

Supplemental Material to:

**Wu Zhong, Haichuan Zhu, Fugeng Sheng, Yonglu Tian,
Jun Zhou, Yingyu Chen, Song Li, and Jian Lin**

**Activation of the MAPK11/12/13/14 (p38 MAPK) pathway
regulates the transcription of autophagy genes in
response to oxidative stress induced by a novel copper
complex in HeLa cells**

Autophagy 2014; 10(7)

<http://dx.doi.org/10.4161/auto.28789>

www.landesbioscience.com/journals/autophagy/article/28789

Table S1. Overview of RNA-Seq results in HeLa cells treated with HYF127c/Cu.
RNA-Seq reports

a

Up-Down-Regulation	Table.S4 (Fold>1, p-value<0.05)	Table.S3 (Fold>1 or Fold<-1, p-value<0.01 and FDR<0.01)
Up	1096	105
Down	4611	547
Total	5707	652

b

Log ₂ (HYF127c/Cu/ Control)	Table S4 (p-value<0.05)	Table.S3 (p-value<0.01and FDR<0.01)
Fold≥10	25	2
5≤Fold≤10	367	31
1≤Fold≤5	704	72
-5≤Fold≤-1	4532	446
-10≤Fold≤-5	79	1

Table S2. The primers used for real-time PCR.

Primes used for Real-Time PCR

primer designation	primer sequence
<i>HSPA1A_fw</i> <i>HSPA1A_re</i>	5'-gtgctgaccaagatgaaggag-3' 5'-gctgcgagtcgttgaagtag-3'
<i>BAG3_fw</i> <i>BAG3_re</i>	5'-ctccattccgggtatacaga-3' 5'-tggtgggtctgtactccc-3'
<i>MAPLC3B_fw</i> <i>MAPLC3B_re</i>	5'-agcagcatccaacaaaatc-3' 5'-ctgtgtccgttcaccaacag-3'
<i>ACTIN_fw</i> <i>ACTIN_re</i>	5'-ggaacgggtgaaggtgacagc-3' 5'-aatcaaagtcctcggccaca-3'
<i>BECN1_fw</i> <i>BECN1_re</i>	5'-caagatcctggaccgtgtca-3' 5'-tggcactttctgtgacatca-3'
<i>MTOR_fw</i> <i>MTOR_re</i>	5'-ggaggctgatggacacaaat-3' 5'-ctgtgtccccgttttcta-3'
<i>SQSTM1_fw</i> <i>SQSTM1_re</i>	5'-agctgcctgtaccacatc-3' 5'-ggggatgctttgaactgg-3'

<i>MCL1_fw</i> <i>MCL1_re</i>	5'-cggtaatcggactcaacctc-3' 5'-cctccttctccgtagcca-3'
<i>ATG7_fw</i> <i>ATG7_re</i>	5'-gatccggggattctttcacg-3' 5'-cagcaatgtaagaccagtcagt-3'
<i>ATG16L1_fw</i> <i>ATG16L1_re</i>	5'-tgccctgcagatcacttttac-3' 5'-gagtcgcttagtggtgctc-3'
<i>BCL2L1_fw</i> <i>BCL2L1_re</i>	5'-atcaatggcaacctcctg-3' 5'-ttgtctacgctttccacgca-3'
<i>BCL2_fw</i> <i>BCL2_re</i>	5'-tgtggatgactgagtacctaacc-3' 5'-cagccaggagaaatcaaacagagg-3'
<i>ATG5_fw</i> <i>ATG5_re</i>	5'-tggatttcgttatatccccttag-3' 5'-cctagtgtgtgcaactgtcca-3'

Figure S1

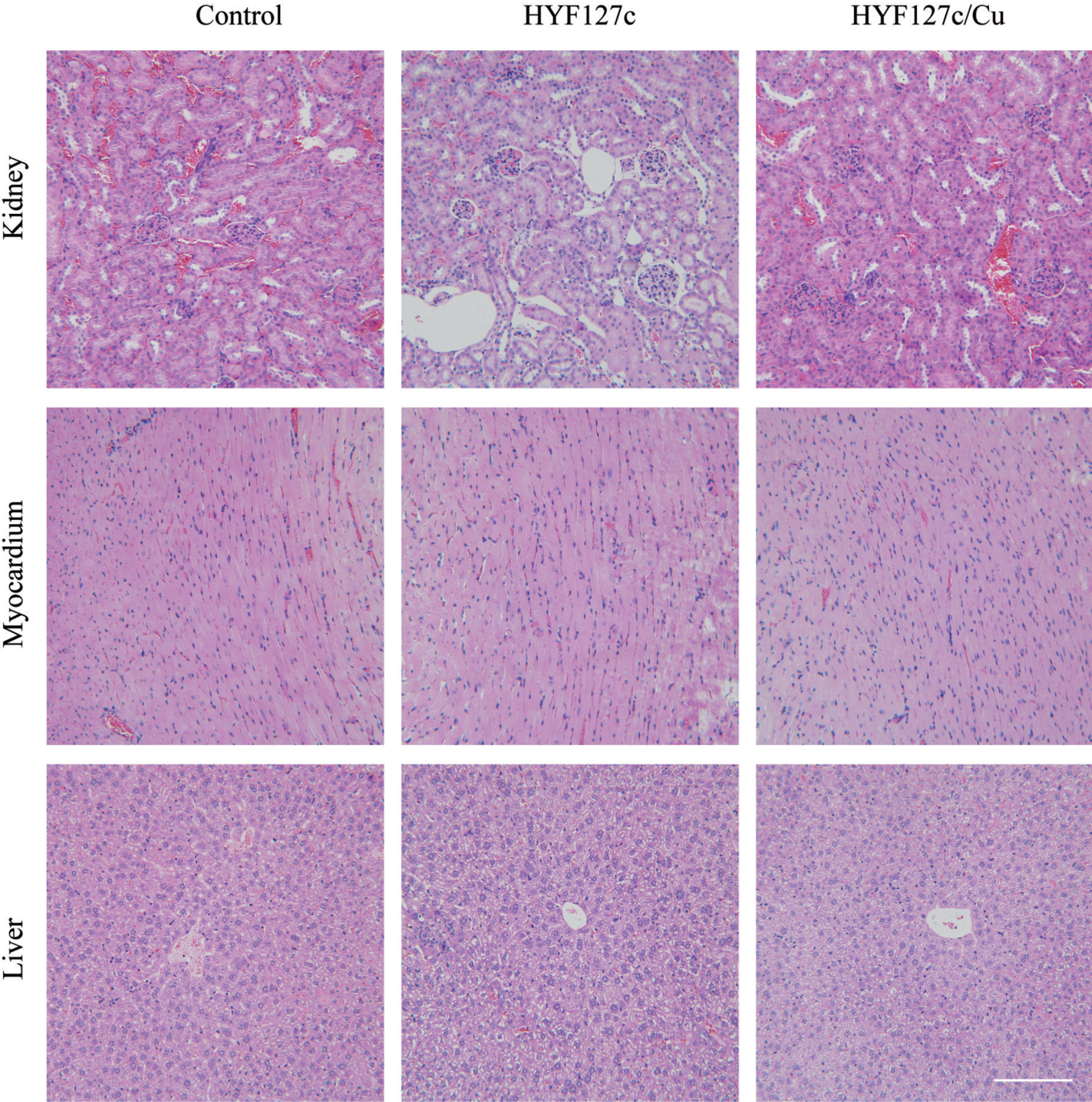


Figure S2

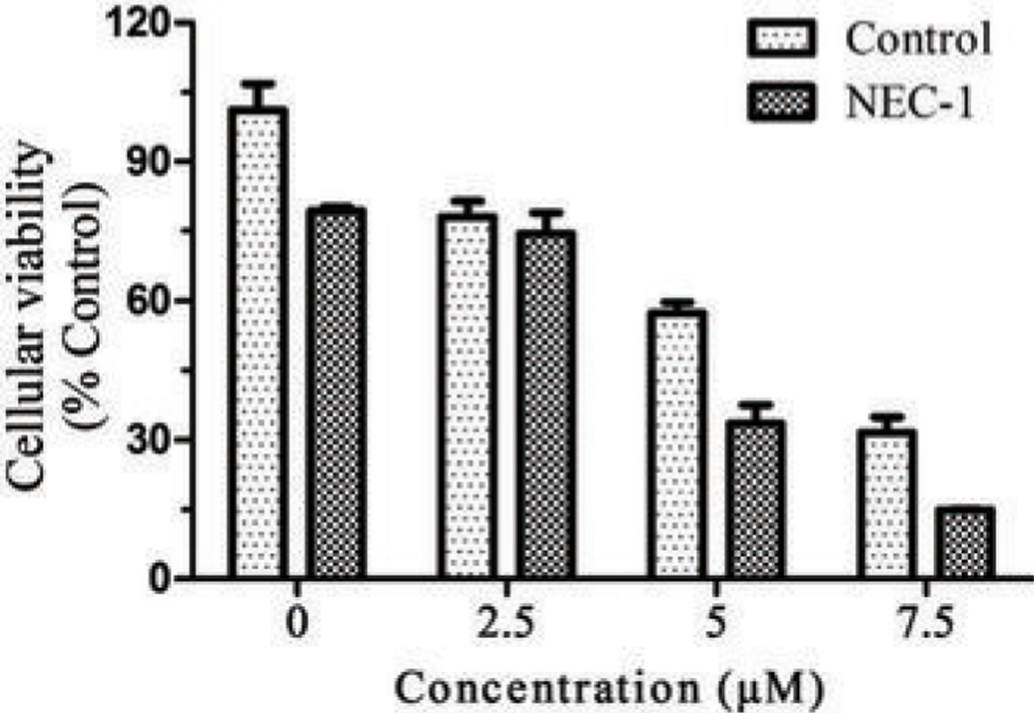


Figure S3

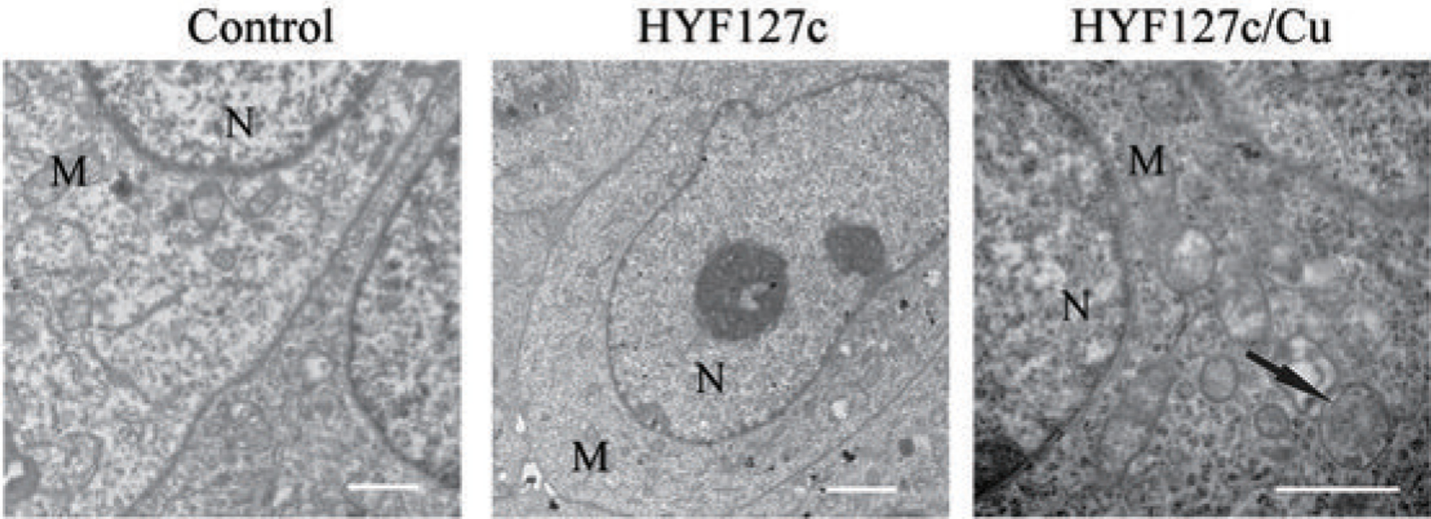


Figure S4

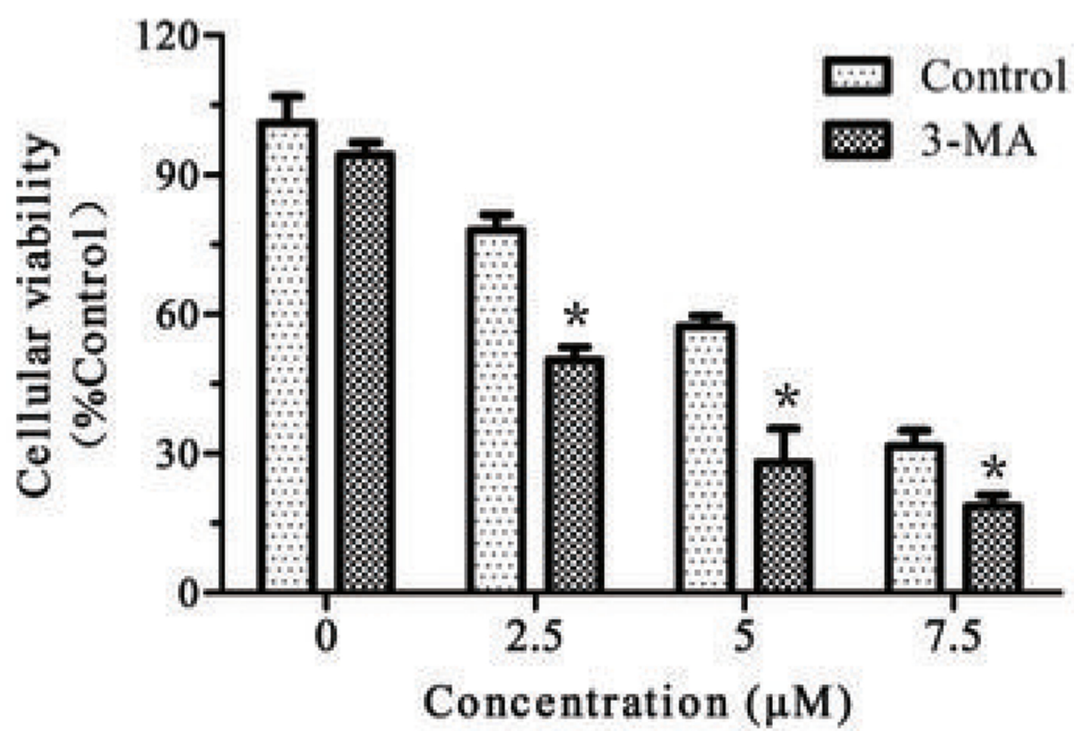


Figure S5

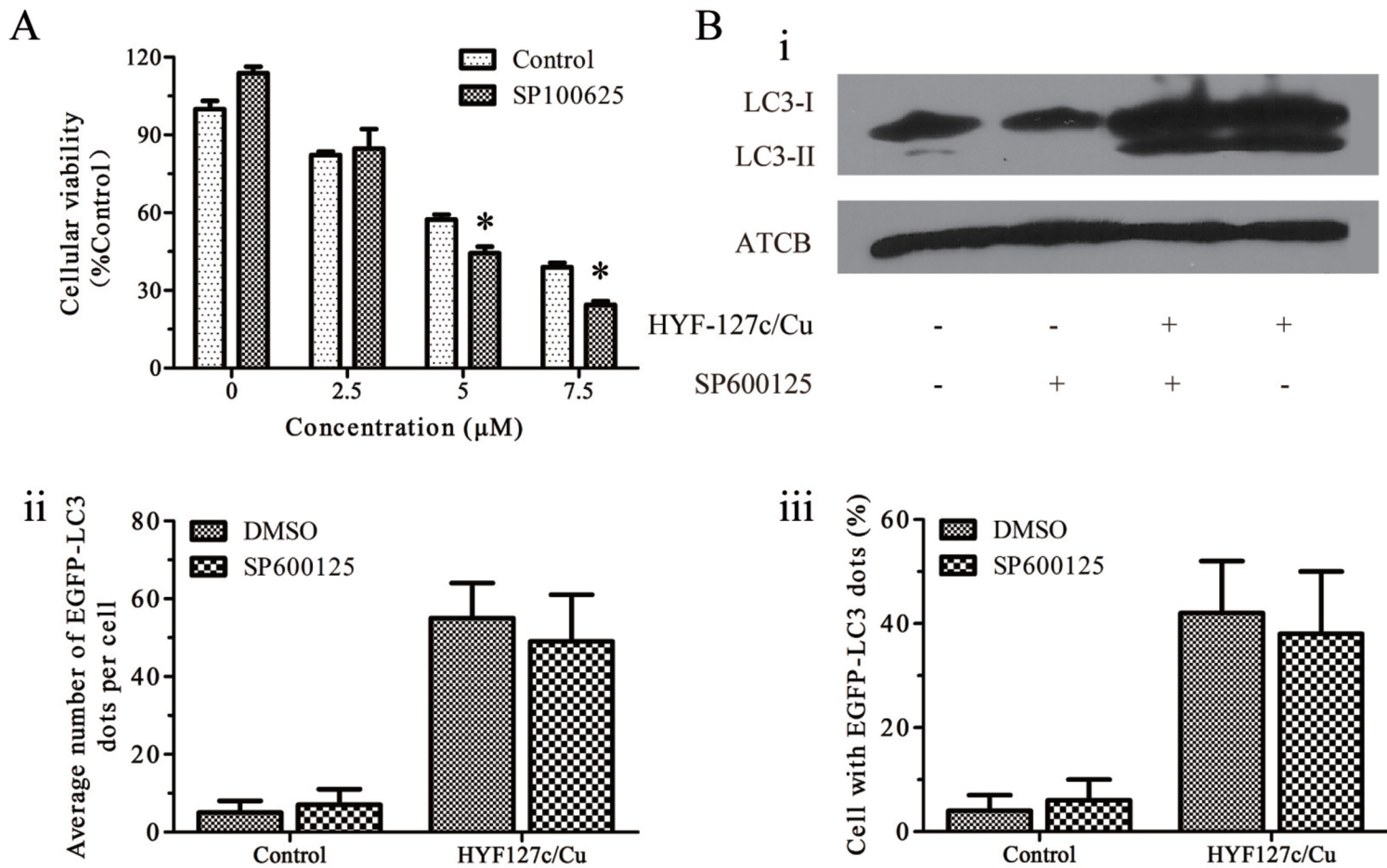


Figure S1. The histological results from kidney, myocardium, and liver in nude mice treated with HYF127c/Cu or HYF127c.

Figure S2. The effect of the combination of necrostatin-1 and HYF127c/Cu on cellular viability in HeLa cells, (n=3).

Figure S3. Electron microscopy images showing extensive cytoplasm vacuolization enclosed in a double membrane in HYF127c/Cu-treated tumors. Electron microscopy image of an untreated tumor is also shown for comparison. The double membrane of the autophagic vacuoles is indicated by a black arrow. N, nucleus; M, mitochondrion. Bar: 0.5 μ m.

Figure S4. The effect of the combination of 3-MA and HYF127c/Cu on cellular viability of HeLa cells (n=3, *: $P < 0.05$).

Figure S5. The effect of SP600125 on HYF127c/Cu-treated cells. **(A)** The effect of SP600125 on the viability of HYF127c/Cu-treated cells (n=3, *: $P < 0.05$). **(B)** The effect of SP600125 on autophagy in HYF127c/Cu-treated cells. (i) Western blotting showed that SP600125 had no effect on LC3-I conversion. SP600125 had no effect on the percentage of cells with EGFP-LC3 dots (ii) and the average number of EGFP-LC3 dots in cells (iii) (n=3, *: $P < 0.05$).