Lactoferrin and lactoferricin endocytosis halt *Giardia* cell growth and prevent infective cyst production

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Running Head: Giardicidal action of lactoferrin and its N-peptide

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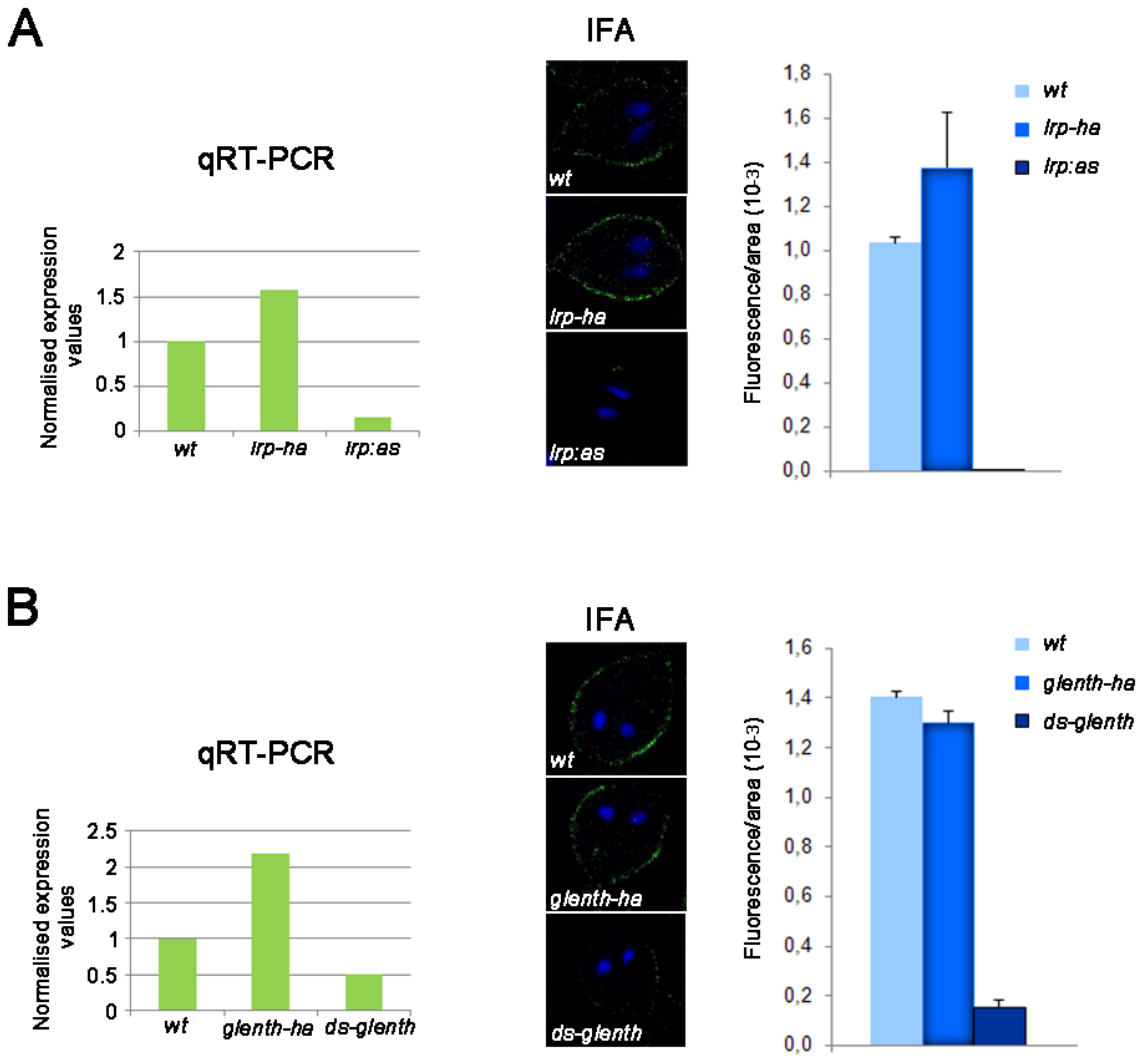
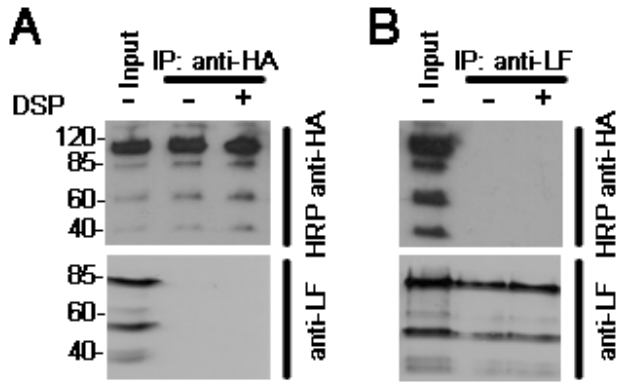
Supplementary Figure S1: GlLRP and GlENTHp are involved in the endocytosis of Bodipy-LDL.



GlLRP and GlENTHp are involved in the endocytosis of Bodipy-LDL. (A) qRT-PCR: *lrp* gene expression in *wild-type* (wt), *lrp-ha*, and *lrp:as* trophozoites. Expression was normalized to the *gdh* housekeeping gene. Error bars indicate standard deviation for experiments with more than one trial. IFA: Confocal fluorescence microscopy of Bodipy-LDL endocytosis (up to 30 min) in *wild-type* (wt), *lrp-ha*, and *lrp:as* trophozoites. Nuclear DNA was labeled with DAPI (blue). Bars: Histograms of fluorescent images (fluorescence/area x 10^3) show the relative amount of fluorescent Bodipy-LDL internalized by parasites in control (*wild-type*-wt-) and transgenic cells (*lrp–ha* and *lrp:as*).
(B) qRT-PCR: *enth* gene expression in *wild-type* (wt), *glenth-ha*, and *ds-glenth* trophozoites. Expression was normalized to the *gdh* housekeeping gene. Error bars indicate standard deviation for experiments with more than one trial. IFA: Confocal fluorescence microscopy of Bodipy-LDL endocytosis (up to 30 min) in *wild-type* (wt), *glenth-ha*, and *ds-glenth* trophozoites. Nuclear DNA was labeled with DAPI (blue). Bars: Histograms of fluorescent images (fluorescence/area x 10^3) show the relative amount of fluorescent Bodipy-LDL internalized by parasites in control (*wild-type*-wt-) and transgenic cells (*glenth-ha* and *ds-glenth*).

Supplementary Figure S2: LRP-HA - bLF immunoprecipitation and immunoblotting.



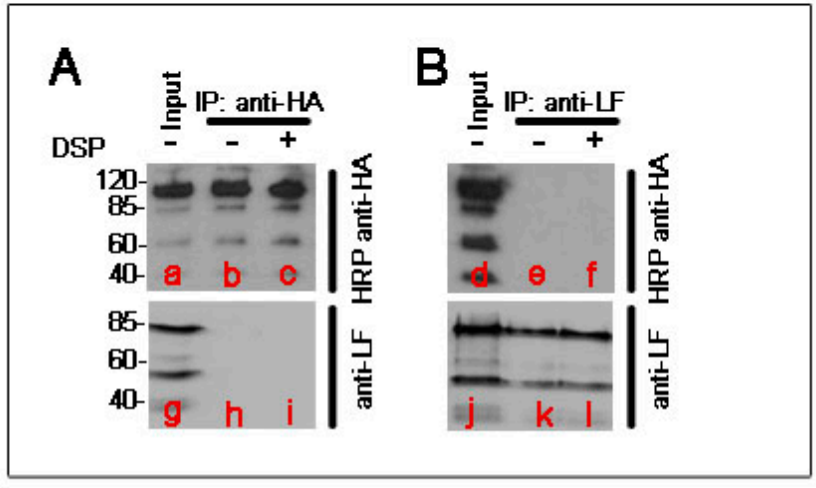
LRP-HA - bLF immunoprecipitation (IPP) and immunoblotting: (A) Whole-cell extracts were isolated from *lrp-ha* trophozoites exposed to bLF for 30 min. Immunoprecipitations of LRP-HA were carried out using anti-HA mouse mAb (SIGMA) and immunoblotted using HRP-labelled anti-HA mouse mAb (SIGMA) to detect LRP-HA or rabbit anti-LF polyclonal antibody (Fab Gennix) to detect bLF. (B) A reverse immunoprecipitation assay was conducted from *lrp-ha* trophozoites exposed to bLF for 30 min using the rabbit anti-LF polyclonal antibody and immunoblotted using the rabbit anti-LF polyclonal antibody to detect bLF or the HRP-labelled anti-HA mouse mAb to detect LRP-HA. IPP was performed as described by Krtková et al. 2017, using the DSP cross-linker. All experiments were repeated three times with different sets of samples and nonspecific IgGs were used as a negative control for immunoprecipitation. Input: *lrp-ha* trophozoites extracts exposed to bLF for 30 min.

Krtkova, J. *et al.* 14-3-3 Regulates Actin Filament Formation in the Deep-Branching Eukaryote Giardia lamblia. *mSphere* **2**, doi:10.1128/mSphere.00248-17 (2017).

Figure S3. Full Western blot scans for Supplementary Fig. 2.
The full scans for each blot are shown on the bottom panels.

Each sample is explained in the Fig. S2 and denoted with the letters a to i.

Fig S2



Full WB scans