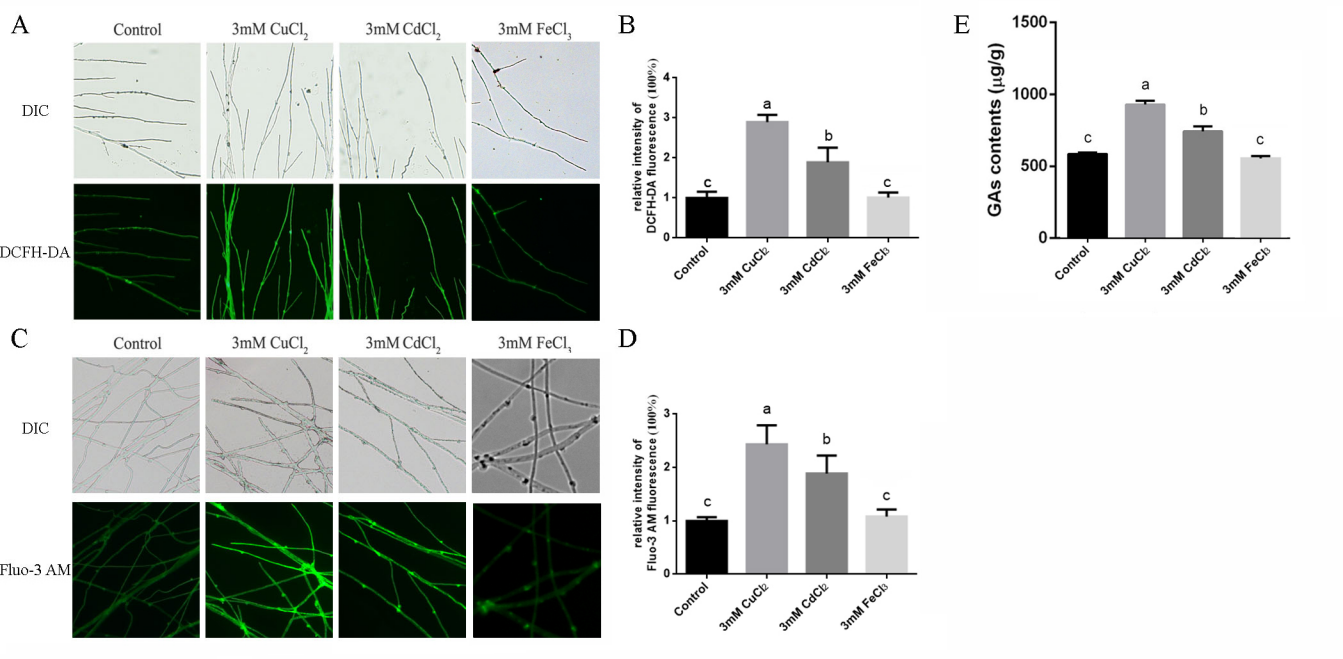
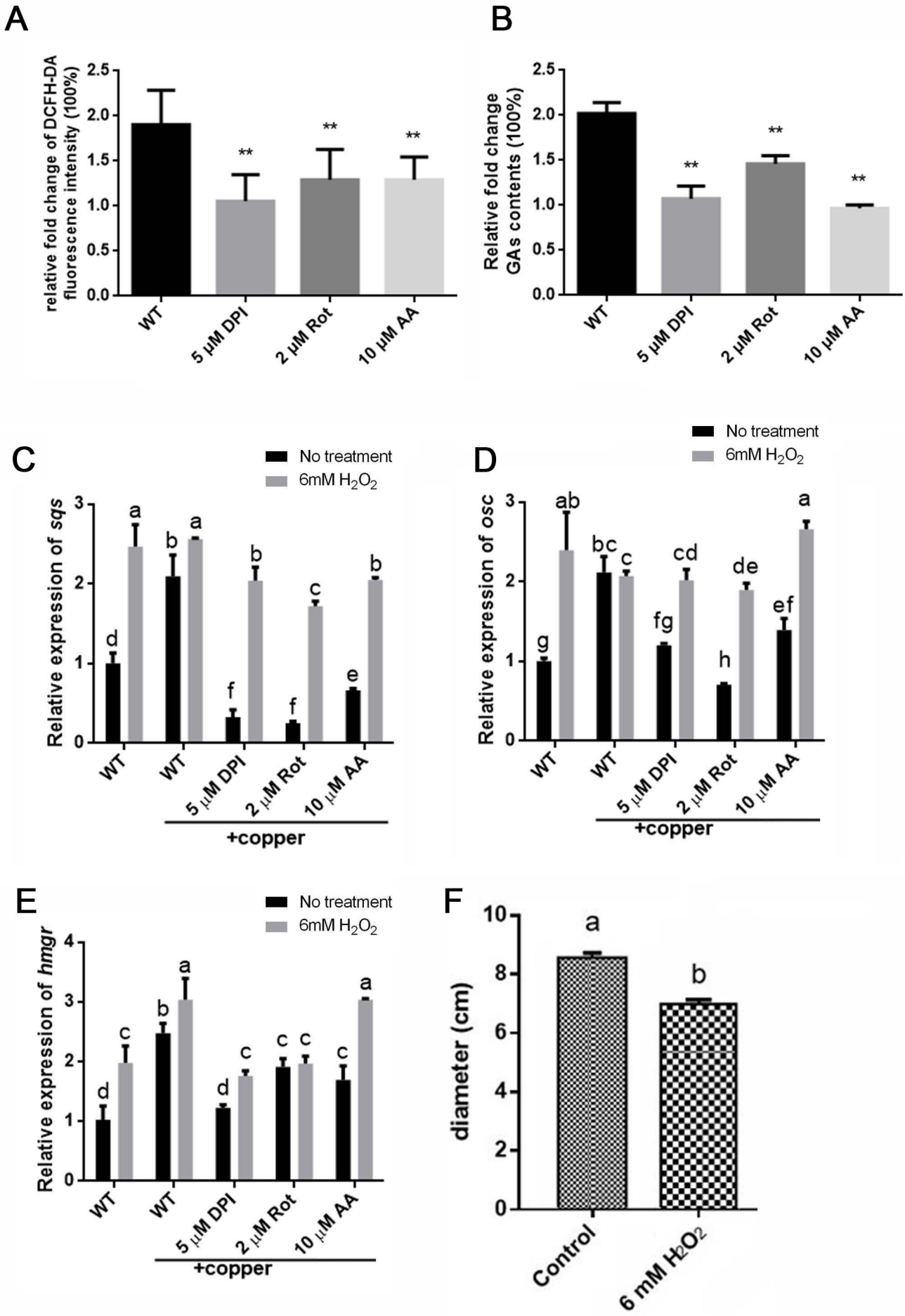
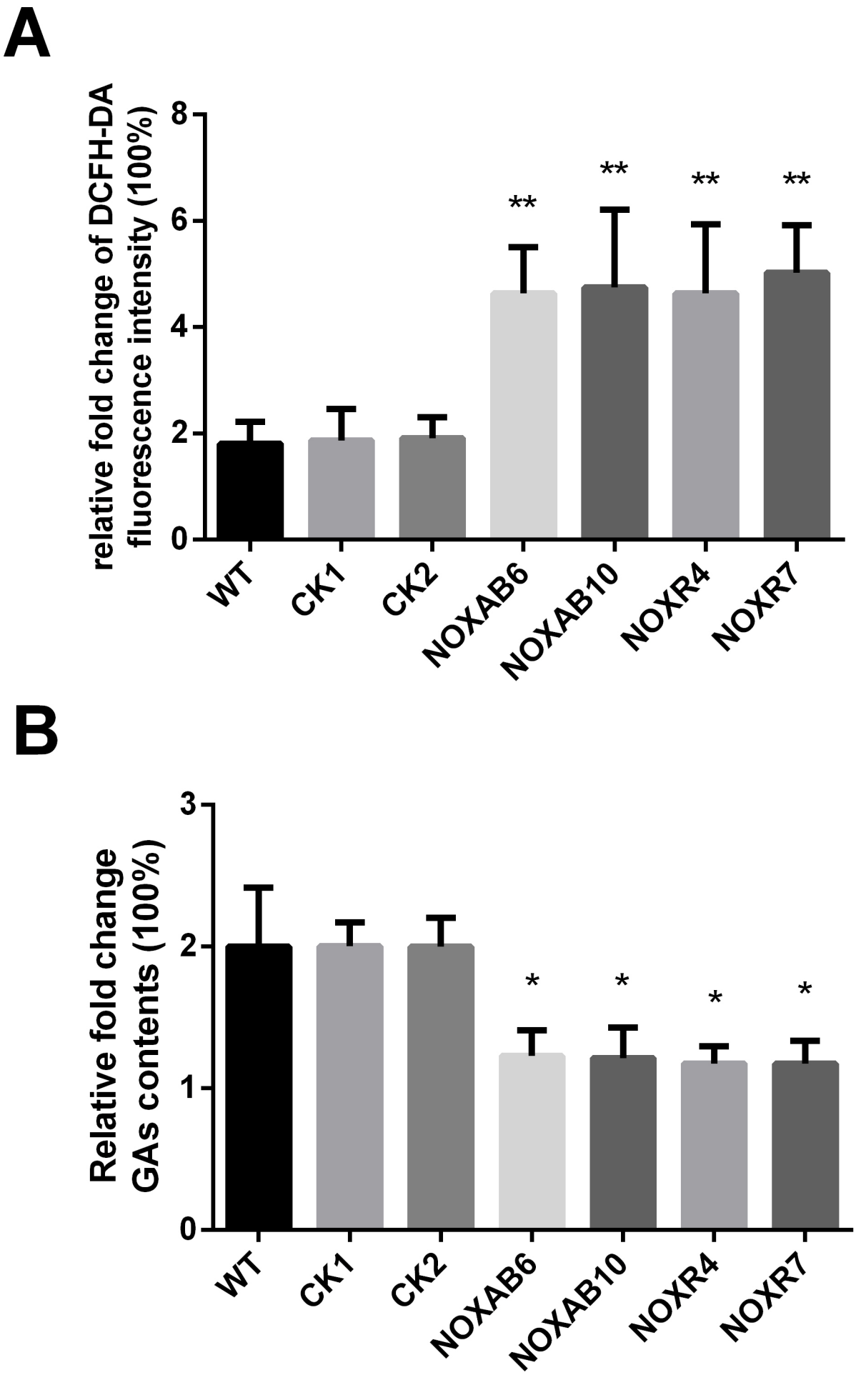
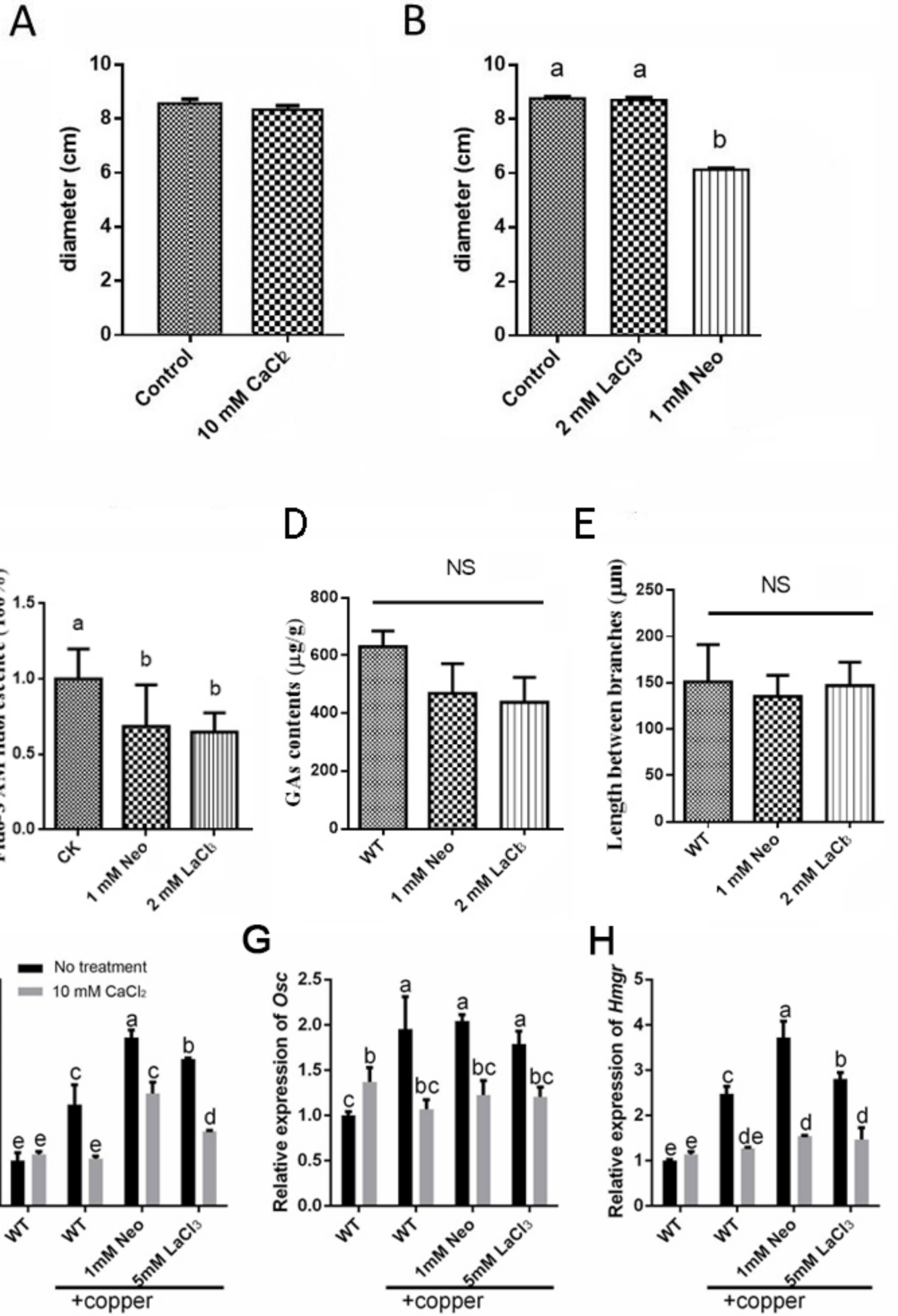
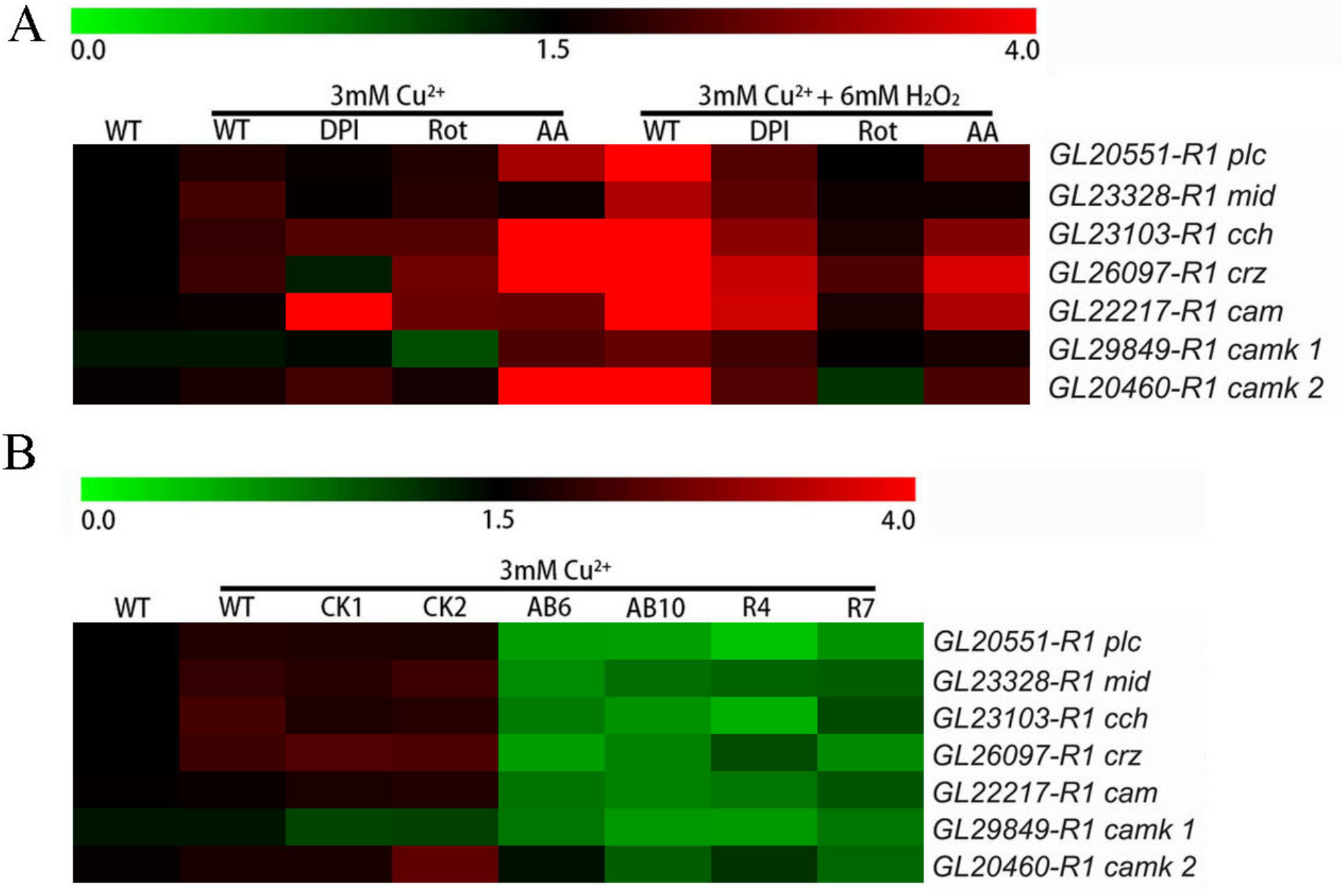
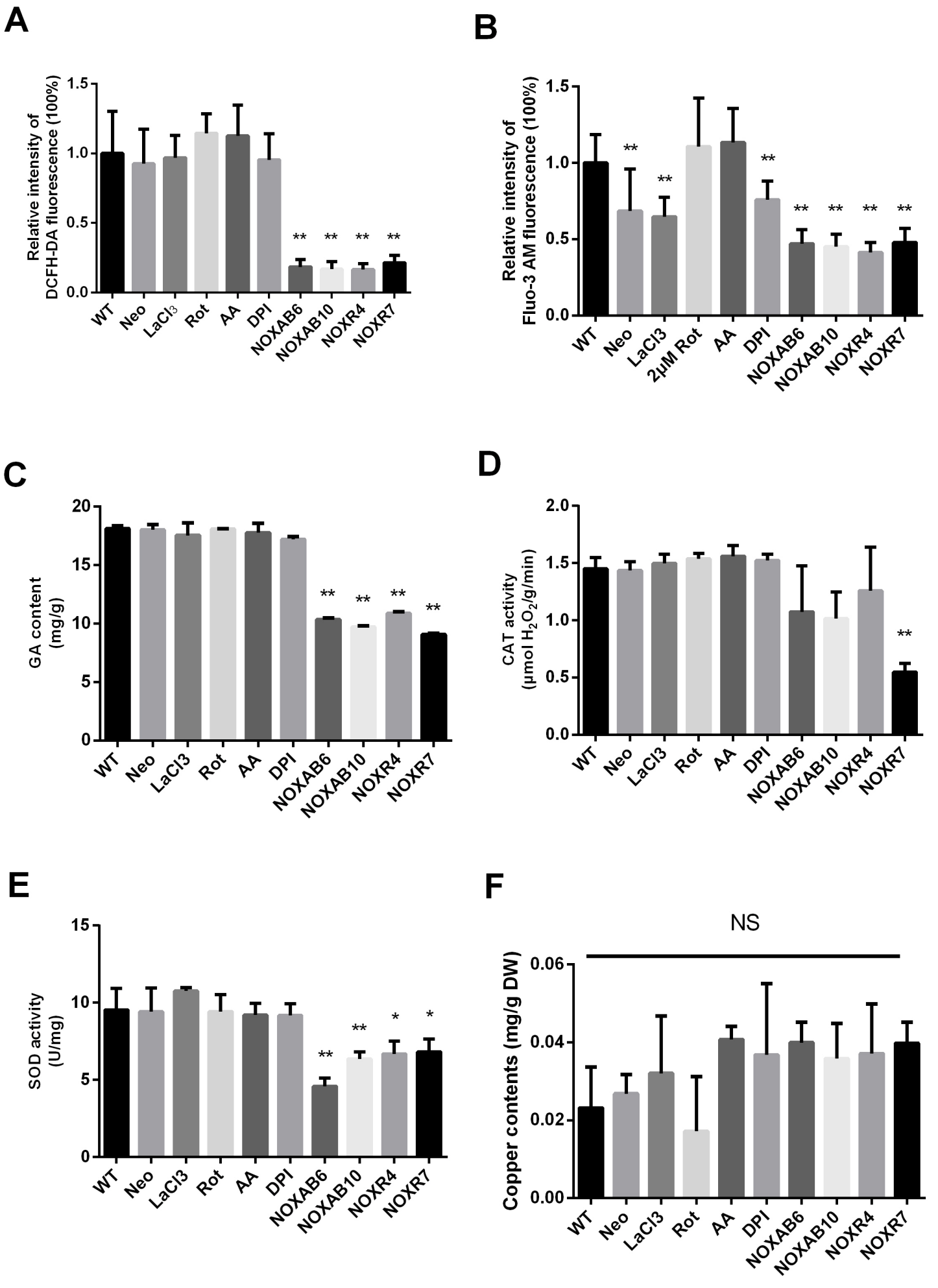

A
Control
3mM CuCl2
3mM CdCl2
3mM FeCl3
DIC
DCFH-DA
B
relative intensity of DCFH-DA fluorescence (100%)
a
b
c
Control
3mM CuCl2
3mM CdCl2
3mM FeCl3
C
Control
3mM CuCl2
3mM CdCl2
3mM FeCl3
DIC
Fluo-3 AM
D
relative intensity of Fluo-3 AM fluorescence (100%)
a
b
c
Control
3mM CuCl2
3mM CdCl2
3mM FeCl3
E
GAs contents (μg/g)
1500
1000
500
0
a
b
c
c
Control
3mM CuCl2
3mM CdCl2
3mM FeCl3


A
relative fold change of DCFH-DA fluorescence intensity (100%)
2.5
2.0
1.5
1.0
0.5
0.0
**
**
**
WT
5 μM DPI
2 μM Rot
10 μM AA
B
Relative fold change GAs contents (100%)
2.5
2.0
1.5
1.0
0.5
0.0
**
**
**
WT
5 μM DPI
2 μM Rot
10 μM AA
C
No treatment
6mM H2O2
Relative expression of sqs
3
2
1
0
d
a
b
a
f
b
f
c
e
b
WT
WT
5 μM DPI
2 μM Rot
10 μM AA
+copper
D
No treatment
6mM H2O2
Relative expression of osc
3
2
1
0
g
ab
bc
c
fg
cd
h
de
ef
a
WT
WT
5 μM DPI
2 μM Rot
10 μM AA
+copper
E
No treatment
6mM H2O2
Relative expression of hmgr
4
3
2
1
0
d
c
b
a
d
c
c
c
c
a
WT
WT
5 μM DPI
2 μM Rot
10 μM AA
+copper
F
diameter (cm)
10
8
6
4
2
0
a
b
Control
6 mM H2O2


A
relative fold change of DCFH-DA fluorescence intensity (100%)
0
2
4
6
8
**
**
**
**
WT
CK1
CK2
NOXAB6
NOXAB10
NOXR4
NOXR7
B
Relative fold change GAs contents (100%)
0
1
2
3
*
*
*
*
WT
CK1
CK2
NOXAB6
NOXAB10
NOXR4
NOXR7


A
diameter (cm)
Control
10 mM CaCl2
B
a
a
b
diameter (cm)
Control
2 mM LaCl3
1 mM Neo
C
Relative intensity of Fluo-3 AM fluorescence (100%)
a
b
b
CK
1 mM Neo
2 mM LaCl3
D
NS
GAs contents (μg/g)
WT
1 mM Neo
2 mM LaCl3
E
NS
Length between branches (μm)
WT
1 mM Neo
2 mM LaCl3
F
No treatment
10 mM CaCl2
Relative expression of Sqs
WT
WT
1mM Neo
5mM LaCl3
+copper
G
Relative expression of Osc
WT
WT
1mM Neo
5mM LaCl3
+copper
H
Relative expression of Hmgr
WT
WT
1mM Neo
5mM LaCl3
+copper


A
0.0
1.5
4.0
3mM Cu2+
3mM Cu2+ + 6mM H2O2
WT
WT
DPI
Rot
AA
WT
DPI
Rot
AA
GL20551-R1 plc
GL23328-R1 mid
GL23103-R1 cch
GL26097-R1 crz
GL22217-R1 cam
GL29849-R1 camk 1
GL20460-R1 camk 2
B
0.0
1.5
4.0
3mM Cu2+
WT
WT
CK1
CK2
AB6
AB10
R4
R7
GL20551-R1 plc
GL23328-R1 mid
GL23103-R1 cch
GL26097-R1 crz
GL22217-R1 cam
GL29849-R1 camk 1
GL20460-R1 camk 2


A
Relative intensity of DCFH-DA fluorescence (100%)
1.5
1.0
0.5
0.0
**
**
**
**
WT
Neo
LaCl3
Rot
AA
DPI
NOXAB6
NOXAB10
NOXR4
NOXR7
B
Relative intensity of Fluo-3 AM fluorescence (100%)
1.5
1.0
0.5
0.0
**
**
**
**
**
**
WT
Neo
LaCl3
2μM Rot
AA
DPI
NOXAB6
NOXAB10
NOXR4
NOXR7
C
GA content (mg/g)
20
15
10
5
0
**
**
**
**
WT
Neo
LaCl3
Rot
AA
DPI
NOXAB6
NOXAB10
NOXR4
NOXR7
D
CAT activity (μmol H_2O_2/g/min)
2.0
1.5
1.0
0.5
0.0
**
WT
Neo
LaCl3
Rot
AA
DPI
NOXAB6
NOXAB10
NOXR4
NOXR7
E
SOD activity (U/mg)
15
10
5
0
**
**
*
*
WT
Neo
LaCl3
Rot
AA
DPI
NOXAB6
NOXAB10
NOXR4
NOXR7
F
NS
Copper contents (mg/g DW)
0.06
0.04
0.02
0.00
WT
Neo
LaCl3
Rot
AA
DPI
NOXAB6
NOXAB10
NOXR4
NOXR7

Supplemental Figures Legends

FIG. S1 Effect of Cd^{2+} and Fe^{3+} on the GAs, ROS and Ca^{2+} contents in *G. lucidum*

G. lucidum wild-type strain was cultured on CYM plates for 5 days with different concentrations of copper. Aerial hyphae were removed from actively growing colonies, suspended in fluorescent dye DCFH-DA for cytosolic ROS detection (A) or Fluo-3AM (B) for the free cytosolic Ca^{2+} detection for 30 min at 37 °C. The hyphae were washed three times with PBS to remove excess fluorophore. The green fluorescence DCF- and Fluo 3-labeled cells were visualized by CLSM, with the green fluorescence intensity representing the levels of cytosolic ROS and Ca^{2+}. DIC denotes differential interference contrast. Scale bar = 100 μm. C and D. Changes in the cytosolic ROS and Ca^{2+} fluorescence ratio in copper-stressed *G. lucidum*. The Y-axis is the fluorescence ratio measured by CLSM, and the X-axis represents the different concentrations of copper. E. The wild-type strain was cultured in CYM liquid with shaking at 28 °C. On the 4th day, 3 mM Cu^{2+}, 3 mM Cd^{2+}, and 3 mM Fe^{3+} were added. On the 7th day, the sample were analyzed. Within each set of experiments, different letters indicate significant differences between the lines ($P < 0.05$, according to one-way ANOVA multiple comparison test).

FIG. S2 The effect of ROS-related reagents on fold change of ROS contents and GAs contents, expression of GAs-related genes in *G. lucidum* under copper stress

A, B. The effect of DPI, Rot, and AA on fold change of ROS and GAs contents induced by copper treated. C-E. The relative expression of genes encoding key enzymes in the GAs biosynthetic pathway was measured in *G. lucidum* mycelia with ROS-related inhibitor, H_2O_2 and 3 mM copper treatment. The Y axis is the relative expression of *Sqs* (C), *Osc* (D) and *Hmgr* (E) measured by RT-PCR, and the X axis is the different treatments. F. The effect of H_2O_2 on hyphal growth without copper treated. All values are the mean ± SD of three independent experiments. Within each set of experiments, different letters

indicate significant differences between the lines ($P < 0.05$, according to one-way ANOVA multiple comparison test).

FIG. S3 The effect of NOX silencing on fold change of ROS contents and GAs contents, expression of GAs-related genes in *G. lucidum* under copper stress

A, B. The effect of NOX silencing on fold change of ROS and GAs contents induced by copper treated. All values are the mean ± SD of three independent experiments. Within each set of experiments, different letters indicate significant differences between the lines ($P < 0.05$, according to one-way ANOVA multiple comparison test).

FIG. S4 The effect of Ca^{2+}-related reagents on hyphal growth, Ca^{2+} contents, GAs contents and expression of GAs-related genes in *G. lucidum* under copper stress

A, B. The effect of $CaCl_2$, $LaCl_3$, and Neo on hyphal growth without copper treated. C-D. The effect of $LaCl_3$, and Neo on fold change of Ca^{2+} and GAs contents induced by copper treated. E. The effect of $LaCl_3$, and Neo on hyphal branching without copper treated. F-H. The relative expression of genes encoding key enzymes in the GAs biosynthetic pathway was measured in *G. lucidum* mycelia with Ca^{2+}-related inhibitor, $CaCl_2$ and 3 mM copper treatment. The Y axis is the relative expression of *Sqs* (F), *Osc* (G) and *Hmgr* (H) measured by RT-PCR, and the X axis is the different treatments. All values are the mean ± SD of three independent experiments. Within each set of experiments, different letters indicate significant differences between the lines ($P < 0.05$, according to one-way ANOVA multiple comparison test).

FIG. S5 The effect of reagents on genes expression in *G. lucidum* under copper stress

The expression levels of certain ROS and Ca^{2+} signaling-related genes were measured after the hyphae were treated with different treatments (A. 3 mM copper and 6 mM H2O2 treatment with ROS related

reagents; B. 3mM treatment on Nox-silenced strains). Relevant genes were detected using real-time PCR, reference gene was used as *actin* gene, and the results are presented as a heatmap generated using GENESPRINGGX 7.3.1 software (Agilent Technologies). Relative expression is shown as a mean value from 0.0 to 4.0 in green to red.

FIG. S6 The effect on ROS, Ca2+, and GAs contents, antioxidant activities, and copper contents in *G. lucidum* under different agents without copper treated

A. Measurement of ROS contents by DCFH-DA staining to detect the effect of different agents and NOX-silencing without copper treated on ROS contents. B Measurement of Ca^{2+} contents by Fluo-3 AM staining to detect the effect of different agents and NOX-silencing without copper treated on Ca^{2+} contents. C. Measurement of GAs contents to detect the effect of different agents and NOX-silencing without copper treated on GAs contents. D, E. Measurement of CAT and SOD activities to detect the effect of different agents and NOX-silencing without copper treated on antioxidant activities. E. Measurement of copper contents to detect the effect of different agents and NOX-silencing without copper treated. Within each set of experiments, different letters indicate significant differences between the lines ($P < 0.05$, according to one-way ANOVA multiple comparison test).