Title: Domestication is associated with differential expression of pikeperch egg proteins involved in metabolism, immune response and protein folding

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Journal: Animal

**Supplementary Material S1 - Detailed methods**

Two-dimensional differential in-gel electrophoresis(2D DIGE) and image analysis

Protein labelling with CyDye DIGE fluor and 2D electrophoresis slices were performed under the same conditions as described by Nynca et al., 2015. Briefly, an aliquot of 50 μg of protein from each sample of (i) domesticated pikeperch fish (DF) eggs extract (n=6) and (ii) wild pikeperch fish (WF) eggs extract (n=6) was labelled with CyDye DIGE Fluor minimal dyes (GE Healthcare, Uppsala, Sweden) at a concentration of 400 pmol dye/50 μg of protein. Cy2 was used to label the internal standard, which was created by mixing equal amounts of protein from DF and WF eggs, and Cy3 and Cy5 to label individual egg extracts of DF and WF. A dye swap (Cy3/Cy5) was performed between DF and WF egg extract samples to exclude dye bias. An internal standard was created by mixing equal amounts of protein from the egg of domesticated (DF) and wild pikeperch fish (WF). Differentially labeled samples were mixed together as follows:

|  |  |  |  |
| --- | --- | --- | --- |
| Gel no. | Cy2 (50µg) | Cy3 (50µg) | Cy5 (50µg) |
| 1 | Pooled Standard | egg extract of DF 1 | egg extract of WF 1 |
| 2 | Pooled Standard | egg extract of DF 2 | egg extract of WF 2 |
| 3 | Pooled Standard | egg extract of DF 3 | egg extract of WF 3 |
| 4 | Pooled Standard | egg extract of WF 4 | egg extract of DF 4 |
| 5 | Pooled Standard | egg extract of WF 5 | egg extract of DF 5 |
| 6 | Pooled Standard | egg extract of WF 6 | egg extract of DF 6 |

In each gel therefore egg extract from DF (Cy3 or Cy5) and WF fish (Cy5 or Cy3) and internal standard (Cy2) were separated. The samples were loaded onto immobiline DryStrip gels strips (24 cm, pH 3 to 10 non-linear; GE, Healthcare).

Isoelectric focusing was performed with an IPGphor isoelectric focusing unit (GE Healthcare), and SDS-PAGE was run using the ETTAN Dalt six electrophoresis unit (GE Healthcare) as described by Nynca et al., 2015. The CyDye-labelled gels were analysed by post-run fluorescence imaging using the Typhoon FLA 9500 (GE Healthcare) with parameters recommended for 2D DIGE experiments by the manufacturer. The Cy2-, Cy3-, and Cy5-labeled images were at excitation and emission values of 488/520, 532/580, and 633/670 nm, respectively. Intragel spot detection and quantification and intergel matching and quantification were achieved using the differential in-gel analysis (DIA) and biological variation analysis (BVA) modules of DeCyder software (version 5.0; GE Healthcare). Differences in the abundance levels of proteins between DF and WF egg extracts were considered to be relevant if 1) the corresponding spots were detected in all gels, 2) a Student’s t-test was significant at the P ≤ 0.05 level (including a false discovery rate correction), and 3) the intensity ratio of spots showed at least a 1.3-fold increase or decrease in their relative abundance. After DIGE analysis, gels were stained with Coomassie Brilliant Blue R-250 (Bio-Rad, Hercules, CA, USA).

Matrix-assisted laser desorption/ionization time-of-flight/time-of-flight mass spectrometry (MALDI TOF/TOF) protein identification

Spots of interest were cut from the gel and prepared for identification as previously described by Nynca et al. (2015). Differentially abundant protein spots of interest were manually excised from the gels and in-gel digested with trypsin. Briefly, gel plugs were destained with 30% acetonitrile in 100mM ammonium bicarbonate for 30 min and vacuum dried. Then, digestion solution (20 ng/μl trypsin, Promega, Madison, WI, USA) in 20mM ammonium bicarbonate was added, and the samples were digested at 37 °C overnight. After digestion, peptides were extracted with 0.1% trifluoroacetic acid (TFA) and then concentrated and desalted using Zip-Tip C18 pipette tips (Millipore, Billerica, MA, USA)). Equal volumes of sample and α-cyano-4-hydroxycinnamic acid matrix (5 mg/mL) were mixed and spotted on the plate (MTP384 GrandSteel). Matrix-assisted laser desorption/ionization time-of-flight/time-of-flight (MALDI-TOF/TOF) mass spectrometry (MS) analysis was accomplished using a MALDI-TOF/TOF mass spectrometer (Autoflex Speed; Bruker Daltonics, Bremen, Germany). Collected MS and tandem mass spectrometry LIFT spectra of selected ions were externally calibrated using monoisotopic protonated ion peptide calibration standards (Bruker Daltonics) and imported to Bio-Tools (Bruker Daltonics).MS peptide mass fingerprint and fragment spectra from each individual spot were combined and used to search against the NCBI database using Mascot (version 2.4; Matrix Science Ltd, London, UK) with the following criteria: enzyme, trypsin; fixed modification, carbamidomethylation (C); and variable modifications, oxidation (M) peptide mass tolerance of 100 ppm, fragment mass tolerance of 0.9 Da, and 1 missed cleavage allowed. The search results were filtered with a significant threshold of p < 0.05 and a Mascot ion score cut-off of ≥30 for at least two peptides.

LIFT - is not an abbreviation; it is a proprietary trademarked term of Bruker Daltonics used with their TOF/TOF mass spectrometer to describe the process of elevating the potential of the collision cell above that of the ion source

Statistical analysis

All of the results of egg quality parameters (survival rate, hatching rate) are presented as the means ± SD. For statistical procedures, percentage data of the survival rate and hatching were transformed by arcsine square-root transformation. Then data were subjected to a Student’s t-test for unpaired samples. All analyses were performed at a significance level of 0.05 using GraphPad Prism software (GraphPad Software Inc. San Diego, CA, USA).

**Supplementary Material S2** – Quality parameters of eggs obtained from domesticated and wild caught pikeperch - raw data

|  |  |  |
| --- | --- | --- |
| Eggs | Survival rate (72 h post fertilization; %) | Hatching Rate (%) |
| Domesticated fish | 85 | 67 |
|  | 90 | 67 |
|  | 91 | 74 |
|  | 89 | 77 |
|  | 96 | 86 |
|  | 91 | 87 |
|  | 89 | 64 |
|  | 91 | 66 |
| Average | 90.2 | 73.5 |
| Standard deviation | 2.9 | 9.2 |
| Wild fish | 91 | 79 |
|  | 92 | 68 |
|  | 89 | 85 |
|  | 95 | 89 |
|  | 97 | 87 |
|  | 87 | 83 |
|  | 89 | 79 |
| Average | 91.4 | 81.4 |
| Standard deviation | 3.6 | 7.0 |

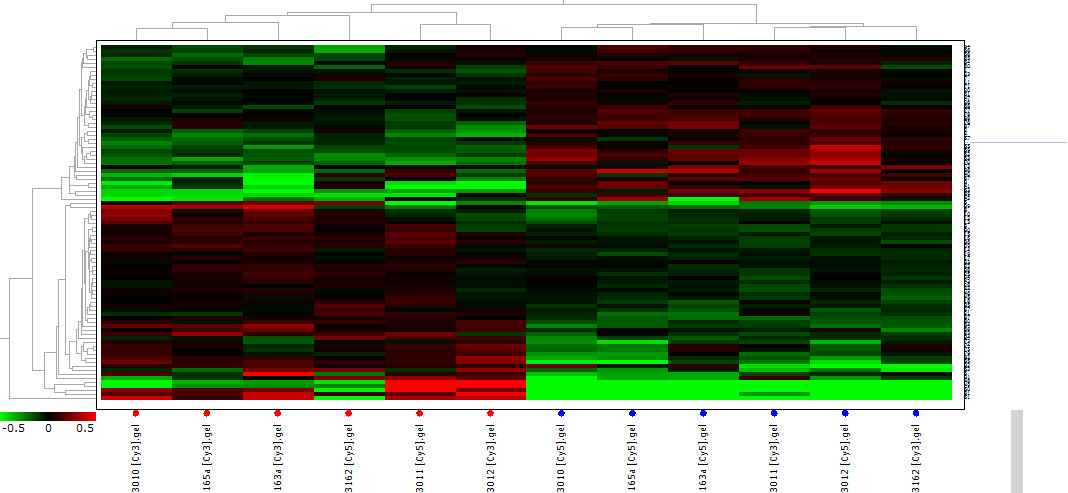
**Supplementary Table S1** Functional enrichement Biological process (species *Homo sapiens*; https://string-db.org/)

|  |  |  |
| --- | --- | --- |
| More in wild | | |
| #term ID | term description | matching proteins in your network (labels) |
| GO:0002224 | toll-like receptor signaling pathway | *COLEC12,HSP90B1,HSPD1* |
| GO:0006457 | protein folding | *HSP90B1,HSPA5,HSPA9,HSPD1,* |
| GO:0006810 | transport | *ALDOC,ATP5A1,COLEC12,HSP90B1,HSPA5,HSP, HSPD1,NME2,RPS21,SERPINB1A9,* |
| GO:0006986 | response to unfolded protein | *HSP90B1,HSPA5,HSPD1* |
| GO:0009152 | purine ribonucleotide biosynthetic process | *ALDOC,ATP5A1,NME2* |
| GO:0009206 | purine ribonucleoside triphosphate biosynthetic process | *ALDOC,ATP5A1,NME2* |
| GO:0017144 | drug metabolic process | *ALDOC,ATP5A1,IL4I1,NCAN,NQO1* |
| GO:0034975 | protein folding in endoplasmic reticulum | *HSP90B1,HSPA5* |
| GO:0036500 | ATF6-mediated unfolded protein response | *HSP90B1,HSPA5* |
| GO:0043066 | negative regulation of apoptotic process | *HSP90B1,HSPA5,HSPA9,HSPD1,NME2,NQO1* |
| GO:0009205 | purine ribonucleoside triphosphate metabolic process | *ALDOC,ATP5A1,NME2* |
| GO:1901575 | organic substance catabolic process | *ALDOC,HSP90B1,HSPA5,IL4I1,NCAN,RPS21* |
| GO:0002366 | leukocyte activation involved in immune response | *ALDOC,HSPD1,NME2,SERPINB1* |
| GO:0002376 | immune system process | *ALDOC,COLEC12,HSP90B1,HSPA9,HSPD1,NME2,SERPINB1* |
| GO:0002443 | leukocyte mediated immunity | *ALDOC,HSPD1,NME2,SERPINB1* |
| GO:0006165 | nucleoside diphosphate phosphorylation | *ALDOC,NME2* |
| GO:0030433 | ubiquitin-dependent ERAD pathway | *HSP90B1,HSPA5* |
| GO:0044248 | cellular catabolic process | *ALDOC,HSP90B1,HSPA5,IL4I1,NCAN,RPS21* |
| GO:0048519 | negative regulation of biological process | *ATP5A1,HSP90B1,HSPA5,HSPA9,HSPD1,NME2,NQO1,RPS21,SERPINB1,STMN1* |
| GO:1901137 | carbohydrate derivative biosynthetic process | *ALDOC,ATP5A1,NCAN,NME2* |
| GO:0006754 | ATP biosynthetic process | *ALDOC,ATP5A1* |
| GO:0035690 | cellular response to drug | *HSP90B1,HSPA5,NQO1* |
| GO:1990542 | mitochondrial transmembrane transport | *ATP5A1,HSPD1* |
| GO:0048523 | negative regulation of cellular process | *ATP5A1,HSP90B1,HSPA5,HSPA9,HSPD1,NME2,NQO1,SERPINB1,STMN1* |
| GO:0009168 | purine ribonucleoside monophosphate biosynthetic process | *ALDOC,ATP5A1* |
| GO:0032091 | negative regulation of protein binding | *HSPA5,STMN1* |
| GO:0034613 | cellular protein localization | *HSP90B1,HSPA5,HSPA9,HSPD1,RPS21* |
| GO:0046907 | intracellular transport | *ATP5A1,HSP90B1,HSPA9,HSPD1,RPS21* |
| GO:1901566 | organonitrogen compound biosynthetic process | *ALDOC,ATP5A1,NCAN,NME2,RPS21* |
| GO:0006886 | intracellular protein transport | *HSP90B1,HSPA9,HSPD1,RPS21* |
| GO:0071236 | cellular response to antibiotic | *HSPA5,NQO1* |
| more in domesticated | | |
| #term ID | term description | matching proteins in your network (labels) |
| GO:0009124 | nucleoside monophosphate biosynthetic process | *ATIC,ATP5B,ENO3,GAPDH,GAPDHS,PGK1,SHMT1* |
| GO:0017144 | drug metabolic process | *ALDH2,ATP5B,BHMT,CKB,ENO3,GAPDH,GAPDHS,IL4I1,MDH1,PGK1,SHMT1* |
| GO:0046364 | monosaccharide biosynthetic process | *ENO3,GAPDH,GAPDHS,MDH1,PGK1,TKT* |
| GO:0006734 | NADH metabolic process | *ENO3,GAPDH,GAPDHS,MDH1,PGK1* |
| GO:0006094 | gluconeogenesis | *ENO3,GAPDH,GAPDHS,MDH1,PGK1* |
| GO:0006458 | 'de novo' protein folding | *CCT2,CCT3,CCT6A,CCT7,HSPD1* |
| GO:0006732 | coenzyme metabolic process | *ATIC,ENO3,GAPDH,GAPDHS,MDH1,PGK1,SHMT1,TKT* |
| GO:0009168 | purine ribonucleoside monophosphate biosynthetic process | *ATIC,ATP5B,ENO3,GAPDH,GAPDHS,PGK1* |
| GO:0019752 | carboxylic acid metabolic process | *ALDH6A1,ATIC,BHMT,CKB,ENO3,GAPDH,GAPDHS,IL4I1,MDH1,PGK1,SHMT1* |
| GO:0044281 | small molecule metabolic process | *ALDH2,ALDH6A1,ATIC,ATP5B,BHMT,CKB,ENO3,GAPDH,GAPDHS,IL4I1,MDH1,PGK1, SHMT1,TKT* |
| GO:0046496 | nicotinamide nucleotide metabolic process | *ENO3,GAPDH,GAPDHS,MDH1,PGK1,TKT* |
| GO:0055086 | nucleobase-containing small molecule metabolic process | *ALDH6A1,ATIC,ATP5B,ENO3,GAPDH,GAPDHS,MDH1,PGK1,SHMT1,TKT* |
| GO:0072522 | purine-containing compound biosynthetic process | *ATIC,ATP5B,ENO3,GAPDH,GAPDHS,PGK1,SHMT1* |
| GO:1904851 | positive regulation of establishment of protein localization to telomere | *CCT2,CCT3,CCT6A,CCT7* |
| GO:0044282 | small molecule catabolic process | *ALDH2,ALDH6A1,ENO3,GAPDH,GAPDHS,IL4I1,PGK1,SHMT1* |
| GO:0044283 | small molecule biosynthetic process | *ATIC,BHMT,ENO3,GAPDH,GAPDHS,MDH1,PGK1,SHMT1,TKT* |
| GO:0009117 | nucleotide metabolic process | *ATIC,ATP5B,ENO3,GAPDH,GAPDHS,MDH1,PGK1,SHMT1,TKT* |
| GO:0061077 | chaperone-mediated protein folding | *CCT2,CCT3,CCT6A,CCT7,HSPD1* |
| GO:0009123 | nucleoside monophosphate metabolic process | *ATIC,ATP5B,ENO3,GAPDH,GAPDHS,PGK1,SHMT1* |
| GO:0006735 | NADH regeneration | *ENO3,GAPDH,GAPDHS,PGK1* |
| GO:0006754 | ATP biosynthetic process | *ATP5B,ENO3,GAPDH,GAPDHS,PGK1* |
| GO:0061621 | canonical glycolysis | *ENO3,GAPDH,GAPDHS,PGK1* |
| GO:0061718 | glucose catabolic process to pyruvate | *ENO3,GAPDH,GAPDHS,PGK1* |
| GO:0009165 | nucleotide biosynthetic process | *ATIC,ATP5B,ENO3,GAPDH,GAPDHS,PGK1,SHMT1* |
| GO:0050821 | protein stabilization | *CCT2,CCT3,CCT6A,CCT7,GAPDH,HSPD1* |
| GO:0046394 | carboxylic acid biosynthetic process | *ATIC,BHMT,ENO3,GAPDH,GAPDHS,PGK1,SHMT1* |
| GO:0009152 | purine ribonucleotide biosynthetic process | *ATIC,ATP5B,ENO3,GAPDH,GAPDHS,PGK1* |
| GO:0032212 | positive regulation of telomere maintenance via telomerase | *CCT2,CCT3,CCT6A,CCT7* |
| GO:0005996 | monosaccharide metabolic process | *ENO3,GAPDH,GAPDHS,MDH1,PGK1,TKT* |
| GO:0006757 | ATP generation from ADP | *ENO3,GAPDH,GAPDHS,PGK1* |
| GO:0042866 | pyruvate biosynthetic process | *ENO3,GAPDH,GAPDHS,PGK1* |
| GO:0006006 | glucose metabolic process | *ENO3,GAPDH,GAPDHS,MDH1,PGK1* |
| GO:0090407 | organophosphate biosynthetic process | *ATIC,ATP5B,ENO3,GAPDH,GAPDHS,PGK1,SHMT1,TKT* |
| GO:0006091 | generation of precursor metabolites and energy | *ALDH2,ATP5B,ENO3,GAPDH,GAPDHS,MDH1,PGK1* |
| GO:0009167 | purine ribonucleoside monophosphate metabolic process | *ATIC,ATP5B,ENO3,GAPDH,GAPDHS,PGK1* |
| GO:1901137 | carbohydrate derivative biosynthetic process | *ATIC,ATP5B,ENO3,GAPDH,GAPDHS,PGK1,SHMT1,TKT* |
| GO:0009435 | NAD biosynthetic process | *ENO3,GAPDH,GAPDHS,PGK1* |
| GO:0019693 | ribose phosphate metabolic process | *ATIC,ATP5B,ENO3,GAPDH,GAPDHS,PGK1,TKT* |
| GO:0005975 | carbohydrate metabolic process | *ALDH2,ENO3,GAPDH,GAPDHS,MDH1,PGK1,TKT* |
| GO:0072521 | purine-containing compound metabolic process | *ATIC,ATP5B,ENO3,GAPDH,GAPDHS,PGK1,SHMT1* |
| GO:0006090 | pyruvate metabolic process | *ENO3,GAPDH,GAPDHS,PGK1* |
| GO:0009108 | coenzyme biosynthetic process | *ATIC,ENO3,GAPDH,GAPDHS,PGK1* |
| GO:0046034 | ATP metabolic process | *ATP5B,ENO3,GAPDH,GAPDHS,PGK1* |
| GO:1901605 | alpha-amino acid metabolic process | *ALDH6A1,BHMT,CKB,IL4I1,SHMT1* |
| GO:0009166 | nucleotide catabolic process | *ENO3,GAPDH,GAPDHS,PGK1* |
| GO:0055114 | oxidation-reduction process | *ALDH2,ALDH6A1,ENO3,GAPDH,GAPDHS,IL4I1,MDH1,PGK1* |
| GO:0009150 | purine ribonucleotide metabolic process | *ATIC,ATP5B,ENO3,GAPDH,GAPDHS,PGK1* |
| GO:0007339 | binding of sperm to zona pellucida | *CCT2,CCT3,CCT7* |
| GO:0044270 | cellular nitrogen compound catabolic process | *ALDH6A1,BHMT,ENO3,GAPDH,GAPDHS,PGK1* |
| GO:1901998 | toxin transport | *CCT2,CCT3,CCT7* |
| GO:1901361 | organic cyclic compound catabolic process | *ALDH6A1,ENO3,GAPDH,GAPDHS,IL4I1,PGK1* |
| GO:0008037 | cell recognition | *CCT2,CCT3,CCT7,COLEC12* |
| GO:1901575 | organic substance catabolic process | *ALDH2,ALDH6A1,BHMT,ENO3,GAPDH,GAPDHS,IL4I1,PGK1,SHMT1* |
| GO:0006575 | cellular modified amino acid metabolic process | *ATIC,BHMT,CKB,SHMT1* |  |
| GO:0006796 | phosphate-containing compound metabolic process | *ATIC,ATP5B,CKB,ENO3,GAPDH,GAPDHS,MDH1,PGK1,SHMT1,TKT* |
| GO:0044248 | cellular catabolic process | *ALDH2,ALDH6A1,BHMT,ENO3,GAPDH,GAPDHS,IL4I1,PGK1,SHMT1* |
| GO:1904951 | positive regulation of establishment of protein localization | *CCT2,CCT3,CCT6A,CCT7,GAPDH* |
| GO:0051702 | interaction with symbiont | *GAPDH,HSPD1,PTX3* |  |  |
| GO:1901566 | organonitrogen compound biosynthetic process | *ATIC,ATP5B,BHMT,ENO3,GAPDH,GAPDHS,PGK1,SHMT1* |
| GO:0046700 | heterocycle catabolic process | *ALDH6A1,ENO3,GAPDH,GAPDHS,PGK1* |
| GO:0019439 | aromatic compound catabolic process | *ENO3,GAPDH,GAPDHS,IL4I1,PGK1* |
| GO:0051131 | chaperone-mediated protein complex assembly | *CCT2,HSPD1* |  |  |
| GO:1903827 | regulation of cellular protein localization | *ACTB,CCT2,CCT3,CCT6A,CCT7* |  |
| GO:0006577 | amino-acid betaine metabolic process | *BHMT,SHMT1* |  |  |
| GO:0043648 | dicarboxylic acid metabolic process | *ATIC,MDH1,SHMT1* |  |  |
| GO:1901606 | alpha-amino acid catabolic process | *ALDH6A1,IL4I1,SHMT1* |  |
| GO:0046653 | tetrahydrofolate metabolic process | *ATIC,SHMT1* |  |  |
| GO:1901564 | organonitrogen compound metabolic process | *ACTB,ALDH6A1,ATIC,ATP5B,BHMT,CKB,ENO3,GAPDH,GAPDHS,HSPD1,IL4I1,MDH1,PGK1,SHMT1,TKT* |
| GO:0009063 | cellular amino acid catabolic process | *ALDH6A1,IL4I1,SHMT1* |  |
| GO:0032880 | regulation of protein localization | *ACTB,CCT2,CCT3,CCT6A,CCT7,GAPDH* |
| GO:0009112 | nucleobase metabolic process | *ALDH6A1,SHMT1* |  |  |
| GO:0042398 | cellular modified amino acid biosynthetic process | *ATIC,SHMT1* |  |  |
| GO:0006909 | phagocytosis | *ACTB,COLEC12,PTX3* |  |  |
| GO:0065008 | regulation of biological quality | *ATP5B,BHMT,CCT2,CCT3,CCT6A,CCT7,CKB,GAPDH,HSPD1,PTX3,SHMT1* |
| GO:1901607 | alpha-amino acid biosynthetic process | *BHMT,SHMT1* |  |  |
| GO:0009620 | response to fungus | *GAPDH,PTX3* |  |  |
| GO:0016310 | phosphorylation | *ATP5B,CKB,ENO3,GAPDH,GAPDHS,PGK1* |
| GO:0051851 | modification by host of symbiont morphology or physiology | *GAPDH,PTX3* |  |  |
| GO:0008652 | cellular amino acid biosynthetic process | *BHMT,SHMT1* |  |  |
| GO:0002252 | immune effector process | *ACTB,CCT2,HSPD1,PTX3,TUBB4B* |
| GO:1990542 | mitochondrial transmembrane transport | *ATP5B,HSPD1* |  |  |
| GO:0002224 | toll-like receptor signaling pathway | *COLEC12,HSPD1* |  |  |
| GO:0042133 | neurotransmitter metabolic process | *BHMT,SHMT1* |  |  |
| GO:0001505 | regulation of neurotransmitter levels | *BHMT,PTX3,SHMT1* |  |  |
| GO:0006725 | cellular aromatic compound metabolic process | *ALDH6A1,ATIC,ATP5B,ENO3,GAPDH,GAPDHS,HSPD1,IL4I1,MDH1,PGK1,SHMT1,TKT* |
| GO:0072527 | pyrimidine-containing compound metabolic process | *ALDH6A1,SHMT1* |  |  |
| GO:0002366 | leukocyte activation involved in immune response | *CCT2,HSPD1,PTX3,TUBB4B* |  |
| GO:0021549 | cerebellum development | *ATIC,CKB* |  |  |  |
| GO:0002443 | leukocyte mediated immunity | *CCT2,HSPD1,PTX3,TUBB4B* |  |
| GO:0002757 | immune response-activating signal transduction | *ACTB,COLEC12,HSPD1* |  |
| GO:1901360 | organic cyclic compound metabolic process | *ALDH6A1,ATIC,ATP5B,ENO3,GAPDH,GAPDHS,HSPD1,IL4I1,MDH1,PGK1,SHMT1,TKT* |
| GO:0032879 | regulation of localization | *ACTB,ATP5B,CCT2,CCT3,CCT6A,CCT7,GAPDH,PTX3* |
| GO:0042737 | drug catabolic process | *ALDH2,IL4I1* |  |  |
| GO:0009987 | cellular process | *ACTB,ALDH2,ALDH6A1,ATIC,ATP5B,BHMT,CCT2,CCT3,CCT6A,CCT7,CKB,COLEC12,ENO3,GAPDH,GAPDHS,HSPD1,IL4I1,MDH1,PGK1,PTX3,SHMT1,TKT,TUBB4B* |
| GO:0045087 | innate immune response | *COLEC12,GAPDH,PTX3,TUBB4B* |  |

ATF 6 - Activating transcription factor 6

ERAD - endoplasmic reticulum-associated

NADH - Nicotinamide adenine dinucleotide

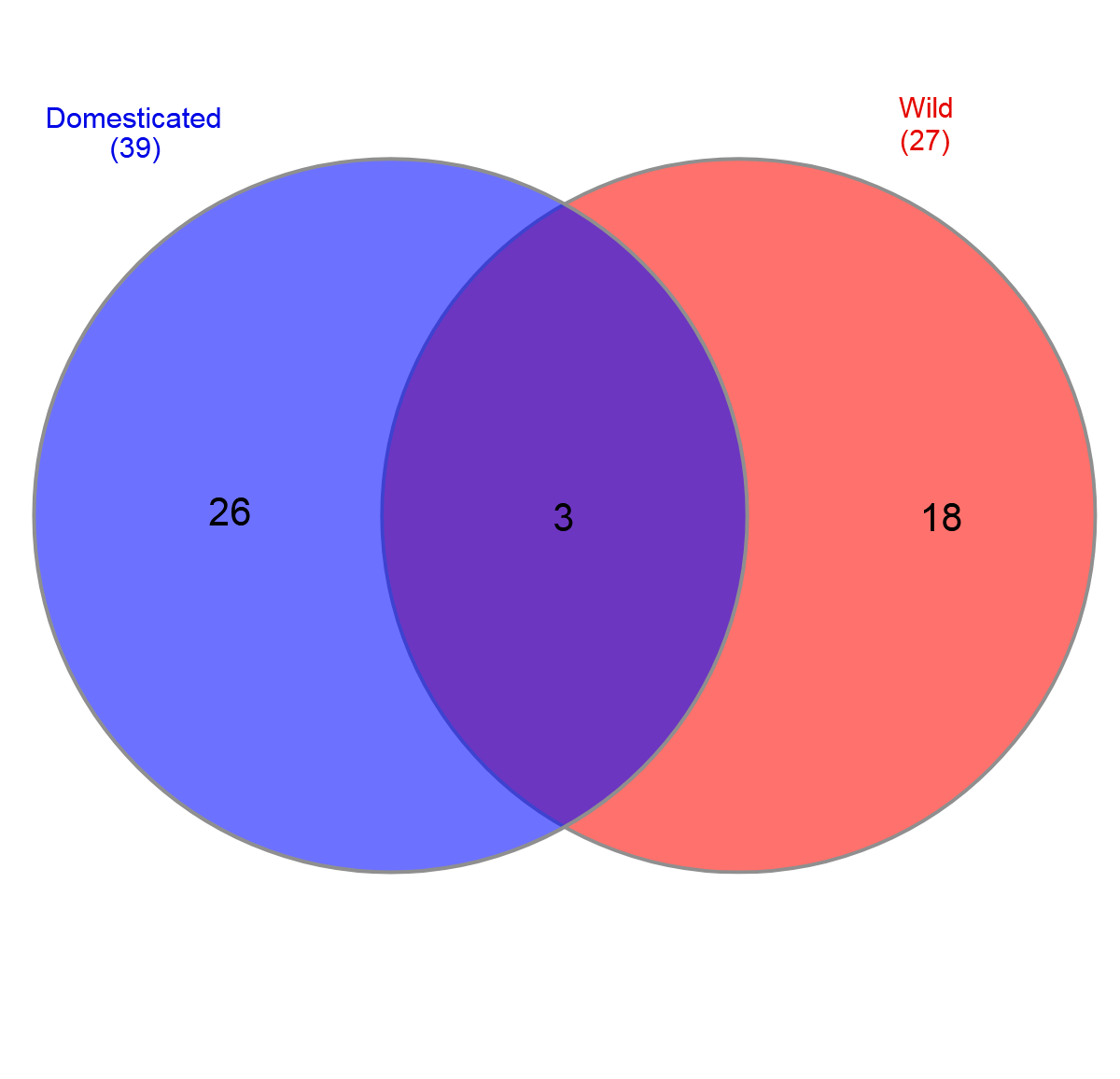


1 2 3 4 5 6 1 2 3 4 5 6

Domesticated Wild

sample

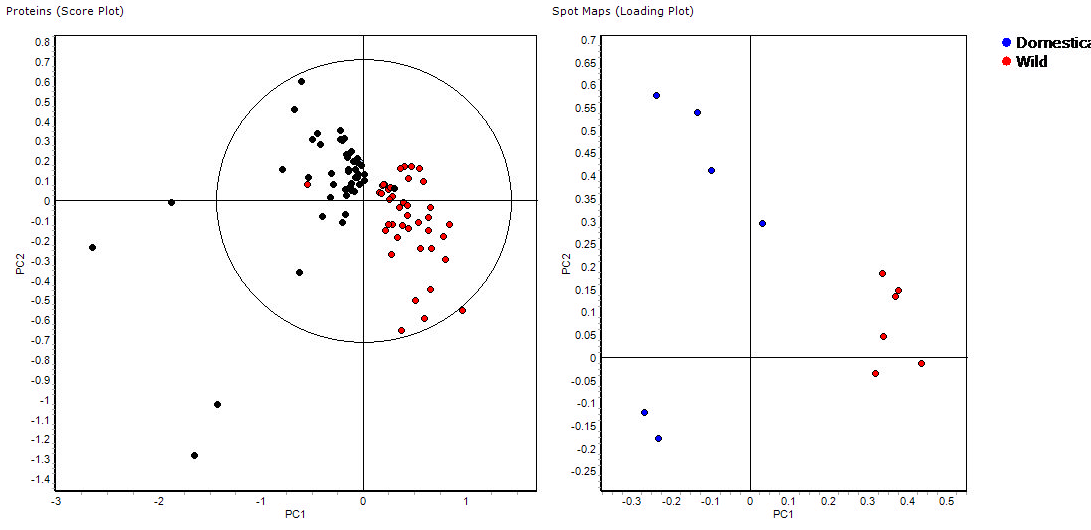
**Supplementary Figure S1** Hierarchical clustering heatmap of differentially expressed proteins in eggs of domesticated females (a) compared with wild-caught (b), as indicated under each column of clusters. Levels of protein expression are indicated on the color scale to the left down with numbers indicating the fold difference from the grand mean for all fish shown in black and indicated by the zero value). Red: increased expression levels; Green: decreased expression levels. The clustering of individual proteins with respect to their similarity in changes of expression between individual fish is represented by the dendrogram to the left. The dendrogram at the top shows similarities in protein expression patterns between individual fish regardless of group.



**Supplementary Figure S2** Venn diagram displaying the protein overlap between eggs form domesticated (blue) and wild pikeperch females (red).

B

A



**Domesticated**

**Wild**

14.2%

14.2%

62.3%

62.3%

**Supplementary Figure S3** A principal component analysis (PCA) score plot obtained for protein of wild-caught and domesticated pikeperch eggs showing alterations in protein abundance. Replicates (n=6 in each group) of different egg samples are represented as: black circles, eggs of domesticated pikeperch eggs; red circles, eggs of wild-caught pikeperch eggs. Spatial distribution of protein spots (A) and gel samples (B).