SUPPLEMENTARY INFORMATION

MATERIAL AND METHODS

Generation of expression constructs. The coding regions of the C57BL/6 IRG genes were amplified as described previously (Boehm *et al*, 1998) and cloned into the SalI site of pGW1H (British Biotech), pGEX-4T-2 (GE Healthcare), pEGFP-C3 (BD Biosciences), pGAD and pGBD (James et al, 1996). Irgb6-FLAG was cloned SalI-EcoRI into pGW1H. The native IRG open reading frames were cloned into pGene/V5-His (Invitrogen) as follows: Irgm1 and –a6 by BamHI-NotI digestion from the pGEX-4T-2-IRG constructs and Irgm2, -m3, and –d by EcoRI-ApaI restriction from the respective pEGFP-C3 constructs. Restriction enzymes were from New England Biolabs. Mutations and epitope tags were introduced by conventional PCR or according to the ”QuikChange” site-directed mutagenesis kit (Stratagene) protocol. The ctag1 C-terminal epitope tag replaces the last two residues (RN) of Irga6 with the sequence KLGRLERPHRD (formerly IIGP1-m, (Uthaiah *et al*, 2003)). In case of Irgd-ctag1 the same 13 residues were added after the last amino acid of the protein. The bacterially expressed Irga6 proteins all carry the extension GSPGIPGSTT at the N terminus due to cleavage of the GST fusion by thrombin. All constructs were verified by sequencing.

Primers (Operon) used were:

		5’-3’ sequence
Irga6-K82A	fwd	gggagacggg atcaggggcgtccagcttcatcaataccc
	rev	gggtattgatgaagctggacgcccctgatcccgtctccc
Irga6-S83N	fwd	ggagacgggatcagggaagaacagcttcatcaataccctg
	rev	cagggtattgatgaagctgttcttccctgatcccgtctcc
Irgb6-K69A	fwd	ggaaacaggcgcaggggcgtccactttcatcaatacc
	rev	ggtattgatgaaagtggacgcccctgcgcctgtttcc
Irgb6-S70N	fwd	gga aacaggcgcagggaagaacactttcatcaatacc
	rev	ggtattgatgaaagtgttcttccctgcgcctgtttcc
Irgm1-S90N	fwd	gggactctggcaatggcatgaattctttcatcaatgcacttcg
	rev	cgaagtgcattgatgaaagaattcatgccattgccagagtccc
Irgm2-S78N/Irgm3-S98N	fwd	gggactctggcaatggcatgaattctttcatcaatgcccttagg
	rev	cctaagggcattgatgaaagaattcatgccattgccagagtccc
Irga6	fwd	ccccccccgtcgaccaccatgggtcagctgttctcttcacctaag
Irga6-ctag1	rev	ccccccccgtcgactcagtcacgatgcggccgctcgagtcggcctag
Irgb6	fwd	ccccccccgtcgaccaccatggcttg ggcctccagc
Irgb6-FLAG	rev	ccccccccgaattcctacttgtcatcgtcgtccttgtaatcaccggatccagcttcccagtactcgggggg
Irgd-ctag1	fwd	gaaacagtaaatgttgccaaactaggccgactcgagcggccgcatcgtgactgagtggtcgacctgcagg
	rev	cctgcaggtcgaccactcagtcacgatgcggccgctcgagtcggcctagtttggcaacatttactgtttc

SDS-PAGE and WB. Fibroblasts were lysed in 1% Triton X100/PBS/Complete Mini Protease Inhibitor Cocktail, EDTA free (Roche). Postnuclear supernatants were subjected to SDS-PAGE

and WB. Membranes were probed for IRG proteins with the indicated primary and HRP-coupled secondary antibodies for chemiluminescence.

Cell proliferation assay: CellTiter 96 AQueous One Solution Cell Proliferation Assay Kit (Promega) was used according to the manufacturer's instructions to determine the influence of the expression of IRG proteins on cell growth and survival in IFNγ- (200 U/ml) and Mifepristone-induced (10^{-9} M) gs3T3WT and -Irga6 cells ($1x10^3$ cells/96well) relative to untreated cells.

Electron microscopy: Stable NIH/3T3 cell lines constitutively expressing Irga6 and Irga6-S83N (J. Zerrahn, unpublished) were fixed in 2.5% glutaraldehyde (vol/vol) in 0.1 M sodium cacodylate buffer (pH 7.2) and washed three times for 10 min in 0.1 M sodium cacodylate buffer (pH 7.2). Secondary fixation was performed in 1% (wt/vol) osmium tetroxide in water for 120 min. After three washes in distilled water for 5 min, samples were dehydrated by sequential incubation in 30%, 50%, 70%, 95% and three times in 100% acetone for 20 min each. Samples were infiltrated by incubation in 2 parts acetone/1 part Spurr resin, followed by 1 part acetone/2 parts Spurr resin (2 hours each) and 100% Spurr resin twice for 24h. Samples embedded in 100% Spurr resin polymerised for 24 hours at 60°C. Ultrathin sections were cut using a Reichart OMU4 Ultracut ultramicrotome (Leica Microsystems). Sections were stained in uranyl acetate and lead citrate and examined and imaged using a JEOL 100CX transmission electron microscope (JEOL).

FIGURE LEGENDS

Fig S1. Cells expressing Irga6 in absence of IFNγ grow normally. gs3T3WT **(A)** and gs3T3-Irga6 cells **(B)** were induced with IFNγ or Mifepristone or left untreated over a period of 10 days. Cell proliferation was determined daily by a colorimetric assay. Error bars: standard deviation of quadruplicated samples. Comparable results were obtained with other clones expressing Irga6, and with gs3T3-Irgm2 and -Irgm3 clones.

Fig S2. Cells expressing Irga6 alone show an enlarged ER lumen. Electron microscopy of NIH/3T3 cells **(A)** and stable transfectants constitutively expressing Irga6-S83N **(B)** and Irga6WT **(C)** respectively. Blue arrows indicate normal ER morphology (A and B), red arrows the enlarged ER lumen observed in cells expressing Irga6WT (C). N: nucleus, M: mitochondrion, ER: endoplasmic reticulum.

Fig S3. Expression levels of Irga6 following IFNγ and Mifepristone-induction are comparable. gs3T3 **(A)** and gs3T3-Irga6 cells **(B)** were stimulated with IFNγ or Mifepristone for 24 h or left untreated. Irga6 was detected from lysate equivalents of 1.5, 3 and $6x10^4$ cells in immunoblot with 165 AS. An additional band of unknown identity at 55 kDa is frequently seen following Mifepristone-induction and rarely in IFNγ-treated cells.

Fig. S4. Partial colocalisation of Irga6 and Irgm3 on ER membranes. Localisation of Irga6 **(A)** and Irgm3 **(B)** in IFNγ-induced gs3T3 cells detected with 165 AS and αIGTP mAb, respectively. Irga6 and Irgm3 largely colocalise at the ER though Irgm3 additionally associates with unidentified globular structures in the cytoplasm. **(C)** Overlay with DAPI.

Tab. SI. Differential behaviour of the IRG proteins.

		Localisation in uninfected cells				**PV association in *Toxoplasma* infected cells**				
		Compartment	-IFNγ	+3GMS	Nucleotide dependent	-IFNγ	+IFNγ	+5IRGs	+3GMS	Nucleotide dependent
GMS	Irgm1	Golgi/endolysomes	WT	WT	no	no	no	----	----	----
	Irgm2	Golgi	WT	WT	no	no	yes	yes	no	yes
	Irgm3	ER	WT	WT	no	no	yes	yes	no	yes
GKS	Irga6	ER/cytosol	aggr.	WT	yes	no	yes	yes	yes ↓	yes
	Irgb6	ER/cytosol	aggr.	WT	yes	yes ↓	yes	yes	yes ↓	yes

Summary of the influence of IFNγ, nucleotide and additional IRG proteins on the subcellular localisation of Irgm1, Irgm2, Irgm3, Irga6 and Irgb6 in the absence and presence *T. gondii* infection (aggr.: aggregated; ↓: less than in presence of IFNγ).