iScience, Volume *24*

Supplemental information

High resistance to *Toxoplasma gondii* infection in inducible nitric oxide synthase knockout rats

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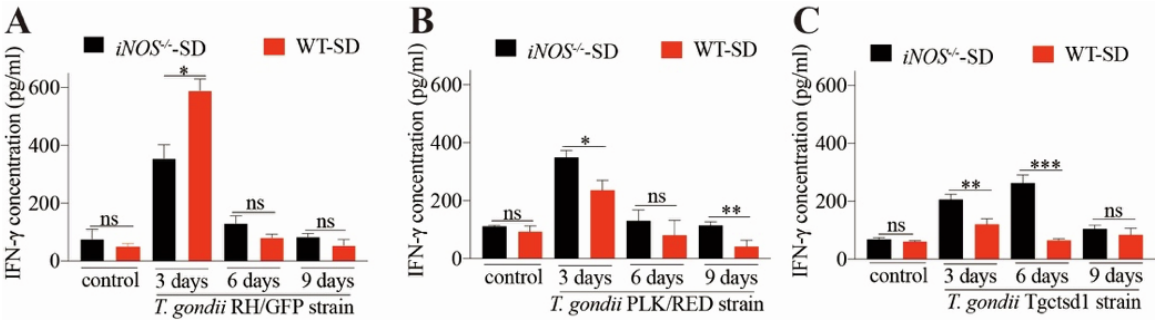

Figure S1. Measurement of IFN-γ content in rat sera, Related to Figure 1. (**A-C**) IFN-γ content was measured after being infected with the *T. gondii* RH/GFP (**A**), PLK/RED (**B**) and Tgctsd1 (**C**) strains. Rats were i.p. infected with 10^7 *T. gondii* tachyzoites and tail blood was collected at 3, 6 and 9 dpi; control groups were i.p. injected with equal volumes of PBS, n = 3. All data are presented as mean ± SD and are representative of three independent experiments. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns: no significant difference.

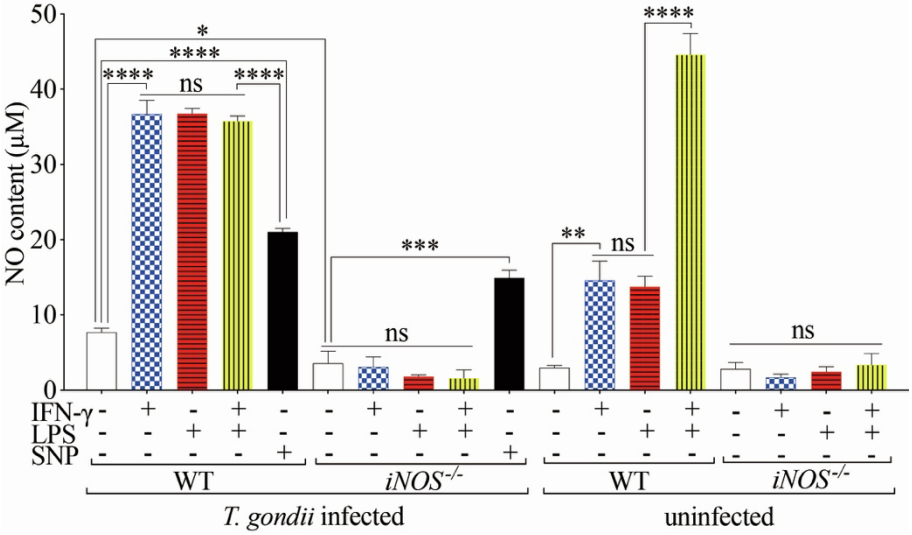

Figure S2. NO production in the supernatants of SD rat PMs preparations, Related to Figure 2. Supernatants were obtained at 24 hpi with *T. gondii* RH/GFP strain. MOI = 2, in 24-well plates, 5×10^5 cells per well. All data are presented as mean ± SD and are representative of three independent experiments. n = 3; *, p <0.5; **, p <0.01; ***, p <0.001; ****, p < 0.0001; ns, no significant difference. LPS, Lipopolysaccharide; IFN-γ, interferon-gamma; SNP, sodium nitroprusside.

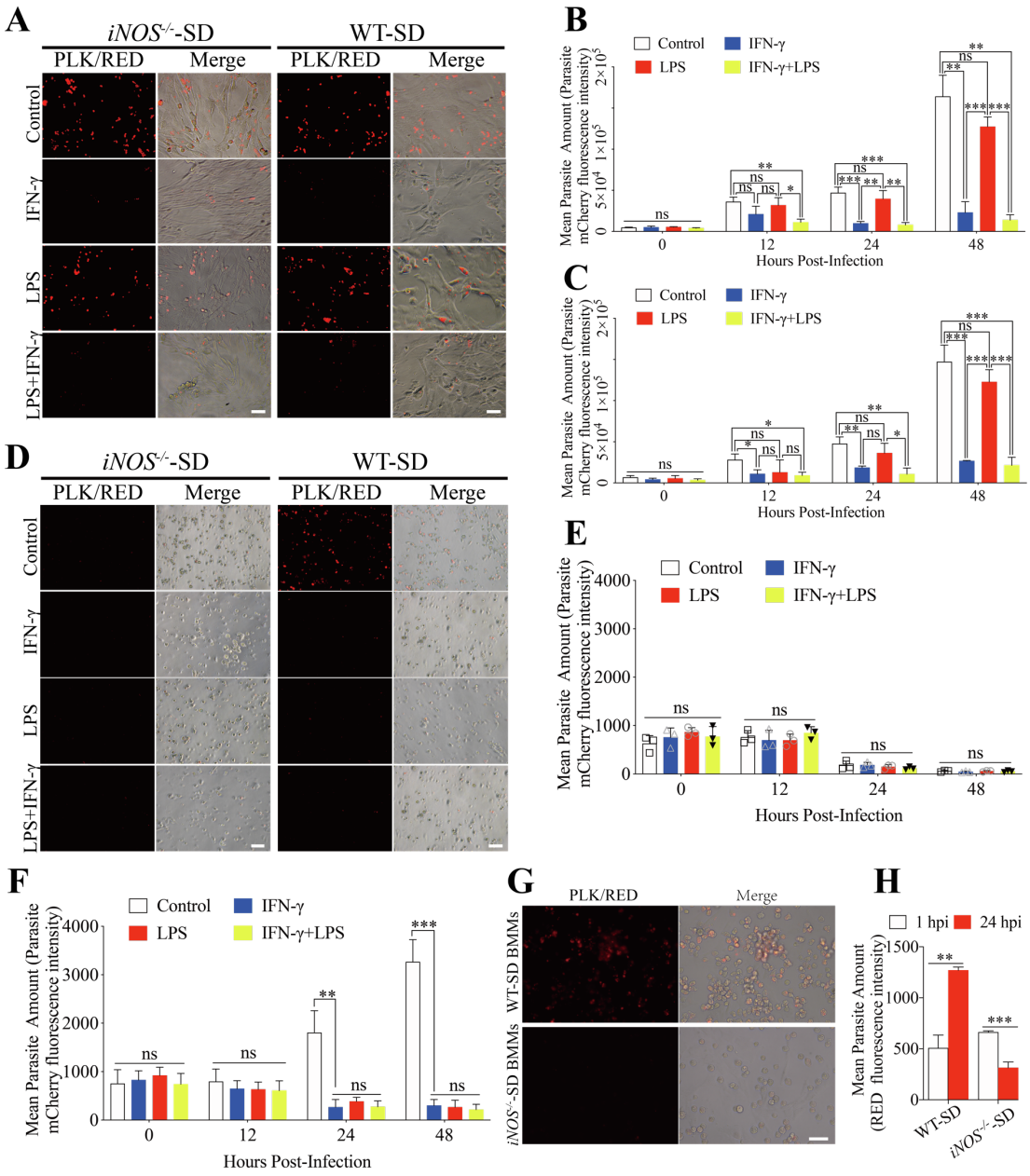

Figure S3. Images and quantification of *T. gondii* infection of rat NMFCs, rat PMs and BMDMs with the PLK/RED strain, **Related to Figure 2.** (**A**) *T. gondii* PLK/RED strain tachyzoites in SD rat NMFCs at 48 hpi. (**B** and **C**) The mean parasite load in *iNOS*$^{-/-}$-SD (**B**) and WT-SD (**C**) rat NMFCs. (**D**) *T. gondii* PLK/RED tachyzoites in SD rat PMs at 48 hpi. (**E** and **F**) The mean parasite load in *iNOS*$^{-/-}$-SD (**E**) and WT-SD (**F**) rat PMs (MOI = 2, cells were transferred into a 24-well plate, 5×10^5 per well, n = 3. (**G**) BMDM cells at 24 hpi. (**H**) The mean parasite load in SD rat BMDMs. Scale bar, 50 μm. MOI = 2, cells were transferred into 24-well plates, 5×10^5 per well. All data are presented as mean ± SD and are representative of three independent experiments, in triplicate, n = 3. **, $p < 0.01$; ***, $p < 0.001$.

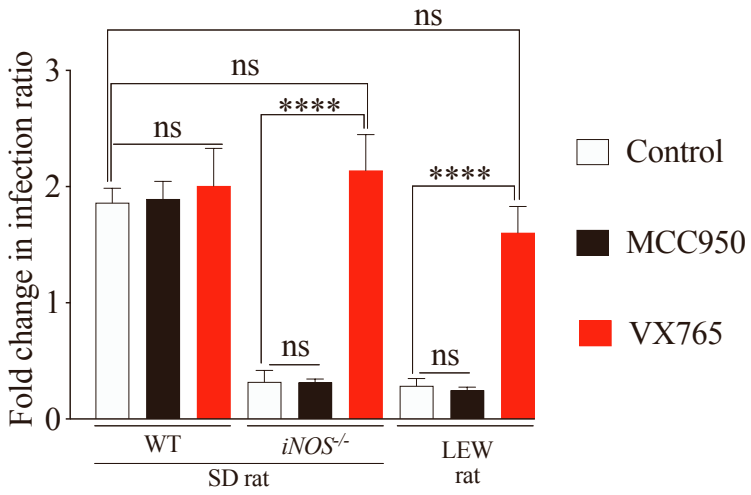

Figure S4. Changes in infection ratio of rat PMs with *T. gondii* after inhibition of caspase 1 or caspase 3, Related to Figure 4. The changes in infection ratio of *T. gondii* RH/GFP strain were counted at 24 hpi, cells and expressed as a fold change. Cells were transferred in 24-well plates with 5×10^5 parasites per well; Rat PMs were pre-treated for 2h ahead of a 1h *T. gondii* invasion incubation and this was continued for the following 24h with the addition of inhibitors of caspase 1 (VX765 at 50 μM) or caspase 3 (MCC950 at 10 μM); MOI = 2. All data are presented as mean ± SD and are representative of three independent experiments, four replicates, n = 3; ****, $p<0.0001$; ns, no significant difference.

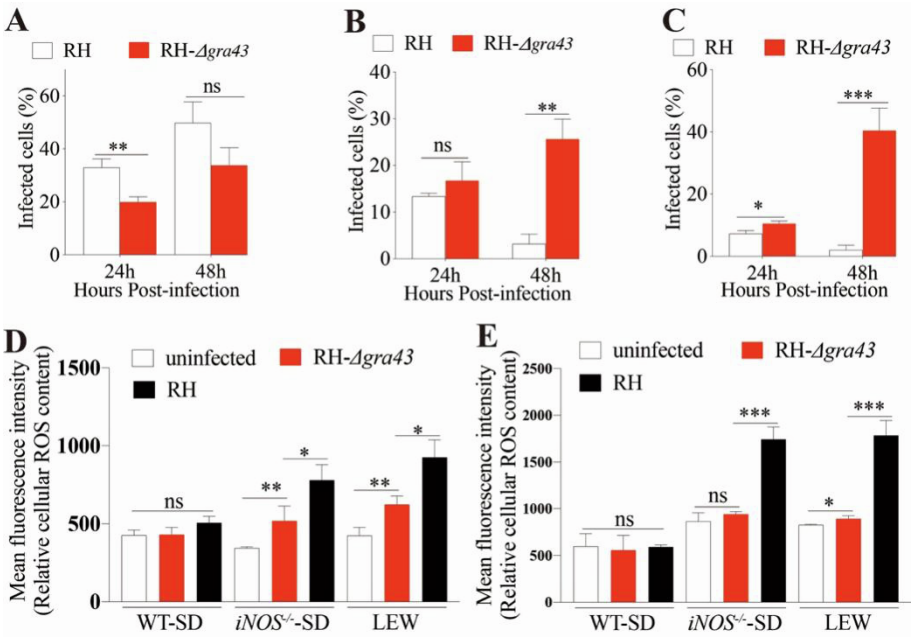

Figure S5. The infection ratio and ROS abundance in rat PMs infected with Δ*gra43 T. gondii*, Related to Figure 2 and Figure 4. (**A**-**C**) The infection ratio in WT-SD (**A**), *iNOS*$^{-/-}$-SD (**B**) and LEW (C) rat PMs infected with *T. gondii* RH-*Δgra43* or RH srain. (**D** and **E**) The relative cellular ROS content in PMs after infection with *T. gondii* RH-*Δgra43* or RH strain at 1 hpi (**D**) and 3 hpi (**E**), following 30 min incubation with DCFH-DA. Cells were transferred in 24 well-plates, seeded at 5×10^5 per well, MOI = 2. All data are presented as mean ± SD and are representative of three independent experiments, n = 3. **, $p < 0.01$; ***, $p < 0.001$; ns, no significant difference.

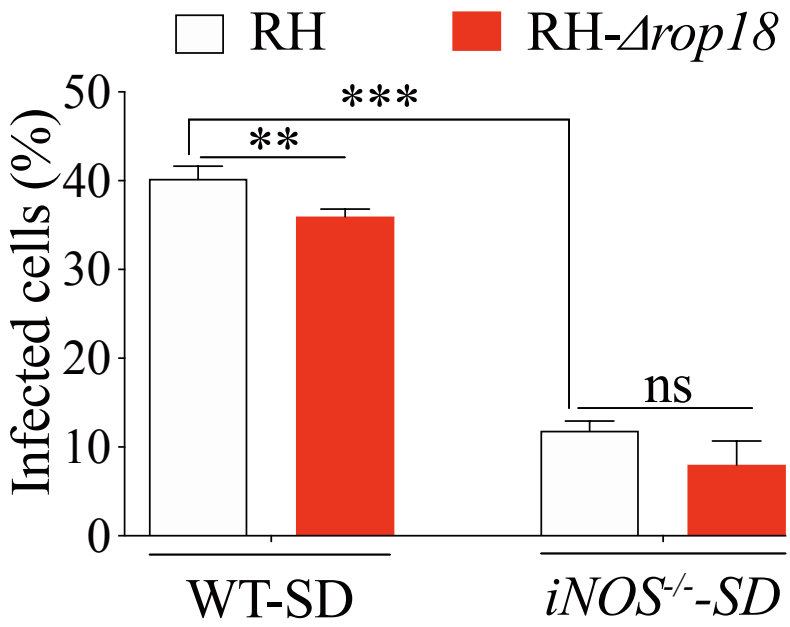

Figure S6. The infection ratio of SD rat PMs infected with *T. gondii* RH-*Δrop18*, Related to Figure 2. Rat PMs were infected with *T. gondii* RH-*Δrop18* or RH strain at 24 hpi; adherent cells were established in a 24 well plate, 5×10^5 per well, MOI = 2. All data are presented as mean ± SD and are representative of three independent experiments, n = 3. **, $p < 0.01$; ***, $p < 0.001$; ns, no significant difference.

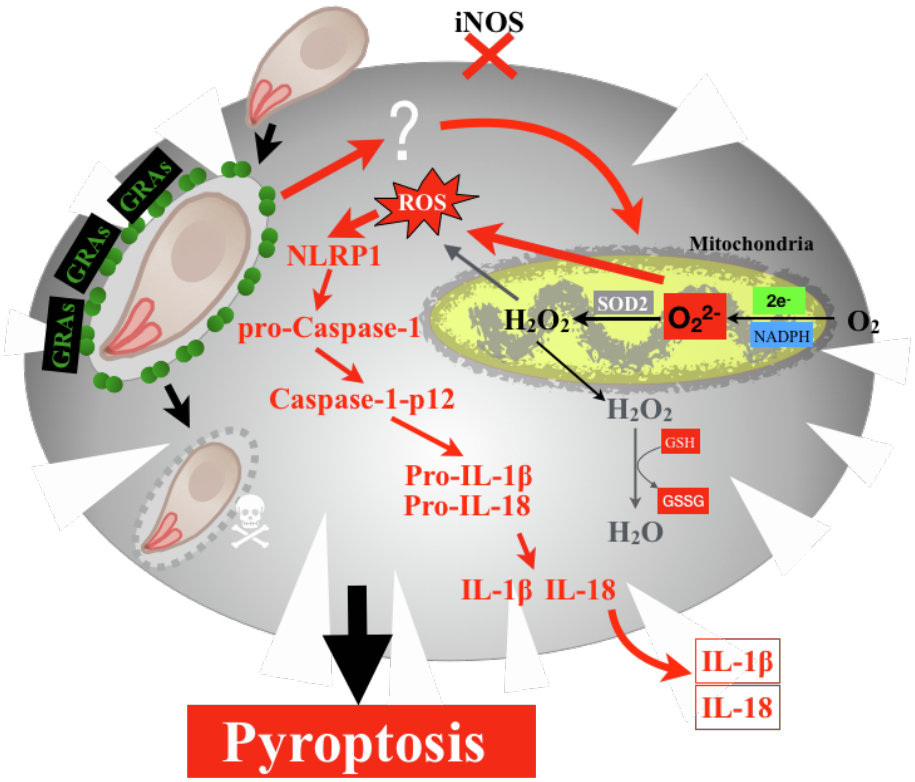

Figure S7. Summary of the cell pyroptosis pathway induced by *T. gondii* infection in *iNOS*$^{-/-}$-SD rat PMs, Related to Figure 4. *T. gondii* invades and forms a parasitophorous vacuole. Rat iNOS is induced to produce NO to provide self-defense. If this level of defense is missing, such as in the knockout rat, a novel alternative method comes into play. With the secretion of dense granule proteins (GRAs) from *T. gondii* and the lack of iNOS, the rat mitochondrion is over-activated and generates a reactive oxygen species (ROS) burst with the depletion of SOD2. The ROS burst may kill *T. gondii* directly, but more importantly, it induces pyroptosis. Pyroptosis then leads to the host cell disruption, together with destruction of the parasitophorous vacuole and this significantly limits the growth of the parasite.

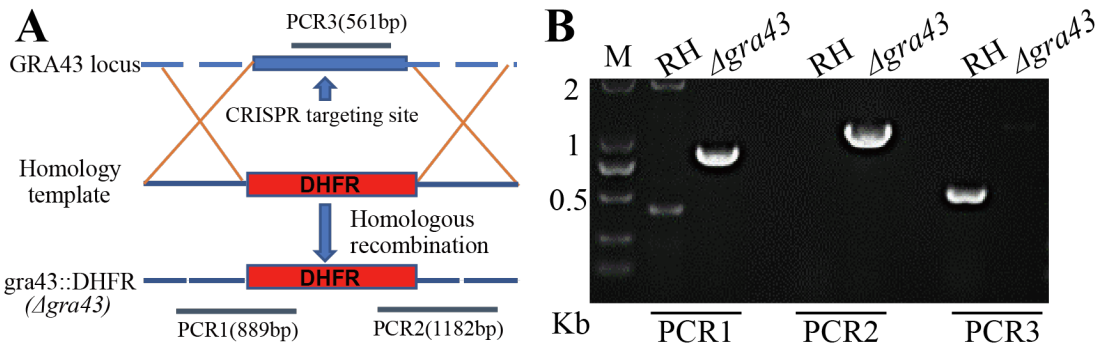

Figure S8. Schematic diagram of the CRISPR/CAS9 strategy and diagnostic PCRs for identifying the *T. gondii* Gra43 knockout, Related to STAR Methods. (**A**) Schematic diagram showing the inactivation of GRA43 by inserting pyrimethamine-resistant DHFR (DHFR*). Transfection of the sgGRA43 together with an amplicon containing a DHFR*-expressing cassette flanked by homology regions to GRA43 was used to generate gene disruptions by insertion. The purple bar in the GRA43 gene represents the region targeted by the sgRNA. (**B**) Diagnostic PCRs of the *T. gondii* RH strain and RH-Δ*gra43* strain. The PCR1, PCR2 and PCR3 were performed to confirm the knock-out of *GRA43* in the *T. gondii* RH strain.

Table S1. Transcript fold change of TRAF6 and MyD88 in *iNOS*$^{-/-}$-SD versus WT-SD rat. Related to Figure 4.

Gene ID	Gene name	Fold change in expression (P)			
		iNOS$^{-/-}$$_{T.\ g}$/WT$_{T.\ g}$	*iNOS*$^{-/-}$$_{T.\ g}$/WT$_{PBS}$	WT$_{T.\ g}$/WT$_{PBS}$	*iNOS*$^{-/-}$$_{PBS}$/WT$_{PBS}$
ENSRNOG00000004639	TRAF6	2.460867906 (3.01E-07)	7.99512761 (3.18E-22)	3.24449304 (3.53E-05)	ns
ENSRNOG00000013634	MyD88	3.026277582 (2.70E-10)	13.4817743 (5.20E-33)	4.4394018 (3.53E-05)	ns

RPKM, Reads Per Kilobase per Million mapped reads; ns, no significant difference; P, p value; *iNOS*$^{-/-}$$_{T.\ g}$ and WT$_{T.\ g}$, *T. gondii*-inoculated *iNOS*$^{-/-}$-SD and WT-SD rats, respectively; *iNOS*$^{-/-}$$_{PBS}$ and WT$_{PBS}$, PBS-inoculated *iNOS*$^{-/-}$-SD and WT-SD rats, respectively, n≥3.

Table S2. Primers used to construct plasmids and Diagnostic PCRs, Related to STAR Methods.

Name	Sequence	Description
gRNA-CRISPR-F	GAATCTCACCCGGCCATATTGTTTTAGAGCTAGAAATAGC	GRA43 specific CRISPR plasmid construction
gRNA-CRISPR-R	AACTTGACATCCCCATTTAC	
5-GRA43F	GACCATGATTACGCCAAGCTTATCCGAATGCGTGACTTCAGC	Amplification of the 5' homology arm of GRA43
5-GRA43R	ATTTACAGCCTGGCTTGGTGGTGGTTATATGAGTCTC	
GRA43-DHFRF	CACCAAGCCAGGCTGTAAATCCCGTGAGTC	Amplification of the DHFR from pUPRT::DHFR-D
GRA43-DHFRR	CGATTGTAGTCGATTCCGTCAGCGGTCTGTC	
3-GRA43F	GACGGAATCGACTACAATCGCCAGCTTGAAAC	Amplification of the 3' homology arm of GRA43
3-GRA43R	GGTACCCGGGGATCCTCTAGATCGTACTGTCGCCGTATCTTTTG	
GRA-P1F	TCAGCACGAATCATACCTCAGA	PCR1 for verifying the Δ*gra43* mutation
GRA-P1R	AGACAAGACGCAGACGCATA	
GRA-P2F	ACTTGTTGTGCCAGTTCTACG	PCR2 for verifying the Δ*gra43* mutation
GRA-P2R	CTGCGGTTAATCGGTCCTTC	
GRA-inF	GTTGGTGGTAGGCGTGGAA	PCR3 for verifying the Δ*gra43* mutation
GRA-inR	TCTTGTGTTGACTGGGCATTG	