Additional preliminary work from the applicant's laboratory relating to the continuation project B3.

The following figures illustrate some of the main findings derived from the present project period of project B3.

Figure 1

We cloned and sequenced the three genomic regions carrying resistance genes of the IRG family from the CAST/EiJ genome from a BAC library. CAST/EiJ is a representative of the Mus musculus castaneus subspecies derived from South-East Asia. The sequenced regions are compared to the corresponding regions of the laboratory mouse genome by means of a dot matrix at high stringency. **The short segment of Chr 11 carrying Irgb10, Irgm3 and Irgm2 (indicated by red dots) shows excellent co-linearity (Fig 1a) as expected within a species. In the two long segments on Chromosomes 11 and 18, which contain the majority of IRG genes (Fig 1b, c) there is massive reorganization and many genes present in C57BL/6 are lost in CAST/EiJ (indicated by positions of blue vertical lines).**

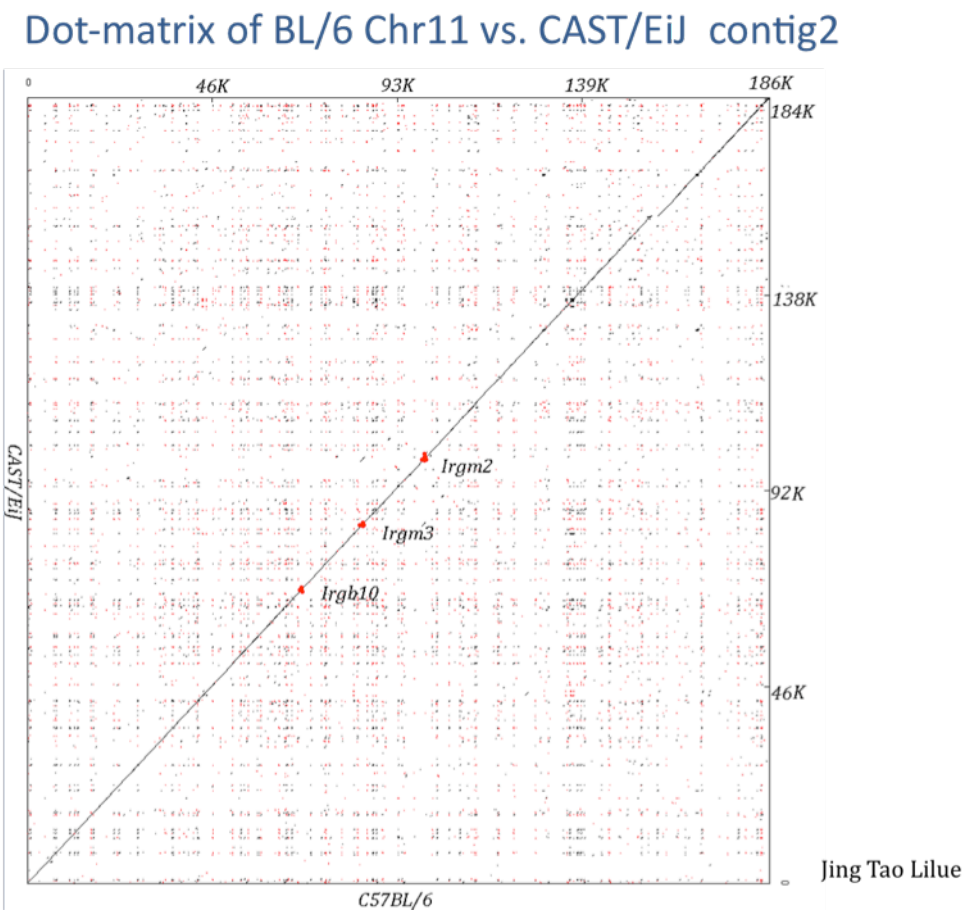

Fig 1A

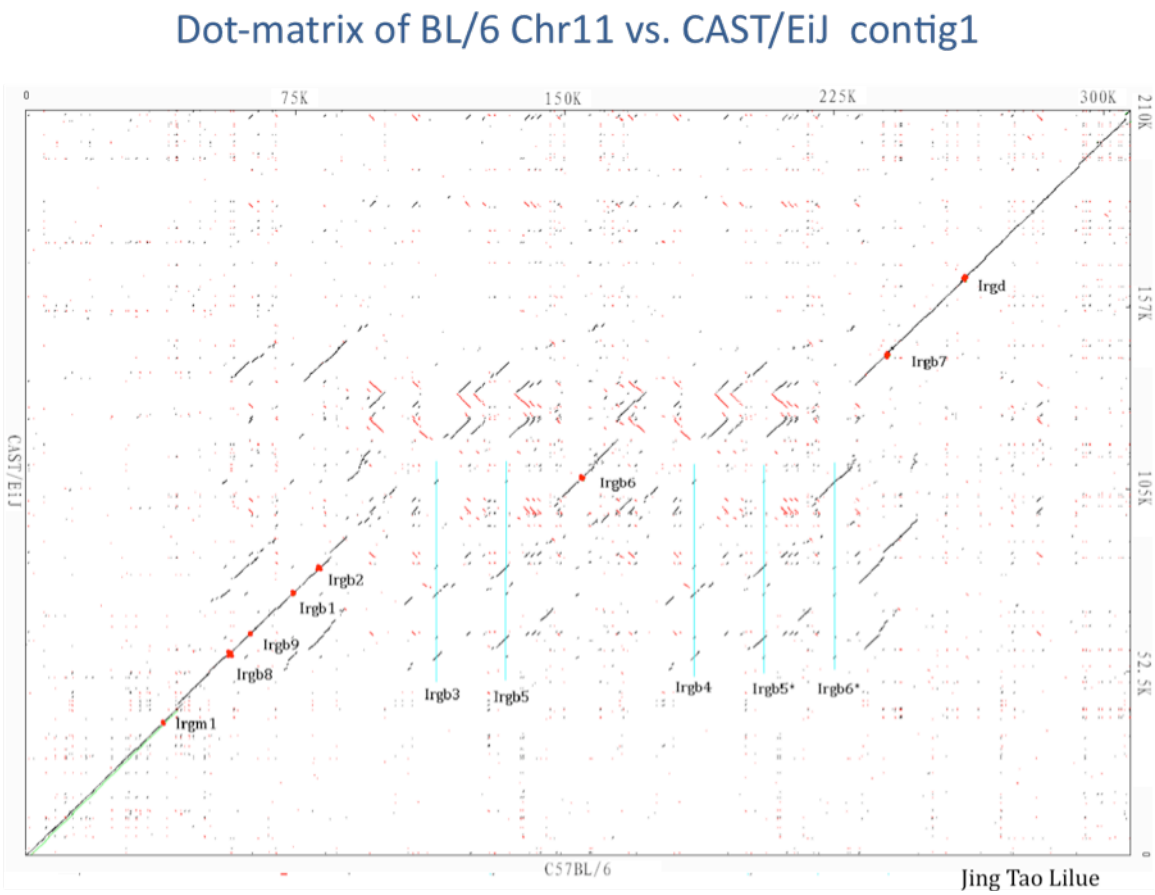
Dot-matrix of BL/6 Chr11 vs. CAST/EiJ contig1
0
75K
150K
225K
300K
210K
157K
105K
52.5K
0
CAST/EiJ
C57BL/6
Irgm1
Irgb8
Irgb9
Irgb1
Irgb2
Irgb3
Irgb5
Irgb6
Irgb4
Irgb5*
Irgb6*
Irgb7
Irgd
Jing Tao Lilue

Fig 1B

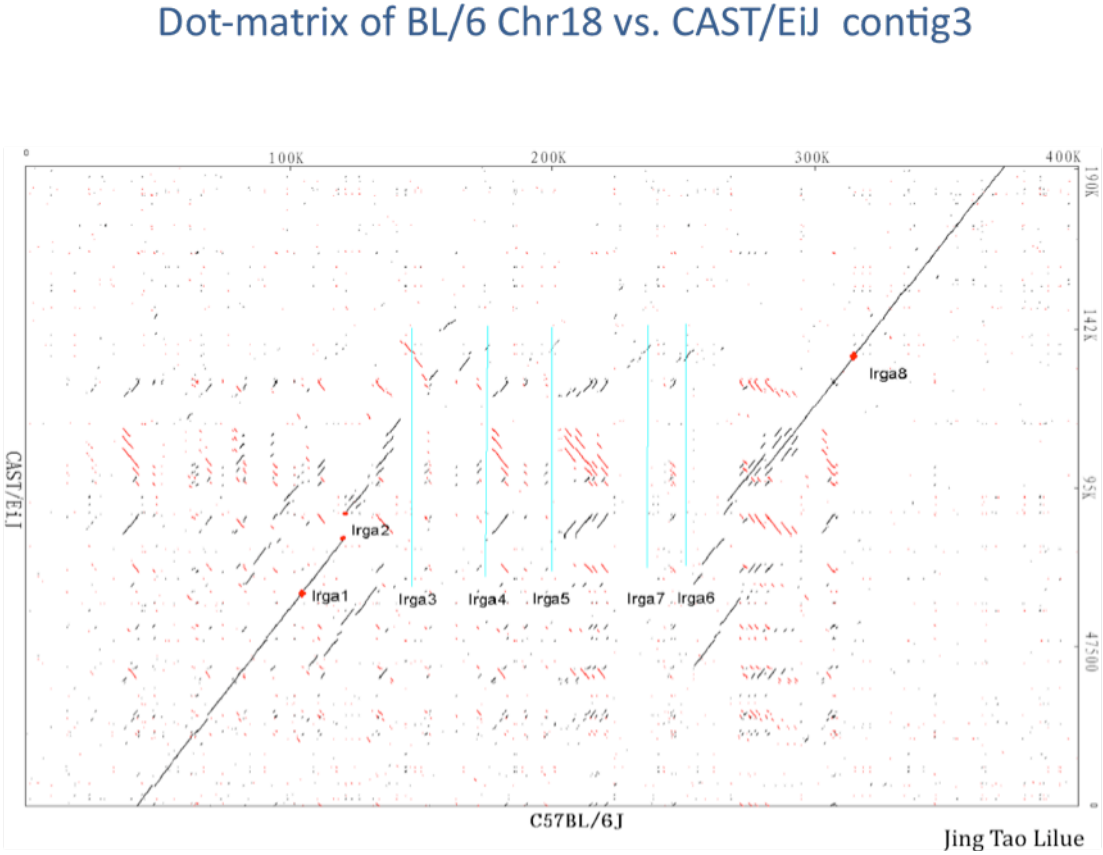
Dot-matrix of BL/6 Chr18 vs. CAST/EiJ contig3
100K
200K
300K
400K
190K
142K
95K
47500
0
CAST/EiJ
C57BL/6J
Irga1
Irga2
Irga3
Irga4
Irga5
Irga7
Irga6
Irga8
Jing Tao Lilue

Fig 1C

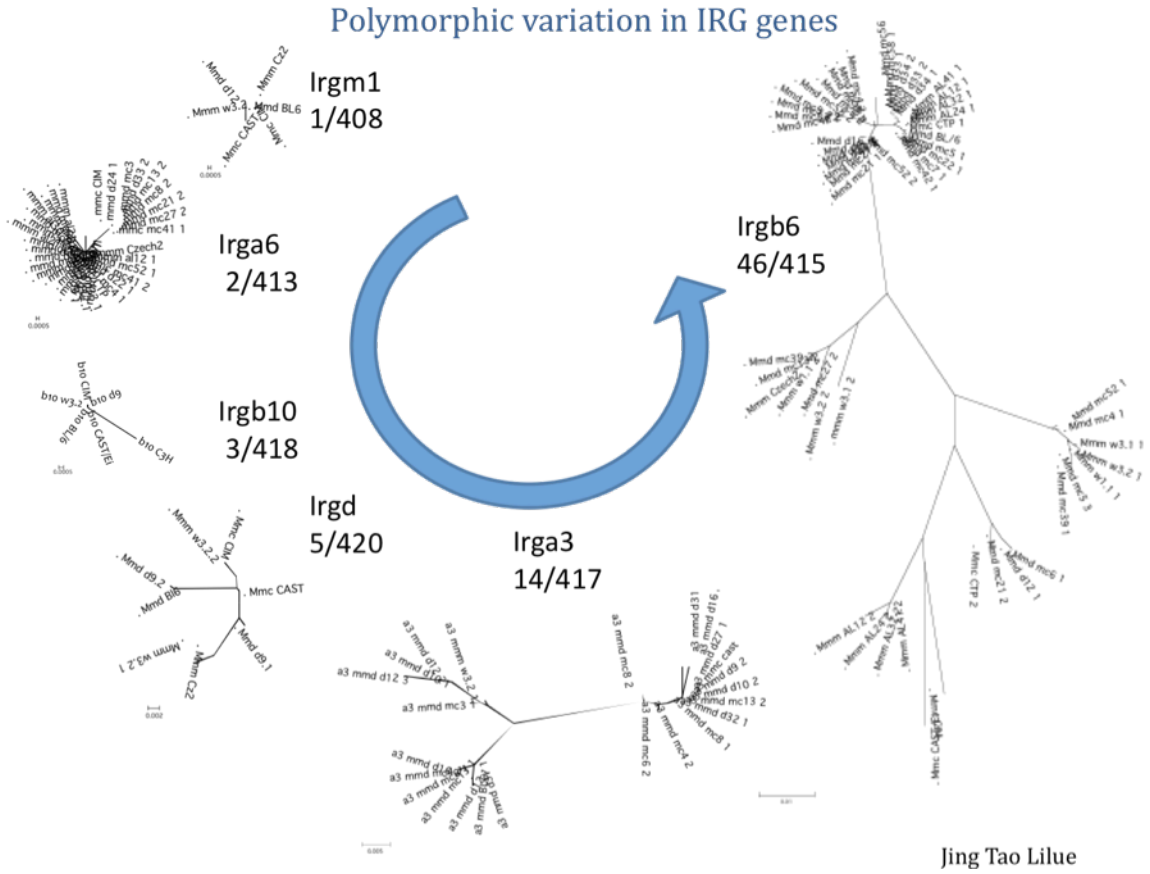

Fig 2

Unrooted trees of several mouse IRG genes depicting polymorphic variation at the amino acid level recovered from wild Eurasian alleles. In each case the first figure given is the number of variant residues found, the second is the total length of the protein. From the alleles so far recovered Irgb6 and Irga3 show considerable polymorphic coding variation.

Irgb6	CIM	CAST	AL12	CzII	CTP
BL/6	0.893	1.175	1.018	1.018	0.798
CIM		1.816	0.911	0.757	0.504
CAST			1.634	0.818	0.717
AL				0.935	0.829
CzII					0.761

Fig 3

dN/dS ratios (calculated by PAML v4.1 Yang Z, Nielsen R (2000)) for some wild Eurasian alleles of Irgb6 (below). Several values are greater than 1.0.

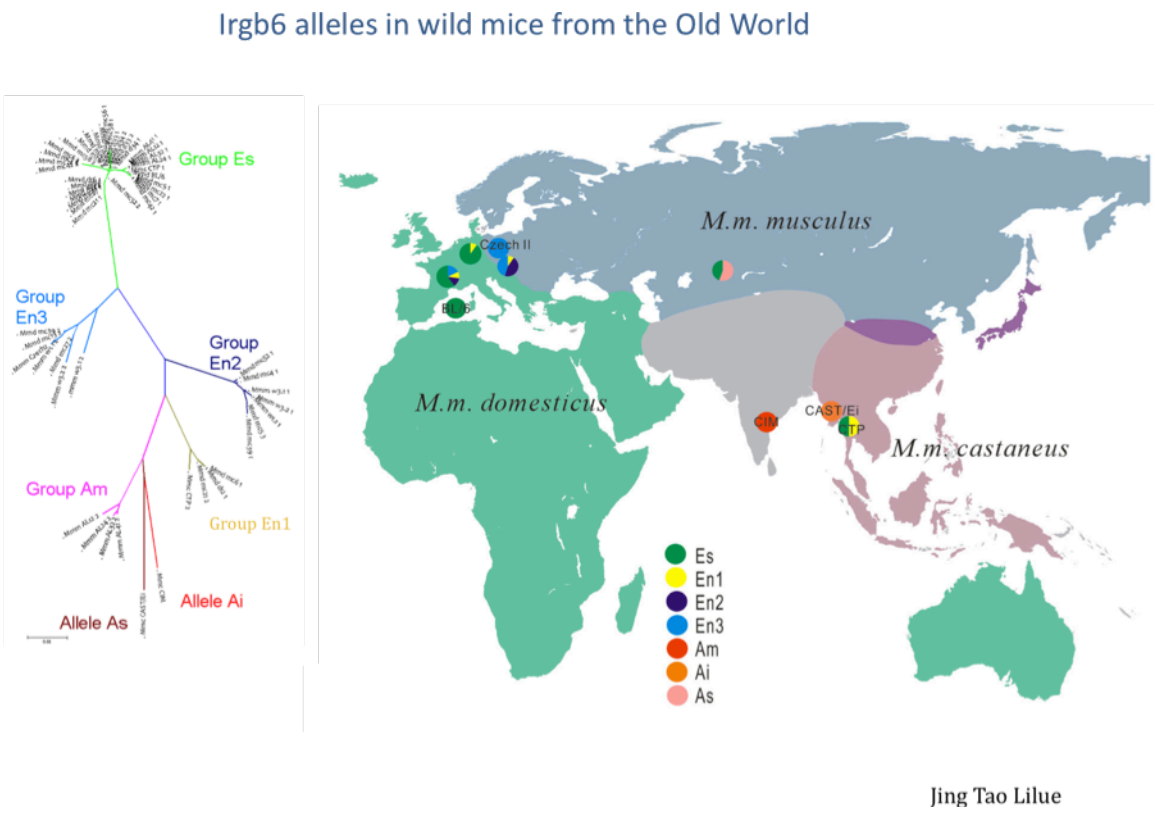

Fig 4

The alleles of Irgb6 were clustered and coloured according to the unrooted tree and representatives of the clusters mapped onto the collection sites according to their frequency, in the coloured pie-charts. Many alleles show broad Eurasian distributions not corresponding with the geographic domains associated with the three Mus musculus sub-species.

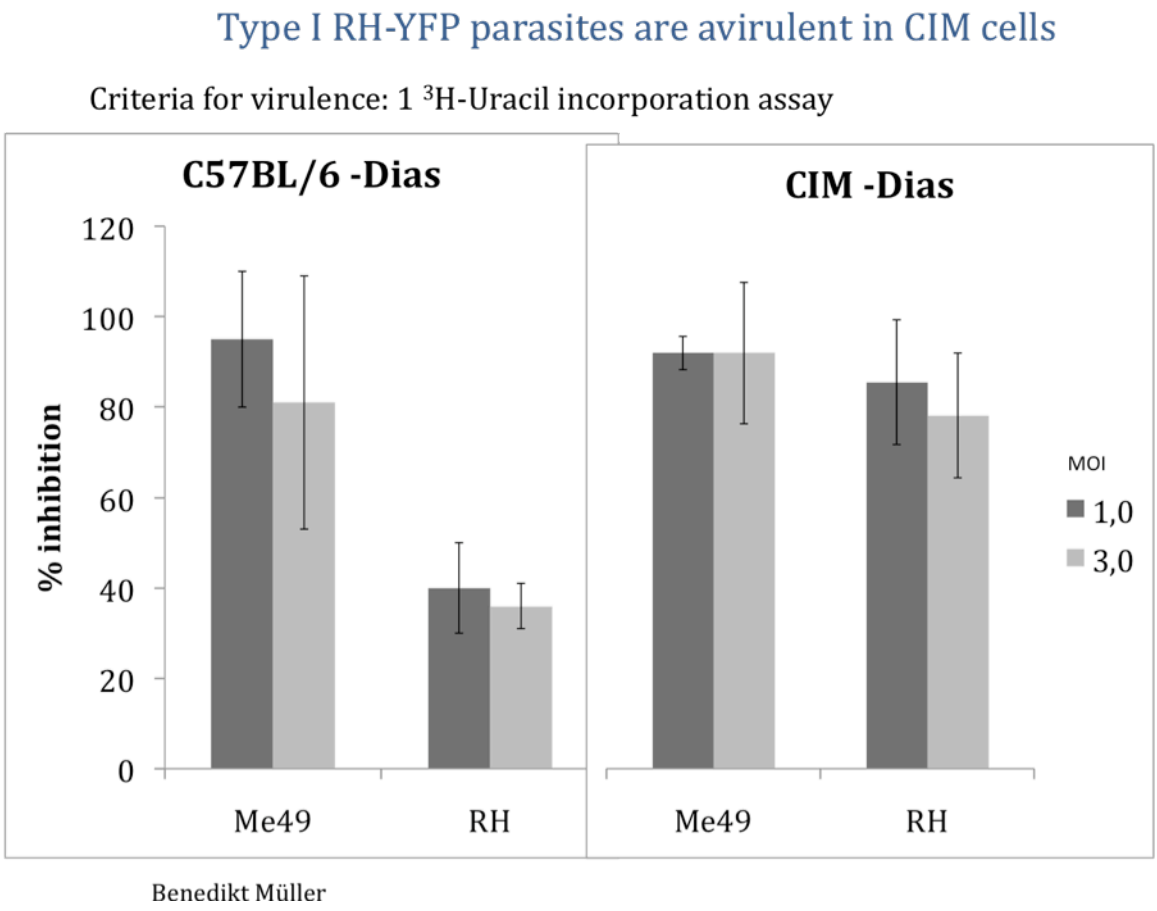

Fig 5. The proliferation of virulent RH strain T. gondii is well-controlled in cells from CIM strain mice

The proliferation of avirulent (ME49) and virulent (RH) strains of Tg in IFNg-induced diaphragm cells (Dias) was measured by the incorporation of 3H-uracil at 48 hr after infection. Data are recorded as inhibition of growth relative to growth in cells not induced by IFNg. In cells from the laboratory strain C57BL/6 (right-hand panel), control of RH strain growth is weak (c. 35%), while in cells from the wild-derived (S. Indian) CIM strain (left-hand panel) RH is controlled essentially as well as the avirulent strain ME49 (c. 80% vs c. 90%)

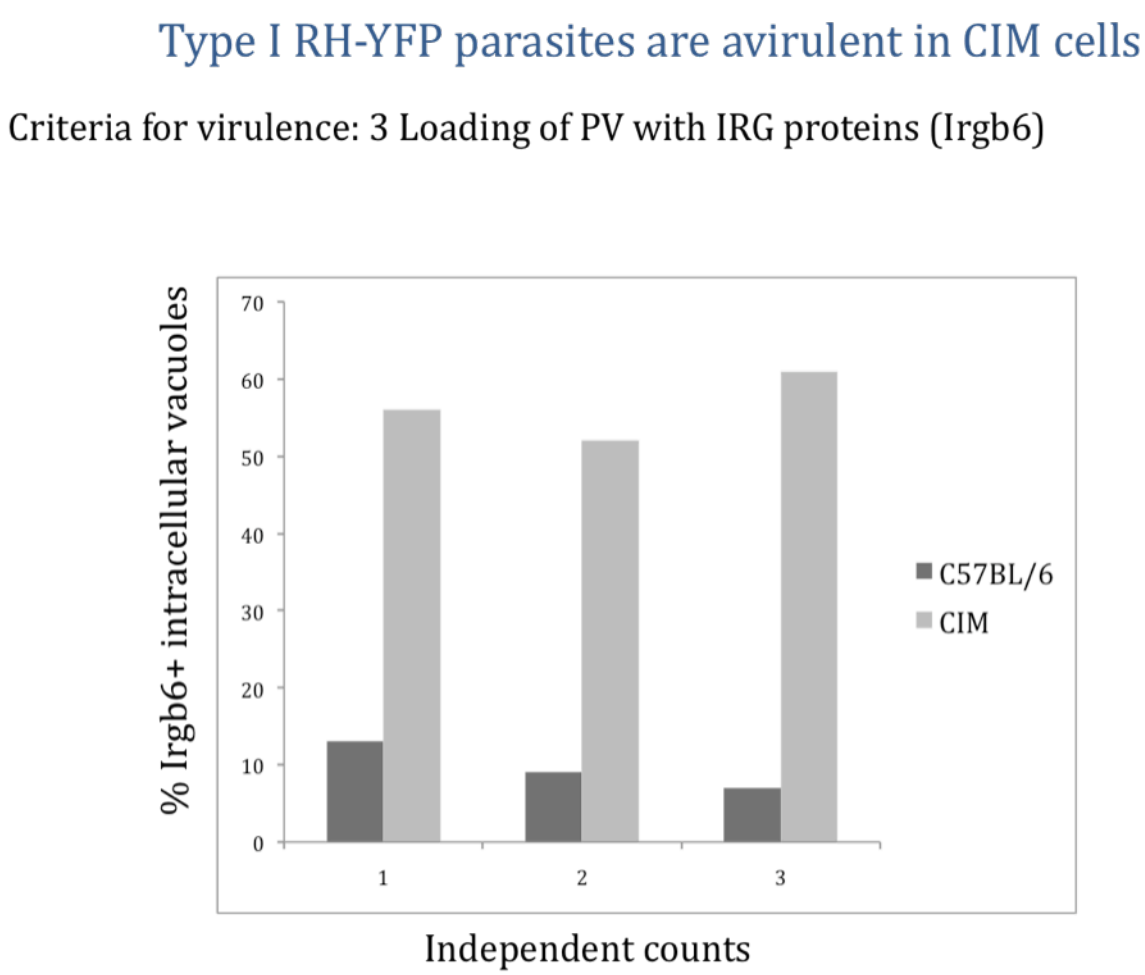

Fig 6

IRG proteins (Irgb6) load efficiently on to the vacuoles of virulent RH strain T. gondii infecting IFNg-induced diaphragm cells from the wild-derived CIM strain, but not from the C57BL/6 strain. Counts from immunofluorescent preparations in 3 independent experiments* (>200 intracellular vacuoles counted in each case). Thus the control of virulent T. gondii by CIM strain cells is reflected in differential behaviour of the IRG proteins.

***Counts made blind on coded preparations by two independent observers**

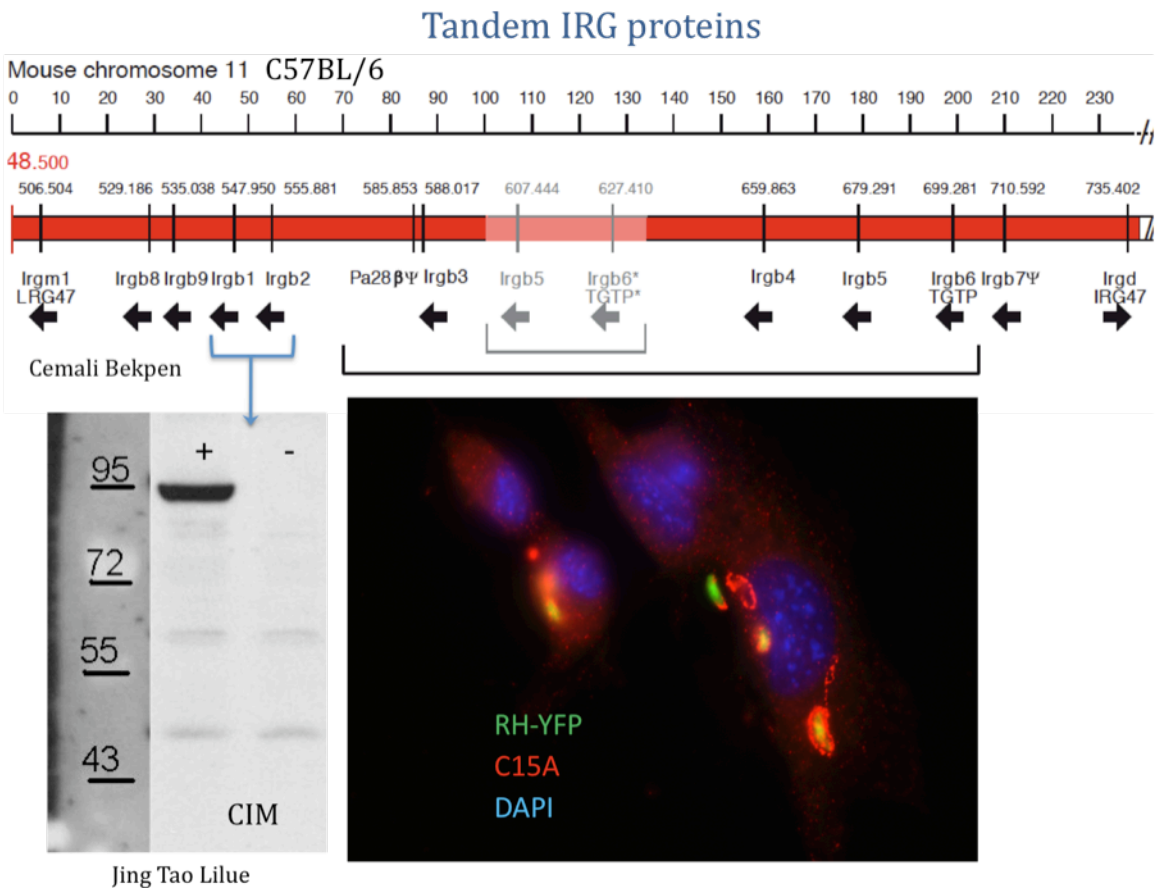

Fig 7

The anomalous "tandem" 94 kDa IRG proteins, whose existence we predicted in 2005 (Bekpen et al, Genome Biology) seem to have special properties in resistance of CIM to virulent T. gondii. C15A is a rabbit antiserum made against the C-terminus of the tandem Irgb2/b1 (seen in the map of chromosome 11 from C57BL/6, above. The antiserum detects an IFNg-dependent, 94 kDa protein on a Western blot of cells from CIM strain mice. In immunofluorescence, C15A (red) stains the parasitophorous vacuoles of infecting virulent RH-YFP strain T. gondii (green). More than 95% of RH vacuoles are coated with C15A in CIM cells. Nuclei of cells are stained blue.