Supplemental Materials

Molecular Biology of the Cell

Heaslip et al.

Supplemental File.

Supplemental Figure Legends.

Figure S1. SAG1ΔGPI-GFP and anti-Gra6 co-localize to the dense granules. Fluorescence image of an extracellular parasite showing co-localization between SAG1ΔGPI-GFP and dense granule protein anti-Gra6. Right panels are 3x magnification of area in the white box. *Green:* SAG1ΔGPI-GFP. *Red:* anti-Gra6. *Cyan:* anti-GAP45 to highlight the parasite periphery.

Figure S2. Analysis of dense granule motions using MSD. (A) Fluorescence image of parasites with GFP labeled dense granules (green) and mCherryFP labeled microtubules (red). Yellow arrow indicates region of the parasite used to make Kymograph. Kymograph, which depicts the spatial position (x-axis) along the trajectory of a granule over time (y-axis), shows a dense granule moving bidirectionally towards the apical (A) and then basal (B) end of the parasite. mCherryFP-Tubulin expression allows the unambiguous identification of the parasites apical end (white arrow heads). (B) Example trajectories and MSD plots on a log-log axes (large graphs) and linear axes (insets) for stationary (red), diffusive-like (blue) and directed (green) dense granule trajectories. Diffusive exponent, α, which is the slope of the log-log MSD indicated. (C) Bar chart indicates directionality of dense granule motions.

Figure S3. Creation and analysis of LoxP-Actin parasite lines. (A) Strategy for creating *LoxP-Actin* and conditional *ΔActin* parasite lines. Adapted from Andenmatten et al 2013. (B) Fluorescence image of untreated and rapamycin treated *LoxP-Actin* parasites. 20% of vacuoles contained at least one parasite (white arrow head) that contain no dense granules. *Green:* YFP. *Red:* SAG1ΔGPI-mCherryFP. *Cyan*: anti-actin.

Figure S4. Protein sequence analysis of TgMyoF. (A) Alignment of TgMyoF (EPT25279.1) with Mouse Myosin Va (NP_034994.2), Myosin VI (Q9UM54.4), Myosin VII (XP_644171.1), Myosin X (CAB56466.2) motor domains. 3 unique inserts in TgMyoF motor domain are highlighted with an orange box. Unique insert in Myosin VI (insert 1) is highlighted with a blue box. P-loop, Loop1 and Switch 1, conserved sequences that determine the kinetics of nucleotide binding to and release from the motor's active site, are highlighted with red boxes. NCBI accession numbers are shown in brackets. (B) Alignment of the IQ motifs of TgMyoF and Myosin Va. Each IQ motifs are highlighted with a green box. (C) Prediction of coiled-coiled regions in TgMyoF and Myosin Va. Green, blue and red lines indicates scan region of 14, 21 and 28 residues respectively.

Figure S5. Creation of LoxP-TgMyoF. Schematic of TgMyoF genomic locus in parental and *LoxP-TgMyoF* parasites. Primer binding sites (F1/F2 and R1/R2) are indicated. (B) Genomic PCR analysis of parental and *LoxP-TgMyoF* parasites lines. (C) Fluorescence image of control and rapamycin treated *LoxP-TgMyoF* parasites indicating accumulation of the microneme protein AMA-1 in the residual body upon loss of functional TgMyoF. Residual body indicated with *. *Green:* TgMyoF-EmeraldFP. *Red:* anti-GAP45 highlighted parasite periphery. *Cyan*: anti-AMA-1 for visualizing the micronemes.

Figure S6. eGFP-TgMyoF localizes to the parasite cytosol. Fluorescence image of intracellular parasites ectopically expressing eGFP-TgMyoF. *Green:* eGFP-TgMyoF. *Red:* anti-GAP45 to highlight parasite periphery.

Figure S7. *TgMyoF-ΔCT* parasites have a replication defect. (A) Immunofluorescence images of parental (top), *LoxP-TgMyoF* (middle) and *TgMyoF-ΔCT*/apicoplast positive (bottom) parasites 40 hours after host cell invasion. Parasites were stained with anti-GFP/Alexa 488 conjugate (left), anti-HSP60 as a marker for the apicoplast (pink) and IMC1 (blue) to highlight the parasite periphery (right). (B) Comparison of the intracellular growth of parental, *LoxP-TgMyoF*, *TgMyoF-ΔCT*/apicoplast-positive and *TgMyoF-ΔCT*/apicoplast-negative parasite strains 40 hours after infection. In both parental and *LoxP-TgMyoF* parasite lines 60% of parasite vacuoles contain 32 or more parasites. Both *TgMyoF-ΔCT* populations have a severe replication defect and vacuoles containing ≥32 parasites were never observed, and less than 10% contained 16 parasites/vacuole. In each case ~25% of vacuoles contained "odd" numbers of parasites (i.e. – parasite number per vacuole was not an integral to the power of 2) and are disorganized within the vacuole (A; bottom panel). Thus the replication defect was equally severe in *TgMyoF-ΔCT /apicoplast positive* and *TgMyoF-ΔCT /apicoplast negative* parasites indicating that loss of apicoplast inheritance contributes to but cannot fully account for the replication defect observed in the *TgMyoF-ΔCT* parasites. Thus, other defects associated with loss of TgMyoF, including but not limited to dense granule trafficking likely also contributes to the lethality of this parasite line.

Supplemental Movie Legends.

Movie S1. Dense granule dynamics in intracellular *T. gondii* parasites expressing SAG1ΔGPI-GFP. Imaging speed 10fps, playback 6x real time.

Movie S2. Dense granule dynamics in intracellular *T. gondii* parasites expressing SAG1ΔGPI-GFP. Microtubules were depolymerized with oryzalin *(left)*, actin was depolymerized with cytochalasin D *(middle)* and actin was stabilized with jasplakinolide *(right)*. Imaging speed 10fps, playback 6x real time.

Movie S3. Dense granule dynamics in untreated *(left)* and rapamycin (*right)* treated *LoxP-Actin* parasites expressing SAG1ΔGPI-mCherryFP. Imaging speed 10fps, playback 6x real time.

Movie S4. Dense granule dynamics in untreated *(left)* and rapamycin (*right)* treated *LoxP-TgMyoF* parasites expressing SAG1ΔGPI-mCherryFP. Imaging speed 10fps, playback 6x real time.

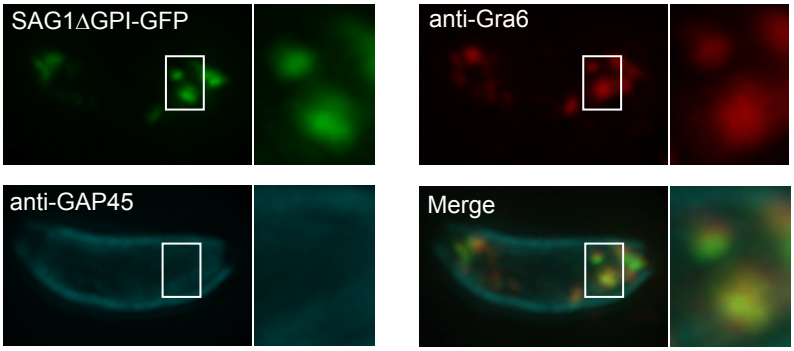
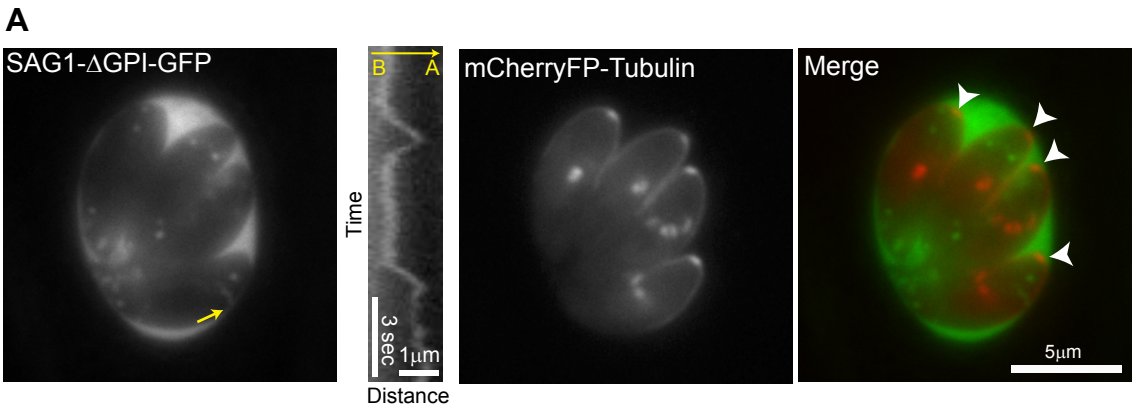
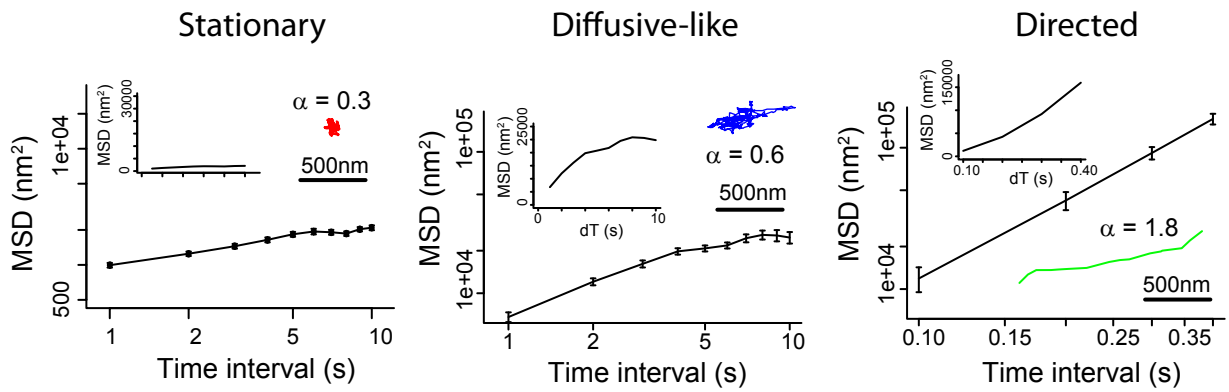


Figure S1


A
SAG1-ΔGPI-GFP
B
A
Time
3 sec
1μm
Distance
mCherryFP-Tubulin
Merge
5μm


B
Stationary
Diffusive-like
Directed
MSD (nm²)
α = 0.3
500nm
Time interval (s)
α = 0.6
dT (s)
α = 1.8

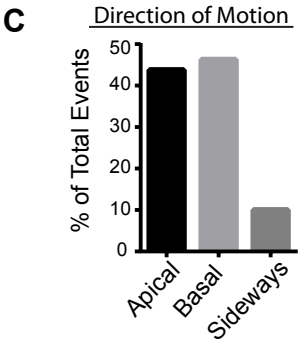

C
Direction of Motion
% of Total Events
Apical
Basal
Sideways

Figure S2

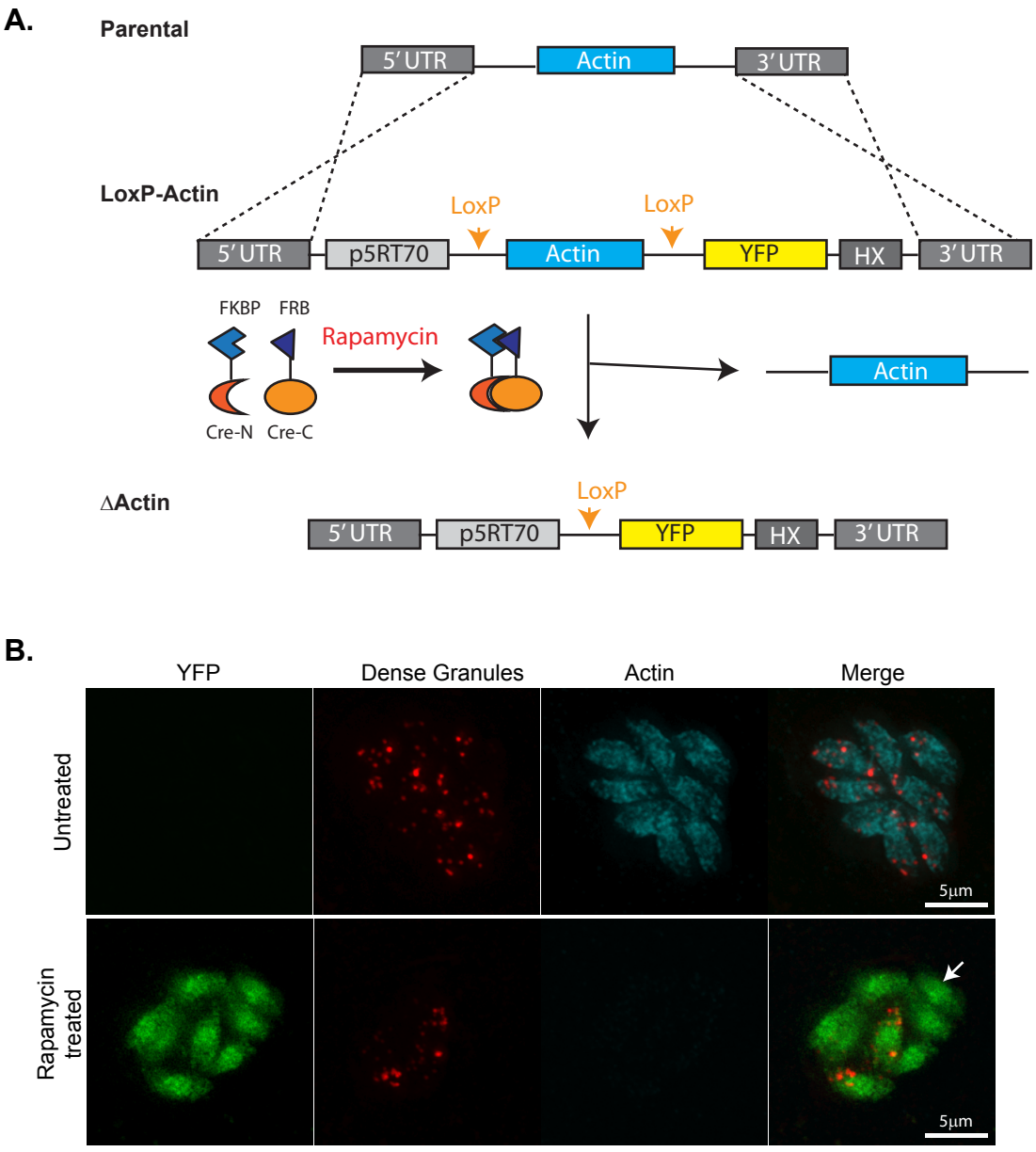
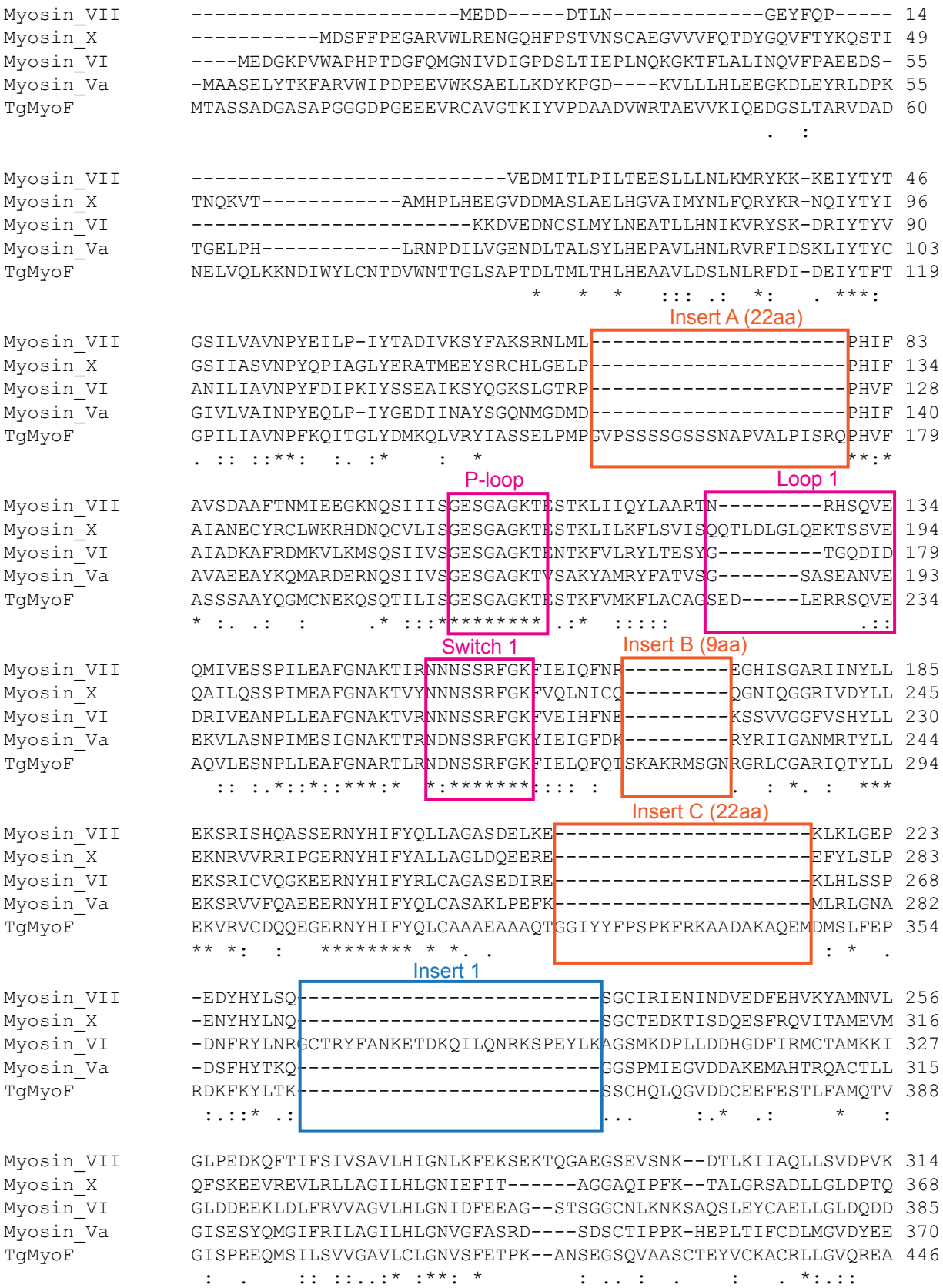

A.
Parental
5′ UTR
Actin
3′ UTR
LoxP-Actin
LoxP
LoxP
5′ UTR
p5RT70
Actin
YFP
HX
3′ UTR
FKBP
FRB
Rapamycin
Cre-N
Cre-C
Actin
ΔActin
LoxP
5′ UTR
p5RT70
YFP
HX
3′ UTR
B.
YFP
Dense Granules
Actin
Merge
Untreated
Rapamycin treated
5µm
5µm

Figure S3

A


Insert A (22aa)
P-loop
Loop 1
Switch 1
Insert B (9aa)
Insert C (22aa)
Insert 1

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Myosin_VII      LETCLTIRHVLI-----RGQNFVIPLKVNEAEDTRDSLAKALYGNVFNWLVVFINS---- 365
Myosin_X        LTDALTQRSMIL-----RGEEILTPLSVQQAVDSRDSLAMALYARCFEWVIKKINS---- 419
Myosin_VI       LRVSLTTRVMLTTAGGTKGTVIKVPLKVEQANNARDALAKTVYSHLFDHVVNRVN----- 440
Myosin_Va       MCHWLCHRKLAT-----ATETYIKPISKLQATNARDALAKHIYAKLFNWIVDHVNQ---- 421
TgMyoF          LQEAMCYRTIKT-----AHESYRKPLKTDEAWEMKDALCRALYGCLFLQVVAKTNASIGY 501
                :   :  * :             *:.  :* : :*:*.  :*.  *  ::   *

Myosin_VII      --KIHKPQKNSTFIGVLDIFGFENFKKNSFEQFCINFANEKLQQHFNQHIFKLEQEEYEK 423
Myosin_X        --RIKG-KDDFKSIGILDIFGFENFEVNHFEQFNINYANEKLQEYFNKHIFSLEQLEYSR 476
Myosin_VI       --QCFPFETSSYFIGVLDIAGFEYFEHNSFEQFCINYCNEKLQQFFNERILKEEQELYQK 498
Myosin_Va       --ALHSAVKQHSFIGVLDIYGFETFEINSFEQFCINYANEKLQQQFNMHVFKLEQEEYMK 479
TgMyoF          LKEVQSADDLLLFCGVLDIFGFECFQFNSFEQLCINFTNERLQNFFNTFVFKCEEELYRA 561
                             *:*** *** *: * ***: **: **:**: **  ::. *:  *

Myosin_VII      EKINWSKIVYNDNQECLDLIEKRPLGILSLLDEESRFPQATDLTYLDKLHTNHEKH-PYY 482
Myosin_X        EGLVWEDIDWIDNGECLDLIEKK-LRLLALINEESHFPQATDSTLLEKLHSQHANN-HFY 534
Myosin_VI       EGLGVNEVHYVDNQDCIDLIEAKLVGILDILDEENRLPQPSDQHFTSAVHQKHKDH-FRL 557
Myosin_Va       EQIPWTLIDFYDNQPCINLIESK-LGILDLLDEECKMPKGTDDTWAQKLYNTHLNKCALF 538
TgMyoF          EGIQWNPLDFPDNADCVALLQEKPLGLFSMLDEECMVPAGKDRGFNNKVCQKHGGHKRFG 621
                * :    : : **  *: *:: : : :: :::**  .*  .*    . :   *  :

Myosin_VII      EKPRRS----------KNTFVVKHYAGEVHYDTQGFLDKNKDTVSDDLSSLLQGSKSKFI 532
Myosin_X        VKPRVA----------VNNFGVKHYAGEVQYDVRGILEKNRDTFRDDLLNLLRESRFDFI 584
Myosin_VI       TIPRKSKLAVHRNIRDDEGFIIRHFAGAVCYETTQFVEKNNDALHMSLESLICESRDKFI 617
Myosin_Va       EKPRMS----------NKAFIIKHFADKVEYQCEGFLEKNKDTVFEEQIKVLKSSKFKML 588
TgMyoF          VIKTKP-----------NCFVVHHFAGSVEYCSDGFLEKNKDQLSVDLQEAVKASTIPFV 670
                     .           : * ::*:*. * *    :::**.* .  .  . :  *   ::
                                                Loop 2
Myosin_VII      IELFTPP----------------------REEGDDSDKGREKKKTTAGQTFKTQLQSLIN 570
Myosin_X        YDLFEHVS---------------------SRNNQDTLKCGSKHRRPTVSSQFKDSLHSLMA 624
Myosin_VI       RELFESS----------------------TNNNKDTKQKAGKLSFISVGNKFKTQLNLLLD 656
Myosin_Va       PELFQDDEKAISPTSATSSGRTPLTRVPVKPTKGRPGQTAKEHKKTVGHQFRNSLHLLME 648
TgMyoF          SNLFSAFL---------------------NRGTAEDGSGKKRKFVTVSSEFREQLGALME 709
                 :**                         .          :    :..  *: .*  *:

Myosin_VII      ILSSTQPHYVRCIKPNTTKEPAVYDRELIQAQLRYAGMMETIRIRKLGYPIRHTHKEFRD 630
Myosin_X        TLSSSNPFFVRCIKPNTQKMPDQFDQVVVLNQLRYSGMLETVRIRKAGYAVRRPFQDFYK 684
Myosin_VI       KLRSTGASFIRCIKPNLKMTSHHFEGAQILSQLQCSGMVSVLDLMQGGYPSRASFHELYN 716
Myosin_Va       TLNATTPHYVRCIKPNDFKFPFTFDEKRAVQQLRACGVLETIRISAAGFPSRWTYQEFFS 708
TgMyoF          TVDKTAPHFIRCIKPNPQNLPDLFDRATVNEQLRYGGVLQAVQVSRAGYPVRLSHRDCFF 769
                 :  : . ::******    .  ::      **:  *::..: :   *:. * ..::

Myosin_VII      RYLILD----------YRARSTDHKQTCAGLINLLSGTGGLER---------DEWQLGNT 671
Myosin_X        RYKVLMR---------NLALPDDIRGKCTVLLQVYDASN-------------SEWQLGKT 722
Myosin_VI       MYKKYMP---------DKLARLDPRLFCKALFKALGLNE-------------NDYKFGLT 754
Myosin_Va       RYRVLMK---------QKDVLGDRKQTCKNVLEKLILDK-------------DKYQFGKT 746
TgMyoF          DYRALADGALAAQLSQGTETPEAWRGRAEALLRHLDEKLKLDRRKKETDSPDRTWAVGKT 829
                 *                      :  .  ::.                    : .* *

Myosin_VII      KVFIR 676
Myosin_X        KVFLR 727
Myosin_VI       KVFFR 759
Myosin_Va       KIFFR 751
TgMyoF          LCFFK 834
                  *::
```

B

```
                                                IQ-1
TgMyoF      KTLCFFKNEAYEILSANLMSVRVAAATAIEARYKCFVQRRFFLMYRQTVV 877
MyoVa       KTKIFFRAGQVAYLEKLRADKLRAACIRIQKTIRGWLLRKRYLCMQRAAI 794
            **  **:      *.    .   **.  *:   : :: *: :*  ::::.:
                IQ-2                IQ-3
TgMyoF      FLQSHIRMFLCKLEAQRLRESRAARRVENFMRGAVARLRYLRTLENIRRI 927
MyoVa       TVQRYVRGYQARCYAKFLRRTKAATTIQKYWRMYVVRRRYKIRRAATIVI 844
             :* ::* : .:  *: **.::**  :::: *  *.* **         *
                IQ-4                IQ-5
TgMyoF      QAAWRGKQTRSQLRDRKLEEAASKIQAFWKMHKQRMFYTNLKKASTIAQL 977
MyoVa       QSYLRGYLTRNRYRKILREYKAVIIQKRVRGWLARTHYKRTMKAIVYLQC 894
            *:  **  **.: *.  *  *  **   :    * .*.  ** .  *
                IQ-6
TgMyoF      KWKRILARRMLRRLR 990
MyoVa       CFRRMMAKRELKKLK 907
             ::*::*:* *::*:
```

C

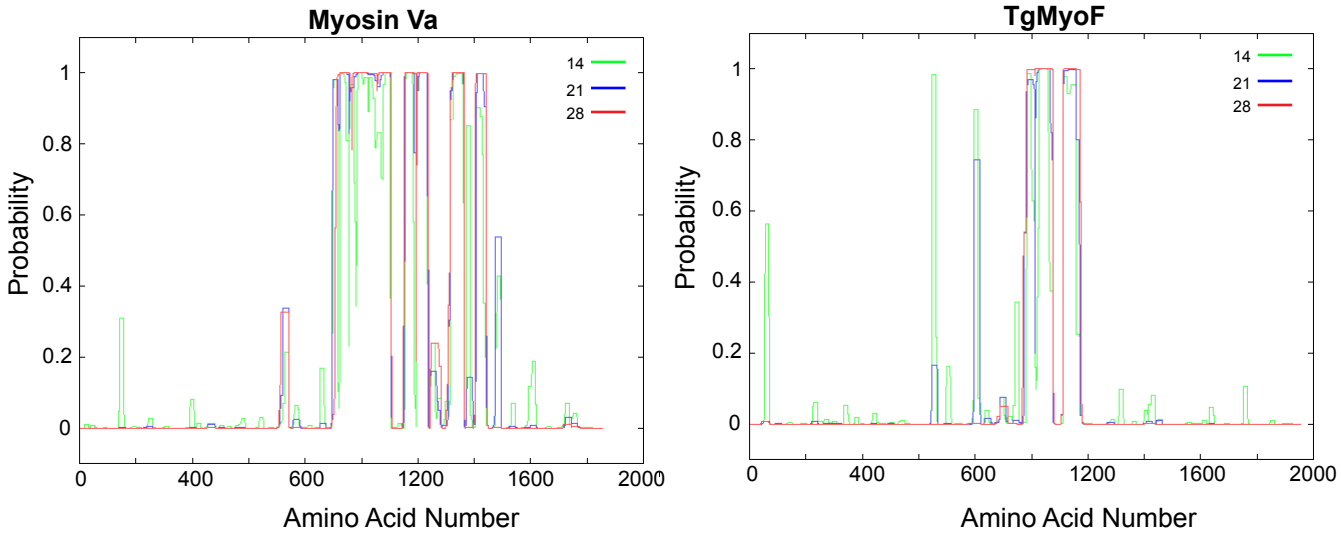
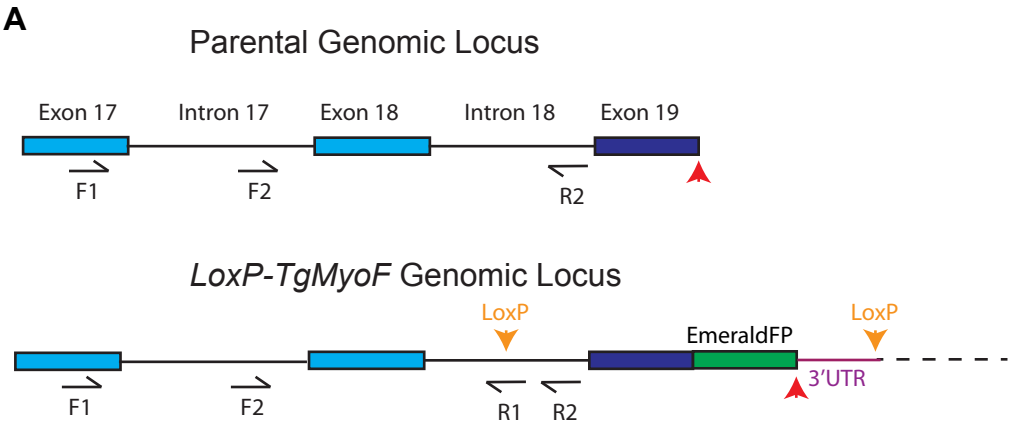
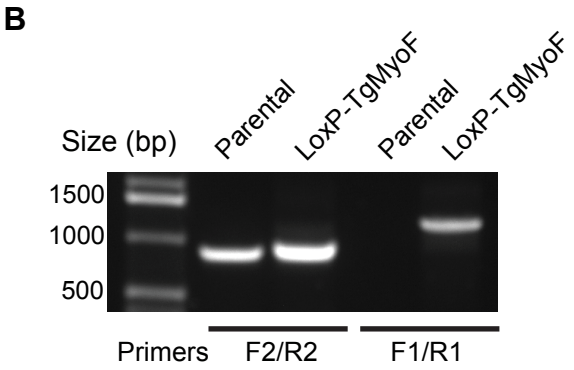


Figure S4


A
Parental Genomic Locus
Exon 17
Intron 17
Exon 18
Intron 18
Exon 19
F1
F2
R2
LoxP-TgMyoF Genomic Locus
LoxP
EmeraldFP
LoxP
3'UTR
F1
F2
R1
R2


B
Size (bp)
Parental
LoxP-TgMyoF
Parental
LoxP-TgMyoF
1500
1000
500
Primers
F2/R2
F1/R1

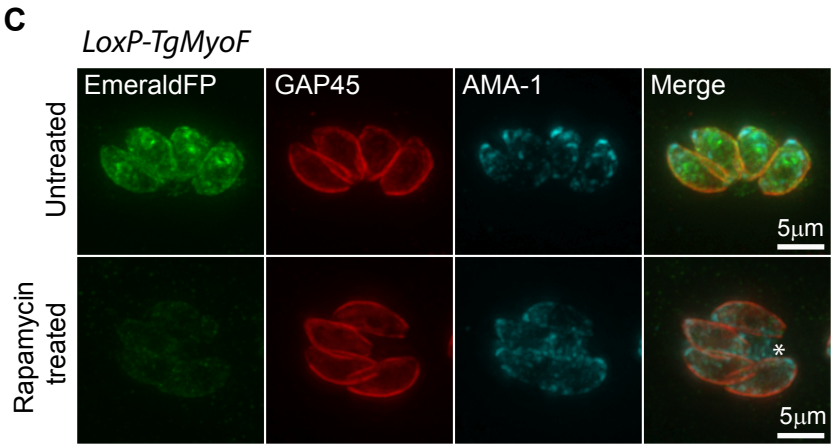

C
LoxP-TgMyoF
EmeraldFP
GAP45
AMA-1
Merge
Untreated
Rapamycin treated
5µm
*
5µm

Figure S5

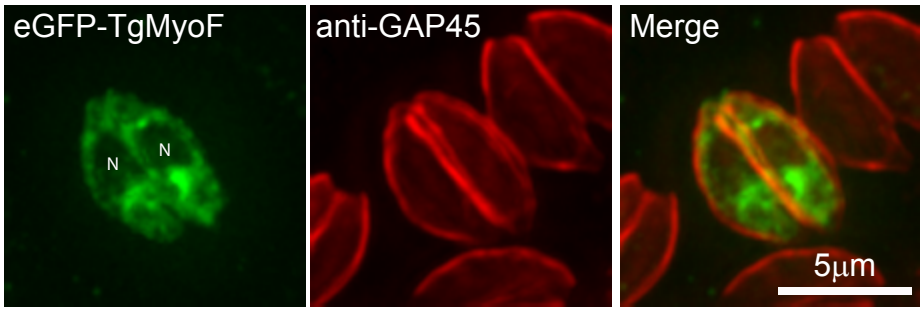


Figure S6

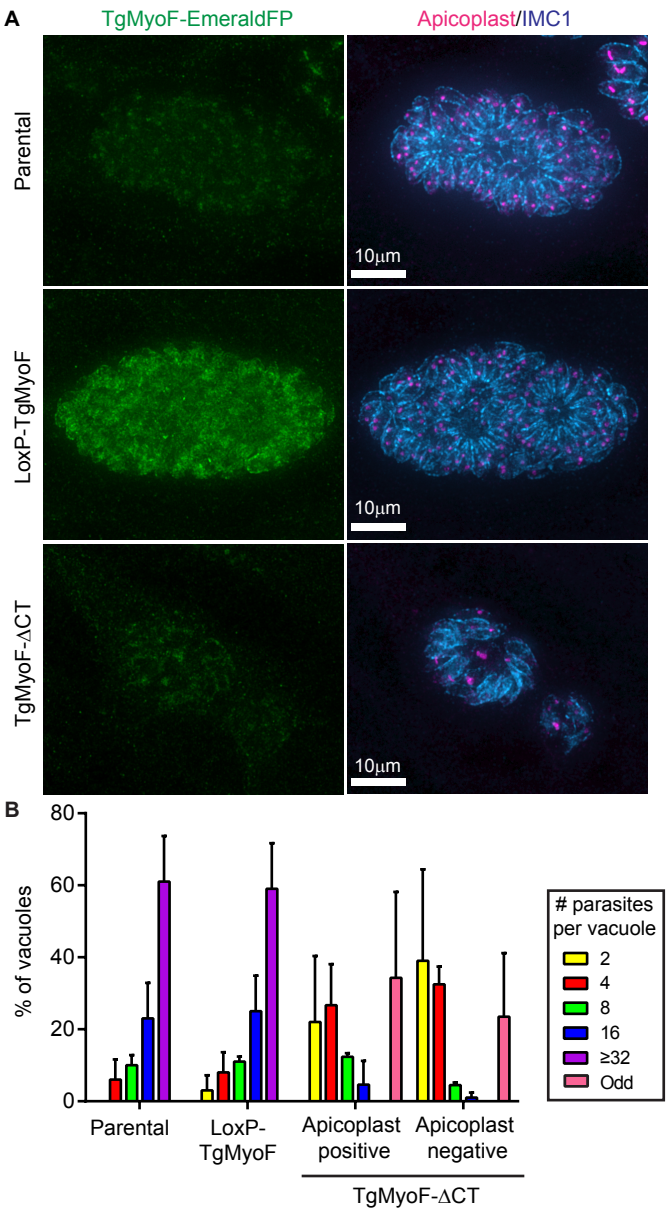

A
TgMyoF-EmeraldFP
Apicoplast/IMC1
Parental
LoxP-TgMyoF
TgMyoF-ΔCT
10μm
10μm
10μm
B
% of vacuoles
80
60
40
20
0
Parental
LoxP-TgMyoF
Apicoplast positive
Apicoplast negative
TgMyoF-ΔCT
parasites per vacuole
2
4
8
16
≥32
Odd

Figure S7

	# Events	% Stationary	% Diffusive-like	% Directed	Run-length ± SEM (nm)	Velocity ± SEM (nm/s)	Diffusion Coefficient ± error (nm^2/s)
Control	201	11	50	39	715 ± 82	486 ± 19	3135 ± 110
Oryzalin	277	4	54	42	604 ± 57	391 ± 19*	3032 ± 110
Cytochalasin D	80	46	53	1	N/A	N/A	835 ± 53**
Jasplakinolide	70	26	63	11	321 ± 130**	190 ± 12**	1586 ± 63**
***LoxP Actin*: Untreated**	392	15	48	37	622 ± 54	423 ± 13	2176 ± 91
***LoxP Actin:* Rapamycin Treated**	239	33	57	10	240 ± 64**	205 ± 18**	820 ± 71**
LoxP TgMyoF: Untreated	122	32	61	7	813 ± 124	443 ± 22	2203 ± 107
***LoxP TgMyoF*: Rapamycin Treated**	115	12	53	35	198 ± 66**	172 ± 9**	902 ± 61**

Table S1: Summary of results. Asterisks indicates that run-lengths, velocities and diffusion coefficients are significantly lower than control (** = $p<0.001$) (* = $p<0.05$).

Primer	Sequence
F1	CGTCGTCGAGTGTATCTACGG
F2	ACTGAGAGTTTCTGTTTTTCCT
R1	TAATGTATGCTATACGAAGTTA
R2	AAGAAGCACTCGAGTCCATTTC
NheI-SAG1-F	ATGCGCTAGCATGTTTCCGAAGGCAGTGAGAC
BglII-SAG1-287R	ATGCAGATCTTGCAGCCCCGGCAAACTCCA
TgMyoF qPCR F	GGAGAGCGGAGCAGGCAAGACAGAAA
TgMyoF qPCR R	TCGGGGAAGGGAAGTAATAGATGC
Tubulin qPCR F	CGCCACGGCCGCTACCTGACT
Tubulin qPCR R	TACGCGCCTTCCTCTGCACCC

Table S2. Primer sequences. Restriction sites are underlined.