The Role of Potassium and Host Calcium Signaling in *Toxoplasma gondii* egress

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Supporting Information
Supplemental Figures:

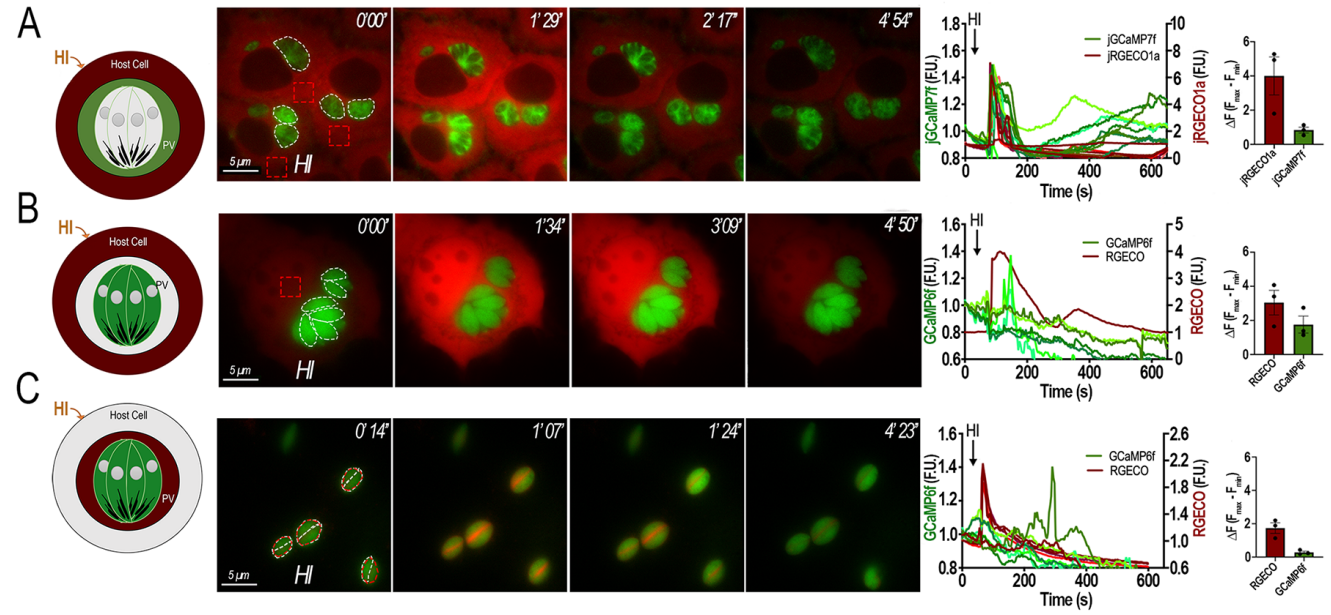

Fig. S1. Impact of host histamine signaling on *T. gondii* Ca^{2+}: **A,** stable HeLa cells expressing jRGECO1a were infected with tachyzoites expressing jGCaMP7f localized to the PV. Dashed white outlines indicates the area used for the green fluorescence (PVs) analysis and the red squares show the region of the host cell used for the analysis of the jRGECO1a fluorescence shown in the graphs. **B,** HeLa cells transiently expressing the red GECI RGECO infected with tachyzoites expressing cytosolic GCaMP6f. Dashed white outlines show the parasites used for the fluorescence analysis shown in the graphs and the dashed red square shows the region of the host cell used to analyze red fluorescence. **C**, HeLa cells infected with tachyzoites expressing cytosolic GCaMP6f and RGECO in the PV. Dashed white outlines indicate parasites and dashed red outlines indicate the PV region used to analyze the fluorescence shown in the graph. Under all conditions 100 µM Histamine was added at the 1 min mark and the response recorded. Numbers at the upper right of each panel indicate the time frame of the video. Tracings to the right of each panel shows green fluorescence (GCaMP6f or jGCaMP7f) and red fluorescence (jRGECO1a or RGECO) fluctuations with the scales for the green fluorescence shown on the left Y axis and for the red fluorescence on the right Y scale. Ca^{2+} fluctuations of PVs are shown in A (*green*) and C (*red*). Single parasites fluctuations are shown in B and C (*green*). Host cytosolic changes are shown in A and B (*red*). Bar graphs represent quantification of the average ΔF values of a minimum of three independent trials (*red*, jRGECO1a or RGECO) (*green*, GCaMP6f or jGCaMP7f) with Standard Error of the Mean (S.E.M.) represented as error bars.

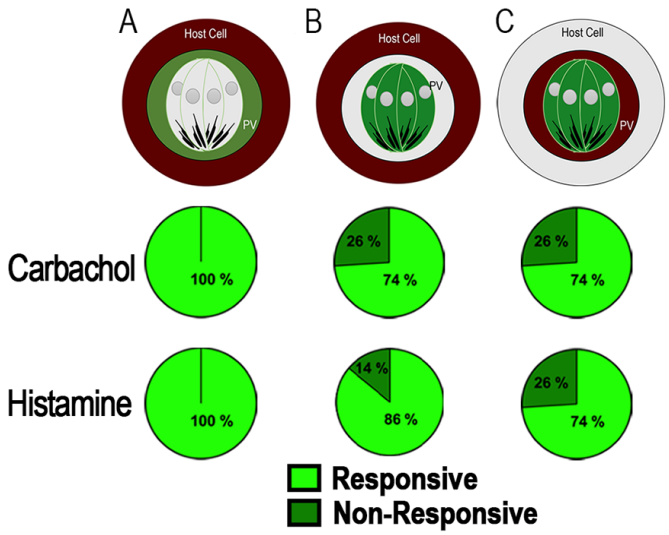

Figure S2. Quantification of parasite responsiveness after addition of carbachol or histamine: A, Quantification of responsive vs. non-responsive PVs from Figs 1A (carbachol) and S1A (histamine). 100% of PVs respond to the addition of either agonist. **B**, Quantification of the % of responsive tachyzoites expressing cytosolic GCaMP6f to carbachol or histamine. The experiment are the ones presented in Fig. 1B and S1B. **C**, % of tachyzoites responding to Carbachol or Histamine addition. The experimental conditions are the ones from Figs 1C and S1C. Light green portions are used to represent the percentage of responsive cells and dark green portions are used to represent the percentage of non-responsive cells.

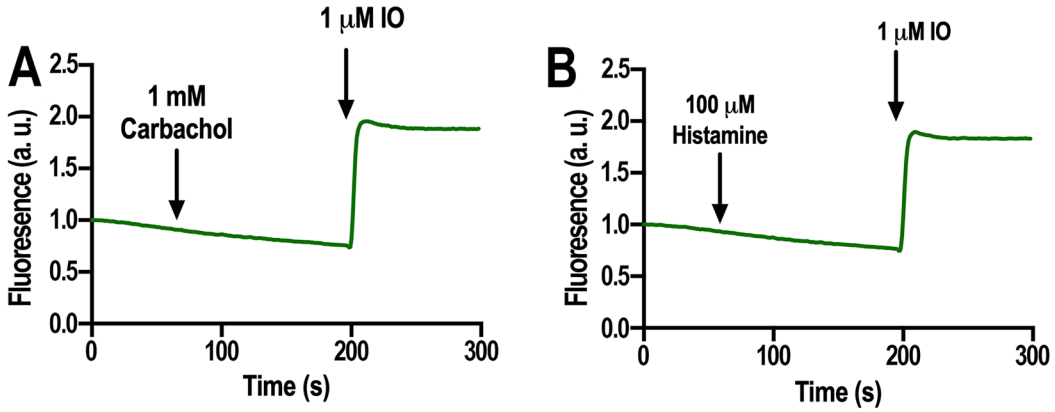

Figure S3. Extracellular tachyzoites expressing cytosolic GCaMP6f do not respond to carbachol or histamine: The fluorescence changes of 2 x 10^7 cells/mL of GCaMP6f tachyzoites resuspended in Ringer Buffer supplemented with 2 mM Ca^{2+} were analyzed in a fluorescence spectrophotometer, Hitachi 7000. Excitation was set at 485 nm and emission at 520 nm. Traces shown are an average of three independent experiments conducted on separate cell preparations. (A) Addition of 1 mM Carbachol (A) or 100 μM Histamine (B) at 60 seconds lead to no increase in the GCaMP6f signal and at 200 second 1 μM of Ionomycin was added as control for responsiveness.

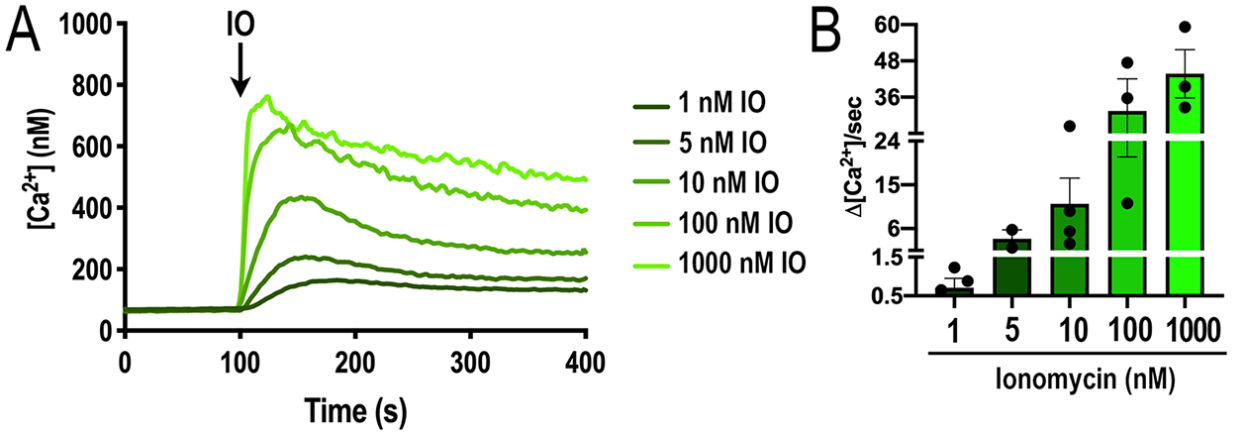

Fig S4. Calibration of Ca^{2+} threshold: (A) RH tachyzoites (5 x 10^7) were loaded with FURA2-AM and resuspended in 2.5 mL of Ringer's Buffer supplemented with 100 µM EGTA. The cytosolic Ca^{2+} response to the addition of various concentrations of ionomycin is shown. The figure shows the average of at least 2 trials of each condition; (B) Quantification of ionomycin titration curve. The first 15 seconds of the slope after addition of ionomycin was determined and averaged from at least two independent trials

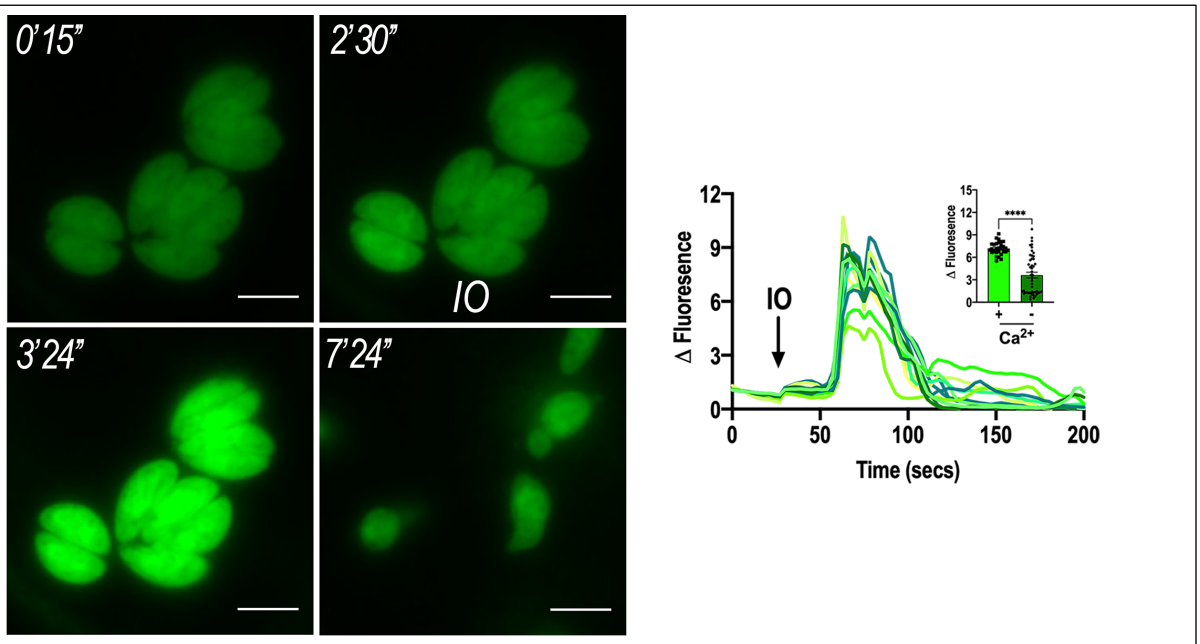

Fig. S5. Ionomycin Induced Egress in Ca^{2+} Free Buffer: A) Hela cells were infected with GCaMP6f-expressing tachyzoites in buffer supplemented with 100 µM EGTA to create a Ca^{2+}-free extracellular buffer. 1 µM Ionomycin was added to trigger egress. B) Representative fluorescence tracings of ionomycin addition in Ca^{2+} free buffer. Inset: quantification of ionomycin induced egress between Ca^{2+}-rich and Ca^{2+}-free media. Error bars represent the Standard Error of the Mean (S.E.M.)

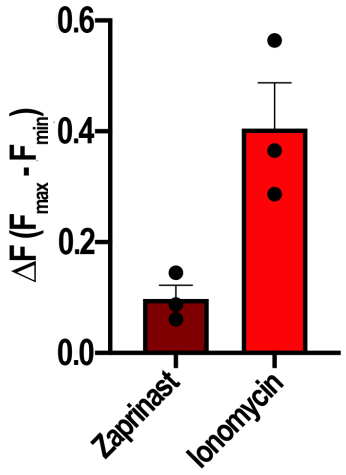

Fig S6. Off targeting effect of zaprinast on host cells: HeLa cells stably expressing jRGECO1a were used for video microscopy analysis. 100 µM Zaprinast was added at approximately 60 seconds and 1 µM ionomycin was added at 300 seconds. ΔF represents the difference between the maximum fluorescence and minimum fluorescence 10 seconds before and 10 seconds after addition of either zaprinast or ionomycin

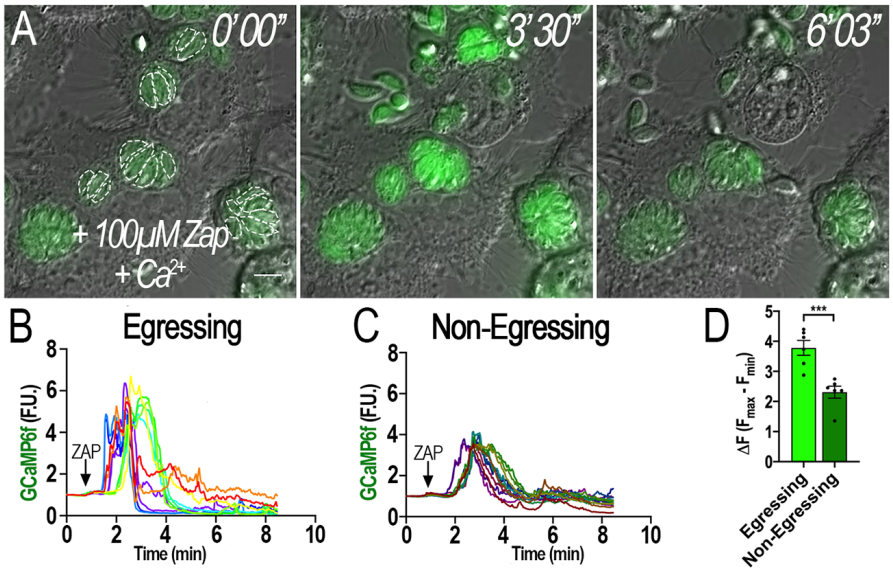

Fig S7. Parasites that do not show the second Ca^{2+} peak are less likely to egress. A, Egress was induced in Hela cells infected with ΔPLP1 GCaMP6f parasites using 100 µM zaprinast; **B and C,** Representative fluorescence tracings of ΔPLP1 GCaMP6f after addition of 100 µM Zaprinast for egress and non-egressing parasites respectively. Notice, two peaks are evident for the parasites that were successfully able to egress and only one peak was visible for the parasites that were unable to egress. **D**, Quantification of egressing and non-egressing ΔPLP1 GCaMP6f parasites. The average ΔFluorescence (F_{max} - F_{min}) of at least 10 cells per either egressing and non-egressing were averaged for 3 independent trials.

Legends for Supplemental Videos

Video S1. Carbachol addition in GCaMP6f Infected Host Cells: Hela cells transiently expressing cytosolic localized RGECO were infected with cytosolic GCaMPf6 expressing parasites. 1 mM Carbachol was added at approximately the 1 min mark. (Fig. 1B shows still images from this video).

Video S2. Thapsigargin stimulation in LAR-GECO expressing host cells: Hela cells transiently expressing the low affinity mitochondrial Ca^{2+} indicator LAR-GECO1.2 were infected with cytosolic GCaMP6f expressing parasite. 2 μM Thapsigargin was added at approximately the 1 min mark. (Fig. 2B shows still images from this video).

Video S3. Two-peaks of Ca^{2+} contribute to parasite egress: Hela cells infected with cytosolic GCaMP6f parasites were treated with 100 μM of Zaprinast in media supplemented with 2 mM Ca^{2+}. (Fig. 3E shows still images from this video).

Video S4. Natural egress in cpd 1 synchronized parasites: Hela cells stably expressing cytosolic jRGECO1a infected with cytosolic GCaMP6f expressing parasites. After 24 h post infection 1 μM cpd1 was added. 24 hr post cpd1 addition parasite egress was imaged after cpd1 wash off. (Fig. 4A shows still images from this video).

Video S5. Whole-cell patch under high K^+ and high Ca^{2+} conditions: Hela cells infected with GCaMP6f parasites were whole-cell patched with a patch pipette solution containing 140 mM K^+ and 10 μM Ca^{2+}. Note parasite egress is slow, methodical, and occurring one-by-one in comparison to detergents and ionophores. Video presented in real time. (Fig. 6B shows still images from this video).

Video S6. Whole-cell patch under low K^+ and high Ca^{2+} conditions: Hela cells infected with GCaMP6f parasites were whole-cell patched with a patch pipette solution comprising 10 mM K^+, 130 mM choline chloride, and 10 μM Ca^{2+}. Parasite egress was slower in comparison to detergents and ionophores, but more rapidly in comparison to high K^+ conditions. Parasite egress occurred almost explosively (all parasites egressed at once) under these conditions. Video presented in real time. (Fig. 6C shows still images from this video).

Supplemental Tables

Table S1: Genetically Encoded Calcium Indicators (GECI) used in this study

GECI	Kd (nM)	Dynamic Range	Use	Source or Reference
GCaMP6f	375	51.8	Standard, well established GECI, with fast on-off kinetics. We previously used this GECI to study *T. gondii* Ca^{2+} dynamics	[1]
R-GECO	482	16	First generation red GECI, enables multi-color imaging when co-expressed with GCaMP6f or jGCaMP7f	[2]
jRGECO1a	148	12	Second generation red GECI with improved kinetics and enhanced sensitivity	[3]
jGCaMP7f	174	30.2	Successor to GCaMP6f with improved sensitivity and faster on/off kinetics	[4]
LAR-GECO1.2	12,000	8.7	Low affinity red GECI with optimized kinetics/sensitivity for the mitochondria of mammalian cells. Host mitochondria surrounds the PV and this indicator permits to see Ca^{2+} fluxes through the host mitochondria	[5]

Table S2: Parasite strains generated and used in this work

Strains	Characteristics	Source/Ref
RH with cytosolic GCaMP6f	Non-selectable drug RH strain expressing GCaMP6f in the cytosol	[6]
RH PV-jGCaMP7f	Expression of jGCaMP7f in the lumen of the PV	This study
RH PV-RGECO *Cyto*-GCaMP6f	Expression of PV-RGECO in the lumen of the PV and cytosolic expression of GCaMP6f	[6]

Table S3: Plasmids used for this work

Plasmid	Characteristics	Source or Reference
pGP-CMV-GCaMP6f	Transient expression of the GECI GCaMP6f in mammalian cells	[1]
pCMV-NLS-R-GECO	Transient expression of the GECI R-GECO in mammalian cells	[2]
pGP-CMV-NES-jRGECO1a	Transient expression of the GECI jRGECO1a in mammalian cells	[3]
pGP-CMV-jGCaMP7f	Transient expression of the GECI jGCaMP7f in mammalian cells	[4]
pCMV-mito-LAR-GECO1.2	Transient expression of the GECI LAR-GECO1.2 in the mitochondria of mammalian cells	[5]
ptub_IEα-DsRed_DHFR_sag1CATsag1	Plasmid vector for expressing proteins in the lumen of the PV	[7]
pCTH3	Random Integration of a chloramphenicol selection plasmid expressing 3xHA epitopes	[8]
ptub_IEα-R-GECO_DHFR_sag1CATsag1	Expressing the GECI R-GECO in the lumen of the PV	[6]
pUltra	Lentivirus expression of a gene of interest	[9]
jRGECO1a-pUltra	Lentivirus expression of the Red GECI jRGECO1a	This study

Table S4: Oligonucleotide primers used in this work

To construct PV-jGCaMP7f		
SV229-jGCaMP7f-GIB-FWD	GGGCTGCAatgggttctcatcatcatc	This study
SV72-jRCaMP1b-Gib-R	ccgggacgtcgtacgggtacctaggCTTCGCTGTCATCATTTGTAC	This study
SV77-P30-Gib-Fwd	TTTCTTGAATTCCCTTTTAGATCTATGTTTCCGAAGGCAGTGAGACG	This study
SV-228-P30-Gib-Rev	gaacccatTGCAGCCCCGGCAAACTC	This study
SV225-pCTH3-Gib-FWD	cagcgaagCctaggtacccgtacgac	This study
SV226-Tub-Gib-Rev	CGGAAACATAGATCtaaaagggaattcaagaaaaaatg	This study
To construct jRGECO1a-pUltra		
SV155V2-jRGECO1a-AgeI-Fwd	aCCGGTATGCTGCAGAACGAGCTTGCTCTTA	This study
SV156-jRGECO1a-ECORI-Rev	GAATTCGCCTACTTCGCTGTCATCATTTGTACA	This study

References for supplemental Tables:

1. Akerboom J, Carreras Calderon N, Tian L, Wabnig S, Prigge M, Tolo J, et al. Genetically encoded calcium indicators for multi-color neural activity imaging and combination with optogenetics. Front Mol Neurosci. 2013;6:2. Epub 2013/03/06. doi: 10.3389/fnmol.2013.00002. PubMed PMID: 23459413; PubMed Central PMCID: PMCPMC3586699.
2. Zhao Y, Araki S, Wu J, Teramoto T, Chang YF, Nakano M, et al. An expanded palette of genetically encoded Ca(2)(+) indicators. Science. 2011;333(6051):1888-91. Epub 2011/09/10. doi: 10.1126/science.1208592. PubMed PMID: 21903779.
3. Dana H, Mohar B, Sun Y, Narayan S, Gordus A, Hasseman JP, et al. Sensitive red protein calcium indicators for imaging neural activity. Elife. 2016;5. Epub 2016/03/25. doi: 10.7554/eLife.12727. PubMed PMID: 27011354; PubMed Central PMCID: PMCPMC4846379.
4. Dana H, Sun Y, Mohar B, Hulse BK, Kerlin AM, Hasseman JP, et al. High-performance calcium sensors for imaging activity in neuronal populations and microcompartments. Nat Methods. 2019;16(7):649-57. Epub 2019/06/19. doi: 10.1038/s41592-019-0435-6. PubMed PMID: 31209382.
5. Wu J, Prole DL, Shen Y, Lin Z, Gnanasekaran A, Liu Y, et al. Red fluorescent genetically encoded Ca2+ indicators for use in mitochondria and endoplasmic reticulum. Biochem J. 2014;464(1):13-22. Epub 2014/08/29. doi: 10.1042/BJ20140931. PubMed PMID: 25164254; PubMed Central PMCID: PMCPMC4214425.
6. Borges-Pereira L, Budu A, McKnight CA, Moore CA, Vella SA, Hortua Triana MA, et al. Calcium Signaling throughout the Toxoplasma gondii Lytic Cycle: A STUDY USING

GENETICALLY ENCODED CALCIUM INDICATORS. J Biol Chem. 2015;290(45):26914-26. Epub 2015/09/17. doi: 10.1074/jbc.M115.652511. PubMed PMID: 26374900; PubMed Central PMCID: PMCPMC4646405.
7. Kafsack BF, Pena JD, Coppens I, Ravindran S, Boothroyd JC, Carruthers VB. Rapid membrane disruption by a perforin-like protein facilitates parasite exit from host cells. Science. 2009;323(5913):530-3. Epub 2008/12/20. doi: 10.1126/science.1165740. PubMed PMID: 19095897; PubMed Central PMCID: PMCPMC2662845.
8. Chasen NM, Stasic AJ, Asady B, Coppens I, Moreno SNJ. The Vacuolar Zinc Transporter TgZnT Protects Toxoplasma gondii from Zinc Toxicity. mSphere. 2019;4(3). Epub 2019/05/24. doi: 10.1128/mSphere.00086-19. PubMed PMID: 31118298; PubMed Central PMCID: PMCPMC6531880.
9. Lou YL, Guo F, Liu F, Gao FL, Zhang PQ, Niu X, et al. miR-210 activates notch signaling pathway in angiogenesis induced by cerebral ischemia. Mol Cell Biochem. 2012;370(1-2):45-51. Epub 2012/07/27. doi: 10.1007/s11010-012-1396-6. PubMed PMID: 22833359.