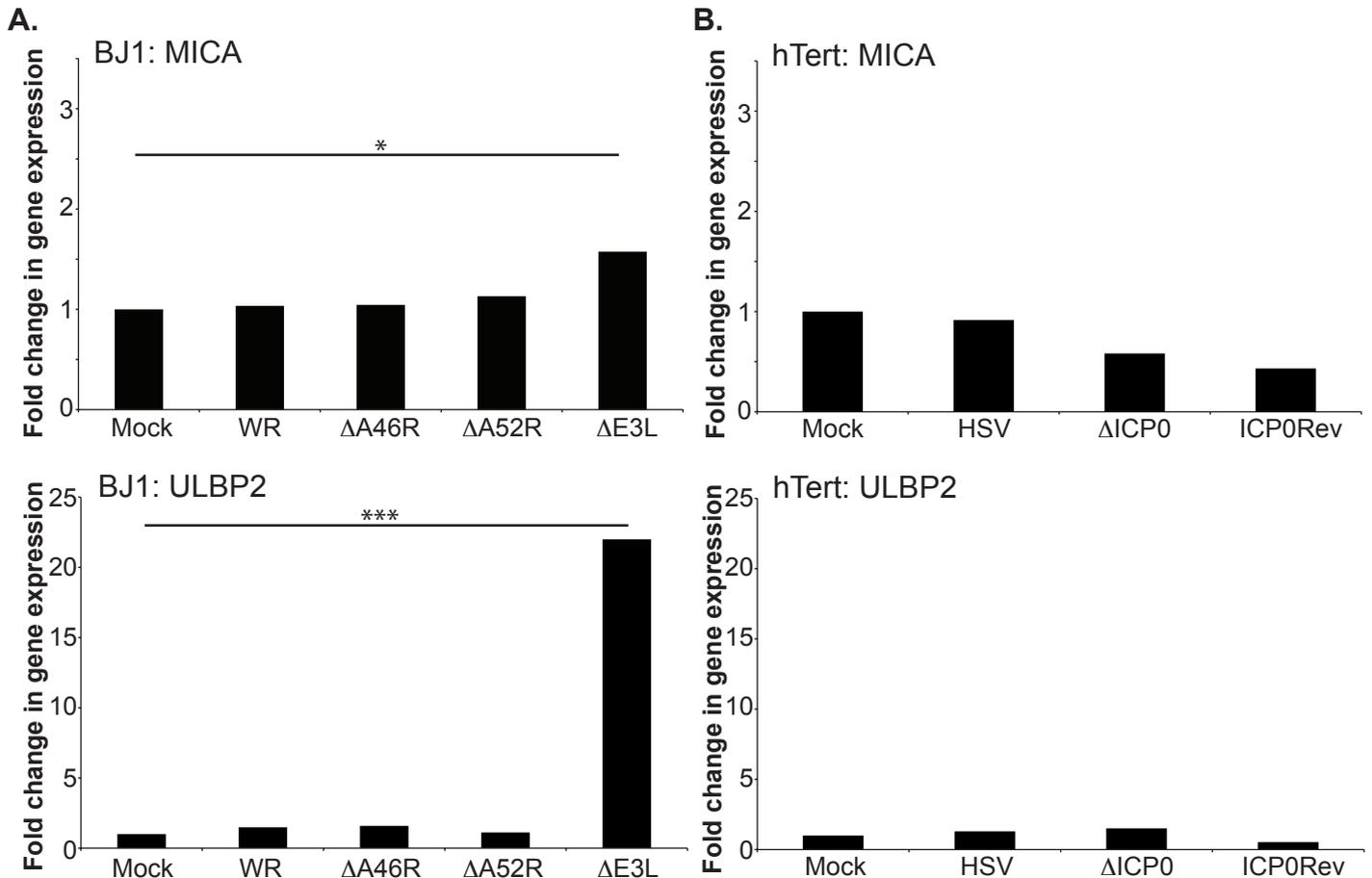


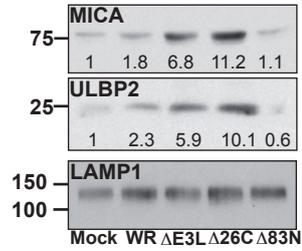
## Supplementary Figure 1



### Supplementary Figure 1

A. BJ1 fibroblasts were infected with the indicated virus or left uninfected and cultured overnight. Total RNA was extracted and qRT-PCR was used to assay MICA and ULBP2 expression. The results are expressed as fold-change in gene expression compared to uninfected cells (4 expts). B. hTert fibroblasts were infected with the indicated virus or left uninfected and cultured overnight. Total RNA was extracted and qRT-PCR was used to assay MICA and ULBP2 expression. The results are expressed as fold-change in gene expression compared to uninfected cells.

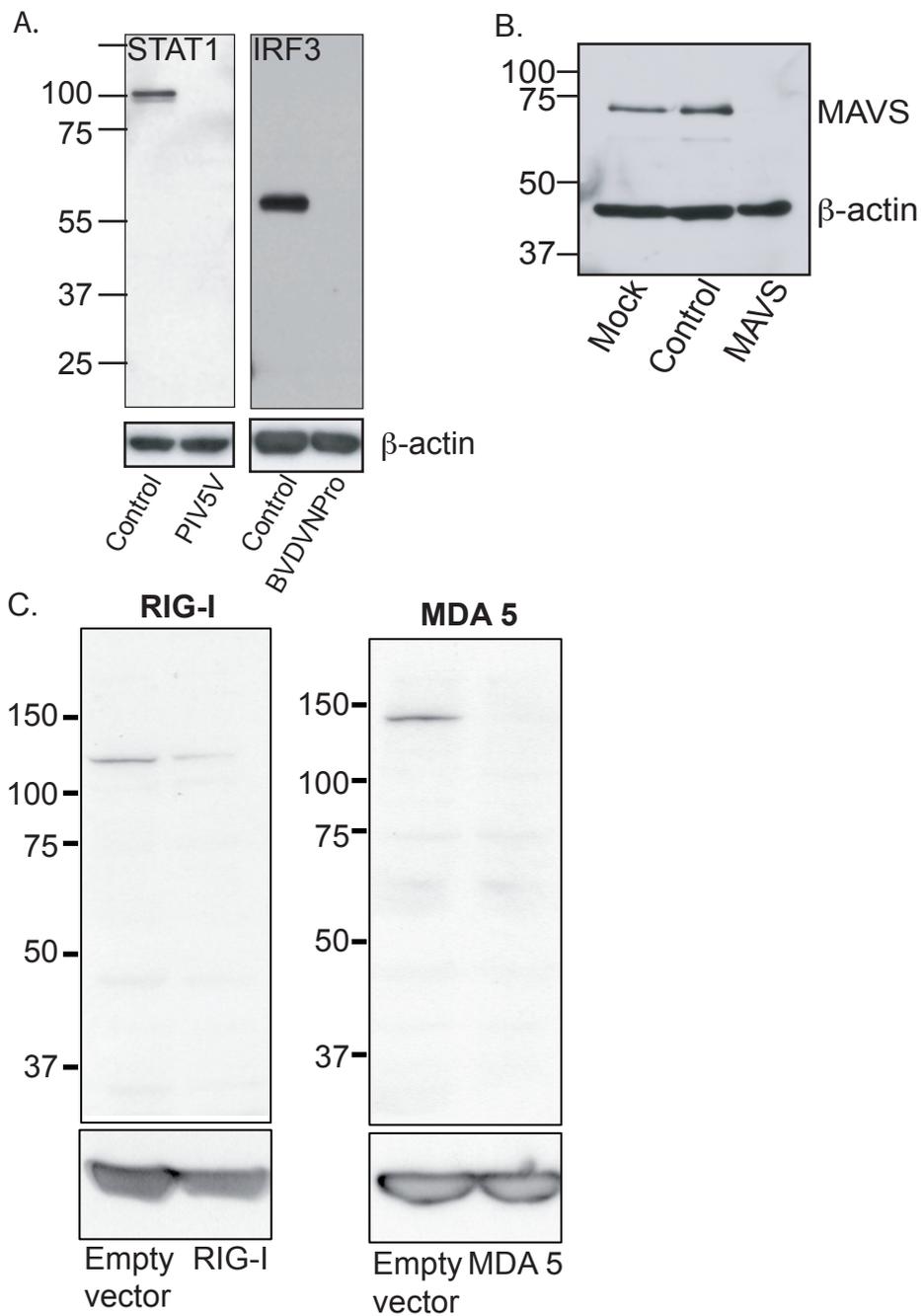
## Supplementary Figure 2



### Supplementary Figure 2

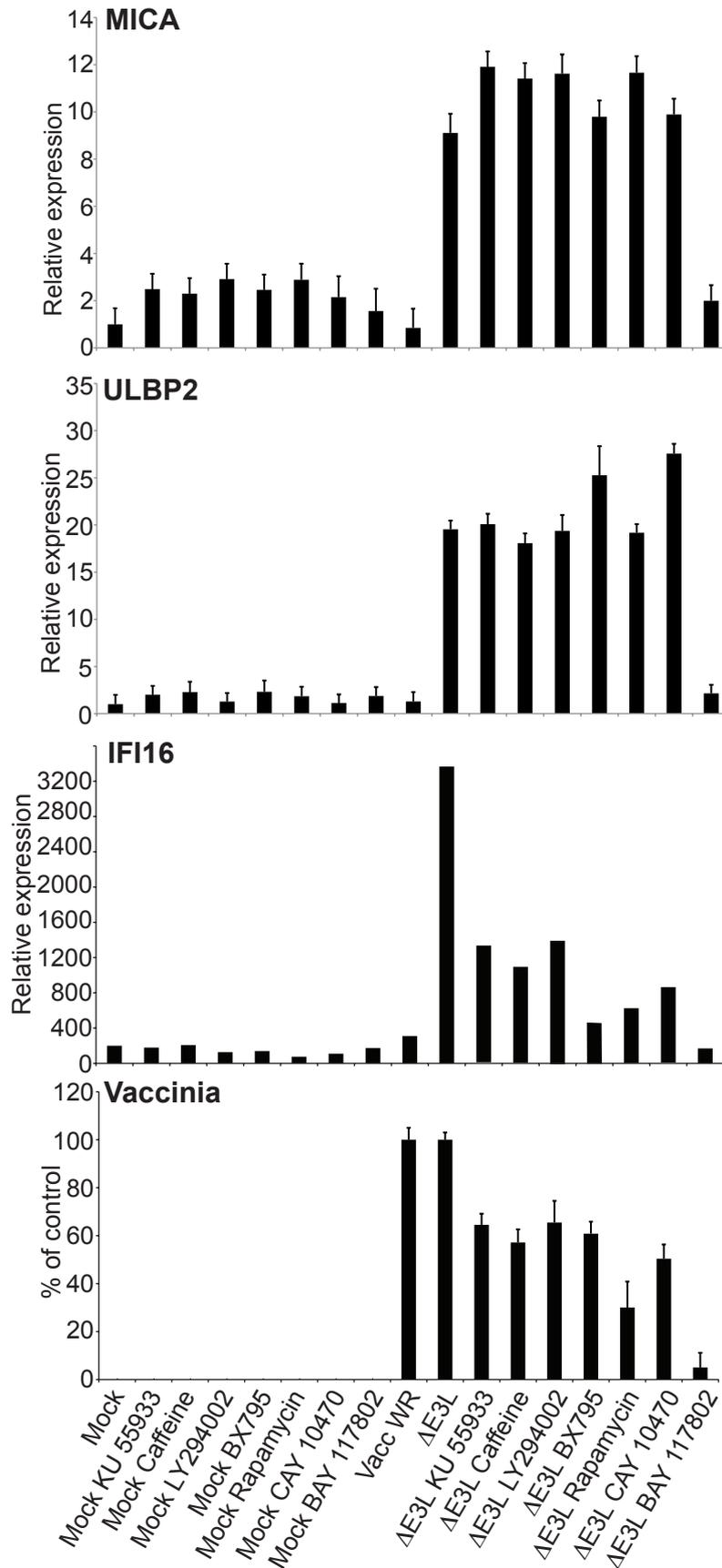
Western blot analysis of MICA and ULBP2 expression in membrane preparations of fibroblasts infected with the indicated viruses, LAMP1 was used as a loading control. The numbers underneath the westerns are the quantitation (in arbitrary units) of the MICA and ULBP2 bands, relative to the LAMP1 loading control (3 expts).

### Supplementary Figure 3



A. Western blot analysis of STAT1 and IRF3 expression in fibroblasts stably transduced with lentiviral constructs that express the PIV5V and BVDV NPro proteins respectively. B. Western blot analysis of MAVS expression in untransduced fibroblasts; fibroblasts transduced with a lentivirus expressing an shRNA plasmid targetting a sequence from bacteria (*Thermotoga* sp.), used as a negative control and fibroblasts transduced with shRNA targeting MAVS. C. RIG-I and MDA 5 expression in control (transduced with empty vector) and fibroblasts where RIG-I and MDA 5 expression had been targetted using CRISPR/Cas9 technology.

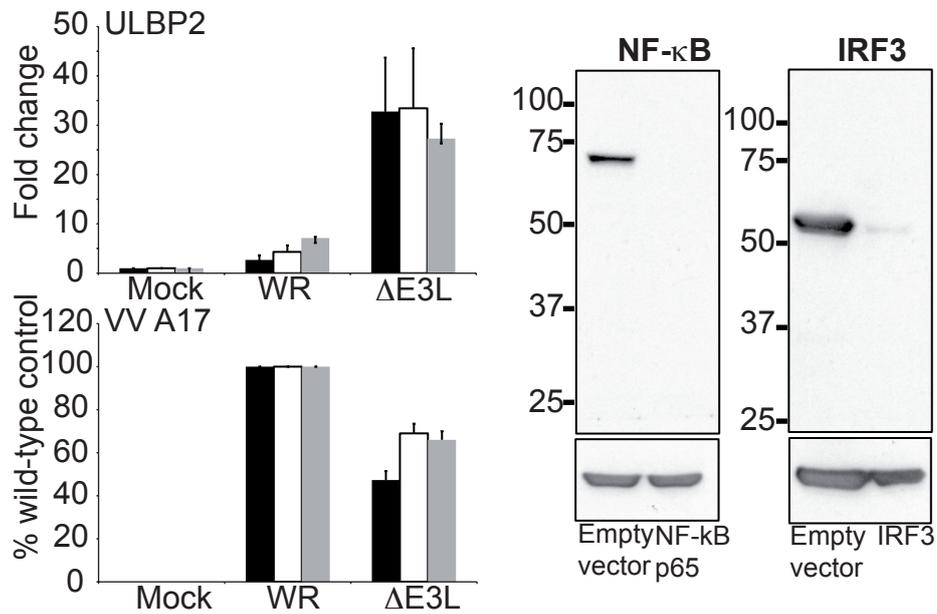
### Supplementary Figure 4



### Supplementary Figure 4

Fibroblasts were pretreated with the indicated inhibitor for 30 min and then either left uninfected or infected with the indicated virus in the presence or absence of the indicated inhibitor. qRT-PCR was used to assay the expression of (A) MICA, (B) ULBP2, (C) IFI16 and (D) VV A17.

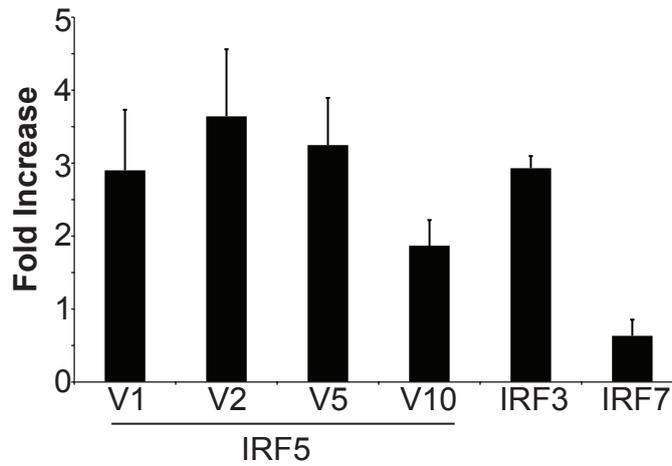
### Supplementary Figure 5



### Supplementary Figure 5

The effects of infection with the indicated viruses on ULBP2 and VV A17 expression in control fibroblasts (transfected with empty vector) or fibroblasts where IRF3 or NF-κB expression had been silenced using CRISPR/Cas9 technology (4 expts).

### Supplementary Figure 6



### Supplementary Figure 6

293T cells were co-transfected with Firefly luciferase reporter plasmid containing the wild-type ULBP2 promoter, renilla luciferase and expression vectors for the indicated IRF5 isoform, IRF3 or IRF7. After 24 h, cells were lysed and the Renilla and Firefly luciferase activities were measured by the dual luciferase assay (6 expts). The data were normalised using the Renilla luciferase levels.

<b>Target</b>	<b>Oligo sequence</b>	
MICA	ccttgccatgaacgtcagg cctctgaggcctcrctgcg	(Welte et al., 2003)
MICB	accttggtatgaacgtcaca ccctctgagacctcgctgca	(Welte et al., 2003)
ULBP1	caagtgaggagaattaataccattgag tgttggttgagtaaagagga	(Ebihara et al., 2007)
ULBP2	caagtgaggagcaccactcg cagatgccagggaggatgaagc	(Wittenbrink et al., 2009)
ULBP3	cctgatgcacaggaagaagag tatggcttgggttgagctaag	(Welte et al., 2003)
IFI16	caaccaaagaaaaggctgga ggaggagctgacaatgaggt	This paper
VV A17	atgagttatttaagatattacaatgctt tcgtcagtatttaaaactgttaaattgtggt	(Myskiw et al., 2009)
VV B10R	caaaatgcaggggtacaacaaca caatgaatccttagtattgccaacg	(Kulesh et al., 2004)
VV HA	catcatctggaattgtcactactaaa acggccgacaataataattaatgc	(de Souza Trindade et al., 2008)
<b>shRNA</b>		
MAVS1(+)	ccggtccagaggagaatgagtataagtcaagagact tatactcattctcctctgggttttg	
MAVS1(-)	aattcaaaaaccagaggagaatgagtataagtctcttg aactatactcattctcctctgga	
MAVS2(+)	ccggtttaccaaggggttgatataattcaagagaatata tccaacccttggtaaattttg	
MAVS2(-)	aattcaaaaattaccaaggggttgatataattctcttgaa atataccaacccttggtaaaa	
MAVS3(+)	ccggtatgtggatgtgtagagattctcaagagagaat ctctacaacatccacatttttg	
MAVS3(-)	aattcaaaaatgtggatgtgtagagattctctcttgaa gaatctctacaacatccacata	
<b>gRNA</b>		All this paper
RIG-I (1)	caccgggattatatccggaagaccc aaacgggtcttccggatataatccc	
RIG-I (2)	caccgagcagcgacgcagcctgcaag aaactgcaggctgctgctgctc	
MDA-5 (1)	caccgtggttgactcgggaattcg aaaccgaattcccaggtccaaccac	
MDA-5 (2)	caccgcagacgagaattccgctat aaacatagcggaaattctcgctc	
IRF3 (1)	caccgttgaagcacggcctacggc aaacgccgtaggccgtgctccaac	
IRF3 (2)	caccgcacgcgcttccgcatccctt aaacaagggatgcggaagcgcgtgc	
NF- $\kappa$ B (1)	caccgcaagtgcgaggggcgctccg aaaccggagcggcccctcgacttgc	
NF- $\kappa$ B (2)	caccgctacaagtgcgaggggcgct aaacagcggcccctcgacttgc	

de Souza Trindade, G., Y. Li, V.A. Olson, G. Emerson, R.L. Regnery, F.G. da Fonseca, E.G. Kroon, and I. Damon. 2008. Real-time PCR assay to identify variants of

- Vaccinia virus: implications for the diagnosis of bovine vaccinia in Brazil. *J Virol Methods* 152:63-71.
- Ebihara, T., H. Masuda, T. Akazawa, M. Shingai, H. Kikuta, T. Ariga, M. Matsumoto, and T. Seya. 2007. Induction of NKG2D ligands on human dendritic cells by TLR ligand stimulation and RNA virus infection. *Int Immunol* 19:1145-1155.
- Kulesh, D.A., R.O. Baker, B.M. Loveless, D. Norwood, S.H. Zwiers, E. Mucker, C. Hartmann, R. Herrera, D. Miller, D. Christensen, L.P. Wasieloski, Jr., J. Huggins, and P.B. Jahrling. 2004. Smallpox and pan-orthopox virus detection by real-time 3'-minor groove binder TaqMan assays on the roche LightCycler and the Cepheid smart Cycloer platforms. *J Clin Microbiol* 42:601-609.
- Myskiw, C., J. Arsenio, R. van Bruggen, Y. Deschambault, and J. Cao. 2009. Vaccinia virus E3 suppresses expression of diverse cytokines through inhibition of the PKR, NF-kappaB, and IRF3 pathways. *J Virol* 83:6757-6768.
- Welte, S.A., C. Sinzger, S.Z. Lutz, H. Singh-Jasuja, K.L. Sampaio, U. Eknigk, H.G. Rammensee, and A. Steinle. 2003. Selective intracellular retention of virally induced NKG2D ligands by the human cytomegalovirus UL16 glycoprotein. *Eur J Immunol* 33:194-203.
- Wittenbrink, M., J. Spreu, and A. Steinle. 2009. Differential NKG2D binding to highly related human NKG2D ligands ULBP2 and RAET1G is determined by a single amino acid in the alpha2 domain. *Eur J Immunol* 39:1642-1651.