1 Transfected *Naegleria gruberi* was incubated in selection media for seven days at 28°C, in 2 or 4 well Permanox chamber slides (Sigma Aldrich C6682/6932).

2 For mitochondrial observation, Mitotracker Red CMXROS (ThermoFisher Scientific M7512) was added to wells containing *N. gruberi* to reach a final concentration of 200nM and incubated for a further full hour.

3 Media was then aspirated from the wells and replaced with a room temperature PBS wash. The PBS wash was removed and replaced a further two times.

4 After the final PBS wash, freshly prepared paraformaldehyde at room temperature and a w/v concentration of 3% was added to the wells and incubated in fume cupboard for 15 minutes.

5 The parasformaldehyde was then removed and replaced with a further 3 rounds of PBS wash and one final wash with
6 The chambers were then removed from the slide and dried by gently tapping against an absorbent material such as laboratory tissue paper.

7 Mounting media containing DAPI (Sigma Aldrich F6057) was then added to the slides, appropriate cover slips mounted and sealed with nail varnish before visualisation on a fluorescence microscope.