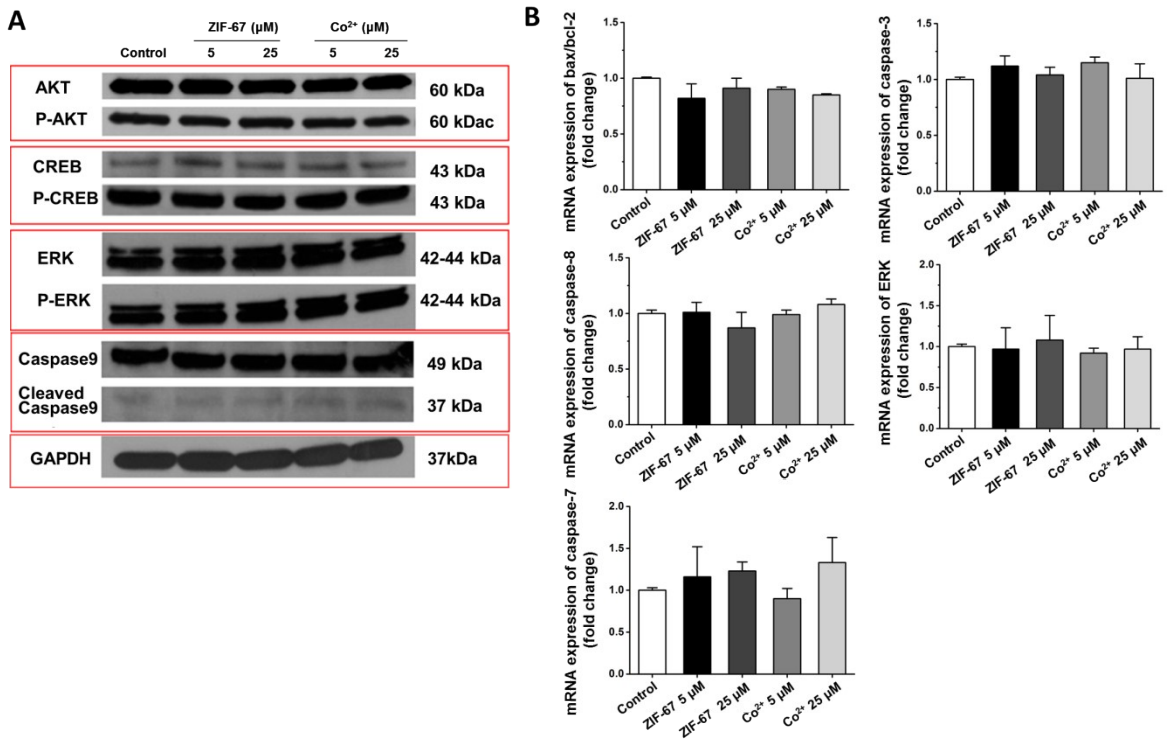

Figure S7. ZIF-67-induced apoptosis was independent of the caspase pathway. (A) The protein levels of AKT, P-AKT, CREB, P-CREB, ERK, P-ERK, caspase-9, and cleaved caspase-9 of whole BV2 cells were detected by Western blotting, with GAPDH as an internal control. (B) The mRNA expressions of caspase-dependent apoptosis-related genes (bax/bcl-2, caspase-3, caspase-8, ERK, and caspase-7) in BV2 cells treated with ZIF-67 and Co^{2+} were examined using RT-qPCR. Data are shown as means ± SD.

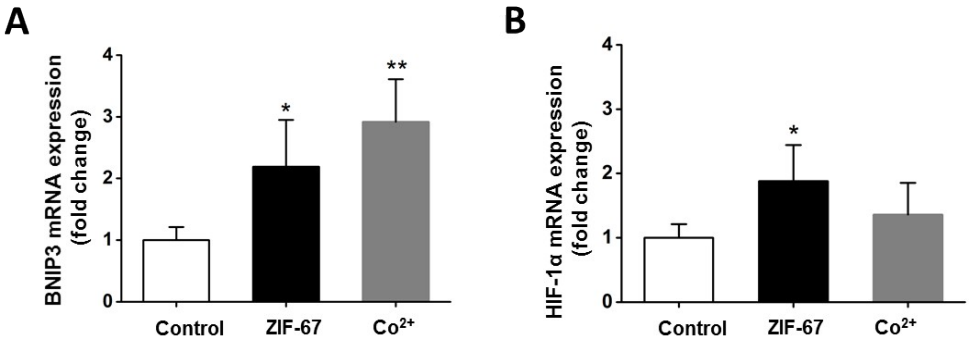

Figure S8. The mRNA expression of BNIP3 and HIF-1α following ZIF-67 and Co^{2+} administration in *in vivo* rat model. Data are shown as means ±SD (n= 6 rats/group). $^{*}p < 0.05$ and $^{**}p < 0.01$ compared with the control group.

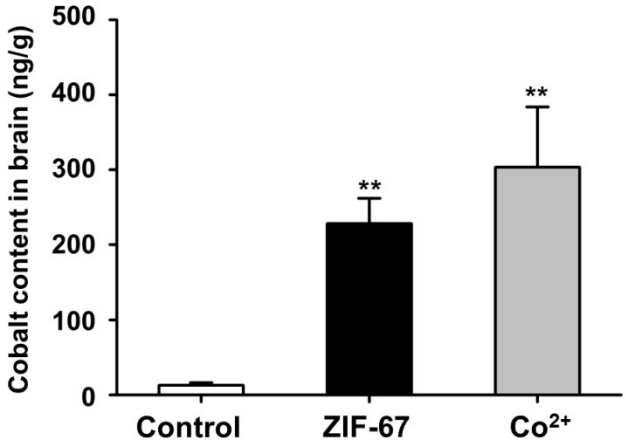

Figure S9. Cobalt content in brain after daily intranasal administration (50 mg/kg bw/d) for 3 d. Data are shown as means ± SD. (n= 6 mice/group). ** $p < 0.01$ compared with the control group.

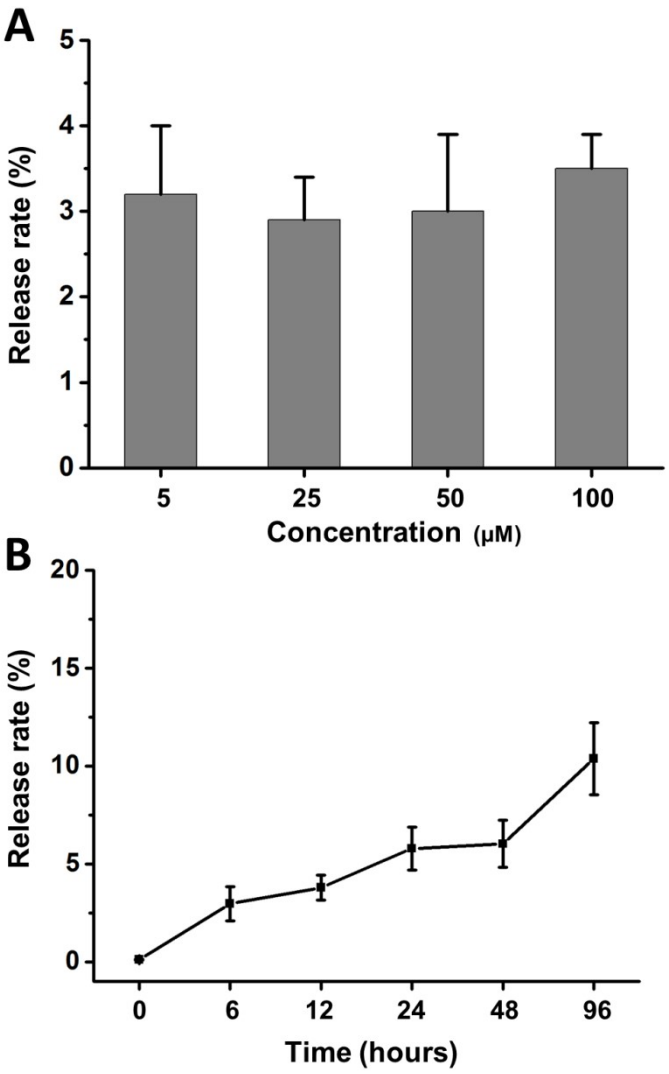

Figure S10. The release rates of cobalt ions from ZIF-67 in DMEM with 10% serum at different concentrations for 24 h (A) and at 50 μM for different time intervals (B). The rate is defined as a ratio of the released cobalt content over the pristine cobalt content.

III. Supplementary Table

Table S1. List of primer sequences of selected genes in RT-qPCR

Gene	Forward (5′→3′)	Reverse (5′→3′)
Ccl4	TTCCTGCTGTTTCTCTTACACCT	CTGTCTGCCTCTTTTGGTCAG
DDx58	GAAGAGCCAGAGTGTCAGAATC	AGCTCCAGTTGGTAATTTCTTGG
Trex1	CGTCAACGCTTCGATGACAAC	CTCAGCCTAGCAAGCTCTGT
Slc2a1	CAGTTCGGCTATAACACTGGTG	GCCCCCGACAGAGAAGATG
Egln3	AGGCAATGGTGGCTTGCTATC	GCGTCCCAATTCTTATTCAGGT
Tnfrsf9	CGTGCAGAACTCCTGTGATAAC	GTCCACCTATGCTGGAGAAGG
BNIP3	TCCTGGGTAGAACTGCACTTC	GCTGGGCATCCAACAGTATTT
HIF-1α	ACCTTCATCGGAAACTCCAAAG	CTGTTAGGCTGGGAAAAGTTAGG
GAPDH	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA

IV. References

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