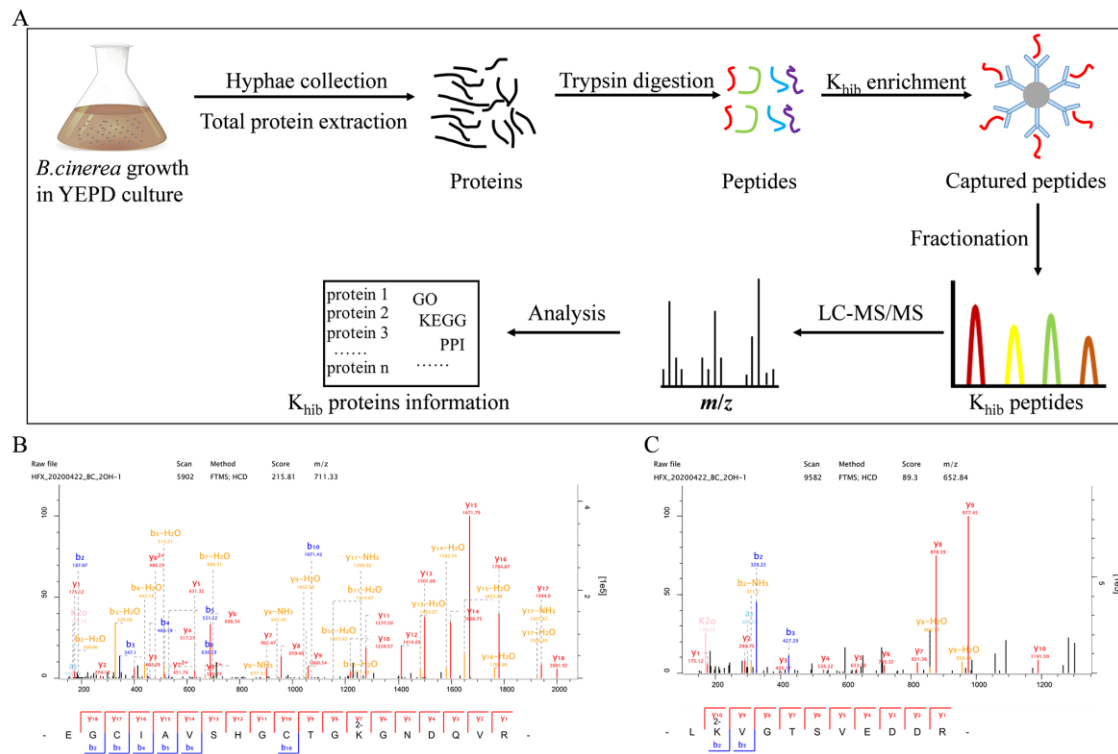
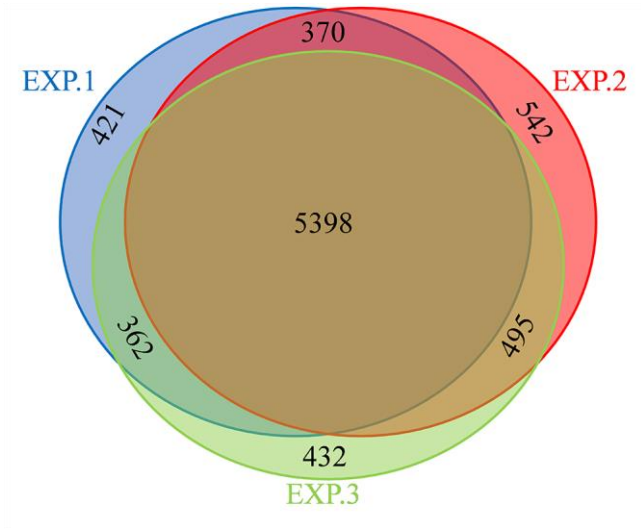


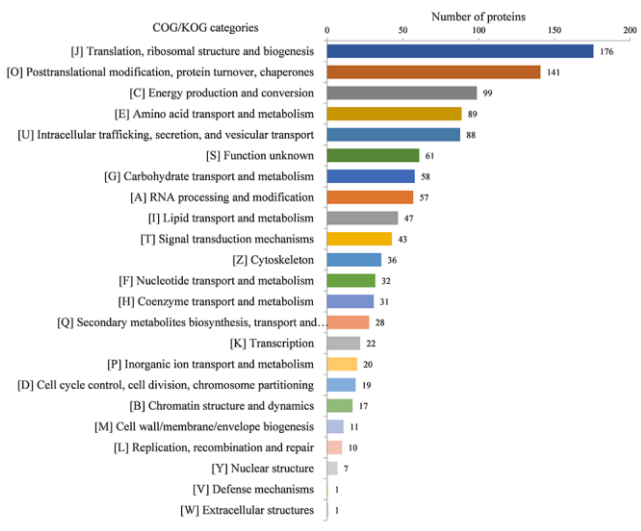
## Supplementary Material



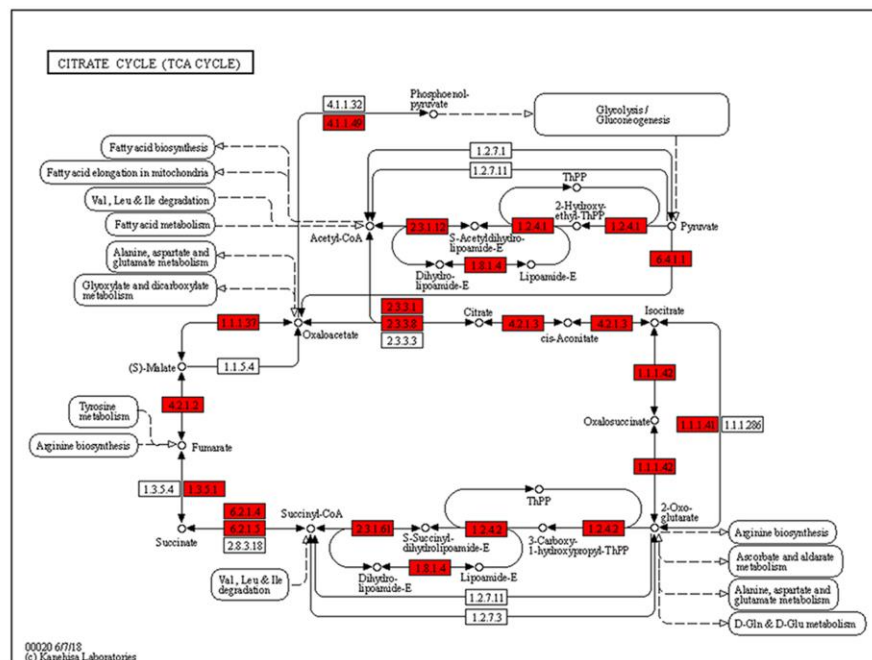
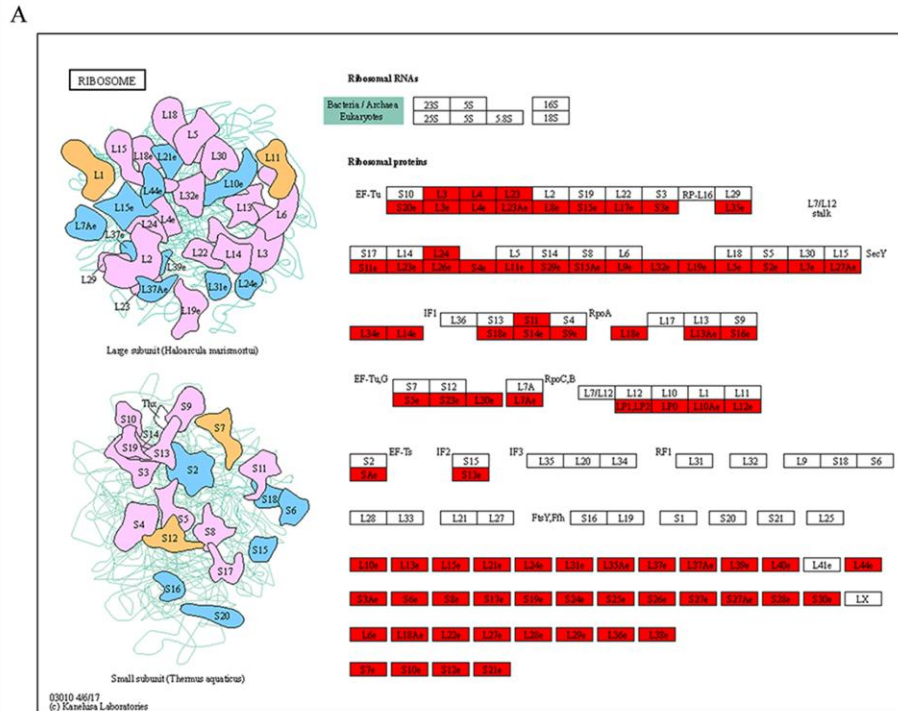
**Supplementary Figure 1.** Proteome-wide analysis workflow and two representative MS/MS spectra. **(A)** The workflow of lysine 2-hydroxyisobutyrylation ( $K_{hib}$ ) analysis for total proteins extracted from *Botrytis cinerea* hyphae using affinity enrichment and high-resolution LC-MS/MS methods. **(B)** and **(C)** The MS/MS spectra of peptide EGCIAVSHGCTG $K_{hib}$ GNDQVR with a 2-hydroxyisobutyrylated site at K120 in Bcass1 (A0A384J6I8) and peptide LK $K_{hib}$ VGTSVEDDR with a 2-hydroxyisobutyrylated site at K194 in Bclgd1 (A0A384J6S6).



**Supplementary Figure 2.** Venn diagram of identified  $K_{hib}$  sites from three biological repeats. Overlapped areas represent that identified  $K_{hib}$  sites were repeated in different biological repeats.



**Supplementary Figure 3.** Functional classification analysis based on Clusters of Orthologous Groups/EuKaryotic Orthologous Groups (COG/KOG) database. The value side the histogram represents protein number of the indicating terms.



**Supplementary Figure 4.** Two representatives of enriched KEGG pathways. (A) Ribosome pathway (map03010). (B) Citrate cycle (TCA cycle) pathway (map00020). The identified  $K_{hib}$  proteins were highlighted in red.

**Supplementary Table 1.** K<sub>hib</sub> sites identified in EXP.1 (biological repeat experiment 1).

**Supplementary Table 2.** K<sub>hib</sub> sites identified in EXP.2 (biological repeat experiment 2).

**Supplementary Table 3.** K<sub>hib</sub> sites identified in EXP.3 (biological repeat experiment 3).

**Supplementary Table 4.** Total sites of K<sub>hib</sub> identified in *B.cinerea* hyphae. All of the sites were detected in 3 biological repeats.

**Supplementary Table 5.** Orthologs of K<sub>hib</sub> proteins identified from *B.cinerea* in different species. The species include *Homo sapiens*, *Oryza sativa* subsp. japonica, *Physcomitrella patens*, *Saccharomyces cerevisiae* and *Toxoplasma gondii*.

**Supplementary Table 6.** Information of K<sub>hib</sub> proteins identified from *B.cinerea* classified into different GO terms.

**Supplementary Table 7.** Information of K<sub>hib</sub> proteins identified from *B.cinerea* classified into different COG/KOG terms.

**Supplementary Table 8.** Subcellular localization details of K<sub>hib</sub> proteins identified from *B.cinerea*.

**Supplementary Table 9.** Details of GO enrichment analysis of identified K<sub>hib</sub> proteins in *B.cinerea*. The GO terms were filtrated with Fisher's exact test P value less than 0.05.

**Supplementary Table 10.** Details of PPI analysis of identified K<sub>hib</sub> proteins in *B.cinerea*. The table includes 506 clustered proteins and 389 unclustered prtoeisms.

**Supplementary Table 11.** Details of 37 identified K<sub>hib</sub> proteins involved in pathogenicity of *B.cinerea*.