Supporting information

Figure S1. Observation of vimentin rearrangement in HFF cells infected with *T. gondii*. HFF cells were infected with *T. gondii* RH strain for 18 h or uninfected (controls), and then fixed with paraformaldehyde. An indirect immunofluorescence assay (IFA) was performed. The results of IFA demonstrate that host cell vimentin was rearranged and accumulated around the *T. gondii* parasitophorous vacuoles (arrowheads). This phenomenon was not observed in uninfected cells.

Figure S2. Host cell vimentin had no obvious effect on the proliferation of *T. gondii*. A. qRT-PCR was performed to verify the knockdown of vimentin in HFF cells treated with siRNA (t test, ***p ≤ 0.001). B. Knockdown of vimentin was also demonstrated by Western Blot. C. Number of tachyzoites per parasitophorous vacuole (PV) in untreated, ctrl siRNA-treated, and vimentin siRNA treated cells. The number of vacuoles containing one, two, four, or eight parasites was visualized under a fluorescence microscope (100×). Means ± SD combined from three independent experiments, each performed in triplicate, were analyzed by two-way ANOVA. No significant difference was found among these three groups, hence these data clearly demonstrated that vimentin expression levels did not affect the proliferation of *T. gondii*. 
### Table S1. Plasmids used in this study

| Plasmid          | Description                                                                 | Used for                                                                 |
|------------------|-----------------------------------------------------------------------------|--------------------------------------------------------------------------|
| pBlunt-Vim       | The coding sequence (CDS) of vimentin was cloned into pEASY-Blunt (TransGen Biotech, Beijing, China) | Amplification template of vimentin for subsequent experiments             |
| pEYFP-Vim        | The CDS without TAA was cloned into pECFP-C1                                | FRET                                                                     |
| pECFP-ROP18      | The CDS without TAA was cloned into pEYFP-C1                                |                                                                          |
| pcDNA3.1-vim-HA  | Vimentin with HA tag was cloned into pcDNA3.1(+)                           | Vimentin expression in cells                                             |
| pcDNA3.1-ROP18-flag | ROP18 with 3xflag tag was cloned into pcDNA3.1(+)                         | ROP18 expression in cells                                               |
| pSAG1::Cas9-U6::sgROP18 & pSAG1::Cas9-U6::sgROP18-in | Cas9 expressed from the SAG1 promoter and CRISPR gRNA targeting ROP18 produced from the U6 promoter | CRISPR plasmid targeting rop18 for the knockin of eGFP-flag |
| pBlue-5’-ROP18-homo | A 990bp fragment upstream the gRNA target in rop18 gene cloned into the vector pBlue-script II SK(-) | Homologous template for the disruption of T. gondii rop18 |
| pBlue-5’-3’-ROP18-homo | A 850bp fragment downstream the gRNA target in rop18 gene cloned into the vector pBlue-5’-ROP18-homo | Homologous template for the disruption of T. gondii rop18 |
| pBlue-5’-3’-ROP18-homo-DHFR-TS | DHFR-TS cassette for drug screen flanked by two homology arms cloned into the vector pBlue-5’-3’-ROP18-homo | Homologous template for the tagging of endogenous rop18 with DHFR-TS |
| pBlue-donor-eGFP-ROP18 | Fragments upstream and downstream the gRNA, eGFP-FLAG, DHFR-TS, SAG1-3’-UTR were cloned into the vector pBlue-script II SK(-) | Homologous template for the tagging of endogenous rop18 with GFP |
| pBlue-p24        | Fragment p24 promoter was cloned into pBlue-script II SK(-)                 | To generate T. gondii RH/GFP parasite                                    |
| pBlue-p24-eGFP   | Fragment eGFP-SAG1-3’-UTR was cloned into pBlue-p24                         |                                                                          |
| pBlue-p24-eGFP-DHFR-TS | Fragments DHFR-TS was cloned into pBlue-p24-eGFP                           |                                                                          |
| pET28a-vim       | Vim was cloned into the vector pET28a(+)                                   | Purification of protein vimentin                                         |
| pGEX-ROP18       | ROP18 (starting from Glu83 based on the second ATG) with tag of flag and his was cloned into the vector pGEX-4T-2 | Purification of protein ROP18                                             |
| pGEX-6-His       | His tag was inserted into plasmid pGEX-4T-2                                | Purification of protein                                                  |
Table S2. Primers used in this study

| primers          | Sequence                                      | Used for                                                                 |
|------------------|-----------------------------------------------|--------------------------------------------------------------------------|
| Vimentin-F       | ATGTCCACCAGGTCCGTGTC                         | To amplify fragment of *vimentin* for pBlunt-Vim cloning                 |
| Vimentin-R       | TTATTCAGGTCATCGTGATGCTG                      |                                                                          |
| Vimentin-F'      | ACGGTGTCAGATGTCACCAGGTCCGTGTC                | To amplify fragment of *vimentin* for pEYFP-Vim cloning                  |
| Vimentin-HA-R    | TCCCCCGGGTTAAGGATCTGGAAATCGTATGGATTCAAGGTCATCGTGAG |                                                                          |
| ROP18-F          | ACGGTGTCACATGTTGTCGTAACA                    | To amplify fragment of *rop18* for pECFP-ROP18 cloning                   |
| ROP18-FLAG-R     | TCCCCGCGGTTCATTTGCTGTCGC                    |                                                                          |
| Vimentin-F''     | CCCAGCTATGTCCACCAGGTCCGT                    | To amplify fragment of vimentin for pcDNA3.1-vim-HA cloning              |
| Vimentin-HA-R'   | CGGGATCCTTAAGGCTAAATCTGGAACATCG             |                                                                          |
| ROP18-F'         | CCCAAGCTTATGTTGTCGTAACA                    | To amplify fragment of ROP18 for pcDNA3.1-ROP18-FLAG cloning            |
| ROP18-FLAG-R'    | CGGGATCCTTAATCGTACGTGC                    |                                                                          |
| SgRop18-F        | TACGCGTACCGTGCAGGAGTTTTCAGGTAAATAGC         | Q5 mutagenesis changing the gRNA in pSAG1::CAS9-U6::sgUPRT for the disruption of *rop18* |
| SgRop18-R        | AACCTGACATCCCCATTAC                         |                                                                          |
| SgROP18-F-in     | GCCGACCAGGACGCCTCGAGTTTTCAGGTAAATAGC        | Q5 mutagenesis changing the gRNA in pSAG1::CAS9-U6::sgUPRT for the knockin.of.eGFP-FLAG.fused.to.*rop18* |
| SgROP18-R-in     | AACCTGACATCCCCATTAC                         |                                                                          |
| 5'-Homo-F-ROP18  | CCCAGCTTGGAGTTGACAGGAGTCCACAGGG             | To amplify fragment of 5'-homo for the knockout of *rop18*               |
| 5'-Homo-R-ROP18  | CGGGATCAGAGGTCGCCAGTGGACATC                 |                                                                          |
| 3'-Homo-F-ROP18  | CGGGATCCTTGAGGTTAGCACCCTTGCGCTGCTG         | To amplify fragment of 3'-homo for the knockout of *rop18*               |
| 3'-Homo-R-ROP18  | GCTCTAGAGATGACTGAGCTGGCTGGCTGCTG           |                                                                          |
| DHFR-TS-F        | CGGGATCAGAGGTCGCCAGTGGAGTACG               | To amplify fragment of DHFR-TS drug screen cassette for the knockout of *rop18* |
| DHFR-TS-R        | CGGGATCAGAGGTCGCCAGTGGAGTACG               |                                                                          |
| 5'-Homo-F-ROP18-in | GGCCGCGGCCGCGCGGGTGGAGTACG               | To amplify fragment of 5'-homo to tag endogenous *rop18* with eGFP-FLAG  |
| 5'-Homo-R-ROP18-in | CATCAGAGGTCGCCAGTGGAGTACG               |                                                                          |
| eGFP-F-in        | TCTCCACACAGAAGGCGCGGCA                    | To amplify fragment of eGFP for the                                       |
| Gene Segment                | Primer Sequence                                                                 | Function/Target                                                                 |
|-----------------------------|---------------------------------------------------------------------------------|--------------------------------------------------------------------------------|
| eGFP-R-in                   | GGATGGTGAGCAAGGGCGAG                                                            | Knock-in of eGFP-FLAG                                                          |
| SAG1-3'-UTR-F-in            | CGTCATCGTCTTTGTAATCAATATC ATGATCTTCTAGTCTCCGTCGAG GCTCTTATAGTCTCTTCTACAGCTC GTCCATGCC | To amplify fragment of SAG1-3'-UTR for the knock-in of eGFP-FLAG               |
| SAG1-3'-UTR-R-in            | CCTGGCCGAAGCTTAGCTCCACCC CGGTGAGCC                                               |                                                                                  |
| DHFR-TS-F-in                | CGCGGTGGTAGCTGAGTCGCC AGGCGTGA                                                  | To amplify fragment of DHFR-TS drug screen cassette for the knock-in of eGFP-FLAG |
| DHFR-TS-R-in                | TATGACGATTTAAATACGTAGGA TTTCACTCCTGCAAGTGC                                      |                                                                                  |
| 3'-homo-F-ROP18-in          | CAGGATGAAATCTCTACGTATTAA ATCGTCAATAAGCGAATAAACAG                                 | To amplify fragment of 3'-homo to tag endogenous rop18 with eGFP-FLAG          |
| 3'-homo-R-ROP18-in          | GGTGCCGCCCGCTCTAGAACTAG TGGATCCTAGTAGGTTATGAA AGACAG                             |                                                                                  |
| Donor-knockout-F            | GCAATTTGACACGACGGACGAG                                                          | To amplify fragment of homologous template for rop18 disruption                 |
| Donor-knockout-R            | GGATGCGTGGCTGCTCCCTCTAAC                                                        |                                                                                  |
| PCR1-F                      | CACATCAATGTGTGTGTCAGGC                                                          | PCR1                                                                            |
| PCR1-R                      | GAATGACATGCTAGGCTTACAC                                                          |                                                                                  |
| PCR2-F                      | CAGGATGCTTACACGAGGACC                                                           | PCR2                                                                            |
| PCR2-R                      | GAATGACATGCTAGGCTTACAC                                                          |                                                                                  |
| PCR3-F                      | GAGACTGTCACACGCTCGG                                                             | PCR3                                                                            |
| PCR3-R                      | GCGACAGTGCTAAGTTATCCAG                                                         |                                                                                  |
| PCR4-F-in                   | CTGTCCGGACAGACAGGCTG                                                           | PCR4                                                                            |
| PCR4-R-in                   | GCAGATGACATCAGGTCTGCC                                                          |                                                                                  |
| PCR5-F-in                   | AGCAGATGCTCAGACGACC                                                            | PCR5                                                                            |
| PCR5-R-in                   | CAGGCAAGTGCTCCTCAGTCC                                                          |                                                                                  |
| PCR6-F-in                   | GCAGAGGGAGATCTCGCTGTC                                                          | PCR6                                                                            |
| PCR6-R-in                   | CTAGGCAACGAGCTGAGTGGAG                                                        |                                                                                  |
| P24-F                       | GCGGGGCTCCGAGGCTGTAGA CTGTAGCT                                              | To amplify the promoter of P24                                                 |
| P24-R                       | CGGATGCTTTGCTGTTGCTCTTTCC AAAG                                                  |                                                                                  |
| eGFP-SAG1-3'-UTR-F-in       | CGGGATCCGTGAGACAAGGGCG                                                        | To amplify fragment of eGFP-SAG1-3'-UTR                                       |
| eGFP-SAG1-3'-UTR-R          | GACTAGTCTCGGGGGGGGCAAAGA ATTG                                                 |                                                                                  |
| DHFR-TS-F’                   | GCTCTAGAAAGGCTTCCAGGCT GTAAAATC                                               | To amplify fragment of DHFR-TS drug screen cassette for the generation of RH/GFP |
| DHFR-TS-R’                   | ATAAAGAATGGCGGCGGGGAATTGTCCGTAAGTGAAG                                          |                                                                                  |
| actin-q-F                    | ACTCTTCAGCCAGGTCCTCCAGC                                                        | Human actin amplification for qRT-PCR                                          |
| actin-q-R                    | TCTCCTTCAGCTCAGTGCC                                                          |                                                                                  |
| vim-q-F                      | CACTGAGTACGGGAGACAGG                                                          | Human vimentin amplification for qRT-PCR                                        |
| vim-q-R                      | GAAGGTTGACGAGGCAGTTCC                                                          |                                                                                  |
| actin-KM-F                   | GCCCTTCTCTTGGGTATGGAA                                                          | Mouse actin amplification for qRT-PCR                                          |
| actin-KM-R                   | CAGCTGAGGATGATCGCC                                                             |                                                                                  |
| vim-KM-F                     | TGAGATCGCCAGACTACAGGA                                                          | Mouse vimentin amplification for qRT-PCR                                        |
| vim-KM-R                     | TTGCGCTCTGAGAAAAGTGC                                                          |                                                                                  |
Table S3. Sequences of vimentin specific siRNA

| siRNA | sequence |
|-------|----------|
| siRNA1 | CAGACAGGAUGUUGACAAGUCGUCU  
AGACGCAUUGUCAACAUCCUGUCUG |
| siRNA2 | GGCAUGCUUGACCUUGAACGCAAA  
UUUGCUUCAAGGUCAAGCGGC |
| siRNA3 | AAACUAAGAGGACAGAUUUAACAA  
UUGUAACUGUCAUCUACUAGUU |

Table S4. Formula of Sensitized Emission method

| Variable | Meaning of variable |
|----------|---------------------|
| a        | Donor(CFP) channel image of Donor excited, with Donor only dyed (Background correction done) |
| b        | Acceptor (YFP) channel image of Donor excited, with Donor only dyed (Background correction done) |
| c        | Acceptor channel image of Donor excited, with Acceptor only dyed (Background correction done) |
| d        | Acceptor channel image of Acceptor excited, with Acceptor only dyed (Background correction done) |
| e        | Donor channel image of Donor excited, with Donor and Acceptor dyed (Background correction done) |
| f        | Acceptor channel image of Donor excited, with Donor and Acceptor dyed (Background correction done) |
| g        | Acceptor channel image of Acceptor excited, with Donor and Acceptor dyed (Background correction done) |

| Variable | Meaning of variable |
|----------|---------------------|
| DSBT¹  | Donor Spectral Bleed-through |
| ASBT²  | Acceptor Spectral Bleed-through |
| PFRET³ | Precision FRET (Correction done FRET image) |

\[
\psi_{dd} = \left( \frac{\text{HV of Donor}}{\text{HV of Acceptor}} \right) \times \left( \frac{\text{Spectral sensitivity of Donor}}{\text{Spectral sensitivity of Acceptor}} \right)
\]

\[
\psi_{ea} = \frac{Q_a}{Q_d}
\]

\[Q_a \] Acceptor quantum yield

\[Q_d \] Donor quantum yield

\[R_0 \] Forster Distance

\[1 \text{DSBT}=\frac{b}{a} \times e; \quad 2\text{ASBT}=\frac{c}{d} \times g; \quad 3\text{PFRET}=f-\text{DSBT}-\text{ASBT};\]
FRET Efficiency = \(1 - \left(\frac{e^{\frac{e}{e^{+PFRET} \times (\frac{\Psi_{dd}}{\Psi_{aa}}) \times (\frac{Q_{d}}{Q_{a}})}}}{e^{+PFRET} \times (\frac{\Psi_{dd}}{\Psi_{aa}}) \times (\frac{Q_{d}}{Q_{a}})}\right)\)

Distance = \(R_0 \left(\left(\frac{1}{E}\right) - 1\right)^{1/6}\)
Supplemental Materials and Methods

Antibodies used in this study

Monoclonal primary antibodies

Mouse (mAb) anti-vimentin (Abcam, ab8978, 1:2000); rabbit (Rb) anti-vimentin (Abcam, ab92547, 1:2000); mAb anti-DDDDk (ABclonal, AE005, 1:2000) and Rb anti-beta-actin (CST, 4970, 1:1000) were used for Western Blotting (WB). mAb anti-vimentin (1:250), mAb anti-SAG1 (1:50) and Rb anti-vimentin (1:250) were used for immunofluorescence (IF). mAb anti-vimentin (1:100); anti-FLAG® M2 (Sigma, F1804, 1:100); and anti-DDDDK (1:100) were used for immunoprecipitation (IP) or Co-IP.

Polyclonal primary antibodies

Rb anti-DDDDK (ABclonal, AE004, 1:2000); Rb anti-Phospho Ser/Thr (Abcam, ab17464, 1:1000), Rb anti-ROP2 (1:1000) were used for WB. Anti-ROP2 (1:100) was used for IF.

Secondary antibodies

Secondary antibodies conjugated with HRP, goat anti-mouse IgG-HRP (Santa Cruz, sc2005, 1:2000) and goat anti-rabbit IgG-HRP (Santa Cruz, sc2004, 1:2000) were used for WB detection and those conjugated with goat anti-rabbit IgG, F(ab’)_2-TRITC (Santa Cruz, sc3841, 1:200), goat anti-rabbit IgG-FITC (Santa Cruz, sc2012, 1:200), and goat anti-mouse IgG-R (Santa Cruz, sc2092, 1:200) were used for IF.

Plasmid construction

Vimentin cDNA was amplified by PCR with Pfu DNA polymerase (TransGen Biotech) using the primers indicated in Table S2 and cloned into pEASY-Blunt (TransGen Biotech) for plasmid construction. To perform fluorescence resonance energy transfer (FRET) experiments, vimentin and ROP18 cDNA fragments were inserted into the SalI/Sacl sites of
the plasmids pEYFP-N1 and pECFP-C1, respectively. Vimentin-HA and ROP18-3×flag were cloned into pcDNA3.1 (+) digested with HindIII/BamHI.

To disrupt the rop18 gene in the RH strain, a CRISPR plasmid, pSAG1::CAS9-U6::sgROP18, was generated by replacing the UPRT targeting gRNA in pSAG1::CAS9-U6::sgUPRT [1] with a specific rop18 targeting gRNA sequence, by Q5 DNA polymerase mutagenesis (NEB). A homologous template (pBlue-5'-3'-ROP18-homo-DHFR-TS) was also generated to promote efficient recombinant insertion of the DHFR-TS cassette into the rop18 locus and disrupt the expression of rop18. To generate this homologous template, 5′-homo, 3′-homo, and DHFR-TS cassette were amplified using RH genomic DNA and plasmid pYFP-LIC-DHFR (Addgene, 83114), respectively as templates, using the primers indicated in Table S2. Briefly, a 990bp fragment homologous to the sequence upstream of the gRNA target rop18 gene was cloned into the HindIII/EcoRV sites of the pBlue-script SK II (-) plasmid to generate pBlue-5′-ROP18-homo, and an 850bp fragment homologous to the downstream of the gRNA target rop18 gene was then cloned into the BamHI/XbaI sites of pBlue-5′-ROP18-homo to form pBlue-5′-3′-ROP18-homo. Next, a DHFR-TS cassette was cloned into the EcoRV/BamHI sites of the pBlue-5′-3′-ROP18-homo plasmid to form a recombinant plasmid which was used for electrotransformation of tachyzoites.

To generate the recombinant RH strain expressing C-terminally eGFP-FLAG-tagged ROP18, a CRISPR plasmid, pSAG1::CAS9-U6::sgROP18-in, expressing an sgRNA targeting downstream of the TAA stop codon of the rop18 gene was first generated. Second, a recombinant plasmid containing homologous template for the eGFP-FLAG fusion expression at the C-terminus of ROP18 and insertion of a DHFR-TS cassette was generated. To construct this plasmid, fragments of the 5′-homo-in, 3′-homo-in, and DHFR-TS cassette were amplified from genomic DNA of RH strain and pYFP-LIC-DHFR, eGFP, and SAG1-3′-UTR plasmids were amplified using pSAG1::CAS9-U6::sgUPRT as template; all primers are provided in Table S2. In brief, a 996bp fragment (5′ flanking region)
homologous to the sequence upstream of the rop18 stop codon (TAA), a 1038bp fragment (3’ flanking region) homologous to sequence downstream of the rop18 sgRNA target site, eGFP, SAG1-3’-UTR, and the DHFR-TS cassette were amplified and inserted into pBlue-script II SK(-), using NEBuilder® HiFi DNA Assembly Master Mix (NEB, E5520).

To generate the pBlue-p24-eGFP-DHFR-TS plasmid, the p24 promoter was amplified from RH strain parasites and eGFP-SAG1-3’-UTR and DHFR-TS were amplified using the templates described above with the primers indicated in Table S2. Briefly, p24 promoter, eGFP-SAG1-3’-UTR, and DHFR-TS fragments were cloned into the Apal/BamHI, BamHI/Spel and XbaI/NotI sites of the plasmid pBluescript II SK(-), respectively. The recombinant plasmid was then linearized with Apal prior to transfection into parasites, to generate a strain ectopically expressing GFP, RH/GFP.

**DNA and siRNA transfection**

Cos7 cells were seeded in 6-well plates and 3 μg of plasmid DNA per well were used for transfection with Lipofectamine® 2000 (Invitrogen, 11668019), following the protocol provided by the manufacturer. For transfection of cells in T-75 culture flasks and 12-well plates, 18μg or 1μg (per well), respectively, of plasmid DNA were used.

Three vimentin specific siRNAs, VIMHSS111286 (siRNA1), VIMHSS111287 (siRNA2), and VIMHSS187671 (siRNA3) (Invitrogen), and a negative control siRNA (12935-300, Invitrogen) were transfected into HFF cells using Lipofectamine® 2000 reagent following the protocol provided by the manufacturer. Information of used siRNAs was shown in Table S3. Before transfection, red fluorescent control siRNA (Invitrogen, 14750100) was used to optimize the transfection conditions and 90pmol/well (3μl/well) Lipofectamine® 2000 was used in subsequent experiments. After siRNA transfection for different periods of time (1, 2, 3, 5, and 7d), cells were collected to extract total RNA and protein to identify the transcription and expression levels of vimentin. After evaluation by qRT-PCR and western blotting, the most efficient siRNA (siRNA2) was chosen for use in subsequent experiments.
**Purification of recombinant ROP18, vimentin and GST**

rop18 was amplified from T. gondii RH strain genomic DNA, starting from Glu83, based on the second ATG [2]. Nucleotides encoding the 6× His tag were incorporated into the reverse primer to generate a rop18 cDNA encoding a protein with 6×His residues at the C-terminus. The ROP18-His fragment was then subcloned into pGEX-4T-2, and expressed as a fusion protein with an N-terminal GST tag, in E.coli BL21-CodonPlus (DE3)-RIPL (Microgene, Shanghai, China), by overnight induction with 1mM isopropyl-β-D-thiogalactopyranoside (IPTG) at 18°C [2, 3]. Cells were lysed and the GST-ROP18-His fusion protein purified using a Ni-NTA fast start kit (Qiagen), according to the manufacturer’s instructions. Nucleotides encoding the 6× His tag were cloned into plasmid pGEX-4T-2 at the 3′ terminal of GST nucleotides by Q5 DNA polymerase mutagenesis (NEB) and this recombinant plasmid was transformed into E. coli BL21 cells. Bacterial expression of GST was then induced with 1mM IPTG at 37°C for 4h and then purified by Ni-NTA fast start kit as above description.

Full-length human vimentin cDNA was cloned into the pET28a (+) vector and this recombinant plasmid was transformed into E. coli BL21 (DE3) cells. Bacterial expression of vimentin was then induced with 1mM IPTG at 37°C for 4h. Cells were collected by centrifugation, resuspended in PBS with protease inhibitors (TransGene, China), and lysed by sonication. Vimentin was purified as previously described [4]. Briefly, lysates were centrifuged and the insoluble fraction resuspended in 20ml dissolving buffer 1 (Triton X-100, 200mM NaCl, 10mM EDTA, 50mM Tris-Cl, pH 8.0), homogenized and re-centrifuged at 10,000g for 20min at 4°C three times. The pellet was resuspended in dissolving buffer 2 (10mM EDTA, 50mM Tris-Cl, pH 8.0), homogenized and re-centrifuged as previously indicated. After resuspension in dissolving buffer 3 (8M urea, 200mM EDTA, 5mM DTT, 200mM Tris-Cl, pH 8.0) and incubated overnight at 4°C, soluble vimentin was collected by centrifugation (27,300g for 30min at 4°C) and the urea removed from the samples by step-wise dialysis into dialysis buffer 1 (4M urea, 5mM DTT, 10mM Tris-Cl, pH 8.0) for 4h, followed by dialysis buffer 2 (2M urea, 5mM DTT, 10mM Tris-Cl, pH 8) for another 4h, and
finally dialysis buffer 3 (10mM Tris-Cl, pH 7.0) overnight. The dialysis product was then centrifuged at 1,000g at 4°C for 10 min, the supernatant was collected for further analysis with precipitate discard.

References:

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