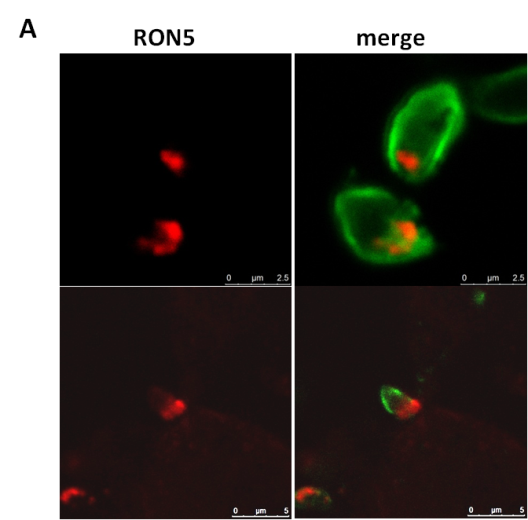
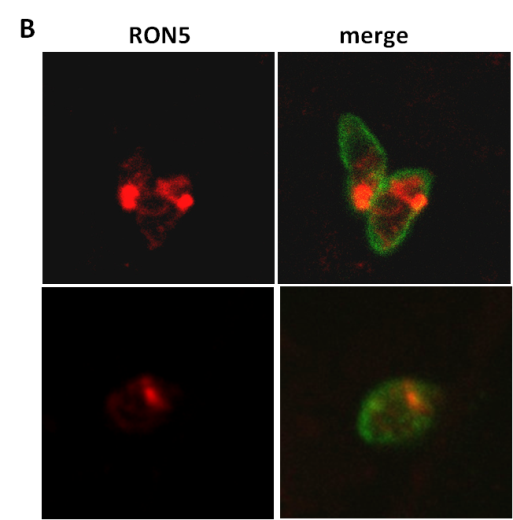
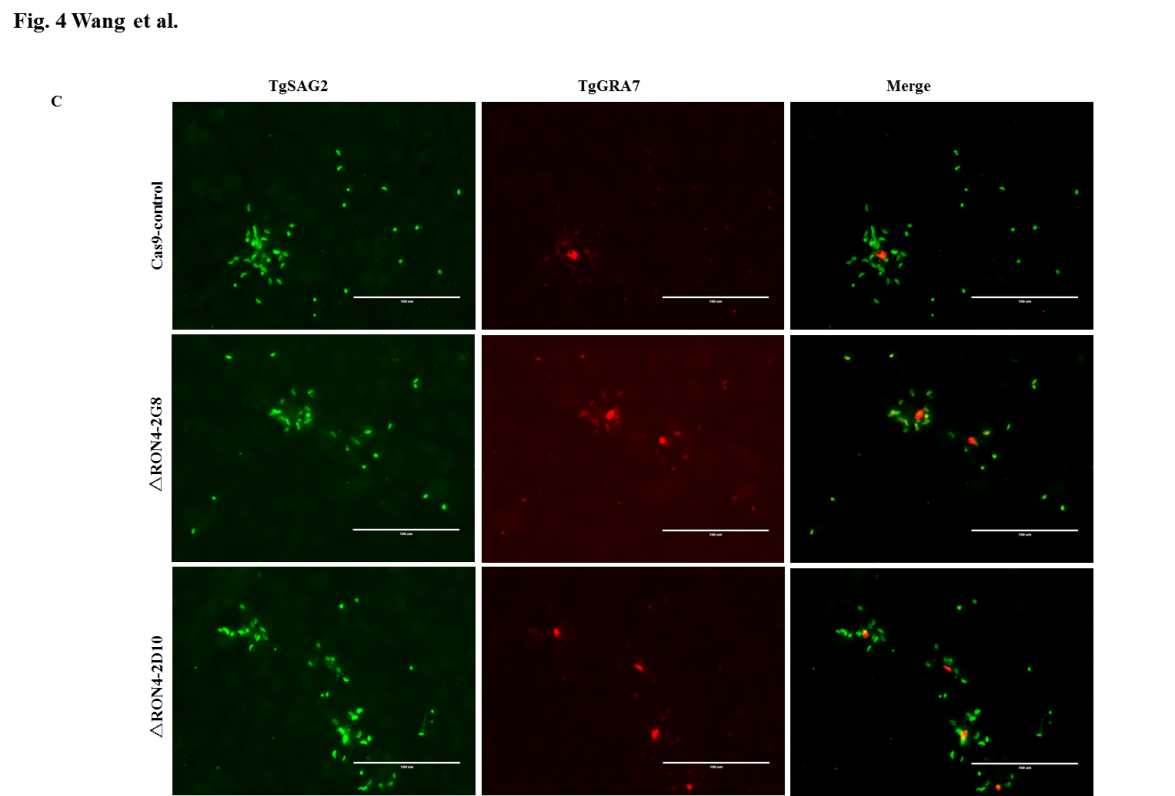


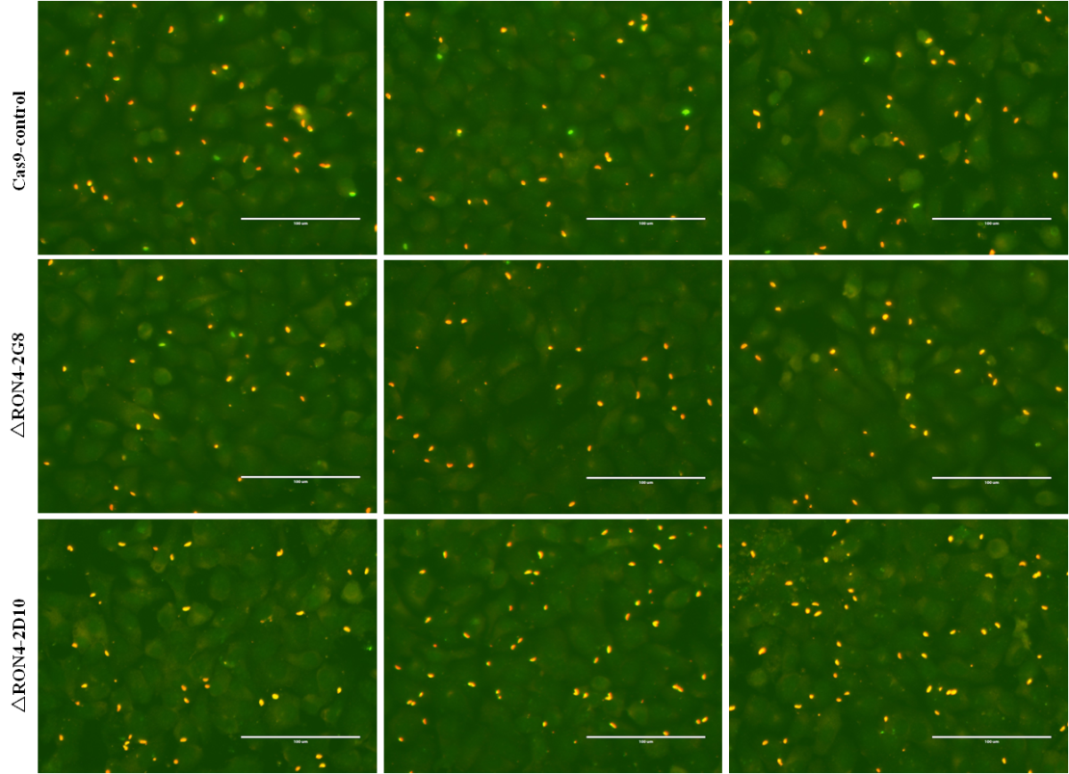
Supplementary Fig. 1 Schematic diagram of the pCD-RON4.

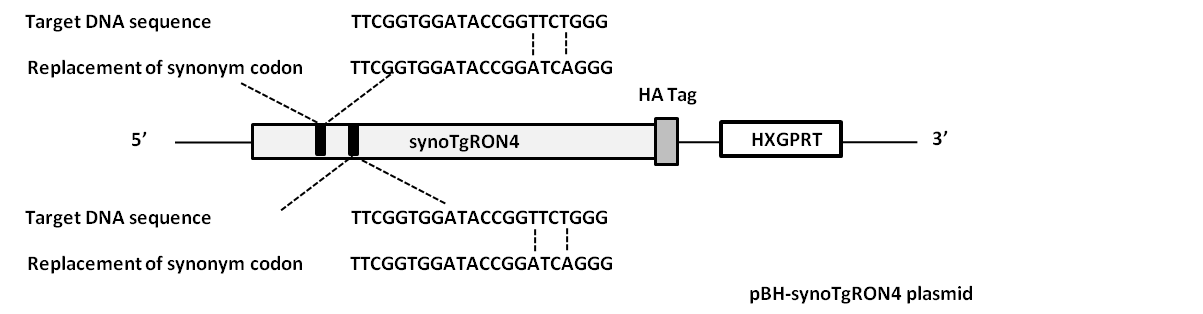
Supplementary Fig. 2 Localization of TgRON5 in wild-type parasites (A) and TgRON4-deficient parasites (B). Red: mouse anti-RON5 antibody detected by Alexa647-anti-mouse IgG. Green: rabbit anti-SAG2 antibody detected by Alexa488-anti-rabbit IgG.



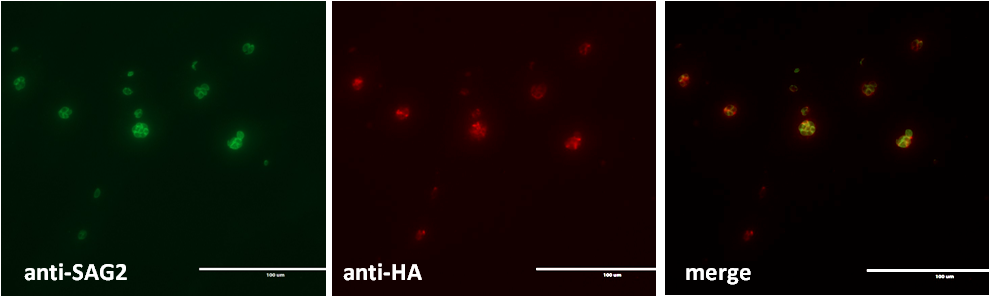
Supplementary Fig. 3 Representative view of egress assay. Red: mouse anti-GRA7 antibody detected by Alexa647-anti-mouse IgG. Green: rabbit anti-SAG2 antibody detected by Alexa488-anti-rabbit IgG.



Supplementary Fig. 4 Representative view of invasion assays. Red: rabbit anti-SAG2 antibody detected by Alexa647-anti-rabbit IgG. Green: rabbit anti-SAG2 antibody detected by Alexa488-anti-rabbit IgG.



Supplementary Fig. 5 Schematic diagram of the TgRON4 complementary expression plasmid



Supplementary Fig. 6 IFAs analysis of complement synoTgRON4 in TgRON4-deficient strains. Green: rabbit anti-SAG2 antibody detected by Alexa488-anti-rabbit IgG. Red: mouse anti-HA antibody detected by Alexa594-anti-mouse IgG.

**Table S1. Primers utilized in this study**

All primers are in the 5’-3’ orientation.

|  |  |
| --- | --- |
| **Name** | **Primer Sequence** |
| Cas9-control Forward | CCACTAGTTCTAGAGCGGCCGTTTAAACGAGCTCCAAGTAAGCAGAAG |
| Cas9-control Reverse | CCGTATTACCGCCTTTGAGTGTTTAAACGAGCTGATACCGCTCGCC |
| ROP16 Forward | CTTTCACCCTCGCATCT |
| ROP16 Reverse | ACGATGGGACGCTGTAT |
| Actin Forward | GCGCGACATCAAGGAGAAGC |
| Actin Reverse | CATCGGGCAATTCATAGGAC |
| rRON4t- Forward | GATCTGGTTCCGCGTGGATCCAGTCCGCCAACGCCTGCT |
| rRON4t- Reverse | GTCACGATGCGGCCGCTCGAGTCAAGAAGCAGTTTTCTG |
| rRON5t- Forward | GATCTGGTTCCGCGTGGATCCGGCAACCTAGTATGTG |
| rRON5t- Reverse | GTCACGATGCGGCCGCTCGAGCAGATTGTGTCTCGGG |
| P1 | TTAACGCGTAAAATGTTTGCTTGCCAACAG |
| P2 | CCAGCTTACCTTCCTGAGTTTGTCGAAGTT |
| P3 | CAGCTGCTTCATCACTTTGTCGTC |
| P4 | CATCTTCCGCACGTCGTACACCTT |
| P5 | CCACCCCGGTGAACAGCTCCTCGC |
| P6 | GGATCGATCCCCCGGGCTGCAGGA |