Figure S1. CEN2 depletion does not affect the distribution of ISP1, rhoptries, or dense granules. (A) Images of cKD parasites treated for 120 h with ATc (-Shld1/+ATc), labeled with antibodies for ISP1 (red), a marker for the apical cap, and IMC1 (green), a marker for the cortex of mature and daughter parasites. Scale bar $=2 \mu \mathrm{~m}$.
(B-D) Images of RH $\Delta h x$ parasites (WT, B), and cKD parasites cultured with Shld1 (+Shld1/ATc, C), or treated for 48 h with ATc (-Shld1/+ATc, D), labeled with antibodies for the rhoptries. Red: anti-RON2-4, a marker for the rhoptry neck. Green: anti-ROP2,3,4, markers for the rhoptry bulb. Scale bars $=2 \mu \mathrm{~m}$.
(E-G) Images of RH $\Delta h x$ parasites (WT, E), and cKD parasites cultured with Shld1 (+Shld1/ATc, F), or treated for 48 h with ATc (-Shld1/+ATc, G), labeled with antibodies for GRA8 (red), a marker for the dense granules, and IMC1 (green). Scale bars $=2 \mu \mathrm{~m}$.

Figure S2. CEN2 depletion does not have a major impact on construction of the basal complex or inheritance of the apicoplast.
Representative images of cKD parasites cultured with Shld1 (+Shld1/-ATc, A\&C) or treated with ATc (-Shld1/+ATc) for $84 \mathrm{~h}(\mathrm{~B})$ or $87 \mathrm{~h}(\mathrm{D})$. The parasites were labeled with antibodies for IMC1 (green), and IAP1 (A\&B, red), a marker for the basal complex, or acyl carrier protein (ACP, C\&D, red), a marker for the apicoplast. Scale bars $=2 \mu \mathrm{~m}$.

Table S1. Primers used in this study.

## Figure S


cKD -Shld1/+ATc

| B anti-RON2-4 <br> WT | anti-ROP2,3,4 | Merge |  |
| :---: | :---: | :---: | :---: |
| $\overline{\mathbf{C}}$ cKD +Shld1/-ATc | $*^{64} \cdot t$ | $0^{6} x$ |  |
| $\begin{array}{cc} D & \cdots \\ c k i \\ c K D & -S h I d 1 /+A T c \end{array}$ | $=\underbrace{}_{n=1}$ | $n=p$ |  |
| E anti-GRA8 <br> WT | anti-IMC1 | Merge | DIC <br> Merge |
| cKD +ShId1/-ATc | $\begin{aligned} & (1) \\ & (818) \end{aligned}$ |  |  |
| G <br> cKD -Shld1/+ATc |  | (1) |  |

Figure S2

A anti-IAP1
is

| cKD | +Shld1/-ATc |
| :--- | ---: |
| B | anti-IAP1 |


$2 \mu$

## cKD -Shld1/+ATc



0

2
-
cKD
-Shld1/+ATC


Table S1. Primers used in this study.

| Name | Sequence (5' ${ }^{\prime}$ to $\mathbf{3}^{\prime}$ ) |
| :--- | :--- |
| S1 | ACTGGCTAGCCAAGGCTGTCGATTCAACAGAGAGC |
| S2 | AGTCGCGGCCGCCTCGCACTTTTCGCAGGGCATCTTG |
| S3 | CGATGGATCCCAGCGAGGAGCACTGCGAGGGGCGAG |
| S4 | CTAAAGGGAACAAAAGCTGGGTACCGGTACCGGGCCCCCCCTCG |
| S5 | CTCCGGCTTGCAACCAAGGACCCGTAATACGACTCACTATAGGGC |
| S6 | ATCGAGGACCCCTGATGAACTTGGCTTATTCAT |
| S7 | GCGCAGATCTGCCAATTTACTGACCGTACACC |
| S8 | CAGCGAGGAGCACTGCGAGG |
| S9 | GATCGCTTCTCGGTTCCTACCCTG |
| AS1 | ACTGGGGCCCCCTGTGCCCCAAAATGTACCGGAGGC |
| AS2 | ACTGGAATTCGCTCGACAAAAAAAAGGCCAAATGTA |
| AS3 | ACGTCTTAAGTCACGGGAAAGTCTTCTTGGTCATGATCG |
| AS4 | CTGCAGGAATTCGATATCAAGCTTAACCGGTTCGACTAAAACAAC |
| AS5 | CGCCCTTGCTCACCATTTTGCTAGCTTTGTCGAAAAAGGGAATTCG |
| AS6 | ATCGGCTAGCGGATCTAAAAGGGAAT |
| AS7 | GCGCCTTAAGCTAGGTGGCGACCGGTCCATCGCCAT |
| AS8 | CAGCTTCGCGGTAATGGCGT |
| AS9 | CCTGTTACGAACGCAAAGATGTGT |

