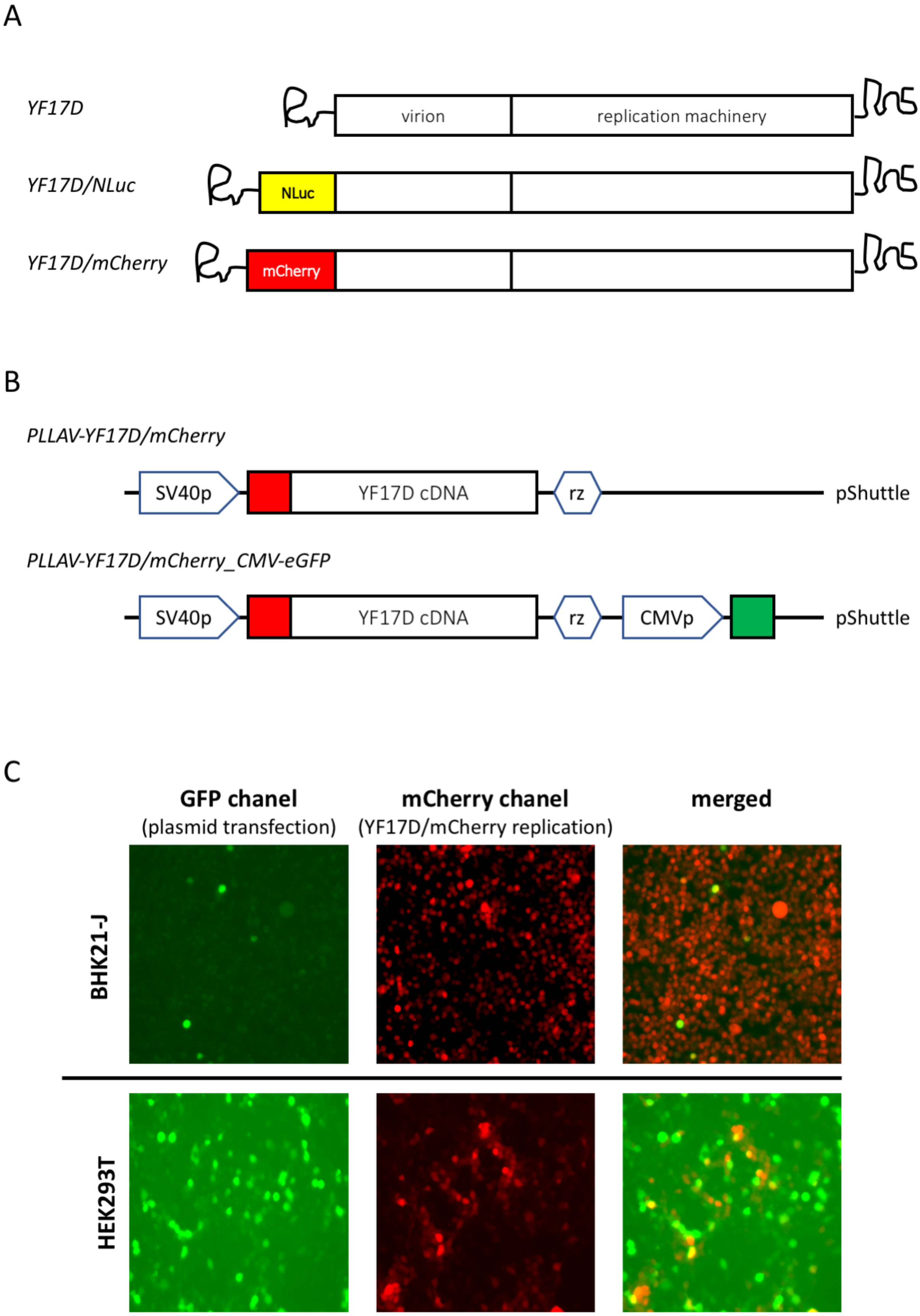
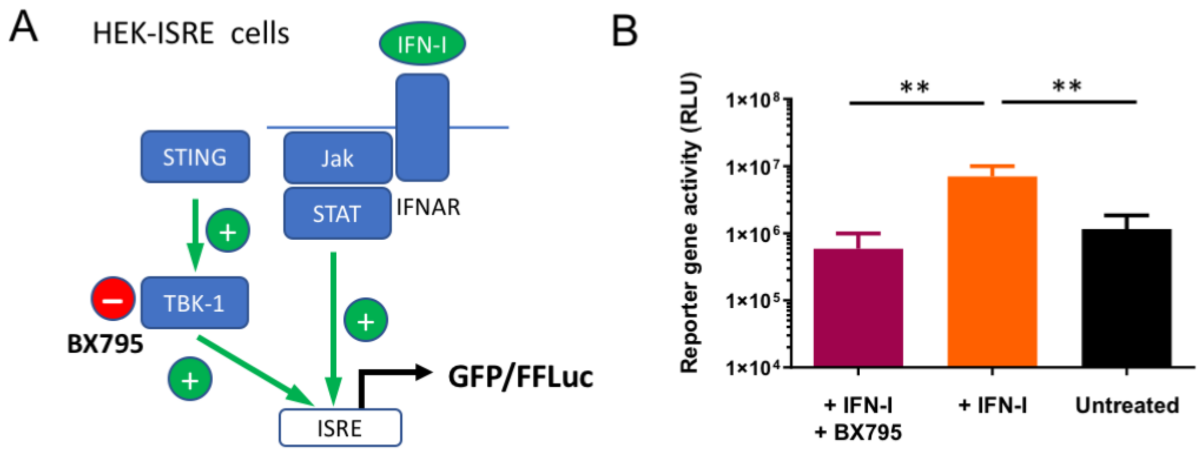
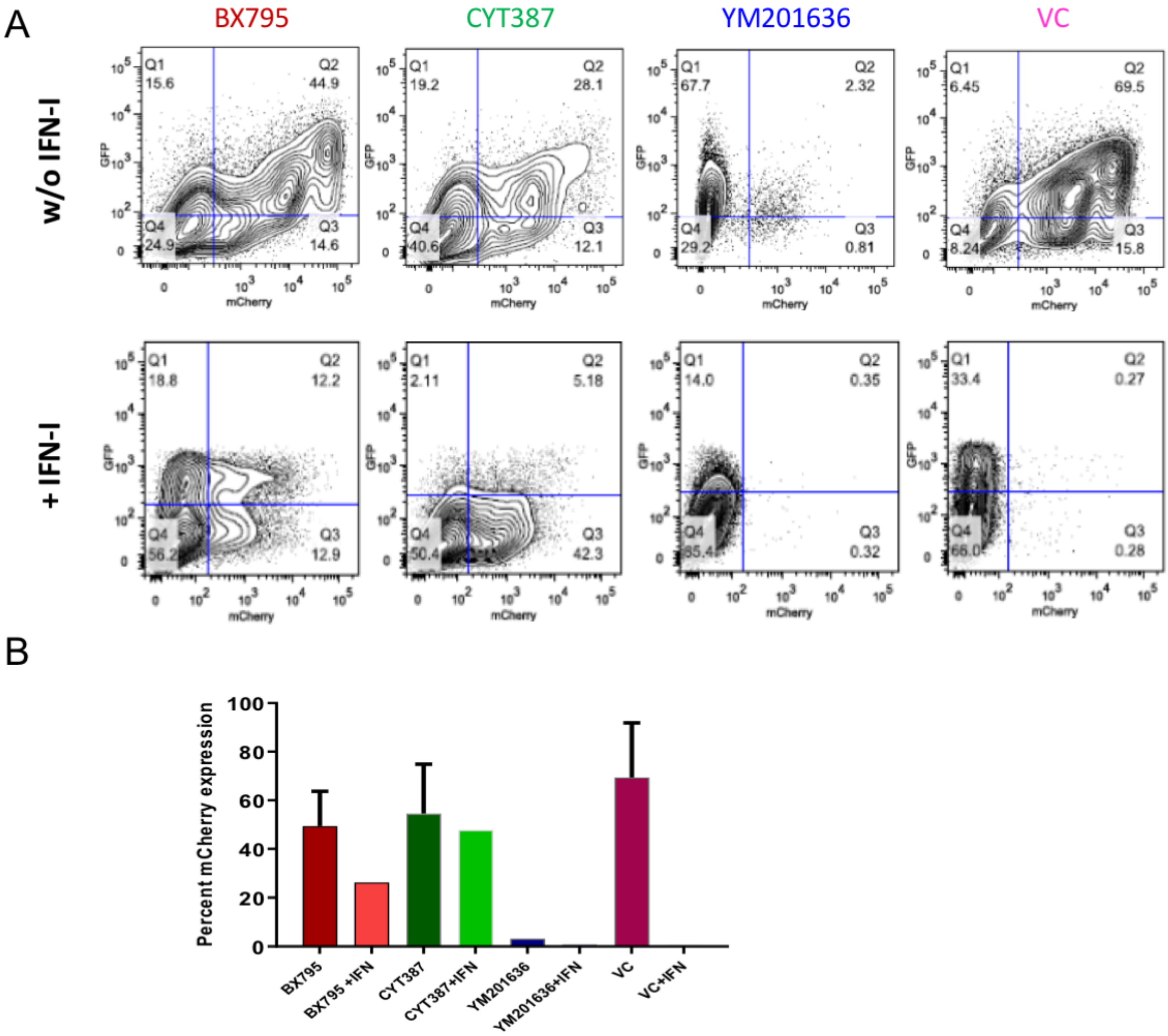


A
YF17D
virion
replication machinery
YF17D/NLuc
NLuc
YF17D/mCherry
mCherry
B
PLLAV-YF17D/mCherry
SV40p
YF17D cDNA
rz
pShuttle
PLLAV-YF17D/mCherry_CMV-eGFP
SV40p
YF17D cDNA
rz
CMVp
pShuttle
C
GFP chanel
(plasmid transfection)
mCherry chanel
(YF17D/mCherry replication)
merged
BHK21-J
HEK293T

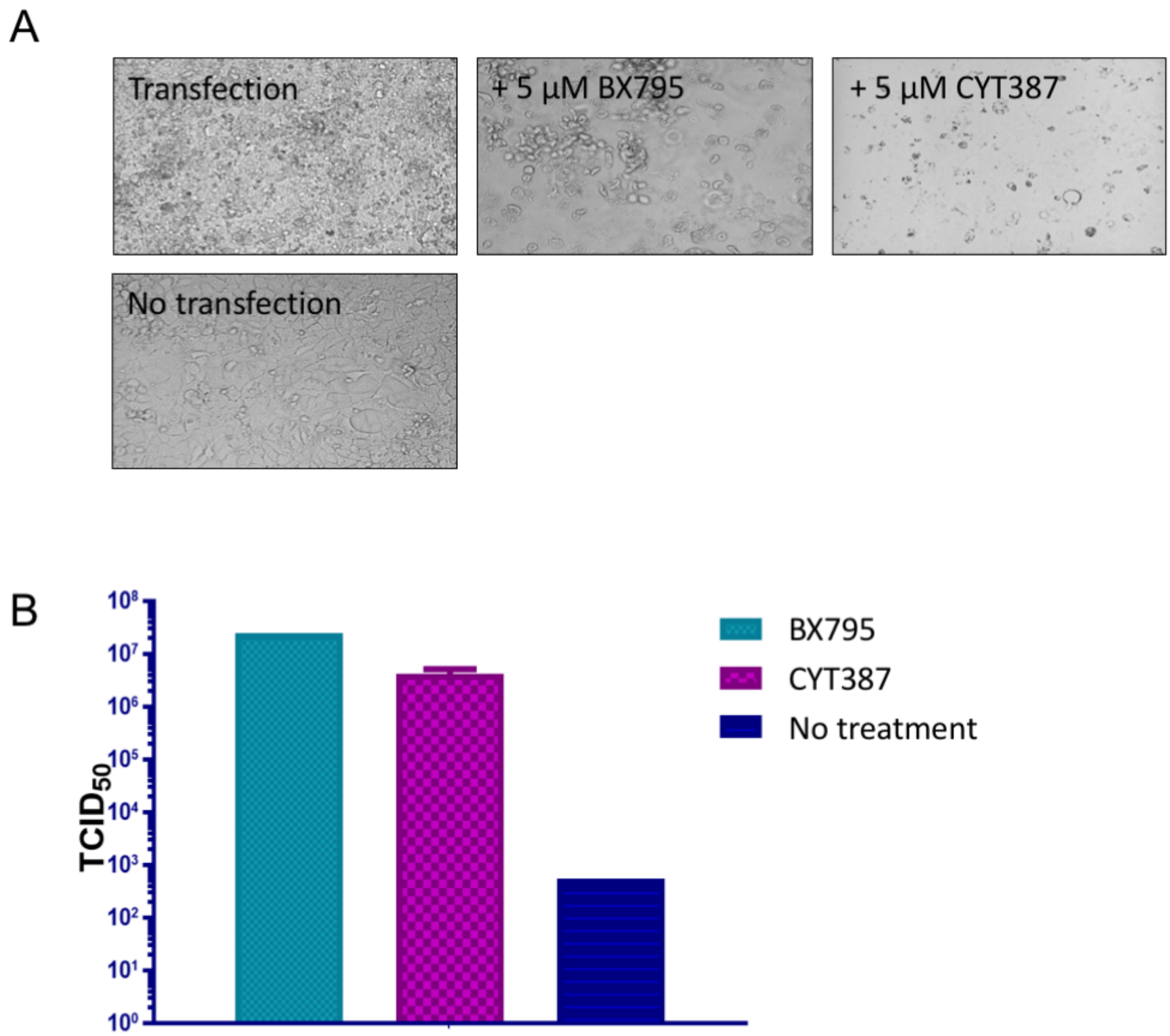
Supplementary Figure 1 (A) Schematic representation of the YF17D virus genome and the NLuc (*yellow box*; Kum *et al.* 2020) and mCherry (*red box*; Kum *et al.* 2018) expressing derivatives thereof. **(B)** Schematic representation of the YF17D/mCherry cDNA expressing plasmid vector PLLAV-YF17D/mCherry (Kum *et al.* 2018) and its derivative PLLAV-YF17D/mCherry_CMV-eGFP expressing eGFP (*green box*) from a second cistron, embedded in its plasmid backbone (*pShuttle*). SV40p, Simian virus 40 origin/promoter; Rz, hepatitis delta virus ribozyme; CMVp, cytomegalovirus immediate-early promoter. **(C)** Fluorescence micrograph showing BHK21-J (*upper panel*) and HEK293T cells (*lower*) transfected with PLLAV-YF17D/mCherry_CMV-eGFP (three days post tranfection). Highly permissive BHK21-J cell cultures (Lindenbach & Rice 1997) show relatively few transfected (eGFP, *green*) cells yet massive amplification and wide spread of virus progeny (YF17D/mCherry, *red*). In HEK293T, that are *bona fide* cGAS knockout cells (Sun *et al.* 2013) and are hence easier to transfect (more and brighter green cells), YF17D/mCherry replicates to lesser extent (fewer red cells). Few cells stain for both markers (yellow when merged), illustrating that PLLAV transfection and YF17D replication are not mutually exclusive; at least not in the absence of cGAS expression.



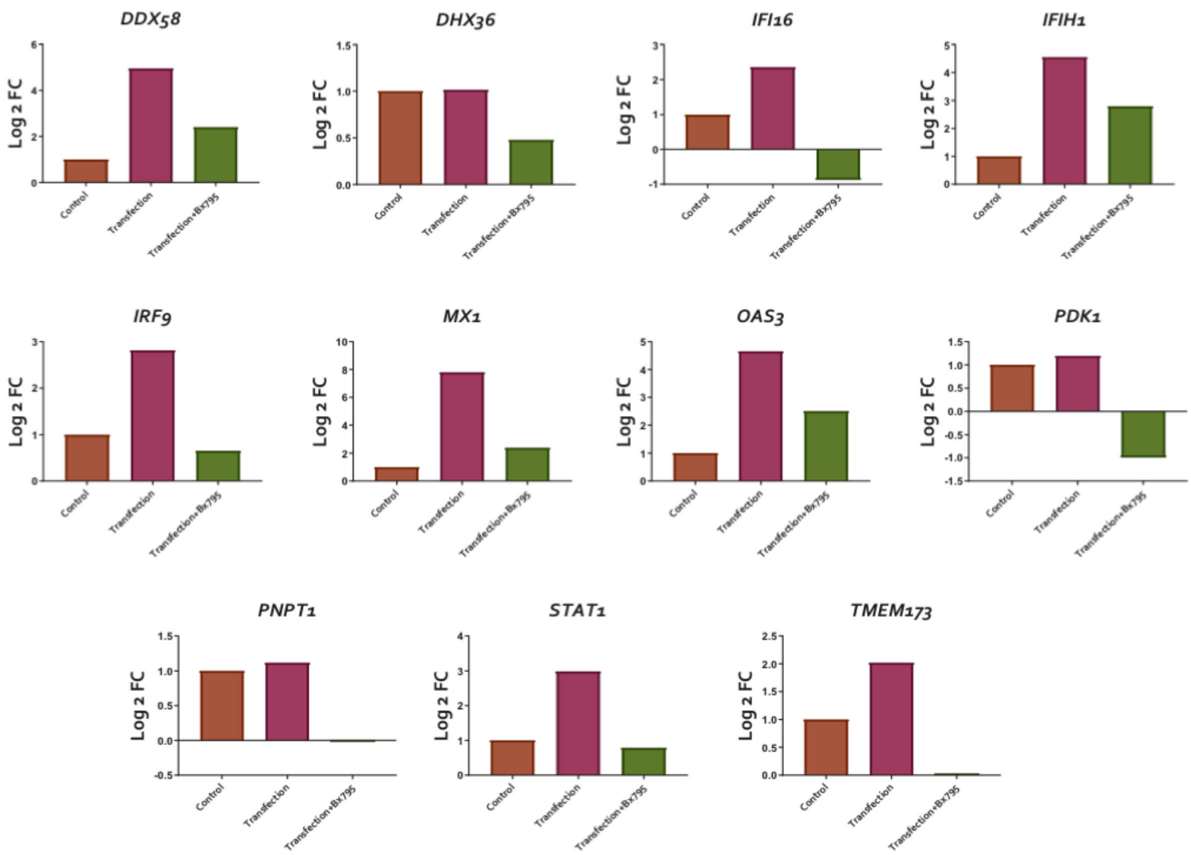
Supplementary Figure 2. Validation of HEK-ISRE reporter cells. (A) Schematic representation of the inhibition of TBK-1 by BX795 in HEK-ISRE reporter cells. **(B)** IFN-I dependent reporter gene expression in HEK-ISRE cells. Treatment with 10 IU mL^{-1} IFN-I (+*IFN-I*) leads to a significant >10x induction (**, p>0.01) of the IRSE-dependent FFLuc expression (*RLU, relative light units*) as compared to non-treated cells. Treatment with 5µM BX795 counteracts this reporter gene induction fully reverting the thus detected IFN-I activity.



Supplementary Figure 3. Re-evaluation of HEK-ISRE reporter cells infected with YF17D/mCherry. (A) Flow cytometry analysis of HEK-ISRE shown in fluorescence micrographs in FIGURE 3. Cells were gated for mCherry (infection, *x-axis*) and GFP (ISRE activation, *y-axis*). **(B)** Quantitation of the fraction of infected cells in (A) when treated with and without IFN-I, plus different small molecule inhibitors with proviral activity. Treatment with 10 IU mL^{-1} IFN-I was sufficient to ablate YF17D/mCherry replication. The TBK-1 inhibitors BX795 and CYT387 could revert the IFN-I mediated antiviral. Data represent mean +SD of three independent experiments.



Supplementary Figure 4. (A) CPE induced in A549 cells following PLLAV-YF17D/mCherry transfection. The TBK-1 inhibitors BX795 and CYT387 (both at 5µM) enhanced the microscopically observed CPE (i.e. a destruction of the cell monolayer) that may result from active YF17D/mCherry replication in A549 cells. In absence of the inhibitors no CPE could be observed. **(B) Titration of the infectious virus progeny produced in A549 cells following PLLAV-YF17D/mCherry transfection.** The TBK-1 inhibitors BX795 and CYT387 (both at 5µM) increased the virus yields from transfected A549 cells by about 4 Log10. Data represent mean +SD of three independent experiments.



Supplementary Figure 5. Differentially expressed genes (DEG) resulting from transfection of A549 cells, or resulting from transfection and concomitant treatment with the TBK-1 inhibitors BX795. Plasmid transfection lead to an upregulation of several marker genes (see Supplementary Table 2). Likewise treatment with 5µM BX795 could revert DEG expression for many prominent ISG (e.g. Mx1, OAS3, STAT1) to baseline levels (untreated cell controls); some DEG, including key factors involved in IFN-I expression (e.g. TMEM173/STING, IRF9), others less characterized (some involved in cellular RNA turnover, e.g. DHX36, PNPT2) even below basal level. Data represent means of three independent experiments. Log2 FC, Log2 fold change.

Supplementary Table 1: RT-qPCR Taqman assays for analysis of DEG in PLLAV transfected cells

Supplementary Table 1

Gene of Interest	**Proposed MoA in immune response to YF17D**	**Reference**	**Assay ID**
18s rRNA	House keeping gene		Hs99999901_s1
GAPDH	House keeping gene		*Hs02786624_g1*
AIM2	Cytosolic DNA sensor	Yu and Levine[1]	*Hs00915710_m1*
AKT1	Controls innate immune cell development and function	Zhang et al[2]	*Hs00178289_m1*
CHUK (IKK)	Inhibitor of nuclear factor kappa-B kinase subunit alpha (IKK-α)	Llona-Minguez et al[3]	*Hs00989497_m1*
CXCL11	Interferon inducible T cell alpha chemoattractant	Ventor et al[4]	*Hs00171138_m1*
DHX36	Enhances RIG-I Signaling	Yoo et al[5]	*Hs00325797_m1*
DDX58	Antiviral, IFN signalling , innate immune receptor	Pulendran et al[6], Querec et al[7]	*Hs01061436_m1*
EIF2AK 2	Antiviral, IFN signalling	Querec et al[7] Gaucher et al[8]	*Hs00169345_m1*
IFI16	Interferon Gamma Inducible Protein 16	Trapani et al[9]	*Hs00986757_m1*
IFIH1	Antiviral, IFN signalling, innate immune receptor	Pulendran et al[6] Querec et al[7]	*Hs00223420_m1*
IFT122	Intraflagellar Transport 122	Boubakri et al[10]	*Hs00217508_m1*
IRF3	Interferon regulatory factor 3	Collins et al[11]	*Hs01547283_m1*
IRF9	Antiviral, IFN signalling	Gaucher et al[8]	*Hs00196051_m1*
MB21D1(cGAS)	Cytosolic DNA sensor	Liang et al[12]	*Hs00403553_m1*
MRE11	Double strand break repair nuclease	Shibata et al[13]	*Hs00967437_m1*
OAS1	Antiviral, IFN signalling	Gaucher et al[8]	*Hs00973635_m1*

OAS3	Antiviral, IFN signalling	Gaucher et al[8]	*Hs00196324_m1*
MX1	Antiviral, IFN signalling	Querec et al[7], Gaucher et al[8]	*Hs00895608_m1*
PDK1	Key role in regulation of glucose and fatty acid metabolism	Tan et al[14]	*Hs01561847_m1*
PDPK1	Important role in the signalling pathways	Sato et al[15]	*Hs00928927_m1*
PLSCR1	Antiviral, IFN signalling	Querec et al[7], Gaucher et al[8]	*Hs01062171_m1*
PNPT1	Antiviral, IFN signalling , innate immune receptor	Pulendran et al[6], Querec et al[7]	*Hs01105971_m1*
STAT1	Key regulators of the early innate immune response	Decker et al[16]	*Hs01013996_m1*
STAT3	Control of inflammation and immunity	Hillmer et al[17]	*Hs00374280_m1*
TBK1	Cytosolic DNA sensor	Lam et al[18]	*Hs00179410_m1*
TLR3	pattern-recognition receptors (PRRs)	Yu and Levine[1]	*Hs01551079_g1*
TLR9	Cytosolic DNA sensor	Yu and Levine[1]	*Hs00370913_s1*
TMEM173(STING)	Stimulator of IFN response	Lam et al[18]	*Hs00736955_g1*
TP53	Tumor protein 53	Matlashewski et al[19]	*Hs01034249_m1*
TRAF6	TNF receptor associated factor (TRAF)	Youseff et al[20]	*Hs00939742_g1*

Extra references to Supplementary Table 1

1. Yu M, Levine SJ. Toll-like receptor 3, RIG-I-like receptors and the NLRP3 inflammasome: Key modulators of innate immune responses to double-stranded RNA viruses. Cytokine and Growth Factor Reviews. 2011. doi:10.1016/j.cytogfr.2011.02.001

2. Zhang Y, Wang X, Yang H, Liu H, Lu Y, Han L, Liu G. Kinase AKT controls innate immune cell development and function. Immunology. 2013. doi:10.1111/imm.12123

3. Llona-Minguez S, Baiget J, Mackay SP. Small-molecule inhibitors of IκB kinase (IKK) and IKK-related kinases. Pharmaceutical Patent Analyst. 2013. doi:10.4155/ppa.13.31

4. Venter M, Myers TG, Wilson MA, Kindt TJ, Paweska JT, Burt FJ, Leman PA, Swanepoel R. Gene expression in mice infected with West Nile virus strains of different neurovirulence. Virology. 2005. doi:10.1016/j.virol.2005.07.013

5. Yoo JS, Takahasi K, Ng CS, Ouda R, Onomoto K, Yoneyama M, Lai JC, Lattmann S, Nagamine Y, Matsui T, et al. DHX36 Enhances RIG-I Signaling by Facilitating PKR-Mediated Antiviral Stress Granule Formation. PLoS Pathogens. 2014. doi:10.1371/journal.ppat.1004012

6. Pulendran B. Learning immunology from the yellow fever vaccine: Innate immunity to systems vaccinology. Nature Reviews Immunology. 2009. doi:10.1038/nri2629

7. Querec TD, Akondy RS, Lee EK, Cao W, Nakaya HI, Teuwen D, Pirani A, Gernert K, Deng J, Marzolf B, et al. Systems biology approach predicts immunogenicity of the yellow fever vaccine in humans. Nature Immunology. 2009. doi:10.1038/ni.1688

8. Gaucher D, Therrien R, Kettaf N, Angermann BR, Boucher G, Filali-Mouhim A, Moser JM, Mehta RS, Drake DR, Castro E, et al. Yellow fever vaccine induces integrated multilineage and polyfunctional immune responses. Journal of Experimental Medicine. 2008. doi:10.1084/jem.20082292

9. Trapani JA, Dawson M, Apostolidis VA, Browne KA. Genomic organization of IFI16, an interferon-inducible gene whose expression is associated with human myeloid cell differentiation: correlation of predicted protein domains with exon organization. Immunogenetics. 1994. doi:10.1007/BF00177824

10. Boubakri M, Chaya T, Hirata H, Kajimura N, Kuwahara R, Ueno A, Malicki J, Furukawa T, Omori Y. Loss of ift122, a Retrograde Intraflagellar Transport (IFT) complex component, leads to slow, progressive photoreceptor degeneration due to inefficient opsin transport. Journal of Biological Chemistry. 2016. doi:10.1074/jbc.M116.738658

11. Collins SE, Noyce RS, Mossman KL. Innate Cellular Response to Virus Particle Entry Requires IRF3 but Not Virus Replication. Journal of Virology. 2004. doi:10.1128/jvi.78.4.1706-1717.2004

12. Liang Y, Peng H. STING-cytosolic DNA sensing: The backbone for an effective tumor radiation therapy. Annals of Translational Medicine. 2016. doi:10.3978/j.issn.2305-5839.2015.12.48

13. Shibata A, Moiani D, Arvai AS, Perry J, Harding SM, Genois MM, Maity R, van Rossum-Fikkert S, Kertokalio A, Romoli F, et al. DNA Double-Strand Break Repair Pathway Choice Is Directed by Distinct MRE11 Nuclease Activities. Molecular Cell. 2014. doi:10.1016/j.molcel.2013.11.003

14. Tan J, Li Z, Lee PL, Guan P, Aau MY, Lee ST, Feng M, Lim CZ, Lee EYJ, Wee ZN, et al. PDK1 signaling toward PLK1-MYC activation confers oncogenic transformation, tumor-initiating cell activation, and resistance to mTOR-targeted therapy. Cancer Discovery. 2013. doi:10.1158/2159-8290.CD-12-0595

15. Sato S, Fujita N, Tsuruo T. Regulation of kinase activity of 3-phosphoinositide-dependent protein kinase-1 by binding to 14-3-3. Journal of Biological Chemistry. 2002. doi:10.1074/jbc.M205141200

16. Decker T, Stockinger S, Karaghiosoff M, Müller M, Kovarik P. IFNs and STATs in innate immunity to microorganisms. Journal of Clinical Investigation. 2002. doi:10.1172/jci15770

17. Hillmer EJ, Zhang H, Li HS, Watowich SS. STAT3 signaling in immunity. Cytokine and Growth Factor Reviews. 2016. doi:10.1016/j.cytogfr.2016.05.001