Macrophage scavenger receptor 1 controls Chikungunya virus infection through autophagy in mice

Long Yang, Tingting Geng, Guang Yang, Jinzhu Ma, Leilei Wang, Harshada Ketkhar, Duomeng Yang, Tao Lin, Jesse Hwang, Shu Zhu, Yanlin Wang, Jianfeng Dai, Fuping You, Gong Cheng, Antony Vella, Richard. A Flavell, Erol Fikrig, Penghua Wang

This Supplementary Information contains Supplementary Figs.1-3 and Supplementary Table 1.

Supplementary Fig.1



Supplementary Fig.1 MSR1 is dispensable for CHIKV-induced type I IFN response. a, b) 8 weeks old, sex-matched, C57BL/6 (WT) and Msr1 deficient ($Msr1^{-/-}$) mice were infected with CHIKV. Quantitative RT-PCR analyses of selected immune gene expression in **a**) whole blood cells and **b**) the ankle joints. *, P<0.05, **, P<0.01 (non-parametric Mann-Whitney U test). **c**) Quantitative RT-PCR analyses of the select immune genes in primary bone marrow-derived macrophages infected with CHIKV at a multiplicity of infection (MOI) of 10. N=2. *, P<0.05 (two-way ANOVA). Each dot represents one mouse/biological repeat; the small horizontal line indicates the median of the result. The data are representative of two independent experiments.

Supplementary Fig.2



Supplementary Fig.2 P62 expression is inducible by CHIKV. **a)** Immunoblots showing p62 levels in primary bone marrow derived mouse macrophages (BMDM) at various times after CHIKV at a multiplicity of infection (MOI) of 10. **b**) Microscopic images of immunofluorescent staining for P62 in trophoblasts without (mock) /with CHIKV at a MOI of 0.5 for 12hrs. P62 was stained by a rabbit antibody, followed by an Alexa Fluor-594 (red) conjugated secondary antibody. The cells were counterstained for nuclei by DAPI (blue). The images were acquired using a Zeiss LSM 880 confocal microscope (400x). Scale bar: 20µM. Quantitative RT-PCR analyses of **c**) *p62* and *Lc3b* mRNA in BMDMs infected with CHIKV (MOI=10), **d**) *P62* mRNA in human trophoblasts infected with CHIKV (MOI=0.5). Bars: mean + s.e.m, n=2.

Supplementary Fig.3





Full uncropped immunoblot images from Fig.3



Full uncropped immunoblot images from Fig.4



Full uncropped immunoblot images from Fig.5



Full uncropped immunoblot images from Fig.6

Supplementary Fig.2 a Msr1 p62 Actin

Full uncropped immunoblot images from Supplementary Fig.2

Supplementary Fig.3 Full uncropped immunoblot images from main and supplementary figures.

Supplementary Table 1

Gene symbol	Species	Forward primer (5'-3')	Reverse primer (5'-3')	Remark		
Primers for qPCR						
MSR1	Hs	TGCACAAGGCAGCTCAC TTTGG	GTGCAAGTGACTCCAG CATCTTC			
Msr1	Mm	AGTGCTGTCTTCTTTAC CAGC	GTGAGGAAGGGATGCT GTA			
nsP2	CHIKV	GGCAGTGGTCCCAGATA ATTCAAG	GCTGTCTAGATCCACC CCATACATG			
Lc3b	Mm	GTCCTGGACAAGACCAA GTTCC	CCATTCACCAGGAGGA AGAAGG			
Sqstm1/p62	Mm	GCTCTTCGGAAGTCAGC AAACC	GCAGTTTCCCGACTCC ATCTGT			
LC3B	Hs	GAGAAGCAGCTTCCTGT TCTGG	GTGTCCGTTCACCAAC AGGAAG			
SQSTM1/p62	Hs	TGTGTAGCGTCTGCGAG GGAAA	AGTGTCCGTGTTTCAC CTTCCG			
Tnfa	Mm	CTCCAGGCGGTGCCTAT GT	GAAGAGCGTGGTGGC CC	Probe: CAGCCTCTTCTC ATTCCTGCTTGT GGC		
lfna	Mm	CTTCCACAGGATCACTG TGTACCT	TTCTGCTCTGACCACCT CCC	Probe: AGAGAGAAGAAA CACAGCCCCTGT GCC		
lfnb1	Mm	CTGGAGCAGCTGAATG GAAAG	CTTCTCCGTCATCTCCA TAGGG	Probe: CAACCTCACCTA CAGGGCGGACTT CAAG		
Actb	Mm	AGAGGGAAATCGTGCGT GAC	CAATAGTGATGATGAC CTGGCCGT	Probe: CACTGCCGCATC CTCTTCCTCCC		
ACTB	Hs	ATCCTGGCCTCGCTGTC CAC	GGGCCGGACTCGTCAT AC			

Guide RNA for CRISPR-Cas9 knockout

ATG16L1	Hs	GGGGGAAGTCAGCGGC GCGG	
ATG12	Hs	GTCTTCCGCTGCAGTTT CCC	
MSR1	Hs	TGATCTCAGACTGAAAG ATT	
SQSTM1/P6 2	Hs	CGAGCCTGAGGCGGAA GCCG	

Supplementary Table 1 Oligo DNA sequences. Homo sapiens (Hs), Mus musculus (Mm), Chikungunya virus (CHIKV).