The Surface Antigen 1(SAG1) of Toxoplasma Gondii Interacts With RACK1 Inducing Autophagy and Maintaining The Viability of Host Cells

Ni An
Jinzhou Medical University
Bing Li
Jinzhou Medical University
Yu-ming Liu
Nanjing Agricultural University
Zhi-xuan Zhang
Jinzhou Medical University
Rongjian Su (teachersurongjian@126.com)
Jinzhou medical university
Xiao-gang Liu
Jinzhou Medical University

Research

Keywords: T.gondii, SAG1, RCAK1, non-canonical autophagy

Posted Date: January 28th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-154045/v1

Abstract

Background

It has been well known that the surface antigen 1(SAG 1) of T.gondii plays an important role in the invasion of Tachyzoite into host cells. However whether it also play a role in the intracellular parasitism of T.gondii remains unclear. The main purpose of this study was to determine the effect of SAG1 on host cells and investigate the underlying mechanism.

Methods

SAG1 was overexpressed in human embryonic kidney cell 293(HEK) by transfection. Autophagy was determined by fluorescent microscope and flow cytometry (FCM) in HEK293 cells co-transfected with Flag-SAG1 and EGFP-LC3. The interaction of SAG1 and RACK1 was measured by co-immunoprecipitation(Co-IP), GST pulldown and fluorescent microscope. The expression of cytokines including IL-1β, IL-6, and IL-12 was determined by qRT-PCR. The expression of LC3, Ki67 and RACK1 was detected by Western blot. Cellular senescence was measured by β-galactosidase staining.

Results

We found that overexpression of SAG1 in human embryonic kidney cells (HEK293) induces non-canonical autophagy and inhibition of autophagy using hydroxychloroquine (HCQ) significantly decreases the cell viability of HEK293 cells. Mechanically, we identified RACK1, an intracellular multifunctional protein as a binding partner of SAG1. Depletion of RACK1 inhibited SAG1 induces non-autophagy and decreases the enhanced expression of cytokines including IL-1β, IL-6, IL-12 and TNF-α in SAG1 overexpressing cells.

Conclusion

These data showed that SAG1 could induce non-canonical autophagy and facilitate the expression of IL-1β, IL-6, IL-12 and TNF-α by interacting with RACK1, maintaining the viability of host cells. Our results suggests a new contribution of SAG1 in the intracellular parasitism.

Background

Toxoplasma gondii (T. gondii) is a common intracellular protozoan parasite, causing 30% of the global human population infection worldwide[1]. T.gondii exists in the body of host in the forms of tachyzoites and bradyzoite[2]. The tachyzoites infection is regarded as the main cause of toxoplasmosis[3]. The surface antigen1 (SAG1) of T.gondii is a kind of GPI-anchored proteins and expressed exclusively on the surface of tachyzoites[4]. Many data have demonstrated that SAG1 plays important roles in the invasion of tachyzoites to host cells. Blockade of SAG1 using specific monoclonal antibody inhibits the attachment of tachyzoites to host cells[5]. Recent advances demonstrated that *T.gondii* could manipulate the cellular process of host cells for its intracellular parasitism[6]. For example, *T.gondii* could maintain

the viability of host cells by inhibiting cell apoptosis under adverse conditions such as CTL-mediated cytotoxicity, IL-2 deprivation, gamma irradiation, UV irradiation, making host cells suitable for its intracellular survival[7].

The responses of host cells to *T.gondii* infection play essential roles in the pathogenicity of *T.gondii*[8]. So far, little is known about the mechanism by which *T. gondii* exploits host cell processes to their own advantage. Autophagy is an evolutionarily process by which cellular materials are delivered to the lysosomes for disposal[9]. Many data have demonstrated the roles of autophagy in many cellular processes including T.gondii infection of host cells. Based on the mechanism by which autophagy is initiated, it can be divided into the canonical autophagy and the non-canonical autophagy[10]. It has been reported that *T. gondii* infection could induce autophagy in many mammalian cells including Hela cells and murine bone marrow derived macrophages(BMDMs), suggesting the critical role of autophagy in antiparasitic responses of host cells[11]. For example, IFN-γ could induce non-canonical autophagy by recruitment of γ-aminobutyric acid (GABA) receptor-associated protein-like 2 (GABARAPL2), causing growth restriction of *T.gondii* in Hela cells[12].[13]Abrogation of autophagy by ATG5 knockout in T.gondii infected intestinal cells aggravates INF-γinduced inflammation, causing the destruction of intestinal structure [14]. These data suggest that autophagy may play a protective role on host cells during *T.gondii* infection.

Receptor for activated C kinase 1(RACK1) is an intracellular scaffolding protein and plays critical roles in the regulation of many essential cellular process including cell growth, cell adhesion and migration[15]. Serveral lines of evidence have demonstrated that RACK1 is involved in the regulation of autophagy by facilitating the formation of autophagosome in vivo and in vitro[16]. For example, RACK1 could induce autophagy in human cancer cells and autophagy induced by RACK1 could maintain the viability of cancer cells and inhibits cell apoptosis[17].

This study was therefore designed to investigate whether SAG1 could induce autophagy, maintaining the viability of host cells in the process of *T.gondii* infection. We demonstrated that SAG1 could induce non-canonical autophagy therefore maintain the viability of host cells. Further analysis showed that SAG1 could interact with RACK1 and knockdown of RACK1 inhibits autophagy induced by SAG1.

Methods

Cell culture

Human embryonic kidney cell line 293T (HEK293T) was purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). The cells were cultured in DMEM medium supplemented with 10% FBS, 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin at 37°C, 5% CO2 -95% O2.

Transfection

The SAG1 recombinants were kindly given by Dr. Na Yang (Shenyang Agricultural University). Transfection was performed according to the instruction of Polyfectine (PEI) transfection reagent (Biowit Technologies, China). Confluent HEK293 cells cultured in a 60 cm2 dish were transfected with 10 μg recombinant plasmid plus 20 μl Polyfectine(PEI) transfection reagent. The transfection efficiency was determined by western blot after 48 hours of transfection. The recombinant plasmids we used were pEGFP-N1, pEGFP-N1-SAG1-GT1, pcDNA3.1 (+)-Flag-SAG1-GT1, pcDNA3.1 (+)-Flag-SAG1-RH, pcDNA3.1 (+)-HA-RACK1, pcDNA3.1 (+)-mCherry-RACK1. The SAG1 positive cells were collected by FACS sorting of HEK293 cells transfected with EGFP-SAG1 recombinant plasmid and amplified under normal culture condition. Cells transfected with pEGFP-N1 were also sorted and used as control.

Mass spectrum

HEK293 cells transfected with Flag tagged SAG1 from GT1 or RH strains were collected after 48 hours of transfection. The expression of Flag-SAG1 from GT1 or RH strains were determined using western blot. The anti-Flag immunoprecipitation was performed and the precipitates were subjected to Mass Spectrum analysis and the possible binding proteins of SAG1 were identified.

Western blot

Preparation of whole cell lysates and western blot analysis was performed as described. The primary antibodies used in this study were anti-EGFP (1:1000, Origene, USA), anti-HA (1:1000, Origene, USA), anti-Flag (1:1000, Santa Cruz, USA), anti-LC3 (1:1000, Cell signaling, USA), anti-RACK1 (1:1000, Cell signaling, USA) and anti-Actin (1:1000, Santa Cruz, USA).

Flow cytometry analysis of canonical and non-canonical autophagy

Flow cytometry analysis of canonical and non-canonical autophagy were performed as previously described [18]. Briefly, HEK293 cells were co-transfected using Flag/EGFP-LC3 or Flag-SAG1/EGFP-LC3. After 48 hours, Cells were treated with baflomycin A (BAF1) or hydroxychloroquine (HCQ) for 2 hours. Control cells (CON) were treated with DMSO. The mean fluorescent intensity (MFI) was detected using flow cytometry. MFIs of HCQ or BAF1 treated cells represents total autophagy and canonical autophagy level respectively. Canonical autophagy was referred as MFI_{BAF1}-MFI_{con}/MFI_{HCQ}-MFI_{con} and noncanonical autophagy as 1-MFI_{BAF1}- MFI_{con}/MFI_{HCQ}- MFI_{con}.

Anti-Flag immunoprecipitation

Cell lysates contain 1000 μg of total protein from HEK293 cells co-transfected with Flag-SAG1 and HA-RACK1 were pre-cleared with 50 μl of protein A-Sepharose beads(Santa Cruz, USA) for 1 h at 4°C and incubated with 5 μg of anti-Flag antibody overnight at 4°C on a rotator. The total volume of this reaction was 1ml. Following antibody incubation, 100 μl of protein A sepharose beads (50% slurry) were added and rotated at 4°C for 3 hours. The beads were then centrifuged at 12,000g for 3 minutes and washed for 3 times with 1% NP40 lysis buffer. The precipitates were eluted by adding of 50 μl of 1× SDS-PAGE

sample loading buffer (50 mm Tris-HCl, pH 6.8, 100 mm DTT, 2% SDS, 0.1% bromphenol blue, 10% glycerol), followed by boiling at 100°C for 5 min. The supernatant obtained after centrifugation was resolved by 10% SDS-PAGE and subjected to Western blot analysis using anti-HA antibody.

GST pulldown assay

Whole cell lysates containing 1000 μg of total protein were incubated with GST tagged SAG1 protein from GT1 strain or GST (negative control) overnight at 4°C on a rotator(20 μg each), subsequentially add 50 μl of glutathione-Sepharose 4B (50% slurry) (GE Healthcare, USA) 3 hours at 4°C on a rotator. The resins were then washed 5 times with ice-cold lysis buffer. Binding Proteins were eluted by boiling for 5 min and centrifuged for 3 min at 12,000 g. The supernatant was resolved by SDS-PAGE and subjected to Western blot analysis using anti-RACK1 antibody as detecting antibody.

Fluorescencent microscopy

Confluent HEK293 cells were co-transfected with pEGFP-N1-SAG1and pcDNA3.1 (+)-mCherry-RACK1 using PEI transfection reagent in a 35 cm2 culture dish. The transfection system were as follows: 4 μg of pEGFP-N1-SAG1 and 8 μg of mCherry-RACK1 plus 20 μl of PEI. After 48 hours of transfection, the distribution of SAG1 and RACK1 was observed by fluorescent microscope.

qRT-PCR analysis

Total RNA was isolated from HEK293 cells transfected with Flag-SAG1. The levels of IL-1β, IL-6, IL-12, and TNF-α mRNA were determined by qPCR. The primers we used were as previously reported [5], [19].

Senescence-Associated β-Galactosidase Stain

Cellular senescence-associated β-galactosidase staining was conducted using a senescence β-galactosidase staining kit (Beyotime, Shanghai, China) following the manufacturer's instruction. Briefly, Cells in a 6-well culture plate were washed with ice cold PBS and fixed with fixing solution for 30 minutes. After being washed with PBS 3 times, 1 ml of staining solution was added to each well and incubated at 37°C overnight. The staining status was observed and photographed using an inverted microscope.

MTT assay

MTT analysis was performed as previously reported. Briefly, Cells were seeded in a 96-well plate (5000 cells/well) and cultured for 48 h, subsequently 50 μl MTT (0.5 mg/ml) (3-(4, 5 dimethylthiazol-2-yl)-2, 5-di-phenyltetrazolium bromide, Sigma, USA) was added to each well and incubated for another 2 h. The dye was dissolved with DMSO and the absorbance was measured at 570nm using a Microplate Reader.

Statistical analysis

All data are presented as mean ± standard deviation (SD). Each experiment was carried out at least in triplicates. Two-tailed student's t-test was performed to analyze the difference between two groups and

one way ANOVA was used to evaluate the difference of multiple groups. A value of $p < 0.05$ was considered statistically significant.

Results

The SAG1-GT1 protein induces the noncanonical autophagy in HEK293 cells

To investigate whether SAG1 from GT1 strain could induce autophagy in host cells. Using HEK293T as tool cells, we monitored the status of autophagy induction by co-transfection Flag-SAG1 with EGFP-LC3 (Fig.1A). Laser confocal microscopy observation showed that transfection of Flag-SAG1 significantly elevated the number of autophagosomes in HEK293 cells as compared with control cells, indicating that SAG1 could induce autophagy in host cells (Fig.1B).

Recent advances have demonstrated the critical roles of noncanonical autophagy in pathogen infection. For this reason, we next determined the characteristic of autophagy induced by SAG1. HEK293 cells co-transfected with Flag-SAG1 and EGFP-LC3 were treated with BAF1 which inhibits canonical autophagy or HCQ which inhibits canonical and non-canonical autophagy equally for 12 hours and the mean fluorescent intensity(MFI) was determined by flow cytometry(Martinez-Martin et al., 2017) (Fig.1C). The MFI ratio of BAF1 treated cells with that of HCQ treated cells in Flag-SAG1-GT1 transfected cells was significantly higher than that in Mock cells (Fig.1D), indicating that SAG1 protein induces the noncanonical autophagy. Western blot analysis of LC3II levels further confirmed this conclusion (Fig.1E).

Inhibition of SAG1-GT1 induced autophagy decreases the viability of host cells

To address the effect of SAG1 induced autophagy on the viability of host cells, we transfected HEK293 cells with EGFP-SAG1 (pEGFP-N1-SAG1-GT1). SAG1 positive cells (SAG1+) were collected by fluorescent-activated cell sorting (FACS) (Fig.2A). Cells expressing EGFP (GFP+) were also sorted as negative control. The expression status of SAG1 in this population was examined by western blotting (Fig.2B). The SAG1+ and GFP+ cells were treated with HCQ for 48 hours. MTT assay showed that the SAG1+ cells treated with HCQ exhibited a obvious growth delay as compared with control cells, whereas treatment of GFP+ cells with HCQ had no effect on cell viability in relative to corresponding control cells. This results revealed that autophagy induced by SAG1 could maintain the viability of HEK293 cells, indicating the protective role of SAG1-GT1 induced autophagy on host cells (Fig.2C). Moreover, β-galactosidase staining revealed that HCQ treatment significantly elevated the percentage of β-galactosidase positive cells in SAG1+ cells, indicating inhibition of autophagy causes the senescence of host cells (Fig.2D).

The SAG1 protein binds physically with RACK1

To elucidate the molecular mechanism underlying these phenomena, we tried to screen the SAG1 binding partners in HEK293 cells. To address this question, anti-Flag immunoprecipitation assay was performed in the lysis of HEK293 cells transfected with Flag-SAG1. The precipitations were subjected to mass spectrum analysis. Subtracted the proteins common with control group, 11 common SAG1 interacting

proteins were identified in the samples of HEK293 cells transfected with SAG1-GT1 or Flag-SAG1-RH (Fig.3A). Based on the m/z ratio of these targeted protein, we identified RACK1 as a binding partner of SAG1 (Fig.3B).Taken the function of SAG1 and the cellular localization of RACK1 into consideration, we chose RACK1, which play crucial functions in the regulation of autophagy, for further research.

We then aimed to verify the interaction between SAG1 and RACK1 using anti-flag co-immunoprecipitation. The results showed that RACK1 co-precipitated with flag-SAG1 (Fig.4A). Moreover, we investigated whether SAG1 could interact directly with RACK1. To address this question, GST-pulldown assay was performed in the whole cell lysis from HEK293 using recombinant GST-SAG1 as baiting proteins. Subsequent western blotting analysis revealed that RACK1 was detected in the precipitations of recombinant GST-SAG1 (Fig.4B). The interaction was further confirmed by the co-localization of SAG1 and RACK1 in SAG1 positive cells transfected with mCherry-RACK1 (Fig.4C). Taken together, these data indicate that SAG1 could bind physically with RACK1 in HEK293 cells.

Depletion of RACK1 negatively regulates autophagy and cell viability

Having demonstrated the interaction of SAG1 and RACK1, we then investigated the functional link of in SAG1 and RACK1. We first examined RACK1 levels in SAG1+ and GFP+ cells mentioned above and found that RACK1 level in SAG1+ cells was markedly higher than that in GFP- cells, suggesting a positive correlation between SAG1 and RACK1 (Fig. 5A). We also found increased autophagy marker LC3-II level and Ki67 level which is regarded as the marker of cell viability in the SAG1+ cells as compared with the GFP+ cells (Fig. 5A). We next investigated whether SAG1 regulates the expression of RACK1. Western blot analysis revealed that transfection of Flag-SAG1 significantly elevated the expression of RACK1 and LC3-II levels in a dose dependent manner, suggesting that SAG1 could regulate the expression RACK1, activating autophagy (Fig.5B). Moreover, we depleted RACK1 level in in the SAG1 positive cells and western blot analysis revealed that depletion of RACK1 significantly decreased LC3-II and Ki67 levels (Fig.5C). β-galactosidase staining also demonstrated that knockdown of RACK1 in the SAG1+ cells decreased the viability of host cells (Fig.5D and E).

Depletion of RACK1 reduces the SAG1-GT1 induced expression of pro-inflammatory cytokines.

Many data have emphasized the immune modulation roles of SAG1 on host cells, however whether SAG1 affects the secretion of pro-inflammatory cytokines remains to be addressed. For this purpose, we compared the levels of IL-1β, IL-6, IL-12, and TNF-α at mRNA level between SAG1 positive and negative cells. We found that the mRNA levels of these pro-inflammatory cytokines in SAG1 positive cells were significantly higher than those in SAG1 negative cells (Fig.6A).

To determine the role of RACK1 in SAG1 induced pro-inflammatory cytokines production, we knocked down RACK1 level in SAG1 positive cells using siRNAs against RACK1 and found that depletion of RACK1 in SAG1 positive cells obviously decreased the IL-1β, IL-6, IL-12, and TNF-α mRNA levels induced by SAG1 overexpression, (Fig.6B). Taken together, these data suggested that Depletion of RACK1 reduces the SAG1-GT1 induced expression of pro-inflammatory cytokines.

Discussion

Upon infected by *T.gondii*, host cells trigger autophagy to eliminate intracellular parasite [20]. As an important surface antigen, SAG1 play important roles in the invasion of *T.gondii* into host cells [21]. However, little is known about the role of SAG1 in autophagy induction. In the present study, we found that SAG1 could induce autophagy in host cells by ectopically expressed SAG1 in HEK293 cells. Furthermore, we treated HEK293 cells using autophagy inhibitor BAF1 and HCQ respectively and analyzed the characteristic of autophagy induced by SAG1 and demonstrated that SAG1 induces autophagy in a non-canonical manner. These data indicated that SAG1 could activate non-canonical autophagy and this finding expands the knowledge of the function of SAG1 during the process of *T.gondii* infection. Researches by other groups have demonstrated the critical role of non-canonical autophagy in the anti-parasitic response in IFN-γ activated macrophages in vitro. The major virulence factors ROP18 and ROP5 determines the fate of parasites in IFN-γ activated macrophages via ATG5 dependent mechanism, indicating that host cells rely on ATG5 for maintaining homeostasis[22].

Because *T.gondii* is an obligatory intracellular parasite, manipulating the cellular process of host cells is very crucial for its own intracellular survive[7]. From the view of pathogen, it is essential for *T.gondii* evade the autophagic degradation of host cells and simultaneously maintain the viability of host cells. Nowadays it has been reported that *T.gondii* could protect itself from autophagic degradation by multiple mechanisms. For example, T.gondii infection causes the prolonged activation of pro-proliferation signaling pathways including EGFR and FAK signaling pathway protecting it from autophagic degradation, maintaining the viability of host cells[23]. However, from the point of host cells, pathogen invasion including bacteria and parasites causes the apoptosis of host cells in most conditions[24]. Because autophagy and apoptosis play important roles for the successful intracellular parasitism of *T.gondii*, it is essential for *T.gondii* to maintain the viability of host cells to satisfy its survive need. We found in this study that inhibition of SAG1 induced autophagy with HCQ in HEK293 cells significantly decreased cell viability and induced cell senescence, indicating the protective role of autophagy induced by SAG1 on the viability of host cells.

RACK1 is a very important intracellular scaffold proteins and play important roles in the regulation of cell viability and immune modulation [17]. In this study, we found that SAG1 could interact with RACK1 and elevate the level of RACK1. Knockdown of RACK1 in SAG1 overexpressing HEK293 cells decreases autophagy level therefore decreasing the viability of HEK293 cells. This data suggested that the critical role of RACK1 in autophagy induced by SAG1.However, the mechanism by which SAG1 regulates the extent and function of RACK1 remains to be further investigated in our future research.

Host cells could secret pro-inflammatory cytokines including INF-γ and TNF-α during *T.gondii* infection by which *T.gondii* could maintain the viability of host cells. INF-γ and TNF-α secreted by host cells infected by *T.gondii* could promote the proliferation of host cells and inhibit the apoptosis of host cells. We found that SAG1 protein could facilitate the secretion of pro-inflammatory cytokines including IL-1β, IL-6, IL-10, IL-12, INF-γ and TNF-α. Knockdown of RACK1 in SAG1 overexpressing cells significantly inhibited the

expression of these pro-inflammatory cytokines. This results indicate that SAG1 could maintain the viability of host cells by promoting RACK1 mediated production of the pro-inflammatory cytokines besides autophagy induction.

Taken together, our data proposed a new points about the role of SAG1 in T.gondii infection. SAG1 plays critical roles not only in the invasion of T.gondii into host cells, but also maintain the viability of host cells by inducing the noncanonical autophagy and promoting the secretion of pro-inflammatory cytokines. These findings hints that unraveling the mechanism of SAG1 induced autophagy may provide new insight in the prevention of T. gondii infection and new ideas for the treatment of toxoplasmosis.

Availability of data and materials

All data are included in this published article

Declarations

Acknowledgements

We thank the staffs of life science institution of Jinzhou medical university for the technical support

Funding

This research was supported by grants of the National Key Research and Development Program of China (2017YFD0500400).

Author information

Affiliations

Life Science institution, Jinzhou Medical University, Jinzhou, Liaoning province, China, 121001

Ni An, Rong-jian Su

Animal Husbandry and Veterinary Medicine College, Jinzhou Medical University, Jinzhou, Liaoning province, China, 121001

Bing Li, Zhi-xuan Zhang, Xiao-gang Liu

Veternary Medicine College, Nanjing Agricultural University, Nanjing, Jiangsu province, China

Yu-mind Liu

Contributions

LXG and SRJ designed this study and wrote this article. AN, LB performed the experiments and analyzed the data. ZZX, LYM performed the experiments. AN, LB contributed equally to this work

Corresponding author

Correspondence to Rong-jian Su and Xiao-gang Liu

Ethics Declarations

Ethics approval and consent to participate

Not applicable

Consent to publish

All the authors agree to the terms of the BioMed Central Copyright and License Agreement and approve publication of this paper

Competing interests

The authors declare they have no conflicting results

References

1. Gisbert Algaba I, Verhaegen B, Murat JB, et al. Molecular Study of Toxoplasma gondii Isolates Originating from Humans and Organic Pigs in Belgium. Foodborne Pathog Dis.2020. https://doi.org/10.1089/fpd.2019.2675
2. Wang Z-D, Wang S-C, Liu H-H, et al. Prevalence and burden of Toxoplasma gondii infection in HIV-infected people: a systematic review and meta-analysis. Lancet HIV. 2017; 4:e177–e188. https://doi.org/10.1016/S2352-3018(17)30005-X
3. Hanifehpour H, Samsam Shariat SK, Ghafari MS, et al. Serological and Molecular Diagnosis of Toxoplasma gondii Infections in Thalassemia Patients. Iran J Parasitol.2019; 14:20–28
4. Lekutis C, Ferguson DJ, Grigg ME, et al. Surface antigens of Toxoplasma gondii: variations on a theme. Int J Parasitol. 2001; 31:1285–1292. https://doi.org/10.1016/s0020-7519(01)00261-2
5. Lai M-Y, Abdul-Majid N, Lau Y-L. Identification of Host Proteins Interacting with Toxoplasma gondii SAG1 by Yeast Two-Hybrid Assay. Acta Parasitol.2019; 64:575–581. https://doi.org/10.2478/s11686-019-00066-4
6. Zhu W, Li J, Pappoe F. Strategies Developed by Toxoplasma gondii to Survive in the Host. Front Microbiol.2019; 10:899. https://doi.org/10.3389/fmicb.2019.00899
7. Zhou L-J, Chen M, Puthiyakunnon S, et al. Toxoplasma gondii ROP18 inhibits human glioblastoma cell apoptosis through a mitochondrial pathway by targeting host cell P2X1. Parasit Vectors. 2019; 12:284. https://doi.org/10.1186/s13071-019-3529-1
8. Hisaeda H, Sakai T, Ishikawa H, et al. Heat shock protein 65 induced by gammadelta T cells prevents apoptosis of macrophages and contributes to host defense in mice infected with Toxoplasma gondii. J Immunol.1997; 159:2375–2381

9. Fracchiolla D, Martens S. Sorting out “non-canonical” autophagy. EMBO J.2018; 37. https://doi.org/10.15252/embj.201798895
10. Dupont N, Nascimbeni AC, Morel E, Codogno P. Molecular Mechanisms of Noncanonical Autophagy. Int Rev Cell Mol Biol.2017; 328:1–23. https://doi.org/10.1016/bs.ircmb.2016.08.001
11. Lee J, Choi J-W, Han HY, et al. 4-Hydroxybenzaldehyde Restricts the Intracellular Growth of Toxoplasma gondii by Inducing SIRT1-Mediated Autophagy in Macrophages. Korean J Parasitol. 2020; 58:7–14. https://doi.org/10.3347/kjp.2020.58.1.7
12. Chu JQ, Jing KP, Gao X, et al. Toxoplasma gondii induces autophagy and apoptosis in human umbilical cord mesenchymal stem cells via downregulation of Mcl-1. Cell Cycle. 2017; 16:477–486. https://doi.org/10.1080/15384101.2017.1281484
13. Zhu YC, He Y, Liu JF, Chen J. Adjuvantic cytokine IL-33 improves the protective immunity of cocktailed DNA vaccine of ROP5 and ROP18 against toxoplasma gondii infection in mice. Parasite. 2020; 27:26. https://doi.org/10.1051/parasite/2020021
14. Burger E, Araujo A, López-Yglesias A, et al. Loss of Paneth Cell Autophagy Causes Acute Susceptibility to Toxoplasma gondii-Mediated Inflammation. Cell Host Microbe.2018; 23:177-190.e4. https://doi.org/10.1016/j.chom.2018.01.001
15. Duff D, Long A. Roles for RACK1 in cancer cell migration and invasion. Cell Signal. 2017; 35:250–255. https://doi.org/10.1016/j.cellsig.2017.03.005
16. Zhao Y, Wang Q, Qiu G, et al. RACK1 Promotes Autophagy by Enhancing the Atg14L-Beclin 1-Vps34-Vps15 Complex Formation upon Phosphorylation by AMPK. Cell Rep. 2015; 13:1407–1417. https://doi.org/10.1016/j.celrep.2015.10.011
17. Xiao T, Zhu W, Huang W, et al. RACK1 promotes tumorigenicity of colon cancer by inducing cell autophagy. Cell Death Dis.2018; 9:1148. https://doi.org/10.1038/s41419-018-1113-9
18. Martinez-Martin N, Maldonado P, Gasparrini F, et al. A switch from canonical to noncanonical autophagy shapes B cell responses. Science. 2017; 355:641–647. https://doi.org/10.1126/science.aal3908
19. Ouhara K, Munenaga S, Kajiya M, et al. The induced RNA-binding protein, HuR, targets 3’-UTR region of IL-6 mRNA and enhances its stabilization in periodontitis. Clin Exp Immunol. 2018; 192:325–336. https://doi.org/10.1111/cei.13110
20. Gao D, Zhang J, Zhao J, et al. Autophagy activated by Toxoplasma gondii infection in turn facilitates Toxoplasma gondii proliferation. Parasitol Res. 2014; 113:2053–2058. https://doi.org/10.1007/s00436-014-3853-5
21. Kobayashi M, Aosai F, Hata H, et al. Toxoplasma gondii: difference of invasion into tissue of digestive organs between susceptible and resistant strain and influence of IFN-gamma in mice inoculated with the cysts perorally. J Parasitol.1999; 85:973–975.
22. Selleck EM, Orchard RC, Lassen KG, et al. A Noncanonical Autophagy Pathway Restricts Toxoplasma gondii Growth in a Strain-Specific Manner in IFN-γ-Activated Human Cells. mBio. 2015; 6:e01157-01115. https://doi.org/10.1128/mBio.01157-15

23. Lopez Corcino Y, Gonzalez Ferrer S, Mantilla LE, et al. Toxoplasma gondii induces prolonged host epidermal growth factor receptor signalling to prevent parasite elimination by autophagy: Perspectives for in vivo control of the parasite. Cell Microbiol. 2019; 21:e13084. https://doi.org/10.1111/cmi.13084
24. Mammari N, Halabi MA, Yaacoub S, et al. Toxoplasma gondii Modulates the Host Cell Responses: An Overview of Apoptosis Pathways. BioMed Res Int. 2019; 6152489. https://doi.org/10.1155/2019/6152489

Figures

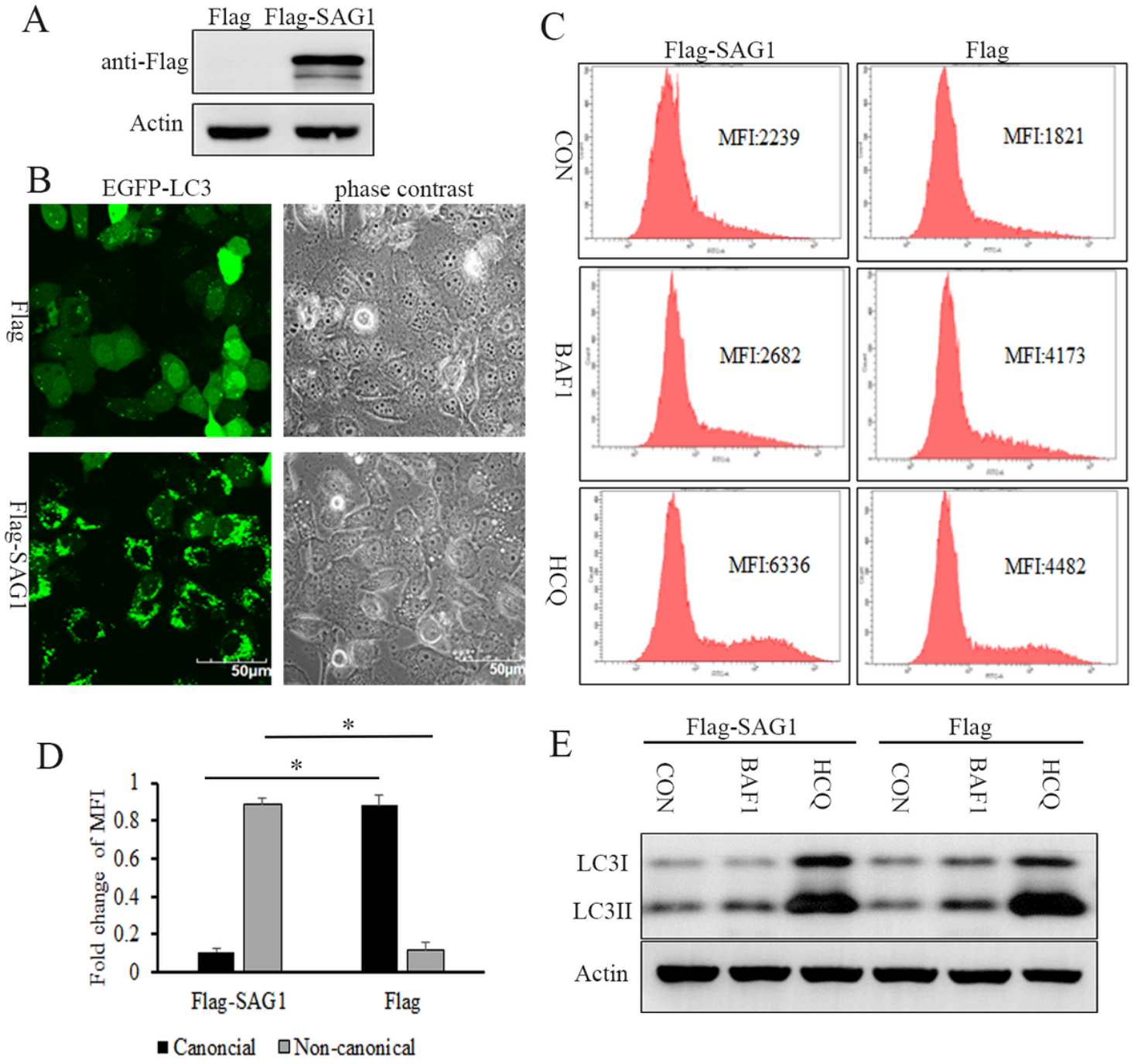


Figure 1

overexpression of SAG1 in HEK293 cells induces non-canonical autophagy. A. Western blot analysis of Flag-SAG1 levels in HEK293 cells. B. Confocal microscopy observation of the formation of autophagosomes in HEK293 cells co-transfected with Flag tagged SAG1 and EGFP-LC3. HEK293 cells co-transfected with blank vector and EGFP-LC3 were used as control (Scale bar: 50 μM). C. Flow cytometry analysis of autophagy status in HEK293 cells co-transfected with Flag tagged SAG1 and EGFP-LC3 treated with autophagy inhibitors HCQ and BAF1 respectively. The autophagy levels were represented by

mean fluorescent intensity (MFI). D. Schematic show of the status of canonical and non-canonical autophagy in HEK293 cells overexpressing SAG1. E. Western blot analysis of LC3 activation in Flag-SAG1 transfected HEK293 cells treated with HCQ or BAF1 respectively. These experiments were repeated for three times and the data was represented by mean±SD.

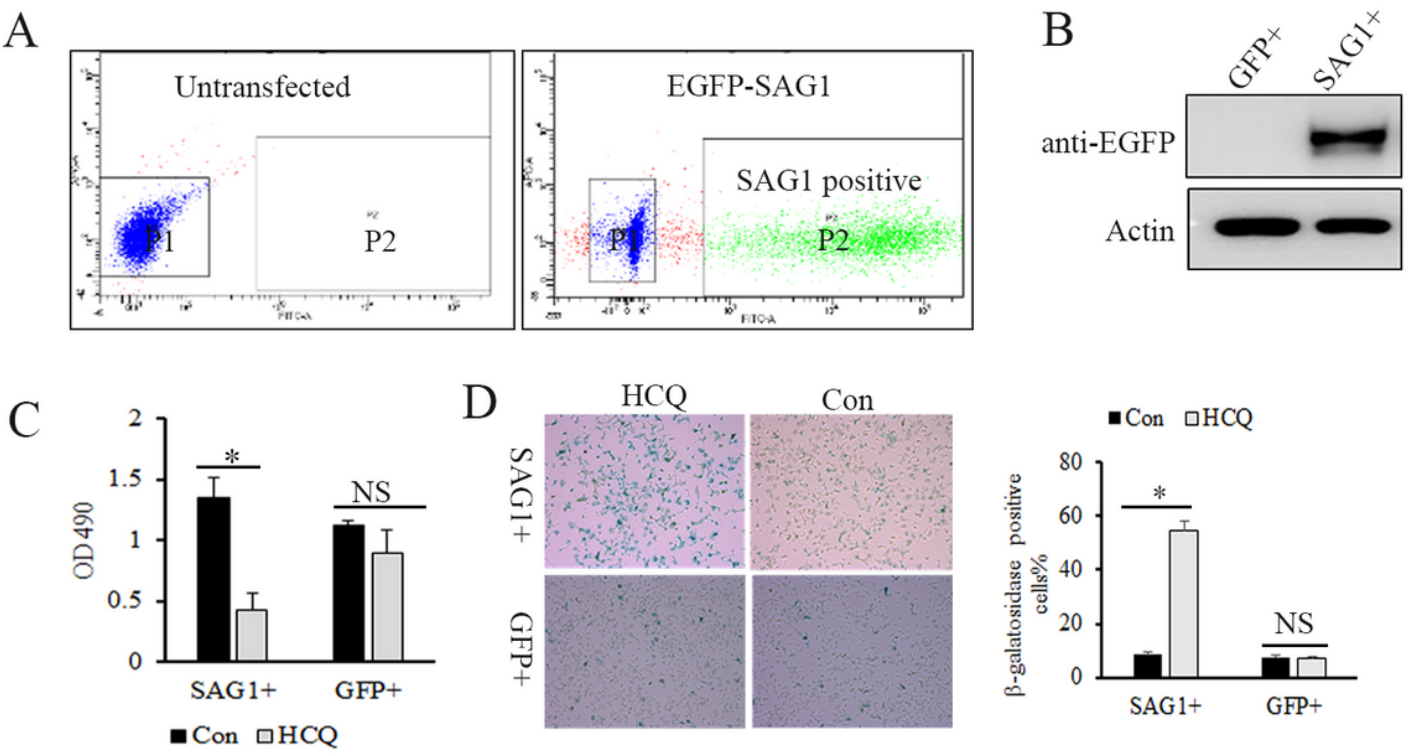
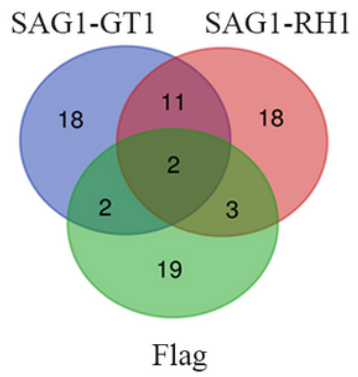


Figure 2

Autophagy induced by SAG1 maintains cell viability of host cells. A. FACS Sorting of HEK293 cells overexpressing EGFP-SAG1(SAG1+). B. Western blot analysis of EGFP-SAG1 level in SAG1+ cells. C. Inhibition of autophagy by HCQ decreased the viability of SAG1 positive cells, whereas did not affect the viability of control cells(GFP+). D. Inhibition of autophagy in SAG1+ cells induced cell senescence, however had little effect on control cells. These experiments were repeated for three times and the data was represented by mean±SD.

A



B

Acession	Peptide sequence	Unique peptide
sp|O43390|HNRPR_HUMAN	DLYEDELVPLFEK,TGYTLDVTTGQR,SFSEFGKLER	3
	EIVLAKPPDK,TGYTLDVTTGQR,NLATIVTEEILEK,DLYEDELVPLFEK	4
sp|P22087|FBRL_HUMAN	NLVPGESVYGEKR,RVSISEGDDKIEYR,ANCIDSTASAEAVFASEVKK,VSISEGDDKIEYI	4
	DHAVVVGVYRPPPK,VSISEGDDKIEYR,RVSISEGDDKIEYR	3
sp|O76031|CLPX_HUMAN	SNILLLGPTGSGK,TLLAQTLAK	2
	TLLAQTLAK,SNILLLGPTGSGK	2
sp|P62424|RL7A_HUMAN	AGVNTVTTLVENK,TCTTVAFTQVNSEDKGALAK	2
	AGVNTVTTLVENK	1
sp|P63244|RACK1_HUMAN	FSPNSSNPIIVSCGWDK,IIVDELKQEVISTSSK	2
	LWNTLGVCK,IIVDELKQEVISTSSK	2
sp|P62241|RS8_HUMAN	QWYESHYALPLGR,IIDVVYNASNNELVR,ISSLLEEQFQQGK	3
	ISSLLEEQFQQGK,IIDVVYNASNNELVR,LDVGNFSWGSECCTR	3
sp|Q71U36|TBA1A_HUMAN	TIQFVDWCPTGFK	1
	VGINYQPPTVVPGGDLAK,QLFHPEQLITGKEDAANNYAR,DVNAAIATIK,AVFVDLEP	4
sp|P61353|RL27_HUMAN	YSVDIPLDK	1
	YSVDIPLDK	1
sp|Q02543|RL18A_HUMAN	DLTTAGAVTQCYR	1
	DLTTAGAVTQCYR	1
sp|P46777|RL5_HUMAN	VTNRDIICQIAYAR	1
	VGLTNYAAAYCTGLLLAR	1
sp|P62753|RS6_HUMAN	DIPGLTDTTVPR,KGEKDIPGLTDTTVPR,LNISFPATGCQK,KLFNLSKEDDVR,LFNLSKE	5
	KGEKDIPGLTDTTVPR	1
	VAVFGAGGVGK	1

Figure 3

Mass spectrum analysis of the SAG1 interacting proteins in HEK293 cells. A. Venn diagram of the status of SAG1 interacting proteins by anti-Flag immunoprecipitation in HEK293 cells. B. List of SAG1 interacting proteins in HEK293 cells obtained by Mass Spectrum.

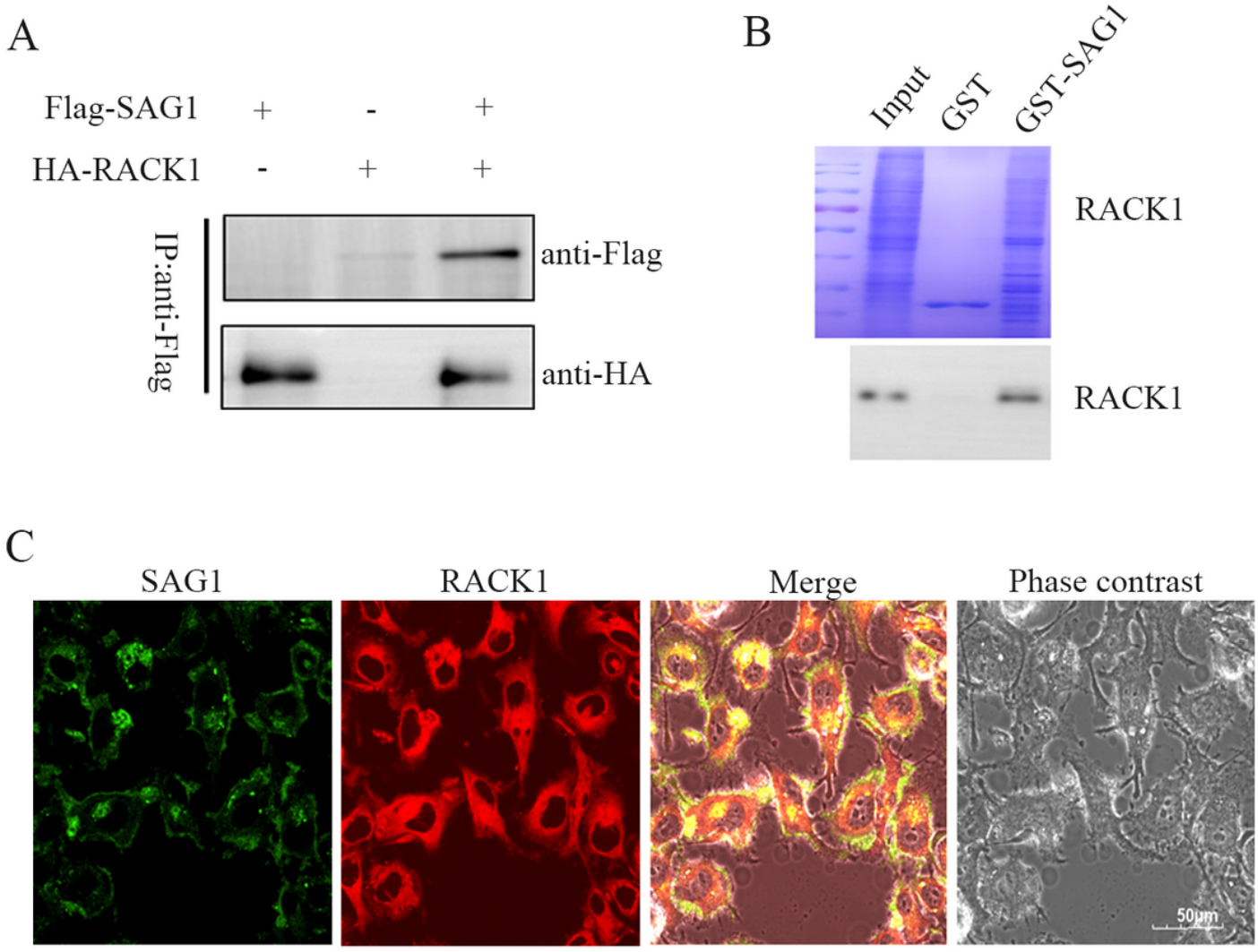


Figure 4

SAG1-GT1 interacted physically with RACK1 in HEK293 cells. A. Co-immunoprecipitation of the interaction of SAG1 and RACK1 in HEK293 cells using anti-Flag immunoprecipitation. B. GST pulldown analysis of SAG1 and RACK1 in HEK293 cells using GST tagged SAG1 as bait. C. Immunofluorescence observation of the co-localization of SAG1 and RACK1 in HEK293 cells co-transfected with EGFP tagged SAG1 and mCherry tagged RACK1 (Scale bar: 50μM).

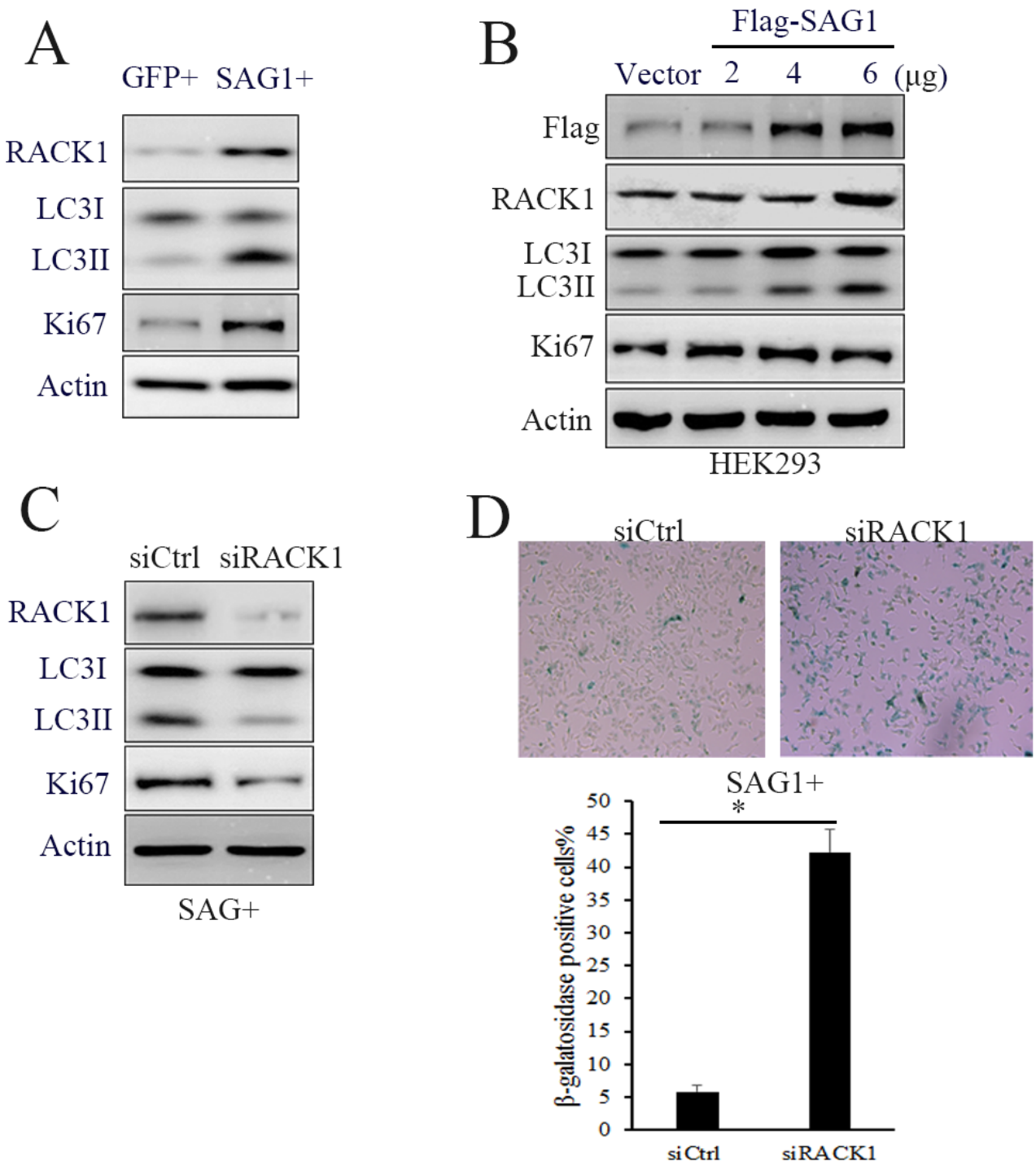


Figure 5

Depletion of RACK1 inhibits SAG1 induced autophagy and maintains the viability of host cells. A. Western blot analysis of RACK, LC3-II and Ki67 levels in SAG1+ and control cells. B. Western blot analysis of RACK, LC3-II and Ki67 levels in HEK293 cells transfected with increasing amount of Flag-tagged SAG1 (2-6 μg). Cells transfected with blank vector were used as control. C. Western blot analysis of the effect of RACK1 depletion on the LC3-II and Ki67 levels in the SAG1+ cells. D. β-galatosidase analysis of the effect

of RACK1 depletion on the viability of the SAG1+ cells. These experiments were repeated for three times and the data was represented by mean±SD.

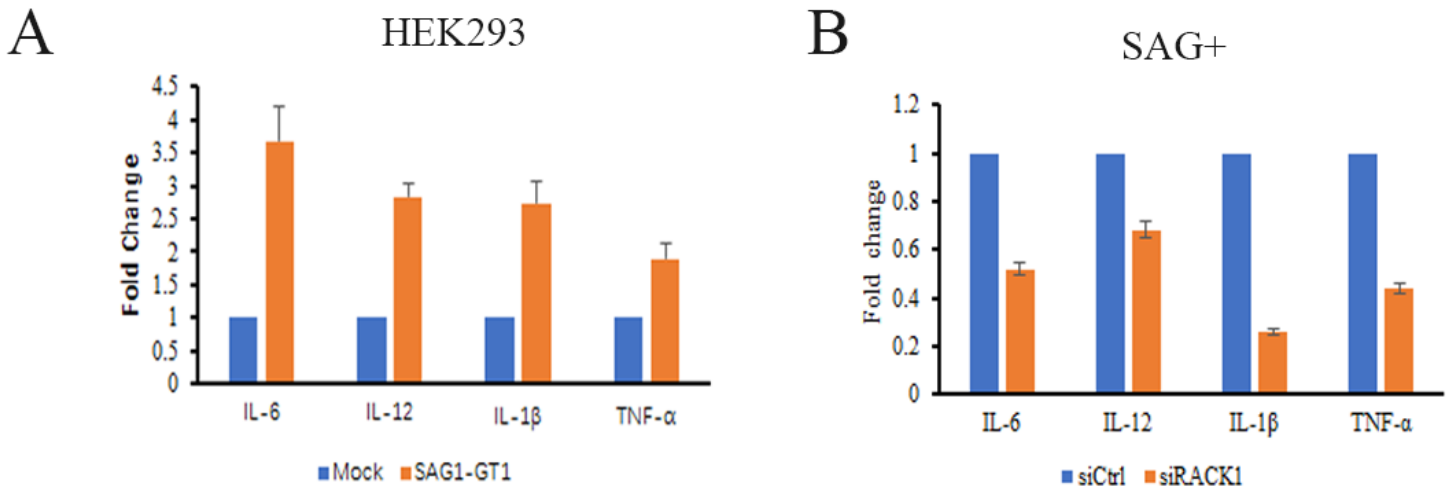


Figure 6

Depletion of RACK1 reduces the SAG1 induced expression of pro-inflammatory cytokines. A. qPCR analysis of the levels of pro-inflammatory cytokines including IL-1β, IL-6, IL-12 and TNF-α in SAG1+ cells and mock cells. B. qPCR analysis of the effect of RACK1 knockdown on the expression of pro-inflammatory cytokines in SAG1+ cells. These experiments were repeated for three times and the data was represented by mean±SD.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supp1.tif