## Copper depletion inhibits $CoCl_2$ -induced aggressive phenotype of MCF-7 cells via downregulation of HIF-1 and inhibition of Snail/Twist-mediated epithelial-mesenchymal transition

Shun Li <sup>1</sup>, Jing Zhang <sup>1</sup>, Hong Yang <sup>1,2</sup>, Chunhui Wu <sup>1,2</sup>, Xitong Dang <sup>3</sup>, Yiyao Liu <sup>1,2 \*</sup>

<sup>1</sup> Department of Biophysics, School of Life Science and Technology, <sup>2</sup> Center for Information in Biomedicine, University of Electronic Science and Technology of China, Chengdu 610054, Sichuan, P.R. China. <sup>3</sup> Division of Trauma, Surgical Critical Care and Burns, University of California San Diego, CA 92103, USA.

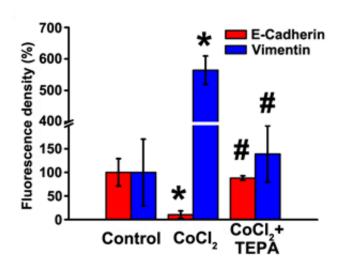
Table S1. Primer sequences for real time-PCR analysis

Gene	Primer sequence $(5' \rightarrow 3')$
β-actin	Forward: GGCATCCTCACCCTGAAGTA
	Reverse: GGGGTGTTGAAGGTCTCAAA
vimentin	Forward: CTCCGGGAGAAATTGCAGGA
	Reverse: TTCAAGGTCAAGACGTGCCA
VEGFa	Forward: GGCTTTTCTGTGGCAAGAGGTT
	Reverse: CGCTGATGTTTGCTCAAGTGGTC
fibronectin	Forward: GACCTATCCAAGCTCAAGTGGT
	Reverse: ATCCAAGGTTTCTGGGTGGG
Snail	Forward: TGCCCTCAAGATGCACATCCGA
	Reverse: GGGACAGGAGAAGGGCTTCTC
Twist1	Forward: GCCAGGTACATCGACTTCCTCT
	Reverse: TCCATCCTCCAGACCGAGAAGG

## Sequences used for Ctr1 silence

Primer sequence (5'→3'): GGAAGAAGGCAGUGGUAGUdTdT

Quantitative analysis of immunofluorescence data with CoCl2 or TEPA treatment.



**Figure S1.** The fluorescence density of E-cadherin and vimentin were measured in each groups. Data was shown as mean  $\pm$  SD of three independent experiments. p<0.05 was considered statistically significant (\*compared to the control group and #compared to the CoCl<sub>2</sub> group).

Gene silence efficacy validation of Ctr1 by western blot.



**Figure S2.** Gene silence efficacy of *Ctr1*. *Ctr1* expression was assayed by western blot after transfection and continued to culture for 24 h.

## Morphology of MCF-7 cells under TEPA treatment only.

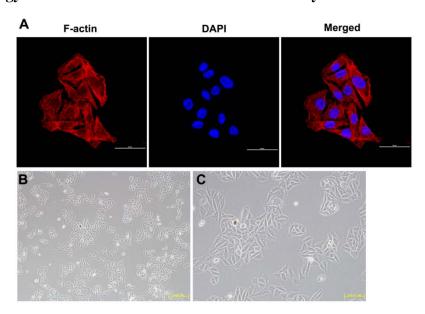


Figure S3. Morphology of MCF-7 cells under TEPA treatment only. (A)

Spindle-shaped cells were not observed after TEPA treatment for 24 h.

F-actin was stained by TRITC-phalloidin (red). Scale bar=50 μm. (B) and

(C) The morphology of MCF-7 cells were visualized by phase-contrast.