



Supplementary Figure 1: *EhMSP-1* silencing reduced F-actin dot formation in HM-1:IMSS trophozoites. HM-1:IMSS empty vector control and *EhMSP-1* silenced trophozoites were incubated on a FN-coated surface for 30 minutes, followed by staining with Alexa 488-labeled Phalloidin to visualize F-actin. 19 Random images were taken for each cell type and the proportion of trophozoites forming F-actin dots in each image was recorded. Each point in the graph denotes data for a single image field. Error bar denotes SD and * denotes a p value of 0.003 (unpaired t test).

Supplementary Video 1: Dynamics of amoebic F-actin dots on a FN-coated surface. We incubated GFP-tagged LifeAct peptide expressing trophozoites on FN and then captured images in the GFP channel every 4 seconds for 5 minutes. The objective was focused on a plane adjacent to the glass surface. The video is a compilation of all the images, rendering at 10 frames per second.

Supplementary Video 2: Dynamics of amoebic F-actin dots in Matrigel. We mixed trophozoites expressing GFP-tagged LifeAct peptide with Matrigel and then imaged after one hour when the Matrigel solidified. Images in the GFP channel were taken every 2 seconds for 3 minutes. The video is a compilation of all the images, rendering at 5 frames per second.