Supplementary material

*Li*ZIP3 is a cellular zinc transporter that mediates the tightly regulated import of zinc in *Leishmania infantum* parasites

Table S1 – Primers used in qRT-PCR.

Primer	Sequence
P1	CTGAACGGCAAGCTGACTGGTAT
P2	TGTAGCCGAGAATGCCCTTCAT
P3	CTTCATCATTCTCGCTGCATCG
P4	ATCGCAAACAGAAACGCCCA
P5	ATATCACTGGAGCTTGCGCTGAT
P6	AAGAGGGAGCATTGGGATGATG
P7	ATAAACACGAGCGACCGATTCC

P1/P2 and P3/P4 amplify a portion of *LiGAPDH* and *LiZIP3* mRNAs, respectively; P5/P6 amplify the end of the coding region of the *LiZIP3* mRNA and the beginning of the endogenous 3' untranslated region (UTR); P5/P7 amplify the end of the coding region of the *LiZIP3* mRNA and the beginning of the 3' UTR of the pXG*LiZIP3* vector.

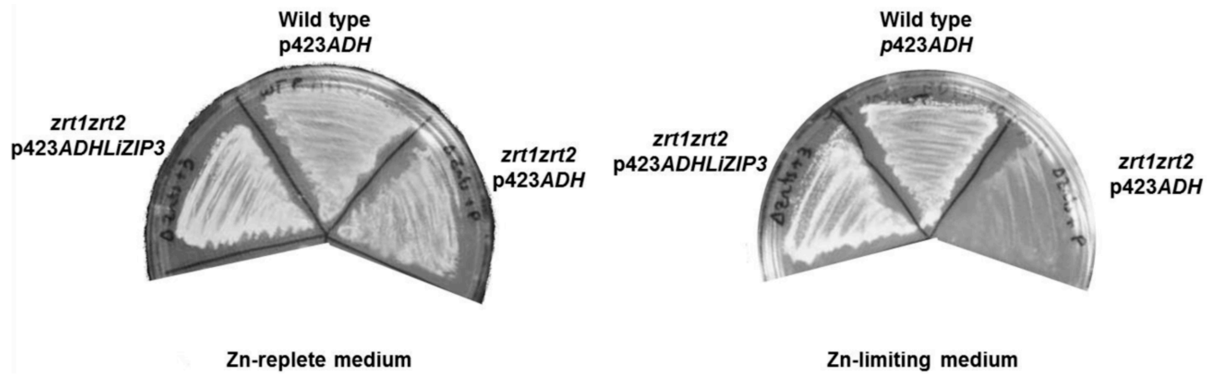

Fig. S1 – Expression of *Li*ZIP3 in the *zrt1zrt2* mutant strain, defective in high- and low-affinity zinc transport systems, rescues the growth defect phenotype in zinc-limiting medium. The yeast mutant strain *zrt1zrt2* (ZHY3), deficient in the high- and low-affinity zinc transport systems *Sc*ZRT1 and *Sc*ZRT2, was transformed with the empty vector p423*ADH* or the vector containing the *LiZIP3* gene, p423*ADHLiZIP3*. The ability of *Li*ZIP3-complemented yeast to grow in zinc-limiting medium was compared to that of the wild type strain (DY1457) and of the mutant strain carrying the empty vector. Mutant strains were able to grow in zinc-replete medium (SD-galactose); however, in zinc-limiting conditions (SD-galactose, 10 μM EDTA), growth was only observed in the *Li*ZIP3-complemented yeast.

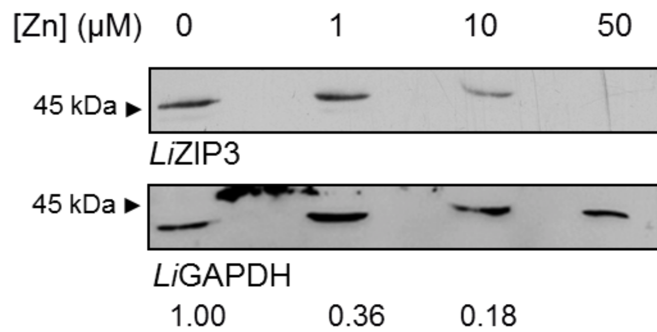

Fig. S2 – Dose-response expression of *Li*ZIP3 to the presence of zinc. Concentrations of zinc from 1 to 50 μM were tested for their ability to decrease *Li*ZIP3 protein level 24 h upon metal supplementation. The numbers bellow the blots refer to the values obtained by densitometry analysis in which the intensity of the *Li*ZIP3 band in the presence of zinc was compared to the intensity in the absence of the metal, after correcting for the loading. Expression of *Li*GAPDH was used as loading control.

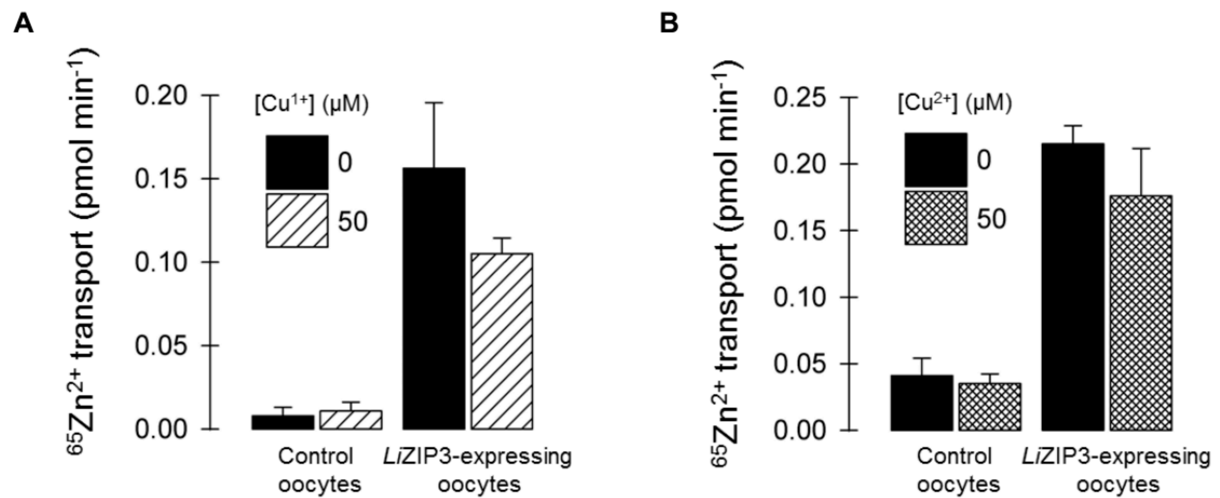

Fig. S3 – Effect of copper on *Li*ZIP3-mediated zinc transport in *X. laevis* oocytes. The uptake of 5 µM $^{65}Zn^{2+}$ was measured in control oocytes and oocytes expressing *Li*ZIP3 at pH 7.5 in the absence or presence of (A) 50 µM Cu^{1+} (n = 19–22) or of (B) 50 µM Cu^{2+} (n = 12–14) in the presence of 100 µM *L*-histidine and, in (A) only, 100 µM *L*-ascorbic acid. Two-way ANOVA revealed interactions (A, $P < 0.001$ and B, $P = 0.006$). Data in A and B were derived from independent preparations.

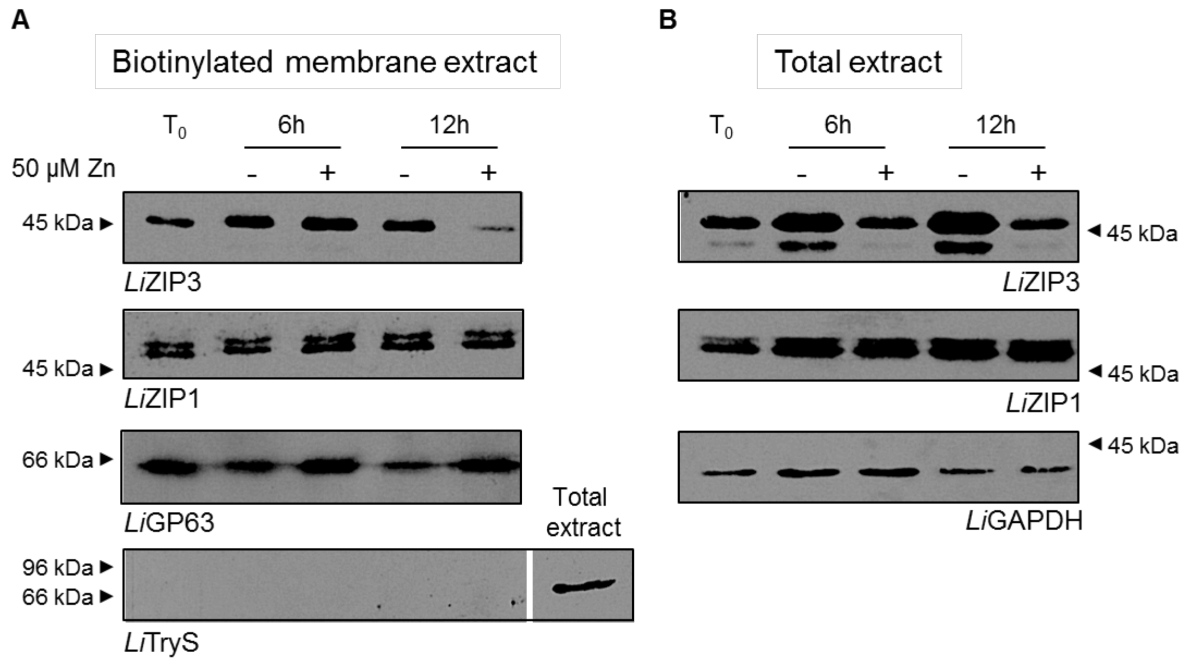

Fig. S4 – Original order of the samples run in blots of Fig. 6A and 6B.

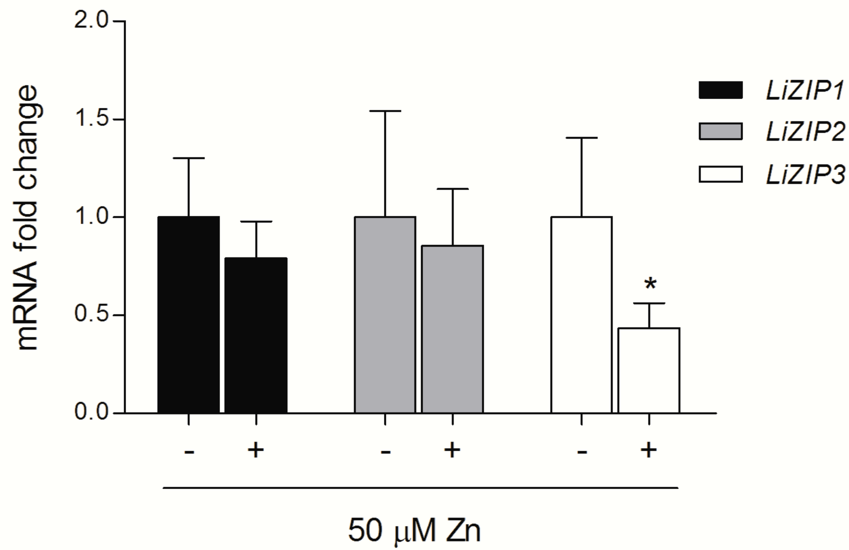

Fig. S5 - The effect of zinc on *LiZIP3* mRNA is specific to this transporter. Fifty micromolar zinc were added for 24 h to cultures of promastigotes grown for 48 h in normal medium. Changes in *LiZIP1*, *LiZIP2* and *LiZIP3* mRNA caused by zinc supplementation were measured by qRT-PCR (mean ± SD, $n = 4$; asterisks indicate significant differences between cultures not supplemented and supplemented with zinc, * $P < 0.05$, Student's t test).

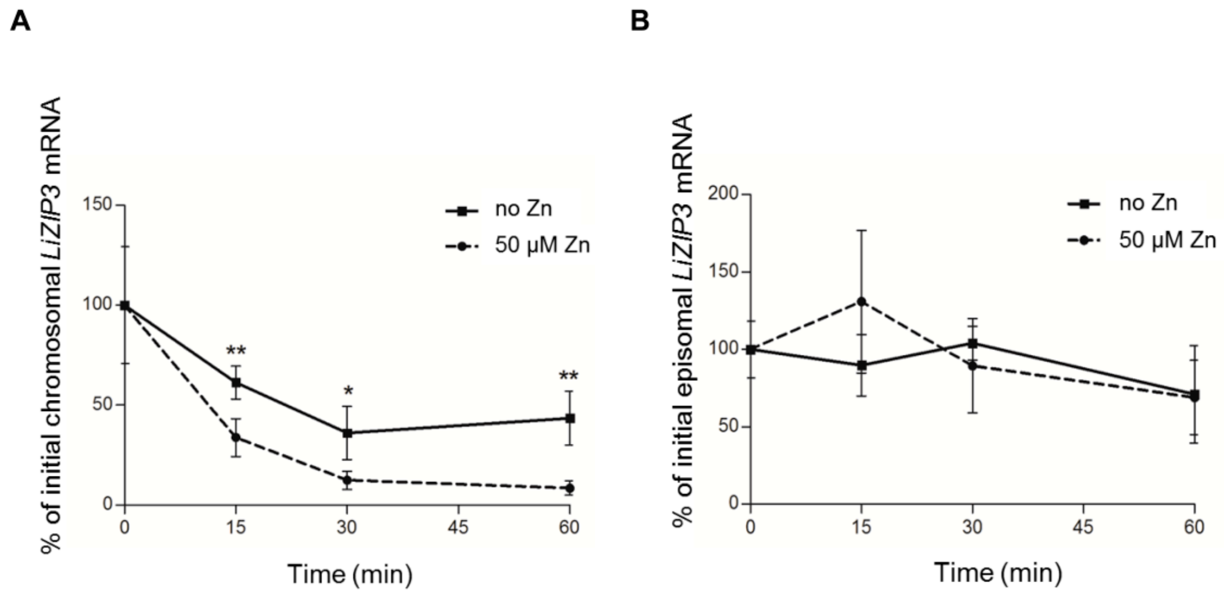

Fig. S6 – Zinc regulates *LiZIP3* mRNA stability through elements in the UTRs. Parasites carrying an extra-chromosomal copy of *LiZIP3* devoid of its own UTRs (pXG*LiZIP3*) were used to analyse the role of the endogenous UTRs in the regulation of *LiZIP3* mRNA by zinc. Fifty micromolar of zinc was added to a culture of pXG*LiZIP3* parasites in the presence of 10 μg mL^{-1} actinomycin D and the effect of the metal on the degradation of the *LiZIP3* mRNA derived from (A) the chromosome and (B) the episome was measured by qRT-PCR at several time-points (mean ± SD, $n = 3$; asterisks indicate significant differences in each time-point, * $P < 0.05$, ** $P < 0.01$, Student's *t* test). The chromosome- and episome-derived *LiZIP3* transcripts were distinguished by using primer P5, complementary to the end of *LiZIP3*, in conjunction with the anti-sense primers P6 and P7, annealing to the beginning of the endogenous and episomal 3' UTRs, respectively.

Supplementary experimental procedures

*Construction of the p423*ADHLiZIP3 *plasmid*

To produce the p423*ADHLiZIP3* construct, the *LiZIP3* open reading frame (ORF) was PCR-amplified from *L. infantum* genomic DNA using primers 5'-cggactagtATGGCGCAACCCACCCTC-3' and 5'-caccgctcgagCATCAGCCACTTCCCGATG-3' (clamp sequences in lower case and restriction sites underlined) and cloned into the *Hin*dIII and *Xho*I sites of pBluescript II KS+ (Stratagene). We verified the insertion of the cloned PCR product by DNA sequencing. *LiZIP3* was subsequently removed from pBluescript II KS+ by *Spe*I and *Xho*I restriction and cloned into p423*ADH* (Mumberg *et al.*, 1995).

Functional expression in the S. cerevisiae zrt1zrt2 *strain*

The yeast strain *zrt1zrt2* (ZHY3, *MATα ade6 can1 his3 leu2 trp1 ura3 zrt1::LEU2 zrt2::HIS3*) (Zhao and Eide, 1996b) was grown in SD-galactose medium supplemented with the required amino acids. Zinc-limiting medium was obtained by addition of 10 μM EDTA.