Toxoplasma gondii prevalent in China: *genotypes and Its interactions with host macrophages*

International Congress on Parasitology,
Philadelphia, Aug. 24-26， 2015



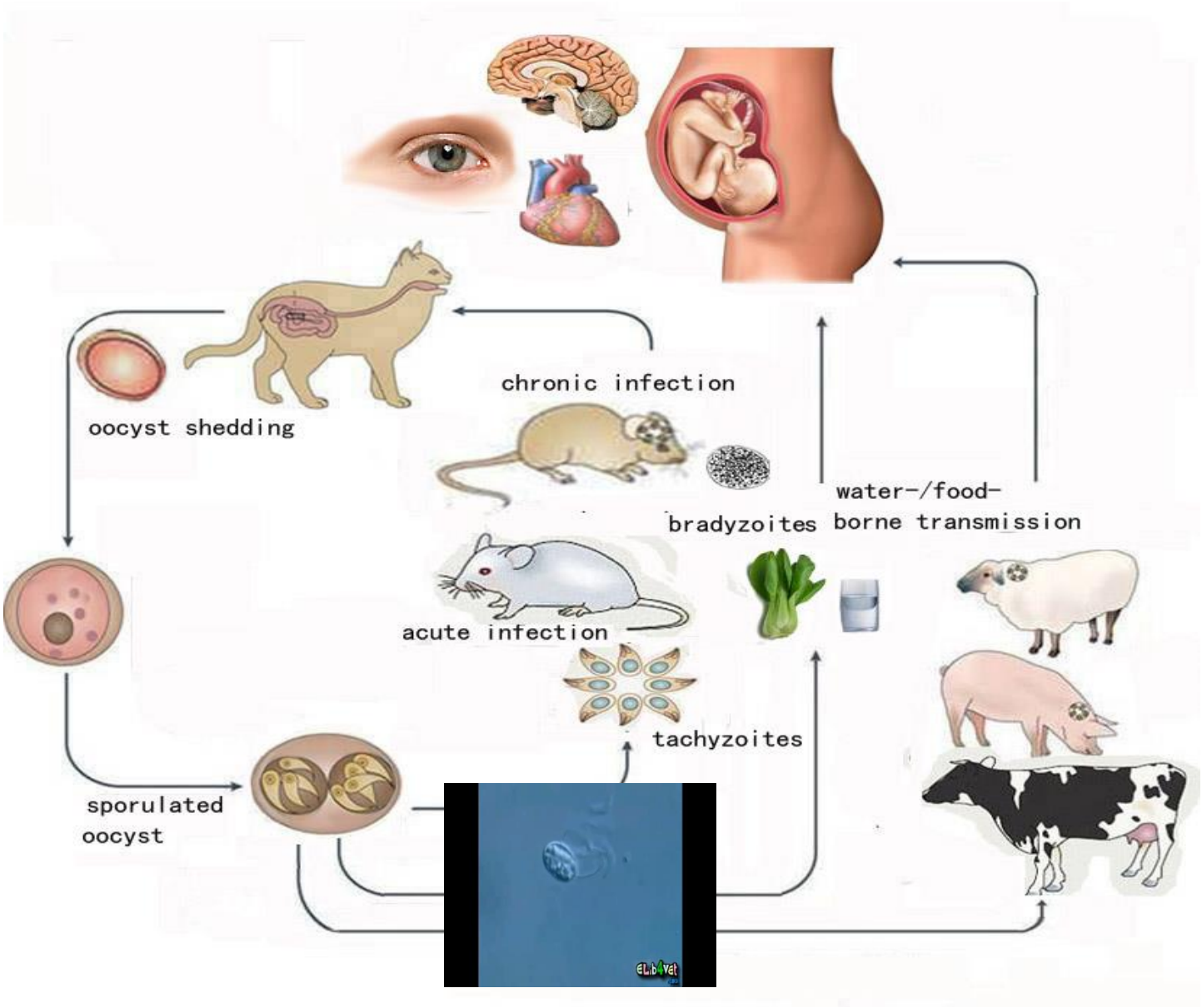
Jilong Shen，沈 继 龙

Dept. of Parasitology, Anhui Medical University

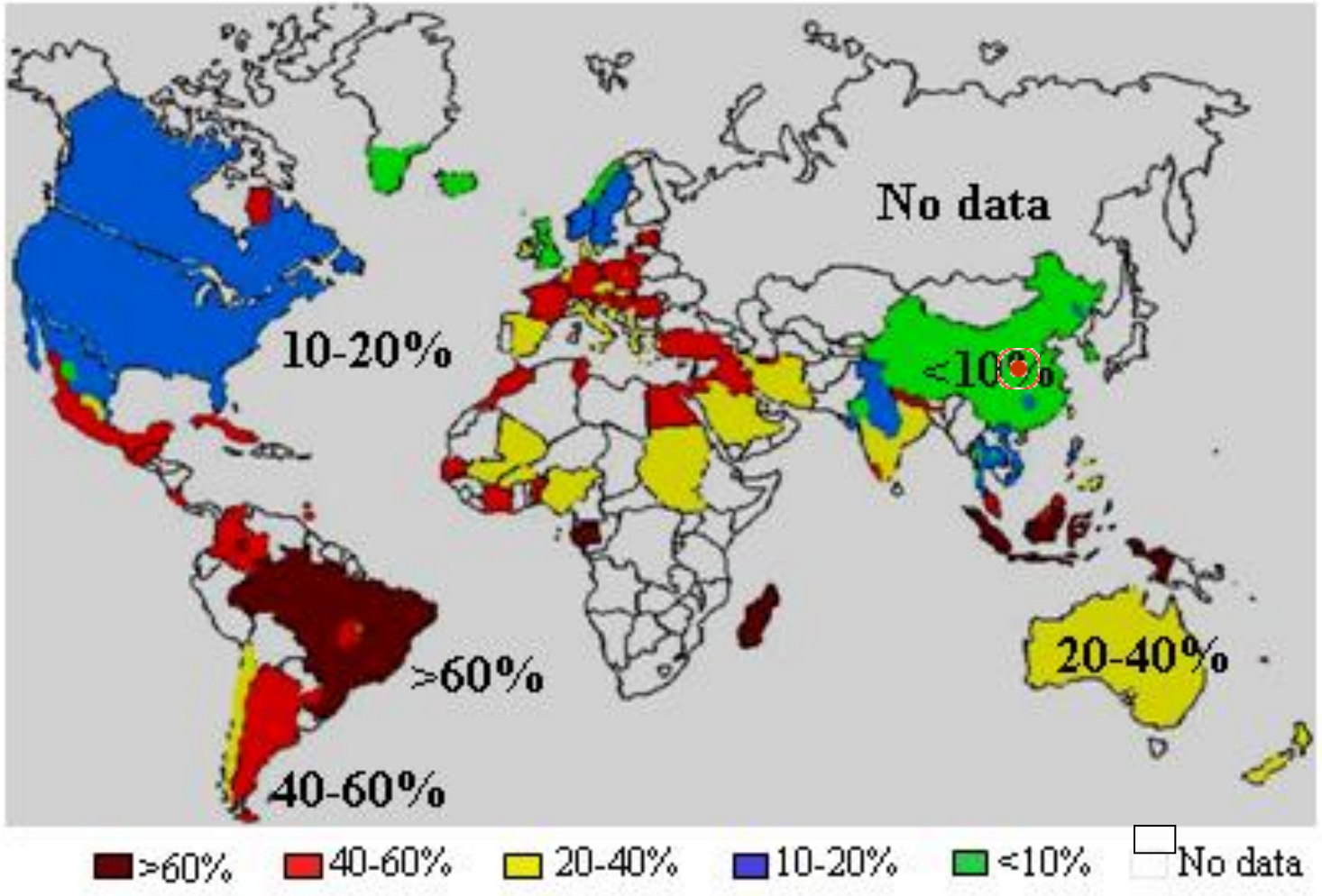
Laboratories of Pathogen Biology and Zoonoses

Anhui, China

Life cycle of *T.gondii*



Prevalence of human *Toxoplasma* infection across the world



Pappas et al., Int. J. Parasitol. 2009. 39:1385-1394.

Toxoplasmosis: a foodborne disease threatening humans health







Losses of livestocks

Immunocompetent

Asymptomatic infection



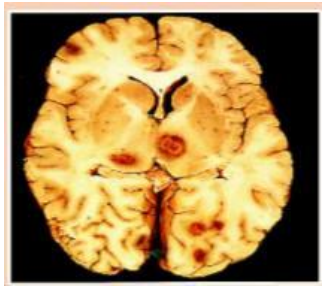
Immunocompetent

Schizophrenia (depression, suicide), other mental disorders



Immunocompromised

Reactivated toxoplasmosis (encephalitis, etc.)



Pregnant

abnormal pregnancy, congenital toxoplasmosis



Questions to be answered:

- **1. How about the population structure of *T.gondii* in china?**
- **2. What is the medical significance of the dominant genotype (virulence and effectors polymorphisms)?**
- **3. What is the mechanism by which the parasite is able to duplicate within its host macrophages (immune evasion)?**

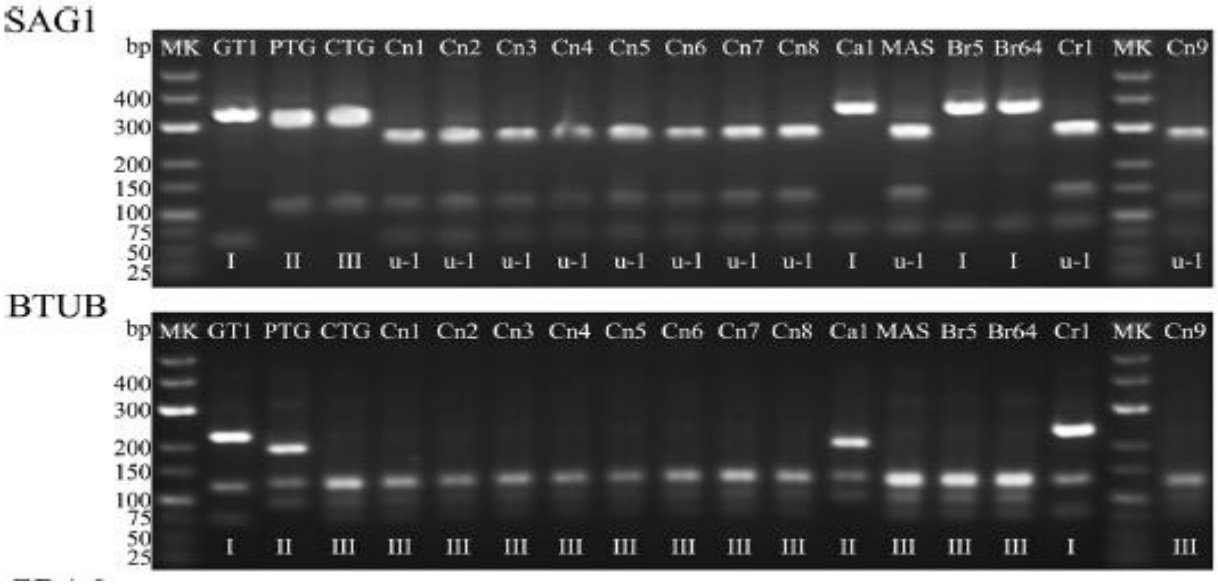
1 Genotypes and clonal lineage dominantly prevalent in China

One species worldwide: *T. gondii*

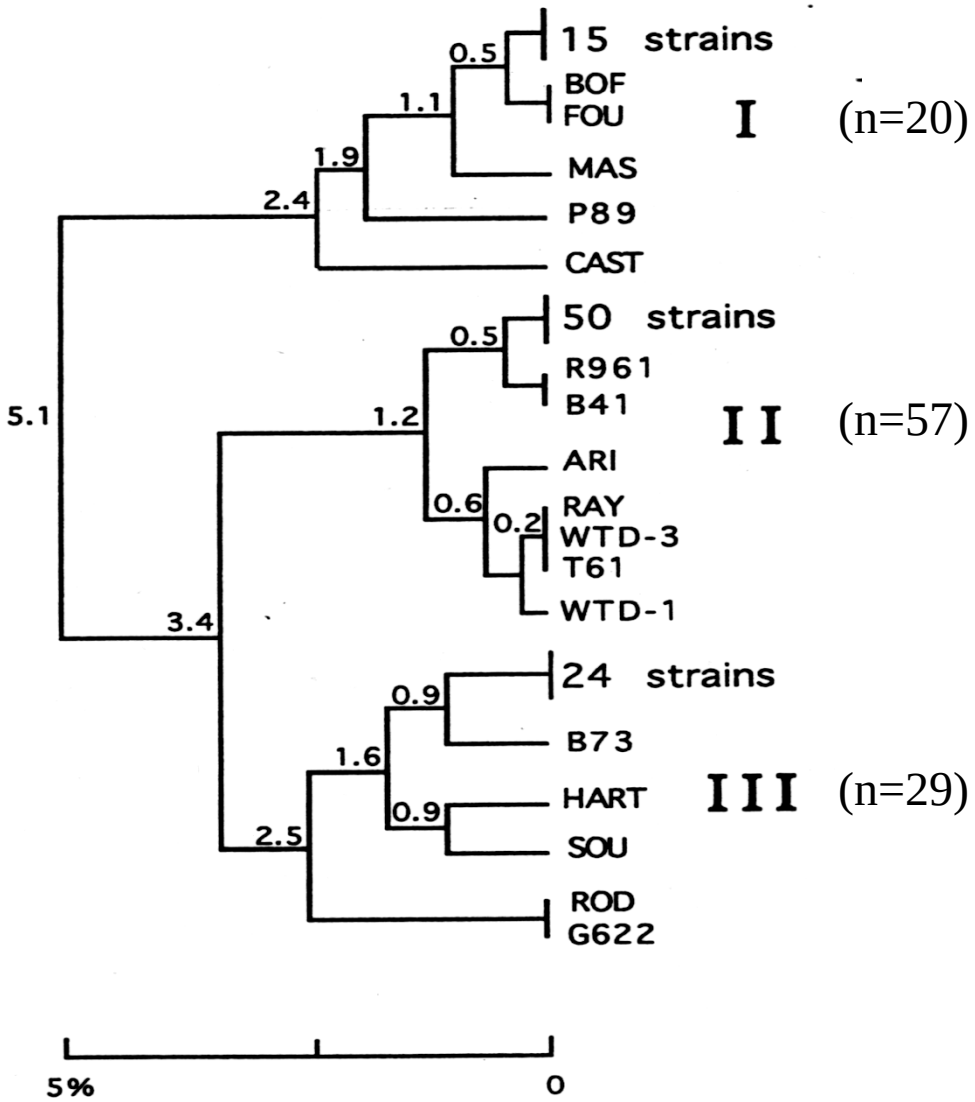
High genetic diversity:ToxoDB#231 (Jun. 2014)

Multiloci (9 markers)

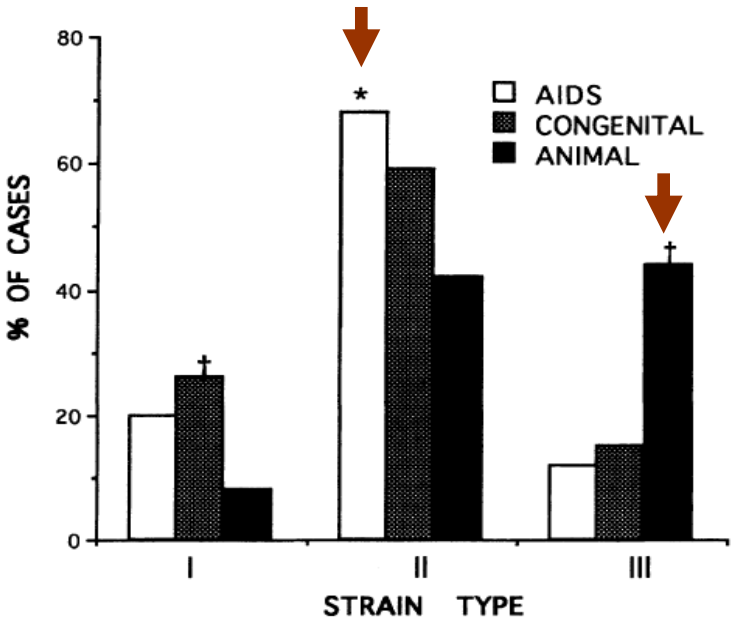
Typing techniques (PCR-RFLP, MS）



clonal population structure of *T.gondii* in human and domestic animals in North America and Europe.

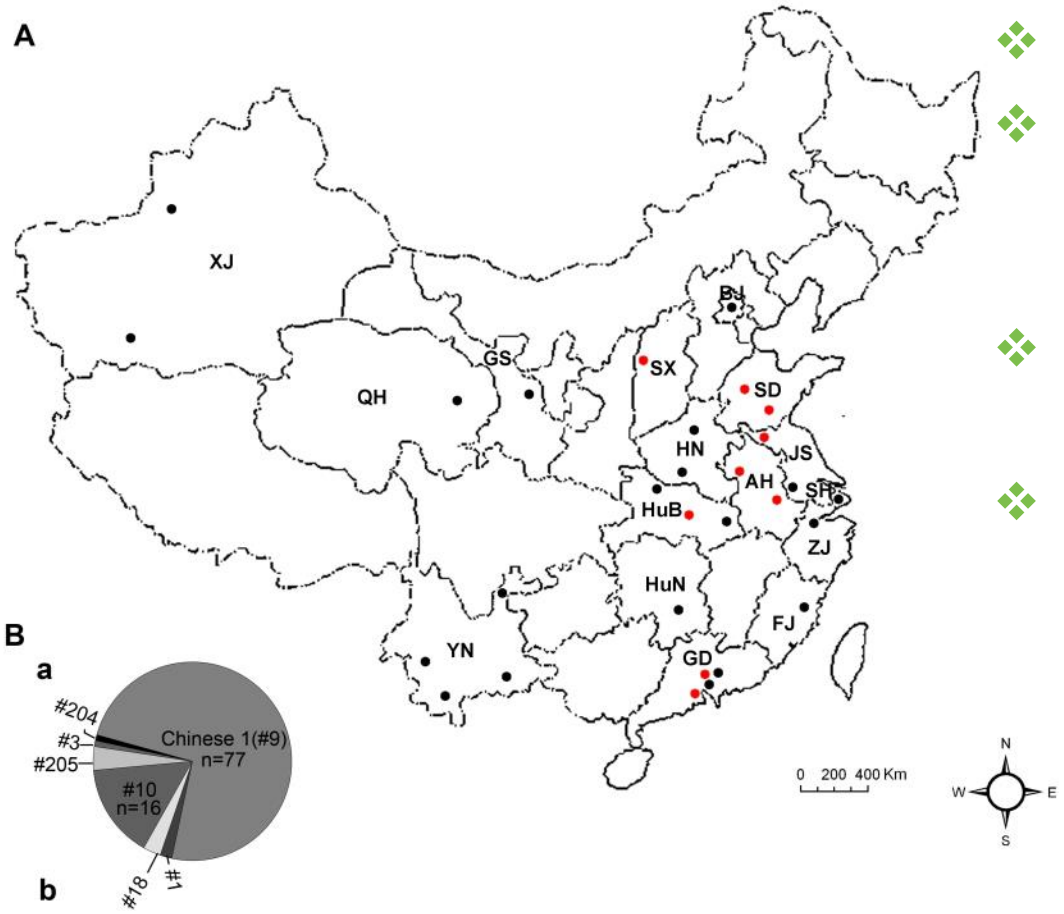


Six PCR-RFLP makers (mutation rate 10^{-8})



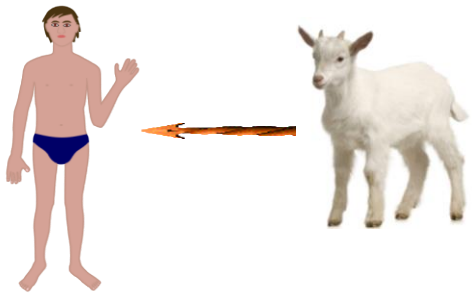
Howe, D.K. and Sibley, L.D. 1995. J. Infect. Dis. 172:1561-1566.

Genetic structure of *Toxoplasma* in China



- **46 isolates from animals**
- **13 samples from human**
- **7 types were found [*ToxoDB#9, type, I, type II, #203, #205, #213, and #215*].**
- **ToxoDB#9 is dominantly defined and termed as Chinese 1**
- **Strains of type Chinese 1 account for 73.8% in animals and 76.9%in human.**

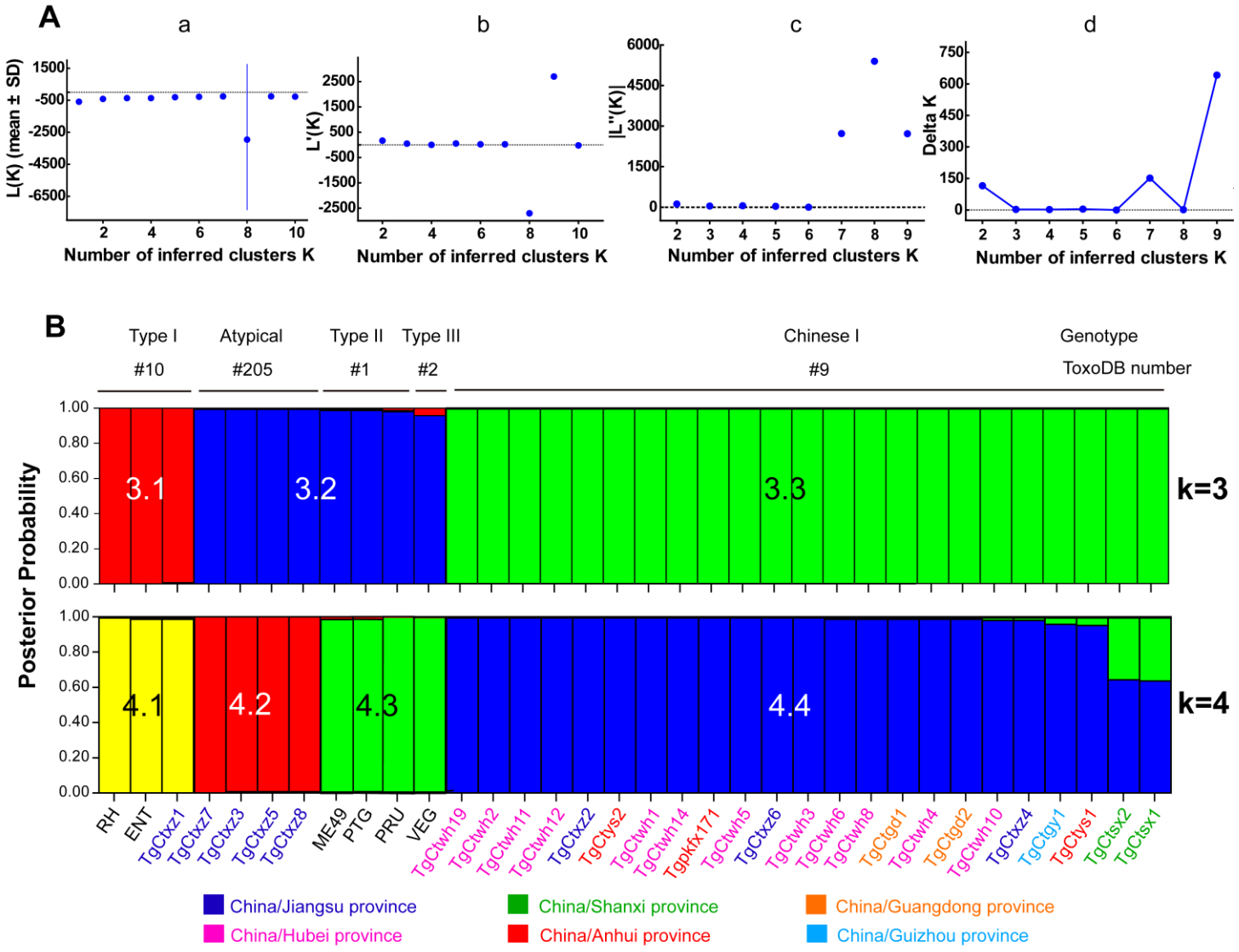
human infections come from animals?



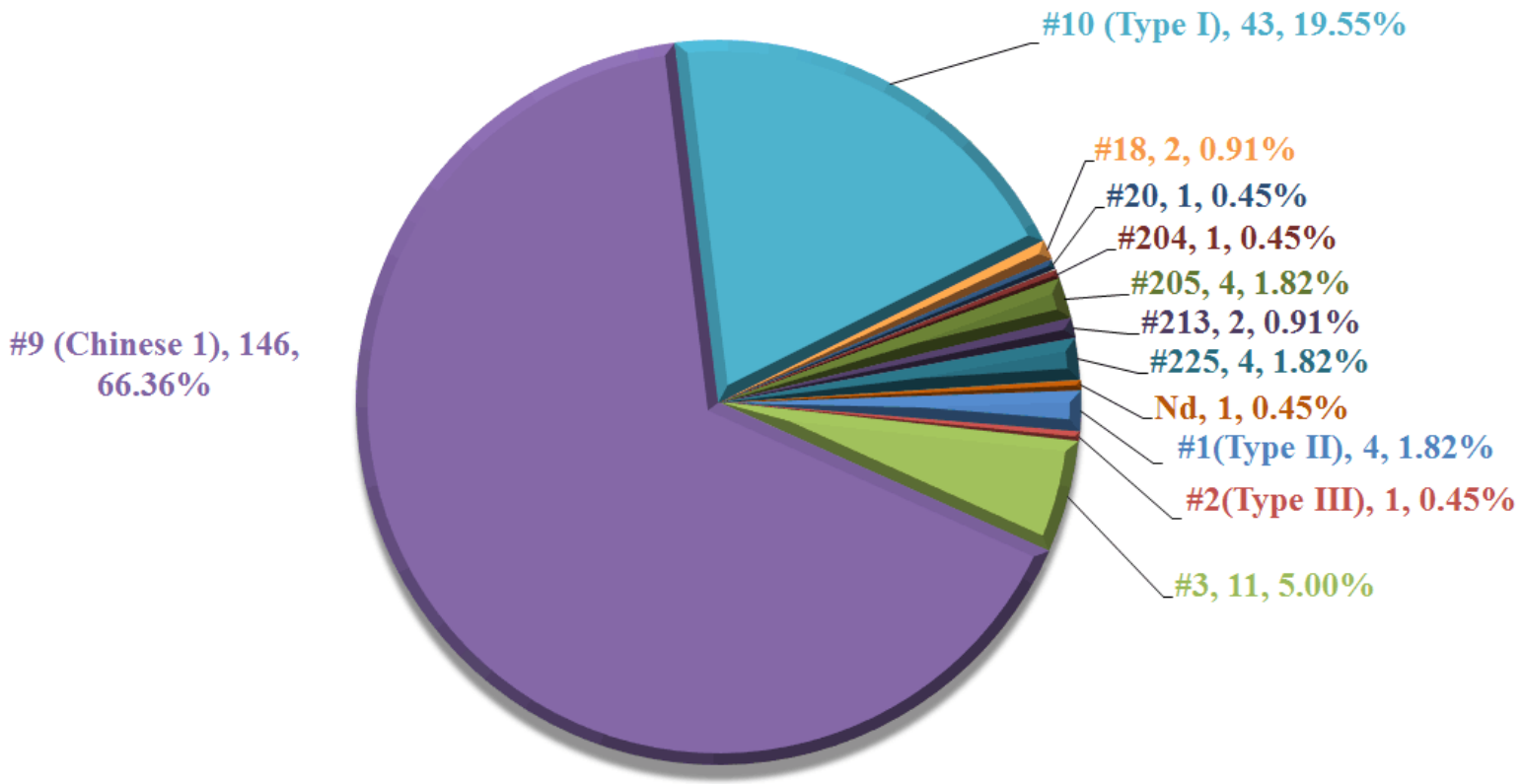
PCR-RFLP genotyping (isolates from Anhui and Hubei)

Isolate	SAG_2	SAG_3	BTUB	GRA_6	L358	PK1	C22-8	C29-2	Apico	genotype
RH	I	I	I	I	I	I	I	I	I	I
PRU	II	II	II	II	II	II	II	II	I	II
CTG	III	III	III	III	III	III	III	III	I	III
Tgysc1	II	III	III	II	II	II	II	II	I	
Tgysc2	II	III	III	II	II	II	II	II	I	
Tgwhc1	II	III	III	II	II	II	II	II	I	
Tgwhc2	II	III	III	II	II	II	II	II	I	
Tgwhc3	II	III	III	II	II	II	II	II	I	
Tgwhc4	II	III	III	II	II	II	II	II	I	
Tgwhc5	II	III	III	II	II	II	II	II	I	
Tgwhc6	II	III	III	II	II	II	II	II	I	Chin 1
Tgwhc7	II	III	III	II	II	II	II	II	I	
Tgwhc8	II	III	III	II	II	II	II	II	I	
Cop I	II	III	III	II	II	II	II	II	I	
Cop II	II	III	III	II	II	II	II	II	I	
CopIII	II	III	III	II	II	II	II	II	I	
CopIV	II	III	III	II	II	II	II	II	I	

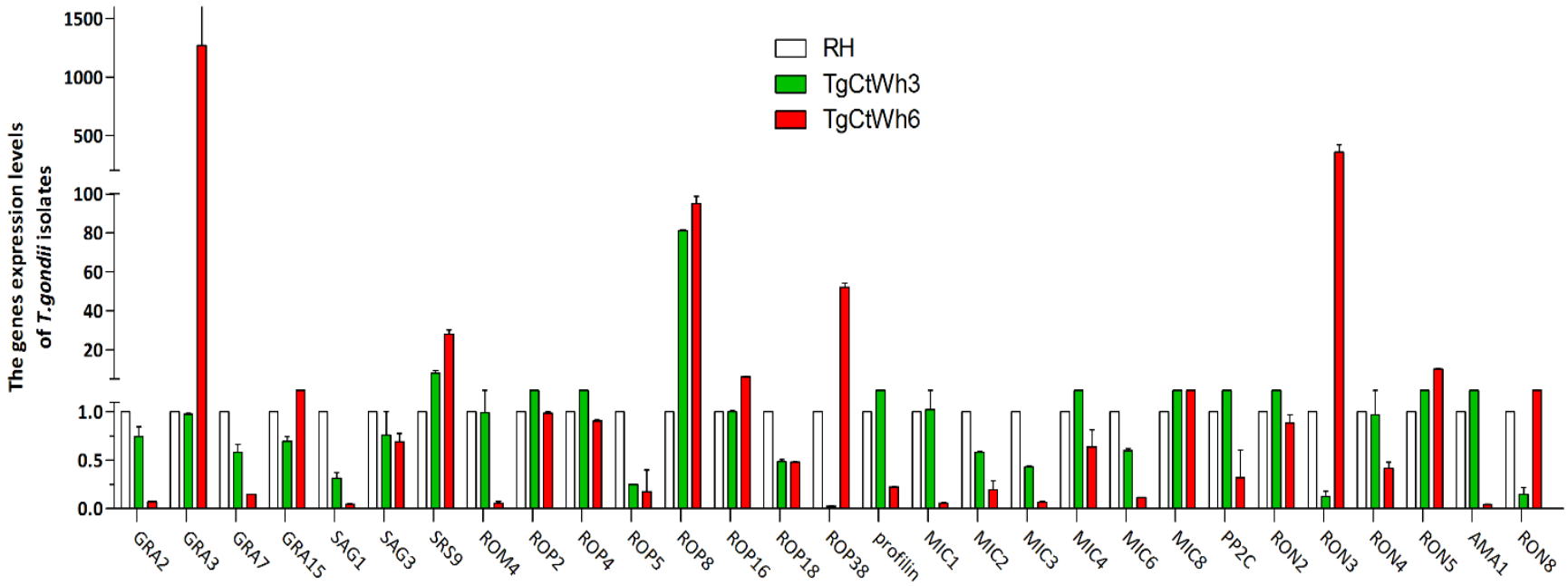
Microsatelite (MS) genotyping for confirmation



Twelve genotypes of T. gondii defined with PCR-RFLP at 10 loci from 220 isolates in China

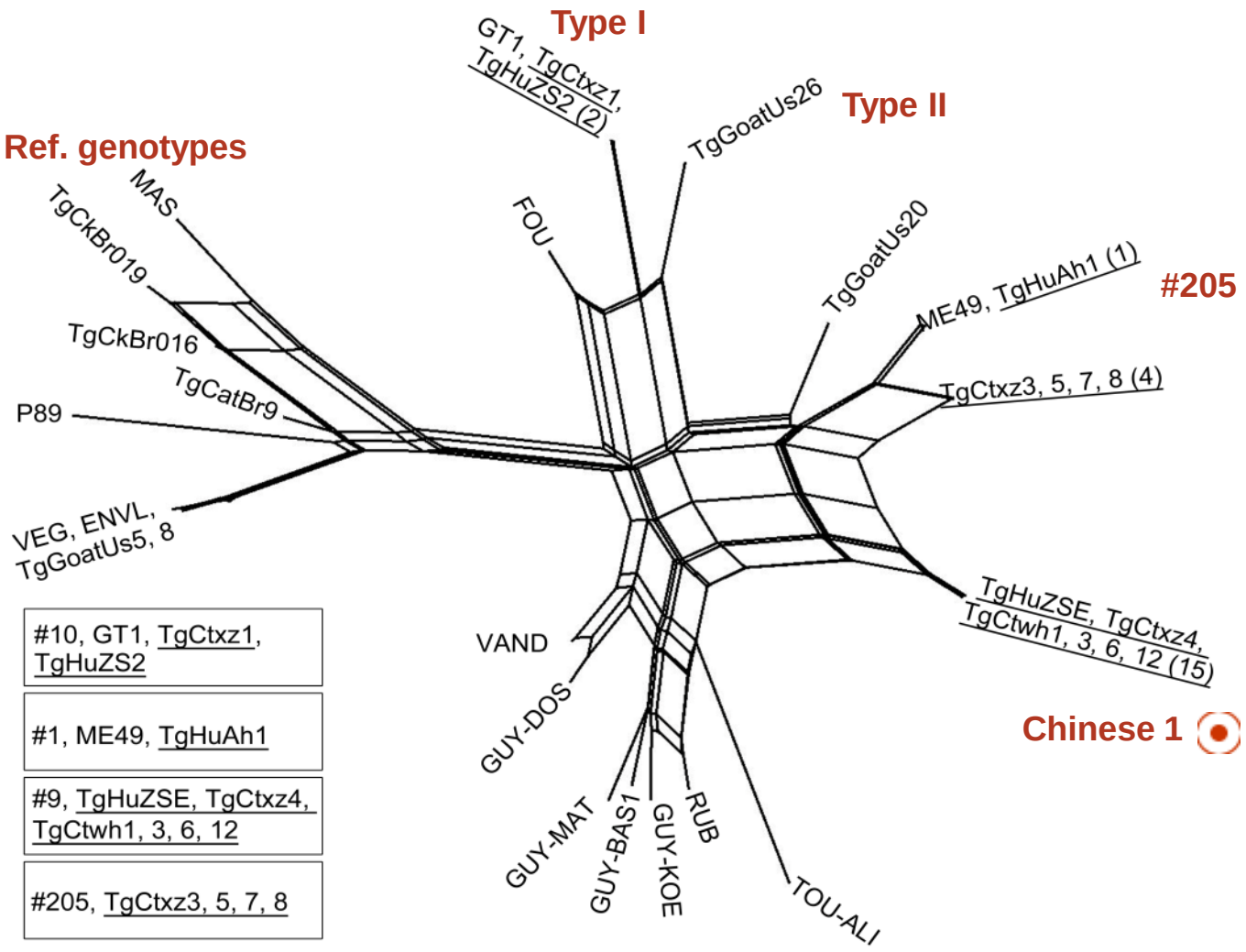


GRA3, ROP38, RON3: What do they mean?



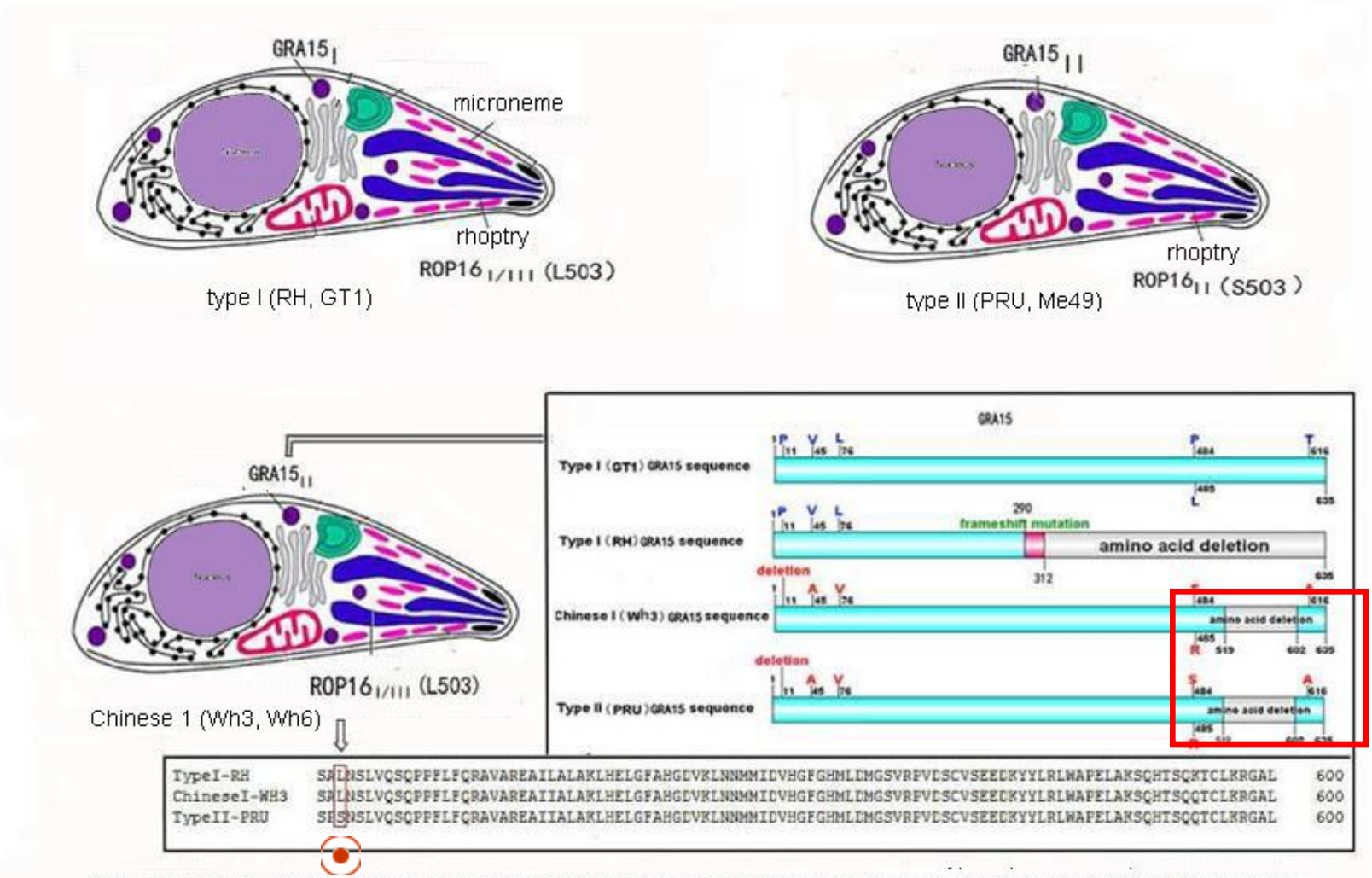
Gene expression profiles （29 factors） among the three strains

It shows a significant increase of RON3 and GRA3 expression inWh6 strain.



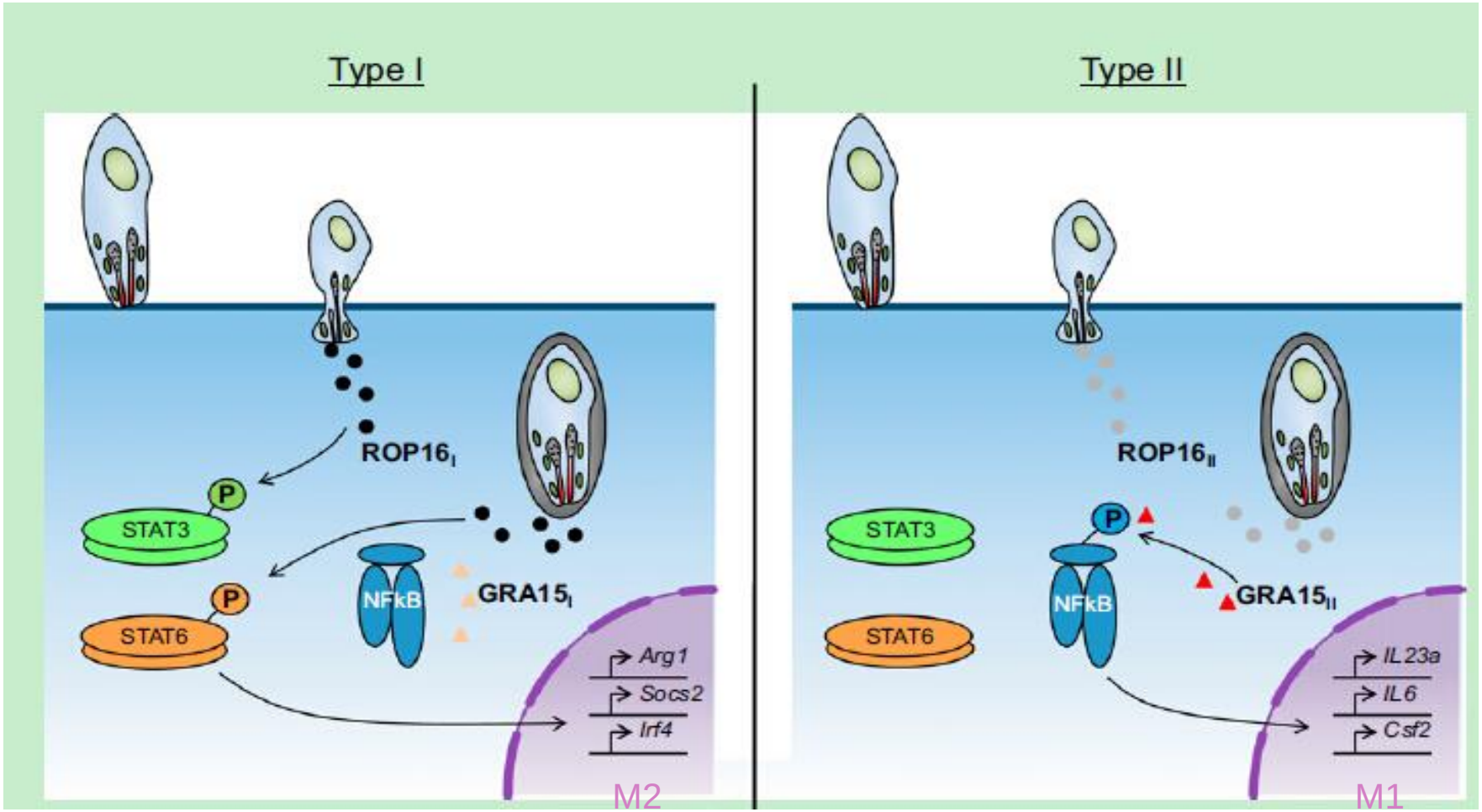
- Instead of types I, II, and III commonly found in Europe and North America, type Chinese 1 dominates in China mainland.

Type Chinese 1 strains share polymorphic ROP16I/III with type I/III, and GRA15II with type II strains



Type Chinese 1 strains share polymorphic rop16I/III with type I and gra15II with type II strains (showing rop16 L503 and the deletion of C terminal portion of gra15II

What is the medical significance of type Chinese 1 strains?



Genotype-associated macrophage polarization and its pathogenesis

Cell Host & Microbe， 2011

2. High- and low- virulent strains of type Chinese 1

Isollates	Locations	Mortality of mice (%)	Survival days postinfection	genotypes	Comments
RH		100 (10/10)	5-7	#10, Type I	Contr
PRU		0 (0/10)	Survived	#3, Type II	Contr
Wh6	Wuhan, Hubei	0 (0/10)	Survived	Chin 1	Pres Res
Wh3	Wuhan, Hubei	100 (10/10)	6-8	Chin 1	Pres Res
Wh14	Wuhan, Hubei	100 (10/10)	5-6	Chin 1	Pres Res
Ctsd2	Jinan, Shandong	10 (1/10)	Survived	Chin 1	Pres Res
Ctsd3	Jinan, Shandong	0 (0/10)	Survived	Chin 1	Pres Res
Ctxz1	Xuzhou, Jiangsu	100 (10/10)	3-4	#10, Type I	Pres Res
Ctxz5	Xuzhou, Jiangsu	100 (10/10)	5-6	#205	Pres Res
Ctxz8	Xuzhou, Jiangsu	100 (10/10)	5-7	#205	Pres Res



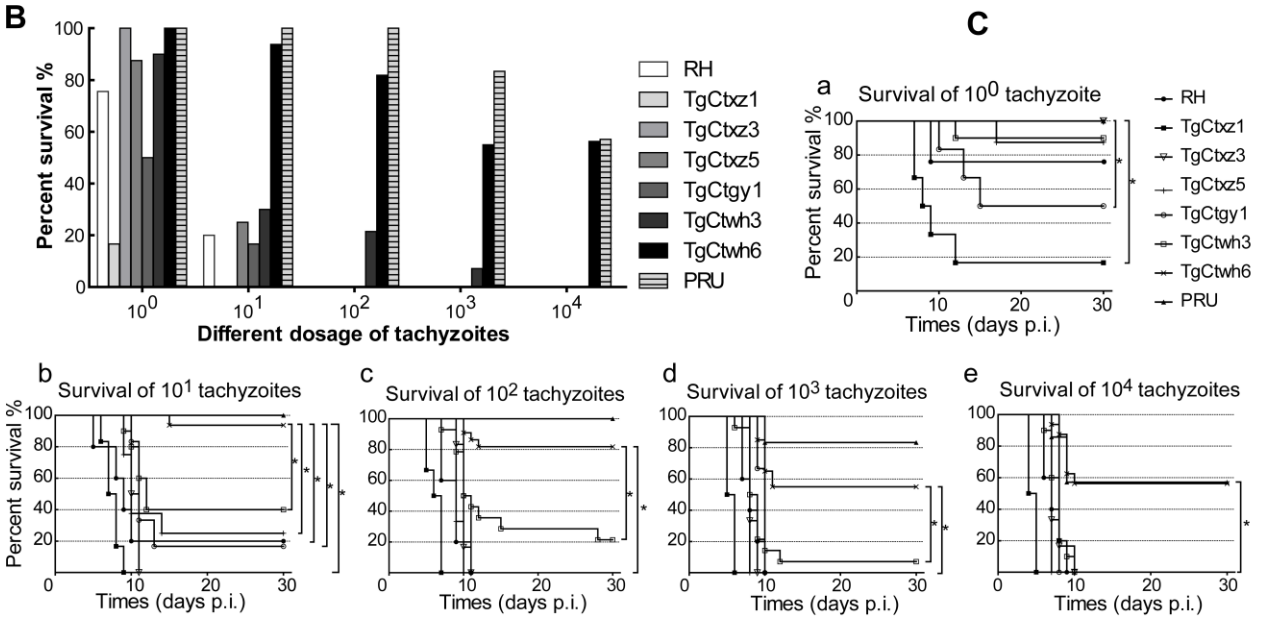
Different virulence was identified in the isolates of genotype Chinese 1.

Wh3 and Wh6 differ in virulence compared with RH or PRU in mice

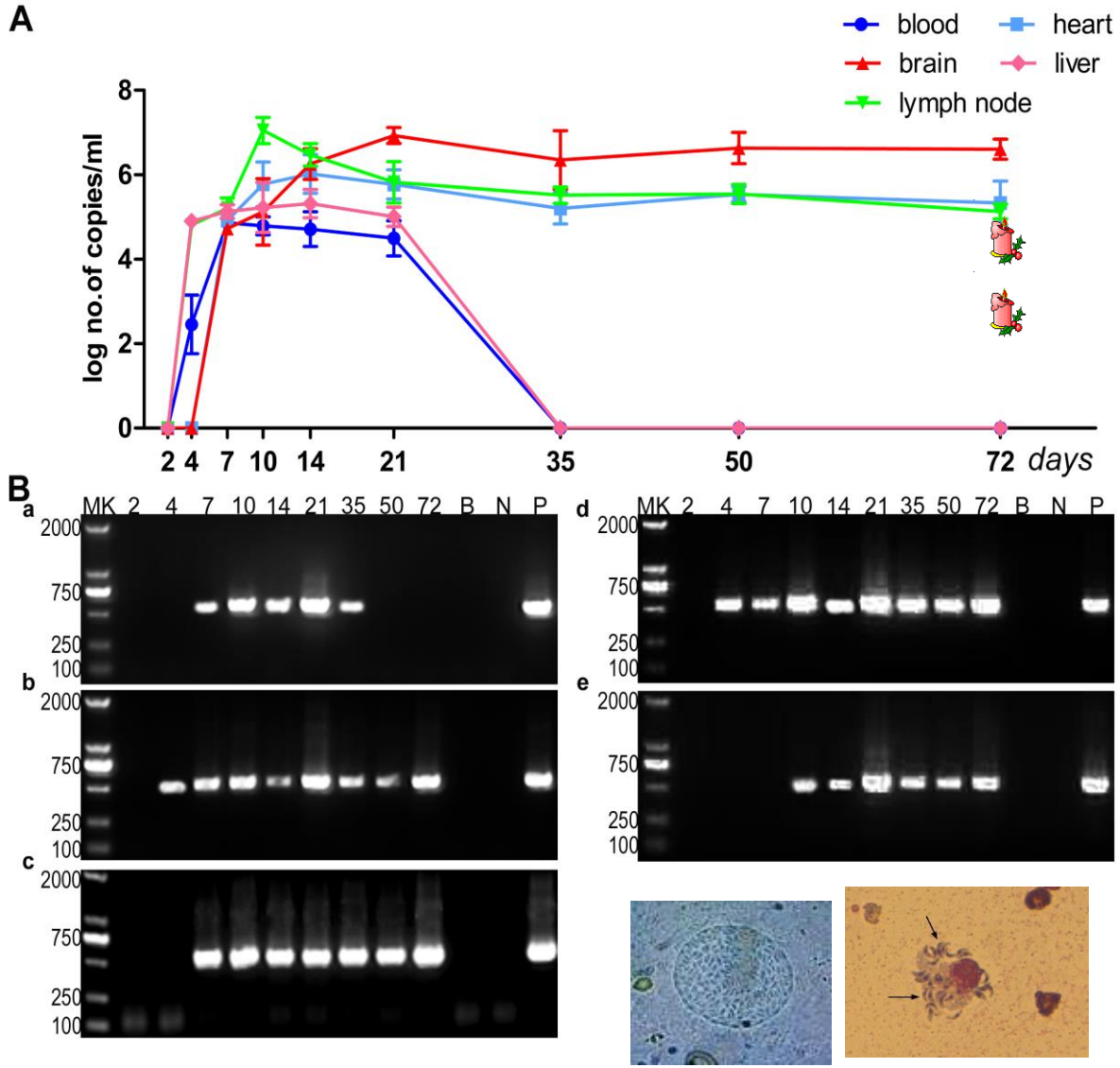
A Summary of mouse virulence of representative strains with different dosage of *T.gondii* tachyzoites

		Toxoplasma gondii isolates							
		RH	TgCtxz1	TgCtxz3	TgCtxz5	TgCtgy1	TgCtwh3	TgCtwh6	PRU
% Mortality in mice inoculated with different dosage of tachyzoites (No.died/ No.infected)	10^0	25 (1/4)	83.3 (5/6)	0 (0/4)	12.5 (1/6)	50 (3/6)	10 (1/10)	0 (0/10)	0 (0/5)
	10^1	80 (4/5)	100 (6/6)	100 (6/6)	75 (6/8)	83.3 (5/6)	60 (6/10)	6.2 (1/16)	0 (0/6)
	10^2	100 (5/5)	100 (6/6)	100 (6/6)	100 (6/6)	100 (6/6)	78.6 (11/14)	18.2 (4/22)	0 (0/6)
	10^3	100 (5/5)	100 (6/6)	100 (6/6)	100 (6/6)	100 (6/6)	92.9 (13/14)	45 (9/20)	16.7 (1/6)
	10^4	100 (5/5)	100 (6/6)	100 (6/6)	100 (6/6)	100 (6/6)	100 (10/10)	43.7 (7/16)	42.9 (3/7)
Cyst found in mice		No	No	No	No	No	No	Yes	Yes
Haplogroup &		C 3.1	C 3.1	C 3.2	C 3.2	C 3.3	C 3.3	C 3.3	C 3.2
Genotypes (ToxoDB)		Type Ⅰ(#10)	Type Ⅰ(#10)	Atypical (#205)	Atypical (#205)	Chinese 1(#9)	Chinese 1(#9)	Chinese 1(#9)	Type Ⅱ(#1)
Location		USA	Easten China, Xuzhou	Easten China, Xuzhou	Easten China, Xuzhou	Westen China, Guiyang	Central China, Wuhan	Central China, Wuhan	France





Time phase of tissue location following Wh6 cyst infection in mouse (DNA copy number tested by q-PCR)



- 50 cysts (Wh6)
- A: DNA copy No. in 5 mice
- B: *T.g* PCR products（529bp） in the brains and peritoneal exudates

a: blood

b: heart

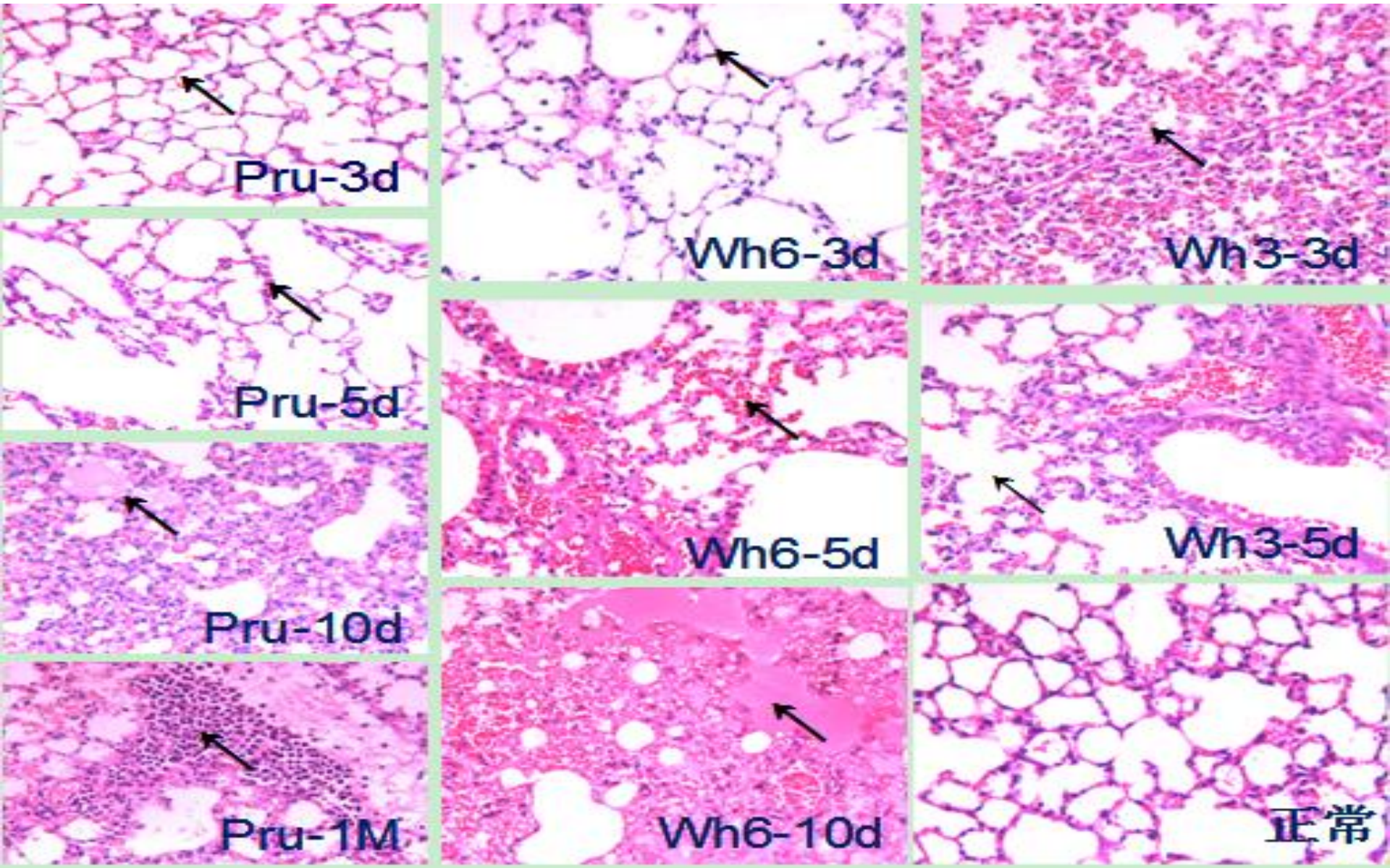
c: brain

d: liver

e: lymphonode

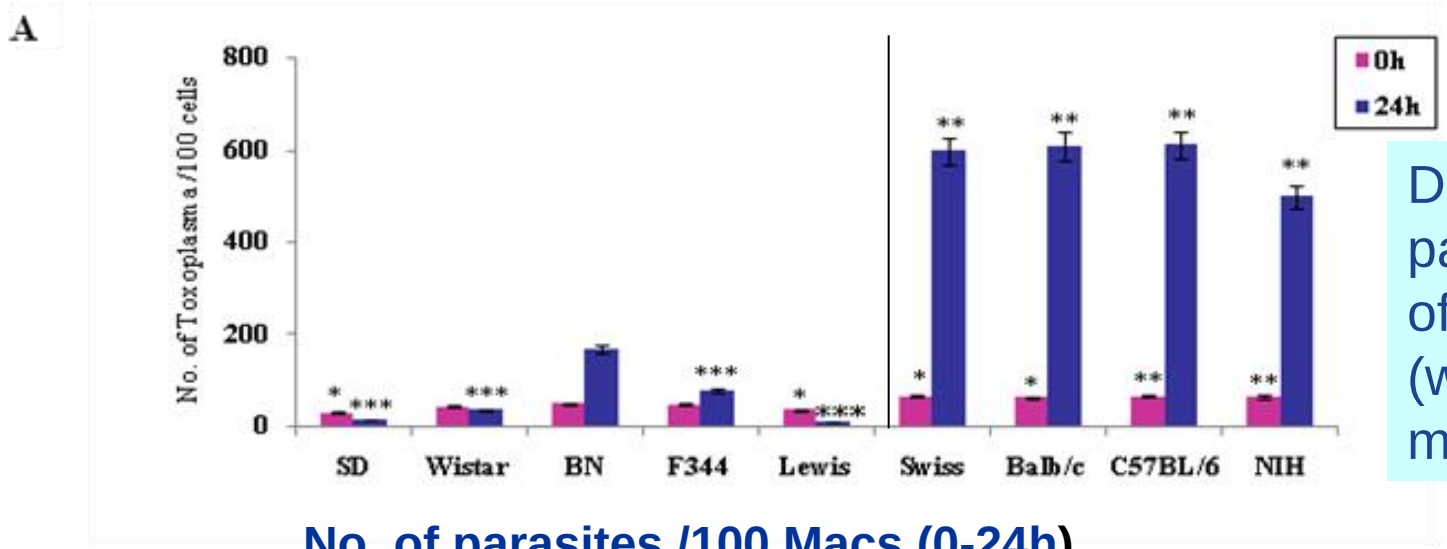
(B, control；N, negative control；P, positive control)

Inflammation Pathology of lung tissues of mouse following infection of T.gondii type II PRU and type Chinese 1 Wh6 and Wh3 strains (HE staining)



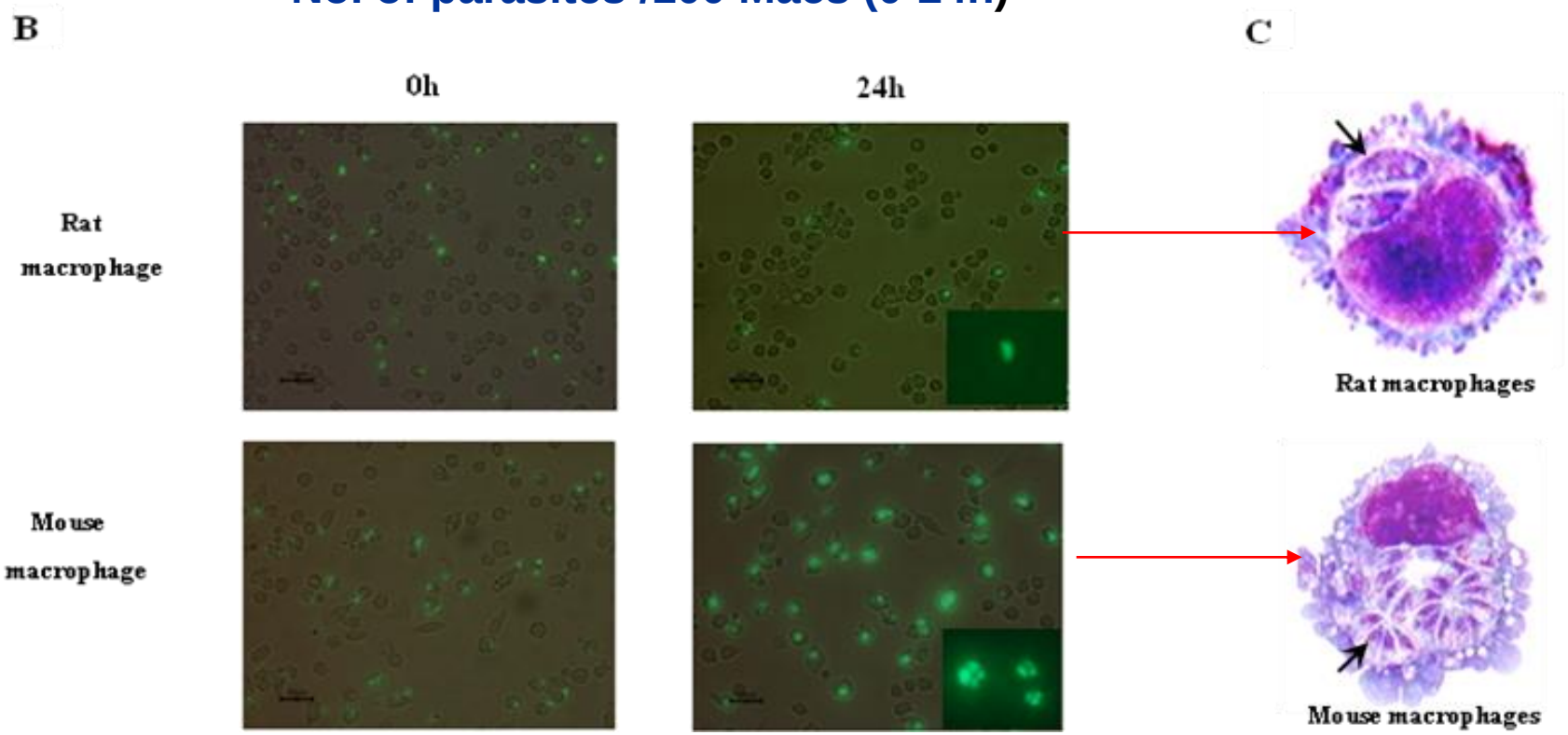
3. Interactions of *T.gondii* with its host macrophages: Resistance/susceptibility and immune evasion

(1) Host resistance or susceptibility depends on the balance of iNOS/NO and Arginase of macrophages

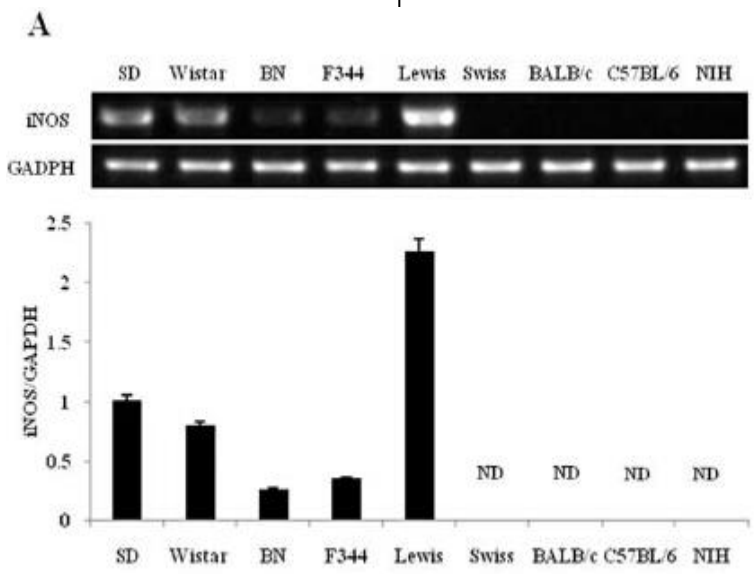


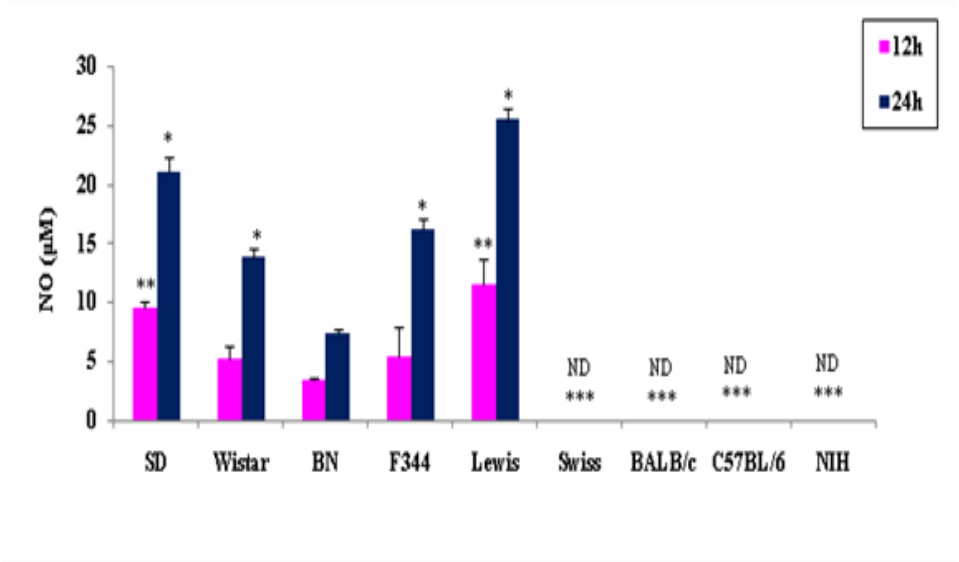
Duplication of parasites in Macs of rats and mice (with fluorescent microscopy)

No. of parasites /100 Macs (0-24h)

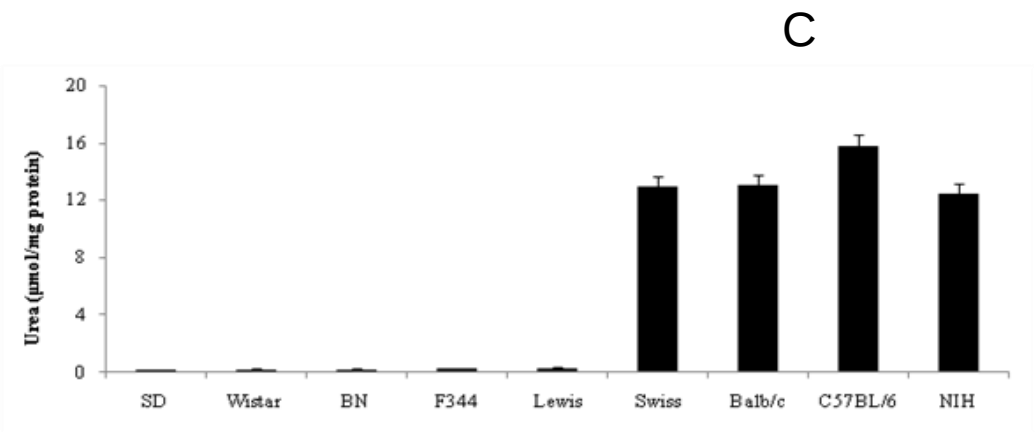


RT-PCR

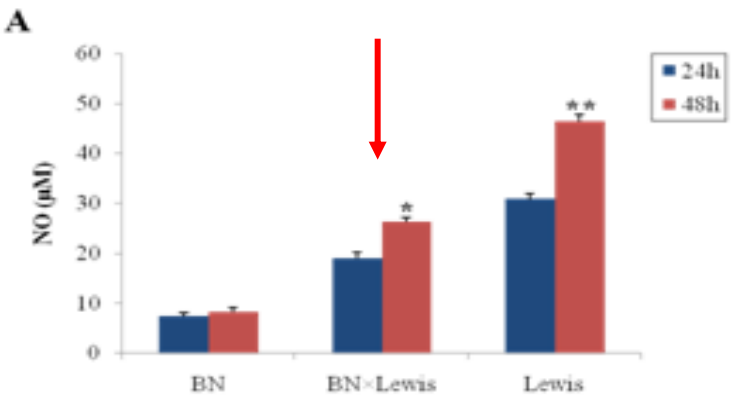


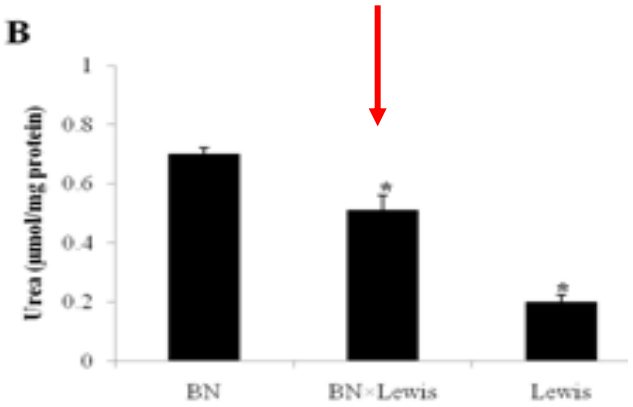


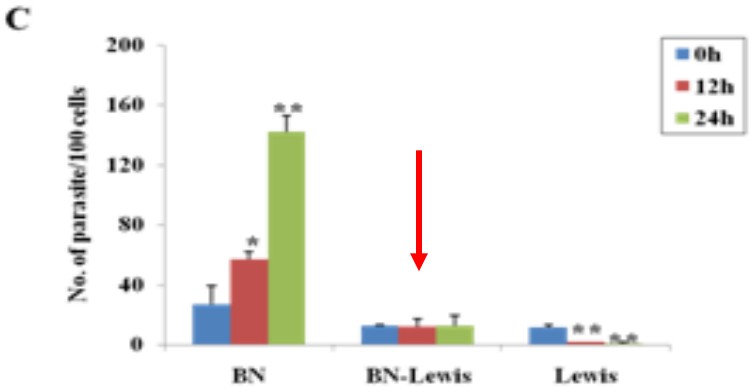
Concentration of iNOS and NO generated in the Macs of rats or mice (24h cell culture)

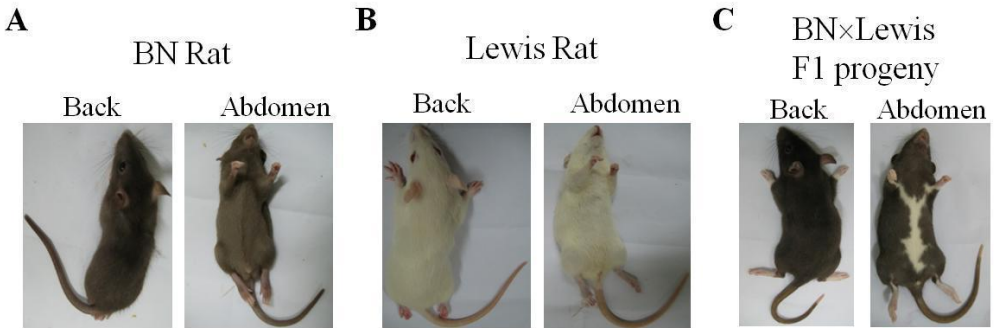


High expression of Arg-1 in peritoneal Macs of mice







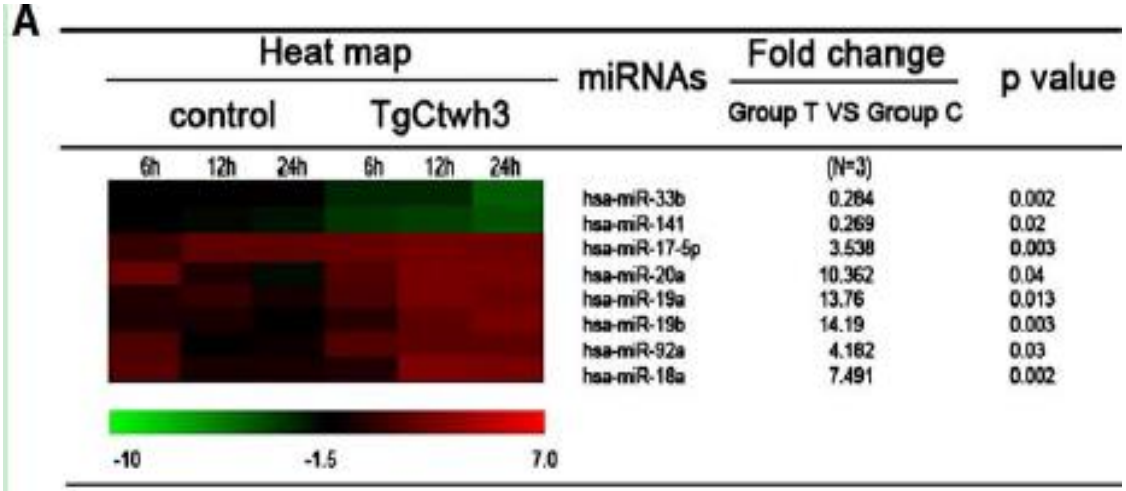


BN rat produced less NO but more urea, whereas Lewis rat secreted more NO but low urea.

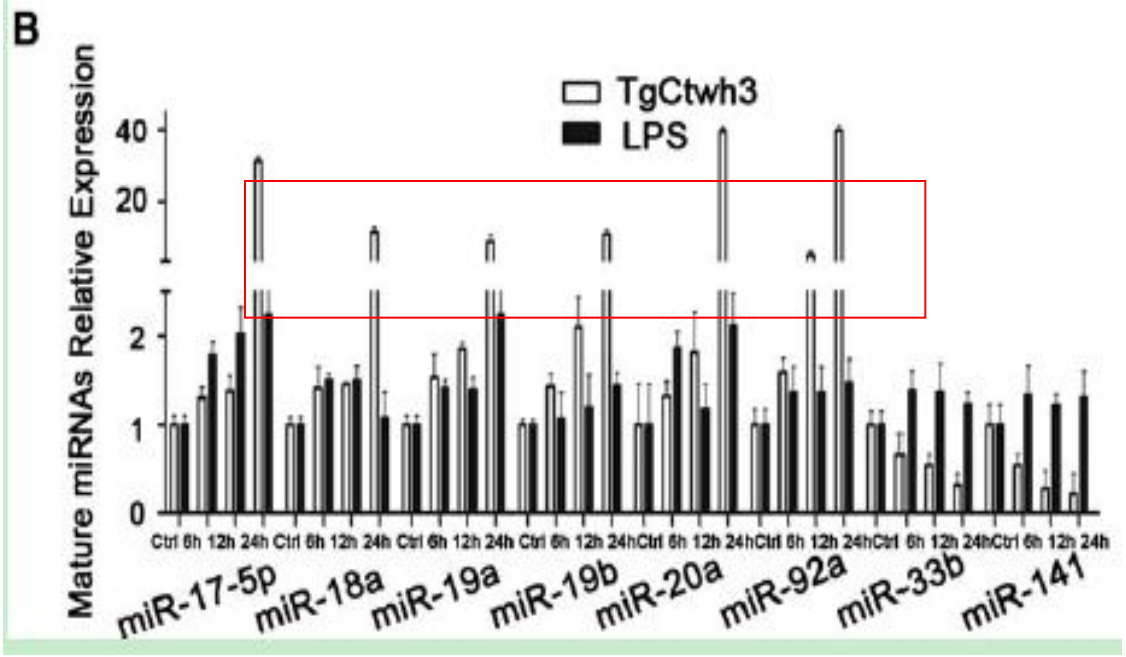
Nitric oxide produced by F1 falls in between its parents.

(2) Stat3-MiR17s-Bim signaling associated inhibition of Macs apoptosis

A



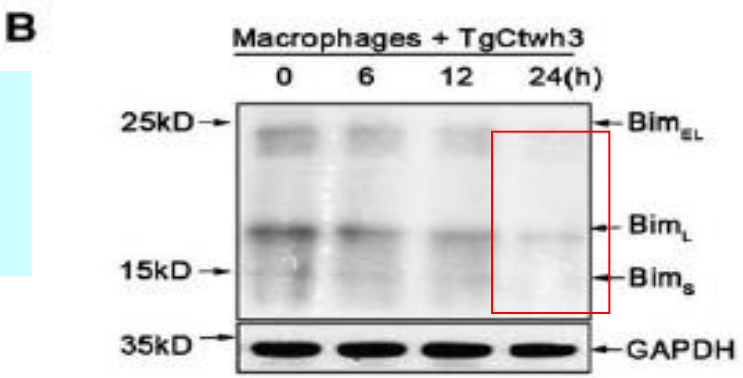
B

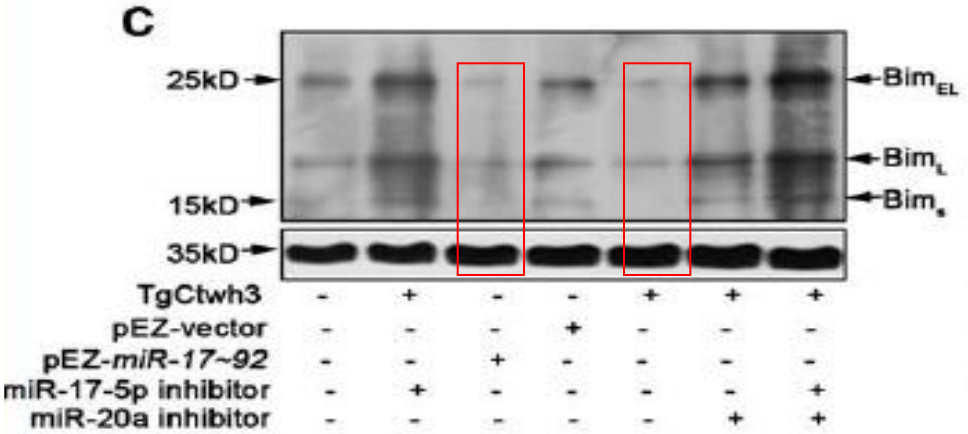




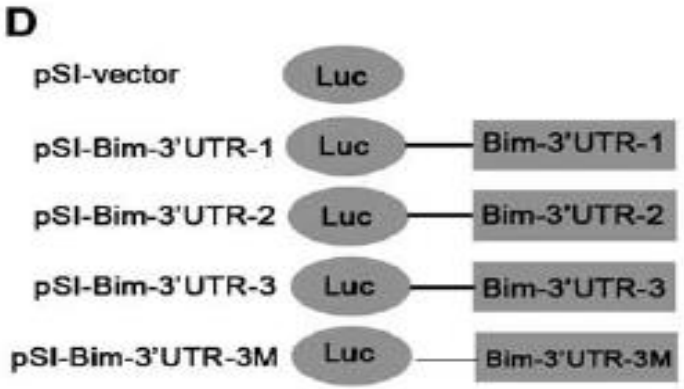
Chips- based identification of microRNA 17-92a in Tg infected human PBMCs verified by qRT-PCR.

T.g infection inhibited expression of BIM



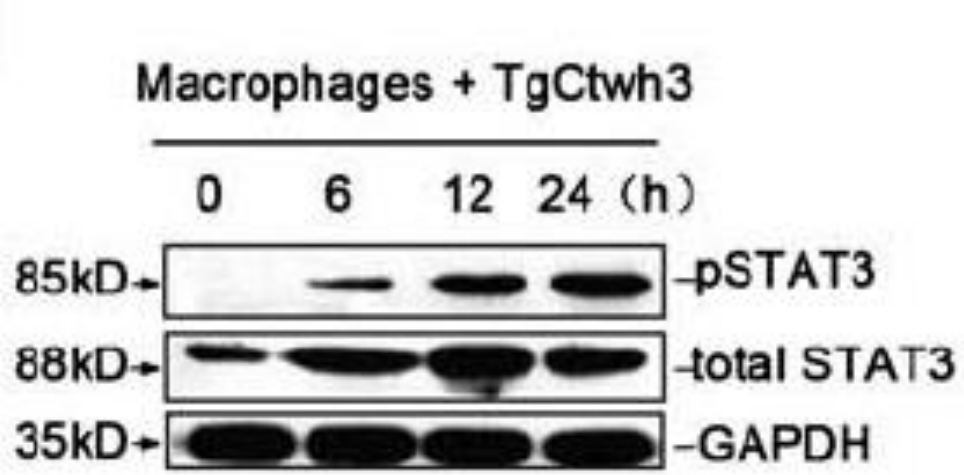


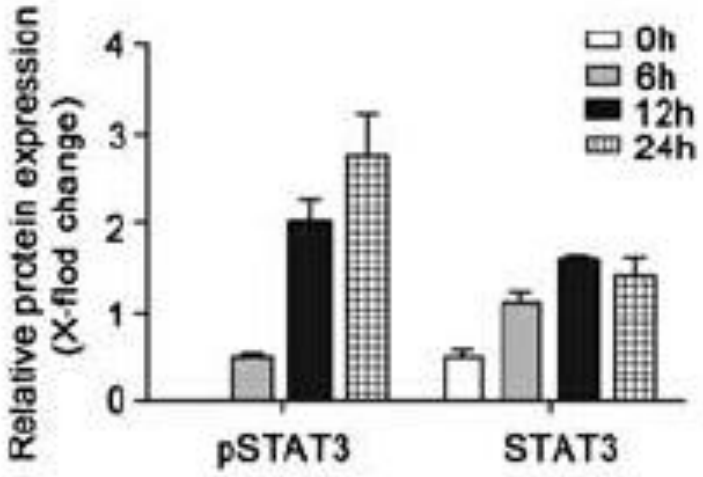
Similar to WT T.g infection, miR17s transfection inhibited BIM expression.



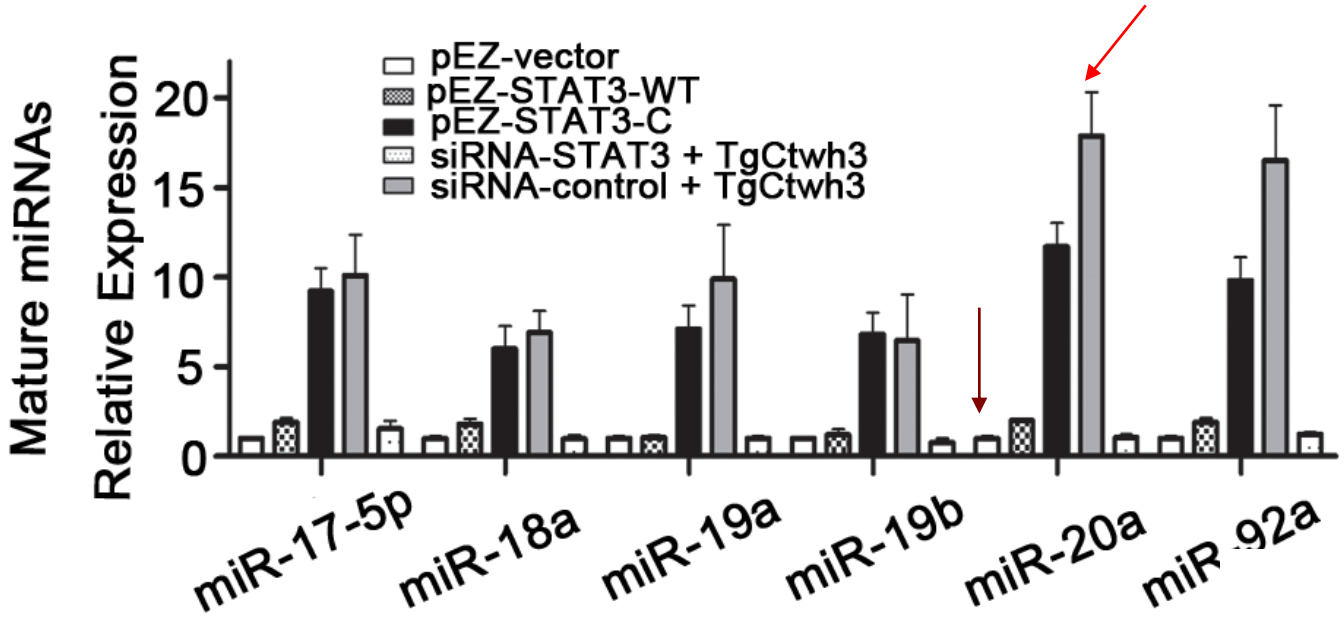
Constructs of luciferase reporter plasmids

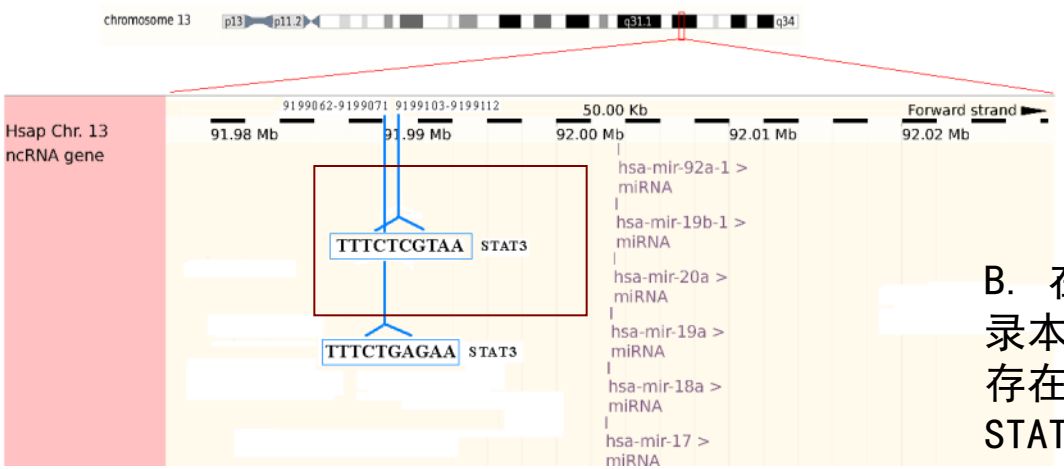
Wh3 infection upregulated expression of pSTAT3

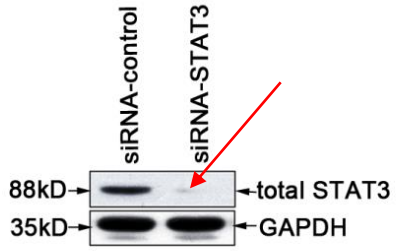




A. Wh3 infection upregulated miR17-92 transcription,which was downregulated following STAT3 siRNA silencing

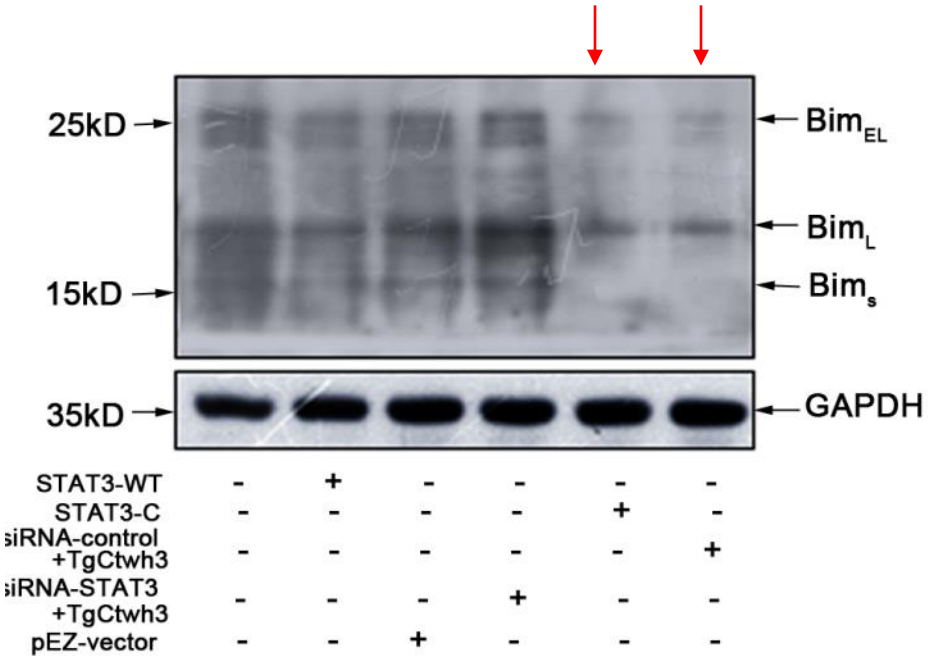


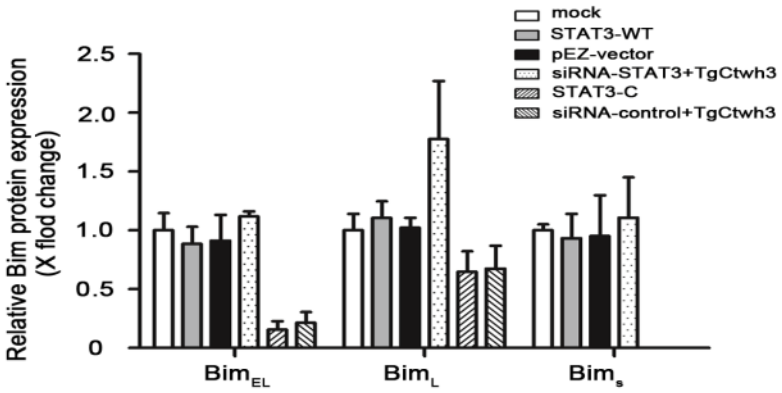


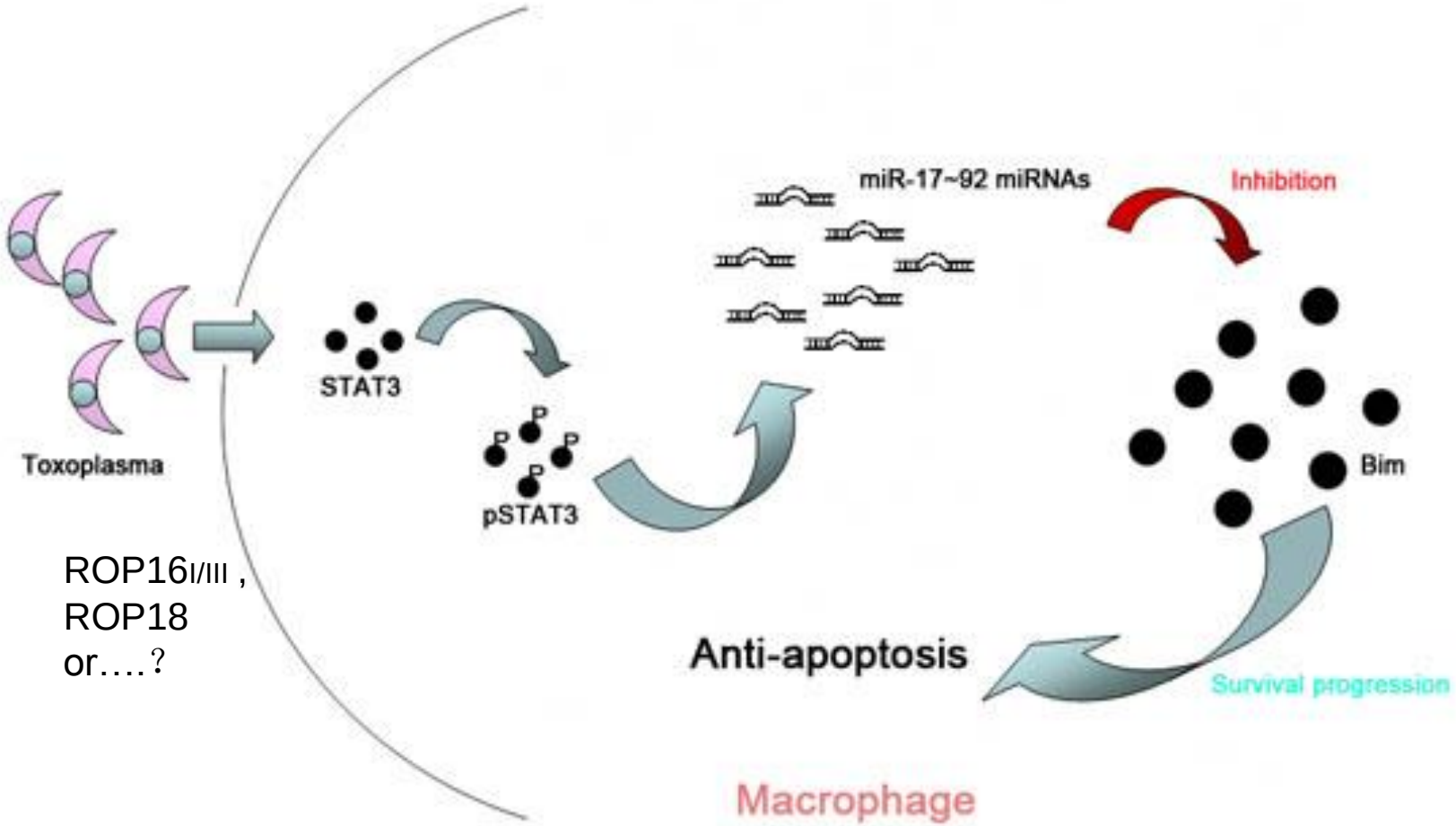


B. 在miR17~92基因簇转录本的上游启动子区域，存在两个高度保守的STAT3结合位点

Wh3 infection and pSTAT3 remarkably inhibited expression of Bim，while inhibition of pSTAT3 enhanced Bim expression





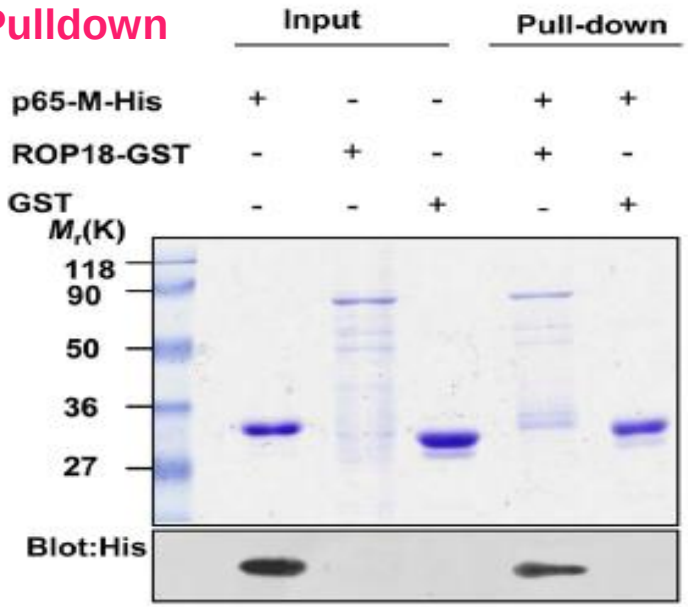


ROP16I/III ,
ROP18
or….?

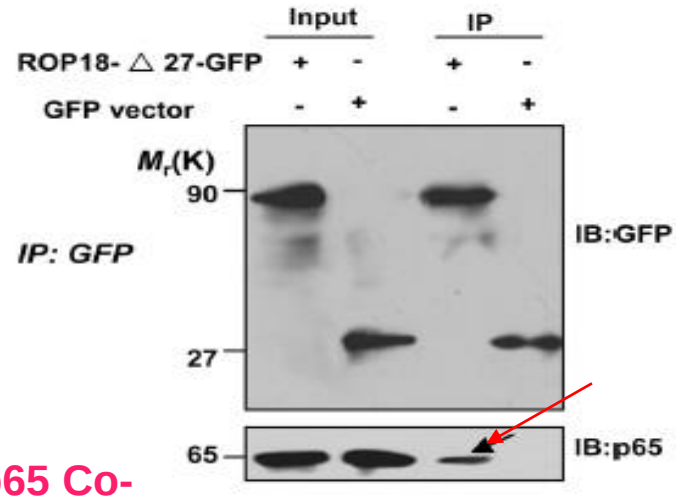
Stat3-MiR17s-Bim signaling is involved in inhibitory apoptosis in infected macrophages, a putative mechanism of immune evasion.

(3)*T.gondii* ROP18 degraded p65 and inhibited activation of NF-κB，driving polarization of the host macrophages to M2 (alternatively activated macrophage)

C Pulldown

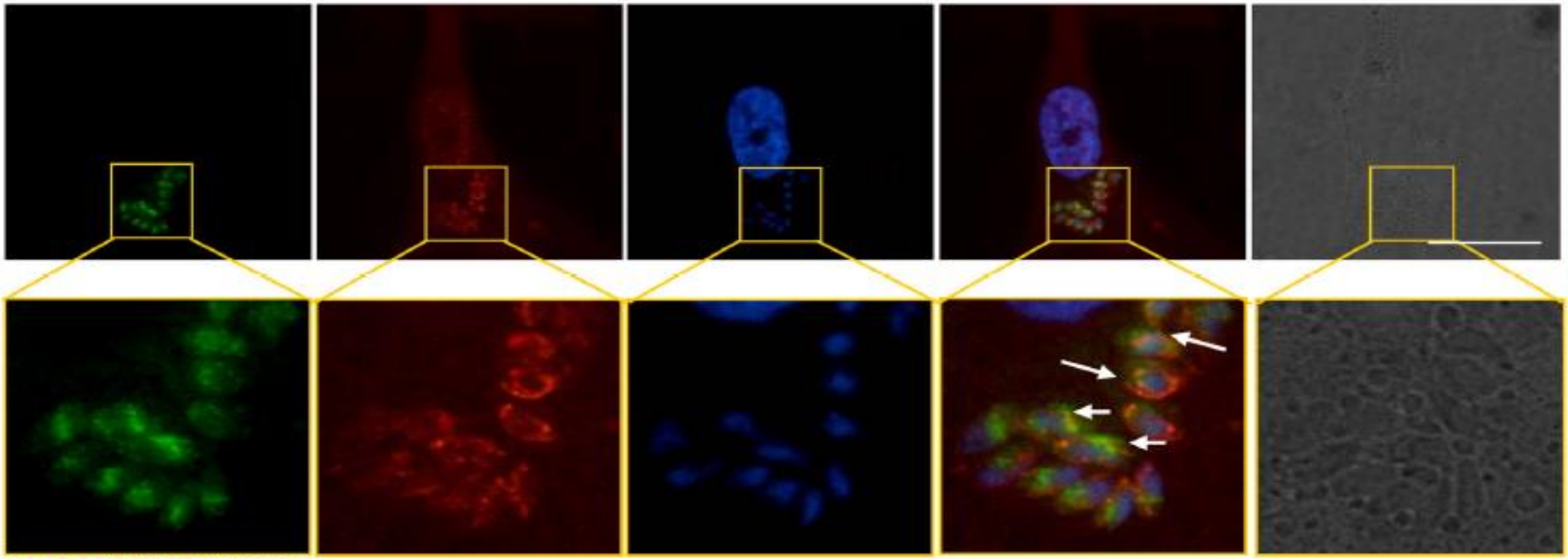


D Co-IP

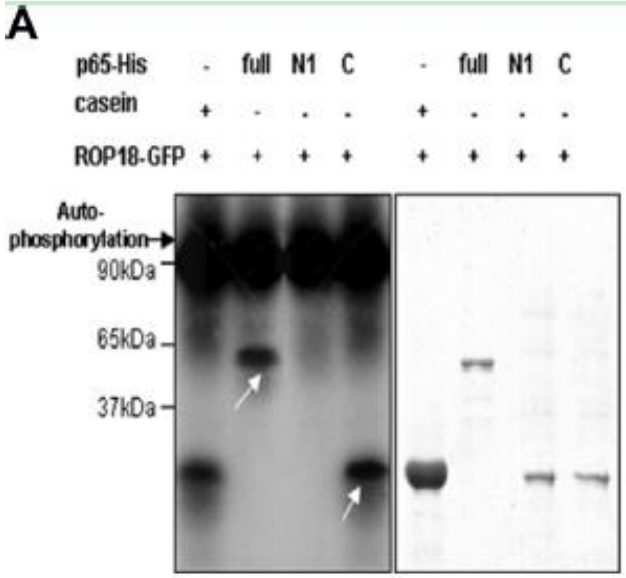


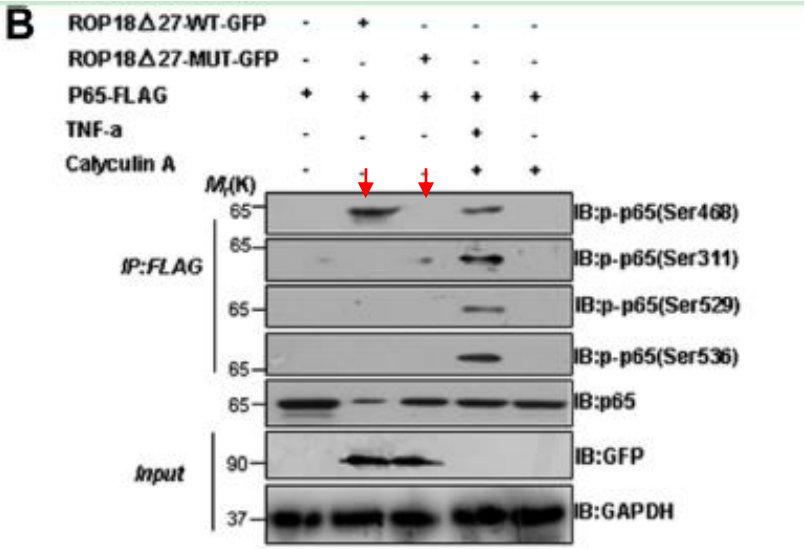
ROP18/p65 Co-localization

E



ROP18 directly phosphorylates C-terminus of NF-kB p65

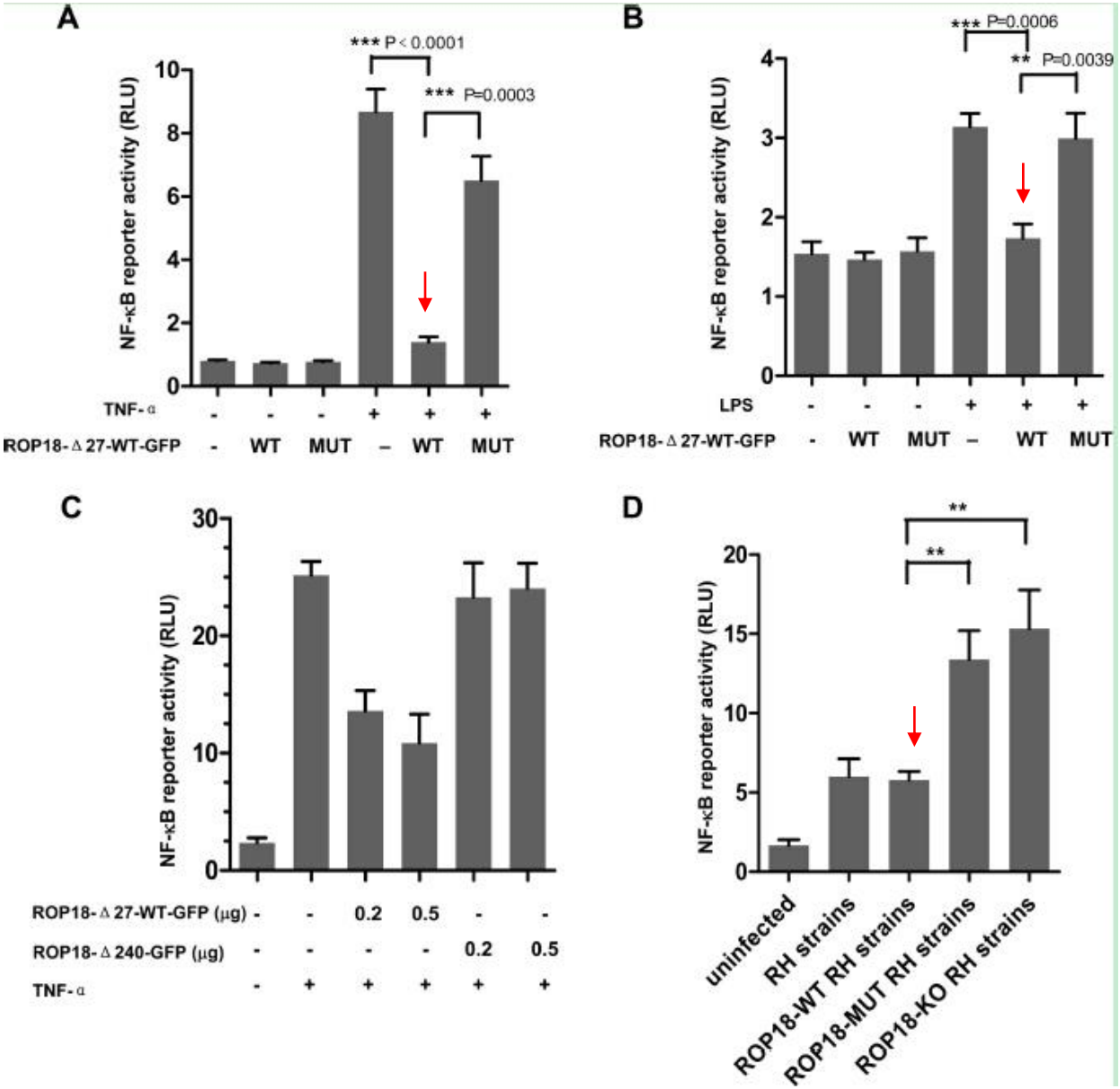




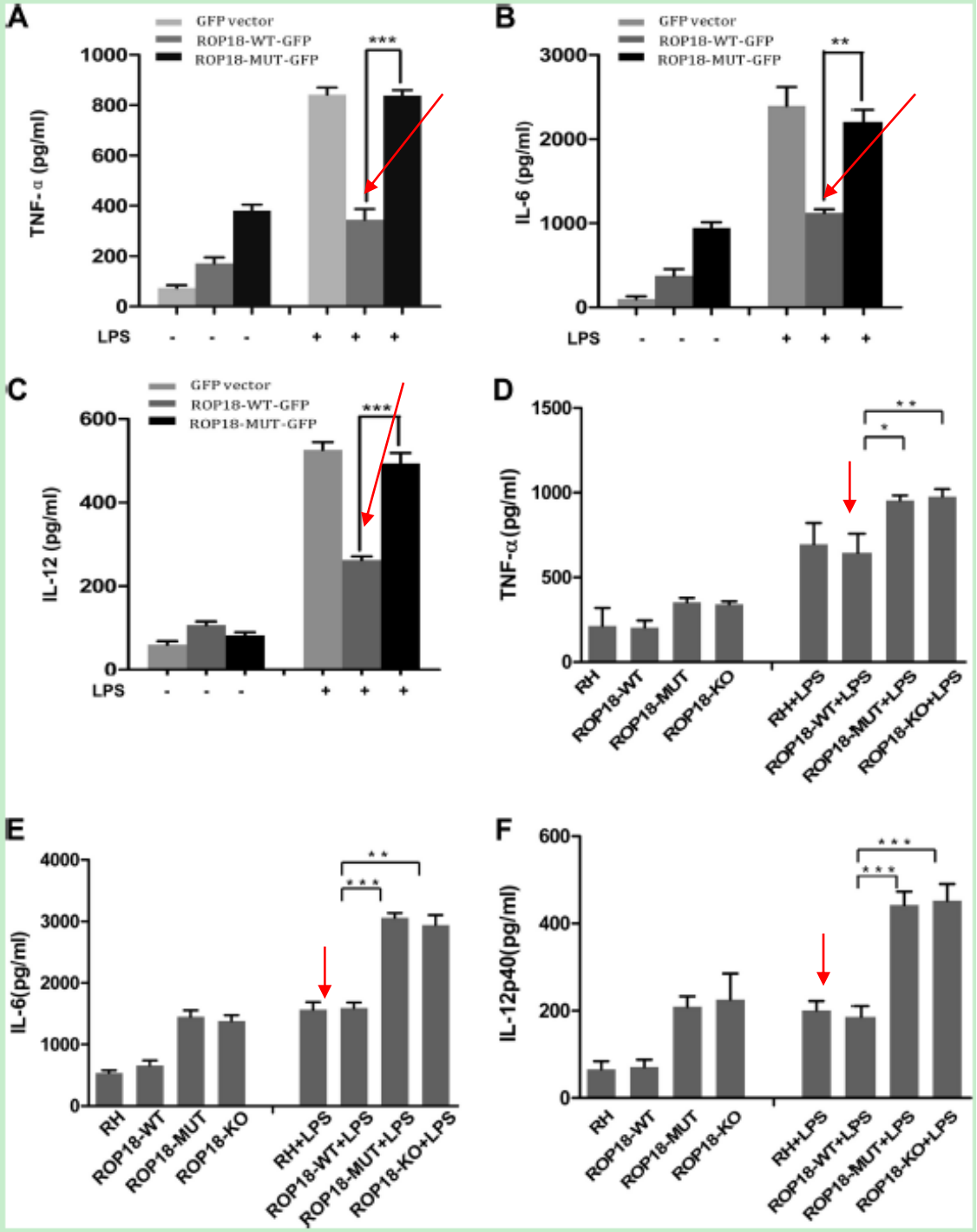
ROP18 phosphorylated the full length and C terminus of p65 （white arrow)）

It is ROP18-WT rather than ROP18-Mut that phosphorylated p65（red arrow）

Kinase activity of ROP18 suppresses NF-B activation



A，B，C: When Macs were transfected with ROP18-WT and –MUT plasmids，no reactivation of NF-κB was noted (showing low expression of TNF-a,IL-6, and IL-12.



D,E,F:When Macs were infected with WT,*rop18*-mutant or –knockout strains of parasites, NF-κB was disabled and TNF-a, IL-6, and IL-12 were downregulated (M2 polarization）

Summary

1. Population structure of *T.gondii* in China differs greatly from other continents of the world, type Chinese 1 dominates in China mainland;

2.Chinese 1 strains share ROP16I/III with type I and GRA15II with type II although Chinese 1 possesses both virulent and avirulent strains;

3. Resistance/susceptibility depends on the ratio of iNOS/NO and Arginase in macrophages of rats and mice;

4. Stat3-MiR17s-Bim signaling is involved in inhibition of apoptosis of human macrophages; ROP18 phosphoryletes p65, resulting in degradation of NF-kB and reduction of inflammation cytokines, driving the macrophages to polarization of M2.

The mechanisms might provide an explanation for the immune evasion of the intracellular apicomplexan.

Acknowledgments

- Special thanks are given to Prof. C. Su at the University of Tennessee, USA, for his kind help with the reference DNA samples and technical support
- This work was funded by National Basic Research Program of china (973 Project, 2010CB530001) and National Science Foundation of China(81471983)
- Appreciation is given to the colleagues and collaborators who are dedicated to this work

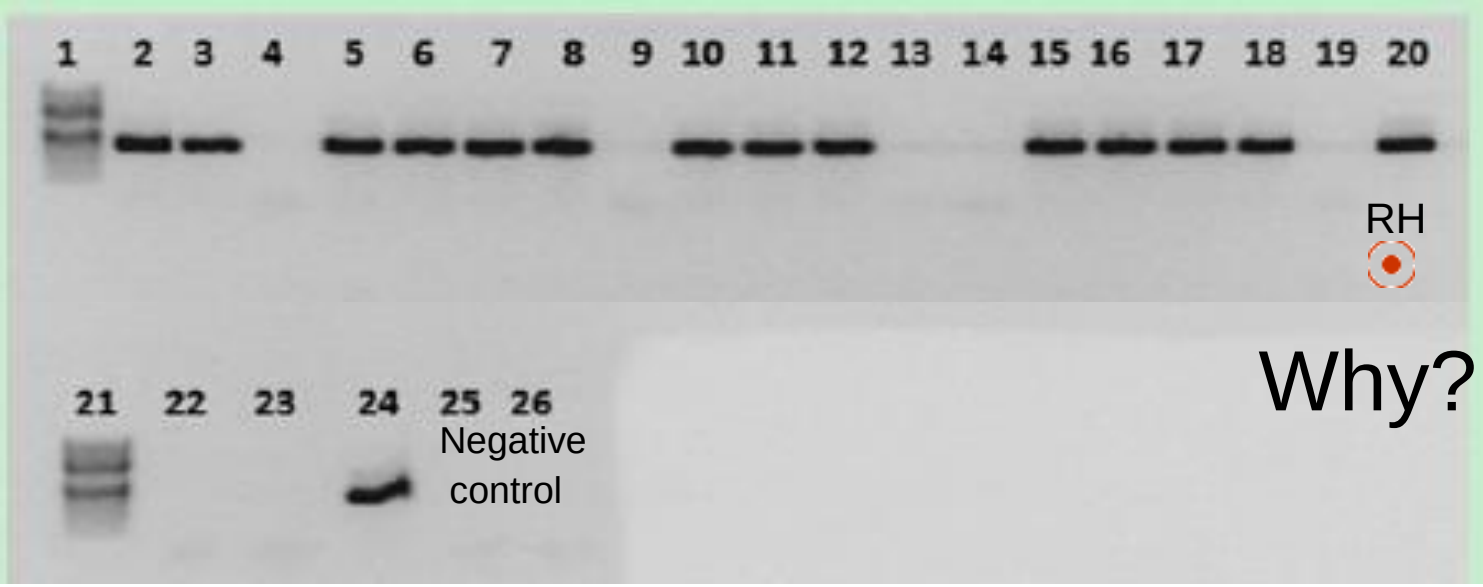


We love the fascinating organism since it knows how to change the situation which is not just.

Thanks for your patience



Striking divergence in Toxo *rop16* sequences from human (virulent) and meat samples (avirulent) in Colombia

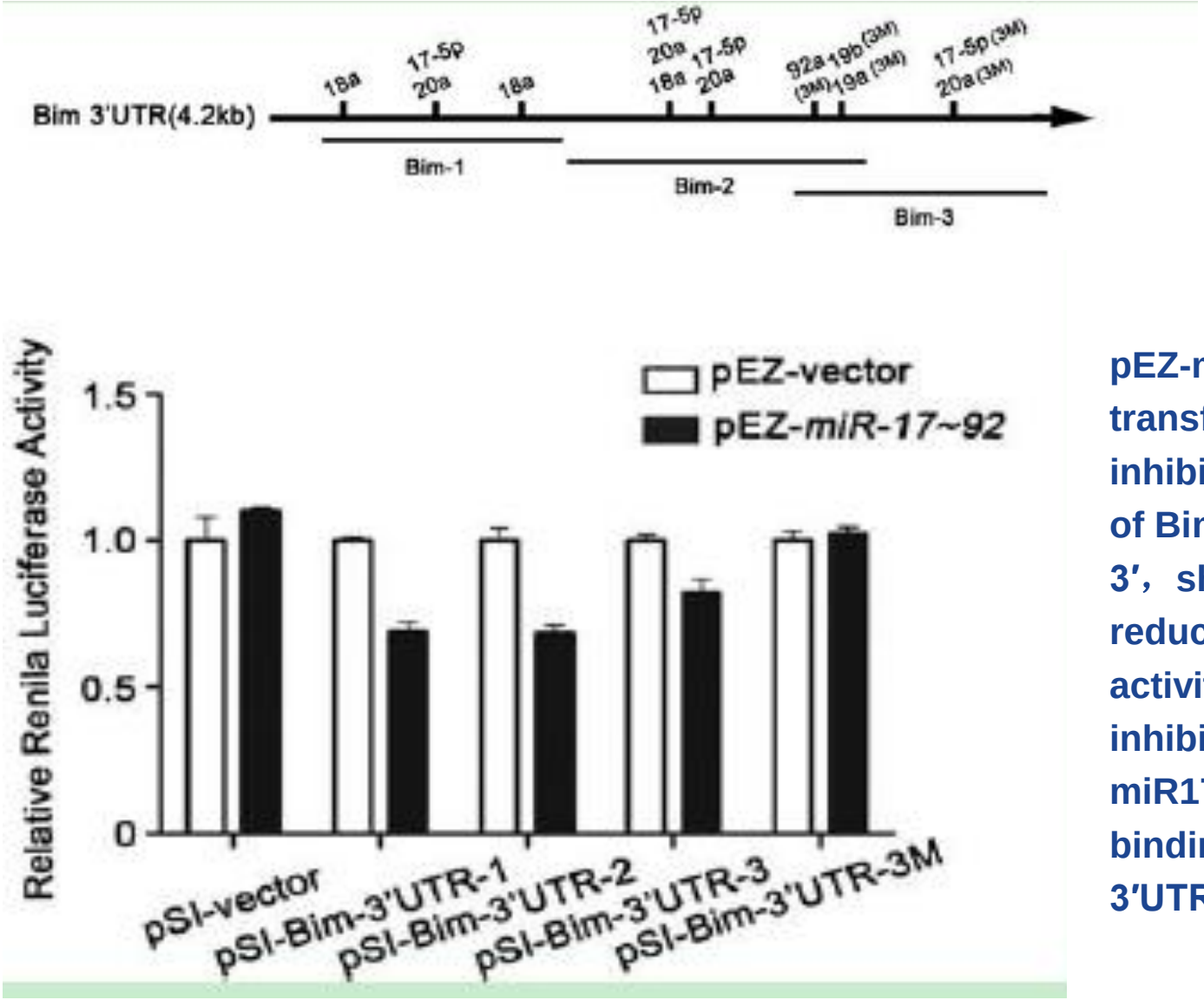


ROP16I/III sequences (mouse-virulent strains) were noted in 45.5%-83.3% patients and soldiers.

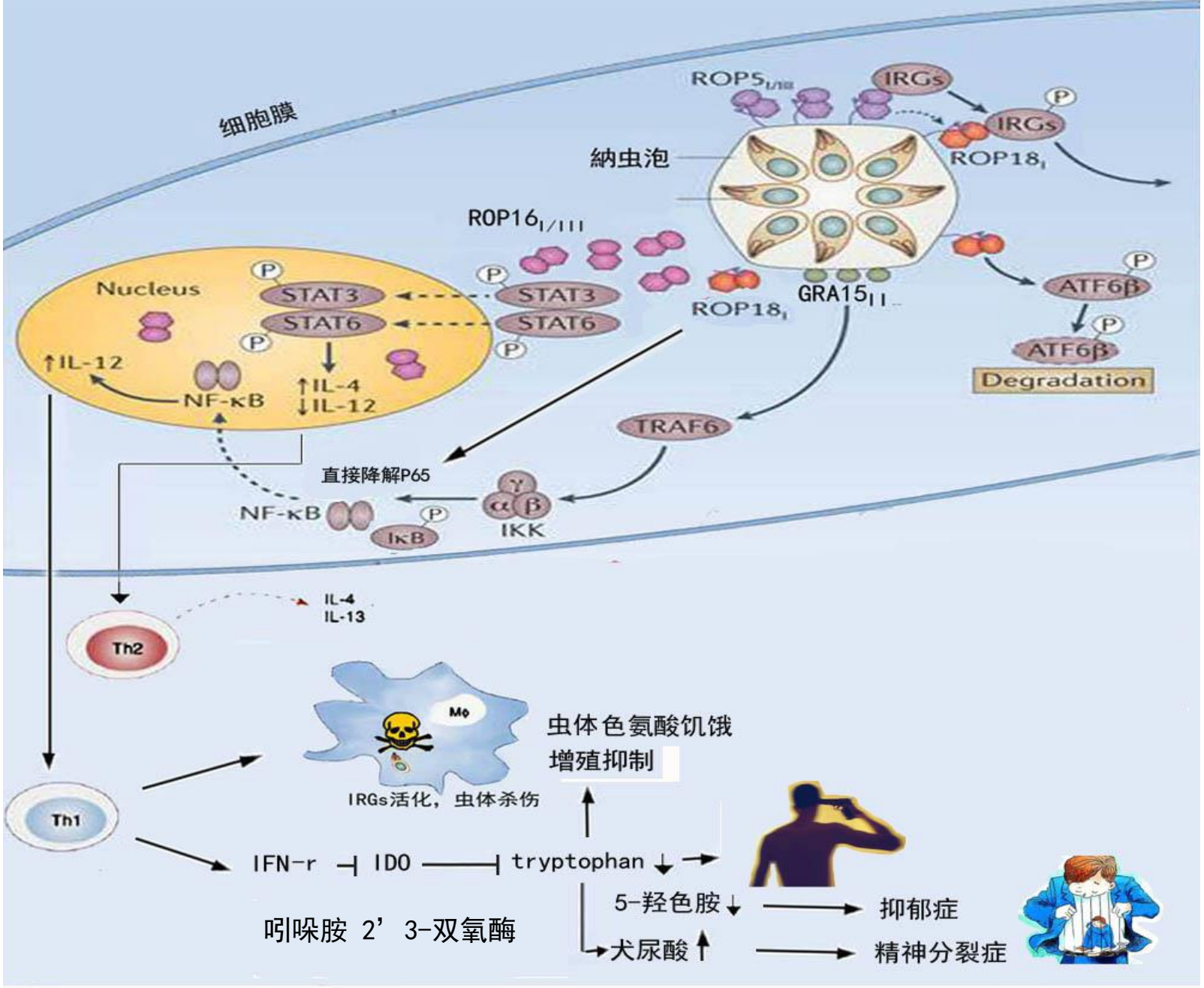
Of note, ROP16II sequences (mouse avirulent type II) were seen in 7 of 7 (100%) from meat samples.

Only 11 of 104 (10.5%) serum samples had specific antibodies against mouse-virulent ROP16I peptide.

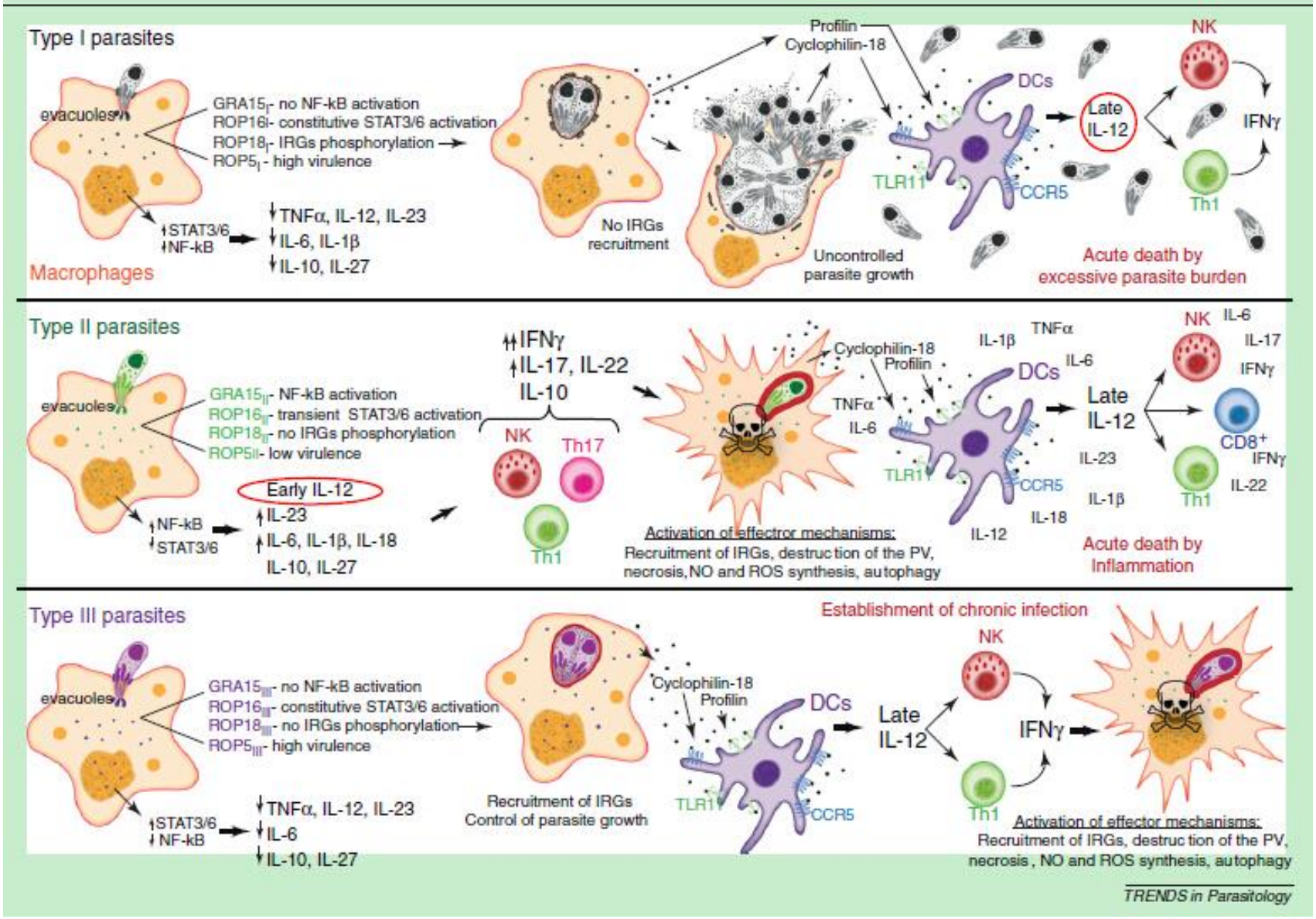
Catalina Alvarez et al. JID, 2015:211

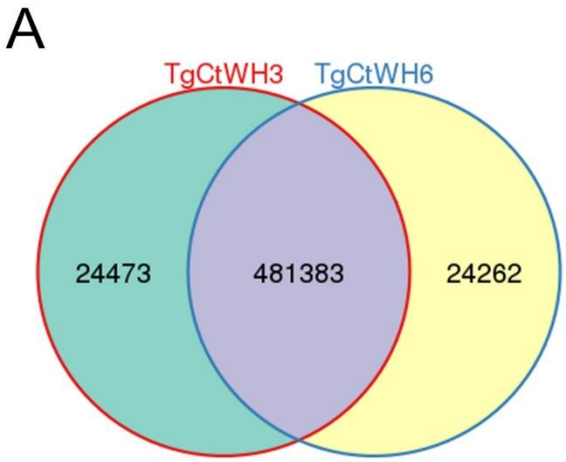


pEZ-miR-17~92 transfection inhibited activity of Bim-3′UTR-1，2，3′，showing reduced luciferase activity and inhibited effect of miR17~92 binding to Bim-3′UTR.

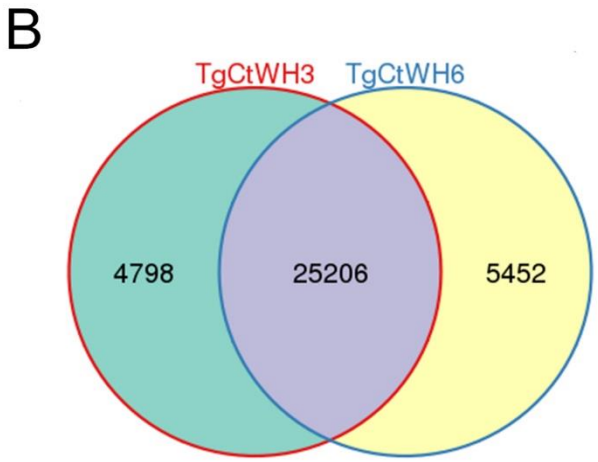


弓形虫感染诱导的IFN-r介导的精神分裂症可能的机制

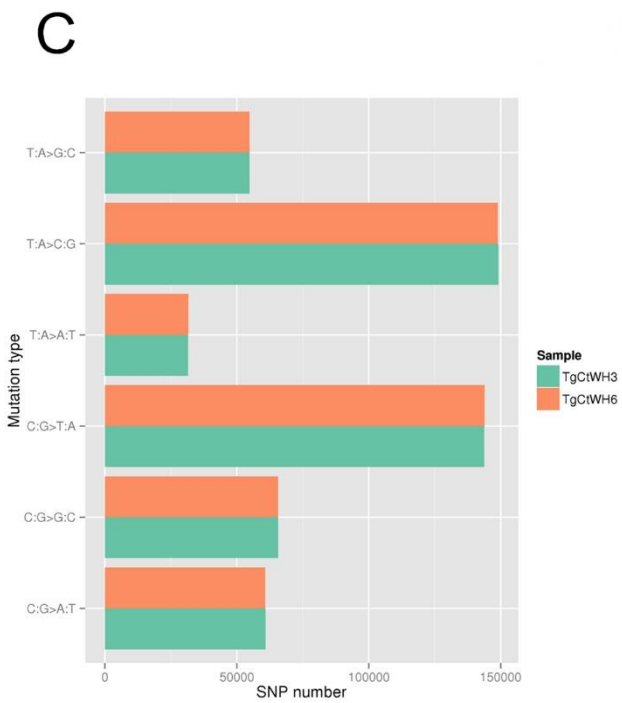

Type I parasites
evacuoles
GRA15I- no NF-kB activation
ROP16I- constitutive STAT3/6 activation
ROP18I- IRGs phosphorylation
ROP5I - high virulence
STAT3/6
NF-kB
TNFα, IL-12, IL-23
IL-6, IL-1β
IL-10, IL-27
Macrophages
No IRGs recruitment
Profilin
Cyclophilin-18
DCs
TLR11
CCR5
Late IL-12
NK
IFNγ
Th1
Uncontrolled parasite growth
Acute death by excessive parasite burden
Type II parasites
evacuoles
GRA15II- NF-kB activation
ROP16II- transient STAT3/6 activation
ROP18II- no IRGs phosphorylation
ROP5II- low virulence
NF-kB
STAT3/6
Early IL-12
IL-23
IL-6, IL-1β, IL-18
IL-10, IL-27
IFNγ
IL-17, IL-22
IL-10
NK
Th17
Th1
Cyclophilin-18
Profilin
DCs
TLR11
CCR5
TNFα
IL-1β
IL-6
IL-23
IL-18
IL-12
Late IL-12
NK
IL-6
IL-17
IFNγ
CD8+
IFNγ
IL-22
Th1
Activation of effector mechanisms:
Recruitment of IRGs, destruction of the PV, necrosis, NO and ROS synthesis, autophagy
Acute death by Inflammation
Type III parasites
evacuoles
GRA15III- no NF-kB activation
ROP16III- constitutive STAT3/6 activation
ROP18III- no IRGs phosphorylation
ROP5III- high virulence
STAT3/6
NF-kB
TNFα, IL-12, IL-23
IL-6
IL-10, IL-27
Recruitment of IRGs
Control of parasite growth
Cyclophilin-18
Profilin
DCs
TLR11
CCR5
Late IL-12
NK
IFNγ
Th1
Establishment of chronic infection
Activation of effector mechanisms:
Recruitment of IRGs, destruction of the PV, necrosis, NO and ROS synthesis, autophagy
TRENDS in Parasitology

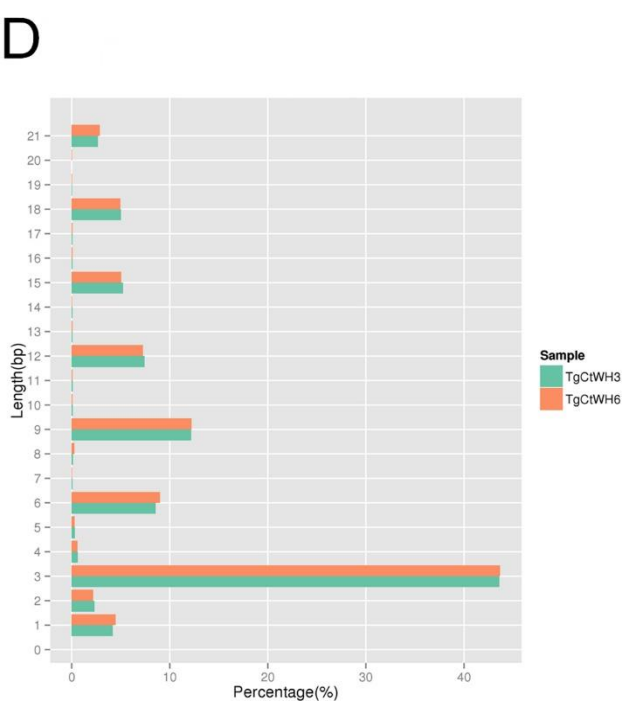


SNPs



Indels





SNPs and Indels comparison and distribution

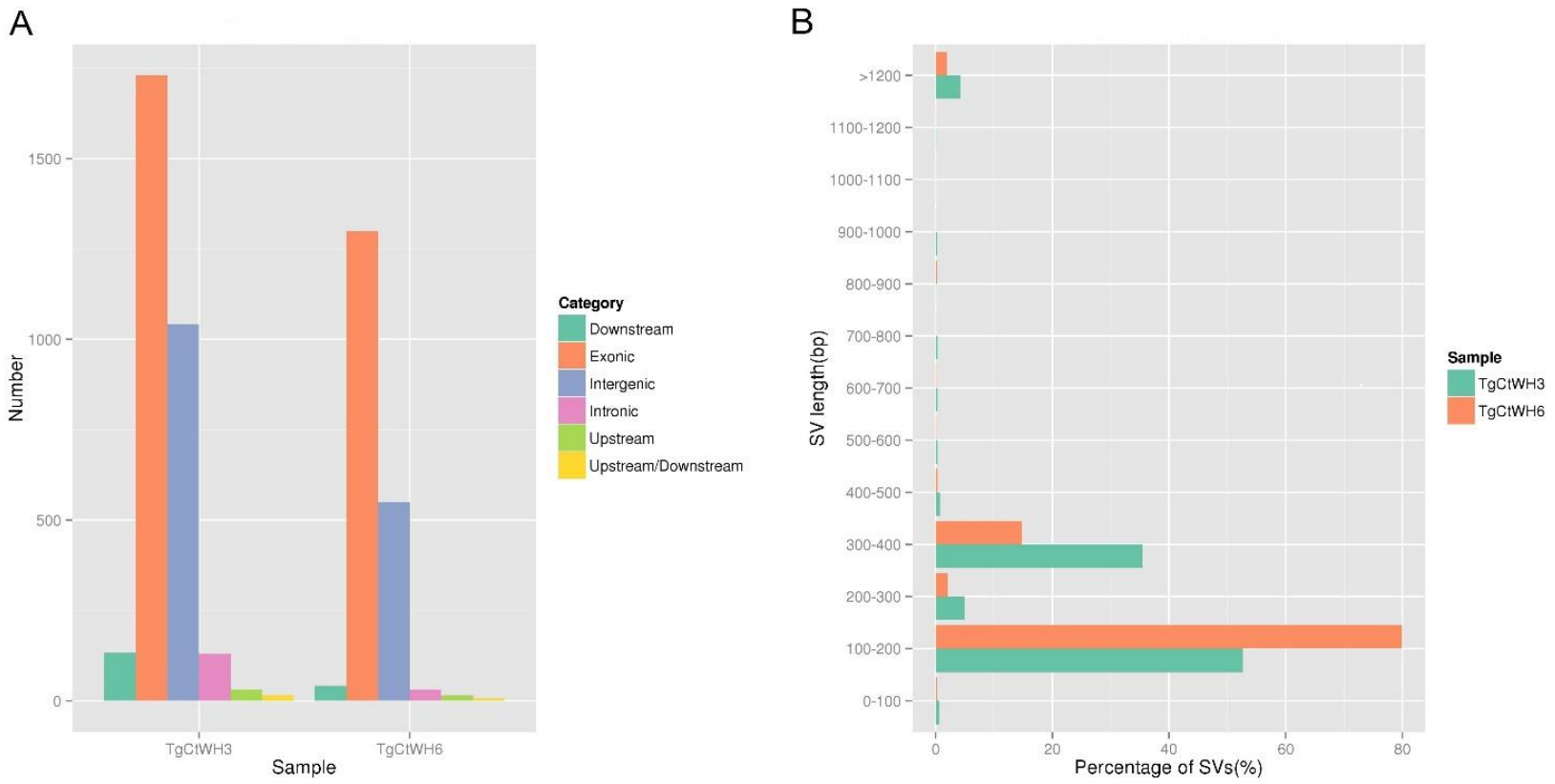
(A) Venn diagram of shared and unique SNPs between two samples and the GT1 reference genome.

(B) Venn diagram of shared and unique Indels between two samples and the GT1 reference genome.

(C) SNP mutation type distribution of TgCtWh3 and TgCtWh6

(D) CDS InDel length distribution of TgCtWh3 and TgCtWh6

Next generation sequencing of Wh3 and Wh6



CN(copy number)Vs and S(structure)Vs distribution

(A) Distribution of CNVs annotation result

(B) Distribution of SVs length

表2 中国的弓形虫优势基因型*T. gondii* genotypes in China

虫株	host	SAG1	SAG2	SAG3	BTUB	GRA6	C22-8	C29-2	L358	PK1	Apic	ToxoDB
ZS, ZS1 (n=2) （n=11）	Human	μ-1	Π	ШI	ШI	Π	Π	ШI	Π	Π	I	**#9 (Chin 1)**
TgCtPRC2, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17; TgCtwh1, 2, 3, 4, 5, 6, 7, 8, TgCtgd1, 2, TgCtsx1, 2, TgCtys1, 2; TgC1, 2, 3, 4, 5, 6, 7, 8 , TgCatBj1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 (n=46)	Cat	μ-1	Π	ШI	ШI	Π	Π	ШI	Π	Π	I	**#9 (Chin 1)**
GYS, PYS, ZC, FS, NY5, NY19, NY11, NY8, NY13, LC-13, PE-18, ZT-11, WS-29, WS-30 (n=14)	Pig	μ-1	Π	ШI	ШI	Π	Π	ШI	Π	Π	I	**#9 (Chin 1)**
SH (n=1)	Humn	I	I	I	I	I	I	I	I	I	I	**#10 (Type I)**
CN, NT, TgPHs1, TgPXx,TgPXd, TgPNx,TgPLh, TgPxda,Tgpxdb1, Tgpyh, TgPNY, TgPGZ (n=12)	Pig	I	I	I	I	I	I	I	I	I	I	**#10 (Type I)**

表3 中国的优势弓形虫基因型

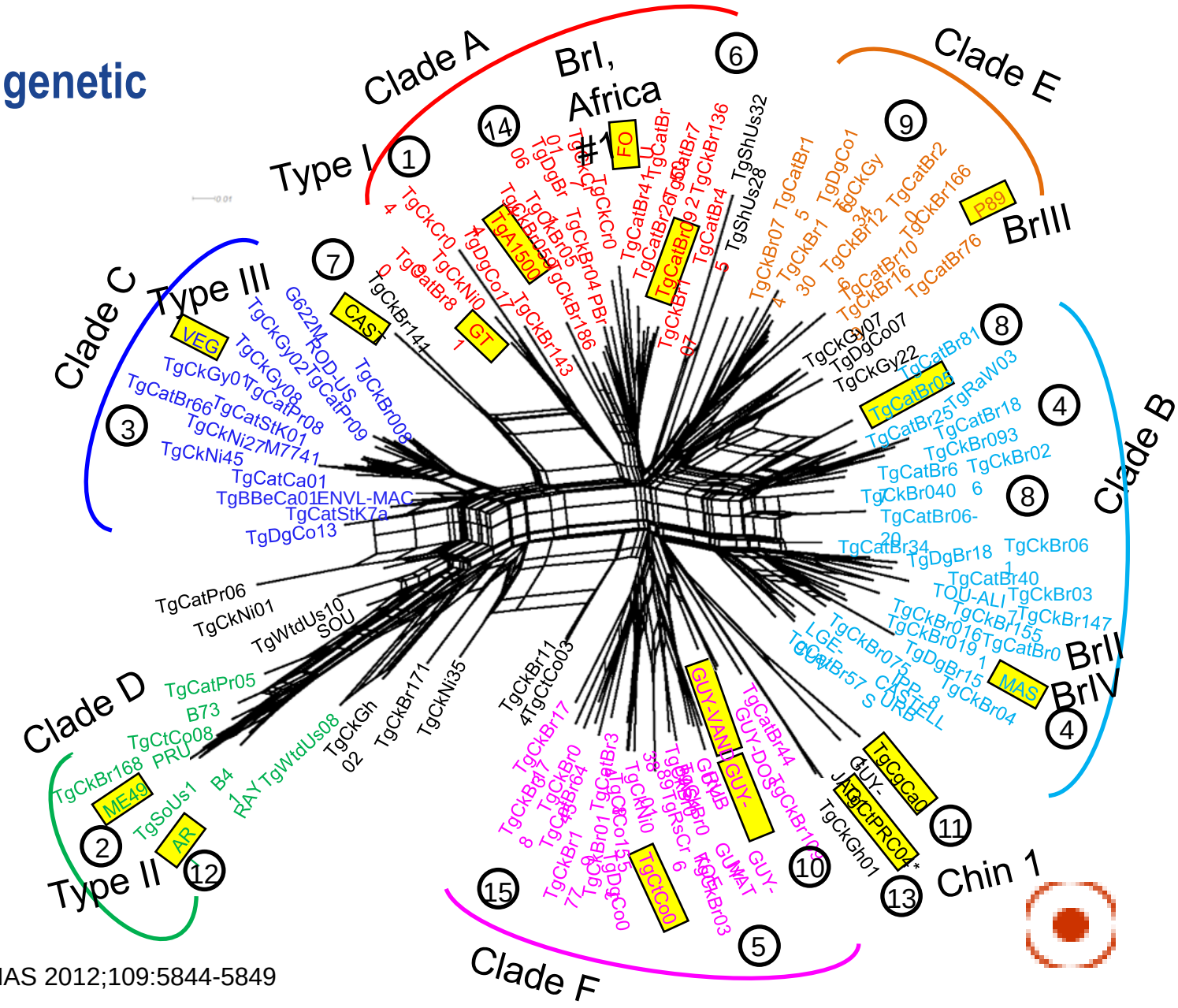
虫 株	宿 主	SAG1	SAG2	SAG3	BTUB	GRA6	c22-8	c29-2	L358	PK1	Apico	ToxoDB
S10 (n=1)	麻雀	I	I	I	I	I	I	I	I	I	I	**#10 (Type I)**
TgCtPRC1,3 (n=2)	猫	I	I	III	I	III	II	I	III	III	I	**#18**
QHO (n=1)	绵羊	II	II	II	II	II	II	II	II	II	I	**#3 (Type II)**
JSEM1 (n=1)	麻雀	II	II	II	II	II	II	II	II	II	I	**#3 (Type II)**
H2 (n=1)	野鸡	II	II	II	II	II	II	II	II	II	I	**#3 (Type II)**
Tgpkf171（n=416）	市售猪肉											**#213 #9(Chin 1）**
SHR	家兔	III	III	III	III	III	III	III	III	III	I	**#2（Type III)**

Global *T.g* genetic diversity

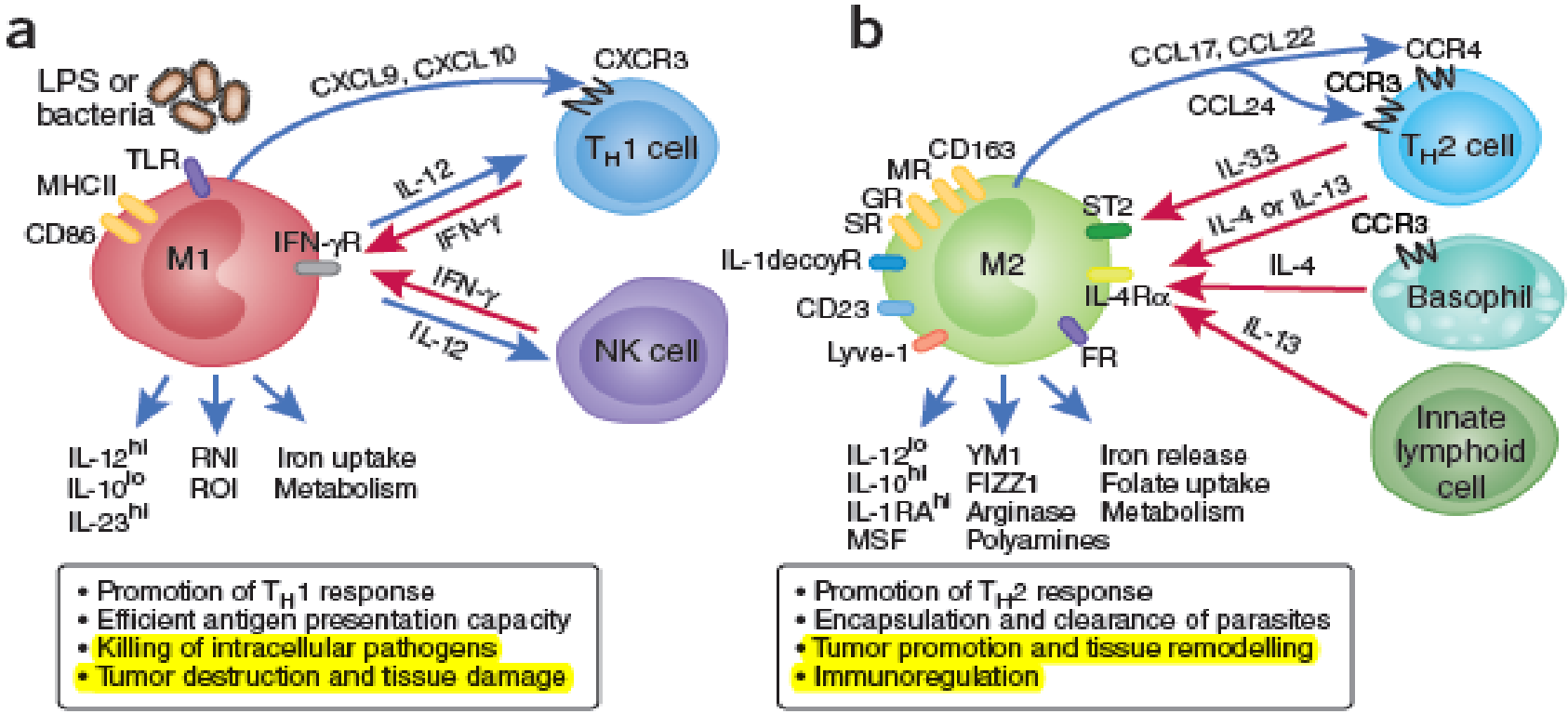
Toxo12

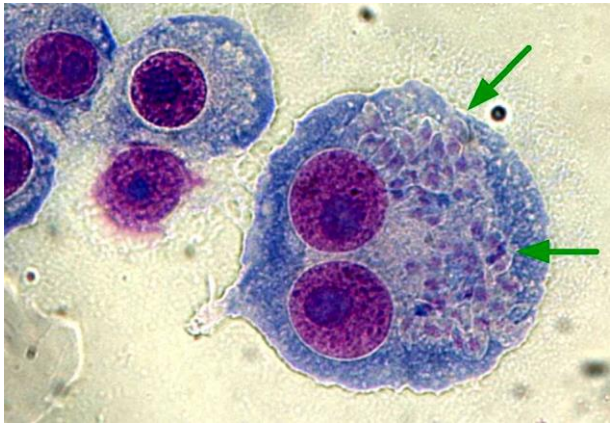
Presented By
Dr. C Su

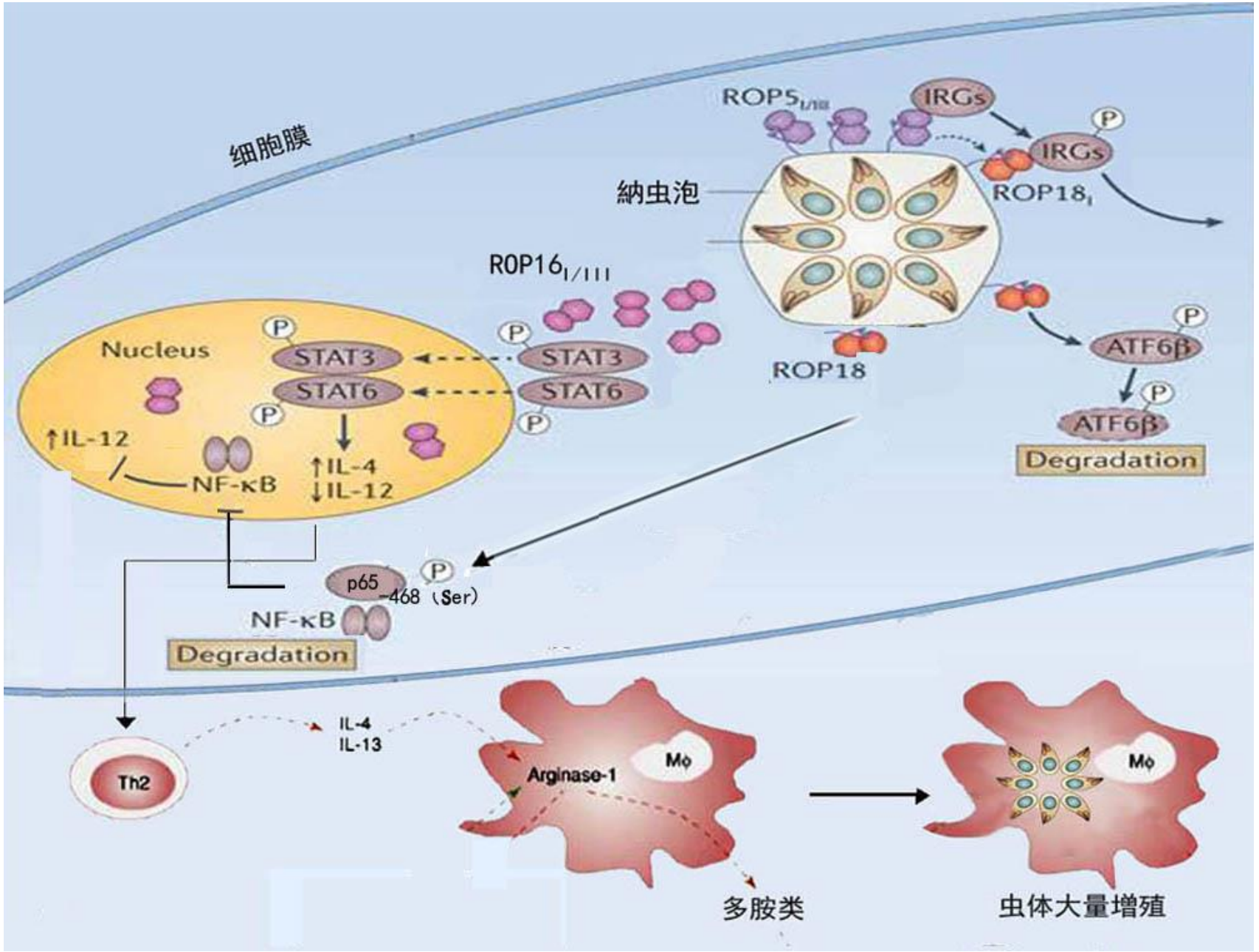
At Intl Confer
Toxo, Oxford
2013



PNAS 2012;109:5844-5849


a
LPS or bacteria
TLR
MHCII
CD86
M1
CXCL9, CXCL10
CXCR3
T_H1 cell
IL-12
IFN-γ
IFN-γR
IFN-γ
IL-12
NK cell
$IL\text{-}12^{hi}$
$IL\text{-}10^{lo}$
$IL\text{-}23^{hi}$
RNI
ROI
Iron uptake
Metabolism
• Promotion of T_H1 response
• Efficient antigen presentation capacity
• Killing of intracellular pathogens
• Tumor destruction and tissue damage
b
CCL17, CCL22
CCR4
CCL24
CCR3
T_H2 cell
IL-33
IL-4 or IL-13
CCR3
IL-4
Basophil
IL-13
Innate lymphoid cell
CD163
MR
GR
SR
IL-1decoyR
CD23
Lyve-1
M2
ST2
IL-4Rα
FR
$IL\text{-}12^{lo}$
$IL\text{-}10^{hi}$
$IL\text{-}1RA^{hi}$
MSF
YM1
FIZZ1
Arginase
Polyamines
Iron release
Folate uptake
Metabolism
• Promotion of T_H2 response
• Encapsulation and clearance of parasites
• Tumor promotion and tissue remodelling
• Immunoregulation





ROP18-induced p65 degradation resulted in inhibition of NF-κB, driving Macs polarization to M2

近2年的代表性论文：

1. Jian Du et al. J Bio Chem, 2014 Mar 19. [Epub ahead of print] doi: 10.1074/jbc.M113.544718 (IF=4.651)

2. Lin Wang et al. PLoS ONE, 2013 Jan, 8(1): e53483. (IF=4.285)

3. Xu X et al. Experimental Parasitology 154 (2015) 51–61 (IF:1.638)

4. Hua Wang et al. International Journal of Food Microbiology, 2012, 157(3): 393-397. (IF=3. 425)

5. Yihong Cai et al. Cell Signal, 2014 Jun, 26(6): 1204-1212. (IF=4.304)

6. Lanting Kong et al. Acta Tropica, 2015; 143: 1–7 (IF;2.27)

7. Yihua Zhang et al. Parasites & Vectors 2014, 7:372 (IF:3.256)

8. Teng Wang et al. Parasitology, 2014, doi:10.1017 /S0031182014000183 (IF:2.56)

9. Lin Wang et al. Parasites & Vectors, 2015;8:162 (IF:3.256)

10.Qian Zhang et al. Journal of Parasitology, 2015; Apr 14. [Epub ahead of print] (IF:1.227)

11. Jie Zhou et al. Parasites & Vectors, 2015; 8:73 (IF:3.256)

12. Min Li et al. Parasites & Vectors, 2014; 7:133 (IF:3.256)

13. Aimei Zhang et al. Parasites & Vectors, 2013, 6:308 (IF:3.256)

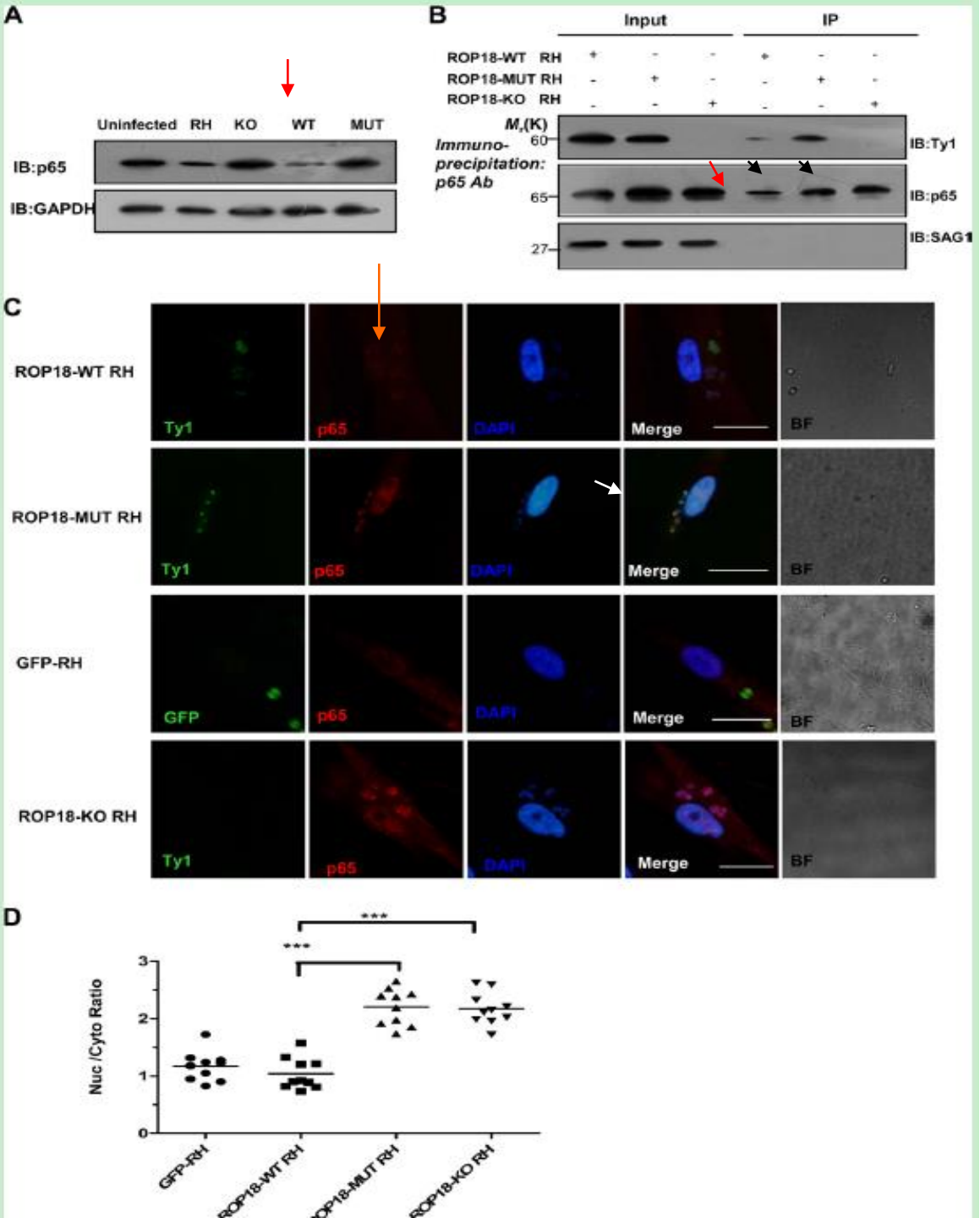
14.Tingting Liu et al. Parasitol Res 2013; 112:3019–3027 (IF:2.10)

15.Xingxing Huo et al. Parasitol Res 2013; 112:3041–3051 (IF:2.10)

16.Yihong Cail et al. Parasites & Vectors 2013, 6:356 (IF:3.256)

17. Li Yu et al. Parasitol Res DOI 10.1007/s00436-012-3187-0 (IF:2.10)

WT Toxo degraded HFF p65



ROP18-WT (neither -mutant nor –knockout) degraded p65

IF indicated degradation of p65 by ROP18

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- And Natiaonal Science Foundation of China(81471983)
- Thanks are given to the colleagues and collaborators who are dedicated to this work

