Quantification of levels of MIC2 in the secreted fractions upon A23187 stimulation in the RHΔhx parental and TKO parasites in 3 independent biological replicates. For each sample, the MIC2 secretion is normalized against tubulin in the pellet from the same sample.

Parasite strain	replicate 1	replicate 2	replicate 3	average ± *SEM*
RHΔ*hx*	0.30	0.44	0.68	0.48±0.14
TKO	0.21	0.31	0.42	0.31±0.07

Fig. S1. (Blot Transparency)

Top: Image of Western blot that contains data displayed in Fig 6C. Western blot of the secreted (supernatant) and unsecreted (pellet) fractions of RH*Δhx* and TKO parasites with (+) or without (-) A23187 treatment. The blot was probed by antibodies against MIC2 and tubulin. Lanes for molecular weight ladders are included.

Bottom: Quantification of levels of MIC2 in the secreted fractions upon A23187 stimulation in the RHΔ*hx* parental and TKO parasites in 3 independent biological replicates.

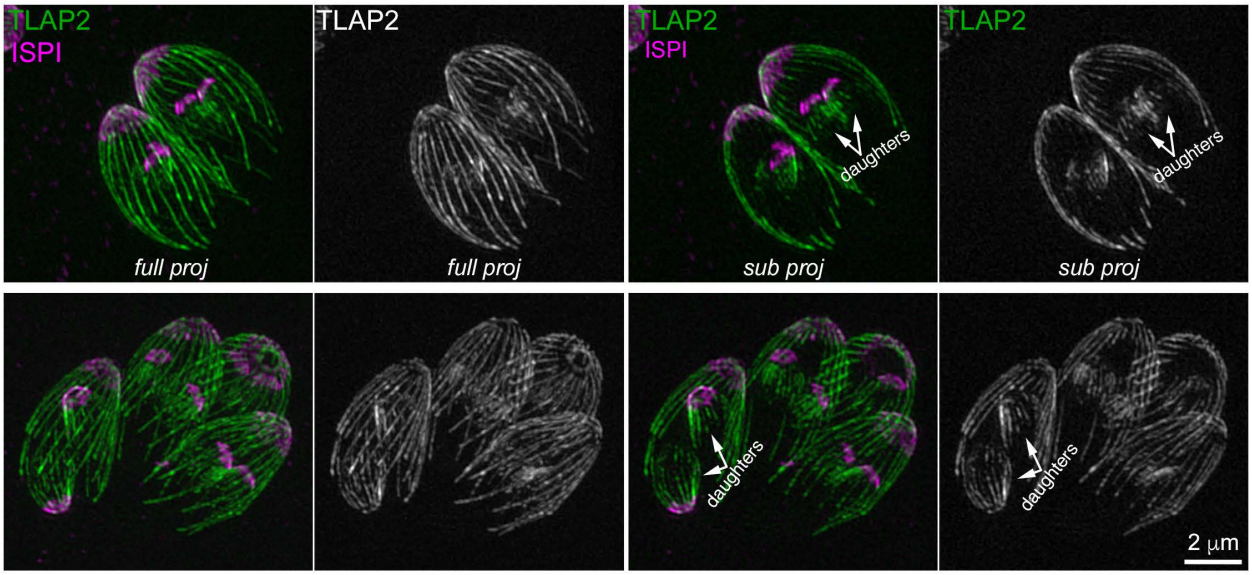

Fig. S2. Full and subset projections of 3D-SIM image stacks of dividing intracellular *mEmeraldFP-TLAP2* knock-in parasites showing that TLAP2 is recruited to the daughter cortical microtubules. Green and grayscale: mEmerald-FP-TLAP2. Magenta: apical cortex labeling with a mouse anti-ISP1 antibody. Parasites at an earlier (top) and a later stage (bottom) of daughter formation are shown.

Table S1. Numbers of data points

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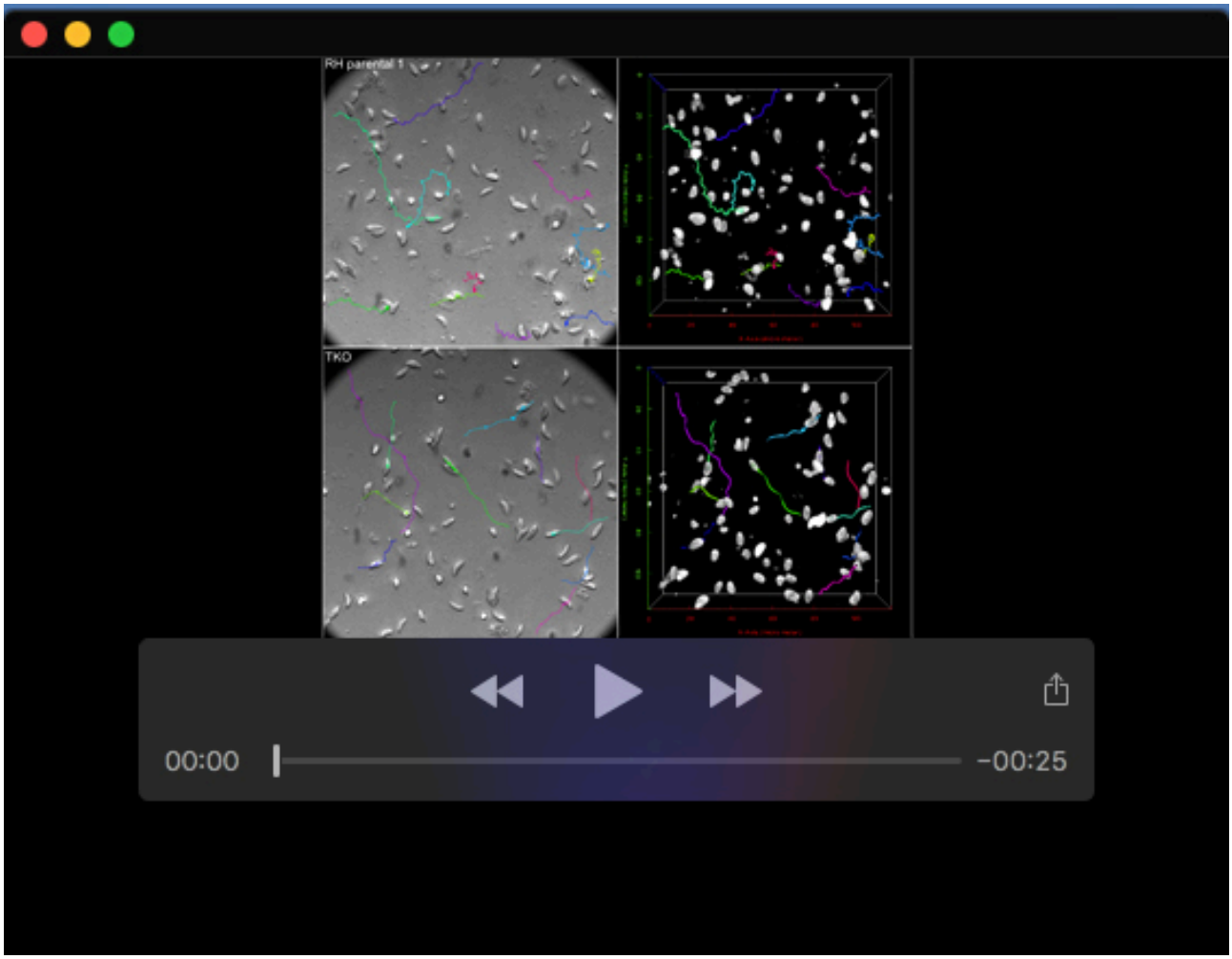
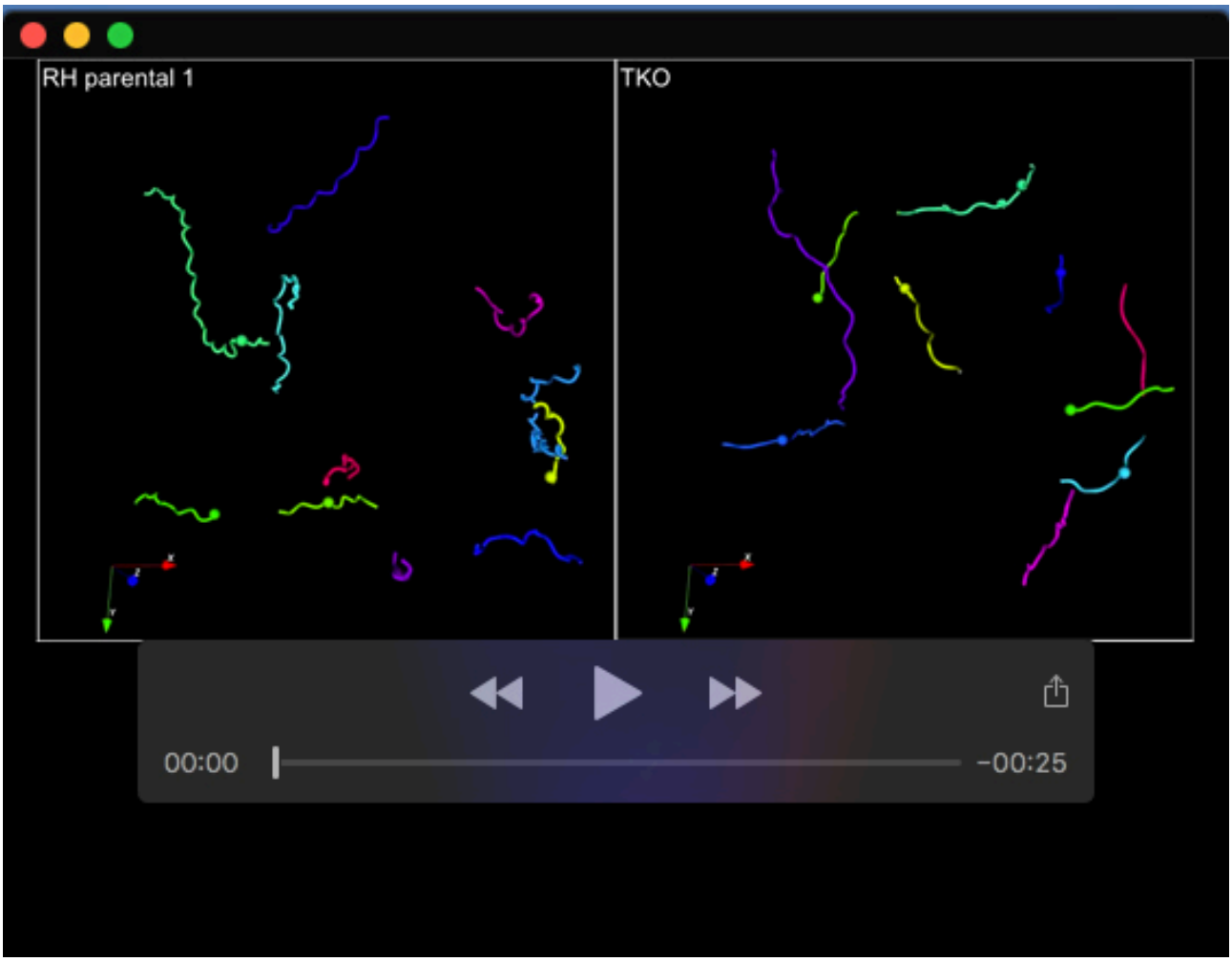
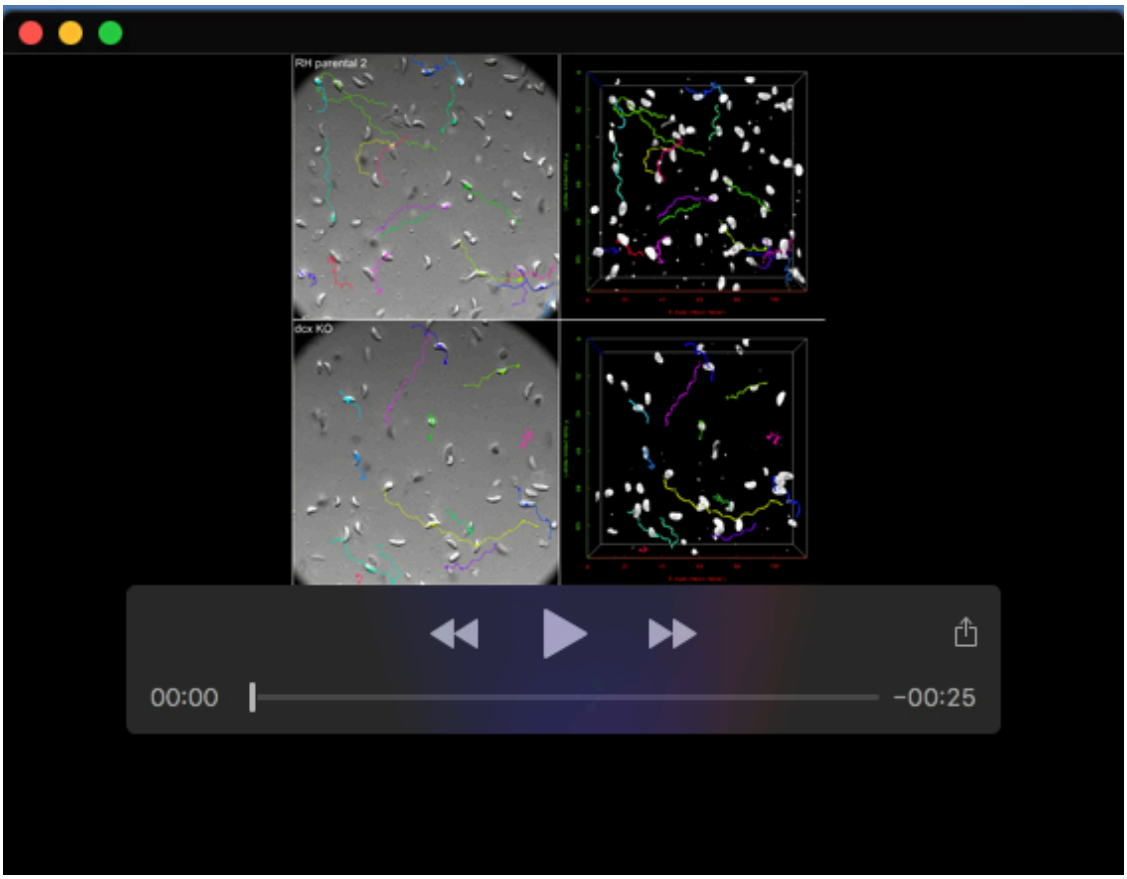


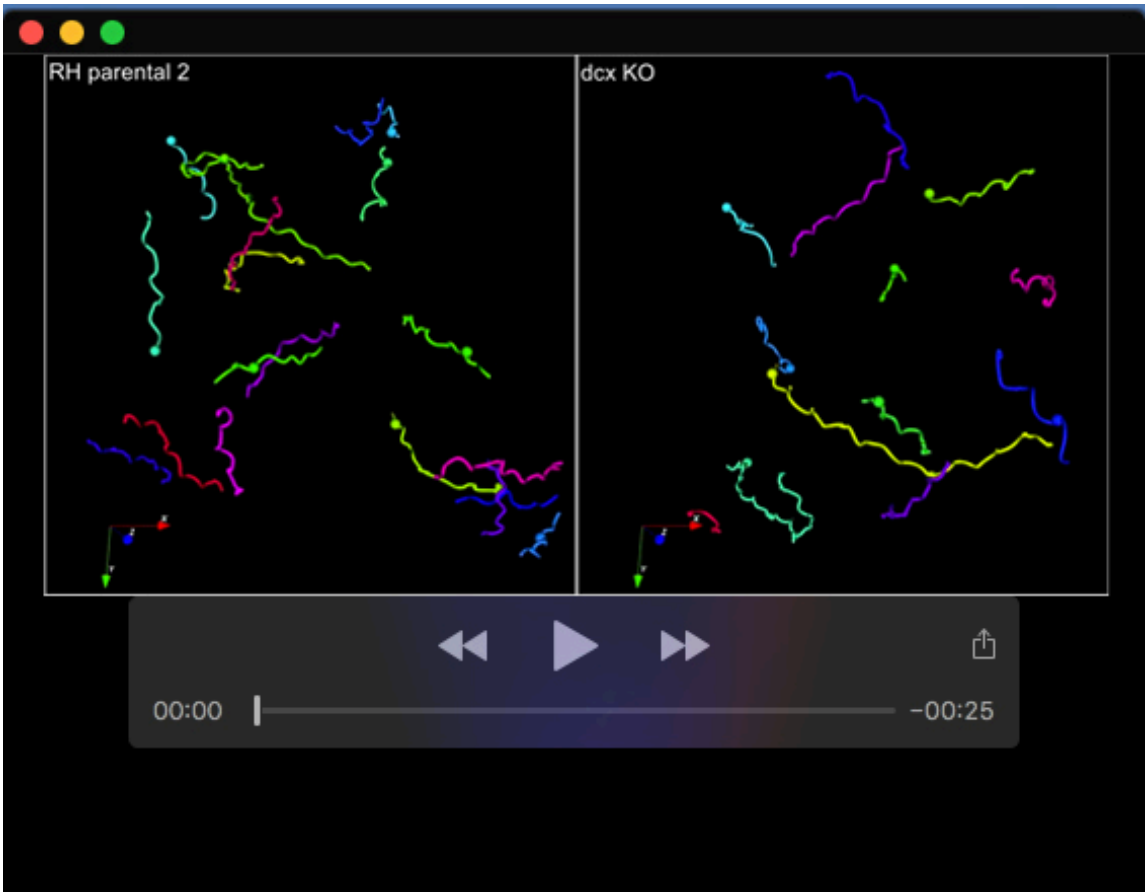
Movie 1. Time-lapse microscopy of 3-D motility in 50% Matrigel for the RH*Δhx* (RH parental 1) and TKO parasites. *Left:* Videos of projected DIC images (projections of 3-D stack generated by Stack focuser in ImageJ/Fiji). *Right:* Corresponding processed images with a subset of 3-D trajectories. Time interval: ~ 1.36 sec. Video speed: 10 frames/s.



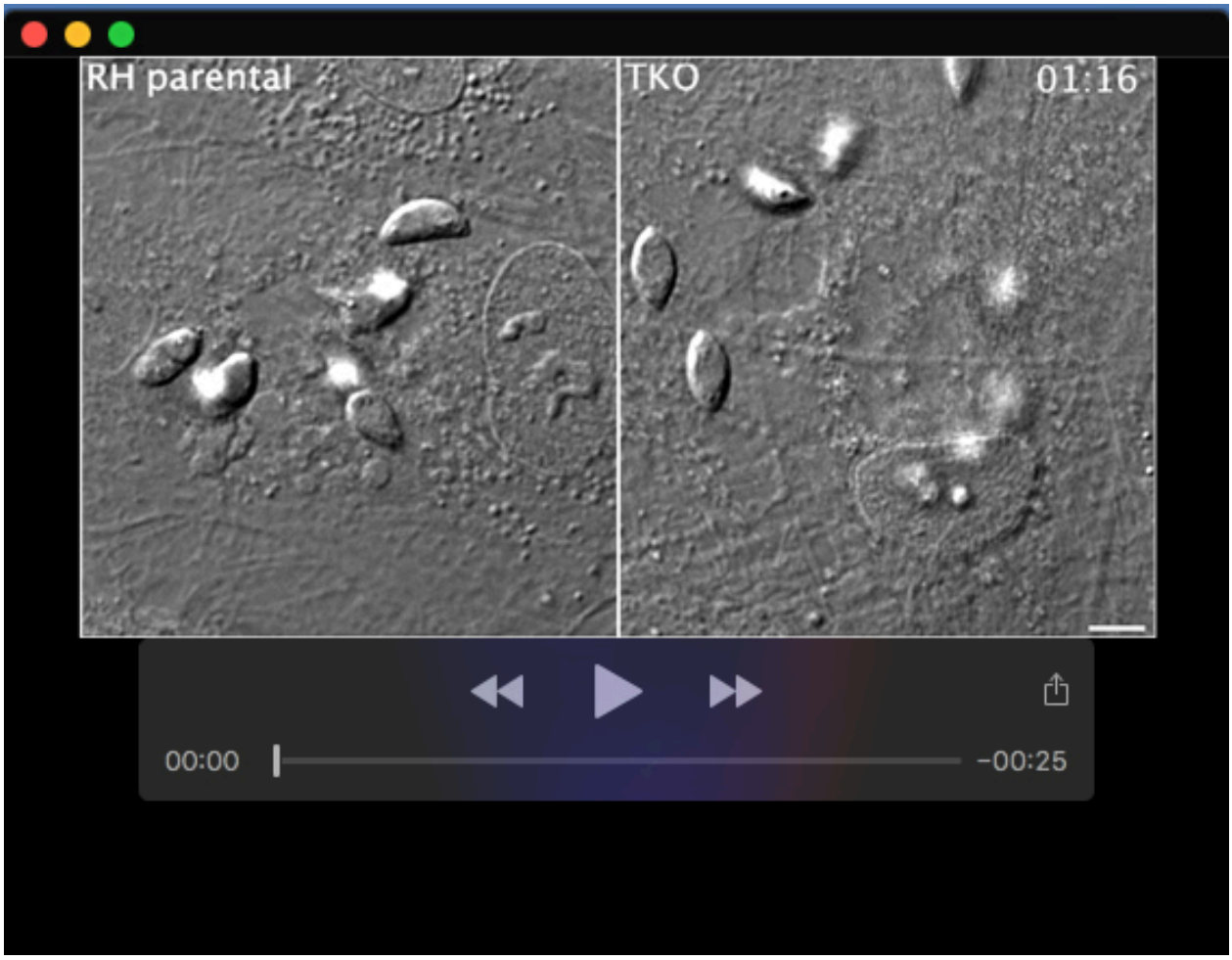
Movie 2. Rotational view of 3-D trajectories for the RH*Δhx* (RH parental 1) and TKO parasites included in Video S1. The traveling sphere associated with each track represents the centroid of the parasite traveling along that path. Length of arrow= 16 μm.



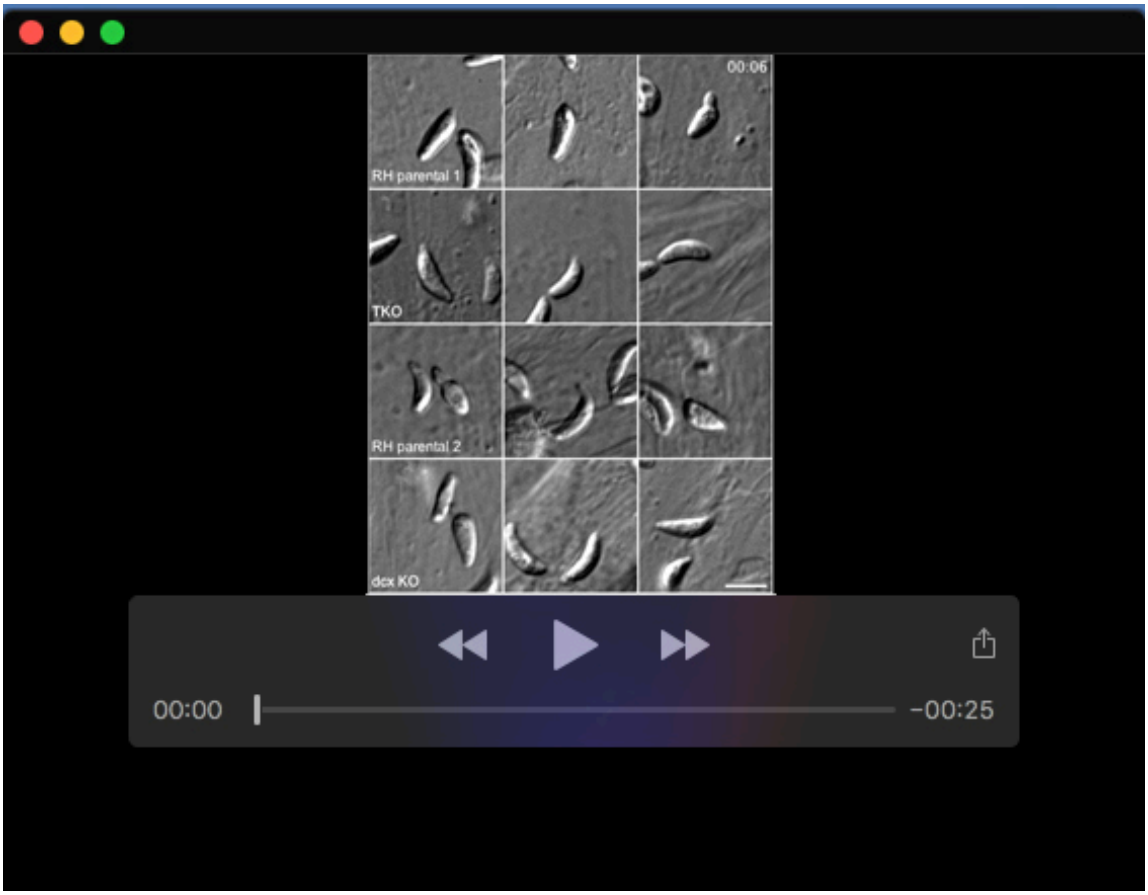
Movie 3. Time-lapse microscopy of 3-D motility in 50% Matrigel for the RH*Δku80* (RH parental 2) and *Δdcx* (*dcx KO*) parasites. *Left:* Videos of projected DIC images (projections of 3-D stack generated by Stack focuser in ImageJ/Fiji). *Right:* Corresponding processed images with a subset of 3-D trajectories. Time interval: ~ 1.36 sec. Video speed: 10 frames/s.



Movie 4. Rotational view of 3-D trajectories for the RH*Δku80* (RH parental 2) and *Δdcx* (*dcx KO*) parasites included in Video S3. The traveling sphere associated with each track represents the centroid of the parasite traveling along that path. Length of arrow= 16 μm.



Movie 5. Time-lapse microscopy of A23187 induced-egress for RH*Δhx* (RH parental) and TKO parasites. A23187 was added at the beginning of the movies to a final concentration of 5 µM. The intracellular wild-type and TKO parasite cultures were placed at 7°C for 1 hr prior to the egress experiment, which was conducted at 37°C. Time interval: 1 sec. Video speed: 60 frames/s. Scale bar: 5 µm.



Movie 6. Time-lapse microscopy of host cell entry for RH*Δhx* (RH parental 1), TKO and RH*Δku80* (RH parental 2) and *Δdcx* (*dcx KO*) parasites. Fig 6F includes images selected from the timelapses in the leftmost column. Time interval: 1 sec. Video speed: 6 frames/s. Scale bar: 5 µm.