

## Description of Additional Supplementary Files.

### File name: Supplementary Data 1

**Description: Mutations found within the coding regions of the 8 independent clones**

- 1a. Mutations in the coding regions of Clone 5E4.
- 1b. Mutations in the coding regions of Clone 8E7.
- 1c. Mutations in the coding regions of Clone 1D10.
- 1d. Mutations in the coding regions of Clone 1E9.
- 1e. Mutations in the coding regions of Clone 1B8.
- 1f. Mutations in the coding regions of Clone 2H11.
- 1g. Mutations in the coding regions of Clone 5G9.
- 1h. Mutations in the coding regions of Clone 5G11.

### File name: Supplementary Data 2

**Description: Differential gene expression and differential translational efficiency analyses for total RNA sequencing (RNA) and ribosome profiling (Ribo) of ME49 $\Delta$ ku80 (WT) and ME49 $\Delta$ ku80 $\Delta$ eif1.2 parasites after 24 h under unstressed or alkaline-stressed conditions, as related to Fig. 5 and Supplementary Figs. 8 and 9.**

- 2a. Differential gene expression between unstressed  $\Delta$ eif1.2 and unstressed WT parasites.
- 2b. Differential gene expression between stressed  $\Delta$ eif1.2 and stressed WT parasites.
- 2c. Differential gene expression between stressed and unstressed WT parasites.
- 2d. Differential gene expression between stressed and unstressed  $\Delta$ eif1.2 parasites.
- 2e. Differential translational efficiency between unstressed  $\Delta$ eif1.2 and unstressed WT parasites.
- 2f. Differences in translational efficiency between stressed and unstressed WT parasites.
- 2g. Differences in translational efficiency between stressed and unstressed  $\Delta$ eif1.2 parasites.

**2a-d**, the  $P_{\text{adj}}$  values were derived using DESeq2, employing two-sided tests with adjustments for multiple comparisons. **2e-g**, the  $P_{\text{adj}}$  values were derived using DESeq2 and edgeR in Riborex, employing two-sided tests with adjustment for multiple comparisons.

**File name: Supplementary Data 3**

**Description: Primers, gRNAs, repair templates, antibodies, other staining reagents, and cross-referenced genes used in this study.**

**3a.** Primers for qPCR and qRT-PCR.

**3b.** Primers used for cloning and genotyping.

**3d.** Primary, secondary antibodies and other staining reagents.

**3e.** Genes exhibiting a fold change of  $\geq 5$ -fold in chronic versus acute infection ( $P$  value  $<0.05$  and  $Q$  value  $<0.05$ ; *Pittman et al.*).

**3f.** Genes exhibiting a fold change of  $\geq 5$ -fold in acute vs chronic infection ( $P$  value  $<0.05$  and  $Q$  value  $<0.05$ ; *Pittman et al.*).

**3g.** Genes exhibiting a fold change of  $\geq 5$ -fold between tissue cysts and in vitro tachyzoites of *T. gondii* ( $P_{\text{adj}} < 0.05$ ; *Ramakrishnan et al.*).

**3h.** Genes exhibiting a fold change of  $\geq 5$ -fold between in vitro tachyzoites and tissue cysts of *T. gondii* ( $P_{\text{adj}} < 0.05$ ; *Ramakrishnan et al.*).

**3e,f.** According to *Pittman et al.*, the  $P$  and  $Q$  values were derived using Cuffdiff, employing one-sided tests with adjustments for multiple comparisons. **3g,h.** According to *Ramakrishnan et al.*, the  $P_{\text{adj}}$  values were derived using DESeq2, employing two-sided tests with adjustments for multiple comparisons.