

Supplemental materials

Table S1. Primers used in this study.

| Purpose | Name | Sequence |
|--|-----------------------|---|
| Generate vector to tag endogenous TgEFP1 | EFP1HA forward | ccccccgcgttcctgccaccaagctcgccaggctgt |
| | EFP1HA reverse | accgttctcgccagttcacCAtCctcaaGtgcataagaaggaa |
| Generate pSag1-Cas9-U6-sg1EFP1 by using Q5 mutagenesis | EFP1SG1 forward | ccaacggagagtttagagctagaaatagc |
| | EFP1SG1 reverse | agtcaactcaactgacatccccattac |
| Amplifying donor template for knockout clone 1 | DonorKO1 forward | ggacgaagacgacgaggcctcatccgcgaggaattcatgaaacaa aagtgtattctgaagaag |
| | DonorKO1 reverse | tgcctttccctcctgtgttcctgctttgacgcctagcggaaagatccgat cttgc |
| Generate pSag1-Cas9-U6-sg2EFP1 by using Q5 mutagenesis | EFP1SG2 forward | ttccggctaggtttagagctagaaatagc |
| | EFP1SG2 reverse | gaaagaaaacaactgacatccccattac |
| Amplifying donor template for knockout clone 1 | DonorKO2 forward | aggcccgagtgacgcatccgaaatcgaaacacgcgaactgaacaa aagtgtattctgaagaag |
| | DonorKO2 reverse | cgcattctgcgagagtcaagagatccatctgggtggagtgcggaaagat ccgatctg |
| Amplifying TgEFP1 gDNA to make complementing construct | TgEFP1comp WT forward | ggaggacggaaattcaaggagatgggacggcg |
| | TgEFP1comp WT reverse | gttccctaggaaattcaatagctgtatgatacataactcgcatatt |
| Deleting signal peptide from complementing construct | ΔSP forward | ccaccatgttcgcgcgcctggcgtcg |
| | ΔSP reverse | gcgcgaacatggcggcaggaagcgc |
| Generation of D97A complementation construct using Q5 | D97A forward | catggagtacgcaccaacggag |
| | D97A reverse | aattcctcgccatgtggatgaag |
| Generation of D129A complementation construct using Q5 | D129A forward | ccggcttagtggcatccaaccaag |
| | D129A reverse | aagaaaagaaaacagttcgc |

| | | |
|--|--------------|---|
| Targeting complementing template to Ku80 locus | KU80 forward | gtccccggttcgccctcagcacacacacatgacgtacatcgaggga acaaaagctgggtac |
| | KU80 reverse | ggatagctccattgttctgatgggaactattccgacattacattcatcctg caagtgcataagaaggaa |

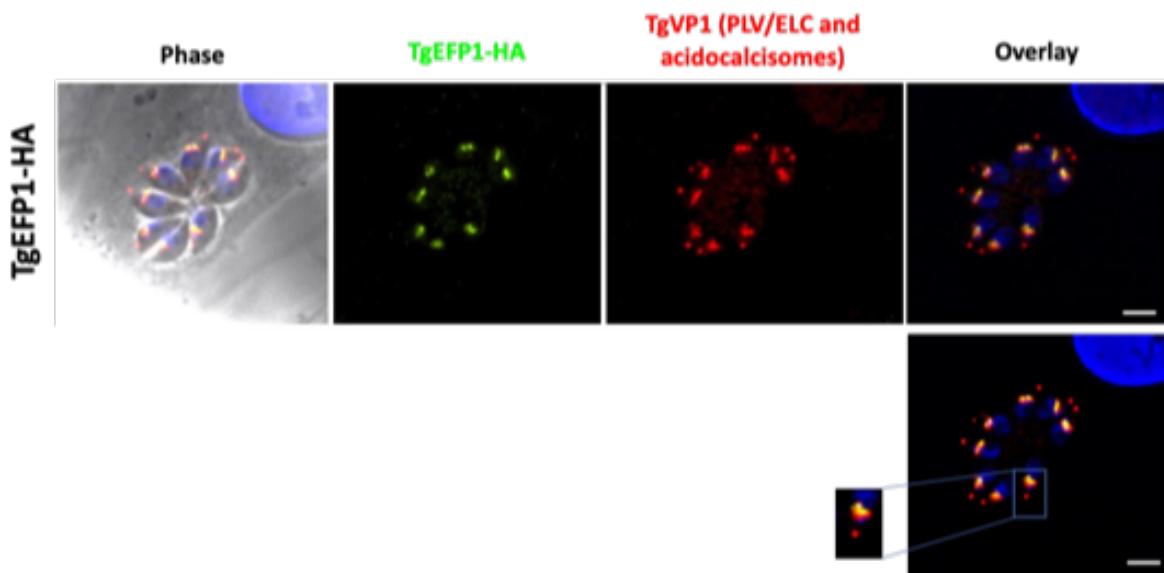


Figure S1: TgEFP1 co-localizes with TgVP1 only at the PLV/ELC and not at the acidocalcisome. Intracellular parasites stained for TgEFP1-HA (green) and TgVP1 (red). Overlay shows co-localization (yellow) of TgEFP1 and TgVP1 at the PLV/ELC. TgVP1 localization is also observed at the acidocalcisomes which is apically localized (red). Scale bars represent 2μM.