Supporting information

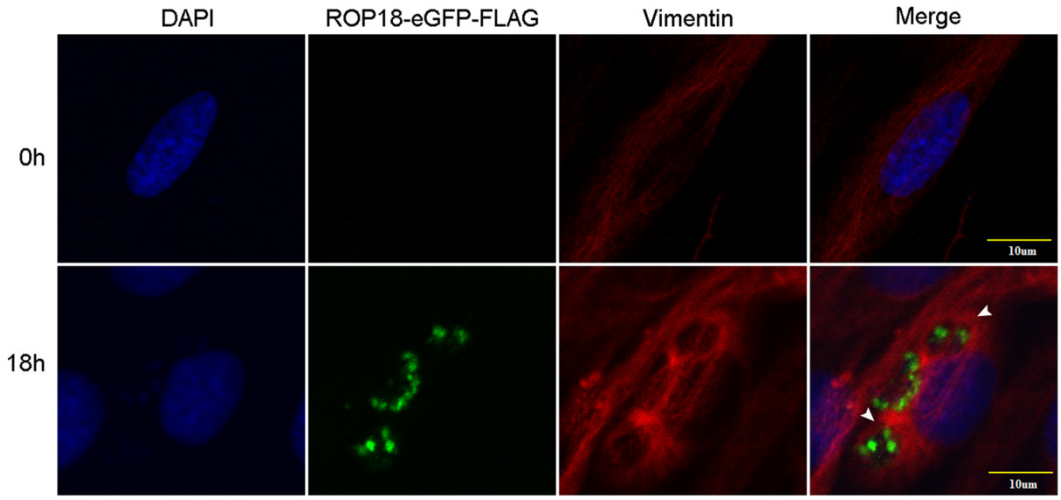

Figure S1. Observation of vimentin rearrangement in HFF cells infected with*T. gondii*. HFF cells were infected with *T. gondii* RH strain for 18 h or uninfected (controls), and then fixed with paraformaldehyde. An indirect immunofluorescence assay (IFA) was performed. The results of IFA demonstrate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

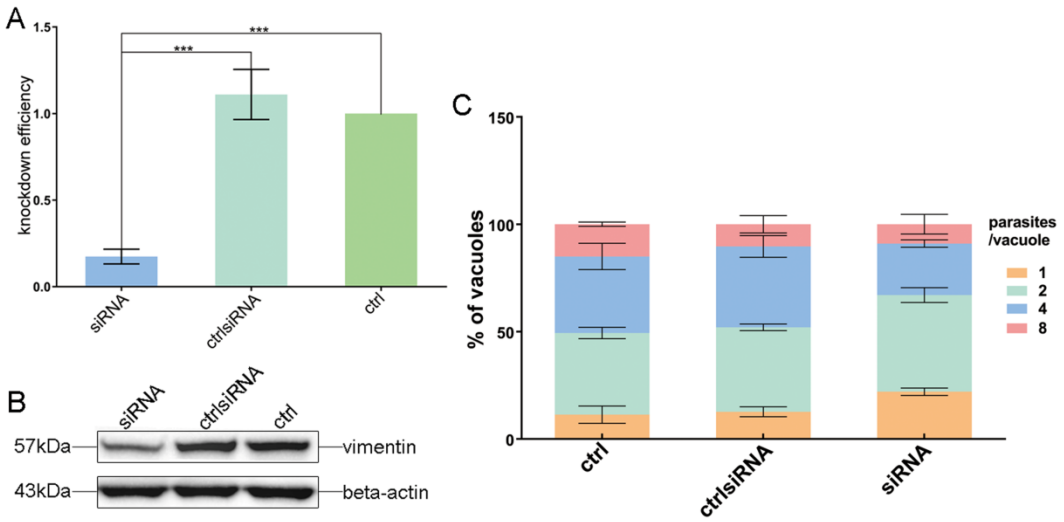

Figure S2. Host cell vimentin had no obvious effect on the proliferation of *T. gondii*.A. qRT-PCR was performed to verify the knockdown of vimentin in HFF cells treated with siRNA (t test, ***$p \leq 0.001$). B. Knockdown of vimentin was also demonstrated by Western Blot. C. Number of tachyzoites per 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

Table S2. Primers used in this study

Table S3. Information of siRNA used in this study

Table S4. Formula information of Sensitized Emission method used in this study.

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 3×flag tag was cloned into pcDNA3.1(+)	ROP18 expression in cells
pSAG1::Cas9-U6::sgROP18	Cas9 expressed from the SAG1 promoter and CRISPR gRNA targeting ROP18 produced from the U6 promoter	CRISPR plasmid targeting *rop18*
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	
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	
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 p Blue-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

		GST

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	TTATTCAAGGTCATCGTGATGCTG	
Vimentin-F’	ACGCGTCGACATGTCCACCAGGTCCGTGTC	To amplify fragment of *vimentin* for pEYFP-Vim cloning
Vimentin-HA-R	TCCCCGCGGTTAAGCGTAATCTGGAACATCGTATGGGTATTCAAGGTCATCGTGATGCTGAG	
ROP18-F	ACGCGTCGACATGTTTTCGGTACAGCGGCC	To amplify fragment of *rop18* for pECFP-ROP18 cloning
ROP18-flag-R	TCCCCGCGGTTACTTATCGTCATCGTCTTTGTAATCAATATCATGATCCTTGTAGTCTCCGTCGTGGTCCTTATAGTCTTCTGTGTGGAGATGTTCCTGC	
Vimentin-F’’	CCCAAGCTTATGTCCACCAGGTCCGTG	To amplify fragment of vimentin for pcDNA3.1-vim-HA cloning
Vimentin-HA-R’	CGCGGATCCTTAAGCGTAATCTGGAACATCG	
ROP18-F’	CCCAAGCTTATGTTTTCGGTACAGCG	To amplify fragment of ROP18 for pcDNA3.1-ROP18-flag cloning
ROP18-flag-R’	CGCGGATCCTTACTTATCGTCATCGTCTTTG	
SgRop18-F	TACGCGTACCGTCGTCCGAAGTTTTAGAGCTAGAAATAGC	Q5 mutagenesis changing the gRNA in pSAG1::CAS9-U6::sgUPRT for the disruption of *rop18*
SgRop18-R	AACTTGACATCCCCATTTAC	
SgROP18-F-in	GCCGACCGAACGCCTCTCGAGTTTTAGAGCTAGAAATAGC	Q5 mutagenesis changing the gRNA in pSAG1::CAS9-U6::sgUPRT for the knockin.of.eGFP-FLAG.fused.to.*rop18*
SgROP18-R-in	AACTTGACATCCCCATTTAC	
5'-homo-F-ROP18	CCCAAGCTTGCAGTTGCACAGGGACGACG	To amply fragment of 5’-homo for the knockout of *rop18*
5'-homo-R-ROP18	CGGATATCAGAGGTGGCCGCTGTACCG	
3'-homo-F-ROP18	CGGGATCCTGGGTTTAGCGACTCTTCTCCC	To amply fragment of 3’-homo for the knockout of *rop18*
3'-homo-R-ROP18	GCTCTAGAGGATGCTGGCTGTCCCTCTAAC	
DHFR-TS-F	CGGATATCAAGCTTCGCCAGGCTGTAAATC	To amply fragment of DHFR-TS drug screen cassette for the knockout of *rop18*
DHFR-TS-R	CGGGATCCCAGGAATTCATCCTGCAAGTGC	
5'-homo-F-ROP18-in	GGGCCCCCCCTCGAGGTCGACGGTATCGATACTTAAGCGACTGGATAGAATTCTGAC	To amply fragment of 5’-homo to tag endogenous *rop18* with eGFP-FLAG
5'-homo-R-ROP18-in	CATCCATGGCGCGCCTTCTGTGTGGAGATGTTC	
eGFP-F-in	TCTCCACACAGAAGGCGCGCCAT	To amply fragment of eGFP for the

	GGATGGTGAGCAAGGGCGAG	knockin of eGFP-FLAG
eGFP-R-in	CGTCATCGTCTTTGTAATCAATATC ATGATCCTTGTAGTCTCCGTCGTG GTCCTTATAGTCCTTGTACAGCTC GTCCATGCC	
SAG1-3'-UTR-F-in	TGATATTGATTACAAAGACGATGA CGATAAGTAGTTAATTAATCACCG TTGTGCTCACTTCTCAAATCG	To amplify fragment of SAG1-3’-UTR for the knockin of eGFP-FLAG
sAG1-3'-UTR-R-in	CCTGGCGAAGCTTAGCTCCACCG CGGTGGCG	
DHFR-TS-F-in	CCGCGGTGGAGCTAAGCTTCGCC AGGCTGTA	To amplify fragment of DHFR-TS drug screen cassette for the knockin of eGFP-FLAG
DHFR-TS-R-in	TATGACGATTTAAATACGTAGGAA TTCATCCTGCAAGTGC	
3'-homo-F-ROP18-in	CAGGATGAATTCCTACGTATTTAA ATCGTCATAAGCGAATTAAAACAG	To amplify fragment of 3’-homo to tag endogenous *rop18* with eGFP-FLAG
3'-homo-R-ROP18-in	GGTGGCGGCCGCTCTAGAACTAG TGGATCCGTAGTTGTTCATATGAA AGACAG	
Donor-knockout-F	GCAGTTGCACAGGGACGACG	To amplify fragment of homologous template for *rop18* disruption
Donor-knockout-R	GGATGCTGGCTGTCCCTCTAAC	
PCR1-F	CACATCAATGTGTGTGTACATGC	PCR1
PCR1-R	GAATGACATGCTAGCGTTCAAC	
PCR2-F	CACGCATGTCTACACGAACC	PCR2
PCR2-R	GACTGGATAGGTACATTCGAACG	
PCR3-F	GAGACTGTCACAGCTCGTCG	PCR3
PCR3-R	GCGACAGTCAGAATTCTATCCAG	
PCR4-F-in	CTGTCCGTACGACAGAGGCTTG	PCR4
PCR4-R-in	GCAGATGAACTTCAGGGTCAGC	
PCR5-F-in	ACGCATGTCTACACGAACCATG	PCR5
PCR5-R-in	CAGGCAGAGTTCTCCATCTTCG	
PCR6-F-in	GCAGAGGGAGTCGTCCTGCTAC	PCR6
PCR6-R-in	CTAGGCAACGCCAGATGGATAG	
P24-F	GCGGGCCCTCGAAGGCTGTAGTA CTGGTGCT	To amplify the promoter of *P24*
P24-R	CGGGATCCCTTGCTTGATTTCTTC AAAG	
eGFP-SAG1-3’-UTR-F	CGGGATCCGTGAGCAAGGGCG	To amplify fragment of eGFP-SAG1-3’-UTR
eGFP-SAG1-3’-UTR-R	GACTAGTCTCGGGGGGGCAAGA ATTG	
DHFR-TS-F’	GCTCTAGAAAGCTTCGCCAGGCT GTAAATC	To amplify fragment of DHFR-TS drug screen cassette for the generation of RH/GFP
DHFR-TS-R’	ATAAGAATGCGGCCGCGGAATTC ATCCTGCAAGTGCATAGAAG	
actin-q-F	ACTCTTCCAGCCTTCCTTCC	Human actin amplification for qRT-PCR
actin-q-R	TCTCCTTCTGCATCCTGTCG	
vim-q-F	CACTGAGTACCGGAGACAGG	Human vimentin amplification for qRT-PCR
vim-q-R	GAAGGTGACGAGCCATTTCC	
actin-KM-F	GCCTTCCTTCTTGGGTATGGAA	Mouse actin amplification for qRT-PCR
actin-KM-R	CAGCTCAGTAACAGTCCGCC	
vim-KM-F	TGAGATCGCCACCTACAGGA	Mouse vimentin amplification for qRT-PCR
vim-KM-R	TTGCGCTCCTGAAAAACTGC	

vim-full-F	GCATCATATGTCCACCAGGTCCGTG	To amplify fragment of vimentin for pET28a-Vim cloning
vim-full-R	CCCGGATCCTTATTCAAGGTCATCGTGATGC	
ROP18-his-F	CGGGATCCATGGAAAGGGCTCAACACC	To amplify fragment of *rop18* for pGST-ROP18 cloning
ROP18-his-R	ACGCGTCGACTTAGTGATGATGATGATGATGCTTGTCATCGTCATCCTTG	
GST-His-F	TCATCACTAAGTCGACTCGAGCGGCCGC	To insert 6×His into pGST-4T-2 for the purification of GST
GST-His-R	TGATGATGATGGGATCCACGCGGAACCAGATC	

Table S3. Sequences of vimentin specific siRNA

siRNA	sequence
siRNA1	CAGACAGGAUGUUGACAAUGCGUCU
	AGACGCAUUGUCAACAUCCUGUCUG
siRNA2	GGCACGUCUUGACCUUGAACGCAAA
	UUUGCGUUCAAGGUCAAGACGUGCC
siRNA3	AAACUAGAGAUGGACAGGUUAUCAA
	UUGAUAACCUGUCCAUCUCUAGUUU

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)
DSBT[1]	Donor Spectral Bleed-through
ASBT[2]	Acceptor Spectral Bleed-through
PFRET[3]	Precision FRET (Correction done FRET image)
$\frac{\Psi_{dd}}{\Psi_{aa}}$	$\left(\frac{\text{HV of Donor}}{\text{HV of Acceptor}}\right) \times \left(\frac{\text{Spectral sensitivity of Donor}}{\text{Spectral sensitivity of Accptor}}\right)$
Q_a	Acceptor quantum yield
Q_d	Donor quantum yield
R_0	Forster Distance

[1] DSBT=$\left(\frac{b}{a}\right) \times e$; [2]ASBT=$(\frac{c}{d})\times g$; [3]PFRET=f-DSBT-ASBT;

FRET Efficiency=$1-\left[\frac{e}{e+PFRET\times\left(\frac{\Psi_{dd}}{\Psi_{aa}}\right)\times\left(\frac{Q_d}{Q_a}\right)}\right]$

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 *Sal*I/*Sac*II sites of

the plasmids pEYFP-N1 and pECFP-C1, respectively. Vimentin-HA and ROP18-3×flag were cloned into pcDNA3.1 (+) digested with *Hind*III/*Bam*HI.

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 *Hind*III/*EcoR*V 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 *Bam*HI/*Xba*I sites of pBlue-5′-ROP18-homo to form pBlue-5′-3′-ROP18-homo. Next, a DHFR-TS cassette was cloned into the *EcoR*V/*Bam*HI 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 *Apa*I/*Bam*HI, *Bam*HI/*Spe*I and *Xba*I/*Not*I sites of the plasmid pBluescript II SK(-), respectively. The recombinant plasmid was then linearized with *Apa*I 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 with1mM 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 at10,000g 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 10min, the supernatant was collected for further analysis with precipitate discard.

References:

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3. Steinfeldt T, Konen-Waisman S, Tong L, et al. Phosphorylation of mouse immunity-related GTPase (IRG) resistance proteins is an evasion strategy for virulent *Toxoplasma gondii*. PLoS Biol. 2010; 8: e1000576.

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