Supporting Information

Fluorescent Nanoprobe based on Azoreductase-Responsive Metal-Organic Frameworks for Imaging of VEGF mRNA under Hypoxic Conditions

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1. Construction of crystal structure of AMOFs

Poor crystallinity of AMOFs did not allow the determination of the crystal structure through single crystal diffraction. Therefore, we constructed the structure of AMOF through a molecular simulation strategy. The initial structure of AMOF could be derived from the referenced crystal structure (ref: CrystEngComm, 2016, 18, 1282-1294) by replacing the aluminium atoms with irons, and 4,4'-biphenyldicarboxylate with 4,4'-azobisbenzoate, respectively. The final crystal structure is obtained through relaxing the initial structure with the universal force field (UFF). The experimental PXRD pattern is consistent with that of the simulated one, and only slightly positively shift was observed, which is possibly because of lattice contraction resulted from the isomerism of azobenzene groups.

2. Synthesis of AMOF@MBs-2/CPPs

100 μM CPPs were added to PBS buffer with containing the above synthesized 0.25 mg mL^{-1} AMOF@MBs or AMOF@MBs-2, and then followed by centrifugation and washing. The obtained production was stored in refrigerator at 4 °C.

3. qPCR

Total cellular RNA was extracted from the corresponding cells using Trizol reagent (Sangon Co.Ltd., Shanghai, China) according to the indicated protocol. The cDNA samples were prepared by using the reverse transcription (RT) reaction with AMV First Strand cDNA Synthesis Kit (BBI, Toronto, Canada). RT-PCR analysis of mRNA was performed with SG Fast qPCR Master Mix (2X) (BBI), according to the indicated protocol on a LightCycler480 Software Setup (Roche). The primers (from 5' to 3') used in this experiment were:

VEGF forward: 5' -GGCTGACTCTAGAATTTCTGGAATCT-3';

VEGF reverse: 5' -GTGGTACAATCATTCCTTGTGCTT-3'.

Table S1 Oligonucleotides Sequences Used in This Work

Name	Sequence (5’ --3’)
MB	Cy5-CGGTTGACTCCTCAGTGGGCACACACAACCG-TAMRA
MB-2	TAMRA-CGGTTGACTCCTCAGTGGGCACACACAACCG
VEGF mRNA	GUGUGUGCCCACUGAGGAGUC
single-base mismatch mRNA	GUGUGUGCCCUCUGAGGAGUC
c-myc mRNA	CCUCAACGUUAGCUUCACCAA
Her-2/neu mRNA	CACAGACAUGAAGCUGC

Supplementary Figures

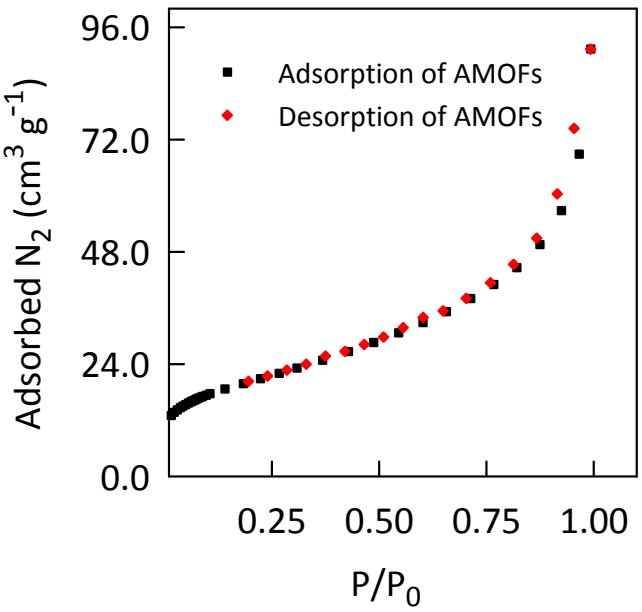

Fig. S1 Nitrogen adsorption-desorption isotherms of AMOFs. BET analysis of the data gave a surface area of 78 m^2 g^{-1} for AMOFs.

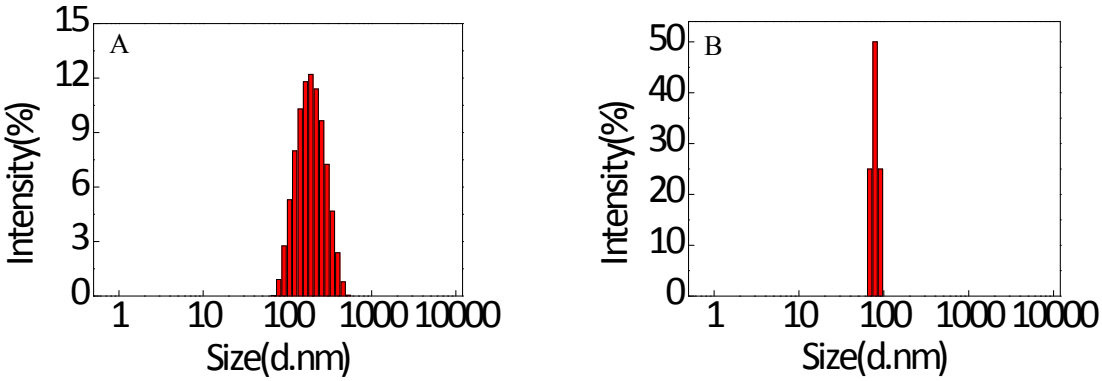

Fig. S2 Hydration particle size of AMOF@MBs before (A) and after (B) azoreductase addition.

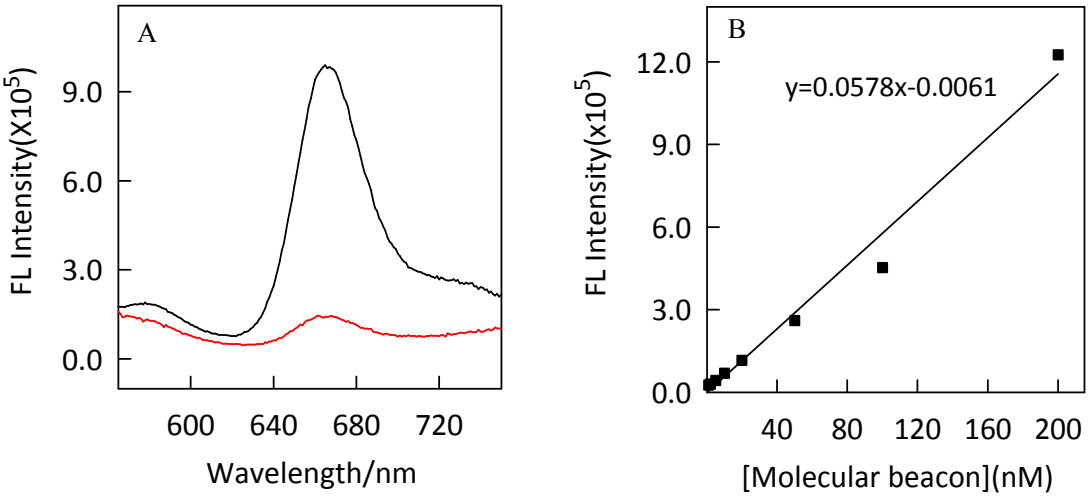

Fig. S3 (A) The loading efficiency of MB was calculated by the fluorescence intensity of Cy5 before (black curve) and after incubated with AMOFs (red curve). (B) Standard curve based on fluorescence intensity of Cy5. The excitation wavelength was 548 nm.

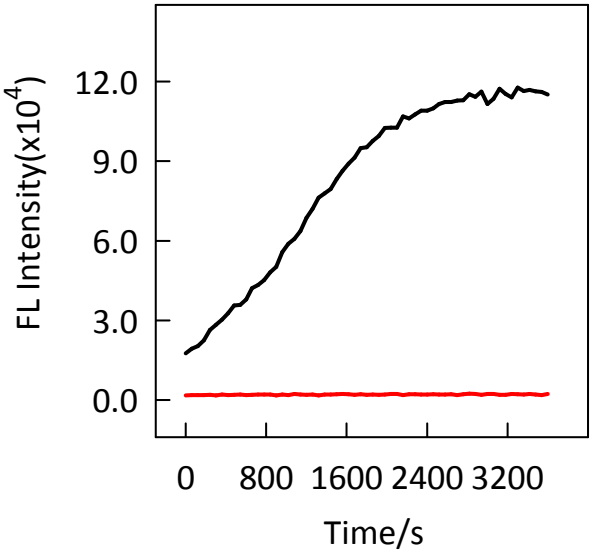

Fig. S4 Fluorescence changes with time of free MB (black curve) and AMOF@MBs (red curve) after the addition of DNase I (0.1 U mL^{-1}) in assay buffer (10 mM Tris, 2.5 mM $MgCl_2$, and 0.5 mM $CaCl_2$, pH =7.5) for 1 h. The excitation wavelength was 548 nm, and the emission wavelength was 580 nm.

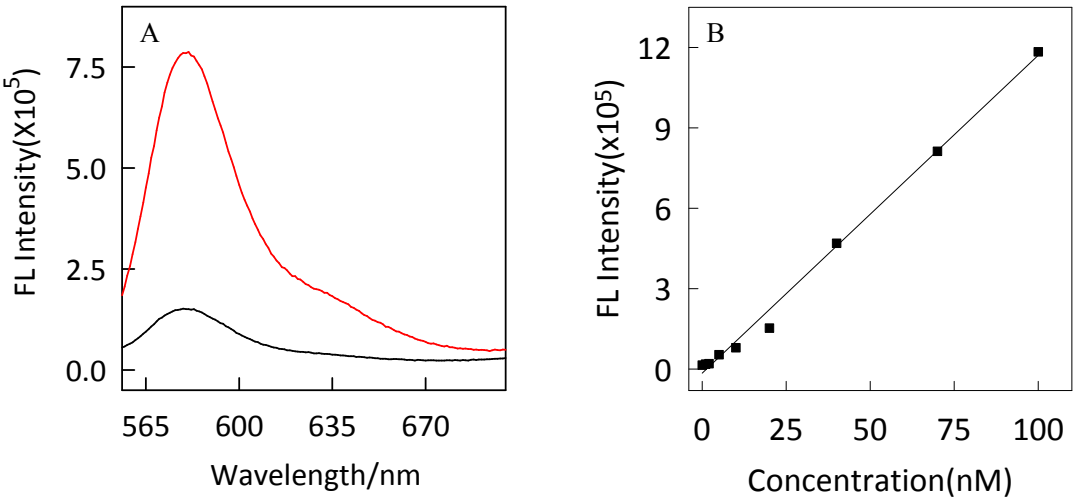

Fig. S5 (A) Black curve represents that our fabricated nanoprobe (AMOF@MBs-2) under normoxic conditions. Red curve represents the fluorescence recovery of AMOF@MBs-2 under hypoxia conditions. (B) Standard curve based on fluorescence intensity of MB-2. The excitation wavelength was 548 nm. The release rate of MB-2 was calculated to be approximately 84.27%.

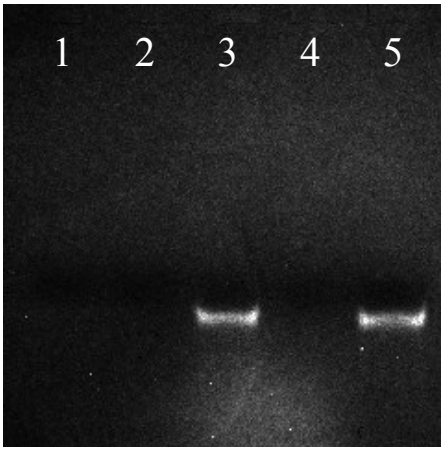

Fig. S6 15% PAGE. Lane 1: The supernatant collected from the solution containing nanoprobe in the absence of VEGF mRNA under normoxia condition (20% O_2) after centrifugation; Lane 2: The supernatant collected from the solution containing nanoprobe in the absence of VEGF mRNA under hypoxia condition after centrifugation; Lane 3: The supernatant collected from the solution containing nanoprobe in the presence of VEGF mRNA under hypoxia conditions after centrifugation; Lane 4: The supernatant collected from the solution containing nanoprobe in the presence of VEGF mRNA upon inactive rat liver microsomes addition; Lane 5: The band produced by hybridization of MB and VEGF mRNA.

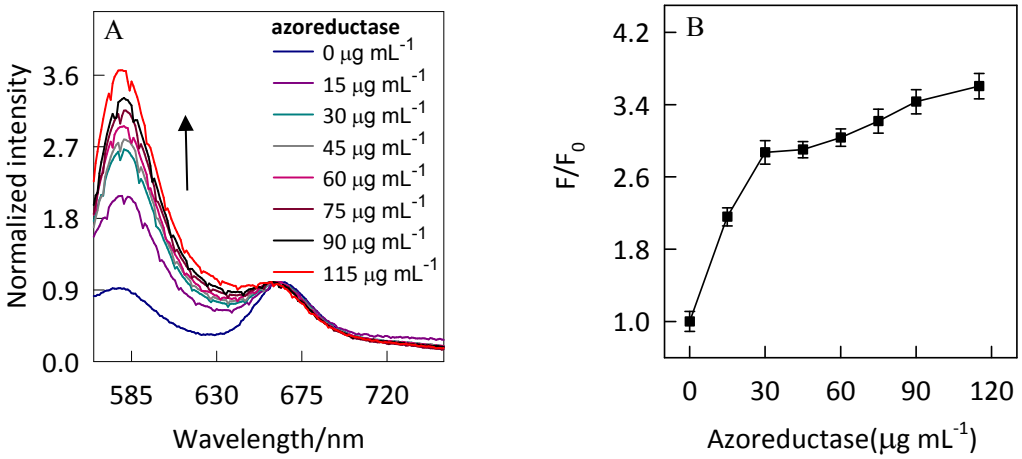

Fig. S7 (A) Fluorescence emission spectra of the released MB attained from AMOF@MBs with varied rat liver microsomes (0, 15, 30, 45, 60, 75, 90, 115 μg mL^{-1}) and NADPH (50 μM) under hypoxic condition in the presence of VEGF mRNA (100 nM). The fluorescence intensity of Cy5 normalized to 1. (B) Calibration curve of fluorescence signal enhancement (F/F_0, where F and F_0 represents the fluorescence of TAMRA) versus the different concentrations of rat liver microsomes. The excitation wavelength was 548 nm.

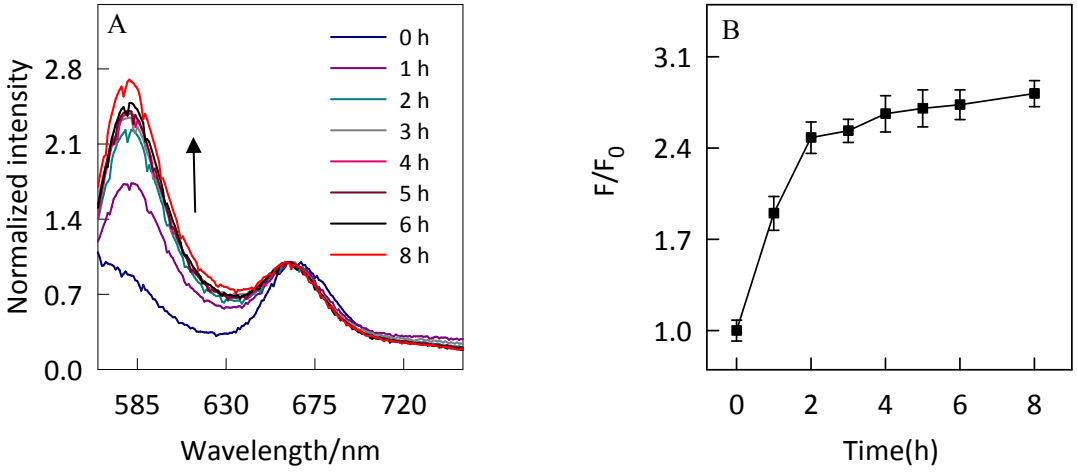

Fig. S8 (A) Fluorescence emission spectra of the released MB attained from AMOF@MBs with varied reaction time (0, 1, 2, 3, 4, 5, 6, 8 h) in the presence of rat liver microsomes (75 μg mL^{-1}) and NADPH (50 μM) under hypoxic condition followed by addition of VEGF mRNA (100 nM). The fluorescence intensity of Cy5 normalized to 1. (B) Calibration curve of fluorescence signal enhancement (F/F_0, where F and F_0 represents the fluorescence of TAMRA after and before rat liver microsomes addition with varied reaction time.

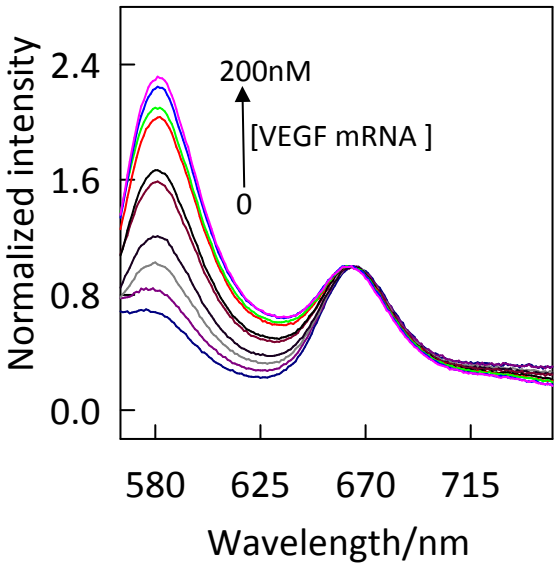

Fig. S9 Fluorescence spectra of AMOF@MBs as functions of different concentrations of VEGF mRNA (0-200 nM) under hypoxic conditions. The fluorescence intensity of Cy5 normalized to 1. The excitation wavelength was 548 nm. Hypoxic conditions: bubbling nitrogen gas into the reaction solution containing rat liver microsomes (75 μg mL^{-1}) and NADPH (50 μM).

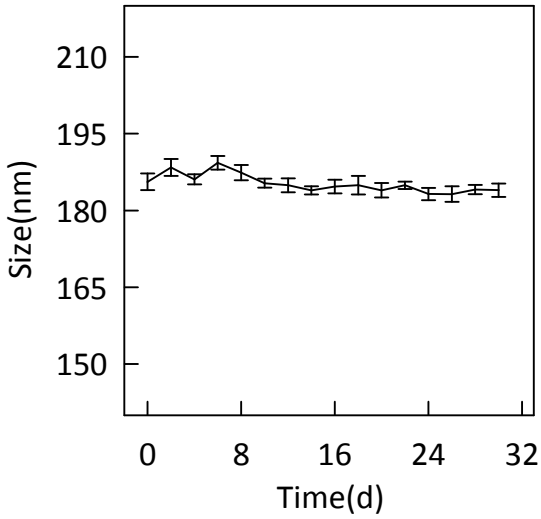

Fig. S10 Hydrodynamic size distribution of AMOF@MBs/CPPs in cell medium supplemented with 10 % fetal bovine serum.

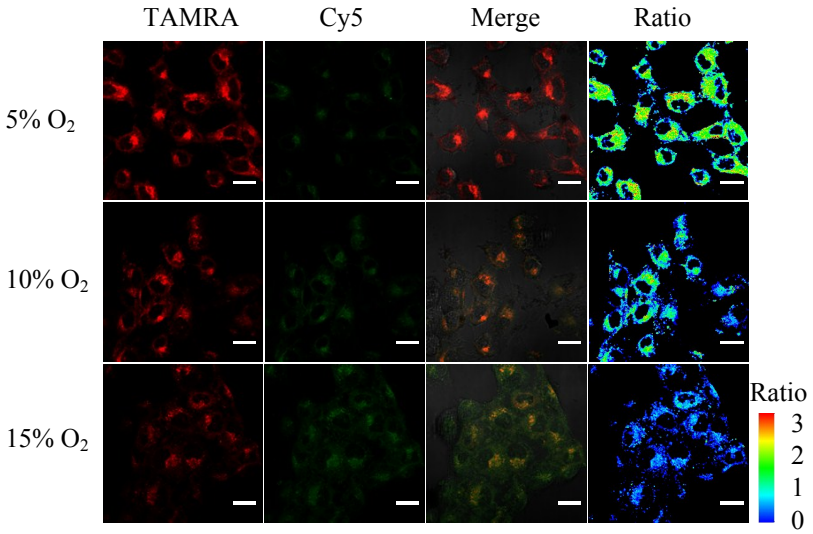

Fig. S11 Confocal microscopy images of the AMOF@MBs/CPPs-incubated (0.25 mg mL^{-1}, 2 h) HeLa cells following treatment with different concentration of O_2 (5 %, 10 %, 15 %) for 8 h. Scale bar: 20 μm.

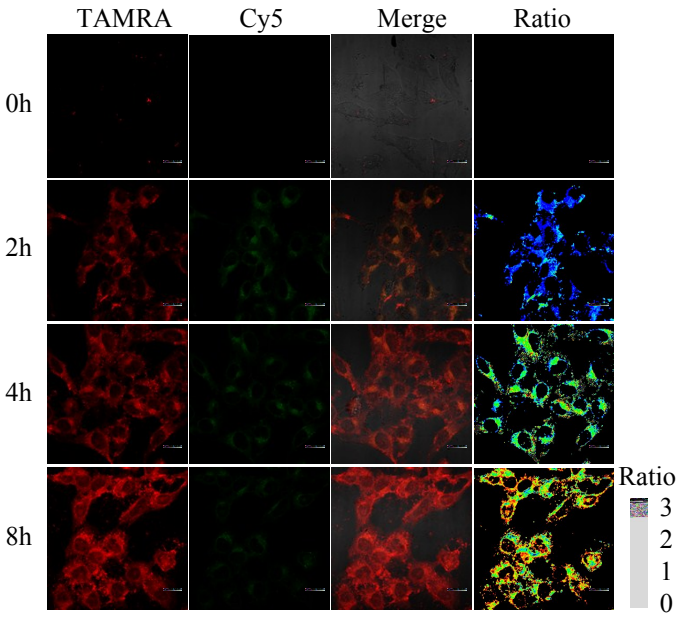

Fig. S12 Confocal microscopy images of the AMOF@MBs/CPPs-incubated (0.25 mg mL^{-1}) HeLa cells following treatment with 1 % O_2 for varied hypoxia time (0, 2, 4, 8 h). Scale bar: 20μm.

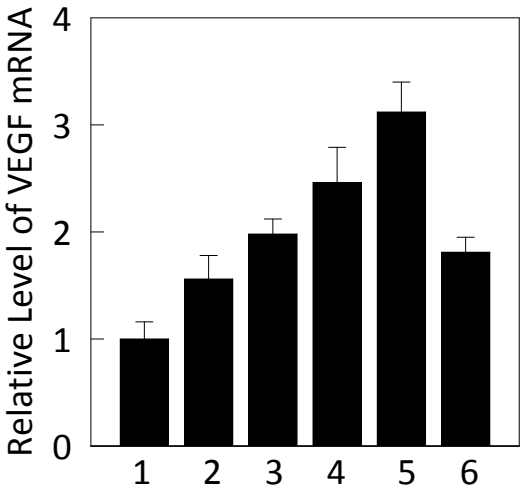

Fig. S13 qPCR results of VEGF mRNA in HeLa cells which were upon different treatments. 1: Under normoxia condition (20 % O_2) for 8 h; 2: Under hypoxia condition (15 % O_2) for 8 h; 3: Under hypoxia condition (10 % O_2) for 8 h; 4: Under hypoxia condition (5 % O_2) for 8 h; 5: Under hypoxia condition (1% O_2) for 8 h; 6: Under hypoxia condition (1% O_2) for 8 h and simultaneously treated with 100 μM resveratrol.

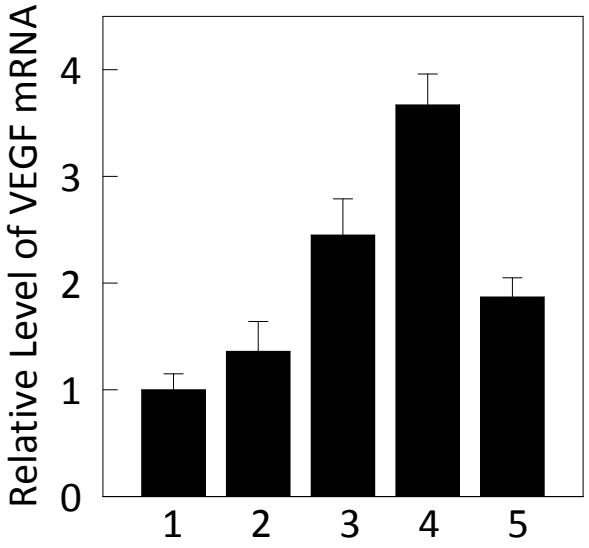

Fig. S14 qPCR results of VEGF mRNA in HeLa cells which were treated with $CoCl_2$ for different concentrations. 1: 0 μM $CoCl_2$ treatment; 2: 100 μM $CoCl_2$ treatment; 3: 200 μM $CoCl_2$ treatment; 4: 400 μM $CoCl_2$ treatment; 5: 400 μM $CoCl_2$ treatment simultaneously treated with 100 μM resveratrol.