*Supporting information*

# Characterization of a unique attachment organelle: Single-cell force spectroscopy of *Giardia duodenalis* trophozoites

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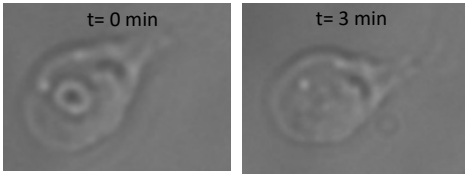


**Figure S1. Viability and reattachment capacities of FluidFM-released *G. duodenalis* trophozoites.** After FluidFM measurements, the individual *G. duodenalis* trophozoites were immediately released from the micropipette by positive pressure of the FluidFM. Trophozoites were either swimming in the liquid environment, or reattached to the glass surface when they were in close proximity to the material. Time chronology of a single trophozoite (500x total magnification), which reattached after release (indicated by the area of aspiration) from the FluidFM micropipette. Area of aspiration was quickly remodelled by the trophozoite, indicating its viability.

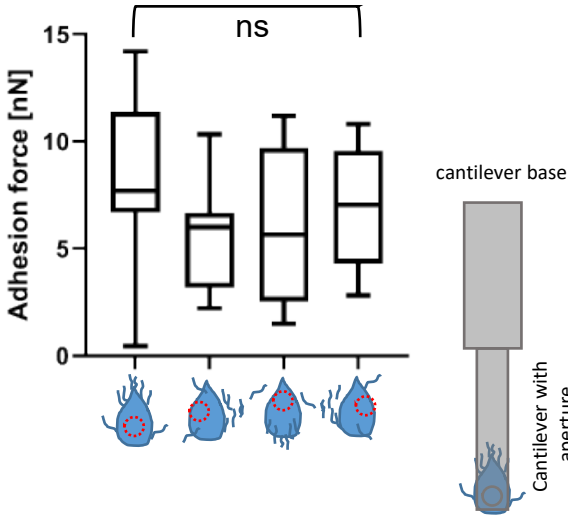


**Figure S2. Adhesion forces of *G. duodenalis* trophozoites from different pulling orientations.** (Left) Adhesion forces of adherent *G. duodenalis* trophozoites on glass-bottom cell culture dishes were tested by FluidFM technology. Schematic top view of settled trophozoites with different xy-orientations (*x-axis*) as they were approached by the aperture of the FluidFM micropipette (*red circles*). (Right) Schematic top view of the micropipette with the cantilever base and the cantilever with aperture. The aperture (*grey circle*) of the micropipette is at the bottom side of the micropipette. Exemplary micropipette positioning on a "down"-orientated trophozoite is shown. Trophozoite orientations from left to right: "down" (n=22), "left" (n=12), "up" (n=8), "right" (n=16). ns, not significant. Kruskal-Wallis test followed by multiple comparison analysis.

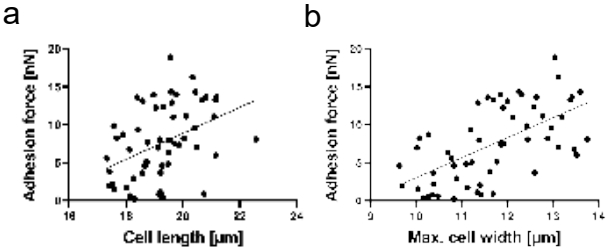


**Figure S3. Correlation between the adhesion force and cell length or width of *G. duodenalis* trophozoites.** Individual *G. duodenalis* trophozoites were seeded on glass-bottom cell culture dishes before they were probed for adhesion forces by FluidFM technology. Prior to the pulling and detachment of individual trophozoites by FluidFM micropipette, trophozoite lengths or widths were determined at 500x total magnification with Zeiss Axiovert 10 and ZEN lite software. Scatter dot plot with simple linear regression $R^2$ of (a) Adhesion force vs. maximum cell length (n=57, $R^2$=0.16) and (b) Adhesion force vs. cell width (n=58; $R^2$=0.37).

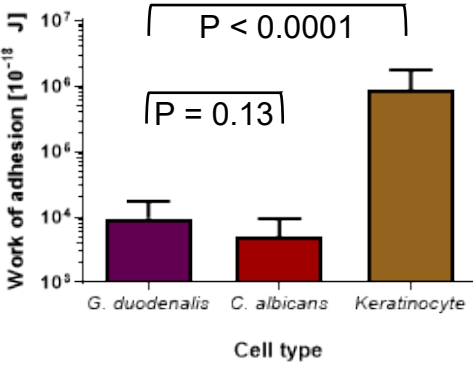


**Figure S4. De-adhesion work of *G. duodenalis* compared to *C. albicans* and human oral keratinocytes on glass.** Individual *G. duodenalis* trophozoites, *C. albicans* yeast cells and human oral keratinocytes were seeded on glass and were detached from the substratum by FluidFM. Depicted is the de-adhesion work of pooled *G. duodenalis* trophozoites (n=107), *C. albicans* yeast cells (n=20), and human oral keratinocytes (n=20) in a bar plot with standard deviation for each species. Kruskal-Wallis test followed by multiple comparison analysis.