

Appendix

Appendix A Specific primer pairs used for qPCR expression analysis.

Appendix B Overview of quantitative proteomic analysis. (A) Repeatability between two biological replicates at Cd treatment time points of 12, 24, 48, and 72 h; (B) Basic iTRAQ information statistics of two replicates; (C) Distribution of proteins identified among different molecular weights (Means±SD, two replicates); (D) Sequence coverage of proteins by the identified peptides (two replicates' average).

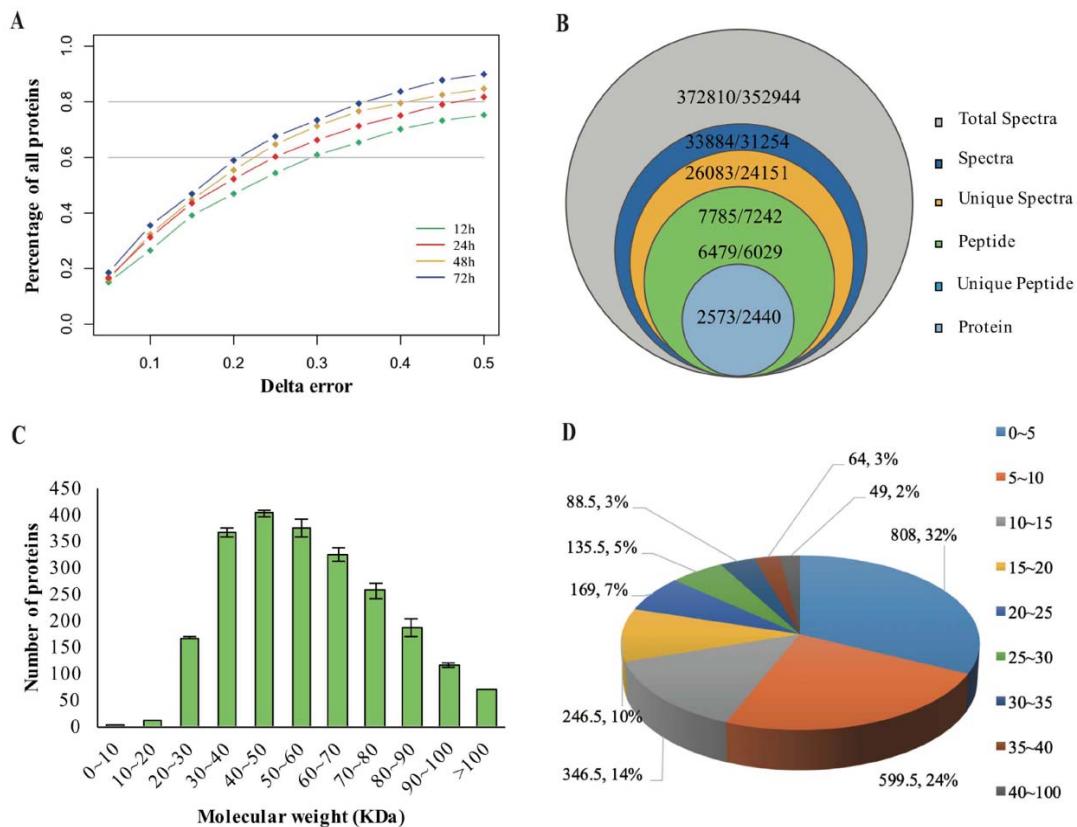
Appendix C Proteomic variation among differential time points of Cd treatment. Venn diagram of the differentially expressed proteins at 12, 24, 48 and 72 h (Left: up-regulated; right: down-regulated).

Appendix D Gene ontology categories for up-regulated (A) and down-regulated (B) proteins. The top 20 enriched GO-terms in three different clusters were shown. Green bar represented the percentage of genes compared with reference, brown bar represented the percentage of genes in differentially abundant proteins.

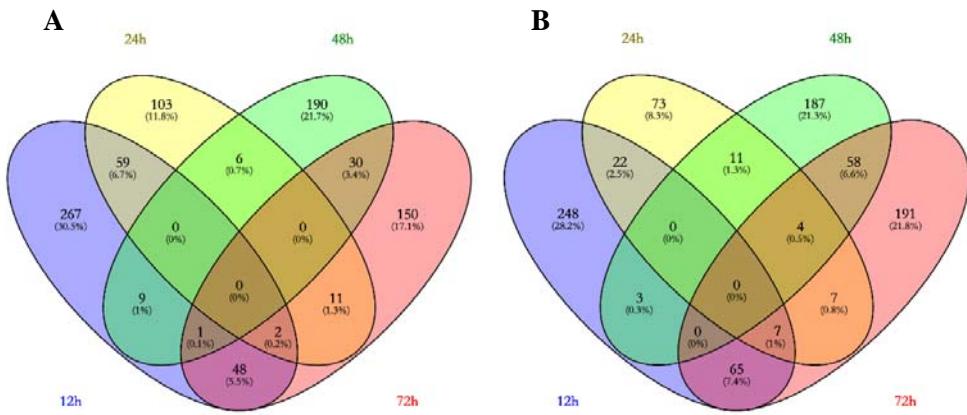
Appendix E Many up-regulated or down-regulated KEGG pathways of candidate regulated proteins in response to cadmium. The input number means the number of proteins found in this study matching with the pathway. The input number means the number of proteins found in this study matching with the pathway.

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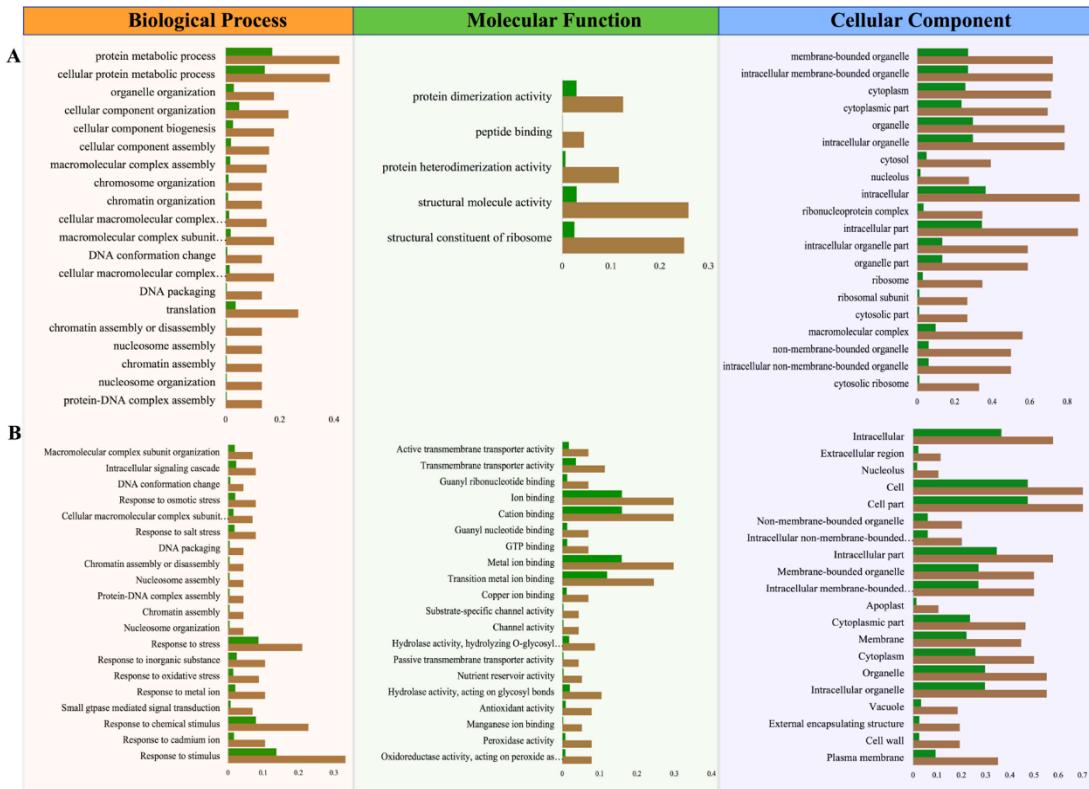
| Gene | Forward primer (5'-3') | Reverse primer (5'-3') | Size (bp) |
|----------------------|------------------------|--------------------------|-----------|
| <i>GRMZM2G158034</i> | GGCAGAGGGACTCCTAAATGTA | TGGATGATGTCACACGAACACT | 121 |
| <i>GRMZM2G308687</i> | CGGGAGTAATAATGACGAACCA | ACAATAGAAAATACAACGACGACG | 100 |
| <i>GRMZM2G039639</i> | CCATCGAGCTGCTCAAACATA | CCAACCTTGAAGCTAGAAGAACAA | 164 |
| <i>GRMZM2G047055</i> | GCCGCTTCTCCTATTCACTCA | CCTCATCAGGCATCTCATTAAAAA | 113 |
| <i>GRMZM2G059693</i> | CGTAGCTTTGGATTGCATGTT | GGAGAGGATGCATATATTCAACAA | 127 |
| <i>GRMZM2G146670</i> | GTCCTAGCATGACTGTTGGGT | TCCTCAATGAAACATTTCGCA | 124 |
| <i>GRMZM2G150616</i> | GGCATCTTGTTGCTGTAGTCA | CGAGCTTGAATTATTGTGAAGGT | 122 |
| <i>GRMZM2G156861</i> | GCACTAGTTGTTGCGTTCC | GCCTTATTACAAACAGTCCTCACG | 122 |
| <i>GRMZM2G156684</i> | GCTTTGAGGAGCGTGTCTG | ATCAAATGCTATTGCTGCACAT | 125 |
| <i>GRMZM2G178817</i> | CGTTTCAGGTGGACAAGAAGG | TGCATAAAACAATGACAATAGCG | 103 |
| <i>gpn1</i> | TGACCAAGGTGAAGAGCACTGT | CAAATCTCACGTGGCTATGAAAC | 134 |



Appendix B Overview of quantitative proteomic analysis. (A) Repeatability between two biological replicates at Cd treatment time points of 12, 24, 48, and 72 h; (B) Basic iTRAQ information statistics of two replicates; (C) Distribution of proteins identified among different molecular weights (Means \pm SD, two replicates); (D) Sequence coverage of proteins by the identified peptides (two replicates' average).



Appendix C Proteomic variation among differential time points of Cd treatment. Venn diagram of the differentially expressed proteins at 12, 24, 48 and 72 h (Left: up-regulated; right: down-regulated).



Appendix D Gene ontology categories for up-regulated (A) and down-regulated (B) proteins. The top 20 enriched GO-terms in three different clusters were shown. Green bar represented the percentage of genes compared with reference, brown bar represented the percentage of genes in differentially abundant proteins.

Appendix E Many up-regulated or down-regulated KEGG pathways of candidate regulated proteins in response to cadmium. The input number means the number of proteins found in this study matching with the pathway. The input number means the number of proteins found in this study matching with the pathway.

| KEGG pathway | Regulation | Pathway ID | Input number | Background number | p-Value | Corrected p-Value |
|---|------------|------------|--------------|-------------------|------------|-------------------|
| Ribosome | Up | zma03010 | 34 | 509 | 3.71E-24 | 1.30E-22 |
| Proteasome | Up | zma03050 | 5 | 99 | 0.00070267 | 0.01229672 |
| 2-Oxocarboxylic acid metabolism | Up | zma01210 | 1 | 86 | 0.44792178 | 0.67497933 |
| Glutathione metabolism | Up | zma00480 | 3 | 146 | 0.08075043 | 0.67497933 |
| Protein processing in endoplasmic reticulum | Up | zma04141 | 4 | 333 | 0.19570278 | 0.67497933 |
| Alanine, aspartate and glutamate metabolism | Up | zma00250 | 1 | 82 | 0.43259041 | 0.67497933 |
| Arginine and proline metabolism | Up | zma00330 | 2 | 111 | 0.17867168 | 0.67497933 |
| Biosynthesis of amino acids | Up | zma01230 | 4 | 378 | 0.26070606 | 0.67497933 |
| beta-Alanine metabolism | Up | zma00410 | 1 | 57 | 0.32675592 | 0.67497933 |
| Phenylalanine, tyrosine and tryptophan biosynthesis | Up | zma00400 | 1 | 75 | 0.40473979 | 0.67497933 |
| Cyanoamino acid metabolism | Up | zma00460 | 1 | 58 | 0.33134212 | 0.67497933 |
| Fructose and mannose metabolism | Up | zma00051 | 1 | 88 | 0.45543296 | 0.67497933 |
| Galactose metabolism | Up | zma00052 | 1 | 85 | 0.44412783 | 0.67497933 |
| Pentose and glucuronate interconversions | Up | zma00040 | 1 | 68 | 0.37553664 | 0.67497933 |
| Glyoxylate and dicarboxylate metabolism | Up | zma00630 | 1 | 90 | 0.46284297 | 0.67497933 |
| Pentose phosphate pathway | Up | zma00030 | 1 | 78 | 0.41683771 | 0.67497933 |
| Nitrogen metabolism | Up | zma00910 | 1 | 54 | 0.31280969 | 0.67497933 |
| One carbon pool by folate | Up | zma00670 | 1 | 21 | 0.13919481 | 0.67497933 |
| Pantothenate and CoA biosynthesis | Up | zma00770 | 1 | 34 | 0.21224131 | 0.67497933 |
| Photosynthesis | Up | zma00195 | 2 | 103 | 0.15941616 | 0.67497933 |
| Plant-pathogen interaction | Up | zma04626 | 2 | 223 | 0.45298524 | 0.67497933 |
| Selenocompound metabolism | Up | zma00450 | 1 | 30 | 0.19044451 | 0.67497933 |
| SNARE interactions in vesicular transport | Up | zma04130 | 1 | 51 | 0.29857753 | 0.67497933 |
| Ubiquitin mediated proteolysis | Up | zma04120 | 3 | 193 | 0.14820339 | 0.67497933 |
| Glycine, serine and threonine metabolism | Up | zma00260 | 1 | 104 | 0.51199127 | 0.71678778 |
| Cysteine and methionine metabolism | Up | zma00270 | 1 | 119 | 0.55971656 | 0.7534646 |
| Pyrimidine metabolism | Up | zma00240 | 1 | 181 | 0.71259287 | 0.83135834 |
| Carbon metabolism | Up | zma01200 | 2 | 346 | 0.68768864 | 0.83135834 |
| KEGG pathway | Regulation | Pathway ID | Input | Background | p-Value | Corrected |

| | | | number | number | | <i>p</i> -Value |
|---|------|----------|--------|--------|------------|-----------------|
| Amino sugar and nucleotide sugar metabolism | Up | zma00520 | 1 | 168 | 0.68565737 | 0.83135834 |
| RNA degradation | Up | zma03018 | 1 | 181 | 0.71259287 | 0.83135834 |
| Starch and sucrose metabolism | Up | zma00500 | 1 | 226 | 0.78935491 | 0.89120716 |
| RNA transport | Up | zma03013 | 1 | 252 | 0.82405014 | 0.90130484 |
| Plant hormone signal transduction | Up | zma04075 | 1 | 371 | 0.92311867 | 0.97906526 |
| Metabolic pathways | Up | zma01100 | 10 | 2519 | 0.98459453 | 0.98459453 |
| Biosynthesis of secondary metabolites | Up | zma01110 | 5 | 1349 | 0.95878445 | 0.98459453 |
| Phenylpropanoid biosynthesis | Down | zma00940 | 10 | 232 | 3.86E-06 | 0.00010409 |
| Phenylalanine metabolism | Down | zma00360 | 8 | 182 | 3.30E-05 | 0.00044601 |
| Endocytosis | Down | zma04144 | 6 | 207 | 0.00266976 | 0.0240278 |
| Glycosaminoglycan degradation | Down | zma00531 | 2 | 19 | 0.0081315 | 0.05488763 |
| Oxidative phosphorylation | Down | zma00190 | 4 | 180 | 0.03213356 | 0.17352124 |
| Cyanoamino acid metabolism | Down | zma00460 | 2 | 58 | 0.05822745 | 0.26202351 |
| Biosynthesis of secondary metabolites | Down | zma01110 | 13 | 1349 | 0.10353342 | 0.31555499 |
| Spliceosome | Down | zma03040 | 4 | 271 | 0.10477546 | 0.31555499 |
| Linoleic acid metabolism | Down | zma00591 | 1 | 16 | 0.105185 | 0.31555499 |
| Ribosome | Down | zma03010 | 6 | 509 | 0.11934696 | 0.32223679 |
| Lysine biosynthesis | Down | zma00300 | 1 | 27 | 0.1673323 | 0.41072473 |
| Starch and sucrose metabolism | Down | zma00500 | 3 | 226 | 0.18754652 | 0.42197968 |
| Metabolic pathways | Down | zma01100 | 19 | 2519 | 0.2892173 | 0.5861479 |
| Amino sugar and nucleotide sugar metabolism | Down | zma00520 | 2 | 168 | 0.30392854 | 0.5861479 |
| Inositol phosphate metabolism | Down | zma00562 | 1 | 83 | 0.42329491 | 0.74577337 |
| Fructose and mannose metabolism | Down | zma00051 | 1 | 88 | 0.44193977 | 0.74577337 |
| Carbon fixation in photosynthetic organisms | Down | zma00710 | 1 | 115 | 0.53277831 | 0.81739235 |
| Cysteine and methionine metabolism | Down | zma00270 | 1 | 119 | 0.54492824 | 0.81739235 |
| Glutathione metabolism | Down | zma00480 | 1 | 146 | 0.6191484 | 0.83645544 |
| Phagosome | Down | zma04145 | 1 | 147 | 0.621654 | 0.83645544 |
| Pyrimidine metabolism | Down | zma00240 | 1 | 181 | 0.6977929 | 0.83645544 |
| Glycolysis / Gluconeogenesis | Down | zma00010 | 1 | 186 | 0.70762885 | 0.83645544 |
| Biosynthesis of amino acids | Down | zma01230 | 2 | 378 | 0.71253612 | 0.83645544 |
| Purine metabolism | Down | zma00230 | 1 | 234 | 0.78732039 | 0.87620113 |
| RNA transport | Down | zma03013 | 1 | 252 | 0.81129735 | 0.87620113 |
| Protein processing in endoplasmic reticulum | Down | zma04141 | 1 | 333 | 0.89004883 | 0.89920778 |
| Carbon metabolism | Down | zma01200 | 1 | 346 | 0.89920778 | 0.89920778 |

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