

Supplementary Data

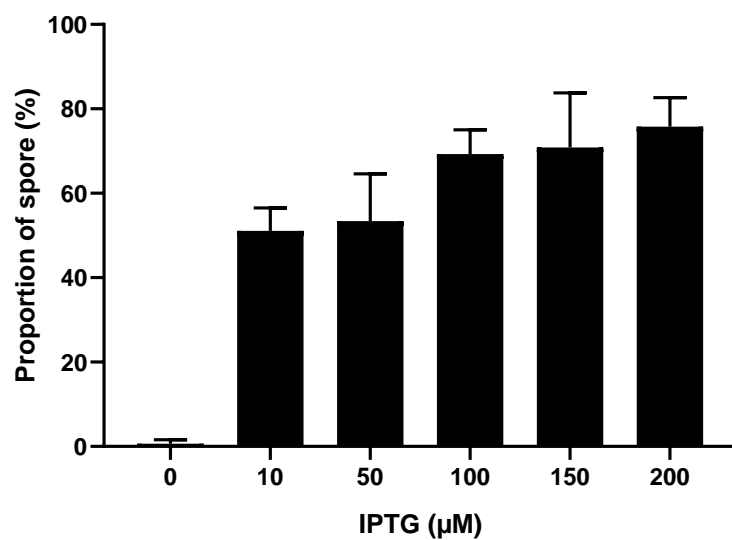
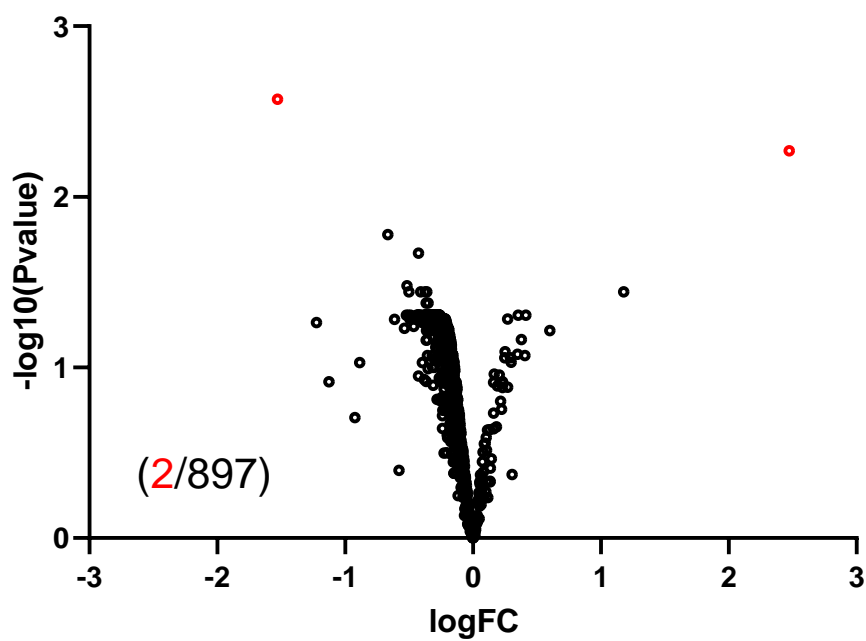


Figure S1. The sporulation efficiency of the mutant strain with induction of *kinA* at different concentrations of IPTG after 8 h of glucose dilution.

A



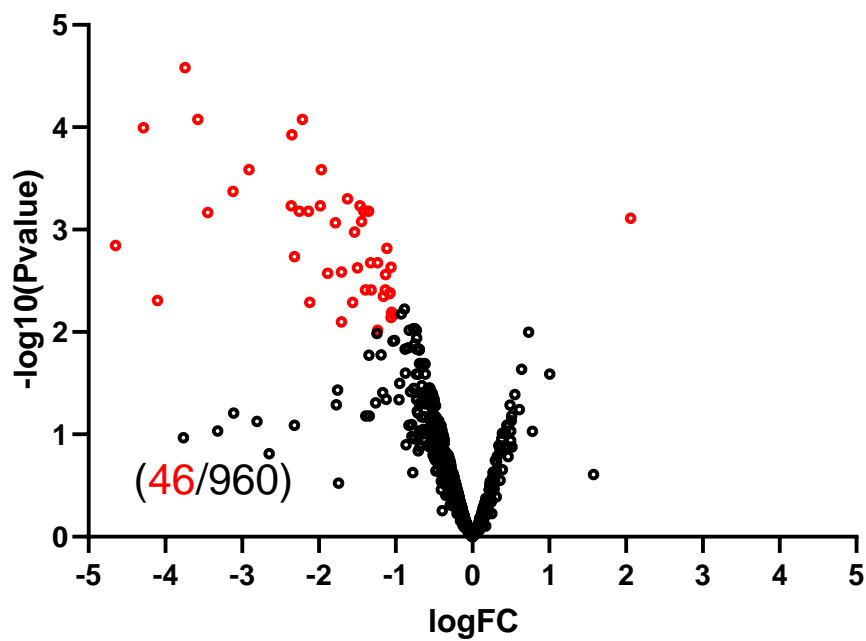
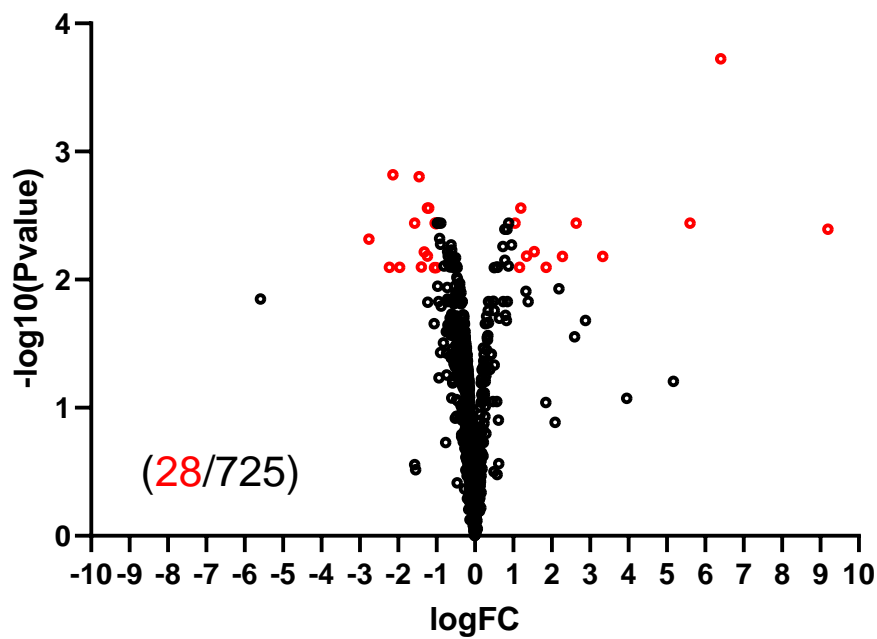
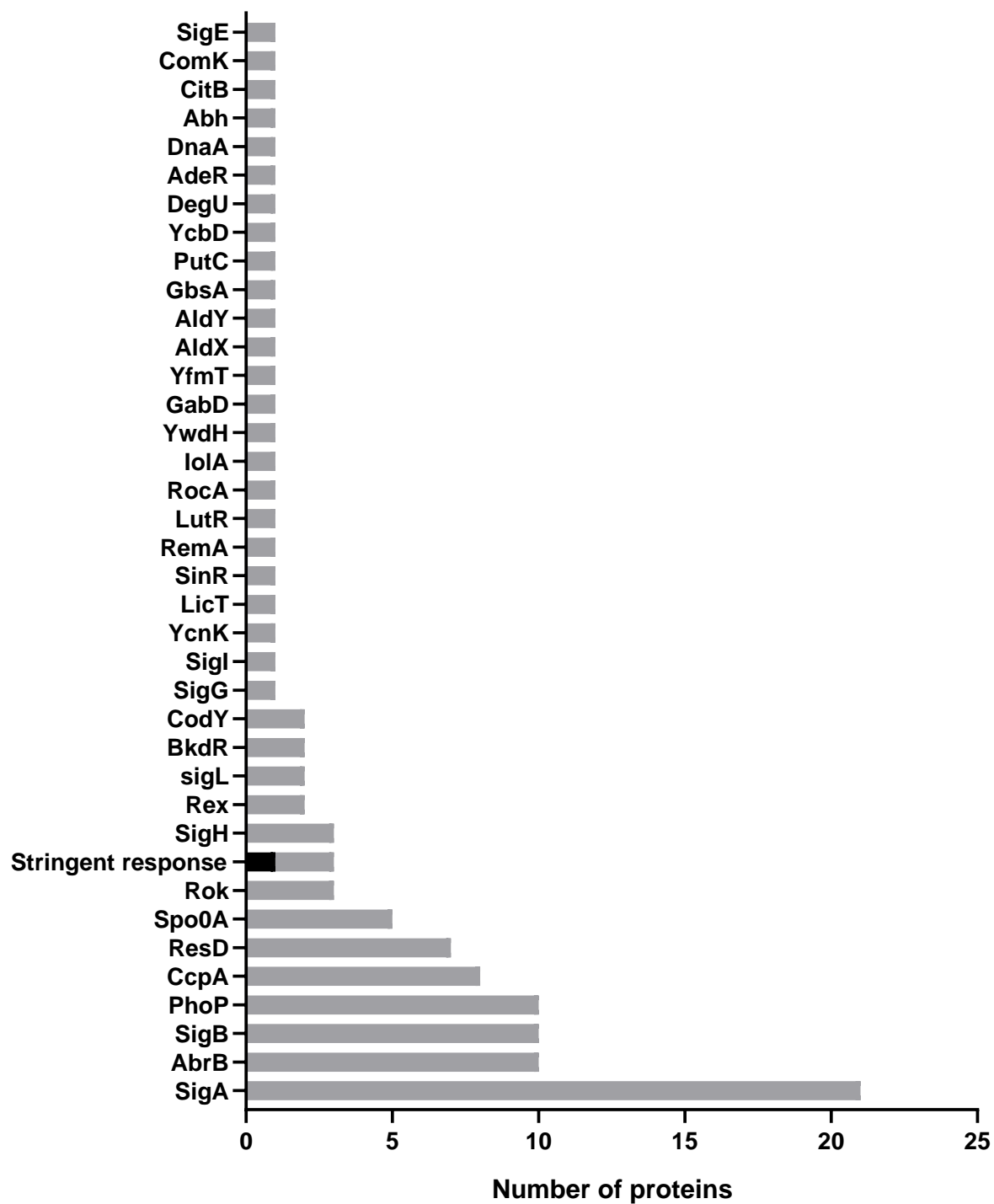
B**C**

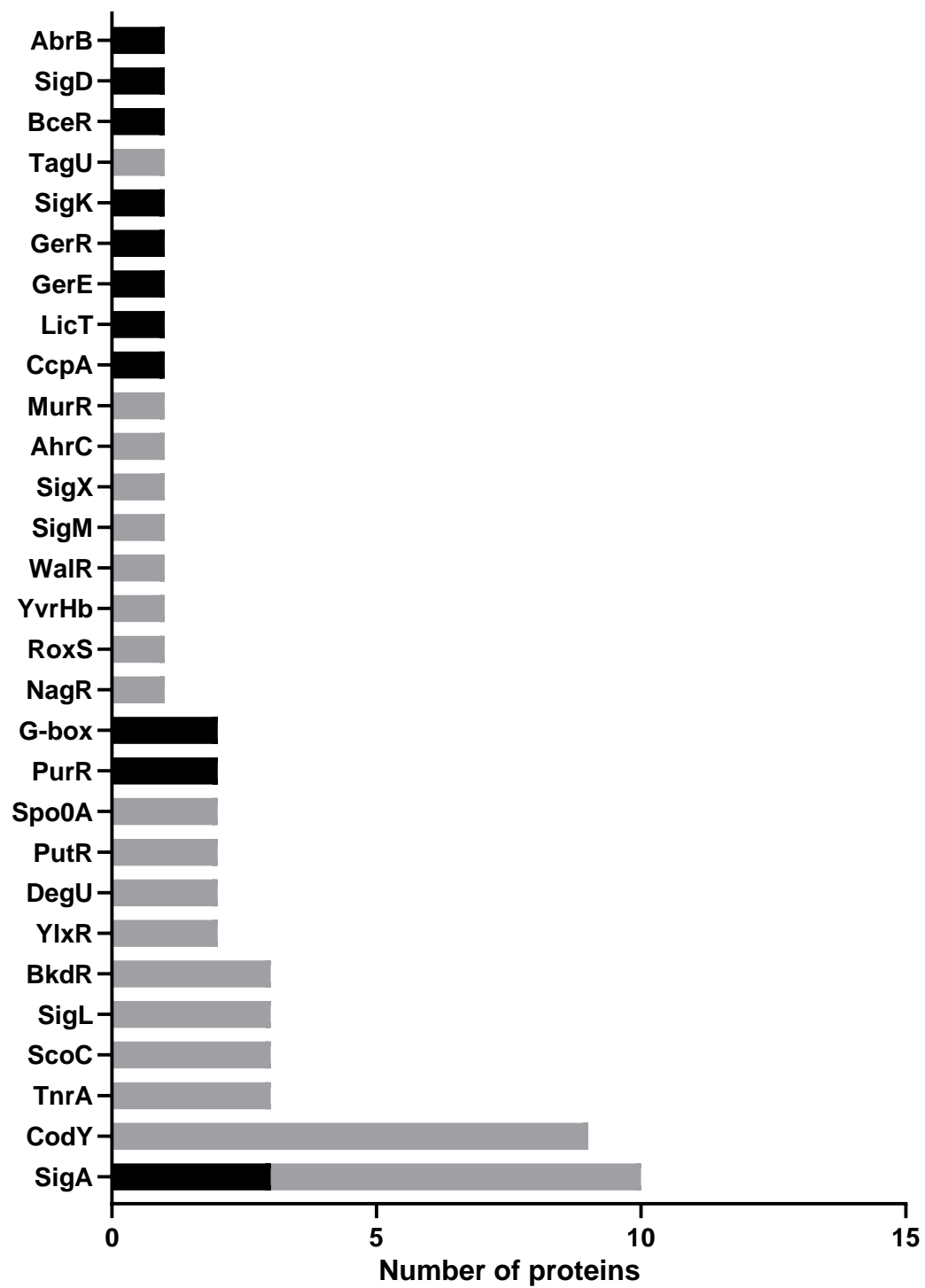
Figure S2. Volcano plots of proteomic comparison of spores or cells. X-axis indicates the average of \log_2 isotopic ratios from the replicates. Negative values indicate downregulation and positive

values indicate upregulation, Y axis is $-\log_{10}$ p-value. Dots in red indicate differentially expressed proteins. Dots in black indicate proteins that are not significant changed in protein expression. Numbers in red and black in brackets are the number of differentially expressed proteins and total quantified proteins, respectively. **(A)** WT+ spores are compared to WT- spores. **(B)** M- cells are compared to WT- cells. **(C)** M- spores are compared to WT- spores.

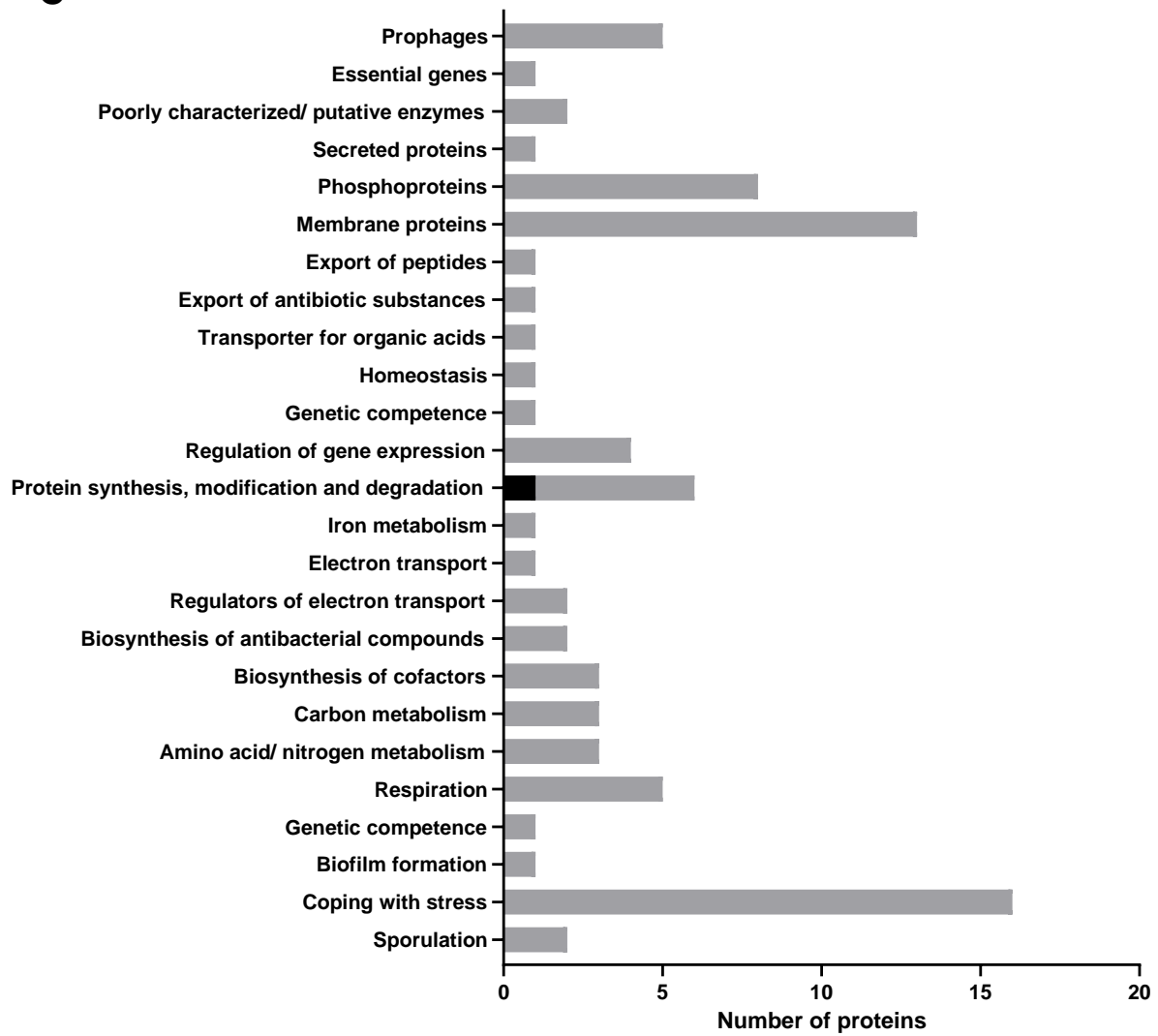
A



B



C



D

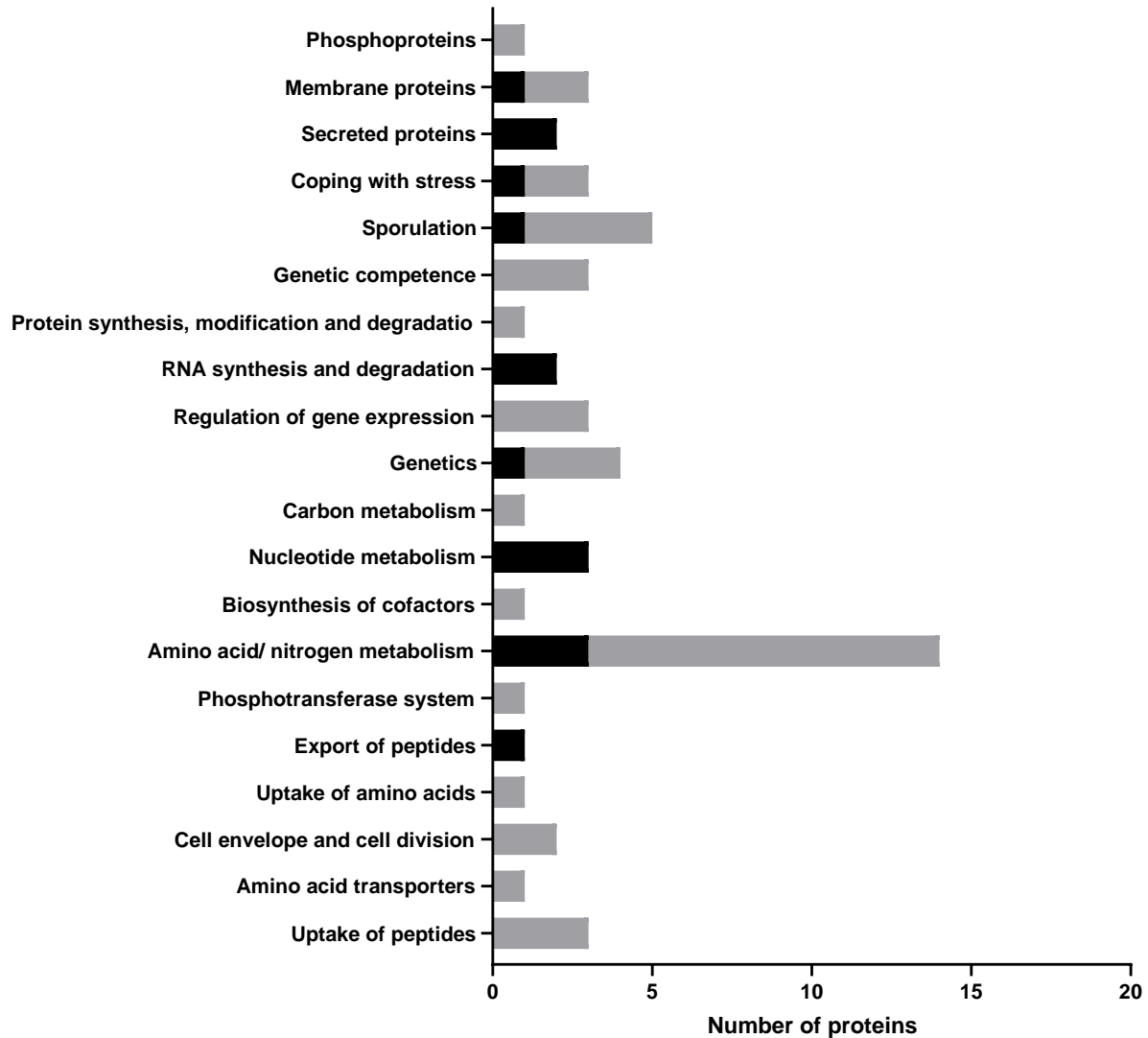


Figure S3. Regulators and functional classification of differentially expressed proteins. X-axis indicates the number of proteins classified into a regulatory or functional categories on the y-axis. Note that every protein could occupy more than one regulatory or functional category. Bars in black and gray indicate number of upregulated and downregulated proteins, separately (A) Regulators of differentially expressed proteins in M- cells compared to WT- cells. (B) Regulators of differentially expressed proteins in M- spores compared to WT- spores. (C) and (D) are the corresponding functional classification to (A) and (B) and their respective comparisons.

Table S2. Differentially expressed carbon and amino acid/ nitrogen metabolism related proteins in *kinA*-overexpressed spores of *B. subtilis*

| Protein | Log ₂ (M+/M-) | Log ₂ (M+/M-) | function | product |
|--|--------------------------|--------------------------|---|--|
| Upregulation | | | | |
| Both quantified in comparison with M- and WT+ spores | | | | |
| AroA | 2.45 | 1.89 | biosynthesis of aromatic amino acids | 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase |
| Quantified in comparison with M- spores | | | | |
| AnsB | 1.14 | | aspartate degradation | L-aspartase |
| Quantified in comparison with WT+ spores | | | | |
| ThrC | | 1.44 | biosynthesis of threonine | threonine synthase |
| Downregulation | | | | |
| Both quantified in comparison with M- and WT+ spores | | | | |
| AcsA | -3.29 | -3.45 | utilization of acetate, fatty acids | acetyl-CoA synthetase |
| ArtP | -2.05 | -1.17 | arginine uptake | high affinity arginine ABC transporter (ATP-binding protein) |
| BdhA | -1.01 | -1.46 | overflow metabolism, fermentation | acetoin/ butanediol dehydrogenase |
| CitZ | -2.29 | -2.05 | TCA cycle | citrate synthase II |
| DppA | -3.11 | -2.70 | degradation of cell wall peptides | D-alanyl-aminopeptidase |
| DppE | -3.75 | -1.58 | uptake of dipeptides | dipeptide ABC transporter (dipeptide-binding protein) |
| GlpK | -2.33 | -2.04 | glycerol utilization | glycerol kinase |
| MelA | -1.19 | -1.14 | utilization of melibiose and raffinose family oligosaccharides (raffinose, stachyose) | alpha-galactosidase |

| | | | | |
|--|-------|-------|--|--|
| OppA | -4.16 | -2.53 | initiation of sporulation, competence development | oligopeptide ABC transporter (binding protein) |
| OppD | -2.93 | -1.42 | initiation of sporulation, competence development | oligopeptide ABC transporter (ATP-binding protein) |
| OppF | -3.04 | -1.84 | initiation of sporulation, competence development | oligopeptide ABC transporter (ATP-binding protein) |
| RbsD | -2.61 | -2.99 | ribose uptake | ribose ABC transporter (membrane protein) |
| RbsK | -2.26 | -2.28 | ribose utilization | ribokinase |
| Quantified in comparison with M- spores | | | | |
| ArgC | -1.51 | | biosynthesis of arginine | N-acetyl-g-glutamyl- phosphate reductase |
| ArtM | -1.52 | | arginine uptake | high affinity arginine ABC transporter (ATP-binding protein) |
| ArtQ | -1.66 | | arginine uptake | high affinity arginine ABC transporter (permease) |
| BfmBA B | -1.06 | | utilization of branched-chain keto acids | 2-oxoisovalerate dehydrogenase (E1 beta subunit) |
| BfmBB | -1.12 | | utilization of branched-chain keto acids | 2-oxoisovalerate dehydrogenase (E2 subunit, lipoamide acyltransferase) |
| BkdAA | -1.04 | | utilization of branched-chain keto acids | 2-oxoisovalerate dehydrogenase (E1 alpha subunit) |
| CitB | -2.63 | | TCA cycle | aconitase, trigger enzyme |
| GabD | -1.26 | | utilization of gamma-amino butyric acid | succinate-semialdehyde dehydrogenase (NADP) |
| GalE | -1.37 | | galactose utilization | UDP glucose 4-epimerase |
| GcvPB | -1.94 | | glycine utilization | glycine decarboxylase (subunit 2) |

| | | | |
|---|-------|---|---|
| GcvT | -1.69 | glycine utilization | aminomethyltransferase (glycine cleavage system protein T) |
| GlnH | -2.49 | glutamine uptake | glutamine ABC transporter (binding protein) |
| GlpD | -1.28 | glycerol utilization | glycerol-3-phosphate dehydrogenase (menaquinone 7) |
| HutG | -4.32 | histidine utilization | formiminoglutamate hydrolase |
| HutI | -4.89 | histidine utilization | imidazolone-5-propionate hydrolase |
| IolS | -1.24 | unknown, may be involved in myo-inositol catabolism | unknown |
| KduI | -1.07 | utilization of galacturonic acid | 5-keto-4-deoxyuronate isomerase |
| LpdV | -1.22 | utilization of branched-chain keto acids | 2-oxoisovalerate dehydrogenase (E3 subunit, dihydrolipoamide dehydrogenase) |
| NagP | -1.87 | N-acetylglucosamine uptake and phosphorylation | N-acetylglucosamine-specific PTS, EIICB |
| PutC | -1.54 | proline utilization | 1-pyrroline-5-carboxylate dehydrogenase |
| RbsB | -1.90 | ribose uptake | ribose ABC transporter (binding protein) |
| YqiT | -1.42 | utilization of branched-chain keto acids | valine dehydrogenase, isoleucine dehydrogenase, L-leucine dehydrogenase |
| Quantified in comparison with WT+ spores | | | |
| BglH | -1.74 | salicin utilization | phospho-beta-glucosidase |
| FruK | -1.26 | fructose utilization | fructose-1-phosphate kinase |
| HisB | -1.13 | biosynthesis of histidine | imidazoleglycerol-phosphate dehydratase |
| IlvD | -1.14 | biosynthesis of branched- | dihydroxy-acid dehydratase |

| | | | |
|-------------|-------|---|---|
| | | chain amino acids | (2,3-dihydroxy-3-methylbutanoate, 2,3-dihydroxy-3-methylpentanoate) |
| LicB | -1.26 | lichenan uptake and phosphorylation, control of LicR activity | lichenan-specific PTS, EIIB component |
| OppC | -1.52 | initiation of sporulation, competence development | oligopeptide ABC transporter (permease) |
| Pel | -2.20 | degradation of polygalacturonic acid | pectate lyase C |
| ProA | -1.00 | biosynthesis of proline | glutamate-5-semialdehyde dehydrogenase |
| RocA | -2.74 | arginine, ornithine and citrulline utilization | 3-hydroxy-1-pyrroline-5-carboxylate dehydrogenase |
| SdhA | -2.55 | TCA cycle | succinate dehydrogenase (flavoprotein subunit) |
| SerC | -1.25 | biosynthesis of serine | 3-phosphoserine aminotransferase |
| YckE | -1.19 | utilization of aryl--glucosides | aryl--glucosidase |
| YvdF | -3.22 | starch and maltodextrin utilization | glucan 1,4-alpha-maltohydrolase, neopullulanase, maltogenic amylase |

Table S3. Differentially expressed proteins associated with coping of stress in *kinA*-overexpressed mutant spores of *B. subtilis*

| Protein | Log ₂ (M+/M-) | Log ₂ (M+/WT+) | function | product |
|---|--------------------------|---------------------------|---|--------------------------------------|
| Upregulation | | | | |
| Both quantified in comparison with M- and WT+ spores | | | | |
| yjqC | 1.66 | 2.49 | protection of the spore | spore coat protein |
| Quantified in comparison with WT+ spores | | | | |
| skfA | | 4.26 | killing of sister cells | spore killing factor |
| skfE | | 1.13 | export of the spore killing factor SkfA | ABC transporter (binding protein) |
| yzkI | | 3.44 | unknown | unknown |
| Downregulation | | | | |
| Both quantified in comparison with M- and WT+ spores | | | | |
| yfkD | -1.39 | -1.60 | unknown | unknown |
| yfhM | -1.82 | -1.46 | survival of ethanol stress | epoxide hydrolase |
| ydaF | -1.83 | -1.78 | unknown | unknown |
| Quantified in comparison with M- spores | | | | |
| yvlB | -1.15 | | unknown | unknown |
| yuaG | -2.22 | | involved in the control of membrane fluidity | membrane-associated scaffold protein |
| yraA | -1.31 | | detoxification of methylglyoxal | glyoxalase III-like enzyme |
| yqfA | -1.20 | | resistance against sublancin | flotillin-like protein |
| yceD | -1.44 | | required for survival of ethanol stress | unknown |
| pbpE | -2.33 | | endopeptidase | penicillin-binding protein PBP 4* |
| katA | -1.41 | | detoxification (degradation) of hydrogen peroxide | vegetative catalase |

| | | | |
|---|-------|--|--|
| gabD | -1.26 | utilization of gamma-amino butyric acid | succinate-semialdehyde dehydrogenase (NADP) |
| Quantified in comparison with WT+ spores | | | |
| bceA | -2.95 | export of toxic peptides | bacitracin ABC transporter (ATP-binding protein) |
| sdpI | -1.99 | protection against SdpC | immunity protein |
| albC | -3.83 | export of antilisterial bacteriocin (subtilisin) | ABC transporter (ATP-binding protein) |
| proA | -1.00 | biosynthesis of proline | glutamate-5-semialdehyde dehydrogenase |
| rpmEB | -1.07 | survival of salt stress | accessory ribosomal protein |
| yoaF | -1.19 | unknown | unknown |
| ydhE | -1.18 | unknown | unknown |
| srfAA | -1.63 | antibiotic synthesis, biocontrol of fungal growth | surfactin synthetase / competence |
| phoR | -1.93 | regulation of phosphate metabolism | two-component sensor kinase |
| phoP | -1.55 | regulation of phosphate metabolism (phoA, phoB, phoD, resABCDE, tagA-tagB, tagDEF, [tuaA-H]) | two-component response regulator (OmpR family) |
| ctsR | -1.45 | regulation of protein degradation | transcription repressor |
| bceB | -3.16 | export of toxic peptides | bacitracin ABC transporter (permease) |

Table S4. Differentially expressed proteins associated with coping of stress in *kinA* mutant cells of *B. subtilis* without induction

| Protein | Log ₂ (M -/WT-) | function | product |
|---------|-------------------------------|----------|---------|
|---------|-------------------------------|----------|---------|

| | | | |
|------|-------|---|--|
| AlbA | -4.65 | antilisterial bacteriocin (subtilisin) production | radical S-adenosylmethionine enzyme |
| AlbC | -2.90 | export of antilisterial bacteriocin (subtilisin) | ABC transporter (ATP-binding protein) |
| Ctc | -2.14 | translation (under stress conditions) | ribosomal protein |
| Dps | -1.33 | iron storage, survival of stress conditions | mini-ferritin |
| GsiB | -3.74 | response to water deficits | general stress protein |
| IolW | -1.40 | utilization of scyllo-inosose | scyllo-inositol dehydrogenase |
| OhrB | -3.12 | organic peroxide resistance | unknown |
| RsbV | -1.24 | control of SigB activity | anti-anti-SigB |
| RsbW | -1.07 | control of SigB activity | anti-sigma factor, protein serine kinase |
| SkfB | -1.35 | maturation of spore killing factor | AdoMet radical sactisynthase |
| SkfC | -1.46 | may be involved in spore killing | unknown |
| SkfE | -1.63 | export of the spore killing factor SkfA | ABC transporter (binding protein) |
| YtxG | -1.14 | unknown | unknown |
| YtxH | -1.13 | unknown | unknown |
| YvyD | -1.71 | dimerization of ribosomes in the stationary phase, protection against paraquat stress | ribosome hibernation promoting factor |
| YxxD | -1.05 | inhibition of the cytotoxic activity of YxiD | antitoxin |

Table S5. Differentially expressed proteins associated with amino/nitrogen metabolism in *kinA* mutant spores of *B. subtilis* without induction

| protein | Log ₂ (M-/WT-) | function | product |
|-------------|---------------------------|--------------------------------------|--------------------------------|
| AroB | 1.04 | biosynthesis of aromatic amino acids | 3-dehydroquinate synthase |
| AroF | 1.55 | biosynthesis of aromatic amino acids | chorismate synthase |
| SerA | 1.35 | biosynthesis of serine | phosphoglycerate dehydrogenase |

| | | | |
|--------------|-------|---|--|
| ArgC | -1.02 | biosynthesis of arginine | N-acetyl-g-glutamyl-phosphate reductase |
| ARTP | -1.32 | arginine uptake | high affinity arginine ABC transporter (ATP-binding protein) |
| BkdAB | -1.01 | utilization of branched-chain keto acids | 2-oxoisovalerate dehydrogenase (E1 beta subunit) |
| BkdB | -1.04 | utilization of branched-chain keto acids | 2-oxoisovalerate dehydrogenase (E2 subunit, lipoamide acyltransferase) |
| Buk | -1.05 | utilization of branched-chain keto acids | butyrate kinase |
| oppA | -1.45 | initiation of sporulation, competence development | oligopeptide ABC transporter (binding protein) |
| OPPD | -1.57 | initiation of sporulation, competence development | oligopeptide ABC transporter (ATP-binding protein) |
| oppF | -1.40 | initiation of sporulation, competence development | oligopeptide ABC transporter (ATP-binding protein) |
| NagP | -1.21 | N-acetylglucosamine uptake and phosphorylation | N-acetylglucosamine-specific PTS, EIICB |
| PutC | -2.14 | proline utilization | 1-pyrroline-5-carboxylate dehydrogenase |
| PUTP | -2.23 | proline uptake | high affinity proline permease |