Transcriptomics throughout the life cycle of *Leishmania infantum*: high down-regulation rate in the amastigote stage.

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ABSTRACT

Leishmania infantum is the causative agent of zoonotic visceral leishmaniasis in the Mediterranean Basin. The promastigote and amastigote stages alternate in the life cycle of the parasite, developing inside the sand-fly gut and inside mammalian phagocytic cells respectively. High-throughput genomic and proteomic analyses have not focused their attention on promastigote development, though partial approaches have been made in *L. major* and *L. braziliensis*. For this reason we have studied the expression modulation of an ethiological agent of visceral leishmaniasis throughout the life cycle, which has been performed by means of complete genomic microarrays. In the context of constitutive genome expression in *Leishmania* spp. described elsewhere and confirmed here (5.7%), we have found a down-regulation rate of 68% in the amastigote stage, which has been contrasted by binomial tests and includes the down-regulation of genes involved in translation and ribosome biogenesis. These findings are consistent with the hypothesis of pre-adaptation of the parasite to intracellular survival at this stage.

Key words: *Leishmania infantum*, life cycle, genomic DNA microarrays, amastigote, down-regulation.

1. INTRODUCTION

Leishmania infantum is the ethiological agent of zoonotic visceral leishmaniasis in Mediterranean areas. Domestic dogs are the main reservoir of the parasite (Alvar et al., 2004) and an increase in co-infection with HIV has been registered (Pasquau et al., 2005). The dimorphic life cycle of the parasite consists of promastigote development to an infective stage inside the gut of phlebotominae sand-flies followed by transmission to the definitive mammalian host dermis and survival of non-motile amastigotes inside phagocytes (reviewed in (Handman, 2001)). Promastigote development inside the vector gut was mimicked in axenic culture (Neal and Miles, 1963; Lemma and Schiller, 1964; Steiger and Steiger, 1976, 1977; Berens and Marr, 1978) and the biology of the promastigote stage has been widely studied, but only under these conditions. On the other hand, amastigotes from mammalian phagocytes are not widely recognised as being equivalent to axenic amastigotes, a controversial issue (e.g. (Gupta et al., 2001; Debrabant et al., 2004)) which was resolved by the finding of considerable differences between axenic and intracellular amastigotes in the gene expression profile (Holzer et al., 2006; Rochette et al., 2009). Therefore, lesion-derived amastigotes and *in vitro*-cultured macrophage-derived amastigotes are generally more suitable for the study of parasite biology. Nevertheless, noticeable infection rates have been reported for axenic amastigote experimental models (Debrabant et al., 2004).

Comparing expression profiling of amastigotes with logarithmic (Akopyants et al., 2004; Almeida et al., 2004; Holzer et al., 2006; Leifso et al., 2007) and stationary phase promastigotes (Akopyants et al., 2004; Almeida et al., 2004; Saxena et al., 2007; Srividya et al., 2007; Rochette et al., 2009) has been performed using microarray technology. However, surprisingly gene expression modulation during promastigote differentiation has not aroused much interest as only three expression profile analyses have been performed by partial genome microarrays so far. Namely, 188 (Saxena et al., 2003) and 22 (Almeida et al., 2004) differentially regulated genes were found for logarithmic *vs.* stationary phase promastigotes (heterogeneous populations) and 36 (Akopyants et al., 2004) for procyclic *vs.* metacyclic promastigotes in *L. major*. Subsequently, 22 differentially regulated genes have been found in *L. braziliensis* promastigotes (Depledge et al., 2009). We reported the first procyclic *vs.* metacyclic promastigote transcriptomic analysis by means of whole-genome shotgun

DNA microarrays which produced 300 differentially regulated genes in a species causing visceral leishmaniasis (Alcolea et al., 2009). A reduced number of differentially regulated genes has also been described elsewhere (Holzer et al., 2006; Cohen-Freue et al., 2007; Leifso et al., 2007), which supports the hypothesis of constitutive gene expression in *Leishmania* spp. with changes in gene expression modulation in a reduced number of genes. In order to assess gene expression profiles throughout the *L. infantum* life cycle, we have given the results of microarray hybridization assays for transcriptome comparison of stationary *vs.* logarithmic phase promastigotes (S/L), stationary phase promastigotes *vs.* intracellular amastigotes (S/A) and logarithmic phase promastigotes *vs.* intracellular amastigotes (L/A). As a result, a set of differentially regulated genes has been found throughout the life cycle of the parasite in a variety of biochemical and physiological processes (transport, movement and cytoskeletal functions, sugar and lipid metabolism, oxidative phosphorylation, amino acid and polyamine metabolism, DNA replication, gene expression, protein maturation and secretory pathway). The differential regulation pattern of the HASP/SHERP cluster is consistent with specificity for the stationary-phase but not the metacyclic stage, except for HASPB, which is up-regulated in amastigotes. Previously, these and other genes were reported to be metacyclic specific, but transcript analysis was only for heterogeneous populations in the stationary phase instead of purified procyclic and metacyclic subpopulations (Saxena et al., 2003; Almeida et al., 2004). Consequently, specific markers for the metacyclic stage have yet to be found, so the terms metacyclic and stationary phase promastigote should be used rigorously. For this reason, a comparison between procyclic-metacyclic (Alcolea et al., 2009) and logarithmic-stationary phase promastigote expression profiles is described in this study, which should be useful to distinguish between such terms.

In the context of constitutive genome expression in *Leishmania* spp. (Holzer et al., 2006; Cohen-Freue et al., 2007; Leifso et al., 2007), transcriptomic analysis results suggest a greater down-regulation rate in the amastigote stage with respect to logarithmic phase promastigotes provided that the set of down-regulated genes is notably greater and contains a wide variety of ribosomal constituents and translation initiation factors. In this study we have performed binomial contrasts for gene modulation data sets contained in this manuscript and published elsewhere for several *Leishmania* spp. that confirm a significantly greater down-regulation rate in amastigotes. These

findings have led us to conclude that down-regulation prevails over up-regulation in the amastigote stage and supports a previously reported hypothesis (Depledge et al., 2009) about the limited dynamic modulation of gene expression for the adaptation of amastigotes to the intraphagolysosomal environment.

2. MATERIAL AND METHODS

2.1 Promastigote culture and amastigote isolation from in vitro infected macrophages.

L. infantum isolate M/CAN/ES/98/10445 (zymodeme MON-1) was cultured in RPMI 1640 supplemented with L-glutamine (Cambrex, Karlskoga, Sweden), 10% heat inactivated foetal bovine serum (HIFBS) (Cambrex) and 100 μg/ml streptomycin – 100 IU/ml penicillin (Cambrex) (complete medium) at 27 ºC and a starting density of 2 x 10^6 promastigotes/ml. Experiments were performed on three replicate early passage (9^{th} for replicate 1 and 3 and 8^{th} for replicate 2) axenically cultured preinocula, originally obtained from the gut of experimentally infected sand-flies, axenized and frozen for storage as described (Moreno et al., 2007). Cell density was assessed daily and promastigotes were recovered on days 2 and 7 and washed in PBS by centrifugation at 2000g for 10 min.

U937 human monocyte-like cell line was cultured at 37 ºC in a 5% CO_2 atmosphere at a starting density of 10^5 cells/ml in complete medium and harvested at 250g after 72 h. Cells were then stimulated with 20 ng/ml phorbol 12-myristate 13-acetate (PMA) in complete medium for 72 h (Minta and Pambrun, 1985). After washing mildly with RPMI supplemented with L-glutamine (Cambrex), adhered cells were recovered by shaking the flask vigorously 10-20 times in the presence of 0.5g/l trypsin, 0.2g/l EDTA (Trypsin-Versene, Cambrex). The trypsin was subsequently inactivated by adding one volume of complete medium and then the cells were harvested. Macrophage infections were performed by incubating 20 x 10^6 promastigotes/ml:10^6 macrophages/ml at 37 ºC for 2 h in complete medium. This incubation was carried out in a water bath and tubes were mildly agitated at 15 min intervals. After that, the mixture was centrifuged at 250g for 10 min and incubated in fresh complete medium for 2 hours in the presence of 5% CO_2. This step was repeated after 16 hours and incubations continued until 72 h. After infected macrophage recovery, amastigotes were obtained by

macrophage lysis with 0.5% SDS in RPMI with vigorous agitation for 1 min followed by centrifugation at 13000g for 1 min (Hart et al., 1981). Aliquots of the infected macrophage culture were fixed for modified Giemsa staining and indirect immunofluorescence assays (IFA) before macrophage lysis. In order to perform IFA, cells were fixed with acetone:methanol (1:1) at -20 ºC for 10 min at a density of 2 x 10^4/5 µl drop. Then, they were incubated with SIM 6.11.2.1. monoclonal anti-gp63 IgG, SIM 110 monoclonal IgG antibody against soluble leishmanial antigens (SLA), anti-rabbit complement factor H antibody or PBS at 37 ºC for 30 min in a hydration chamber, washed three times by mild agitation in PBS for 10 min, incubated with fluorescein isotiocyanate (FITC)-conjugated goat anti-mouse IgG (Serotec, Raleigh, NC) and 0.1% Evans' Blue (Fisher, Pittsburgh, PA). Washes were repeated and preparations mounted with 90% glycerol. Negative controls were anti-rabbit complement factor H monoclonal IgG and PBS.

2.2 Microarray hybridization assays, analysis of selected clone sequences and validation.

Genomic DNA was isolated from non-infected U937 cells as described (Alcolea et al., 2009) and directly labelled with Cy5 (350 µM dATP, dCTP, dGTP and (1/3 Cy5-dUTP + 2/3 dTTP)) using a GenomiPhi™ DNA amplification kit (GE Healthcare). RNA isolation, quality assessment and amplification, labelled cDNA synthesis, *L. infantum* random genome microarray hybridization, normalization of raw data, statistical analysis, clone sequencing, assembling and mapping of DNA sequences and validation by qRT-PCR were performed as described previously (Alcolea et al., 2009). Briefly, hybridization experiments were as follows: Cy5-labelled cDNA from stationary-phase promastigotes (day 6) vs. Cy3-labelled cDNA from logarithmic-phase promastigotes (day 2) (S/L); Cy5-cDNA from stationary-phase promastigotes (day 6) vs. Cy3-cDNA from amastigotes (S/A); and Cy5-cDNA from logarithmic-phase promastigotes (day 2) vs. Cy3-cDNA from amastigotes (L/A). U937 Cy5-labelled DNA was hybridized following the same procedure. LOWESS per pin algorithm was used to normalize medians of raw fluorescence intensity values and ratios after background substraction. The criteria for a given spot to be considered as differentially regulated were: (i) $F \geq 1.7$ (Cy5/Cy3 ratio if Cy5 > Cy3) or ≤ -1.7 (-Cy3/Cy5 ratio if Cy3 > Cy5), (ii) total relative fluorescence

intensity value > 5000 FU and (iii) $p < 0.05$ (adjusted by FDR). The ends of the selected clones were sequenced with m13-pUC18 oligonucleotides and aligned with the *L. infantum* genome project sequence in General Feature Format (GFF) deposited in a GBrowse database. Forward and reverse reads were mapped to define the boundaries of the clones in the genome. Depending on mapping and assembling outcomes, clones were classified as type a (congruent alignments, one single pair of alignments), type b (congruent alignments, more than one pair of alignments) and type c (uncongruent alignments or lack of one insert end read). Clones containing more than one gene sequence and type *c* clones needed to be analysed by qRT-PCR by the SYBR-Green method to sort out which genes are differentially regulated (Alcolea et al., 2009). Reaction and quantification protocol and primers are provided in Table S1. Glimmer 3.0 annotations were performed when clones were mapped against a genomic sequence that did not contain any annotated gene. Stage-specifically regulated genes were re-annotated and analysed with BLAST2GO to establish a molecular function and biological process GO term distribution among them according to α-score (Conesa et al., 2005).

2.3. Binomial test.

Differential regulation of genes is a dichotomous variable defined by two categories: down-regulation and up-regulation. Transcriptome analysis by means of DNA microarray hybridization and proteome approaches are independent random samplings carried out simultaneously for hundreds or thousands of genes and proteins from the same genome and proteome respectively. Therefore, this variable follows a binomial distribution B (n, p), in which the defining parameters are n = number of trials and p = probability of one out of the two events; let p be the probability of down-regulation and q = 1-p the probability of up-regulation. The null hypothesis of absence of significant differences between the number of up- and down-regulated genes (H_0: $p_0 = q_0$) in a given set of differentially regulated genes is suitable for contrasting by means of a binomial test. When $np > 10$ and $nq > 10$, B (n, p) can be approximated to a normal distribution $N(np, (npq)^{1/2})$ according to the Laplace-De Moivre theorem. This distribution can be typified by a standard normal N(0, 1) when $n > 50$, in which $Z = [(p-p_0)/(p_0q_0/n)^{1/2}]$. When these procedures are not permitted, an exact binomial test can be applied: calculate $P(X \geq r) = [P(X = r) + P(X = r + 1) + \ldots + P(X = n)]$, where r is the number of times the event

with probability p occurred and and $P(X = r) = [n!p^r q^{n-r}]/[r!(n-r)!]$. See the table of frequencies in Table S2. The fold-change cutoff described in 2.2 was applied to all the differentially regulated gene data sets prior to contrasting them with the hypothesis using the binomial test.

3. RESULTS AND DISCUSSION

3.1. Overview of the microarray hybridization analysis.

The infection of differentiated U937 cells with promastigotes to obtain intracellular amastigotes was checked by gp63 IFA and the absence of contaminant promastigotes by promastigote surface antigen (PSA or gp46) IFA (Fig. 1). Randomly sheared genomic DNA from the host cells was hybridized with *L. infantum* genomic microarrays in order to be able to discard cross-hybridizing spots in the subsequent microarray analysis. Because of the efficient amastigote isolation procedure based on mild macrophage lysis (Hart et al., 1981), cross-hybridization was not found under the hybridization conditions described (Fig. S2), considering the intensity cutoff value (see 2.2.) after the substraction of the local background median. S/L, S/A and L/A high throughput transcriptomic analyses have been carried out by hybridizing of labelled cDNAs from the main life cycle stages with shotgun genomic microarrays as previously described (Alcolea et al., 2009) (Figs. 2, S1 and S2). 20% differentially regulated gene outputs have been validated by qRT-PCR. As in previous reports, we have observed a correlation between qRT-PCR, microarray analysis and co-regulation of adjacent genes in some of the clones by means of this technique, which is consistent with a limited rate of RNA co-regulation, as already reported (Saxena et al., 2007).

We have detected 222 differentially regulated genes throughout promastigote differentiation (S/L), 210 in S/A differentiation and 303 in the amastigote-to-promastigote transition (L/A) (Fig. 2 and 3). Previously, we compared procyclic and metacyclic promastigote homogeneous subpopulations in the stationary phase (Alcolea et al., 2009) but now we have come to realize by means of Venn diagrams that metacyclic promastigotes up-regulate a distinct set of genes rather than the whole population in the stationary phase, as well as stationary phase procyclics compared to logarithmic phase promastigotes (all procyclics) (Fig. 3A). When considered together with the HASP/SHERP cluster expression profile (see below)**,** it becomes clear that the term metacyclic promastigote should

223 be more rigorously cited in literature and more clearly distinguished from the term stationary phase
224 promastigote. The degree of differentiation is higher in purified metacyclic fractions than in
225 heterogeneous populations of stationary phase promastigotes. Nevertheless, the transcriptome
226 information extracted from both stages is complementary. Gene sets described in this report and in the
227 procyclic-metacyclic transcriptome analysis (Alcolea et al., 2009) have been grouped according to
228 their annotated functions (Table 1). Differentially regulated genes with a more or less known function
229 are described as non-redundant in tables 2-4. In Tables S3-S8 the following are described: conserved
230 hypothetical genes, hypothetical genes of unknown function, unknown genes, pseudogenes and
231 unresolved clones (containing more than one gene annotation and/or type *c* clones (Alcolea et al.,
232 2009) or lacking gene annotations). We have observed a higher rate of hypothetical proteins and
233 unknown genes up-regulated in the amastigote stage. Additionally, some clones presumably contain
234 differentially regulated genes that have not yet been annotated in the *L. infantum* genome sequence
235 project (Tables S4, S6 and S8). This fact has been observed in all the experiments and strongly
236 suggests that gene annotation of the *L. infantum* genome is still not complete and therefore,
237 oligonucleotide microarrays do not represent all genes.

238 To some extent, there is a similarity in the expression profile of some genes between in vitro-
239 cultured macrophage infection-derived amastigotes and temperature plus pH shift-induced amastigote-
240 like forms as previously described (Alcolea et al., 2010), which confirms the relevance of these factors
241 in the differentiation of promastigotes to amastigotes. Nevertheless, axenic and intracellular
242 amastigote expression profiles have been described as being considerably different (Holzer et al.,
243 2006; Rochette et al., 2009) despite the similarities found. With respect to promastigote
244 differentiation, only four reports have been published (Saxena et al., 2003; Akopyants et al., 2004;
245 Almeida et al., 2004; Alcolea et al., 2009), and in all of them the analysis of the procyclic-metacyclic
246 promastigote expression profile has been carried out with whole-genome microarrays and in a species
247 that cause visceral leishmaniasis.

248

249 *3.2. Gene expression modulation throughout the life cycle of Leishmania infantum.*

Microarray hybridizations with logarithmic and stationary phase promastigote cDNAs have been reported only in four studies (Saxena et al., 2003; Akopyants et al., 2004; Almeida et al., 2004; Depledge et al., 2009), and even then only the procyclic stage of promastigote development has been considered in the majority of amastigote vs. promastigote transcriptome analyses (Holzer et al., 2006; Leifso et al., 2007; Srividya et al., 2007; Rochette et al., 2009). Our description of the transcriptome variation in the life cycle of the *L. infantum* is illustrated in Figs. 4, 5, S3 and S4. Biological processes and molecular functions from the GO database were annotated on genes with BLAST2GO software, and this analysis has provided an overview of the transcriptome variations (Figs. 4 and S3). Activities related with gene expression seem to be more activated in stationary phase promastigotes with respect to the two other stages analysed, provided that α-score values indicate that biological processes such as translation and ribosome biogenesis biological processes are highly represented at this stage with respect to logarithmic phase promastigotes (S/L) and intracellular amastigotes (S/A). These terms are related to structural constituents of ribosomes and molecular functions like RNA binding. On the other hand, the dividing stages of the parasite that survive in their respective hosts after the establishment of infection up-regulate genes related to cellular biosynthetic processes and other metabolic processes. There are few GO annotations made on genes up-regulated in the amastigote stage due to the more reduced numbers found and the significant proportion of genes of unknown function among them (Fig. S3).

DNA packaging, epigenetics, replication and nucleotide metabolism. Genes from the four histone families have been found to be differentially regulated. Some of them and histone deacetylase (H-deAc) are up-regulated in L/A, while other histone genes show different regulation patterns (see more details in Supplementary data file 3), which suggest different epigenetic regulation mechanisms between the promastigote and the amastigote stage. Structural maintenance of chromosome 3 protein gene (smc3) is down-regulated in S/L and is involved in chromosome organization during cell division. With regards to DNA replication, we have detected the up-regulation in S/A of a minichromosome maintenance complex protein (mcm) involved in replication initiation and down-regulation in S/L of DNA polymerase I α catalytic subunit (cDNAPol IαB) and mitochondrial DNA polymerase I protein D (mPol ID). Furthermore, mPol ID is up-regulated in L/A and consequently, its

278 greatest transcript levels are reached in logarithmic-phase promastigotes, which suggests a greater
279 genome replication rate at this stage.

280 Adenosine kinase domain-containing nucleoside diphosphate kinase b genes (Ndkb) are up-
281 regulated in S/A and were previously found to be up-regulated in the procyclic subpopulation with
282 respect to the metacyclic from the population in stationary phase (Alcolea et al., 2009). Another
283 differentially regulated gene involved in nucleotide metabolism is the phosphoribosyl pyrophosphate
284 synthetase gene (PRPPS), which is specifically down-regulated in S/A. Regarding tandemly
285 positioned 3'-nucleotidase/nuclease (3'NT/Nase) genes, up-regulation has been detected in S/A. This
286 gene is specifically down-regulated by the effect of temperature increase combined with medium
287 acidification (Alcolea et al., 2010). Likewise, its overexpression in logarithmic-phase promastigotes
288 and absence in amastigotes have been described elsewhere (Sopwith et al., 2002). PRPPS down-
289 regulation and Ndkb plus 3'NTase/Nase up-regulation in S/A are consistent with a higher purine
290 nucleoside uptake combined with a higher rate of nucleoside triphosphate biosynthesis in stationary
291 phase promastigotes, which is probably related to a higher genome replication rate.

292 *Gene expression, protein maturation and transport and secretory pathway*. The following four
293 genes are differentially regulated at the transcriptional and post-transcriptional levels: DNA-directed
294 RNA polymerase II subunit 9 gene which is down-regulated in S/L; U5 RNA-splicing factor that is
295 up-regulated in S/L (suggesting changes in small nucleolar RNA (snRNA) processing in stationary-
296 phase promastigotes); a component of a nucleolar small ribonucleoprotein (snRNP) which is up-
297 regulated in L/A; and splicing factor 3A that is down-regulated in L/A. At the translational level, 16
298 ribosomal component transcripts and 4 genes involved in translation initiation (initiation factors IF2
299 and IF2α, IF3s8 and IF5a) are up-regulated in L/A. Despite 12 ribosomal proteins being up-regulated
300 in S/L and 23 in S/A, none of the translation regulation factors up-regulated in L/A have been found to
301 be differentially regulated at this stage. However, an eukaryotic translation initiation factor (EIF) and a
302 GTP-binding elongation factor from Tu family (EF-Tu) are down-regulated (further details in
303 Supplementary data file 3), which suggests changes in the global gene expression rate at the level of
304 protein biosynthesis.

Endoribonuclease L-PSP (pb5) is involved in single-stranded mRNA cleavage according to GO and we have found its up-regulation in L/A and S/A. Previously, L-PSP (pb5) was found to be significantly down-regulated in mature amastigotes (Rosenzweig et al., 2008) and may be involved in specific post-transcriptional regulation of gene expression. The functional characterization of L-PSP would be helpful for understanding gene expression regulation in trypanosomatids. The P1/S1 nuclease gene may be involved in specific gene expression regulation during the amastigote stage, as it is down-regulated in S/A. Several minicircle sequences are up-regulated in S/L, in L/A and in metacyclic PNA^- compared to procyclic PNA^+ stationary phase promastigotes (Alcolea et al., 2009). As a consequence, the RNA editing process seems to increase in differentiated stages of the life cycle: metacyclic promastigotes and amastigotes.

Peptidyl-prolyl cis-trans isomerases prevent adenosine kinase domain (and consequently Ndkb) aggregation (Sen et al., 2007) and accelerate protein folding by catalyzing the cis-trans isomerization of proline imidic peptide bonds in oligopeptides (Chakraborty et al., 2004). Two FKBP-type (FKBP) and a cyclophilin-type (Cph) peptityl-prolyl cis-trans isomerase are up-regulated in L/A and were also found as up-regulated in stationary phase procyclic promastigotes (Alcolea et al., 2009). In other words, the parasite down-regulates these genes in the ongoing differentiation process, which suggests that protein refolding and oligomerization via peptidyl-prolyl cis-trans isomerases is more relevant in developing promastigotes.

In the dolichol-anchored oligosaccharide biosynthetic process, the first UDP-activated monosaccharide residue is added by the enzyme UDP-N-acetylglucosamine:dolichyl-phosphate N-acetylglucosamine phosphotransferase (UDP-NAGDPT) (EC 2.7.8.15), which is down-regulated in amastigotes (L/A and S/A). This finding indicates that N-glycosylation of proteins is less abundant in amastigotes than in promastigotes. Two genes indirectly involved in protein glycosylation are also differentially regulated, namely phosphomanomutase (PMM) which is up-regulated in S/A, and glucose-6-phosphate N-acetyl transferase (GNAT) that is also up-regulated in S/A but down-regulated in S/L. The N-acetylglucosaminylphosphatidylinositol de-N-acetylase gene (NAGPID) is up-regulated in L/A and is involved in the second step of glycosylphosphatidylinositol (GPI) anchor biosynthesis in all eukaryotes (Doering et al., 1989), which anchors lipophosphoglycan (LPG) in *Leishmania* spp.

(reviewed by (Ilgoutz and McConville, 2001)). Indeed, the LPG biosynthetic protein 2 (*lpg2*) is up-regulated in L/A. Apart from that, the α subunit of the protein farnesyltransferase gene (FNTA) is down-regulated in S/L, which suggests that prenylation is less common in stationary than in logarithmic phase promastigotes. In addition, an isoprenylcysteine methyltransferase (ICMT) gene has been found to be down-regulated in S/A.

With regard to vesicle coating, the syntaxin gene (Sx) is down-regulated in S/L whereas a clathrin heavy chain gene (clathrin-H), an adaptor complex subunit medium chain 3 (Apcx3) and a vesicle-associated membrane protein gene (*vamp*) are up-regulated in L/A. *vamp* is also up-regulated in S/A and down-regulated in S/L. Sx, clathrin-H, Apcx3 and *vamp* expression profiles suggest that vesicle trafficking activity reaches its greatest intensity in immature promastigotes, which would be consistent with the up-regulation of already mentioned genes involved in LPG and protein maturation in that stage which respect to amastigotes and/or stationary phase promastigotes.

Hexose metabolism, oxidative phosphorylation and transport. Two tandemly positioned glucose transporter genes (GT) are up-regulated in S/L, S/A and L/A. Consequently, glucose uptake is expected to reach its highest levels in stationary-phase promastigotes. GT down-regulation is specifically induced by the exposure to concomitant temperature increase and acidification (Alcolea et al., 2010). Regarding the subsequent Embden-Meyerhoff pathway, fructose 1,6-bisphosphate aldolase (ALD) and 2,3-bisphosphoglycerate-independent phosphoglycerate mutase (PGM^{BPI}) have been found to be up-regulated in S/A. ALD, PGM^{BPI} and glycosomal malate dehydrogenase (gMDH) genes were also found to be repressed by temperature plus pH shift (Alcolea et al., 2010). On the other hand, we have detected the down-regulation in L/A of a catabolite regulation-dependent PGM gene. As far as the Krebs cycle (TCA) is concerned, GDP-forming succinyl-CoA ligase (SCL) is up-regulated in S/A. Conversely, a 4-methyl-5-(beta-hydroxyethyl)-thiazole monophosphate synthesis protein (MPET) is up-regulated in L/A and down-regulated in S/L. This gene is required for thiamine pyrophosphate (TPP) biosynthesis and acts as an important cofactor precursor for the pyruvate dehydrogenase complex (PDH). It was previously found to be down-regulated in metacyclic *vs.* procyclic promastigotes (Alcolea et al., 2009). This means that the highest expression levels of METP are reached in procyclic/logarithmic-phase promastigotes. Gluconeogenesis activation is expected to take

place in stationary-phase promastigotes, because glycosomal phosphoenolpyruvate carboxykinase (gPEPCK) is up-regulated in S/L. Furthermore, two genes involved in glycogen biosynthesis are up-regulated in S/A, specifically phosphoglucomutase (PGluM) and glycogen sintase kinase (GSK).

The mitochondrial inner membrane ADP/ATP translocases 1and 2 (ANC1 and ANC2) are up-regulated in L/A. ANC1 and ANC2 carry ATP from the mitochondrial matrix into the inter-membrane space and transports ADP back (Cozens et al., 1989). Moreover, two distinct non-ligated ATPase subunit 9 genes (F_0s9) are up-regulated in L/A. These genes are involved in ATP synthesis coupled to the respiratory electron transport chain according to the annotations made with BLAST2GO, PFAM and InterPro databases (see Supplementary data file 3). In addition, three respiratory electron transport chain complex III components are differentially regulated throughout the parasite life cycle, namely cytochrome c oxidase complex subunits V (coxV) , VI (coxVI) and VIII (coxVIII). Subunits coxV and coxVIII are up-regulated in L/A, coxV is down-regulated in S/L and coxVI is up-regulated in S/A. These facts suggest a decrease in respiratory electron transport chain and oxidative phosphorylation activities in amastigotes with respect to logarithmic-phase promastigotes. Other ATPase genes are also differentially regulated, as well as several ABC transporter genes (further details in Supplementary data file 3). For instance, the sodium stibogluconate resistance protein (SbGRP) and multidrug resistance protein (MRP) are down-regulated in amastigotes. Other kinds of transporters are also differentially regulated in the life cycle, specifically, a vacuolar-type proton-translocating pyrophosphatase (vH^+-PPase) that is up-regulated in S/A, Tim17 which is up-regulated in L/A, and an amino acid permease gene that is up-regulated in S/L and down-regulated in S/A (see details in Supplementary data file 3).

Porphyrin biosynthesis. *Leishmania* parasites are auxotrophes for porphyrins, although the last three enzymes of porphyrin biosynthetic pathway are coded in the genome of the parasite. Amastigotes uptake phorphyrins from the host cell by protein endocytosis or as a free group (McConville et al., 2007), while logarithmic-phase promastigotes up-regulate coproporphyrinogen III oxidase (C(III)O) and protoporphyrinogen oxidase (PO) genes, but not ferrochelatase. In fact, C(III)O and PO are up-regulated in L/A, S/A and down-regulated in S/L. As a consequence, the highest transcript levels of both genes are reached in stationary-phase promastigotes. The combined exposure

389 to temperature increase and pH decrease is sufficient for their down-regulation (Alcolea et al., 2010).
390 These observations suggest that promastigotes synthesize porphyrins from a precursor inside the gut of
391 the sand-fly vector thus highlighting the importance of heme group biosynthesis in that environment
392 for survival.

393 *Aminoacid and polyamine metabolism and redox homeostasis.* Cystathionine γ-lyase (CGL),
394 involved in Cys, Ile, Thr and Ser metabolism, is down-regulated in S/L. CGL reaction product L-
395 cystathionine connects with methionine metabolism, in which S-adenosyl-L-methionine decarboxylase
396 (SAMDC) is S/A-up-regulated. SAMDC is responsible for the rate limiting reaction of S-adenosyl-
397 methionine decarboxylation into S-adenosyl-methioninamine in the biosynthetic pathway of
398 polyamines in mammals and nematodes (Da'dara and Walter, 1998). Additionally, the putrescine
399 biosynthetic agmatinase gene is up-regulated in S/A and L/A. Products of the previous reactions, S-
400 adenosyl-methioninamine and putrescine, are the substrates for spermidine synthase (SpS), which is
401 down-regulated in S/L. A spermidine molecule is coupled to two glutathione molecules in the
402 trypanothione molecule. The trypanothione reductase gene (TR) is down-regulated in L/A, as well as
403 by the combined effect of temperature and pH shift (Alcolea et al., 2010). Consequently, these data do
404 not clarify if there is preferential regulation of polyamine or of any amino acid biosynthetic pathway in
405 *L. infantum*. In order to better understand these findings, the generation of knockout parasites for these
406 genes could be helpful, and especially for grouping genes with the same expression pattern (CGL and
407 SpS; SAMDC, agm and NdKb).

408 *Lipid metabolism*. The triacylglycerol (TAG) lipase (TGL) and lipase precursor-like genes are
409 located in tandem and are down-regulated in S/L. Two other different lipase genes show different
410 expression patterns, one being down-regulated in S/A and the other one in L/A. In spite of this, all
411 these TGL develop triacylglycerol lipase activity according to GO analysis and catalytic activity
412 annotation EC 3.1.1.3., which could be explained by the preferential regulation of each TGL isoform
413 for each of the main developmental stages. In a previous characterization of temperature and pH
414 influence on differentiation (Alcolea et al., 2010), we detected the up-regulation of one of these TGLs.
415 FA from TAG degradation could be destined either to β-ox and to prostaglandin or sphingolipid
416 biosyinthesis processes. The prostaglandin F synthase gene (PGFS) is down-regulated in S/L and up-

417 regulated in S/A and was also found as down-regulated in both PNA^- (metacyclic) promastigotes
418 (Alcolea et al., 2009) and temperature-pH-generated amastigote-like forms (Alcolea et al., 2010),
419 which support the previous suggestion of a prostaglandin F2α role as a competence factor for
420 promastigotes inside the sand-fly vector (Kabututu et al., 2002). In sphingolipid biosynthesis,
421 dihydroceramide is reduced to ceramide by sphingolipid δ-4 desaturase, a gene down-regulated in S/L
422 but up-regulated in L/A, in S/A and in axenically-cultured amastigotes obtained by a combined
423 temperature and pH shift (Alcolea et al., 2010). As a result, *L. infantum* promastigotes reach their
424 highest levels of sphingolipid δ-4 desaturase transcripts in the logarithmic phase, which suggests an
425 increase in inositol phosphoceramide glycan-based anchoring of surface proteins during this stage. As
426 observed previously (Alcolea et al., 2010), there is a lack of correlation between PGFS/sphingolipid δ-
427 4 desaturase down-regulation and TGL up-regulation in amastigotes. Conversely, these three genes are
428 up-regulated in the logarithmic phase with respect to stationary phase promastigotes, which is
429 consistent with a preferential destination of FA from TGL-mediated degradation to these pathways
430 and a higher biosynthetic rate of prostaglandins and sphingolipids in logarithmic phase promastigotes.
431 The 3-hydroxymethylglutaryl-CoA reductase gene (3-HMG-CoA reductase) has been found to be
432 down-regulated in L/A and is specifically up-regulated by temperature plus pH shift (Alcolea et al.,
433 2010). C8-sterol isomerase is down-regulated in S/L. No differential regulation of any other gene
434 involved in ergosterol biosynthesis has been detected in promastigotes and amastigotes. Meanwhile,
435 the lathosterol oxidase gene is up-regulated in S/A, as well as by temperature and pH shift (Alcolea et
436 al., 2010).

437 *Proteolysis.* Various protease genes are up-regulated in the differentiated stages (see
438 Supplementary data file 3). Furthermore, several proteins related to ubiquitin-proteasome protein
439 degradation machinery are differentially regulated in the life cycle progression. The proteasome α2
440 subunit takes part in ubiquitin-dependent protein catabolic processes and presents threonine-type
441 endopeptidase activity. With regards to our findings, this gene and the ubiquitin-conjugating enzyme
442 E2 gene (UBC2) are up-regulated in L/A in agreement with previous reports (Rosenzweig et al.,
443 2008). Proteins destined to proteasome-mediated degradation are ubiquitinylated by ubiquitin ligase

(E3) after conjugation of E2 with ubiquitin. Two ubiquitin-like genes (Ubq) are also up-regulated in L/A and a distinct ubq in S/L. Considered together, these data suggest that ubiquitin labelling for protein degradation increases with amastigote to promastigote differentiation, except for the third ubq isoform mentioned. Ubiquitin molecules may act as rapid markers for protein degradation and also as chaperones for correct protein folding (Rawlings and Barrett, 1994), a possibility that would offer a feasible explanation for distinct expression regulation patterns found in different ubq genes. Different patterns have been found for another proteasome complex-related genes such as proteasome regulatory non-ATPase subunit LinJ07_V3.1300 (rS-non-ATPase) and proteasome β3 subunit (LinJ28_V3.0110) genes which are down-regulated in S/L and and cysteine peptidase, ClanCA, family C12 (UHC-C12) that is up-regulated in S/L. UHC-C12 shows ubiquitin C-terminal phosphohydrolase activity (EC. 1.4.19.12) for the release of ubiquitin residues from tagged proteins. Further research on the regulation of proteasome-mediated degradation would be useful for studying the relationship between differentiation and protein degradation throughout the *Leishmania* spp. life cycle.

Signal transduction and cell cycle regulation. Despite the fact that both logarithmic phase promastigotes and amastigotes are dividing forms, the differences found between both stages in the gene expression pattern of a cdc-2 related kinase and three mitogen activated protein kinase (MAPK) genes suggest a different division rate or cell cycle regulation or both (see details in Supplementary data file 3). Some proteins involved in signal transduction processes have been found to be differentially regulated. Firstly, a beta propeller protein (β-prop) and an ADP-ribosylation factor (ADPrf) are up-regulated in L/A. β-prop is a transducin because it contains a G-beta repeat structure (PF00400) and ADPrf has GTP binding and small GTPase activity (GO). Moreover, protein phoshatase 2C (PP2C) and catalytic subunit of PP1 (cPP1) are up-regulated in L/A and cPP1 is also up-regulated in S/A but down-regulated in S/L. Adenylate cyclase and five protein kinase (PK) genes including GSK have also been found to be differentially regulated (Supplementary data file 3). Calcium-dependent cysteine-type endopeptidase (C2cp, μ-calpain) (E.C. 3.4.22.52) is up-regulated in metacyclic promastigotes (Alcolea et al., 2009), L/A and S/A, thus lending support to the suggestion that the highest C2cp transcript levels are reached in the metacyclic stage as would be expected and

indicating its possible involvement in morphological changes. Two other peptidase genes involved in signal transduction have been found to be differentially regulated, specifically, the presenilin-like aspartic peptidase gene from A22A family (A22Aap) which is up-regulated in S/A and serine peptidase E from S51 family (SerP51) in S/L as mentioned above.

Cytoskeleton and movement. Three paraflagellar rod components (PAR4, PFR and PFR1D) are up-regulated in L/A. PFR and PFR1D are structural components of the flagellar macromolecular complex and bind calmodulin while PAR4, also up-regulated in S/A, is involved in flagellum biogenesis and organization according to GO analysis. A set of dynein genes have also been found to be differentially regulated. Among flagellar dynein light chain DLC1 family members, we have detected the up-regulation in L/A and the down-regulation in S/L of dynein light chain lc6 flagellar outer arm type 1 (DynLC6) and a dynein light chain (DynLC). Two non-flagellar dyneins have also been detected as being up-regulated in L/A. Firstly, a dynein light chain protein from the Tctex-1 family (DLCTctex-1), and secondly a light chain 2b of cytoplasmic dynein (Dyn2bLC7_18.1010), which belongs to the Roadblock/LC7 family (PF03259) and is involved in vesicle and organelle transport inside the cytoplasm by microtubule de/polymerization. Two more dynein genes of unknown location are differentially regulated. One is a dynein heavy chain gene (DynH) that is up-regulated in S/A and the other is a dynein-associated roadblock protein-like protein (DynLC7) which is down-regulated in S/L. An α-tubulin and a microtubule-associated protein with unknown function (MTap) are up-regulated in L/A. On the other hand, an actin gene is down-regulated in L/A and S/A. As mentioned above, C2cp is up-regulated in S/L, S/A and L/A and is involved in cytoskeletal remodelling. Finally, a centrosome-associated caltractin gene is up-regulated in S/L. To sum up, some genes related with the flagellar and paraflagellar structures are down-regulated in amastigotes.

Surface molecules and genes of unknown function. The hydrophilic surface protein (HASP)/SHERP cluster located in chromosome 23 is differentially regulated during the progression of the life cycle of the parasite. The HASPA1 hydrophilic surface protein of unknown function is up-regulated in S/L, S/A and L/A, with the resulting highest expression level in stationary phase promastigotes. The HASPB gene is down-regulated in S/A but and was found to be up-regulated in temperature/pH-induced amastigote-like forms (Alcolea et al., 2010), whereas HASPA2 is up-

regulated in S/L. SHERP genes LinJ23_V3.1210/30 (both located between HASPA1, HASPA2 and HASPB) are up-regulated in S/L and the latter is also up-regulated in S/A. No differences were detected between procyclic (PNA^+) and metacyclic (PNA^-) stationary-phase promastigotes (Alcolea et al., 2009). Despite previous statements (Saxena et al., 2003), all the data suggest that these genes are not adequate markers for metacyclic promastigotes, because they are not strictly specific to the stage. Conversely, they are stationary phase promastigote markers, except for HASPB. In addition to surface molecules coded by HASP/SHERP cluster, three surface antigen-like proteins of unknown function (SALp) are differentially regulated: one of them is up-regulated in L/A and S/A and down-regulated in S/L; another SALp is up-regulated in L/A, whilst SALp2 is down-regulated in L/A. With regards to the A2-A2rel cluster, the A2 gene is down-regulated in L/A and S/A. This gene was spotted as a control on the genomic microarrays and it is up-regulated in the amastigote stage as expected. The 3'a2rel gene is also down-regulated in L/A and S/A and it has been found to be up-regulated in S/L. 5'a2rel-related is up-regulated in S/A. A2rel genes flank the A2 gene and are not exclusive to the amastigote stage, as a difference with A2 gene, only found to be up-regulated in amastigotes and temperature plus pH shift-induced amastigote-like forms (Alcolea et al., 2010).

Several genes from the amastin-tuzin clusters are differentially regulated. Some amastin genes are not amastigote-specific in spite of their up-regulation during this stage. The function of these genes has not been characterized to date so their characterization together with knowledge of their expression modulation would contribute to the understanding of their role in the parasite's physiology. Finally, the high rate of hypothetical and unknown gene up-regulation in amastigotes reported in this study and elsewhere (e.g. (Rochette et al., 2009)) is specially intriguing.

3.3. Gene down-regulation prevails over up-regulation in the amastigote stage.

In 2007, Leifso *et al*. concluded that the genome of *Leishmania* spp. is constitutively expressed, given their own finding of only ≈3% differentially regulated genes in a microarray-based transcriptomic analysis of an *L. major* amastigote expression profile referred to the logarithmic phase promastigote stage (Leifso et al., 2007). Prior to this, however, the data obtained in an expression profiling of *L. major* and *L. mexicana* amastigote-to-promastigote (Holzer et al., 2006) pointed to the

same differential regulation rate. Both analyses were performed by means of oligonucleotide microarrays covering the entire set of annotated genes in the *L. major* genome project, which are suitable for global gene expression modulation estimation, when compared with low coverage oligonucleotide, shotgun genomic microarray transcriptomic or proteomic approaches (Table 5). Subsequent studies of the expression profiles of metacyclic promastigotes and temperature-pH shift-induced axenic amastigotes with whole-genome shotgun genomic DNA microarrays (Alcolea et al., 2009; Alcolea et al., 2010) also highlighted the constitutive expression of the *L. infantum* genome, which has been further confirmed in the analysis reported in this study by means of the same microarrays for logarithmic, stationary phase promastigotes and intracellular amastigotes. A higher rate of differential regulation in axenic amastigotes was found by means of oligonucleotide microarrays with complete coverage of annotated genes in the *L. infantum* genome project (Rochette et al., 2009), although the intensity value cutoff was not considered in that microarray analysis nor in the *L. mexicana* and *L. major* reports (Holzer et al., 2006; Leifso et al., 2007). This fact must be taken into account as it may have led to an overestimation of the differential expression rate.

In the context of the hypothesis of constitutive gene expression in *Leishmania* spp., the differential expression profile that we have found for genes involved in translation (see *Gene expression, protein maturation, secretory pathway and nucleotide metabolism* subsection in the previous section) supports the suggestion that translation is less active in intracellular amastigotes than in promastigotes, with the greatest levels of gene expression in logarithmic-phase promastigotes. Furthermore, this reduced translational activity is consistent with the initial observation of a smaller number of up-regulated than down-regulated genes in amastigotes referred to logarithmic- and stationary-phase promastigotes (dL/A and uL/A, respectively). Considering the completeness of the genomic DNA microarrays used for this transcriptomic analysis, the results suggest that down-regulation prevails over up-regulation in amastigote differentiation. The specific numbers of differentially regulated genes found is 224 up-regulated *vs.* 80 down-regulated in L/A and 129 up-regulated *vs.* 81 down-regulated in S/A (Tables 2-4; summarized in tables 1 and 5 and Fig. 3). In addition, data sets contrasting sharply the variety of down-regulated genes with the very limited amount of up-regulated genes in amastigotes have been published previously elsewhere, except for

555 some analyses performed by partial genome approaches (reviewed in Table 5). On the basis of these
556 observations, we were able to subject the null hypothesis of lack of significant differences between up-
557 and down-regulation in amastigotes to a binomial test. Individually, six reports on the expression
558 profile of the amastigote stage compared with logarithmic or stationary phase promastigotes or both in
559 several *Leishmania* spp. showed a highly significant predominance of down-regulated over up-
560 regulated genes in amastigotes (reviewed in Table 5). More up-regulated genes in amastigotes were
561 found in two out of three partial transcriptomic analyses carried out in *L. major* (Akopyants et al.,
562 2004; Leifso et al., 2007), while fewer up-regulated genes were described in another report on the
563 same species (Almeida et al., 2004). According to the binomial test outcome, none of these differences
564 are statistically significant. This was not the case of the higher number of up-regulated proteins found
565 in a partial proteomic and transcriptomic analysis in *L. infantum* (McNicoll et al., 2006), for which we
566 have only calculated significance of the differences found in the protein set. This was because of the
567 lack of independence in sampling of the transcripts reported, as only transcripts corresponding to the
568 outcomes of the proteomic analysis were analysed. Higher but statistically non-significant amastigote-
569 like (axenic amastigote) down-regulation rates have been observed (Table 5) for previous data sets
570 (Rochette et al., 2009; Alcolea et al., 2010). The outputs were respectively: lack of statistical
571 significance of the differences between up- and down-regulated gene sets; predominance of down-
572 regulation; and greater abundance of up-regulation. To summarize, statistically significant higher
573 down-regulation rates have been found for intracellular amastigote stages, with two exceptions, and
574 lack of significant differences in axenic amastigotes. Interestingly, the only high throughput proteomic
575 approach performed in the genus *Leishmania* (Rosenzweig et al., 2008) revealed a data set in which
576 the down-regulation rate in amastigotes is significantly greater (Table 5). All the intracellular
577 amastigote expression profiles described by high throughput transcriptomic and proteomic analyses
578 are consistent with the predominance of down-regulation.

579 Intraspecies binomial tests were also performed, as microarray and proteomic data mining are
580 equivalent to independent sampling procedures from unknown populations of genomic sequences and
581 protein sets in terms of expression rate. In spite of the exceptions described, the intraspecific binomial
582 tests have revealed highly significant differences between up-regulation and down-regulation in *L.*

major, *L. mexicana*, *L. donovani* and *L. infantum* amastigotes, both including and excluding axenic amastigotes. Therefore, the binomial tests performed for each individual data subset and the intraspecific sets point to a significantly higher frequency of down-regulation in amastigotes. The interspecific global binomial test has revealed very significant differences between down- and up-regulated genes in amastigotes, with a clear predominance of down-regulation. A global test was also performed by separating L/A and S/A comparisons in order to exclude a possible influence of promastigote differentiation, but the null hypothesis was rejected again with a very low p-value. These statistical analyses of differential regulation rates and down-regulation in amastigotes of a considerable set of genes related with gene expression regulation clearly show that the down-regulation rate is higher than the up-regulation rate in intracellular amastigotes whether at the post-transcriptional or the post-translational level. These findings provide additional evidence in support of the hypothesis of amastigote preadaptation for intracellular survival in addition to the limited dynamic modulation of gene expression at the post-transcriptional level (Depledge et al., 2009) in different *Leishmania* spp. including the *L. (Leishmania)* subgenus causative of both human cutaneous and visceral leishmaniasis in the Old and New World.

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LEGEND TO FIGURES

Fig. 1. Isolation and characterization of the promastigote and amastigote populations. (A) Average growth curve of the replicate promastigote cultures. (B-G) Cultures of stimulated U937 lines infected with promastigotes for the preparation of intracellular amastigotes. (B) Modified Giemsa staining. (C) PBS as negative control for the FITC-conjugated anti-mouse IgG secondary antibody. (D) Monoclonal anti-rabbit complement factor H primary antibody negative control. (E) SIM110 monoclonal anti-SLA as positive control. (F) SIM 6.11.2.1. monoclonal anti-gp63 IgG. (G) Purified monoclonal anti-gp46 IgG. Evan's Blue was used as the contrast staining in C-G.

Fig. 2. Average M/A scatter plots of three-replicate microarray hybridization analyses. The stages compared are S/L, S/A and L/A and the respective cDNA samples were labelled as Cy5/Cy3. M = ($\log_2$Ri−$\log_2$Gi) and A = [($\log_2$Ri+$\log_2$Gi)/2], where R and G are respectively red (Cy5) and green (Cy3) intensity values. Red spots correspond to selected DNA fragments containing a gene up-

752 regulated at least 1.7 times and green spots represent those down-regulated at least 1.7 times. Further
753 criteria for spot selection are detailed in Materials and methods section. Standard deviations are
754 shown.
755
756 **Fig. 3. Overview of gene expression modulation throughout the life cycle of *L. infantum*.** The
757 number of differentially regulated genes is indicated in each of the transitions between the main stages
758 of the cycle. (A) The microarray hybridization analysis S/L has resulted in 96 genes up-regulated in
759 logarithmic phase promastigotes and 114 in stationary phase promastigotes. Venn diagrams for the
760 comparison of the transcriptome analysis output for logarithmic/stationary phase promastigotes and
761 for the previously reported for procyclic/metacyclic analysis (Alcolea et al., 2009) clearly show that
762 both gene expression profiles are very dissimilar. Indeed, only 6 and 5 genes respectively are
763 coincident between the two expression profiles. (B) Gene expresion profiling of the S-A transition
764 shows that 129 genes are up-regulated in S/A whereas 81 are down-regulated. gp63 IFA was
765 performed to check U937 infections for the purification of intracellular amastigotes, which appear in
766 green colour in the micrograph to contrast with the red staining (Evans' Blue) of the host cell. (C) 129
767 genes are up-regulated in the transition of amastigotes to logarithmic phase promastigotes whereas 79
768 are down-regulated. Table 1 breaks down genes into each stage according to annotation status.
769
770 **Fig. 4. Multilevel sector charts of α-scores for GO terms annotated in down-regulated genes in**
771 **amastigotes.** GO term nodes at different level in the ontology have been filtered by an α-score value
772 of 2 with BLAST2GO and represented in these diagrams. (A) and (B) are respectively the sector charts
773 for biological process and molecular function terms annotated on genes up-regulated in L/A. (C) and
774 (D) are referred to S/A. Terms related to gene expression and its regulation are highly represented at
775 both the biological process and the molecular function level in the promastigote with respect to the
776 amastigote stage.
777

Fig. 5. Diagram of the differential expression profiles of S/L, S/A and L/A in *L. infantum*. Proteins coded by stage-specifically regulated genes are represented in the leishmanial expression and secretory pathways, plasma membrane, cytosol and organelles. Functional relations are also shown whenever possible. See abbreviations in the text. (A) Gene expression, secretory pathway and ubiquitin-proteasome degradation. (B) Metabolism and kDNA-related genes. (C) Surface molecules, cytoskeleton, vessicle trafficking, signal transduction and cell cycle regulation.

Table 1. Differential gene regulation in *Leishmania infantum*: status of gene annotations. The number of differentially regulated genes in each of the main stages is given in the table. A breakdown of genes according to the presence or absence of annotated gene functions and gene annotation in the sequence contained in the clone is described (each clone without gene annotation presumably contains a differentially regulated gene).

Annotation status		Number of differentially regulated genes								
		dS/L	*uS/L*	*dSmet/ SPro*	*uSmet/ Spro*	*uS/A*	*dS/A*	*uL/A*	*dL/A*	*Total*
Genes with annotated function		41	56	52	36	72	30	104	13	404
Hypothetical protein genes		17	15	37	44	22	27	50	26	238
Unknown genes		0	0	0	0	0	0	2	0	2
Pseudogenes		1	0	0	0	0	0	1	0	2
Minicircle sequences		0	2	0	5	0	1	1	0	9
Unresolved clones	containing annotated gene functions	23	21	7	9	4	18	18	18	118
	lacking annotated gene sequences	20	25	26	84	31	5	48	22	262
Total		102	119	122	178	129	81	224	79	1035

d, down-regulated; u, up-regulated; dS/L, genes down-regulated in stationary versus logarithmic phase promastigotes; uS/L, genes up-regulated in stationary versus logarithmic phase promastigotes; dSmet/pro, genes down-regulated in the subpopulation of metacyclic promastigotes with respect to the procyclic promastigotes from the promastigote population in stationary phase; uSmet/pro, genes up-regulated in the subpopulation of metacyclic promastigotes with respect to the procyclic promastigotes from the promastigote population in stationary phase; uS/A, up-regulation in stationary phase promastigotes versus amastigotes; dS/A, down-regulated genes in stationary phase promastigotes versus amastigotes; dL/A, genes down-regulated in logarithmic phase promastigotes versus amastigotes; uL/A, genes up-regulated in logarithmic phase promastigotes versus amastigotes.

Table 2. Differentially regulated genes in stationary versus logarithmic phase promastigotes S/L. The features described are: clone number; F (up-regulation if F > 1.7 and down-regulation if F < -1.7); base-two logarithmic scale F and S.D. values; *p*; GenBank GSS accession numbers; e-values; Def., clone definition according to mapping outcomes *a*, *b* or *c* (see explanation in 2.2.); Id., annotation in the *L. infantum* genome sequence; annotated gene function; relative quantitative real time reverse transcription-PCR (qRT-PCR). When a given clone overlaps with more than one annotation (asterisk in Def., only clones checked for differential regulation using qRT-PCR appear in this table.

Clone	F	$Log_2F \pm SD$	P	GenBank GSS	e-value		Def.	Id.	Annotated Gene Function	qRT-PCR	
					Fw	Rv				+/-	F ± SD
Lin14E10	1.92	1.0 ± 0.2	0.039	**GS882298**	0	7e-170	b	LinJ31_V3.1930	Ubiquitin fusion protein (ubq)		N.D.
Lin15B2	1.73	0.7 ± 0.2	0.045	**GS882299**	2e-77	2e-80	b	LinJ34_V3.2680	Regulatory subunit of protein kinase A-like protein (rPKA)		N.D.
Lin18F11	2.11	0.9 ± 0.2	0.010	**GS882300**	0	5e-137	b	LinJ31_V3.3380	Sodium stibogluconate resistance protein, putative (SbGRP)		N.D.
Lin50G2	1.87	0.9 ± 0.1	0.007	**GS882301**	0	0	b	LinJ34_V3.2660	Amastin-like surface protein, putative		N.D.
Lin54D12	2.31	1.2 ± 0.3	0.018	**GS882302**	5e-137	-	b	LinJ13_V3.1130	40S ribosomal protein S4, putative (S4)		N.D.
Lin54H9[a]	3.41	1.8 ± 0.3	0.007	**GS882303**	0	0	a	LinJ32_V3.0460	40S ribosomal protein S2 (S2)		N.D.
Lin63B7	2.33	1.2 ± 0.4	0.027	**GS882304**	1e-103	1e-103	b	LinJ15_V3.1200	60S acidic ribosomal protein P2		N.D.
Lin63F3	2.48	1.3 ± 0.3	0.021	**GS882305**	0	0	a	LinJ36_V3.6550	Glucose transporter, lmgt2, putative (GT)	+	6.6 ± 1.0
								LinJ36_V3.6560	Glucose transporter, putative (GT)	+	4.3 ±1.3
Lin65G6	1.78	0.8 ± 0.3	0.037	**GS882306**	0	0	b	LinJ08_V3.0690	Amastin-like protein		N.D.
								LinJ08_V3.0700	Amastin-like protein		N.D.
Lin67B7[a]	2.52	1.3 ± 0.5	0.039	**GS882307**	0	0	b	LinJ29_V3.1160	Ribosomal protein L1a, putative (L1a)	+	7.4 ± 0.9
Lin82G7	1.87	0.9 ± 0.4	0.047	**GS882308**	0	0	b	LinJ24_V3.0420	Cysteine peptidase, Clan CA, family C12, putative (UHC-C12)	+	3.1 ± 0.6
Lin83D1	1.88	0.9 ± 0.1	0.005	**GS882309**	0	0	b	LinJ08_V3.0700	Amastin-like protein		N.D.
								LinJ08_V3.0710	Amastin-like protein		N.D.
Lin86B4	3.58	1.8 ± 0.7	0.047	**GS882310**	0	0	b	LinJ36_V3.6550	Glucose transporter, lmgt2, putative (GT)	+	6.6 ± 1.0
Lin90H12	3.06	1.6 ± 0.4	0.016	**GS882311**	0	0	b	LinJ36_V3.0990	40S ribosomal protein S18, putative (S18)		N.D.
Lin91F9[a]	2.14	1.1 ± 0.4	0.034	**GS882312**	2e-105	-	c	LinJ31_V3.1660	Putative 3 ketoacyl coa thiolase-like protein (Thiol I)	+	4.6 ± 0.3
Lin92F7	1.70	0.8 ± 0.2	0.019	**GS882313**	0	0	b	LinJ35_V3.0400	40S ribosomal protein S3a, putative (S3a)		N.D.
Lin92H2	1.72	0.8 ± 0.2	0.020	**GS882314**	0	0	b	LinJ36_V3.2560	Caltractin, putative		N.D.
Lin96F4	1.78	0.8 ± 0.1	0.015	**GS882315**	0	0	b	LinJ26_V3.0370	Hypothetical protein, conserved		N.D.
								LinJ26_V3.0380	RET2,RNA editing complex MP57		?
Lin96G5	1.81	0.9 ± 0.1	0.007	**GS882316**	0	0	b	LinJ36_V3.3940	40S ribosomal protein S27-1, putative (S27-1)		N.D.
Lin101B5	2.06	1.0 ± 0.4	0.048	**GS882317**	0	0	b	LinJ09_V3.0650	Serine peptidase family S51, peptidase E, putative (SerP51)	+	14.0 ± 0.4
Lin101D5	1.75	0.8 ± 0.1	0.004	**GS882318**	4e-39	0	b	LinJ27_V3.2500	Glycosomal phosphoenolpyruvate carboxykinase, putative (gPEPCK)		N.D.
Lin113A12	2.02	1.0 ± 0.3	0.032	**GS882319**	2e-65	2e-40	b	LinJ23_V3.1200	Hydrophilic surface protein 2 (HASPA1)	+	13.1 ± 1.7
								LinJ23_V3.1210	SHERP	+	2.5 ± 0.1
								LinJ23_V3.1220	Hydrophilic surface protein (HASPB)	+	4.9 ± 0.5
Lin118C1	2.40	1.3 ± 0.4	0.030	**GS882320**	0	0	b	LinJ08_V3.0700	Amastin-like protein		N.D.
Lin127E8	1.77	0.8 ± 0.2	0.030	**GS882321**	0	0	b	LinJ35_V3.1870	60S ribosomal protein L5, putative (L5)		N.D.
Lin128D7	2.01	1.0 ± 0.2	0.039	**GS882322**	0	0	b	LinJ21_V3.2150	40S ribosomal protein S6, putative (S6)		N.D.
Lin129H10	2.55	1.3 ± 0.3	0.020	**GS882323**	0	0	b	LinJ08_V3.0960	Cathepsin L-like protease (Cathepsin-L)		N.D.
Lin147H2	1.84	0.9 ± 0.2	0.032	**GS882324**	0	0	b	LinJ34_V3.1160	Tuzin-like protein		N.D.
Lin149H2	1.87	0.9 ± 0.3	0.029	**GS882325**	0	0	a	LinJ20_V3.1210	Cysteine peptidase, Clan CA, family C2, putative (C2cp)		N.D.
								LinJ20_V3.1220	Cysteine peptidase, Clan CA, family C2, putative (C2cp)		N.D.
Lin156F3[a]	1.73	0.8 ± 0.3	0.045	**GS882326**	0	0	c	LinJ29_V3.3010	Amastin, putative	+	2.1 ± 0.3
								LinJ29_V3.3020	Tuzin-like protein, putative	+	4.2 ± 0.6
Lin160E1[a]	1.76	0.8 ± 0.3	0.032	**GS882327**	0	0	b	LinJ35_V3.1870	60S ribosomal protein L5, putative		N.D.
Lin163A7	2.11	1.1 ± 0.1	0.006	**GS882328**	0	2e-170	b	LinJ23_V3.1220	Hydrophilic surface protein (HASPB)	+	4.9 ± 0.5
								LinJ23_V3.1230	SHERP	+	3.8 ± 0.2
								LinJ23_V3.1240	Hydrophilic surface protein 2 (HASPA2)	+	7.4 ± 0.8
Lin166B10	1.95	1.0 ± 0.2	0.018	**GS882329**	1e-26	0	b	LinJ31_V3.1850	Amino acid permease (aap)		N.D.
Lin189A7[a]	1.74	0.8 ± 0.3	0.044	**GS882330**	0	0	a	LinJ34_V3.2730	Ribosomal protein L3, putative (L3)		N.D.
Lin165D2	3.90	2.0 ± 0.3	0.005	**GS882331**	0	0	b	LinJ23_V3.1230	SHERP	+	3.8 ± 0.2
Lin194E2[a]	1.70	0.8 ± 0.1	0.008	**GS882332**	0	0	c	LinJ08_V3.0730	Tuzin, putative	+	2.4 ± 0.1
Lin194F11	1.96	1.0 ± 0.2	0.022	**GS882333**	0	0	b	LinJ31_V3.0950	Sodium stibogluconate resistance protein, putative (SbGRP)	+	7.6 ± 1.0
Lin196A12[a]	2.10	1.1 ± 0.3	0.031	**GS882334**	0	0	b	LinJ29_V3.1170/80	Ribosomal protein L1a, putative (L1a)		N.D.
Lin210C4	1.70	0.8 ± 0.3	0.045	**GS882335**	0	0	b	LinJ08_V3.0690	Amastin-like protein		N.D.
Lin219E1	1.73	0.8 ± 0.1	0.006	**GS882336**	0	0	b	LinJ32_V3.3330	Ribosomal protein L3, putative (L3)		N.D.
Lin231G5	2.17	1.1 ± 0.1	0.001	**GS882337**	0	0	b	LinJ35_V3.2040	60S ribosomal protein L32 (L32)		N.D.
Lin245E2	2.03	1.0 ± 0.2	0.034	**GS882338**	0	4e-39	b	LinJ22_V3.0680	3´a2rel-related protein (3'a2rel)		N.D.
Lin251A12	1.73	0.8 ± 0.1	0.004	**GS882339**	7e-124	0	c	LinJ34_V3.2660	Amastin-like surface protein, putative		N.D.
Lin251A8	1.78	0.8 ± 0.2	0.034	**GS882340**	0	0	b	LinJ23_V3.0860	3-ketoacyl-Coa thiolase-like protein (Thiol I)		N.D.
Lin253D4	1.92	0.9 ± 0.2	0.010	**GS882341**	0	0	b	LinJ34_V3.2660	Amastin-like surface protein, putative		N.D.
Lin274G6	2.58	1.4 ± 0.5	0.034	**GS882342**	0	0	b	LinJ08_V3.0680	Amastin-like protein		N.D.
								LinJ08_V3.0690	Amastin-like protein		N.D.
Lin302G5	2.35	1.2 ± 0.2	0.006	**GS882343**	4e-119	1e-45	a	LinJ35_V3.4000	U5 snRNA-associate splicing factor (U5-sn-RNA-sf)		N.D.
Lin306F2	1.86	0.9 ± 0.2	0.043	**GS882344**	0	0	b	LinJ22_V3.1410	40S ribosomal protein L14, putative (L14)		N.D.
Lin10E1	-2.00	-1.0 ± 0.3	0.038	**GS882345**	0	0	a	LinJ35_V3.1740	Dynein-associated roadblock protein-like protein		N.D.
Lin19C6[a]	-1.75	-0.8 ± 0.0	0.002	**GS882346**	0	0	b	LinJ15_V3.0240	Protein phosphatase 1 catalitic subunit, putative (cPP1)	+	-3.1 ± 0.4
Lin24E4	-2.25	-1.2 ± 0.1	0.005	**GS882347**	0	0	b	LinJ20_V3.0310	Developmentally regulated phosphoprotein-like protein (DRPP)	+	-5.5 ± 0.6
Lin27B2	-2.36	-1.3 ± 0.4	0.038	**GS882348**	0	0	b	LinJ35_V3.1230	Short chain dehydrogenase, putative (SCDH)		N.D.
								LinJ35_V3.1240	Short chain dehydrogenase, putative (SCDH)		N.D.
Lin31C11	-1.78	-0.8 ± 0.1	0.009	**GS882349**	0	0	b	LinJ35_V3.3280	Cystathionine gamma lyase, putative (CGL)		N.D.
Lin35B2	-1.74	-0.8 ± 0.2	0.045	**GS882350**	0	0	b	LinJ05_V3.1210	Surface antigen-like protein (SALp)		N.D.

Lin49B3	-2.05	-1.0 ± 0.2	0.020	**GS882351**	0	0	b	LinJ31_V3.0860	Triacylglycerol lipase-like protein (TGL)	+	-8.0 ± 0.2
								LinJ31_V3.0870	Lipase precursor-like protein (TGL)	+	-3.2 ± 0.1
Lin54C2	-1.74	-0.8 ± 0.2	0.028	**GS882352**	0	0	a	LinJ24_V3.2410	Mitogen-activated protein kinase (MAPK)		N.D.
Lin54F7	-1.83	-0.8 ± 0.1	0.010	**GS882353**	0	0	a	LinJ27_V3.1070	Histone H1, putative (H1)		N.D.
Lin58H5	-2.21	-1.1 ± 0.0	0.000	**GS882354**	0	0	b	LinJ31_V3.2400	3,2 trans-enoyl-CoA isomerase, mitochondrial precursor, putative (ECH)		N.D.
Lin59B2[a]	-2.50	-1.3 ± 0.4	0.042	**GS882355**	0	0	b	LinJ30_V3.0790	4-methyl-5(beta-hydroxyethyl)-thiazole monophosphate synthesis protein, putative (MPETs)	+	-4.5 ± 0.5
Lin76A1	-1.97	-2.0 ± 0.1	0.003	**GS882356**	0	0	b	LinJ31_V3.3320	Histone H4 (H4)		N.D.
Lin77B1[a]	-2.27	-1.2 ± 0.1	0.013	**GS882357**	0	0	b	LinJ21_V3.0080	Syntaxin, putative (Sx)	+	-1.8 ± 0.0
Lin80B3	-1.93	-0.9 ± 0.2	0.028	**GS882358**	0	0	b	LinJ28_V3.3250	Glucose 6-phophate N-acetyl transferase (GNAT)		N.D.
Lin88B2	-1.79	-0.8 ± 0.2	0.015	**GS882359**	0	0	b	LinJ10_V3.1070	Histone H3 (H3)		N.D.
Lin90H10	-1.74	-0.8 ± 0.2	0.022	**GS882360**	0	0	a	LinJ07_V3.1300	Proteasome regulatory non ATP-ase subunit, putative (rS-non-ATPase)		N.D.
Lin91E7	-1.87	-0.9 ± 0.2	0.035	**GS882361**	5e-155	0	a	LinJ29_V3.2250	C8-sterol isomerase-like protein		N.D.
Lin100D11	-2.01	-1.0 ± 0.1	0.001	**GS882362**	3e-27	0	b	LinJ04_V3.0570	Spermidine synthase, putative (SpS)		N.D.
Lin100F4[a]	-1.75	-0.8 ± 0.3	0.043	**GS882363**	0	0	a	LinJ28_V3.2280	Dynein light chain lc6, flagellar outer arm, putative (DynLC6)	+	-2.4 ± 0.3
Lin104C1[a]	-1.73	-0.8 ± 0.1	0.011	**GS882364**	0	1e-116	c	LinJ33_V3.2930	GTP-binding elongation factor Tu family protein, putative (EF-Tu)	+	-3.4 ± 0.4
Lin106C12[a]	-3.52	-1.8 ± 0.2	0.008	**GS882365**	0	0	b	LinJ08_V3.0010	Structural maintenance of chromosome 3 protein, putative (smc3)	+	-2.2 ± 0.1
								LinJ08_V3.0030	Vesicle associated membrane protein, putative (vamp)	+	-6.3 ± 1.5
Lin109C7	-2.06	-1.0 ± 0.4	0.040	**GS882366**	0	0	a	LinJ22_V3.1300	Cyclophilin, putative (Cph)		N.D.
Lin109F4	-1.70	-0.8 ± 0.1	0.007	**GS882367**	0	0	a	LinJ06_V3.1330	Coproporphyrinogen III oxidase, putative (C(III)O)	+	-4.8 ± 0.2
								LinJ06_V3.1340	Protoporphyrinogen oxidase-like protein (PO)	+	-9.6 ± 0.5
Lin111F3	-1.91	-0.9 ± 0.1	0.008	**GS882368**	0	0	a	LinJ31_V3.2210	Protein farnesyltransferase alpha subunit, putative (FTase-α)		N.D.
Lin144F11	-1.77	-0.8 ± 0.2	0.021	**GS882369**	2e-164	2e-142	a	LinJ31_V3.2210	Prostaglandin F2-alpha synthase (PGFS)		N.D.
Lin147D2	-2.04	-1.0 ± 0.3	0.032	**GS882370**	0	0	a	LinJ28_V3.0110	Proteasome beta 3 subunit, putative (β3)	+	-1.9 ± 0.0
								LinJ28_V3.0120	DNA directed RNA polymerase II, subunit 9, putative (RNAPol II-s9)	+	-2.1 ± 0.2
Lin187C7	-1.92	-0.9 ± 0.1	0.002	**GS882371**	0	0	b	LinJ26_V3.1680	Sphingolipid delta 4 desaturase, putative	+	-2.6 ± 0.1
								LinJ26_V3.1690	Cytochrome c oxidase subunit V, putative (coxV)	+	
Lin203B5[a]	-2.03	-1.0 ± 0.2	0.014	**GS882372**	0	2e-176	c	LinJ16_V3.0950	Dynein light chain, putative (DLC)	+	-11.0 ± 0.2
Lin237D5	-1.53	-0.6 ± 0.1	0.017	**GS882373**	1e-122	0	b	LinJ22_V3.1300	Cyclophilin, putative (Cph)		N.D.
Lin239H2[a]	-1.78	-0.8 ± 0.1	0.001	**GS882374**	0	0	b	LinJ30_V3.0800	4- methyl-5 (betahydroxyethyl)-thiazole monophosphate synthesis protein, putative (MPETs)	+	-4.5 ± 0.5
Lin271C2[a]	-1.95	-1.0 ± 0.2	0.013	**GS882375**	1e-069	1e-165	b	LinJ28_V3.0090	Adenylate cyclase-like protein (AC)	+	-2.7 ± 0.3
Lin276H10[a]	-1.95	-1.0 ± 0.2	0.027	**GS882376**	0	0	b	LinJ19_V3.1520	Eukaryotic translation initiation factor-like protein (EIF)	+	-7.3 ± 0.3
Lin294G4	-2.65	-1.4 ± 0.2	0.009	**GS882377**	0	0	b	LinJ16_V3.1640	DNA polymerase I alpha catalytic subunit, putative (cDNAPol IαB)		N.D.
Lin310C4	-1.85	-0.9 ± 0.2	0.010	**GS882378**	0	0	b	LinJ22_V3.1300	Cyclophilin, putative (Cph)		N.D.

[a]Clones containing more than one gene fragment; only the differentially regulated gene is indicated.

Table 3. Differentially regulated genes in stationary phase promastigotes versus amastigotes S/A. The features described are: clone number; F (up-regulation if F > 0 and down-regulation if F < 0); base-two logarithmic scale F and S.D. values; *P*; GenBank GSS accession numbers; e-values; Def., clone definition according to mapping outcomes *a*, *b* or *c* (see brief explanation in 2.2); Id., annotation in the *L. infantum* genome sequence; annotated gene function; relative quantitative real time reverse transcription-PCR (qRT-PCR). When a given clone overlaps with more than one annotation ([a]), only clones checked for differential regulation using qRT-PCR appear in this table. cLinA2 and cLdoA2 were spotted onto microarrays as control genes.

Clone	F	$Log_2F \pm SD$	P	GenBank GSS	e-value Fw	e-value Rv	Def.	Id.	Annotated Gene Function	qRT-PCR +/-	qRT-PCR F ± SD
Lin9B6[a]	2.26	1.2 ± 0.0	0.012	GS882379	0	0	c	LinJ21_V3.0700	Phosphoglucomutase (PGluM)	+	5.7 ± 0.4
Lin16B12	2.11	1.1 ± 0.1	0.048	GS882380	0	0	b	LinJ25_V3.1850	3-oxo-5-alpha-steroid 4-dehydrogenase, putative (steroid DH)		N.D.
Lin18F11	2.11	0.9 ± 0.2	0.010	GS882300	0	5e-137	b	LinJ31_V3.3380	Sodium stibogluconate resistance protein, putative (SbGRP)		N.D.
Lin28C5[a]	5.78	2.5 ± 0.1	0.013	GS882381	0	0	b	LinJ26_V3.1670	Sphingolipid delta-4 desaturase	+	10.1 ± 0.9
Lin31H9[a]	2.12	1.1 ± 0.1	0.049	GS882382	0	0	b	LinJ26_V3.1000	Dynein heavy chain, putative (DynH)	+	15.6 ± 0.8
Lin33C4[a]	3.09	1.6 ± 0.0	0.011	GS882383	0	0	c	LinJ22_V3.0340	40S ribosomal protein S15, putative (S15)	+	37.0 ± 1.1
Lin34B10	2.22	1.2 ± 0.0	0.014	GS882384	0	0	b	LinJ22_V3.0660	5'a2rel-related protein (5'a2rel)		N.D.
Lin35B2	6.66	2.7 ± 0.2	0.032	GS882385	0	0	b	LinJ05_V3.1210	Surface antigen-like protein (SALp)	+	6.7 ± 1.3
Lin40G12[a]	1.92	0.9 ± 0.2	0.013	GS598916	2e-161	0	b	LinJ23_V3.1560	Lathosterol oxidase-like protein	+	3.4 ± 0.1
Lin42F4	3.52	1.8 ± 0.1	0.013	GS882386	1e-94	0	a	LinJ32_V3.4040	Cysteine peptidase, Clan CA, family C54, putative (C54cp)	+	5.0 ± 0.2
								LinJ32_V3.4050	60S ribosomal protein L2, putative (L2)	+	2.1 ± 0.3
Lin47D8	4.00	2.0 ± 0.5	0.023	GS598917	0	0	a	LinJ06_V3.1330	Coproporphyrinogen III oxidase, putative C(IIIO)	+	14.2 ± 0.3
								LinJ06_V3.1340	Protoporphyrinogen oxidase-like protein (PO)	+	4.7 ± 0.3
Lin50G5	2.02	1.0 ± 0.0	0.008	GS882387	0	0	b	LinJ21_V3.2080	Cytochrome c oxidase subunit VI, putative (coxVI)		N.D.
Lin50H7	2.32	1.2 ± 0.1	0.005	GS598919	7e-164	0	b	LinJ28_V3.2380	2,3-bisphosphoglycerate-independent phosphoglycerate mutase-like protein (PGM^{BPI})	+	7.6 ± 1.0
								LinJ28_V3.2390	Cyclin dependent kinase-binding protein, putative (cdkbp)	+	2.9 ± 0.4
Lin58H6	2.34	1.2 ± 0.1	0.032	GS882388	0	0	b	LinJ08_V3.0030	Vessicle-associated membrane protein, putative (vamp)		N.D.
Lin61F2[a]	3.38	1.8 ± 0.0	0.001	GS882389	0	0	b	LinJ15_V3.0220	60S ribosomal protein L13a, putative (L13a)	+	8.5 ± 0.4
								LinJ15_V3.0240	Protein phosphatase 1 catalytic subunit (cPP1)	+	7.4 ± 0.2
Lin63F3	5.33	2.4 ± 0.1	0.011	GS882390	0	0	a	LinJ36_V3.6550	Glucose transporter lmgt2, putative (GT)	+	6.6 ± 0.5
								LinJ36_V3.6560	Glucose transporter, putative (GT)	+	5.1 ± 0.3
Lin69B5[a]	2.11	1.1 ± 0.1	0.039	GS882391	0	0	b	LinJ33_V3.0770	60S ribosomal protein L6, putative (L6)	+	1.8 ± 0.1
Lin75E6	2.71	1.4 ± 0.1	0.019	GS882392	0	0	b	LinJ18_V3.0270	Glycogen synthase kinase, putative (GSK)		N.D.
Lin80B3	2.06	1.0 ± 0.3	0.024	GS598924	0	0	b	LinJ28_V3.3250	Glucose-6-phosphate-N-acetyltransferase, putative (GNAT)		N.D.
Lin80E12[a]	2.47	1.3 ± 0.0	0.007	GS882393	0	0	c	LinJ35_V3.0600	60S ribosomal protein L18a, putative (L18a)	+	18.0 ± 1.3
Lin86F11[a]	2.09	1.1 ± 0.1	0.039	GS882394	0	0	b	LinJ30_V3.3390	60S ribosomal protein L9, putative (L9)	+	6.4 ± 0.3
Lin87B9[a]	2.41	1.3 ± 0.0	0.002	GS882395	0	0	b	LinJ36_V3.4730	60S ribosomal protein L18, putative (L18)	+	3.0 ± 0.1
Lin87F1[a]	3.74	1.9 ± 0.1	0.030	GS882396	0	0	b	LinJ13_V3.1120	40S ribosomal protein S4, putative (S4)	+	7.4 ± 0.3
Lin89F9[a]	1.78	0.8 ± 0.1	0.048	GS882397	0	0	b	LinJ31_V3.2370	3'-nucleotidase/nuclease, putative (3'NTase/Nase)	+	4.9 ± 0.4
								LinJ31_V3.2380	3'-nucleotidase/nuclease precursor, putative (3'NTase/Nase)	+	20.7 ± 0.8
Lin89H11	1.95	1.1 ± 0.0	0.011	GS882398	0	0	b	LinJ31_V3.1240	Vacuolar-type proton translocating pyrophosphatase 1, putative (vH^+-PPase)	+	16.4 ± 3.1
Lin92F7	2.38	1.3 ± 0.1	0.020	GS882399	0	0	b	LinJ35_V3.0400	40S ribosomal protein S3a, putative (S3a)		N.D.
Lin94A5[a]	2.28	1.2 ± 0.1	0.027	GS882400	0	0	b	LinJ23_V3.0220	Endoribonuclease L-PSP (pb5), putative	+	9.3 ± 0.2
Lin95F9	2.82	1.5 ± 0.1	0.027	GS882401	0	0	b	LinJ28_V3.2360	Ribosomal protein S29, putative (S29)		N.D.
Lin100F8	3.55	1.8 ± 0.0	0.009	GS882402	0	7e-167	b	LinJ35_V3.3330	60S ribosomal subunit protein L31, putative (L31)		N.D.
								LinJ35_V3.3340	60S ribosomal subunit protein L31, putative (L31)		N.D.
Lin102E4	5.59	2.5 ± 0.0	0.005	GS882403	9e-154	0	b	LinJ23_V3.1200	Hydrophilic surface protein, putative (HASPA1)	+	5.2 ± 0.6
Lin104C3	2.37	1.2 ± 0.1	0.030	GS882404	6e-47	0	b	LinJ32_V3.3100/10	Nucleoside diphosphate kinase b, putative (Ndkb)	+	14.0 ± 0.0
								LinJ32_V3.3120	Minochromosome maintenance (MCM) complex protein (mmc)	+	3.1 ± 0.7
Lin105A3	2.22	1.1 ± 0.1	0.005	GS598937	0	0	b	LinJ36_V3.1320	Fructose-1,6-bisphosphate aldolase (ALD)		N.D.
Lin105C8[a]	3.52	1.8 ± 0.1	0.023	GS882405	0	0	b	LinJ33_V3.0960	40S ribosomal protein S3, putative (S3)	+	2.2 ± 0.1
Lin106B3	1.74	0.8 ± 0.0	0.023	GS882406	0	0	a	LinJ36_V3.3100	Succinyl-CoA ligase (GDP-forming) beta-chain, putative (SCL)	+	3.5 ± 0.5
Lin107C12	2.70	1.4 ± 0.1	0.040	GS882407	7e-130	2e-127	b	LinJ11_V3.1180	40S ribosomal protein S15a, putative (S15a)	+	1.9 ± 0.2
Lin111F3	2.09	1.1 ± 0.4	0.035	GS598941	0	0	a	LinJ31_V3.2210	Prostaglandin F2α synthetase (PGFS)		N.D.
Lin112H5[a]	3.40	1.8 ± 0.1	0.025	GS882408	0	0	a	LinJ15_V3.1600	Presenilin-like aspartic peptidase, clan AD, family A22A, putative (A22A)	+	2.4 ± 0.3
Lin123E8	2.31	1.2 ± 0.1	0.048	GS882409	5e-171	0	b	LinJ26_V3.0160	60S ribosomal protein L7, putative (L7)		N.D.
Lin126D1	2.26	1.2 ± 0.0	0.010	GS882410	0	0	b	LinJ35_V3.2080	Calcium motive P-type ATPase, putative ($Ca2^+$-ATPase)	+	47.8 ± 8.0
Lin130H10	1.71	0.8 ± 0.1	0.033	GS882411	0	0	b	LinJ28_V3.0210	Histone H2B variant (H2B)	+	10.3 ± 0.2
Lin135F6[a]	1.73	0.8 ± 0.0	0.007	GS882412	0	0	b	LinJ29_V3.1920	40S ribosomal protein S15a, putative (S15a)	+	2.0 ± 0.3
Lin137D6	1.87	0.9 ± 0.0	0.004	GS882413	0	0	b	LinJ30_V3.3160	S-adenosylmethionine decarboxylase proenzyme-like protein (SAMDC)	+	5.8 ± 1.0
Lin139B3	3.13	1.3 ± 0.0	0.011	GS882414	0	0	b	LinJ16_V3.0470	60S ribosomal protein L21, putative (L21)	+	2.5 ± 0.4
Lin142E2[a]	2.55	1.4 ± 0.1	0.043	GS882415	0	0	c	LinJ21_V3.1290	60S ribosomal protein L9, putative (L9)	+	3.6 ± 0.5
								LinJ21_V3.1300	40S ribosomal protein S23, putative (S23)	+	6.8 ± 0.6
Lin146F6[a]	1.71	0.8 ± 0.1	0.049	GS882416	0	0	b	LinJ36_V3.4390	UDP-N-acetylglucosamine-dolichyl-phosphaten-acetylglucosaminephosphotransferase (UDP-NAGDPT)	+	56.1 ± 4.6
Lin148E9[a]	3.29	1.7 ± 0.0	0.004	GS882417	0	0	a	LinJ06_V3.0410	60S ribosomal protein L19, putative (L19)	+	3.3 ± 0.3
Lin149H2	3.12	1.6 ± 0.1	0.036	GS882418	0	0	b	LinJ20_V3.1210	Cysteine peptidase, Clan CA, family C2, putative (C2cp)		N.D.
								LinJ20_V3.1220	Cysteine peptidase, Clan CA, family C2, putative (C2cp)		N.D.

Lin153B7[a]	3.15	1.7 ± 0.1	0.022	**GS882419**	0	0	b	LinJ21_V3.1790	40S ribosomal protein S11, putative (S11)	+	2.4 ±0.1
Lin159D1[a]	2.35	1.2 ± 0.1	0.039	**GS882420**	0	0	b	LinJ01_V3.0430/40	Ribosomal protein S7, putative (S7)	+	6.3 ± 0.6
Lin183A2[a]	3.22	1.7 ± 0.1	0.031	**GS882421**	0	0	b	LinJ23_V3.0080	Agmatinase-like protein (agm)	+	5.9 ± 0.1
Lin188G6	2.23	1.2 ± 0.1	0.035	**GS882422**	0	0	b	LinJ28_V3.1950	Heat shock protein 70, putative		N.D.
								LinJ28_V3.1960	Heat shock protein 70, putative		N.D.
Lin193E6	4.28	2.1 ± 0.1	0.027	**GS882423**	0	0	b	LinJ23_V3.1230	SHERP	+	6.3 ± 0.5
Lin193F5[a]	1.75	0.8 ± 0.1	0.045	**GS882424**	0	0	b	LinJ36_V3.2070	Phosphomannomutase, putative (PMM)	+	7.1 ± 0.3
Lin196A12[a]	1.88	0.9 ± 0.0	0.018	**GS882425**	0	0	b	LinJ29_V3.1170/80	Ribosomal protein L1a, putative (L1a)	+	1.8 ± 0.1
Lin205H12[a]	3.99	2.0 ± 0.0	0.002	**GS882426**	0	0	b	LinJ32_V3.0930	60S ribosomal protein L18a, putative (L18a)	+	3.8 ± 0.5
Lin208G9[a]	1.92	0.9 ± 0.1	0.025	**GS882427**	2e-59	0	b	LinJ19_V3.0040	Histone H2B (H2B)	+	2.6 ± 0.2
								LinJ19_V3.0050	40S ribosomal protein S2, putative (S2)	+	4.1 ± 0.4
Lin214D6[a]	3.07	1.6 ± 0.2	0.044	**GS882428**	0	0	b	LinJ05_V3.0040	Paraflagellar rod component par4, putative (PAR4)	+	11.0 ±2.3
Lin219A10[a]	1.89	0.9 ± 0.1	0.004	**GS598961**	0	0	b	LinJ19_V3.0710	Glycosomal malate dehydrogenase (gMDH)	+	2.2 ± 0.3
Lin228A12	1.78	0.8 ± 0.1	0.037	**GS882429**	0	0	b	LinJ30_V3.3650	40S ribosomal protein S14 (S14)	+	4.5 ± 0.3
Lin239A10	1.94	1.0 ± 0.0	0.040	**GS882430**	0	0	a	LinJ24_V3.2340	Fatty acid desaturase, putative	+	8.2 ± 1.0
Lin11D7	-2.53	-1.3 ± 0.3	0.020	**GS598854**	0	0	b	LinJ31_V3.0460	Amastin, putative		N.D.
Lin22E12	-2.79	-1.5 ± 0.1	0.001	**GS598856**	0	0	b	LinJ31_V3.1850	Amino acid permease (aap)		N.D.
Lin25C5[a]	-2.39	-1.3 ± 0.1	0.023	**GS882431**	0	0	b	LinJ07_V3.0360	Phosphatidylglycerophosphate synthase-like protein (PLD)	+	-6.3 ± 0.2
Lin26B8	-2.19	-1.1 ± 0.0	0.013	**GS882432**	4e-162	9e-37	b	LinJ29_V3.2850	Actin-like protein, putative (Actin)		N.D.
Lin35E9	-3.63	-1.9 ± 0.1	0.014	**GS882433**	0	0	b	LinJ17_V3.1520	Otubain cysteine peptidase, Clan CA, family C65, putative	+	-13.9 ± 0.4
Lin36B8	-1.99	-1.0 ± 0.2	0.015	**GS598859**	0	0	b	LinJ30_V3.3230	3-hydroxy-3-methyglutaryl-CoA reductase, putative		N.D.
Lin48F2	-2.61	-1.4 ± 0.0	0.012	**GS882434**	0	0	a	LinJ31_V3.2590	Ferredoxin, 2fe-2s-like protein (Fd)		N.D.
								LinJ31_V3.2600	Ferredoxin, 2fe-2s-like protein (Fd)		N.D.
Lin62D3[a]	-1.92	-0.9 ± 0.4	0.040	**GS598862**	0	0	b	LinJ05_V3.0350	Trypanothione reductase (TR)	+	-25.7 ± 1.4
Lin76D1	-4.41	-2.1 ± 0.3	0.054	**GS882435**	0	0	b	LinJ08_V3.0520	Ribose-phosphate pyrophosphokinase, putative (PRPPS)		N.D.
Lin83D1	-2.36	-1.2 ± 0.1	0.049	**GS882309**	0	0	b	LinJ08_V3.0700/10	Amastin-like protein		N.D.
Lin103F1	-1.83	-0.9 ± 0.0	0.004	**GS882436**	0	0	a	LinJ35_V3.3080	Prenyl protein specific carboxyl methyltransferase, putative (Isoprenyl cysteine methyltransferase, ICMT)		N.D.
Lin113C3	-1.87	-0.9 ± 0.1	0.040	**GS598876**	3e-74	0	a	LinJ14_V3.1440	Pteridine transporter (PT3)	+	-3.4 ± 0.2
								LinJ14_V3.1450	Myo-inositol-1-phosphate synthase (INO1)	+	-6.4 ± 0.2
Lin123H10[a]	-1.81	-0.9 ± 0.1	0.035	**GS882437**	0	0	b	LinJ30_V3.1520	P1/S1 nuclease (P1/S1)	+	-3.0 ± 0.1
Lin142C1[a]	-1.72	-0.8 ± 0.1	0.003	**GS882438**	4e-159	9e-111	c	LinJ16_V3.0550	Aspartate carbamoyltransferase, putative (AspCT)	+	-4.3 ± 0.6
Lin142D7	-1.87	-0.9 ± 0.1	0.033	**GS882439**	0	0	a	LinJ36_V3.6760	MAP-kinase homologue (MAPKh)		N.D.
Lin165E2	-3.48	-1.8 ± 0.2	0.004	**GS598885**	0	0	b	LinJ22_V3.0680	3'a2rel-related protein (3'a2rel)	+	-9.3 ± 0.5
Lin197A12	-1.95	-1.0 ± 0.2	0.016	**GS598890**	0	0	a	LinJ31_V3.2540	Lipase, putative (TGL)		N.D.
Lin201F12[a]	-1.79	-0.8 ± 0.0	0.018	**GS882440**	0	0	c	LinJ29_V3.1270	Lipase domain protein, putative (TGL)	+	-2.6 ± 0.3
Lin210C4	-2.09	-1.1 ± 0.0	0.013	**GS598893**	0	0	b	LinJ08_V3.0690	Amastin-like protein		N.D.
Lin234D7	-1.93	-1.0 ± 0.0	0.005	**GS882441**	9e-151	2e-118	b	LinJ11_V3.1220	ABC transporter-like protein (ABC)	+	-3.4 ± 0.2
								LinJ11_V3.1230	ABC1 transporter, putative (ABC1)	+	-4.1 ± 0.1
Lin238D6	-2.34	-1.2 ± 0.1	0.041	**GS882442**	0	0	a	LinJ30_V3.1820	Splicing factor 3A, putative (Sf3A)		N.D.
Lin254D11	-2.30	-1.2 ± 0.1	0.041	**GS882443**	7e-59	0	b	LinJ11_V3.1250	ABC transporter-like protein (ABC)		N.D.
Lin264E5[a]	-1.73	-0.8 ± 0.1	0.044	**GS882444**	0	0	a	LinJ32_V3.1080	Protein kinase, putative	+	-5.0 ± 0.5
Lin267D9[a]	-2.06	-1.0 ± 0.2	0.010	**GS598898**	9e-111	0	b	LinJ16_V3.0590	Carbamoyl-phosphate synthetase, putative (CPS)	+	-2.9 ± 0.4
Lin294A10	-1.89	-0.9 ± 0.0	0.004	**GS882445**	0	0	a	LinJ35_V3.1420	Threonyl-tRNA synthetase, putative (^{Thr}tS)		N.D.
Lin298D3	-4.39	-2.1 ± 0.0	0.009	**GS882446**	0	0	a	LinJ17_V3.1520	Otubain cysteine peptidase, Clan CA, family C65, putative	+	-13.9 ± 0.4
Lin310F2	-2.34	-1.2 ± 0.5	0.046	**GS598904**	0	0	b	LinJ23_V3.1220	Hydrophilic surface protein (HASPB)		N.D.
cLinA2	-2.45	-1.3 ± 0.0	0.000	**S69693**	-	-	-	-	*L. infantum* A2 gene – DNA microarray control spot		X
cLdoA2	-2.01	-1.0 ± 0.1	0.021	-	-	-	-	-	*L. donovani* A2 gene – DNA microarray control spot		X

Table 4. Differentially regulated genes in logarithmic phase promastigotes versus amastigotes L/A. The features described are: clone number; F (up-regulation if F > 0 and down-regulation if F < 0); base-two logarithmic scale F and S.D. values; *p*; GenBank GSS accession numbers; e-values; Def., clone definition according to mapping outcomes *a*, *b* or *c* (see brief explanation in the text); Id., annotation in the *L. infantum* genome sequence; annotated gene function; relative quantitative real time reverse transcription PCR (qRT-PCR). When a given clone overlaps with more than one annotation ([a]), only qRT-PCR checked clones for differential regulation appear in this table.

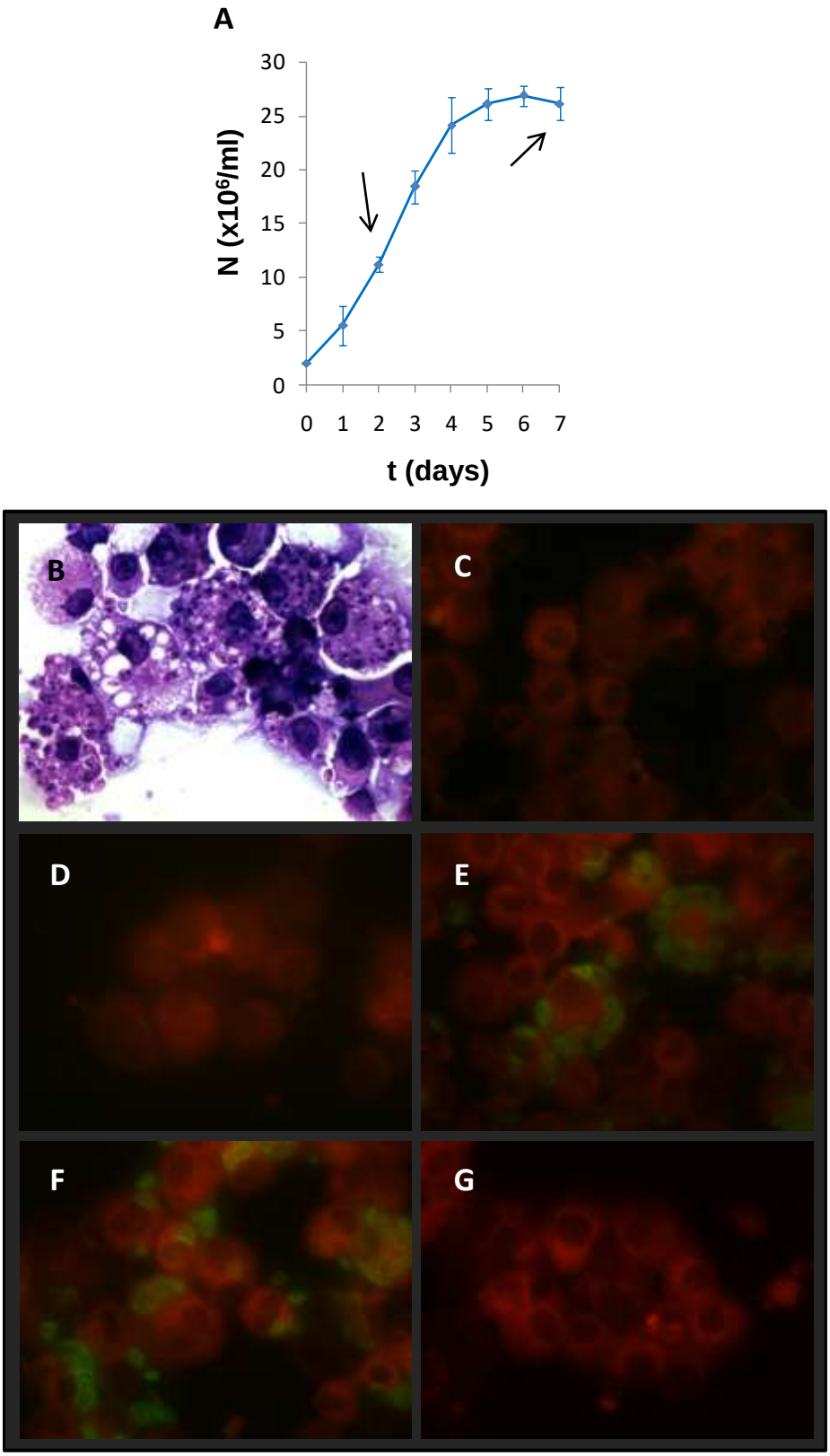
Clone	F	$Log_2F \pm SD$	*p*	GenBank GSS	e-value Fw	e-value Rv	Def.	Id.	Annotated Gene Function	qRT-PCR +/-	qRT-PCR F ± SD
Lin18F11	2.11	0.9 ± 0.2	0.010	GS882300	0	5e-137	b	LinJ31_V3.3380	Sodium stibogluconate resistance protein, putative (SbGRP)		N.D.
Lin54C2[a]	3.45	1.8 ± 0.6	0.039	GS882447	0	0	a	LinJ24_V3.2410	Mitogen-activated protein kinase (MAPK)	+	2.4 ± 0.1
Lin54D2	2.17	1.1 ± 0.2	0.016	GS882448	1e-149	0	b	LinJ36_V3.1700	Clathrin heavy chain, putative (Clathrin-H)		N.D.
Lin55A8	2.02	1.0 ± 0.2	0.011	GS882449	0	0	b	LinJ19_V3.0200	ADP/ATP translocase1, putative (ANC2)		N.D.
Lin63F3	2.87	1.5 ± 0.2	0.008	GS882450	0	0	a	LinJ36_V3.6550	Glucose transporter, lmgt2, putative (GT)	+	4.1 ± 0.2
								LinJ36_V3.6560	Glucose transporter, putative (GT)	+	5.2 ± 0.1
Lin70E4	2.38	1.3 ± 0.4	0.037	GS882451	0	0	b	LinJ26_V3.0450	ATPase subunit 9, putative (F_0s9)		N.D.
Lin52F10[a]	2.23	1.2 ± 0.3	0.020	GS882452	0	0	c	LinJ23_V3.0230	ABC transporter/multidrug resistance protein/p-glycoprotein, putative (MRP)	+	7.4 ± 0.8
Lin51E2	2.06	1.0 ± 0.3	0.035	GS882453	3e-061	0	a	LinJ36_V3.0020	Histone H4 (H4)		N.D.
Lin45G3	3.06	1.6 ± 0.3	0.012	GS882454	0	0	a	LinJ08_V3.0030	Vesicle-associated membrane protein, putative (vamp)	+	6.1 ± 0.3
Lin44G7	2.14	1.1 ± 0.4	0.046	GS882455	4e-076	4e-063	a	LinJ08_V3.0730/40	Tuzin, putative	+	3.3 ± 0.2
Lin44D8	2.27	1.2 ± 0.3	0.021	GS882456	1e-143	8e-068	b	LinJ13_V3.1120	40S ribosomal proteinS4, putative (S4)		N.D.
Lin38C2	5.35	2.4 ± 0.7	0.030	GS882457	0	7e-130	b	LinJ12_V3.0230	Thiolase protein-like protein (Thiol I)		N.D.
Lin34D12	2.68	1.4 ± 0.2	0.008	GS882458	0	2e-173	a	LinJ33_V3.0960	40S ribosomal protein S3, putative (S3)		N.D.
Lin28C5[a]	5.08	2.3 ± 0.2	0.003	GS882459	1e-162	-	c	LinJ32_V3.1770	Protein phosphatase 2C, putative (PP2C)	+	17.9 ± 1.8
Lin24E4[a]	4.00	2.0 ± 0.2	0.003	GS882460	0	0	b	LinJ20_V3.0310	Developmentally regulated phosphoprotein-like protein (DRPP)	+	9.9 ± 0.7
Lin18H3[a]	2.05	1.0 ± 0.3	0.029	GS882461	0	0	b	LinJ15_V3.0240	Protein phosphatase 1 catalitic subunit, putative (cPP1)	+	5.9 ± 0.3
Lin12G8[a]	2.85	1.5 ± 0.6	0.047	GS882462	6e-087	1e-125	b	LinJ28_V3.2650	Ribosomal protein S29, putative (S29)	+	2.8 ± 0.3
Lin17D4[a]	2.47	1.3 ± 0.4	0.035	GS882463	0	0	b	LinJ09_V3.0060	N-acetylglucosaminylphophatidylinositoldeacetylase (NAGPID)	+	5.5 ± 0.4
Lin87B9[a]	2.06	1.0 ± 0.2	0.012	GS882464	0	0	b	LinJ36_V3.4730	60S Ribosomal protein L18, putative (L18)	+	6.1 ± 0.3
Lin93C5[a]	2.16	1.1 ± 0.4	0.038	GS882465	0	4e-128	b	LinJ13_V3.1450	Alpha tubulin (α-tub)	+	22.7 ± 1.3
Lin94A5[a]	2.06	1.0 ± 0.2	0.007	GS882466	0	0	b	LinJ23_V3.0220	Endoribonuclease (pb5), putative (L-PSP (pb5))	+	8.2 ± 0.4
Lin95F11	2.42	1.3 ± 0.5	0.039	GS882467	0	0	b	LinJ28_V3.2360	Ribosomal protein S29, putative (S29)		N.D.
Lin100F8[a]	2.24	1.2 ± 0.3	0.017	GS882468	0	0	a	LinJ28_V3.2280	Dynein light chain lc6, flagellar outer arm, putative (DynLC6)	+	17.9 ± 0.8
Lin100F4	2.12	1.1 ± 0.2	0.010	GS882469	0	5e-174	b	LinJ35_V3.3330	60S Ribosomal subunit protein L31, putative (L31)		N.D.
								LinJ35_V3.3340	60S Ribosomal subunit protein L31, putative (L31)		N.D.
Lin102E4	4.74	2.2 ± 0.7	0.035	GS882470	1e-122	3e-138	b	LinJ23_V3.1200	Hydrophilic surface protein 2 (HASPA1)		N.D.
Lin105C8[a]	3.25	1.7 ± 0.4	0.015	GS882471	0	0	b	LinJ33_V3.0960	40S ribosomal protein S3, putative		N.D.
Lin105G12	2.32	1.2 ± 0.4	0.041	GS882472	2e-25	2e-105	b	LinJ13_V3.1370	Mitochondrial DNA polymerase I protein D, putative (mPol ID)		N.D.
Lin106F5[a]	2.10	1.1 ± 0.1	0.005	GS882473	0	0	b	LinJ21_V3.2070	Proteasome alpha 2 subunit, putative (α2)	+	2.6 ± 0.1
Lin107A8[a]	2.46	1.3 ± 0.5	0.042	GS882474	0	3e-175	a	LinJ23_V3.0040	Beta propeller protein, putative (β-prop)	+	3.2 ± 0.4
Lin107C2	4.11	2.0 ± 0.3	0.005	GS882475	0	0	b	LinJ08_V3.1000	Histone deacetylase, putative (H-deAc)		N.D.
Lin108H5[a]	3.10	1.6 ± 0.2	0.007	GS882476	0	0	b	LinJ36_V3.0250	Peptidyl-prolyl cis-trans isomerase, putative (FKPB)	+	6.9 ± 0.3
Lin111D8	3.26	1.7 ± 0.2	0.003	GS882477	0	0	a	LinJ08_V3.1000	Histone deacetylase, putative (H-deAc)		N.D.
Lin113A4	7.54	2.9 ± 0.4	0.005	GS882478	0	0	a	LinJ19_V3.1480	MAP kinase (MAPK)		N.D.
Lin113E12[a]	2.61	1.4 ± 0.5	0.035	GS882479	0	0	b	LinJ05_V3.1060	ATPase, putative (AFG1)	+	5.7 ± 0.3
Lin113H11	2.20	1.1 ± 0.1	0.005	GS882480	0	0	b	LinJ36_V3.1700	Clathrin heavy chain, putative		N.D.
Lin114D8[a]	2.78	1.5 ± 0.1	0.003	GS882481	0	0	c	LinJ09_V3.1390	Paraflagellar rod component, putative (PFR)	+	29.0 ± 2.5
Lin126D1	2.08	1.1 ± 0.3	0.019	GS882482	0	0	b	LinJ35_V3.2080	Calcium motive P-type ATPase, putative (Ca^2+-ATPase)	+	4.4 ± 0.1
Lin128D8[a]	2.20	1.1 ± 0.2	0.010	GS882483	0	0	a	LinJ23_V3.1430	Membrane-bound acid phosphatase 2 (MBAP2)	+	8.0 ± 1.1
Lin131A7[a]	2.82	1.5 ± 0.2	0.005	GS882484	0	0	b	LinJ36_V3.7320	Eukaryotic translation initiation factor 3 subunit 8, putative (IF3s8)	+	3.9 ± 0.3
Lin134C1[a]	2.35	1.2 ± 0.2	0.006	GS882485	0	0	b	LinJ26_V3.2230	Ribosomal protein , putative	+	5.1 ± 0.2
Lin134F4[a]	2.19	1.1 ± 0.3	0.020	GS882486	3e-83	1e-137	a	LinJ09_V3.1190	Inner membrane preprotein translocase Tim17 (Tim17)	+	7.4 ± 0.4
Lin135F1[a]	2.27	1.2 ± 0.2	0.014	GS882487	0	0	b	LinJ23_V3.0040	Beta propeller protein, putative (β-prop)	+	3.2 ± 0.4
Lin137D6	2.30	1.2 ± 0.4	0.043	GS882488	0	0	b	LinJ08_V3.0960	Cathepsin L-like protease (Cathepsin-L)		N.D.
Lin138C1[a]	2.32	1.2 ± 0.4	0.035	GS882489	0	0	b	LinJ24_V3.1380	Translation initiation factor IF-2, putative (IF2)	+	7.2 ± 0.3
Lin141B7[a]	2.22	1.2 ± 0.2	0.008	GS882490	0	0	b	LinJ23_V3.0040	Beta propeller protein, putative (β-prop)	+	3.2 ± 0.4
								LinJ23_V3.0060	Peptidyl-prolyl cis-trans isomerase, putative (FKBP)	+	5.1 ± 0.3
Lin142C8	3.65	1.9 ± 0.2	0.005	GS882491	0	0	a	LinJ21_V3.0800	60S ribosomal protein L36, putative (L36)	+	10.2 ± 0.4
								LinJ21_V3.0820	ATPase subunit 9, putative (F_0s9)	+	3.1 ± 0.2
Lin146F6[a]	2.62	1.4 ± 0.2	0.008	GS882492	6e-155	0	b	LinJ36_V3.4390	UDP-N-acetylglucosamine-dolichyl-phosphaten-acetylglucosaminephosphotransferase (UDP-NAGDPT)	+	12.7 ± 1.0
Lin147E10[a]	2.38	1.2 ± 0.5	0.041	GS882493	0	0	c	LinJ34_V3.4290	Lipophosphoglycan biosynthetic protein (lpg2)	+	8.6 ± 0.0
Lin148E9[a]	2.27	1.2 ± 0.2	0.012	GS882494	0	0	b	LinJ06_V3.0410	60S ribosomal protein L19, putative (L19)	+	17.2 ± 0.3
Lin153A3	2.36	1.2 ± 0.2	0.010	GS882495	0	0	b	LinJ05_V3.1210	Surface antigen-like protein (SALp)	+	5.0 ± 0.8
Lin153B7	2.21	1.1 ± 0.2	0.015	GS882496	0	0	b	LinJ21_V3.1790	40S ribosomal protein S11, putative (S11)	+	3.2 ± 0.1
Lin161C9	6.98	2.8 ± 0.5	0.012	GS882497	0	2e-167	b	LinJ26_V3.1670	Sphingolipid delta 4 desaturase, putative		N.D.
Lin163A6	2.08	1.1 ± 0.2	0.014	GS882498	0	0	b	LinJ09_V3.0170	Microtubule associated protein-like protein (MTap)		N.D.
								LinJ09_V3.0180	Microtubule associated protein-like protein (MTap)		N.D.
								LinJ09_V3.0190	Microtubule associated protein-like protein (MTap)		N.D.
Lin170B7	2.01	1.0 ± 0.2	0.018	GS882499	0	0	b	LinJ23_V3.0860	3-ketoacyl-Coa thiolase-like protein (Thiol I)		N.D.

Lin182F2	3.14	1.7 ± 0.2	0.004	**GS882500**	0	0	b	LinJ25_V3.0740	Eukaryotic initiation factor 5a, putative (IF5a)		N.D.
								LinJ25_V3.0750	Eukaryotic initiation factor 5a, putative (IF5a)		N.D.
Lin197F11	4.68	2.2 ± 0.3	0.004	**GS882501**	0	0	b	LinJ26_V3.1680	Sphingolipid delta 4 desaturase, putative		N.D.
								LinJ26_V3.1690	Cytochrome c oxidase subunit V, putative (coxV)	+	6.1 ± 0.2
Lin203B5[a]	2.71	1.4 ± 0.1	0.002	**GS882502**	0	0	b	LinJ16_V3.0950	Dynein light chain, putative (DLC)	+	163 ± 17
Lin210A3[a]	2.02	1.0 ± 0.1	0.003	**GS882503**	0	0	a	LinJ28_V3.2280	Dynein light chain lc6, flagellar outer arm, putative (DynLC6)	+	20.4 ± 0.5
Lin210F9[a]	2.10	1.1 ± 0.1	0.004	**GS882504**	2e-176	0	b	LinJ13_V3.0460	40S ribosomal protein S12, putative (S12)	+	6.7 ± 0.3
Lin212A6	2.26	1.2 ± 0.4	0.032	**GS882505**	2e-102	2e-93	b	LinJ32_V3.0460	40S ribosomal protein S2 (S2)		N.D.
Lin213D1[a]	2.95	1.6 ± 0.3	0.016	**GS882506**	0	0	b	LinJ23_V3.0080	Agmatinase-like protein (agm)	+	4.4 ± 0.6
Lin214D6[a]	4.33	2.1 ± 0.3	0.005	**GS882507**	0	0	b	LinJ05_V3.0040	Paraflagellar rod component par4, putative (PAR4)	+	1.8 ± 0.0
Lin217C10[a]	2.28	1.2 ± 0.2	0.012	**GS882508**	0	0	b	LinJ19_V3.0050	40S ribosomal protein S2 (S2)		N.D.
Lin223H2[a]	3.17	1.7 ± 0.7	0.049	**GS882509**	0	0	b	LinJ19_V3.0170	MAP kinase, putative (MAPK)	+	5.7 ± 0.3
								LinJ19_V3.0190	ADP/ATP translocase 1, putative (ANC1)	+	2.2 ± 0.1
Lin226A10[a]	2.09	1.1 ± 0.2	0.018	**GS882510**	0	0	b	LinJ26_V3.0160	60S ribosomal protein L7, putative (L7)	+	8.8 ± 2.0
Lin226F1	5.60	2.5 ± 0.5	0.011	**GS882511**	0	0	b	LinJ31_V3.1660	Putative 3-ketoacyl-CoA thiolase-like protein (Thiol I)		N.D.
Lin226F2	2.07	1.0 ± 0.3	0.021	**GS882512**	0	0	b	LinJ05_V3.0420	Dynein-light chain-protein, putative (DLC)		N.D.
Lin228D4[a]	2.27	1.2 ± 0.4	0.031	**GS882513**	0	0	a	LinJ19_V3.0090	Fibrillarin, putative (Fibril)	+	13.7 ± 0.1
Lin231A5	2.89	1.5 ± 0.5	0.030	**GS882514**	0	0	b	LinJ35_V3.1310	Ubiquitin-conjugating enzyme E2, putative (UBC2)		N.D.
Lin231E8[a]	2.03	1.0 ± 0.1	0.005	**GS882515**	0	0	b	LinJ16_V3.0600	Histone H3, putative (H3)	+	2.0 ± 0.2
Lin236H2	3.45	1.8 ± 0.4	0.014	**GS882516**	0	1e-147	b	LinJ29_V3.1860	Histone H2A, putative (H2A)		N.D.
								LinJ29_V3.1870	Histone H2A, putative (H2A)		N.D.
Lin237D5	2.72	1.4 ± 0.3	0.016	**GS882517**	0	0	b	LinJ22_V3.1300	Cyclophilin, putative (Cph)		N.D.
Lin239H2[a]	4.20	2.1 ± 0.3	0.005	**GS882518**	0	0	b	LinJ30_V3.0800	4-methyl-5(beta-hydroxyethyl)-thiazole monophosphate synthesis protein, putative (MPETs)	+	35.0 ± 1.9
Lin241B10	2.15	1.1 ± 0.1	0.003	**GS882519**	0	0	b	LinJ34_V3.2420	Adaptor complex subunit medium chain 3, putative (Apcx3)		N.D.
Lin276F6	2.54	1.3 ± 0.2	0.009	**GS882520**	0	0	b	LinJ35_V3.2370	Protein kinase, putative (PK)		N.D.
Lin276F9[a]	2.44	1.3 ± 0.2	0.006	**GS882521**	0	-	c	LinJ24_V3.2230	60S ribosomal protein L12 putative (L12)	+	1.8 ± 0.1
Lin277E3	2.28	1.2 ± 0.4	0.035	**GS882522**	0	0	b	LinJ36_V3.0590	Ubiquitin-like protein, putative (ubq)	+	2.4 ± 0.3
								LinJ36_V3.0600	Cdc2-related kinase (cdc2)	+	3.0 ± 0.5
Lin280E9[a]	2.41	1.3 ± 0.2	0.011	**GS882523**	0	0	a	LinJ31_V3.1600	Cytochrome c oxidase VIII (cox VIII), putative	+	2.7 ± 0.2
Lin282B6	3.14	1.6 ± 0.3	0.008	**GS882524**	0	0	b	LinJ03_V3.0960	Elongation initiation factor 2 alpha subunit, putative (IF2α)		N.D.
Lin288C8[a]	2.23	1.2 ± 0.2	0.006		0	-	c	LinJ27_V3.1120	Histone H1, putative (H1)	+	14.7 ± 0.5
Lin294C9	4.26	2.1 ± 0.5	0.020	**GS882525**	0	1e-82	a	LinJ04_V3.0330	ADP-ribosylation factor, putative (ADPrf)		N.D.
Lin295C8[a]	2.02	1.0 ± 0.3	0.030	**GS882526**	0	0	b	LinJ31_V3.2070	Ubiquitin-fusion protein (ubq)	+	4.9 ± 0.2
Lin301B5	3.68	1.9 ± 0.7	0.041	**GS882527**	0	0	b	LinJ18_V3.1010	Dynein light chain 2b, cytoplasmic, putative (Dyn2bLC7)		N.D.
Lin309E11	2.74	1.5 ± 0.5	0.031	**GS882528**	0	0	b	LinJ20_V3.1220	Cysteine peptidase, Clan A, family C2, putative (C2cp)		N.D.
Lin241H6	2.67	1.4 ± 0.4	0.031	**GS882529**	0	0	b	LinJ33_V3.3380	60S ribosomal protein L44, putative (L44)	+	3.5 ± 0.1
								LinJ33_V3.3390	H1 histone-like protein (H1)	+	1.9 ± 0.1
Lin249D12[a]	2.57	1.4 ± 0.1	0.002	**GS882530**	-	0	c	LinJ26_V3.0160	60S ribosomal protein L7, putative (L7)	+	6.0 ± 0.3
Lin251A8	2.12	1.1 ± 0.2	0.011	**GS882531**	0	0	b	LinJ23_V3.0860	3-Ketoacyl –CoA thiolase-like protein (Thiol I)		N.D.
Lin253A10[a]	2.85	1.5 ± 0.2	0.007	**GS882532**	0	0	c	LinJ04_V3.0460	60S ribosomal protein L11 (L11)		N.D.
								LinJ06_V3.1330	Coproporphyrinogen III oxidase, putative (C(III)O)	+	3.2 ± 0.2
								LinJ06_V3.1340	Protoporphyrinogen oxidase-like protein (PO)	+	6.7 ± 0.2
Lin256B3	2.50	1.3 ± 0.2	0.005	**GS882533**	2e-31	0	a	LinJ35_V3.0670	RING finger protein, conserved		N.D.
Lin260G2	2.24	1.2 ± 0.4	0.045	**GS882534**	0	4e-156	b	LinJ26_V3.1670	Sphingolipid delta 4 desaturase, putative		N.D.
Lin265E2	2.29	1.2 ± 0.2	0.008	**GS882535**	9e-154	0	b	LinJ29_V3.1880	Paraflagellar rod protein 1D, putative (PFR1D)	+	15.2 ± 0.7
Lin268E7	3.62	1.9 ± 0.4	0.014	**GS882536**	0	0	b	LinJ27_V3.0300	Acyl carrier protein, putative (ACP)		N.D.
Lin270A3[a]	3.08	1.6 ± 0.6	0.041	**GS882537**	0	0	b	LinJ23_V3.0060	Peptidyl-prolyl cis-trans isomerase, putative (FKBP)	+	2.5 ± 0.1
								LinJ23_V3.0080	Agmatinase-like protein (agm)	+	3.3 ± 0.0
Lin11D7	-2.53	-1.3 ± 0.3	0.020	**GS882538**	0	0	b	LinJ31_V3.0460	Amastin, putative		N.D.
Lin12D9	-1.75	-0.8 ± 0.2	0.026	**GS882539**	0	0	b	LinJ27_V3.1900	FtsJ cell division protein, putative		N.D.
Lin25B12[a]	-1.88	-0.9 ± 0.3	0.038	**GS882540**	0	-	c	LinJ29_V3.2310	GTP-binding protein, putative	+	-15.3 ± 1.1
Lin26B8	-2.10	-1.1 ± 0.2	0.009	**GS882541**	6e-158	5e-35	b	LinJ29_V3.2850	Actin-like protein, putative		N.D.
Lin130C5	-18.35	-4.2 ± 1.3	0.028	**GS882542**	0	0	a	LinJ36_V3.4270	Phosphoglycerate mutase-like protein (PGM)		N.D.
Lin142C1[a]	-1.72	-0.8 ± 0.1	0.003	**GS882438**	4e-159	9e-111	c	LinJ16_V3.0550	Aspartate carbamoyltransferase, putative (AspCT)	+	-3.6 ± 0.4
Lin239B8	-1.82	-0.9 ± 0.3	0.032	**GS882543**	4e-162	6e-112	b	LinJ32_V3.1080	Protein kinase, putative (PK)	+	-2.8 ± 0.5
Lin245E2	-2.03	-1.0 ± 0.3	0.032	**GS882544**	0	0	b	LinJ22_V3.0680	3'a2rel-related protein (3'a2rel)		N.D.
Lin247B12	-1.82	-0.9 ± 0.2	0.017	**GS882545**	0	2e-121	b	LinJ12_V3.0690	Surface antigen protein 2 precursor (SALp2)		N.D.
Lin254D11	-2.49	-1.3 ± 0.3	0.020	**GS882546**	0	0	b	LinJ11_V3.1250	ABC transporter-like protein (ABC)		N.D.
Lin259H9[a]	-2.34	-1.2 ± 0.4	0.036	**GS882547**	0	1e-168	c	LinJ29_V3.1370	Lipase domain protein, putative (TGL)	+	-1.9 ± 0.1
Lin298D3	-2.13	-1.1 ± 0.4	0.049	**GS882548**	0	0	a	LinJ17_V3.1520	Otubain cysteine peptidase, Clan CA, family 65, putative		N.D.
cLinA2	-3.11	-1.3 ± 0.0	0.000	**S69693**	-	-	-	-	*L. infantum* A2 gene – DNA microarray control spot		X
cLdoA2	-2.54	-1.0 ± 0.1	0.021	-	-	-	-	-	*L. donovani* A2 gene – DNA microarray control spot		X

Table 5. Intra- and inter-species analyses of differential gene regulation in *Leishmania* spp. amastigotes. A review of non-redundant absolute and relative frequencies of up- and down-regulated genes in amastigotes are shown in the table for each of the expression profile analyses performed to date for this stage which referred to logarithmic and/or stationary phase promastigotes. Significance tests of the binomially distributed random variable of differential regulation in amastigotes were performed for each of the individual expression profiling reports, as well as intra- and inter-specifically, either including or separating logarithmic phase promastigotes versus amastigotes L/A and stationary phase promastigotes versus amastigotes S/A profiles.

Legend: (1) Functional genomics (partial shotgun DNA microarrays); (2) functional genomics (partial cDNA microarrays); (3) functional genomics (complete oligonucleotide microarrays); (4) proteomics; (5) functional genomics (complete shotgun DNA microarrays); (6) functional genomics (partial oligonucleotide microarrays); (a) coverage rate calculated by Clarke and Carbon equation for shotgun DNA microarrays with 5% probability of not finding any sequence; (b) oligonucleotide sequences or expressed sequence tags (ESTs) representing genes and excluding intergenic regions. Complete oligonucleotide microarrays probably do not represent 100% of genes because clones containing genic sequences not annotated in the genome have been detected by means of shotgun DNA microarrays; (c) this report; (d) axenic amastigotes (amastigote-like forms); (e) amastigote-like forms (axenic) obtained by the unique effect of simultaneous temperature and pH shift; (f) predominance of up-regulation in amastigotes without significant statistical difference; (g) significant difference between both groups with predominance of up-regulation in amastigotes; (h) binomial test without approximation to the normal distribution, as $np \leq 10$ and/or $nq \leq 10$. *Statistical significance. Three binomial tests: two independent tests for L/A and S/A respectively and one global test for L/A + S/A.

Species	Approach.	Genome coverage	References	Global gene differential regulation	Up-regulated genes in amastigotes			Down-regulated genes in amastigotes			*Binomial test (P-value)*	
					dL/A	dS/A	dL/A + dS/A	uL/A	uS/A	uL/A + uS/A		
L. major	(1)	16.1% (b)	Akopyants et al., 2004	15% of 10%	29 (23.8%)	7 (5.7%)	34 (27.9%)	26 (21.3%)	19 (15.6%)	7 (5.7%)	0.051 (f)	0.016
	(2)	22.4% (b)	Almeida et al., 2004	22.2% of 7.2%	-	-	58 (44%)	13 (9.8%)	23 (17.4%)	38 (28.8%)	0.081	
	(3)	100% (b)	Leifso et al., 2007	1.9%	87 (56.1%)	-	-	68 (43.9%)	-	-	0.063 (f)	
L. mexicana	(3)	100% (b)	Holzer et al., 2006	2.9%	28 (11.9%)	-	-	208 (88.1%)	-	-	<0.001	<0.001
				0.6%	14 (27.4%)(d)	-	-	37 (72.6%)(d)	-	-	<0.001	
L. donovani	(1)	6.5% (a)	Srividya et al., 2007	6.5% of 4.6%	-	15 (60%)	-	-	10 (40%)	-	0.212 (h)	<0.001
	(4)	-	Rosenzweig et al., 2008	53.6% of 11.2%	-	173 (35%)	-	-	321 (65%)	-	<0.001	
L. infantum	(4)	-	McNicoll et al., 2006	100% of 0.9%	-	53 (72.6%)	-	-	20 (27.4%)	-	<0.001 (g)	<0.001
	(4)	-	Leifso et al., 2007	68.9% of 1.1%	8 (12.7%)	-	-	55 (87.3%)	-	-	<0.001 (h)	
	(3)	100% (b)	Rochette et al., 2009	5.8%	-	217 (45.5%)	-	-	260 (54.5%)	-	0.024	
				11.2%	-	443 (48.1%)(d)	-	-	478 (51.8%)(d)	-	0.125	
	(5)	100% (a)	Alcolea et al.	2.2%	-	80 (44.4%)(e)	-	-	100 (66.6%)(e)	-	0.069	
			(c) [what is this?]DATA FROM THIS MANUSCRIPT. IT IS DEFINED IN THE TABLE LEGEND. THIS IS CORRECT	5.7%	66 (14.4%)	68 (14.6%)	13 (2.8%)	189 (40.5%)	94 (20.2%)	35 (7.5%)	<0.001*	
L. braziliensis	(6)	8.3% (b)	Depledge et al., 2009	8.1% of 8.3%	10 (12.8%)	35 (63.6%)	3 (5.4%)	3 (5.4%)	1 (1.8%)	3 (5.4%)	<0.001 (g, h)	
Global binomial test for interspecies L/A differential regulation (p-value)									<0.001			
Global binomial test for interspecies S/A differential regulation (p-value)									<0.001			
Global binomial test for promastigote/amastigote differential regulation (p-value)									<0.001			


A
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N (x10⁶/ml)
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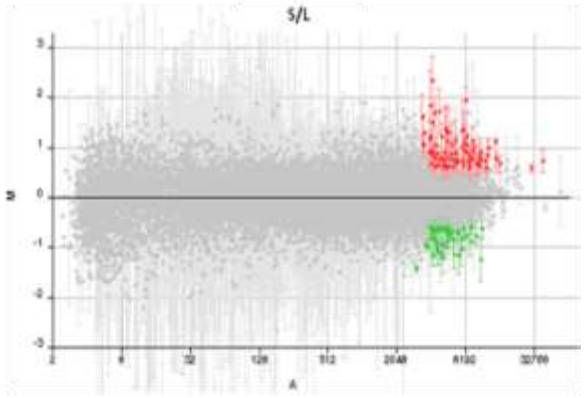
S/L
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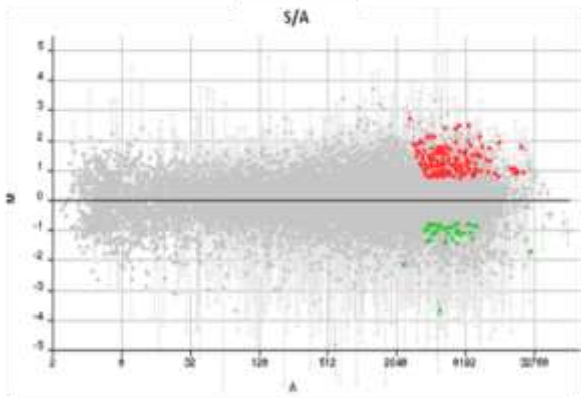
S/A
M
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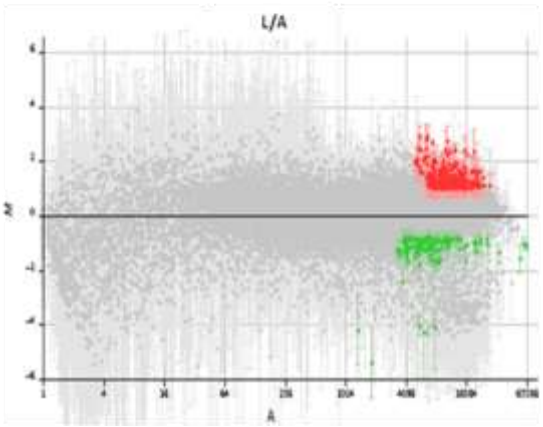
L/A
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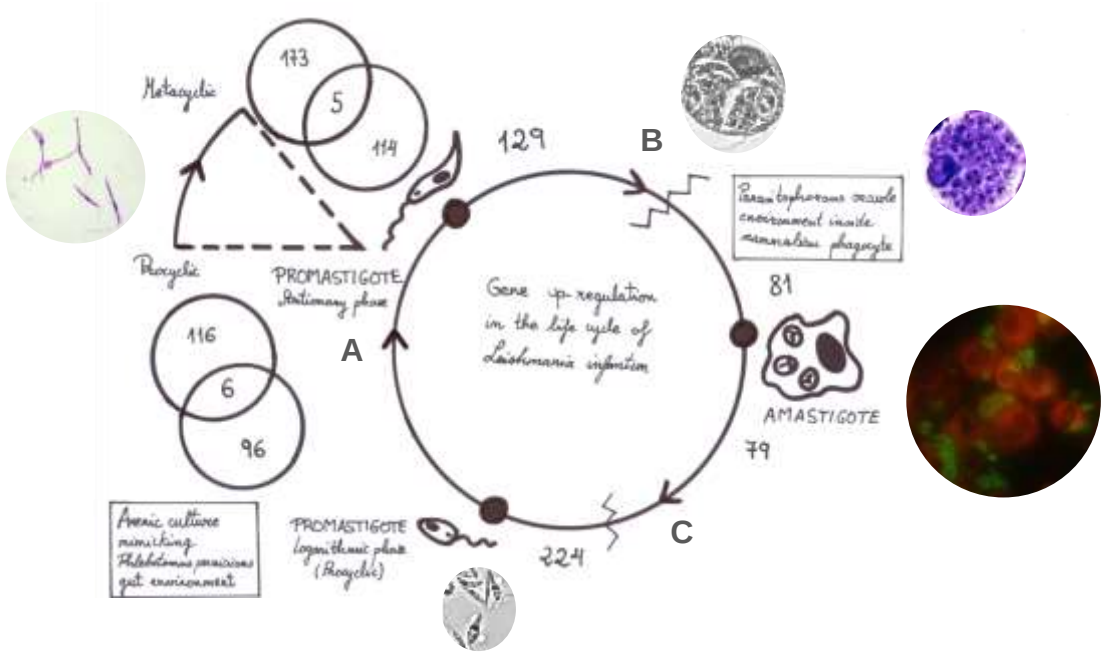
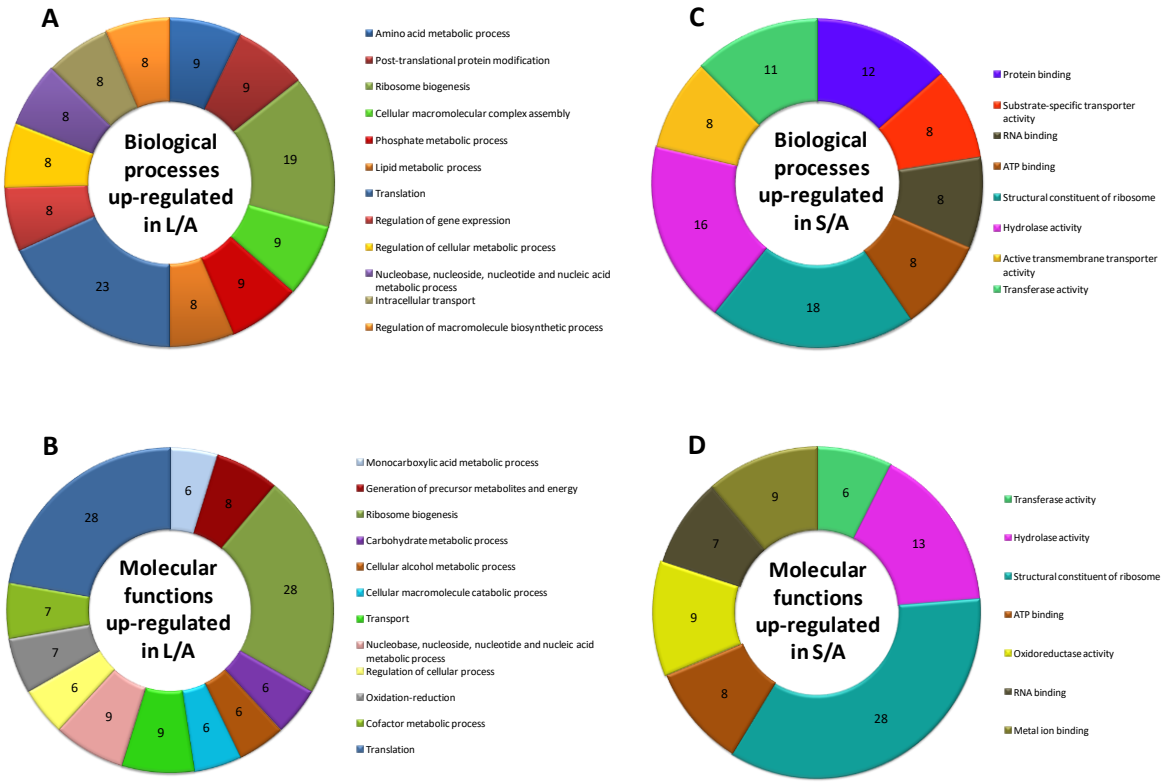

Metacyclic
173
5
114
Procyclic
116
6
96
PROMASTIGOTE
Stationary phase
A
129
B
Gene up-regulation in the life cycle of Leishmania infantum
81
AMASTIGOTE
79
C
224
PROMASTIGOTE
logarithmic phase
(Procyclic)
Axenic cultivare mimicking Phlebotomus promicrops gut environment
Parasitophorous vacuole environment inside mammalian phagocyte


A
Biological processes up-regulated in L/A
9
9
19
9
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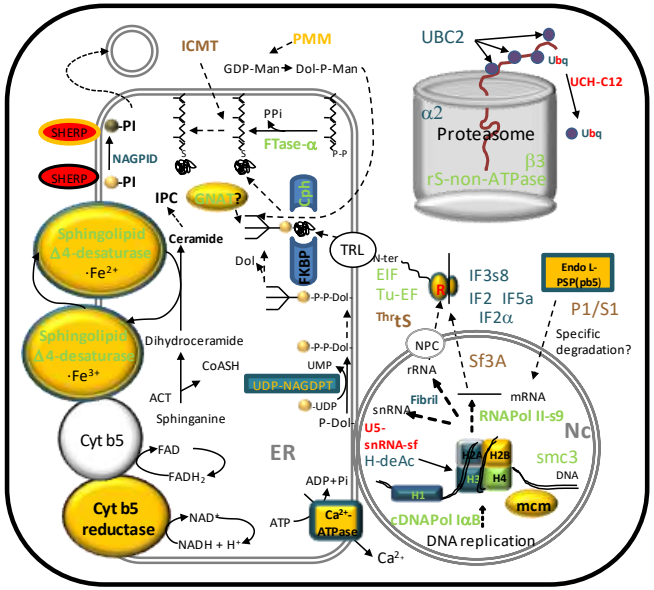
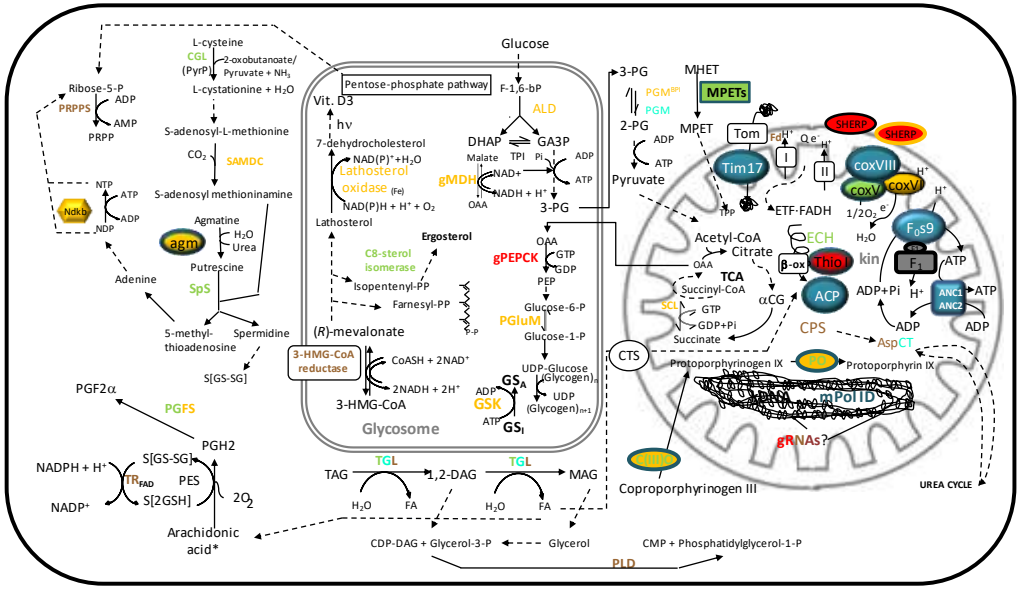
8
23
8
8
8
8
8
Amino acid metabolic process
Post-translational protein modification
Ribosome biogenesis
Cellular macromolecular complex assembly
Phosphate metabolic process
Lipid metabolic process
Translation
Regulation of gene expression
Regulation of cellular metabolic process
Nucleobase, nucleoside, nucleotide and nucleic acid metabolic process
Intracellular transport
Regulation of macromolecule biosynthetic process
C
Biological processes up-regulated in S/A
12
8
8
8
18
16
8
11
Protein binding
Substrate-specific transporter activity
RNA binding
ATP binding
Structural constituent of ribosome
Hydrolase activity
Active transmembrane transporter activity
Transferase activity
B
Molecular functions up-regulated in L/A
6
8
28
6
6
6
9
9
6
7
7
28
Monocarboxylic acid metabolic process
Generation of precursor metabolites and energy
Ribosome biogenesis
Carbohydrate metabolic process
Cellular alcohol metabolic process
Cellular macromolecule catabolic process
Transport
Nucleobase, nucleoside, nucleotide and nucleic acid metabolic process
Regulation of cellular process
Oxidation-reduction
Cofactor metabolic process
Translation
D
Molecular functions up-regulated in S/A
6
13
28
8
9
7
9
Transferase activity
Hydrolase activity
Structural constituent of ribosome
ATP binding
Oxidoreductase activity
RNA binding
Metal ion binding

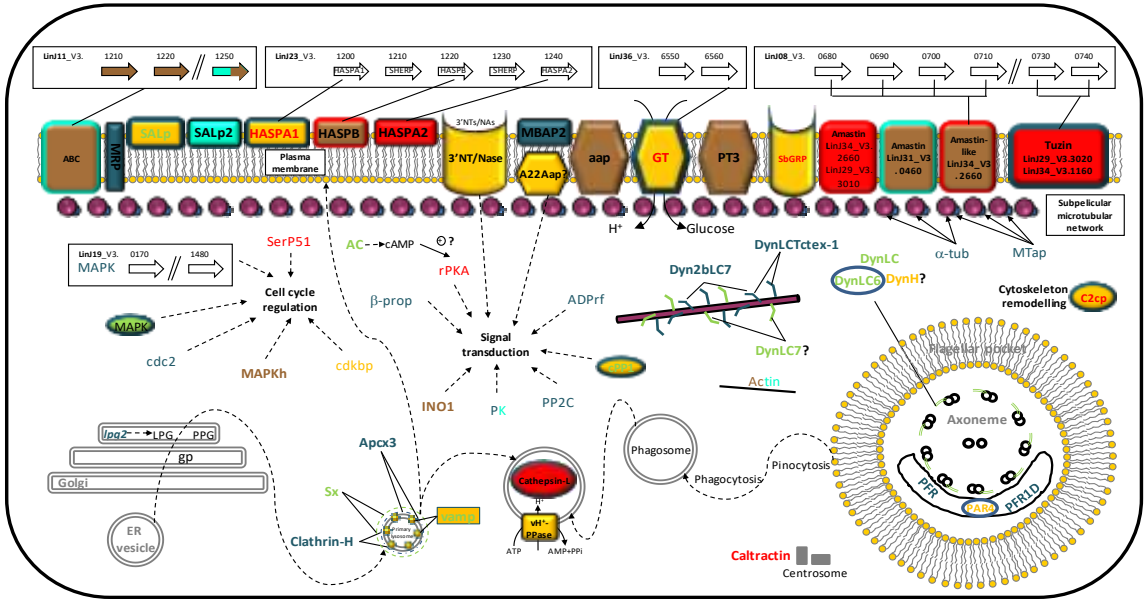
A. Gene expression, secretory pathway and ubiquitin-proteasome degradation.



B. Metabolism and kDNA-related genes.



C . Surface molecules, cytoskeleton, vesicle trafficking, signal transduction and cell cycle regulation.



Down-regulated in S/L

Up-regulated in S/L

Down-regulated in S/A

Up-regulated in S/A

Down-regulated in L/A

Up-regulated in L/A

SUPPLEMENTARY DATA FILE 1

Fig. S1. A replicate of *L. infantum* microarray hybridization experiment with L/A (Cy5/Cy3) cDNA samples.

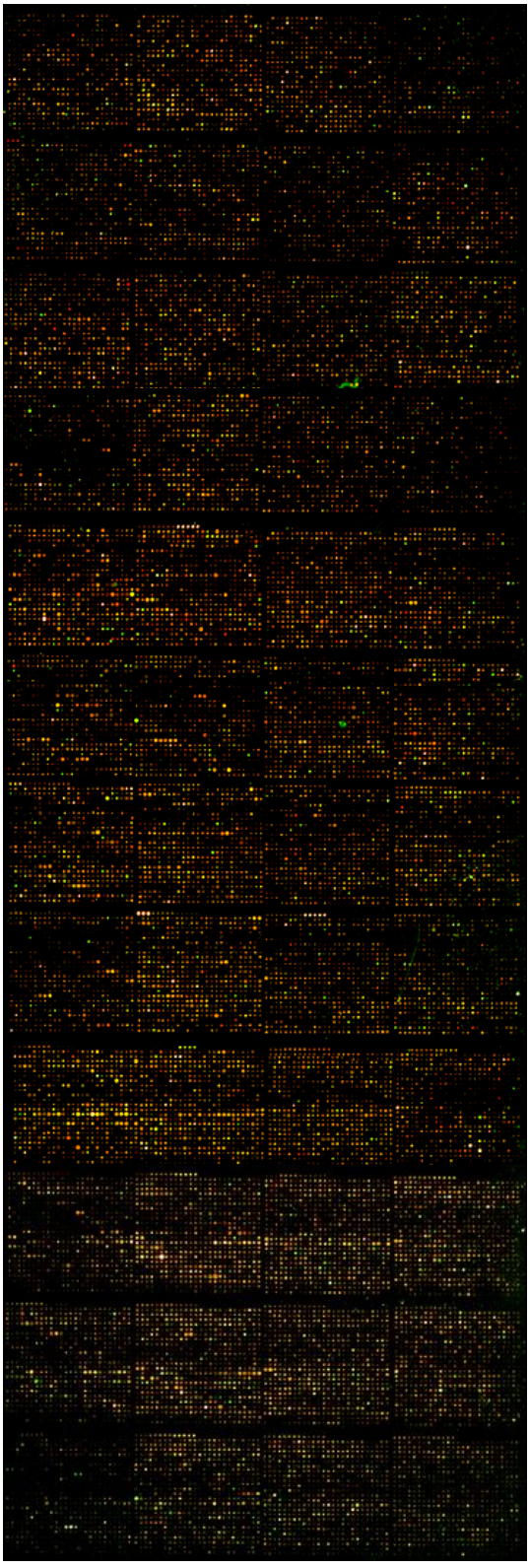


Fig. S2. Raw image of microarray hybridization analysis of *Leishmania*-U937 cell line cross-hybridization. Genomic DNA was isolated from U937 cells. Then, it was randomly sheared by sonication and labelled with Cy5 by GenomiPhi™ DNA Amplification Kit (GE Healthcare). Hybridizations with *L. infantum* genomic microarrays were performed under the specific conditions described in the MS for *L. infantum* microarray-cDNA. Cross-hybridization was not found once the local background median was substracted and intensity value cutoff was applied.

Table S1. **Primers for qRT-PCR reactions.** Annealing temperature for each oligonucleotide pair was optimized by temperature gradients checked by agarose gel electrophoresis, ensuring the absence of primer dimmers and unspecific sequences together with melting curve analysis. Sequence specificity was checked by product sequencing. Thermal cycling was: 95 ºC for 5' + 40x (95 ºC for 30'' + 50-60ºC for 30'' + 72ºC for 30'' data acquisition) + 72 ºC for 5' + melting curve [95 ºC for 1' + 80x (55→95 ºC for 10'' (+0.5 ºC/step) with data acquisition)]. Quantification method: efficiency corrected ΔCt method after elimination of outliers (CV > 20%).

Annotation	Annotated gene function	Forward (5'-3')	Reverse (5'-3')
LinJ36_V3.6550	Glucose transporter, lmgt2, putative (GT)	TGACGATCCTGCTTGGGATT	CAGGCCAAGACTGCCCATGA
LinJ36_V3.6560	Glucose transporter, putative (GT)	CGGCAAGGCTCCCTCATAAT	CGAGGAATCTGGTGCGGTGT
LinJ29_V3.1160	Ribosomal protein L1a, putative (L1a)	ATCTGGACGAAGTCGGCGTT	ACGTCCGTGTTCGTCAGCAT
LinJ24_V3.0420	Cysteine peptidase, Clan CA, family C12, putative (UHC-C12)	AGGCGACGGAGGCGAACTAT	CGTGACCGGGTCGTCCACAA
LinJ31_V3.1660	Putative 3 ketoacyl-Coa thiolase-like protein (Thiol I)	GGCTGAAAAGGCGGGGTACT	CTTTTTGCCCGAACTCCGAA
LinJ09_V3.0650	Serine peptidase family S51, peptidase E, putative (SerP51)	GCAATACCTCGCCCACTTCA	CAAACGGATCCAGCACCACT
LinJ23_V3.1200	Hydrophilic surface protein 2 (HASPA1)	ATGGGAAGCTCCTGCACGAA	CATGGATGCCGTCCTTCTCA
LinJ23_V3.1210	SHERP	GAGCGACAATGCGCACAACA	TTACGAGCCGCCGCTTATCT
LinJ23_V3.1220	Hydrophilic surface protein (HASPB)	AATGACGGCGATGGCCCTAA	GCCACCGCCGTCATTTTTCT
LinJ23_V3.1230	SHERP	CGGCGGAGACGAGCGACAAT	CCGTGCTGCCCACTGCATCA
LinJ23_V3.1240	Hydrophilic surface protein 2 (HASPA2)	TGCTGACCACTGCCAGCGTT	TGCCAAACTCTCGGCGATGA
LinJ29_V3.3010	Amastin, putative	GCCGCGACCGTTTCCTCCTT	ACGCAGAGACGACGAAGAA
LinJ29_V3.3020	Tuzin-like protein, putative	AGGGCGCCATCAGCTGCATT	GCACACCCTCGCCCTTCGAT
LinJ08_V3.0730	Tuzin, putative	CTGCCACGGCTCGATTTCTA	GTTCGTCCCCATCGTTTCAA
LinJ31_V3.0950	Sodium stibogluconate resistance protein, putative (SbGRP)	AGACCTCCACCTCAGCGATGA	GAACTCGGCGCTCTCAGGTAT
LinJ15_V3.0240	Protein phosphatase 1 catalitic subunit, putative (cPP1)	AAAGCGGCATTTGCTCACCAT	TCCAACGTGATGATGCTGCAT
LinJ20_V3.0310	Developmentally regulated phosphoprotein-like protein (DRPP)	AGTCAGCAAACCAGCTCTGCA	CGCGTACCGTAGCCCTCCAT
LinJ31_V3.0860	Triacylglycerol lipase-like protein (TGL)	AGCACGTCCCCTATGAGGTT	GATGCTTGCACGATGCATGT
LinJ30_V3.0790	4-methyl-5(beta-hydroxyethyl)-thiazole monophosphate synthesis protein, putative (MPETs)	ATGCCAGGAGCCGTCCATCT	CAGCAGCCCCATAGGAGCAA
LinJ21_V3.0080	Syntaxin, putative (Sx)	GCGCGAAACAGGCCTCCACA	CACCTGGACGCCTGAGATGT
LinJ28_V3.2280	Dynein light chain lc6, flagellar outer arm, putative (DynLC6)	TGGCGCGACGGTGAAGCTAT	ACCAAAGTTCCGGCCAACAA
LinJ33_V3.2930	GTP-binding elongation factor Tu family protein, putative (EF-Tu)	TGTGTCTCAAGAAGGAAAAA	GCCGCTGATCGTCGTTGAGT
LinJ08_V3.0010	Structural maintenance of chromosome 3 protein, putative (smc3)	ATCACGGAGGTGCTCAACGA	TCTTCAGCTCCCGCACTGTT
LinJ08_V3.0030	Vesicle associated membrane protein, putative (vamp)	TTAGCGCGTGGTGACCGTAT	TGATCCGCCAGAGTGGAAGA
LinJ06_V3.1330	Coproporphyrinogen III oxidase, putative (C(III)O)	CCGGCGCAAGAACACCATA	GACTGGAGGCCGAACTTCGT
LinJ06_V3.1340	Protoporphyrinogen oxidase-like protein (PO)	GAAGGCGAATGCAGCAGAGAA	GCCGCTGACATGCCGACTAT
LinJ28_V3.0110	Proteasome beta 3 subunit, putative (b3)	TGAGAGCTTGCACGGCATGT	TGCACCGTAGCCGGAGAGA
LinJ28_V3.0120	DNA directed RNA polymerase II, subunit 9, putative (RNAPol II-s9)	CTGCGCTTCGAGCTGTTCGA	ACAGGGTCGGTGGACAGGAT
LinJ26_V3.1690	Cytochrome c oxidase subunit V, putative (coxV)	GAGATGGATGGCTGCAAGCT	CCTCCCACTTCATCGTCTCT
LinJ16_V3.0950	Dynein light chain, putative (DLC)	CGTTGGAGAAGCACGAGGATA	CACAATGCCAGGAAGGTCCAA
LinJ28_V3.0090	Adenylate cyclase-like protein (AC)	TACCTCTTCCGCAGCGTTGA	CAGGCCGACATCGCTCACTA
LinJ19_V3.1520	Eukaryotic translation initiation factor-like protein (EIF)	AAGGCCAGGGCACAGAAAAT	GGCAGGATGGTGCAGTTGAA
LinJ21_V3.0700	Phosphoglucomutase (PGluM)	GCTACAGGCGGGACGCATAAT	GAGTGGAATGATCCGCGATGA
LinJ26_V3.1670	Sphingolipid delta-4 desaturase	ACGTTGATCTGCACCGTACCA	ACCCGTCGATGTCGGCAGTA
LinJ26_V3.1000	Dynein heavy chain, putative (DynH)	TCTTTCCGCAAGGGTTTCTCA	TCCTCCGGCAGCTCCTAACA
LinJ22_V3.0340	40S ribosomal protein S15, putative (S15)	GCGCTGAGCGAGGAGGAGTT	CTCGCCAACCTTACGTGCT
LinJ05_V3.1210	Surface antigen-like protein (SALp)	ACCTTCCCAGCTTCCCTGAA	AGCAGCCACCAGGTTCGATT
LinJ23_V3.1560	Lathosterol oxidase-like protein	TCCACCACGAACTGTCCAACT	CTCGTACACGGGGTCCACATA
LinJ32_V3.4040	Cysteine peptidase, Clan CA, family C54, putative (C54cp)	CCAATTCGCTCGTGGCGTTT	GCCACAGAAACGACGCCATT
LinJ32_V3.4050	60S ribosomal protein L2, putative (L2)	ATCATCTCGCACAACCACGA	CGGAAGCGGTAGAACGAGTT
LinJ28_V3.2380	2,3-bisphosphoglycerate-independent phosphoglycerate mutase-like protein (PGM^{BPI})	GCTACAGGCGGGACGCATAAT	GAGTGGAATGATCCGCGATGA
LinJ28_V3.2390	Cyclin dependent kinase-binding protein, putative (cdkbp)	ATCGACCCGACAGAGCTGCAT	CGTCTCGGCGTTGTTGAAACT
LinJ15_V3.0220	60S ribosomal protein L13a, putative (L13a)	GGAGATCCGCAACAAGATCA	GTAGGCCACCAGCGAGTTAA
LinJ33_V3.0770	60S ribosomal protein L6, putative (L6)	AGGTCGACATCTCCAGCGTT	ATGAAGTCGCCCTCGCTCTT
LinJ35_V3.0600	60S ribosomal protein L18a, putative (L18a)	AGCCAGGCGTACCACGACCT	TCCGGGATGCTCTTCACGTT
LinJ30_V3.3390	60S ribosomal protein L9, putative (L9)	CCCGTCCAAGGTCAAGGATG	GACCAGGCACATCTGGTGCA
LinJ36_V3.4730	60S ribosomal protein L18, putative (L18)	TGGTCGGTGACGTCCTGGAT	TTGCGACCACGCAGCAGGTA
LinJ13_V3.1120	40S ribosomal protein S4, putative (S4)	TGCGTGGACCTGATCAAGAA	CGCTCCTGGATCACATTCAT
LinJ31_V3.2370	3′-nucleotidase/nuclease, putative (3′NTase/Nase)	GGCTGAGGTGCACAACCACT	GGGCGACGTGCTCATAGGAA
LinJ31_V3.2380	3′-nucleotidase/nuclease precursor, putative (3′NTase/Nase)	CAACACCACCATTGGGCACA	TAAATCCAGTGCGATCGGCT
LinJ31_V3.1240	Vacuolar-type proton translocating pyrophosphatase 1, putative (vH^+-PPase)	ATGGCTATCTCGGCCTCCAA	GCCGGGCCTGAGGTATCCT
LinJ23_V3.0220	Endoribonuclease L-PSP (pb5), putative	ACTCGCTGACGGCTGAGGAT	ACAAGCGGGTGGTCATGGAA
LinJ32_V3.3100	Nucleoside diphosphate kinase b, putative (Ndkb)	GACTCACAGCCTGGCACGAT	CTCATCCGCCTTGAACCAAAA
LinJ32_V3.3120	Minochromosome maintenance (MCM) complex protein (mmc)	GGATAGACCGCCACGGATA	CGGAGAGATCGACGGATGAA
LinJ33_V3.0960	40S ribosomal protein S3, putative (S3)	GGGCATCATCCGCTACGTCA	GCGGAGTCGACGAACGACTT
LinJ36_V3.3100	Succinyl-CoA ligase (GDP-forming) beta-chain, putative (SCL)	CCAACTTCCTCGACGCTGGT	TTGCGCTATGACATCGCAGT
LinJ11_V3.1180	40S ribosomal protein S15a, putative (S15a)	ATGATGAGCGTTCTCGCGAA	CTTGCCAGCGCGATGGTCAT
LinJ15_V3.1600	Presenilin-like aspartic peptidase, clan AD, family A22A, putative (A22A)	CACGCCACTCCATTCAAGCT	CCGCCAGGATGCTCACCATA
LinJ35_V3.2080	Calcium motive P-type ATPase, putative ($Ca2^+$-ATPase)	GCGCTGGCTCGACAACAAGT	GTGATCATGCTGTGTACAAA
LinJ28_V3.0210	Histone H2B variant (H2B)	GACTCTGACAGCTCGCGAAA	TTGTACTTGGCGACAGCCTT
LinJ29_V3.1920	40S ribosomal protein S15a, putative (S15a)	CCTGAACAAGTGCGGTGCTA	GAGGTCGTCAGCACCACGAA
LinJ30_V3.3160	S-adenosylmethionine decarboxylase proenzyme-like protein (SAMDC)	GGTGGATGGTCATCACTGCT	CACTTTGCAGCGGCGTGGAAA
LinJ16_V3.0470	60S ribosomal protein L21, putative (L21)	GTCGGCGACTACGTCGATGT	GGTGCTCCTTCGCCTTGAAT
LinJ21_V3.1290	60S ribosomal protein L9, putative (L9)	TTCACGGTGATTCGCTGGTT	CACCTTGAAGCGGTAGCCCT
LinJ21_V3.1300	40S ribosomal protein S23, putative (S23)	GTGCGTGTGCAGCTGATCAA	CACCAGCACCTCGTCGTTCT
LinJ36_V3.4390	UDP-N-acetylglucosamine-dolichyl-phosphaten-acetylglucosaminephosphotransferase (UDP-NAGDPT)	TGCCGCAGCTCTTCAGCATT	GATCGCCCACGTCAGCTTCT
LinJ06_V3.0410	60S ribosomal protein L19, putative (L19)	GTGTTCCGCAACAAGCGCAA	TTGCGAGCCTTGTTGCGGTT
LinJ21_V3.1790	40S ribosomal protein S11, putative (S11)	TGAGGTCGTCATTGGCCAGT	GGCGAACTTCTTGCCCATCT
LinJ01_V3.0430	Ribosomal protein S7, putative (S7)	CGCCCGACGGACGTGTACAA	CGGGGGTCCAGGAACACCTT
LinJ23_V3.0080	Agmatinase-like protein (agm)	CGCGACCTGTTGCACATCAT	GCCATCGCTACGGCACATAT
LinJ36_V3.2070	Phosphomannomutase, putative (PMM)	AGGCGGTCAAATCAGTTTCGA	TAATCGTTGCCGCCCTCTGA
LinJ19_V3.0040	Histone H2B (H2B)	CGTGATGGAGCGCATCTGCA	GCCTTCGTGCCCTCAGCCAT
LinJ19_V3.0050	40S ribosomal protein S2, putative (S2)	AGATCTACCCCGTGCAGAAA	TGTGGCTCACCGATCTTGTT
LinJ05_V3.0040	Paraflagellar rod component par4, putative (PAR4)	AAGGACGACGGTATGAGCA	TCAGTGCCCCGAAGTTGCTCT
LinJ19_V3.0710	Glycosomal malate dehydrogenase (gMDH)	ACGGGTAAACCGCTGGTGTA	ACTCGCTGCCCTTCACGATA
LinJ30_V3.3650	40S ribosomal protein S14 (S14)	AAGAAGGGCGACCTCGTTTA	CTCCCGGTGACCTTCACGTAT
LinJ24_V3.2340	Fatty acid desaturase, putative	TCACCTTCGGGAGGGCTAT	GCGCGGATGATCCACTTGGT
LinJ07_V3.0360	Phosphatidylglycerophosphate synthase-like protein (PLD)	TCGTCTGCCCCAAGGAGTTT	CCTTCTCCACCACCCAACGT
LinJ17_V3.1520	Otubain cysteine peptidase, Clan CA, family C65, putative	GAAGGGTGTGGCCGACAAGA	TGCAGGTAAGCGGAGATCAA
LinJ05_V3.0350	Trypanothione reductase (TR)	ACGGCGAGGTTCTGGGTGTT	TCCGATGGTGCTGTGGAAGT
LinJ14_V3.1440	Pteridine transporter (PT3)	GCCGCTGACGAAGCCACTGA	TCCGCCTTCGTCGTCGTTGAA
LinJ14_V3.1450	Myo-inositol-1-phosphate synthase (INO1)	CTCATGGAGCGCGTCACGAT	CATGGCACGCAGCAGCAGTTCT
LinJ30_V3.1520	P1/S1 nuclease (P1/S1)	GTGGACGTGATGGCGATTCAT	GACACGCTTGCATCGAGCAA
LinJ16_V3.0550	Aspartate carbamoyltransferase, putative (AspCT)	ACGGTGCATTCGCTGCTGAA	GAGGCCACACCCTGCTGAA
LinJ22_V3.0680	3'a2rel-related protein (3'a2rel)	ACGCGGTACAGCGAAAGGT	CAGCGTCGAGGTTCAGCCAA
LinJ11_V3.1220	ABC transporter-like protein (ABC)	CAGGTGGTCTTCCTGGACGA	CACCATGATCGCCACAATGT
LinJ16_V3.0590	Carbamoyl-phosphate synthetase, putative (CPS)	GCTACACCGGCTTCTCCTTT	ACGCGTGATCTTCGTCATCT
LinJ24_V3.2410	Mitogen-activated protein kinase (MAPK)	TACGCGGAGTGTGCGTTTCGA	TTCCCGATCATCCCAGTTGA
LinJ23_V3.0230	ABC transporter/multidrug resistance protein/p-glycoprotein, putative (MRP)	GGGAGCGGGTTCATCCTGAT	TGCAGCCGGTGCGCGATCGT
LinJ32_V3.1770	Protein phosphatase 2C, putative (PP2C)	GAGGTAGCGCAGCAGCTTGT	GGCCTTTCGCTTTCTTGTCGT
LinJ28_V3.2650	Ribosomal protein S29, putative (S29)	GCATCTCGACCAGTGGCGTT	CGCCTTCTGGTTGGAGCAGA
LinJ09_V3.0060	N-acetylglucosaminylphophatidylinositoldeacetylase (NAGPID)	GTTGGACTGCTGGCGGTTCT	CAGACTGATGCGGGTGGAAT
LinJ13_V3.1450	Alpha tubulin (a-tub)	ACGTACCGCCAGCTGTTCAA	CTGGAGACCCGTGCAGTTGT
LinJ21_V3.2070	Proteasome alpha 2 subunit, putative (a2)	ACGCTAAAGGAGGGCTTTGA	CGACGCTCAGGACTTCGAAA
LinJ23_V3.0040	Beta propeller protein, putative (b-prop)	CCCGATGTCCTCGTTTCCAC	AAGCGAAGAACGCGCACAAT
LinJ36_V3.0250	Peptidyl-prolyl cis-trans isomerase, putative (FKPB)	ACCGTGCACTGCACCGGGTA	CTCATCCCAGCCGCGAATCA
LinJ05_V3.1060	ATPase, putative (AFG1)	TCTGATGTGGCACACGCCAT	ACAATGACGTCGCGCCCAAA
LinJ09_V3.1390	Paraflagellar rod component, putative (PFR)	GACCACGAGGCTGACGAGTT	GTCTCGGTTATGCTGCGCAA
LinJ23_V3.1430	Membrane-bound acid phosphatase 2 (MBAP2)	TGCGTATGTATTGCCCCTTCA	GAGCCCCGATGCAGAAGGTT
LinJ36_V3.7320	Eukaryotic translation initiation factor 3 subunit 8, putative (IF3s8)	GAGGCCCACCAGCTCTTCTA	GGGCCTCAGTTGAAGGCTAA
LinJ26_V3.2230	Ribosomal protein , putative	TGCGTGAAGGTGAAGCACAA	GCAGTCACGGCGATCTTCTT
LinJ09_V3.1190	Inner membrane preprotein translocase Tim17 (Tim17)	GATGGTGGCCAAGGACAGCA	AAGCCGAAGAAGGCGAAGCT
LinJ24_V3.1380	Translation initiation factor IF-2, putative (IF2)	CGACTCGCTTGAATGCCAACT	GCGAGGTACGCACCAATGGA
LinJ21_V3.0800	60S ribosomal protein L36, putative (L36)	CGCGCTGTGAAGGCTATCAT	GCTTCTTCTTGGCCGCAGTA
LinJ21_V3.0820	ATPase subunit 9, putative (F_0s9)	GCCATCTTTGGCTGCTTGCT	CATCTTGGTCAGGTTGGGCT
LinJ34_V3.4290	Lipophosphoglycan biosynthetic protein (lpg2)	GGCAGCAATCGGTGACACAA	CATTGTGGTGGGCGAGGTTA
LinJ13_V3.0460	40S ribosomal protein S12, putative (S12)	AGGAGAACGTCGTGGTTGAT	CTCCTCATCCTCGCAGTCAT
LinJ19_V3.0170	MAP kinase, putative (MAPK)	TCTGCGCTGTACGCTGGCAT	GGGCTGCTGTTGCTGGTCAA
LinJ19_V3.0190	ADP/ATP translocase 1, putative (ANC1)	GACGGCCTGGTAGGTCTGTA	ATCCAGCCCAGCATGAAGTT
LinJ26_V3.0160	60S ribosomal protein L7, putative (L7)	TGCGCGGAGGATATGGTGAA	CGCCACCTTCCACGAAGTGA
LinJ19_V3.0090	Fibrillarin, putative (Fibril)	ATCAAGGCGAACTGCATCGA	AGAAGCTGAAGGATTCCGGC
LinJ16_V3.0600	Histone H3, putative (H3)	ACGTCGAAGAAGAGCAAGAA	ATCAGCAGGCTCGTACTCTT
LinJ24_V3.2230	60S ribosomal protein L12 putative (L12)	CGCAGTGTGACGCACGAGGT	TTCGCTCGCCTTCCAGACGT
LinJ36_V3.0590	Ubiquitin-like protein, putative (ubq)	AGCAACCCCCAGTTTATGCA	AGCTTCCCATCATTGCCATT
LinJ36_V3.0600	Cdc2-related kinase (cdc2)	TGGAGTGTCGGCTGCATTTT	GCGTCCCCAGCACTTGAAAA
LinJ31_V3.1600	Cytochrome c oxidase VIII (cox VIII), putative	GATCCCTACCGCCTCACCAA	TCCGGCCAATCCATCATGTA
LinJ27_V3.1120	Histone H1, putative (H1)	GGCGGTGAGGAAGGTGGCTA	GCGACCTTCTTCGCGACCTT
LinJ31_V3.2070	Ubiquitin-fusion protein (ubq)	CCGCACGCTCTCGGACTACA	GCCTTCTTGCGGCAGTTCGT
LinJ33_V3.3380	60S ribosomal protein L44, putative (L44)	TGCAACGCACACAAGTCGTT	CCTGGCGGATAGACTTGCAA
LinJ33_V3.3390	H1 histone-like protein (H1)	TCCGCACGCAGAAGATGGAA	TTCGACGGGCGAGACTTCAA
LinJ29_V3.1880	Paraflagellar rod protein 1D, putative (PFR1D)	ATCTGGACGAAGTCGGCGTT	GGCAGCGTGAAGCCCTTCTT
LinJ23_V3.0060	Peptidyl-prolyl cis-trans isomerase, putative (FKBP)	TGAGCGACTGGGATCGTCTT	GGTGTTGCCGCTGTTCCTTA
LinJ29_V3.2310	GTP-binding protein, putative	GCGCGCGTTCGCGAATATCT	ATCCCTGCCGCCCCTCTTGT
LinJ32_V3.1080	Protein kinase, putative (PK)	TCCGCAGCGTCTGTGTCGAT	TGGCGCTTGTGGAACGTCTT
LinJ29_V3.1370	Lipase domain protein, putative (TGL)	TGAGAGCGACGTTGTCGTGAA	ATCGCTGTCGGCTTCACAATA
Ref. gene	Li18S	TGCGACAAGTGCTTTCCCAT	GGCGCCTTTGAGGGGTTTA

Table S2. Table of differential regulation frequencies for S/A and L/A transcriptome analyses in *Leishmania* sp. n is the total number of differentially regulated genes; p is the frequency of down-regulated genes in amastigotes and q = 1-p is the frequency of up-regulated genes in amastigotes.

Ref. MS	n	p	Q	np	nq	Approx.
Akopyants et al., 2004	122	0.426	0.574	52.0	70.0	Yes
Almeida et al., 2004	132	0.561	0.439	74.0	58.0	Yes
Leifso et al., 2007	155	0.439	0.561	68.0	87.0	Yes
Holzer et al., 2006	236	0.881	0.119	207.9	28.1	Yes
	51	0.725	0.275	37.0	14.0	Yes
Srividya et al., 2007	25	0.400	0.600	10	15.0	No
Rosenzweig et al., 2008	494	0.650	0.350	321.1	173.0	Yes
McNicoll et al., 2006	73	0.274	0.826	20.0	60.3	Yes
Leifso et al., 2007	55	0.855	0.145	47.0	8.0	No
Rochette et al., 2009	477	0.545	0.455	260.0	217.0	Yes
	921	0.519	0.481	478.0	443.0	Yes
Alcolea et al., 2010	180	0.555	0.445	99.9	80.1	Yes
This manuscript	345	0.696	0.304	240.0	105.0	Yes
Depledge et al., 2009	55	0.873	0.127	48.0	7.0	No

SUPPLEMENTARY DATA FILE 2

Table S3. Hypothetical and unknown protein genes that are differentially regulated in S/L.
F < -1.7 indicate gene down-regulation and F > 1.7 up-regulation.

Clone	*Fold change*	*$Log_2R \pm SD$*	*p*	*NCBI Acc. No.*	*E-value*		*Clone def.*	*Annotation*	*Gene function*
					F	*R*			
Lin21A6	1.93	0.9 ± 0.2	0.017	GS882549	0	0	a	LinJ07_V3.1060	Hypothetical protein, unknown function
Lin43D9	2.08	1.1 ± 0.3	0.031	GS882550	0	1e-180	b	LinJ08_V3.0280	Hypothetical protein, conserved
								LinJ35_V3.4550	Hypothetical protein, conserved
								LinJ35_V3.4560	Hypothetical protein, conserved
Lin86C2	1.97	1.0 ± 0.2	0.018	GS882551	-	5e-137	b	LinJ04_V3.0540	Hypothetical protein, conserved
Lin106B12	1.85	0.9 ± 0.1	0.002	GS882552	2e-105	-	b	LinJ29_V3.2330	Hypothetical protein, unknown function
Lin111G5	1.74	0.8 ± 0.1	0.014	GS882553	0	0	b	LinJ12_V3.0090	Hypothetical protein, conserved
Lin121C5	1.96	1.0 ± 0.2	0.022	GS882554	0	0	b	LinJ06_V3.1290	Hypothetical protein, conserved
Lin138B4	1.76	0.8 ± 0.2	0.028	GS882556	0	0	b	LinJ32_V3.3700	Hypothetical protein, conserved
								LinJ32_V3.3710	Hypothetical protein, unknown function
Lin139E2	1.74	0.8 ± 0.2	0.045	GS882557	0	0	b	LinJ06_V3.1360	Hypothetical protein, conserved
Lin142D1	2.00	1.0 ± 0.1	0.004	GS882558	2e-74	2e-108	b	LinJ08_V3.1090	Hypothetical protein, conserved
								LinJ08_V3.1100	Hypothetical protein, conserved
Lin147B4	1.79	0.8 ± 0.2	0.027	GS882559	1e-134	0	b	LinJ08_V3.1090	Hypothetical protein, conserved
								LinJ08_V3.1100	Hypothetical protein, conserved
Lin163A9	1.94	0.9 ± 0.2	0.034	S19A3	0	0	b	LinJ30_V3.2310	Hypothetical protein, conserved
								LinJ30_V3.2320	Hypothetical protein, conserved
								LinJ30_V3.2330	Hypothetical protein, conserved
Lin165D2	1.81	0.9 ± 0.2	0.023	GS882560	0	0	b	LinJ30_V3.2310	Hypothetical protein, conserved
								LinJ30_V3.2320	Hypothetical protein, conserved
								LinJ30_V3.2330	Hypothetical protein, conserved
Lin169B8	1.74	0.8 ± 0.2	0.023	GS882561	0	0	b	LinJ24_V3.2320	Hypothetical predicted multi-pass transmembrane protein
								LinJ24_V3.2330	Hypothetical protein, conserved
Lin173H5	1.93	0.9 ± 0.2	0.038	GS882562	0	0	b	LinJ31_V3.2340	Hypothetical protein, conserved
Lin238G11	2.05	1.0 ± 0.4	0.044	GS882563	6e-158	0	b	LinJ12_V3.0090	Hypothetical protein, conserved
Lin312C2	1.84	0.9 ± 0.1	0.002	GS882564	0	0	b	LinJ27_V3.0120	Hypothetical protein, conserved
Lin12F8	-1.91	-0.9 ± 0.0	0.001	GS882565	0	0	b	LinJ28_V3.0820	Hypothetical protein, conserved
Lin13A1	-1.93	-0.9± 0.3	0.031	GS882566	6e-44	4e-14	b	LinJ28_V3.3260	Hypothetical protein, conserved
Lin16A6	-1.97	-1.0 ± 0.4	0.051	GS882567	0	0	a	LinJ33_V3.1740	Hypothetical protein, conserved
								LinJ33_V3.1750	Hypothetical protein, conserved
Lin27H9	-1.74	-0.8 ± 0.2	0.044	GS882568	0	0	b	LinJ17_V3.0010	Hypothetical protein, conserved
Lin41A10	-1.75	-0.8 ± 0.3	0.040	GS882569	0	0	b	LinJ09_V3.1610	Hypothetical protein, conserved
								LinJ09_V3.1620	Hypothetical protein, conserved
								LinJ09_V3.1630	Hypothetical protein, conserved
Lin44H5	-1.92	-0.9 ± 0.2	0.051	GS882570	0	0	b	LinJ31_V3.0090	Hypothetical protein, conserved
								LinJ31_V3.0100	Hypothetical protein, conserved
Lin47D11	-1.71	-0.8 ± 0.2	0.032	GS882571	0	0	b	LinJ30_V3.2860	Hypothetical protein, conserved
								LinJ30_V3.2870	Hypothetical protein, conserved
Lin50C5	-1.79	-0.8 ± 0.0	0.002	GS882572	0	0	b	LinJ20_V3.0560	Hypothetical protein, conserved
Lin83E8	-1.93	-0.9 ± 0.2	0.021	GS882573	0	0	b	LinJ35_V3.0140	Hypothetical protein, conserved
								LinJ35_V3.0150	Hypothetical protein, conserved
								LinJ35_V3.0160	Hypothetical protein, conserved
Lin122B10	-1.75	-0.8 ± 0.2	0.027	GS882576	0	0	a	LinJ29_V3.2070	Hypothetical protein, conserved
Lin159H6	-1.86	-0.9 ± 0.3	0.044	GS882577	0	0	b	LinJ18_V3.1630	Hypothetical protein, conserved
								LinJ18_V3.1640	Hypothetical protein, conserved
Lin173C7	-2.08	-1.0 ± 0.1	0.009	GS882578	0	2e-170	b	LinJ33_V3.0650	Hypothetical protein, conserved
								LinJ33_V3.0660	Hypothetical protein, conserved
								LinJ33_V3.0670	Hypothetical protein, conserved
Lin204D8	-2.05	-1.0 ± 0.1	0.006	GS882579	0	0	b	LinJ33_V3.0650	Hypothetical protein, conserved
								LinJ33_V3.0660	Hypothetical protein, conserved
								LinJ33_V3.0670	Hypothetical protein, conserved
Lin223E7	-1.92	-0.9 ± 0.1	0.019	GS882581	0	0	b	LinJ28_V3.2040	Hypothetical protein, conserved
Lin239G8	-1.74	-0.8 ± 0.2	0.005	GS882582	0	0	b	LinJ32_V3.3180	Hypothetical protein, conserved (pseudogene)
Lin276F12	-1.75	-0.8 ± 0.2	0.034	GS882583	0	0	b	LinJ34_V3.0730	Hypothetical protein, conserved
								LinJ34_V3.0740	Hypothetical protein, conserved
Lin283E8	-2.05	-1.0 ± 0.2	0.000	GS882585	0	0	b	LinJ31_V3.1110	Hypothetical protein, conserved
Lin292E2	-1.89	-0.9 ± 0.2	0.029	GS882586	0	0	b	LinJ36_V3.6610	Hypothetical protein, conserved
								LinJ36_V3.6620	Hypothetical protein, conserved
Lin309E6	-1.75	-0.8 ± 0.2	0.020	GS882587	0	0	b	LinJ35_V3.2950	Hypothetical protein, conserved
								LinJ35_V3.2960	Hypothetical protein, conserved

Table S4. Unresolved clones that contain differentially regulated genes in S/L. F < -1.7 indicate gene down-regulation and F > 1.7 up-regulation.

Clone	*Fold change*	*$Log_2R \pm SD$*	*p*	*NCBI Acc. No.*	*E-value*		*Clone def.*	*Annotation*	*Gene function*
					F	*R*			
Lin13H10	1.96	0.7 ± 0.2	0.015	GS882588	0	0	b		
Lin33C4	1.74	0.8 ± 0.1	0.005	GS882589	0	0	c	LinJ36_V3.3500	Hypothetical transmembrane protein
								LinJ36_V3.3510	Hypothetical protein, conserved
								LinJ22_V3.0340	40S ribosomal protein S15, putative
								LinJ22_V3.0350	Hypothetical protein, conserved
								LinJ22_V3.0360	Hypothetical protein, conserved
Lin37A10	1.72	0.8 ± 0.2	0.022	GS882590	0	0	c	LinJ32_V3.3560	Hypothetical protein, conserved
								LinJ32_V.33570	Chloride channel protein, putative
Lin43B1	2.28	1.2 ± 0.3	0.025	GS882591	-	4e-76	b		
Lin48E3	1.71	0.8 ± 0.1	0.009	GS882592	0	0	a	LinJ28_V3.1030	Oxidorreductase-like protein
								LinJ28_V3.1040	Hypothetical protein, conserved
								LinJ28_V3.1050	40S ribosomal protein S14
Lin50E2	1.88	0.9 ± 0.2	0.012	GS882593	4e-23	0	b	LinJ07_V3.0540	Hypothetical protein, conserved
								LinJ07_V3.0550	60S ribosomal protein L7a, putative
Lin62H12	1.70	0.8 ± 0.0	0.001	GS882594	0	0	c		
Lin72H4	2.42	1.3 ± 0.4	0.025	GS882595	-	3e-135	b		
Lin73E5	1.75	0.8 ± 0.2	0.024	GS882596	0	0	b	LinJ36_V3.2010	Hypothetical protein, conserved
								LinJ36_V3.2020	60S ribosomal protein L37a
Lin76C12	1.74	0.8 ± 0.3	0.055	GS882597	0	0	c	LinJ08_V3.0910	Hypothetical protein, conserved
								LinJ34_V3.3450	Transmembrane/endomembrane-like protein
Lin77H11	2.05	1.0 ± 0.2	0.018	GS882598	0	0	c	LinJ31_V3.1930	Ubiquitin-fusion protein
								LinJ31_V3.1090	Hypothetical protein, conserved
Lin81H2	2.52	1.3 ± 0.4	0.030	GS882599	0	0	b	LinJ24_V3.2290	Hypothetical protein, conserved
								LinJ24_V3.2300	60S ribosomal protein L12, putative
								LinJ24_V3.2310	Hypothetical protein, conserved
								LinJ24_V3.2320	Hypothetical protein, conserved
Lin91F10	2.84	1.5 ± 0.4	0.019	GS882600	-	5e-72	c		
Lin105B7	1.57	0.6 ± 0.1	0.015	GS882601	5e-134	3e-163	b	LinJ11_V3.1220	Hypothetical protein, conserved
								LinJ11_V3.1230	60S ribosomal protein L28, putative
								LinJ11_V3.1240	Protein transport protein sec31, putative
Lin106D5	2.60	1.4 ± 0.1	0.003	GS882602	0	0	b		
Lin113G1	1.78	0.8 ± 0.2	0.034	GS882603	0	0	b		
Lin115E3	1.73	0.8 ± 0.1	0.010	GS882604	0	0	b	LinJ35_V3.4430	DNA polymerase epsilon catalytic subunit, putative
								LinJ35_V3.4440	Hypothetical protein, conserved
								LinJ35_v3.4450	Hypothetical protein, conserved
Lin125E7	1.78	0.8 ± 0.1	0.003	GS882605	4e-131	4e-131	b		
Lin126D11	1.70	0.8 ± 0.3	0.029	GS882606	5e-32	8e-37	b		
Lin129G3	1.75	0.8 ± 0.2	0.029	GS882555	0	0	c	LinJ33_V3.1570	Hypothetical protein, conserved
								LinJ22_V3.1080	Hypothetical protein, conserved
Lin135F11	1.90	0.9 ± 0.2	0.032	GS882607	4e-48	1e-44	b		
Lin137A7	1.80	0.8 ± 0.2	0.014	GS882608	1e-66	0	b		
Lin139B3	1.93	0.9 ± 0.1	0.015	GS882609	0	0	b	LinJ16_V3.0460	Hypothetical protein, conserved
								LinJ16_V3.0470	60S ribosomal protein L21, putative
Lin159D1	1.72	0.8 ± 0.2	0.035	GS882610	0	0	b	LinJ01_V3.0430	Ribosomal protein S7, putative
								LinJ01_V3.0440	Ribosomal protein S7, putative
								LinJ01_V3.0440	Hypothetical protein, conserved
Lin165H2	2.29	1.2 ± 0.1	0.001	GS882611	3e-131	7e-136	b		
Lin174G2	1.92	0.9 ± 0.1	0.001	GS882612	0	0	b		
Lin177D10	2.02	1.0 ± 0.3	0.024	GS882613	3e-126	0	b		
Lin194D11	1.52	0.6 ± 0.2	0.031	GS882614	0	0	b		
Lin199E11	3.05	1.6 ± 0.5	0.026	GS882615	0	5e-103	b		
Lin200A6	1.71	0.8 ± 0.1	0.014	GS882616	0	6e-161	b		
Lin201H8	1.77	0.8 ± 0.3	0.034	GS882617	0	0	c	LinJ36_V3.3400	Hypothetical protein, unknown function
								LinJ36_V3.3410	Hypothetical protein, conserved
Lin209G3	1.78	0.8 ± 0.3	0.037	GS882618	0	0	c	LinJ17_V3.0230	Hypothetical protein, conserved
								LinJ17_V3.0240	Hypothetical protein, unknown function
Lin213A1	1.98	1.0 ± 0.3	0.033	GS882619	6e-118	9e-114	b		
Lin217F1	1.75	0.8 ± 0.3	0.044	GS882620	0	0	b		
Lin218D10	1.92	0.9 ± 0.2	0.016	GS882621	0	0	b		
Lin227D10	1.96	1.0 ± 0.4	0.051	GS882622	0	0	b		
Lin228C8	2.18	1.1 ± 0.3	0.019	GS882623	0	0	c	LinJ33_V3.0850	Hypothetical protein, conserved
Lin232B5	1.70	0.8 ± 0.1	0.010	GS882624	0	0	b		
Lin241B9	1.83	0.9 ± 0.3	0.003	GS882625	0	0	b		
Lin256D7	1.73	0.8 ± 0.1	0.005	GS882626	0	0	b		
Lin264F3	1.71	0.8 ± 0.2	0.040	GS882627	2e-160	0	b	LinJ22_V3.1480	CCR4 associated factor, putative
								LinJ22_V3.1490	Hypothetical protein, conserved
Lin269B5	1.73	0.8 ± 0.2	0.027	GS882628	4e-11	5e-35	a	LinJ22_V3.1480	CCR4 associated factor, putative
								LinJ22_V3.1490	Hypothetical protein, conserved
Lin276C7	2.03	0.9 ± 0.1	0.013	GS882629	0	0	b		
Lin279A11	1.74	0.8 ± 0.1	0.011	GS882630	0	0	b		
Lin283D9	1.90	0.9 ± 0.1	0.005	GS882631	0	0	c	LinJ28_V3.2050	Zinc transporter, putative
								LinJ28_V3.2060	Zinc transporter, putative
Lin16E9	-1.71	-0.8 ± 0.1	0.014	GS882632	3e-52	1e-57	b		
Lin21D12	-1.78	-0.8 ± 0.3	0.029	GS882633	0	0	b	LinJ29_V3.2450	Hypothetical protein, conserved
								LinJ29_V3.2460	Hypothetical protein, conserved
								LinJ29_V3.2470	Metallo peptidase, Clan MH, Family M20
Lin21G5	-1.82	-0.9 ± 0.2	0.016	GS882634	0	0	b		
Lin35B5	-1.75	-0.8 ± 0.3	0.051	GS882350	1e-131	7e-130	a		
Lin52F10	-2.24	-1.2 ± 0.2	0.008	GS882635	1e-100	2e-154	b		
Lin56H12	-1.71	-0.8 ± 0.2	0.017	S20C9	5e-180	0	b		
Lin65D5	-1.92	-0.9 ± 0.2	0.018	S20C12	0	0	c	LinJ35_V3.1010	Hypothetical protein, conserved
								LinJ35_V3.1020	Casein kinase I, putative
								LinJ35_V3.1030	Hypothetical protein, unknown function
Lin76D2	-1.99	-1.0 ± 0.1	0.002	GS882636	7e-59	7e-59	b		
Lin78B12	-1.75	-0.8 ± 0.1	0.008	S20D4	0	0	b		
Lin80G6	-2.14	-1.1 ± 0.3	0.032	GS882637	0	0	b		
Lin86D3	-1.72	-0.8 ± 0.1	0.010	GS882638	0	0	b		
Lin88H3	-1.75	-0.8 ± 0.1	0.012	GS882574	0	0	c	LinJ31_V3.1160	Hypothetical protein, conserved

								LinJ31_V3.1170	Hypothetical protein, unknown function
Lin93C7	-1.95	-1.0 ± 0.3	0.026	GS882639	0	0	b	LinJ18_V3.1630	Hypothetical protein, conserved
								LinJ18_V3.1640	Hypothetical protein, conserved
								LinJ18_V3.1650	Chaperone protein DNAJ, putative
Lin94H3	-1.85	-0.9 ± 0.3	0.032	GS882640	0	5e-180	b	LinJ22_V3.1480	CCR4 associated factor, putative
								LinJ22_V3.1490	Hypothetical protein, conserved
								LinJ22_V3.1500	Hypothetical protein, unknown function
Lin102E2	-1.79	-0.8 ± 0.2	0.023	GS882575	0	0	c	LinJ18_V3.0710	Hypothetical protein, unknown function
								LinJ18_V3.0720	Hypothetical protein, conserved
								LinJ26_V3.0800	Hypothetical protein, conserved
								LinJ26_V3.0810	Hypothetical protein, conserved
Lin105E9	-1.83	-0.9 ± 0.1	0.004	GS882641	0	0	a		
Lin109C6	-2.24	-1.2 ± 0.4	0.033	GS882642	0	3e-169	a		
Lin110F4	-1.88	-0.9 ± 0.1	0.009	GS882643	0	2e-176	a	LinJ31_V3.1620	Hypothetical protein, conserved
								LinJ31_V3.1630	Hypothetical protein, conserved
								LinJ31_V3.1640	Dipthine synthase-like protein
Lin111A10	-1.70	-0.8 ± 0.1	0.005	GS882644	0	0	b		
Lin112H3	-1.70	-0.8 ± 0.2	0.019	GS882645	0	2e-151	c	LinJ29_V3.2070	
Lin116C2	-2.16	-1.1 ± 0.2	0.014	GS882646	0	0	a		
Lin118B11	-1.84	-0.9 ± 0.2	0.023	GS882647	0	4e-119	a		
Lin119A2	-1.88	-0.9 ± 0.2	0.029	GS882648	0	0	b	LinJ29_V3.1930	
Lin122E10	-1.79	-0.8 ± 0.3	0.051	GS882649	0	0	b	LinJ24_V3.2350	Notchless homolog, putative
								LinJ24_V3.2360	Zinc-finger multi-pass transmembrane protein
								LinJ24_V3.2370	DNA excision repair protein, putative
Lin124E11	-1.70	-0.8 ± 0.1	0.006	GS882650	0	0	b	LinJ36_V3.7230	Hypothetical protein, conserved
								LinJ36_V3.7240	T-complex protein 1 (theta subunit), putative
Lin127A1	-1.85	-0.9 ± 0.1	0.006	GS882651	0	0	b		
Lin135B3	-2.10	-1.0 ± 0.1	0.010	GS882652	0	0	b	LinJ30_V3.0750	Hypothetical protein, conserved
								LinJ30_V3.0760	Co-chaperone GrpE, putative
								LinJ30_V3.0770	Unknown
Lin138B12	-1.85	-0.9 ± 0.4	0.054	GS882653	0	0	a	LinJ15_V3.1600	Presenilin-like aspartic peptidase, clan AD, family A22A, putative
								LinJ15_V3.1610	Hypothetical protein, conserved
								LinJ15_V3.1620	Hypothetical protein, conserved
Lin145E5	-1.76	-0.8 ± 0.1	0.07	GS882654	0	0	c	LinJ15_V3.1520	Hypothetical protein, conserved
								LinJ15_V3.1530	NPH2/RS6-like protein
								LinJ15_V3.1540	cAMP-specific phosphodiesterase, putative
Lin203E9	-1.88	-0.9 ± 0.3	0.024	GS882655	1e-149	2e-145	a		
Lin204F3	-1.76	-0.8 ± 0.2	0.040	GS882656	0	0	b		
Lin209B12	-2.29	-1.2 ± 0.3	0.024	GS882580	0	0	c	LinJ35_V3.4170	Hypothetical protein, conserved
								LinJ35_V3.4180	Hypothetical protein, conserved
Lin237G11	-1.77	-0.8 ± 0.2	0.001	GS882657	7e-164	1e-140	b		
Lin261A10	-2.67	-1.4 ± 0.1	0.039	GS882658	0	0	b	LinJ22_V3.1480	CCR4 associated factor, putative
								LinJ22_V3.1490	Hypothetical protein, conserved
Lin270C4	-1.96	-1.0 ± 0.3	0.054	GS882659	3e-27	1e-11	b		
Lin270F7	-1.75	-0.8 ± 0.2	0.018	GS882660	0	5e-60	b		
Lin268H6	-1.84	-0.9 ± 0.2	0.048	GS882661	0	0	a	LinJ10_V3.0650	Hypothetical protein, conserved
Lin282A4	-1.75	-0.8 ± 0.2	0.043	GS882584	0	0	b	LinJ29_V3.1160	Ribosomal protein L1a, putative
								LinJ29_V3.1170	Ribosomal protein L1a, putative
								LinJ29_V3.1180	Ribosomal protein L1a, putative
								LinJ29_V3.1190	Hypothetical protein, conserved
Lin287F2	-1.85	-0.9 ± 0.2	0.041	GS882662	0	0	b	LinJ33_V3.1900	Hypothetical protein, conserved
								LinJ33_V3.1910	Hypothetical protein, conserved
								LinJ33_V3.1920	Hypothetical protein, conserved
								LinJ33_V3.1930	Dual-specificity protein kinase, putative
Lin288C8	-1.85	-0.9 ± 0.2	0.004	GS882663	1e-122	0	b	LinJ27_V3.1120	Histone h1, putative
								LinJ27_V3.1130	Carboxypeptidase, putative
Lin292F2	-1.83	-0.9 ± 0.3	0.043	GS882664	0	0	b		
Lin302C2	-1.75	-0.8 ± 0.2	0.002	GS882665	9e-154	0	b	LinJ15_V3.1140	Tryparedoxin peroxidase
								LinJ15_V3.1150	Hypothetical protein, conserved

Table S5. Hypothetical and unknown protein genes that are differentially regulated in S/A. F < -1.7 indicate gene down-regulation and F > 1.7 up-regulation.

Clone	*Fold change*	*$Log_2R \pm SD$*	*p*	*NCBI Acc. No.*	*E-value*		*Clone def.*	*Annotation*	*Gene function*
					F	*R*			
Lin44G7	2.75	1.5 ± 0.0	0.007	GS882666	0	0	b	LinJ31_V3.0960	Hypothetical protein, conserved
								LinJ31_V3.0970	Hypothetical protein, conserved
Lin45A12	1.76	0.8 ± 0.1	0.041	GS882667	0	0	b	LinJ25_V3.0660	Hypothetical protein, conserved
								LinJ25_V3.0670	Hypothetical protein, conserved
Lin53F1	3.78	1.9 ± 0.2	0.049	GS882668	0	0	b	LinJ33_V3.0950	Hypothetical protein, conserved
								LinJ33_V3.0960	Hypothetical protein, conserved
Lin93F9	2.04	1.0 ± 0.1	0.032	GS882670	0	0	b	LinJ34_V3.2140	Hypothetical protein, conserved
Lin94G6	1.84	0.9 ± 0.1	0.045	GS882671	0	0	b	LinJ30_V3.3250	Hypothetical protein, conserved
								LinJ30-V3.3260	Hypothetical protein, conserved
								LinJ30_V3.3270	Hypothetical protein, conserved
Lin119E7	1.87	0.9 ± 0.0	0.017	GS882672	0	0	b	LinJ31_V3.1480	Hypothetical protein, unknown function
Lin123C12	2.61	1.4 ± 0.0	0.006	GS882673	0	0	b	LinJ35_V3.0190	Hypothetical protein, conserved
Lin134A3	2.75	1.5 ± 0.1	0.045	GS882675	0	0	b	LinJ04_V3.0630	Hypothetical protein, conserved
								LinJ04_V3.0640	Hypothetical protein
Lin158A10	2.51	1.3 ± 0.0	0.016	GS882676	0	0	b	LinJ23_V3.0870	Hypothetical protein, conserved
Lin163E7	3.65	1.9 ± 0.0	0.012	GS882677	0	0	a	LinJ06_V3.0270	Hypothetical protein, conserved
Lin172G11	1.78	0.8 ± 0.1	0.035	GS882678	0	0	b	LinJ30_V3.3400	Hypothetical protein, conserved
Lin189D8	1.74	0.8 ± 0.1	0.048	GS882679	0	0	a	LinJ26_V3.1840	Hypothetical protein, conserved
								LinJ26_V3.1850	Hypothetical protein, conserved
Lin200B11	1.82	0.9 ± 0.0	0.018	GS882680	0	0	b	LinJ29_V3.1030	Hypothetical protein, conserved
Lin203A1	2.92	1.5 ± 0.1	0.016	GS882681	7e-130	7e-133	a	LinJ06_V3.1360	Hypothetical protein, conserved
Lin205H2	1.88	0.9 ± 0.1	0.027	GS882682	1e-180	0	b	LinJ23_V3.0130	Hypothetical protein, conserved
Lin206H3	3.42	1.8 ± 0.1	0.026	GS882683	0	0	b	LinJ10_V3.1370	Hypothetical protein, conserved
Lin208D6	2.70	1.4 ± 0.1	0.042	GS882684	0	0	a	LinJ29_V3.1150	Hypothetical protein, conserved
Lin239F8	2.06	1.1 ± 0.1	0.036	GS882686	0	2e-151	b	LinJ32_V3.3360	Hypothetical protein, conserve
								LinJ32_V3.3370	Hypothetical protein, conserved
Lin252G12	1.91	0.9 ± 0.1	0.039	GS882687	1e-97	4e-88	b	LinJ02_V3.0310	Hypothetical protein, conserved
								Lin02_V3.0320	Hypothetical protein, conserved
Lin228E5	2.65	1.4 ± 0.1	0.042	GS882688	7e-56	7e-56	b	LinJ06_V3.1290	Hypothetical protein, conserved
Lin236A4	1.73	0.8 ± 0.0	0.022	GS882689	0	3e-166	b	LinJ35_V3.4620	Hypothetical protein, conserved
								LinJ35_V3.4630	Hypothetical protein, conserved
								LinJ35_V3.4640	Hypothetical protein, conserved
Lin243B8	1.79	0.8 ± 0.1	0.049	GS882690	0	0	b	LinJ06_V3.1350	Hypothetical protein, unknown function
Lin14A9	-1.75	-0.8 ± 0.0	0.024	GS882691	1e-91	4e-91	b	LinJ23_V3.1170	Hypothetical protein, unknown function
Lin16D7	-1.79	-0.8 ± 0.1	0.035	GS882692	1e-168	0	b	LinJ36_V3.5780	Hypothetical protein, conserved
								LinJ36_V3.5790	Hypothetical protein, conserved
Lin22E1	-1.73	-0.8 ± 0.0	0.013	GS882693	5e-171	0	b	LinJ19_V3.0490	Hypothetical protein, conserved
Lin31C4	-1.71	-0.8 ± 0.1	0.047	GS882694	0	0	b	LinJ11_V3.1360	Hypothetical protein, conserved
								LinJ11_V3.1370	Hypothetical protein, conserved
Lin36D11	-1.74	-0.8 ± 0.1	0.038	GS882695	5e-35	8e-34	b	LinJ36_V3.0850	Hypothetical protein, unknown function
Lin44E11	-2.13	-1.1 ± 0.1	0.028	GS882696	0	0	b	LinJ20_V3.0020	Hypothetical protein, conserved
Lin47E12	-1.72	-0.8 ± 0.0	0.021	GS882697	1e-143	1e-143	b	LinJ16_V3.1060	Hypothetical protein, conserved
Lin62C5	-1.91	-0.9 ± 0.1	0.038	GS882698	0	4e-82	b	LinJ13_V3.0880	Hypothetical protein, conserved
Lin94C9	-2.41	-1.3 ± 0.1	0.029	GS882699	0	0	a	LinJ32_V3.0220	Hypothetical protein, conserved
Lin106F12	-1.84	-0.9 ± 0.0	0.008	GS882700	0	0	a	LinJ36_V3.0850	Hypothetical protein, unknown function
Lin110H2	-1.73	-0.8 ± 0.0	0.017	GS882702	0	0	b	LinJ36_V3.3060	Hypothetical protein, conserved
								LinJ36_V3.3070	Hypothetical protein, conserved
Lin125F4	-2.26	-1.2 ± 0.1	0.049	GS882703	0	0	b	LinJ34_V3.2530	Hypothetical protein, conserved
								LinJ34_V3.2540	Hypothetical protein, conserved
Lin126C12	-2.11	-1.1 ± 0.1	0.043	GS882704	0	0	b	LinJ29_V3.2720	Hypothetical protein, conserved
								LinJ29_V3.2730	Hypothetical protein, conserved
Lin131E1	-1.71	-0.8 ± 0.1	0.052	GS882705	0	0	a	LinJ15_V3.1270	Hypothetical protein, conserved
Lin131F10	-2.16	-1.1 ± 0.0	0.020	GS882706	0	0	b	LinJ32_V3.3160	Hypothetical protein, conserved
Lin138D2	-1.76	-0.8 ± 0.0	0.021	GS882707	0	0	b	LinJ07_V3.0630	Hypothetical protein, conserved
								LinJ07_V3.0640	Hypothetical protein, conserved
Lin139B8	-2.75	-1.5 ± 0.1	0.026	GS882708	0	0	a	LinJ33_V3.3280	Hypothetical protein, conserved
Lin144H7	-1.84	-0.9 ± 0.1	0.044	GS882709	0	0	b	LinJ31_V3.1210	Hypothetical protein, unknown function
Lin154G1	-330	-1.7 ± 0.0	0.001	GS882710	0	2e-179	b	LinJ30_V3.2310	Hypothetical protein, conserved
								LinJ30_V3.2320	Hypothetical protein, conserved
								LinJ30_V3.2330	Hypothetical protein, conserved
Lin158B7	-2.18	-1.1 ± 0.0	0.020	GS882711	0	0	b	LinJ31_V3.0250	Hypothetical protein, conserved
Lin167F2	-1.88	-0.9 ± 0.1	0.037	GS882712	0	0	b	LinJ28_V3.1770	Hypothetical protein, conserved
								LinJ28_V3.1780	Hypothetical protein, conserved
Lin182G8	-1.97	-1.0 ± 0.1	0.038	GS882713	0	0	b	Lin36_V3.6410	Hypothetical protein, conserved
								LinJ36_V3.6420	Hypothetical protein, conserved
Lin194D5	-2.56	-1.4 ± 0.1	0.037	GS882714	0	0	b	LinJ30_V3.0810	Hypothetical protein, conserved
Lin210A10	-1.73	-0.8 ± 0.0	0.000	GS882716	0	0	b	LinJ27_V3.0020	Hypothetical protein, conserved
								LinJ27_V3.0030	Hypothetical protein, conserved
Lin222B7	-1.75	-0.8 ± 0.0	0.018	GS882717	1e-168	0	b	LinJ21_V3.1070	Hypothetical protein, conserved
								LinJ21_V3.1080	Hypothetical protein, conserved
								LinJ21_V3.1090	Hypothetical protein, conserved
Lin281H8	-28.20	-4.8 ± 0.6	0.026	GS882718	4e-14	4e-156	b	LinJ03_V3.0540	Hypothetical protein

Table S6. Unresolved clones that contain differentially regulated genes in S/A. F < -1.7 indicate gene down-regulation and F > 1.7 up-regulation.

Clone	*Fold change*	*$Log_2R \pm SD$*	*p*	*NCBI Acc. No.*	*E-value*		*Clone def.*	*Annotation*	*Gene function*	*qrtRT-PCR F.ch. ± SD*
					F	*R*				
Lin10F7	2.85	1.5 ± 0.1	0.031	GS882719	0	0	b			
Lin11G12	1.91	0.9 ± 0.0	0.011	GS882720	6e-41	9e-40	b			
Lin13H10	1.84	0.9 ± 0.1	0.043	GS882721	0	0	b			
Lin46E9	1.79	0.8 ± 0.1	0.031	S23B9	1e-94	1e-94	b			
Lin48F10	2.11	1.1 ± 0.0	0.016	GS882722	0	7e-136	b			
Lin56G3	2.81	1.5 ± 0.0	0.042	GS882723	4e-45	2e-59	a			
Lin63A10	3.81	1.9 ± 0.0	0.000	GS882724	0	0	b			
Lin65B9	2.41	1.3 ± 0.1	0.026	GS882725	0	0	b			
Lin72E2	1.95	1.1 ± 0.1	0.046	GS882726	7e-173	0	b			
Lin79C10	2.99	1.6 ± 0.0	0.002	GS882727	0	0	b			
Lin81H6	2.27	1.2 ± 0.1	0.044	GS882728	0	0	b			
Lin86D3	5.17	2.01 ± 0.0	0.006	GS882729	0	0	b			
Lin91F9	3.03	1.6 ± 0.1	0.017	GS882669	0	0	c	LinJ31_V3.1630	Hypothetical protein, unknown function	N.D.
								LinJ31_V3.1640	Dipthine synthase-like protein	- -1.6 ± 0.3
								LinJ31_V3.1650	Hypothetical protein, conserved	N.D.
								LinJ35_V3.5080	Hypothetical protein, conserved	N.D.
Lin92C9	1.71	0.8 ± 0.0	0.027	GS882730	0	0	b			
Lin96B8	2.72	1.4 ± 0.2	0.042	GS882731	0	0	b			
Lin97A12	2.66	1.4 ± 0.0	0.016	GS882732	0	0	b			
Lin101D2	1.77	0.8 ± 0.1	0.031	GS882733	0	0	b			
Lin122C8	2.90	1.5 ± 0.2	0.049	GS882734	0	0	b			
Lin122H12	4.47	2.2 ± 0.1	0.014	GS882735	0	0	b			
Lin123C9	2.52	1.3 ± 0.1	0.032	GS882736	0	0	b			
Lin124D12	2.38	1.3 ± 0.1	0.027	GS882737	0	0	b			
Lin125E7	3.16	1.7 ± 0.0	0.003	GS882738	0	0	c			
Lin132B12	3.44	1.8 ± 0.1	0.024	GS882674	0	0	c	LinJ06_V3.0810	Hypothetical protein, unknown function	N.D.
								LinJ31_V3.2090	Hypothetical protein, unknown function	N.D.
Lin165B11	2.20	1.1 ± 0.1	0.033	GS882739	0	0	b			
Lin194F7	3.02	1.6 ± 0.0	0.009	GS882740	0	0	b			
Lin200G6	1.86	0.9 ± 0.1	0.027	GS882741	0	0	b			
Lin209B6	2.99	1.6 ± 0.1	0.040	GS882742	0	0	b			
Lin204G10	2.68	1.4 ± 0.0	0.006	GS882743	0	0	b			
Lin210A5	2.30	1.2 ± 0.0	0.015	GS882744	0	2e-148	b	LinJ30_V3.2060	Succinyl-diaminopimelate desuccinylase-like protein	N.D.
								LinJ30_V3.2070	Ubiquitin fusion protein	N.D.
Lin225G6	3.55	1.8 ± 0.2	0.042	GS882745	0	2e-151	b			
Lin228A2	4.30	2.1 ± 0.1	0.014	GS882685	2e-176	8e-179	c	LinJ27_V3.2470	Hypothetical protein, conserved	N.D.
Lin241G4	1.99	1.0 ± 0.0	0.005	GS882746	0	0	b			
Lin242C5	2.20	1.1 ± 0.1	0.025	GS882747	0	0	b			
Lin251E12	1.90	0.9 ± 0.1	0.034	GS882748	0	0	b			
Lin252C12	1.99	1.0 ± 0.0	0.005	GS882749	3e-175	1e-100	b			
Lin9B10	-1.99	-1.0 ± 0.1	0.032	S25A1	0	0	c	LinJ32_V3.3810	3-hydroxyisobutyryl-coenzyme a hydrolase-like protein	N.D.
								LinJ27_V3.0300	Acyl carrier protein, putative	N.D.
								LinJ27_V3.0310	Methylmalonyl-coenzyme a mutase, putative	N.D
Lin13E5	-2.37	-1.2 ± 0.1	0.045	GS882750	0	0	c	LinJ24_V3.0530	DNAJ domain protein, putative	N.D.
								LinJ24_V3.0540	Hypothetical protein, conserved	N.D.
								LinJ24_V3.0550	Hypothetical protein, unknown function	N.D.
								LinJ31_V3.2910	Hypothetical protein, conserved	N.D.
								LinJ31_V3.2920	Hypothetical protein, conserved	N.D.
Lin22G2	-12.66	-3.7 ± 0.1	0.018	GS882751	0	0	b			
Lin24G2	-1.91	-0.9 ± 0.0	0.007	GS882752	0	0	b	LinJ32_V3.1250	Hypothetical protein, conserved	N.D.
								LinJ32_V3.1260	Proteasome regulatory non-ATP-ase subunit, putative	N.D.
								LinJ32_V3.1270	Hypothetical protein, conserved	N.D.
Lin31C6	-1.83	-0.9 ± 0.1	0.032	GS882753	0	0	b	LinJ15_V3.1630	Protein kinase, putative	N.D.
								LinJ15_V3.1640	Condensin subunit 1, putative	N.D.
Lin48B7	-2.10	-1.1 ± 0.1	0.041	GS882754	0	0	b	LinJ36_V3.6250	Hypothetical protein, conserved	N.D.
								LinJ36_V3.6260	I6202.3-like protein	N.D.
Lin99F1	-1.72	-0.8 ± 0.0	0.004	GS882755	0	0	b	LinJ30_V3.3430	PAS-domain containing phosphoglycerate kinase, putative	N.D.
								LinJ30_V3.3440	CAS/CSE/importing domain protein, putative	N.D.
Lin107F4	-1.77	-0.8 ± 0.0	0.001	GS882701	0	0	b	LinJ18_V3.0780	DNA-directed RNA polymerase II, putative	- -1.1 ± 0.2
								LinJ18_V3.0790	DNA-directed RNA polymerase II, putative	- -1.1 ± 0.2
								LinJ18_V3.0800	Hypothetical protein, conserved	N.D.
Lin118F12	-2.06	-1.0 ± 0.1	0.035	GS882756	1e-146	3e-147	b			
Lin122G6	-2.57	-1.4 ± 0.0	0.012	GS882757	0	0	c	LinJ27_V3.1930	Hypothetical protein, conserved	N.D.
								LinJ27_V3.1940	D-lactate dehydrogenase-like protein	- 1.3 ± 0.0
								LinJ27_V3.1950	Branched-chain aminoacid aminotransferase, putative	N.D.
								LinJ27_V3.1960	Hypothetical protein, conserved	N.D.
Lin124E11	-2.04	-1.0 ± 0.1	0.038	GS882758	0	0	b	LinJ36_V3.7230	Hypothetical protein, conserved	N.D.
								LinJ36_V3.7240	T-complex protein 1 (theta subunit), putative	N.D.
Lin125G2	-2.56	-1.4 ± 0.1	0.045	GS882759	0	0	c	LinJ31_V3.3240	Hypothetical protein, conserved	N.D.
								LinJ31_V3.3250	Phosphatidylethanolaminen-methyltransferase-like protein	- -1.5 ± 0.1
								LinJ36_V3.1950	Hypothetical protein, conserved	N.D.
								LinJ36_V3.1960	Hypothetical protein, conserved	N.D.
Lin146F1	-2.19	-1.1 ± 0.0	0.010	GS882760	4e-91	4e-91	b			
Lin152A7	-2.31	-1.2 ± 0.1	0.030	GS882761	0	0	c	LinJ27_V3.1920	Hypothetical protein, conserved	N.D.
								LinJ27_V3.1930	Hypothetical protein, conserved	N.D.
								LinJ27_V3.1950	Branched-chain amino acid aminotransferase, putative	N.D.
								LinJ27_V3.1960	Hypothetical protein, conserved	N.D.
Lin161C6	-1.89	-0.9 ± 0.1	0.046	GS882762	0	0	b	LinJ35_V3.4910	Proteasome alpha-1 subunit, putative	N.D.
								LinJ35_V3.4920	Hypothetical protein, conserved	N.D.
Lin164G2	-2.00	-1.0 ± 0.0	0.019	GS882763	0	0	b	LinJ29_V3.0070	Syntaxin, putative	N.D.
								LinJ29_V3.0080	Hypothetical protein, conserved	N.D.
Lin168B7	1.73	0.8 ± 0.1	0.046	GS882764	-	3e-154	c	LinJ23_V3.0620	Oxidoreductase-like protein	N.D.
								LinJ23_V3.0630	Oxidoreductase-like protein	N.D.
Lin190D7	-2.14	-1.1 ± 0.1	0.031	GS882765	0	0	b	LinJ29_V3.0900	Hypothetical protein, unknown function	N.D.
								LinJ29_V3.0910	Hypothetical protein, conserved	N.D.

								LinJ29_V3.0920	Guanine deaminase, putative	N.D.	
Lin199F4	-1.91	-0.9 ± 0.0	0.022	GS882715	0	0	a	LinJ07_v3.1270	Hypothetical protein, conserved	N.D.	
								LinJ07_V3.1280	ATPase subunit 9, putative, pseudogene	N.D.	
Lin223G12	-1.99	-1.0 ± 0.1	0.042	GS882766	0	0	c	LinJ27_V3.1930	Hypothetical protein, conserved	N.D.	
								LinJ27_V3.1940	D-lactate dehydrogenase-like protein	-	1.3 ± 0.0
								LinJ27_V3.1950	Branched-chain amino acid aminotransferase, putative	N.D.	
								LinJ27_V3.1960	Hypothetical protein, conserved	N.D.	
Lin227H5	-1.71	-0.8 ± 0.0	0.016	GS882767	2e-105	3e-110	a				
Lin290F10	-2.87	-1.5 ± 0.0	0.006	GS882768							
Lin298C1	-1.71	-0.8 ± 0.1	0.037	GS882770	0	0	a	LinJ16_V3.1630	Endoplasmic reticulum oxidoreductin, putative	N.D.	
								LinJ16_V3.1640	DNA polymerase I alpha catalytic subunit, putative	N.D.	
Lin306G4	-2.13	-1.1 ± 0.0	0.013	GS882769	0	0	b				

Table S7. Hypothetical and unknown protein genes that are differentially regulated in L/A. F < -1.7 indicate gene down-regulation and F > 1.7 up-regulation.

Clone	*Fold change*	*$Log_2R \pm SD$*	*p*	*NCBI Acc. No.*	*E-value*		*Clone def.*	*Annotation*	*Gene function*
					F	*R*			
Lin21C1	2.19	1.1 ± 0.4	0.049	GS882777	0	0	b	LinJ23_V3.0100	Hypothetical protein, conserved
								LinJ23_V3.0110	Hypothetical protein, unknown function
Lin26G3	2.57	1.4 ± 0.1	0.001	GS882776	6e-161	7e-59	a	LinJ36_V3.5190	Hypothetical protein, unknown function
Lin47D11	2.12	1.1 ± 0.2	0.015	GS882775	0	0	b	LinJ30_V3.2860	Hypothetical protein, conserved
								LinJ30_V3.2870	Hypothetical protein, conserved
Lin47F5	3.20	1.7 ± 0.4	0.015	GS882774	0	0	a	LinJ02_V3.0060	Hypothetical protein, conserved
Lin70C4	2.86	1.5 ± 0.4	0.027	GS882771	0	0	b	Lin J32_V3.0370	Hypothetical protein, conserved
Lin77G12	2.02	1.0 ± 0.3	0.026	GS882773	0	0	b	LinJ32_V3.1720	Hypothetical protein, conserved
Lin83E8	2.21	1.1 ± 0.1	0.005	GS882778	0	0	b	LinJ35_V3.0140	Hypothetical protein, conserved
								LinJ35_V3.0150	Hypothetical protein, conserved
								LinJ35_V3.0160	Hypothetical protein, conserved
Lin109E2	2.04	1.0 ± 0.3	0.025	GS882779	0	0	b	LinJ31_V3.1420	Hypothetical protein, conserved
Lin119E7	2.39	1.3 ± 0.4	0.025	GS882780	0	0	b	LinJ31_V3.1480	Hypothetical protein, unknown function
Lin121C6	3.18	1.7 ± 0.0	0.000	GS882781	0	0	b	LinJ09_V3.1600	Hypothetical protein, conserved
								LinJ09_V3.1610	Hypothetical protein, conserved
								LinJ09_V3.1620	Hypothetical protein, conserved
Lin128C11	2.21	1.1 ± 0.1	0.002	GS882783	1e-168	1e-168	b	LinJ26_V3.2300	Hypothetical protein, conserved
Lin130D8	2.64	1.4 ± 0.1	0.002	GS882784	0	0	b	LinJ09_V3.0910	Hypothetical protein, conserved
Lin131G3	2.93	1.6 ± 0.3	0.013	GS882785	6e-121	0	b	LinJ35_V3.3770	Hypothetical protein, conserved
Lin132B12	2.13	1.1 ± 0.2	0.016	GS882786	0	8e-179	b	LinJ06_V3.0810	Hypothetical protein, unknown function
								LinJ31_V3.2090	Hypothetical protein, unknown function
Lin133F9	3.63	1.9 ± 0.4	0.017	GS882787	0	0	b	LinJ35_V3.5310	Hypothetical protein, conserved
Lin134A3	2.45	1.3 ± 0.2	0.011	GS882788	0	0	b	LinJ04_V3.0630	Hypothetical protein, conserved
								LinJ04_V3.0640	Hypothetical protein
Lin136D8	6.75	2.8 ± 0.6	0.014	GS882789	0	0	b	LinJ33_V3.0650	Hypothetical protein, conserved
								LinJ33_V3.0660	Hypothetical protein, conserved
								LinJ33_V3.0670	Hypothetical protein, conserved
Lin143H10	2.04	1.0 ± 0.1	0.005	GS882790	0	0	a	LinJ31_V3.0690	Hypothetical protein, conserved
Lin147B4	2.04	1.0 ± 0.3	0.032	GS882792	0	1e-134	b	LinJ08_V3.1090	Hypothetical protein, conserved
								LinJ08_V3.1100	Hypothetical protein, conserved
Lin157C6	2.24	1.2 ± 0.3	0.019	GS882793	0	0	b	LinJ26_V3.2580	Hypothetical protein, conserved
Lin158A10	2.19	1.1 ± 0.2	0.016	GS882794	0	0	b	LinJ23_V3.0870	Hypothetical protein, conserved
Lin158F5	2.45	1.3 ± 0.1	0.002	GS882795	0	0	b	LinJ31_V3.1620	Hypothetical protein, conserved
								LinJ31_V3.1630	Hypothetical protein, unknown function
Lin160E1	3.69	1.9 ± 0.6	0.034	GS882796	0	2e-111	b	LinJ30_V3.2310	Hypothetical protein, conserved
								LinJ30_V3.2320	Hypothetical protein, conserved
								LinJ30_V3.2330	Hypothetical protein, conserved
Lin163E7	2.89	1.5 ± 0.5	0.029	GS882797	0	0	a	LinJ06_V3.0270	Hypothetical protein, conserved
Lin166D11	2.50	1.3 ± 0.3	0.012	GS882798	0	0	b	LinJ23_V3.1350	Hypothetical protein, conserved
								LinJ23_V3.1360	Hypothetical protein, conserved
Lin166H10	3.87	2.0 ± 0.7	0.038	GS882799	0	0	a	LinJ30_V3.2310	Hypothetical protein, conserved
								LinJ30_V3.2320	Hypothetical protein, conserved
								LinJ30_V3.2330	Hypothetical protein, conserved
Lin177B9	2.01	1.0 ± 0.3	0.020	GS882800	0	0	b	LinJ26_V3.2580	Hypothetical protein, conserved
Lin194B7	2.04	1.0 ± 0.2	0.012	GS882801	0	0	b	LinJ23_V3.1830	Hypothetical protein, unknown function
Lin200D7	2.17	1.1 ± 0.0	0.000	GS882802	0	0	b	LinJ36_V3.0520	Hypothetical protein, conserved
Lin200F4	2.00	1.0 ± 0.4	0.042	GS882803	0	0	b	LinJ31_V3.2080	Hypothetical protein, unknown function
Lin201C10	2.26	1.2 ± 0.3	0.017	GS882804	2e-102	7e-130	b	LinJ34_V3.2770	Hypothetical protein, conserved
								LinJ34_V3.2780	Hypothetical protein, conserved
Lin203A1	2.77	1.5 ± 0.6	0.044		5e-134	7e-130	a	LinJ06_V3.1360	Hypothetical protein, conserved
Lin204D8	7.49	2.9 ± 0.4	0.005	GS882806	0	0	b	LinJ33_V3.0650	Hypothetical protein, conserved
								LinJ33_V3.0660	Hypothetical protein, conserved
								LinJ33_V3.0670	Hypothetical protein, conserved
Lin209B12	5.35	2.4 ± 0.1	0.000	GS882807	7e-93	0	a	LinJ35_V3.4190	Conserved Hypothetical protein
Lin215A9	2.15	1.1 ± 0.4	0.038	GS882808	0	0	b	LinJ10_V3.1230	Hypothetical protein, conserved
								LinJ10_V3.1240	Hypothetical protein, conserved
								LinJ36_V3.2290	Hypothetical protein, conserved
Lin226F12	2.16	1.1 ± 0.3	0.024	GS882810	0	0	b	LinJ27_V3.2090	Hypothetical protein, conserved
Lin226G2	2.08	1.1 ± 0.3	0.029	GS882811	0	0	b	LinJ23_V3.1330	Hypothetical protein, unknown function
Lin230F8	2.45	1.3 ± 0.5	0.042	GS882812	0	0	b	LinJ06_V3.1350	Hypothetical protein, unknown function
Lin230H7	4.56	2.2 ± 0.6	0.027	GS882813	0	5e-103	b	LinJ08_V3.0020	Hypothetical protein, conserved
Lin233D10	2.24	1.2 ± 0.3	0.027	GS882814	0	-	b	LinJ13_V3.0320	Hypothetical protein, conserved
								LinJ13_V3.0330	Unknown
Lin238B10	2.43	1.3 ± 0.4	0.028	GS882815	0	0	a	LinJ34_V3.0770	Hypothetical protein, conserved
Lin239G8	3.25	1.7 ± 0.6	0.043	GS882816	0	0	b	LinJ32_V3.3180	Hypothetical protein, conserved (pseudogene)
Lin288E4	2.80	1.5 ± 0.5	0.042	GS882817	0	1e-140	b	LinJ21_V3.1920	Hypothetical protein unknown function
Lin289C9	2.48	1.3 ± 0.4	0.036	GS882818	0	0	b	LinJ28_V3.1110	Hypothetical protein, conserved
Lin289G12	2.07	1.1 ± 0.3	0.027	GS882819	0	0	b	LinJ31_V3.1760	Hypothetical protein, conserved
Lin246C5	2.60	1.4 ± 0.4	0.031	GS882820	0	0	b	LinJ08_V3.1010	Hypothetical protein, conserved
Lin250G5	2.82	1.5 ± 0.1	0.002	GS882821	0	0	b	LinJ09_V3.1610	Hypothetical protein, conserved
Lin265F5	2.53	1.3 ± 0.4	0.029	GS882823	0	5e-171	b	LinJ13_V3.0330	Unknown
Lin269B4	3.11	1.6 ± 0.5	0.032	GS882824	0	0	b	LinJ26_V3.2300	Hypothetical protein, conserved
Lin271B5	2.06	1.0 ± 0.4	0.042	GS882825	0	0	b	LinJ09_V3.0020	Hypothetical protein, conserved
Lin14C11	-1.90	-0.9 ± 0.1	0.003	GS882826	0	0	b	LinJ24_V3.1740	Hypothetical protein. conserved
								LinJ24_V3.1750	Hypothetical protein. conserved
Lin23A8	-1.77	-0.8 ± 0.1	0.009	GS882827	0	0	b	LinJ13_V3.0890	Hypothetical protein. conserved
								LinJ13_V3.0900	Hypothetical protein. conserved
Lin23E10	-2.65	-1.4 ± 0.3	0.019	GS882828	0	2e-81	a	LinJ31_V3.1230	Hypothetical protein. conserved
Lin29B5	-1.86	-0.9 ± 0.1	0.007	GS882829	0	0	b	LinJ29_V3.1200	Hypothetical protein. conserved
Lin31C7	-1.86	-0.9 ± 0.2	0.013	S28C12	0	0	b	LinJ29_V3.1200	Hypothetical protein. conserved
Lin62C5	-1.73	-0.8 ± 0.2	0.013	GS882830	3e-67	0	b	LinJ13_V3.0880	Hypothetical protein. conserved
Lin129H5	-2.04	-1.0 ± 0.4	0.048	GS882831	0	0	b	LinJ16_V3.1110	Hypothetical protein, conserved
Lin134B11	-1.93	-0.9 ± 0.4	0.054	GS882832	0	0	b	LinJ29_V3.0110	Hypothetical protein, conserved
Lin135A8	-1.98	-1.0 ± 0.3	0.033	GS882833	0	0	b	LinJ35_V3.4270	Hypothetical protein, unknown function
Lin138D2	-1.78	-0.8 ± 0.2	0.022	GS882834	0	0	b	LinJ07_V3.0630	Hypothetical protein, conserved
								LinJ07_V3.0640	Hypothetical protein, conserved
Lin139B8	-2.16	-1.1 ± 0.5	0.053	GS882835	0	0	b	LinJ33_V3.3280	Hypothetical protein, conserved
Lin139F9	-2.19	-1.1 ± 0.5	0.052	GS882836	0	0	a	LinJ31_V3.3110	Hypothetical protein, unknown function
								LinJ31_V3.3120	Hypothetical protein, unknown function

Lin154A5	-2.25	-1.2 ± 0.2	0.006	GS882837	0	0	b	LinJ10_V3.0720	Hypothetical protein, conserved
Lin154G1	-2.92	-1.5 ± 0.5	0.033	GS882838	0	0	b	LinJ30_V3.2310	Hypothetical protein, conserved
								LinJ30_V3.2310	Hypothetical protein, conserved
								LinJ30_V3.2310	Hypothetical protein, conserved
Lin171B2	-2.92	-1.5 ± 0.2	0.005	GS882839	3e-135	0	b	LinJ24_V3.0560	Hypothetical protein, conserved
Lin177B5	-2.47	-1.3 ± 0.2	0.007	GS882840	5e-94	0	b	LinJ24_V3.1970	Hypothetical protein, conserved
Lin183A3	-1.95	-1.0 ± 0.4	0.045	GS882841	0	0	b	LinJ24_V3.2250	Hypothetical protein, conserved
Lin209F8	-1.92	-0.9 ± 0.3	0.028	GS882842	0	0	b	LinJ31_V3.1270	Hypothetical protein, conserved
Lin234B2	-1.73	-0.8 ± 0.2	0.025	GS882844	0	4e-159	b	LinJ28_V3.0960	Hypothetical protein, conserved
Lin236E4	-1.96	-1.0 ± 0.3	0.023	GS882845	0	0	a	LinJ33_V3.3280	Hypothetical protein, conserved
								LinJ33_V3.3290	Hypothetical protein, conserved
Lin241H12	-2.31	-1.2 ± 0.5	0.049	GS882846	0	0	b	LinJ34_V3.1120	Hypothetical protein, conserved
Lin243C7	-1.79	-0.8 ± 0.2	0.014	GS882847	5e-109	1e-48	b	LinJ02_V3.0280	Hypothetical protein, conserved
Lin267A6	-2.21	-1.1 ± 0.2	0.001	GS882848	0	0	b	LinJ06_V3.1270	Hypothetical protein, unknown function
Lin274C4	-1.87	-0.9 ± 0.2	0.017	GS882849	0	0	b	LinJ07_V3.0630	Hypothetical protein, conserved
Lin280G2	-2.29	-1.2 ± 0.3	0.017	GS882850	0	0	b	LinJ31_V3.1470	Hypothetical protein, unknown function
Lin281H8	-42.32	-5.4 ± 1.3	0.018	GS882851	8e-68	0	b	LinJ03_V3.0540	Hypothetical protein
Lin284E12	-2.09	-1.1 ± 0.2	0.011	GS882852	3e-70	8e-176	b	LinJ03_V3.0540	Hypothetical protein
Lin287C10	-2.50	-1.3 ± 0.4	0.026	GS882853	0	0	b	LinJ32_V3.3600	Hypothetical protein, conserved
								LinJ32_V3.3610	Hypothetical protein, conserved

Table S8. Unresolved clones that contain differentially regulated genes in L/A. F < -1.7 indicate gene down-regulation and F > 1.7 up-regulation.

Clone	*F*	*$Log_2F \pm SD$*	*p*	*GenBank*	*e-value* *Fw*	*Rv*	*Def.*	*Id.*	*Annotated Gene Function (GO terms in additional Fig N., additional file N)*	*qRT-PCR* +/-	F ± SD
Lin7D12	2.53	1.3 ± 0.5	0.042	GS882871	0	-	c				
Lin9F12	2.42	1.3 ± 0.4	0.029	GS882870	1e-91	6e-121	b				
Lin12G9	2.34	1.2 ± 0.1	0.002	GS882869	3e-27	3e-27	b				
Lin13B7	3.25	1.7 ± 0.5	0.030	GS882868	0	0	b				
Lin24C4	5.35	2.4 ± 0.5	0.012	GS882867	0	0	a				
Lin25B5	3.02	1.6 ± 0.5	0.030	GS882866	0	0	b				
Lin32B5	2.77	1.5 ± 0.5	0.038	GS882865	1e-29	1e-23	b				
Lin34D7	2.09	1.1 ± 0.2	0.010	GS882864	1e-128	3e-018	b				
Lin49F3	2.22	1.2 ± 0.2	0.013	GS882876	7e-136	0	b	LinJ27_V3.0020	Hypothetical protein, conserved	N.D.	
								LinJ27_V3.0030	Hypothetical protein, conserved	N.D.	
								LinJ27_V3.0040	Metallo-peptidase, Clan M-, Family M48	N.D.	
Lin53D8	2.15	1.1 ± 0.3	0.029	GS882863	0	0	b				
Lin55B1	3.04	1.6 ± 0.3	0.011	GS882855	0	0	b				
Lin56G3	2.73	1.4 ± 0.5	0.036	GS882856	4e-45	2e-59	a				
Lin56G7	2.43	1.3 ± 0.0	0.000	GS882857	0	0	b				
Lin63A10	2.40	1.3 ± 0.1	0.004	GS882858	0	0	b				
Lin67B10	3.37	1.8 ± 0.3	0.011	GS882859	0	0	b				
Lin72E2	2.04	1.0 ± 0.2	0.015	GS882860	7e-173	0	b				
Lin74D2	2.44	1.3 ± 0.4	0.033	GS882861	0	0	b				
Lin76A8	3.05	1.6 ± 0.5	0.033	GS882862	0	0	b				
Lin77D11	2.06	1.0 ± 0.2	0.015	GS882772	0	0	c	LinJ32_V3.1580	Hypothetical protein	N.D.	
								LinJ32_V3.1590	Hypothetical protein, conserved	N.D.	
								LinJ10_V3.0460	Hypothetical protein, unknown function	N.D.	
Lin81B5	2.05	1.0 ± 0.1	0.003	GS882872	0	0	b				
Lin81H6	2.47	1.3 ± 0.2	0.006	GS882873	0	0	b				
Lin86D3	6.41	2.7 ± 0.1	0.001	GS882874	0	0	b				
Lin93C7	2.34	1.2 ± 0.3	0.020	GS882875	0	0	b	LinJ18_V3.1630	Hypothetical protein, conserved	N.D.	
								LinJ18_V3.1640	Hypothetical protein, conserved	N.D.	
								LinJ18_V3.1650	Chaperone protein DNAJ, putative	N.D.	
Lin93D7	2.26	1.2 ± 0.3	0.016	GS882890	0	0	b				
Lin96B8	2.22	1.1 ± 0.0	0.000	GS882891	0	0	b				
Lin100F11	2.57	1.4 ± 0.3	0.014	GS882892	0	5e-137	b				
Lin105H10	2.04	1.0 ± 0.3	0.022	GS882877	0	0	b	LinJ18_V3. 0570	Hypothetical protein, conserved	N.D.	
Lin109E12	2.06	1.0 ± 0.2	0.008	GS882893	3e-166	0	b				
Lin111F8	2.07	1.0 ± 0.1	0.003	GS882894	1e-140	1e-137	b				
Lin122B2	2.11	1.1 ± 0.2	0.014	GS882878	0	0	b	LinJ19_V3.1490	Oxidoreductase-like protein	N.D.	
								LinJ19_V3. 1500	Hypothetical protein, conserved	N.D.	
Lin122H12	2.09	1.1 ± 0.3	0.023	GS882895	0	0	b				
Lin124D12	2.43	1.3 ± 0.1	0.001	GS882896	0	0	b				
Lin 125A7	2.16	1.1 ± 0.3	0.022	GS882782	0	0	c	LinJ31_V3.1360	Hypothetical protein, conserved	N.D.	
Lin127A1	2.94	1.6 ± 0.1	0.000	GS882897	0	0	b				
Lin131C1	2.23	1.2 ± 0.4	0.044	GS882879	0	0	b	LinJ10_V3.0080	Hypothetical protein, conserved	N.D.	
								LinJ10_V3.0090	WD-repeat containing protein	N.D.	
Lin132C4	2.32	1.2 ± 0.2	0.009	GS882880	0	0	a	LinJ21_V3.2040	Hypothetical protein, conserved	N.D.	
								LinJ21_V3.2050	RNA-binding regulatory protein (pumilio family), putative	N.D.	
Lin132C5	2.16	1.1 ± 0.3	0.028	GS882898	0	0	a				
Lin139B3	3.12	1.6 ± 0.5	0.030	GS882881	0	0	b	LinJ16_V3.0460	Hypothetical protein, conserved	N.D.	
								LinJ16_V3.0470	60S ribosomal protein L21, putative	N.D.	
Lin145E8	4.08	2.0 ± 0.2	0.004	GS882791	0	-	c	LinJ23_V3.1380	Hypothetical protein, unknown function	N.D.	
Lin149C8	2.48	1.3 ± 0.5	0.042	GS882899	0	0	a				
Lin166G8	2.54	1.3 ± 0.2	0.005	GS882900	0	0	b				
Lin174G2	2.68	1.4 ± 0.6	0.049	GS882901	0	0	b				
Lin189F2	2.09	1.1 ± 0.3	0.025	GS882902	0	0	b				
Lin202D11	2.62	1.4 ± 0.3	0.013	GS882882	0	0	b	LinJ06_V3.0340	Serine peptidase, clan SC, family S9A-like protein	N.D.	
								LinJ06_V3.0350	NAD(p)-dependent steroid dehydrogenase-like protein	N.D.	
								LinJ06_V3.0360	Hypothetical protein, conserved	N.D.	
Lin202G8	20.1	1.0 ± 0.3	0.033	GS882883	0	0	b	LinJ06_V3.0340	Serine peptidase, clan SC, family S9A-like protein	N.D.	
								LinJ06_V3.0350	NAD(p)-dependent steroid dehydrogenase-like protein	N.D.	
								LinJ06_V3.0360	Hypothetical protein, conserved	N.D.	
Lin205G10	2.69	1.4 ± 0.4	0.029	GS882903	0	0	a				
Lin209B6	2.34	1.2 ± 0.1	0.001	GS882904	0	0	b				
Lin209H1	2.50	1.3 ± 0.2	0.012	GS882884	0	0	a	LinJ20_V3.0030	Hypothetical protein, conserved	N.D.	
								LinJ20_V3.0040	Phosphate-repressible phosphate permease	N.D.	
Lin211G3	2.58	1.4 ± 0.1	0.002	GS882885	5e-171	0	b	LinJ27_V3.0610	Hypothetical protein, conserved	N.D.	
								LinJ27_V3.0620	Small GTP-binding protein Rab1, putative	N.D.	
Lin213C10	2.38	1.3 ± 0.2	0.010	GS882905	8e-37	0	b				
Lin215C12	2.34	1.2 ± 0.3	0.024	GS882906	6e-87	4e-91	a				
Lin216D9	3.12	1.6 ± 0.2	0.004	GS882907	0	0	b				
Lin219D1	1.81	0.9 ± 0.3	0.037	GS882843	0	0	c	LinJ02_V3.0720	Hypothetical protein, conserved	N.D.	
								LinJ02_V3.0730	Hypothetical protein, conserved	N.D.	
								LinJ02_V3.0740	Hypothetical protein, conserved	N.D.	
								LinJ27_V3.2470	Hypothetical protein, conserved	N.D.	
Lin224B12	2.71	1.4 ± 0.3	0.010	GS882886	0	0	b	LinJ33_V3.0220	Hypothetical protein, unknown function	N.D.	
Lin225C1	2.30	1.2 ± 0.3	0.016	GS882809	2e-71	3e-83	b	LinJ29_V3.1250	Tryparedoxin	-	
								LinJ29_V3.1260	Hypothetical protein, conserved	N.D.	
Lin225G6	2.24	1.2 ± 0.4	0.044	GS882908	0	1e-156	b				
Lin229G11	3.05	1.6 ± 0.2	0.004	GS882887	0	0	a	LinJ23_V3.0340	Tryptophanyl-tRNA synthetase, putative	N.D.	
								LinJ23_V3.0350	Hypothetical protein, conserved	N.D.	
Lin241G4	2.34	1.2 ± 0.3	0.021	GS882909	3e-178	0	b				
Lin252G9	2.22	1.1 ± 0.4	0.038	GS882822	-	0	c	LinJ31_V3.1340	Hypothetical protein, conserved	N.D.	
Lin280B7	3.86	1.9 ± 0.6	0.030	GS882910	0	0	b				
Lin284G3	2.15	1.1 ± 0.4	0.041	GS882911	0	2e-176	b				
Lin288F7	3.69	1.9 ± 0.3	0.006	GS882912	0	0	b				

Lin252E9	2.44	1.3 ± 0.5	0.039	GS882913	0	0	b			
Lin256D11	3.81	1.9 ± 0.4	0.016	GS882914	0	0	b			
Lin257F10	2.25	1.2 ± 0.2	0.011	GS882915	0	0	b			
Lin271H5	2.11	1.1 ± 0.0	0.001	GS882916	5e-137	1e-137	b			
Lin272G8	2.57	1.4 ± 0.0	0000	GS882917	0	0	b			
Lin302C2	2.55	1.4 ± 0.5	0.037	GS882888	7e-161	0	b	LinJ15_V3.1140	Tryparedoxin peroxidase	N.D.
								LinJ15_V3.1150	Hypothetical protein, conserved	N.D.
Lin309G6	2.17	1.1 ± 0.4	0.046	GS882889	0	0	a	LinJ34_V3.2580	Methyltransferase-like protein	N.D.
								LinJ34_V3.2590	Hypothetical protein, conserved	N.D.
Lin4B9	-2.40	-1.3 ± 0.2	0.05	GS882941	0	0	c	LinJ29_V3.1450	Amastin-like protein	N.D.
								LinJ06_V3.1280	Hypothetical protein, conserved	N.D.
								LinJ06_V3.1290	Hypothetical protein, conserved	N.D.
								LinJ06_V3.1300	Hypothetical protein, conserved	N.D.
Lin7H4	-3.07	-1.6 ± 0.1	0.001	GS882942	5e-119	-	c	LinJ29_V3.3010	Amastin, putative	N.D.
								LinJ29_V3.3020	Tuzin-like protein, putative	N.D.
Lin8D4	-2.00	-1.0 ± 0.3	0.024	GS882918	0	0	b			
Lin22G2	-16.58	-4.1 ± 0.4	0.003	GS882919	0	0	b			
Lin25B8	-2.08	-1.1 ± 0.1	0.003	GS882920	0	6e-161	b			
Lin25B12	-1.88	-0.9 ± 0.3	0.038	S28B4	0	-	c	Lin29_V3.2310	GTP-binding protein, putative	N.D.
								Lin29_V3.2320	Hypothetical protein, conserved	N.D.
Lin25C5	-2.70	-1.4 ± 0.0	0.000	GS882921	0	4e-113	b			
Lin32G7	-2.17	-1.1 ± 0.1	0.003	GS882922	5e-134	0	b			
Lin37B3	-2.14	-1.1 ± 0.4	0.047	GS882923	-	4e-141	c	LinJ31_V3.3100	Hypothetical protein. conserved	N.D.
								LinJ31_V3.3110	Hypothetical protein. unknown function	N.D.
Lin37E5	-1.88	-0.9 ± 0.3	0.028		0	0	a	LinJ27_V3.0970	Hypothetical protein, conserved	N.D.
								LinJ27_V3.0980	gBP21, MRP1	N.D.
Lin46H3	-2.16	-1.1 ± 0.2	0.012	GS882943	1e-128	0	c	LinJ27_V3.1950	Branched-chain aminoacid aminotransferase, putative	N.D.
								LinJ27_V3.1960	Hypothetical protein, conserved	N.D.
								Lin35_V3.3620	Hypothetical protein, conserved	N.D.
Lin58D8	-1.72	-0.8 ± 0.2	0.020	GS882944	0	0	b	LinJ15_V3.0340	Hypothetical protein, conserved	N.D.
								LinJ15_V3.0350	Ecotin, putative	N.D.
								LinJ15_V3.0360	Hypothetical protein, unknown function	N.D.
Lin129F11	-3.19	-1.7 ± 0.6	0.035	GS882945	-	0	c	LinJ22_V3.0630	Serine/threonine protein kinase SOS2, putative	N.D.
								LinJ22_V3.0640	Hypothetical protein, conserved	N.D.
Lin137B5	-1.83	-0.9 ± 0.3	0.032	GS882924	0	0	b	LinJ35_V3.1930	Hypothetical protein, conserved	N.D.
								LinJ35_V3.1940	Hypothetical protein, conserved	N.D.
								LinJ35_V3.1950	Ribosomal protein L32-like protein	N.D.
Lin144G1	-1.93	-0.9 ± 0.3	0.026	GS882946	0	0	c	LinJ36_V3.0120	Hypothetical protein, conserved	N.D.
								LinJ36_V3.0130	mRNA capping methyltransferase, putative	N.D.
								LinJ31_V3.1450	Hypothetical protein, conserved	N.D.
								LinJ31_V3.1460	P-glycoprotein-like protein	N.D.
Lin162A9	-1.73	-0.8 ± 0.2	0.014	GS882947	0	0	b	LinJ22_V3.0470	Hypothetical protein, conserved	N.D.
								LinJ22_V3.0480	Ubiquitin-conjugating enzyme-like protein	N.D.
Lin164F3	-2.28	-1.2 ± 0.4	0.038	GS882925	0	0	b			
Lin165D2	-2.10	-1.1 ± 0.4	0.045	GS882926	0	7e-127	b			
Lin172A9	-2.00	-1.0 ± 0.4	0.047	GS882948	0	0	a	LinJ06_V3.1200	Hypothetical protein, conserved	N.D.
								LinJ06_V3.1210	Nuclear transport factor 2 protein (NTF2), putative	N.D.
Lin184D12	-2.27	-1.2 ± 0.5	0.047	GS882927	0	0	b			
Lin198E5	-1.75	-0.8 ± 0.1	0.002	GS882928	0	3e-150	b			
Lin199B4	-1.92	-0.9 ± 0.2	0.017	GS882929	0	9e-148	b			
Lin200H6	-12.07	-3.6 ± 1.5	0.055	GS882930	3e-36	7e-59	b			
Lin201F12	-1.79	-0.8 ± 0.3	0.038	GS882949	0	0	b	LinJ36_V3.6360	Hypothetical protein, conserved	N.D.
								LinJ36_V3.6360	Centrin, putative	N.D.
Lin209D12	-1.77	-0.8 ± 0.1	0.006	GS882931	4e-45	1e-60	b			
Lin211C5	-1.74	-0.8 ± 0.2	0.022	GS882932	0	0	b			
Lin216A8	-1.81	-0.9 ± 0.3	0.029	GS882933	0	0	a			
Lin224B5	-1.75	-0.8 ± 0.2	0.017	GS882950	0	0	b	LinJ16_V3.0720	Hypothetical protein, conserved	N.D.
								LinJ16_V3.0730	Cysteine peptidase, Clan CA, family C19, putative	N.D.
Lin226G7	-1.84	-0.9 ± 0.3	0.041	GS882951	0	0	c	LinJ15_V3.0490	Hypothetical protein	N.D.
								LinJ22_V3.1120	Polyprotein, putative	N.D.
Lin244G7	-3.25	-1.7 ± 0.7	0.046	GS882934	3e-67	7e-65	a			
Lin301G2	-2.51	-1.3 ± 0.5	0.047	GS882935	0	0	b			
Lin285C9	-2.15	-1.1 ± 0.1	0.004	GS882936	0	0	b			
Lin287B10	-3.36	-1.7 ± 0.3	0.008	GS882937	0	0	b			
Lin294H2	-1.75	-0.8 ± 0.3	0.048	GS882938	0	0	b			
Lin297C3	-2.52	-1.3 ± 0.1	0.002	GS882952	0	0	b	LinJ36_V3.6750	Hypothetical protein, conserved	N.D.
								LinJ36_V3.6760	MAP kinase, putative	N.D.
Lin297H12	-2.18	-1.1 ± 0.2	0.015	GS882939	0	0	b			
Lin300A12	-1.81	-0.9 ± 0.3	0.029	GS882940	0	6e-115	b			
Lin301A6	-1.80	-0.8 ± 0.4	0.053	GS882854	6e-118	0	c	LinJ26_V3.2190	Hypothetical protein, conserved	N.D.
								LinJ26_V3.2200	Hypothetical protein, conserved	N.D.
								LinJ26_V3.2210	Hypothetical protein, conserved	N.D.
								LinJ26_V3.2220	Hypothetical protein, conserved	N.D.

Table S9. Clones that probably contain differentially regulated gRNA genes from minicircle sequences.

Clone		*F*	*$Log_2F \pm SD$*	*p*	*GenBank*	*Content*
Lin100B7	uS/L	1.72	0.8 ± 0.2	0.029	GS882953	Contig 917. Possible minicircle sequence.
Lin110D9		1.75	0.8 ± 0.1	0.006	GS882954	Contig 957. Possible minicircle sequence.
Lin117A10		1.73	0.8 ± 0.2	0.023	GS882955	Contig 957. Possible minicircle sequence.
Lin232H8		2.01	1.0 ± 0.1	0.004	GS882956	Contig 957. Possible minicircle sequence.
Lin228H1	dS/A	-2.10	-1.1 ± 0.1	0.036	GS882957	Contig 957. Possible minicircle sequence.
Lin43C12	dL/A	-19.32	-4.3 ± 1.7	0.048	GS882958	Contig 957. Possible minicircle sequence.

SUPPLEMENTARY DATA FILE 3

Fig. S3. Multilevel sector charts of α-scores for GO terms annotated in genes.(A, C, E, G) Biological process terms; (B, D, F, H) Molecular function terms. (A, B) uS/L; (C, D) dS/L; (E, F) dS/A; (G, H) dL/A.

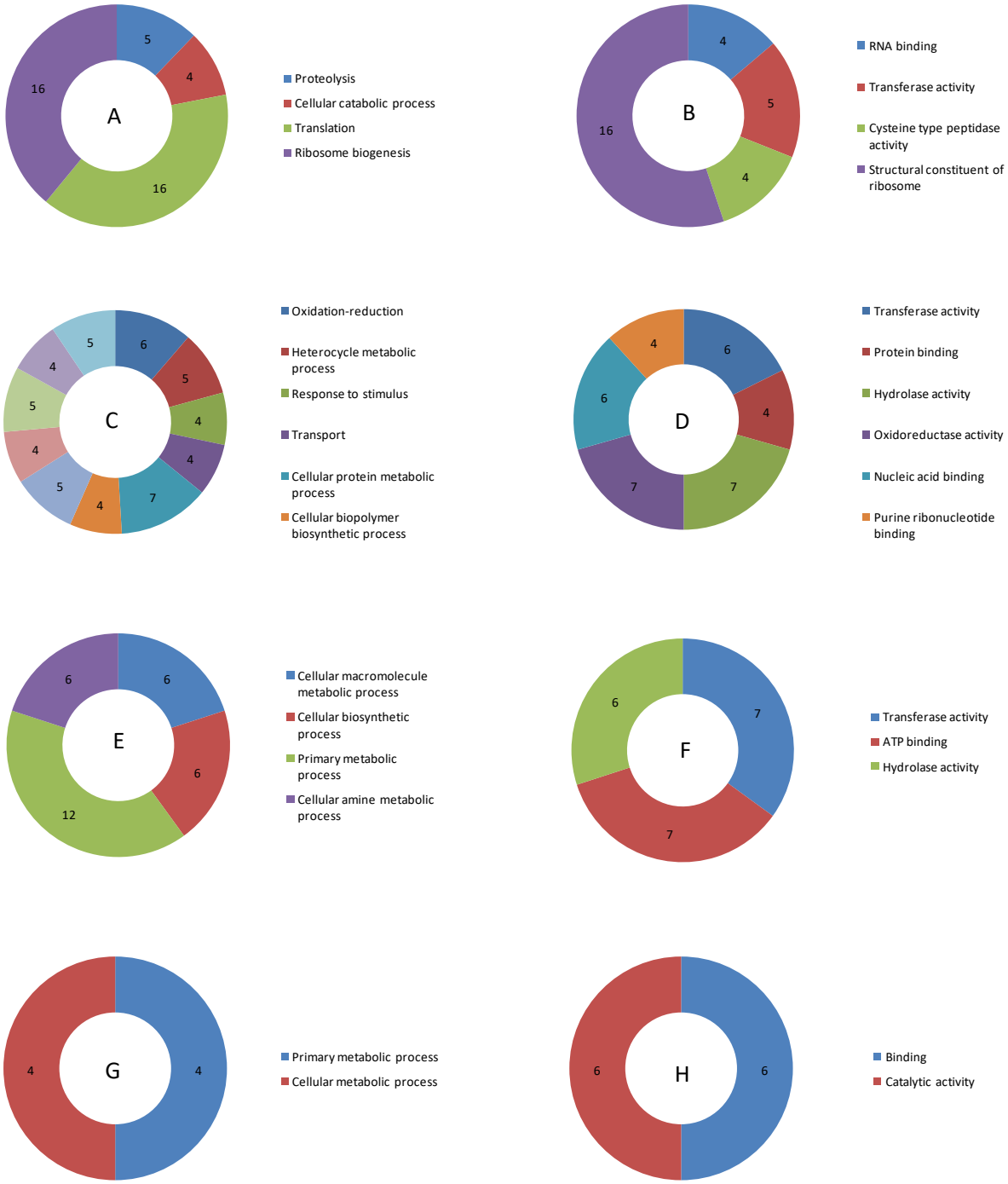
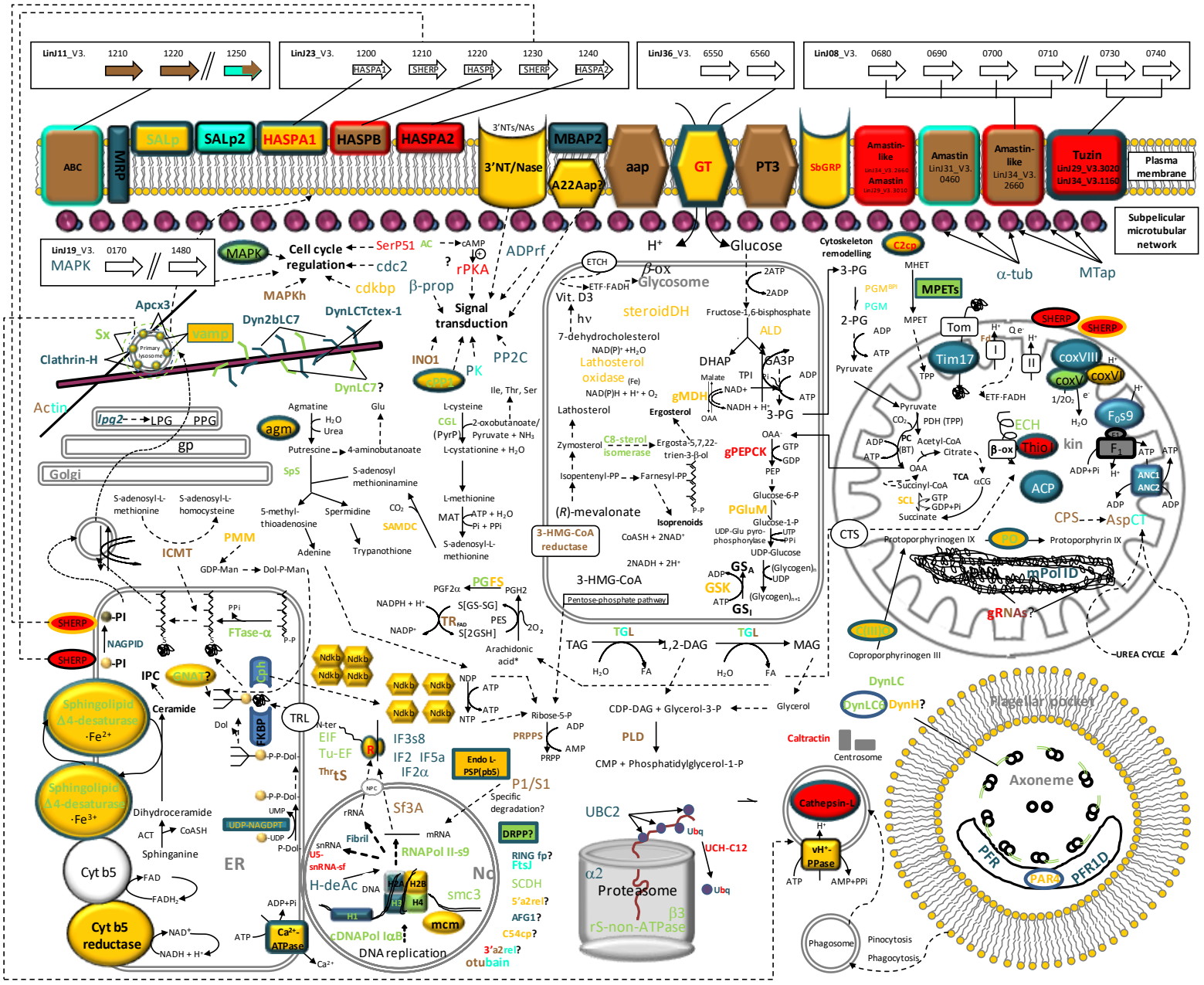


Fig. S4. Complete scheme of S/L, S/A and L/A expression profiles.

Colour legend: down-regulated in S/L; up-regulated in S/L; up-regulated in S/A; down-regulated in S/A; down-regulated in L/A; up-regulated in L/A. Abbreviations are defined in the text and in additional file.

Abbreviations: 3'NT/Nase, 3'-nucleotidase/nuclease; 3-HMG-CoA, 3-hydroxymethyl glutaryl-CoA; αCG, α-cetoglutarate; α-tub, α-tubulina; A22ap, presenilin-like aspartic peptidase, clanAD, family A22A; aap, amino acid permease; ABC, ABC transporter; AC, adenylate cyclase; ADPrf, ADP ribosylation factor; ACP, acyl carrier protein; AFG1, AFG1 ATPase; agm, agmatinase; ALD, fructose-1,6, bisphosphate aldolase; ANC, ADP/ATP translocase; AspCT, aspartate carbamoyl transferase; *β-ox*, FA β-oxidation; β-prop, β-propeller protein; BT, biotin; C(III)O, coproporphyrinogen III oxidase; C2cp, calpain-like cysteine peptidase, Clan CA, family C2; C54cp, cysteine peptidase, family C54; cdc, cyclin dependent protein kinase; cdkbp, cdc binding protein; cDNAPol I, DNA polimerase I, catalytic subunit; CGL, cystathionine γ-liase; Clathrin-H, clathrin heavy chain; cox, cytochrome c oxidase; Cph, cyclophilin; cPP1, protein phosphatase 1 catalytic subunit; CPS, carbamoyl phosphate synthetase; cyt b5, cytochrome b5; DRPP, developmentally regulated phosphoprotein-like protein; DynLC, dynein light chain; ECH, enoyl-CoA hydratase/isomerase; EIF, eukaryotic translation initiation factor; EndoL-PSP (pb5); endoribonuclease L-PSP (pb5); ETF, electron transfer flavoprotein; F_0s9F9, ATP synthetase regulatory subunit F_0; Fd, ferredoxin; Fibril, fibrilarin; FKBP, FK-506 binding protein; FTase-α, farnesyltransferase a; FtsJ, cell división protein; gMDH, mitocondrial malate dehydrogenase; GNAT, glucose-6-phosphate N-acetyltransferase; gPEPCK, glycosomal phosphoenolpyruvate carboxykinase; gRNA, guide RNA; GS, glycogen synthase; GSK, GS kinase; GT, H^+-glucose symporter; H1, H2A, H2B, H3, H4, histone superfamily genes; HASP, hydrophilic surface protein; H-deAC, histone deacetylase; ICMT, isoprenylcysteine methyltransferase; IPC, inositol phosphoceramide; kDNA, kinetoplast DNA; LPG, lipophosphoglycan; lpg2, lpg biosynthetic protein 2; MAPK(h), mytogen activated protein kinase (homologue); MBAP, membrane-bound acid phosphatase; mcm, minichromosome maintenance protein; MPETs, 4-methyl-(5-β-hydroxyethyl)thiazole monophosphate synthesis protein; mPol ID, mitocondrial DNA polymerase ID; MRP, multidrug resistance protein; Mtap, microtubule associated protein; Ndkb, nucleoside diphosphate kinase b; OAA, oxalacetate; P1/S1, P1/S1 nuclease; PC, pyruvate carboxylase; PDH, pyruvate dehydrogenase complex; P-Dol, dolichol phosphate; PES, prostaglandin peroxide synthase; PFR, PAR4, paraflagellar rod proteins; PGFS, prostaglandin F synthetase; PGluM, phosphoglucomutase; PGM, phosphoglycerate mutase; PGM^{BPI}, PG- independent PGM; PI, phosphatidylinositol; PK, protein kinase; INO1, *mio*-inositol-1-phosphate synthase; PLD, phospholipase D; PMM, phosphomannomutase; PO, protophorphyrinogen oxidase; PP2C, protein phosphatase 2C; PPG, proteophosphoglycan; PRPPS, phosphoribosyl pyrophosphate synthetase; PT3, pteridine transporter 3; RNAPol, RNA polymerase; rPKA, protein kinase A regulatory subunit; rS-non-ATPase, proteasome regulatory non-ATPase subunit; SALp, surface antigen-like protein; SAMDC, S-adenosyl-methionine decarboxylase; SbGRP, sodium stibogluconate resistance protein; SCDH, short chain dehydrogenase; SCL, succinyl-CoA ligase; SerP51, serine peptidase, family S51, peptidase E; Sf3A, splicing factor3A; smc3, structural maintenance of chromosome 3 protein; SpS, spermidine synthase; steroidDH, 3-oxo-5-α-steroid dehydrogenase; Sx, syntaxin; Apcx3, adaptor complex medium chain subunit 3; Thio, thiolase; ^{Thr}tS, threonyl-tRNA synthetase; Tim17, mitochondrial inner membrane translocase; Tom, mitochondrial outer membrane translocase; TPI, triose phosphate isomerase; TR, trypanothione reductase; TRL, translocon; U5-snRNA-sf, small nuclear RNA splicing factor U5; UBC2, ubiquitin conjugating enzyme E2; Ubq, ubiquitin; UCH-C12, cysteine peptidase, Clan CA, family C12; vamp, vesicle associated membrane protein; vH^+-PPase, vacuolar-type proton-translocating pyrophosphatase.


LinJ11_V.3. 1210 1220 1250
LinJ23_V.3. 1200 1210 1220 1230 1240
HASPA1
SHERP
HASPB
SHERP
HASPA2
LinJ36_V.3. 6550 6560
LinJ08_V.3. 0680 0690 0700 0710 0730 0740
ABC
MRP
SALp
SALp2
HASPA1
HASPB
HASPA2
3'NTs/NAs
3'NT/Nase
MBAP2
A22Aap?
aap
GT
PT3
SbGRP
Amastin-like LinJ34_V3.2660 Amastin LinJ29_V3.3010
Amastin LinJ31_V3.0460
Amastin-like LinJ34_V3.2660
Tuzin LinJ29_V3.3020 LinJ34_V3.1160
Plasma membrane
Subpelicular microtubular network
LinJ19_V.3. 0170 1480
MAPK
Cell cycle regulation
SerP51
AC
cAMP
cdc2
cdkbp
β-prop
?
rPKA
ADPrf
H+
Glucose
Cytoskeleton remodelling
C2cp
α-tub
MTap
MAPKh
Signal transduction
Apcx3
Sx
vamp
Dyn2bLC7
DynLCTctex-1
Clathrin-H
Primary lysosome
Actin
DynLC7?
INO1
PK
PP2C
Ile, Thr, Ser
lpg2
LPG
PPG
gp
Golgi
agm
Agmatine
H2O
Urea
Putrescine
4-aminobutanoate
Glu
L-cysteine
CGL (PyrP)
2-oxobutanoate/ Pyruvate + NH3
L-cystationine + H2O
SpS
S-adenosyl methioninamine
S-adenosyl-L-methionine
S-adenosyl-L-homocysteine
5-methyl-thioadenosine
Spermidine
Trypanothione
CO2
SAMDC
MAT
ATP + H2O
Pi + PPi
L-methionine
S-adenosyl-L-methionine
PMM
ICMT
GDP-Man
Dol-P-Man
Adenine
ETC
β-ox
Glycosome
ETF-FADH
Vit. D3
hν
7-dehydrocholesterol
steroidDH
NAD(P)+ +H2O
Lathosterol oxidase (Fe)
NAD(P)H + H+ + O2
Lathosterol
Ergosterol
gMDH
Zymosterol
C8-sterol isomerase
Ergosta-5,7,22-trien-3-β-ol
Isopentenyl-PP
Farnesyl-PP
P-P
Isoprenoids
(R)-mevalonate
3-HMG-CoA reductase
CoASH + 2NAD+
2NADH + 2H+
3-HMG-CoA
Pentose-phosphate pathway
Fructose-1,6-bisphosphate
2ATP
2ADP
ALD
DHAP
TPI
GA3P
Malate
NAD+
NADH + H+
ADP
ATP
3-PG
OAA
gPEPCK
GTP
GDP
PEP
Glucose-6-P
PGluM
Glucose-1-P
UDP-Glu pyro-phosphorylase
UTP
PPi
UDP-Glucose
GSA
GSK
GSI
(Glycogen)n
(Glycogen)n+1
PGFS
PGF2α
PGH2
NADPH + H+
NADP+
TRFAD
S[GS-SG]
S[2GSH]
PES
2O2
Arachidonic acid*
TAG
TGL
H2O
FA
1,2-DAG
MAG
CDP-DAG + Glycerol-3-P
Glycerol
PLD
CMP + Phosphatidylglycerol-1-P
3-PG
PGM BPI
PGM
2-PG
ADP
ATP
Pyruvate
MHET
MPETs
MPET
Tom
Tim17
ETF-FADH
I
II
SHERP
coxVIII
coxV
coxV
1/2O2
H2O
F0s9
F1
ATP
ADP+Pi
ANC1
ANC2
ADP
ATP
CO2
PDH (TPP)
PC (BT)
Acetyl-CoA
Citrate
OAA
TCA
αCG
Succinyl-CoA
SCL
GTP
GDP+Pi
Succinate
ECH
β-ox
ThioI
kin
ACP
CPS
AspCT
CTS
Protoporphyrinogen IX
PPO
Protoporphyrin IX
mPolID
gRNAs?
FECH
Coproporphyrinogen III
UREA CYCLE
PI
NAGPID
SHERP
SHERP
PI
IPC
GNAT?
FTase-α
PPi
P-P
Cpn
FKBP
Sphingolipid Δ4-desaturase ·Fe2+
Sphingolipid Δ4-desaturase ·Fe3+
Ceramide
Dol
P-P-Dol
TRL
N-ter
EIF
Tu-EF
ThrtS
Ndkb
NDP
ATP
NTP
Ribose-5-P
PRPPS
ADP
AMP
PRPP
IF3s8
IF2
IF5a
IF2α
Endo L-PSP(pb5)
P1/S1
Specific degradation?
DRPP?
RING fp?
FtsJ
SCDH
5'a2rel?
AFG1?
C54cp?
3'a2rel?
otubain
Dihydroceramide
ACT
CoASH
Sphinganine
UMP
UDP-NAGDPT
UDP
P-Dol
ER
Cyt b5
FAD
FADH2
Cyt b5 reductase
NAD+
NADH + H+
ADP+Pi
ATP
Ca2+-ATPase
Ca2+
NPC
rRNA
Sf3A
mRNA
Fibril
snRNA
U5-snRNA-sf
RNAPol II-s9
H-deAc
DNA
H2A
H2B
H3
H4
H1
Nc
smc3
mcm
cDNAPol IαB
DNA replication
UBC2
Ubq
UCH-C12
α2
Proteasome
β3
rS-non-ATPase
Ubq
DynLC
DynLCc
DynH?
Flagellar pocket
Caltractin
Centrosome
Axoneme
PFR
PAR4
PFR1D
Cathepsin-L
H+
vH+-PPase
ATP
AMP+PPi
Phagosome
Pinocytosis
Phagocytosis

Additional information about S/L, S/A and L/A expression profiles.

Epigenetics, replication and nucleotide metabolism. Histone genes H1 (LinJ27_V3.1120 and LinJ33_V3.3390), H2A (LinJ29_V3.1860/70), H3 (LinJ16_V3.0600) and H4 (36_V3.0020) are up-regulated in L/A. H1 LinJ27_V3.1070, H3 LinJ10_V3.1070 and H4 LinJ31_V3.3320 are down-regulated in S/L and H2B (LinJ19_V3.0040 and LinJ28_V3.0210) is up-regulated in S/A.

Gene expression, protein maturation and transport and secretory pathway. At the transcriptional and post-transcriptional processes, four genes are differentially regulated in the ongoing of the life cycle. To begin with, DNA-directed RNA polymerase II subunit 9 gene is down-regulated in S/L. On the other hand, U5 RNA-splicing factor is up-regulated in S/L, what suggests changes in small nucleolar RNA (snRNA) processing in stationary-phase promastigotes. Meanwhile, a component of a nucleolar small nuclear ribonucleoprotein (snRNP) involved in ribosomal RNA processing (Jansen et al., 1991), is up-regulated in L/A, in contrast to splicing factor 3A, which is down-regulated in L/A. At the translational level, a considerable number of ribosomal components (40S ribosomal S2, S3, S4, S11, S12, S29; 60S ribosomal proteins L7, L11, L12, L18, L19, L31, L36, L38, L44) and four genes involved in translation initiation (initiation factors IF2 and IF2α, eukaryotic translation initiation factor 3 subunit 8 IF3s8 and eukaryotic initiation factor 5a IF5a) are up-regulated in L/A. Despite some ribosomal proteins are up-regulated in S/L (40S ribosomal proteins S2, S3a, S4, S6, S18, S27-1; 60S ribosomal proteins L1a, L3, L5, L14, L32; and acidic ribosomal protein P2) and in S/A (40S ribosomal proteins S2, S3, S3a, S4, S7, S11, S14, S15a, S23, S24 and S29; 60S ribosomal proteins L1a, L2, L3a, L6, L7, L9, L13a, L18, L18a, L19, L21 and L31), none of the translation regulation factors mentioned have been found to be differentially regulated. An eukaryotic translation initiaton factor (EIF) and a GTP-binding elongation factor from Tu family (EF-Tu) are down-regulated in S/L instead.

Hexose metabolism, oxidative phosphorylation and transport. Two non-ligated ATPase subunit 9 genes (F_0s9) are up-regulated in L/A. ATP synthesis-coupled proton transport biological function GO term has been annotated on both sequences by means of BLAST2GO software and hydrogen ion transporting ATP synthase activity (rotational mechanism) is the molecular function term associated to these sequences. PFAM and InterPro domain annotations correspond respectively to ATP synthase subunit C and ATPase F_0 complex subunit C. This subunit is included in the ATP synthase complex and contributes to ATP biosynthesis by rotation driven by the proton electrochemical potential gradient. In addition to mitochondrial ATP synthetase complex, two ATPase genes have been found to be up-regulated in L/A: an AFG1-like ATPase and a calcium-motive P-type ATPase (Ca^{2+}-P-ATPase). Ca^{2+}-P-ATPase is also up-regulated in S/A and belongs to the P-type (E1-E2-type) ATPase superfamily of cation transport enzymes and is included in Ca^{2+}-transporting ATPase group (Fagan and Saier, 1994). In addition, six genes that belong to the ATP binding cassette (ABC) superfamily have been detected to be differentially regulated: an ABC transporter/multidrug resistance protein/p-glycoprotein (MRP) is up-regulated in L/A; two sodium stibogluconate-resistance protein genes (SbGRP) are up-regulated in S/L and one of them is down-regulated in S/A and specifically by combined temperature and pH shift as previously reported (Alcolea et al., 2009a); and three different tandemly mapping ABC transporter-like genes are down-regulated in S/A and one of them also in L/A. Other kinds of transporters are differentially regulated in the life cycle. To begin with, a vacuolar-type proton-translocating pyrophosphatase (vH^+-PPase) is up-regulated in S/A and is involved in the establishment of proton electrochemical potential gradient between the vacuole lumen and the cytosol. Mitochondrial inner membrane pre-protein translocase complex subunit (PF02466) Tim17 is up-regulated in L/A. Finally, aminoacid permease LinJ31_V3.1850 (aap) is up-regulated in S/L and down-regulated in S/A, what suggests that aap transcript levels increase in the progression of the life cycle from procyclic

promastigotes to amastigotes. The up-regulation of aap in the amastigote stage is due to the specific influence of temperature and pH shift (Alcolea et al., 2009a).

As a summary, SCL, several glycolytic and glycogen biosynthetic genes are up-regulated in S/A, gPEPCK is up-regulated in S/L and some genes related with electron transport-coupled oxidative phosphorylation are up-regulated in L/A. Apart from that, amastigotes up-regulate an ABC transporter cluster and down-regulate SbGRP and MRP genes from the same superfamily.

Lipid metabolism. 3,2-*trans*-enoyl-CoA hidratase isomerase gene (ECH) is down-regulated in S/L. ECH is involved in unsaturated fatty acid oxidation β-ox. Consequently, unsaturated FA may be destined to degradation inside the mitochondrial or glycosomal lumen or to arachidonic acid derivative biosynthetic processes, such prostaglandins. In fact, prostaglandin F synthase gene (PGFS) is also down-regulated in S/L. In addition, PGFS is up-regulated in S/A according to this analysis and was also found as down-regulated in PNA^- (metacyclic) promastigotes (Alcolea et al., 2009b) and in temperature-pH-generated amastigote-like forms (Alcolea et al., 2009a), which globally supports the previous suggestion of prostaglandin F2α role as a competence factor for promastigotes inside the sand-fly vector (Kabututu et al., 2002). Three 3-ketoacyl-CoA thiolase genes (Thiol I) are up-regulated in L/A and two of them also in S/L. These genes are involved in the reversible addition of acetyl-CoA groups to acyl groups in β-ox, as well as in branched chain aminoacid catabolism. ECH (isoform with unsaturated FA substrates) and Thiol I gene up-regulation is not correlated, which could be due to a different regulation of unsaturated and saturated FA β-ox pathways depending on the developmental stage. Regarding FA biosynthetic processes, acyl carrier protein gene (ACP) is up-regulated in L/A.

Proteolysis. A set of protease genes are differentially regulated. Cathepsin L-like protease LinJ08_V3.0960 (Cathepsin-L) is up-regulated in L/A and S/L; AUT2/APG4/ATG4 cysteine peptidase Clan CA family C54 protein (C54cp) is up-regulated in S/A; otubain cysteine peptidase, clan CA, family C65, of unknown function is down-regulated in S/A and L/A; and serine peptidase S51 (SerP51) is up-regulated in S/L and also metacyclic promastigotes (Alcolea et al., 2009b).

Signal transduction and cell cycle regulation. A variety of genes related to signal transduction and cell cycle regulation have been annotated in the genome sequences of three *Leishmania* species. In addition, *L. major*, *Trypanosoma brucei* and *T. cruzi* kinomes have been compared (Parsons et al., 2005) and protein kinases and their potential usefulness as drug targets have been reviewed (Naula et al., 2005). However, cell cycle regulation and signal transduction processes are poorly understood in *Leishmania* spp. because signaling pathways have not been elucidated yet. Differential gene expression data constitute the first step for a better understanding of the pathways that signal for the response to each stimulus in the ongoing of the differentiation processes. According to the microarray hybridization assays, a cdc2-related kinase and three mitogen activated protein kinase genes (MAPK) are up-regulated under L/A. One out of these MAPK is down-regulated and a cyclin-dependent kinase-binding protein gene (cdkbp) is up-regulated in S/L. In addition, a MAPK homologue (MAPKh) is down-regulated in S/A. Dealing with signal transduction, a beta propeller protein (-prop) containing WD40 repeats in circular beta propeller structure (IPR001680) is up-regulated in L/A. Four protein kinase genes (PK) have also been detected to be differentially regulated in the development of the parasite's biological cycle, each of them in a distinct stage. As an example, regulatory subunit of a protein kinase A-like protein (rPKA) is up-regulated in S/L, which is related to cAMP signaling pathway in other eukaryotes. In spite of this, a non-receptor type adenylate cyclase gene (AC) is down-regulated in S/L, what again evidences the need for studying signal transduction processes in *Leishmania* parasites. GSK is up-regulated in S/A as mentioned in the manuscript. -calpain is up-regulated in L/A and S/A. Two other peptidase genes involved in signal transduction have been found as differentially regulated:

presenilin-like aspartic peptidase gene from A22A family (A22Aap) is up-regulated in S/A and serine peptidase E from S51 family (SerP51) in S/L.

Surface molecules and genes of unknown function. Genes from the amastin-tuzin cluster located in chromosome 8 are differentially regulated. some tuzin genes are up-regulated in L/A and some amastin-like genes down-regulated in S/A and up-regulated in S/L. Other amastin gene with the same pattern is LinJ34_V3.2660, while amastin LinJ34_V3.0460 is down-regulated in S/A and L/A. Moreover, two additional tuzin genes are up-regulated in S/L, as well as amastin LinJ19_V3.3010. Other genes of unknown function in the parasite not yet mentioned and their specific differential regulation are: a RING finger protein (RINGfp), up-regulated in L/A; two adjoining short chain dehydrogenase genes (SCDH), down-regulated in S/L; developmentally regulated phosphoprotein-like protein, up-regulated in L/A and down-regulated in S/L; and FtsJ cell division protein (FtsJ), down-regulated in L/A. Finally, a great set of hypothetical and unknown proteins are differentially regulated throughout the parasite's life cycle. In fact, about one half of the total number of annotated genes in the *Leishmania* spp. genomes are not associated to known functions, which highlights the need for characterizing these genes to improve the knowledge on the parasite's biology.

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