High-throughput functionalization of the *Toxoplasma* kinome uncovers a novel regulator of invasion and egress

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SUPPLEMENTAL TABLES

Table S1. Combined results from the HiT screens summarizing data from arrayed and pooled analyses.

Table S2. Oligos and plasmids used in this study.

SUPPLEMENTAL FIGURES

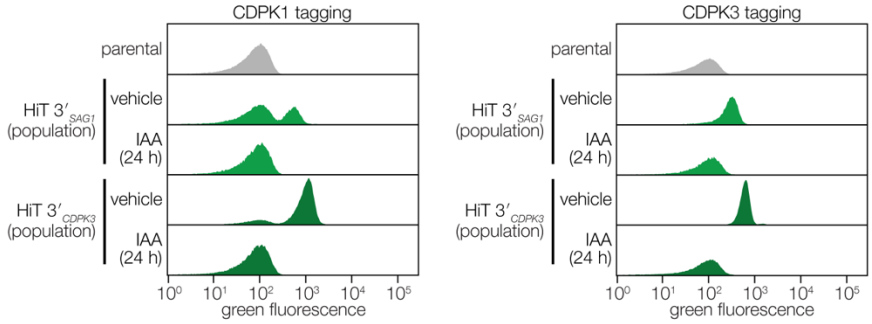
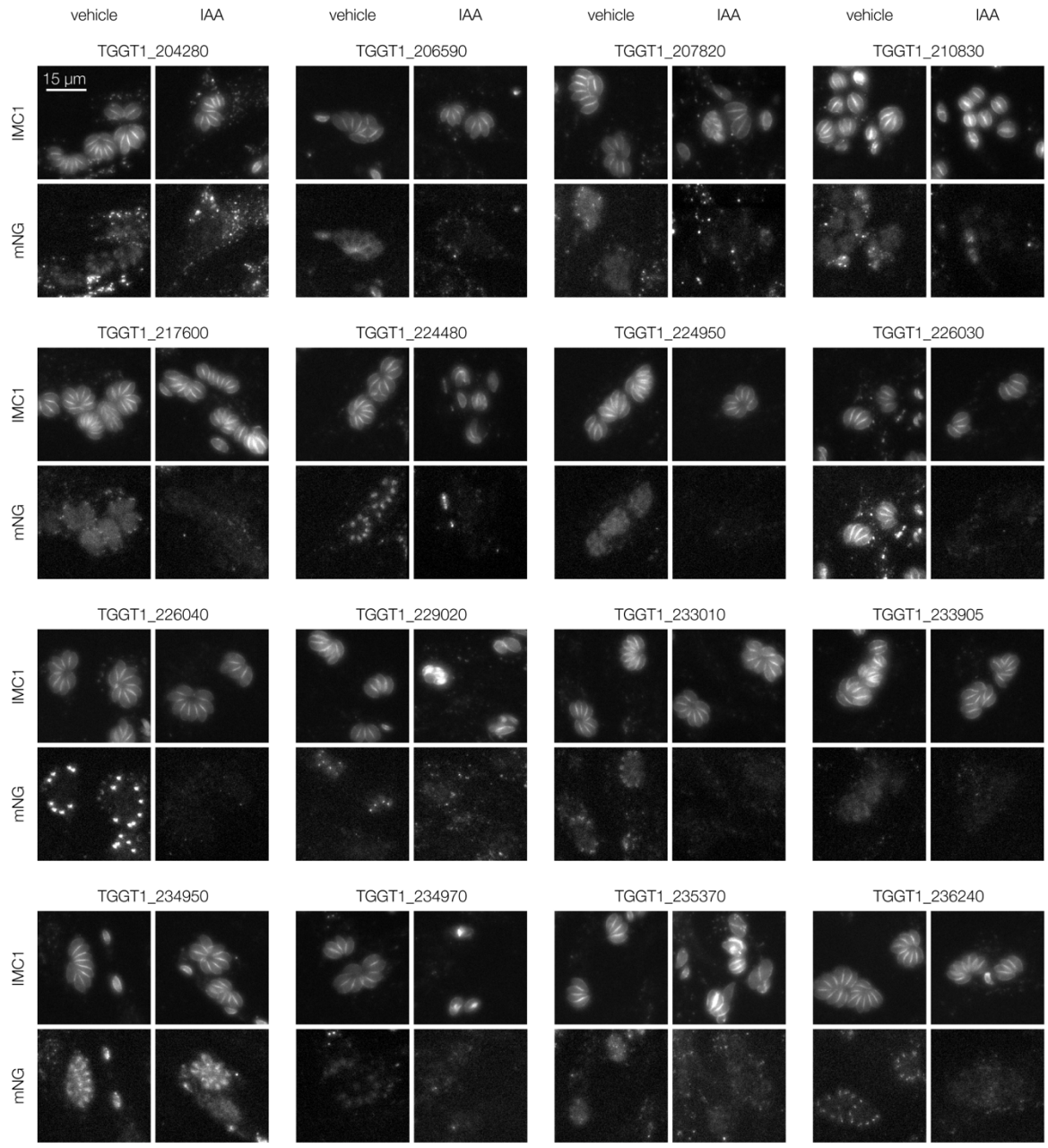
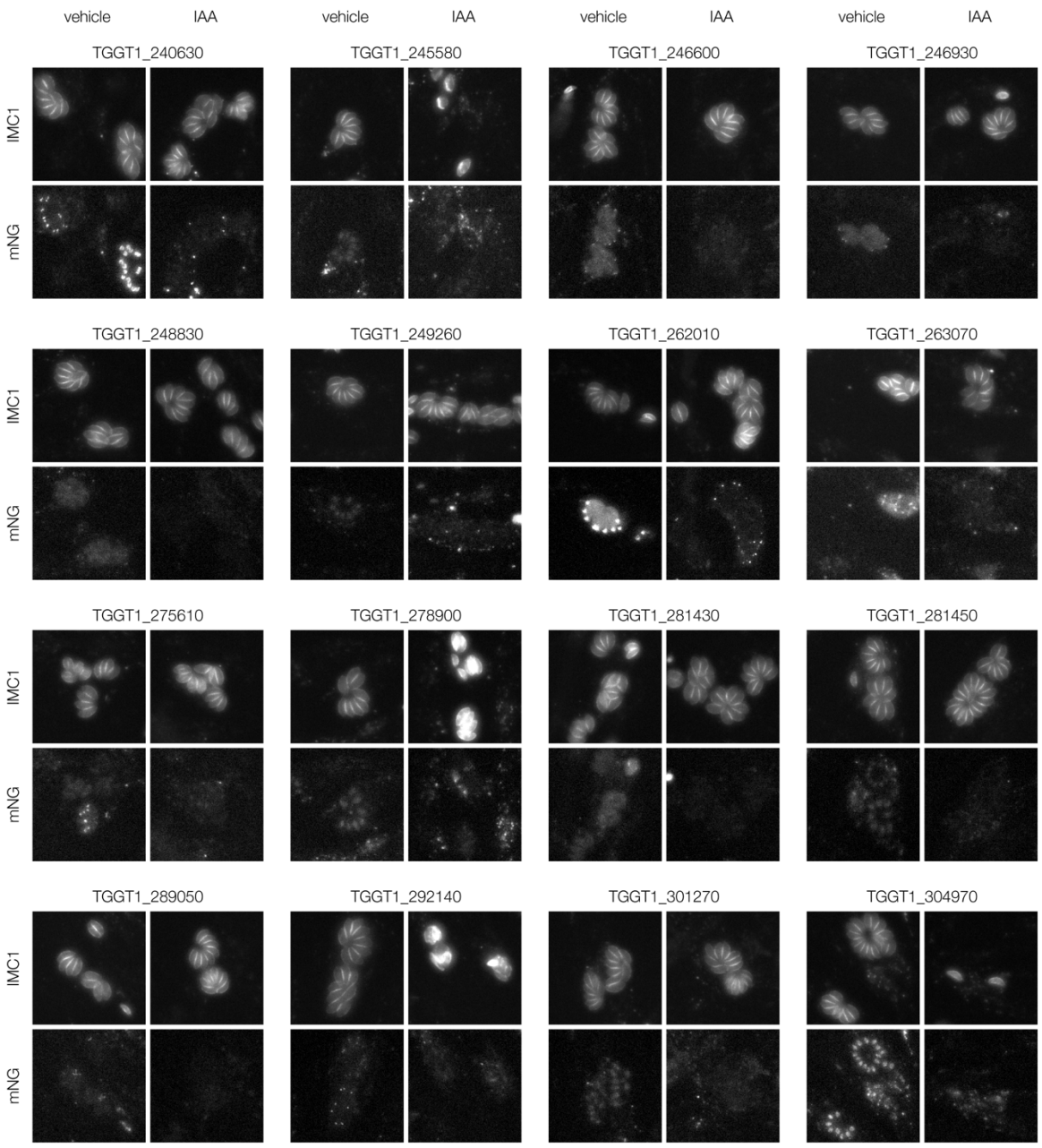


Figure S1. HiT vector-transfected populations are IAA-responsive. IAA regulation of HiT vector-transfected populations. Transfected populations were treated with IAA or PBS 3 hours post-infection. After 24 hours of IAA or PBS treatment populations were analyzed by flow cytometry.


vehicle
IAA
vehicle
IAA
vehicle
IAA
vehicle
IAA
TGGT1_204280
TGGT1_206590
TGGT1_207820
TGGT1_210830
15 µm
IMC1
mNG
TGGT1_217600
TGGT1_224480
TGGT1_224950
TGGT1_226030
IMC1
mNG
TGGT1_226040
TGGT1_229020
TGGT1_233010
TGGT1_233905
IMC1
mNG
TGGT1_234950
TGGT1_234970
TGGT1_235370
TGGT1_236240
IMC1
mNG


vehicle
IAA
vehicle
IAA
vehicle
IAA
vehicle
IAA
TGGT1_240630
TGGT1_245580
TGGT1_246600
TGGT1_246930
IMC1
mNG
TGGT1_248830
TGGT1_249260
TGGT1_262010
TGGT1_263070
IMC1
mNG
TGGT1_275610
TGGT1_278900
TGGT1_281430
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mNG
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TGGT1_301270
TGGT1_304970
IMC1
mNG

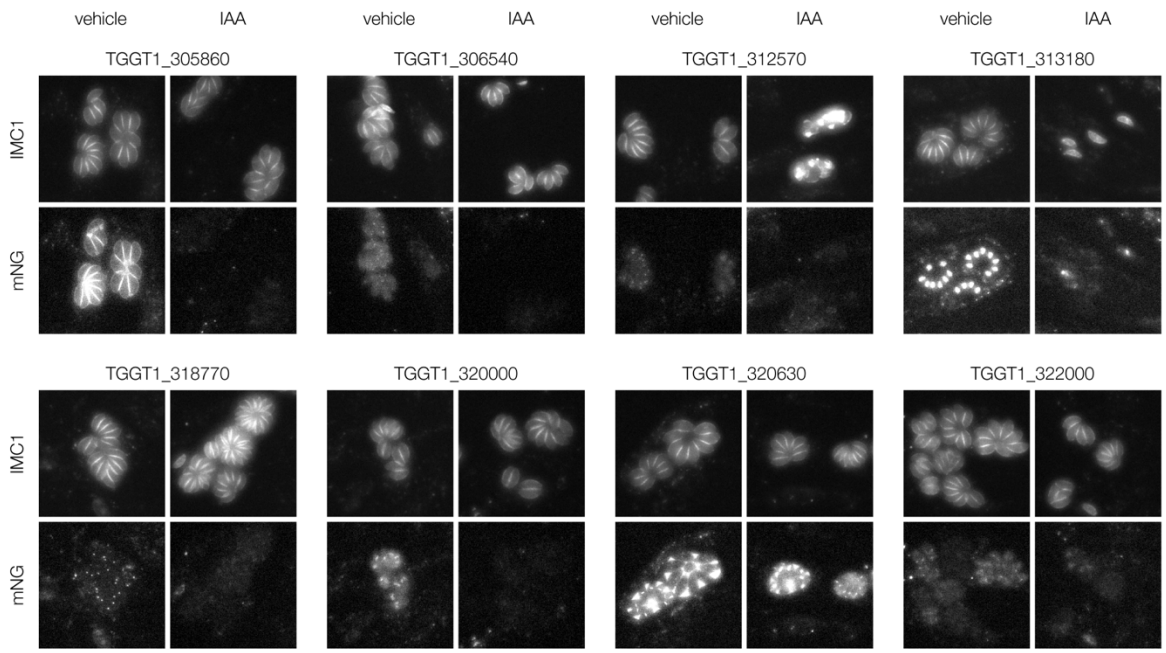

Figure S2. Representative images of assigned localizations within the array by widefield microscopy. Widefield microscopy of representative clones with assigned localizations. Localizations were assigned to a gene if half or more of single-integrated wells for that gene displayed consistent localizations. The maximum IMC1-tdTomato and mNeonGreen markers are displayed for cultures treated with either IAA or vehicle for 24 hours.

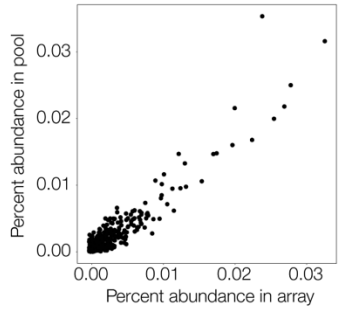

Figure S3. Abundance of gRNAs captured in the array correlates strongly with their presence in the pooled population. Percent abundance of each gRNA captured in the array versus the percent abundance of each gRNA in the pooled population from which they were subcloned. Spearman correlation coefficient = 0.77.

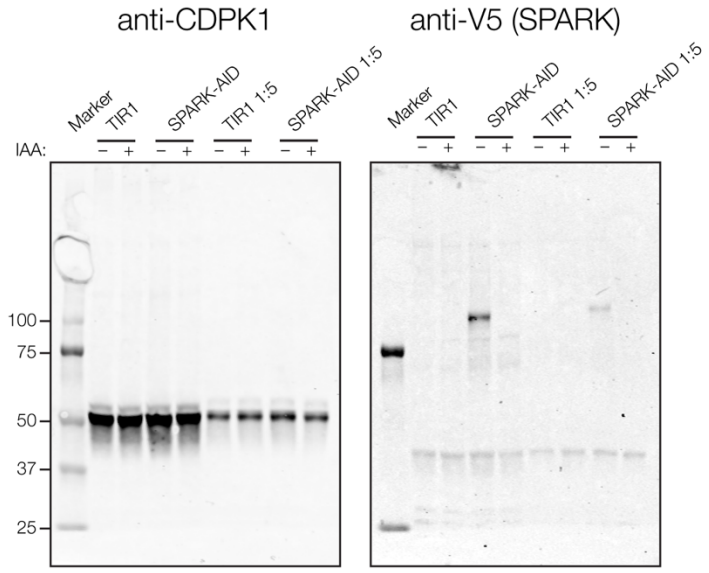

Figure S4. SPARK-AID depletion monitored by immunoblot. Full immunoblot from Figure 5D. SPARK-AID was detected using an anti-V5 antibody and CDPK1 was used as a loading control.

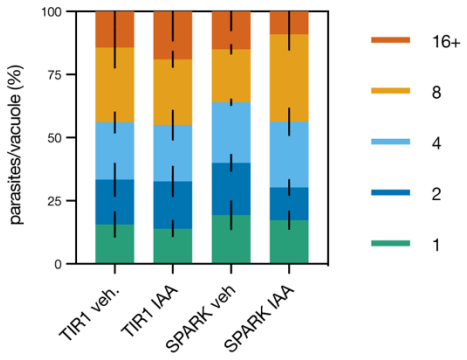

Figure S5. Knockdown of SPARK does not lead to a replication defect. Replication assay of SPARK-AID parasites. Parasites were treated with either IAA or vehicle at 3 hours post-invasion and incubated for 24 hours. Parasites were stained and imaged as 4x4 fields. The number of parasites per vacuole were counted until 100 vacuoles had been counted for a given sample. (N=3).

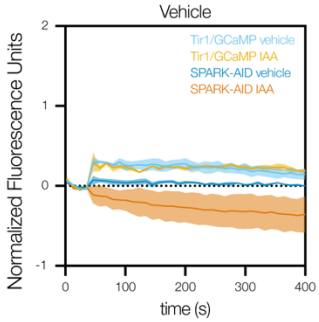

Figure S6. Vehicle-treated extracellular GCaMP6f/SPARK-AID parasites in basal Ca^{2+} buffer. Vehicle trace from **Figure 5I**. Extracellular parasites were treated with vehicle and the fluorescence response was quantified and normalized as previously described.