# Identification of potential mitochondrial CLPXP protease interactors and substrates suggest its central role in energy metabolism

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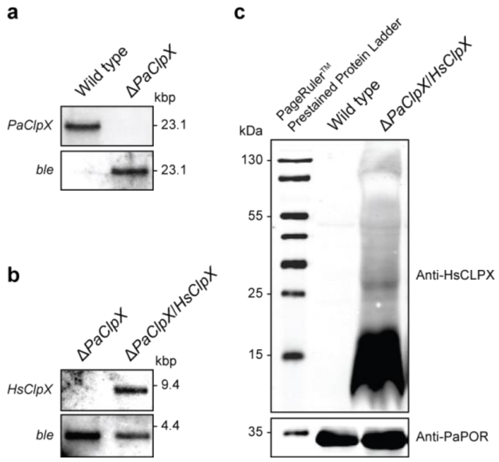
## SUPPLEMENTARY INFORMATION

Supplementary Figures 1 to 2

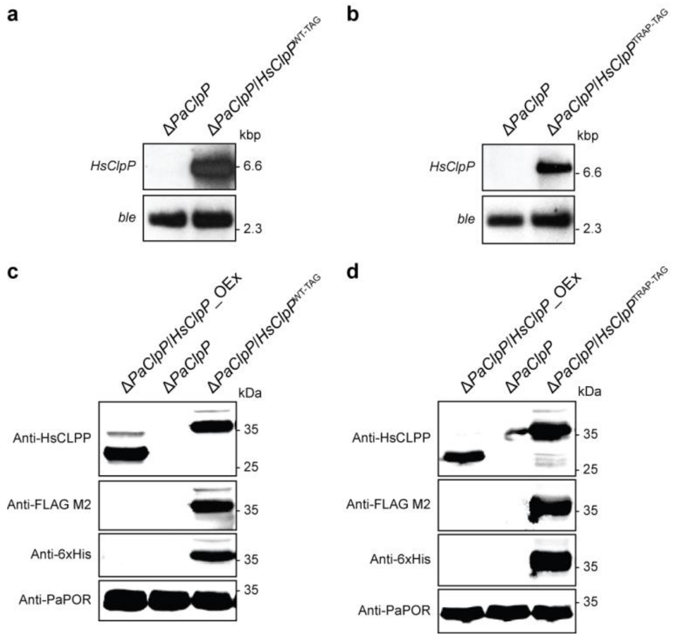
Supplementary Tables 1 to 5

## SUPPLEMENTARY FIGURES



**Supplementary Figure 1 | Human CLPX is not stable in *Podospora anserina* mitochondria.**

**a**, Southern blot analysis of MunI-digested genomic DNA (gDNA) from a *P. anserina* wild-type and *PaClpX* deletion strain (Δ*PaClpX*). The *PaClpX*-specific probe detects the 17,715 bp fragment containing *PaClpX* only in wild-type gDNA. A 17,767 bp fragment containing the phleomycin resistance gene (*ble*) is detected by a *ble*-specific hybridization probe only in Δ*PaClpX* gDNA. **b**, Southern blot analysis of HindIII-digested gDNA with an *HsClpX*-specific hybridization probe verifying the *PaClpX* deletion strain containing an expression construct coding for human CLPX (Δ*PaClpX*/*HsClpX*). **c**, Western blot analysis of mitochondrial protein extracts from the indicated strains using an HsCLPX-specific antibody. Mature HsCLPX has a predicted mass of ~63 kDa. No corresponding signal is detectable in mitochondrial protein extracts from Δ*PaClpX*/*HsClpX*. PaPORIN was detected as a loading control.



**Supplementary Figure 2 | Verification of *PaClpP* deletion strains with human CLPP variants for use in a CLPP substrate-trapping assay.**

**a**, Southern blot analysis of HindIII-digested gDNA with a *HsClpP*-specific hybridization probe verifying presence of an expression construct coding for C-terminally 3xFLAG-6xHis-tagged wild-type human CLPP in Δ*PaClpP*/*HsClpP*$^{\text{WT-TAG}}$. *ble*, phleomycin resistance gene. **b**, Verification of Δ*PaClpP*/*HsClpP*$^{\text{TRAP-TAG}}$ by Southern blot analysis as in a. **c**, Confirmation of the presence of affinity-tagged wild-type human CLPP in Δ*PaClpP*/*HsClpP*$^{\text{WT-TAG}}$ mitochondria by western blot analysis of mitochondrial protein extracts from the indicated strains using different antibodies. Mitochondrial protein extract from a strain expressing untagged wild-type human CLPP (Δ*PaClpP*/*HsClpP*_OEx) was used for comparison. PaPORIN was detected as a loading control. **d**, Confirmation of the presence of affinity-tagged inactive human CLPP in Δ*PaClpP*/*HsClpP*$^{\text{TRAP-TAG}}$ mitochondria by western blot analysis as in c.

# SUPPLEMENTARY TABLES

**Supplementary Table 1 | All proteins identified in the background control sample (Δ*PaClpP*) as well as in the samples containing HsCLPP$^{\text{WT-TAG}}$ or HsCLPP$^{\text{TRAP-TAG}}$.**

| *P. anserina* ID* | Protein | Avg. Number of Unique Peptides Δ*PaClpP* | Avg. Number of Unique Peptides HsCLPP$^{\text{WT-TAG}}$ | Avg. Number of Unique Peptides HsCLPP$^{\text{TRAP-TAG}}$ |
|---|---|---|---|---|
| Pa_6_2570 | Putative Mitochondrial of precursor Heat shock protein SSC1 | 2.3 | **27.0 (x 11.6)** | **28.3 (x 12.1)** |
| Pa_3_10800 | Putative Sulfide:quinone oxidoreductase, mitochondrial precursor | 14.0 | 16.0 | 18.7 |
| Pa_1_8390 | Putative mitochondrial ATP synthase subunit alpha precursor | 13.0 | 15.7 | 12.7 |
| Pa_6_5610 | Putative Mitochondrial precursor of ATP synthase subunit beta | 8.7 | 13.3 | 14.7 |
| Pa_3_10150 | Putative ADP,ATP carrier protein | 9.0 | 10.0 | 9.3 |
| Pa_3_4870 | Putative NADH-ubiquinone oxidoreductase 78 kDa subunit mitochondrial precursor | 2.7 | 3.7 | **21.0 (x 7.9)** |
| Pa_3_10790 | Putative Beta-lactamase hydrolase-like protein | 1.3 | **11.3 (x 8.5)** | **13.0 (x 9.8)** |
| Pa_2_9780 | Putative outer mitochondrial membrane protein porin | 7.7 | 9.0 | 8.3 |
| Pa_1_5850 | Putative mitochondrial isocitrate dehydrogenase [NAD] subunit 1 precursor | 6.7 | 9.0 | 8.3 |
| Pa_2_12010 | Putative Elongation factor Tu, mitochondrial precursor | 1.7 | **8.7 (x 5.2)** | **10.0 (x 6.0)** |
| Pa_1_9290 | Putative mitochondrial succinate dehydrogenase [ubiquinone] flavoprotein subunit precursor | 5.7 | 7.7 | 6.7 |
| Pa_6_8690 | Putative protein of unknown function | 7.3 | 8.7 | 3.7 |
| Pa_6_5140 | Putative Mitochondrial precursor of phosphate carrier protein | 6.0 | 6.7 | 6.0 |
| Pa_6_2730 | Putative Isocitrate dehydrogenase [NAD] subunit 2 | 0.7 | **7.3 (x 11.0)** | **6.3 (x 9.5)** |
| Pa_6_4730 | Putative Mitochondrial precursor of Cytochrome b-c1 complex subunit 2 | 4.0 | 6.0 | 4.0 |
| Pa_1_17000 | Putative Woronin body major protein precursor HEX1 | 3.0 | 4.7 | 4.3 |
| Pa_1_13390 | Putative lipoamide acyltransferase component of branched-chain alpha-keto acid dehydrogenase complex | 3.0 | 3.3 | 4.3 |
| Pa_3_6450 | Putative mitochondrial acetolactate synthase small subunit precursor | 1.7 | 5.0 | 3.7 |
| Pa_6_3510 | Putative ATP-dependent RNA helicase mss116 | 2.0 | 4.0 | 4.3 |
| Pa_1_17630 | Putative mitochondrial ATP synthase subunit 4 precursor | 2.0 | 4.0 | 3.0 |
| Pa_2_11540 | Putative Cytochrome c oxidase polypeptide VI, mitochondrial precursor | 2.7 | 2.7 | 3.3 |
| Pa_3_3200 | Putative Acetolactate synthase catalytic subunit, mitochondrial precursor | 3.0 | 2.0 | 3.7 |
| Pa_3_8090 | Putative mitochondrial ATP synthase subunit 5 precursor | 2.7 | 3.0 | 2.7 |
| Pa_1_7770 | Putative protein of unknown function | 2.3 | 3.0 | 2.3 |
| Pa_7_570 | Putative cytochrome c oxidase polypeptide IV | 2.3 | 2.3 | 3.0 |
| Pa_1_9450 | Putative mitochondrial ATP synthase gamma chain precursor | 2.0 | 2.7 | 2.3 |
| Pa_2_1420 | Putative mitochondrial LSU ribosomal protein L17 precursor | 3.3 | 1.7 | 1.7 |
| Pa_2_4980 | Putative 3-hydroxybutyryl-CoA dehydrogenase | 0.7 | 2.0 | **3.3 (x 5.0)** |
| Pa_1_19590 | Putative protein of unknown function | 1.3 | 2.3 | 2.3 |
| Pa_6_5480 | Cytochrome c oxidase polypeptide 5 encoded by the cox5 gene | 1.7 | 1.3 | 1.7 |
| Pa_3_11310 | Putative mitochondrial LSU ribosomal protein L24 precursor | 2.0 | 2.7 | 0.0 |
| Pa_1_6980 | Putative prohibitin-2 | 1.3 | 1.7 | 1.3 |
| Pa_3_9080 | Putative mitochondrial LSU ribosomal protein L16 precursor | 1.3 | 1.3 | 1.7 |
| Pa_1_6520 | Putative mitochondrial-processing peptidase subunit beta precursor | 2.0 | 1.3 | 0.7 |
| Pa_1_12550 | Cytochrome c& encoded by the cyc1 gene | 1.0 | 1.3 | 1.3 |
| Pa_1_19720 | Cytosolic translation elongation factor 1 subunit A (eEF1A) encoded by the AS4 gene | 1.0 | 1.3 | 1.3 |
| Pa_3_6870 | Putative protein of unknown function | 1.0 | 1.3 | 1.3 |
| Pa_2_12340 | Putative protein of unknown function | 1.0 | 1.3 | 1.0 |
| Pa_7_8595 | Putative protein of unknown function | 1.3 | 1.0 | 1.0 |
| Pa_mito_cox2 | Cytochrome c oxidase subunit 2 | 1.0 | 1.0 | 1.3 |
| Pa_1_10210 | Putative protein of unknown function | 1.0 | 1.0 | 1.0 |
| Pa_1_4450 | Putative protein of unknown function | 1.0 | 1.0 | 1.0 |
| Pa_3_7390 | Putative Kinesin-like protein | 1.0 | 1.0 | 1.0 |
| Pa_5_5380 | Putative protein of unknown function | 1.0 | 1.0 | 1.0 |
| Pa_5_9300 | Putative protein of unknown function | 1.0 | 1.0 | 1.0 |
| Pa_4_5910 | Putative Stomatin-like protein | 1.3 | 1.0 | 0.7 |
| Pa_7_2170 | Putative mitochondrial ATP synthase delta chain precursor | 0.7 | 1.0 | 1.3 |
| Pa_3_6770 | Putative mitochondrial DNA replication protein YHM2 | 0.7 | 1.0 | 1.0 |
| Pa_3_4100 | Putative NADH-ubiquinone oxidoreductase 29.9 kDa subunit mitochondrial precursor | 1.0 | 1.0 | 0.0 |
| Pa_2_11200 | Putative actin | 1.0 | 0.0 | 0.7 |
| Pa_4_7790 | Putative Ubiquitin | 0.7 | 0.7 | 0.0 |
| Pa_5_6010 | Putative NADH-ubiquinone oxidoreductase 40 kDa subunit | 1.3 | 0.0 | 0.0 |
| Pa_1_13340 | mtHMG1 mitochondrial protein encoded by the mthmg1 gene | 1.0 | 0.0 | 0.0 |

**P. anserina* IDs correspond to the '*P. anserina* Genome Project' database release version 6.32 (downloaded from http://podospora.igmors.u-psud.fr/)

For each protein, the *P. anserina* ID, protein name (based on the annotation from the '*P. anserina* Genome Project' database) and average numbers of unique peptides identified by MS analysis across all biological replicates of the respective sample are listed. Proteins that were highly enriched in the samples containing HsCLPP$^{\text{WT-TAG}}$ or HsCLPP$^{\text{TRAP-TAG}}$ over the background control sample (using spectral counting, enrichment factor > 3) are in bold (enrichment factor in parentheses).

**Supplementary Table 2 | Human homologues of proteins co-purifying with HsCLPP$^{\text{WT-TAG}}$ and HsCLPP$^{\text{TRAP-TAG}}$ in *P. anserina*.**

| *P. anserina* ID* | Avg. Number of Unique Peptides HsCLPP$^{\text{WT-TAG}}$ | Avg. Number of Unique Peptides HsCLPP$^{\text{TRAP-TAG}}$ | % Identity/% Positives *H. sapiens* homologue | E-Value | Swiss-Prot ID | Gene |
|---|---|---|---|---|---|---|
| **Chaperones and Protein Import** | | | | | | |
| Pa_6_2570 | 27.0 | 28.3 | 66/81 | 0.0 | P38646 | HSPA9 |
| Pa_6_5750 | 16.7 | 13.7 | 57/75 | 0.0 | P10809 | HSPD1 |
| Pa_2_9700 | 14.3 | 15.3 | 29/47 | 2.8E-71 | O94826 | TOMM70A |
| Pa_2_10580 | 9.0 | 9.3 | 30/48 | 1.5E-25 | O96008 | TOMM40 |
| Pa_6_1920 | 3.0 | 2.7 | 25/42 | 1.9E-7 | Q15388 | TOMM20 |
| Pa_2_12760 | 1.7 | 1.3 | 57/76 | 3.8E-102 | P35232 | PHB |
| **Metabolism** | | | | | | |
| Pa_1_22300 | 12.0 | 15.3 | 52/66 | 0.0 | P23378 | GLDC |
| Pa_3_10790 | 11.3 | 13.0 | 29/44 | 6.6E-19 | O95571 | ETHE1 |
| Pa_5_5970 | 10.0 | 9.0 | 68/81 | 0.0 | Q99798 | ACO2 |
| Pa_3_11290 | 8.7 | 9.3 | 63/77 | 1.1E-175 | P00505 | GOT2 |
| Pa_6_1590 | 8.0 | 7.0 | 53/72 | 1.9E-136 | P24752 | ACAT1 |
| Pa_3_6780 | 7.3 | 14.0 | 65/81 | 0.0 | O75390 | CS |
| Pa_6_2730 | 7.3 | 6.3 | 62/76 | 3.4E-137 | P50213 | IDH3A |
| Pa_3_2310 | 6.7 | 12.0 | 47/63 | 1.8E-121 | P10515 | DLAT |
| Pa_5_11920 | 5.7 | 5.0 | 35/52 | 1.7E-47 | Q13825 | AUH |
| Pa_2_1050 | 5.7 | 4.3 | 90/96 | 0.0 | P78827† | ilv-2 |
| Pa_6_10000 | 5.0 | 3.7 | 90/95 | 0.0 | C7C436† | mcsA |
| Pa_1_13140 | 4.7 | 6.3 | 53/72 | 0.0 | P31327 | CPS1 |
| Pa_3_7700 | 4.3 | 7.7 | 59/76 | 6.6E-172 | Q9P2R7 | SUCLA2 |
| Pa_1_3450 | 4.0 | 6.0 | 32/49 | 1.7E-28 | Q92506 | HSD17B8 |
| Pa_1_17280 | 3.3 | 5.0 | 58/73 | 0.0 | Q12428† | PDH1 |
| Pa_1_14630 | 3.0 | 6.3 | 25/45 | 4.1E-25 | Q8N159 | NAGS |
| Pa_4_7010 | 3.0 | 3.7 | 31/51 | 1.9E-30 | Q16698 | DECR1 |
| Pa_3_10910 | 3.0 | 3.3 | 86/92 | 0.0 | P15937† | acu-8 |
| Pa_4_8600 | 3.0 | 3.0 | 44/61 | 4.4E-91 | O15382 | BCAT2 |
| Pa_2_6200 | 2.3 | 5.0 | 38/60 | 4.2E-25 | P23434 | GCSH |
| Pa_6_8420 | 2.3 | 4.0 | 52/72 | 4.0E-137 | P45954 | ACADSB |
| Pa_2_430 | 2.3 | 3.3 | 71/86 | 0.0 | Q9Y697 | NFS1 |
| Pa_3_2600 | 2.0 | 5.3 | 57/71 | 9.9E-117 | P40926 | MDH2 |
| Pa_1_15690 | 2.0 | 3.7 | 54/65 | 0.0 | Q10341† | cys2 |
| Pa_2_4980 | 2.0 | 3.3 | 39/51 | 2.3E-54 | Q16836 | HADH |
| Pa_3_1420 | 2.0 | 2.0 | 34/51 | 2.4E-34 | Q9UHQ9 | CYB5R1 |
| Pa_3_9430 | 1.7 | 3.0 | 31/48 | 1.8E-56 | P04181 | OAT |
| Pa_4_3040 | 1.7 | 1.7 | 63/75 | 0.0 | P48735 | IDH2 |
| Pa_1_7660 | 1.7 | 1.3 | 58/72 | 0.0 | Q02252 | ALDH6A1 |
| Pa_7_10210 | 1.3 | 3.0 | 54/72 | 3.7E-87 | P30084 | ECHS1 |
| Pa_1_1980 | 1.3 | 1.7 | 55/70 | 0.0 | P51649 | ALDH5A1 |
| Pa_4_660 | 1.3 | 1.3 | 57/74 | 0.0 | P34897 | SHMT2 |
| **Electron Transport Chain and Respiration** | | | | | | |
| Pa_1_14370 | 2.7 | 2.3 | 26/50 | 2.1E-11 | O75947 | ATP5H |
| Pa_4_7160 | 2.0 | 4.7 | 57/71 | 1.3E-75 | O75489 | NDUFS3 |
| Pa_1_8620 | 2.0 | 1.7 | 72/86 | 0.0 | O75306 | NDUFS2 |
| Pa_5_7500 | 1.3 | 1.7 | 42/65 | 5.5E-17 | O14561 | NDUFAB1 |
| Pa_6_240 | 1.3 | 1.3 | 56/73 | 4.2E-74 | P47985 | UQCRFS1 |
| **Other Pathways** | | | | | | |
| Pa_2_12010 | 8.7 | 10.0 | 55/73 | 3.2E-160 | P49411 | TUFM |
| Pa_4_1130 | 2.3 | 2.3 | nhd | - | - | Pa_4_1130 |
| Pa_5_8240 | 2.0 | 2.7 | 47/59 | 2.1E-38 | P30044 | PRDX5 |
| Pa_6_8740 | 2.0 | 2.3 | 41/55 | 2.9E-15 | P10599 | TXN |

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†If no human homologue was determinable, a homologue from a fungal species was selected for reference

For each protein, the *P. anserina* ID, average number of unique peptides identified by MS analysis across all biological replicates of the respective sample, % of identical and positive amino acids compared with the human homologue's matched region, corresponding BLAST search E-value as well as Swiss-Prot ID and gene name of the human homologue are listed. Categories (e.g. 'Metabolism') were assigned based on annotations from the Swiss-Prot database and the general literature. nhd, no homologue determinable

**Supplementary Table 3 | Human homologues of potential CLPXP substrates in *P. anserina*.**

| *P. anserina* ID* | Avg. Number of Unique Peptides | % Identity/% Positives *H. sapiens* homologue | E-Value | Swiss-Prot ID | Gene |
|---|---|---|---|---|---|
| **Chaperones** | | | | | |
| Pa_6_5510 | 7.3 | 38/54 | 5.0E-45 | Q8NBU5 | ATAD1 |
| Pa_6_5590 | 3.7 | 36/52 | 2.5E-76 | O76031 | CLPX |
| **Metabolism** | | | | | |
| Pa_6_5560 | 15.7 | 46/64 | 0.0 | Q02218 | OGDH |
| Pa_6_1640 | 7.7 | 42/63 | 5.8E-93 | P31327 | CPS1 |
| Pa_7_9520 | 7.3 | 32/46 | 1.3E-29 | O00330 | PDHX |
| Pa_5_5370 | 5.0 | 54/67 | 3.6E-126 | P36957 | DLST |
| Pa_7_10050 | 4.0 | 54/70 | 4.7E-123 | P08559 | PDHA1 |
| Pa_1_13750 | 2.7 | 46/61 | 1.6E-94 | P48728 | AMT |
| Pa_5_5810 | 2.3 | 58/73 | 0.0 | P09622 | DLD |
| Pa_1_15800 | 2.0 | 63/80 | 1.0E-141 | P11177 | PDHB |
| Pa_1_20100 | 1.3 | 60/72 | 7E-144 | P26440 | IVD |
| Pa_3_9520 | 1.3 | 45/61 | 1.6E-88 | P35914 | HMGCL |
| **Electron Transport Chain and Respiration** | | | | | |
| Pa_3_4870 | 21.0 | 64/69 | 0.0 | P28331 | NDUFS1 |
| Pa_4_7950 | 3.7 | 75/86 | 0.0 | P49821 | NDUFV1 |
| Pa_5_9670 | 3.3 | 41/57 | 2.9E-91 | Q5T2R2 | PDSS1 |
| **Other Pathways** | | | | | |
| Pa_3_11170 | 10.7 | 61/78 | 5.4E-142 | O59778† | bio2 |
| Pa_1_18430 | 2.7 | 30/45 | 3.0E-19 | P22626 | HNRNPA2B1 |
| Pa_2_10680 | 2.0 | 52/74 | 5.9E-37 | Q86SX6 | GLRX5 |
| Pa_1_6330 | 1.7 | 54/72 | 0.0 | Q96RP9 | GFM1 |
| Pa_5_2590 | 1.7 | 46/69 | 2.7E-5 | G2TRP3† | ymr31 |

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†If no human homologue was determinable, a homologue from a fungal species was selected for reference

For each protein, the *P. anserina* ID, average number of unique peptides identified by MS analysis across all biological replicates, % of identical and positive amino acids compared with the human homologue's matched region, corresponding BLAST search E-value as well as Swiss-Prot ID and gene name of the human homologue are listed. Categories (e.g. 'Metabolism') were assigned based on annotations from the Swiss-Prot database and the general literature.

**Supplementary Table 4 | Full GO enrichment analysis (biological process) of human homologues of proteins co-purifying with HsCLPP$^{\text{WT-TAG}}$ and HsCLPP$^{\text{TRAP-TAG}}$ in *P. anserina*.**

| GO Term | Count | Expected | Fold Enrichment | *P*-value |
|---|---|---|---|---|
| carboxylic acid metabolic process | 27 | 1.7 | > 5 | 4.4E-24 |
| small molecule metabolic process | 34 | 4.4 | > 5 | 6.1E-23 |
| oxoacid metabolic process | 27 | 1.9 | > 5 | 1.3E-22 |
| organic acid metabolic process | 27 | 1.9 | > 5 | 2.2E-22 |
| oxidation-reduction process | 25 | 2.0 | > 5 | 4.1E-19 |
| single-organism metabolic process | 37 | 8.5 | 4.4 | 1.6E-17 |
| cellular respiration | 13 | 0.3 | > 5 | 9.7E-14 |
| organic acid catabolic process | 13 | 0.4 | > 5 | 4.8E-13 |
| carboxylic acid catabolic process | 13 | 0.4 | > 5 | 4.8E-13 |
| cellular amino acid metabolic process | 15 | 0.7 | > 5 | 7.7E-13 |
| organonitrogen compound metabolic process | 22 | 2.7 | > 5 | 6.7E-12 |
| energy derivation by oxidation of organic compounds | 14 | 0.7 | > 5 | 1.2E-11 |
| small molecule catabolic process | 13 | 0.5 | > 5 | 1.7E-11 |
| cellular metabolic process | 40 | 16.6 | 2.4 | 3.8E-11 |
| primary metabolic process | 40 | 16.8 | 2.4 | 6.0E-11 |
| **cellular amino acid catabolic process** | 10 | 0.2 | > 5 | 1.4E-10 |
| **generation of precursor metabolites and energy** | 14 | 0.8 | > 5 | 1.6E-10 |
| organic substance metabolic process | 40 | 17.4 | 2.3 | 2.6E-10 |
| alpha-amino acid metabolic process | 12 | 0.5 | > 5 | 3.6E-10 |
| metabolic process | 41 | 19.6 | 2.1 | 5.4E-10 |
| **tricarboxylic acid cycle** | 7 | 0.1 | > 5 | 1.7E-09 |
| citrate metabolic process | 7 | 0.1 | > 5 | 4.1E-09 |
| tricarboxylic acid metabolic process | 7 | 0.1 | > 5 | 9.1E-09 |
| aerobic respiration | 7 | 0.1 | > 5 | 1.1E-07 |
| single-organism catabolic process | 15 | 1.6 | > 5 | 1.2E-07 |
| small molecule biosynthetic process | 11 | 0.8 | > 5 | 1.8E-06 |
| branched-chain amino acid catabolic process | 5 | 0.0 | > 5 | 3.5E-06 |
| organonitrogen compound catabolic process | 10 | 0.6 | > 5 | 3.9E-06 |
| branched-chain amino acid metabolic process | 5 | 0.1 | > 5 | 9.1E-06 |
| single-organism process | 41 | 24.9 | 1.7 | 9.9E-06 |
| organic acid biosynthetic process | 9 | 0.5 | > 5 | 2.1E-05 |
| carboxylic acid biosynthetic process | 9 | 0.5 | > 5 | 2.1E-05 |
| single-organism cellular process | 39 | 22.3 | 1.8 | 6.1E-05 |
| cellular amino acid biosynthetic process | 6 | 0.2 | > 5 | 1.5E-04 |
| cellular catabolic process | 15 | 2.7 | > 5 | 1.9E-04 |
| alpha-amino acid catabolic process | 6 | 0.2 | > 5 | 2.1E-04 |
| dicarboxylic acid metabolic process | 6 | 0.2 | > 5 | 2.2E-04 |
| monocarboxylic acid metabolic process | 10 | 1.0 | > 5 | 2.7E-04 |
| single-organism biosynthetic process | 14 | 2.6 | > 5 | 8.4E-04 |
| **respiratory electron transport chain** | 6 | 0.2 | > 5 | 1.3E-03 |
| electron transport chain | 6 | 0.3 | > 5 | 1.4E-03 |
| glutamine family amino acid metabolic process | 5 | 0.1 | > 5 | 1.7E-03 |
| catabolic process | 15 | 3.3 | 4.5 | 2.7E-03 |
| alpha-amino acid biosynthetic process | 5 | 0.2 | > 5 | 3.1E-03 |
| organic substance catabolic process | 14 | 2.9 | 4.9 | 3.1E-03 |
| monocarboxylic acid catabolic process | 5 | 0.2 | > 5 | 5.4E-03 |
| nucleobase-containing small molecule metabolic process | 9 | 1.1 | > 5 | 6.7E-03 |
| serine family amino acid metabolic process | 4 | 0.1 | > 5 | 9.9E-03 |
| nitrogen compound metabolic process | 25 | 10.4 | 2.4 | 1.1E-02 |
| **fatty acid metabolic process** | 7 | 0.6 | > 5 | 1.2E-02 |
| cellular process | 40 | 27.7 | 1.4 | 1.7E-02 |
| organonitrogen compound biosynthetic process | 9 | 1.2 | > 5 | 1.7E-02 |
| protein targeting to mitochondrion | 4 | 0.1 | > 5 | 3.7E-02 |
| establishment of protein localization to mitochondrion | 4 | 0.1 | > 5 | 4.6E-02 |

Gene ontology (GO) enrichment analysis was performed online (http://amigo.geneontology.org/rte). Input were the Swiss-Prot IDs of all 41 human homologues of proteins co-purifying with HsCLPP$^{\text{TRAP-TAG}}$ and HsCLPP$^{\text{WT-TAG}}$ in *P. anserina* (see Supplementary Table 1). Only results with a *P*-value < 5.0E-02 are displayed. Selected representative or descriptive GO terms included in Fig. 3a are in bold.

Analysis Type: PANTHER Overrepresentation Test (release 20150430) | Annotation Version and Release Date: GO Ontology database; Released 2015-05-09 | Reference List: *Homo sapiens* (all genes in database) | Annotation Data Set: GO biological process complete | Bonferroni correction: true

**Supplementary Table 5 | Full GO enrichment analysis (biological process) of human homologues of potential CLPXP substrates in *P. anserina*.**

| GO Term | Count | Expected | Fold Enrichment | *P*-value |
|---|---|---|---|---|
| **tricarboxylic acid cycle** | 5 | 0.0 | > 5 | 2.4E-07 |
| **cellular respiration** | 7 | 0.1 | > 5 | 3.8E-07 |
| citrate metabolic process | 5 | 0.0 | > 5 | 4.6E-07 |
| alpha-amino acid catabolic process | 6 | 0.1 | > 5 | 6.3E-07 |
| energy derivation by oxidation of organic compounds | 8 | 0.3 | > 5 | 6.8E-07 |
| tricarboxylic acid metabolic process | 5 | 0.0 | > 5 | 8.2E-07 |
| carboxylic acid metabolic process | 10 | 0.7 | > 5 | 1.4E-06 |
| regulation of acetyl-CoA biosynthetic process from pyruvate | 4 | 0.0 | > 5 | 2.0E-06 |
| **cellular amino acid catabolic process** | 6 | 0.1 | > 5 | 2.1E-06 |
| small molecule metabolic process | 13 | 1.8 | > 5 | 2.6E-06 |
| regulation of acyl-CoA biosynthetic process | 4 | 0.0 | > 5 | 2.7E-06 |
| **generation of precursor metabolites and energy** | 8 | 0.3 | > 5 | 3.0E-06 |
| alpha-amino acid metabolic process | 7 | 0.2 | > 5 | 4.5E-06 |
| aerobic respiration | 5 | 0.0 | > 5 | 4.9E-06 |
| oxoacid metabolic process | 10 | 0.8 | > 5 | 5.0E-06 |
| organic acid metabolic process | 10 | 0.8 | > 5 | 6.0E-06 |
| oxidation-reduction process | 10 | 0.8 | > 5 | 7.1E-06 |
| regulation of sulfur metabolic process | 4 | 0.0 | > 5 | 1.2E-05 |
| acyl-CoA metabolic process | 5 | 0.1 | > 5 | 1.3E-05 |
| thioester metabolic process | 5 | 0.1 | > 5 | 1.3E-05 |
| pyruvate metabolic process | 5 | 0.1 | > 5 | 2.2E-05 |
| single-organism metabolic process | 15 | 3.5 | 4.3 | 3.8E-05 |
| cellular amino acid metabolic process | 7 | 0.3 | > 5 | 4.8E-05 |
| organic acid catabolic process | 6 | 0.2 | > 5 | 6.0E-05 |
| carboxylic acid catabolic process | 6 | 0.2 | > 5 | 6.0E-05 |
| organonitrogen compound metabolic process | 10 | 1.1 | > 5 | 1.6E-04 |
| coenzyme metabolic process | 6 | 0.2 | > 5 | 2.2E-04 |
| regulation of coenzyme metabolic process | 4 | 0.0 | > 5 | 2.9E-04 |
| regulation of cofactor metabolic process | 4 | 0.0 | > 5 | 2.9E-04 |
| small molecule catabolic process | 6 | 0.2 | > 5 | 3.2E-04 |
| lysine catabolic process | 3 | 0.0 | > 5 | 4.2E-04 |
| lysine metabolic process | 3 | 0.0 | > 5 | 4.2E-04 |
| single-organism catabolic process | 8 | 0.7 | > 5 | 6.7E-04 |
| cofactor metabolic process | 6 | 0.3 | > 5 | 8.1E-04 |
| organonitrogen compound catabolic process | 6 | 0.3 | > 5 | 1.1E-03 |
| sulfur compound metabolic process | 6 | 0.3 | > 5 | 1.3E-03 |
| aspartate family amino acid catabolic process | 3 | 0.0 | > 5 | 2.8E-03 |
| purine ribonucleoside monophosphate metabolic process | 5 | 0.2 | > 5 | 3.2E-03 |
| regulation of fatty acid metabolic process | 4 | 0.1 | > 5 | 3.3E-03 |
| purine nucleoside monophosphate metabolic process | 5 | 0.2 | > 5 | 3.3E-03 |
| acetyl-CoA metabolic process | 3 | 0.0 | > 5 | 3.9E-03 |
| branched-chain amino acid catabolic process | 3 | 0.0 | > 5 | 3.9E-03 |
| ribonucleoside monophosphate metabolic process | 5 | 0.2 | > 5 | 4.3E-03 |
| organic substance catabolic process | 9 | 1.2 | > 5 | 4.6E-03 |
| nucleoside monophosphate metabolic process | 5 | 0.2 | > 5 | 5.6E-03 |
| branched-chain amino acid metabolic process | 3 | 0.0 | > 5 | 6.9E-03 |
| **acetyl-CoA biosynthetic process from pyruvate** | 2 | 0.0 | > 5 | 9.5E-03 |
| catabolic process | 9 | 1.4 | > 5 | 1.5E-02 |
| purine ribonucleoside metabolic process | 5 | 0.2 | > 5 | 1.6E-02 |
| purine nucleoside metabolic process | 5 | 0.2 | > 5 | 1.7E-02 |
| nucleobase-containing small molecule metabolic process | 6 | 0.4 | > 5 | 2.2E-02 |
| ribonucleoside metabolic process | 5 | 0.2 | > 5 | 2.4E-02 |
| purine ribonucleotide metabolic process | 5 | 0.3 | > 5 | 2.7E-02 |
| metabolic process | 17 | 8.1 | 2.1 | 2.7E-02 |
| ribonucleotide metabolic process | 5 | 0.3 | > 5 | 3.4E-02 |
| nucleoside metabolic process | 5 | 0.3 | > 5 | 3.5E-02 |
| purine nucleotide metabolic process | 5 | 0.3 | > 5 | 3.7E-02 |
| cellular catabolic process | 8 | 1.1 | > 5 | 3.9E-02 |
| ribose phosphate metabolic process | 5 | 0.3 | > 5 | 4.0E-02 |
| glycosyl compound metabolic process | 5 | 0.3 | > 5 | 4.6E-02 |
| phosphorus metabolic process | 9 | 1.6 | > 5 | 4.7E-02 |

Gene ontology (GO) enrichment analysis was performed online (http://amigo.geneontology.org/rte). Input were the Swiss-Prot IDs of all 18 human homologues of potential CLPXP candidate substrates identified in *P. anserina* (see Supplementary Table 2). Only results with a *P*-value < 5.0E-02 are displayed. Selected representative or descriptive GO terms included in Fig. 3b are in bold.

Analysis Type: PANTHER Overrepresentation Test (release 20150430) | Annotation Version and Release Date: GO Ontology database; Released 2015-05-09 | Reference List: *Homo sapiens* (all genes in database) | Annotation Data Set: GO biological process complete | Bonferroni correction: true