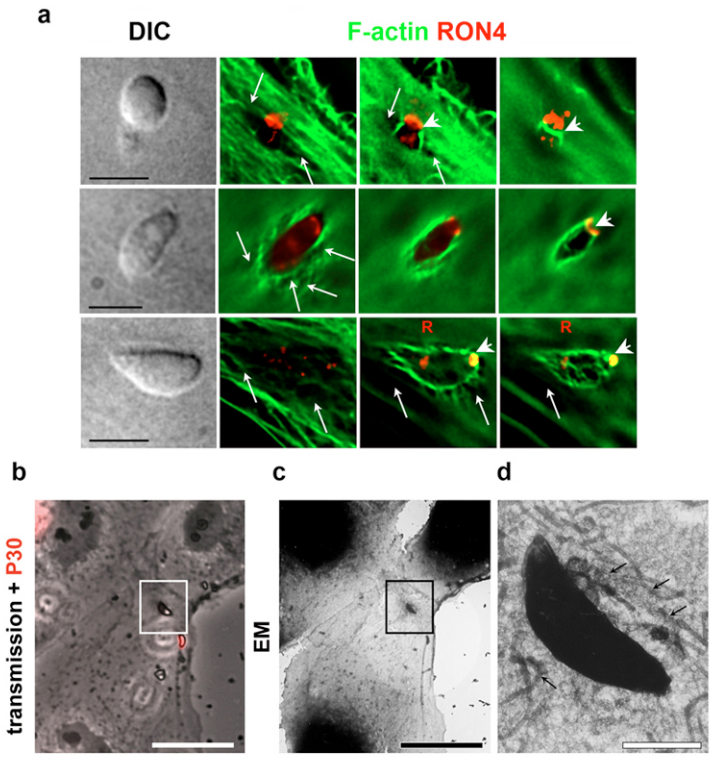

Fig. S1. Fluorescence microscopy and correlative fluorescence and transmission electron microscopy of invading and internalized tachyzoites in Ptk1 cells. (**A**) Confocal microscopy of host cell F-actin (green) at the site of entering parasites for 3 different planes. The RON4 labeling (red) delineates the zoite-cell junction (white arrowhead) and stains the rhoptries. Scale bar, 5 mm. (**B**) Overlay of P30 fluorescence image (red) and a phase contrast image. Scale bar, 30 mm. (**C**) Transmission electron micrograph of the same field of view as shown in b. (**D**) High resolution electron micrograph of fully-internalized tachyzoite identified by the absence of P30 fluorescence in b. Scale bar, 2 mm. Black arrows indicate the accumulation of endoplasmic reticulum in the vicinity of the parasite.

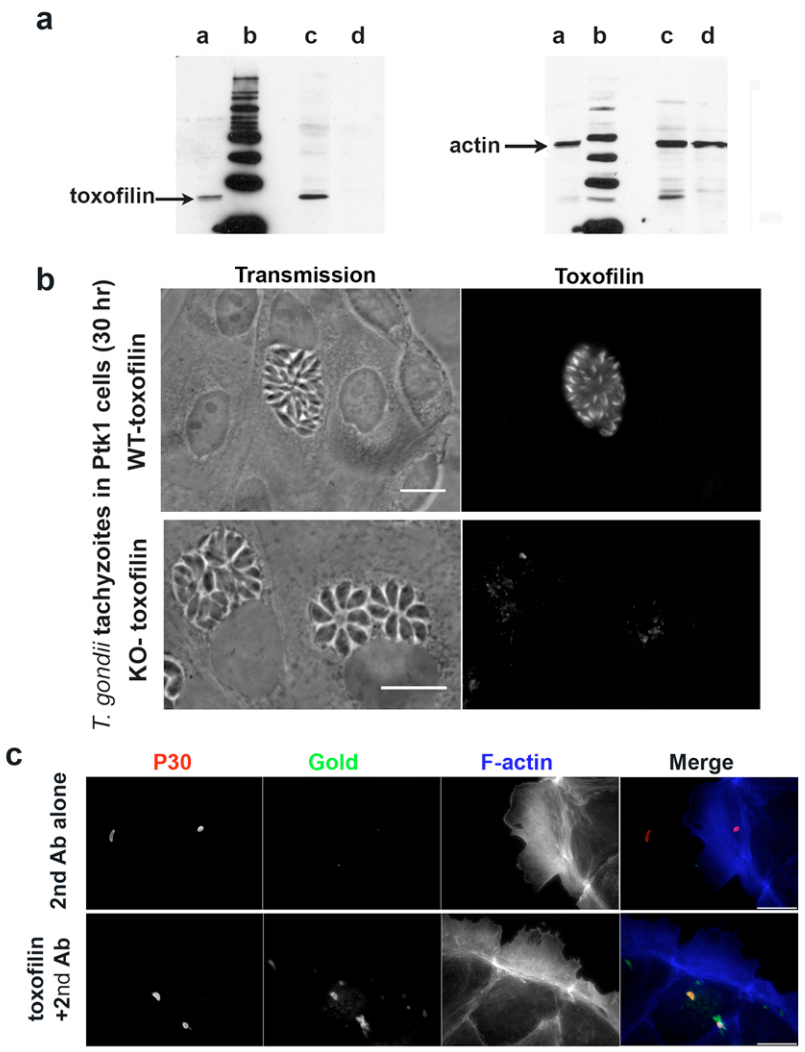

Fig. S2. Toxofilin signals visualized by immunofluorescence and immunogold microscopy are specific. (A) Western blot of total extract of free WT-toxofilin tachyzoites (10^6) (lane a) and HFF cells (~2×10^5) infected with WT-toxofilin (lane c) and KO toxofilin (lane d) tachyzoites; lane b presents the molecular weight markers. The membrane was probed first using the anti-toxofilin antibodies (left panel) and then anti-Tg actin antibodies (right panel) **(B,C)** Invasion assay was performed on PtK1 cells. (B) Toxofilin was detected using anti-toxofilin antibodies followed by Alexa Fluor 488. Scale bar, 10 mm. (C) Cells were prepared as for immunogold EM experiment. Extracellular tachyzoites were detected using anti-P30 antibodies and Alexa Fluor 568 anti-mouse secondary antibodies (shown in red in the merge column). Toxofilin was detected using anti-toxofilin antibodies followed by Alexa Fluor 488-15 nm gold anti-rabbit antibodies (shown in green in the merge column). In control, secondary Alexa Fluor 488-15 nm gold anti-rabbit antibodies were used alone without anti-toxofilin. F-actin was visualized with Alexa Fluor 350-phalloidin. Scale bar, 20 mm.

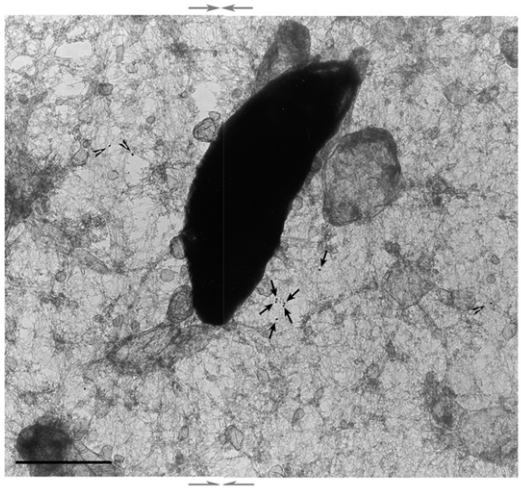

Fig. S3. A larger field of view than that the one presented in Fig. 3A-E showing gold labeling of toxofilin at the onset of Ptk1 cell invasion by a tachyzoite. The figure is reconstituted from 2 separate electron microscopy micrographs (see grey arrows at the edge of the figure) and shows that toxofilin secretion is specifically localized in the vicinity of the parasite. Scale bar, 1 mm. The view is rotated by 20° counterclockwise from the view in Fig. 3A,B. The arrows point to the gold labels depicted in Fig. 3A-E. The three additional black arrowheads point to gold labels outside the field of view shown in Fig. 3.

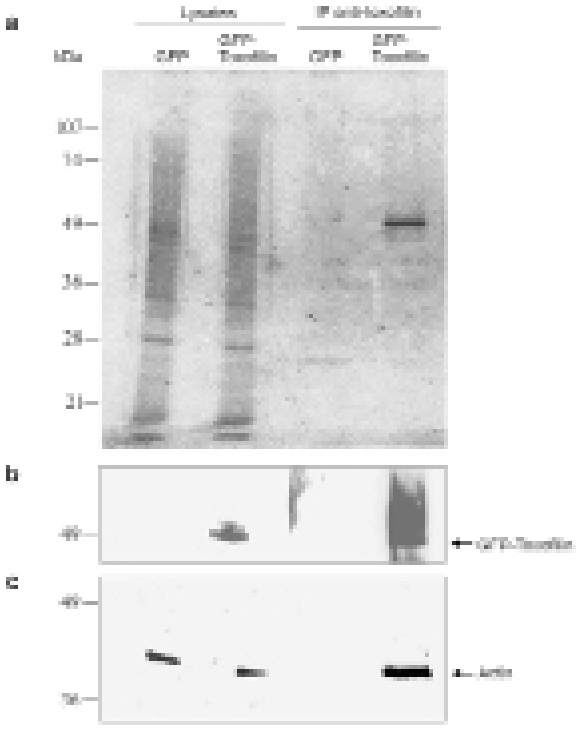

Fig. S4. Toxofilin is phosphorylated when expressed in cells. HeLa cells were transfected with GFP alone or GFP-toxofilin and labeled with ^{32}P. Toxofilin was immunoprecipitated from cell lysates. (**A**) Radioactive scan of whole cell lysates and immunopreciptation eluates. (**B**) Western blot using anti-Toxofilin. (**C**) Western-blot using anti-actin.

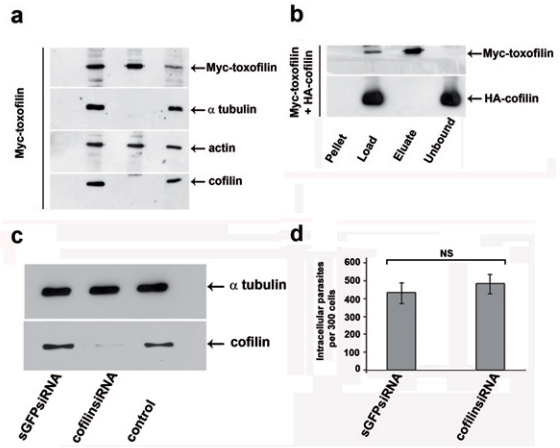

Fig. S5. Toxofilin upregulates actin turnover independently of host cell cofilin. HEK293 cells were transfected with myc-toxofilin **(A)**, or with both myc-toxofilin and HA-cofilin **(B)** to detect putative partners. Lysates were subjected to immunoprecipitation using anti-myc antibodies. (A) Immunoprecipitation of toxofilin does not pull down endogenous cofilin, nor tubulin, but co-purifies with actin as expected. (B) Ectopically expressed HA-cofilin is not co-precipitating with myc-toxofilin. **(C)** HeLa cells were specifically silenced for cofilin as controlled using western blot, and **(D)** invasion assays were performed on control and silenced cells: no significant differences were observed in the number of infected cells (P>0.05, Student t-test).