1 **Screening the *Toxoplasma* kinome with high-throughput tagging (HiT) identifies a regulator of**
2 **invasion and egress**

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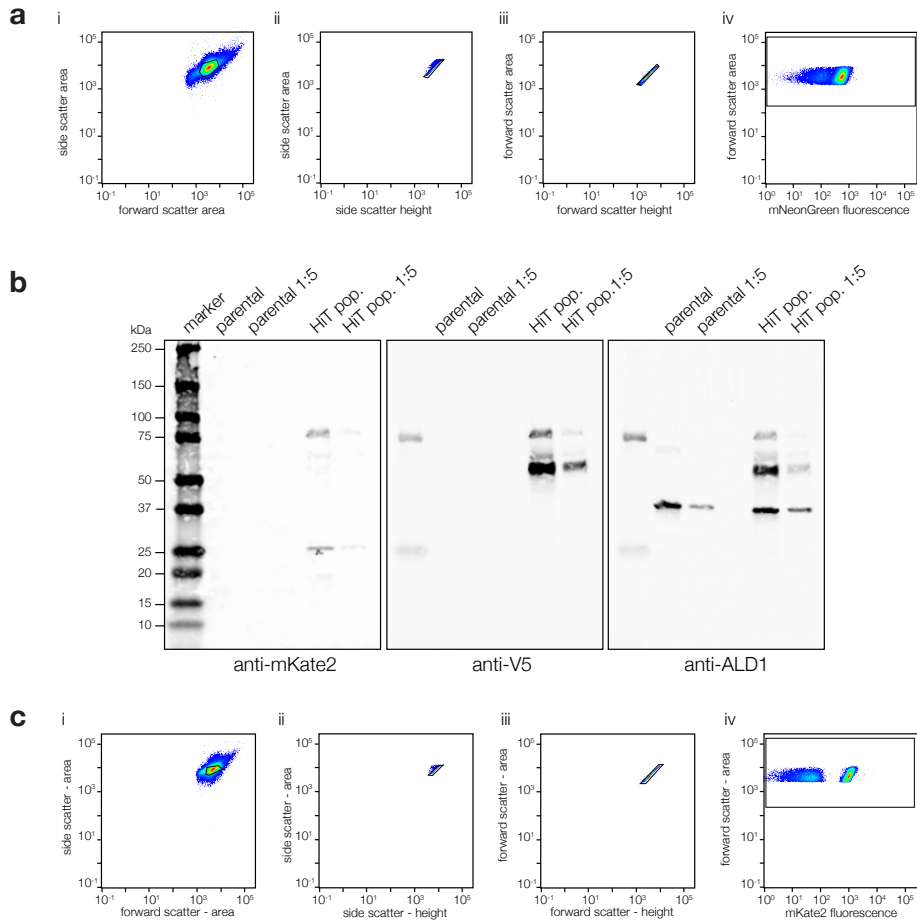
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8 **SUPPLEMENTARY TABLES**

9 **Supplementary Table 1.** Oligos and plasmids used in this study.

10 **Supplementary Table 2.** Combined results from the HiT screens summarizing data from arrayed and
11 pooled analyses.

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14 **SUPPLEMENTARY FIGURES**

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anti-mKate2   anti-V5   anti-ALD1
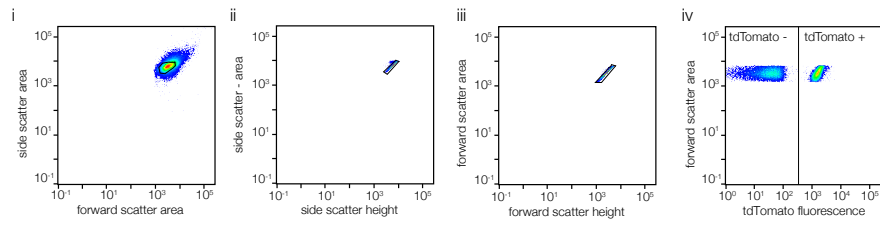
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16 **Supplementary Figure 1. Flow cytometry gating strategies and complete immunoblot of transfected**
17 **HiT populations. a**, Flow cytometry gating strategy for **Fig. 1b**, **Fig. 1l**, and **Extended Data Fig. 1d**.
18 Events were subsequently gated for toxoplasma (i) and single cells (ii–iii). Events were used to create
19 histograms of mNeonGreen fluorescence in **Fig. 1b**, **Fig. 1l**, and **Extended Data Fig. 1d** (iv). Example
20 pseudocolor density plots are provided from the CDPK3 HiT $3'_{CDPK3}$ population of **Fig. 1b**. **b**, Complete
21 immunoblot from **Fig. 1f**. CDPK1-V5-T2A was detected using an anti-V5 antibody, mKate2 was detected
22 using an anti-RFP antibody, and ALD1 was used as a loading control. (**c**) Flow cytometry gating strategy
23 for **Fig. 1e**. Events were subsequently gated for toxoplasma (i) and single cells (ii–iii). Events were used to
24 create a histogram of mKate2 fluorescence in **Fig. 1e** (iv). Example pseudocolor density plots are provided
25 from the CDPK1 HiT $3'_{CDPK3}$ population of **Fig. 1e**.

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Supplementary Figure 2. Flow cytometry gating strategy of competition experiments. Flow cytometry gating strategy for **Fig. 4b**. Events were subsequently gated for toxoplasma (i) and single cells (ii–iii). Events were binned as either tdTomato positive or tdTomato negative using the TIR1 and TIR1/IMC1-tdTomato strains as references (iv). Example pseudocolor density plots are provided from the starting population of the WT sample.

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anti-CDPK1   anti-V5 (SPARK)
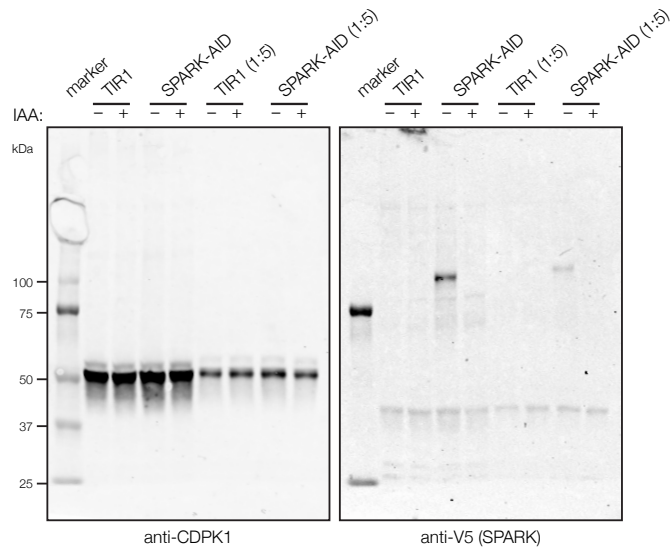
35

36 **Supplementary Figure 3. Complete immunoblot of SPARK knockdown.** Full immunoblot from **Fig. 5d**.
37 SPARK-AID was detected using an anti-V5 antibody and CDPK1 was used as a loading control.