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Gingival exudate dynamics implicate inhibition of the alternative complement pathway in the protective action of the C3 inhibitor Cp40 in non-human primate periodontitis

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Abstract

Background and Aim: Periodontitis is a prevalent chronic inflammatory disease associated with dysbiosis. Although complement inhibition has been successfully used to treat periodontitis in animal models, studies globally analysing inflamed tissue proteins to glean insight into possible mechanisms of action are missing. Using quantitative shotgun proteomics, we aimed to investigate differences in composition of inflammatory gingival tissue exudate ('gingival crevicular fluid'; GCF), before and after local administration of an inhibitor of the central complement component, C3, in non-human primates.

Methods: The C3 inhibitor, Cp40 (also known as AMY-101) was administered locally in the maxillary gingival tissue of cynomolgus monkeys with established periodontitis, either once a week ('1x treatment'; n=5 animals) or three times per week ('3x-treatment'; n=10 animals), for six weeks followed by another six weeks of observation in the absence of treatment. 45 GCF samples were processed for FASP digestion and liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. Data were processed using the ProgenesisQI software. The statistical

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Conflict of Interest Disclosure

J.D.L. is the founder of Amyndas Pharmaceuticals, which is developing complement inhibitors (including third-generation compstatin analogs such as AMY-101), and is the inventor of the compstatin technology licensed to Apellis Pharmaceuticals (4(1MeW)7W/POT-4/APL-1 and PEGylated derivatives). J.D.L. and G.H. are inventors of a joint patent that describes the use of complement inhibitors for therapeutic purposes in periodontitis.

significance of differences between the groups was determined by RM-ANOVA and a protein expression change was considered as a true regulation at >2 -fold and $p < 0.05$. The human orthologues were subjected to Gene Ontology analyses using Panther. Data are available via ProteomeXchange with identifier PXD009502.

Results: 573 proteins with >2 peptides were longitudinally quantified. Both 3x and 1x administration of Cp40 resulted in significant down-regulation of dozens of proteins during the 6-week course of treatment as compared to baseline. Following drug withdrawal at 6 weeks, more than 50% of the down-regulated proteins showed increased levels at week 12. The top scored pathway was ‘complement activation, alternative pathway’ and several proteins involved in this pathway were down-regulated at 6-weeks.

Conclusion: We mapped the proteomic fingerprint changes in local tissue exudate of cynomolgus monkey periodontitis in response to C3 inhibition and identified the alternative pathway of complement activation and leukocyte degranulation as main targets, which are thus likely to play significant roles in periodontal disease pathogenesis. Label-free quantitative proteomics strategies utilizing GCF are powerful tools for the identification of treatment targets and providing insights into disease mechanisms.

Keywords

Gingival crevicular fluid; GCF; gingival inflammation; periodontitis; proteome; complement; alternative complement pathway; primates; label-free quantitation; Cp40

Introduction

Complement is an interactive network of soluble, cell surface-associated and intracellular molecules that activate, amplify, and regulate immune and inflammatory responses (1, 2). The complement system contains approximately 50 proteins; in addition to C1–9, the classic serum proteins, the system includes pattern-recognition molecules, convertases and other proteases, receptors and regulators. The complement cascade is triggered through distinct initiation mechanisms, the classical, lectin, or alternative. The classical and lectin pathways are activated following the binding of pattern-recognition molecules (*e.g.*, C1q and mannose-binding lectin, respectively) to immune complexes (classical pathway) or to carbohydrate moieties exposed on microbes or altered/damaged host cells (lectin pathway). The alternative pathway is activated by a tick-over mechanism but also amplifies the initial response induced by the classical and lectin pathways. All three initiation mechanisms converge at C3, the activation of which leads to the generation of effectors that promote pathogen phagocytosis (via C3b opsonization), induce chemotaxis and inflammation (via the anaphylatoxins C3a and C5a), and lyse susceptible microbial pathogens (via the C5b-9 membrane attack complex; MAC). Furthermore, complement cross-talks with and amplifies Toll-like receptor-mediated immune and inflammatory responses and integrates innate and adaptive immunity (2, 3). Although complement plays a crucial role in immunity and host-microbe homeostasis, when it is overactivated or dysregulated owing to host genetic or microbial virulence factors, complement can switch from a homeostatic to a pathological effector in a number of diseases, including cancer or inflammatory disorders such as periodontitis (4–7).

Periodontal disease is a prevalent chronic inflammatory disease associated with dysbiosis that leads to the destruction of the tooth-supporting tissues such as gingiva, periodontal ligament and alveolar bone (8). Early clinical studies have correlated clinical periodontal inflammation and tissue destruction with increased complement activity in the gingival crevicular fluid (GCF), the inflammatory exudate that bathes the periodontal pocket (9–11). More recently, animal model-based mechanistic studies have shown that the complement system is involved both in the dysbiotic transformation of the periodontal microbiota and in the destructive inflammatory response that drives periodontitis (12, 13).

This knowledge was exploited for the development of a possible anti-inflammatory treatment in periodontitis and recent studies reported favourable clinical changes in response to complement inhibition by compstatin Cp40 in experimentally induced or naturally-occurring periodontitis in non-human primates (14–16). Cp40, also known as AMY-101 (Amyndas Pharmaceuticals), binds exclusively human or non-human primate C3 and blocks its interaction with and cleavage by the C3 convertase into its active fragments (C3a and C3b), thereby preventing amplification of complement activation and effector generation regardless of the initiation mechanism of complement activation (2, 3). Maekawa et al showed that locally administered Cp40 can prevent experimental periodontitis or reverse pre-existing chronic periodontal inflammation by causing a significant reduction in clinical indices that measure periodontal inflammation (gingival index and bleeding on probing) or tissue destruction (probing pocket depth and clinical attachment level). This clinical improvement persisted for at least 6 weeks after drug withdrawal (albeit at reduced efficacy) and was consistent with significantly decreased numbers of osteoclasts in bone biopsies (14, 15).

Despite this progress using complement inhibitors in preclinical models as a potential treatment approach for periodontitis, studies globally analyzing inflamed tissue proteins to better understand disease mechanisms are missing. Such studies are necessary especially since complement is tightly interconnected with and regulates other components of the immune system (2). For the unbiased characterization and cataloguing of diverse proteins in biological fluids, mass-spectrometry based shotgun proteomic strategies, especially quantitative ones, have become the method of choice (17). Non-targeted quantitative proteome analysis of local tissue exudates may lead to discovery of a number of novel processes that might be regulated by complement (18, 19). In this line, the non-invasively accessible GCF contains an ideal proteome of gingival inflammation as it carries the soluble immunological content of the periodontal micro-anatomical niche (20). So far, the studies in humans demonstrated that quantitative proteomic platforms are able to facilitate characterization and concurrent quantitative analysis of proteome in periodontal health and disease (18, 19, 21, 22). However, to date, in-depth analysis of oral fluids proteome in non-human primates has not been reported. We reasoned that untargeted shotgun proteome analysis of the collected GCF samples may lead to improved understanding of the host response to treatment with Cp40 and possible discovery of a number of novel processes that might be regulated by complement. Using label-free quantitative proteomics (LFQ), the present study aimed to investigate differences in protein composition of GCF before and after local administration of Cp40 (AMY-101), a specific and potent inhibitor of the central complement component C3 in naturally-occurring periodontitis in non-human primates.

Experimental Section

GCF sampling & processing

The C3 inhibitor Cp40 (an improved analog of the original compstatin (23) was administered locally in the maxillary gingival tissue of adult systemically healthy cynomolgus monkeys (*Macaca fascicularis*) with established periodontitis, either once a week ('1x treatment'; n=5 animals) or three times per week ('3x-treatment'; n=10 animals), for six weeks followed by another six weeks of observation in the absence of treatment as described earlier (14). The study was approved by the Institutional Animal Care and Use Committees of the University of Pennsylvania and of the Simian Conservation Breeding and Research Center (SICONBREC; Makati City, Philippines) where the animal study was performed. For GCF collection, the preselected two teeth (with deepest periodontal pocket in the mouth) were isolated with cotton rolls after drying with gentle air. Sterile methylcellulose strips (Periopaper, Oraflow, New York, USA) were then gently placed in the mesio-buccal site for 60 seconds. Samples free of blood contamination were placed in Eppendorph tubes which were frozen at -80°C before use. The two samples collected from each animal were pooled into one tube.

Protein digestion and C18 clean up

GCF samples were eluted in phosphate-buffered saline (PBS), pH 7.2) supplemented with the EDTA-free Protease Inhibitor Cocktail (Sigma-Aldrich, Dorset, UK) by centrifuging at $2,000\times g$ for 15 minutes at 4°C . Cell-free supernatants were processed for measurement of total protein concentration by the Qubit fluorometer assay kit (Fisher Scientific, Wohlen, Switzerland). In the '3x-treatment' group, there was an increase in the mean total protein concentrations at 6 weeks ($348.6\text{ }\mu\text{g/ml}$ [SD 147.0]) compared to baseline ($240.7\text{ }\mu\text{g/ml}$ [SD 258.8]) or the 12-week timepoint ($262.0\text{ }\mu\text{g/ml}$ [SD 247.7]) but the differences did not reach statistical significance ($p>0.05$). In the '1x-treatment' group, the pattern was similar over time and the mean total protein concentrations were $137.5\text{ }\mu\text{g/ml}$ (SD 78.04), $174.1\text{ }\mu\text{g/ml}$ (SD 87.3) and $94.7\text{ }\mu\text{g/ml}$ (SD 29.8), for baseline, 6 weeks, and 12 weeks, respectively (no significant differences among the groups). The individual sample variations, ranged from $36.8\text{ }\mu\text{g/ml}$ to $785.8\text{ }\mu\text{g/ml}$ in the group that was treated three times per week ('3x-treatment') and from $53.4\text{ }\mu\text{g/ml}$ to $242.5\text{ }\mu\text{g/ml}$ in the group that was treated once a week ('1x-treatment'). An amount of 7–15 μg of total protein from the GCF samples was then subjected to filter device with relative molecular mass cut offs of 30,000, for the digestion and detergent removing as described previously (24). Briefly, the supernatants were denatured with 8M Urea in 100mM Tris/HCl buffer, alkylated with 0.05M iodoacetamide, washed by 0.5M NaCl, then digested with trypsin in 0.05M triethylammonium bicarbonate (1:50 w/w) overnight at 37°C . The digested peptides were collected by centrifugation and the reactions were stopped by adding trifluoroacetic acid (TFA) to a final concentration of 0.5%. Digested solutions were desalted using reverse phase cartridges Finisterre SPE C18 (Wicom International AG). Briefly, SPE C18 cartridges were first wet with 100% methanol, followed with 60% of acetonitrile (ACN) in 0.1% TFA, equilibrated with 3% of ACN in 0.1% TFA. Then, the digested solution was loaded on cartridges, washed with 3% ACN in 0.1% TFA, eluted with 0.5 ml of 60% ACN in 0.1% TFA, dried in vacuum centrifuge, and

solubilized with 30 μ l 3% ACN in 0.1% formic acid. The desalted samples were stored at -20°C until further use.

LC-MS/MS analysis and database search

The samples were injected into an Orbitrap Fusion mass spectrometer (Thermo Fisher Scientific) for proteomic analysis. The peptides were separated on an Easy nano-flow HPLC system (Thermo Fisher Scientific) coupled to a fused silica emitter (15-cm long, 75- μ m diameter) packed with a ReproSil-Pur C18-AQ 120 A and 1.9 μ m resin (Dr. Maisch HPLC GmbH). The linear gradient of acetonitrile/water (acetonitrile gradient from 2 to 35 % in 80 minutes) with 0.1 % formic acid was used to separate peptide at a flow rate of 300 nl/min. Mass spectra were acquired in a data-dependent manner, with an automatic switch between MS and MS/MS using a top 10 method. Then, these spectra were acquired in the Orbitrap analyzer at a mass range of 300– 1700 m/z . The higher energy collisional dissociation (HCD) peptide fragments that acquired at 28 normalized collision energy were analyzed at high resolution in the Orbitrap.

Protein identification and quantification

The raw files from the mass spectrometer were uploaded onto the ProgenesisQI for proteomics software package (version 4.1, Nonlinear Dynamics, Newcastle upon Tyne, UK). The LC-MS data were aligned according to the manufacturer's specifications. From each ProgenesisPeptideIon (default sensitivity in peak picking) a maximum of the top five tandem mass spectra were exported using charge deconvolution and deisotoping option and a maximum number of 200 peaks per MS/MS. The Mascot generic file (.mgf) was searched with Mascot Server v.2.4.1 (www.matrixscience.com) using the parameters 10ppm for precursor ion mass tolerance, 0.7 Da for fragment ion tolerance. Trypsin was used as the protein-cleaving enzyme, two missed cleavages as well as semi-tryptic peptides were allowed. Carbamidomethylation of cysteine was specified as a fixed modification, and oxidation of methionine was selected as variable modifications. Searched was a forward and reversed Macaca database (downloaded on 10/08/2015 from uniprot) concatenated to 260 known mass spectrometry contaminants in order to evaluate the false discovery rate using the target-decoy strategy (25). The mascot result was loaded into Scaffold v4.2.1 using 10% proteinFDR and 5% peptideFDR thresholds and protein cluster analysis. The spectrum report was exported and loaded into ProgenesisQI for proteomics. Normalization was adjusted to only peptides from Albumin proteins. For protein quantification, the average of the normalized abundance from the most intense N peptide ions of each protein group were calculated individually for each sample. This generates the normalised quantitative protein abundance. For statistical testing the parametric test (analysis of variance) on the transformed (hyperbolic arcsine transformation) normalised protein abundance was applied.

Bioinformatic data analysis

Human Swissprot database was used for orthologue mapping human protein homologs of the macaque GCF proteins. Blast mapping was successful in more than 99% of the listed macaque proteins as there were only 3 proteins which did not have a blast mapping. Mapped proteins with an eValue smaller 0.0001 were considered as human orthologue of the target Macaque protein. The representative human orthologues were used in the following

bioinformatics. Human orthologues of mapped macaque proteins were subjected to Protein ANalysis THrough Evolutionary Relationships (PANTHER) Gene Ontology (GO) classifications to investigate Biological processes, molecular function and cellular compartment <http://www.pantherdb.org/tools/>. Enrichment analysis was performed to determine the significant enriched GO terms in biological process with fold change ≥ 8 and P value $< 10e-7$. Heat map, bar plots and histograms were generated by customized R scripts.

Statistical analysis

Significant differences between the groups for total protein concentrations were determined using the repeated-measures analysis of variance and the Bonferroni post-hoc test to compare differences between groups in GraphPad Software (GraphPad Software, La Jolla, USA). Differences were considered statistically significant at $p < 0.05$. For quantitative protein expression analysis, significant differences between the pair-wise group comparisons were done in the Progenesis LC-MS using normalized protein abundances in arcsinh transformation. Differences were considered as statistically significant at fold-change ≥ 2 , p -value < 0.05 .

Results

Clinical findings

The clinical findings of the study that provided the GCF samples for proteomic analysis were reported in an earlier publication (14). Briefly, in both groups, treatment with Cp40 resulted in a significant reduction in gingival indices and probing pocket depth and clinical attachment level. This clinical improvement persisted, albeit at reduced efficacy, for at least 6 weeks after drug withdrawal.

Qualitative protein survey in Macaque GCF (Protein identification)

The workflow is outlined in Figure 1A. Mascot search results at ion score cut-off value of 30 identified 168785 high confidence unique peptides based on the target-decoy search against *M. fascicularis* database and these peptides assembled to 1236 proteins with minimum 2 peptides at FDR of 2.2 % and 0.1% at protein and peptide level, respectively (Supplementary Table 1). As FDR's were lower than 5%, the quality of data was high. Molecular weight of the identified proteins ranged from 4 to 523 kDa. The most abundant proteins based on total number of unique peptides were C3, PZP-like alpha-2-macroglobulin, desmoplakin, cytokeratins and albumin. There was considerable variation in the number of identified proteins among different macaques and within individual macaques across time points or treatment types. The number of identified proteins among individual GCF samples varied from 38 to 369 (Supplementary Table 1). Among the total 1236 proteins, 534 and 399 proteins were overlapped between the time points in the two treatment groups (Figure 1B, Figure 1C). The number of proteins identified reduced from baseline (Week 0) to Week 6 and Week 12 in the '3x-treatment' group (Figure 1B). In the '1x-treatment' group, the number of identified proteins was highest at Week 12 while there was a decline in protein identification from baseline to Week 6 (Figure 1C).

Comparative Quantitative Abundance Analysis of GCF proteins in response C3 treatment

Progenesis LCMS software was carried out for quantitative analysis of a total of 45 LC-MS/MS files. For protein expression analysis, significant differences between the pair-wise group comparisons were done in Progenesis LC-MS using normalized protein abundances in arcsinh transformation. Differences were considered as statistically significant at a p value of <0.05 . A total of 573 proteins were identified and quantified by LFQ with high confidence ($FDR < 0.05$). These protein lists are provided in Supplementary Table 2 and Supplementary Table 3 and they describe protein IDs, protein names, identified unique peptide for each protein, the normalized abundance intensities of each identified protein during the experimental time points. Based on the mean abundance across the samples at baseline, albumin was the most abundant protein quantified and abundance of the quantified proteins spanned across 5 orders of magnitude. The top 5 most highly expressed proteins (human orthologues) were albumin, Keratin-type II cytoskeletal 6A, Keratin-type I cytoskeletal 16, Ig gamma-1 chain C region and annexin-A1, known constituents of human GCF proteome (19). The 5 proteins with the lowest abundance included gamma-glutamylcyclotransferase, apolipoprotein B-100, bifunctional purine biosynthesis protein, thioredoxin-dependent peroxide reductase, neutrophil cytosol factor 1.

Gene Ontology (GO) mapping of quantified GCF proteins

To determine which proteins are highly enriched in GCF of systemically healthy adult cynomolgus monkeys, we used the PANTHER Classification System to investigate the protein function based on their cellular component, biological process and molecular functions (<http://pantherdb.org/>.) In total, 95.5% of the listed proteins (547 out of 573) were functionally annotated by PANTHER (Figure 2). Among them, 38.40% and 25.10% of all proteins in cellular component were Cell part (Panther GO: 0044464) and organelle (Panther GO: 0043226) respectively (Figure 2A). 27.40% and 24.80% of all proteins involved in biological process involved cellular process (Panther GO: 0051179) and metabolic process (Panther GO: 0008152) respectively (Figure 2B). Particularly, 'immune system process' (Panther GO: 0002376) accounted for 6% of total proteins involved in biological process (Figure 2B). Finally, 41.40% and 35.80% of total proteins in categories of molecular functions were in the category of 'Binding' (Panther GO: 0005488) and catalytic activity (Panther GO: 0003824) respectively (Figure 2C). Within the 'immune system process', macrophage activation accounted for 31.8% (Figure 2D). To further survey the biological process of the quantified proteins, functional enrichment analysis for total 547 was performed (Figure 3). Totally, 36 GO terms were significantly enriched with minimum 8-fold change as compared to the background and p value with 10^{-7} . Interestingly, we found that the 'alternative pathway of complement activation' was the top enriched pathway; the 'classical pathway of complement activation' and the 'pathway of complement activation regulation' were also significantly enriched, indicating that complement in general and specifically the alternative pathway plays an important role in natural periodontitis (Figure 3).

Fold changes in abundance intensity were calculated based on value log-ratios between pairs of time points. A protein expression change was considered as a true regulation at ≥ 2 -fold and $p < 0.05$. In the '3x-treatment' group, a comparison in protein expression abundance

between baseline and 6 weeks identified a total of 128 regulated proteins, 97 % of them were down-regulated in response to intervention (Figure 4A). The human orthologues of the upregulated proteins included tetranectin (involved in bone mineralization), mannose-binding lectin (a pattern-recognition molecule with direct antimicrobial functions and also involved in the initiation of the lectin pathway of complement) and vitronectin (a cell adhesion and spreading factor and a complement regulator that prevents MAC assembly). Following drug withdrawal at 6 weeks, 173 proteins out of 573 showed increased levels at week 12, whereas only two were down-regulated (Table 1 and Figure 4B). Calnexin (calcium-binding protein) and Apo(a) (serine proteinase activity) were down-regulated by 8.6-fold and 19.2-fold, respectively. Moreover, 95 proteins were down-regulated at 6 weeks and then up-regulated upon drug withdrawal at 12 weeks (Figure 4C). Gene ontology enrichment analysis of the 95 proteins were performed using PANTHER. The analysis revealed that the top enriched GO terms were *regulated exocytosis*, *exocytosis and leukocyte degranulation*, represented by 31–36 proteins (The full list is given as supplemental Table S-5).

In the ‘1x-treatment’ group, 252 (44% of those quantified) proteins were down-regulated at 6 weeks after intervention compared to baseline and 318 (55 of those quantified) were up-regulated following drug withdrawal at 12 weeks (Table 2 and Figure 5A-B). Among them, 108 proteins were specifically up-regulated but not down-regulated in Week 6 Vs. Week 0, whereas 42 proteins act in the opposite way (Figure 5C). Moreover, 210 proteins were down-regulated at 6 weeks and then up-regulated upon drug withdrawal at 12 weeks (Figure 5C). Gene ontology enrichment analysis of the 210 proteins were performed using PANTHER. Similar to the ‘3x-treatment group’, the analysis revealed that the top enriched GO terms were *regulated exocytosis*, *exocytosis and leukocyte degranulation*, represented by 51–61 proteins (The full list is given as supplemental Table S-6).

To identify differences between the two treatment modalities (‘1x-treatment’ vs ‘3x-treatment’), we compared their respective GCF proteome at 0, 6 and 12 weeks. At baseline, there were little differences between the two indicated intervention groups as only 6 out of 573 proteins showed significant difference (Table 3). Pairwise comparisons revealed significant regulation of 30 proteins at 6 weeks and 93 proteins at 12 weeks. At 6 weeks, 17 proteins were significantly lower in the ‘3x-treatment’ group compared to the ‘1x-treatment’ group. The top 5 regulated proteins included cell cycle control protein 50A (6.3-fold), dual oxidase 1 (5.2-fold), oral keratin-type II cytoskeletal 2 (4.8-fold), annexin A2 (3.9-fold), keratin, type II cytoskeletal 6 (3.9-fold). At 12 weeks, the relative fold change differences were more pronounced, as levels of 93 proteins were lower in the ‘3x-treatment’ group versus ‘1x-treatment’ group. The top regulated ones were BPI fold-containing family B member 1 (role innate immune response in mucosa, bactericidal), basic salivary proline-rich protein 2, semenogelin-2, calnexin, AP-1 complex subunit beta-1.

Next, to address how Cp40 treatment with 1 and 3 doses affect the protein level of the regulation of the alternative pathway of complement activation, the classical pathway of complement activation and the regulation of complement activation, we compared and visualized the proteins abundance using heatmap (Figure 6). We found CO9 (Complement component C9a; Complement component C9b), CO5 (Complement C5), CO3 (Complement

C3), CFAH (Complement factor H) and CO8B (Complement component C8 beta chain) from alternative activation pathway were significantly down-regulated by both 1x and 3x doses of Cp40 treatment (Figure 6A). IC1 (Plasma protease C1 inhibitor), CO4A (Complement C4-A), IGHM (Ig mu chain C region), IGHA2 (Ig alpha-2 chain C region), IGHG1 (Ig gamma-1 chain C region) and CO7 (Complement component C7) from classical activation pathway were also significantly down-regulated by both 1 x and 3 x doses of CP40 treatment (Figure 6B). Taken together, these data suggest that Cp40 treatment can suppress natural periodontal disease through downregulation of a number of components involved in complement activation (Figure 6C).

Discussion

By application of label-free shotgun proteomics combined with bioinformatics tools, we were able to shed light on the involvement of both alternative and classical complement pathway in naturally occurring periodontitis in a non-human primate model. However, the alternative pathway of complement was the most enriched pathway. This study is the first to characterize the whole proteome of C3-mediated local inflammation in naturally occurring periodontitis in primates. The advantage conferred by utilizing a label-free shotgun proteomic platform was to simultaneously identify and quantify 573 proteins with more than two peptides, leading to the discovery of a large number of proteins and processes involved in established disease. By use of GO annotations, we were able to show that among 573 proteins, the alternative pathway of complement activation had the highest fold change of enrichment, providing proteomic proof of alternative pathway involvement in periodontal disease pathogenesis. On the basis of the present findings, one can propose that local C3 inhibition in the absence of conventional treatments has considerable impact on the proteomic profile of local tissue exudatome, including the complement system itself, where important components were downregulated possibly as a consequence of Cp40-mediated inhibition of periodontal inflammation (Figure 7). In this regard, inflammatory pathways were shown to upregulate the expression of complement-associated proteins, such as Factor B, a critical component of the alternative pathway (26–28).

The number of identified or quantified proteins in this study was considerably higher than that reported earlier in the human or canine GCF (18, 19, 29). Comparison of the GCF proteome between the two intervention groups indicated only small differences at baseline, which allows for un-biased comparison the two treatment regimens (1 x and 3 x doses). Proteome changes in GCF were more pronounced in the '3x-treatment' group, therefore further data will be discussed in this context. Of the 573 individual proteins that could be quantified in total, 125 proteins in the '3x-treatment' group were down-regulated at 6-weeks, whereas three proteins were identified as upregulated, including tetranectin, mannan-binding lectin and vitronectin. Whereas the latter two proteins are linked to the complement pathway and its regulation, tetranectin is a plasminogen-binding protein that has important roles in wound-healing processes, especially in early bone remineralization as a bone matrix protein (30, 31). Vitronectin is a multifunctional human glycoprotein expressed also by extrahepatic cells with a significant role in cell migration and adhesion, tissue repair and downregulation of MAC formation (32, 33). Significant changes in proteins that are involved in tissue repair and bone mineralization in response to Cp40 treatment may suggest that complement

inhibition leads to restoration of homeostasis and bone tissue healing (Figure 7). This possible function would be in addition to the documented Cp40-mediated inhibition of clinical inflammation, which correlates with suppression of osteoclastogenesis and alveolar bone loss as well as a decrease in the RANKL/OPG ratio in the GCF (15). The critical involvement of C3 in periodontal disease pathogenesis is also supported by earlier findings in a human experimental gingivitis study where C3 activation in the GCF correlated with dental plaque biofilm accumulation (9). Conversely, C3 activation in the GCF of periodontitis patients was suppressed in response to periodontal therapy that resolved clinical inflammation (34). Similarities in the GCF content of humans and non-human primates are not surprising as primates share clinical, microbiological and histological features of human periodontitis, which makes them an excellent model to study the human disease (35–39).

The next analyses were conducted to survey the biological process of the quantified proteins by use of PANTHER database. Although, thirty-six GO terms were significantly enriched, as alluded to above, the alternative pathway of complement activation was the most represented one, followed by the classical pathway of complement activation. These results support earlier observations that the complement system and predominantly the alternative pathway is activated in the gingival crevice/periodontal pocket in human periodontitis patients (11, 34, 40). Those studies demonstrated conversion of C3 proactivator (Factor B) to C3-activator Bb in GCF of periodontitis patients but not in healthy controls (11, 34, 40). In line with these earlier reports, the current data from non-human primates also showed that C3 inhibition (at both doses of Cp40) down-regulated the GCF levels of Factor B and properdin, which are key regulators of the alternative complement pathway. Deposition of properdin, a serum glycoprotein that can both initiate and positively regulate the alternative pathway (41, 42), was also reported in biopsies from patients with periodontitis (43). Properdin is unique among complement proteins in that it is primarily produced by leukocytes (44). The cellular source of properdin in GCF could be infiltrating neutrophils as they are known to contain large stores of it, which are rapidly released upon stimulation with a variety of agonists, including C5a, TNF- α , and IL-8. Thus, the downregulation of properdin levels in the GCF following treatment with Cp40 is likely, at least in part, due to inhibition of inflammatory activity (*e.g.*, C5a generation) that triggers properdin release from neutrophils.

Furthermore, longitudinal comparisons allowed us to assess the protein expression changes following drug withdrawal. The earlier data indicated that the protective effects of Cp40 persist in the periodontium as all clinical indices remained at significantly lower levels relative to their corresponding baselines (14). Compared to the relatively 'rigid' clinical indices, the GFC proteome showed a more dynamic pattern when the drug was discontinued. 95 proteins were found to be the most dynamic ones as being down-regulated at 6 weeks after treatment, and then following drug withdrawal at 12 weeks showing significantly increased levels. Gene ontology analysis of these proteins suggested that Cp40 is able to regulate exocytosis of a wide range of proteins into GCF. The exocytosis of granules (or degranulation) containing pre-formed mediators, such as proteases, lipases, and inflammatory mediators by neutrophils is an essential element of host defense against microbial infection but can also instigate tissue damage in inflammatory diseases (45, 46) including periodontitis (47, 48). As neutrophils are responsible for significant tissue damage

in human periodontitis through the release of cytotoxic molecules and tissue-degrading proteases (49–52), the ability of Cp40 to inhibit exocytosis is likely a host protective mechanism. Pharmacological inhibitors of neutrophil exocytosis are promising effective anti-inflammatory molecules with potential applications in clinical conditions in which neutrophil secretion is abnormally up-regulated (53, 54). The present data suggest that complement inhibition by Cp40 may regulate this neutrophil function in the setting of periodontitis.

Conclusions

Taken together, these data suggest that the ability of Cp40 treatment to suppress natural periodontal disease in non-human primates is mediated, in great part, through inhibition of the alternative pathway of complement activation. Label-free quantitative proteomics approaches utilizing GCF are thus useful tools for providing insights into periodontal disease mechanisms and potentially identifying new treatment targets.

Data deposition

The mass spectrometry proteomics data have been deposited to the PRIDE Archive (<http://www.ebi.ac.uk/pride/archive/>) via the PRIDE (55) repository with the data set identifier PXD009502.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

A) Schematic representation of the protein discovery and quantification workflow by use of label-free quantitative proteomics (LFQ) in uncovering *Macaca* gingival crevicular fluid (GCF) proteome. The C3 inhibitor Cp40 was administered locally in the maxillary gingival tissue of adult systemically healthy cynomolgus monkeys (*Macaca fascicularis*) with established periodontitis, once a week ('1x treatment'; n=5 animals) or three times per week ('3x-treatment'; n=10 animals) for six weeks (end of treatment) followed by another six weeks of observation in the absence of treatment (end of monitoring). More details can be found under Materials and Methods. **B)** Qualitative protein survey in Macaque GCF in the '3 x treatment' group. **C)** Qualitative protein survey in Macaque GCF in the '1 x treatment' group.

Figure 2.

Gene Ontology (GO) mapping of quantified GCF proteins. Gene ontology analysis of total 573 proteins involved in GCF from monkey natural periodontitis using PANTHER program. PANTHER Classification System was used to investigate the protein function based on their cellular component (A), biological process (B) and molecular function (C) and immune system process (D).

Figure 3.
Gene ontology analysis of GCF proteome from monkey natural periodontitis using PANTHER program. Bar plot showing the enrichment of PANTHER biological pathways. Fold change of enrichment and p value are shown (cutoff: fold change ≥ 8 , $p \leq 10^{-7}$).

Figure 4.

Scatter plot of GCF proteome in response to the ‘3x-treatment’ of Cp40 at **A)** Week 6 Vs. Week 0 **B)** Week 12 Vs. Week 6. Red dots: fold change ≥ 2 and p -value < 0.05 ; blue dots: fold change ≥ 0.5 and $p < 0.05$ **C)** Venn diagram showing overlapping between significantly down-regulated proteins of Week 6 Vs. Week 0 (blue) and significantly up-regulated proteins in Week 12 Vs. Week 6 (red).

Figure 5.

Scatter plot of GCF proteome in response to the '1x-treatment' of CP40 at **A)** Week 6 Vs. Week 0 **B)** week12 Vs. Week 6. (red dots: fold change ≥ 2 and p -value < 0.05 ; blue dots: fold change ≥ 0.5 and $p < 0.05$). **C)** Venn diagram showing overlapping between significant down-regulated proteins of Week 6 Vs. Week 0 (blue) and significantly up-regulated proteins in Week 12 Vs. Week 6 (red). Among them, 210 proteins down-regulated first and then up-regulated (the common changed proteins).

Figure 6.

Heat map showing the fold change ($\log_2(\text{FC})$) of complement activation related proteins in total 573 proteins involved in GCF from monkey natural periodontitis according to PANTHER GO database. Alternative activation pathway (A), classical activation pathway (B) and complement activation (C). The red color indicates up-regulation, the blue color indicates down-regulation.

Figure 7.

The C3 inhibitor Cp40 and its targets in the setting of periodontitis. Complement is involved both in the dysbiotic transformation of the periodontal microbiota and in the inflammatory process that leads to the degradation of periodontal tissue which in turn exacerbates dysbiosis, leading to the generation of a vicious cycle of dysbiosis and destructive inflammation. Complement inhibition using the C3 inhibitor Cp40 (AMY-101) can block both dysbiosis and destructive inflammation, thereby reversing periodontitis.

Table 1.

Comparative Quantitative Abundance Analysis of GCF proteins in response to the ‘3x-treatment’ of Cp40 at Week 6 Vs. Week 0, Week 12 Vs. Week 0, at Week 12 Vs. Week 6. Proteins are considered regulated if fold change 0.5 or 2 and $p < 0.05$. Fold changes are converted to log2 value.

Accession	Week 6 Vs Week 0			Week 12 Vs Week 0			Week 12 Vs Week 6		
	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend
A0A023JCR5*	0.01	-1.85	down	0.84	-0.10		0.00	1.75	up
ACTN1*	0.04	-3.56	down	0.98	-0.47		0.01	3.08	up
AK1C1*	0.08	-2.71		0.91	-0.77		0.04	1.94	up
ANXA5*	0.01	-4.64	down	0.87	-0.85		0.00	3.79	up
CAN1	0.03	-3.33	down	0.83	-0.39		0.00	2.94	up
CAP1*	0.04	-2.94	down	0.56	-0.01		0.01	2.93	up
CATB*	0.15	-0.71		0.32	0.67		0.04	1.38	up
CATC*	0.08	-1.97		0.53	0.43		0.02	2.40	up
CAZA2*	0.06	-2.07		0.87	-0.69		0.02	1.39	up
CDC42*	0.11	-3.03		0.82	-0.63		0.03	2.41	up
EF1G	0.07	-3.43		0.92	-0.30		0.04	3.13	up
ENPL	0.08	-1.73		0.82	-0.26		0.04	1.47	up
EST1	0.03	-3.67	down	0.85	-0.90		0.01	2.77	up
G6PI*	0.27	-0.98		0.36	0.57		0.05	1.55	up
G7NTM4	0.02	-3.94	down	0.82	-0.69		0.01	3.25	up
G7NTU4	0.05	-4.13		0.86	-0.98		0.03	3.15	up
G7NU00	0.15	-1.67		0.60	0.36		0.04	2.03	up
G7NU30*	0.03	-3.05	down	0.61	0.01		0.00	3.06	up
G7NUG4	0.03	-3.87	down	0.90	-0.34		0.01	3.53	up
G7NUR3*	0.05	-4.10	down	0.67	-0.15		0.00	3.96	up
G7NUS8	0.04	-3.26	down	0.75	-0.30		0.01	2.96	up
G7NV01*	0.01	-2.62	down	0.95	-0.15		0.01	2.47	up
G7NV78*	0.02	-4.90	Down	0.61	-0.78		0.04	4.12	up
G7NVC3*	0.05	-1.00	Down	0.34	0.42		0.61	1.43	
G7NVF8	0.02	-2.81	Down	0.71	0.21		0.00	3.03	up
G7NW34*	0.03	-4.07	Down	0.88	-1.30		0.01	2.76	up
G7NW65	0.07	-1.89		0.96	-0.48		0.03	1.41	up
G7NY31	0.02	-2.33	down	0.98	-0.12		0.01	2.21	up
G7NY46	0.00	1.66	up	0.52	0.77		0.02	-0.90	
G7NYC5	0.03	-3.59	down	0.50	-0.97		0.20	2.61	
G7NYN6	0.03	1.00	up	0.08	1.04		0.78	0.04	
G7NYU6	0.13	-3.31		0.60	-0.61		0.01	2.70	up
G7NYZ9*	0.11	-4.91		0.76	-0.04		0.02	4.87	up

Accession	Week 6 Vs Week 0			Week 12 Vs Week 0			Week 12 Vs Week 6		
	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend
G7P161	0.05	-2.82	down	0.78	-0.13		0.01	2.69	up
G7P197	0.06	-2.88		0.99	-0.32		0.04	2.56	up
G7P2M3*	0.01	-4.65	down	0.98	0.57		0.01	5.22	up
G7P2N0*	0.03	-3.39	down	0.71	-1.42		0.01	1.97	up
G7P2N7*	0.03	-2.91	down	0.42	-1.30		0.12	1.61	
G7P350	0.04	-3.84	down	0.68	-1.49		0.04	2.35	up
G7P3D8*	0.05	-1.77		0.40	0.55		0.01	2.32	up
G7P407	0.03	-2.96	down	0.79	-0.41		0.01	2.55	up
G7P448	0.15	-2.80		0.40	-0.38		0.01	2.42	up
G7P458	0.03	-3.45	down	0.96	-0.55		0.01	2.90	up
G7P474	0.55	0.24		0.04	1.48	up	0.09	1.24	
G7P4B6	0.02	-4.07	down	0.67	-1.65		0.01	2.42	up
G7P4D2	0.03	-5.37	down	0.60	-0.73		0.11	4.64	
G7P4J4	0.05	-1.64	down	0.43	-0.89		0.14	0.75	
G7P4U1*	0.03	-1.90	down	0.90	0.14		0.02	2.03	up
G7P536	0.03	-4.63	down	1.00	-1.56		0.01	3.07	up
G7P597	0.08	-3.59		0.82	-0.24		0.02	3.35	up
G7P5E2	0.23	-2.09		0.38	0.51		0.03	2.60	up
G7P5L5	0.01	-1.94	down	0.72	0.42		0.01	2.36	up
G7P631	0.40	-2.10		0.53	-0.73		0.05	1.37	up
G7P728	0.69	-0.09		0.12	-3.20		0.03	-3.11	down
G7P7Z5	0.02	-4.87	down	0.28	-1.75		0.30	3.12	
G7P8A1*	0.04	-5.00	down	0.63	-1.89		0.06	3.11	
G7P8B7	0.04	-3.19	down	0.99	-0.25		0.03	2.95	up
G7P8Z4*	0.05	-1.16	down	0.40	0.25		0.01	1.41	up
G7P916*	0.02	-2.29	down	0.80	-0.03		0.01	2.27	up
G7P9A1	0.09	-3.08		0.62	-0.35		0.03	2.74	up
G7P9G4	0.07	-2.41		0.92	-0.27		0.04	2.14	up
G7P9Y7	0.05	-4.78	down	0.81	-1.20		0.03	3.58	up
G7PAB8	0.12	-2.12		0.79	-0.43		0.04	1.69	up
G7PB74	0.03	-2.83	down	0.96	-0.73		0.01	2.10	up
G7PBD8	0.06	-1.93		0.74	-0.03		0.02	1.90	up
G7PBL1	0.03	-4.70	down	0.79	-0.96		0.02	3.74	up
G7PCD1	0.04	-4.86	down	0.65	-1.59		0.04	3.27	up
G7PCF6	0.05	-2.22	down	0.81	-0.22		0.01	2.00	up
G7PCH4*	0.02	-2.79	down	0.76	-0.11		0.00	2.67	up
G7PDX8*	0.02	-3.87	down	0.79	-0.19		0.00	3.68	up
G7PE53	0.07	-2.31		0.72	-0.22		0.02	2.09	up

Accession	Week 6 Vs Week 0			Week 12 Vs Week 0			Week 12 Vs Week 6		
	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend
G7PE64	0.05	-2.26		0.86	-0.44		0.01	1.82	up
G7PEA9	0.01	-3.07	down	0.41	-0.84		0.06	2.23	
G7PEY1	0.02	-3.34	down	0.89	-0.55		0.01	2.79	up
G7PEZ9	0.02	-3.57	down	0.96	-0.40		0.01	3.17	up
G7PF52	0.07	-3.39		0.94	-0.87		0.03	2.52	up
G7PFC3	0.05	-1.76		0.82	0.03		0.03	1.79	up
G7PFC9	0.09	-3.01		0.96	-0.94		0.03	2.08	up
G7PFP5	0.02	-5.49	down	0.92	-1.03		0.01	4.47	up
G7PG41	0.03	-2.42	down	0.64	0.14		0.01	2.56	up
G7PGB0*	0.05	-1.70		0.72	0.05		0.02	1.75	up
G7PGE6	0.10	-4.13		0.98	-1.45		0.03	2.68	up
G7PGT2	0.04	-4.41	down	0.71	-1.25		0.06	3.15	
G7PIC9*	0.10	-5.33		0.96	-0.50		0.04	4.83	up
G7PID3	0.05	-4.02	down	0.85	-0.52		0.01	3.50	up
G7PID4	0.12	-3.04		0.71	0.38		0.04	3.42	up
G7PID9	0.03	-4.86	down	0.69	-0.99		0.03	3.87	up
G7PIJ9*	0.01	-3.78	down	0.52	-0.89		0.02	2.89	up
G7PIP1	0.04	-2.75	down	0.70	-0.26		0.08	2.49	
G7PIJ36	0.14	-1.87		0.39	-0.05		0.01	1.82	up
G7PJE5	0.09	-5.36		0.88	-1.27		0.04	4.09	up
G7PJR1	0.05	-3.76		0.99	-1.18		0.02	2.58	up
G7PJR6	0.07	-1.96		0.92	-0.07		0.04	1.89	up
G7PJX4	0.04	-3.89	down	0.99	-0.99		0.01	2.91	up
G7PJY3	0.14	-2.22		0.50	0.56		0.02	2.78	up
G7PKG8	0.03	-3.11	down	0.52	-0.37		0.17	2.74	
G7PLB1	0.07	-2.54		0.72	-0.35		0.02	2.19	up
G7PLE9	0.03	-2.13	down	0.59	-0.15		0.01	1.99	up
G7PM16	0.03	-3.00	down	0.64	0.07		0.01	3.07	up
G7PM19	0.20	-2.16		0.56	-0.11		0.04	2.05	up
G7PMB2*	0.02	-2.53	down	0.69	0.19		0.01	2.72	up
G7PME6	0.05	-6.72	down	0.55	-4.18		0.06	2.53	
G7PMM0	0.02	-3.12	down	0.93	-0.48		0.01	2.64	up
G7PNK2*	0.12	-2.53		0.56	-0.05		0.02	2.49	up
G7PP62	0.24	-4.35		0.70	-1.87		0.04	2.48	up
G7PPB6	0.11	-2.00		0.86	-0.89		0.03	1.11	up
G7PQF0	0.02	-3.06	down	0.90	-0.35		0.01	2.71	up
G7PR30	0.20	-6.30		0.79	-1.54		0.04	4.77	up
G7PR71	0.03	-5.77	down	0.40	-2.94		0.05	2.83	up

Accession	Week 6 Vs Week 0			Week 12 Vs Week 0			Week 12 Vs Week 6		
	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend
G7PRG1	0.01	-2.98	down	0.97	-0.12		0.01	2.86	up
G7PRJ1	0.08	-3.50		0.97	-0.74		0.04	2.76	up
G7PRP9*	0.02	-4.25	down	0.82	-0.54		0.02	3.71	up
G7PRZ9*	0.04	-3.26	down	0.93	-0.23		0.03	3.03	up
G7PS17*	0.03	-3.52	down	0.68	-1.07		0.03	2.45	up
G7PSJ7	0.03	-4.44	down	0.93	-1.16		0.01	3.28	up
G7PSZ1*	0.07	-2.37		0.83	-0.18		0.04	2.20	up
G7PT41	0.01	-2.75	down	0.83	0.18		0.01	2.92	up
G7PT92*	0.04	-2.87	down	0.94	-1.03		0.01	1.84	up
G7PTC0	0.04	-5.37	down	0.51	-1.00		0.19	4.36	
G7PTL5	0.03	-3.51	down	0.79	-0.51		0.00	3.00	up
G7PTR1*	0.03	-4.57	down	0.83	-0.94		0.03	3.63	up
G7PTW8	0.01	1.21	up	0.12	1.22		0.39	0.01	
G7PUC0	0.06	-3.55		0.74	-0.41		0.01	3.15	up
G7PUF4	0.02	-3.46	down	0.65	-0.08		0.00	3.39	up
G7PUH3*	0.06	-4.08		0.87	-0.33		0.01	3.75	up
G7PUP9	0.06	-4.38		0.89	-0.29		0.02	4.09	up
G7PUS4	0.04	-2.99	down	0.97	-0.64		0.02	2.35	up
G7PUW6*	0.01	-2.67	down	0.73	-0.47		0.00	2.20	up
G7PW62	0.03	-2.64	down	0.82	-0.60		0.01	2.03	up
G7PW68	0.12	-2.84		0.53	-0.58		0.02	2.26	up
G7PWK4	0.28	-5.88		0.62	-0.09		0.03	5.79	up
G7PWW0*	0.03	-3.84	down	0.94	-0.84		0.02	3.00	up
G7PWX3	0.04	-2.72	down	0.95	-0.19		0.02	2.53	up
G7PXC3	0.19	-1.92		0.58	0.15		0.04	2.07	up
G7PXH3	0.04	-3.55	down	0.92	-0.59		0.03	2.95	up
G7PXI7	0.02	-4.26	down	0.59	-0.43		0.15	3.83	
G7PXJ8	0.04	-5.19	down	0.49	-0.71		0.24	4.48	
G7PY93	0.02	-3.64	down	0.96	-0.79		0.01	2.85	up
G7PYE1*	0.01	-3.44	down	0.78	-0.72		0.00	2.72	up
G7PYG8	0.02	-4.39	down	0.39	-0.92		0.16	3.47	
G7PZB9	0.15	-0.42		0.34	0.59		0.05	1.01	up
G7PZH6*	0.13	-2.05		0.75	-0.16		0.03	1.89	up
G7PZZ8	0.00	-5.69	down	0.13	-0.78		0.28	4.91	
G7Q069	0.04	-5.40	down	0.56	-0.89		0.24	4.51	
G7Q0F5	0.03	-5.18	down	0.67	-1.18		0.05	4.00	
G7Q0J9	0.08	-3.02		0.73	-0.33		0.01	2.69	up
G7Q0V3	0.30	-1.87		0.42	0.24		0.04	2.11	up

Accession	Week 6 Vs Week 0			Week 12 Vs Week 0			Week 12 Vs Week 6		
	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend
G7Q0W5	0.04	-2.07	down	0.67	-0.28		0.01	1.79	up
G7Q1A4*	0.02	-3.60	down	0.52	-1.97		0.03	1.64	up
G7Q216	0.15	-2.15		0.58	-0.08		0.02	2.07	up
G7Q228	0.09	-2.25		0.60	-0.06		0.01	2.19	up
G7Q2W9*	0.07	-2.40		0.45	0.50		0.01	2.90	up
G7Q337*	0.17	-1.05		0.48	0.26		0.05	1.31	up
G7Q3I7*	0.09	-1.88		0.75	-0.41		0.04	1.47	up
G8F2B0	0.10	-2.01		0.71	0.04		0.02	2.05	up
G8F2D0*	0.07	-2.68		0.66	-0.58		0.01	2.10	up
G8F2E1*	0.17	1.27		0.05	2.04	up	0.48	0.77	
G8F2G9	0.05	-3.23		0.89	-0.66		0.01	2.57	up
G8F2T1	0.03	-4.08	down	0.78	-1.02		0.03	3.05	up
G8F340	0.10	-1.50		0.01	-3.38	down	0.57	-1.88	
G8F3M1	0.06	-2.60		0.85	-0.03		0.02	2.57	up
G8F3W4*	0.03	-2.66	down	0.97	-0.31		0.02	2.34	up
G8F3X8	0.12	-1.66		0.53	-0.07		0.02	1.59	up
G8F406	0.07	-5.22		0.98	-0.41		0.03	4.81	up
G8F4N6	0.03	-2.61	down	0.85	-0.46		0.06	2.15	
G8F4W0	0.04	-3.47	down	0.78	0.08		0.09	3.56	
G8F4Z4	0.09	-4.74		0.60	-1.15		0.01	3.59	up
G8F5G1	0.00	-3.16	down	0.70	-0.37		0.01	2.79	up
G8F5P8	0.08	-2.79		0.77	-0.51		0.01	2.28	up
G8F637	0.04	-2.33	down	0.80	-0.11		0.01	2.22	up
G8F646*	0.14	-3.07		0.47	-0.18		0.02	2.89	up
G8F672	0.05	-4.69	down	0.72	-1.62		0.05	3.07	
HS90A*	0.03	-2.96	down	0.79	-0.41		0.01	2.55	up
I7G7C5*	0.08	-2.24		0.79	0.20		0.04	2.44	up
I7G7S3*	0.01	-4.47	down	0.80	-0.98		0.01	3.49	up
I7G8U9*	0.02	-3.18	down	0.81	-0.31		0.00	2.87	up
I7G9P9*	0.23	-1.46		0.46	0.18		0.04	1.65	up
I7G9S0	0.05	-2.69	down	0.69	-0.02		0.01	2.67	up
I7GH17*	0.03	-3.81	down	0.63	-1.38		0.03	2.43	up
I7GH35*	0.02	-3.99	down	0.86	-0.91		0.00	3.08	up
I7GH92*	0.17	-3.96		0.66	-0.29		0.04	3.67	up
I7GHF1	0.05	-4.24	down	0.66	-1.57		0.08	2.67	
I7GHV0*	0.02	-3.67	down	0.51	-0.73		0.07	2.94	
I7GIB8*	0.02	-2.96	down	1.00	-0.34		0.02	2.61	up
I7GK56	0.04	-3.22	down	0.41	-2.11		0.06	1.11	

Accession	Week 6 Vs Week 0			Week 12 Vs Week 0			Week 12 Vs Week 6		
	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend
I7GK91*	0.03	-2.67	down	0.93	-0.26		0.03	2.41	up
I7GKE9	0.06	-3.38		0.75	-0.15		0.02	3.24	up
I7GL64*	0.05	-3.42		0.92	-0.43		0.04	2.99	up
I7GMW7*	0.02	-3.09	down	0.49	-1.13		0.33	1.97	
NHRF1	0.01	-4.67	down	0.61	-0.43		0.06	4.23	
PGM1*	0.16	-2.88		0.61	-0.36		0.03	2.52	up
PRDX2*	0.04	-1.08	down	0.80	0.28		0.14	1.36	
PRDX6	0.02	-2.01	down	0.87	0.28		0.01	2.30	up
Q25N99*	0.03	-4.06	down	0.91	-0.41		0.03	3.65	up
Q28911	0.24	0.22		0.44	-4.05		0.04	-4.27	down
Q2PFZ6	0.03	-2.49	down	0.88	0.19		0.02	2.68	up
Q4R4M2*	0.31	-2.63		0.42	0.30		0.05	2.92	up
Q4R4P0*	0.05	-3.21	down	0.85	-1.13		0.03	2.08	up
Q4R4Y1*	0.01	-5.26	down	0.39	-1.81		0.30	3.45	
Q4R5L3*	0.05	-1.51		0.64	0.22		0.02	1.73	up
Q4R5N7	0.06	-2.57		0.79	-0.24		0.02	2.33	up
Q4R5Q1*	0.04	-3.52	down	0.79	-0.37		0.00	3.16	up
Q4R7X1*	0.03	-2.67	down	0.65	-0.06		0.00	2.61	up
Q5ISS9	0.01	-3.92	down	0.84	-1.05		0.01	2.87	up
Q5IST7	0.02	-3.62	down	0.78	-1.15		0.02	2.48	up
Q8WNT7	0.04	-2.49	down	0.44	0.14		0.00	2.63	up
Q95LN9	0.02	-3.13	down	0.91	-0.56		0.00	2.58	up
RL18*	0.05	-5.60	down	0.47	-1.40		0.22	4.20	
SEMG2	0.05	-7.71	down	0.69	-4.64		0.01	3.08	up
TBB2A*	0.02	-3.99	down	0.86	-0.91		0.00	3.08	up
TPIS	0.05	-2.00	down	0.63	0.02		0.01	2.03	up

Table 2.

Comparative Quantitative Abundance Analysis of GCF proteins in response to the ‘1x-treatment’ of Cp40 at Week 6 Vs. Week 0, Week 12 Vs. Week 0, at Week 12 Vs. Week 6. Proteins are considered regulated if fold change ≥ 0.5 or ≤ 2 and $p < 0.05$. Fold changes are converted to log2 value.

Accession	Week6 Vs Week0			Week12 Vs Week0			Week12 Vs Week6		
	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend
A0A023JBX1 *	0.03	-2.19	down	0.26	1.03		0.01	3.22	up
A0A023JCR5 *	0.14	-1.13		0.17	0.73		0.00	1.87	up
ACTN1 *	0.01	-2.69	down	0.67	0.47		0.01	3.17	up
AK1C1 *	0.01	-3.70	down	0.85	-0.21		0.00	3.49	up
ANXA5 *	0.06	-1.93		0.69	0.40		0.03	2.33	up
CALR	0.09	-1.84		0.59	0.41		0.04	2.25	up
CAMP	0.03	-1.55	down	0.50	-0.20		0.16	1.34	
CAN1	0.05	-1.74		0.30	0.90		0.01	2.65	up
CAN2 *	0.01	-2.55	down	0.19	1.63		0.00	4.18	up
CAP1 *	0.03	-1.94	down	0.47	0.76		0.02	2.70	up
CATC *	0.07	-2.11		0.69	0.29		0.05	2.41	up
CAZA2 *	0.01	-2.03	down	0.38	0.51		0.01	2.54	up
CBR1 *	0.06	-3.12		0.31	0.32		0.00	3.43	up
CDC42 *	0.00	-3.11	down	0.34	0.61		0.00	3.73	up
CISY *	0.14	-1.16		0.20	2.00		0.04	3.16	up
EF1G	0.02	-2.22	down	0.50	0.82		0.02	3.04	up
ENPL	0.05	-1.40		0.07	1.11		0.01	2.51	up
EST1	0.06	-2.26		0.64	0.40		0.04	2.66	up
G0YW28 *	0.00	-2.75	down	0.86	0.39		0.00	3.14	up
G6PI *	0.08	-1.15		0.02	1.30	up	0.01	2.46	up
G7NTC9	0.03	-2.83	down	0.58	-0.36		0.08	2.48	
G7NTM4	0.13	-3.43		0.55	0.11		0.02	3.54	up
G7NTU2	0.04	-2.90	down	0.82	1.21		0.09	4.11	
G7NTU4	0.03	-2.00	down	0.59	1.24		0.05	3.24	up
G7NU00	0.08	-1.07		0.35	0.97		0.04	2.04	up
G7NU30 *	0.03	-1.77	down	0.21	1.60		0.01	3.36	up
G7NUF2	0.15	-3.76		0.40	1.80		0.03	5.56	up
G7NUG4	0.29	-1.93		0.33	0.65		0.02	2.58	up
G7NUG8	0.19	-2.16		0.28	1.07		0.03	3.22	up
G7NUH0	0.01	-3.29	down	0.86	2.18		0.05	5.47	up
G7NUQ0	0.01	-2.49	down	0.46	-0.43		0.07	2.06	

Accession	Week6 Vs Week0			Week12 Vs Week0			Week12 Vs Week6		
	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend
G7NUR3 *	0.02	-1.79	down	0.36	0.90		0.01	2.69	up
G7NUS8	0.01	-2.06	down	0.56	0.68		0.01	2.74	up
G7NUU5	0.05	-4.14	down	0.96	-0.13		0.02	4.01	up
G7NUU7	0.02	-3.19	down	0.64	-0.16		0.09	3.03	
G7NV01 *	0.03	-2.43	down	0.70	0.37		0.02	2.79	up
G7NV22	0.00	-2.68	down	0.09	1.92		0.00	4.60	up
G7NV51 *	0.11	-1.70		0.03	1.51	up	0.00	3.20	up
G7NV78 *	0.03	-3.92	down	0.87	0.15		0.04	4.08	up
G7NV87 *	0.06	-5.86		0.75	-0.54		0.01	5.31	up
G7NV96	0.01	-2.62	down	0.76	0.94		0.03	3.56	up
G7NVF8	0.05	-1.39	down	0.94	0.35		0.08	1.74	
G7NVM5	0.02	-2.40	down	0.06	1.54		0.00	3.94	up
G7NW00	0.01	-3.09	down	0.85	0.94		0.09	4.04	
G7NW34 *	0.02	-2.28	down	0.77	0.54		0.03	2.82	up
G7NW65	0.01	-2.15	down	0.18	0.84		0.00	2.99	up
G7NW99	0.02	-3.49	down	0.82	2.48		0.39	5.98	
G7NWB8	0.00	-3.61	down	0.44	1.22		0.00	4.83	up
G7NWU6	0.83	-2.99		0.25	1.54		0.00	4.54	up
G7NX83	0.02	-3.51	down	0.77	1.17		0.04	4.68	up
G7NXH7	0.03	-3.77	down	0.51	0.65		0.01	4.42	up
G7NYC5	0.02	-3.44	down	0.35	1.17		0.00	4.62	up
G7NYM3	0.00	-4.51	down	0.68	-0.30		0.04	4.22	up
G7NYZ9 *	0.12	-4.04		0.44	0.24		0.01	4.28	up
G7NZC2	0.00	-3.42	down	0.60	-0.21		0.01	3.20	up
G7NZN7 *	0.02	-3.00	down	0.90	0.79		0.02	3.79	up
G7P028	0.02	-3.50	down	0.71	1.25		0.02	4.75	up
G7P049	0.04	-2.10	down	0.86	0.72		0.07	2.82	
G7P0A3	0.08	-1.09		0.15	0.97		0.02	2.06	up
G7P0H3	0.15	-4.77		0.65	-0.21		0.01	4.56	up
G7P0L2	0.03	-3.69	down	0.51	0.15		0.01	3.84	up
G7P0P7	0.00	-3.48	down	0.68	1.19		0.01	4.66	up
G7P114	0.03	-3.23	down	0.36	0.27		0.00	3.50	up
G7P161	0.01	-2.63	down	0.38	1.05		0.01	3.68	up
G7P164	0.04	-1.74	down	0.38	0.99		0.02	2.73	up
G7P186	0.27	-7.41		0.19	-0.44		0.00	6.97	up
G7P197	0.00	-2.97	down	0.44	0.98		0.00	3.94	up

Accession	Week6 Vs Week0			Week12 Vs Week0			Week12 Vs Week6		
	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend
G7P1J3	0.01	-1.76	down	0.21	0.57		0.00	2.34	up
G7P2M3 *	0.03	-3.35	down	0.74	0.74		0.05	4.09	
G7P2N0 *	0.30	-0.86		0.25	1.17		0.05	2.03	up
G7P360	0.03	-2.09	down	0.39	1.27		0.02	3.36	up
G7P3D8 *	0.00	-2.14	down	0.69	0.62		0.01	2.75	up
G7P3R4	0.09	-4.62		0.45	0.98		0.01	5.60	up
G7P407	0.07	-1.42		0.15	1.10		0.01	2.52	up
G7P448	0.02	-4.36	down	0.17	1.17		0.01	5.53	up
G7P458	0.10	-2.55		0.14	1.16		0.00	3.70	up
G7P474	0.03	-1.45	down	0.65	0.08		0.10	1.52	
G7P489 *	0.98	-2.75		0.45	-0.49		0.02	2.26	up
G7P4B6	0.03	-1.91	down	0.75	0.28		0.02	2.19	up
G7P4D2	0.03	-3.36	down	0.56	0.61		0.02	3.97	up
G7P4N8 *	0.04	-1.58	down	0.12	1.34		0.01	2.92	up
G7P4U1 *	0.01	-1.65	down	0.83	0.46		0.04	2.10	up
G7P536	0.00	-2.17	down	0.46	0.97		0.01	3.14	up
G7P597	0.09	-2.66		0.60	0.72		0.04	3.39	up
G7P5F7	0.00	-2.32	down	0.03	1.99	up	0.00	4.32	up
G7P5Q3	0.94	-5.17		0.26	-0.30		0.01	4.88	up
G7P5Z0	0.22	-4.47		0.35	-0.47		0.01	4.00	up
G7P631	0.18	-1.64		0.28	1.87		0.04	3.51	up
G7P6M8 *	0.02	-1.45	down	0.69	0.89		0.03	2.34	up
G7P6Z7	0.05	-1.86		0.11	1.20		0.00	3.05	up
G7P703	0.00	-4.97	down	0.19	-1.66		0.10	3.30	
G7P728	0.13	-3.08		0.31	2.36		0.01	5.44	up
G7P7E9	0.02	1.48	up	0.05	3.65		0.47	2.17	
G7P7G3	0.02	-2.28	down	0.31	1.33		0.01	3.61	up
G7P7Z5	0.06	-4.24		0.62	0.55		0.03	4.80	up
G7P8A1 *	0.03	-2.38	down	0.91	1.13		0.09	3.51	
G7P8B7	0.05	-1.43	down	0.32	1.59		0.03	3.02	up
G7P8Q6	0.01	-3.07	down	0.58	-0.22		0.11	2.86	
G7P916 *	0.09	-1.57		0.23	0.65		0.01	2.22	up
G7P987 *	0.07	-2.18		0.18	0.86		0.01	3.04	up
G7P9G4	0.01	-2.56	down	0.92	0.06		0.01	2.61	up
G7P9Y7	0.07	-2.54		0.56	0.85		0.04	3.39	up
G7P9Y9	0.08	-4.50		0.64	0.46		0.04	4.95	up

Accession	Week6 Vs Week0			Week12 Vs Week0			Week12 Vs Week6		
	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend
G7PAB8	0.02	-1.80	down	0.44	0.72		0.02	2.52	up
G7PAU7	0.19	-8.89		0.53	-2.53		0.00	6.36	up
G7PAV9	0.03	-4.02	down	0.93	1.20		0.07	5.23	
G7PB74	0.01	-2.34	down	0.96	0.26		0.02	2.60	up
G7PB96	0.02	-2.92	down	0.94	0.74		0.05	3.66	up
G7PBH4	0.00	-2.76	down	0.41	1.36		0.01	4.12	up
G7PBL1	0.03	-2.54	down	0.69	0.74		0.04	3.28	up
G7PBN2	0.11	-3.88		0.43	1.35		0.02	5.24	up
G7PBW8 *	0.03	-1.99	down	0.10	3.00		0.01	4.99	up
G7PC52 *	0.01	-5.20	down	0.58	-1.75		0.02	3.45	up
G7PC54 *	0.09	-2.36		0.39	0.23		0.01	2.59	up
G7PCF6	0.03	-1.69	down	0.12	1.56		0.01	3.25	up
G7PCU4 *	0.11	-1.17		0.00	2.71	up	0.00	3.88	up
G7PD43	0.04	-2.71	down	0.07	2.24		0.00	4.95	up
G7PDX8 *	0.00	-2.67	down	0.30	1.08		0.00	3.75	up
G7PE50	0.04	-2.21	down	0.96	1.00		0.11	3.21	
G7PE53	0.01	-2.00	down	0.31	0.88		0.00	2.87	up
G7PE64	0.11	-1.38		0.13	2.84		0.02	4.22	up
G7PEA9	0.03	-1.34	down	0.14	1.52		0.02	2.86	up
G7PED5	0.04	-2.70	down	0.37	0.79		0.02	3.49	up
G7PEG3 *	0.00	-2.46	down	0.47	-0.12		0.08	2.34	
G7PEJ9	0.04	-2.30	down	0.74	0.50		0.04	2.80	up
G7PEV4	0.00	-2.66	down	0.28	1.03		0.00	3.69	up
G7PEX7 *	0.03	-2.15	down	0.81	1.20		0.06	3.35	
G7PEY1	0.03	-2.72	down	0.60	0.65		0.02	3.36	up
G7PEZ9	0.01	-2.86	down	0.91	0.00		0.01	2.86	up
G7PF52	0.03	-2.02	down	0.48	0.79		0.02	2.81	up
G7PFC3	0.06	-1.48		0.34	0.98		0.03	2.46	up
G7PFC9	0.00	-1.88	down	0.12	1.03		0.00	2.91	up
G7PFM1	0.11	-2.76		0.16	1.43		0.00	4.19	up
G7PFN4 *	0.05	-3.30		0.27	1.96		0.01	5.26	up
G7FP5	0.12	-1.17		0.25	1.70		0.04	2.87	up
G7PG41	0.02	-1.68	down	0.93	0.37		0.04	2.05	up
G7PGB0 *	0.44	-1.03		0.06	1.96		0.00	2.98	up
G7PGB3 *	0.01	-2.74	down	0.89	-0.13		0.02	2.61	up
G7PGI0	0.19	-2.42		0.91	-0.92		0.01	1.50	up

Accession	Week6 Vs Week0			Week12 Vs Week0			Week12 Vs Week6		
	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend
G7PGI9	0.02	-1.97	down	0.70	0.61		0.03	2.59	up
G7PGT2	0.04	-2.97	down	0.95	0.41		0.10	3.38	
G7PHE1	0.01	-4.29	down	0.88	0.23		0.02	4.52	up
G7PHH9 [*]	0.36	-2.39		0.16	2.15		0.02	4.55	up
G7PHU7	0.03	-4.31	down	0.91	-0.59		0.03	3.72	up
G7PI76 [*]	0.02	-3.95	down	0.87	0.73		0.03	4.69	up
G7PIA8	0.53	-4.06		0.22	2.60		0.00	6.66	up
G7PID3	0.09	-2.34		0.74	0.05		0.02	2.39	up
G7PID4	0.06	-2.24		0.84	0.10		0.04	2.33	up
G7PID6	0.06	-2.70		0.75	0.45		0.04	3.15	up
G7PID7	0.04	-3.22	down	0.83	0.50		0.06	3.73	
G7PID9	0.02	-3.24	down	0.92	0.04		0.07	3.27	
G7PIG1 [*]	0.03	-2.53	down	0.15	1.38		0.01	3.91	up
G7PII6	0.01	-3.14	down	0.86	0.58		0.07	3.73	
G7PIJ9 [*]	0.11	-2.91		0.30	1.62		0.02	4.53	up
G7PIP1	0.00	-1.98	down	0.44	0.86		0.01	2.83	up
G7PIP6 [*]	0.02	-2.14	down	0.72	0.52		0.02	2.66	up
G7PIY5	0.01	-3.13	down	0.72	0.81		0.01	3.94	up
G7PJ36	0.06	-1.28		0.18	1.24		0.02	2.52	up
G7PJD6	0.05	-1.69		0.36	1.55		0.03	3.24	up
G7PJR1	0.02	-2.72	down	0.78	-0.40		0.03	2.32	up
G7PJX4	0.05	-1.53	down	0.60	0.66		0.04	2.19	up
G7PJY3	0.01	-1.67	down	0.24	1.09		0.01	2.76	up
G7PL94 [*]	0.05	-2.64	down	0.61	-0.06		0.21	2.59	
G7PLB1	0.03	-1.87	down	0.18	0.91		0.00	2.78	up
G7PLC0 [*]	0.02	-2.00	down	0.76	0.81		0.04	2.81	up
G7PLD3	0.17	-2.17		0.07	4.00		0.01	6.17	up
G7PLD4	0.02	-1.33	down	0.61	0.35		0.01	1.68	up
G7PLE9	0.03	-1.36	down	0.05	0.84		0.00	2.20	up
G7PLL6	0.01	-3.55	down	0.83	0.38		0.01	3.94	up
G7PM16	0.25	-1.76		0.27	0.95		0.03	2.71	up
G7PMB2 [*]	0.02	-2.17	down	0.09	1.14		0.00	3.31	up
G7PMB3	0.09	-1.12		0.13	1.12		0.03	2.23	up
G7PME1	0.02	-3.76	down	0.62	0.73		0.01	4.49	up
G7PME6	0.05	-6.61	down	0.88	-3.08		0.02	3.53	up
G7PMM0	0.01	-1.96	down	0.33	1.02		0.01	2.98	up

Accession	Week6 Vs Week0			Week12 Vs Week0			Week12 Vs Week6		
	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend
G7PN00	0.01	-2.82	down	0.61	1.14		0.02	3.96	up
G7PNK2*	0.05	-1.90	down	0.98	0.13		0.06	2.03	
G7PNN3	0.02	-2.37	down	0.68	0.31		0.01	2.68	up
G7PP62	0.02	-2.51	down	0.88	0.47		0.04	2.98	up
G7PP83	0.01	-5.15	down	0.97	0.33		0.03	5.47	up
G7PPB6	0.02	-3.53	down	0.96	0.28		0.11	3.81	
G7PPR6*	0.01	-3.35	down	0.23	1.26		0.00	4.61	up
G7PPY5	0.07	-1.43		0.02	1.64	up	0.01	3.07	up
G7PQF0	0.01	-2.07	down	0.37	0.85		0.01	2.92	up
G7PQY3*	0.02	-3.58	down	0.42	-1.48		0.02	2.10	up
G7PR30	0.03	-3.10	down	0.95	1.80		0.12	4.91	
G7PR71	0.00	-4.79	down	0.10	-1.62		0.02	3.17	up
G7PRF3*	0.01	-3.14	down	0.85	0.11		0.01	3.25	up
G7PRG1	0.07	-1.22		0.38	0.99		0.04	2.21	up
G7PRJ1	0.00	-3.12	down	0.94	0.04		0.01	3.16	up
G7PRL7	0.34	-0.51		0.02	-1.26	down	0.17	-0.76	
G7PRP9*	0.01	-3.25	down	0.76	0.24		0.01	3.49	up
G7PRZ9*	0.02	-2.45	down	0.12	1.46		0.00	3.91	up
G7PS17*	0.03	-2.52	down	0.39	0.61		0.01	3.13	up
G7PSJ7	0.04	-2.21	down	0.52	0.69		0.03	2.90	up
G7PSZ1*	0.03	-1.87	down	0.22	1.02		0.01	2.88	up
G7PT00	0.05	2.27	up	0.12	1.58		0.47	-0.68	
G7PT41	0.03	-2.36	down	0.36	0.53		0.00	2.88	up
G7PT85	0.01	-5.17	down	0.57	-0.21		0.03	4.96	up
G7PT99	0.05	-2.77	down	0.76	-0.16		0.02	2.61	up
G7PTC0	0.07	-4.81		0.76	0.16		0.04	4.97	up
G7PTE0	0.01	-2.28	down	0.86	0.50		0.07	2.78	
G7PTL5	0.00	-1.98	down	0.32	1.03		0.01	3.01	up
G7PTR1*	0.05	-2.12	down	0.75	0.71		0.05	2.83	
G7PTX4*	0.01	-2.49	down	0.14	1.57		0.00	4.05	up
G7PU56	0.08	-1.50		0.13	1.21		0.01	2.71	up
G7PUC0	0.06	-1.75		0.33	1.22		0.02	2.96	up
G7PUH3*	0.02	-3.88	down	0.74	0.26		0.02	4.13	up
G7PUP9	0.07	-2.80		0.87	-0.14		0.03	2.66	up
G7PUS2*	0.02	-2.91	down	0.28	2.73		0.02	5.64	up
G7PUS3*	0.04	-4.32	down	1.00	-0.50		0.05	3.82	

Accession	Week6 Vs Week0			Week12 Vs Week0			Week12 Vs Week6		
	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend
G7PUS4	0.05	-3.00	down	0.77	0.21		0.02	3.20	up
G7PUS5	0.05	-3.57		0.81	0.08		0.05	3.66	up
G7PVR8	0.07	-3.17		0.66	-0.09		0.02	3.07	up
G7PVV5	0.02	-2.42	down	0.27	1.14		0.01	3.56	up
G7PW62	0.05	-1.52		0.20	1.19		0.01	2.71	up
G7PW68	0.42	-1.16		0.18	0.74		0.04	1.90	up
G7PW69	0.03	-2.07	down	0.53	0.46		0.01	2.53	up
G7PWX3	0.03	-2.86	down	0.79	-0.13		0.01	2.72	up
G7PWX6	0.02	-2.68	down	0.88	0.80		0.04	3.47	up
G7PX62	0.10	-2.50		0.55	-0.21		0.02	2.29	up
G7PXC3	0.04	-2.17	down	0.12	1.47		0.01	3.64	up
G7PXH3	0.00	-2.96	down	0.67	0.45		0.00	3.41	up
G7PXH4	0.02	-4.26	down	0.87	-0.30		0.04	3.96	up
G7PXI7	0.04	-3.29	down	0.64	0.29		0.02	3.58	up
G7PXJ2 *	0.00	-2.94	down	0.67	0.74		0.01	3.68	up
G7P XK8	0.01	-4.92	down	0.06	-2.10		0.58	2.82	
G7PXM0	0.34	-1.29		0.17	0.87		0.01	2.16	up
G7PXN2	0.01	-2.62	down	0.62	1.10		0.01	3.72	up
G7PXP4	0.00	-3.83	down	0.52	1.00		0.00	4.83	up
G7PXS2	0.04	-2.22	down	0.79	1.06		0.08	3.28	
G7PXX2	0.00	-3.38	down	0.64	0.40		0.00	3.78	up
G7PY92	0.12	-1.19		0.32	0.45		0.03	1.64	up
G7PYE1 *	0.03	-2.67	down	0.77	-0.15		0.01	2.52	up
G7PYG1	0.02	-3.54	down	0.87	1.29		0.10	4.82	
G7PYG8	0.04	-2.01	down	0.26	1.50		0.02	3.51	up
G7PYP2 *	0.01	-4.50	down	0.52	0.24		0.00	4.74	up
G7PZ75	0.01	-3.86	down	0.94	1.14		0.04	5.00	up
G7PZH6 *	0.07	-1.20		0.28	0.74		0.02	1.95	up
G7PZT1	0.10	-1.36		0.20	0.91		0.02	2.28	up
G7PZZ0	0.03	-2.19	down	0.44	1.00		0.01	3.19	up
G7PZZ8	0.02	-4.91	down	1.00	0.58		0.10	5.49	
G7Q017 *	0.03	-4.17	down	0.67	-1.75		0.00	2.42	up
G7Q062	0.05	1.15		0.03	1.87	up	0.46	0.73	
G7Q098	0.02	-2.40	down	0.78	0.38		0.03	2.78	up
G7Q0J9	0.03	-2.07	down	0.72	0.16		0.02	2.23	up
G7Q0W5	0.01	-2.45	down	0.25	0.74		0.00	3.18	up
G7Q1A4 *	0.03	-3.38	down	0.88	0.03		0.04	3.41	up

Accession	Week6 Vs Week0			Week12 Vs Week0			Week12 Vs Week6		
	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend
G7Q1J3	0.07	-0.88		0.01	-1.27	down	0.44	-0.39	
G7Q1Y5	0.01	-4.75	down	0.90	0.41		0.04	5.16	up
G7Q216	0.01	-2.04	down	0.58	0.65		0.01	2.69	up
G7Q221 *	0.00	-2.90	down	0.35	1.35		0.00	4.25	up
G7Q228	0.03	-1.53	down	0.23	0.95		0.01	2.48	up
G7Q2L0	0.00	-3.56	down	0.20	0.98		0.00	4.54	up
G7Q2W9 *	0.02	-1.84	down	0.07	1.07		0.00	2.91	up
G7Q337 *	0.00	-2.39	down	0.14	1.15		0.00	3.54	up
G7Q351 *	0.04	-3.17	down	0.62	-1.24		0.03	1.93	up
G7Q361	0.04	-4.67	down	0.85	-0.62		0.08	4.05	
G7Q3I2 *	0.02	-2.08	down	0.45	0.90		0.02	2.98	up
G7Q3I7 *	0.33	-0.78		0.07	1.99		0.02	2.77	up
G8F2B0	0.03	-1.68	down	0.14	1.21		0.01	2.89	up
G8F2D0 *	0.18	-1.93		0.10	0.91		0.01	2.84	up
G8F2G9	0.03	-2.63	down	0.51	0.67		0.02	3.29	up
G8F2T1	0.02	-2.27	down	0.38	0.90		0.01	3.16	up
G8F2X3 *	0.03	-1.82	down	0.60	1.31		0.04	3.14	up
G8F3A2	0.05	-2.39	down	0.32	1.84		0.02	4.24	up
G8F3M1	0.00	-2.32	down	0.03	1.90	up	0.00	4.22	up
G8F3W4 *	0.00	-2.91	down	0.39	0.60		0.00	3.51	up
G8F3X1	0.01	-1.91	down	0.26	1.35		0.01	3.26	up
G8F3X8	0.02	-2.20	down	0.69	0.16		0.01	2.36	up
G8F4A5	0.02	-3.01	down	0.85	0.41		0.03	3.41	up
G8F4M8 *	0.02	-4.97	down	0.72	-0.47		0.05	4.49	up
G8F4N6	0.08	-1.94		0.34	0.54		0.01	2.48	up
G8F4W0	0.01	-2.54	down	0.75	-0.07		0.05	2.48	
G8F4Z2 *	0.19	-2.24		0.28	0.59		0.04	2.84	up
G8F4Z4	0.14	-1.09		0.03	2.76	up	0.01	3.84	up
G8F5B0	0.00	-3.20	down	0.11	1.34		0.00	4.54	up
G8F5G1	0.01	-1.89	down	0.38	1.14		0.02	3.04	up
G8F5I4	0.08	-1.27		0.09	1.12		0.01	2.39	up
G8F5P8	0.06	-1.83		0.31	0.95		0.01	2.78	up
G8F5U5	0.23	-1.36		0.17	2.57		0.04	3.94	up
G8F5Y2	0.04	-2.34	down	0.49	1.48		0.04	3.82	up
G8F637	0.06	-1.59		0.26	0.99		0.02	2.59	up
G8F646 *	0.03	-1.86	down	0.58	0.86		0.03	2.72	up

Accession	Week6 Vs Week0			Week12 Vs Week0			Week12 Vs Week6		
	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend
G8F694	0.52	1.46		0.01	1.83	up	0.30	0.37	
G8F6C0	0.08	-1.59		0.16	1.43		0.01	3.02	up
HBA	0.03	-4.17	down	0.67	-1.75		0.00	2.42	up
HNRPK *	0.20	-4.59		0.34	1.57		0.02	6.17	up
HS90A *	0.07	-1.42		0.15	1.10		0.01	2.52	up
I7G286	0.00	-4.72	down	0.59	0.99		0.00	5.70	up
I7G469	0.00	-4.61	down	0.68	-0.04		0.03	4.57	up
I7G5A5 *	0.03	-2.31	down	0.89	0.79		0.14	3.10	
I7G7A9	0.00	-3.58	down	0.44	1.26		0.00	4.84	up
I7G7C5 *	0.16	-1.55		0.22	1.35		0.03	2.90	up
I7G7L7	0.44	-0.93		0.09	4.25		0.03	5.18	up
I7G7S3 *	0.04	-2.70	down	0.33	0.99		0.01	3.69	up
I7G8U7 *	0.01	-4.81	down	0.81	-0.26		0.06	4.55	
I7G8U9 *	0.04	-1.93	down	0.16	1.24		0.01	3.17	up
I7G9P9 *	0.01	-2.21	down	0.21	0.61		0.00	2.83	up
I7G9Q9 *	0.00	-3.62	down	0.84	0.62		0.01	4.24	up
I7G9S0	0.07	-1.56		0.17	1.19		0.01	2.75	up
I7GAK8	0.01	-2.90	down	0.75	0.63		0.01	3.53	up
I7GEN1 *	0.04	-1.81	down	0.20	1.04		0.01	2.85	up
I7GF08 *	0.00	-3.39	down	0.71	0.30		0.00	3.69	up
I7GH17 *	0.02	-2.47	down	0.81	0.25		0.02	2.71	up
I7GH35 *	0.13	-2.57		0.51	0.55		0.02	3.12	up
I7GH92 *	0.17	-1.40		0.32	1.57		0.05	2.97	up
I7GHA4	0.02	-3.01	down	0.98	-0.24		0.01	2.76	up
I7GHD6	0.15	-1.95		0.47	0.31		0.02	2.26	up
I7GHF1	0.03	-2.55	down	0.87	0.84		0.17	3.38	
I7GHV0 *	0.01	-3.37	down	0.66	1.10		0.02	4.47	up
I7GIB8 *	0.03	-1.55	down	0.26	0.83		0.01	2.37	up
I7GIP6	0.01	-3.81	down	0.60	-0.43		0.05	3.38	
I7GJP1	0.03	-1.64	down	0.61	0.43		0.02	2.07	up
I7GK56	0.02	-2.04	down	0.60	0.59		0.01	2.64	up
I7GK91 *	0.04	-2.06	down	0.22	0.71		0.01	2.77	up
I7GKE9	0.20	-1.07		0.06	2.16		0.01	3.23	up
I7GKJ1 *	0.00	-2.66	down	0.91	0.76		0.03	3.42	up
I7GKQ8 *	0.33	-2.75		0.12	0.63		0.04	3.39	up

Accession	Week6 Vs Week0			Week12 Vs Week0			Week12 Vs Week6		
	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend
I7GL64 *	0.05	-1.95	down	0.40	1.28		0.03	3.23	up
I7GL88	0.02	-2.22	down	0.38	0.81		0.01	3.03	up
I7GPN4	0.09	-2.89		0.70	0.07		0.02	2.96	up
IF4A2 *	0.05	-2.70	down	0.55	0.21		0.01	2.90	up
MIF *	0.01	-2.65	down	0.27	0.81		0.00	3.46	up
MNDA *	0.05	-2.78	down	0.43	1.51		0.01	4.28	up
NHRF1	0.00	-3.74	down	0.58	1.51		0.01	5.25	up
PEBP1	0.03	-2.35	down	0.55	-0.50		0.08	1.85	
PGM1 *	0.11	-1.71		0.07	1.57		0.00	3.28	up
PRDX2 *	0.02	-2.32	down	0.71	0.46		0.02	2.78	up
PRDX6	0.03	-2.45	down	0.22	0.53		0.00	2.97	up
Q18ND3	0.01	-2.05	down	0.97	0.47		0.04	2.52	up
Q25N99 *	0.03	-2.76	down	0.81	-0.11		0.05	2.65	
Q2PFQ8 *	0.08	-4.19		0.39	-0.40		0.01	3.79	up
Q2PFT0 *	0.00	-1.88	down	0.54	1.26		0.02	3.14	up
Q4R3L3 *	0.01	-3.71	down	0.94	0.84		0.04	4.55	up
Q4R3S2 *	0.02	-2.99	down	0.74	-0.14		0.05	2.85	up
Q4R432	0.21	-3.51		0.49	0.60		0.04	4.10	up
Q4R4M2 *	0.03	-1.96	down	0.53	1.27		0.03	3.23	up
Q4R4P0 *	0.06	-1.94		0.78	0.12		0.04	2.07	up
Q4R4U0	0.04	-1.75	down	0.53	0.55		0.02	2.30	up
Q4R4V0 *	0.05	-0.88		0.10	1.46		0.01	2.34	up
Q4R5G4 *	0.01	-2.75	down	0.43	0.56		0.00	3.31	up
Q4R5L3 *	0.07	-1.08		0.06	1.42		0.01	2.50	up
Q4R5N5	0.00	-3.31	down	0.14	0.98		0.00	4.29	up
Q4R5N7	0.11	-1.00		0.05	1.34		0.01	2.34	up
Q4R5Q1 *	0.02	-2.01	down	0.25	1.22		0.01	3.22	up
Q4R6D8	0.02	-2.13	down	0.84	0.18		0.02	2.31	up
Q4R810	0.04	-1.95	down	0.37	0.81		0.02	2.76	up
Q4R829	0.04	-1.72	down	0.09	1.46		0.01	3.18	up
Q4R8U5	0.01	-1.87	down	0.63	-0.07		0.05	1.80	
Q5ISL3 *	0.06	-4.42		0.88	0.38		0.05	4.80	up
Q5ISS9	0.08	-3.31		0.52	-0.03		0.00	3.28	up
Q5IST7	0.00	-3.02	down	0.84	0.16		0.00	3.19	up
Q5IST8 *	0.02	-3.75	down	0.21	1.69		0.01	5.44	up

Accession	Week6 Vs Week0			Week12 Vs Week0			Week12 Vs Week6		
	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend
Q8WNT7	0.02	-1.81	down	0.17	1.15		0.01	2.96	up
Q95LN9	0.01	-2.43	down	0.35	0.62		0.00	3.05	up
RL18 *	0.08	-3.52		0.52	1.21		0.04	4.73	up
RPN1	0.01	-4.19	down	0.66	-0.22		0.05	3.98	up
SAHH	0.03	-1.54	down	0.10	1.12		0.01	2.66	up
SEMG2	0.03	-4.17	down	0.20	2.97		0.01	7.14	up
SODC	0.00	-5.66	down	0.13	-0.98		0.01	4.68	up
SPB6 *	0.01	-3.83	down	0.83	0.15		0.03	3.98	up
TBA1B *	0.04	-2.88	down	0.62	0.85		0.02	3.73	up
TBB2A *	0.13	-2.57		0.51	0.55		0.02	3.12	up
TPIS	0.01	-2.41	down	0.78	0.34		0.01	2.75	up
UBE2N *	0.28	-0.78		0.07	1.31		0.04	2.09	up

*. More than one proteins assigned. Details please see supplement table S2

Table 3:

Comparative Quantitative Abundance Analysis of GCF proteins in response to ‘1x-treatment’ vs ‘3x-treatment’ of Cp40. Fold changes are converted to log2 value and proteins are considered regulated if fold change ≥ 0.5 or ≤ -0.5 and $p < 0.05$.

Accession	Week0			Week6			Week12		
	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend
ANXA5 *	0.61	-0.81		0.03	1.90	up	0.26	0.43	
CAN2 *	0.21	0.11		0.66	0.27		0.03	2.24	up
CATB *	0.06	2.58		0.21	1.21		0.03	1.60	up
CAZA2 *	0.24	0.04		0.82	0.09		0.02	1.24	up
CDC42 *	0.21	0.18		0.47	0.10		0.04	1.42	up
CRYAB *	0.12	0.69		0.05	1.85	up	0.08	1.85	
ENPL	0.25	-0.12		0.41	0.21		0.04	1.26	up
G7NTC9	0.01	2.59	up	0.82	-0.03		0.54	0.37	
G7NTD4	0.45	-0.26		0.26	1.47		0.01	3.31	up
G7NUQ0	0.40	-0.30		0.37	0.21		0.02	2.82	up
G7NUR3 *	0.31	-0.46		0.03	1.86	up	0.12	0.59	
G7NUU5	0.18	2.64		0.03	-2.83	down	0.08	1.98	
G7NV22	0.29	0.05		0.68	-1.10		0.05	1.64	up
G7NV40	0.21	0.20		0.30	1.25		0.02	2.58	up
G7NV96	0.15	0.69		0.93	-0.70		0.05	2.45	up
G7NW65	0.29	0.03		0.90	-0.23		0.02	1.35	up
G7NY46	0.35	0.58		0.03	-1.37	down	0.95	0.08	
G7NYM1	0.81	0.04		0.65	0.43		0.03	1.43	up
G7NYN6	0.62	-0.36		0.00	-1.89	down	0.09	-1.24	
G7P0L2	0.22	0.55		0.39	0.73		0.05	1.64	up
G7P1J3	0.27	-0.40		0.27	0.30		0.02	1.66	up
G7P3D8 *	0.05	1.08	up	0.18	0.72		0.14	1.15	
G7P3G7 *	0.45	-2.04		0.03	2.67	up	0.18	0.68	
G7P407	0.33	-0.15		0.03	1.38	up	0.05	1.35	
G7P448	0.21	0.27		0.63	-1.29		0.04	1.82	up
G7P4N8 *	0.11	0.63		0.48	0.76		0.02	1.97	up
G7P5F7	0.21	0.30		0.50	0.11		0.01	2.79	up
G7P5Q3	0.97	-0.37		0.40	0.81		0.03	1.86	up
G7P728	0.75	-0.79		0.03	-3.78	down	0.01	4.77	up
G7P747 *	0.95	0.11		0.97	-1.66		0.02	4.06	up
G7P987 *	0.52	0.25		0.80	-0.01		0.04	1.63	up

Accession	Week0			Week6			Week12		
	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend
G7P9E9	0.50	-0.34		0.35	2.41		0.04	3.66	up
G7PA06 *	0.06	1.69		0.61	1.23		0.05	1.33	up
G7PAB8	0.16	0.23		0.45	0.54		0.04	1.38	up
G7PB90	0.54	-0.31		0.01	2.38	up	0.53	-0.31	
G7PBL1	0.37	-0.17		0.03	1.99	up	0.10	1.53	
G7PBW8 *	0.23	0.12		0.56	-0.37		0.03	2.49	up
G7PC52 *	0.85	-0.52		0.01	-4.50	down	0.97	-2.07	
G7PCF6	0.25	0.28		0.21	0.81		0.02	2.06	up
G7PCU4 *	0.28	0.16		0.80	0.77		0.01	1.95	up
G7PD43	0.71	-1.32		0.69	-0.06		0.03	2.21	up
G7PE64	0.59	-0.19		0.09	0.69		0.02	3.09	up
G7PED5	0.14	1.38		0.23	-0.74		0.02	2.16	up
G7PFC9	0.35	-0.46		0.19	0.67		0.03	1.51	up
G7PFM1	0.23	1.30		0.47	-0.29		0.01	2.93	up
G7PFN4 *	0.31	-0.61		0.70	-0.16		0.03	2.65	up
G7PGB0 *	0.95	-0.30		0.35	0.37		0.03	1.60	up
G7PGL4	0.57	-0.54		0.35	1.21		0.03	9.66	up
G7PGL7	0.42	0.47		0.19	2.85		0.01	2.44	up
G7PHH9 *	0.70	0.91		0.41	-0.24		0.00	4.38	up
G7PIC9 *	0.31	0.36		0.02	1.98	up	0.16	0.99	
G7PID3	0.39	-0.09		0.03	1.59	up	0.22	0.47	
G7PID5	0.35	0.32		0.03	2.28	up	0.15	0.81	
G7PID9	0.33	0.22		0.05	1.84	up	0.16	1.25	
G7PIG1 *	0.24	-0.46		0.86	1.09		0.03	1.85	up
G7PJD6	0.24	0.68		0.49	0.39		0.04	2.16	up
G7PJR5	0.75	0.12		0.03	1.31	up	0.73	-0.70	
G7PLB1	0.21	0.33		0.17	1.00		0.02	1.59	up
G7PLD3	0.84	-0.64		0.02	-4.46	down	0.05	3.07	up
G7PLL6	0.11	0.47		0.84	-1.03		0.04	1.89	up
G7PMB2 *	0.34	0.19		0.28	0.55		0.05	1.14	up
G7PMB3	0.23	0.17		0.75	-3.21		0.05	1.30	up
G7PN00	0.06	0.75		0.24	0.79		0.04	2.47	up
G7PP62	0.10	-0.20		0.11	1.64		0.03	2.14	up
G7PPY5	0.35	0.15		0.60	-0.54		0.01	2.20	up
G7PQY3 *	0.98	-1.03		0.01	-5.63	down	0.57	-4.07	
G7PRF3 *	0.18	0.06		0.41	-1.68		0.04	1.85	up

Accession	Week0			Week6			Week12		
	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend
G7PRZ9 *	0.31	-0.19		0.68	0.62		0.04	1.50	up
G7PT85	0.41	-1.03		0.01	-8.08	down	0.44	-0.75	
G7PTX4 *	0.24	0.63		0.78	-0.91		0.02	2.57	up
G7PU56	0.20	0.32		0.24	0.09		0.05	1.47	up
G7PUF6	0.62	-0.41		0.16	1.82		0.02	2.46	up
G7PUP9	0.37	-0.07		0.03	1.51	up	0.26	0.09	
G7PVV5	0.21	0.34		0.09	2.32		0.01	2.99	up
G7PW68	0.29	0.33		0.09	2.01		0.02	1.65	up
G7PX62	0.29	0.46		0.78	0.08		0.03	1.48	up
G7PXC3	0.12	0.61		0.92	0.36		0.02	1.93	up
G7PXJ2 *	0.04	1.41	up	0.81	-0.44		0.06	1.80	
G7PY92	0.92	-0.79		0.85	-0.17		0.05	1.16	up
G7PYE1 *	0.38	0.49		0.03	1.25	up	0.10	1.05	
G7PZ75	0.51	0.59		0.03	-3.69	down	0.35	0.95	
G7PZT1	0.57	-1.15		0.82	0.08		0.01	1.89	up
G7Q017 *	0.76	-0.76		0.02	-5.57	down	0.51	-4.24	
G7Q062	0.04	-2.36	down	0.23	0.55		0.11	1.11	
G7Q2L0	0.23	0.28		0.75	-0.94		0.03	1.63	up
G7Q337 *	0.12	0.64		0.93	-0.70		0.04	1.53	up
G7Q3I7 *	0.89	-0.45		0.64	0.65		0.01	1.95	up
G8F2B0	0.28	0.20		0.32	0.52		0.05	1.37	up
G8F2D0 *	0.45	0.32		0.28	1.07		0.02	1.81	up
G8F3M1	0.22	0.10		0.22	0.38		0.01	2.03	up
G8F3W4 *	0.20	0.32		0.61	0.07		0.04	1.24	up
G8F3X1	0.43	-0.05		0.79	-0.36		0.03	2.09	up
G8F4W8	0.04	-1.05	down	0.52	-0.43		0.12	-1.74	
G8F4Z2 *	0.39	0.40		0.98	0.41		0.04	1.98	up
G8F4Z4	0.27	-0.60		0.05	3.05		0.01	3.31	up
G8F5B0	0.14	0.96		0.46	0.26		0.04	2.05	up
HBA	0.76	-0.76		0.02	-5.57	down	0.51	-4.24	
HNRPK *	0.54	1.12		0.28	-1.89		0.01	4.00	up
HS90A *	0.33	-0.15		0.03	1.38	up	0.05	1.35	
I7G286	0.11	0.72		0.04	-3.25	down	0.00	3.54	up
I7G7C5 *	0.29	0.53		0.35	1.22		0.05	1.68	up
I7G7L7	0.68	-0.56		0.82	0.99		0.03	3.86	up

Accession	Week0			Week6			Week12		
	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend	Anova (p)	Log2 FC	Trend
I7GEN1 *	0.46	-0.26		0.41	-0.49		0.02	1.90	up
I7GH35 *	0.74	-0.21		0.03	1.22	up	0.16	1.25	
I7GHA4	0.07	1.62		1.00	-0.41		0.05	1.40	up
I7GIB8 *	0.17	0.29		0.11	1.70		0.03	1.46	up
I7GK56	0.68	-1.13		0.52	0.05		0.05	1.58	up
I7GKE9	0.47	-0.50		0.08	1.82		0.05	1.81	up
I7GL88	0.29	0.12		0.61	0.19		0.04	1.62	up
I7GPN4	0.24	3.21		0.16	0.35		0.05	3.09	up
MNDA *	0.25	1.39		0.81	-0.66		0.01	3.84	up
Q29427	0.37	-0.28		0.60	0.50		0.01	6.94	up
Q4R3L3 *	0.15	1.13		0.12	-2.69		0.01	3.90	up
Q4R4V0 *	0.09	1.06		0.35	1.57		0.01	2.87	up
Q4R5L3 *	0.19	0.41		0.34	0.83		0.02	1.61	up
Q4R5N5	0.20	0.41		0.68	-0.74		0.01	1.91	up
Q4R5Q1 *	0.28	-0.09		0.17	1.43		0.05	1.50	up
Q4R6D8	0.15	0.20		0.37	1.13		0.04	1.70	up
Q4R829	0.23	0.24		0.82	-0.10		0.02	2.02	up
Q7M2S8 *	0.35	0.18		0.54	-0.13		0.01	4.69	up
SEMG2	0.57	-2.70		0.20	0.84		0.01	4.91	up
SODC	0.04	1.34	up	0.00	-3.79	down	0.71	0.46	
TBB2A *	0.74	-0.21		0.03	1.22	up	0.16	1.25	
TCPQ	0.15	0.51		0.42	2.06		0.03	1.77	up
UBE2N *	0.15	1.04		0.66	0.75		0.02	1.53	up

*. More than one proteins assigned. Details please see supplement Table S4