Supplementary Information

SI-1: Detailed experimental procedures

1. The Se-enriched *G. lucidum* sample was put into the star ball mill and an appropriate amount of ultra-pure water was added to dissolve it. The solid-liquid material ratio was 1:25 for ball grinding. After ball milling, the sample was centrifuged for separation (5000 r/min, 30min). Then the supernatant was collected and stored in a refrigerator at 4°C, and the sediment was used for next step. Two forms of selenium including $SeCys_2$ (15.1μg/g) and selenite (47.7 μg/g) were detected in the supernatant.

2. 0.2g sediment sample was added into the following five buffer solutions (5 mL): Deionized water (DW), Tris-HCl buffer (pH=2.1) (T-H/2.1), Phosphate buffer (pH=7.5) (P/7.5), Tris-HCl buffer (pH=7.5) (T-H/7.5) and Glycine-HCl buffer (pH=2.1) (G-H/2.1), respectively. After fully oscillation (37 °C/250rpm for 3 hours), the solutions were centrifuged at 5000rpm for 10 minutes, and the supernatants and residues were collected for Se determination, respectively.

3. 0.2g sediment sample was treated by the following twelve sequential extraction methods, respectively:

A-Phosphate (pH 7.5), trypsin: 5 ml of 0.1 mol/l Phosphate buffer (pH 7.5) containing 45 mg of trypsin and stirred at 37°C/250rpm for 20h. Then the solution was centrifuged at 5000rpm for 10 minutes to collect

the supernatant for Se determination.

B-Phosphate (pH 7.5), protease XIV: 5 ml of 0.1 mol/l Phosphate buffer (pH 7.5) containing 0.5 mg of protease XIV and stirred at 37°C/250 rpm for 8 h. Then the solution was centrifuged at 5000rpm for 10 minutes to collect the supernatant for Se determination.

C-Tris-HCl (pH 7.5), trypsin: 5 ml of 0.05 mol/l Tris-HCl buffer (pH7.5) containing 45 mg of trypsin and stirred at 37°C/250 rpm for 20h. Then the solution was centrifuged at 5000rpm for 10 minutes to collect the supernatant for Se determination.

D-Tris-HCl (pH 7.5), protease XIV: 5 ml of 0.05 mol/l Tris-HCl buffer (pH 7.5) containing 0.5 mg of protease XIV and stirred at 37°C at 250rpm for 8 h. Then the solution was centrifuged at 5000rpm for 10 minutes to collect the supernatant for Se determination.

E-Tris-HCl (pH 7.5), protease K; Phosphate (pH 7.5), protease XIV: Step1 — 5 ml of 0.05mol/l Tris-HCl buffer (pH 7.5) containing 0.5 mg of protease K and stirred at 50°C/250 rpm for 14h. Then the solution was centrifuged at 5000rpm for 10 minutes to collect the supernatant for Se determination. Step 2 — The sediment in Step 1 was extracted in 5 ml of 0.1 mol/l phosphate buffer (pH 7.5) containing 0.5 mg of protease XIV and stirred at 37°C/250 rpm for 8 h. Then the solution was centrifuged at 5000rpm for 10 minutes to collect the supernatant for Se determination.

F-Tris-HCl (pH 7.5), protease K; Tris-HCl (pH 7.5), protease XIV: Step1 — 5 ml of 0.05mol/L Tris-HCl buffer (pH 7.5) containing 0.5 mg of protease K and stirred at 50°C/250rpm for 14h. Then the solution was centrifuged at 5000rpm for 10 minutes to collect the supernatant for Se determination. Step 2 — The sediment in Step 1 was extracted in 5 ml of 0.1 mol/L Tris-HCl buffer (pH7.5) containing 0.5 mg of protease XIV and stirred at 37°C/250 rpm for 8 h. Then the solution was centrifuged at 5000rpm for 10 minutes to collect the supernatant for Se determination.

G-Glycine (pH 2.1), pepsin; Phosphate (pH 7.5), trypsin: Step1 — 5 ml of 0.05 mol/L Glycine-HCl buffer (pH 2.1) containing 45 mg of pepsin and stirred at 37°C/250 rpm for 20h. Then the solution was centrifuged at 5000rpm for 10 minutes to collect the supernatant for Se determination. Step 2 — The sediment in Step 1 was extracted in 5 ml of 0.1 mol/L phosphate buffer (pH 7.5) containing 45 mg of trypsin and stirred at 37°C/250 rpm for 20h. Then the solution was centrifuged at 5000rpm for 10 minutes to collect the supernatant for Se determination.

H-Phosphate (pH 7.5), protease K; Phosphate (pH 7.5), protease XIV: Step1 — 5 ml of 0.1 mol/L Phosphate buffer (pH 7.5) containing 0.5 mg of protease K and stirred at 50°C/250 rpm for 14h. Then the solution was centrifuged at 5000rpm for 10 minutes to collect the supernatant for Se determination. Step 2 — The sediment in Step 1 was extracted in 5 ml

of 0.1 mol/l Phosphate buffer (pH 7.5) containing 0.5 mg of protease XIV and stirred at 37°C/250rpm for 8 h. Then the solution was centrifuged at 5000rpm for 10 minutes to collect the supernatant for Se determination.

I-Phosphate (pH 7.5), protease K; Tris-HCl (pH 7.5), protease XIV: Step1 — 5 ml of 0.1 mol/l Phosphate buffer (pH 7.5) containing 0.5 mg of protease K and stirred at 50°C at 250 rpm for 14h. Then the solution was centrifuged at 5000rpm for 10 minutes to collect the supernatant for Se determination. Step 2 — The sediment in Step 1 was extracted in 5 ml of 0.1 mol/L Tris-HCl buffer (pH 7.5) containing 0.5 mg of protease XIV and stirred at 37°C/250 rpm for 8 h. Then the solution was centrifuged at 5000rpm for 10 minutes to collect the supernatant for Se determination.

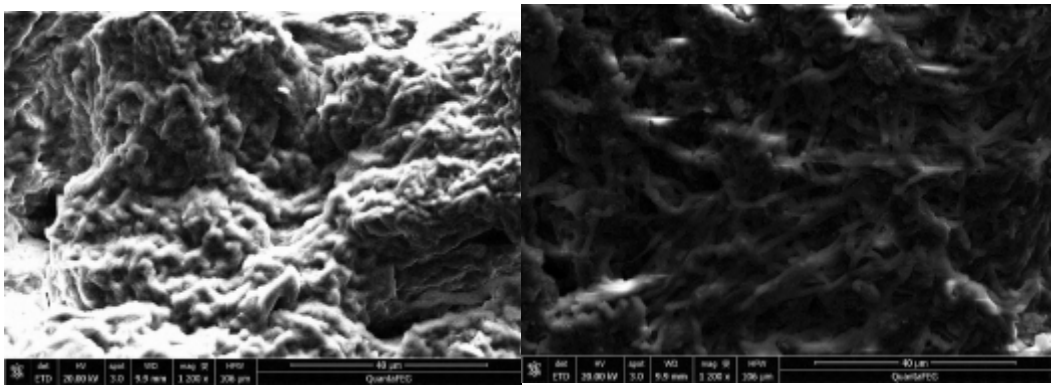
J-Glycine (pH 2.1), pepsin; Tris-HCl (pH 7.5), trypsin: Step1 — 5 ml of 0.05mol/L Glycine-HCl buffer (pH 2.1) containing 45 mg of pepsin and stirred at 37°C/250 rpm for 20h. Then the solution was centrifuged at 5000rpm for 10 minutes to collect the supernatant for Se determination. Step 2 — The sediment in Step 1 was extracted in 5 ml of 0.1 mol/L Tris-HCl buffer (pH 7.5) containing 45 mg of trypsin and stirred at 37°C/250 rpm for 20h. Then the solution was centrifuged at 5000rpm for 10 minutes to collect the supernatant for Se determination.

K-Tris-HCl (pH 2.1), pepsin; Phosphate (pH 7.5), trypsin: Step 1 — 5

ml of 0.05 mol/L Tris-HCl buffer (pH 2.1) containing 45 mg of pepsin and stirred at 37°C/250 rpm for 20 h. Then the solution was centrifuged at 5000rpm for 10 minutes to collect the supernatant for Se determination. Step 2 — The sediment in Step 1 was extracted in 5 ml of 0.1 mol/L phosphate buffer (pH 7.5) containing 45 mg of trypsin, and stirred at 37°C/250 rpm for 20 h. Then the solution was centrifuged at 5000rpm for 10 minutes to collect the supernatant for Se determination.

L-Tris-HCl (pH 2.1), pepsin; Tris-HCl (pH 7.5), trypsin: Step 1 — 5ml of 0.05 mol/l Tris-HCl buffer (pH 2.1) containing 45 mg of pepsin and stirred at 37°C/250 rpm for 20 h. Then the solution was centrifuged at 5000rpm for 10 minutes to collect the supernatant for Se determination. Step 2 — The sediment in Step 1 was extracted in 5 ml of 0.1 mol/l Tris-HCl buffer (pH 7.5) containing 45 mg of trypsin and stirred at 37°C at 250 rpm for 20 h. Then the solution was centrifuged at 5000rpm for 10 minutes to collect the supernatant for Se determination.

Figure S1. SEM (scanning electron microscope) photos on Se-enriched *Ganoderma lucidum* and normal *Ganoderma lucidum*



Note: left one is Se-enriched *Ganoderma lucidum* and right one is normal

Table S1. Se speciation and their percentages in high Se-enriched *Ganoderma lucidum* via 12 sequential extraction methods.

Sample Preparation	$SeCys_2$ (μg/g)	SeMecys (μg/g)	SeMet (μg/g)	Selenite (μg/g)	Sum (μg/g)
A	<D.L.	<D.L.	2.4±0.4 (100%)	<D.L.	2.4
B	<D.L.	1.2±0.1 (6.7%)	<D.L.	16.7±0.8 (92.3%)	17.9
C	<D.L.	<D.L.	3±0.7 (100%)	<D.L.	3
D	<D.L.	2.1±0.1 (13.0%)	<D.L.	14±1.1 (87.0%)	16.1
E-step1	<D.L.	<D.L.	1.5±0.2	10.8±2.3	12.3
E-step2	<D.L.	<D.L.	<D.L.	0.7±0.1	0.7
E-overall	<D.L.	<D.L.	1.5±0.2 (11.6%)	11.5±2.4 (89.4%)	12.9
F-step1	<D.L.	<D.L.	1.5±0.2	10.8±2.3	12.3
F-step2	<D.L.	<D.L.	0.8±0.1	<D.L.	0.8
F-overall	<D.L.	<D.L.	2.3±0.3 (17.7%)	10.8±2.3 (82.3%)	13
G-step1	<D.L.	<D.L.	1.7±0.4	<D.L.	1.7
G-step2	<D.L.	10.7±0.1	<D.L.	<D.L.	10.7
G-overall	<D.L.	10.7±0.1 (86.3%)	1.7±0.4 (13.7%)	<D.L.	12.4
H-step1	<D.L.	<D.L.	1.4±0.5	16.4±2.2	17.8
H-step1	<D.L.	<D.L.	<D.L.	1.1±0.1	1.1
H-overall	<D.L.	<D.L.	1.4±0.5 (7.4%)	17.5±2.3 (92.6%)	18.9
I-step1	<D.L.	<D.L.	1.4±0.5	16.4±2.2	17.8
I-step2	<D.L.	<D.L.	<D.L.	1.4±0.2	1.4
I-overall	<D.L.	<D.L.	1.4±0.5 (7.3%)	17.8±2.4 (92.7%)	19.2
J-step1	<D.L.	<D.L.	1.7±0.4	<D.L.	1.7
J-step2	<D.L.	11.1±2.9	1.1±0.1	<D.L.	12.2
J-overall	<D.L.	11.1±2.9 (79.9%)	2.8±0.5 (20.1%)	<D.L.	13.9
K-step1	<D.L.	1.3±0.4	2.6±0.8	1.5±0.9	5.4
K-step2	<D.L.	7.8±2.2	1.2±0.1	<D.L.	9
K-overall	<D.L.	9.1±2.8 (63.2%)	3.8±0.9 (26.4%)	1.5±0.9 (10.4%)	14.4
L-step1	<D.L.	1.3±0.4	2.6±0.8	1.5±0.9	5.4
L-step2	<D.L.	12±0.6	1.2±0.2	<D.L.	13.3
L-overall	<D.L.	13.3±1.0 (71.1%)	3.8±1.0 (20.3%)	1.5±0.9 (8.6%)	18.7

Note: A-Tris-HCl (pH 2.1), pepsin; Phosphate (pH 7.5), trypsin; Tris-HCl (pH 2.1), pepsin; Tris-HCl (pH 7.5); Phosphate (pH 7.5), protease XIV; Phosphate (pH 7.5), protease K; Phosphate (pH 7.5), protease XIV; Phosphate (pH 7.5), protease K; Tris-HCl (pH 7.5), protease XIV; Phosphate (pH 7.5), trypsin; Tris-HCl (pH 7.5), protease XIV; Tris-HCl (pH 7.5), protease K; Phosphate (pH

7.5), protease XIV; Tris-HCl (pH 7.5), protease K; Tris-HCl (pH 7.5), protease XIV; Tris-HCl (pH 7.5), trypsin; Glycine (pH 2.1), pepsin; Phosphate (pH 7.5), trypsin; Glycine (pH 2.1), pepsin; Tris-HCl (pH 7.5), trypsin.